

Functional Genomics Characterization of Six4 During Skeletal Myogenesis

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Abstract

Adult skeletal muscles can regenerate after injury due to the presence of satellite cells, a quiescent population of myogenic progenitor cells characterized by expressing the transcription factor Pax7. Once activated, satellite cells repair the muscle damage and replenish the stem cell niche due to the coordinated function of several transcription factors including Pax7 and the myogenic regulatory factors (MRFs). MRFs are skeletal muscle-specific transcription factors that can convert non-muscle cells into the myogenic lineage. MRFs are known to cooperate with other transcription factors in regulating the complex transcriptional network driving myogenic differentiation of muscle progenitors. The Six4 transcription factor emerges as a strong candidate for cooperating with MRFs. Six4 is expressed in skeletal muscles; the lack of a muscle development phenotype in Six4-null mice has been attributed to compensation by other Six family members. However, this did not exclude a critical role for Six4 during muscle development as Six1;Six4 double mutant mice show a more severe muscle phenotype than Six1 mutant mice. Nevertheless, the role of Six4 during adult muscle regeneration has never been addressed. I combined a partial loss-of-function of Six4 with high-throughput approaches to address the role of Six4 during adult skeletal muscle regeneration. I observed an important function of Six4 during muscle regeneration in vivo and in in vitro cell models. Using RNA interference assays against Six4 in tibialis anterior muscle regeneration after cardiotoxin-induced muscle damage, I observed for the first time that Six4 plays a role in proper muscle regeneration. The ability of the MRF MyoD, a central regulator of skeletal myogenesis, to convert a non-muscle cell model into the myogenic lineage was impaired with attenuated Six4 expression. I employed genome-wide approaches by combining ChIP-sequencing with gene expression profiling and identified a set of muscle genes coordinately regulated by both Six4 and MyoD. Throughout

the genome, the cooperation between Six4 and MyoD was associated with binding of the H3K27me3 demethylase Utx and depletion of the H3K27me3 repressive chromatin mark. Together, these results reveal an important role for Six4 during adult muscle regeneration, and suggest a widespread mechanism of cooperation between Six4 and MyoD that correlates with modifying the epigenetic landscape of the regulatory regions of a large set of genes needed for efficient myogenesis.

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List of Abbreviations

AD	transcriptional activation domain
Afap111,	actin filament associated protein 1-like1
Ankrd1	ankyrin repeat domain 1
APC	anaphase-promoting complex
APCcdh1	CDH1 dependent Anaphase-Promoting Complex
Ash2L	Ash2 (absent, Small, Or Homeotic)-Like (Drosophila)
ATCC	American Type Culture Collection,
ATP2a1	calcium transporting ATPase, fast twitch 1
Baf60c	Brg1-associated factor 60C
bHLH	basic-helix-loop-helix
BMP-4	bone morphogenetic protein 4
Brg1	transcription activator BRG1 or ATP-dependent helicase Smarca4
Cacng1	calcium channel, voltage-dependent, gamma subunit 1
Capn2	calpain-2 catalytic subunit
Ccl3	C-C motif chemokine 3
Cd68	cluster of differentiation 68, also known as macrosialin
ChIP	chromatin immunoprecipitation
ChIP-on-chip	chromatin immunoprecipitation followed by microarray (chip)
ChIP-seq	chromatin immunoprecipitation followed high throughput sequencing
Chrna1	cholinergic receptor, alpha 1 subunit
Chrng	cholinergic receptor, gamma subunit
c-Met	Met proto-oncogene
CSA	cross-sectional area
CTX	cardiotoxin
Dach	dachshund
dKO	double knockout
DM	differentiation medium
DMEM	Dulbecco's Modified Eagle's Medium with high glucose
DSHB	Developmental Studies Hybridoma Bank,
Dusp6	dual-specificity Phosphatase
eMHC	embryonic MHC
Eya3	eyes absent homolog 3
FAPs	fibro/ adipogenic progenitors
FBS	fetal bovine serum
FDR	false discovery rate
FGF	fibroblast growth factors
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GM	growth medium
Gpr20	G protein-coupled receptor 20
H&E	Hematoxylin and eosin
H3K27ac	histone 3 acetylated on lysine 27

H3K27me3	histone 3 trimethylated on lysine 27
H3K36me3	histone 3 trimethylated on lysine 36
H3K4me1	histone 3 monomethylated on lysine 4
H3K4me3	histone 3 trimethylated on lysine 4
H3K9ac	histone 3 acetylated on lysine 9
H4R3me2	histone 4 dimethylated on arginine 3
HD	Six-type homeodomain
Hes6	hairy and enhancer of split 6
Hey1	hairy and enhancer of split-related 1
Heyl	hairy and enhancer of split-related 3
HGF	hepatocyte growth factor
HoxD10	homeobox protein Hox-D10
Hrc	histidine rich calcium binding protein
IF	immunofluorescence
IGF1	insulin-like growth factor 1
IL1 β	interleukin-1 beta
IL4	interleukin-4
IL6	interleukin-6
IP	immunoprecipitation
IVT	<i>in vitro</i> translated
KDM6	lysine demethylase family 6
KMT2	lysine methyltransferase family 2
KO	knockout
Lbx1	ladybird homeobox homolog 1
Ldb3	Lim domain binding 3
Luc	firefly luciferase
MACS	Model-based Analysis of ChIP-Seq
MAPK	mitogen-activated protein Kinase
MB	myoblasts
Mef2a	myocyte-specific enhancer factor 2A
Mef2c	myocyte-specific enhancer factor 2C
MHC	myosin heavy chain
MLL	histone-lysine N-methyltransferase MLL
MRF	Myogenic Regulatory Factor
Mrf4	myogenic regulatory factor 4
MT	myotubes
Myc	v-Myc avian myelocytomatosis viral oncogene homolog
Myf5	myogenic factor 5
Myh3	myosin-3
Mylpf	myosin regulatory light chain 2, skeletal muscle
MyoD	myogenic differentiation 1 (also known as Myod1)
MyoD-CER	MyoD core enhancer region
Myog	myogenin

Myoz2	myozenin 2
NeuroD2	neurogenic differentiation factor 2
Nfat	nuclear factor of activated T-cells
Obscn	obscurin
P/S	Penicillin/Streptomycin
PAVIS	Peak Annotation and Visualization
Pax3	paired box protein Pax-3
Pax7	paired box protein Pax-7
PBS	phosphate-buffered saline
PBS-T	PBS, 0.1% Triton X-100 (v/v)
Pbx1	pre B-cell leukemia transcription factor 1
Pde4dip	phosphodiesterase 4D interacting protein
PDGF	platelet-derived growth factor
Pdgfra	alpha-type platelet-derived growth factor receptor
PEI	polyethylenimine
Pkia	cAMP-dependent protein kinase inhibitor alpha
PRC2	Polycomb repressor complex 2
Prkri	protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)
Prmt5	Protein arginine N-methyltransferase 5
Psm6	proteasome subunit, beta type, 6
PTM	post-translational modifications
Ptpc	receptor-type tyrosine-protein phosphatase C, also known as CD45
PWM	position weight matrix
Ren	Renilla luciferase
RNA pol II	RNA polymerase II
RT	room temperature
SD	Six domain
Setd7	SET domain containing (lysine methyltransferase) 7
shLuc	shRNA directed against the irrelevant Luciferase gene
shNS	control scrambled shRNA
shRNA	Short hairpin RNA
shSix1	shRNA directed against Six1
shSix4	shRNA directed against Six4
siRNA	small interfering RNA
Six1	homeobox protein SIX1
Six4	homeobox protein SIX4
Sox11	SRY (sex determining region Y)-Box 11
Spry1	Sprouty homolog 1
SWI/SNF	SWItch/Sucrose Non-Fermentable
TA	tibialis anterior
Tbp	TATA-box-binding protein

TBS	Tris-buffered saline,
TF	transcription factors
TGF β	transforming growth factor beta
TNF α	tumor necrosis factor alpha
Tnni1	troponin I, skeletal, slow 1
Tnni2	troponin I, skeletal, fast 2
Tnnt2	troponin C2, fast
Trim54	tripartite motif containing 54, also known as Murf3
TSS	transcriptional start site
Utx	Ubiquitously-transcribed tetratricopeptide repeat protein, X-linked
V109E	valine residue mutated to a glutamic acid residue
Vgll2	vestigial-like gene 2
Wt	wild type

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Chapter 1: General Introduction

Skeletal muscle is a highly intricate and heterogeneous tissue comprising almost half of the body mass (1). Skeletal muscles are responsible for locomotion, maintaining posture and enabling breathing, made possible due to the presence of contractile proteins within muscle fibers, which are also called myofibers. Myofibers are formed by the fusion of many myogenic precursor cells named myoblasts in a process known as myogenesis. Vertebrate skeletal myogenesis is a highly regulated process involving the expression of a large set of genes that enable the process and allow muscle function throughout sequential developmental stages from embryonic to adult. The expression of these genes is precisely coordinated by a group of transcription factors (TFs) acting independently, synergistically or antagonistically in a temporally-regulated manner to ensure proper muscle formation and regeneration. Improper expression or malfunction of one or more of these factors or genes regulated by them could be embryonically lethal or result in devastating skeletal muscle abnormalities. Comprehensive understanding of the role of all players of muscle formation can help find ways to cure diseases such as muscular dystrophies and muscle wasting with old age and ensure a better lifestyle.

1.1 Skeletal myogenesis

1.1.1 Embryonic and fetal myogenesis

All vertebrate skeletal muscles are derived from the mesoderm, one of the three early germ layers formed during embryogenesis. All skeletal muscles, except for some head muscles, are formed from progenitor cells within somites (2). Somites are spherical epithelial structures that bud off from the paraxial mesoderm all along the neural tube following an anterior-posterior progression. Somites can give rise to distinct cell lineages: skeletal muscles, axial skeleton and dorsal dermis, as well as smooth muscles, endothelial cells and

brown fat. The dorsal portion of the somite or the dermomyotome and an underlying compartment, known as the myotome, are the source of skeletal muscles (3). The myotome is initially formed when post-mitotic myogenic cells (or myocytes) expressing muscle-specific myogenic TFs translocate from the lateral edges of the dermomyotome directly underneath, thus contributing to the first trunk fibers formed (4). At later stages, myogenic proliferative progenitor cells invade the myotome from the medial edge of the dermomyotome and form secondary fibers of the myotome (5, 6). In absence of the myotome, myogenesis still proceeds relatively unaffected (7). Muscles are formed from the dermomyotome successively as the embryo develops in two sequential stages designated as embryonic and fetal myogenesis (Fig. 1.1A). Embryonic myogenesis forms the primary muscle fibers while fetal myogenesis adds fibers to the embryonic ones in a process referred to as secondary myogenesis, thus contributing to muscle growth and maturation. Innervation also occurs during the same period (8). Prenatal and postnatal myogenesis have been attributed to muscle progenitors arising from the central domain of the dermomyotome co-expressing the paired-box homeodomain TFs Pax3 and Pax7 (5, 6, 9). Alternatively, progenitor cells expressing Pax3 and originating from the hypaxial domain of the dermomyotome contribute to limb progenitor cells (10) (Figure 1.1B). Lineage-tracing and ablation experiments focusing on these progenitor cells showed Pax3 requirement for embryonic myogenesis and Pax3/Pax7 redundant function during fetal myogenesis (11). At the end of fetal myogenesis satellite cells, which are also derived from these Pax3⁺/Pax7⁺ or Pax3⁺ progenitor cells, assume their position between the myofiber plasma membrane and the basal lamina and are responsible for postnatal muscle growth and regeneration (5, 6, 10, 11).

The dermomyotome is nominally divided into epaxial and hypaxial domains marked by the expression of the transcription factors En1 and Sim1 respectively (12), and named accor-

Figure 1-1 Skeletal myogenesis in the mouse

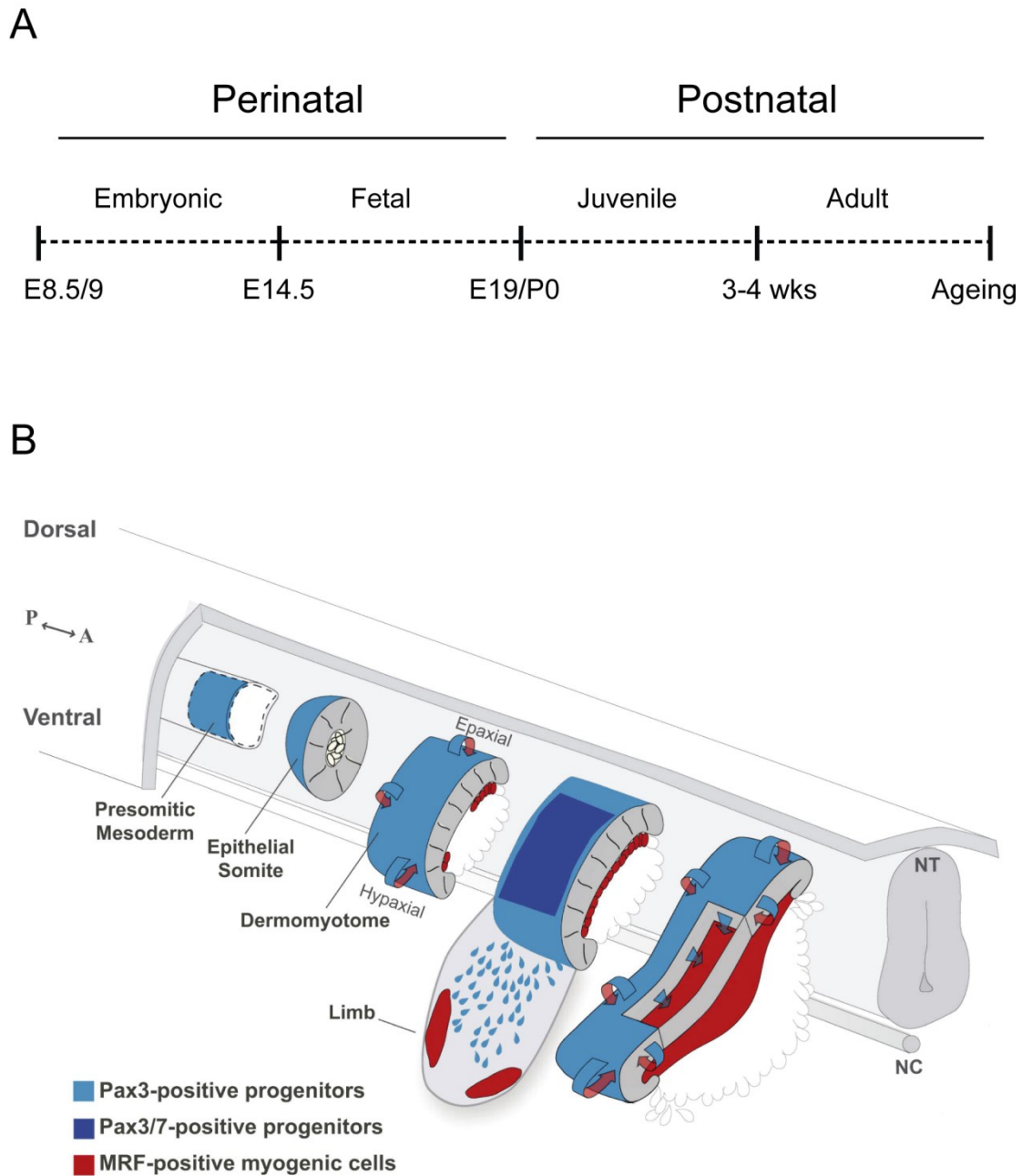


Figure 1.1. Skeletal myogenesis in the mouse

A) Scheme of the timeline of different stages of murine skeletal myogenesis.

B) Prenatal skeletal myogenesis showing Pax3-positive progenitor cells derived from the ventrolateral lip of the dermomyotome to form the limb muscles. The migrating progenitors remain MRF-negative till they reach their destination. A, anterior; P, posterior; NT, neural tube; NC, notochord.

Fig. 1B is modified from Buckingham and Rigby, 2014 (14).

ding to the innervation pattern of distinct muscles derived from these domains (4). Cells from the dorsomedial lip of the dermomyotome migrate underneath and form epaxial muscles which are located dorsally and they include the deep back muscles. Cells from the ventrolateral lip of the dermomyotome, on the other hand, form the hypaxial muscles which are located ventrally and laterally. These cells migrate underneath and form the diaphragm and the body wall muscles, or delaminate and migrate laterally to form the limb muscles (Fig. 1.1B) (3, 13). In both epaxial and hypaxial muscles, myogenic commitment and the onset of myogenic differentiation are marked by the expression of the myogenic regulatory factors (MRFs) Myf5 and MyoD (14).

Signals from surrounding tissues affect prenatal skeletal myogenesis. For example, signals from the neural tube and the notochord can initiate epaxial myogenesis, while those from the overlying surface ectoderm and neighbouring lateral plate mesoderm affect hypaxial myogenesis (8). Both canonical/ β -catenin and non-canonical/ β -catenin-independent Wnt signalling produced by the neural tube and surface ectoderm activate embryonic myogenesis by regulating the formation of the dermomyotome and the myotome (15-17). For example, non-canonical Wnt11 and Wnt6 are required to maintain the essential epithelial nature of the dorsomedial and ventrolateral lips of the dermomyotome respectively (16, 17), while canonical Wnt/ β -catenin signalling mediated by Wnt1, Wnt3a, Wnt4 or Wnt7a are important for muscle determination (inducing MyoD or Myf5 expression) in addition to the myotome and the dermomyotome formation (15, 18-21). Sonic hedgehog signals secreted by the notochord and the neural tube are important for epaxial muscle specification, activation of Myf5 expression (22) and even for later stages of hypaxial myogenesis (23). Notch and fibroblast growth factors (FGFs) signalling seem to exert opposing function to that of Wnt

and Sonic hedgehog by mediating expansion of the progenitor cell pool and sometimes by inhibiting cell determination in the myogenic lineage (14, 24).

Along with the extrinsic signals, different intrinsic signals are involved in the spatiotemporal regulation of developmental myogenesis. Many TFs regulate epaxial and hypaxial myogenesis such as the paired domain TFs Pax3 and Pax7, the *sine oculis*-related homeobox TFs Six1 and Six4, and their eyes absent (Eya) cofactors, Eya1 and Eya2 (4). Limb muscle formation is a special case because it requires long-range migration of the precursor cells. These cells give rise mainly to skeletal muscles, yet they don't express the skeletal muscle specific MRFs until they reach their target destination. Limb muscle formation occurs in four sequential steps: 1) generation of muscle precursor cells in the dermomyotome, 2) delamination and migration of these progenitor cells to their correct destination, 3) cell proliferation, and 4) cell differentiation into muscle (25). Pax3 seems to be involved in regulating the first three steps. Myogenic progenitor cells destined to the limb muscles are marked by expression of the paired domain TF Pax3 (26). Pax3 transactivates its downstream target gene coding for the receptor tyrosine kinase *c-Met* (27) which in turn regulates progenitor cell delamination and migration (26, 28-30), guided by interaction with its ligand, the hepatocyte growth factor (HGF) which is expressed in the neighbouring mesenchymal cells throughout the migration route (25). Another Pax3 target is the homeobox TF Lbx1 (31). Lbx1 is exclusively expressed in the hypaxial migrating myogenic cells and plays important role in the migration but not in the delamination of these cells (32). Lbx1 activates the chemokine receptor CXCR4 which interacts with its ligand SDF1 present in the adjacent limb mesenchyme (33). Once they reach their destination, progenitor cells expressing Pax3 will give rise to myogenic but not endothelial cells (11). These cells then proliferate due to the signalling activity of FGFs (34) and β -catenin (11). Pax3 plays a role in

this signalling pathway by controlling the FGF receptor Fgfr4 (35). Finally, Pax3 induces Myf5 and MyoD expression (35-37) and gets down-regulated with the expression of the differentiation myogenic factors (26). With the expression of Myf5 and MyoD, the precursor cells become committed to the myogenic lineage as myoblasts which can proliferate extensively before exiting the cell cycle and differentiating into myofibers. Not all myogenic progenitor cells differentiate; few cells remain as reservoirs and take their place between the myofiber plasmalemma and the newly formed basal lamina as quiescent satellite cells at the end of the fetal stage of myogenesis. These cells will contribute to muscle growth and regeneration during postnatal life (5, 6, 8, 10).

1.1.2 Postnatal myogenesis and muscle regeneration

Postnatal myogenesis takes place during the first postnatal day and onward and it represents two phases of myogenesis: neonatal or juvenile myogenesis and muscle regeneration. Juvenile myogenesis occurs during the first three weeks of a mouse life allowing muscle growth and maturation (38) and resulting in approximately threefold increase in muscle mass (39). Muscle regeneration, on the other hand, occurs in response to extensive physical exercises, injury or disease. The ability of muscle to regenerate was first reported by Studitsky when he minced an entire muscle and replaced it in situ (40). Both juvenile myogenesis and muscle regeneration are possible due to the presence of satellite cells, muscle stem cells named after their anatomical location between the muscle fibre plasmalemma and the surrounding basal lamina (41). Other cells have been reported to contribute to muscle regeneration including bone marrow-derived progenitors (42, 43) pericytes (44), PW1-expressing interstitial cells (45), mesoangioblasts (46), AC133⁺ stem cells (47) and muscle side population expressing Syndecan-4 and Pax7 (48). However, the fact that no regeneration was observed after ablation of satellite cells argues against a

contribution of these cell types, or others remaining to be characterized, to adult muscle repair after injury. The cells perhaps play a minor, supportive role during regeneration instead (49-52). In the absence of satellite cells, muscle regeneration will be impaired and the injured skeletal muscle tissue will be infiltrated instead by inflammatory and adipogenic cells (49).

Satellite cells originate from somites (5, 6, 9, 10, 53). After the juvenile phase, satellite cells number drops from 30% of total myonuclei per myofiber to less than 5% (39) and they become mitotically quiescent under normal physiological conditions (54), an essential step to maintain the satellite cells function until they are needed for tissue homeostasis or repair. Satellite cells enter a reversible quiescent state maintained by an upregulation of a large set of genes including inhibitors of the cell cycle and myogenesis in comparison to cycling myoblasts as revealed by microarray analysis (55, 56). The Notch signalling pathway, activated by the forkhead TF Foxo3 (57), induces the expression of Hes1 (also known as Hey1) and Hes3 (or Hairy and Enhancer of Split-related 1 and 3 respectively) which are involved in shifting satellite cells into quiescence (58). Under resting healthy state, all satellite cells of adult muscles are characterized by the expression of Pax7 (59) and absence of MyoD expression (60), while satellite cells of a few trunk muscles, notably the diaphragm, also express Pax3 (61, 62). Pax7 function is indispensable for adult muscle regeneration (49-52, 59, 62, 63). Upon muscle injury, the damaged tissue will release different growth factors like insulin-like growth factor 1 (IGF1) (64) that will inhibit the cell cycle inhibitor p27^{Kip1} (65) and promote entry of the quiescent satellite cells into the cell cycle (66). Regenerating muscle tissue also expresses high level of FGF2 which will activate the ERK1/2 pathway and control the transition of satellite cells from G1 into the S phase (67). Consequently, the activated satellite cells will undergo mitosis giving rise to myoblasts, highly proliferative

myogenic progenitor cells expressing the MRFs Myf5 and MyoD (68-70). These cells can extensively proliferate especially between the second and fifth day after a massive injury (71, 72) so that they can replace all the myofiber myonuclei within 4-5 days (73). The p38 α / β family of mitogen-activated protein kinases (MAPKs) reversibly regulates the transition of satellite cells from quiescence to activation by inducing MyoD expression and regulating proliferation of satellite cells (74). The p38 α MAPK activates JNK/cJun pathway and this in turn activates cyclin D1 and promotes cell cycle progression (75).

As stem cells, satellite cells can undergo symmetrical cell division generating two identical daughter cells, or an asymmetrical cell division generating one daughter cell committed to differentiate and one self-renewing daughter cell. The satellite cells have an outstanding ability to self-renew providing myogenic cells for differentiation while maintaining a homeostatic level of uncommitted mother cells (76). They can undergo several cycles of regeneration while maintaining a constant number of satellite cells (77). The nature of satellite cells division is tightly regulated and controlled by different mechanisms including the satellite cells niche components. For example, the cytokine Wnt7a, acting through the non-canonical Wnt pathway, signals through its receptor Frizzled7 to promote satellite cells pool expansion by stimulating symmetrical division of Myf5-negative satellite stem cells (78). The extracellular matrix glycoprotein fibronectin stimulates Wnt7a signalling by binding Frizzled7-Syndecan-4 co-receptor complex (77). Moreover, the ECM component Collagen VI is a key component of satellite cell niche playing a critical role in the satellite cells self-renewal (79).

Both Pax7 and Pax3 can upregulate genes promoting cell proliferation and repress genes involved in terminal muscle differentiation (80, 81). Among these genes are Myf5 and MyoD

which are rapidly induced upon satellite cells activation (82, 83) by both Pax7 and Pax3 (36, 84, 85). A subpopulation of satellite cells will down-regulate the expression of MyoD and return to quiescence to maintain the satellite cell pool (69), in a process dependent on the expression of Sprouty1, a receptor tyrosine kinase signalling inhibitor, which promotes cell cycle exit by inhibiting the ERK signalling pathway (86). Notch signalling activity is also needed for satellite cells return to quiescence (87) by inhibiting MyoD expression (88), inhibiting Mef2c function and its cooperation with MyoD and myogenin (89), promoting Pax7 expression (90), and regulating the expression of the basal lamina components and adhesion molecules (91). Angiopoietin1/ Tie2 signalling acting through ERK1/2 pathway promotes the return of a subset of satellite cells to quiescence (92). Dusp6 also acts through ERK pathway to ensure the return of satellite cells to quiescence (93). However, the majority of activated myogenic cells or myoblasts will undergo several rounds of cell divisions (73, 94) before they down-regulate Pax7 expression (81) and upregulate the expression of the MRFs myogenin and MRF4 to execute terminal muscle differentiation. Terminal muscle differentiation involves exiting the cell cycle followed by fusion of myocytes with the host fibers or with each other to form new myofibers to regenerate the damaged tissue (95). The main steps of muscle regeneration are summarized in Fig. 1.2.

Maintenance of the satellite cell pool can be attributed in part to the heterogeneity of their population presumed to contain only a small proportion of self-renewing “true” stem cells and a large proportion of myogenic progenitor cells capable of limited rounds of cell division before their terminal differentiation into multinucleated myofibers (96). These cells seem to differ in their proliferation capabilities and self-renewal potentials (97), fidelity in retaining the parental DNA stands (98, 99), commitment to the myogenic lineage (86, 100), and transplantation potential (76, 86). Satellite cells are a mixture of 80% fast-dividing cells

Figure 1-2 Adult skeletal muscle regeneration

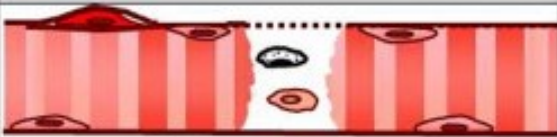
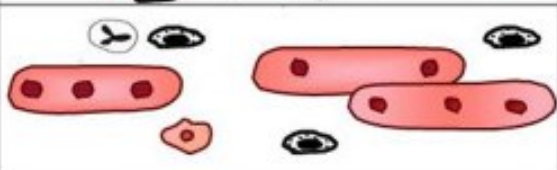
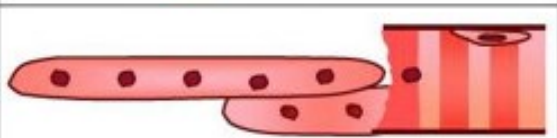
	~ Day	Activity	Schematic representation
Inflammation & proliferation	0	Injury - membrane damage. Necrosis and myoblast activation	
	1-7	Inflammation and myoblast proliferation	
Formation, growth and maturation of myotubes and myofibres	3-7	Myoblasts differentiate and form myotubes Myotubes fuse together	
	7-10	Myotubes fuse with end of damaged myofibres	
	7-21+	Growth and maturation of regenerated portion of myofibre	

Figure 1.2. Adult skeletal muscle regeneration.

Summary of the different steps during injury-induced muscle regeneration (95).

and 20% of slow-dividing reserve cells as revealed by radioisotope-labelling of growing rat muscles (97). They represent two populations based on genetic lineage tracing of Myf5 expression: 1) satellite cells that have never expressed Myf5 and these are the “true” satellite cells representing 10% of the satellite cell population, 2) satellite cells that have expressed Myf5 and these are the committed myogenic cells representing 90% of the satellite cell population (86). Their commitment to the myogenic lineage was correlated with asymmetrical sequestration of the polarity-establishing Par complex and subsequent induction of the p38 α / β MAPK signalling pathway in the myogenic committed cells leading to MyoD induction (100) and epigenetic silencing of Pax7 expression (101). They differ in their expression level of Pax7 with Pax7-high satellite cells being more resistant to differentiation with lower metabolic activity and slower proliferation compared to Pax7-low satellite cells. They also exhibit asymmetric DNA segregation during cell division compared to random DNA segregation observed in Pax7-low satellite cells (102).

In their niche, satellite cells are surrounded by different cells and tissues that affect their function under normal quiescent homeostatic conditions and upon exposure to damage. In addition to the myofiber extracellular matrix of basal lamina, satellite cells are usually close to capillaries and often interact with endothelial cells during differentiation (103). Different types of interstitial cells, fibrogenic cells and immune cells are found spread across the adult muscle tissue under normal physiological conditions (77). Macrophages, mast cells as well as monocytes are the most abundant resident immune cells (104). Resident circulatory monocytes can act as patrolling cells under healthy conditions checking for any potential tissue damage and secreting chemoattractive molecules upon muscle injury (105). Several types of immune cells coordinate their function coincidentally or sequentially to ensure

removal of the damaged cells, regeneration of the muscle tissue and restoration of tissue homeostasis (77). Chemokines and cytokines produced by resident immune cells attract neutrophils (106, 107) and eosinophils (108) and these in turn will secrete other cytokines and chemokines to recruit more monocytes (109, 110) or stimulate fibro/adipocyte progenitors that help clear the cellular debris (108).

After the first day of injury, monocytes become the predominant immune cells in the damaged muscle tissue. Two different subtypes of monocytes infiltrate the muscle tissue: Ly-6C⁺ monocytes which predominate in the first days after the damage, and Ly-6C⁻ monocytes that predominate later on during the regenerative stage (111). Once monocytes invade the tissue, they differentiate into macrophages and consequently there are two main subtypes of macrophages: the pro-inflammatory M1 macrophages regulating the early phase of muscle damage, and the anti-inflammatory M2 macrophages regulating the second regenerative phase of muscle damage (111-113). M1 macrophages secrete pro-inflammatory cytokines such as IL1 β , IL6 and TNF α which promote proliferation of myogenic cells and limit their differentiation, while M2 macrophages secrete IL4 and IGF1 and these promote myogenic differentiation (112, 114, 115). Therefore, the various populations of immune cells perform specific functions at specific stages during muscle regeneration. Impaired function, imbalance in immune cell populations or even a sustained or delayed signal of any of them can impair muscle regeneration (113, 116-119).

1.2 Transcription factors regulating myogenesis

Both prenatal and postnatal myogenesis are possible due to the coordinated function of several extrinsic and intrinsic TFs and cofactors acting temporally and sequentially to ensure proper muscle formation. Several families of TFs are critical for muscle formation; among

the most important ones are the Pax TFs, the MRFs and the Six TFs. The expression profile of these TFs during myogenesis is summarized in Fig. 1.3.

1.2.1 Pax3 and Pax7

Pax proteins are a family of TFs characterized by the presence of a paired domain, an octapeptide motif, and sometimes a complete or partial homeobox domain. The family comprises nine members playing key roles in the development of various tissues and organs during embryogenesis, with Pax3 and Pax7 being crucial for skeletal muscle development (120). The main function of this family of TFs is to maintain stem cell properties by limiting lineage specification and inhibiting apoptosis and terminal differentiation (121). Both Pax3 and Pax7 are involved in myogenic lineage specification, with Pax3 acting primarily during prenatal stages of myogenesis (122) and Pax7 acting primarily during postnatal stages of myogenesis (59). Pax3 is expressed in mice as early as E8 (day 8 post coitus) at the level of the entire somite, and then it becomes restricted to the dermomyotome and later to the dorsomedial and ventrolateral dermomyotomal lips. Pax7 expression starts later (E9) and it is restricted to the central region of the myotome. At the limb level, Pax3 is also expressed before Pax7 and its expression is downregulated before birth when MyoD and myogenin are turned on (26, 28, 62, 122). However, some body wall satellite cells (62) and some adult myogenic interstitial cells (123) remain Pax3-positive. Pax7, in turn, maintains its expression during fetal and adult myogenesis (62) marking the satellite cell population (59). Unlike MRFs, Pax3 and Pax7 are not muscle-specific TFs as they are also expressed in the dorsal neural tube, neural crest and some areas of the brain (14).

The importance of Pax3 and Pax7 function during myogenesis was revealed by loss-of-function and gain-of-function experiments. Pax3 mutant mice or *spotch* mice have dramatic

Figure 1-3 Main transcription factors controlling skeletal myogenesis

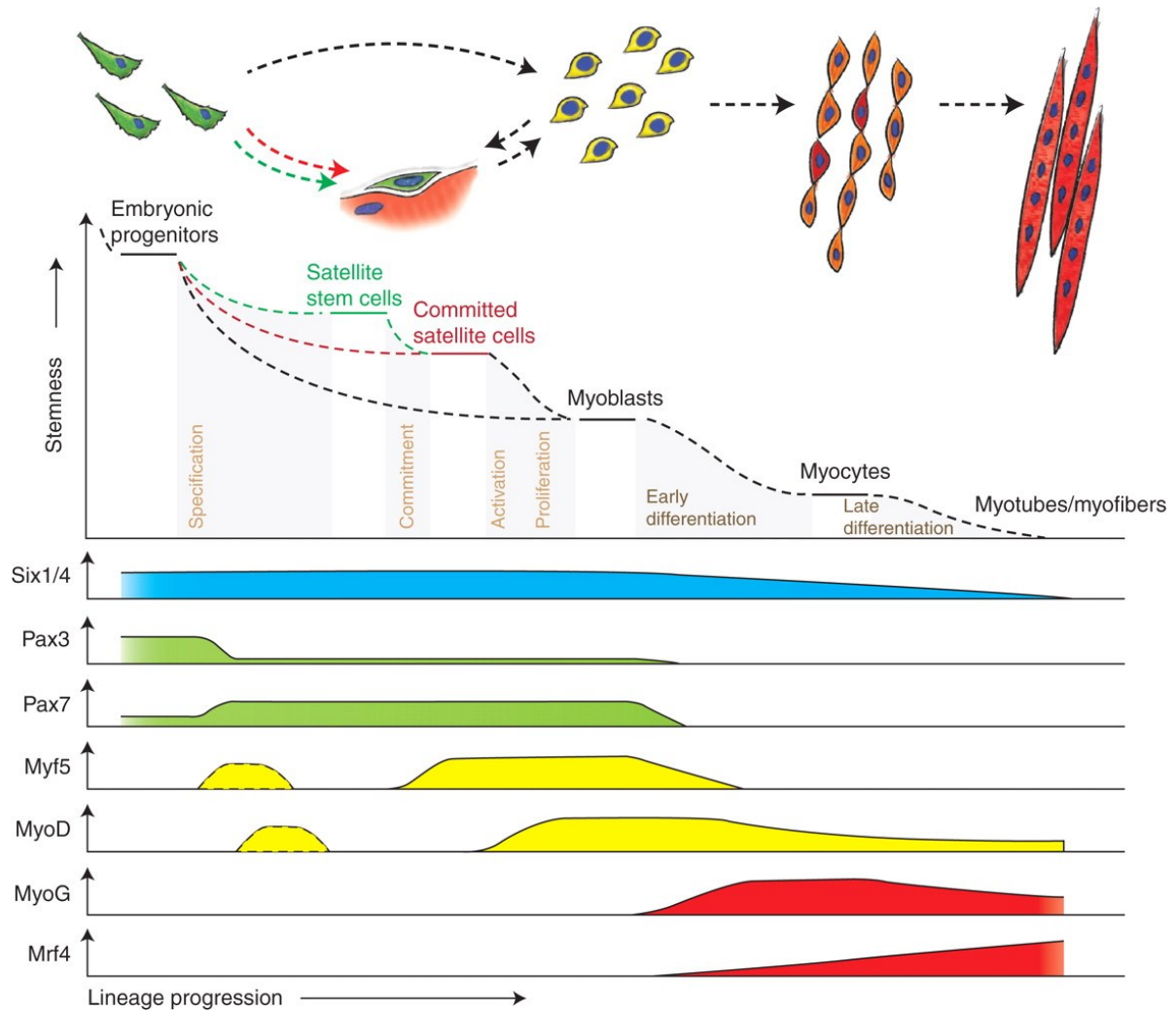


Figure 1.3. Main transcription factors controlling skeletal myogenesis.

During myogenic progenitor cell differentiation into myofibers, Pax3 and Pax7 specify the myogenic progenitors while MRFs commit them into the myogenic lineage and control their further differentiation into myotubes. Six1 and Six4 are expressed during all the steps of muscle formation (24).

function and gain-of-function experiments. Pax3 mutant mice or *spotch* mice have dramatic loss of limb muscles but intact trunk muscles (26, 29) implicating the critical role of Pax3 in limb muscle formation, in part by regulating limb muscle progenitor migration and c-Met expression (28). The role of Pax3 in migration of muscle progenitors cannot be compensated by replacing Pax3 with Pax7 (30). Gain-of-function of Pax3 in mice by replacing Pax3 with Pax3-FKHR, a chimeric protein present in 60% of alveolar rhabdomyosarcomas, rescues the defects observed in Pax3-loss-of-function by activating c-Met signalling (124). Moreover, gain-of-function experiments showed that Pax3 is necessary and sufficient to drive the skeletal myogenic program in a model of pluripotent stem cells by inducing the expression of Six1 with its cofactor Eya2 before MyoD and myogenin induction (125). Indeed, Pax3 expression in ES cells enhanced the ability to form paraxial mesoderm and skeletal muscles and showed successful engraftment into *mdx* mice, an animal model of Duchenne's muscular dystrophy (126).

Pax7 loss-of-function experiments confirmed that Pax7 is dispensable for embryonic and fetal myogenesis, but plays a crucial role during adult muscle regeneration (49, 59, 62, 63). This role was recently challenged by the unexpected finding that only juvenile muscle growth is compromised by eliminating Pax7-expressing satellite cells, while adult muscle regeneration remains unaffected (127). However, this finding was re-addressed lately by two other groups showing an indispensable role for Pax7 in adult muscle regeneration (52, 128). Similar to Pax3^{-/-} mice, Pax3^{-/-};Pax7^{-/-} mice lack limb muscles; however, they also lack embryonic and fetal axial or trunk muscles (5). This is attributed to the lack of Pax3⁺/Pax7⁺ progenitor cells responsible for all embryonic and fetal myogenesis in axial and limb muscles and the source of satellite cells and adult myogenesis. In absence of Pax3 and Pax7, these

progenitor cells assume a non-myogenic lineage. However, generation of the primary myotome is unaffected in these animals (6, 9).

Although binding identical DNA motifs with different affinities, Pax7 and Pax3 display different genomic DNA binding profiles and gene regulation during myogenesis (80), partially explaining their non-redundant functions. This can explain why Pax7 cannot compensate for Pax3 function in limb muscle progenitor migration (30) and Pax3 cannot compensate for Pax7 anti-apoptotic function in satellite cells (62). Pax7 plays a critical role in regulating transcription in adult skeletal muscle cells partly by controlling the expression of the MRF Myf5 where Pax7 binds to a paired motif in the Myf5 enhancer (129). Pax7 recruits the MLL histone methyltransferase mediated by a Pax3/7 binding protein bridging Pax7 and Wdr5 complex (130) to the promoter of *Myf5* (83), a function that requires methylation of Pax7 by the arginine methyltransferase Carn1 (131).

1.2.2 Myogenic regulatory factors (MRFs)

Although different upstream transcriptional regulatory networks might control the formation of different muscles during embryogenesis, all of them eventually get committed to form muscles upon the expression of skeletal muscle-specific family of transcription factors known as MRFs. MRFs are a family of basic-helix-loop-helix (bHLH) domain-containing TFs comprising four members: myogenic factor 5 (Myf5; (132)), myogenic differentiation 1 (Myod1, also known as MyoD; (133)), Mrf4 (also known as Myf6 or herculin; (134-136)) and myogenin (Myog; (137)). MyoD was the first MRF to be identified by subtractive hybridization of myogenic specific transcripts able to convert fibroblasts into muscle cells (133, 138), a revolutionary discovery that pioneered a new era of cell and tissue re-programming. The quest to identify a single transcription factor that is able to enforce the myogenic program in non-muscle cells was based on the ability of the DNA demethylating

agent 5-azacytidine to generate clones in C3H 10T1/2 fibroblasts with a skeletal muscle phenotype (139). In fact, the Myod gene is heavily methylated in C3H 10T1/2 cells but it becomes demethylated and expressed upon 5-azacytidine treatment (140). Subsequently, the other MRFs were shown to be able to convert fibroblasts into the myogenic lineage with varying potentials (132, 134-137). Additionally, MyoD was able to convert several cell types derived from the three different embryonic germ layers into muscles, including different fibroblast cell lines, primary dermal fibroblasts, adipocytes, primary chondrocytes, smooth muscle, retinal pigmented epithelial cells, as well as melanoma, neuroblastoma, osteosarcoma cells, and a hepatoma cell line (141, 142). The ability of MyoD to fully induce the myogenic program in all these cells suggested that MyoD is the “master regulatory gene” of myogenesis and if other tissue-specific factors are required during muscle differentiation, they must be activated by MyoD (141, 143). However, not all cells are convertible by MyoD as different cell types have been reported to be resistant to myogenic conversion such as P19, CV1, HeLa and HepG2 cells as well as primary fibroblasts/C3H 10T1/2 hybrid cells (141, 144-146).

Loss-of-function animal studies displayed a redundant function among the MRFs in regulating skeletal myogenesis and distinguished two main MRFs functions: 1) myogenic commitment performed mainly by Myf5 and MyoD, and 2) myogenic differentiation performed by MyoD, Myog, and Mrf4. Myf5 is the first MRF expressed during mouse embryogenesis and its expression appears first before myotome formation in the epaxial myogenic precursor cells arising from the dorsomedial lip of the dermomyotome and later on in the myotome. MyoD expression is activated after Myf5 in the hypaxial and then in the epaxial dermomyotome (147). Mice null for Myf5 (148) or MyoD alleles (149) have normal muscle development, with mild defects in trunk muscles in Myf5 mutant mice and delayed

onset of limbs, branchial arches, and tongue and diaphragm muscles in the MyoD mutant mice (150). These studies implicated a redundant function between Myf5 and MyoD in myogenic commitment. An initial report on mice lacking both Myf5 and MyoD demonstrated a complete lack of skeletal muscles (151); however, a later study clarified that targeting Myf5 resulted in a perturbed expression of Mrf4 and when the expression of Mrf4 was partially rescued, myogenesis was consequently rescued. This observation together with the lack of muscles in Myf5;MyoD;Mrf4 mutant mice implicated a role of Mrf4 in myogenic determination in conditions where Myf5 and MyoD are both missing. This function agrees with the pattern of Mrf4 expression during embryogenesis as it appears before MyoD expression in epaxial and hypaxial somites during the embryonic phase of muscle formation and then reappears later during the fetal stage (147). Furthermore, redundancy between Myf5 and MyoD function is attributed to the presence of two myogenic cell lineages: a Myf5-dependent and a Myf5-independent MyoD-expressing myoblasts based on lineage tracing and ablation experiments (152, 153). In fact, Pax3 and Myf5 can induce MyoD independently in the epaxial muscles (37) whereas Pax3 induces MyoD expression in the hypaxial muscles indirectly by direct activation of Myf5 expression (36). This is manifested by the lack of MyoD expression and trunk muscle formation in mice with mutations in both Pax3 and Myf5 (37).

Myog knockout (KO) mice, on the other hand, display a severe reduction of all skeletal muscles due to defects in myogenic cell differentiation without affecting myoblast formation (154, 155) implicating that Myog is needed only for prenatal myogenic differentiation. This is further supported by the observation that Myog was not able to compensate for Myf5 function in myogenic determination when Myog was substituted into the Myf5 locus in MyoD mutant mice (156). However, the role played by Myog in embryonic myogenesis

seems to be less critical postnatal as conditional inactivation of Myog using tamoxifen-induced Cre-mediated excision of Myog alleles in all tissues did not affect hindlimb and tongue muscle formation (157). Mrf4 KO mice have a transient defect in myotome formation that is compensated for at later stages (158, 159). Ablation of Mrf4-positive cells implicated a similar function to Myog in terminal muscle differentiation where all differentiated myofibers were lacking (152). This is further confirmed by the ability of a *Myog*-promoter-*Mrf4* transgene to partially rescue the muscle defects observed in Myog mutant mice (160).

The MRFs are a subfamily of the large bHLH structural group of TFs which contains more than 100 members (161) playing critical roles in the differentiation of many cell lineages like the neurogenic differentiation factor (NeuroD) subfamily in neuronal specification and differentiation, and the E-protein subfamily in lymphocyte differentiation (162). MRFs function by binding DNA as heterodimers with the constitutively expressed E-proteins (163-165). The MRFs contain a conserved bHLH domain, a 68-amino acid domain that is necessary and sufficient to drive myogenesis (143). Binding to DNA occurs at a specific DNA motif known as the Ebox with the consensus CANNTG sequence (where ‘N’ means any nucleotide; (166)), and is mediated through the MRFs basic region. Heterodimerization with the ubiquitously expressed E-proteins is mediated through the helix-loop-helix domain (143). In addition, MyoD, Myf5 and Mrf4 but not Myog have a conserved histidine- and cysteine-rich (H/C) region and an alpha-helix in the C-terminal region (helix III). The H/C region and helix III present in MyoD allows MyoD to induce genes that are present in inaccessible chromatin state by interaction with the Pbx/Meis complex (162, 167) and by initiating chromatin remodelling through interaction with the SWI/SNF chromatin remodelling complex (168-170) and the histone acetyltransferase p300 (171).

Since their discovery as members of the large bHLH family of TFs, extensive research

has been conducted to understand how MRFs can turn on specifically the myogenic program in cells but no other programs such as the neurogenic program like the NeuroD subfamily members. All bHLH TFs bind the Ebox motif which is quite abundant across the genome. One of the early findings by Weintraub's group is the presence of a 'myogenic code' in all MRFs that is essential for myogenic conversion. Three amino acids (Ala-Thr-Lys) in the myogenic code are critical for myogenic specificity, two of which are present in the basic region and one amino acid in the junction between the basic region and Helix 1 (172). The myogenic code is necessary for activation of muscle-specific genes (172, 173). The myogenic code in MyoD is required for binding a non-canonical Ebox in the *Myog* promoter and for forming a complex with Pbx/Meis (174). In addition, recent genomic mapping of MyoD during myogenesis and NeuroD2 during neurogenesis revealed different preference of the central dinucleotides of the Ebox. While loci bound uniquely by MyoD tend to have GG central dinucleotides, those uniquely bound by NeuroD have central GA dinucleotides. These unique binding events are associated with gene transcription during myogenesis and neurogenesis, respectively. Nevertheless, some loci are bound by both transcription factors and these tend to have GC central dinucleotides and they are associated with regional epigenetic modifications (145).

Moreover, although MyoD is expressed in myoblasts, it can induce different genes sequentially during myogenesis (175-177). The ability of MyoD to induce its target genes at the proper time is regulated by different mechanisms. A few myogenic inhibitors have been identified that can either dimerize with MyoD itself or sequester the E-proteins from MyoD in myoblasts such as the Id family of proteins, Twist, and MyoR, thus preventing MyoD from binding the Ebox and inducing its target genes (reviewed in (165)). MyoD requires the presence of two Eboxes for proper reporter plasmid activation in vitro (178), but the Eboxes

are quite abundant across the genome and many of them are not even accessible to MyoD due to chromatin folding into higher-order structure. The homeodomain protein Pbx plays a role in marking and directing MyoD to the promoters of genes to be expressed (167, 179). MyoD will then recruit p300 and SWI/SNF complex and this will make the chromatin accessible to the transcriptional apparatus. Moreover, cooperation and interaction with other co-activators, signalling pathways and TFs play a critical role in specifying temporal gene expression. For example, p38 kinase signalling pathway cooperates with MyoD in regulating the expression of a set of late muscle structural genes (175). Among the TFs reported to cooperate with MyoD are the MADS-box factor 2 (Mef2) (180), the nuclear factor of activated T-cells (Nfat) (181), Six4 (182) and others. Cooperation between MyoD and Mef2 or Six TF is further implicated by the enrichment of their DNA binding sites among MyoD target genes that are induced during C2C12 differentiation (183, 184). Many of these cooperating factors have been shown to be important for myogenesis through loss-of-function animal studies as in the case of Mef2c KO mice (185, 186), Nfat KO mice (187), and Six1 KO mice (188).

1.2.3 Six homeobox transcription factors

The mammalian sine oculis-related homeobox (Six) TFs are named after the *Drosophila* homologue *sine oculis* (*so*) gene, a key determinant of the *Drosophila* entire visual system development (189-191). The *Drosophila* Six family contains two additional members: *optix* and *DSix4* (192, 193), those are also involved in eye development (193), muscles, somatic cells of the gonads and body fat formation (194, 195). The mammalian Six family contains six members, Six1 (196, 197), Six2 (196, 198, 199), Six3 (198, 200, 201), Six4 (202, 203), Six5 (198) and Six6 (204, 205). The Six proteins share two conserved domains: a Six-type homeodomain and a Six domain (Fig. 1.4) (206). The Six-type homeodomain contains a

helix-turn-helix motif (207) and allows binding to DNA. Unlike most of the typical homeodomains, the Six-type homeodomain lacks two highly conserved amino acid residues, an arginine at position 5 and a glutamine at position 12 in helix 1 (206). Initial reports identified the DNA binding site of Six1/2/4/5 as MEF3 site (TCAGGTTC) (203, 208-212), while later reports refined the consensus of the DNA binding site of the mouse Six proteins as GGGTATCA using protein binding microarrays (213) or an optimized MEF3 (TCaggTTtC, where a capital letter means that the nucleotide is strongly preferred) using Six1 ChIP-on chip data (182, 214). Unlike the other Six factors, Six3 binds the traditional ATTA homeodomain core recognition sequence (215). Sixty-two percent (37 amino acids out of 60) of the homeodomain residues that are critical for DNA binding are absolutely conserved in all fly and mouse proteins (207). Additionally, there are four amino acids within the homeodomain that further divide the Six factors into 3 subgroups, Six1/Six2, Six4/Six5 and Six3/Six6 (192).

The Six domain is present N-terminal to the homeodomain and it contains 110-115 amino acids (196, 198, 203) with 42% of the residues being absolutely conserved among the Six proteins (207). The Six domain mediates protein-protein interactions with other cofactors like the Eya co-activators (216-218), Groucho/TLE family of transcriptional repressors (215, 219, 220), and dachshund (Dach) family of co-repressors (221, 222). Besides the conserved homeodomain and Six domain, the Six proteins contain N-terminal and C-terminal segments that vary considerably in length. However, the C-terminal segment length is conserved within the Six subgroups (206). The role of the N-terminal segment is still not fully characterized, but the last 194 amino acids of the C-terminal domains of the murine Six4 (198), last 150 amino acids of Six5 (218) and the last 60 amino acids of Six2 (212) function as activation domains in mammalian reporter assays when fused to the Gal4 DNA binding

Figure 1-4 Six factors share a conserved Six domain and a conserved Six-type homeodomain

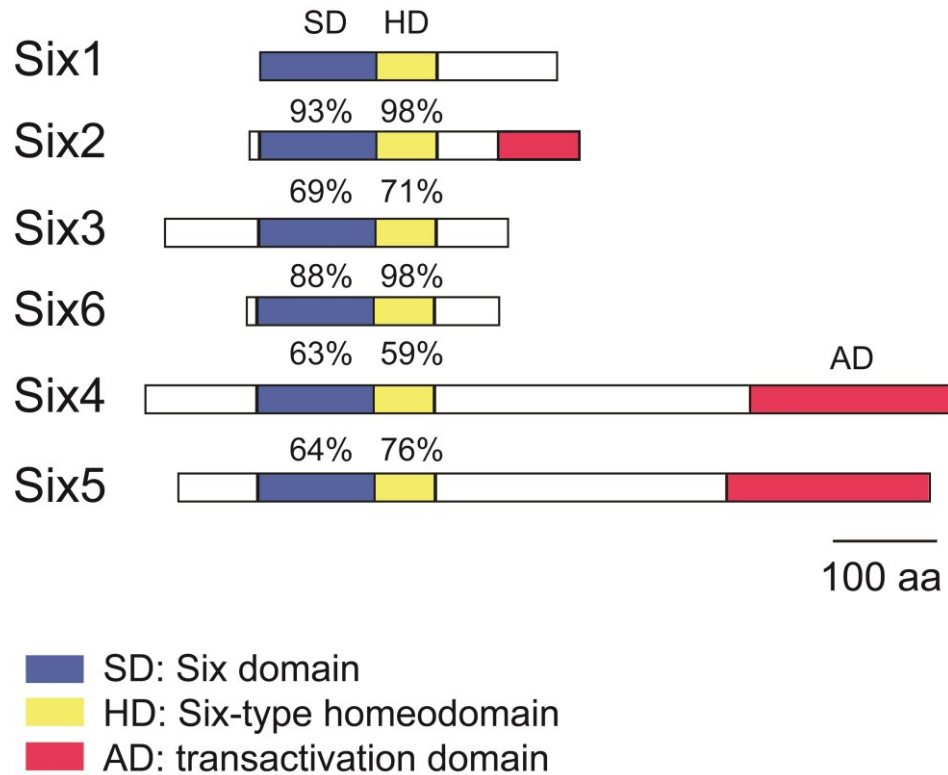


Figure 1.4. Six factors share a conserved Six domain and a conserved Six-type homeodomain.

A schematic representation of the mouse Six4 protein showing the conserved Six domain (SD) and Six-type homeodomain (HD) in addition to the reported transactivation domain (AD). Conservation percentages indicated above each protein are relative to the Six domain and homeodomain of Six1. The figure is reproduced from Kawakami et al, 2000 (206) with the modification listed in (212).

domain.

The murine Six factors show a widespread expression pattern in many organs and tissues during embryogenesis. Six3 and Six6 seem to have restricted expression in certain areas of the developing eye and brain, specifically in the optic vesicle, optic chiasma, nasal placodes, ventral forebrain and Rathke's pouch (204, 223). On the contrary, Six1, Six2, Six4 and Six5 show a broader expression pattern with Six2 being expressed in the head and limb mesoderm, branchial arches, fore- and midgut, nephrotomes, and genital eminence (196). Six1 and Six4 appear to be co-expressed in the same organs and tissues including: sensory organs such as taste buds otic and nasal placodes, branchial arches, Rathke's pouch, dorsal root ganglia, somites, limb buds, nephrogenic cords, and male gonads (196, 224-226). Six4 is also expressed in the retina (227). In addition, Six5 is expressed in neural and sensory tissues, skeletal muscle, cartilage, kidney, liver, heart and lung (224, 228, 229).

The broad expression profile of the Six factors during embryogenesis implicates a potential function in the development of various organs and tissues. This is further confirmed by loss-of-function and gain-of-function animal studies. Among the Six factors, Six1, Six2, Six4 and Six5 are expressed in skeletal muscles and their precursors (196, 224). Early work indicating a role of Six factors in regulating vertebrate skeletal myogenesis include the finding that Six1 and Six4 bind upstream of the *Myog* promoter and regulate its expression (208); Six4 and Six5 cooperate with the Eya co-activators in regulating this promoter (218). Gain-of-function experiments in chick embryos showed that ectopic expression of Six1 together with Eya2 induces the expression of the MRFs MyoD and Myog and the terminal muscle differentiation marker myosin heavy chain (MHC) in somite explants (217). The role of Six factors in regulating skeletal myogenesis was further confirmed in Six factors KO mice. Six5 mutant mice have no apparent abnormalities in skeletal muscle development or

function, but other defects associated with myotonic dystrophy are observed such as cataract formation (230, 231) and defects in male reproductive system development (230). Similarly, Six4 mutant mice have no defects in skeletal muscle formation or any other organs or tissues (232) implicating a potential redundancy between Six4 and Six5 in regulating muscle formation. This is important considering that both proteins belong to the same Six subgroup characterized by a long C-terminal region with a transactivation activity (206). On the contrary, Six1 mutant mice display a severe hypaxial muscle hypoplasia with a delayed expression of MyoD and Myog in the limb buds. Six1 mutant mice die at birth due to a severe rib deformation and the lack of muscles within the diaphragm (188).

The importance of Six4 during prenatal muscle development comes from Six1;Six4 double KO (dKO) mice. In Six1 mutant mice, only differentiation of hypaxial muscle progenitor cells is impaired without any defect in their specification, proliferation or migration (188). In the Six1;Six4 dKO mice, on the other hand, formation of both epaxial and hypaxial muscles is impaired, with a more drastic effect on the hypaxial ones (233), as some epaxial and craniofacial muscles remain intact due to a compensatory activation of MyoD by Six2 (234). Specification, migration and differentiation of progenitor cells are impaired. The expression of all MRFs is either completely abolished or reduced (233, 235) and that of Pax3 together with its downstream targets cMet and Lbx1, which regulate migration of myogenic precursors, is reduced as well (233). However, the muscle phenotype observed in the Six1;Six4 dKO is more severe than the one observed in Pax3 mutant mice, implicating that Six1 and Six4 regulate muscle formation through two pathways, a Pax3-dependent pathway and a Pax3-independent but Myf5-dependent one. Further characterization of Six1;Six4 dKO mice showed a unique function of Six1 and Six4 in the early activation of fast-twitch muscle genes in a mixed population of fast/slow myocytes of

the primary myotome (236). The remaining epaxial muscles in these mice are composed of myofibers lacking fast-type genes and expressing predominantly the slow muscle genes (237). In addition to muscle defects, Six1;Six4 dKO mice lack ears (233), display severe defects in kidney (238), the trigeminal ganglia (239) and male gonads development (226).

Therefore, unlike the MRFs, the murine Six1 and Six4 are expressed in many organs and tissues. Unlike Pax3, they are involved in the formation of all skeletal muscles, even head muscles where Pax3 is not expressed (233, 240). Unlike the MRFs, Pax3 and Pax7, Six1 and Six4 are expressed throughout skeletal muscle formation, from the specification of progenitor cells to terminal muscle differentiation, prenatally and postnatal (Fig. 1.3) (24). In adult mice, Six1 and Six4 are expressed in quiescent, activated and differentiated satellite cells (241, 242). Conditional KO of Six1 in satellite cells impairs muscle regeneration and disrupts satellite cell homeostasis by blocking the return of satellite cells to quiescence after regeneration (92). Among the genes deregulated in Six1;Six4 dKO muscle tissues is the Dual-specificity Phosphatase 6, *Dusp6* (237), a negative regulator of ERK1/2 signalling pathway which permits the return of satellite cells to quiescence (92). Nevertheless, the role that Six4 could play during muscle regeneration has never been reported, in part due to the lack of skeletal muscle phenotype in Six4 null mice (232).

In addition to their role in organ and tissue development, the Six factors are reported to regulate the cell cycle progression. Six1 regulates G2/M (243) and G1/S transition by directly controlling the expression of cyclin A (244) and the *Drosophila cdc25* homolog, *string*, (245), or by activating the expression of c-Myc (221) and cyclin D1 (246) respectively. Six6 also regulates G1/S transition by inhibiting the expression of the cyclin-dependent kinase inhibitor p27^{kip1} during retina and pituitary development (222). As regulators of the cell cycle, misregulation of the Six factors is implicated in cancer. Six1 is

highly expressed in human mammary carcinoma cells (243), probably through gene amplification (247), and its overexpression is sufficient to induce malignant transformation (244, 248) by increasing TGF- β signalling leading to epithelial to mesenchymal transition (249). Six1 is also elevated in chronic myelogenous leukemia, lung carcinoma, colorectal adenocarcinoma, Burkitt's lymphoma (243), hepatocellular carcinoma (250), ovarian carcinoma (251), Wilms' tumor (252), cervical cancer (253), malignant peripheral nerve sheath (254) and rhabdomyosarcoma (255). Indeed, its overexpression is associated with increased malignancy and worse prognosis in different types of cancers including brain, cervical, prostate, colon, kidney, liver (256), Wilms' tumor (257) and lung adenocarcinoma (258). As for Six4, no clear involvement of its function in cancer has been reported except for an amplification of its gene in cells derived from the metastatic pleural effusions of a breast cancer patient (247).

Expression of the Six factors is regulated by at least three different mechanisms: 1) transcriptional level, 2) post-translational modifications, and 3) proteasome-mediated degradation. Human Six1 expression in mammary carcinoma seems to be regulated during the cell cycle (243), in part by the transcription factor E2F1 (259). Human Six1 is phosphorylated during G2/M transition by casein kinase 2 and this phosphorylation reduces the ability of Six1 to bind to the MEF3 site in the Aldolase A promoter (260). Finally, human Six1 is degraded in an ubiquitin-proteasome-dependent manner by the anaphase-promoting complex, APC^{Cdh1} during the late mitotic phase of the cell cycle (261).

Finally, the Six factors act in collaboration with other cofactors and chromatin modifying enzymes to regulate myogenesis. Eya co-activators and Six factors form a regulatory complex that seems to be conserved through evolution, from *Drosophila* to

human, and in different developmental processes. The Eya cofactors were first discovered as key determinants of *Drosophila* eye development, together with Six and Pax TFs (reviewed in (262)). The Eya cofactors are protein tyrosine phosphatases (221, 263) expressed in almost the same tissues and organs as Six factors (216). *Eya1*^{-/-}*Eya2*^{+/-} mice display a skeletal muscle phenotype comparable to *Six1*^{-/-} mice, while *Eya1*^{-/-}*Eya2*^{-/-} mice have a more drastic phenotype where the expression of Pax3 is severely reduced, and consequently hypaxial myogenesis is totally lost (264). The Six factors cooperate with Eya cofactors in regulating expression of genes involved in different biological processes including kidney, sensory organs development and skeletal muscle formation (217, 218, 221, 265). Indeed, Six factors directly interact with Eya cofactors (216) and translocate them from the cytoplasm to the nucleus (218). *Eya1* and *Six1* recruit the SWI/SNF chromatin-remodelling complex to activate genes involved in neurogenesis through interaction with Brg1 and Baf170 (266). Moreover, Eya phosphatase activity is required to switch Dach-mediated repression of Six target genes into activation by recruiting co-activators like the CREB binding protein (221) known to interact with both Eya and Dach (267). Although Dach function is often correlated with gene repression (222) Dach, Eya and Six cooperate to regulate the expression of Pax3 during myogenesis (217). In addition, Ski cofactor interacts with *Six1* and *Eya3* to regulate the expression of Myog during muscle differentiation (268), although the mode of action in this case is not well established.

Furthermore, the Six proteins cooperate with other TFs to regulate different biological processes. *Six1* cooperates with Sox2 to regulate neuronal differentiation (266, 269). In mammalian cell transcriptional reporter assays, *Six4* but not *Six1* cooperates with MyoD in inducing Myog reporter activity and with MyoD and Myog in activating reporter plasmids containing MEF3 and Ebox (182). *Six4* recruits Utx, the demethylase of histone 3

trimethylated on lysine 27 (H3K27me3), to the regulatory regions of Myog and muscle creatine kinase during muscle differentiation (270). Besides, Six4 recruits the SWI/SNF remodelling complex component Baf60c to the regulatory region of the Akt signalling pathway activator Deptor, thus enhancing the glycolytic metabolism in skeletal muscles of type 2 diabetic mice (271).

Over more than two decades since their discovery, numerous attempts using different approaches have been performed to comprehensively understand the role of the Six factors during organogenesis. Though these trials enhanced our knowledge of the general function of these factors, the progress in identifying the target genes regulated by these factors under certain conditions has been slow. Among the target genes directly regulated by Six factors during myogenesis are the MRFs *Myf5* (235), *Myog* (208), and *Myod* (234, 242), as well as *Pax3* (264), muscle creatine kinase (270), *Gdnf*, and *c-Myc* (221).

1.3 Gene expression and chromatin modifications

Induction of gene expression during muscle formation or in response to any other external stimulus is a highly complex process. This complexity is partly dependent on the packaging of DNA within eukaryotic cells into chromatin structure composed of repeating units of nucleosomes which essentially form a barrier to the transcriptional apparatus. Nucleosomes are formed by the wrapping of approximately 147 base pairs of DNA around an octamer of core histone proteins consisting of a tetramer of H3 and H4 flanked by two H2A-H2B dimers (272). Nucleosomes are separated by a linker DNA, which may associate with histone H1. Chromatin is a highly heterogeneous structure displaying two main genomic regions: a highly compacted region known as heterochromatin and a relatively uncondensed transcriptionally permissive region known as euchromatin (273).

In its simplest representation, gene expression is induced when a TF binds to a specific

DNA motif near the promoters of its target genes leading to eventual recruitment of RNA polymerase II (RNA pol II) and the general transcriptional factors thus forming the pre-initiation complex that will initiate gene transcription. However, RNA pol II itself gets stalled more than once during transcription and needs to be phosphorylated in order to translocate across the gene body, aided by nucleosome remodellers that move or displace histone octamers from DNA. Gene transcription can be simplified as a multistep process involving different regulators including several TFs, cofactors, chromatin remodelling complexes, chromatin modifying enzymes in addition to the basic transcriptional machinery (reviewed in (274)). This complex multi-steps process usually requires the cooperative function of more than one TF, each contributing a specific function such as recruitment of the chromatin remodelling complex that will result in a certain gene induction output. It is the combined functions of these TFs that can lead to a maximal gene induction, higher than the sum of their individual gene induction outputs (275).

The canonical core histone proteins (H2A, H2B, H3 and H4) and histone variants (such as H3.1, H3.3 and HTZ.1) are subject to diverse post-translational modifications (PTMs) including acetylation, methylation, phosphorylation, ubiquitylation and SUMOylation that sometimes act as epigenetic marks that influence gene activation or repression. Most of these PTMs occur at the histone N-terminal tails, with lysine residue being the key modified amino acid undergoing acetylation, methylation, ubiquitylation and SUMOylation (276). Acetylation of histone H3 and H4 is generally associated with gene activation, whereas SUMOylation seems to be associated with gene repression. However, methylation and ubiquitylation could be either activating or repressing depending on the specific modified residue. In the case of lysine methylation, the extent of this modification such as mono-, di-, or tri-methylation can also have an influence (276). For example, H3K4me3 and H4K4me2

are associated with accessible chromatin structures and gene activation and they are usually present at promoters of transcribed genes (277, 278), whereas H3K36me3 and H3K79me3 mark actively transcribed regions or gene bodies (279). On the contrary, H3K27me3 marks silenced promoters, whereas H3K9me3 and H4K20me3 mark transcriptionally inert pericentromeric heterochromatin (280). However, the same PTM can correlate with opposite gene expression patterns depending on the genomic location of the histones modified. For example H3K4me1 is usually associated with gene expression when it localizes to gene enhancers (281, 282), but it is associated with gene silencing when it localizes to gene promoters in myoblast cells (283).

In general, histones PTMs are reversible. Acetylation of histone H3 and H4 is catalyzed by histone acetyl transferases and removed by histone deacetylases (284). Histone lysine methylation, on the other hand, is catalyzed by lysine methyltransferases and removed by lysine demethylases (reviewed in (276)). Of peculiar function during skeletal myogenesis are two groups of antagonizing proteins: the Trithorax group and the Polycomb group. The Trithorax group adds the activation mark of H3K4me3, catalyzed by Set-domain-containing lysine methyltransferase family 2 (KMT2) members that are encoded by six genes in the mouse (MLL1–4, SET1a, and SET1b). In contrast, the Polycomb group adds the repressive mark of H3K27me3 which is catalyzed by Set-domain-containing Ezh2 subunit of the polycomb repressor complex 2 (PRC2) (reviewed in (285)). For gene transcription to occur, the repressive H3K27me3 mark should be removed and this is catalyzed by the Jumonji domain-containing lysine demethylase family 6 (KDM6) members Utx, Uty, and Jmjd3 ((286-288)). Members of the KDM6 and KMT2 families work together to antagonize the repressive function of PRC2.

1.4 Functional genomics and skeletal myogenesis

The advance in high-throughput genomics approaches enhanced our understanding of skeletal myogenesis. The combined data generated using gene expression profiling, RNA-sequencing, chromatin immunoprecipitation followed by either microarray (ChIP-on-chip) or high throughput sequencing (ChIP-seq) in the past thirteen years of research improved significantly our knowledge of the key regulators of myogenesis (reviewed in (289)). With more data generated every year, the panoramic picture of all the steps and all the players involved in myogenesis is starting to take shape.

1.4.1 Gene expression profiling

Since the first gene expression profiling microarray (290), there has been a great advance in the technology. Instead of testing the expression of 48 genes, now we can quantify the expression of all the genes in the genome. Pioneering gene expression profiling experiments were performed on the myoblast cell line C2C12 (291) during their differentiation from myoblasts into myotubes (176, 177), and on the fibroblast cell line C3H 10T1/2 upon their myogenic conversion by MyoD (175). In addition to profiling genes using *in vitro* cell models of muscle differentiation, several attempts aimed at looking at the expression of genes that are regulated *in vivo* either during muscle regeneration or in the case of gain-of-function (or loss-of-function) of a certain TF by using the entire muscle tissues. However, gene expression profiling trials performed on the entire muscle tissues were challenging as many cells, mainly immune cells, contributed to the expression data of myogenic cells (292). Nevertheless, expression profiling of Six1/Six4 dKO embryos revealed the important function of these TFs in activating the fast-type gene program in the embryonic myofibers (236, 237). However, the number of discovered genes identified using this *in vivo* tissue model was relatively modest.

Expression profiling combined with a TF loss-of-function was also performed on cell lines and primary myoblasts. MyoD-regulated genes were identified in primary myoblasts isolated from MyoD mutant mice compared to control mice (183), and from C2C12 cell line in the case of targeting the expression level of the MRF Myog, Six1, Six4 or both Six1 and Six4 (182). Likewise, expression profiling combined with a TF gain-of-function was performed in fibroblast cells overexpressing Pax3-FKHR (293), C2C12 cells overexpressing Pax7-Flag (83), primary myoblasts overexpressing either Pax3- or Pax7-cTAP (80), and in C3H 10T1/2 fibroblasts converted to myocytes in the presence of MyoD (175). However, these experiments do not distinguish between a direct and indirect target of the TF as many TFs can induce other factors which in turn can regulate the expression of these genes, as it is the case with MyoD and its target Myog. To bypass this problem, expression profiling was performed in the presence or absence of the protein synthesis inhibitor cyclohexamide (175). Alternatively, gene expression profiling is often combined with TF binding profiling using ChIP-on-chip or ChIP-seq with the aim of identifying genes directly regulated by a certain TF.

1.4.2 ChIP-on-chip and ChIP-seq

1.4.2.1 Genomic mapping of TFs

Since the invention of ChIP-on-chip (294) and ChIP-seq (295, 296), numerous studies were performed to map TFs and the epigenetic landscape during myogenesis. The first ChIP-on-chip attempts mapped the binding of MyoD and Myog at the proximal regulatory regions of a set of genes in C2C12 cells (183, 297), or in fibroblasts converted into the myogenic lineage (297). Mef2c was also mapped in C2C12 cells (183), and RNA pol II in the myogenic-converted fibroblasts (297) at the proximal promoters of select genes. These experiments showed that although MyoD and Myog bind the same DNA motif and a set of

common loci; yet, each one has its own unique target genes where Myog mainly binds to the late muscle genes. Later ChIP-on-chip experiments mapped the binding profile of Six1 during C2C12 differentiation from myoblasts to myotubes (182).

Using ChIP-seq, MyoD was shown to bind to more than 20,000 binding sites in C2C12 myoblasts or myotubes and in fibroblasts expressing MyoD (298). Thousands of MyoD binding events were constitutive in myoblasts and myotubes. Caltech data under the ENCODE project identified the same binding profile for MyoD (ENCODE project, <http://encodeproject.org/ENCODE>), and a higher number of unique binding sites (~40,000) for MyoD was identified in C2C12 myotubes by another group (299). On the contrary, others reported a smaller list of MyoD targets in primary myoblasts and fibroblasts expressing MyoD by using ChIP-seq based on chromatin tandem affinity purification, a method to map the most confident binding sites (300). Although most MyoD binding events did not correlate with differential expression between myotubes and myoblasts, it correlated well with an increase in histone H4 acetylation, a histone mark associated with transcribed genes (298). Similar to MyoD, the binding profile of Myog revealed a large number of binding events (~35,000) in C2C12 myotubes (299). Additionally, other TFs were mapped during myogenesis including Myf5, Snail1 (300), Pax3, Pax7 (80), Mef2d isoforms (301), Mef2a (using ChIP-exo, (302)), and YY1 (303).

TF genomic binding profiling reinforced previous findings and clarified some of the dilemmas in understanding differential functions of certain TFs or even the same TF under different conditions. For example, the dispensable function of Pax3 in postnatal myogenesis and the indispensable function of Pax7 in protecting satellite cells from apoptosis can be explained by the significant difference in their binding profiles in adult primary myoblasts. While Pax7 binds to more than 50,000 genomic loci, Pax3 binding is restricted to only 4,600

loci. Moreover, Pax7 binding events have been annotated to genes promoting myoblast growth and inhibiting their differentiation (80). One of the biggest conundrums in myogenesis is how MyoD activates different genes sequentially though it binds many of these genes in both myoblasts and myotubes (298). This issue is answered partly by the cooperation of MyoD with different TFs and cofactors at different stages during myogenesis such as Mef2 TFs and the Six factors. This finding is confirmed by the significant genomic overlap of MyoD with Mef2c (183), Mef2d (301) and Six1 (182). Interestingly, one of the two Mef2d isoforms is able to recruit Ash2L promoting transactivation of late muscle genes (301) in a p38-dependent manner (304). Moreover, Six4 recruits the demethylase of the repressive histone mark of H3K27me3, Utx, to the regulatory regions of Myog and muscle creatine kinase as an initial step in their activation. Whether this function of Six4 can explain genome-wide induction of MyoD-induced genes during differentiation awaits further genomic mapping of Six4 and is the focus of the current thesis.

1.4.2.2 Genomic mapping of histone modifications

As mentioned earlier, the histone modifications are a key determinant of the TF binding activity as the histone proteins undergo different modifications that correlate with gene transcriptional activation or repression. In fact, different histone marks can distinguish an enhancer from a promoter or a gene body; some reflect an active transcription, gene silencing or even a bivalent “paused” activation/silencing signature waiting for the proper cue (reviewed in (305)). Genomic mapping of the chromatin marks and chromatin modifying enzymes has been extensively studied during myogenesis. Among the chromatin marks mapped in C2C12 cells are those associated with promoters and enhancers including H3K4me1 (histone 3 monomethylated on lysine 4), H3K4me2, H3K4me3, H3K9ac (histone 3 acetylated on lysine 9), H4K12ac, H3K18ac (306) and H3K27ac (307), in addition to

marks associated with gene bodies like H3K36me3 (306) and H2Bub (308), and those associated with euchromatin and heterocromatin signatures such as H3K9me3 and H3K27me3 (306). Besides C2C12 cells, histone marks in quiescent and activated satellite cells from young and old mice have been profiled including H3K4me3, H3K27me3 and H3K36me3 (56).

In addition to histone marks, genomic mapping of chromatin modifying enzymes has been performed using *in vitro* myogenic cell models, and together with the histone marks they defined the genomic landscape during muscle formation. For example, mapping the histone acetyltransferase p300 and its acetylated histone mark H3K27ac (307), together with RNA pol II and H3K4me1 (306) defined transcriptionally active enhancers which showed a significant correlation and dependence on MyoD binding events (298, 307). An interesting genome-wide mapping of the H3K27me3 demethylase Utx showed a significant overlap between Utx binding profile and the histone chaperone Spt6. Spt6 is not only a transcriptional elongation factor that facilitates RNA pol II movement during transcription, but it also associates with Utx thus allowing the removal of H3K27me3 mark during gene transcription (309). Interestingly, Utx is recruited to the promoters of Myog and muscle creatine kinase by Six4. Whether Six4 is involved in recruiting Utx to all Utx-bound genes induced during myogenesis has never been investigated.

1.5 Hypothesis and aims

Six4 and Six1 play an important role during prenatal muscle formation from the specification of progenitor cells to muscle differentiation, including a function upstream of Pax3 and MRFs. The role of Six1 and Six4 during muscle regeneration is beginning to be addressed as these factors are expressed in quiescent, activated satellite cells and in their differentiated progeny. A role of Six1 in maintaining satellite cells homeostasis has been

recently reported. Six4 mutant mice display no obvious developmental phenotype, and a potential role for Six4 in adult muscle regeneration has not been reported. Recently, our lab has shown an important role of Six4 during C2C12 myoblasts differentiation and a synergy between Six4 and MyoD in inducing *Myog* reporter plasmid. Considering that Six1 and Six4 share some transcriptional targets, and that Six1 and MyoD binding profiles largely overlap, it is possible that Six4 also overlaps with MyoD.

Based on these data, I hypothesized that Six4 plays important role during adult muscle regeneration in part by cooperating with the MRF MyoD in regulating a set of genes needed for proper muscle formation. My work has focused on determining whether Six4 plays an important role during adult muscle differentiation *in vitro* and muscle regeneration *in vivo* and on elucidating its molecular mechanism of action. My thesis reports the results of experiments on four fronts: 1) determining whether Six4 is needed for adult muscle formation by manipulating an *in vitro* cell model of MyoD-induced myogenic conversion. This model was used to identify target genes regulated by Six4 as comprehensively as possible by using gene expression profiling. The role of Six4 during muscle regeneration was addressed using cardiotoxin-induced muscle regeneration combined with Six4 partial loss-of-function; 2) identifying target genes of Six4 during skeletal myogenesis by performing ChIP-seq to map the genomic Six4 binding profile during the differentiation of C2C12 myoblast cell line, a well-used cell model of muscle differentiation; 3) defining a set of muscle genes directly regulated by both Six4 and MyoD by combining their genomics data; 4) establishing a connection between Utx and the set of Six4 and MyoD common targets.

N.B. The result chapters (3-5) are published in abridged form in Chakroun et al, 2015 (310).

Chapter 2: Materials and Methods

2.1 Antibodies

Rabbit antibody against mouse Six4 was produced by immunization with a recombinant protein representing amino acids 672 to 775 of mouse Six4, and was purified by affinity as previously described (182). Specificity of the purified antibody was confirmed by immunoprecipitating all the murine Six family proteins tagged with a triple Flag epitope produced in rabbit reticulocyte lysates (Promega), followed by western blot probing with anti-Flag antibody; only Six4 was immunoprecipitated by the purified antibody. Other primary antibodies used include rabbit anti-Six4 (amino acids 414-775; (182)), rabbit anti-Six1 (182), anti-MHC (MF20 clone, obtained from the Developmental Studies Hybridoma Bank, DSHB), anti-Myog (clone F5D, DSHB, contributed by W. Wright), anti-beta-tubulin (clone E7, DSHB), rabbit and mouse anti-MyoD (Santa Cruz, sc-760x and sc-32758x), anti-embryonic MHC (eMHC, clone fl.652-b, DSHB), anti-Pax7 (clone Pax7-b, DSHB), mouse anti-Flag (Sigma, F1804), goat anti-Myc (Santa Cruz, sc-789X), rabbit anti-Prmt5 (Millipore, 07-405; Abcam, ab109451), and goat anti-Eya3 (Santa Cruz, sc-15101). Secondary antibodies used in western blot experiments were all coupled to horseradish peroxidase (HRP) and purchased from Promega (anti-mouse or anti-rabbit IgG), or from Thermo Scientific (anti-goat IgG). Anti-mouse coupled to FITC (Jackson ImmunoResearch), anti-mouse coupled to Alexa Fluor 488 (Jackson ImmunoResearch), and anti-rabbit coupled to Alexa Fluor 594 (Invitrogen) were used in immunofluorescence (IF) experiments.

2.2 Muscle injury and *in vivo* siRNA delivery

All surgical procedures were performed aseptically in compliance with the University of Ottawa Animal Care and Use Committee following the Guidelines of the Canadian Council on Animal Care and the Animals for Research Act. Seven-week-old C57BL/6 female mice

(Charles River, Canada) were anesthetized with isoflurane. After shaving both legs, the left *tibialis anterior* (TA) muscle was injected with 30 µl of cardiotoxin (CTX, Latoxan, France, 10 mM in phosphate-buffered saline (PBS)), while the right TA muscle was injected with 30 µl of PBS as a control. For time course immunostaining, mice were sacrificed by cervical dislocation as resting muscle, or 4 and 7 days after CTX injection. For eMHC immunostaining and hematoxylin and eosin (H&E) staining, both legs were injected with CTX followed by small interfering RNA (siRNA) targeting Six4 (siSix4#2, sequence available in Table A2.1) or control siRNA following a modified protocol used by other groups (130, 303). Briefly, 7.5 µl of siRNA (20 µM) was mixed with 7.5 µl of Opti-MEM medium (Invitrogen), and 3 µl of Lipofectamine RNAiMax (Invitrogen) was mixed with 12 µl of Opti-MEM medium. Then, the diluted siRNA and Lipofectamine were mixed together and incubated for 20 minutes (min.) at room temperature (RT) before injection into TA muscles. The first siRNA injection was delivered 6 hours after CTX injection, and was then repeated every 2 days. siSix4 was injected into one TA, while control siRNA was injected into the contralateral TA. At least 5 mice were used for each time point tested. Mice were sacrificed after 2 siRNA injections 4 days post-CTX injection for eMHC immunostaining or after 3 siRNA injections 7 days post-CTX injection for H&E staining.

2.3 TA immunostaining

After cervical dislocation, TA muscles were dissected and fixed in 2 % (w/v) paraformaldehyde in PBS for 30 min. at 4°C. Muscles were washed 2 times for 5 min. in cold PBS (4°C) and quenched with 0.25 M glycine in PBS 2 times for 10 min. on ice. Muscles were then incubated in 70% cold ethanol and processed for paraffin embedding and sectioning. Slides were deparaffinized at RT for 3 times 10 min. in xylene, 2 times 5 min. in

100% ethanol, 2 min. in 95% ethanol, 2 min. in 80% ethanol, and 30 seconds in 70% ethanol. Slides were then kept in distilled water until antigen retrieval which was performed by incubating the slides in citrate buffer (10 mM citric acid, 0.05% Tween 20 (v/v), pH 6.0) for 20 min at 98°C, followed by cooling down to RT in the citrate buffer.

Sections were quickly rinsed with PBS-T (PBS, 0.1% Triton X-100 (v/v)) and blocked in 3% bovine serum albumin (w/v) in PBS-T for 1 hour at RT. Sections were then incubated with primary antibodies against Six4 (1:100 v/v, rabbit polyclonal), MyoD (20 µg/ml, mouse monoclonal), Pax7 (13 µg/ml, mouse monoclonal), Myog (26 µg/ml, mouse monoclonal), or eMHC (33 µg/ml, mouse monoclonal) in blocking buffer for 1 hour at RT, and rinsed 4 times 3 min. with PBS-T. Alternatively, sections without primary antibodies were included, to confirm specificity of the signal obtained. Sections were then incubated with secondary antibodies conjugated to Alexa Fluor 594 (1:1000, donkey anti-rabbit IgG) and with Alexa Fluor 488 (1:400, goat anti-mouse IgG sub1 specific) in blocking buffer for 40 min. at RT in the dark, and rinsed with PBS-T. Sections were rinsed with PBS, then deionized water, and with 70% ethanol before incubation with 0.3% Sudan Black (w/v, Sigma) in 70% ethanol for 10 min. Slides were finally washed with PBS followed by deionized water; air-dried and mounted with ProLong Gold antifade reagent with DAPI (Invitrogen).

Day 4 eMHC IF Images were acquired with a Carl Zeiss Axiovision Observer Z1 microscope and the Axiovision MosaiX tiling and stitching feature was used to capture a panorama of the entire muscle at 20X magnification and an exposure time of 800 ms for the Alexa Fluor 488 Green channel, 100 ms for the DAPI channel and 40 ms for the bright field channel. The Axiovision Outline tool was used to manually measure all nucleated fluorescent fibers.

2.4 Hematoxylin and eosin (H&E) staining

Mice were sacrificed after 3 injections of siRNA, 7 days after CTX injection. Paraffin sections were prepared as above, and left and right TA muscles were set side-by-side on microscope slides. After deparaffinization, slides were incubated in hematoxylin solution (Fisher Scientific) for 4 min. at RT, rinsed with water for 1 min., acidified 70% ethanol for 25-40 seconds, water for 1 min., and then incubated with eosin (Fisher Scientific) for 1 min. Slides were rinsed again with: water for 1 min., 70% ethanol for 30 seconds, 80% ethanol for 2 min., 95% ethanol for 2 min., 100% ethanol for 2 times 5 min., and finally with xylene for 2 times 10 min. Slides were then mounted with xylene based permount and multiple images of the muscle sections were acquired using Carl Zeiss Axiovision Imager A2 microscope at 10X magnification. Stitching the images was done using FIJI software's Grid/Collection stitching plugin. For each mouse, single panoramic images containing the entire control and experimental muscle sections were adjusted for equal levels and contrast in Adobe Photoshop CS6, and areas without myofibers or with poor staining were masked. CellProfiler (311) and its “UnmixColors” module were programmed to analyze the color of H&E images, to automatically identify the outlines of myofibers and nuclei, and to report the surface area of fibers containing at least one centrally-located nucleus (defined as nuclei whose outline is not in contact with the myofiber outline).

2.5 Cell culture and stable cell lines

C2C12 myoblasts (American Type Culture Collection, ATCC) were grown in Growth Medium (GM) containing Dulbecco's Modified Eagle's Medium with high glucose (DMEM), supplemented with 10% Fetal Bovine Serum (FBS); 1% L-Glutamine and 1% Penicillin/Streptomycin (P/S) until confluent. Once confluent, the cells were induced to differentiate for 24, 48 or 96 hours by replacing the medium with differentiation medium

(DM) containing DMEM supplemented with 2% Horse Serum; 1% L-Glutamine and 1% P/S. C2C12 cells differentiated for 96 hours were harvested by differential trypsinization to separate myotubes from reserve cells. Human embryonic kidney carcinoma cells (HEK 293T) and C3H 10T1/2 fibroblasts (ATCC) were grown in DMEM supplemented with 10% FBS, 1% L-glutamine and 1% P/S. Primary myoblasts were prepared as described by Rando and Blau (312) with the modifications described in (242, 313). Primary myoblasts were cultured on Matrigel (Corning)-coated dishes in DMEM (ATCC) supplemented with 20% FBS, 10% donor equine serum; 1% P/S, 10 ng/ml basic FGF (Peprotech) and 2 ng/ml HGF (Peprotech). Cells were induced to differentiate in DMEM supplemented with 10% donor equine serum and 1% P/S. All cells were cultured in a humidified water-jacketed incubator at 37°C with 5% CO₂.

2.5.1 Knockdown cell lines

Short hairpin RNA (shRNA)-mediated knockdown of Six4 in C2C12 and C3H 10T1/2 cells was mediated by pSUPER.retro vector (Oligoengine) containing different sequences targeting murine Six4 (shSix4#1: GCAAGCAAATGTACTGTG; shSix4#2: GCAAGGGACATGAGGATTT; shSix4#3: GGATCTACCTCCCAGGATG; shSix4#4: GAGACGGTGTATTGTTTCA; shSix4#5: GATGAAGACATGCAAGAC), murine Six1 (shSix1#1: GCTGAAAGCGCACTACGTG; shSix1#2: GAAAGGGAGAACACCGAAA; shSix1#3: AGAATCAACTCTCTCCTCT; shSix1#4: GGGAGTGGTACGCGCACAA; shSix1#5: CTTGAGCACGCTCTCGTTCTT), or control scrambled sequences (shNS: CAACAGATGAAGAGCACCAA; shLuc: CTGACGCGGAATACTTCGA) that have no significant homology to the mouse genome. Retroviruses carrying the indicated shRNA vectors were produced using Phoenix cells according to the manufacturer's protocol (Orbigen) and were used to infect C2C12 or C3H 10T1/2 cells. Selection of the cells was done using 2 µg/mL puromycin

(Sigma) starting 2 days after infecting the cells and for a total duration of 3-4 days, after which the cells were maintained in medium containing a lower concentration of puromycin (0.5 µg/mL).

2.5.2 Overexpression cell lines

For overexpression of 3xFlag-Six4-myc in C2C12, we amplified the complete coding DNA sequence of Six4 by PCR from cDNA isolated from C2C12 cells using the following primers: ATAATGCGGCCGCGTCCTCTTCCTCCCCACCGGGCAGATT and GTCGTCTCTAGATAAGTCTTGCATGTCTTCATCC. After cloning Six4 coding DNA sequence into pBABE-puro (Addgene), retroviruses were produced and used to infect C2C12 myoblasts. Selection of infected cells was performed as discussed above.

2.6 RNAi in cultured cells

C2C12 myoblasts were seeded at 100,000 cells in 6-well plates (Corning), transfected with Stealth siRNA duplexes targeting Six4 (siSix4#1, siSix4#2, siSix4#3; sequences are available in Table A2.1) or control non-silencing siRNA (Invitrogen) using Lipofectamine RNAiMax (Invitrogen) following the manufacturer's protocol. Once confluent, cells were induced to differentiate for 24 hours before RNA and protein extraction.

2.7 Myogenic conversion assay

C3H 10T1/2 cell lines were seeded at 50,000 cells on 6-well plates and infected with retroviruses expressing either 3xFlag-MyoD-myc or empty plasmids. After 24 hours, cells were switched to DM for 24 hours for RNA and protein extraction, or for 48 hours for protein extraction. For IF, cells were seeded at 10,000 cells on tissue culture-treated coverslips (Nunc) pre-coated with collagen type I (Roche) in 24-well plates. Retroviral infection was performed and cells were differentiated for 48 hours. IF detection of MHC was performed as described earlier (182). Percent differentiation was calculated as the percentage

of nuclei within MHC-positive cytoplasm over the total number of nuclei. Fusion index was calculated as the percent of nuclei in myotubes (MHC-positive cytoplasm with at least 3 nuclei) over the total number of nuclei.

pBABE-3xFlag-MyoD-myc-puro plasmid was subcloned from p3xFlag-MyoD-myc plasmid described previously (182).

2.8 Immunoprecipitation (IP) of *in vitro*-translated proteins

Murine Six family proteins tagged with a triple Flag epitope were subcloned from p3xFlag-myc plasmid (Sigma) into pBluescript plasmid and used to produce 3x (Flag-tagged) *in vitro*-translated (IVT) Six family proteins in rabbit reticulocyte lysates (Promega) according to the manufacturer's recommendations. The expression level of different IVT 3xFlag-Six proteins was estimated by running western blot and probing with anti-Flag antibody. Based on western blot normalization, 5 µl of IVT Six1, Six2, Six3 or Six5, or 45 µl of either Six4 or Six5 were used in IP. IP was performed in a total volume of 225 µl in RIPA buffer (50 mM Hepes pH 7.5, 500 mM LiCl, 1mM EDTA, 0.7% sodium deoxycholate, 1% NP-40) supplemented with protease inhibitors cocktail (pepstatin, leupeptin, aprotinin A, and AEBSF) and 2 µl of anti-Six4 antibody for 2 hours at 4°C. Protein A sepharose beads were added for another 2 hours at 4°C, followed by 7 times washing in RIPA buffer, and elution using T50E10S1 (50 mM Tris pH 8.0, 10 mM EDTA, and 1% (w/v) SDS). Protein eluate and input samples were run using SDS-PAGE and their membranes were probed with anti-Flag antibody.

2.9 Co-immunoprecipitation of Six4-myc and Flag-Utx-myc

Full length and different mutants of Six4 protein were cloned into CMV-myc plasmid using primers listed in Table A2.1 (Appendix 2). p3x-Flag-Utx-myc was constructed by

subcloning the coding sequence of human Utx (clone BC093868) into p3xFlag-myc (Sigma). Different CMV-myc plasmids of Six4 mutants were co-transfected with Flag-Utx-myc or empty plasmid into HEK 293T cells seeded in 100 mm dishes using polyethylenimine (PEI, Sigma-Aldrich) as a transfection reagent (314). Alternatively, Flag-Utx-myc was co-transfected with CMV-GFP-myc plasmid as a control. Once confluent, cells were split into 3x150 mm dishes and left to become confluent. Freshly prepared and dialyzed nuclear extracts were prepared and immunoprecipitated as explained before (270) using 2 µg of anti-Flag antibody (pre-coupled to Dynabeads M-280 Sheep anti-Mouse IgG (Invitrogen) per IP condition for 1-2 hours, followed by 4 times washes for 10 min. in wash buffer (20 mM Hepes pH 7.6, 300 mM KCl, 0.5 mM EDTA, 10% glycerol and 0.1% Nonidet-40). Finally proteins were eluted with 6 M urea 0.1% (w/v) SDS in 20 mM Tris pH 6.8, 5X Laemmli sample buffer, and β-mercaptoethanol at ratio of 7:2:1 respectively. Eluate and input samples were run on SDS-PAGE and the membranes were probed with goat anti-myc antibody (Santa Cruz).

2.10 Immunoprecipitation of Flag-Six4-myc

Full length Six4 protein, Six4 expressing amino acids 1-424 and Six4 V109E mutant were cloned into p3xFlag-myc plasmid (Sigma) using primers listed in Table A2.1 (Appendix 2). Full length Flag-Six4-myc or the Six4 mutants mentioned above were transfected into HEK 293T cells seeded in 100 mm dishes using PEI as a transfection reagent (314). Once confluent, cells were washed with PBS, scraped, suspended in 5x the cell pellet volumes of a lysis buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 2 mM EDTA, 0.2% Triton-X 100, 15% glycerol and protease inhibitors (pepstatin, leupeptin, aprotinin A and PMSF) and incubated on ice for 10 min. while mixing occasionally. The cell suspensions

were then homogenized in a pre-chilled Dounce homogenizer with 20 strokes using pestle B and incubated for 10 more min. on ice followed by spinning down in a microcentrifuge at full speed for 15 min. at 4°C. After preclearing with protein A sepharose beads (Invitrogen) for 1-2 hours at 4°C, 1 µg of anti-Flag antibody was added to 1 mg of cell lysates and incubated at 4°C for an overnight. Protein A sepharose beads were then added and samples were incubated for 1-2 hours at 4°C. Precipitated proteins were washed with the lysis buffer 4 times for 10 min. each and eluted with 25 µl of T₅₀E₁₀S₁ at 65 °C for 20 min. while flicking occasionally. Eluate and input samples were run on SDS-PAGE and the membranes were probed with goat anti-myc or anti-Eya3 antibody (Santa Cruz).

2.11 SDS-PAGE and western blot

Total protein lysates were prepared either from TRIzol (Invitrogen) according to the manufacturer's protocol or in lysis buffer containing 20 mM Tris pH 6.8, 6 M urea and 0.1% SDS as described previously (182). After transfer, membranes were blocked in 5% milk in Tris-buffered saline (TBS), incubated with primary antibodies overnight, washed 3 times with TTTBS (TBS with 0.05% TritonX-100 and 0.05% Tween 20), incubated with HRP-tagged secondary antibody for 1 hour, washed, and the signal was developed using SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemicals).

2.12 Reverse transcription followed by quantitative PCR (RT-qPCR)

Total RNA was isolated from C3H 10T1/2 cells using the Absolutely RNA Miniprep Kit (Stratagene) following the manufacturer's protocol including DNase I treatment. Total RNA was isolated from C2C12 cells using TRIzol (Invitrogen). TA muscles were first homogenized in TRIzol using polytron followed by RNA extraction using TRIzol protocol and DNase I treatment (Invitrogen) following the manufacturer's protocol. Reverse-

transcriptase PCR was performed using the SuperScript® First-Strand Synthesis System for RT-PCR (Invitrogen) using random hexamers as primers. qPCR was performed as explained previously (182). The expression levels of genes in C2C12 and C3H 10T1/2 cells were normalized to the *Rps26* gene expression levels, while those in TA muscles were normalized to a geometric mean of expression levels of a set of housekeeping genes with comparable expression levels including *Rps26*, *Tbp*, *Actb* and *Rplp0* (315).

2.13 Gene expression profiling

Gene expression profiling was performed on C3H 10T1/2 cell lines 48 hours following myogenic conversion and 24 hours after inducing differentiation as described previously using Agilent 4x44k Mouse Genome microarrays (182). Raw processed probe intensity data were filtered for present probes by keeping only probes present in at least 6 replicates out of 12 total replicates (shLuc + pBABE, shSix4#4 + pBABE, shLuc + pBABE-MyoD, and shSix4#4 + pBABE-MyoD). Filtered data were further analyzed using Expander 6.5 (316) where they were log-transformed, normalized by quantile and then tested for statistical significance using unpaired student's t-test. Finally normalized gene expression data were visualized using Java Treeview (317) as fold change of shLuc + pBABE.

2.14 ChIP and ChIP-sequencing

ChIP assays and quantitative real-time PCR assays were performed as described before (182) using anti-Six4 or anti-MyoD (Santa Cruz, sc-760x) antibodies. PCR primers are listed in Table A2.1. ChIP-sequencing experiments were performed on various C2C12 cell lines differentiated for 24 hours as described before (182) using anti-Six4 antibody (shNS and shSix4#4 C2C12 cell line), anti-Flag antibody (Flag-Six4 C2C12, and parental C2C12), or normal rabbit IgG (parental C2C12). TruSeq gDNA libraries and sequencing on HiSeq2000 at 4 samples per lane were prepared by the McGill University and Génome Québec

Innovation Centre. Further analysis and alignment of ChIP-seq data was performed as discussed before (182). Six4-bound peaks were called using MACS v2.0 (318) versus different controls: IgG ChIP-seq, input sample, or no control. Only peaks that were identified by all three peak calling methods were retained in the final list of peaks. Finally, peaks discovered in the IgG sample run against the input sample were filtered out.

De novo motif discovery was performed using Amadeus (319) with the top peaks from the anti-Six4 experiment. Matching background sequences were selected using CisGenome (320). The program was run with “large” searches looking for 12 base pair motifs. The background sequence of the search was set as 10x the size of the Six4-bound sequences randomly selected using CisGenome (320). Once the position weight matrix (PWM) of each motif was obtained, the motif was mapped in all Six4-bound peaks using CisGenome to get a representative PWM of all Six4-bound peaks. PWMs were represented graphically using the Weblogo program (321). Motif abundance within Six4-bound peaks versus matching non-bound control regions was calculated using CisGenome (320) as described previously (214).

To assign Six4 binding loci to target genes, first we annotated Six4-bound peaks using different tools including PAVIS (322) and a custom Perl script using the “Known genes” annotation table from the UCSC genome browser (323). For every binding event, we considered all genes within 50 kb upstream or downstream of the peak summit, but finally only one gene was assigned per binding event. We considered the closest (smallest distance between peak summit and gene transcriptional start site, TSS) regulated gene by Six4 knockdown (gene expression profiling data on myogenic conversion, and C2C12 cells in case of Six4 knockdown (182)) to be the most likely target gene. If none of the close genes is affected by Six4 knockdown, then the gene with the closest TSS to the peak summit was assigned to the peak. Accordingly, some peaks were not assigned to any genes as we did not

find any gene within 50 kb upstream or downstream of the peak summit, while some genes were assigned to more than one peak. Once genes were assigned per peaks, their functional categories were represented using David Bioinformatics online tool v6.7 (324).

Finally, ChIP-seq read density heat maps were generated using seqMINER (325). First, the bed file of the Six4 peaks identified in shNS C2C12 or MyoD peaks (298) were sorted according to the read density of the central 200 bp around either MyoD or Six4 summits in the anti-Six4 ChIP-seq in shNS C2C12 cell line. Then, the heat maps of all ChIP-seq data presented in this paper were visualized using seqMINER with contrast setting matching the ChIP-seq read counts in order to normalize the heat maps. The output read density files from seqMINER were used to create the average read density boxplots. The heat map of the difference in shNS and shSix4 read density was generated using bedtools genomecov (bedtools.readthedocs.org/en/latest/content/tools/genomecov.html) which covers the read density of ChIP-seq bedgraph file at every single base pair specified by a bed file. The genome coverage output file was then visualized using Java Treeview (317).

2.15 Reporter constructs and reporter assays

Regulatory regions bound by both Six4 and MyoD were amplified from C2C12 genomic DNA and cloned upstream of the firefly luciferase gene in pGL3-Basic reporter plasmid (Promega). Mutations of the MEF3 motifs in these regulatory regions were introduced by sequential overlapping mutagenic PCR reactions. Primers are listed in Table A2.1. Luciferase reporter assays were performed on HEK 293T cells as explained before (182) using PEI as a transfection reagent following a protocol described previously (314).

The Luciferase reporter assays involve multiple controls which are used for normalization. First, all cells were co-transfected with plasmids carrying Renilla luciferase driven by the CMV promoter, which controls for cell number and transfection efficiency.

Second, cells not co-transfected with plasmids overexpressing MyoD or Six4 were co-transfected with an empty expression plasmid, which controls for basal promoter activity. To measure the effect of normalization procedures we used the noise (average divided by standard deviation) among replicate values for all promoters when cells were co-transfected with both MyoD and Six4 because these are most representative of variability in the dataset. The first normalization step was to divide all firefly luciferase (Luc) measurements by corresponding Renilla (Ren); this reduced average noise among replicates from 0.535 to 0.3890. The second step was to divide all Luc/Ren values for a given replicate to the corresponding Luc/Ren value representing basal promoter activity; this further reduced average noise to 0.186. Data normalized by both steps were used to calculate synergy and the individual effects of MyoD and Six4.

To calculate synergy values, we treated the calculation for additive synergy equivalently to the test for additive genetic interactions (326). The derivation is as follows. In the absence of synergy, the effect of a TF on promoter activity is expected to be the same regardless of the presence of a second TF. This assumption can be represented as $\alpha_{TF1} - \alpha_{bas} = \alpha_{TF1,TF2} - \alpha_{TF2}$, where α_{TF1} and α_{TF2} represent test promoter activities when the first (TF1) or second (TF2) TF is overexpressed, respectively; α_{bas} is the basal test promoter activity when no TFs are over-expressed and $\alpha_{TF1,TF2}$ is the activity when both TFs are overexpressed. Based on this assumption, the predicted promoter activity when both TFs are overexpressed but have no synergy is $pred_{\alpha,TF1,TF2} = m_{\alpha,TF1} + m_{\alpha,TF2} - m_{\alpha,bas}$, where m is the measured promoter activity. We can determine the magnitude of positive synergy by comparing the measured promoter activity when both TFs are overexpressed to the predicted value: synergy = $m_{\alpha,TF1,TF2} - pred_{\alpha,TF1,TF2}$.

2.16 Utx inhibition

C2C12 myoblasts were seeded at 100,000 cells in 6-well plates. Once confluent, cells were induced to differentiate for 24 hours in the presence of different concentrations (10 and 25 μ M) of GSK-J4 (R&D Systems) or in the presence of DMSO as vehicle. Cells were harvested for RNA and protein extraction using TRIzol.

2.17 Genomic data deposition

All ChIP-seq and DNA microarray data are deposited in the NCBI GEO database under accession number GSE66319 and GSE66901.

Chapter 3: Genome-Wide Cooperation between Six4 and MyoD in Regulating Adult Muscle Differentiation

3.1 Introduction

Skeletal myogenesis, whether prenatal or postnatal, is regulated by a coordinated function of several TFs with Pax3 and Pax7 specifying the muscle progenitor cells, and MRFs controlling their myogenic commitment and differentiation (reviewed in (14)). However, the complex transcriptional machinery regulating sequential steps during muscle differentiation involves other TFs besides MRFs. In fact, various families of TFs cooperate with MRFs to ensure a proper timely expression of genes regulating myogenesis. Among the TFs reported to cooperate with MyoD in regulating myogenesis are the Mef2 (180, 327) and Nfat family members (181). In addition, we have reported cooperation between Six4 and MyoD, and Six4 and Myog in inducing a luciferase reporter plasmid containing tandem copies of MEF3 and Ebox motifs. We have also shown cooperation between Six4 and MyoD in inducing a *Myog* proximal promoter reporter plasmid (182). Six4 and Six1 are involved in regulating many steps during prenatal myogenesis as early as specification of progenitor cells to muscle differentiation at their designed territories (233). A role for Six1 during *in vitro* adult muscle formation and *in vivo* muscle regeneration has recently been documented (182, 93). Six4 is also needed for *in vitro* adult skeletal myogenesis by regulating the expression of a group of genes needed for proper C2C12 cells differentiation as assayed by gene expression profiling performed after RNAi-mediated knockdown of Six4 (182). To identify genes directly regulated by Six4, I used high-throughput genomics approaches to map the binding sites of Six4 during muscle differentiation and to determine, as comprehensively as possible, the target genes regulated by Six4 during MyoD-induced myogenic conversion. The combination of these two approaches with the available data

about MyoD allowed the identification of genes concomitantly bound and potentially cooperatively regulated by both Six4 and MyoD.

3.2 Results

3.2.1 Six4 is required for efficient MyoD-induced myogenic conversion

In order to study the role of Six4 during adult myogenesis, I started by using the well-studied myogenic conversion assay where ectopic MyoD can convert the fibroblast cell line C3H 10T1/2 into myoblasts . Although C3H 10T1/2 cells are isolated from a mouse embryo (328), once converted into the myogenic lineage they represent adult myogenesis. This model has been used previously to study cooperation between MyoD and other TFs including members of the Mef2 (180), Nfat families (181), as well as vestigial-like gene 2 (Vgll2, (329)). To study a potential role of Six4 in regulating myogenic conversion and cooperating with MyoD, I first measured Six4 expression in C3H 10T1/2 fibroblasts using western blotting. Since Six4 is expressed in these cells (Fig. 3.1A), I used retroviruses expressing different shRNA sequences to stably knockdown Six4 expression in C3H 10T1/2 fibroblasts (Fig. 3.1B and C). I tested a number of RNA hairpins hoping to identify the one with the best knockdown of Six4 and no cross-reactivity towards Six1. I included Six1 in these experiments because I was interested in comparing the effect of Six4 knockdown to that of Six1, which has a known role in adult skeletal myogenesis. For this reason, stable C3H 10T1/2 cell lines with attenuated expression of either Six1 or Six4 were established using the shRNA retroviruses (Fig. 3.1B and C). One of the control scrambled shRNA (shNS) downregulated Six1 and Six4 expression, probably through off-target effects or other means, while shLuc, directed against the irrelevant Luciferase gene, did not appreciably affect the expression of Six1 or Six4 when compared with the non-transfected cells. For further experiments, only shLuc was used as a control shRNA. shSix1#4 (and 4b which

represents another biological replicate) as well as shSix1#5 affected the expression of both Six4 and Six1 and were not used for any further experiments. shSix1#2 and #3 had the most pronounced effect on Six1 knockdown without affecting Six4 expression, and consequently they were used as Six1 knockdown cell lines in subsequent experiments. Likewise, shSix4#1 affected both Six1 and Six4 while shSix4#2 had no effect on Six4 expression. shSix4#3, #4 and #5 significantly reduced the expression of Six4 without substantially affecting Six1 expression compared to shLuc; consequently, they were used in further experiments.

The C3H 10T1/2 cell lines were then used in the myogenic conversion assay where MyoD expression in the shLuc control cell line resulted in the induction of Myog expression (Fig. 3.2A), an early myogenic differentiation marker, and MHC (Fig. 3.2B), a marker of terminal muscle differentiation, 24 and 48 hours after inducing differentiation respectively. Both Myog and MHC expression were attenuated in the shSix4#4 cell line used (Fig. 3.2A and B). IF on representative cell lines 48 hours after inducing differentiation using an antibody against MHC revealed a decrease in myotubes formation in both shSix4#4 and shSix1#3 cell lines compared to shLuc cells (Fig. 3.2C). This is further represented by the significant reduction in the percent of differentiation (counting all nuclei within MHC⁺ cells relative to the total number of nuclei) and myoblast fusion index (counting all nuclei within MHC⁺ myotubes having at least three nuclei relative to all nuclei) in all shSix1 and shSix4 cell lines tested, except for shSix4#3, compared to the control shLuc cell line (Fig. 3.2D). shSix4#3 cell line shows the same trend of reduced percent of differentiation and fusion index like the other cell lines tested but it misses the statistical test cut-off. In summary, these data confirm the important role of Six1 and Six4 during adult myogenesis represented by the myogenic conversion assay and complements previous data using C2C12 myoblasts.

Figure 3-1 Establishing C3H 10T1/2 cell lines with attenuated expression of Six1 or Six4

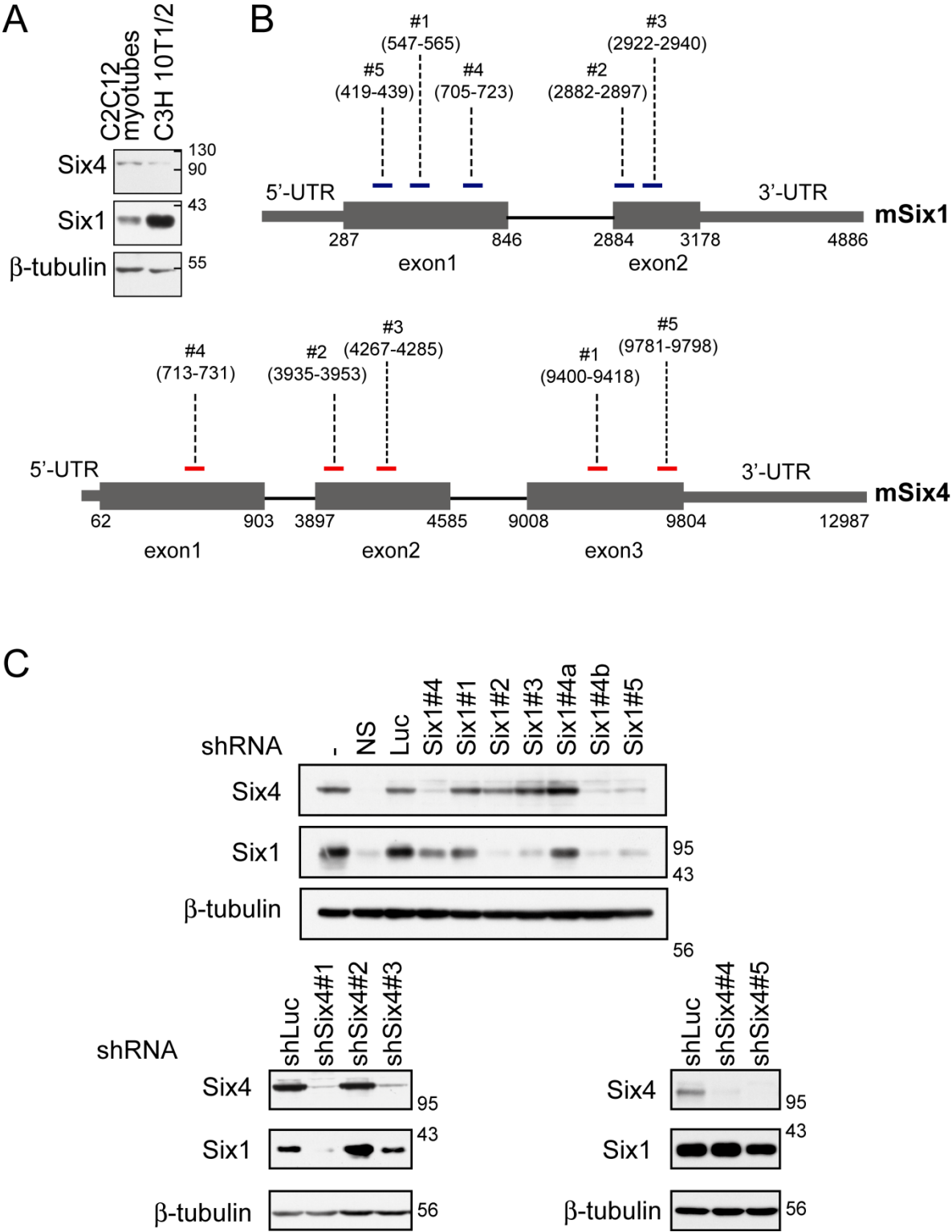


Figure 3.1. Establishing C3H 10T1/2 cell lines with attenuated expression of Six1 or Six4.

A) Western blot on total protein extracts from C2C12 myotubes and C3H 10T1/2 cells showing expression of Six1 and Six4. B) Graphical representation of shRNA sequences targeting Six1 and Six4 transcripts. C) Western blot on total protein extracts from the stable C3H 10T1/2 cell lines established using the different shRNA sequences. shSix1#4a and 4b are two separate biological replicates of cells transfected with the shRNA sequence #4. β -tubulin is shown as loading control.

Figure 3-2 Impaired myogenic conversion in shSix1 and shSix4 C3H 10T1/2 cell lines

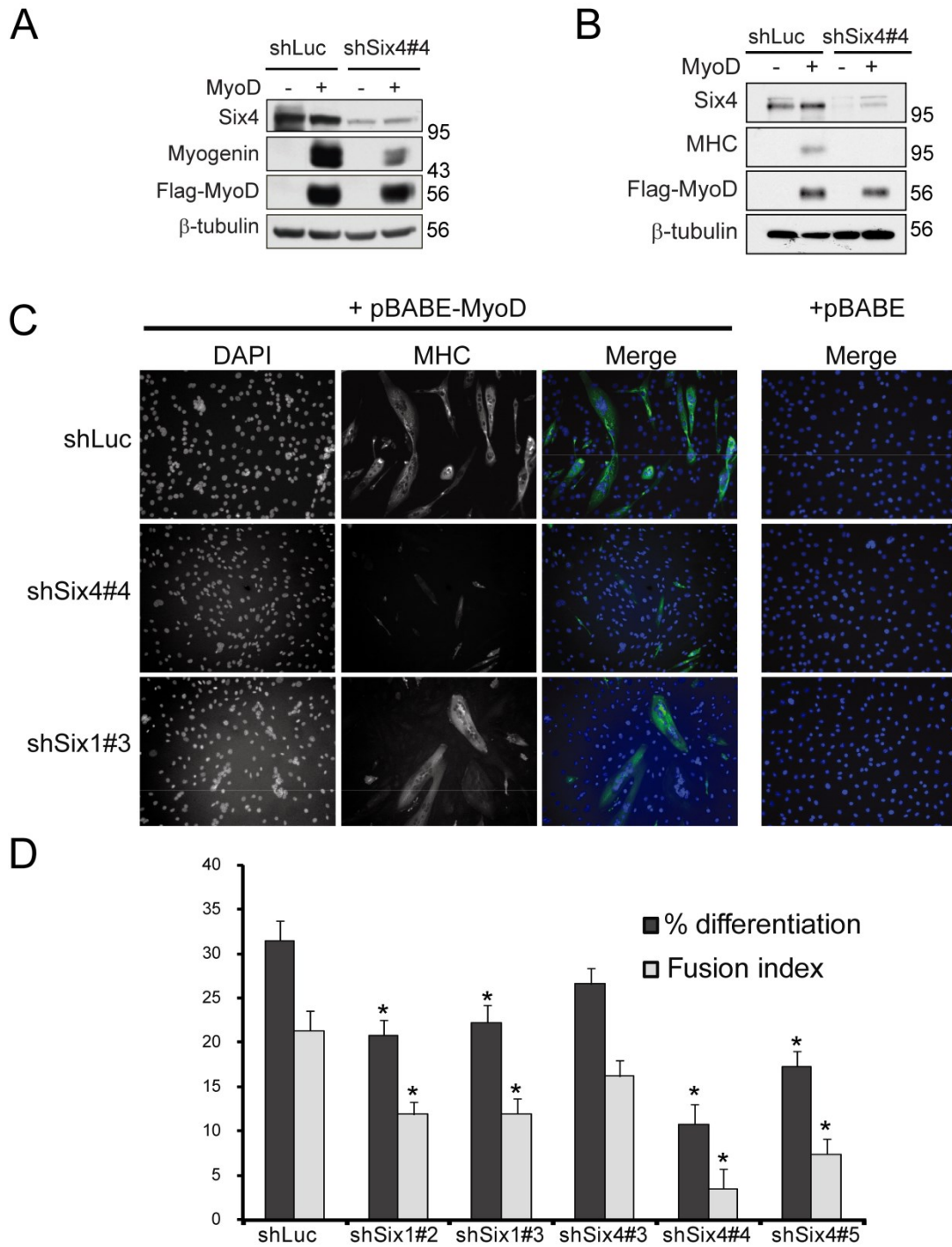


Figure 3.2. Impaired myogenic conversion in shSix1 and shSix4 C3H 10T1/2 cell lines.

A) Western blots on total protein extracts from representative cell lines 24 hours after inducing differentiation in presence or absence of ectopic MyoD. B) Western blot on total protein extracts from representative cell lines 48 hours after inducing differentiation in presence or absence of ectopic MyoD. β -tubulin is shown as loading control. C) IF on representative C3H 10T1/2 cell lines used in the myogenic conversion assay. In the merged images, DAPI is represented by blue staining while MHC is green. D) Percent of differentiation and fusion index are significantly impaired in Six1 and Six4 knockdown cell lines used ($n = 5$, * $p < 0.05$ using one-tailed unpaired student's t-test).

3.2.2 Six4 regulates a set of genes needed for efficient MyoD-induced myogenic conversion

We have used RNA interference assays coupled to gene expression profiling in order to identify genes regulated by Six4 during C2C12 myoblast differentiation. By using the C2C12 cell model, we were able to identify genes regulated by Six4 irrespective of their regulation by MyoD. To complement the C2C12 cell model and to identify Six4 target genes impaired during MyoD-induced myogenic conversion, I performed gene expression profiling on shSix4#4 or shLuc cells in the presence or absence of MyoD 24 hours after inducing differentiation. A large set of genes (2730 probes, 1944 unique genes) were significantly induced or downregulated ($p < 0.05$, fold change between shLuc + pBABE and shLuc + pBABE-MyoD ≥ 1.5) during MyoD-induced myogenic conversion (Appendix 2, Table A2.2). Many genes (723 genes) were also significantly affected by Six4 knockdown ($p < 0.05$, fold change between shLuc + pBABE-MyoD and shSix4 + pBABE-MyoD ≥ 1.5 ; Appendix 2, Table A2.3). These genes were clustered into four groups of co-expressed genes (Fig. 3.3). Cluster 1 contains 355 genes normally induced by MyoD but with attenuated induction in the cells with Six4 knockdown. This cluster counts many genes involved in muscle development or contraction, including *Myog*, multiple myosin heavy (*Myh1*, *Myh2*, *Myh6*, *Myh7*, and *Myh8*) or light chain genes (*Mylpf*, *Myl1*, *Myl3*, and *Myl4*), troponin genes (*Tnnc2*, *Tnni1*, *Tnni3*, *Tnnt1*, *Tnnt2*, and *Tnnt3*) and several others (Appendix 1, Table A2.4 contains a complete list of biological processes enriched in this cluster). Cluster 2 contains 193 genes normally downregulated by MyoD but with less pronounced reduction in the cells with Six4 knockdown. Genes in this cluster are enriched for different biological processes, including cell adhesion, signal transduction and proliferation (Appendix 2, Table A2.5). On the contrary, clusters 3 and 4 are composed of 175 genes affected by shSix4 independently of

Figure 3-3 Genes modulated by Six4 during myogenic conversion

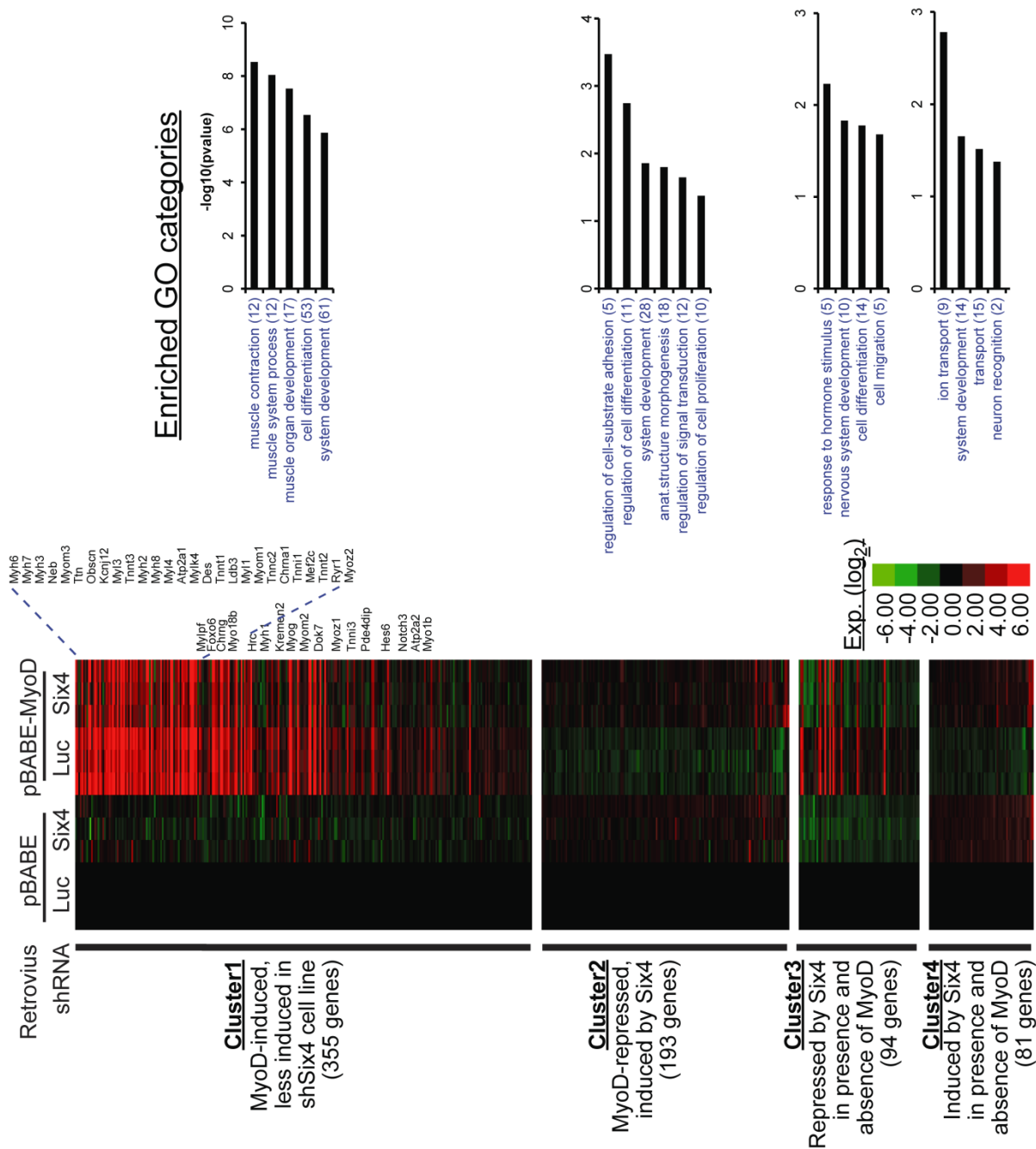


Figure 3.3. Genes modulated by Six4 during myogenic conversion.

Heat map on all genes significantly modulated by Six4 knockdown during myogenic conversion 24 hours after inducing differentiation on either shLuc or shSix4#4 cell lines in the presence or absence of MyoD ($p < 0.05$, fold change between shLuc + MyoD and shSix4 + MyoD ≥ 1.5). Heat map is presented as logarithm (base 2) of fold change of shLuc + pBABE sorted in decreasing order of Six4 effect on MyoD-regulated genes ($n = 3$ biological replicates for each condition). For each cluster, select biological processes from gene ontology that are significantly enriched ($p < 0.05$) are indicated, with the total number of genes in each process given in parentheses.

MyoD expression. Cluster 3 contains genes involved in different biological processes such as nervous system development, cell differentiation and cell migration (Appendix 2, Table A2.6), while genes in cluster 4 are enriched for biological processes such as ion transport, system development and neuron recognition (Appendix 2, Table A2.7). Together, these data indicate that MyoD cannot fully drive myogenic conversion without proper expression levels of Six4, and suggest that Six4 is a necessary myogenic TF co-regulating with MyoD the expression of a large set of muscle genes.

3.3.3 Generating a specific anti-Six4 antibody

To identify genes bound by and potentially regulated by Six4 during muscle differentiation, I set out to perform chromatin immunoprecipitation followed by high-throughput DNA sequencing (ChIP-seq) of Six4 in C2C12 myoblasts (291), an *in vitro* model of muscle differentiation, often used in large genome-scale experiments (183, 298, 301, 306, 307) as these cells do not undergo spontaneous differentiation. C2C12 cells can be maintained as myoblasts or induced to differentiate all the way to myotubes simply by altering the composition of the culture medium (Fig. 3.4A, (182)). C2C12 myoblasts (MB) differentiate into myocytes marked by the expression of Myog 24 hours after inducing differentiation (T24) followed by myotubes formation (T48 and later time points) marked by the activation of MHC expression (182). Six1 and Six4 are expressed throughout C2C12 cell differentiation from myoblasts to myotubes with maximal expression of Six4 at T24 (Fig. 3.4A, Liu et al, 2010).

