

The Regulation of Skeletal Myogenesis by C/EBP β : Lessons from small muscles and big tumours

Hamood AlSudais

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Department of Cellular and Molecular Medicine
Faculty of Medicine
University of Ottawa

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AUTHORIZATION

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ABSTRACT

Skeletal muscle associated disorders are correlated with significant morbidity, including frailty, fatigue, reduced mobility and poor resistance to treatments as well as mental health repercussions resulting from a loss of independence. Thus, conditions affecting skeletal muscle put considerable pressure on the health care system. In response to injury, skeletal muscle can regenerate and the molecular mechanisms underlying this unique process has been the subject of intense research with the goal of developing better treatment modalities for muscle-related diseases. Our laboratory has previously demonstrated that C/EBP β is a negative regulator of postnatal myogenic differentiation. Expressed in muscle satellite cells (MuSCs), the primary source of regenerative potential in skeletal muscle, C/EBP β inhibits entry into the myogenic differentiation program and is required for MuSC self-renewal after injury. Despite the important role of C/EBP β in muscle homeostasis, little is known about the genes it regulates. To better understand how C/EBP β regulates these processes, I used both a candidate-based approach to identify the inhibitor of DNA binding and differentiation protein ID3 as a C/EBP β target gene that mediates inhibition of myogenic differentiation, and an unbiased approach using RNA-seq. I compared gene expression profiles from C2C12 myoblasts overexpressing C/EBP β to control cells under growth and differentiation conditions. I observed that more than 20% of the molecular signature found in quiescent MuSCs is regulated by C/EBP β . Caveolin-1 was implicated as a direct target of C/EBP β and part of the molecular mechanism by which C/EBP β maintains MuSCs quiescence. Interestingly, the RNA-seq data identified numerous C/EBP β -regulated secreted proteins including growth factors and cytokines. Co-culture experiments indicate that secreted proteins mediate the inhibition of cell differentiation and fusion, suggesting that C/EBP β functions in an autocrine and paracrine fashion to influence

activation of myoblasts in the absence of cell-to-cell contact. Given the role of C/EBP β in regulating secretory proteins that inhibit myogenic differentiation, I examined the requirement of C/EBP β in the expression of anti-myogenic proteins secreted by cancer cells that affect MuSCs function and drive the development of cancer cachexia. Indeed, the expression of C/EBP β in cancer cells was found to be required for the production of a cachexia-inducing secretome by tumours *in vitro* and *in vivo*. Furthermore, C/EBP β was found to be sufficient to convert non-cachectic tumours into cachexia-inducing ones. In comparing differentially expressed C/EBP β -regulated secreted protein transcripts from our RNA-seq data to that from 27 different types of human cancers revealed an ~18% similarity between C/EBP β -regulated secreted proteins and those enriched in cachectic tumours including pancreatic, gastric and brain cancers. Three of these C/EBP β -regulated secreted proteins (SERPINF1, TNFRSF11B and CD93) were tested further and found to be inducers of muscle atrophy. This work provides molecular insight into the role of C/EBP β in the regulation of MuSC function and the regulation of cachexia-inducing factors by tumours, placing C/EBP β as a novel therapeutic target for the treatment of cancer cachexia and other muscle-related diseases.

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LIST OF ABBREVIATIONS

ANG1	Angiopoietin 1
ASCs	Activated muscle satellite cells
ATFs	Activating Transcription Factors
BaCl ₂	Barium Chloride
bFGF	basic fibroblast growth factor
bHLH	basic helix-loop-helix
BMP4	Bone Morphogenetic Protein
BrdU	Bromodeoxyuridine
bZIP	basic Leucine Zipper
c-Fos	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit
c-Jun	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
C/EBPs	CCAAT/Enhancer Binding proteins
<i>Camk2g</i>	Calcium/Calmodulin Dependent Protein Kinase II Gamma
CAV-1	Caveolin-1 protein
Cav1sh	Cells transduced with lentiviral particles targeting caveolin-1
<i>Ccl</i>	Crystallin Gamma C
<i>Ccl2</i>	C-C Motif Chemokine Ligand 2
<i>Ccl9</i>	C-C Motif Chemokine Ligand 9
CD93	CD93 Molecule
ChIP	Chromatin immunoprecipitation
cKO	Conditional Knock-Out
COVID-19	The Coronavirus disease 2019

CREB	cAMP Response Element-binding protein
<i>Csf1</i>	Colony Stimulating Factor 1
CTX	Cardiotoxin
<i>Cxcl14</i>	C-X-C Motif Chemokine Ligand 14
CYPB	Cyclophilin B
D%	Differentiation percentage
DAPI	4',6-diamidino-2-phenylindole
DLL4	Delta-like 4
DM	Differentiation media
DMD	Duchene Muscular Dystrophy
DMEM	Dulbecco modified eagle medium
<i>Dpt</i>	Dermatopontin
ECM	Extracellular matrix
EDL	Extensor digitorum longus
EdU	5-Ethynyl-2'-deoxyuridine
eIF-2a	Eukaryotic translation initiation factor 2A
eIF-4E	Eukaryotic Translation Initiation Factor 4E
EL4	mouse T cell lymphoma cell line
Esr1	The gene encoding Estrogen receptor α
FACS	Fluorescent Activated Cell Sorting
FAPs	Fibro-Adipogenic Progenitors
FBS	Fetal Bovine Serum
<i>Fgf10</i>	Fibroblast Growth Factor 10

<i>Fgf2</i>	Fibroblast Growth Factor 2
<i>Fgf7</i>	Fibroblast Growth Factor 7
<i>Fgfr2</i>	Fibroblast Growth Factor Receptor 2
<i>Fgfr3</i>	Fibroblast Growth Factor Receptor 3
FI	Fusion index
FoxO	Forkhead Box protein
<i>Fstll</i>	Follistatin Like 1
GBM	Glioblastoma multiforme
GEO	Gene Expression Omnibus
GFP	Green fluorescent protein
GM	Growth media
GO	Gene Ontology
GR	Glucocorticoid Receptor
<i>Grem1</i>	Gremlin 1, DAN Family BMP Antagonist
GTE _x	Genotype-Tissue Expression
HCSD	Human Cancer Secretome Database
HGF	Hepatocyte Growth Factor
HS	Horse Serum
ID	Inhibitor of DNA binding and differentiation proteins
<i>Id1</i>	Inhibitor of DNA binding and differentiation protein 1
<i>Id2</i>	Inhibitor of DNA binding and differentiation protein 2
<i>Id3</i>	Inhibitor of DNA binding and differentiation protein 3
<i>Id4</i>	Inhibitor of DNA binding and differentiation protein 4

IFN γ	Interferon Gamma
IGF1	Insulin Like Growth Factor 1
<i>Igfbp5</i>	Insulin Like Growth Factor Binding Protein 5
IL-1 β	Interleukin 1 beta
IL-4	Interleukin 1 4
IL-6	Interleukin 6
KO	Knockout
LAP	Liver activator protein
LAP*	Liver activator protein star
LGG	brain lower grade glioma
LIP	Liver inhibitory protein
LLC	Lewis Lung Carcinoma
LPS	Lipopolysaccharide
Maf	MAF BZIP Transcription Factor
MAFbx	Muscle atrophy F-box
MCK	Muscle creatine kinase
MCP-1	Monocyte chemoattractant proteins 1
MDM2	Mouse Double Minute 2 homolog
mdx	X chromosome-linked muscular dystrophy, a mouse model for DMD
<i>Mef2c</i>	Myocyte Enhancer Factor 2C
MIP-2	Macrophage inflammatory protein 2
<i>Mmp13</i>	Matrix Metalloproteinase 13

<i>Mmp2</i>	Matrix Metallopeptidase 2
<i>Mmp3</i>	Matrix metallopeptidase 3
MRF4	Muscle-Specific Regulatory Factor 4, now known as MYF6
MRFs	Muscle regulatory factors
MSC	mesenchymal stem cell
mTORC	Mechanistic Target Of Rapamycin Kinase
MuRF1	Muscle RING finger protein 1
MuSCs	Muscle satellite cells
MYF5	Myogenic Factor 5
<i>Myh1</i>	Myosin heavy chain 1
MyHC	Myosin heavy chain protein
MYOD	Myogenic differentiation 1
<i>Myod1</i>	Myogenic Differentiation 1 gene
MYOG	Myogenin
<i>Neb</i>	Nebulin
NF-kB	Nuclear Factor Kappa B Subunit 1
P21	Post-natal day 21
p38 MAPK	p38 mitogen-activated protein kinases
P49	Post-natal day 49
P56	Post-natal day 56
PAAD	Pancreatic adenocarcinoma
PAX3	Paired-box protein-3
PAX7	Paired-box protein-7

PBS	Phosphate-buffered saline
PC3, PC-3	Prostate cancer cell line
PFA	Paraformaldehyde
PKR	RNA-dependent protein kinase
pLX	basic retroviral vector with 5'LTR promoter
pLXSN	basic retroviral vector with 5'LTR promoter
<i>Ppara</i>	Peroxisome Proliferator Activated Receptor Alpha
<i>Pparg</i>	Peroxisome Proliferator Activated Receptor Gamma
QSCs	Quiescent muscle satellite cells
RNA-seq	RNA Sequencing
RPMI 1640	Roswell Park memorial institute medium
RT-qPCR	Real-Time quantitative Polymerase Chain Reaction
RUNX1	RUNX Family Transcription Factor 1
scRNA-seq	Single-cell RNA-seq
SCs	Satellite cells
SERPINF1	Serpin Family F Member 1
shCav1	shRNA construct targeting Caveolin-1
shCtl	shRNA construct not targeting a specific gene (control)
shId1	shRNA construct targeting <i>Id1</i>
shId3	shRNA construct targeting <i>Id3</i>
shRNA	short hairpin RNA
sh β	shRNA against <i>Cebpb</i>
<i>Six5</i>	SIX Homeobox 5

SKOV-3	Human ovary adenocarcinoma cell lines
<i>Snai2</i>	Snail Family Transcriptional Repressor 2
snRNA-seq	Single-nucleus RNA-seq
STAD	Stomach adenocarcinoma
TA	Tibialis anterior
TBP	TATA-binding protein
<i>Tcf4</i>	Transcription Factor 4
TCGA	Cancer Genome Atlas
TNF α	Tumor necrosis factor alpha
TNFRSF11B	TNF Receptor Superfamily Member 11b
<i>Tnni2</i>	Troponin I2, Fast Skeletal Type
<i>Tnni3</i>	Troponin T3, Fast Skeletal Type
TSS	Transcription start site
UPS	Ubiquitin-proteasome system
VEGF	Vascular Endothelial Growth Factor
WHO	The World Health Organization
WISP1	WNT1 inducible signaling pathway protein 1
WNT4	Wnt Family Member 4
WT	Wild-type
YFP	Yellow fluorescent protein

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CHAPTER ONE: INTRODUCTION

Skeletal muscle accounts for more than 40% of body mass. Skeletal muscle controls body movement, breathing, maintains energy homeostasis and protects internal organs through the direct actions of muscle contraction and its influence on other tissues. Diseases that affect muscle result in reduced quality of life and affect all age groups. The World Health Organization (WHO) estimates that 1 in 3 to 1 in 5 people suffer from a musculoskeletal condition, which includes disorders affecting the muscle, joints, bones and spine (Briggs et al. 2018). These skeletal muscle-associated disorders result in a significant reduction in mobility and are linked with frailty, depression and the development of secondary chronic health conditions; all of which put pressure on the health system (Briggs et al. 2016). Further, skeletal muscle wasting can occur secondary to numerous diseases, particularly those with important inflammatory components, such as cancer, AIDS, kidney disease, arthritis and autoimmune disorders (Shen et al. 2019). In response to injury, skeletal muscle can regenerate using a population of tissue-specific stem cells called satellite cells to promote repair (Mauro 1961; Bischoff 1975), and these have been at the centre of intense regenerative medicine research. Indeed, a better understanding of skeletal muscle development and regenerative processes is key to finding treatments for muscle-related diseases. In this thesis, the mechanism by which C/EBP β , a bzip family transcription factor, inhibits myogenic differentiation and regulates muscle satellite cell (MuSC) quiescence and activation are investigated. Furthermore, my findings in muscle cells lead to the identification of a role for C/EBP β in the production of tumour-derived cachexia-inducing factors and thus identifies C/EBP β as a novel and interesting therapeutic target for the treatment of muscle atrophy associated with cancer (cancer cachexia), which is a major health challenge with no treatment as of yet.

1.1. Skeletal Muscle Regeneration and the muscle satellite cells

Skeletal muscles retain the ability to regenerate throughout the lifespan of an organism and this ability was demonstrated experimentally 75 years ago. Of note, three papers by Le Gros Clark *et al* described muscle regeneration in rabbit and rat muscles after crush injury, ischemia or muscle grafting (Clark and Blomfield 1945; Clark 1946; Clark and Wajda 1947). These studies were followed by milestone studies of muscle regeneration by Studitsky and collaborators, who demonstrated that when dissected muscle is minced into a semi-liquid mixture and then transplanted back into the site of removal, the muscle regenerates within a few weeks (Studitsky 1964). Skeletal muscle's regenerative capacity is dependent on the presence of progenitor cells that are located between the basal membrane and the sarcolemma of muscle fibers (Mauro 1961; Bischoff 1975). Based on their anatomical location, these progenitor cells were called satellite cells (found around myofibers) and are considered the primary source of regenerative potential in skeletal muscle (Mauro 1961; Bischoff 1975; Reznik 1969; Moss and Leblond 1970). The muscle satellite cell (MuSC) population is heterogeneous but is characterized by the expression of Paired-box protein-7 (PAX7) (Seale et al. 2000). MuSCs are a small population of undifferentiated muscle progenitor cells, quiescent in homeostatic muscle, representing approximately 5% of all muscle cells and the quiescent MuSCs pool is established between days P49 and P56 (White et al. 2010; Gattazzo et al. 2020). MuSCs are activated in response to muscle injury to proliferate, differentiate and fuse to repair damaged muscle (Snow 1977; Bischoff 1975). MuSCs must self-renew to maintain the MuSCs pool, allowing for the regeneration process to occur multiple times over the lifespan of an animal (Collins et al. 2005; Kuang et al. 2007). A defect in any of these steps results in impaired muscle regeneration after injury. In 2011, multiple groups ablated the MuSC population using Cre-lox recombination technology (Sambasivan et al. 2011; Murphy

et al. 2011; McCarthy et al. 2011; Lepper et al. 2011). The elimination of *Pax7*-expressing cell (MuSCs) from mice using diphtheria toxin expression driven by the *Pax7* promoter caused a complete loss of muscle regeneration and was associated with increased fibrosis and fatty tissue in injured muscle (Sambasivan et al. 2011; Murphy et al. 2011; McCarthy et al. 2011; Lepper et al. 2011). The defective regeneration in these mice was rescued by the transplantation of donor MuSCs (Sambasivan et al. 2011), indicating that MuSCs are indispensable for muscle regeneration and that other muscle resident cell populations (non-MuSCs) are unable to fully regenerate damaged muscle.

In addition to the extraordinary ability of MuSCs to repair extensive muscle damage, the capacity to self-renew allows transplanted MuSCs to repopulate the stem cell niche in injured skeletal muscle. In particular, Collins *et al.*, showed that the transplantation of a single myofiber with only seven associated MuSCs into irradiation-depleted host muscle (lacking MuSCs) lead to the generation of more than 100 myofibers and a repopulation of the host MuSC pool (Collins et al. 2005). Similarly, when donor GFP⁺ MuSCs are isolated and transplanted into the muscle of dystrophic mice, the transplanted MuSCs contributed to the formation of GFP⁺ fibers and, more importantly, some of these cells maintained the expression of *Pax7* indicating their ability to self-renew (Montarras 2005). This ability to self-renew allows muscle to accommodate multiple rounds of injury; for example, tibialis anterior (TA) muscle of a mouse can regenerate after up to 50 serial injuries (Luz et al. 2002).

1.1.2. PAX7 and MuSC function

The paired-box proteins are a group of nine transcription factors that play a role in the development of various organs. PAX3 and PAX7 are essential for skeletal muscle development and all skeletal muscle precursors express at least one of them (Relaix et al. 2004,

2005, 2006). During development, skeletal muscle originates from the mesoderm and more specifically from the somite, a segmental mesoderm structure (Chal and Pourquié 2017). Precursor muscle cells that express the Paired-box protein-3 (PAX3) in the somite migrate to the sites of limb and trunk and differentiate to form the skeletal muscle of the trunk and limbs (Chal and Pourquié 2017; Buckingham et al. 2003). PAX7 protein is structurally similar to PAX3 and both bind to the same DNA elements (Soleimani et al. 2012a). Knockout studies showed that while PAX3 is essential for fetal skeletal muscle development, PAX7 is indispensable for postnatal skeletal muscle growth and MuSC function. Lineage tracing experiments showed that about 95% of limb muscle originates from *Pax3*-expressing cells (Schienda et al. 2006). PAX3 null mice do not survive and fail to form limb muscles due to a defective migration of precursor cells from the somite (Goulding et al. 1993; Daston et al. 1996), while mice lacking the expression of *Pax7* gene develop muscle normally but fail to maintain post-natal muscle growth and die within 3 weeks after birth (Mansouri et al. 1996; Kuang et al. 2006; Seale et al. 2004). Approximately 5-10% of *Pax7*-null mice survive to adulthood with reduced MuSC numbers and these MuSCs, when isolated, fail to proliferate and differentiate efficiently in cultures (Kuang et al. 2006; Oustanina et al. 2004). Furthermore, muscle fibers in PAX7-null mice are smaller than those of control mice and this is associated with reduced numbers of myonuclei (Kuang et al. 2006; Relaix et al. 2006; Seale et al. 2000). The reduced number of MuSCs in PAX7-null mice is attributed to increased apoptosis in MuSCs lacking *Pax7* rather than to a failure of specification (Oustanina et al. 2004; Relaix et al. 2006).

The role of PAX7 in the function of adult MuSCs was further examined using an inducible conditional knockout of the *Pax7* gene in the cells that express it. Lepper *et al*

showed that inducing *Pax7* knockout in MuSC, by the administration of tamoxifen at birth, mimics the earlier observations of defective muscle growth in the germline *Pax7* knockout (Lepper et al. 2009). However, when the *Pax7* knockout is induced in MuSCs in 2 to 3-week-old mice, limb muscles grow and regenerate normally (Lepper et al. 2009). The authors concluded that PAX7 is not essential for adult muscle regeneration as measured by the diameter of regenerating fibers 10 days post injury (Lepper et al. 2009); however, these findings were later challenged (von Maltzahn et al. 2013; Günther et al. 2013). Using the same alleles that Lepper *et al* generated (Lepper et al. 2009), the Rudnicki group showed that regeneration is defective in mice that were administrated tamoxifen between postnatal day 40 and 44 and kept on tamoxifen diet for the rest of the experimental time course (von Maltzahn et al. 2013), suggesting that the Lepper findings were due to recombination escapers that continued to express *Pax7*. Gunther *et al* used a different *Pax7*-Cre allele and confirmed the requirement of PAX7 expression in MuSCs for proper regeneration and the maintenance of the MuSC population (Günther et al. 2013).

In vitro studies were in line with the *in vivo* data that showed the requirement of *Pax7* in MuSC self-renewal and skeletal muscle regeneration. Loss of *Pax7* in primary myoblasts resulted in defective cell expansion in culture; however, when plated at high confluency they differentiated normally (von Maltzahn et al. 2013). Furthermore, knockdown of *Pax7* in cultured myofibers resulted in the decrease of MuSC numbers and an increase in myogenin-expressing cells, a marker of myogenic differentiation (von Maltzahn et al. 2013). Another study showed that the loss of *Pax7* in primary myoblasts reduced the uptake of 5-ethynyl-2'-deoxyuridine (EdU) by these myoblasts as compared to controls indicating a defective proliferation process (Günther et al. 2013). Furthermore, this defective proliferation was

accompanied with premature differentiation (Günther et al. 2013). In contrast, PAX7-overexpressing myoblasts showed increased EdU-incorporation as compared to controls (Günther et al. 2013). These findings showed that *Pax7* expression is required for proper MuSC function and skeletal muscle regeneration.

1.1.3. The Muscle Regulatory Factors and Myogenesis

Skeletal myogenesis is controlled primarily by the coordinated expression and activity of a group of transcription factors that are downstream of PAX3 and PAX7 called the muscle regulatory factors (MRFs) namely MYOD, MYF5, myogenin (MYOG) and MRF4 (now called Myf6) (Weintraub et al. 1991). MRFs are basic helix-loop-helix proteins that heterodimerize with E proteins to bind a specific DNA motif (CANNTG), the E-box, in their target gene promoters and enhancers (Weintraub et al. 1991). The first steps to identifying the molecular mechanisms of skeletal myogenesis began in non-myogenic cells. In 1977, fibroblast cells were transformed into myogenic cells after the exposure to an anti-neoplastic nucleoside analogue, 5-azacytidine, a demethylating agent (Constantinides et al. 1977; Jones and Taylor 1980). These findings opened the door for the discovery of the MRFs. In the search for genes that regulate skeletal myogenesis, *Myod1* was the first member of MRFs family to be discovered 35 years ago (Lassar et al. 1986; Davis et al. 1987). Three members were identified shortly thereafter (Edmondson and Olson 1989; Braun et al. 1989; Rhodes and Konieczny 1989). The overexpression of any of these genes is sufficient to convert fibroblasts into myoblasts, suggesting that MRFs are the core of the transcriptional network that governs skeletal myogenesis (Davis et al. 1987; Tapscott et al. 1988; Thayer et al. 1989; Braun et al. 1989; Rhodes and Konieczny 1989).

Knockout studies of the MRFs in mice illustrated the coordinated regulation of skeletal myogenesis by these genes during development. The knockout of MYOD in mice did not affect normal skeletal muscle development and mice were viable and fertile (Rudnicki et al. 1992). Interestingly, *Myf5* mRNA was elevated in these mice suggesting that MYF5 compensates for MYOD loss (Rudnicki et al. 1992). Similar to MYOD null mice, MYF5 null mice developed normal skeletal muscle, however they died prematurely due to rib malformations that prevented proper lung function (Braun et al. 1992, 1994; Rudnicki et al. 1993). There were no observed changes in *Myod1* activation nor the subsequent induction of myogenin expression in MYF5-null mice (Rudnicki et al. 1993), suggesting that the activation of MYOD is independent of MYF5 expression and that MYOD can compensate for MYF5 loss (Rudnicki et al. 1993). When both MYOD and MYF5 were knocked out in mice, a complete loss of skeletal muscle was observed, and pups died soon after birth (Rudnicki et al. 1993). Furthermore, the presence of one functional allele of *Myod1* in *Myf5*-null mice was enough to allow for the normal formation of skeletal muscle (Rudnicki et al. 1993). On the other hand, *Myod1*-null mice with one functional allele of *Myf5* formed skeletal muscle with fewer fibers and died after birth despite normal rib formation (Rudnicki et al. 1993). Later work showed that mice with double knockout of MYOD and MYF5 developed normal skeletal muscle (Kassar-Duchossoy et al. 2004). The discrepancy between the two studies (Rudnicki et al. 1993; Kassar-Duchossoy et al. 2004) was explained by the effect of *Myf5* deletion on the expression of *Mrf4* which is located upstream of *Myf5* on chromosome 10 in mice (Kassar-Duchossoy et al. 2004). Kassar-Duchossoy *et al* used three different *Myf5* mutants and showed that skeletal muscle develops normally in MYOD/MYF5 double knockout mice only when *Mrf4* expression is unperturbed (Kassar-Duchossoy et al. 2004). Thus MYOD, MYF5 and MRF4 are involved in the determination process (Kassar-Duchossoy et al. 2004).

Three MRF4 knockout mice were generated and they presented different phenotypes, likely due the effect on *Myf5* expression when *Mrf4* is mutated given that these two genes are adjacent. The first knockout model of MRF4 resulted in defective myotomal myogenesis, back muscle development and rib formation which caused death at birth (Braun and Arnold 1995). A second knockout model resulted in a weak phenotype in which mice were viable with few misshaped ribs (Zhang et al. 1995). The third *Mrf4*-mutant allele showed an intermediate phenotype in terms of rib formation and pups died after birth (Patapoutian et al. 1995). Indeed, the expression of *Myf5* mRNA was reduced in *Mrf4*-mutant mice which correlates with rib malformations, suggesting that inhibited *Myf5* expression may explain part of the phenotype of this model (Braun and Arnold 1995; Zhang et al. 1995; Hernández-Hernández et al. 2017).

The knockout of myogenin in mice resulted in perinatal death due to lack of functional skeletal muscle (Hasty et al. 1993; Nabeshima et al. 1993). Interestingly, MYOG-null mice formed MYOD-expressing myoblasts but these myoblasts failed to express myosin heavy chain and actin, two markers of terminal myogenic differentiation (Nabeshima et al. 1993). Furthermore, MYOG-null mice had decreased levels of *Mrf4* and muscle creatine kinase (MCK) mRNA supporting the lack of mature muscle tissue observed in these mice (Hasty et al. 1993). These findings suggest that myogenin expression is not required for the specification of muscle cells but is essential for myogenic differentiation.

In addition to their role in pre-natal myogenesis, the involvement of these MRFs in the maintenance of MuSCs and regeneration of skeletal muscle after birth was described in multiple studies. After muscle injury, the first MRFs to be activated are MYF5 and MYOD suggesting a role of MYF5 and MYOD in the expansion of MuSCs before cell-cycle exit and terminal myogenic differentiation (Zammit et al. 2006; Crist et al. 2012; Asfour et al. 2018)

MYF5-null mice have normal skeletal muscle up to 7 months of age, at which time fibrosis was observed in TA muscles indicating chronic degeneration/regeneration processes (Gayraud-Morel et al. 2007). Furthermore, increased TA fiber diameter was reported in MYF5-null mice (Gayraud-Morel et al. 2007). In young MYF5-null mice, muscle appeared normal but when challenged with muscle injury, a delay in regeneration accompanied with fibrotic and fatty tissue accumulation was observed (Gayraud-Morel et al. 2007). When MYF5-null primary myoblasts are isolated and cultured, no significant changes in proliferation and differentiation were reported (Gayraud-Morel et al. 2007). However, another study illustrated that MYF5-null myoblasts have reduced proliferation (reduced BrdU-incorporation) and undergo precocious differentiation (Montarras et al. 2000). These results indicate that MYF5 expression is required for muscle maintenance postnatally and is required for proper myogenesis.

The role of MYOD in adult myogenesis was illustrated by the Rudnicki group by crossing MYOD-null mice (Rudnicki et al. 1992) with mdx mice, a model for Duchenne Muscular Dystrophy (DMD) in which the dystrophin gene is mutated (Bulfield et al. 1984). Megeney *et al* showed that the loss of MYOD in mdx mice resulted in a worsening of the mdx phenotype as illustrated by reduced fiber size, muscle mass and survival (Megeney et al. 1996). Indeed, when the muscle of MYOD-null mice was injured, regeneration was defective indicating a role for MYOD in MuSC myogenesis (Megeney et al. 1996). Furthermore, multiple studies reported that despite the increased numbers of MuSCs in MYOD-null mice, these cells had reduced proliferation and differentiation capabilities suggesting that these cells favour self-renewal over commitment to regeneration (Megeney et al. 1996; Sabourin et al. 1999; Yablonka-Reuveni et al. 1999; Cornelison et al. 2000).

To examine the role of myogenin in postnatal myogenesis, mice with a conditional knockout of myogenin were generated and the activation of the Cre recombinase was induced on embryonic day 15.5, after developmental primary myogenesis occurs (Knapp 2006; Meadows et al. 2008). Surprisingly, when *Myog* is deleted after embryonic myogenesis, no changes in the histological appearance of muscle were observed; however, adult-age mice were smaller than controls (Knapp 2006; Meadows et al. 2008). More strikingly, despite the reported requirement of MYOG for myogenic differentiation, isolated myoblasts from MYOG-null mice differentiated normally, though expressed increased levels of *Myf5*, *Myod1* and *Mrf4* mRNA suggesting that other MRFs can compensate for the loss of MYOG (Meadows et al. 2008). Thus, while myogenin is required for embryonic myogenesis, it is dispensable for muscle growth and regeneration after birth.

A recently published data showed that *Mrf4* knockout mice are born with normal skeletal muscle and have no significant defect in muscle development or regeneration in adulthood (Lazure et al. 2020).

1.1.4. Role of MRFs in the regulation of MuSCs quiescence, activation and self-renewal

When MuSCs are activated, they have two possible fates: to self-renew or to commit to myogenic differentiation. Studies have suggested that MuSC fate is decided by both cell-intrinsic and extrinsic factors, with the latter provided by the MuSC niche (local microenvironment) (Dumont et al. 2015). The MRFs, among other factors, play a role in MuSC fate decisions and maintain the balance between MuSC quiescence, activation and self-renewal which is critical for proper muscle regeneration (Dumont et al. 2015).

For MuSCs to maintain quiescence, they are expected to express genes that inhibit the cell cycle and inhibit the expression of genes that promote the cell cycle progression. Thus, quiescent PAX7-expressing MuSCs do not express any of the MRFs proteins (Zammit et al. 2002; Crist et al. 2012). However, in response to injury, MYF5 and MYOD are induced to allow for the progression of cell cycle (Bischoff 1986; Zammit et al. 2002; Dumont et al. 2015). Indeed, MuSCs isolated from MYF5-null mice have a reduced proliferative capacity and differentiate precociously (Montarras et al. 2000). Multiple studies reported that MYOD-null mice have increased numbers of MuSCs with reduced differentiation capacity causing defective regeneration (Megeney et al. 1996; Sabourin et al. 1999; Yablonka-Reuveni et al. 1999; Cornelison et al. 2000), which suggests that these cells are not fully committed for myogenesis but rather remain as stem cells.

The MuSC population is heterogenous (Collins et al. 2005; Kuang et al. 2007; Troy et al. 2012) and whether this heterogeneity controls MuSC fate was intensely investigated. In 2007, Kuang *et al* reported that MuSCs are heterogenous for *Myf5* expression (Kuang et al. 2007). Using a *Myf5*-cre/ROSA26-YFP mouse model, in which only cells that have expressed *Myf5* mRNA at any stage in life would express YFP, researchers illustrated that about 10% of MuSCs have never expressed *Myf5* (*Pax7*^{+/}/*Myf5*⁻) (Kuang et al. 2007). Transplantation experiments revealed that both MuSC populations (*Pax7*^{+/}/*Myf5*⁻ and *Pax7*^{+/}/*Myf5*⁺) were able to undergo myogenic differentiation (Kuang et al. 2007). However, while the transplanted *Pax7*^{+/}/*Myf5*⁻ contributed extensively to the MuSC pool, the *Pax7*^{+/}/*Myf5*⁺ cells contributed mainly to terminal differentiation, without producing additional precursors (Kuang et al. 2007). Interestingly, while both MuSC populations undergo symmetric division to give rise to identical daughter cells, *Pax7*^{+/}/*Myf5*⁻ MuSCs also undergo asymmetric division to produce one *Pax7*^{+/}/*Myf5*⁻ cell and one *Pax7*^{+/}/*Myf5*⁺ cell (Kuang et al. 2007). The symmetric division

of *Pax7*⁺/*Myf5*⁻ MuSCs was proposed to be the primary mechanism of MuSC self-renewal (Kuang et al. 2007). Similarly, asymmetrical division giving rise to one daughter cell that is PAX7⁺/MYOD⁻ (self-renewing MuSC) and one daughter cell that is PAX7⁺/MYOD⁺ (proliferating myoblasts) has also been proposed to underlie self-renewal (Troy et al. 2012). Multiple reports established that MuSC cell cycle status can be identified based on MYOD protein expression along with PAX7 protein expression: self-renewal/quiescence (PAX7⁺/MYOD⁻), proliferation (PAX7⁺/MYOD⁺) and differentiation (PAX7⁻/MYOD⁺) (Halevy et al. 2004; Olguin and Olwin 2004; Zammit et al. 2004; Troy et al. 2012).

The role of the other MRFs, MRF4 and myogenin, in the regulation of MuSC cell cycle status was recently reported (Lazure et al. 2020; Ganassi et al. 2020). Both myogenin and MRF4 appear to have a role in the regulation of the MuSCs niche that is required for the maintenance of MuSC quiescence, activation and self-renewal. The MuSC niche is the local microenvironment that consists of the basal lamina and myofiber that surrounds and interacts with MuSCs through various signaling pathways (Kuang et al. 2008; Brun et al. 2017; Mashinchian et al. 2018). In muscle, MRF4 was shown to regulate the transcription of various myokines and secreted proteins (Lazure et al. 2020). Myofibers from MRF4-null mice have decreased expression of various secreted proteins suggesting a change in the MuSC niche that could affect MuSC status and a depletion of the MuSC population with age caused by loss of quiescence and spontaneous differentiation (Lazure et al. 2020). Transplantation experiments indicated that, unlike wild-type (WT) host mice, MRF4-null mice had reduced retention of donor GFP⁺ MuSCs. Further, supplementation with chick embryo extract could rescue MuSCs in myofibers cultures from MRF4 KO mice (Lazure et al. 2020), suggesting that MRF4 maintains the MuSC pool mainly through the regulation of the niche (Lazure et al. 2020).

The role of myogenin in the regulation of MuSC quiescence and activation was illustrated in the zebrafish model where MYOG-null fish are viable (Ganassi et al. 2020). Isolated MYOG-null adult myofibers had increased MuSC numbers that were mispositioned on myofibers, and were primed for activation and entry to the cell cycle (Ganassi et al. 2020). These data suggested that MYOG regulates MuSC localization, MuSC:niche interaction, and the niche cues, thereby regulating the maintenance of muscle and MuSC homeostasis (Ganassi et al. 2020) .

1.1.5. Contributors to the MuSCs niche

The interaction between MuSCs and niche factors has been shown to play a major role in the regulation of MuSC heterogeneity. The source of these niche factors is the muscle fiber and surrounding muscle-resident cells such as endothelial cells, fibroblasts, fibro-adipogenic progenitors (FAPs) and immune cells.

Myofiber-secreted factors such as Wnt Family Member 4 (WNT4) and Delta-like 4 (DLL4) are required for the maintenance of MuSC quiescence (Eliazer et al. 2019, 2020). Multiple studies have demonstrated that the Wnt signaling pathway regulates MuSC commitment, self-renewal and differentiation during regeneration (Brack et al. 2008, 2009; Le Grand et al. 2009; Jones et al. 2015; Parisi et al. 2015; Rudolf et al. 2016; Lacour et al. 2017). Eliazer *et al* showed that *Wnt4* is highly expressed in muscle fibers and its level decreases during regeneration (Eliazer et al. 2019). Using various inducible knockout approaches, authors targeted WNT4 in the MuSC niche and illustrated the requirement of WNT4 to maintain MuSC quiescence (Eliazer et al. 2019). Depletion of WNT4 expression in muscle fibers accelerated the transition of MuSCs from quiescent to activated (Eliazer et al. 2019).

Conversely, WNT4 gain-of-function in the myofiber locked most MuSCs in the quiescent state and inhibited muscle regeneration due to the lack of MuSC expansion (Eliazer et al. 2019). Another example of niche factors that regulate MuSC function is the delta-like canonical Notch ligand 4 (DLL4). Through single-nucleus RNA-seq (snRNA-seq), recent published data illustrates that myonuclei of a single myofiber are transcriptionally heterogeneous (Chemello et al. 2020; Dos Santos et al. 2020; Kim et al. 2020; Petrany et al. 2020). Interestingly, a recent preprint report from the Brack group illustrated that niche factor localization and composition along the muscle fiber controls MuSC heterogeneity (Eliazer et al. 2020). For example DLL4 expression is unequally distributed along the muscle fiber and MuSCs that are located in low DLL4 regions are primed for activation at the onset of injury (Eliazer et al. 2020). Thus, *Dll4* deletion in myofibers depletes the MuSC pool which confirms the requirement of the niche DLL4 for MuSC self-renewal (Eliazer et al. 2020).

Early reports demonstrated a positive correlation between MuSC numbers in a muscle fiber and the degree of capillarization surrounding the fiber (Latroche et al. 2015; Christov et al. 2007). Indeed, MuSCs are found in close proximity to endothelial cells and the cross talk between these two populations was recently shown *in vivo* (Verma et al. 2018). Verma *et al.* showed that MuSCs secrete Vascular Endothelial Growth Factor (VEGF), a potent pro-angiogenic factor, that is required to recruit endothelial cell in close proximity to MuSCs (Verma et al. 2018). The recruitment of endothelial cells near MuSCs maintains the quiescent state of MuSCs through the interaction between the endothelial-derived DLL4 and the NOTCH receptor on MuSCs (Verma et al. 2018). These data indicate that multiple sources exist for the niche factor DLL4; the myonuclei (Eliazer et al. 2020) and endothelial cells (Verma et al. 2018).

The supportive role of cells other than MuSC in muscle regeneration has been further reinforced. Fibroblast ablation in mice by diphtheria toxin driven by the *Tcf4* promoter, reduced MuSC proliferation and promoted premature differentiation, resulting in smaller regenerating fibers and a depletion of the MuSC pool (Murphy et al. 2011). *In vitro* co-culture experiments indicate that the fibroblast-derived secretome inhibits myogenic differentiation (Rao et al. 2013), and fibroblasts play a major role in the regulation of the skeletal muscle extracellular matrix (ECM), which undergoes extensive remodeling during muscle regeneration (Zou et al. 2008; Thomas et al. 2015; Urciuolo et al. 2013). Fibroblasts secrete various collagens such as collagen VI, and in mice lacking collagen VI, both muscle regeneration and MuSC self-renewal are defective (Urciuolo et al. 2013). Furthermore, fibroblasts secrete factors that promote MuSCs quiescence such as angiopoietin 1 (ANG1), which can stimulate *Pax7* expression (Abou-Khalil et al. 2009).

Fibro/adipogenic progenitors (FAPs) are PDGFR α -expressing progenitors capable of differentiating into fibroblasts and adipocytes (Uezumi et al. 2010). In response to injury, quiescent FAPs are activated and expand and while they do not form myofibers, they facilitate MuSC expansion and differentiation (Uezumi et al. 2010; Woszczyzna et al. 2019). FAPs, through the secretion of factors that modulate and remodel the ECM, establish the niche for MuSC expansion and myofiber formation (Uezumi et al. 2010). Recent work from the Rando group showed that the depletion of FAPs in skeletal muscle not only affected muscle regeneration, but also affected MuSC number and muscle size (Woszczyzna et al. 2019). Elimination of FAPs from skeletal muscle tissue caused a significant reduction in MuSC numbers in uninjured muscle with age indicating that FAPs are required to maintain the MuSC pool (Woszczyzna et al. 2019). Moreover, skeletal muscle atrophy and reduced function were observed in skeletal muscle after FAPs depletion (Woszczyzna et al. 2019).

Immune cells are other important players in the regulation of the MuSCs niche during muscle regeneration and disease. After muscle damage, resident immune cells attract neutrophils through cytokine and chemokine secretion, for example tumor necrosis factor (TNF- α), interleukin 6 (IL6) and macrophage inflammatory protein 2 (MIP-2) (Wang and Thorlacius 2005; Brigitte et al. 2010; Dinulovic et al. 2017). Recruited neutrophils then secrete factors; such as, monocyte chemoattractant protein 1 (MCP-1) and other cytokines to recruit monocytes to the damaged site, where they differentiate into M1 or M2 macrophages (Scapini et al. 2000; Dinulovic et al. 2017). At the early stages of injury, M1 macrophages are predominant and they secrete pro-inflammatory cytokines such as TNF α and IL-1 β , known to promote MuSC proliferation and inhibit myogenic differentiation (Dinulovic et al. 2017). Indeed, the depletion of M1 macrophages in regenerating muscle reduces MuSC proliferation (Segawa et al. 2008; Deng et al. 2012; Summan et al. 2006; Dinulovic et al. 2017). The removal of damaged tissue debris by M1 macrophages changes the niche conditions to allow for the expansion of anti-inflammatory M2 macrophages. M2 macrophages secrete factors that promote MuSC differentiation such as the insulin growth factor 1 (IGF1), and IL-4 (Dinulovic et al. 2017; Arnold et al. 2007). Indeed, the suppression of the transition from M1 to M2 macrophages impairs muscle regeneration (Dinulovic et al. 2017; Segawa et al. 2008; Deng et al. 2012; Summan et al. 2006).

The various contributors to the MuSC niche highlight the complex combination of niche factors required to maintain and determine MuSC fate. These findings advance the idea of the regulation of the MuSC niche as a therapeutic approach for the treatment of muscle diseases. For example, systemic inflammation is one of the hallmarks of cancer cachexia and

the circulating cytokines are expected to interact with, and influence, the MuSC niche in a way that contributes to the pathogenesis of this disorder.

1.2. Cancer cachexia and inflammatory cytokines

The word cachexia is derived from the Greek words “Kakos” and “hexia” which together mean “bad condition” (Donohoe et al. 2011). Cancer cachexia’s earliest definition was “a wasting syndrome involving loss of muscle and fat directly caused by tumour factors, or indirectly caused by an aberrant host response to tumour presence” (MacDonald et al. 2003). Since then, the definition has been updated to reveal the complexity of this paraneoplastic syndrome as a “multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment” (Fearon et al. 2011). This definition highlights the intricacy of cancer cachexia aetiology which is a result of multiple interactions between tumour and the host factors (Donohoe et al. 2011). Moreover, it makes a distinction between cancer cachexia and other conditions, such as anorexia, starvation, depression and malabsorption all of which can affect the body weight (Evans et al. 2008). Cancer cachexia is estimated to affect more than 80% of advanced cancer patients and it is associated with resistance to chemotherapy, decreased quality of life and survival (Anker et al. 2019; von Haehling and Anker 2010). The two hallmarks of cancer cachexia are skeletal muscle atrophy and systemic inflammation (Onesti and Guttridge 2014). Tumour cells produce elevated levels of pro-inflammatory factors such as IL-6, TNF- α , IFN- γ and IL-1 β (Deans et al. 2006; Donohoe et al. 2011). These factors are not only procachectic (Jiang and Clemens 2006), but also stimulate a host inflammatory response leading to the amplification of the inflammatory

signal (Yasumoto et al. 1995; Donohoe et al. 2011). In rodents, the administration of IL-6, IL-1 or TNF- α in the absence of tumors induces cachexia (Fong et al. 1989; Ling et al. 1997; Chen et al. 2016) and targeting these factors in tumor-bearing mice with neutralizing antibodies attenuates cachexia (Zaki et al. 2004; Gelin et al. 1991). Several clinical studies have shown the association between increased inflammatory factors and cachexia in patients with various cancer types (reviewed in (Prado and Qian 2019)).

In skeletal muscle, inflammatory cytokines cause an imbalance between muscle protein degradation and protein synthesis through the activation of catabolic pathways mediated by nuclear factor kappa B (NF- κ B), FoxO and the ubiquitin-proteasome system (UPS) pathway among others (Hasselgren and Fischer 2001; Reid and Li 2001; Li and Reid 2000; Patel and Patel 2017). Two E3 ubiquitin ligases, atrogin-1 (also, known as muscle atrophy F-box, MAFbx) and the muscle RING finger protein 1 (MuRF1) are required for skeletal muscle protein degradation via the UPS in muscle atrophy (Bodine 2001; Gomes et al. 2001). In various muscle-atrophy animal models, both MuRF1 and atrogin-1 are elevated and muscle atrophy is prevented in mice lacking either one (Cao et al. 2005; Gomes et al. 2001; Bodine 2001; Li et al. 2008). MuRF1, but not atrogin-1, targets myosin heavy chain for degradation in denervated muscle and in myotubes after glucocorticoid treatment (Cohen et al. 2009; Clarke et al. 2007). Atrogin-1 was shown to target MYOD directly for degradation (Lagirand-Cantaloube et al. 2009). Furthermore, in myostatin-induced skeletal muscle atrophy, atrogin-1 showed elevated interaction with various proteins including cytoskeleton and sarcomeric proteins including myosin heavy chain and desmin (Lokireddy et al. 2012).

Taken together, inflammatory cytokines are a major driver of cancer cachexia and thus are promising therapeutic targets.

1.3. CCAAT/Enhancer Binding Proteins

CCAAT/Enhancer Binding Proteins (C/EBPs) are a family of six transcriptional factors of the basic-leucine zipper (bZIP) named C/EBP α , β , γ , δ , ϵ and C/EBP-homologous protein (CHOP, or DDIT3) that are involved in the regulation of a variety of cellular activities including inflammation, cell cycle progression and differentiation (Tsukada et al. 2011; Ramji and Foka 2002; Cao et al. 1991; Pulido-Salgado et al. 2015). While all C/EBPs share similar structure of the C-terminal domain, which contains a bZIP domain housing the DNA-binding region and a leucine zipper motif, they have divergent N-termini. In the N-terminal domain, the activation domain, responsible for activation of transcription, is found in all family members except C/EBP γ , which acts as a dominant negative (Ramji and Foka 2002; Cooper et al. 1995). The C/EBP family can form homodimers or heterodimers with each other, other bZIP family members as well as non-bZIP proteins (Pulido-Salgado et al. 2015). Despite what their name suggests, the ideal C/EBP consensus sequence is TT(G/A)CGCAA, in its simplified form (Pulido-Salgado et al. 2015). RNA-seq data from humans and mice showed that C/EBP members, except *CEBPE*, are expressed in almost all tested tissues (Habuka et al. 2015; Merkin et al. 2012) (Figure 1 and 2).

1.3.1. CCAAT/Enhancer Binding Protein Beta

C/EBP β was first identified as a regulator of IL-6 signaling in the immune system but since has been implicated in the regulation of numerous biological processes outside of the immune system (Ramji and Foka 2002). C/EBP β is encoded by an intronless gene that, following leaky ribosomal scanning, produces three protein isoforms: LAP* (Liver activator protein star, LAP1, 38 kDa), LAP (Liver activator protein, LAP2, 35 kDa) and LIP (Liver inhibitory protein, 20 kDa) (Descombes and Schibler 1991). Among the three isoforms of

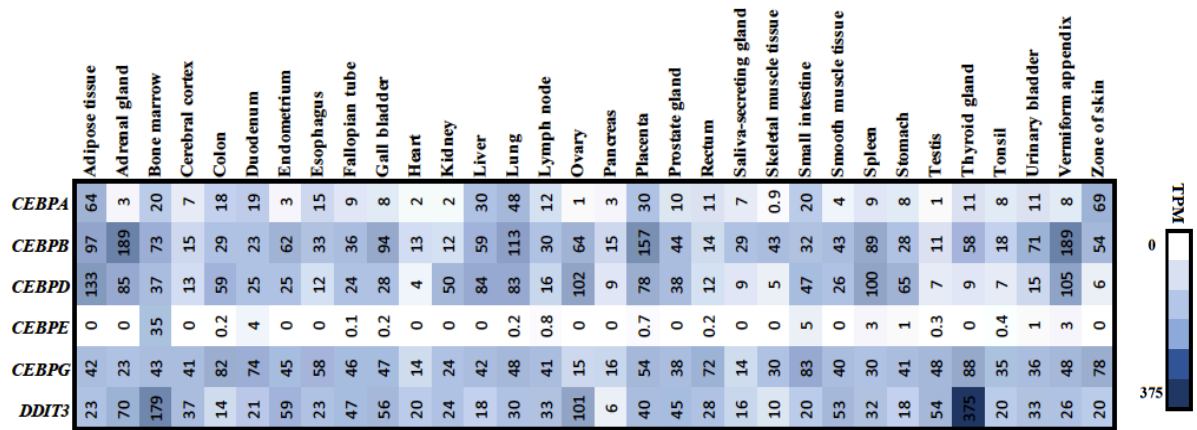


Figure 1. *CEBP* family member gene expression in 32 different human tissues. Expression values of *CEBPA*, *CEBPB*, *CEBPD*, *CEBPE*, *CEBPG* and *DDIT3* genes in various human tissues from 122 individuals obtained from the Expression Atlas (Papatheodorou et al. 2019) using the RNA-seq dataset E-MTAB-2836 (Habuka et al., 2015). Gene expression values are presented as Transcript Per Million (TPM).

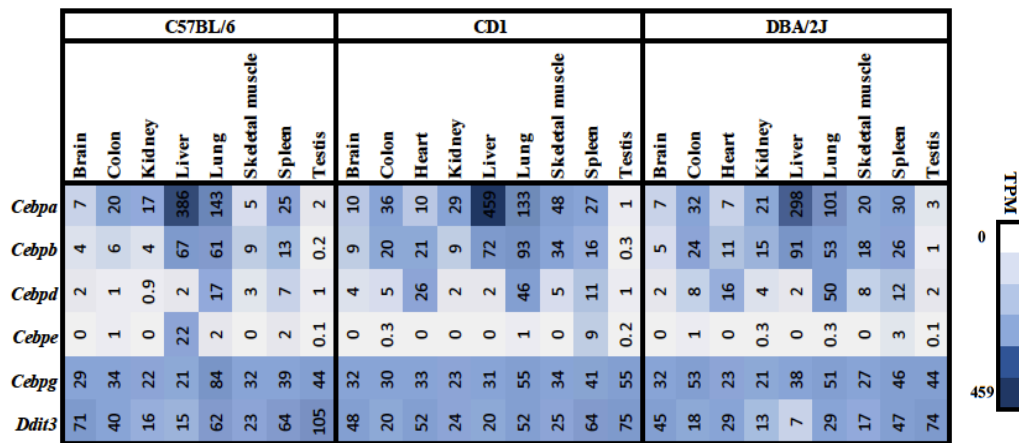


Figure 2. *Cebp* family member gene expression in 9 different mouse tissues from 3 mouse strains. Expression values of *Cebpa*, *Cebpb*, *Cebpd*, *Cebpe*, *Cebpg* and *Ddit3* genes in various tissues from 3 different mouse strains were obtained from the Expression Atlas (Papatheodorou et al. 2019) using the RNA-seq dataset E-MTAB-2801 (Merkin et al., 2012). Gene expression values are presented as Transcript Per Million (TPM).

C/EBP β , LIP lacks the activation domain and thus acts as a dominant negative C/EBP transcription factor (Descombes and Schibler 1991). Prior to DNA binding, C/EBP β proteins homodimerize or heterodimerize with other C/EBPs, members of bZIP family (such as c-Fos, c-Jun, CREBs, ATFs and Maf) and some non-bZIP (such as, NF-kB, glucocorticoid receptor (GR), PPAR α and RUNX1) (Grigoryan et al. 2009; Newman 2003; Pulido-Salgado et al. 2015; Tsukada et al. 2011; Diehl and Hannink 1994; Dooher et al. 2011; Faggioli 2004; Ruocco et al. 1996; Johansson-Haque et al. 2008; Ugarte et al. 2012). These various protein-protein interactions lead to changes in C/EBP β transactivation potential and expand the C/EBP β -regulated genes to include genes with no C/EBP binding sites (Tsukada et al. 2011; Huber et al. 2012; Pulido-Salgado et al. 2015).

The knockout of C/EBP β in mice highlights the wide range of cellular activities that are regulated by C/EBP β . Knockout mice were not produced at a Mendelian ratio (Screpanti et al. 1995) and this was further investigated by Croniger *et al* who reported the existence of two phenotypes in these mice (Croniger et al. 1997). While half of the C/EBP β -null mice survive (phenotype A), the other half die at birth due to hypoglycemia that is caused by defective liver function (phenotype B) (Croniger et al. 1997). Surviving C/EBP β -null mice, with age (after 12 weeks) developed skin lesions that appeared earlier in mice that were exposed to pathogens (Screpanti et al. 1995). Internal organ examination showed enlargement of the spleen and peripheral lymph nodes in C/EBP β -null mice and an imbalance in the distribution of T- and B-lymphocytes (Screpanti et al. 1995). Indeed, these defects render C/EBP β -null mice immunocompromised (Screpanti et al. 1995; Tanaka et al. 1995). C/EBP β -null mice also exhibited reduced liver regeneration attributed to defective liver cell proliferation after partial hepatectomy (Greenbaum et al. 1998), fasting hypoglycemia (Croniger et al. 2001), female sterility (Sterneck et al. 1997), defective mammary gland

development (Robinson et al. 1998), reduced fat mass and body weight (Tanaka et al. 1997; Staiger et al. 2009), resistance to diet-induced-obesity and inflammation (Millward et al. 2007; Rahman et al. 2007, 2012), defective and reduced brown fat (Tanaka et al. 1997; Carmona et al. 2005), delayed bone formation (Staiger et al. 2009) and resistance to skin cancers (Zhu et al. 2002). Other groups reported an association between C/EBP β and the expression of cytokines (reviewed in (Huber et al. 2012)). Interestingly, acute-phase protein expression was decreased in livers of C/EBP β -null mice as compared to controls (Cappelletti et al. 1996) and as such the implication of C/EBP β in the regulation of the inflammatory response likely contributes to the resistance of C/EBP β -null mice to tumor-induced muscle atrophy (Zhang et al. 2011).

1.3.2. CCAAT/Enhancer Binding Protein Beta is a negative regulator of skeletal myogenesis

While C/EBP β knockout mice showed no apparent skeletal muscle defects, the role of C/EBP β in skeletal muscle and, in particular MuSCs, was not known. In 2012, data from our laboratory showed that C/EBP β is expressed in MuSCs and acts as a repressor of myogenic differentiation (Marchildon et al. 2012). While C/EBP β is expressed in undifferentiated myoblasts, its expression is rapidly decreased at the onset of differentiation through proteolytic degradation mediated by the E3 Ubiquitin ligase MDM2 (Fu et al. 2015). Myogenic differentiation was inhibited in myoblasts overexpressing C/EBP β , at least in part, through the direct upregulation of *Pax7* expression and the suppression of MYOD and myogenin (Marchildon et al. 2012). When isolated and cultured, C/EBP β -null murine primary myoblasts underwent precocious differentiation with increased fusion (Marchildon et al. 2012). The conditional knockout of C/EBP β in *Pax7*-expressing cells *in utero* resulted in fiber

hypertrophy in male mice at postnatal day 21 mimicking the observed enhanced fusion in cultured cells (Marchildon et al. 2012). Moreover, when C/EBP β was conditionally depleted in MuSCs prior to BaCl₂ muscle injury, regeneration was enhanced producing larger myofibers (Marchildon et al. 2012). We later showed that this enhanced repair after injury was at the expense of MuSC self-renewal and this resulted in impaired muscle regeneration after a second injury (Lala-Tabbert et al. 2016a). Loss of C/EBP β in MuSCs caused a reduction in the self-renewing MuSCs population (PAX7+/MYOD-) and increase in the differentiating MuSCs (PAX7-/MYOD+) and myogenin-expressing cells (Lala-Tabbert et al. 2016a). These results highlighted the critical role of C/EBP β in the regulation of MuSC activation, self-renewal and differentiation (Figure 3).

1.3.3. CCAAT/Enhancer Binding Protein Beta expression and cancer cachexia

The earliest evidence of the association between C/EBP β expression in muscle and atrophy stemmed from the observation that C/EBP β levels were increased in dexamethasone (a synthetic glucocorticoid)-induced muscle atrophy and that muscle atrophy was prevented by the knockdown of C/EBP β (Gonnella et al. 2011). Published data during the same time showed that the binding of C/EBP β to the promoter of the atrogin-1 gene is required for tumour-induced muscle atrophy and mice lacking C/EBP β were spared from tumour-induced muscle wasting (Zhang et al. 2011). With the variety and complexity of changes that are observed in C/EBP β -null mice, the observed muscle protection from tumour-induced atrophy might result from the loss of C/EBP β in muscle, reduced systemic inflammation, or abnormalities in other niche cell populations in these mice. With this in mind, data from our laboratory and others demonstrated that tumour-bearing mice exhibit a defective regeneration after injury (Marchildon et al. 2015; He et al. 2013). Furthermore, isolated primary myoblasts

from tumour-bearing mice failed to differentiate efficiently *ex vivo* and this was associated with elevated levels of C/EBP β (Marchildon et al. 2015, 2016). In cultured cells, C/EBP β -null primary myoblasts were spared from the inhibited differentiation after exposure to conditioned medium from cancer cells (Marchildon et al. 2015). However, *in vivo*, the conditional knockout of C/EBP β in MuSCs failed to fully rescue the defective muscle regeneration in tumour-bearing mice and showed reduced number of regenerating fibers as compared to controls (Marchildon et al. 2016). These unexpected results were explained by the requirement for C/EBP β expression in MuSCs to protect these cells from apoptosis in the context of cancer cachexia (Marchildon et al. 2016). These reports illustrate the complexity of the regulation of MuSCs functions by C/EBP β in healthy and diseased muscles and demonstrated the need to better understand the molecular mechanism by which C/EBP β regulates MuSC functions.

1.4. Rationale and Hypothesis

Previous data from our laboratory demonstrated that C/EBP β is a negative regulator of MuSC activation and differentiation (Marchildon et al. 2012; Fu et al. 2015; Lala-Tabbert et al. 2016a). Furthermore, C/EBP β expression is stimulated in atrophic muscle and its upregulation in muscle cells is required for cancer-induced atrophy (Zhang et al. 2011; Marchildon et al. 2015, 2016). The molecular mechanism by which C/EBP β regulates these processes remains largely unexplored. *I hypothesized that C/EBP β regulates skeletal muscle homeostasis and MuSC entry into the myogenic differentiation program by stimulating the expression of genes that inhibit myogenic differentiation.* Identifying C/EBP β -regulated genes in muscle would allow for a better understanding of MuSC function, including its role in skeletal muscle atrophy and regeneration.

The *specific aims* of this thesis were: **(1)** to identify the molecular mechanism by which C/EBP β regulates MuSC homeostasis and differentiation using a candidate base approach, and an unbiased approach using RNA-seq comparing gene expression profile from myoblasts overexpressing C/EBP β to control cells under growth and differentiation conditions; **(2)** to apply the obtained knowledge from the identified C/EBP β -target genes to better understand genes that drive the development of cancer cachexia.

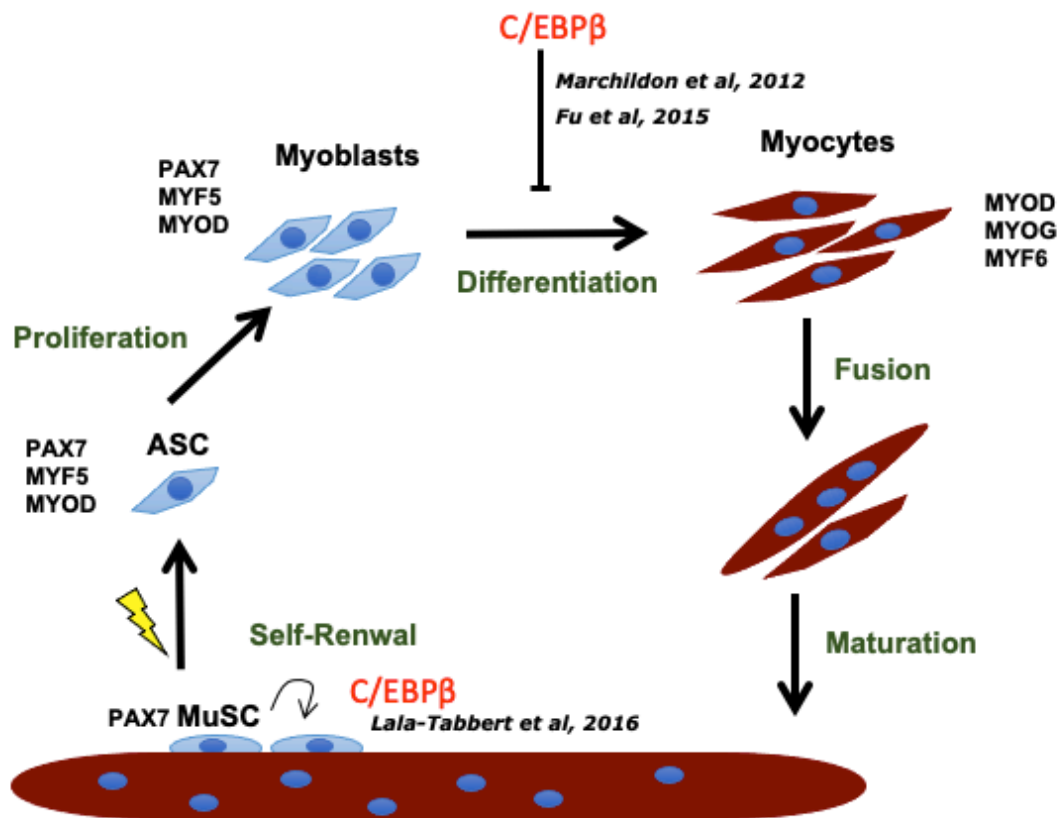


Figure 3. The role of C/EBPβ in MuSCs during Adult Myogenesis. MuSCs are indispensable for skeletal muscle regeneration and in response to injury, these quiescent PAX7-expressing cells are activated (ASC) and upregulate MYF5 and MYOD proteins to allow for myoblast expansion. Subsequently, myoblasts upregulate MYOG to stimulate differentiation and fusion. During MuSC activation, a subpopulation of MuSCs undergo self-renewal to maintain the MuSC quiescent population. C/EBPβ acts as a negative regulator of myogenic differentiation, at least in part, by the upregulation of PAX7, downregulation of MYOD and inhibition of *Myog* transactivation. Moreover, expression of C/EBPβ in MuSCs is required for their self-renewal.

CHAPTER TWO: CCAAT/ENHANCER BINDING PROTEIN β INHIBITS MYOGENIC DIFFERENTIATION VIA ID3

CCAAT/Enhancer Binding Protein β inhibits myogenic differentiation via ID3

Hamood AlSudais^a, Neena Lala-Tabbert^b and Nadine Wiper-Bergeron^{b,*}

^aGraduate Program in Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Rm 2541, Ottawa, Ontario, Canada, K1H 8M5

^bDepartment of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Rm 2539, Ottawa, Ontario, Canada, K1H 8M5

*To whom correspondence should be addressed: Dr. Nadine Wiper-Bergeron, 451 Smyth Road, Room 2539, Ottawa, Ontario, Canada, K1H 8M5 Tel: 1-613-562-5800 ext. 8176, Email:

Nadine.WiperBergeron@uottawa.ca

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Abstract

Myogenesis is regulated by the coordinated expression of muscle regulatory factors, a family of transcription factors that includes MYOD, MYF5, myogenin and MRF4. Muscle regulatory factors are basic helix-loop-helix transcription factors that heterodimerize with E proteins to bind the regulatory regions of target genes. Their activity can be inhibited by members of the Inhibitor of DNA binding and differentiation (ID) family, which bind E-proteins with high affinity, thereby preventing muscle regulatory factor-dependent transcriptional responses. CCAAT/Enhancer Binding protein beta (C/EBP β) is a transcription factor expressed in myogenic precursor cells that acts to inhibit myogenic differentiation, though the mechanism remains poorly understood. We identify *Id3* as a novel C/EBP β target gene that inhibits myogenic differentiation. Overexpression of C/EBP β stimulates *Id3* mRNA and protein expression, and is required for C/EBP β -mediated inhibition of myogenic differentiation. Misexpression of C/EBP β in myogenic precursors, such as in models of cancer cachexia, prevents the differentiation of myogenic precursors and we show that loss of *Id3* rescues differentiation under these conditions, suggesting that the stimulation of *Id3* expression by C/EBP β is an important mechanism by which C/EBP β inhibits myogenic differentiation.

Introduction

Skeletal muscle tissue retains the ability to regenerate throughout the lifespan of an organism and this ability is dependent on the presence of a small population of skeletal muscle stem cells called satellite cells. Normally quiescent, in response to injury satellite cells can become activated to proliferate, differentiate and fuse to repair damaged muscle (Bischoff 1975; Mauro 1961). Myogenic differentiation is controlled by a group of four basic helix-loop-helix (bHLH) transcription factors called muscle regulatory factors (MRFs) whose coordinated expression drives muscle-specific gene expression (Weintraub et al. 1991; Asfour et al. 2018). MYF5 and MYOD are expressed during activation and proliferation of satellite cells and their expression is followed by myogenin (MYOG) and MRF4 which drive terminal differentiation (Cornelison and Wold 1997). To exert their actions, MRFs heterodimerize with E-proteins to bind the regulatory regions of their target genes.

The activity of the myogenic regulatory factors can be inhibited by members of the inhibitor of DNA binding and differentiation (ID) family (Benezra et al. 1990; Sun et al. 1991; Christy et al. 1991; Jen et al. 1992; Davis et al. 1990; Yokoyama and Asahara 2011). The ID proteins, encoded by four genes (*Id1-4*), are members of the HLH superfamily; however, unlike bHLH transcription factors, they lack a DNA-binding domain and thus form inactive heterodimers with other bHLH members (Benezra et al. 1990; Sun et al. 1991). ID proteins bind strongly to E-proteins preventing their dimerization with MRFs and thereby inhibiting the activation of MRF target genes during myogenesis (Benezra et al. 1990; Sun et al. 1991; Christy et al. 1991; Jen et al. 1992). ID1, ID2 and ID3 can inhibit myogenic differentiation while ID4 is without effect (Melnikova and Christy 1996; Atherton et al. 1996; Melnikova et al. 1999; Jen et al. 1992).

CCAAT/Enhancer Binding proteins (C/EBPs) are a family of six bzip transcription factors that are involved in the regulation of cellular functions such as apoptosis, differentiation and cell growth (Tsukada et al. 2011; Ramji and Foka 2002; Cao et al. 1991). C/EBP β plays a significant role in the regulation of mesenchymal stem cell fate including repression of osteoblastogenesis and stimulation of adipogenesis (Wiper-Bergeron et al. 2007b; Darlington et al. 1998; Wiper-Bergeron et al. 2007a; Cao et al. 1991). In addition to these roles, C/EBP β is expressed in quiescent and proliferating skeletal muscle satellite cells and is rapidly decreased upon activation by the ubiquitin-proteasome system to allow for myogenic differentiation (Fu et al. 2015; Marchildon et al. 2012). Overexpression of C/EBP β in myoblasts blocks their differentiation, while loss of C/EBP β expression promotes precocious differentiation and larger myotubes *in vitro* (Marchildon et al. 2012). *In vivo*, loss of C/EBP β in satellite cells results in larger myofibers and enhanced regenerative capacity after a single injury (Marchildon et al. 2012). However, in this model, muscle regeneration was defective after a second injury due to impaired self-renewal and a reduction in the satellite cell pool supporting repair (Lala-Tabbert et al. 2016a). Although the role of C/EBP β in satellite cell function has been elucidated, the specific mechanism by which C/EBP β inhibits myogenic differentiation remains unknown.

C/EBP β expression can interfere with the transcriptional activity of MYOD and has been shown to regulate the expression of *Id1* and *Id2* in the immune system (Saisanit and Sun 1997; Xu et al. 2003; Karaya et al. 2005). In this study, we examined a potential role for ID proteins in the C/EBP β -mediated inhibition of myogenic differentiation. We find that C/EBP β stimulates *Id3* and *Id1* expression in differentiating myoblasts, but appears to only regulate *Id3* transcription directly. Knockdown of *Id3* or *Id1* rescues myogenic differentiation in cells

overexpressing C/EBP β , though neither could rescue fusion. Further, *Id3* is the most highly expressed Id family member in myoblasts, and only knockdown of ID3 could enhance myogenic differentiation in control cells.

Results

ID proteins are expressed in proliferating myoblasts and are downregulated with differentiation. ID1, ID2 and ID3 are known inhibitors of myogenic differentiation (Jen et al. 1992; Melnikova and Christy 1996; Melnikova et al. 1999; Atherton et al. 1996). Their expression patterns in proliferating myoblasts and during myogenic differentiation were evaluated in the myoblast cell line C2C12 and in primary myoblasts isolated from mouse hindlimb muscle. In C2C12 cells, *Id1*, *Id2*, and *Id3* mRNAs were all detected in proliferating cells cultured in growth medium (Fig. 4A). All were rapidly and substantially downregulated 12 hours after induction to differentiate (Fig. 4A) and remained low up to 96 hours in differentiation medium. By contrast, myogenin (*Myog*) expression, a marker of terminal myogenic differentiation that is not expressed in proliferating myoblasts, was significantly induced after 48 hours in differentiation medium confirming differentiation (Fig. 4B). Assessment of protein expression patterns revealed that in accordance with their anti-myogenic roles, ID1, ID3 and C/EBP β protein expression was highest in growth medium. Induction to differentiate caused a rapid downregulation of ID1 and ID3, evident 12 hours after switching to differentiation medium (Fig. 4C). C/EBP β expression was also downregulated by 12 hours in differentiation medium, in accordance with published observations (Marchildon et al. 2012; Fu et al. 2015). By contrast, myogenin expression was detected as early as 12 hours following induction to differentiate and levels peaked at the 24 hour time point (Fig. 4C). The structural protein, myosin heavy chain, was detected beginning at 24 hours after induction and

continued to increase over the course of the experiment. ID2 protein levels were not examined as *Id2* mRNA was weakly expressed in comparison to *Id1* and *Id3* and a reliable antibody was not found (Figure 4A).

While C2C12 myoblasts are a useful cell line to study myogenic differentiation, they are polyploid and have inactivation of the p19/Arf locus (Pajcini et al. 2010) and thus do not always recapitulate events *in vivo*. Therefore we assessed ID expression in primary myoblasts. Consistent with our findings in C2C12 myoblasts, *Id1*, *Id2*, and *Id3* were all downregulated as early as 12 hours after induction to differentiate, with *Id2* expressed at lower levels (Figure 4D). The time course of myogenic differentiation is condensed in primary myoblasts as compared to C2C12 cells with the expression of myogenin mRNA and protein levels upregulated 6 hours after the addition of differentiation media (Figure 4E, F). ID1, ID3 and C/EBP β protein, while high in growth medium, were rapidly downregulated 6 hours after induction of differentiation (Figure 4F). Consistent with differentiation, myosin heavy chain expression increased after myogenin expression and following downregulation of ID1 and ID3 expression (Figure 4F).

C/EBP β regulates the expression of *Id3*. Given that C/EBP β can inhibit myogenic differentiation and can regulate the expression of *Id1* and *Id2* in the immune system (Saisanit and Sun 1997; Xu et al. 2003; Karaya et al. 2005), we examined *Id* gene expression in C2C12 myoblasts retrovirally transduced to express C/EBP β (β) with an empty virus (pLXSN) under growth and differentiation conditions (Fig. 5). Overexpression of C/EBP β was confirmed by western blot in proliferating cells (Figure 5A). The expression of *Id1*, *Id2* and *Id3* in these cells was examined under growth and differentiation conditions (Fig. 5B). In proliferating cells, none of the ID genes were significantly upregulated. However, in differentiating cells, only

Id3 mRNA expression was increased in cells overexpressing C/EBP β as compared to empty virus controls (Figure 5B).

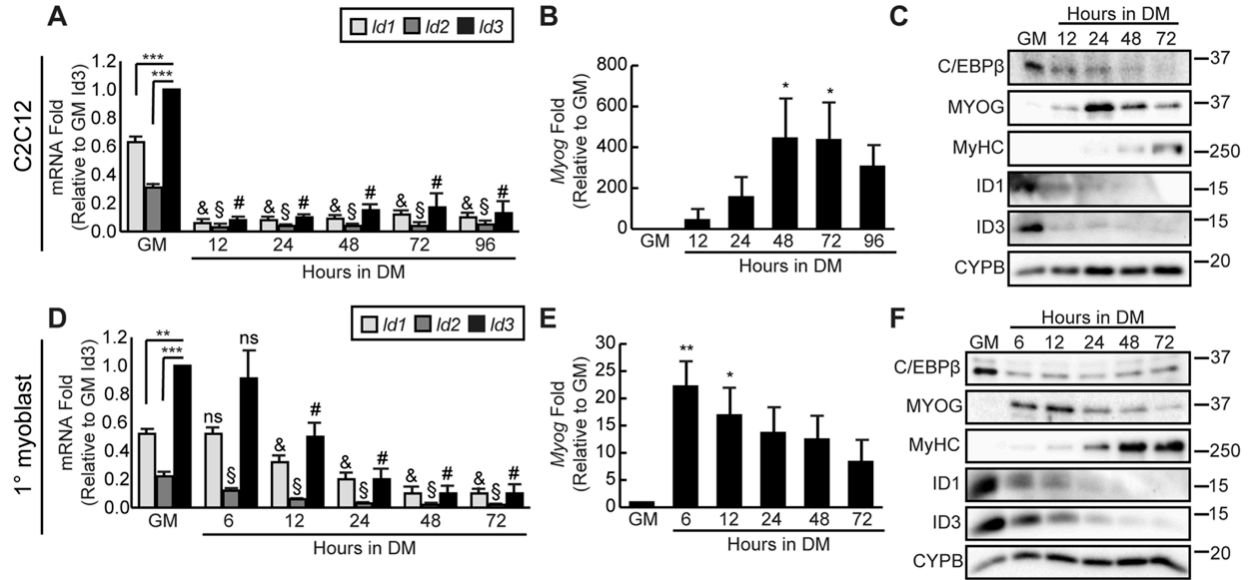


Figure 4. ID protein expression is regulated during myogenic differentiation. (A) RT-qPCR analysis of *Id1*, *Id2* and *Id3* expression in C2C12 myoblasts in growth medium (GM) and after induction to differentiate (DM). Data is presented as the fold change relative to *Id3* expression in GM. Bars are the mean $\pm$ SEM, $n=4$, *** $p<0.001$ for comparisons in GM. & $p<0.0001$ relative to *Id1* expression in GM. § $p<0.0001$ relative to *Id2* expression in GM. # $p<0.0001$ relative to *Id3* expression in GM. **(B)** *Myog* mRNA expression in cells cultured as in (A) presented as the fold change relative to GM condition. **(C)** C/EBP β , myogenin (MYOG), myosin heavy chain (MyHC), ID1 and ID3 protein expression in C2C12 myoblasts cultured as in (A). Cyclophilin B (CYPB) is a loading control. **(D)** RT-qPCR analysis of *Id1*, *Id2* and *Id3* expression in proliferating (GM) and differentiating primary myoblasts (6-72h). Data is presented as the fold change relative to *Id3* expression in GM. $n=3$, ** $p<0.01$, *** $p<0.001$ for comparisons in GM. & $p<0.001$ relative to *Id1* expression in GM. § $p<0.001$

relative to *Id2* expression in GM. # $p < 0.001$ relative to *Id3* expression in GM. ns is not statistically significant relative to GM values. **(E)** *Myog* mRNA expression in primary myoblasts cultured as in (D) and presented as the fold change relative to its expression in GM. **(F)** C/EBP β , myogenin (MYOG), myosin heavy chain (MyHC), ID1 and ID3 protein expression in primary myoblasts cultured as in (D).

However, western blot analysis revealed upregulation of ID1 and ID3 protein in differentiating C/EBP β -overexpressing cells (Figure 5C,D). Increased ID protein expression in C/EBP β -overexpressing myoblasts correlated with a reduction in myogenin protein expression as compared to controls (Figure 5C,E).

Conversely, in primary myoblasts isolated from a conditional null mouse where *Cebpb* is excised in muscle precursor cells (*Cebpb*^{fl/fl}*Pax7*^{CreER/wt} (C/EBP β ^{-/-})), *Id1* and *Id3* mRNA expression were reduced as compared to non-Cre expressing littermate controls (*Cebpb*^{fl/fl}*Pax7*^{wt/wt} (wild-type)) (Figure 5F). The expression of *Id2* was not significantly affected by knockdown of *Cebpb* expression. Taken together, these data suggest that C/EBP β regulates *Id1* and *Id3* expression in myoblasts.

Loss of Id3 rescues C/EBP β -mediated inhibition of myogenic differentiation. Given that *Id3* is the most abundantly expressed ID family member in myoblasts and was most upregulated by C/EBP β (Figure 4A,D, 5B-D), we first examined a potential role for ID3 in the inhibition of myogenic differentiation by C/EBP β . C/EBP β -overexpressing myoblasts and empty virus controls were retrovirally transduced with shRNA targeting *Id3* mRNA (shId3) or with non-silencing shRNA (shCtl) and induced to differentiate. RT-qPCR confirmed both the overexpression of *Cebpb* and knockdown of *Id3* (Figure 6A,B). While *Id3* mRNA expression was reduced approximately 50% in pLXSN-controls by the *Id3*-targetting shRNA, the expression of *Id1* and *Id2* were not affected (Figure 6B,C). Overexpression of C/EBP β increased *Id3* expression in cultures transduced with the shCtl construct, and this was reduced approximately 80% by the shId3 construct (Figure 6B). *Id1* expression was also significantly increased by C/EBP β overexpression, but returned to baseline levels with addition of the shId3 construct (Fig. 6C). Given that the shId3 construct does not affect *Id1* and or *Id2* expression

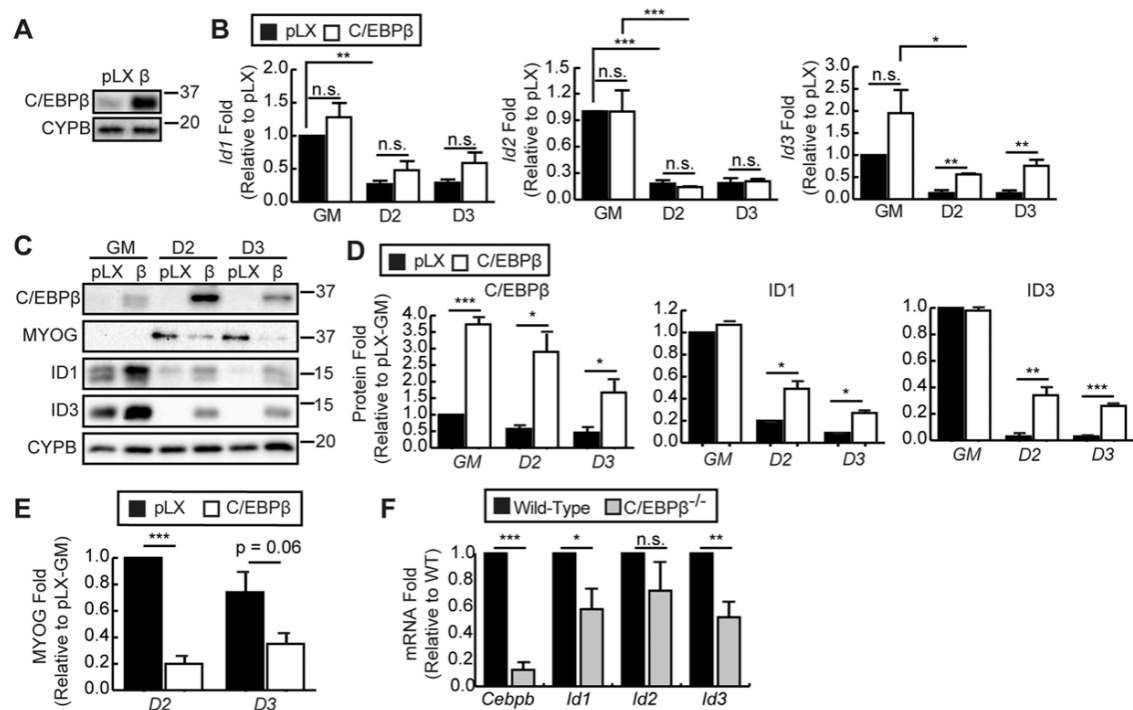


Figure 5. C/EBPβ regulates ID3 and ID1 expression. (A) C/EBPβ expression in proliferating C2C12 myoblasts that were retrovirally transduced with empty virus (pLX) or to express C/EBPβ (β). Cyclophilin B (CYPB) is a loading control. (B) Id1, Id2 and Id3 mRNA expression in cells transduced as in (A) in growth medium (GM) or cultured in differentiation medium for two (D2) or three days (D3) (n=4). (C) C/EBPβ, myogenin (MYOG), ID1 and ID3 protein expression in C2C12 myoblasts transduced and cultured as in (B). (D) Quantification of C/EBPβ, ID1 and ID3 expression from (D) presented as fold change relative to pLX GM conditions after normalization to CYPB expression (n=4). (E) Quantification of MYOG expression from (C) presented as fold change relative to pLXSN after two days (D2) of differentiation (n=4). (F) Cebpb, Id1, Id2 and Id3 expression in proliferating primary myoblasts isolated from Cebpbfl/flPax7wt/wt (wild-type) and Cebpbfl/flPax7CreER/wt (C/EBPβ^{-/-}) mice (n=5). Bars are the mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, n.s. is not significant (two-tailed Student's t-test or two-way ANOVA as required).

in the absence of C/EBP β -overexpression, the reduction in *Id1* expression could be a consequence of enhanced differentiation, which is accompanied by downregulation of ID expression. To assess the efficacy of myogenic differentiation in these cultures, myosin heavy chain (MyHC) immunostaining was performed (Fig. 6D). Knockdown of *Id3* in empty vector controls increased the percentage of nuclei found in MyHC-expressing cells after 2 and three days in differentiation medium (Figure 6D,E). Consistent with our previous findings (Marchildon et al. 2012), the differentiation index (% nuclei in MyHC⁺ cells/total nuclei) was reduced when C/EBP β was overexpressed and this inhibition was rescued when *Id3* was knocked down (Figure 6D,E), restoring the differentiation index to the level of controls at both time points. However, loss of *Id3* failed to rescue fusion (the average number of myonuclei/MyHC⁺ cells) in C/EBP β -overexpressing cells (Figure 6D,F) indicating that C/EBP β has a role in negatively regulating cell fusion independently of ID3. Western blot analysis revealed that the knockdown of *Id3* expression in empty vector control cells (pLXSN) increased the expression of myogenin after one day in differentiating medium by ~ 2.5 fold (Figure 6G,H). Additionally, while myogenin expression is decreased approximately 60% in C/EBP β -overexpressing cells on day one of differentiation, this was rescued to the levels of pLXSN controls with loss of *Id3* (Figure 6G,H). On day two of differentiation, myogenin expression was no longer statistically different with knockdown of *Id3*, resulting in a normalization of its expression across experimental conditions at this time point (Figure 6H). However, the loss of *Id3* enhanced the expression of MyHC in pLXSN control cells and restored the defective MyHC expression observed in C/EBP β -overexpressing cells on day two of differentiation (Figure 6G,I).

