

Snf2l Regulates Foxg1 Expression to Control Cortical Progenitor Cell Proliferation and
Differentiation

Chelsea Pamela McGregor

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements for the degree of
Master of Science

Biochemistry, Microbiology and Immunology
Faculty of Medicine
University of Ottawa

© Chelsea Pamela McGregor, Ottawa, Canada, 2012

Abstract

Over the past five years the role of epigenetic modifiers in brain development has become increasingly evident. In this regard, Snf2l, a homolog of the chromatin remodeling protein ISWI, was shown to have enriched expression in the brain and be important for neuronal differentiation. Mice lacking functional Snf2l have hypercellularity of the cerebral cortex due to increased cell cycle re-entry. In this thesis I demonstrate the effects of Snf2l-ablation on cortical progenitor cells including increased proliferation and cell cycle deregulation, the consequence of which is a delay in neuronal migration and altered numbers of mature cortical neurons. This phenotype arises from increased expression of Foxg1, a winged-helix repressor expressed in the forebrain and anterior optic vesicle. Moreover, genetically reducing its overexpression rescues the Snf2l-ablated phenotype. Snf2l is bound directly to a promoter region of Foxg1 suggesting that it acts as a repressive regulator *in vivo* and is an important factor in forebrain differentiation.

Acknowledgments

First I would like to thank my supervisor David Picketts for giving me the opportunity to work in a great research environment. My time here has provided me with an opportunity to learn more than I expected. Thank you to a great supervisor who often seemed to know better than myself what I was capable of.

It is said that you spend more time with your colleagues than with your own family and I couldn't have asked for a better lab 'family'. Thank you to everyone who was willing to discuss research, offer solutions through the roadblocks, and provide some fun and companionship through the years. It would have been a much less enjoyable experience without Darren Yip, Keqin Yan, Pamela Lagali, Mike Huh, Matt Todd, Matias Alvarez-Saavedra, Chantal Medina, Melissa Geoffroy, Emma Goodall, Maureen Curtin, Stephen Rennick and Tina Price-O'Dea.

Last but not least, thank you to my family whose unwavering support I have always counted on, even though it may have sounded like I was speaking in a foreign language at times. To my husband, Kevin, thank you for putting up with all of those late nights in the lab. Your support and belief in what I do mean a lot to me.

Table of Contents

Abstract	ii
Acknowledgments.....	iii
Table of Contents	iv
List of Abbreviations.....	vii
List of Figures	ix
List of Tables	x
1.0 Introduction.....	1
1.1 Development of the Cerebral Cortex	1
1.2 Control of Cell Number in the Cerebral Cortex	9
1.3 Foxg1	18
1.4 Epigenetic Mechanisms and Brain Development.....	21
1.5 ISWI.....	29
1.6 Ex6DEL Mouse Model	35
1.7 Rationale and Aims.....	40
2.0 Methods	41
2.1 Reagents	41
2.2 Mouse Lines	41
2.3 Generation/Dissection of Embryos.....	41

2.4 Forebrain Dissection	42
2.5 Genotyping	42
2.6 Chromatin Immunoprecipitation	43
2.7 Q-PCR.....	45
2.8 qRT-PCR.....	46
2.9 Semi-Quantitative PCR.....	47
2.10 BrdU-Birthdating	50
2.11 Calculation of Cell Kinetics Using IdU/BrdU Double Labelling...	50
2.12 Cryosectioning.....	50
2.13 Cortical Thickness Measurements	53
2.14 Immunohistochemistry.....	54
2.15 <i>In Situ</i> Hybridization.....	55
2.16 TUNEL Staining	56
2.17 Neural Stem Cell Culture Generation.....	57
2.18 Western Blot.....	57
3.0 Results	59
3.1 Characterization of Changes in Foxg1 Expression	59
3.2 Effects of Snf2l Ablation on the Neural Progenitor Population.....	64
3.3 Altered Generation and Timing of Cortical Layers.....	80

3.4 Snf2l Binding to the Foxg1 Locus.....	93
4.0 Discussion.....	99
4.1 Altered Cell Cycle and Progenitor Cell Kinetics	99
4.2 Differentiation Timing and Cell Fate Determination	103
4.3 Snf2l Directly Affects Foxg1 Transcription	105
4.4 Snf2l and its Cofactors in Transcriptional Regulation	108
References	111
Curriculum Vitae	144

List of Abbreviations

Ac: acetylated
ACF: ATP-dependent chromatin-assembly and remodeling factor
ATP: adenosine triphosphate
ATRX: Alpha-Thalassemia Mental Retardation, X-linked
BMP: Bone Morphogenetic Protein
BP: basal progenitor
BPTF: bromodomain and PHD finger transcription factor
BrdU: bromodeoxyuridine
Brg1: Brahma-Related Gene 1
Brm: Brahma
CECR2: Cat Eye Syndrome Chromosome Region, Candidate 2
CDK: cyclin-dependent kinase
CERF: CECR2-containing Remodeling Factor
ChIP: chromatin immunoprecipitation
CHRAC: Chromatin Accessibility Complex
CKI: cyclin-dependent kinase inhibitor
CNS: central nervous system
CP: cortical plate
DNA: deoxyribonucleic acid
DNMT: DNA Methyltransferase
E: embryonic day
Ex6DEL: Snf2l exon 6 deleted
Foxg1: Forkhead Box G1
GABA: gamma-aminobutyric acid
GFAP: Glial Fibrillary Acidic Protein
H3: Histone 3
H4: Histone 4
IdU: iododeoxyuridine
IHC: immunohistochemistry
IPC: intermediate progenitor cell

ISWI: Imitation Switch
IZ: intermediate zone
K: Lysine
kDa: kilodalton
LV: lateral ventricle
MBD: methyl-binding domain
Me: methylated
MeCP2: Methyl CpG Binding Protein 2
mRNA: messenger ribonucleic acid
NoRC: Nucleolar Remodeling Complex
NURF: Nucleosome Remodeling Factor
P: post-natal day
PCR: polymerase chain reaction
Q-PCR: quantitative-polymerase chain reaction
Rb: Retinoblastoma protein
RG: radial glia
RSF: Remodeling and Spacing Factor
RT-PCR: reverse transcriptase- polymerase chain reaction
SEM: standard error of the mean
Shh: sonic hedgehog
Snf2h: Sucrose Non-Fermenting 2 Homolog
Snf2l: Sucrose Non-Fermenting 2-Like
SVZ: subventricular zone
Tc: cell cycle length
TGF- β : Transforming Growth Factor- β
Ts: S-phase length
TSS: transcriptional start site
VZ: ventricular zone
WICH: WSTF-ISWI Chromatin Remodeling Complex
Wnt: Wingless
WT: wild type

List of Figures

Figure 1. Anatomy and development of the forebrain	3
Figure 2. Progenitors and Development of the Cerebral Cortex	6
Figure 3. Epigenetic modification of the genome.....	25
Figure 4. Ex6DEL phenotype	37
Figure 5. Ex6DEL cortical tissue has increased Foxg1 expression and its genetic reduction rescues the hypercellularity and proliferation phenotype	39
Figure 6. Double-thymidine analog labeling of neural precursors	52
Figure 7. Increased Foxg1 expression in the Ex6DEL cerebral cortex.....	61
Figure 8. Genetic rescue of Foxg1 restores expression levels.....	63
Figure 9. Increased ventricular zone in Ex6DEL embryonic cortex.....	67
Figure 10. Increased intermediate progenitor cell proliferation	70
Figure 11. Ex6DEL embryos show dysregulation of cell cycle timing in cortical progenitors	74
Figure 12. Genetic rescue Foxg1 incompletely rescues dysregulation of cell cycle timing in cortical progenitors.....	77
Figure 13. Rescue of progenitor cell phenotypes is not due to apoptosis	79
Figure 14. Ex6DEL mice have increased cortical thickness	82
Figure 15. Differences in laminar marker expression in Ex6DEL cortex.....	85
Figure 16. Delayed neurogenesis of Layer VI.....	87

Figure 17. Delayed neurogenesis of Layer II/III	90
Figure 18. Differentiation timing of Layer V neurons is rescued by a decrease in Foxg1 expression.....	92
Figure 19. Snf2l binds directly to a Foxg1 promoter region	95
Figure 20. Snf2l regulates expression of both Foxg1 Transcripts	98

List of Tables

Table 1. Cell cycle regulators in cortical development	13
Table 2. Associated functions of common histone modifications	26
Table 3. Primers used for Q-PCR ChIP analysis	46
Table 4. Primary antibodies used for IHC	54

1.0 Introduction

1.1 Development of the Cerebral Cortex

The cerebral cortex is responsible for many higher brain functions such as perception, emotion and language, among others, which have evolved in primates (review of association cortex function in Pandya and Seltzer, 1982). The cortex, which has occupied an increasing percentage of brain volume throughout vertebrate evolution, is derived from an increasing cell expansion of the anterior neuroepithelium (Diamond and Hall, 1969).

The nervous system is derived from the neuroepithelium, a compartment of the ectoderm. During embryogenesis it folds up to form a tube termed the neural tube which is the basis of the brain and spinal cord of the central nervous system (CNS). At the rostral end of the neural tube three vesicles (prosencephalon, mesencephalon and rhombencephalon) represent the beginnings of the forebrain, midbrain and hindbrain, respectively. The forebrain can be further subdivided into the diencephalon, hypothalamus, retina and telencephalon (reviewed in Price and Willshaw, 2000) [Figure 1].

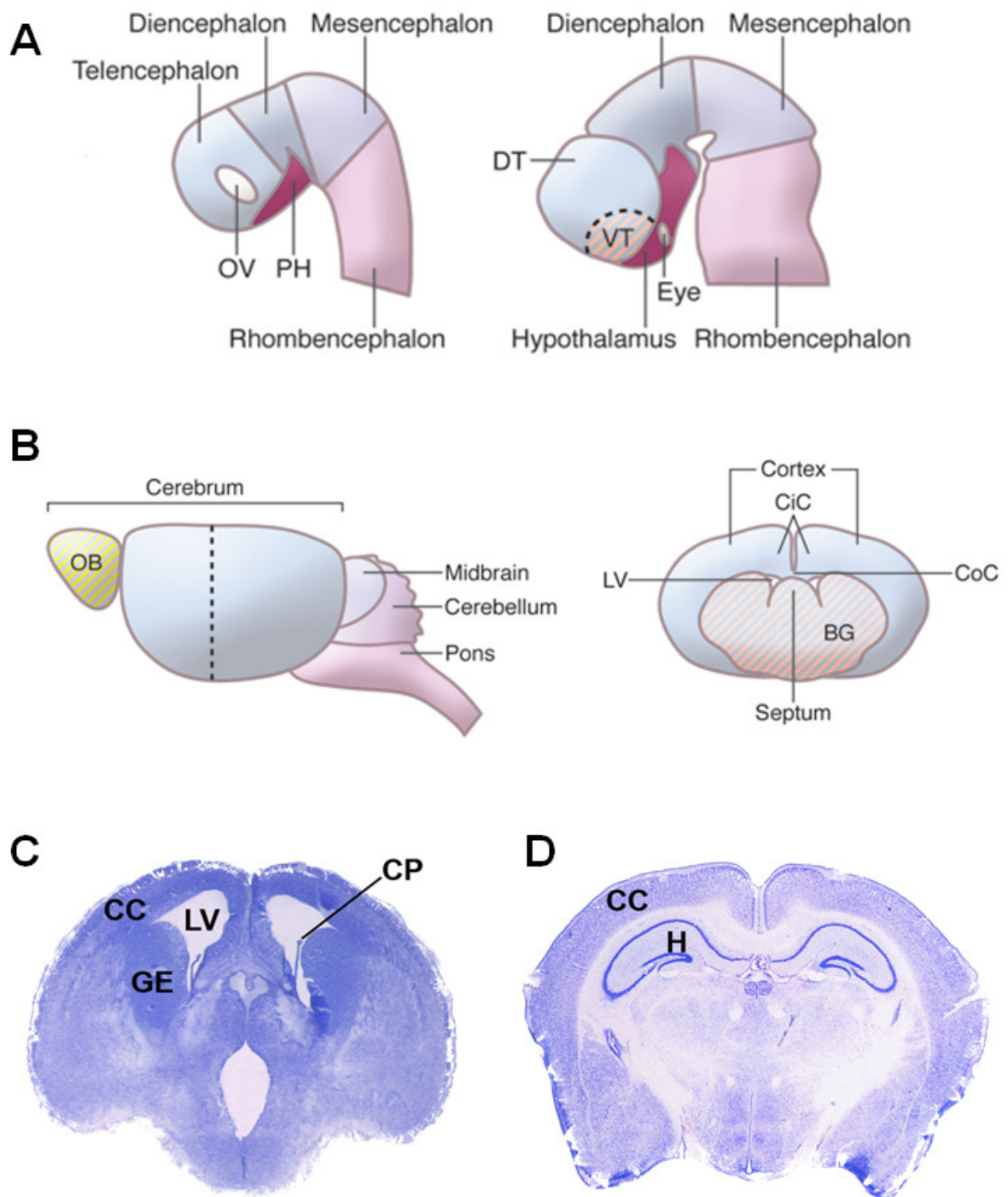
Among the first cell types generated from the neuroepithelium of the dorsal telencephalon are radial glia (RG), which are apparent in the forebrain by embryonic day 10 (E10) in mice (Edwards et al., 1990). These cells are critically important for corticogenesis, as they are involved in the generation of neuronal and glial cell types in the cortex and direct radial migration (Noctor et al., 2002; Rakic, 1972). Radial glia can be identified by certain molecular markers and their unique morphology. They have been defined immunohistochemically by Pax6 expression (Götz et al., 1998). They have a long radial process containing multiple microtubules that terminates in branched endfeet at the pial surface.