We have previously mapped the genomic binding profile of Six1 during C2C12 cell differentiation (MB, T24 and myotubes) using ChIP-on-chip approach. Six1 was found to bind to a large set of genes regulating muscle formation or function (182). Six4 was shown to bind to some of these genes including *Myog*, *Ldb3* (Lim domain binding 3), *Chrng* (Choline-

Figure 3-4 Six4 binds to the regulatory regions of Six1 target genes during C2C12 myoblast differentiation

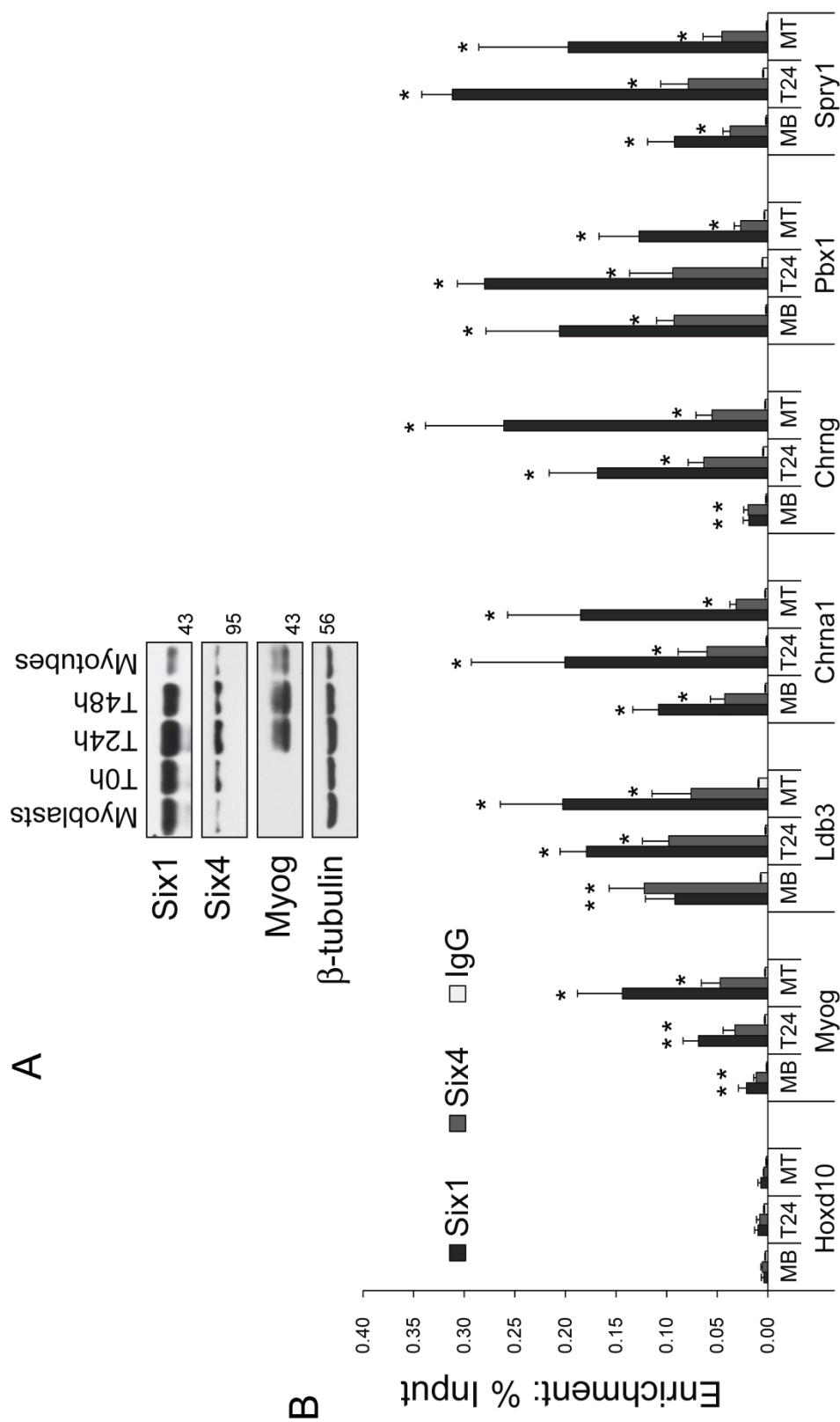


Figure 3.4 Six4 binds to the regulatory regions of Six1 target genes during C2C12 myoblast differentiation.

A) Western blot on total protein extracts harvested from C2C12 myoblasts, or 0, 24, 48 and 96 hours (Myotubes) after inducing differentiation. β -tubulin is shown as loading control. B) ChIP on C2C12 myoblasts (MB), at T24 or myotubes (MT) immunoprecipitated with anti-Six1, anti-Six4 or ctrl IgG. * $p < 0.05$ compared to the negative control locus *HoxD10* or Ctrl IgG ChIP, $n = 3$.

rgic receptor, gamma subunit), *Chrna1* (Cholinergic receptor, alpha 1 subunit), *Spry1* (Sprouty homolog 1), and *Pbx1* (Pre B-cell leukemia transcription factor 1) during the three tested time points of C2C12 differentiation (Fig. 3.4B, (182)). The binding of Six4 to Six1 target loci was believed to be a real binding event as potential cross-reactivity between anti-Six4 antibody and Six1 protein during ChIP is unlikely because the Six1 protein is undetectable in the anti-Six4 ChIP eluates by western blot (Fig. 3.5A, (182)).

Although ChIP followed by western blot did not show a cross-reactivity of anti-Six4 antibody with Six1 protein (Fig. 3.5A), IP performed on *in vitro*-translated (IVT) Flag-tagged Six1 or Six4 proteins with the anti-Six1 or anti-Six4 antibody followed by western blot using anti-Flag antibody showed that anti-Six4 antibody cross-reacts with Six1 protein but not vice versa (Fig 3.5B). Because it is essential to use a specific antibody before undertaking genome-wide ChIP studies, I set out to design a new rabbit anti-Six4 antibody.

Anti-Six4 antibody was generated against the C-terminal domain of Six4 protein starting from amino acid 414 till the last amino acid 775 (182, 208). Although this domain is not present in the Six1 protein, there are seven amino acids (PLTSSLV, amino acids 600-606) that are shared with Six1 protein and it would be preferable to avoid them in the new Six4 immunogen (Fig. 3.6). Among the Six factors, only Six5 has a long C-terminal domain (206); yet, this C-terminal domain is not homologous to Six4 C-terminal domain (Fig. 3.7). I therefore chose a new Six4 immunogen that starts after the three common amino acids between Six4 and Six5 (LLS) starting from amino acid 672 till amino acid 775. The newly designed rabbit anti-Six4 antibody was tested against all the Six family proteins expressed in reticulocyte lysates as Flag-tagged and immunoprecipitated using the new anti-Six4 antibody. Western blot performed on the IP eluates showed no cross-reactivity with the new

Figure 3-5 Cross-reactivity of the anti-Six4 antibody

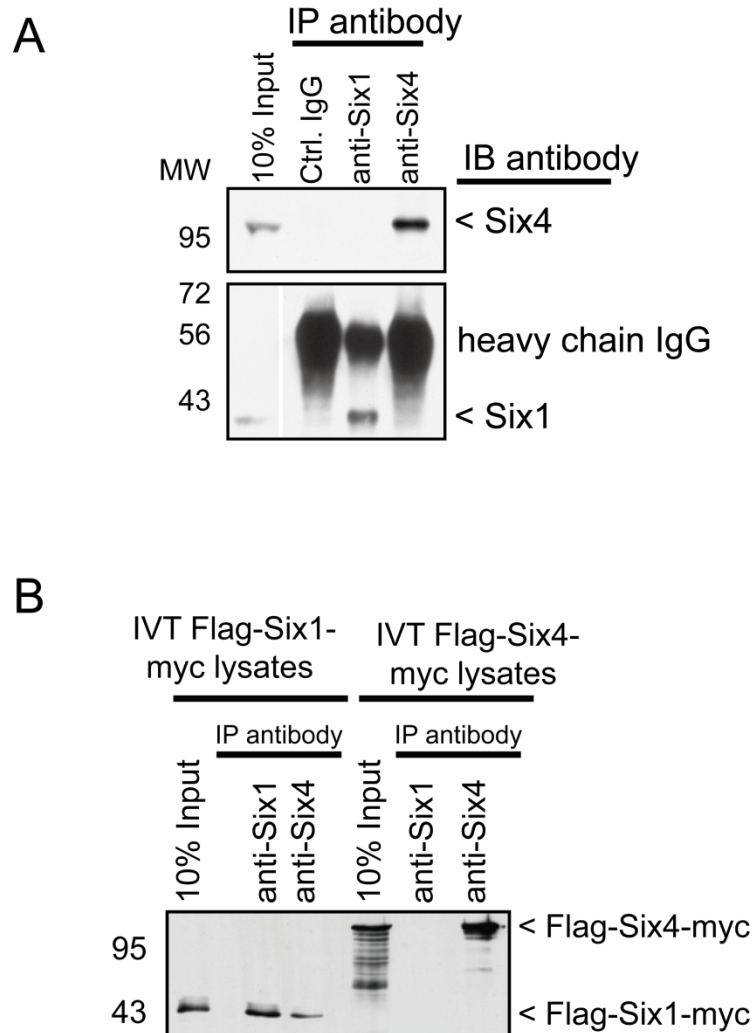


Figure 3.5. Cross-reactivity of the anti-Six4 antibody.

A) Western blot on ChIP samples from C2C12 24 hours after inducing differentiation immunoprecipitated with the indicated antibodies and probed with either anti-Six1 or anti-Six4 antibody. B) Western blot on *in vitro*-translated (IVT) Flag-Six1-myc and IVT Flag-Six4-myc expressed in reticulocyte lysates and immunoprecipitated using either anti-Six1 or anti-Six4 antibody. Membrane was probed with anti-Flag antibody.

[illegible]

Pairwise alignment of the murine Six1 and Six4 proteins using ClustalW2. Anti-Six1 antibody was raised against the entire Six1 protein while anti-Six4 immunogen starts at amino acids 414 to 775. The first three amino acids 'FKV' of the immunogen are highlighted in yellow. The amino acids highlighted in purple indicate identical residues between Six1 and Six4 proteins. “*” indicates identical amino acids, while “:” and “.” indicates amino acids with similar properties.

Figure 3-7 Pairwise alignment of the murine Six4 and Six5 proteins

```

Six4  MSSSSPTGQIASAADIKQENGMEASASEGQEAHREVAGGAAAGLSPPAPAPFFLEPGDAAA 60
Six5  -MATSPAEPsAGPAARG-----EAAAATEEQEEEARQLLQTLQAAEGEAAAAGAGDAAA 53
      ::*: : *..* .*: . * :* * *... . . *****

Six4  ASRVSREEGAAAAGAADQVQLHSELLGRHQHAAAQPPPLAFSPDHVACVCEALQQGGNLD 120
Six5  AADSGSPSGPGSP-----RETVEVPTGLRFSPEQVACVCEALLQAGHAG 98
      *: . .*.:. : : . . * ***:***** *: : .

Six4  RLARFLWSLPQSDLLRGNESLLKARALVAFHQGIYPELYSILESHSFESANHPLLQQLWY 180
Six5  RLSRFLGALPPAERLRGSDPVLRRARLVAFORGEYAELYQLLESRPFAAHHAFLQDLYL 158
      *:*** :** : : ***.:.*:*****:* *.***.***:*. :*:.*:***:

Six4  KARYTEAERARGRPLGAVDKYRLRRKFPLPRTIWDGEETVYCFKEKSRNALKELYKQNRY 240
Six5  RARYHEAERARGRALGAVDKYRLRKKFPLPKTIWDGEETVYCFKERSRAALKACYRGNRY 218
      :*** *****.*****.*****.*****.*****.*** ** * : ***

Six4  PSPAEKRHLAKITGLSLTQVSNWFKNRRQRDRNPSE--TQSKSESDGNPSTEDESSKGHE 298
Six5  PTPDEKRRLATLTGLSLTQVSNWFKNRRQRDRGTGTGGGAPCKSESDGNPTTEDESSRSPE 278
      *: * ***:**.:*****. : : .*****:*****:. *

Six4  DLSPHPLSGASDGVTNLSLSSHVEPVYMQQIGNAKISLSSSGVLLNGSLVPASTSPVFLN 358
Six5  DLRGVASMAAEAPAQs-----SIFLAGATSPATCPASSSILVNGSFLAASSPPAVLL 331
      **. * *:. : : : : : : : : : : : : : : : : : : : : :

Six4  GNSFIQGHNGVILNGLNVGNTQTVSLNPPKMSSNIVNGIAMTDILGSTSQDVKEFKVLQ 418
Six5  NGSPVIINSLALGENSSLGPLLLTGGSAPQPQPSLQGVSEAKNSLVLDPQTGEVRLDEAQ 391
      ..* : : . . : : . : * . . .*: : : : * . * . : : . . . : . *

Six4  SSAVNSAATTSYSPSAPVSFPGIIPCTEVKREGIQTVASQDGGSVVTFFTPQVINQYGIV 478
Six5  SEAP---ETKGVHGTGTEEIPGALPQVVPGPPASTFPLTPG-----AVPAVAAPQVVP 442
      *. * *.. : : .** : * . .*. * * :*. :

Six4  QIPNSGANGQFLNGSIGFSPLQLPPVSVAASQGNLS--VTPSTSDGSTFTSEPATVQH GK 536
Six5  LSPSSGYPTGLSPTSRLNLPQVVPTSQVVTLPQAVGPLQLLAAGPGSPVKVAAAAGPTN 502
      *.** : * :. *: *. * .. : : : : : : : .*. :

Six4  LFLSPLTPSAVVYTVPNSGQTVGAVKQEGLERGLVFSQLMPVNHSAQVNASLSENLSGS 596
Six5  VHLLNSSVGVTALQLPSSTAPGNFLLANPVSGSPIVTGVAVQQGKIILTATFPTSM LVSQ 562
      :.* : .... :*. * . . : : . . : : : : : : : : : : : : : * ..

Six4  GLHPLTSSLVNVSAAHGFSLTPPTLLNPTELNPDLAESQPVSA PVASKCTVSSVSNTNYA 656
Six5  VLPPAPSLALPLKQEP--AITVPEGALPVGPSPTLPEGHTLGP ISTQLPPASVVTSGTS 620
      * * . * : : : : : * * . * *. * : : : : : : : : : : : : :

Six4  TLQNCSLIPGQDLLSGPMTQAALGEIVPTAEEQVSHASTAVHQDFVREQRLVLQSVPNIK 716
Six5  LPFSP---DSSGLLSFSAPLPEGLMLSPAAPVWPAGLELSTGVEGLGTQATHTVLR LP 677
      . ...***. : . * :..* * *. : . . . : * :

Six4  ENFLQNSENKATNNMLMLDSKSKYVLDGMVEAGCEDLGTDKKELAKLQTVQLDEDMQDL 775
Six5  DDPDPQG-----LLLGATTGTEVD-----EGLEAEAKVLTQLQSVVPEEPLEL- 719
      : * . : : * : : : * * : : * * : * : * : : : :

```

Figure 3.7. Pairwise alignment of the murine Six4 and Six5 proteins.

New Six4 immunogen starts right after the three amino acids “LLS” highlighted in purple i.e. from amino acids 672 to 775.

antibody (Fig. 3.8A). Similar to the old anti-Six4 antibody, the new anti-Six4 antibody was able to immunoprecipitate Six4 in ChIP experiment showing a similar enrichment level to the old antibody (Fig. 3.8B). For further experiments, only the new anti-Six4 antibody was used.

In summary, these data demonstrate by using small-scale ChIP experiments that Six4 directly binds the regulatory regions of some muscle genes as reported by us and by other groups (182, 236, 270, 271). This opens the door to performing a comprehensive identification of Six4-bound loci during myoblast differentiation.

3.3.4 Genome-wide mapping of Six4 binding profile during myoblast differentiation

Since Six4 is expressed in C2C12 MB and its expression levels increase upon inducing differentiation in these cells, peaking at T24 post-differentiation (Fig. 3.4A), I performed ChIP-seq at T24. To obtain a controlled set of Six4-bound loci, I established C2C12 cell lines with either a stable knockdown of Six4 expression level (Fig. 3.9A) or a stable overexpression of Flag-Six4-myc (Fig 3.9B). shSix4#4 C2C12 line had the most pronounced effect on Six4 knockdown (Fig 3.9A) without having an effect on the expression of Six1 or MyoD at T24 (Fig. 3.9C). As a control, I performed ChIP-seq using the custom-made anti-Six4 antibody in the shSix4#4 cell line in comparison to shNS control cell line. As a second control, I performed ChIP-seq using the anti-Flag antibody in Flag-Six4-myc C2C12 cells. Finally, I also performed control ChIP-seq experiments using normal rabbit IgG and anti-Flag antibodies in parental C2C12 cells.

Using stringent analysis criteria (described in details in chapter 2), I identified a total of 58,528 Six4 binding peaks in the shNS C2C12 cell line. Although this is relatively a large set of bound loci, they represent high confidence Six4 binding events as evident by the ChIP-seq read densities heat map (Fig. 3.10). Six4 binding at peaks identified in the shNS C2C12 is

Figure 3-8 Specificity of the newly custom-made rabbit anti-Six4 antibody

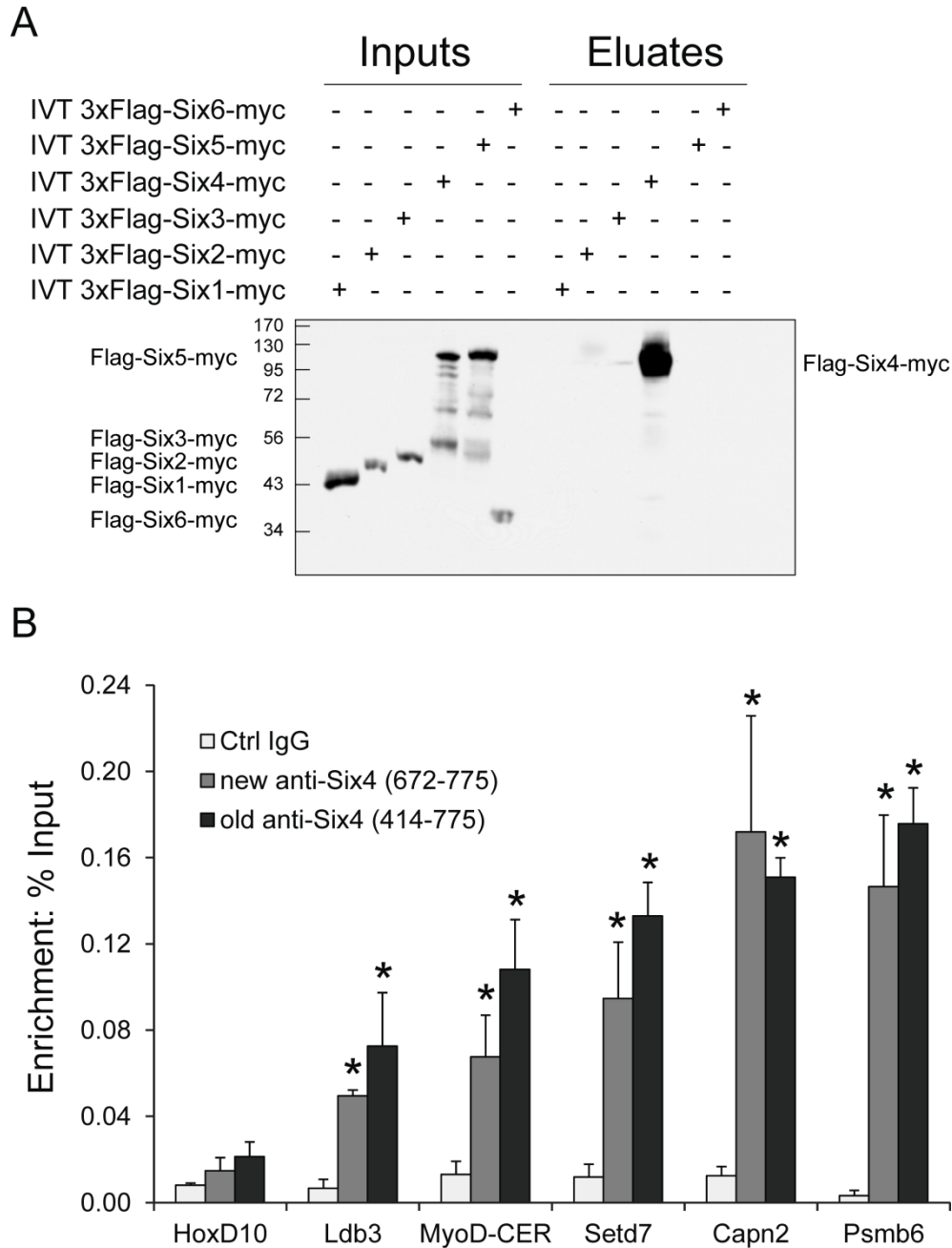


Figure 3.8. Specificity of the newly custom-made rabbit anti-Six4 antibody.

A) *In vitro*-translated 3xFlag-tagged full length mouse Six family proteins were expressed in reticulocyte lysates and immunoprecipitated using the new rabbit anti-Six4 (amino acids 672-775) antibody. Western blot membrane was probed with an anti-Flag antibody. B) ChIP on C2C12 cells at T24 using the new and old (amino acids 414-775) anti-Six4 antibodies. *, $p < 0.05$ compared to Ctrl IgG ChIP and *HoxD10*, $n = 3$.

Figure 3-9 C2C12 cell lines used in ChIP-seq

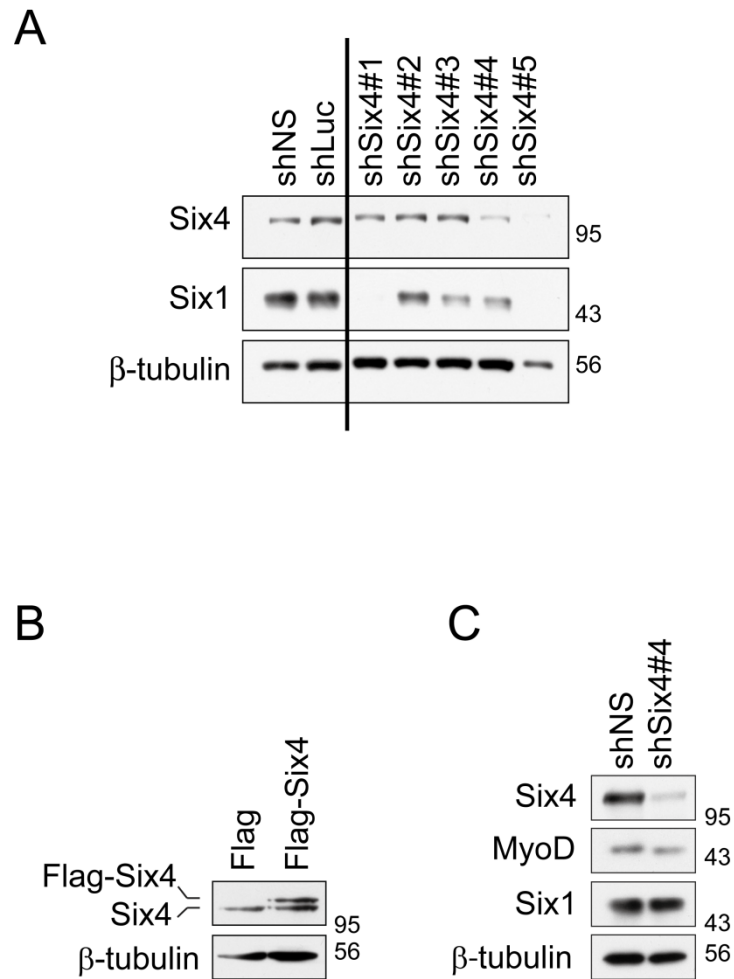


Figure 3.9. C2C12 cell lines used in ChIP-seq.

A) Western blot on total protein extracts harvested from C2C12 cell lines established using the shRNA sequences described in Figure 3.1 and harvested as myoblasts. The black line separates lanes that are not contiguous on the film but they are from the same film and same exposure. B) Western blot on total protein extracts from C2C12 cell line overexpressing Flag-Six4-myc or empty expression plasmid. C) Western blot on total protein extracts from shSix4#4 C2C12 cell line harvested at T24, n = 3. β -tubulin is shown as loading control.

associated with a decrease in read density (i.e. peak height) of the corresponding peak in the shSix4 cell line which is more evident by the plot of the difference in read densities of shNS and shSix4 cell lines (Fig. 3.10). Besides, Six4 binding is paralleled by binding of Flag-Six4, although the anti-Flag antibody did not give us the same degree of enrichment as the anti-Six4 antibody (Fig. 3.10). This is further supported by the boxplot of the ChIP-seq mean signal around peaks summit where the binding of Flag-Six4, as well as the decrease of ChIP-seq read density in the shSix4 dataset are observed only at Six4 peaks, but not at control regions not bound by Six4 (Fig. 3.11A). One of these peaks is represented by Six4 binding at the 5'UTR of one of the isoforms of the *Pde4dip* (Phosphodiesterase 4D Interacting Protein) gene (Fig. 3.11C). Both anti-Six4 and Flag-Six4 antibodies yielded a distinguishable ChIP-seq peak, and the peak height dropped by approximately 70% with Six4 knockdown (normalized read density of 11.41 in shNS and 3.63 in shSix4), further suggesting that it represents a high confidence Six4 binding site.

To assign genes to Six4 peaks, I first used PAVIS (322) to annotate Six4 peaks to genes within 50 kilobase pairs (kb) of the transcriptional start site (TSS) and 10 kb of the transcriptional end site. PAVIS allows annotation of each peak to a single gene with the TSS closest to the peak summit. Based on these criteria, most of the Six4 binding events are relatively close to the TSS of genes; yet, a significant proportion of Six4 binding sites (17%) are found more than 50 kb away from any gene start site (Fig. 3.12A). When the Six4-bound loci are broken down based on their location versus genes, 60.3% of these peaks are present within genes (exons, introns, 5'UTR and 3'UTR), while the rest are intergenic, falling in proximal promoters or in more distal regions (Fig. 3.12B).

Distinct chromatin marks are associated with various genomic regions and reflect different gene expression patterns (reviewed in (305)). I next analyzed the various categories

Figure 3-10 Read density plots of ChIP-seq in C2C12 cell lines

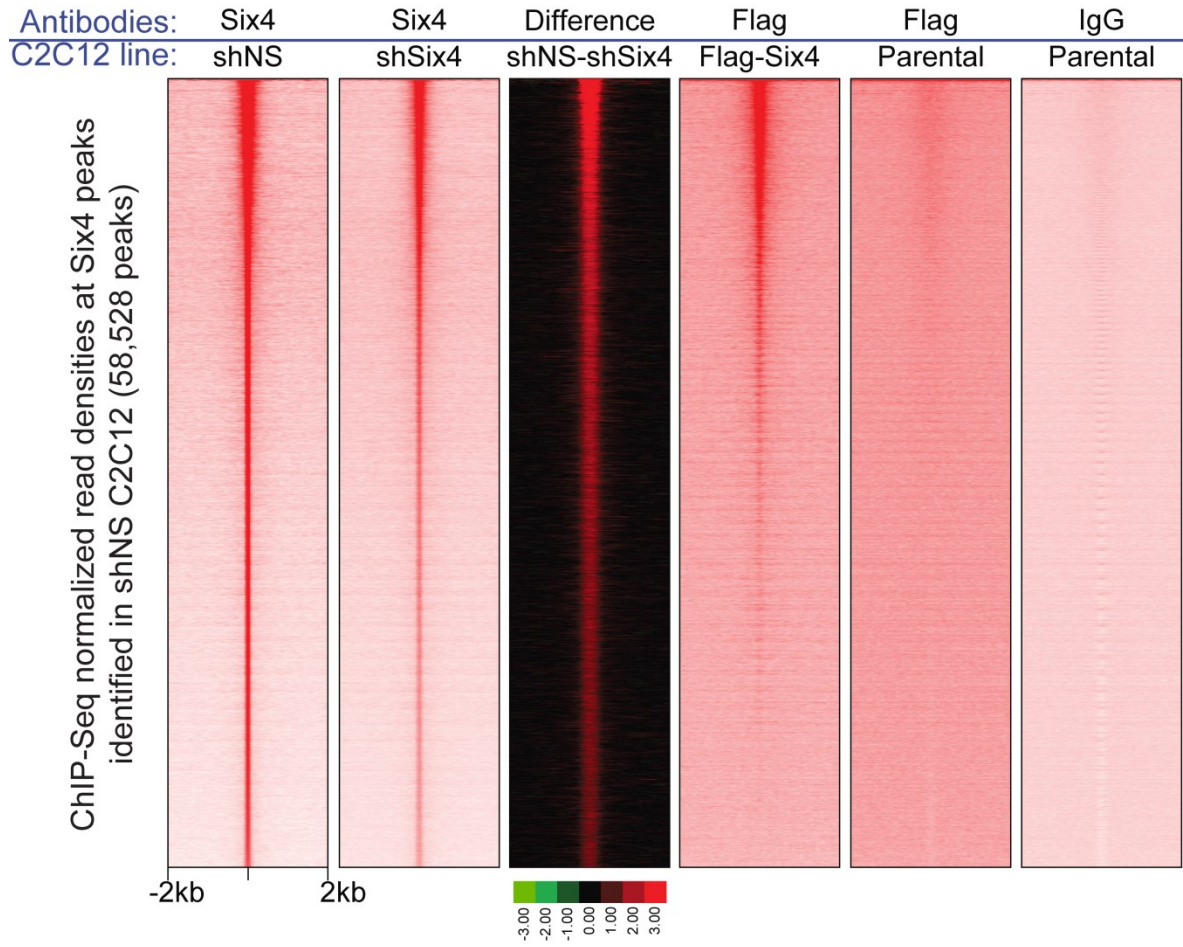


Figure 3.10. Read density plots of ChIP-seq in C2C12 cell lines.

Heat maps of Six4 ChIP-seq read densities in C2C12 cell lines at Six4 peaks identified and sorted by decreasing peak height in the anti-Six4 experiment in shNS C2C12 cell line. All plots were generated using seqMINER (325) except for the difference in read densities of shNS and shSix4 ChIP-seq which was generated using bedtools genomecov. Stronger color intensity represents higher ChIP-seq read density. Colorbar of the heat map of the difference in the read densities of shNS and shSix4 is represented underneath. Black means no difference in binding, red means weaker binding after Six4 knockdown, and green represents weaker binding in control conditions. The red color predominates throughout.



A) Mean read densities of Six4 ChIP-seq in shNS, Flag-Six4, and parental C2C12 cell lines 200 bp around the summit of Six4 peaks identified in shNS cell line, or in negative control regions. B) An example of Six4 binding close to the TSS of *Pde4dip* gene. Only two mRNA isoforms of *Pde4dip* are shown for clarity and Six4 binds close to the TSS of one of them.

of Six4 binding sites in relation to distinct chromatin features mapped in the C2C12 cells including RNA pol II and histone marks associated with gene expression such as H3K4me3 and H3K9ac or gene repression such as H3K27me3 (306). Six4 binding sites at proximal promoters seem to have the most distinguishable profile of these marks, with the strongest enrichment for H3K4me3 and the lowest amount of H3K27me3. H3K36me3 accumulates within the body of genes that are actively transcribed and seems to be higher in Six4 binding sites falling inside genes (Fig. 3.12C).

Although PAVIS is a useful binding site annotation tool, it has a drawback of assigning only one gene per binding site. To circumvent this limitation, we devised an algorithm (implemented in Perl) that uses the “Known genes” annotation table from the UCSC genome browser (323) and assigns several genes to a single binding site within a pre-set distance window from the binding site peak. By using this script, I identified all genes within 50 kb window from each peak summit. Next, I combined ChIP-seq annotation table with gene expression profiling data performed during C3H 10T1/2 myogenic conversion (discussed earlier) and in differentiating C2C12 myoblasts targeted by siSix4 (182). I set an algorithm to locate the TSS of genes whose expression is deregulated by the knockdown of Six4 in differentiating C2C12 myoblasts (182) or in fibroblasts undergoing myogenic conversion (from Fig. 3.3). If no deregulated gene existed within this window, then the gene whose TSS is closest to the peak summit was assigned. Approximately 74% of the ChIP-seq peaks (43,540 peaks) were annotated to a total of 14,637 unique genes (data available in (310)). Functional annotation term enrichment analysis revealed that Six4 target genes are involved in various biological processes including muscle development, represented by 438 genes, regulation of transcription, represented by 1149 genes, and cell proliferation by 1261 genes (Table 3.1; a complete list of enriched functional annotation terms is available in Table A2.8).

Figure 3-12 Six4 peaks annotation

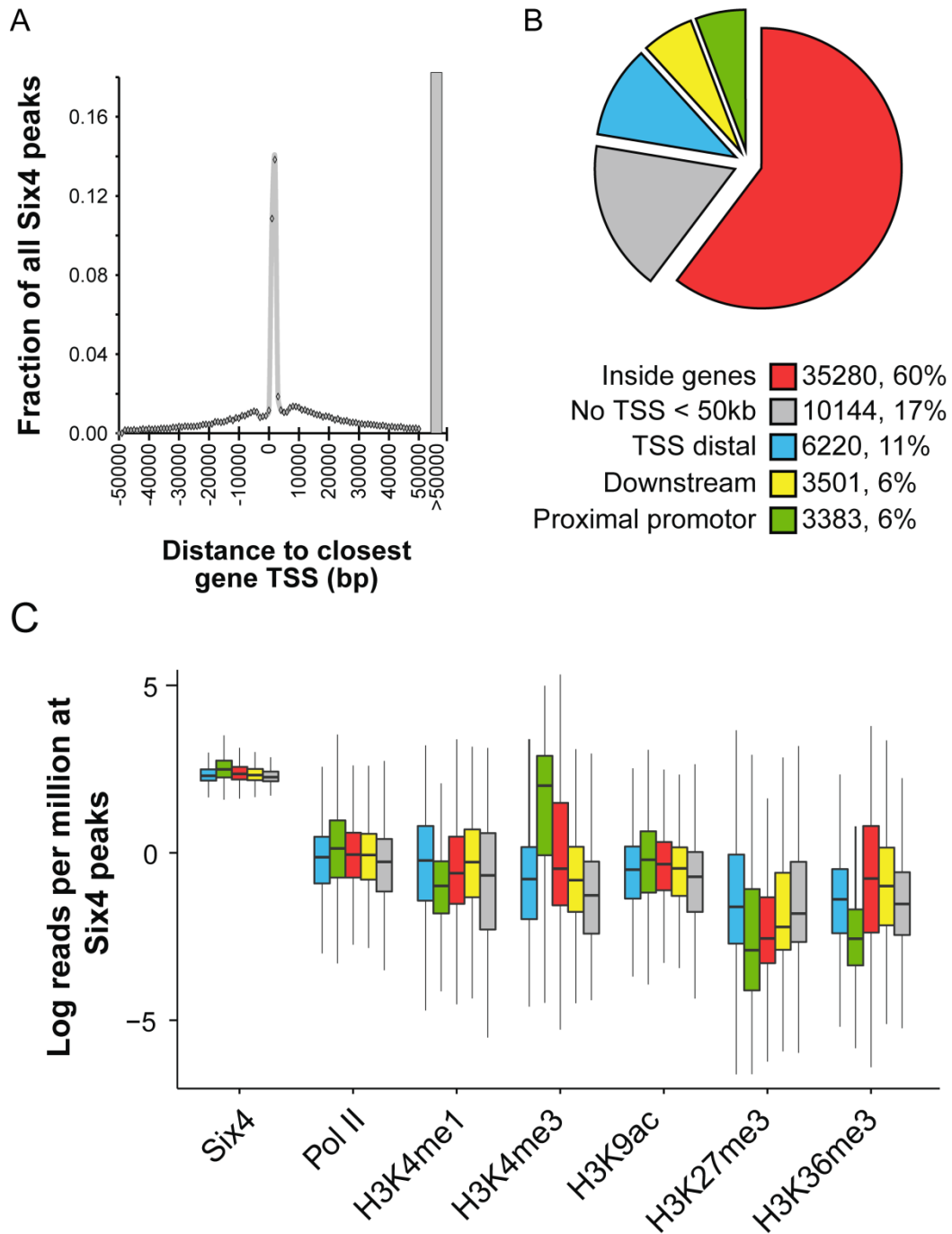


Figure 3.12. Six4 peaks annotation.

A) Distribution of Six4 binding sites with regards to the closest gene transcriptional start sites (TSS). B) Distribution of Six4 peaks identified in shNS C2C12 cell line (58,528 peaks) in relation to their distance from known genes using PAVIS (322). C) Average ChIP-seq read density of histone marks and RNA pol II at Six4 peaks separated according to their location with regards to known genes, based on panel B.

Table 1 3-1 Selected gene ontology biological processes significantly enriched among all Six4-bound genes

Table 3.1 Selected gene ontology biological processes significantly enriched among all Six4-bound genes.

Rank	Biological process	Six4-bound	Total in genome	FDR ^a	Examples
1	cell development	1396	1970	1.36E-42	Cdx2, Bdnf, Atoh1, Nkx3-2, Bmp4, Runx2, Ctnnb1, Cux1, Numb, Gas1, Map3k1, Sfrp2, Robo1
2	neurogenesis	1050	1441	2.44E-39	Eya1, Etv4, Fgf8, Gli2, Rora, Tbx6, Gsk3b, Cdk5, Gdf6, Neurod1, Neurog2, Heyl
10	reg. of transcript. from RNA pol. II promoter	1149	1629	1.70E-33	Atf1, Bach1, Prdm1, Bmi1, Chd2, Klf6, Hoxb1, Foxo3, Rxra, Smarce1, Sox14,
16	cardiovascular system development	679	905	2.74E-31	Gata4, Mef2a, Cited1, Nr4a1, Notch2, Tbx1, Tbx5, Tead1, Hand1, Lbx1, Smyd1
34	cellular response to growth factor stimulus	510	665	3.16E-27	Acvr1, Rhoa, Bmpr1a, Cd44, Mapk14, ErbB4, Mtor, Itga5, Smad3, Tgfbr3, Sos1
37	muscle structure development	438	558	3.99E-27	Bmp2, Camk2d, Dmpk, Foxc1, Six1, Six4, Rbpj, Mef2c, Myog, Myf5, Nfatc4, Sox8
58	cell proliferation	1261	1887	6.10E-22	Cdc20, Cdc25c, Ccnd3, Bub1b, Cdk4, Cdkn1c, E2f3, Fosl2, Jak2, Nfya
59	cell-cell signaling	887	1281	1.14E-21	Bmp2, Chrna1, Chrmg, Gabra3, Gnai1, Inhbb, Irs1, Itgb2, Pdgfa, Fgfr1, Fgf20
82	programmed cell death	1218	1832	5.29E-20	Casp3, Casp8, E2f2, Gadd4b, Nfkb1, Fadd, Tnfrsf18, Ripk2, Traf4
89	cell migration	748	1073	2.23E-19	Angpt1, Rhog, Egfr, Ccr4, EphA1, Ephb3, Cxcr2, Met, Myo1c, Ccl4, Sema5a, Kif20b
97	actin filament-based process	439	592	1.44E-18	Actn1, Actn2, Arhgap6, Cav3, Des, Myh3, Arhgef5, Rock2, Racgap1, Tmod3

^a The *false discovery rate* values given are for multiple hypothesis testing by the algorithm of Benjamini and Hochberg. The background set of genes used in these calculations was the entire genome.

Other enriched categories include neurogenesis, represented by 1050 genes, and cardiovascular development, represented by 679 genes. The enrichment of these categories can reflect genes involved in more than one pathway such as *Eya1* and *Hey1* which are involved in neurogenesis and myogenesis. This is not an unexpected finding as Six4 is involved in regulating many developmental processes in addition to myogenesis.

Some Six factors are known to bind to the MEF3 DNA sequence motif, and we reported previously a MEF3 motif that better represents the Six1 binding profile based on Six1 ChIP-on-chip in C2C12 cells (182, 214). I performed a *de novo* motif search among the top (highest confidence peaks with the lowest MACS p value) Six4-bound sequences and discovered a MEF3-like motif (Fig 3.13A) very similar to that we found for Six1 (182, 214). Mapping this motif in all Six4 binding sites identified in ChIP-seq and in matched negative control loci revealed that it is strongly enriched among Six4-bound regions ($p < 3 \times 10^{-16}$ by hypergeometric distribution test) with the strongest enrichment being among the highest confidence (lowest MACS p value) target loci (Fig. 3.13B).

3.3.5 Six4 expression, binding profile and functional overlap with MyoD

The myogenic conversion data (Figs 3.2 and 3.3, Table A2.3) implicates a potential cooperation between Six4 and MyoD in regulating myogenesis. Moreover, *de novo* motif search among the highest confidence Six4-bound peaks identified several motifs enriched in these sequences including the Ebox motif, the DNA binding site of MyoD (Fig. 3.13C). Similar to the MEF3 motif, the Ebox motif is overabundant among the top Six4 peaks (Fig. 3.13D, $p = 9.99 \times 10^{-16}$, hypergeometric test) with a distribution comparable to that of the MEF3 motif (Fig. 3.13B). Additionally, the genomic binding profiles of Six4 and MyoD overlap considerably with a total of 9,422 bound by both Six4 and MyoD in myotubes representing 4,733 unique genes (Fig. 3.13E). These common peaks represent 36% of the

Figure 3-13 A significant overlap between Six4 and MyoD peaks

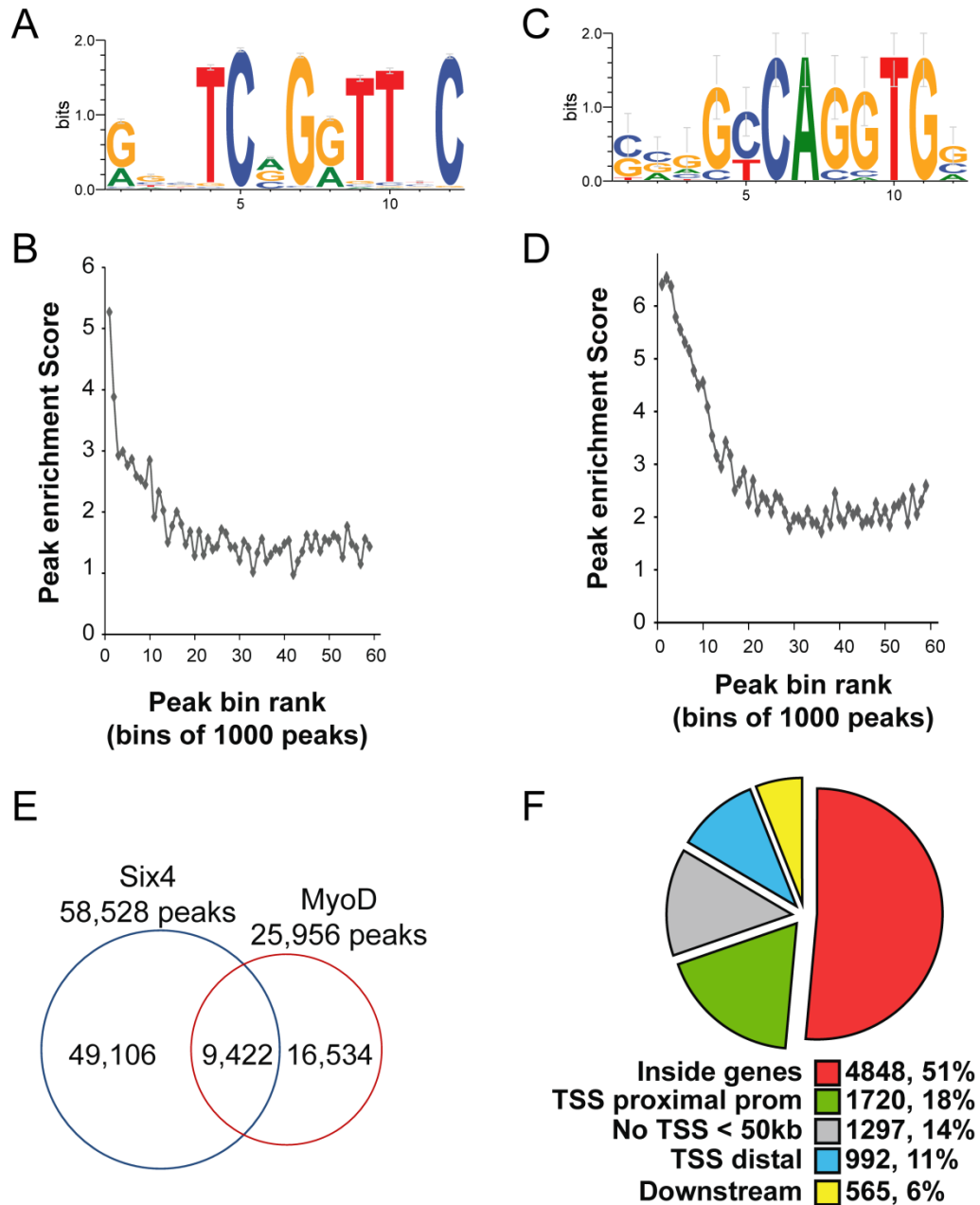


Figure 3.13. A significant overlap between Six4 and MyoD peaks.

A) Logo representation of the de novo MEF3 motif identified within Six4 bound regions. B) MEF3 enrichment curve among Six4 peaks in bins of 1000 sorted in order of increasing MACS p-value. C) Ebox motif is enriched (D) among Six4 peaks. E) Overlap between Six4 peaks identified in the anti-Six4 experiment in shNS C2C12 cell line and MyoD peaks identified in Cao et al, 2010 (298) using UCSC genome table browser. Six4 and MyoD binding sites are considered to overlap if their respective center points are separated by less than 1 kb. F) Distribution of Six4 binding sites with regards to assigned target genes, as determined by PAVIS (322).

peaks bound by MyoD and 16% of the peaks bound by Six4. When considering the distance of these joint Six4-MyoD target sites from the TSS of closest known genes (Fig. 3.13F), those binding within genes are still the most abundant, but with an increase in the proportion of the sites binding within proximal promoters compared to the entire set of Six4 targets from 6% to 18% (compare Fig. 3.13F with 3.12B). Representative examples of Six4 and MyoD binding at proximal promoters and distal regulatory regions of common genes are shown in Fig. 3.14A compared to regulatory regions bound only by Six4 (Fig. 3.14B). In each case, both Flag-Six4 and Six4 show binding at these loci, and the Six4 peak height drops significantly in shSix4 cell line compared to shNS cell line.

Binding of Six4 and MyoD at a select number of common loci was confirmed by ChIP assays followed by qPCR (qChIP) performed in the shNS C2C12 cells at T24 (Fig. 3.15A). Among the selected regulatory regions are those of *Psm6* (Proteasome subunit, beta type, 6) and *Setd7* (SET domain containing (lysine methyltransferase) 7) which show significant ChIP enrichment only for Six4 and not for MyoD, as these two loci are bound only by Six4 but not by MyoD based on ChIP-seq data. Instead, a significant binding of both Six4 and MyoD was detected at the regulatory regions of a set of their common targets including muscle genes and developmentally important TFs in agreement with ChIP-seq data. Among the selected muscle genes are *Chrng*, *Chrna1*, *Cacng1* (calcium channel, voltage-dependent, gamma subunit 1), *Afap1l1* (actin filament associated protein 1-like1), *ATP2a1* (calcium transporting ATPase, fast twitch 1), *Obscn* (obscurin), *Hrc* (histidine rich calcium binding protein), *Myoz2* (myozenin 2), *Pde4dip* (phosphodiesterase 4D interacting protein), *Trim54* (tripartite motif containing 54, also known as *Murf3*) and *Myog*. Among the developmentally important transcription factors are *Myog*, *Hey1*, and *Hes6* (hairy and enhancer of split 6). On

Figure 3-14 Six4 binding profile overlaps with that of MyoD

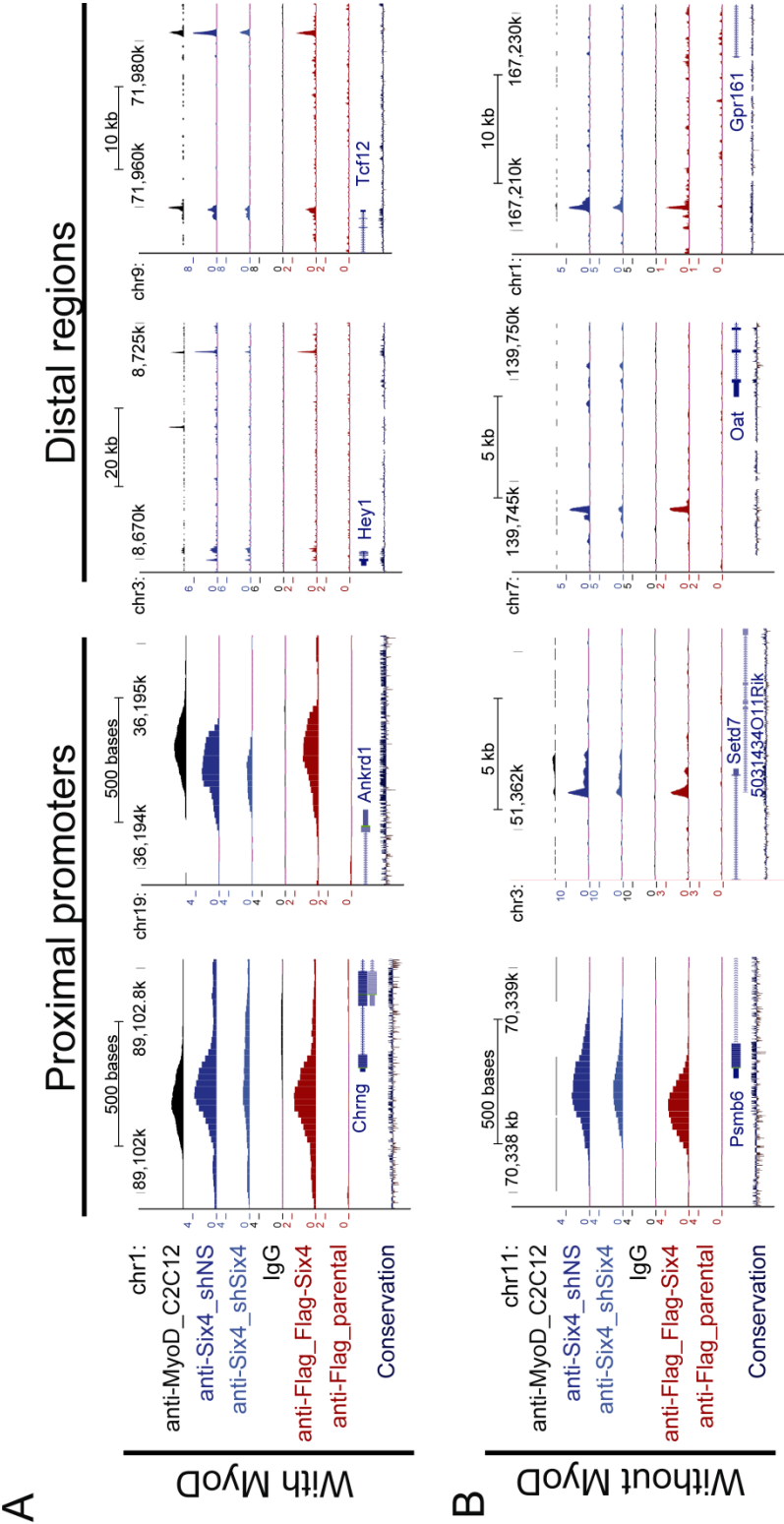


Figure 3.14. Six4 binding profile overlaps with that of MyoD.

A) Examples of Six4 and MyoD binding at common loci, either at proximal promoters or at more distal regions.
B) Examples of Six4 binding sites (gene-proximal or -distal) that do not overlap with MyoD binding sites.

the contrary, no significant enrichment of either Six4 or MyoD was detected at two negative controls, the promoter of *HoxD10* (homeobox protein Hox-D10) and a gene desert locus found on chromosome 15.

To further confirm that Six4 binding at the selected loci represents a true binding event, ChIP followed by gene-specific qPCR was performed in the shSix4 C2C12. A reduction in the enrichment level at all tested Six4-bound loci was observed in shSix4 cells compared to shNS cells (Fig. 3.16). Six4 binding by ChIP was not restricted to C2C12 myoblasts as it was also observed in myotubes obtained by differentiating primary mouse myoblasts isolated from adult mouse hindlimbs (Fig. 3.15B). To summarize, by using well-controlled ChIP-seq parameters, I have identified a comprehensive set of Six4 transcriptional targets in differentiating adult muscle cells, a substantial proportion of it is likely to be under a putative combined regulation of Six4 and MyoD.

3.3.6 Transcriptional synergy between Six4 and MyoD

To test the ability of Six4 and MyoD to induce the expression of their joint target genes, I cloned the regulatory regions (as defined by the Six4 and MyoD ChIP-seq peaks) of selected targets into a reporter plasmid upstream of the firefly luciferase reporter gene, and performed promoter reporter assays to evaluate the activity of Six4 and MyoD on these regulatory regions. Reporter assays were performed in HEK 293T cells as these cells express no MyoD and very little Six4 proteins, so the effect of ectopic expression of these two transcription factors can be robustly detected. Luciferase activity was measured in cells transfected with the promoter reporter plasmids, with or without co-transfection of expression plasmids for Six4, MyoD/ HEB- β (MyoD along with its partner E-protein HEB- β), or both Six4 and MyoD/HEB- β . When expressed individually, Six4 and MyoD significantly activated transcription from the cloned promoters above baseline levels (black

Figure 3-15 qChIP confirming overlap between Six4 and MyoD binding profiles

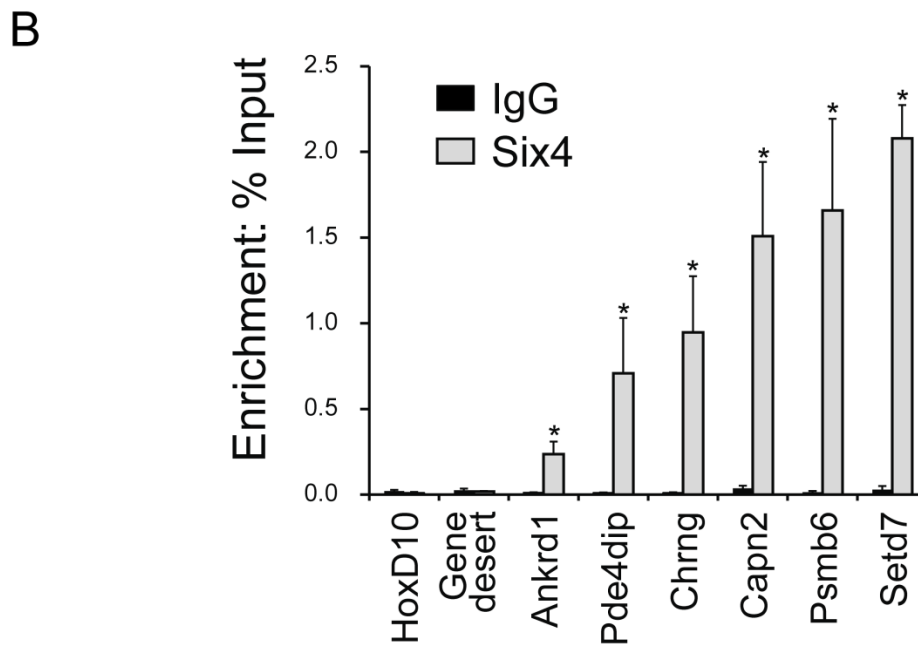
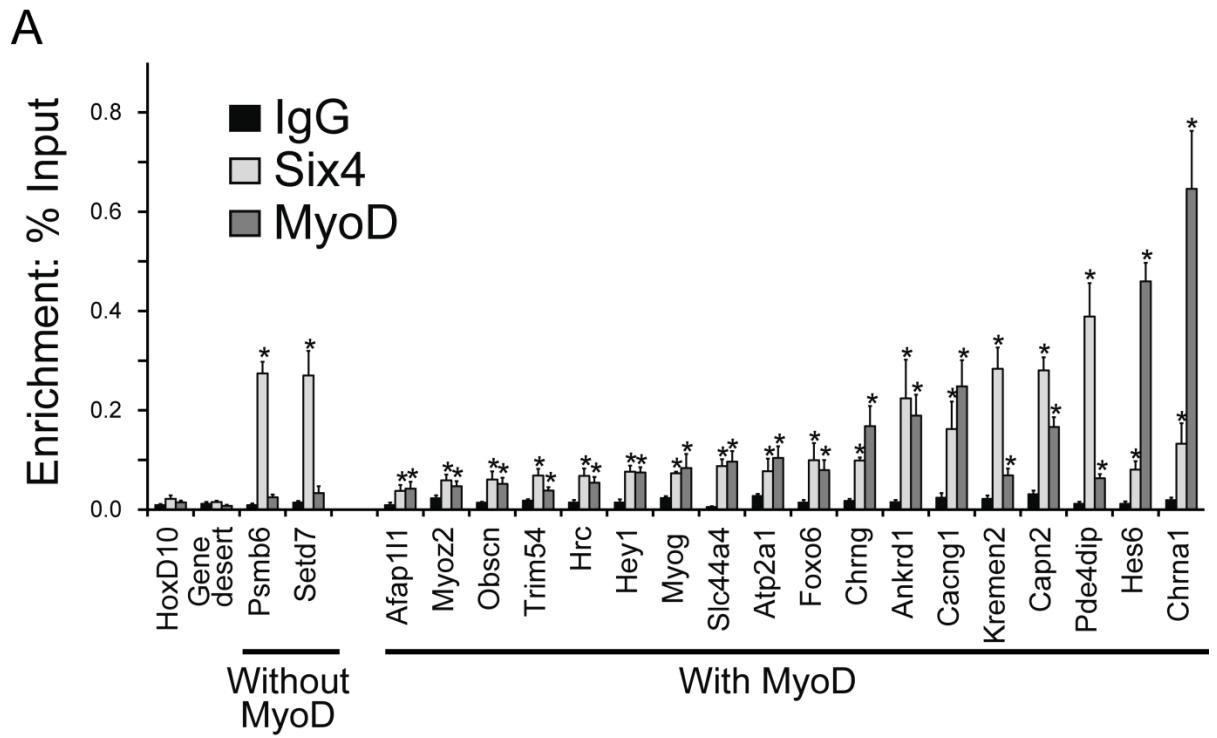


Figure 3.15. qChIP confirming overlap between Six4 and MyoD binding profiles.

A) Binding of Six4 and MyoD is confirmed by ChIP-qPCR to negative and Six4-only or joint Six4- and MyoD-bound loci, in C2C12 cells at T24 (n is between 4 and 6 biological replicates). The y-axis represents enrichment of transcription factor binding expressed as percent of input material. Error bars represent the standard error of the mean. Asterisks indicate that by one-tailed unpaired student's t-tests ($p < 0.05$), the ChIP enrichment using the specific antibody is significantly higher than ChIP using normal rabbit IgG, and is also significantly higher at the tested target loci than at the two negative control loci (*HoxD10* and gene desert located on chromosome 15). B) ChIP assays for Six4 performed in myotubes prepared from primary mouse myoblasts ($n = 3$), and represented as in panel A.

Figure 3-16 ChIP-qPCR in shNS and shSix4 C2C12 cells

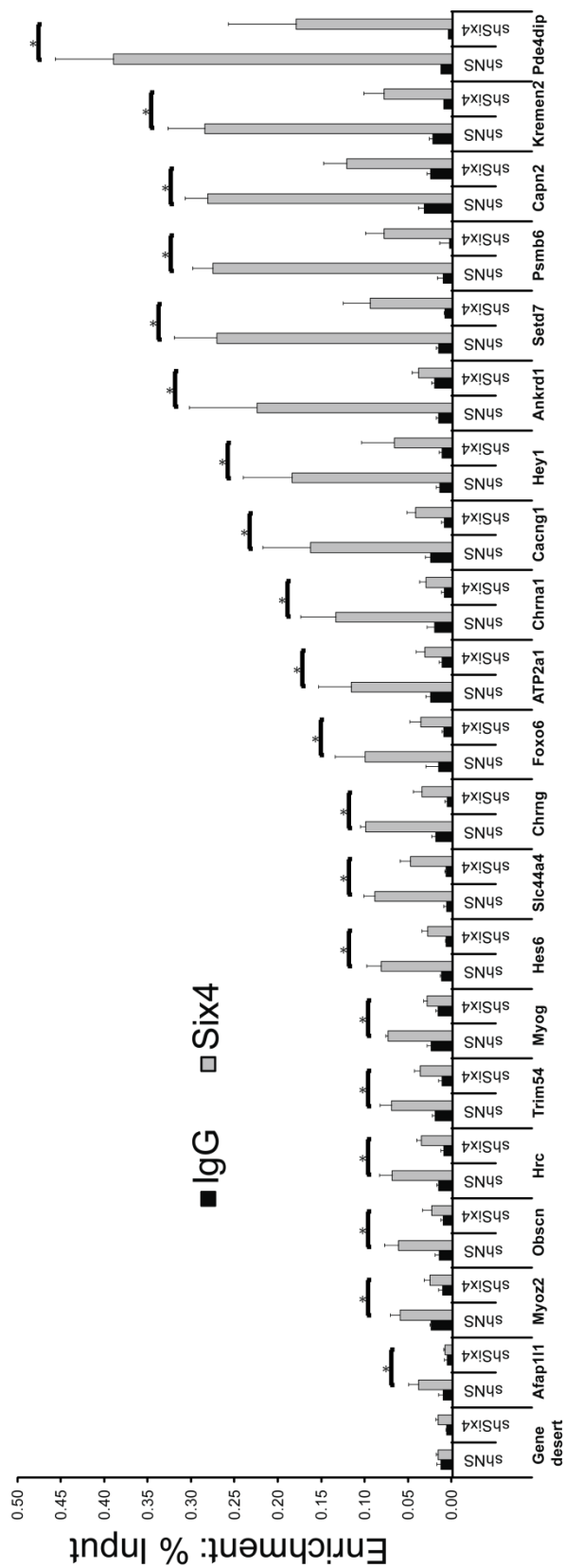


Figure 3.16. ChIP-qPCR in shNS and shSix4 C2C12 cells.
The ChIP experiments performed 24 hours after inducing differentiation confirm that these loci are bound by Six4. n is between 4 and 6 biological replicates. Asterisks indicate $p < 0.05$ compared to shNS using one-tailed unpaired student's t-test.

dashed lines, Fig. 3.17). When Six4 and MyoD were co-expressed, a transcriptional synergy was observed (more-than-additive effect, red dashed lines, Fig. 3.17) between them on all the tested promoters. The assays were also performed on reporter constructs containing point mutations of MEF3 sites where I observed an abolishment of the synergy between Six4 and MyoD. This confirms that the synergy is due to direct binding of Six4 to the tested promoters and rules out indirect effects. These results extend our previous observation of cooperation between Six4 and MyoD in regulating transcription from the *Myog* promoter to demonstrate that this cooperation occurs at many more gene promoters, suggesting that the transcriptional synergy between Six4 and MyoD might reflect a widespread phenomenon.

3.3.7 Six4 and MyoD co-regulate gene expression during myogenic differentiation

Based on the presented results so far, Six4 and MyoD seem to exert a combined, cooperative function in regulating numerous target genes during myogenesis. By combining Six4 and MyoD ChIP-seq, I found 7,684 unique genes bound by both TFs. To identify the genes bound and regulated by both TFs, Six4 and MyoD co-bound loci were further filtered by combining ChIP-seq data with gene expression profiling of C2C12 myoblasts undergoing differentiation (T24) while being subjected to siRNA-mediated knockdown of MyoD (our unpublished data). I also included expression profiling data from the myogenic conversion (discussed earlier) and data from C2C12 at T24 subjected to siRNA-mediated Six4 knockdown (182). Based on this, I identified a set of 2,534 peaks annotated to 1,453 genes bound by both TFs and likely regulated by both of them. Among these peaks, there are 665 peaks annotated to 366 genes with expression levels significantly affected by both Six4 and MyoD knockdown ($p < 0.05$), with a fold change difference between cells with Six4 knockdown and control cells of at least 1.5, and a fold change difference between cells with MyoD knockdown (or ectopic expression in fibroblasts) and control cells of at least 1.5

Figure 3-17 Transcriptional synergy between Six4 and MyoD

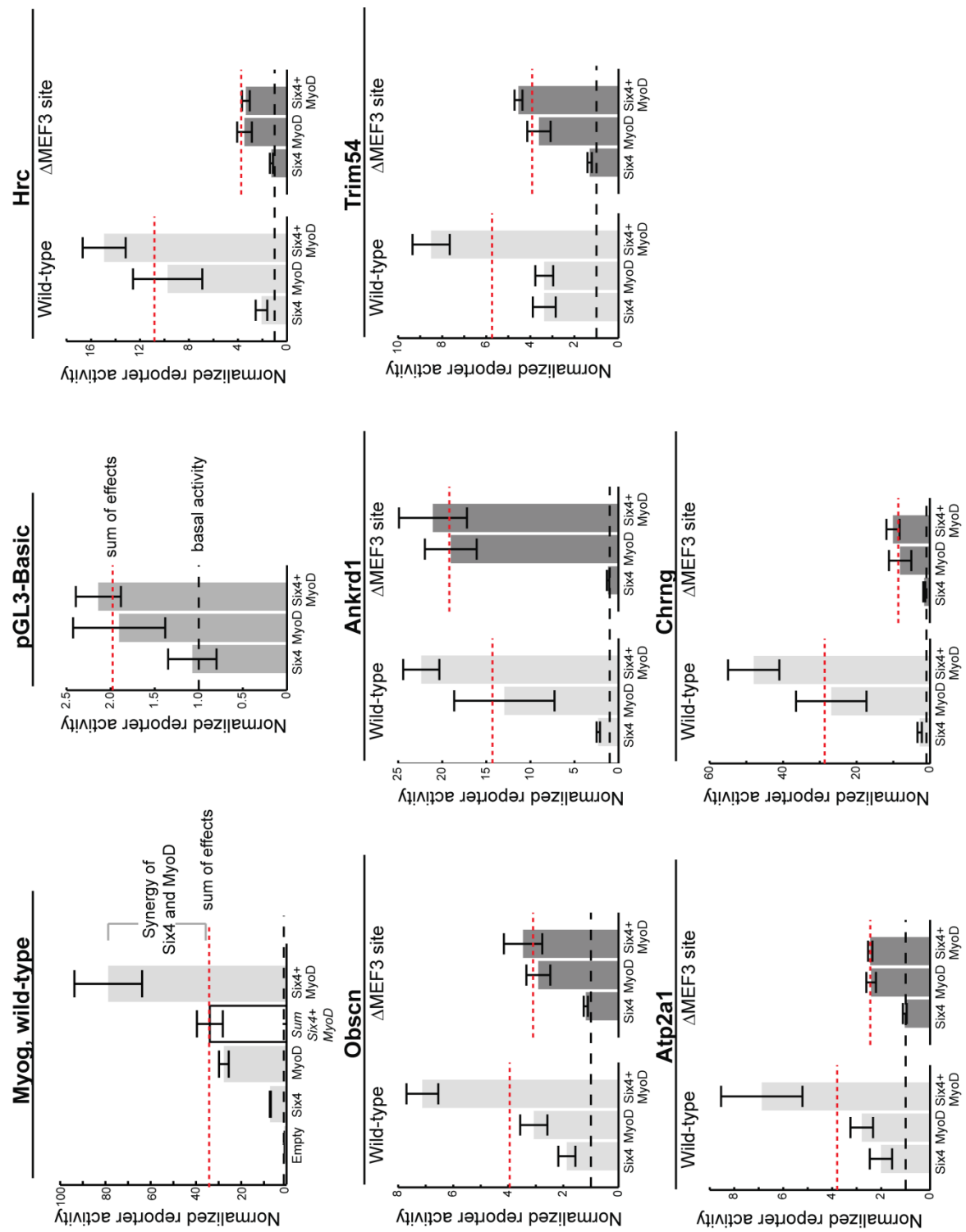


Figure 3.17. Transcriptional synergy between Six4 and MyoD.

HEK 293T cells were transfected with the indicated firefly luciferase reporter constructs containing Six4- and MyoD-bound regulatory regions, either in wild-type or MEF3 site-mutant form. The effect of co-transfecting expression plasmids for Six4, MyoD, or both, is shown. The basal activity, in absence of co-expressed transcription factor, is set to a value of 1 and is represented by a black dash line. A synergistic effect of Six4 and MyoD on each reporter is observed when both factors together induce reporter activity above the "sum of effects", represented by red dashed line, and was calculated based on the individual effects of Six4 and MyoD, as detailed in the Materials and Methods chapter. For each tested reporter, synergy between Six4 and MyoD is observed, and is abolished by mutation of the MEF3 site. Error bars represent 95% confidence intervals based on biological replicates of the data (between 4 and 6 replicates, depending on the reporter).

(Appendix 2, Table A2.9). Functional annotation term enrichment analysis on these 366 genes revealed a specific enrichment of biological processes involving muscle contraction and development (Table 3.2).

From this list of genes, I selected a number of genes to confirm their regulation by Six4 and MyoD in C2C12 cells harvested at T24. The three different siRNAs targeting Six4 were effective in reducing the protein levels of Six4 without affecting Six1 or MyoD levels (Fig. 3.18A). Six1 and Six5 mRNA expression levels were also unaffected by either Six4 or MyoD knockdown (Fig. 3.18B) when quantified by RT-qPCR, while the mRNA levels of the joint Six4 and MyoD targets were lower in cells with Six4 or MyoD knockdown. As predicted from gene expression profiling, these data confirm the ability of Six4 and MyoD to regulate the expression of several genes identified in their common bound-regulated genes list.

3.3 Conclusion

The data presented in this chapter reveals a genomic cooperation between Six4 and MyoD in regulating a core set of genes needed for proper muscle differentiation. Although cooperation between MyoD and other TFs has been previously reported using small-scale experimental approaches (181, 327), the results presented in this chapter reveal an important genome-wide report of cooperation between MyoD and a TF. The myogenic conversion assay coupled to gene expression profiling not only reinforces our previous observation of an important function of Six4 during C2C12 myoblasts differentiation (182), but it extends our knowledge to demonstrate that the ability of MyoD, the master regulator of skeletal myogenesis, to convert fibroblasts into muscles is impaired without proper Six4 expression due to attenuated expression of a large set of muscle genes co-regulated by both TFs (Fig. 3.3, Table A2.3). Although a few genes were identified previously by our group and others to

Table 2 3-2 Selected gene ontology biological processes significantly enriched among genes bound by Six4 and MyoD and deregulated when either factor is knocked-down

Table 3.2 Selected gene ontology biological processes significantly enriched among genes bound by Six4 and MyoD and deregulated when either factor is knocked-down.

Name	Six4- and MyoD-regulated	Total in genome	FDR	Examples
muscle system process	37	352	2.31E-14	Pin1, Cnn1, Htr2b, Lmcd1, Camta2, Camk2b, Akap13
muscle contraction	31	295	6.5E-12	Tpm1, Pgam2, Chrna1, Chrng, Myh3, Myl3, Myl4, Des, Tmod1, Tnni1, Tnni2,
muscle structure development	38	558	1.77E-09	Sox8, Sort1, Heg1, Myoz1, Bin1, Megf10, Myog, Popdc2, Mamstr, Ldb3, Lef1
actin filament-based process	33	592	2.67E-06	Eps8, Cfl2, Obscn, Rapgef3, Enah, Inpp1, Dbn1, Jak2, Cdk5r1
calcium ion transport	20	355	0.001128	Ryr1, Pde2a, S100a1, Wfs1, Mylk, Bspry, Jph2, Ubash3b

Figure 3-18 Six4 and MyoD jointly regulate muscle gene expression during myogenic differentiation

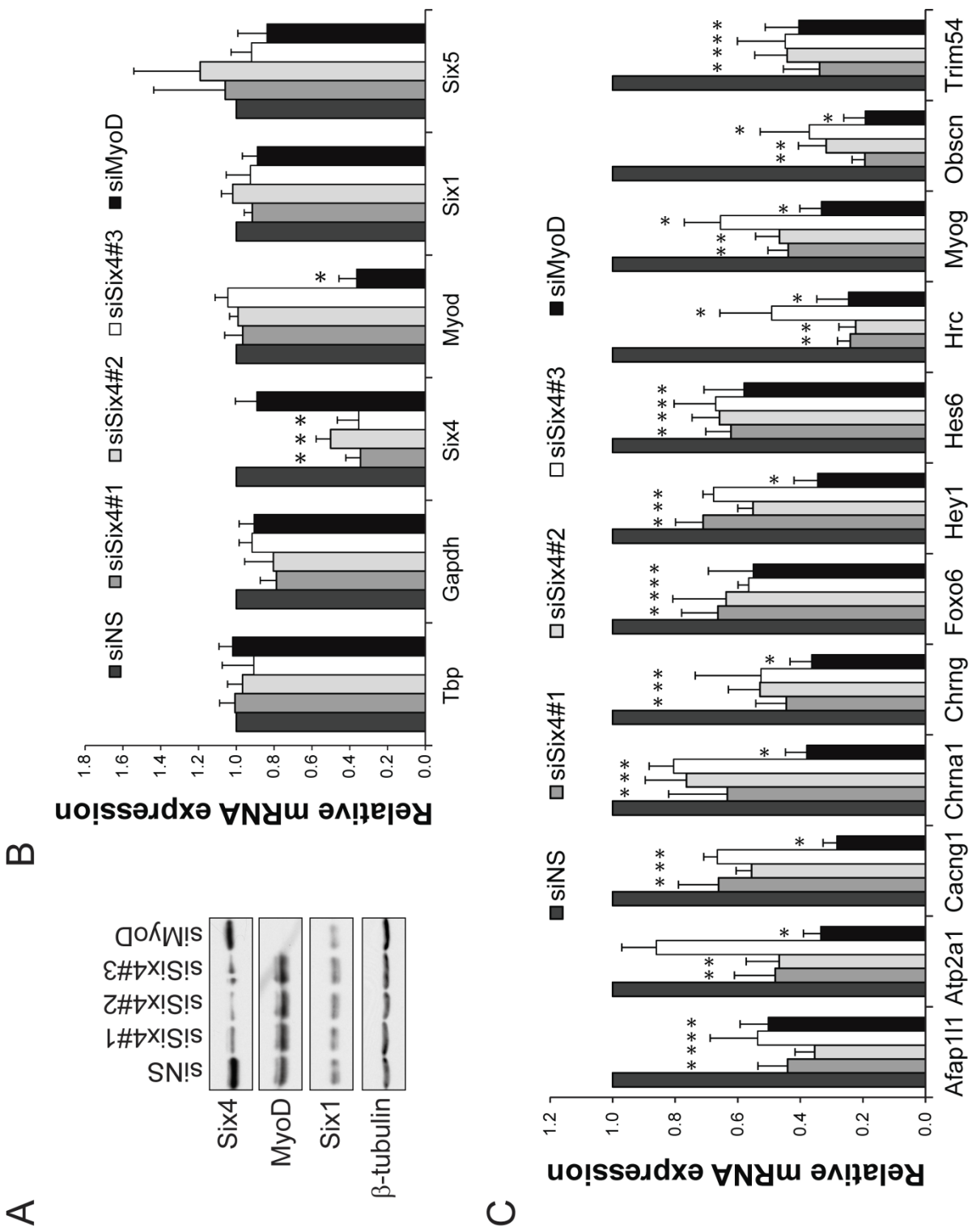


Figure 3.18. Six4 and MyoD jointly regulate muscle gene expression during myogenic differentiation.

A) Western blot on total protein extracts from C2C12 cells in presence of different siRNA targeting Six4, MyoD or non-silencing control siRNA. B) RT-qPCR on C2C12 cells in presence of siRNA against Six4, MyoD or non-silencing control siRNA showing mRNA levels of *Myod* and of TFs of the Six family (n = 3-6). * $p < 0.05$ compared to siNS-transfected samples. C) Same as panel B, but showing the expression of genes jointly regulated by Six4 and MyoD.

be directly bound by Six4 using small-scale experimental approaches (182, 236, 270, 271), the ChIP-seq data presented in this chapter is the first high-throughput experiment identifying a high confidence set of Six4 transcriptional targets during myoblast differentiation. These Six4 targets are characterized by enrichment of genes involved in muscle structure development and actin filament-based process, in addition to many other biological processes (Table 3.1). A substantial overlap between Six4 genomic binding profile and that of MyoD was observed; accounting for 36% of the total MyoD-bound loci (Fig. 3.13E) with a significant enrichment of the Ebox binding site of MyoD (Fig. 3.13D), further implicating that these two TFs can work together in regulating skeletal muscle differentiation. To test this possibility, I combined two high-throughput experimental approaches including Six4 and MyoD DNA binding and expression data and identified a large group of genes bound and potentially regulated by both TFs during myoblast differentiation (Table A2.8). By using small-scale experimental approaches, I was able to confirm: 1) direct binding of Six4 and MyoD to a select number of genes including genes of the contractile apparatus, neuromuscular junction genes and developmentally regulated TFs (Fig. 3.15); 2) the regulation of these genes by Six4 and MyoD (Fig. 3.18); and 3) a transcriptional synergy between Six4 and MyoD in regulating transcription from reporter plasmids containing Six4- and MyoD-bound regulatory regions of these genes, a synergy that was dependent on an intact MEF3 motif (Fig. 3.17). Together, these data demonstrate that MyoD needs proper expression level of Six4 in order to cooperatively drive skeletal myogenesis using *in vitro* models of muscle differentiation by regulating a large set of genes involved in this process.

Chapter 4: Genome-Wide Association between Six4, MyoD and the Histone Demethylase Utx during Myogenesis

4.1 Introduction

In the previous chapter, I have established a genome-wide map of Six4 and MyoD cooperation in regulating gene expression during adult myogenesis. However, the mechanism underlying this cooperation is still to be explored. Genome-wide studies revealed an increase in histone H4 acetylation, a histone mark associated with transcribed genes and with MyoD binding (298). Additionally, MyoD binding at distal regulatory regions affect p300 binding and presence of H3K27ac mark, thus defining transcriptionally active enhancers (307). Since no genome-wide mapping has been conducted previously on Six4 in myogenic cells, our knowledge of the genomic landscape associated with Six4 binding is very limited. However, small scale experiments revealed two interesting potential candidates: the H3K27me3 demethylase Utx and the SWI/SNF remodelling complex component Baf60c. Six4 was reported to recruit Baf60c to the regulatory region of *Deptor*, an Akt signalling pathway activator, thus enhancing the glycolytic metabolism in skeletal muscles of type 2 diabetic mice (271). Six4 was also reported to recruit Utx to the regulatory regions of two important muscle genes, *Myog* and muscle creatine kinase (270). Genome-wide mapping of Utx in C2C12 myotubes revealed its association with the transcriptional elongation factor Spt6, thus facilitating Utx movement ahead of the transcriptional machinery to remove the chromatin repressive mark of H3K27me3 (309). Utx plays important role during embryonic organogenesis especially in the heart and hematopoietic tissues and Utx mutant mice are embryonically lethal (330, 331). To address the potential involvement of Utx in the genome-wide cooperation between Six4 and MyoD, I performed

bioinformatics analyses to correlate binding profiles of Six4, MyoD and Utx and studied potential regulation of the joint targets of Six4 and MyoD by Utx.

4.2 Results

4.2.1 Utx binding profile overlaps with that of Six4 at MyoD target loci

The potential role of Utx in the genome-wide regulation of the joint targets between Six4 and MyoD was examined by comparing the ChIP-seq binding profiles of Utx (309), MyoD (298) and Six4. First, I compared Utx binding at Six4- (identified in this study) and MyoD-bound loci (298) by dividing these loci into three categories: 1) Six4-bound loci that are not bound by MyoD (Six4 - MyoD), 2) Six4-bound loci that are bound by MyoD (Six4 + MyoD), and 3) MyoD-bound loci that are not bound by Six4 (MyoD – Six4). Based on these categories, I found that there is a notable enrichment for Utx ChIP-seq reads at these three categories compared to negative control loci, with the highest Utx recruitment being at loci bound by both TFs (Fig. 4.1A). Second, I correlated the ChIP-seq binding profile of Utx with that of Six4 at all MyoD-bound loci, by ranking the 25,956 binding sites of MyoD (298) in decreasing order of Six4 binding intensity (Fig. 4.1B). This gave read density profiles where MyoD binding intensity is uniformly distributed, and where Six4 binding goes from very strong to weak and then absent. This analysis revealed that the binding profile of Utx is strikingly similar to that of Six4, among MyoD target sites (Fig. 4.1B, Pearson correlation coefficient of 0.604). I then separated these ranked MyoD binding sites into 5 clusters of equal size (Figure 4.1B) and analyzed ChIP-seq density for MyoD, Six4 and Utx using boxplots (Fig. 4.2). Similar to the heat maps (Fig. 4.1B), this showed that while MyoD binding is essentially invariant in all 5 clusters, Utx binding follows the same trend as Six4 and decreases markedly from one cluster to the next, being highest at cluster 1 (Fig. 4.2). In contrast, I found an inverse correlation between Six4 binding intensity and H3K27me3 abun-

Figure 4-1 Utx is associated with Six4-bound MyoD target genes

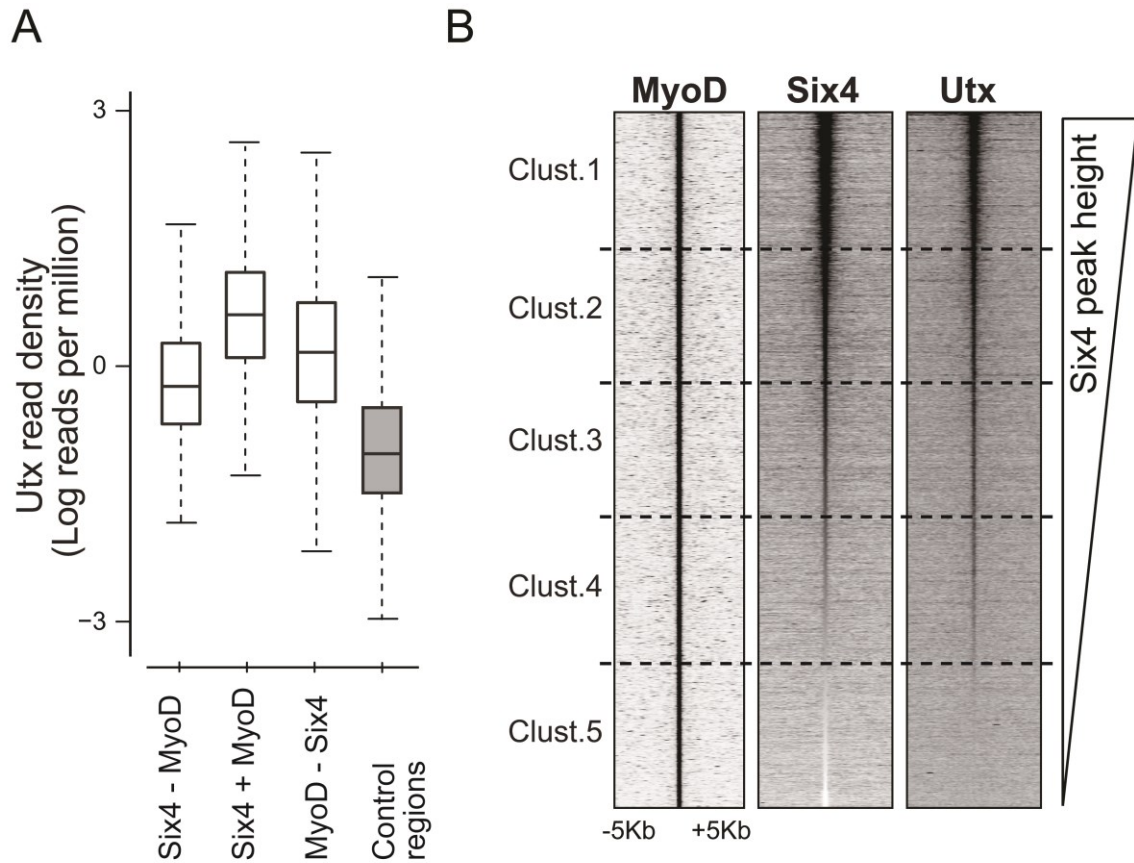


Figure 4.1. Utx is associated with Six4-bound MyoD target genes.

A) Boxplots showing the anti-Utx ChIP-seq read density (309) at loci bound by Six4 alone, MyoD alone, by both Six4 and MyoD, and at negative control regions not bound by either of the two transcription factors. B) Heat map of MyoD, Utx and Six4 ChIP-seq read densities at the 25,956 MyoD-bound loci in C2C12 myotubes (298) sorted in decreasing order of Six4 read density and divided into five equal clusters.

Figure 4-2 Utx binds to Six4-bound MyoD target genes

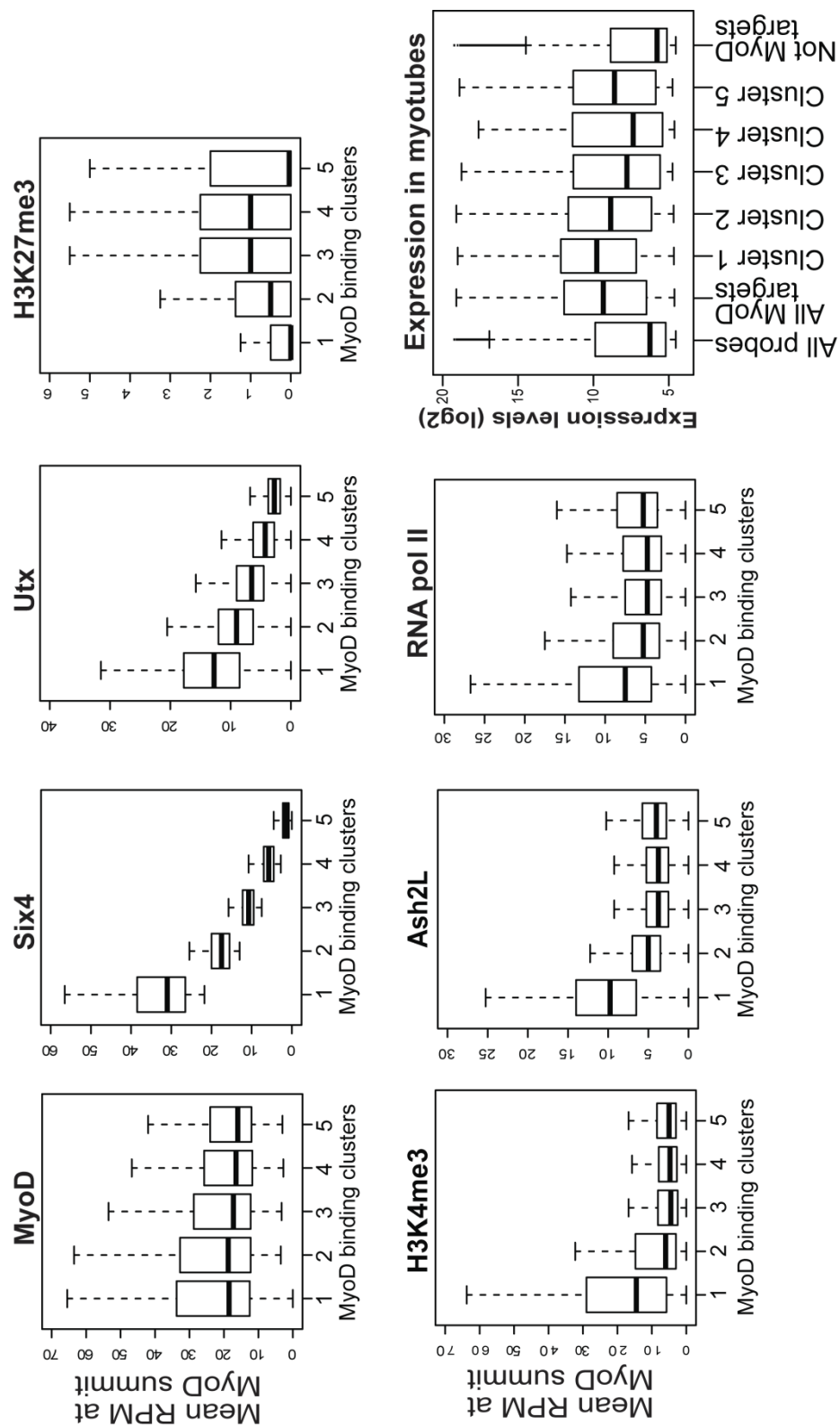


Figure 4.2. Utx binds to Six4-bound MyoD target genes.

Mean read density plots of ChIP-seq data of Utx (309), H3K27me3, H3K4me3, RNA pol II (306) and Ash2L (our unpublished data) at the 5 clusters, and the expression levels (our unpublished data) in myotubes of the genes annotated to the bound loci in the 5 different clusters.

dance (306) at MyoD target sites, which is consistent with the fact that Utx enzymatically erases this repressive mark. When I considered the enrichment of H3K4me3 and RNA pol II (306) across the five clusters of MyoD targets, I found an association profile similar to that of Utx, where their abundance is highest at MyoD target loci that are strongly bound by Six4. Likewise, Ash2L, a component of the MLL complex that adds the H3K4me3 activation mark, displays a similar binding trend to H3K4me3, Utx and Six4. Finally, the expression of MyoD-bound genes was found to be highest in clusters 1 and 2, which are characterized with the highest levels of Six4 and Utx binding, in agreement with the assumption that Six4 recruits Utx to cooperate with MyoD to mediate gene activation (Fig. 4.2). This analysis revealed that the association between Utx and Six4 is connected to the transcriptional activity of MyoD target gene during myogenic differentiation and that this phenomenon is not restricted to a few loci but is instead widespread, occurring genome-wide.

4.2.2 Utx regulates the expression of genes jointly modulated by Six4 and MyoD

After establishing a positive correlation between Utx and Six4 binding at MyoD target genes with chromatin marks of actively transcribed genes and with higher gene expression level, I set out to determine if Utx demethylase activity is required for the transcriptional activation of Six4 and MyoD target genes. For this purpose, I used GSK-J4, a small-molecule inhibitor of Utx demethylase activity (332). Twenty-four hours after inducing C2C12 differentiation in the presence of GSK-J4, I observed a reduction in Myog protein expression levels (Fig. 4.3A) and in its mRNA levels (Fig. 4.3B). Likewise, I found that all of the Six4 and MyoD common target genes tested were comparably affected by Utx inhibition (Fig. 4.3B). However, Myog itself is a TF and many of the genes tested are Myog targets based on gene expression profiling performed in C2C12 myoblasts differentiated for 24 hours in presence of siRNA targeting Myog (182). To rule out the possibility that all these

Figure 4-3 Utx is needed for the expression of Six4 and MyoD joint target genes

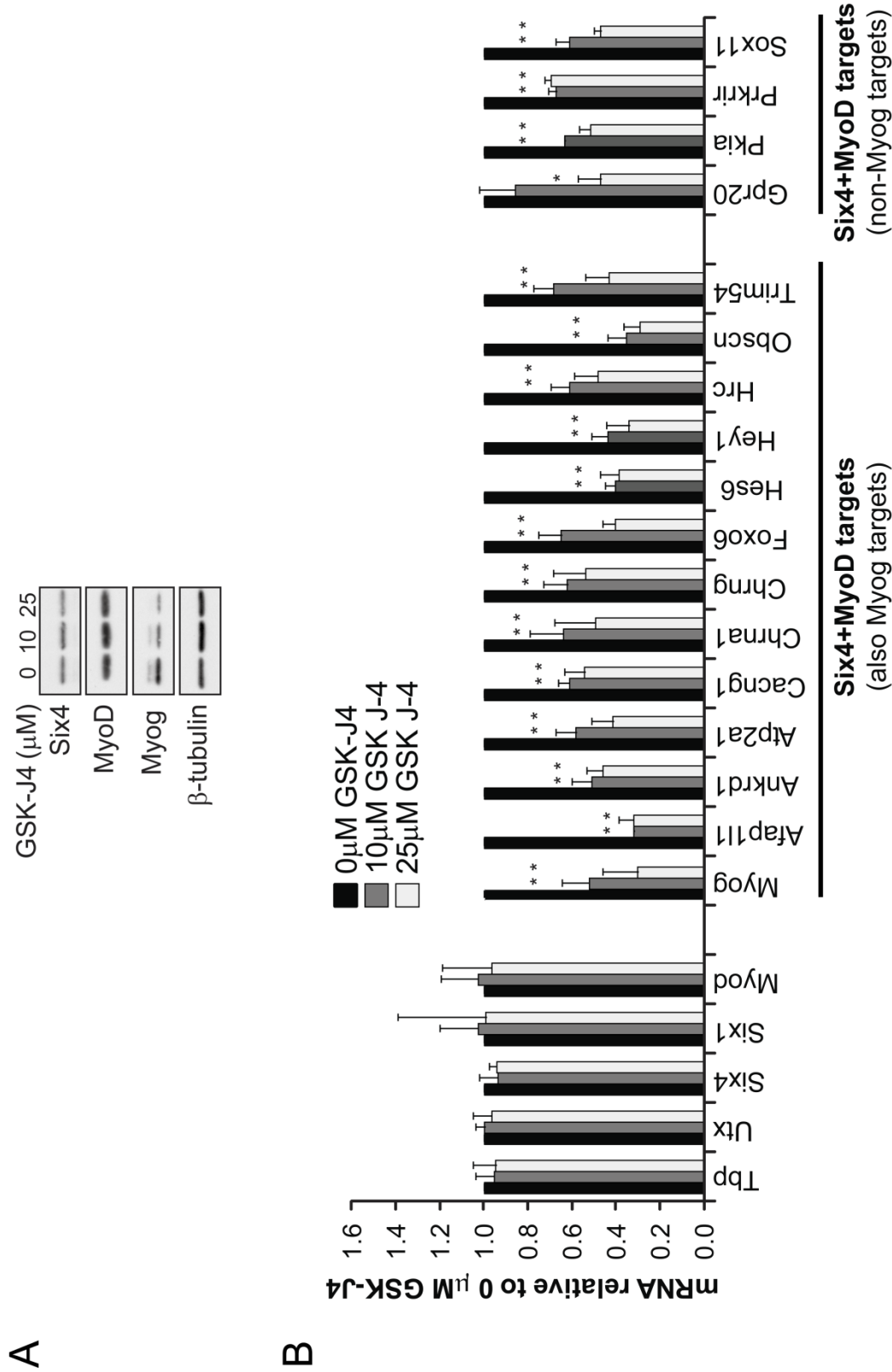


Figure 4.3. Utx is needed for the expression of Six4 and MyoD joint target genes.

A) Western blot on total protein extracts from C2C12 cell line 24 hours after inducing differentiation in the presence of varying concentrations of the Utx inhibitor GSK-J4. B) RT-qPCR on expression levels of several genes under the same condition as in panel A. Expression levels of genes that are also regulated by myogenin and genes not regulated by myogenin are shown as assessed by RT-qPCR. RT-qPCR data were normalized to the expression levels in the vehicle-treated cells (0 μ M). (n = 3). * p < 0.05 compared to 0 μ M GSK-J4 using two-tailed unpaired student's t-test.

gene deregulation events are due to the failure to activate Myog, I selected a few genes that are induced by Six4 and MyoD but not by Myog based on gene expression profiling data in C2C12 at T24 (182). *Gpr20* (G protein-coupled receptor 20), *Sox11* (SRY (sex determining region Y)-Box 11), *Pkia* (cAMP-dependent protein kinase inhibitor alpha) and *Prkrir* (protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)) represent a few of these genes (Fig. 4.4B). They are bound by Six4 and MyoD based on ChIP-seq data (fig. 4.4A) and are downregulated by knocking down either Six4 or MyoD using siRNA interference in C2C12 at T24 (Fig. 4.4C). Together, these data indicate that Utx presence at MyoD target genes is strongly associated with the recruitment of Six4, and that both Six4 binding (Fig. 3.18C) and Utx activity (Fig. 4.3B) are necessary for the transcriptional induction of a large set of MyoD target genes during myogenic differentiation.

4.2.3 Mapping Six4 interaction domain with Utx

Six4 is the largest Six family member characterized by a long C-terminal domain with a reported transactivation activity mapped to its last 194 amino acids when fused to Gal4 DNA binding domain (203). Besides the conserved Six domain (SD), the Six-type homeodomain (HD) and the transcriptional activation domain (AD), the Six4 protein remains poorly characterized (Fig. 4.5A). The Six factors interact with Eya cofactors (217, 218, 221). Mutations in Six1 protein associated with branchio-oto-renal syndrome have been identified including a mutation in the SD that converts a valine residue into a glutamic acid residue V17E (333). This mutation completely abolishes interaction between Six1 with Eya cofactors (334). Based on sequence homology, this valine residue is mapped to amino acid 109 in the Six4 protein (Figure 4.5B). To mimic V17E mutation in Six1, I mutated valine 109 into glutamic acid (V109E) and expressed this mutant in HEK 293T cells as Flag-tagged at its N-

Figure 4-4 Some genes regulated by Six4/MyoD/Utx are not regulated by myogenin

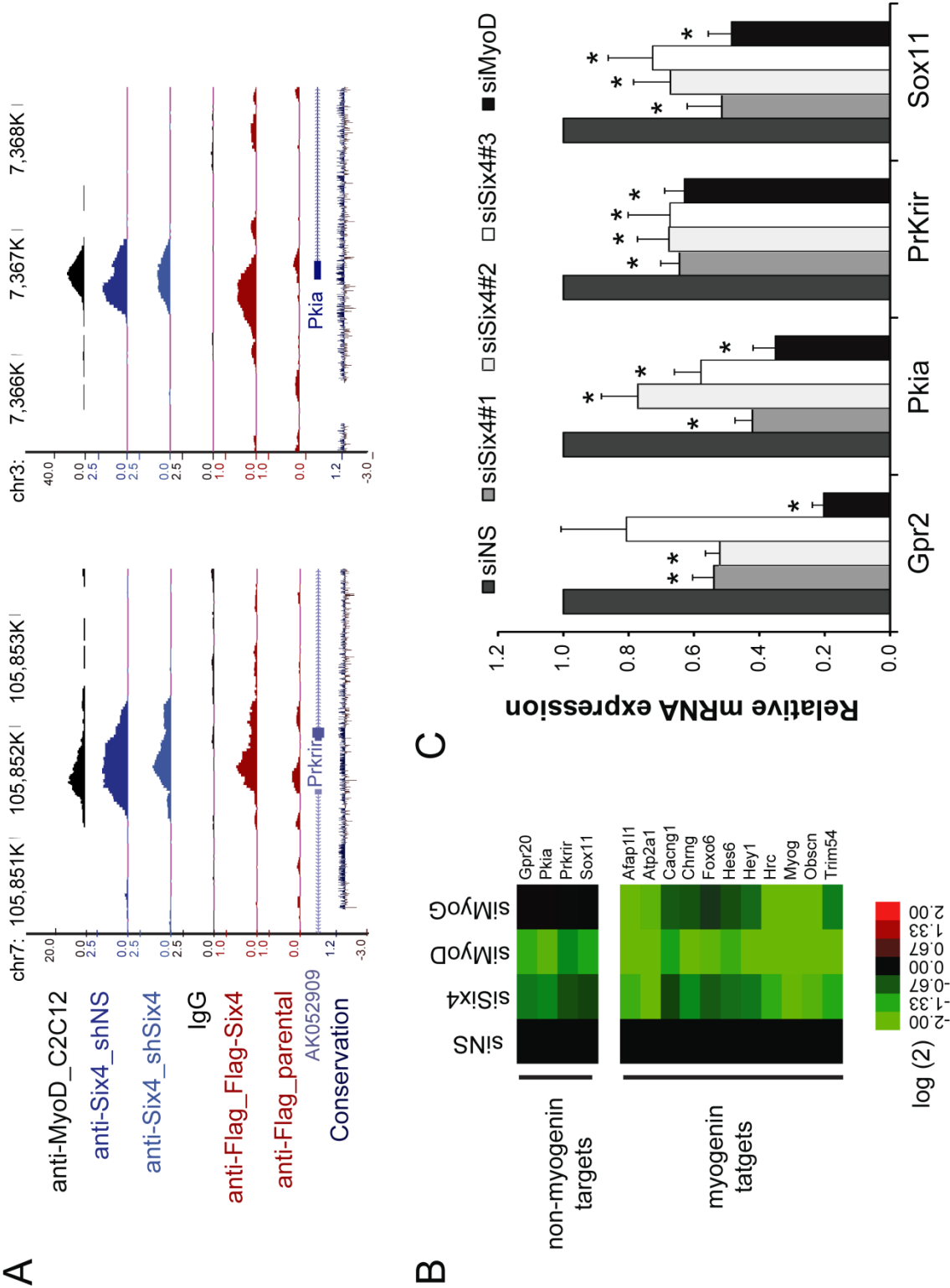


Figure 4.4. Some genes regulated by Six4/MyoD/Utx are not regulated by myogenin.

A) *Prkrir* and *Pkia* are bound by Six4 and MyoD. B) Heat map on C2C12 cell line at T24 (182) confirming regulation of some of these genes by Six4 and MyoD but not by myogenin. Data are presented as fold change of C2C12 cell line in the presence of non-specific scramble siRNA. C) RT-qPCR on C2C12 cell line at T24 in presence of siRNA targeting Six4, MyoD or a non-specific scramble siRNA (n is between 3 and 5). Asterisks indicate $p < 0.05$ compared to siNS using one-tailed unpaired student's t-test. These results suggest that the down-regulation of Six4/MyoD/Utx target genes is not merely due to the down-regulation of myogenin, which is one of their targets.

terminus and myc-tagged at its C-terminus (Flag-Six4-myc V108E) in comparison to wild type (wt) Six4 (Flag-Six4-myc) or Six4 truncated protein expressing amino acids 1-424. IP experiments performed on total protein extracts from HEK 293 T cells expressing Flag-Six4-myc, Flag-Six4-myc V109E, Flag-Six4-myc 1-424 or empty expression plasmid confirmed the ability of Six4 to interact with Eya3, as reported for other Six factors (217, 218, 221), and the decrease in interaction in the V109E mutant as predicted based on sequence homology between Six4 and Six1 (Figure 4.5C). As expected, the interaction with Eya3 was greatly decreased with V109E mutation, and surprisingly, the findings also suggest that the Six4 truncated protein lacking the C-terminal domain (1-424) is also impaired. This suggests that valine 109 might not be the only amino acid involved in Six4-Eya3 interaction. Additional experiments are required to map the amino acids in the C-terminal domain that might be involved in the interaction between Six4 and Eya proteins.

Moreover, Six4 was reported to interact with Utx in pull-down experiments using nuclear extracts from C2C12 cells (270). I used co-IP experiments in order to map Six4 interacting domain with Utx. Wild type (wt) mouse Six4, V109E Six4 mutant and various Six4 truncated proteins were cloned into an expression vector where transcription is driven by a CMV promoter and the protein is expressed as a fusion with myc tag at its C-terminal end, while Utx was cloned into an expression vector driven by a CMV promoter and expressing Utx as a fusion protein with 3xFlag tag at its N-terminal end and myc tag at its C-terminus. Six4-myc, wt or mutant, was co-transfected with either Flag-Utx-myc or empty plasmid into HEK 293T cells. Alternatively, GFP-myc was co-transfected with Flag-Utx-myc as a negative control (Fig. 4.6, Fig. 4.7). Based on co-IP experiments, wt Six4, Six4 V109E mutant and a truncated Six4 containing amino acids 1-424, all interact with Flag-Utx-myc. Six4 interaction with Flag-Utx-myc is lost when a truncated Six4 protein containing

Figure 4-5 Six4 valine 109 is required for interaction with Eya cofactor

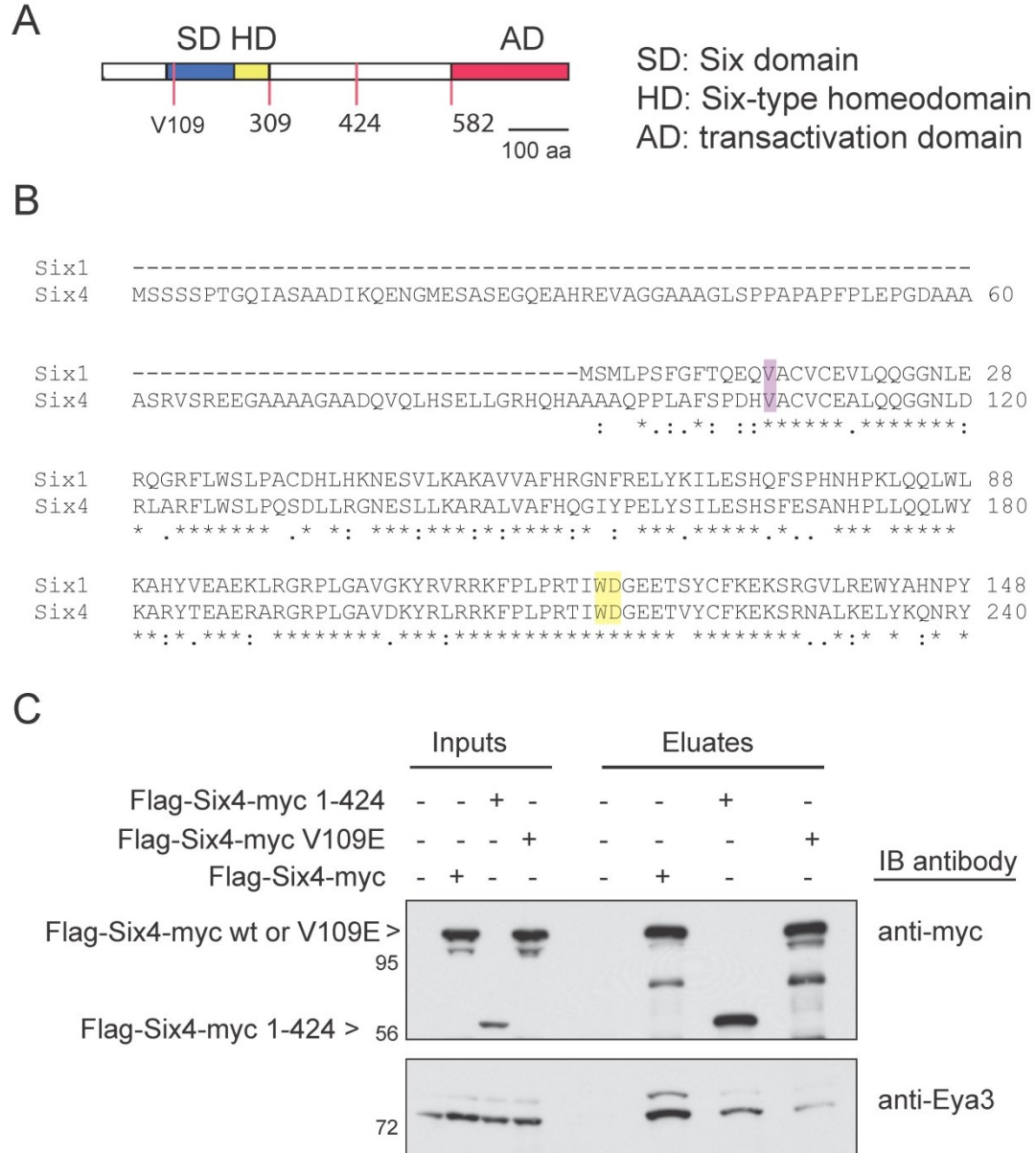


Figure 4.5. Six4 valine 109 is required for interaction with Eya cofactor.

A) A schematic representation of the mouse Six4 protein showing the conserved Six domain (SD) and the Six-type homeodomain (HD) in addition to the reported transactivation domain (AD). B) Pairwise alignment of the conserved Six domain of the murine Six1 and Six4 proteins using ClustalW2 showing conservation of amino acid residues surrounding Six4 V109 which is highlighted in purple. Amino acids highlighted in yellow indicate the last two amino acids of the Six domain. C) Western blot on total protein extracts from HEK 293T cells transfected with the indicated plasmids and immunoprecipitated with anti-Flag antibody. Membranes were probed with the indicated antibodies.

amino acids 1-309 is overexpressed (Fig. 4.6B) indicating that the amino acids from 425-775 are not necessary for this interaction and suggesting that amino acids 309-424 are necessary for this interaction. This was further corroborated by the pull down of a Six4 mutant protein containing amino acids 217-775 with Flag-Utx-myc (Fig. 4.6B). However, Six4 (amino acids 217-583) and Six4 (amino acids 217-424) did not interact with Flag-Utx-myc (Fig. 4.7B) suggesting that 309-424 is necessary but may not be sufficient for this interaction. To verify that amino acids 309-424 are required for this interaction, I tried to clone the entire C-terminal domain into the CMV-myc plasmid. However, I was not able to detect the expression of any Six4 mutant protein starting at amino acid 309 (309-424, 309-583 and 309-775; data not shown). As a substitute for this mutant protein, I cloned amino acids 280-775. To ensure that the protein will translocate to the nucleus, I also included a nuclear localization signal (NLS) at the N-terminal end of amino acids 280-775 considering that previous work by another group showed the presence of NLS in the SD and the HD (218). However the expression levels of this Six4 mutant protein (Fig. 4.7A) and of Six4-myc 424-775 (Fig. 4.7B) were too low to detect any potential interaction with Flag-Utx-myc. Finally, GFP-myc was not pulled-down with Flag-Utx-myc (Fig. 4.6B and Fig. 4.7A and B) indicating that Six4-Utx interaction is specific. Together, these data show that the C-terminal domain of Six4 (amino acids 424-775) including the reported transactivation domain is not required for the interaction between Six4 and Utx and that amino acids 309-424 are necessary but not sufficient for this interaction.

4.3 Conclusion

The data presented in this chapter shows a strong positive correlation between genomic Utx binding profile and that of Six4 at MyoD-bound peaks. This correlation is associated

Figure 4-6 Six4 C-terminal domain is not required for Six4 Utx interaction

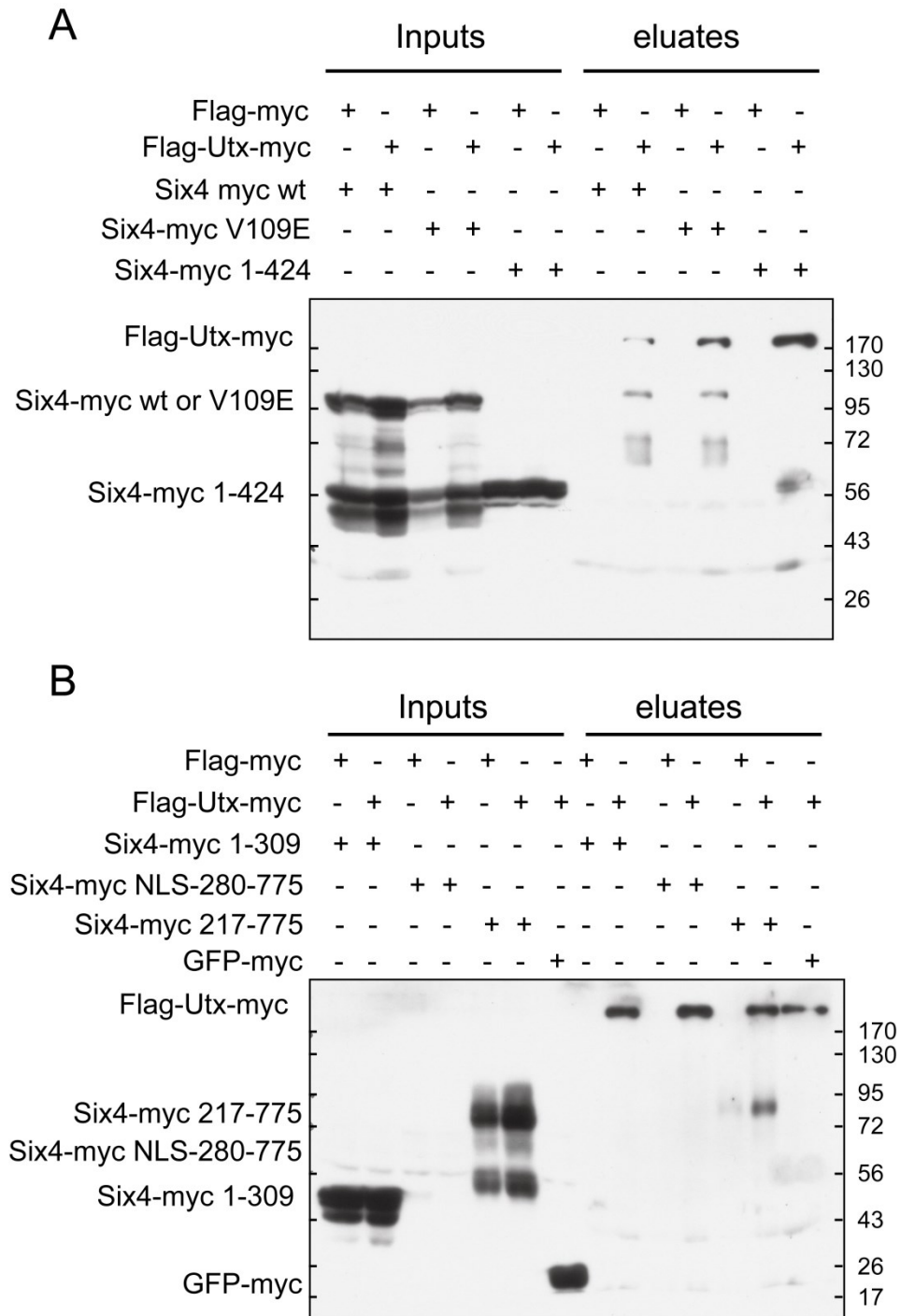


Figure 4.6. Six4 C-terminal domain is not required for Six4 Utx interaction.

A) and B) Flag-Utx-myc was co-expressed with different Six4 mutant proteins as myc-tagged in HEK 293T cells and immunoprecipitated using mouse anti-Flag antibody. Membranes were probed using goat anti-myc antibody. Flag-Utx-myc was not detected in the input lysates of these blots, possibly because of too low expression levels.

Figure 4-7 Amino acids 309-424 of Six4 protein are necessary for Six4-Utx interaction

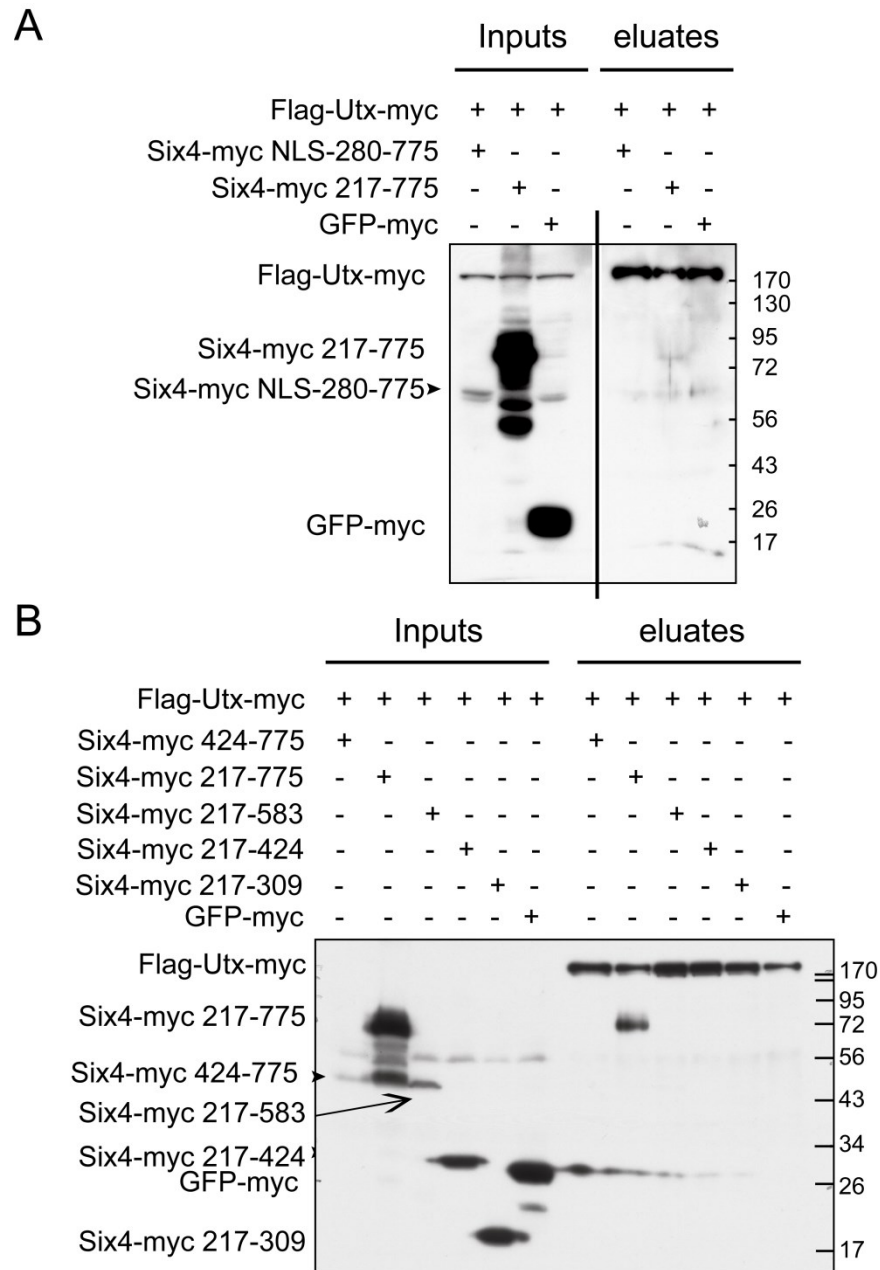


Figure 4.7. Amino acids 309-424 of Six4 protein are necessary for Six4-Utx interaction.

A) and B) Flag-Utx-myc was co-expressed with different Six4 mutant proteins as myc-tagged in HEK 293T cells and immunoprecipitated using mouse anti-Flag antibody. Membranes were probed using goat anti-myc antibody. A) Six4-myc NLS-280-775 is expressed at low level. B) Six4-myc 424-775 and 217-583 are expressed at low levels while Six4-myc 217-424 and 217-309 are expressed at high levels but are not pulled down by Flag-Utx-myc. Flag-Utx-myc was not detected in the input lysates of this blot, possibly because of too low expression levels.

with the same trend of binding of active transcription marks such as RNA pol II, Ash2L and H3K4me3. As expected, Utx binding was negatively correlated with the presence of H3K27me3 as this histone mark is erased by Utx. The significance of the association between Six4 and Utx binding at MyoD peaks is further reinforced by the higher expression level of MyoD target genes bound by Six4 and Utx compared to genes that are not bound by them. The requirement of Utx in inducing the expression of the joint Six4 and MyoD target genes is confirmed by the decrease in their transcript levels when the demethylase activity of Utx was inhibited. Although Utx interaction with Six4 does not require the reported transactivation domain of Six4 (203), it involves a previously uncharacterized part of the protein containing amino acids 309-424. Further analysis of the function of these amino acids and the rest of Six4 C-terminal domain will help us understand the significance of this interaction. However, our experiments and work done by others (270) did not explore if the interaction between Six4 and Utx is direct or mediated through other binding proteins. If other binding proteins are involved in mediating Six4-Utx interaction, the SWI/SNF remodelling complex proteins emerge as potential candidates, as Six4 was reported to interact with Baf60c in HEK 293 cells (271), while Utx was reported to interact with Brg1 in T cells (335) and HEK 293 cells (330). Additional experiments to identify potential Six4- and Utx-binding protein(s) should help clarify these questions.

Chapter 5: Six4 is Necessary for Proper Muscle Regeneration *in Vivo*

5.1 Introduction

Adult skeletal muscles undergo regeneration upon exposure to injury or myotrauma. Skeletal muscle regeneration occurs due to the ability of satellite cells to get activated upon myotrauma and to proliferate extensively before they subsequently fuse together to repair the damaged muscle (336). Myotrauma can be extrinsic, due to physical injury, or intrinsic, due to a genetic predisposition to myofiber fragility (as seen in Duchenne muscular dystrophy). Muscle regeneration involves two interdependent phases: 1) an inflammatory and degenerative phase aiming at clearing the damaged tissues mainly through phagocytosis within the first few days; and 2) a regenerative phase aiming at rebuilding the muscle tissue through revascularization and formation of new myofibers, and this is completed within 7-10 days after injury. Subsequently, the muscle will restore its normal size and contractile function through maturation and innervation (reviewed in (337, 338)). Muscle regeneration is a highly synchronized process regulated by the interplay between various types of cells including myogenic and inflammatory cells acting through secreted factors or TFs (77, 95). During the first day of myotrauma, degeneration is characterized by necrosis of the muscle fibers evident by the disruption of the myofiber sarcolemma leading to increased myofiber permeability and infiltration of inflammatory cells (77, 95). Neutrophils are the first inflammatory cells to infiltrate the damaged tissue, peaking in number in the first 1-6 hours post-injury. By day 2, monocytes and macrophages become the dominant immune cells at the site of muscle injury (339). Concomitantly, quiescent satellite cells get activated within the first day, and enter a proliferative phase by day 2 before they fuse to form myotubes between days 3 and 7. Later on, the newly formed myotubes will fuse with the ends of the damaged myofibers, and this will be completed by day 10, accompanied by a decrease in

inflammation. Finally, the newly formed myofibers will undergo hypertrophy and mature to retain their adult size by three weeks. Regenerated myofibers are characterized by the presence of centrally-located myonuclei that will persist for more than one month (95).

Different animal models exist to study muscle regeneration, and the choice of a model depends on the question addressed and the animal species used (reviewed in (340)). Among the models used is mechanical damage to the muscle by inflicting crushing or contusion (341-343), thermal injury (by using hot or cold probes), muscle grafting as well as muscle denervation, ischemia and others. The major problems associated with these models are reproducibility of inflicting the same lesions from one animal to the next, the limited size of the muscle to be grafted and the technical challenges associated with denervation and ischemia in small animals like mice (340). Animal models with abnormal regeneration due to defects in specific genes are also available. The most commonly studied is the *mdx* mouse, a spontaneously occurring mouse with a mutation in the dystrophin gene, often used as a model of Duchenne muscular dystrophy (344, 345). One of the easiest and most reproducible models to induce muscle regeneration is through the use of myotoxins which are mainly injected into the *tibialis anterior* (TA) muscle for ease access. Myotoxic agents could be divided into three main categories: local anesthetics, toxins and other chemical agents. The most commonly used local anesthetic for experimental studies is bupivacaine (Marcaine) which destroys a large number of muscle fibers by a single injection. However, Marcaine use is limited since it is highly toxic in rats, but much less toxic in mice (340). The use of toxins, mainly venom myotoxins (346, 347), is the most widespread method for inducing muscle regeneration. Cardiotoxin (CTX) is a peptide extracted from cobra snake venom that acts as a protein kinase C-specific inhibitor and causes depolarization and disruption of the plasma membrane of skeletal muscle cells, activation of tissue phospholipase C and lysis of various

cell types (348, 349); notexin, in turn, is a phospholipase A2 toxin derived from a wide variety of snake venoms (346, 350) and acts by inhibiting the release of the neurotransmitter acetylcholine (347). CTX, together with other venom toxins, are the most widely-used muscle damaging agents when massive and complete regenerative response is desired due to their ability to act as chemotactic factors enhancing macrophage invasion and satellite cell activation without causing serious damage to the basal lamina or microcirculation (347). In addition, a variety of chemical agents are used in inducing muscle regeneration including barium chloride (351) which causes death of the myofibers without damaging the basement membrane. Barium chloride does not stimulate immune cell infiltration, as is the case with venom myotoxins (339), and can be used in injury models when the contribution of the immune cells needs to be controlled (351).

Like embryonic and adult muscle differentiation, muscle regeneration requires the coordinated function of several TFs expressed in satellite cells and their differentiated cells, notably Pax7 and MRFs (reviewed in (77, 337, 348)). The Six factors play a critical role during prenatal myogenesis, and a role for Six1 during muscle regeneration has been recently reported (92). Similar to Six1, Six4 is also expressed in adult quiescent, activated and differentiated satellite cells (241); however, its role during *in vivo* muscle regeneration has not been reported. Impairment of myogenic conversion with the knockdown of Six4 (Fig. 3.2 and 3.3) and the need for a proper expression level of Six4 for C2C12 myoblast differentiation and activation of muscle genes (such as those important for muscle contraction, response to nerve stimuli, energy metabolism (Fig. 3.18)) suggest a role for Six4 in controlling adult skeletal muscle regeneration. To address the role of Six4 during adult muscle regeneration, I employed siRNA interference assays to target Six4 expression during CTX-induced muscle damage.

5.2 Results

5.2.1 Six4 is expressed in satellite cells and their differentiated progeny

To study the role of Six4 during muscle regeneration, I used the TA muscle as a model since this muscle is well-established for this field of research due to two main considerations. First, its ease of access as its size is large enough to acquire sufficient amount of material to inject and small enough to induce massive degeneration upon CTX injection. Second, data obtained using this model can be compared to those obtained in other studies. I first examined the expression profile of Six4 protein in the TA muscles of adult mice at different time points following CTX-induced muscle injury. Paraffin-embedded sections from resting uninjured TA muscles and from TA muscles at 4 days (day of maximal myoblasts proliferation) and 7 days (when myotubes are formed and the contractile apparatus is maturing) after CTX injection were co-stained with a mouse antibody recognizing the satellite cell marker Pax7 and with the custom-made rabbit antibody specific to Six4. In line with what others have reported (241), I found that Six4 is expressed in satellite cells of resting muscle and in activated myogenic cells 4 and 7 days post-CTX (Fig. 5.1). In resting muscle, all Pax7-positive cells have essentially detectable expression level of Six4 protein. Additionally, Six4 was detected in the myonuclei of undamaged fibers (white arrows). In regenerating muscle 4 or 7 days after CTX injection, I found again that essentially all Pax7-positive cells are also Six4-positive. Additionally, Six4 was also detected in the centrally-located nuclei of regenerating fibers. The staining signal of Six4 in regenerating tissue was consistently stronger than the one observed in quiescent satellite cells, with more cells per field being Six4-positive. These results indicate that Six4 is expressed in quiescent satellite cells, with increased expression during muscle regeneration.