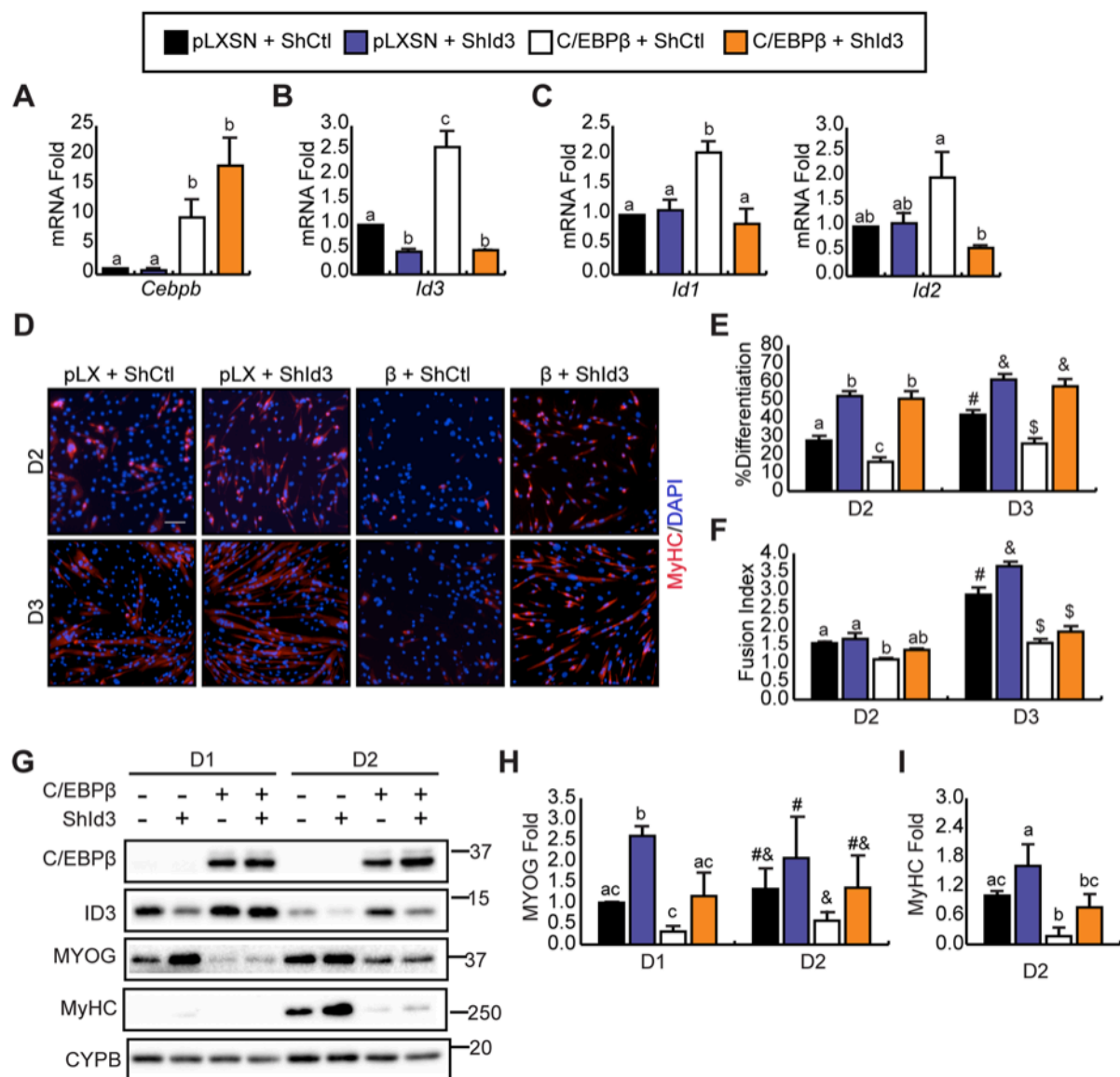


Figure 6. Inhibition of *Id3* expression rescues C/EBPβ-mediated inhibition of myogenic differentiation. C2C12 myoblasts expressing C/EBPβ or control plasmid (pLXSN) were retrovirally transduced to express a control shRNA (ShCtl) or a shRNA directed against *Id3* (ShId3) to create pooled stable cell lines. RT-qPCR analysis of (A) *Cebpb*, (B) *Id3*, and (C) *Id1* and *Id2* expression in myoblasts cultured in differentiation medium for two days. Data presented relative to pLXSN + ShCtl (n=3). (D) MyHC (red) immunostaining of differentiating cells from (A) two (D2) and three (D3) days after induction. Nuclei are

counterstained with DAPI (blue). Scale bar = 50 μ m. **(E)** Differentiation index (# of MyHC⁺ cells relative to the total nuclei) from (D) (n=3). **(F)** Fusion index (average # of nuclei per MyHC⁺ cell) (n=3). **(G)** C/EBP β , ID3, Myogenin (MYOG), myosin heavy chain (MyHC) protein expression in pooled stable myoblasts differentiated for one (D1) or two days (D2). Cyclophilin B (CYPB) is used as a loading control. **(H)** Quantification of MYOG protein expression from (G), normalized to CYPB expression and presented as fold change relative to pLXSN+ShCtl cells after one day (D1) of differentiation (n=3). **(I)** Quantification of MyHC western blot from (G), normalized to CYPB expression and presented as fold change relative to pLXSN+ShCtl cells after two days (D2) of differentiation (n=4). Bars are the mean $\pm$ SEM. Means with different letters or symbols are significantly different from one another, $p < 0.05$ (ANOVA, Tukey post-hoc test).

Since ID1 protein levels are affected by overexpression of C/EBP β , we assessed whether knockdown *Id1* could also rescue C/EBP β -mediated inhibition of myogenic differentiation (Figure. S1). C2C12 myoblasts overexpressing C/EBP β and their empty vector controls (pLXSN) were transduced to express a shRNA targeting *Id1* (shId1) or a non-targeting control (shCtl) (Figure. S1A). After 3 days of differentiation, myosin heavy chain immunostaining revealed that, in contrast to knockdown of *Id3*, knockdown of *Id1* did not enhance myogenic differentiation in pLXSN control cells as measured by the differentiation and fusion indices (Figure S1B,C,D). However, the differentiation index of C/EBP β -overexpressing cells was restored to normal levels with knockdown of *Id1*. Similar to shId3 knockdown, the knockdown of *Id1* failed to rescue the defective fusion index in C/EBP β -overexpressing cells. These data highlight the dominant role of ID3 over the other ID family members, at least in normal cells, as only knockdown of *Id3* in control cells enhanced differentiation and fusion.

To determine if C/EBP β directly regulates transcription of the *Id3* gene, we used published ChIP-seq signal tracks from proliferating and differentiating C2C12 myoblasts (GSE36024) to identify C/EBP β occupancy peaks at the promoter and two additional upstream regions (Figure 7A-D). *In silico* analysis of the regulatory region of *Id3* revealed that six putative C/EBP binding sites are located within -1 kb of the transcription start site (TSS), three of which are found within -300 bp of the TSS (Figure 7D). We also found C/EBP β motifs at approximately -26 kb and -20 kb from the TSS (Figure 7B,C). ChIP-qPCR analysis in differentiating myoblasts retrovirally transduced with empty virus (pLXSN) or to overexpress C/EBP β (β) confirmed the binding of C/EBP β at the -26 kb upstream region (Figure 7E and inset). C/EBP β occupancy of the -20 kb and promoter region of *Id3* in empty virus control

cultures was not significantly different from that of a negative region (Figure 7E). However, in C/EBP β -overexpressing cells, C/EBP β recruitment at all *Id3* regulatory regions examined

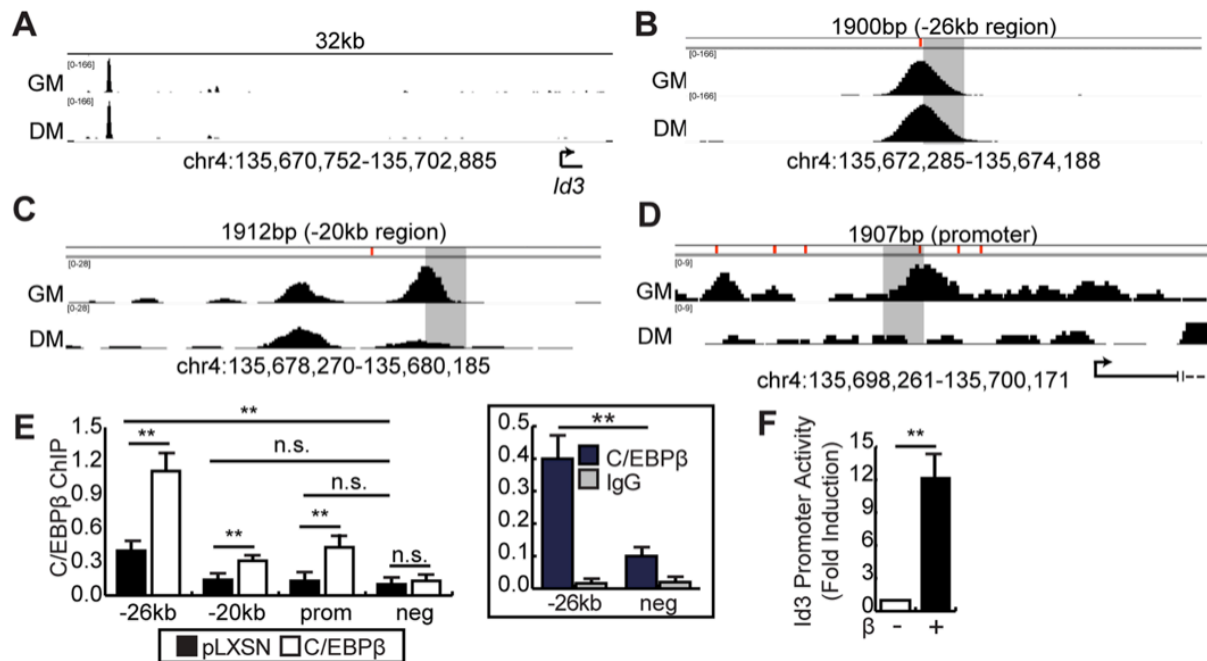


Figure 7. C/EBP β is a direct transcriptional regulator of *Id3*. (A) *Id3* regulatory region (Chr4:135,670,752-135,702,885) with C/EBP β (GSE36024) binding peaks indicated in proliferating (GM) and differentiating (DM) C2C12 myoblasts. C/EBP β (GSE36024) binding peaks at (B) the -26kb upstream region of *Id3* (Chr4:135,672,285-135,674,188), (C) the -20kb upstream region of *Id3* (Chr4:135,678,270-135,680,185) and (D) the promoter region of *Id3* (Chr4:135,698,261-135,700,171). C/EBP motifs are indicated by vertical dash marks above each histogram and the targeted region by qPCR is highlighted in grey. (E) qPCR-ChIP analysis of C/EBP β recruitment to the *Id3* regulatory regions in C2C12 cells retrovirally transduced with empty virus (pLXSN) or to express C/EBP β and differentiated for three days. qPCR-ChIP data is shown as copy number as compared to a standard curve of 10% input of each condition. (n=4). (Boxed) qPCR-ChIP data of C/EBP β occupancy in pLXSN cells at the

-26kb region and the negative region presented with IgG control and shown as copy numbers in relation to the 10% input of each condition (n=4). **(F)** *Id3* promoter activity in a transient transcription assay where the *Id3* promoter (-935 to +13) drives expression of luciferase. Proliferating C2C12 cells were transiently transfected with the reporter construct and to express C/EBP β and luciferase activity measured and shown relative to non-C/EBP β expressing controls (n=4). Bars are the mean $\pm$ SEM, **p<0.01, n.s. is not significant (two-tailed Student's t-test).

was enriched as compared to pLXSN (Figure 7E) suggesting that overexpression, which occurs *in vivo* with sarcopenia and cachexia (Marchildon et al. 2015; Giresi et al. 2005), can force C/EBP β onto elements that are not normally occupied. To confirm specificity, we repeated the ChIP in primary myoblasts isolated from a conditional null mouse where *Cebpb* is excised in muscle precursor cells (*Cebpb*^{fl/fl}*Pax7*^{CreER/wt} (C/EBP β ^{-/-})) (Figure. S2A). In this experiments, C/EBP β recruitment to the -26kb region of the *Id3* gene was significantly reduced in C/EBP β ^{-/-} cells when compared to WT controls, while other regions which did not show significant C/EBP β recruitment were unaffected (Figures S2A, 7E). To confirm the direct regulation of *Id3* by C/EBP β , we examined the ability of C/EBP β to activate the *Id3* promoter in a luciferase reporter assay (Kumar et al. 2009). In cells transiently expressing C/EBP β , *Id3* promoter activity was increased ~12 fold indicating that *Id3* is a novel direct transcriptional target of C/EBP β in myoblasts (Figure 7F).

Given that ID1 protein levels are increased with C/EBP β overexpression, we examined C/EBP β recruitment to the *Id1* promoter region. In contrast to the *Id3* promoter, where overexpression of C/EBP β increased its recruitment, the *Id1* promoter was no different than a chromatin region devoid of C/EBP β peaks (Figure S2B).

Loss of Id3 rescues myogenic differentiation in cancer cachexia. We have previously shown that exposure of myoblasts to conditioned medium from cachexia-inducing tumours, can upregulate C/EBP β expression and inhibit myogenic differentiation. Indeed, loss of C/EBP β rescues myoblast differentiation in this model system (Marchildon et al. 2015). Given that ID3 is a downstream of C/EBP β and loss of ID3 expression increases myogenin expression and promotes myogenic differentiation (Figure 6), we asked if the knockdown of ID3 could rescue differentiation in an *in vitro* cancer cachexia model (Jiang and Clemens 2006). C2C12

myoblasts were grown in conditioned media (CM) from a cachexia-inducing prostate cancer cell line (PC3) or proliferating C2C12 cells (mock) for 48 hours prior to induction to differentiate. Differentiation was then induced in the absence of conditioned medium for up to 3 days. Inhibition of myogenic differentiation in cells pre-treated with PC3-conditioned media was confirmed by MyHC staining (Figure 8A). We next asked if knockdown of ID3 could rescue the defective differentiation seen in myoblasts pre-treated with PC3-conditioned media. Under differentiation conditions, knockdown of ID3 stimulated myosin heavy chain protein expression in control cells and in cells pre-cultured in PC3-conditioned media as compared to control shRNA (Figure 8B). Indeed, pre-treatment with PC3-conditioned medium inhibited differentiation in shCtl cells but knockdown of ID3 rescued this defect in differentiation, most prominently on day 3 (Figure 8C,D). Furthermore, while pre-treatment with PC3 conditioned medium reduced myotube size in shCtl cells on day 3, this effect was rescued in cultures lacking ID3, suggesting that the blockade in myogenic differentiation observed in cancer cachexia is mediated through misexpression of ID3 (Figure 8C,E). Pre-treatment with PC3-conditioned medium did not influence cell density in these experiments, nor did knockdown of *Id3* (Figure 8F).

Discussion

Herein, we demonstrate that C/EBP β can upregulate *Id1* and *Id3* but not *Id2* expression in myoblasts. We find that *Id3* is the most abundant Id family member expressed in myoblasts and the stimulation of *Id3* expression by ectopic C/EBP β is more pronounced than that of *Id1*. We demonstrate that C/EBP β binds the regulatory region of *Id3* (promoter and two upstream regions) and is a direct target of C/EBP β in myoblasts. Moreover, the knockdown of *Id3*

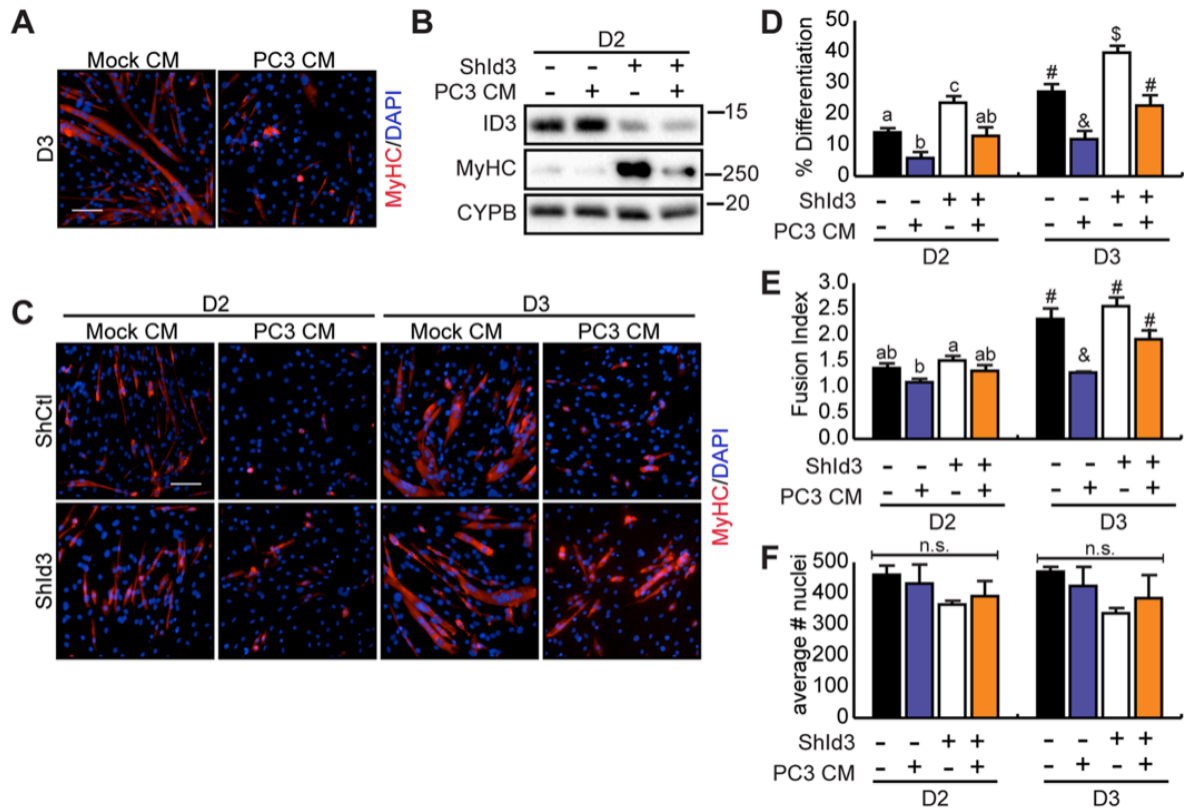


Figure 8. Loss of ID3 rescues myogenic differentiation in an *in vitro* model of cachexia.

(A) Representative images of myosin heavy chain (red) immunostaining of C2C12 myoblasts that grown in C2C12-conditioned media (Mock CM) or PC3-conditioned media (PC3 CM) for 48h then differentiated in fresh medium for three days. Nuclei are counterstained with DAPI (blue). Scale bar =50 μ m. (B) ID3 and myosin heavy chain (MyHC) protein expression in C2C12 cells retrovirally transduced to express a control shRNA or a shRNA directed against *Id3* (ShId3). Myoblasts were treated as in A and collected as differentiating cells (D2). (C) Myosin heavy chain immunostaining of C2C12 cells transduced as in (B) and treated and cultured as in (A). (D,E) Differentiation index (# of nuclei in MyHC-expressing cells/ total nuclei) and fusion index (average # of nuclei per MyHC+ cell) for (C) (n=4). (F) Average number of nuclei per field of view in cultures from (C). Data information: Bars are the mean

± SEM. Means with different letters or symbols are significantly different from one another, $p < 0.05$ (ANOVA, Bonferroni's multiple comparison test).

rescues the inhibition of myogenic differentiation in C/EBP β -overexpressing cells, placing *Id3* as a major effector of C/EBP β action in this system.

While C/EBP β is a known transcriptional regulator of both *Id1* and *Id2* in the immune system (Saisanit and Sun 1997; Xu et al. 2003; Karaya et al. 2005), changes in C/EBP β expression did not affect *Id2* expression in myogenic cells, suggesting cell-type specific regulation of these genes by C/EBP β . Interestingly, *Pax7* is also a direct target of C/EBP β (Marchildon et al. 2012) and PAX7 has been shown to also regulate *Id3* expression (Kumar et al. 2009), suggesting that C/EBP β can regulate *Id3* expression directly as well as indirectly through PAX7.

While all ID proteins bind E-proteins with high affinity and thus can interfere with the actions of bHLH transcription factors, in muscle, their effects are not entirely overlapping. For example, ID1 and ID2 appear to inhibit MYOD and MYF5 action but not that of MYOG or MRF4, while ID3 showed a weak inhibition of all MRFs (Langlands et al. 1997) suggesting that ID3 can function both in early and late myogenic differentiation if it is expressed, as occurs in cells overexpressing C/EBP β and in our model of cancer cachexia. While the overexpression of ID2 but not ID3 in myogenic cells has been shown to inhibit the induction of myogenin expression (Melnikova et al. 1999; Melnikova and Christy 1996) we find that knockdown of *Id3* increases myogenin protein expression even in the presence of ectopic C/EBP β . Further, we have shown that C/EBP β can reduce MYOD activity, an upstream regulator of myogenin (Marchildon et al. 2012) though whether this effect is mediated directly by ID3 remains to be investigated. As such, our findings suggest that the misexpression of ID3 could therefore have a more potent impact of the progression of differentiation by targeting the late regulators of differentiation.

The shRNA used to knockdown ID3 expression is specific and failed to decrease the mRNA expression of *Id1* or *Id2* in control cultures. However, in the presence of ectopic C/EBP β , the expression of the *Id3*-targeting shRNA did reduce *Id1* mRNA, suggesting that the loss of ID3 in these cells restores the myogenic differentiation program including the downregulation of ID1 expression. Indeed, knockdown of *Id1* in C2C12 cells overexpressing C/EBP β was able to rescue differentiation but not fusion, but was without effect in control cells. While these findings would suggest that ID3 is the dominant ID protein in myoblasts under normal physiological conditions, in disease states where C/EBP β expression is induced in myoblasts, for example cachexia, both *Id1* and *Id3* upregulation are likely to contribute. While we did not see recruitment of C/EBP β to the *Id1* promoter, this analysis did not include possible enhancer regions. Another condition that is associated with poor muscle regeneration and differentiation is sarcopenia, the loss of muscle mass that accompanies aging. It has been reported that the expression of *Cebpb* in primary myoblasts increases with mouse age (3-week, 3-month and 18-month of age) (Price et al. 2014), and *Cebpb* is considered part of a molecular signature of muscle aging (Giresi et al. 2005). Interestingly, analysis of the raw microarray data set from Price, Von Maltzahn et al (Price et al. 2014), revealed that along with upregulation of *Cebpb* in aging primary myoblasts, there is upregulation of *Id1* and *Id3*, but not *Id2*, consistent with our findings. These findings suggest that ID3 is an interesting therapeutic target to restore differentiation function in aged myoblasts.

Despite restoring myogenic marker expression, knockdown of *Id3* was not able to restore fusion, suggesting that C/EBP β acts to limit myotube size in a mechanism that is independent of ID3. In cancer cachexia however, where treatment of proliferating myoblasts with conditioned medium from the PC3 tumour is known to transiently upregulate C/EBP β

expression and to inhibit myogenic differentiation (Marchildon et al. 2015), knockdown of ID3 rescues both differentiation and fusion. Indeed, given the design of the experiment with pre-treatment causing differentiation defects, it is possible that at the time of fusion, the cells have recovered from the cachectic insult. Examination of this in co-culture experiments would clarify the role for ID3 in this context.

Experimental procedures

Cell Culture. Mouse primary myoblasts were isolated and cultured as previously described (Marchildon et al. 2012). Briefly, hindlimb muscles of adult mice (aged 6-8 weeks) were dissected and digested with collagenase/Dispase (Roche). Isolated cells were then pre-plated to remove fibroblasts and primary myoblasts were maintained on matrigel-coated plates in Dulbecco modified eagle medium DMEM (Wisent) supplemented with 20% fetal bovine serum (FBS) (Wisent), 10% horse serum (HS) (Sigma), 10 ng/ml basic fibroblast growth factor (bFGF) and 2 ng/ml hepatocyte growth factor (HGF) (Peprotech). To induce differentiation, confluent cultures were switched to differentiation media (DMEM, 2% FBS and 10% HS). *Primary* myoblasts from *Cebpb*^{fl/fl}*Pax7*^{wt/wt} (WT) or *Cebpb*^{fl/fl}*Pax7*^{CreER/wt} (cKO) mice isolated as previously described (Marchildon et al. 2012), were treated with 2 μ M 4-OH tamoxifen (Sigma) for 48h to induce excision of *Cebpb* in Cre-expressing cells. C2C12 myoblasts (ATCC) were grown in DMEM supplemented with 10% FBS (GM, growth media) and differentiation was induced by switching confluent cells to DMEM supplemented with 2% HS. PC-3 human prostate cancer cells (ATCC) were maintained in Roswell Park memorial institute medium (RPMI 1640) (Sigma) supplemented with 10% FBS. For *in vitro* cachexia experiments, conditioned media was collected from 90% confluent PC-3 cells after two days

and added to C2C12 growth media (1:1 ratio) with fresh medium (Jiang and Clemens 2006). After two days in conditioned media, C2C12s were switched to fresh differentiation media.

All animal experiments were approved by the University of Ottawa Animal Care Committee and all procedures were performed in accordance with the regulations set out by the Canadian Council on Animal Care.

Retroviral Infection. Replication incompetent retroviruses were generated in Phoenix Amphi packaging cells (ATCC) as previously described (Marchildon et al. 2012) using pLXSN-C/EBP β plasmid. Following retroviral infection of C2C12 myoblasts with empty virus (pLXSN) or virus to express C/EBP β (pLXSN-C/EBP β), pooled stable cells were selected based on neomycin (Wisent) resistance. Knockdown of *Id3* was accomplished by retrovirally transducing C2C12 myoblasts with a sequence targeting *Id3* (pRS-shId3, Origene, TR501033), *Id1* (pRS-shId1, Origene, TR511687) or a non-targeting control sequence (shCtl, Origene) and stable cells generated based on puromycin (Wisent) resistance.

Immunofluorescence. Cells were fixed in ice-cold 100% methanol and then permeabilized with PBS containing 0.5% Triton X-100 (Bioshop). Cells were incubated in primary antibody (MF20, DSHB) overnight at 4° C followed by secondary antibody (anti-mouse Cy3, Jackson ImmunoResearch) incubation. Nuclei were counterstained with 0.5 μ g/ml DAPI. Images were captured using a Zeiss AxioObserver D1 microscope (Zeiss) at the 10X objective. ImageJ software was used for the quantitative analysis of MyHC stained cells. Representative images were cropped using Adobe Photoshop.

Western Analysis. Whole cell extracts were prepared from primary myoblasts or C2C12 myoblasts. Equal amount of proteins were resolved on a 15% SDS-PAGE gel and then transferred to a PVDF membrane (Bio-Rad). Membranes were then probed with the following primary antibodies: C/EBP β (E299, ab32358), ID1 (Biocheck, BCH-1/37-2), ID3 (Biocheck, BCH-4/17-3), myogenin (F5D, DSHB), MyHC (MF20, DSHB) and Cyclophilin B (ab16045). The ChemiDocTM MP system (Bio-Rad) was used to detect chemiluminescence. Resultant images were cropped in Adobe Photoshop.

Real-time quantitative PCR. RNA was isolated using RNeasy kit (Qiagen) as per manufacturer's protocol. The remaining DNA was digested with DNase (Ambion) and DNA-digested RNA was used to make cDNA using iScriptTM cDNA Synthesis Kit (Bio-Rad) following the manufacturer's protocol. RT-qPCR reactions were performed using iTaqTM Universal SYBR[®] Green Supermix (Bio-Rad) on the CFX96 platform (Bio-Rad). Relative transcript expression was calculated using the $\Delta\Delta C_t$ method after normalizing each to 18S rRNA. The primer sequences used in this study are as followed: *Cebpb-F*: TCGAACCCGCGGACTGCAAG *Cebpb-R*: CGACGACGACGTGGACAGGC *Id1-F*: GAGGCGGCATGTGTTCCA *Id1-R*: CTCTGGAGGCTGAAAGGTGG *Id2-F*: GGACTCGCATCCCACTATCG *Id2-R*: GATGCCTGCAAGGACAGGAT *Id3-F*: AGCTCACTCCGGAACCTTGTG *Id3-R*: GTTCAGTCCTTCTCTCGGGC *Myog-F*: ATCGCGCTCCTCCTGGTTGA *Myog-R*: CTGGGGACCCCTGAGCATTG *18s-F*: CGCCGCTAGAGGTGAAATC *18s-R*: CCAGTCGGCATCGTTTATGG

Chromatin Immunoprecipitation. C2C12 myoblasts were crosslinked with 1% formaldehyde for 30 min then sonicated for 30 cycles (30 sec ON/OFF) using Diagenode

Bioruptor[®]. Equal amounts of chromatin was used to perform Immunoprecipitation (ChIP) analysis as previously described (Lala-Tabbert et al. 2016a) using C/EBP β (C-19, Santa Cruz Biotech, Sc-150) or rabbit polyclonal IgG (Invitrogen). Data are presented as copy numbers as compared to a standard curve that was generated using 10% input of each sample. The primer sequences used are as followed: *Id3*_-26kb (chr4:135,672,285-135,674,188) F:GGCTGTTCGTTGACCTTGTTT R: AGGGAATCGTGACGGTTGG, *Id3*_-20kb (chr4:135,678,270-135,680,185) F: TTCGAAAGGCTTCCGGGCTAA R: TCCCTGCGACCCAAAGCTTAC, *Id3*_promoter (chr4:135,698,261-135,700,171) F: AGTTCTCGGTGGAAACGGTC R:CTAGGCGCTGAGATTGCAGA, *Id1*_promoter (chr2:152,736,188-152,736,308) F: TTTGAACGTTCTGAACCCGC R: GGCTGAGAACAGAGTGTGGG, Negative region (chr11:71,360,398-71,360,930) F: TCCCAGCTCACAGGCTAGAA R: AATGCAGAGCAGAAGGGGTC.

Luciferase Assay. The -934/+13bp *Id3*-luciferase reporter construct was kindly provided by Dr. Andrew Lasser(Kumar et al. 2009). Briefly, C2C12 cells were transiently transfected with the *Id3*-luc reporter construct and a constitutively active RSV- β -galactosidase reporter in the presence or absence of mammalian expression plasmid for C/EBP β using FuGENE HD (Promega). Cells were collected 48h post-transfection to assess luciferase activity using the Dual-Luciferase Reporter Assay kit (Promega) according to manufacturer's instructions. Luciferase activity was measured using a Monolight 2010 luminometer (Analytical Luminescence laboratory) and corrected for transfection efficiency with β -gal activity.

Statistical Analysis. Two means were compared by Student's t-test for a minimum of three biological repeats. For multiple means, one-way or two-way ANOVA was conducted with

appropriate post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Other comparisons are explained in the relevant legends. For multiple comparisons, means indicated by different letters or symbols are statistically different from one another with a $p < 0.05$ as a minimum cutoff.

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Competing interests statement.

The authors declare that they have no conflicts of interest.

Author contributions statement.

HA contributed to the conception and design, collection, assembly, analysis and interpretation of data, the manuscript writing and final approval of the manuscript. NLT contributed to the collection, assembly and the analysis of the data, the manuscript writing and final approval of the manuscript. NWB contributed to the conception and design, analysis and interpretation of data, financial support, the manuscript writing, and the final approval of manuscript.

Data availability.

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files)

**CHAPTER THREE: CCAAT/ENHANCER-BINDING PROTEIN BETA
PROMOTES MUSCLE STEM CELL QUIESCENCE THROUGH
REGULATION OF QUIESCENCE-ASSOCIATED GENES**

**CCAAT/Enhancer Binding Protein β Promotes Muscle Stem Cell Quiescence through
Regulation of Quiescence-Associated Genes**

Neena Lala-Tabbert^{a,c}, Hamood AlSudais^b, François Marchildon^{b,d}, Dechen Fu^{a,e} and Nadine
Wiper-Bergeron^{a,1}

^aDepartment of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa,
451 Smyth Road, Rm 2541, Ottawa, Ontario, Canada, K1H 8M5

^bGraduate Program in Cellular and Molecular Medicine, Faculty of Medicine, University of
Ottawa, 451 Smyth Road, Rm 2539, Ottawa, Ontario, Canada, K1H 8M5

^cCurrent Affiliation: Apoptosis Research Centre, Children's Hospital of Eastern Ontario
Research Institute, 401 Smyth Road, Ottawa, ON, K1H 8L1, Canada

^dCurrent Affiliation: Laboratory of Molecular Metabolism, The Rockefeller University, 1230
York Avenue, Box 223, New York, NY USA 10065

^eCurrent Affiliation: Cleveland Clinic, Lerner Research Institute, 9500 Euclid Avenue, NE6-
251, Cleveland, Ohio, USA 44195

¹To whom correspondence should be addressed: Nadine Wiper-Bergeron PhD, 451 Smyth
Road, Room 2539, Ottawa, Ontario, Canada, K1H 8M5 Tel: 1-613-562-5800 ext. 8176, Email:
Nadine.WiperBergeron@uottawa.ca

Running Title: C/EBP β regulates muscle satellite cell quiescence

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Author contributions

Neena Lala-Tabbert: Conception and design, collection of data, Data analysis and interpretation, Manuscript writing, Final approval of manuscript

Hamood AlSudais: Conception and design, collection of data, Data analysis and interpretation, Manuscript writing, Final approval of manuscript

François Marchildon: Collection of data, Provision of study materials, Final approval of manuscript

Dechen Fu: Collection of data, Data analysis and interpretation, Final approval of manuscript

Nadine Wiper-Bergeron: Conception and design, Financial support, Manuscript writing, Final approval of manuscript

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Keywords: muscle satellite cells; CCAAT-Enhancer-Binding Protein-beta; myoblasts, muscle; skeletal; cell differentiation; cell proliferation; quiescence; regeneration

Abstract

Regeneration of skeletal muscle depends on resident muscle stem cells called satellite cells that in healthy, uninjured muscle remain quiescent (non-cycling). After activation and expansion of satellite cells post-injury, satellite cell numbers return to uninjured levels and return to mitotic quiescence. Here, we show that the transcription factor CCAAT/Enhancer Binding Protein beta (C/EBP β) is required to maintain quiescence of satellite cells in uninjured muscle. We show that C/EBP β is expressed in quiescent satellite cells *in vivo* and upregulated in non-cycling myoblasts *in vitro*. Loss of C/EBP β in satellite cells promotes their premature exit from quiescence resulting in spontaneous activation and differentiation of the stem cell pool. Forced expression of C/EBP β in myoblasts inhibits proliferation by upregulation of 28 quiescence-associated genes. Further, we find that Caveolin-1 is a direct transcriptional target of C/EBP β and is required for cell cycle exit in muscle satellite cells expressing C/EBP β . The induction of mitotic quiescence is considered necessary for the long-term maintenance of adult stem cell populations with dysregulation driving increased differentiation of progenitors and depletion of the stem cell pool. Our findings place C/EBP β as an important transcriptional regulator of muscle satellite cell quiescence.

Significance Statement

Little is known about the mechanisms regulating satellite cell quiescence. Here, Lala-Tabbert et al use an unbiased approach to show that C/EBP β -overexpression induces expression of quiescence-associated genes in myoblasts. The authors identify Caveolin-1 as a target of C/EBP β required for cell growth arrest *in vitro*. Furthermore, *in vivo*, C/EBP β is required to maintain muscle satellite cell quiescence in steady state adult muscle.

Introduction

Most adult mammalian tissues contain resident stem cells. These cells are an important reserve for tissue regeneration, providing differentiated cells upon activation while retaining the ability to repopulate the stem cell pool (self-renewal). In tissues with low turnover such as skeletal muscle, adult stem cells remain quiescent (in the G0 or G1 state of cell cycle) for extended periods of time until activated for regeneration and repair (Arai et al. 2004; Cotsarelis et al. 1990; Li and Clevers 2010; Schultz et al. 1978). Loss or dysregulation of quiescence in stem cells can result in an imbalance, characterized by an increase in differentiating progenitor cells and depletion of the stem cell pool (Cheung et al. 2012; Orford and Scadden 2008).

Muscle stem cells, called satellite cells (MuSCs), ensure the regeneration of skeletal muscle (Collins et al. 2005; Sambasivan et al. 2011). Though a heterogeneous population, these cells express PAX7 and are maintained in a quiescent state in resting muscle. Despite the importance of MuSC quiescence in muscle homeostasis, the mechanisms that maintain quiescence are only partially understood. Regulators of MuSC quiescence include Notch signaling (Bjornson et al. 2012; Baghdadi et al. 2018), miRNAs (Cheung et al. 2012; Baghdadi et al. 2018), CDK inhibitors (Chakkalakal et al. 2014), the translation initiation factor eIF2 α (Zismanov et al. 2016), Suv4-20h1 (Boonsanay et al. 2016) and the secreted factor, Oncostatin M (Sampath et al. 2018).

CCAAT/Enhancer Binding Protein β (C/EBP β) is a member of the C/EBP family of basic leucine zipper (bzip) transcription factors that is involved in several cellular processes such as growth and differentiation (Ramji and Foka 2002; Tsukada et al. 2011). C/EBP β 's function in cell proliferation is very complex and highly context specific, having both inhibitory and stimulatory effects. For example, C/EBP β -null mice have enhanced hematopoiesis and mild epidermal hyperplasia, suggesting that C/EBP β inhibits expansion of lymphoid cells and

keratinocytes, respectively (Screpanti et al. 1995; Zhu et al. 1999). By contrast, C/EBP β -null mammary epithelial cells have impaired proliferation and disrupted ductal morphogenesis (Robinson et al. 1998) and C/EBP β has been shown to be essential for mitotic clonal expansion during adipogenesis (Tang et al. 2003; Zhang et al. 2004), suggesting that the regulation of proliferation by C/EBP β is highly cell type specific.

In skeletal muscle, C/EBP β is expressed in MuSCs in resting muscle and when satellite cells are isolated and induced to differentiate, C/EBP β expression is rapidly downregulated (Marchildon et al. 2012; Fu et al. 2015; AlSudais et al. 2018). Persistent expression of C/EBP β in myoblasts upregulates ID3 expression and blocks differentiation, locking cells in a PAX7^{high}MYOD^{low} undifferentiated state (Marchildon et al. 2012; AlSudais et al. 2018). We have also shown that during regeneration, loss of C/EBP β in SCs leads to increased differentiation and fusion at the expense of SC self-renewal (Lala-Tabbert et al. 2016a).

In this study, we examined the mechanism by which C/EBP β maintains MuSC quiescence in uninjured adult muscle. We demonstrate that C/EBP β is highly expressed in quiescent MuSCs and is upregulated in growth arrested myoblasts. In resting muscle, we show that loss of C/EBP β in PAX7⁺ cells results in exit from quiescence leading to stem cell pool depletion. When overexpressed, C/EBP β promotes cell cycle arrest in proliferating C2C12 myoblasts and mediates quiescence through upregulation of several MuSC quiescence-related genes. Of these genes, we identified *Cav1* as direct transcriptional target of C/EBP β required for C/EBP β -induced cell cycle arrest.

Materials and Methods

Mice and Animal Care. All animal work was performed in accordance with the guidelines set out by the Canadian Council on Animal Care and was approved by the University of Ottawa Animal Care Committee. C57BL/6-Tg (human ubiquitin C–green fluorescent protein [GFP]) 30Scha/J mice (GFP mice) aged 6 weeks and C57BL/6J mice aged 4 weeks were obtained from the Jackson Laboratory. C/EBP $\beta^{fl/fl}$ Pax7 $^{+/+}$ (control) and conditional null C/EBP $\beta^{-/-}$ Pax7 $^{CreER-/+}$ (C/EBP $\beta^{-/-}$) mice were previously described (Marchildon et al. 2012). Activation of CreERtm *in utero* was achieved by a single gavage of 2.5 mg of tamoxifen (dissolved in corn oil) of pregnant dams when pregnancy was at E15.5. All animals were housed in a controlled facility (22°C with 30% relative humidity on 12 hours light/dark cycle) and provided with food and water ad libitum.

Primary and C2C12 Myoblast Culture. Primary myoblasts were isolated as described previously (Motohashi et al. 2014). Briefly, hind limb muscles of adult (6 to 8 weeks of age) mice were dissected and digested with collagenase/dispase (Roche), and enriched for SCs by magnetic-activated cell sorting (MACS). Primary myoblasts were cultured in growth media (Dulbecco's Modified Eagle Medium containing 20% fetal bovine serum (FBS), 10% horse serum (HS)) supplemented with 10ng/ml basic fibroblast growth factor (bFGF) and 2ng/ml hepatocyte growth factor (HGF) (Peprotech). To induce CreERtm activity in culture, primary myoblasts were treated with 4-OH tamoxifen (2uM dissolved in 100% ethanol; Sigma-Aldrich) for 48 hours.

C2C12 murine myoblasts were retrovirally transduced with empty vector (pLXSN) or to express C/EBP β (C/EBP β) and selected based on G418 resistance and maintained in growth medium (DMEM with 10% FBS). Finally, pLXSN and pLXSN-C/EBP β expressing C2C12s

were transduced with lentiviral particles targeting caveolin-1 (Cav1Sh) or non-targeting control (ShCtl) (Santa Cruz Biotechnology) to create pooled stable lines.

Induction of mitotic quiescence was achieved by a 48 hour culture in methionine-depleted DMEM supplemented with 1% FBS for 48 hours as previously described (Zhang et al. 2010; Kitzmann et al. 1998).

Flow Cytometry. Primary myoblast and C2C12 myoblast cultures were fixed with cold 70% ethanol overnight. Cells were then washed and stained with propidium iodide (50ug/ml; Molecular Probes) and analyzed in a CyANTM ADP Analyzer with data analysis using Kaluza[®] Flow Analysis Software (Beckman Coulter).

Western Analysis. Whole cell extracts from C2C12s and primary myoblasts were resolved on a 12% SDS-PAGE gel, transferred to a PVDF membrane and probed with specific antibodies: C/EBP β (E299; Abcam), Caveolin-1 (N-20; Santa Cruz), and Cyclophilin B (Abcam). Chemiluminescence was detected with the ChemiDocTM MP System (Bio-Rad Laboratories).

Real-time quantitative PCR. Total RNA was isolated from C2C12s and primary myoblasts using the RNeasy kit (QIAGEN), treated with DNase (Ambion) and reverse transcribed using iScript cDNA synthesis kit (Bio-Rad Laboratories) following manufacturers' protocols. RT-qPCRs were performed using iTaq Universal SYBR[®] Green Supermix (Bio-Rad Laboratories) on a CFX96TM Real-Time PCR Detection System (Bio-Rad Laboratories). The $\Delta\Delta C_t$ method was used to determine relative fold induction after normalization with 18S rRNA.

Chromatin Immunoprecipitation. C2C12 myoblasts were crosslinked for 30 min at room temperature with 1% formaldehyde and sonicated with a Diagenode bioruptor[®]. Chromatin immunoprecipitation (ChIP) analysis was performed as described (Wiper-Bergeron et al., 2003) using antibodies against C/EBP β (C-19; Santa Cruz) or rabbit IgG as negative control.

Protein G conjugated Dynabeads (Invitrogen) were used to pull down immunoconjugates and DNA fragments were purified using the Qiaquick PCR purification kit (Qiagen). A standard curve was generated using input DNA for each immunoprecipitate and quantification was determined as the abundance of target DNA to the input DNA and presented as fold difference to negative control (IgG). Primer sequences for qPCR amplification were forward: 5-ATGGAGCCTCCCTGTTGTTG-3 and reverse: 5-ATGTGTGGGGACCCAAATCC-3.

Myoblast Transplantation. Muscle regeneration in 5-week-old C57BL/6 mice was induced by injecting 30 μ l of 10 μ M cardiotoxin (CTX; Sigma-Aldrich) into the midbelly of both TA muscles. The next day, 10^5 GFP donor myoblasts retrovirally transduced to express pLXSN and C/EBP β were injected into the right and left TA of the CTX-injured mice, respectively. Mice were sacrificed 2 weeks after transplantation, and the TAs were collected, embedded, in optical cutting temperature (OCT) compound, flash frozen in isopentane cooled by liquid nitrogen, and sectioned (8 mm thick) for immunohistochemistry.

Immunofluorescence. Myoblasts were fixed in 2% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and blocked with 0.3% Triton X-100 and 10% goat serum. Cryosections were fixed in 4% PFA and processed for antigen retrieval in citrate buffer at 95°C for 20 minutes. Sections were permeabilized with 0.5% Triton X-100 PBS and blocked in 0.1% Triton X-100, 5% donkey serum (Cederlane) prior to incubation with primary antibody overnight at 4°C. Cells and sections were then incubated with biotin anti-mouse (for Pax7 staining) or secondary antibodies conjugated to a fluorescent dye (Cy3, Alexa 488 or Alexa Flour 647; all from Jackson ImmunoResearch). Nuclei were counterstained with DAPI (0.5 μ g/ml). Primary antibodies used were: Pax7-c (DSHB), C/EBP β (C-19; Santa Cruz), MyoD (C-20; Santa Cruz), myogenin (M-225; Santa Cruz), Ki67 (Abcam), GFP (abcam),

caveolin-1 (N-20; Santa Cruz), GFP (Abcam), Pax3/7 (H-208; Santa Cruz) and laminin (AL-4; Millipore).

BrdU labelling. Proliferating myoblasts were pulsed with 10 μ M BrdU (Sigma) for 6 hours and fixed with 2% paraformaldehyde for 15 min then 95% methanol for 30 min. Cells were permeabilized in 1N HCL for 10 min followed by 2N HCL for an additional 10 min. Following incubation in 0.1M Sodium Borate (pH 8.5) for 12 min, cells were blocked for 1 hour and incubated with anti-BrdU-biotin overnight. Detection was achieved using streptavidin-conjugated secondary antibody.

Microscope Image Acquisition. Digital images were taken at room temperature with a Leica DM 3000B microscope and Infinity-3 camera (Lumenera). The Infinity Capture imaging software (Lumenera) was used to take images which were later composed and edited in paint.net.

RNA-Sequencing Sample Preparation and Analysis. RNA was extracted from proliferating C2C12 myoblasts retrovirally transduced to overexpress C/EBP β or express empty vector using RNeasy kit. Total RNA was sent to Genome Quebec for NEB mRNA stranded library preparation which were then sequenced using the Illumina HiSeq2500 SR50. Reads were analysed by the Ottawa Bioinformatics core facility at the Sprott Center for Stem Cell Research in the Ottawa Hospital Research Institute. Briefly, reads were mapped to the mouse genome (mm10, GRCm38) using HISAT2 with more than 94.5% of reads mapping to the mouse genome. Mapped reads were then counted using featureCounts and differentially expressed genes were generated using DESeq2 package. Functional analysis (gene ontology (GO)) analysis of differentially expressed genes were performed using the web-based DAVID 6.8 (Huang et al. 2009a).

Differential Expression Analysis. All used microarray datasets were publicly available in the GEO database (GSE3483, GSE15155, GSE47177). QSCs were obtained from freshly isolated MuSCs. ASCs were obtained from cultured cells after isolation in GSE3483 and GSE1515, and 60 hr post BaCl₂-injury in GSE47177. Differentially expressed genes between QSC and ASC in each dataset were determined using the interactive web tool GEO2R. We compared genes that were differentially expressed in QSC with $\text{Log}_2\text{FC} \geq 2$ to differentially expressed genes in C/EBP β -overexpressing C2C12s with $\text{Log}_2\text{FC} \geq 1$. Differentially expressed genes between paraformaldehyde (PFA)-fixed and unfixed MuSCs from RNA-Seq performed by Machado et al were obtained from the original publication (Machado et al. 2017) and a cut-off of 2-fold was used for comparison with differentially expressed genes in C/EBP β -overexpressing C2C12s. Venn diagram for genes shared between datasets were detected and prepared using web-based tools (bioinformatics.psb.ugent.be/webtools/Venn/ and genevenn.sourceforge.net/index.htm). The list of cell proliferation genes was obtained from the Mouse Genome Informatics (MGD, www.informatics.jax.org/vocab/gene_ontology).

Single cell RNA-seq analysis. Filtered raw barcode/feature (cell/gene) count matrices were downloaded from GEO for two samples of homeostatic MuSCs (accessions GSM3614994, GSM3614995) (Dell'Orso et al. 2019). Matrices were loaded into Seurat v3.2.2 running under R v4.0.2, merged, and normalized using the Seurat NormalizeData (Butler et al. 2018) function with default parameters. Normalized UMI count matrices were exported for further visualization. Data were processed by the Ottawa Bioinformatics core facility at the Sprott Center for Stem Cell Research in the Ottawa Hospital Research Institute.

Statistical Analysis. Statistical analysis was performed using GraphPad Prism Software (version 8.4.3). A two-tailed student's t test was performed when comparing one experimental condition to the control. Significance is indicated as * <0.05 , ** <0.01 and *** <0.001 . One-

way ANOVA was used to determine significance between three or more experimental conditions. When significance was determined by the one-way ANOVA, a Tukey's post hoc test was used to determine the conditions that were significant. All experiments are representative of a minimum of three biological replicates and presented at the mean $\pm$ standard error mean (SEM). Flow cytometry data was analyzed using Chi-square test to compare proportions.

Data Availability Statement. The data that support the findings of this study are derived in part from resources available in the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) with reference numbers GSE3483 (Fukada et al.), GSE15155 (Pallafacchina et al.), GSE47177 (Liu et al.), GSE103164 (Machado et al.) and GSE126834 (Dell'Orso et al). Original RNA-seq data was deposited to GEO with accession number GSE152051.

Results

C/EBP β is required for SC quiescence. Overexpression of C/EBP β increases PAX7 expression while decreasing MYOD protein expression (Marchildon et al. 2012) and this profile coupled with low Ki67 expression is characteristic of cells that self-renew under differentiation conditions to return to quiescence (Motohashi and Asakura 2014; Ono et al. 2012). We first examined the expression of *Cebpb* in a published single cell RNA-Seq of freshly isolated and FACS-purified mouse MuSCs (Dell'Orso et al. 2019). This analysis indicated that *Cebpb* is expressed in almost all (~88%) *Pax7*⁺ cells (Fig. 9A). Next, we examined whether quiescent MuSCs express C/EBP β protein by immunostaining tibialis anterior (TA) muscle from control mice at postnatal day 56. We found that C/EBP β is

expressed in PAX7-positive cells (Fig. 9B), confirming our previous findings (Marchildon et al. 2012). To determine if C/EBP β is regulated during the transition from activation to quiescence, we used a C2C12 myoblast model in which we induced reversible cell cycle arrest by methionine-depletion. This halts the cell cycle and prevents myogenic differentiation (Kitzmann et al. 1998). G0/G1 arrest was confirmed by cell cycle analysis, with approximately 93% of cells in G0/G1 versus approximately 63% in growth medium (GM) (Fig. 9C). Interestingly, C/EBP β expression was higher in methionine-depleted cells when compared to proliferating C2C12s (GM) (Fig. 9D, E), suggesting that C/EBP β is upregulated when proliferating myoblasts are induced to exit the cell cycle.

To examine the effect of C/EBP β -deficiency on myoblast proliferation, we isolated primary myoblasts from conditional knockout mice (*Cebpb^{fl/fl}Pax7^{CreER/+}*; C/EBP β ^{-/-}) in which C/EBP β expression is abrogated in *Pax7*⁺ cells following activation of the CreER recombinase with tamoxifen, and controls (*Cebpb^{fl/fl}Pax7^{+/+}*; wild-type). Quantitative reverse transcription polymerase chain reaction (RT-qPCR) and western blot analysis confirmed efficient knockdown of C/EBP β when freshly isolated myoblasts were cultured in tamoxifen (Fig. 10A-C). As shown previously, of the three possible C/EBP β protein isoforms, the activating Liver Activator Protein (LAP) isoform is predominantly expressed in myoblasts (Marchildon et al. 2012). MuSC-derived myoblasts from conditional knockout and wild-type mice were cultured in high serum (growth medium, GM) and 24 hours after plating, cell proliferation was assessed by Ki67 immunostaining (Fig. 10D). Loss of C/EBP β resulted in a larger fraction of cycling cells in C/EBP β ^{-/-} cultures (58%) as compared to control cultures (46%) (Fig. 10D,E). Further, a larger percentage of cells were in S phase, as measured by BrdU incorporation after a 6 hours pulse (Fig. 10F). Cell cycle kinetics was also assessed by flow cytometry (Fig. 10G) and

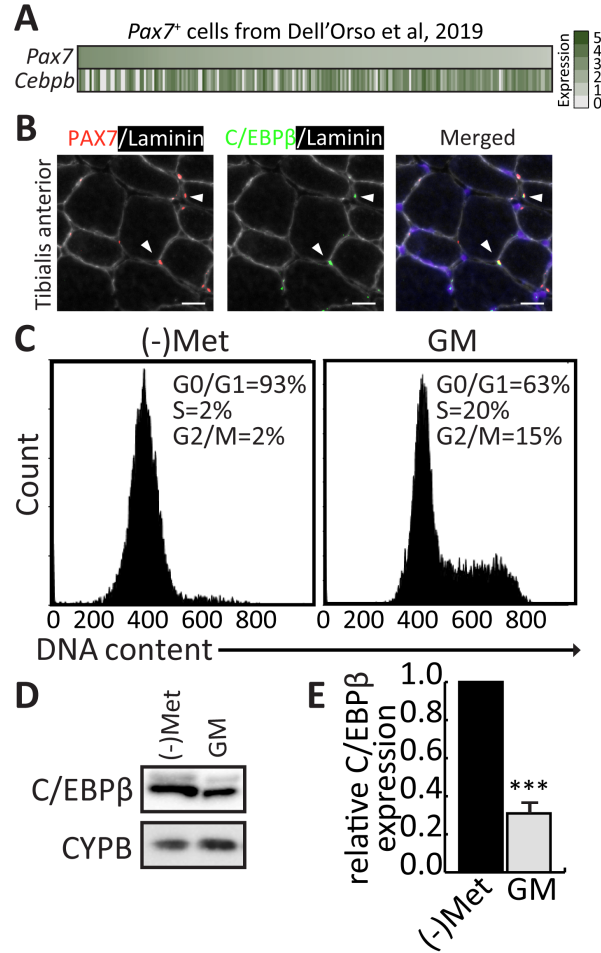


Figure 9. C/EBPβ is expressed in growth arrested myoblasts. (A) Heatmap of the expression of *Pax7* and *Cebpb* in *Pax7*-expressing cells from published scRNA-Seq of FACS-isolated MuSCs (VCAM⁺/CD31⁻/CD45⁻/Sca1⁻) (Dell'Orso et al, 2019). (B) Representative pictures of uninjured TA sections stained for PAX7 (red), C/EBPβ (green) and Laminin (white) from mice at P56. Nuclei were counterstained with DAPI (blue). Solid arrow heads (►) mark PAX7⁺/C/EBPβ⁺ satellite cells. Scale bar: 20μm. (C) Flow cytometric analysis of DNA content in C2C12 myoblasts cultured in growth medium (GM) or methionine-depleted ((-)Met) media to induce G0 arrest. A minimum of 10,000 events were counted per sample. ***p<0.0001, Chi-square test to compare proportions. (D) Representative western blots of C/EBPβ expression from cells cultured as in (C). Cyclophilin B (CYPB) is a loading control.

(E) Quantification of C/EBP β protein expression normalized with CYPB and relative to (-)
Met. Data information: Data in panel E are presented as the mean $\pm$ SEM, ***p<0.001
(Student's t-test).

revealed that C/EBP β -deficient cells had fewer cells in the G0/G1 phase (51%) and more cells in the S (21%) and G2/M phase (25%) compared to control myoblasts (62%, 15% and 20%, respectively) (Fig. 10G) confirming the Ki67 and BrdU incorporation data (Fig. 10D,E).

To confirm these results *in vivo*, the MuSC population was assessed in wild-type and conditional null (C/EBP β ^{-/-}) muscle following excision of *Cebpb* *in utero* by a single tamoxifen gavage of pregnant dams at E15.5 as previously described (Marchildon et al. 2012). Adult mice were sacrificed at postnatal day 56 (P56) and knockdown of C/EBP β was confirmed by scoring the number of C/EBP β ⁺/PAX7⁺ cells in the TA muscle as a function of area (Fig. 10H). We assessed the total number of PAX7⁺ cells in TA muscle and found that the number of PAX7⁺ cells was significantly reduced in C/EBP β ^{-/-} muscle compared to control littermates (Fig. 10I, J) suggesting that C/EBP β is required for the maintenance of MuSCs in uninjured adult muscle. To examine the cell cycle state of these PAX7⁺ MuSCs, we performed dual immunohistochemistry (IHC) of control and C/EBP β ^{-/-} TA muscle sections for PAX7 and Ki67. We expected the majority of PAX7⁺ MuSCs to be quiescent (Ki67⁻) at P56; however, we saw a >2-fold increase in the number of cycling (Ki67⁺) cells in C/EBP β ^{-/-} muscle compared to control muscle (Fig. 10K), suggesting that MuSCs continue to cycle when *Cebpb* is absent from PAX7⁺ cells, whereas the majority of MuSCs are quiescent (Ki67⁻) in control muscle. To determine if C/EBP β deficiency in MuSCs of resting muscle leads to activation and precocious differentiation, muscle sections were co-immunostained for PAX7 and MYOD. While only 11% of PAX7⁺ cells were MYOD⁺ in control muscle, 42% of PAX7⁺ cells were also MYOD⁺ in C/EBP β ^{-/-} muscle, suggesting that MuSCs activate and proliferate in the absence of C/EBP β (Fig. 10L). Taken together these results suggest that C/EBP β is required to maintain the quiescent state of MuSCs in healthy, uninjured muscle of adult mice. Depletion

of C/EBP β in MuSCs results in their spontaneous exit from quiescence, proliferation and differentiation leading to a reduction of the MuSC pool.

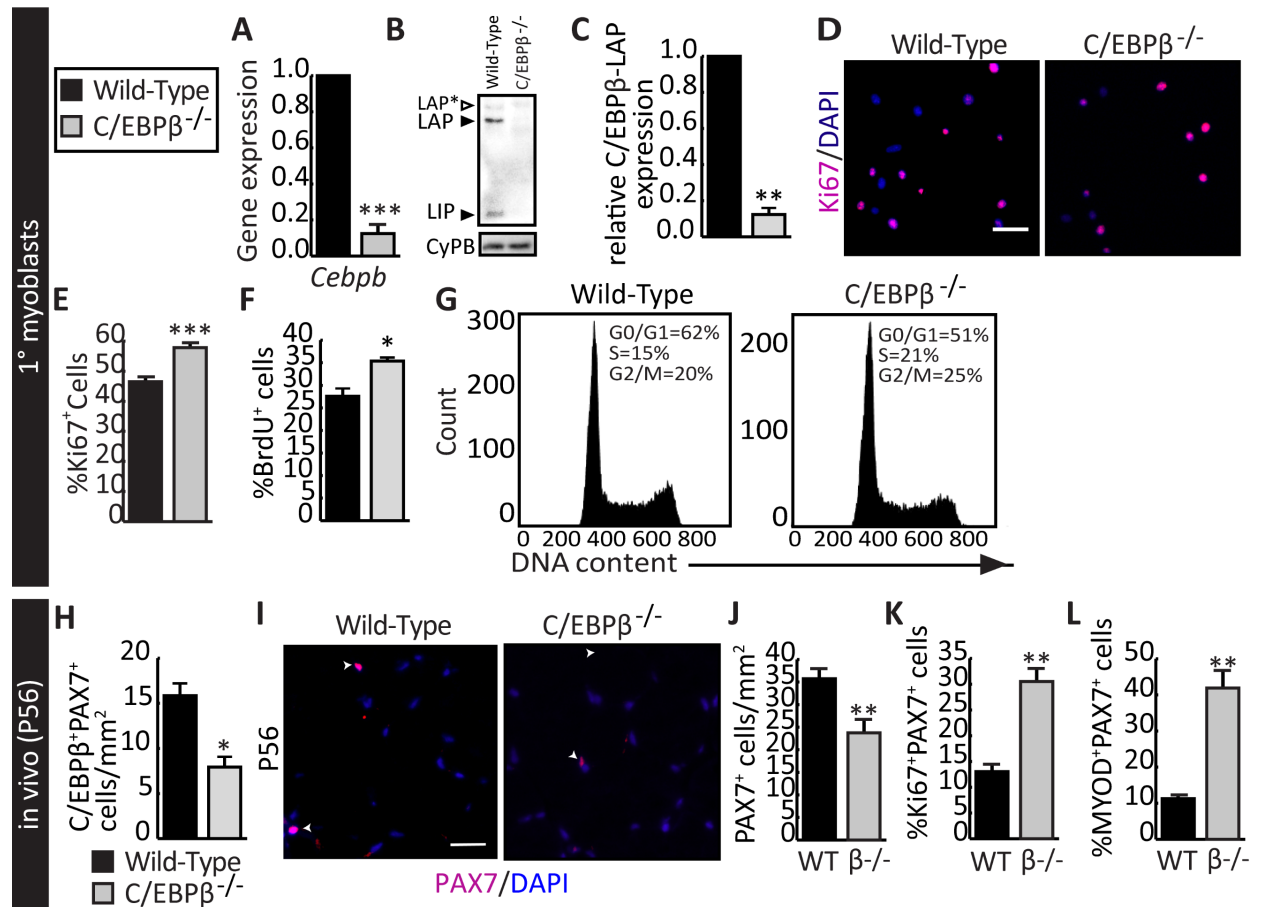


Figure 10. Loss of C/EBP β leads to premature activation of myogenic progenitors. (A) *Cebpb* mRNA expression in primary myoblasts isolated from wild-type and conditional C/EBP β knockout (C/EBP β ^{-/-}) mice (n=3). **(B)** Representative western blots of C/EBP β protein isoform expression (LAP*, LAP and LIP) in myoblasts from wild-type and C/EBP β ^{-/-} mice. Cyclophilin B (CyPB) is a loading control. **(C)** Quantification of C/EBP β LAP isoform expression normalized to CyPB, shown relative to wild-type. **(D)** Representative images of wild-type and C/EBP β ^{-/-} myoblasts cultured in growth medium and stained with Ki67 (red)

and DAPI (blue). Scale bar = 50 μ m. (E) Percent Ki67⁺ primary myoblasts relative to total cells from (D). (n=3) (F) Percent BrdU⁺ primary myoblasts relative to total cells (n=3). (G) Flow cytometric analysis of DNA content in wild-type (left) and C/EBP β ^{-/-} primary myoblasts. A minimum of 10,000 events were counted per sample. ****p<0.0001, Chi-square test to compare proportions. (H) Number of C/EBP β ⁺PAX7⁺ cells per mm² of TA muscle from wild-type and C/EBP β conditional knockout (C/EBP β ^{-/-}) mice at post-natal day 56 (P56) (n=3 mouse pairs). (I) Representative pictures of PAX7⁺ cells (red) in TA muscle sections from wild-type and C/EBP β ^{-/-} mice at P56. Scale bar = 20 μ m. (J) Number of PAX7⁺ cells per area (mm²) at P56 (n=3 mouse pairs). (K) Percentage of Ki67⁺/PAX7⁺ cells relative to total PAX7⁺ population in muscle from (I). (L) Percentage of MYOD⁺/PAX7⁺ cells relative to total PAX7⁺ population in TA muscle of wild-type and C/EBP β ^{-/-} mice at P56 (n=3 mouse pairs). Data information: In A, C, E, F, H, J-L, data are presented as the mean $\pm$ SEM, *p<0.05, **p<0.01, ***p<0.001 (Student's t-test).

C/EBP β induces quiescence in cycling myoblasts. To determine if C/EBP β expression modifies myoblast cell cycle kinetics to promote quiescence, C2C12s retrovirally transduced with empty vector (pLXSN) or to express C/EBP β were cultured under high serum conditions (GM) for 24 hours. RT-qPCR confirmed overexpression of *Cebpb* (Fig. 11A). In cells overexpressing C/EBP β , only ~43% of cells were cycling (Ki67⁺) whereas, ~70% of cells were cycling in pLXSN controls (Fig. 11B and C), suggesting that C/EBP β promotes cell cycle exit under growth conditions. Further, BrdU incorporation after a 6-hour pulse was reduced by >20% when C/EBP β was overexpressed (Fig. 11D).

To determine the effect of constitutive expression of C/EBP β on satellite cells *in vivo*, donor myoblasts were isolated from GFP mice and retrovirally transduced with empty vector (pLXSN) or to express C/EBP β . Overexpression of C/EBP β was confirmed by western blot analysis (Fig. 11E). Equal number of cells were transplanted into regenerating TA muscles of C57BL/6 mice and allowed to engraft for two weeks. The number of GFP⁺ fibers with centrally located nuclei in the TA was assessed to determine the myogenic potential of transplanted myoblasts (Fig. 11F,G). C/EBP β -overexpressing myoblasts gave rise to significantly fewer regenerating fibers compared to pLXSN myoblasts (Fig. 11G), consistent with our previous results showing that C/EBP β inhibits myoblast differentiation *in vitro* (Lala-Tabbert et al. 2016b; Marchildon et al. 2012; AlSudais et al. 2018; Fu et al. 2015). In line with a requirement for C/EBP β in maintaining MuSC quiescence (Fig. 10), there were more PAX7⁺/GFP⁺ cells in muscle transplanted with C/EBP β -overexpressing myoblasts (~12.5%) compared to muscles transplanted with pLXSN myoblasts (~5%) (Fig. 11H). Furthermore, constitutive expression of C/EBP β in myoblasts inhibited their ability to proliferate *in vivo*, as demonstrated by decreased numbers of Ki67⁺/GFP⁺ cells compared to control myoblasts (Fig. 11I). To confirm that C/EBP β overexpression in C2C12s and primary myoblast does not induce senescence, we

performed senescence-associated (SA) β -galactosidase (β -gal) staining (Fig. S3). SA β -gal⁺ cells were only present in etoposide-treated myoblasts and were not present in either pLXSN or C/EBP β -overexpressing C2C12s or primary myoblasts confirming that these cells are not senescent (Fig. S3). Together, these experiments show that C/EBP β can inhibit proliferation of myoblasts and improve engraftment of primary myoblasts into the MuSC niche *in vivo*.

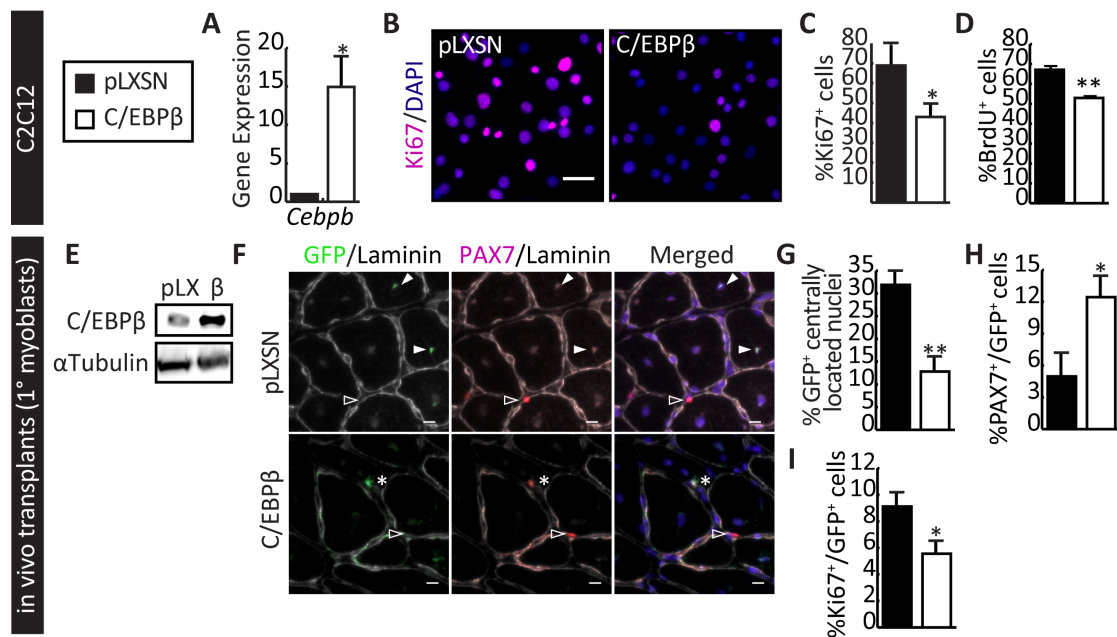


Figure 11. C/EBP β induces quiescence in myogenic progenitors. (A) *Cebpb* mRNA expression in pLXSN and C/EBP β C2C12 myoblasts cultured in growth medium (n=5). (B) Representative images of pLXSN and C/EBP β C2C12 myoblasts stained with Ki67 (red) and DAPI (blue). Scale bar = 50 μ m. (C) Percent Ki67⁺ C2C12 myoblasts relative to total nuclei (n=4). (D) Percent BrdU⁺ C2C12 myoblasts relative to total cells (n=3). (E) Representative western blot of C/EBP β expression in GFP⁺ primary myoblasts retrovirally transduced to overexpress C/EBP β (C/EBP β) or with empty vector (pLXSN) used for transplantation

experiments in (F). α Tubulin is a loading control. **(F)** Representative images of TA sections from C57BL/6 mice transplanted with primary myoblasts transduced as in (E). Sections were stained for GFP (green), PAX7 (red), LAMININ (white) and DAPI (blue). Solid arrow heads (►) mark GFP⁺ cells, open arrow heads (◄) mark PAX7⁺ cells and asterisks (*) mark GFP⁺/PAX7⁺ cells. Scale bar =10 μ m. **(G)** Percent of centrally located GFP⁺ nuclei relative to total number of centrally located nuclei (n=4 mouse pairs). **(H)** Percent of PAX7⁺/GFP⁺ cells relative to total number of PAX7⁺ cells (n=4 mouse pairs). **(I)** Percent of Ki67⁺/GFP⁺ cells relative to total number of GFP⁺ cells (n=4 mouse pairs). Data information: In A, C, D, G-I, data are presented as the mean $\pm$ SEM, *p<0.05, **p<0.01 (Student's t-test).

C/EBP β regulates the expression of genes associated with MuSC quiescence. Given the *in vivo* effect of C/EBP β loss- and gain-of-function on MuSCs quiescence (Fig. 10 and 11), we sought to identify potential mediators of C/EBP β -induced cell cycle arrest. We performed RNA-seq on C2C12 myoblasts retrovirally transduced to express C/EBP β or with empty vector. In myoblasts overexpressing C/EBP β , 1351 coding genes were differentially expressed (≥ 2 -fold change, $p < 0.05$) compared to controls. Of these genes, 831 (62%) were upregulated and 520 (38%) were downregulated in C/EBP β -overexpressing cells (Fig. 12A). In accordance with a negative role for C/EBP β in myogenic differentiation (Marchildon et al. 2012; AlSudais et al. 2018; Fu et al. 2015), GO analysis of downregulated genes showed that the top terms were associated with cell differentiation, muscle contraction and development (26% of downregulated genes) (Fig. 12A). Interestingly, 178 genes involved in cell proliferation showed altered expression in C/EBP β -overexpressing myoblasts (121 upregulated and 57 downregulated) (Fig. 12A). These data suggest that C/EBP β inhibits myoblast proliferation and induces growth arrest through the transcriptional regulation of cell proliferation genes.

To identify C/EBP β -regulated genes with a potential role in the regulation of MuSC quiescence, we compared our RNA-seq data with three different microarray datasets (Fukada et al (GSE3483), Pallafacchina et al (GSE15155), Lui et al (GSE47177) that compared gene expression in quiescent MuSC (QSC) to activated MuSC (ASC) (Fukada et al. 2007; Pallafacchina et al. 2010; Liu et al. 2013). In all three datasets, C/EBP β expression is upregulated in quiescent MuSCs compared to activated MuSCs independently confirming our findings. Our analysis showed that there is 21-24% similarity between genes that are differentially expressed in C/EBP β -overexpressing myoblasts and QSCs, compared to control myoblasts and ASCs, respectively (Fig. 12B, C and Supplemental Table 1-4). In GSE3483 (Fukada et al. 2007), 988 genes were upregulated by more than 4-fold in QSC, of which 175

(17.7%) were also upregulated in C/EBP β -overexpressing myoblasts (Fig. 12B and Supplemental Table 2). Similarly, 190 (17.3%) of 1110 QSC-upregulated genes from GSE15155 (Pallafacchina et al. 2010) and 84 (20.3%) of 413 QSC-upregulated genes from GSE47177 (Liu et al. 2013) were also upregulated in C/EBP β -overexpressing myoblasts (Fig. 12B and Supplemental Table 3 and 4). Moreover, 6% (52 of 871), 4.2% (40 of 950) and 4.2% (19 of 448) of QSC-downregulated genes from GSE3483, GSE15155 and GSE47177 were also downregulated in C/EBP β -overexpressing myoblasts (Fig. 12C and Supplemental Table 2-4). Further comparisons identified 58 genes (including C/EBP β) that were upregulated and 6 genes that were downregulated in C/EBP β -overexpressing myoblasts and QSCs in all datasets (Fig 12B and C). Previous analysis of QSC datasets revealed significant differences in the molecular signature of QSC due to the heterogeneity in experimental, biological and technical conditions between studies (Pietrosemoli et al. 2017; Machado et al. 2017; Fukada et al. 2007; Liu et al. 2013; Pallafacchina et al. 2010). It has been suggested that the isolation method can affect the transcriptome of QSCs due to activation during MuSC purification (Machado et al. 2017; Pietrosemoli et al. 2017). Despite the heterogeneity in the molecular signature of QSC in each dataset, C/EBP β was upregulated in all the examined datasets and potentially regulates more than 20% of top QSC-associated genes (Fig. 12B and C). A study by Machado et al reported significant transcriptional changes between unfixed and *in situ* paraformaldehyde (PFA)-fixed FACS-isolated MuSCs (Machado et al. 2017). Thus, we compared C/EBP β -regulated genes from our RNA-seq to PFA-fixed MuSCs (true quiescent MuSCs) genes. Interestingly, 17% of C/EBP β -upregulated genes are upregulated in “true quiescent MuSCs” and 14% of C/EBP β -down regulated genes are downregulated in “true quiescent MuSCs” (Fig. 12D and Supplemental Table 5). To include all potential C/EBP β -regulated QSC-associated genes, we focused our analysis on genes that were upregulated in

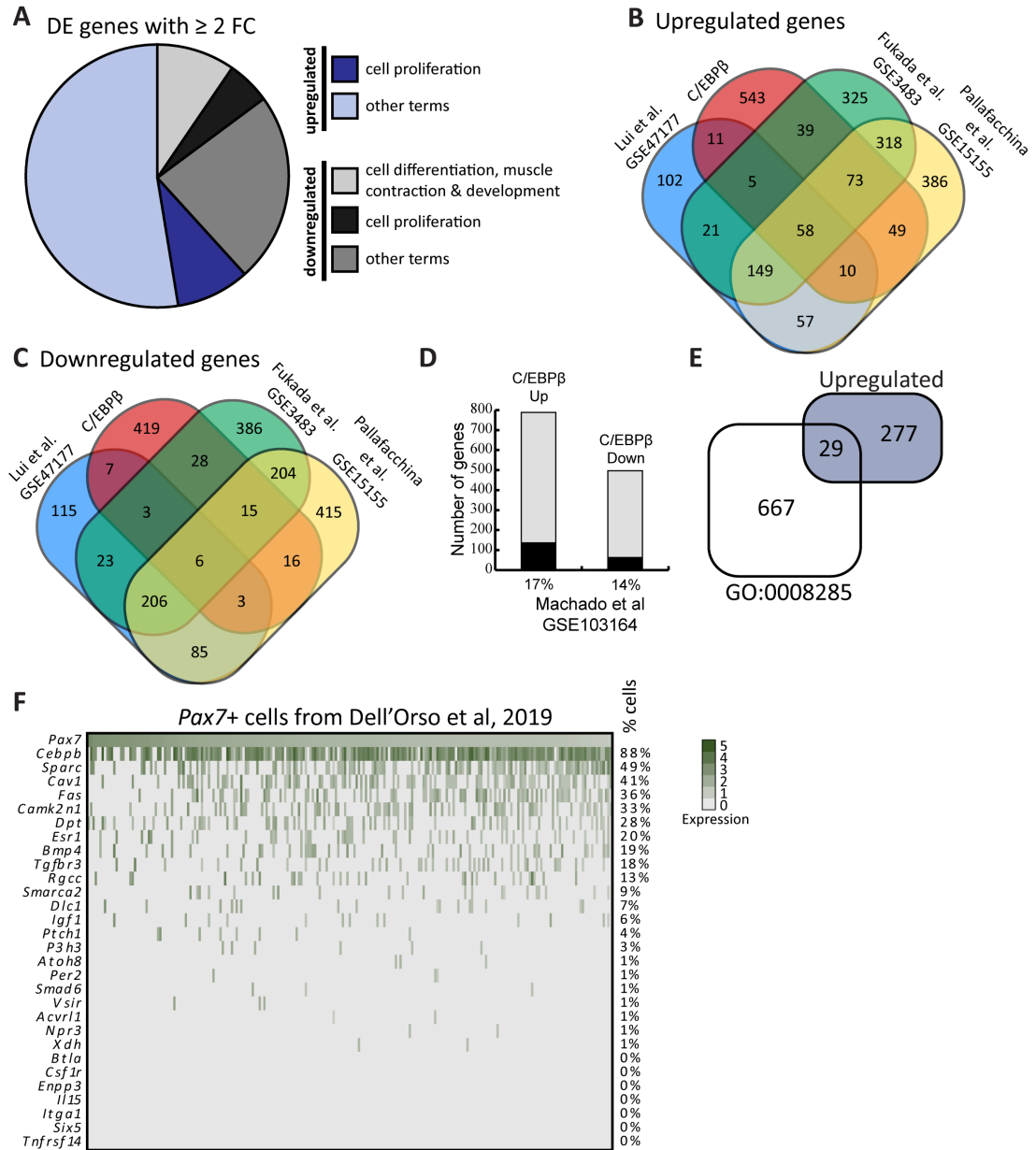


Figure 12. C/EBP β regulates MuSC quiescence-associated genes. (A) Pie chart of differentially expressed (DE) genes with ≥ 2 -fold change (FC) and adjusted p-value ≤ 0.05 from RNA-seq analysis of C/EBP β -overexpressing C2C12 myoblasts compared to empty vector control. Percentage of selected gene-ontology is included. (B) Venn-diagram of significantly upregulated genes with ≥ 2 -fold change in C/EBP β -overexpressing C2C12 myoblasts compared to empty vector control (red) and ≥ 4 -fold change in QSC compared to

ASC in GSE3483 (Fukada et al) (green), GSE15155 (Pallafacchina et al) (yellow) and GSE47177 (Lui et al) (blue). **(C)** Venn-diagram of significantly downregulated genes with ≥ 2 -fold change in C/EBP β -overexpressing C2C12s and ≥ 4 -fold change QSC datasets as in (B). **(D)** Percentage of genes shared between C/EBP β -regulated genes from (A) and PFA-fixed “true quiescent” MuSCs (GSE103164) (Machado et al). **(E)** Venn-diagram of overlap between the 306 upregulated genes in C/EBP β -overexpressing C2C12s and any one QSC datasets (GSE3483, GSE15155, GSE47177, GSE103164) and 696 genes that are annotated as negative regulators of cell proliferation (GO:0008258). **(F)** Heatmap of the expression of the 29 genes identified in (E) in *Pax7*-expressing cells from published scRNA-Seq of FACS-isolated MuSCs (VCAM⁺/CD31⁻/CD45⁻/Sca1⁻) (Dell’Orso et al). The proportion of Pax7⁺ cells expressing an individual gene is included.