Figure 1. Anatomy and development of the forebrain.

(A) The rostral end of the neurotube forms three vesicles: prosencephalon, mesencephalon and rhombencephalon. The prosencephalon, or forebrain, gets further regionalized into telencephalon, diencephalon, optic vesicle (OV), and prospective hypothalamus (PH). Approximately at E10.5 in the mouse the expanding telencephalon bifurcates dorsally to form two hemispheres and gets patterned into dorsal telencephalon (DT) and ventral telencephalon (VT). From Geng and Oliver, 2009.

(B) Lateral view (Left) and coronal section (Right) of adult mouse brain. Black dashed line on lateral view indicates the location of coronal section shown. OB; olfactory bulb; BG, basal ganglia; CiC, cingulate cortex; CoC, corpus callosum; LV, lateral ventricle. From Geng and Oliver, 2009.

(C) Nissl-stained coronal sections of embryonic day 15.5 (C) and adult brain (D) showing the location of important structures at each stage. CC, cerebral cortex; CP, choroid plexus; GE, ganglionic eminence; H, hippocampus; LV, lateral ventricle. Figure adapted from Lagali et al., 2010.



The nuclei of RG are located in the ventricular zone, a germinal zone adjacent to the lateral ventricle (Rakic, 1972). Here they form a pseudo-stratified epithelium where the apical-basal position of the nucleus is dependent on cell cycle stage (Sauer, 1935). Mitosis occurs at the apical membrane, primarily with a cleavage plane perpendicular to the ventricular surface (Noctor et al., 2008). During the early half of corticogenesis most of these divisions are symmetric producing new radial glia but during the latter half (after embryonic day 16 in rats) the majority become asymmetric divisions producing intermediate progenitor cells (IPC), nascent neurons and eventually glial cells (Noctor et al., 2008) [Figure 2A]. Newly-formed IPCs and neurons use the radial fibres of RG as a guide for migration towards the cortical plate (Rakic, 1972).

As corticogenesis proceeds, IPCs migrate primarily to another proliferative region superficial to the ventricular zone, the subventricular zone, but can also be found in the adjacent regions of the ventricular zone and intermediate zone (Kowalczyk et al., 2009). IPCs, identified by their expression of *Svet1* and *Tbr2*, are a morphologically heterogeneous population with either a radial or multipolar morphology (Englund et al., 2005; Kowalczyk et al., 2009; Tarabykin et al., 2001).

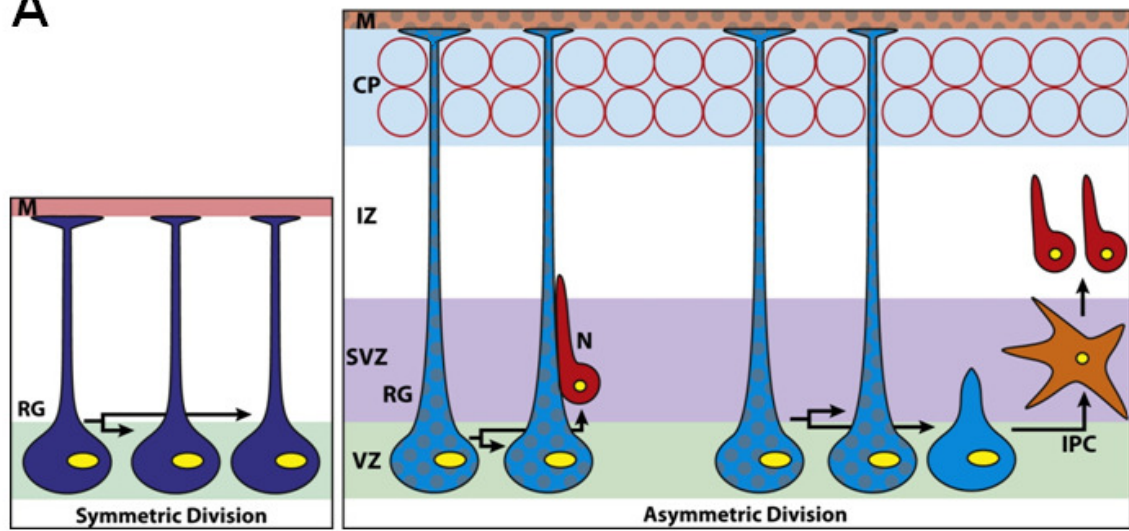
The subventricular zone becomes more prominent throughout corticogenesis as the ventricular zone begins to decrease (Bayer et al., 1991) suggesting that IPCs have played a significant role in cerebral hemisphere expansion in mammals (Martínez-Cerdeño et al., 2006). One study suggests that greater than 80% of cortical projection neurons are derived from this population (Kowalczyk et al., 2009). Intermediate progenitors undergo a finite number of symmetrical abventricular mitosis to produce either two daughter IPCs

Figure 2. Progenitors and Development of the Cerebral Cortex.

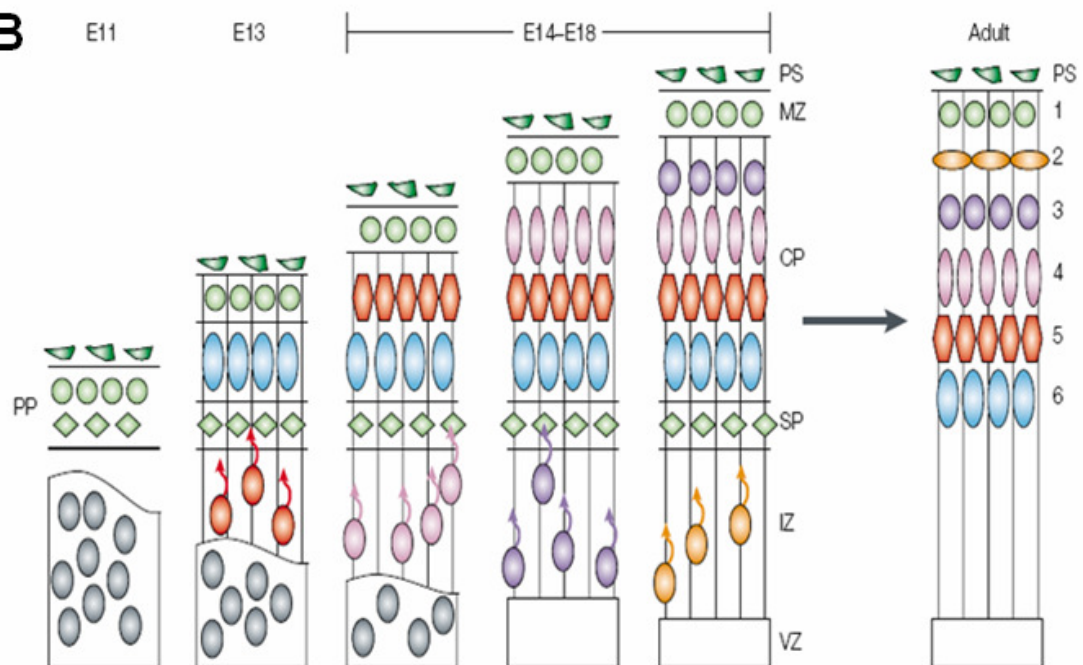
(A) The left panel depicts stem cells in the ventricular zone (VZ), which include radial glia (RG; dark-blue cells) that undergo symmetric divisions to form two undifferentiated daughter cells that re-enter the cell cycle. A later stage in development is depicted in the right panel. Radial glia cells begin to undergo more asymmetrical divisions to result in a proliferating radial glia cell and either a neuron (N, red cell) or intermediate progenitor cell (IPC; orange cell) cell. The majority of IPCs go through a finite number of symmetrical abventricular mitoses to produce either two daughter IPCs (not shown) or nascent neurons (shown). M, marginal zone; SVZ, subventricular zone; IZ, intermediate zone; and CP, cortical plate. Open red circles in the CP represent neurons. Figure adapted from Sockanathan and Gaiano, 2009.

(B) Neurons of the cerebral cortex originate in the germinal region (VZ) adjacent to the lateral ventricle before migrating through the intermediate zone (IZ) towards the pial surface (PS). Nascent neurons first settle between layers of the preplate (PP) with subsequent waves migrating past the earlier-born layer to create a laminar structure with layers 1-6 in the cortical plate (CP). SP, subplate; MZ, marginal zone. Figure adapted from Gupta et al., 2002.

A



B



or nascent neurons (Noctor et al., 2008) [Figure 2A].

The founder neurons of the cerebral cortex first form a structure termed the preplate that is present below the pial surface (Luskin and Shatz, 1985; Stewart and Pearlman, 1987; Zecevic et al., 1999). The preplate divides into two separate layers, the subplate and the marginal zone, when migrating neurons of the cortical plate take their place between them [Figure 2B]. The subplate layer is a heterogeneous layer of neurons that remains apical to the cortical plate (Del Rio et al., 2000). This population is transient in higher mammals, where the few remaining cells become interstitial white matter cells (Kostovic and Rakic, 1990), but has been shown to persist in rodents (Robertson et al., 2000). The subplate directs cortical afferents and extending thalamocortical efferents into the more superficial layers of the cortex (De Carlos and O'Leary, 1992; Ghosh and Shatz, 1993; McConnell et al., 1989). The superficial layer of preplate origin is the marginal zone which represents layer I of the cortex. It is populated primarily by Cajal-Retzius cells that are born before the subplate neurons on embryonic days 10 and 11 in mice (Bayer and Altman, 1990; García-Moreno et al., 2007). Cajal-Retzius cells have a horizontal orientation in rodents but appear to have an increasingly complex organization in primates (Zecevic and Rakic, 2001). Many functions have been elucidated for these neurons: maintaining radial glial identity, orienting the leading edge of radially migrating neurons, acting as a stop signal for migration and controlling superficial dendritic maturation (Chai et al., 2009; Chameau et al., 2009; Supèr et al., 2000). Most of these functions are performed by the glycoprotein Reelin which is secreted by the Cajal-Retzius cells and is a hallmark of their identity (Ogawa et al., 1995). As a demonstration of its importance, Reeler mice which do not produce Reelin have defects in their cortex where the preplate does not split and cortical lamination is reversed and poorly organized, which manifests as impaired locomotion and abnormal social behaviour (Alter et

al., 1968; Caviness Jr, 1982; Caviness Jr and Sidman, 1973; Falconer, 1951; Mikoshiba et al., 1980; Sheppard and Pearlman, 1997). The subplate and marginal zone prepare the cortex for the migration, differentiation and synaptogenesis of cortical projection neurons of the cortical plate.

The cortical plate is a mosaic of a variety of neuronal types with different expression and connection patterns within a laminated 6-layer structure. Aside from layer I, the layers of the cortex are formed sequentially in an ‘inside-out’ fashion where early born neurons form the deeper layers and the later born neurons migrate past them to form the more superficial layers [Figure 2B] (Angevine Jr and Sidman, 1961). Multiple glutamatergic projection neuron types can have cell bodies residing within the same cortical layer. Projection neurons can be broadly classified into callosal projection neurons, which extend axons through the corpus callosum and reside primarily in layers II/III, V, and VI, or corticofugal projection neurons, which project to subcerebral targets in the thalamus, brainstem and spinal cord with various types located throughout layers II to VI (reviewed in Molyneaux et al., 2007). These connections are responsible for effecting the functions of the cerebral cortex, however, there are other cell types found in the cortical plate that fine-tune and support the function of projection neurons; interneurons and glial cells. Interneurons are GABAergic inhibitory neurons that form local connections within the cortical plate. Unlike projection neurons that migrate radially from the dorsal germinal zones, the majority of interneurons arrive in the cortex via a tangential migration from the ganglionic eminences (Wonders and Anderson, 2005). Their correct localization is dependent on signals from projection neuron subtypes with which they interact to control firing rates and develop local networks during periods of developmental plasticity (Corbin and Butt, 2011; Hensch, 2005; Lodato et al., 2011).

Abnormal interneuron function has been shown to contribute to disease states in humans such as epilepsy and schizophrenia (Benes and Berretta, 2001; Jones-Davis et al., 2009).

As neurogenesis nears completion in the late prenatal period, gliogenesis takes over to produce astrocytes and oligodendrocytes. Astrocytes and oligodendrocytes are generated sequentially from the germinal zones, with *in vitro* evidence suggesting oligodendrocytes are also produced from progenitors of the basal forebrain (Culican et al., 1990; He et al., 2001; Parnavelas, 1999; Schmechel and Rakic, 1979). Both of these lineages support neuronal function of the cerebral cortex. Glial cell differentiation finalizes the differentiation of the most complex and advanced neural structure of the animal kingdom. While much of the morphological differentiation has been elucidated, a lot remains to be discovered about the complex molecular pathways and extrinsic signaling cues that co-ordinate to direct this process.