Figure 5-1 Six4 is expressed in satellite cells during CTX-induced muscle regeneration

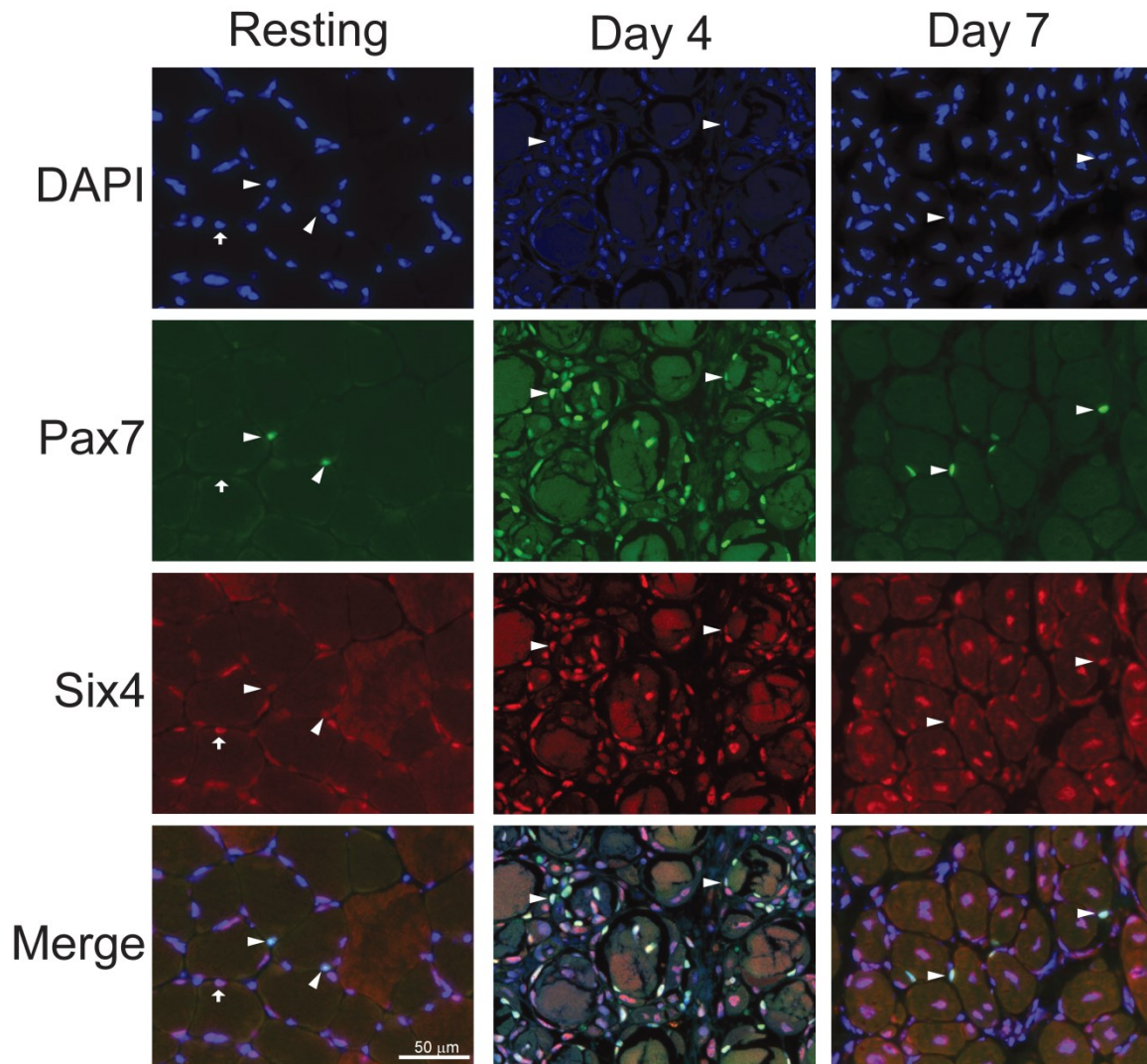


Figure 5.1. Six4 is expressed in satellite cells during CTX-induced muscle regeneration. Co-immunostaining for Six4 and Pax7 on sections from resting or regenerating TA muscles 4 or 7 days after CTX-induced injury. All cells expressing Pax7 express Six4. White arrowheads indicate examples of these cells, while white arrows indicate a likely myonucleus expressing only Six4.

Once satellite cells get activated, they express MyoD followed by Myog upon differentiation into myocytes (69). I examined the expression of Six4 in activated satellite cells (MyoD-positive) and in differentiating satellite cells (Myog-positive) by performing co-staining of Six4 with MyoD or Myog. I found that the expression profiles of the Six4 and MyoD proteins (Fig. 5.2A) and that of Six4 and Myog (Fig. 5.2B) greatly overlap during adult muscle regeneration, since we observed marked co-staining in small mononucleated cells (presumptive activated and differentiating satellite cells respectively), especially at day 4. At 7 days post-CTX, the expression levels of MyoD (Fig. 5.2A) and Myog (Fig. 5.2B) decrease in the newly repaired myofibers (with centrally-located nuclei), but co-expression with Six4, whose expression remains high in these cells, is still clearly noticeable. Thus, although Six4 and MyoD do not appear to be expressed in exactly the same profile during regeneration, they are co-expressed in myogenic cells undergoing differentiation during muscle repair.

5.2.2 Six4 is required for the expression of Six4/MyoD target genes *in vivo*

To examine the expression of Six4 and MyoD joint target genes during muscle regeneration, I performed RT-qPCR on RNA extracted from entire TA muscles 4 days after CTX or vehicle injection. As expected, the expression of *Pax7* is higher in regenerating, compared to resting, muscles (Fig. 5.3A) reflecting the increase in satellite cell numbers. Similarly, *Myod* and *Myog* expression increases in regenerating muscles (Fig. 5.3A) indicating the initiation of satellite cell differentiation. The expression of *Six4* and *Six1* also increases during regeneration as predicted from the immunostaining of Six4 (Fig. 5.1 and 5.2) and as published previously (241, 242) most likely due in part to increase in satellite cell numbers. The expression levels of the macrophage and immune cell genes *Ptprc* (receptor-type tyrosine-protein phosphatase C, also known as CD45), *Cd68* (cluster of differentiation

Figure 5-2 Six4 is expressed in differentiated satellite cells

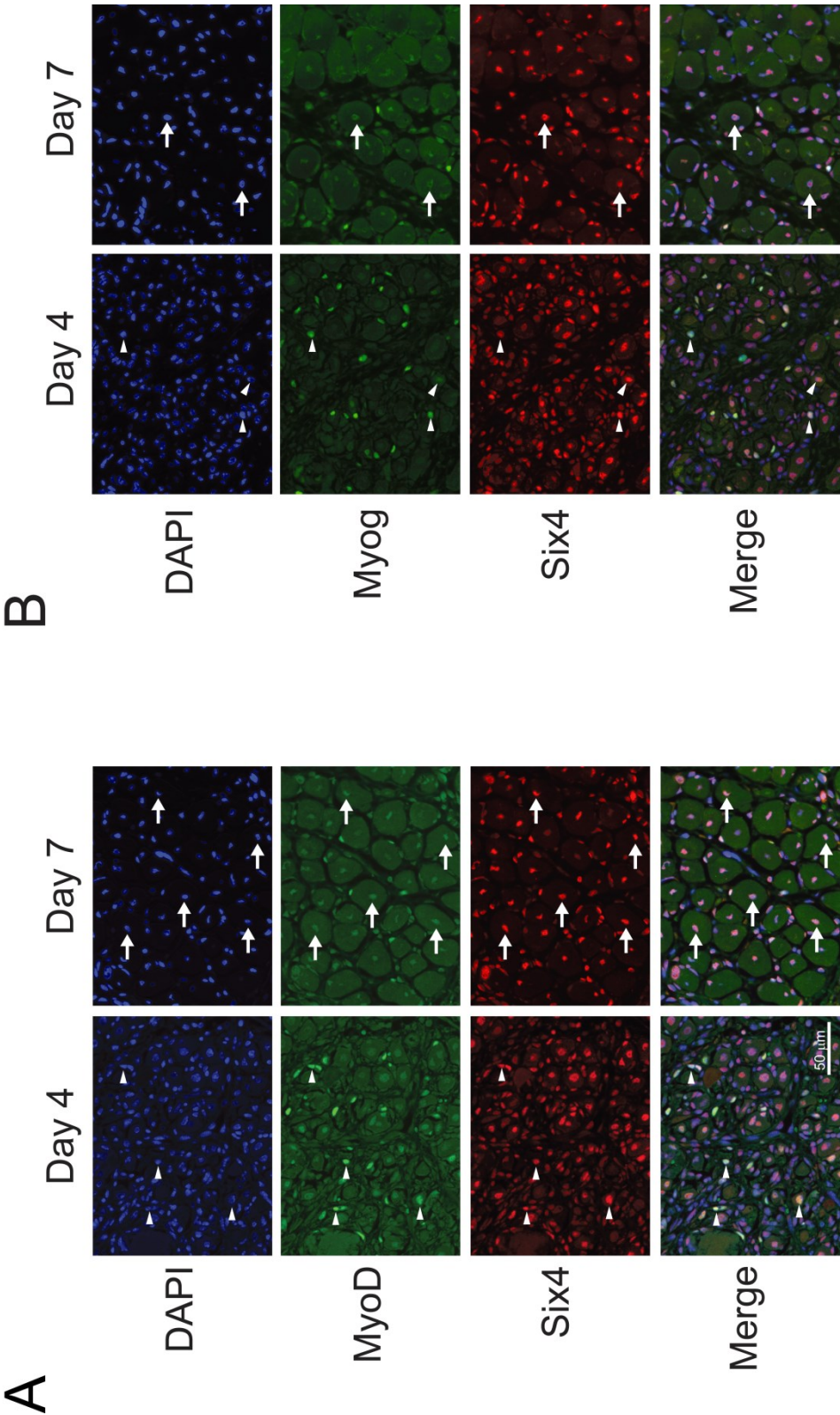


Figure 5.2. Six4 is expressed in differentiated satellite cells.

A) Co-immunostaining for Six4 and MyoD or B) Six4 and Myog on sections from regenerating TA muscles 4 or 7 days after CTX-induced injury. White arrowheads indicate a few of the small mononucleated cells that co-express both TFs, arrows point to newly regenerated myofibers (with central nuclei) where MyoD and Six4 (A) or Myog and Six4 (B) are both expressed.

68, also known as macrosialin) and *Ccl3* (C-C motif chemokine 3) also increase reflecting the transient increase in the abundance of macrophage cells within injured muscles (118, 352). In addition, the transcript level of the *Pdgfra* (alpha-type platelet-derived growth factor receptor), a gene that specifically marks fibro/ adipogenic progenitors (FAPs) which promote the differentiation of myogenic cells (353), increases in the regenerating muscles. Similarly, the expression levels of *Myh3* (myosin-3, coding for embryonic MHC (eMHC) a marker of regenerating myofibers, Fig. 5.3A) and other joint targets of Six4 and MyoD (Fig. 5.3B and C) are higher in the regenerating muscle samples.

The expression of Six4 in muscle stem cells and the increase in its expression during regeneration together with the fact that Six4 is needed for in vitro myogenesis (chapters 3 and (182)) suggest a role for Six4 in the regeneration process, most likely by directly regulating the expression of target genes shared with MyoD. To study the role of Six4 during muscle regeneration, I injected siRNA against Six4 intramuscularly 6 hours following CTX injection in order to knockdown Six4 expression in activated satellite cells similar to what has been reported by others (130, 303, 354) and as represented in Fig 5.4A. Quantitative RT-PCR revealed that 4 days after the initial injury, this procedure yields a significant decrease in the abundance of Six4 mRNA (Fig. 5.4B). Importantly, Six4 knockdown did not affect the expression of Six1 and Six5, the other Six family members relevant to skeletal muscle indicating that the knockdown of Six4 is specific and excludes any compensatory upregulation of these related genes. The transcript levels of Pax7 were also unaffected by Six4 knockdown implicating that the role of Six4 during muscle regeneration is not simply due to modulating the expression levels of this critical TF or the number of cells that express it. Likewise, a panel of genes from muscle-infiltrating immune cells was also unaffected, which is consistent with the fact that these genes are not targets of Six4, as Six4 is not

Figure 5-3 Expression of Six4-regulated genes *in vivo* during CTX-induced muscle regeneration

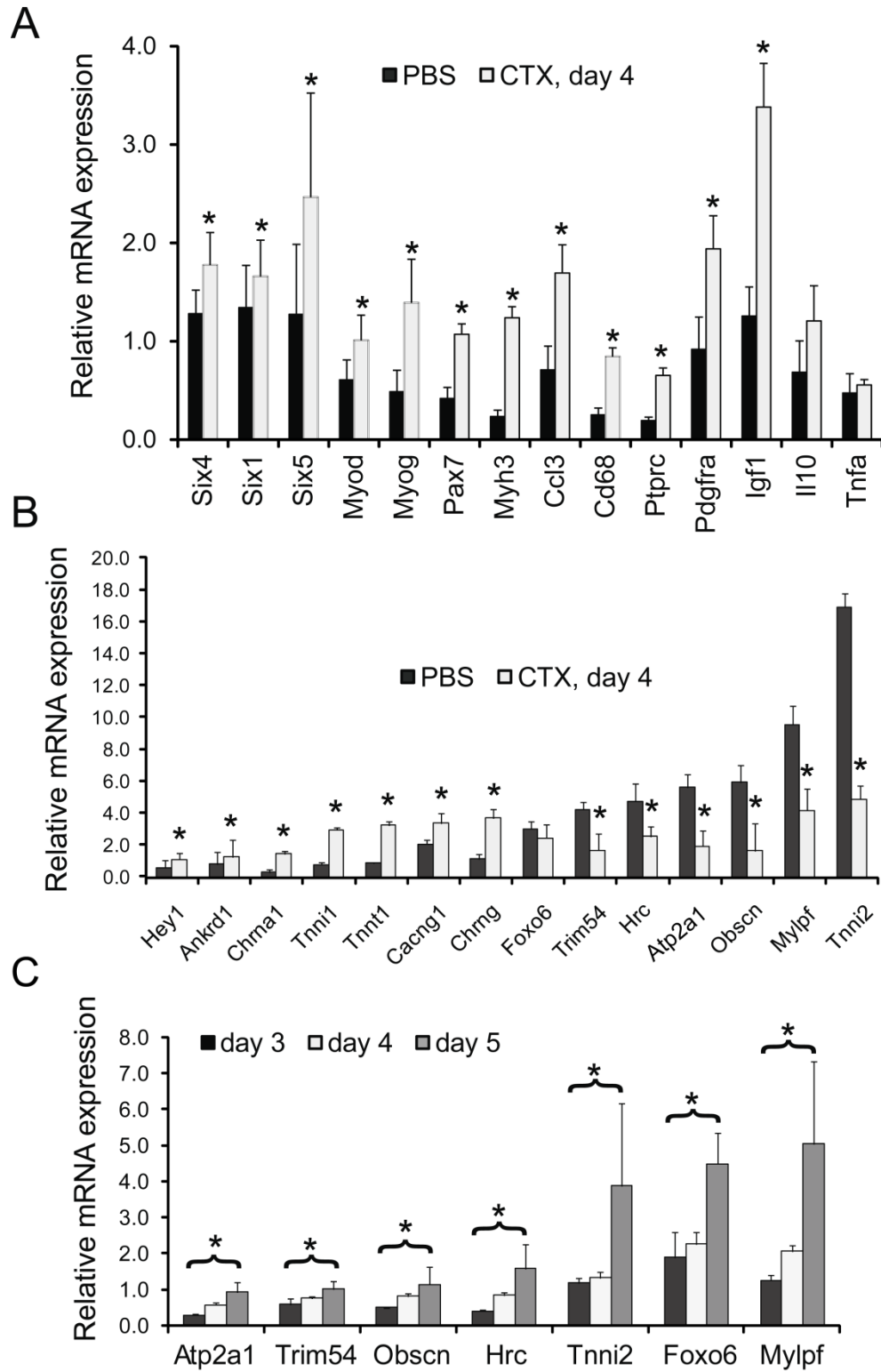


Figure 5.3. Expression of Six4-regulated genes *in vivo* during CTX-induced muscle regeneration.

A) and B) RT-PCR quantitation of the mRNA abundance of muscle TFs and genes marking regenerating muscle, in TA muscles harvested 4 days after CTX injury. $n = 8$; *, $p < 0.05$ using two-tailed unpaired t-test compared to PBS-treated muscles. C) RT-qPCR at 3, 4 or 5 days after CTX injection ($n \geq 3$). *, $p < 0.05$ using one-way ANOVA test.

Figure 5-4 Six4 regulates the expression of Six4/MyoD target genes during muscle regeneration

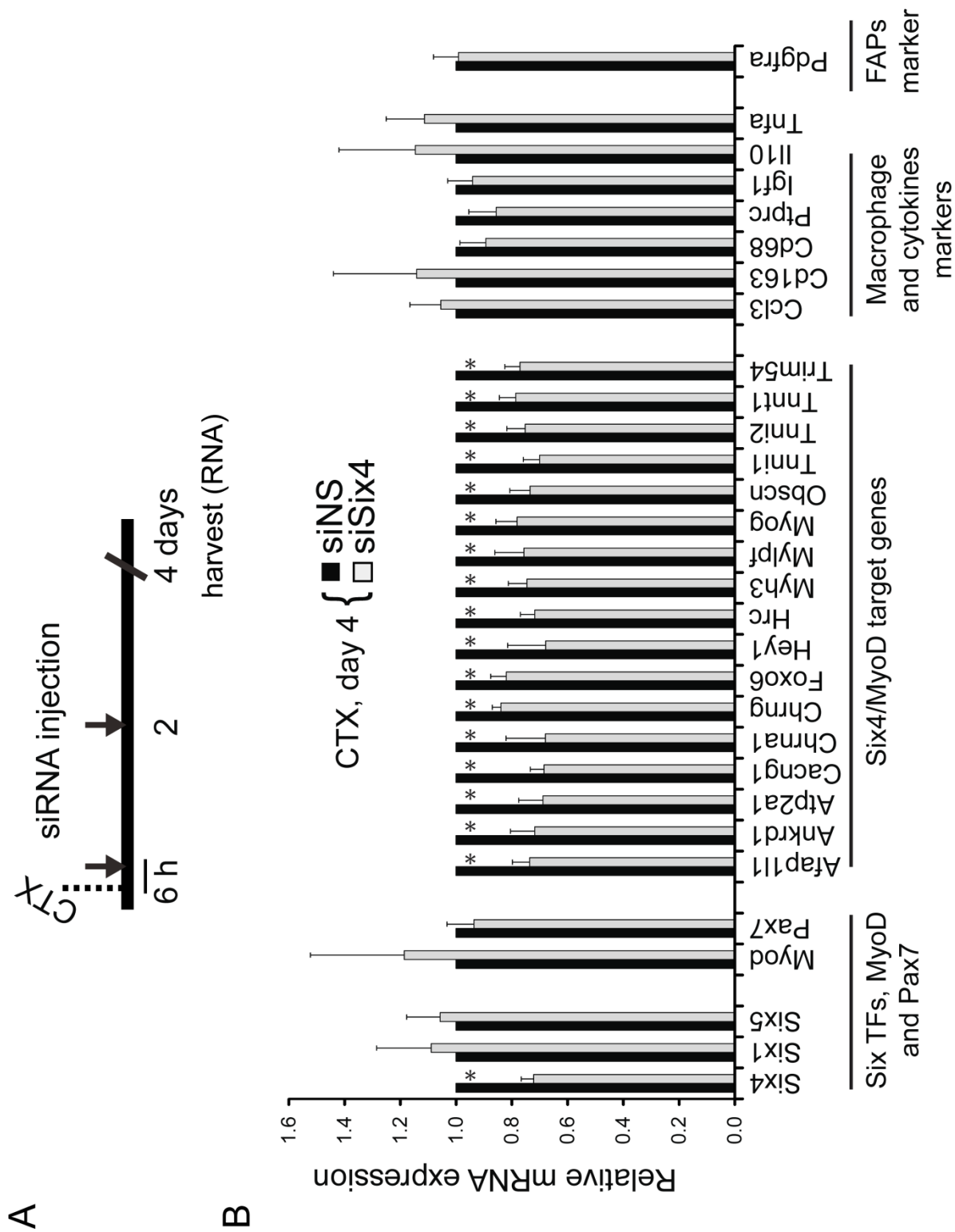


Figure 5.4. Six4 regulates the expression of Six4/MyoD target genes during muscle regeneration.

A) Schematic representation of the timeline of CTX and siRNA injection into TA muscle.

B) RT-PCR quantitation of mRNA abundance in TA muscles that have been injured by CTX and treated with siRNA duplexes against Six4 or a non-silencing control. mRNA abundance is shown relative to that in the siNS-treated muscle and is normalized to the geometric mean of 4 control genes, as outlined in the materials and methods section. $n = 5$; *, $p < 0.05$ by paired t-test. The results reveal that the siSix4 duplex is specific to Six4 and does not lead to a compensatory upregulation of Six1 or Six4 expression. They also show that Six4 is needed for appropriate expression of a panel of joint targets of Six4 and MyoD.

Data from gene expression profiling by GeneAtlas using 3 different probes 1425767-at, 1439753_x_at, and 1456862_at. The dataset is available online at the BioGPS website. Rectangles indicate expression of Six4 in the C2C12 cell line (blue) and macrophages (red).

expressed in immune cells (Fig. 5.5, outlined in red; compare to C2C12 myoblasts and C3H 10T1/2 fibroblasts which are outlined in blue). Importantly however, the expression levels of the joint Six4 and MyoD target genes were decreased significantly by Six4 knockdown (Fig. 5.4B), consistent with the data obtained in C2C12 myoblasts (Fig. 3.18). Previous reports showed a role of Six4 in regulating fast-twitch muscle genes (Richard et al., 2011; Grifone et al., 2004; Niro et al., 2010). In this model, both fast-twitch muscle genes (e.g. *ATP2a1*, *Tnni2*, and *Myh2*) and slow-twitch muscle genes (e.g. *Tnni1* and *Tnnt2*) were equally affected by Six4 knockdown (Fig. 5.4B). Together, these results indicate that Six4 regulates the expression of target genes involved in myogenesis during muscle regeneration.

5.2.3 Six4 is needed for proper muscle regeneration *in vivo*

Finally, I examined the effect of Six4 knockdown on the regenerating muscle morphology. For this purpose, I used the same approach of knocking down the expression of Six4 in CTX-damaged TA muscle as outlined in Fig. 5.6A. To study the morphology of regenerating myofibers, I stained muscle sections subjected to 2 injections of siSix4 or siNS (6 and 48 hours post-CTX) with eMHC, a marker of the regenerating muscle fibers (Fig. 5.6B). By capturing panoramic pictures of the entire TA muscle sections and measuring the cross-sectional area (CSA) of every eMHC-positive (eMHC+) myofiber (Fig. 5.6C and D), I observed that TA muscles treated with siSix4 have a higher proportion of smaller CSA ($\leq 75 \mu\text{m}^2$) eMHC+ myofibers and a lower proportion of the larger CSA ($> 75 \mu\text{m}^2$) eMHC+ myofibers compared to control muscles (Fig. 5.6D). The regeneration defects observed with Six4 knockdown were persistent 7 days after injury when muscle sections were stained with H&E (Fig. 5.7A). Again, by measuring the CSA of all regenerating myofibers (with centrally-located nuclei) within TA muscles, we observed a higher proportion of smaller

Figure 5-6 Six4 is needed for proper muscle regeneration *in vivo*

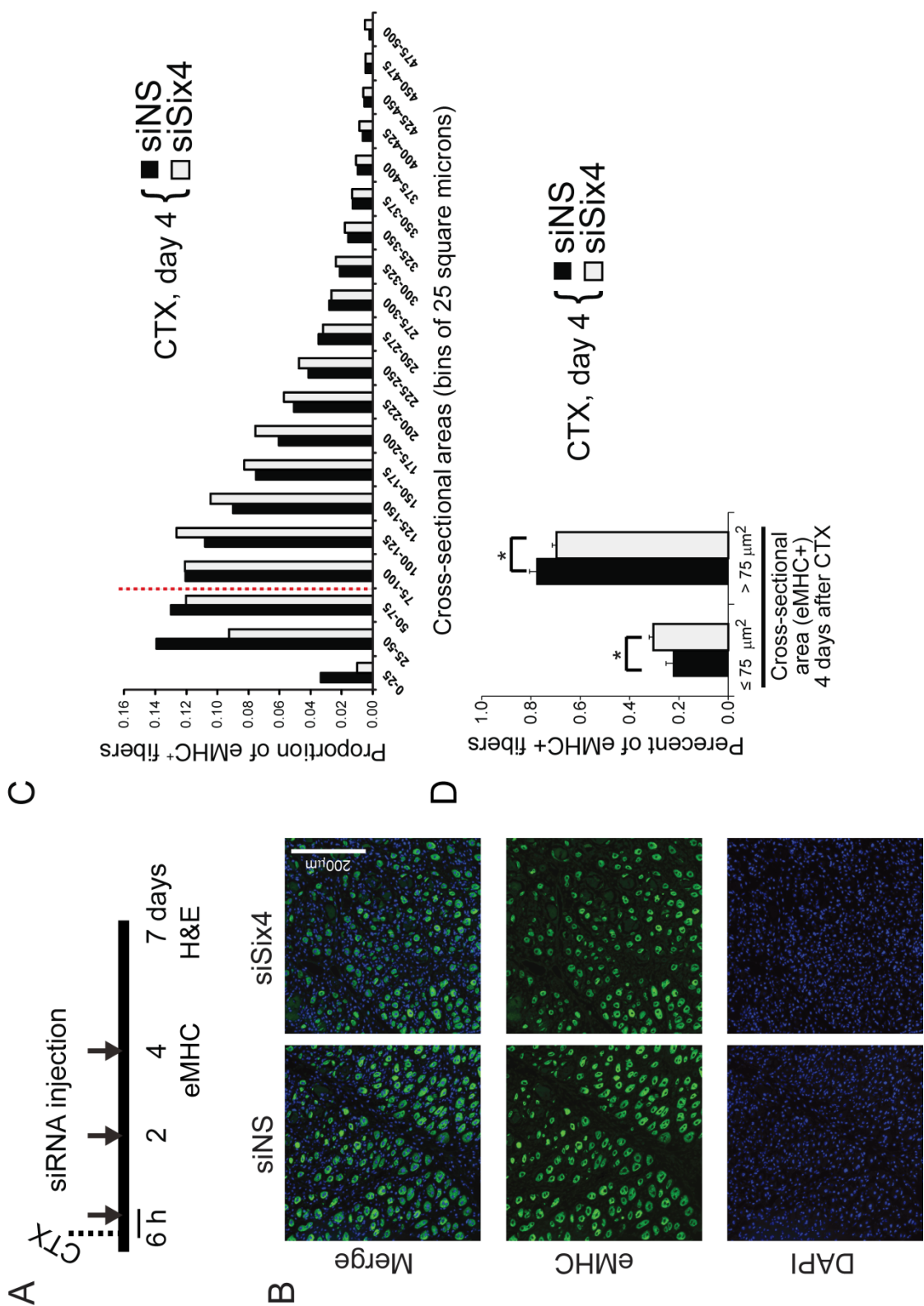


Figure 5.6. Six4 is needed for proper muscle regeneration *in vivo*.

A) Schematic representation of the timeline of CTX and siRNA injection into TA muscle. B) Immunofluorescence showing staining of eMHC (green) and DAPI (blue) on TA muscles 4 days after CTX-induced muscle regeneration in the presence of siRNA targeting Six4 (siSix4) or with a non-silencing control siRNA (siNS). C) Distribution of eMHC⁺ myofiber cross-sectional areas (CSA), in bins of 25 square microns, for TA muscles treated with siRNA duplexes against Six4 or a non-silencing duplex, 4 days after injury (related to panel A). The red dotted line indicates the point at which the distributions between siNS and siSix4-treated tissues cross each other, and was used for panel D. D) Proportion of eMHC⁺ myofibers with the indicated CSA range, 4 days after injury, in siRNA-treated samples. n = 6; *, p < 0.05 by paired t-test.

Figure 5-7 Impaired muscle regeneration with knockdown of Six4 *in vivo*

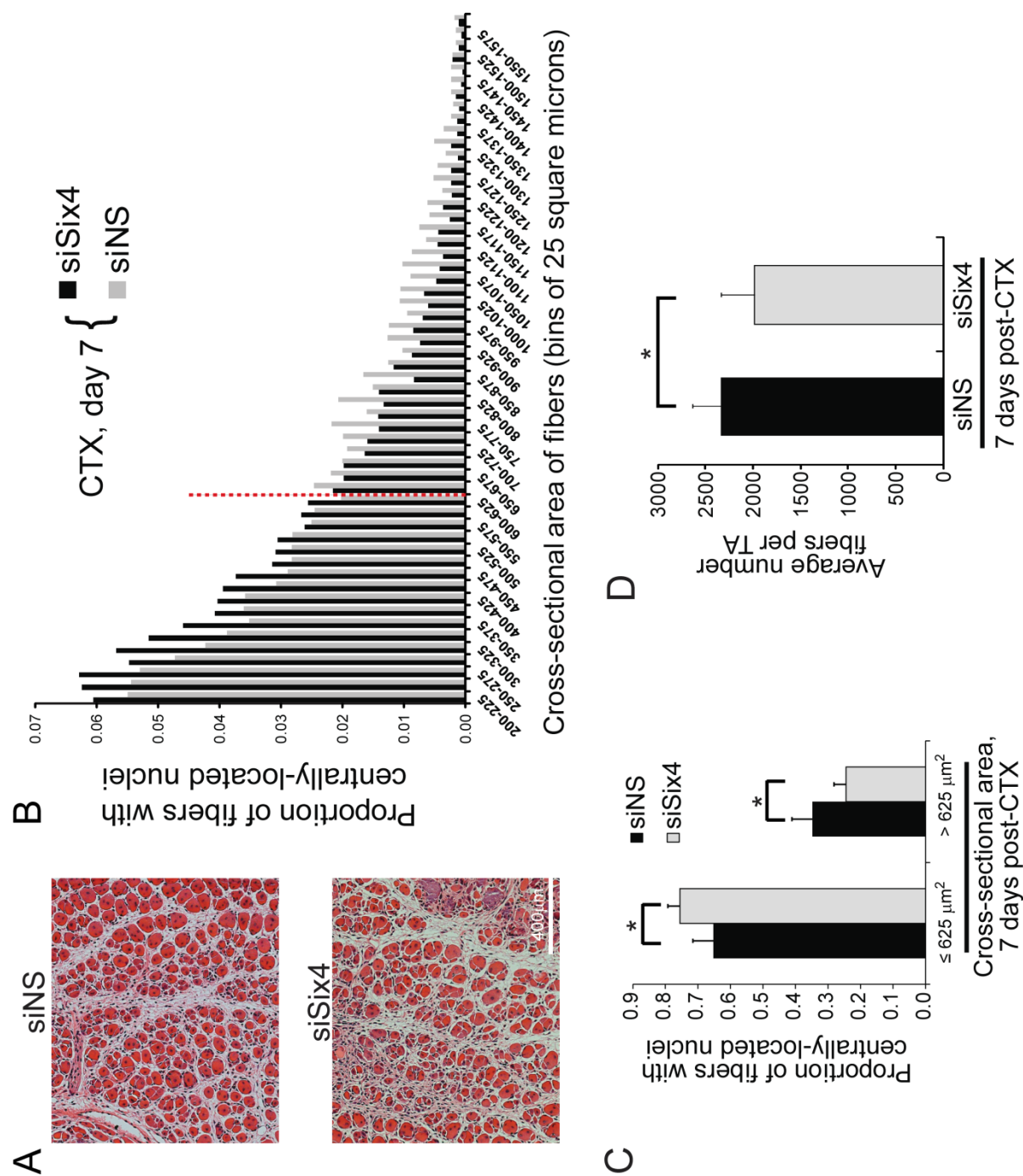


Figure 5.7. Impaired muscle regeneration with knockdown of Six4 *in vivo*.

A) Representative images of H&E performed 7 days post-CTX induced muscle regeneration in the presence of siSix4 or siNS. B) Distribution of myofiber cross-sectional areas (CSA), in bins of 25 square microns, for TA muscles treated with siRNA duplexes against Six4 or a non-silencing duplex, 7 days after injury. The red dotted line indicates the point at which the distributions between siNS and siSix4-treated tissues cross each other, and was used for panel C. C) Proportion of myofibers (centrally-nucleated) with a given CSA range, 7 days after injury and siRNA-treatment. $n = 6$; *, $p < 0.05$ by paired t-test. D) Average number of fibers with centrally-located nuclei, 7 days after CTX injection, with or without Six4 knockdown ($n = 6$; * $p = 0.05$ using paired t-test). Error bars represent standard errors of the mean.

(CSA $\leq 625 \mu\text{m}^2$) myofibers and a lower proportion of the larger (CSA $>625 \mu\text{m}^2$) myofibers in muscles treated with siSix4 compared to control muscles (Figs. 5.7B and C). In addition, siSix4 treatment reduced not only the CSA of myofibers but also the total number of fibers per muscle, where muscles treated with siSix4 had significantly lower average numbers of myofibers (1983 fibers, on average) compared to controls (average of 2336 fibers, Fig. 5.7D). In summary, the results of these experiments indicate that proper Six4 expression is required for efficient muscle regeneration.

5.3 Conclusion

By using partial-loss-of-function of Six4 during CTX-induced muscle regeneration, I present the first evidence of a role of Six4 during adult muscle regeneration *in vivo*, as assessed by fiber number and size measurements and marker gene expression assays. In accordance with what others have shown (241), I found that Six4 is expressed in quiescent satellite cells (Fig. 5.1) and its expression increases during CTX-induced muscle regeneration as evident by the increase in the overall immunostaining signal and by the apparent increase in the number of cells expressing Six4 per field (Fig. 5.1). This is further supported by the increase in Six4 transcript level in the entire TA muscle (Fig. 5.3A). The increase in the transcript levels of the other Six factors expressed in the muscle, Six1 and Six5 (Fig. 5.3A), during muscle regeneration was not affected by Six4 knockdown, suggesting that these factors are neither regulated by Six4 nor compensating for its impaired expression. Since Six1;Six4 dKO mice had an exacerbated muscle defects (233) compared to Six1 KO mice (188), a possible compensation for Six4 function, most likely by Six1 or Six5, in Six4 KO mice was suggested (232). However, the role of Six4 during adult muscle regeneration cannot be entirely replaced by either Six1 or Six5; acute partial loss-of-function

of Six4 leads to impaired muscle regeneration characterized by a smaller number of myofibers, an increased proportion of the small myofibers and a lower numbers of the larger ones. Besides, the expression levels of a set of muscle genes involved in muscle structure, contractile function, and neuromuscular junction are reduced by Six4 knockdown. A defective inflammatory response can delay muscle regeneration (113, 116-119). The possibility that the impaired muscle regeneration by Six4 knockdown is due to perturbed immune response is eliminated as Six4 is not expressed in the immune cells (Fig. 5.5) and the expression levels of markers of the immune cells and cytokines produced by them are not affected by Six4 knockdown (Fig. 5.4B). These results reveal an important function of Six4 during adult muscle regeneration as opposed to a possible compensated role during prenatal muscle formation and raise the question of the mechanism(s) that can differentiate between these two functions. The H3K27me3 demethylase Utx could explain the function of Six4 during muscle regeneration since the global levels of H3K27me3 seem to increase with age (56). Moreover, Utx is needed for the expression of a set of Six4/MyoD common target genes including those impaired in muscle regeneration with partial-loss-of-function of Six4 (Fig. 4.3B and 5.4B). Therefore, Six4 is required not only for regulating adult myogenesis using *in vitro* models of myogenic conversion and myoblasts differentiation, but also for adult muscle regeneration *in vivo*.

Chapter 6: General Discussion

As an early transcribed TF during embryogenesis of different organs and tissues (196, 224, 227), Six4 was anticipated to play a critical role in the development of organs and tissues where it is expressed, especially in skeletal muscles (208). Six4 is the largest Six family member characterized by an activation domain distinguishing it from the co-expressed family member Six1 (203). The anticipated role of Six4 during prenatal myogenesis led to the generation of Six4 mutant mice (232) earlier than Six1 mutant mice (188, 221). The lack of muscle defects in Six4 mutant mice (232), which was attributed to a compensation by other Six family members, did not discourage researchers from further investigating its role in combination with knocking out Six1, the Six family member shown to be critical for embryonic myogenesis (188, 221). The phenotype of the Six1;Six4 double mutant mice (233), in comparison to Six1 mutant mice (188, 221), were indicative of an important function of Six4 during prenatal muscle formation. Six1 seems to play an uncompensated unique function during hypaxial muscle differentiation. However, the functions of both Six1 and Six4 are required during myotome muscle formation, myogenic progenitor specification, and hypaxial myogenic progenitor delamination and migration. In as much as these results reflected an important function of Six4 during prenatal muscle development, it implied that Six4 does not have its unique function in this process; rather, it co-regulates with Six1 an unknown number of target genes critical for muscle formation. If other Six4 target genes are not regulated by Six1, they could be regulated by Six5, the other Six factor expressed in skeletal muscle. Preliminary ChIP-seq data on Six1 (Blais's lab unpublished data) and Six4 (this study) reveal a binding site for both Six1 and Six4 at a Six5 regulatory region implicating that Six1 could be involved indirectly in regulating Six4 target genes by regulating Six5 expression. Although Six2 has been shown to be upregulated in the

embryos when both Six1 and Six4 are knocked out, the embryonic regions where this upregulation occurs are limited to the dorsal part of the most caudal somites and the branchial arches that give rise to head and neck muscles (234).

Recently, our lab revealed an important function of Six4 during C2C12 myoblast differentiation and identified a set of genes downregulated by the knockdown of the expression level of either Six1 or Six4 (182). These results, together with a recently documented role of Six1 during *in vivo* muscle regeneration (92), implicated a potential role of Six4 during *in vivo* muscle regeneration and motivated me to carry out a thorough investigation of Six4 function during adult myogenesis. The results of this comprehensive study provide a map of the Six4 binding profile in the context of myogenesis. It reveals a widespread cooperation between Six4 and MyoD in regulating adult myogenesis and presents the first evidence of a novel role of Six4 during adult muscle regeneration.

6.1 Six4 is an important myogenic factor during *in vitro* adult muscle differentiation

Myogenic conversion assay has been used to study cooperation between MyoD and other TFs where an augmented myogenic conversion was observed when both TFs are expressed in comparison to myogenic conversion when only MyoD is expressed (327, 329). For example, overexpression of Mef2c or Vgll2 in fibroblasts synergizes with MyoD to induce myogenesis, and this can be attributed partly to the lack of expression of Mef2c (327) and Vgll2 (329) in C3H 10T1/2 cells prior to the introduction of MyoD. In the work presented here, I used the same model of myogenic conversion combined to gene expression profiling in order to identify a core set of genes regulated by potential cooperation between Six4 and MyoD. However, instead of co-expressing Six4 and MyoD, I knocked down the expression of Six4 while inducing ectopic MyoD expression in these cells. A similar approach was used by others where an inhibitor of p38 kinase was used during MyoD-

induced myogenic conversion in order to identify a set of late muscle genes cooperatively regulated by MyoD and p38 signalling kinase (175). This assay confirmed our previous observation of an important uncompensated role of Six4 during adult muscle differentiation (Fig. 3.2; (182)). Additionally, the findings I have made bring a nuance to the well-accepted notion of MyoD as a master regulator of skeletal myogenesis (141). For MyoD to function as a master regulator of C3H 10T1/2 fibroblasts myogenic conversion, it needs proper expression levels of Six4. The enrichment of muscle-related functional categories, such as muscle contraction, muscle system process and muscle organ development, among Six4-regulated MyoD-induced genes indicates that Six4 is an important myogenic factor that needs to be expressed at proper levels to enable MyoD to perform its function as a master regulator of skeletal myogenesis. Although this approach was successful and I identified a total of 548 genes co-regulated by MyoD and Six4 (Table A2.3.; 355 genes potentially induced by both transcription factors and 193 genes potentially repressed by them), this approach does not really indicate a direct regulation by both TFs. Myog is expressed during MyoD-induced myogenic conversion of C3H 10T1/2 as early as 8 hours (355). Myog can regulate many of the genes co-regulated by Six4 and MyoD (182). Consequently, the cooperation between Six4 and MyoD cannot be distinguished from that between Six4 and Myog. This is especially important to consider, since we have previously shown that Six4 and Myog can cooperatively activate a reporter plasmid containing tandem copies of Ebox and MEF3 (182). Therefore, while this assay showed that Six4 is necessary for myogenic conversion, it did not allow me to distinguish whether Six4 is cooperating with MyoD and/or Myog.

6.2 Six4 cooperates with MyoD in regulating myoblast differentiation

Although the gene expression profiling performed during the myogenic conversion

assay suggests an importance of Six4 during adult myogenesis, it does not allow distinguishing direct from indirect cooperation between Six4 and MyoD in regulating the set of identified common genes. To thoroughly characterize the role of Six4 during adult myogenesis, it is critical to identify the transcriptional targets of Six4 and distinguish between directly and indirectly regulated genes. This requires a combination of genome-wide binding study of Six4 and gene expression profiling of genes regulated by loss-of-function of Six4. Gene expression profiling was performed by our lab and others (182, 236, 237) and in this study (Fig. 3.3 and Table A2.3), yet a comprehensive Six4-binding profile experiment is still lacking. To get a controlled set of Six4-bound loci, I performed Six4 genomic binding profile using two different antibodies, a custom-made specific anti-Six4 antibody and an anti-Flag antibody, in different C2C12 myoblasts cell lines undergoing differentiation. The number of Six4-bound peaks identified in this experiment was higher than the ones identified for MyoD in differentiating C2C12 cells in any of the four ChIP-seq experiments performed by four independent groups (ENCODE project, <http://encodeproject.org/ENCODE> (298-300)). Although the number of Six4-bound loci is more than two times the number of genes in the mouse genome, other studies reported a comparatively large number of sequence-specific TF-bound loci as it is the case with Pax7 in adult primary myoblasts (52,683 bound loci; (80)), MyoD in MEFs during myogenic conversion (67,000-124,000 bound loci depending on the p value threshold; (145)), and NeuroD2 in P19 cells (35,000-72,000 bound loci depending on p value threshold; (145)). Many parameters can affect the number of peaks identified including the TF per se, as well as a large number of technical variables such as the antibody used, the cell model, the choice of peak calling method and thresholds, making it difficult to compare different experiments. However, the 58,528 Six4-bound loci identified in this study using high stringent MACS calling parameters represent high confidence peaks

for the following reasons. First, the binding of Six4 (using the anti-Six4 antibody) was associated with the binding of Flag-Six4 (using the anti-Flag antibody). Second, the read density of Six4 binding drops in shSix4 cell line compared to shNS control cell line (Fig. 3.10 and 3.11A). Finally, small scale gene-specific ChIP assay experiments performed on representative peaks confirmed that they are real Six4 peaks (Fig. 3.15 and 3.16).

A TF can bind at a gene promoter, enhancer, within a gene body or even sometimes at genomic regions far from known genes (see Fig. 3.12A and B). Often a single bound-locus is surrounded by many genes. This makes it difficult to assign a certain TF binding event with the regulation of a specific gene. Although there is no general rule to assign a TF binding at a certain locus to one of the nearby genes, the most common approach is to assign the closest gene (based on distance from gene TSS to the peak summit, (356)) or the closest regulated gene (regulated by ablation of the TF) to a bound locus (357). For Six4-bound loci, I combined ChIP-seq data with gene expression data performed in C2C12 myoblasts in presence of siSix4 at T24 (182) and myogenic conversion gene expression and assigned each locus to the closest regulated gene if one was found; otherwise, it was assigned to the closest gene. Out of the 14,637 unique genes bound by Six4 only 897 genes were significantly regulated by Six4 knockdown ($p < 0.05$, with a fold change difference between cells with Six4 knockdown and control cells of at least 1.5), indicating that most of Six4 binding events are not associated with gene induction or repression. The same observation of relatively low number of regulated genes in comparison to a large set of bound loci was reported for other TFs such as Pax7 in primary myoblasts (1279 regulated genes in 52,683 bound loci; (80)), MyoD in MEFs during myogenic conversion (990 upregulated genes in 124,000 bound loci; (145)) and NeuroD2 in P19 cells (810 regulated genes in 72,000 bound-loci; (145)). It is not clear what the significance of Six4 binding at these loci is. However, they could be

associated with genes that could be regulated at a different stage of myogenic differentiation such as myoblasts or myotubes, genes regulated after specific stimuli that muscle cells might be subjected to (calcium concentration fluctuations, mechanical stress such as stretch, hypoxia, and others), or they could be genes related to other biological processes such as neurogenesis, spermatogenesis and others where Six4 plays an important function. If true, this would mean that Six4 could bind to certain loci irrespective of the tissue type similar to what we reported before for Six1 using ChIP-on-chip approach where we observed a significant enrichment of biological processes related to kidney and sensory organs development where Six1 is known to play a critical role in their development (182). The fact that this binding is not correlated with gene expression could be due to the absence of a tissue-specific partner TF, such as NeuroD2 for neurogenesis target genes, which is not expressed in myogenic cells.

Nevertheless, our model of identifying Six4 regulated genes is useful but not ideal compared to a Six4 KO model. In both C2C12 cells (182) and C3H 10T1/2 myogenic conversion (this study), we were able to reduce the expression level of Six4 only partially and consequently we could be missing many genes that are only deregulated in the complete absence of Six4. Alternatively, some Six4 target genes might be regulated by another compensatory mechanism. Although I did not notice an increase in the abundance of the other Six factors after the knockdown of Six4, I cannot rule out the possibility that the DNA binding sites left vacant by the knockdown of Six4 are bound by another TF that accomplishes the same function (i.e. functional compensation without upregulation). Finally, I focused on assigning Six4-bound loci only to coding transcripts and the possibility of Six4 binding and regulating small RNAs and long non-coding RNAs, which are transcribed across enhancers (358, 359), is not included in this study.

Analysis of Six4-bound peaks revealed a significant enrichment of muscle structure development category represented by 438 genes (Table 3.1) confirming the participation of Six4 in the regulation of myogenesis. Cell proliferation category is also enriched among Six4-bound peaks and it is represented by 1261 genes. This category is very important during myogenesis as many genes driving the cell cycle are repressed while inhibitors of the cell cycle get expressed when myoblasts start differentiating (176, 177). Additionally, Six4 peaks showed a notable overlap with those bound by MyoD (298) representing 36% of all MyoD-bound peaks (Fig. 3.13). Six4-bound peaks showed a significant enrichment of the Ebox motif (Fig. 13D) with central GG dinucleotide (Fig. 3.13C) being dominant among Six4 and MyoD common peaks. This GG-rich Ebox motif is found at lineage-specific genes associated with gene transcription upon MyoD binding (145). These results do not only implicate a potential cooperation between Six4 and MyoD in regulating a large set of genes, they also indicate that the genes potentially regulated by these two TFs are likely to be induced during myoblast differentiation and are probably muscle-related genes. Indeed, Six4 and MyoD common target genes that are bound and significantly regulated by Six4 and MyoD (366 unique genes; Table A2.9, indicated by “+++” with regulation by both Six4 and MyoD) based on gene expression profiling data (performed during C3H 10T1/2 myogenic conversion and in C2C12 myoblasts undergoing differentiation in presence of siRNA targeting Six4 or MyoD) showed a significant enrichment of several muscle-related biological processes including muscle system process, muscle contraction, muscle structure development, actin filament-based process and calcium ion transport (Table 3.2). It is noteworthy that the genes commonly bound and significantly regulated by both Six4 and MyoD represent a substantial fraction (41%) of the total genes bound and significantly regulated by Six4 (336 genes out of 897 genes). The remaining 59% of Six4 target genes

could be either non-muscle genes that are either induced by Six4 alone or in collaboration with another TF, whether it is muscle-specific or not, or they are muscle genes that require the combined function of Six4 and a muscle-specific TF different from MyoD. If another myogenic TF cooperates with Six4 in co-regulating the rest of Six4 target genes during myogenesis, then Myog is the most likely candidate. Myog is expressed in C2C12 cells differentiated for 24 hours. It regulates many of Six1 and Six4 target genes and seems to bind many of their regulatory regions (182). Finally, Myog synergizes with Six4 in reporter plasmid assay. Further analysis of Six4 (this study) and Myog ChIP-seq data (Mousavi et al, Mol Cell, 2013; ENCODE project) in combination with gene expression profiling of Six4 in case of partial-loss-of function (this study, (182)) and Myog in case of siRNA knockdown (182) will help clarify this issue.

The cooperation between Six4 and MyoD in regulating myogenesis was further confirmed by performing reporter assays on select regulatory regions of joint target genes including genes of the contractile apparatus, neuromuscular junction and developmentally regulated TFs. These regions are bound by Six4 and MyoD based on ChIP-seq and on confirmatory ChIP followed by gene-specific qPCR (Fig. 3.15 and 3.16). The associated genes are affected both when MyoD is knocked-down and when Six4 is knocked-down (Fig. 3.18). The reporter assays revealed that Six4 and MyoD are involved in a transcriptional synergy in activating reporter plasmids containing the regulatory regions of these joint target genes. The synergy is evident by the significantly higher transcriptional effect when both Six4 and MyoD are present compared to the sum of their individual effects. This synergy depends on direct binding of Six4 to these regulatory regions since mutating the MEF3 site, the DNA binding site of Six4, abolishes the transcriptional synergy between Six4 and MyoD (Fig. 3.17). Transcriptional synergy between MyoD and other TFs was reported using similar

assays and included various Mef2 isoforms (327, 360) and Nfatc3 (181). The ability of two TFs to synergize in activating a reporter plasmid reflects the complexity of gene transcription which is usually regulated by the combined action of more than one TF, each contributing a specific function (275). MyoD is known to recruit certain co-activators such as p300 and PCAF histone acetyltransferase to target promoters, and to interact with TAF3 (TATA-binding protein Associated Factor 3) thus enhancing transcriptional activity (171, 361, 362). Six4, in turn, can recruit the co-activator Eya (262) or Utx (270), and I have shown that the demethylase activity of Utx is needed for the expression of the joint targets of Six4 and MyoD (Fig. 4.3B).

6.3 Utx displays a widespread overlap with Six4 binding profile and functions at MyoD-bound loci

Although MyoD genomic binding profile during myogenic differentiation is highly correlated with a general regional increase in H4 acetylation, a histone mark associated with actively transcribed genes, only some MyoD binding events are accompanied with gene regulation (298). The presence of Six4 and Utx at MyoD-bound loci (Fig. 4.1A) may be what distinguishes the “bound” from the “bound and regulated” MyoD targets. The interest in Utx comes from an early study showing that transcriptionally inactive muscle genes in myoblasts are repressed by trimethylation of H3K27, and that this mark is removed upon differentiation (363). This was followed by another study showing the removal of the H3K27me3 repressive mark from the regulatory regions of Myog and muscle creatine kinase by Utx, which is recruited to these regulatory regions by Six4 (270). My results demonstrate that the association between Six4 and Utx is widespread, occurring at a set of MyoD loci characterized by Six4 binding (Fig. 4.1B). The significance of my findings is in allowing us to distinguish between two groups of MyoD target loci: 1) Six4-bound loci which are

associated with binding of Utx, Ash2L and RNA pol II, as well as the presence of active methylation mark, and a low levels of repressive methylation mark; and 2) loci not bound by Six4 characterized by low or no binding of Utx, Ash2L and RNA pol II, as well as low levels of active chromatin mark and relatively higher levels of repressive chromatin mark (Fig. 4.2).

As predicted from the enrichment of the Ebox motif with central GG dinucleotide associated with gene transcription and from the high level of active chromatin marks with low level of repressive ones, MyoD and Six4 bound loci have comparatively higher gene expression levels (Fig. 4.2). This is further supported by the finding that pharmacological inhibition of Utx impairs myoblast differentiation (by reducing Myog expression) and attenuates the induction of a set of Six4 and MyoD common target genes (Fig. 4.3). Therefore, these results suggest an important function of Six4, a non-muscle specific TF, in defining the function of the muscle-specific factor MyoD by binding a subset of its target genes, those that are repressed by H3K27me3 in myoblasts, and allowing their induction possibly by recruiting the demethylase Utx.

The widespread function of Utx during *in vitro* muscle differentiation raises the question of a similar function during *in vivo* muscle regeneration. A role for Utx in embryonic myogenesis has not been reported because the early embryonic lethality caused by Utx nullizygosity precludes the study of skeletal myogenesis. These mutant mice suffer from defects in several organs including heart and hematopoietic tissues at a time point before which skeletal muscle develops (330, 331). However, Utx is expressed in somites, limb buds and the myotome (330), and targeting Utx with morpholino oligonucleotides in zebrafish causes defect in somite development (287). This suggests that Utx may be active in developing muscles. The fact that satellite cells isolated from old mice have higher global

levels of H3K27me3 relative to satellite cells isolated from young adult mice (56) reflects an increase in H3K27me3 mark with age. It is possible that newborn and developing mice have very little H3K27me3 mark, and therefore may not need Utx for gene activation. However in adult mice, there is a substantial amount of H3K27me3 which needs to be actively removed. Therefore Six4 (and possibly Utx) may be required only during adult myogenesis. Future experiments addressing the role of Utx during muscle regeneration will help clarify this possibility. If true, this suggests that Six4 would be unique, among Six family members expressed in satellite cells, in being essential for the recruitment of Utx to MyoD target genes.

This association between a sequence-specific TF and Utx is reminiscent of the situation prevailing in other systems, though there are no other reports of a genome-wide level correlation between Utx and TF function. Utx was reported to trigger heart development through its interaction with the cardiac TFs Tbx5, Nkx2.5, and GATA4, and binding a handful of cardiac enhancers (330). Similarly, UTX was shown to interact with RAR α and to occupy several promoters of RAR α target genes leading to activation and proper differentiation of leukemic cells (364). p53, in turn, was reported to recruit Utx and Jmjd3 to the regulatory regions of many developmental transcription factors leading to gene activation during human embryonic stem cell differentiation (365). This might constitute a general mechanism for gene activation during the differentiation of tissue precursors. It is tempting to speculate that a similar connection between Six4 and Utx might also exist in neuronal, kidney, olfactory, prostate precursor cells, gonads and other tissues where we know Six4 is expressed, especially that Utx is ubiquitously expressed in somatic cells and gonads (366-368).

An unexpected finding in this study is the observation that Utx interaction with Six4 (Fig. 4.6 and 4.7) does not involve the previously reported transactivation domain of Six4 (198). However, the Six4 protein is still poorly characterized at the structure-function level and further experiments are required to understand the function of the Six4 protein containing amino acids 309-424 that are necessary but not sufficient for interaction with Utx. Although Six4 forms a stable complex with Utx (270), it is still unclear if it does so through a direct interaction or by recruiting a Utx-binding protein. If Six4-Utx interaction is mediated through another binding protein, the SWI/SNF remodelling complex proteins emerge as potential mediators, as Six4 has been reported to interact with Baf60c, a subunit of the SWI/SNF complex, in HEK 293 cells (271). Utx, on the other hand, has been reported to interact with Brg1 in T cells (335) and HEK 293 cells (330). Further identification of potential Six4- and Utx-binding protein(s) should help address these questions. An interesting finding is that Six4-Eya3 interaction seems to depend partly on Six4 C-terminal domain (Fig.4.5). Additional experiments are needed to confirm this interaction and explore if Eya3 function is needed for inducing Six4 and MyoD common targets or Six4 unique targets and to determine if Eya3 participates in the same Six4 complex that also contains Utx, or if instead the two are mutually exclusive partners of Six4.

6.4 Six4 is required for proper *in vivo* muscle regeneration

My results present the first evidence of an important role of Six4 during *in vivo* adult muscle regeneration. Several TFs coordinate their functions to ensure proper muscle regeneration including Pax7 and MRFs (reviewed in (14, 77)). Among the Six factors, a function for Six1 during muscle regeneration has been recently reported whereby Six1 regulates the return of satellite cells to quiescence, thus maintaining their homeostatic level (92). However, a potential role of Six4 during muscle regeneration has never been reported,

though it is expressed in quiescent, activated and differentiated satellite cells (241). In this study, I confirmed the report of Yajima et al (241), who showed that Six4 is expressed in quiescent satellite cells of resting adult muscles (Fig. 5.1), but with increased expression during muscle regeneration (Fig. 5.1 and 5.3A). By inducing partial loss-of-function of Six4 in activated satellite cells by intramuscular knockdown during CTX-induced muscle regeneration, I was able to demonstrate that optimal levels of Six4 are necessary for the process of muscle regeneration as assessed by fiber size measurements and marker gene expression assays. Intramuscular knockdown of Six4 in CTX-damaged TA muscles resulted in a smaller number of myofibers (Fig. 5.7D) with prevalence of the small myofibers (Fig. 5.6 and 5.7A, B and C) compared to contralateral control muscles (injected with siNS duplexes). This phenotype was associated with the decrease in expression levels of a number of muscle genes (Fig. 5.4B) involved in muscle structure (*Afp1l1* and *Obscn*), contractile function (*ATP2a1*, *Cacng1*, *Hrc*, *Myh3*, *Mylpf*, *Tnni1*, *Tnni2* and *Tnnt2*), and neuromuscular junction (*Chrng* and *Chrna1*), as well as TFs involved in muscle differentiation and regeneration or satellite cell quiescence (*Myog* and *Hey1*).

The defects observed in muscle regeneration due to partial loss-of-function of Six4 cannot be attributed to a defect in the expression of other Six factors as the expression of neither Six1 nor Six5 changes upon intramuscular knockdown of Six4 (Fig. 5.4B). Likewise, the possibility that the defect is due to impaired or delayed inflammatory response (113, 116-119) is also unlikely, for two main reasons. First, Six4 is not expressed in immune cells (Fig. 5.5). Second, the expression levels of genes prototypical of the inflammatory response were unchanged in the samples upon Six4 knockdown (Fig. 5.4B). It is worth mentioning that the immune cells prevailing at day 4 post-CTX-damage are mainly macrophages of both the pro-inflammatory (M1) and the anti-inflammatory (M2) subtypes. M1 macrophages predominate

at 1 day after CTX-damage, but they start transitioning into M2 macrophages between days 2-3 (111). For effective muscle regeneration, the number, time of subtype switch and the ratio of M1 to M2 macrophages should be maintained (117-119). In conditions of Six4 knockdown, we can presume that the total number of M1 or M2 macrophages is unchanged since the expression levels of markers of all macrophages (Ptpcr-positive and CD68-positive), M1 (CCL3-positive) or M2 macrophages (CD163-positive) were unchanged (Fig. 5.4). Likewise, the expression levels of cytokines produced by M1 macrophages (*Tnfa*) or M2 macrophages (*Il10* and *Igfl*) were not affected by Six4 knockdown (Fig. 5.4). In addition to immune cells, FAPs are most abundant at day 4. These cells secrete IL6 which will promote the differentiation of myogenic cells (353). Like the immune cell markers, the transcript level of the *Pdgfra*, a gene that specifically marks FAPs in muscle, was unaffected by Six4 knockdown (Fig. 5.4), suggesting that Six4 knockdown did not affect regeneration through a deregulation of FAPs number. Therefore, the defects observed with Six4 knockdown during muscle regeneration can be attributed to a defective differentiation of myogenic cells.

6.5 Six4 function in prenatal versus postnatal myogenesis

The results presented above reveal an uncompensated function of Six4 during *in vivo* adult muscle regeneration and adult muscle differentiation using two *in vitro* models: C2C12 myoblasts differentiation and C3H 10T1/2 myogenic conversion. These results are in apparent contrast with the observation that the embryonic development and juvenile growth of skeletal muscles are seemingly normal in Six4-deficient mice (232). It may be difficult to compare prenatal myogenesis with adult muscle regeneration since they are derived from different progenitor cells. While prenatal myofibers are derived from either embryonic (E10.5-12.5) or fetal myoblasts (E14.5-17.5), adult myofibers are derived from satellite cells.

These three different classes of myogenic precursor cells display differences in morphology when explanted in culture with embryonic myoblasts being elongated, fetal myoblasts being triangular while satellite cells have a rounded shape. They respond differently to signalling molecules such TGF β , BMP-4 (bone morphogenetic protein 4) and Notch. Satellite cells have a high binding capacity to PDGF (platelet-derived growth factor), early expression of acetylcholine receptors and acetyl-cholinesterase and more importantly different expression pattern of several muscle specific genes. The differences are reflected by differential expression of many genes when assayed by microarray analysis (reviewed in (8)) and by the different target genes of Pax7 and Pax3 which are also expressed differently in these cells (80). The observation that Six1;Six4 dKO mice have an aggravated muscle phenotype compared to Six1 KO mice suggests a compensation by other Six family members. Both Six1 and Six5 are potential candidates to compensate for Six4 since Six1 has the same tissue expression pattern as Six4, while Six5 is co-expressed with Six4 in several tissues (228) and has a long C-terminal domain with a reported transactivation activity similar to Six4 (198, 206, 218).

Postnatal myogenesis contributes to both juvenile muscle growth and adult muscle regeneration in case of injury, and both occur through the differentiation of satellite cells (38, 369-371). Yet, juvenile muscle growth proceeds unaffected in Six4 KO mice (232) while adult muscle regeneration is impaired with partial loss-of-function of Six4 (this study). It could be that our approach of using siRNA to induce acute partial knockdown of Six4 is very different from Six4 mutant mice; the siRNA is very fast, acute, with little time for compensation to occur. Inducing muscle regeneration in adult Six4 mutant mice and/or

studying the role of Six4 during juvenile muscle growth by inducing partial loss of function will help resolve this issue.

Alternatively, potential compensation by other Six factors during embryonic juvenile myogenesis and lack of such compensation during adult myogenesis could explain this discrepancy. This possibility suggests a unique function of Six4 during adult myogenesis which is in agreement with the recent findings that Six4 functions in the differentiation of isolated adult satellite cells is different from the one reported for Six1 and Six5 (241). While Six4 knockdown inhibited satellite cell differentiation, knockdown of either Six1 or Six5 did not significantly affect satellite cell differentiation; instead, Six5 knockdown increased satellite cell proliferation (241). However, in the context of adult muscle regeneration, conditional KO of Six1 in satellite cells 3 days before inducing CTX-regeneration in TA muscles, resulted in impaired myogenesis with smaller regenerating myofibers (92), which is an effect comparable to what I observed with Six4 knockdown. Additionally, Six1 KO resulted in failure of satellite cells to return to quiescence due to impaired expression of *Dusp6*. Although I did not explore the role of *Dusp6* during muscle regeneration with partial Six4 loss-of-function and did not look at quiescence, a similar function to Six1 could be predicted for Six4 based on the fact that *Dusp6* was identified as a Six1/Six4-regulated gene in gene expression profiling performed on Six1;Six4 dKO mice in comparison to Six1^{-/+}; Six4^{-/+} mice (237). Moreover, *Dusp6* gene is co-regulated and bound by both Six4 and MyoD based on my ChIP-seq and gene expression profiling data (Table A2.9). This implicates a similar function of Six1 and Six4 at least in regulating *Dusp6*, yet Six4 does not compensate for Six1 absence in the Six1 conditional KO during CTX-induced muscle regeneration. Besides *Dusp6*, many genes that are downregulated during muscle regeneration with Six4 knockdown are bound by Six1 in differentiated C2C12 cells (182) and are

downregulated by Six1 knockdown both in differentiated C2C12 cells (182) and in Six1;Six4 dKO mice (237). Consequently, they are likely to be deregulated by Six1 knockdown or KO during *in vivo* muscle regeneration. Apparently, the combined functions of both Six1 and Six4 are needed to regulate a set of genes during muscle regeneration. The lack of compensation by Six1 during muscle regeneration could be attributed to the unique function of Six4 in recruiting Utx to the regulatory regions of target genes. Further identification of Six1- and Six4- interacting proteins can help identify potential key modulators of Six1 and Six4 function during adult muscle regeneration and may help us explain if Six1 and Six4 regulate the expression of the same target genes through different mechanisms.

6.6 Six4 function beyond myogenesis

Out of the 14,637 unique genes annotated to Six4-bound peaks, only 3,863 genes are regulated by Six4 while the rest are annotated to Six4 peaks based solely on their proximity to the Six4 binding site. In addition to skeletal muscle related biological processes, several functional categories are enriched among Six4-bound peaks such as neurogenesis (Table 3.1; Table A2.8), brain development, sensory organ development, and reproductive system development. This might reflect the role of Six4 in the development of sensory organs including olfactory placodes (372, 373), gustatory papilla (374), as well as brain (375), sensory neurons (239) and male gonads (226). Some genes may be involved in more than one biological process. Such is the case of *Hey1*, a gene involved in both myogenesis and neurogenesis. Indeed, *Hey1* is among the genes that are regulated by Six4 during *in vitro* muscle formation (Fig. 3.18) and *in vivo* muscle regeneration (Fig. 5.4B). Nevertheless, many genes bound by Six4 are not induced or repressed in C2C12 cells implicating that Six4 can bind its target genes in a model system where these genes are not normally expressed. Their expression, however, might be dependent on the binding of the sequence-specific TF

driving such biological process. For example, although *Neurod1* and *Elavl4* regulatory regions are bound by Six4 in muscle cells, the expression of these genes does not seem to be controlled by Six4 in this tissue. It could be that these genes are bound and regulated by Six4 in the neuronal cells where they are normally expressed, along with some tissue specific TF, partner of Six4. Specifically, these genes are bound and regulated by NeuroD2 during P19 neurogenic conversion (145). NeuroD2 is a bHLH factor like MyoD and its role in neurogenesis is analogous to that of MyoD in myogenesis; it could cooperate with Six4 in regulating the expression of these two genes and others. The identification of binding sites of Six4 in other tissues, and identification of partner TFs of Six4 in such tissues, would be extremely interesting to pursue.

6.7 Concluding remarks

This study provides the first functional genomic investigation of Six4 in context of adult myogenesis, with emphasis on its function during adult muscle regeneration. Many Six4-regulated genes are identified and further experimentation will help understand their role in the regeneration process. Six4 seems to regulate many genes involved in this process in cooperation with MyoD. Although the significance of the Utx-Six4 interaction awaits further experiments, my work has established that Utx binding profile and function overlap with Six4 during muscle differentiation.

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Contributions of Collaborators

Imane Chakroun and Alexandre Blais designed the experiments and performed bioinformatics analyses. Imane Chakroun, Dabo Yang and John Girgis did the animal work. Dabo Yang performed eMHC immunostaining on tissues. Dabo Yang and John Girgis performed H&E staining, while Alexandre Blais, Dabo Yang and John Girgis analyzed images of eMHC and H&E staining. Imane Chakroun and Atchayaa Gunasekharan (under the supervision of Imane Chakroun) performed luciferase assays while Hilary Phenix analyzed luciferase data. Brahim Redouane performed IP experiments on Prmt5 and Alphonse Chu performed gene expression profiling on MyoD in C2C12 cells. Imane Chakroun, Yubing Liu and Alexandre Blais performed ChIP experiments using the old Six4 antibody (Fig. 3.4). The rest of experiments including cloning, gene expression profiling, ChIP and ChIP-seq were performed by Imane Chakroun.

Appendix 1: Prmt5 Regulates Skeletal Muscle Differentiation by Cooperating with Six4

A1.1 Abstract

Prmt5 is a histone and non-histone modifying enzyme that can mono or symmetrically dimethylate arginine residues resulting in transcriptional activation, repression or regulation of the function of its non-histone target proteins. Prmt5 is dispensable for prenatal skeletal myogenesis but it plays a critical role during adult muscle regeneration, in part by epigenetic silencing of the cell cycle inhibitor p21 in adult satellite cells, thus promoting their proliferation. However, Prmt5 plays a critical role in the muscle regeneration process aside from the satellite cell proliferation. This could be attributed partly to the ability of Prmt5 to regulate adult myogenic differentiation by controlling myogenin expression in activated satellite cells and in *in vitro* models of myogenic differentiation. A full understanding of the role of Prmt5 in muscle regeneration is delayed by the lack of a comprehensive knowledge of Prmt5-regulated target genes in this process. By using a high-throughput genomic mapping approach, I identified a novel set of Prmt5-bound loci that display a significant overlap with Six4-bound loci. Prmt5 binding correlates with Six4 binding at MyoD peaks implicating a potential cooperation between Six4 and Prmt5 in regulating MyoD target genes.

A1.2 Introduction

Protein arginine methylation is a PTM that marks histone and other cellular proteins. It is catalyzed by a group of enzymes known as protein arginine methyltransferases (PRMTs) which are classified into three subgroups depending on the arginine modification they catalyze. Type I PRMTs includes Prmt1-4, Prmt6 and Prmt8 and these can transfer a methyl group to the terminal nitrogen of the guanidine group within an arginine residue (omega- N^G -monomethylarginine or MMA) as well as two asymmetric methyl groups to generate omega- N^G , N^G – dimethylarginine (aDMA). Type II PRMTs, on the other hand, includes Prmt5 and

Prmt9 and these catalyze symmetric omega - N^G , N^G – dimethylarginine (sDMA) and MMA (reviewed in (1-3)). Prmt7 belongs to type III PRMTs and it can only catalyze MMA (4). Two enzymes can remove the methyl group from the arginine residues: the peptidylarginine deiminase Pad4 (5) which can deiminate MMA residues into citrulline, and the arginine demethylase Jmjd6 which can regenerate arginine residues from H4R3me2 (histone 4 dimethylated on arginine 3) and from the symmetric and asymmetric forms of H3R2me2 (6). Recent reports revealed that Jmjd6 can also demethylate methylated arginine residues in non-histone proteins including Hsp70 (7), estrogen receptor alpha (8) and RNA helicase A (9) as well as the methyl group on the γ -phosphate 7SK snRNA (10).

PRMTs demonstrate a widespread expression pattern in many organs and tissues. They are present in both the nucleus and the cytoplasm and can methylate a variety of proteins including histones, TFs, co-activators and many others. Consequently, they are involved in regulating various biological processes including epigenetic modifications, regulation of transcription, cell cycle, apoptosis, DNA repair, signal transduction, RNA processing, stem cell proliferation, primordial germ cell specification, and spliceosome assembly (1, 2).

Prmt4 (also known as Carm1 or the coactivator associated arginine methyltransferase 1) and Prmt5 are the two arginine methyltransferases with reported roles during skeletal myogenesis. Prmt4 has been reported to methylate Pax7 allowing it to recruit the MLL histone methyltransferase complex to *Myf5* promoter (11, 12). Additionally, Prmt4 is needed for the expression of late muscle genes (13, 14). Prmt5 was initially identified as Jak2-binding protein 1 (JBP1; (15)). It is the primary mammalian enzyme responsible for type II arginine methyltransferase activity. It is involved in activating Myog expression in MyoD-induced myogenic conversion (16). Recently, Prmt5 has been reported to regulate adult mouse satellite cell proliferation by epigenetic repression of the cell cycle inhibitor p21 (17).

Transcriptional co-repression was assumed to be the main function of Prmt5 since its reported synergy with the TF E2F1 in repressing cyclin E1 (*Ccne1*) expression (18). This initial report was followed by others showing co-repression in association with other TFs and repressor complexes including the SWI/SNF complex (19-21), Blimp1 (22), and Snail (23). However, other reports showed a transcriptional activation function for Prmt5 in inducing Myog (16) and IL2 (24) expression in MyoD-induced myogenic conversion and mitogen-activated Jurkat T cells and human peripheral blood lymphocytes respectively. Considering the many proteins modified by Prmt5, this enzyme is involved in many biological processes including DNA repair (25), ribosome biogenesis (26), structure of the Golgi apparatus (27), lipid and glucose metabolism (28, 29), myogenic differentiation (13, 14, 16, 30, 31), keratinocyte and nerve cell differentiation (32-34) and germ cell specification and development (22, 35-37), as well as signalling pathways, cell cycle and cell death reflecting a role of Prmt5 in tumorigenesis (23, 38-44). Indeed, Prmt5 levels are elevated in gastric, ovarian, breast, lung, colorectal and bladder cancer, as well as lymphoid and lymphoma, glioblastoma multiforme, melanoma, and germ cell tumors. Sometimes, Prmt5 expression is associated with poor prognosis (1, 2, 45). The recent identification of potent and selective Prmt5 inhibitors seems to be promising in treating lymphomas (46, 47). Additionally, Prmt5 is crucial for the early steps of embryonic mouse development and Prmt5 mutant mice are deficient in producing pluripotent stem cells and consequently they die at an early embryonic stage (48).

Prmt5 can function by forming complexes with other cofactors that target it to different histones, nuclear factors and other substrates in both the nucleus and the cytoplasm. In the nucleus, Prmt5 can either activate or repress gene expression. For example, Prmt5 binding to CoPR5 (cooperator of Prmt5) is associated with transcriptional repression of *Ccne1* by

favouring methylation of H4R3 over H3R8 (49) or by transcriptional activation of *Myog* (30). Similarly, Prmt5 interaction with hSWI/SNF ATP-dependent chromatin remodelling proteins can result in transcriptional activation of *Myog* (16) or repression of *St7* (suppressor of tumorigenicity 7) and *Nm23* (nonmetastatic 23) tumor suppressor genes associated with promotion of a tumorigenic state in NIH3T3 cells (19, 50). Prmt5 can also associate with regulators of transcription elongation (51). In the cytoplasm, Prmt5 in association with the WD-repeat methylosome protein 50 (MEP50), the most common cofactor of Prmt5, can methylate a variety of cytosolic proteins including spliceosomal Sm-class ribonucleoproteins (52) as well as small RNA-binding proteins called Piwi which are present exclusively in germ cells (53).

Prmt5 symmetrically dimethylates H2AR3, H4R3, H3R2 and H3R8 resulting in various transcriptional regulatory outcomes (15, 18-22, 54-56). Symmetrical dimethylation of H3R2 leads to recruitment of Wdr5 and the MLL complex leading to consequent methylation of H3K4 (57). Alternatively, Prmt5 can regulate transcription by methylating transcription factors such as Pax3 (58), NF- κ B (29), p53 (40), GATA4 (59) and E2F-1 (60, 61). MyoD, Myog and Mef2D have been shown to interact with Prmt5 in vitro (13) but no one has reported arginine methylation of any of these TFs by Prmt5 or any other family member. Recently, our lab showed an interaction between Prmt5 and Flag-Six4 in differentiated C2C12 using mass spectrometry (B. Redouane, Blais' lab unpublished data) implicating that Six4 and Prmt5 could cooperate in regulating transcriptional events during myogenesis. To test this possibility, I used high-throughput approaches to map the genomic binding profile of Six4 and Prmt5 during primary myoblast differentiation in order to identify a core set of genes bound and potentially regulated by both Six4 and Prmt5.

A1.3 Results

A1.3.1 Prmt5 is recruited to Six4-bound loci in differentiated C2C12 myoblasts

Since Prmt5 was identified as a Six4 interacting protein by performing mass spectrometry on nuclear extracts from differentiated C2C12 cells overexpressing Flag-Six4-myc and immunoprecipitated using anti-Flag antibody, it was important to confirm that this interaction is specific especially that Prmt5 has been reported to be a contaminant in several affinity-purified Flag-tagged complexes (62). IP performed on total protein extracts from C2C12 cells at T24 using either anti-Six4 or anti-Prmt5 antibody confirmed that endogenous Six4 interacts with endogenous Prmt5 (Fig. A1.1A). By using ChIP followed by gene-specific qPCR, I was able to show that Prmt5 is recruited to the regulatory regions of a few of Six4-bound loci identified using ChIP-seq performed in C2C12 cells including *Psm6*, *Setd7* and *Lef1* (lymphoid enhancer-binding factor 1). Like Six4, Prmt5 did not bind to the gene desert locus on Chromosome 15 or the regulatory region of *Hoxd10*, the two negative loci tested (Fig. A1.1B). *Ccne1*, one of Prmt5 target genes (18), was not bound by Six4 but was bound by Prmt5, as expected (Fig. A1.1B).

A1.3.2 Prmt5 interacts with Six4 in primary myotubes

To study the role of Prmt5 during skeletal myogenesis, I used primary myoblasts which are isolated from the mouse hindlimb muscles (63-65) and differentiated into myotubes in culture. Primary myoblasts represent a more physiologically relevant model to study skeletal myogenesis than C2C12 myoblasts, though they are more difficult to manipulate due to their higher rate of spontaneous differentiation. Western blot performed on primary myoblasts under non-confluent conditions, confluent (T0), or induced to differentiate for 24 (T24) or

Figure A1-1 Prmt5 is recruited to Six4-bound loci

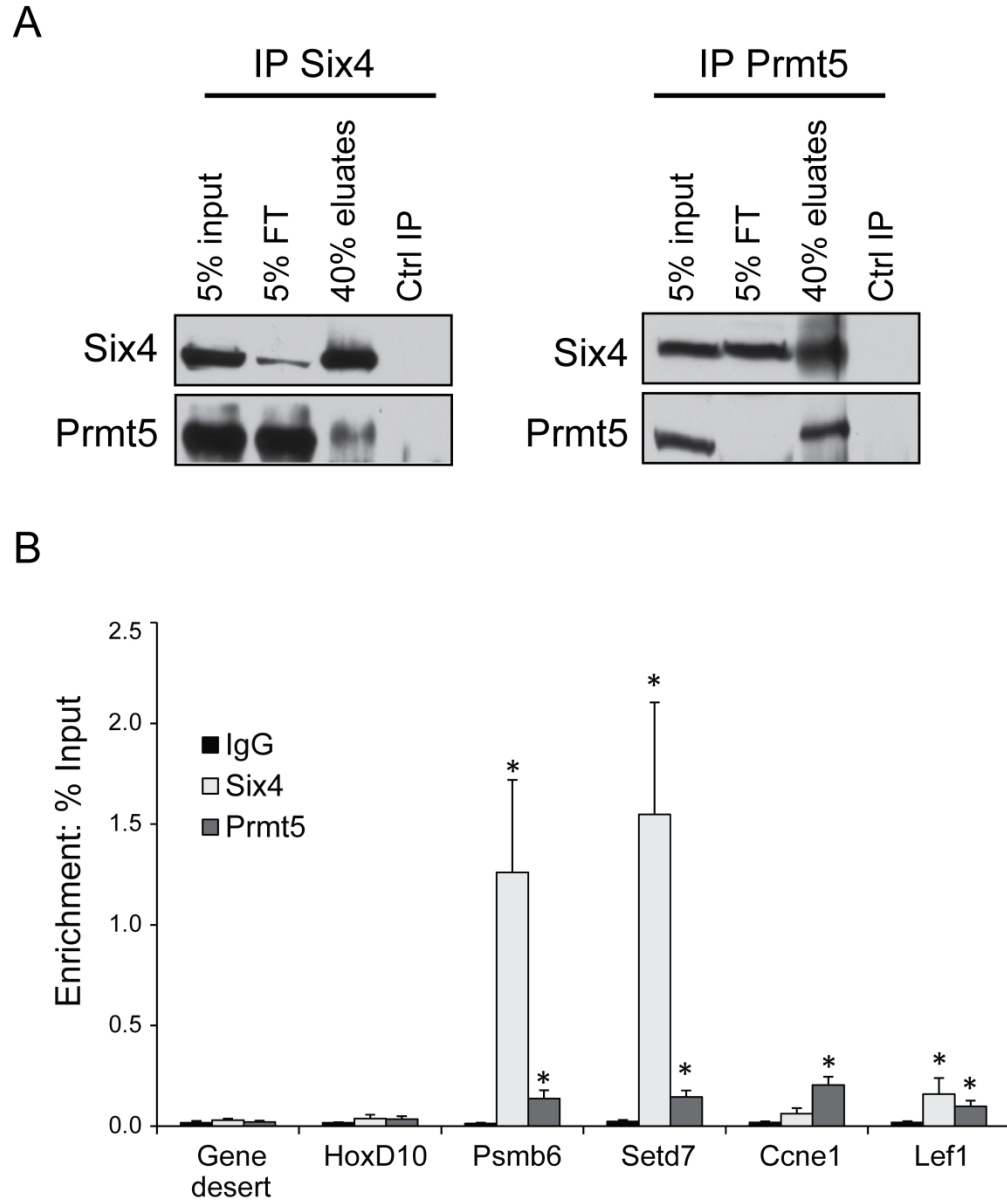


Figure A1.1. Prmt5 is recruited to Six4-bound loci.

A) Total protein extracts from C2C12 cells at T24 immunoprecipitated with either anti-Six4 or anti-Prmt5 antibody and probed with the indicated antibodies.

B) ChIP on C2C12 cells at T24 using normal rabbit IgG, anti-Six4 or anti-Prmt5 antibody. n = 4, * p < 0.04 using one-tailed unpaired t-test compared to IgG or to the two negative loci tested, gene desert and *HoxD10*.

48 hours (T48, or myotubes, MT) reveals that Six4 is expressed in these cells with levels that decline by T48 (Fig. A1.2A). Six1 and MyoD protein expression levels follow the same pattern as Six4. Myog is expressed in these cells upon inducing differentiation while MHC appears later. Instead, Prmt5 is expressed ubiquitously during primary myoblast differentiation (Fig. A1.2A). IP experiments performed on total protein extracts isolated from non-confluent primary MB or MT reveal that Six4 interacts with Prmt5 in primary MT but not in primary MB (Fig. A1.2B). For this reason, further experiments on Prmt5 were performed in primary MT.

A1.3.3 Genome-wide mapping of Prmt5 in primary myotubes

Since the interaction between Six4 and Prmt5 occurs specifically in primary MT (Fig. A1.2B), I performed ChIP-seq in these cells using anti-Prmt5 antibody and identified a total of 5,808 peaks bound by Prmt5. By using PAVIS to annotate peaks to nearby genes within 50 kb upstream or downstream of the peak summit, I found that 98% (5,693 peaks) of all the peaks bound by Prmt5 are annotated to nearby genes represented by 5,168 unique genes. Although the majority of these peaks are bound upstream from the TSS of the closest gene (56.9%), still a significant portion of these peaks (33.6%) are found at the 5'-UTR or within the gene body (introns and exons, Fig. A1.3A). Examples of these peaks are represented in Fig. A1.3B and C. In line with the reported role of Prmt5 in cell cycle, cell death, metabolic processes, organelle organizations, signalling pathways, DNA repair and genome organization, these biological processes are enriched among Prmt5-bound peaks (Table A1.1) (23, 25, 27-29, 38-44).

A1.3.4 A significant overlap between Six4 and Prmt5 binding profiles

To study if Prmt5-bound peaks show any overlap with Six4-bound peaks in differentiating C2C12 cells, first, I tested the ability of Prmt5 to bind the same loci identified

Figure A1-2 Prmt5 interacts with Six4 in primary myotubes

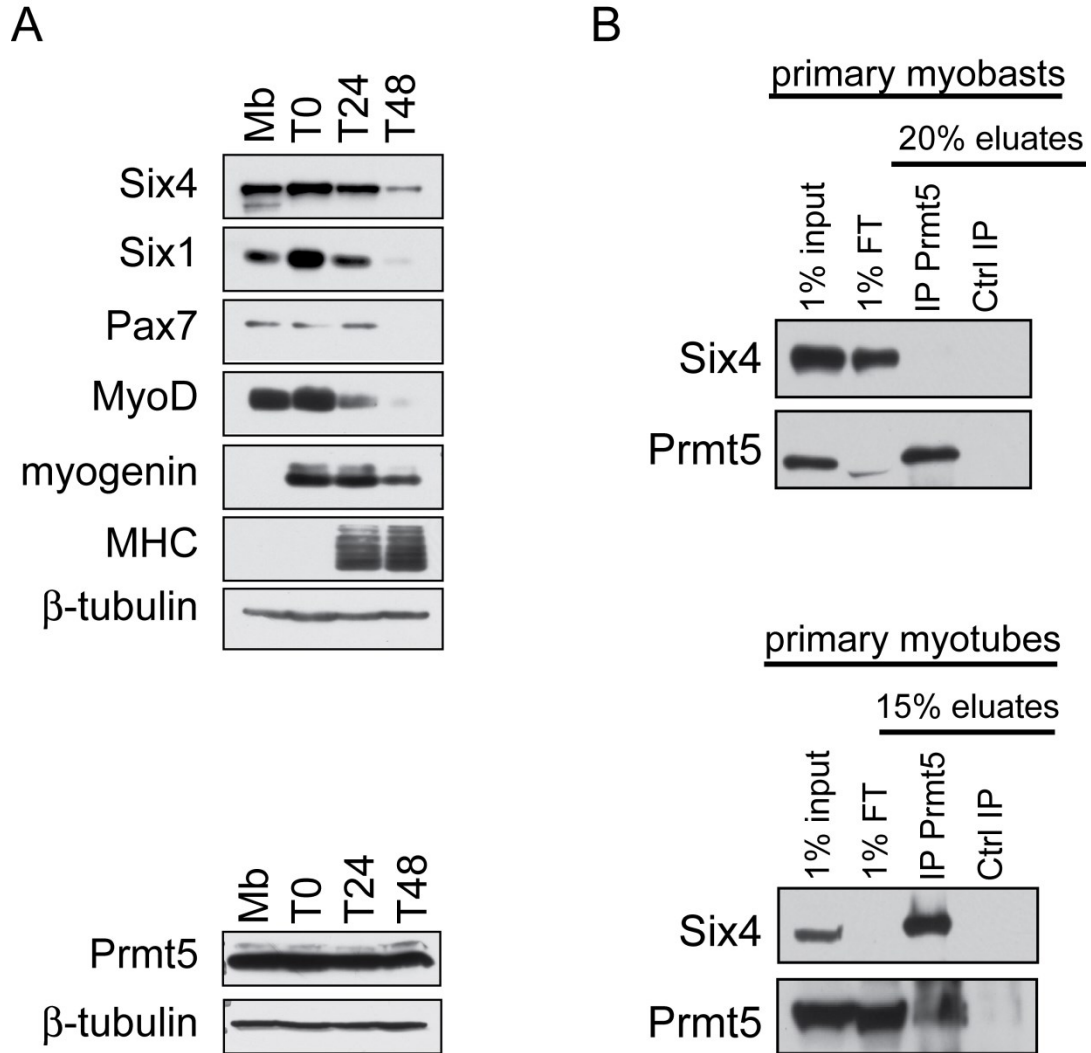


Figure A1.2. Prmt5 interacts with Six4 in primary myotubes.

A) Western blot on total protein extracts from non-confluent primary myoblasts (MB), or myoblasts differentiated for the indicated time points. Membranes were probed with the indicated antibodies.

B) Western blot on total protein extracts from primary myoblasts or myotubes (T48) immunoprecipitated with anti-Prmt5 or normal rabbit IgG and probed with the indicated antibodies.

Figure A1-3 Prmt5 ChIP-seq in primary myotubes

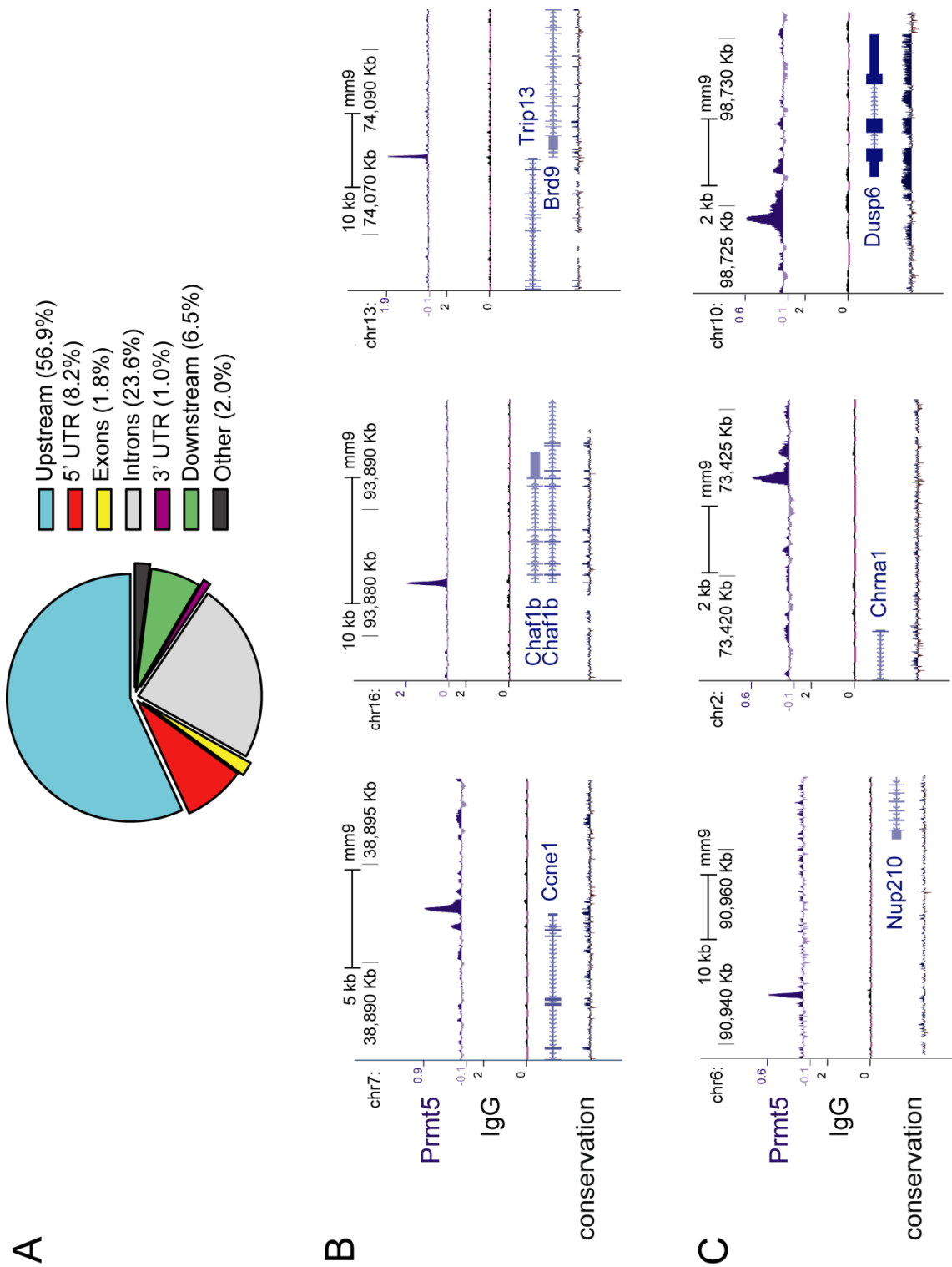


Figure A1.3. Prmt5 ChIP-seq in primary myotubes.

A) Distribution of Prmt5 peaks identified in primary myotubes (5,808 peaks) in relation to their distance from known genes using PAVIS (70).

B) Examples of Prmt5 binding at TSS of genes. Some of the isoforms of *Trip13* and *Brd9* mRNA were removed for clarity.

C) Examples of Prmt5 binding upstream of genes. Some of the isoforms of *Nup210* mRNA were removed for clarity.

Table 3 A1.1 Select gene ontology biological processes significantly enriched among Prmt5-bound loci in primary myotubes

Table A1.1 Select gene ontology biological processes significantly enriched among Prmt5-bound loci in primary myotubes.

Category	Number of targets	p-value	Example gene products
metabolic process	1976	5.19E-47	Btd, Ascc1, Gart, Map3K7, Rorb, Usp53
cell cycle	215	5.26E-12	Cdca2, Ccne1, Cdc6, Bub3, Wee1, Incenp
organelle organization	332	1.20E-09	Als2, Dlc1, Pdlim7, Epc1, Rock2, Prdm9
gene expression	695	5.38E-07	Med22, Mef2b, Klf16, Foxd2, Phf20, Hif
intracellular transport	143	2.10E-06	Xpo1, Aplg2, Slc25a10, Copz1, Hook2, Nxf1
regulation of cell death	171	6.73E-05	Tgfb1, Jag2, Fas, Adora1, Casp9, Traf4
actin filament-based process	63	1.62E-04	Tln1, Lima1, Fhod1, Eln, Tnnt2, Myh9
cellular response to stimulus	164	3.08E-04	Hmgn1, Xrcc3, Ldha, Foxo3, Hif1a, Kif22
lipid biosynthetic process	92	4.54E-04	Cyp51, Cyb5R3, Gdf1, Hexb, Ptgs1, Pgap3
cytoskeleton organization	103	4.81E-04	Tnnt2, Pdgfb, Actn1, Tubg1, Cnp, Coro1a
Chromosome organization	122	1.01E-03	Rad51c, Cbx4, Kdm1b, Smarcd2, Atf6b
Golgi vesicle transport	23	1.77E-03	Pacs1, Sec23a, Colga5, Prkci, Bet1, Ergic1
blastocyst development	22	7.29E-03	Srf, Taf8, Tgfbr1, Junb, Cul3, Sp1, Nasp

in primary MT in another myogenic cell model, the C2C12 cells. ChIP followed by gene-specific qPCR performed in C2C12 cells at T24 and MB confirmed the ability of Prmt5 to bind to the regulatory sites of a set of genes identified in primary MT (Fig. A1.4). Among the genes tested are a few cell cycle genes and TFs and genes involved in chromatin assembly such as *Ccne1*, *Cdca2* (cell division cycle-associated protein 2), *Cdc6* (cell division control protein 6 homolog isoform), *Kif14* (kinesin-like protein KIF14), *Bub1* (mitotic checkpoint serine/threonine-protein), *E2f1*, *Mybl2* (myb-related protein B), *Asf1b* (anti-silencing function protein 1 homolog B), *Trip13* (thyroid receptor-interacting protein 13), *Chaf1b* (chromatin assembly factor 1 subunit B), and *Anxa11* (annexin A11). Second, by comparing Prmt5-bound loci in primary MT to Six4-bound loci in differentiating C2C12 cells (58,528 loci), I found that 85% of all Prmt5 peaks are also bound by Six4 and 35% of them are bound by both Six4 and MyoD (Fig. A1.5A). Similar to what I observed with Utx binding profile (Fig. 4.1 and 2.2), Prmt5 binding seems to correlate positively with Six4 binding at MyoD-identified peaks (Fig. A1.5B and C), thus implicating that Six4 and Prmt5 could work together in regulating the expression of a large set of genes during myoblast differentiation including a set of MyoD target genes.

A1.4 Discussion

The role of Prmt5 as an epigenetic modifying enzyme has been studied extensively in the past few years. These studies have resulted in the identification of some target genes regulated as a consequence of either an epigenetic modification of the histone proteins flanking their regulatory regions or of the TF involved in controlling their expression (reviewed in (2, 3)). The few genome-wide studies performed so far looked at the histone marks modified by Prmt5 specifically H4R3me2s (histone 4 symmetrically dimethylated at

Figure A1-4 Prmt5-bound loci in primary myotubes are also bound by Prmt5 in C2C12 cells

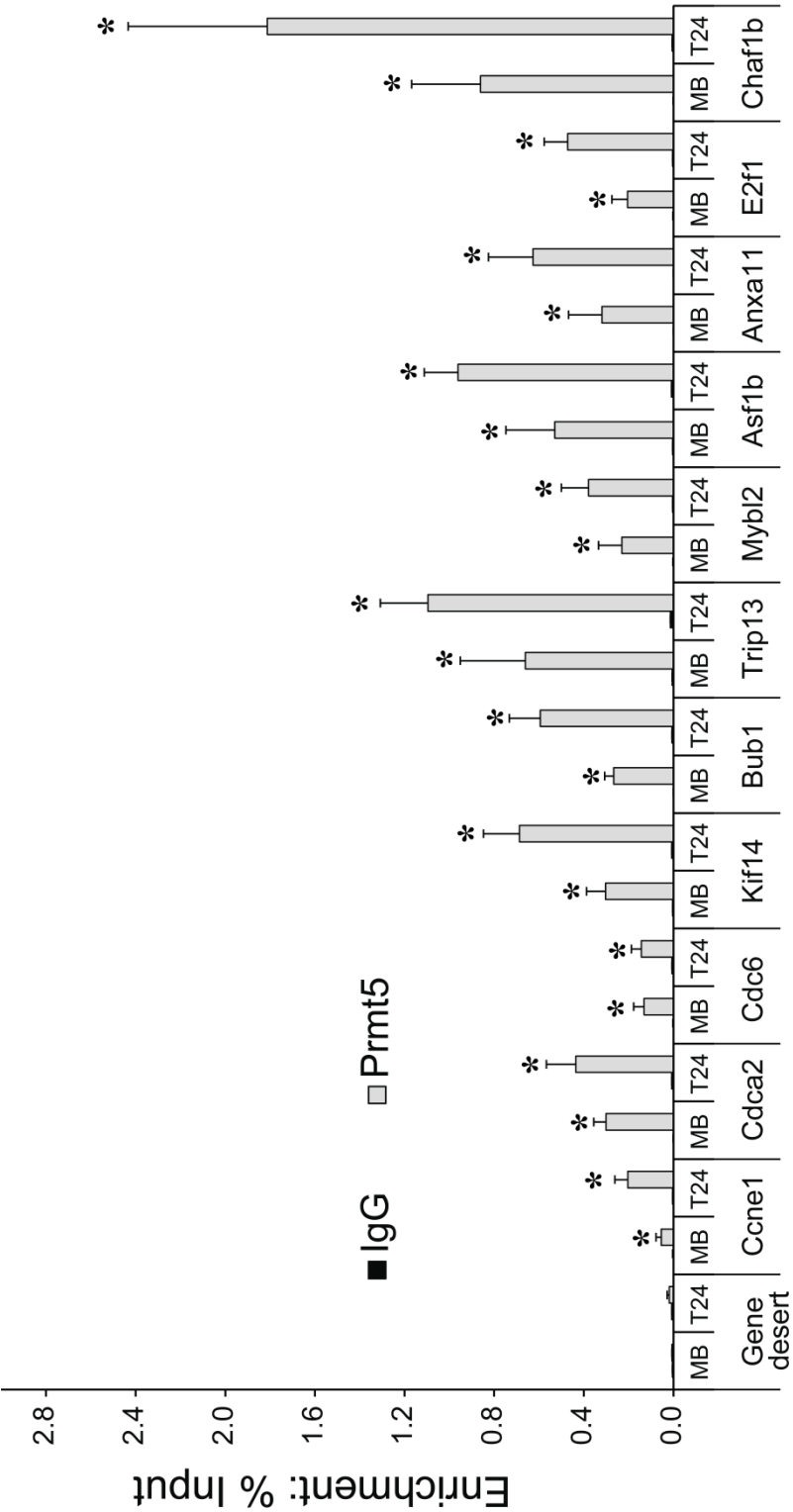


Figure A1.4. Prmt5-bound loci in primary myotubes are also bound by Prmt5 in C2C12 cells.
ChIP on Prmt5 in C2C12 at MB or T24 showing a significant enrichment of Prmt5 binding at all loci tested relative to IgG Ctrl and to the gene desert negative locus. n=4, * p < 0.05.

Figure A1-5 Prmt5 binding overlaps significantly with Six4 binding in differentiated C2C12 cells

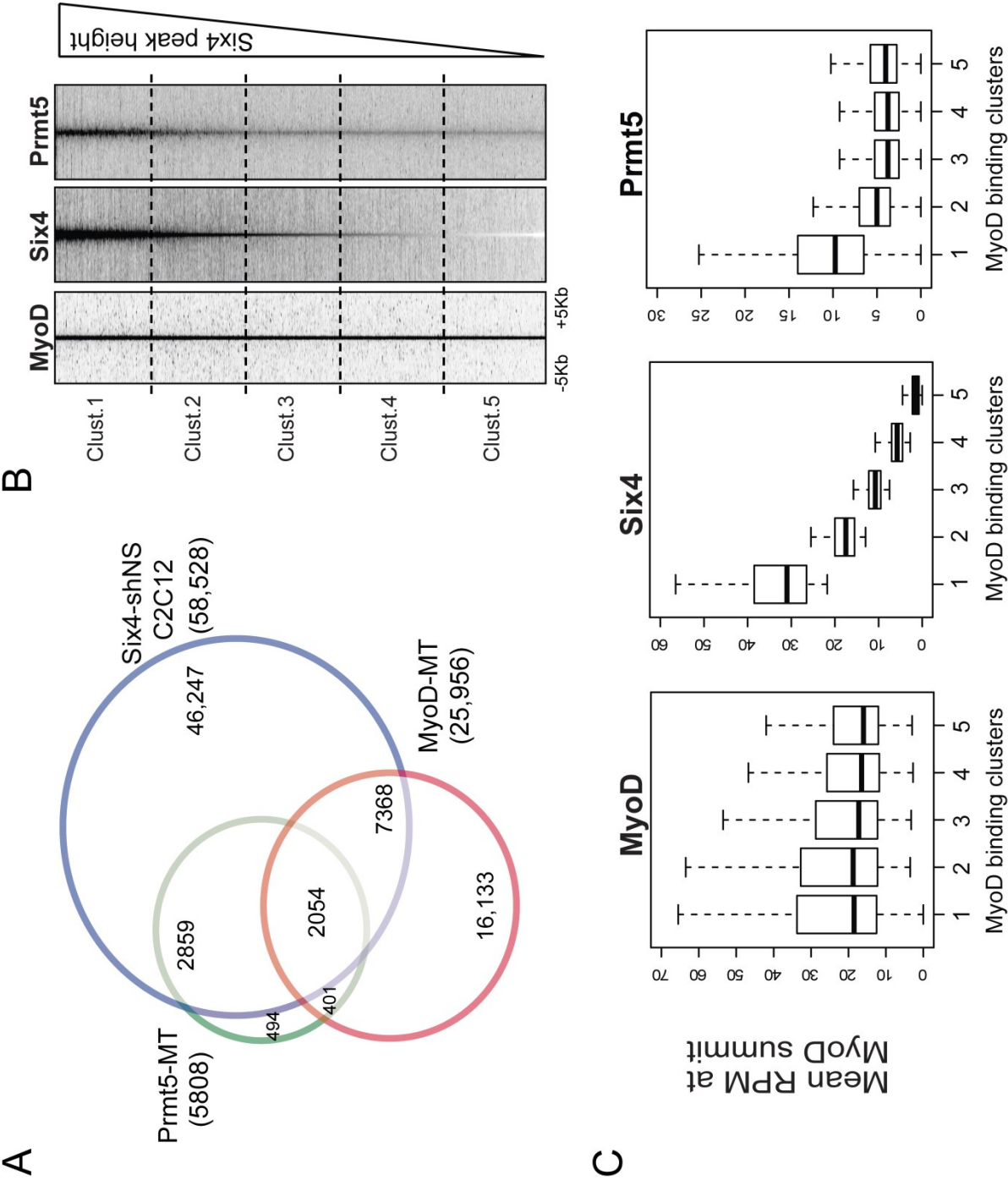


Figure A1.5. Prmt5 binding overlaps significantly with Six4 binding in differentiated C2C12 cells.

A) Venn diagram showing the overlap between Prmt5-bound loci in primary myotubes with Six4- and MyoD-bound loci in differentiated C2C12 cells.

B) Heat map of MyoD and Six4 ChIP-seq read densities at the 25,956 MyoD-bound loci in C2C12 myotubes (71) sorted in decreasing order of Six4 read density and divided into five equal clusters.

C) Mean read density plots of ChIP-seq data of MyoD (71), Six4 in shNS C2C12 cells and Prmt5 at the 5 clusters of MyoD peaks.

arginine 3) and H3R2me2s (57, 66, 67) but not at the binding of Prmt5 per se, making this study the first genome-wide mapping of Prmt5 binding profile.

Recently, a role of Prmt5 has been reported during adult muscle regeneration. According to this study, Prmt5 contributes to regeneration partly by maintaining the proliferation of the satellite cell pool through silencing the cell cycle inhibitor p21. However, inactivation of p21 in Prmt5 mutant mice did not restore muscle regeneration indicating a function of Prmt5 in this process beyond p21 and reflecting the need to identify other Prmt5-regulated genes during myogenesis in order to fully understand its role in this process. In this study, I addressed the role of Prmt5 using an *in vitro* model of muscle regeneration and identified a novel set of genes that are bound and potentially regulated by Prmt5. These genes were involved in several biological processes including muscle-related ones such actin filament-based process and cytoskeleton organization. Other biological processes are enriched such as metabolic processes, cell cycle and gene expression reflecting the diverse functions of Prmt5 (Table A1.1). However, due to the lack of gene expression profiling data of Prmt5-loss-of function in primary myotubes, I was not able to identify the genes that are bound and regulated by Prmt5. Nevertheless, Prmt5 binding profile showed a significant overlap with that of Six4 with 85% of all Prmt5-bound peaks being bound by Six4 and 35% of them are also bound by MyoD (Fig. A1.6A). It is not surprising with this notable overlap with Six4-bound loci that many of the biological processes enriched among Prmt5 peaks (Table A1.1) are also enriched among Six4 peaks (Table 3.1; Table A2.8). Additionally, Prmt5 binding profile correlated well with MyoD peaks in cluster 1 (Fig. A1.6B) that has higher Six4 binding and higher expression levels in C2C12 myotubes (Fig. 4.2) suggesting that Prmt5-bound genes might be induced during differentiation.

It is worth mentioning that Prmt5 function during fetal myogenesis of Pax7-positive cells seems to be dispensable (17), and Six4 function during prenatal and juvenile myogenesis is also dispensable (68). However, proper expression levels of both Six4 and Prmt5 (17) are needed for muscle regeneration. The observation that Six4 interacts with Prmt5 (Fig A1.2B) and binds 85% of Prmt5-bound loci (Fig. A1.6A) suggest that both Six4 and Prmt5 could work together in regulating adult muscle regeneration. With a recent report showing a methylation of arginine 200 in human Six4 (69), which is conserved in the mouse Six4, it will be interesting to investigate if Prmt5 methylates Six4 and the function of this modification.

Interestingly, Prmt5 binding at MyoD peaks seems to correlate well with Six4 and Utx binding at cluster1 which is also associated with RNA pol II and Ash2L binding, presence of an active chromatin mark and a low level of repressive chromatin mark as well as higher transcription levels of assigned genes. This implicates that Prmt5 binding there is correlated most likely with gene induction. Epigenetic modifications by Prmt5 can result in either gene silencing (18-23) or induction (16, 24) depending on the arginine residue it modifies. Notably, symmetrical dimethylation of H3R2 (H3R2me2s) by Prmt5 enhances the binding of Wdr5, a component of the MLL complex that catalyzes H3K trimethylation and contains Ash2L (57). It could be that recruitment of Prmt5 to cluster 1 loci is associated specifically with H3R2me2s, and the role of Six4 could be to recruit Prmt5 to these loci to catalyze this modification. Further gene-specific ChIP experiments on Prmt5 and its various histone marks (H3R2me2s, H4R3me2s and H3R8me2s) as well as the active chromatin mark H3K4me3 and the repressive chromatin mark of H3K27me3 in the C2C12 cell lines (shSix4 and shNS C2C12 cells) will help clarify this possibility and the link between Six4, Prmt5 and Utx. Potential cooperation between Prmt5 and Six4 (or MyoD) and the need for Prmt5

methyltransferase activity in possible induction of Cluster 1 genes could be addressed by using the specific inhibitor of Prmt5, GSK3235025 (46), in reporter assays and quantification of mRNA transcript levels of cluster 1 genes.