C/EBP β -overexpressing myoblasts and in QSC from any one dataset (Fig. 12B,D), resulting in a list of 306 genes (of which 44% are found in true quiescent MuSCs) which were then compared to mouse genes that are annotated as negative regulators of cell proliferation (GO:0008285) (Fig. 12E). We identified 29 (28 excluding *Cebpb*) genes that negatively regulate cell proliferation and are upregulated in C/EBP β -overexpressing cells. Among these 28 genes, several known inhibitors of MuSC proliferation were identified, including *Dpt*, *Esr1*, *Six5* and *Cav1* (Fig. 12E, F) (Collins et al. 2019; Kim et al. 2019; Yajima et al. 2010; Volonte et al. 2005; Gnocchi et al. 2009). Next, we sought to evaluate the proportion of MuSCs that express any of these 28 genes. Using published single cells-RNAseq data from FACS-isolated MuSCs, we found that the majority of these genes (19 of 29) are detected in less than 10% of *Pax7*-expressing cells (Fig. 12F), indicating that these 19 genes are expressed at a low level and would not be efficiently detected during sequencing. Interestingly, after *Cebpb* that is expressed in more than 88% of MuSCs, *Sparc* expression was found in 49% of *Pax7*⁺ cells and *Cav1* in 41% (Fig. 12F). The role of the Secreted Protein Acidic and Rich in Cysteine (*Sparc*) gene product in myogenic differentiation is unclear. Ectopic expression of SPARC in myoblasts has been shown to inhibit myogenic differentiation (Petersson et al. 2013), while other reports found that SPARC promotes differentiation (Munk et al. 2019; Motamed et al. 2003). Caveolin-1 (*Cav1*), however, is a structural protein component of caveolae that has been identified as a surface marker of quiescent satellite cells (Volonte et al. 2005; Gnocchi et al. 2009). A recent study examining human MuSC heterogeneity identified a population of CAV1⁺ MuSCs (CXCR4/CD29/CD56/CAV1) that were resistant to activation upon isolation and showed improved engraftment after transplantation (Barruet et al. 2020), a phenotype similar to C/EBP β -overexpressing primary myoblasts (Fig. 11) (Lala-Tabbert et al. 2016b). Thus, we next investigated the role of *Cav1* in the C/EBP β -growth arrest phenotype.

C/EBP β regulates Caveolin-1 expression. To examine whether C/EBP β regulates MuSC quiescence via the regulation of *Cav1*, we first performed RT-qPCR and western blot analysis on extracts from C/EBP β -overexpressing or control C2C12s cultured in growth medium. C/EBP β overexpression stimulated *Cav1* gene expression and CAV1 protein expression (Fig. 13A-C), validating our RNA-seq data. To examine whether the change in CAV1 expression is due to an increase in the number of CAV1⁺ cells or due to an increase in CAV1 expression in each cell, we performed immunostaining on C/EBP β -overexpressing or empty vector control C2C12s (Fig. 13D). Interestingly, there were more CAV1⁺ cells in C/EBP β -overexpressing cultures compared to control and these cells expressed more CAV1 protein (Fig. 13D, E).

Since *Cav1* expression was stimulated in C/EBP β -overexpressing cells, we examined whether C/EBP β can bind to the regulatory regions of *Cav1*. *In silico* analysis of the *Cav1* regulatory region identified 12 putative C/EBP β binding motifs. Using publicly available C/EBP β ChIP-seq reads from C2C12 myoblasts (GSE36024), we identified a C/EBP β binding peak 25bp upstream of a TATA-binding protein (TBP) motif, near six of the predicted C/EBP β binding sites (Fig. 13F, inset). Chromatin immunoprecipitation (ChIP) analysis performed in pLXSN and C/EBP β -overexpressing C2C12s revealed that C/EBP β occupies the *Cav1* regulatory region in pLXSN C2C12s and to a greater extent in C/EBP β -overexpressing C2C12s (Fig. 13G), suggesting that C/EBP β is a transcriptional regulator of *Cav1*.

Given that the Caveolin-1 gene is regulated by C/EBP β *in vitro*, TA muscle sections from wild-type (WT) and conditional knockout (C/EBP β ^{-/-}) animals at P56 were immunostained for CAV1 to determine if C/EBP β regulates CAV1 expression *in vivo*. Consistent with our *in vitro* data, we observed a significant reduction in the number of CAV1⁺ cells among the MuSC (PAX7⁺) population in the TA of C/EBP β conditional knockout animals compared to controls

(Fig. 13H, I). Taken together, these data confirm the regulation of Caveolin-1 expression by C/EBP β in MuSC.

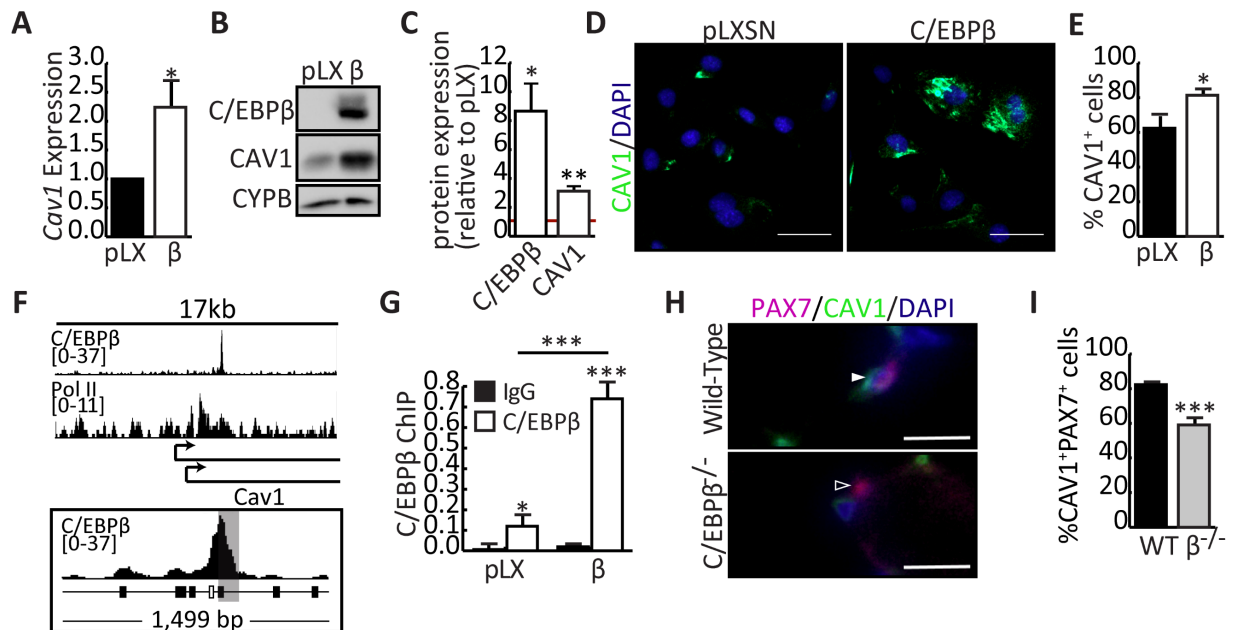


Figure 13. C/EBP β is a transcriptional regulator of Caveolin-1. (A) RT-qPCR for *Cav1* expression in empty vector (pLX) and C/EBP β -overexpressing (β) C2C12 myoblasts cultured in GM (n=5). (B) Representative western blot of C/EBP β and Caveolin-1 (CAV1) expression in control (pLX) and C/EBP β -overexpressing (β) C2C12 myoblasts cultured in GM. CYPB is a loading control. (C) Quantification of C/EBP β and CAV1 protein expression normalized with CYPB and relative to control (pLXSN) (n=4). (D) Representative images of empty vector and C/EBP β -overexpressing C2C12 myoblasts stained with CAV1 (green) and DAPI (blue). Scale bar = 50 μ m. (E) Percent CAV1⁺ C2C12 myoblasts relative to total nuclei (n=4). (F) *Cav1* regulatory region (Chr6:17,250,000-17,266,000) with C/EBP β (GSE36024) and RNA Pol II (GSE25308) binding peaks indicated. Region Chr6:17,258,600-17,260,000 is expanded in the inset to show putative C/EBP binding motifs (black boxes), the TBP motif (white box)

and the amplified region (grey box). **(G)** ChIP analysis of C/EBP β occupancy on the *Cav1* promoter in empty vector (pLX) and C/EBP β -overexpressing C2C12 (β) myoblasts. RT-qPCR data is shown as a percent enrichment relative to 10% input used for immunoprecipitation for each condition (n=4). **(H)** Representative images of TA muscle sections from wild-type and C/EBP $\beta^{-/-}$ conditional knockout mice at post-natal day 56. Sections stained with CAV1 (green), PAX7 (red) and DAPI (blue). Solid arrowhead (►) marks CAV1⁺/PAX7⁺ cells and the open arrowhead (◄) marks CAV1⁻/PAX7⁺ cells. Scale bar = 10 μ m. **(I)** Percent of CAV1⁺ cells among the PAX7⁺ population (n=3 mouse pairs). Data information: In A, C, E, G and I, data are presented as the mean $\pm$ SEM, *p<0.05, **p<0.01, ***p<0.001 (Student's t-test).

C/EBP β -mediated quiescence is dependent on Caveolin-1 expression. To determine if Caveolin-1 is required for C/EBP β -induced cell cycle arrest in C2C12 myoblasts, control (pLXSN) and C/EBP β -overexpressing C2C12 cells were lentivirally transduced to express a scrambled shRNA construct (ShCtl) or a shRNA construct targeting Caveolin-1 (ShCav1). RT-qPCR and western blot analysis confirmed C/EBP β overexpression and knockdown of Caveolin-1 (Fig. 14A-E). To determine if C/EBP β inhibits myoblast proliferation through Caveolin-1, C2C12 pooled stable lines were stained for Ki67. The number of proliferating (Ki67⁺) cells was rescued when Caveolin-1 was knocked down in C/EBP β -overexpressing myoblasts (Fig. 14F), suggesting that C/EBP β promotes cell cycle arrest in myoblasts through upregulation of Caveolin-1. This model is further supported by a rescue of BrdU incorporation in C/EBP β overexpressing cells when Caveolin-1 is also knocked down (Fig. 14G). Collectively, our data indicates that C/EBP β regulates the maintenance of quiescence MuSCs via the regulation of Caveolin-1 expression and potentially other QSC-related genes.

Discussion

In this study, we examined the role of C/EBP β in the regulation of MuSC quiescence and myoblast proliferation. We found that C/EBP β expression is induced in growth arrested C2C12 myoblasts. *In vivo*, loss of C/EBP β in MuSC leads to their exit from quiescence resulting in their precocious differentiation and depletion of the stem cell pool. Furthermore, we found that overexpression of C/EBP β in myoblasts induced cell cycle withdrawal and upregulated several genes associated with QSCs including *Cav1*. C/EBP β -induced cell cycle arrest was dependent on direct transcriptional regulation of *Cav1* gene expression.

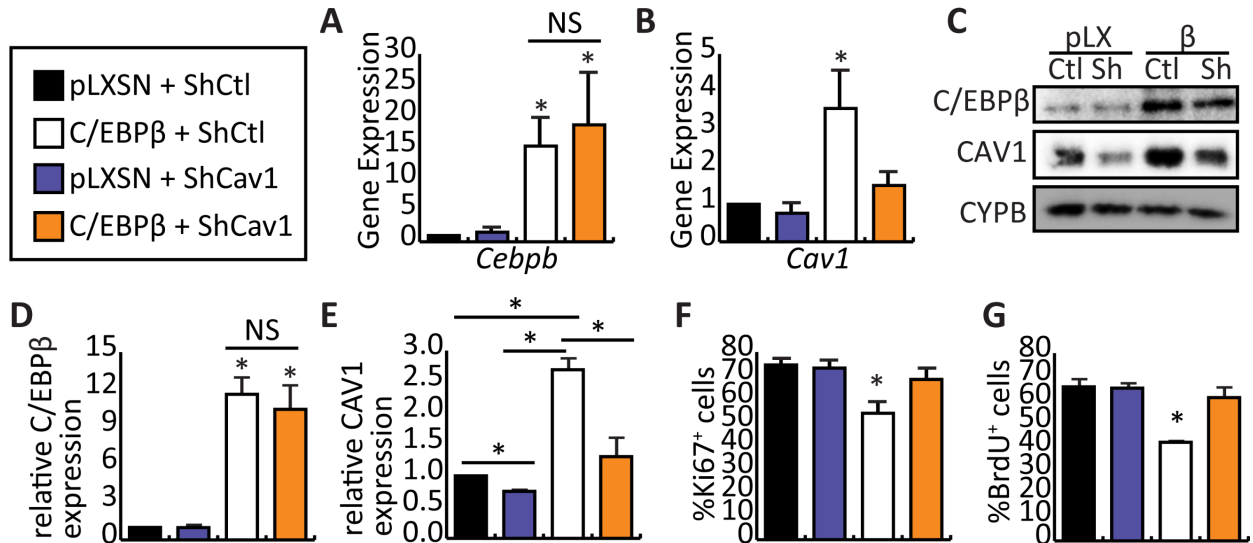


Figure 14. C/EBPβ-induced growth arrest is dependent on Caveolin-1 expression. C2C12 myoblasts expressing C/EBPβ (β) and controls (pLX) were transduced with lentiviral particles to express a control shRNA (ShCtl) or a shRNA directed against caveolin-1 (ShCav1) to create pooled stable cell lines. Cells were cultured in growth media. RT-qPCR analysis of (A) *Cebpb* and (B) *Cav1* expression in cells cultured in GM (n=6). (C) Representative western blots of C/EBPβ and CAV1 expression. CyPB is a loading control. Quantification of (D) C/EBPβ and (E) CAV1 protein expression normalized with CyPB and relative to pLXSN + shCtl (n=4). (F) Percent of Ki67⁺ cells relative to total nuclei (n=3). (G) Percent of BrdU⁺ cells relative to total nuclei (n=3). Data information: In A, B, D-G, data are presented as the mean ± SEM. For panels A, B, D, F and G, *p<0.05 in comparison to all other conditions except where explicitly indicated as not significant (NS) (ANOVA, Tukey's multiple comparison test). For panel E, *p<0.05 for the comparisons indicated (ANOVA, Tukey's multiple comparison test).

Caveolin-1 has been shown to be expressed in quiescent mouse MuSCs and downregulated upon MuSC activation (Kann and Krauss 2019; Gnocchi et al. 2009; Volonte et al. 2005). Furthermore, a recent study identified a population of CAV1⁺ human MuSCs (Hu-MuSCs; CXCR4/CD29/CD56/CAV1) that were slower to divide, had decreased MYOD expression and were more efficient at repopulating the MuSC niche after transplantation compared to CAV1⁻ Hu-MuSCs (Barruet et al. 2020). Interestingly, the characteristics of CAV1⁺ Hu-MuSCs are identical to C/EBP β -overexpressing myoblasts (Fig. 11) (Lala-Tabbert et al. 2016b). In this study, we show that C/EBP β induces cell cycle arrest through upregulation of Caveolin-1 in cycling C2C12 myoblasts. In addition to MuSCs, Caveolin-1 has been implicated in the maintenance of quiescence in a number of adult stem cells including hematopoietic stem cells (Bai et al. 2014). In its absence, expansion of the hematopoietic stem cell pool with disrupted function occurred (Bai et al. 2014). Furthermore, the Caveolin-1 null mouse has expansion of several stem cell populations including mammary stem cells and neural stem cells (Jasmin et al. 2009; Sotgia et al. 2005). Interestingly, C/EBP β has also been implicated in the maintenance of mammary stem cells and neural stem cells, suggesting that C/EBP β may be an upstream regulator of Caveolin-1 in a number of adult stem cell populations (LaMarca et al. 2010; Obora et al. 2017; Zhang et al. 2012).

In addition to *Cav1* upregulation, our RNA-Seq analysis identified 27 other potential C/EBP β target genes with putative roles in MuSC quiescence (Fig. 12). Using publicly available ChIP-seq signal tracks from proliferating C2C12 myoblasts (GSE36024), we examined C/EBP β occupancy of the regulatory regions of these 27 genes (Supplemental Table 6). C/EBP β occupancy peaks, whose position is reported as distance to the closest transcription start site (TSS), were found for 22 of the candidate genes. Of these, *Dpt* and *Esr1* have been shown to be negative regulators of myoblast proliferation. Single cell RNA-seq data from

FACS-isolated MuSCs (Dell'Orso et al. 2019) indicated that 28% and 20% of *Pax7*-expressing cells also express *Dpt* and *Esr1*, respectively (Fig 12F). *Dpt* encodes for the tyrosine-rich non-collagenous matrix protein, Dermatotontin that has been shown to increase cell adhesion and inhibit proliferation of C2C12s (Kim et al. 2019). *Esr1*, the gene encoding Estrogen receptor α (ER α) is the most prominent estrogen receptor gene expressed by MuSCs (Collins et al. 2019). Conditional knockout of *Esr1* in PAX7⁺ cells results in fewer MuSCs in uninjured muscle and ESR1-null MuSCs showed reduced engraftment into the stem cell niche (Collins et al. 2019). While likely not a direct target of C/EBP β , *Six5* negatively regulates myoblast proliferation and myogenin expression (Yajima et al. 2010). Interestingly, these genes appear to have the same function in MuSCs as C/EBP β and could potentially be downstream regulators of C/EBP β action in MuSCs. Further work will address the role of these targets in the maintenance of satellite cell quiescence.

It is important to mention that there are two factors that would impact the sensitivity of the comparison of our RNA-seq with the three published datasets (GSE3483, GSE47177, GSE15155) (Fukada et al. 2007; Pallafacchina et al. 2010; Liu et al. 2013). First, our data set was generated from C2C12 myoblasts, a proliferating myoblast cell line, whereas the three publicly available datasets were obtained from primary myoblasts. Second, the publicly available datasets were generated by microarray, whereas we used RNA sequencing which is more robust. To overcome these discrepancies, we focused only on QSC-regulated genes with a 4-fold change in expression which we then compared to C/EBP β -regulated genes with a 2-fold change in expression compared to controls. Previous analysis of various QSC datasets failed to identify a clear molecular signature of QSC suggesting dataset heterogeneity due to variations in experimental (isolation methods), biological (source of MuSCs) and technical (type of analysis) conditions (Liu et al. 2013; Pallafacchina et al. 2010; Fukada et al. 2007;

Machado et al. 2017; Pietrosemoli et al. 2017). Our current analysis indicates that in spite of these discrepancies, we were able to identify upregulation of C/EBP β in all QSC datasets examined (GSE47177, GSE15155, GSE3483) and we identified 28 (14 of which are enriched in PFA-fixed quiescent MuSCs, GSE103164) additional gene that were consistently up-regulated in at least two datasets (Fig. 12D and E); thus, validating C/EBP β as a bona fide and important regulator of MuSC quiescence.

Quiescence is considered an efficient approach to long term maintenance of adult stem cell populations, and the molecular mechanisms governing the induction of quiescence likely also regulate the activation of stem cell populations and their self-renewal. In skeletal muscle, aging triggers MuSC dysfunction characterized by a switch from mitotic quiescence to a pre-senescent state, resulting in blunted regenerative responses and the development of sarcopenia (Sousa-Victor et al. 2014). In this study, we show that C/EBP β expression is required for the maintenance of MuSC quiescence in steady state adult skeletal muscle; however, increased *Cebpb* expression is also observed in MuSCs of aged mice (Price et al. 2014; Giresi et al. 2005). Recently, the activation trajectories and kinetics from quiescence to activation was studied in old and young mice (Kimmel et al. 2020). While old and young MuSCs follow the same trajectory from quiescence to activation, the kinetics of this process is slower in aged cells. Our analysis of the datasets indicates that old MuSCs have higher expression of *Cebpb*, and while activation leads to a downregulation of *Cebpb* expression, aged MuSCs have higher *Cebpb* levels than young cells. Thus, considering our findings, higher *Cebpb* expression in aged MuSCs could contribute to the reduced kinetics activation and differentiation kinetics observed. In addition, the truncated dominant negative C/EBP β isoform liver-enriched inhibitory protein (LIP) has been shown to be upregulated in old mice (20-22 months) and depletion of this isoform increased health-span (fitter and more resistant to cancer) and

survival of old mice (Müller et al. 2018). LIP results from alternative translation initiation of *Cebpb* mRNA which is enhanced by mTORC1 activity (Jundt et al. 2005; Zidek et al. 2015b). Interestingly inhibition of mTORC1 activity prevents senescence in a number of stem cell populations including MuSCs (García-Prat et al. 2016; Iglesias-Bartolome et al. 2012; He et al. 2020). Therefore, changes in systemic factors (such as mTORC1 signaling) could convert C/EBP β -induced quiescence to senescence in sarcopenia.

Conclusion

In skeletal muscle, satellite cells exist in a quiescent state for extended periods of time and the maintenance of satellite cell quiescence is critical for long-term tissue homeostasis, with loss of quiescence leading to depletion of the stem cell pool. Despite the importance of maintaining stem cell quiescence under steady-state conditions, little is known about the mechanisms regulating satellite cell quiescence. Using both *in vitro* and *in vivo* experiments, we find that loss of C/EBP β expression in satellite cells increases their activation and spontaneous differentiation and depletes the stem cell pool in uninjured muscle; whereas, overexpression of C/EBP β induces cell growth arrest. Further, we demonstrate that C/EBP β regulates the expression of several genes associated with satellite cell quiescence and that C/EBP β -induced cell cycle arrest in myoblasts is dependent on the upregulation of Caveolin-1, a novel transcriptional target of C/EBP β . Together, our data identifies C/EBP β as a novel and important regulator of satellite cell quiescence.

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Conflict of interest. The authors declare that they have no conflict of interest.

**CHAPTER FOUR: C/EBP β PROMOTES THE EXPRESSION OF
ATROPHY-INDUCING FACTORS BY TUMOURS AND IS A
CENTRAL REGULATOR OF CANCER CACHEXIA**

**C/EBP β promotes the expression of atrophy-inducing factors by tumours and is a
central regulator of cancer cachexia**

Hamood AlSudais^a, Rashida Rajgara^a and Nadine Wiper-Bergeron^{b,1}

^aGraduate Program in Cellular and Molecular Medicine, Faculty of Medicine, University of
Ottawa, 451 Smyth Road, Rm 2539, Ottawa, Ontario, Canada, K1H 8M5

^bDepartment of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa,
451 Smyth Road, Rm 2541, Ottawa, Ontario, Canada, K1H 8M5

¹To whom correspondence should be addressed: Nadine Wiper-Bergeron PhD, 451 Smyth
Road, Room 2539, Ottawa, Ontario, Canada, K1H 8M5 Tel: 1-613-562-5800 ext. 8176, Email:
Nadine.WiperBergeron@uottawa.ca

Abstract

Background

Loss of skeletal muscle mass is a feature of diseases with strong inflammatory components such as cancer cachexia and is associated with frailty and increased morbidity. In response to secreted factors from cachexia-inducing tumours, the expression of CCAAT/Enhancer Binding protein β (C/EBP β) is stimulated in muscle, leading to both myofiber atrophy and the inhibition of muscle regeneration. C/EBP β is a transcription factor involved in the regulation of immune responses and can regulate many secreted factors, including cytokines. Since tumour-secreted factors drive cachexia and aggressive tumours have higher expression of C/EBP β , we examined a potential role for C/EBP β in the expression of tumour-derived cachexia-inducing factors.

Methods

We used gain- and loss-of-function approaches *in vitro* and *in vivo* to evaluate the role of tumour C/EBP β expression on the secretion of cachexia-inducing factors.

Results

We report that C/EBP β overexpression upregulates the expression of 260 secreted protein genes, resulting in a secretome that inhibits myogenic differentiation (-31%, $p < 0.05$) and myotube maturation (-38% (fusion index), $p < 0.05$). We find that knockdown of C/EBP β in cachexia-inducing Lewis Lung Carcinoma cells restores myogenic differentiation (+25%, $p < 0.0001$) and prevents myotube atrophy (+100%, $p < 0.05$) in conditioned medium experiments, and *in vivo*, prevents muscle wasting (-51% for small myofibers versus controls, $p < 0.01$; +140% for large myofibers, $p < 0.01$). Conversely, overexpression of C/EBP β in non-cachectic tumours converts their secretome into a cachexia-inducing one, resulting in both

myotube atrophy (-45%, $p < 0.05$) and inhibition of differentiation in culture (+15 to 30%, $p < 0.05$) and muscle wasting *in vivo* (+89% small fibers, $p < 0.05$; -73% large fibers, $p = 0.09$). Comparison of the differently expressed transcripts coding for secreted proteins in C/EBP β -overexpressing myoblasts with the secretome from 27 different types of human cancers revealed ~18% similarity between C/EBP β -regulated secreted proteins and those secreted by highly cachectic tumors (brain, pancreatic and stomach cancers). At the protein level, we identified 16 novel secreted factors that are present in human cancer secretomes and are upregulated by C/EBP β . Of these, we tested the effect of 3 factors (SERPINF1, TNFRSF11B and CD93) on myotubes and found all had atrophic potential (-30% for myotube diameter, $p < 0.05$).

Conclusion

We find that C/EBP β is necessary and sufficient to induce the secretion of cachexia-inducing factors by cancer cells and loss of C/EBP β in tumors attenuates muscle atrophy in an animal model of cancer cachexia. Our findings establish C/EBP β as a central regulator of cancer cachexia and an important therapeutic target.

Keywords: CCAAT-Enhancer-Binding Proteins; Muscular Atrophy; Cachexia; muscle, skeletal; secreted proteins; Carcinoma, Lewis lung.

Introduction

Skeletal muscle atrophy that is not reversible with nutrition supplementation and systemic inflammation are the two main features of cancer cachexia, a paraneoplastic syndrome affecting approximately 80% of advanced cancer patients and accounting for >20% of cancer mortalities (Tisdale 2009; Anker et al. 2019; von Haehling and Anker 2010; Onesti and Guttridge 2014). Cancer cachexia leads to significant morbidity, poor tolerance to chemotherapy and decreased survival (Anker et al. 2019), and thus therapeutic advances in this area are fundamental to improving both patient quality of life and anti-cancer treatment efficacy. The cause of cachexia is multifactorial and results from a complex interaction between the tumour and the host (Donohoe et al. 2011). Pro-inflammatory cytokines such as $\text{TNF}\alpha$, IL-6, IL-1 β and IFN γ are known inducers of cachexia and their expression is increased in cachexia, however, the inhibition of any one single factor has failed to yield therapeutic benefits in all cancer patients (Jiang and Clemens 2006; Prado and Qian 2019). Indeed, human cancers produce a complex and highly variable secretome making the identification of a single pathway responsible for the development of cachexia challenging (Prado and Qian 2019; Porporato 2016). Thus, the identification of a hierarchical transcription factor responsible for the production of the cachexia-inducing secretome has tremendous therapeutic potential.

Skeletal muscle tissue retains the ability to regenerate throughout the lifespan of an organism and this ability is dependent on the presence of a small population of skeletal muscle stem cells, called satellite cells. In response to injury, satellite cells become activated, proliferate, differentiate and fuse to repair damaged muscle (Bischoff 1975; Mauro 1961). The process of regeneration is controlled by a group of four transcription factors called the muscle regulatory factors (MRFs) named MYOD, MYF5, myogenin and MRF4 (Weintraub et al.

1991). After muscle injury, MYF5 and MYOD are the first MRFs to be expressed, followed by myogenin and MRF4, which drive the gene expression program required for terminal differentiation (Cornelison and Wold 1997).

CCAAT/Enhancer Binding proteins (C/EBPs) are a family of six bzip transcription factors that are involved in the regulation of a variety of cellular activities including apoptosis, differentiation, cell growth and the immune response (Tsukada et al. 2011; Ramji and Foka 2002; Cao et al. 1991; Wang et al. 2019). One family member, C/EBP β , plays a significant role in the regulation of mesenchymal stem cell fate, particularly as a repressor of osteoblastogenesis and an activator of adipogenesis (Cao et al. 1991; Darlington et al. 1998; Wipier-Bergeron et al. 2007b, 2007a). Further, C/EBP β has been implicated in the regulation of the inflammatory response and our lab has demonstrated that it is critical for skeletal muscle homeostasis (Lala-Tabbert et al. 2016a; Marchildon et al. 2012; Wang et al. 2019; Zhang et al. 2011). In healthy muscle, C/EBP β is expressed in muscle satellite cells and acts as a negative regulator of myogenic differentiation (AlSudais et al. 2018; Fu et al. 2015; Marchildon et al. 2012). Overexpression of C/EBP β inhibits myogenic differentiation in both mouse primary myoblasts and C2C12 myoblasts, while loss of C/EBP β expression in muscle satellite cells leads to precocious differentiation, increased fusion, larger myofibers *in vivo* and enhanced regenerative capacity (Marchildon et al. 2012). These C/EBP β -dependent effects on myogenic differentiation are mediated, at least in part, through the regulation of PAX7, MYOD, MYOG, and the inhibitor of DNA binding and differentiation protein (ID) proteins. In particular, C/EBP β downregulates MYOD and MYOG expression, which promote myogenic differentiation while upregulating the negative regulators *Pax7* (Marchildon et al. 2012) and ID3 (AlSudais et al. 2018). In the context of cancer cachexia, our lab has demonstrated that the expression of C/EBP β is stimulated in myoblasts and in satellite cells

that are exposed to conditioned medium from cachexia-inducing tumours (Marchildon et al. 2015). The persistent elevated expression of C/EBP β in myoblasts in this context inhibits myogenic differentiation which is rescued by the knockdown of C/EBP β in culture (Marchildon et al. 2015). *In vivo*, muscle regeneration is impaired in cachectic animals and is associated with an elevated number of muscle satellite cells (PAX7+) that express higher levels of C/EBP β when compared to controls (Marchildon et al. 2016, 2015). Using an RNA-seq dataset generated by our lab to identify the molecular mechanism by which C/EBP β inhibits myogenic differentiation, we performed gene ontology analysis on genes upregulated by C/EBP β in myoblasts and found the term “secreted” among the top 5 terms identified, suggesting that C/EBP β is a major regulator of the myoblast secretome. Within the category of secreted factors, the majority coded for cytokines and chemokines. Indeed, C/EBP β was first identified as a transcription factor that binds to the regulatory regions of *Il6*, and since has been shown to stimulate acute-phase inflammatory gene expression as well as the expression of the cytokine genes *Tnf*, *Il8* and *Csf3* (Poli et al. 1990; Akira et al. 1990). The exposure of myoblasts to cytokines such as IL-6, TNF- α , IFN- γ and IL-1 β is known to block myogenic differentiation (Jiang and Clemens 2006), suggesting that C/EBP β functions can function in both an autocrine and paracrine fashion to inhibit myogenic differentiation. Interestingly, multiple studies demonstrate that cytokines are key players in the pathogenesis of cancer cachexia (Jiang and Clemens 2006) and it is reported that C/EBP β is expressed in most cancers (Klijn et al. 2014). Further, C/EBP β expression in human cancers is associated with aggressiveness and poor outcomes (Oya et al. 2003; Rask et al. 2000; Homma et al. 2006). Thus, we investigated the role of C/EBP β expression in tumours on the development of cachexia and the production of cachexia-inducing secreted factors in culture and *in vivo*.

Methods

Cell culture

C2C12 myoblasts (ATCC) were grown in DMEM (Wisent) supplemented with 10% FBS (Wisent) (GM, Growth media). To induce differentiation, growth media was changed to low serum differentiation media (DM: DMEM supplemented with 2% horse serum (Sigma)). Lewis Lung Carcinoma (LLC) and SKOV-3 human ovary adenocarcinoma cell lines were purchased from ATCC and cultured in DMEM (Wisent) supplemented with 10% FBS (Wisent). The mouse T cell lymphoma cell line (EL4, ATCC) was cultured in DMEM (Wisent) and supplemented with 10% horse serum (Sigma). All cultures were supplemented with 1% penicillin/streptomycin.

For the assessment of cell growth, cells were plated at equal numbers and allowed to grow for 24h after which cells were counted using a hemacytometer or stained with crystal violet (0.03%) and eluted in 1% SDS. Optical density was measured at 570 nm as previously described (Lala-Tabbert et al. 2016b) and presented as fold over controls.

For *in vitro* cachexia experiments, conditioned medium from cancer cell lines was prepared as previously described in Brown *et al* (Brown et al. 2017) with the following modifications. Cancer cells (LLC, SKOV3 and EL4) were grown to ~80% confluency (or 5×10^5 cells/ml for EL4), after which fresh media was added. Conditioned media was collected after 2 days. Conditioned media was added to C2C12 for two days during proliferation (in GM) to measure effects on myogenic differentiation or after formation of myotubes (4 days after differentiation, D4) to measure atrophy at a 1:1 ratio with regular C2C12 media.

For co-culture experiments, C2C12 cells were grown in wells and cancer cells in inserts and the cell secretome was exchanged through a 0.4 μ m membrane without cell-cell contact (Corning™ Transwell™, C3412).

For treatment with recombinant proteins, C2C12s were grown and differentiated for 4 days after which media was changed and supplemented with recombinant SERPINF1 (0.5 μ g/ml, 8295-SF, R&D Systems), TNFRSF11B (10 ng/ml, 6945-OS, R&D Systems), CD93 (6 μ g/ml, 1696-CD, R&D Systems), vehicle (0.1% BSA in PBS) or left untreated for 2 days.

Retroviral infection

Phoenix Ampho packaging cells (ATCC) were used to generate replication incompetent retroviruses as previously described (Marchildon et al. 2012). Luc-pSUPER-Retro-GFP/Neo plasmid (ShLuc, shCtl) was a gift from Dr. William Kaelin (Addgene plasmid # 22133; <http://n2t.net/addgene:22133>; RRID:Addgene_22133) (Li et al. 2007). pSUPER-Retro-GFP/Neo-shCebpb was a gift from Dr. Bruce Spiegelman (Kajimura et al. 2009). For expression of C/EBP β , cDNA encoding full-length C/EBP β was cloned into the pLXSN vector (Clontech) as previously described (Wiper-Bergeron et al. 2007a). Following retroviral infection, cells were maintained in media containing G418 (Wisent) for at least 7 days.

RNA-Sequencing Sample Preparation and Analysis.

RNA extraction, sequencing and analysis was performed as previously described (Lala-Tabbert et al. 2020). Briefly, total RNA was extracted from proliferating C/EBP β -overexpressing C2C12 myoblasts or controls using the RNeasy kit (Qiagen). Gene-coding standard library preparation (NEB) and sequencing using the Illumina HiSeq2500 SR50 was

performed by Genome Quebec. Reads were analyzed by the Ottawa Bioinformatics core facility at the Sprott Center for Stem Cell Research at the Ottawa Hospital Research Institute as previously reported (Lala-Tabbert et al. 2020). Functional analysis (gene ontology (GO)) of differentially expressed genes were performed using the web-based DAVID 6.8 (Huang et al. 2009b, 2009a). Raw and processed data are deposited in the GEO database (GSE152051) (Lala-Tabbert et al. 2020).

Immunofluorescence

Cells were fixed in ice-cold methanol, incubated with primary antibody against myosin heavy chain (MyHC) (MF20, DSHB, 1:50) overnight at 4 °C followed by incubation with secondary anti-mouse Cy3 antibody (anti-mouse Cy3, Jackson ImmunoResearch, 1:500) at room temperature. Nuclei were counterstained with DAPI. A AxioObserver D1 microscope (Zeiss) was used to capture random images with the 10X objective. All quantification analysis of MyHC was performed using Fiji (ImageJ). Two parameters were used to evaluate myogenic differentiation in 5 random pictures from each condition: the percent differentiation (# of nuclei expressing MyHC/total nuclei number) and the fusion index (average number of nuclei per fiber). For myotube atrophy, myotube diameter was measured for each cell in 5 random pictures from each condition and presented as the average pixel number (arbitrary units).

Western Analysis

Whole cell extracts were prepared from C2C12, LLC, SKOV3 and EL4 cell lines and from LLC and EL4 inoculated tumors using whole cell extract buffer (IPH) as described (Lamarche et al. 2021). Protein concentrations were estimated using Coomassie Plus

(Bradford) assay (ThermoFisher) and equal amount of proteins were resolved on a 15% SDS-PAGE gel and then transferred to a PVDF membrane (Bio-Rad). Membranes were blocked then probed with primary antibodies: C/EBP β (E299, Abcam ab32358, 1:500), Myogenin (F5D, DSHB, 1:200) and Cyclophilin B (Abcam, ab16045, 1:5000). The ChemiDoc™ MP system (Bio-Rad) was used to detect chemiluminescence. Protein quantification and blot cropping were performed using Image Lab software (Bio-Rad).

RT² Profiler PCR Array

C2C12 cells differentiated for 4 days or after induction of atrophy for 2 additional days with conditioned medium from LLC were collected in RLT buffer (Qiagen) and RNA extraction was performed using RNeasy kit (Qiagen) with in-column DNA digestion as per manufacturer's protocol. RNA was quantified by Nanodrop (Thermo Scientific) and cDNA was prepared using iScript™ cDNA Synthesis Kit (Bio-Rad) following the manufacturer's protocol. Gene expression profiles were tested using the real-time RT² Profiler PCR Array (QIAGEN, PAMM-099Z, Skeletal Muscle: Myogenesis and Myopathy RT² Profiler PCR Array) in combination with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). Ct values table was uploaded on to the data analysis web portal at <http://www.qiagen.com/geneglobe> and fold change/regulation was calculated using $\Delta\Delta C_t$ method. Heatmap was prepared using Microsoft Excel.

Mice, Animal Care and LLC-tumour graft.

All animal work was performed in accordance with the guidelines set out by the Canadian Council on Animal Care and was approved by the University of Ottawa Animal Care

Committee. All animals were housed in a controlled facility (22°C with 30% relative humidity on a 12-hour light/dark cycle) and provided with food and water ad libitum. Six-week-old C57BL/6 female mice (Charles River) were inoculated subcutaneously with 5×10^5 LLC cells (in 100 μ l PBS) that were retrovirally transfected to express shRNA against *Cebpb* (sh β) or against luciferase as a control (shCtl), or with 1×10^6 EL4 cells that were retrovirally transfected to express C/EBP β (EL4- β) or with empty vector (pLX). Sham controls were injected with PBS. The tumour growth occurred over 3 weeks and mice were monitored every 2 days for wellness. Three weeks post-inoculation, mice were sacrificed and tibialis anterior (TA), soleus, extensor digitorum longus (EDL), gastrocnemius muscles and tumors were harvested and weighed. The tibialis anterior muscles were embedded in O.C.T and flash frozen in isopentane in liquid nitrogen for sectioning and staining with H&E. Images for H&E stained sections were taken by the EVOS M7000 imaging system (Invitrogen) and myofiber cross-sectional area was measured using Fiji (ImageJ).

Proteome Profiler Mouse XL Cytokine Array profiler

Protein lysates from C2C12 and LLC cells were prepared using lysis buffer 17 (R&D Systems, Cat#895943) supplemented with 10 μ g/ml Aprotinin, 10 μ g/ml Leupeptin and 10 μ g/ml Pepstatin following manufacturer's protocol. Protein concentrations were estimated using Coomassie Plus (Bradford) assay (ThermoFisher). Total protein (300 μ g) from each condition was incubated overnight at 4 °C with a nitrocellulose membrane containing 111 different capture antibodies for mouse cytokines printed in duplicate (Mouse XL Cytokine Array Kit, ARY028, R&D systems). After incubation with the detection antibody and streptavidin-horseradish peroxidase (HRP), the ChemiDoc™ MP system (Bio-Rad) was used

to detect chemiluminescence. Pixel densities of detected spots on each membrane were measured and analyzed using the software Quick spots (HLImage++, Western Vision Software, <https://www.wvision.com/QuickSpots.html>). Pixel densities were presented as log₂ fold change relative to respective controls and the heatmap was prepared using Microsoft Excel.

Statistical Analysis

To determine significance between a test group and a control, a two-tailed unpaired Student's t test was used. To determine the significance for experiments with three or more conditions, a one-way ANOVA was used and if significant differences were identified, multiple comparison analysis was performed using a Tukey's post hoc test. All statistical analysis was performed using GraphPad Prism 7. On graphs, significance is indicated as * <0.05 , ** <0.01 and *** <0.001 or as indicated in figure legends. All experiments represent a minimum of three biological replicates and are shown as the mean $\pm$ standard error mean (SEM).

Figure preparation

Photomicrographs were processed using Adobe Photoshop CS5.1™ and figure assembled in Adobe Illustrator CS5.1™.

Results

C/EBP β regulates the expression of secreted protein genes that inhibit myogenic differentiation. In cancer cachexia, regenerative responses are inhibited, resulting in an increase in PAX7⁺ satellite cells that fail to differentiate (He et al. 2013; Marchildon et al. 2015, 2016). Central to their failure to differentiate is the misexpression of C/EBP β , whose downregulation is required for efficient myogenic differentiation (Marchildon et al. 2015, 2016). To identify novel C/EBP β target genes in myogenic cells, we performed an RNA-seq experiment on proliferating C2C12 myoblasts retrovirally transduced to express C/EBP β or with empty vector (Lala-Tabbert et al. 2020). The RNA-seq identified 2210 protein-coding genes that were significantly differentially expressed (≥ 1.5 -fold difference) in C/EBP β -expressing cells as compared to controls (Supplemental Table 7 and 8). More than half (58%) of the 2210 differentially expressed genes were upregulated in C/EBP β -expressing cells (Figure 15A, Supplemental Table 7). Gene ontology analysis of the upregulated genes revealed that the GO term “secreted” was among the top five terms (Figure 15B), representing 20% of the genes interrogated. The majority of these secreted genes were cytokines and of the known mouse cytokine genes (134 genes) (Ge et al. 2013), 66 (49%) were detected in C2C12 cells and of these, 36 were upregulated by at least 1.5-fold in C/EBP β -overexpressing cells as compared to controls (Figure 15C). Of the 36 upregulated cytokines in C/EBP β -overexpressing myoblasts, more than 30% are known inhibitors of myogenesis, including *Tnf* (Jiang and Clemens 2006), *Il1b* (Jiang and Clemens 2006), *Cxcl14* (Waldemer-Streyer et al. 2017), *Cxcl12* (Odemis et al. 2007) and *Csfl* (Borycki et al. 1995).

In addition to cytokine genes, growth factor and growth factor receptor genes (*Hgf*, *Fgf2*, *Fgf7*, *Fgf10*, *Fgfr2*, and *Fgfr3*) were also upregulated by C/EBP β overexpression (Figure 15D). Both HGF and FGF are known to regulate myogenic differentiation such that treatment

of C2C12 myoblasts with HGF and FGF increases cell proliferation and inhibits myogenin expression and subsequent differentiation (Bandow et al. 2004).

The upregulation of a large number of genes coding for secreted proteins by C/EBP β suggests that they may play a mechanistic role in the inhibition of myogenesis by C/EBP β via an autocrine and/or paracrine pathways. To test whether secreted proteins produced by C/EBP β -overexpressing cells were responsible for the C/EBP β -mediated inhibition of cell differentiation and fusion, we used an *in vitro* co-culture system which allows secreted factors to be shared between two cell types without permitting cell-cell contact). Wild type (WT) C2C12 cells were co-cultured with C2C12 myoblasts retrovirally transduced to express C/EBP β (β) or with empty virus (pLX) grown on inserts to allow for the exchange of the secretome. Cells proliferated for two days in growth medium before the induction of differentiation in low serum media in the continual presence of secreted factors from C/EBP β -overexpressing cells or empty virus controls. As compared to controls, C2C12 cells co-cultured with C/EBP β -expressing cells had a 24% reduction in differentiation as measured by the percentage differentiation (% of nuclei in myosin heavy chain expressing cells/total number of DAPI stained nuclei) and a 38% reduction in myotube size, as measured by the fusion index (number of nuclei in myotubes/number of myotubes) after 3 days in differentiation medium (Fig. 15E-G). These results, taken together, suggest that the upregulation of secreted proteins by C/EBP β is central to the molecular mechanism by which C/EBP β inhibits myogenic differentiation.

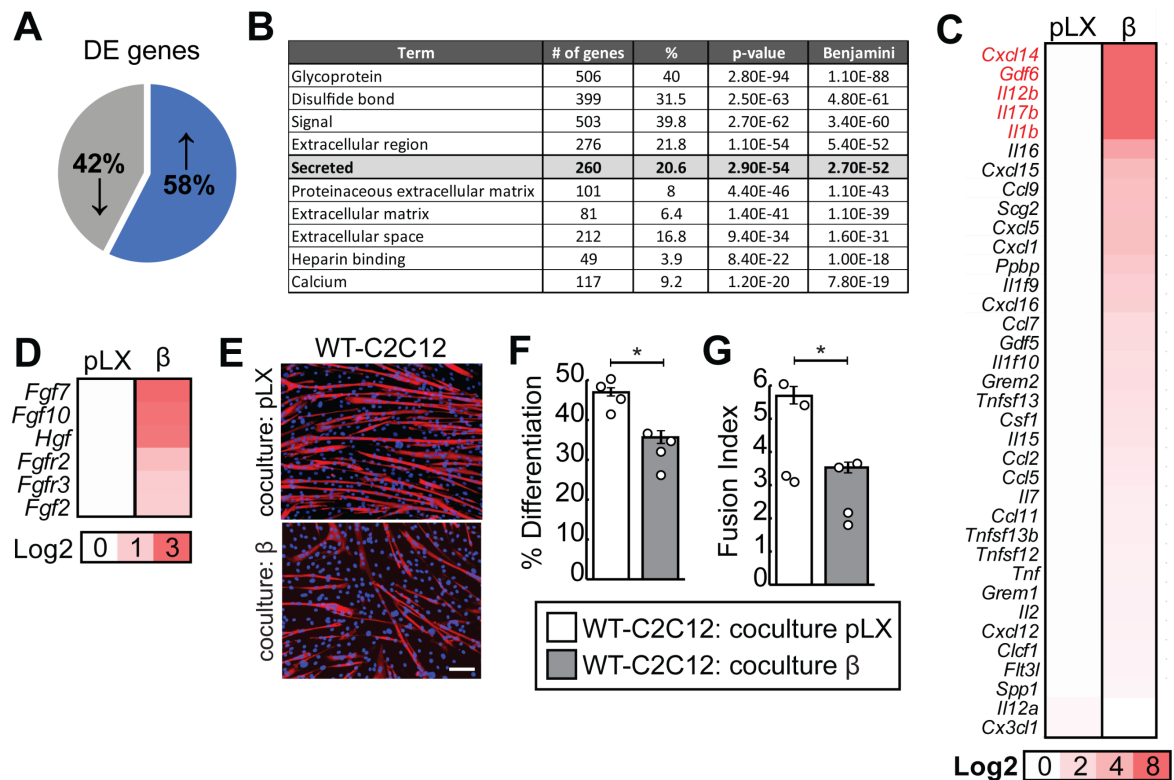


Figure 15. C/EBP β upregulates genes coding for secreted proteins that inhibit myogenesis. RNA-seq was performed on proliferating C2C12 cells retrovirally transduced with empty vector (pLX) or to express C/EBP β (β). **(A)** A pie chart showing the direction of change for genes that are significantly ($p_{adj} \leq 0.05$ and ≥ 1.5 -Fold change) differentially expressed (DE) in C/EBP β -overexpressing myoblasts as compared to empty vector controls (pLX). **(B)** Top 10 terms produced by Gene Ontology analysis of the 1273 upregulated genes in C/EBP β -overexpressing myoblasts from (A). **(C)** Heatmap of the expression of 35 cytokine genes that are upregulated in C/EBP β -overexpressing cells as compared to pLX. Genes highlighted in red are not detected in pLX conditions but induced in myoblasts overexpressing C/EBP β . **(D)** Heatmap for the expression of growth factors (*Hgf* and *Fgf*) and FGF receptors genes in control (pLX) and C/EBP β -overexpressing cells. **(E)** MyHC (red) immunostaining of WT-C2C12 cells cocultured with C2C12 cells retrovirally transduced with empty vector (pLX)

or to express C/EBP β (β) in growth medium for 2 days followed by differentiation medium for another 3 days. Nuclei are counterstained with DAPI (blue). Scale bar = 100 μ m. **(F)** Percent differentiation (# of nuclei in MyHC⁺ cells relative to the total nuclei) in cells cultured as in (E) (n=4). **(G)** Fusion index (average # of nuclei per MyHC⁺ cell) for cells cultured as in (E) (n=4). Data information: for panels E and F, data are presented as the mean $\pm$ SEM. * $p \leq 0.05$ (Student's t-test).

C/EBP β is required for the secretion of cachexia-inducing factors by Lewis Lung Carcinoma (LLC) cells. Given that C/EBP β is upregulated in a variety of cancer cells (Klijn et al. 2014) and upregulates secreted proteins that inhibit myogenic differentiation (Figure 15), we evaluated a potential role for C/EBP β in the expression of cachexia-inducing factors by tumours. We used a well-described *in vitro* cachexia model in which myotubes are incubated with conditioned medium from Lewis lung carcinoma cells (LLC) for two days. This treatment leads to a reduction in myotube size (atrophy) in response to cachexia-inducing factors in the conditioned medium produced by the cancer cells (Zhang et al. 2011). To test if C/EBP β is required for the secretion of cachexia-inducing factors by LLC cells, we retrovirally transduced LLC cells to express a shRNA against *Cebpb* (LLCsh β) or to express a shRNA against luciferase (LLCshCtl) and confirmed the knockdown of C/EBP β by western blotting (Figure 16A). Pooled stable LLC cells (sh β and shCtl) were grown for 48hrs after which conditioned medium was collected. C2C12 cells were grown and differentiated normally for four days and then treated with conditioned medium from LLC-shCtl cells, LLC-sh β cells or with fresh media as an untreated control for two days (Figure 16B). Immunostaining for myosin heavy chain was performed on myotubes at day 4 (before treatment) and on day 6 (two days after treatment) and myotube maturation was evaluated by measuring the myotube diameter. As expected, incubation of C2C12 myotubes with conditioned media from LLC-shCtl cells resulted in a >60% reduction in myotube diameter as compared to the unconditioned control (Figure 16C, D). Moreover, myotubes incubated with conditioned medium from LLC-shCtl were approximately 20% smaller than untreated myotubes on day 4 condition indicating that LLC-shCtl conditioned media not only prevented the maturation of myotubes but also caused atrophy (Figure 16C, D). More importantly, conditioned media from LLC-sh β cells did not result in a significant reduction in myotube diameter when compared to unconditioned

control (Figure 16C, D), indicating that tumour expression of C/EBP β is necessary for the production of cachexia-inducing factors by LLC cells.

To quantify this effect, we performed an RT-qPCR array comparing the expression of 84 genes involved in myogenesis and myopathy in C2C12 cells at day 4 (before treatment) and at day 6 (two days after treatment with either unconditioned medium, LLC-shCtl or LLC-sh β conditioned media). Treatment of C2C12s with conditioned media from LLC-shCtl resulted in the downregulation of 24 genes and the upregulation of 2 genes as compared to untreated C2C12s at day 6 using a 1.5-fold cut-off (Figure 16E, Supplemental Table 9). Among these 24 downregulated genes we found well-known regulators of myogenic differentiation and myotube maturation including *Myh1*, *Mef2c*, *Myf6*, *Pparg*, *Igf1*, *Camk2g*, *Tnnt3*, *Tnni2* and *Neb* (Figure 16E, Supplemental Table 9). Interestingly, cells incubated with conditioned media from LLC-sh β cells showed a partial rescue of the expression of 23 of these genes, with their expression returning almost to untreated levels (Figure 16E and Supplemental Table 9). These findings confirm the critical requirement for C/EBP β in the production of a secretome that promotes wasting.

Only two genes were found to be upregulated in myotubes treated with LLC-shCtl conditioned media, *Igfbp5* and *Pax7* (Figure 16E and Supplemental Table 9). The role of *Igfbp5* in myogenic differentiation is complicated and dependent on the ratio of IGF1 and IGFBP5 expression (Ren et al. 2008; James et al. 1996). However, the overexpression of *Pax7* is known to be induced by exposure to conditioned medium from tumours or cancer patient serum (He et al. 2013), consistent with our observations.

Since the culture density can affect the concentration of secreted proteins found in conditioned medium and C/EBP β has been implicated in the regulation of proliferation, we assessed the growth of LLC-shCtl and LLC-sh β cells. Knockdown of C/EBP β in LLC cells

did not significantly affect cell proliferation as measured by cell counting and crystal violet staining (Figure 16F). Thus, the ability of C/EBP β to promote myotube atrophy is not due to changes in culture density used to produce conditioned medium, but rather regulation of the LLC secretome itself.

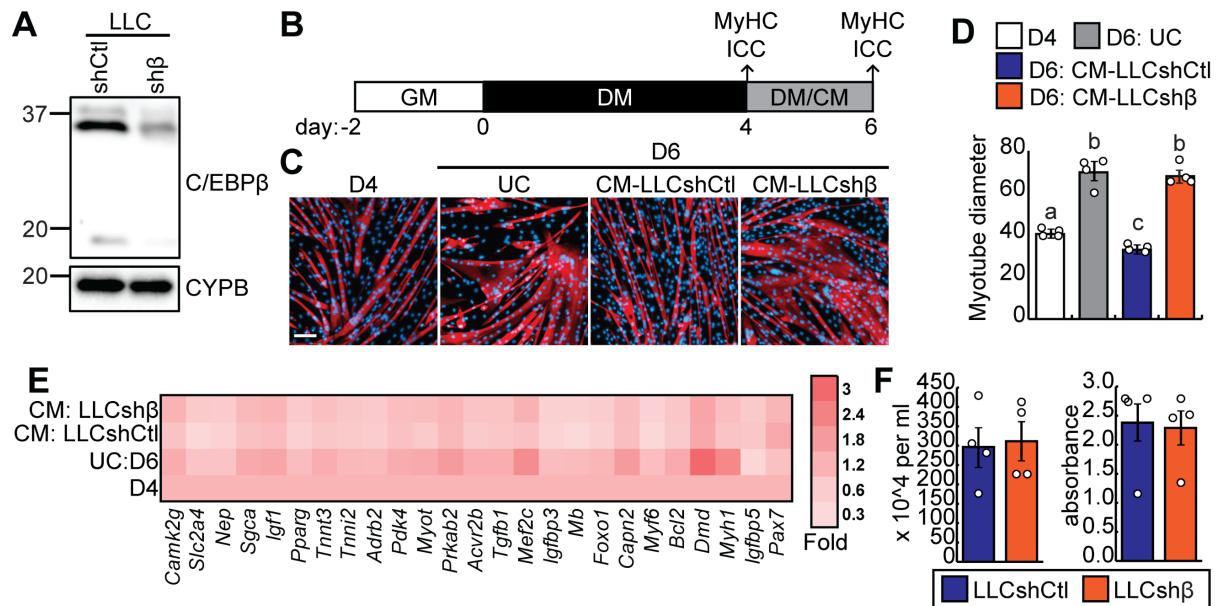


Figure 16. C/EBP β is required for the expression of atrophy-inducing proteins by LLC cells. (A) C/EBP β protein expression (isoforms LAP* at 37 kDa, LAP at 36 kDa and LIP at 17 kDa) in LLC retrovirally transduced with a control shRNA directed against luciferase (shCtl) or one directed against *Cebpb* (sh β). Cyclophilin B (CYPB) is used as a loading control. (B) Schematic representation of the experimental system. C2C12 myoblasts were grown to confluency in growth medium (GM) and differentiated in low serum medium (differentiation medium, DM) for 4 days (D4). They were then treated for two additional days with conditioned medium (CM) (DM: LLC, 1:1) from LLC-shCtl or sh β cells. (C) Myosin heavy chain immunostaining (red) of myotubes on day 4 of differentiation (D4), day 6 of differentiation

(D6) after treatment with unconditioned medium (UC) or CM for two days (D6 LLC). Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μm . **(D)** Average myotube diameter of cells treated as in (C) in pixels (n=4). **(E)** Heatmap of differentially regulated genes in C2C12 myotubes treated with CM from LLC-shCtl as compared to untreated cells (D6) determined by RNA array for myogenesis and myopathy-related genes (RT2 Profiler Array, Qiagen). Relative gene expression values for myotubes treated with LLC-sh β is included for comparison. All data are presented as relative to D4 samples. The isolated mRNA was from 3 pooled trials. **(F)** Cell numbers (right) and crystal violet absorbance (left) of LLC-shCtl and LLC-sh β cell cultures after 48h in growth medium (n=4). Data information: For panels D and F, bars are the mean $\pm$ SEM. Means with different letters are significantly different from one another at a cut-off of $p \leq 0.05$ (one-way ANOVA).

Secreted factors from cancer cells not only cause muscle atrophy but can also inhibit satellite cell function (Jiang and Clemens 2006; Marchildon et al. 2015) and this can be tested using an *in vitro* cachexia model in which myoblasts are grown in conditioned medium from cancer cells prior to differentiation (Jiang and Clemens 2006; Marchildon et al. 2015). To assess the importance of C/EBP β expression in tumour cells on the inhibition of myogenic differentiation, conditioned medium from LLC-shCtl or LLC-sh β cells was used to treat sub-confluent C2C12 myoblasts for two days in growth medium (1:1) after which differentiation was induced in fresh medium for three days (Figure 17A,B). The effect of conditioned medium on myoblast differentiation was evaluated using myosin heavy chain immunostaining and the calculation of the differentiation and fusion indices. As expected, myoblasts that were incubated with conditioned medium from LLC-shCtl cells had a 25% reduction in the differentiation index and ~50% reduction in fusion index as compared to unconditioned control (Figure 17C, D). Myoblasts incubated with conditioned medium from LLC-sh β cells, by contrast, differentiated normally, however, the myotubes produced were smaller, reflected by a ~30% reduction in the fusion index (Figure 17C, D). While fusion was significantly inhibited by pre-treatment with conditioned medium from LLC-sh β cells, the cultures still produced larger myotubes than those exposed to LLC-shCtl cells, indicating a partial rescue of cell size. Furthermore, while the expression of myogenin, a differentiation marker, was reduced by ~60% in cells that were exposed to conditioned media from LLC-shCtl cells as compared to untreated controls, incubation with conditioned media from LLC-sh β cells did not affect expression of this marker (Figure 17E, F). Taken together, these data suggest that C/EBP β expression in LLC cells is required to produce cachexia-inducing factors that cause muscle atrophy and inhibit myogenic differentiation.

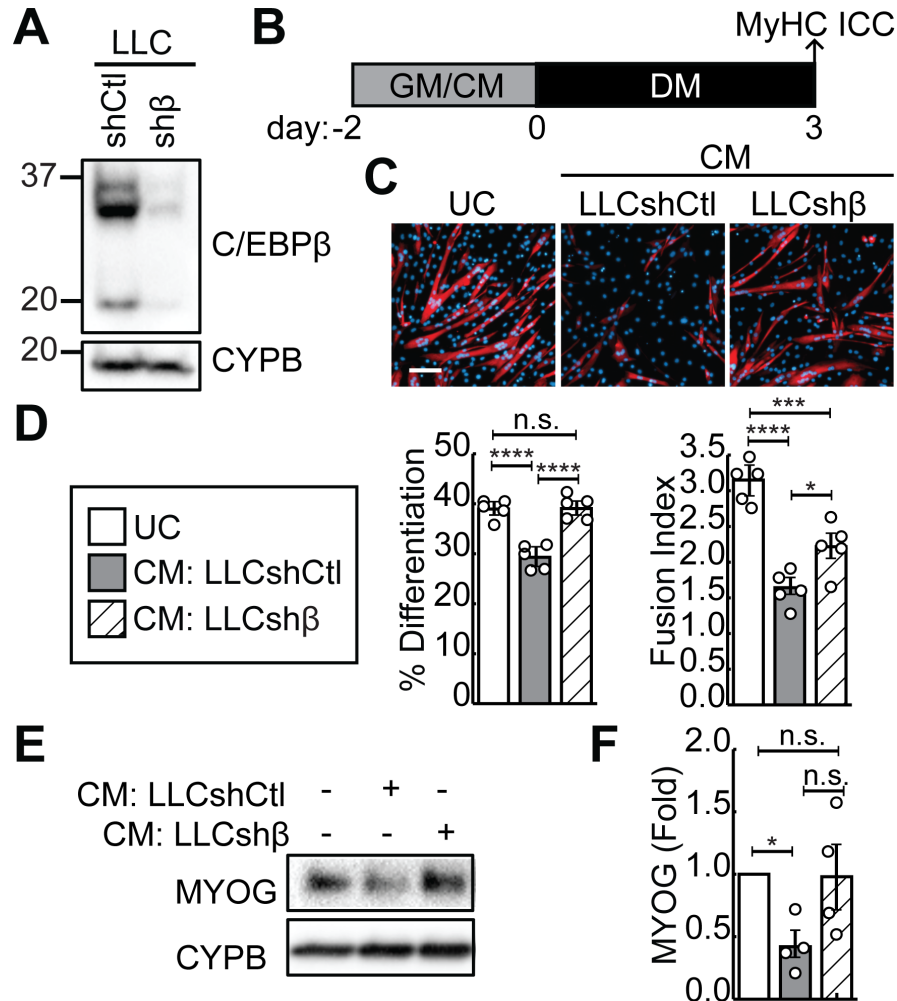


Figure 17. C/EBPβ is required for the expression of anti-myogenic proteins by LLC cells.

(A) C/EBPβ protein expression in LLC-shCtl and LLC-shβ cells. Cyclophilin B (CYPB) is a loading control. (B) Schematic representation of the experimental system. C2C12 myoblasts were grown for 48h in growth medium supplemented with conditioned medium (1:1) from LLC cells that were retrovirally transduced to express a shRNA directed against *Cebpb* (shβ) or against luciferase (ShCtl) and then differentiated in the absence of CM for 3 days (DM). (C) Immunostaining for myosin heavy chain (red) on day 3 of differentiation of cells treated as in (B). Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μm. (D) Percent

differentiation and fusion index (FI) for cells treated as in (B, C) (n=5). **(E)** Myogenin protein expression on day 1 of differentiation for C2C12 cells treated as in (B). Cyclophilin B (CYPB) is a loading control. **(F)** Quantification of Myogenin protein expression from (E) (n=4). Data information: For panels D and F, bars are the mean $\pm$ SEM, * $p \leq 0.05$, ** $p \leq 0.01$ (Student t-test).

C/EBP β converts the secretome of non-cachectic tumours to one that promotes wasting.

To determine if C/EBP β is sufficient to generate an atrophy-inducing secretome, we retrovirally transduced two cancer cell lines that are classified as non-cachectic (Zhang et al. 2017; Marchildon et al. 2015), the human ovarian adenocarcinoma cell line SKOV3 and the mouse T cell lymphoblastoma cell line EL4, to overexpress C/EBP β (SKOV3- β and EL4- β) or with empty virus (SKOV3-pLX and EL4-pLX). Overexpression of C/EBP β was confirmed by western blotting (Figure 18A, E). To assess the ability of conditioned media from SKOV3 and EL4-derived cultures to induce myotube atrophy, C2C12 myotubes were treated with conditioned media or fresh media for two days (Figure 18B). Immunostaining for myosin heavy chain was performed on myotubes at day 4 (before treatment) and at day 6 (two days after treatment) and myotube maturation was evaluated by measuring cell diameter. While conditioned medium from SKOV3-pLX cells had no effect on C2C12 myotube size as compared to untreated controls, SKOV3- β conditioned medium resulted in a 45% reduction in myotube diameter (Figure 18C, D). Similarly, while treatment of C2C12 myotubes with conditioned medium from EL4-pLX cells resulted in only a ~15% reduction in myotube diameter as compared to unconditioned controls on day 6, EL4- β conditioned medium induced a 33% reduction in myotube size as compared to myotubes conditioned with EL4-pLX, a reduction of 44% from untreated controls (Figure 18F, G). These data indicate that C/EBP β is sufficient to convert a cancer cell-derived secretome from atrophy-neutral to an atrophy-inducing one.

To evaluate the effect of C/EBP β expression in cancer cells on the differentiation of myoblasts, sub-confluent C2C12 myoblasts were incubated with conditioned medium from SKOV3-pLX cells, SKOV3- β cells or with unconditioned media for two days in growth

medium then differentiated in fresh media for three days (Figure 19A, B). Following differentiation, cells were immunostained for myosin heavy chain and the differentiation and

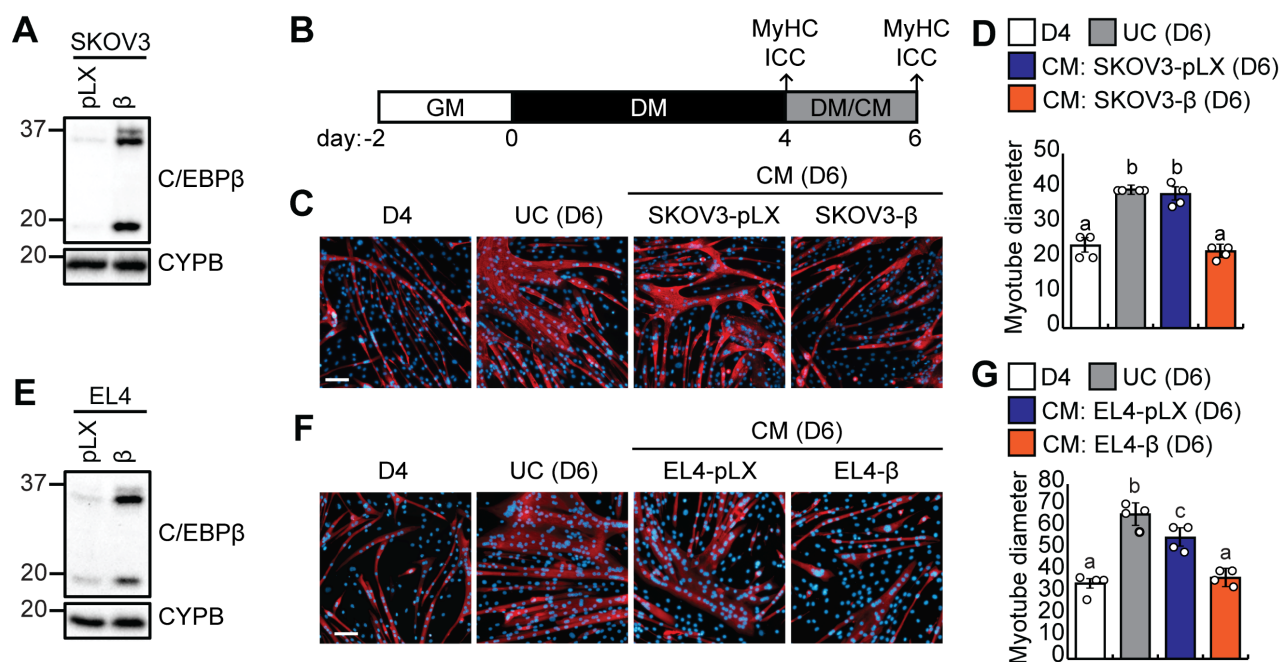


Figure 18. C/EBPβ expression converts non-atrophy inducing secretomes to atrophy-inducing ones. (A) C/EBPβ protein expression in SKOV3 cells retrovirally transduced to express C/EBPβ (SKOV3-β) or with empty virus (SKOV3-pLX). Cyclophilin B (CYPB) is a loading control. (B) Schematic representation of the experimental system. C2C12 myoblasts were grown to confluency in growth medium (GM) and differentiated in low serum medium (DM) for 4 days (D4). Differentiated myotubes were then treated for two days with conditioned medium (CM) from tumour cells (SKOV3 or EL4) retrovirally transduced to express C/EBPβ or with empty vector in DM. (C) Myosin heavy chain immunostaining (red) of D4 myotubes and myotubes after SKOV3 CM treatment for two days (CM (D6)). Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μm. (D) Average myotube diameter of

cells from (C) in pixels (n=4). **(E)** C/EBP β protein expression in EL4 cells retrovirally transduced to express C/EBP β (EL4- β) or with empty virus (EL4-pLX). Cyclophilin B (CYPB) is used as a loading control. **(F)** Myosin heavy chain immunostaining of D4 myotubes and myotubes after a two-day treatment with EL4 CM (CM (D6)). Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μ m. **(G)** Average myotube diameter of cells from (F) in pixels (n=4). Data information: For panels D and G, bars are the mean $\pm$ SEM. Means with different letters are significantly different from one another at a minimum cut-off of $p \leq 0.0001$ for panel D or $p < 0.05$ (panel G) (one-way ANOVA).

fusion indices were calculated. While myoblasts treated with conditioned medium from SKOV3-pLX cells had a differentiation index comparable to unconditioned controls, myoblasts treated with SKOV3- β conditioned medium showed a significant reduction in the percentage of differentiated cells (30% reduction as compared to SKOV3-pLX) (Figure 19C, D). Interestingly, the fusion index was reduced in myoblasts that were treated with either SKOV3-pLX or SKOV3- β as compared to unconditioned control, suggesting that C/EBP β does not regulate secreted factors that influence myoblast fusion (Figure 19C, D). Consistent with our observations using SKOV3 cells, conditioned medium from EL4-pLX cells did not adversely affect differentiation (Figure 19E-G). However, when myoblasts were pre-treated with conditioned medium from EL4 cells overexpressing C/EBP β , the percent differentiation was significantly reduced, implying that the effect of C/EBP β expression on the cancer secretome is applicable to various cell types and cancers (Figure 19E-G). Cell fusion was significantly reduced by pre-treatment by EL4-pLX cells, and this effect was increased when C/EBP β was overexpressed, similar to what was observed with SKOV3 cells (Figure 19G). Taken together, these data suggest that C/EBP β expression is sufficient to produce a secretome that inhibits myogenic differentiation. Further, our results suggest that tumours produce factors in a non-C/EBP β -dependent fashion that inhibit myoblast fusion.

Loss of C/EBP β in cancer cells attenuates muscle atrophy in an in vivo model of cachexia. To understand the role of tumour C/EBP β expression on the pathogenesis of cancer cachexia, mice were injected subcutaneously with 5×10^5 LLC cells that were retrovirally transduced with shRNA targeting *Cebpb* mRNA (LLC-sh β) or to express a shRNA against luciferase (LLC-shCtl) as control. Sham controls were injected with PBS. Tumours were allowed to grow and induce cachexia for three weeks, after which the tumours and TA muscles were

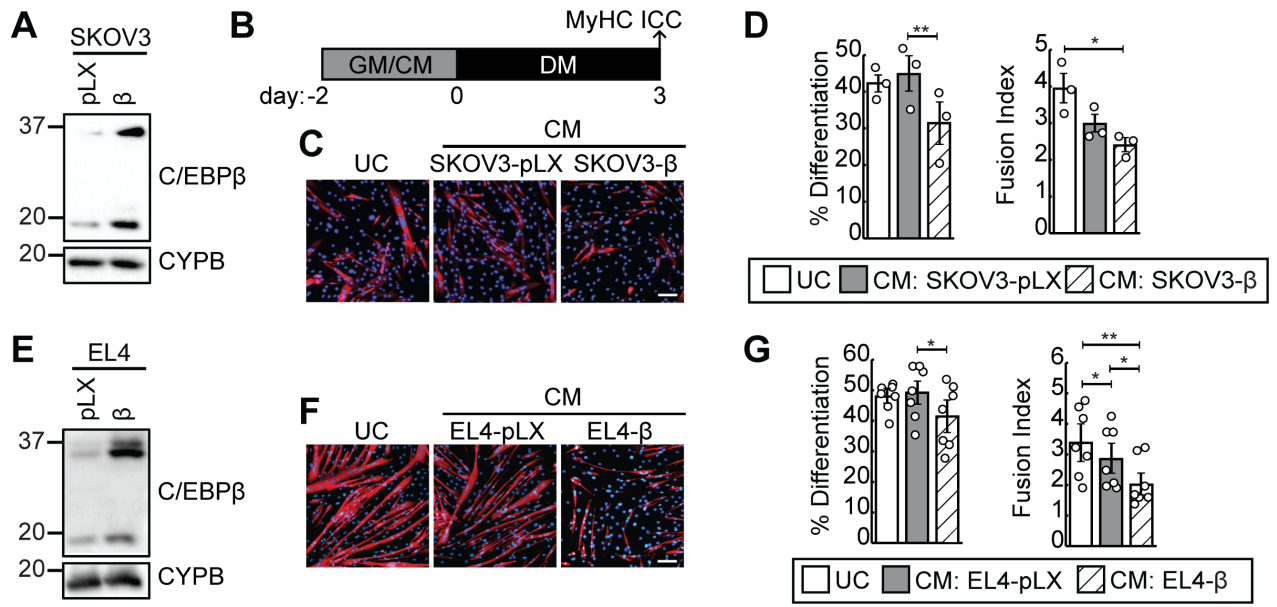


Figure 19. C/EBP β converts the SKOV3 and EL4 secretomes from myogenesis-permissive to non-permissive. (A) C/EBP β protein expression in SKOV3-pLX and SKOV3- β cells. Cyclophilin B (CYPB) is a loading control. (B) Schematic representation of the experiment timeline. C2C12 myoblasts were cultured for 48h in growth medium: conditioned medium (1:1) from SKOV3 cells or EL4 cells that were retrovirally transduced to express C/EBP β (SKOV3- β , EL4- β) or with empty vector (SKOV3-pLX, EL4-pLX) and then differentiated for 3 days in regular DM. (C) Myosin heavy chain immunostaining of D3 differentiated myoblasts from cells cultured as in (B) in the presence of conditioned medium from SKOV3-pLX or β cells. Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μ m. (D) Percent differentiation and fusion index (n=3) from cells cultured as in (C). (E) C/EBP β protein expression in EL4-pLX and EL4- β cells. Cyclophilin B (CypB) is a loading control. (F) Myosin heavy chain immunostaining of day 3 differentiated myoblasts after 48h of proliferation in the presence of EL4 conditioned medium. Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μ m. (G) Percentage of differentiated cells and fusion index from

cells cultured as in (F) (n=7). Data information: For panels D and G, bars are the mean $\pm$ SEM, *p $\leq$ 0.05, **p $\leq$ 0.01 (one-way ANOVA).

harvested from sham and tumour-bearing mice. The knockdown of C/EBP β in tumours was confirmed by western blotting (Figure 20A). As expected, we observed a significant reduction in the myofiber cross-sectional area in TA sections from LLC-shCtl-bearing mice as compared to sham controls (Figure 20B, C). However, fiber XSA from LLC-sh β -bearing mice was comparable to that of sham controls (Figure 20B, C), supporting a role for tumour C/EBP β expression in the development of cancer cachexia. To further visualize the differences in fibers size between all the examined conditions, we analyzed the distribution of myofiber XSA for the TA muscle. We observed a trend towards increased smaller fibers ($<1200 \mu\text{m}^2$) and decreased larger fibers ($>2500\mu\text{m}^2$) for muscles from LLC-shCtl-bearing mice as compared to sham controls (Figure 20D, E), whereas muscle fibers from LLC-sh β -bearing mice had a distribution of fiber sizes similar to sham controls (Figure 20D, E). When comparing the mass of dissected tumours from experimental animals, we observed $\sim 46\%$ reduction in the weight of LLC-sh β tumours as compared to LLC-shCtl tumours, which could contribute to attenuation of cachexia observed (Figure 20F). To explore this possibility, we plotted the tumour mass against the average TA muscle fiber XSA for each mouse, revealing no strong correlation between tumour size and muscle wasting (Figure 20G). For example, both the smallest and largest LLC-shCtl tumours resulted in fiber XSA of $\sim 1500 \mu\text{m}^2$, and all LLC-sh β tumours, regardless of size, promoted larger myofiber XSAs (Figure 20G). Taken together, these data suggest that the loss of C/EBP β in LLC cells attenuates muscle atrophy *in vivo*.

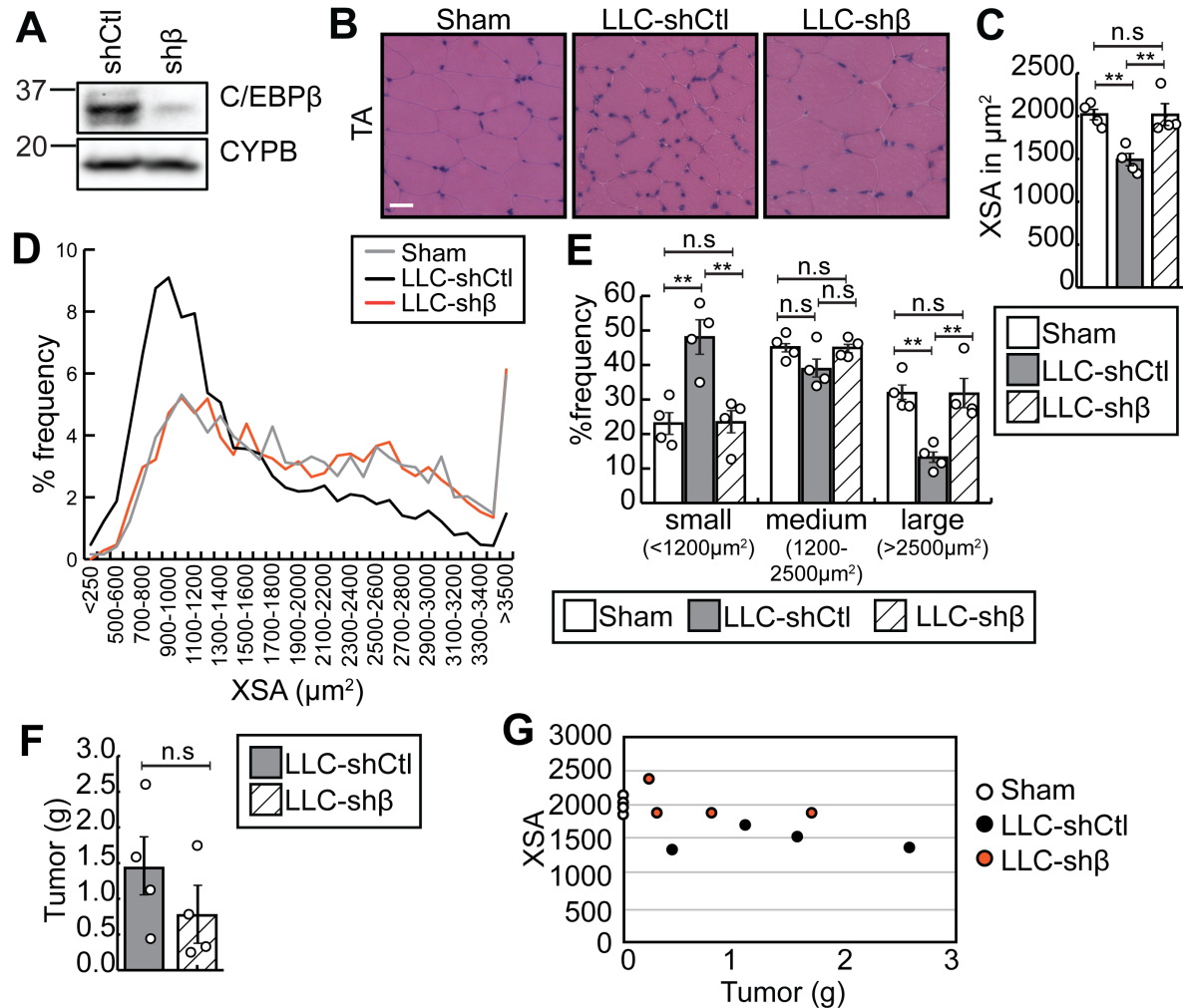


Figure 20. Loss of C/EBPβ expression in tumours attenuates muscle atrophy in a mouse model of cachexia. Mice were inoculated subcutaneously into the right flank with 5×10^5 Lewis Lung Carcinoma (LLC) cells that were retrovirally transduced to express shRNA against *Cebpb* (LLC-shβ) or against luciferase as control (LLC-shCtl). Sham mice received PSB alone. Tumours were allowed to grow for 3 weeks to induce cachexia. **(A)** Representative western blot of C/EBPβ protein expression in LLC-shCtl and LLC-shβ tumours 3 weeks post inoculation. Cyclophilin B (CYPB) is a loading control. **(B)** H&E staining of cross-sections of TA muscle from sham and tumour-bearing mice. Scale bar = 30 μm. **(C)** Average fiber cross sectional area (XSA) in the TA muscle from (B) (n=4). **(D)** Frequency distribution of myofiber

XSA in the TA muscle. **(E)** Frequency of muscle cross-sectional areas grouped into small fibers ($<1200\mu\text{m}^2$), medium-sized fibers (between $1200\text{-}2500\mu\text{m}^2$) and large fibers ($>2500\mu\text{m}^2$) from (D). **(F)** LLC-shCtl and LLC-sh β tumour mass 3 weeks after inoculation (n=4 per condition). **(G)** Average fiber cross sectional area (XSA) from TA muscle plotted against tumour mass. Data information: For panels C, E and F, bars represent the mean $\pm$ SEM, *p<0.05, **p<0.01 (one-way ANOVA).