1.2 Control of Cell Number in the Cerebral Cortex

Many genetic pathways are involved in determining brain size. Misregulation of any of these can lead to functional and pathological consequences. Both increases and decreases in cortical size in humans and murine models are caused by the loss of genes that affect cell death cascades, cell cycle regulation and self-renewal and differentiation pathways.

Cell death is important as a natural refining process during the development of the nervous system (Buss and Oppenheim, 2004) and can occur as a downstream effect of developmental aberrations or pathologies. In the cortex, naturally occurring cell death can be detected in all regions but is more prevalent in proliferating populations during embryonic development (Blaschke et al., 1998; Thomaïdou et al., 1997) and peaks in the post-natal cortical plate around the first week of life in rats (Ferrer et al., 1994). Many of the transgenic mouse models that eliminate factors important for developmental cell death have devastating

neuronal phenotypes. The elimination of pro-apoptotic factors caspase-3 (cysteine-aspartic protease 3), caspase-9, and Apaf-1 (Apoptotic protease activating factor 1) leads to an overgrowth of the cortex, with exencephaly of the forebrain in the severe cases, due to a lack of cell death or caspase activation (Cecconi et al., 1998; Kuida et al., 1998; Kuida et al., 1996; Yoshida et al., 1998). In each of the above mutants, a thickening of the germinal zones was observed in histological sections (Kuida et al., 1998; Kuida et al., 1996; Yoshida et al., 1998). An increase in Bromodeoxyuridine labeling is observed in Apaf-1 mutant forebrain during development which the authors suggest is due to increased survival of proliferative progenitors (De Zio et al., 2005; Yoshida et al., 1998). Ablating both Bax (Bcl-2 associated X) and Bak (Bcl-2 antagonist/killer) in mice produces a subtler phenotype where an enlargement of the brain, thickening of the cerebral cortex and persistence of SVZ progenitor cells was observed (Lindsten et al., 2003; Lindsten et al., 2000). Before the onset of neurogenesis, progenitors experience exponential growth and elimination of just three progenitor cells from the neuroepithelium at this early time point can result in the loss of 192 progenitors after six cell divisions (Haydar et al., 1999). This illustrates the importance of programmed cell death in defining cell number in the cerebral cortex. However, the proliferative properties of progenitor cells as they progress through exponential growth and then into neurogenic divisions can be just as critical.

As neurons terminally differentiate they exit the cell cycle, however, studies show that fine-tuning the cell cycle also has consequences on cell fate decisions. As neuronal differentiation progresses the cell cycle lengthens, primarily due to a four-fold lengthening of the G1 phase (Takahashi et al., 1995). More recent evidence has also found that both apical and basal progenitors shorten their S-phase length when they become committed to a neurogenic lineage (Arai et al., 2011).

The timing and progression of the cell cycle is determined primarily by the CDK (cyclin-dependent kinase) and cyclin families. Cyclins are expressed in a transient manner and activate specific CDKs which allows passage through cell cycle check-points such as cell size, DNA damage and chromosome spindle attachment (Sánchez and Dynlacht, 2005). Aberrant activity of these groups of proteins has consequences for cell proliferation, genomic stability and organism survival (i.e. cancer, neurodegenerative disorders) (Malumbres and Barbacid, 2009; Wang et al., 2009). Evidence that the cell cycle plays a role in cell fate changes first came from treatment of whole embryo cultures treated with a chemical inhibitor (olomoucine) of cdk2/CyclinE or A and cdk1/CyclinB which act at G1/S and G2/M transitions. Olomoucine treated embryos had a G2+M+G1 time that was 2 hours longer than controls and a premature switch of progenitors from proliferative to neurogenic divisions in the cortical neuroepithelium (Calegari and Huttner, 2003). This study has been followed by others that concentrate on specific factors or complexes known to affect cell cycle progression, particularly those that act during G1 phase due to its known importance in neurogenesis. Overexpression of cdk4/cyclinD, but not cdk2/cyclinE, increased the proliferative index of E13.5 cortex and shortened the cell cycle by 10% (G1 phase by 30%). This shortening was accompanied by an increase in the basal progenitor population of the subventricular zone, a delay in neurogenesis and a wider cortical surface at post-natal day (P) 0. When the complex was targeted by shRNA the opposite effects were observed (Lange et al., 2009). Work by Pilaz *et al* has shown that ectopic expression of cyclin D1 or E1 alone can induce similar changes. Induction of cyclin activity during the latter half of corticogenesis (E15) translates to a shift in cell fate where layers 2/3 are favoured (Pilaz et al., 2009). Presumably, the alteration in progenitor pool observed in transient expression studies would translate to a more marked change in cortical size in a transgenic animal

model. Cyclin D2 (cD2) is localized to the subventricular zone and is upregulated at the transition between radial glia and intermediate progenitor, suggesting unique developmental functions (Glickstein et al., 2007; Glickstein et al., 2009). Animals that are null for cD2 have reduced cortical volume and progenitor cell populations due to reduced proliferation. At the level of the cell cycle, cD2-null progenitors in the VZ have an elongated G1 phase, consistent with premature cell cycle-exit, and also a shortened S phase (Glickstein et al., 2009). These studies show that positive or negative changes on cell cycle length due to manipulation of CDK/Cyclin activity have concordant changes on the renewal/differentiation balance and cellular output of cortical progenitor cells.

Other components of cell cycle regulatory pathways can also modulate cortical development. One of the most important downstream targets of G1 phase CDK/Cyclin complexes is the retinoblastoma tumour suppressor protein pRb. It acts upon terminal neuronal differentiation and cell cycle exit in neuronal precursors (Slack et al., 1998). When pRb is conditionally deleted in the neocortex or CNS there are ectopic mitoses present which leads to a 30% increase in cerebral hemisphere volume at E16.5 or a 27% increase in brain weight at E18.5 (Ferguson et al., 2002; MacPherson et al., 2003).

Cyclin-dependent kinase inhibitors (CKI) act to titrate the activity of CDK/Cyclin complexes and play a key role in the development of the nervous system (reviewed in Cunningham and Roussel, 2001). The CKI p21^{Cip1} is a downstream mediator of antiproliferative TGF- β signaling in organotypic slice culture (Siegenthaler and Miller, 2005). *In vivo*, p57^{Kip2} is expressed primarily in the VZ earlier in corticogenesis and later co-localized with select populations undergoing differentiation suggesting a possible developmental role (Ye et al., 2009). Its overexpression can induce cell cycle arrest and neuronal differentiation more effectively than p27^{Kip1} after *in vivo* electroporation (Tury et

al., 2011). The effect of removing p57^{Kip2} expression on overall cell number during corticogenesis remains to be validated. The most studied of the CKIs is p27^{Kip1}, where knock-out animals exhibit increased production of late-born neurons (Fero et al., 1996; Goto et al., 2004). Overexpression was found to have the opposite effect on layer output due to an increased probability of cell cycle exit (Mitsubishi and Takahashi, 2009; Tarui et al., 2005). There was a trend towards having an elongated G1 phase and cell cycle length; however, none of the results were statistically significant (Tarui et al., 2005). It is also worth noting that roles for p27 in neuronal differentiation independent of its cell cycle regulation have been found which could complicate the interpretations of the above studies (Nguyen et al., 2006). In *Xenopus*, p27 is essential for neurogenesis demonstrating that a role in neuronal development is evolutionarily conserved (Vernon et al., 2003). Overall, many important regulators of the cell cycle have been found implicated in terminal differentiation and neuronal output in the cerebral cortex [summarized in Table 1].

Table 1. Cell Cycle Regulators in the development of the Cerebral Cortex

Cell Cycle Regulator	Role in Forebrain Development	Reference
cdk4/cyclin D	Controls cell cycle length, proliferation index, neurogenesis timing and cortical volume	Glickstein et al., 2009; Lange et al., 2009; Pilaz et al., 2009
cdk2/cyclin E1	Effects self-renewal/differentiation decision in cortical progenitors, helps determine early to late-born neuronal fate ratios	Pilaz et al., 2009
pRb	Determination of terminal differentiation, cerebral hemisphere volume	Ferguson et al., 2002; MacPherson et al., 2003; Slack et al., 1998
p21 ^{Cip1}	Mediates antiproliferative TGF- β signaling	Siegenthaler and Miller, 2005
p27 ^{Kip1}	Controls cell cycle length and terminal differentiation, helps determine early to late-born neuronal fate ratios, essential for neurogenesis	Fero et al., 1996; Goto et al., 2004; Mitsubishi and Takahashi, 2009; Tarui et al., 2005; Vernon et al.,

	in <i>Xenopus</i>	2003
p57 ^{Kip2}	Induces cell cycle arrest in the ventricular zone	Tury et al., 2011; Ye et al., 2009

Cell output of the cerebral cortex is controlled by both the speed and type of cell division (Caviness Jr et al., 2003). Several important pathways interact to balance self-renewal with differentiation, and pattern the forebrain. Among these are the well studied Wnt (Wingless), Shh (Sonic Hedgehog), and TGF- β (Transforming Growth Factor- β)/BMP (Bone Morphogenetic Protein) signaling cascades. Each of these morphogens is secreted from different signaling centers and was identified foremost for their role in patterning of the embryo. Early in embryogenesis, Wnts are responsible for early establishment of the anterior-posterior axis and later are secreted from the cortical hem and are required specifically for the proliferation of hippocampal progenitors (for a complete review of Wnt signaling in neural development see Freese et al., 2010). Wnt ligands interact with frizzled and lipoprotein receptor-related protein (LRP) 5/6 coreceptors at the cell surface and the resulting cascade stabilizes β -Catenin so it can act as a coactivator of the TCF/LEF (T-Cell Factor/Lymphoid Enhancer Factor) family of transcription factors (MacDonald et al., 2009). Aside from its patterning roles, the canonical Wnt signaling pathway is important for control of growth of the dorsal telencephalon. In a dual role, earlier progenitors are encouraged to expand and self-renew whereas intermediate progenitors in the upper SVZ undergo neuronal differentiation in response to Wnt activity (Chenn and Walsh, 2002; Hirabayashi et al., 2004; Munji et al., 2011). Consequently, in vivo electroporation of Wnt3a or transgenic expression of a stabilized form of β -Catenin results in expansion of progenitor populations and increased cortical surface area, sometimes accompanied by gyrations or neuronal heterotopias (Chenn and Walsh, 2002, 2003; Munji et al., 2011; Woodhead et al., 2006). Wnt

and TGF- β signaling use cross-talk to finely tune their activity and are able to co-regulate the expression of important developmental genes (Chesnutt et al., 2004; Falk et al., 2008; Guo and Wang, 2009). The TGF- β family of ligands, which includes BMPs, are important for dorso-ventral patterning of the neural tube and in the dorsal telencephalon are expressed at the dorsal midline, where the choroid plexus fails to develop in its absence (Chesnutt et al., 2004; Hébert et al., 2002). To initiate the TGF- β /BMP cascade, intracellular dimerization of ligands is required to produce a mature signaling molecule. They are recognized by a heterotetramer of Type I and II receptor subunits. Once bound, the receptor complex phosphorylates Smads1, 5 and 8 which then associate with Smad4 and translocate to the nucleus to act as transcriptional regulators (Zeng et al., 2010). Components of TGF- β and BMP signaling are found in the cerebral cortex during both development and postnatally and control differentiation timing in neural precursor cells (Miller, 2003; Panchision et al., 2001; Sun et al., 2011; Tomoda et al., 1996). TGF- β 1 is able to induce the differentiation of cultured radial glia into astrocytes and in organotypic slice cultures it decreases proliferating ventricular zone cells and increases the proportion of cells exiting the cell cycle by 20% (Siegenthaler and Miller, 2005; Stipursky and Gomes, 2007). This is accomplished via increased expression of the CKI p21 (Siegenthaler and Miller, 2005). The *in vivo* function of the other TGF- β isoforms (2 and 3) were examined via double knock-out mice and found to have increased proliferation and a decrease in neuronal output (Vogel et al., 2010). The evidence available suggests TGF- β signaling plays a general role in control of terminal differentiation of cortical progenitors. Studies examining BMP function have also identified a primarily anti-proliferative role for this signaling pathway separate from its patterning abilities. BMP4 stops cell division and can induce co-expression of Nestin, a progenitor cell marker, with GFAP (Glial Fibrillary Acidic Protein) in rat neural stem cell cultures (Sun et