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Appendix 2: Supplementary Tables

Table 4 A2-1 List of oligonucleotide sequences

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
siSix4#1	Sense	GCAAGUACUUCACCUGUCUCCUUA	siRNA
siSix4#2	Sense	UAAGGAAGACAGGUGAAGUACUUGC	siRNA
siSix4#3	Sense	GGGACACAAUGGAGUUAUCCUAAU	siRNA
siMyod	Sense	GAGCGCUCUCCACAGACAUU	siRNA
HoxD10	Forward	GAGAAATCGGACTCACCTTCC	ChIP
	Reverse	CACATACCCAGGCAGAACG	ChIP
Gene Desert 15	Forward	TCCTCCCCATCTGTGTCATC	ChIP
	Reverse	GGATCCATCACCATCAATAACC	ChIP
Foxo6	Forward	ATCTGCTTAGCTGGCAGGTG	ChIP
	Reverse	GCCACCACCTCCTCTTGTAAC	ChIP
Trim54	Forward	TGGGAAGTGGGAATGTCACAC	ChIP
	Reverse	GCAGCACTGTTTGTCCCTTC	ChIP
Slc44a4	Forward	GAGGGGGAGCGTGAAATAG	ChIP
	Reverse	GTAAAGGGCTGGACAGTCG	ChIP
Pde4dip	Forward	GCTCAGAGCTGACGCAAAG	ChIP
	Reverse	CGGACTACATCTCCCAGAGG	ChIP
Obscn	Forward	TCCAAAATAGCCCTGGTGTC	ChIP
	Reverse	GTCTTCCTGCTCCTGACCAC	ChIP
Kremen2	Forward	TGCTTGGGAAGCCTAGAAAC	ChIP
	Reverse	GTTGACACTGCGCAGAGAAG	ChIP
Hrc	Forward	TTGGTGGGGACAGCTGAG	ChIP
	Reverse	AACTCAGATCTGGGGACAGG	ChIP
Hey1	Forward	TTAGCTGCTGAGGGCTGAC	ChIP
	Reverse	ACAGCCGGTCTCATAGGAAG	ChIP
Hes6	Forward	CACTACCCCCAGTCCCTCTC	ChIP
	Reverse	TCAAGTTACTGGGGCCAGAC	ChIP
Afap111	Forward	TGCTATAAAAAGCCCCTTGG	ChIP
	Reverse	GGCACACATTGCAGTCATTC	ChIP
Setd7	Forward	GCGGAGAACTCAGCAAAAC	ChIP
	Reverse	ATATCACCGCCTGGGTGTC	ChIP
Psm6	Forward	GACACACTGCCGTAAAGTGC	ChIP
	Reverse	CTTCTGGGAGCGAAGCTG	ChIP
Capn2	Forward	TGCAGTTTTTGGTTGCACTC	ChIP
	Reverse	GCCAGCTACTGCCACAGTC	ChIP
Ankrd1	Forward	GCGTATGCGAATCAGGAAG	ChIP
	Reverse	CGATACGTGGGATGACTCG	ChIP
Atp2a1	Forward	AGAACAGGAGGGAGGTAGGG	ChIP
	Reverse	ATTTGCTCCTTGGCCTTTG	ChIP
Chrna1	Forward	ATTGTCTTACATCACCTGTGTGC	ChIP

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
	Reverse	TGTTAAGCAAGGCACGTCTG	ChIP
Cacng1	Forward	GACTTCTATTAATGCTTGTCAGTAGGC	ChIP
	Reverse	GGGTATTTCTAGCCCTCAGATG	ChIP
Chrng	Forward	AGAGGTGTAGGACCGAAGCA	ChIP
	Reverse	GCTGGGCTCAGATTACATGG	ChIP
Myoz2	Forward	CGGCGAGTAGTTTGGTAATTG	ChIP
	Reverse	CTTGGCTTGGTTGGTTTCTC	ChIP
Myog	Forward	AGAGGGAAGGGGAATCACAT	ChIP
	Reverse	TGGCATGAACCCAGAGATAA	ChIP
Spry1	Forward	GATGCAGGAAGATGACATGC	ChIP
	Reverse	TCGTAAGGGCTCTGGTTAC	ChIP
Ldb3	Forward	CCTCCCCAGGGACTATATTAGC	ChIP
	Reverse	ACCGGAGGAGTCACGTTG	ChIP
Pbx1	Forward	GAATGGCTGTGGAACAAACC	ChIP
	Reverse	GCTGGCAGGTTTTCACTCC	ChIP
Trim54	Forward	GACGACGGTACCATTCCATTTCTGAAT CATGCACTCTCTAGGAG	Reporter assay
	Reverse	GACGACAAGCTTGACTGGATTCTTGG GATCCCGGCTATTG	Reporter assay
Obscn	Forward	GACGACGGTACCTTCTGGTTCTGTGTG GTGGTCCTGGGTCC	Reporter assay
	Reverse	GACGACAAGCTTGACTGGGCCTAGCTA GCCTGGGGTCTG	Reporter assay
Hrc	Forward	GACGACCTCGAGCAGAAAGAGAGAGAG AGAAGAATCAAAGAG	Reporter assay
	Reverse	GACGACAAGCTTGGCTGAACGTTTAGA GTGTCTTGCTGGG	Reporter assay
Ankrd1	Forward	GACGACGGTACCGGCCGCCACACTGTT CTA	Reporter assay
	Reverse	GACGACCTCGAGGATGAACCCCTGGAA CTGG	Reporter assay
Atp2a1	Forward	GACGACGCTAGCTGGGGCTTCTTTTTG TTCTG	Reporter assay
	Reverse	GACGACCTCGAGGCAGGGGGAGTAAGG ATCTC	Reporter assay
Chrng	Forward	GACGACGCTAGCGAGATATGATGGGGC TGAGG	Reporter assay
	Reverse	GACGACAGATCTAAGAGGAGCTGAGGT CTCTGG	Reporter assay
Chrng_Mef3_mut	Forward	CGTCCCCTGGCTGGCCCATGAGAGAGA AGCCCAGCATTGTACATATCC	Reporter assay
	Reverse	GGATATGTACAATGCTGGGCTTCTCTC TCATGGGCCAGCCAGGGGACG	Reporter assay
Atp2a1_Mef3_mut	Forward	CAGAGCAGCTGCTCTTTGGACGATGCT GCCACCCAGGCCAGGCCAGCC	Reporter assay

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
	Reverse	GGCTGGCCTGGCCTGGGTGGCAGCATCG TCCAAAGAGCAGCTGCTCTG	Reporter assay
Trim54_Mef3_mut	Forward	CTCAGTCCGGGGACAGCTGTGGCGCACC ATAGATGAGCTAAATTGGGAAGT	Reporter assay
	Reverse	CAGTTCCCAATTTAGCTCATCTATGG TGCGCCACAGCTGTCCCCGGACTGAG	Reporter assay
Hrc_Mef3_mut	Forward	GTGGGGACAGCTGAGGCCTACTATCCT GCGCCCCCAGCACCTCCCCCTGTT	Reporter assay
	Reverse	AACAGGGGGGAGGTGCTGGGGGCGCAGG ATAGTAGGCCTCAGCTGTCCCCAC	Reporter assay
Obscn_Mef3_ mut#1	Forward	GGACAGCCAGAGGGGTACTCACTCTTG CCTCCAGGAAAGAATTTCCCG	Reporter assay
	Reverse	CGGGAAATTCTTTCTGGAGGCAAGAG TGAGTACCCCTCTGGCTGTCC	Reporter assay
Obscn_Mef3_ mut#2	Forward	CCTCCTTGGGACTGAGGGTCAATCTGA CTCCTGGACAGTGACACCAGG	Reporter assay
	Reverse	CCTGGTGTCACTGTCCAGGAGTCAGAT TGACCCTCAGTCCCAAGGAGG	Reporter assay
Ankrd1_Mef3_mut	Forward	CTGCCAAAGGAATGACCCTCGCACAA TTGTTCTGATTTCGCATACGCC	Reporter assay
	Reverse	GGCGTATGCGAATCAGGAACAATTGT GCGAGGGTCATTCTTTGGCAG	Reporter assay
Foxo6	Forward	TCAAGGATAAAGGCGACAGC	RT-qPCR
	Reverse	AGTGCCCTCGTTCTGCAC	RT-qPCR
Trim54	Forward	AAGCCCGTGGTGATCTTG	RT-qPCR
	Reverse	GGGATTGCCACAGAGGATTAG	RT-qPCR
Obscn	Forward	CTGGTGGTCGTGAGAGAGC	RT-qPCR
	Reverse	CTCGCACTGGAATGTAGCAG	RT-qPCR
Kremen2	Forward	ACATCTGCAGGGCTTCCTC	RT-qPCR
	Reverse	CTGGAAGCATTCCGACAAG	RT-qPCR
Hrc	Forward	CATTATCCCAAACCACTCG	RT-qPCR
	Reverse	CCTGCTGACCTGTGACCTC	RT-qPCR
Hey1	Forward	CGAGGTGGAAAAGGAGAGC	RT-qPCR
	Reverse	GTCTTTTCCTGGCCAAAACC	RT-qPCR
Hes6	Forward	GGCCAGGAGGATGAGGAC	RT-qPCR
	Reverse	TCCTGAAGACTCTCGTTGATCC	RT-qPCR
Afap111	Forward	CAGAACTCAGACTCCGACAGC	RT-qPCR

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
	Reverse	CTTCCCTTTGCCATGTTAC	RT-qPCR
Myog	Forward	CAGTGAATGCAACTCCCA	RT-qPCR
	Reverse	ACCCAGCCTGACAGACAATC	RT-qPCR
Ankrd1	Forward	CTGTGAGGCTGAACCGCTAT	RT-qPCR
	Reverse	GCATTCTCCTTGAGGCTGTC	RT-qPCR
Atp2a1	Forward	TGTCCCTCCACTTCCTCATC	RT-qPCR
	Reverse	TCCGAGCAATGAACTTGAGA	RT-qPCR
Chrng	Forward	GGACCTGATGCGAACTACG	RT-qPCR
	Reverse	CCTCCTCTCGTTCATTCAGG	RT-qPCR
Utx	Forward	GCCACAGACCTAGTCCTCAGA	RT-qPCR
	Reverse	GTCTTGAGGCGGACATTTA	RT-qPCR
Tbp	Forward	TCATGGACCAGAACACAGC	RT-qPCR
	Reverse	GCTGTGGAGTAAGTCCTGTGC	RT-qPCR
Rplp0	Forward	GAACTGCTGCCTCACATCC	RT-qPCR
	Reverse	TGGAAGAAGGAGGTCTTCTCG	RT-qPCR
Rps26	Forward	GCCATCCATAGCAAGGTTGT	RT-qPCR
	Reverse	GCCTCTTTACATGGGCTTG	RT-qPCR
Actnb	Forward	CAGCTTCTTTGCAGCTCCTT	RT-qPCR
	Reverse	CACGATGGAGGGGAATACAG	RT-qPCR
Cacng1	Forward	ATCTGCGCATTTCTGTCCTT	RT-qPCR
	Reverse	TCCTCGCTGTCAATCATACG	RT-qPCR
Six5	Forward	GCCTCTCGCTTACACAGGTC	RT-qPCR
	Reverse	GGACTCATCCTCCGTAGTGG	RT-qPCR
Chrna1	Forward	ATCACCGTCATCGTCATCAA	RT-qPCR
	Reverse	GGCTTCCCAGAGATGTCAGA	RT-qPCR
Myod	Forward	CCCCGGCGGCAGAATGGCTACG	RT-qPCR
	Reverse	GGTCTGGGTTCCTGTTCTGTGT	RT-qPCR
Six1	Forward	TTTTACGCAAGAGCAAGTGG	RT-qPCR
	Reverse	CTCTCGTTCTTGTGCAGGTG	RT-qPCR
Six4	Forward	CGAGACCCAGTCCAAAAGC	RT-qPCR
	Reverse	GCTAGAGAGGCTGAGGTTGG	RT-qPCR
Gpr20	Forward	TGGGTCCACAGACCTAGAGC	RT-qPCR
	Reverse	AGGGCATGTCAGTGGTCAG	RT-qPCR
Sox11	Forward	TTCGAGTTCCCCGACTACTG	RT-qPCR
	Reverse	GCGCCTCTCAATACGTGAAC	RT-qPCR
Prkrir	Forward	CTAGTCCTTATCGGACAGTCCTC	RT-qPCR
	Reverse	GTCTGCTATGCGGGTTGTTC	RT-qPCR
Pkia	Forward	CCTGGTTTCCTCTGCAAGTG	RT-qPCR
	Reverse	TTCGGTGGAGCTTCTCTGTC	RT-qPCR
Tnnt1	Forward	TGAACTCCGGATTCTGTCTG	RT-qPCR
	Reverse	GCTGGTGGATCCATTCTGAC	RT-qPCR
Tnni1	Forward	CTGCTAAGTCCCCGACCTC	RT-qPCR

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
	Reverse	GGCACGAGCAGAAAGATAGG	RT-qPCR
Tnni2	Forward	AGATCGACGTGGCTGAAGAG	RT-qPCR
	Reverse	TTGCCCTCAGGTCAAATAG	RT-qPCR
Mylpf	Forward	TGACCATGTTTGGGGAAAAG	RT-qPCR
	Reverse	TTTTTGATGGTGCCCTTCC	RT-qPCR
Myh3	Forward	TTTGATGCCAAAACCTACTGC	RT-qPCR
	Reverse	ATGGCATACACGTCCCTCTGG	RT-qPCR
Pax7	Forward	CTTACTGCCACCCACCTAC	RT-qPCR
	Reverse	GGCCAGGTAATCAACAGCAG	RT-qPCR
Il10	Forward	CATCGATTCTCCCCTGTG	RT-qPCR
	Reverse	CATGGCCTTG TAGACACCTTG	RT-qPCR
Tnfa	Forward	CCACCACGCTCTTCTGTCTAC	RT-qPCR
	Reverse	ATGAGAGGGAGGCCATTTG	RT-qPCR
Ptprc	Forward	GCAGAAACAGAAGATGTGGTTG	RT-qPCR
	Reverse	TGGCGATGATGTCATAGAGG	RT-qPCR
Cd68	Forward	CAGCTGTTCACCTTGACCTG	RT-qPCR
	Reverse	CAATGATGAGAGGCAGCAAG	RT-qPCR
Cd163	Forward	GGGGCAAGCATAACTGTGAC	RT-qPCR
	Reverse	TCCTGAACATCTGGACACTCC	RT-qPCR
Ccl3	Forward	GCCCTTGCTGTTCTTCTCTG	RT-qPCR
	Reverse	GGAATCTTCCGGCTGTAGG	RT-qPCR
Igfl	Forward	GACCGAGGGGCTTTTACTTC	RT-qPCR
	Reverse	GCAACACTCATCCACAATGC	RT-qPCR
mSix4-CDS	Forward	AATAATGCGGCCGCTCCTCTTCCTCC CCCACCGGGCAGATT	IP
	Reverse	GTCGTCTCTAGATAAGTCTTGCATGTC TTCATCC	IP
mSix4V109E	Forward	GTCGCTGCGAATGCGAGGCG	IP
	Reverse	CGCCTCGCATTCGAGGCGAC	IP
mSix4_delta309	Reverse	GTCGTCTCTAGATGCGCCTGAAAGTGG ATGAGG	IP
mSix4_delta424	Reverse	GTCGTCTCTAGAGTTCACAGCAGAAC TCTGGAG	IP
mSix4_delta583	Reverse	GACGACCTCGAGTTATGCACTGTGATTGACA GGCATC	IP
Six4_aa424	Forward	GACGATGCGGCCGCATCAGCAGCCACC ACCTCCTAC	IP
mSix4_aa217	Forward	GATGATGCGGCCGAGAGGAGACGGTGTAT TGTTTC	IP
mSix4_NLS-280	Forward	CGACGGATCCACCATGGCGGCCGCAAGC CCCAAGAAGAAGCGGAAGGTGGAGGCCA GCGGAGGAGGAAAAAGCGAATCGGATG GCAAC	IP
human_Utx mSix4_NLS-280	Forward	GACGACGCGGCCGCGAAATCCTGCGGAG TGTCGCTCG	IP
	Reverse	GACGACTCTAGAAGATGAGGCGGATGG	IP

Table A2.1 List of oligonucleotide sequences			
Oligo	Strand	SEQUENCE	Use
		TAATGG	

Table 5 A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion								
ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P378173	1110012L19Rik	68618	NM_026787	13.50	12.88	13.43	13.19	No
A_52_P552547	1110054M08Rik	68841	NR_037954	9.02	8.23	9.54	9.12	Yes
A_51_P492446	1110057K04Rik	68832	NM_172401	12.81	12.21	12.60	12.31	No
A_52_P354119	1190001L17Rik	68923	AK004434	6.78	6.16	6.80	6.29	No
A_52_P394291	1190002N15Rik	68861	NM_001033145	10.44	11.04	10.28	10.65	No
A_51_P234044	1190005I06Rik	68918	NM_197988	11.44	10.72	11.44	10.68	No
A_52_P148184	1600014C10Rik	72244	BC092099	9.70	9.00	9.68	9.29	No
A_51_P147064	1600014C23Rik	72240	NM_028164	6.42	5.44	5.79	5.40	No
A_52_P418515	1700007G11Rik	75784	NM_001024614	2.78	5.60	3.07	4.83	No
A_52_P30632	1700007K13Rik	69327	NM_027040	9.38	8.51	8.80	8.14	No
A_51_P110914	1700010D01Rik	76386	NM_029590	3.75	6.18	3.61	5.16	No
A_51_P324742	1700013G24Rik	69380	NM_027063	4.20	5.93	3.75	5.71	No
A_51_P513979	1700026L06Rik	69987	NM_027283	9.26	8.42	8.53	7.84	Yes
A_51_P231946	1700029P11Rik	66346	NM_025503	7.25	6.48	6.68	6.57	No
A_52_P150436	1700086L19Rik	74284	NR_030733	4.87	6.47	4.66	5.74	Yes
A_51_P299858	1700125H20Rik	73634	NM_028589	7.27	6.21	6.94	6.21	No
A_51_P502075	1810009J06Rik	73626	NM_023707	4.48	3.40	4.55	4.31	Yes
A_51_P301499	1810027L02Rik	69177	AK007620	6.29	5.57	5.86	5.77	No
A_52_P660047	1810034E14Rik	66503	NR_045798	5.10	4.22	5.62	4.86	Yes
A_51_P268673	2210016H18Rik	70125	AK008737	6.86	5.65	7.01	6.00	No
A_51_P270558	2210409D07Rik	70157	NR_045360	2.46	7.50	2.52	6.40	No
A_52_P627727	2210409E12Rik	72381	NR_003492	6.51	5.65	6.45	5.88	No
A_51_P199352	2310015B20Rik	69563	NR_038041	6.39	13.04	5.67	12.53	No
A_52_P587606	2310022A10Rik	66367	NM_175107	4.16	5.56	4.43	5.48	No
A_51_P507172	2310022A10Rik	66367	NM_175107	10.90	12.00	11.02	11.60	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P324391	2310058N22Rik	71921	AK009987	5.62	6.49	5.22	6.35	No
A_52_P638513	2310061J03Rik	66391	NR_027965	10.30	9.43	10.53	10.05	No
A_52_P445622	2610002M06Rik	67028	NM_025921	8.57	7.76	8.69	8.39	Yes
A_51_P347875	2610305D13Rik	112422	NM_145078	10.35	9.69	10.46	10.09	No
A_51_P199175	2700038G22Rik	67194	NR_045040	5.47	6.17	5.37	6.28	No
A_52_P324656	2700046G09Rik	67188	NR_033198	9.60	8.97	9.45	8.83	No
A_52_P59070	2810403D21Rik	69964	NR_015493	5.56	4.44	5.47	5.02	No
A_52_P27694	2810408A11Rik	70419	XR_388526	9.01	8.14	8.54	8.02	No
A_51_P308290	2810468N07Rik	72834	AK013390	3.23	4.58	3.33	4.44	No
A_52_P206669	2900011G08Rik	72933	AK013526	4.95	6.24	4.69	5.42	No
A_51_P264656	2900011O08Rik	67254	NM_144518	4.73	3.83	5.62	5.10	Yes
A_52_P459048	2900011O08Rik	67254	NM_144518	5.36	3.25	5.16	4.49	No
A_51_P339744	3010003L21Rik	109163	XR_378198	7.55	8.19	7.51	8.01	No
A_52_P500622	3110057O12Rik	269423	NM_026622	4.91	4.19	5.15	4.49	No
A_52_P156034	3830408C21Rik	100040322	NR_015471	5.83	4.90	5.96	5.74	Yes
A_51_P158007	4833439L19Rik	97820	NM_133797	13.75	14.40	13.77	14.15	No
A_52_P499240	4930412C18Rik	320152	AK054421	6.84	6.08	6.85	6.34	No
A_51_P195557	4930429B21Rik	67576	NR_027966	4.57	5.40	5.00	5.50	No
A_51_P137018	4930438A08Rik	73988	XM_003689172	1.95	5.82	2.28	5.64	No
A_52_P215263	4930556M19Rik	75259	NR_045065	4.91	3.94	5.07	4.36	No
A_52_P1003306	4930557B21Rik	75308	AK016159	4.14	3.30	4.76	3.69	No
A_52_P148972	4930581F22Rik	78934	NR_029475	6.57	5.84	6.74	6.57	No
A_51_P408749	4933439C10Rik	74476	NR_015585	4.28	2.31	4.76	4.30	Yes
A_51_P264132	5430416O09Rik	71406	NR_033355	3.47	4.91	3.74	4.73	No
A_51_P333460	5430427O19Rik	71398	NM_001163539	6.20	5.61	6.17	5.43	No
A_51_P446886	5730409E04Rik	230757	NM_001145950	12.68	12.06	12.51	12.13	No
A_52_P593212	5730446D14Rik	66640	BC094279	9.49	8.00	9.20	8.28	No
A_51_P317512	5730508B09Rik	70617	NM_027482	7.62	9.07	7.36	8.32	No
A_52_P23308	5730508B09Rik	70617	NM_027482	6.71	8.07	6.48	7.46	No
A_52_P105020	5830428M24Rik	76062	NR_038060	4.88	3.40	4.36	3.49	No
A_51_P334490	6430548M08Rik	234797	NM_172286	8.25	8.99	8.34	8.83	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P251672	6430548M08Rik	234797	NM_172286	8.42	9.38	8.44	8.91	No
A_51_P446825	6430573F11Rik	319582	NM_176952	2.88	6.69	2.23	4.77	Yes
A_51_P493771	8030462N17Rik	212163	AK163840	5.09	6.26	5.00	5.55	No
A_51_P384051	8430436N08Rik	71510	NR_040645	7.23	6.09	7.24	6.48	No
A_52_P553589	9030617O03Rik	217830	XR_381840	8.94	8.26	8.69	8.60	No
A_52_P170173	9330102E08Rik	330427	NR_077223	5.26	6.42	5.46	5.91	No
A_51_P209921	9330159F19Rik	212448	NM_001162537	5.56	8.60	4.64	7.92	No
A_51_P423804	9530010C24Rik	109279	AK079182	6.01	4.85	5.84	5.01	No
A_51_P303791	9530076L18	328192	AK035610	7.12	6.22	6.99	6.19	No
A_52_P62775	A230057D06Rik	319893	NR_015533	5.52	4.27	6.04	5.29	No
A_52_P38117	A530016L24Rik	319942	NM_177039	7.03	5.63	6.55	5.68	No
A_52_P620472	A830082K12Rik	320174	NR_045195	7.13	6.13	7.66	7.62	Yes
A_51_P261931	A930003A15Rik	68162	NR_015488	1.79	7.92	1.93	6.80	Yes
A_52_P672532	Aamdc	66273	NM_001177946	12.49	13.99	12.38	13.42	No
A_51_P209401	Aamdc	66273	NM_001177946	9.89	11.29	9.72	10.59	No
A_52_P267505	Abca8b	27404	NM_013851	7.58	6.30	8.05	6.98	No
A_51_P309338	Abcb11	27413	NM_021022	7.20	6.48	6.59	5.78	Yes
A_51_P161342	Abcb6	74104	NM_023732	8.67	10.45	8.60	9.77	No
A_52_P628117	Abcc5	27416	NM_013790	9.39	8.27	9.65	9.10	No
A_52_P292428	Abhd13	68904	NM_026868	8.68	9.43	8.89	9.12	No
A_51_P270083	Abhd14b	76491	NM_029631	10.31	9.34	10.73	10.21	No
A_52_P640512	Ablim3	319713	NM_198649	3.59	9.49	3.39	7.87	No
A_52_P502032	Abr	109934	NM_198018	8.99	10.36	9.36	10.06	No
A_52_P348648	Abra	223513	NM_175456	2.14	8.83	2.56	8.16	No
A_51_P384113	Acad12	338350	NM_178799	10.18	9.51	10.13	9.55	No
A_51_P130757	Acer3	66190	NM_025408	10.13	9.49	10.33	9.81	No
A_52_P675395	Ackr3	12778	NM_007722	12.04	12.72	12.21	12.43	No
A_51_P451574	Acot1	26897	NM_012006	9.45	8.74	9.08	8.41	No
A_51_P206708	Acox2	93732	NM_053115	7.31	6.60	7.35	6.45	No
A_51_P444335	Acp6	66659	NM_019800	7.60	9.62	7.21	8.41	No
A_51_P356265	Acrbp	54137	NM_016845	4.02	3.08	3.65	4.70	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P496956	Acsbg1	94180	NM_053178	8.76	6.74	8.48	6.95	No
A_51_P496432	Acs11	14081	NM_007981	11.11	12.19	11.35	11.70	No
A_52_P597618	Acs11	14081	NM_007981	7.21	8.27	7.57	7.97	No
A_51_P463452	Acs11	14081	NM_007981	7.00	8.14	7.41	7.79	No
A_51_P511560	Acs13	74205	NM_001033606	10.68	12.47	10.75	11.91	No
A_52_P78203	Acs14	50790	NM_207625	11.77	13.45	11.63	13.10	No
A_51_P268154	Acs14	50790	NM_207625	9.68	10.99	9.71	10.74	No
A_51_P246854	Acta1	11459	NM_009606	12.16	15.07	11.65	14.26	No
A_52_P239556	Acta2	11475	NM_007392	7.53	8.90	6.50	8.21	Yes
A_52_P210078	Acta2	11475	NM_007392	11.94	13.57	11.14	12.92	Yes
A_51_P149469	Actc1	11464	NM_009608	14.03	15.76	13.53	14.88	No
A_51_P241269	Actg2	11468	NM_009610	13.20	14.28	12.86	13.68	No
A_52_P656699	Actn3	11474	NM_013456	9.69	12.47	8.50	11.18	No
A_51_P320668	Actr3b	242894	XM_006535714	6.43	5.84	6.56	6.10	No
A_51_P232399	Acy3	71670	NM_027857	8.50	7.77	7.89	7.35	No
A_52_P290457	Adam12	11489	NM_007400	3.16	6.12	3.48	5.38	Yes
A_51_P510882	Adam12	11489	NM_007400	3.73	6.73	4.69	6.26	No
A_52_P277205	Adam23	23792	NM_011780	7.92	7.12	8.54	8.26	No
A_52_P333289	Adam33	110751	NM_033615	4.68	3.85	4.45	4.27	No
A_51_P319917	Adam8	11501	NM_007403	9.07	12.48	10.11	13.25	Yes
A_52_P213932	Adamts1	11504	NM_009621	11.37	10.30	11.56	10.32	No
A_51_P367780	Adamts12	77794	NM_029981	4.35	6.48	4.19	6.20	No
A_51_P295621	Adamts13	269959	AK020361	11.38	10.45	11.23	10.50	No
A_51_P213077	Adamts13	269959	AK020361	5.83	4.51	5.71	4.80	No
A_51_P185575	Adamts14	229595	NM_144899	8.90	7.98	9.79	9.15	Yes
A_52_P407280	Adamts15	66548	NR_104339	8.21	7.46	8.72	8.12	Yes
A_51_P259603	Adcyap1r1	11517	NM_007407	4.47	9.71	5.69	10.31	No
A_52_P615225	Adcyap1r1	11517	NM_007407	4.98	6.22	4.72	6.72	No
A_52_P629895	Adh1	11522	NM_007409	8.41	7.07	7.91	6.71	No
A_51_P428555	Adh1	11522	NM_007409	7.23	6.17	6.96	5.91	No
A_51_P233797	Adh7	11529	NM_009626	9.15	8.34	9.32	8.78	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P661412	Adora1	11539	NM_001008533	2.19	8.23	2.68	7.60	No
A_52_P172272	Adora1	11539	NM_001008533	6.61	9.09	6.49	8.50	No
A_52_P419455	Adora2b	11541	NM_007413	6.98	7.76	7.92	8.75	No
A_51_P382789	Adora3	11542	NM_027025	3.94	8.75	3.25	6.39	Yes
A_52_P289022	Adprh	11544	NM_007414	5.74	6.50	5.99	6.39	No
A_51_P338615	Adprh	11544	NM_007414	10.57	11.49	10.62	11.02	No
A_52_P586865	Afap111	106877	NM_178928	3.18	7.44	4.14	5.76	No
A_51_P303739	Afap111	106877	NM_178928	3.31	8.51	3.87	6.73	No
A_51_P197596	Afap112	226250	NM_146102	7.15	6.34	7.77	6.93	Yes
A_51_P183283	Aff2	14266	NM_008032	5.83	4.12	5.76	5.05	No
A_51_P388329	Aff3	16764	NM_001290814	7.74	6.88	7.14	6.42	No
A_51_P486121	Aff3	16764	NM_001290814	9.76	8.22	9.46	7.61	No
A_52_P231041	Agap1	347722	AK083235	10.47	11.84	10.65	11.60	No
A_51_P488121	Agap2	216439	NM_001033263	4.07	6.03	4.41	5.68	No
A_51_P334444	Agl	77559	AK036225	10.12	10.98	10.06	10.59	No
A_52_P101221	Ago2	239528	NM_153178	9.62	10.82	9.59	9.77	Yes
A_51_P180001	Ago2	239528	NM_153178	10.72	11.78	10.55	10.78	Yes
A_52_P101218	Ago2	239528	NM_153178	6.59	7.76	6.67	6.83	Yes
A_51_P166107	Ago2	239528	NM_153178	13.02	13.95	13.01	13.50	No
A_51_P425490	Agpat3	28169	NM_053014	14.30	15.09	14.48	14.92	No
A_52_P523569	Agpat3	28169	NM_053014	12.59	13.55	13.05	13.51	No
A_52_P552550	Agtrap	11610	NM_009642	9.98	9.38	9.90	9.31	No
A_51_P278675	Ahnak	66395	NM_009643	7.27	6.46	7.41	6.70	No
A_51_P235297	Ahsa2	268390	NM_172391	6.98	7.91	6.80	7.87	No
A_52_P458000	AI467606	101602	NM_178901	3.06	7.09	2.62	6.64	No
A_51_P472829	Aif11	108897	NM_145144	8.95	12.78	9.21	12.98	No
A_51_P144052	Aim1	11630	NM_172393	10.90	9.87	10.66	9.93	No
A_52_P649568	Aipl1	114230	NM_053245	6.02	5.35	5.17	4.24	No
A_51_P101006	Ak8	68870	NM_001033874	6.36	7.89	5.62	7.25	No
A_51_P179647	Akap17b	338351	NM_001081956	7.95	7.27	7.50	6.95	No
A_51_P224362	Akap6	238161	NM_198111	9.96	11.10	10.12	10.74	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P237575	Akirin1	68050	NM_023423	12.60	13.52	12.87	13.47	No
A_52_P142208	Akirin1	68050	NM_023423	9.79	10.68	9.94	10.58	No
A_51_P486618	Akr1b10	67861	AK052576	11.95	12.91	11.91	12.35	No
A_52_P257058	Akr1b10	67861	AK052576	4.69	5.70	5.11	4.95	No
A_52_P346706	Akr1b3	11677	NM_009658	11.98	13.64	12.14	13.12	No
A_51_P284177	Akr1c14	105387	NM_134072	9.80	8.68	9.76	9.21	No
A_51_P408044	Akt2	11652	NM_007434	10.09	11.41	10.43	10.88	No
A_52_P2977	Akt2	11652	NM_007434	11.57	12.77	11.66	12.19	No
A_51_P215887	Aldh18a1	56454	NM_019698	10.45	9.63	10.59	10.18	No
A_51_P334942	Aldh1a1	11668	NM_013467	6.23	7.12	6.40	7.13	No
A_51_P383399	Aldh1a7	26358	NM_011921	6.14	7.95	6.49	7.48	No
A_52_P498193	Aldh1l2	216188	NM_153543	11.03	10.31	11.42	10.66	No
A_51_P216905	Aldoa	11674	NM_007438	16.10	17.03	16.23	16.58	No
A_52_P98387	Aldoa	11674	NM_007438	16.38	17.48	16.53	17.05	No
A_52_P241612	Aldoat2	79459	NM_001277340	8.65	9.87	8.76	9.16	Yes
A_52_P522023	Alkbh1	211064	NM_001102565	7.14	7.86	7.51	7.82	No
A_51_P447258	Alox12b	11686	NM_009659	8.37	7.72	8.60	8.14	No
A_51_P247249	Alox5	11689	NM_009662	3.52	6.33	3.26	6.65	No
A_51_P118885	Amacr	17117	NM_008537	12.07	14.41	12.20	13.79	No
A_51_P391159	Ang	11727	NM_007447	7.56	6.43	7.12	6.81	No
A_52_P98778	Ang4	219033	NM_177544	6.77	5.91	6.72	6.25	No
A_51_P338443	Angptl4	57875	NM_020581	14.24	12.99	14.49	14.47	Yes
A_51_P269375	Ank1	11733	NM_031158	7.58	11.63	7.19	9.97	Yes
A_52_P562817	Ank1	11733	NM_031158	6.17	11.28	6.04	9.90	Yes
A_52_P655842	Ank1	11733	NM_031158	3.23	7.90	3.30	6.84	No
A_52_P120803	Ankrd1	107765	NM_013468	8.67	12.82	8.37	12.35	No
A_51_P499530	Ankrd10	102334	NM_133971	8.46	10.67	8.83	10.31	No
A_52_P956125	Ankrd12	106585	NM_001025572	4.56	3.20	4.57	4.38	Yes
A_51_P230722	Ankrd12	106585	NM_001025572	10.50	9.84	10.79	10.60	No
A_51_P442206	Ankrd42	73845	NM_028665	9.37	8.72	9.41	8.95	No
A_51_P218402	Ankrd44	329154	AK038343	5.31	6.77	5.49	6.04	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P62037	Anxa2	12306	NM_007585	15.94	15.19	16.23	15.75	No
A_51_P451032	Anxa3	11745	NM_013470	15.01	14.07	14.97	14.59	No
A_51_P368696	Apaf1	11783	NM_001042558	6.94	6.32	6.99	6.82	No
A_52_P300981	Apbb1ip	54519	NM_019456	4.42	3.74	4.54	4.10	No
A_51_P473576	Apex1	11792	NM_009687	12.30	13.06	12.46	12.93	No
A_51_P209327	Apln	30878	NM_013912	9.64	8.84	9.25	8.91	No
A_51_P359315	Apobec2	11811	NM_009694	2.63	9.23	2.31	7.53	Yes
A_51_P203182	Apobr	171504	NM_138310	7.99	6.54	8.78	7.55	Yes
A_51_P342387	Apoh	11818	NM_013475	7.08	6.31	7.42	7.01	Yes
A_51_P497724	Apol7a	75761	NM_029419	7.74	6.48	7.42	6.53	No
A_51_P239177	Apol7c	108956	NM_175391	6.97	6.10	6.67	5.63	No
A_51_P500215	Apol9b	71898	NM_173743	11.43	10.13	11.24	10.38	No
A_51_P265685	Apoo	68316	NM_026673	12.41	13.18	12.24	12.68	No
A_51_P207601	Aqp5	11830	NM_009701	10.74	9.71	10.49	9.59	No
A_52_P20357	Aqr	11834	NM_009702	10.11	10.78	10.17	10.58	No
A_52_P195018	Arap3	106952	NM_139206	5.65	4.50	4.84	4.27	No
A_52_P482897	Areg	11839	NM_009704	4.36	6.06	4.10	6.94	No
A_52_P147992	Arglu1	234023	NM_176849	4.77	5.51	4.66	5.12	No
A_52_P527106	Arhgap12	75415	NM_001039692	10.88	9.75	10.49	9.99	No
A_51_P438165	Arhgap22	239027	NM_153800	11.77	12.85	12.23	13.96	Yes
A_52_P105151	Arhgap39	223666	NM_198420	7.10	8.58	7.58	8.61	No
A_51_P267904	Arhgap42	71544	NM_027823	6.09	5.42	6.38	6.14	No
A_51_P172085	Arhgdig	14570	NM_008113	6.08	9.47	7.24	9.50	No
A_52_P50325	Arhgdig	14570	NM_008113	5.54	8.36	6.16	8.43	No
A_52_P647900	Arhgef2	16800	NM_008487	10.83	11.54	10.99	11.28	No
A_51_P451516	Arhgef25	52666	NM_028027	16.09	15.43	16.00	15.52	No
A_51_P188772	Arhgef28	110596	NM_012026	11.61	10.53	11.52	10.75	No
A_51_P380337	Arhgef3	71704	NM_027871	7.03	7.80	7.28	7.19	Yes
A_52_P290020	Arhgef6	73341	NM_152801	3.40	7.00	2.92	5.41	Yes
A_51_P159565	Arhgef9	236915	NM_001033329	8.48	7.78	8.74	7.99	No
A_51_P485828	Arid3a	13496	NM_007880	9.11	10.48	9.01	10.05	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P482990	Arid5a	214855	NM_145996	5.74	8.69	5.65	7.75	Yes
A_52_P350512	Arid5a	214855	NM_145996	5.62	8.45	5.91	8.03	No
A_52_P669843	Arih2	23807	NM_011790	6.56	7.25	6.91	7.18	No
A_52_P155849	Arl4a	11861	NM_001039515	7.75	8.38	8.05	8.22	No
A_52_P86704	Arl4a	11861	NM_001039515	5.70	6.48	5.73	6.01	No
A_51_P169409	Arl5a	75423	NM_182994	8.85	9.63	8.32	8.73	Yes
A_51_P259902	Arl6ip5	65106	NM_022992	14.56	16.00	14.77	15.82	No
A_52_P38208	Arpp21	74100	NM_033264	2.90	6.58	4.30	4.78	Yes
A_52_P582732	Arpp21	74100	NM_033264	2.50	7.13	2.55	5.68	No
A_52_P188018	Arrdc1	215705	NM_178408	8.70	7.95	8.68	8.28	No
A_51_P383524	Art3	109979	NM_181728	2.56	8.07	2.51	6.32	Yes
A_52_P176405	Arx	11878	NM_007492	2.15	7.68	2.52	6.72	No
A_52_P105509	Asah2	54447	NM_018830	7.80	7.18	8.13	7.91	Yes
A_51_P435239	Asap1	13196	NM_010026	12.08	11.44	12.15	11.64	No
A_51_P502203	Asap3	230837	NM_001008232	8.44	7.77	8.58	8.09	No
A_51_P115715	Asb2	65256	NM_023049	3.13	10.21	1.85	8.98	Yes
A_51_P170827	Asb5	76294	NM_029569	4.88	7.50	4.71	7.50	No
A_51_P247963	Asb9	69299	NM_027027	7.70	6.82	6.58	6.25	Yes
A_52_P672399	Ascc1	69090	NM_026937	7.01	6.35	6.81	6.54	No
A_52_P209832	Asic1	11419	NM_009597	8.01	6.98	9.14	8.17	No
A_51_P259975	Aspa	11484	NM_023113	7.71	8.56	7.91	8.58	No
A_52_P625171	Asph	65973	NM_023066	7.08	9.08	6.97	7.75	Yes
A_51_P338713	Ass1	11898	M31690	9.53	10.47	9.74	11.09	Yes
A_52_P361165	Ass1	11898	M31690	9.71	10.46	9.98	11.17	No
A_51_P269216	Atf5	107503	NM_030693	15.87	14.79	16.04	15.14	No
A_52_P464346	Atf6	226641	NM_001081304	9.42	10.31	9.60	10.16	No
A_51_P129108	Atf6	226641	NM_001081304	9.69	10.65	9.55	10.32	No
A_51_P376789	Athl1	212974	NM_145387	10.94	10.34	11.02	10.63	No
A_51_P493012	Atl1	73991	NM_178628	5.89	5.00	5.60	5.12	No
A_52_P526396	Atl1	73991	NM_178628	7.60	6.80	7.67	7.00	No
A_52_P669510	Atp11a	50770	NM_015804	6.43	7.29	7.08	7.81	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P215815	Atp11c	320940	NM_001037863	7.20	6.19	7.20	6.91	Yes
A_51_P300817	Atp1a1	11928	NM_144900	15.08	16.06	15.34	15.63	No
A_52_P515497	Atp1a1	11928	NM_144900	14.85	15.76	15.21	15.54	No
A_52_P227267	Atp1a2	98660	NM_178405	3.78	6.71	2.91	5.11	No
A_51_P151484	Atp1b1	11931	NM_009721	11.53	10.90	12.04	11.70	No
A_52_P572950	Atp1b3	11933	NM_007502	8.53	9.47	8.77	8.96	No
A_51_P520384	Atp1b3	11933	NM_007502	12.60	13.46	12.77	13.28	No
A_51_P520378	Atp1b3	11933	NM_007502	12.08	13.03	12.22	12.53	No
A_52_P686130	Atp2a1	11937	NM_007504	1.96	10.59	2.08	8.72	Yes
A_51_P244856	Atp2a1	11937	NM_007504	5.53	9.54	5.46	7.85	Yes
A_51_P451075	Atp2a2	11938	NM_009722	9.64	11.11	9.52	10.34	Yes
A_52_P168097	Atp2a2	11938	NM_009722	7.03	7.89	6.95	7.43	No
A_51_P156526	Atp2b3	320707	XM_006528086	2.43	6.12	3.20	4.82	Yes
A_51_P294849	Atp5g3	228033	NM_175015	16.41	17.09	16.50	16.82	No
A_52_P591542	Atp8b2	54667	NM_001081182	12.29	11.53	12.51	12.05	No
A_51_P382524	Atp8b2	54667	NM_001081182	5.90	5.08	5.88	5.42	No
A_51_P118664	Atrx	22589	NM_009530	6.76	6.12	6.67	6.52	No
A_52_P607514	Atxn7l1	380753	NM_001033436	4.03	4.71	4.05	4.27	No
A_51_P204261	Atxn7l3	217218	NM_001098836	8.76	7.83	8.57	7.91	No
A_51_P474151	Awat2	245532	NM_177746	3.67	8.54	3.98	7.02	Yes
A_52_P54176	Axin2	12006	NM_015732	2.83	5.73	4.14	6.24	No
A_52_P24631	Azin1	54375	NM_018745	10.15	11.04	10.32	10.63	No
A_52_P628521	B130052P14Rik	105442	AK045264	5.73	6.38	5.38	5.47	Yes
A_51_P424954	B230114P17Rik	402735	AK045415	5.19	3.68	4.99	4.46	No
A_52_P504268	B3galnt1	26879	NM_020026	12.12	11.40	12.15	11.68	No
A_51_P167105	B3galt4	54218	NM_019420	7.71	6.98	7.68	7.06	No
A_51_P301435	B3galt5	93961	NM_033149	2.41	7.73	2.87	6.88	No
A_51_P481221	Bace2	56175	NM_019517	5.68	7.28	5.80	6.43	No
A_51_P376934	Bace2	56175	NM_019517	6.10	7.50	6.11	7.18	No
A_52_P38436	Baiap2	108100	NM_001037754	9.37	8.65	9.47	8.98	No
A_52_P290202	Baiap2	108100	NM_001037754	9.77	9.06	9.99	9.53	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P195374	Baiap211	66898	NM_025833	5.31	9.86	4.71	8.83	Yes
A_52_P138096	Baiap211	66898	NM_025833	2.67	6.41	2.75	5.48	No
A_51_P248819	Bambi	68010	NM_026505	9.62	8.82	9.59	9.10	No
A_51_P248819	Bambi	68010	NM_026505	9.61	8.93	9.57	9.17	No
A_51_P248819	Bambi	68010	NM_026505	9.59	8.91	9.53	9.08	No
A_51_P248819	Bambi	68010	NM_026505	9.58	8.91	9.51	9.19	No
A_51_P248819	Bambi	68010	NM_026505	9.65	9.00	9.66	9.29	No
A_51_P248819	Bambi	68010	NM_026505	9.62	9.01	9.65	9.25	No
A_51_P248819	Bambi	68010	NM_026505	9.66	9.05	9.67	9.25	No
A_51_P248819	Bambi	68010	NM_026505	9.65	9.05	9.70	9.29	No
A_51_P248819	Bambi	68010	NM_026505	9.64	9.04	9.68	9.27	No
A_52_P26794	Baspl	70350	NM_027395	8.63	7.96	8.01	7.56	No
A_52_P1061446	Baz1a	217578	NM_013815	7.72	9.43	7.74	8.44	Yes
A_51_P280761	Baz1a	217578	NM_013815	11.12	12.49	10.72	11.78	Yes
A_51_P374651	Baz1a	217578	NM_013815	8.13	9.27	8.25	9.15	No
A_51_P248122	Bbc3	170770	NM_133234	12.25	11.55	12.30	11.80	No
A_52_P606638	Bbip1	100503572	BC037041	8.16	7.19	7.87	7.64	No
A_52_P674196	Bbs9	319845	NM_181316	10.51	9.75	10.46	10.01	No
A_51_P253019	BC010981	407830	AK171523	10.27	11.24	9.82	10.88	No
A_51_P253084	BC022960	237246	XM_006529013	7.17	6.07	7.29	6.37	No
A_51_P174407	BC028528	229600	XM_006501376	9.25	8.61	9.25	8.95	No
A_52_P561690	BC034090	207792	XM_148974	3.63	6.38	2.87	6.19	No
A_51_P164255	BC051019	57355	NM_001040700	2.38	7.06	2.53	7.16	No
A_51_P173601	Bcam	57278	NM_020486	3.11	4.70	3.80	3.12	No
A_52_P448266	Bcat2	12036	NM_009737	9.23	8.64	9.44	9.05	No
A_52_P161495	Bcl6	12053	NM_009744	12.18	13.33	12.31	13.22	No
A_51_P155997	Bcl7a	77045	NM_029850	8.59	10.45	8.20	9.59	Yes
A_52_P629625	Bend6	320705	AK045534	9.41	8.71	9.61	8.82	No
A_51_P508781	Best1	24115	NM_011913	5.66	8.53	5.32	7.37	No
A_51_P279154	Bex1	19716	NM_009052	3.32	8.20	2.69	7.33	No
A_51_P323081	Bhlhb9	70237	NM_198161	8.57	9.39	8.27	8.77	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P272553	Bhlhe40	20893	NM_011498	8.28	9.02	8.40	9.28	No
A_51_P451176	Bhlhe41	79362	NM_024469	4.49	6.28	4.51	5.84	No
A_51_P249627	Bicd2	76895	NM_029791	10.20	11.47	10.25	11.05	No
A_52_P297398	Bicd2	76895	NM_029791	12.52	13.59	12.53	13.46	No
A_51_P350513	Bin1	30948	NM_009668	13.67	15.32	13.84	14.88	No
A_52_P384956	Bin1	30948	NM_009668	8.51	10.53	8.90	9.99	No
A_51_P104670	Bop1	12181	NM_013481	11.57	12.69	11.62	12.38	No
A_52_P238846	Bpnt1	23827	NM_011794	9.28	10.27	8.89	9.82	No
A_51_P178575	Brd3	67382	AF269193	12.06	12.75	11.98	12.28	No
A_51_P421559	Bspry	192120	NM_138653	4.85	8.68	5.00	7.05	Yes
A_51_P217509	Btbd11	74007	NM_028709	7.05	8.21	7.69	8.19	No
A_52_P381553	Btbd17	72014	NM_028055	3.78	11.96	4.42	10.22	Yes
A_51_P251293	Btbd3	228662	NM_145534	6.35	5.54	6.39	5.74	No
A_52_P672689	Btc	12223	NM_007568	5.29	3.21	6.43	5.18	Yes
A_52_P436258	Btg1	12226	NM_007569	9.10	9.88	9.44	9.32	No
A_51_P402359	Btla	208154	NM_001037719	2.39	5.54	2.86	3.25	Yes
A_52_P337126	Bves	23828	NM_024285	9.86	11.42	9.44	10.78	No
A_52_P269396	Bysl	53414	NM_016859	9.08	10.10	9.37	9.82	No
A_52_P131836	Bysl	53414	NM_016859	11.92	12.81	12.17	12.60	No
A_51_P503993	Bzrap1	207777	NM_172449	7.21	6.37	6.83	6.54	No
A_52_P174872	C030015A19Rik	77418	AK028820	8.04	7.32	8.12	7.93	Yes
A_52_P341733	C130013H08Rik	100327264	AK081392	4.34	5.96	4.05	5.59	No
A_52_P371135	C130050O18Rik	319772	NM_177000	6.14	5.36	5.66	5.11	No
A_51_P503303	C1galt1c1	59048	NM_021550	12.09	12.75	12.09	12.61	No
A_51_P466162	C1qbp	12261	NM_007573	14.00	14.73	14.10	14.67	No
A_52_P385546	C1ql3	227580	NM_153155	5.37	3.68	5.75	4.57	No
A_52_P588633	C1qtnf3	81799	NM_030888	3.95	8.99	3.36	6.51	Yes
A_51_P425071	C1qtnf3	81799	NM_030888	3.74	7.78	4.60	5.49	Yes
A_52_P584458	C230091D08Rik	330544	AK140886	8.14	8.84	7.93	8.42	No
A_51_P368743	C630043F03Rik	68285	NR_027923	6.32	8.17	6.94	7.62	No
A_51_P155550	C78339	97863	XM_006516985	12.11	12.91	12.15	12.36	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P1064707	C79468	97294	CF745569	8.72	9.96	9.05	9.91	No
A_52_P370225	C8g	69379	NM_027062	8.18	7.29	8.21	7.65	No
A_51_P444565	C920025E04Rik	667803	NM_001271005	12.67	11.81	12.93	12.58	No
A_51_P200339	Cachd1	320508	AK046180	9.47	8.63	9.91	9.52	Yes
A_51_P103541	Cacna1s	12292	NM_001081023	6.16	10.46	5.93	8.74	Yes
A_51_P478138	Cacnb1	12295	NM_031173	9.08	10.32	9.06	9.61	Yes
A_51_P210286	Cacng1	12299	NM_007582	2.22	10.34	4.20	8.87	No
A_51_P324524	Calcoco2	76815	NM_001271018	5.41	4.65	5.26	4.94	No
A_51_P225186	Calclrl	54598	NM_018782	13.37	12.68	13.02	12.57	No
A_52_P367760	Calml4	75600	NM_138304	12.69	11.50	12.25	11.37	No
A_52_P189707	Camk2a	12322	NM_177407	6.97	9.23	5.95	8.21	Yes
A_51_P208330	Camkk1	55984	NM_018883	11.15	12.08	11.58	12.15	No
A_51_P314045	Camsap3	69697	NM_027171	6.84	5.80	6.70	5.92	No
A_51_P141071	Cand2	67088	NM_025958	7.77	9.93	7.15	8.66	Yes
A_52_P501589	Cand2	67088	NM_025958	6.52	8.33	6.03	7.26	Yes
A_52_P148069	Cap2	67252	NM_026056	2.29	7.15	2.83	5.82	No
A_51_P273921	Capg	12332	NM_007599	14.07	13.34	14.24	13.87	No
A_51_P480013	Car11	12348	NM_009800	7.59	6.89	7.48	7.22	No
A_51_P153765	Car13	71934	NM_024495	13.04	12.33	13.14	12.79	No
A_51_P455647	Car2	12349	NM_009801	4.95	8.29	5.48	7.96	No
A_51_P431329	Car3	12350	NM_007606	4.50	10.94	4.00	9.59	Yes
A_52_P310511	Car6	12353	NM_009802	5.10	3.66	6.48	4.62	Yes
A_51_P174696	Casq2	12373	NM_009814	3.71	10.64	3.66	9.14	Yes
A_51_P519295	Cast	12380	NM_009817	12.15	11.38	12.25	11.68	No
A_52_P548913	Cast	12380	NM_009817	8.16	7.32	8.06	7.20	No
A_52_P638903	Cast	12380	NM_009817	11.26	10.33	11.28	10.93	No
A_51_P480290	Cas2l	69743	NM_027195	4.53	9.97	4.26	8.18	Yes
A_52_P306845	Cav1	12389	NM_007616	13.41	12.79	13.26	13.05	No
A_51_P279552	Cav2	12390	NM_016900	11.17	10.21	11.45	11.09	Yes
A_51_P279552	Cav2	12390	NM_016900	11.02	10.24	11.48	11.11	Yes
A_51_P279552	Cav2	12390	NM_016900	11.23	10.23	11.47	11.09	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P279552	Cav2	12390	NM_016900	11.21	10.29	11.48	11.13	Yes
A_51_P279552	Cav2	12390	NM_016900	11.24	10.23	11.47	11.04	Yes
A_51_P279552	Cav2	12390	NM_016900	10.95	10.13	11.38	10.94	Yes
A_51_P279552	Cav2	12390	NM_016900	11.12	10.34	11.49	11.13	Yes
A_51_P279552	Cav2	12390	NM_016900	11.14	10.15	11.34	10.93	Yes
A_51_P279552	Cav2	12390	NM_016900	11.15	10.16	11.38	10.93	Yes
A_51_P279552	Cav2	12390	NM_016900	11.23	10.20	11.43	10.96	Yes
A_51_P255304	Cav3	12391	NM_007617	2.00	8.54	2.36	6.49	No
A_52_P13802	Cbfa2t3	12398	NM_009824	2.32	8.86	2.66	7.34	Yes
A_51_P380750	Cbfa2t3	12398	NM_009824	2.24	9.72	2.31	8.30	Yes
A_51_P238425	Cbr2	12409	NM_007621	16.21	15.62	15.56	15.01	Yes
A_52_P676573	Cbx5	12419	NM_007626	10.65	11.53	10.22	10.82	No
A_51_P221428	Cbx6	494448	NM_028763	12.80	11.99	12.93	12.50	No
A_52_P89273	Cbx8	30951	XM_006533519	10.85	10.23	10.88	10.46	No
A_52_P170945	Ccdc106	232821	NM_146178	7.01	6.34	6.85	6.32	No
A_51_P317031	Ccdc109b	66815	NM_025779	8.65	7.54	8.72	8.23	No
A_51_P159610	Ccdc120	54648	NM_207202	7.17	6.38	7.36	6.96	No
A_51_P430033	Ccdc13	100502861	NM_028384	5.21	3.15	5.68	4.55	No
A_51_P193395	Ccdc134	76457	NM_172428	9.73	11.95	9.59	11.20	No
A_51_P135732	Ccdc136	232664	NM_001201378	11.09	10.47	11.17	10.83	No
A_52_P623211	Ccdc141	545428	NM_001025576	5.06	9.57	5.73	8.65	Yes
A_51_P132170	Ccdc141	545428	NM_001025576	3.55	6.51	3.75	5.76	No
A_52_P347401	Ccdc155	384619	NM_201374	5.97	6.62	5.73	6.31	No
A_51_P378932	Ccdc159	67119	NM_025977	8.93	8.24	9.09	8.59	No
A_51_P378934	Ccdc159	67119	NM_025977	9.89	9.19	10.04	9.41	No
A_51_P426724	Ccdc17	622665	NM_001037916	7.55	6.50	7.76	6.82	No
A_51_P303371	Ccdc23	69216	NM_024462	14.67	16.47	14.45	15.62	Yes
A_52_P57686	Ccdc28a	215814	NM_144820	10.67	10.03	10.56	10.23	No
A_51_P174720	Ccdc43	52715	NM_025918	8.59	9.24	8.65	8.97	No
A_52_P181605	Ccdc50	67501	NM_001289436	8.40	7.60	8.37	8.28	Yes
A_51_P108901	Ccdc86	108673	NM_023731	11.10	12.35	11.13	12.00	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P1171934	Ccdc88a	108686	AK042672	6.25	5.43	5.95	5.73	No
A_52_P322141	Ccdc88b	78317	NM_001081291	6.68	8.05	6.29	6.72	Yes
A_51_P468362	Ccdc88c	68339	XM_006516187	4.57	10.46	4.53	9.60	Yes
A_52_P315988	Ccdc88c	68339	XM_006516187	2.24	6.78	3.40	5.49	No
A_52_P665242	Ccdc90b	66365	NM_025515	11.00	10.42	10.90	10.41	No
A_52_P18116	Ccl24	56221	NM_019577	2.34	6.58	2.29	4.47	No
A_51_P460954	Ccl6	20305	NM_009139	5.88	5.03	4.99	4.31	Yes
A_52_P514391	Ccnd2	12444	NM_009829	13.42	12.62	12.94	12.47	No
A_51_P238448	Ccnd3	12445	NM_007632	13.41	14.36	13.52	13.99	No
A_51_P360165	Ccng2	12452	NM_007635	13.31	12.60	12.83	12.69	No
A_52_P625745	Ccnl2	56036	NM_207678	6.27	7.02	6.15	6.42	Yes
A_51_P520139	Ccpgl1os	546143	NM_001198789	10.17	9.41	10.07	9.77	No
A_51_P189777	Ccrn4l	12457	NM_009834	8.70	10.02	8.54	10.10	No
A_51_P172853	Cd14	12475	NM_009841	9.20	8.49	9.59	9.28	Yes
A_51_P333780	Cd164	53599	NM_016898	13.03	14.51	12.98	13.68	Yes
A_52_P482983	Cd164	53599	NM_016898	14.66	16.01	14.62	15.31	Yes
A_52_P220090	Cd164	53599	NM_016898	8.69	10.28	8.71	9.60	Yes
A_51_P517430	Cd1d1	12479	NM_007639	12.05	11.28	11.92	11.47	No
A_52_P5454	Cd248	70445	NM_054042	13.17	12.28	13.20	12.46	No
A_52_P244193	Cd24a	12484	NM_009846	10.39	9.08	9.00	8.28	Yes
A_51_P375138	Cd36	12491	L23108	2.48	5.61	1.99	4.14	No
A_51_P317443	Cd3eap	70333	NM_145822	9.81	10.47	9.89	10.20	No
A_52_P166435	Cd47	16423	NM_010581	11.62	10.94	11.32	10.95	No
A_51_P200535	Cd47	16423	NM_010581	10.04	9.24	9.82	9.41	No
A_51_P393934	Cd82	12521	NM_007656	11.48	14.77	11.21	14.12	No
A_51_P238722	Cd93	17064	NM_010740	4.77	6.11	3.62	5.00	Yes
A_51_P366542	Cd97	26364	NM_011925	12.21	12.86	12.23	13.05	No
A_52_P469502	Cda	72269	NM_028176	9.63	8.57	9.84	8.90	No
A_52_P105765	Cdc42bpg	240505	NM_001033342	4.49	3.72	4.50	4.31	No
A_52_P340700	Cdc42se1	57912	NM_172395	10.48	11.17	10.60	10.86	No
A_52_P378372	Cdc42se2	72729	NM_178626	4.61	5.38	4.79	5.07	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P340013	Cdc42se2	72729	NM_178626	8.91	9.58	9.04	9.47	No
A_51_P481930	Cdh15	12555	NM_007662	5.18	14.03	4.65	13.19	No
A_52_P630905	Cdh18	320865	NM_001081299	6.33	5.52	6.69	6.21	Yes
A_52_P416341	Cdh23	22295	NM_023370	3.29	5.15	3.37	5.21	No
A_52_P371129	Cdk5r1	12569	NM_009871	7.11	11.82	6.84	10.48	Yes
A_51_P236412	Cdkl2	53886	NM_016912	9.75	9.15	9.69	9.17	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.08	14.62	13.38	14.29	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.14	14.63	13.42	14.29	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.26	14.72	13.42	14.36	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.28	14.73	13.42	14.34	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.23	14.60	13.37	14.26	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.23	14.60	13.35	14.31	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.28	14.60	13.37	14.30	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.14	14.44	13.39	14.37	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.37	14.51	13.42	14.32	No
A_51_P363947	Cdkn1a	12575	NM_007669	13.19	14.30	13.32	14.27	No
A_51_P144813	Cdkn2a	12578	NM_009877	9.26	8.36	8.87	8.58	No
A_51_P144813	Cdkn2a	12578	NM_009877	9.30	8.62	8.95	8.67	No
A_51_P144813	Cdkn2a	12578	NM_009877	9.30	8.68	9.01	8.76	No
A_52_P498241	Cdkn2b	12579	NM_007670	12.93	12.17	13.16	12.54	No
A_51_P479645	Cdnf	227526	NM_177647	7.65	7.00	7.81	6.96	No
A_52_P569998	Cdsn	386463	NM_001008424	10.76	10.13	11.72	11.12	Yes
A_51_P444447	Cebpd	12609	NM_007679	13.79	13.17	13.93	13.85	Yes
A_51_P204845	Celf1	13046	NM_017368	11.66	12.99	11.13	12.04	Yes
A_52_P243542	Celf2	14007	NM_001110229	6.95	9.39	6.24	8.67	Yes
A_51_P182471	Celf2	14007	NM_001110229	11.87	13.65	11.72	13.19	No
A_52_P499551	Celf2	14007	NM_001110229	4.01	6.03	4.20	5.16	No
A_52_P100909	Celf2	14007	NM_001110229	7.98	9.70	8.04	9.37	No
A_51_P125607	Celf5	319586	NM_176954	6.00	5.33	5.89	5.05	No
A_51_P456114	Cemip	80982	AK018112	9.35	10.13	8.20	9.04	Yes
A_52_P31011	Cep162	382090	NM_199316	9.47	8.54	9.34	8.98	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P161037	Cep170	545389	BC054781	10.10	9.33	10.21	9.68	No
A_51_P212271	Cep41	83922	NM_031998	8.73	9.52	8.67	9.08	No
A_52_P559545	Cercam	99151	NM_207298	5.15	4.14	5.55	4.81	No
A_51_P296528	Cers4	67260	NM_026058	10.64	10.04	10.69	10.20	No
A_52_P309381	Cfh	12628	M12660	13.15	12.38	12.68	12.25	No
A_52_P183598	Cfhr2	545366	NM_001025575	7.31	6.08	6.69	5.90	No
A_51_P302149	Cfl2	12632	NM_007688	10.40	11.37	9.03	10.22	Yes
A_51_P254234	Chchd4	72170	NM_133928	9.82	11.23	9.93	10.89	No
A_52_P476902	Chd6	71389	NM_173368	5.23	4.44	5.04	4.63	No
A_52_P84315	Chd7	320790	NM_001277149	6.95	11.18	6.51	9.88	Yes
A_51_P507307	Chd7	320790	NM_001277149	2.61	5.92	1.85	4.64	Yes
A_52_P70035	Chd9	109151	NM_177224	5.70	5.02	5.65	5.20	No
A_51_P362066	Chil1	12654	NM_007695	2.49	5.50	4.10	5.44	No
A_51_P142923	Chka	12660	BC056758	7.69	8.60	7.88	9.12	No
A_51_P335758	Chn1	108699	NM_029716	3.36	6.08	4.20	5.86	No
A_52_P118310	Chn1	108699	NM_029716	5.95	7.89	5.73	7.52	No
A_51_P505571	Chrdl2	69121	NM_133709	2.17	8.14	2.64	6.98	Yes
A_52_P189725	Chrm1	11435	NM_007389	2.64	11.63	2.69	10.03	Yes
A_52_P533707	Chrm1	11435	NM_007389	3.21	13.97	3.28	12.51	Yes
A_52_P213550	Chrm5	110835	NM_176844	6.66	5.37	7.03	5.90	No
A_51_P475342	Chrmb1	11443	NM_009601	5.06	10.00	5.07	9.33	No
A_51_P450929	Chrmd	11447	NM_021600	6.34	10.38	5.94	9.24	No
A_51_P288009	Chrmg	11449	NM_009604	2.91	15.03	2.49	13.73	Yes
A_51_P288010	Chrmg	11449	NM_009604	4.11	9.81	3.50	8.49	No
A_51_P464023	Chst12	59031	NM_021528	11.81	11.05	12.07	11.54	No
A_52_P176409	Chst12	59031	NM_021528	12.32	11.51	12.47	11.82	No
A_51_P465128	Chst15	77590	NM_029935	7.00	8.40	6.95	7.65	Yes
A_51_P277006	Chst8	68947	NM_175140	4.59	8.68	4.99	6.04	Yes
A_52_P512365	Cideb	12684	NM_009894	4.21	3.01	4.15	3.42	No
A_51_P512969	Cilp2	68709	NM_026818	4.84	7.50	4.53	6.05	Yes
A_51_P138152	Cisd1	52637	NM_134007	13.88	14.48	13.97	14.29	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P425772	Ckb	12709	NM_021273	10.44	12.78	9.76	11.54	Yes
A_52_P385606	Ckb	12709	NM_021273	8.62	11.03	7.91	9.78	No
A_51_P380807	Ckm	12715	NM_007710	3.39	10.20	2.86	8.65	No
A_52_P151974	Clcn5	12728	XM_006527563	11.20	13.13	11.05	12.96	No
A_51_P506236	Clcn5	12728	XM_006527563	7.08	9.08	6.71	8.66	No
A_51_P171332	Cldn19	242653	NM_001038590	7.40	5.42	7.04	5.76	No
A_52_P23661	Cldn19	242653	NM_001038590	6.84	5.33	6.94	5.64	No
A_51_P354272	Cluh	74148	NM_001081158	11.84	13.53	12.19	13.02	No
A_52_P156452	Cmah	12763	NM_007717	3.75	6.30	3.52	5.89	No
A_51_P438924	Cmtm5	67272	NM_026066	3.54	5.86	5.64	5.07	No
A_52_P375931	Cmtr2	234728	NM_146215	6.65	7.71	7.22	7.57	No
A_52_P74653	Cnnm3	94218	NM_001039551	7.14	8.00	7.60	7.56	No
A_52_P436464	Cnot6l	231464	NM_144910	10.72	12.61	10.70	11.96	Yes
A_51_P208031	Cnot6l	231464	NM_144910	7.25	9.04	7.22	8.37	No
A_51_P403378	Cnr1	12801	NM_007726	5.13	8.91	5.42	7.19	Yes
A_52_P196138	Cntrl	26920	NM_001290635	7.44	6.64	7.02	6.72	No
A_52_P451485	Cobl	12808	NM_172496	4.86	6.63	5.04	5.85	Yes
A_51_P358344	Col17a1	12821	NM_007732	7.55	6.68	7.41	6.91	No
A_51_P435380	Col26a1	140709	NM_024474	5.30	10.17	4.83	9.77	No
A_52_P216436	Col2a1	12824	NM_031163	13.03	12.14	13.25	12.29	No
A_51_P409010	Comp	12845	NM_016685	7.38	6.17	6.93	6.21	No
A_51_P255765	Coq10b	67876	NM_001039710	8.86	9.47	8.79	9.08	No
A_52_P366525	Coq10b	67876	NM_001039710	9.79	10.41	9.78	10.37	No
A_51_P300277	Coro1a	12721	NM_009898	6.70	5.94	6.66	6.32	No
A_52_P538995	Coro2b	235431	NM_175484	6.91	8.44	6.99	8.03	No
A_51_P509997	Cox6a2	12862	NM_009943	8.41	10.30	8.98	9.05	Yes
A_52_P423814	Cox8b	12869	NM_007751	2.50	11.23	2.61	9.61	Yes
A_52_P161237	Cpa1	109697	NM_025350	9.08	10.59	8.05	9.19	Yes
A_52_P158376	Cpa4	71791	NM_027926	4.50	7.19	4.25	6.76	No
A_52_P462296	Cpeb1	12877	NM_007755	7.73	7.14	8.34	8.04	No
A_52_P535212	Cpeb3	208922	NM_198300	7.65	9.35	7.77	8.46	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P261059	Cpne2	234577	NM_153507	10.65	10.00	10.24	9.91	No
A_51_P427563	Cpne8	66871	NM_025815	9.89	9.10	10.17	9.62	No
A_52_P383653	Cpne8	66871	NM_025815	8.69	7.60	8.86	8.09	No
A_51_P521283	Crabp2	12904	NM_007759	6.40	8.61	6.07	7.70	Yes
A_51_P242265	Cradd	12905	NM_009950	10.89	10.30	10.51	10.27	No
A_52_P343856	Creb3l2	208647	XM_006505824	10.41	9.49	10.18	9.73	No
A_51_P500643	Creb3l2	208647	XM_006505824	13.25	12.54	13.12	12.68	No
A_51_P497882	Creb3l4	78284	NM_030080	7.48	6.64	7.41	7.03	No
A_51_P499979	Crebl2	232430	BF535186	7.10	6.16	7.35	6.90	No
A_51_P496640	Crim1	50766	NM_015800	9.98	8.88	10.16	9.25	No
A_52_P46447	Crim1	50766	NM_015800	12.03	11.05	11.97	11.13	No
A_51_P314397	Crip2	68337	NM_024223	13.36	14.64	13.19	13.92	Yes
A_52_P106482	Cryab	12955	NM_009964	12.66	14.08	12.55	13.23	Yes
A_51_P504423	Cryab	12955	NM_009964	9.71	10.65	9.56	10.27	No
A_51_P183561	Csdc2	105859	NM_145473	5.16	6.05	5.94	5.69	No
A_51_P248705	Cse1l	110750	NM_023565	11.25	11.92	11.22	11.65	No
A_51_P341465	Csf2ra	12982	NM_009970	6.20	5.43	5.76	5.31	No
A_52_P199446	Csmd3	239420	NM_001081391	6.13	5.54	6.91	6.37	Yes
A_52_P278064	Cspg4	121021	NM_139001	13.26	12.32	13.50	12.47	No
A_51_P160824	Cspg4	121021	NM_139001	12.37	11.59	12.61	11.63	No
A_52_P437662	Cspg5	29873	NM_013884	4.90	6.77	4.89	6.26	No
A_52_P604629	Csrnp1	215418	NM_153287	8.61	9.41	8.55	9.46	No
A_51_P466448	Csrnp2	207785	NM_153407	6.72	7.36	6.42	7.03	No
A_51_P300337	Csrp2	13008	NM_007792	17.17	16.13	17.08	16.43	No
A_51_P133684	Csrp3	13009	NM_013808	2.86	7.05	2.72	6.33	No
A_51_P284426	Cstad	78617	NM_030137	10.64	9.61	10.46	9.72	No
A_52_P235442	Ctif	269037	NM_201354	9.18	9.85	9.33	9.72	No
A_51_P335849	Ctnnbip1	67087	NM_023465	12.82	12.23	12.83	12.45	No
A_51_P398887	Ctns	83429	NM_031251	11.98	11.38	11.37	11.09	No
A_51_P485542	Ctps2	55936	NM_018737	13.94	13.29	13.86	13.52	No
A_52_P264924	Ctsw	13041	NM_009985	4.81	6.22	4.19	6.57	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P106779	Cttnbp2	30785	NM_080285	6.79	5.49	7.25	6.37	No
A_51_P115953	Cttn3	629147	NM_001134697	3.14	7.60	3.23	6.15	Yes
A_51_P379409	Cuedc1	103841	NM_198013	10.00	9.32	9.98	9.62	No
A_51_P378789	Cxcl13	55985	NM_018866	3.10	7.24	2.58	7.31	No
A_52_P585124	Cxcr4	12767	NM_009911	2.59	8.19	3.44	7.46	No
A_52_P565353	Cxcr4	12767	NM_009911	2.65	6.05	2.89	5.52	No
A_51_P311434	Cyb5a	109672	NM_025797	13.68	12.92	13.77	13.19	No
A_51_P295610	Cyc1	66445	NM_025567	13.04	14.16	13.15	13.85	No
A_51_P261671	Cycs	13063	NM_007808	11.50	12.75	11.27	12.28	No
A_52_P82247	Cycs	13063	NM_007808	12.17	13.36	11.93	12.92	No
A_51_P329291	Cygb	114886	NM_030206	6.08	7.07	5.80	6.63	No
A_52_P134083	Cyhr1	54151	NM_001276321	12.00	11.34	11.87	11.55	No
A_51_P516097	Cyld	74256	NM_173369	10.22	11.37	10.11	11.13	No
A_51_P300001	Cyld	74256	NM_173369	8.14	8.98	8.13	8.81	No
A_51_P501844	Cyp26b1	232174	NM_175475	12.23	11.04	12.87	11.41	No
A_51_P352763	Cyp2b19	13090	NM_007814	6.72	6.09	6.87	6.69	No
A_52_P231292	Cyp2j6	13110	NM_010008	7.48	6.78	7.36	6.87	No
A_51_P210603	Cyp4f13	170716	NM_130882	10.07	9.32	10.10	9.72	No
A_52_P164161	Cyp51	13121	NM_020010	10.28	11.02	10.41	10.54	No
A_52_P350111	Cyth1	19157	NM_011180	8.99	10.05	8.82	9.17	Yes
A_51_P314701	Cyth1	19157	NM_011180	3.62	5.11	4.25	4.52	No
A_52_P102722	D030025P21Rik	100303738	NR_028577	5.61	6.44	6.19	6.68	No
A_52_P329164	D130020L05Rik	319760	NR_038050	7.68	6.96	7.96	7.47	No
A_51_P215143	D430006K04	328861	AK084893	7.26	6.50	7.31	6.37	No
A_52_P97699	D430019H16Rik	268595	NM_001252508	2.75	6.90	2.35	5.30	Yes
A_52_P619911	Dact2	240025	NM_172826	8.26	6.87	7.14	5.53	No
A_51_P126198	Dapk2	13143	NM_010019	5.24	10.09	4.88	10.31	No
A_51_P244824	Dapp1	26377	NM_011932	8.29	7.62	8.62	8.22	Yes
A_51_P359919	Dars	226414	NM_177445	11.12	11.77	11.22	11.62	No
A_51_P446232	Dbn1	56320	NM_019813	9.77	11.16	9.48	10.17	Yes
A_51_P497280	Dbndd1	72185	NM_028146	6.98	12.25	7.10	11.22	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P484289	Dbn1	72185	NM_028146	3.80	7.00	4.73	5.89	No
A_51_P170641	Dcaf4	73828	NM_030246	10.67	10.09	10.79	10.40	No
A_51_P370561	Dcald	68087	NM_026551	10.70	11.88	10.66	11.57	No
A_52_P3825	Ddb2	107986	NM_028119	7.77	6.99	7.79	7.31	No
A_52_P470150	Ddc	13195	NM_016672	5.29	9.91	4.40	8.58	Yes
A_51_P126702	Ddit4l	73284	NM_030143	10.09	9.33	10.33	9.71	No
A_51_P161582	Ddr1	12305	L57509	13.37	14.20	13.32	13.83	No
A_52_P424037	Ddx21	56200	NM_019553	7.99	9.23	8.59	9.31	No
A_52_P372032	Ddx21	56200	NM_019553	10.68	11.63	10.66	11.48	No
A_52_P279143	Ddx21	56200	NM_019553	8.20	9.25	8.21	8.57	No
A_52_P262801	Ddx26b	236790	NM_172779	4.12	2.82	5.09	4.91	Yes
A_51_P337662	Ddx26b	236790	NM_172779	8.94	8.33	8.98	8.92	No
A_51_P394154	Ddx51	69663	NM_027156	10.49	11.98	10.57	11.35	Yes
A_51_P357996	Defb3	27358	NM_013756	7.01	6.31	6.61	6.34	No
A_52_P350950	Dennd2c	329727	NM_177857	7.57	6.84	7.52	7.12	No
A_51_P445841	Deptor	97998	NM_145470	7.63	9.01	7.68	8.48	No
A_52_P661503	Deptor	97998	NM_145470	9.58	10.87	9.61	10.53	No
A_51_P335969	Des	13346	NM_010043	3.36	9.69	3.04	7.63	Yes
A_51_P335969	Des	13346	NM_010043	3.34	9.56	3.23	7.57	Yes
A_51_P335969	Des	13346	NM_010043	3.65	9.67	3.06	7.69	Yes
A_51_P335969	Des	13346	NM_010043	3.29	9.57	3.07	7.63	Yes
A_51_P335969	Des	13346	NM_010043	3.30	9.58	3.02	7.64	Yes
A_51_P335969	Des	13346	NM_010043	3.61	9.62	3.20	7.70	Yes
A_51_P335969	Des	13346	NM_010043	3.42	9.52	3.09	7.62	Yes
A_51_P335969	Des	13346	NM_010043	3.65	9.61	3.58	7.71	Yes
A_51_P335969	Des	13346	NM_010043	3.34	9.60	2.86	7.73	Yes
A_51_P335969	Des	13346	NM_010043	3.56	9.47	2.89	7.74	Yes
A_52_P582858	Dgkd	227333	NM_177646	11.47	12.27	11.43	11.99	No
A_51_P136411	Dgki	320127	NM_001081206	6.45	5.55	6.43	4.95	No
A_51_P505156	Dgkz	104418	NM_138306	8.90	9.78	8.90	9.60	No
A_51_P290986	Dhcr7	13360	NM_007856	11.93	12.68	12.06	12.08	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P535946	Dhcr7	13360	NM_007856	7.62	8.40	7.83	7.84	No
A_51_P312437	Dhrs7	66375	NM_025522	13.28	12.56	13.34	12.90	No
A_52_P290926	Dhrs7c	68460	NM_001013013	2.92	8.78	3.02	6.60	Yes
A_51_P188993	Dhrs7c	68460	NM_001013013	2.17	7.99	2.47	6.26	No
A_52_P572476	Dhrs9	241452	AK089247	7.92	6.76	7.39	6.47	No
A_51_P285997	Dhx32	101437	NM_133941	11.43	12.28	11.61	12.27	No
A_52_P286342	Dhx33	216877	NM_178367	6.09	6.87	6.49	6.79	No
A_51_P474188	Dhx57	106794	NM_198942	11.74	11.11	11.68	11.28	No
A_52_P223809	Dhx58	80861	NM_030150	7.18	6.28	7.02	6.35	No
A_52_P242251	Diexf	215193	NM_145415	7.74	9.03	7.99	8.82	No
A_51_P208603	Dio2	13371	NM_010050	3.20	5.70	3.17	6.10	No
A_51_P468876	Dixdc1	330938	BC024834	10.46	9.69	10.70	10.07	No
A_52_P406828	Dkc1	245474	NM_001030307	8.31	9.16	8.42	9.00	No
A_52_P374642	Dlat	235339	NM_145614	10.83	11.51	11.19	11.50	No
A_52_P600038	Dlgap4	228836	NM_146128	9.76	10.58	9.61	10.09	No
A_51_P306017	Dll1	13388	NM_007865	4.69	10.66	4.31	9.73	Yes
A_51_P306017	Dll1	13388	NM_007865	4.94	10.67	4.15	9.78	Yes
A_51_P306017	Dll1	13388	NM_007865	5.01	10.70	4.17	9.87	Yes
A_51_P306017	Dll1	13388	NM_007865	4.70	10.75	4.38	9.93	Yes
A_51_P306017	Dll1	13388	NM_007865	5.01	10.73	4.34	9.93	Yes
A_51_P306017	Dll1	13388	NM_007865	5.27	10.73	4.27	9.94	Yes
A_51_P306017	Dll1	13388	NM_007865	4.61	10.56	4.13	9.79	Yes
A_51_P306017	Dll1	13388	NM_007865	4.84	10.58	4.19	9.81	Yes
A_51_P306017	Dll1	13388	NM_007865	4.63	10.57	4.48	9.84	Yes
A_51_P306017	Dll1	13388	NM_007865	5.18	10.54	4.36	9.96	No
A_51_P264769	Dmp1	13406	NM_016779	4.80	7.02	5.39	8.35	Yes
A_51_P407213	Dmpk	13400	NM_032418	5.64	7.94	5.86	7.64	No
A_52_P643375	Dmpk	13400	NM_032418	10.04	12.52	10.22	12.34	No
A_52_P157673	Dmpk	13400	NM_032418	6.63	7.78	6.59	7.59	No
A_52_P512274	Dnah2	327954	NM_001081330	5.37	4.63	4.50	3.77	No
A_52_P592230	Dnaja4	58233	NM_021422	9.25	8.42	8.92	8.24	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P304264	Dnajb5	56323	NM_019874	5.88	7.14	5.48	6.45	No
A_52_P348704	Dnajb5	56323	NM_019874	6.33	8.39	6.20	7.83	No
A_51_P227112	Dnajb5	56323	NM_019874	6.30	7.95	6.07	7.19	No
A_51_P398235	Dnajc11	230935	NM_172704	9.22	9.85	9.32	9.64	No
A_51_P449956	Dnajc7	56354	NM_019795	12.14	12.76	12.02	12.49	No
A_51_P219868	Dnm1	13429	NM_010065	10.59	9.30	11.26	10.75	No
A_51_P173735	Dock11	75974	NM_001009947	11.02	11.78	11.06	11.78	No
A_52_P578790	Dok3	27261	NM_013739	3.91	6.54	3.93	5.25	Yes
A_51_P155458	Dok7	231134	XM_006503901	4.82	14.18	4.64	13.17	Yes
A_51_P322941	Dok7	231134	XM_006503901	2.66	9.16	3.18	8.28	Yes
A_51_P227345	Dpep1	13479	NM_007876	6.95	8.96	6.72	7.79	No
A_52_P591166	Dpysl2	12934	NM_009955	14.34	13.32	13.99	13.64	No
A_52_P92161	Drp2	13497	NM_010078	3.48	7.41	3.68	6.72	No
A_51_P189943	Dscam	13508	NM_031174	3.02	7.68	3.13	4.83	Yes
A_52_P39056	Dsel	319901	NM_001081316	5.56	4.63	5.52	4.99	No
A_52_P660693	Dsel	319901	NM_001081316	11.88	11.24	11.85	11.56	No
A_52_P572765	Dtna	13527	NM_207650	3.70	7.49	3.69	6.61	Yes
A_51_P184300	Dtna	13527	NM_207650	5.00	7.11	5.46	6.64	No
A_52_P108607	Dtna	13527	NM_207650	7.33	8.19	7.62	8.42	No
A_52_P558259	Dtna	13527	NM_207650	8.49	9.28	8.55	9.53	No
A_52_P523422	Dtnb	13528	NM_007886	9.80	9.17	9.99	9.70	No
A_51_P475138	Duox1	99439	NM_001099297	5.75	5.04	5.62	5.25	No
A_51_P475138	Duox1	99439	NM_001099297	5.65	5.04	5.43	5.46	No
A_52_P534361	Duoxa1	213696	NM_145395	7.71	11.36	6.38	10.58	No
A_51_P384243	Dusp13	27389	NM_001007268	2.75	6.44	2.67	5.95	No
A_52_P399934	Dusp2	13537	NM_010090	8.18	10.34	8.03	9.92	No
A_51_P235088	Dusp27	240892	NM_001033344	6.42	10.35	5.33	9.36	No
A_52_P486175	Dusp27	240892	NM_001033344	5.28	9.60	4.44	8.62	No
A_51_P347333	Dusp4	319520	AK012530	7.92	10.34	7.98	9.37	Yes
A_51_P518051	Dusp4	319520	AK012530	5.28	8.04	5.27	6.82	No
A_51_P114314	Dusp8	18218	NM_008748	8.84	8.16	8.82	8.38	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P188046	Dync1i1	13426	NM_010063	10.81	13.09	10.72	12.42	No
A_51_P227962	Dynlrb2	75465	NM_029297	5.38	4.72	5.15	4.63	No
A_51_P519108	Dzank1	241688	NM_172859	8.26	7.45	7.88	7.24	No
A_51_P105822	E230025N22Rik	240216	NM_172831	6.34	5.65	6.22	5.91	No
A_51_P368009	E2f2	242705	NM_177733	8.39	12.54	7.45	11.64	No
A_51_P157524	E2f4	104394	NM_148952	8.34	9.29	8.84	9.61	No
A_51_P431531	E2f8	108961	NM_001013368	8.38	9.11	7.93	8.54	No
A_52_P672722	E430018J23Rik	101604	BC010442	7.57	6.90	7.62	7.30	No
A_51_P386562	Ebf2	13592	NM_010095	6.47	5.64	6.11	5.29	No
A_52_P271572	Ece2	107522	NM_139293	8.43	7.67	8.78	8.28	No
A_51_P505472	Ece2	107522	NM_139293	7.95	7.04	8.04	7.51	No
A_51_P477414	Echdc2	52430	NM_026728	12.97	12.25	12.88	12.45	No
A_51_P405397	Ecm1	13601	NM_007899	15.10	14.45	15.69	15.11	Yes
A_51_P115005	Edn1	13614	NM_010104	8.18	7.24	7.83	7.38	No
A_51_P317191	Eepd1	67484	NM_026189	5.48	7.46	4.67	6.41	No
A_51_P160544	Efemp2	58859	NM_021474	14.75	14.16	14.78	14.36	No
A_52_P429308	Efhd2	27984	NM_025994	12.02	13.29	12.05	12.83	No
A_51_P266683	Efna1	13636	NM_010107	8.63	7.50	8.76	8.23	Yes
A_51_P380970	Efnb2	13642	NM_010111	8.12	6.59	7.70	6.73	No
A_52_P340073	Efnb2	13642	NM_010111	12.86	11.88	12.79	11.94	No
A_52_P586851	Efs	13644	NM_010112	5.74	6.82	5.17	5.85	Yes
A_51_P412914	Efs	13644	NM_010112	6.39	7.28	5.89	6.49	Yes
A_52_P38627	Egf	13645	NM_010113	3.85	5.94	4.42	5.82	No
A_52_P38627	Egf	13645	NM_010113	3.46	5.47	4.41	5.63	No
A_52_P38627	Egf	13645	NM_010113	3.81	5.67	5.30	5.75	No
A_52_P38627	Egf	13645	NM_010113	3.86	5.71	5.50	5.78	No
A_52_P38627	Egf	13645	NM_010113	3.62	5.45	4.23	5.64	No
A_52_P38627	Egf	13645	NM_010113	3.73	5.52	4.67	5.76	No
A_52_P38627	Egf	13645	NM_010113	3.94	5.73	4.38	5.73	No
A_52_P38627	Egf	13645	NM_010113	3.64	5.43	4.88	5.53	No
A_52_P38627	Egf	13645	NM_010113	4.20	5.62	4.64	5.71	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P56471	Egfr	13649	NM_207655	9.11	8.45	9.12	8.47	No
A_52_P440836	Egr3	13655	NM_001289925	6.00	7.96	6.52	8.32	No
A_51_P222475	Ehbp111	114601	NM_053252	12.42	13.27	12.47	13.07	No
A_51_P455807	Ehd4	98878	NM_133838	13.66	14.48	13.64	14.20	No
A_52_P190921	Eif2ak1	15467	AK032508	8.68	9.48	8.57	8.97	No
A_51_P385773	Eif2ak1	15467	AK032508	9.55	10.21	9.58	9.88	No
A_51_P253642	Eif2ak3	13666	AK041698	9.20	8.55	9.47	8.97	No
A_51_P211341	Eif3j1	78655	NM_144545	11.30	11.90	11.30	11.73	No
A_51_P266168	Eif4e2	26987	XM_006529670	13.17	13.80	13.37	13.72	No
A_52_P48155	Eif5a	276770	NM_181582	12.25	13.06	12.59	12.77	No
A_51_P211923	Eif5a2	208691	NM_177586	7.74	7.14	7.92	7.57	No
A_52_P429534	Elavl1	15568	NM_010485	8.65	9.63	8.37	9.01	Yes
A_52_P613688	Elmo1	140580	NM_198093	4.63	6.84	4.98	6.30	No
A_51_P398673	Elmsan1	238317	NM_001163502	8.85	9.67	8.74	9.08	Yes
A_52_P354682	Elov17	74559	NM_029001	10.74	9.44	11.20	10.40	Yes
A_51_P382849	Emb	13723	NM_010330	12.06	11.24	11.89	11.37	No
A_52_P10622	Emb	13723	NM_010330	9.23	8.25	9.30	8.74	No
A_52_P527800	Emilin2	246707	NM_145158	13.43	12.83	14.08	13.81	Yes
A_51_P203062	Enah	13800	NM_010135	10.78	11.89	10.55	11.20	Yes
A_52_P748882	Eno2	13807	NM_013509	7.82	7.18	7.89	7.70	No
A_51_P328539	Eno3	13808	NM_007933	10.69	14.51	10.07	13.95	No
A_51_P112932	Entpd2	12496	NM_009849	3.76	2.19	4.49	3.29	No
A_52_P674382	Epb4.113	13823	AK086340	4.06	6.15	4.38	5.60	No
A_52_P286098	Epb4.114b	54357	NM_019427	12.64	11.40	12.63	11.94	No
A_52_P180518	Epb4.115	226352	NM_145506	5.68	6.69	6.03	6.57	No
A_51_P280404	Epdr1	105298	NM_134065	10.41	9.55	11.27	10.77	Yes
A_52_P577388	Epdr1	105298	NM_134065	13.86	12.90	14.43	14.00	No
A_52_P504787	Epha7	13841	NM_001122889	9.78	9.07	9.88	9.14	No
A_52_P45596	Ephb4	13846	L25761	6.25	5.61	6.60	5.93	No
A_52_P542612	Ephb4	13846	L25761	10.41	9.69	10.31	9.90	No
A_51_P309850	Ephb6	13848	NM_007680	9.30	8.19	9.35	8.56	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P508289	Ephx1	13849	NM_010145	14.23	13.58	14.28	13.79	No
A_51_P188111	Eps8	13860	NM_007945	8.08	7.04	8.95	8.63	Yes
A_51_P188114	Eps8	13860	NM_007945	8.16	7.27	9.04	8.67	Yes
A_52_P37757	Eps8l1	67425	NM_026146	5.95	5.10	5.64	4.72	No
A_51_P225484	Epyc	13516	NM_007884	7.68	6.10	6.30	5.07	Yes
A_52_P303274	Epyc	13516	NM_007884	9.02	7.57	7.69	6.88	Yes
A_52_P432949	ErbB3	13867	BC049279	5.48	11.30	5.78	9.82	Yes
A_52_P39765	Erc1	111173	NM_178085	12.00	11.37	11.79	11.32	No
A_52_P598912	Erc1	111173	NM_178085	8.27	7.67	7.97	7.18	No
A_52_P471227	Ercc8	71991	NM_028042	4.93	5.74	5.25	5.40	No
A_51_P391716	Ermap	27028	NM_013848	2.66	7.28	3.07	5.31	Yes
A_52_P237077	Esr1	13982	NM_007956	2.09	5.20	3.73	4.73	No
A_52_P237077	Esr1	13982	NM_007956	2.37	5.25	3.03	5.35	No
A_52_P237077	Esr1	13982	NM_007956	2.61	5.39	3.15	4.97	No
A_52_P237077	Esr1	13982	NM_007956	2.59	5.24	3.54	4.64	No
A_52_P237077	Esr1	13982	NM_007956	2.79	5.42	3.52	4.94	No
A_51_P238183	Etnk2	214253	AK031665	5.04	4.21	5.07	3.95	No
A_52_P194250	Ets1	23871	NM_011808	9.27	8.47	8.92	8.63	No
A_52_P514488	Exoc3l	277978	NM_177788	3.91	6.50	4.05	5.78	No
A_52_P631499	Exoc6	107371	XM_006526591	9.02	10.10	8.88	9.83	No
A_51_P416243	Exosc9	50911	NM_019393	10.41	11.65	10.24	11.23	No
A_51_P480311	F2	14061	NM_010168	2.51	6.64	2.72	5.50	No
A_52_P811856	F420014N23Rik	100038591	NR_045715	7.88	7.11	7.84	7.66	No
A_52_P226348	F730043M19Rik	320046	AK089514	5.80	6.39	6.37	6.95	No
A_51_P386660	Faah	14073	NM_010173	7.44	6.34	7.64	6.62	No
A_52_P487615	Fam105a	223433	NM_198301	5.38	4.54	6.14	5.49	No
A_52_P87900	Fam107a	268709	NM_183187	6.58	5.56	7.86	6.34	No
A_51_P303180	Fam114a1	68303	NM_026667	11.96	11.28	12.37	11.84	No
A_52_P742248	Fam117b	72750	NM_001037725	12.15	11.53	11.55	11.31	No
A_51_P425824	Fam132a	67389	NM_026125	8.83	8.05	8.84	8.49	No
A_52_P110002	Fam136a	66488	NM_025591	9.29	10.10	9.45	9.79	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P356343	Fam13a	58909	NM_153574	5.57	9.56	5.23	7.84	Yes
A_51_P329949	Fam13a	58909	NM_153574	6.35	10.22	6.06	8.81	Yes
A_52_P539343	Fam154b	330577	AK007315	6.72	5.83	6.91	5.96	No
A_52_P235954	Fam160b1	226252	NM_145505	7.90	7.17	7.82	7.33	No
A_51_P118246	Fam161a	73873	NM_028672	10.46	9.65	10.37	9.71	No
A_52_P470116	Fam161b	217705	NM_172581	6.99	6.15	6.22	5.76	No
A_51_P455620	Fam167a	219148	NM_177628	2.27	6.39	3.80	6.48	No
A_52_P501077	Fam171a2	217219	NM_199200	13.90	14.68	13.73	14.31	No
A_52_P9289	Fam172a	68675	NM_001163420	6.43	5.75	6.36	5.90	No
A_52_P75808	Fam177a	73385	AK052876	6.22	5.45	6.33	5.90	No
A_52_P426870	Fam178a	226151	NM_001081225	9.46	10.76	9.56	10.57	No
A_52_P123843	Fam178a	226151	NM_001081225	6.88	9.06	6.76	8.50	No
A_51_P207706	Fam180a	208164	NM_173375	4.40	2.53	5.16	3.90	No
A_51_P106527	Fam195a	68241	NM_026633	9.73	10.96	9.96	10.78	No
A_51_P205326	Fam198a	245050	NM_177743	5.13	3.70	5.20	4.38	No
A_51_P317620	Fam19a2	268354	NM_182807	3.15	5.31	3.81	4.64	No
A_51_P424878	Fam213b	66469	NM_025582	8.24	10.36	7.92	8.84	Yes
A_52_P63709	Fam49b	223601	NM_144846	7.90	9.86	7.66	9.15	Yes
A_51_P374190	Fam53b	77938	NM_212473	5.95	6.59	6.06	6.17	No
A_52_P85292	Fam53b	77938	NM_212473	6.24	6.89	6.45	6.63	No
A_51_P521001	Fam63a	75007	NM_133858	6.29	5.70	5.92	6.04	No
A_52_P370534	Fam65b	193385	NM_029679	6.40	8.85	7.05	8.45	No
A_51_P122217	Fam65b	193385	NM_029679	5.52	8.64	6.51	8.01	No
A_52_P129867	Fastkd1	320720	NM_177244	7.41	8.68	7.49	8.33	No
A_51_P448866	Fastkd5	380601	NM_198176	9.03	9.83	9.10	9.57	No
A_51_P349142	Fbl	14113	NM_007991	14.49	15.23	14.50	15.08	No
A_52_P590535	Fbln2	14115	NM_007992	15.56	14.94	15.60	14.91	No
A_51_P186703	Fbln5	23876	AK090129	16.57	15.96	16.74	16.32	No
A_52_P559817	Fbln7	70370	NM_024237	8.00	7.14	8.08	7.16	No
A_51_P427132	Fbxl13	320118	NM_177076	5.21	7.89	5.20	8.00	No
A_52_P604735	Fbxl2	72179	XR_379879	5.51	6.35	6.12	6.38	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P500996	Fbxl20	72194	NM_028149	9.38	8.71	9.67	9.52	Yes
A_51_P149373	Fbxl20	72194	NM_028149	11.78	11.11	11.69	11.46	No
A_52_P41239	Fbxl21	213311	NM_178674	5.19	4.15	5.17	4.29	No
A_51_P466685	Fbxl22	74165	NM_175206	3.41	7.01	3.73	5.65	Yes
A_52_P646783	Fbxl22	74165	NM_175206	7.76	9.90	6.86	8.72	No
A_52_P423364	Fbxl7	448987	NM_176959	14.54	13.47	14.33	13.68	No
A_51_P423666	Fbxo17	50760	NM_015796	9.69	10.90	9.56	10.20	No
A_51_P324551	Fbxo25	66822	NM_025785	13.78	13.09	13.80	13.41	No
A_51_P429770	Fcer1a	14125	NM_010184	6.14	5.45	5.97	5.65	No
A_51_P161890	Fcgrt	14132	NM_010189	13.49	12.75	13.55	13.02	No
A_52_P136138	Fdft1	14137	NM_010191	5.06	6.17	5.73	5.36	No
A_51_P485946	Fdft1	14137	NM_010191	12.07	12.98	12.12	12.28	No
A_51_P285770	Fem1a	14154	AK005979	12.68	13.56	12.89	13.15	No
A_51_P352924	Fem1b	14155	NM_010193	9.43	10.60	9.66	10.31	No
A_52_P635338	Fes	14159	NM_010194	10.94	10.11	10.78	10.26	No
A_52_P365011	Fes	14159	NM_010194	9.09	8.22	9.00	8.51	No
A_52_P639229	Fgd4	224014	NM_139232	5.33	6.95	6.05	6.83	No
A_52_P454815	Fgf11	14166	NM_010198	3.72	6.30	4.00	5.75	No
A_51_P315925	Fgf21	56636	NM_020013	3.77	5.82	2.85	4.86	Yes
A_52_P235347	Fgf21	56636	NM_020013	4.87	7.84	4.48	7.03	Yes
A_51_P302377	Fgfbp1	14181	NM_008009	2.99	6.49	3.00	6.41	No
A_51_P255805	Fhdc1	229474	NM_001033301	9.54	8.58	9.66	9.09	No
A_51_P164530	Fhit	14198	NM_010210	3.49	5.60	4.41	4.44	Yes
A_51_P495379	Fhod1	234686	NM_177699	12.06	11.23	12.09	11.57	No
A_52_P326548	Fhod3	225288	NM_175276	6.25	8.93	6.88	8.39	No
A_51_P271417	Fibcd1	98970	NM_178887	6.90	5.55	5.61	5.18	Yes
A_51_P117739	Figf	14205	NM_010216	13.16	11.88	12.78	11.97	No
A_52_P358505	Filip1	70598	XM_006511442	2.59	7.85	2.42	5.78	Yes
A_51_P333438	Filip1	70598	XM_006511442	2.51	9.85	2.86	8.43	Yes
A_51_P293784	Fkbp1b	14226	NM_016863	6.15	5.41	6.08	5.57	No
A_52_P821	Fli1	14247	NM_008026	8.02	7.35	7.96	7.55	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P205907	Flnc	68794	NM_001081185	11.51	12.51	11.65	12.21	No
A_52_P61735	Flnc	68794	NM_001081185	13.26	14.17	13.41	13.96	No
A_51_P349844	Flnc	68794	NM_001081185	6.79	7.66	6.85	7.46	No
A_51_P423091	Flot1	14251	NM_008027	11.84	11.08	11.69	11.27	No
A_51_P142295	Flrt3	71436	NM_178382	8.69	7.67	8.62	7.88	No
A_52_P520859	Flt1	14254	NM_010228	8.12	7.36	8.30	7.69	No
A_51_P398723	Flt1	14254	NM_010228	10.31	9.66	10.35	9.77	No
A_51_P374337	Flt3l	14256	NM_013520	7.39	6.26	7.43	6.79	No
A_52_P508991	Fmo1	14261	NM_010231	9.06	11.52	8.45	10.13	Yes
A_52_P460767	Fmo5	14263	NM_010232	7.20	6.21	7.18	6.51	No
A_51_P207622	Fmod	14264	NM_021355	5.98	5.31	5.51	5.13	No
A_52_P113000	Fnbp1l	214459	NM_153118	12.12	11.52	11.52	11.32	No
A_52_P433029	Fndc1	68655	NM_001081416	5.12	4.43	5.26	4.91	No
A_51_P153423	Fndc1	68655	NM_001081416	5.15	7.65	5.02	6.63	No
A_52_P186485	Fndc3a	319448	NM_207636	7.99	6.96	8.22	7.36	No
A_52_P213696	Fndc5	384061	NM_027402	5.64	13.27	4.84	11.57	Yes
A_51_P354913	Fndc5	384061	NM_027402	5.32	12.74	5.57	11.09	Yes
A_52_P494930	Fnip2	329679	NM_001162999	9.42	8.54	9.35	9.09	No
A_52_P202276	Focad	230393	NM_001081184	10.77	11.74	11.04	11.42	No
A_51_P196444	Foxc2	14234	NM_013519	12.01	11.08	11.87	11.41	No
A_52_P118970	Foxd2os	100040736	NR_030721	10.38	9.66	10.65	10.16	No
A_51_P432567	Foxg1	15228	NM_008241	13.66	12.99	13.93	13.60	No
A_52_P453884	Foxo1	56458	NM_019739	10.30	11.30	10.43	11.00	No
A_51_P138044	Foxo1	56458	NM_019739	10.88	11.87	10.89	11.46	No
A_52_P589568	Foxo6	329934	NM_194060	9.81	13.45	9.92	12.15	Yes
A_51_P233753	Foxp2	114142	NM_053242	4.45	2.56	4.92	4.74	Yes
A_52_P162957	Frat2	212398	NM_177603	9.20	8.26	9.09	8.62	No
A_51_P466288	Frmd5	228564	XM_006499275	3.30	5.35	2.68	5.37	No
A_52_P70856	Frmpd1	666060	NM_001081172	2.25	11.12	1.95	10.00	Yes
A_51_P286748	Frzb	20378	NM_011356	4.90	3.41	5.71	3.44	No
A_52_P168338	Fundc2	67391	NM_026126	6.85	7.53	7.10	7.35	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P657593	Fxr1	14359	XM_006535400	9.15	10.05	8.94	9.83	No
A_52_P472302	Fxyd6	59095	NM_022004	7.31	11.12	6.55	9.08	Yes
A_52_P457376	Fyb	23880	NM_011815	3.00	4.41	3.31	6.00	Yes
A_51_P177667	Fyn	14360	U70324	7.99	9.26	8.60	9.37	No
A_51_P177667	Fyn	14360	U70324	8.03	9.28	8.49	9.27	No
A_51_P177667	Fyn	14360	U70324	8.03	9.27	8.54	9.30	No
A_51_P177667	Fyn	14360	U70324	7.98	9.17	8.41	9.16	No
A_51_P177667	Fyn	14360	U70324	8.12	9.26	8.42	9.25	No
A_51_P177667	Fyn	14360	U70324	8.13	9.23	8.42	9.22	No
A_51_P177667	Fyn	14360	U70324	8.19	9.29	8.57	9.21	No
A_51_P177667	Fyn	14360	U70324	8.33	9.42	8.51	9.24	No
A_51_P177667	Fyn	14360	U70324	8.07	9.14	8.43	9.13	No
A_51_P177667	Fyn	14360	U70324	8.10	9.08	8.54	9.25	No
A_52_P597634	Fzd1	14362	NM_021457	14.03	13.34	13.92	13.38	No
A_52_P168231	Fzd3	14365	NM_021458	4.04	5.21	4.33	4.87	No
A_51_P190863	Fzd3	14365	NM_021458	6.31	7.39	6.47	6.86	No
A_52_P681391	G0s2	14373	NM_008059	7.37	9.11	6.87	7.95	Yes
A_52_P433817	Gab3	210710	NM_181584	6.35	5.41	6.56	5.73	No
A_52_P558188	Gabarap	56486	NM_019749	14.39	13.81	14.40	14.10	No
A_52_P264550	Gabra3	14396	NM_008067	5.79	5.09	6.22	5.72	Yes
A_52_P1197913	Gadd45b	17873	NM_008655	11.85	12.77	11.33	12.03	Yes
A_51_P500984	Gadd45b	17873	NM_008655	12.17	13.28	11.93	12.75	No
A_51_P315904	Gadd45g	23882	NM_011817	13.36	14.91	13.17	14.49	No
A_52_P94830	Galnt16	108760	NM_001081421	5.99	7.88	6.21	6.70	No
A_52_P296338	Galnt18	233733	NM_173739	6.45	7.71	5.85	6.91	Yes
A_51_P263290	Galnt9	231605	NM_198306	2.00	7.52	2.31	5.95	Yes
A_51_P484869	Gamt	14431	NM_010255	13.14	14.28	13.43	13.83	No
A_51_P484869	Gamt	14431	NM_010255	13.12	14.27	13.40	13.84	No
A_51_P484869	Gamt	14431	NM_010255	13.07	14.11	13.34	13.77	No
A_51_P484869	Gamt	14431	NM_010255	13.22	14.22	13.41	13.87	No
A_51_P484869	Gamt	14431	NM_010255	13.17	14.22	13.36	13.85	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P484869	Gamt	14431	NM_010255	13.11	14.11	13.29	13.86	No
A_51_P484869	Gamt	14431	NM_010255	13.13	14.18	13.28	13.81	No
A_51_P484869	Gamt	14431	NM_010255	13.21	14.22	13.35	13.79	No
A_51_P484869	Gamt	14431	NM_010255	13.21	14.18	13.35	13.85	No
A_51_P484869	Gamt	14431	NM_010255	13.20	14.12	13.34	13.68	No
A_52_P589321	Gapdh	14433	NM_008084	2.75	4.56	2.75	4.29	No
A_51_P265695	Gapdhs	14447	NM_008085	3.77	4.80	3.99	4.74	No
A_51_P265695	Gapdhs	14447	NM_008085	3.82	4.62	4.32	4.48	No
A_51_P265695	Gapdhs	14447	NM_008085	4.24	4.99	4.29	4.59	No
A_51_P274992	Gar1	68147	NM_026578	8.49	9.34	8.73	9.20	No
A_52_P639774	Gart	14450	NM_010256	8.97	9.79	9.05	9.59	No
A_52_P670095	Gata1	14460	NM_008089	4.60	3.62	4.70	3.75	No
A_52_P670095	Gata1	14460	NM_008089	4.75	3.98	4.52	4.17	No
A_52_P670095	Gata1	14460	NM_008089	3.80	3.09	4.47	3.67	No
A_51_P461319	Gatm	67092	NM_025961	1.91	8.84	2.12	7.57	Yes
A_52_P666026	Gatsl2	80909	BC026208	4.38	6.15	5.11	5.78	No
A_52_P89267	Gatsl2	80909	BC026208	8.55	9.89	8.56	9.48	No
A_51_P517051	Gatsl3	71962	NM_028022	5.90	5.07	6.33	5.59	No
A_51_P203955	Gbp2	14469	NM_010260	10.58	9.57	10.19	9.45	No
A_51_P398766	Gbp2b	14468	NM_010259	8.69	7.62	8.39	7.59	No
A_51_P483159	Gchfr	320415	NM_177157	13.19	12.46	13.34	13.03	No
A_51_P198694	Gckr	231103	NM_144909	2.69	5.44	3.43	2.97	Yes
A_51_P464300	Gdf1	14559	NM_008107	11.43	12.49	11.60	12.02	No
A_52_P28960	Gdf6	242316	NM_013526	6.25	6.87	4.54	5.69	Yes
A_52_P236088	Gdpl1	66569	NM_025638	7.80	8.63	7.59	8.33	No
A_52_P75465	Gdpl1	66569	NM_025638	10.29	11.43	10.44	11.41	No
A_51_P426875	Gdpl3	68616	NM_024228	4.66	3.73	4.79	4.25	No
A_51_P318192	Gem	14579	NM_010276	6.33	8.27	7.17	8.84	No
A_52_P314923	Gemin5	216766	NM_172558	7.92	8.65	8.10	8.59	No
A_51_P284937	Gfm1	28030	NM_138591	9.20	9.86	9.36	9.78	No
A_52_P398998	Gfra1	14585	NM_010279	5.28	7.02	4.81	6.49	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P311022	Gfra1	14585	NM_010279	5.72	7.95	5.29	6.67	No
A_52_P117352	Gja4	14612	NM_008120	9.00	8.32	9.11	8.39	No
A_52_P525317	Gja5	14613	NM_008121	3.05	6.70	3.38	4.55	Yes
A_51_P385639	Gjb5	14622	NM_010291	9.47	8.08	8.74	7.36	Yes
A_51_P157185	Gjd4	225152	NM_153086	2.10	7.50	1.66	5.77	Yes
A_51_P337543	Glee	93683	NM_033320	11.85	12.53	10.65	11.33	Yes
A_52_P673978	Glee	93683	NM_033320	4.95	5.89	5.57	5.58	No
A_51_P511546	Gldc	104174	NM_138595	3.40	6.60	3.47	5.80	No
A_51_P125882	Glp2r	93896	NM_175681	6.34	5.63	6.23	5.99	No
A_51_P142767	Glt8d2	74782	NM_029102	9.84	8.69	10.10	9.37	No
A_52_P57582	Gm11127	100529082	NM_001199967	11.44	10.86	11.79	11.66	Yes
A_52_P70216	Gm12942	100039968	NM_001099319	10.18	9.24	9.97	9.32	No
A_51_P375631	Gm13999	100036539	AK016792	7.89	7.25	7.47	7.36	No
A_52_P287313	Gm1661	381544	NM_001145637	5.24	3.23	5.56	5.29	No
A_52_P545512	Gm16880	100503474	AK086995	4.38	3.40	3.84	3.93	No
A_52_P353417	Gm2a	14667	NM_010299	8.53	7.67	8.91	8.55	Yes
A_52_P683895	Gm5069	277333	NR_003623	14.87	15.51	14.78	15.22	No
A_51_P437538	Gm5124	331392	NR_045668	11.22	11.86	11.13	11.75	No
A_51_P458983	Gm5617	434402	NM_001004191	9.16	8.52	9.22	8.62	No
A_51_P424256	Gm6211	621312	XR_002190	13.22	13.83	13.25	13.84	No
A_51_P291460	Gm6498	624367	NR_003630	14.70	15.35	14.49	14.88	No
A_51_P247496	Gm9767	100040851	NR_028030	3.75	7.12	4.18	5.82	Yes
A_52_P463271	Gmids	218138	NM_146041	9.60	8.87	9.71	9.33	No
A_51_P495986	Gmpr	66355	NM_025508	10.72	11.90	10.17	10.75	No
A_52_P640474	Gnb4	14696	NM_013531	8.38	7.58	8.47	7.90	No
A_52_P331883	Gng7	14708	NM_001038655	6.58	5.97	6.48	5.87	No
A_51_P377376	Gnl3	30877	NM_153547	9.06	10.13	9.49	10.21	No
A_52_P607372	Golim4	73124	NM_175193	8.06	7.44	8.09	7.52	No
A_52_P820985	Golph3l	229593	NM_146133	10.42	9.68	10.33	9.96	No
A_51_P370700	Got1	14718	NM_010324	12.01	12.89	12.00	12.54	No
A_51_P276943	Gpc1	14733	NM_016696	15.00	16.59	15.19	16.42	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P251833	Gpha2	170458	NM_130453	8.17	7.44	7.71	7.48	No
A_51_P462102	Gpr125	70693	NM_133911	11.88	12.61	11.92	12.27	No
A_51_P281912	Gpr137c	70713	AK082691	3.46	4.53	4.15	4.05	No
A_51_P497993	Gpr155	68526	NM_001190297	9.16	10.25	9.40	9.47	Yes
A_52_P558988	Gpr155	68526	NM_001190297	6.44	7.20	6.52	6.58	No
A_52_P375503	Gpr161	240888	XM_006496850	5.01	4.15	4.72	4.62	No
A_52_P635015	Gpr39	71111	NM_027677	6.86	4.95	6.88	5.74	No
A_52_P317393	Gpr56	14766	NM_018882	7.96	10.35	7.73	9.14	Yes
A_51_P362373	Gprasp2	245607	NM_001163015	3.06	6.58	2.52	5.67	Yes
A_51_P309988	Gprc5c	70355	NM_147217	3.26	8.46	2.83	6.69	Yes
A_52_P405460	Gprc5c	70355	NM_147217	3.76	6.13	3.76	4.76	No
A_52_P554703	Gprin3	243385	NM_183183	9.75	8.83	9.57	8.61	No
A_51_P493886	Gpt2	108682	NM_173866	10.40	12.78	10.55	11.79	Yes
A_51_P461364	Gpx7	67305	NM_024198	3.82	5.35	5.09	4.22	Yes
A_52_P325116	Gramd1b	235283	XM_006510235	10.30	12.30	9.47	11.12	Yes
A_51_P221008	Gramd1b	235283	XM_006510235	10.40	12.32	9.61	11.16	Yes
A_52_P5696	Gramd1b	235283	XM_006510235	4.68	5.87	4.76	5.31	No
A_52_P619738	Gramd3	107022	NM_026240	9.52	8.89	9.54	9.24	No
A_51_P187151	Grb10	14783	NM_010345	7.73	8.41	7.41	7.53	Yes
A_51_P152990	Grem2	23893	NM_011825	6.36	5.67	6.02	5.74	No
A_51_P192800	Gm	14824	NM_008175	14.58	13.96	14.41	13.97	No
A_51_P109144	Grtp1	66790	NM_025768	5.62	7.94	5.40	7.32	No
A_51_P479311	Gstm1	14862	NM_010358	15.73	14.93	15.83	15.20	No
A_52_P415215	Gstm1	14862	NM_010358	11.42	10.51	11.42	10.85	No
A_51_P284486	Gstm2	14863	NM_008183	14.32	13.68	14.08	13.45	No
A_51_P260169	Gstm5	14866	NM_010360	12.82	12.22	12.71	12.32	No
A_51_P114002	Gstm7	68312	AK002213	5.97	5.24	5.47	5.38	No
A_51_P179664	Gstt1	14871	NM_008185	8.43	7.32	8.23	7.45	No
A_51_P387670	Gtpbp4	69237	NM_027000	11.25	11.88	11.17	11.22	Yes
A_52_P479522	Gulp1	70676	NM_028450	7.88	6.75	7.94	7.19	No
A_52_P228932	Gys1	14936	NM_030678	8.55	9.35	8.72	8.77	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P571350	H19	14955	AK003142	7.02	14.26	6.65	12.69	Yes
A_51_P142196	H19	14955	AK003142	8.33	15.55	8.34	14.04	Yes
A_51_P487668	H2afy3	67552	NR_003523	14.26	13.66	13.82	13.54	No
A_51_P473734	H2-DMa	14998	NM_010386	9.60	8.85	9.36	8.80	No
A_52_P146877	H2-DMb1	14999	NM_010387	6.90	5.88	5.86	5.33	Yes
A_52_P474242	H2-K1	14972	M69070	10.85	10.14	11.07	10.81	No
A_51_P469968	H2-M3	14991	NM_013819	9.47	8.60	9.38	8.92	No
A_51_P237754	H2-T23	15040	NM_010398	13.02	12.35	13.30	12.97	No
A_51_P138909	H60c	670558	NM_001204916	5.15	3.98	4.80	4.42	No
A_51_P297679	Hcls1	15163	NM_008225	7.64	6.90	7.04	6.37	No
A_51_P213334	Hdac11	232232	NM_144919	7.15	8.92	7.18	8.16	No
A_52_P435016	Hdac7	56233	NM_019572	11.24	10.55	11.27	10.69	No
A_51_P254656	Hdc	15186	NM_008230	7.21	6.16	6.71	6.10	No
A_51_P362531	Heatr1	217995	NM_144835	9.85	10.64	9.93	10.62	No
A_52_P430283	Heatr2	433956	NM_001081265	10.29	10.93	10.25	10.64	No
A_51_P430002	Heatr5a	320487	NM_177171	5.71	4.86	5.31	4.68	No
A_51_P111018	Heatr5b	320473	BC019508	8.90	8.23	8.72	7.86	No
A_51_P437050	Heg1	77446	XM_006522715	7.95	8.69	7.87	8.07	Yes
A_52_P520375	Heg1	77446	XM_006522715	4.56	5.36	4.81	5.12	No
A_52_P321358	Helz2	229003	NM_183162	6.98	6.23	7.33	6.79	No
A_51_P249313	Herc3	73998	NM_028705	7.20	8.78	6.96	8.04	Yes
A_52_P679860	Herc6	67138	NM_025992	5.80	5.07	5.55	5.00	No
A_51_P498093	Hes1	15205	NM_008235	7.30	8.61	7.06	8.35	No
A_51_P429366	Hes6	55927	NM_019479	12.87	15.45	12.80	14.61	Yes
A_52_P512301	Hes6	55927	NM_019479	8.62	11.16	8.38	10.24	No
A_52_P85403	Hexim1	192231	NM_138753	12.95	13.59	13.18	13.30	No
A_51_P258409	Hey1	15213	NM_010423	8.11	10.95	8.18	10.32	No
A_52_P337259	Hey1	56198	NM_013905	4.12	5.77	4.09	5.58	No
A_51_P206824	Hfe2	69585	NM_027126	2.42	11.14	2.50	9.27	Yes
A_51_P484998	Hgf	15234	AK082461	11.27	10.39	11.34	10.96	No
A_51_P263310	Higd1a	56295	NM_019814	7.59	8.35	7.07	7.26	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P290819	Hint3	66847	NM_025798	11.43	10.70	11.34	10.80	No
A_51_P166023	Hip1	215114	NM_146001	8.37	10.01	7.82	8.66	No
A_52_P11174	Hip1	215114	NM_146001	10.52	12.46	10.40	11.47	No
A_51_P516756	Hipk3	15259	NM_010434	7.70	8.99	8.05	8.83	No
A_52_P109010	Hipk3	15259	NM_010434	13.00	13.78	13.09	13.40	No
A_52_P49712	Hira	15260	NM_010435	3.46	4.94	4.32	4.61	No
A_51_P443754	Hivep2	15273	NM_010437	6.86	8.47	6.93	7.80	No
A_51_P179701	Hlx	15284	NM_008250	7.77	6.94	7.67	7.44	No
A_51_P449580	Hmga2-ps1	15365	NR_037996	7.18	6.38	7.95	7.24	Yes
A_52_P498608	Hmgn5	50887	NM_016710	9.58	10.95	8.89	9.55	Yes
A_51_P457331	Hmgn5	50887	NM_016710	8.73	10.14	8.23	9.20	Yes
A_51_P476091	Hnrnpdl	50926	NM_016690	12.58	13.18	12.41	12.82	No
A_52_P292693	Hnrnpdl	50926	NM_016690	10.15	10.79	10.13	10.33	No
A_52_P573366	Hnrnpul1	232989	NM_178089	6.52	7.33	6.44	7.04	No
A_52_P151211	Homer1	26556	NM_152134	6.93	8.53	7.23	8.09	No
A_51_P387160	Homer1	26556	NM_152134	3.78	5.23	4.03	4.86	No
A_52_P37991	Homer1	26556	NM_152134	5.03	6.63	5.22	6.43	No
A_51_P352303	Homer2	26557	NM_011983	3.13	4.78	2.75	3.61	Yes
A_52_P535623	Hook1	77963	NM_030014	5.84	5.13	5.33	5.37	No
A_52_P422675	Hook3	320191	NM_207659	10.51	9.88	10.58	10.22	No
A_51_P235945	Hp	15439	NM_017370	9.67	7.73	8.47	7.22	No
A_51_P434483	Hpn	15451	NM_008281	4.53	3.65	4.45	4.87	No
A_51_P159113	Hr	15460	NM_021877	5.69	4.57	5.64	4.91	No
A_51_P267751	Hrc	15464	NM_010473	2.86	11.84	2.80	10.62	Yes
A_52_P59681	Hrsp12	15473	NM_008287	10.95	10.31	11.24	10.77	No
A_51_P469522	Hs2st1	23908	NM_011828	9.58	11.25	9.94	11.04	No
A_51_P127297	Hsd11b1	15483	NM_008288	8.12	10.30	7.19	9.83	Yes
A_51_P435911	Hsd3b7	101502	NM_133943	11.20	10.53	11.14	10.74	No
A_51_P130332	Hspa12b	72630	NM_028306	8.99	8.18	8.62	8.24	No
A_51_P453149	Hspa2	15512	NM_008301	8.62	7.96	8.47	8.03	No
A_52_P652704	Hspa4l	18415	NM_011020	8.53	9.23	8.49	9.23	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P520687	Hspa9	15526	NM_010481	10.74	11.75	11.33	11.30	No
A_52_P404533	Hspb1	15507	NM_013560	9.09	10.59	8.35	9.74	No
A_52_P222624	Hspb2	69253	NM_024441	8.17	10.90	7.67	9.57	Yes
A_51_P322138	Hspb2	69253	NM_024441	3.54	6.32	4.03	5.21	Yes
A_51_P419007	Hspb3	56534	NM_019960	2.55	6.29	3.12	5.22	No
A_51_P346445	Hspb7	29818	NM_013868	3.30	5.97	2.62	4.09	Yes
A_51_P346445	Hspb7	29818	NM_013868	3.34	5.89	2.74	4.03	Yes
A_51_P346445	Hspb7	29818	NM_013868	3.17	5.78	2.57	3.97	Yes
A_51_P346445	Hspb7	29818	NM_013868	2.67	5.89	3.08	4.14	Yes
A_51_P346445	Hspb7	29818	NM_013868	3.27	5.91	2.28	4.42	Yes
A_51_P110455	Hsph1	15505	NM_013559	12.84	13.64	12.80	13.37	No
A_52_P181925	Htr2b	15559	NM_008311	4.78	7.55	4.06	5.91	Yes
A_51_P225224	Htra1	56213	NM_019564	13.55	12.71	13.76	13.30	Yes
A_52_P207314	Htra4	330723	NM_001081187	5.14	4.14	4.63	4.14	No
A_51_P331831	Hvcn1	74096	NM_001042489	6.96	5.81	7.03	6.19	No
A_51_P479408	Hyal1	15586	NM_008317	7.80	7.05	8.26	7.58	No
A_52_P144173	Hyal1	15586	NM_008317	8.09	6.92	8.48	8.07	No
A_52_P247462	Hykk	235386	NM_177351	8.76	7.81	8.60	7.73	No
A_52_P663686	I830012O16Rik	667370	NM_001005858	7.13	5.75	7.32	6.50	Yes
A_51_P408506	Icam1	15894	BC008626	4.32	6.49	4.07	5.83	No
A_51_P408506	Icam1	15894	BC008626	4.00	5.78	4.04	5.76	No
A_51_P408506	Icam1	15894	BC008626	4.46	6.00	4.25	5.67	No
A_51_P408506	Icam1	15894	BC008626	4.37	5.90	4.30	5.92	No
A_51_P408506	Icam1	15894	BC008626	4.34	5.81	4.40	5.71	No
A_51_P408506	Icam1	15894	BC008626	4.32	5.70	4.24	5.57	No
A_51_P408506	Icam1	15894	BC008626	4.51	5.85	4.33	5.74	No
A_51_P408506	Icam1	15894	BC008626	4.56	5.80	4.25	5.75	No
A_51_P408506	Icam1	15894	BC008626	4.68	5.88	4.48	5.78	No
A_51_P408506	Icam1	15894	BC008626	4.67	5.60	4.36	5.58	No
A_52_P240542	Id2	15902	NM_010496	12.62	13.57	12.86	13.59	No
A_51_P219074	Id2	15902	NM_010496	11.49	12.27	11.55	12.33	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P268559	Idh3a	67834	NM_029573	11.40	12.23	11.62	11.99	No
A_52_P123018	Ids	15931	NM_010498	14.85	14.00	14.89	14.40	No
A_51_P317321	Iffo1	320678	AK044382	9.97	13.11	10.01	12.21	Yes
A_52_P655008	Iffo1	320678	AK044382	5.88	8.30	5.56	6.89	No
A_51_P237856	Ifi203	15950	NM_001045481	9.03	8.18	9.24	8.70	No
A_51_P387810	Ifih1	71586	NM_027835	8.06	7.11	7.99	7.58	No
A_51_P359570	Ifit3	15959	NM_010501	10.68	9.44	10.68	9.75	No
A_51_P294555	Ifitm6	213002	NM_001033632	5.00	4.36	4.40	3.89	No
A_51_P436401	Ifna5	15968	NM_010505	5.67	4.94	4.72	4.86	No
A_51_P172251	Ifrd2	15983	NM_025903	10.95	11.65	11.14	11.61	No
A_52_P261409	Ift172	67661	NM_026298	8.25	7.66	7.94	7.57	No
A_51_P147942	Igfl	16000	NM_010512	9.38	11.01	9.60	10.70	No
A_51_P147942	Igfl	16000	NM_010512	9.36	10.83	9.49	10.49	No
A_51_P147942	Igfl	16000	NM_010512	9.38	11.00	9.50	10.63	No
A_51_P147942	Igfl	16000	NM_010512	9.45	10.83	9.54	10.60	No
A_51_P147942	Igfl	16000	NM_010512	9.47	11.02	9.55	10.59	No
A_51_P147942	Igfl	16000	NM_010512	9.45	10.85	9.51	10.56	No
A_51_P147942	Igfl	16000	NM_010512	9.48	10.94	9.54	10.64	No
A_51_P147942	Igfl	16000	NM_010512	9.46	10.87	9.52	10.56	No
A_51_P147942	Igfl	16000	NM_010512	9.45	10.81	9.47	10.52	No
A_51_P147942	Igfl	16000	NM_010512	9.52	10.70	9.53	10.68	No
A_52_P536434	Igfl	16000	NM_010512	9.26	10.63	9.35	10.34	No
A_51_P516826	Igf2	16002	NM_010514	7.09	16.08	6.99	14.95	No
A_51_P516826	Igf2	16002	NM_010514	7.14	16.01	6.79	14.88	No
A_51_P516826	Igf2	16002	NM_010514	7.27	16.11	7.00	14.98	No
A_51_P516826	Igf2	16002	NM_010514	7.35	16.15	6.90	14.99	No
A_51_P516826	Igf2	16002	NM_010514	7.23	16.02	6.93	14.88	No
A_51_P516826	Igf2	16002	NM_010514	7.34	16.12	6.93	14.91	No
A_51_P516826	Igf2	16002	NM_010514	7.31	16.07	7.07	14.98	No
A_51_P516826	Igf2	16002	NM_010514	7.36	16.08	6.99	14.93	No
A_51_P516826	Igf2	16002	NM_010514	7.40	16.09	6.87	14.92	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P516826	Igf2	16002	NM_010514	7.38	16.05	6.71	14.99	No
A_51_P516833	Igf2	16002	NM_010514	5.15	14.33	5.18	13.11	No
A_51_P447545	Igfbp1	16006	NM_008341	2.41	8.24	2.34	7.00	Yes
A_52_P253179	Igfbp3	16009	NM_008343	7.09	10.90	9.35	11.48	Yes
A_52_P281702	Igfbp5	16011	NM_010518	4.65	9.93	4.91	8.77	Yes
A_51_P374571	Igfbp6	16012	X81584	5.64	4.64	5.82	5.10	No
A_52_P429964	Igsf10	242050	NM_001162884	5.12	3.87	4.04	3.33	No
A_51_P327632	Igsf3	78908	NM_207205	9.19	8.53	8.98	8.38	No
A_51_P152404	Ikbip	67454	NM_026166	14.05	13.43	14.08	13.88	No
A_51_P222280	Ikbke	56489	NM_019777	7.78	6.99	8.11	7.70	Yes
A_51_P353946	Il11ra1	16157	NM_010549	16.34	15.44	16.01	15.56	No
A_51_P353947	Il11ra1	16157	NM_010549	14.46	13.73	14.41	13.77	No
A_52_P442228	Il12a	16159	NM_008351	3.81	5.87	3.82	6.19	No
A_52_P552194	Il13ra1	16164	NM_133990	4.74	5.50	4.95	5.73	No
A_52_P143976	Il17rc	171095	NM_134159	14.13	15.05	14.19	14.53	No
A_51_P153565	Il18	16173	NM_008360	9.90	8.85	9.33	8.94	No
A_51_P153565	Il18	16173	NM_008360	9.81	8.85	9.23	9.00	No
A_51_P153565	Il18	16173	NM_008360	9.77	8.82	9.26	8.90	No
A_51_P153565	Il18	16173	NM_008360	9.83	8.91	9.31	9.02	No
A_51_P153565	Il18	16173	NM_008360	9.71	8.88	9.26	8.96	No
A_51_P153565	Il18	16173	NM_008360	9.69	8.87	9.33	9.05	No
A_51_P153565	Il18	16173	NM_008360	9.84	9.02	9.36	9.15	No
A_51_P153565	Il18	16173	NM_008360	9.76	8.95	9.34	9.08	No
A_51_P153565	Il18	16173	NM_008360	9.81	9.04	9.39	9.06	No
A_51_P153565	Il18	16173	NM_008360	9.61	8.91	9.24	8.90	No
A_51_P122321	Il33	77125	NM_133775	5.62	6.78	5.18	9.06	Yes
A_51_P268234	Il34	76527	NM_029646	6.23	7.50	6.18	7.22	No
A_51_P217218	Il6	16193	NM_031168	4.01	7.51	4.16	6.97	No
A_51_P217218	Il6	16193	NM_031168	3.99	7.29	4.19	7.15	No
A_51_P217218	Il6	16193	NM_031168	3.92	7.11	3.66	7.06	No
A_51_P217218	Il6	16193	NM_031168	4.24	7.43	4.02	7.03	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P217218	Il6	16193	NM_031168	4.20	7.39	3.83	7.11	No
A_51_P217218	Il6	16193	NM_031168	3.86	7.05	4.28	6.93	No
A_51_P217218	Il6	16193	NM_031168	4.07	7.16	4.13	7.01	No
A_51_P217218	Il6	16193	NM_031168	4.05	7.02	3.70	6.86	No
A_51_P217218	Il6	16193	NM_031168	4.69	7.27	5.35	7.15	No
A_51_P217218	Il6	16193	NM_031168	4.68	7.07	5.93	6.93	No
A_51_P438905	Inadl	12695	NM_172696	6.96	8.43	7.23	7.68	Yes
A_52_P402885	Inpp1	16329	AK009002	8.33	7.21	8.70	8.05	No
A_52_P119510	Inpp1	16329	AK009002	6.37	5.42	6.53	5.80	No
A_52_P330289	Inpp4b	234515	NM_001024617	2.76	7.17	3.58	6.00	Yes
A_51_P225933	Inpp4b	234515	NM_001024617	5.49	9.71	4.81	8.62	Yes
A_51_P142334	Inpp1l	16332	NM_010567	12.67	14.49	12.62	13.74	Yes
A_51_P163238	Ip6k3	271424	NM_173027	3.41	5.82	3.40	5.90	No
A_51_P347823	Ipo4	75751	NM_024267	12.36	12.99	12.68	13.04	No
A_52_P99531	Ippk	75678	NM_199056	9.16	9.84	9.21	9.69	No
A_52_P194289	Iqsec1	232227	NM_001134384	9.67	10.37	9.45	9.80	No
A_52_P425860	Iqsec1	232227	NM_001134384	11.09	11.68	11.06	11.47	No
A_51_P363749	Irf6	54139	NM_016851	3.67	5.97	2.90	5.15	No
A_51_P127367	Irf9	16391	NM_008394	9.88	9.20	9.78	9.12	No
A_51_P416295	Irgm2	54396	NM_019440	8.88	7.88	8.56	7.76	No
A_52_P635312	Irx2	16372	NM_010574	7.05	6.41	7.27	6.62	No
A_52_P463936	Isg15	100038882	NM_015783	8.43	7.49	7.85	7.17	No
A_52_P81270	Itga6	16403	NM_008397	7.77	9.68	8.65	9.43	Yes
A_51_P262401	Itga6	16403	NM_008397	9.84	11.68	10.59	11.46	No
A_51_P181671	Itga7	16404	L23423	7.13	11.00	6.40	10.85	No
A_52_P413034	Itga7	16404	L23423	4.81	10.78	5.25	10.59	No
A_51_P352076	Itgae	16407	NM_008399	6.15	5.02	5.95	5.28	No
A_51_P382484	Itgav	16410	AK171739	11.63	10.94	11.37	11.05	No
A_52_P298088	Itgb1bp2	26549	NM_013712	2.76	7.39	2.70	6.08	No
A_52_P143287	Itgb4	192897	NM_001005608	9.67	8.54	9.80	8.74	No
A_51_P408382	Itgb4	192897	NM_001005608	9.89	8.68	9.94	8.79	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P195107	Itgb6	16420	NM_021359	3.50	10.59	4.41	9.92	Yes
A_51_P161054	Itgb6	16420	NM_021359	2.70	8.85	2.80	7.72	No
A_52_P507736	Itgb8	320910	XM_006516016	6.96	6.24	7.27	7.12	Yes
A_52_P629324	Itgb8	320910	XM_006516016	4.66	3.37	4.60	4.37	No
A_52_P50623	Itk	16428	AK037554	10.66	9.99	10.91	10.32	No
A_51_P519314	Itpa	16434	NM_025922	11.84	12.43	11.71	12.12	No
A_51_P480408	Itp2	16439	NM_019923	4.26	3.35	4.61	4.03	No
A_51_P104897	Itp3	16440	NM_080553	14.90	14.12	14.97	14.41	No
A_52_P337427	Izumo4	71564	NM_027829	9.50	8.57	9.70	9.22	No
A_51_P451338	Jag2	16450	NM_010588	3.14	6.18	3.11	5.43	No
A_51_P483231	Jak2	16452	NM_008413	9.07	8.42	9.67	9.21	Yes
A_52_P512847	Jak3	16453	NM_010589	6.05	4.90	5.40	5.38	No
A_52_P374846	Jam2	67374	NM_023844	4.16	6.35	5.28	6.37	No
A_51_P418375	Jam2	67374	NM_023844	9.62	10.94	9.91	10.80	No
A_51_P358354	Jam3	83964	NM_023277	11.90	12.90	12.27	12.53	No
A_52_P389131	Jam3	83964	NM_023277	3.66	5.14	4.27	4.41	No
A_52_P408411	Jazf1	231986	NM_001168277	8.35	7.40	8.16	7.47	No
A_51_P325491	Josd2	66124	NM_025368	11.99	12.85	11.98	12.41	No
A_51_P218653	Jph2	59091	NM_021566	2.40	7.12	3.05	5.84	Yes
A_52_P142143	Jph2	59091	NM_021566	4.59	9.44	3.25	8.00	No
A_51_P191262	Jsrp1	71912	NM_028001	3.55	10.90	3.70	9.66	No
A_51_P159201	Junb	16477	NM_008416	13.42	14.23	13.79	14.34	No
A_51_P159194	Junb	16477	NM_008416	13.43	14.08	13.63	14.08	No
A_52_P401386	Jup	16480	NM_010593	7.00	6.13	7.03	6.61	No
A_51_P394384	Katmb11	72425	NM_024254	8.38	9.39	8.23	9.43	No
A_51_P394387	Katmb11	72425	NM_024254	9.19	10.31	9.16	10.28	No
A_52_P374157	Kbtbd8	243574	NM_001008785	3.27	5.68	3.29	4.40	Yes
A_52_P326513	Kcna4	16492	NM_021275	5.23	3.84	5.96	4.78	Yes
A_52_P350554	Kcnb1	16500	NM_008420	3.24	4.58	3.87	3.50	No
A_51_P507333	Kcnc2	268345	NM_001025581	6.02	4.67	6.50	5.99	Yes
A_52_P545132	Kcnc2	268345	NM_001025581	6.33	5.23	6.59	5.83	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P153132	Kcne2	246133	NM_134110	4.56	3.17	4.08	2.97	No
A_52_P185255	Kcnip3	56461	NM_019789	7.94	6.86	8.14	7.32	No
A_51_P325941	Kcnj12	16515	NM_010603	3.53	6.77	2.31	4.56	Yes
A_52_P509421	Kcnt2	240776	NM_001081027	4.07	2.53	4.79	3.64	Yes
A_52_P224348	Kctd12b	207474	NM_175429	9.27	8.56	8.30	7.84	Yes
A_51_P147422	Kctd19	279499	NM_177791	6.55	5.84	7.11	6.53	No
A_51_P107782	Kdsr	70750	NM_027534	11.40	10.69	11.84	11.54	Yes
A_51_P123655	Kera	16545	NM_008438	5.81	3.98	4.89	4.07	No
A_52_P72434	Khk	16548	NM_008439	9.70	8.86	9.66	9.14	No
A_51_P459465	Kidins220	77480	NM_001081378	4.63	6.90	5.18	6.22	No
A_52_P220858	Kif24	109242	NM_024241	5.27	6.69	5.74	6.02	No
A_51_P103586	Kif26b	269152	AK052046	6.34	8.06	7.11	7.25	No
A_51_P179258	Kif26b	269152	AK052046	7.58	9.33	7.55	8.72	No
A_52_P10195	Kifc3	16582	NM_010631	6.29	8.12	6.18	7.41	Yes
A_51_P114297	Kifc3	16582	NM_010631	8.71	10.57	8.72	9.99	No
A_51_P133936	Kitl	17311	NM_013598	12.07	10.34	12.35	11.05	No
A_51_P133936	Kitl	17311	NM_013598	11.93	10.29	12.38	11.24	No
A_51_P133936	Kitl	17311	NM_013598	12.17	10.68	12.37	11.24	No
A_51_P133936	Kitl	17311	NM_013598	12.01	10.53	12.38	11.26	No
A_51_P133936	Kitl	17311	NM_013598	12.15	10.73	12.41	11.24	No
A_51_P133936	Kitl	17311	NM_013598	12.12	10.72	12.41	11.21	No
A_51_P133936	Kitl	17311	NM_013598	12.11	10.74	12.41	11.30	No
A_51_P133936	Kitl	17311	NM_013598	11.97	10.69	12.39	11.23	No
A_51_P133936	Kitl	17311	NM_013598	11.87	10.63	12.28	11.14	No
A_51_P133936	Kitl	17311	NM_013598	11.90	10.73	12.38	11.16	No
A_51_P347547	Klf10	21847	NM_013692	9.79	11.44	9.89	11.38	No
A_52_P817975	Klf12	16597	XM_006518623	10.87	9.99	10.68	10.16	No
A_52_P617636	Klf12	16597	XM_006518623	9.08	7.97	9.24	8.43	No
A_52_P64383	Klf15	66277	NM_023184	5.74	3.80	5.40	4.90	No
A_51_P507290	Klf5	12224	NM_009769	9.14	10.86	9.28	10.41	No
A_51_P451346	Klf6	23849	NM_011803	14.42	13.65	14.27	13.91	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P206861	Klf8	245671	NM_173780	8.55	7.87	8.49	8.27	No
A_52_P675996	Klf9	16601	NM_010638	7.79	6.96	8.00	7.13	No
A_51_P412200	Klf9	16601	NM_010638	9.04	8.17	9.21	8.50	No
A_51_P259118	Klhl1	93688	NM_053105	7.98	6.85	7.90	7.48	No
A_51_P521304	Klhl13	67455	NM_026167	2.27	6.91	2.27	5.36	No
A_51_P150905	Klhl22	224023	NM_145479	12.08	13.05	12.22	12.38	Yes
A_52_P550094	Klhl24	75785	NM_029436	8.53	7.84	8.40	8.29	No
A_52_P348250	Klhl29	208439	NM_001164493	5.19	6.66	5.89	6.20	No
A_51_P205215	Klhl30	70788	NM_027551	6.39	10.46	5.00	9.35	Yes
A_52_P90684	Klhl31	244923	NM_172925	3.27	8.11	3.17	6.46	No
A_51_P292447	Klhl36	234796	NM_146219	9.39	8.63	9.50	8.92	No
A_51_P235139	Klhl40	72330	NM_028202	3.65	9.83	3.28	9.25	No
A_52_P647740	Klhl41	228003	NM_001081087	2.54	9.95	2.76	8.62	Yes
A_51_P176474	Kndc1	76484	NM_177261	4.48	1.86	4.19	4.04	Yes
A_51_P256682	Kpna1	16646	NM_008465	9.01	9.74	8.06	8.81	Yes
A_51_P390239	Kremen1	84035	NM_032396	7.25	8.47	7.39	8.09	No
A_52_P143056	Kremen1	84035	NM_032396	6.06	6.84	6.23	6.72	No
A_51_P287691	Kremen2	73016	NM_028416	2.10	6.11	2.44	4.97	Yes
A_51_P111285	Krt10	16661	NM_010660	8.70	10.09	8.96	9.73	No
A_51_P482128	Krt15	16665	NM_008469	10.82	9.23	10.42	8.90	No
A_51_P152023	Krt80	74127	XM_006521487	5.20	6.86	5.25	7.32	No
A_52_P479051	Krtap6-1	16700	NM_010672	5.44	4.76	4.78	5.25	No
A_52_P114414	Ksr1	16706	XM_006532339	2.70	6.98	3.29	5.74	Yes
A_52_P666855	Ksr1	16706	XM_006532339	5.35	7.05	5.96	6.56	No
A_51_P139410	L1cam	16728	NM_008478	4.38	3.67	5.90	5.36	Yes
A_51_P408989	L3hypdh	67217	NM_026038	9.45	8.68	9.26	8.85	No
A_51_P191865	Lama2	16773	U12147	6.81	8.81	7.59	8.24	No
A_51_P191865	Lama2	16773	U12147	6.79	8.69	6.85	8.28	No
A_51_P191865	Lama2	16773	U12147	7.02	8.84	7.38	8.23	No
A_51_P191865	Lama2	16773	U12147	6.95	8.68	6.88	8.28	No
A_51_P191865	Lama2	16773	U12147	6.92	8.65	6.82	8.20	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P191865	Lama2	16773	U12147	6.88	8.54	6.75	8.16	No
A_51_P191865	Lama2	16773	U12147	6.84	8.47	6.74	8.19	No
A_51_P191865	Lama2	16773	U12147	6.77	8.40	6.92	8.29	No
A_51_P191865	Lama2	16773	U12147	7.01	8.57	6.83	8.18	No
A_51_P191865	Lama2	16773	U12147	7.29	8.62	6.89	8.27	No
A_52_P652859	Lama2	16773	U12147	4.89	6.97	4.96	6.67	No
A_51_P411061	Lama5	16776	NM_001081171	8.89	11.96	7.24	11.01	No
A_51_P248044	Lamb2	16779	NM_008483	13.40	12.64	13.16	12.61	No
A_51_P174943	Lamc2	16782	NM_008485	7.25	7.95	7.10	7.87	No
A_52_P142807	Lamp3	239739	NM_177356	9.71	10.48	9.87	10.50	No
A_52_P572284	Laptn4a	17775	NM_008640	15.15	14.52	15.04	14.54	No
A_52_P772918	Larp1	73158	NM_028451	8.18	9.02	8.84	9.07	No
A_52_P239726	Larp4	207214	BC075726	8.36	8.97	8.30	8.68	No
A_52_P1029978	Laspl	16796	NM_010688	16.12	15.30	15.97	15.40	No
A_52_P567306	Laspl	16796	NM_010688	5.80	5.07	6.08	5.08	No
A_51_P454008	Lbp	16803	NM_008489	7.01	6.03	5.72	4.61	Yes
A_51_P165602	Lbx2	16815	NM_010692	7.76	7.11	7.75	7.52	No
A_52_P389874	Ldb3	24131	NM_011918	6.66	9.32	5.95	7.48	Yes
A_51_P265351	Ldb3	24131	NM_011918	3.34	6.06	2.90	4.56	Yes
A_51_P265348	Ldb3	24131	NM_011918	5.63	7.20	5.37	5.99	Yes
A_51_P501145	Lef1	16842	NM_010703	7.80	9.00	8.43	9.09	No
A_51_P420400	Lef1	16842	NM_010703	6.49	7.67	7.60	7.78	No
A_52_P563375	Lgals2	107753	NM_025622	6.36	5.42	6.26	5.54	No
A_51_P302327	Lgals7	16858	NM_008496	7.95	6.59	6.95	5.77	Yes
A_51_P442746	Lgr4	107515	NM_172671	11.39	10.77	10.95	10.30	No
A_52_P497908	Lhx6	16874	NM_008500	10.10	9.25	10.03	9.83	No
A_52_P579352	Lifr	16880	NM_013584	7.12	8.18	6.75	7.69	No
A_51_P113182	Lifr	16880	NM_013584	5.83	6.63	5.45	6.29	No
A_52_P438170	Lifr	16880	NM_013584	4.04	5.54	4.21	4.69	No
A_51_P436491	Lingo3	237403	NM_001013758	3.08	7.13	3.04	5.64	Yes
A_51_P459661	Lipa	16889	NM_021460	11.37	10.59	11.49	10.78	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P435366	Lipe	16890	NM_010719	6.89	6.21	6.87	6.55	No
A_52_P58594	Lipo1	381236	XM_006527164	10.35	9.69	10.37	9.97	No
A_51_P348749	Llgl2	217325	NM_145438	6.95	6.37	7.46	7.15	Yes
A_51_P367263	Lman11	235416	NM_199222	5.94	8.44	6.07	7.63	No
A_52_P112651	Lman2	66890	NM_025828	9.67	10.33	9.37	9.76	No
A_51_P200867	Lmf1	76483	BC020104	12.96	13.83	12.97	13.41	No
A_52_P426554	Lmnb2	16907	NM_010722	10.15	13.23	9.58	12.16	Yes
A_52_P460319	Lmnb2	16907	NM_010722	7.11	9.46	6.53	8.47	No
A_51_P427964	Lmod3	320502	NM_001081157	5.67	13.90	5.55	12.90	Yes
A_52_P450965	LOC102635200	102635200	XR_397441	4.94	4.14	4.51	4.33	No
A_52_P1083765	LOC102636154	102636154	XR_379389	8.94	8.31	9.25	8.88	No
A_52_P900367	LOC102640237	102640237	XR_377736	4.71	5.76	4.90	4.96	Yes
A_51_P300493	Lox	16948	NM_010728	14.99	14.14	15.26	14.39	No
A_52_P480351	Lox12	94352	NM_033325	6.79	6.17	6.32	5.78	No
A_52_P460537	Lpar2	53978	NM_020028	7.42	6.35	7.16	6.67	No
A_51_P134452	Lpar3	65086	NM_022983	5.67	6.82	4.90	6.97	No
A_52_P184606	Lphn3	319387	XM_006534969	5.58	4.62	5.65	5.37	Yes
A_52_P576258	Lphn3	319387	XM_006534969	10.58	9.80	10.80	10.30	No
A_51_P259296	Lpl	16956	NM_008509	4.44	6.77	5.19	7.58	No
A_51_P306067	Lpp	210126	NM_178665	13.27	12.67	13.04	12.74	No
A_52_P32971	Lpp	210126	NM_178665	7.60	7.00	7.57	7.04	No
A_52_P389957	Lrch1	380916	XM_006519154	5.28	6.60	4.89	5.96	Yes
A_51_P116137	Lrig1	16206	NM_008377	10.36	11.76	10.29	11.65	No
A_51_P442990	Lrpprc	72416	NM_028233	12.31	13.10	12.35	12.77	No
A_51_P337210	Lrrc4	192198	NM_138682	3.37	5.29	3.59	4.12	Yes
A_52_P375546	Lrrc46	69297	NM_027026	7.22	6.18	6.53	6.15	No
A_51_P420841	Lrrc8b	433926	AK054249	8.75	9.66	8.59	9.34	No
A_51_P310298	Lrrc8d	231549	NM_178701	8.19	8.83	8.46	8.85	No
A_51_P107738	Lrrc8e	72267	NM_028175	4.60	3.47	5.07	4.53	Yes
A_52_P518764	Lrrccl	71710	NM_028915	8.04	7.28	8.00	7.47	No
A_51_P290788	Lrrk2	66725	NM_025730	9.61	8.82	9.86	9.35	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P453374	Lrrn4cl	68852	NM_001013019	11.14	9.88	11.43	11.02	No
A_51_P460798	Lsmp	268890	XM_006522201	5.80	4.68	5.04	4.92	No
A_52_P422755	Lsm6	78651	NM_030145	9.48	10.09	9.25	9.52	No
A_52_P655743	Lsm6	78651	NM_030145	12.83	13.82	12.63	13.23	No
A_51_P152802	Lsm6	78651	NM_030145	7.51	8.40	7.45	7.79	No
A_52_P63044	Lsp1	16985	NM_019391	9.06	9.74	8.34	8.78	Yes
A_51_P197816	Ltv1	353258	AK011491	7.06	7.91	7.21	7.72	No
A_51_P167527	Lum	17022	NM_008524	10.86	9.62	8.92	8.29	Yes
A_51_P265495	Ly6a	110454	NM_010738	12.11	11.06	11.98	11.03	No
A_52_P230938	Ly6c1	17067	NM_010741	8.56	7.53	8.49	7.71	No
A_51_P197528	Ly6c2	100041546	NM_001099217	13.47	12.45	13.29	12.46	No
A_51_P517695	Ly6f	17071	NM_008530	6.69	5.56	6.47	5.48	No
A_51_P420918	Ly6i	57248	NM_020498	8.76	7.62	8.55	7.74	No
A_51_P452189	Lyar	17089	NM_025281	10.63	11.22	10.72	11.33	No
A_52_P376169	Lypd6	320343	NM_177139	3.98	7.94	3.71	6.77	Yes
A_51_P105068	Lypd6b	71897	NM_027990	2.60	5.94	2.83	4.28	No
A_52_P540462	Lyrm9	66274	NM_001076681	6.63	7.52	6.32	6.67	Yes
A_52_P393120	Lyrm9	66274	NM_001076681	4.39	5.17	4.61	4.86	No
A_52_P238027	Lyz2	17105	AK159276	7.49	6.79	7.01	6.43	No
A_51_P453818	Lzts1	211134	XM_006509608	7.10	10.56	5.70	9.51	No
A_52_P120022	Lzts3	241638	NM_197945	10.20	12.46	10.56	11.51	Yes
A_51_P243514	Macc1	238455	NM_001163136	4.64	7.63	3.89	7.74	No
A_51_P213030	Macrocl	107227	NM_134147	10.61	12.28	10.86	12.11	No
A_52_P559957	Macrocl	107227	NM_134147	10.78	12.45	11.15	12.48	No
A_51_P121635	Maged2	80884	NM_030700	14.72	14.06	14.19	13.74	No
A_51_P497692	Mageh1	75625	NM_023788	9.45	8.81	9.34	8.88	No
A_51_P259995	Mak16	67920	NM_026453	9.62	10.38	9.74	10.12	No
A_52_P108169	Mamstr	74490	NM_172418	7.04	8.35	7.12	7.54	Yes
A_51_P122141	Mamstr	74490	NM_172418	4.62	6.69	5.09	5.88	Yes
A_51_P324923	Man2a1	17158	NM_008549	14.68	13.80	14.41	14.05	No
A_51_P205139	Map1b	17755	NM_008634	5.14	7.19	5.22	6.34	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P431870	Map1s	270058	NM_173013	10.82	11.62	11.06	11.53	No
A_51_P241074	Map2k1	26395	NM_008927	10.59	11.54	11.08	11.49	No
A_51_P377789	Map3k12	26404	NM_009582	10.83	10.16	11.00	10.84	Yes
A_52_P364130	Map3k14	53859	NM_016896	9.00	10.31	9.33	10.77	No
A_52_P528772	Map4	17758	AK019079	6.90	10.14	6.32	8.46	No
A_51_P465571	Map7d2	78283	NM_001081124	5.75	4.95	5.85	5.08	No
A_52_P540360	Mapk15	332110	NM_177922	5.01	4.37	4.75	4.53	No
A_51_P158628	Mapk3	26417	NM_011952	12.59	11.85	12.43	11.98	No
A_51_P158628	Mapk3	26417	NM_011952	12.57	11.86	12.44	12.02	No
A_51_P158628	Mapk3	26417	NM_011952	12.71	12.01	12.48	12.06	No
A_51_P158628	Mapk3	26417	NM_011952	12.68	11.98	12.54	12.10	No
A_51_P158628	Mapk3	26417	NM_011952	12.57	11.88	12.48	12.00	No
A_51_P158628	Mapk3	26417	NM_011952	12.46	11.77	12.40	12.01	No
A_51_P158628	Mapk3	26417	NM_011952	12.58	11.91	12.52	12.06	No
A_51_P158628	Mapk3	26417	NM_011952	12.53	11.87	12.48	12.13	No
A_52_P243249	Mapk3	26417	NM_011952	12.73	12.02	12.65	12.15	No
A_52_P349477	Mapk8ip1	19099	NM_011162	3.72	5.21	3.68	4.09	No
A_51_P438149	Mapre2	212307	NM_153058	12.85	11.81	12.49	11.97	No
A_52_P588298	Marf1	223989	AK035704	4.88	4.22	4.50	4.57	No
A_52_P541175	Marveld1	277010	NM_183195	11.86	11.26	12.09	11.63	No
A_51_P158814	Marveld1	277010	NM_183195	6.92	6.05	7.13	6.63	No
A_52_P247185	Masp1	17174	XM_006521828	14.59	13.61	14.52	13.99	No
A_52_P297093	Matn3	17182	NM_010770	4.21	4.96	2.83	2.50	Yes
A_51_P193185	Mb	17189	NM_013593	3.48	10.78	2.80	8.82	No
A_52_P228437	Mbnl1	56758	XM_006501794	8.76	7.95	8.23	7.60	No
A_51_P474709	Mcu	215999	NM_001033259	11.10	11.81	11.37	11.49	No
A_52_P599264	Mdfic	16543	NM_175088	10.39	9.66	10.08	9.80	No
A_51_P490823	Me2	107029	XM_006525496	9.21	9.92	9.46	9.73	No
A_52_P370731	Meaf6	70088	AK039242	3.42	4.33	4.10	3.78	No
A_51_P116027	Med27	68975	NM_026896	12.33	12.93	12.23	12.55	No
A_52_P290579	Mef2a	17258	NM_001033713	5.49	7.51	5.34	6.77	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P175157	Mef2a	17258	NM_001033713	9.59	11.89	9.68	11.39	No
A_52_P311841	Mef2a	17258	NM_001033713	5.76	7.99	5.84	7.18	No
A_51_P135802	Mef2c	17260	NM_025282	8.20	9.69	7.73	8.14	Yes
A_51_P504672	Mef2d	17261	AK141270	6.24	7.39	6.44	7.16	No
A_51_P394676	Mef2d	17261	AK141270	12.52	13.38	12.46	13.08	No
A_51_P514070	Megf10	70417	NM_001001979	10.33	12.82	10.19	12.02	Yes
A_52_P449718	Megf6	230971	NM_001162977	4.21	6.68	3.15	5.23	No
A_52_P575691	Meis3	17537	NM_008627	7.87	7.24	8.01	7.61	No
A_52_P667222	Memol	76890	NM_133771	10.63	11.46	10.74	11.21	No
A_52_P205695	Mettl7a1	70152	NM_027334	9.35	8.36	9.52	8.64	No
A_52_P458177	Mettl7a2	393082	AB099518	6.51	5.16	6.36	5.54	No
A_52_P319066	Mex3b	108797	NM_175366	4.27	4.93	3.45	3.72	Yes
A_52_P474266	Mfap3l	71306	NM_001177881	6.37	8.60	7.26	9.09	Yes
A_52_P634185	Mfap3l	71306	NM_001177881	2.90	4.98	3.89	5.55	No
A_52_P139569	Mfap3l	71306	NM_001177881	9.34	11.56	9.80	12.14	No
A_51_P195244	Mfap4	76293	NM_029568	9.52	11.10	7.43	8.86	Yes
A_51_P494825	Mfhas1	52065	NM_001081279	12.03	10.97	11.54	11.21	No
A_51_P324351	Mfi2	30060	NM_013900	8.68	6.70	8.81	7.34	No
A_51_P279437	Mfsd2a	76574	NM_029662	5.76	7.40	6.12	7.44	No
A_52_P658320	Mfsd7a	243197	NM_172883	3.48	5.47	4.22	5.07	No
A_51_P386899	Mfsd7c	217721	NM_145447	3.45	4.72	3.78	3.67	Yes
A_52_P485850	Mgam	232714	NM_001171003	2.47	4.46	3.15	5.29	No
A_51_P291260	Mgam	232714	NM_001171003	7.10	8.47	7.17	9.02	No
A_51_P495360	Mgat3	17309	NM_010795	7.63	6.63	6.87	6.31	No
A_52_P81461	Mgea5	76055	NM_023799	12.82	12.23	12.69	12.53	No
A_51_P287810	Mgst1	56615	NM_019946	7.70	6.97	6.91	6.51	No
A_51_P150120	Mgst2	211666	NM_174995	8.47	6.91	7.42	6.42	No
A_51_P215077	Mgst3	66447	AK019197	11.93	10.92	11.82	11.19	No
A_51_P253824	Mgst3	66447	AK019197	10.80	9.68	10.90	10.25	No
A_51_P276051	Micall2	231830	NM_174850	12.46	11.45	12.49	11.73	No
A_52_P232470	Mief2	237781	NM_001009927	6.54	7.30	6.71	6.91	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P185871	Mkrl1	54484	NM_018810	11.18	10.50	10.96	10.52	No
A_51_P342031	Mkrl3	22652	NM_011746	3.65	5.41	3.37	5.01	No
A_51_P379660	Mllt11	56772	NM_019914	10.87	12.36	10.64	11.88	No
A_52_P518808	Mmd	67468	AK007961	7.65	8.61	7.31	8.54	No
A_52_P429723	Mme	17380	NM_008604	8.49	7.36	8.27	7.48	No
A_51_P217517	Mmel1	27390	NM_013783	6.99	9.43	6.86	7.81	Yes
A_52_P15507	Mmgt2	216829	NM_175002	10.22	9.26	9.78	9.10	No
A_51_P220062	Mmp15	17388	NM_008609	6.23	8.29	6.52	7.69	No
A_52_P975	Mmp16	17389	NM_019724	13.84	12.89	14.09	13.25	No
A_51_P502132	Mmp23	26561	NM_011985	6.96	6.14	6.70	6.24	No
A_51_P414396	Mmrn2	105450	NM_153127	8.93	7.90	8.23	7.63	No
A_52_P477431	Mnt	17428	NM_010813	12.73	11.93	12.69	12.30	No
A_51_P470205	Mob3b	214944	AK089781	5.72	4.22	5.96	5.29	No
A_51_P140321	Mocos	68591	NM_026779	9.60	8.52	10.05	9.22	Yes
A_52_P86935	Mocos	68591	NM_026779	6.99	6.11	7.57	6.49	No
A_52_P579441	Morc3	338467	NM_001045529	10.17	10.77	10.12	10.52	No
A_52_P36928	Morn2	378462	NM_194269	9.25	8.60	9.19	8.62	No
A_52_P45170	Mospd1	70380	NM_027409	11.64	12.46	11.45	11.84	Yes
A_51_P411217	Mospd1	70380	NM_027409	7.33	8.14	7.54	7.70	No
A_51_P368660	Mphosph10	67973	NM_026483	7.69	8.28	7.81	7.67	Yes
A_52_P134170	Mphosph10	67973	NM_026483	6.78	7.52	7.06	7.04	No
A_51_P462978	Mpp2	50997	NM_016695	6.43	5.82	6.72	6.07	No
A_51_P228295	Mpz11	68481	NM_001001880	15.31	14.43	15.47	14.73	No
A_51_P160913	Mr1	15064	NM_008209	9.14	8.26	9.10	8.59	No
A_51_P519700	Mras	17532	NM_008624	12.07	11.33	11.88	11.23	No
A_52_P203798	Mras	17532	NM_008624	8.04	7.37	8.00	7.64	No
A_51_P519696	Mras	17532	NM_008624	10.66	9.86	10.59	10.06	No
A_52_P535782	Mri1	67873	AK047287	4.13	4.89	4.82	5.30	No
A_52_P186686	Mro	71263	NM_027741	2.88	6.76	2.55	6.16	No
A_52_P547478	Mrpl15	27395	NM_025300	10.49	11.40	10.82	11.28	No
A_51_P362292	Ms4a10	69826	NM_023529	2.93	6.88	2.70	5.76	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P148744	Msantdl	403174	NM_207277	5.00	7.28	5.07	6.05	Yes
A_52_P326240	Msh5	17687	NM_013600	5.40	4.58	5.39	4.84	No
A_51_P429865	Msh5	17687	NM_013600	5.69	4.86	5.12	4.67	No
A_52_P780821	Msi2	76626	AK031365	9.21	11.23	9.06	10.06	Yes
A_52_P386979	Msi2	76626	AK031365	8.58	10.85	8.96	10.15	Yes
A_51_P221449	Msi2	76626	AK031365	7.18	9.15	7.01	8.32	No
A_52_P628060	Msi2	76626	AK031365	8.05	10.15	8.04	9.35	No
A_52_P580159	Mtcl1	68617	AK051750	8.76	9.58	9.48	9.93	No
A_51_P195622	Mtdh	67154	NM_026002	9.45	10.11	9.70	10.12	No
A_51_P175699	Mtfp1	67900	NM_026443	9.15	10.03	9.05	9.39	No
A_51_P392928	Mthfd1	108156	NM_138745	11.33	12.14	11.49	11.88	No
A_51_P138189	Mthfs	107885	NM_026829	11.63	10.90	11.65	11.33	No
A_52_P350537	Mtmr11	194126	NM_181409	9.56	8.86	9.57	9.17	No
A_52_P296208	Mtrr	210009	NM_172480	5.41	6.23	5.47	6.19	No
A_52_P421947	Mtss1	211401	NM_144800	4.32	6.41	3.28	5.12	Yes
A_52_P601055	Mtss1	211401	NM_144800	8.98	10.61	7.97	9.49	Yes
A_51_P201338	Mtss1	211401	NM_144800	5.36	7.99	5.34	6.90	Yes
A_52_P211197	Mturn	68235	NM_001289740	8.33	7.32	8.59	7.97	Yes
A_51_P445487	Mturn	68235	NM_001289740	10.16	9.29	10.08	9.42	No
A_51_P218910	Mturn	68235	NM_001289740	9.88	9.15	10.01	9.63	No
A_51_P426130	Muc5ac	17833	NM_010844	5.97	5.05	5.20	5.27	No
A_51_P378029	Murc	68016	NM_026509	9.33	12.27	8.63	11.87	No
A_52_P335064	Mustn1	66175	NM_181390	8.85	10.40	8.84	10.24	No
A_51_P514085	Mx2	17858	NM_013606	7.04	6.14	6.52	6.06	No
A_52_P230650	Mxd4	17122	AK040356	12.59	11.77	12.34	11.85	No
A_51_P294994	Mxi1	17859	NM_001008542	10.18	9.57	10.36	9.92	No
A_52_P440284	Mxra7	67622	NM_026280	11.39	13.14	11.15	12.44	No
A_51_P356579	Mxra7	67622	NM_026280	8.69	9.64	8.73	9.62	No
A_51_P275072	Myadml2	68515	NM_026751	2.54	6.15	3.14	5.47	No
A_51_P137121	Mybph	53311	NM_016749	2.06	12.53	2.24	11.11	No
A_51_P137125	Mybph	53311	NM_016749	3.84	12.79	3.81	11.37	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P93284	Mycl	16918	NM_008506	4.43	12.09	4.05	11.27	No
A_51_P281844	Myef2	17876	NM_010852	11.77	12.46	11.48	12.21	No
A_51_P364788	Myh1	17879	NM_030679	4.02	5.98	3.70	4.77	Yes
A_51_P192042	Myh1	17879	NM_030679	2.99	12.92	2.11	11.32	No
A_51_P395652	Myh2	17882	NM_001039545	2.18	8.30	2.33	6.37	Yes
A_52_P247953	Myh3	17883	NM_001099635	2.32	14.21	2.82	11.67	Yes
A_51_P167668	Myh3	17883	NM_001099635	2.14	14.75	2.27	12.39	Yes
A_51_P338072	Myh4	17884	NM_010855	2.02	5.77	3.74	4.36	No
A_51_P512210	Myh6	17888	NM_010856	2.23	8.73	2.65	5.65	Yes
A_51_P143162	Myh7	140781	NM_080728	6.18	14.14	5.50	11.23	Yes
A_52_P88033	Myh7	140781	NM_080728	5.88	14.32	5.12	11.42	Yes
A_51_P143296	Myh8	17885	NM_177369	2.61	12.74	3.36	10.83	Yes
A_51_P416858	Myl1	17901	NM_021285	2.43	14.47	2.43	12.65	Yes
A_52_P273120	Myl1	17901	NM_021285	2.30	10.09	2.84	7.95	No
A_51_P150480	Myl12a	67268	NM_026064	10.09	10.78	10.04	10.41	No
A_51_P177210	Myl3	17897	NM_010859	4.05	12.22	4.32	10.28	Yes
A_51_P413130	Myl4	17896	NM_010858	8.29	11.84	8.14	9.96	Yes
A_52_P544523	Myl4	17896	NM_010858	8.16	12.02	8.10	10.23	Yes
A_52_P145912	Mylk	107589	NM_139300	8.45	9.12	7.38	7.78	Yes
A_52_P575217	Mylk	107589	NM_139300	8.48	9.20	7.63	8.01	Yes
A_52_P1011632	Mylk4	238564	NM_001166030	2.60	10.81	2.73	8.94	Yes
A_52_P383572	Mylpf	17907	NM_016754	6.20	15.73	5.52	14.37	Yes
A_52_P685422	Myo10	17909	NM_019472	10.82	11.98	10.64	11.56	No
A_51_P421780	Myo18b	74376	AK016515	2.33	9.88	2.46	8.62	Yes
A_51_P126476	Myo1b	17912	NM_010863	11.36	10.49	10.74	10.28	No
A_52_P361673	Myo1b	17912	NM_010863	10.36	9.55	9.80	8.87	No
A_51_P126484	Myo1b	17912	NM_010863	10.88	10.15	10.40	10.07	No
A_52_P412408	Myo1h	231646	NM_001164573	5.23	4.43	5.08	5.39	Yes
A_52_P66130	Myo5b	17919	NM_201600	2.39	5.65	3.47	4.40	No
A_52_P564653	Myo6	17920	U49739	8.71	7.88	8.53	7.49	No
A_51_P245391	Myo9a	270163	NM_173018	5.43	4.64	5.70	5.28	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P14881	Myo9b	17925	NM_015742	5.93	5.24	5.81	5.38	No
A_51_P351166	Myod1	17927	NM_010866	5.95	9.90	6.92	8.65	No
A_51_P351166	Myod1	17927	NM_010866	6.26	10.07	6.88	8.53	No
A_51_P351166	Myod1	17927	NM_010866	6.14	9.89	7.08	8.76	No
A_51_P351166	Myod1	17927	NM_010866	6.15	9.87	7.02	8.30	No
A_51_P351166	Myod1	17927	NM_010866	6.20	9.90	6.94	8.54	No
A_51_P351166	Myod1	17927	NM_010866	6.40	9.91	7.00	8.66	No
A_51_P351166	Myod1	17927	NM_010866	6.42	9.87	7.10	8.54	No
A_51_P351166	Myod1	17927	NM_010866	6.66	9.96	7.22	8.63	No
A_51_P351166	Myod1	17927	NM_010866	6.75	9.94	7.01	8.47	No
A_51_P351166	Myod1	17927	NM_010866	6.86	9.98	7.14	8.41	No
A_51_P102122	Myog	17928	NM_031189	3.38	17.69	3.00	16.34	Yes
A_51_P102122	Myog	17928	NM_031189	3.45	17.42	3.31	16.33	Yes
A_51_P102122	Myog	17928	NM_031189	3.26	17.46	3.25	16.39	Yes
A_51_P102122	Myog	17928	NM_031189	3.75	17.46	3.08	16.40	Yes
A_51_P102122	Myog	17928	NM_031189	3.48	17.40	3.60	16.36	Yes
A_51_P102122	Myog	17928	NM_031189	3.59	17.45	3.26	16.41	Yes
A_51_P102122	Myog	17928	NM_031189	3.74	17.50	3.20	16.47	Yes
A_51_P102122	Myog	17928	NM_031189	3.56	17.43	3.13	16.41	Yes
A_51_P102122	Myog	17928	NM_031189	3.41	17.44	2.73	16.45	Yes
A_51_P102122	Myog	17928	NM_031189	3.50	17.34	3.14	16.44	Yes
A_51_P302520	Myom1	17929	NM_010867	3.84	9.64	3.54	8.02	Yes
A_51_P431785	Myom2	17930	NM_008664	6.85	13.84	6.35	12.82	Yes
A_51_P268983	Myom3	242702	NM_001085509	3.76	13.05	3.54	10.70	Yes
A_51_P379750	Myoz1	59011	NM_021508	4.30	6.09	4.04	5.16	Yes
A_52_P305767	Myoz1	59011	NM_021508	3.74	5.32	3.36	4.60	No
A_51_P248638	Myoz2	59006	NM_021503	3.17	8.24	4.24	6.76	Yes
A_52_P577853	Mysm1	320713	NM_177239	7.98	7.35	8.40	8.10	Yes
A_51_P437883	Mzfl	109889	NM_145819	8.46	7.68	8.14	7.63	No
A_51_P208931	N4bp3	212706	NM_145974	9.12	11.21	9.37	10.97	No
A_52_P191341	Naa15	74838	NM_053089	7.15	7.95	7.12	7.56	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P4657	Naa25	231713	BC048860	8.12	9.20	8.45	8.78	No
A_52_P440881	Naa25	231713	BC048860	8.41	9.46	8.81	9.26	No
A_52_P208665	Naa50	72117	NM_028108	11.43	12.35	11.26	11.93	No
A_51_P393587	Naa50	72117	NM_028108	11.08	12.04	11.10	11.84	No
A_51_P265026	Naip2	17948	NM_010872	7.00	6.00	8.30	7.56	Yes
A_51_P294233	Nanog	71950	NM_028016	2.90	5.89	4.89	4.73	No
A_51_P366207	Nap1l2	17954	NM_008671	6.17	7.02	6.21	6.74	No
A_51_P414115	Naprt1	223646	NM_172607	10.75	9.84	10.66	10.04	No
A_51_P124606	Nat2	17961	NM_010874	11.05	10.17	11.22	10.46	No
A_52_P201551	Nav1	215690	NM_173437	9.28	10.36	9.19	9.71	No
A_52_P627327	Nav3	260315	XM_006513678	8.82	9.75	8.99	9.65	No
A_52_P613255	Ncam1	17967	NM_001113204	10.49	11.52	10.41	10.98	No
A_52_P291924	Ncam1	17967	NM_001113204	5.09	7.82	5.82	7.22	No
A_52_P291924	Ncam1	17967	NM_001113204	5.59	7.90	6.09	7.19	No
A_52_P291924	Ncam1	17967	NM_001113204	5.47	7.78	5.83	7.13	No
A_52_P291924	Ncam1	17967	NM_001113204	5.69	7.92	6.03	7.16	No
A_52_P291924	Ncam1	17967	NM_001113204	5.63	7.79	5.94	7.20	No
A_52_P291924	Ncam1	17967	NM_001113204	5.75	7.92	6.03	7.32	No
A_52_P291924	Ncam1	17967	NM_001113204	5.49	7.64	5.83	7.21	No
A_52_P291924	Ncam1	17967	NM_001113204	5.68	7.69	5.85	7.21	No
A_52_P291924	Ncam1	17967	NM_001113204	5.87	7.88	5.89	7.34	No
A_52_P291924	Ncam1	17967	NM_001113204	5.71	7.67	5.89	7.16	No
A_52_P382754	Ncam1	17967	NM_001113204	9.23	12.31	9.57	11.39	No
A_52_P684037	Ncam1	17967	NM_001113204	5.29	7.58	5.63	6.99	No
A_51_P380309	Ncam1	17967	NM_001113204	12.45	13.59	12.40	13.31	No
A_52_P668157	Ncam1	17967	NM_001113204	3.95	5.34	3.87	4.93	No
A_52_P492989	Ncan	13004	NM_007789	4.81	3.87	4.77	4.42	No
A_51_P405606	Ndrp1	17988	NM_008681	13.97	12.72	14.21	13.15	No
A_51_P458540	Ndufa12	66414	NM_025551	11.58	12.23	11.73	12.10	No
A_51_P458538	Ndufa12	66414	NM_025551	13.30	13.88	13.35	13.72	No
A_52_P303235	Ndufab1	70316	NM_028177	8.59	9.37	8.65	9.27	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P1093473	Ndufab1	70316	NM_028177	13.08	13.72	13.11	13.55	No
A_51_P162098	Ndufaf4	68493	NM_026742	10.93	12.07	10.85	11.55	No
A_52_P325265	Ndufaf4	68493	NM_026742	5.47	7.00	5.66	6.28	No
A_52_P287387	Ndufaf4	68493	NM_026742	6.23	7.02	6.22	6.43	No
A_51_P395014	Ndufs3	68349	NM_026688	13.65	14.30	13.91	14.16	No
A_51_P495331	Neb	17996	NM_010889	2.39	10.13	3.27	7.61	Yes
A_51_P218535	Neb	17996	NM_010889	2.49	8.55	2.31	6.29	Yes
A_51_P339932	Neb	17996	NM_010889	2.43	6.45	2.15	4.79	Yes
A_52_P2632	Negr1	320840	NM_177274	6.23	4.51	7.28	6.97	No
A_51_P452323	Nes	18008	NM_016701	9.34	13.36	8.99	12.32	Yes
A_51_P452323	Nes	18008	NM_016701	9.22	13.06	8.91	12.25	Yes
A_51_P452323	Nes	18008	NM_016701	9.20	13.05	8.87	12.25	Yes
A_51_P452323	Nes	18008	NM_016701	9.23	13.12	8.94	12.33	Yes
A_51_P452323	Nes	18008	NM_016701	9.21	12.99	8.81	12.21	Yes
A_51_P452323	Nes	18008	NM_016701	9.21	13.13	8.92	12.37	Yes
A_51_P452323	Nes	18008	NM_016701	9.16	13.06	8.89	12.30	Yes
A_51_P452323	Nes	18008	NM_016701	9.18	13.01	8.85	12.25	Yes
A_51_P452323	Nes	18008	NM_016701	9.20	13.06	8.87	12.33	Yes
A_51_P452323	Nes	18008	NM_016701	9.25	13.06	8.85	12.36	Yes
A_51_P460143	Neu2	23956	NM_015750	8.81	7.72	8.65	7.81	No
A_52_P339011	Neurl1a	18011	NM_021360	7.55	11.85	7.92	10.70	No
A_52_P135736	Neurl1a	18011	NM_021360	4.24	8.23	4.39	7.13	No
A_52_P367520	Nexn	68810	NM_199465	11.06	12.87	10.84	12.40	No
A_52_P236448	Ngfr	18053	NM_033217	3.97	6.81	5.01	4.96	Yes
A_52_P236448	Ngfr	18053	NM_033217	3.88	6.76	3.80	5.03	Yes
A_52_P236448	Ngfr	18053	NM_033217	8.17	6.71	3.94	5.05	Yes
A_52_P236448	Ngfr	18053	NM_033217	4.87	6.91	4.61	5.26	Yes
A_52_P236448	Ngfr	18053	NM_033217	5.19	6.94	4.74	5.40	Yes
A_52_P236448	Ngfr	18053	NM_033217	5.48	6.90	4.64	5.36	Yes
A_52_P236448	Ngfr	18053	NM_033217	4.96	6.91	4.13	5.38	Yes
A_52_P236448	Ngfr	18053	NM_033217	5.20	6.96	4.68	5.44	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P236448	Ngfr	18053	NM_033217	5.02	7.03	4.84	5.51	Yes
A_52_P236448	Ngfr	18053	NM_033217	5.01	6.90	4.74	5.54	Yes
A_52_P313813	Nhp2	52530	NM_026631	12.29	13.37	12.26	13.16	No
A_51_P329975	Ninj2	29862	NM_016718	5.46	4.88	5.21	5.26	No
A_52_P987292	Nip7	66164	NM_025391	5.42	6.92	5.04	6.19	Yes
A_51_P231597	Nkain3	269513	AK051427	8.08	7.35	7.91	7.59	No
A_51_P124285	Nkd1	93960	NM_027280	10.66	12.06	9.51	10.68	No
A_52_P493925	Nkd1	93960	NM_027280	6.86	8.40	5.89	7.03	No
A_51_P500226	Nkiras2	71966	NM_028024	11.15	12.03	10.88	11.27	Yes
A_52_P145066	Nkiras2	71966	NM_028024	7.65	8.49	7.61	7.93	No
A_51_P438002	Nmb	68039	NM_026523	8.52	7.84	8.46	8.12	No
A_51_P125067	Nmi	64685	NM_019401	11.44	10.64	11.37	10.64	No
A_51_P369762	Nmrk2	69564	NM_027120	3.24	11.38	3.19	10.38	No
A_51_P347764	Noc3l	57753	NM_021315	6.98	7.78	7.28	7.86	No
A_51_P181691	Nod2	257632	NM_145857	2.43	6.98	2.07	6.05	Yes
A_51_P332228	Nol4	319211	NM_199024	4.43	5.11	4.27	5.80	Yes
A_51_P250832	Nol6	230082	NM_139236	7.29	7.88	7.66	7.74	No
A_51_P366277	Nol8	70930	NM_001271397	10.26	10.99	10.24	10.61	No
A_52_P89099	Nolc1	70769	NM_001039352	7.76	8.73	7.86	8.47	No
A_52_P463109	Nolc1	70769	NM_001039352	10.85	11.65	10.88	11.35	No
A_51_P258078	Nop2	110109	NM_138747	12.91	14.23	13.12	13.95	No
A_51_P187902	Nop56	67134	XM_006500062	13.68	14.29	13.59	14.31	No
A_52_P183088	Notch1	18128	Z11886	7.86	9.25	7.81	8.84	No
A_51_P508510	Notch1	18128	Z11886	8.84	10.33	8.91	9.82	No
A_51_P508510	Notch1	18128	Z11886	8.87	10.35	8.92	9.88	No
A_51_P508510	Notch1	18128	Z11886	8.77	10.23	8.82	9.74	No
A_51_P508510	Notch1	18128	Z11886	8.88	10.33	8.88	9.82	No
A_51_P508510	Notch1	18128	Z11886	8.86	10.29	8.88	9.78	No
A_51_P508510	Notch1	18128	Z11886	8.88	10.30	8.87	9.81	No
A_51_P508510	Notch1	18128	Z11886	8.88	10.25	8.81	9.74	No
A_51_P508510	Notch1	18128	Z11886	8.83	10.19	8.88	9.77	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P508510	Notch1	18128	Z11886	8.96	10.32	8.89	9.77	No
A_51_P508510	Notch1	18128	Z11886	8.89	10.24	8.93	9.86	No
A_52_P625940	Noxo1	71893	NM_027988	9.09	8.45	9.26	8.65	No
A_51_P232556	Nphp3	74025	NM_028721	8.56	7.69	8.59	8.30	No
A_52_P565549	Npr2	230103	NM_173788	4.67	3.91	4.69	4.44	No
A_51_P169783	Nprl3	17168	NM_181569	6.85	8.14	7.00	7.75	No
A_52_P122891	Npsr1	319239	NM_175678	4.21	7.67	4.63	6.03	Yes
A_51_P424338	Nqo1	18104	NM_008706	12.23	11.38	12.29	11.25	No
A_52_P35581	Nqo2	18105	NM_020282	6.88	6.27	6.71	6.31	No
A_51_P229536	Nqo2	18105	NM_020282	12.20	11.49	11.91	11.42	No
A_52_P494622	Nr4a2	18227	NM_013613	9.23	8.41	9.36	8.69	No
A_52_P28844	Nrap	18175	NM_008733	3.91	5.93	4.01	5.65	No
A_51_P180413	Nrcam	319504	NM_176930	5.02	7.64	5.57	7.67	No
A_52_P328064	Nrg1	211323	XM_006509089	5.24	4.31	4.96	4.37	No
A_52_P158772	Nrg1	211323	XM_006509089	9.85	8.99	9.45	9.00	No
A_52_P52635	Nrg1	211323	XM_006509089	8.29	7.52	8.17	7.77	No
A_52_P369769	Nrg1	211323	XM_006509089	9.09	8.46	8.98	8.53	No
A_52_P86892	Nrn11	234700	NM_175024	5.61	4.53	5.09	4.60	No
A_51_P448784	Nrsn1	22360	NM_009513	8.04	6.84	7.49	6.70	No
A_51_P163953	Nsg2	18197	NM_008741	6.34	10.10	5.12	8.63	Yes
A_51_P367025	Nsun7	70918	XM_006504105	5.15	3.90	5.68	5.19	No
A_52_P423849	Nt5c	50773	NM_015807	11.86	11.14	11.88	11.54	No
A_51_P425696	Nt5c2	76952	AK038326	9.54	10.60	9.58	10.14	No
A_52_P574668	Nt5e	23959	NM_011851	5.48	4.69	5.55	4.79	No
A_52_P359819	Ntf5	78405	NM_198190	7.03	6.04	7.05	6.67	Yes
A_51_P497661	Ntn4	57764	NM_021320	4.42	5.63	4.80	4.87	No
A_51_P193336	Nucb2	53322	NM_001130479	11.60	10.96	11.74	11.45	No
A_51_P148670	Nudt7	67528	NM_024437	10.10	9.38	10.17	9.63	No
A_52_P136914	Nudt7	67528	NM_024437	7.32	6.49	7.61	6.89	No
A_51_P420037	Nup210	54563	NM_018815	2.65	8.88	2.79	7.34	Yes
A_51_P219291	Nup93	71805	NM_172410	10.68	11.32	10.48	10.60	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P324754	Nupr1l	69034	NM_026916	8.07	7.30	8.23	7.76	No
A_52_P279579	Nyap1	243300	NM_175521	9.67	8.64	9.66	9.05	No
A_51_P428529	Oas1c	114643	NM_033541	8.57	7.41	8.78	7.70	No
A_51_P154842	Oas1f	243262	NM_145153	7.31	6.54	7.19	6.59	No
A_51_P387123	Oas12	23962	NM_011854	6.89	5.84	6.66	5.88	No
A_52_P642453	Obscn	380698	NM_199152	2.05	8.13	2.32	5.91	Yes
A_51_P479583	Obsl1	98733	NM_178884	13.39	14.35	13.36	13.75	No
A_52_P175342	Ogfr1l	70155	NM_001081079	9.83	8.07	9.72	8.58	No
A_51_P347312	Ogfr1l	70155	NM_001081079	11.43	10.28	11.45	10.46	No
A_51_P137236	Olfm1	56177	NM_019498	8.13	11.25	7.63	10.55	No
A_52_P454183	Olfm12b	320078	NM_177068	8.34	12.13	8.11	11.54	No
A_51_P282584	Olfm12b	320078	NM_177068	7.09	10.93	6.82	10.31	No
A_51_P191782	Olfm13	99543	NM_133859	11.85	11.13	11.40	10.61	No
A_51_P255646	Olf1065	258403	NM_146408	6.40	7.45	6.33	6.88	No
A_52_P646552	Olf128	383243	NM_206816	5.72	5.12	5.14	5.04	No
A_52_P219853	Olf1381	258461	NM_146469	6.04	5.30	5.41	5.49	No
A_51_P156196	Olf603	259073	NM_147070	4.31	3.43	4.20	3.98	No
A_51_P235764	Olf659	259052	NM_147050	2.24	5.34	3.48	4.25	Yes
A_52_P405994	Orai1	109305	NM_175423	8.25	9.64	8.47	9.05	No
A_51_P166244	Osbp11a	64291	NM_207530	13.16	12.37	12.98	12.61	No
A_52_P275354	Osbp17	71240	AK017095	10.93	10.24	10.86	10.36	No
A_51_P277345	Ostf1	20409	NM_017375	12.64	11.93	12.75	12.11	No
A_51_P107326	Oxct1	67041	NM_024188	15.65	15.07	15.34	14.94	No
A_52_P585509	Oxr1	170719	AK040881	4.74	5.67	5.49	5.37	No
A_51_P519420	P2rx5	94045	NM_033321	3.17	4.90	3.22	3.55	No
A_52_P587738	P2ry2	18442	NM_008773	8.73	10.21	9.12	9.86	No
A_51_P287635	P2ry2	18442	NM_008773	4.45	6.30	5.70	5.84	No
A_52_P365896	Pa2g4	18813	NM_011119	8.45	9.24	8.61	9.26	No
A_51_P390446	Pacs2	217893	NM_001291444	12.42	13.08	12.57	12.99	No
A_52_P545393	Pacsin2	23970	AF128535	10.52	11.13	10.67	10.90	No
A_51_P345896	Pafah2	100163	NM_133880	12.48	11.87	12.53	12.04	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P159060	Paip1	218693	NM_145457	9.35	8.42	9.55	9.04	No
A_51_P171883	Palmd	114301	NM_023245	5.14	8.31	4.57	7.28	No
A_51_P366513	Papss2	23972	NM_011864	7.23	6.19	7.69	6.76	No
A_51_P430973	Paqr7	71904	NM_027995	6.16	5.50	6.06	5.58	No
A_51_P422540	Paqr8	74229	NM_028829	10.42	9.42	10.72	9.85	No
A_51_P114693	Parm1	231440	AK161172	8.85	13.93	7.58	11.56	Yes
A_51_P114577	Parm1	231440	AK161172	5.64	10.32	5.89	8.01	Yes
A_51_P129199	Parp10	671535	NM_001163576	10.18	9.41	9.85	9.53	No
A_52_P593379	Pax9	18511	NM_011041	8.03	7.32	8.47	7.96	Yes
A_51_P324450	Pbp2	76400	NM_029595	3.96	3.36	4.16	3.49	No
A_52_P543460	Pbx1	18514	AK144446	9.29	8.64	9.60	8.82	No
A_52_P197199	Pcdh9	211712	AY861425	10.73	9.97	10.92	10.69	Yes
A_51_P475704	Pcdh9	211712	AY861425	7.77	6.81	7.91	7.32	No
A_51_P245789	Pcolce2	76477	NM_029620	9.19	7.65	9.73	8.64	No
A_51_P361150	Pcp4l1	66425	NM_025557	8.09	7.17	7.33	7.16	No
A_52_P496202	Pcsk1n	30052	NM_013892	8.93	10.17	8.99	9.41	No
A_51_P480578	Pcx	18563	NM_008797	12.57	13.21	12.51	12.60	Yes
A_52_P573	Pde11a	241489	NM_001081033	4.39	3.26	3.87	3.77	No
A_51_P120024	Pde2a	207728	NM_001008548	2.43	9.91	2.42	8.22	Yes
A_52_P502828	Pde2a	207728	NM_001008548	5.02	7.22	5.24	6.11	Yes
A_51_P489488	Pde4dip	83679	NM_178080	9.41	11.54	9.55	10.64	Yes
A_52_P674593	Pde5a	242202	NM_153422	8.25	6.90	7.48	6.22	No
A_52_P618427	Pdgfb	18591	NM_011057	5.81	6.96	5.51	7.03	No
A_51_P175262	Pdgfb	18591	NM_011057	3.81	5.20	4.01	5.47	No
A_52_P636948	Pdgfd	71785	NM_027924	9.01	8.29	9.39	9.32	Yes
A_52_P264283	Pdgfra	18595	NM_011058	5.87	4.68	6.23	5.45	No
A_52_P568792	Pdhb	68263	NM_024221	12.64	13.42	12.60	13.05	No
A_51_P321921	Pdhb	68263	NM_024221	12.14	12.99	12.16	12.61	No
A_51_P350453	Pdk4	27273	NM_013743	12.92	12.25	13.54	13.19	Yes
A_51_P472726	Pdlim2	213019	NM_145978	15.69	15.00	15.46	15.08	No
A_51_P157677	Pdlim3	53318	NM_016798	3.86	10.14	2.46	9.17	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P579531	Pdlim3	53318	NM_016798	4.78	10.68	3.95	9.71	No
A_51_P470432	Pdlim4	30794	AK035513	9.29	11.42	8.38	10.97	Yes
A_52_P149438	Pdlim5	56376	NM_019809	6.70	8.61	6.72	7.86	No
A_51_P506284	Pdlim7	67399	NM_026131	8.01	9.42	7.56	8.66	Yes
A_52_P489193	Pdlim7	67399	NM_026131	9.82	11.37	9.48	10.60	No
A_52_P112816	Pdssl	56075	NM_019501	6.61	7.62	6.49	7.28	No
A_52_P359502	Pdxk	216134	NM_172134	13.92	13.23	13.64	13.18	No
A_52_P609120	Pdyp	57028	NM_020271	7.77	9.20	7.80	8.66	No
A_51_P266695	Peli3	240518	NM_172835	7.63	6.77	7.58	7.06	No
A_52_P286166	Penk	18619	NM_001002927	8.68	7.61	9.45	8.31	No
A_51_P303397	Pepd	18624	NM_008820	12.88	13.66	12.77	13.39	No
A_52_P79889	Perl	18626	NM_011065	9.37	8.69	9.41	9.04	No
A_51_P317941	Perp	64058	NM_022032	9.39	8.30	8.78	7.86	No
A_52_P118958	Pex19	19298	NM_023041	10.64	11.57	10.71	11.06	No
A_51_P427177	Pex19	19298	NM_023041	9.11	9.91	9.11	9.54	No
A_51_P480861	Pga5	58803	NM_021453	4.17	5.60	4.60	4.90	No
A_51_P264495	Pgam2	56012	NM_018870	6.92	12.06	6.18	10.07	Yes
A_51_P226740	Pgap2	233575	NM_145583	9.25	8.59	8.87	8.56	No
A_52_P341449	Pgm3	109785	NM_028352	11.18	10.56	11.14	10.74	No
A_52_P96772	Pgp	67078	NM_025954	8.52	9.90	8.32	9.05	No
A_51_P466964	Pgp	67078	NM_025954	12.86	13.96	12.79	13.31	No
A_51_P144581	Pgrmc1	53328	NM_016783	14.21	13.48	14.06	13.63	No
A_51_P165934	Phb	18673	NM_008831	11.17	11.86	11.14	11.70	No
A_52_P380369	Phf11d	219132	BC030186	7.86	6.85	7.68	7.02	No
A_52_P218584	Phf201l	239510	NM_001081409	6.18	6.92	6.30	6.60	No
A_52_P125533	Phip	83946	NM_001081216	7.20	6.32	6.82	6.44	No
A_52_P126045	Phtf2	68770	NM_172992	7.08	7.80	7.08	7.76	No
A_52_P583433	Pign	27392	NM_013784	9.64	8.81	8.92	8.54	No
A_51_P186487	Pik3c2b	240752	AK049181	3.37	6.04	3.35	6.01	No
A_51_P463428	Pik3ip1	216505	AF528078	12.52	11.59	12.58	11.95	No
A_52_P484787	Pin1	23988	NM_023371	4.72	3.70	4.26	4.48	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P271468	Pip4k2a	18718	NM_008845	8.51	9.65	8.71	9.55	No
A_51_P386612	Pir	69656	NM_027153	11.16	10.44	11.18	10.68	No
A_52_P609109	Pitpnc1	71795	NM_145823	7.29	6.61	7.64	7.39	Yes
A_51_P501897	Pitx2	18741	NM_001042502	5.91	7.07	5.98	6.34	No
A_52_P141123	Pja1	18744	NM_008853	9.81	10.63	9.80	10.07	No
A_52_P234729	Pkd2	18764	NM_008861	13.13	12.39	13.00	12.61	No
A_51_P395258	Pkia	18767	M63554	6.15	8.96	5.92	8.23	No
A_52_P314129	Pkia	18767	M63554	9.29	12.14	9.37	11.35	No
A_52_P328249	Pkm	18746	NM_011099	5.21	5.99	5.22	5.95	No
A_52_P6442	Pknox2	208076	NM_001029838	3.96	6.85	3.56	5.73	No
A_51_P381618	Pla1a	85031	NM_134102	5.24	7.02	5.80	7.74	No
A_51_P402760	Pla2g4a	18783	NM_008869	12.18	11.18	12.22	11.74	No
A_51_P215627	Plac9a	211623	NM_207229	11.49	10.65	11.57	11.05	No
A_52_P686218	Plb1	665270	NM_001081407	9.49	8.41	8.16	7.30	Yes
A_52_P18262	Plcb1	18795	NM_019677	7.58	6.24	8.11	7.10	Yes
A_51_P284665	Plcb1	18795	NM_019677	8.79	7.12	9.12	7.52	No
A_52_P351574	Plcb1	18795	NM_019677	8.79	7.10	8.78	7.01	No
A_51_P137029	Plce1	74055	AK035546	6.98	6.09	7.23	6.46	No
A_51_P287617	Plekhh1	211945	NM_181073	6.53	10.32	6.57	9.30	Yes
A_52_P439502	Plekhh1	211945	NM_181073	2.91	7.89	2.60	6.71	No
A_51_P502194	Plekhh3	241075	NM_001039493	4.22	5.16	5.70	4.63	No
A_51_P509971	Plekho1	67220	NM_023320	12.31	12.96	11.82	12.37	Yes
A_51_P258150	Plin2	11520	NM_007408	15.41	14.73	15.33	15.02	No
A_51_P495780	Plin4	57435	NM_020568	12.36	10.79	12.59	11.50	No
A_51_P290576	Plk2	20620	NM_152804	13.03	12.30	13.33	12.96	No
A_51_P271614	Pls3	102866	NM_145629	14.58	15.65	14.59	15.07	No
A_51_P229675	Plscr1	22038	NM_011636	12.24	11.27	12.00	11.65	No
A_51_P229676	Plscr1	22038	NM_011636	12.13	11.44	11.96	11.52	No
A_52_P654841	Plscr1	22038	NM_011636	11.28	10.68	11.29	10.78	No
A_51_P110529	Plxna2	18845	NM_008882	9.34	10.56	9.87	10.70	No
A_51_P229602	Plxna2	18845	NM_008882	11.75	12.85	12.13	12.97	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P232748	Plxnb3	140571	NM_019587	4.67	7.22	5.08	6.62	No
A_51_P258529	Pmp22	18858	AK035065	14.45	13.85	14.10	13.73	No
A_52_P1067763	Pmp22	18858	AK035065	3.01	5.82	2.54	5.61	No
A_52_P642836	Pnmal2	434128	NM_001099636	3.87	4.69	3.71	4.26	No
A_51_P192589	Pno1	66249	NM_025443	12.13	13.17	12.39	13.13	No
A_51_P488890	Pnp	18950	NM_013632	11.89	11.26	11.91	11.50	No
A_51_P246754	Pnp0	103711	NM_134021	8.91	8.22	9.33	8.97	Yes
A_52_P460095	Polk	27015	NM_012048	3.56	2.59	3.70	3.69	No
A_51_P397493	Polm	54125	NM_017401	12.02	11.29	11.63	11.50	No
A_51_P236197	Polr1a	20019	AK087773	12.17	13.05	12.14	12.69	No
A_51_P410949	Polr3g	67486	NM_001081176	7.78	9.09	7.48	8.43	No
A_52_P662785	Polr3g	67486	NM_001081176	3.45	6.19	3.58	5.27	No
A_51_P484919	Polrmt	216151	NM_172551	11.50	12.21	11.61	11.99	No
A_51_P256665	Pon3	269823	NM_173006	10.96	9.96	11.01	10.65	Yes
A_52_P662226	Pon3	269823	NM_173006	10.53	9.68	10.69	10.30	Yes
A_51_P224311	Popdc2	64082	NM_022318	6.24	10.38	6.28	9.16	Yes
A_52_P486279	Popdc3	78977	NM_024286	6.34	9.19	6.42	8.16	No
A_51_P189985	Ppan	235036	NM_145610	11.55	12.33	11.76	12.33	No
A_51_P433026	Ppapdc2	74411	NM_028922	9.14	8.51	9.25	8.82	No
A_51_P321374	Ppapdc3	227721	NM_145521	6.31	11.15	6.18	9.52	Yes
A_51_P106799	Pparg	19016	NM_011146	12.16	10.90	12.15	11.42	No
A_51_P106799	Pparg	19016	NM_011146	11.89	10.70	12.11	11.44	No
A_51_P106799	Pparg	19016	NM_011146	11.90	10.84	12.14	11.44	No
A_51_P106799	Pparg	19016	NM_011146	11.87	10.83	12.10	11.40	No
A_51_P106799	Pparg	19016	NM_011146	11.85	10.83	12.08	11.39	No
A_51_P106799	Pparg	19016	NM_011146	11.87	10.90	12.14	11.37	No
A_51_P106799	Pparg	19016	NM_011146	11.88	10.93	12.18	11.49	No
A_51_P106799	Pparg	19016	NM_011146	11.79	10.84	12.10	11.43	No
A_51_P106799	Pparg	19016	NM_011146	11.74	10.80	12.08	11.39	No
A_51_P106799	Pparg	19016	NM_011146	11.89	11.00	12.19	11.54	No
A_51_P294891	Ppargclb	170826	NM_133249	5.33	11.00	5.69	10.32	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P526724	Ppargc1b	170826	NM_133249	3.14	9.52	3.58	8.66	No
A_52_P293222	Ppargc1b	170826	NM_133249	2.63	8.69	2.80	7.60	No
A_51_P296905	Ppat	231327	NM_172146	9.37	10.00	9.09	9.64	No
A_51_P428372	Ppbbp	57349	NM_023785	5.20	4.11	5.38	4.31	No
A_52_P239922	Ppefl	237178	NM_011147	4.39	2.84	4.72	3.30	No
A_51_P316311	Ppfia4	68507	NM_001144855	3.77	11.79	3.06	10.51	Yes
A_51_P101879	Ppid	67738	NM_026352	11.90	12.68	11.87	12.61	No
A_52_P485700	Ppid	67738	NM_026352	12.39	13.24	12.37	13.07	No
A_51_P308590	Ppif	105675	NM_134084	7.99	9.24	8.34	8.95	No
A_52_P130415	Ppip5k2	227399	NM_173760	6.87	6.20	6.78	6.39	No
A_51_P164835	Ppl	19041	NM_008909	6.34	5.25	6.57	5.96	Yes
A_52_P262883	Ppp1r14b	18938	NM_008889	13.68	15.19	13.89	14.98	No
A_51_P133037	Ppp1r14b	18938	NM_008889	15.90	17.13	16.03	17.07	No
A_51_P239386	Ppp1r14c	76142	NM_133485	3.89	5.90	4.23	5.18	Yes
A_52_P601192	Ppp2r1b	73699	NM_001034085	9.79	10.50	9.07	9.68	Yes
A_51_P136810	Ppp3r2	19059	NM_001004025	6.26	5.45	5.83	5.77	No
A_51_P332652	Pqlc3	217430	NM_172574	11.92	11.15	12.27	11.62	No
A_52_P597338	Praf2	54637	NM_138602	9.50	8.71	9.58	9.10	No
A_51_P309501	Praf2	54637	NM_138602	11.39	10.65	11.42	10.98	No
A_51_P297011	Prcep	72461	NM_028243	12.28	11.66	12.22	11.83	No
A_51_P487331	Prdm10	382066	XM_006510465	6.17	5.58	6.27	5.78	No
A_51_P167117	Prdm11	100042784	AK078754	4.36	3.65	4.14	4.19	No
A_51_P200819	Prdx4	53381	AK165551	14.77	13.84	15.14	14.75	Yes
A_52_P313861	Prdx4	53381	AK165551	6.71	6.01	6.68	6.50	No
A_52_P90124	Prep	19072	NM_011156	12.48	13.35	12.27	13.02	No
A_51_P409893	Prkar2a	19087	NM_008924	8.09	9.83	8.84	9.48	No
A_51_P217295	Prkar2a	19087	NM_008924	3.22	5.01	4.05	5.00	No
A_51_P221062	Prkar2b	19088	NM_011158	13.74	14.99	13.73	14.53	No
A_51_P460734	Prkcd	18753	NM_011103	11.06	10.44	10.87	10.54	No
A_51_P180974	Prkcdbp	109042	NM_028444	14.69	14.07	14.75	14.04	No
A_51_P179864	Prkx	19108	NM_016979	10.30	11.99	10.47	11.57	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P458279	Prlr	19116	NM_011169	7.34	8.64	7.40	8.21	No
A_52_P121761	Prmt3	71974	AK049836	7.16	8.87	7.07	8.36	No
A_51_P101317	Prmt3	71974	AK049836	8.76	9.64	9.06	9.57	No
A_51_P295967	Proc	19123	NM_001042768	5.85	4.99	5.07	5.09	No
A_51_P382152	Procr	19124	NM_011171	11.46	10.72	11.96	11.48	Yes
A_52_P62270	Prpf18	67229	NM_026045	6.10	6.75	6.52	6.39	No
A_51_P369270	Prrc2b	227723	NM_172661	5.20	6.13	5.00	5.47	Yes
A_52_P497188	Prrgl	546336	NM_001289683	10.97	10.04	10.93	10.55	No
A_51_P284233	Prrg2	65116	NM_022999	11.36	10.77	11.47	11.10	No
A_52_P329917	Prrg2	65116	NM_022999	11.69	11.03	11.80	11.34	No
A_51_P209771	Prrx1	18933	NM_001025570	14.51	13.83	14.16	13.73	No
A_51_P397437	Prss46	74306	NM_183103	2.45	6.38	2.88	5.39	No
A_52_P501167	Prss53	330657	NM_001081268	6.50	5.84	6.22	5.98	No
A_51_P431539	Prss56	69453	NM_027084	3.17	6.60	3.47	6.07	No
A_52_P674087	Prtg	235472	NM_175485	8.31	7.46	8.50	8.10	No
A_51_P209818	Prtn3	19152	NM_011178	4.82	4.17	4.36	4.25	No
A_51_P326932	Prune2	353211	NM_181348	11.24	11.84	11.35	11.95	No
A_52_P523368	Psap11	76943	NM_175249	5.39	4.76	6.00	5.04	No
A_51_P104960	Psmc1	19179	NM_008947	13.47	14.08	13.45	13.71	No
A_51_P280971	Psmc3	19182	NM_008948	13.05	13.68	13.02	13.40	No
A_52_P49727	Psmc1	70247	NM_027357	12.86	13.52	12.81	13.09	No
A_51_P361535	Psme2	19188	NM_011190	14.97	14.04	14.72	14.46	No
A_51_P430263	Psph	100678	NM_133900	11.80	11.10	11.99	11.50	No
A_51_P409694	Psrc1	56742	NM_019976	12.77	12.17	12.58	12.50	No
A_52_P431872	Ptcd3	69956	NM_027275	12.87	13.60	12.95	13.45	No
A_51_P161257	Ptchd4	627626	NM_028474	4.19	2.81	4.27	4.29	No
A_51_P157406	Ptgds	19215	NM_008963	5.13	8.47	4.97	8.27	No
A_51_P372819	Ptgis	19223	NM_008968	10.27	11.70	10.54	11.53	No
A_51_P444437	Ptgr1	67103	NM_025968	14.15	13.38	13.78	13.40	No
A_51_P279100	Ptgs1	19224	NM_008969	13.77	13.00	13.86	13.41	No
A_51_P129360	Pthlh	19227	NM_008970	5.11	7.50	6.33	7.65	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P129363	Pthlh	19227	NM_008970	4.79	6.99	5.73	7.12	No
A_51_P484378	Ptpdc1	218232	NM_207232	6.60	5.35	6.48	5.93	No
A_51_P393699	Ptpla	30963	NM_013935	12.02	13.82	11.53	13.33	No
A_51_P463120	Ptplad2	66775	XM_006538189	13.15	12.34	13.16	12.66	No
A_51_P269793	Ptplad2	66775	XM_006538189	13.49	12.39	13.48	12.89	No
A_51_P455208	Ptpn13	19249	NM_011204	10.32	9.39	10.53	9.91	No
A_51_P236118	Ptpn18	19253	U52523	5.13	4.00	4.56	3.48	No
A_52_P114722	Ptpn6	15170	NM_013545	6.37	4.97	5.67	4.85	No
A_52_P605266	Ptpnd	19266	D13903	3.12	6.04	3.57	5.84	No
A_51_P468231	Ptpnd	19266	D13903	2.61	5.33	2.92	5.19	No
A_51_P206153	Ptpnd	19266	D13903	3.99	6.25	4.20	5.93	No
A_52_P148883	Ptpnf	19268	NM_011213	13.43	12.37	13.75	13.28	No
A_52_P1012802	Ptpnj	19271	NM_008982	8.93	8.13	8.98	8.55	No
A_51_P468381	Purb	19291	NM_011221	11.44	13.14	11.28	12.24	Yes
A_52_P236883	Purb	19291	NM_011221	10.63	12.46	10.49	11.66	Yes
A_51_P433091	Purb	19291	NM_011221	12.88	14.63	12.81	14.13	No
A_51_P256439	Pus7	78697	NM_178403	9.42	10.08	9.91	10.01	No
A_51_P500156	Pvalb	19293	NM_013645	2.04	6.99	2.26	4.98	Yes
A_51_P237097	Pvr	52118	AK040334	5.28	6.38	5.46	6.25	No
A_52_P162697	Pvr	52118	AK040334	9.26	10.65	9.49	10.74	No
A_52_P628590	Pvr	52118	AK040334	8.92	10.48	9.02	10.15	No
A_52_P162695	Pvr	52118	AK040334	10.98	12.50	11.04	12.52	No
A_52_P225377	Pvr	52118	AK040334	5.53	6.30	5.45	6.05	No
A_51_P476129	Pwp2	110816	NM_029546	11.36	12.02	11.46	11.93	No
A_52_P152476	Pwp2	110816	NM_029546	10.46	11.13	10.54	10.98	No
A_51_P219662	Pxdc1	66895	NM_025831	7.49	6.78	7.79	7.19	No
A_51_P422194	Pxt1	69307	NM_153390	5.01	3.68	4.19	4.24	No
A_52_P350664	Pygb	110078	NM_153781	11.47	10.70	11.45	10.77	No
A_51_P434670	Qk	19317	NM_021881	10.97	12.60	11.06	12.37	No
A_52_P120719	Rab27b	80718	NM_030554	7.74	7.00	7.35	6.72	No
A_52_P319361	Rab30	75985	NM_029494	9.52	8.25	9.27	8.56	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P293688	Rab32	67844	NM_026405	12.73	11.85	13.01	12.37	No
A_52_P247057	Rab36	76877	NM_029781	5.49	4.76	5.62	4.58	No
A_52_P450918	Rab3il1	74760	BC020147	8.51	7.64	8.32	7.71	No
A_51_P248031	Rab42	242681	XM_006538847	5.78	5.17	5.77	5.28	No
A_51_P276418	Rab6b	270192	NM_173781	10.02	9.08	10.86	10.55	Yes
A_52_P214678	Rab9	56382	NM_019773	13.53	12.88	13.64	13.24	No
A_51_P431888	Radil	231858	NM_178702	11.44	10.84	11.78	11.23	No
A_51_P431894	Radil	231858	NM_178702	10.77	10.00	10.94	10.47	No
A_51_P256771	Raet1a	19368	NM_009016	7.85	7.11	7.67	7.23	No
A_52_P10063	Raet1e	379043	NM_198193	9.40	8.68	9.40	8.94	No
A_51_P274789	Rai14	75646	NM_030690	9.65	8.96	9.65	9.14	No
A_52_P645008	Ralgps1	241308	NM_175211	3.48	4.46	4.38	4.07	No
A_51_P115817	Ralgps2	78255	NM_023884	9.37	11.13	9.27	10.47	Yes
A_51_P190106	Ralgps2	78255	NM_023884	9.06	10.78	8.93	9.91	No
A_52_P276537	Ralgps2	78255	NM_023884	7.30	8.91	7.29	8.53	No
A_51_P227090	Ramp3	56089	NM_019511	8.49	11.92	9.16	11.19	No
A_51_P416771	Ranbp3l	223332	NM_198024	4.92	4.23	5.04	4.64	No
A_52_P39756	Rap1gap2	380711	NM_001015046	7.04	9.81	6.90	9.05	No
A_51_P297799	Rap1gap2	380711	NM_001015046	5.67	8.73	5.85	7.68	No
A_52_P336290	Rap2a	76108	AK018024	12.30	11.47	12.41	11.94	No
A_51_P116906	Rapgef3	223864	NM_144850	10.72	9.80	10.12	9.54	No
A_51_P385974	Rapsn	19400	NM_009023	3.84	9.21	3.32	8.89	No
A_51_P202440	Rarb	218772	NM_011243	5.44	6.87	4.82	5.57	Yes
A_51_P202440	Rarb	218772	NM_011243	5.11	6.78	5.36	5.48	Yes
A_51_P202440	Rarb	218772	NM_011243	5.41	6.92	4.77	5.68	Yes
A_51_P202440	Rarb	218772	NM_011243	4.86	6.69	5.00	5.47	Yes
A_51_P202440	Rarb	218772	NM_011243	4.90	6.88	4.61	5.66	Yes
A_51_P202440	Rarb	218772	NM_011243	5.26	6.75	5.08	5.56	Yes
A_51_P202440	Rarb	218772	NM_011243	5.40	6.94	4.99	5.76	Yes
A_51_P202440	Rarb	218772	NM_011243	5.24	6.86	4.81	5.69	Yes
A_51_P202440	Rarb	218772	NM_011243	5.36	6.77	4.97	5.63	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P202440	Rarb	218772	NM_011243	5.20	6.50	4.70	5.62	Yes
A_51_P401184	Rarres1	109222	NM_001164763	5.96	5.28	6.72	6.06	Yes
A_51_P381558	Rasa4	54153	NM_133914	12.29	11.69	12.44	12.27	Yes
A_52_P198435	Rasgrp3	240168	NM_207246	7.75	7.04	7.12	6.73	No
A_51_P105927	Rasl12	70784	NM_001033158	6.66	8.60	6.68	6.97	Yes
A_52_P460703	Rassf3	192678	NM_138956	7.61	8.92	7.64	8.55	No
A_52_P596592	Rassf9	237504	NM_146240	7.87	6.82	7.78	6.83	No
A_52_P419873	Rbl1	19645	NM_009029	4.28	6.73	4.00	5.16	No
A_51_P136888	Rbl1	19645	NM_009029	11.28	13.73	11.19	12.85	No
A_51_P136888	Rbl1	19645	NM_009029	11.16	13.47	11.16	12.81	No
A_51_P136888	Rbl1	19645	NM_009029	11.45	13.67	11.19	12.81	No
A_51_P136888	Rbl1	19645	NM_009029	11.50	13.71	11.18	12.87	No
A_51_P136888	Rbl1	19645	NM_009029	11.49	13.69	11.23	12.91	No
A_51_P136888	Rbl1	19645	NM_009029	11.47	13.66	11.16	12.83	No
A_51_P136888	Rbl1	19645	NM_009029	11.48	13.66	11.17	12.84	No
A_51_P136888	Rbl1	19645	NM_009029	11.48	13.61	11.11	12.81	No
A_51_P136888	Rbl1	19645	NM_009029	11.54	13.67	11.19	12.87	No
A_51_P136888	Rbl1	19645	NM_009029	11.33	13.43	11.08	12.77	No
A_51_P508864	Rbfox1	268859	NM_021477	3.24	6.62	3.40	5.85	No
A_52_P598492	Rbm20	73713	NM_001170847	4.63	6.92	6.37	6.11	No
A_51_P487832	Rbm24	666794	NM_001081425	2.55	10.28	2.60	8.09	Yes
A_52_P920158	Rbm24	666794	NM_001081425	3.19	12.35	3.21	10.57	Yes
A_51_P418737	Rbm33	381626	AK014289	5.73	6.84	5.26	6.07	No
A_51_P262670	Rbm38	56190	NM_019547	5.43	7.32	5.53	6.44	No
A_51_P166818	Rbm43	71684	NM_030243	9.20	8.34	9.27	8.62	No
A_52_P100028	Rbpj	19664	NM_009035	11.44	12.06	11.50	11.84	No
A_52_P144032	Rbpms	19663	NM_019733	6.79	5.88	6.16	5.47	No
A_52_P223618	Rcn3	52377	NM_026555	13.83	13.14	13.93	13.62	No
A_51_P479618	Rdh5	19682	NM_134006	10.39	11.11	10.40	11.69	No
A_51_P508341	Reep1	52250	NM_178608	6.08	10.56	5.47	9.40	Yes
A_51_P439299	Relt	320100	NM_177073	6.32	7.50	5.95	6.60	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P316661	Ren1	19701	NM_031192	5.12	3.27	5.23	4.51	No
A_51_P305508	Rerg	232441	NM_181988	4.19	5.30	4.13	5.22	No
A_51_P245631	Rftn2	74013	NM_028713	10.08	9.23	9.78	9.54	No
A_51_P485260	Rgl1	19731	NM_016846	11.72	10.95	11.81	11.43	No
A_52_P20075	Rgs12	71729	NM_173402	7.67	6.71	7.16	6.95	No
A_51_P389957	Rgs14	51791	NM_016758	5.74	7.08	6.19	6.55	No
A_51_P249286	Rgs16	19734	NM_011267	3.49	9.79	3.73	8.26	Yes
A_51_P244792	Rgs17	56533	NM_001161822	8.51	7.33	8.55	7.82	No
A_52_P843282	Rgs17	56533	NM_001161822	6.84	5.79	6.34	5.92	No
A_52_P378968	Rgs2	19735	NM_009061	7.03	7.93	7.03	7.89	No
A_51_P402165	Rgs7	24012	NM_011880	5.09	3.61	5.29	4.06	No
A_52_P635598	Rgs8	67792	NM_026380	3.71	4.53	3.81	3.82	No
A_51_P135012	Rhpn2	52428	NM_027897	7.87	6.75	8.41	7.54	Yes
A_51_P163188	Rin2	74030	NM_028724	11.22	10.57	11.42	10.88	No
A_52_P174775	Ripk1	19766	NM_009068	9.96	9.18	10.07	9.88	Yes
A_51_P349528	Rnase10	75019	NM_029145	8.02	7.00	7.69	7.23	No
A_52_P262511	Rnase4	58809	NM_021472	12.47	11.75	12.55	11.90	No
A_51_P237383	Rnase4	58809	NM_021472	13.84	12.89	13.81	13.12	No
A_51_P268117	Rnfl1	29864	NM_013876	12.26	13.11	12.63	13.03	No
A_51_P266847	Rnfl26	70294	NM_144528	11.58	12.55	11.60	12.19	No
A_51_P398517	Rnfl30	59044	NM_021540	12.07	11.46	12.39	12.08	No
A_52_P228702	Rnfl50	330812	NM_177378	5.57	4.54	5.75	5.62	No
A_52_P683038	Rnfl70	77733	NM_029965	9.38	8.70	9.22	8.75	No
A_51_P500661	Rnfl82	328234	NM_183204	3.64	5.53	3.30	5.39	No
A_51_P159503	Rnf213	672511	XM_006534630	10.77	10.07	10.94	10.60	No
A_52_P3845	Rnf217	268291	NM_001146349	9.18	10.81	9.43	10.50	No
A_51_P450854	Rnf217	268291	NM_001146349	7.56	9.33	7.99	9.00	No
A_52_P43111	Rnf7	19823	NM_011279	12.11	13.30	12.55	13.00	No
A_51_P414166	Rnf7	19823	NM_011279	13.86	14.78	13.94	14.48	No
A_52_P191527	Rnmt	67897	NM_026440	8.43	9.26	8.71	9.02	No
A_51_P375187	Rpf2	67239	NM_001042556	12.12	13.11	12.10	12.89	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P506674	Rpp38	227522	NM_001013376	9.81	10.53	9.80	10.21	No
A_52_P491433	Rps15a	267019	NM_170669	3.05	5.23	3.34	4.12	Yes
A_51_P320466	Rps6ka2	20112	NM_011299	6.64	9.15	7.05	8.41	No
A_51_P413031	Rptor	74370	NM_028898	7.17	8.23	7.37	7.84	No
A_52_P229320	Rptor	74370	NM_028898	12.36	13.20	12.45	12.99	No
A_52_P490032	Rragd	52187	AK081204	4.17	9.28	5.30	8.53	No
A_51_P457989	Rragd	52187	AK081204	6.61	11.47	7.52	10.77	No
A_51_P115768	Rragd	52187	AK081204	8.14	9.92	8.37	9.66	No
A_51_P112912	Rras2	66922	NM_025846	12.34	13.18	12.25	13.00	No
A_52_P558859	Rras2	66922	NM_025846	9.90	10.83	10.00	10.74	No
A_51_P314679	Rrn3	106298	NM_001039521	11.74	12.36	11.82	12.17	No
A_52_P458229	Rrp1b	72462	NM_028244	7.60	8.35	7.54	8.17	No
A_51_P361906	Rrp7a	74778	NM_029101	11.83	12.78	12.01	12.38	No
A_52_P579640	Rrs1	59014	NM_021511	10.21	11.09	10.18	10.90	No
A_51_P209930	Rtn2	20167	NM_013648	6.99	11.96	6.25	11.09	Yes
A_51_P304170	Rtp4	67775	NM_023386	11.50	10.59	11.27	10.60	No
A_52_P249544	Rufy3	52822	NM_027530	2.37	5.45	3.08	4.14	No
A_51_P206585	Runx1	12394	NM_001111023	6.47	9.45	5.93	8.95	No
A_52_P202358	Runx1	12394	NM_001111023	7.71	8.54	7.72	8.49	No
A_52_P612137	Runx1t1	12395	NM_009822	11.74	10.40	11.41	10.73	No
A_51_P230942	Runx2	12393	NM_009820	11.79	10.62	11.61	11.17	No
A_51_P230942	Runx2	12393	NM_009820	11.88	10.79	11.57	11.16	No
A_51_P230942	Runx2	12393	NM_009820	11.78	10.72	11.60	11.24	No
A_51_P230942	Runx2	12393	NM_009820	11.91	10.85	11.60	11.14	No
A_51_P230942	Runx2	12393	NM_009820	11.88	10.83	11.62	11.24	No
A_51_P230942	Runx2	12393	NM_009820	11.78	10.75	11.59	11.08	No
A_51_P230942	Runx2	12393	NM_009820	11.97	10.95	11.70	11.36	No
A_51_P230942	Runx2	12393	NM_009820	11.82	10.83	11.62	11.10	No
A_51_P230942	Runx2	12393	NM_009820	11.94	10.97	11.64	11.27	No
A_51_P230942	Runx2	12393	NM_009820	11.83	10.91	11.68	11.25	No
A_52_P255406	Rxfp3	239336	NM_178717	2.88	7.06	2.86	6.38	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P416074	Ryr1	20190	NM_009109	2.69	10.31	2.79	8.82	Yes
A_52_P391110	Ryr1	20190	NM_009109	2.97	10.44	4.00	9.20	Yes
A_51_P172475	S100a1	20193	NM_011309	8.73	7.79	9.05	8.47	Yes
A_51_P108812	S100a5	20199	NM_011312	5.25	3.74	4.86	4.68	Yes
A_51_P208083	S100b	20203	NM_009115	13.24	11.45	13.12	11.89	No
A_51_P374999	S100bp	74648	NM_029036	5.61	6.69	5.55	6.18	No
A_52_P532227	S1pr1	13609	NM_007901	9.87	9.10	9.94	9.51	No
A_52_P578043	Sacs	50720	XM_006519226	7.78	10.22	7.99	9.17	Yes
A_51_P384306	Sacs	50720	XM_006519226	4.00	5.71	4.00	4.49	No
A_51_P101137	Samd14	217125	NM_146025	10.89	10.26	10.72	10.34	No
A_52_P198898	Samd5	320825	NM_177271	4.03	2.32	5.42	4.24	Yes
A_51_P432199	Sap30	60406	NM_021788	9.20	10.03	9.00	9.75	No
A_51_P498152	Sat2	69215	NM_026991	10.56	9.97	10.41	10.03	No
A_52_P502547	Scai	320271	NM_178778	7.38	6.61	7.60	7.29	Yes
A_52_P182813	Scai	320271	NM_178778	6.22	5.43	6.33	5.92	No
A_51_P163252	Schip1	30953	NM_013928	5.73	7.03	5.68	6.84	No
A_51_P206635	Sclt1	67161	NM_001081411	7.82	7.19	7.50	7.18	No
A_52_P519737	Scml4	268297	NM_172938	5.40	7.88	4.52	7.24	No
A_51_P184331	Scn3b	235281	NM_178227	3.70	5.49	3.75	4.57	No
A_52_P113611	Scn5a	20271	NM_021544	2.81	10.02	2.78	9.90	No
A_52_P683315	Scrib	105782	NM_134089	13.41	14.93	13.52	14.21	Yes
A_51_P234359	Sct	20287	NM_011328	5.05	6.27	4.83	5.39	No
A_51_P380432	Scx	20289	NM_198885	11.74	14.48	11.26	14.00	No
A_51_P249494	Sdcbp	53378	NM_016807	15.55	14.96	15.46	15.06	No
A_51_P260871	Sdhd	66925	NM_025848	13.69	14.32	13.83	14.18	No
A_52_P72839	Sec23a	20334	NM_009147	10.81	10.22	10.88	10.57	No
A_51_P139578	Secisbp2l	70354	NM_177608	7.16	6.38	7.38	6.88	No
A_52_P649623	Sema3d	108151	NM_028882	7.09	8.56	7.05	8.51	No
A_51_P389814	Sema3f	20350	NM_011349	10.54	9.56	10.32	9.74	No
A_52_P498231	Sema3f	20350	NM_011349	9.49	8.58	9.29	8.57	No
A_52_P312102	Sema3g	218877	XM_006518821	7.38	8.81	7.14	8.97	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P409076	Sema5a	20356	NM_009154	10.29	10.90	10.58	10.97	No
A_51_P352245	Sema6a	20358	NM_018744	1.96	7.61	2.28	7.11	No
A_51_P417952	Sema6b	20359	NM_013662	5.33	7.39	5.16	5.74	Yes
A_52_P410765	Sema7a	20361	NM_011352	9.39	10.80	10.21	12.20	Yes
A_51_P383991	Sept4	18952	NM_011129	3.37	7.29	2.53	5.61	Yes
A_51_P325318	Serinc2	230779	NM_172702	8.38	12.05	6.99	10.98	No
A_51_P191375	Serpinb6a	20719	NM_009254	14.09	13.28	14.18	13.64	No
A_51_P406328	Serpinb6c	97848	AF425084	11.27	10.65	11.43	11.05	No
A_52_P602669	Serpinb6d	238568	NM_001076790	11.18	10.53	11.46	11.06	No
A_52_P544885	Serpinb8	20725	NM_011459	7.48	6.85	7.29	6.86	No
A_52_P220573	Serpini1	20713	NM_009250	8.04	6.92	7.55	6.85	No
A_52_P154710	Sesn2	230784	AK170547	14.64	13.88	14.49	14.06	No
A_51_P246773	Sesn3	75747	AK033655	10.94	9.84	10.61	10.08	No
A_52_P236777	Setd3	52690	NM_028262	3.67	4.80	3.07	3.98	No
A_51_P352296	Sfrp1	20377	NM_013834	9.86	8.90	9.48	8.92	No
A_51_P335491	Sgca	20391	NM_009161	7.92	11.62	7.36	11.34	No
A_51_P194273	Sgcg	24053	NM_011892	8.70	10.57	8.97	9.84	No
A_52_P127069	Sgk3	170755	NM_133220	6.80	6.08	6.98	6.44	No
A_51_P211998	Sgms2	74442	NM_028943	6.72	5.89	7.02	6.73	No
A_51_P187346	Sgms2	74442	NM_028943	8.85	8.01	8.82	8.39	No
A_52_P576100	Sh2b1	20399	NM_011363	11.63	12.31	11.81	12.38	No
A_51_P404023	Sh2b2	23921	NM_018825	7.20	7.93	7.33	7.86	No
A_52_P301312	Sh2d4b	328381	XM_006519136	4.21	6.90	4.10	5.98	Yes
A_51_P342906	Sh3bp1	20401	NM_009164	10.76	10.08	10.68	10.44	No
A_52_P273169	Sh3d19	27059	NM_001082414	7.30	6.31	7.45	6.72	No
A_51_P223315	Sh3glb1	54673	NM_019464	5.95	8.21	6.39	7.94	No
A_52_P218100	Sh3glb1	54673	NM_019464	8.46	10.45	8.45	9.96	No
A_52_P696044	Shb	230126	NM_001033306	8.13	9.36	8.13	9.40	No
A_51_P408649	Shisa2	219134	NM_145463	2.29	8.06	2.65	6.22	Yes
A_51_P238523	Shisa4	77552	NM_175259	13.24	12.42	12.98	12.37	No
A_52_P512817	Shisa5	66940	NM_025858	15.97	15.24	16.02	15.56	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P543869	Shpk	74637	NM_029031	7.49	6.74	7.91	7.06	No
A_51_P444005	Shroom1	71774	NM_027917	9.77	8.91	9.50	8.77	No
A_51_P311995	Shroom2	110380	NM_172441	3.70	5.26	3.54	4.90	No
A_51_P183803	Siah2	20439	NM_009174	8.30	9.03	8.54	8.87	No
A_51_P328725	Sik1	17691	NM_010831	7.49	8.65	7.06	8.14	No
A_52_P26318	Sipa1l2	244668	NM_001081337	8.89	9.80	8.94	9.51	No
A_52_P170635	Sirpa	19261	NM_007547	12.10	11.31	12.21	11.69	No
A_51_P110068	Sirt2	64383	NM_022432	11.04	11.95	11.38	11.69	No
A_52_P547447	Sirt2	64383	NM_022432	11.42	12.19	11.65	11.89	No
A_52_P656024	Sirt2	64383	NM_022432	10.02	10.74	10.04	10.33	No
A_52_P342015	Sirt5	68346	NM_178848	7.08	6.33	7.16	6.54	No
A_51_P411909	Sirt5	68346	NM_178848	10.76	10.18	10.74	10.25	No
A_52_P607702	Six4	20474	XM_006515632	10.02	10.76	8.70	9.45	Yes
A_52_P455643	Skida1	72668	NM_028317	4.82	2.98	4.68	4.93	Yes
A_51_P118132	Skil	20482	NM_011386	9.17	10.00	8.78	9.86	No
A_52_P597461	Skil	20482	NM_011386	3.82	5.31	3.85	4.78	No
A_52_P513177	Sla	20491	NM_001029841	3.79	4.95	3.31	4.53	No
A_51_P275527	Slc12a2	20496	NM_009194	10.52	11.61	10.51	10.79	No
A_52_P557129	Slc12a5	57138	NM_020333	5.87	6.88	5.60	5.98	Yes
A_51_P267278	Slc15a2	57738	NM_021301	5.57	4.50	6.96	6.27	Yes
A_51_P352324	Slc16a2	20502	NM_009197	7.99	9.21	7.54	8.16	No
A_51_P340653	Slc16a4	229699	NM_146136	9.54	8.10	8.59	7.67	Yes
A_52_P598885	Slc16a4	229699	NM_146136	6.06	4.96	5.58	4.42	No
A_51_P432180	Slc16a6	104681	NM_001029842	7.50	10.58	6.78	9.51	Yes
A_52_P579933	Slc16a6	104681	NM_001029842	2.83	6.67	3.63	5.68	Yes
A_51_P408631	Slc20a1	20515	NM_015747	11.30	12.92	11.33	13.21	No
A_51_P473940	Slc22a15	242126	AK029299	8.93	8.33	9.19	8.67	No
A_51_P111902	Slc22a17	59049	NM_021551	4.76	5.44	4.48	5.07	No
A_52_P640221	Slc22a18	18400	NM_001042760	6.28	5.66	6.14	6.05	No
A_51_P502225	Slc22a4	30805	NM_019687	2.77	9.56	3.46	9.45	No
A_51_P268496	Slc24a3	94249	NM_053195	11.07	12.13	11.48	12.03	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P404904	Slc25a10	27376	NM_013770	11.96	12.57	12.20	12.54	No
A_52_P359088	Slc25a25	227731	NM_146118	3.79	4.57	4.38	4.81	No
A_52_P590756	Slc25a35	71998	NM_028048	6.76	6.12	6.82	6.54	No
A_51_P113403	Slc26a11	268512	NM_178743	10.30	9.53	10.43	9.97	No
A_51_P224164	Slc26a4	23985	NM_011867	2.36	5.42	3.50	5.18	No
A_51_P289836	Slc29a1	63959	NM_022880	13.67	15.74	13.91	15.47	No
A_51_P304125	Slc29a2	13340	NM_007854	6.18	7.65	6.52	8.12	No
A_51_P185465	Slc29a3	71279	NM_023596	9.15	8.17	8.95	8.10	No
A_52_P607255	Slc2a13	239606	NM_001033633	9.73	8.47	9.97	9.25	No
A_51_P268831	Slc2a6	227659	NM_172659	6.65	9.32	7.10	9.54	No
A_51_P261092	Slc2a9	117591	NM_145559	6.10	5.37	5.51	5.02	No
A_52_P147666	Slc30a7	66500	NM_023214	8.33	7.62	8.61	8.34	Yes
A_52_P83193	Slc30a7	66500	NM_023214	9.71	9.08	9.51	9.32	No
A_52_P193194	Slc35d2	70484	NM_001001321	9.23	8.51	8.95	8.46	No
A_52_P124083	Slc35e2	320541	AK087552	4.37	5.11	4.57	4.75	No
A_51_P259774	Slc37a2	56857	NM_020258	5.67	8.57	5.23	7.17	No
A_51_P259773	Slc37a2	56857	NM_020258	4.75	7.46	4.73	6.41	No
A_52_P232648	Slc38a1	105727	NM_134086	5.31	5.99	4.86	5.50	No
A_51_P295701	Slc39a13	68427	NM_026721	12.75	12.12	12.94	12.75	Yes
A_51_P389988	Slc40a1	53945	NM_016917	7.56	8.82	7.24	8.61	No
A_52_P578562	Slc41a1	98396	NM_173865	8.79	9.74	9.20	9.46	No
A_52_P74368	Slc43a2	215113	NM_173388	4.14	5.49	4.41	4.70	No
A_52_P84901	Slc44a1	100434	NM_133891	9.75	10.81	9.73	10.44	No
A_51_P169374	Slc52a3	69698	NM_027172	6.75	5.84	5.93	5.15	No
A_52_P283055	Slc5a3	53881	NM_017391	10.14	9.12	10.52	10.04	Yes
A_51_P112762	Slc5a3	53881	NM_017391	6.65	5.49	6.75	6.39	No
A_52_P128180	Slc5a7	63993	NM_022025	4.58	3.40	5.59	5.38	Yes
A_52_P221824	Slc6a13	14412	NM_144512	6.27	7.09	6.43	6.69	No
A_51_P351872	Slc6a9	14664	NM_008135	14.69	13.68	14.96	14.14	No
A_51_P273639	Slc7a5	20539	NM_011404	12.75	13.54	12.85	12.98	No
A_52_P474145	Slc7a9	30962	NM_021291	5.33	3.88	3.82	3.94	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P397983	Slc9a2	226999	AK077026	3.48	6.26	3.28	5.77	No
A_52_P241676	Slc9a3r2	65962	NM_023449	9.67	8.89	9.08	8.62	No
A_52_P375323	Slc9a9	331004	NM_177909	4.50	5.96	4.50	5.75	No
A_52_P255782	Slco2b1	101488	NM_175316	2.55	7.53	3.07	7.14	No
A_51_P194415	Slco3a1	108116	NM_023908	11.15	12.43	11.40	12.26	No
A_52_P413395	Sln	66402	NM_025540	2.20	7.95	2.35	6.15	Yes
A_51_P359046	Slurp1	57277	NM_020519	4.34	3.20	4.05	3.98	No
A_51_P461404	Smarca1	93761	NM_053123	9.82	8.82	9.79	9.24	No
A_52_P283041	Smarca1	93761	NM_053123	10.45	9.45	10.45	9.85	No
A_51_P504037	Smarca2	67155	NM_011416	9.88	8.85	9.85	8.75	No
A_51_P423880	Smardc3	66993	NM_025891	11.67	12.73	11.80	12.66	No
A_52_P551011	Smim1	68859	NM_001163721	8.05	6.91	7.76	7.11	No
A_52_P295104	Smim5	66528	NM_183259	4.39	11.42	5.48	9.45	Yes
A_51_P443403	Smoc1	64075	NM_022316	3.61	5.99	3.48	5.19	No
A_52_P646762	Smoc2	64074	NM_022315	12.91	11.71	12.75	11.63	No
A_51_P213045	Smoc2	64074	NM_022315	11.64	10.49	11.75	10.65	No
A_51_P130595	Smpd5	100503915	NM_001195537	8.15	7.52	7.60	7.11	No
A_51_P104478	Smpx	66106	NM_001252591	4.00	11.10	2.68	8.79	Yes
A_52_P395149	Smtnl2	276829	NM_177776	2.62	7.22	2.80	5.78	Yes
A_52_P69740	Smurf2	66313	NM_025481	9.07	8.43	9.36	8.91	No
A_52_P472660	Smurf2	66313	NM_025481	11.01	10.30	11.26	10.77	No
A_51_P305547	Snai2	20583	NM_011415	14.48	13.35	14.45	14.02	No
A_51_P244061	Snape5	330959	NM_183316	12.40	11.53	12.27	11.57	No
A_52_P59264	Sncap	67847	NM_026408	6.79	5.68	6.00	5.15	No
A_52_P670812	Snhg18	100616095	NR_038186	12.50	11.78	12.31	12.02	No
A_51_P225948	Snx33	235406	NM_175483	11.17	10.53	11.22	10.85	No
A_51_P253117	Sorbs1	20411	NM_178362	11.62	12.45	11.46	12.20	No
A_52_P652999	Sorbs1	20411	NM_178362	6.68	7.33	6.56	7.17	No
A_51_P230287	Sorcs2	81840	NM_030889	11.05	12.05	11.50	12.24	No
A_51_P273667	Sox17	20671	NM_011441	5.64	5.00	5.60	5.12	No
A_52_P269672	Sox8	20681	NM_011447	2.46	9.06	3.61	8.02	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P269667	Sox8	20681	NM_011447	2.48	6.69	2.55	6.13	No
A_51_P375963	Spaca3	75622	NM_029367	4.61	3.63	3.78	3.45	No
A_52_P305279	Spata13	219140	NM_001033272	6.51	7.40	6.01	6.58	Yes
A_52_P219966	Spg21	27965	NM_138584	10.16	12.72	10.66	11.78	No
A_52_P400436	Sphk1	20698	NM_025367	9.05	8.13	9.21	8.75	No
A_52_P282838	Spin2c	278240	NM_001005370	9.43	8.75	9.14	8.55	No
A_51_P112592	Spire2	234857	NM_172287	6.51	7.49	6.44	7.23	No
A_52_P186033	Spn	20737	NM_009259	8.63	7.86	7.12	6.59	Yes
A_51_P123745	Spns2	216892	NM_153060	6.73	5.86	7.62	6.22	Yes
A_52_P381484	Spon2	100689	NM_133903	12.41	11.20	12.29	11.36	No
A_51_P434017	Spon2	100689	NM_133903	10.91	9.72	10.76	9.85	No
A_51_P490136	Spry1	24063	NM_011896	8.27	10.72	8.38	11.00	No
A_52_P204311	Spry4	24066	NM_011898	3.34	7.68	4.23	6.34	No
A_51_P393862	Spryd4	66701	NM_025716	9.36	10.38	9.01	9.52	Yes
A_52_P600547	Src	20779	NM_009271	9.50	8.69	9.51	9.15	No
A_52_P600547	Src	20779	NM_009271	9.45	8.70	9.49	9.15	No
A_52_P600547	Src	20779	NM_009271	9.43	8.70	9.75	9.01	No
A_52_P600547	Src	20779	NM_009271	9.50	8.77	9.44	9.08	No
A_52_P600547	Src	20779	NM_009271	9.55	8.84	9.45	9.08	No
A_52_P600547	Src	20779	NM_009271	9.42	8.72	9.45	9.08	No
A_52_P600547	Src	20779	NM_009271	9.48	8.78	9.53	9.15	No
A_52_P600547	Src	20779	NM_009271	9.45	8.78	9.49	9.09	No
A_52_P600547	Src	20779	NM_009271	9.39	8.75	9.46	9.09	No
A_52_P600547	Src	20779	NM_009271	9.40	8.82	9.54	9.16	No
A_52_P473344	Srebf2	20788	NM_033218	14.92	15.52	14.81	14.96	No
A_52_P665742	Srebf2	20788	NM_033218	10.12	11.11	10.18	10.92	No
A_51_P100289	Srf	20807	NM_020493	11.64	12.35	11.42	12.25	No
A_51_P395864	Srgap1	117600	NM_001081037	10.26	9.22	10.04	9.56	No
A_52_P454622	Srgap1	117600	NM_001081037	5.46	3.92	5.35	4.37	No
A_51_P489313	Sri	109552	NM_025618	13.81	13.16	13.83	13.42	No
A_51_P332742	Srl	106393	NM_175347	5.92	11.41	5.34	9.90	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P375862	Srpk3	56504	NM_019684	1.99	9.02	2.09	8.09	No
A_51_P425047	SrpX2	68792	NM_026838	7.92	9.26	7.67	9.10	No
A_52_P297032	Srxn1	76650	NM_029688	11.86	11.26	12.22	12.04	Yes
A_51_P387971	Ssh3	245857	NM_198113	6.97	6.26	7.31	6.91	Yes
A_51_P238165	Sstr2	20606	NM_009217	4.16	6.01	4.00	5.39	No
A_51_P330369	Ssx2ip	99167	NM_138744	9.61	10.45	9.89	10.41	No
A_51_P483473	St3gal5	20454	NM_011375	13.54	14.69	13.59	14.32	No
A_52_P449104	St6galnac4	20448	NM_011373	8.50	10.20	8.50	9.58	Yes
A_51_P260578	St8sia2	20450	NM_009181	5.13	7.49	5.52	6.47	Yes
A_51_P236267	St8sia4	20452	NM_009183	2.87	6.53	2.92	5.88	No
A_51_P218292	Stac	20840	NM_016853	5.36	3.40	4.10	3.83	Yes
A_51_P177261	Stac3	237611	NM_177707	7.87	15.10	7.50	14.33	No
A_51_P242663	Stam	20844	NM_011484	9.54	10.61	9.81	10.57	No
A_52_P435118	Stard13	243362	NM_146258	5.84	7.24	6.08	6.72	No
A_52_P481182	Stard5	170460	NM_023377	6.50	5.76	6.89	6.42	No
A_51_P390496	Stard7	99138	NM_139308	11.12	11.83	11.27	11.42	No
A_52_P496503	Stat1	20846	NM_009283	10.98	10.26	10.86	10.45	No
A_52_P496503	Stat1	20846	NM_009283	11.05	10.36	10.85	10.41	No
A_52_P496503	Stat1	20846	NM_009283	10.97	10.31	10.80	10.43	No
A_52_P496503	Stat1	20846	NM_009283	11.01	10.37	10.81	10.50	No
A_52_P496503	Stat1	20846	NM_009283	11.01	10.38	10.87	10.45	No
A_52_P496503	Stat1	20846	NM_009283	10.94	10.32	10.79	10.34	No
A_52_P496503	Stat1	20846	NM_009283	10.93	10.33	10.84	10.39	No
A_52_P496503	Stat1	20846	NM_009283	10.87	10.27	10.79	10.33	No
A_51_P167754	Stk11	20869	AK172528	12.48	13.14	12.77	13.10	No
A_52_P305061	Stk32a	269019	NM_178749	4.35	3.48	4.94	3.49	No
A_51_P294807	Stom	13830	NM_013515	11.23	10.64	11.16	10.85	No
A_51_P515242	Strip2	320609	NM_177204	6.33	8.53	5.63	8.77	No
A_51_P252061	Strn	268980	XM_006524357	4.75	3.90	5.24	4.11	No
A_51_P484009	Stx2	13852	NM_007941	12.37	11.77	12.60	12.22	No
A_52_P533792	Sucla2	20916	NM_011506	13.79	14.48	13.68	14.03	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P111554	Sucla2	20916	NM_011506	11.42	12.12	11.38	11.81	No
A_52_P488666	Susd2	71733	NM_027890	6.80	4.90	6.33	5.22	No
A_52_P69160	Swap70	20947	NM_009302	10.81	10.07	10.55	10.19	No
A_51_P278994	Swap70	20947	NM_009302	11.91	11.19	11.55	11.26	No
A_51_P516724	Sync	68828	NM_023485	6.62	8.54	6.94	7.97	No
A_52_P562467	Sync	68828	NM_023485	9.33	11.16	9.45	10.73	No
A_51_P314186	Syne1	64009	NM_001079686	6.34	7.86	6.96	7.03	Yes
A_52_P335984	Syne1	64009	NM_001079686	8.27	9.31	8.64	8.72	No
A_51_P119776	Synpo2	118449	AK035258	7.97	9.03	8.30	8.84	No
A_52_P516312	Synpo2l	68760	NM_175132	3.67	7.66	3.24	6.51	No
A_51_P119749	Synpo2l	68760	NM_175132	8.03	15.25	8.06	14.41	No
A_52_P453833	Sypl	19027	AK035670	12.00	12.70	11.58	12.08	Yes
A_51_P125935	Syt11	229521	NM_018804	7.09	6.50	7.38	6.90	No
A_51_P384831	Syt12	171180	NM_134164	6.48	7.96	6.87	7.84	No
A_52_P253044	Syt13	80976	NM_030725	9.03	10.66	9.28	11.08	No
A_51_P290921	Syt12	83671	NM_001040087	7.54	9.40	8.32	9.92	No
A_51_P486144	Tagap1	380608	NM_147155	8.08	8.68	8.00	8.22	No
A_52_P385594	Tal2	21350	NM_009317	3.66	5.82	4.04	5.32	No
A_51_P377547	Tank	21353	NM_011529	11.33	12.15	11.20	11.98	No
A_51_P304500	Tapbp	21356	NM_001025313	13.41	12.56	13.50	12.94	No
A_51_P508919	Tas1r1	110326	NM_031867	3.82	8.15	4.33	7.39	No
A_52_P402331	Tbc1d1	57915	NM_019636	6.37	7.13	5.47	6.14	Yes
A_51_P448005	Tbc1d8	54610	NM_018775	3.08	4.83	2.64	3.70	Yes
A_52_P1026830	Tbc1d8b	245638	NM_001081499	10.71	10.09	10.46	10.20	No
A_51_P461828	Tbp	21374	NM_013684	7.12	6.42	7.08	6.98	No
A_51_P349776	Tceal3	594844	NM_001029978	7.47	8.34	7.20	7.52	Yes
A_52_P1034723	Tceal7	100040972	NM_001127169	2.23	7.05	2.72	5.95	No
A_52_P87395	Tcf12	21406	AK035173	8.80	9.64	8.61	9.26	No
A_51_P310135	Tcf12	21406	AK035173	9.51	10.63	9.61	10.62	No
A_51_P280401	Tcta	102791	NM_133986	9.79	9.01	9.89	8.97	No
A_52_P348287	Tcte3	21647	U21674	5.67	6.58	6.23	6.44	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P184672	Tcte3	21647	U21674	6.45	7.18	6.76	6.87	No
A_51_P307589	Tdrd9	74691	NM_029056	3.65	6.76	3.77	6.11	No
A_51_P179041	Tead4	21679	NM_011567	6.69	8.65	7.10	8.10	No
A_51_P197645	Tekt5	70426	NM_001291001	4.45	6.49	3.81	5.58	No
A_51_P395473	Tenc1	209039	NM_153533	10.93	10.26	11.18	10.86	Yes
A_52_P206429	Tenm3	23965	NM_011857	3.62	5.16	2.77	3.97	No
A_52_P74441	Tes	21753	NM_207176	9.15	8.17	8.53	8.14	No
A_51_P483436	Tex36	73808	NM_028654	3.66	4.62	3.91	4.35	No
A_52_P205831	Tfap2a	21418	NM_011547	9.05	8.30	9.39	9.09	Yes
A_52_P228236	Tfrc	22042	NM_011638	7.86	10.34	7.96	9.79	No
A_52_P228236	Tfrc	22042	NM_011638	8.08	10.33	8.12	9.86	No
A_52_P228236	Tfrc	22042	NM_011638	8.07	10.22	8.00	9.77	No
A_52_P228236	Tfrc	22042	NM_011638	8.07	10.23	8.07	9.80	No
A_52_P228236	Tfrc	22042	NM_011638	8.03	10.18	7.93	9.82	No
A_52_P228236	Tfrc	22042	NM_011638	8.04	10.19	7.98	9.73	No
A_52_P228236	Tfrc	22042	NM_011638	8.13	10.27	8.10	9.87	No
A_52_P228236	Tfrc	22042	NM_011638	8.12	10.22	8.08	9.74	No
A_52_P228236	Tfrc	22042	NM_011638	8.13	10.14	8.05	9.82	No
A_52_P228236	Tfrc	22042	NM_011638	8.40	10.32	8.09	9.85	No
A_51_P415126	Tgfa	21802	NM_031199	4.67	4.03	4.83	4.43	No
A_51_P415126	Tgfa	21802	NM_031199	4.68	4.05	4.83	4.42	No
A_52_P949440	Tgfbli1	21804	NM_001289550	9.75	8.95	9.74	9.10	No
A_51_P182796	Tgm2	21817	NM_009373	6.06	5.20	5.84	5.22	No
A_52_P220879	Tgm2	21817	NM_009373	10.76	9.86	10.84	10.03	No
A_52_P363951	Thbs1	21825	NM_011580	10.17	11.03	9.21	10.41	Yes
A_52_P487599	Thnsl2	232078	NM_178413	8.42	7.65	8.31	7.75	No
A_51_P344878	Thop1	50492	NM_022653	10.76	12.26	10.62	11.45	No
A_52_P173526	Thsd7a	330267	NM_001164805	9.07	8.27	9.19	8.61	No
A_52_P117334	Thumpd3	14911	NM_008188	10.02	10.68	10.19	10.40	No
A_51_P232142	Thumpd3	14911	NM_008188	11.24	11.97	11.43	11.79	No
A_52_P479249	Thyl	21838	NM_009382	13.40	12.81	13.86	13.48	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P371974	Tiam1	21844	NM_009384	8.10	9.37	8.06	8.96	No
A_51_P294017	Ticam1	106759	NM_174989	9.86	8.89	9.93	9.53	Yes
A_51_P294020	Ticam1	106759	NM_174989	9.77	8.86	9.92	9.49	Yes
A_51_P249989	Tifa	211550	NM_145133	8.17	7.53	8.03	7.87	No
A_51_P348624	Timm10	30059	NM_013899	11.91	13.10	11.81	12.75	No
A_51_P511623	Timm23	53600	NM_016897	13.11	13.94	13.35	13.84	No
A_51_P373043	Timm8a1	30058	NM_013898	11.15	12.39	11.53	12.44	No
A_51_P283004	Tinag	26944	NM_012033	9.60	8.49	8.90	8.33	No
A_52_P676956	Tirap	117149	NM_001177845	9.30	8.53	9.35	9.14	No
A_51_P278686	Tirap	117149	NM_001177845	9.83	9.05	9.85	9.52	No
A_52_P679101	Tjp2	21873	NM_011597	6.43	7.65	6.33	7.36	No
A_51_P394515	Tkt	21881	NM_009388	15.31	14.61	15.31	14.83	No
A_52_P330694	Tle1	21885	NM_011599	10.29	11.78	10.67	11.53	No
A_51_P439446	Tll1	21892	U34042	6.85	6.05	6.49	6.04	No
A_51_P452629	Tlr2	24088	NM_011905	10.86	9.38	10.78	10.01	No
A_51_P452629	Tlr2	24088	NM_011905	10.84	9.53	10.77	9.95	No
A_51_P452629	Tlr2	24088	NM_011905	10.90	9.60	10.81	10.10	No
A_51_P452629	Tlr2	24088	NM_011905	10.88	9.59	10.79	10.04	No
A_51_P452629	Tlr2	24088	NM_011905	10.81	9.53	10.71	10.02	No
A_51_P452629	Tlr2	24088	NM_011905	10.98	9.71	10.82	10.08	No
A_51_P452629	Tlr2	24088	NM_011905	10.81	9.54	10.82	10.13	No
A_51_P452629	Tlr2	24088	NM_011905	10.81	9.70	10.76	10.07	No
A_51_P452629	Tlr2	24088	NM_011905	10.78	9.70	10.81	10.13	No
A_51_P452629	Tlr2	24088	NM_011905	10.82	9.76	10.88	10.20	No
A_52_P85174	Tlr3	142980	NM_126166	5.72	5.01	6.04	5.23	No
A_52_P117393	Tlr6	21899	NM_011604	9.70	9.04	9.79	9.35	No
A_51_P401683	Tm6sf1	107769	NM_145375	3.56	8.18	3.33	5.89	Yes
A_52_P237948	Tm6sf1	107769	NM_145375	3.27	8.14	3.56	6.53	Yes
A_52_P988932	Tm9sf1	74140	NM_028780	3.94	4.95	4.25	4.60	No
A_52_P16877	Tmcc3	319880	NM_172051	7.79	9.42	8.04	8.37	Yes
A_51_P189272	Tmcc3	319880	NM_172051	12.32	13.46	12.34	12.79	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P416521	Tmcc3	319880	NM_172051	12.99	14.37	13.19	13.56	No
A_52_P78064	Tmco4	77056	NM_029857	6.30	5.59	6.10	5.81	No
A_52_P517029	Tmeff2	56363	NM_019790	3.32	4.95	4.45	5.72	Yes
A_52_P229052	Tmeff2	56363	NM_019790	8.09	9.01	8.77	9.95	No
A_51_P376959	Tmeff2	56363	NM_019790	8.87	9.86	9.48	10.57	No
A_51_P444669	Tmem106a	217203	NM_144830	8.95	8.00	8.72	7.97	No
A_51_P114049	Tmem109	68539	NM_134142	15.68	16.64	15.37	16.13	No
A_51_P164987	Tmem120b	330189	NM_001039723	8.02	10.57	8.40	9.85	No
A_51_P421664	Tmem123	71929	NM_133739	12.08	13.29	11.88	12.73	No
A_52_P101982	Tmem129	68366	NM_026698	11.41	12.65	11.55	12.13	No
A_51_P468037	Tmem129	68366	NM_026698	7.41	8.61	7.52	8.19	No
A_52_P101399	Tmem130	243339	NM_177735	3.80	5.23	3.23	5.10	No
A_52_P463977	Tmem140	68487	NM_197986	11.47	10.61	11.85	11.53	No
A_52_P571371	Tmem144	70652	NM_027495	5.54	4.70	4.16	4.11	No
A_52_P339154	Tmem14a	75712	NM_029398	9.27	8.43	9.33	8.72	No
A_51_P422893	Tmem14a	75712	NM_029398	11.79	10.72	11.68	11.14	No
A_51_P412579	Tmem182	381339	XM_006496124	2.38	11.12	2.80	10.23	No
A_52_P8685	Tmem183a	57439	NM_020588	10.24	11.08	10.39	10.94	No
A_51_P515532	Tmem200a	77220	NM_029881	7.29	9.73	7.41	8.78	No
A_51_P384441	Tmem201	230917	NM_177672	7.43	8.87	7.27	7.93	Yes
A_51_P241843	Tmem202	73893	NM_178388	7.54	6.93	7.96	7.58	Yes
A_51_P331207	Tmem218	66279	NM_025464	12.25	11.52	12.09	11.71	No
A_52_P220783	Tmem229b	268567	NM_178745	8.96	10.34	9.27	9.88	No
A_51_P155813	Tmem255a	245386	NM_172930	3.49	6.25	3.49	6.48	No
A_51_P303725	Tmem38a	74166	NM_144534	10.80	13.48	10.62	12.30	Yes
A_52_P434729	Tmem38a	74166	NM_144534	8.70	11.64	8.62	10.38	No
A_51_P181865	Tmem38b	52076	NM_028053	5.61	8.80	6.12	8.14	No
A_51_P152216	Tmem41a	66664	NM_025693	11.16	11.77	11.38	11.60	No
A_51_P268953	Tmem64	100201	NM_181401	12.84	14.12	12.71	13.96	No
A_52_P10017	Tmem64	100201	NM_181401	11.05	12.09	11.17	12.00	No
A_51_P175945	Tmem74	239408	AK045145	5.73	4.44	6.09	5.23	Yes