Overexpression of C/EBP β in non-cachectic tumours promotes muscle atrophy *in vivo*.

Next, we tested if the overexpression of C/EBP β in EL4 cancer cells was sufficient to cause muscle wasting *in vivo*. Mice were injected subcutaneously with 1×10^6 EL4 cells retrovirally transduced to overexpress C/EBP β (EL4- β) or with empty vector (EL4-pLX). Sham controls were injected with PBS. Tumors were allowed to grow for three weeks after which the tumour and TA muscles were harvested. The overexpression of C/EBP β in the tumour was confirmed by western blotting (Figure 21A). As previously reported (Zhang et al. 2017), we did not observe a reduction in average TA myofiber XSA in EL4-pLX-bearing mice as compared to sham controls (Figure 21B-E). Expression of C/EBP β in EL4 tumours did not produce a statistically significant reduction in average myofiber XSA, however a reduction in the fiber XSA distribution was observed (Figure 21B-E), characterized by a shift towards smaller fibers in EL4- β tumour-bearing mice as compared to EL4-pLX and sham controls (Fig. 21D, E). In particular, we observed a significant increase in the proportion of small fibers ($<1200\mu\text{m}^2$) in EL4- β tumour-bearing mice, consistent with increased muscle wasting (Figure 21E). The overexpression of C/EBP β in EL4 cells did not result in an increase in tumour mass as compared to controls (Figure 21F). Taken together, our data indicates that increased C/EBP β expression in non-cachectic tumours (EL4) promotes muscle atrophy.

C/EBP β -regulated secretome shares similarity with the secretome of various cancer types. Given that our data suggests that (i) C/EBP β regulates secreted proteins that inhibit myogenesis and causes muscle atrophy, (ii) about 80% of patients with advanced cancers are cachectic and (iii) C/EBP β is expressed in most cancer types, we sought to compare secreted proteins that are dysregulated in myoblasts overexpressing C/EBP β to those dysregulated in various tumours. To do so, we used data that was generated by the Nielsen group

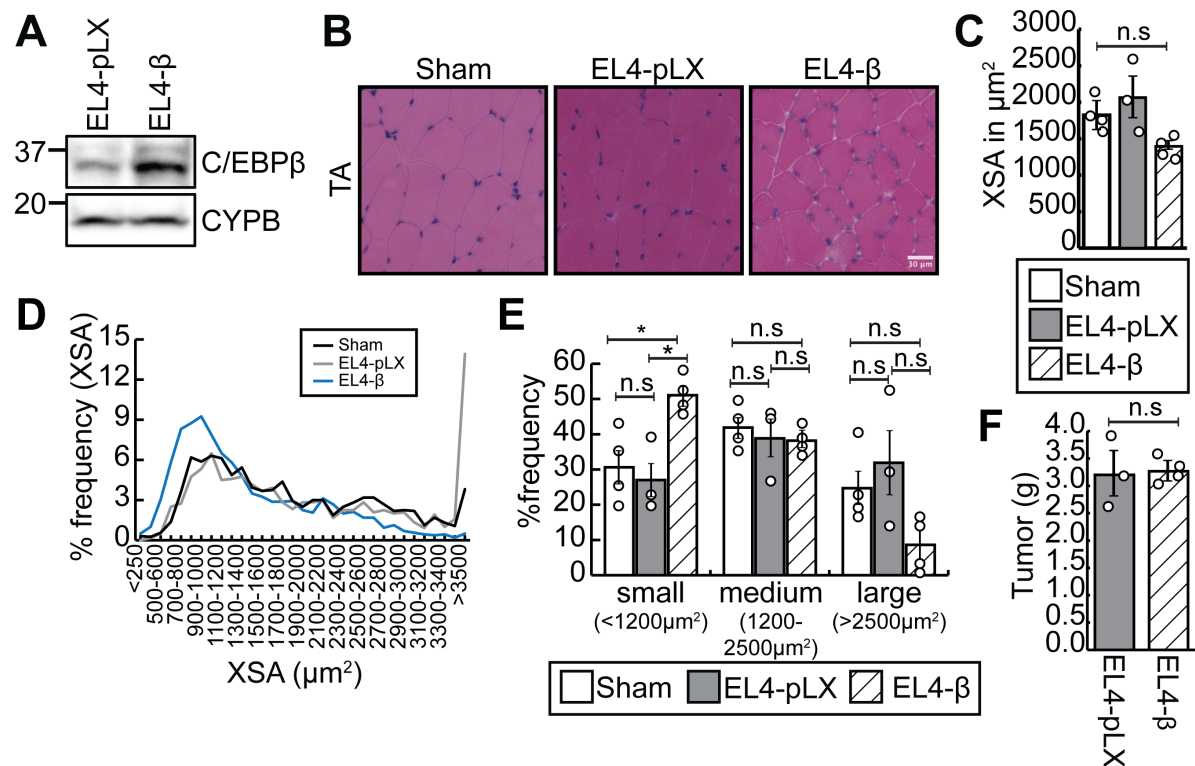


Figure 21. C/EBPβ overexpression converts non-cachectic tumours into atrophy-inducing ones *in vivo*. Mice were inoculated with 1×10^6 lymphoblastoma (EL4) cells that were retrovirally transduced to express C/EBPβ (EL4-β) or with empty vector (EL4-pLX) subcutaneously into the right flank and tumours were allowed to grow for 3 weeks. PBS alone was used for sham injections. **(A)** Representative western blot of C/EBPβ protein expression in EL4-pLX and EL4-β tumours 3 weeks post inoculation. Cyclophilin B (CYPB) is a loading control. **(B)** H&E staining of the TA muscle 3 weeks post-inoculation with EL4 cells. Scale bar = 30 μm **(C)** Average TA fiber cross sectional area (XSA) from (B) (n=4 except EL4-pLX n=3). **(D)** Frequency distribution of myofiber XSA from (C). **(E)** Frequency of muscle cross-sectional areas grouped into small fibers (<1200 μm²), medium-sized fibers (between 1200-2500 μm²) and large fibers (>2500 μm²) from (D). **(F)** EL4-pLX (n=3) and EL4-β (n=4) tumour

mass. Data information: For panels C, E and F, bars represent the mean $\pm$ SEM, * $p \leq 0.05$ (C-E: one-way ANOVA; F: two-tailed Student's t test).

(Robinson et al. 2019) in which they compared the transcripts of secreted proteins from 27 different types of tumours obtained from the Cancer Genome Atlas (TCGA) to tumour equivalent healthy tissue profiles from the Genotype-Tissue Expression (GTEx) database (Robinson et al. 2019). Two lists of secreted proteins were used in their analysis, (1) proteins with a N-terminal signal peptide and annotated as “secreted” in the Uniprot database (a set of 1810 genes) and (2) proteins detected experimentally in the secretome of any data set available in the Human Cancer Secretome Database (HCSD) (a set of 6543 genes, ~800 overlapping with the first list) (Robinson et al. 2019). Thus, we compared differentially expressed genes from these human cancers to our list of differentially expressed genes from mouse C/EBP β -overexpressing myoblasts. We only used genes from the two lists (secreted, 1810 genes and HCSD, 6543 genes) that are also expressed in mouse myoblasts. While only 45% of the “secreted” list of genes were detected in mouse myoblasts, 82% of the HCSD list were detected. Thus, the HCSD list provides a more appropriate measure of the similarity between cancer secretome and C/EBP β -regulated secreted proteins in mouse myoblasts. We performed the analysis using a cut-off of 2-fold difference for significantly differentially expressed genes. Our analysis indicates that the similarity between C/EBP β -regulated secreted proteins and secreted proteins from any cancer subtype (Robinson et al. 2019) ranged from 10-19% using the HCSD lists and from 18-40% using the “secreted” list (Table 1 and 2). Differentially expressed secreted proteins from Glioblastoma multiforme (GBM) and brain lower grade glioma (LGG) had the highest similarity to C/EBP β -regulated secreted proteins (19%). Pancreatic adenocarcinoma (PAAD) (with 18% similarity) and stomach adenocarcinoma (STAD) (with 17% similarity) ranked third and fourth, respectively (Table 1 and 2). Interestingly, GBM, PAAD and STAD are known to cause cancer cachexia (Cui et al. 2019; Bachmann et al. 2009; Li et al. 2015) and their secretome likely plays a role in that process.

Table 1: C/EBP β -regulated secreted proteins share some similarity with the secretome of various cancer types using HCSD list. Differentially expressed (DE, 2-fold cut-off) genes in β -overexpressing cells from our RNA-seq were compared to DE genes in various cancer types as compared to normal tissue (Nielsen's group). Analysis was done on secreted genes from the HCSD database that are expressed in C2C12 cells.

Organ	Cancer type	% of upregulated genes	% of downregulated genes	Total % (up+down)
Brain	GBM	11	8	19
Brain	LGG	10	9	19
Pancreas	PAAD	13	5	18
Stomach	STAD	10	7	17
Kidney	KIRC	11	6	17
Thyroid	THCA	8	8	16
Salivary gland	HNSC	7	8	15
Kidney	KIRP	9	6	15
Adrenal gland	ACC	8	7	15
Esophagus	ESCA	8	7	15
Adrenal gland	PCPG	10	5	15
Lung	LUAD	6	7	13
Liver	CHOL	8	5	13
Breast	BRCA	7	6	13
Testis	TGCT	6	7	13
Colon	COAD	5	7	12
Colon	READ	5	7	12
Liver	LIHC	8	4	12
Bladder	BLCA	6	6	12
Cervix uteri	CESC	6	6	12
Lung	LUSC	5	6	11
Prostate	PRAD	5	6	11
Ovary	OV	7	4	11
Skin	SKCM	6	5	11
Uterus	UCEC	6	5	11
Kidney	KICH	5	5	10
Uterus	UCS	6	4	10

Table 2: C/EBP β -regulated secreted proteins share some similarity with the secretome of various cancer types using “secreted” list. Differentially expressed (DE, 2-fold cut-off) genes in β -overexpressing cells from our RNA-seq were compared to DE genes in various cancer types as compared to normal tissue (Nielsen’s group). Analysis was done on secreted genes that are annotated “secreted” in the Uniprot database that are expressed in C2C12 cells.

Organ	Cancer type	% of upregulated genes	% of downregulated genes	Total % (up+down)
Brain	GBM	32	8	40
Brain	LGG	29	7	36
Colon	COAD	28	6	34
Pancreas	PAAD	28	5	33
Stomach	STAD	28	4	32
Colon	READ	27	5	32
Salivary gland	HNSC	26	5	31
Kidney	KIRC	26	5	31
Liver	LIHC	26	5	31
Bladder	BLCA	25	6	31
Esophagus	ESCA	26	5	31
Testis	TGCT	26	4	30
Breast	BRCA	25	4	29
Lung	LUAD	23	4	27
Lung	LUSC	24	3	27
Liver	CHOL	22	5	27
Thyroid	THCA	21	6	27
Uterus	UCS	23	4	27
Adrenal gland	PCPG	23	3	26
Kidney	KICH	20	5	25
Adrenal gland	ACC	21	3	24
Ovary	OV	20	4	24
Prostate	PRAD	20	3	23
Kidney	KIRP	19	4	23
Cervix uteri	CESC	18	4	22
Skin	SKCM	6	12	18
Uterus	UCEC	19	4	11

Taking into consideration the fact that most advanced cancers lead to cachexia, we generated lists of upregulated and down-regulated secreted proteins in C/EBP β -overexpressing myoblasts and in at least one type of cancer. This resulted in 314 upregulated and 173 downregulated genes (Supplemental Table 10 and 11). Gene ontology analysis of the 314 upregulated genes showed terms associated with proliferation, differentiation, migration, angiogenesis and the inflammatory response (Supplemental Table 12). Gene ontology analysis of the 174 downregulated genes showed terms associated with muscle contraction, developmental and muscle proteins, migration, and angiogenesis (Supplemental Table 13).

C/EBP β stimulates the expression of SERPINF1, TNFRSF11B and CD93 to promote muscle atrophy. Given that our data showed that C/EBP β upregulates several cytokines (Figure 15C), we assessed their protein expression in C2C12 myoblasts retrovirally transduced to overexpress C/EBP β (C2C12- β) or with empty vector (C2C12-pLX) and LLC cells retrovirally transduced to express a shRNA against *Cebpb* (LLC-sh β) or to express an shRNA against luciferase (LLC-shCtl). Lysates from C2C12-pLX, C2C12- β , LLC-shCtl & LLC-sh β were incubated with membranes capturing antibodies against 111 mouse cytokines (the proteome Profiler mouse XL Cytokine Array, R&D, ARY028) (Figure 22A, B). The overexpression of C/EBP β in C2C12s resulted in the upregulation of 95 cytokines (51 proteins ≥ 1.5 -fold) and the downregulation of 16 cytokines (2 proteins ≥ 1.5 -fold) out of the 111 tested factors (Figure 22C and Supplemental Table 14). The knockdown of C/EBP β in LLC cells resulted in the downregulation of 101 cytokines (31 proteins ≥ 1.5 -fold) and the upregulation of 10 cytokines (1 protein ≥ 1.5 fold) out of the 111 tested cytokines as compared to controls (Figure 22C and Supplemental Table 14). By comparing cytokines that are upregulated in

C2C12- β cells and downregulated in LLC-sh β cells using a 1.5-fold cut-off, we narrowed the list to 16 candidate proteins (Figure 22D). From the 16 candidate cytokines, the expression of 10 were upregulated at the mRNA level in at least 2 tumour types from the 27 tumours that were analyzed and reported by the Nielsen group (Robinson et al. 2019) (Figure 8E). Osteoprotegerin (TNFRSF11B) was found to be upregulated in 15 types of cancers followed by the matrix metalloproteinase 3 (MMP3) which was upregulated in 10 tumours. WNT1 inducible signaling pathway protein 1 (WISP1, Cellular communication network factor 4 CCN4) and CD93 were upregulated in 8 tumours each (Figure 22E). To the best of our knowledge, a role for 15 of the 16 candidate cytokines identified has not been described in muscle atrophy (exception is *Serpinf1*). We selected three secreted factors (TNFRSF11B, SERPINF1 and CD93) based on fold change in C/EBP β -modified cells (Figure 22A-D) and their expression in human cachectic tumours to examine their role in muscle atrophy, as a proof-of-concept (Figure 22E). TNFRSF11B (Osteoprotegerin, OPG) is upregulated in 15 tumours including brain, colon, pancreatic and stomach cancers that are known as cachexia-inducing cancers (Cui et al. 2019; Bachmann et al. 2009; Li et al. 2015). SERPINF1 (Pigment Epithelium Derived Factor member 1, PEDF) and CD93 are upregulated in 6 and 8 tumours, respectively, including brain and pancreatic tumours which are known to lead to cancer cachexia (Cui et al. 2019; Bachmann et al. 2009; Li et al. 2015). To test the effect of SERPINF1, TNFRSF11B and CD93 on myotubes, C2C12 myotubes were treated with recombinant proteins of SERPINF1 (0.5 μ g/ml, ED₅₀ is 0.1-0.6 μ g/ml (Ek et al. 2007)), TNFRSF11B (10 ng/ml, ED₅₀ is 2-10 ng/ml), CD93 (6 μ g/ml, ED₅₀ is 6-30 μ g/ml), vehicle or as untreated controls for two days (Figure 22F). Immunostaining for myosin heavy chain was performed on myotubes at day 4 (before treatment) and at day 6 (two days after treatment) and

myotube size was evaluated by measuring the myotube width (diameter). Myotubes that were treated with any recombinant protein (SERPINF1, TNFRSF11B or CD93) showed a ~30% reduction in myotube diameter as compared to vehicle and untreated control (Figure 22F, G). Taken together, our data shows that these three C/EBP β -regulated secreted factors promote atrophy and support the model that C/EBP β is a central regulatory factor in the development of a cachexia-inducing secretome by human cancers.

Discussion

In this study, we identified C/EBP β as a critical regulator of cachexia-inducing factor expression by cancer cells, leading to both an inhibition of myogenesis and muscle atrophy. C/EBP β expression in tumours was found to be necessary and sufficient for the development of cachexia in tumour-bearing animals. Stimulation of C/EBP β expression drives the expression of a large number of secreted factors, including many cytokines, and we found that SERPINF1, TNFRSF11B and CD93 can all drive atrophy in culture. These findings are in line with the reported role of C/EBP β in the regulation of the immune response via the regulation of cytokines (Popeijus and van der Krieken 2016; Akira et al. 1990).

While our work advances C/EBP β as a central regulator of cachexia-inducing factors expression in tumours, cachexia is a multi-system syndrome and the role for C/EBP β extends beyond the tumour. First, we have demonstrated that tumour-bearing animals have higher levels of C/EBP β in their muscle satellite cells, which prevents regenerative responses from effectively counteracting the atrophy (Marchildon et al. 2015, 2016). Further, C/EBP β expression is induced by exposure to conditioned medium from certain tumours, suggesting that one of the effects of the cancer cell secretome is to induce C/EBP β expression in other

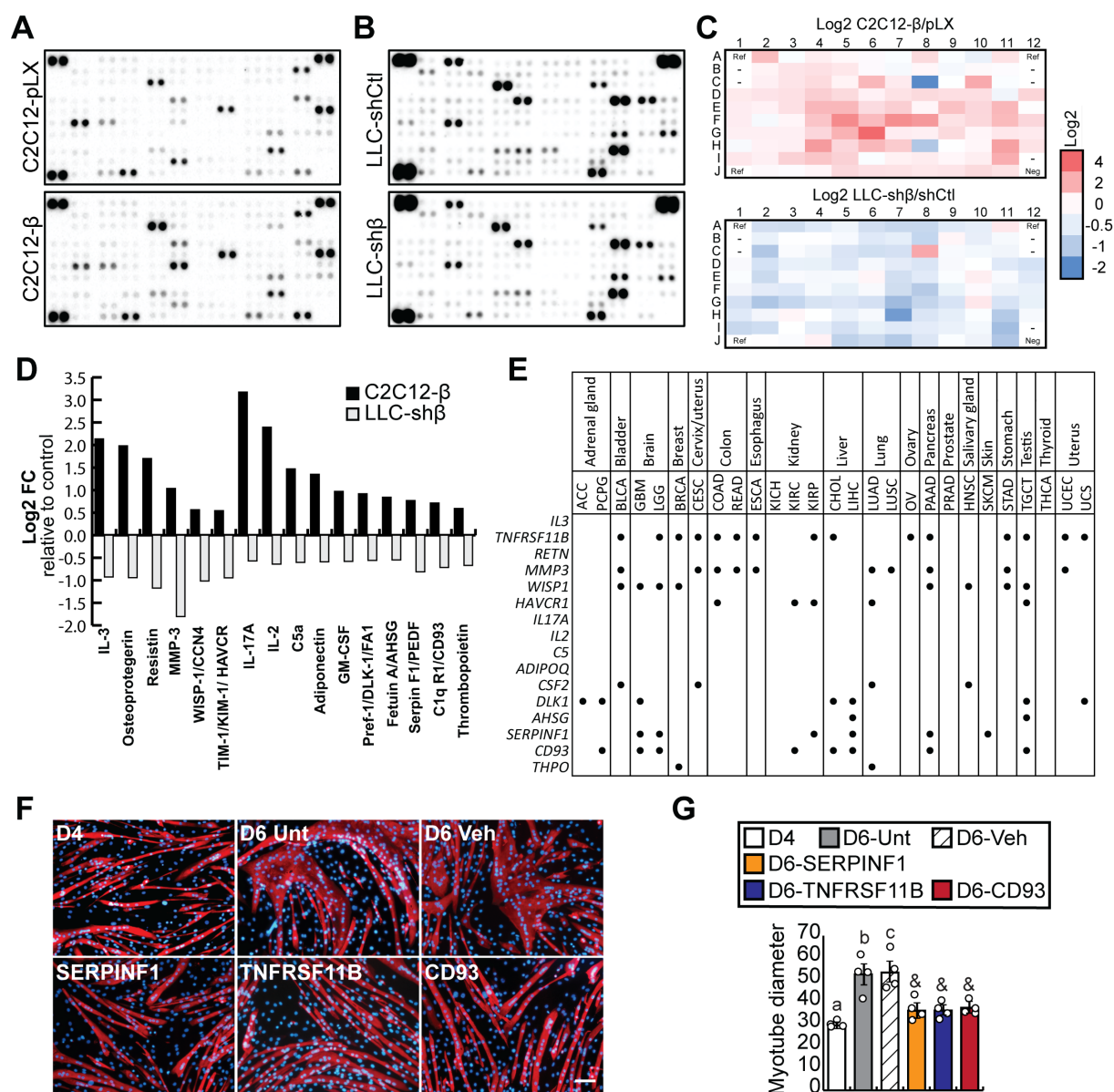


Figure 22. C/EBP β regulates atrophy-inducing cytokines. (A) Lysates from proliferating C2C12 cells retrovirally transduced with empty vector (pLX) or to express C/EBP β (β) were used to interrogate the expression of 111 mouse cytokines using the proteome Profiler mouse XL Cytokine Array (R&D, ARY028). (B) Lysates from LLC cells retrovirally transduced to express a shRNA directed against *Cebpb* (sh β) or against luciferase (ShCtl) were used to test the expression of 111 mouse cytokines. (C) Expression heatmap for (A) and (B) presented as

Log2 fold relative to respective controls (C2C12-pLX and LLC-shCtl). **(D)** Expression of 16 proteins from (C) that are upregulated in C2C12- β as compared to C2C12-pLX and downregulated in LLC-sh β as compared to LLC-shCtl by ≥ 1.5 -fold change. **(E)** Tumours in which the mRNA expression of the 16 cytokines in (D) are upregulated, generated using the 27 human cancer data sets from the Nielson's group [55]. **(F)** Myosin heavy chain immunostaining of C2C12 myoblasts differentiated for four days (D4) and then treated for two days (until D6) with recombinant SERPINF1 (0.5 μ g/ml), TNFRSF11B (10 ng/ml), CD93 (6 μ g/ml), vehicle or left untreated (Unt). Nuclei are counterstained with DAPI (Blue). Scale bar = 100 μ m. **(G)** Average myotube diameter of cells from (F) in pixels (n=4). Bars represent the mean $\pm$ SEM. Means with different letters or symbols are significantly different from one another, at a minimum of $p \leq 0.05$ (One-way ANOVA).

tissues, including muscle. In skeletal muscle, this upregulation can inhibit regeneration, and induce the expression of atrogen-1 in myofibers and contribute to wasting (Zhang et al. 2011; Marchildon et al. 2015, 2016). Further, the expression of C/EBP β in muscle myoblasts promotes the production of a cachexia-inducing secretome that acts in an autocrine and paracrine fashion. While we and others showed the requirement of C/EBP β expression in muscle cells for the development of cachexia (Zhang et al. 2011; Marchildon et al. 2015), it remains unknown if fully differentiated C/EBP β -null muscle would be resistant to atrophy. Indeed, while C/EBP β knockout mice are resistant to tumor-induced-atrophy (Zhang et al. 2011), these mice are immunocompromised and would not be expected to produce the systemic inflammation required for the development of cachexia. Thus, a muscle-specific C/EBP β knockout model would allow the full exploration of the importance of C/EBP β in skeletal muscle for the wasting seen in cachexia.

C/EBP β knockout studies have demonstrated that this factor is indispensable for the development of immune cells and the immune response (Screpanti et al. 1995; Huber et al. 2012), however, no specific report has investigated the association between C/EBP β -regulated pro-inflammatory genes and the development of cancer cachexia. Factors that are derived from tumor cells and immune cells are well established as inducers of muscle atrophy and yet, therapies that target individual factors such as TNF α , IL-1 α or IL-6 have failed to demonstrate strong therapeutic benefit in clinical trials for the treatment of cachexia (reviewed in (Prado and Qian 2019)). However, treatments that target multiple cytokines have proven more effective (Hickish et al. 2017; Gordon et al. 2005). For example, thalidomide is a glutamic acid derivative that acts as an anti-inflammatory (reduces cytokines such as TNF α and IL-6), immunomodulator (reduces NF-kB) and anti-angiogenic (reduces VEGF and FGF) agent

(Franks et al. 2004). While its mechanism of action is not fully understood, in a randomized trial testing the efficacy of thalidomide in patients with inoperable pancreatic cancer with >10% weight loss, the use of thalidomide (200 mg/daily) for 24 weeks spared the patient's body weight and bone free muscle mass loss as compared to the placebo group (Gordon et al. 2005). Interestingly, our findings demonstrate that C/EBP β is a regulator of the majority of cytokines, placing it as a central regulator of cytokine production in the context of cachexia (Figure 22), and thus is an important therapeutic target for the treatment of this paraneoplastic syndrome. Furthermore, given that C/EBP β also regulates other factors involved in tumor growth, such as HGF and FGFs, and the angiogenic factors VEGF and SERPINF1 (Figure 15, Figure 22, Supplemental Table 7 and 14), targeting C/EBP β in cancer patients could, in addition to preventing or treating cancer cachexia, could also limit tumor growth. Both outcomes have tremendous therapeutic potential for cancer patients and offer the possibility of potent complementary treatment in conjunction with current standard of care.

Recently, proteomics were used to compare plasma proteins from tumour-bearing (LLC-bearing) and sham mice, finding 39 up-regulated proteins in plasma of cachectic mice (Lim et al. 2020). Of these, the transcripts of 11 are also upregulated in myoblasts-overexpressing C/EBP β (≥ 1.5 -fold) as compared to control. Furthermore, clinical studies, mainly in pancreatic and gastrointestinal cancer patients, report a correlation between secreted factors and the cachectic state (reviewed in (Dolly et al. 2020)). Some of these factors are (i) markers of inflammation; such as, interleukins (IL-1, IL-6, IL-8, IL-10) and tumour necrosis factor (TNF α), (ii) members of TGF β family, such as myostatin, (iii) tumour-secreted factors; such as, VEGF and (iv) markers of adipose tissue lipolysis such as leptin, adiponectin, free fatty acids and resistin. Interestingly, some of these factors were tested in our cytokine array, and

IL-10, VEGF, adiponectin and resistin are all upregulated in myoblasts overexpressing C/EBP β and downregulated in LLC cells following C/EBP β knockdown (Figure 22A-E, Supplemental Table 14). Our RNA-seq data suggests that some of these factors are transcriptional targets of C/EBP β (Supplemental Table 7) and their association with cachexia from clinical trials highlights the importance of targeting C/EBP β expression as a therapeutic approach towards treating cachexia.

Ethics statement:

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle* (Haehling et al. 2019).

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Conflict of interest:

None declared

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Author contributions:

H.A. contributed to the conception and design, collection of data, data analysis and interpretation, the manuscript writing, final approval of manuscript. R.R. contributed to the collection of data, final approval of manuscript. N.W.B contributed to the conception and design, analysis and interpretation of data, financial support, the manuscript writing, the final approval of manuscript.

CHAPTER FIVE: GENERAL DISCUSSION

Subsequent to the discovery of the inhibitory effect of C/EBP β on myogenic differentiation (Marchildon et al. 2012; Fu et al. 2015) and C/EBP β requirement for MuSC self-renewal (Lala-Tabbert et al. 2016a), my thesis work focused on the molecular mechanism by which C/EBP β regulates these different aspects of myogenesis. We showed first, using a candidate-based approach that C/EBP β directly upregulates *Id3* to block myoblast differentiation (AlSudais et al. 2018). Next, we used RNA-seq as an unbiased approach to identify C/EBP β -target genes in myoblasts. Findings from our RNA-seq coupled with published data provided an explanation for the phenotypes we observed in C/EBP β -null MuSCs. Furthermore, our RNA-seq data suggested that C/EBP β functions in an autocrine and paracrine fashion to influence myoblast function, a finding which was extended to cancer cells and their anti-myogenic secretome. We found that C/EBP β regulates the expression of anti-myogenic and pro-atrophic factors by tumours. We illustrated that the loss of C/EBP β in cachectic tumours prevents muscle atrophy and that its induction in non-cachectic tumours convert these tumours into cachexia-inducing ones, placing C/EBP β as a central regulator of muscle wasting in the context of cancer.

5.1. C/EBP β regulates inhibitors of myoblasts proliferation and differentiation

We performed RNA-seq on RNA isolated from C/EBP β -overexpressing cells and control cells during proliferation and two days after differentiation (GSE152054). Combined analysis of differentially expressed genes in C/EBP β -overexpressing cells at both time points revealed that 3149 genes are differentially expressed, of which 1400 are upregulated protein coding genes (Supplemental Table 15). Approximately 41% of the upregulated genes are implicated in cell differentiation or proliferation. For example, 94 genes from the list were

identified as negative regulators of cell differentiation, which is in accordance with the negative role for C/EBP β in myogenic differentiation. Some of these genes are known inhibitors of myogenic differentiation such as; the inhibitor of DNA binding 1 and 3 (*Id1*, *Id3*) (AlSudais et al. 2018; Jen et al. 1992), *Snai2* (Soleimani et al. 2012b), growth factor genes (*Hgf*, *Fgf2*, *Fgf10* and *Fgf7*) (Clegg et al. 1987; Jarocha et al. 2014; Bandow et al. 2004; Walker et al. 2015) and the bone morphogenetic protein (*Bmp4*) (Dahlqvist 2003). Moreover, 89 genes from the list were identified as negative regulators of cell proliferation, which is concordant with a role for C/EBP β in the maintenance of the MuSC population, self-renewal and quiescence. Examples of genes with a reported negative role in myoblast proliferation are *Cav1* (Lala-Tabbert et al. 2020), *Six5* (Yajima et al. 2010), *Grem1* (Frank et al. 2006) and *Dpt* (Kim et al. 2019). These data support our findings and suggests that the effect of C/EBP β on myogenesis is mediated through the regulation of a large gene expression program that regulates different aspects of myogenesis.

5.2. C/EBP β target genes appear to be cell type dependent

The effects of C/EBP β are cell-type dependent as it acts as an inhibitor of differentiation in osteoblastogenesis and myogenesis and promotes differentiation in adipogenesis. The cell-type specific effects can be attributed to post-translational modifications of C/EBP β , the presence of co-expressed binding partners, and the chromatin accessibility of C/EBP β -target genes. In myotubes, the phosphorylation of C/EBP β by p38 MAPK increases C/EBP β binding to its DNA-binding motif in the promoter of the atrogin-1 gene (Zhang et al. 2011). Furthermore, in preadipocytes that are induced to differentiate, C/EBP β phosphorylation is required for its DNA-binding activity and thus the transactivation

of *Cebpa* and *Pparg* expression (Tang et al. 2005). Whether post-translational modification of C/EBP β dictates what motifs it binds to and thus the genes it regulates, or its binding partners, is yet to be studied.

The dimerization of C/EBP β with different transcription factors gives C/EBP β the access, presumably, to interact with novel binding sites lacking a consensus binding site. The C/EBPs are able to dimerize with members of the bZIP family as well as non-bZIP family members (Grigoryan et al. 2009; Newman 2003; Pulido-Salgado et al. 2015; Tsukada et al. 2011; Diehl and Hannink 1994; Dooher et al. 2011; Faggioli 2004; Ruocco et al. 1996; Johansson-Haque et al. 2008; Ugarte et al. 2012) and such dimerization is dependent on the co-expression of these proteins in each cell type. For example, our hypothesis that C/EBP β regulates the expression of ID proteins, was initially focused on *Id2* given that it is a known target of C/EBP β in immune cells (Karaya et al. 2005). In myoblasts however, we showed that *Id3* and *Id1*, but not *Id2*, are regulated by C/EBP β in gain and loss of function experiments (AlSudais et al. 2018). This discrepancy in the regulation of *Id2* by C/EBP β in myoblasts and immune cells cannot be attributed to the accessibility of the *Id2* locus in myoblast as the expression of *Id2*, while lower than that of *Id3* and *Id1*, is still detected at high levels in our RNA-seq data. Thus, the finding is most likely a result of C/EBP β post-translational modifications and/or the co-expression of other transcription factors necessary for regulation of *Id2* in myoblasts.

Another layer of complexity in the regulation of C/EBP β transcriptional activity is the expression of three protein isoforms that are translated from the same mRNA. The LAP isoform lacks the first 21 amino acid and thus has only two activation domains, whereas LAP* has three. The LIP isoform is deficient in the first 151 amino acids, and thus lacks all the activation domains and acts as a dominant negative for C/EBPs. The ratio of C/EBP β isoforms

is not constant and is changed during cellular processes such as the acute phase response, liver development and regeneration and neoplastic transformation of mammary epithelial cells (An et al. 1996; Luedde et al. 2004; Eaton et al. 2001). The ratio of these isoforms is therefore critical to control the relative expression of C/EBP β target genes, whose expression can be negatively regulated by the presence of the LIP isoform. In myoblasts and healthy muscle, the LAP isoform is the predominant isoform of C/EBP β that is expressed (Marchildon et al. 2012; Alsudais and Wiper-Bergeron 2019; Lala-Tabbert et al. 2020) and while the change in the ratio of C/EBP β isoforms in disease states has not been thoroughly investigated yet, it could represent a mechanism for muscle disease pathogenesis. The regulation of the C/EBP β isoform ratio was shown to be controlled by various signaling pathways that regulate the expression and activity of eukaryotic translation initiation factors (eIFs) (Calkhoven et al. 2000). For example, RNA-dependent protein kinase (PKR) phosphorylation events lead to the inactivation of eIF-2 α , thereby affecting the initiation of translation (Calkhoven et al. 2000). Calkhoven *et al* used mutated forms of eIF-2 α that are resistant to phosphorylation to show that high activity of eIF-2 shifts the C/EBP β isoform ratio towards the dominant negative isoform, LIP (Calkhoven et al. 2000). It has also been shown that the mTOR signaling pathway promotes eIF-4E activity and that the inhibition of mTOR by rapamycin shifts C/EBP β isoform ratio to the transcriptionally active isoforms (LAP* and LAP) (Calkhoven et al. 2000; Slink and Leutz 2010, 2012). Taken together, these reports highlight the complexity of C/EBP β regulation. A careful evaluation of C/EBP β isoform ratio and its target genes in different cell types and under different disease and stress conditions would be critical before targeting C/EBP β to treat inflammatory conditions such as cachexia. One approach would be to evaluate the effect of the overexpression of each isoform individually on gene expression and various cellular activities. It is important to mention that in all the experimental models that have been

used in this thesis, the LAP isoform was consistently the predominantly expressed C/EBP β isoform. Thus, we can speculate that our observed effects are, largely, the result of the activating isoform of C/EBP β .

It is expected that the output of C/EBP β activity on target genes in a given tissue will be the result of the integration of multiple regulatory inputs. For example, C/EBP β mRNA and protein expression are stimulated in response to glucocorticoids (Gonnella et al. 2011) and while C/EBP β activates pro-inflammatory pathways, glucocorticoids suppress inflammation. This contradiction, and the mechanism underlying the different responses remains to be fully elucidated and it is unclear whether the isoform ratio is impacted by glucocorticoids. Interestingly, both C/EBP β and glucocorticoid receptor activities are modulated by the mTOR signaling pathway. The inhibition of mTOR pathway by rapamycin was shown to block glucocorticoids anti-inflammatory action in myeloid immune cells (Weichhart et al. 2011). In rapamycin-treated mice, the expression of C/EBP β inhibitory isoform (LIP) is reduced in liver and epididymal white adipose tissue shifting the C/EBP β isoform ratio towards activators (LAP and LAP*) (Zidek et al. 2015a). If the same effect of rapamycin on C/EBP β occurs in immune cells, this would suggest that the blockage of mTOR pathway facilitates the transcription of C/EBP β inflammatory gene targets. In skeletal muscle, a combined treatment of myotubes with rapamycin and the synthetic glucocorticoid, dexamethasone, synergistically stimulated glucocorticoid-induced atrophy and enhanced the expression of GR-target genes such as MuRF1, atrogin-1 and the Foxo genes (Shimizu et al. 2011). Interestingly, our RNA-seq data confirmed previous reports that atrogin-1 and other atrophy genes are direct transcriptional targets of C/EBP β . Interestingly, Gonnella *et al* showed that C/EBP β expression is required for dexamethasone-induced muscle atrophy and the expression of

atrophy genes (Gonnella et al. 2011). These data show that signaling pathways can modulate the activity of C/EBP β .

5.3. C/EBP β is a regulator of cytokine expression, the muscle niche and beyond

The ability of MuSCs to repair damaged muscle is interrupted in some pathological and physiological conditions. Recent studies suggest that in some of these conditions, the defective MuSC functions are due, in large part, to extrinsic factors rather than intrinsic factors (Price et al. 2014; Chakkalakal et al. 2012; Sousa-Victor et al. 2014; Blau et al. 2015; Wong et al. 2020). Quiescent MuSCs are protected, to an extent, from the anti-myogenic environment present in some pathological and physiological conditions as they remain in their dormant state waiting for the right microenvironmental signal to begin muscle repair (Latil et al. 2012; Wong et al. 2020). For instance, Pomerantz and colleagues showed that MuSCs isolated from atrophic denervated gluteus maximus muscles from patients who suffered a complete spinal cord injury 20 years prior to muscle donation were viable and when transplanted into the TA of nude mice, formed muscle fibers and populated the muscle niche (Wong et al. 2020). More strikingly, isolated MuSCs from humans 17 days post mortem were viable and able to differentiate and fuse to form myotubes in culture (Latil et al. 2012). Furthermore, transplantation of MuSCs from mice 4-day post mortem were able to robustly regenerate cardiotoxin-injured TA muscles in living mice (Latil et al. 2012). These findings illustrate the potential of restoring MuSC functions in various disease models through the regulation and restoration of the normal MuSCs niche. Such modulation can be achieved by the use of pharmacological inhibitors of pathways that promote MuSC activation, pharmacological activators of inhibited pathways in the diseased muscle niche or by the regulation of a transcription factor, such as C/EBP β , that modulates both such pathways.

The role of C/EBP β in the regulation of the immune system and cytokine expression is well-established. In the current work, we illustrated that C/EBP β is indispensable for the production of a cachexia-inducing secretome by cancer cells. We showed that C/EBP β modulates several intrinsic factors (such as *Pax7*, *Cav1* and *Id3*) (Marchildon et al. 2012; Lala-Tabbert et al. 2020; AlSudais et al. 2018), and in an autocrine and paracrine fashion regulates MuSC function. The ability of C/EBP β to regulate secreted factors in different cell types demonstrates the critical importance of studying this transcription factor in cells that are part of the MuSC niche. After muscle injury, the proliferation and expansion of MuSCs requires the function of pro-inflammatory macrophages (M1) and their differentiation and fusion into myotubes is promoted by M2 macrophages (Arnold et al. 2007). In mice with mutated CREB binding sites in the *Cebpb* promoter, muscle regeneration was defective, a phenotype attributed to the defective transition from M1 to M2 macrophages during regeneration (Ruffell et al. 2009). The CREB binding site in *Cebpb* promoter is required for the induction of *Cebpb* expression in macrophages in response to LPS stimulation (Ruffell et al. 2009). The authors reported that the reduced expression of *Cebpb* in M1 macrophages did not cause major changes in the expression of the major pro-inflammatory cytokines (*Il1*, *Il6*, *Tnfa* and *Il2*), but prevented the induction of M2-specific factors *Msr1*, *Il10*, *Il13ra* and *Arg1* (Ruffell et al. 2009). Thus, they concluded that the defect is not attributed to a reduction in pro-inflammatory factors produced by M1 macrophages that have reduced expression of C/EBP β , but rather a failure to induce M2-specific factors (Ruffell et al. 2009). While these findings appear to be the opposite of what our findings suggest, the mutated *Cebpb* promoter did not significantly change the basal expression of *Cebpb* in tested cells but attenuated the stimulation of *Cebpb* in response to LPS. The effect of this after muscle injury is not reported. Further, the experiment was performed in mice with a global mutation of the *Cebpb* promoter and yet, other cells in the

microenvironment of muscle were not tested for changes in their functions during regeneration. Finally, the tested pro-inflammatory factors were limited to only 4 factors rather than a broad unbiased screen and was further restricted to evaluation of the cross-sectional area of regenerating fibers and not the MuSC compartment. Given that C/EBP β is expressed in various cell types, many of which are contributors to the MuSC niche, studying the role of C/EBP β in the regulation of secreted factors in these cells and their effect on MuSC function would provide better understanding of inflammatory diseases and their treatment.

In cachexia, systemic inflammation affects the muscle microenvironment and thus promotes MuSC activation and expansion but limits their differentiation leading to defective regeneration (Talbert and Guttridge 2016; He et al. 2013). Our current work illustrates that elevated levels of C/EBP β in myoblasts and cancer cells shifts their secretome into one that inhibits differentiation, and that the inhibition of differentiation is dependent on these secreted factors. Thus, the induction of C/EBP β in other muscle resident cell types could result in the production of similar anti-myogenic factors, leading to an amplification of the pro-cachectic signal (Figure 23). For example, elevated levels of IL-6, TNF α and C-reactive protein, among others, are observed in obese individuals and contributes to the chronic inflammation seen in these patients (González-Plaza et al. 2018; Castro et al. 2017; Stępień et al. 2014; Fujita et al. 2019). Transcriptional profiling of subcutaneous adipose tissue from obese female subjects before and two-years after bariatric surgery showed a connection between systemic inflammation and elevated levels of CEBPB expression prior to surgery (GSE66921) (González-Plaza et al. 2018). Indeed C/EBP β expression is required for adipogenesis (Tanaka et al. 1997; Staiger et al. 2009; Millward et al. 2007; Rahman et al. 2007, 2012) and its role in the regulation of adipocyte secreted factors (cytokines and adipokines) is yet to be fully

investigated; however, knockout of *Cebpb* in mice renders them resistant to diet-induced obesity (Millward et al. 2007; Rahman et al. 2007, 2012). However, our current work in myoblasts and cancer along with the reported role of C/EBP β in the immune system supports a similar role for C/EBP β in the regulation of cytokines in other cell types. Interestingly, microarray data from epididymal adipose tissue isolated from mice that were fed with either a normal or high-fat diet for 24 weeks showed increased expression of both *Cebpb* and

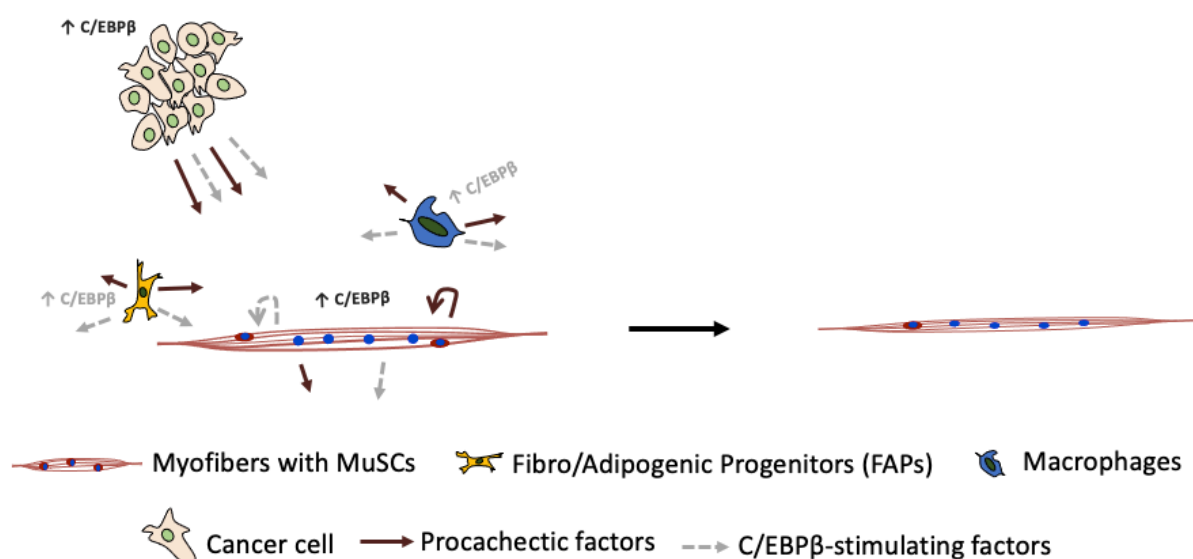


Figure 23. Proposed role for C/EBP β in the tumour anti-myogenic signal amplification in the muscle microenvironment. C/EBP β regulates the production of procachectic factors and C/EBP β -stimulating factors by cancer cells. These C/EBP β -regulated factors in the tumour can potentially stimulate C/EBP β expression in various cells in the muscle microenvironment causing an amplification of the tumour's atrophy-inducing and anti-myogenesis signal which can worsen muscle atrophy.

pro-inflammatory secreted protein genes in mice fed with high-fat diet (Kwon et al. 2012; Do et al. 2011). Furthermore, GO analysis of significantly upregulated genes in epididymal adipose tissue from mice on a high-fat diet indicated that immunity and immune and inflammatory response terms are among the top enriched terms. Intriguingly, 9% of the upregulated genes are annotated as “secreted” based on the UniProt database (Bateman et al. 2021) and 30% of these secreted protein genes are also upregulated in C/EBP β -overexpressing myoblasts, including *Cxcl14*, *Fstl1*, *Mmp2*, *Mmp3*, *Mmp13*, *Ccl2*, *Ccl* and *Ccl9*. These findings are suggestive of an association between C/EBP β expression and the elevated levels of pro-inflammatory genes in obesity and further investigation is needed to confirm the impact, if any, of obesity and systemic inflammation on MuSCs functions and muscle niche.

In addition to pathological conditions, physiological conditions, such as aging, have been associated with elevated *Cebpb* expression and inflammation (Price et al. 2014). Whether C/EBP β contributes to the inflammation observed with aging is yet to be investigated, however, *Cebpb* has been identified as part of the molecular signature for sarcopenia in muscle (Giresi et al. 2005; Price et al. 2014). In muscle from aged mice, recent scRNA-seq data showed that while aged MuSCs follow the same trajectory of activation, proliferation and differentiation as young MuSCs, they do so at a slower rate (Kimmel et al. 2020). An earlier study showed that a parabiotic pairing of young and old mice restored the defective function of aged MuSCs and muscle regeneration in old mice (Conboy et al. 2005). Furthermore, the inflammatory signal in the old niche has been linked to intrinsic MuSC defects that is associated with elevated levels of *Cebpb* in aged mice (Price et al. 2014; Chakkalakal et al. 2012; Sousa-Victor et al. 2014; Blau et al. 2015). These reports suggest that the niche of aged mice is largely responsible for defective MuSC functions in aged mice. We can speculate that secreted factors from different cell compartments contribute to the anti-myogenic niche for

MuSCs and my findings suggest that elevated levels of C/EBP β is central to this process. These observations support the notion of amplification of the anti-myogenic signal in a C/EBP β -dependent manner. A quick approach to test the potential of this hypothesis is through the use of scRNA-seq on muscle samples from cachectic, obese and old-aged mice. This would allow examination of the association between *Cebpb* expression and secreted protein expression in all muscle-resident cells in different pathological and physiological conditions. Such work would directly address the potential of targeting C/EBP β in these conditions to restore the normal MuSC niche.

5.4. C/EBP β and the tumour niche

The tumour microenvironment, or niche, consists of various cell types including the heterogenous population of cancer cells as well as non-malignant cells such as immune cells, fibroblasts and adipocytes that support tumour progression (Balkwill et al. 2012; Arneth 2019; Anderson and Simon 2020). The interaction between cancer cells and surrounding cells is mediated by secreted factors including growth factors, cytokines, chemokines and other factors that are produced by cancer and surrounding cells (Balkwill et al. 2012; Arneth 2019; Anderson and Simon 2020). Immune cells infiltrate the tumour area and while they initially fight tumour cells, evidence suggests that their secretome is converted into a tumour-promoting one (Balkwill et al. 2012). In response to paracrine signalling from cancer cells, cancer-associated fibroblasts secrete factors that promote tumour growth (Li and Wang 2011; Sugimoto et al. 2006; Balkwill et al. 2012), including a pro-inflammatory gene expression profile that supports growth of pancreatic, breast and skin cancers in mouse models (Orimo et al. 2005; Erez et al. 2010; Balkwill et al. 2012). Another cell type present in some tumour

microenvironments is adipocytes, which secrete adipokines and pro-inflammatory factors and provide fatty acids to fuel and promote tumour growth (Nieman et al. 2011; Hanahan and Coussens 2012; Balkwill et al. 2012; Gao et al. 2020). Interestingly, others and we have shown that C/EBP β expression is stimulated by tumour cytokines (Zhang et al. 2011; Marchildon et al. 2016, 2015) and in a feed forward loop, C/EBP β can stimulate the expression of these cytokines (Poli 1998; Cardinaux et al. 2000; Cloutier et al. 2009). Thus, it is of great therapeutic importance to investigate C/EBP β 's role in the regulation of the tumour-promoting secretome in the tumour microenvironment, as it can easily serve to amplify the pro-inflammatory environment and enhance the muscle wasting capacity. Interestingly, the RNA-seq from myoblasts overexpressing C/EBP β identified potential C/EBP β -target genes with known tumour-supportive roles in the tumour microenvironment. For example, various cytokines (Figure 15), growth factors (Figure 15), and angiogenic factors such as *Vegfa*, *Vegfb* and *Vegfd* are significantly upregulated in myoblasts overexpressing C/EBP β (Supplemental Table 7). Furthermore, the cytokines profiler array in myoblasts and tumour cells (Figure 22) identified C/EBP β -regulated factors such as VEGF, PDGF, TNF α and resistin. All these factors contribute to tumour development by promoting tumour growth, metastasis or vascularization (Whiteside 2008; Balkwill et al. 2012; Anderson and Simon 2020; Gao et al. 2020). Interestingly, a recent report showed that neutralization of the adipocyte-derived factor resistin (one of our identified C/EBP β -regulated factors, Figure 22) reduces breast tumour size, weight and tumour progression in mice (Gao et al. 2020). In humans, resistin has been reported to be secreted mainly by macrophages (Patel et al. 2003; Fain et al. 2003), but other reports showed that it is expressed and secreted by isolated adipocytes as well (Degawa-Yamauchi et al. 2003; McTernan et al. 2003; Gao et al. 2020). We report here that resistin is expressed in

a C/EBP β -dependent fashion in both murine myoblasts and lung cancer cells (Figure 22). Previous studies on different cancer cells showed the involvement of C/EBP β in tumour functions such as migration, invasion, growth and chemoresistance (Pal et al. 2009; Carro et al. 2010; Xia et al. 2015; Regalo et al. 2016; Gardiner et al. 2017; Armstrong et al. 2009; Ji et al. 2018; Uddin et al. 2019). These studies were focused on the role of C/EBP β in the regulation of intrinsic cancer cell function. Taken together, these reports and our current findings indicate that C/EBP β is a regulator of tumour-promoting factors (growth and angiogenic factors), which can act as a signal amplifier in other tissues through the upregulation of C/EBP β and the associated secretome. Indeed, investigating the role of C/EBP β in the regulation of secreted factors in all tumour cell types is important to critically evaluate the potential of targeting C/EBP β systemically for the treatment of both cachexia and cancer.

5.5. Cytokine storm and C/EBP β

The term “Cytokine storm” became a hot topic for most media platforms during the emergence of the COVID-19 pandemic in the year 2020. While the term received attention in 2020, it was used first in 1993 (Ferrara et al. 1993) to describe “the uncontrolled systemic inflammatory response in graft versus host disease” (Turnquist et al. 2020). It was later used to describe the uncontrolled overproduction of cytokines in both infectious and non-infectious diseases (Turnquist et al. 2020). The cytokine storm phenomenon was reported in gravely ill COVID-19 patients and is believed to be the cause of serious illness following infection causing organ failure, coagulopathy and vascular leakage leading to death (Turnquist et al. 2020; Fajgenbaum and June 2020; Tang et al. 2020; Hojyo et al. 2020). Interestingly, IL-6 has been identified as a central player in the uncontrolled production of cytokines in this syndrome

(Turnquist et al. 2020; Hojyo et al. 2020; Zhou et al. 2020; Liu et al. 2020). While being stimulated by other cytokines such as IL-1 β and TNF α , IL-6 activates JAK-STAT3 signaling, which in turn leads to the activation of the IL-6-STAT3-NF-kB pathway that stimulates pro-inflammatory cytokines transcription (Turnquist et al. 2020). Our current work on muscle and cancer cells suggests that C/EBP β is a regulator of cytokine secretion and may play a role in the development of the “cytokine storm” via IL-6 and IL-1 β production. A recent RNA-seq dataset from human induced pluripotent stem cell-derived lung alveolar type 2 cell and human primary alveolar type 2 cell alveolospheres that were infected with SARS-CoV-2 showed a rapid transcriptional shift to an inflammatory phenotype in infected cells (Huang et al. 2020; Katsura et al. 2020). Interestingly, the expression level of the *CEBPB* transcript was elevated by ~2-fold in infected cells as compared to controls (Huang et al. 2020; Katsura et al. 2020). The previous reports that proposed a role of C/EBP β in the immune response and our current work showing that the modulation of C/EBP β expression in myoblasts and in cancer cells affects cytokine expression through a mechanism that can be potentially amplified by other cell types suggest the potential of targeting C/EBP β for conditions with hyperactive immune responses such as COVID-19, cancer and cachexia.

5.6. Conclusions:

Findings of this study illustrate the molecular mechanism by which C/EBP β regulates MuSC quiescence, activation, self-renewal and differentiation (Figure 24). We show that C/EBP β regulates myoblast differentiation in an autocrine and paracrine fashion and applied the knowledge obtained to identify C/EBP β as a central player in the regulation of the tumour atrophy-inducing secretome (Figure 25). These findings suggest that C/EBP β is a hierarchical

regulator of cancer cachexia given its role in the muscle and tumour and thus is a potential therapeutic target in cancer cachexia.

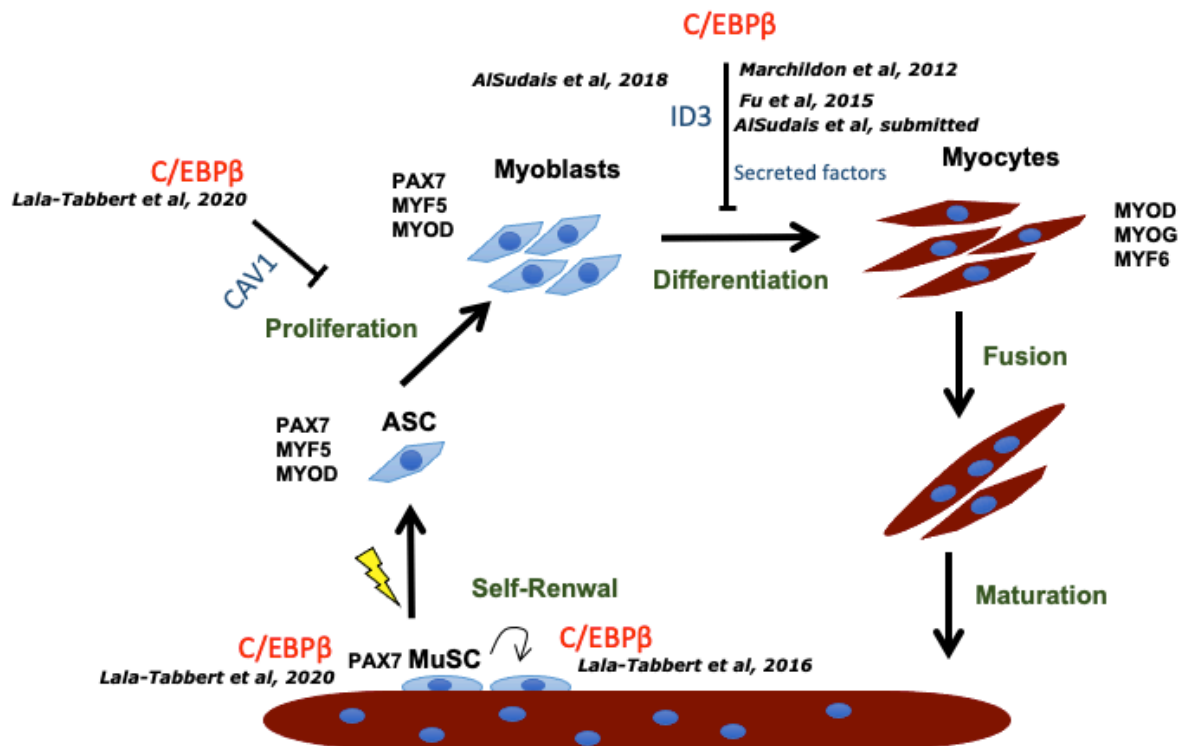


Figure 24. The role of C/EBPβ in the regulation of MuSCs functions. C/EBPβ inhibits myogenic differentiation through the regulation of various differentiation associated genes. C/EBPβ downregulates MYOD and MYOG and upregulates PAX7, the inhibitor of DNA-binding protein 3 (ID3) and anti-myogenic secreted factors. In addition to differentiation regulator genes, C/EBPβ upregulates negative regulators of proliferation such as *Cav1* to inhibit myoblast proliferation, maintains MuSC quiescence and promote MuSC self-renewal.

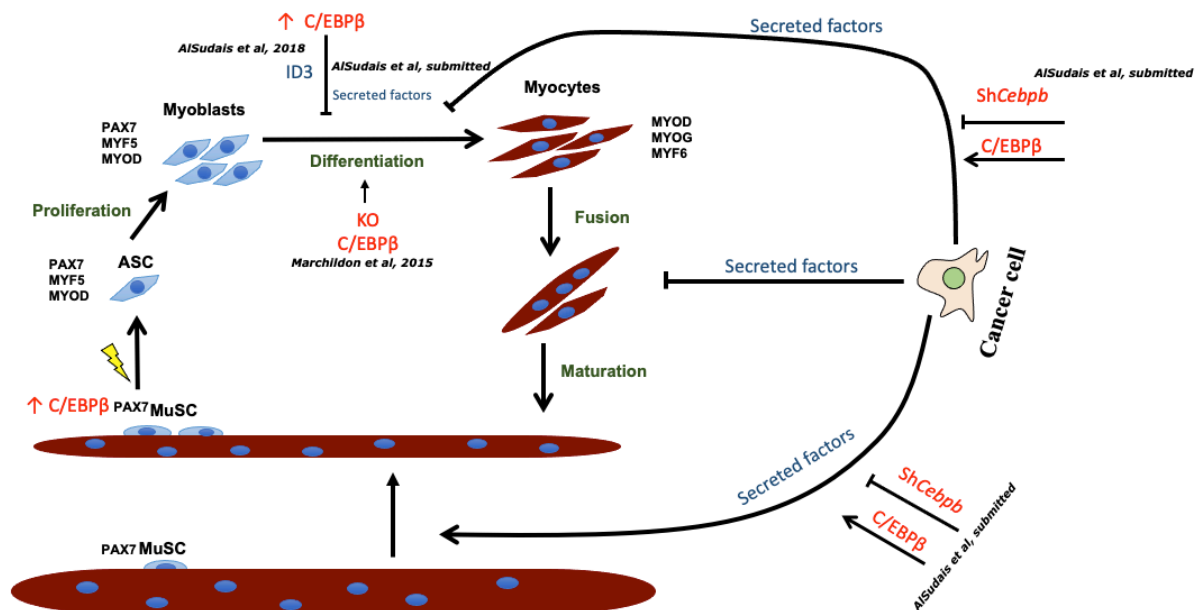


Figure 25. The role of C/EBPβ in cancer cachexia. Exposure of MuSCs to cancer cell secretome stimulates C/EBPβ expression in MuSCs, which prevents their progression to differentiation, which can be rescued by C/EBPβ knockout in muscle cells. Moreover, C/EBPβ plays a central role in the regulation of cancer cell anti-myogenic and atrophy-inducing secreted proteins. Loss of C/EBPβ in cachectic tumours such as LLC converts them to non-cachectic. Conversely, inducing C/EBPβ expression in non-cachectic tumours such as SKOV-3 and EL4 is sufficient to convert them to cachectic tumours.

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Appendix one: Supplemental Figures

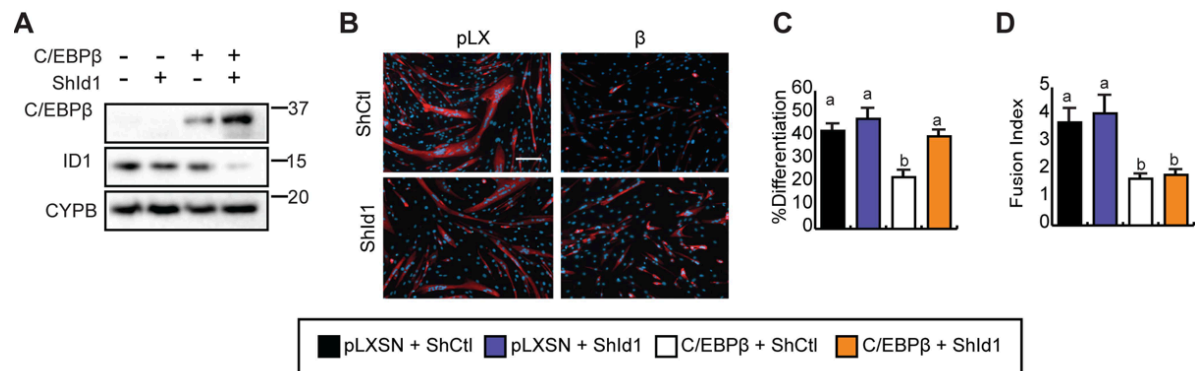


Figure S 1. Knockdown of Id1 rescues differentiation but not fusion in C/EBPβ-overexpressing cells. (A) Representative western blot of C/EBPβ and ID1 expression in C2C12 myoblasts transduced to express C/EBPβ (β) or with empty virus (pLX) and a shRNA targeting *Id1* (shId1) or a non-targeting control (shCtl). Cyclophilin B (CYPB) is a loading control. (B) Immunostaining for myosin heavy chain (MyHC) in cultures derived as in (A) and differentiated for 3 days. DAPI stains nuclei blue. Scale bar = 50μm. (C) Differentiation index (#nuclei in MyHC+ cells/ total nuclei) in cells differentiated as in (B). (D) Fusion index (average # nuclei/MyHC+ cells). Bars are means + SEM, n=4. Means indicated with different letters are significantly different from one another, with a minimum cut-off of p<0.05.

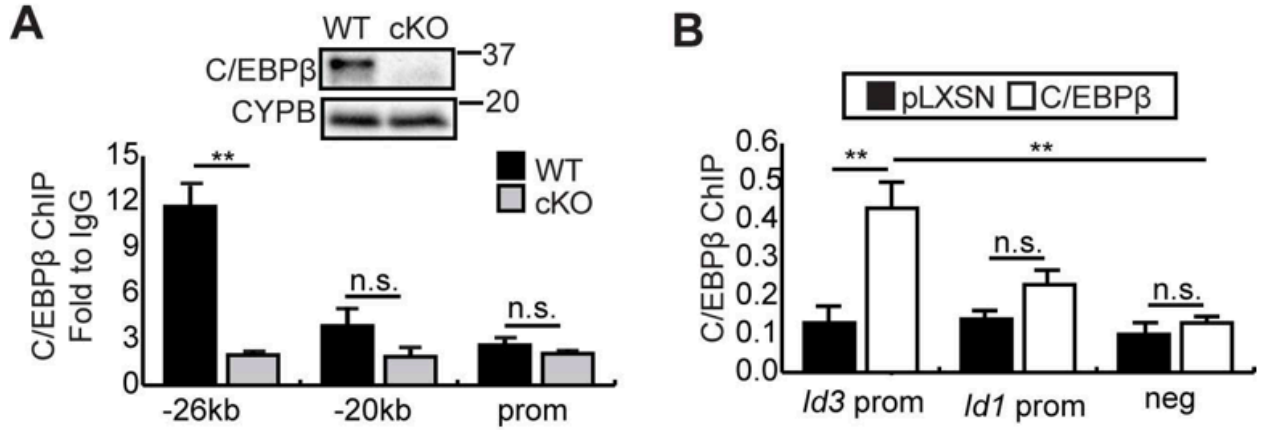


Figure S2. C/EBPβ regulates ID3 expression. (A) qPCR-ChIP analysis of C/EBPβ recruitment to the *Id3* regulatory regions in C/EBPβ^{-/-} (cKO) and WT control primary myoblasts and differentiated for one days. Recruitment is shown relative to IgG non-specific pulldown control. (n=2). Western blot validating C/EBPβ expression is shown as an inset. Cyclophilin B (CYPB) is a loading control. (B) qPCR-ChIP analysis of C/EBPβ recruitment to the *Id3* promoter, the *Id1* promoter and a negative region devoid of C/EBP response elements in C2C12 cells retrovirally transduced with empty virus (pLXSN) or to express C/EBPβ and differentiated for three days. qPCR-ChIP data is shown as copy number as compared to a standard curve of 10% input of each condition. **p<0.01, n=4. n.s. is not significant.

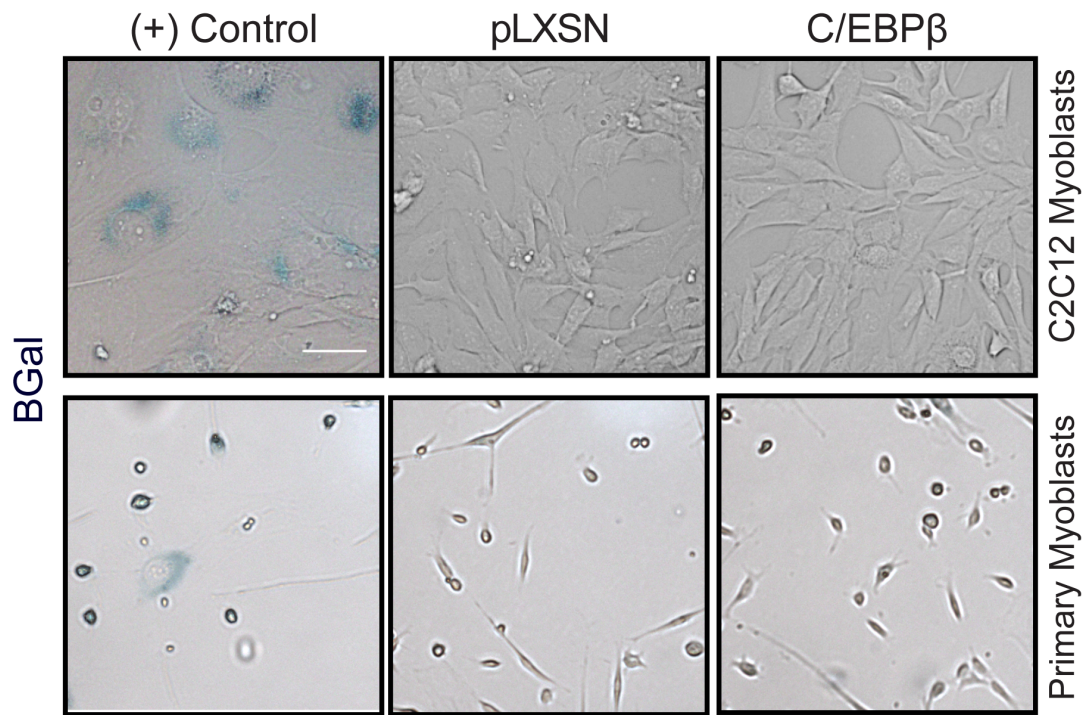


Figure S3. C/EBP β induces quiescence in myogenic progenitors. Representative bright field images of pLXSN and C/EBP β C2C12 myoblasts stained with SA β -galactosidase (blue). C2C12 myoblasts were treated with etoposide to induce senescence in (+) control cells. Scale bar = 50 μ m

Appendix Two: Supplemental Tables

Supplemental Table 1. Differentially expressed genes in C/EBP β -overexpressing C2C12s as compared to control with 2-Fold cut-off and p.adj ≤ 0.05 .