al., 2011). Similarly, BMP7 exposure *in vivo* induced premature differentiation of radial glia into astrocytes and increased the transition from radial glia to intermediate progenitor (Ortega and Alcántara, 2010)). Another ligand, BMP2, preferentially induces neuronal differentiation and decreases proliferation *in vitro* (Mabie et al., 1999). The variety of BMP ligands expressed in the forebrain affect specific cell types and offer clues as to how one signaling pathway can precisely control multiple differentiation processes. Using different isoforms to control differentiation also extends to the level of BMP receptors. Panchision *et al* propose that BMP Receptor Type IA (BMPR-1A), which is expressed in all proliferative populations of the neural tube, promotes proliferation and induces expression of BMPR-1B. When BMPR-1B activity meets a certain threshold it induces terminal differentiation (Panchision et al., 2001). The complexity and redundancy of the BMP pathway make it difficult to precisely define its function in the control of self-renewal and differentiation but the Sonic Hedgehog (Shh) pathway has been studied *in vivo* with the aid of many good transgenic models. Shh is a morphogen secreted from the ventral side of the neural tube and counteracts the dorsal signaling of Wnt and BMP via mutual inhibition (Guo and Wang, 2009; Huang et al., 2007; Ulloa et al., 2007; Ulloa and Martí, 2010). Secreted Shh binds the transmembrane protein Patched (Ptc) and stops its inhibition of G-protein coupled receptor family member Smoothened (Smo). Downstream of Smo activation, the transcriptional regulation controlled by Shh is mainly mediated by the three mammalian Gli transcription factors. Gli1 acts only as an activator and is transcriptionally activated by the other Gli proteins. Gli2 and 3 can be processed to become repressors by proteasomal digestion of their C-terminal domain. In the absence of Shh ligand, the Gli transcription factors are phosphorylated by PKA which leads to bTrCP binding and targeting to the proteasome. Gli1 and Gli2 tend to be completely degraded but the repressor form of Gli3 is released and is free

to repress target genes. While the mechanisms are not completely understood, when Smo is activated by hedgehog signaling it stops Gli activator degradation and causes the release of the suppressor Sufu allowing Gli translocation to the nucleus (for reviews see Ayers and Thérond, 2010; Cohen Jr, 2010; Ingham et al., 2011). Complete knock-out of Shh in murine embryos results in severe patterning defects and demonstrates the requirement for Shh signaling in development and/or maintenance of the midline structures of the brain (Chiang et al., 1996). Later in the telencephalon, Shh is required for ventral fate specification specifically for interneuron marker expression in the medial ganglionic eminence (Fuccillo et al., 2004; Machold et al., 2003; Xu et al., 2005). Low levels of Shh activity also act as an endogenous mitogen in the cerebral cortex starting at approximately E13 in mice (Dahmane et al., 2001; Komada et al., 2008). Tbr2-positive progenitor cell numbers and their proliferation index is increased in models of Shh activation leading to a thickening of the cortical plate (Komada et al., 2008; Shikata et al., 2011). The opposite effect was seen in models of Shh inactivation (Dahmane et al., 2001; Komada et al., 2008). There is some evidence to demonstrate that Shh signaling is affecting cell cycle regulation in neural progenitors. Cell cycle is elongated in proliferating cells of the cerebral cortex in Shh and Smo conditional knock-outs (Komada et al., 2008). In chick embryo neural tube, expression of repressive Gli caused an accumulation of cells in G1 where expression of Cyclin D1 was found to be modulated (Cayuso et al., 2006). The requirement for Shh extends postnatally to the maintenance of stem cell niches (ie. Subventricular Zone) (Balordi and Fishell, 2007; Machold et al., 2003). Signaling pathways present in the cortex cooperate to regulate proliferation/differentiation of neural and glial progenitors throughout development and sometimes into adulthood. These pathways combined with intrinsic genetic factors and proteins important for control of cell death and cell cycle dictate the final size and shape of

the cerebral cortex. Characterization of downstream effectors may continue to shed light on the complex process of corticogenesis.

1.3 Foxg1

Brain development requires a multitude of intrinsic genetic pathways and transcription factors. These transcription factors are involved in spatial and cell type specification, which guides primordial cells of the neuroepithelium into the broad array of specified neural structures found in the adult brain. The contribution of transcription factors to brain development has been reviewed elsewhere (Guillemot, 2007; Hevner, 2006; Molyneaux et al., 2007), while this chapter is on the role of one transcription factor, Foxg1, in forebrain development.

The forkhead homeobox (Fox) family of transcriptional regulators is involved in the downstream regulation of many signaling pathways in a wide variety of tissue and cell types (reviewed in Wijchers et al., 2006). Forkhead box G1 (Foxg1), formerly called Brain Factor-1 (BF-1), is crucial for the development of anterior neural tube structures including the retina and optic chiasm, olfactory epithelium, inner ear, and forebrain (Duggan et al., 2008; Huh et al., 1999; Kawauchi et al., 2009; Pauley et al., 2006; Pratt et al., 2004; Xuan et al., 1995). It was first discovered based on the homology of its DNA binding domain to other mammalian forkhead proteins (Tao and Lai, 1992). The *Foxg1* gene is found on chromosome 12qB3 in mice, 14q12 in humans, and encodes a 481 amino acid protein (Li et al., 1996; Wiese et al., 1995). Interestingly, two Foxg1 transcripts have been identified in mice that contain the same open reading frame located in Exon 2. Class 2 transcripts contain a non-coding Exon 1 but Class 1 transcripts are transcribed from a promoter region within Intron 1 and are more abundant (Li et al., 1996). Foxg1 expression is enriched in the anterior neural structures such as the forebrain and retina in rodents but is also found in the testis in humans (Hatini et al.,

1994; Obendorf et al., 2007; Tao and Lai, 1992). Transcript levels are also higher in the developing brain than in the adult brain (Tao and Lai, 1992). Its expression patterns along with a high degree of evolutionary conservation strongly suggest that Foxg1 plays an important developmental role (Bourguignon et al., 1998; Koinuma et al., 2003; Mondal et al., 2007; Murphy et al., 1994; Zhao et al., 2009). Targeted disruption of the *Foxg1* allele in mice resulted in perinatal lethality and a severe reduction in the size of the cerebral hemispheres. Histological analysis of the cerebral cortex in these animals revealed a lack of development of both the ventral and dorsal telencephalon due to precocious differentiation of neural progenitors that depleted their population (Martynoga et al., 2005; Xuan et al., 1995). Foxg1 activity is dosage dependent, where haploinsufficiency causes a reduction in the volume of the cortex and hippocampus, a depletion of intermediate progenitor cells, an elongated cell cycle length and increased p21 expression (Eagleson et al., 2007; Siegenthaler et al., 2008). Foxg1 heterozygous mice also have postnatal deficits in hippocampal neurogenesis and exhibit hyperlocomotion and memory deficits (Shen et al., 2006a). Less is known about the effects of overexpression in mammals. In chick embryos, retroviral expression of FoxG1 in the neural tube leads to a thickening of the neuroepithelium and, in *Xenopus*, exogenous FoxG1 expands the neural plate by specifying more cells to a neuronal fate and increasing proliferation (Ahlgren et al., 2003; Hardcastle and Papalopulu, 2000). Humans are also sensitive to FOXG1 dosage since heterozygosity and duplications have been linked to syndromic mental retardation (congenital variant of Rett Syndrome and West Syndrome, respectively) and FOXG1 has been found upregulated in hepatoblastoma, medulloblastoma and ovarian cancer (Adesina et al., 2007a; Adesina et al., 2007b; Ariani et al., 2008; Brunetti-Pierri et al., 2011; Chan et al., 2009; Kortüm et al., 2011; Mencarelli et al., 2009; Papa et al., 2008; Shoichet et al., 2005; Striano et al., 2011; Tohyama et al., 2011).

Aside from growth control, Foxg1 also functions in forebrain patterning, fate determination and neuronal protection. In Foxg1 mutants there is an excess of Reelin expressing neurons in the cortical plate due to abnormal medial-lateral patterning (Hanashima et al., 2004; Muzio and Mallamaci, 2005). Both Wnt and BMP signaling are expanded in the dorsal telencephalon whereas the anterior expression domain Fgf8 is reduced and ventral Shh signal is absent (Dou et al., 1999; Hanashima et al., 2002; Huh et al., 1999; Martynoga et al., 2005; Muzio and Mallamaci, 2005). In the ventral portion of the telencephalon (ganglionic eminences) Foxg1 signal is autonomously required for cells to adopt ventral cell fates even in the presence of normal morphogenetic gradients (Manuel et al., 2010). A study of progenitors treated with Foxg1 short-hairpin RNA *in vitro* demonstrates altered intrinsic cell fate decisions and a resetting of the timing mechanisms in early progenitors (Shen et al., 2006b). Foxg1 also encourages self-renewal and expansion of neural progenitors while blocking gliogenesis (Brancaccio et al., 2010). In post-mitotic cerebellar granule neurons, Foxg1 promotes survival downstream of Insulin-like Growth Factor 1/Akt signaling (Dastidar et al., 2011). How Foxg1 is able to carry out all of these varying functions with precision is not completely understood and its downstream targets still require characterization.

Foxg1 protein contains a central winged-helix DNA binding domain known as the Forkhead domain and a c-terminal end that is capable of protein interaction (Bourguignon et al., 1998; Li et al., 1995; Rodriguez et al., 2001; Tao and Lai, 1992). Introducing a DNA-binding mutant expressed in a similar pattern to the wildtype protein restores the proliferative ability of progenitor cells in the dorsal telencephalon of Foxg1-null mice but is unable to rescue premature differentiation or the lack of ganglionic eminence development suggesting separate mechanisms of action *in vivo* (Hanashima et al., 2002). Foxg1 can repress

expression of p21, p27 and Wnt8b and upregulate Pax6 via different mechanisms (Danesin et al., 2009; Hardcastle and Papalopulu, 2000; Manuel et al., 2011; Siegenthaler et al., 2008). It can act as a transcriptional repressor on its own or act in concert with other cofactors (Bourguignon et al., 1998; Li et al., 1995). Foxg1 recruits co-repressors Groucho/Transducin-like Enhancer of split (TLE) that negatively regulate the differentiation of progenitor cells in the telencephalon (Roth et al., 2010; Yao et al., 2001). This interaction is inhibited by association of Foxg1 with Grg6 and shows a pathway where both DNA binding ability and protein interaction are required for Foxg1 transcriptional repression (Marçal et al., 2005). Protein interactions alone are responsible for repression of TGF- β /BMP differentiation-inducing signaling. The C-terminal end of Foxg1 associates with Fast-2 (a SMAD DNA-binding partner), SMADs and FoxO proteins directly (Dou et al., 2000; Rodriguez et al., 2001; Seoane et al., 2004).

Foxg1 is upregulated when there is an overexpression of Bmi1 (B lymphoma Mo-MLV insertion region 1 homolog) in cortical progenitor cells and its DNA-binding domain is required to maintain self-renewal and multipotency signaled by Bmi1 (Fasano et al., 2009). Foxg1 can also be regulated via intracellular localization. Phosphorylation at Threonine 226 induced by FGF signaling promotes nuclear export and neuronal differentiation. On the other hand, CKI phosphorylation of Serine 19 promotes nuclear import (Regad et al., 2007). Finally, Foxg1 activity is controlled epigenetically by microRNA-9 opening the door to the possibility of other regulatory mechanisms (Bredenkamp et al., 2007; Shibata et al., 2008; Shibata et al., 2011).

1.4 Epigenetic Mechanisms and Brain Development

A broad definition of epigenetics is the “mechanisms of temporal and spatial control of gene activity of complex organisms” outside of Mendelian genetics (Holliday, 1990). This

involves mechanisms that can alter mRNA transcript stability or chromatin structure.

Chromatin facilitates approximately 2 meters of DNA to be packaged into a single nucleus but can also function as a regulatory mechanism controlling access to the DNA (Van Holde and Isenberg, 1975). The basic subunit of chromatin is the nucleosome which consists of 147 base pairs of DNA wrapped around a histone protein octamer of two copies each of histones H2A, H2B, H3, and H4 (Kornberg, 1974; Luger et al., 1997). The interaction of DNA with the histone proteins is stabilized multiple ways, including hydrogen bonds, hydrophobic and van der Waals forces (De Santis et al., 1973). A fifth histone type, linker histone H1, is bound outside of the core octamer at the entry/exit site of the linker DNA and is proposed to stabilize nucleosome spacing and higher-order compaction (reviewed in Happel and Doenecke, 2009; Routh et al., 2008). Higher-order chromatin structure has generally been identified as variants of a '30 nm fibre' where nucleosomes are packaged into helical structures whose actual diameter depends on linker DNA length (Robinson et al., 2006; Routh et al., 2008; Schalch et al., 2005). These chromatin fibres are organized into loops of about 60 kilobases that anchor on the nuclear matrix and can be gathered together to define interphase chromosome structure (Pienta and Coffey, 1984).