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P196388	Tmem8	60455	NM_021793	9.72	9.13	10.12	9.65	No
A_51_P261835	Tmem8c	66139	NM_025376	8.57	13.33	9.33	12.01	Yes
A_51_P297069	Tmod1	21916	NM_021883	3.46	10.96	4.74	9.65	Yes
A_51_P297068	Tmod1	21916	NM_021883	2.11	9.01	2.76	7.68	No
A_51_P234488	Tmod2	50876	NM_001038710	7.01	6.36	7.55	6.53	No
A_52_P451614	Tmod2	50876	NM_001038710	8.73	8.05	8.92	8.22	No
A_51_P516741	Tmprss5	80893	NM_030709	3.55	5.61	3.55	4.75	No
A_51_P288916	Tmtc2	278279	NM_177368	4.08	8.06	3.43	6.47	No
A_52_P412417	Tnfaip3	21929	NM_009397	8.68	9.95	8.50	9.86	No
A_51_P435968	Tnfaip8	106869	NM_134131	10.63	9.70	10.67	10.25	No
A_51_P131408	Tnfrsf12a	27279	NM_013749	12.36	13.49	12.69	13.66	No
A_51_P399305	Tnfrsf19	29820	NM_013869	2.69	6.66	2.30	4.81	Yes
A_51_P174061	Tnik	665113	NM_026910	2.53	7.77	2.83	5.75	Yes
A_52_P663757	Tnik	665113	NM_026910	3.73	7.31	2.94	6.36	Yes
A_51_P461894	Tnnc1	21924	NM_009393	6.68	12.82	6.68	11.47	No
A_51_P353232	Tnnc2	21925	NM_009394	6.42	16.75	6.41	15.14	Yes
A_52_P657360	Tnni1	21952	NM_021467	9.51	13.54	9.53	11.96	Yes
A_51_P435704	Tnni1	21952	NM_021467	8.61	12.33	8.58	10.96	No
A_51_P355122	Tnni2	21953	NM_009405	7.87	14.19	7.06	12.95	Yes
A_51_P362627	Tnnt1	21955	NM_011618	7.41	14.30	6.61	12.45	Yes
A_51_P338262	Tnnt2	21956	NM_011619	6.95	15.99	6.86	14.50	Yes
A_51_P345699	Tnnt3	21957	NM_011620	5.77	13.86	5.11	11.92	Yes
A_52_P622418	Tnnt3	21957	NM_011620	5.53	14.36	4.82	12.49	Yes
A_51_P276404	Top1	21969	AK083930	11.57	12.54	11.46	11.81	No
A_51_P410918	Tor4a	227612	NM_146115	9.57	8.83	9.78	9.06	No
A_52_P561073	Tox	252838	NM_145711	7.98	6.03	7.92	6.64	No
A_52_P591017	Tpbg	21983	NM_011627	11.16	10.44	11.21	11.04	Yes
A_51_P162209	Tpbg	21983	NM_011627	8.36	7.71	8.56	8.28	No
A_52_P476560	Tpcn1	252972	AK077411	11.94	12.91	12.05	12.82	No
A_51_P325173	Tpm1	22003	NM_024427	8.68	14.11	8.64	13.26	Yes
A_52_P532910	Tpm1	22003	NM_024427	13.81	14.71	13.93	14.48	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P315976	Tpm2	22004	NM_009416	14.06	15.61	14.14	15.17	No
A_51_P176661	Tprn	97031	NM_175286	10.75	10.06	10.73	10.31	No
A_52_P727235	Trabd2b	666048	NM_001085549	7.76	7.10	8.04	7.20	No
A_51_P243808	Traf3ip3	215243	NM_153137	3.64	5.76	3.89	5.00	No
A_51_P106059	Traf4	22032	NM_009423	13.10	14.39	13.00	14.01	No
A_52_P78403	Traf4	22032	NM_009423	9.31	10.67	9.22	10.18	No
A_51_P102631	Trappc2	66226	AK077643	9.80	10.41	10.08	10.32	No
A_52_P771589	Trdn	76757	AF223416	2.89	4.41	2.79	3.96	No
A_51_P249608	Trdn	76757	AF223416	2.32	8.68	2.84	8.41	No
A_52_P268563	Trdn	76757	AF223416	2.37	8.76	3.14	8.18	No
A_52_P457967	Trerf1	224829	AK035176	7.66	6.85	7.58	7.15	No
A_51_P331570	Trib3	228775	NM_175093	11.30	10.26	11.93	10.54	No
A_51_P114714	Trim13	66597	NM_023233	9.48	10.12	9.62	9.95	No
A_52_P367034	Trim34a	94094	NM_030684	4.81	3.97	4.82	3.90	No
A_51_P181175	Trim54	58522	NM_021447	6.94	12.94	6.40	11.60	No
A_51_P181785	Trim56	384309	NM_201373	10.55	9.72	10.69	10.42	Yes
A_52_P517224	Trim63	433766	NM_001039048	2.28	8.28	2.52	6.81	No
A_51_P158103	Triobp	110253	NM_001024716	9.98	9.15	10.25	9.66	No
A_51_P132551	Trip10	106628	NM_134125	8.29	7.70	8.26	7.97	No
A_51_P296878	Triqk	208820	NM_173746	10.49	9.55	10.29	9.69	No
A_51_P137317	Trit1	66966	NM_025873	8.59	10.02	8.74	9.56	No
A_52_P45797	Trmt10a	108943	NM_175389	7.24	8.16	7.55	8.09	No
A_51_P126310	Trmt10a	108943	NM_175389	9.30	9.95	9.21	9.83	No
A_51_P325152	Trmt61a	328162	NM_177374	8.89	10.32	9.08	10.15	No
A_52_P642039	Trmt61b	68789	NR_027952	4.42	5.28	5.09	4.96	No
A_52_P391624	Trnp1	69539	NM_001081156	13.83	13.18	14.35	13.86	Yes
A_51_P226184	Trove2	20822	NM_013835	8.41	7.82	8.24	7.85	No
A_51_P175580	Trp53inp1	60599	NM_021897	13.91	13.31	12.70	12.39	Yes
A_52_P547315	Trpc1	22063	NM_011643	7.05	6.44	7.43	7.10	Yes
A_51_P269203	Trpv4	63873	NM_022017	12.83	11.98	12.69	12.00	No
A_51_P110725	Tsc22d2	72033	NM_001081229	8.34	7.71	8.15	8.01	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P706621	Tsc22d2	72033	NM_001081229	9.09	8.44	8.93	8.82	No
A_52_P600518	Tsc22d3	14605	NM_001077364	8.60	9.34	8.63	8.76	No
A_52_P384392	Tsc22d4	78829	NM_023910	10.73	10.10	10.55	9.76	No
A_51_P387379	Tshz3	243931	NM_172298	10.01	11.23	9.69	10.85	No
A_51_P374707	Tspan12	269831	NM_173007	8.81	10.50	8.92	10.69	No
A_51_P334308	Tspan14	52588	NM_145928	12.95	14.05	12.96	13.47	No
A_52_P657376	Tspan15	70423	NM_197996	9.83	9.07	10.10	9.62	No
A_51_P367240	Tspan33	232670	NM_146173	3.88	13.10	2.97	11.89	Yes
A_52_P398158	Tspan33	232670	NM_146173	2.30	7.35	2.83	5.86	No
A_51_P509609	Tspan4	64540	NM_053082	15.56	14.79	15.27	14.89	No
A_51_P360077	Tspan9	109246	NM_175414	7.56	8.46	7.39	8.26	No
A_52_P324825	Tspan9	109246	NM_175414	13.81	14.79	13.73	14.53	No
A_52_P448357	Tspyl4	72480	NM_030203	6.14	5.38	6.21	5.37	No
A_51_P409132	Tsr1	104662	NM_177325	9.08	10.28	9.50	10.02	No
A_52_P221900	Tsr1	104662	NM_177325	10.31	11.42	10.52	11.05	No
A_51_P164788	Tssk4	71099	NM_027673	6.83	6.14	7.24	6.81	Yes
A_51_P292490	Ttbk2	140810	NM_001024856	9.79	9.15	9.61	9.37	No
A_52_P327118	Ttc12	235330	NM_172770	8.86	8.25	9.04	8.62	No
A_52_P96748	Ttc30b	72421	NM_028235	10.09	9.31	10.09	9.55	No
A_52_P239752	Ttc39c	72747	XM_006526284	5.79	4.42	5.48	5.35	No
A_51_P180891	Ttc39c	72747	XM_006526284	12.35	11.65	12.50	11.89	No
A_52_P21	Ttc9	69480	NM_001033149	5.43	7.24	6.02	7.40	No
A_51_P286878	Ttll11	74410	NM_029774	6.65	7.84	6.61	7.31	No
A_52_P316824	Ttll5	320244	NM_001081423	10.06	10.79	9.83	10.78	No
A_51_P512629	Ttll5	320244	NM_001081423	9.74	10.71	9.68	10.63	No
A_51_P401792	Ttn	22138	NM_011652	2.88	11.65	3.85	9.42	Yes
A_52_P454295	Ttn	22138	NM_011652	2.27	8.26	3.26	6.21	Yes
A_51_P148509	Ttn	22138	NM_011652	3.69	9.93	3.70	8.57	Yes
A_51_P219312	Ttn	22138	NM_011652	5.55	10.28	5.81	9.18	Yes
A_51_P258817	Ttpa	50500	NM_015767	4.43	5.72	4.61	5.77	No
A_52_P29610	Ttpa	50500	NM_015767	4.10	5.62	4.33	5.51	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P145404	Tuba3a	22144	NM_009446	4.13	5.14	4.42	5.35	No
A_51_P514300	Tuba4a	22145	NM_009447	10.91	12.41	11.05	12.20	No
A_52_P409498	Tuba4a	22145	NM_009447	9.78	11.12	9.89	10.84	No
A_51_P421140	Tubb6	67951	NM_026473	15.25	16.73	15.10	16.43	No
A_51_P389017	Tulp2	56734	NM_008807	6.80	5.19	6.65	5.39	No
A_51_P165504	Twist2	13345	NM_007855	9.30	8.69	9.69	9.27	No
A_51_P316816	Uba7	74153	NM_023738	9.11	8.32	9.49	9.00	No
A_51_P504478	Ubac1	98766	NM_133835	12.52	13.51	12.65	13.22	No
A_52_P585021	Ubap2l	74383	NM_028475	5.77	6.39	5.91	6.39	No
A_52_P476754	Ubash3b	72828	NM_176860	8.66	10.36	8.67	9.57	Yes
A_51_P430450	Ube2f	67921	AK030594	8.38	9.22	8.68	9.10	No
A_51_P430445	Ube2f	67921	AK030594	11.38	12.06	11.49	11.95	No
A_52_P244064	Ube2g2	22213	NM_019803	12.79	13.79	12.69	13.39	No
A_51_P248441	Ube2g2	22213	NM_019803	7.28	8.49	7.30	8.00	No
A_52_P214740	Ube2l6	56791	NM_019949	8.76	8.09	9.03	8.24	No
A_52_P188134	Ubl5	66177	NM_025401	8.06	7.21	7.56	6.94	No
A_51_P303217	Ucma	68527	NM_026754	5.28	12.38	5.57	11.31	Yes
A_51_P297105	Ucp2	22228	NM_011671	2.72	5.45	2.55	4.64	No
A_51_P332717	Ugcg	22234	NM_011673	7.98	9.08	7.98	9.15	No
A_51_P308362	Ugt1a6b	394435	NM_201410	14.06	12.96	14.17	13.51	No
A_51_P478419	Unc45b	217012	NM_178680	2.57	13.01	2.09	11.37	Yes
A_52_P541095	Unc5a	107448	NM_153131	4.75	7.53	4.61	6.29	Yes
A_51_P469449	Ung	22256	NM_001040691	9.23	10.00	8.66	9.55	No
A_51_P341285	Uqcc1	56046	NM_018888	10.42	11.13	10.44	10.72	No
A_52_P468537	Uqcc1	56046	NM_018888	9.80	10.42	9.88	10.14	No
A_52_P171692	Urah	76974	AK004470	6.39	5.24	5.11	4.81	Yes
A_51_P389479	Urah	76974	AK004470	7.67	6.68	7.22	7.01	No
A_51_P288419	Usp13	72607	AV347341	7.03	7.73	6.15	7.16	No
A_51_P302273	Usp17la	13531	NM_007887	6.05	5.17	5.47	4.83	No
A_51_P164219	Usp18	24110	NM_011909	7.46	6.45	6.82	5.60	Yes
A_51_P172663	Usp2	53376	NM_198092	7.02	8.33	7.35	8.02	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P255489	Usp2	53376	NM_198092	4.16	5.53	4.51	5.19	No
A_51_P122085	Usp20	74270	NM_028846	12.00	12.81	12.07	12.54	No
A_52_P452019	Usp31	76179	NM_001033173	7.13	7.76	7.29	8.00	No
A_51_P153693	Utp14b	195434	NM_001001981	5.43	6.56	6.39	5.90	Yes
A_52_P281924	Vamp5	53620	NM_016872	8.89	10.62	8.88	10.33	No
A_52_P1037117	Vangl1	229658	NM_177545	7.56	6.88	7.52	6.92	No
A_52_P350687	Vangl1	229658	NM_177545	8.92	8.07	8.88	8.27	No
A_51_P452203	Vash2	226841	NM_144879	8.74	10.01	8.75	9.37	No
A_51_P487298	Vasn	246154	NM_139307	14.11	13.44	14.23	13.94	No
A_52_P219314	Vasp	22323	NM_009499	7.54	9.16	7.83	8.47	No
A_51_P179131	Vasp	22323	NM_009499	9.40	10.63	9.45	10.05	No
A_52_P613596	Vav3	57257	NM_020505	8.71	8.04	9.37	8.93	No
A_51_P210956	Vcam1	22329	NM_011693	13.14	12.46	12.98	13.07	No
A_52_P334562	Vdr	22337	NM_009504	11.11	10.36	11.38	10.94	No
A_51_P491648	Vgl12	215031	NM_153786	2.56	6.32	2.75	5.95	No
A_51_P417077	Vill	22351	NM_001164567	10.12	9.46	9.67	9.26	No
A_51_P395555	Vkorc1	27973	NM_178600	14.83	14.24	14.81	14.39	No
A_51_P240329	Vma21	67048	NM_001290781	9.67	10.84	9.45	10.11	Yes
A_52_P224373	Vma21	67048	NM_001290781	10.57	11.95	10.67	11.56	No
A_51_P494863	Vmac	106639	NM_178926	10.12	9.36	10.21	9.65	No
A_51_P424532	Vnn1	22361	NM_011704	5.83	4.93	5.37	5.04	No
A_51_P400217	Vpreb1	22362	NM_016982	9.05	7.93	8.95	8.32	No
A_52_P561650	Vwal	246228	NM_147776	2.96	5.14	3.25	4.65	No
A_51_P103397	Vwf	22371	NM_011708	3.66	5.30	2.89	4.59	Yes
A_52_P436590	Wbscr17	212996	NM_145218	3.53	4.59	3.99	5.34	No
A_51_P288558	Wdr16	71860	NM_027963	2.84	7.42	2.42	5.30	Yes
A_51_P489108	Wdr19	213081	AK052303	12.54	11.81	12.28	11.96	No
A_52_P355057	Wdr4	57773	NM_021322	11.26	12.06	11.72	12.12	No
A_52_P384901	Wdr43	72515	NM_175639	10.31	11.14	10.60	11.16	No
A_51_P118650	Wdr6	83669	NM_031392	12.78	11.97	12.24	11.70	No
A_52_P663303	Wdr60	217935	AK046399	10.24	9.55	9.94	9.43	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P492245	Wdr61	66317	NM_001025375	14.86	13.69	15.18	14.27	No
A_51_P492765	Wdr75	73674	NM_028599	11.02	11.83	11.33	11.59	No
A_52_P443330	Wdr77	70465	NM_027432	6.39	7.56	6.25	6.96	Yes
A_52_P393056	Wdr77	70465	NM_027432	8.18	9.26	8.61	8.97	No
A_52_P529486	Wdr77	70465	NM_027432	9.61	10.65	9.98	10.44	No
A_51_P463552	Wdr78	242584	NM_146254	10.82	9.82	10.39	9.91	No
A_51_P216593	Wfs1	22393	NM_011716	12.22	13.23	12.22	12.62	Yes
A_51_P303620	Whrn	73750	AK039124	10.36	11.08	10.37	11.21	No
A_52_P14321	Whsc1	107823	NM_001081102	6.48	7.37	6.59	7.25	No
A_51_P220343	Wisp1	22402	NM_018865	12.25	13.00	11.91	12.73	No
A_51_P486217	Wnk4	69847	NM_175638	7.87	6.63	8.07	6.97	No
A_52_P61864	Wnt2	22413	NM_023653	8.79	7.03	8.73	7.44	No
A_52_P250865	Wnt5a	22418	XM_006518925	10.07	8.95	9.95	9.58	No
A_51_P137991	Wnt5b	22419	NM_009525	11.69	10.04	11.54	10.16	No
A_52_P85805	Wnt5b	22419	NM_009525	9.14	7.64	9.10	7.75	No
A_51_P375453	Wnt9a	216795	NM_139298	8.73	10.01	8.82	9.86	No
A_51_P432520	Wrnip1	78903	NM_030215	11.55	12.36	11.70	12.03	No
A_52_P513281	Wrnip1	78903	NM_030215	10.74	11.58	10.99	11.34	No
A_51_P386358	Wwp2	66894	NM_025830	14.63	13.88	14.39	13.88	No
A_51_P494403	Xaf1	327959	NM_001037713	9.67	8.66	9.19	8.32	No
A_51_P119239	Xirp1	22437	NM_011724	4.03	8.10	4.38	7.13	No
A_51_P168792	Xylb	102448	BC026406	9.41	8.82	9.51	9.25	No
A_52_P217838	Ybx1	22608	NM_011732	8.00	8.67	7.54	8.23	No
A_51_P311977	Ybx3	56449	NM_011733	9.11	10.07	9.43	9.73	No
A_51_P305731	Yipf2	74766	NM_138303	9.99	9.03	10.14	9.40	No
A_52_P534150	Yipf2	74766	NM_138303	10.40	9.65	10.40	9.74	No
A_51_P186961	Yipf4	67864	NM_026417	10.65	10.04	10.61	10.26	No
A_52_P305890	Yipf5	67180	NM_023311	13.40	12.71	13.45	13.15	No
A_52_P127465	Ypel1	106369	NM_023249	8.74	7.35	8.36	7.93	No
A_52_P631307	Ypel4	241525	XM_006499466	7.21	6.24	7.51	7.02	No
A_52_P254231	Yrdc	230734	NM_153566	9.51	10.67	9.87	10.53	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P218563	Yrdc	230734	NM_153566	8.71	9.48	8.95	9.49	No
A_51_P388042	Zbtb18	30928	NM_013915	9.37	12.90	9.22	11.95	No
A_51_P108131	Zbtb4	75580	NM_029348	12.54	11.89	12.56	12.17	No
A_51_P381178	Zbtb46	72147	NM_028125	3.81	5.73	3.39	4.44	Yes
A_52_P65237	Zbtb7c	207259	NM_145356	3.93	6.09	3.26	5.49	No
A_52_P281941	Zdhhc2	70546	XM_006509487	9.37	8.72	9.70	9.15	No
A_52_P77983	Zfand5	22682	NM_009551	10.70	11.60	10.76	11.34	No
A_52_P233664	Zfhx3	11906	NM_007496	9.83	9.09	9.50	9.12	No
A_51_P342556	Zfhx4	80892	NM_030708	5.23	4.29	5.22	4.68	No
A_52_P250517	Zfp106	20402	NM_011743	6.44	7.78	6.36	7.13	Yes
A_51_P390826	Zfp106	20402	NM_011743	8.78	10.25	8.86	9.69	No
A_52_P468937	Zfp106	20402	NM_011743	5.13	7.21	5.67	6.48	No
A_52_P644354	Zfp106	20402	NM_011743	10.88	11.90	11.05	11.45	No
A_52_P451345	Zfp148	22661	NM_011749	9.89	9.26	9.42	9.17	No
A_52_P290221	Zfp182	319535	NM_001111076	7.19	6.52	7.17	6.89	No
A_52_P384264	Zfp185	22673	NM_009549	7.46	6.47	6.56	6.26	Yes
A_51_P137808	Zfp26	22688	NM_011753	3.61	4.31	4.61	4.35	Yes
A_51_P250445	Zfp276	57247	NM_020497	8.92	9.70	9.24	9.78	No
A_52_P664404	Zfp286	192651	NM_138949	7.52	6.94	7.31	7.16	No
A_52_P664405	Zfp286	192651	NM_138949	7.49	6.90	7.71	7.01	No
A_52_P597603	Zfp369	170936	AK082535	4.14	3.13	4.17	3.09	No
A_52_P282091	Zfp386	56220	AK043310	5.72	6.69	6.20	7.00	No
A_52_P5823	Zfp420	233058	AK029387	4.30	5.52	4.41	5.33	No
A_51_P262268	Zfp473	243963	NM_178734	4.60	3.77	4.11	4.23	No
A_51_P126626	Zfp503	218820	NM_145459	14.15	13.45	14.20	13.78	No
A_52_P137901	Zfp516	329003	NM_183033	8.78	8.10	8.86	8.54	No
A_52_P254275	Zfp52	22710	NM_144515	10.33	9.48	10.10	9.95	No
A_51_P122762	Zfp618	72701	XM_006538287	4.37	3.06	3.57	3.39	No
A_51_P106144	Zfp64	22722	NM_009564	12.15	11.50	12.03	11.97	No
A_52_P532957	Zfp667	384763	NM_001024928	7.92	8.86	7.90	8.58	No
A_52_P79506	Zfp667	384763	NM_001024928	5.76	6.98	6.00	6.57	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P612636	Zfp697	242109	AK082993	5.37	6.16	5.68	6.45	No
A_52_P65077	Zfp820	75424	NM_029281	9.29	8.66	9.61	9.17	No
A_51_P100379	Zfp947	210853	NM_177596	7.49	6.87	7.59	7.07	No
A_51_P123731	Zfr2	103406	NM_001034895	7.09	6.05	7.19	6.55	No
A_51_P381230	Zhx2	387609	NM_199449	4.59	5.99	4.62	4.98	Yes
A_52_P321325	Zhx3	320799	NM_177263	11.27	12.07	11.45	12.08	No
A_52_P234039	Znrf3	407821	NM_001290501	6.98	7.66	7.21	7.67	No
A_52_P42762	Zp3	22788	NM_011776	6.93	5.96	6.91	6.26	No
A_51_P152973	Zp3	22788	NM_011776	6.39	5.25	6.40	5.68	No
A_51_P354345	Zpbp	53604	NM_015785	6.23	5.51	5.98	5.73	No
A_51_P382805	Zpbp2	69376	NM_027061	6.92	6.26	6.77	6.44	No
A_52_P663011	Zswim6	67263	NM_145456	9.22	10.26	9.08	9.70	No
A_51_P215995	Zswim6	67263	NM_145456	10.61	11.62	10.58	11.32	No
A_52_P620240			AK044380	4.29	2.67	4.82	4.55	Yes
A_52_P299231			XM_006513371	7.02	11.01	7.41	9.34	Yes
A_52_P196271			AK034076	7.00	6.19	7.47	7.53	Yes
A_52_P189050			AK161939	8.92	11.45	8.75	10.25	Yes
A_51_P319662			AK054460	4.19	5.05	4.68	3.90	Yes
A_51_P254634			AK046342	3.44	6.35	3.14	5.22	Yes
A_51_P256798				3.37	5.89	3.74	4.84	Yes
A_51_P491279			AK020378	5.49	6.43	5.07	5.41	Yes
A_52_P558290			AK031509	4.36	3.37	5.01	4.37	Yes
A_52_P609913			AK081298	3.71	5.27	4.24	4.31	Yes
A_52_P488011				8.93	8.32	9.36	9.21	Yes
A_52_P1197653			CB590277	6.69	6.10	6.80	6.88	Yes
A_51_P237746			AK004226	12.66	12.07	13.23	12.84	Yes
A_52_P883941			AK048677	8.85	9.62	8.32	8.90	Yes
A_52_P651784				9.91	10.59	9.96	9.92	Yes
A_52_P335218				7.67	6.53	6.82	5.89	Yes
A_52_P613087				8.09	7.16	8.18	7.76	Yes
A_52_P152859			AK037803	8.06	7.39	7.54	7.37	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P374395				7.55	6.75	7.11	6.97	No
A_52_P273438				7.33	7.97	7.59	7.95	No
A_51_P205000			AF000427	9.01	8.36	8.87	8.49	No
A_52_P182118				7.23	6.47	7.12	6.66	No
A_51_P183701				5.49	4.73	5.38	5.20	No
A_52_P344978				9.24	9.87	9.18	9.45	No
A_52_P657133				7.18	6.39	6.30	6.43	No
A_51_P513369			AK010841	5.47	6.37	5.92	6.58	No
A_51_P114407				11.03	12.74	10.45	12.11	No
A_52_P237440				4.43	5.23	4.67	5.00	No
A_52_P2659				7.23	9.52	6.38	8.28	No
A_51_P401471			AK011951	10.54	11.15	10.06	10.67	No
A_52_P1132371			AK082068	5.11	4.49	4.77	4.39	No
A_52_P618580			AK132852	5.33	5.96	5.66	6.01	No
A_52_P1100772			AK089422	6.57	5.64	6.84	5.95	No
A_51_P315849				8.83	7.94	8.42	8.09	No
A_52_P871130			BB526037	3.62	5.22	4.59	4.92	No
A_52_P885203				5.50	4.32	5.08	4.39	No
A_52_P510790				9.56	8.44	9.09	7.88	No
A_52_P31625			AK079835	4.83	5.60	4.60	5.23	No
A_52_P860150			AK086814	6.18	5.15	5.86	5.65	No
A_52_P52182				3.50	5.92	4.34	5.31	No
A_52_P883263			AK034726	5.60	4.60	4.85	4.77	No
A_51_P355094				4.90	3.25	4.15	4.02	No
A_52_P441305				5.66	5.04	5.46	5.23	No
A_52_P245766				10.79	11.63	11.08	11.45	No
A_52_P615247			XR_387206	4.17	3.02	3.73	3.58	No
A_52_P645675				4.51	3.75	4.04	3.95	No
A_52_P1067988			AK046257	6.33	5.15	6.98	5.93	No
A_52_P1147868			AK035470	7.27	8.01	7.52	7.77	No
A_52_P231697			AK078537	5.73	4.46	6.21	5.76	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P361951				10.99	11.74	10.76	11.28	No
A_52_P175748				5.44	7.57	5.90	7.05	No
A_52_P190286				5.92	6.93	6.44	7.14	No
A_52_P1132727			AK076363	4.78	4.05	4.52	4.20	No
A_52_P255224			AK032716	4.83	4.17	4.56	3.86	No
A_51_P502068				11.47	10.82	11.58	11.17	No
A_52_P1076740			AK082805	6.65	8.08	6.98	8.29	No
A_52_P707683			AK087612	4.65	2.98	4.27	3.82	No
A_51_P164256				2.16	5.84	2.50	5.85	No
A_51_P326826			AK090125	6.02	6.66	6.22	6.28	No
A_52_P1052802			AK089990	5.90	5.01	5.84	5.40	No
A_52_P615958				9.70	10.36	9.54	10.11	No
A_52_P532370				10.41	11.17	10.18	10.71	No
A_51_P124315			XM_006498501	5.93	6.99	5.54	6.57	No
A_52_P1367			AK162987	6.38	5.55	6.10	5.78	No
A_51_P310886			AK047015	5.26	6.57	4.87	5.76	No
A_51_P362801				5.15	4.26	4.96	4.62	No
A_52_P788961				9.13	8.18	9.34	8.60	No
A_52_P949155				7.50	8.99	7.70	8.75	No
A_52_P1004880				3.16	12.91	2.90	11.81	No
A_52_P390448				6.92	8.17	7.17	7.64	No
A_51_P478544				11.58	12.18	11.49	11.96	No
A_52_P368126			AK162351	3.08	4.42	2.78	2.95	No
A_52_P55489				12.80	13.50	12.63	13.13	No
A_52_P112591			U07662	3.29	5.10	2.88	4.09	No
AT_ssM_AB_5				14.46	15.23	14.54	14.90	No
AT_ssM_AB_5				14.58	15.23	14.51	14.94	No
AT_ssM_AB_5				14.61	15.25	14.45	14.94	No
AT_ssM_AB_5				14.61	15.24	14.51	14.86	No
A_52_P432937				11.98	12.72	11.86	12.47	No
A_52_P716154			AV242843	6.84	7.66	6.95	7.37	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P1021832				10.67	13.12	10.60	12.54	No
A_52_P653054				10.14	8.62	10.00	9.11	No
A_52_P347078			AK089832	7.70	7.12	7.87	7.39	No
A_52_P604345			AK049762	6.19	5.06	5.96	5.47	No
A_52_P555089				12.28	12.99	12.10	12.66	No
A_52_P348667			AK031090	6.35	5.52	6.42	5.59	No
A_51_P262453			AK021406	5.07	4.29	5.24	3.98	No
A_52_P1196772			AK087259	10.83	9.83	10.96	10.32	No
A_52_P662961			AK013898	3.93	7.43	4.11	6.37	No
A_52_P101838				3.83	5.14	4.00	4.53	No
A_51_P153702				6.15	4.91	5.92	5.41	No
A_52_P1196662			AK086825	3.48	4.36	3.67	4.02	No
A_52_P795929			AK081614	4.23	6.26	4.04	5.42	No
A_52_P624998				6.96	7.78	7.04	7.62	No
A_52_P1077539				4.18	5.30	4.08	4.69	No
A_52_P61549				14.38	15.09	14.26	14.76	No
A_52_P190059				8.88	9.63	8.94	9.46	No
A_52_P110913			AK087420	3.50	5.51	3.30	5.25	No
A_52_P654437				13.18	13.93	13.10	13.63	No
A_52_P167500				9.56	10.37	9.62	10.12	No
A_52_P664044			CN524910	5.12	7.58	5.24	7.14	No
A_52_P661496				6.15	7.11	6.11	6.68	No
A_52_P932240			AK082846	4.83	3.80	4.89	4.17	No
A_52_P764017				5.71	6.75	5.68	6.52	No
AT_ssM_GD_5				11.73	12.63	11.70	12.26	No
AT_ssM_GD_5				11.77	12.61	11.72	12.23	No
AT_ssM_GD_5				11.90	12.64	11.68	12.21	No
AT_ssM_GD_5				11.93	12.64	11.64	12.15	No
A_52_P272054				11.46	12.30	11.42	12.01	No
A_51_P114126			AK012034	3.91	5.69	3.88	4.75	No
AT_ssM_TR_3				3.44	5.92	3.95	5.55	No