Upregulated in C/EBP β -overexpressing C2C12s as compared to controls with 2-Fold cut-off				
<i>1110012L19Rik</i>	<i>1700024P16Rik</i>	<i>1700034E13Rik</i>	<i>2310007B03Rik</i>	<i>4930426L09Rik</i>
<i>4930486L24Rik</i>	<i>5730559C18Rik</i>	<i>A530084C06Rik</i>	<i>A930018M24Rik</i>	<i>Abat</i>
<i>Abca1</i>	<i>Abca8a</i>	<i>Abca9</i>	<i>Abcd2</i>	<i>Abcg1</i>
<i>Abhd3</i>	<i>Ackr4</i>	<i>Acot1</i>	<i>Acp5</i>	<i>Acpp</i>
<i>Acta2</i>	<i>Actg2</i>	<i>Acvrl1</i>	<i>Adam12</i>	<i>Adam23</i>
<i>Adamts1</i>	<i>Adamts12</i>	<i>Adamts15</i>	<i>Adamts16</i>	<i>Adamts2</i>
<i>Adamts3</i>	<i>Adamts5</i>	<i>Adamts9</i>	<i>Adamts13</i>	<i>Adamts14</i>
<i>Adcy3</i>	<i>Adcy4</i>	<i>Adgb</i>	<i>Adgrf4</i>	<i>Adgrg2</i>
<i>Adgrg3</i>	<i>Adgrg5</i>	<i>Adgrl2</i>	<i>Adgrl4</i>	<i>Adhl</i>
<i>Adm</i>	<i>Adra1d</i>	<i>Aebp1</i>	<i>Aff3</i>	<i>Agap2</i>
<i>Agt</i>	<i>Ak4</i>	<i>Akr1c12</i>	<i>Akr1c18</i>	<i>Aldh1a3</i>
<i>Aldh1l1</i>	<i>Alox8</i>	<i>Alpk1</i>	<i>Alpl</i>	<i>Angpt4</i>
<i>Angptl7</i>	<i>Ankrd55</i>	<i>Aoc3</i>	<i>Apba1</i>	<i>Apbb1ip</i>
<i>Aplp1</i>	<i>Apobec1</i>	<i>Apoc1</i>	<i>Apoll0b</i>	<i>Apol6</i>
<i>Arhgap24</i>	<i>Arhgap26</i>	<i>Arhgef6</i>	<i>Arrdc4</i>	<i>Asb2</i>
<i>Aspg</i>	<i>Asphd2</i>	<i>Aspn</i>	<i>Ass1</i>	<i>Atoh8</i>
<i>Atp6v0d2</i>	<i>Atp8b1</i>	<i>Atp8b4</i>	<i>Avpr1a</i>	<i>AW011738</i>
<i>Azin2</i>	<i>B230217C12Rik</i>	<i>B3galt1</i>	<i>Bach2</i>	<i>Bambi</i>
<i>Bcl3</i>	<i>Bcl6b</i>	<i>Bdkrb1</i>	<i>Best1</i>	<i>Bgn</i>
<i>Bicc1</i>	<i>Bmp4</i>	<i>Bmpr1b</i>	<i>Bnc1</i>	<i>Boc</i>
<i>Bpifb1</i>	<i>Bpifc</i>	<i>Brinp3</i>	<i>Btla</i>	<i>Clqtnf1</i>
<i>Clra</i>	<i>Clrb</i>	<i>Clrl</i>	<i>Cls1</i>	<i>Cls2</i>
<i>C3</i>	<i>Cables1</i>	<i>Cacna1a</i>	<i>Cacna1d</i>	<i>Cacna1g</i>
<i>Cacng7</i>	<i>Camk2n1</i>	<i>Camp</i>	<i>Capn6</i>	<i>Caps2</i>
<i>Car11</i>	<i>Car9</i>	<i>Casp1</i>	<i>Casp14</i>	<i>Casp4</i>
<i>Cav1</i>	<i>Cbs</i>	<i>Ccdc102a</i>	<i>Ccdc60</i>	<i>Ccdc85a</i>
<i>Ccdc88b</i>	<i>Cck</i>	<i>Ccl2</i>	<i>Ccl7</i>	<i>Ccl9</i>
<i>Ccr1</i>	<i>Cd24a</i>	<i>Cd302</i>	<i>Cd33</i>	<i>Cd74</i>
<i>Cd93</i>	<i>Cdh11</i>	<i>Cdh17</i>	<i>Cdh6</i>	<i>Cebpb</i>
<i>Celsr1</i>	<i>Cemip</i>	<i>Ces1g</i>	<i>Ces2c</i>	<i>Ces2g</i>
<i>Cfap100</i>	<i>Cfap161</i>	<i>Cfap46</i>	<i>Cfap69</i>	<i>Cfh</i>
<i>Cgrefl</i>	<i>Chrdl1</i>	<i>Chst12</i>	<i>Chsy3</i>	<i>Cilp</i>
<i>Clca2</i>	<i>Clec4e</i>	<i>Clu</i>	<i>Cnn1</i>	<i>Cnr1</i>
<i>Cnrip1</i>	<i>Cntn1</i>	<i>Coll0a1</i>	<i>Coll1a1</i>	<i>Coll1a2</i>
<i>Coll2a1</i>	<i>Colla1</i>	<i>Colla2</i>	<i>Col24a1</i>	<i>Col25a1</i>
<i>Col27a1</i>	<i>Col3a1</i>	<i>Col5a1</i>	<i>Col5a2</i>	<i>Col6a1</i>

<i>Col6a2</i>	<i>Col6a4</i>	<i>Colec12</i>	<i>Copz2</i>	<i>Cpa6</i>
<i>Cpm</i>	<i>Cpt1c</i>	<i>Creb3l1</i>	<i>Crispld2</i>	<i>Crlf1</i>
<i>Crocc2</i>	<i>Csf1</i>	<i>Csf1r</i>	<i>Csf2rb</i>	<i>Csf2rb2</i>
<i>Ctgf</i>	<i>Cthrc1</i>	<i>Ctla2a</i>	<i>Ctla2b</i>	<i>Ctsf</i>
<i>Cxcl1</i>	<i>Cxcl14</i>	<i>Cxcl16</i>	<i>Cxcl5</i>	<i>Cyb5a</i>
<i>Cyp11b1</i>	<i>Cyp2d22</i>	<i>Cyp2f2</i>	<i>Cys1</i>	<i>Dact1</i>
<i>Dchs1</i>	<i>Dchs2</i>	<i>Dclk3</i>	<i>Ddx58</i>	<i>Dennd2d</i>
<i>Dhrs3</i>	<i>Dio2</i>	<i>Dkk3</i>	<i>Dlc1</i>	<i>Dlgap2</i>
<i>Dpt</i>	<i>Draxin</i>	<i>Ebfl</i>	<i>Ecm2</i>	<i>Ednra</i>
<i>Efemp1</i>	<i>Efs</i>	<i>Egfr</i>	<i>Ehd3</i>	<i>Eln</i>
<i>Emb</i>	<i>Emid1</i>	<i>Emilin1</i>	<i>Emilin2</i>	<i>Enah</i>
<i>Enox1</i>	<i>Enpep</i>	<i>Enpp1</i>	<i>Enpp3</i>	<i>Ephb2</i>
<i>Ephx2</i>	<i>Epm2a</i>	<i>Ereg</i>	<i>Erv3</i>	<i>Esr1</i>
<i>Esyt3</i>	<i>Eva1a</i>	<i>F3</i>	<i>F5</i>	<i>F7</i>
<i>Fabp4</i>	<i>Fabp7</i>	<i>Fads6</i>	<i>Fam105a</i>	<i>Fam110c</i>
<i>Fam114a1</i>	<i>Fam13a</i>	<i>Fam150a</i>	<i>Fam161a</i>	<i>Fam167b</i>
<i>Fam181b</i>	<i>Fam20a</i>	<i>Fam43a</i>	<i>Fam46a</i>	<i>Fam84a</i>
<i>Far2</i>	<i>Fas</i>	<i>Fat2</i>	<i>Fat4</i>	<i>Fbln1</i>
<i>Fbln5</i>	<i>Fbln7</i>	<i>Fcgr3</i>	<i>Fcor</i>	<i>Fcrlb</i>
<i>Fgf10</i>	<i>Fgf7</i>	<i>Fgfr2</i>	<i>Fibin</i>	<i>Figl2</i>
<i>Fmo1</i>	<i>Fmo2</i>	<i>Fmod</i>	<i>Fosl2</i>	<i>Foxc1</i>
<i>Foxf2</i>	<i>Foxq1</i>	<i>Fst</i>	<i>Fstl1</i>	<i>Fxyd5</i>
<i>Fzd4</i>	<i>Fzd8</i>	<i>Gas6</i>	<i>Gata6</i>	<i>Gbp9</i>
<i>Gda</i>	<i>Gdf6</i>	<i>Gdpc2</i>	<i>Gfpt2</i>	<i>Ggt5</i>
<i>Ggt7</i>	<i>Gimap9</i>	<i>Gjb3</i>	<i>Gjb4</i>	<i>Gjb5</i>
<i>Gli2</i>	<i>Glis1</i>	<i>Glis3</i>	<i>Glra3</i>	<i>Glrx</i>
<i>Gm10638</i>	<i>Gm2115</i>	<i>Gm42878</i>	<i>Gm5662</i>	<i>Gm8909</i>
<i>Gng11</i>	<i>Gng2</i>	<i>Gpm6b</i>	<i>Gpr153</i>	<i>Gpr176</i>
<i>Gpr35</i>	<i>Gprin3</i>	<i>Gpsm3</i>	<i>Gpx3</i>	<i>Gramd2</i>
<i>Grb14</i>	<i>Greb1l</i>	<i>Grhl1</i>	<i>Grk5</i>	<i>Grpr</i>
<i>Gstk1</i>	<i>Gstt1</i>	<i>Gucy2d</i>	<i>Gucy2e</i>	<i>H2-D1</i>
<i>H2-DMa</i>	<i>H2-DMb1</i>	<i>H2-DMb2</i>	<i>H2-K1</i>	<i>H2-Q1</i>
<i>H2-Q2</i>	<i>H2-Q4</i>	<i>H2-Q6</i>	<i>H2-Q7</i>	<i>Hao1</i>
<i>Has2</i>	<i>Hectd2</i>	<i>Heph</i>	<i>Hfe</i>	<i>Hgf</i>
<i>Hlf</i>	<i>Hmcn2</i>	<i>Hmgcll1</i>	<i>Hp</i>	<i>Hpgd</i>
<i>Hsd11b1</i>	<i>Htr2a</i>	<i>Htr2b</i>	<i>Htr7</i>	<i>Icam5</i>
<i>Icosl</i>	<i>Id4</i>	<i>Igf1</i>	<i>Igf2</i>	<i>Igfbp4</i>
<i>Igfbp7</i>	<i>Igsf10</i>	<i>Il11ra1</i>	<i>Il13ra2</i>	<i>Il15</i>
<i>Il16</i>	<i>Il17re</i>	<i>Il18rap</i>	<i>Il1f9</i>	<i>Il1r1</i>
<i>Il1rap</i>	<i>Il1rl1</i>	<i>Il1rn</i>	<i>Inhba</i>	<i>Inhbb</i>

<i>Insc</i>	<i>Irf5</i>	<i>Islr</i>	<i>Ism1</i>	<i>Itga1</i>
<i>Itga11</i>	<i>Itga8</i>	<i>Itga9</i>	<i>Itih2</i>	<i>Itih5</i>
<i>Itpkb</i>	<i>Izumo4</i>	<i>Jag1</i>	<i>Kalrn</i>	<i>Kank4</i>
<i>Kazald1</i>	<i>Kcna4</i>	<i>Kcnab2</i>	<i>Kcnc2</i>	<i>Kcnd1</i>
<i>Kcnh1</i>	<i>Kcnj15</i>	<i>Kcnj16</i>	<i>Kcnj8</i>	<i>Kcnmb1</i>
<i>Kcnt2</i>	<i>Kctd15</i>	<i>Kdm7a</i>	<i>Kif1a</i>	<i>Kif21b</i>
<i>Kif26b</i>	<i>Kif5a</i>	<i>Klf15</i>	<i>Klhl33</i>	<i>Klhl6</i>
<i>Klra2</i>	<i>Kng1</i>	<i>Kng2</i>	<i>Ksr1</i>	<i>Lama4</i>
<i>Lce1g</i>	<i>Lcp1</i>	<i>Lgals3bp</i>	<i>Lgalsl</i>	<i>Lgr4</i>
<i>Lilrb4a</i>	<i>Lipn</i>	<i>Lmcd1</i>	<i>Lmo2</i>	<i>Lmod1</i>
<i>Lox</i>	<i>Lpl</i>	<i>Lrrc32</i>	<i>Lrrc4c</i>	<i>Lrrc74b</i>
<i>Lrrc75b</i>	<i>Ltbp2</i>	<i>Ltc4s</i>	<i>Ly6d</i>	<i>Lypd6b</i>
<i>Lyz2</i>	<i>Map3k5</i>	<i>Mapk13</i>	<i>Mark1</i>	<i>Masp1</i>
<i>Mc5r</i>	<i>Mcpt1</i>	<i>Mdfi</i>	<i>Mdga1</i>	<i>Megf6</i>
<i>Mfap2</i>	<i>Mfap5</i>	<i>Mgarp</i>	<i>Mgst1</i>	<i>Mgst2</i>
<i>Mkx</i>	<i>Mmp13</i>	<i>Mmp19</i>	<i>Mmp2</i>	<i>Mmp23</i>
<i>Mmp3</i>	<i>Mmp8</i>	<i>Mn1</i>	<i>Mogat2</i>	<i>Mrgprf</i>
<i>Ms4a4d</i>	<i>Mst1</i>	<i>Musk</i>	<i>Mxd4</i>	<i>Myl9</i>
<i>Mylk</i>	<i>Mylk3</i>	<i>N4bp211</i>	<i>Naalad2</i>	<i>Naaladl2</i>
<i>Naip5</i>	<i>Ncf2</i>	<i>Ncf4</i>	<i>Nebi</i>	<i>Neto2</i>
<i>Nfatc4</i>	<i>Nhs</i>	<i>Nid2</i>	<i>Nkd2</i>	<i>Nkx2-5</i>
<i>Nlrc5</i>	<i>Nlrp4e</i>	<i>Nnmt</i>	<i>Nod1</i>	<i>Nol4l</i>
<i>Nos2</i>	<i>Notch4</i>	<i>Notum</i>	<i>Npas4</i>	<i>Npr3</i>
<i>Npy1r</i>	<i>Nrep</i>	<i>Nrg1</i>	<i>Nrg2</i>	<i>Nrn1</i>
<i>Nsg1</i>	<i>Nt5e</i>	<i>Ntsr2</i>	<i>Nxf7</i>	<i>Nxph2</i>
<i>Nynrin</i>	<i>Olfm13</i>	<i>Olftr810</i>	<i>Olr1</i>	<i>Optc</i>
<i>Orm1</i>	<i>Orm2</i>	<i>Orm3</i>	<i>Osmr</i>	<i>Otor</i>
<i>Oxtr</i>	<i>P3h3</i>	<i>P4ha3</i>	<i>Palld</i>	<i>Panx3</i>
<i>Paox</i>	<i>Papss2</i>	<i>Parm1</i>	<i>Parp14</i>	<i>Pawr</i>
<i>Pcdh18</i>	<i>Pcsk2</i>	<i>Pcsk6</i>	<i>Pde1a</i>	<i>Pde3a</i>
<i>Pde4a</i>	<i>Pde7b</i>	<i>Pdgfra</i>	<i>Pdgfrb</i>	<i>Pdgfrl</i>
<i>Peg10</i>	<i>Per2</i>	<i>Pex5l</i>	<i>Pfkfb3</i>	<i>Phyhd1</i>
<i>Phyhipl</i>	<i>Pi15</i>	<i>Pi16</i>	<i>Pid1</i>	<i>Pilra</i>
<i>Pitpnc1</i>	<i>Pla2g2d</i>	<i>Pla2g5</i>	<i>Plb1</i>	<i>Plcb1</i>
<i>Plce1</i>	<i>Plek</i>	<i>Plekha6</i>	<i>Plekhg4</i>	<i>Plekhn1</i>
<i>Plin1</i>	<i>Plpp3</i>	<i>Plxdc2</i>	<i>Plxna4</i>	<i>Podnl1</i>
<i>Podxl2</i>	<i>Postn</i>	<i>Ppbb</i>	<i>Ppef2</i>	<i>Ppp1r14a</i>
<i>Ppp1r3c</i>	<i>Prdm1</i>	<i>Prelp</i>	<i>Prg4</i>	<i>Prickle2</i>
<i>Prickle3</i>	<i>Prkd1</i>	<i>Prokr1</i>	<i>Prr5l</i>	<i>Prr9</i>
<i>Prrg1</i>	<i>Psmb8</i>	<i>Psmb9</i>	<i>Ptch1</i>	<i>Ptch2</i>

<i>Ptchd4</i>	<i>Ptger1</i>	<i>Ptges</i>	<i>Ptgfr</i>	<i>Ptgs2</i>
<i>Ptn</i>	<i>Ptpu</i>	<i>Ptx3</i>	<i>Pxylp1</i>	<i>Rab32</i>
<i>Ranbp31</i>	<i>Rap1gap</i>	<i>Rapgef3</i>	<i>Rarres2</i>	<i>Rasgrf2</i>
<i>Rasgrp3</i>	<i>Rasl11a</i>	<i>Rasl12</i>	<i>Rassf2</i>	<i>Rassf5</i>
<i>Rassf6</i>	<i>Rbm43</i>	<i>Rbp1</i>	<i>Rcn3</i>	<i>Rdh12</i>
<i>Relt</i>	<i>Rffl</i>	<i>Rgag1</i>	<i>Rgag4</i>	<i>Rgcc</i>
<i>Rgs4</i>	<i>Rhbd13</i>	<i>Rhoj</i>	<i>Rnase10</i>	<i>Rnase2a</i>
<i>Rnase4</i>	<i>Ror1</i>	<i>Ror2</i>	<i>Rora</i>	<i>Rspo1</i>
<i>Rspo2</i>	<i>Rtn4rl1</i>	<i>Rtn4rl2</i>	<i>S100a8</i>	<i>S1pr1</i>
<i>Saa3</i>	<i>Sardh</i>	<i>Sash1</i>	<i>Sbsn</i>	<i>Scara3</i>
<i>Scd3</i>	<i>Scg2</i>	<i>Sdc3</i>	<i>Sec16b</i>	<i>Selenop</i>
<i>Sema3g</i>	<i>Sema4a</i>	<i>Sema6d</i>	<i>Serpina12</i>	<i>Serpina3g</i>
<i>Serpinb1b</i>	<i>Serpine2</i>	<i>Serping1</i>	<i>Sesn3</i>	<i>Sfrp1</i>
<i>Sfrp2</i>	<i>Sgpp2</i>	<i>Sh3gl3</i>	<i>Sh3rf2</i>	<i>Shroom3</i>
<i>Siglecg</i>	<i>Six5</i>	<i>Slamf8</i>	<i>Slc10a6</i>	<i>Slc11a1</i>
<i>Slc16a11</i>	<i>Slc16a3</i>	<i>Slc18a1</i>	<i>Slc1a3</i>	<i>Slc1a6</i>
<i>Slc24a3</i>	<i>Slc25a34</i>	<i>Slc2a10</i>	<i>Slc36a2</i>	<i>Slc43a2</i>
<i>Slc44a3</i>	<i>Slc44a5</i>	<i>Slc46a3</i>	<i>Slc6a12</i>	<i>Slc6a2</i>
<i>Slc6a7</i>	<i>Slc7a2</i>	<i>Slc8a1</i>	<i>Slc9b2</i>	<i>Slfn4</i>
<i>Slpi</i>	<i>Smad6</i>	<i>Smad7</i>	<i>Smad9</i>	<i>Smarca2</i>
<i>Smoc1</i>	<i>Smoc2</i>	<i>Smpd3</i>	<i>Smpd13a</i>	<i>Smpd13b</i>
<i>Snai1</i>	<i>Sncaip</i>	<i>Sned1</i>	<i>Snhg11</i>	<i>Sntg2</i>
<i>Sod3</i>	<i>Sox18</i>	<i>Sp100</i>	<i>Sparc</i>	<i>Sparcl1</i>
<i>Spata13</i>	<i>Spats2l</i>	<i>Sprr2h</i>	<i>Sqrdl</i>	<i>Srd5a2</i>
<i>Srgn</i>	<i>Srpx2</i>	<i>St8sia4</i>	<i>Stard8</i>	<i>Stc1</i>
<i>Susd2</i>	<i>Svep1</i>	<i>Tacstd2</i>	<i>Tagln</i>	<i>Tap1</i>
<i>Tap2</i>	<i>Tcam1</i>	<i>Tgfb1</i>	<i>Tgfbr3</i>	<i>Tgfbr3l</i>
<i>Tgm5</i>	<i>Thbd</i>	<i>Thbs1</i>	<i>Thbs2</i>	<i>Thsd1</i>
<i>Thsd7a</i>	<i>Tiam1</i>	<i>Tifa</i>	<i>Tle6</i>	<i>Tlr2</i>
<i>Tlr7</i>	<i>Tmem154</i>	<i>Tmem176a</i>	<i>Tmem176b</i>	<i>Tmem185a</i>
<i>Tmem268</i>	<i>Tmem45a</i>	<i>Tmprss6</i>	<i>Tnfaip6</i>	<i>Tnfrsf14</i>
<i>Tnfrsf1b</i>	<i>Tnfrsf21</i>	<i>Tns4</i>	<i>Tnxb</i>	<i>Tpsab1</i>
<i>Trabd2b</i>	<i>Trhde</i>	<i>Trib2</i>	<i>Trim12c</i>	<i>Trpv4</i>
<i>Try5</i>	<i>Tusc5</i>	<i>Txnip</i>	<i>U90926</i>	<i>Uba7</i>
<i>Ugt1a6a</i>	<i>Unc13a</i>	<i>Unc5c</i>	<i>Ush1g</i>	<i>Vdr</i>
<i>Vegfd</i>	<i>Vgll3</i>	<i>Vipr2</i>	<i>Vit</i>	<i>Vldlr</i>
<i>Vsir</i>	<i>Vwf</i>	<i>Wdr27</i>	<i>Wdr66</i>	<i>Wdr93</i>
<i>Wfdc21</i>	<i>Whrn</i>	<i>Wisp2</i>	<i>Wnk4</i>	<i>Wnt5b</i>
<i>Xdh</i>	<i>Xkr5</i>	<i>Xkrx</i>	<i>Ypel2</i>	<i>Ypel4</i>
<i>Zc2hc1a</i>	<i>Zfp105</i>	<i>Zfp423</i>	<i>Zfp467</i>	<i>Zfp474</i>

Downregulated in C/EBPβ-overexpressing C2C12s as compared to controls with 2-Fold cut-off

<i>1810011O10Rik</i>	<i>2210011C24Rik</i>	<i>4930523C07Rik</i>	<i>8430408G22Rik</i>	<i>Ablim3</i>
<i>Abra</i>	<i>Ackr3</i>	<i>Acsl6</i>	<i>Acta1</i>	<i>Actc1</i>
<i>Actn2</i>	<i>Adam8</i>	<i>Add2</i>	<i>Adgrg6</i>	<i>Adgrl3</i>
<i>Adora1</i>	<i>Agmat</i>	<i>Aig1</i>	<i>Ak5</i>	<i>Akap6</i>
<i>Aknad1</i>	<i>Alox12</i>	<i>Alox5</i>	<i>Alpk3</i>	<i>Amigo2</i>
<i>Ank1</i>	<i>Ankrd35</i>	<i>Ano5</i>	<i>Apobec2</i>	<i>Apod</i>
<i>Apol9b</i>	<i>Ar</i>	<i>Areg</i>	<i>Arhgap22</i>	<i>Arhgap27</i>
<i>Arhgap6</i>	<i>Arl14epl</i>	<i>Art1</i>	<i>Arx</i>	<i>Aspa</i>
<i>Atf3</i>	<i>Atg9b</i>	<i>Atp2a1</i>	<i>Atp2b1</i>	<i>Atp6v0e2</i>
<i>AU018091</i>	<i>AU021092</i>	<i>B3gnt5</i>	<i>B4galnt3</i>	<i>Bace2</i>
<i>Baiap2l1</i>	<i>Bik</i>	<i>Blnk</i>	<i>Btbd11</i>	<i>Btbd17</i>
<i>Btc</i>	<i>C3ar1</i>	<i>Cacna1s</i>	<i>Cadm4</i>	<i>Calcb</i>
<i>Calcr1</i>	<i>Camk2a</i>	<i>Cap2</i>	<i>Car8</i>	<i>Casq2</i>
<i>Cbfa2t3</i>	<i>Ccdc141</i>	<i>Ccdc162</i>	<i>Ccdc3</i>	<i>Ccdc73</i>
<i>Ccnd2</i>	<i>Cd200r2</i>	<i>Cd200r3</i>	<i>Cd300lb</i>	<i>Cd36</i>
<i>Cdh1</i>	<i>Cdh15</i>	<i>Cdh18</i>	<i>Cdk6</i>	<i>Chchd10</i>
<i>Chdh</i>	<i>Chil5</i>	<i>Chmp4c</i>	<i>Chn2</i>	<i>Chrnbl</i>
<i>Chrnd</i>	<i>Chrng</i>	<i>Ckb</i>	<i>Ckm</i>	<i>Clca3b</i>
<i>Clca4b</i>	<i>Clcn5</i>	<i>Cldn9</i>	<i>Clic5</i>	<i>Clip4</i>
<i>Cmb1</i>	<i>Cmklr1</i>	<i>Cntnap4</i>	<i>Col18a1</i>	<i>Col23a1</i>
<i>Cox8b</i>	<i>Cpn1</i>	<i>Cr2</i>	<i>Csn3</i>	<i>Csrp3</i>
<i>Cst6</i>	<i>Ctrb1</i>	<i>Cubn</i>	<i>Cxcl13</i>	<i>Cystm1</i>
<i>Dact2</i>	<i>Dbnadd1</i>	<i>Ddc</i>	<i>Dhrs7c</i>	<i>Diras1</i>
<i>Dkk2</i>	<i>Dlgap1</i>	<i>Dll1</i>	<i>Dmp1</i>	<i>Dnm3</i>
<i>Dock8</i>	<i>Dok7</i>	<i>Duox1</i>	<i>Dusp22</i>	<i>Dusp4</i>
<i>Dusp6</i>	<i>Dynap</i>	<i>Dync1i1</i>	<i>E2f2</i>	<i>Eaf2</i>
<i>Eda2r</i>	<i>Edil3</i>	<i>Efhdl</i>	<i>Efna5</i>	<i>Eml5</i>
<i>Epb41l4b</i>	<i>Epha4</i>	<i>Eps8</i>	<i>Erb3</i>	<i>F2</i>
<i>F2rl1</i>	<i>Fam107a</i>	<i>Fam167a</i>	<i>Fam180a</i>	<i>Fam189a2</i>
<i>Fam213a</i>	<i>Fam65b</i>	<i>Fam81a</i>	<i>Fam83b</i>	<i>Fbxl22</i>
<i>Fbxl7</i>	<i>Fbxo40</i>	<i>Fcna</i>	<i>Fer1l6</i>	<i>Fgd4</i>
<i>Fgf21</i>	<i>Fgf5</i>	<i>Fgf3p1</i>	<i>Fgl2</i>	<i>Filip1</i>
<i>Fmn1l</i>	<i>Fn3k</i>	<i>Fndc5</i>	<i>Foxa2</i>	<i>Foxo6</i>
<i>Frmd4b</i>	<i>Frmpd1</i>	<i>Fry</i>	<i>Gadd45a</i>	<i>Galnt6</i>
<i>Gap43</i>	<i>Gatm</i>	<i>Gent3</i>	<i>Glrp1</i>	<i>Gm13889</i>
<i>Gm14137</i>	<i>Gm42517</i>	<i>Gm7325</i>	<i>Gpnm3</i>	<i>Gpr137b</i>
<i>Gpr179</i>	<i>Gprc5a</i>	<i>Gprc5c</i>	<i>Gprin1</i>	<i>Grip1</i>

<i>Grtp1</i>	<i>Gsta1</i>	<i>Gsta2</i>	<i>Gsta4</i>	<i>Hap1</i>
<i>Has1</i>	<i>Havcr2</i>	<i>Hdc</i>	<i>Heyl</i>	<i>Hfe2</i>
<i>Hhip</i>	<i>Hmga1</i>	<i>Hmga1-rs1</i>	<i>Hmga2</i>	<i>Hpd1</i>
<i>Hrc</i>	<i>Hspb1</i>	<i>Ifitm1</i>	<i>Igf2bp2</i>	<i>Igf2bp3</i>
<i>Igfbp5</i>	<i>Igsf11</i>	<i>Igsf5</i>	<i>Igsf9</i>	<i>Il17rb</i>
<i>Il17rd</i>	<i>Il34</i>	<i>Illdr2</i>	<i>Inpp4b</i>	<i>Ip6k3</i>
<i>Iqcd</i>	<i>Irf6</i>	<i>Itga10</i>	<i>Itga4</i>	<i>Itga6</i>
<i>Itga7</i>	<i>Itgb7</i>	<i>Itgb8</i>	<i>Itgb11</i>	<i>Jam2</i>
<i>Jup</i>	<i>Kcnj12</i>	<i>Kcnk5</i>	<i>Klf12</i>	<i>Klf4</i>
<i>Klf5</i>	<i>Klhdc8a</i>	<i>Klhl30</i>	<i>Klhl31</i>	<i>Klhl40</i>
<i>Klhl41</i>	<i>Klk10</i>	<i>Krt13</i>	<i>Krt19</i>	<i>L1cam</i>
<i>Lama3</i>	<i>Lbh</i>	<i>Lbx1</i>	<i>Lef1</i>	<i>Lingo2</i>
<i>Lipg</i>	<i>Lix1</i>	<i>Lmod2</i>	<i>Lmod3</i>	<i>Lpar2</i>
<i>Lrrc4</i>	<i>Lrrc8b</i>	<i>Lrrc8d</i>	<i>Lvrn</i>	<i>Lypd6</i>
<i>Maats1</i>	<i>Macc1</i>	<i>11-Mar</i>	<i>Mamstr</i>	<i>Man1a</i>
<i>Map2</i>	<i>Map3k7cl</i>	<i>Map3k9</i>	<i>Mapk4</i>	<i>Mapt</i>
<i>Mb</i>	<i>Mboat1</i>	<i>Mctp1</i>	<i>Mef2c</i>	<i>Megf10</i>
<i>Mertk</i>	<i>Mest</i>	<i>Mfap3l</i>	<i>Mfsd6</i>	<i>Mmp10</i>
<i>Mrln</i>	<i>Msc</i>	<i>Msln</i>	<i>Mybpc1</i>	<i>Mybph</i>
<i>Myh1</i>	<i>Myh2</i>	<i>Myh3</i>	<i>Myh7</i>	<i>Myh8</i>
<i>Myl1</i>	<i>Mylk2</i>	<i>Mylk4</i>	<i>Mylpf</i>	<i>Myo18b</i>
<i>Myo7b</i>	<i>Myog</i>	<i>Myom3</i>	<i>Myoz2</i>	<i>Mypn</i>
<i>Naip1</i>	<i>Nalcn</i>	<i>Nbeal2</i>	<i>Ndr4</i>	<i>Ndst4</i>
<i>Neb</i>	<i>Nefl</i>	<i>Nefm</i>	<i>Neu2</i>	<i>Ngef</i>
<i>Ngfr</i>	<i>Nhs11</i>	<i>Nkain1</i>	<i>Nmrk2</i>	<i>Nov</i>
<i>Npas2</i>	<i>Nppb</i>	<i>Npr1</i>	<i>Nptx1</i>	<i>Nr3c2</i>
<i>Nrarp</i>	<i>Nrcam</i>	<i>Oas2</i>	<i>Obscn</i>	<i>Ocln</i>
<i>Olfm1</i>	<i>Olfml2a</i>	<i>Opn3</i>	<i>Osbp2</i>	<i>Otog</i>
<i>Otop1</i>	<i>Pacrg</i>	<i>Padi2</i>	<i>Pag1</i>	<i>Paqr8</i>
<i>Pard3b</i>	<i>Pard6b</i>	<i>Patj</i>	<i>Pax8</i>	<i>Pcbd1</i>
<i>Pcdh20</i>	<i>Pcd1b</i>	<i>Pclo</i>	<i>Pde5a</i>	<i>Pdgfb</i>
<i>Pdgfd</i>	<i>Pdlim3</i>	<i>Perp</i>	<i>Pgm5</i>	<i>Phex</i>
<i>Pik3r5</i>	<i>Pip5k1b</i>	<i>Pitx3</i>	<i>Pknox2</i>	<i>Plcg2</i>
<i>Plcl2</i>	<i>Plekha7</i>	<i>Plet1</i>	<i>Pmaip1</i>	<i>Podxl</i>
<i>Popdc3</i>	<i>Pou4f1</i>	<i>Ppargc1a</i>	<i>Ppef1</i>	<i>Ppfia4</i>
<i>Ppm1e</i>	<i>Ppp2r2b</i>	<i>Prdm8</i>	<i>Prkag3</i>	<i>Prkcb</i>
<i>Prl2c2</i>	<i>Prl2c3</i>	<i>Prl2c5</i>	<i>Prrg4</i>	<i>Ptprd</i>
<i>Qpct</i>	<i>R3hdml</i>	<i>Ralgps2</i>	<i>Rasgef1a</i>	<i>Rbm24</i>
<i>Rec114</i>	<i>Reep1</i>	<i>Rgs17</i>	<i>Rhbd12</i>	<i>Rhpn2</i>
<i>Rnf144b</i>	<i>Rnf183</i>	<i>Rorb</i>	<i>Rph3al</i>	<i>Rpp25</i>

<i>Rps6ka2</i>	<i>Rragd</i>	<i>Rsad2</i>	<i>Rundc3a</i>	<i>Runx1t1</i>
<i>Ryr1</i>	<i>Slpr3</i>	<i>Samd12</i>	<i>Sbk3</i>	<i>Scml4</i>
<i>Scn10a</i>	<i>Scn4a</i>	<i>Scn5a</i>	<i>Scn7a</i>	<i>Sema3e</i>
<i>Sema4f</i>	<i>Sema6a</i>	<i>Sgcg</i>	<i>Sh3bgr</i>	<i>Siglec15</i>
<i>Slc13a1</i>	<i>Slc14a1</i>	<i>Slc16a10</i>	<i>Slc22a23</i>	<i>Slc25a23</i>
<i>Slc29a2</i>	<i>Slc40a1</i>	<i>Slc44a4</i>	<i>Slc9a2</i>	<i>Smim5</i>
<i>Smpx</i>	<i>Smyd1</i>	<i>Sncg</i>	<i>Sox11</i>	<i>Sox8</i>
<i>Sox9</i>	<i>Sp8</i>	<i>Sprr1a</i>	<i>Spry1</i>	<i>Srgap1</i>
<i>Srl</i>	<i>Srpk3</i>	<i>St6gal1</i>	<i>Stac3</i>	<i>Stc2</i>
<i>Stmn2</i>	<i>Stmnd1</i>	<i>Suox</i>	<i>Susd5</i>	<i>Sv2b</i>
<i>Sybu</i>	<i>Syngr1</i>	<i>Synpo2l</i>	<i>Tbc1d8</i>	<i>Tbc1d9</i>
<i>Tcap</i>	<i>Tceal7</i>	<i>Tcf7</i>	<i>Tcte2</i>	<i>Tec</i>
<i>Tecrl</i>	<i>Tenm4</i>	<i>Tgfa</i>	<i>Tigit</i>	<i>Tlx2</i>
<i>Tm4sf4</i>	<i>Tm6sf1</i>	<i>Tmcc3</i>	<i>Tmem158</i>	<i>Tmem182</i>
<i>Tmem184a</i>	<i>Tmem200a</i>	<i>Tmem8c</i>	<i>Tmod1</i>	<i>Tmprss11b</i>
<i>Tmprss11f</i>	<i>Tnik</i>	<i>Tnnc2</i>	<i>Tnni1</i>	<i>Tnni2</i>
<i>Tnnt1</i>	<i>Tnnt3</i>	<i>Traf1</i>	<i>Trdn</i>	<i>Trim54</i>
<i>Trim55</i>	<i>Trim63</i>	<i>Trim72</i>	<i>Tslp</i>	<i>Tspan13</i>
<i>Tspan15</i>	<i>Tspan32</i>	<i>Tspan7</i>	<i>Tspan8</i>	<i>Ttc9</i>
<i>Ttn</i>	<i>Ubash3b</i>	<i>Uchl1</i>	<i>Unc13c</i>	<i>Unc45b</i>
<i>Upk1b</i>	<i>Upp1</i>	<i>Usp18</i>	<i>Usp43</i>	<i>Vgll2</i>
<i>Vmn2r78</i>	<i>Vmn2r79</i>	<i>Wnt7b</i>	<i>Wwc1</i>	<i>Xirp1</i>
<i>Xirp2</i>	<i>Zdhhc15</i>			

Supplemental Table 2. Differentially expressed genes in QSC (GSE3483) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off.

Upregulated genes in QSC (GSE3483) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>Abat</i>	<i>Abca1</i>	<i>Abca8a</i>	<i>Abcg1</i>	<i>Acpp</i>
<i>Adamts1</i>	<i>Adamts15</i>	<i>Adamts5</i>	<i>Adamts14</i>	<i>Adcy4</i>
<i>Adgr14</i>	<i>Adh1</i>	<i>Alpl</i>	<i>Aoc3</i>	<i>Apobec1</i>
<i>Arhgap24</i>	<i>Arrdc4</i>	<i>Aspn</i>	<i>Bach2</i>	<i>Bambi</i>
<i>Bcl3</i>	<i>Bdkrb1</i>	<i>Bgn</i>	<i>Bicc1</i>	<i>Bmp4</i>
<i>Clra</i>	<i>Clsl</i>	<i>C3</i>	<i>Cables1</i>	<i>Cacna1d</i>
<i>Capn6</i>	<i>Casp4</i>	<i>Cbs</i>	<i>Cd302</i>	<i>Cd93</i>
<i>Cebpb</i>	<i>Ces2g</i>	<i>Cfh</i>	<i>Clu</i>	<i>Colla1</i>
<i>Colla2</i>	<i>Col3a1</i>	<i>Col5a1</i>	<i>Col6a1</i>	<i>Col6a2</i>
<i>Colec12</i>	<i>Crlf1</i>	<i>Ctgf</i>	<i>Ctla2a</i>	<i>Cxcl1</i>
<i>Cxcl14</i>	<i>Cyp2d22</i>	<i>Cys1</i>	<i>Dhrs3</i>	<i>Dpt</i>
<i>Efemp1</i>	<i>Efs</i>	<i>Egfr</i>	<i>Eln</i>	<i>Emilin1</i>
<i>Enpep</i>	<i>Enpp3</i>	<i>Eva1a</i>	<i>Fabp4</i>	<i>Fas</i>
<i>Fbln7</i>	<i>Fibin</i>	<i>Fmo1</i>	<i>Fmo2</i>	<i>Fmod</i>
<i>Fstl1</i>	<i>Fzd4</i>	<i>Gfpt2</i>	<i>Gli2</i>	<i>Gpm6b</i>
<i>Gpx3</i>	<i>Gstt1</i>	<i>H2-D1</i>	<i>H2-K1</i>	<i>Heph</i>
<i>Hfe</i>	<i>Hlf</i>	<i>Hsd11b1</i>	<i>Icosl</i>	<i>Igf1</i>
<i>Igfbp4</i>	<i>Islr</i>	<i>Itga8</i>	<i>Itih5</i>	<i>Itpkb</i>
<i>Jag1</i>	<i>Kcnj8</i>	<i>Klf15</i>	<i>Knlg1</i>	<i>Ksr1</i>
<i>Lama4</i>	<i>Lcp1</i>	<i>Lgals3bp</i>	<i>Lgalsl</i>	<i>Lmo2</i>
<i>Lpl</i>	<i>Map3k5</i>	<i>Mfap2</i>	<i>Mgst1</i>	<i>Mmp2</i>
<i>Ms4a4d</i>	<i>Mxd4</i>	<i>Mylk</i>	<i>N4bp2l1</i>	<i>Naalad2</i>
<i>Npr3</i>	<i>Nrep</i>	<i>Olfm13</i>	<i>Osmr</i>	<i>P3h3</i>
<i>Papss2</i>	<i>Parp14</i>	<i>Pde7b</i>	<i>Pdgfra</i>	<i>Pdgfrb</i>
<i>Per2</i>	<i>Pitpnc1</i>	<i>Plce1</i>	<i>Plek</i>	<i>Plekha6</i>
<i>Plpp3</i>	<i>Plxdc2</i>	<i>Ppp1r14a</i>	<i>Ppp1r3c</i>	<i>Prelp</i>
<i>Prg4</i>	<i>Psmb8</i>	<i>Rapgef3</i>	<i>Rarres2</i>	<i>Rbm43</i>
<i>Rcn3</i>	<i>Rgcc</i>	<i>Rhbd13</i>	<i>Rhoj</i>	<i>Rnase4</i>
<i>Rora</i>	<i>Slpr1</i>	<i>Sash1</i>	<i>Scara3</i>	<i>Serping1</i>
<i>Six5</i>	<i>Slc10a6</i>	<i>Slc2a10</i>	<i>Smarca2</i>	<i>Smoc2</i>
<i>Sod3</i>	<i>Sox18</i>	<i>Sp100</i>	<i>Sparcl1</i>	<i>Spats2l</i>
<i>Srgn</i>	<i>Stard8</i>	<i>Susd2</i>	<i>Svep1</i>	<i>Tagln</i>
<i>Tgfbi</i>	<i>Thbd</i>	<i>Thbs2</i>	<i>Tifa</i>	<i>Tmem45a</i>
<i>Tnfaip6</i>	<i>Tnxb</i>	<i>Txnip</i>	<i>Uba7</i>	<i>Vit</i>
<i>Vldlr</i>	<i>Vsir</i>	<i>Vwf</i>	<i>Xdh</i>	<i>Zfp423</i>

Downregulated genes in QSC (GSE3483) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
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<i>Apobec2</i>	<i>Areg</i>	<i>B3gnt5</i>	<i>Baiap2l1</i>	<i>Ccnd2</i>
<i>Chchd10</i>	<i>Chrnd</i>	<i>Chrng</i>	<i>Cst6</i>	<i>Diras1</i>
<i>Dock8</i>	<i>Eps8</i>	<i>Gsta2</i>	<i>Gsta4</i>	<i>Hmga2</i>
<i>Itga6</i>	<i>Jam2</i>	<i>Klf5</i>	<i>Klhdc8a</i>	<i>Lbh</i>
<i>Myh3</i>	<i>Ndr4</i>	<i>Nefl</i>	<i>Nefm</i>	<i>Nkain1</i>
<i>Nptx1</i>	<i>Nrcam</i>	<i>Opn3</i>	<i>Otog</i>	<i>Phex</i>
<i>Plet1</i>	<i>Pmaip1</i>	<i>Ppargc1a</i>	<i>Ppfia4</i>	<i>Prkcb</i>
<i>Qpct</i>	<i>Rgs17</i>	<i>Sema3e</i>	<i>Slc14a1</i>	<i>Smyd1</i>
<i>Sox11</i>	<i>Stc2</i>	<i>Tmem158</i>	<i>Tmem184a</i>	<i>Tmem8c</i>
<i>Tnni1</i>	<i>Tnnt1</i>	<i>Trim54</i>	<i>Ttn</i>	<i>Uchl1</i>
<i>Wwc1</i>	<i>Xirp1</i>			

Supplemental Table 3. Differentially expressed genes in QSC (GSE15155) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off.

Upregulated genes in QSC (GSE15155) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>A930018M24Rik</i>	<i>Abat</i>	<i>Abca1</i>	<i>Abca8a</i>	<i>Abca9</i>
<i>Acvr1l</i>	<i>Adamts1</i>	<i>Adamts12</i>	<i>Adamts15</i>	<i>Adamts5</i>
<i>Adamts9</i>	<i>Adamtsl4</i>	<i>Adgrl4</i>	<i>Adhl</i>	<i>Arhgap24</i>
<i>Arhgef6</i>	<i>Asb2</i>	<i>Aspn</i>	<i>Bach2</i>	<i>Bambi</i>
<i>Bcl3</i>	<i>Bdkrb1</i>	<i>Bgn</i>	<i>Bicc1</i>	<i>Bmp4</i>
<i>Clra</i>	<i>Cls1</i>	<i>C3</i>	<i>Cables1</i>	<i>Camk2n1</i>
<i>Capn6</i>	<i>Casp4</i>	<i>Cav1</i>	<i>Cd302</i>	<i>Cd93</i>
<i>Cebpb</i>	<i>Cfh</i>	<i>Cilp</i>	<i>Clu</i>	<i>Colla1</i>
<i>Colla2</i>	<i>Col3a1</i>	<i>Col5a1</i>	<i>Col6a1</i>	<i>Col6a2</i>
<i>Colec12</i>	<i>Copz2</i>	<i>Crispld2</i>	<i>Crlf1</i>	<i>Csflr</i>
<i>Csf2rb2</i>	<i>Ctla2a</i>	<i>Ctla2b</i>	<i>Cxcl1</i>	<i>Cyp2d22</i>
<i>Cys1</i>	<i>Dclk3</i>	<i>Ddx58</i>	<i>Dhrs3</i>	<i>Dio2</i>
<i>Dlc1</i>	<i>Dpt</i>	<i>Ecm2</i>	<i>Efemp1</i>	<i>Efs</i>
<i>Egfr</i>	<i>Enpep</i>	<i>Enpp3</i>	<i>Ephx2</i>	<i>Esr1</i>
<i>Fabp4</i>	<i>Fam114a1</i>	<i>Fam43a</i>	<i>Fas</i>	<i>Fbln5</i>
<i>Fibin</i>	<i>Fmo2</i>	<i>Fmod</i>	<i>Fosl2</i>	<i>Fstl1</i>
<i>Fzd4</i>	<i>Gda</i>	<i>Gfpt2</i>	<i>Gli2</i>	<i>Glis3</i>
<i>Gng11</i>	<i>Gpm6b</i>	<i>Gpx3</i>	<i>Gstt1</i>	<i>H2-D1</i>
<i>H2-K1</i>	<i>Hfe</i>	<i>Igf1</i>	<i>Igfbp4</i>	<i>Inhbb</i>
<i>Islr</i>	<i>Itga1</i>	<i>Itga8</i>	<i>Itih5</i>	<i>Itpkb</i>
<i>Kcnj8</i>	<i>Kdm7a</i>	<i>Klf15</i>	<i>Ksr1</i>	<i>Lama4</i>
<i>Lgalsl</i>	<i>Lmcd1</i>	<i>Lmo2</i>	<i>Lpl</i>	<i>Lyz2</i>
<i>Map3k5</i>	<i>Mfap5</i>	<i>Mgst1</i>	<i>Mmp2</i>	<i>Ms4a4d</i>
<i>Mxd4</i>	<i>Mylk</i>	<i>N4bp2l1</i>	<i>Naalad2</i>	<i>Nebl</i>
<i>Nnmt</i>	<i>Nrep</i>	<i>Olfml3</i>	<i>Osmr</i>	<i>Papss2</i>
<i>Parp14</i>	<i>Pcdh18</i>	<i>Pde7b</i>	<i>Pdgfrb</i>	<i>Pdgfrl</i>
<i>Per2</i>	<i>Pitpnc1</i>	<i>Plcel</i>	<i>Plpp3</i>	<i>Plxdc2</i>
<i>Podxl2</i>	<i>Postn</i>	<i>Ppp1r14a</i>	<i>Ppp1r3c</i>	<i>Prelp</i>
<i>Prg4</i>	<i>Prickle2</i>	<i>Psmb9</i>	<i>Ptx3</i>	<i>Rapgef3</i>
<i>Rarres2</i>	<i>Rasgrf2</i>	<i>Rasgrp3</i>	<i>Rbm43</i>	<i>Rcn3</i>
<i>Rgcc</i>	<i>Rgs4</i>	<i>Rhbdl3</i>	<i>Rhoj</i>	<i>Rnase4</i>
<i>Ror1</i>	<i>Rora</i>	<i>Slpr1</i>	<i>Sash1</i>	<i>Sbsn</i>
<i>Scara3</i>	<i>Sema3g</i>	<i>Serping1</i>	<i>Slc10a6</i>	<i>Slc43a2</i>
<i>Smarca2</i>	<i>Smoc2</i>	<i>Sod3</i>	<i>Sox18</i>	<i>Sparc</i>
<i>Sparcl1</i>	<i>Spats2l</i>	<i>Sqrdl</i>	<i>Srgn</i>	<i>St8sia4</i>

<i>Tagln</i>	<i>Tgfb1</i>	<i>Tgfbr3</i>	<i>Thbs2</i>	<i>Tnfaip6</i>
<i>Tnxb</i>	<i>Trib2</i>	<i>Trim12c</i>	<i>Txnip</i>	<i>Vldlr</i>
<i>Vsir</i>	<i>Vwf</i>	<i>Xdh</i>	<i>Ypel2</i>	<i>Ypel4</i>

Downregulated genes in QSC (GSE15155) with 4-fold cut-off and in C/EBPβ-overexpressing cells with 2-Fold cut-off

<i>Areg</i>	<i>Arhgap22</i>	<i>B3gnt5</i>	<i>Baiap2l1</i>	<i>Btbd11</i>
<i>Cdk6</i>	<i>Chrng</i>	<i>Coll8a1</i>	<i>Cst6</i>	<i>Cubn</i>
<i>Dmpl</i>	<i>Dock8</i>	<i>Dok7</i>	<i>Dusp4</i>	<i>Dynap</i>
<i>E2f2</i>	<i>Eps8</i>	<i>Gsta2</i>	<i>Gsta4</i>	<i>Hmga2</i>
<i>Hpd1</i>	<i>Lbh</i>	<i>Macc1</i>	<i>Mfap3l</i>	<i>Myh3</i>
<i>Ndr4</i>	<i>Nptx1</i>	<i>Nrcam</i>	<i>Opn3</i>	<i>Otog</i>
<i>Prkcb</i>	<i>Rbm24</i>	<i>Rgs17</i>	<i>Sema3e</i>	<i>Slc22a23</i>
<i>Synpo2l</i>	<i>Tcf7</i>	<i>Ubash3b</i>	<i>Wnt7b</i>	<i>Wwcl</i>

Supplemental Table 4. Differentially expressed genes in QSC (GSE47177) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off.

Upregulated genes in QSC (GSE47177) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>Abat</i>	<i>Abca8a</i>	<i>Adamts1</i>	<i>Adamts5</i>	<i>Adhl</i>
<i>Alpk1</i>	<i>Arhgap24</i>	<i>Arhgef6</i>	<i>Aspn</i>	<i>Bdkrb1</i>
<i>Biccl</i>	<i>Bmp4</i>	<i>Clrb</i>	<i>Clsl</i>	<i>C3</i>
<i>Cables1</i>	<i>Cacna1d</i>	<i>Camk2n1</i>	<i>Casp4</i>	<i>Cebpb</i>
<i>Cfh</i>	<i>Clu</i>	<i>Col6a1</i>	<i>Cpm</i>	<i>Crlf1</i>
<i>Ctgf</i>	<i>Cyp2d22</i>	<i>Dcl1</i>	<i>Dpt</i>	<i>Ecm2</i>
<i>Enpep</i>	<i>Esr1</i>	<i>Fas</i>	<i>Fibin</i>	<i>Fmod</i>
<i>Fzd4</i>	<i>Gas6</i>	<i>Gfpt2</i>	<i>Gpx3</i>	<i>H2-Q4</i>
<i>Hmcn2</i>	<i>Hsd11b1</i>	<i>Igf1</i>	<i>Igfbp4</i>	<i>Itga1</i>
<i>Itga8</i>	<i>Itih5</i>	<i>Kcnj8</i>	<i>Kcnt2</i>	<i>Ksr1</i>
<i>Map3k5</i>	<i>Mgarp</i>	<i>Mmp2</i>	<i>Ms4a4d</i>	<i>Naalad2</i>
<i>Npy1r</i>	<i>Nrep</i>	<i>Osmr</i>	<i>Parp14</i>	<i>Pde3a</i>
<i>Pde7b</i>	<i>Pdgfrb</i>	<i>Pdgfrl</i>	<i>Plpp3</i>	<i>Plxdc2</i>
<i>Ppp1r14a</i>	<i>Prelp</i>	<i>Rarres2</i>	<i>Rgs4</i>	<i>Rhoj</i>
<i>Ror1</i>	<i>Rora</i>	<i>Serping1</i>	<i>Slc10a6</i>	<i>Sparcl1</i>
<i>Svepl</i>	<i>Tagln</i>	<i>Tgfbr3</i>	<i>Thbs2</i>	<i>Tifa</i>
<i>Tnfaip6</i>	<i>Tnxb</i>	<i>Txnip</i>	<i>Vgll3</i>	

Downregulated genes in QSC (GSE47177) with 4-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>Adam8</i>	<i>Alox5</i>	<i>B3gnt5</i>	<i>Baiap2l1</i>	<i>C3ar1</i>
<i>Ccdc141</i>	<i>Cdk6</i>	<i>Chrnd</i>	<i>Chrng</i>	<i>Dynap</i>
<i>Hmga2</i>	<i>Klhl41</i>	<i>Mfap3l</i>	<i>Nrcam</i>	<i>Prrg4</i>
<i>Sema3e</i>	<i>Smyd1</i>	<i>Trafl</i>	<i>Ttn</i>	

Supplemental Table 5. Differentially expressed genes in Fixed-MuSCs "true quiescent" (GSE103164) with 2-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off.

Upregulated genes in Fixed-MuSCs "true quiescent" (GSE103164) with 2-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>1110012L19Rik</i>	<i>A930018M24Rik</i>	<i>AW011738</i>	<i>Abat</i>	<i>Abca8a</i>
<i>Abca9</i>	<i>Ackr4</i>	<i>Acot1</i>	<i>Adamts12</i>	<i>Adamts15</i>
<i>Adamts5</i>	<i>Adcy3</i>	<i>Adh1</i>	<i>Aldh1l1</i>	<i>Alpk1</i>
<i>Apoc1</i>	<i>Arhgef6</i>	<i>Asb2</i>	<i>Asphd2</i>	<i>Atoh8</i>
<i>Azin2</i>	<i>B230217C12Rik</i>	<i>B3galt1</i>	<i>Bicc1</i>	<i>Btla</i>
<i>Cables1</i>	<i>Cacna1a</i>	<i>Cacna1d</i>	<i>Capn6</i>	<i>Car11</i>
<i>Chst12</i>	<i>Cnrip1</i>	<i>Col10a1</i>	<i>Copz2</i>	<i>Cpt1c</i>
<i>Crlf1</i>	<i>Cyb5a</i>	<i>Cyp2d22</i>	<i>Dclk3</i>	<i>Dhrs3</i>
<i>Draxin</i>	<i>Ecm2</i>	<i>Ednra</i>	<i>Efs</i>	<i>Ehd3</i>
<i>Eln</i>	<i>Epm2a</i>	<i>Esr1</i>	<i>Fads6</i>	<i>Fam114a1</i>
<i>Fam13a</i>	<i>Fam181b</i>	<i>Fam20a</i>	<i>Fibin</i>	<i>Fzd4</i>
<i>Gas6</i>	<i>Gdf6</i>	<i>Gli2</i>	<i>Gng11</i>	<i>Gstk1</i>
<i>Gstt1</i>	<i>Hfe</i>	<i>Hlf</i>	<i>Hsd11b1</i>	<i>Icosl</i>
<i>Igf1</i>	<i>Igfbp4</i>	<i>Il11ra1</i>	<i>Il15</i>	<i>Inhbb</i>
<i>Itga8</i>	<i>Itih5</i>	<i>Izumo4</i>	<i>Kcnj8</i>	<i>Kctd15</i>
<i>Kif21b</i>	<i>Klf15</i>	<i>Klhl33</i>	<i>Ksr1</i>	<i>Lgalsl</i>
<i>Lpl</i>	<i>Mfap2</i>	<i>Mgst1</i>	<i>Mrgprf</i>	<i>Ms4a4d</i>
<i>Nod1</i>	<i>Npr3</i>	<i>Nynrin</i>	<i>P3h3</i>	<i>Paox</i>
<i>Pde7b</i>	<i>Pdgfrl</i>	<i>Plek</i>	<i>Plekha6</i>	<i>Plekhn1</i>
<i>Podxl2</i>	<i>Ppp1r14a</i>	<i>Prep</i>	<i>Prickle3</i>	<i>Prkd1</i>
<i>Prrg1</i>	<i>Psmb9</i>	<i>Ptch1</i>	<i>Ptgfr</i>	<i>Pxylp1</i>
<i>Rarres2</i>	<i>Rbm43</i>	<i>Rhoj</i>	<i>Ror1</i>	<i>Rora</i>
<i>Sash1</i>	<i>Scara3</i>	<i>Sec16b</i>	<i>Six5</i>	<i>Slc7a2</i>
<i>Smad6</i>	<i>Smarca2</i>	<i>Sparc</i>	<i>Spats2l</i>	<i>Sqrdl</i>
<i>Srpx2</i>	<i>Svepl</i>	<i>Tgfbr3</i>	<i>Tgfbr3l</i>	<i>Tmem176a</i>
<i>Tmem176b</i>	<i>Tnfrsf14</i>	<i>Tnxb</i>	<i>Trim12c</i>	<i>Txnip</i>
<i>Vegfd</i>	<i>Zc2hc1a</i>	<i>Zfp105</i>	<i>Zfp467</i>	<i>Zic4</i>
<i>Zkscan7</i>				
Downregulated genes in Fixed-MuSCs "true quiescent" (GSE103164) with 2-fold cut-off and in C/EBP β -overexpressing cells with 2-Fold cut-off				
<i>1810011O10Rik</i>	<i>Acta1</i>	<i>Areg</i>	<i>Arhgap22</i>	<i>Art1</i>
<i>Atf3</i>	<i>Atg9b</i>	<i>Atp2b1</i>	<i>Cadm4</i>	<i>Ccnd2</i>
<i>Cdk6</i>	<i>Ckm</i>	<i>Col23a1</i>	<i>Cystm1</i>	<i>Duox1</i>
<i>Dusp4</i>	<i>Dusp6</i>	<i>Eda2r</i>	<i>Fgl2</i>	<i>Gadd45a</i>

<i>Gm13889</i>	<i>Gprc5a</i>	<i>Hap1</i>	<i>Has1</i>	<i>Hmgal</i>
<i>Hmgal-rs1</i>	<i>Hrc</i>	<i>Hspb1</i>	<i>Igf2bp2</i>	<i>Itga6</i>
<i>Kcnk5</i>	<i>Klf4</i>	<i>Klf5</i>	<i>Klhl40</i>	<i>Lmod3</i>
<i>Lpar2</i>	<i>Lrrc8b</i>	<i>Msc</i>	<i>Myl1</i>	<i>Mylpf</i>
<i>Neu2</i>	<i>Nmrk2</i>	<i>Nrarp</i>	<i>Opn3</i>	<i>Pmaip1</i>
<i>Rsad2</i>	<i>Sema4f</i>	<i>Slc22a23</i>	<i>Slc44a4</i>	<i>Sox11</i>
<i>Sox9</i>	<i>Tcf7</i>	<i>Tm4sf4</i>	<i>Tmem184a</i>	<i>Tmod1</i>
<i>Tnni2</i>	<i>Tnni2</i>	<i>Tnnt3</i>	<i>Trim72</i>	<i>Ubash3b</i>
<i>Upp1</i>	<i>Vgll2</i>	<i>Xirp1</i>		

Supplemental Table 6. Presence of C/EBP β peaks in regulatory regions of putative quiescence genes using C/EBP β ChIP-seq dataset in C2C12s (GSE36024). Occupancy peaks are reported in distance to the closest transcription start site (TSS).

Gene	Distance from TSS (KB)	C/EBP β peak value	Gene ID
<i>Smarca2</i>	0	136	NM_026003
<i>Itga1</i>	0.1	17	NM_001033228
	4.5	25	
<i>Xdh</i>	0.107	15	NM_011723
	8.5	36	
<i>Esr1</i>	0.768	18	NM_007956
	166	53	
<i>Cav1</i>	1.7	37	NM_001243064
<i>Dpt</i>	2	44	NM_019759
<i>Vsir</i>	2.5	12	NM_028732
<i>Per2</i>	2.77	16	NM_011066
<i>Tgfbr3</i>	-6	23	NM_011578
<i>Enpp3</i>	-6.187	12	NM_134005
<i>Sparc</i>	-8.34	19	NM_009242
	5.564	30	
<i>Npr3</i>	-8.8	23	NM_008728
<i>Dlc1</i>	-10	23	NM_001194941
<i>Rgcc</i>	-11	16	NM_025427
<i>Igfl</i>	21	79	NM_010512
<i>Csflr</i>	22	22	NM_001037859
<i>Bmp4</i>	-122	32	NM_007554
	-129	41	
	-144	33	
<i>Atoh8</i>	-30	17	NM_153778
<i>Btla</i>	0.28	9	NM_001037719
<i>Il15</i>	19	21	NM_01254747
<i>Ptch1</i>	0.7	15	NM_008957
<i>Smad6</i>	0	156	NM_008542
<i>Six5</i>	No strong C/EBP β binding		NM_011383
<i>P3h3</i>	No strong C/EBP β binding		NM_013534
<i>Acvr1l</i>	No strong C/EBP β binding		NM_009612
<i>Camk2n1</i>	No strong C/EBP β binding		NM_025451
<i>Fas</i>	No strong C/EBP β binding		NM_007987
<i>Tnfrsf14</i>	No strong C/EBP β binding		NM_178931

Supplemental Table 7. Significantly upregulated genes in C/EBP β -overexpressing C2C12 myoblasts as compared to control with Log₂ ≥ 0.531 (Protein coding genes).

Upregulated in C/EBP β -overexpressing C2C12s as compared to controls with Log ₂ ≥ 0.531				
<i>1110012L19Rik</i>	<i>Adamts16</i>	<i>Amotl2</i>	<i>Atp6v0d2</i>	<i>Cacna1a</i>
<i>1700024P16Rik</i>	<i>Adamts2</i>	<i>Angpt4</i>	<i>Atp6v1g2</i>	<i>Cacna1d</i>
<i>1700034E13Rik</i>	<i>Adamts3</i>	<i>Angptl7</i>	<i>Atp8b1</i>	<i>Cacna1g</i>
<i>2310007B03Rik</i>	<i>Adamts5</i>	<i>Ankrd55</i>	<i>Atp8b4</i>	<i>Cacng7</i>
<i>2510009E07Rik</i>	<i>Adamts6</i>	<i>Antxr1</i>	<i>Atxn1</i>	<i>Cald1</i>
<i>2610008E11Rik</i>	<i>Adamts9</i>	<i>Aoc3</i>	<i>Atxn7l1</i>	<i>Calr</i>
<i>4921524J17Rik</i>	<i>Adamts13</i>	<i>Aox1</i>	<i>Avpr1a</i>	<i>Camk2n1</i>
<i>4930426L09Rik</i>	<i>Adamts14</i>	<i>Apba1</i>	<i>AW011738</i>	<i>Camp</i>
<i>4930486L24Rik</i>	<i>Adarb1</i>	<i>Apbb1ip</i>	<i>Azin2</i>	<i>Capn6</i>
<i>5730409E04Rik</i>	<i>Adcy3</i>	<i>Apcdd1</i>	<i>B230217C12Rik</i>	<i>Caps2</i>
<i>5730559C18Rik</i>	<i>Adcy4</i>	<i>Apln</i>	<i>B2m</i>	<i>Car11</i>
<i>A230050P20Rik</i>	<i>Adgb</i>	<i>Aplp1</i>	<i>B3galt1</i>	<i>Car9</i>
<i>A430105I19Rik</i>	<i>Adgra2</i>	<i>Apobec1</i>	<i>Bach2</i>	<i>Carmil1</i>
<i>A530084C06Rik</i>	<i>Adgrf4</i>	<i>Apobr</i>	<i>Bambi</i>	<i>Casp1</i>
<i>A930018M24Rik</i>	<i>Adgrg1</i>	<i>Apoc1</i>	<i>BC028528</i>	<i>Casp12</i>
<i>Abat</i>	<i>Adgrg2</i>	<i>Apoe</i>	<i>Bcl3</i>	<i>Casp14</i>
<i>Abca1</i>	<i>Adgrg3</i>	<i>Apol10b</i>	<i>Bcl6b</i>	<i>Casp4</i>
<i>Abca8a</i>	<i>Adgrg5</i>	<i>Apol6</i>	<i>Bdh2</i>	<i>Catsper2</i>
<i>Abca9</i>	<i>Adgrl1</i>	<i>Arap3</i>	<i>Bdkrb1</i>	<i>Cav1</i>
<i>Abcb1b</i>	<i>Adgrl2</i>	<i>Arhgap24</i>	<i>Bdnf</i>	<i>Cblb</i>
<i>Abcd2</i>	<i>Adgrl4</i>	<i>Arhgap26</i>	<i>Best1</i>	<i>Cbs</i>
<i>Abcg1</i>	<i>Adhl</i>	<i>Arhgap42</i>	<i>Bgn</i>	<i>Cbx4</i>
<i>Abhd14b</i>	<i>Adm</i>	<i>Arhgef19</i>	<i>Bicc1</i>	<i>Cc2d1a</i>
<i>Abhd3</i>	<i>Adra1b</i>	<i>Arhgef25</i>	<i>Bmf</i>	<i>Ccdc102a</i>
<i>Acad11</i>	<i>Adra1d</i>	<i>Arhgef26</i>	<i>Bmp4</i>	<i>Ccdc60</i>
<i>Acadsb</i>	<i>Aebp1</i>	<i>Arhgef6</i>	<i>Bmpr1b</i>	<i>Ccdc65</i>
<i>Ackr4</i>	<i>Aff3</i>	<i>Arid3a</i>	<i>Bnc1</i>	<i>Ccdc85a</i>
<i>Acot1</i>	<i>Agap2</i>	<i>Arid5b</i>	<i>Bnip3</i>	<i>Ccdc88b</i>
<i>Acot2</i>	<i>Agt</i>	<i>Arl4d</i>	<i>Boc</i>	<i>Cck</i>
<i>Acp5</i>	<i>Ahrr</i>	<i>Arnt2</i>	<i>Bpifb1</i>	<i>Ccl2</i>
<i>Acpp</i>	<i>Ak4</i>	<i>Arrb1</i>	<i>Bpifc</i>	<i>Ccl7</i>
<i>Acsf2</i>	<i>Akna</i>	<i>Arrdc4</i>	<i>Brinp3</i>	<i>Ccl9</i>
<i>Acs11</i>	<i>Akr1c12</i>	<i>Arsj</i>	<i>Btla</i>	<i>Ccr1</i>
<i>Acss2</i>	<i>Akr1c13</i>	<i>Arxes2</i>	<i>Clqtnf1</i>	<i>Cd14</i>
<i>Acta2</i>	<i>Akr1c14</i>	<i>Asb2</i>	<i>Clqtnf12</i>	<i>Cd1d1</i>
<i>Actg2</i>	<i>Akr1c18</i>	<i>Aspg</i>	<i>Clqtnf6</i>	<i>Cd24a</i>

<i>Acvr1l</i>	<i>Aldh1a3</i>	<i>Asphd2</i>	<i>Clra</i>	<i>Cd302</i>
<i>Adam12</i>	<i>Aldh1l1</i>	<i>Aspn</i>	<i>Clrb</i>	<i>Cd33</i>
<i>Adam23</i>	<i>Aldoc</i>	<i>Asrgl1</i>	<i>Clrl</i>	<i>Cd55</i>
<i>Adamts1</i>	<i>Alox8</i>	<i>Ass1</i>	<i>Clsl</i>	<i>Cd74</i>
<i>Adamts10</i>	<i>Alpk1</i>	<i>Atoh8</i>	<i>Clss2</i>	<i>Cd93</i>
<i>Adamts12</i>	<i>Alpl</i>	<i>Atp11a</i>	<i>C3</i>	<i>Cdh11</i>
<i>Adamts15</i>	<i>Amigo3</i>	<i>Atp2b4</i>	<i>Cables1</i>	<i>Cdh17</i>
<i>Cdh23</i>	<i>Col27a1</i>	<i>Cxx1c</i>	<i>Ecm1</i>	<i>Fam13a</i>
<i>Cdh6</i>	<i>Col3a1</i>	<i>Cyb5a</i>	<i>Ecm2</i>	<i>Fam150a</i>
<i>Cdkn1c</i>	<i>Col5a1</i>	<i>Cyp11b1</i>	<i>Ednra</i>	<i>Fam161a</i>
<i>Cdnf</i>	<i>Col5a2</i>	<i>Cyp1b1</i>	<i>Eepd1</i>	<i>Fam167b</i>
<i>Cebpa</i>	<i>Col5a3</i>	<i>Cyp2d22</i>	<i>Efemp1</i>	<i>Fam181b</i>
<i>Cebpb</i>	<i>Col6a1</i>	<i>Cyp2f2</i>	<i>Efemp2</i>	<i>Fam20a</i>
<i>Celsr1</i>	<i>Col6a2</i>	<i>Cyp2j6</i>	<i>Efs</i>	<i>Fam214a</i>
<i>Cemip</i>	<i>Col6a3</i>	<i>Cys1</i>	<i>Egfr</i>	<i>Fam26e</i>
<i>Cers4</i>	<i>Col6a4</i>	<i>Dact1</i>	<i>Ehd3</i>	<i>Fam43a</i>
<i>Ces1g</i>	<i>Col7a1</i>	<i>Dcaf15</i>	<i>Ell2</i>	<i>Fam46a</i>
<i>Ces2c</i>	<i>Colec12</i>	<i>Dchs1</i>	<i>Elmod2</i>	<i>Fam46c</i>
<i>Ces2g</i>	<i>Copz2</i>	<i>Dchs2</i>	<i>Eln</i>	<i>Fam69b</i>
<i>Cfap100</i>	<i>Cotl1</i>	<i>Dclk1</i>	<i>Emb</i>	<i>Fam84a</i>
<i>Cfap161</i>	<i>Cp</i>	<i>Dclk3</i>	<i>Emid1</i>	<i>Far2</i>
<i>Cfap46</i>	<i>Cpa6</i>	<i>Dcxr</i>	<i>Emilin1</i>	<i>Fas</i>
<i>Cfap69</i>	<i>Cpeb3</i>	<i>Ddit4</i>	<i>Emilin2</i>	<i>Fat1</i>
<i>Cfh</i>	<i>Cpm</i>	<i>Ddx58</i>	<i>Enah</i>	<i>Fat2</i>
<i>Cgref1</i>	<i>Cpq</i>	<i>Defb42</i>	<i>Eng</i>	<i>Fat4</i>
<i>Chd3</i>	<i>Cpt1a</i>	<i>Dennd2d</i>	<i>Enox1</i>	<i>Fbln1</i>
<i>Chd9</i>	<i>Cpt1c</i>	<i>Dennd4a</i>	<i>Enpep</i>	<i>Fbln2</i>
<i>Chrdl1</i>	<i>Cpz</i>	<i>Dhrs3</i>	<i>Enpp1</i>	<i>Fbln5</i>
<i>Chst12</i>	<i>Creb3l1</i>	<i>Dhx58</i>	<i>Enpp2</i>	<i>Fbln7</i>
<i>Chsy3</i>	<i>Crim1</i>	<i>Dio2</i>	<i>Enpp3</i>	<i>Fbn1</i>
<i>Cilp</i>	<i>Crispld2</i>	<i>Dkk3</i>	<i>Epha1</i>	<i>Fbxl20</i>
<i>Clca2</i>	<i>Crlf1</i>	<i>Dlc1</i>	<i>Ephb2</i>	<i>Fbxo32</i>
<i>Clcf1</i>	<i>Crocc2</i>	<i>Dlgap2</i>	<i>Ephx2</i>	<i>Fcgr3</i>
<i>Clec4e</i>	<i>Csad</i>	<i>Dlk2</i>	<i>Epm2a</i>	<i>Fcgrt</i>
<i>Clip2</i>	<i>Csfl</i>	<i>Dlx1</i>	<i>Ereg</i>	<i>Fcor</i>
<i>Clip3</i>	<i>Csflr</i>	<i>Dlx2</i>	<i>Erv3</i>	<i>Fcrlb</i>
<i>Clmp</i>	<i>Csf2rb</i>	<i>Dnmt3l</i>	<i>Esr1</i>	<i>Fgf10</i>
<i>Clu</i>	<i>Csf2rb2</i>	<i>Dock10</i>	<i>Esyt3</i>	<i>Fgf2</i>
<i>Cnn1</i>	<i>Csrp2</i>	<i>Dock4</i>	<i>Ets1</i>	<i>Fgf7</i>
<i>Cnr1</i>	<i>Ctgf</i>	<i>Dok4</i>	<i>Etv1</i>	<i>Fgfr2</i>

<i>Cnrip1</i>	<i>Cthrc1</i>	<i>Dpep1</i>	<i>Evala</i>	<i>Fgfr3</i>
<i>Cntn1</i>	<i>Ctla2a</i>	<i>Dpt</i>	<i>Evalb</i>	<i>Fhod3</i>
<i>Cntnap1</i>	<i>Ctla2b</i>	<i>Dram1</i>	<i>F3</i>	<i>Fibcd1</i>
<i>Col10a1</i>	<i>Ctps2</i>	<i>Draxin</i>	<i>F5</i>	<i>Fibin</i>
<i>Col11a1</i>	<i>Ctsf</i>	<i>Dse</i>	<i>F7</i>	<i>Figl2</i>
<i>Col11a2</i>	<i>Ctsf</i>	<i>Dusp23</i>	<i>Fabp4</i>	<i>Fkbp14</i>
<i>Col12a1</i>	<i>Cxadr</i>	<i>Dysf</i>	<i>Fabp7</i>	<i>Fkbp7</i>
<i>Col16a1</i>	<i>Cxcl1</i>	<i>E130311K13Rik</i>	<i>Fads6</i>	<i>Flt3l</i>
<i>Colla1</i>	<i>Cxcl12</i>	<i>Ebfl</i>	<i>Fam105a</i>	<i>Fmo1</i>
<i>Colla2</i>	<i>Cxcl14</i>	<i>Ece2</i>	<i>Fam110c</i>	<i>Fmo2</i>
<i>Col24a1</i>	<i>Cxcl16</i>	<i>Echdc3</i>	<i>Fam114a1</i>	<i>Fmod</i>
<i>Col25a1</i>	<i>Cxcl5</i>	<i>Echs1</i>	<i>Fam129a</i>	<i>Fosl2</i>
<i>Foxc1</i>	<i>Gm35339</i>	<i>H2-T23</i>	<i>Ikzf2</i>	<i>Kcnh2</i>
<i>Foxf2</i>	<i>Gm42878</i>	<i>H6pd</i>	<i>Il11ra1</i>	<i>Kcnj15</i>
<i>Foxl1</i>	<i>Gm5662</i>	<i>Hacd4</i>	<i>Il13ra2</i>	<i>Kcnj16</i>
<i>Foxo1</i>	<i>Gm8909</i>	<i>Hao1</i>	<i>Il15</i>	<i>Kcnj2</i>
<i>Foxq1</i>	<i>Gm996</i>	<i>Hapln4</i>	<i>Il16</i>	<i>Kcnj8</i>
<i>Foxred2</i>	<i>Gnao1</i>	<i>Has2</i>	<i>Il17ra</i>	<i>Kcnmb1</i>
<i>Fst</i>	<i>Gngl1</i>	<i>Hectd2</i>	<i>Il17re</i>	<i>Kcnn1</i>
<i>Fstl1</i>	<i>Gng2</i>	<i>Heph</i>	<i>Il18rap</i>	<i>Kcnt2</i>
<i>Fstl3</i>	<i>Gp1bb</i>	<i>Herpud1</i>	<i>Il1f9</i>	<i>Kctd15</i>
<i>Fuom</i>	<i>Gpc6</i>	<i>Hfe</i>	<i>Il1r1</i>	<i>Kdelr3</i>
<i>Fxyd5</i>	<i>Gpm6b</i>	<i>Hgf</i>	<i>Il1rap</i>	<i>Kdm5b</i>
<i>Fycol</i>	<i>Gpr153</i>	<i>Hivep3</i>	<i>Il1rl1</i>	<i>Kdm7a</i>
<i>Fzd1</i>	<i>Gpr176</i>	<i>Hlf</i>	<i>Il1rn</i>	<i>Khk</i>
<i>Fzd4</i>	<i>Gpr35</i>	<i>Hmcn2</i>	<i>Il7</i>	<i>Kif1a</i>
<i>Fzd8</i>	<i>Gprin3</i>	<i>Hmgcll1</i>	<i>Inhba</i>	<i>Kif21b</i>
<i>Gabre</i>	<i>Gpsm3</i>	<i>Hoxa3</i>	<i>Inhbb</i>	<i>Kif26b</i>
<i>Gas1</i>	<i>Gpx3</i>	<i>Hoxc13</i>	<i>Insc</i>	<i>Kif5a</i>
<i>Gas6</i>	<i>Gramd2</i>	<i>Hoxc8</i>	<i>Ints6l</i>	<i>Kifc3</i>
<i>Gata2</i>	<i>Grb14</i>	<i>Hp</i>	<i>Irak2</i>	<i>Klf15</i>
<i>Gata6</i>	<i>Greb1l</i>	<i>Hpgd</i>	<i>Irak3</i>	<i>Klf9</i>
<i>Gatb</i>	<i>Grem1</i>	<i>Hpse</i>	<i>Irf5</i>	<i>Klhl33</i>
<i>Gbp9</i>	<i>Grhl1</i>	<i>Hsd11b1</i>	<i>Isg20</i>	<i>Klhl5</i>
<i>Gcnt2</i>	<i>Grin2d</i>	<i>Hspb6</i>	<i>Islr</i>	<i>Klhl6</i>
<i>Gda</i>	<i>Grk5</i>	<i>Htr2a</i>	<i>Ism1</i>	<i>Klra2</i>
<i>Gdf6</i>	<i>Grpr</i>	<i>Htr2b</i>	<i>Itga1</i>	<i>Kng1</i>
<i>Gdpd2</i>	<i>Gsdmd</i>	<i>Htr7</i>	<i>Itga11</i>	<i>Kng2</i>
<i>Gfpt2</i>	<i>Gse1</i>	<i>Htra3</i>	<i>Itga5</i>	<i>Krbal</i>
<i>Gfra4</i>	<i>Gstk1</i>	<i>Icam5</i>	<i>Itga8</i>	<i>Krtcap3</i>

<i>Ggn</i>	<i>Gstm2</i>	<i>Icosl</i>	<i>Itga9</i>	<i>Ksr1</i>
<i>Ggt5</i>	<i>Gstt1</i>	<i>Id1</i>	<i>Itih2</i>	<i>Lama4</i>
<i>Ggt7</i>	<i>Gucy2d</i>	<i>Id3</i>	<i>Itih5</i>	<i>Larp6</i>
<i>Gimap9</i>	<i>Gucy2e</i>	<i>Id4</i>	<i>Itpkb</i>	<i>Lcelg</i>
<i>Ginm1</i>	<i>Gulp1</i>	<i>Idh1</i>	<i>Izumo4</i>	<i>Lcp1</i>
<i>Gjb3</i>	<i>Gys1</i>	<i>Idua</i>	<i>Jag1</i>	<i>Lctl</i>
<i>Gjb4</i>	<i>H2-D1</i>	<i>Ifi202b</i>	<i>Jak3</i>	<i>Ldhb</i>
<i>Gjb5</i>	<i>H2-DMa</i>	<i>Ifi204</i>	<i>Junb</i>	<i>Ldlrad4</i>
<i>Gli2</i>	<i>H2-DMb1</i>	<i>Ifi27</i>	<i>Kalrn</i>	<i>Lgals3bp</i>
<i>Glis1</i>	<i>H2-DMb2</i>	<i>Igf1</i>	<i>Kank4</i>	<i>Lgals9</i>
<i>Glis3</i>	<i>H2-K1</i>	<i>Igf2</i>	<i>Kat6b</i>	<i>Lgalsl</i>
<i>Glra3</i>	<i>H2-Q1</i>	<i>Igfbp4</i>	<i>Kazald1</i>	<i>Lgr4</i>
<i>Glrx</i>	<i>H2-Q2</i>	<i>Igfbp6</i>	<i>Kcna4</i>	<i>Lifr</i>
<i>Glyctk</i>	<i>H2-Q4</i>	<i>Igfbp7</i>	<i>Kcnab2</i>	<i>Lilrb4a</i>
<i>Gm10638</i>	<i>H2-Q6</i>	<i>Igsf10</i>	<i>Kcnc2</i>	<i>Limk2</i>
<i>Gm2115</i>	<i>H2-Q7</i>	<i>Igsf3</i>	<i>Kcnd1</i>	<i>Lipn</i>
<i>Gm21992</i>	<i>H2-T22</i>	<i>Ikbke</i>	<i>Kcnhl</i>	<i>Lmcd1</i>
<i>Lmo2</i>	<i>Mgst1</i>	<i>Neto2</i>	<i>Optrn</i>	<i>Per2</i>
<i>Lmod1</i>	<i>Mgst2</i>	<i>Nfatc1</i>	<i>Orai2</i>	<i>Per3</i>
<i>Lmtk3</i>	<i>Micall2</i>	<i>Nfatc4</i>	<i>Orm1</i>	<i>Pex11a</i>
<i>Lox</i>	<i>Mitf</i>	<i>Nfil3</i>	<i>Orm2</i>	<i>Pex5l</i>
<i>Loxl1</i>	<i>Mkx</i>	<i>Nfix</i>	<i>Orm3</i>	<i>Pfkfb3</i>
<i>Loxl3</i>	<i>Mmaa</i>	<i>Nhs</i>	<i>Osmr</i>	<i>Phkb</i>
<i>Loxl4</i>	<i>Mmp13</i>	<i>Nid2</i>	<i>Otor</i>	<i>Phldb2</i>
<i>Lpar1</i>	<i>Mmp14</i>	<i>Nkd2</i>	<i>Oxtr</i>	<i>Phyhd1</i>
<i>Lpar4</i>	<i>Mmp19</i>	<i>Nktr</i>	<i>P2rx6</i>	<i>Phyhipl</i>
<i>Lpin1</i>	<i>Mmp2</i>	<i>Nkx2-5</i>	<i>P2rx7</i>	<i>Pi15</i>
<i>Lpl</i>	<i>Mmp23</i>	<i>Nlrc5</i>	<i>P3h1</i>	<i>Pi16</i>
<i>Lrrc32</i>	<i>Mmp3</i>	<i>Nlrp4e</i>	<i>P3h3</i>	<i>Pianp</i>
<i>Lrrc4c</i>	<i>Mmp8</i>	<i>Nlrx1</i>	<i>P4ha1</i>	<i>Pidl</i>
<i>Lrrc74b</i>	<i>Mn1</i>	<i>Nnmt</i>	<i>P4ha2</i>	<i>Piezo2</i>
<i>Lrrc75b</i>	<i>Mogat2</i>	<i>Nod1</i>	<i>P4ha3</i>	<i>Pik3cd</i>
<i>Ltbp2</i>	<i>Mpp2</i>	<i>Nol4l</i>	<i>Pak3</i>	<i>Pik3ip1</i>
<i>Ltc4s</i>	<i>Mr1</i>	<i>Nos1</i>	<i>Palld</i>	<i>Pilra</i>
<i>Ly6d</i>	<i>Mrc2</i>	<i>Nos2</i>	<i>Panx3</i>	<i>Pitpnc1</i>
<i>Lypd6b</i>	<i>Mrgprf</i>	<i>Notch4</i>	<i>Paox</i>	<i>Pkd2</i>
<i>Lysmd2</i>	<i>Ms4a4d</i>	<i>Notum</i>	<i>Papss2</i>	<i>Pkdcc</i>
<i>Lyz2</i>	<i>Msrbb3</i>	<i>Npas4</i>	<i>Parm1</i>	<i>Pkp2</i>
<i>Maf</i>	<i>Mst1</i>	<i>Nphp3</i>	<i>Parp10</i>	<i>Pla2g2d</i>
<i>Maged1</i>	<i>Musk</i>	<i>Nphp4</i>	<i>Parp14</i>	<i>Pla2g5</i>

<i>Maged2</i>	<i>Mustn1</i>	<i>Npr3</i>	<i>Pawr</i>	<i>Plat</i>
<i>Man2b2</i>	<i>Mxd4</i>	<i>Npy1r</i>	<i>Pax7</i>	<i>Plb1</i>
<i>Map3k20</i>	<i>Mxra8</i>	<i>Nrep</i>	<i>Pcbp3</i>	<i>Plcb1</i>
<i>Map3k5</i>	<i>Myf5</i>	<i>Nrg1</i>	<i>Pcdh18</i>	<i>Plcd1</i>
<i>Map7d3</i>	<i>Myl9</i>	<i>Nrg2</i>	<i>Pcdh7</i>	<i>Plce1</i>
<i>Mapk13</i>	<i>Mylk</i>	<i>Nrn1</i>	<i>Pcdhgc5</i>	<i>Plek</i>
<i>Mark1</i>	<i>Mylk3</i>	<i>Nrp1</i>	<i>Pcsk2</i>	<i>Plekha6</i>
<i>Masp1</i>	<i>Myo1d</i>	<i>Nsg1</i>	<i>Pcsk6</i>	<i>Plekhg4</i>
<i>Matn2</i>	<i>N4bp2l1</i>	<i>Nt5e</i>	<i>Pdcd4</i>	<i>Plekhn1</i>
<i>Mbnl3</i>	<i>Naaa</i>	<i>Ntsr2</i>	<i>Pde10a</i>	<i>Plin1</i>
<i>Mbp</i>	<i>Naalad2</i>	<i>Nucb2</i>	<i>Pde1a</i>	<i>Plin4</i>
<i>Mc5r</i>	<i>Naaladl2</i>	<i>Nxf3</i>	<i>Pde3a</i>	<i>Plod1</i>
<i>Mcam</i>	<i>Nacad</i>	<i>Nxf7</i>	<i>Pde4a</i>	<i>Plod2</i>
<i>Mcpt1</i>	<i>Nadk2</i>	<i>Nxph2</i>	<i>Pde7b</i>	<i>Plpp3</i>
<i>Mdfi</i>	<i>Naip2</i>	<i>Nynrin</i>	<i>Pdgfra</i>	<i>Plxdc2</i>
<i>Mdga1</i>	<i>Naip5</i>	<i>Ogn</i>	<i>Pdgfrb</i>	<i>Plxna4</i>
<i>Medag</i>	<i>Napepld</i>	<i>Olfml3</i>	<i>Pdgfrl</i>	<i>Podnl1</i>
<i>Megf6</i>	<i>Nav2</i>	<i>Olf810</i>	<i>Pdk1</i>	<i>Podxl2</i>
<i>Mettl7a1</i>	<i>Ncf2</i>	<i>Olr1</i>	<i>Pdk2</i>	<i>Polm</i>
<i>Mfap2</i>	<i>Ncf4</i>	<i>Omd</i>	<i>Pear1</i>	<i>Porcn</i>
<i>Mfap5</i>	<i>Ndst3</i>	<i>Oplah</i>	<i>Peg10</i>	<i>Postn</i>
<i>Mgarp</i>	<i>Nebl</i>	<i>Optc</i>	<i>Peli3</i>	<i>Ppbp</i>
<i>Ppef2</i>	<i>Rab11a</i>	<i>Rnd3</i>	<i>Serpinf1</i>	<i>Slc46a3</i>
<i>Ppp1r14a</i>	<i>Rab11fip2</i>	<i>Rnf165</i>	<i>Serping1</i>	<i>Slc6a12</i>
<i>Ppp1r26</i>	<i>Rab20</i>	<i>Rnf207</i>	<i>Serpinh1</i>	<i>Slc6a17</i>
<i>Ppp1r3c</i>	<i>Rab32</i>	<i>Rnft2</i>	<i>Sesn3</i>	<i>Slc6a2</i>
<i>Ppp3ca</i>	<i>Rab39b</i>	<i>Ror1</i>	<i>Sfmbt2</i>	<i>Slc6a7</i>
<i>Ppt2</i>	<i>Rab3il1</i>	<i>Ror2</i>	<i>Sfrp1</i>	<i>Slc7a2</i>
<i>Prdm1</i>	<i>Rab6b</i>	<i>Rora</i>	<i>Sfrp2</i>	<i>Slc7a7</i>
<i>Prdx5</i>	<i>Ranbp3l</i>	<i>Rps6ka1</i>	<i>Sgca</i>	<i>Slc8a1</i>
<i>Prelp</i>	<i>Rap1gap</i>	<i>Rrbp1</i>	<i>Sgpp2</i>	<i>Slc9b2</i>
<i>Prex1</i>	<i>Rap1gap2</i>	<i>Rsbn1</i>	<i>Sh2d6</i>	<i>Slfn4</i>
<i>Prg4</i>	<i>Rapgef3</i>	<i>Rspo1</i>	<i>Sh3gl3</i>	<i>Slpi</i>
<i>Prickle1</i>	<i>Rarres2</i>	<i>Rspo2</i>	<i>Sh3rf2</i>	<i>Smad1</i>
<i>Prickle2</i>	<i>Rasgrf2</i>	<i>Rtn4rl1</i>	<i>Sh3rf3</i>	<i>Smad6</i>
<i>Prickle3</i>	<i>Rasgrp3</i>	<i>Rtn4rl2</i>	<i>Shank2</i>	<i>Smad7</i>
<i>Prkd1</i>	<i>Rasl11a</i>	<i>Rtp4</i>	<i>Shpk</i>	<i>Smad9</i>
<i>Prkg1</i>	<i>Rasl12</i>	<i>Runx2</i>	<i>Shroom1</i>	<i>Smarca2</i>
<i>Prokr1</i>	<i>Rassf2</i>	<i>Ryr3</i>	<i>Shroom3</i>	<i>Smoc1</i>
<i>Pros1</i>	<i>Rassf5</i>	<i>S100a8</i>	<i>Siglecg</i>	<i>Smoc2</i>

<i>Prr5l</i>	<i>Rassf6</i>	<i>Slpr1</i>	<i>Sipa1l2</i>	<i>Smpd3</i>
<i>Prr7</i>	<i>Rbm12b1</i>	<i>Saa3</i>	<i>Sirpa</i>	<i>Smpdl3a</i>
<i>Prr9</i>	<i>Rbm43</i>	<i>Samd9l</i>	<i>Six5</i>	<i>Smpdl3b</i>
<i>Prrgl</i>	<i>Rbp1</i>	<i>Sardh</i>	<i>Slamf8</i>	<i>Snai1</i>
<i>Psemb8</i>	<i>Rbpms</i>	<i>Sash1</i>	<i>Slc10a6</i>	<i>Snai2</i>
<i>Psemb9</i>	<i>Rcn3</i>	<i>Sat2</i>	<i>Slc11a1</i>	<i>Sncaip</i>
<i>Psrc1</i>	<i>Rdh12</i>	<i>Sbsn</i>	<i>Slc16a11</i>	<i>Sned1</i>
<i>Pstpip1</i>	<i>Reck</i>	<i>Scara3</i>	<i>Slc16a3</i>	<i>Snhg11</i>
<i>Ptch1</i>	<i>Relt</i>	<i>Scarf2</i>	<i>Slc18a1</i>	<i>Sntg2</i>
<i>Ptch2</i>	<i>Ret</i>	<i>Scd3</i>	<i>Slc1a3</i>	<i>Snx32</i>
<i>Ptchd4</i>	<i>Rffl</i>	<i>Scg2</i>	<i>Slc1a4</i>	<i>Snx7</i>
<i>Ptger1</i>	<i>Rftn2</i>	<i>Scn1b</i>	<i>Slc1a6</i>	<i>Socs2</i>
<i>Ptges</i>	<i>Rfx5</i>	<i>Scoc</i>	<i>Slc22a21</i>	<i>Sod3</i>
<i>Ptgfr</i>	<i>Rgag1</i>	<i>Sdc1</i>	<i>Slc22a4</i>	<i>Sox12</i>
<i>Ptgfrn</i>	<i>Rgag4</i>	<i>Sdc3</i>	<i>Slc24a3</i>	<i>Sox18</i>
<i>Ptgs2</i>	<i>Rgcc</i>	<i>Sec16b</i>	<i>Slc25a27</i>	<i>Sp100</i>
<i>Ptk7</i>	<i>Rgl1</i>	<i>Selenom</i>	<i>Slc25a34</i>	<i>Spag1</i>
<i>Ptn</i>	<i>Rgs11</i>	<i>Selenop</i>	<i>Slc2a10</i>	<i>Sparc</i>
<i>Ptprf</i>	<i>Rgs16</i>	<i>Sema3f</i>	<i>Slc2a13</i>	<i>Sparcl1</i>
<i>Ptprn</i>	<i>Rgs4</i>	<i>Sema3g</i>	<i>Slc36a1</i>	<i>Spata13</i>
<i>Ptpru</i>	<i>Rhbdl3</i>	<i>Sema4a</i>	<i>Slc36a2</i>	<i>Spats2l</i>
<i>Ptx3</i>	<i>Rhof</i>	<i>Sema6d</i>	<i>Slc38a10</i>	<i>Sphk1</i>
<i>Pxylp1</i>	<i>Rhoj</i>	<i>Serpina12</i>	<i>Slc39a13</i>	<i>Spon2</i>
<i>Pycr1</i>	<i>Ripk2</i>	<i>Serpina3g</i>	<i>Slc43a1</i>	<i>Sprrr2h</i>
<i>Pygo1</i>	<i>Rnase10</i>	<i>Serpina1b</i>	<i>Slc43a2</i>	<i>Sqrdl</i>
<i>Pyroxd2</i>	<i>Rnase2a</i>	<i>Serpine1</i>	<i>Slc44a3</i>	<i>Srd5a2</i>
<i>Qsox1</i>	<i>Rnase4</i>	<i>Serpine2</i>	<i>Slc44a5</i>	<i>Srfbp1</i>
<i>Srgn</i>	<i>Thsd1</i>	<i>Tril</i>	<i>Ypel3</i>	<i>Zfp503</i>
<i>Srpx2</i>	<i>Thsd7a</i>	<i>Trim12c</i>	<i>Ypel4</i>	<i>Zfp532</i>
<i>Ssc5d</i>	<i>Tiam1</i>	<i>Trim62</i>	<i>Zc2hc1a</i>	<i>Zfp608</i>
<i>St5</i>	<i>Tiam2</i>	<i>Trim7</i>	<i>Zc3h12a</i>	<i>Zfp810</i>
<i>St8sia4</i>	<i>Tifa</i>	<i>Trps1</i>	<i>Zcwpw1</i>	<i>Zfp950</i>
<i>Stard5</i>	<i>Timp3</i>	<i>Trpv4</i>	<i>Zfp105</i>	<i>Zfr2</i>
<i>Stard8</i>	<i>Tle2</i>	<i>Try5</i>	<i>Zfp112</i>	<i>Zic4</i>
<i>Stc1</i>	<i>Tle6</i>	<i>Tspan2</i>	<i>Zfp287</i>	<i>Zkscan7</i>
<i>Steap1</i>	<i>Tln2</i>	<i>Ttc28</i>	<i>Zfp330</i>	<i>Zswim4</i>
<i>Steap2</i>	<i>Tlr2</i>	<i>Tusc5</i>	<i>Zfp423</i>	<i>Zswim6</i>
<i>Susd1</i>	<i>Tlr6</i>	<i>Typ23a</i>	<i>Zfp467</i>	<i>Xkrx</i>
<i>Susd2</i>	<i>Tlr7</i>	<i>Txnip</i>	<i>Zfp474</i>	<i>Ypel2</i>
<i>Svep1</i>	<i>Tmc4</i>	<i>U90926</i>		

<i>Svil</i>	<i>Tmed3</i>	<i>Uaca</i>
<i>Syn3</i>	<i>Tmeff1</i>	<i>Uba7</i>
<i>Syngap1</i>	<i>Tmem106b</i>	<i>Ugp2</i>
<i>Synm</i>	<i>Tmem119</i>	<i>Ugt1a6a</i>
<i>Synpo</i>	<i>Tmem154</i>	<i>Unc13a</i>
<i>Synpo2</i>	<i>Tmem169</i>	<i>Unc5b</i>
<i>Tacstd2</i>	<i>Tmem176a</i>	<i>Unc5c</i>
<i>Tagln</i>	<i>Tmem176b</i>	<i>Ush1g</i>
<i>Tap1</i>	<i>Tmem185a</i>	<i>Vcan</i>
<i>Tap2</i>	<i>Tmem2</i>	<i>Vdr</i>
<i>Tbcd12</i>	<i>Tmem268</i>	<i>Vegfa</i>
<i>Tbcd12b</i>	<i>Tmem45a</i>	<i>Vegfd</i>
<i>Tbx1</i>	<i>Tmem53</i>	<i>Vgll3</i>
<i>Tbx18</i>	<i>Tmem56</i>	<i>Vipr2</i>
<i>Tbx3</i>	<i>Tmem86a</i>	<i>Vit</i>
<i>Tcaf2</i>	<i>Tmprss6</i>	<i>Vkorc1</i>
<i>Tcam1</i>	<i>Tnfaip6</i>	<i>Vldlr</i>
<i>Tcea2</i>	<i>Tnfaip8</i>	<i>Vsir</i>
<i>Tcp1l12</i>	<i>Tnfrsf14</i>	<i>Vwa1</i>
<i>Tcta</i>	<i>Tnfrsf1b</i>	<i>Vwf</i>
<i>Tead3</i>	<i>Tnfrsf21</i>	<i>Wdr27</i>
<i>Tet2</i>	<i>Tnfrsf25</i>	<i>Wdr66</i>
<i>Tgfb2</i>	<i>Tnfsf12</i>	<i>Wdr93</i>
<i>Tgfb3</i>	<i>Tnrc18</i>	<i>Wfdc21</i>
<i>Tgfb1</i>	<i>Tns4</i>	<i>Whrn</i>
<i>Tgfbr3</i>	<i>Tnxb</i>	<i>Wisp2</i>
<i>Tgfbr3l</i>	<i>Tpm2</i>	<i>Wnk4</i>
<i>Tgm5</i>	<i>Tpsab1</i>	<i>Wnt5b</i>
<i>Thbd</i>	<i>Trabd2b</i>	<i>Xdh</i>
<i>Thbs1</i>	<i>Traf3ip2</i>	<i>Xkr5</i>
<i>Thbs2</i>	<i>Trhde</i>	
<i>Thra</i>	<i>Trib2</i>	

Supplemental Table 8. Significantly downregulated genes in C/EBP β -overexpressing C2C12 myoblasts as compared to control with Log2 ≥ 0.531 (Protein coding genes).