There are several ways the cell can alter chromatin structure at a basic level including DNA methylation, histone modifications and chromatin remodeling complexes [Figure 3A]. DNA methylation plays an important role in transcriptional regulation. The addition of a methyl group to cytosine to form 5-methylcytosine occurs at CpG dinucleotide consensus sites that are commonly found grouped into CpG islands. They are found in or near 70% of promoters of human genes (Saxonov et al., 2006). DNA methylation is mediated by DNA methyltransferases (DNMTs). DNMT3a and DNMT3b are *de novo* methyltransferases that can introduce new methylation marks whereas DNMT1 is required for maintenance of

methylation during DNA replication. DNA methylation is associated with transcriptional repression due to both steric hindrance created by the methyl groups themselves and via methyl-binding domain (MBD) proteins (reviewed in Klose and Bird, 2006). Methylation patterns in the cerebral cortex are dynamic during development and throughout the lifespan of humans and vary according to brain region (Ladd-Acosta et al., 2007; Siegmund et al., 2007). Additionally, both Rett syndrome and immunodeficiency, centromere instability, facial anomalies (ICF) syndrome in humans are associated with intellectual disability and mutations in MeCP2 (a MBD protein) and DNMT3B respectively suggesting an important role for DNA methylation in the CNS (Amir et al., 1999; Xu et al., 1999). Hypomethylation of neurons in the forebrain results in neuronal death, underdeveloped neurons and a lack of long term signal potentiation in some pathways (Fan et al., 2001; Feng et al., 2010; Hutnick et al., 2009). Modulating expression of downstream MBD protein function has similar effects, and in the case of MeCP2 specifically causes defects in dendritic spine morphology and density and axonal organization (Belichenko et al., 2009; Chapleau et al., 2009; Chen et al., 2001; Martìn Caballero et al., 2009; Smrt et al., 2007).

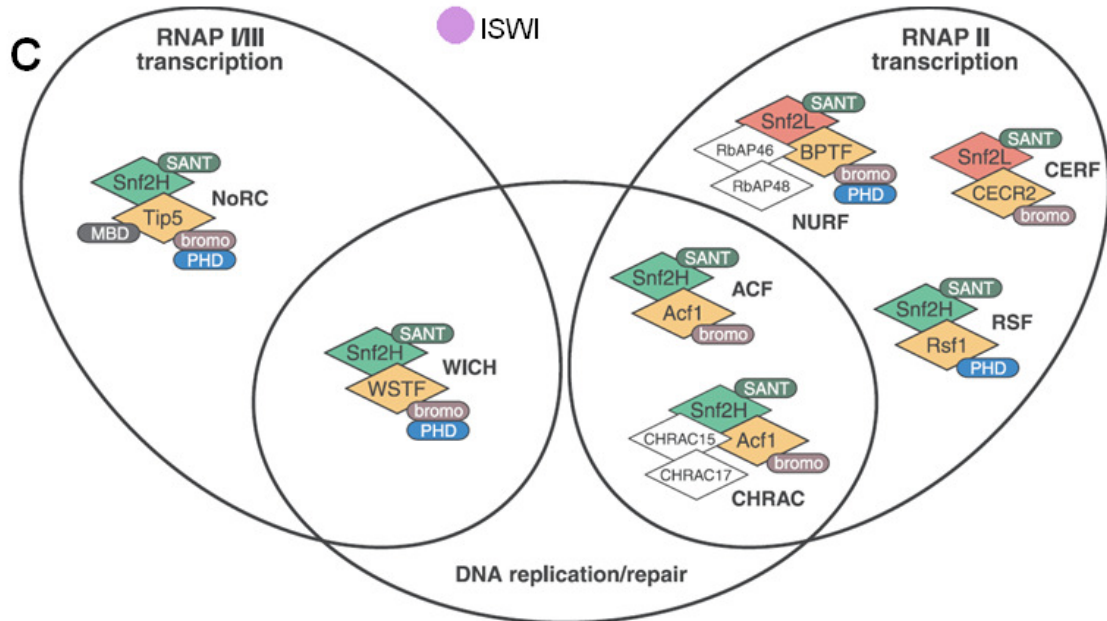
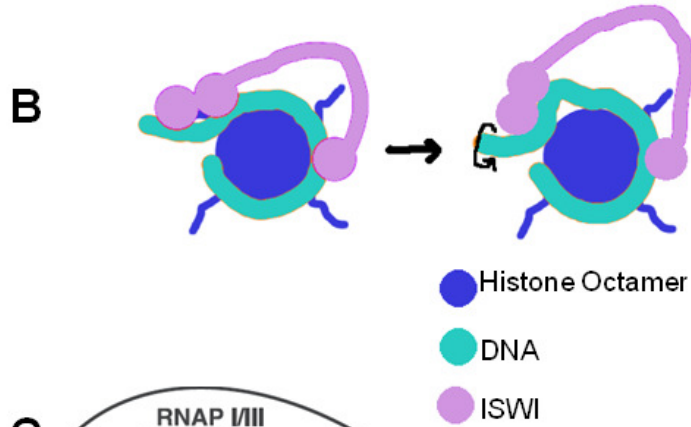
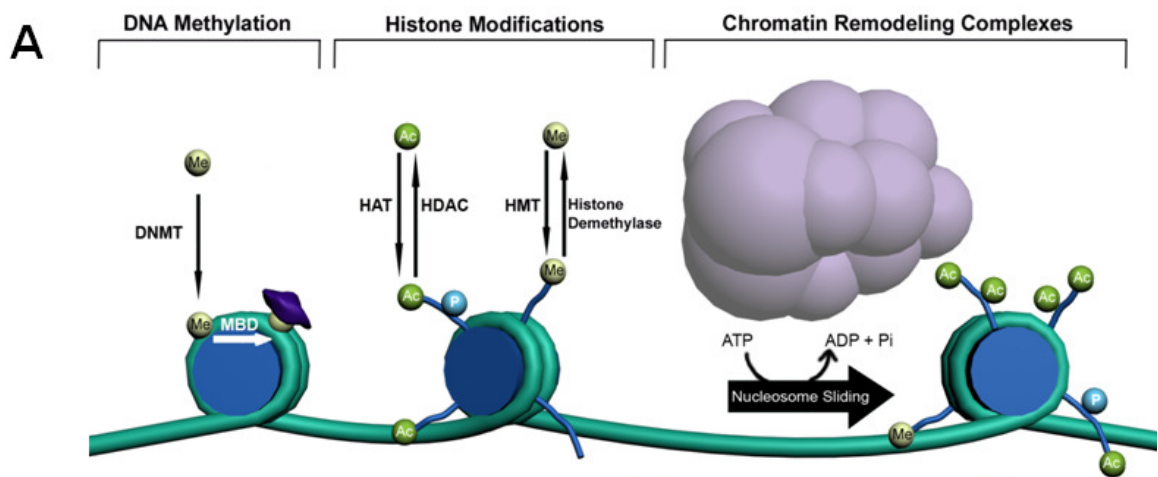
Histone proteins residues are subjected to post-translational covalent modification, particularly exposed amino-terminal tails and carboxy-terminal regions. The range of modifications possible includes acetylation (ac), methylation (me), phosphorylation, ADP ribosylation, ubiquitination and sumoylation and each has their own set of enzymes responsible for catalyzing the addition or removal of these moieties [Figure 2A]. The combinations of post-translational modifications present on a given nucleosome define a 'code' which determines the recruitment of chromatin-associating factors (reviewed in Campos and Reinberg, 2009). Many marks have become associated with particular functions

Figure 3. Epigenetic modification of the genome.

(A) The mammalian genome is compacted into chromatin which can be modified by DNA methylation, histone covalent modification and chromatin remodeling (Left to Right). The methylation of CpG dinucleotides is catalyzed by DNA Methyltransferases (DNMTs) and be recognized and bound by Methyl Binding Domain proteins (MBD). DNA methylation mediates repression through steric hindrance and recruitment of other remodeling factors. Exposed histone tails can be covalently modified by acetylation (Ac), methylation (Me), phosphorylation (P), ubiquitination, ADP ribosylation, and sumoylation. The addition or removal of each of these marks is mediated by a distinct set of enzymes. Histone acetylation is added by Histone Acetyltransferases (HAT) and removed from Histone Deacetylases (HDAC). Similarly the addition/removal of methylation is carried out by Histone Methyltransferases (HMT) and Histone Demethylases, respectively. The code that is created by different patterns of histone modification is read by other factors; among these are chromatin remodeling factors. Chromatin remodeling factors use the energy generated from ATP hydrolysis to slide, assemble or evict nucleosomes. From Lagali et al., 2010.

(B) The ISWI family of chromatin remodeling proteins disrupts DNA-histone interactions at entry to the nucleosome and new bonds are formed with extranucleosomal DNA. This creates a bulge of DNA that can be propagated through the nucleosome resulting in a shift in position of the histone octamer in relation to DNA sequence. Adapted from Längst and Becker, 2001b, 2004.

(C) The mammalian ISWI orthologs Snf2l and Snf2h associate with multiple complexes which guide their associations with chromatin (often via bromodomains or plant homeodomains [PHD]) and modulate their function. This allows ISWI complexes to play diverse roles in transcriptional control, DNA replication and DNA repair response. From Erdel and Rippe, 2011b.



in defining chromatin domains, transcriptional regulation, DNA repair, DNA replication and even splicing [Table 2].

Table 2. Associated functions of common histone modifications

Type of Modification	Associated with...	Reference
H3K4me3	Transcriptional activation, active promoters	Heintzman et al., 2007
H3K9me3	Transcriptional repression	Bannister et al., 2001
H3K27me3	Transcriptional repression	Boyer et al., 2006; Lee et al., 2006; Roh et al., 2006
H3K36me3	Exon marking	Kolasinska-Zwierz et al., 2009; Schwartz et al., 2009
H3K9ac	Transcriptional Activation	Roh et al., 2006; Vermeulen et al., 2007
H3K14ac	Transcriptional Activation	LeRoy et al., 2008; Roh et al., 2006; Vermeulen et al., 2007
H4K20me3, me1/2	Heterochromatin, DNA repair	Barski et al., 2007; Botuyan et al., 2006
H4K16ac	Chromatin decondensation	Shogren-Knaak et al., 2006
H2A.X S139 Phos	DNA repair	Rogakou et al., 1998

Table 2. Many histone tail covalent modifications are associated with a common function. This table summarizes some of the most prevalent in the literature.

Given the broad range of modifications possible, their role in brain development is complex and has not been completely elucidated (Lee and Lee, 2010). Examples of epigenetic mediators important in neuronal development are repressor element-1 silencing transcription (REST)/ Corepressor for element-1-silencing transcription factor (CoREST), Bmi1 and Ezh2. REST/CoREST are transcriptional repressors that can interact or operate in separate complexes involved in recruiting, among others, histone deacetylases HDAC1 and HDAC2, Sin3, MeCP2, histone methyltransferases G9a and Suv39 h1 and histone demethylase LSD1 (for a review of function and associating factors see Qureshi et al., 2010). REST represses the expression of neuronal genes in non-neuronal tissue and neural stem cells (Chen et al., 1998). During neuronal differentiation levels of REST become

successively lower to allow neuronal commitment and then terminal differentiation (Ballas et al., 2005). When REST expression is maintained during corticogenesis, neural fate can be specified but neuronal differentiation and migration are delayed (Mandel et al., 2011). In vitro evidence also points to a role for REST in mediating neuronal subtype specification (Abrajano et al., 2009). CoREST remains associated with chromatin independently of REST and controls a different gene network as well, one that focuses on neural stem cell multipotency and lineage restriction (Abrajano et al., 2010; Ballas et al., 2005). REST and CoREST may also interact with Polycomb group (PcG) proteins, Bmi1 and Ezh2 (Qureshi et al., 2010). The Polycomb group proteins form two core Polycomb Repressive Complexes (PRC). PRC1 contains Bmi1/Mel18, HPH, Ring1/Ring1B, and Cbx/Hpc proteins and PRC2 contains Eed, Suz12, Ezh2, and Rbap48/46. Together PRC1 and PRC2 mediate histone deacetylation and histone H3 Lysine 27 trimethylation (H3K27me3). H3K27me3 is mediated by methyltransferase activity of Ezh2 followed by transcriptional repression possibly by interfering with chromatin remodeling activity (reviewed in Lin et al., 2011). Deletion of Ezh2 (enhancer of Zeste homolog 2) in the cerebral cortex ablate H3K27me3 in neural progenitors and the resulting neurons. Ezh2-null cortical apical progenitors over-produce intermediate progenitors and neurons at the expense of self-renewal and cause an advance in the differentiation timeline of cortical plate neurons (Pereira et al., 2010). Polycomb group protein Bmi1 is also important for progenitor self-renewal. Its presence is required to downregulate p16^{Ink4}, p19 and the p21^{Cip1}/Retinoblastoma pathways in order to maintain the self-renewal and proliferation of neural stem cells during development and postnatally (Fasano et al., 2007; Molofsky et al., 2003; Wang et al., 2010; Zencak et al., 2005). Mediators of histone post-translational modifications are important for the maintenance of stem cells and cell fate determination.