Table A2.2 List of genes significantly induced or downregulated by MyoD during C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				Six4 target
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
AT_ssM_TR_3				3.57	5.75	3.53	5.23	No
AT_ssM_TR_3				3.67	5.67	3.63	5.25	No
AT_ssM_TR_3				3.97	5.80	4.09	5.24	No
A_52_P987461			XR_387845	6.84	6.13	6.85	6.26	No
A_52_P1164120			AK047247	3.97	4.99	3.98	4.67	No
A_52_P465669				5.84	5.06	5.83	5.47	No
A_52_P229210				12.77	13.54	12.76	13.26	No
A_52_P941191				9.51	8.59	9.50	8.90	No

Table 6 A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P100298	Stx3	20908	AK137098	5.42	4.20	6.11	5.67	2
A_51_P101347	Pls1	102502	NM_001033210	5.96	5.12	7.01	6.51	2
A_51_P101929	Lgals3	16854	NM_010705	13.76	13.50	14.45	14.43	4
A_51_P102122	Myog	17928	NM_031189	3.41	17.44	2.73	16.45	1
A_51_P102471	Pglyrp2	57757	NM_021319	7.05	6.75	5.69	5.50	3
A_51_P103237	Hunk	26559	NM_015755	5.58	6.14	4.30	3.94	3
A_51_P103397	Vwf	22371	NM_011708	3.66	5.30	2.89	4.59	1
A_51_P103541	Cacna1s	12292	NM_001081023	6.16	10.46	5.93	8.74	1
A_51_P104012	Tmem158	72309	NM_001002267	11.86	11.49	12.29	12.12	2
A_51_P104077	Usp13	72607	AV347341	5.31	5.71	4.77	5.01	1
A_51_P104478	Smpx	66106	NM_001252591	4.00	11.10	2.68	8.79	1
A_51_P104487	Chma4	11438	NM_015730	4.87	5.28	4.18	4.65	1
A_51_P105927	Rasl12	70784	NM_001033158	6.66	8.60	6.68	6.97	1
A_51_P106538	Htra3	78558	NM_001042615	9.26	9.45	7.67	8.15	3
A_51_P107738	Lrrc8e	72267	NM_028175	4.60	3.47	5.07	4.53	2
A_51_P107782	Kdsr	70750	NM_027534	11.40	10.69	11.84	11.54	4
A_51_P114067	Ncs1	14299	NM_019681	7.23	6.66	7.76	7.25	4

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P114462	Ccl17	20295	NM_011332	5.53	5.56	5.43	6.21	2
A_51_P114693	Parm1	231440	AK161172	8.85	13.93	7.58	11.56	3
A_51_P114878	Ikbkap	230233	NM_026079	12.13	12.67	11.56	11.92	1
A_51_P115715	Asb2	65256	NM_023049	3.13	10.21	1.85	8.98	3
A_51_P115817	Ralgps2	78255	NM_023884	9.37	11.13	9.27	10.47	1
A_51_P115953	Ctxn3	629147	NM_001134697	3.14	7.60	3.23	6.15	1
A_51_P116157				8.17	8.10	7.29	7.50	3
A_51_P116601	A330021E22Rik	207686	NM_172447	8.82	8.37	9.05	9.04	4
A_51_P118237	Sgk1	20393	NM_011361	11.66	11.59	12.06	12.44	2
A_51_P118468	Cep170	545389	BC054781	3.99	3.08	4.00	4.01	2
A_51_P120024	Pde2a	207728	NM_001008548	2.43	9.91	2.42	8.22	1
A_51_P120717	Lmnbl	16906	NM_010721	12.60	12.66	11.64	11.89	1
A_51_P122071	Muc5b	74180	NM_028801	4.59	3.83	5.09	4.44	2
A_51_P122321	Il33	77125	NM_133775	5.62	6.78	5.18	9.06	2
A_51_P125205	Aqp1	11826	NM_007472	14.78	13.83	15.42	15.04	2
A_51_P135012	Rhpn2	52428	NM_027897	7.87	6.75	8.41	7.54	2
A_51_P135802	Mef2c	17260	NM_025282	8.20	9.69	7.73	8.14	1
A_51_P139410	L1cam	16728	NM_008478	4.38	3.67	5.90	5.36	4
A_51_P140321	Mocos	68591	NM_026779	9.60	8.52	10.05	9.22	2
A_51_P141071	Cand2	67088	NM_025958	7.77	9.93	7.15	8.66	1
A_51_P142334	Inpp1l	16332	NM_010567	12.67	14.49	12.62	13.74	1
A_51_P143152	Luzp1	269593	NM_024452	9.89	9.97	9.21	9.37	3
A_51_P143162	Myh7	140781	NM_080728	6.18	14.14	5.50	11.23	1
A_51_P143296	Myh8	17885	NM_177369	2.61	12.74	3.36	10.83	1
A_51_P144349	Dtx4	207521	NM_172442	11.49	11.14	11.69	11.91	2
A_51_P148744	Msantdl	403174	NM_207277	5.00	7.28	5.07	6.05	1
A_51_P149868	Irx1	16371	NM_010573	6.14	5.62	6.56	6.32	2
A_51_P150905	Klhl22	224023	NM_145479	12.08	13.05	12.22	12.38	1
A_51_P152444	4930465K10Rik	67640	NR_027978	4.31	4.87	4.09	3.91	1
A_51_P153693	Utp14b	195434	NM_001001981	5.43	6.56	6.39	5.90	1
A_51_P154753	Klc3	232943	NM_146182	6.77	6.34	7.38	7.15	4
A_51_P155458	Dok7	231134	XM_006503901	4.82	14.18	4.64	13.17	1
A_51_P155997	Bcl7a	77045	NM_029850	8.59	10.45	8.20	9.59	1
A_51_P156526	Atp2b3	320707	XM_006528086	2.43	6.12	3.20	4.82	1
A_51_P157185	Gjd4	225152	NM_153086	2.10	7.50	1.66	5.77	1
A_51_P159610	Ccdc120	54648	NM_207202	7.17	6.38	7.36	6.96	2
A_51_P160625	Wapal	218914	NM_001004436	9.98	10.15	9.85	9.47	1
A_51_P162894	4933405L10Rik	71046	NM_027655	4.44	3.88	4.71	4.60	2
A_51_P163146	Sema3a	20346	NM_009152	8.30	8.29	9.00	9.27	4

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P163953	Nsg2	18197	NM_008741	6.34	10.10	5.12	8.63	1
A_51_P164530	Fhit	14198	NM_010210	3.49	5.60	4.41	4.44	1
A_51_P164835	Ppl	19041	NM_008909	6.34	5.25	6.57	5.96	2
A_51_P165082	Tmem191c	224019	NM_177473	7.63	8.36	8.28	9.20	2
A_51_P166648	Evalc	70967	XR_397180	11.14	10.74	11.95	11.70	4
A_51_P167132	Usp27x	54651	NM_019461	4.43	4.22	5.33	5.35	4
A_51_P169255	Olfrl90	258392	NM_146397	5.79	4.90	5.76	5.55	2
A_51_P169409	Arl5a	75423	NM_182994	8.85	9.63	8.32	8.73	3
A_51_P169588	Dhx38	64340	NM_178380	4.83	5.67	4.99	4.81	1
A_51_P170807	LOC102641377	102641377	XR_402119	8.36	7.87	8.83	8.57	4
A_51_P172131	Lurap1l	52829	NM_026821	12.50	12.15	12.84	12.76	2
A_51_P172475	S100a1	20193	NM_011309	8.73	7.79	9.05	8.47	2
A_51_P172502	Cxcl12	20315	NM_001012477	14.74	14.21	13.65	13.28	3
A_51_P172853	Cd14	12475	NM_009841	9.20	8.49	9.59	9.28	2
A_51_P173692	Lingo4	320747	NM_177250	7.41	7.40	6.18	6.41	3
A_51_P174061	Tnik	665113	NM_026910	2.53	7.77	2.83	5.75	1
A_51_P174192	Cenpe	229841	NM_173762	6.26	6.42	5.34	4.75	1
A_51_P174696	Casq2	12373	NM_009814	3.71	10.64	3.66	9.14	1
A_51_P175945	Tmem74	239408	AK045145	5.73	4.44	6.09	5.23	2
A_51_P176474	Kndc1	76484	NM_177261	4.48	1.86	4.19	4.04	2
A_51_P177210	Myl3	17897	NM_010859	4.05	12.22	4.32	10.28	1
A_51_P177371	Prnd	26434	NM_023043	4.32	4.22	3.65	3.34	1
A_51_P181691	Nod2	257632	NM_145857	2.43	6.98	2.07	6.05	1
A_51_P181785	Trim56	384309	NM_201373	10.55	9.72	10.69	10.42	2
A_51_P181922	Yjefn3	234365	XM_006509732	9.27	9.61	8.89	8.90	1
A_51_P185575	Adamtsl4	229595	NM_144899	8.90	7.98	9.79	9.15	4
A_51_P187151	Grb10	14783	NM_010345	7.73	8.41	7.41	7.53	1
A_51_P188114	Eps8	13860	NM_007945	8.16	7.27	9.04	8.67	4
A_51_P188911	Galnt12	230145	NM_172693	8.91	8.35	9.74	9.38	4
A_51_P189943	Dscam	13508	NM_031174	3.02	7.68	3.13	4.83	1
A_51_P191463	H2-M2	14990	XM_006525458	10.08	9.63	10.45	10.28	2
A_51_P195244	Mfap4	76293	NM_029568	9.52	11.10	7.43	8.86	1
A_51_P195374	Baiap2l1	66898	NM_025833	5.31	9.86	4.71	8.83	3
A_51_P195506	Csfl	12977	NM_007778	12.38	12.18	12.71	12.89	2
A_51_P196726	Itih2	16425	NM_010582	9.70	9.09	10.65	10.03	4
A_51_P196925	Cx3cl1	20312	NM_009142	12.30	12.42	12.64	13.06	4
A_51_P197596	Afap1l2	226250	NM_146102	7.15	6.34	7.77	6.93	4
A_51_P197773	Parp6	67287	NM_029922	9.28	8.86	9.61	9.48	2
A_51_P198694	Gckr	231103	NM_144909	2.69	5.44	3.43	2.97	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P199314	Slc38a2	67760	AK164568	7.83	7.29	7.18	6.62	3
A_51_P200068	Arf3	11842	NM_007478	11.01	11.01	10.53	10.29	1
A_51_P200339	Cachd1	320508	AK046180	9.47	8.63	9.91	9.52	4
A_51_P200819	Prdx4	53381	AK165551	14.77	13.84	15.14	14.75	2
A_51_P202440	Rarb	218772	NM_011243	5.26	6.75	5.08	5.56	3
A_51_P202801	Abcb9	56325	NM_019875	9.40	9.95	8.80	8.82	1
A_51_P203062	Enah	13800	NM_010135	10.78	11.89	10.55	11.20	1
A_51_P203182	Apobr	171504	NM_138310	7.99	6.54	8.78	7.55	2
A_51_P203850	4930539H15Rik	78188	AK019727	6.71	6.49	7.02	7.22	2
A_51_P204845	Celf1	13046	NM_017368	11.66	12.99	11.13	12.04	1
A_51_P205139	Map1b	17755	NM_008634	5.14	7.19	5.22	6.34	1
A_51_P206037	Peg3	18616	NM_008817	3.65	4.35	4.54	2.22	1
A_51_P206824	Hfe2	69585	NM_027126	2.42	11.14	2.50	9.27	1
A_51_P207934	Enpp4	224794	NM_199016	7.28	7.44	6.87	6.54	1
A_51_P208121	Klhl25	207952	NM_029652	10.41	10.64	9.99	9.94	1
A_51_P209782	Cyp2c44	226143	NM_001001446	5.53	5.21	6.25	4.53	1
A_51_P209930	Rtn2	20167	NM_013648	6.99	11.96	6.25	11.09	1
A_51_P212980	Spat9	75571	NM_029343	6.37	5.50	6.68	6.30	2
A_51_P213928	Nap1l3	54561	NM_138742	5.32	5.05	4.86	4.31	3
A_51_P214516	Nipsnap1	18082	NM_008698	10.12	9.70	10.58	10.37	4
A_51_P215815	Atp11c	320940	NM_001037863	7.20	6.19	7.20	6.91	2
A_51_P216593	Wfs1	22393	NM_011716	12.22	13.23	12.22	12.62	1
A_51_P217498	Slc2a4	20528	NM_009204	4.97	6.68	2.79	5.01	3
A_51_P217517	Mmel1	27390	NM_013783	6.99	9.43	6.86	7.81	1
A_51_P218402	Ankrd44	329154	AK038343	5.31	6.77	5.49	6.04	1
A_51_P218612	Mtr	238505	NM_001081128	5.29	5.44	4.91	4.80	1
A_51_P218653	Jph2	59091	NM_021566	2.40	7.12	3.05	5.84	1
A_51_P220162	Notch3	18131	NM_008716	11.07	11.53	10.26	10.73	1
A_51_P220370	Msl3l2	73390	NM_001163833	4.57	5.16	4.67	3.59	1
A_51_P221223	Scg5	20394	NM_009162	7.10	6.74	8.35	8.69	2
A_51_P221781	Zfp33b	69623	XM_006535329	4.17	3.68	4.48	4.33	2
A_51_P222280	Ikbke	56489	NM_019777	7.78	6.99	8.11	7.70	2
A_51_P223025	Dock5	68813	NM_177780	10.07	9.55	10.58	10.30	4
A_51_P224311	Popdc2	64082	NM_022318	6.24	10.38	6.28	9.16	1
A_51_P225224	Htra1	56213	NM_019564	13.55	12.71	13.76	13.30	2
A_51_P226269	Rgcc	66214	NM_025427	12.26	11.89	11.53	10.95	1
A_51_P229925	Chst7	60322	NM_021715	11.61	11.08	12.34	11.81	2
A_51_P230269	H2-Q10	15007	NM_010391	3.80	3.86	6.27	5.05	2
A_51_P231499	Enpp1	18605	AK014707	10.73	10.77	11.26	11.43	4

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P232187	Vwa3a	233813	AK086942	4.25	4.76	6.02	4.17	1
A_51_P232868	Neurl1b	240055	NM_001081656	10.91	10.95	9.17	9.42	3
A_51_P233753	Foxp2	114142	NM_053242	4.45	2.56	4.92	4.74	2
A_51_P234864	Sfxn5	94282	NM_178639	7.86	7.70	8.43	8.64	4
A_51_P235764	Olfir659	259052	NM_147050	2.24	5.34	3.48	4.25	1
A_51_P238425	Cbr2	12409	NM_007621	16.21	15.62	15.56	15.01	3
A_51_P238722	Cd93	17064	NM_010740	4.77	6.11	3.62	5.00	3
A_51_P239386	Ppp1r14c	76142	NM_133485	3.89	5.90	4.23	5.18	1
A_51_P240329	Vma21	67048	NM_001290781	9.67	10.84	9.45	10.11	1
A_51_P240453	Nusap1	108907	NM_133851	11.79	11.76	10.47	10.61	1
A_51_P240801	Tmem173	72512	NM_028261	8.15	7.85	7.12	7.08	3
A_51_P241843	Tmem202	73893	NM_178388	7.54	6.93	7.96	7.58	2
A_51_P244154	Lrrc8b	433926	AK054249	6.90	7.57	6.73	6.79	1
A_51_P246754	Pnpo	103711	NM_134021	8.91	8.22	9.33	8.97	2
A_51_P247496	Gm9767	100040851	NR_028030	3.75	7.12	4.18	5.82	1
A_51_P248638	Myoz2	59006	NM_021503	3.17	8.24	4.24	6.76	1
A_51_P249286	Rgs16	19734	NM_011267	3.49	9.79	3.73	8.26	1
A_51_P249313	Herc3	73998	NM_028705	7.20	8.78	6.96	8.04	1
A_51_P254262	Nxph3	104079	NM_130858	7.25	6.87	7.50	7.58	2
A_51_P254634			AK046342	3.44	6.35	3.14	5.22	1
A_51_P256066	Tiam2	24001	NM_011878	11.82	11.31	12.18	12.10	2
A_51_P256682	Kpna1	16646	NM_008465	9.01	9.74	8.06	8.81	3
A_51_P256798				3.37	5.89	3.74	4.84	1
A_51_P258894	Chst2	54371	NM_018763	8.47	8.14	8.99	8.91	2
A_51_P259064	Ube2d3	66105	AK020443	10.46	10.57	9.65	9.83	1
A_51_P260578	St8sia2	20450	NM_009181	5.13	7.49	5.52	6.47	1
A_51_P261835	Tmem8c	66139	NM_025376	8.57	13.33	9.33	12.01	1
A_51_P261931	A930003A15Rik	68162	NR_015488	1.79	7.92	1.93	6.80	1
A_51_P261991	Bdnf	12064	NM_007540	10.84	10.39	11.60	11.17	2
A_51_P262079	H2-Q7	15018	NM_010394	11.86	11.33	12.16	12.04	2
A_51_P262766	Ccnd1	12443	S78355	12.96	12.57	13.01	13.21	2
A_51_P263290	Galnt9	231605	NM_198306	2.00	7.52	2.31	5.95	3
A_51_P263310	Higd1a	56295	NM_019814	7.59	8.35	7.07	7.26	3
A_51_P264495	Pgam2	56012	NM_018870	6.92	12.06	6.18	10.07	3
A_51_P264656	2900011O08Rik	67254	NM_144518	4.73	3.83	5.62	5.10	4
A_51_P264769	Dmp1	13406	NM_016779	4.80	7.02	5.39	8.35	2
A_51_P265026	Naip2	17948	NM_010872	7.00	6.00	8.30	7.56	4
A_51_P266683	Efnal	13636	NM_010107	8.63	7.50	8.76	8.23	2
A_51_P266861	Me1	17436	NM_008615	11.87	11.92	10.13	10.25	3

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P267278	Slc15a2	57738	NM_021301	5.57	4.50	6.96	6.27	4
A_51_P267751	Hrc	15464	NM_010473	2.86	11.84	2.80	10.62	1
A_51_P268331	Rnf128	66889	NM_023270	8.27	8.44	8.82	9.20	4
A_51_P268983	Myom3	242702	NM_001085509	3.76	13.05	3.54	10.70	1
A_51_P269375	Ank1	11733	NM_031158	7.58	11.63	7.19	9.97	1
A_51_P271503	Il1r1	16177	NM_008362	10.31	10.13	10.49	10.75	2
A_51_P272283	Cmb1	69574	NM_181588	9.42	9.65	8.14	8.79	3
A_51_P276418	Rab6b	270192	NM_173781	10.02	9.08	10.86	10.55	4
A_51_P277006	Chst8	68947	NM_175140	4.59	8.68	4.99	6.04	1
A_51_P279552	Cav2	12390	NM_016900	11.23	10.20	11.43	10.96	4
A_51_P280404	Epdr1	105298	NM_134065	10.41	9.55	11.27	10.77	2
A_51_P281380	Tspan5	56224	NM_019571	11.44	11.35	11.88	11.95	2
A_51_P281716	Ltbp1	268977	NM_019919	8.81	8.24	9.40	9.03	4
A_51_P282144	Adam23	23792	NM_011780	9.37	8.84	10.12	9.64	4
A_51_P282594	Svop	68666	NM_026805	5.64	5.02	4.08	3.31	3
A_51_P282983	Slc39a14	213053	NM_144808	9.64	9.32	9.94	10.07	2
A_51_P283473	Fibin	67606	NM_026271	11.47	11.93	10.40	10.50	1
A_51_P283499	Drd4	13491	NM_007878	3.72	5.08	3.62	2.66	1
A_51_P286073	4930463O16Rik	75804	NR_108059	5.37	5.18	6.91	7.17	4
A_51_P287617	Plekhh1	211945	NM_181073	6.53	10.32	6.57	9.30	1
A_51_P287691	Kremen2	73016	NM_028416	2.10	6.11	2.44	4.97	1
A_51_P288009	Chrng	11449	NM_009604	2.91	15.03	2.49	13.73	1
A_51_P288558	Wdr16	71860	NM_027963	2.84	7.42	2.42	5.30	1
A_51_P290065	Gap43	14432	NM_008083	5.33	4.87	6.65	5.96	4
A_51_P290986	Dhcr7	13360	NM_007856	11.93	12.68	12.06	12.08	1
A_51_P293218	Pan3	72587	NM_028291	10.70	10.34	11.12	11.08	2
A_51_P294020	Ticam1	106759	NM_174989	9.77	8.86	9.92	9.49	2
A_51_P294346	Mis18bp1	217653	AK035117	6.55	6.54	5.27	5.53	1
A_51_P294891	Ppargc1b	170826	NM_133249	5.33	11.00	5.69	10.32	1
A_51_P297069	Tmod1	21916	NM_021883	3.46	10.96	4.74	9.65	3
A_51_P302520	Myom1	17929	NM_010867	3.84	9.64	3.54	8.02	1
A_51_P302566	Maob	109731	NM_172778	7.92	7.74	6.90	6.62	3
A_51_P302856	Ugt2b37	112417	NM_053215	3.49	3.59	5.92	6.31	4
A_51_P303217	Ucma	68527	NM_026754	5.28	12.38	5.57	11.31	1
A_51_P303371	Ccdc23	69216	NM_024462	14.67	16.47	14.45	15.62	3
A_51_P303725	Tmem38a	74166	NM_144534	10.80	13.48	10.62	12.30	1
A_51_P306017	Dll1	13388	NM_007865	5.18	10.54	4.36	9.96	3
A_51_P306183	Mafk	17135	NM_010757	8.24	8.13	8.63	8.73	2
A_51_P309589	2700099C18Rik	77022	NR_024720	7.97	8.06	7.02	6.93	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P309988	Gprc5c	70355	NM_147217	3.26	8.46	2.83	6.69	1
A_51_P310594	Aldh3a1	11670	NM_007436	5.68	5.82	6.07	6.44	2
A_51_P312121	Xdh	22436	XR_385314	4.18	2.72	5.15	4.80	2
A_51_P312497	Atp7a	11977	AK033254	11.07	11.52	10.80	10.91	1
A_51_P314186	Syne1	64009	NM_001079686	6.34	7.86	6.96	7.03	1
A_51_P314397	Crip2	68337	NM_024223	13.36	14.64	13.19	13.92	1
A_51_P315391	Txlnb	378431	NM_138628	4.99	6.15	4.40	5.17	1
A_51_P315925	Fgf21	56636	NM_020013	3.77	5.82	2.85	4.86	1
A_51_P316311	Ppfia4	68507	NM_001144855	3.77	11.79	3.06	10.51	1
A_51_P317321	Iffo1	320678	AK044382	9.97	13.11	10.01	12.21	1
A_51_P318933	Camk2b	12323	NM_007595	4.65	4.41	4.12	3.80	1
A_51_P319662			AK054460	4.19	5.05	4.68	3.90	1
A_51_P321374	Ppapdc3	227721	NM_145521	6.31	11.15	6.18	9.52	1
A_51_P323081	Bhlhb9	70237	NM_198161	8.57	9.39	8.27	8.77	1
A_51_P325173	Tpm1	22003	NM_024427	8.68	14.11	8.64	13.26	1
A_51_P325941	Kcnj12	16515	NM_010603	3.53	6.77	2.31	4.56	1
A_51_P326191	Serpina3g	20715	NM_009251	6.18	6.34	7.01	7.86	2
A_51_P326259				3.74	3.79	4.31	4.56	4
A_51_P326425	Arfp2	76932	NM_029802	8.38	8.06	8.79	8.81	4
A_51_P327869				5.22	5.04	4.36	3.34	1
A_51_P327874	Pth1r	19228	NM_011199	6.36	6.25	5.90	5.15	1
A_51_P329733	Csnk1g1	214897	NM_173185	4.44	3.97	4.94	4.94	2
A_51_P331781	Fam151b	73942	NM_001163627	4.09	3.16	5.12	4.51	2
A_51_P332141	Cln2	12724	AF097415	8.63	8.29	8.97	8.95	2
A_51_P332185	Mbd5	109241	NM_029924	4.04	2.91	4.54	5.14	2
A_51_P332228	Nol4	319211	NM_199024	4.43	5.11	4.27	5.80	2
A_51_P332742	Srl	106393	NM_175347	5.92	11.41	5.34	9.90	1
A_51_P333518	Sh3rf2	269016	NM_172966	5.65	5.19	4.41	3.44	3
A_51_P333780	Cd164	53599	NM_016898	13.03	14.51	12.98	13.68	1
A_51_P334308	Tspan14	52588	NM_145928	12.95	14.05	12.96	13.47	1
A_51_P335104	Slx1b	75764	NM_029420	9.46	8.89	9.49	9.50	2
A_51_P335969	Des	13346	NM_010043	3.61	9.62	3.20	7.70	1
A_51_P336391	Tmem18	211986	NM_172049	11.81	11.27	10.58	10.27	3
A_51_P337210	Lrrc4	192198	NM_138682	3.37	5.29	3.59	4.12	1
A_51_P337543	Glce	93683	NM_033320	11.85	12.53	10.65	11.33	3
A_51_P338262	Tnnt2	21956	NM_011619	6.95	15.99	6.86	14.50	1
A_51_P338443	Angptl4	57875	NM_020581	14.24	12.99	14.49	14.47	2
A_51_P338713	Ass1	11898	M31690	9.53	10.47	9.74	11.09	2
A_51_P342387	Apoh	11818	NM_013475	7.08	6.31	7.42	7.01	2

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P343429	Slc25a37	67712	NM_026331	9.67	9.40	9.95	9.99	4
A_51_P343833	Traf1	22029	NM_009421	7.11	7.35	6.95	8.72	2
A_51_P345699	Tnnt3	21957	NM_011620	5.77	13.86	5.11	11.92	1
A_51_P346445	Hspb7	29818	NM_013868	3.17	5.78	2.57	3.97	1
A_51_P347333	Dusp4	319520	AK012530	7.92	10.34	7.98	9.37	1
A_51_P348749	Llgl2	217325	NM_145438	6.95	6.37	7.46	7.15	2
A_51_P349772	Tceal3	594844	NM_001029978	8.56	9.15	7.88	8.32	1
A_51_P350453	Pdk4	27273	NM_013743	12.92	12.25	13.54	13.19	4
A_51_P350817	Cnn1	12797	NM_009922	9.71	9.26	7.61	7.32	3
A_51_P352303	Homer2	26557	NM_011983	3.13	4.78	2.75	3.61	1
A_51_P353232	Tnnc2	21925	NM_009394	6.42	16.75	6.41	15.14	1
A_51_P355122	Tnni2	21953	NM_009405	7.87	14.19	7.06	12.95	3
A_51_P356265	Acrbp	54137	NM_016845	4.02	3.08	3.65	4.70	2
A_51_P356620	Rab39b	67790	NM_175122	6.26	5.68	6.95	6.45	4
A_51_P357759	Pld2	18806	NM_008876	9.58	9.07	10.10	9.74	2
A_51_P359272	Alcam	11658	NM_009655	9.55	8.83	10.39	9.78	2
A_51_P359315	Apobec2	11811	NM_009694	2.63	9.23	2.31	7.53	1
A_51_P362373	Gprasp2	245607	NM_001163015	3.06	6.58	2.52	5.67	1
A_51_P362399	Lig4	319583	NM_176953	8.39	9.03	8.31	8.18	1
A_51_P362627	Tnnt1	21955	NM_011618	7.41	14.30	6.61	12.45	1
A_51_P364788	Myh1	17879	NM_030679	4.02	5.98	3.70	4.77	1
A_51_P366157	Igsf1	209268	NM_183336	6.68	6.09	7.91	7.47	4
A_51_P367240	Tspan33	232670	NM_146173	3.88	13.10	2.97	11.89	1
A_51_P367434	Tmed5	73130	NM_028876	11.42	11.19	11.91	11.88	2
A_51_P368660	Mphosph10	67973	NM_026483	7.69	8.28	7.81	7.67	1
A_51_P369270	Prrc2b	227723	NM_172661	5.20	6.13	5.00	5.47	1
A_51_P377789	Map3k12	26404	NM_009582	10.83	10.16	11.00	10.84	2
A_51_P379171	LOC102637942	102637942	XR_374584	7.50	7.04	7.96	7.82	2
A_51_P379750	Myoz1	59011	NM_021508	4.30	6.09	4.04	5.16	1
A_51_P381178	Zbtb46	72147	NM_028125	3.81	5.73	3.39	4.44	1
A_51_P381230	Zhx2	387609	NM_199449	4.59	5.99	4.62	4.98	1
A_51_P381260	Fxyd5	18301	NM_008761	10.11	9.67	10.83	10.55	4
A_51_P381558	Rasa4	54153	NM_133914	12.29	11.69	12.44	12.27	2
A_51_P382152	Procr	19124	NM_011171	11.46	10.72	11.96	11.48	4
A_51_P382789	Adora3	11542	NM_027025	3.94	8.75	3.25	6.39	1
A_51_P383524	Art3	109979	NM_181728	2.56	8.07	2.51	6.32	1
A_51_P383991	Sept4	18952	NM_011129	3.37	7.29	2.53	5.61	1
A_51_P384441	Tmem201	230917	NM_177672	7.43	8.87	7.27	7.93	1
A_51_P384693	Kirrel3	67703	NM_026324	4.76	3.93	6.26	5.61	4

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P385639	Gjb5	14622	NM_010291	9.47	8.08	8.74	7.36	1
A_51_P386899	Mfsd7c	217721	NM_145447	3.45	4.72	3.78	3.67	1
A_51_P387670	Gtpbp4	69237	NM_027000	11.25	11.88	11.17	11.22	1
A_51_P387971	Ssh3	245857	NM_198113	6.97	6.26	7.31	6.91	2
A_51_P388478	Efnb1	13641	NM_010110	10.45	10.68	10.14	9.85	1
A_51_P390124	Naa16	66897	NM_025832	9.06	9.01	9.27	9.62	2
A_51_P390334	2900026A02Rik	243219	NM_172884	8.75	8.68	7.84	7.81	3
A_51_P390967	Slc43a3	58207	NM_021398	12.26	12.16	11.65	11.24	1
A_51_P391716	Ermap	27028	NM_013848	2.66	7.28	3.07	5.31	1
A_51_P393862	Spryd4	66701	NM_025716	9.36	10.38	9.01	9.52	1
A_51_P394154	Ddx51	69663	NM_027156	10.49	11.98	10.57	11.35	1
A_51_P395473	Tenc1	209039	NM_153533	10.93	10.26	11.18	10.86	4
A_51_P395652	Myh2	17882	NM_001039545	2.18	8.30	2.33	6.37	1
A_51_P397394	Cobll1	319876	NM_177025	7.36	7.15	7.81	7.98	4
A_51_P397834	Dpm1	13480	NM_010072	7.91	7.87	7.05	7.21	3
A_51_P398673	Elmsan1	238317	NM_001163502	8.85	9.67	8.74	9.08	1
A_51_P399305	Tnfrsf19	29820	NM_013869	2.69	6.66	2.30	4.81	1
A_51_P400944	Rprd1a	225283	NM_144861	8.06	7.96	7.05	7.27	3
A_51_P401683	Tm6sf1	107769	NM_145375	3.56	8.18	3.33	5.89	1
A_51_P401792	Ttn	22138	NM_011652	2.88	11.65	3.85	9.42	1
A_51_P402193	Map3k1	26401	NM_011945	9.39	9.63	8.99	9.02	1
A_51_P402359	Btla	208154	NM_001037719	2.39	5.54	2.86	3.25	1
A_51_P402575	Mtel1	68617	AK051750	5.92	6.35	6.73	7.00	4
A_51_P402583	Gpld1	14756	NM_008156	5.15	4.62	5.68	5.72	2
A_51_P403378	Cnr1	12801	NM_007726	5.13	8.91	5.42	7.19	1
A_51_P404815	Apol6	71939	NM_028010	8.45	8.61	7.42	7.63	3
A_51_P405397	Ecm1	13601	NM_007899	15.10	14.45	15.69	15.11	4
A_51_P406557	Al464131	329828	NM_001085515	11.44	12.06	11.61	11.41	1
A_51_P408649	Shisa2	219134	NM_145463	2.29	8.06	2.65	6.22	1
A_51_P408749	4933439C10Rik	74476	NR_015585	4.28	2.31	4.76	4.30	2
A_51_P413130	Myl4	17896	NM_010858	8.29	11.84	8.14	9.96	1
A_51_P415247			AK015886	9.45	9.26	8.81	8.59	1
A_51_P416074	Ryr1	20190	NM_009109	2.69	10.31	2.79	8.82	1
A_51_P416126	Chd3	216848	NM_146019	11.06	10.82	10.69	10.10	1
A_51_P416858	Myl1	17901	NM_021285	2.43	14.47	2.43	12.65	1
A_51_P417720	Itga11	319480	NM_176922	11.15	11.79	10.42	10.58	3
A_51_P417952	Sema6b	20359	NM_013662	5.33	7.39	5.16	5.74	1
A_51_P419017	Pde10a	23984	NM_011866	7.46	7.03	8.00	7.67	4
A_51_P420037	Nup210	54563	NM_018815	2.65	8.88	2.79	7.34	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P421559	Bspry	192120	NM_138653	4.85	8.68	5.00	7.05	1
A_51_P421780	Myo18b	74376	AK016515	2.33	9.88	2.46	8.62	1
A_51_P423308	Igfbp2	16008	NM_008342	7.92	7.88	6.70	6.87	3
A_51_P424878	Fam213b	66469	NM_025582	8.24	10.36	7.92	8.84	1
A_51_P425680	Ivd	56357	NM_019826	11.65	11.95	11.49	11.35	1
A_51_P425772	Ckb	12709	NM_021273	10.44	12.78	9.76	11.54	3
A_51_P427964	Lmod3	320502	NM_001081157	5.67	13.90	5.55	12.90	1
A_51_P429366	Hes6	55927	NM_019479	12.87	15.45	12.80	14.61	1
A_51_P430900	Dusp1	19252	NM_013642	10.76	10.71	11.16	11.43	2
A_51_P431329	Car3	12350	NM_007606	4.50	10.94	4.00	9.59	1
A_51_P431433	Tmem2	83921	NM_031997	10.30	10.71	10.24	10.03	1
A_51_P431785	Myom2	17930	NM_008664	6.85	13.84	6.35	12.82	1
A_51_P432180	Slc16a6	104681	NM_001029842	7.50	10.58	6.78	9.51	1
A_51_P433870	Vegfc	22341	NM_009506	9.22	9.13	9.21	10.22	2
A_51_P434036	Gsap	212167	NM_175437	5.50	5.13	5.67	6.15	2
A_51_P436491	Lingo3	237403	NM_001013758	3.08	7.13	3.04	5.64	1
A_51_P437050	Heg1	77446	XM_006522715	7.95	8.69	7.87	8.07	1
A_51_P437978	Agtr2	11609	NM_007429	3.62	2.69	6.55	6.33	4
A_51_P438165	Arhgap22	239027	NM_153800	11.77	12.85	12.23	13.96	4
A_51_P438905	Inadl	12695	NM_172696	6.96	8.43	7.23	7.68	1
A_51_P439210	Kcnh5	238271	NM_172805	3.30	3.64	5.04	4.97	4
A_51_P444447	Cebpd	12609	NM_007679	13.79	13.17	13.93	13.85	2
A_51_P444696	Cdca2	108912	NM_175384	8.36	8.61	7.19	7.26	1
A_51_P445765	Lrp8	16975	NM_001080926	6.76	6.81	7.24	7.61	2
A_51_P446232	Dbn1	56320	NM_019813	9.77	11.16	9.48	10.17	1
A_51_P446825	6430573F11Rik	319582	NM_176952	2.88	6.69	2.23	4.77	1
A_51_P447545	Igfbp1	16006	NM_008341	2.41	8.24	2.34	7.00	1
A_51_P448005	Tbc1d8	54610	NM_018775	3.08	4.83	2.64	3.70	1
A_51_P449795	Crip1	12925	M13018	5.29	5.64	4.49	4.04	1
A_51_P450057	Cit	12704	AK047894	7.60	7.39	6.73	6.62	1
A_51_P450888	Rtn4r	65079	NM_022982	4.27	3.79	4.00	2.76	1
A_51_P451075	Atp2a2	11938	NM_009722	9.64	11.11	9.52	10.34	1
A_51_P452323	Nes	18008	NM_016701	9.20	13.05	8.87	12.25	1
A_51_P452702	Akap13	75547	NM_029332	4.78	5.13	4.64	4.39	1
A_51_P456114	Cemip	80982	AK018112	9.35	10.13	8.20	9.04	3
A_51_P456657	Tgfr2	228839	NM_173396	10.99	11.64	10.30	10.63	1
A_51_P460954	Ccl6	20305	NM_009139	5.88	5.03	4.99	4.31	1
A_51_P461319	Gatm	67092	NM_025961	1.91	8.84	2.12	7.57	3
A_51_P461364	Gpx7	67305	NM_024198	3.82	5.35	5.09	4.22	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P462428	Galnt15	78754	AK019470	6.93	5.25	6.80	6.62	2
A_51_P465128	Chst15	77590	NM_029935	7.00	8.40	6.95	7.65	1
A_51_P465758	Gm16499	100049166	BC006619	4.82	4.19	2.89	2.58	3
A_51_P466685	Fbxl22	74165	NM_175206	3.41	7.01	3.73	5.65	1
A_51_P468362	Ccdc88c	68339	XM_006516187	4.57	10.46	4.53	9.60	1
A_51_P468381	Purb	19291	NM_011221	11.44	13.14	11.28	12.24	1
A_51_P474151	Awat2	245532	NM_177746	3.67	8.54	3.98	7.02	1
A_51_P476618	Slc12a8	171286	NM_134251	5.96	5.90	5.71	5.17	1
A_51_P476820	Calr3	73316	NM_028500	6.29	5.78	6.60	6.41	2
A_51_P478138	Cacnb1	12295	NM_031173	9.08	10.32	9.06	9.61	1
A_51_P478419	Unc45b	217012	NM_178680	2.57	13.01	2.09	11.37	1
A_51_P478952	N4bp211	100637	NM_133898	4.78	4.23	5.50	5.41	2
A_51_P480202	Dlx2	13392	NM_010054	9.84	9.45	10.07	10.05	2
A_51_P480290	Casz1	69743	NM_027195	4.53	9.97	4.26	8.18	1
A_51_P480578	Pcx	18563	NM_008797	12.57	13.21	12.51	12.60	1
A_51_P482990	Arid5a	214855	NM_145996	5.74	8.69	5.65	7.75	1
A_51_P483231	Jak2	16452	NM_008413	9.07	8.42	9.67	9.21	4
A_51_P484289	Slc7a6	330836	NM_178798	9.17	8.87	9.55	9.61	2
A_51_P487832	Rbm24	666794	NM_001081425	2.55	10.28	2.60	8.09	1
A_51_P488554	3010026O09Rik	68067	NM_026543	10.17	10.40	9.68	9.52	1
A_51_P488780	Krt28	70843	XM_006534220	3.34	3.83	3.20	2.66	1
A_51_P488991	Oaf	102644	NM_178644	12.26	11.80	12.83	12.47	4
A_51_P489488	Pde4dip	83679	NM_178080	9.41	11.54	9.55	10.64	1
A_51_P489503	Notum	77583	NM_175263	4.46	3.64	4.97	4.75	2
A_51_P490997	Gas2l1	78926	NM_144560	12.03	11.55	12.15	12.23	2
A_51_P491279			AK020378	5.49	6.43	5.07	5.41	1
A_51_P493886	Gpt2	108682	NM_173866	10.40	12.78	10.55	11.79	1
A_51_P493987	Moxd1	59012	NM_021509	4.27	4.63	4.97	5.66	4
A_51_P495331	Neb	17996	NM_010889	2.39	10.13	3.27	7.61	1
A_51_P496562	Slit2	20563	NM_178804	8.92	8.53	9.83	9.58	2
A_51_P497280	Dbn1d1	72185	NM_028146	6.98	12.25	7.10	11.22	1
A_51_P497993	Gpr155	68526	NM_001190297	9.16	10.25	9.40	9.47	1
A_51_P498322	Rasa1	218397	NM_145452	9.10	8.79	9.20	9.55	2
A_51_P499551	Cd276	102657	NM_133983	8.12	7.61	8.55	8.40	2
A_51_P500226	Nkiras2	71966	NM_028024	11.15	12.03	10.88	11.27	1
A_51_P500996	Fbxl20	72194	NM_028149	9.38	8.71	9.67	9.52	2
A_51_P502075	1810009J06Rik	73626	NM_023707	4.48	3.40	4.55	4.31	2
A_51_P502437	Cacna2d3	12294	NM_009785	4.67	5.11	5.58	6.56	2
A_51_P505571	Chrdl2	69121	NM_133709	2.17	8.14	2.64	6.98	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_51_P506284	Pdlim7	67399	NM_026131	8.01	9.42	7.56	8.66	3
A_51_P506397	Edil3	13612	NM_001037987	3.84	3.47	5.00	6.02	2
A_51_P508341	Reep1	52250	NM_178608	6.08	10.56	5.47	9.40	3
A_51_P509997	Cox6a2	12862	NM_009943	8.41	10.30	8.98	9.05	1
A_51_P511102	Cttnbp2nl	80281	NM_030249	8.16	7.71	8.45	8.33	2
A_51_P512210	Myh6	17888	NM_010856	2.23	8.73	2.65	5.65	1
A_51_P512969	Cilp2	68709	NM_026818	4.84	7.50	4.53	6.05	1
A_51_P513449	Pogz	229584	NM_172683	10.13	10.12	9.40	9.40	3
A_51_P513979	1700026L06Rik	69987	NM_027283	9.26	8.42	8.53	7.84	1
A_51_P514070	Megf10	70417	NM_001001979	10.33	12.82	10.19	12.02	1
A_51_P514126	Nfya	18044	NM_001110832	6.90	6.96	6.76	6.31	1
A_51_P515883	Plac8	231507	NM_139198	5.72	5.61	4.30	3.90	3
A_51_P516133	Hist1h1c	50708	AK216922	12.62	12.93	12.53	12.23	1
A_51_P521084	Fam107b	66540	NM_025626	9.13	9.45	8.66	8.74	3
A_51_P521283	Crabp2	12904	NM_007759	6.40	8.61	6.07	7.70	1
A_52_P1011632	Mylk4	238564	NM_001166030	2.60	10.81	2.73	8.94	1
A_52_P101221	Ago2	239528	NM_153178	9.62	10.82	9.59	9.77	1
A_52_P10195	Kifc3	16582	NM_010631	6.29	8.12	6.18	7.41	1
A_52_P103569	Fam124b	241128	NM_173425	4.42	3.96	4.22	4.60	2
A_52_P104214	Tspan32	27027	NM_020286	5.93	6.24	5.16	5.13	1
A_52_P105509	Asah2	54447	NM_018830	7.80	7.18	8.13	7.91	2
A_52_P1061446	Baz1a	217578	NM_013815	7.72	9.43	7.74	8.44	1
A_52_P106482	Cryab	12955	NM_009964	12.66	14.08	12.55	13.23	1
A_52_P106805	Mtf2	17765	S78454	7.71	8.04	7.73	7.38	1
A_52_P108169	Mamstr	74490	NM_172418	7.04	8.35	7.12	7.54	1
A_52_P1131944	A130019P10Rik	399617	AK037456	4.62	3.76	3.97	4.64	2
A_52_P114414	Ksr1	16706	XM_006532339	2.70	6.98	3.29	5.74	1
A_52_P115253	Kif20b	240641	NM_183046	6.86	6.77	5.71	5.50	1
A_52_P117420	Tacc1	320165	NM_177089	8.87	9.40	8.05	8.50	1
A_52_P1197653			CB590277	6.69	6.10	6.80	6.88	2
A_52_P1197913	Gadd45b	17873	NM_008655	11.85	12.77	11.33	12.03	1
A_52_P120022	Lzts3	241638	NM_197945	10.20	12.46	10.56	11.51	1
A_52_P122891	Npsr1	319239	NM_175678	4.21	7.67	4.63	6.03	1
A_52_P124352	Ifrd1	15982	NM_013562	5.09	5.89	5.22	5.23	1
A_52_P128032	3830406C13Rik	218734	NM_178141	9.10	8.68	8.29	8.06	3
A_52_P128180	Slc5a7	63993	NM_022025	4.58	3.40	5.59	5.38	4
A_52_P130727	Ldoc1l	223732	NM_177630	6.04	6.22	5.60	5.51	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P130912	Pcdh7	54216	NM_001122758	4.10	4.08	4.47	5.01	2
A_52_P132353			AK085783	4.91	3.65	4.68	4.89	2
A_52_P132507	Prune	229589	NM_173347	7.33	7.55	7.18	6.88	1
A_52_P13802	Cbfa2t3	12398	NM_009824	2.32	8.86	2.66	7.34	1
A_52_P140005	Nipal1	70701	NM_001081205	4.54	3.82	5.91	5.54	4
A_52_P14456	Srrm4	68955	NM_026886	6.57	6.01	6.74	6.74	2
A_52_P145912	Mylk	107589	NM_139300	8.45	9.12	7.38	7.78	3
A_52_P147666	Slc30a7	66500	NM_023214	8.33	7.62	8.61	8.34	2
A_52_P150436	1700086L19Rik	74284	NR_030733	4.87	6.47	4.66	5.74	1
A_52_P15073	Mettl20	320204	NM_177101	5.58	5.58	5.99	6.20	2
A_52_P151362	Mboat2	67216	XM_006515167	6.79	7.72	5.41	6.26	3
A_52_P153019	Ptgfr	19220	AK086563	6.49	6.15	7.48	7.37	4
A_52_P156034	3830408C21Rik	100040322	NR_015471	5.83	4.90	5.96	5.74	2
A_52_P161237	Cpa1	109697	NM_025350	9.08	10.59	8.05	9.19	1
A_52_P161630	St6gal1	20440	NM_145933	9.87	10.04	9.51	9.38	1
A_52_P164797	Lct	226413	NM_001081078	5.19	5.66	4.71	4.97	1
A_52_P16877	Tmcc3	319880	NM_172051	7.79	9.42	8.04	8.37	1
A_52_P169567	Elp4	77766	NM_023876	8.32	8.08	7.55	7.48	3
A_52_P174775	Ripk1	19766	NM_009068	9.96	9.18	10.07	9.88	2
A_52_P174872	C030015A19Rik	77418	AK028820	8.04	7.32	8.12	7.93	2
A_52_P176634	Taf9b	407786	NM_001001176	6.50	7.00	5.43	5.16	3
A_52_P181605	Ccdc50	67501	NM_001289436	8.40	7.60	8.37	8.28	2
A_52_P181925	Htr2b	15559	NM_008311	4.78	7.55	4.06	5.91	1
A_52_P184606	Lphn3	319387	XM_006534969	5.58	4.62	5.65	5.37	2
A_52_P186033	Spn	20737	NM_009259	8.63	7.86	7.12	6.59	3
A_52_P189050			AK161939	8.92	11.45	8.75	10.25	1
A_52_P189707	Camk2a	12322	NM_177407	6.97	9.23	5.95	8.21	3
A_52_P189725	Chrna1	11435	NM_007389	2.64	11.63	2.69	10.03	1
A_52_P195535	2210418O10Rik	100380879	BC048160	6.17	5.31	6.15	6.21	2
A_52_P196271			AK034076	7.00	6.19	7.47	7.53	2
A_52_P197199	Pcdh9	211712	AY861425	10.73	9.97	10.92	10.69	2
A_52_P198898	Samd5	320825	NM_177271	4.03	2.32	5.42	4.24	4
A_52_P199446	Csmd3	239420	NM_001081391	6.13	5.54	6.91	6.37	4
A_52_P205282	Huwe1	59026	NM_021523	8.44	8.52	8.17	7.56	1
A_52_P205831	Tfap2a	21418	NM_011547	9.05	8.30	9.39	9.09	2
A_52_P211197	Mturn	68235	NM_001289740	8.33	7.32	8.59	7.97	2
A_52_P213696	Fndc5	384061	NM_027402	5.64	13.27	4.84	11.57	1
A_52_P217675	Ankrd32	105377	BC009101	5.89	6.06	5.13	5.08	1
A_52_P221457	Whsc1	107823	NM_001081102	4.55	5.36	4.71	4.32	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P222073	Ackr2	59289	NM_021609	4.97	5.46	4.62	4.77	1
A_52_P222624	Hspb2	69253	NM_024441	8.17	10.90	7.67	9.57	1
A_52_P222775	Map3k6	53608	NM_016693	7.19	6.71	7.84	7.52	4
A_52_P224348	Kctd12b	207474	NM_175429	9.27	8.56	8.30	7.84	3
A_52_P227880	Cenpf	108000	NM_001081363	6.22	6.27	4.31	5.09	1
A_52_P228377	Anks1b	77531	NM_001177397	4.51	3.70	5.23	5.01	4
A_52_P229572	Lrch2	210297	NM_001081173	7.29	7.15	7.72	7.73	2
A_52_P229972	Slc22a1	20517	NM_009202	3.88	3.42	4.36	4.23	4
A_52_P23413	D8Ert82e	244418	NM_172911	7.40	8.15	6.98	7.54	1
A_52_P236448	Ngfr	18053	NM_033217	5.20	6.96	4.68	5.44	3
A_52_P239556	Acta2	11475	NM_007392	7.53	8.90	6.50	8.21	3
A_52_P241612	Aldoat2	79459	NM_001277340	8.65	9.87	8.76	9.16	1
A_52_P241742	Toporsos	100504309	AK008077	10.45	10.09	10.91	10.68	2
A_52_P243542	Celf2	14007	NM_001110229	6.95	9.39	6.24	8.67	1
A_52_P244005	Cd80	12519	XM_006521739	7.54	7.28	7.72	7.92	2
A_52_P244193	Cd24a	12484	NM_009846	10.39	9.08	9.00	8.28	3
A_52_P247953	Myh3	17883	NM_001099635	2.32	14.21	2.82	11.67	1
A_52_P250517	Zfp106	20402	NM_011743	6.44	7.78	6.36	7.13	1
A_52_P255959	Ccnblip1	239083	NM_001111119	9.11	8.88	8.35	8.26	3
A_52_P256182				7.96	7.68	8.48	8.54	2
A_52_P258004	Sys1	66460	NM_025575	5.56	5.41	5.67	6.00	2
A_52_P261843	Zdhhc18	503610	NM_001017968	7.91	7.73	8.35	8.38	2
A_52_P261846	Col18a1	12822	NM_009929	10.99	11.11	11.49	11.81	2
A_52_P262801	Ddx26b	236790	NM_172779	4.12	2.82	5.09	4.91	2
A_52_P264550	Gabra3	14396	NM_008067	5.79	5.09	6.22	5.72	2
A_52_P269672	Sox8	20681	NM_011447	2.46	9.06	3.61	8.02	1
A_52_P27482	Rtkn2	170799	NM_001081346	7.59	7.20	6.60	6.03	1
A_52_P281702	Igfbp5	16011	NM_010518	4.65	9.93	4.91	8.77	1
A_52_P282058	Col8a1	12837	NM_007739	10.03	9.49	8.68	8.35	3
A_52_P283055	Slc5a3	53881	NM_017391	10.14	9.12	10.52	10.04	2
A_52_P28960	Gdf6	242316	NM_013526	6.25	6.87	4.54	5.69	3
A_52_P290020	Arhgef6	73341	NM_152801	3.40	7.00	2.92	5.41	3
A_52_P290457	Adam12	11489	NM_007400	3.16	6.12	3.48	5.38	1
A_52_P290926	Dhrs7c	68460	NM_001013013	2.92	8.78	3.02	6.60	1
A_52_P292826	Gns	75612	AK040524	4.92	4.54	4.97	5.22	2
A_52_P295104	Smim5	66528	NM_183259	4.39	11.42	5.48	9.45	1
A_52_P296338	Galnt18	233733	NM_173739	6.45	7.71	5.85	6.91	1
A_52_P297032	Srxn1	76650	NM_029688	11.86	11.26	12.22	12.04	2
A_52_P297093	Matn3	17182	NM_010770	4.21	4.96	2.83	2.50	3

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P298465	Racgap1	26934	NM_012025	8.96	9.02	7.74	7.77	1
A_52_P299231			XM_006513371	7.02	11.01	7.41	9.34	1
A_52_P300730	Hmga2	15364	NM_010441	10.58	10.66	11.23	11.91	2
A_52_P300786	Naip5	17951	NM_010870	5.08	4.32	5.65	5.20	2
A_52_P301312	Sh2d4b	328381	XM_006519136	4.21	6.90	4.10	5.98	1
A_52_P305279	Spta13	219140	NM_001033272	6.51	7.40	6.01	6.58	1
A_52_P308465	Plxnb1	235611	NM_172775	5.80	5.95	5.34	5.30	1
A_52_P310511	Car6	12353	NM_009802	5.10	3.66	6.48	4.62	2
A_52_P313279	H2-L	14980	NM_001267808	12.36	11.92	12.67	12.60	2
A_52_P316584	Pot1b	72836	AK013364	3.66	2.86	4.06	4.49	2
A_52_P317393	Gpr56	14766	NM_018882	7.96	10.35	7.73	9.14	1
A_52_P319066	Mex3b	108797	NM_175366	4.27	4.93	3.45	3.72	1
A_52_P322141	Ccdc88b	78317	NM_001081291	6.68	8.05	6.29	6.72	1
A_52_P322555	Pex13	72129	AK037872	7.96	7.61	7.22	6.95	3
A_52_P325116	Gramd1b	235283	XM_006510235	10.30	12.30	9.47	11.12	3
A_52_P325900	Cdc14a	229776	AK032515	8.19	7.87	8.39	8.77	2
A_52_P327110	Bean1	65115	NM_001141922	4.70	4.83	5.44	5.75	2
A_52_P329851	Ctbs	74245	NM_028836	6.47	5.93	6.90	6.52	2
A_52_P330289	Inpp4b	234515	NM_001024617	2.76	7.17	3.58	6.00	1
A_52_P33192	Palld	72333	XM_006509503	9.72	9.81	9.12	9.19	3
A_52_P337297	Amd2	100041585	NM_007444	11.76	12.17	11.50	11.54	1
A_52_P338066	Ubd	24108	NM_023137	3.51	4.62	4.75	7.00	4
A_52_P338530	Zdhhc20	75965	NM_029492	9.80	9.69	8.53	8.72	3
A_52_P342159	Nfatc4	73181	NM_023699	12.25	12.08	11.19	11.25	1
A_52_P342828	Fez1	235180	XM_006510229	11.57	11.06	12.60	12.23	4
A_52_P34420	Brpf3	268936	NM_001081315	6.08	5.58	6.03	6.29	2
A_52_P349143	Dtwd2	68857	NM_026854	6.64	6.65	5.92	5.79	1
A_52_P350111	Cyth1	19157	NM_011180	8.99	10.05	8.82	9.17	1
A_52_P353417	Gm2a	14667	NM_010299	8.53	7.67	8.91	8.55	2
A_52_P354682	Elov17	74559	NM_029001	10.74	9.44	11.20	10.40	4
A_52_P355751	Gm3383	100041515	NM_001291093	4.96	5.00	5.41	5.87	2
A_52_P355934	Myo1b	17912	NM_010863	6.62	6.13	6.12	5.36	1
A_52_P356343	Fam13a	58909	NM_153574	5.57	9.56	5.23	7.84	1
A_52_P358505	Filip1	70598	XM_006511442	2.59	7.85	2.42	5.78	1
A_52_P359819	Ntf5	78405	NM_198190	7.03	6.04	7.05	6.67	2
A_52_P371063	Iqcc	230767	NM_198026	7.60	7.24	8.07	7.86	2
A_52_P371129	Cdk5r1	12569	NM_009871	7.11	11.82	6.84	10.48	1
A_52_P374157	Kbtbd8	243574	NM_001008785	3.27	5.68	3.29	4.40	1
A_52_P376169	Lypd6	320343	NM_177139	3.98	7.94	3.71	6.77	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P37826	Dapk1	69635	NM_029653	6.26	6.37	5.74	5.71	1
A_52_P379531	Timeless	21853	NM_011589	9.86	10.21	8.82	8.85	1
A_52_P381553	Btbd17	72014	NM_028055	3.78	11.96	4.42	10.22	1
A_52_P38208	Arpp21	74100	NM_033264	2.90	6.58	4.30	4.78	1
A_52_P383572	Mylpf	17907	NM_016754	6.20	15.73	5.52	14.37	1
A_52_P384718	Cdh10	320873	U69137	8.14	7.36	9.04	8.45	4
A_52_P389874	Ldb3	24131	NM_011918	6.66	9.32	5.95	7.48	1
A_52_P389957	Lrch1	380916	XM_006519154	5.28	6.60	4.89	5.96	3
A_52_P395149	Smtnl2	276829	NM_177776	2.62	7.22	2.80	5.78	1
A_52_P402331	Tbc1d1	57915	NM_019636	6.37	7.13	5.47	6.14	3
A_52_P407280	Adamts15	66548	NR_104339	8.21	7.46	8.72	8.12	4
A_52_P410765	Sema7a	20361	NM_011352	9.39	10.80	10.21	12.20	4
A_52_P411921	D7Ert443e	71007	NM_001199941	4.13	5.65	4.06	2.94	1
A_52_P412408	Myo1h	231646	NM_001164573	5.23	4.43	5.08	5.39	2
A_52_P413395	Sln	66402	NM_025540	2.20	7.95	2.35	6.15	1
A_52_P413976	9430091E24Rik	434350	NR_040363	5.89	5.72	6.43	6.31	2
A_52_P415299	Top2a	21973	NM_011623	9.69	9.75	8.26	8.07	1
A_52_P421713	9930012K11Rik	268759	NM_001004155	8.76	8.37	9.35	8.97	2
A_52_P421947	Mtss1	211401	NM_144800	4.32	6.41	3.28	5.12	1
A_52_P423814	Cox8b	12869	NM_007751	2.50	11.23	2.61	9.61	1
A_52_P426554	Lmn2	16907	NM_010722	10.15	13.23	9.58	12.16	1
A_52_P426698	Sema3b	20347	NM_001042779	10.08	10.52	9.57	9.75	3
A_52_P429534	Elavl1	15568	NM_010485	8.65	9.63	8.37	9.01	1
A_52_P432949	Erbb3	13867	BC049279	5.48	11.30	5.78	9.82	1
A_52_P436464	Cnot6l	231464	NM_144910	10.72	12.61	10.70	11.96	1
A_52_P437363	Ppp1r12a	17931	XM_006513326	8.17	7.94	7.45	7.01	3
A_52_P440640			AK038400	4.24	3.63	4.26	4.33	2
A_52_P442722	Nol8	70930	NM_001271397	6.17	6.41	5.54	5.55	1
A_52_P443330	Wdr77	70465	NM_027432	6.39	7.56	6.25	6.96	1
A_52_P443846	Ppfibp1	67533	NM_026221	8.95	8.72	8.20	7.79	3
A_52_P445622	2610002M06Rik	67028	NM_025921	8.57	7.76	8.69	8.39	2
A_52_P449104	St6galnac4	20448	NM_011373	8.50	10.20	8.50	9.58	1
A_52_P451485	Cobl	12808	NM_172496	4.86	6.63	5.04	5.85	1
A_52_P45170	Mospd1	70380	NM_027409	11.64	12.46	11.45	11.84	1
A_52_P453833	Sypl	19027	AK035670	12.00	12.70	11.58	12.08	1
A_52_P455643	Skida1	72668	NM_028317	4.82	2.98	4.68	4.93	2
A_52_P456779	Gm16794	100504734	XR_105305	3.32	3.12	4.32	3.85	2
A_52_P457376	Fyb	23880	NM_011815	3.00	4.41	3.31	6.00	2
A_52_P465986	Efcab2	68226	NM_026626	6.52	6.49	6.27	5.67	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P466542	Zfp809	235047	NM_172763	6.16	5.86	6.81	6.48	4
A_52_P470150	Ddc	13195	NM_016672	5.29	9.91	4.40	8.58	1
A_52_P472302	Fxyd6	59095	NM_022004	7.31	11.12	6.55	9.08	1
A_52_P476731	Fam110c	104943	NM_027828	5.15	4.59	6.15	6.04	2
A_52_P476754	Ubash3b	72828	NM_176860	8.66	10.36	8.67	9.57	1
A_52_P48569	Slc38a4	69354	NM_027052	6.77	6.53	6.13	5.83	1
A_52_P488011				8.93	8.32	9.36	9.21	2
A_52_P488092	Etl4	208618	NM_001081006	8.38	7.93	8.73	8.63	2
A_52_P491433	Rps15a	267019	NM_170669	3.05	5.23	3.34	4.12	1
A_52_P492550	Ttc7	225049	NM_028639	3.86	3.81	4.54	4.57	4
A_52_P495699	Zfp599	235048	NM_181419	6.64	6.32	6.69	6.95	2
A_52_P496142	Col4a2	12827	NM_009932	11.11	11.27	10.01	10.17	3
A_52_P498608	Hmgn5	50887	NM_016710	9.58	10.95	8.89	9.55	1
A_52_P502547	Scai	320271	NM_178778	7.38	6.61	7.60	7.29	2
A_52_P503334	Erlin2	244373	NM_153592	11.22	11.32	10.48	10.57	3
A_52_P507736	Itgb8	320910	XM_006516016	6.96	6.24	7.27	7.12	2
A_52_P508991	Fmo1	14261	NM_010231	9.06	11.52	8.45	10.13	1
A_52_P509421	Kcnt2	240776	NM_001081027	4.07	2.53	4.79	3.64	4
A_52_P510107	Nudt15	214254	NM_172527	6.82	7.36	6.32	6.49	3
A_52_P510215	Traf6	22034	NM_009424	7.96	8.22	7.47	7.50	1
A_52_P517029	Tmeff2	56363	NM_019790	3.32	4.95	4.45	5.72	4
A_52_P520495	Vcam1	22329	NM_011693	8.24	7.69	8.51	8.34	2
A_52_P525317	Gja5	14613	NM_008121	3.05	6.70	3.38	4.55	1
A_52_P527800	Emilin2	246707	NM_145158	13.43	12.83	14.08	13.81	4
A_52_P534870	Bcl2	12043	NM_009741	9.21	8.63	9.51	9.34	2
A_52_P535212	Cpeb3	208922	NM_198300	7.65	9.35	7.77	8.46	1
A_52_P539414	Gtf2h3	209357	NM_181410	10.02	10.28	9.61	9.65	3
A_52_P540462	Lym9	66274	NM_001076681	6.63	7.52	6.32	6.67	1
A_52_P541095	Unc5a	107448	NM_153131	4.75	7.53	4.61	6.29	1
A_52_P545132	Kcnc2	268345	NM_001025581	6.33	5.23	6.59	5.83	2
A_52_P545810	Lrrfip1	16978	NM_008515	12.18	11.71	12.55	12.48	2
A_52_P547315	Trpc1	22063	NM_011643	7.05	6.44	7.43	7.10	2
A_52_P551098	Gpr126	215798	NM_001002268	5.29	4.99	5.02	4.09	1
A_52_P551476	Raph1	77300	NM_001045513	5.10	5.19	5.90	5.79	4
A_52_P552547	1110054M08Rik	68841	NR_037954	9.02	8.23	9.54	9.12	2
A_52_P557129	Slc12a5	57138	NM_020333	5.87	6.88	5.60	5.98	1
A_52_P563123	Tnni3	21954	NM_009406	5.03	5.44	4.77	4.53	1
A_52_P569998	Cdsn	386463	NM_001008424	10.76	10.13	11.72	11.12	4
A_52_P571350	H19	14955	AK003142	7.02	14.26	6.65	12.69	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P572765	Dtna	13527	NM_207650	3.70	7.49	3.69	6.61	1
A_52_P57582	Gm11127	100529082	NM_001199967	11.44	10.86	11.79	11.66	2
A_52_P577205	Arhgef3	71704	NM_027871	6.53	7.03	6.66	6.23	1
A_52_P577853	Mysm1	320713	NM_177239	7.98	7.35	8.40	8.10	4
A_52_P578043	Sacs	50720	XM_006519226	7.78	10.22	7.99	9.17	1
A_52_P578790	Dok3	27261	NM_013739	3.91	6.54	3.93	5.25	1
A_52_P578985	Uap111	227620	NM_001033293	5.88	5.70	6.28	6.51	2
A_52_P579411	Nxpe3	385658	NM_001134457	4.28	4.49	5.54	5.27	2
A_52_P580773			AK172242	4.63	4.21	4.78	4.85	2
A_52_P584415	Cars2	71941	NM_024248	6.97	6.82	7.27	7.41	2
A_52_P586851	Efs	13644	NM_010112	5.74	6.82	5.17	5.85	3
A_52_P588633	C1qtnf3	81799	NM_030888	3.95	8.99	3.36	6.51	1
A_52_P589568	Foxo6	329934	NM_194060	9.81	13.45	9.92	12.15	1
A_52_P590396	Sort1	20661	NM_019972	7.57	8.27	6.23	7.20	3
A_52_P59335	Ankef1	319196	AK029804	4.39	3.73	4.39	4.55	2
A_52_P597608	Stk16	20872	NM_011494	5.47	5.32	5.45	5.91	2
A_52_P601192	Ppp2r1b	73699	NM_001034085	9.79	10.50	9.07	9.68	3
A_52_P601497	Trim39	79263	NM_024468	6.67	6.10	7.12	6.72	4
A_52_P604195	Mbp	17196	NM_001025245	5.93	5.99	4.36	4.22	3
A_52_P607103	Pik3r3	18710	NM_181585	11.80	11.62	10.93	10.82	3
A_52_P607702	Six4	20474	XM_006515632	10.02	10.76	8.70	9.45	3
A_52_P609109	Pitpnc1	71795	NM_145823	7.29	6.61	7.64	7.39	2
A_52_P6092	Fam84b	399603	NM_001162926	6.39	5.70	7.48	7.18	4
A_52_P609913			AK081298	3.71	5.27	4.24	4.31	1
A_52_P613087				8.09	7.16	8.18	7.76	2
A_52_P618173	Limch1	77569	NM_001001980	4.67	4.81	3.55	3.35	1
A_52_P620154	Rgmb	68799	AK028583	3.55	4.43	3.75	5.32	2
A_52_P620240			AK044380	4.29	2.67	4.82	4.55	4
A_52_P620472	A830082K12Rik	320174	NR_045195	7.13	6.13	7.66	7.62	4
A_52_P622641	Ppfibp2	19024	NM_008905	4.08	4.29	2.87	3.09	1
A_52_P623211	Ccdc141	545428	NM_001025576	5.06	9.57	5.73	8.65	1
A_52_P625171	Asph	65973	NM_023066	7.08	9.08	6.97	7.75	1
A_52_P625215	Wfikkn2	278507	NM_181819	5.12	4.69	4.41	3.84	3
A_52_P625745	Ccnl2	56036	NM_207678	6.27	7.02	6.15	6.42	1
A_52_P628521	B130052P14Rik	105442	AK045264	5.73	6.38	5.38	5.47	3
A_52_P63044	Lsp1	16985	NM_019391	9.06	9.74	8.34	8.78	3
A_52_P630905	Cdh18	320865	NM_001081299	6.33	5.52	6.69	6.21	2
A_52_P632878			AK173262	2.72	3.08	5.11	5.56	4
A_52_P63343	Ciart	229599	NM_001033302	6.35	6.55	6.11	5.89	1

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P636199	Smc2	14211	NM_008017	10.90	11.17	10.16	9.97	1
A_52_P636948	Pdgfd	71785	NM_027924	9.01	8.29	9.39	9.32	2
A_52_P63709	Fam49b	223601	NM_144846	7.90	9.86	7.66	9.15	1
A_52_P638459	Ccl5	20304	NM_013653	5.57	5.76	6.15	8.22	2
A_52_P639343	Fam221a	231946	NM_172727	5.41	5.37	4.83	4.74	3
A_52_P640386	Usp53	99526	XM_006502419	4.32	4.21	5.38	5.02	4
A_52_P642453	Obscn	380698	NM_199152	2.05	8.13	2.32	5.91	1
A_52_P64364	Ikbkb	16150	NM_010546	9.86	9.98	9.48	9.37	1
A_52_P647260	Synpo2	118449	AK035258	3.94	3.92	4.59	4.84	2
A_52_P647740	Klhl41	228003	NM_001081087	2.54	9.95	2.76	8.62	1
A_52_P651784				9.91	10.59	9.96	9.92	1
A_52_P657360	Tnni1	21952	NM_021467	9.51	13.54	9.53	11.96	1
A_52_P660047	1810034E14Rik	66503	NR_045798	5.10	4.22	5.62	4.86	2
A_52_P662142	BC061212	381724	NM_198667	5.01	4.69	3.90	2.97	1
A_52_P662226	Pon3	269823	NM_173006	10.53	9.68	10.69	10.30	2
A_52_P663686	I830012O16Rik	667370	NM_001005858	7.13	5.75	7.32	6.50	2
A_52_P666779	Ptpn6	15170	NM_013545	6.77	6.48	7.16	7.12	2
A_52_P668030	Mrpl44	69163	NM_001081210	8.29	8.60	7.71	7.62	1
A_52_P668812	Sgol2	68549	NM_199007	10.64	10.33	9.11	9.15	1
A_52_P672689	Btc	12223	NM_007568	5.29	3.21	6.43	5.18	4
A_52_P677364	Bzw2	66912	NM_025840	10.29	10.67	9.36	9.60	3
A_52_P679612	Aff2	14266	NM_008032	3.02	1.98	3.79	4.27	2
A_52_P681391	G0s2	14373	NM_008059	7.37	9.11	6.87	7.95	1
A_52_P683315	Scrib	105782	NM_134089	13.41	14.93	13.52	14.21	1
A_52_P686130	Atp2a1	11937	NM_007504	1.96	10.59	2.08	8.72	1
A_52_P686218	Plb1	665270	NM_001081407	9.49	8.41	8.16	7.30	3
A_52_P70856	Frmpd1	666060	NM_001081172	2.25	11.12	1.95	10.00	1
A_52_P731128			AK040300	6.18	5.50	6.50	6.12	2
A_52_P74183	Riok1	71340	NM_024242	9.55	9.43	8.99	8.38	3
A_52_P780821	Msi2	76626	AK031365	9.21	11.23	9.06	10.06	1
A_52_P826021	B3galt1	26877	NM_020283	7.72	7.44	7.95	8.25	2
A_52_P827311	Lurap1	68075	AK047461	5.16	4.75	5.58	5.61	2
A_52_P84315	Chd7	320790	NM_001277149	6.95	11.18	6.51	9.88	1
A_52_P85200	Cln4-2	12727	NM_011334	11.30	11.53	10.90	10.90	1
A_52_P86872	Itgav	16410	AK171739	4.11	3.14	4.34	3.85	2
A_52_P87839	Sema3c	20348	NM_013657	7.67	6.98	8.40	7.80	2
A_52_P883941			AK048677	8.85	9.62	8.32	8.90	3
A_52_P900367	LOC102640237	102640237	XR_377736	4.71	5.76	4.90	4.96	1
A_52_P90805	Lrrc15	74488	NM_028973	5.99	5.84	4.73	3.93	3

Table A2.3 List of genes significantly modulated by Six4 knockdown during MyoD-induced C3H 10T1/2 myogenic conversion

ProbeID	Gene Symbol	GeneID	Genebank	Normalized expression Values				cluster #
				shLuc + pBabe	shLuc + MyoD	shSix4 + pBabe	shSix4 + MyoD	
A_52_P92121	Ankrd33b	67434	NM_027496	5.71	5.60	6.24	6.32	2
A_52_P932421	Gm10941	100169878	NR_026944	8.33	8.24	8.67	9.08	2
A_52_P93393	Gxylt2	232313	NM_198612	11.99	11.87	11.62	11.29	1
A_52_P956125	Ankrd12	106585	NM_001025572	4.56	3.20	4.57	4.38	2
A_52_P97699	D430019H16Rik	268595	NM_001252508	2.75	6.90	2.35	5.30	1
A_52_P987292	Nip7	66164	NM_025391	5.42	6.92	5.04	6.19	1

Table 7 A2.4 Gene expression profiling cluster 1 gene ontology analysis

Table A2.4 Gene expression profiling cluster 1 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0006936~muscle contraction	12	216	60	13138	2.96E-09
GO:0003012~muscle system process	12	219	67	13451	9.13E-09
GO:0007517~muscle organ development	17	202	176	12272	2.97E-08
GO:0030154~cell differentiation	53	219	1569	13451	2.92E-07
GO:0048731~system development	61	219	2027	13451	1.36E-06
GO:0006941~striated muscle contraction	7	202	24	12272	1.89E-06
GO:0055002~striated muscle cell development	9	202	54	12272	2.46E-06
GO:0055001~muscle cell development	9	202	61	12272	6.31E-06
GO:0014706~striated muscle tissue development	12	202	127	12272	7.34E-06
GO:0060537~muscle tissue development	12	202	136	12272	1.41E-05
GO:0048468~cell development	26	219	611	13451	1.93E-05
GO:0048513~organ development	50	219	1663	13451	1.95E-05
GO:0042692~muscle cell differentiation	11	216	117	13138	2.17E-05
GO:0048468~cell development	26	216	611	13138	2.24E-05
GO:0048513~organ development	50	216	1663	13138	2.46E-05
GO:0031032~actomyosin structure organization	6	216	25	13138	4.56E-05
GO:0007507~heart development	14	202	223	12272	7.78E-05
GO:0051146~striated muscle cell differentiation	9	202	89	12272	1.02E-04
GO:0048747~muscle fiber development	6	216	38	13138	3.62E-04
GO:0045214~sarcomere organization	4	202	11	12272	6.48E-04
GO:0009888~tissue development	23	219	638	13451	6.89E-04
GO:0048738~cardiac muscle tissue development	6	202	57	12272	0.00235

Table A2.4 Gene expression profiling cluster 1 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0030239~myofibril assembly	4	216	19	13138	0.00345
GO:0055082~cellular chemical homeostasis	12	219	268	13451	0.00435
GO:0006937~regulation of muscle contraction	5	216	41	13138	0.0044
GO:0055082~cellular chemical homeostasis	12	202	268	12272	0.00467
GO:0015674~di-, tri-valent inorganic cation transport	9	202	161	12272	0.00501
GO:0030036~actin cytoskeleton organization	9	219	165	13451	0.00545
GO:0007399~nervous system development	25	216	854	13138	0.00667
GO:0007010~cytoskeleton organization	13	219	326	13451	0.00703
GO:0060538~skeletal muscle organ development	6	202	74	12272	0.00725
GO:0008016~regulation of heart contraction	5	216	50	13138	0.00892
GO:0050801~ion homeostasis	12	202	293	12272	0.00903
GO:0006873~cellular ion homeostasis	11	216	261	13138	0.01086
GO:0051640~organelle localization	5	219	54	13451	0.01128
GO:0006811~ion transport	21	219	712	13451	0.01179
GO:0009790~embryonic development	20	219	670	13451	0.01273
GO:0030001~metal ion transport	15	202	442	12272	0.01405
GO:0000087~M phase of mitotic cell cycle	9	216	194	13138	0.01452
GO:0009408~response to heat	4	219	32	13451	0.01474
GO:0051239~regulation of multicellular organismal process	22	219	778	13451	0.01507
GO:0009408~response to heat	4	216	32	13138	0.01512
GO:0048741~skeletal muscle fiber development	4	202	32	12272	0.01514
GO:0044057~regulation of system process	9	219	201	13451	0.01669
GO:0048878~chemical homeostasis	13	216	365	13138	0.01714
GO:0051656~establishment of organelle localization	4	219	34	13451	0.01737
GO:0022403~cell cycle phase	12	219	328	13451	0.01808
GO:0000279~M phase	11	216	283	13138	0.01813
GO:0000278~mitotic cell cycle	10	219	244	13451	0.01819
GO:0000279~M phase	11	202	283	12272	0.01822
GO:0007512~adult heart development	3	202	13	12272	0.01849
GO:0010927~cellular component assembly involved in morphogenesis	4	219	36	13451	0.02024
GO:0006812~cation transport	16	202	515	12272	0.02225
GO:0016358~dendrite development	4	219	39	13451	0.02501
GO:0019725~cellular homeostasis	12	216	343	13138	0.02577
GO:0007242~intracellular signaling cascade	24	202	915	12272	0.02614

Table A2.4 Gene expression profiling cluster 1 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0022402~cell cycle process	13	219	393	13451	0.0268
GO:0001503~ossification	6	216	106	13138	0.03003
GO:0003007~heart morphogenesis	5	216	74	13138	0.03299
GO:0060415~muscle tissue morphogenesis	3	216	18	13138	0.03434
GO:0055008~cardiac muscle tissue morphogenesis	3	202	18	12272	0.03438
GO:0032886~regulation of microtubule-based process	4	219	45	13451	0.0362
GO:0000280~nuclear division	8	216	190	13138	0.03671
GO:0007067~mitosis	8	216	190	13138	0.03671
GO:0030003~cellular cation homeostasis	7	202	152	12272	0.03895
GO:0048729~tissue morphogenesis	9	219	238	13451	0.04026
GO:0048285~organelle fission	8	219	197	13451	0.04131
GO:0060348~bone development	6	219	118	13451	0.04295
GO:0007525~somatic muscle development	2	202	3	12272	0.04834
GO:0030261~chromosome condensation	3	219	22	13451	0.04883
GO:0065003~macromolecular complex assembly	11	219	338	13451	0.04895
GO:0009266~response to temperature stimulus	4	219	51	13451	0.04954
GO:0007528~neuromuscular junction development	3	216	23	13138	0.05385
GO:0022008~neurogenesis	15	216	537	13138	0.05813
GO:0032989~cellular component morphogenesis	11	219	351	13451	0.06006
GO:0048739~cardiac muscle fiber development	2	219	4	13451	0.06328
GO:0031175~neuron projection development	8	219	218	13451	0.06402
GO:0051276~chromosome organization	12	219	404	13451	0.06516
GO:0000910~cytokinesis	3	219	27	13451	0.07043
GO:0001701~in utero embryonic development	9	202	267	12272	0.07227
GO:0009636~response to toxin	3	219	29	13451	0.07978
GO:0051056~regulation of small GTPase mediated signal transduction	8	202	228	12272	0.08097
GO:0042401~biogenic amine biosynthetic process	3	202	29	12272	0.08123
GO:0043009~chordate embryonic development	12	216	421	13138	0.08552
GO:0002700~regulation of production of molecular mediator of immune response	3	216	30	13138	0.08601
GO:0009792~embryonic development ending in birth or egg hatching	12	219	425	13451	0.08605
GO:0048519~negative regulation of biological process	29	219	1326	13451	0.08779

Table A2.4 Gene expression profiling cluster 1 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0009792~embryonic development ending in birth or egg hatching	12	216	425	13138	0.0898
GO:0048863~stem cell differentiation	3	216	31	13138	0.09099
GO:0009653~anatomical structure morphogenesis	25	219	1114	13451	0.09227
GO:0042592~homeostatic process	15	219	584	13451	0.09318
GO:0009144~purine nucleoside triphosphate metabolic process	5	202	106	12272	0.09621
GO:0001501~skeletal system development	9	216	285	13138	0.0967
GO:0042127~regulation of cell proliferation	14	219	538	13451	0.09894

Table 8 A2.5 Gene expression profiling cluster 2 gene ontology analysis**Table A2.5 Gene expression profiling cluster 2 gene ontology analysis**

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0010810~regulation of cell-substrate adhesion	5	104	40	12272	3.39E-04
GO:0045595~regulation of cell differentiation	11	114	386	13138	1.80E-03
GO:0051239~regulation of multicellular organismal process	16	118	778	13451	3.02E-03
GO:0050767~regulation of neurogenesis	6	104	132	12272	5.04E-03
GO:0001952~regulation of cell-matrix adhesion	3	104	14	12272	5.94E-03
GO:0007611~learning or memory	5	104	89	12272	6.62E-03
GO:0030155~regulation of cell adhesion	5	114	94	13138	8.74E-03
GO:0051960~regulation of nervous system development	6	114	148	13138	9.00E-03
GO:0002474~antigen processing and presentation of peptide antigen via MHC class I	3	114	17	13138	9.17E-03
GO:0060284~regulation of cell development	6	104	159	12272	1.08E-02
GO:0001656~metanephros development	4	104	58	12272	1.27E-02
GO:0051240~positive regulation of multicellular organismal process	6	114	163	13138	1.33E-02
GO:0048513~organ development	24	114	1663	13138	1.36E-02
GO:0048731~system development	28	118	2027	13451	1.39E-02
GO:0007399~nervous system development	15	114	854	13138	1.47E-02
GO:0010646~regulation of cell communication	14	114	771	13138	1.50E-02
GO:0009653~anatomical structure morphogenesis	18	118	1114	13451	1.59E-02

Table A2.5 Gene expression profiling cluster 2 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0006952~defense response	10	118	448	13451	1.62E-02
GO:0010811~positive regulation of cell-substrate adhesion	3	104	27	12272	2.14E-02
GO:0009966~regulation of signal transduction	12	104	661	12272	2.26E-02
GO:0048732~gland development	6	104	197	12272	2.50E-02
GO:0050793~regulation of developmental process	11	118	568	13451	2.62E-02
GO:0060429~epithelium development	7	104	271	12272	2.65E-02
GO:0032944~regulation of mononuclear cell proliferation	4	104	80	12272	2.96E-02
GO:0050670~regulation of lymphocyte proliferation	4	104	80	12272	2.96E-02
GO:0016311~dephosphorylation	5	104	141	12272	3.08E-02
GO:0034097~response to cytokine stimulus	3	118	32	13451	3.14E-02
GO:0070663~regulation of leukocyte proliferation	4	104	82	12272	3.15E-02
GO:0008284~positive regulation of cell proliferation	7	104	284	12272	3.23E-02
GO:0048518~positive regulation of biological process	21	118	1493	13451	3.26E-02
GO:0060249~anatomical structure homeostasis	4	114	83	13138	3.47E-02
GO:0006796~phosphate metabolic process	14	114	866	13138	3.48E-02
GO:0048002~antigen processing and presentation of peptide antigen	3	118	35	13451	3.70E-02
GO:0048646~anatomical structure formation involved in morphogenesis	8	114	365	13138	3.80E-02
GO:0030335~positive regulation of cell migration	3	104	37	12272	3.84E-02
GO:0046668~regulation of retinal cell programmed cell death	2	104	5	12272	4.13E-02
GO:0009888~tissue development	11	104	638	12272	4.14E-02
GO:0030334~regulation of cell migration	4	104	92	12272	4.22E-02
GO:0042127~regulation of cell proliferation	10	114	538	13138	4.22E-02
GO:0051056~regulation of small GTPase mediated signal transduction	6	104	228	12272	4.29E-02
GO:0051272~positive regulation of cell motion	3	104	41	12272	4.63E-02
GO:0022037~metencephalon development	3	114	41	13138	4.84E-02
GO:0045785~positive regulation of cell adhesion	3	104	43	12272	5.04E-02
GO:0045664~regulation of neuron differentiation	4	104	102	12272	5.43E-02
GO:0040017~positive regulation of locomotion	3	114	44	13138	5.49E-02
GO:0009968~negative regulation of signal transduction	5	104	171	12272	5.57E-02
GO:0030182~neuron differentiation	8	114	399	13138	5.65E-02
GO:0048699~generation of neurons	9	104	497	12272	5.73E-02

Table A2.5 Gene expression profiling cluster 2 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0002009~morphogenesis of an epithelium	5	104	173	12272	5.77E-02
GO:0045597~positive regulation of cell differentiation	5	104	175	12272	5.97E-02
GO:0001822~kidney development	4	104	107	12272	6.10E-02
GO:0051270~regulation of cell motion	4	114	107	13138	6.47E-02
GO:0001894~tissue homeostasis	3	104	50	12272	6.59E-02
GO:0043524~negative regulation of neuron apoptosis	3	104	50	12272	6.59E-02
GO:0048857~neural nucleus development	2	118	8	13451	6.75E-02
GO:0007160~cell-matrix adhesion	3	114	50	13138	6.88E-02
GO:0040012~regulation of locomotion	4	118	110	13451	7.10E-02
GO:0010648~negative regulation of cell communication	5	104	186	12272	7.13E-02
GO:0030278~regulation of ossification	3	104	53	12272	7.29E-02
GO:0022603~regulation of anatomical structure morphogenesis	5	114	187	13138	7.77E-02
GO:0035295~tube development	6	118	264	13451	8.05E-02
GO:0022008~neurogenesis	9	104	537	12272	8.17E-02
GO:0009611~response to wounding	7	118	347	13451	8.22E-02
GO:0006869~lipid transport	4	114	119	13138	8.30E-02
GO:0048514~blood vessel morphogenesis	5	104	198	12272	8.52E-02
GO:0031589~cell-substrate adhesion	3	118	57	13451	8.79E-02
GO:0001763~morphogenesis of a branching structure	4	114	125	13138	9.30E-02
GO:0001819~positive regulation of cytokine production	3	104	62	12272	9.54E-02
GO:0006665~sphingolipid metabolic process	3	104	62	12272	9.54E-02

Table 9 A2.6 Gene expression profiling cluster 3 gene ontology analysis**Table A2.6 Gene expression profiling cluster 3 gene ontology analysis**

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0002831~regulation of response to biotic stimulus	2	60	13	13451	5.56E-02
GO:0002861~regulation of inflammatory response to antigenic stimulus	2	55	18	12272	7.64E-02
GO:0006461~protein complex assembly	4	60	227	13451	7.75E-02
GO:0006928~cell motion	6	60	367	13451	2.21E-02

Table A2.6 Gene expression profiling cluster 3 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0007399~nervous system development	10	61	854	13138	1.48E-02
GO:0007417~central nervous system development	5	55	369	12272	7.87E-02
GO:0009725~response to hormone stimulus	5	60	165	13451	5.88E-03
GO:0010942~positive regulation of cell death	4	60	252	13451	9.85E-02
GO:0016477~cell migration	5	60	240	13451	2.09E-02
GO:0022008~neurogenesis	6	55	537	12272	8.63E-02
GO:0030154~cell differentiation	14	60	1569	13451	1.67E-02
GO:0030155~regulation of cell adhesion	3	60	94	13451	6.40E-02
GO:0031294~lymphocyte costimulation	2	55	8	12272	3.47E-02
GO:0031295~T cell costimulation	2	60	8	13451	3.46E-02
GO:0032101~regulation of response to external stimulus	3	60	103	13451	7.50E-02
GO:0042692~muscle cell differentiation	3	61	117	13138	9.98E-02
GO:0043065~positive regulation of apoptosis	4	55	248	12272	9.56E-02
GO:0043068~positive regulation of programmed cell death	4	55	250	12272	9.73E-02
GO:0043434~response to peptide hormone stimulus	3	61	95	13138	6.99E-02
GO:0048731~system development	16	60	2027	13451	2.59E-02
GO:0048870~cell motility	5	60	284	13451	3.58E-02
GO:0051093~negative regulation of developmental process	4	60	226	13451	7.67E-02
GO:0051146~striated muscle cell differentiation	3	55	89	12272	5.84E-02
GO:0065003~macromolecular complex assembly	5	60	338	13451	6.07E-02

Table 10 A2.7 Gene expression profiling cluster 4 gene ontology analysis**Table A2.7 Gene expression profiling cluster 4 gene ontology analysis**

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0002027~regulation of heart rate	2	43	16	12272	5.34E-02
GO:0006810~transport	15	49	2342	13451	3.06E-02
GO:0006811~ion transport	9	49	712	13451	3.41E-03
GO:0006812~cation transport	8	43	515	12272	1.65E-03
GO:0007411~axon guidance	3	49	98	13451	4.77E-02
GO:0008038~neuron recognition	2	49	12	13451	4.20E-02
GO:0015674~di-, tri-valent inorganic cation transport	4	43	161	12272	1.75E-02
GO:0015695~organic cation transport	2	43	13	12272	4.36E-02
GO:0030001~metal ion transport	7	43	442	12272	3.68E-03

Table A2.7 Gene expression profiling cluster 4 gene ontology analysis

Gene Ontology ID and Term	Count	List Total	Pop Hits	Pop Total	P Value
GO:0042060~wound healing	3	50	112	13138	6.54E-02
GO:0043632~modification-dependent macromolecule catabolic process	5	43	508	12272	9.45E-02
GO:0048519~negative regulation of biological process	9	49	1326	13451	9.55E-02
GO:0048731~system development	14	49	2027	13451	2.23E-02
GO:0051239~regulation of multicellular organismal process	7	49	778	13451	5.71E-02
GO:0055085~transmembrane transport	5	49	460	13451	8.09E-02

Table 11 A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes				
Rank	Biological process	Six4-bound	Total in genome	FDR
1	cell development	1396	1970	1.36E-42
2	neurogenesis	1050	1441	2.44E-39
3	generation of neurons	998	1362	4.74E-39
4	enzyme linked receptor protein signaling pathway	777	1028	1.28E-37
5	neuron differentiation	923	1257	1.33E-36
6	regulation of developmental process	1333	1912	2.36E-35
7	positive regulation of gene expression	966	1332	5.90E-35
8	cellular component morphogenesis	906	1242	3.35E-34
9	tissue development	1254	1794	5.83E-34
10	regulation of transcription from RNA polymerase II promoter	1149	1629	1.70E-33
11	positive regulation of transcription, DNA-templated	887	1221	1.93E-32
12	cell morphogenesis	851	1165	1.93E-32
13	transcription from RNA polymerase II promoter	1249	1797	3.62E-32
14	regulation of multicellular organismal development	1042	1469	1.02E-31
15	circulatory system development	679	905	2.74E-31
16	cardiovascular system development	679	905	2.74E-31
17	positive regulation of cellular biosynthetic process	1087	1547	1.36E-30
18	cell projection organization	867	1201	3.23E-30
19	neuron development	743	1009	5.38E-30

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
20	cell morphogenesis involved in differentiation	636	845	6.18E-30
21	positive regulation of macromolecule biosynthetic process	1014	1436	1.03E-29
22	positive regulation of RNA biosynthetic process	904	1262	1.03E-29
23	positive regulation of nucleobase-containing compound metabolic process	1021	1448	1.39E-29
24	positive regulation of biosynthetic process	1102	1578	1.78E-29
25	positive regulation of RNA metabolic process	917	1284	1.83E-29
26	positive regulation of nitrogen compound metabolic process	1043	1484	1.84E-29
27	phosphorylation	1338	1963	7.42E-29
28	neuron projection development	654	878	1.07E-28
29	embryo development	781	1075	1.31E-28
30	regulation of cellular component organization	1193	1733	4.22E-28
31	cellular response to organic substance	1257	1840	1.42E-27
32	response to endogenous stimulus	998	1424	2.06E-27
33	protein phosphorylation	1023	1464	2.06E-27
34	cellular response to growth factor stimulus	510	665	3.16E-27
35	response to growth factor	521	682	3.99E-27
36	anatomical structure formation involved in morphogenesis	734	1009	3.99E-27
37	muscle structure development	438	558	3.99E-27
38	transmembrane receptor protein tyrosine kinase signaling pathway	549	726	1.04E-26
39	tissue morphogenesis	445	572	5.03E-26
40	movement of cell or subcellular component	1164	1703	1.61E-25
41	organ morphogenesis	671	920	3.43E-25
42	neuron projection morphogenesis	500	658	4.49E-25
43	regulation of cell differentiation	977	1405	5.66E-25
44	negative regulation of metabolic process	1270	1880	7.03E-25
45	cell projection morphogenesis	606	821	7.47E-25
46	positive regulation of transcription from RNA polymerase II promoter	671	922	8.40E-25
47	positive regulation of molecular function	1106	1616	1.69E-24
48	negative regulation of cellular metabolic process	1171	1723	2.83E-24
49	cell part morphogenesis	616	840	4.50E-24

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
50	muscle organ development	301	368	9.18E-24
51	regulation of phosphorus metabolic process	1280	1905	1.05E-23
52	central nervous system development	627	859	1.15E-23
53	cell morphogenesis involved in neuron differentiation	490	649	1.56E-23
54	tube development	472	622	1.84E-23
55	regulation of phosphate metabolic process	1270	1892	2.91E-23
56	single-organism organelle organization	1265	1887	7.96E-23
57	negative regulation of macromolecule metabolic process	1163	1721	1.02E-22
58	cell proliferation	1261	1887	6.10E-22
59	cell-cell signaling	887	1281	1.14E-21
60	locomotion	1036	1522	1.31E-21
61	axon development	446	590	1.52E-21
62	regulation of intracellular signal transduction	1033	1518	1.71E-21
63	cell cycle	1089	1611	3.23E-21
64	positive regulation of cell communication	832	1196	3.60E-21
65	vasculature development	448	595	4.62E-21
66	positive regulation of developmental process	652	911	4.65E-21
67	regulation of cell development	535	729	5.90E-21
68	positive regulation of signaling	826	1188	6.34E-21
69	axonogenesis	427	564	7.41E-21
70	regulation of anatomical structure morphogenesis	558	766	1.12E-20
71	negative regulation of biosynthetic process	853	1233	1.17E-20
72	muscle tissue development	299	374	1.17E-20
73	heart development	368	476	1.18E-20
74	epithelium development	776	1110	1.24E-20
75	striated muscle tissue development	287	357	1.66E-20
76	negative regulation of cellular biosynthetic process	839	1212	1.92E-20
77	positive regulation of signal transduction	780	1118	2.28E-20
78	embryonic morphogenesis	434	577	2.72E-20
79	morphogenesis of an epithelium	356	460	4.02E-20
80	regulation of transport	1026	1517	4.52E-20
81	blood vessel development	426	566	5.23E-20

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
82	programmed cell death	1218	1832	5.29E-20
83	negative regulation of signaling	691	979	6.25E-20
84	brain development	478	646	6.48E-20
85	regulation of cellular protein metabolic process	1088	1620	7.68E-20
86	cellular response to endogenous stimulus	705	1002	8.38E-20
87	apoptotic process	1201	1806	9.04E-20
88	negative regulation of cell communication	691	981	1.37E-19
89	cell migration	748	1073	2.23E-19
90	regulation of nervous system development	472	640	3.46E-19
91	regulation of cell proliferation	973	1439	6.24E-19
92	positive regulation of catalytic activity	931	1371	6.79E-19
93	negative regulation of cellular macromolecule biosynthetic process	768	1108	6.82E-19
94	negative regulation of macromolecule biosynthetic process	801	1161	6.82E-19
95	growth	665	944	7.55E-19
96	negative regulation of nucleobase-containing compound metabolic process	790	1145	1.25E-18
97	actin filament-based process	439	592	1.44E-18
98	negative regulation of nitrogen compound metabolic process	804	1168	1.46E-18
99	negative regulation of signal transduction	655	930	1.58E-18
100	regulation of phosphorylation	851	1248	6.24E-18
101	regulation of cell death	983	1466	1.76E-17
102	negative regulation of response to stimulus	769	1120	2.95E-17
103	regulation of neurogenesis	422	572	3.76E-17
104	regulation of protein modification process	873	1290	5.08E-17
105	axon guidance	297	383	5.51E-17
106	neuron projection guidance	297	383	5.51E-17
107	blood vessel morphogenesis	374	500	8.81E-17
108	embryo development ending in birth or egg hatching	480	664	1.02E-16
109	sensory organ development	390	525	1.16E-16
110	regulation of neuron differentiation	351	466	1.56E-16
111	behavior	494	687	1.68E-16
112	embryonic organ development	334	441	2.46E-16

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
113	positive regulation of cell differentiation	483	671	2.90E-16
114	gland development	322	424	4.89E-16
115	chordate embryonic development	472	655	5.01E-16
116	synaptic transmission	556	788	7.05E-16
117	positive regulation of response to stimulus	1047	1584	7.15E-16
118	cytoskeleton organization	680	987	1.06E-15
119	cell motility	793	1170	1.21E-15
120	localization of cell	793	1170	1.21E-15
121	negative regulation of transcription, DNA-templated	678	985	1.61E-15
122	forebrain development	291	380	2.54E-15
123	regulation of protein phosphorylation	698	1019	3.01E-15
124	negative regulation of gene expression	762	1123	3.51E-15
125	negative regulation of RNA biosynthetic process	689	1005	3.55E-15
126	mitotic cell cycle	640	927	4.73E-15
127	regulation of programmed cell death	926	1392	5.15E-15
128	organophosphate metabolic process	1275	1971	6.21E-15
129	negative regulation of RNA metabolic process	709	1039	6.27E-15
130	regulation of apoptotic process	915	1376	9.24E-15
131	actin cytoskeleton organization	374	509	9.80E-15
132	response to oxygen-containing compound	877	1314	1.00E-14
133	organophosphate catabolic process	817	1218	1.92E-14
134	cellular response to stress	980	1489	4.87E-14
135	skeletal system development	342	463	5.46E-14
136	muscle cell differentiation	255	331	5.89E-14
137	stem cell differentiation	250	324	8.04E-14
138	protein localization	1260	1957	8.34E-14
139	carbohydrate derivative catabolic process	817	1224	1.15E-13
140	small GTPase mediated signal transduction	517	739	1.15E-13
141	response to organonitrogen compound	537	771	1.20E-13
142	nucleoside phosphate catabolic process	780	1164	1.31E-13
143	regulation of growth	446	627	1.34E-13
144	nucleotide catabolic process	775	1156	1.36E-13
145	wound healing	480	681	1.50E-13

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
146	response to nitrogen compound	577	836	1.70E-13
147	purine nucleotide catabolic process	761	1134	1.74E-13
148	negative regulation of transcription from RNA polymerase II promoter	469	664	1.75E-13
149	cell cycle process	800	1198	1.86E-13
150	developmental growth	261	342	1.94E-13
151	regulation of cellular localization	661	972	2.03E-13
152	purine-containing compound catabolic process	764	1140	2.37E-13
153	positive regulation of cell proliferation	563	816	4.00E-13
154	regulation of cell projection organization	304	409	5.02E-13
155	transmembrane receptor protein serine/threonine kinase signaling pathway	250	327	5.02E-13
156	tube morphogenesis	275	365	5.69E-13
157	positive regulation of protein metabolic process	734	1094	5.74E-13
158	signal transduction by protein phosphorylation	485	693	7.22E-13
159	response to hormone	596	871	7.66E-13
160	ribonucleotide catabolic process	727	1084	8.75E-13
161	cell fate commitment	205	261	1.02E-12
162	purine ribonucleotide catabolic process	726	1083	1.05E-12
163	positive regulation of phosphorylation	559	813	1.31E-12
164	regulation of catabolic process	629	926	1.32E-12
165	organonitrogen compound catabolic process	931	1421	1.33E-12
166	angiogenesis	304	411	1.39E-12
167	regulation of neuron projection development	245	322	2.29E-12
168	regulation of cell cycle	587	860	2.46E-12
169	cellular nitrogen compound catabolic process	994	1529	3.19E-12
170	Wnt signaling pathway	265	353	3.39E-12
171	protein modification by small protein conjugation or removal	533	774	3.39E-12
172	nucleoside catabolic process	728	1091	4.15E-12
173	epithelial tube morphogenesis	262	349	4.65E-12
174	Ras protein signal transduction	375	524	5.03E-12
175	regulation of cellular catabolic process	572	838	5.06E-12
176	positive regulation of cellular protein metabolic process	668	994	5.48E-12

Table A2.8 Gene ontology biological processes significantly enriched among all Six4-bound genes

Rank	Biological process	Six4-bound	Total in genome	FDR
177	peptidyl-amino acid modification	632	936	6.52E-12
178	reproductive structure development	333	459	6.52E-12
179	chemotaxis	461	661	7.37E-12
180	cell-substrate adhesion	207	267	7.75E-12
181	neurotrophin TRK receptor signaling pathway	217	282	7.96E-12
182	aromatic compound catabolic process	994	1533	7.96E-12
183	neurotrophin signaling pathway	219	285	7.96E-12
184	heterocycle catabolic process	993	1532	9.30E-12
185	reproductive system development	335	463	9.62E-12
186	taxis	461	662	1.05E-11
187	glycosyl compound catabolic process	730	1098	1.14E-11
188	single-organism catabolic process	1242	1950	1.17E-11
189	purine nucleoside catabolic process	711	1067	1.17E-11
190	purine ribonucleoside catabolic process	711	1067	1.17E-11
191	MAPK cascade	455	653	1.27E-11
192	nucleobase-containing small molecule metabolic process	1079	1677	1.27E-11
193	nucleoside triphosphate catabolic process	707	1061	1.36E-11
194	ion transport	960	1479	1.41E-11
195	nucleobase-containing compound catabolic process	963	1484	1.41E-11
196	striated muscle cell differentiation	191	244	1.47E-11
197	cell division	533	779	1.74E-11
198	regulation of response to stress	672	1005	1.92E-11
199	negative regulation of cell death	552	810	1.93E-11
200	response to wounding	824	1255	2.01E-11