Downregulated in C/EBP β -overexpressing C2C12s as compared to controls with Log2 ≤ -0.531				
<i>1190002N15Rik</i>	<i>Akirin1</i>	<i>Asah2</i>	<i>Carhsp1</i>	<i>Chrng</i>
<i>1700030J22Rik</i>	<i>Aknad1</i>	<i>Aspa</i>	<i>Cask</i>	<i>Cilp2</i>
<i>1700037H04Rik</i>	<i>Alcam</i>	<i>Atf3</i>	<i>Casq2</i>	<i>Ckb</i>
<i>1700088E04Rik</i>	<i>Aldh3b1</i>	<i>Atg9b</i>	<i>Cbfa2t3</i>	<i>Ckm</i>
<i>1810011O10Rik</i>	<i>Alox12</i>	<i>Atmin</i>	<i>Ccbe1</i>	<i>Clca3b</i>
<i>2010111I01Rik</i>	<i>Alox5</i>	<i>Atp1a2</i>	<i>Ccdc141</i>	<i>Clca4b</i>
<i>2210011C24Rik</i>	<i>Alpk2</i>	<i>Atp1a3</i>	<i>Ccdc162</i>	<i>Clcn3</i>
<i>4930523C07Rik</i>	<i>Alpk3</i>	<i>Atp2a1</i>	<i>Ccdc181</i>	<i>Clcn5</i>
<i>6430548M08Rik</i>	<i>Als2cl</i>	<i>Atp2b1</i>	<i>Ccdc3</i>	<i>Cldn15</i>
<i>8430408G22Rik</i>	<i>Amigo2</i>	<i>Atp6v0e2</i>	<i>Ccdc73</i>	<i>Cldn9</i>
<i>A930004D18Rik</i>	<i>Angptl4</i>	<i>Atp9a</i>	<i>Ccnd1</i>	<i>Clic5</i>
<i>Abcg2</i>	<i>Ank1</i>	<i>AU018091</i>	<i>Ccnd2</i>	<i>Clip4</i>
<i>Abl2</i>	<i>Ank2</i>	<i>AU021092</i>	<i>Cd200r2</i>	<i>Cmb1</i>
<i>Ablim3</i>	<i>Ankrd29</i>	<i>AU040320</i>	<i>Cd200r3</i>	<i>Cmklr1</i>
<i>Abra</i>	<i>Ankrd33b</i>	<i>Avil</i>	<i>Cd28</i>	<i>Cngb1</i>
<i>Acad10</i>	<i>Ankrd35</i>	<i>B3gnt5</i>	<i>Cd300lb</i>	<i>Cnih4</i>
<i>Acadl</i>	<i>Ankrd6</i>	<i>B4galnt3</i>	<i>Cd34</i>	<i>Cntnap4</i>
<i>Ackr3</i>	<i>Ankrd9</i>	<i>B4galt4</i>	<i>Cd36</i>	<i>Col18a1</i>
<i>Acsbg1</i>	<i>Ano5</i>	<i>Bace2</i>	<i>Cd44</i>	<i>Col23a1</i>
<i>Acs16</i>	<i>Anpep</i>	<i>Bach1</i>	<i>Cd53</i>	<i>Col4a5</i>
<i>Acta1</i>	<i>Antxr2</i>	<i>Baiap211</i>	<i>Cd81</i>	<i>Cox8b</i>
<i>Actc1</i>	<i>Anxa1</i>	<i>Bbc3</i>	<i>Cdc42ep2</i>	<i>Cpn1</i>
<i>Actn2</i>	<i>Apobec2</i>	<i>BC022687</i>	<i>Cdh1</i>	<i>Cr2</i>
<i>Actr3b</i>	<i>Apod</i>	<i>BC052040</i>	<i>Cdh13</i>	<i>Creb5</i>
<i>Acvr2a</i>	<i>Apol9b</i>	<i>Bicd1</i>	<i>Cdh15</i>	<i>Cryab</i>
<i>Adam8</i>	<i>Aqp1</i>	<i>Bik</i>	<i>Cdh18</i>	<i>Csn3</i>
<i>Adamts14</i>	<i>Ar</i>	<i>Blnk</i>	<i>Cdk6</i>	<i>Csrp3</i>
<i>Adamts15</i>	<i>Arc</i>	<i>Btbd11</i>	<i>Cdkn1a</i>	<i>Cst6</i>
<i>Add2</i>	<i>Areg</i>	<i>Btbd17</i>	<i>Celf2</i>	<i>Ctnnal1</i>
<i>Add3</i>	<i>Arhgap18</i>	<i>Btc</i>	<i>Cenpv</i>	<i>Ctrb1</i>
<i>Adgrg6</i>	<i>Arhgap19</i>	<i>Bves</i>	<i>Chac1</i>	<i>Cubn</i>
<i>Adgrl3</i>	<i>Arhgap22</i>	<i>C3ar1</i>	<i>Chchd10</i>	<i>Cxcl13</i>
<i>Adora1</i>	<i>Arhgap27</i>	<i>Cachd1</i>	<i>Chchd6</i>	<i>Cystm1</i>
<i>Adora2b</i>	<i>Arhgap32</i>	<i>Cacna1s</i>	<i>Chdh</i>	<i>D330045A20Rik</i>
<i>Aes</i>	<i>Arhgap44</i>	<i>Cacng1</i>	<i>Chil5</i>	<i>Daam1</i>
<i>Agap1</i>	<i>Arhgap45</i>	<i>Cadm1</i>	<i>Chka</i>	<i>Dact2</i>

<i>Agmat</i>	<i>Arhgap6</i>	<i>Cadm4</i>	<i>Chml</i>	<i>Dbnadd1</i>
<i>AI464131</i>	<i>Arl14epl</i>	<i>Calcb</i>	<i>Chmp4c</i>	<i>Ddc</i>
<i>AI467606</i>	<i>Arl4c</i>	<i>Calcr1</i>	<i>Chn1</i>	<i>Dennd2a</i>
<i>Aif1l</i>	<i>Arpp21</i>	<i>Camk2a</i>	<i>Chn2</i>	<i>Des</i>
<i>Aigl</i>	<i>Art1</i>	<i>Cap2</i>	<i>Chrna1</i>	<i>Dhrs7c</i>
<i>Ak5</i>	<i>Art3</i>	<i>Car2</i>	<i>Chrnbl</i>	<i>Dhx34</i>
<i>Akap6</i>	<i>Arx</i>	<i>Car8</i>	<i>Chrnd</i>	<i>Diras1</i>
<i>Dkk2</i>	<i>Epb41</i>	<i>Foxo6</i>	<i>Gsta4</i>	<i>Irf6</i>
<i>Dlgap1</i>	<i>Epb41l4b</i>	<i>Frmd4b</i>	<i>Hap1</i>	<i>Irs1</i>
<i>Dll1</i>	<i>Epb41l5</i>	<i>Frmpd1</i>	<i>Has1</i>	<i>Itga10</i>
<i>Dlx3</i>	<i>Epha4</i>	<i>Fry</i>	<i>Havcr2</i>	<i>Itga3</i>
<i>Dmbx1</i>	<i>Eps8</i>	<i>Gadd45a</i>	<i>Hdac9</i>	<i>Itga4</i>
<i>Dmp1</i>	<i>Eps8l2</i>	<i>Galnt6</i>	<i>Hdc</i>	<i>Itga6</i>
<i>Dnm3</i>	<i>Erb3</i>	<i>Galnt7</i>	<i>Hebp1</i>	<i>Itga7</i>
<i>Dock8</i>	<i>Ero1lb</i>	<i>Gap43</i>	<i>Hes1</i>	<i>Itgb7</i>
<i>Dock9</i>	<i>Etfbkmt</i>	<i>Gas2</i>	<i>Hes6</i>	<i>Itgb8</i>
<i>Dok7</i>	<i>Ethel</i>	<i>Gata4</i>	<i>Hey1</i>	<i>Itgbl1</i>
<i>Dtna</i>	<i>Etv4</i>	<i>Gatm</i>	<i>Heyl</i>	<i>Jade3</i>
<i>Duox1</i>	<i>Exoc6</i>	<i>Gch1</i>	<i>Hfe2</i>	<i>Jag2</i>
<i>Duoxa1</i>	<i>Ezr</i>	<i>Gchfr</i>	<i>Hhip</i>	<i>Jam2</i>
<i>Dusp10</i>	<i>F2</i>	<i>Gcnt3</i>	<i>Hmga1</i>	<i>Jsrp1</i>
<i>Dusp13</i>	<i>F2rl1</i>	<i>Gcsh</i>	<i>Hmga1-rs1</i>	<i>Jund</i>
<i>Dusp14</i>	<i>Fam107a</i>	<i>Gdf15</i>	<i>Hmga2</i>	<i>Jup</i>
<i>Dusp18</i>	<i>Fam167a</i>	<i>Gdpd1</i>	<i>Hmgn3</i>	<i>Kank3</i>
<i>Dusp22</i>	<i>Fam171b</i>	<i>Gdpd5</i>	<i>Hn1</i>	<i>Katnbl1</i>
<i>Dusp27</i>	<i>Fam180a</i>	<i>Gfod1</i>	<i>Hpcal1</i>	<i>Kcnf1</i>
<i>Dusp3</i>	<i>Fam189a2</i>	<i>Gfra1</i>	<i>Hpd1</i>	<i>Kcnj12</i>
<i>Dusp4</i>	<i>Fam189b</i>	<i>Gjc1</i>	<i>Hrc</i>	<i>Kcnk5</i>
<i>Dusp5</i>	<i>Fam198b</i>	<i>Glpr1</i>	<i>Hs2st1</i>	<i>Kcnmb4</i>
<i>Dusp6</i>	<i>Fam213a</i>	<i>Glrp1</i>	<i>Hsdl1</i>	<i>Kcnn4</i>
<i>Dynap</i>	<i>Fam65b</i>	<i>Gm13889</i>	<i>Hspa12b</i>	<i>Khdrbs3</i>
<i>Dync1i1</i>	<i>Fam71f1</i>	<i>Gm14137</i>	<i>Hspb1</i>	<i>Kif24</i>
<i>Dyrk2</i>	<i>Fam81a</i>	<i>Gm42517</i>	<i>Htatip2</i>	<i>Kif5c</i>
<i>Dyrk3</i>	<i>Fam83b</i>	<i>Gm7325</i>	<i>Ifitm1</i>	<i>Klf12</i>
<i>E2f2</i>	<i>Fbxl22</i>	<i>Gnail</i>	<i>Igdcc4</i>	<i>Klf2</i>
<i>E330009J07Rik</i>	<i>Fbxl7</i>	<i>Gnaz</i>	<i>Igf2bp2</i>	<i>Klf4</i>
<i>Eaf2</i>	<i>Fbxo40</i>	<i>Got1</i>	<i>Igf2bp3</i>	<i>Klf5</i>
<i>Eda2r</i>	<i>Fcna</i>	<i>Gpd2</i>	<i>Igfbp2</i>	<i>Klhdc8a</i>
<i>Edil3</i>	<i>Fer1l6</i>	<i>Gpnmb</i>	<i>Igfbp3</i>	<i>Klhl30</i>
<i>Efhdl</i>	<i>Fgd4</i>	<i>Gpr137b</i>	<i>Igfbp5</i>	<i>Klhl31</i>

<i>Efh2</i>	<i>Fgf11</i>	<i>Gpr179</i>	<i>Igsf11</i>	<i>Klhl40</i>
<i>Efna5</i>	<i>Fgf21</i>	<i>Gprc5a</i>	<i>Igsf5</i>	<i>Klhl41</i>
<i>Efnb2</i>	<i>Fgf5</i>	<i>Gprc5b</i>	<i>Igsf9</i>	<i>Klk10</i>
<i>Egln3</i>	<i>Fgfbp1</i>	<i>Gprc5c</i>	<i>Il17rb</i>	<i>Kras</i>
<i>Ehd4</i>	<i>Fgl2</i>	<i>Gprin1</i>	<i>Il17rd</i>	<i>Krt13</i>
<i>Eif4ebp1</i>	<i>Filip1</i>	<i>Grasp</i>	<i>Il2rg</i>	<i>Krt14</i>
<i>Eif4ebp2</i>	<i>Flrt2</i>	<i>Gria1</i>	<i>Il34</i>	<i>Krt19</i>
<i>Eif6</i>	<i>Flt1</i>	<i>Grip1</i>	<i>Ildr2</i>	<i>Krt80</i>
<i>Eml5</i>	<i>Fmn1</i>	<i>Grtp1</i>	<i>Inpp4b</i>	<i>L1cam</i>
<i>Emp3</i>	<i>Fn3k</i>	<i>Gsr</i>	<i>Intu</i>	<i>L3mbtl3</i>
<i>Endod1</i>	<i>Fndc5</i>	<i>Gsta1</i>	<i>Ip6k3</i>	<i>Lama3</i>
<i>Enpp5</i>	<i>Foxa2</i>	<i>Gsta2</i>	<i>Iqcd</i>	<i>Lamb3</i>
<i>Lamc2</i>	<i>Me2</i>	<i>Myog</i>	<i>Oas2</i>	<i>Pkp4</i>
<i>Lbh</i>	<i>Mecom</i>	<i>Myom2</i>	<i>Obscn</i>	<i>Plau</i>
<i>Lbx1</i>	<i>Mef2a</i>	<i>Myom3</i>	<i>Ociad2</i>	<i>Plcg2</i>
<i>Lef1</i>	<i>Mef2c</i>	<i>Myoz2</i>	<i>Ocln</i>	<i>Plcl1</i>
<i>Lgals3</i>	<i>Megf10</i>	<i>Mypn</i>	<i>Odc1</i>	<i>Plcl2</i>
<i>Lif</i>	<i>Meis2</i>	<i>N4bp3</i>	<i>Olfm1</i>	<i>Plekha7</i>
<i>Lingo2</i>	<i>Mertk</i>	<i>Naip1</i>	<i>Olfml2a</i>	<i>Plekhh1</i>
<i>Lipg</i>	<i>Mest</i>	<i>Nalcn</i>	<i>Opn3</i>	<i>Plekhf2</i>
<i>Lix1</i>	<i>Mfap3l</i>	<i>Nap1l2</i>	<i>Osbp2</i>	<i>Plekhh1</i>
<i>Lmln</i>	<i>Mfsd2a</i>	<i>Nbeal2</i>	<i>Otog</i>	<i>Plet1</i>
<i>Lmo7</i>	<i>Mfsd6</i>	<i>Ncdn</i>	<i>Otop1</i>	<i>Plk2</i>
<i>Lmod2</i>	<i>Mfsd9</i>	<i>Nceh1</i>	<i>P2ry2</i>	<i>Plp2</i>
<i>Lmod3</i>	<i>Mgam</i>	<i>Ncoa7</i>	<i>Pacrg</i>	<i>Plpp2</i>
<i>Lpar2</i>	<i>Mgat3</i>	<i>Ndr2</i>	<i>Pacsin2</i>	<i>Plppr3</i>
<i>Lrba</i>	<i>Mgp</i>	<i>Ndr2</i>	<i>Padi2</i>	<i>Plscr1</i>
<i>Lrrc1</i>	<i>Mgst3</i>	<i>Ndst4</i>	<i>Pag1</i>	<i>Pmaip1</i>
<i>Lrrc14b</i>	<i>Mkl1</i>	<i>Neb</i>	<i>Palm3</i>	<i>Pnma12</i>
<i>Lrrc20</i>	<i>Mmp10</i>	<i>Nefl</i>	<i>Panx1</i>	<i>Pnpla2</i>
<i>Lrrc4</i>	<i>Mpp6</i>	<i>Nefm</i>	<i>Paqr8</i>	<i>Poc1b</i>
<i>Lrrc8b</i>	<i>Mrln</i>	<i>Neol</i>	<i>Pard3b</i>	<i>Podxl</i>
<i>Lrrc8c</i>	<i>Mrpl38</i>	<i>Neu2</i>	<i>Pard6b</i>	<i>Polr3g</i>
<i>Lrrc8d</i>	<i>Mrps27</i>	<i>Neurl1a</i>	<i>Patj</i>	<i>Popdc3</i>
<i>Lrrn1</i>	<i>Msc</i>	<i>Ngef</i>	<i>Pax5</i>	<i>Pou4f1</i>
<i>Lvrn</i>	<i>Msi2</i>	<i>Ngfr</i>	<i>Pax8</i>	<i>Ppal</i>
<i>Lypd6</i>	<i>Msln</i>	<i>Nhs1</i>	<i>Pcbd1</i>	<i>Ppargc1a</i>
<i>Maats1</i>	<i>Msr2</i>	<i>Nid1</i>	<i>Pcdh19</i>	<i>Ppargc1b</i>
<i>Macc1</i>	<i>Msx2</i>	<i>Nkain1</i>	<i>Pcdh20</i>	<i>Ppef1</i>
<i>Mal</i>	<i>Mtcl1</i>	<i>Nmrk2</i>	<i>Pced1b</i>	<i>Ppfia4</i>

<i>Mamdc2</i>	<i>Mtss1</i>	<i>Nmt2</i>	<i>Pclo</i>	<i>Ppm1e</i>
<i>Mamstr</i>	<i>Mxra7</i>	<i>Noct</i>	<i>Pde5a</i>	<i>Ppm1l</i>
<i>Man1a</i>	<i>Mybpc1</i>	<i>Nog</i>	<i>Pdgfb</i>	<i>Ppp1r14b</i>
<i>Manba</i>	<i>Mybph</i>	<i>Nol4</i>	<i>Pdgfd</i>	<i>Ppp1r2</i>
<i>Map2</i>	<i>Mycl</i>	<i>Nov</i>	<i>Pdlim1</i>	<i>Ppp2r2b</i>
<i>Map3k7cl</i>	<i>Myh1</i>	<i>Npas2</i>	<i>Pdlim3</i>	<i>Prdm8</i>
<i>Map3k9</i>	<i>Myh2</i>	<i>Nppb</i>	<i>Perp</i>	<i>Prkag3</i>
<i>Mapk12</i>	<i>Myh3</i>	<i>Npr1</i>	<i>Pgm5</i>	<i>Prkcb</i>
<i>Mapk4</i>	<i>Myh7</i>	<i>Nptx1</i>	<i>Phex</i>	<i>Prkg2</i>
<i>Mapt</i>	<i>Myh8</i>	<i>Nqo1</i>	<i>Phgdh</i>	<i>Prl2c2</i>
<i>March9</i>	<i>Myl1</i>	<i>Nr3c2</i>	<i>Pik3r5</i>	<i>Prl2c3</i>
<i>March11</i>	<i>Myl6b</i>	<i>Nr4a1</i>	<i>Pip5k1b</i>	<i>Prl2c5</i>
<i>Mb</i>	<i>Mylk2</i>	<i>Nrarp</i>	<i>Pitpnm2</i>	<i>Prmt2</i>
<i>Mboat1</i>	<i>Mylk4</i>	<i>Nrcam</i>	<i>Pitx3</i>	<i>Prrg4</i>
<i>Mboat2</i>	<i>Mylpf</i>	<i>Ntn1</i>	<i>Pkig</i>	<i>Prrt4</i>
<i>Mctp1</i>	<i>Myo18b</i>	<i>Ntn4</i>	<i>Pknox2</i>	<i>Psat1</i>
<i>Mcu</i>	<i>Myo7b</i>	<i>Nudt12</i>	<i>Pkp1</i>	<i>Psmd8</i>
<i>Ptcd2</i>	<i>Rras2</i>	<i>Slc30a4</i>	<i>Stmn4</i>	<i>Tmem37</i>
<i>Ptprd</i>	<i>Rsad2</i>	<i>Slc35e4</i>	<i>Stmnd1</i>	<i>Tmem38a</i>
<i>Ptrh1</i>	<i>Rubcnl</i>	<i>Slc39a10</i>	<i>Stn1</i>	<i>Tmem8c</i>
<i>Pvr</i>	<i>Rundc3a</i>	<i>Slc40a1</i>	<i>Stom</i>	<i>Tmod1</i>
<i>Pxdc1</i>	<i>Runx1t1</i>	<i>Slc44a4</i>	<i>Ston2</i>	<i>Tmpo</i>
<i>Pxn</i>	<i>Ryr1</i>	<i>Slc4a11</i>	<i>Strbp</i>	<i>Tmprss11b</i>
<i>Pygm</i>	<i>Slpr3</i>	<i>Slc7a5</i>	<i>Strip2</i>	<i>Tmprss11f</i>
<i>Qpct</i>	<i>Samd12</i>	<i>Slc9a2</i>	<i>Suox</i>	<i>Tmsb4x</i>
<i>R3hdml</i>	<i>Sbk3</i>	<i>Slc9a3r2</i>	<i>Susd5</i>	<i>Tnfaip3</i>
<i>Rab27b</i>	<i>Scml4</i>	<i>Slco3a1</i>	<i>Sv2b</i>	<i>Tnik</i>
<i>Rab4a</i>	<i>Scn10a</i>	<i>Slco4a1</i>	<i>Sybu</i>	<i>Tnnc2</i>
<i>Rad23b</i>	<i>Scn4a</i>	<i>Smim5</i>	<i>Syn1</i>	<i>Tnni1</i>
<i>Ralb</i>	<i>Scn5a</i>	<i>Smpx</i>	<i>Syngri1</i>	<i>Tnni2</i>
<i>Ralgps2</i>	<i>Scn7a</i>	<i>Sms</i>	<i>Synpo2l</i>	<i>Tnnt1</i>
<i>Rasal2</i>	<i>Scrn1</i>	<i>Smyd1</i>	<i>Syt13</i>	<i>Tnnt2</i>
<i>Rasgef1a</i>	<i>Sdpr</i>	<i>Sncg</i>	<i>Syt1l</i>	<i>Tnnt3</i>
<i>Rasl11b</i>	<i>Sema3a</i>	<i>Sntb1</i>	<i>Tbcl1d8</i>	<i>Tom1l1</i>
<i>Rassf3</i>	<i>Sema3c</i>	<i>Sobp</i>	<i>Tbcl1d9</i>	<i>Tomm5</i>
<i>Rassf4</i>	<i>Sema3d</i>	<i>Soga3</i>	<i>Tcap</i>	<i>Tpcn1</i>
<i>Rassf7</i>	<i>Sema3e</i>	<i>Sorbs2</i>	<i>Tceal7</i>	<i>Tpd52</i>
<i>Rbm24</i>	<i>Sema4c</i>	<i>Sorcs2</i>	<i>Tcf7</i>	<i>Tppp3</i>
<i>Rbm38</i>	<i>Sema4f</i>	<i>Sort1</i>	<i>Tcte2</i>	<i>Traf1</i>
<i>Rbpms2</i>	<i>Sema5a</i>	<i>Sowahc</i>	<i>Tead2</i>	

<i>Rcan2</i>	<i>Sema6a</i>	<i>Sox11</i>	<i>Tead4</i>	<i>Trdn</i>
<i>Rec114</i>	<i>Serpinb6a</i>	<i>Sox13</i>	<i>Tec</i>	
<i>Reep1</i>	<i>Serpinb9b</i>	<i>Sox8</i>	<i>Timm8a1</i>	
<i>Rfx2</i>	<i>Sgcg</i>	<i>Sox9</i>	<i>Tinagl1</i>	
<i>Rgs17</i>	<i>Sgms2</i>	<i>Sp8</i>	<i>Tiparp</i>	
<i>Rhbdl2</i>	<i>Sgpl1</i>	<i>Spire2</i>	<i>Tjp2</i>	
<i>Rhob</i>	<i>Sh3bgr</i>	<i>Sprr1a</i>	<i>Tlx2</i>	
<i>Rhou</i>	<i>Sh3bp2</i>	<i>Spry1</i>	<i>Tm4sf1</i>	
<i>Rhpn2</i>	<i>Shb</i>	<i>Sptbn2</i>	<i>Tm4sf4</i>	
<i>Rin1</i>	<i>Shmt2</i>	<i>Src</i>	<i>Tm6sf1</i>	
<i>Rin3</i>	<i>Siah2</i>	<i>Srd5a1</i>	<i>Tmcc3</i>	
<i>Rnd2</i>	<i>Siglec15</i>	<i>Srgap1</i>	<i>Tmem144</i>	
<i>Rnf128</i>	<i>Slc13a1</i>	<i>Srl</i>	<i>Tmem158</i>	
<i>Rnf144b</i>	<i>Slc14a1</i>	<i>Srpk3</i>	<i>Tmem182</i>	
<i>Rnf183</i>	<i>Slc16a1</i>	<i>Ssfa2</i>	<i>Tmem184a</i>	
<i>Rnf217</i>	<i>Slc16a10</i>	<i>St6gal1</i>	<i>Tmem200a</i>	
<i>Rnh1</i>	<i>Slc16a13</i>	<i>Stac3</i>	<i>Tmem238</i>	
<i>Rorb</i>	<i>Slc22a23</i>	<i>Stap2</i>	<i>Trim8</i>	
<i>Rph3al</i>	<i>Slc25a13</i>	<i>Stard10</i>	<i>Trnp1</i>	
<i>Rpp25</i>	<i>Slc25a23</i>	<i>Stau2</i>	<i>Trove2</i>	
<i>Rps6ka2</i>	<i>Slc25a29</i>	<i>Stc2</i>	<i>Tsc22d1</i>	
<i>Rragd</i>	<i>Slc29a2</i>	<i>Stmn2</i>	<i>Tslp</i>	
<i>Ttc9</i>	<i>Zfp697</i>	<i>Wnt7b</i>	<i>Tspan13</i>	
<i>Ttll1</i>	<i>Zfpm1</i>	<i>Wrb</i>	<i>Tspan14</i>	
<i>Ttll11</i>	<i>Usp13</i>	<i>Wwc1</i>	<i>Tspan15</i>	
<i>Ttn</i>	<i>Usp15</i>	<i>Xirp1</i>	<i>Tspan32</i>	
<i>Tuba4a</i>	<i>Usp18</i>	<i>Xirp2</i>	<i>Tspan5</i>	
<i>Tubb3</i>	<i>Usp2</i>	<i>Xrcc5</i>	<i>Tspan7</i>	
<i>Tuft1</i>	<i>Usp43</i>	<i>Zc2hc1c</i>	<i>Tspan8</i>	
<i>Uap1l1</i>	<i>Usp6nl</i>	<i>Zdhhc15</i>	<i>Tspyl5</i>	
<i>Ubash3b</i>	<i>Vac14</i>	<i>Zdhhc2</i>	<i>Trib3</i>	
<i>Ube2d1</i>	<i>Vat1</i>	<i>Zfmx2</i>	<i>Trim13</i>	
<i>Uchl1</i>	<i>Vgll2</i>	<i>Zfp2</i>	<i>Trim16</i>	
<i>Ugcg</i>	<i>Vim</i>	<i>Zfp36l2</i>	<i>Trim36</i>	
<i>Unc13c</i>	<i>Vmn2r78</i>	<i>Zfp622</i>	<i>Trim54</i>	
<i>Unc45b</i>	<i>Vmn2r79</i>	<i>Tenm4</i>	<i>Trim55</i>	
<i>Upk1b</i>	<i>Vps37b</i>	<i>Tgfa</i>	<i>Trim63</i>	
<i>Upp1</i>	<i>Wdr36</i>	<i>Thpo</i>	<i>Trim72</i>	
<i>Uqcrfs1</i>	<i>Wfs1</i>	<i>Tigit</i>	<i>Traf4</i>	

Supplemental Table 9. Skeletal Muscle: Myogenesis and Myopathy RT-qPCR array for myotubes treated with or without conditioned media from LLC-shCtl or LLC-sh β . Fold regulation and Ct values of 84 tested genes in untreated C2C12 myotubes (D6) and myotubes treated with LLC-shCtl or LLC-sh β relative to D4 myotubes.

Position	Symbol	Fold Regulation			
		D4	D6 UNT	D6-LLC shctl	D6-LLC sh β
C10	<i>Igf2</i>	1	-2.59	-2.19	-2.1
C12	<i>Igfbp5</i>	1	-2.39	-1.46	-1.53
E08	<i>Myog</i>	1	-2.23	-1.48	-1.69
A06	<i>Agrn</i>	1	-1.86	-1.45	-1.38
G11	<i>Ttn</i>	1	-1.69	-2.41	-2.11
D07	<i>Mapk14</i>	1	-1.45	-1.22	-1.28
F11	<i>Prkag3</i>	1	-1.45	-1.53	-1.46
G03	<i>Slc2a4</i>	1	-1.38	-2.57	-1.64
B02	<i>Capn3</i>	1	-1.37	-1.63	-1.43
C02	<i>Dysf</i>	1	-1.35	-1.44	-1.55
E10	<i>Neb</i>	1	-1.35	-2.07	-1.66
B05	<i>Cav1</i>	1	-1.28	-1.32	-1.44
E12	<i>Nos2</i>	1	-1.28	-1.46	-1.03
A07	<i>Akt1</i>	1	-1.26	-1.19	-1.31
A11	<i>Bmp4</i>	1	-1.24	-1.3	-1.18
C01	<i>Dmpk</i>	1	-1.24	-1.19	-1.27
F02	<i>Pax7</i>	1	-1.23	1.23	-1.02
F05	<i>Ppargc1a</i>	1	-1.23	-1.74	-1.89
E04	<i>Myf6</i>	1	-1.2	-2.63	-2.14
A09	<i>Atp2a1</i>	1	-1.19	-1.59	-1.39
F04	<i>Pparg</i>	1	-1.19	-1.98	-1.59
C11	<i>Igfbp3</i>	1	-1.17	-2.29	-1.97
G12	<i>Utrn</i>	1	-1.15	-1.53	-1.47
E07	<i>Myod1</i>	1	-1.13	-1.38	-1.34
D10	<i>Mb</i>	1	-1.12	-2.98	-1.91
E11	<i>Nfkb1</i>	1	-1.12	-1.08	-1.12
A05	<i>Adrb2</i>	1	-1.11	-1.75	-1.58
D01	<i>Ikbkb</i>	1	-1.08	-1.01	-1.07
G07	<i>Tnni2</i>	1	-1.06	-1.64	-1.51
A02	<i>Actn3</i>	1	-1.02	1.04	-1.08
C05	<i>Foxo1</i>	1	-1.02	-1.92	-1.76

After the removal of genes with ct values >33: Lep, mmp9, Mstn, Pax3, adipog & Tnf

D08	<i>Mapk3</i>	1	-1.01	-1.11	1.01
G06	<i>Tnnc1</i>	1	-1.01	-1.36	-1.25
G08	<i>Tnnt1</i>	1	-1.01	-1.24	-1.17
B08	<i>Cs</i>	1	-1	-1.15	-1.01
D05	<i>Lmna</i>	1	1.01	-1.22	-1.27
E06	<i>Myh2</i>	1	1.03	-1.14	-1.04
A08	<i>Akt2</i>	1	1.04	1.05	-1.23
B10	<i>Dagl</i>	1	1.04	-1.26	-1.16
E03	<i>Myf5</i>	1	1.05	1.22	1.09
D02	<i>Il1b</i>	1	1.06	1.45	1.12
C07	<i>Hdac5</i>	1	1.07	-1.29	-1.17
B09	<i>Ctnnb1</i>	1	1.1	1.09	1.09
E02	<i>Musk</i>	1	1.1	-1.2	-1.14
F12	<i>Rhoa</i>	1	1.1	-1.04	1.01
G10	<i>Trim63</i>	1	1.11	-1.08	-1.01
F06	<i>Ppargc1b</i>	1	1.12	-1	-1.03
B11	<i>Des</i>	1	1.13	1.11	1.09
A03	<i>Acvr2b</i>	1	1.14	-1.6	-1.36
G09	<i>Tnnt3</i>	1	1.14	-1.48	-1.22
G04	<i>Tgfb1</i>	1	1.15	-1.48	-1.38
B06	<i>Cav3</i>	1	1.16	1.03	1.17
A01	<i>Acta1</i>	1	1.18	-1.08	1.09
A12	<i>Camk2g</i>	1	1.19	-1.32	1.03
F03	<i>Pdk4</i>	1	1.19	-1.3	-1.2
F10	<i>Prkag1</i>	1	1.19	1.01	1.1
A10	<i>Bcl2</i>	1	1.21	-1.6	-1.48
C04	<i>Fgf2</i>	1	1.21	-1.02	-1.1
C03	<i>Fbxo32</i>	1	1.22	1.72	1.6
G02	<i>Sgca</i>	1	1.23	-1.28	-1.06
C08	<i>Hk2</i>	1	1.24	-1.04	-1.01
E09	<i>Myot</i>	1	1.24	-1.63	-1.17
D06	<i>Mapk1</i>	1	1.25	1.07	1.11
B03	<i>Casp3</i>	1	1.29	1.17	1.09
C09	<i>Igfl</i>	1	1.31	-1.19	1
F07	<i>Ppp3ca</i>	1	1.35	1.03	1.26
B07	<i>Cryab</i>	1	1.39	1.1	1.15
F08	<i>Prkaa1</i>	1	1.41	1.08	1.11
C06	<i>Foxo3</i>	1	1.43	1.39	1.33
B01	<i>Capn2</i>	1	1.48	-1.2	-1.18
F09	<i>Prkab2</i>	1	1.49	-1.06	-1.01
D09	<i>Mapk8</i>	1	1.57	1.24	1.3

G01	<i>Rps6kb1</i>	1	1.61	1.23	1.21
B04	<i>Cast</i>	1	1.67	1.19	1.17
D11	<i>Mef2c</i>	1	1.77	-1.3	1.06
E05	<i>Myh1</i>	1	1.85	-1.4	-1.2
B12	<i>Dmd</i>	1	2.41	1.25	1.29
D03	<i>Il6</i>	1	4.54	2.78	2.66
A04	<i>Adipoq</i>				
D04	<i>Lep</i>				
D12	<i>Mmp9</i>				
E01	<i>Mstn</i>				
F01	<i>Pax3</i>				
G05	<i>Tnf</i>				

Position	Symbol	AVG Ct			
		D4	D6 UNT	D6-LLC shCtl	D6-LLC shß
A01	Acta1	17.58	17.03	17.38	17.08
A02	Actn3	21.76	21.49	21.39	21.50
A03	Acvr2b	28.56	28.07	28.93	28.63
A04	Adipoq	35.00	35.00	35.00	34.76
A05	Adrb2	26.43	26.28	26.93	26.72
A06	Aggrn	28.79	29.39	29.02	28.89
A07	Akt1	25.17	25.21	25.12	25.18
A08	Akt2	25.49	25.14	25.11	25.41
A09	Atp2a1	20.35	20.30	20.70	20.45
A10	Bcl2	27.81	27.23	28.17	28.00
A11	Bmp4	25.17	25.17	25.24	25.03
A12	Camk2g	24.15	23.59	24.24	23.73
B01	Capn2	24.24	23.38	24.19	24.11
B02	Capn3	25.19	25.34	25.59	25.33
B03	Casp3	27.20	26.53	26.66	26.70
B04	Cast	25.40	24.36	24.84	24.81
B05	Cav1	24.81	24.87	24.90	24.96
B06	Cav3	23.32	22.80	22.97	22.73
B07	Cryab	19.79	19.02	19.34	19.21
B08	Cs	24.80	24.50	24.70	24.44
B09	Ctnnb1	24.26	23.82	23.82	23.76
B10	Dag1	21.84	21.49	21.87	21.69

B11	Des	21.12	20.65	20.66	20.63
B12	Dmd	27.76	26.19	27.13	27.03
C01	Dmpk	24.72	24.72	24.66	24.69
C02	Dysf	26.85	26.99	27.07	27.11
C03	Fbxo32	26.55	25.95	25.45	25.49
C04	Fgf2	29.28	28.71	29.00	29.04
C05	Foxo1	25.49	25.22	26.12	25.93
C06	Foxo3	27.09	26.27	26.30	26.30
C07	Hdac5	25.29	24.89	25.34	25.15
C08	Hk2	26.71	26.11	26.46	26.35
C09	Igf1	26.60	25.91	26.54	26.22
C10	Igf2	24.57	25.64	25.39	25.27
C11	Igfbp3	26.55	26.48	27.44	27.16
C12	Igfbp5	21.22	22.17	21.45	21.46
D01	Ikbkb	27.93	27.74	27.64	27.65
D02	Il1b	31.60	31.22	30.75	31.06
D03	Il6	32.57	30.08	30.78	30.78
D04	Lep	34.49	33.84	34.32	33.52
D05	Lmna	22.74	22.42	22.72	22.71
D06	Mapk1	24.56	23.94	24.15	24.04
D07	Mapk14	27.09	27.32	27.06	27.07
D08	Mapk3	27.15	26.86	26.99	26.77
D09	Mapk8	26.30	25.35	25.68	25.55
D10	Mb	20.07	19.93	21.33	20.63
D11	Mef2c	23.02	21.89	23.08	22.56
D12	Mmp9	33.81	32.62	32.75	32.25
E01	Mstn	35.00	34.29	34.70	33.06
E02	Musk	24.22	23.78	24.17	24.03
E03	Myf5	28.48	28.10	27.88	27.99
E04	Myf6	28.07	28.04	29.16	28.80
E05	Myh1	21.72	20.53	21.90	21.61
E06	Myh2	21.71	21.37	21.59	21.39
E07	Myod1	23.19	23.06	23.35	23.25
E08	Myog	22.15	23.00	22.41	22.53
E09	Myot	26.67	26.07	27.07	26.53
E10	Neb	21.14	21.28	21.88	21.50
E11	Nfkb1	24.94	24.79	24.74	24.73
E12	Nos2	31.59	31.65	31.83	31.26
F01	Pax3	34.73	32.08	35.00	34.77
F02	Pax7	29.94	29.94	29.33	29.60
F03	Pdk4	28.19	27.64	28.25	28.08

F04	Pparg	27.75	27.70	28.42	28.04
F05	Ppargc1a	28.39	28.38	28.88	28.94
F06	Ppargc1b	27.77	27.30	27.47	27.45
F07	Ppp3ca	23.73	23.00	23.37	23.02
F08	Prkaa1	25.90	25.11	25.48	25.38
F09	Prkab2	27.04	26.17	26.82	26.69
F10	Prkag1	25.06	24.51	24.74	24.55
F11	Prkag3	24.11	24.34	24.41	24.28
F12	Rhoa	21.71	21.28	21.45	21.33
G01	Rps6kb1	25.82	24.84	25.21	25.17
G02	Sgca	22.55	21.95	22.60	22.27
G03	Slc2a4	30.64	30.80	31.69	30.98
G04	Tgfb1	25.36	24.86	25.61	25.46
G05	Tnf	34.94	31.01	35.00	34.82
G06	Tnnc1	18.10	17.81	18.24	18.05
G07	Tnni2	19.35	19.13	19.75	19.57
G08	Tnnt1	19.62	19.33	19.62	19.47
G09	Tnnt3	18.84	18.35	19.10	18.75
G10	Trim63	24.29	23.84	24.09	23.93
G11	Ttn	22.63	23.09	23.59	23.33
G12	Utrn	25.40	25.30	25.71	25.59
H01	Actb	20.59	20.59	20.11	19.77
H02	B2m	20.83	20.83	20.95	20.86
H03	Gapdh	20.26	20.04	20.10	20.61
H04	Gusb	26.52	25.98	26.13	26.01
H05	Hsp90ab1	20.17	19.42	19.54	19.26

27 human cancer types		314 genes (from "secreted"list) that are upregulated in our RNA-seq data of C2C12-β myoblasts (GSE152051) as compared to control and also, upregulated in at least 1 type of human cancer from the data that was analyzed by the Nielson's group (Robinson et al., 2019) with 2-fold cut-off																											
		Adrenal gland		Bladder	Brain		Breast	Cervix uteri	Colon		Esophagus	KICH	KIRC	Kidney		Liver		Lung		Ovary	Pancreas	Prostate	Salivary gland	Skin	Stomach	Testis	Thyroid	Uterus	# tumors per genes
genes from all cancer types & β-GM (no duplicates)		ACC	PCPG	BLCA	GBM	LGSG	BRCA	CESC	COAD	READ	ESCA	KICH	KIRC	KIRP	LIHC	LUAD	LUSC	OV	PANL	PRAD	HNSC	SKCM	STAD	TGCT	THCA	UCEC	UCS		
314 genes		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
upregulated		ACC	PCPG	BLCA	GBM	LGSG	BRCA	CESC	COAD	READ	ESCA	KICH	KIRC	KIRP	LIHC	LUAD	LUSC	OV	PANL	PRAD	HNSC	SKCM	STAD	TGCT	THCA	UCEC	UCS		
PTGES		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25	
CTHRC1		1		1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	23	
COL1A1		1	1		1	1		1	1	1	1	1	1		1	1	1	1	1		1		1	1	1	1	1	22	
NETO2		1		1			1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	22	
IGF2		1	1	1	1	1		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	
LGALS3BP				1	1	1		1	1	1	1		1	1	1	1	1	1	1	1		1	1	1	1	1	1	21	
NPR3		1			1	1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	
POSTN		1	1		1	1		1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	21	
TNFRSF21		1	1	1		1		1	1	1	1			1	1	1	1	1	1		1	1	1	1	1	1	1	21	
ADAM12		1	1	1	1		1		1	1	1		1		1	1	1	1	1		1	1	1	1	1	1	1	20	
APOC1				1	1		1	1	1	1	1		1	1	1		1	1	1	1	1		1	1	1	1	1	20	
EPHB2		1			1	1		1	1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	20	
MFAP2			1		1	1			1	1	1				1	1	1	1	1	1	1		1	1	1	1	1	20	
OLR1		1		1	1		1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20	
PSMB9				1	1	1		1	1	1	1		1	1	1	1	1	1	1		1	1	1	1	1	1	1	20	
COL11A1		1		1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	19	
F5			1			1		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	19	
NAALADL2			1		1	1		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	19	
PODXL2			1		1		1	1	1	1	1																		

	APLP1	1	1		1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	13
	BAMBI				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	13
	CXCL5		1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	13
	IL1RAP		1	1	1		1		1		1		1	1	1	1	1	1	1	1	1	1	13
	IL1RN		1	1		1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	13
	LYPD6B		1			1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	13
	SFRP2			1	1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	13
	XDH		1			1	1			1		1	1	1	1	1	1	1	1	1	1	1	13
	ACPP	1	1	1			1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	12
	AGAP2	1	1	1			1			1		1	1	1	1	1	1	1	1	1	1	1	12
	AGT			1	1	1		1	1	1		1		1	1	1	1	1	1	1	1	1	12
	BGN		1	1	1	1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	12
	CAV1	1	1		1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	12
	CDH11	1	1		1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	12
	COL5A2	1	1		1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	12
	FAT2			1			1		1				1	1	1	1	1	1	1	1	1	1	12
	ITIH2					1			1		1	1	1	1	1	1	1	1	1	1	1	1	12
	NGF2		1	1	1	1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	12
	RBP1	1			1		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	12
	SHROOM3		1			1	1			1		1	1	1	1	1	1	1	1	1	1	1	12
	THBS2			1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	12
	ASS1	1				1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	11
	GDA		1			1				1		1	1	1	1	1	1	1	1	1	1	1	11
	JAG1	1	1		1	1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	11
	LOX	1		1	1		1		1		1		1	1	1	1	1	1	1	1	1	1	11
	LY6D	1		1			1	1					1	1	1	1	1	1	1	1	1	1	11
	SPARC	1		1	1	1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	11
	TNFAIP6	1	1	1	1					1	1		1	1	1	1	1	1	1	1	1	1	11
	ADAM23		1					1			1	1	1	1	1	1	1	1	1	1	1	1	10
	ADAMTS16		1			1					1	1	1	1	1	1	1	1	1	1	1	1	10
	DPEP1		1			1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	10
	EMID1	1				1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	10
	EREG		1				1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	10
	FST		1	1	1	1		1		1		1	1	1	1	1	1	1	1	1	1	1	10
	LAMA4	1	1		1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	10
	LTBP2	1	1		1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	10
	MMP3		1			1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	10
	NRG1	1	1	1				1	1		1		1	1	1	1	1	1	1	1	1	1	10
	NTSE		1		1	1			1				1	1	1	1	1	1	1	1	1	1	10
	SMOC1	1	1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10
	ALDH1A3	1						1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
	ASPN	1	1	1	1	1		1		1	1	1	1	1	1	1	1	1	1	1	1	1	9
	CSF1R			1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
	ENPP3					1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	9
	FAM181B	1	1		1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	9
	INHBB	1	1						1	1		1	1	1	1	1	1	1	1	1	1	1	9
	S100A8		1	1		1					1	1	1	1	1	1	1	1	1	1	1	1	9
	WNT5B	1	1	1						1	1	1	1	1	1	1	1	1	1	1	1	1	9
	AK4	1	1			1					1	1	1	1	1	1	1	1	1	1	1	1	8
	APBB1P			1	1				1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	CACNA1D				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	CCL2		1	1	1		1				1	1	1	1	1	1	1	1	1	1	1	1	8
	CD93			1	1					1		1	1	1	1	1	1	1	1	1	1	1	8
	CLCA2	1		1		1	1		1			1	1	1	1	1	1	1	1	1	1	1	8
	COL12A1		1	1							1	1	1	1	1	1	1	1	1	1	1	1	8
	COL5A1			1		1					1	1	1	1	1	1	1	1	1	1	1	1	8
	EGFR			1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	F7				1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	8
	FBLN7	1		1					1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	FZD8	1				1					1	1	1	1	1	1	1	1	1	1	1	1	8
	ICAM5	1							1			1	1	1	1	1	1	1	1	1	1	1	8
	ITIH5	1	1						1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	MASP1	1			1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	8
	MYL9	1		1						1		1	1	1	1	1	1	1	1	1	1	1	8
	PI15							1				1	1	1	1	1	1	1	1	1	1	1	8
	PTN	1	1	1	1	1						1	1	1	1	1	1	1	1	1	1	1	8
	VWF	1	1		1					1	1	1	1	1	1	1	1	1	1	1	1	1	8
	WDR93		1							1		1	1	1	1	1	1	1	1	1	1	1	8
	ADAMTS15		1	1	1			1	1	1		1	1	1	1	1	1	1	1	1	1	1	7
	BMP4	1			1		1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	7
	CASP1			1			1	1		1		1	1	1	1	1	1	1	1	1	1	1	7
	CASP14		1			1	1		1	1		1	1	1	1	1	1	1	1	1	1	1	7
	CFAP46		1					1	1			1	1	1	1	1	1	1	1	1	1	1	7
	EMILIN2			1						1	1	1	1	1	1	1	1	1	1	1	1	1	7
	HP			1						1		1	1	1	1	1	1	1	1	1	1	1	7
	PDGFRL		1	1						1	1	1	1	1	1	1	1	1	1	1	1	1	7
	ROR1	1		1						1	1	1	1	1	1	1	1	1	1	1	1	1	7
	SBSN		1									1	1	1	1	1	1	1	1	1	1	1	7
	SEMA4A		1			1	1				1	1	1	1	1	1	1	1	1	1	1	1	7
	SMOC2	1								1	1	1	1	1	1	1	1	1	1	1	1	1	7
	SPATS2L									1		1	1	1	1	1	1	1	1	1	1	1	7
	SRPX2			1	1		1	1			1		1	1	1	1	1	1	1	1	1	1	7
	TIFA			1		1	1		1		1		1	1	1	1	1	1	1	1	1	1	7
	TRHDE	1							1	1	1	1	1	1	1	1	1	1	1	1	1	1	7

ADAMTS2			1			1				1	1	1	1	1	1	1	1	6
ALPL		1			1													6
C3			1	1			1	1							1	1		6
CACNA1A	1								1	1	1							6
CASP4		1	1		1	1									1		1	6
CLU			1	1				1	1		1							6
CNTN1	1								1	1	1	1	1			1		6
COLEC12						1	1	1	1	1		1	1			1		6
CPA6					1	1		1					1				1	6
FIBIN			1	1				1	1		1	1			1			6
FMOD			1	1				1			1	1	1		1			6
GDF6	1						1	1			1		1			1		6
GPM6B	1										1			1		1	1	6
IGFBP7		1	1	1							1	1		1	1			6
IL17RE	1	1				1			1		1				1		1	6
ISM1	1										1	1			1		1	6
KIF21B						1	1	1				1			1		1	6
MXK				1	1			1						1			1	6
MMP13		1			1	1				1	1			1				6
NNMT			1	1				1	1			1			1			6
PARP14					1		1				1		1		1			6
PCSK2	1									1		1		1		1		6
PLXDC2				1				1	1	1		1			1			6
SFRP1	1	1								1		1			1	1		6
SUSD2				1	1				1		1	1	1		1			6
THBD			1	1		1					1	1		1				6
THSD7A	1	1		1			1		1		1	1		1				6
TIAM1	1										1	1		1		1		6
ADAMTS5	1	1					1				1	1		1		1		5
ADM		1										1		1		1		5
AEBP1			1	1					1			1		1				5
COL11A2	1											1		1			1	5
COL24A1	1	1			1							1		1				5
CPM	1			1		1				1						1		5
CRLF1										1	1				1	1		5
DKK3	1								1	1				1				5
DOCK10							1	1				1		1		1		5
FABP7			1		1			1						1		1		5
FBLN1			1						1	1		1			1			5
FGFR2				1					1		1				1	1		5
HPGD	1										1	1	1		1			5
LMCD1		1							1	1		1	1					5
MFAP5			1						1		1	1			1			5
MGST1								1		1		1			1		1	5
MMP2			1	1					1		1	1		1		1		5
ORM1						1	1					1				1	1	5
PTPRU	1	1						1						1		1		5
PTX3			1	1							1				1			5
RGAG1	1	1			1		1	1		1					1			5
SELENOP							1	1		1				1		1		5
SRGN			1								1	1	1		1	1		5
TNFRSF18			1			1		1			1	1	1		1	1		5
UNCSC		1			1							1		1			1	5
ABAT	1	1			1						1	1						4
ACTA2			1					1				1						4
ADAMTS3	1		1				1	1	1									4
ANGPT4	1			1				1	1									4
ANGPTL7	1											1			1			4
BPIFB1		1			1	1									1			4
CACNA1G	1											1					1	4
CHST12		1							1						1			4
COL6A1			1								1		1		1			4
COL6A2			1								1	1		1	1			4
DDX58				1							1		1		1			4
EFEMP1			1						1			1	1		1			4
EHD3	1									1	1	1			1			4
ELN	1		1												1			4
EMILIN1			1	1								1			1			4
ENAH				1	1				1	1								4
FAM150A						1				1				1		1		4
FSTL1			1					1				1			1			4
GLRX						1					1	1			1		1	4
HEPH									1			1			1			4
HMCN2	1			1						1		1			1			4
ISLR			1						1			1			1			4
ITGA1			1						1			1			1			4
KCNAB2	1											1		1		1		4
LGALS1		1						1						1		1		4
LPL			1	1						1				1				4
MARK1	1								1		1			1				4
MMP19			1	1								1			1			4
OLFML3			1	1								1			1			4
ORM2					1	1				1			1			1		4
PLEKHA6	1				1					1		1						4
PRG4			1					1				1	1				1	4
RAB32			1								1	1		1				4
RCN3		1	1									1						4
SPARCL1							1	1		1	1							4
VLDLR	1						1	1						1				4
WISP2	1						1	1	1									4

ADAMTS9			1				1								1		3
ARHGEF6			1	1											1		3
BCL3			1		1												3
BICC1							1	1	1		1						3
BRINP3				1								1				1	3
CIQTNF1			1	1							1						3
CCL7										1	1		1				3
CFAP100											1					1	3
CILP	1		1								1						3
CNRIP1		1									1				1		3
COL25A1										1				1		1	3
CROCC2	1										1					1	3
CSF1			1	1											1		3
CTGF			1	1								1					3
ECM2			1	1								1					3
ENPP1			1		1										1		3
FABP4						1			1					1			3
FAM161A		1								1				1			3
FAM20A			1									1				1	3
FAS			1									1		1			3
HGF			1									1			1		3
IGFBP4			1									1			1		3
NID2			1										1		1		3
PI16			1	1								1					3
PLA2G5			1				1					1					3
RARRES2			1					1							1		3
RSPO1		1									1					1	3
RTN4RL1						1			1						1		3
SEMA3G		1								1					1		3
THBS1			1								1				1		3
ADAMTS14											1				1		2
ADGRL2															1		2
C1RL			1												1		2
CAMP				1			1								1		2
CKK		1															2
CFH											1					1	2
CTSG								1									2
CYB5A											1		1			1	2
FAT4								1							1		2
FBLN5			1												1		2
FGF7							1								1		2
GAS6		1					1										2
GFPT2								1				1					2
GSTK1			1								1						2
KNR1									1						1		2
MEGF6								1				1					2
PALLD			1									1					2
PLB1							1					1					2
SERPINA12				1					1								2
SERPING1			1												1		2
SMPDL3A							1								1		2
SP100			1												1		2
SQRDL			1												1		2
STARD8		1													1		2
SVEP1							1								1		2
TAGLN			1								1						2
TGMS										1						1	2
TPSAB1		1	1														2

Supplemental Table 11. List of 173 genes coding for secreted proteins that are downregulated in C/EBP β -overexpressing myoblasts and in human cancers. List of 173 downregulated genes in C/EBP β -overexpressing myoblasts (with ≥ 2 -fold change and p.adj ≤ 0.05) and in at least 1 type of human cancer from (Robinson et al. 2019).

27 cancer types		173 genes (from "secreted" list) that are downregulated in our RNA-seq data of C2C12-β myoblasts (GSE152051) as compared to control and also, downregulated in at least 1 type of human cancer from the data that was analyzed by the Nielson's group (Robinson et al., 2019) with 2-fold cut-off																												
genes from all cancer types & β-GM (no duplicates)		Adrenal gland		Bladder		Brain		Breast	Cervix uteri	Colon	Esophagus	Kidney			Liver		Lung		Ovary	pancreas	prostate	Salivary gland			skin	stomach	testis	thyroid	uterus	# tumors per genes
173 genes		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27		
downregulated		ACC	PCPG	BLCA	GBM	LGG	BRCA	CESC	COAD	READ	ESCA	KICH	KIRC	KIRP	CHOL	LIHC	LUAD	LUSC	OV	PAAD	PRAD	HNSC	SKCM	STAD	TGCT	THCA	UCEC	UCS		
	EML5	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	24	
	ACTA1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22	
	ASPA	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22	
	TTN	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	
	CKM	1	1		1	1			1	1	1	1			1		1	1	1	1	1	1	1	1	1	1	1	1	19	
	APOD	1		1			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	
	ACTC1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1		1	1	1	17	
	FAM180A	1	1	1			1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1		1	1	1	17	
	FNDC5	1			1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	16	
	PGM5		1	1			1	1	1	1	1				1		1	1	1	1	1	1	1		1	1	1	1	16	
	TMOD1	1		1			1	1	1	1	1			1	1	1	1	1	1	1	1	1	1	1		1	1	1	16	
	EFHD1			1	1			1	1	1	1		1	1	1				1	1	1	1	1				1	1	15	
	FRY			1	1			1	1	1	1		1		1		1	1	1	1	1	1	1			1	1	1	15	
	NR3C2			1	1		1	1	1	1	1			1	1		1	1	1	1	1	1	1		1		1	1	15	
	CAMK2A			1	1	1		1	1	1	1		1				1	1	1	1	1	1		1		1	1	1	14	
	ITGA7			1			1	1	1	1	1						1	1	1	1	1	1		1		1	1	1	14	
	SYNGR1				1	1		1	1	1	1						1	1	1	1	1	1		1		1	1	1	14	
	ACTN2				1			1	1	1	1	1					1	1	1	1	1	1		1		1	1	1	13	
	DLL1						1	1	1	1	1			1		1	1	1	1	1	1		1		1	1	1		13	
	HHIP		1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1		1		1	1	1		13	
	MB	1			1	1				1	1		1			1	1	1	1	1		1		1		1	1		13	
	OLFM1			1	1	1	1		1	1	1	1							1	1	1	1		1		1	1	1	13	
	TSLP						1		1	1	1	1		1		1	1	1	1	1	1		1		1	1	1	1	13	
	GATM		1						1	1	1	1	1	1	1				1	1	1	1	1		1				12	
	KRT13	1	1		1	1			1	1	1	1			1				1	1	1	1	1		1		1		12	
	MYH7	1							1	1	1				1		1	1	1	1	1	1	1	1	1	1	1		12	
	NBEAL2	1	1			1			1	1	1		1	1		1	1	1	1	1	1	1	1	1	1	1	1		12	
	NDRG4				1		1		1	1	1	1			1		1	1	1	1	1	1	1	1	1	1	1	1	12	
	TSPAN7			1	1		1	1	1	1	1	1		1			1	1	1	1	1	1	1	1	1	1	1	1	12	
	AKAP6			1	1			1	1	1	1						1		1	1	1	1		1			1	1	11	
	IGFBP5	1		1				1	1	1	1			1					1	1	1	1		1			1	1	11	
	MERTK		1	1			1	1	1	1	1				1				1	1	1	1		1			1	1	11	
	MYH1				1	1	1			1	1						1					1	1		1			1	11	
	PDGFD	1	1	1			1	1	1	1	1				1				1	1	1	1	1	1	1			1	11	
	SCN4A					1	1		1	1	1				1				1	1	1	1	1	1	1			1	11	
	TSPAN8		1		1	1					1	1	1	1					1	1	1	1	1	1			1	1	11	
	CCDC3				1	1	1	1				1	1	1			1		1	1	1	1	1			1	1		10	
	CHN2		1		1	1					1	1			1		1		1	1	1	1				1	1		10	
	L1CAM	1			1	1			1	1	1	1		1		1		1	1		1	1			1	1	1		10	
	PDLIM3			1				1	1	1	1	1		1			1	1	1		1	1			1	1	1	1	10	
	SNCG		1		1	1	1	1	1	1	1	1									1	1	1	1		1	1	1	10	
	NGFR	1		1			1		1	1	1			1		1					1	1					1	1	9	
	MAPT			1				1	1	1	1	1									1	1					1	1	9	
	MYH2				1	1			1	1	1	1			1		1				1	1	1	1	1	1		1	9	
	MYLPF				1	1	1				1	1					1			1	1	1	1	1	1			1	9	
	NEB	1	1		1		1					1	1				1				1	1	1	1				1	9	
	NEFM				1	1		1	1	1	1	1									1	1			1			1	9	
	PDE5A			1				1	1	1	1	1					1	1	1		1	1					1	1	9	
	PLCL2				1		1		1	1	1	1					1	1	1		1	1					1	1	9	
	SEMA6A		1				1	1	1	1	1	1					1	1	1	1	1	1			1			1	9	
	AMIGO2							1	1	1	1								1	1	1	1	1				1	1	8	
	CAP2			1	1	1		1	1	1	1	1								1	1	1	1				1	1	8	
	EFNA5				1	1			1	1	1	1										1	1				1		8	
	EPS8			1			1	1												1	1	1					1	1	8	
	GSTA1		1									1		1	1				1	1	1			1	1			1	8	
	ITGBL1	1				1		1							1			1		1	1	1	1		1	1	1	1	8	
	MYO7B	1					1						1	1				1	1		1	1				1	1	1	8	
	PTPRD			1	1			1			1						1	1	1	1		1					1	1	8	
	STMN2	1			1	1			1	1	1	1						1	1	1				1				1	8	
	TMEM200A	1	1				1					1							1				1				1	1	8	

[illegible]

Supplemental Table 12. GO analysis for the 314 upregulated genes from Supplemental Table 10. Genes are upregulated in C/EBP β -overexpressing myoblasts (with ≥ 2 -fold change and $p_{\text{adj}} \leq 0.05$) and in at least 1 type of human cancer from (Robinson et al. 2019).

Term	Count	%	PValue	Bonferroni	Benjamini	FDR
Secreted	160	51	1.78E-95	4.46E-93	4.46E-93	2.32E-92
signal peptide	213	68	6.96E-95	8.18E-92	8.18E-92	1.12E-91
GO:0005576~extracellular region	168	54	3.25E-94	7.64E-92	7.64E-92	4.18E-91
Signal	227	73	4.94E-90	1.23E-87	6.17E-88	6.41E-87
Glycoprotein	207	66	8.30E-85	2.07E-82	6.91E-83	1.08E-81
Disulfide bond	188	60	1.40E-81	3.49E-79	8.73E-80	1.81E-78
glycosylation site:N-linked (GlcNAc...)	195	62	3.18E-66	3.73E-63	1.87E-63	5.12E-63
disulfide bond	159	51	2.45E-58	2.87E-55	9.58E-56	3.94E-55
GO:0005578~proteinaceous extracellular matrix	69	22	3.09E-58	7.27E-56	3.63E-56	3.98E-55
GO:0005615~extracellular space	122	39	7.13E-56	1.67E-53	5.58E-54	9.17E-53
Extracellular matrix	58	19	1.00E-54	2.50E-52	5.01E-53	1.30E-51
GO:0031012~extracellular matrix	55	18	3.63E-42	8.52E-40	2.13E-40	4.66E-39
GO:0008201~heparin binding	35	11	9.24E-30	4.01E-27	4.01E-27	1.30E-26
Calcium	55	18	7.70E-22	1.93E-19	3.21E-20	1.00E-18
IPR009030:Insulin-like growth factor binding protein, N-terminal	26	8.3	4.85E-21	2.98E-18	2.98E-18	7.18E-18
IPR000884:Thrombospondin, type 1 repeat	20	6.4	7.36E-20	4.52E-17	2.26E-17	1.09E-16
Cell adhesion	40	13	7.68E-20	1.92E-17	2.74E-18	9.98E-17
GO:0070062~extracellular exosome	104	33	1.21E-19	2.84E-17	5.68E-18	1.55E-16
GO:0007155~cell adhesion	43	14	3.61E-19	7.03E-16	7.03E-16	6.19E-16
GO:0005581~collagen trimer	20	6.4	2.53E-17	5.95E-15	9.92E-16	3.26E-14
Heparin-binding	18	5.8	7.45E-17	2.78E-14	3.44E-15	1.44E-13
Collagen	19	6.1	9.55E-17	2.78E-14	3.11E-15	1.44E-13
SM00209:TSP1	19	6.1	4.30E-16	6.71E-14	6.71E-14	5.33E-13
IPR001881:EGF-like calcium-binding	21	6.7	2.31E-15	1.43E-12	4.77E-13	3.45E-12
IPR000742:Epidermal growth factor-like domain	26	8.3	1.33E-14	8.18E-12	2.05E-12	1.97E-11
IPR008160:Collagen triple helix repeat	17	5.4	1.51E-14	9.27E-12	1.85E-12	2.24E-11
IPR013032:EGF-like, conserved site	24	7.7	1.76E-14	1.08E-11	1.81E-12	2.62E-11
Hydroxylation	17	5.4	6.65E-14	1.66E-11	1.66E-12	8.64E-11
GO:0005509~calcium ion binding	43	14	7.01E-14	3.05E-11	1.52E-11	9.90E-11
EGF-like domain	24	7.7	7.74E-14	1.93E-11	1.76E-12	1.01E-10
GO:0009986~cell surface	40	13	1.83E-13	4.29E-11	6.13E-12	2.35E-10

IPR018097:EGF-like calcium-binding, conserved site	17	5.4	8.38E-13	5.15E-10	7.35E-11	1.24E-09
IPR000152:EGF-type aspartate/asparagine hydroxylation site	17	5.4	9.89E-13	6.07E-10	7.59E-11	1.46E-09
domain:EGF-like 3; calcium-binding	13	4.2	1.25E-12	1.46E-09	3.66E-10	2.01E-09
SM00179:EGF CA	21	6.7	1.82E-12	2.74E-10	1.37E-10	2.17E-09
short sequence motif:Cell attachment site	16	5.1	2.79E-12	3.28E-09	6.56E-10	4.50E-09
Cleavage on pair of basic residues	23	7.3	5.27E-12	1.32E-09	1.10E-10	6.84E-09
GO:0030199~collagen fibril organization	12	3.8	1.81E-11	3.53E-08	1.76E-08	3.10E-08
SM00181:EGF	23	7.3	3.28E-11	4.96E-09	1.65E-09	3.92E-08
domain:Fibrillar collagen NC1	8	2.6	3.64E-11	4.27E-08	7.12E-09	5.87E-08
IPR000885:Fibrillar collagen, C-terminal	8	2.6	4.27E-11	2.62E-08	2.91E-09	6.33E-08
GO:0030198~extracellular matrix organization	17	5.4	4.42E-11	8.61E-08	2.87E-08	7.58E-08
domain:VWFC	9	2.9	1.09E-10	1.28E-07	1.83E-08	1.76E-07
Zymogen	20	6.4	1.40E-10	3.49E-08	2.69E-09	1.82E-07
mmu04512:ECM-receptor interaction	15	4.8	1.71E-10	3.18E-08	3.18E-08	2.12E-07
mmu04510:Focal adhesion	21	6.7	1.98E-10	3.68E-08	1.84E-08	2.45E-07
SM00038:COLFI	8	2.6	5.55E-10	8.38E-08	2.09E-08	6.63E-07
GO:0005201~extracellular matrix structural constituent	11	3.5	5.86E-10	2.54E-07	8.48E-08	8.27E-07
GO:0008237~metallopeptidase activity	18	5.8	6.39E-10	2.77E-07	6.94E-08	9.02E-07
IPR001791:Laminin G domain	12	3.8	6.94E-10	4.26E-07	4.26E-08	1.03E-06
domain:EGF-like 2; calcium-binding	12	3.8	6.99E-10	8.22E-07	1.03E-07	1.13E-06
GO:0008233~peptidase activity	30	9.6	3.27E-09	1.42E-06	2.84E-07	4.62E-06
mmu04151:PI3K-Akt signaling pathway	25	8	3.31E-09	6.15E-07	2.05E-07	4.09E-06
GO:0032496~response to lipopolysaccharide	19	6.1	3.45E-09	6.72E-06	1.68E-06	5.92E-06
domain:TSP type-1 2	10	3.2	5.24E-09	6.15E-06	6.84E-07	8.45E-06
domain:TSP type-1 1	10	3.2	5.24E-09	6.15E-06	6.84E-07	8.45E-06
IPR001007:von Willebrand factor, type C	10	3.2	6.46E-09	3.97E-06	3.61E-07	9.58E-06
compositionally biased region:Cys-rich	16	5.1	6.94E-09	8.15E-06	8.15E-07	1.12E-05
GO:0050840~extracellular matrix binding	9	2.9	8.14E-09	3.53E-06	5.89E-07	1.15E-05
region of interest:Triple-helical region	8	2.6	8.61E-09	1.01E-05	9.20E-07	1.39E-05
GO:0008083~growth factor activity	16	5.1	9.60E-09	4.16E-06	5.95E-07	1.35E-05
Metalloprotease	15	4.8	1.18E-08	2.94E-06	2.10E-07	1.53E-05
IPR024079:Metallopeptidase, catalytic domain	13	4.2	1.87E-08	1.15E-05	9.55E-07	2.76E-05
mmu04974:Protein digestion and absorption	13	4.2	2.25E-08	4.19E-06	1.05E-06	2.79E-05
Growth factor	14	4.5	2.30E-08	5.75E-06	3.84E-07	2.99E-05
propeptide:C-terminal propeptide	6	1.9	2.45E-08	2.88E-05	2.40E-06	3.95E-05
domain:EGF-like 5; calcium-binding	9	2.9	2.90E-08	3.40E-05	2.62E-06	4.67E-05
SM00214:VWC	10	3.2	3.34E-08	5.05E-06	1.01E-06	4.00E-05

IPR010294:ADAM-TS Spacer 1	8	2.6	3.80E-08	2.33E-05	1.80E-06	5.63E-05
IPR013273:Peptidase M12B, ADAM-TS	8	2.6	3.80E-08	2.33E-05	1.80E-06	5.63E-05
domain:EGF-like 4; calcium-binding	9	2.9	3.81E-08	4.47E-05	3.20E-06	6.14E-05
Protease	27	8.6	3.97E-08	9.93E-06	6.21E-07	5.16E-05
Inflammatory response	15	4.8	5.34E-08	1.34E-05	7.86E-07	6.94E-05
domain:EGF-like 6; calcium-binding	8	2.6	6.18E-08	7.26E-05	4.84E-06	9.96E-05
GO:0004222~metalloendopeptidase activity	14	4.5	7.33E-08	3.18E-05	3.97E-06	1.03E-04
GO:0006508~proteolysis	30	9.6	8.13E-08	1.58E-04	3.17E-05	1.39E-04
domain:TSP type-1	7	2.2	1.36E-07	1.60E-04	9.98E-06	2.19E-04
short sequence motif:Cysteine switch	9	2.9	2.01E-07	2.37E-04	1.39E-05	3.25E-04
GO:0030335~positive regulation of cell migration	17	5.4	2.05E-07	3.99E-04	6.64E-05	3.51E-04
GO:0006954~inflammatory response	22	7	2.07E-07	4.02E-04	5.75E-05	3.54E-04
domain:TSP type-1 3	8	2.6	2.11E-07	2.48E-04	1.38E-05	3.41E-04
IPR010909:PLAC	7	2.2	2.19E-07	1.34E-04	9.59E-06	3.24E-04
GO:0008284~positive regulation of cell proliferation	28	8.9	2.35E-07	4.57E-04	5.71E-05	4.02E-04
GO:0005604~basement membrane	12	3.8	3.29E-07	7.72E-05	9.65E-06	4.23E-04
GO:0005518~collagen binding	10	3.2	3.33E-07	1.44E-04	1.60E-05	4.69E-04
GO:0001525~angiogenesis	18	5.8	3.69E-07	7.19E-04	7.99E-05	6.33E-04
GO:0001503~ossification	12	3.8	4.21E-07	8.20E-04	8.20E-05	7.22E-04
domain:TSP N-terminal	7	2.2	4.41E-07	5.19E-04	2.73E-05	7.12E-04
Sulfation	8	2.6	5.17E-07	1.29E-04	7.18E-06	6.71E-04
IPR013320:Concanavalin A-like lectin/glucanase, subgroup	16	5.1	5.63E-07	3.45E-04	2.30E-05	8.34E-04
mmu04060:Cytokine-cytokine receptor interaction	18	5.8	5.65E-07	1.05E-04	2.10E-05	7.00E-04
GO:0016485~protein processing	11	3.5	1.24E-06	0.00241	2.20E-04	0.00213
IPR002870:Peptidase M12B, propeptide	9	2.9	1.30E-06	8.01E-04	5.01E-05	0.00193
Pyrrolidone carboxylic acid	9	2.9	1.41E-06	3.53E-04	1.86E-05	0.00184
IPR001590:Peptidase M12B, ADAM/reprolysin	9	2.9	1.50E-06	9.23E-04	5.43E-05	0.00223
GO:0016525~negative regulation of angiogenesis	10	3.2	2.31E-06	0.00448	3.74E-04	0.00395
Proteoglycan	8	2.6	2.53E-06	6.31E-04	3.16E-05	0.00328
GO:0042060~wound healing	11	3.5	2.83E-06	0.00549	4.23E-04	0.00485
GO:0031100~organ regeneration	9	2.9	3.05E-06	0.00592	4.24E-04	0.00522
IPR003598:Immunoglobulin subtype 2	16	5.1	3.13E-06	0.00192	1.07E-04	0.00464
Immunity	20	6.4	3.35E-06	8.37E-04	3.99E-05	0.00435
IPR011489:EMI domain	6	1.9	3.51E-06	0.00215	1.14E-04	0.00521
IPR000716:Thyroglobulin type-1	6	1.9	3.51E-06	0.00215	1.14E-04	0.00521
GPI-anchor	12	3.8	3.93E-06	9.83E-04	4.47E-05	0.00511

domain:EGF-like 1; calcium-binding	6	1.9	4.51E-06	0.00528	2.65E-04	0.00727
mmu04610:Complement and coagulation cascades	10	3.2	4.67E-06	8.69E-04	1.45E-04	0.00579
SM00210:TSPN	7	2.2	4.89E-06	7.38E-04	1.23E-04	0.00584
GO:0004252~serine-type endopeptidase activity	15	4.8	7.13E-06	0.00309	3.09E-04	0.01006
GO:0005125~cytokine activity	15	4.8	7.93E-06	0.00344	3.13E-04	0.0112
GO:0007596~blood coagulation	10	3.2	8.42E-06	0.01627	0.00109	0.01444
region of interest:Heparin-binding	6	1.9	8.61E-06	0.01006	4.81E-04	0.01388
domain:EMI	6	1.9	8.61E-06	0.01006	4.81E-04	0.01388
SM00280:KAZAL	8	2.6	8.91E-06	0.00135	1.92E-04	0.01066
GO:0005102~receptor binding	21	6.7	9.15E-06	0.00396	3.31E-04	0.01292
GO:0006955~immune response	17	5.4	9.54E-06	0.0184	0.00116	0.01635
IPR026823:Complement C1r-like EGF domain	6	1.9	1.10E-05	0.00674	3.38E-04	0.01631
mmu04611:Platelet activation	12	3.8	1.19E-05	0.00222	3.17E-04	0.01479
GO:0090263~positive regulation of canonical Wnt signaling pathway	9	2.9	1.20E-05	0.02309	0.00137	0.02057
Immunoglobulin domain	21	6.7	1.29E-05	0.00322	1.40E-04	0.01675
GO:0031225~anchored component of membrane	12	3.8	1.33E-05	0.00311	3.46E-04	0.01705
IPR002035:von Willebrand factor, type A	10	3.2	1.52E-05	0.00928	4.44E-04	0.0225
GO:0002376~immune system process	20	6.4	1.61E-05	0.03091	0.00174	0.02763
Hydrolase	45	14	1.74E-05	0.00433	1.81E-04	0.02257
IPR002350:Kazal domain	8	2.6	1.92E-05	0.01172	5.36E-04	0.02844
SM00211:TY	6	1.9	2.10E-05	0.00317	3.97E-04	0.02513
GO:0019221~cytokine-mediated signaling pathway	12	3.8	2.56E-05	0.04868	0.00262	0.04392
GO:0048407~platelet-derived growth factor binding	5	1.6	2.71E-05	0.01168	9.03E-04	0.03819
GO:0005178~integrin binding	10	3.2	2.87E-05	0.01237	8.89E-04	0.04047
Hemostasis	7	2.2	2.94E-05	0.00731	2.94E-04	0.03814
Blood coagulation	7	2.2	2.94E-05	0.00731	2.94E-04	0.03814
IPR015369:Follistatin/Osteonectin EGF domain	4	1.3	3.06E-05	0.01858	8.15E-04	0.04525
Innate immunity	14	4.5	3.20E-05	0.00796	3.07E-04	0.0415
GO:0030324~lung development	11	3.5	3.34E-05	0.0629	0.00324	0.05717
topological domain:Extracellular	63	20	3.45E-05	0.03969	0.00184	0.05557
domain:Disintegrin	7	2.2	3.45E-05	0.0397	0.00176	0.05558
GO:0050731~positive regulation of peptidyl-tyrosine phosphorylation	10	3.2	3.76E-05	0.07064	0.00348	0.06446
GO:0042056~chemoattractant activity	6	1.9	3.85E-05	0.01657	0.00111	0.05432
domain:Peptidase M12B	7	2.2	4.02E-05	0.04612	0.00197	0.06478
domain:Kazal-like	7	2.2	4.02E-05	0.04612	0.00197	0.06478