Histone modifications can also serve to recruit chromatin remodeling complexes. SNF2 chromatin remodeling factors are a superfamily of enzymes containing a Switching/Sucrose non-fermenting-2 (SWI/SNF2)-like DNA translocase/helicase motif with the ability to alter chromatin structure using the energy derived from ATP hydrolysis. There are 23 subfamilies of chromatin remodeling factors within this superfamily. With the creation of transgenic mouse models, the importance of chromatin remodeling factors in neural development is emerging. *ATRX* was identified as the gene mutated in α -thalassemia mental retardation X-linked (ATR-X) syndrome (Gibbons et al., 1995). Subsequent studies in mice show that ablation of *ATRX* in the forebrain reduces progenitor cell survival due to activation of p53 and interrupts cellular migration leading to hypocellularity of multiple layers in the cerebral cortex and loss of the dentate gyrus in the hippocampus (Bérubé et al., 2005; Seah et al., 2008). There are two mammalian SWI/SNF family homologs, Brm (Brahma) and Brg1 (Brahma-related gene 1) (Chiba et al., 1994; Khavari et al., 1993; Randazzo et al., 1994). Brm is dispensable for normal development but does play a role in cell cycle control, while Brg1 is required for proper development from early embryogenesis (Bultman et al., 2000; Reyes et al., 1998). Initial studies of Brg1 in neural stem cells and its targeted deletion in neural tissue generated conflicting results. Brg1 was shown to be required for neuronal differentiation by mediating the transcriptional activity of neuregulin-related-1 and NeuroD and resulting in more progenitor cells in developing *Xenopus* but induced premature neural differentiation when ablated in murine brain (Matsumoto et al., 2006; Seo et al., 2005). These results can perhaps be reconciled by the fact that BAF subunits of the complex associated with Brg1 switch during neuronal development. This switching allows Brg1 complexes to control neural progenitor proliferation and self-renewal and

determine the timing of neural differentiation (Lessard et al., 2007). In my thesis I identified a novel role for the ISWI family member, Snf2l, in regulating CNS growth and development.

1.5 ISWI

The SWI/SNF SF2 subfamily is a group of chromatin remodeling proteins that associates with co-factors in large functional complexes, some greater than 1 MD in size (Mohrmann et al., 2004; Peterson et al., 1994; Wang, 2003). SWI/SNF complexes can slide nucleosomes along the DNA or evict them (Brown et al., 2011; Gutiérrez et al., 2007; Montel et al., 2007; Whitehouse et al., 1999). To target sites of nucleosome remodeling the SNF/SWI proteins use their bromodomain, located on the C-terminal end, to increase binding to acetylated H3 and H4 and facilitate remodeling activity there (Awad and Hassan, 2008). The ISWI (imitation switch) protein was originally identified in *Drosophila* based on its homology to the SWI/SNF protein Brahma (Elfring et al., 1994). While ISWI contains the SWI2/SNF2 ATPase-Helicase domain common to all SF2 family members, it does not contain the bromodomain found in SWI/SNF proteins placing it in a separate subfamily (Flaus and Owen-Hughes, 2011). Instead, it is characterized by the presence of C-terminal HAND, SANT (Swi3, Ada2, NCoR, TFIIB) and SLIDE (SANT-Like ISWI) domains that mediate interaction with substrate nucleosomes (Grüne et al., 2003). The HAND domain interacts with nucleosomal DNA near the entry/exit site and is postulated to give ISWI a preferred directionality for nucleosome movement (Dang and Bartholomew, 2007; Grüne et al., 2003; Längst and Becker, 2001a). The SLIDE domain contacts extranucleosomal DNA and due to its proximity could be responsible for recognition of the epitope at the base of histone H4 that is critical for stimulating nucleosome remodeling (Clapier et al., 2002; Dang and Bartholomew, 2007; Dang et al., 2006; Grüne et al., 2003). The SANT domain is less critical for chromatin remodeling as it can still occur *in vitro* in Δ SANT deletion mutants (

Grüne et al., 2003). Its binding target has not been confirmed. The conserved ATPase domain associates with DNA close to the dyad axis of the nucleosome (Dang and Bartholomew, 2007; Grüne et al., 2003; Schwanbeck et al., 2004). Nucleosome movement induced by ISWI occurs in *cis* while maintaining histone octamer integrity (Hamiche et al., 1999). Observation of incremental movements up to 10 base pairs suggests that nucleosomes are being slid along the DNA (Gangaraju et al., 2009; Hamiche et al., 1999; Schwanbeck et al., 2004). The proposed mechanism for the nucleosome sliding is one where DNA-histone bonds are disrupted at entry to the nucleosome and new bonds are formed with extranucleosomal DNA. This creates a bulge of DNA that can be propagated through the nucleosome resulting in a shift in position of the histone octamer in relation to DNA sequence [Figure 3B] (Längst and Becker, 2004). ISWI slides nucleosomes along DNA while still maintaining their structure (Kassabov et al., 2002). The differences in the sliding orientation and spacing of arrays are determined not only by the ATPase subunit but also the proteins that associate with it (Fan et al., 2005; He et al., 2006; Rippe et al., 2007).

ISWI orthologs have been identified in several model organisms, from yeast to humans, indicating an important cellular function. Humans and mice have two ISWI homologs: Snf2h (Sucrose non-fermenting 2 homolog) and Snf2l (Sucrose non-fermenting 2-like) (Aihara et al., 1998; Dirscherl and Krebs, 2004; Lazzaro and Picketts, 2001; Okabe et al., 1992).

Similar to SWI/SNF, the ISWI homologs exist in multi-protein complexes. ISWI proteins function within multiple chromatin remodeling complexes that differ by organism. *Drosophila* ISWI has been found in three different complexes: CHRAC (chromatin accessibility complex), ACF (ATP-dependent chromatin-assembly and remodeling factor), and NURF (nucleosome remodeling factor) (Ito et al., 1997; Tsukiyama and Wu, 1995;

Varga-Weisz et al., 1997). Mammalian complexes are more numerous and complicated by the presence of two ISWI homologs [Figure 3C]. Snf2h associated complexes are RSF (remodeling and spacing factor) where it is associated with Rsf1, NoRC (nucleolar remodeling complex) where it interacts with Tip5, WICH (WSTF-ISWI chromatin remodeling complex) that contains WSTF (Williams Syndrome Transcription Factor), ACF and CHRAC that both contain Acf1 but differ by the addition of histone-fold proteins CHRAC 15 and 17 (Bozhenok et al., 2002; LeRoy et al., 1998; Poot et al., 2000; Strohner et al., 2001). Snf2l has been associated with only two complexes in mammalian cells: NURF and CERF (CECR2-containing remodeling factor). Mammalian NURF has homologous subunits to its counterpart in *Drosophila* with BPTF (bromodomain and PHD finger transcription factor) and Retinoblastoma-Associated Protein (RbAP) 46/48 (Barak et al., 2003). CERF contains Snf2l and transcription factor CECR2 (cat eye syndrome chromosome region, candidate 2) (Banting et al., 2005). Interestingly, recent work has shown that CECR2 associates predominantly with Snf2h in the testis raising the possibility of ISWI subunit switching in different biological contexts (Thompson et al., 2012).

ISWI remodelers constantly scan chromatin via transient interactions and remain associated longer at sites of active remodeling (Erdel et al., 2010). The mechanisms that stably target them to specific sites within the chromatin are diverse and are often due to specificities of their associated factors (reviewed in Erdel and Rippe, 2011b). While analysis of genome-wide ISWI binding sites fails to reveal a global recognition sequence, Acf1 can target chromatin remodeling activity based on a 40 base pair sequence element and demonstrates that targeting based on DNA sequence is possible (Rippe et al., 2007; Sala et al., 2011). Binding can also be directed by histone post-translational modifications. Several ISWI-associated proteins contain bromodomains or plant homeodomains (PHD) known to

associate with acetylated or methylated histone residues, respectively. As an example, the PHD finger of BPTF is able to recruit NURF to sites of active transcription due to a preference for trimethylated lysine 4 of histone H3 (Wysocka et al., 2006). Additionally, the bromodomains of BPTF and Tip5 can recognize histone H4 acetylated at lysine position 16 (Kwon et al., 2009; Zhou and Grummt, 2005). Specific histone variants can be recognized by chromatin remodeling complexes providing information about the processes they are involved in. WSTF interacts with histone H2A.X and phosphorylates tyrosine 142 which participates in the DNA damage response (Xiao et al., 2009). CENP-A is an H3 variant specific to the centromere (Palmer et al., 1991). RSF has been found associated with CENP-A in immunoprecipitation experiments where it contributes to CENP-A integration into the centromere (Perpelescu et al., 2009). Different targeting strategies contribute to the diversity of biological functions performed by chromatin remodeling factors.

ISWI complexes have roles in many crucial DNA-related processes such as transcriptional activation or repression, DNA repair, replication and chromosomal structure (Reviewed in Dirscherl and Krebs, 2004; Erdel and Rippe, 2011b). In *Drosophila*, ISWI binds both intergenic and genic regions of the genome but was often found enriched at gene regulatory regions in the proximity of the TSS (Sala et al., 2011). ISWI-bound genes and those whose expression is changed at least 1.5X-fold in ISWI-ablated larvae show an overlap of 50% but when the fold change is increased to 4X that number drops to only 10% demonstrating that the nucleosome positioning induced by ISWI leads to mild expression changes (Sala et al., 2011). ISWI has been implicated in controlling the expression of patterning genes in several organisms. Ultrabithorax expression is encouraged by ISWI in *Drosophila* and Engrailed is upregulated by both *Drosophila* ISWI and NURF in human culture cells via binding to its promoter (Barak et al., 2003; Deuring et al., 2000). ACF

antagonizes Wingless/Wnt targets by interfering with TCF binding to target genes in the absence of active signaling but when the pathway is activated NURF acts as a transcriptional co-activator (Liu et al., 2008; Song et al., 2009). Additionally, *Xenopus* ISWI binds to and represses BMP4 and upregulates Sox9, Shh and Pax6 (Dirscherl et al., 2005). ACF/CHRAC and WICH have been implicated in the DNA damage response and make contributions beyond nucleosome repositioning. Snf2l, Snf2h and Acf1 become mobilized to sites of DNA damage within 10 seconds (Erdel and Rippe, 2011a). Reduced expression of Snf2h or Acf1 renders cells hypersensitive to DNA double-strand breaks (Lan et al., 2010). While ATPase activity of Snf2h is required for repair, Acf1 additionally interacts with and recruits KU70 to double-strand breaks (Lan et al., 2010). The phosphorylation of H2A.X on tyrosine 142 by WSTF also contributes to the maintenance DNA repair foci (Xiao et al., 2009). To regulate DNA replication, ISW2, a yeast ISWI complex, localizes to replication sites suggesting a role in fork progression (Vincent et al., 2008). When Acf1 is depleted in mammalian cells they experience delays in late S-phase when heterochromatin is typically replicated suggesting that ACF is required for replication through heterochromatin (Collins et al., 2002). Later work by the same group showed recruitment of WICH to the DNA clamp, Proliferating Cell Nuclear Antigen (PCNA), primarily for the maintenance of chromatin structure of the nascent strands (Poot et al., 2004). Once DNA replication has occurred a cell is capable of dividing its genome between two daughter cells through mitosis. Centromeres are required to equally segregate chromosomes. In the absence of the ISWI complex RSF, CENP-A is not integrated into centromeres and results in accumulation of cells in prometaphase and disorganization of the mitotic spindle (Perpelescu et al., 2009). ISWI also maintains spindle microtubules during anaphase by acting as a RanGTP-dependent microtubule-associated protein (Yokoyama et al., 2009). Global condensation of chromatin is

also regulated by *Drosophila* ISWI promoting association of linker histone H1 (Corona et al., 2007; Siriaco et al., 2009).

The *in vivo* roles of ISWI have been examined in *Drosophila* and *Xenopus*. In *Drosophila*, ISWI is highly expressed in both oocytes and early embryos. As embryogenesis continues expression becomes confined to the CNS and gonads before dropping below *in situ* detection limits. Trace amounts of ISWI transcript are found in pupae and adult female flies (Elfring et al., 1994). Individuals lacking ISWI do not survive past late larval or early pupal stage. Expression of a dominant negative protein in restricted patterns allowed for survival but resulted in defective development of the affected structures due to a lack of cell viability (Deuring et al., 2000). Along this line, ISWI was found to be necessary for the early stages of oogenesis and the proper response of female germline stem cells to BMP signaling (Deuring et al., 2000; Xi and Xie, 2005). Studies in *Xenopus* reveal a specific requirement for ISWI in neural development. Many ISWI morpholino-injected embryos had gastrulation or neural tube closure defects. Those that escaped early catastrophic defects still had obvious spinal deformities and reduced brain and eye development (Dirscherl et al., 2005). In all of the above studies ISWI activity was completely ablated but in more complex mammalian systems the differential functions of Snf2h and Snf2l *in vivo* are still being dissected.

Snf2h is found throughout the different tissues in the body but has an association with proliferating cell populations (Lazzaro and Picketts, 2001). Lack of Snf2h causes S-phase defects in cells and Snf2h-null blastocysts fail to survive due to growth arrest and cell death (Stopka and Skoultschi, 2003; Xiao et al., 2009). The Snf2l homolog is a 24 exon protein located on the X chromosome whose basal promoter expression is controlled by CREB and Sp1 transcription factors in humans (Okabe et al., 1992; Xia et al., 2008). Snf2l expression is enriched in the brain and reproductive organs in mice (Lazzaro and Picketts,

2001). Human SNF2L is ubiquitously expressed and its activity is regulated by expressing a SNF2L+exon13 isoform that lacks enzymatic activity in non-neuronal tissues (Barak et al., 2004). Snf2l activity has been shown to be important for reproductive function in mammals. It is upregulated in response to luteinizing hormone in granulosa cells where it binds to the proximal promoter of StAR (steroidogenic acute regulatory protein) and activates its expression, an important step in formation of the corpus luteum (Lazzaro et al., 2006). In the nervous system, Snf2l expression is increased during neuronal differentiation and promotes neurite extension outgrowth (Barak et al., 2003; Lazzaro and Picketts, 2001).