Table 12 A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr7	52590081	52590082	15464	Hrc	NM_010473	-557	7.24	4.67	+++	+++
chr3	135100765	135101764	66105	Ube2d3	NM_025356	5	310.69	61.51	+++	+++
chr3	97571415	97572414	83679	Pde4dip	NM_178080	23	220.04	31.95	+++	+++
chr17	23908133	23909132	73016	Kremen2	NM_028416	-25837	166.63	28.34	+++	+++
chr15	75910869	75911868	105782	Scrib	NM_134089	-11209	123.25	29.03	+++	+++
chr17	71339623	71340622	17929	Myom1	NM_010867	-28774	120.10	31.03	+++	+++
chr5	17079995	17080994	20348	Sema3c	NM_013657	-139	104.54	20.24	+++	+++
chr3	101408156	101409155	11928	Atp1a1	NM_144900	-76	103.69	27.80	+++	+++
chr4	94645302	94646301	320713	Mysm1	NM_177239	-11	99.46	28.71	+++	+++
chr1	93310325	93311324	55927	Hes6	AK146192	-1026	93.67	20.49	+++	+++
chr16	4559232	4560231	106393	Srl	NM_175347	-36679	89.64	26.56	+++	+++
chr2	90910006	90911005	68427	Slc39a13	NM_001290765	-34	86.61	19.38	+++	+++
chr1	79772151	79773150	69163	Mrpl44	NM_01081210	59	86.06	23.22	+++	+++
chr8	90885581	90886580	11513	Adcy7	BC057682	49983	78.70	16.58	+++	+++
chr1	89101786	89102785	11449	Chrng	NM_009604	-100	76.87	23.69	+++	+++
chr18	61558161	61559160	170826	Ppargc1b	NM_133249	1425	76.87	23.69	+++	+++
chr14	56261829	56262828	71099	Tssk4	NM_029596	-6690	74.43	19.31	+++	+++
chr3	90294507	90295506	20193	S100a1	NM_011309	23246	73.74	22.97	+++	+++
chr18	32539660	32540659	30948	Bin1	NM_001083334	3290	72.89	18.99	+++	+++
chr17	9048841	9049840	23984	Pde10a	NM_011866	6492	72.46	21.34	+++	+++
chr9	110684092	110685091	17897	Myl3	NM_010859	18408	70.07	18.46	+++	+++
chr4	9595967	9596966	65973	Asph	NM_001290367	25	67.98	20.37	+++	+++
chr4	149610735	149611734	12353	Car6	NM_009802	-35991	66.21	16.04	+++	+++
chr15	97614245	97615244	223864	Rapgef3	NM_144850	-16648	65.84	13.60	+++	+++
chr2	163212198	163213197	59091	Jph2	NM_021566	10989	65.60	18.32	+++	+++
chr17	56357743	56358742	14154	Fem1a	NM_010192	-37973	63.13	17.15	+++	+++
chr4	62162881	62163880	192120	Bspry	AK035588	22281	62.45	11.82	+++	+++
chr17	35137344	35138343	17687	Msh5	AK132626	32397	62.13	19.98	+++	+++
chr19	57051113	57052112	226250	Afap1l2	NM_001177796	31453	62.11	19.07	+++	+++
chr11	115760900	115761899	66528	2210020M01Rik	NM_183259	-53	62.05	14.07	+++	+++
chr4	119959964	119960963	329934	Foxo6	NM_194060	-598	58.52	19.38	+++	+++
chr6	125115252	125116251	320678	Iffo1	NM_001039669	20494	57.71	16.09	+++	+++
chr11	69714663	69715662	20528	Slc2a4	M23383	46474	57.68	18.93	+++	+++
chr4	119949915	119950914	329934	Foxo6	NM_194060	9452	57.12	13.30	+++	+++
chr11	107340249	107341248	71795	Pitpnc1	NM_145823	-8715	55.64	16.61	+++	+++
chr2	164257932	164258931	66460	Sys1	AK032073	-28039	54.10	18.30	+++	+++
chr3	87709625	87710624	12904	Crabp2	NM_007759	-42490	54.10	18.30	+++	+++
chr1	93355649	93356648	55927	Hes6	AK146192	-46350	53.72	18.09	+++	+++
chr11	78639513	78640512	66274	1810012P15Rik	NM_001076681	-84	53.71	15.30	+++	+++
chr15	101903766	101904765	209039	Tenc1	NM_153533	-26578	53.19	15.78	+++	+++
chr11	82721455	82722454	217012	Unc45b	NM_178680	-2800	52.64	17.94	+++	+++
chr15	97642266	97643265	223864	Rapgef3	NM_144850	-44669	52.28	12.53	+++	+++
chr5	36015390	36016389	78558	Htra3	NM_001042615	6542	52.09	13.59	+++	+++
chr2	151930761	151931760	76650	Srxn1	NM_029688	-205	51.20	17.58	+++	+++
chr5	36012421	36013420	78558	Htra3	NM_001042615	9511	50.83	17.38	+++	+++
chr2	120389078	120390077	20402	Zfp106	NM_011743	-11	49.76	17.23	+++	+++
chr17	66460470	66461469	106585	Ankrd12	AK012645	-34584	48.38	12.92	+++	+++
chr2	17948738	17949737	72668	2810030E01Rik	NM_028317	20839	48.21	14.46	+++	+++
chr10	41238633	41239632	53599	Cd164	NM_016898	-173	47.16	12.70	+++	+++
chr10	127984239	127985238	13867	Erbp3	NM_010153	41819	46.93	15.31	+++	+++
chr7	133571784	133572783	11937	Atp2a1	NM_007504	34304	46.58	15.52	+++	+++
chr6	54631163	54632162	68235	Mturn	NM_001289741	46	45.59	15.00	+++	+++
chr9	40608417	40609416	15481	Hspa8	AK004608	-439	45.50	16.15	+++	+++
chr14	34026677	34027676	239027	Arhgap22	NM_153800	-2832	44.74	12.25	+++	+++
chr3	54915471	54916470	320604	Ccdc169	NM_001290138	-25290	44.09	15.79	+++	+++
chr2	117937010	117938009	21825	Thbs1	NM_011580	-148	43.84	14.86	+++	+++
chr19	29325655	29326654	16452	Jak2	NM_001048177	-163	43.54	15.48	+++	+++
chr8	90795990	90796989	11513	Adcy7	NM_007406	189	42.95	10.99	+++	+++
chr3	87786861	87787860	18008	Nes	NM_016701	12347	42.70	15.43	+++	+++
chr8	24049471	24050470	11733	Ank1	NM_001277280	-35337	42.70	15.43	+++	+++
chr11	95381742	95382741	104079	Nxph3	NM_130858	-6363	42.60	14.59	+++	+++
chr15	66709066	66710065	22402	Wispl	NM_018865	-13389	42.58	13.83	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr13	34436688	34437687	73102	Slc22a23	NM_001033167	-137	42.53	14.55	+++	+++
chr2	73417939	73418938	11435	Chrna1	NM_007389	-76	42.35	11.81	+++	+++
chr16	30235972	30236971	74488	Lrrc15	NM_028973	46869	42.31	14.82	+++	+++
chr12	85534385	85535384	238317	C130039O16Rik	NM_001163502	81	42.13	12.92	+++	+++
chr18	57292530	57293529	70417	Megf10	NM_001001979	287	41.60	14.83	+++	+++
chr17	15962301	15963300	68799	Rgmb	NM_178615	750	41.32	15.07	+++	+++
chr4	119921178	119922177	329934	Foxo6	NM_194060	38189	41.32	15.07	+++	+++
chr8	126001395	126002394	72185	Dnndd1	NM_001170976	37461	41.32	15.07	+++	+++
chr9	59333998	59334997	73893	Tmem202	NM_178388	38811	41.32	15.07	+++	+++
chr9	43874599	43875598	21838	Thy1	NM_009382	23633	41.20	14.22	+++	+++
chr10	80401645	80402644	17873	Gadd45b	NM_008655	9310	40.64	13.57	+++	+++
chr13	100211294	100212293	17755	Mtap1b	NM_008634	2629	40.29	13.75	+++	+++
chr16	4521370	4522369	106393	Srl	NM_175347	1184	39.95	14.71	+++	+++
chr4	124801673	124802672	70088	Meaf6	NM_027310	39783	39.95	14.71	+++	+++
chr9	40128630	40129629	235283	Gramd1b	NM_172768	24746	39.95	14.71	+++	+++
chr16	52452801	52453800	11658	Alcam	AY644766	-191	38.98	13.43	+++	+++
chr15	102346941	102347940	26404	Map3k12	NM_001163643	-6	38.88	12.68	+++	+++
chr1	51972362	51973361	17912	Myo1b	NM_001290982	-40	38.59	14.35	+++	+++
chr8	125184727	125185726	12398	Cbfa2t3	NM_177289	16849	38.59	14.35	+++	+++
chr1	60623245	60624244	77300	Raph1	NM_001045513	-136	38.56	13.58	+++	+++
chr10	76592938	76593937	12822	Col18a1	NM_001109991	-16988	38.56	13.58	+++	+++
chr14	70575418	70576417	268759	9930012K11Rik	NM_001112735	-16609	38.56	13.58	+++	+++
chr7	52689662	52690661	14936	Gys1	NM_030678	-47	38.56	13.58	+++	+++
chr8	26385176	26386175	320165	Tacc1	AK190590	-34303	38.56	13.58	+++	+++
chr15	58829164	58830163	211401	Mtss1	NM_144800	42489	38.47	12.82	+++	+++
chr12	55935574	55936573	12632	Cfl2	NM_007688	27791	38.42	12.13	+++	+++
chr17	29088934	29089933	69307	Pxt1	NM_153390	-10227	38.42	12.13	+++	+++
chr8	9977272	9978271	319583	Lig4	NM_176953	-1449	38.29	13.81	+++	+++
chr3	103538298	103539297	99543	Olfml3	AK143728	3094	37.68	10.92	+++	+++
chr8	88078685	88079684	108682	Gpt2	NM_173866	35880	37.30	10.49	+++	+++
chr16	4550744	4551743	106393	Srl	NM_175347	-28191	37.24	14.00	+++	+++
chr10	79850083	79851082	66548	Adamtsl5	NR_104339	-39390	36.97	13.47	+++	+++
chr5	36013886	36014885	78558	Htra3	NM_001042615	8046	36.97	13.47	+++	+++
chr16	27388868	27389867	67501	Ccdc50	NM_026202	306	36.53	10.69	+++	+++
chr17	34092309	34093308	14972	H2-K1	BC080756	41892	36.41	12.81	+++	+++
chr15	101935547	101936546	209039	Tenc1	NM_153533	1192	36.21	13.06	+++	+++
chr12	102267107	102268106	68339	Ccdc88c	NM_026681	-414	36.03	12.97	+++	+++
chr10	41272328	41273327	53599	Cd164	NM_016898	33523	35.99	11.61	+++	+++
chr11	69734438	69735437	20528	Slc2a4	M23383	26699	35.99	11.61	+++	+++
chr18	24761928	24762927	68591	Mocos	NM_026779	-49764	35.99	11.61	+++	+++
chr6	97160272	97161271	320502	Lmod3	NM_001081157	42003	35.99	11.61	+++	+++
chr1	36364217	36365216	214855	Arid5a	NM_145996	140	35.96	12.24	+++	+++
chr17	34161046	34162045	14972	H2-K1	NM_001001892	-24268	35.96	12.93	+++	+++
chr1	164789528	164790527	14261	Fmo1	NM_010231	58	35.90	13.64	+++	+++
chr1	137662125	137663124	21952	Tnni1	NM_021467	-33373	35.55	12.36	+++	+++
chr1	137667166	137668165	21952	Tnni1	NM_021467	-28332	35.39	10.47	+++	+++
chr5	136592037	136593036	54153	Rasa4	NM_133914	32752	35.39	10.47	+++	+++
chr13	32893424	32894423	238564	Mylk4	NM_001166030	-20276	35.17	9.64	+++	+++
chr15	102348299	102349298	26404	Map3k12	NM_001163643	-1364	34.80	11.34	+++	+++
chr12	114377942	114378941	68337	Crip2	NM_024223	-5	34.68	12.61	+++	+++
chr13	100284482	100285481	17755	Mtap1b	NM_008634	1576	34.68	12.61	+++	+++
chr19	4509629	4510628	18563	Pcx	NM_001162946	-343	34.68	12.61	+++	+++
chr3	130814537	130815536	16842	Lef1	NM_001276403	-672	34.68	12.61	+++	+++
chr8	4238460	4239459	72267	Lrrc8e	NM_028175	12134	34.68	12.61	+++	+++
chr10	80423270	80424269	17873	Gadd45b	NM_008655	30935	34.57	13.28	+++	+++
chr4	132806183	132807182	53608	Map3k6	NM_016693	9951	34.57	13.28	+++	+++
chr8	28087016	28088015	244373	Erlin2	NM_153592	-46815	34.57	13.28	+++	+++
chr14	34060541	34061540	239027	Arhgap22	NM_153800	31033	34.40	11.46	+++	+++
chr2	164266342	164267341	66460	Sys1	AK032073	-19629	34.01	12.97	+++	+++
chr2	90780012	90781011	13046	Celf1	NM_001244891	-42	33.88	12.18	+++	+++
chr12	56618667	56619666	79459	Aldoat2	NM_001277340	-47025	33.61	11.08	+++	+++
chr3	87733647	87734646	12904	Crabp2	NM_007759	-18468	33.61	11.08	+++	+++
chr16	15887049	15888048	12609	Cebpd	NM_007679	171	33.41	12.28	+++	+++
chr3	14885735	14886734	12350	Car3	AK017227	22698	33.41	12.28	+++	+++
chr11	102316953	102317952	217219	Fam171a2	NM_199200	-8476	33.25	12.92	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr17	33896570	33897569	57875	Angptl4	AK134419	21451	33.25	12.92	+++	+++
chr11	6381356	6382355	19291	Purb	NM_011221	-5936	33.08	12.46	+++	+++
chr11	72303039	72304038	216892	Spns2	NM_153060	-133	33.08	12.46	+++	+++
chr2	60526711	60527710	16420	Itgb6	AK019511	-15400	32.74	11.27	+++	+++
chr2	6793365	6794364	14007	Cellf2	NM_010160	12178	32.73	12.27	+++	+++
chr11	80289826	80290825	12569	Cdk5r1	NM_009871	-222	32.71	12.62	+++	+++
chr12	104597298	104598297	65256	Asb2	NM_023049	-3587	32.71	12.62	+++	+++
chr4	11083233	11084232	60599	Trp53inp1	NM_021897	146	32.71	12.62	+++	+++
chr17	32279840	32280839	18131	Notch3	NM_008716	23458	32.43	10.81	+++	+++
chr9	66859987	66860986	22003	Tpm1	AK179259	20185	32.43	10.81	+++	+++
chr2	169956324	169957323	228913	Zfp217	NM_001033299	-104	32.30	12.04	+++	+++
chr13	55551544	55552543	56320	Dbn1	NM_019813	37394	32.15	11.96	+++	+++
chr9	66896424	66897423	22003	Tpm1	NM_001164255	97	32.15	11.96	+++	+++
chr12	102246113	102247112	68339	Ccdc88c	NM_026681	20581	31.95	12.56	+++	+++
chr12	112929311	112930310	12709	Ckb	NM_021273	-19262	31.95	12.56	+++	+++
chr5	34124980	34125979	107823	Whsc1	NM_001177884	-37913	31.83	11.46	+++	+++
chr16	35982986	35983985	16646	Kpna1	NM_008465	38	31.81	12.12	+++	+++
chr6	58781310	58782309	73998	Herc3	NM_028705	-73	31.42	12.27	+++	+++
chr10	93977473	93978472	319880	Tmcc3	NM_172051	372	31.39	11.56	+++	+++
chr4	46100453	46101452	21916	Tmod1	NM_021883	10926	31.08	11.07	+++	+++
chr1	89086584	89087583	11449	Chrng	NM_009604	-15302	30.90	11.64	+++	+++
chr2	52280018	52281017	75423	Arl5a	NM_182994	-124	30.90	11.64	+++	+++
chr5	123794723	123795722	77045	Bcl7a	NM_029850	767	30.90	11.64	+++	+++
chr14	79700903	79701902	66214	1190002H23Rik	NM_025427	40	30.66	12.20	+++	+++
chr2	164311311	164312310	66460	Sys1	NM_025575	25019	30.66	12.20	+++	+++
chr4	119955333	119956332	329934	Foxo6	NM_194060	4034	30.66	12.20	+++	+++
chr4	155154699	155155698	56036	Ccnl2	AK048244	-31399	30.66	12.20	+++	+++
chr7	149718506	149719505	21957	Tnnt3	NM_001163670	31482	30.66	12.20	+++	+++
chr2	122455992	122456991	67092	Gatm	NM_025961	-19479	30.62	11.16	+++	+++
chr1	108655958	108656957	70750	Kdsr	NM_027534	-139	30.17	11.25	+++	+++
chr13	75844914	75845913	192657	Ell2	NM_138953	483	30.17	11.25	+++	+++
chr15	58886071	58887070	211401	Mtss1	NM_144800	-14419	30.17	11.25	+++	+++
chr18	32306795	32307794	19123	Proc	NM_001042767	-8071	30.17	11.25	+++	+++
chr5	145118648	145119647	66898	Baiap211	NM_025833	-167	30.17	11.25	+++	+++
chr8	91220865	91221864	257632	Nod2	DQ289013	44602	30.16	10.03	+++	+++
chr18	32070130	32071129	225341	Lims2	NM_144862	-20531	30.14	11.92	+++	+++
chr1	75314065	75315064	13346	Des	NM_010043	-42354	30.10	10.29	+++	+++
chr11	58976379	58977378	380698	Obscn	NM_001171512	-27002	30.10	10.29	+++	+++
chr11	80241821	80242820	12569	Cdk5r1	NM_009871	-48227	29.88	10.78	+++	+++
chr1	78653717	78654716	195434	Utp14b	NM_001001981	-183	29.88	11.77	+++	+++
chr3	96361260	96362259	69585	Hfe2	NM_027126	32653	29.87	8.68	+++	+++
chr15	7068614	7069613	16880	Lifr	NM_001113386	-10458	29.83	9.36	+++	+++
chr16	36828057	36829056	57738	Slc15a2	NM_021301	-43509	29.83	9.36	+++	+++
chr18	23199468	23200467	319211	Nol4	NM_199024	187	29.67	11.31	+++	+++
chr5	140266928	140267927	17135	Mafk	NM_010757	-62	29.67	11.31	+++	+++
chr15	72984796	72985795	239528	Eif2c2	NM_153178	30082	29.52	7.60	+++	+++
chr15	98529542	98530541	66120	Fkbp11	BC002311	28588	29.41	10.85	+++	+++
chr4	124743377	124744376	70088	Meaf6	NM_001290701	-18501	29.38	11.84	+++	+++
chr4	133035318	133036317	69539	Trnp1	NM_001081156	18648	29.38	11.84	+++	+++
chr4	155186159	155187158	56036	Ccnl2	AK048244	62	29.38	11.84	+++	+++
chr7	138079146	138080145	56213	Htra1	NM_019564	-71	29.38	11.84	+++	+++
chr7	28337689	28338688	11652	Akt2	NM_001110208	-38390	29.38	11.84	+++	+++
chr8	37157495	37158494	244418	D8Ert82e	NM_172911	114	29.38	11.84	+++	+++
chr2	39045912	39046911	320271	Scai	NM_178778	-162	29.29	11.45	+++	+++
chr8	26350801	26351800	320165	Tacc1	AK190590	73	29.29	11.45	+++	+++
chr7	133604210	133605209	11937	Atp2a1	NM_007504	1878	28.96	10.02	+++	+++
chr2	130489819	130490818	241638	Lzts3	NM_001291027	-21739	28.88	11.57	+++	+++
chr15	97612912	97613911	223864	Rapgef3	NM_144850	-15315	28.85	9.43	+++	+++
chr14	34063056	34064055	239027	Arhgap22	NM_153800	33548	28.71	11.47	+++	+++
chr18	32322577	32323576	19123	Proc	NM_001042767	-23853	28.50	11.02	+++	+++
chr10	90545402	90546401	67454	Ikbp1	NM_027078	119	28.44	10.99	+++	+++
chr4	45150159	45151158	666060	Frmpd1	NM_001081172	-47119	28.44	10.99	+++	+++
chr2	17947710	17948709	72668	2810030E01Rik	NM_028317	21867	28.11	11.48	+++	+++
chr5	91568171	91569170	11839	Areg	NM_009704	31	28.11	11.48	+++	+++
chr5	96591123	96592122	231464	Cnot61	NM_144910	-614	28.11	11.48	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr7	28327365	28328364	11652	Akt2	NM_001110208	-48714	28.11	11.48	+++	+++
chr1	75340529	75341528	13346	Des	NM_010043	-15890	27.88	9.79	+++	+++
chr11	54910845	54911844	14667	Gm2a	NM_010299	-274	27.82	9.76	+++	+++
chr11	118151061	118152060	19157	Cyth1	NM_011180	-41655	27.68	8.91	+++	+++
chr17	26644686	26645685	19252	Dusp1	NM_013642	232	27.68	8.91	+++	+++
chr7	150969596	150970595	13360	Dhcr7	NM_007856	-38976	27.68	8.91	+++	+++
chr4	124791549	124792548	70088	Meaf6	NM_027310	29659	27.63	11.22	+++	+++
chr5	137547605	137548604	384309	Trim56	NM_201373	43973	27.23	10.67	+++	+++
chr7	148045605	148046604	12869	Cox8b	NM_007751	40241	27.23	10.67	+++	+++
chr9	66891127	66892126	22003	Tpm1	NM_001164256	52	27.23	10.67	+++	+++
chr15	57524807	57525806	387609	Zhx2	NM_199449	-915	27.11	10.29	+++	+++
chr17	32300832	32301831	18131	Notch3	NM_008716	2466	27.04	10.25	+++	+++
chr9	66359214	66360213	74165	Fbxl22	NM_175206	2687	27.04	10.25	+++	+++
chr1	136185896	136186895	17928	Myog	NM_031189	-185	26.85	11.13	+++	+++
chr1	89081037	89082036	11449	Chrng	NM_009604	-20849	26.85	11.13	+++	+++
chr11	6375612	6376611	19291	Purb	NM_011221	-192	26.85	11.13	+++	+++
chr2	32404927	32405926	20448	St6galnac4	NM_001276425	-37171	26.85	11.13	+++	+++
chr4	8737910	8738909	320790	Chd7	BC034239	-45408	26.85	11.13	+++	+++
chr6	30632124	30633123	109697	Cpa1	NM_025350	43404	26.85	11.13	+++	+++
chr7	52844226	52845225	56636	Fgf21	NM_020013	26135	26.85	11.13	+++	+++
chr1	75396137	75397136	13346	Des	NM_010043	39719	26.70	9.50	+++	+++
chr15	31382828	31383827	223455	March6	AK218077	16105	26.70	9.50	+++	+++
chr8	109554695	109555694	66164	Nip7	NM_025391	-25582	26.70	9.50	+++	+++
chr15	78034270	78035269	19293	Pvalb	NM_013645	-103	26.39	10.86	+++	+++
chr18	24867379	24868378	68591	Mocos	NM_026779	49403	26.09	10.37	+++	+++
chr2	163223337	163224336	59091	Jph2	NM_001205076	-108	26.03	10.34	+++	+++
chr5	37380011	37381010	22393	Wfs1	NM_011716	-290	26.03	10.34	+++	+++
chr17	33918148	33919147	57875	Angptl4	AK134419	-128	26.01	10.33	+++	+++
chr18	61554320	61555319	170826	Ppargc1b	NM_133249	5266	25.87	9.95	+++	+++
chr5	20649998	20650997	212167	Pion	NM_175437	-41587	25.80	7.90	+++	+++
chr14	21521694	21522693	59011	Myoz1	NM_021508	-46432	25.73	9.58	+++	+++
chr9	57819393	57820392	20361	Sema7a	NM_011352	31952	25.62	10.44	+++	+++
chr1	92894916	92895915	16978	Lrrfip1	NM_001111312	113	25.61	10.77	+++	+++
chr11	102268381	102269380	217219	Fam171a2	NM_199200	40097	25.61	10.77	+++	+++
chr11	72291561	72292560	216892	Spns2	NM_001276383	-3532	25.61	10.77	+++	+++
chr15	98557655	98558654	66120	Fkbp11	BC002311	475	25.61	10.77	+++	+++
chr7	150970659	150971658	13360	Dhcr7	NM_007856	-37913	25.61	10.77	+++	+++
chr11	5737547	5738546	56012	Pgam2	NM_018870	-34248	25.59	9.23	+++	+++
chr11	67779547	67780546	71860	Wdr16	NM_027963	-903	25.59	9.23	+++	+++
chr11	96838385	96839384	103711	Pnpo	NM_134021	-33552	25.59	9.23	+++	+++
chr15	78035419	78036418	19293	Pvalb	NM_013645	912	25.55	10.73	+++	+++
chr13	100282821	100283820	17755	Mtap1b	NM_008634	3237	25.38	10.00	+++	+++
chr15	63829337	63830336	223601	Fam49b	NM_144846	-313	25.23	7.31	+++	+++
chr4	155213308	155214307	56036	Ccnl2	AK048244	27211	24.85	10.02	+++	+++
chr5	122951605	122952604	11938	Atp2a2	NM_001110140	130	24.85	10.02	+++	+++
chr9	50559966	50560965	69253	Hspb2	NM_001164708	-7	24.85	10.02	+++	+++
chr14	61351336	61352335	219140	Spata13	NM_001033272	12	24.72	9.65	+++	+++
chr7	134397197	134398196	17907	Mylpf	NM_016754	42576	24.72	9.65	+++	+++
chr19	11844380	11845379	n/a	AK044380	AK044380	-47855	24.53	8.24	+++	+++
chr2	164859883	164860882	57138	Slc12a5	BC057624	41244	24.53	8.24	+++	+++
chr14	61791393	61792392	50720	Sacs	NM_172809	67	24.39	10.41	+++	+++
chr16	33739598	33740597	77446	Heg1	AK043598	-15538	24.39	10.41	+++	+++
chr3	87774566	87775565	18008	Nes	NM_016701	52	24.39	10.41	+++	+++
chr4	118867373	118868372	69216	Ccdc23	NM_024462	-42	24.39	10.41	+++	+++
chr5	81448448	81449447	319387	Lphn3	NM_198702	-648	24.39	10.41	+++	+++
chr5	140270690	140271689	17135	Mafk	NM_010757	3701	24.33	10.37	+++	+++
chr3	95086840	95087839	229589	Prune	NM_173347	-1342	24.22	9.69	+++	+++
chr17	25712240	25713239	20681	Sox8	NM_011447	-5109	23.88	10.12	+++	+++
chr1	93309491	93310490	55927	Hes6	AK146192	-192	23.83	7.52	+++	+++
chr8	98239335	98240334	234593	Ndrp4	NM_001195006	12899	23.83	7.52	+++	+++
chr12	102235519	102236518	68339	Ccdc88c	BC027658	-15452	23.73	9.72	+++	+++
chr12	8976303	8977302	17182	Matn3	NM_010770	22069	23.73	9.72	+++	+++
chr13	32867613	32868612	238564	Mylk4	NM_001166030	5536	23.68	9.70	+++	+++
chr14	56254140	56255139	71099	Tssk4	NM_029596	-14379	23.58	9.35	+++	+++
chr6	5446009	5447008	27273	Pdk4	NM_013743	-231	23.46	8.48	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr1	92949825	92950824	16978	Lrrfip1	NM_008515	305	23.40	8.70	+++	+++
chr6	5247809	5248808	269823	Pon3	NM_173006	-42076	23.40	8.70	+++	+++
chr8	96696150	96697149	17750	Mt2	NM_008630	133	23.35	6.97	+++	+++
chr12	25515778	25516777	67216	Mboat2	AK033106	-186	23.18	10.05	+++	+++
chr13	75934882	75935881	192657	Ell2	NM_138953	36057	23.18	10.05	+++	+++
chr2	163179189	163180188	59091	Jph2	NM_021566	43998	23.18	10.05	+++	+++
chr5	38581415	38582414	18196	Nsg1	NM_010942	-31209	23.18	10.05	+++	+++
chr7	148127233	148128232	12869	Cox8b	NM_007751	-41388	23.18	10.05	+++	+++
chr8	125175061	125176060	12398	Cbfa2t3	NM_177289	26515	23.18	10.05	+++	+++
chr1	75356296	75357295	13346	Des	NM_010043	-123	22.86	7.33	+++	+++
chr7	28392304	28393303	11652	Akt2	NM_007434	450	22.76	9.81	+++	+++
chr6	97155214	97156213	320502	Lmod3	NM_001081157	47061	22.69	9.17	+++	+++
chr11	78959526	78960525	16706	Ksr1	NM_013571	-170	22.52	9.37	+++	+++
chr11	97882881	97883880	12295	Cacnb1	NM_145121	561	22.52	9.37	+++	+++
chr14	55512157	55513156	13644	Efs	NM_010112	32969	22.52	9.37	+++	+++
chr17	34087735	34088734	14972	H2-K1	BC080756	46466	22.52	9.37	+++	+++
chr3	138404545	138405544	56224	Tspan5	BC046784	-114	22.52	9.37	+++	+++
chr3	95116248	95117247	229589	Prune	NM_173347	-30750	22.52	9.37	+++	+++
chr19	4231651	4232650	245857	Ssh3	NM_198113	37022	22.49	7.80	+++	+++
chr4	46051718	46052717	21916	Tmod1	NM_021883	125	22.49	7.80	+++	+++
chr15	97644914	97645913	223864	Rapgef3	NM_144850	-47317	22.33	8.44	+++	+++
chr2	164610670	164611669	21925	Tnnc2	NM_009394	-5936	22.33	8.44	+++	+++
chr5	34162303	34163302	107823	Whsc1	NM_001177884	-590	22.33	8.44	+++	+++
chr1	178765300	178766299	15365	Hmga2-ps1	NR_037996	465	22.07	9.43	+++	+++
chr8	90806364	90807363	11513	Adcy7	NM_001109756	-27	21.99	7.80	+++	+++
chr11	120558684	120559683	12409	Cbr2	NM_007621	34152	21.98	9.69	+++	+++
chr11	67734551	67735550	71860	Wdr16	NM_027963	44094	21.98	9.69	+++	+++
chr12	120476794	120477793	320910	Itgb8	AK034644	-545	21.98	9.69	+++	+++
chr18	36919545	36920544	12475	Cd14	NM_009841	-33737	21.98	9.69	+++	+++
chr2	164594797	164595796	21925	Tnnc2	NM_009394	9938	21.98	9.69	+++	+++
chr6	125444344	125445343	22371	Vwf	AK170399	-37761	21.98	9.69	+++	+++
chr7	108986258	108987257	16332	Inpp1l	NM_010567	-18	21.98	9.69	+++	+++
chr7	134376221	134377220	17907	Mylpf	NM_016754	21600	21.98	9.69	+++	+++
chr7	4452874	4453873	21955	Tnnt1	NM_011618	14204	21.98	9.69	+++	+++
chr9	57787548	57788547	20361	Sema7a	NM_011352	107	21.98	9.69	+++	+++
chr3	135085669	135086668	66105	Ube2d3	NM_025356	-15092	21.93	9.06	+++	+++
chr11	70468732	70469731	216874	Camta2	NM_001190378	28047	21.90	7.13	+++	+++
chr11	108226046	108227045	11818	Apoh	NM_013475	-30065	21.86	8.74	+++	+++
chr8	96696649	96697648	17750	Mt2	NM_008630	632	21.86	8.21	+++	+++
chr4	136025530	136026529	269593	Luzp1	NM_024452	355	21.86	9.62	+++	+++
chr11	5661866	5662865	56012	Pgam2	NM_018870	41434	21.84	8.46	+++	+++
chr11	104469118	104470117	17896	Myl4	NM_010858	30901	21.72	9.54	+++	+++
chr1	195098921	195099920	14373	G0s2	NM_008059	-39	21.58	9.46	+++	+++
chr11	97855497	97856496	12295	Cacnb1	NM_001159320	23690	21.58	9.46	+++	+++
chr17	23836691	23837690	73016	Kremen2	NM_028416	45606	21.58	9.46	+++	+++
chr9	50692882	50693881	73699	Ppp2r1b	NM_028614	28343	21.58	9.46	+++	+++
chr8	98201808	98202807	234593	Ndr4	NM_001195006	324	21.49	7.57	+++	+++
chr11	78832587	78833586	16706	Ksr1	AK137532	40252	21.38	9.05	+++	+++
chr17	8716971	8717970	23984	Pde10a	NM_011866	-2195	21.38	9.05	+++	+++
chr2	31304668	31305667	11898	Ass1	NM_007494	-20622	21.38	9.05	+++	+++
chr2	69551049	69552048	228003	Kbtbd10	NM_001081087	43373	21.38	9.05	+++	+++
chr11	78564687	78565686	n/a	AK136065	AK136065	-10582	21.34	8.74	+++	+++
chr11	97896973	97897972	12295	Cacnb1	NM_145121	-13532	21.34	8.74	+++	+++
chr11	11708553	11709552	13195	Ddc	AK050963	13872	21.27	8.18	+++	+++
chr14	75282349	75283348	380916	Lrch1	AK161894	16681	21.27	8.18	+++	+++
chr15	66723372	66724371	22402	Wisp1	NM_018865	918	21.27	8.18	+++	+++
chr4	8777207	8778206	320790	Chd7	BC034239	-6111	21.27	8.18	+++	+++
chr5	123795670	123796669	77045	Bel1a	NM_029850	1714	21.27	8.18	+++	+++
chr2	32029275	32030274	227723	Prrc2b	NM_001159634	-8868	20.92	9.09	+++	+++
chr15	58838074	58839073	211401	Mtss1	NM_144800	33579	20.81	8.75	+++	+++
chr10	79810712	79811711	66548	Adamts15	NR_104339	-19	20.80	9.33	+++	+++
chr11	118073353	118074352	19157	Cyth1	NM_001112700	204	20.80	9.33	+++	+++
chr11	6374478	6375477	19291	Purb	NM_011221	943	20.80	9.33	+++	+++
chr16	34936928	34937927	107589	Mylk	NM_139300	37101	20.80	9.33	+++	+++
chr2	26913618	26914617	66139	Tmem8c	NM_001159602	9271	20.80	9.33	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr4	128790021	128791020	384061	Fndc5	NM_027402	-23783	20.80	9.33	+++	+++
chr4	149147477	149148476	230917	Tmem201	NM_001284270	-35800	20.80	9.33	+++	+++
chr5	36055991	36056990	78558	Htra3	NM_001042615	-34060	20.80	9.33	+++	+++
chr7	28363155	28364154	11652	Akt2	NM_001110208	-12924	20.80	9.33	+++	+++
chr4	155207718	155208717	56036	Ccnl2	AK048244	21621	20.78	8.19	+++	+++
chr14	75283803	75284802	380916	Lrch1	AK161894	15227	20.77	8.45	+++	+++
chr15	31274125	31275124	67434	Ankrd33b	NM_026153	22890	20.50	7.35	+++	+++
chr1	75358383	75359382	13346	Des	NM_010043	1965	20.41	9.11	+++	+++
chr5	91946157	91947156	231440	Parm1	NM_145562	-69	20.29	8.75	+++	+++
chr11	95394822	95395821	104079	Nxph3	NM_130858	-19443	20.25	8.73	+++	+++
chr13	55671413	55672412	27261	Dok3	AK154750	-41996	20.25	8.73	+++	+++
chr18	32550691	32551690	30948	Bin1	NM_001083334	14321	20.25	8.73	+++	+++
chr2	164261385	164262384	66460	Sys1	AK032073	-24586	20.25	8.73	+++	+++
chr4	136068492	136069491	269593	Luzp1	NM_024452	-27279	20.25	8.73	+++	+++
chr4	46129280	46130279	21916	Tmod1	NM_021883	39753	20.25	8.73	+++	+++
chr7	149615885	149616884	21953	Tnni2	NM_009405	-11989	20.25	8.73	+++	+++
chr17	25676186	25677185	20681	Sox8	NM_011447	30946	20.24	8.44	+++	+++
chr14	55495572	55496571	13644	Efs	NM_010112	49554	20.22	7.91	+++	+++
chr15	75845225	75846224	105782	Scrib	NM_134089	47753	20.22	7.91	+++	+++
chr3	108086327	108087326	20661	Sort1	NM_001271599	-155	20.22	7.91	+++	+++
chr7	135078537	135079536	233908	Fus	NM_139149	-31956	20.22	7.91	+++	+++
chr1	183949816	183950815	13800	Enah	AK020248	-205	20.01	6.75	+++	+++
chr8	91170792	91171791	257632	Nod2	NM_145857	47	20.01	6.75	+++	+++
chr11	108285977	108286976	11818	ApoH	NM_013475	29867	19.71	8.44	+++	+++
chr16	92057681	92058680	53881	Slc5a3	NM_017391	-386	19.71	8.44	+++	+++
chr12	112943110	112944109	12709	Ckb	NM_021273	-33061	19.64	8.97	+++	+++
chr16	38368118	38369117	64082	Popdc2	NM_022318	6360	19.64	8.97	+++	+++
chr2	181193755	181194754	72147	Zbtb46	NM_028125	-124	19.64	8.97	+++	+++
chr2	25036010	25037009	227612	A830007P12Rik	NM_146115	15824	19.64	8.97	+++	+++
chr2	32438613	32439612	20448	St6galnac4	NM_001276425	-3485	19.64	8.97	+++	+++
chr3	101902833	101903832	12373	Casq2	NM_009814	12901	19.64	8.97	+++	+++
chr5	111127291	111128290	69663	Ddx51	NM_027156	45322	19.64	8.97	+++	+++
chr5	64550782	64551781	57915	Tbc1d1	NM_019636	-168	19.64	8.97	+++	+++
chr7	108598696	108599695	207728	Pde2a	NM_001243758	163	19.64	8.97	+++	+++
chr7	82600413	82601412	75547	Akap13	AK006382	160	19.64	8.97	+++	+++
chr10	5151210	5152209	64009	Syne1	NM_001079686	-124	19.52	7.13	+++	+++
chr13	34094197	34095196	19766	Ripk1	NM_009068	-46	19.52	7.13	+++	+++
chr2	25083753	25084752	227612	A830007P12Rik	NM_146115	-31920	19.52	7.13	+++	+++
chr6	137467888	137468887	13860	Eps8	NM_007945	26	19.52	7.13	+++	+++
chr8	90825202	90826201	11513	Adcy7	NM_001037724	7600	19.52	7.13	+++	+++
chr15	31355511	31356510	223455	March6	AK218077	43422	19.30	8.49	+++	+++
chr1	183958441	183959440	13800	Enah	AK020248	-8830	19.26	8.76	+++	+++
chr14	27927490	27928489	71704	Arhgef3	NM_027871	-95	19.19	7.65	+++	+++
chr1	137675150	137676149	21952	Tnni1	NM_021467	-20348	19.14	8.41	+++	+++
chr4	11599620	11600619	14579	Gem	NM_010276	-31474	19.14	8.41	+++	+++
chr1	136229226	136230225	68507	Ppfia4	NM_001144855	-221	19.09	6.56	+++	+++
chr14	70568887	70569886	268759	9930012K11Rik	NM_001112735	-10078	18.88	7.27	+++	+++
chr10	21643062	21644061	20393	Sgk1	AK043284	41573	18.83	8.52	+++	+++
chr14	35378516	35379515	24131	Ldb3	NM_001039074	22778	18.83	6.12	+++	+++
chr4	41449715	41450714	329828	AI464131	NM_001085515	-106	18.77	7.44	+++	+++
chr11	5737040	5738039	56012	Pgam2	NM_018870	-33741	18.65	8.42	+++	+++
chr17	32309817	32310816	18131	Notch3	NM_008716	-6520	18.65	8.42	+++	+++
chr18	36894613	36895612	12475	Cd14	NM_009841	-8805	18.65	8.42	+++	+++
chr2	60390619	60391618	18779	Pla2r1	NM_008867	200	18.63	7.87	+++	+++
chr18	32547218	32548217	30948	Bin1	NM_001083334	10848	18.62	8.12	+++	+++
chr8	9975710	9976709	319583	Lig4	NM_176953	114	18.62	8.12	+++	+++
chr11	11772749	11773748	13195	Ddc	NM_016672	17163	18.49	8.61	+++	+++
chr13	109004141	109005140	74559	Elov17	NM_029001	44	18.49	8.61	+++	+++
chr13	22036387	22037386	193452	Zfp184	AK036164	-76	18.49	8.61	+++	+++
chr14	34171411	34172410	239027	Arhgap22	NM_153800	38958	18.49	8.61	+++	+++
chr17	56292750	56293749	20359	Sema6b	NM_001130456	-13484	18.49	8.61	+++	+++
chr19	37248161	37249160	208922	Cpeb3	NM_001290826	1893	18.49	8.61	+++	+++
chr2	164256840	164257839	66460	Sys1	AK032073	-29131	18.49	8.61	+++	+++
chr4	148179357	148180356	69743	Cas21	AK144492	1357	18.49	8.61	+++	+++
chr4	45197149	45198148	666060	Frmpd1	NM_001081172	-129	18.49	8.61	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr6	125031583	125032582	54137	Acrbp	NM_001127340	33698	18.49	8.61	+++	+++
chr15	31304650	31305649	67434	Ankrd33b	NM_026153	-7636	18.32	8.51	+++	+++
chr1	93294921	93295920	55927	Hes6	AK146192	14379	18.17	7.39	+++	+++
chr11	87405069	87406068	18952	Sept4	NM_011129	10937	18.17	7.39	+++	+++
chr4	46107992	46108991	21916	Tmod1	NM_021883	18465	18.17	7.39	+++	+++
chr7	135381146	135382145	12862	Cox6a2	NM_009943	-31766	18.17	7.39	+++	+++
chr12	33638701	33639700	19027	Syp1	NM_198710	151	18.13	7.61	+++	+++
chr3	90340706	90341705	20193	S100a1	NM_011309	-22954	18.09	7.84	+++	+++
chr11	97920686	97921685	12295	Cacnb1	NM_145121	-37245	18.04	8.08	+++	+++
chr2	158084158	158085157	16803	Lbp	NM_008489	-47571	18.04	8.08	+++	+++
chr1	89072140	89073139	11449	Chrng	NM_009604	-29746	17.61	6.68	+++	+++
chr11	58795090	58796089	380698	Obscn	BC044882	47499	17.61	6.68	+++	+++
chr2	32023087	32024086	227723	Prrc2b	NM_001159634	-15056	17.61	6.68	+++	+++
chr4	132796577	132797576	53608	Map3k6	NM_016693	345	17.61	6.68	+++	+++
chr2	163222947	163223946	59091	Jph2	NM_021566	240	17.58	7.57	+++	+++
chr1	93248968	93249967	70788	Klhl30	NM_027551	1819	17.55	8.08	+++	+++
chr10	80376528	80377527	16907	Lmn2	NM_010722	3963	17.55	8.08	+++	+++
chr1	137936900	137937899	12292	Cacna1s	NM_001081023	-12078	17.36	8.25	+++	+++
chr1	183984839	183985838	13800	Enah	AK020248	-35228	17.36	8.25	+++	+++
chr14	41707624	41708623	328381	Sh2d4b	NM_027816	-1870	17.36	8.25	+++	+++
chr2	77009339	77010338	545428	Ccdc141	NM_001025576	-1147	17.36	8.25	+++	+++
chr3	97671463	97672462	83679	Pde4dip	NM_178080	273	17.36	8.25	+++	+++
chr6	29643616	29644615	232670	Tspan33	NM_001301407	-98	17.36	8.25	+++	+++
chr7	133604803	133605802	11937	Atp2a1	NM_007504	1285	17.36	8.25	+++	+++
chr7	19876196	19877195	20167	Rtn2	NM_001025364	249	17.36	8.25	+++	+++
chr8	125163581	125164580	12398	Cbfa2t3	NM_177289	37995	17.36	8.25	+++	+++
chr8	24168914	24169913	11733	Ank1	NM_031158	668	17.36	8.25	+++	+++
chr15	31338791	31339790	67434	Ankrd33b	NM_026153	-41777	17.31	8.23	+++	+++
chr8	97516128	97517127	14766	Gpr56	NM_018882	8330	17.29	7.42	+++	+++
chr10	80384484	80385483	16907	Lmn2	NM_010722	-3994	17.17	7.12	+++	+++
chr13	55566319	55567318	56320	Dbn1	NM_019813	22619	17.17	7.12	+++	+++
chr15	78008469	78009468	19293	Pvalb	NM_013645	25614	17.17	7.12	+++	+++
chr15	58861397	58862396	211401	Mtss1	NM_144800	10256	17.15	7.35	+++	+++
chr14	35175963	35176962	105450	Mmrn2	NM_153127	-12227	17.11	7.33	+++	+++
chr15	66722635	66723634	22402	Wisp1	NM_018865	181	17.11	5.78	+++	+++
chr1	133135318	133136317	56489	Ikbke	NM_019777	37708	17.03	7.54	+++	+++
chr3	95505735	95506734	229595	Adamtsl4	NM_001301705	-14395	17.02	8.06	+++	+++
chr18	32306271	32307270	19123	Proc	NM_001042767	-7547	17.00	7.78	+++	+++
chr2	155710773	155711772	56046	Uqc	NM_018888	44774	16.96	7.76	+++	+++
chr2	90922749	90923748	68427	Slc39a13	NM_001290765	-12777	16.96	7.76	+++	+++
chr5	136570334	136571333	54153	Rasa4	NM_133914	11049	16.96	7.76	+++	+++
chr8	26384466	26385465	320165	Tacc1	AK190590	-33593	16.93	6.78	+++	+++
chr12	102224340	102225339	68339	Ccdc88c	BC027658	-4273	16.67	6.46	+++	+++
chr14	70569802	70570801	268759	9930012K11Rik	NM_001112735	-10993	16.67	6.46	+++	+++
chr19	3999872	4000871	71670	Acy3	NM_027857	13712	16.67	6.46	+++	+++
chr2	26934365	26935364	66139	Tmem8c	NM_025376	-7184	16.67	6.46	+++	+++
chr8	96710162	96711161	17750	Mt2	NM_008630	14145	16.67	6.46	+++	+++
chr11	115873294	115874293	192897	Itgb4	AK140269	4999	16.49	7.50	+++	+++
chr6	49317345	49318344	231946	D330028D13Rik	NM_172727	108	16.46	7.75	+++	+++
chr11	114656363	114657362	72014	Btdb17	NM_028055	344	16.25	7.90	+++	+++
chr16	5159104	5160103	19041	Ppl	NM_008909	-27030	16.25	7.90	+++	+++
chr2	164277291	164278290	66460	Sys1	AK032073	-8680	16.25	7.90	+++	+++
chr3	101375105	101376104	11928	Atp1a1	NM_144900	32976	16.25	7.90	+++	+++
chr4	45283621	45284620	666060	Frmpd1	NM_001081172	41428	16.25	7.90	+++	+++
chr5	122963510	122964509	11938	Atp2a2	NM_001110140	-11776	16.25	7.90	+++	+++
chr5	136580986	136581985	54153	Rasa4	NM_133914	21701	16.25	7.90	+++	+++
chr9	108956626	108957625	235611	Plxnb1	NM_172775	-40824	16.25	7.90	+++	+++
chr11	109300806	109301805	104681	Slc16a6	NM_134038	33238	16.18	6.86	+++	+++
chr6	91106386	91107385	54563	Nup210	NM_018815	-40066	16.18	6.86	+++	+++
chr7	148041667	148042666	12869	Cox8b	NM_007751	44179	16.18	6.86	+++	+++
chr1	39535198	39536197	54610	Tbc1d8	NM_018775	-106	16.10	7.05	+++	+++
chr1	36364977	36365976	214855	Arid5a	NM_145996	900	16.00	7.24	+++	+++
chr10	79830528	79831527	66548	Adamtsl5	NR_104339	-19835	16.00	7.24	+++	+++
chr15	31248776	31249775	67434	Ankrd33b	NM_026153	48239	16.00	7.24	+++	+++
chr7	139547662	139548661	77590	Chst15	NM_029935	-39324	15.94	7.46	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr1	75389362	75390361	13346	Des	NM_010043	32944	15.92	7.71	+++	+++
chr1	75367656	75368655	13346	Des	NM_010043	11238	15.90	7.44	+++	+++
chr1	92871235	92872234	16978	Lrrfip1	NM_001111312	-23569	15.90	7.44	+++	+++
chr11	114691230	114692229	70355	Gprc5c	NM_001110337	-21116	15.90	7.44	+++	+++
chr16	34902103	34903102	107589	Mylk	NM_139300	2276	15.90	7.44	+++	+++
chr16	38369861	38370860	64082	Popdc2	NM_022318	8103	15.90	7.44	+++	+++
chr4	140960039	140961038	29818	Hspb7	NM_013868	-16155	15.90	7.44	+++	+++
chr5	145131493	145132492	66898	Baiap21l	NM_025833	-13012	15.90	7.44	+++	+++
chr7	31843804	31844803	18301	Fxyd5	NM_001287217	-16954	15.90	7.44	+++	+++
chr8	97571850	97572849	14766	Gpr56	NM_018882	46024	15.85	6.69	+++	+++
chr3	90390433	90391432	20199	Sl00a5	NM_011312	-21511	15.75	6.24	+++	+++
chr19	4282576	4283575	245857	Ssh3	NM_198113	-13904	15.54	6.99	+++	+++
chr5	36029396	36030395	78558	Htra3	NM_001042615	-7465	15.45	7.19	+++	+++
chr17	56322795	56323794	20359	Sema6b	NM_001130456	-43529	15.43	5.44	+++	+++
chr11	109286527	109287526	104681	Slc16a6	NM_134038	47517	15.43	5.17	+++	+++
chr9	20446359	20447358	23988	Pin1	NM_023371	-9715	15.41	6.48	+++	+++
chr3	54902597	54903596	320604	Ccdc169	NM_001290138	-38164	15.39	7.41	+++	+++
chr11	5695240	5696239	56012	Pgam2	NM_018870	8060	15.21	6.59	+++	+++
chr14	34044875	34045874	239027	Arhgap22	NM_153800	15367	15.21	6.59	+++	+++
chr14	7526817	75267816	380916	Lrch1	AK161894	32213	15.21	6.59	+++	+++
chr16	34806981	34807980	107589	Mylk	NM_139300	22446	15.21	6.59	+++	+++
chr7	133768646	133769645	171504	Apob48r	NM_138310	40625	15.21	6.59	+++	+++
chr7	150193653	150194652	27027	Tspan32	NM_001128082	2590	15.21	6.59	+++	+++
chr1	89142383	89143382	11449	Chrng	X03819	40234	15.16	7.54	+++	+++
chr11	114733990	114734989	70355	Gprc5c	NM_147217	21147	15.16	7.54	+++	+++
chr11	66894468	66895467	17883	Myh3	DQ021875	3133	15.16	7.54	+++	+++
chr11	72191515	72192514	276829	Smtnl2	NM_177776	33201	15.16	7.54	+++	+++
chr12	80129758	80130757	211945	Plekhh1	NM_181073	109	15.16	7.54	+++	+++
chr16	38360006	38361005	64082	Popdc2	NM_022318	-1753	15.16	7.54	+++	+++
chr4	119915009	119916008	329934	Foxo6	NM_194060	44358	15.16	7.54	+++	+++
chr4	8773053	8774052	320790	Chd7	BC034239	-10265	15.16	7.54	+++	+++
chr7	134234487	134235486	20737	Spn	NM_009259	46345	15.16	7.54	+++	+++
chr7	29688531	29689530	16858	Lgals7	NM_008496	39627	15.16	7.54	+++	+++
chr8	26366353	26367352	320165	Tacc1	AK190590	-15480	15.16	7.54	+++	+++
chr1	92869516	92870515	16978	Lrrfip1	NM_001111312	-25288	14.86	7.11	+++	+++
chr10	6979464	6980463	76142	Ppp1r14c	NM_133485	428	14.86	7.11	+++	+++
chr16	34860273	34861272	107589	Mylk	NM_139300	-39555	14.86	7.11	+++	+++
chr18	61123035	61124034	12322	Camk2a	NM_001286809	328	14.86	7.11	+++	+++
chr4	148288715	148289714	69743	Cas21	NM_027195	-17652	14.86	7.11	+++	+++
chr4	62121081	62122080	192120	Bspry	AK035588	-19520	14.86	7.11	+++	+++
chr5	35250474	35251473	403174	A930005I04Rik	NM_207277	-7590	14.86	7.11	+++	+++
chr7	139507216	139508215	77590	Chst15	NM_029935	679	14.86	7.11	+++	+++
chr8	74189261	74190260	16453	Jak3	NM_001190830	-10521	14.86	7.11	+++	+++
chr3	95754506	95755505	67220	Plekho1	NM_023320	44757	14.85	7.36	+++	+++
chr7	149660708	149661707	16985	Lsp1	NM_001271523	-1710	14.85	7.36	+++	+++
chr9	43886597	43887596	21838	Thy1	NM_009382	35631	14.84	6.01	+++	+++
chr17	26621619	26622618	19252	Dusp1	NM_013642	23299	14.67	5.59	+++	+++
chr10	127985749	127986748	13867	Erbp3	NM_010153	40309	14.64	5.01	+++	+++
chr15	101896289	101897288	209039	Tenc1	NM_153533	-34055	12.86	6.48	+++	+++
chr10	93986273	93987272	319880	Tmcc3	NM_172051	9172	12.83	6.47	+++	+++
chr11	5703229	5704228	56012	Pgam2	NM_018870	71	12.83	6.47	+++	+++
chr14	75321323	75322322	380916	Lrch1	AK161894	-22294	12.83	6.47	+++	+++
chr2	158083661	158084660	16803	Lbp	NM_008489	-48068	12.83	6.47	+++	+++
chr2	60553513	60554512	16420	Itgb6	NM_001159564	6648	12.83	6.47	+++	+++
chr7	88715944	88716943	26557	Homer2	NM_001164087	47348	12.83	6.47	+++	+++
chr9	21861815	21862814	12797	Cnn1	NM_009922	-41382	12.83	6.47	+++	+++
chr16	34879526	34880525	107589	Mylk	NM_139300	-20302	12.76	6.66	+++	+++
chr6	91089549	91090548	54563	Nup210	NM_018815	-23229	12.76	6.66	+++	+++
chr6	95048262	95049261	243574	Kbtbd8	NM_001008785	-19138	12.76	6.66	+++	+++
chr8	26321649	26322648	320165	Tacc1	AK046384	-10228	12.53	6.53	+++	+++
chr1	136140440	136141439	17928	Myog	NM_031189	-45641	12.40	5.80	+++	+++
chr1	155550435	155551434	19734	Rgs16	NM_011267	-36548	12.40	5.80	+++	+++
chr11	104438334	104439333	17896	Myl4	NM_010858	117	12.40	5.80	+++	+++
chr15	97557126	97558125	223864	Rapgef3	NM_144850	40472	12.40	5.80	+++	+++
chr17	15479693	15480692	13388	Dill1	NM_007865	32595	12.40	5.80	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr7	133709341	133710340	171504	Apob48r	NM_138310	-18681	12.40	5.80	+++	+++
chr11	11744055	11745054	13195	Ddc	AK050963	-21631	12.36	4.56	+++	+++
chr17	25707071	25708070	20681	Sox8	NM_011447	61	12.24	4.76	+++	+++
chr1	137761820	137762819	21956	Tnnt2	NM_001130176	24883	12.23	5.92	+++	+++
chr3	95471663	95472662	229595	Adamtsl4	NM_144899	19619	12.23	5.92	+++	+++
chr1	63580558	63581557	23792	Adam23	AY545641	-33365	12.22	5.35	+++	+++
chr10	41209583	41210582	53599	Cd164	NM_016898	-29223	12.22	5.35	+++	+++
chr16	14003998	14004997	67254	2900011O08Rik	NM_144518	17769	12.22	5.35	+++	+++
chr18	61081351	61082350	12322	Camk2a	NM_177407	-3435	12.22	5.35	+++	+++
chr4	149126157	149127156	230917	Tmem201	NM_001284270	-14480	12.22	5.35	+++	+++
chr9	59381384	59382383	73893	Tmem202	NM_178388	-8576	12.22	5.35	+++	+++
chr8	90778547	90779546	11513	Adcy7	NM_007406	-17255	12.04	6.03	+++	+++
chr12	104576215	104577214	65256	Asb2	NM_023049	-204	12.00	6.46	+++	+++
chr17	35607209	35608208	15007	H2-Q10	NM_010391	676	12.00	6.46	+++	+++
chr2	155787371	155788370	56046	Uqcc	NM_018888	-31825	12.00	6.46	+++	+++
chr2	26940138	26941137	66139	Tmem8c	NM_025376	-12957	12.00	6.46	+++	+++
chr2	6642409	6643408	14007	Celf2	NM_001160292	-250	12.00	6.46	+++	+++
chr5	37392034	37393033	22393	Wfs1	NM_011716	-12313	12.00	6.46	+++	+++
chr6	54655382	54656381	68235	Mturn	NM_001289741	24265	12.00	6.46	+++	+++
chr6	91066424	91067423	54563	Nup210	NM_018815	-104	12.00	6.46	+++	+++
chr9	41007600	41008599	72828	Ubash3b	NM_176860	-42523	12.00	6.46	+++	+++
chr12	85521645	85522644	238317	C130039O16Rik	NM_001163502	12821	11.92	6.18	+++	+++
chr6	121160290	121161289	24110	Usp18	NM_011909	-35134	11.85	6.14	+++	+++
chr8	126038246	126039245	72185	Dnndd1	NM_001170976	610	11.85	6.14	+++	+++
chr2	60535977	60536976	16420	Itgb6	NM_001159564	24184	11.76	5.48	+++	+++
chr5	37347573	37348572	22393	Wfs1	NM_011716	32149	11.70	6.28	+++	+++
chr17	15944000	15944999	68799	Rgmb	NM_178615	19051	11.67	5.83	+++	+++
chr2	155781730	155782729	56046	Uqcc	NM_018888	-26184	11.53	5.56	+++	+++
chr1	136217176	136218175	68507	Ppfia4	NM_001144855	11830	11.50	5.54	+++	+++
chr10	127647830	127648829	66701	Spryd4	NM_025716	521	11.50	5.54	+++	+++
chr15	58835383	58836382	211401	Mtss1	NM_144800	36270	11.50	5.54	+++	+++
chr17	35688501	35689500	386463	Cdsn	NM_001008424	-72	11.50	5.54	+++	+++
chr17	48576713	48577712	11811	Apobec2	NM_009694	-5160	11.50	5.54	+++	+++
chr19	34326477	34327476	11475	Acta2	NM_007392	2850	11.50	5.54	+++	+++
chr8	75107078	75108077	74166	Tmem38a	NM_144534	11577	11.50	5.54	+++	+++
chr18	23197404	23198403	319211	Nol4	NM_001161483	-740	11.48	4.59	+++	+++
chr15	7034700	7035699	16880	Lifr	NM_001113386	-44372	11.38	5.12	+++	+++
chr7	28345037	28346036	11652	Akt2	NM_001110208	-31042	11.31	5.64	+++	+++
chr1	195164209	195165208	16780	Lamb3	NM_001277928	36273	11.11	4.12	+++	+++
chr1	137946578	137947577	12292	Cacna1s	NM_001081023	-2400	11.11	5.73	+++	+++
chr1	182864752	182865751	208795	Tmem63a	NM_144794	-7397	11.00	6.10	+++	+++
chr11	78868472	78869471	16706	Ksr1	AK137532	4367	11.00	6.10	+++	+++
chr13	100271818	100272817	17755	Mtap1b	NM_008634	14240	11.00	6.10	+++	+++
chr13	46513184	46514183	666794	Rbm24	NM_001081425	16	11.00	6.10	+++	+++
chr13	46523969	46524968	666794	Rbm24	NM_001081425	10801	11.00	6.10	+++	+++
chr2	158142452	158143451	16803	Lbp	NM_008489	10724	11.00	6.10	+++	+++
chr2	168537879	168538878	11981	Atp9a	NM_015731	21366	11.00	6.10	+++	+++
chr5	32485180	32486179	665270	Plb1	NM_030072	-49401	11.00	6.10	+++	+++
chr7	147126036	147127035	76484	Kndc1	AK169316	17086	11.00	6.10	+++	+++
chr7	82599785	82600784	75547	Akap13	NM_029332	-135	11.00	6.10	+++	+++
chr9	50530062	50531061	12955	Cryab	NM_001289782	-28868	11.00	6.10	+++	+++
chr9	65245714	65246713	70784	Rasl12	NM_001033158	-81	11.00	6.10	+++	+++
chr16	57754893	57755892	12837	Col8a1	NM_007739	-543	10.89	5.82	+++	+++
chr17	26616183	26617182	19252	Dusp1	NM_013642	28735	10.89	5.82	+++	+++
chr17	36160934	36161933	100529082	Gm11127	NM_001199967	33883	10.89	5.82	+++	+++
chr4	9570348	9571347	65973	Asph	NM_001177854	17	10.89	5.82	+++	+++
chr9	65228520	65229519	70784	Rasl12	NM_001033158	-17275	10.89	5.82	+++	+++
chr11	11736514	11737513	13195	Ddc	AK050963	-14090	10.75	5.96	+++	+++
chr11	80321979	80322978	12569	Cdk5r1	NM_009871	31931	10.75	5.96	+++	+++
chr11	80303049	80304048	12569	Cdk5r1	NM_009871	13001	10.73	4.42	+++	+++
chr11	60851366	60852365	16515	Kcnj12	NM_001267593	15801	10.62	5.28	+++	+++
chr12	87046456	87047455	217721	Mfsd7c	NM_145447	-40533	10.62	5.28	+++	+++
chr19	60949983	60950982	14773	Grk5	NM_018869	-15169	10.61	4.63	+++	+++
chr5	114806292	114807291	231646	Myo1h	BC144870	-32	10.61	4.63	+++	+++
chr9	43092088	43093087	102644	Oaf	NM_178644	-44689	10.61	4.63	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr1	195172838	195173837	16780	Lamb3	NM_001277928	44902	10.56	4.90	+++	+++
chr15	73004335	73005334	239528	Eif2c2	NM_153178	10543	10.56	4.90	+++	+++
chr4	11618631	11619630	14579	Gem	NM_010276	-12463	10.35	5.73	+++	+++
chr6	30630088	30631087	109697	Cpa1	NM_025350	41368	10.35	5.73	+++	+++
chr11	104485736	104486735	17896	Myl4	NM_010858	47519	10.32	4.95	+++	+++
chr3	122988174	122989173	118449	Synpo2	NM_080451	-49607	10.19	5.43	+++	+++
chr12	33614391	33615390	19027	Sypl	NM_013635	-23919	10.02	5.74	+++	+++
chr14	61730987	61731986	50720	Sacs	NM_172809	-25807	10.02	5.74	+++	+++
chr16	90407891	90408890	26559	Hunk	NM_015755	21750	10.02	5.74	+++	+++
chr2	155728732	155729731	56046	Uqcc	NM_018888	26815	10.02	5.74	+++	+++
chr2	31950982	31951981	227721	Ppapdc3	NM_145521	312	10.02	5.74	+++	+++
chr6	71624474	71625473	52250	Reep1	NM_178608	-32880	10.02	5.74	+++	+++
chr7	19825932	19826931	20167	Rtn2	NM_013648	-41584	10.02	5.74	+++	+++
chr9	43071925	43072924	102644	Oaf	NM_178644	-24526	10.02	5.74	+++	+++
chr1	93346852	93347851	55927	Hes6	AK146192	-37553	9.95	5.50	+++	+++
chr6	29622562	29623561	232670	Tspan33	NM_001301407	-21152	9.95	5.50	+++	+++
chr7	52898483	52899482	74490	Mamstr	AK036109	1836	9.95	5.50	+++	+++
chr4	132750149	132751148	53608	Map3k6	NM_016693	-46084	9.85	5.24	+++	+++
chr11	107237161	107238160	71795	Pitpnc1	AK003844	-38710	9.76	5.01	+++	+++
chr11	11724145	11725144	13195	Ddc	AK050963	-1721	9.76	5.01	+++	+++
chr6	112223261	112224260	30937	Lmed1	NM_144799	10	9.75	4.68	+++	+++
chr13	32870447	32871446	238564	Mylk4	NM_001166030	2702	9.67	5.54	+++	+++
chr7	29792307	29793306	20190	Ryr1	AK042482	38896	9.54	5.07	+++	+++
chr11	101782434	101783433	17285	Meox1	NM_010791	-27266	9.41	5.39	+++	+++
chr17	35188205	35189204	17687	Msh5	NM_001146215	-5037	9.10	4.24	+++	+++
chr11	101719737	101720736	17285	Meox1	NM_010791	35432	9.06	5.38	+++	+++
chr4	41492690	41493689	329828	Al464131	NM_001085515	-43081	9.06	5.38	+++	+++
chr7	107157892	107158891	69121	Chrdl2	NM_001291320	-36	9.06	5.38	+++	+++
chr9	62013716	62014715	93683	Glce	NM_033320	-43800	9.06	5.38	+++	+++
chr13	55037183	55038182	107448	Unc5a	NM_153131	-13110	9.04	5.17	+++	+++
chr14	34091174	34092173	239027	Arhgap22	AK052541	-27630	9.04	5.17	+++	+++
chr5	64587791	64588790	57915	Tbc1d1	NM_001289514	-33305	9.04	5.17	+++	+++
chr7	135136668	135137667	233908	Fus	AK086587	21868	9.04	5.17	+++	+++
chr10	80275856	80276855	237403	Lingo3	NM_001013758	30429	8.97	4.46	+++	+++
chr11	11755422	11756421	13195	Ddc	AK050963	-32998	8.97	4.46	+++	+++
chr11	8031919	80312918	12569	Cdk5r1	NM_009871	21871	8.97	4.46	+++	+++
chr15	57558414	57559413	387609	Zhx2	NM_199449	32693	8.97	4.46	+++	+++
chr5	35251233	35252232	403174	A930005104Rik	NM_207277	-6831	8.97	4.46	+++	+++
chr11	5937022	5938021	12323	Camk2b	NM_001174054	28230	8.93	4.75	+++	+++
chr11	66888142	66889141	17883	Myh3	AY963798	-3160	8.93	4.75	+++	+++
chr15	63862451	63863450	223601	Fam49b	NM_144846	29060	8.93	4.75	+++	+++
chr13	100298147	100299146	17755	Mtap1b	NM_008634	-12090	8.59	3.91	+++	+++
chr13	100241778	100242777	17755	Mtap1b	NM_008634	-27856	8.43	4.82	+++	+++
chr16	17735978	17736977	224023	Klhl22	NM_145479	-23236	8.40	4.99	+++	+++
chr15	66717297	66718296	22402	Wisp1	NM_018865	-5158	8.38	4.05	+++	+++
chr6	54655048	54656047	68235	Mturn	NM_001289741	23931	8.38	4.05	+++	+++
chr1	88001115	88002114	15559	Htr2b	NM_008311	6077	8.15	4.85	+++	+++
chr6	28811976	28812975	192198	Lrrc4	NM_138682	-30729	8.15	4.85	+++	+++
chr11	114751308	114752307	70355	Gprc5c	NM_147217	38465	8.13	5.02	+++	+++
chr14	21469804	21470803	59011	Myoz1	NM_021508	5459	8.13	5.02	+++	+++
chr3	67296687	67297686	109222	Rarres1	NM_001164763	22259	8.13	5.02	+++	+++
chr6	97202520	97203519	320502	Lmod3	NM_001081157	-246	8.13	5.02	+++	+++
chr9	50560977	50561976	12955	Cryab	NM_009964	353	8.13	5.02	+++	+++
chr15	58923548	58924547	211401	Mtss1	NM_144800	-10467	8.11	4.48	+++	+++
chr6	112195279	112196278	30937	Lmed1	NM_144799	-27973	7.94	4.91	+++	+++
chr8	88024646	88025645	108682	Gpt2	NM_173866	8631	7.85	3.43	+++	+++
chr7	90043801	90044800	108797	Mex3b	NM_175366	28459	7.62	4.72	+++	+++
chr11	101754657	101755656	17285	Meox1	NM_010791	512	7.47	4.01	+++	+++
chr13	55670680	55671679	27261	Dok3	AK154750	-41263	7.47	4.01	+++	+++
chr4	108034889	108035888	67305	Gpx7	NM_024198	43930	7.47	4.01	+++	+++
chr9	40128131	40129130	235283	Gramd1b	NM_172768	25245	7.47	4.01	+++	+++
chr3	105628165	105629164	11542	Adora3	NM_027025	-45111	7.32	4.22	+++	+++
chr3	95110800	95111799	229589	Prune	NM_173347	-25302	7.32	4.22	+++	+++
chr4	108078907	108079906	67305	Gpx7	NM_024198	-89	7.31	4.37	+++	+++
chr1	195127771	195128770	16780	Lamb3	NM_008484	84	7.29	4.53	+++	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr3	107063368	107064367	229699	Slc16a4	NM_146136	-30342	7.29	4.53	+++	+++
chr11	102353531	102354530	217219	Fam171a2	NM_199200	-45054	7.24	4.67	+++	+++
chr3	96854750	96855749	14613	Gja5	NM_008121	18912	7.24	4.67	+++	+++
chr9	112109912	112110911	74100	Arpp21	NM_001177617	9354	7.24	4.67	+++	+++
chr1	195142906	195143905	16780	Lamb3	NM_001277928	14970	6.56	3.96	+++	+++
chr6	71673272	71674271	52250	Reep1	NM_178608	15919	6.46	4.20	+++	+++
chr17	26626719	26627718	19252	Dusp1	NM_013642	18199	6.37	4.31	+++	+++
chr2	113668658	113669657	20394	Scg5	NM_009162	91	6.37	4.31	+++	+++
chr7	125278403	125279402	267019	Rps15a	NM_170669	-19242	6.37	4.31	+++	+++
chr1	155593579	155594578	19734	Rgs16	NM_011267	6597	5.74	3.04	+++	+++
chr15	10881711	10882710	81799	C1qtnf3	NM_001204134	100	5.72	3.40	+++	+++
chr2	60555195	60556194	16420	Itgb6	NM_001159564	4966	5.66	3.88	+++	+++
chr2	4871735	4872734	68527	Ucma	NM_026754	-24933	5.54	3.95	+++	+++
chr9	20446678	20447677	23988	Pin1	NM_023371	-9396	5.22	2.65	+++	+++
chr3	137614631	137615630	26377	Dapp1	NM_011932	29383	5.12	3.43	+++	+++
chr11	11756577	11757576	13195	Ddc	NM_016672	33335	5.07	3.08	+++	+++
chr17	25667159	25668158	20681	Sox8	NM_011447	39973	4.28	2.72	+++	+++
chr1	158860979	158861978	78255	Ralgs2	NM_023884	7471	3.80	2.90	+++	+++
chr15	98593177	98594176	11842	Arf3	NM_007478	-128	249.69	50.69	+++	+
chr6	124695105	124696104	15170	Ptpn6	NM_001077705	-6878	124.84	34.09	+++	+
chr8	97361724	97362723	20295	Ccl17	NM_011332	27872	122.43	27.70	+++	+
chr14	73924231	73925230	214254	Nudt15	NM_172527	23319	111.83	29.42	+++	+
chr13	101262076	101263075	17940	Naip5	BC047215	-39757	70.07	18.46	+++	+
chr11	69208834	69209833	216848	Chd3	NM_146019	-26406	60.87	18.43	+++	+
chr6	101327591	101328590	55983	Pdzrn3	NM_018884	-200	58.87	19.12	+++	+
chr12	31268836	31269835	211986	Tmem18	NM_172049	29	57.52	16.37	+++	+
chr8	108673732	108674731	330836	Slc7a6	NM_178798	-18543	55.56	18.66	+++	+
chr12	3235317	3236316	74173	1700012B15Rik	NR_015551	27	55.03	15.56	+++	+
chr11	69230630	69231629	216848	Chd3	NM_146019	-48202	54.02	16.58	+++	+
chr12	4923634	4924633	217379	Ubxn2a	NM_145441	-9808	51.20	17.58	+++	+
chr11	70375860	70376859	18806	Pld2	NM_001302475	22587	47.23	15.08	+++	+
chr2	48804452	48805451	109241	Mbd5	NM_029924	-76	47.23	15.08	+++	+
chr5	105844072	105845071	433926	Lrrc8b	NM_001033550	-222	44.09	15.79	+++	+
chr5	124561788	124562787	56325	Abcb9	NM_019875	-16481	44.09	15.79	+++	+
chr8	49075550	49076549	52357	Wwc2	NM_133791	-145	43.31	15.36	+++	+
chr6	34730081	34731080	76223	Agbl3	NM_178630	150	42.60	14.59	+++	+
chr8	97380881	97381880	20295	Ccl17	NM_011332	47029	42.35	11.81	+++	+
chr2	156688082	156689081	228839	Tgfr2	AK157623	22770	41.32	15.07	+++	+
chr9	107514662	107515661	20347	Sema3b	NM_009153	-3590	39.35	13.27	+++	+
chr10	40068370	40069369	11702	Amd2	NM_007444	-46878	37.47	13.74	+++	+
chr11	4998994	4999993	78926	Gas2l1	NM_001190408	-34164	36.75	13.72	+++	+
chr11	70353040	70354039	18806	Pld2	NM_008876	-126	35.90	13.64	+++	+
chr9	66766818	66767817	235442	Rab8b	NM_173413	195	35.90	13.64	+++	+
chr8	112132076	112133075	64340	Dhx38	AK172917	-43075	34.80	11.34	+++	+
chr11	115699900	115700899	217325	Llgl2	NM_145438	15029	34.68	12.61	+++	+
chr11	120519894	120520893	77583	Notum	NM_175263	-1036	33.25	12.92	+++	+
chr17	36407809	36408808	79263	Trim39	NM_178281	70	32.99	12.77	+++	+
chr17	3331382	3332381	24001	Tiam2	NM_001122998	5310	31.39	11.56	+++	+
chr6	42214680	42215679	12366	Casp2	NM_007610	143	29.18	10.43	+++	+
chr16	21204175	21205174	13845	Ephb3	NM_010143	-193	28.88	11.57	+++	+
chr11	69179600	69180599	216848	Chd3	NM_146019	2829	28.70	10.49	+++	+
chr11	120494308	120495307	77583	Notum	NM_175263	24551	28.54	11.38	+++	+
chr2	118213890	118214889	381413	Gpr176	NM_201367	-15235	28.11	11.48	+++	+
chr11	69178208	69179207	216848	Chd3	NM_146019	4221	27.82	9.76	+++	+
chr4	80557465	80558464	52829	D4Bwg0951e	NM_026821	1376	27.38	9.82	+++	+
chr11	115675475	115676474	217325	Llgl2	NM_145438	-9397	27.29	10.70	+++	+
chr17	36388888	36389887	79263	Trim39	NM_178281	16624	26.85	11.13	+++	+
chr11	69224226	69225225	216848	Chd3	NM_146019	-41798	26.70	9.22	+++	+
chr11	69181799	69182798	216848	Chd3	NM_146019	630	25.61	10.77	+++	+
chr11	98926986	98927985	21973	Top2a	NM_011623	-41983	24.85	10.02	+++	+
chr1	178743787	178744786	545389	Cep170	NM_001099637	-7032	24.49	8.97	+++	+
chr10	14264766	14265765	215798	Gpr126	NM_001002268	-424	24.39	10.41	+++	+
chr5	114501830	114502829	68666	Svop	NM_026805	39060	24.39	10.41	+++	+
chr7	152100524	152101523	12443	Cend1	NM_007631	24807	24.39	10.41	+++	+
chr9	107528709	107529708	20347	Sema3b	NM_009153	-17637	24.09	9.32	+++	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr15	98637914	98638913	11842	Arf3	NM_007478	-44865	23.68	9.70	+++	+
chr9	107521524	107522523	20347	Sema3b	NM_009153	-10452	23.68	9.70	+++	+
chr11	69170836	69171835	216848	Chd3	BC030435	-9224	23.40	8.70	+++	+
chr9	121799376	121800375	59289	Ackr2	NM_001276719	-7597	23.18	10.05	+++	+
chr4	47069730	47070729	230145	Galnt12	NM_172693	-34595	22.76	9.81	+++	+
chr9	123168561	123169560	72309	Tmem158	NM_001002267	847	22.33	8.44	+++	+
chr2	35904234	35905233	75495	Morn5	NM_029309	-259	21.93	9.06	+++	+
chr2	31114070	31115069	14299	Nes1	NM_019681	13128	20.80	9.33	+++	+
chr15	96560028	96561027	67760	Slc38a2	BC027259	-30399	20.34	7.73	+++	+
chr11	120508448	120509447	77583	Notum	NM_175263	10411	19.64	8.97	+++	+
chr12	4913737	4914736	217379	Ubxn2a	NM_145441	90	19.64	8.97	+++	+
chr5	139884967	139885966	76854	Gpr30	NM_029771	-13666	19.64	8.97	+++	+
chr16	17275914	17276913	224019	Tmem191c	NM_177473	22	19.14	8.41	+++	+
chr4	107475340	107476339	16975	Lrp8	NM_001080926	977	17.54	7.81	+++	+
chr2	118198130	118199129	381413	Gpr176	NM_201367	526	17.03	7.54	+++	+
chr5	139874080	139875079	76854	Gpr30	NM_029771	-24553	17.02	8.06	+++	+
chr10	40021820	40022819	11702	Amd2	NM_007444	-328	16.67	6.46	+++	+
chr11	115674904	115675903	217325	Llgl2	NM_145438	-9968	16.67	6.46	+++	+
chr11	4915395	4916394	78926	Gas2l1	NM_030228	49203	16.55	7.28	+++	+
chr14	69884803	69885802	67712	Slc25a37	AK046008	17858	16.25	7.90	+++	+
chr5	139856115	139857114	76854	Gpr30	NM_029771	-42518	15.90	7.44	+++	+
chr9	36604233	36605232	235180	Fez1	NM_183171	-35536	15.52	5.78	+++	+
chr4	56863277	56864276	230233	Ikbkap	NM_026079	-48574	15.16	7.54	+++	+
chr9	34750221	34751220	67703	Kirrel3	AK122566	31353	15.16	7.54	+++	+
chr2	71432194	71433193	13392	Dlx2	NM_010054	-47883	12.00	6.46	+++	+
chr11	120508758	120509757	77583	Notum	NM_175263	10101	11.85	6.14	+++	+
chr2	3600275	3601274	66540	Fam107b	NM_025626	-29955	11.50	5.54	+++	+
chr6	108785548	108786547	192193	Edem1	NM_138677	7414	11.50	5.54	+++	+
chr17	3442374	3443373	24001	Tiam2	NM_011878	-39441	10.62	5.28	+++	+
chr6	108786050	108787049	192193	Edem1	NM_138677	7916	9.98	5.51	+++	+
chr10	18150351	18151350	215819	Nhs1	NM_173390	23371	9.76	5.01	+++	+
chr1	72871002	72872001	16008	Igfbp2	NM_008342	426	9.75	4.68	+++	+
chr5	137718518	137719517	22051	Trip6	NM_011639	36452	9.30	5.13	+++	+
chr2	25174255	25175254	227620	Uap1l1	AK131159	46392	9.06	5.19	+++	+
chr11	4916247	4917246	78926	Gas2l1	NM_030228	48351	8.21	4.23	+++	+
chr4	80510168	80511167	52829	D4Bwg0951e	NM_026821	-45922	8.11	4.48	+++	+
chr6	86721119	86722118	23885	Gmcl1	NM_011818	-38247	7.74	4.79	+++	+
chr1	40339770	40340769	16177	Il1r1	NM_001123382	16840	7.32	4.22	+++	+
chr9	110634926	110635925	19228	Pth1r	NM_001083936	9689	6.99	3.66	+++	+
chr5	89358400	89359399	54403	Slc4a4	NM_018760	42616	4.44	3.17	+++	+
chr13	63916044	63917043	76251	0610007P08Rik	NM_001013608	-84	221.35	50.86	+	+++
chr9	71980445	71981444	21406	Tcf12	NM_011544	-21319	216.93	51.80	+	+++
chr4	151412914	151413913	74035	Nol9	NM_028727	-22	199.06	48.81	+	+++
chr11	84683859	84684858	70325	Pigw	NM_027388	9322	154.84	40.19	+	+++
chr4	132478248	132479247	230789	Fam76a	NM_145553	-282	146.16	32.97	+	+++
chr10	8238295	8239294	338362	Ust	NM_177387	-172	138.40	30.33	+	+++
chr15	38447579	38448578	54375	Azin1	NM_018745	943	131.87	23.91	+	+++
chr3	105489911	105490910	109050	6530418L21Rik	NM_175398	-17106	131.85	33.30	+	+++
chr6	40420865	40421864	243780	E330009J07Rik	NM_175528	-35233	128.09	29.87	+	+++
chr6	83004091	83005090	16950	Lox13	NM_013586	20374	123.12	33.73	+	+++
chr3	94778247	94779246	20341	Selenbp1	NM_009150	41743	118.43	30.71	+	+++
chr2	30296927	30297926	12908	Crat	NM_007760	-26136	109.94	25.59	+	+++
chr3	144382967	144383966	54673	Sh3glb1	NM_001282037	-168	100.07	28.50	+	+++
chr10	126949582	126950581	237611	Stac3	NM_177707	11310	99.46	28.71	+	+++
chr9	49606616	49607615	17967	Ncam1	NM_001081445	59	98.23	25.05	+	+++
chr2	26758041	26759040	20933	Med22	NM_001033908	7622	89.35	24.89	+	+++
chr19	4125344	4126343	58988	Rps6kb2	NM_021485	37402	84.67	23.92	+	+++
chr10	126702878	126703877	16572	Kif5a	NM_001039000	-2959	84.48	22.43	+	+++
chr13	46764265	46765264	97863	C78339	NM_001033192	-126	78.52	22.63	+	+++
chr1	194955999	194956998	54139	Irf6	NM_016851	-22807	75.69	22.57	+	+++
chr16	14705481	14706480	20583	Snai2	NM_011415	30	74.07	21.72	+	+++
chr11	101633449	101634448	18612	Etv4	NM_008815	11713	74.04	21.24	+	+++
chr11	29273096	29274095	108686	Ccdc88a	NM_176841	-179	73.48	15.81	+	+++
chr6	113255996	113256995	18294	Ogg1	AK167456	-20474	71.99	18.20	+	+++
chr9	95411857	95412856	67958	2610101N10Rik	NM_026476	59	69.46	20.69	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr8	124473990	124474989	20539	Slc7a5	NM_011404	-42904	69.10	21.89	+	+++
chr15	85709172	85710171	29870	Gtse1	NM_013882	19297	68.78	18.25	+	+++
chr17	75864482	75865481	240168	Rasgrp3	NM_207246	105	66.04	21.17	+	+++
chr16	23107070	23108069	13682	Eif4a2	NM_001123037	19	65.89	17.67	+	+++
chr15	99532051	99533050	11419	Accn2	NM_009597	9750	64.52	20.81	+	+++
chr15	76341880	76342879	30840	Fbxl6	AK040458	26785	63.58	15.59	+	+++
chr13	114408359	114409358	56534	Hspb3	NM_019960	45026	63.42	13.26	+	+++
chr1	36613981	36614980	78321	Ankrd23	BC022973	-18278	62.72	16.23	+	+++
chr10	5958975	5959974	83397	Akap12	NM_031185	39299	62.44	18.39	+	+++
chr19	5444625	5445624	14283	Fos11	NM_010235	-2573	60.41	16.62	+	+++
chr15	74539385	74540384	57277	Slurp1	NM_020519	18572	59.75	15.95	+	+++
chr15	79963008	79964007	17309	Mgat3	NM_001795	-40643	59.60	17.79	+	+++
chr10	116457832	116458831	12461	Cct2	NM_007636	42539	59.21	18.43	+	+++
chr12	70472541	70473540	66244	Sdcccag1	NM_025441	-14888	59.16	19.28	+	+++
chr4	63707916	63708915	21923	Tnc	NM_011607	-367	58.20	17.49	+	+++
chr15	27524409	27525408	11732	Ank	NM_020332	23708	58.11	15.91	+	+++
chr8	82162733	82163732	73945	Otud4	NM_001081164	-342	57.24	14.53	+	+++
chr2	91803369	91804368	104418	Dgkz	NM_138306	-149	56.35	17.78	+	+++
chr12	33832031	33833030	320046	F730043M19Rik	AK089514	-69	55.56	18.66	+	+++
chr7	53608281	53609280	17927	Myod1	NM_010866	-23063	55.56	18.66	+	+++
chr8	122232774	122233773	67866	Wfdc1	NM_023395	43010	55.56	18.66	+	+++
chr14	41768887	41769886	52588	Tspan14	NM_145928	-7889	55.46	18.60	+	+++
chr15	74550997	74551996	57277	Slurp1	NM_020519	6960	55.02	17.50	+	+++
chr15	61818754	61819753	17869	Myc	NM_001177354	278	54.93	15.88	+	+++
chr3	127540040	127541039	211550	Tifa	NM_145133	46242	54.93	17.46	+	+++
chr14	60869837	60870836	71844	Nupl1	NM_170591	-122	54.76	18.22	+	+++
chr12	60112557	60113556	20334	Sec23a	NM_009147	-53	54.61	17.71	+	+++
chr10	79533653	79534652	20869	Stk11	NR_126043	-44395	54.60	14.03	+	+++
chr3	28633270	28634269	208691	Eif5a2	NM_177586	-46463	53.31	17.87	+	+++
chr9	86360227	86361226	70348	Ube2cbp	NM_027394	-2204	53.31	17.87	+	+++
chr10	79611854	79612853	20869	Stk11	NM_011492	33074	52.65	16.28	+	+++
chr10	81014013	81015012	114606	Tle6	NM_053254	45578	52.11	16.81	+	+++
chr11	119128782	119129781	207592	Tbc1d16	NM_172443	-39469	52.11	16.81	+	+++
chr16	32877261	32878260	69823	Fyttl1	NM_001159349	-109	51.87	17.52	+	+++
chr14	32064095	32065094	21924	Tnnc1	NM_009393	43098	51.20	17.58	+	+++
chr4	53452574	53453573	100434	Slc44a1	NM_001159633	-211	51.20	17.58	+	+++
chr8	26830018	26831017	71910	Ppapdc1b	NM_028000	-2	51.20	17.58	+	+++
chr9	71959110	71960109	21406	Tcf12	NM_011544	17	51.20	17.58	+	+++
chr1	95202019	95203018	269224	Pask	NM_080850	36847	50.85	13.37	+	+++
chr13	101500406	101501405	26886	Cenph	NM_021886	44949	50.72	16.49	+	+++
chr9	121627651	121628650	18087	Nktr	NM_010918	-148	50.61	16.84	+	+++
chr10	79623838	79624837	20869	Stk11	NM_011492	45058	50.21	15.44	+	+++
chr17	35977750	35978749	15937	Ier3	NM_133662	19593	50.21	15.44	+	+++
chr8	75877144	75878143	16795	Large	NM_010687	-1189	50.21	15.44	+	+++
chr15	78869074	78870073	26912	Gcat	NM_001161712	8271	50.00	16.52	+	+++
chr16	10366245	10367244	70426	Tekt5	NM_001099275	28797	49.94	16.90	+	+++
chr12	81890970	81891969	217684	4933426M11Rik	NM_178682	-76	49.92	15.68	+	+++
chr10	79627415	79628414	20869	Stk11	NM_011492	48635	49.78	14.51	+	+++
chr11	118226168	118227167	58251	BC100451	NM_021440	-22812	49.78	14.51	+	+++
chr15	57972256	57973255	76773	Wdyhv1	NM_029734	-235	49.78	14.51	+	+++
chr10	128328505	128329504	19682	Rdh5	NM_134006	27349	49.76	17.23	+	+++
chr15	87454161	87455160	106014	Fam19a5	NM_134096	-999	49.76	17.23	+	+++
chr14	60883435	60884434	71844	Nupl1	NM_170591	-13720	49.61	13.14	+	+++
chr11	94851772	94852771	20391	Sgca	NM_009161	-14631	49.42	16.21	+	+++
chr11	94789850	94790849	20391	Sgca	NM_001136080	44443	49.33	16.16	+	+++
chr15	93105219	93106218	223827	Gxylt1	NM_001033275	-204	48.89	15.54	+	+++
chr10	128346378	128347377	19682	Rdh5	NM_134006	9476	48.87	15.15	+	+++
chr10	27337066	27338065	16773	Lama2	AK042011	-818	47.95	15.84	+	+++
chr3	137806015	137807014	17777	Mttp	NM_001163457	-163	47.95	15.84	+	+++
chr10	50312010	50313009	77987	Ascc3	NM_198007	36	47.81	16.17	+	+++
chr3	143867625	143868624	16911	Lmo4	NM_010723	95	47.61	16.47	+	+++
chr4	144829033	144830032	21938	Tnfrsf1b	NM_011610	7241	47.56	11.76	+	+++
chr15	94419833	94420832	320709	Tmem117	AK034024	-39283	47.54	14.86	+	+++
chr17	74694369	74695368	76890	Memo1	NM_133771	-666	47.16	12.70	+	+++
chr19	10067198	10068197	24115	Best1	NM_011913	8426	47.16	12.70	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr1	180457646	180458645	269152	Kif26b	NM_001161665	-1110	46.91	16.51	+	+++
chr17	12511228	12512227	26407	Map3k4	NM_011948	-202	46.91	16.51	+	+++
chr2	143835726	143836725	81910	Rrbp1	AF273691	774	46.91	16.51	+	+++
chr11	94850683	94851682	20391	Sgca	NM_009161	-13542	46.66	15.56	+	+++
chr19	5487529	5488528	75555	1700020D05Rik	NM_023781	15759	45.95	12.48	+	+++
chr15	103102944	103103943	12419	Cbx5	NM_001076789	-33197	45.54	13.21	+	+++
chr11	74462485	74463484	74148	1300001I01Rik	NM_001081158	-12	45.50	16.15	+	+++
chr17	32029325	32030324	17691	Sik1	NM_010831	-37088	45.50	16.15	+	+++
chr7	104099463	104100462	244141	Nars2	NM_153591	-74	45.50	16.15	+	+++
chr9	35007030	35008029	117149	Tirap	NM_054096	21	45.50	16.15	+	+++
chr10	127962289	127963288	216459	Myl6b	NM_172259	-27048	45.22	15.19	+	+++
chr10	80637381	80638380	69564	Itgb1bp3	NM_027120	26902	44.77	14.57	+	+++
chr17	24551028	24552027	13177	Dci	NM_010023	-12100	44.64	13.45	+	+++
chr8	74134475	74135474	26457	Slc27a1	NM_011977	30561	44.64	13.45	+	+++
chr1	74432858	74433857	18173	Slc11a1	NM_013612	11582	44.09	15.79	+	+++
chr5	88993461	88994460	52822	Rufy3	NM_027530	-104	44.09	15.79	+	+++
chr11	88725531	88726530	11640	Akap1	NM_001042541	-131	43.96	13.81	+	+++
chr1	137595572	137596571	n/a	Nav1	AK158038	-11390	43.87	14.87	+	+++
chr13	43493220	43494219	68346	Sirt5	NM_178848	27633	43.87	14.87	+	+++
chr4	151382419	151383418	110326	Tas1r1	NM_031867	29681	43.38	13.19	+	+++
chr5	31251694	31252693	57742	Abhd1	NR_003522	-245	43.38	13.19	+	+++
chr19	58528462	58529461	14585	Gfra1	NM_010279	123	43.13	14.87	+	+++
chr15	85641479	85642478	29870	Gtse1	NM_001168672	-48158	42.97	15.17	+	+++
chr8	97855714	97856713	17388	Mmp15	NM_008609	-20023	42.76	9.07	+	+++
chr4	151453726	151454725	74035	Nol9	NM_028727	40791	42.53	14.55	+	+++
chr19	6276184	6277183	240505	Cdc42bpg	NM_001033342	-29773	42.35	11.81	+	+++
chr15	102109500	102110499	105988	Esp11	NM_001014976	-16724	41.80	13.10	+	+++
chr15	38400171	38401170	54375	Azin1	NM_018745	48351	41.40	13.25	+	+++
chr14	32019441	32020440	21924	Tnnc1	NM_009393	-1557	41.32	15.07	+	+++
chr3	58380402	58381401	69227	2810407C02Rik	NM_001040396	323	41.32	15.07	+	+++
chr4	151447428	151448427	74035	Nol9	NM_028727	34493	41.32	15.07	+	+++
chr11	29593209	29594208	68585	Rtn4	NM_194051	-65	41.20	14.22	+	+++
chr10	80164512	80165511	71564	Izumo4	NM_027829	-426	41.17	11.58	+	+++
chr15	27395776	27396775	11732	Ank	NM_020332	-156	41.17	11.58	+	+++
chr6	106749651	106750650	58799	Crbn	NM_175357	-83	41.17	11.58	+	+++
chr5	67009654	67010653	11787	Apb2	NM_001201414	-98	40.96	14.48	+	+++
chr11	97627388	97628387	16796	Laspl	NM_010688	-33098	40.00	11.36	+	+++
chr12	110030735	110031734	214663	Slc25a29	NM_181328	42852	40.00	11.36	+	+++
chr17	71964988	71965987	68789	Trmt61b	AK004173	-17387	39.95	14.71	+	+++
chr19	46592727	46593726	93679	Trim8	NM_053100	17090	39.95	14.71	+	+++
chr8	74119552	74120551	26457	Slc27a1	NM_011977	15638	39.95	14.71	+	+++
chr3	151928308	151929307	68810	Nexn	NM_199465	477	39.94	13.94	+	+++
chr1	196802080	196803079	12490	Cd34	NM_133654	37566	39.87	13.90	+	+++
chr6	34827681	34828680	78412	3110062M04Rik	NM_001135611	-116	39.87	13.90	+	+++
chr2	80420927	80421926	50884	Nckap1	NM_001290745	-88	39.35	13.27	+	+++
chr2	91846849	91847848	26427	Creb3l1	NM_011957	16979	39.12	13.87	+	+++
chr15	79921141	79922140	20972	Syng1	NM_009303	-123	38.90	14.14	+	+++
chr4	151444400	151445399	74035	Nol9	NM_028727	31465	38.88	12.68	+	+++
chr4	48292125	48293124	76299	Erp44	NM_029572	-164	38.63	13.61	+	+++
chr15	76352239	76353238	30840	Fbxl6	AK040458	16426	38.59	14.35	+	+++
chr5	135964506	135965505	215114	Hip1	NM_146001	-30894	38.59	14.35	+	+++
chr7	106591397	106592396	233552	Gdpd5	NM_201352	-5452	38.59	14.35	+	+++
chr8	125374221	125375220	12555	Cdh15	NM_007662	2448	38.59	14.35	+	+++
chr13	21622420	21623419	93681	Zfp192	NM_139141	61	38.56	13.58	+	+++
chr16	55973477	55974476	66391	2310061J03Rik	NR_027965	754	38.56	13.58	+	+++
chr19	10762859	10763858	58803	Pga5	NM_021453	-10798	38.56	13.58	+	+++
chr10	127935295	127936294	216459	Myl6b	NM_172259	-54	38.29	13.81	+	+++
chr12	15823329	15824328	217410	Trib2	NM_144551	-238	38.29	13.81	+	+++
chr13	120197446	120198445	18115	Nnt	AK168736	-1	37.68	10.92	+	+++
chr15	57907380	57908379	70472	Atad2	BC032211	22452	37.63	12.40	+	+++
chr9	108468788	108469787	23918	Impdh2	NM_011830	6457	37.26	13.25	+	+++
chr17	87361865	87362864	104215	Rhoq	NM_145491	-86	37.24	14.00	+	+++
chr19	46575461	46576460	93679	Trim8	NM_053100	-177	37.24	14.00	+	+++
chr4	128964040	128965039	68828	Sync	NM_023485	-325	37.24	14.00	+	+++
chr9	90057148	90058147	108153	Adamts7	BC076575	-168	37.24	14.00	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr15	103002608	103003607	12419	Cbx5	NM_001110216	42676	37.21	12.53	+	+++
chr4	133842724	133843723	433766	Trim63	NM_001039048	-27811	37.20	11.87	+	+++
chr17	27702277	27703276	106672	Al413582	NM_001002895	-105	36.81	12.66	+	+++
chr11	119804057	119805056	108100	Baiap2	NM_001037755	152	36.75	13.72	+	+++
chr1	137584322	137585321	n/a	Nav1	AK158038	-140	36.64	13.67	+	+++
chr8	59966249	59967248	97165	Hmgb2	NM_008252	-23891	36.64	13.67	+	+++
chr3	105516899	105517898	109050	6530418L21Rik	NM_175398	9883	36.41	12.81	+	+++
chr1	95238978	95239977	269224	Pask	NM_008050	-113	35.99	11.61	+	+++
chr5	33580245	33581244	100689	Spon2	NM_133903	-19858	35.96	12.93	+	+++
chr11	116704462	116705461	67622	Mxra7	NM_026280	-15602	35.90	13.64	+	+++
chr3	7366028	7367027	18767	Pkia	NM_008862	-76	35.90	13.64	+	+++
chr6	34267074	34268073	11677	Akr1b3	NM_009658	-85	35.90	13.64	+	+++
chr7	53596608	53597607	17927	Myod1	NM_010866	-34736	35.90	13.64	+	+++
chr18	35090965	35091964	15526	Hspa9	NM_010481	22541	35.86	13.24	+	+++
chr6	42299030	42300029	13835	Epha1	AK017662	23738	35.81	11.22	+	+++
chr4	117508719	117509718	14664	Slc6a9	NM_008135	1357	35.32	13.32	+	+++
chr11	101645865	101646864	18612	Etv4	AK158497	-203	35.17	9.64	+	+++
chr11	116714480	116715479	67622	Mxra7	NM_026280	-25620	35.14	12.50	+	+++
chr18	9449866	9450865	67974	Ccny	NM_026484	-218	35.14	12.50	+	+++
chr15	78858398	78859397	26912	Geat	NM_001161712	-2406	35.03	13.16	+	+++
chr2	91852656	91853655	26427	Creb3l1	NM_011957	11172	34.87	11.38	+	+++
chr10	82161688	82162687	74782	Glt8d2	NM_029102	-8793	34.75	12.64	+	+++
chr1	78533764	78534763	23874	Farsb	NM_001278075	-48792	34.68	12.61	+	+++
chr4	15498266	15499265	320541	Slc35e2	NM_177186	22714	34.68	12.61	+	+++
chr11	69360056	69361055	327954	Dnahc2	NM_001081330	2055	34.57	13.28	+	+++
chr19	5490188	5491187	75555	1700020D05Rik	NM_023781	13100	34.57	13.28	+	+++
chr2	165817626	165818625	17979	Ncoa3	NM_008679	-11	34.57	13.28	+	+++
chr4	132439923	132440922	230789	Fam76a	NM_145553	38044	34.57	13.28	+	+++
chr5	121834906	121835905	231713	Naa25	AK052060	-12585	34.57	13.28	+	+++
chr8	71707701	71708700	211134	Lzts1	NM_199364	-43349	34.57	13.28	+	+++
chr9	86358013	86359012	70348	Ube2cbp	NM_027394	11	34.57	13.28	+	+++
chr9	90096036	90097035	108153	Adamts7	NM_001003911	27227	34.57	13.28	+	+++
chr1	157080798	157081797	n/a	BC034090	AK078517	-1428	34.40	11.46	+	+++
chr13	51943703	51944702	23882	Gadd45g	NM_011817	2160	34.31	12.41	+	+++
chr2	29724152	29725151	99151	Cercam	NM_207298	-362	34.30	12.06	+	+++
chr13	98010595	98011594	15212	Hexb	NM_010422	-42783	34.28	12.75	+	+++
chr15	79663773	79664772	494448	Cbx6	NM_028763	491	34.28	12.75	+	+++
chr10	80568226	80569225	50492	Thop1	NM_022653	35899	34.26	10.25	+	+++
chr10	128359738	128360737	19682	Rdh5	NM_134006	-3885	34.14	12.32	+	+++
chr1	36619798	36620797	78321	Adamts23	BC022973	-24095	34.09	9.45	+	+++
chr11	115235127	115236126	237988	Cdr2l	NM_001080929	-7603	33.95	11.55	+	+++
chr10	128375228	128376227	19682	Rdh5	NM_134006	-19375	33.68	11.11	+	+++
chr5	125955618	125956617	78894	Aacs	NM_030210	-125	33.61	11.08	+	+++
chr9	49604923	49605922	17967	Ncam1	NM_001081445	1752	33.61	11.08	+	+++
chr15	79989473	79990472	17309	Mgat3	NM_010795	-14178	33.41	12.28	+	+++
chr3	14578263	14579262	13559	E2f5	NM_007892	93	33.41	12.28	+	+++
chr11	118946662	118947661	30951	Cbx8	NM_013926	-44935	33.25	12.92	+	+++
chr17	35966336	35967335	15937	Ier3	NM_133662	8179	33.25	12.92	+	+++
chr2	35960359	35961358	16874	Lhx6	NM_008500	70	33.25	12.92	+	+++
chr4	135730302	135731301	242705	E2f2	NM_177733	2494	33.25	12.92	+	+++
chr8	46970198	46971197	53318	Pdlim3	NM_016798	-141	33.25	12.92	+	+++
chr9	123021759	123022758	21922	Clec3b	NM_011606	-37805	33.25	12.92	+	+++
chr8	86752450	86753449	67873	Mri1	NM_026423	28274	33.14	10.02	+	+++
chr17	27261749	27262748	271424	Ip6k3	NM_173027	42461	33.08	12.46	+	+++
chr19	5529014	5530013	75555	1700020D05Rik	NM_023781	-25727	32.74	11.27	+	+++
chr12	25393007	25394006	20135	Rrm2	NM_009104	389	32.63	11.87	+	+++
chr13	104968563	104969562	66975	2410002O22Rik	NM_025879	-517	32.43	12.47	+	+++
chr2	109757360	109758359	107515	Lgr4	NM_172671	57	32.43	10.81	+	+++
chr4	149328695	149329694	74646	Spsb1	NM_029035	-80	32.43	10.81	+	+++
chr8	86733976	86734975	67873	Mri1	NM_026423	46748	32.43	8.05	+	+++
chr11	118188504	118189503	58251	BC100451	NM_021440	14845	32.15	11.96	+	+++
chr18	60779001	60780000	104027	Synpo	NM_001109975	4459	32.15	11.96	+	+++
chr19	42109547	42110546	56642	Ankrd2	NM_020033	-481	32.15	11.96	+	+++
chr9	119892453	119893452	22437	Xirp1	NM_011724	39764	32.15	11.96	+	+++
chr9	7763593	7764592	71929	Tmem123	AK202070	17	32.15	11.96	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr4	135695828	135696827	242705	E2f2	NM_177733	-31981	32.03	9.80	+	+++
chr3	58218121	58219120	72033	Tsc22d2	NM_001081229	-990	31.96	9.06	+	+++
chr10	128132862	128133861	19344	Rab5b	NM_177411	-38	31.95	12.56	+	+++
chr12	114175883	114176882	16450	Jag2	NM_010588	-8677	31.95	12.56	+	+++
chr5	28397250	28398249	231070	Insig1	NM_153526	-202	31.95	12.56	+	+++
chr8	125375296	125376295	12555	Cdh15	NM_007662	3523	31.95	12.56	+	+++
chr9	58046923	58047922	26968	Islr	NM_012043	-40395	31.95	12.56	+	+++
chr4	154975385	154976384	320541	Slc35e2	NM_177186	-168	31.83	11.46	+	+++
chr4	33118126	33119125	52187	AK081204	AK081204	10992	31.83	11.46	+	+++
chr1	172585392	172586391	320078	Olfml2b	NM_177068	11230	31.82	10.52	+	+++
chr2	91787419	91788418	104418	Dgkz	NM_001166597	-1376	31.45	7.90	+	+++
chr11	121065374	121066373	67608	Narf	NM_026272	-32676	31.33	10.58	+	+++
chr2	92871774	92872773	100042784	Prdm11	NM_001177536	14028	31.08	11.07	+	+++
chr5	138186620	138187619	243300	6430598A04Rik	NM_175521	-4285	30.92	9.58	+	+++
chr11	119852299	119853298	11302	AatK	AK147239	25674	30.90	11.64	+	+++
chr11	74369237	74370236	380711	Rap1gap2	NM_001015046	33924	30.90	11.64	+	+++
chr17	14302196	14303195	240025	Dact2	NM_172826	38143	30.90	11.64	+	+++
chr8	107778113	107779112	277978	Exoc3l	NM_177788	41386	30.90	11.64	+	+++
chr9	25288647	25289646	67484	Eepd1	NM_026189	-35	30.90	11.64	+	+++
chr9	59598546	59599545	270163	Myo9a	NM_173018	66	30.90	11.64	+	+++
chr12	110073646	110074645	214663	Slc25a29	NM_181328	-60	30.66	12.20	+	+++
chr12	3349714	3350713	16570	Kif3c	NM_008445	-14918	30.66	12.20	+	+++
chr12	99975339	99976338	24000	Ptpn21	NM_001146199	-224	30.66	12.20	+	+++
chr17	24591803	24592802	67078	Pgp	NM_025954	-15115	30.66	12.20	+	+++
chr18	60755374	60756373	104027	Synpo	AK049810	4278	30.66	12.20	+	+++
chr19	46564806	46565805	93679	Trim8	NM_053100	-10832	30.66	12.20	+	+++
chr19	6305983	6306982	240505	Cdc42bpg	NM_001033342	27	30.66	12.20	+	+++
chr2	167057318	167058317	19223	Ptgis	NM_008968	8220	30.66	12.20	+	+++
chr3	143864921	143865920	16911	Lmo4	NM_001161769	-61	30.66	12.20	+	+++
chr7	53605169	53606168	17927	Myod1	NM_010866	-26175	30.66	12.20	+	+++
chr11	74738860	74739859	103677	Smg6	NM_001002764	-14	30.63	11.83	+	+++
chr15	27525062	27526061	11732	Ank	NM_020332	24361	30.36	10.71	+	+++
chr13	81849419	81850418	67486	Polr3g	NM_001081176	94	30.17	11.25	+	+++
chr18	76255391	76256390	207259	Zbtb7c	NM_145356	36780	30.17	11.25	+	+++
chr9	90098903	90099902	108153	Adamts7	NM_001003911	30094	30.17	11.25	+	+++
chr19	10087168	10088167	24115	Best1	NM_011913	-11545	30.14	11.92	+	+++
chr6	51419992	51420991	53379	Hnrnpa2b1	NM_016806	-599	30.13	11.56	+	+++
chr11	69650869	69651868	11443	Chrnbl	NM_009601	-41930	30.10	10.29	+	+++
chr15	61816483	61817482	17869	Myc	NM_001177353	88	30.10	10.89	+	+++
chr10	81037613	81038612	114606	Tle6	NM_053254	21978	29.88	10.78	+	+++
chr17	35958244	35959243	15937	Ier3	NM_133662	87	29.87	8.68	+	+++
chr13	51940608	51941607	23882	Gadd45g	NM_011817	-936	29.83	9.36	+	+++
chr8	124425387	124426386	20539	Slc7a5	NM_011404	5700	29.83	9.36	+	+++
chr16	20611134	20612133	107522	Ece2	NM_177941	-40	29.73	11.35	+	+++
chr1	135837784	135838783	116847	Prelp	NM_054077	-20306	29.67	10.37	+	+++
chr15	54943493	54944492	97998	Deptor	NM_145470	-45	29.67	10.37	+	+++
chr11	119051464	119052463	207592	Tbc1d16	NM_172443	37850	29.67	11.31	+	+++
chr2	130921804	130922803	72630	Hspa12b	NM_028306	-30844	29.67	11.31	+	+++
chr7	20402459	20403458	57278	Bcam	NM_020486	-47078	29.67	11.31	+	+++
chr11	119794158	119795157	108100	Baiap2	NM_001037755	-9748	29.53	10.01	+	+++
chr2	76207527	76208526	241489	Pde11a	NM_001081033	-31317	29.43	9.69	+	+++
chr11	103173028	103174027	53859	Map3k14	NM_016896	-44813	29.41	10.85	+	+++
chr5	36508282	36509281	76943	Psap1l	NM_175249	-37888	29.41	10.85	+	+++
chr1	138579721	138580720	100503474	Gm16880	NR_037986	-12567	29.38	11.50	+	+++
chr11	70050973	70051972	216867	Slc16a11	AB041591	24062	29.38	11.84	+	+++
chr12	110070203	110071202	214663	Slc25a29	NM_181328	3384	29.38	11.84	+	+++
chr13	85328522	85329521	66671	Ccnh	NM_023243	-60	29.38	11.84	+	+++
chr17	7408916	7409915	20112	Rps6ka2	NM_011299	34953	29.38	11.84	+	+++
chr19	25035651	25036650	76088	Dock8	AK050543	-37868	29.38	11.84	+	+++
chr4	117555418	117556417	14664	Slc6a9	NM_008135	48056	29.38	11.84	+	+++
chr4	117918003	117919002	19268	Ptprf	NM_011213	45500	29.38	11.84	+	+++
chr6	113276413	113277412	18294	Ogg1	AK167456	-57	29.38	11.84	+	+++
chr7	107613901	107614900	22228	Ucp1	NM_011671	-27453	29.38	11.84	+	+++
chr9	51771133	51772132	14148	Fdx1	NM_007996	6	29.38	11.84	+	+++
chr10	127926843	127927842	216459	Myl6b	NM_172259	8399	28.96	10.02	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr13	8869777	8870776	207615	Wdr37	NM_172445	12	28.96	10.02	+	+++
chr19	10028547	10029546	24115	Best1	NM_011913	47077	28.96	10.02	+	+++
chr2	93302170	93303169	12521	Cd82	NM_001271430	-11	28.96	10.02	+	+++
chr3	90068575	90069574	78284	Creb3l4	NM_030080	-21641	28.96	10.02	+	+++
chr4	151460534	151461533	74035	Nol9	NM_028727	47599	28.96	10.02	+	+++
chr15	78812901	78813900	26912	Gcat	NM_001161712	-47903	28.91	9.72	+	+++
chr17	47978890	47979889	17240	Mdfi	NM_001109973	-7750	28.88	11.57	+	+++
chr2	14976340	14977339	75869	Arl5b	AY974590	-149	28.85	9.43	+	+++
chr17	29215469	29216468	12575	Cdkn1a	NM_001111099	-11962	28.75	9.13	+	+++
chr10	79723080	79724079	14431	Gamt	NM_010255	134	28.50	11.02	+	+++
chr10	79722199	79723198	14431	Gamt	NM_010255	1015	28.44	10.99	+	+++
chr11	121098150	121099149	67608	Narf	NM_026272	101	28.44	10.99	+	+++
chr12	79326699	79327698	268566	Gphn	NM_145965	-443	28.44	10.99	+	+++
chr19	42244601	42245600	277010	Marveld1	NM_183195	23223	28.44	10.99	+	+++
chr6	131337985	131338984	56449	Csda	NM_011733	-17	28.44	10.99	+	+++
chr6	50405729	50406728	71720	Osbpl3	NM_001163645	-60	28.40	9.75	+	+++
chr13	46714748	46715747	67252	Cap2	NM_026056	10313	28.22	10.55	+	+++
chr4	42962678	42963677	56323	Dnajb5	NM_019874	-90	28.22	10.55	+	+++
chr8	11634953	11635952	102334	Ankrd10	NM_001167967	302	28.22	10.55	+	+++
chr1	179371645	179372644	30928	Zfp238	NM_013915	-393	28.11	11.48	+	+++
chr12	45429584	45430583	319504	Nrcam	AK048567	213	28.11	11.48	+	+++
chr15	79634775	79635774	494448	Cbx6	NM_028763	29489	28.11	11.48	+	+++
chr17	27692909	27693908	15361	Hmga1	NM_001166541	-110	28.11	11.48	+	+++
chr19	6046489	6047488	12333	Capn1	NM_001110504	-31164	28.11	11.48	+	+++
chr7	119459458	119460457	320878	Mical2	NM_001193305	416	28.11	11.48	+	+++
chr3	105519030	105520029	109050	6530418L21Rik	NM_175398	12014	28.04	11.45	+	+++
chr11	69616983	69617982	11443	Chrb1	NM_009601	-8044	27.82	9.76	+	+++
chr3	89197149	89198148	54124	Cks1b	NM_016904	24565	27.82	9.76	+	+++
chr6	30419166	30420165	72649	Tmem209	NM_178625	40041	27.82	9.76	+	+++
chr15	91402852	91403851	239606	Slc2a13	NM_001033633	341	27.80	9.47	+	+++
chr11	68935251	68936250	11686	Alox12b	NM_009659	-34823	27.75	10.62	+	+++
chr14	45838912	45839911	70713	Gpr137c	NM_027518	21	27.68	8.91	+	+++
chr19	5939841	5940840	104252	Cdc42ep2	NM_026772	-15525	27.68	8.91	+	+++
chr4	46662680	46663679	381605	Tbc1d2	NM_198664	-109	27.68	8.91	+	+++
chr17	71947583	71948582	68789	Trmt61b	AK004173	19	27.63	11.22	+	+++
chr3	27081260	27082259	320024	Nceh1	NM_178772	-166	27.63	11.22	+	+++
chr12	60113892	60114891	20334	Sec23a	NM_009147	-1388	27.53	10.20	+	+++
chr15	81957935	81958934	76457	Ccdc134	AK132115	84	27.53	10.20	+	+++
chr1	153191298	153192297	117198	Ivns1abp	NM_001039511	171	27.29	10.70	+	+++
chr10	42892853	42893852	109205	Sobp	NM_175407	984	27.29	10.70	+	+++
chr11	62061897	62062896	11541	Adora2b	NM_007413	-89	27.29	10.70	+	+++
chr11	118917319	118918318	30951	Cbx8	NM_013926	-15592	27.23	10.67	+	+++
chr11	74451096	74452095	74148	1300001101Rik	NM_001081158	-11401	27.23	10.67	+	+++
chr9	49345569	49346568	17967	Ncam1	X59306	5179	27.23	10.67	+	+++
chr15	103045280	103046279	12419	Cbx5	NM_001110216	4	27.21	8.95	+	+++
chr12	99984617	99985616	24000	Ptpn21	NM_001146199	-9502	27.04	10.25	+	+++
chr1	74378832	74379831	18173	Slc11a1	NM_013612	-42445	26.87	9.86	+	+++
chr11	77978448	77979447	22032	Traf4	NM_009423	105	26.85	11.13	+	+++
chr17	27278592	27279591	271424	Ip6k3	NM_173027	25618	26.85	11.13	+	+++
chr19	4876082	4877081	11474	Actn3	NM_013456	1303	26.85	11.13	+	+++
chr2	127096070	127097069	99138	Stard7	NM_139308	606	26.85	11.13	+	+++
chr2	30214370	30215369	12908	Crat	NM_007760	47330	26.85	11.13	+	+++
chr3	131005697	131006696	74442	Sgms2	NM_028943	41645	26.85	11.13	+	+++
chr3	86027330	86028329	80877	Lrba	NM_001077687	-326	26.85	11.13	+	+++
chr9	121668787	121669786	72330	Kbtbd5	NM_028202	-17438	26.85	11.13	+	+++
chr9	37383852	37384851	22619	Siae	NM_011734	-37080	26.85	11.13	+	+++
chr11	115280930	115281929	237988	Cdr2l	NM_001080929	38201	26.83	10.78	+	+++
chr1	55109006	55110005	81898	Sf3b1	NM_031179	-25184	26.70	9.50	+	+++
chr8	107994957	107995956	67971	Tppp3	NM_026481	-135	26.70	9.50	+	+++
chr8	72519663	72520662	234366	Gatad2a	NM_001113345	116	26.70	9.50	+	+++
chr15	78286160	78287159	71753	Tmprss6	EU190436	9114	26.70	9.22	+	+++
chr14	21365249	21366248	74843	Zmynd17	NM_029104	-49626	26.62	8.69	+	+++
chr19	42222515	42223514	277010	Marveld1	NM_183195	1137	26.56	10.31	+	+++
chr2	18313972	18314971	13418	Dnajc1	NM_007869	-15	26.39	10.86	+	+++
chr11	118198725	118199724	58251	BC100451	NM_021440	4624	26.24	9.55	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr1	157071010	157072009	n/a	BC034090	AK078517	8361	26.03	10.34	+	+++
chr17	24606987	24607986	67078	Pgp	NM_025954	70	26.03	10.34	+	+++
chr17	45873155	45874154	n/a	1600014C23Rik	AK005450	-2862	26.03	10.34	+	+++
chr2	56968707	56969706	18227	Nr4a2	BC144854	-1758	26.03	10.34	+	+++
chr7	72017338	72018337	66647	Ndn12	NM_023239	89	26.03	10.34	+	+++
chr9	36953673	36954672	208076	Pknox2	NM_148950	727	26.03	10.34	+	+++
chr12	40764119	40765118	11861	Arl4a	NM_001039515	-45	25.87	9.95	+	+++
chr4	102791635	102792634	242584	Wdr78	NM_146254	-5231	25.87	9.95	+	+++
chr2	152462066	152463065	266620	Defb36	NM_001037247	32504	25.65	9.26	+	+++
chr10	79861807	79862806	216166	Plk5	NM_183152	43104	25.62	10.44	+	+++
chr1	137487087	137488086	215690	Nav1	NM_173437	-5301	25.61	10.77	+	+++
chr10	79563327	79564327	20869	Stk11	NR_126043	-14720	25.61	10.77	+	+++
chr11	118183039	118184038	58251	BC100451	NM_021440	20310	25.61	10.77	+	+++
chr11	118215501	118216500	58251	BC100451	NM_021440	-12145	25.61	10.77	+	+++
chr17	27257192	27258191	271424	Ip6k3	NM_173027	47018	25.61	10.77	+	+++
chr2	160435403	160436402	21969	Top1	AK166844	-35730	25.61	10.77	+	+++
chr4	151451819	151452818	74035	Nol9	NM_028727	38884	25.61	10.77	+	+++
chr7	56136121	56137120	108961	E2f8	NM_001013368	-210	25.61	10.77	+	+++
chr13	51941434	51942433	23882	Gadd45g	NM_011817	-110	25.60	8.97	+	+++
chr16	44140304	44141303	72117	Naa50	NM_028108	883	25.60	8.97	+	+++
chr2	167103869	167104868	19223	Ptgis	NM_008968	-38332	25.59	9.23	+	+++
chr2	179962047	179963046	16776	Lama5	NM_001081171	-1983	25.59	9.23	+	+++
chr11	103020051	103021050	192231	Hexim1	NM_138753	42913	25.57	8.47	+	+++
chr4	135720592	135721591	242705	E2f2	NM_177733	-7217	25.57	8.47	+	+++
chr5	124889327	124890326	67956	Setd8	NM_030241	-112	25.07	8.98	+	+++
chr12	8647203	8648202	328059	Slc7a15	NM_177802	-41831	24.90	10.05	+	+++
chr1	66862676	66863675	68691	1110028C15Rik	NM_177645	860	24.85	10.02	+	+++
chr13	74944928	74945927	12380	Cast	NM_009817	-59	24.85	10.02	+	+++
chr19	47323334	47324333	18011	Neurl1a	NM_001163480	9025	24.85	10.02	+	+++
chr2	25110503	25111502	97031	Tprn	NM_175286	-7115	24.85	10.02	+	+++
chr2	26865042	26866041	227659	Slc2a6	AK163445	17977	24.85	10.02	+	+++
chr3	122432869	122433868	242202	Pde5a	AK031275	230	24.85	10.02	+	+++
chr7	73437530	73438529	233328	Lrrk1	AK081849	-27573	24.85	10.02	+	+++
chr2	71710774	71711773	16403	Itga6	NM_008397	46495	24.81	7.71	+	+++
chr8	87600597	87601596	212999	Tnpo2	NM_001122843	40031	24.81	7.71	+	+++
chr13	21597871	21598871	93681	Tfp192	NM_139141	20519	24.72	9.65	+	+++
chr9	58022389	58023388	26968	Islr	NM_012043	-15861	24.72	9.65	+	+++
chr11	102864232	102865231	68087	Deakd	NM_026551	-5870	24.69	9.94	+	+++
chr11	118224024	118225023	58251	BC100451	NM_021440	-20668	24.53	8.24	+	+++
chr7	57103005	57104004	71974	Prmt3	BC050775	19204	24.53	8.24	+	+++
chr15	38471927	38472926	54375	Azin1	NM_018745	-23406	24.49	8.97	+	+++
chr17	24768976	24769975	65962	Slc9a3r2	NM_023449	12427	24.49	8.97	+	+++
chr3	127598798	127599797	70617	5730508B09Rik	BC144728	-57	24.49	8.97	+	+++
chr7	74517268	74518267	17258	Mef2a	AK153751	-24	24.49	8.97	+	+++
chr9	62676132	62677131	14155	Fem1b	NM_010193	-17177	24.49	8.97	+	+++
chr10	79549272	79550271	20869	Stk11	NR_126043	-28776	24.42	10.10	+	+++
chr1	137594705	137595704	n/a	Nav1	AK158038	-10523	24.39	10.41	+	+++
chr1	155488418	155489417	67792	Rgs8	NM_026380	-11249	24.39	10.41	+	+++
chr11	69651625	69652624	11443	Chrn1	NM_009601	-42686	24.39	10.41	+	+++
chr17	27290183	27291182	271424	Ip6k3	NM_173027	14027	24.39	10.41	+	+++
chr2	128524105	128525104	17289	Mertk	NM_008587	-128	24.39	10.41	+	+++
chr2	166712964	166713963	110750	Cse1l	BC145393	-18132	24.39	10.41	+	+++
chr6	30418533	30419532	72649	Tmem209	NM_178625	40674	24.39	10.41	+	+++
chr8	126414873	126415872	11459	Acta1	NM_009606	3264	24.39	10.41	+	+++
chr8	13199932	13200931	66790	Grtp1	NM_025768	193	24.39	10.41	+	+++
chr11	60541592	60542591	237781	Smcr7	NM_001009927	193	23.95	10.16	+	+++
chr6	90713694	90714693	232227	lqsec1	NM_001134384	45924	23.83	7.52	+	+++
chr12	33860448	33861447	320046	F730043M19Rik	AK089514	-28486	23.81	10.08	+	+++
chr9	20938377	20939376	18577	Pde4a	NM_183408	-31281	23.73	10.04	+	+++
chr15	76368811	76369810	30840	Fbxl6	NM_013909	-135	23.73	9.72	+	+++
chr8	74045238	74046237	26457	Slc27a1	NM_011977	-47088	23.73	9.72	+	+++
chr14	51550362	51551361	18950	Pnp	NM_013632	-13116	23.68	9.70	+	+++
chr16	97547245	97548244	56175	Bace2	NM_019517	-30590	23.68	9.70	+	+++
chr17	14839444	14840443	21826	Thbs2	NM_011581	-8675	23.68	9.70	+	+++
chr18	23961953	23962952	212307	Mapre2	NM_153058	-18	23.68	9.70	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	152452636	152453635	266620	Defb36	NM_001037247	23074	23.68	9.70	+	+++
chr2	160446439	160447438	21969	Top1	AK166844	-24694	23.68	9.70	+	+++
chr3	128916494	128917493	18741	Pitx2	NM_001042502	140	23.68	9.70	+	+++
chr5	98459741	98460740	71914	Antxr2	NM_133738	-260	23.68	9.70	+	+++
chr10	120913050	120914049	192678	Rassf3	NM_138956	-244	23.58	9.35	+	+++
chr1	74443625	74444624	18173	Slc11a1	NM_013612	22349	23.51	8.02	+	+++
chr11	72974017	72975016	94045	P2rx5	NM_033321	486	23.51	8.02	+	+++
chr11	35651508	35652507	104458	Rars	NM_025936	-3978	23.40	8.70	+	+++
chr12	113826375	113827374	11565	Adssl1	NM_007421	-31383	23.40	8.70	+	+++
chr3	88489395	88490394	19769	Rit1	AK018785	-30881	23.40	8.70	+	+++
chr6	90714476	90715475	232227	Iqsec1	NM_001134384	45142	23.24	9.77	+	+++
chr1	157059407	157060406	n/a	BC034090	AK078517	19964	23.18	10.05	+	+++
chr11	115964959	115965958	70450	Unc13d	BC046519	-26184	23.18	10.05	+	+++
chr11	68901552	68902551	20877	Aurkb	NM_011496	42908	23.18	10.05	+	+++
chr17	74737861	74738860	76890	Memo1	NM_133771	-44158	23.18	10.05	+	+++
chr19	10979381	10980380	68539	Tmem109	NM_134142	-23648	23.18	10.05	+	+++
chr19	40345232	40346231	54132	Pdlim1	NM_016861	375	23.18	10.05	+	+++
chr19	46558355	46559354	93679	Trim8	NM_053100	-17283	23.18	10.05	+	+++
chr19	5488847	5489846	75555	1700020D05Rik	NM_023781	14441	23.18	10.05	+	+++
chr19	6321752	6322751	240505	Cdc42bpg	NM_001033342	15796	23.18	10.05	+	+++
chr2	3200708	3201707	18108	Nmt2	NM_001290370	-276	23.18	10.05	+	+++
chr4	135733243	135734242	242705	E2f2	NM_177733	5435	23.18	10.05	+	+++
chr4	33070180	33071179	52187	Rragd	NM_027491	708	23.18	10.05	+	+++
chr4	46614561	46615560	381605	Tbc1d2	NM_198664	48011	23.18	10.05	+	+++
chr6	113268673	113269672	18294	Ogg1	AK167456	-7797	23.18	10.05	+	+++
chr6	42642421	42643420	232748	Fam115c	NM_146174	-47881	23.18	10.05	+	+++
chr6	72488272	72489271	12332	Capg	NM_007599	-5661	23.18	10.05	+	+++
chr8	72876402	72877401	14559	Gdfl	NM_008107	23239	23.18	10.05	+	+++
chr8	8689921	8690920	13642	Efnb2	NM_010111	-29648	23.07	9.37	+	+++
chr3	142058212	142059211	56376	Pdlim5	NM_001190853	-52	22.95	8.24	+	+++
chr15	101212935	101213934	110310	Krt7	NM_033073	-29399	22.90	9.89	+	+++
chr5	136091610	136092609	56221	Ccl24	NM_019577	-43197	22.86	7.33	+	+++
chr14	32007779	32008778	21924	Tnnc1	NM_009393	-13219	22.83	9.54	+	+++
chr1	137571965	137572964	n/a	Nav1	AK158038	12218	22.76	9.81	+	+++
chr10	79255658	79256657	73106	Prss11	NM_001042710	-4371	22.57	9.40	+	+++
chr11	118901743	118902742	30951	Cbx8	NM_013926	-16	22.57	9.40	+	+++
chr11	98671011	98672010	268480	Rapgef1l	NM_001080925	-26588	22.57	9.40	+	+++
chr18	76219740	76220739	207259	Zbtb7c	NM_145356	1129	22.57	9.40	+	+++
chr19	42202968	42203967	277010	Marveld1	NM_183195	-18411	22.57	9.40	+	+++
chr1	137179885	137180884	93689	Lmod1	NM_053106	-41005	22.52	9.37	+	+++
chr11	103002095	103003094	192231	Hexim1	NM_138753	24957	22.52	9.37	+	+++
chr17	74714984	74715983	76890	Memo1	NM_133771	-21281	22.52	9.37	+	+++
chr6	115910819	115911818	67784	Plxnd1	NM_026376	33705	22.52	9.37	+	+++
chr2	25127407	25128406	97031	Tprn	NM_175286	9790	22.49	7.80	+	+++
chr4	144809184	144810183	21938	Tnfrsf1b	NM_011610	27090	22.45	9.05	+	+++
chr8	47555859	47556858	14081	Acs1l	NM_007981	-37	22.41	8.23	+	+++
chr3	102906185	102907184	329727	Dennd2c	NM_177857	-24794	22.39	8.74	+	+++
chr1	137326351	137327350	215690	Nav1	AK147844	34485	22.33	8.44	+	+++
chr11	62094378	62095377	11541	Adora2b	NM_007413	32393	22.33	8.44	+	+++
chr14	26614716	26615715	11744	Anxa11	NM_013469	-46425	22.33	8.44	+	+++
chr19	10969218	10970217	68539	Tmem109	NM_134142	-13485	22.33	8.44	+	+++
chr5	31262160	31263159	57742	Abhd1	NR_003522	10222	22.33	8.44	+	+++
chr15	38403154	38404153	54375	Azin1	NM_018745	45368	22.27	8.95	+	+++
chr10	126817695	126818694	14632	Gli1	AK157048	-39253	22.07	9.43	+	+++
chr1	157062292	157063291	n/a	BC034090	AK078517	17079	21.98	9.69	+	+++
chr10	80655674	80656673	69564	Itgb1bp3	NM_027120	8609	21.98	9.69	+	+++
chr11	94819429	94820428	20391	Sgca	NM_001136080	14864	21.98	9.69	+	+++
chr14	73725087	73726086	19645	Rb1	NM_009029	12	21.98	9.69	+	+++
chr3	129672451	129673450	66815	Ccdc109b	NM_025779	174	21.98	9.69	+	+++
chr3	89233752	89234751	54124	Cks1b	NM_016904	-12039	21.98	9.69	+	+++
chr7	37493281	37494280	243931	Tshz3	NM_172298	10645	21.98	9.69	+	+++
chr7	37494058	37495057	243931	Tshz3	NM_172298	11422	21.98	9.69	+	+++
chr11	96882626	96883625	83395	Sp6	NM_031183	3071	21.93	9.06	+	+++
chr17	50647906	50648905	224860	Plcl2	NM_013880	-466	21.93	9.06	+	+++
chr19	4916649	4917648	11474	Actn3	NM_013456	-39265	21.93	9.06	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	157971687	157972686	21817	Tgm2	NM_009373	-59	21.90	7.13	+	+++
chr13	33096102	33097101	20708	Serpinb6b	NM_011454	39221	21.86	8.74	+	+++
chr1	137566666	137567665	n/a	Nav1	AK158038	17517	21.65	9.50	+	+++
chr19	40686311	40687310	67590	Tctn3	NM_026260	-106	21.58	9.46	+	+++
chr5	92566569	92567568	56041	Uso1	NM_019490	106	21.58	9.46	+	+++
chr8	47002347	47003346	53318	Pdlim3	NM_016798	32009	21.58	9.46	+	+++
chr1	74437655	74438654	18173	Slc11a1	NM_013612	16379	21.49	7.57	+	+++
chr10	42708135	42709134	268297	Scml4	NM_172938	48949	21.49	7.57	+	+++
chr4	144836272	144837271	21938	Tnfrsf1b	NM_011610	2	21.49	7.57	+	+++
chr6	97128685	97129684	101351	A130022J15Rik	NM_175313	-30308	21.49	7.57	+	+++
chr9	75257295	75258294	50772	Mapk6	NM_015806	27	21.49	7.57	+	+++
chr8	13214532	13214532	66790	Grtp1	NM_025768	-13409	21.42	9.08	+	+++
chr10	79688665	79689664	14431	Gamt	NM_010255	34549	21.38	9.05	+	+++
chr15	94458893	94459892	320709	Tmem117	AK034024	-223	21.38	9.05	+	+++
chr16	32643548	32644547	22042	Tfrc	NM_011638	19521	21.38	9.05	+	+++
chr2	128700940	128701939	70370	Fbln7	NM_024237	11773	21.38	9.05	+	+++
chr3	105507739	105508738	109050	6530418L21Rik	NM_175398	723	21.38	9.05	+	+++
chr4	128963249	128964248	68828	Sync	NM_023485	-1116	21.38	9.05	+	+++
chr1	172574214	172575213	320078	Olfml2b	NM_177068	52	21.34	8.74	+	+++
chr10	80655068	80656067	69564	Itgb1bp3	NM_027120	9215	21.34	8.74	+	+++
chr4	122693444	122694443	16918	Mycl1	NM_008506	20603	21.34	8.74	+	+++
chr16	36071266	36072265	69544	Wdr5b	NM_027113	30491	21.27	8.18	+	+++
chr17	14339836	14340835	240025	Dact2	NM_172826	503	21.27	8.18	+	+++
chr2	49597081	49598080	71897	Lypd6b	NM_027990	-45625	21.27	8.18	+	+++
chr4	129952057	129953056	230779	Serinc2	NM_001253386	-93	21.27	8.18	+	+++
chr1	36620618	36621617	78321	Ankrd23	BC022973	-24915	20.95	6.94	+	+++
chr8	25560700	25561699	69068	1810011O10Rik	NM_026931	-11782	20.92	9.09	+	+++
chr15	79968639	79969638	17309	Mgat3	NM_010795	-35012	20.82	7.96	+	+++
chr1	89504039	89505038	23956	Neu2	NM_015750	13415	20.81	8.75	+	+++
chr1	137460055	137461054	215690	Nav1	NM_173437	21378	20.80	9.33	+	+++
chr13	54189393	54190392	14057	Sfxn1	NM_027324	22680	20.80	9.33	+	+++
chr13	74599876	74600875	235956	Zfp825	NM_146231	31023	20.80	9.33	+	+++
chr18	75963987	75964986	207259	Zbtb7c	NM_145356	-15345	20.80	9.33	+	+++
chr19	46573933	46574932	93679	Trim8	NM_053100	-1705	20.80	9.33	+	+++
chr3	55030388	55031387	13175	Dclk1	NM_019978	-15560	20.80	9.33	+	+++
chr4	53838404	53839403	52076	Tmem38b	NM_028053	-13	20.80	9.33	+	+++
chr5	138219561	138220560	243300	6430598A04Rik	NM_175521	-37226	20.80	9.33	+	+++
chr6	120413086	120414085	16172	Il17ra	NM_008359	372	20.80	9.33	+	+++
chr7	140967867	140968866	101437	Dhx32	NM_001286032	120	20.80	9.33	+	+++
chr7	6237544	6238543	384763	Zfp667	NM_001024928	-138	20.80	9.33	+	+++
chr8	74061106	74062105	26457	Slc27a1	NM_011977	-31220	20.80	9.33	+	+++
chr12	103545873	103546872	217835	Rin3	NM_001161365	24523	20.77	8.45	+	+++
chr10	128354899	128355898	19682	Rdh5	NM_134006	955	20.50	7.35	+	+++
chr11	107592611	107593610	12299	Cacng1	NM_007582	-15321	20.50	7.35	+	+++
chr11	69608718	69609717	11443	Chrnbl	NM_009601	222	20.50	7.35	+	+++
chr3	8666792	8667791	15213	Hey1	NM_010423	-254	20.50	7.35	+	+++
chr7	20334629	20335628	57278	Bcam	NM_020486	20753	20.50	7.35	+	+++
chr19	53785023	53786022	73713	Rbm20	NM_001170847	33728	20.41	8.82	+	+++
chr10	79240324	79241323	73106	Prssl1	NM_001042710	10964	20.41	9.11	+	+++
chr11	75365044	75366043	215113	slc43a2	BC050243	-13965	20.41	9.11	+	+++
chr17	12520363	12521362	26407	Map3k4	NM_011948	-9337	20.41	9.11	+	+++
chr3	54611042	54612041	170767	Rfxap	NM_133231	172	20.29	8.75	+	+++
chr5	129513800	129514799	13852	Stx2	NM_007941	140	20.29	8.75	+	+++
chr11	5421764	5422763	22433	Xbp1	NM_013842	1295	20.27	7.94	+	+++
chr11	102859405	102860404	68087	Deakd	NM_026551	-1043	20.25	8.73	+	+++
chr12	41172112	41173111	238130	Dock4	NM_172803	-28	20.25	8.73	+	+++
chr19	5456700	5457699	14283	Fosl1	NM_010235	9503	20.25	8.73	+	+++
chr2	179942355	179943354	16776	Lama5	NM_001081171	17710	20.25	8.73	+	+++
chr3	69402028	69403027	26879	B3galnt1	NM_020026	256	20.25	8.73	+	+++
chr6	84538086	84539085	232174	Cyp26b1	NM_175475	47	20.25	8.73	+	+++
chr1	23389765	23390764	70155	Ogfrl1	NM_001081079	-251	20.22	7.91	+	+++
chr11	5420662	5421661	22433	Xbp1	NM_013842	193	20.22	7.91	+	+++
chr11	69828317	69829316	13385	Dlg4	NM_001109752	-3290	20.22	7.91	+	+++
chr13	31724018	31725017	14238	Foxf2	NM_010225	6834	20.22	7.91	+	+++
chr15	25556534	25557533	17909	Myo10	NM_019472	4730	19.72	7.91	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr14	32010670	32011669	21924	Tnnc1	NM_009393	-10328	19.71	6.29	+	+++
chr8	87583543	87584542	212999	Tnpo2	NM_001122843	22977	19.71	6.29	+	+++
chr8	90661396	90662395	234549	Heatr3	NM_172757	113	19.71	6.29	+	+++
chr16	14705893	14706892	20583	Snai2	NM_011415	442	19.69	8.16	+	+++
chr1	89166853	89167852	98363	Efh1	NM_028889	6415	19.64	8.97	+	+++
chr10	126817099	126818098	14632	Gli1	AK157048	-38657	19.64	8.97	+	+++
chr10	93103503	93104502	57764	Ntn4	NM_021320	210	19.64	8.97	+	+++
chr11	74466585	74467584	74148	1300001101Rik	NM_001081158	4089	19.64	8.97	+	+++
chr13	54166808	54167807	14057	Sfxn1	NM_027324	95	19.64	8.97	+	+++
chr2	179948881	179949880	16776	Lama5	NM_001081171	11184	19.64	8.97	+	+++
chr3	19593606	19594605	381485	Trim55	NM_001081281	49647	19.64	8.97	+	+++
chr4	122638051	122639050	76574	Mfsd2a	NM_029662	-120	19.64	8.97	+	+++
chr5	123086656	123087655	18439	P2rx7	NM_001038887	-6764	19.64	8.97	+	+++
chr6	120443523	120444522	16172	Il17ra	NM_008359	30809	19.64	8.97	+	+++
chr7	119372215	119373214	320878	Mical2	NM_177282	3366	19.64	8.97	+	+++
chr7	73388372	73389371	233328	Lrrk1	AK081849	21586	19.64	8.97	+	+++
chr7	90780033	90781032	170460	Stard5	NM_023377	7	19.64	8.97	+	+++
chr12	114167967	114168966	16450	Jag2	NM_010588	-761	19.52	7.13	+	+++
chr2	30271043	30272042	12908	Crat	NM_007760	-252	19.52	7.13	+	+++
chr17	14809059	14810058	21826	Thbs2	NM_011581	21711	19.52	8.91	+	+++
chr10	80489818	80490817	208666	Diras1	NM_145217	-2196	19.26	8.76	+	+++
chr4	135728181	135729180	242705	E2f2	NM_177733	373	19.26	8.76	+	+++
chr10	80450504	80451503	208666	Diras1	NM_145217	37119	19.19	7.65	+	+++
chr13	43460469	43461468	68346	Sirt5	NM_178848	95	19.19	7.65	+	+++
chr15	103000074	103001073	12419	Cbx5	NM_001110216	45210	19.19	7.65	+	+++
chr2	28083459	28084458	56177	Olfm1	NM_019498	22751	19.19	7.65	+	+++
chr9	119919744	119920743	22437	Xirp1	NM_011724	12473	19.19	7.65	+	+++
chr19	47296735	47297734	18011	Neur11a	NM_001163480	-6098	19.18	7.89	+	+++
chr10	128141170	128142169	19344	Rab5b	NM_177411	-8346	19.14	8.41	+	+++
chr10	42707196	42708195	268297	Scml4	NM_172938	48010	19.14	8.41	+	+++
chr10	94406940	94407939	54712	Plxnc1	NM_018797	-228	19.14	8.41	+	+++
chr11	75401455	75402454	215113	Slc43a2	NM_173388	21616	19.14	8.41	+	+++
chr19	42091238	42092237	56642	Ankrd2	NM_020033	-18790	19.14	8.41	+	+++
chr4	3605382	3606381	17096	Lyn	BC003253	615	19.14	8.41	+	+++
chr7	149246438	149247437	18218	Dusp8	NM_008748	29234	19.14	8.41	+	+++
chr8	107799914	107800913	277978	Exoc3l	NM_177788	19585	19.14	8.41	+	+++
chr8	97876320	97877319	17388	Mmp15	NM_008609	584	18.70	5.77	+	+++
chr18	74022887	74023886	71263	Mro	NM_027741	4347	18.65	8.42	+	+++
chr2	25316047	25317046	227622	BC029214	NM_153557	68	18.63	7.87	+	+++
chr10	80532489	80533488	50492	Thop1	NM_022653	162	18.62	8.12	+	+++
chr1	136311589	136312588	240756	Klhl12	NM_153128	-40043	18.56	6.91	+	+++
chr10	81021556	81022555	114606	Tle6	NM_053254	38035	18.56	6.91	+	+++
chr15	27610900	27611899	223433	Fam105a	NM_001242424	-103	18.56	6.91	+	+++
chr9	121613575	121614574	18087	Nktr	NM_010918	-14224	18.56	6.91	+	+++
chr1	36636510	36637509	78321	Ankrd23	BC022973	-40807	18.49	8.61	+	+++
chr12	77694492	77695491	263406	Plekkg3	AK172977	24230	18.49	8.61	+	+++
chr12	81870317	81871316	217684	4933426M11Rik	NM_178682	-20729	18.49	8.61	+	+++
chr13	46620918	46621917	67252	Cap2	AK049813	24147	18.49	8.61	+	+++
chr18	23910459	23911458	212307	Mapre2	NM_001162941	126	18.49	8.61	+	+++
chr18	76231673	76232672	207259	Zbtb7c	NM_145356	13062	18.49	8.61	+	+++
chr2	122124096	122125095	213696	Duoxa1	NM_145395	14871	18.49	8.61	+	+++
chr2	91514551	91515550	14061	F2	NM_010168	-38480	18.49	8.61	+	+++
chr3	32264051	32265050	22401	Zmat3	NM_009517	37	18.49	8.61	+	+++
chr4	149264492	149265491	74646	Spsb1	NM_029035	18849	18.49	8.61	+	+++
chr4	150183085	150184084	74155	Errfi1	NM_133753	-44443	18.49	8.61	+	+++
chr5	145844334	145845333	11867	Arpe1b	NM_023142	-30291	18.49	8.61	+	+++
chr7	107622963	107623962	22228	Ucp2	NM_011671	-18391	18.49	8.61	+	+++
chr7	25184249	25185248	16534	Kcnn4	NM_008433	21090	18.49	8.61	+	+++
chr7	29595955	29596954	16855	Lgals4	NM_010706	-22556	18.49	8.61	+	+++
chr8	122385366	122386365	72042	Cotl1	NM_028071	-21387	18.49	8.61	+	+++
chr8	126177739	126178738	69581	Rhou	NM_133955	411	18.49	8.61	+	+++
chr5	66936551	66937550	11787	Apbb2	AK207143	-2195	18.24	7.92	+	+++
chr1	137220818	137221817	93689	Lmod1	NM_053106	-72	18.17	7.39	+	+++
chr15	78309688	78310687	71753	Tmprss6	NM_027902	-11124	18.17	7.39	+	+++
chr15	81766833	81767832	105859	Csdc2	NM_145473	145	18.17	7.39	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr17	25868712	25869711	214901	Chtf18	NM_145409	-4852	18.17	7.39	+	+++
chr2	32236786	32237785	13429	Dnm1	NR_125959	-28437	18.17	7.39	+	+++
chr8	71258606	71259605	234356	Csgalnact1	NM_001252623	-61	18.17	7.39	+	+++
chr17	87336771	87337770	104215	Rhoq	NM_145491	-25180	18.13	7.61	+	+++
chr13	51975084	51976083	23882	Gadd45g	NM_011817	33541	18.13	8.41	+	+++
chr17	5491871	5492870	224454	Zdhhc14	NM_146073	-229	18.13	8.41	+	+++
chr1	134215491	134216490	213417	Klhdc8a	NM_144810	20789	18.04	8.08	+	+++
chr11	100489783	100490782	71966	Nkiras2	NM_028024	6015	18.04	8.08	+	+++
chr19	47242492	47243491	18011	Neurl1a	NM_021360	-10318	18.04	8.08	+	+++
chr2	153318017	153319016	329540	8430427H17Rik	AK029844	-264	18.04	8.08	+	+++
chr5	134575048	134576047	80909	Gatsl2	NM_030719	-70	18.04	8.08	+	+++
chr7	119369662	119370661	320878	Mical2	NM_177282	813	18.04	8.08	+	+++
chr10	77706673	77707672	216131	Trappc10	NM_001081055	215	17.66	7.37	+	+++
chr10	79727112	79728111	14431	Gamt	NM_010255	-3899	17.61	6.68	+	+++
chr6	34860196	34861195	78412	3110062M04Rik	NM_001135611	-32631	17.61	6.68	+	+++
chr5	123464576	123465575	109305	Orail	NM_175423	-7	17.58	7.57	+	+++
chr6	90661123	90662122	232227	Iqsec1	NM_001134383	4901	17.58	7.57	+	+++
chr1	138033923	138034922	16565	Kif21b	NM_001039472	6446	17.55	8.08	+	+++
chr12	70602483	70603482	76062	5830428M24Rik	NR_038060	-26446	17.55	8.08	+	+++
chr5	135726603	135727602	14371	Fzd9	NM_010246	-186	17.55	8.08	+	+++
chr1	135814023	135815022	116847	Prelp	NM_054077	3456	17.54	7.81	+	+++
chr2	14970323	14971322	75869	Arl5b	AY974590	-6166	17.54	7.81	+	+++
chr19	41875932	41876931	71085	Arhgap19	NM_027667	143	17.36	8.25	+	+++
chr2	122185990	122186989	213696	Duoxa1	NM_145395	-47024	17.36	8.25	+	+++
chr3	102538313	102539312	70747	Tspan2	NM_027533	121	17.36	8.25	+	+++
chr7	20335205	20336204	57278	Beam	NM_020486	20177	17.36	8.25	+	+++
chr7	31911439	31912438	15451	Hpn	NM_008281	-11630	17.36	8.25	+	+++
chr9	37062511	37063510	56857	Slc37a2	NM_001145960	313	17.36	8.25	+	+++
chr9	43901775	43902774	53376	Usp2	NM_198091	9273	17.36	8.25	+	+++
chr17	7373386	7374385	20112	Rps6ka2	NM_011299	-578	17.34	6.98	+	+++
chr2	160697713	160698712	64899	Lpin3	NM_022883	-8193	17.34	6.98	+	+++
chr18	76218863	76219862	207259	Zbtb7c	NM_145356	252	17.21	7.14	+	+++
chr13	52636443	52637442	20963	Syk	AK138741	-41863	17.19	8.16	+	+++
chr12	17006589	17007588	217430	Pqlc3	NM_001161111	-165	17.17	7.12	+	+++
chr4	135761651	135762650	242705	E2f2	NM_177733	33843	17.17	7.12	+	+++
chr4	154937304	154938303	320541	Slc35e2	NM_177186	-37721	17.17	7.12	+	+++
chr9	34983056	34984055	117149	Tirap	NM_001177847	23935	17.17	7.12	+	+++
chr19	53786696	53787695	73713	Rbm20	NM_001170847	35401	17.11	7.33	+	+++
chr16	18788381	18789380	12741	Cldn5	NM_013805	11942	17.02	8.06	+	+++
chr19	10955380	10956379	68539	Tmem109	NM_134142	354	17.02	8.06	+	+++
chr11	74200386	74201385	380711	Rap1gap2	NM_001015046	20641	17.00	7.78	+	+++
chr12	82732128	82733127	69480	Ttc9	NM_001033149	273	17.00	7.78	+	+++
chr13	38012318	38013317	68750	Rreb1	NM_001177869	32079	17.00	7.78	+	+++
chr1	134195306	134196305	213417	Klhdc8a	NM_144810	604	16.96	7.76	+	+++
chr14	66586850	66587849	12759	Clu	NM_013492	31	16.96	7.76	+	+++
chr7	119323351	119324350	320878	Mical2	NM_177282	-45499	16.96	7.76	+	+++
chr11	102845709	102846708	68087	Dcaad	NM_026551	12654	16.67	6.46	+	+++
chr16	44139474	44140473	72117	Naa50	NM_028108	53	16.67	6.46	+	+++
chr2	120833755	120834754	96957	Tmem62	NM_175285	30889	16.67	6.46	+	+++
chr8	97665647	97666646	16582	Kifc3	NM_010631	294	16.67	6.46	+	+++
chr1	78528409	78529408	23874	Farsb	NM_001278075	-43437	16.65	7.10	+	+++
chr11	121248993	121249992	217371	Rab40b	NM_139147	73	16.55	7.28	+	+++
chr15	76364065	76365064	30840	Fbxl6	AK040458	4600	16.49	7.50	+	+++
chr2	28101280	28102279	56177	Olfm1	NM_019498	40572	16.49	7.50	+	+++
chr1	134189064	134190063	213417	Klhdc8a	NM_144810	-5639	16.46	7.75	+	+++
chr11	68872633	68873632	20877	Aurkb	NM_011496	13989	16.46	7.75	+	+++
chr11	118914147	118915146	30951	Cbx8	NM_013926	-12420	16.39	5.98	+	+++
chr17	47929087	47930086	17240	Mdfr	NM_010783	40582	16.39	5.98	+	+++
chr1	138032906	138033905	16565	Kif21b	NM_001039472	5429	16.25	7.90	+	+++
chr11	103079052	103080051	53859	Map3k14	NM_016896	24152	16.25	7.90	+	+++
chr11	62633506	62634505	94092	Trim16	NM_053169	252	16.25	7.90	+	+++
chr11	96858708	96859707	83395	Sp6	NM_031183	-15675	16.25	7.90	+	+++
chr12	103540293	103541292	217835	Rin3	NM_001161365	18943	16.25	7.90	+	+++
chr13	46708878	46709877	67252	Cap2	NM_026056	4443	16.25	7.90	+	+++
chr13	46715529	46716528	67252	Cap2	NM_026056	11094	16.25	7.90	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr16	31200155	31201154	78618	Acap2	NM_030138	670	16.25	7.90	+	+++
chr2	12932254	12933253	227580	C1ql3	NM_153155	-263	16.25	7.90	+	+++
chr2	129427359	129428358	19261	Sirpa	NM_001177647	8929	16.25	7.90	+	+++
chr5	123525783	123526782	330189	Tmem120b	NM_001039723	-1	16.25	7.90	+	+++
chr7	119479989	119480988	320878	Mical2	NM_001193305	20947	16.25	7.90	+	+++
chr7	63304974	63305973	233280	Nipa1	NM_153578	-30531	16.25	7.90	+	+++
chr9	49293649	49294648	235330	Ttc12	NM_172770	182	16.25	7.90	+	+++
chr11	53876716	53877715	30794	Pdlim4	NM_019417	5304	16.22	6.88	+	+++
chr11	107589575	107590574	12299	Cacng1	NM_007582	-12285	16.18	6.86	+	+++
chr19	58527728	58528727	14585	Gfra1	NM_010279	857	16.18	6.86	+	+++
chr2	151787976	151788975	69698	2310046K01Rik	NM_027172	-33771	16.18	6.86	+	+++
chr3	88520319	88521318	19769	Rit1	AK018785	44	16.18	6.86	+	+++
chr3	105512943	105513942	109050	6530418L21Rik	NM_175398	5927	16.15	7.84	+	+++
chr13	24729960	24730959	193385	Fam65b	NM_029679	-57	16.00	7.24	+	+++
chr19	46697296	46698295	13074	Cyp17a1	NM_007809	49695	15.94	7.46	+	+++
chr6	135245240	135246239	76400	Pbp2	NM_029595	14663	15.92	7.71	+	+++
chr14	64069601	64070600	219148	Fam167a	NM_177628	14871	15.90	7.44	+	+++
chr16	45408315	45409314	17470	Cd200	NM_010818	352	15.90	7.44	+	+++
chr4	122624070	122625069	76574	Mfsd2a	NM_029662	13862	15.90	7.44	+	+++
chr11	115947949	115948948	70450	Unc13d	BC046519	-9174	15.75	6.24	+	+++
chr15	101200292	101201291	110310	Krt7	NM_033073	-42042	15.75	6.24	+	+++
chr6	91141825	91142824	232232	Hdac11	AK181630	26771	15.55	7.50	+	+++
chr15	25567538	25568537	17909	Myo10	NM_019472	15734	15.54	6.99	+	+++
chr4	133863061	133864060	433766	Trim63	NM_001039048	-7474	15.54	6.99	+	+++
chr6	34824158	34825157	78412	3110062M04Rik	NM_199145	2457	15.54	6.99	+	+++
chr18	38790940	38791939	24066	Spry4	NM_011898	-30518	15.52	5.78	+	+++
chr19	29037894	29038893	67072	Cdc37l1	NM_001302443	-26448	15.45	7.19	+	+++
chr4	59015437	59016436	67103	Ptgr1	NM_025968	-15987	15.45	7.19	+	+++
chr9	62685970	62686969	14155	Fem1b	NM_010193	-27015	15.45	7.19	+	+++
chr2	4366224	4367223	209630	Frmd4a	AK043369	44703	15.44	7.44	+	+++
chr15	81763226	81764225	105859	Csdc2	NM_145473	-3463	15.40	7.16	+	+++
chr18	75813918	75814917	269037	Gm672	NM_201354	42933	15.39	7.41	+	+++
chr14	9164775	9165774	268709	Fam107a	NM_183187	-15196	15.21	6.59	+	+++
chr15	101835442	101836441	16691	Krt8	NM_031170	-1169	15.21	6.59	+	+++
chr19	14672447	14673446	21888	Tle1	AK017548	-474	15.21	6.59	+	+++
chr8	71659109	71660108	211134	Lzts1	NM_199364	5244	15.21	6.59	+	+++
chr1	137427233	137428232	215690	Nav1	NM_173437	-20705	15.16	7.54	+	+++
chr1	137555890	137556889	n/a	Nav1	AK158038	28293	15.16	7.54	+	+++
chr11	116379255	116380254	20698	Sphk1	NM_001172475	-13470	15.16	7.54	+	+++
chr11	68154210	68155209	18208	Ntn1	NM_008744	45619	15.16	7.54	+	+++
chr11	99846719	99847718	16671	Krt33b	NM_013570	43964	15.16	7.54	+	+++
chr18	60823221	60824220	104027	Synpo	NM_001109975	-33982	15.16	7.54	+	+++
chr2	160428596	160429595	21969	Top1	AK166844	-42537	15.16	7.54	+	+++
chr3	151922218	151923217	68810	Nexn	NM_199465	-6044	15.16	7.54	+	+++
chr4	122632286	122633285	76574	Mfsd2a	NM_029662	5646	15.16	7.54	+	+++
chr5	134574522	134575521	80909	Gatsl2	NM_030719	-596	15.16	7.54	+	+++
chr5	38935359	38936358	117591	Slc2a9	NM_001012363	-42468	15.16	7.54	+	+++
chr6	100681664	100682663	232313	Gxylt2	NM_198612	27437	15.16	7.54	+	+++
chr7	29512312	29513311	50760	Fbxo17	NM_015796	11004	15.16	7.54	+	+++
chr9	62705375	62706374	14155	Fem1b	NM_010193	-46420	15.16	7.54	+	+++
chr1	138580133	138581132	100503474	Gm16880	NR_037986	-12155	15.14	7.27	+	+++
chr1	134229925	134230924	213417	Klhdc8a	NM_144810	35223	15.10	6.76	+	+++
chr10	126705721	126706720	16572	Kif5a	NM_001039000	-5802	15.10	6.76	+	+++
chr1	161161541	161162540	26374	Rfwd2	NM_011931	-416	14.86	7.11	+	+++
chr11	101691081	101692080	18612	Etv4	NM_008815	-44957	14.86	7.11	+	+++
chr3	51505002	51506001	211666	Mgst2	NM_174995	40388	14.86	7.11	+	+++
chr7	106557886	106558885	233552	Gdpd5	NM_201352	28328	14.86	7.11	+	+++
chr8	108042242	108043241	67971	Tppp3	NM_026481	-47420	14.86	7.11	+	+++
chr7	29746508	29747507	26411	Map4k1	AK143215	-20061	14.85	7.36	+	+++
chr10	9620450	9621449	78808	Stxbp5	NM_001081344	-112	14.84	6.01	+	+++
chr17	26337677	26338676	14570	Arhgdig	NM_008113	119	14.84	6.01	+	+++
chr7	29593593	29594592	16855	Lgals4	NM_010706	-24918	14.84	6.01	+	+++
chr9	64956943	64957942	56741	Igdcc4	NM_020043	8142	14.84	6.01	+	+++
chr12	110140827	110141826	22375	Wars	NM_001164314	-8943	14.67	5.59	+	+++
chr9	20953904	20954903	18577	Pde4a	NM_183408	-15754	14.67	5.59	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr10	126962935	126963934	237611	Stac3	NM_177707	24663	12.86	6.48	+	+++
chr10	8218662	8219661	338362	Ust	NM_177387	19462	12.86	6.48	+	+++
chr11	62082249	62083248	11541	Adora2b	NM_007413	20264	12.83	6.47	+	+++
chr14	64088316	64089315	219148	Fam167a	NM_177628	33586	12.83	6.47	+	+++
chr2	49696373	49697372	71897	Lypd6b	NM_027990	-22458	12.83	6.47	+	+++
chr4	144804477	144805476	21938	Tnfrsf1b	NM_011610	31797	12.83	6.47	+	+++
chr7	106508178	106509177	233552	Gdpd5	NM_201352	-21381	12.83	6.47	+	+++
chr7	119368981	119369980	320878	Mical2	NM_177282	132	12.83	6.47	+	+++
chr7	29501407	29502406	50760	Fbxo17	NM_015796	99	12.83	6.47	+	+++
chr7	74529563	74530562	17258	Mef2a	AK153751	-12319	12.83	6.47	+	+++
chr1	135966917	135967916	14264	Fmod	NM_021355	33326	12.76	6.66	+	+++
chr10	5724516	5725515	13982	Esr1	NM_007956	9480	12.76	6.66	+	+++
chr19	53750512	53751511	73713	Rbm20	NM_001170847	-784	12.76	6.66	+	+++
chr8	13200691	13201690	66790	Grtp1	NM_025768	-567	12.76	6.66	+	+++
chr19	58507056	58508055	14585	Gfra1	NM_010279	21529	12.65	6.36	+	+++
chr5	136355413	136356412	15507	Hspb1	NM_013560	-7876	12.44	6.25	+	+++
chr1	145850929	145851928	19735	Rgs2	NM_009061	-150	12.40	5.80	+	+++
chr11	72973388	72974387	94045	P2rx5	NM_033321	-144	12.40	5.80	+	+++
chr13	74944576	74945575	12380	Cast	NM_009817	294	12.40	5.80	+	+++
chr14	41775792	41776791	52588	Tspan14	NM_145928	3805	12.40	5.80	+	+++
chr16	96413457	96414456	93961	B3galt5	NM_001122993	-43451	12.40	5.80	+	+++
chr3	51512375	51513374	211666	Mgst2	NM_174995	47761	12.40	5.80	+	+++
chr6	84537455	84538454	232174	Cyp26b1	NM_175475	678	12.40	5.80	+	+++
chr7	97450446	97451445	83671	Sytl2	NM_001289584	82	12.40	5.80	+	+++
chr9	71673419	71674418	21406	Tcf12	NM_011544	43668	12.40	5.80	+	+++
chr14	76979236	76980235	72661	Serp2	NR_027699	-23040	12.30	6.40	+	+++
chr15	101211844	101212843	110310	Krt7	NM_033073	-30490	12.24	4.76	+	+++
chr1	134242858	134243857	213417	Klhlhc8a	NM_144810	48156	12.22	5.35	+	+++
chr19	5963865	5964864	104252	Cdc42ep2	NM_026772	-39549	12.22	5.35	+	+++
chr1	192796185	192797184	226841	Vash2	NM_144879	6193	12.18	5.01	+	+++
chr1	20941273	20942272	74229	Paqr8	NM_028829	33853	12.00	6.46	+	+++
chr1	40822050	40823049	381339	Tmem182	NM_001081198	-39896	12.00	6.46	+	+++
chr1	89490997	89491996	23956	Neu2	NM_015750	373	12.00	6.46	+	+++
chr11	4151017	4152016	71962	Gatsl3	NM_028022	33264	12.00	6.46	+	+++
chr11	99846011	99847010	16671	Krt33b	NM_013570	44672	12.00	6.46	+	+++
chr12	81858998	81859997	217684	4933426M11Rik	NM_178682	-32048	12.00	6.46	+	+++
chr12	82731217	82732216	69480	Ttc9	NM_001033149	-639	12.00	6.46	+	+++
chr16	10394608	10395607	70426	Tekt5	NM_001099275	434	12.00	6.46	+	+++
chr19	10017033	10018032	16319	Incenp	NM_016692	-43510	12.00	6.46	+	+++
chr2	11387066	113879065	11464	Actc1	NM_009608	-19	12.00	6.46	+	+++
chr4	129959856	129960855	230779	Serinc2	NM_172702	-7526	12.00	6.46	+	+++
chr5	125359962	125360961	269704	Zfp664	NM_001081750	17388	12.00	6.46	+	+++
chr6	55087385	55088384	12922	Crhr2	NM_001288618	-4875	12.00	6.46	+	+++
chr7	119378652	119379651	320878	Mical2	NM_177282	9803	12.00	6.46	+	+++
chr8	126406006	126407005	11459	Acta1	NM_009606	12131	12.00	6.46	+	+++
chr15	97768843	97769842	22337	Vdr	NM_009504	-30616	11.88	6.16	+	+++
chr11	74252335	74253334	380711	Rap1gap2	NM_001015046	-14062	11.85	6.14	+	+++
chr14	9137270	9138269	268709	Fam107a	NM_183187	4521	11.85	6.14	+	+++
chr16	96421689	96422688	93961	B3galt5	NM_001122993	-35219	11.85	6.14	+	+++
chr19	5893564	5894563	104252	Cdc42ep2	NM_026772	24930	11.85	6.14	+	+++
chr5	123087201	123088200	18439	P2rx7	NM_001038887	-6219	11.85	6.14	+	+++
chr5	136364074	136365073	15507	Hspb1	NM_013560	786	11.85	6.14	+	+++
chr7	20362069	20363068	57278	Bcam	NM_020486	-6688	11.85	6.14	+	+++
chr10	12801934	12802933	22634	Plagl1	NM_009538	-8160	11.74	6.31	+	+++
chr18	76256345	76257344	207259	Zbtb7c	NM_145356	37734	11.67	5.83	+	+++
chr5	104854554	104855553	20750	Spp1	NM_001204203	-9076	11.67	5.83	+	+++
chr19	53785639	53786638	73713	Rbm20	NM_001170847	34344	11.53	5.56	+	+++
chr1	135967729	135968728	14264	Fmod	NM_021355	34138	11.50	5.54	+	+++
chr1	155489029	155490028	67792	Rgs8	NM_026380	-10638	11.50	5.54	+	+++
chr14	26661128	26662127	11744	Anxa11	NM_013469	-13	11.50	5.54	+	+++
chr15	81742197	81743196	105859	Csdc2	NM_145473	-24492	11.50	5.54	+	+++
chr17	74608577	74609576	76890	Memo1	NM_133771	7382	11.50	5.54	+	+++
chr4	106200570	106201569	74754	Dher24	NM_053272	-32573	11.50	5.54	+	+++
chr7	140032673	140033672	109359	Fam175b	NM_198017	-17735	11.50	5.54	+	+++
chr8	97672672	97673671	16582	Kifc3	NM_010631	-6732	11.50	5.54	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr9	49624122	49625121	17967	Ncam1	NM_001081445	-17448	11.50	5.54	+	+++
chr19	5429520	5430519	14283	Fosl1	NM_010235	-17678	11.39	4.82	+	+++
chr18	38760309	38761308	24066	Spry4	NM_011898	114	11.38	5.12	+	+++
chr19	5944438	5945437	104252	Cdc42ep2	NM_026772	-20122	11.38	5.12	+	+++
chr9	106349469	106350468	76491	Abhd14b	NM_029631	-1002	11.38	5.12	+	+++
chr19	53723136	53724135	73713	Rbm20	NM_001170847	-28160	11.31	5.64	+	+++
chr5	31418952	31419951	58522	Trim54	NM_021447	468	11.11	5.73	+	+++
chr11	58023999	58024998	16145	Igtp	AK165065	11442	11.00	6.10	+	+++
chr12	82127395	82128394	64075	Smoc1	NM_022316	101	11.00	6.10	+	+++
chr16	31412103	31413102	71911	Bdh1	NM_175177	-9780	11.00	6.10	+	+++
chr2	156226610	156227609	347740	2900097C17Rik	NR_024329	-8395	11.00	6.10	+	+++
chr3	84264597	84265596	229474	Fhdc1	NM_001033301	18317	11.00	6.10	+	+++
chr4	63240392	63241391	230279	6330416G13Rik	NM_144905	18947	11.00	6.10	+	+++
chr5	125371754	125372753	269704	Zfp664	NM_001081750	29180	11.00	6.10	+	+++
chr5	66985322	66986321	11787	Apbb2	NM_001201414	24235	11.00	6.10	+	+++
chr7	106453061	106454060	17760	Mtap6	NM_001043355	37605	11.00	6.10	+	+++
chr8	129587013	129588012	93742	Pard3	NM_033620	-443	11.00	6.10	+	+++
chr9	37055575	37056574	56857	Slc37a2	NM_001145960	7249	11.00	6.10	+	+++
chr12	110174609	110175608	22375	Wars	NM_001164314	-42725	10.92	5.83	+	+++
chr3	58177832	58178831	72033	Tsc22d2	NM_001081229	-41279	10.92	5.83	+	+++
chr11	16700157	16701156	13649	Egfr	NM_007912	48452	10.89	5.82	+	+++
chr12	103533641	103534640	217835	Rin3	NM_001161365	12291	10.89	5.82	+	+++
chr13	55479873	55480872	66890	Lman2	AK028452	-16229	10.89	5.82	+	+++
chr16	22844213	22845212	70573	Tbecd1	NM_001081368	12901	10.89	5.82	+	+++
chr2	156178604	156179603	347740	2900097C17Rik	NR_024329	39612	10.89	5.82	+	+++
chr7	107641202	107642201	22228	Ucp2	NM_011671	-152	10.89	5.82	+	+++
chr7	72017833	72018832	66647	Ndn12	NM_023239	-407	10.89	5.82	+	+++
chr7	97460706	97461705	83671	Sytl2	NM_001289584	10342	10.89	5.82	+	+++
chr8	71243574	71244573	234356	Csgalnact1	NM_172753	14968	10.89	5.82	+	+++
chr11	53888526	53889525	30794	Pdlim4	NM_019417	-6507	10.75	5.96	+	+++
chr11	4128273	4129272	71962	Gatsl3	NM_028022	10520	10.62	5.28	+	+++
chr2	122130747	122131746	213696	Duoxa1	NM_145395	8220	10.62	5.28	+	+++
chr5	138220662	138221661	243300	6430598A04Rik	NM_175521	-38327	10.62	5.28	+	+++
chr9	71673686	71674685	21406	Tcf12	NM_011544	43401	10.62	5.28	+	+++
chr6	90707250	90708249	232227	Iqsec1	NM_001134383	-41227	10.61	4.63	+	+++
chr1	135957055	135958054	14264	Fmod	NM_021355	23464	10.56	4.90	+	+++
chr7	29594122	29595121	16855	Lgals4	NM_010706	-24389	10.56	4.90	+	+++
chr14	63783119	63784118	14137	Fdft1	NM_010191	13710	10.28	5.68	+	+++
chr1	137540376	137541375	215690	Nav1	NM_173437	-37004	10.19	5.43	+	+++
chr19	4175079	4176078	58988	Rps6kb2	NM_021485	-12334	10.19	5.43	+	+++
chr2	157925619	157926618	21817	Tgm2	NM_009373	46010	10.19	5.43	+	+++
chr1	137381441	137382440	215690	Nav1	AK147844	-20606	10.02	5.74	+	+++
chr10	77694605	77695604	216131	Trappc10	NM_001081055	12283	10.02	5.74	+	+++
chr11	88763916	88764915	11640	Akap1	NM_001042541	-38516	10.02	5.74	+	+++
chr16	20604331	20605330	107522	Ece2	NM_177941	-6843	10.02	5.74	+	+++
chr19	11033921	11034920	69826	Ms4a10	AK008019	14740	10.02	5.74	+	+++
chr2	12932781	12933780	227580	C1ql3	NM_153155	-790	10.02	5.74	+	+++
chr2	79493316	79494315	70599	Ssfa2	NM_080558	14478	10.02	5.74	+	+++
chr3	142024760	142025759	56376	Pdlim5	NM_022554	29555	10.02	5.74	+	+++
chr4	59623011	59624010	230259	E130308A19Rik	NM_001015681	-15582	10.02	5.74	+	+++
chr5	53024941	53025940	211006	Sepsecs	NM_172490	35500	10.02	5.74	+	+++
chr5	99368487	99369486	19092	Prkg2	AK165904	3836	10.02	5.74	+	+++
chr6	113226908	113227907	18294	Ogg1	AK167456	-49562	10.02	5.74	+	+++
chr6	115667437	115668436	94346	Tmem40	NM_001168259	17033	10.02	5.74	+	+++
chr6	135245893	135246892	76400	Pbp2	NM_029595	14010	10.02	5.74	+	+++
chr6	37226785	37227784	320127	Dgki	NM_001081206	22692	10.02	5.74	+	+++
chr7	19543170	19544169	272359	Irf2bp1	NM_178757	-45744	10.02	5.74	+	+++
chr7	97450856	97451855	83671	Sytl2	NM_001289584	492	10.02	5.74	+	+++
chr8	126406630	126407629	11459	Acta1	NM_009606	11507	10.02	5.74	+	+++
chr8	71645050	71646049	211134	Lzts1	NM_199364	19303	10.02	5.74	+	+++
chr8	71244309	71245308	234356	Csgalnact1	NM_172753	14233	10.00	4.25	+	+++
chr13	46629144	46630143	67252	Cap2	AK049813	32373	9.95	5.50	+	+++
chr18	60792010	60793009	104027	Synpo	NM_001109975	-2771	9.95	5.50	+	+++
chr2	148197391	148198390	21824	Thbd	NM_009378	36034	9.95	5.50	+	+++
chr5	123526391	123527390	330189	Tmem120b	NM_001039723	608	9.95	5.50	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr5	136379430	136380429	15507	Hspb1	NM_013560	16142	9.95	5.50	+	+++
chr9	62706360	62707359	14155	Fem1b	NM_010193	-47405	9.95	5.50	+	+++
chr9	71722795	71723794	21406	Tcf12	NM_011544	-5709	9.95	5.50	+	+++
chr3	122465991	122466990	242202	Pde5a	AK031275	33352	9.85	5.24	+	+++
chr7	106363564	106364563	233549	Mogat2	NM_177448	23058	9.85	5.24	+	+++
chr6	135317848	135318847	13730	Emp1	NM_001288627	838	9.85	4.43	+	+++
chr3	27094151	27095150	320024	Nceh1	NM_178772	12726	9.76	5.01	+	+++
chr7	71984598	71985597	66647	Ndn12	NM_023239	32829	9.76	5.01	+	+++
chr10	93056401	93057400	57764	Ntn4	NM_021320	-46893	9.75	4.68	+	+++
chr2	128523545	128524544	17289	Mertk	NM_008587	-688	9.75	4.68	+	+++
chr5	125359192	125360191	269704	Zfp664	NM_001081750	16618	9.41	5.39	+	+++
chr9	119920111	119921110	22437	Xirp1	NM_011724	12106	9.29	4.08	+	+++
chr11	107616585	107617584	12299	Cacng1	NM_007582	-39295	9.06	5.19	+	+++
chr11	102573967	102574966	52715	Ccdc43	NM_025918	-15428	9.06	5.38	+	+++
chr14	64088933	64089932	219148	Fam167a	NM_177628	34203	9.06	5.38	+	+++
chr18	61949441	61950440	106877	Afap111	NM_178928	-3625	9.06	5.38	+	+++
chr2	113882669	113883668	11464	Actc1	NM_009608	-4622	9.06	5.38	+	+++
chr3	8698201	8699200	15213	Hey1	NM_010423	-31663	9.06	5.38	+	+++
chr4	58956085	58957084	67103	Ptgr1	NM_025968	43366	9.06	5.38	+	+++
chr5	123455797	123456796	109305	Orail	NM_175423	-8786	9.06	5.38	+	+++
chr7	132746459	132747458	233863	Gtf3c1	AK171723	46533	9.06	5.38	+	+++
chr7	73476761	73477760	233328	Lrrk1	AK040108	10891	9.06	5.38	+	+++
chr7	75408533	75409532	434197	Fam169b	NM_001013811	-9692	9.06	5.38	+	+++
chr9	110741537	110742536	74306	Prss46	NM_183103	-4973	9.04	5.17	+	+++
chr9	25335303	25336302	67484	Eepd1	NM_026189	46622	9.04	5.17	+	+++
chr15	57914052	57915051	70472	Atad2	BC032211	15780	8.98	4.95	+	+++
chr11	119047959	119048958	207592	Tbc1d16	NM_172443	41355	8.97	4.46	+	+++
chr3	51511623	51512622	211666	Mgst2	NM_174995	47009	8.97	4.46	+	+++
chr1	174227615	174228614	98660	Atp1a2	NM_178405	81	8.93	4.75	+	+++
chr14	26661678	26662677	11744	Anxa11	NM_013469	538	8.93	4.75	+	+++
chr4	102476941	102477940	73094	Sgip1	NM_144906	44474	8.93	4.75	+	+++
chr7	6238163	6239162	384763	Zfp667	NM_001024928	482	8.93	4.75	+	+++
chr9	64941828	64942827	56741	Igdcc4	NM_020043	-6974	8.93	4.75	+	+++
chr9	7783520	7784519	71929	Tmem123	AK202070	19944	8.93	4.75	+	+++
chr18	33614841	33615840	27528	D0H4S114	NM_053078	7948	8.85	5.26	+	+++
chr5	31277726	31278725	57742	Abhd1	NR_003522	25788	8.50	5.05	+	+++
chr12	103533192	103534191	217835	Rin3	NM_001161365	11842	8.38	4.05	+	+++
chr2	93310133	93311132	12521	Cd82	NM_001271431	-7526	8.38	4.05	+	+++
chr4	59016011	59017010	67103	Ptgr1	NM_025968	-16561	8.38	4.05	+	+++
chr7	53619216	53620215	17927	Myod1	NM_010866	-12128	8.38	4.05	+	+++
chr8	35088589	35089588	19663	Rbpms	NM_001286168	-48754	8.38	4.05	+	+++
chr9	71722331	71723330	21406	Tcf12	NM_011544	-5245	8.38	4.05	+	+++
chr17	35913563	35914562	15937	Ier3	NM_133662	-44595	8.21	4.23	+	+++
chr2	76208077	76209076	241489	Pde11a	NM_001081033	-31867	8.21	4.23	+	+++
chr13	45619176	45620175	66355	Gmpr	AK188187	3456	8.18	4.86	+	+++
chr1	132358761	132359760	13136	Cd55	NM_010016	57	8.15	4.85	+	+++
chr11	69869903	69870902	13385	Dlg4	NM_001109752	38297	8.15	4.85	+	+++
chr12	113263411	113264410	74691	Tdrd9	BC116656	32025	8.15	4.85	+	+++
chr14	70013851	70014850	94352	Loxl2	NM_033325	5069	8.15	4.85	+	+++
chr19	53966323	53967322	73713	Rbm20	BC038663	49124	8.15	4.85	+	+++
chr12	91975999	91976998	13371	Dio2	NM_010050	380	8.13	5.02	+	+++
chr17	5769966	5770965	224454	Zdhc14	BC021423	38995	8.13	5.02	+	+++
chr4	46676671	46677670	381605	Tbc1d2	NM_198664	-14100	8.13	5.02	+	+++
chr7	123637230	123638229	53322	Nucb2	NM_001130479	-10158	8.13	5.02	+	+++
chr7	149256451	149257450	18218	Dusp8	NM_008748	19221	8.13	5.02	+	+++
chr11	102589717	102590716	52715	Ccdc43	NM_025918	-31178	8.11	4.48	+	+++
chr14	9130638	9131637	268709	Fam107a	NM_183187	11153	8.11	4.48	+	+++
chr9	119910683	119911682	22437	Xirp1	NM_011724	21534	8.11	4.48	+	+++
chr3	90096349	90097348	78284	Creb3l4	NM_030080	-49415	7.67	3.86	+	+++
chr7	119432694	119433693	320878	Mical2	NM_001193305	-26349	7.67	3.86	+	+++
chr11	68220692	68221691	18208	Ntn1	NM_008744	-20864	7.58	4.52	+	+++
chr14	63754104	63755103	14137	Fdft1	NM_010191	42725	7.58	4.52	+	+++
chr16	44526362	44527361	117606	Boc	NM_172506	32122	7.47	4.01	+	+++
chr9	77440080	77441079	244923	Klhl31	NM_172925	-43959	7.32	4.22	+	+++
chr1	89797116	89798115	227333	Dgkd	BC048957	-34241	7.24	4.67	+	+++