Sushi	7	2.2	4.31E-05	0.01073	3.99E-04	0.05604
GO:0070374~positive regulation of ERK1 and ERK2 cascade	13	4.2	5.71E-05	0.10526	0.00504	0.09786
IPR013098:Immunoglobulin I-set	11	3.5	6.42E-05	0.03864	0.00164	0.09504
GO:0031091~platelet alpha granule	5	1.6	6.86E-05	0.01599	0.00161	0.08818
Collagen degradation	5	1.6	7.26E-05	0.01798	6.48E-04	0.09425
GO:0071356~cellular response to tumor necrosis factor	10	3.2	7.37E-05	0.13372	0.00622	0.12629
GO:0001968~fibronectin binding	6	1.9	8.17E-05	0.03485	0.00221	0.11529
propeptide:N-terminal propeptide	4	1.3	8.64E-05	0.09656	0.00405	0.13925
GO:0007599~hemostasis	7	2.2	9.17E-05	0.16348	0.00741	0.15702
Gamma-carboxyglutamic acid	5	1.6	9.23E-05	0.02281	7.96E-04	0.11985
domain:EGF-like 1	10	3.2	9.51E-05	0.10576	0.00429	0.15327
metal ion-binding site:Zinc; in inhibited form	6	1.9	1.03E-04	0.11374	0.00446	0.16556
IPR019577:SPARC/Testican, calcium-binding domain	4	1.3	1.05E-04	0.06222	0.00257	0.15489
SM00327:VWA	9	2.9	1.25E-04	0.01871	0.0021	0.14945
GO:0001568~blood vessel development	8	2.6	1.47E-04	0.24823	0.01135	0.25085
IPR018486:Hemopexin, conserved site	5	1.6	1.48E-04	0.08669	0.00348	0.21856
region of interest:Nonhelical region	4	1.3	1.49E-04	0.16098	0.00625	0.24058
mmu05146:Amoebiasis	10	3.2	1.51E-04	0.02773	0.00351	0.18706
mmu05205:Proteoglycans in cancer	13	4.2	1.55E-04	0.02841	0.0032	0.1917
IPR000436:Sushi/SCR/CCP	7	2.2	1.66E-04	0.09687	0.00377	0.24556
Wnt signaling pathway	11	3.5	1.71E-04	0.04174	0.00142	0.22131
IPR000867:Insulin-like growth factor-binding protein, IGFBP	5	1.6	1.82E-04	0.10599	0.00399	0.26999
domain:IGFBP N-terminal	5	1.6	1.87E-04	0.19736	0.00755	0.30123
SM00408:IGc2	16	5.1	2.05E-04	0.03054	0.0031	0.24526
GO:0001934~positive regulation of protein phosphorylation	12	3.8	2.06E-04	0.33097	0.01534	0.35318
SM00282:LamG	7	2.2	2.10E-04	0.03123	0.00288	0.25092
GO:0070208~protein heterotrimerization	5	1.6	2.13E-04	0.33979	0.01526	0.36483
GO:0090090~negative regulation of canonical Wnt signaling pathway	9	2.9	2.48E-04	0.38297	0.0171	0.42414
mmu04015:Rap1 signaling pathway	13	4.2	2.54E-04	0.04609	0.00471	0.31368
GO:0007267~cell-cell signaling	9	2.9	2.65E-04	0.40327	0.01765	0.45346
Protease inhibitor	9	2.9	2.75E-04	0.06642	0.00221	0.3565
GO:0043410~positive regulation of MAPK cascade	9	2.9	2.83E-04	0.42399	0.01822	0.48441
GO:0016787~hydrolase activity	43	14	2.91E-04	0.11852	0.00739	0.40939
GO:0005539~glycosaminoglycan binding	5	1.6	2.93E-04	0.11927	0.00703	0.41216
domain:Ig-like C2-type	9	2.9	3.12E-04	0.30689	0.01214	0.50177
metal ion-binding site:Calcium 2; via carbonyl oxygen	6	1.9	3.39E-04	0.32822	0.01275	0.54443

domain:Ig-like C2-type 1	10	3.2	3.53E-04	0.33979	0.01289	0.56814
domain:Ig-like C2-type 2	10	3.2	3.74E-04	0.35532	0.01321	0.60062
IPR000585:Hemopexin-like domain	5	1.6	3.82E-04	0.20916	0.00806	0.56463
IPR018487:Hemopexin-like repeats	5	1.6	3.82E-04	0.20916	0.00806	0.56463
GO:0004175~endopeptidase activity	9	2.9	3.98E-04	0.15862	0.00905	0.56007
GO:0005520~insulin-like growth factor binding	5	1.6	4.22E-04	0.16749	0.00912	0.59436
domain:Hemopexin-like 2	5	1.6	4.24E-04	0.3928	0.01457	0.68228
domain:Hemopexin-like 1	5	1.6	4.24E-04	0.3928	0.01457	0.68228
IPR003645:Follistatin-like, N-terminal	4	1.3	4.72E-04	0.25171	0.00962	0.69723
IPR001212:Somatomedin B domain	4	1.3	4.72E-04	0.25171	0.00962	0.69723
Cytokine	11	3.5	4.74E-04	0.11171	0.0037	0.61367
mmu04668:TNF signaling pathway	9	2.9	4.88E-04	0.08681	0.00822	0.60284
lipid moiety-binding region:GPI-anchor amidated serine	7	2.2	5.04E-04	0.44725	0.0168	0.81026
domain:TSP type-1 4	5	1.6	5.07E-04	0.44906	0.01642	0.81473
GO:0009612~response to mechanical stimulus	7	2.2	5.29E-04	0.64293	0.03267	0.90246
GO:0008285~negative regulation of cell proliferation	17	5.4	5.40E-04	0.6509	0.03235	0.92213
GO:0030574~collagen catabolic process	5	1.6	5.49E-04	0.65642	0.03186	0.93604
GO:0010811~positive regulation of cell-substrate adhesion	6	1.9	5.53E-04	0.65967	0.0312	0.94433
GO:0071363~cellular response to growth factor stimulus	7	2.2	5.76E-04	0.67411	0.03153	0.98214
GO:0046426~negative regulation of JAK-STAT cascade	6	1.9	6.18E-04	0.69994	0.03289	1.05408
GO:0043206~extracellular fibril organization	4	1.3	6.25E-04	0.70381	0.03235	1.06538
GO:0010466~negative regulation of peptidase activity	9	2.9	6.26E-04	0.70478	0.0316	1.06825
propeptide:Removed in mature form	13	4.2	6.31E-04	0.52391	0.01986	1.01328
domain:Ig-like C2-type 3	8	2.6	6.39E-04	0.52841	0.01959	1.02616
domain:PLAC	4	1.3	6.70E-04	0.54522	0.02	1.07544
IPR003599:Immunoglobulin subtype	19	6.1	6.85E-04	0.34363	0.01349	1.0108
domain:EGF-like 7; calcium-binding	5	1.6	7.06E-04	0.56387	0.02053	1.13229
GO:0030414~peptidase inhibitor activity	9	2.9	7.43E-04	0.27571	0.01524	1.04348
GO:0051781~positive regulation of cell division	6	1.9	7.64E-04	0.77426	0.03744	1.30165
GO:0044691~tooth eruption	3	1	7.71E-04	0.77741	0.03686	1.31386
GO:0045766~positive regulation of angiogenesis	9	2.9	7.82E-04	0.782	0.03647	1.33198
domain:Follistatin-like	3	1	8.09E-04	0.61385	0.02294	1.29728
metal ion-binding site:Zinc; catalytic	9	2.9	8.20E-04	0.61856	0.02269	1.31391
GO:0035987~endodermal cell differentiation	5	1.6	8.72E-04	0.81711	0.03964	1.48435
GO:0008009~chemokine activity	6	1.9	9.23E-04	0.33031	0.01806	1.29539

GO:0005044~scavenger receptor activity	6	1.9	9.23E-04	0.33031	0.01806	1.29539
GO:1903561~extracellular vesicle	6	1.9	9.62E-04	0.20241	0.02035	1.23045
GO:0051897~positive regulation of protein kinase B signaling	8	2.6	9.92E-04	0.85513	0.04393	1.68625
metal ion-binding site:Calcium 1	6	1.9	0.00104	0.70594	0.02806	1.66561
GO:0030212~hyaluronan metabolic process	4	1.3	0.00106	0.87249	0.04573	1.79665
GO:0030195~negative regulation of blood coagulation	4	1.3	0.00106	0.87249	0.04573	1.79665
GO:0016477~cell migration	11	3.5	0.00111	0.88586	0.04709	1.8924
GO:0072562~blood microparticle	9	2.9	0.00115	0.23629	0.02221	1.46483
IPR018247:EF-Hand 1, calcium-binding site	10	3.2	0.00115	0.50592	0.02179	1.68691
IPR003591:Leucine-rich repeat, typical subtype	10	3.2	0.00115	0.50592	0.02179	1.68691
GO:0017147~Wnt-protein binding	5	1.6	0.0012	0.40576	0.02238	1.67832
Copper	6	1.9	0.0012	0.2601	0.00909	1.55319
GO:0042493~response to drug	15	4.8	0.00129	0.91867	0.05309	2.18466
GO:0001958~endochondral ossification	5	1.6	0.00131	0.92226	0.0529	2.22356
Basement membrane	5	1.6	0.00131	0.28018	0.00962	1.69382
GO:0051384~response to glucocorticoid	7	2.2	0.00133	0.92564	0.0527	2.26174
SM00274:FOLN	4	1.3	0.00139	0.18887	0.01729	1.6437
GO:0071953~elastic fiber	3	1	0.00142	0.28371	0.02534	1.81002
metal ion-binding site:Zinc 2; catalytic	4	1.3	0.00143	0.81288	0.03738	2.27373
Mitogen	5	1.6	0.00146	0.30643	0.0104	1.88345
GO:0042981~regulation of apoptotic process	11	3.5	0.00151	0.94775	0.05846	2.5649
Angiogenesis	8	2.6	0.00152	0.31572	0.01048	1.95215
SM00120:HX	5	1.6	0.00152	0.20539	0.01753	1.80381
GO:0030247~polysaccharide binding	4	1.3	0.00153	0.48588	0.02734	2.14028
domain:SMB 2	3	1	0.0016	0.84786	0.04098	2.55089
domain:Thyroglobulin type-1 2	3	1	0.0016	0.84786	0.04098	2.55089
domain:Thyroglobulin type-1 1	3	1	0.0016	0.84786	0.04098	2.55089
domain:SMB 1	3	1	0.0016	0.84786	0.04098	2.55089
GO:0007160~cell-matrix adhesion	7	2.2	0.00163	0.95862	0.06171	2.76469
GO:0071260~cellular response to mechanical stimulus	7	2.2	0.00163	0.95862	0.06171	2.76469
GO:0050918~positive chemotaxis	4	1.3	0.00164	0.95924	0.06082	2.77763
mmu04014:Ras signaling pathway	12	3.8	0.00165	0.265	0.02533	2.02921
GO:0001501~skeletal system development	8	2.6	0.00176	0.96739	0.06371	2.96853
domain:TSP type-1 6	4	1.3	0.00176	0.8738	0.044	2.80048
IPR006212:Furin-like repeat	4	1.3	0.00182	0.67382	0.03338	2.66709
Chemotaxis	7	2.2	0.00191	0.37976	0.01283	2.4515
GO:0034097~response to cytokine	7	2.2	0.00198	0.97907	0.07036	3.34637

GO:0010759~positive regulation of macrophage chemotaxis	4	1.3	0.002	0.9796	0.06954	3.36839
domain:Collagen-like	5	1.6	0.00206	0.91119	0.05021	3.26805
region of interest:Spacer	4	1.3	0.00214	0.9193	0.05109	3.39508
GO:0005614~interstitial matrix	4	1.3	0.00216	0.39824	0.03563	2.74205
GO:0045087~innate immune response	16	5.1	0.00223	0.98706	0.076	3.75476
GO:0030282~bone mineralization	5	1.6	0.00235	0.98979	0.0786	3.95491
compositionally biased region:Poly-Leu	8	2.6	0.00236	0.93754	0.05502	3.73411
GO:0070051~fibrinogen binding	3	1	0.0024	0.64743	0.04084	3.33339
GO:0001558~regulation of cell growth	6	1.9	0.00242	0.99101	0.07934	4.06317
GO:0016055~Wnt signaling pathway	11	3.5	0.0025	0.99231	0.08051	4.19502
GO:0060355~positive regulation of cell adhesion molecule production	3	1	0.00252	0.9926	0.07979	4.22682
GO:0060665~regulation of branching involved in salivary gland morphogenesis by mesenchymal-epithelial signaling	3	1	0.00252	0.9926	0.07979	4.22682
domain:VWFA 2	4	1.3	0.00257	0.95119	0.05861	4.0594
metal ion-binding site:Calcium 2	6	1.9	0.00268	0.95746	0.06003	4.24021
GO:0050728~negative regulation of inflammatory response	7	2.2	0.00285	0.99613	0.08843	4.77237
GO:0050727~regulation of inflammatory response	6	1.9	0.00302	0.99722	0.09196	5.0484
mmu04350:TGF-beta signaling pathway	7	2.2	0.00305	0.43366	0.04279	3.71508
IPR004074:Interleukin-1 receptor, type I/II	3	1	0.00309	0.85067	0.05439	4.48491
GO:0008217~regulation of blood pressure	6	1.9	0.00324	0.9982	0.09689	5.41044
IPR002477:Peptidoglycan binding-like	4	1.3	0.00341	0.87754	0.05823	4.94098
GO:0050729~positive regulation of inflammatory response	6	1.9	0.00347	0.99886	0.10196	5.78988
GO:0004908~interleukin-1 receptor activity	3	1	0.00356	0.78742	0.05782	4.91073
GO:0005031~tumor necrosis factor-activated receptor activity	4	1.3	0.00362	0.79285	0.05664	4.99074
GO:0045860~positive regulation of protein kinase activity	6	1.9	0.00372	0.99929	0.10717	6.18705
GO:0032964~collagen biosynthetic process	3	1	0.00373	0.99931	0.10603	6.21173
GO:0001725~stress fiber	6	1.9	0.00388	0.59921	0.05913	4.88193
GO:0048146~positive regulation of fibroblast proliferation	6	1.9	0.00398	0.99957	0.11091	6.60225
domain:TSP type-1 5	4	1.3	0.00415	0.99244	0.08966	6.48315
GO:0019955~cytokine binding	4	1.3	0.00418	0.83732	0.0628	5.73454
GO:0010595~positive regulation of endothelial cell migration	5	1.6	0.00422	0.99973	0.11556	6.98691
GO:0043025~neuronal cell body	18	5.8	0.0044	0.64536	0.06274	5.51692
IPR001111:Transforming growth factor-beta, N-terminal	4	1.3	0.00445	0.93529	0.07323	6.39308
IPR001818:Peptidase M10, metallopeptidase	4	1.3	0.00445	0.93529	0.07323	6.39308
IPR021190:Peptidase M10A	4	1.3	0.00445	0.93529	0.07323	6.39308

Developmental protein	25	8	0.00453	0.67843	0.02942	5.72514
GO:0001938~positive regulation of endothelial cell proliferation	6	1.9	0.00453	0.99986	0.12192	7.48787
repeat:LRR 6	10	3.2	0.00462	0.99568	0.09762	7.19838
topological domain:Cytoplasmic	66	21	0.00476	0.99631	0.09856	7.40073
metal ion-binding site:Calcium 3; via carbonyl oxygen	4	1.3	0.00478	0.99642	0.09732	7.43665
domain:Hemopexin-like 4	4	1.3	0.00478	0.99642	0.09732	7.43665
domain:Hemopexin-like 3	4	1.3	0.00478	0.99642	0.09732	7.43665
GO:0001843~neural tube closure	7	2.2	0.00488	0.99993	0.12892	8.04141
GO:0008543~fibroblast growth factor receptor signaling pathway	5	1.6	0.00501	0.99994	0.13041	8.2504
IPR001368:TNFR/NGFR cysteine-rich region	4	1.3	0.00503	0.95479	0.08028	7.19922
IPR020067:Frizzled domain	4	1.3	0.00503	0.95479	0.08028	7.19922
GO:0022617~extracellular matrix disassembly	4	1.3	0.00511	0.99995	0.13115	8.41139
GO:0002244~hematopoietic progenitor cell differentiation	7	2.2	0.00513	0.99996	0.12981	8.43578
GO:0060687~regulation of branching involved in prostate gland morphogenesis	3	1	0.00517	0.99996	0.12919	8.50554
GO:0002544~chronic inflammatory response	3	1	0.00517	0.99996	0.12919	8.50554
GO:0060449~bud elongation involved in lung branching	3	1	0.00517	0.99996	0.12919	8.50554
GO:0009143~nucleoside triphosphate catabolic process	3	1	0.00517	0.99996	0.12919	8.50554
SM00261:FU	4	1.3	0.0052	0.54459	0.05463	6.03764
metal ion-binding site:Calcium 4; via carbonyl oxygen	3	1	0.00542	0.99832	0.10783	8.3945
domain:VWFA 3	3	1	0.00542	0.99832	0.10783	8.3945
SM00032:CCP	6	1.9	0.00567	0.57633	0.05565	6.5736
GO:0045429~positive regulation of nitric oxide biosynthetic process	5	1.6	0.0059	0.99999	0.14418	9.6451
Serine protease inhibitor	6	1.9	0.00608	0.78219	0.03833	7.61444
IPR001811:Chemokine interleukin-8-like domain	5	1.6	0.00615	0.9773	0.09482	8.72985
GO:0042472~inner ear morphogenesis	6	1.9	0.00615	0.99999	0.148	10.0348
IPR013783:Immunoglobulin-like fold	28	8.9	0.00622	0.97826	0.09351	8.82501
GO:0034394~protein localization to cell surface	4	1.3	0.00656	1	0.15522	10.6715
GO:0060441~epithelial tube branching involved in lung morphogenesis	4	1.3	0.00656	1	0.15522	10.6715
GO:0071230~cellular response to amino acid stimulus	8	2.6	0.0069	1	0.16059	11.1887
repeat:LRR 8	8	2.6	0.00697	0.99973	0.13423	10.6619
domain:FZ	4	1.3	0.00702	0.99974	0.13291	10.7306
domain:Cadherin 7	3	1	0.00715	0.99978	0.1332	10.9287
GO:0007568~aging	9	2.9	0.00718	1	0.16452	11.6106

SM00121:IB	4	1.3	0.00718	0.66291	0.0657	8.24924
IPR002077:Voltage-dependent calcium channel, alpha-1 subunit	3	1	0.00721	0.98823	0.10511	10.1644
Biom mineralization	4	1.3	0.00723	0.83702	0.04434	8.99625
IPR011992:EF-hand-like domain	11	3.5	0.00728	0.98876	0.1037	10.265
Metal-binding	64	20	0.00734	0.84143	0.04392	9.12576
GO:0030336~negative regulation of cell migration	7	2.2	0.00747	1	0.1688	12.065
GO:0050679~positive regulation of epithelial cell proliferation	6	1.9	0.00771	1	0.17171	12.425
GO:0000187~activation of MAPK activity	6	1.9	0.00771	1	0.17171	12.425
domain:Ig-like C2-type 5	5	1.6	0.00807	0.99993	0.14669	12.244
GO:0030141~secretory granule	7	2.2	0.00817	0.85465	0.10725	10.0194
GO:0032967~positive regulation of collagen biosynthetic process	4	1.3	0.00823	1	0.18021	13.2107
repeat:LRR 5	10	3.2	0.00844	0.99995	0.15062	12.773
GO:0030971~receptor tyrosine kinase binding	5	1.6	0.00844	0.97479	0.1192	11.2809
GO:0009887~organ morphogenesis	7	2.2	0.00853	1	0.18396	13.6489
GO:0043065~positive regulation of apoptotic process	13	4.2	0.00853	1	0.18204	13.6547
GO:0060326~cell chemotaxis	6	1.9	0.00859	1	0.18116	13.7391
IPR006026:Peptidase, metallopeptidase	4	1.3	0.00861	0.99504	0.11869	12.0186
GO:0005179~hormone activity	7	2.2	0.00865	0.97692	0.11806	11.5347
domain:Cadherin 5	5	1.6	0.00867	0.99996	0.15217	13.1032
GO:0060346~bone trabecula formation	3	1	0.00868	1	0.18103	13.8802
domain:Laminin G-like 1	4	1.3	0.0088	0.99997	0.1519	13.2763
domain:Laminin G-like 2	4	1.3	0.0088	0.99997	0.1519	13.2763
SM00201:SO	3	1	0.00883	0.73814	0.07579	10.0657
IPR003096:Smooth muscle protein/calponin	3	1	0.00892	0.99593	0.12014	12.4368
IPR002138:Peptidase C14, caspase non-catalytic subunit p10	3	1	0.00892	0.99593	0.12014	12.4368
GO:0042476~odontogenesis	4	1.3	0.00915	1	0.18793	14.5804
GO:0051591~response to cAMP	5	1.6	0.00918	1	0.18647	14.6196
GO:0045177~apical part of cell	7	2.2	0.00925	0.88742	0.11427	11.2693
IPR004843:Metallophosphoesterase domain	4	1.3	0.00946	0.99708	0.12421	13.1348
VLDL	3	1	0.00956	0.90938	0.05557	11.7301
mmu04810:Regulation of actin cytoskeleton	10	3.2	0.00987	0.84202	0.12349	11.5619
repeat:LRR 12	6	1.9	0.00998	0.99999	0.16816	14.9304
IPR011029:Death-like domain	6	1.9	0.01	0.99792	0.12818	13.8392
GO:0000902~cell morphogenesis	6	1.9	0.01002	1	0.1998	15.858
GO:0001649~osteoblast differentiation	7	2.2	0.01009	1	0.19893	15.9519
GO:0004714~transmembrane receptor protein tyrosine kinase activity	5	1.6	0.0103	0.98883	0.13497	13.5989

GO:0042802~identical protein binding	19	6.1	0.01044	0.98946	0.13261	13.7613
GO:0051216~cartilage development	6	1.9	0.01054	1	0.2048	16.6032
GO:0048048~embryonic eye morphogenesis	3	1	0.01074	1	0.20622	16.8897
GO:0090197~positive regulation of chemokine secretion	3	1	0.01074	1	0.20622	16.8897
GO:0048245~eosinophil chemotaxis	3	1	0.01074	1	0.20622	16.8897
GO:0071305~cellular response to vitamin D	3	1	0.01074	1	0.20622	16.8897
IPR015917:Peptidase C14, caspase precursor p45, core	3	1	0.0108	0.99873	0.13495	14.8635
IPR018244:Allergen V5/Tpx-1-related, conserved site	3	1	0.0108	0.99873	0.13495	14.8635
IPR015621:Interleukin-1 receptor family	3	1	0.0108	0.99873	0.13495	14.8635
propeptide:Activation peptide	6	1.9	0.01105	1	0.18193	16.3992
GO:0031214~biomineral tissue development	4	1.3	0.01117	1	0.21152	17.5077
metal ion-binding site:Zinc 2; in inhibited form	3	1	0.01125	1	0.18237	16.6697
IPR017948:Transforming growth factor beta, conserved site	4	1.3	0.01131	0.99907	0.13809	15.5095
IPR001073:Complement C1q protein	4	1.3	0.01131	0.99907	0.13809	15.5095
GO:0042127~regulation of cell proliferation	10	3.2	0.01173	1	0.21887	18.3084
domain:C1q	4	1.3	0.01192	1	0.18965	17.5802
domain:VWFA	5	1.6	0.01214	1	0.1903	17.8791
GO:0005515~protein binding	82	26	0.01226	0.99527	0.14977	15.9803
GO:0044344~cellular response to fibroblast growth factor stimulus	4	1.3	0.01226	1	0.2255	19.0595
GO:0001937~negative regulation of endothelial cell proliferation	4	1.3	0.01226	1	0.2255	19.0595
GO:0006935~chemotaxis	7	2.2	0.01231	1	0.22428	19.1342
SM00208:TNFR	4	1.3	0.01232	0.84619	0.09878	13.7756
mmu05323:Rheumatoid arthritis	6	1.9	0.01235	0.90089	0.14281	14.2652
GO:0004860~protein kinase inhibitor activity	5	1.6	0.01241	0.99557	0.14732	16.1558
SM00369:LRR_TYP	10	3.2	0.01244	0.84897	0.0947	13.9
GO:0048471~perinuclear region of cytoplasm	20	6.4	0.01245	0.94733	0.14353	14.8833
domain:EGF-like 2	6	1.9	0.01279	1	0.19688	18.747
IPR017891:Insulin-like growth factor binding protein, N-terminal, Cys-rich conserved site	3	1	0.01284	0.99964	0.15235	17.4229
GO:0001666~response to hypoxia	9	2.9	0.0129	1	0.23158	19.9576
GO:0030728~ovulation	3	1	0.01298	1	0.23072	20.0664
GO:0035413~positive regulation of catenin import into nucleus	3	1	0.01298	1	0.23072	20.0664
GO:0003014~renal system process	3	1	0.01298	1	0.23072	20.0664
GO:0048333~mesodermal cell differentiation	3	1	0.01298	1	0.23072	20.0664
Osteogenesis	4	1.3	0.01317	0.96361	0.07416	15.8174

GO:0010628~positive regulation of gene expression	14	4.5	0.01328	1	0.23326	20.4776
GO:0004867~serine-type endopeptidase inhibitor activity	7	2.2	0.01333	0.99704	0.15325	17.2494
GO:0001541~ovarian follicle development	5	1.6	0.01346	1	0.23399	20.7291
domain:Cadherin 4	5	1.6	0.01375	1	0.20731	20.0025
domain:Ig-like C2-type 4	5	1.6	0.01375	1	0.20731	20.0025
repeat:LRR 4	10	3.2	0.01391	1	0.20693	20.2191
GO:0043588~skin development	5	1.6	0.01428	1	0.24425	21.8483
metal ion-binding site:Calcium 3	4	1.3	0.01431	1	0.20963	20.7404
Leucine-rich repeat	10	3.2	0.01433	0.97289	0.07872	17.0951
IPR001611:Leucine-rich repeat	10	3.2	0.01459	0.99988	0.16821	19.5689
domain:Cadherin 3	5	1.6	0.0146	1	0.21074	21.1067
GO:2001238~positive regulation of extrinsic apoptotic signaling pathway	4	1.3	0.01463	1	0.24732	22.3229
GO:0060349~bone morphogenesis	4	1.3	0.01463	1	0.24732	22.3229
IPR001309:Peptidase C14, ICE, catalytic subunit p20	3	1	0.01503	0.99991	0.16967	20.0943
IPR023413:Green fluorescent protein-like	3	1	0.01503	0.99991	0.16967	20.0943
IPR000566:Lipocalin/cytosolic fatty-acid binding protein domain	5	1.6	0.01524	0.99992	0.16882	20.3524
repeat:LRR 11	6	1.9	0.0154	1	0.21838	22.136
GO:1900025~negative regulation of substrate adhesion-dependent cell spreading	3	1	0.01541	1	0.25661	23.3758
GO:0090331~negative regulation of platelet aggregation	3	1	0.01541	1	0.25661	23.3758
GO:0032930~positive regulation of superoxide anion generation	3	1	0.01541	1	0.25661	23.3758
GO:0071392~cellular response to estradiol stimulus	4	1.3	0.01591	1	0.26145	24.0271
GO:0030308~negative regulation of cell growth	7	2.2	0.01598	1	0.2604	24.1303
domain:Sushi	3	1	0.01614	1	0.225	23.0754
repeat:LRR 7	8	2.6	0.01625	1	0.22376	23.2156
GO:0007275~multicellular organism development	27	8.6	0.01655	1	0.26619	24.8792
IPR001839:Transforming growth factor-beta, C-terminal	4	1.3	0.0168	0.99997	0.18127	22.1937
Hormone	6	1.9	0.01685	0.98571	0.09009	19.8092
IPR007110:Immunoglobulin-like domain	23	7.3	0.0172	0.99998	0.18209	22.6685
domain:Cadherin 1	5	1.6	0.01734	1	0.23429	24.5781
domain:Cadherin 2	5	1.6	0.01734	1	0.23429	24.5781
IPR001283:Allergen V5/Tpx-1-related	3	1	0.01736	0.99998	0.18058	22.8569
IPR014044:CAP domain	3	1	0.01736	0.99998	0.18058	22.8569
Growth factor binding	3	1	0.0176	0.98819	0.09199	20.5999
IPR002048:EF-hand domain	9	2.9	0.01761	0.99998	0.17993	23.1456

mmu00760:Nicotinate and nicotinamide metabolism	4	1.3	0.01779	0.96453	0.18835	19.9346
GO:0090179~planar cell polarity pathway involved in neural tube closure	3	1	0.01803	1	0.28402	26.7842
GO:0002523~leukocyte migration involved in inflammatory response	3	1	0.01803	1	0.28402	26.7842
GO:0043616~keratinocyte proliferation	3	1	0.01803	1	0.28402	26.7842
GO:0042177~negative regulation of protein catabolic process	4	1.3	0.01864	1	0.28987	27.5607
GO:0031069~hair follicle morphogenesis	4	1.3	0.01864	1	0.28987	27.5607
domain:Collagen-like 1	3	1	0.01887	1	0.24943	26.4436
domain:Collagen-like 2	3	1	0.01887	1	0.24943	26.4436
glycosylation site:O-linked (Xyl...) (glycosaminoglycan)	3	1	0.01887	1	0.24943	26.4436
GO:0006469~negative regulation of protein kinase activity	6	1.9	0.01892	1	0.29137	27.9237
IPR021158:Peptidase M10A, cysteine switch, zinc binding site	3	1	0.01984	1	0.19728	25.6912
GO:0014068~positive regulation of phosphatidylinositol 3-kinase signaling	5	1.6	0.01985	1	0.301	29.0793
domain:Sushi 2	4	1.3	0.01986	1	0.25794	27.6329
domain:Sushi 1	4	1.3	0.01986	1	0.25794	27.6329
metal ion-binding site:Calcium 1; via carbonyl oxygen	4	1.3	0.01986	1	0.25794	27.6329
GO:0045786~negative regulation of cell cycle	4	1.3	0.02009	1	0.30181	29.3817
GO:0040007~growth	4	1.3	0.02009	1	0.30181	29.3817
GO:0032924~activin receptor signaling pathway	3	1	0.02081	1	0.30846	30.26
GO:0018108~peptidyl-tyrosine phosphorylation	5	1.6	0.02089	1	0.30718	30.3589
IPR012674:Calycin	5	1.6	0.0214	1	0.20788	27.424
GO:0030177~positive regulation of Wnt signaling pathway	4	1.3	0.02161	1	0.31369	31.2331
GO:0044297~cell body	6	1.9	0.02165	0.99416	0.22676	24.539
GO:0005109~frizzled binding	4	1.3	0.02177	0.99993	0.23308	26.7048
domain:Thyroglobulin type-1	3	1	0.02177	1	0.27627	29.8799
SM00115:CASc	3	1	0.02188	0.96457	0.15381	23.2375
GO:0045121~membrane raft	10	3.2	0.02193	0.99454	0.21975	24.8182
IPR011038:Calycin-like	5	1.6	0.02239	1	0.21315	28.5013
IPR000463:Cytosolic fatty-acid binding	3	1	0.02246	1	0.21054	28.578
metal ion-binding site:Calcium	5	1.6	0.02259	1	0.28216	30.8216
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	6	1.9	0.02306	1	0.32865	32.9592
GO:0043154~negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	5	1.6	0.02307	1	0.32642	32.9698
SM00110:C1Q	4	1.3	0.02316	0.97095	0.15507	24.4343

SM00235:ZnMc	4	1.3	0.02316	0.97095	0.15507	24.4343
Lipoprotein	19	6.1	0.02335	0.99728	0.11811	26.4325
GO:0060445~branching involved in salivary gland morphogenesis	3	1	0.02376	1	0.33206	33.7732
GO:0050673~epithelial cell proliferation	3	1	0.02376	1	0.33206	33.7732
GO:0032355~response to estradiol	6	1.9	0.02395	1	0.33199	34.0007
IPR001314:Peptidase S1A, chymotrypsin-type	7	2.2	0.02412	1	0.22111	30.3567
GO:0051592~response to calcium ion	5	1.6	0.02421	1	0.33261	34.298
GO:0032092~positive regulation of protein binding	5	1.6	0.02421	1	0.33261	34.298
GO:0010976~positive regulation of neuron projection development	7	2.2	0.02468	1	0.33555	34.8345
metal ion-binding site:Copper	3	1	0.02485	1	0.30275	33.3551
IPR006586:ADAM, cysteine-rich	4	1.3	0.02502	1	0.22514	31.3025
IPR009020:Proteinase inhibitor, propeptide	3	1	0.02521	1	0.22344	31.4994
IPR018933:Netrin module, non-TIMP type	3	1	0.02521	1	0.22344	31.4994
domain:EF-hand 2	8	2.6	0.02526	1	0.30389	33.8071
repeat:LRR 3	10	3.2	0.02538	1	0.30203	33.9322
GO:0030593~neutrophil chemotaxis	5	1.6	0.02538	1	0.34109	35.6394
GO:0008360~regulation of cell shape	7	2.2	0.02545	1	0.33957	35.7187
domain:EF-hand 1	8	2.6	0.02592	1	0.30446	34.5248
GO:0007507~heart development	10	3.2	0.02636	1	0.34708	36.7344
GO:0051965~positive regulation of synapse assembly	5	1.6	0.02659	1	0.34728	36.9925
GO:0032270~positive regulation of cellular protein metabolic process	3	1	0.02686	1	0.34792	37.296
IPR020894:Cadherin conserved site	6	1.9	0.0273	1	0.23645	33.6426
GO:0008289~lipid binding	10	3.2	0.02788	1	0.28226	32.904
SM00409:IG	19	6.1	0.02791	0.98608	0.17659	28.7116
IPR015526:Frizzled/secreted frizzled-related protein	3	1	0.02809	1	0.23919	34.4385
domain:VWFA 1	3	1	0.0281	1	0.32252	36.8421
repeat:LRR 10	6	1.9	0.02825	1	0.32088	36.9971
GO:0005245~voltage-gated calcium channel activity	4	1.3	0.0283	1	0.27959	33.3183
GO:0019838~growth factor binding	4	1.3	0.0283	1	0.27959	33.3183
GO:0050680~negative regulation of epithelial cell proliferation	5	1.6	0.02911	1	0.36878	39.727
IPR016666:TGF beta-induced protein bIGH3/osteoblast-specific factor 2	2	0.6	0.02911	1	0.24354	35.452
IPR008673:Microfibril-associated glycoprotein	2	0.6	0.02911	1	0.24354	35.452
IPR001999:Osteonectin-like, conserved site	2	0.6	0.02911	1	0.24354	35.452
IPR017064:Acid sphingomyelinase-like phosphodiesterase, predicted	2	0.6	0.02911	1	0.24354	35.452

mmu04550:Signaling pathways regulating pluripotency of stem cells	7	2.2	0.02919	0.99595	0.27681	30.7089
GO:0048565~digestive tract development	4	1.3	0.03011	1	0.37654	40.7882
GO:0002526~acute inflammatory response	3	1	0.03013	1	0.37436	40.8031
GO:0050678~regulation of epithelial cell proliferation	3	1	0.03013	1	0.37436	40.8031
IPR001254:Peptidase S1	7	2.2	0.03085	1	0.25289	37.1423
GO:0005592~collagen type XI trimer	2	0.6	0.03088	0.99937	0.28474	33.2074
GO:0005584~collagen type I trimer	2	0.6	0.03088	0.99937	0.28474	33.2074
GO:1905286~serine-type peptidase complex	2	0.6	0.03088	0.99937	0.28474	33.2074
GO:1990682~CSF1-CSF1R complex	2	0.6	0.03088	0.99937	0.28474	33.2074
IPR016293:Peptidase M10A, metazoans	3	1	0.0311	1	0.25138	37.3794
GO:0030023~extracellular matrix constituent conferring elasticity	2	0.6	0.03128	1	0.29787	36.1414
GO:0002114~interleukin-33 receptor activity	2	0.6	0.03128	1	0.29787	36.1414
GO:0086059~voltage-gated calcium channel activity involved SA node cell action potential	2	0.6	0.03128	1	0.29787	36.1414
GO:0005160~transforming growth factor beta receptor binding	4	1.3	0.03192	1	0.29669	36.7339
GO:0060595~fibroblast growth factor receptor signaling pathway involved in mammary gland specification	2	0.6	0.03204	1	0.39061	42.7697
GO:1903225~negative regulation of endodermal cell differentiation	2	0.6	0.03204	1	0.39061	42.7697
GO:0061033~secretion by lung epithelial cell involved in lung growth	2	0.6	0.03204	1	0.39061	42.7697
GO:1904956~regulation of midbrain dopaminergic neuron differentiation	2	0.6	0.03204	1	0.39061	42.7697
GO:1902722~positive regulation of prolactin secretion	2	0.6	0.03204	1	0.39061	42.7697
GO:0071307~cellular response to vitamin K	2	0.6	0.03204	1	0.39061	42.7697
GO:0060615~mammary gland bud formation	2	0.6	0.03204	1	0.39061	42.7697
GO:0038145~macrophage colony-stimulating factor signaling pathway	2	0.6	0.03204	1	0.39061	42.7697
GO:0060915~mesenchymal cell differentiation involved in lung development	2	0.6	0.03204	1	0.39061	42.7697
GO:0060667~branch elongation involved in salivary gland morphogenesis	2	0.6	0.03204	1	0.39061	42.7697
GO:0030175~filopodium	5	1.6	0.0321	0.99953	0.28352	34.2809
Serine protease	7	2.2	0.03281	0.99976	0.1595	35.1697
active site:AMP-threonine intermediate	2	0.6	0.03282	1	0.35951	41.6095
region of interest:Nonhelical region (N-terminal)	2	0.6	0.03282	1	0.35951	41.6095
GO:0007179~transforming growth factor beta receptor signaling pathway	5	1.6	0.03314	1	0.39869	43.8758
GO:0035690~cellular response to drug	5	1.6	0.03314	1	0.39869	43.8758
GO:0007204~positive regulation of cytosolic calcium ion concentration	7	2.2	0.03319	1	0.39679	43.9247

GO:0048546~digestive tract morphogenesis	3	1	0.03354	1	0.39771	44.2717
GO:0032331~negative regulation of chondrocyte differentiation	3	1	0.03354	1	0.39771	44.2717
GO:0050930~induction of positive chemotaxis	3	1	0.03354	1	0.39771	44.2717
GO:0051145~smooth muscle cell differentiation	3	1	0.03354	1	0.39771	44.2717
mmu05200:Pathways in cancer	13	4.2	0.03463	0.99858	0.30523	35.3694
GO:0034361~very-low-density lipoprotein particle	3	1	0.03468	0.99975	0.2922	36.4946
SM00198:SCP	3	1	0.03472	0.99518	0.20703	34.454
Disease mutation	5	1.6	0.03573	0.99989	0.16943	37.668
GO:0007173~epidermal growth factor receptor signaling pathway	4	1.3	0.03594	1	0.41719	46.5996
GO:0030334~regulation of cell migration	5	1.6	0.036	1	0.41533	46.6542
GO:0001948~glycoprotein binding	5	1.6	0.03618	1	0.32296	40.5493
mmu05133:Pertussis	5	1.6	0.03618	0.99894	0.30282	36.6404
IPR003961:Fibronectin, type III	8	2.6	0.03649	1	0.28511	42.3465
IPR002126:Cadherin	6	1.9	0.03715	1	0.28603	42.934
GO:0009749~response to glucose	5	1.6	0.03748	1	0.42596	48.0423
IPR008979:Galactose-binding domain-like	5	1.6	0.03776	1	0.28654	43.4633
IPR009003:Trypsin-like cysteine/serine peptidase domain	7	2.2	0.03776	1	0.28317	43.4677
SM00204:TGFB	4	1.3	0.03782	0.99704	0.21541	36.9333
GO:0002062~chondrocyte differentiation	4	1.3	0.038	1	0.4281	48.5241
GO:0048536~spleen development	4	1.3	0.038	1	0.4281	48.5241
repeat:TNFR-Cys 3	3	1	0.03876	1	0.40663	47.1369
IPR008983:Tumour necrosis factor-like domain	4	1.3	0.03879	1	0.28638	44.3563
GO:0042813~Wnt-activated receptor activity	3	1	0.03903	1	0.33726	42.986
GO:0004602~glutathione peroxidase activity	3	1	0.03903	1	0.33726	42.986
repeat:LRR 2	10	3.2	0.03912	1	0.4061	47.4559
repeat:LRR 1	10	3.2	0.03912	1	0.4061	47.4559
IPR015919:Cadherin-like	6	1.9	0.03936	1	0.28659	44.8361
GO:0048661~positive regulation of smooth muscle cell proliferation	5	1.6	0.04055	1	0.44711	50.8084
GO:0071347~cellular response to interleukin-1	5	1.6	0.04055	1	0.44711	50.8084
GO:0014070~response to organic cyclic compound	5	1.6	0.04055	1	0.44711	50.8084
GO:0010596~negative regulation of endothelial cell migration	3	1	0.04078	1	0.44663	51.0138
GO:0061098~positive regulation of protein tyrosine kinase activity	3	1	0.04078	1	0.44663	51.0138
IPR001134:Netrin domain	3	1	0.04082	1	0.29238	46.0736

IPR000834:Peptidase M14, carboxypeptidase A	3	1	0.04082	1	0.29238	46.0736
domain:Peptidase S1	6	1.9	0.04102	1	0.41775	49.1049
Cytoskeleton	3	1	0.04185	0.28967	0.28967	20.8683
GO:0043066~negative regulation of apoptotic process	16	5.1	0.04214	1	0.45524	52.1873
GO:0010862~positive regulation of pathway-restricted SMAD protein phosphorylation	4	1.3	0.04231	1	0.45419	52.3301
GO:0001657~ureteric bud development	4	1.3	0.04231	1	0.45419	52.3301
GO:0010942~positive regulation of cell death	4	1.3	0.04231	1	0.45419	52.3301
GO:0001658~branching involved in ureteric bud morphogenesis	4	1.3	0.04231	1	0.45419	52.3301
domain:EGF-like 8; calcium-binding	3	1	0.04261	1	0.42657	50.4461
IPR000372:Leucine-rich repeat-containing N-terminal	4	1.3	0.04273	1	0.30057	47.6364
GO:0005925~focal adhesion	12	3.8	0.04316	0.99997	0.3395	43.3131
IPR016352:Small leucine-rich proteoglycan, class I, decorin/aspurin/byglycan	2	0.6	0.04335	1	0.30098	48.1419
IPR001500:Alpha-1-acid glycoprotein	2	0.6	0.04335	1	0.30098	48.1419
IPR003410:Hyalin	2	0.6	0.04335	1	0.30098	48.1419
IPR011390:Insulin-like growth factor binding protein-related protein (IGFBP-rP), MAC25	2	0.6	0.04335	1	0.30098	48.1419
GO:0006915~apoptotic process	16	5.1	0.04354	1	0.46159	53.3748
metal ion-binding site:Zinc 1	5	1.6	0.04366	1	0.43111	51.3189
SM00643:C345C	3	1	0.04453	0.99897	0.24052	41.9904
GO:0045600~positive regulation of fat cell differentiation	4	1.3	0.04454	1	0.46699	54.2044
GO:0005588~collagen type V trimer	2	0.6	0.04597	0.99998	0.34645	45.4134
Acute phase	3	1	0.04597	0.99999	0.20966	45.738
GO:0005006~epidermal growth factor-activated receptor activity	2	0.6	0.04655	1	0.38191	48.9706
GO:0035529~NADH pyrophosphatase activity	2	0.6	0.04655	1	0.38191	48.9706
domain:NTR	3	1	0.04659	1	0.4492	53.6668
mmu05144:Malaria	4	1.3	0.04704	0.99987	0.36113	44.9365
GO:0035989~tendon development	2	0.6	0.04767	1	0.48815	56.7059
GO:0048050~post-embryonic eye morphogenesis	2	0.6	0.04767	1	0.48815	56.7059
GO:2000041~negative regulation of planar cell polarity pathway involved in axis elongation	2	0.6	0.04767	1	0.48815	56.7059
GO:0086046~membrane depolarization during SA node cell action potential	2	0.6	0.04767	1	0.48815	56.7059
GO:0002158~osteoclast proliferation	2	0.6	0.04767	1	0.48815	56.7059
GO:0010641~positive regulation of platelet-derived growth factor receptor signaling pathway	2	0.6	0.04767	1	0.48815	56.7059

GO:1903598~positive regulation of gap junction assembly	2	0.6	0.04767	1	0.48815	56.7059
GO:0034447~very-low-density lipoprotein particle clearance	2	0.6	0.04767	1	0.48815	56.7059
GO:0046546~development of primary male sexual characteristics	2	0.6	0.04767	1	0.48815	56.7059
IPR008993:Tissue inhibitor of metalloproteinases-like, OB-fold	3	1	0.04786	1	0.32366	51.6446
GO:0046579~positive regulation of Ras protein signal transduction	3	1	0.04855	1	0.4922	57.3891
GO:0045089~positive regulation of innate immune response	3	1	0.04855	1	0.4922	57.3891
GO:0042311~vasodilation	3	1	0.04855	1	0.4922	57.3891
domain:AMOP	2	0.6	0.04882	1	0.46155	55.3826
repeat:1; approximate	2	0.6	0.04882	1	0.46155	55.3826
domain:Collagen-like 7	2	0.6	0.04882	1	0.46155	55.3826
region of interest:Nonhelical region (C-terminal)	2	0.6	0.04882	1	0.46155	55.3826
region of interest:Nuclease	2	0.6	0.04882	1	0.46155	55.3826
region of interest:Phosphodiesterase	2	0.6	0.04882	1	0.46155	55.3826
domain:Collagen-like 8	2	0.6	0.04882	1	0.46155	55.3826
IPR000859:CUB domain	4	1.3	0.04899	1	0.32657	52.4863
GO:0097190~apoptotic signaling pathway	4	1.3	0.04919	1	0.49443	57.8782
Lipid degradation	5	1.6	0.04939	1	0.21988	48.2138
SM00631:Zn_pept	3	1	0.04977	0.99955	0.25659	45.6846
PIRSF016553:TGF beta-induced protein bIGH3/osteoblast-specific factor 2	2	0.6	0.05028	0.87937	0.87937	37.7952
PIRSF036767:acid sphingomyelinase-like phosphodiesterase	2	0.6	0.05028	0.87937	0.87937	37.7952
repeat:TNFR-Cys 2	3	1	0.0507	1	0.47106	56.7861
repeat:TNFR-Cys 1	3	1	0.0507	1	0.47106	56.7861
IPR013806:Kring-like fold	3	1	0.05153	1	0.33713	54.3336
IPR011641:Tyrosine-protein kinase ephrin type A/B receptor-like	3	1	0.05153	1	0.33713	54.3336
GO:0007219~Notch signaling pathway	6	1.9	0.05224	1	0.51344	60.129
GO:0070509~calcium ion import	3	1	0.05262	1	0.5137	60.4081
Complement pathway	3	1	0.05283	1	0.22969	50.5973
GO:0005891~voltage-gated calcium channel complex	3	1	0.05321	1	0.3787	50.5134
GO:0046872~metal ion binding	65	21	0.05324	1	0.41706	53.8004
repeat:LRR 9	6	1.9	0.05371	1	0.48764	58.9404
GO:0042995~cell projection	18	5.8	0.05393	1	0.37203	50.9901
GO:0004181~metallocarboxypeptidase activity	3	1	0.05442	1	0.41708	54.6065
GO:0008198~ferrous iron binding	3	1	0.05442	1	0.41708	54.6065
domain:EGF-like 9	3	1	0.05494	1	0.49212	59.7935

active site:Charge relay system	8	2.6	0.05504	1	0.48928	59.8625
domain:Fibronectin type-III 2	6	1.9	0.05524	1	0.48713	60.0015
IPR000157:Toll/interleukin-1 receptor homology (TIR) domain	3	1	0.0553	1	0.35375	56.9483
GO:0048705~skeletal system morphogenesis	4	1.3	0.05659	1	0.53774	63.1576
domain:Fibronectin type-III 1	6	1.9	0.0568	1	0.49357	61.0537
GO:0042572~retinol metabolic process	3	1	0.05681	1	0.53673	63.3029
IPR000782:FAS1 domain	2	0.6	0.05738	1	0.36107	58.3374
IPR009017:Green fluorescent protein	2	0.6	0.05738	1	0.36107	58.3374
IPR005533:AMOP	2	0.6	0.05738	1	0.36107	58.3374
IPR006605:G2 nidogen/fibulin G2F	2	0.6	0.05738	1	0.36107	58.3374
IPR008266:Tyrosine-protein kinase, active site	5	1.6	0.05792	1	0.36033	58.6898
GO:0043395~heparan sulfate proteoglycan binding	3	1	0.05856	1	0.43408	57.3279
GO:0004623~phospholipase A2 activity	3	1	0.05856	1	0.43408	57.3279
IPR017850:Alkaline-phosphatase-like, core domain	3	1	0.05916	1	0.36307	59.4826
IPR017849:Alkaline phosphatase-like, alpha/beta/alpha	3	1	0.05916	1	0.36307	59.4826
GO:0070098~chemokine-mediated signaling pathway	4	1.3	0.05917	1	0.54933	64.8453
GO:0009897~external side of plasma membrane	10	3.2	0.0608	1	0.3985	55.3793
GO:0097169~AIM2 inflammasome complex	2	0.6	0.06082	1	0.3883	55.3893
GO:0004551~nucleotide diphosphatase activity	2	0.6	0.06158	1	0.44396	59.2229
GO:0070052~collagen V binding	2	0.6	0.06158	1	0.44396	59.2229
GO:0005111~type 2 fibroblast growth factor receptor binding	2	0.6	0.06158	1	0.44396	59.2229
GO:0004528~phosphodiesterase I activity	2	0.6	0.06158	1	0.44396	59.2229
GO:0031727~CCR2 chemokine receptor binding	2	0.6	0.06158	1	0.44396	59.2229
GO:0030178~negative regulation of Wnt signaling pathway	4	1.3	0.0618	1	0.56311	66.4933
GO:0060348~bone development	4	1.3	0.0618	1	0.56311	66.4933
mmu04010:MAPK signaling pathway	9	2.9	0.06205	0.99999	0.43301	54.7696
mmu05168:Herpes simplex infection	8	2.6	0.06223	0.99999	0.4191	54.8729
domain:EGF-like 4	4	1.3	0.0627	1	0.52571	64.7997
GO:0005504~fatty acid binding	3	1	0.06279	1	0.44366	59.9603
GO:0090175~regulation of establishment of planar polarity	2	0.6	0.06305	1	0.56818	67.249
GO:0060688~regulation of morphogenesis of a branching structure	2	0.6	0.06305	1	0.56818	67.249
GO:0035990~tendon cell differentiation	2	0.6	0.06305	1	0.56818	67.249

GO:1901299~negative regulation of hydrogen peroxide-mediated programmed cell death	2	0.6	0.06305	1	0.56818	67.249
GO:0060279~positive regulation of ovulation	2	0.6	0.06305	1	0.56818	67.249
GO:2000427~positive regulation of apoptotic cell clearance	2	0.6	0.06305	1	0.56818	67.249
GO:0090191~negative regulation of branching involved in ureteric bud morphogenesis	2	0.6	0.06305	1	0.56818	67.249
GO:0045837~negative regulation of membrane potential	2	0.6	0.06305	1	0.56818	67.249
GO:1900020~positive regulation of protein kinase C activity	2	0.6	0.06305	1	0.56818	67.249
SM00608:ACR	4	1.3	0.06311	0.99995	0.30552	54.132
Differentiation	15	4.8	0.06319	1	0.265	57.171
mmu00590:Arachidonic acid metabolism	5	1.6	0.06348	0.99999	0.41162	55.6151
mmu04270:Vascular smooth muscle contraction	6	1.9	0.06363	1	0.3992	55.7007
domain:Sushi 3	3	1	0.06377	1	0.52843	65.44
domain:TIR	3	1	0.06377	1	0.52843	65.44
GO:0007229~integrin-mediated signaling pathway	5	1.6	0.06385	1	0.5705	67.723
m_extrinsicPathway:Extrinsic Prothrombin Activation Pathway	3	1	0.06442	0.99115	0.99115	49.8981
domain:Sushi 17	2	0.6	0.06456	1	0.52952	65.9075
domain:FAS1 2	2	0.6	0.06456	1	0.52952	65.9075
domain:FAS1 1	2	0.6	0.06456	1	0.52952	65.9075
domain:Sushi 20	2	0.6	0.06456	1	0.52952	65.9075
domain:Collagen-like 6	2	0.6	0.06456	1	0.52952	65.9075
region of interest:Collagen-binding	2	0.6	0.06456	1	0.52952	65.9075
domain:Sushi 18	2	0.6	0.06456	1	0.52952	65.9075
domain:Sushi 19	2	0.6	0.06456	1	0.52952	65.9075
domain:Sushi 16	2	0.6	0.06456	1	0.52952	65.9075
domain:FAS1 4	2	0.6	0.06456	1	0.52952	65.9075
domain:FAS1 3	2	0.6	0.06456	1	0.52952	65.9075
IPR013151:Immunoglobulin	4	1.3	0.06521	1	0.38914	63.1775
GO:0045662~negative regulation of myoblast differentiation	3	1	0.06552	1	0.57782	68.6954
GO:0007166~cell surface receptor signaling pathway	8	2.6	0.06558	1	0.57579	68.73
mmu00592:alpha-Linolenic acid metabolism	3	1	0.06606	1	0.39857	57.1029
GO:0004197~cysteine-type endopeptidase activity	4	1.3	0.06612	1	0.45441	61.9192
GO:0008283~cell proliferation	8	2.6	0.06665	1	0.57954	69.3385
GO:0045499~chemorepellent activity	3	1	0.06713	1	0.45294	62.4981

IPR014716:Fibrinogen, alpha/beta/gamma chain, C-terminal globular, subdomain 1	3	1	0.06714	1	0.39472	64.2907
GO:0009611~response to wounding	4	1.3	0.06723	1	0.58046	69.6631
Laminin EGF-like domain	3	1	0.06755	1	0.27661	59.6921
GO:0001889~liver development	5	1.6	0.06794	1	0.58212	70.0591
domain:EGF-like 8	3	1	0.06835	1	0.54718	68.0682
compositionally biased region:Glu-rich	9	2.9	0.07049	1	0.55529	69.2328
domain:Fibronectin type-III 4	4	1.3	0.07118	1	0.55553	69.5974
IPR020436:Somatomedin B, chordata	2	0.6	0.07121	1	0.40987	66.5288
IPR001604:DNA/RNA non-specific endonuclease	2	0.6	0.07121	1	0.40987	66.5288
IPR010600:Inter-alpha-trypsin inhibitor heavy chain, C-terminal	2	0.6	0.07121	1	0.40987	66.5288
IPR011707:Multicopper oxidase, type 3	2	0.6	0.07121	1	0.40987	66.5288
IPR003367:Thrombospondin, type 3-like repeat	2	0.6	0.07121	1	0.40987	66.5288
IPR017897:Thrombospondin, type 3 repeat	2	0.6	0.07121	1	0.40987	66.5288
IPR008859:Thrombospondin, C-terminal	2	0.6	0.07121	1	0.40987	66.5288
IPR015615:Transforming growth factor-beta-related	3	1	0.07126	1	0.40653	66.5576
mmu04621:NOD-like receptor signaling pathway	4	1.3	0.07144	1	0.41155	60.068
GO:0005507~copper ion binding	4	1.3	0.07157	1	0.46846	64.941
SM00063:FRI	3	1	0.07274	0.99999	0.33453	59.4595
GO:0007566~embryo implantation	4	1.3	0.07286	1	0.60633	72.655
Cell junction	15	4.8	0.07398	1	0.29488	63.1583
GO:0005887~integral component of plasma membrane	25	8	0.07422	1	0.44268	62.9207
PIRSF018239:IGFBP-related protein, MAC25 type	2	0.6	0.07448	0.95814	0.79541	50.9488
PIRSF002490:class I small leucine-rich proteoglycan, decorin/asporin/byglycan types	2	0.6	0.07448	0.95814	0.79541	50.9488
PIRSF036899:alpha(1)-acid glycoprotein	2	0.6	0.07448	0.95814	0.79541	50.9488
GO:0086091~regulation of heart rate by cardiac conduction	3	1	0.07463	1	0.61319	73.5373
GO:0055074~calcium ion homeostasis	3	1	0.07463	1	0.61319	73.5373
GO:0072559~NLRP3 inflammasome complex	2	0.6	0.07544	1	0.43786	63.5424
GO:0072558~NLRP1 inflammasome complex	2	0.6	0.07544	1	0.43786	63.5424
IPR023796:Serp domain	4	1.3	0.07545	1	0.42151	68.7208
IPR000215:Serp family	4	1.3	0.07545	1	0.42151	68.7208
GO:0071560~cellular response to transforming growth factor beta stimulus	4	1.3	0.07576	1	0.61659	74.0814
GO:0047429~nucleoside-triphosphate diphosphatase activity	2	0.6	0.07638	1	0.48476	67.4159
Apoptosis	12	3.8	0.07682	1	0.30012	64.5983

GO:0031594~neuromuscular junction	4	1.3	0.07782	1	0.43837	64.732
GO:0003006~developmental process involved in reproduction	2	0.6	0.07819	1	0.62638	75.225
GO:0010453~regulation of cell fate commitment	2	0.6	0.07819	1	0.62638	75.225
GO:0060512~prostate gland morphogenesis	2	0.6	0.07819	1	0.62638	75.225
GO:0010757~negative regulation of plasminogen activation	2	0.6	0.07819	1	0.62638	75.225
GO:0048592~eye morphogenesis	2	0.6	0.07819	1	0.62638	75.225
GO:0001961~positive regulation of cytokine-mediated signaling pathway	2	0.6	0.07819	1	0.62638	75.225
GO:0051150~regulation of smooth muscle cell differentiation	2	0.6	0.07819	1	0.62638	75.225
GO:0006685~sphingomyelin catabolic process	2	0.6	0.07819	1	0.62638	75.225
GO:0060414~aorta smooth muscle tissue morphogenesis	2	0.6	0.07819	1	0.62638	75.225
GO:0071542~dopaminergic neuron differentiation	3	1	0.07934	1	0.6297	75.7495
IPR008996:Cytokine, IL-1-like	3	1	0.07974	1	0.43634	70.8058
repeat:TSP type-3 7	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 3	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 5	2	0.6	0.08004	1	0.59652	73.95
domain:Sushi 14	2	0.6	0.08004	1	0.59652	73.95
domain:Sushi 11	2	0.6	0.08004	1	0.59652	73.95
domain:Sushi 15	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 5	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 1	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 8	2	0.6	0.08004	1	0.59652	73.95
repeat:2-5	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 4	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 1	2	0.6	0.08004	1	0.59652	73.95
domain:Collagen-like 4	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 3	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 4	2	0.6	0.08004	1	0.59652	73.95
domain:TSP C-terminal	2	0.6	0.08004	1	0.59652	73.95
domain:Sushi 12	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 2	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 6	2	0.6	0.08004	1	0.59652	73.95
domain:Collagen-like 5	2	0.6	0.08004	1	0.59652	73.95
repeat:TSP type-3 2	2	0.6	0.08004	1	0.59652	73.95
region of interest:Fatty acid binding	2	0.6	0.08004	1	0.59652	73.95
domain:Plastocyanin-like 6	2	0.6	0.08004	1	0.59652	73.95
domain:Sushi 13	2	0.6	0.08004	1	0.59652	73.95

GO:0042803~protein homodimerization activity	19	6.1	0.08032	1	0.49621	69.3233
GO:0042475~odontogenesis of dentin-containing tooth	4	1.3	0.08169	1	0.63867	76.7915
SM00199:SCY	4	1.3	0.082	1	0.35947	64.0421
PIRSF001191:matrix metalloproteinase, stromelysin type	3	1	0.08241	0.97058	0.69131	54.6828
SM00682:G2F	2	0.6	0.08251	1	0.35172	64.2817
SM00554:FAS1	2	0.6	0.08251	1	0.35172	64.2817
SM00723:AMOP	2	0.6	0.08251	1	0.35172	64.2817
domain:Cadherin 6	3	1	0.0827	1	0.60567	75.1403
IPR000488:Death domain	3	1	0.08409	1	0.45078	72.7851
GO:0032570~response to progesterone	3	1	0.08413	1	0.6477	77.8239
GO:2001244~positive regulation of intrinsic apoptotic signaling pathway	3	1	0.08413	1	0.6477	77.8239
GO:0042517~positive regulation of tyrosine phosphorylation of Stat3 protein	3	1	0.08413	1	0.6477	77.8239
GO:0042327~positive regulation of phosphorylation	3	1	0.08413	1	0.6477	77.8239
IPR023352:Membrane associated eicosanoid/glutathione metabolism-like domain	2	0.6	0.08483	1	0.45017	73.1099
IPR020821:Extracellular Endonuclease, subunit A	2	0.6	0.08483	1	0.45017	73.1099
IPR025155:WxxW domain	2	0.6	0.08483	1	0.45017	73.1099
IPR023827:Peptidase S8, subtilisin, Asp-active site	2	0.6	0.08483	1	0.45017	73.1099
IPR001129:Membrane-associated, eicosanoid/glutathione metabolism (MAPEG) protein	2	0.6	0.08483	1	0.45017	73.1099
IPR017350:Caspase, interleukin-1 beta convertase	2	0.6	0.08483	1	0.45017	73.1099
IPR000557:Calponin repeat	2	0.6	0.08483	1	0.45017	73.1099
SM00255:TIR	3	1	0.08527	1	0.35216	65.5447
GO:0043204~perikaryon	6	1.9	0.08549	1	0.46082	68.3264
GO:0071456~cellular response to hypoxia	5	1.6	0.08563	1	0.65228	78.4404
GO:0046677~response to antibiotic	3	1	0.089	1	0.6649	79.763
GO:0070372~regulation of ERK1 and ERK2 cascade	3	1	0.089	1	0.6649	79.763
GO:0072557~IPAF inflammasome complex	2	0.6	0.08983	1	0.46846	70.2058
GO:0048839~inner ear development	4	1.3	0.09096	1	0.67104	80.4948
domain:Ig-like C2-type 6	3	1	0.09274	1	0.64639	79.1798
domain:Death	3	1	0.09274	1	0.64639	79.1798
GO:0002682~regulation of immune system process	2	0.6	0.09308	1	0.67769	81.2588
GO:0002138~retinoic acid biosynthetic process	2	0.6	0.09308	1	0.67769	81.2588
GO:0010631~epithelial cell migration	2	0.6	0.09308	1	0.67769	81.2588

GO:0048143~astrocyte activation	2	0.6	0.09308	1	0.67769	81.2588
GO:0036006~cellular response to macrophage colony-stimulating factor stimulus	2	0.6	0.09308	1	0.67769	81.2588
GO:0001957~intramembranous ossification	2	0.6	0.09308	1	0.67769	81.2588
GO:1903779~regulation of cardiac conduction	2	0.6	0.09308	1	0.67769	81.2588
GO:2000503~positive regulation of natural killer cell chemotaxis	2	0.6	0.09308	1	0.67769	81.2588
GO:2000052~positive regulation of non-canonical Wnt signaling pathway	2	0.6	0.09308	1	0.67769	81.2588
GO:0006919~activation of cysteine-type endopeptidase activity involved in apoptotic process	4	1.3	0.09414	1	0.67989	81.6326
GO:0030855~epithelial cell differentiation	4	1.3	0.09414	1	0.67989	81.6326
SM00042:CUB	4	1.3	0.09436	1	0.37354	69.4224
GO:0007409~axonogenesis	5	1.6	0.09524	1	0.68217	82.0097
domain:EGF-like 1; atypical	2	0.6	0.09526	1	0.65345	80.0956
glycosylation site:O-linked (Xyl...)(chondroitin sulfate)	2	0.6	0.09526	1	0.65345	80.0956
domain:Sushi 10	2	0.6	0.09526	1	0.65345	80.0956
domain:Ig-like C2-type 9	2	0.6	0.09526	1	0.65345	80.0956
region of interest:B	2	0.6	0.09526	1	0.65345	80.0956
region of interest:Nonhelical region (NC2)	2	0.6	0.09526	1	0.65345	80.0956
domain:Sushi 9	2	0.6	0.09526	1	0.65345	80.0956
domain:EGF-like 3	4	1.3	0.0961	1	0.65354	80.3904
GO:0045669~positive regulation of osteoblast differentiation	4	1.3	0.09737	1	0.68851	82.7226
GO:0016337~single organismal cell-cell adhesion	5	1.6	0.09771	1	0.68775	82.8356
IPR002884:Proprotein convertase, P	2	0.6	0.09826	1	0.49855	78.3973
IPR000020:Anaphylatoxin/fibulin	2	0.6	0.09826	1	0.49855	78.3973
IPR024873:Ectonucleotide pyrophosphatase/phosphodiesterase family	2	0.6	0.09826	1	0.49855	78.3973
IPR014873:Voltage-dependent calcium channel, alpha-1 subunit, IQ domain	2	0.6	0.09826	1	0.49855	78.3973
SM01411:SM01411	3	1	0.09839	1	0.37744	71.0107
GO:0048247~lymphocyte chemotaxis	3	1	0.099	1	0.69064	83.2492
GO:0019731~antibacterial humoral response	3	1	0.099	1	0.69064	83.2492

Supplemental Table 13. GO analysis for the 173 downregulated genes from Supplemental Table 11. Genes downregulated in C/EBP β -overexpressing myoblasts (with ≥ 2 -fold change and $p_{\text{adj}} \leq 0.05$) and in at least 1 type of human cancer from (Robinson et al. 2019).

Term	Count	%	pValue	Bonferroni	Benjamini	FDR
Glycoprotein	72	42	1.78E-14	3.53E-12	3.53E-12	2.22E-11
Secreted	42	24	1.32E-11	2.63E-09	1.32E-09	1.66E-08
GO:0070062~extracellular exosome	57	33	4.76E-11	1.09E-08	1.09E-08	6.10E-08
signal peptide	62	36	2.23E-10	2.36E-07	2.36E-07	3.55E-07
GO:0005576~extracellular region	43	25	5.27E-10	1.21E-07	6.04E-08	6.76E-07
Disulfide bond	56	33	5.43E-10	1.08E-07	3.60E-08	6.80E-07
GO:0030018~Z disc	13	7.6	7.10E-10	1.63E-07	5.42E-08	9.10E-07
Signal	70	41	8.22E-10	1.63E-07	4.09E-08	1.03E-06
disulfide bond	50	29	3.10E-08	3.28E-05	1.64E-05	4.94E-05
glycosylation site:N-linked (GlcNAc...)	62	36	4.20E-08	4.44E-05	1.48E-05	6.69E-05
GO:0006936~muscle contraction	8	4.7	2.48E-07	2.68E-04	2.68E-04	3.96E-04
GO:0030424~axon	16	9.3	7.03E-07	1.61E-04	4.02E-05	9.00E-04
Myosin	7	4.1	1.59E-06	3.16E-04	6.32E-05	1.99E-03
Cell adhesion	16	9.3	2.22E-06	4.42E-04	7.36E-05	2.78E-03
Muscle protein	7	4.1	2.57E-06	5.11E-04	7.31E-05	3.22E-03
GO:0005865~striated muscle thin filament	5	2.9	4.82E-06	1.10E-03	2.21E-04	6.17E-03
GO:0007155~cell adhesion	17	9.9	5.58E-06	6.01E-03	3.01E-03	8.90E-03
Phosphoprotein	86	50	9.12E-06	1.81E-03	2.27E-04	1.14E-02
GO:0005615~extracellular space	31	18	1.07E-05	2.44E-03	4.06E-04	1.36E-02
GO:0005859~muscle myosin complex	4	2.3	1.20E-05	2.75E-03	3.93E-04	1.54E-02
GO:0030016~myofibril	6	3.5	2.22E-05	5.06E-03	6.34E-04	2.84E-02
Actin-binding	11	6.4	2.26E-05	4.49E-03	5.00E-04	2.83E-02
GO:0050839~cell adhesion molecule binding	7	4.1	3.83E-05	1.22E-02	1.22E-02	5.17E-02
Cytoplasm	56	33	4.96E-05	9.82E-03	9.86E-04	6.21E-02
Growth factor	8	4.7	5.12E-05	1.01E-02	9.26E-04	6.41E-02
GO:0031672~A band	5	2.9	5.65E-05	1.29E-02	1.44E-03	7.24E-02
GO:0005856~cytoskeleton	24	14	7.65E-05	1.74E-02	1.75E-03	9.79E-02
GO:0007157~heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	6	3.5	8.92E-05	9.19E-02	3.16E-02	1.42E-01
Calmodulin-binding	8	4.7	9.06E-05	1.79E-02	1.50E-03	1.13E-01

GO:0009986~cell surface	17	9.9	1.02E-04	2.30E-02	2.12E-03	1.30E-01
IPR000742:Epidermal growth factor-like domain	10	5.8	1.17E-04	5.29E-02	5.29E-02	1.67E-01
domain:IQ	6	3.5	1.34E-04	1.32E-01	3.47E-02	2.13E-01
Developmental protein	20	12	1.50E-04	2.93E-02	2.29E-03	1.87E-01
mmu04810:Regulation of actin cytoskeleton	10	5.8	1.52E-04	1.89E-02	1.89E-02	1.75E-01
Mitogen	5	2.9	1.52E-04	2.98E-02	2.16E-03	1.90E-01
Cytoskeleton	21	12	1.77E-04	3.47E-02	2.35E-03	2.22E-01
GO:0003779~actin binding	12	7	2.10E-04	6.51E-02	3.31E-02	2.83E-01
IPR001609:Myosin head, motor domain	5	2.9	2.51E-04	1.10E-01	5.65E-02	3.57E-01
GO:0005178~integrin binding	7	4.1	2.58E-04	7.96E-02	2.73E-02	3.48E-01
GO:0030017~sarcomere	5	2.9	2.73E-04	6.05E-02	5.19E-03	3.49E-01
GO:0008083~growth factor activity	8	4.7	3.04E-04	9.30E-02	2.41E-02	4.10E-01
EGF-like domain	9	5.2	3.09E-04	5.97E-02	3.84E-03	3.87E-01
GO:0008307~structural constituent of muscle	4	2.3	3.47E-04	1.05E-01	2.20E-02	4.68E-01
IPR027401:Myosin-like IQ motif-containing domain	4	2.3	3.64E-04	1.55E-01	5.48E-02	5.17E-01
Motor protein	7	4.1	4.27E-04	8.15E-02	4.99E-03	5.34E-01
IPR002928:Myosin tail	4	2.3	4.30E-04	1.81E-01	4.86E-02	6.11E-01
GO:0030175~filopodium	6	3.5	5.16E-04	1.11E-01	9.05E-03	6.59E-01
GO:0042383~sarcolemma	7	4.1	5.33E-04	1.15E-01	8.68E-03	6.80E-01
SM00242:MYSc	5	2.9	5.46E-04	6.35E-02	6.35E-02	6.25E-01
GO:0034113~heterotypic cell-cell adhesion	4	2.3	5.72E-04	4.61E-01	1.43E-01	9.09E-01
Neurogenesis	9	5.2	5.91E-04	1.11E-01	6.52E-03	7.38E-01
GO:0051781~positive regulation of cell division	5	2.9	6.33E-04	4.95E-01	1.28E-01	1.00E+00
short sequence motif:Cell attachment site	6	3.5	6.70E-04	5.08E-01	1.32E-01	1.06E+00
IPR000048:IQ motif, EF-hand binding site	6	3.5	6.74E-04	2.69E-01	6.07E-02	9.56E-01
GO:0005737~cytoplasm	78	45	7.18E-04	1.52E-01	1.09E-02	9.17E-01
SM00060:FN3	8	4.7	7.36E-04	8.46E-02	4.32E-02	8.42E-01
GO:0030240~skeletal muscle thin filament assembly	3	1.7	7.46E-04	5.54E-01	1.26E-01	1.18E+00
GO:0060048~cardiac muscle contraction	5	2.9	8.10E-04	5.83E-01	1.18E-01	1.28E+00
domain:Fibronectin type-III 2	7	4.1	8.16E-04	5.78E-01	1.34E-01	1.29E+00
domain:Fibronectin type-III 1	7	4.1	8.51E-04	5.93E-01	1.21E-01	1.35E+00
GO:0014704~intercalated disc	5	2.9	9.40E-04	1.94E-01	1.34E-02	1.20E+00
IPR013032:EGF-like, conserved site	8	4.7	9.99E-04	3.71E-01	7.44E-02	1.41E+00
GO:0016459~myosin complex	5	2.9	1.01E-03	2.07E-01	1.35E-02	1.29E+00

GO:0014069~postsynaptic density	9	5.2	1.06E-03	2.16E-01	1.34E-02	1.35E+00
GO:0031674~I band	4	2.3	1.09E-03	2.20E-01	1.30E-02	1.38E+00
GO:0005516~calmodulin binding	8	4.7	1.18E-03	3.15E-01	6.10E-02	1.58E+00
mmu04360:Axon guidance	7	4.1	1.25E-03	1.46E-01	7.59E-02	1.44E+00
mmu04151:PI3K-Akt signaling pathway	11	6.4	1.48E-03	1.70E-01	6.02E-02	1.70E+00
IPR003961:Fibronectin, type III	8	4.7	1.48E-03	4.98E-01	9.38E-02	2.10E+00
GO:0007275~multicellular organism development	20	12	1.64E-03	8.30E-01	1.99E-01	2.59E+00
GO:0044295~axonal growth cone	4	2.3	1.72E-03	3.25E-01	1.95E-02	2.18E+00
GO:0007399~nervous system development	11	6.4	1.80E-03	8.57E-01	1.94E-01	2.83E+00
GO:0044297~cell body	6	3.5	1.83E-03	3.43E-01	1.98E-02	2.32E+00
domain:Myosin head-like	4	2.3	2.08E-03	8.90E-01	2.41E-01	3.26E+00
GO:0045214~sarcomere organization	4	2.3	2.46E-03	9.30E-01	2.34E-01	3.85E+00
IPR010526:Sodium ion transport-associated	3	1.7	2.69E-03	7.13E-01	1.45E-01	3.76E+00
IPR008989:Myosin S1 fragment, N-terminal	3	1.7	2.69E-03	7.13E-01	1.45E-01	3.76E+00
IPR001696:Voltage gated sodium channel, alpha subunit	3	1.7	2.69E-03	7.13E-01	1.45E-01	3.76E+00
mmu04510:Focal adhesion	8	4.7	3.09E-03	3.23E-01	9.30E-02	3.53E+00
IPR003598:Immunoglobulin subtype 2	8	4.7	3.21E-03	7.75E-01	1.53E-01	4.48E+00
GO:0005515~protein binding	52	30	3.94E-03	7.18E-01	1.66E-01	5.19E+00
GO:0048662~negative regulation of smooth muscle cell proliferation	4	2.3	4.41E-03	9.92E-01	3.53E-01	6.81E+00
Cell junction	13	7.6	4.47E-03	5.90E-01	4.58E-02	5.45E+00
GO:0005198~structural molecule activity	8	4.7	5.02E-03	8.01E-01	1.83E-01	6.57E+00
GO:0003774~motor activity	5	2.9	5.25E-03	8.16E-01	1.71E-01	6.86E+00
IPR011993:Pleckstrin homology-like domain	10	5.8	5.39E-03	9.18E-01	2.22E-01	7.41E+00
GO:0022407~regulation of cell-cell adhesion	3	1.7	5.56E-03	9.98E-01	3.95E-01	8.51E+00
IPR004009:Myosin, N-terminal, SH3-like	3	1.7	6.11E-03	9.42E-01	2.28E-01	8.36E+00
GO:0001518~voltage-gated sodium channel complex	3	1.7	6.17E-03	7.58E-01	6.24E-02	7.63E+00
Methylation	16	9.3	6.18E-03	7.09E-01	5.98E-02	7.47E+00
mmu05410:Hypertrophic cardiomyopathy (HCM)	5	2.9	6.25E-03	5.46E-01	1.46E-01	7.01E+00
GO:0051015~actin filament binding	6	3.5	6.33E-03	0.86981	1.84E-01	8.21741
IPR001452:Src homology-3 domain	7	4.1	6.95E-03	9.61E-01	2.36E-01	9.45985
GO:0007173~epidermal growth factor receptor signaling pathway	4	2.3	7.10E-03	1.00E+00	4.47E-01	10.7478
mmu05414:Dilated cardiomyopathy	5	2.9	7.44E-03	6.10E-01	1.45E-01	8.2832
Integrin	4	2.3	7.49E-03	0.77578	6.87E-02	8.98021