1.6 Ex6DEL Mouse Model

In order to study the role of Snf2l *in vivo* our group generated a transgenic mouse with Exon 6, the conserved ATP binding pocket, flanked by *loxP* sites. The floxed allele was then ubiquitously deleted by crossing it with a Gata1-Cre mouse line. The resulting mice produce a chromatin remodeling-deficient Snf2l protein 7 kDa smaller than the wildtype protein [Figure 4A,B]. These mice were viable and are able to propagate the deleted allele to their offspring. This is referred to as the Ex6DEL transgenic line. Ex6DEL mice have an increased brain to body weight ratio in both sexes [Figure 4C]. Heterozygous females are normal suggesting that the modified Snf2l is not acting as a dominant negative. The postnatal cerebral cortex in Ex6DEL mice exhibits hypercellularity and increased cell density with smaller nuclei in cortical plate neurons but normal lamination patterns [Figure 4D,E]. The hypercellularity in the postnatal brain is due to increased proliferation and cell cycle re-entry of progenitors during development and is concurrent with deregulation of cell cycle regulators p16, p21 and p27 [Figures 4F-I and 5A]. The intermediate progenitor population is specifically affected and is increased in number at mid-corticogenesis. To explore the gene

Figure 4. Snf2l Ex6DEL mouse phenotype.

Figures from Yip et al., 2012.

(A) Ex6DEL mice were created using a targeted strategy that deletes the conserved ATP binding pocket (Exon 6) of Snf2l. Schematic of the Snf2l locus, targeted allele, and the Ex6DEL allele (LoxP sites, grey triangles; NeoR, neomycin resistance gene). Below, sequence conservation of the 60 amino acids encoded by exon 6. Lys residue critical for ATPase activity is red.

(B) Snf2l immunoblot of E15 cortical extracts from WT, HET, and Ex6DEL mice. The Ex6DEL allele produces a protein product that is reduced in molecular weight.

(C) Plot of brain weight to body weight ratios for Ex6DEL, WT, or HET mice.

(D,E) Cell counts within a fixed brain volume from P7 (D) or Adult (E) brains.

(F) Increased numbers of mitotic cells in apical and basal progenitors. E15.5 forebrain sections stained for terminally differentiated neurons (Tuj, Red) and mitotic progenitors (PH3, Green) show increased numbers of mitotic cells in apical and basal progenitors. Scale bar = 30µm. (H) Quantification of PH3+ cells to total cells in the VZ of E15 embryos.

(G) Neural progenitors from WT or Ex6DEL mice were pulsed with BrdU at E16.5 then harvested at E17.5 and stained for Ki67 (green), a marker of proliferation, and BrdU (red). Scale bar = 50µm. (I) The proportion of BrdU+/Ki67+ cells were quantified and represent progenitors that re-entered the cell cycle. During development the percentage of cells re-

entering the cells cycle decreases in the WT tissue but remains constant in Ex6DEL tissue. MZ, medial zone; II/III, IV, V/VI: cortical layers II and III, IV, and V and VI, respectively; LV, lateral ventricle; SP, subplate; VZ, ventricular zone; CP, cortical plate; *, p-value <0.05; error bars represent Standard Error of the Mean (SEM).

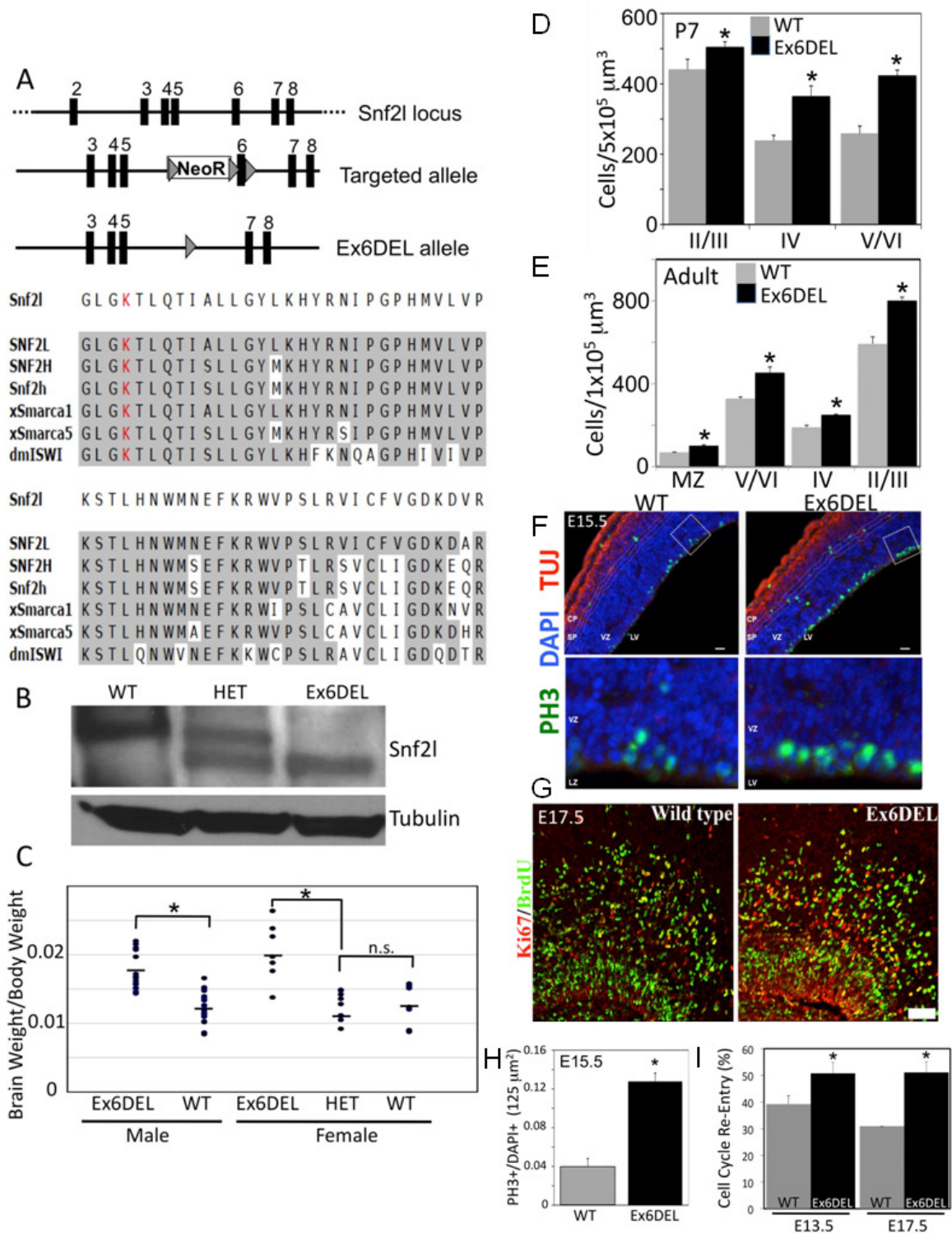


Figure 5. Ex6DEL cortical tissue has increased Foxg1 expression and its genetic reduction rescues the hypercellularity and proliferation phenotype.

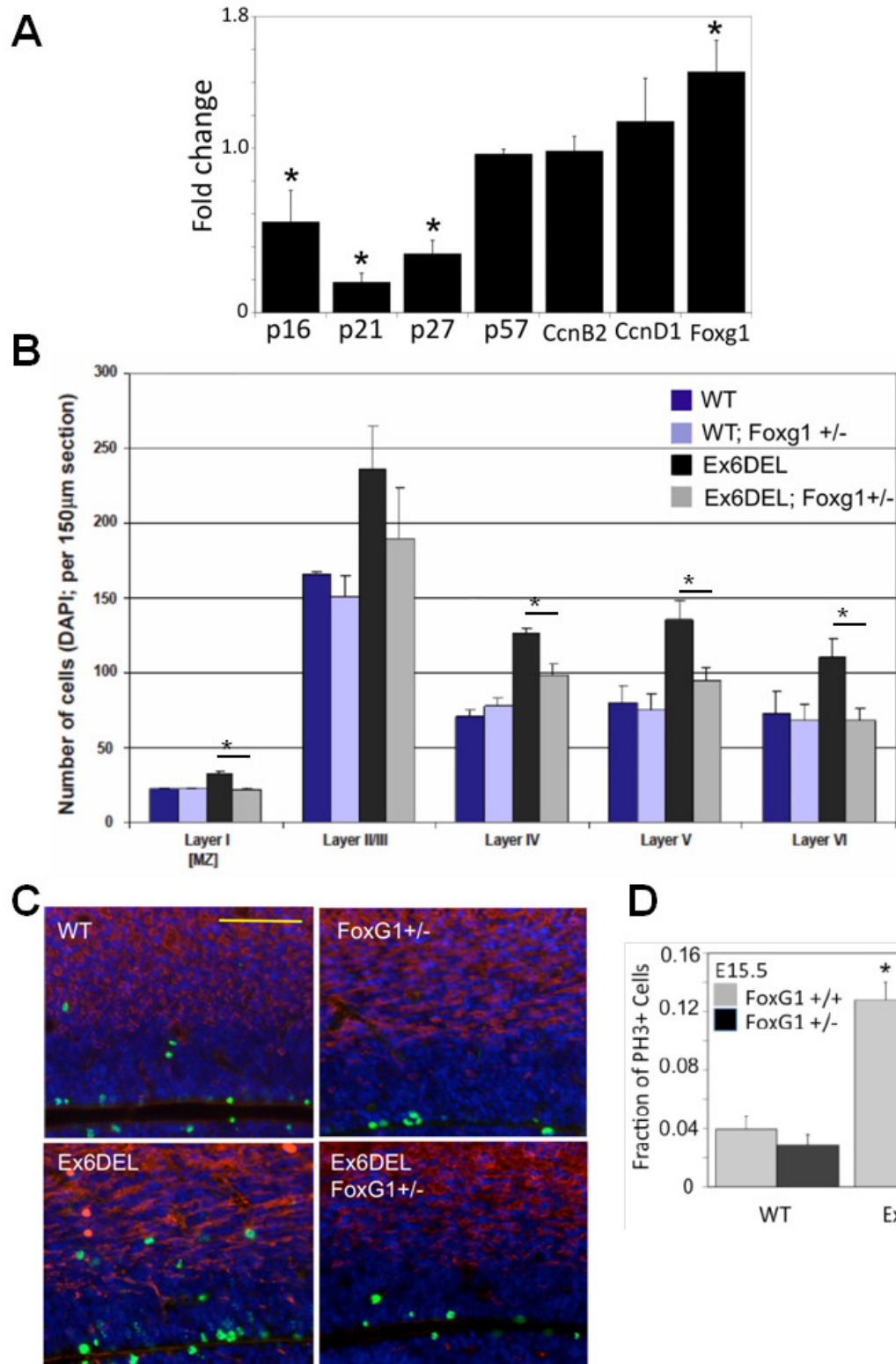
From Yip et al., 2012.

(A) qRT-PCR analysis of Foxg1, cdk inhibitors (p16, p21, p27, p57), and cyclins (Ccnb2, Ccnd1) normalized to WT levels. Foxg1 expression is increased and the Foxg1 target gene p21 is repressed.

(B) Increased cell counts in Ex6DEL mice are rescued in mice with reduced Foxg1 levels. Coronal brain sections (P7.5) from WT, Foxg1^{+/-}, Ex6DEL, and Ex6DEL;Foxg1^{+/-} embryos were stained with DAPI and the cells counted in each neuronal layer. The number of cells within a 150 μ m section were quantified. Consistent with earlier results the Ex6DEL mice showed an increase in cell number in all layers. The increase was rescued in the Ex6DEL; Foxg1^{+/-} animals.

(C) E15.5 cortical sections from WT, Foxg1^{+/-}, Ex6DEL, or Ex6DEL; Foxg1^{+/-} animals stained with PH3 (green) to label mitotic progenitors. Differentiated post-mitotic neurons are stained with Tuj (red). Yellow scale bar = 125 μ m.

(D) Quantification of this experiment. *, p-value <0.05; error bars represent SEM.



expression changes behind the phenotype, microarrays were performed using E15.5 cortical tissue. Altered expression (≥ 2 -fold) of 256 genes (183 decreased; 73 increased) was detected. Among the upregulated genes found in the Ex6DEL cortex is Foxg1. The hypercellularity and increased proliferation of the Ex6DEL cortex can be rescued by genetically reducing Foxg1 expression [Figure 5B-D], providing evidence that Foxg1 and Snf2l operate in the same pathway (Yip et al., 2012).