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	129458864	129459863	19261	Sirpa	NM_001177647	40434	7.24	4.67	+	+++
chr8	71699881	71700880	211134	Lzts1	NM_199364	-35529	7.24	4.67	+	+++
chr1	89743239	89744238	227333	Dgkd	NM_177646	-6123	6.99	3.66	+	+++
chr6	100682917	100683916	232313	Gxylt2	NM_198612	28690	6.99	3.66	+	+++
chr10	45031151	45032150	78977	Popdc3	NM_024286	22541	6.98	3.90	+	+++
chr19	4162864	4163863	58988	Rps6kb2	NM_021485	-119	6.93	3.34	+	+++
chr6	90677745	90678744	232227	lqsec1	NM_001134383	-11722	6.81	4.09	+	+++
chr9	72296041	72297040	17427	Mns1	NM_008613	10206	6.81	4.09	+	+++
chr12	82731581	82732580	69480	Ttc9	NM_001033149	-275	6.75	3.79	+	+++
chr14	61876954	61877953	24053	Sgcg	NM_011892	-127	6.75	3.79	+	+++
chr14	64070457	64071456	219148	Fam167a	NM_177628	15727	6.56	3.96	+	+++
chr3	84264235	84265234	229474	Fhdc1	NM_001033301	18679	6.56	3.96	+	+++
chr13	37974070	37975069	68750	Rreb1	NR_033218	-5733	6.48	4.21	+	+++
chr2	4438927	4439926	209630	Frmd4a	NM_001177844	-41373	6.46	4.20	+	+++
chr6	34295723	34296722	11677	Akr1b3	NM_009658	-28734	6.46	4.20	+	+++
chr10	84883132	84884131	74007	Btbd11	NM_021320	34074	6.37	4.31	+	+++
chr11	102551007	102552006	52715	Ccdc43	AK161543	-1334	6.32	3.47	+	+++
chr4	33144999	33145998	52187	AK081204	AK081204	37865	6.32	3.47	+	+++
chr5	31276836	31277835	57742	Abhd1	NR_003522	24898	6.28	3.95	+	+++
chr3	129384975	129385974	13645	Egf	NM_010113	42530	6.05	3.56	+	+++
chr3	109112522	109113521	57257	Vav3	NM_020505	-30579	5.84	3.70	+	+++
chr10	27336188	27337187	16773	Lama2	AK042011	61	5.82	3.69	+	+++
chr19	56454508	56455507	18175	Nrap	NM_198059	-1354	5.82	3.69	+	+++
chr10	93104321	93105320	57764	Ntn4	NM_021320	1028	5.68	3.28	+	+++
chr16	96442789	96443788	93961	B3galt5	NM_001122993	-14119	5.66	3.88	+	+++
chr6	134767225	134768224	232430	Crebl2	NM_177687	-12492	5.66	3.88	+	+++
chr3	55074201	55075200	13175	Dclk1	NM_019978	28254	5.54	3.95	+	+++
chr11	115228438	115229437	237988	Cdr2l	NM_001080929	-14292	5.40	3.06	+	+++
chr5	66937545	66938544	11787	Apbb2	AK207143	-3189	5.40	3.06	+	+++
chr8	129964638	129965637	93742	Pard3	NM_001013581	35864	4.74	3.59	+	+++
chr1	136057574	136058573	12654	Chi3l1	NM_007695	-20907	4.70	3.44	+	+++
chr9	57315411	57316410	66812	Ppdc	NM_176831	-27990	182.60	42.67	+	+
chr2	29474666	29475665	107746	Rapgef1	NM_001039086	-74	154.21	37.50	+	+
chr15	3532194	3533193	14600	Ghr	NM_001048178	537	151.94	30.77	+	+
chr7	29123314	29124313	22695	Zfp36	NM_011756	40434	128.77	28.75	+	+
chr3	54538865	54539864	55994	Smad9	NM_019483	-20139	109.81	30.49	+	+
chr2	29932645	29933644	263803	Pkn3	AK154801	-118	102.78	29.43	+	+
chr15	81688282	81689281	11429	Aco2	NM_080633	-14111	96.48	17.67	+	+
chr6	114353286	114354285	15465	Hrh1	NM_001252643	127	91.92	22.08	+	+
chr2	127412566	127413565	77721	Mrps5	NM_029963	-96	90.27	22.16	+	+
chr10	17442429	17443428	17684	Cited2	NM_010828	-105	90.13	25.80	+	+
chr1	145549523	145550522	214498	Cdc73	NM_145991	-209	89.64	26.56	+	+
chr8	113240669	113241668	234729	Vac14	AK156589	4113	89.64	26.56	+	+
chr2	22895268	22896267	74159	Acbd5	NM_001102436	-27953	89.01	23.31	+	+
chr19	32831521	32832520	67979	Atad1	NM_026487	-45233	88.79	21.89	+	+
chr8	116962985	116963984	80707	Wwox	AK083096	-67	87.32	21.63	+	+
chr8	125897084	125898083	66855	Tcf25	NM_001286363	-27	87.32	21.63	+	+
chr19	3282471	3283470	12894	Cpt1a	NM_013495	-40330	86.23	24.25	+	+
chr17	56175684	56176683	106766	Stap2	NR_102313	-31155	80.01	22.01	+	+
chr15	77712950	77713949	56551	Txn2	NM_019913	45949	77.16	19.78	+	+
chr1	93146321	93147320	50880	Scly	NM_016717	-48094	76.99	22.31	+	+
chr7	28473789	28474788	22718	Zfp780b	AK044869	-42139	75.30	23.33	+	+
chr5	114221348	114222347	231633	Tmem119	NM_146162	28514	74.78	16.00	+	+
chr1	13579559	13580558	72265	Tram1	NM_028173	-114	73.96	21.66	+	+
chr7	17509069	17510068	19222	Ptgir	AK033567	17731	72.18	22.61	+	+
chr19	44181478	44182477	226144	Erlin1	NM_001164360	-37703	71.43	19.10	+	+
chr3	51212413	51213412	74838	Naa15	NM_053089	-7025	69.62	15.23	+	+
chr12	117643324	117644323	76044	Ncapg2	NM_133762	-51	69.46	20.69	+	+
chr11	98543386	98544385	57911	Gsdma	NM_021347	18222	69.10	21.89	+	+
chr11	61668165	61669164	29869	Ulk2	NM_013881	-71	67.53	16.26	+	+
chr12	117519083	117520082	217935	Wdr60	NM_146039	-18085	66.04	21.17	+	+
chr17	35030878	35031877	70129	Slc44a4	NM_023557	-20033	66.04	21.17	+	+
chr5	101227205	101228204	191578	Helq	NM_001081107	-86	66.04	21.17	+	+
chr17	45700303	45701302	18037	Nfkbie	NM_008690	8139	65.12	20.68	+	+
chr15	5066089	5067088	105787	Prkaa1	NM_001013367	-27272	64.71	19.55	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	105239014	105240013	19672	Rcn1	NM_009037	-38	63.58	15.59	+	+
chr1	174306087	174307086	16524	Kcnj9	NM_008429	-47193	63.01	20.46	+	+
chr11	80022020	80023019	71956	Rnf135	BC145236	25147	62.14	19.53	+	+
chr9	110003076	110004075	17758	Mtap4	NM_008633	38438	61.50	20.10	+	+
chr15	102796506	102797505	15425	Hoxc6	NM_010465	-32139	60.77	16.06	+	+
chr15	81296142	81297141	56438	Rbx1	NM_019712	-104	60.49	19.55	+	+
chr17	43013103	43014102	12488	Cd2ap	NM_009847	-230	58.63	16.91	+	+
chr5	24191261	24192260	72252	1700022A21Rik	NR_003953	40491	58.52	19.38	+	+
chr5	31495038	31496037	192292	Nrbp1	NM_147201	-47753	57.78	18.10	+	+
chr17	45710350	45711349	18037	Nfkbie	NM_008690	18186	57.13	14.48	+	+
chr6	48543351	48544350	71660	Rarres2	NM_027852	-21182	57.04	19.02	+	+
chr15	98748061	98749060	74775	Lmbr11	NM_029098	-32	56.44	17.83	+	+
chr16	93808017	93809016	n/a	AK009785	AK009785	-13384	56.37	15.83	+	+
chr18	69503575	69504574	21413	Tcf4	NM_013685	-71	56.37	15.83	+	+
chr12	77938160	77939159	104886	Rab15	NM_134050	-15149	55.64	16.61	+	+
chr11	77576219	77577218	360013	Myo18a	AK146852	-84	54.48	14.96	+	+
chr11	117710007	117711006	11799	Birc5	NM_001012273	-44	54.10	18.30	+	+
chr17	45692068	45693067	18037	Nfkbie	NM_008690	-97	54.10	18.30	+	+
chr2	118591852	118592851	214240	Disp2	NM_170593	-13103	54.10	18.30	+	+
chr4	108023079	108024078	414872	Zyg11b	NM_001033634	-49884	53.52	17.13	+	+
chr17	45734430	45735429	18037	Nfkbie	NM_008690	42266	53.48	12.72	+	+
chr7	87169759	87170758	209225	Zfp710	NM_001145999	560	52.64	17.94	+	+
chr10	127104292	127105291	20852	Stat6	NM_009284	24751	52.02	17.18	+	+
chr2	166904856	166905855	98999	Znfx1	NM_001033196	-16841	51.20	17.58	+	+
chr1	133106269	133107268	54354	Rassf5	NM_018750	-10094	51.08	14.77	+	+
chr11	22890098	22891097	12464	Cct4	NM_009837	6	51.08	14.77	+	+
chr11	80191048	80192047	70591	5730455P16Rik	NM_027472	-31	50.72	16.49	+	+
chr12	71925825	71926824	319710	Frmd6	NM_028127	-176	50.72	16.49	+	+
chr15	76778512	76779511	117171	1110038F14Rik	NM_054099	39	50.44	17.17	+	+
chr11	51576646	51577645	77371	Sec24a	AK038836	-10	49.36	11.22	+	+
chr11	105042295	105043294	73431	1700052K11Rik	NR_027956	-48	49.33	16.16	+	+
chr16	4213604	4214603	12914	Crebbp	NM_001025432	-700	49.33	16.16	+	+
chr5	143582945	143583944	231861	Tnrc18	NM_178242	-4379	49.28	15.36	+	+
chr11	77304268	77305267	216963	Git1	NM_001004144	-2146	48.33	16.87	+	+
chr6	122258416	122259415	13619	Phc1	S73884	14916	48.04	15.88	+	+
chr12	56565426	56566425	18035	Nfkbia	NM_010907	27709	47.85	16.61	+	+
chr16	32246683	32247682	69576	2310010M20Rik	NM_183283	-24512	46.91	16.51	+	+
chr15	81661542	81662541	11429	Aco2	NM_080633	-40851	46.42	15.83	+	+
chr7	4088044	4089043	243813	Leng9	NM_175529	12931	46.42	15.83	+	+
chr15	99802781	99803780	70281	2310068J16Rik	NR_028124	438	45.62	13.92	+	+
chr7	109146712	109147711	101706	Numa1	NM_133947	399	45.50	16.15	+	+
chr5	138557639	138558638	74570	Zkscan1	NM_029869	24216	45.24	11.38	+	+
chr11	3308734	3309733	71523	8430429K09Rik	NR_028317	-43206	45.22	15.19	+	+
chr15	5093267	5094266	105787	Prkaa1	NM_001013367	-94	45.22	15.19	+	+
chr5	135382712	135383711	76629	Wbscr28	NM_194065	-609	45.22	15.19	+	+
chr10	128242112	128243111	73827	1110012D08Rik	NM_178066	-1186	44.92	15.84	+	+
chr5	31504256	31505255	192292	Nrbp1	NM_147201	-38535	44.64	13.45	+	+
chr6	52100266	52101265	72628	2700086A05Rik	AK146012	-24450	44.64	13.45	+	+
chr17	45733651	45734650	18037	Nfkbie	NM_008690	41487	44.49	15.20	+	+
chr15	76729846	76730845	223669	Zfp7	NM_145916	20595	44.09	11.18	+	+
chr11	115425153	115426152	15374	Hn1	NM_008258	-49969	43.54	12.03	+	+
chr17	48061270	48062269	74123	Foxp4	NM_028767	-189	43.38	13.19	+	+
chr10	110943073	110944072	382421	EG382421	NR_033603	5730	43.31	15.36	+	+
chr13	99423804	99424803	380863	Tmem171	NM_001025606	40483	43.25	14.17	+	+
chr11	3863435	3864434	64934	Pes1	NM_022889	-43	42.92	14.37	+	+
chr2	63935805	63936804	60344	Fign	NM_001267846	-210	42.70	15.43	+	+
chr2	144194225	144195224	228714	Csrp2bp	NM_001166640	7	42.53	14.55	+	+
chr3	138152033	138153032	75624	Metap1	NM_175224	-187	42.53	14.55	+	+
chr15	98461593	98462592	74351	Ddx23	NM_001080981	31203	42.30	13.69	+	+
chr15	53177301	53178300	14042	Ext1	NM_010162	-63	42.27	12.36	+	+
chr10	126415395	126416394	11567	Avil	NM_009635	-21870	42.13	12.92	+	+
chr15	102054664	102055663	16421	Itgb7	NM_013566	7203	41.82	14.95	+	+
chr2	166821361	166822360	98999	Znfx1	NM_001291162	47135	41.75	12.43	+	+
chr9	116083901	116084900	21813	Tgfbr2	NM_009371	81	41.60	14.06	+	+
chr11	86496951	86497950	75909	Tmem49	NM_029478	-127	41.32	15.07	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	152119258	152120257	67231	Tbc1d20	AK036751	151	41.32	15.07	+	+
chr4	140257939	140258938	18602	Padi4	AK169844	47990	41.32	15.07	+	+
chr15	78267049	78268048	246221	Mpst	NM_138670	27138	40.96	12.70	+	+
chr4	122562683	122563682	19063	Ppt1	NM_008917	49714	40.88	12.66	+	+
chr12	3426192	3427191	100043040	1110002L01Rik	AK031200	56	40.68	14.72	+	+
chr11	120050869	120051868	72055	Slc38a10	NM_001164800	-38704	39.95	14.71	+	+
chr2	167513939	167514938	329562	A530013C23Rik	AK166336	-1820	39.95	14.71	+	+
chr5	3152577	31523576	192292	Nrbp1	NM_147201	-20214	39.95	14.71	+	+
chr2	33070910	33071909	26360	Angptl2	NM_011923	-71	39.94	13.94	+	+
chr5	31350277	31351276	330064	Slc5a6	NM_001177621	159	39.93	14.31	+	+
chr1	173340967	173341966	16456	F11r	NM_172647	-26225	39.87	13.90	+	+
chr18	12286527	12287526	76482	3110002H16Rik	NM_029623	-40212	39.87	13.90	+	+
chr2	167505163	167506162	329562	A530013C23Rik	AK166336	-10596	39.87	13.90	+	+
chr5	21207037	21208036	242864	Napepld	NM_178728	-325	39.87	13.90	+	+
chr2	166888156	166889155	98999	Znfx1	NM_001033196	-141	39.65	12.40	+	+
chr2	125450441	125451440	58521	Eid1	NM_025613	-47895	39.35	13.27	+	+
chr15	3532962	3533961	14600	Ghr	NM_001048178	-232	39.26	13.22	+	+
chr15	41619895	41620894	n/a	AK085881	AK085881	63	38.83	11.14	+	+
chr15	81977086	81978085	20788	Sreb12	NM_033218	-113	38.42	12.13	+	+
chr3	144327594	144328593	54673	AK086477	AK086477	24168	38.42	12.13	+	+
chr12	114033622	114034621	217887	BC022687	NM_145450	-13064	38.08	14.08	+	+
chr17	79335861	79336860	73694	2410091C18Rik	NM_028611	-114	38.07	12.96	+	+
chr12	112229993	112230992	217864	Rcor1	NM_198023	-47516	37.98	14.02	+	+
chr7	100023190	100024189	72461	Prp	AK011816	-73	37.98	14.02	+	+
chr2	158620015	158621014	71878	Fam83d	NM_027975	13372	37.69	13.12	+	+
chr8	126544811	126545810	56199	Abcb10	NM_019552	-38289	37.68	10.92	+	+
chr9	24955879	24956878	80517	Herpud2	NM_020586	-99	37.63	12.40	+	+
chr6	57485181	57486180	243382	Ppm1k	NM_175523	-261	37.35	10.03	+	+
chr13	12553296	12554295	56048	Lgals8	NM_001199043	210	37.26	13.25	+	+
chr15	82066719	82067718	66570	Cenpm	NM_001080158	7548	37.26	13.25	+	+
chr1	123223944	123224943	72999	Insig2	NM_133748	-105	37.24	14.00	+	+
chr11	74709560	74710559	97761	Sgsm2	NM_197943	523	37.24	14.00	+	+
chr3	96081581	96082580	97122	Hist2h4	NM_033596	-14841	37.24	14.00	+	+
chr4	107973123	107974122	414872	Zyg11b	NM_001033634	73	37.24	14.00	+	+
chr4	155571434	155572433	231002	Plekhn1	NM_001008233	25614	37.24	14.00	+	+
chr9	72832740	72833739	67685	Dyx1c1	NM_026314	26649	37.24	14.00	+	+
chr11	117784317	117785316	71776	Tha1	AY219872	-49977	37.20	11.87	+	+
chr6	54319584	54320583	78004	Prr15	NM_030024	43079	37.20	11.87	+	+
chr8	125966469	125967468	23854	Def8	NM_054046	84	37.20	11.87	+	+
chr14	62266481	62267480	668253	Dleu2	AK033134	479	37.05	13.89	+	+
chr11	3434947	3435946	29856	Smtn	NM_013870	3849	36.81	12.66	+	+
chr12	56592369	56593368	18035	Nfkb1a	NM_010907	766	36.64	13.67	+	+
chr17	46169297	46170296	22339	Vegfa	NM_001025250	-471	36.39	12.12	+	+
chr11	69418914	69419913	13643	Efnb3	NM_007911	-45675	36.03	12.97	+	+
chr10	85484133	85485132	69754	Fbxo7	NM_153195	-41	35.99	11.61	+	+
chr9	121240884	121241883	67095	Trak1	BC046285	-34692	35.99	11.61	+	+
chr11	75323801	75324800	280408	Rilp	NM_001029938	706	35.96	12.93	+	+
chr2	32568648	32569647	30933	Tor2a	NR_045485	-43606	35.96	12.93	+	+
chr7	148660736	148661735	12476	Cd151	NM_001111050	6549	35.96	12.93	+	+
chr11	101034741	101035740	53321	Cntnap1	NM_016782	-2190	35.90	13.64	+	+
chr17	31806745	31807744	12954	Cryaa	NM_001278570	-7631	35.90	13.64	+	+
chr2	26244201	26245200	67839	Gpsm1	NM_001199146	46893	35.90	13.64	+	+
chr5	24082821	24083820	100910	Chpf2	NM_133913	-9247	35.52	13.43	+	+
chr4	35172402	35173401	73205	3110043O21Rik	NM_001081343	228	35.32	13.32	+	+
chr1	99873148	99874147	18484	Pam	AK049423	189	35.29	12.93	+	+
chr11	21471463	21472462	216560	AV249152	NM_145425	-321	34.75	12.64	+	+
chr9	102528343	102529342	28135	Cep63	NM_001301689	-389	34.75	12.64	+	+
chr18	36719719	36720718	15200	Hbegf	NM_010415	-44760	34.68	12.61	+	+
chr7	38845842	38846841	12447	Ccne1	NM_007633	46168	34.68	12.61	+	+
chr9	15083919	15084918	75316	Taf1d	NM_027261	-26239	34.68	12.61	+	+
chr19	5023747	5024746	225870	Rin1	NM_145495	-26561	34.57	13.28	+	+
chr3	107434098	107435097	11694	Alx3	NM_007441	36650	34.57	13.28	+	+
chr8	108145979	108146978	13018	Ctcf	NM_181322	-13959	34.57	13.28	+	+
chr17	29751358	29752357	381085	Tbc1d22b	BC060066	42025	34.37	12.80	+	+
chr4	155610143	155611142	231002	Plekhn1	BC051407	-7992	34.17	8.84	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr11	72831954	72832953	66874	I200014J11Rik	NM_025818	-28915	34.01	12.97	+	+
chr3	96066616	96067615	97122	Hist2h4	NM_033596	125	34.01	12.97	+	+
chr15	77718358	77719357	56551	Txn2	NM_019913	40541	33.81	11.81	+	+
chr16	17530684	17531683	66863	Lztr1	AK088938	10201	33.68	11.11	+	+
chr11	74711012	74712011	97761	Sgsm2	NM_197943	-930	33.41	12.28	+	+
chr14	9046136	9047135	71393	Kctd6	NM_027782	-26	33.41	12.28	+	+
chr19	4397019	4398018	225876	Kdm2a	NM_001001984	-442	33.41	12.28	+	+
chr7	100022621	100023620	72461	Prp	AK011816	-642	33.41	12.28	+	+
chr2	118570894	118571893	214240	Disp2	NM_170593	-34061	33.25	12.92	+	+
chr2	25283538	25284537	11305	Abca2	NM_007379	-156	33.25	12.92	+	+
chr8	114425662	114426661	56773	Chst5	NM_019950	7938	33.25	12.92	+	+
chr5	34529404	34530403	17122	Mxd4	NM_010753	456	33.08	12.83	+	+
chr15	100298774	100299773	68614	Letmd1	NM_134093	-191	32.98	10.78	+	+
chr10	117146794	117147793	70574	Cpm	NM_027468	34346	32.74	11.27	+	+
chr11	101163081	101164080	56208	Becn1	AK077392	1	32.74	11.27	+	+
chr8	72273259	72274258	66869	Zfp869	NM_181274	-32912	32.50	10.85	+	+
chr1	136852045	136853044	67196	Ube2t	NM_001278115	-6597	32.15	11.96	+	+
chr19	4037285	4038284	29866	Cabp2	NM_001160253	-44703	32.15	11.96	+	+
chr5	112699938	112700937	22022	Tpst2	NM_009419	-5289	32.15	11.96	+	+
chr9	107640177	107641176	83486	Rbm5	NM_148930	14729	32.15	11.96	+	+
chr6	56664569	56665568	78937	Avl9	NM_030235	171	32.12	8.50	+	+
chr6	124880484	124881483	69202	Ptms	NM_026988	-13020	32.07	12.27	+	+
chr15	76701298	76702297	223669	Zfp7	NM_145916	-7908	31.95	12.56	+	+
chr2	118604943	118605942	214240	Disp2	NM_170593	-12	31.95	12.56	+	+
chr7	66483522	66484521	22215	Ube3a	NM_001033962	-98	31.95	12.56	+	+
chr2	22924492	22925491	74159	Acbd5	NM_001102438	401	31.42	12.27	+	+
chr15	102069387	102070386	19411	Rarg	NM_001042727	6897	31.26	10.55	+	+
chr3	108456267	108457266	229725	Clec1	NM_145543	-64	31.26	10.55	+	+
chr16	94790695	94791694	13185	Dscr3	NM_007834	-42959	31.15	12.12	+	+
chr1	95408316	95409315	227377	Farp2	NM_145519	136	31.08	11.07	+	+
chr16	94747728	94748727	13185	Dscr3	NM_007834	9	30.97	12.02	+	+
chr1	87689445	87690444	12283	Cab39	NM_133781	-77	30.92	9.58	+	+
chr19	5724707	5725706	78891	Scyl1	NM_023912	46195	30.84	10.64	+	+
chr10	98725253	98726252	67603	Dusp6	NM_026268	-112	30.80	11.93	+	+
chr11	72047172	72048171	67279	Med31	NM_026068	-18578	30.66	12.20	+	+
chr13	62957330	62958329	14121	Fbp1	NM_019395	31767	30.66	12.20	+	+
chr15	76803929	76804928	117171	I110038F14Rik	NM_054099	25371	30.66	12.20	+	+
chr17	24387707	24388706	12449	Ccnf	NM_007634	148	30.66	12.20	+	+
chr19	6995921	6996920	26379	Esrra	NM_007953	-123	30.66	12.20	+	+
chr4	155576764	155577763	231002	Plekhn1	NM_001008233	20284	30.66	12.20	+	+
chr4	57580366	57581365	242481	Palm2	NM_172868	-254	30.66	12.20	+	+
chr6	8208821	8209820	170772	Glicc1	NM_133236	34	30.66	12.20	+	+
chr8	108384128	108385127	74334	Ranbp10	NM_145824	-33378	30.66	12.20	+	+
chr8	113142055	113143054	234729	Vac14	NM_146216	18	30.66	12.20	+	+
chr6	30846116	30847115	54160	Copg2	NM_017478	145	30.59	12.16	+	+
chr18	34665828	34666827	225358	Fam13b	NM_146084	150	30.55	11.79	+	+
chr5	74038405	74039404	24051	Sgcb	NM_011890	66	30.55	11.79	+	+
chr12	114035596	114036595	217887	BC022687	NM_145450	-11090	30.36	10.71	+	+
chr14	47451318	47452317	63985	Gmfb	NM_022023	-9901	30.36	10.71	+	+
chr11	23397298	23398297	67358	I700093K21Rik	NM_026105	21398	30.17	11.25	+	+
chr4	115500586	115501585	17346	Mknk1	NM_001285487	-10717	30.17	11.25	+	+
chr4	123594099	123595098	54170	Rragc	NM_017475	-77	30.14	11.92	+	+
chr9	47337744	47338743	54725	Cadm1	NM_001025600	-191	29.83	9.36	+	+
chr10	127103055	127104054	20852	Stat6	NM_009284	23514	29.81	11.39	+	+
chr1	193893811	193894810	n/a	AK016486	AK016486	-21942	29.67	11.31	+	+
chr8	114250168	114251167	12927	Bear1	AK138372	5369	29.67	11.31	+	+
chr11	51569169	51570168	77371	Sec24a	BC117906	668	29.38	11.84	+	+
chr12	88540666	88541665	71275	4933437F05Rik	NM_027744	38386	29.38	11.84	+	+
chr16	93831985	93832984	n/a	AK009785	AK009785	-37352	29.38	11.84	+	+
chr17	29686117	29687116	381085	Tbc1d22b	NM_198647	-130	29.38	11.84	+	+
chr2	26788476	26789475	20930	Surf1	NR_073420	-16926	29.38	11.84	+	+
chr7	91030919	91031918	67943	Mesdc2	NM_023403	-9091	29.38	11.84	+	+
chr4	140885600	140886599	13836	Epha2	NM_010139	28946	28.96	10.02	+	+
chr9	57268136	57269135	66812	Ppcdc	NM_176831	19286	28.96	10.02	+	+
chr11	112642633	112643632	217294	BC006965	AK035132	158	28.88	11.57	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr11	16850791	16851790	72735	Eldr	NR_110421	-6	28.88	11.57	+	+
chr19	21346165	21347164	22682	Zfand5	NM_009551	-103	28.70	10.49	+	+
chr5	33361044	33362043	22629	Ywhah	NM_011738	80	28.52	10.10	+	+
chr12	8506123	8507122	11852	Rhob	NM_007483	169	28.50	11.02	+	+
chr2	144266836	144267835	228714	Csrp2bp	AK088564	39254	28.50	11.02	+	+
chr11	6174442	6175441	18293	Ogdh	NM_010956	-16658	28.44	10.99	+	+
chr17	35035225	35036224	70129	Slc44a4	NM_023557	-15686	28.44	10.99	+	+
chr17	47729970	47730969	224836	Usp49	NM_198421	-18075	28.44	10.99	+	+
chr4	141093899	141094898	320456	B330016D10Rik	NR_030695	-7678	28.44	10.99	+	+
chr5	116064535	116065534	231659	Gen111	NM_172719	9331	28.44	10.99	+	+
chr5	37086805	37087804	320661	D5Erttd579e	NM_001081232	-97	28.44	10.99	+	+
chr5	37103913	37104912	320661	D5Erttd579e	NM_001081232	-17205	28.44	10.99	+	+
chr8	108128585	108129584	13018	Ctcf	NM_181322	-31353	28.44	10.99	+	+
chr15	77136658	77137657	93686	Rbfox2	NM_053104	326	28.22	10.55	+	+
chr6	29161561	29162560	23917	Impdh1	NM_011829	211	28.22	10.55	+	+
chr1	34899015	34900014	226970	Arhgef4	NM_183019	40049	28.11	11.48	+	+
chr1	74795009	74796008	241113	Prkag3	NM_153744	21	28.11	11.48	+	+
chr12	4483728	4484727	17977	Ncoa1	NM_010881	-168	28.11	11.48	+	+
chr19	3323521	3324520	12894	Cpt1a	NM_013495	721	28.11	11.48	+	+
chr2	26180978	26181977	67839	Gpsm1	NM_153410	2451	28.11	11.48	+	+
chr5	31543197	31544196	192292	Nrbp1	NM_147201	407	28.11	11.48	+	+
chr7	19678949	19679948	20475	Six5	NM_011383	-444	28.11	11.48	+	+
chr8	114269166	114270165	12927	Bcar1	NM_009954	-1917	28.11	11.48	+	+
chr9	46037647	46038646	66113	Apoa5	NM_080434	-38544	28.11	11.48	+	+
chr15	75799000	75799999	105732	Fam83h	NM_001168253	40429	27.82	9.76	+	+
chr17	25969741	25970740	106618	Wdr90	AK129465	28171	27.82	9.76	+	+
chr9	121250653	121251652	67095	Trak1	BC046285	-24923	27.82	9.76	+	+
chr4	140828904	140829903	13836	Epha2	NM_010139	-27751	27.82	8.29	+	+
chr2	118587206	118588205	214240	Disp2	NM_170593	-17749	27.80	9.47	+	+
chr2	166904128	166905127	98999	Znfx1	NM_001033196	-16113	27.63	11.22	+	+
chr9	102534157	102535156	28135	Cep63	NM_001301689	-6203	27.29	10.70	+	+
chr16	10992682	10993681	56722	Litaf	NM_019980	33	27.28	9.50	+	+
chr11	115375123	115376122	15374	Hn1	NM_008258	62	27.23	10.67	+	+
chr11	50744626	50745625	19127	Prop1	NM_008936	22009	27.23	10.67	+	+
chr19	10783542	10784541	12507	Cd5	NM_007650	29423	27.23	10.67	+	+
chr6	29145608	29146607	23917	Impdh1	NM_011829	16164	27.23	10.67	+	+
chr10	62705984	62706983	68802	Mypn	NM_182992	-39784	26.99	10.86	+	+
chr1	134018221	134019220	18557	Cdk18	NM_008795	17542	26.85	11.13	+	+
chr16	10956798	10957797	56722	Litaf	NM_019980	35917	26.85	11.13	+	+
chr3	110052610	110053609	99890	Prmt6	NM_178891	807	26.85	11.13	+	+
chr8	114255583	114256582	12927	Bcar1	AK138372	-47	26.85	11.13	+	+
chr9	107611503	107612502	83486	Rbm5	NM_148930	43403	26.85	11.13	+	+
chr1	188528784	188529783	21808	Tgfb2	NM_009367	588	26.83	10.78	+	+
chr15	81790765	81791764	29858	Pmm1	NM_013872	33	26.83	10.78	+	+
chr7	27980530	27981529	12587	Mia1	NM_019394	-14862	26.83	10.78	+	+
chr7	35014657	35015656	14751	Gpi1	NM_008155	168	26.83	10.78	+	+
chr11	6191576	6192575	18293	Ogdh	NM_010956	427	26.80	8.10	+	+
chr12	109792491	109793490	68519	Eml1	AK132592	19917	26.70	9.50	+	+
chr14	55172917	55173916	219072	Haus4	NM_145462	-219	26.70	9.50	+	+
chr17	79234314	79235313	19106	Eif2ak2	NM_011163	47099	26.70	9.50	+	+
chr15	100443760	100444759	207818	Smagp	NM_174992	23027	26.67	8.96	+	+
chr8	86522051	86523050	70134	2210011C24Rik	NM_001291292	13069	26.64	9.47	+	+
chr9	108165838	108166837	13138	Dag1	NM_001276485	-49	26.63	11.00	+	+
chr10	80039639	80040638	118445	Klfl6	NM_078477	-98	26.62	8.69	+	+
chr12	56547767	56548766	18035	Nfkbia	NM_010907	45368	26.62	8.69	+	+
chr15	83372057	83373056	223722	Mcat	NM_001030014	13585	26.62	8.69	+	+
chr6	125165121	125166120	21940	Cd27	NM_001042564	21425	26.62	8.69	+	+
chr11	68504378	68505377	77579	Myh10	AK047340	-539	26.56	10.31	+	+
chr17	25998018	25999017	106618	Wdr90	NM_001163766	-58	26.56	10.31	+	+
chr10	80220132	80221131	208266	Dot11	NM_199322	2682	26.39	10.86	+	+
chr13	43399249	43400248	328232	Gfod1	AK053379	-208	26.24	9.55	+	+
chr15	82074728	82075727	66570	Cenpm	NM_178269	-51	26.17	9.24	+	+
chr1	60236631	60237630	269198	Nbeal1	NM_173444	-312	26.03	10.34	+	+
chr12	112127150	112128149	72805	Zfp839	NM_001199785	39162	26.03	10.34	+	+
chr4	131604398	131605397	269587	Epb4.1	NM_001128606	96	26.03	10.34	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr5	149254928	149255927	11987	Slc7a1	NM_007513	-43948	26.03	10.34	+	+
chr6	86142625	86143624	21802	Tgfa	AK165017	-2120	26.03	10.34	+	+
chr7	49666416	4967415	319748	Zfp865	NM_001033383	-5062	26.03	10.34	+	+
chr8	114400199	114401198	56773	Chst5	NM_019950	33401	26.03	10.34	+	+
chr11	112643212	112644211	217294	BC006965	AK035132	-422	25.73	9.58	+	+
chr13	34967190	34968189	19134	Prpf4b	NM_013830	328	25.61	10.77	+	+
chr17	57199322	57200321	66972	Slc25a23	NM_025877	-536	25.61	10.77	+	+
chr2	142726810	142727809	16558	Kif16b	NM_001081133	-110	25.61	10.77	+	+
chr4	120419614	120420613	60613	Kcnq4	NM_001081142	-333	25.61	10.77	+	+
chr4	155405523	155406522	21936	Tnfrsf18	NM_021985	5573	25.61	10.77	+	+
chr5	114239135	114240134	231633	Tmem119	NM_146162	10727	25.61	10.77	+	+
chr8	108120294	108121293	13018	Ctcf	NM_181322	-39644	25.61	10.77	+	+
chr8	116159030	116160029	270096	Mon1b	NM_001048143	45	25.61	10.77	+	+
chr18	61797354	61798353	56069	Il17b	AK003506	-49715	25.60	8.97	+	+
chr10	62891872	62892871	30045	Dnajc12	NM_013888	43062	25.59	9.23	+	+
chr11	95685132	95686131	66610	Abi3	NM_025659	18122	25.59	9.23	+	+
chr15	83401807	83402806	223722	Mcat	NM_001030014	-16166	25.59	9.23	+	+
chr9	70389783	70390782	66660	Met	BC145577	-302	25.59	9.23	+	+
chr2	26206928	26207927	67839	Gpsm1	NM_001199146	9620	25.57	8.47	+	+
chr4	123581576	123582575	54170	Rragc	NM_017475	-12600	25.57	8.47	+	+
chr12	86476748	86477747	73422	Prox2	NM_175198	-29867	25.38	10.00	+	+
chr16	33941136	33942135	545156	Kalrn	AK081504	37177	25.38	10.00	+	+
chr11	75275435	75276434	280408	Rilp	NM_001029938	-47661	25.22	9.61	+	+
chr1	92672190	92673189	n/a	Col6a3	AK076391	31964	24.85	10.02	+	+
chr15	78252706	78253705	246221	Mpst	NM_138670	12795	24.85	10.02	+	+
chr2	29921848	29922847	263803	Pkn3	AK154801	-10915	24.85	10.02	+	+
chr4	140539115	140540114	74772	Atp13a2	NM_001164366	-3173	24.85	10.02	+	+
chr6	116287540	116288539	71779	March8	NM_001302385	1	24.85	10.02	+	+
chr2	121274825	121275824	574418	Serinc4	NM_001025371	7176	24.72	9.65	+	+
chr3	104315726	104316725	269473	Lrig2	NM_001025067	-447	24.72	9.65	+	+
chr7	109054083	109055082	101706	Numa1	NM_133947	-28042	24.72	9.65	+	+
chr2	127073021	127074020	26371	Ciao1	NM_025296	32	24.53	8.24	+	+
chr2	164657948	164658947	75642	1700020C07Rik	NM_029370	-4414	24.53	8.24	+	+
chr2	32612167	32613166	30933	Tor2a	NR_045485	-87	24.53	8.24	+	+
chr15	98464770	98465769	74351	Ddx23	NM_001080981	28026	24.49	8.97	+	+
chr19	4098712	4099711	29866	Cabp2	NM_001160252	15694	24.49	8.97	+	+
chr2	164687408	164688407	75642	1700020C07Rik	NM_029370	-33874	24.49	8.97	+	+
chr10	40446002	40447001	327747	9030224M15Rik	NM_177793	43415	24.39	10.41	+	+
chr11	101328779	101329778	11858	Rnd2	NM_009708	-373	24.39	10.41	+	+
chr11	79997059	79998058	71956	Rnf135	BC145236	186	24.39	10.41	+	+
chr13	115248821	115249820	14313	Fst	NM_001301373	-162	24.39	10.41	+	+
chr15	78258523	78259522	246221	Mpst	NM_138670	18612	24.39	10.41	+	+
chr2	142728353	142729352	16558	Kif16b	NM_001081133	-1653	24.39	10.41	+	+
chr2	181409847	181410846	18389	Oprl1	NR_045541	-39374	24.39	10.41	+	+
chr4	149382721	149383720	100198	H6pd	NM_173371	-89	24.39	10.41	+	+
chr5	150214147	150215146	70717	6330406I15Rik	NM_027519	267	24.39	10.41	+	+
chr7	112729196	112730195	15458	Hpx	NM_017371	18935	24.39	10.41	+	+
chr8	114249691	114250690	12927	Bear1	AK138372	5846	24.39	10.41	+	+
chr12	74140130	74141129	20471	Six1	NM_009189	7070	24.22	9.69	+	+
chr15	75811949	75812948	105732	Fam83h	NM_001168253	27480	24.22	9.69	+	+
chr10	127113618	127114617	20852	Stat6	NM_009284	34077	23.99	8.73	+	+
chr14	62300872	62301871	668253	Dleu2	AK139063	-162	23.99	8.73	+	+
chr3	144342471	144343470	54673	AK086477	AK086477	9291	23.95	10.16	+	+
chr16	31663281	31664280	13383	Dlg1	NM_001252433	-240	23.73	10.04	+	+
chr17	30748986	30749985	109801	Glo1	NM_025374	119	23.70	6.32	+	+
chr3	108204276	108205275	53883	Celsr2	NM_001004177	-2885	23.70	6.32	+	+
chr1	133049238	133050237	54354	Rassf5	NM_018750	46938	23.68	9.70	+	+
chr11	20148760	20149759	216543	Cep68	NM_172260	168	23.68	9.70	+	+
chr18	31963493	31964492	74320	Wdr33	NM_001170970	283	23.68	9.70	+	+
chr2	167486876	167487875	329562	A530013C23Rik	AK166336	-28883	23.68	9.70	+	+
chr15	76161321	76162320	68877	Maf1	NM_001164608	-19903	23.51	8.02	+	+
chr19	4090087	4091086	29866	Cabp2	NM_001160252	7069	23.51	8.02	+	+
chr5	31356610	31357609	330064	Slc5a6	NM_001177622	-5813	23.51	8.02	+	+
chr19	4096792	4097791	29866	Cabp2	NM_001160252	13774	23.40	8.70	+	+
chr2	120178231	120179230	211499	Tmem87a	AK134268	18636	23.40	8.70	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr2	3391461	3392460	50497	Hspa14	AK088996	38126	23.40	8.70	+	+
chr2	58999272	59000271	227937	Pkp4	NM_175464	866	23.40	8.70	+	+
chr8	48952089	48953088	75677	Cldn22	NM_029383	42754	23.40	8.70	+	+
chr11	3351923	3352922	71523	8430429K09Rik	NR_028317	-17	23.35	6.97	+	+
chr11	75299697	75300696	280408	Rilp	NM_001029938	-23399	23.24	9.77	+	+
chr11	96685165	96686164	18023	Nfe2l1	NM_001130451	-224	23.24	9.77	+	+
chr18	34822466	34823465	52563	Cdc23	NM_178347	-11576	23.24	9.77	+	+
chr10	76195310	76196309	12833	Col6a1	NM_009933	-7021	23.18	10.05	+	+
chr11	77276004	77277003	216961	Coro6	BC100532	619	23.18	10.05	+	+
chr13	56702258	56703257	21810	Tgfb1	AK135536	-8206	23.18	10.05	+	+
chr17	35068515	35069514	70129	Slc44a4	NM_023557	17605	23.18	10.05	+	+
chr19	4099610	4100609	29866	Cabp2	NM_001160252	16592	23.18	10.05	+	+
chr19	60887048	60888047	94281	Sfxn4	AK148530	49786	23.18	10.05	+	+
chr5	115956105	115957104	75387	Sirt4	NM_133760	-22112	23.12	10.02	+	+
chr17	45717529	45718528	18037	Nfkbi	NM_008690	25365	23.07	9.37	+	+
chr3	52820545	52821544	108927	Lhfp	NM_175386	-24424	23.07	9.37	+	+
chr17	13896860	13897859	21646	Tcte2	AK005879	1042	23.05	9.98	+	+
chr1	74843770	74844769	241113	Prkag3	NM_153744	-48741	23.03	9.65	+	+
chr13	63653511	63654510	19206	Ptch1	AB164616	11833	23.03	9.65	+	+
chr2	154875915	154876914	269378	Ahecy	NM_016661	23819	22.97	9.03	+	+
chr9	54433684	54434683	56506	Cib2	NM_019686	-26298	22.97	9.03	+	+
chr8	13471948	13472947	14456	Gas6	NM_019521	22088	22.92	8.73	+	+
chr19	5803024	5804023	78891	Scyl1	NM_023912	-32123	22.86	7.33	+	+
chr10	85802104	85803103	21859	Timp3	AK082483	39448	22.76	9.81	+	+
chr4	41222200	41223199	68970	Dcaf12	NM_026893	39235	22.76	9.81	+	+
chr6	83087725	83088724	22377	Wbp1	NM_016757	-16770	22.76	9.81	+	+
chr12	8457117	8458116	11852	Rhob	NM_007483	49175	22.57	9.40	+	+
chr11	3442746	3443745	29856	Smtn	NM_013870	-3951	22.52	9.37	+	+
chr4	154460057	154461056	76866	Morn1	NM_001081100	-129	22.52	9.37	+	+
chr7	4610178	4611177	243819	Ppp6r1	NM_172894	-126	22.52	9.37	+	+
chr8	108378418	108379417	74334	Ranbp10	NM_145824	-27668	22.52	9.37	+	+
chr8	26126664	26127663	11502	Adam9	NM_001270996	231	22.52	9.37	+	+
chr15	77633812	77634811	239552	Apol8	NM_001081970	-46900	22.49	7.80	+	+
chr15	85035906	85036905	14114	Fbln1	NM_010180	-32	22.49	7.80	+	+
chr18	36719015	36720014	15200	Hbegf	NM_010415	-44056	22.49	7.80	+	+
chr9	57353306	57354305	110119	Mpi	NM_025837	46754	22.49	7.80	+	+
chr4	134090486	134091485	69885	2610002D18Rik	NM_001081099	24073	22.45	9.05	+	+
chr17	46297391	46298390	75564	Rsph9	NM_029338	-16744	22.41	8.23	+	+
chr15	76210160	76211159	68877	Maf1	NM_001164608	28937	22.33	8.44	+	+
chr16	4789523	4790522	15369	Hmox2	NM_010443	32958	22.33	8.44	+	+
chr17	31795303	31796302	12954	Cryaa	NM_001278570	-19073	22.33	8.44	+	+
chr2	23010978	23011977	67121	Mastl	NM_025979	67	22.33	8.44	+	+
chr4	154440726	154441725	76866	Morn1	NM_001081100	-19460	22.33	8.44	+	+
chr5	108862967	108863966	243197	Mfsd7	BC094512	14444	22.33	8.44	+	+
chr5	36826551	36827550	66717	Ccdc96	NM_025725	-186	22.33	8.44	+	+
chr9	56007877	56008876	56434	Tspan3	NM_019793	501	22.33	8.44	+	+
chr10	81105603	81106602	70615	Ankrd24	NM_027480	2467	22.07	9.43	+	+
chr11	63734671	63735670	54710	Hs3st3b1	NM_018805	616	21.98	9.69	+	+
chr12	112088080	112089079	72805	Zfp839	NM_001199785	92	21.98	9.69	+	+
chr14	56203512	56204511	68680	Fitm1	NM_026808	9502	21.98	9.69	+	+
chr17	25031693	25032692	16005	Igfals	NM_008340	16479	21.98	9.69	+	+
chr3	20054253	20055252	20585	Hltf	AK079817	46274	21.98	9.69	+	+
chr7	30773707	30774706	72723	Zfp74	BC034883	-35126	21.98	9.69	+	+
chr8	116158550	116159549	270096	Mon1b	NM_001048143	-436	21.98	9.69	+	+
chr9	108006857	108007856	235606	Apeh	NM_146226	-10546	21.98	9.69	+	+
chr1	123263607	123264606	72999	Insig2	NM_178082	-34950	21.93	9.06	+	+
chr11	77304778	77305777	216963	Git1	NM_001004144	-1636	21.93	9.06	+	+
chr6	100783789	100784788	232314	Ppp4r2	NM_182939	658	21.93	9.06	+	+
chr15	83175089	83176088	69291	1700001L05Rik	NR_027980	22139	21.92	8.77	+	+
chr3	88717495	88718494	229524	Mstol	NM_144898	-123	21.90	7.13	+	+
chr15	57684626	57685625	67819	Der1l	NM_024207	38848	21.65	9.50	+	+
chr17	29270824	29271823	442827	Rab44	NM_001002786	-677	21.58	9.46	+	+
chr7	28013122	28014121	12587	Mia1	NM_019394	-47454	21.58	9.46	+	+
chr15	56524235	56525234	15117	Has2	NM_008216	1367	21.49	7.57	+	+
chr19	6984010	6985009	26379	Esrra	NM_007953	11789	21.49	7.57	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr7	38892222	38893221	12447	Ccne1	NM_007633	-213	21.42	9.08	+	+
chr2	164228050	164229049	20971	Sdc4	NM_011521	40139	21.38	9.05	+	+
chr4	154537047	154538046	76866	Morn1	BC048593	39677	21.38	9.05	+	+
chr7	910400299	91041298	67943	Mesdc2	NM_023403	290	21.38	9.05	+	+
chr15	3458845	3459844	14600	Ghr	NM_001048147	-37858	21.34	8.74	+	+
chr17	27759493	27760492	23969	Pacsin1	NM_001286743	-32543	21.31	8.45	+	+
chr13	64254046	64255045	238673	Zfp367	NM_175494	-39	21.27	8.18	+	+
chr4	41221884	41222883	68970	Deaf12	NM_026893	39551	21.27	8.18	+	+
chr5	24096642	24097641	n/a	2010209O12Rik	AK012891	-1429	21.27	8.18	+	+
chr6	90319193	90320192	243537	Uroc1	NM_144940	36411	21.27	8.18	+	+
chr14	55194122	55195121	219072	Haus4	NM_145462	-21424	20.95	6.94	+	+
chr4	154500278	154501277	76866	Morn1	BC048593	2908	20.92	9.09	+	+
chr4	154509911	154510910	76866	Morn1	BC048593	12541	20.92	9.09	+	+
chr5	24110059	24111058	n/a	2010209O12Rik	AK012891	11989	20.92	9.09	+	+
chr1	53841404	53842403	98267	Stk17b	NM_133810	156	20.80	9.33	+	+
chr11	4886284	4887283	11764	Ap1b1	NM_001243044	-43	20.80	9.33	+	+
chr12	85328490	85329489	171210	Acot2	NM_134188	180	20.80	9.33	+	+
chr16	94719165	94720164	13185	Dscr3	NM_007834	28572	20.80	9.33	+	+
chr19	29399417	29400416	27356	Ins16	NM_013754	-109	20.80	9.33	+	+
chr3	19210656	19211655	18583	Pde7a	NM_001122759	167	20.80	9.33	+	+
chr6	57530657	57531656	243382	Ppm1k	NM_175523	-45737	20.80	9.33	+	+
chr7	106330959	106331958	67800	Dgat2	NM_026384	-236	20.80	9.33	+	+
chr7	17459178	17460177	14709	Gng8	NM_010320	-17457	20.80	9.33	+	+
chr7	30022761	30023760	52150	Kcnk6	NM_001033525	-5720	20.80	9.33	+	+
chr9	64659300	64660299	102442	Dennd4a	AK051202	654	20.80	9.33	+	+
chr19	46469228	46470227	54130	Actr1a	NM_016860	498	20.55	9.19	+	+
chr2	180007613	180008612	66481	Rps21	NM_025587	15971	20.41	9.11	+	+
chr4	108000197	108001196	414872	Zyg11b	NM_001033634	-27002	20.29	8.75	+	+
chr12	112403766	112404765	22031	Traf3	NM_001286122	-315	20.25	8.73	+	+
chr17	25980949	25981948	106618	Wdr90	AK129465	16963	20.25	8.73	+	+
chr17	29678028	29679027	381085	Tbc1d22b	NM_198647	-8219	20.25	8.73	+	+
chr19	58935211	58936210	73442	Hspa12a	NM_175199	-237	20.25	8.73	+	+
chr3	36761418	36762417	229227	4932438A13Rik	AK220265	-110	20.25	8.73	+	+
chr10	86353056	86354055	192188	Stab2	AK142339	49920	20.24	8.44	+	+
chr17	46168787	46169786	22339	Vegfa	NM_001025250	40	20.24	8.44	+	+
chr4	144483876	144484875	20148	Dhrs3	NM_011303	1337	20.23	8.17	+	+
chr10	85765200	85766199	21859	Timp3	AK082483	2544	20.22	7.91	+	+
chr11	105820406	105821405	13056	Cyb561	NM_007805	-15445	20.22	7.91	+	+
chr11	94335320	94336319	12291	Cacna1g	NM_001177890	-308	20.22	7.91	+	+
chr14	65270808	65271807	16554	Kif13b	NM_001081177	-60	20.22	7.91	+	+
chr2	118860071	118861070	269336	Ccdc32	NM_199310	-5442	20.22	7.91	+	+
chr14	55135828	55136827	219072	Haus4	NM_145462	36871	19.78	8.76	+	+
chr12	109571972	109572971	68519	Eml1	NM_001043336	-36695	19.71	8.44	+	+
chr11	78158120	78159119	11676	Aldoc	NM_009657	20921	19.69	8.16	+	+
chr10	75022347	75023346	192734	Al646023	NM_198860	229	19.64	8.97	+	+
chr11	101050642	101051641	53321	Cntnap1	NM_016782	13712	19.64	8.97	+	+
chr11	104508360	104509359	16416	Itgb3	NM_016780	10160	19.64	8.97	+	+
chr12	3427170	3428169	100043040	1110002L01Rik	AK031200	-923	19.64	8.97	+	+
chr13	101556101	101557100	268697	Ccnb1	NM_172301	-160	19.64	8.97	+	+
chr16	8671551	8672550	66690	Tmem186	NM_025708	-34257	19.64	8.97	+	+
chr7	30772297	30773296	72723	Zfp74	BC034883	-33716	19.64	8.97	+	+
chr9	121207559	121208558	67095	Trak1	NM_175114	1408	19.64	8.97	+	+
chr2	174263831	174264830	57314	Th11	NM_020580	23027	19.52	7.13	+	+
chr7	54050658	54051657	14884	Gtf2h1	NM_008186	-306	19.52	7.13	+	+
chr15	81190554	81191553	321003	Xpnpep3	AK042110	-39564	19.23	7.67	+	+
chr9	44187032	44188031	71732	Vps11	NM_027889	-17779	19.23	7.67	+	+
chr13	34965768	34966767	19134	Prpf4b	NM_013830	-1095	19.19	7.65	+	+
chr17	46241078	46242077	75564	Rsph9	NM_029338	39570	19.19	7.65	+	+
chr17	47732142	47733141	224836	Usp49	NM_198421	-15903	19.19	7.65	+	+
chr18	69751696	69752695	21413	Tcf4	NM_001083967	-884	19.19	7.65	+	+
chr19	5741338	5742337	78891	Scyl1	NM_023912	29564	19.19	7.65	+	+
chr5	20456652	20457651	242860	Rsbnl1	NM_001080977	489	19.19	7.65	+	+
chr1	140515595	140516594	59125	Nek7	AK165583	179	19.15	8.14	+	+
chr17	13852322	13853321	21646	Tcte2	AK005879	45580	19.15	8.14	+	+
chr6	17413538	17414537	17295	Met	NM_008591	82	19.15	8.14	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr10	85783612	85784611	21859	Timp3	AK082483	20956	19.14	8.41	+	+
chr19	4121055	4122054	29866	Cabp2	NM_001160252	38037	19.14	8.41	+	+
chr3	138189703	138190702	75624	Metap1	NM_175224	-37857	19.14	8.41	+	+
chr5	37113712	37114711	320661	D5Erttd579e	NM_001081232	-27004	19.14	8.41	+	+
chr7	35018705	35019704	14751	Gpi1	NM_008155	-3881	19.14	8.41	+	+
chr13	91946785	91947784	69085	Zcchc9	NM_145453	17	19.09	6.56	+	+
chr15	77628742	77629741	239552	Apol8	NM_001081970	-41830	19.09	6.56	+	+
chr15	76983570	76984569	93686	Rbfox2	NM_175387	173	18.77	7.44	+	+
chr13	64262589	64263588	238673	Zfp367	NM_175494	-8582	18.65	8.42	+	+
chr16	17561594	17562593	224022	Slc7a4	BC016100	14206	18.65	8.42	+	+
chr3	96024924	96025923	97122	Hist2h4	NM_033596	41817	18.65	8.42	+	+
chr5	125087361	125088360	67978	Tctn2	AK032378	39061	18.63	7.87	+	+
chr10	127041302	127042301	20852	Stat6	NM_009284	-38240	18.62	8.12	+	+
chr7	117916940	117917939	11717	Ampd3	NM_009667	1323	18.62	8.12	+	+
chr15	100254043	100255042	18174	Slc11a2	AJ493663	103	18.56	6.91	+	+
chr3	116210595	116211594	13171	Dbt	NM_010022	-4902	18.56	6.91	+	+
chr4	133684000	133684999	230806	Aim11	AK155034	46274	18.56	6.91	+	+
chr10	80237006	80238005	208266	Dot11	NM_199322	-2795	18.49	8.61	+	+
chr14	65285264	65286263	16554	Kif13b	NM_001081177	14397	18.49	8.61	+	+
chr17	6986728	6987727	22350	Ezr	NM_009510	-99	18.49	8.61	+	+
chr19	5348791	5349790	73720	Cst6	NM_028623	284	18.49	8.61	+	+
chr2	131970495	131971494	54338	Slc23a2	NM_018824	-151	18.49	8.61	+	+
chr5	118330639	118331638	18125	Nos1	NM_008712	14292	18.49	8.61	+	+
chr5	149210967	149211966	11987	Slc7a1	NM_007513	14	18.49	8.61	+	+
chr7	29163887	29164886	22695	Zfp36	NM_011756	-140	18.49	8.61	+	+
chr8	123631907	123632906	234814	Mthfsd	NM_001166482	-128	18.49	8.61	+	+
chr8	126506176	126507175	56199	Abcb10	NM_019552	347	18.49	8.61	+	+
chr11	5640721	5641720	76508	2210015D19Rik	NR_015577	-20932	18.21	7.41	+	+
chr11	74684214	74685213	97761	Sgsm2	NM_197943	25869	18.17	7.39	+	+
chr12	86435355	86436354	73422	Prox2	NM_175198	11527	18.17	7.39	+	+
chr18	69752513	69753512	21413	Tcf4	NM_001083967	-67	18.17	7.39	+	+
chr4	154507989	154508988	76866	Morn1	BC048593	10619	18.17	7.39	+	+
chr5	44198863	44199862	242960	Fbx15	NM_001159963	-25975	18.17	7.39	+	+
chr12	56566199	56567198	18035	Nfkb1a	NM_010907	26936	18.13	7.61	+	+
chr4	141091736	141092735	320456	B330016D10Rik	NR_030695	-9841	18.08	8.10	+	+
chr7	52961845	52962844	13170	Dbp	NM_016974	1729	18.08	8.10	+	+
chr1	133092215	133093214	54354	Rassf5	NM_018750	3961	18.04	8.08	+	+
chr10	127091802	127092801	20852	Stat6	NM_009284	12261	18.04	8.08	+	+
chr15	77786719	77787718	56551	Txn2	NM_019913	-27795	18.04	8.08	+	+
chr2	167552320	167553319	329562	A530013C23Rik	NR_015500	36113	18.04	8.08	+	+
chr4	132853353	132854352	269589	Sytl1	NM_031393	-34851	18.04	8.08	+	+
chr5	115246157	115247156	66236	2610524H06Rik	NR_027817	17329	18.04	8.08	+	+
chr6	48487268	48488267	71660	Rarres2	NM_027852	34902	18.04	8.08	+	+
chr6	53237768	53238767	320189	Creb5	AK170766	980	18.04	8.08	+	+
chr7	30768317	30769316	72723	Zfp74	BC034883	-29736	18.04	8.08	+	+
chr15	76993499	76994498	93686	Rbfox2	NM_175387	-9757	17.96	5.95	+	+
chr11	77907679	77908678	14252	Flot2	AK201315	45207	17.90	7.03	+	+
chr11	45878416	45879415	11492	Adam19	BC093480	9428	17.61	6.68	+	+
chr15	77710056	77711055	56551	Txn2	NM_019913	48843	17.61	6.68	+	+
chr5	29704534	29705533	56873	Lmbr1	NM_020295	-104	17.61	6.68	+	+
chr10	126416609	126417608	11567	Avil	NM_009635	-20656	17.55	8.08	+	+
chr12	16877000	16877999	50496	E2f6	AK149061	48602	17.54	7.81	+	+
chr6	86106934	86107933	21802	Tgfa	AK165017	-37811	17.54	7.81	+	+
chr1	133586690	133587689	226421	5430435G22Rik	NM_145509	1919	17.36	8.25	+	+
chr11	77685903	77686902	360013	Myo18a	AY692138	19150	17.36	8.25	+	+
chr15	77785877	77786876	56551	Txn2	NM_019913	-26953	17.36	8.25	+	+
chr4	128919062	128920061	n/a	AK077492	AK077492	-21412	17.36	8.25	+	+
chr7	150933871	150934870	381974	Mrgprg	NM_203492	18528	17.36	8.25	+	+
chr7	36340060	36341059	243923	Rgs9bp	NM_145840	30042	17.36	8.25	+	+
chr7	52032291	52033290	101568	Vrk3	S71575	6573	17.36	8.25	+	+
chr15	98764493	98765492	74775	Lmbr11	NM_029098	-16464	17.28	6.17	+	+
chr19	44322278	44323277	30049	Scd3	NM_024450	10474	17.28	6.17	+	+
chr3	88296209	88297208	20351	Sema4a	NM_001163489	-31605	17.28	6.17	+	+
chr8	72240172	72241171	66869	Zfp869	NM_001039967	130	17.28	6.17	+	+
chr11	98570536	98571535	57911	Gsdma	NM_021347	45372	17.17	7.12	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr17	66086615	66087614	70351	Ppp4r1	NR_104465	-45580	17.17	7.12	+	+
chr19	5802185	5803184	78891	Scyl1	NM_023912	-31284	17.17	7.12	+	+
chr2	23011471	23012470	67121	Mastl	NM_025979	-427	17.17	7.12	+	+
chr3	127223751	127224750	28036	Larp7	NM_138593	32017	17.17	7.12	+	+
chr5	125086376	125087375	67978	Tctn2	AK032378	38076	17.17	7.12	+	+
chr8	95341800	95342799	270084	Lpcat2	NM_173014	-36949	17.11	5.78	+	+
chr11	101023719	101024718	53321	Cntnap1	NM_016782	-13212	17.03	7.54	+	+
chr2	29437583	29438582	107746	Rapgef1	NM_001039086	-37157	17.03	7.54	+	+
chr2	166902531	166903530	98999	Znfx1	NM_001033196	-14516	17.00	7.78	+	+
chr1	137625336	137626335	27280	Phlda3	NM_013750	-36826	16.96	7.76	+	+
chr16	8470713	8471712	268860	Abat	NM_172961	-42309	16.96	7.76	+	+
chr2	118617322	118618321	214240	Disp2	AK035975	12345	16.96	7.76	+	+
chr2	144267190	144268189	228714	Csrp2bp	AK088564	39608	16.96	7.76	+	+
chr3	116215534	116216533	13171	Dbt	NM_010022	38	16.96	7.76	+	+
chr5	109058503	109059502	231580	Gak	NM_001282052	-207	16.96	7.76	+	+
chr5	118323519	118324518	18125	Nos1	NM_008712	7172	16.96	7.76	+	+
chr5	136178421	136179420	215210	Tmem120a	NM_172541	41122	16.96	7.76	+	+
chr7	109213195	109214194	11875	Art5	AJ297548	34411	16.96	7.76	+	+
chr11	77316652	77317651	216963	Git1	NM_001004144	10239	16.67	6.46	+	+
chr2	30141353	30142352	227697	Dolk	NM_177648	22	16.67	6.46	+	+
chr5	34543720	34544719	17122	Mxd4	NM_010753	-13861	16.67	6.46	+	+
chr1	193885238	193886237	n/a	AK016486	AK016486	-30515	16.49	7.50	+	+
chr11	57822876	57823875	73158	Larp1	NM_028451	811	16.39	5.98	+	+
chr5	114249983	114250982	231633	Tmem119	NM_146162	-122	16.39	5.98	+	+
chr10	85530490	85531489	69754	Fbxo7	NM_153195	46057	16.25	7.90	+	+
chr10	86434476	86435475	192188	Stab2	AK142339	-31501	16.25	7.90	+	+
chr11	115147091	115148090	237987	Otop2	NM_172801	-20886	16.25	7.90	+	+
chr11	95666781	95667780	66610	Abi3	NM_025659	36473	16.25	7.90	+	+
chr13	56683189	56684188	21810	Tgfbf1	AK135536	-27275	16.25	7.90	+	+
chr15	78340125	78341124	16185	Il2rb	NM_008368	-15129	16.25	7.90	+	+
chr16	11007974	11008973	56722	Litaf	NM_019980	-15260	16.25	7.90	+	+
chr19	10784199	10785198	12507	Cd5	NM_007650	28766	16.25	7.90	+	+
chr2	127269725	127270724	68126	Fahd2a	NM_029629	77	16.25	7.90	+	+
chr6	87818538	87819537	54161	Copg	NM_017477	-18896	16.25	7.90	+	+
chr7	116895127	116896126	56786	Tmem9b	NM_020050	151	16.25	7.90	+	+
chr7	17489197	17490196	19222	Ptgir	AK033567	-2142	16.25	7.90	+	+
chr9	107640935	107641934	83486	Rbm5	NM_148930	13971	16.25	7.90	+	+
chr11	63733751	63734750	54710	Hs3st3b1	NM_018805	1536	16.18	6.86	+	+
chr12	86462486	86463485	73422	Prox2	NM_175198	-15605	16.18	6.86	+	+
chr4	55428310	55429309	19359	Rad23b	NM_009011	37705	16.18	6.86	+	+
chr12	86434766	86435765	73422	Prox2	NM_175198	12116	15.94	7.46	+	+
chr6	38759500	38760499	15258	Hipk2	NM_001136065	27224	15.92	7.71	+	+
chr9	99143453	99144452	68121	Cep70	AK020622	67	15.92	7.71	+	+
chr11	57821618	57822617	73158	Larp1	NM_028451	-448	15.90	7.44	+	+
chr15	84296981	84297980	72301	1810041L15Rik	NM_001163145	-19954	15.90	7.44	+	+
chr17	57198692	57199691	66972	Slc25a23	NM_025877	95	15.90	7.44	+	+
chr19	41069946	41070945	17060	Blnk	NM_008528	-1421	15.90	7.44	+	+
chr7	150925662	150926661	381974	Mrgprg	NM_203492	26737	15.90	7.44	+	+
chr7	31064943	31065942	233065	Alkbh6	NM_198027	-28329	15.90	7.44	+	+
chr8	107676309	107677308	12400	Cbfb	NM_001161458	-17765	15.90	7.44	+	+
chr11	75276180	75277179	280408	Rilp	NM_001029938	-46916	15.75	6.24	+	+
chr11	95720130	95721129	66610	Abi3	NM_001163464	-16840	15.75	6.24	+	+
chr15	73517640	73518639	239530	Gpr20	NM_173365	19796	15.75	6.24	+	+
chr1	122016931	122017930	170706	Tmem37	NM_019432	-47074	15.52	5.78	+	+
chr11	116942131	116943130	76972	2810008D09Rik	NR_027059	4535	15.52	5.78	+	+
chr2	91366479	91367478	75786	Ckap5	NM_029437	28	15.52	5.78	+	+
chr1	95123641	95124640	208777	Sned1	NM_172463	-8333	15.45	7.19	+	+
chr17	87877612	87878611	12314	Calm2	NM_007589	-31837	15.45	7.19	+	+
chr2	172236303	172237302	20878	Aurka	NM_001291185	-40733	15.21	6.59	+	+
chr3	144306189	144307188	54673	AK086477	AK086477	45573	15.21	6.59	+	+
chr6	33198963	33199962	20336	Exoc4	NM_009148	314	15.21	6.59	+	+
chr7	109148877	109149876	101706	Numa1	NM_133947	2564	15.21	6.59	+	+
chr1	74795442	74796441	241113	Prkag3	NM_153744	-413	15.16	7.54	+	+
chr11	119959828	119960827	72055	Slc38a10	AK195379	11523	15.16	7.54	+	+
chr11	68505600	68506599	77579	Myh10	NM_175260	283	15.16	7.54	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr12	3319078	3320077	19325	Rab10	NM_016676	-9609	15.16	7.54	+	+
chr14	73516586	73517585	105670	Rcbtb2	NM_001170694	-25231	15.16	7.54	+	+
chr16	17560932	17561931	224022	Slc7a4	BC016100	14868	15.16	7.54	+	+
chr2	118616287	118617286	214240	Disp2	AK035975	11310	15.16	7.54	+	+
chr2	125509931	125510930	58521	Eid1	NM_025613	11596	15.16	7.54	+	+
chr2	30121223	30122222	227696	Phyhd1	NM_001252568	1	15.16	7.54	+	+
chr5	114257403	114258402	231633	Tmem119	NM_146162	-7542	15.16	7.54	+	+
chr7	125584961	125585960	110058	Syt17	NM_138649	568	15.16	7.54	+	+
chr8	72611404	72612403	330790	Hapln4	NM_177900	4477	15.16	7.54	+	+
chr8	26153635	26154634	11502	Adam9	NM_001270996	-26741	15.11	7.51	+	+
chr15	76983882	76984881	93686	Rbfox2	NM_175387	-140	15.10	6.76	+	+
chr6	29129048	29130047	23917	Impdh1	NM_011829	32724	14.98	6.93	+	+
chr11	64975859	64976858	216831	Arhgap44	NM_175003	105	14.89	7.13	+	+
chr4	149362584	149363583	100198	H6pd	NM_173371	14207	14.89	7.13	+	+
chr12	4529693	4530692	17977	Ncoa1	NM_010881	-46133	14.86	7.11	+	+
chr17	35262684	35263683	55938	Apom	NM_018816	5514	14.86	7.11	+	+
chr2	167522973	167523972	329562	A530013C23Rik	NR_015500	6766	14.86	7.11	+	+
chr2	93174106	93175105	241556	Tspan18	NM_183180	39	14.86	7.11	+	+
chr4	107972589	107973588	414872	Zyg11b	NM_001033634	607	14.86	7.11	+	+
chr5	36826164	36827163	66717	Ccdc96	NM_025725	-573	14.86	7.11	+	+
chr9	107672699	107673698	83486	Rbm5	BC060985	135	14.86	7.11	+	+
chr13	67406704	67407703	238690	Zfp458	NM_001001152	-27802	14.85	6.42	+	+
chr17	48110572	48111571	74123	Foxp4	NM_028767	-49491	14.85	7.36	+	+
chr5	150180869	150181868	70717	6330406115Rik	NM_027519	-33012	14.85	7.36	+	+
chr4	154547181	154548180	76866	Morn1	BC048593	49811	14.69	6.55	+	+
chr17	7031952	7032951	22350	Ezr	NM_009510	-45323	14.67	5.59	+	+
chr11	72832663	72833662	66874	1200014J11Rik	NM_025818	-28206	14.54	6.70	+	+
chr9	108176600	108177599	13138	Dag1	NM_001276485	-10811	12.88	5.66	+	+
chr1	137637316	137638315	27280	Phlda3	NM_013750	-24846	12.83	6.47	+	+
chr18	61866703	61867702	56069	Il17b	NM_019508	19615	12.83	6.47	+	+
chr4	141074277	141075276	320456	B330016D10Rik	NR_030695	-27300	12.83	6.47	+	+
chr5	115017023	115018022	433940	BC057022	NM_001004180	-737	12.83	6.47	+	+
chr11	79991386	79992385	71956	Rnf135	BC145236	-5488	12.76	6.66	+	+
chr12	112082643	112083642	72805	Zfp839	NM_001199785	-5346	12.76	6.66	+	+
chr13	23653702	23654701	14957	Hist1h1d	NM_145713	7302	12.76	6.66	+	+
chr6	52099583	52100582	72628	2700086A05Rik	AK146012	-25133	12.76	6.66	+	+
chr16	4781262	4782261	15369	Hmox2	NM_010443	24697	12.57	6.55	+	+
chr11	98532901	98533900	57911	Gsdma	NM_021347	7737	12.44	6.25	+	+
chr9	109835120	109836119	17758	Mtap4	NM_008633	1343	12.43	5.82	+	+
chr5	14514635	14515634	26875	Pclo	NM_001110796	218	12.40	5.80	+	+
chr5	150181483	150182482	70717	6330406115Rik	NM_027519	-32398	12.40	5.80	+	+
chr7	52011452	52012451	101568	Vrk3	BC027129	7954	12.40	5.80	+	+
chr7	17460414	17461413	14709	Gng8	NM_010320	-16221	12.23	5.92	+	+
chr15	100319456	100320455	68614	Letmd1	NM_134093	17119	12.22	5.35	+	+
chr15	100565543	100566542	207839	Galnt6	NM_172451	-6236	12.22	5.35	+	+
chr19	58924921	58925920	73442	Hspa12a	NM_175199	10054	12.22	5.35	+	+
chr6	124869735	124870734	69202	Ptms	NM_026988	-2271	12.18	5.01	+	+
chr6	57483929	57484928	243382	Ppm1k	NM_175523	992	12.09	5.65	+	+
chr11	64938042	64939041	216831	Arhgap44	NM_175003	37922	12.00	6.46	+	+
chr11	72811069	72812068	66874	1200014J11Rik	NM_025818	-49800	12.00	6.46	+	+
chr2	25516529	25517528	107771	Bmyc	NM_023326	-45370	12.00	6.46	+	+
chr5	37130043	37131042	320661	D5Ertd579e	NM_001081232	-43335	12.00	6.46	+	+
chr7	100051889	100052888	72461	Prpc	AK011816	28627	12.00	6.46	+	+
chr7	109247904	109248903	11875	Art5	BC144827	152	12.00	6.46	+	+
chr8	32238671	32239670	68867	Rnf122	NM_175136	16854	12.00	6.46	+	+
chr9	121509802	121510801	22354	Vipr1	NM_011703	-41532	12.00	6.46	+	+
chr11	102817992	102818991	70218	Kif18b	NM_197959	-32054	11.85	6.14	+	+
chr18	66609713	66610712	58801	Pmaip1	NM_021451	-8045	11.85	6.14	+	+
chr4	141114966	141115965	320456	B330016D10Rik	NR_030695	13390	11.85	6.14	+	+
chr5	34559990	34560989	17122	Mxd4	NM_010753	-30131	11.85	6.14	+	+
chr7	87802229	87803228	70461	Crtc3	NM_173863	31035	11.85	6.14	+	+
chr9	54635140	54636139	12903	Crabp1	NM_013496	23026	11.85	6.14	+	+
chr1	88551865	88552864	18159	Nppc	NM_010933	14783	11.74	6.31	+	+
chr2	167766788	167767787	69553	AK009597	AK009597	49560	11.61	6.23	+	+
chr11	50079697	50080696	103534	Mgat4b	NM_145926	41361	11.50	5.54	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr15	100546131	100547130	207839	Galnt6	NM_001161768	4278	11.50	5.54	+	+
chr10	87389833	87390832	22172	Tyms-ps	NR_000040	-39083	11.39	4.82	+	+
chr9	117161396	117162395	207181	Rbms3	NM_001172126	-295	11.21	5.39	+	+
chr15	57679281	57680280	67819	Der1l	NM_024207	44193	11.11	5.73	+	+
chr19	9101927	9102926	66395	Ahnak	NM_001039959	28276	11.11	5.73	+	+
chr11	99907294	99908293	16660	Krt31	NM_010659	4072	11.00	6.10	+	+
chr14	55476395	55477394	12050	Bcl2l2	NM_007537	-25367	11.00	6.10	+	+
chr2	59124007	59125006	227937	Pkp4	NM_175464	-21640	11.00	6.10	+	+
chr4	49599390	49600389	67063	2810432L12Rik	NM_025944	10853	11.00	6.10	+	+
chr5	24046338	24047337	100910	Chpf2	NM_133913	-45730	11.00	6.10	+	+
chr7	106774524	106775523	245190	Gm4980	NM_001195529	590	11.00	6.10	+	+
chr7	133919998	133920997	66090	Ypel3	NM_025347	10	11.00	6.10	+	+
chr8	26162118	26163117	11502	Adam9	NM_001270996	-35224	11.00	6.10	+	+
chr16	33244741	33245740	69150	Snx4	NM_080557	-6301	10.89	5.82	+	+
chr8	72606774	72607773	330790	Hapln4	NM_177900	-154	10.89	5.82	+	+
chr12	16857129	16858128	50496	E2f6	AK149061	28731	10.75	5.54	+	+
chr2	29948614	29949613	263803	Pkn3	AK142988	14829	10.73	4.42	+	+
chr15	84280467	84281466	72301	1810041L15Rik	NM_001163145	-3440	10.62	5.28	+	+
chr19	16509975	16510974	14675	Gna14	NM_008137	319	10.62	5.28	+	+
chr5	115941480	115942479	75387	Sirt4	NM_133760	-7487	10.62	5.28	+	+
chr11	63738413	63739412	54710	Hs3st3b1	NM_018805	-3127	10.56	4.90	+	+
chr12	112397788	112398787	22031	Traf3	NM_001286122	-6293	10.56	4.90	+	+
chr17	13851919	13852918	21646	Tcte2	AK005879	45983	10.54	5.62	+	+
chr16	96313141	96314140	93871	Brwd1	NM_145125	-9606	10.35	5.73	+	+
chr2	32575951	32576950	30933	Tor2a	NR_045485	-36303	10.35	5.73	+	+
chr2	29571783	29572782	107746	Rapgef1	NM_001039087	14571	10.34	5.13	+	+
chr15	81820691	81821690	29858	Pmm1	NM_001282040	-29831	10.19	5.43	+	+
chr8	26091926	26092925	11502	Adam9	NM_001270996	34969	10.19	5.43	+	+
chr1	134777486	134778485	16980	Lrrn2	NM_010732	1055	10.02	5.74	+	+
chr11	77700528	77701527	19193	Pipox	NM_008952	6347	10.02	5.74	+	+
chr12	29279296	29280295	71693	Colec11	NM_027866	28360	10.02	5.74	+	+
chr17	24373661	24374660	12449	Ccnf	NM_007634	14194	10.02	5.74	+	+
chr2	93118237	93119236	241556	Tspan18	AK171054	33936	10.02	5.74	+	+
chr5	118347691	118348690	18125	Nos1	NM_008712	31344	10.02	5.74	+	+
chr5	150267725	150268724	75860	4930588N13Rik	NM_029464	25945	10.02	5.74	+	+
chr6	118325715	118326714	213895	Bms1	AK135031	43221	10.02	5.74	+	+
chr7	106774173	106775172	245190	Gm4980	NM_001195529	941	10.02	5.74	+	+
chr7	26461084	26462083	232984	B3gnt8	NM_001036740	48942	10.02	5.74	+	+
chr8	126551366	126552365	56199	Abcb10	NM_019552	-44844	10.02	5.74	+	+
chr14	47473084	47474083	63985	Gmfb	NM_022023	-31667	9.95	5.50	+	+
chr15	73525534	73526533	239530	Gpr20	NM_173365	11902	9.95	5.50	+	+
chr16	94740489	94741488	13185	Dscr3	NM_007834	7248	9.95	5.50	+	+
chr2	131956687	131957686	54338	Slc23a2	NM_018824	13658	9.95	5.50	+	+
chr2	163862123	163863122	58231	Stk4	NM_021420	-37291	9.95	5.50	+	+
chr4	133907690	133908689	56219	Extl1	AK164184	20273	9.95	5.50	+	+
chr10	85758538	85759537	21859	Timp3	AK082483	-4119	9.85	4.43	+	+
chr12	109608555	109609554	68519	Eml1	NM_001043336	-112	9.85	4.43	+	+
chr14	65258070	65259069	16554	Kif13b	NM_001081177	-12798	9.85	4.43	+	+
chr15	76683481	76684480	223669	Zfp7	NM_145916	-25725	9.76	5.01	+	+
chr5	34559442	34560441	17122	Mxd4	NM_010753	-29583	9.76	5.01	+	+
chr8	90462603	90463602	94187	Zfp423	NM_033327	20392	9.75	4.68	+	+
chr6	54227539	54228538	78004	Prr15	NM_030024	-48967	9.73	5.37	+	+
chr5	150256650	150257649	75860	4930588N13Rik	NM_029464	14870	9.63	5.31	+	+
chr7	31344181	31345180	56189	Prodh2	BC052815	49896	9.63	5.31	+	+
chr16	10992049	10993048	56722	Litaf	NM_019980	666	9.50	4.88	+	+
chr9	116127664	116128663	21813	Tgfb2	NM_009371	-43683	9.50	4.88	+	+
chr11	115388876	115389875	15374	Hn1	NM_008258	-13692	9.41	5.39	+	+
chr6	124414318	124415317	232371	C1rl	NM_181344	-28313	9.41	5.39	+	+
chr6	100783056	100784055	232314	Ppp4r2	NM_182939	-76	9.10	4.24	+	+
chr4	47190724	47191723	12819	Col15a1	NM_009928	-29660	9.06	5.19	+	+
chr1	107709802	107710801	21934	Tnfrsf11a	NM_009399	33003	9.06	5.38	+	+
chr11	46209337	46210336	432552	Fam71b	NM_001013783	-8395	9.06	5.38	+	+
chr12	25326846	25327845	12879	Cys1	NM_001004455	39315	9.06	5.38	+	+
chr13	52143577	52144576	74050	4921525O09Rik	NR_045061	-48033	9.06	5.38	+	+
chr18	34706826	34707825	225358	Fam13b	NM_146084	-40849	9.06	5.38	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr4	133608269	133609268	230806	Aim1l	NM_001162970	-15598	9.06	5.38	+	+
chr6	100842038	100843037	232314	Ppp4r2	NM_182939	40060	9.06	5.38	+	+
chr15	57685551	57686550	67819	Der1l	NM_024207	37923	9.04	5.17	+	+
chr15	77159956	77160955	93686	Rbfox2	NM_053104	-22973	9.00	5.15	+	+
chr11	72844786	72845785	66874	1200014J11Rik	NM_025818	-16083	8.97	4.46	+	+
chr2	29488279	29489278	107746	Rapgef1	NM_001039086	13540	8.95	4.76	+	+
chr11	69418478	69419477	13643	Efnb3	NM_007911	-45239	8.93	4.75	+	+
chr13	56727594	56728593	21810	Tgfb1	AK135536	17131	8.93	4.75	+	+
chr15	100559027	100560026	207839	Galnt6	NM_172451	281	8.93	4.75	+	+
chr17	29257143	29258142	442827	Rab44	NM_001002786	-14358	8.93	4.75	+	+
chr17	32154299	32155298	74377	Hsf2bp	NM_028902	16655	8.89	5.28	+	+
chr4	41494801	41500800	76992	Fam219aos	NR_045726	35832	8.85	5.26	+	+
chr7	142742815	142743814	19267	Ptpre	NM_011212	13809	8.85	5.26	+	+
chr9	47580380	47581379	54725	Cadm1	NM_207675	-40976	8.50	5.05	+	+
chr10	62665426	62666425	68802	Mypn	NM_182992	775	8.43	4.82	+	+
chr1	53885663	53886662	98267	Stk17b	NM_133810	-44104	8.21	4.23	+	+
chr19	5016548	5017547	225870	Rin1	NM_145495	-33760	8.21	4.23	+	+
chr6	87738963	87739962	69834	Rab43	NM_133717	20286	8.18	4.86	+	+
chr12	112258996	112259995	217864	Rcor1	NM_198023	-18513	8.15	4.85	+	+
chr16	4469401	4470400	11515	Adcy9	NM_009624	-49403	8.15	4.85	+	+
chr9	121915595	121916594	215494	C85492	NM_153540	-10951	8.15	4.85	+	+
chr9	55937636	55938635	19200	Pstpip1	NM_011193	354	8.15	4.85	+	+
chr12	112381324	112382323	22031	Traf3	NM_001286122	-22757	8.13	5.02	+	+
chr14	48693063	48694062	93834	Peli2	NM_033602	-46981	8.13	5.02	+	+
chr16	34095456	34096455	545156	Kalrn	AK139581	1221	8.13	5.02	+	+
chr7	106812712	106813711	245190	Gm4980	NM_001195529	-37599	8.13	5.02	+	+
chr7	88202056	88203055	116904	Alpk3	NM_054085	71	8.13	5.02	+	+
chr10	127165167	127166166	320080	Zbtb39	NM_198035	-10927	8.11	4.48	+	+
chr11	77585393	77586392	360013	Myo18a	NM_001291212	-4844	8.11	4.48	+	+
chr18	66630172	66631171	58801	Pmaip1	NM_021451	12415	8.11	4.48	+	+
chr2	160750641	160751640	280635	Emilin3	NM_001291145	-13066	8.11	4.48	+	+
chr6	90269551	90270550	71797	Chst13	NM_027928	5129	8.11	4.48	+	+
chr5	21164545	21165544	242864	Napepld	NM_178728	42119	7.94	4.91	+	+
chr18	12326810	12327809	76482	3110002H16Rik	NM_029623	72	7.92	3.74	+	+
chr11	64868616	64869615	216831	Arhgap44	NM_175003	1760	7.67	3.86	+	+
chr15	77736631	77737630	56551	Txn2	NM_019913	22268	7.67	3.86	+	+
chr17	43154128	43155127	94185	Tnfrsf21	NM_178589	1125	7.58	4.52	+	+
chr7	36340459	36341458	243923	Rgs9bp	NM_145840	29643	7.47	4.01	+	+
chr9	119981913	119982912	13051	Cx3cr1	NM_009987	-5013	7.47	4.01	+	+
chr6	57484268	57485267	243382	Ppm1k	NM_175523	653	7.32	4.22	+	+
chr17	31801268	31802267	12954	Cryaa	NM_001278570	-13108	7.29	4.53	+	+
chr1	134749626	134750625	16980	Lrrn2	NM_010732	-26806	7.24	4.67	+	+
chr11	63765437	63766436	54710	Hs3st3b1	NM_018805	-30151	7.24	4.67	+	+
chr4	155418327	155419326	21936	Tnfrsf18	NM_021985	18377	7.24	4.67	+	+
chr17	29693329	29694328	381085	Tbc1d22b	NM_198647	7083	7.05	4.56	+	+
chr1	74800445	74801444	241113	Prkag3	NM_153744	-5416	7.03	4.37	+	+
chr6	29268607	29269606	330277	Fam71f1	NM_207258	-33	6.99	3.66	+	+
chr13	49308593	49309592	66329	Susd3	NM_025491	34440	6.93	3.34	+	+
chr15	71831522	71832521	69700	Col22a1	NM_027174	32636	6.81	4.09	+	+
chr15	100260120	100261119	18174	Slc11a2	AJ493663	-5975	6.75	3.79	+	+
chr2	118844058	118845057	269336	Ccdc32	NM_199310	10534	6.75	3.79	+	+
chr10	39406166	39407165	19714	Rev3l	BC048946	-45300	6.56	3.96	+	+
chr6	125206316	125207315	21940	Cd27	NM_001042564	-19771	6.56	3.96	+	+
chr12	27140700	27141699	108089	Rnf144a	NM_080563	-41079	6.46	4.20	+	+
chr14	65251817	65252816	16554	Kif13b	NM_001081177	-19051	6.37	4.31	+	+
chr7	87797708	87798707	70461	Crtc3	NM_173863	35556	6.37	4.31	+	+
chr8	13503544	13504543	14456	Gas6	NM_019521	-9509	6.37	4.31	+	+
chr5	36799449	36800448	66717	Ccdc96	NM_025725	-27288	6.09	3.02	+	+
chr15	100260239	100261238	18174	Slc11a2	AJ493663	-6094	6.00	3.23	+	+
chr11	77614527	77615526	360013	Myo18a	NM_011586	224	5.68	3.28	+	+
chr18	66646152	66647151	58801	Pmaip1	NM_021451	28395	5.54	3.95	+	+
chr11	64975648	64976647	216831	Arhgap44	NM_175003	316	5.07	3.08	+	+
chr13	73940862	73941861	72293	Nkd2	NM_028186	43718	5.07	3.08	+	+
chr4	49572401	49573400	67063	2810432L12Rik	NM_025944	37842	4.74	3.59	+	+
chr5	53664470	53665469	66278	1810013D10Rik	NM_001145433	6626	4.74	3.59	+	+

Table A2.9 List of Six4 and MyoD co-bound loci annotated to genes regulated by both Six4 and MyoD

chr	start	end	GeneID	Symbol	Accession	Dist. to TSS (bp)	pvalue (-log ₁₀)	Fold change	Reg. by Six4	Reg. By MyoD
chr15	80614816	80615815	213988	Tnrc6b	NM_177124	-13724	4.28	2.72	+	+
chr19	36194010	36195009	107765	Ankrd1	NM_013468	-176	36.25	9.18	+	+++

+, $p < 0.05$ **or** fold change difference between cells with Six4 knockdown (or MyoD knockdown or ectopic expression in fibroblasts) and control cells of at least 1.5

+++, $p < 0.05$ **and** fold change difference between cells with Six4 knockdown (or MyoD knockdown or ectopic expression in fibroblasts) and control cells of at least 1.5