GO:0008360~regulation of cell shape	6	3.5	7.53E-03	1.00E+00	4.42E-01	11.3578
GO:0015629~actin cytoskeleton	7	4.1	7.71E-03	0.83001	7.42E-02	9.43802
m_ ephA4Pathway:Eph Kinases and ephrins support platelet aggregation	3	1.7	7.76E-03	0.30662	3.07E-01	7.1328
IPR013111:EGF, extracellular	3	1.7	7.83E-03	0.97397	2.45E-01	10.5993
mmu04012:ErbB signaling pathway	5	2.9	8.76E-03	6.70E-01	1.47E-01	9.68966
site:Cleavage	4	2.3	9.24E-03	0.99995	6.64E-01	13.732
GO:0016020~membrane	76	44	9.36E-03	0.88402	8.59E-02	11.3547
SM00408:IGc2	8	4.7	9.50E-03	6.82E-01	3.17E-01	10.3788
mmu04530:Tight junction	5	2.9	9.85E-03	0.71271	1.44E-01	10.831
GO:0007411~axon guidance	6	3.5	9.99E-03	1.00E+00	5.15E-01	14.7973
Differentiation	12	7	1.01E-02	8.67E-01	8.75E-02	11.9041
mmu04261:Adrenergic signaling in cardiomyocytes	6	3.5	1.04E-02	0.73187	1.36E-01	11.3949
GO:0031954~positive regulation of protein autophosphorylation	3	1.7	1.06E-02	0.99999	5.13E-01	15.6236
mmu00330:Arginine and proline metabolism	4	2.3	1.07E-02	0.74062	0.12624	11.6645
Immunoglobulin domain	10	5.8	1.10E-02	0.88872	9.11E-02	12.9061
GO:0005102~receptor binding	10	5.8	1.10E-02	0.97139	2.76E-01	13.8839
GO:0043292~contractile fiber	3	1.7	1.13E-02	0.92558	9.87E-02	13.5276
Cleavage on pair of basic residues	7	4.1	1.15E-02	0.89974	9.14E-02	13.4758
GO:0007166~cell surface receptor signaling pathway	7	4.1	1.26E-02	1	0.55479	18.3769
SM00326:SH3	7	4.1	1.27E-02	0.78408	3.18E-01	13.6391
GO:0005248~voltage-gated sodium channel activity	3	1.7	1.33E-02	0.9862	3.00E-01	16.4836
SM00015:IQ	4	2.3	1.35E-02	0.80389	0.27806	14.4304
Cell projection	12	7	1.40E-02	0.93987	1.06E-01	16.2152
GO:0030506~ankyrin binding	3	1.7	1.46E-02	0.99099	3.04E-01	17.9693
repeat:III	3	1.7	1.46E-02	1	7.88E-01	20.8235
repeat:II	3	1.7	1.46E-02	1	0.78798	20.8235
repeat:IV	3	1.7	1.46E-02	1	7.88E-01	20.8235
repeat:I	3	1.7	1.46E-02	1	7.88E-01	20.8235
domain:Fibronectin type-III 4	4	2.3	1.46E-02	1	7.57E-01	20.88
GO:0016337~single organismal cell-cell adhesion	5	2.9	1.49E-02	1	0.59433	21.3149
GO:0031430~M band	3	1.7	1.50E-02	0.9685	1.25E-01	17.5887
GO:0043025~neuronal cell body	11	6.4	1.67E-02	0.97873	1.33E-01	19.3785
GO:0030832~regulation of actin filament length	2	1.2	1.74E-02	1	6.32E-01	24.4283
GO:0086043~bundle of His cell action potential	2	1.2	1.74E-02	1	6.32E-01	24.4283
GO:0031594~neuromuscular junction	4	2.3	1.74E-02	0.98225	1.34E-01	20.1903

chain:Myosin-1	2	1.2	1.76E-02	1	7.90E-01	24.5853
Alternative splicing	49	28	1.77E-02	0.97165	0.12807	20.0872
GO:0009987~cellular process	3	1.7	1.85E-02	1	0.63476	25.721
GO:0005886~plasma membrane	55	32	1.87E-02	0.98658	0.13814	21.4315
Sodium channel	3	1.7	1.88E-02	0.97704	0.13045	21.1417
region of interest:Actin-binding	3	1.7	1.88E-02	1	0.78662	26.0868
GO:0001525~angiogenesis	7	4.1	1.88E-02	1	0.62428	26.1729
GO:0005578~proteinaceous extracellular matrix	8	4.7	1.91E-02	0.98796	0.13699	21.9065
propeptide:Removed in mature form	7	4.1	1.92E-02	1	7.68E-01	26.506
GO:0048146~positive regulation of fibroblast proliferation	4	2.3	1.93E-02	1	0.61548	26.6829
mmu04014:Ras signaling pathway	7	4.1	1.99E-02	0.92044	0.20555	20.7562
GO:0030054~cell junction	13	7.6	2.06E-02	0.99147	0.14246	23.3981
GO:0008284~positive regulation of cell proliferation	11	6.4	2.09E-02	1	6.29E-01	28.5782
SH3 domain	6	3.5	2.09E-02	0.98514	0.13957	23.2702
IPR003599:Immunoglobulin subtype	10	5.8	2.24E-02	0.99997	0.52795	27.5837
GO:0008344~adult locomotory behavior	4	2.3	2.25E-02	1	0.64175	30.4873
mmu05214:Glioma	4	2.3	2.27E-02	0.94473	0.21439	23.3655
GO:0035987~endodermal cell differentiation	3	1.7	2.31E-02	1	6.36E-01	31.1081
GO:0045499~chemorepellent activity	3	1.7	2.35E-02	0.99952	0.42057	27.4813
IPR027324:Microtubule associated protein MAP2/MAP4/Tau	2	1.2	2.36E-02	0.99998	0.52167	28.8019
IPR001084:Tau and MAP proteins tubulin-binding repeat	2	1.2	2.36E-02	0.99998	0.52167	28.8019
GO:0043087~regulation of GTPase activity	4	2.3	2.43E-02	1	0.64001	32.4368
mmu05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	2.3	2.46E-02	0.95658	0.2144	25.0474
GO:0086010~membrane depolarization during action potential	3	1.7	2.47E-02	1	0.63287	32.9251
GO:0050840~extracellular matrix binding	3	1.7	2.52E-02	0.99972	0.42065	29.1326
GO:0030425~dendrite	10	5.8	2.52E-02	0.99711	0.16699	27.8982
GO:0007584~response to nutrient	4	2.3	2.61E-02	1	0.63957	34.4111
repeat:Tau/MAP 1	2	1.2	2.63E-02	1	0.84657	34.5094
repeat:Tau/MAP 3	2	1.2	2.63E-02	1	0.84657	34.5094
repeat:Tau/MAP 2	2	1.2	2.63E-02	1	0.84657	34.5094
GO:0045840~positive regulation of mitotic nuclear division	3	1.7	2.64E-02	1	0.63111	34.7462
IPR002957:Keratin, type I	3	1.7	2.64E-02	1	0.54025	31.7375
IPR013320:Concanavalin A-like lectin/glucanase, subgroup	6	3.5	2.65E-02	1	0.51905	31.7601

GO:0046875~ephrin receptor binding	3	1.7	2.69E-02	0.99984	0.4213	30.7938
IPR004045:Glutathione S-transferase, N-terminal	3	1.7	2.80E-02	1	0.51911	33.2814
GO:0043005~neuron projection	9	5.2	2.82E-02	0.99856	0.17992	30.6639
mmu05218:Melanoma	4	2.3	2.86E-02	0.97408	0.22965	28.518
GO:0005272~sodium channel activity	3	1.7	2.87E-02	0.99991	0.42243	32.4618
IPR013098:Immunoglobulin I-set	5	2.9	2.96E-02	1	0.51935	34.7842
domain:Ig-like C2-type 1	5	2.9	2.96E-02	1	0.86275	38.0176
domain:Fibronectin type-III 3	4	2.3	2.97E-02	1	0.84655	38.1012
m_nfatPathway:NFAT and Hypertrophy of the heart (Transcription in the broken heart)	4	2.3	2.97E-02	0.75777	0.50784	24.9143
GO:0045202~synapse	10	5.8	2.98E-02	0.99902	0.1843	32.123
domain:Ig-like C2-type 2	5	2.9	3.03E-02	1	0.83612	38.7408
GO:0001540~beta-amyloid binding	3	1.7	3.05E-02	0.99995	0.42393	34.1335
mmu05200:Pathways in cancer	9	5.2	3.05E-02	0.97979	0.22903	30.1342
GO:0007160~cell-matrix adhesion	4	2.3	3.09E-02	1	0.67758	39.4218
IPR022413:ATP:guanido phosphotransferase, N-terminal	2	1.2	3.13E-02	1	0.52168	36.4249
IPR000749:ATP:guanido phosphotransferase	2	1.2	3.13E-02	1	0.52168	36.4249
IPR026966:Fibronectin type III C-terminal domain	2	1.2	3.13E-02	1	0.52168	36.4249
IPR022414:ATP:guanido phosphotransferase, catalytic domain	2	1.2	3.13E-02	1	0.52168	36.4249
IPR022415:ATP:guanido phosphotransferase active site	2	1.2	3.13E-02	1	0.52168	36.4249
GO:0005887~integral component of plasma membrane	17	9.9	3.18E-02	0.99939	0.19054	33.8945
SM00181:EGF	6	3.5	3.22E-02	0.9804	0.48075	31.351
Cell membrane	39	23	3.28E-02	0.99869	0.20467	34.1588
GO:0030335~positive regulation of cell migration	6	3.5	3.30E-02	1	0.68923	41.4207
GO:0031175~neuron projection development	5	2.9	3.37E-02	1	0.68643	42.1782
GO:0000902~cell morphogenesis	4	2.3	3.40E-02	1	0.67839	42.454
GO:0005154~epidermal growth factor receptor binding	3	1.7	3.42E-02	0.99999	0.44439	37.4767
GO:0070252~actin-mediated cell contraction	2	1.2	3.45E-02	1	0.67249	42.891
GO:1901897~regulation of relaxation of cardiac muscle	2	1.2	3.45E-02	1	0.67249	42.891
GO:0086016~AV node cell action potential	2	1.2	3.45E-02	1	0.67249	42.891
GO:0030035~microspike assembly	2	1.2	3.45E-02	1	0.67249	42.891
GO:0019228~neuronal action potential	3	1.7	3.55E-02	1	0.67212	43.7912
GO:0007596~blood coagulation	4	2.3	3.73E-02	1	0.68073	45.4852

GO:0051017~actin filament bundle assembly	3	1.7	3.74E-02	1	0.67162	45.5661
domain:EGF-like 2	4	2.3	3.83E-02	1	0.88591	46.2527
topological domain:Extracellular	29	17	3.85E-02	1	0.87454	46.4713
GO:0046982~protein heterodimerization activity	10	5.8	3.88E-02	1	0.47056	41.4295
GO:0002027~regulation of heart rate	3	1.7	3.94E-02	1	0.68106	47.3231
GO:0043204~perikaryon	5	2.9	3.99E-02	0.99991	0.22842	40.6834
GO:0031012~extracellular matrix	7	4.1	4.06E-02	0.99993	0.22643	41.2241
GO:0050808~synapse organization	3	1.7	4.14E-02	1	0.69016	49.0599
GO:0042995~cell projection	12	7	4.23E-02	0.99995	0.22951	42.5525
GO:0036336~dendritic cell migration	2	1.2	4.29E-02	1	0.69457	50.3554
GO:0033634~positive regulation of cell-cell adhesion mediated by integrin	2	1.2	4.29E-02	1	0.69457	50.3554
GO:0090131~mesenchyme migration	2	1.2	4.29E-02	1	0.69457	50.3554
GO:0030241~skeletal muscle myosin thick filament assembly	2	1.2	4.29E-02	1	0.69457	50.3554
Proto-oncogene	4	2.3	4.31E-02	0.99984	0.25334	42.396
GO:0004111~creatine kinase activity	2	1.2	4.34E-02	1	0.49223	45.0414
repeat:WD 17	2	1.2	4.34E-02	1	0.89265	50.6128
IPR008266:Tyrosine-protein kinase, active site	4	2.3	4.40E-02	1	0.63	47.3335
IPR003080:Glutathione S-transferase, alpha class	2	1.2	0.04657	1	0.63421	49.3108
IPR014746:Glutamine synthetase/guanido kinase, catalytic domain	2	1.2	0.04657	1	0.63421	49.3108
IPR015497:Epidermal growth factor receptor ligand	2	1.2	0.04657	1	0.63421	49.3108
GO:0007229~integrin-mediated signaling pathway	4	2.3	0.04804	1	0.72696	54.4164
Hormone	4	2.3	0.04842	0.99995	0.27285	46.292
domain:Fibronectin type-III 5	3	1.7	0.04854	1	0.90843	54.694
mmu04512:ECM-receptor interaction	4	2.3	0.04907	0.99824	0.32717	44.1627
Calcium	12	7	0.04913	0.99996	0.26894	46.7862
IPR016201:Plexin-like fold	3	1.7	0.04938	1	0.64001	51.404
mmu04015:Rap1 signaling pathway	6	3.5	0.04957	0.99835	0.31397	44.5002
GO:0048365~Rac GTPase binding	3	1.7	0.05073	1	0.53219	50.4869
domain:GST C-terminal	3	1.7	0.05074	1	0.90866	56.3329
GO:0005925~focal adhesion	8	4.7	0.05112	0.99999	0.26518	48.946
GO:0006929~substrate-dependent cell migration	2	1.2	0.0513	1	0.74218	56.8444
GO:0038033~positive regulation of endothelial cell chemotaxis by VEGF-activated vascular endothelial growth factor receptor signaling pathway	2	1.2	0.0513	1	0.74218	56.8444

GO:0072126~positive regulation of glomerular mesangial cell proliferation	2	1.2	0.0513	1	0.74218	56.8444
IPR010987:Glutathione S-transferase, C-terminal-like	3	1.7	0.05137	1	0.63922	52.8286
repeat:WD 16	2	1.2	0.05182	1	0.90399	57.1129
GO:0007628~adult walking behavior	3	1.7	0.05203	1	0.73899	57.3678
GO:0030027~lamellipodium	5	2.9	0.05231	1	0.26477	49.7572
Synapse	7	4.1	0.05401	0.99998	0.28454	50.1117
GO:0019899~enzyme binding	8	4.7	0.05424	1	0.54085	52.9027
GO:0048013~ephrin receptor signaling pathway	3	1.7	0.05426	1	0.74605	58.9422
m_dbpbPathway:Transcriptional activation of dbpb from mRNA	2	1.2	0.05476	0.92913	0.58617	41.4278
IPR000299:FERM domain	3	1.7	0.05748	1	0.66671	56.9815
IPR019748:FERM central domain	3	1.7	0.05748	1	0.66671	56.9815
IPR019749:Band 4.1 domain	3	1.7	0.05748	1	0.66671	56.9815
GO:0014037~Schwann cell differentiation	2	1.2	0.0596	1	0.77146	62.4856
domain:SH3	5	2.9	0.05976	1	0.92613	62.4873
repeat:WD 15	2	1.2	0.06019	1	0.91983	62.7577
GO:0009898~cytoplasmic side of plasma membrane	3	1.7	0.06109	1	0.29677	55.4055
GO:0010628~positive regulation of gene expression	8	4.7	0.06127	1	0.77372	63.5405
GO:0035556~intracellular signal transduction	8	4.7	0.06193	1	0.77017	63.9444
GO:0016324~apical plasma membrane	7	4.1	0.06291	1	0.29831	56.5002
GO:0007010~cytoskeleton organization	4	2.3	0.06298	1	0.76892	64.5834
GO:0015631~tubulin binding	3	1.7	0.06465	1	0.59095	59.4378
GO:0030336~negative regulation of cell migration	4	2.3	0.0659	1	0.77776	66.3052
IPR011992:EF-hand-like domain	6	3.5	0.06648	1	0.707	62.475
SM00409:IG	10	5.8	0.06661	0.99974	0.69325	54.6746
GO:0008088~axo-dendritic transport	2	1.2	0.06782	1	0.78091	67.3897
GO:0010642~negative regulation of platelet-derived growth factor receptor signaling pathway	2	1.2	0.06782	1	0.78091	67.3897
GO:0060371~regulation of atrial cardiac muscle cell membrane depolarization	2	1.2	0.06782	1	0.78091	67.3897
GO:0048739~cardiac muscle fiber development	2	1.2	0.06782	1	0.78091	67.3897
GO:0048729~tissue morphogenesis	2	1.2	0.06782	1	0.78091	67.3897
IPR027359:Voltage-dependent potassium channel, four helix bundle domain	3	1.7	0.0682	1	0.70295	63.4493
domain:Ig-like 5	2	1.2	0.06849	1	0.9378	67.6598

IPR000072:Platelet-derived growth factor (PDGF)	2	1.2	0.06903	1	0.69437	63.9138
Hydrolase	19	11	0.07158	1	0.35252	60.5479
GO:0045121~membrane raft	6	3.5	0.07467	1	0.33854	63.0002
domain:EGF-like 1	4	2.3	0.07524	1	0.94781	71.1957
GO:0001778~plasma membrane repair	2	1.2	0.07596	1	0.81262	71.6528
GO:0043569~negative regulation of insulin-like growth factor receptor signaling pathway	2	1.2	0.07596	1	0.81262	71.6528
IPR015812:Integrin beta subunit	2	1.2	0.0764	1	0.71965	67.7786
IPR014766:Carboxypeptidase, regulatory domain	2	1.2	0.0764	1	0.71965	67.7786
GO:0008093~cytoskeletal adaptor activity	2	1.2	0.07671	1	0.64115	65.9583
GO:0008081~phosphoric diester hydrolase activity	3	1.7	0.0771	1	0.62865	66.1508
GO:0030198~extracellular matrix organization	4	2.3	0.0782	1	0.81597	72.7252
GO:0001934~positive regulation of protein phosphorylation	5	2.9	0.07823	1	0.81014	72.7419
GO:0005882~intermediate filament	4	2.3	0.0789	1	0.34802	65.1091
Guanine-nucleotide releasing factor	4	2.3	0.07957	1	0.37589	64.5979
Cell envelope biogenesis, outer membrane	2	1.2	0.07997	0.39354	0.39354	32.8956
GO:0030154~cell differentiation	12	7	0.08046	1	0.81348	73.7752
GO:0043406~positive regulation of MAP kinase activity	3	1.7	0.08087	1	0.80936	73.9598
GO:0004714~transmembrane receptor protein tyrosine kinase activity	3	1.7	0.08228	1	0.63969	68.6259
SM00295:B41	3	1.7	0.08289	0.99997	0.72691	62.9652
GO:0070374~positive regulation of ERK1 and ERK2 cascade	5	2.9	0.08294	1	0.81202	74.8831
Voltage-gated channel	4	2.3	0.08378	1	0.38348	66.5726
GO:0043653~mitochondrial fragmentation involved in apoptotic process	2	1.2	0.08404	1	0.81078	75.3589
GO:0060710~chorio-allantoic fusion	2	1.2	0.08404	1	0.81078	75.3589
GO:0070886~positive regulation of calcineurin-NFAT signaling cascade	2	1.2	0.08404	1	0.81078	75.3589
IPR018039:Intermediate filament protein, conserved site	3	1.7	0.08419	1	0.74339	71.4389
IPR009030:Insulin-like growth factor binding protein, N-terminal	4	2.3	0.08438	1	0.73273	71.5252
SM00141:PDGF	2	1.2	0.08472	0.99998	0.69282	63.8036
domain:Ig-like 4	2	1.2	0.08487	1	0.96054	75.6138
GO:0001756~somitogenesis	3	1.7	0.08607	1	0.81313	76.213
GO:0016477~cell migration	5	2.9	0.0867	1	0.81019	76.4768
Thick filament	2	1.2	0.08684	1	0.3865	67.9433

m_biopeptidesPathway:Bioactive Peptide Induced Signaling Pathway	3	1.7	0.08771	0.98662	0.65992	58.1834
Kinase	10	5.8	0.08772	1	0.38169	68.328
IPR013151:Immunoglobulin	3	1.7	0.08895	1	0.74095	73.4828
GO:0044325~ion channel binding	4	2.3	0.09007	1	0.6611	72.0369
Glycosyltransferase	5	2.9	0.09095	1	0.38525	69.7037
IPR001759:Pentaxin	2	1.2	0.09097	1	0.73845	74.3111
IPR008969:Carboxypeptidase-like, regulatory domain	2	1.2	0.09097	1	0.73845	74.3111
IPR017891:Insulin-like growth factor binding protein, N-terminal, Cys-rich conserved site	2	1.2	0.09097	1	0.73845	74.3111
IPR000906:ZU5	2	1.2	0.09097	1	0.73845	74.3111
ATP-binding	16	9.3	0.09118	1	0.37852	69.8007
GO:0043588~skin development	3	1.7	0.09136	1	0.82203	78.3197
GO:0009611~response to wounding	3	1.7	0.09136	1	0.82203	78.3197
GO:0004713~protein tyrosine kinase activity	4	2.3	0.09179	1	0.65551	72.7423
GO:0051428~peptide hormone receptor binding	2	1.2	0.09295	1	0.64791	73.2095
GO:0016772~transferase activity, transferring phosphorus-containing groups	2	1.2	0.09295	1	0.64791	73.2095
domain:Olfactomedin-like	2	1.2	0.09295	1	0.96785	78.8242
IPR012336:Thioredoxin-like fold	4	2.3	0.09353	1	0.7382	75.3227
IPR018114:Peptidase S1, trypsin family, active site	4	2.3	0.09513	1	0.73427	75.9351
topological domain:Cytoplasmic	33	19	0.09585	1	0.96779	79.8749
GO:0016323~basolateral plasma membrane	5	2.9	0.09716	1	0.40556	73.0031
IPR004001:Actin, conserved site	2	1.2	0.09817	1	0.73601	77.0627
mmu04670:Leukocyte transendothelial migration	4	2.3	0.09825	1	0.51516	69.8098
region of interest:Linker 12	3	1.7	0.09842	1	0.96736	80.7662
GO:0051764~actin crosslink formation	2	1.2	0.09999	1	0.8454	81.3812
GO:0055003~cardiac myofibril assembly	2	1.2	0.09999	1	0.8454	81.3812
GO:0001818~negative regulation of cytokine production	2	1.2	0.09999	1	0.8454	81.3812

Supplemental Table 14. C/EBP β regulates cytokines protein expression in myoblasts and in Lewis lung carcinoma cells. Raw density quantification of blots from the Proteome Profiler Mouse XL Cytokine Array in C2C12-pLX, C2C12- β , LLC-shCtl and LLC-sh β .

Cytokines Array: C2C12s			
SPOTS PAIRS	C2C12plx	C2C12β	Log2 β/pLX
(G11-G12)(IL-17A)	528.93	4770.41	3.172965
(F9-F10)(IL-1 beta/ IL-1F2)	1554.56	8705.89	2.485485
(F13-F14)(IL-2)	648.66	3411.87	2.395028
(F15-F16)(IL-3)	1459.3	6404.5	2.133809
(H7-H8)(LIX)	6037.13	25838.6	2.097593
(H21-H22)(Osteoprotegerin/ TNFRSF11B)	1305.34	5156.66	1.982011
(C19-C20)(CXCL1/KC)	8774.67	30059.56	1.776407
(I21-I22)(Resistin)	1545.19	5015.6	1.698638
(G9-G10)(IL-15)	3067.92	9210.09	1.585954
(C11-C12)(Complement Component C5/C5a)	7999.76	22206.8	1.472973
(H11-H12)(MMP-2)	8592.3	23837.19	1.472098
(E21-E22)(IGFBP-1)	8061.36	22363.06	1.472022
(E9-E10)(G-CSF)	2081.93	5714.56	1.456721
(E7-E8)(Gas 6)	4078.76	11126.07	1.447742
(F21-F22)(IL-6)	1643.38	4322.97	1.395357
(F11-F12)(IL-1ra/IL-1F3)	23808.32	61908.68	1.378676
(A3-A4)(Adiponectin/Acrp30)	5014.48	12791.6	1.351025
(E17-E18)(ICAM-1/CD54)	5091.4	12148.3	1.25462
(F7-F8)(IL-1 alpha/IL-1F1)	6101.35	13931.1	1.191109
(I7-I8)(Periostin/OSF-2)	2741.26	6160.32	1.168166
(H13-H14)(MMP-3)	1539.97	3161.19	1.037566
(D7-D8)(CXCL13/BLC/BCA-1)	3862.37	7583.82	0.973438
(E13-E14)(GM-CSF)	2819.08	5523.74	0.970421
(I5-I6)(Pentraxin 3/ TSG-14)	11261.6	21340.16	0.922159
(I9-I10)(Pref-1/DLK-1/FA1)	7186.86	13577.82	0.917818
(D23-D24)(Fetuin A/AHSG)	15350.84	27575.84	0.845087
(H9-H10)(M-CSF)	24383.45	42951.37	0.816802
(E19-E20)(IFN-gamma)	5199.87	9138.34	0.813457
(G7-G8)(IL-13)	3990.85	7010.97	0.812918
(F23-F24)(IL-7)	16507.19	28941.73	0.810057
(F19-F20)(IL-5)	5349.78	9249.03	0.789823
(J9-J10)(Serpin F1/PEDF)	11578.88	19716.2	0.767886
(G17-G18)(IL-27p28)	8132.18	13738.15	0.756474
(D9-D10)(CXCL16)	5417.55	9064.55	0.742595

(J19-J20)(VEGF)	15448.89	25580.94	0.727566
(D15-D16)(DPPIV/CD26)	7846.81	12914.35	0.718797
(A15-A16)(C1q R1/CD93)	6483.38	10654.06	0.716585
(C7-C8)(Chitinase 3-like 1)	4948.62	8027.76	0.697971
(E11-E12)(GDF-15)	14613.19	23422.25	0.680609
(D19-D20)(Endoglin/CD105)	2047.97	3207.69	0.64734
(D17-D18)(EGF)	4660.89	7170.96	0.621561
(E5-E6)(Flt-3 Ligand)	5594.21	8485.6	0.601082
(J11-J12)(Thrombopoietin)	4746.77	7158.8	0.592772
(B7-B8)(CCL12/MCP-5)	13175.81	19757.43	0.584504
(D5-D6)(CXCL11/I-TAC)	13329.1	19842.48	0.574013
(J21-J22)(WISP-1/CCN4)	40565.92	59949.53	0.56348
(F17-F18)(IL-4)	5593.95	8221.72	0.555573
(D13-D14)(DKK-1)	6989.88	10261.07	0.553842
(A21-A22)(CCL5/RANTES)	9715.22	14177.68	0.545303
(J13-J14)(TIM-1/KIM-1/ HAVCR)	3938.96	5747.57	0.545137
(H23-H24)(PD-ECGF/ Thymidine phosphorylase)	5081.43	7367.99	0.536037
(D3-D4)(CXCL10/IP-10)	7058.63	10011.54	0.504204
(C5-C6)(Chemerin)	8257.53	11640.04	0.495314
(B5-B6)(CCL11/Eotaxin)	8882.98	12497.58	0.492533
(J17-J18)(VCAM-1/CD106)	35702.51	50173.35	0.490896
(J15-J16)(TNF-alpha)	5312.15	7388.11	0.475909
(B9-B10)(CCL17/TARC)	12648.32	17397.6	0.459943
(I1-I2)(PDGF-BB)	13067.31	17880.7	0.452441
(G21-G22)(IL-33)	13738.69	18757.83	0.449248
(D1-D2)(CXCL9/MIG)	8898.02	11838.37	0.411914
(C13-C14)(Complement Factor D)	5671.08	7396.18	0.383157
(G23-G24)(LDL R)	27685.63	35988.88	0.378414
(I15-I16)(RAGE)	4911.15	6344.14	0.369364
(E1-E2)(FGF acidic)	5343.06	6866.64	0.361938
(G13-G14)(IL-22)	1861.3	2375.95	0.352194
(F1-F2)(IGFBP-3)	12896.83	16423.08	0.348708
(A7-A8)(Angiopoietin-1)	14580.02	18342.64	0.331209
(I19-I20)(Reg3G)	7415.64	9307.54	0.327829
(G15-G16)(IL-23)	3636.63	4553.16	0.324266
(C3-C4)(CD160)	8125.92	10167.47	0.323358
(G1-G2)(IL-10)	10387.18	12977.06	0.32116
(I3-I4)(Pentraxin 2/SAP)	6345.26	7807.83	0.299242
(G5-G6)(IL-12p40)	5125.56	6296.25	0.296783
(H5-H6)(Lipocalin-2/NGAL)	1699.3	2077.54	0.289936
(G3-G4)(IL-11)	13492.53	16432.15	0.28436

(I13-I14)(Proprotein Convertase 9/ PCSK9)	11917.92	14492.66	0.28219
(E3-E4)(FGF-21)	12410.28	15034.68	0.276758
(H3-H4)(LIF)	16998.44	20317.56	0.257325
(D11-D12)(Cystatin C)	41812.29	49643.51	0.247678
(C9-C10)(Coagulation Factor III/ Tissue Factor)	54354.47	63677.08	0.228375
(B3-B4)(CCL6/C10)	15535.29	18091.57	0.219768
(E15-E16)(HGF)	53499	62057.24	0.214088
(J7-J8)(Serpine E1/PAI-1)	54196.93	61454.32	0.181303
(F5-F6)(IGFBP-6)	40237.24	45411.85	0.174537
(G19-G20)(IL-28)	38032.54	42873.61	0.172856
(H19-H20)(Osteopontin (OPN))	48589.28	54469.1	0.1648
(B15-B16)(CCL21/6Ckine)	4932.48	5510.17	0.159784
(I17-I18)(RBP4)	7794.14	8704.65	0.159396
(A9-A10)(Angiopoietin-2)	4386.99	4855.78	0.146472
(J3-J4)(E-Selectin/CD62E)	12670.36	13853.11	0.128752
(B19-B20)(CD14)	11393.11	12128.63	0.090255
(A11-A12)(Angiopoietin-like 3)	10815.2	11344.98	0.068994
(B11-B12)(CCL19/MIP-3 beta)	13237.21	13851.04	0.065395
(J5-J6)(P-Selectin/CD62P)	33604.1	34988.94	0.058262
(B21-B22)(CD40/TNFRSF5)	52053.55	52949.25	0.024614
(E23-E24)(IGFBP-2)	63543.64	63517.27	-0.0006
(H17-H18)(Myeloperoxidase)	8102.55	8009.89	-0.01659
(C17-C18)(CX3CL1/Fractalkine)	8650.33	8277.25	-0.0636
(A17-A18)(CCL2/JE/MCP-1)	8047.14	7628.29	-0.07712
(A5-A6)(Amphiregulin)	17219.82	16283.7	-0.08064
(I11-I12)(Proliferin)	48375.31	45349.63	-0.09318
(C21-C22)(CXCL2/MIP-2)	7978.21	7454.27	-0.098
(A13-A14)(BAFF/BLyS/TNFSF13B)	5467.67	5106.43	-0.09861
(H1-H2)(Leptin)	9274.86	8423.6	-0.13889
(B13-B14)(CCL20/MIP-3 alpha)	10897.21	9818.52	-0.15038
(F3-F4)(IGFBP-5)	59017.32	52380.96	-0.1721
(B17-B18)(CCL22/MDC)	13358.37	11787.27	-0.18051
(D21-D22)(Endostatin)	44641.92	38098.4	-0.22867
(A19-A20)(CCL3/CCL4 MIP-1 alpha/beta)	7368.13	6042.87	-0.28606
(H15-H16)(MMP-9)	867.26	432.65	-1.00326
(C15-C16)(C-Reactive Protein/CRP)	388.47	89.63	-2.11575
(J23-J24)(NC)	0	0	
(A23-A24)(Reference Spots)	63738.9	63683.56	
(A1-A2)(Reference Spots)	64056.7	63683.64	
(J1-J2)(Reference Spots)	64086.39	63681.96	

Cytokines Array: LLCs			
SPOTS PAIRS	LLC-shCtl	LLC-sh β	Log2 sh β /shCtl
(H13-H14)(MMP-3)	5340.91	1529.08	-1.80442
(G13-G14)(IL-22)	580.59	249.41	-1.219
(I21-I22)(Resistin)	721.71	320.26	-1.17218
(G3-G4)(IL-11)	2367.14	1163.45	-1.02474
(J21-J22)(WISP-1/CCN4)	2545.68	1258.33	-1.01654
(C3-C4)(CD160)	1371.28	687.61	-0.99586
(J13-J14)(TIM-1/KIM-1/ HAVCR)	1190.47	618.98	-0.94357
(H21-H22)(Osteoprotegerin/ TNFRSF11B)	1037.2	540.26	-0.94097
(F15-F16)(IL-3)	513.37	269.89	-0.92763
(J9-J10)(Serpin F1/PEDF)	3629.34	2065.27	-0.81338
(B15-B16)(CCL21/6CKine)	1711.2	1002.46	-0.77146
(G15-G16)(IL-23)	1195.73	708.39	-0.75528
(G5-G6)(IL-12p40)	1269.75	755.03	-0.74994
(A15-A16)(C1q R1/CD93)	2359.77	1436.97	-0.71562
(I1-I2)(PDGF-BB)	4587.41	2822.16	-0.70088
(A13-A14)(BAFF/BLyS/TNFSF13B)	2129.02	1309.78	-0.70086
(E3-E4)(FGF-21)	2551.85	1594.81	-0.67816
(J11-J12)(Thrombopoietin)	1706.02	1072.41	-0.66978
(A5-A6)(Amphiregulin)	23051.52	14755.54	-0.64361
(F13-F14)(IL-2)	222.88	142.79	-0.64237
(I3-I4)(Pentraxin 2/SAP)	1779.64	1151.86	-0.62762
(C11-C12)(Complement Component C5/C5a)	1723.04	1135.11	-0.60212
(A3-A4)(Adiponectin/Acrp30)	2414.96	1603.12	-0.59112
(A11-A12)(Angiopoietin-like 3)	2258.5	1502.59	-0.58791
(E13-E14)(GM-CSF)	906.51	606.86	-0.57896
(G11-G12)(IL-17A)	230.22	155.21	-0.56879
(G1-G2)(IL-10)	1378.3	932.85	-0.56317
(I9-I10)(Pref-1/DLK-1/FA1)	2644.05	1792.65	-0.56066
(C13-C14)(Complement Factor D)	935.14	634.87	-0.55872
(D3-D4)(CXCL10/IP-10)	2210.93	1509.93	-0.55017
(D23-D24)(Fetuin A/AHSG)	3549.37	2429.95	-0.54664
(G7-G8)(IL-13)	1407.04	980.68	-0.52081
(G17-G18)(IL-27p28)	1639.6	1150.05	-0.51165
(G21-G22)(IL-33)	2398.84	1683.78	-0.51063
(C9-C10)(Coagulation Factor III/ Tissue Factor)	37071.62	26381	-0.49082
(D15-D16)(DPPIV/CD26)	1778.69	1282.56	-0.47179
(H15-H16)(MMP-9)	305.35	220.35	-0.47067

(J15-J16)(TNF-alpha)	1481.3	1073.85	-0.46407
(G9-G10)(IL-15)	1305.71	950.98	-0.45735
(D9-D10)(CXCL16)	2021.7	1473.5	-0.45632
(F5-F6)(IGFBP-6)	15385.45	11452.89	-0.42585
(I13-I14)(Proprotein Convertase 9/ PCSK9)	2161.1	1610.44	-0.42431
(D21-D22)(Endostatin)	14802.84	11078.35	-0.41813
(B11-B12)(CCL19/MIP-3 beta)	2656.12	1991.28	-0.41562
(H1-H2)(Leptin)	1217.75	918.47	-0.40691
(E23-E24)(IGFBP-2)	2200.42	1661.44	-0.40534
(A17-A18)(CCL2/JE/MCP-1)	2137.62	1626.17	-0.39453
(A7-A8)(Angiopoietin-1)	1576.72	1199.97	-0.39393
(E15-E16)(HGF)	1046.43	803.6	-0.38093
(F3-F4)(IGFBP-5)	1389.35	1071.94	-0.37419
(D13-D14)(DKK-1)	1879.27	1450.35	-0.37377
(I15-I16)(RAGE)	1404.29	1091.14	-0.364
(H17-H18)(Myeloperoxidase)	2586.97	2026.55	-0.35224
(B13-B14)(CCL20/MIP-3 alpha)	1470.99	1157.72	-0.3455
(I7-I8)(Periostin/OSF-2)	899.66	711.3	-0.33892
(A19-A20)(CCL3/CCL4 MIP-1 alpha/beta)	1585.66	1262.72	-0.32855
(B19-B20)(CD14)	2997.39	2400.47	-0.32039
(D7-D8)(CXCL13/BLC/BCA-1)	1579.76	1269.3	-0.31567
(F9-F10)(IL-1 beta/ IL-1F2)	1208.01	974.31	-0.31018
(G23-G24)(LDL R)	9619.76	7761.83	-0.3096
(I11-I12)(Proliferin)	3467.46	2801.4	-0.30773
(F23-F24)(IL-7)	3224.76	2619.22	-0.30005
(B3-B4)(CCL6/C10)	5066.68	4116.36	-0.29967
(D5-D6)(CXCL11/I-TAC)	3024.6	2469.21	-0.29269
(I17-I18)(RBP4)	5514.28	4517.25	-0.28773
(H9-H10)(M-CSF)	5671.7	4652.47	-0.28578
(F17-F18)(IL-4)	1167.21	962.34	-0.27845
(F21-F22)(IL-6)	504.66	423.39	-0.25332
(J19-J20)(VEGF)	1821.66	1532.41	-0.24945
(A9-A10)(Angiopoietin-2)	955.19	808.79	-0.24002
(C7-C8)(Chitinase 3-like 1)	1191.8	1011.32	-0.2369
(C21-C22)(CXCL2/MIP-2)	997.87	855.19	-0.22261
(F1-F2)(IGFBP-3)	4037.54	3486.43	-0.21173
(I19-I20)(Reg3G)	2496.88	2166.2	-0.20496
(C19-C20)(CXCL1/KC)	1289.36	1119.39	-0.20394
(H3-H4)(LIF)	3448.9	3008.62	-0.19703
(E7-E8)(Gas 6)	1851.94	1634.39	-0.18029
(D11-D12)(Cystatin C)	21150.23	18676.97	-0.17941

(F7-F8)(IL-1 alpha/IL-1F1)	2036.91	1805.83	-0.17372
(H11-H12)(MMP-2)	6853.3	6084.89	-0.17157
(H23-H24)(PD-ECGF/ Thymidine phosphorylase)	1946.09	1728.4	-0.17114
(E9-E10)(G-CSF)	1905.31	1694.35	-0.16929
(B21-B22)(CD40/TNFRSF5)	1556.11	1386.71	-0.16628
(F11-F12)(IL-1ra/IL-1F3)	1796.81	1626.85	-0.14336
(D17-D18)(EGF)	1351.82	1228.37	-0.13816
(B9-B10)(CCL17/TARC)	2698.41	2455.61	-0.13603
(C17-C18)(CX3CL1/Fractalkine)	8321.52	7627.54	-0.12563
(E21-E22)(IGFBP-1)	1627.22	1493.49	-0.12372
(D1-D2)(CXCL9/MIG)	1652.68	1520.02	-0.12072
(E5-E6)(Flt-3 Ligand)	1643.45	1516.69	-0.1158
(C5-C6)(Chemerin)	1469.24	1364.64	-0.10655
(J5-J6)(P-Selectin/CD62P)	1389.24	1290.45	-0.10642
(H19-H20)(Osteopontin (OPN))	38154.77	35628.56	-0.09883
(E17-E18)(ICAM-1/CD54)	1278.1	1198.6	-0.09265
(E1-E2)(FGF acidic)	1287.13	1223.42	-0.07324
(J17-J18)(VCAM-1/CD106)	25290.44	24063.35	-0.07175
(D19-D20)(Endoglin/CD105)	38667.95	37401.21	-0.04805
(I5-I6)(Pentraxin 3/ TSG-14)	2402.19	2329.54	-0.04431
(H7-H8)(LIX)	1748.36	1713.24	-0.02928
(B7-B8)(CCL12/MCP-5)	3722.14	3661.55	-0.02368
(B5-B6)(CCL11/Eotaxin)	1936.91	1906.48	-0.02285
(J3-J4)(E-Selectin/CD62E)	4535.65	4533.52	-0.00068
(F19-F20)(IL-5)	1925.08	2012	0.063712
(H5-H6)(Lipocalin-2/NGAL)	634.46	715.68	0.173785
(E11-E12)(GDF-15)	1960.62	2258.52	0.204068
(B17-B18)(CCL22/MDC)	1821.07	2106.2	0.209856
(J7-J8)(Serpin E1/PAI-1)	5566.14	6640.35	0.254582
(E19-E20)(IFN-gamma)	2235.52	2832.11	0.341267
(G19-G20)(IL-28)	8732.79	11857.88	0.441332
(A21-A22)(CCL5/RANTES)	786.94	1069.86	0.443096
(C15-C16)(C-Reactive Protein/CRP)	49.94	176.27	1.819519
(J23-J24)(NC)	0	0	
(A1-A2)(Reference Spots)	63203.97	63697.55	
(A23-A24)(Reference Spots)	62986.01	63659.25	
(J1-J2)(Reference Spots)	63656.23	62838.34	

Supplemental Table 15. Significantly upregulated genes in proliferating and differentiating C/EBP β -overexpressing C2C12s as compared to controls. p.adj ≤ 0.05 .

<i>Sept6</i>	<i>Adam23</i>	<i>Alox8</i>	<i>Aspscr1</i>	<i>Btg3</i>
<i>1110012L19Rik</i>	<i>Adamts1</i>	<i>Alpk1</i>	<i>Ass1</i>	<i>Btla</i>
<i>1700017B05Rik</i>	<i>Adamts10</i>	<i>Alpl</i>	<i>Atl3</i>	<i>Clqtnf1</i>
<i>1700034E13Rik</i>	<i>Adamts12</i>	<i>Ampd3</i>	<i>Atoh8</i>	<i>Clqtnf12</i>
<i>2010315B03Rik</i>	<i>Adamts16</i>	<i>Anapc13</i>	<i>Atp2b4</i>	<i>Clqtnf6</i>
<i>2210013O21Rik</i>	<i>Adamts2</i>	<i>Angpt1</i>	<i>Atp2c1</i>	<i>Clra</i>
<i>2510009E07Rik</i>	<i>Adamts3</i>	<i>Angpt4</i>	<i>Atp6v0d2</i>	<i>Clrb</i>
<i>2610008E11Rik</i>	<i>Adamts5</i>	<i>Angptl7</i>	<i>Atp8b1</i>	<i>Clrl</i>
<i>2810021J22Rik</i>	<i>Adamts6</i>	<i>Ankrd55</i>	<i>Atp8b4</i>	<i>Clis1</i>
<i>3830406C13Rik</i>	<i>Adamts9</i>	<i>Ankzf1</i>	<i>Atxn1</i>	<i>Clis2</i>
<i>4930402H24Rik</i>	<i>Adamts14</i>	<i>Anp32a</i>	<i>Atxn7l1</i>	<i>C3</i>
<i>4930486L24Rik</i>	<i>Adarb1</i>	<i>Antxr1</i>	<i>Auh</i>	<i>Cables1</i>
<i>5730409E04Rik</i>	<i>Adcy3</i>	<i>Aoc3</i>	<i>Auts2</i>	<i>Cacna1a</i>
<i>6030419C18Rik</i>	<i>Adcy4</i>	<i>Aox1</i>	<i>Avpr1a</i>	<i>Cacna1d</i>
<i>6030458C11Rik</i>	<i>Adcy6</i>	<i>Apba1</i>	<i>AW011738</i>	<i>Cacna1g</i>
<i>A230050P20Rik</i>	<i>Adgb</i>	<i>Apbb1ip</i>	<i>Azin2</i>	<i>Cacng7</i>
<i>A530084C06Rik</i>	<i>Adgra2</i>	<i>Apcdd1</i>	<i>B230217C12Rik</i>	<i>Cald1</i>
<i>A930018M24Rik</i>	<i>Adgrf4</i>	<i>Apln</i>	<i>B2m</i>	<i>Calm2</i>
<i>Abat</i>	<i>Adgrg3</i>	<i>Aplp1</i>	<i>B3galnt1</i>	<i>Calr</i>
<i>Abca1</i>	<i>Adgrl2</i>	<i>Apobec1</i>	<i>B3galt1</i>	<i>Camk2n1</i>
<i>Abca8a</i>	<i>Adgrl4</i>	<i>Apoc1</i>	<i>B3glct</i>	<i>Camkk2</i>
<i>Abca9</i>	<i>Adhl</i>	<i>Apoe</i>	<i>B3gnt9</i>	<i>Camp</i>
<i>Abcb1b</i>	<i>Adm</i>	<i>Apol10b</i>	<i>Bace1</i>	<i>Camta2</i>
<i>Abcc1</i>	<i>Adpgk</i>	<i>Apol6</i>	<i>Bach2</i>	<i>Capn15</i>
<i>Abcc10</i>	<i>Adra1b</i>	<i>Arap3</i>	<i>Bbs2</i>	<i>Capn6</i>
<i>Abcc4</i>	<i>Adra1d</i>	<i>Arfgap1</i>	<i>BC017158</i>	<i>Car11</i>
<i>Abcd2</i>	<i>Aebp1</i>	<i>Arfgef2</i>	<i>BC028528</i>	<i>Car9</i>
<i>Abcg1</i>	<i>Aff3</i>	<i>Arhgap24</i>	<i>Bcl9l</i>	<i>Carmil1</i>
<i>Abhd14b</i>	<i>Agap2</i>	<i>Arhgap26</i>	<i>Bdh2</i>	<i>Casp1</i>
<i>Abhd18</i>	<i>Agt</i>	<i>Arhgap42</i>	<i>Bdkrb1</i>	<i>Casp12</i>
<i>Abhd3</i>	<i>Ahcyl1</i>	<i>Arhgef19</i>	<i>Best1</i>	<i>Casp14</i>
<i>Acaal1a</i>	<i>Ahrr</i>	<i>Arhgef25</i>	<i>Bgn</i>	<i>Casp4</i>
<i>Acad11</i>	<i>Ak4</i>	<i>Arid3a</i>	<i>Bicc1</i>	<i>Cav1</i>
<i>Acadsb</i>	<i>Akna</i>	<i>Arl4d</i>	<i>Birc2</i>	<i>Cblb</i>
<i>Ackr4</i>	<i>Akr1c12</i>	<i>Arnt2</i>	<i>Bmf</i>	<i>Cbr1</i>
<i>Acot1</i>	<i>Akr1c13</i>	<i>Arrb1</i>	<i>Bmp1</i>	<i>Cbr2</i>
<i>Acp5</i>	<i>Akr1c14</i>	<i>Arrdc4</i>	<i>Bmp4</i>	<i>Cbs</i>

<i>Acpp</i>	<i>Akr1c18</i>	<i>Arsj</i>	<i>Bmpr1b</i>	<i>Cbx4</i>
<i>Acsf2</i>	<i>Aldh1a3</i>	<i>Arvcf</i>	<i>Bnc1</i>	<i>Cc2d1a</i>
<i>Acs11</i>	<i>Aldh111</i>	<i>Arxes2</i>	<i>Boc</i>	<i>Ccdc102a</i>
<i>Actg2</i>	<i>Aldh6a1</i>	<i>Asb2</i>	<i>Brinp3</i>	<i>Ccdc130</i>
<i>Acvrl1</i>	<i>Aldh7a1</i>	<i>Aspg</i>	<i>Brsk1</i>	<i>Ccdc50</i>
<i>Adam12</i>	<i>Aldoc</i>	<i>Asphd2</i>	<i>Btd</i>	<i>Ccdc60</i>
<i>Ccdc65</i>	<i>Clec2d</i>	<i>Crim1</i>	<i>Dio2</i>	<i>Elov15</i>
<i>Ccdc80</i>	<i>Clip2</i>	<i>Crlf1</i>	<i>Disp1</i>	<i>Emb</i>
<i>Ccdc85a</i>	<i>Clip3</i>	<i>Crocc2</i>	<i>Dixdc1</i>	<i>Emid1</i>
<i>Ccdc85b</i>	<i>Clk1</i>	<i>Crtap</i>	<i>Dkk3</i>	<i>Emilin1</i>
<i>Cck</i>	<i>Clk4</i>	<i>Csad</i>	<i>Dlc1</i>	<i>Emilin2</i>
<i>Ccl2</i>	<i>Clmp</i>	<i>Csf1</i>	<i>Dlg3</i>	<i>Enah</i>
<i>Ccl7</i>	<i>Clu</i>	<i>Csf2rb</i>	<i>Dlg4</i>	<i>Enox1</i>
<i>Ccl9</i>	<i>Cnn1</i>	<i>Csf2rb2</i>	<i>Dlgap2</i>	<i>Enpep</i>
<i>Ccng2</i>	<i>Cnrip1</i>	<i>Csrp2</i>	<i>Dlx1</i>	<i>Enpp1</i>
<i>Cd14</i>	<i>Cntn1</i>	<i>Cst3</i>	<i>Dlx2</i>	<i>Enpp2</i>
<i>Cd1d1</i>	<i>Cntnap1</i>	<i>Ctgf</i>	<i>Dnase2a</i>	<i>Enpp3</i>
<i>Cd24a</i>	<i>Col11a1</i>	<i>Ctla2a</i>	<i>Dnmt3l</i>	<i>Epb41l1</i>
<i>Cd302</i>	<i>Col12a1</i>	<i>Ctla2b</i>	<i>Dnpep</i>	<i>Epb41l3</i>
<i>Cd33</i>	<i>Col16a1</i>	<i>Ctps2</i>	<i>Dock10</i>	<i>Epha1</i>
<i>Cd47</i>	<i>Col1a1</i>	<i>Ctsf</i>	<i>Dock4</i>	<i>Ephb6</i>
<i>Cd55</i>	<i>Col1a2</i>	<i>Ctsf</i>	<i>Dok4</i>	<i>Ephx2</i>
<i>Cd59a</i>	<i>Col27a1</i>	<i>Ctso</i>	<i>Dpep1</i>	<i>Epm2a</i>
<i>Cd74</i>	<i>Col3a1</i>	<i>Cux1</i>	<i>Dph5</i>	<i>Erap1</i>
<i>Cd93</i>	<i>Col4a1</i>	<i>Cx3cl1</i>	<i>Dpt</i>	<i>Erbin</i>
<i>Cdh11</i>	<i>Col4a2</i>	<i>Cxadr</i>	<i>Dram1</i>	<i>Ereg</i>
<i>Cdh23</i>	<i>Col5a1</i>	<i>Cxcl1</i>	<i>Draxin</i>	<i>Ermard</i>
<i>Cdk14</i>	<i>Col5a2</i>	<i>Cxcl12</i>	<i>Dse</i>	<i>Erv3</i>
<i>Cdk5rap3</i>	<i>Col5a3</i>	<i>Cxcl14</i>	<i>Dstn</i>	<i>Esr1</i>
<i>Cdnf</i>	<i>Col6a1</i>	<i>Cxcl16</i>	<i>Dtwd1</i>	<i>Esyt2</i>
<i>Cebpa</i>	<i>Col6a2</i>	<i>Cxcl5</i>	<i>Dusp23</i>	<i>Esyt3</i>
<i>Cebpb</i>	<i>Col6a3</i>	<i>Cxx1c</i>	<i>Dzip1</i>	<i>Ets1</i>
<i>Cercam</i>	<i>Col6a4</i>	<i>Cyb5a</i>	<i>E130311K13Rik</i>	<i>Etv1</i>
<i>Ces1g</i>	<i>Col8a1</i>	<i>Cyld</i>	<i>Ebf1</i>	<i>Eva1a</i>
<i>Ces2c</i>	<i>Colec12</i>	<i>Cyp11b1</i>	<i>Ece2</i>	<i>Eva1b</i>
<i>Ces2g</i>	<i>Colgalt1</i>	<i>Cyp1b1</i>	<i>Echdc2</i>	<i>Evc2</i>
<i>Cfap100</i>	<i>Copg1</i>	<i>Cyp2d22</i>	<i>Echdc3</i>	<i>Ext1</i>
<i>Cfap161</i>	<i>Copz2</i>	<i>Cyp2f2</i>	<i>Echs1</i>	<i>F3</i>
<i>Cfap46</i>	<i>Cotl1</i>	<i>Cys1</i>	<i>Ecm1</i>	<i>F5</i>
<i>Cfap69</i>	<i>Cp</i>	<i>Dact1</i>	<i>Ecm2</i>	<i>F7</i>

<i>Cfh</i>	<i>Cpa6</i>	<i>Dchs1</i>	<i>Ednra</i>	<i>Fabp4</i>
<i>Chd9</i>	<i>Cpeb3</i>	<i>Dchs2</i>	<i>Efemp1</i>	<i>Fabp7</i>
<i>Chid1</i>	<i>Cped1</i>	<i>Dclk3</i>	<i>Efemp2</i>	<i>Fam105a</i>
<i>Chpf2</i>	<i>Cpm</i>	<i>Dcxr</i>	<i>Efs</i>	<i>Fam110c</i>
<i>Chrdl1</i>	<i>Cpne8</i>	<i>Ddx58</i>	<i>Egfr</i>	<i>Fam114a1</i>
<i>Chst12</i>	<i>Cpq</i>	<i>Defb42</i>	<i>Ehbp1</i>	<i>Fam118a</i>
<i>Chst15</i>	<i>Cpt1a</i>	<i>Dennd2d</i>	<i>Ehd2</i>	<i>Fam129a</i>
<i>Chsy3</i>	<i>Cpt1c</i>	<i>Dennd4a</i>	<i>Ehd3</i>	<i>Fam131b</i>
<i>Cilp</i>	<i>Cpz</i>	<i>Dhrs3</i>	<i>Eid1</i>	<i>Fam13a</i>
<i>Cited2</i>	<i>Creb3l1</i>	<i>Dhrs7</i>	<i>Ell2</i>	<i>Fam161a</i>
<i>Clca2</i>	<i>Creb3l2</i>	<i>Dhx58</i>	<i>Eln</i>	<i>Fam167b</i>
<i>Fam168a</i>	<i>Foxq1</i>	<i>Gngl1</i>	<i>Hectd2</i>	<i>Il17rc</i>
<i>Fam181b</i>	<i>Foxred2</i>	<i>Gngl2</i>	<i>Heph</i>	<i>Il17re</i>
<i>Fam193b</i>	<i>Frs2</i>	<i>Gng2</i>	<i>Hfe</i>	<i>Il18rap</i>
<i>Fam20a</i>	<i>Fstl1</i>	<i>Got2</i>	<i>Hgf</i>	<i>Il1r1</i>
<i>Fam26e</i>	<i>Fto</i>	<i>Gpc6</i>	<i>Hif1a</i>	<i>Il1rap</i>
<i>Fam3c</i>	<i>Fuom</i>	<i>Gpm6b</i>	<i>Hivep3</i>	<i>Il1rl1</i>
<i>Fam43a</i>	<i>Fuz</i>	<i>Gpr153</i>	<i>Hlf</i>	<i>Il1rn</i>
<i>Fam46a</i>	<i>Fzd1</i>	<i>Gpr176</i>	<i>Hmcn2</i>	<i>Il6ra</i>
<i>Fam69b</i>	<i>Fzd4</i>	<i>Gpr180</i>	<i>Hmgcll1</i>	<i>Il6st</i>
<i>Farp1</i>	<i>Fzd8</i>	<i>Gpr35</i>	<i>Hoxa11</i>	<i>Inhba</i>
<i>Fas</i>	<i>Gab1</i>	<i>Gprin3</i>	<i>Hoxa3</i>	<i>Inhbb</i>
<i>Fat1</i>	<i>Gadd45gip1</i>	<i>Gpsm3</i>	<i>Hoxc6</i>	<i>Inpp5k</i>
<i>Fat2</i>	<i>Gas1</i>	<i>Gpx3</i>	<i>Hoxc8</i>	<i>Insc</i>
<i>Fat4</i>	<i>Gas6</i>	<i>Grb14</i>	<i>Hp</i>	<i>Ints6l</i>
<i>Fbln1</i>	<i>Gata2</i>	<i>Greb1l</i>	<i>Hpgd</i>	<i>Irak2</i>
<i>Fbln2</i>	<i>Gata6</i>	<i>Grem1</i>	<i>Hps4</i>	<i>Irak3</i>
<i>Fbln5</i>	<i>Gatb</i>	<i>Grhl1</i>	<i>Hpse</i>	<i>Irf2</i>
<i>Fbln7</i>	<i>Gbp9</i>	<i>Grin2d</i>	<i>Hsd11b1</i>	<i>Isg20</i>
<i>Fbn1</i>	<i>Gcnt2</i>	<i>Grk5</i>	<i>Hsd17b11</i>	<i>Islr</i>
<i>Fbxl20</i>	<i>Gda</i>	<i>Grpr</i>	<i>Htr7</i>	<i>Ism1</i>
<i>Fbxw9</i>	<i>Gdf6</i>	<i>Gsdmd</i>	<i>Htra1</i>	<i>Itfg1</i>
<i>Fcgr3</i>	<i>Gdpd2</i>	<i>Gstm2</i>	<i>Htra3</i>	<i>Itga1</i>
<i>Fcgrt</i>	<i>Gem</i>	<i>Gstt1</i>	<i>Icam5</i>	<i>Itga11</i>
<i>Fcor</i>	<i>Gfra4</i>	<i>Gucy2d</i>	<i>Icosl</i>	<i>Itga5</i>
<i>Fcrlb</i>	<i>Ggcx</i>	<i>Gucy2e</i>	<i>Id1</i>	<i>Itga8</i>
<i>Fdxr</i>	<i>Ggn</i>	<i>Gulp1</i>	<i>Id3</i>	<i>Itih2</i>
<i>Fgf10</i>	<i>Ggt5</i>	<i>H2-D1</i>	<i>Id4</i>	<i>Itih5</i>
<i>Fgf2</i>	<i>Ggt7</i>	<i>H2-DMa</i>	<i>Idh1</i>	<i>Itm2b</i>
<i>Fgf7</i>	<i>Gimap9</i>	<i>H2-DMb1</i>	<i>Idua</i>	<i>Itm2c</i>

<i>Fgfr2</i>	<i>Ginm1</i>	<i>H2-DMb2</i>	<i>Ifi202b</i>	<i>Itpkb</i>
<i>Fignl2</i>	<i>Gjb5</i>	<i>H2-K1</i>	<i>Ifi204</i>	<i>Itp2</i>
<i>Fkbp10</i>	<i>Glb1l</i>	<i>H2-Q1</i>	<i>Ifnar2</i>	<i>Izumo4</i>
<i>Fkbp11</i>	<i>Gli2</i>	<i>H2-Q2</i>	<i>Igf1</i>	<i>Jag1</i>
<i>Fkbp14</i>	<i>Glis1</i>	<i>H2-Q4</i>	<i>Igfbp4</i>	<i>Jak1</i>
<i>Fkbp7</i>	<i>Glis3</i>	<i>H2-Q6</i>	<i>Igfbp6</i>	<i>Kalrn</i>
<i>Fkbp9</i>	<i>Glra3</i>	<i>H2-Q7</i>	<i>Igfbp7</i>	<i>Kank4</i>
<i>Flt3l</i>	<i>Glrx</i>	<i>H2-T22</i>	<i>Igsf10</i>	<i>Kat6b</i>
<i>Fmo1</i>	<i>Glul</i>	<i>H2-T23</i>	<i>Igsf3</i>	<i>Kazald1</i>
<i>Fmo2</i>	<i>Gm10638</i>	<i>H2afj</i>	<i>Ikbip</i>	<i>Kcna4</i>
<i>Fmod</i>	<i>Gm2115</i>	<i>H6pd</i>	<i>Ikbke</i>	<i>Kcnab2</i>
<i>Fndc3b</i>	<i>Gm8909</i>	<i>Hacd4</i>	<i>Ikf2</i>	<i>Kcnc2</i>
<i>Fosl2</i>	<i>Gm996</i>	<i>Hao1</i>	<i>Il11ra1</i>	<i>Kcnd1</i>
<i>Foxc1</i>	<i>Gmppa</i>	<i>Hbp1</i>	<i>Il13ra1</i>	<i>Kcnj15</i>
<i>Foxf2</i>	<i>Gmppb</i>	<i>Hccs</i>	<i>Il13ra2</i>	<i>Kcnj16</i>
<i>Foxp1</i>	<i>Gnao1</i>	<i>Hdac7</i>	<i>Il16</i>	<i>Kcnj8</i>
<i>Kcnmb1</i>	<i>Lmo4</i>	<i>Mcfd2</i>	<i>Myl9</i>	<i>Npy1r</i>
<i>Kcnn1</i>	<i>Lmod1</i>	<i>Mcpt1</i>	<i>Mylk</i>	<i>Nr2f2</i>
<i>Kcnt2</i>	<i>Lmtk3</i>	<i>Mdfr</i>	<i>Mylk3</i>	<i>Nrep</i>
<i>Kctd1</i>	<i>Lonp2</i>	<i>Mdga1</i>	<i>Myo1d</i>	<i>Nrg1</i>
<i>Kctd15</i>	<i>Lox</i>	<i>Mdm4</i>	<i>Myo5a</i>	<i>Nrg2</i>
<i>Kdelc2</i>	<i>Loxl1</i>	<i>Medag</i>	<i>N4bp1</i>	<i>Nrn1</i>
<i>Kdelr2</i>	<i>Loxl3</i>	<i>Megf6</i>	<i>N4bp2l1</i>	<i>Nrp1</i>
<i>Kdelr3</i>	<i>Lpl</i>	<i>Mettl7a1</i>	<i>N6amt1</i>	<i>Nsg1</i>
<i>Kdm5b</i>	<i>Lrch2</i>	<i>Mfap2</i>	<i>Naaa</i>	<i>Nsun3</i>
<i>Khk</i>	<i>Lrig3</i>	<i>Mfap5</i>	<i>Naalad2</i>	<i>Nt5e</i>
<i>Kif1a</i>	<i>Lrp1</i>	<i>Mgarp</i>	<i>Naaladl2</i>	<i>Nucb1</i>
<i>Kif21b</i>	<i>Lrp5</i>	<i>Mgat1</i>	<i>Nacad</i>	<i>Nucb2</i>
<i>Kif26b</i>	<i>Lrpap1</i>	<i>Mgst1</i>	<i>Nacc2</i>	<i>Nxf3</i>
<i>Kif5a</i>	<i>Lrrc32</i>	<i>Mgst2</i>	<i>Nadk2</i>	<i>Nxph2</i>
<i>Kif7</i>	<i>Lrrc45</i>	<i>Micall2</i>	<i>Naip2</i>	<i>Nynrin</i>
<i>Klf15</i>	<i>Lrrc4c</i>	<i>Mlxip</i>	<i>Naip5</i>	<i>Oat</i>
<i>Klf9</i>	<i>Lrrc75b</i>	<i>Mmaa</i>	<i>Napepld</i>	<i>Ogn</i>
<i>Klhl22</i>	<i>Lrrk2</i>	<i>Mmab</i>	<i>Ncf4</i>	<i>Olfr13</i>
<i>Klhl33</i>	<i>Ltbp1</i>	<i>Mmd</i>	<i>Ndst3</i>	<i>Olfr810</i>
<i>Klhl6</i>	<i>Ltbp2</i>	<i>Mmp13</i>	<i>Nedd4</i>	<i>Olr1</i>
<i>Klra2</i>	<i>Ltbp3</i>	<i>Mmp14</i>	<i>Nfat5</i>	<i>Omd</i>
<i>Kmt2e</i>	<i>Ltbr</i>	<i>Mmp19</i>	<i>Nfatc1</i>	<i>Oplah</i>
<i>Kng1</i>	<i>Ltc4s</i>	<i>Mmp2</i>	<i>Nfatc4</i>	<i>Orai2</i>
<i>Kng2</i>	<i>Lxn</i>	<i>Mmp23</i>	<i>Nfib</i>	<i>Orc6</i>

<i>Krba1</i>	<i>Ly6d</i>	<i>Mmp3</i>	<i>Nfil3</i>	<i>Orm1</i>
<i>Ksr1</i>	<i>Lypd6b</i>	<i>Mmp8</i>	<i>Nfix</i>	<i>Orm2</i>
<i>Lama4</i>	<i>Lysmd2</i>	<i>Mn1</i>	<i>Nfkbia</i>	<i>Orm3</i>
<i>Lamb1</i>	<i>Lyst</i>	<i>Mocs1</i>	<i>Nhs</i>	<i>Osmr</i>
<i>Lamc1</i>	<i>Lyz2</i>	<i>Mogat2</i>	<i>Nid2</i>	<i>Otor</i>
<i>Larp6</i>	<i>Maf</i>	<i>Mpp2</i>	<i>Nkd2</i>	<i>Otx1</i>
<i>Layn</i>	<i>Maged2</i>	<i>Mppe1</i>	<i>Nkx2-5</i>	<i>Oxtr</i>
<i>Lcp1</i>	<i>Man2b1</i>	<i>Mr1</i>	<i>Nlrc5</i>	<i>P2rx6</i>
<i>Ldhb</i>	<i>Man2b2</i>	<i>Mrc2</i>	<i>Nlrp4e</i>	<i>P2rx7</i>
<i>Lgals3bp</i>	<i>Man2c1</i>	<i>Mrgprf</i>	<i>Nlrx1</i>	<i>P3h1</i>
<i>Lgals9</i>	<i>Map3k12</i>	<i>Mri1</i>	<i>Nnmt</i>	<i>P3h3</i>
<i>Lgalsl</i>	<i>Map3k5</i>	<i>Ms4a4d</i>	<i>Nod1</i>	<i>P4ha2</i>
<i>Lgr4</i>	<i>Mapk13</i>	<i>Msrb3</i>	<i>Nol3</i>	<i>P4ha3</i>
<i>Lhfpl2</i>	<i>Mapk1ip1</i>	<i>Mst1</i>	<i>Nol4l</i>	<i>P4hb</i>
<i>Lifr</i>	<i>Mark1</i>	<i>Mthfr</i>	<i>Nos1</i>	<i>Pak3</i>
<i>Lilrb4a</i>	<i>Masp1</i>	<i>Mtmr11</i>	<i>Notum</i>	<i>Paox</i>
<i>Lipn</i>	<i>Matn2</i>	<i>Mturn</i>	<i>Npas4</i>	<i>Papss2</i>
<i>Lix1l</i>	<i>Mbd6</i>	<i>Mxd4</i>	<i>Nphp3</i>	<i>Paqr7</i>
<i>Lman1</i>	<i>Mbnl3</i>	<i>Mxra8</i>	<i>Npr2</i>	<i>Parm1</i>
<i>Lmcd1</i>	<i>Mbp</i>	<i>Mycbp2</i>	<i>Npr3</i>	<i>Parp10</i>
<i>Lmo2</i>	<i>Mc5r</i>	<i>Myf5</i>	<i>Nptn</i>	<i>Parp12</i>
<i>Parp14</i>	<i>Pitpnc1</i>	<i>Ppp1r3c</i>	<i>Rapgef3</i>	<i>Rspo1</i>
<i>Parp16</i>	<i>Pkd2</i>	<i>Ppp1r9b</i>	<i>Rarres2</i>	<i>Rspo2</i>
<i>Parp8</i>	<i>Pkp2</i>	<i>Ppp3ca</i>	<i>Rasgrf2</i>	<i>Rspry1</i>
<i>Parp9</i>	<i>Pla1a</i>	<i>Prdm1</i>	<i>Rasl11a</i>	<i>Rsrp1</i>
<i>Pawr</i>	<i>Pla2g15</i>	<i>Prdx2</i>	<i>Rasl12</i>	<i>Rtn4rl1</i>
<i>Pax7</i>	<i>Pla2g2d</i>	<i>Prdx5</i>	<i>Rassf2</i>	<i>Rtn4rl2</i>
<i>Pcbp3</i>	<i>Pla2g5</i>	<i>Prg4</i>	<i>Rassf5</i>	<i>Rtp4</i>
<i>Pcdh18</i>	<i>Pla2r1</i>	<i>Prickle1</i>	<i>Rassf6</i>	<i>Runx2</i>
<i>Pcolce</i>	<i>Plat</i>	<i>Prickle2</i>	<i>Rbak</i>	<i>Rxra</i>
<i>Pcsk2</i>	<i>Plb1</i>	<i>Prickle3</i>	<i>Rbl2</i>	<i>Ryk</i>
<i>Pcsk6</i>	<i>Plcb1</i>	<i>Prkd1</i>	<i>Rbm43</i>	<i>S100a8</i>
<i>Pcyt2</i>	<i>Plcb4</i>	<i>Prokr1</i>	<i>Rbp1</i>	<i>S1pr1</i>
<i>Pde10a</i>	<i>Plcd1</i>	<i>Pros1</i>	<i>Rbpms</i>	<i>Saa3</i>
<i>Pde1a</i>	<i>Plce1</i>	<i>Prr5l</i>	<i>Rcn1</i>	<i>Samd14</i>
<i>Pde3a</i>	<i>Pld3</i>	<i>Prr7</i>	<i>Rcn3</i>	<i>Samd9l</i>
<i>Pde4a</i>	<i>Plek</i>	<i>Prrc1</i>	<i>Rdh12</i>	<i>Sardh</i>
<i>Pde4d</i>	<i>Plekha6</i>	<i>Prrg1</i>	<i>Reck</i>	<i>Sash1</i>
<i>Pde7b</i>	<i>Plekhg4</i>	<i>Prrx2</i>	<i>Rffl</i>	<i>Scara3</i>
<i>Pdgfra</i>	<i>Plekhn1</i>	<i>Psd</i>	<i>Rgag1</i>	<i>Scarf2</i>

<i>Pdgfrb</i>	<i>Plin1</i>	<i>Psmb8</i>	<i>Rgag4</i>	<i>Scg2</i>
<i>Pdgfrl</i>	<i>Plin4</i>	<i>Psmb9</i>	<i>Rgcc</i>	<i>Scn1b</i>
<i>Pdk1</i>	<i>Plod1</i>	<i>Ptch1</i>	<i>Rgl1</i>	<i>Scp2</i>
<i>Pdlim2</i>	<i>Plod2</i>	<i>Ptch2</i>	<i>Rgs11</i>	<i>Sdc1</i>
<i>Pdpn</i>	<i>Plod3</i>	<i>Ptchd4</i>	<i>Rgs4</i>	<i>Sdc2</i>
<i>Pdxk</i>	<i>Plpp3</i>	<i>Ptger1</i>	<i>Rhbdl3</i>	<i>Sdc3</i>
<i>Peak1</i>	<i>Plpp5</i>	<i>Ptges</i>	<i>Rhof</i>	<i>Sdf4</i>
<i>Peg10</i>	<i>Plppr2</i>	<i>Ptgfr</i>	<i>Rhoj</i>	<i>Sec16b</i>
<i>Peli3</i>	<i>Plxdc2</i>	<i>Ptk7</i>	<i>Rin2</i>	<i>Sec22b</i>
<i>Per2</i>	<i>Plxnd1</i>	<i>Ptn</i>	<i>Ripk2</i>	<i>Sec24d</i>
<i>Per3</i>	<i>Pmp22</i>	<i>Ptpru</i>	<i>Rnase10</i>	<i>Sec31a</i>
<i>Pex5l</i>	<i>Pnp</i>	<i>Ptx3</i>	<i>Rnase2a</i>	<i>Selenop</i>
<i>Pfkfb2</i>	<i>Pnrc1</i>	<i>Pxylp1</i>	<i>Rnase4</i>	<i>Selenos</i>
<i>Pfkfb3</i>	<i>Podnl1</i>	<i>Pygo1</i>	<i>Rnaset2a</i>	<i>Sema3f</i>
<i>Pfkl</i>	<i>Podxl2</i>	<i>Pyroxd2</i>	<i>Rnd3</i>	<i>Sema4a</i>
<i>Pgghg</i>	<i>Polm</i>	<i>Qsox1</i>	<i>Rnf149</i>	<i>Sema6d</i>
<i>Phf21a</i>	<i>Postn</i>	<i>Rab11fip2</i>	<i>Rnf150</i>	<i>Serhl</i>
<i>Phyhd1</i>	<i>Ppard</i>	<i>Rab20</i>	<i>Rnf167</i>	<i>Serpina12</i>
<i>Pi15</i>	<i>Ppbp</i>	<i>Rab23</i>	<i>Rnf19b</i>	<i>Serpina3g</i>
<i>Pi16</i>	<i>Ppcdc</i>	<i>Rab2b</i>	<i>Rnf207</i>	<i>Serpine1</i>
<i>Pianp</i>	<i>Ppef2</i>	<i>Rab32</i>	<i>Ror1</i>	<i>Serpine2</i>
<i>Pidl</i>	<i>Ppib</i>	<i>Rab39b</i>	<i>Ror2</i>	<i>Serpinf1</i>
<i>Piezo2</i>	<i>Ppic</i>	<i>Rab3il1</i>	<i>Rora</i>	<i>Serping1</i>
<i>Pik3cd</i>	<i>Ppm1m</i>	<i>Rab6b</i>	<i>Rpgrip11</i>	<i>Sesn3</i>
<i>Pik3ip1</i>	<i>Ppox</i>	<i>Ranbp31</i>	<i>Rrbp1</i>	<i>Sfmbt2</i>
<i>Pink1</i>	<i>Ppp1r26</i>	<i>Rap1gap</i>	<i>Rsad1</i>	<i>Sfrp1</i>
<i>Sfrp2</i>	<i>Slc6a17</i>	<i>Srm</i>	<i>Tgfbi</i>	<i>Tnfrsf1b</i>
<i>Sh2d6</i>	<i>Slc6a2</i>	<i>Srpx2</i>	<i>Tgfbr1</i>	<i>Tnfrsf21</i>
<i>Sh3bp1</i>	<i>Slc7a2</i>	<i>Ssbp2</i>	<i>Tgfbr3</i>	<i>Tnfsf12</i>
<i>Sh3bp5</i>	<i>Slc7a7</i>	<i>Ssc5d</i>	<i>Tgfbr3l</i>	<i>Tnpo2</i>
<i>Sh3gl1</i>	<i>Slc8a1</i>	<i>Ssr4</i>	<i>Tgm2</i>	<i>Tnrc18</i>
<i>Sh3gl3</i>	<i>Slit2</i>	<i>St5</i>	<i>Tgm5</i>	<i>Tns3</i>
<i>Sh3rf2</i>	<i>Slpi</i>	<i>St6galnac6</i>	<i>Thbd</i>	<i>Tns4</i>
<i>Sh3rf3</i>	<i>Smad1</i>	<i>St8sia4</i>	<i>Thbs1</i>	<i>Tnxb</i>
<i>Shc1</i>	<i>Smad6</i>	<i>Stard5</i>	<i>Thbs2</i>	<i>Tpcn2</i>
<i>Shox2</i>	<i>Smad7</i>	<i>Stard8</i>	<i>Thra</i>	<i>Tpsab1</i>
<i>Shpk</i>	<i>Smad9</i>	<i>Stat1</i>	<i>Thsd1</i>	<i>Tpst1</i>
<i>Siah1a</i>	<i>Smarca2</i>	<i>Stau1</i>	<i>Thsd7a</i>	<i>Trabd2b</i>
<i>Sidt2</i>	<i>Smg6</i>	<i>Stc1</i>	<i>Tiam2</i>	<i>Traf3ip2</i>
<i>Siglecg</i>	<i>Smoc2</i>	<i>Steap1</i>	<i>Tifa</i>	<i>Traf5</i>

<i>Sik2</i>	<i>Smpd3</i>	<i>Steap2</i>	<i>Timp3</i>	<i>Trafd1</i>
<i>Sirpa</i>	<i>Smpdl3a</i>	<i>Stk24</i>	<i>Tkt</i>	<i>Trhde</i>
<i>Six5</i>	<i>Smpdl3b</i>	<i>Stoml1</i>	<i>Tle2</i>	<i>Trib1</i>
<i>Slamf8</i>	<i>Smyd4</i>	<i>Ston1</i>	<i>Tle6</i>	<i>Trib2</i>
<i>Slc10a6</i>	<i>Snai2</i>	<i>Stt3a</i>	<i>Tlr2</i>	<i>Tril</i>
<i>Slc10a7</i>	<i>Snaip</i>	<i>Stx5a</i>	<i>Tlr7</i>	<i>Trim12c</i>
<i>Slc16a11</i>	<i>Sned1</i>	<i>Svep1</i>	<i>Tmed3</i>	<i>Trim44</i>
<i>Slc18a1</i>	<i>Snhg11</i>	<i>Syn3</i>	<i>Tmeff1</i>	<i>Trim62</i>
<i>Slc1a3</i>	<i>Sntg2</i>	<i>Synm</i>	<i>Tmem104</i>	<i>Trmt1</i>
<i>Slc1a6</i>	<i>Snx14</i>	<i>Synpo</i>	<i>Tmem106b</i>	<i>Trmu</i>
<i>Slc22a21</i>	<i>Snx32</i>	<i>Tacstd2</i>	<i>Tmem119</i>	<i>Trps1</i>
<i>Slc22a4</i>	<i>Snx33</i>	<i>Tagln</i>	<i>Tmem154</i>	<i>Trpv4</i>
<i>Slc24a3</i>	<i>Snx7</i>	<i>Tap1</i>	<i>Tmem165</i>	<i>Tsku</i>
<i>Slc25a27</i>	<i>Socs2</i>	<i>Tap2</i>	<i>Tmem169</i>	<i>Tspan17</i>
<i>Slc25a36</i>	<i>Socs3</i>	<i>Tapbp</i>	<i>Tmem176a</i>	<i>Tspan4</i>
<i>Slc25a37</i>	<i>Sod3</i>	<i>Tbc1d12</i>	<i>Tmem176b</i>	<i>Tspyl2</i>
<i>Slc25a45</i>	<i>Sorbs3</i>	<i>Tbc1d2b</i>	<i>Tmem18</i>	<i>Ttc14</i>
<i>Slc2a10</i>	<i>Sox12</i>	<i>Tbc1d8b</i>	<i>Tmem185a</i>	<i>Ttc28</i>
<i>Slc2a13</i>	<i>Sox18</i>	<i>Tbl2</i>	<i>Tmem243</i>	<i>Ttc7</i>
<i>Slc30a7</i>	<i>Sp100</i>	<i>Tbrg1</i>	<i>Tmem268</i>	<i>Tusc5</i>
<i>Slc36a1</i>	<i>Sparc</i>	<i>Tbx1</i>	<i>Tmem39a</i>	<i>Tvp23a</i>
<i>Slc36a2</i>	<i>Sparcl1</i>	<i>Tbx18</i>	<i>Tmem45a</i>	<i>Tvp23b</i>
<i>Slc38a10</i>	<i>Spata13</i>	<i>Tbx3</i>	<i>Tmem53</i>	<i>Txndc5</i>
<i>Slc39a13</i>	<i>Spats2l</i>	<i>Tcaf2</i>	<i>Tmem56</i>	<i>Txnip</i>
<i>Slc41a3</i>	<i>Spon2</i>	<i>Tcea2</i>	<i>Tmem59</i>	<i>Uaca</i>
<i>Slc43a1</i>	<i>Spp1</i>	<i>Tcf7l2</i>	<i>Tmem63a</i>	<i>Uba5</i>
<i>Slc43a3</i>	<i>Sqrdl</i>	<i>Tcn2</i>	<i>Tmem86a</i>	<i>Uba7</i>
<i>Slc44a5</i>	<i>Sra1</i>	<i>Tcp1l1l2</i>	<i>Tmprss6</i>	<i>Ugdh</i>
<i>Slc46a3</i>	<i>Srd5a2</i>	<i>Tcta</i>	<i>Tnfaip6</i>	<i>Ugp2</i>
<i>Slc50a1</i>	<i>Srfbp1</i>	<i>Tep1</i>	<i>Tnfrsf14</i>	<i>Ugt1a6a</i>
<i>Slc6a12</i>	<i>Srgn</i>	<i>Tgfb1l1</i>	<i>Tnfrsf1a</i>	<i>Unc5c</i>
<i>Ush1g</i>	<i>Vkorc1</i>	<i>Whrn</i>	<i>Ypel2</i>	<i>Zfp354c</i>
<i>Uvssa</i>	<i>Vldlr</i>	<i>Wisp2</i>	<i>Ypel3</i>	<i>Zfp423</i>
<i>Vamp2</i>	<i>Vsir</i>	<i>Wnt10b</i>	<i>Ypel4</i>	<i>Zfp467</i>
<i>Vav3</i>	<i>Vwa1</i>	<i>Wnt5b</i>	<i>Zc2hc1a</i>	<i>Zfp474</i>
<i>Vcan</i>	<i>Wdr27</i>	<i>Xbp1</i>	<i>Zcwpw1</i>	<i>Zfp503</i>
<i>Vdr</i>	<i>Wdr66</i>	<i>Xdh</i>	<i>Zdhhc1</i>	<i>Zfp532</i>
<i>Vegfd</i>	<i>Wdr83</i>	<i>Xkrx</i>	<i>Zer1</i>	<i>Zfp608</i>
<i>Vgll3</i>	<i>Wdr83os</i>	<i>Xylt2</i>	<i>Zfp105</i>	<i>Zfp658</i>
<i>Vipr2</i>	<i>Wdr93</i>	<i>Yipf2</i>	<i>Zfp287</i>	<i>Zfp810</i>

<i>Vit</i>	<i>Wfdc21</i>	<i>Yipf5</i>	<i>Zfp330</i>	<i>Zfp9</i>
<i>Zfp950</i>				
<i>Zhx1</i>				
<i>Zic4</i>				
<i>Zkscan7</i>				
<i>Zkscan8</i>				
<i>Zmat1</i>				
<i>Zmiz1</i>				
<i>Zswim4</i>				
<i>Zswim6</i>				