1.7 Rationale and Aims

Snf2l is a regulator of cortical growth and differentiation. The phenotype of mice expressing inactive Snf2l is dependent on Foxg1 overexpression. This tentatively places Snf2l and Foxg1 in the same genetic pathway. However, whether the regulation of Foxg1 is due to a direct interaction is unknown. The broad range of known processes mediated by Foxg1 suggests that other aspects of the Ex6DEL phenotype remain to be elucidated. Further characterization of Ex6DEL neural progenitor phenotypes and how these relate to corticogenesis will provide further insight into the developmental role of this novel interaction. I hypothesize that Foxg1 is a direct target of Snf2l and this regulation allows cortical progenitors to exit the cell cycle and differentiate at the appropriate time and in proper numbers. In order to verify this hypothesis the following experimental aims are proposed:

1. Characterize changes in Foxg1 expression within the cortex.
2. Examine the effects of Snf2l ablation on the neural progenitor population.
3. Determine if the generation and differentiation timing of cortical layers is altered in the Ex6DEL mice.
4. Determine whether Snf2l binds to the Foxg1 locus.

2.0 Methods

2.1 Reagents

Unless otherwise specified, all chemicals and other reagents used were supplied by Fisher scientific or Sigma-Aldrich.

2.2 Mouse Lines

All animal protocols received approval from the University of Ottawa Animal Care Committee, accredited by the Canadian Council on Animal Care. The Snf2l Ex6DEL mouse line used for this study had been previously generated from the cross between a Snf2l Exon 6 Floxed (Snf2l^{f/f}) line maintained on a 129sv background and a Gata1 Cre line maintained on a CD-1 background kindly provided by Dr. S. Orkin (Howard Hughes Medical Institute, Chevy Chase MD, USA). The resulting Snf2l Ex6DEL offspring were fertile and a line of Snf2l-null mice was maintained. Out-crossing of this line was completed with 129sv mice purchased from Charles River (Boston MA). The other transgenic line used in this study was the Foxg1-Cre line, on a C56Bl/6 background, which were generated by the laboratory of Susan K. McConnell (Stanford University, Stanford, CA). These mice are a knock-in Cre and are Foxg1 heterozygous (Hébert and McConnell, 2000).

2.3 Generation/Dissection of Embryos

Embryos were generated by pairing Snf2l Ex6DEL^{-X} females with Snf2l Ex6DEL^{-Y} or Foxg1-Cre^{+/-} males. Females were timed-mated using one of two methods. The first was to pair the females for one evening from approximately 5 P.M. until 9 A.M the following morning which was considered embryonic day (E) 0.5 and monitored for signs of pregnancy. The second method was to pair the females for three consecutive evenings and the presence of a vaginal plug was designated E 0.5. All pregnant mares were sacrificed using CO₂

asphyxiation followed by cervical dislocation just before embryo removal. The mare was laid in a supine position and the fur and upper layers of skin are pulled back exposing the undermost skin layer which is then cut using fine scissors and forceps from just above the anus to the ribcage. The entire string of embryos was removed followed by the removal of individual embryos from the embryonic sacs. Embryos were transferred into a clean 10cm² Petri dish (Fisher) containing Hank's Balanced Salt Solution (Gibco) or 1X PBS [3 mM disodium phosphate heptahydrate, 1 mM potassium phosphate, 225 mM sodium chloride].

2.4 Forebrain Dissection

Curved forceps were used to immobilize the embryo's head, with the forebrain accessible, by placing the tines on either side of the neck below the chin. The uppermost layer of skin was slit directly between the eyes using forceps. Grasping the skin layer posterior to the cortex the layer was gently peeled back to expose the cortex. Each individual hemisphere of the cortex was then removed using forceps.

2.5 Genotyping

To determine the genotype of an embryo a small portion of tissue was removed at the time of dissection. An alkaline lysis reaction was used to extract DNA (Truett et al., 2000). A standard PCR reaction was used to determine the sex of the embryos as well as their transgenic status for *Snf2l* and *Foxg1*. The reaction contents for a single reaction were 1X PCR Buffer (Invitrogen), 1.5 mM magnesium chloride (Invitrogen), 25 nM dNTP (Invitrogen), 0.5 μ M of Primer DNA, 1 μ L Sample DNA and 0.5 μ L Taq Polymerase in a total of 50 μ L. All of the primer sequences used for genotyping were ordered from IDT and are listed below. 20 μ L of each reaction was analyzed by electrophoresis on a 2% agarose gel containing ethidium bromide and imaged using a Gene Genius Bio Imaging System (Syngene).

Sexing Reaction Primers

Fabp1-F	5'-TGGACAGGACTGGACCTCTGCTTTCCTAGA-3'
Fabp1-R	5'-TAGAGCTTTGCCACATCACAGGTCATTTCAG-3'
SRY-F	5'-TTGTCTAGAGAGCATGGAGGGCCATGTCAA-3'
SRY-R	5'-CCACTCCTCTGTGACACTTTAGCCCTCCGA-3'

Snf2l Ex6DEL Primers

Snf2L intron 5-F	5'-CCTGGGCTGGAACCATGATC-3'
Snf2L exon 6-R	5'-CCATGTGGGGTCCAGGAATG-3'
Snf2L intron 6-R	5'-GTATGGACAAGTGTGTGAAGCC-3'

Cre Primers

Cre-F	5'-ATGCTTCTGTCCGTTTGCCG-3'
Cre-R	5'-CCTGTTTTGCACGTTCACCG-3'

2.6 Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) experiments were carried out as previously described with some modification (Zhou et al., 2004). Forebrain tissue was dissected from *Snf2l^{ff}* embryos at embryonic day (E) 15.5 and placed in Hank's Balanced Salt Solution (HBSS) on ice. The tissue was then manually triturated to a single cell suspension and the number of cells quantified (each forebrain generated an average of 5×10^6 cells). Cross-linking was achieved by incubating the cell suspension with 1% Paraformaldehyde for 1 hour on ice. Cells were then pelleted and washed twice in HBSS. Cell pellets were stored in -80°C until enough material was collected to run a ChIP. To begin the ChIP, all cell material (approx. 4×10^7 cells) was combined and then resuspended in 1 mL of Lysis Buffer [50 mM Tris-Cl pH8, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% sodium dodecylsulfate (SDS)] for 1 hour on ice. Note that all steps up to the elution step were carried out on ice or

at 4°C. Lysed material was divided into 6 equal aliquots which were each brought up to a volume of 300 µL, this allows for more efficient sonication. A Vibra Cell sonicator (Sonics & Materials Inc) was used at 35% amplitude for 10 second pulses. Five pulses was usually adequate to generate chromatin fragments of approx. 600 bp, however, 5 µL of each aliquot was run on a 0.8% agarose gel to verify the fragmentation and additional pulses were administered as necessary. The sonicated lysate was cleared of cellular debris by centrifugation for 20 min at 14,000 rpm using an Eppendorf 5417R centrifuge. All of the supernatant was combined to form a homogeneous starting material from which a 1% aliquot is removed to use for quantitation later. The remaining material was divided equally to be used for the specific immunoprecipitation and the negative control. Both aliquots were then diluted 10-fold in ChIP Dilution Buffer [16.7 mM Tris-Cl pH8, 1.2 mM EDTA, 0.1% SDS, 1.1% Triton-X 100 (EMD), 167 mM NaCl] and salmon sperm DNA (ssDNA) and bovine serum albumin (BSA) were added to final concentrations of 0.2 mg/mL and 0.5 mg/mL, respectively. The chromatin was then precleared for an hour using 25 µL of Sepharose G slurry (GE Healthcare) blocked in Low Salt Buffer (20 mM Tris-Cl pH8, 2 mM EDTA, 0.1% SDS, 1% Triton-X 100, 150 mM NaCl) containing 0.2 mg/mL ssDNA and 0.5 mg/mL BSA. The precleared supernatant was then allowed to form antibody complexes with either 1 µg of Sheep anti-Snf2l or Sheep IgG (Sigma) for one hour before adding 25 µL of blocked Sepharose G slurry. This mixture was incubated overnight. The beads were then sequentially washed for 15 min in Low Salt Buffer, High Salt Buffer (20 mM Tris-Cl pH8, 2 mM EDTA, 0.1% SDS, 1% Triton-X 100, 0.5 M NaCl), LiCl Wash Buffer [10 mM Tris-Cl pH8, 1 mM EDTA, 0.25 M Lithium Chloride (BDH), 1% IGEPAL-360, 1% Deoxycholate, Sodium salt] and twice with TE Buffer (10 mM Tris-Cl pH8, 1 mM EDTA). Complexes bound to the beads were then eluted twice with 250 µL of Elution Buffer (0.1% Sodium Bicarbonate, 1%

SDS) at room temperature. To reverse cross-link, 20 μ L of 5 M NaCl was added to each sample before incubating it at 65°C for 6 hours. Eluate and the Input samples were then digested with RNase and Proteinase K (produced in the lab). Finally, DNA was recovered by phenol-chloroform (Applied Biosystems) extraction followed by precipitation with 100 μ L 5M NaCl, 1 μ L 10 mg/mL glycogen (Ambion) and 500 μ L isopropanol overnight at -20°C. Centrifugation at 13,000 rpm for 10 min was used to pellet the DNA which was washed twice in Ethanol and then dried completely before resuspending the eluate samples in 30 μ L and the input sample in 60 μ L HPLC Water (Sigma).

2.7 Q-PCR

Primer sets were designed to span the conserved areas of the Fog1 locus, both 5' and 3' to the coding sequence, and are spaced, on average, 500 bp apart. (Fig. 18A) The maximum amplicon length for these primers was 250 bp. All primer pairs were optimized to give r^2 values greater than 0.99 using the linear amplifying portion of a serial dilution curve and between 90-100% efficiency values by varying primer concentration (see Table 3 for primer pair sequences and their optimal reaction concentrations). Quantitative PCR analysis was run on an MX3000P instrument (Stratagene) using AbsoluteTM QPCR SYBR[®] Green Mix (Thermo Scientific). The volume for each reaction was 25 μ L and contained 12.5 μ L SYBR Green Pre-Mix, 1 μ L ROX (1:500 dilution), 0.8-2 μ L 5 μ M Primer Mix, 0.8 μ L DNA and the remainder HPLC Water. The cycling conditions were: one cycle at 95°C for 10 min and 40 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec. The Ct values of serial dilutions (1%, 0.1% and 0.01%) of input material were graphed and modeled by an exponential equation. Ct values obtained for the IgG and Snf2l pull-down samples were then input into this equation and the corresponding percent input value was calculated.

Table 3. Primers used for Q-PCR ChIP analysis

Primer	Sequence	Optimal Reaction Concentration
Foxg1 R1-F	5'-TTGCGCACATTAGTTTGTCC-3'	400 nM
Foxg1 R1-R	5'-CCCAAAGCCTCCTTTAGTCC-3'	400 nM
Foxg1 R2-F	5'-CCAGTTCTGGGACCACACTT-3'	400 nM
Foxg1 R2-R	5'-CCAGAACCAATTGGGAGAAA-3'	400 nM
Foxg1 R3-F	5'-TGCCAGTGCCACAATGTTAT-3'	400 nM
Foxg1 R3-R	5'-TTAGGGAGCTGCAGGTTGTC-3'	400 nM
Foxg1 R4-F	5'-TGCTGAAATTGCTCGCTCTA-3'	400 nM
Foxg1 R4-R	5'-CTCAGACCGGAAACAAATCC-3'	400 nM
Foxg1 R5-F	5'-ACAATTTTCAAGCCGCCATA-3'	280 nM
Foxg1 R5-R	5'-CCCGTGGAAGAAATTAGCAG-3'	280 nM
Foxg1 R6-F	5'-CTTTCCTCCCTCTTTCTG-3'	400 nM
Foxg1 R6-R	5'-AAAACAGGGCCTATGTGTGG-3'	400 nM
Foxg1 R7-F	5'-GCTGAAGAGGAGGTGGAGTG-3'	160 nM
Foxg1 R7-R	5'-AGCACAGACCCAAACAGTCC-3'	160 nM
Foxg1 R8-F	5'-GGCTAGCCAGACGCTCCT-3'	280 nM
Foxg1 R8-R	5'-AGCTACAGGCGCACACTAGG-3'	280 nM
Foxg1 R9-F	5'-TGATTCCTCAAGTCCTCGTTC-3'	320 nM
Foxg1 R9-R	5'-GGTGGTGATGATGATGGTGA-3'	320 nM
Foxg1 R10-F	5'-TGTGCTATGAATGCTGCAA-3'	320 nM
Foxg1 R10-R	5'-GGGGAGATAGGGCTATTTTACTG-3'	320 nM
Foxg1 R11-F	5'-GAGGTGCAATGTGGGAGAA-3'	160 nM
Foxg1 R11-R	5'-TGAATGAAATGGCAAAGCAG-3'	160 nM

Table 3. Eleven sets of primers were used to amplify regions within and surrounding the Foxg1 locus and detect regions of Snf21 binding. The sequence of each primer is listed along with their final concentration in Q-PCR reaction mixtures.

2.8 qRT-PCR

E15.5 forebrain tissue was dissected as described above, being careful to only remove the cortical portion of the telencephalon. The tissue was then immediately flash-frozen on liquid nitrogen and afterwards placed on dry ice to prevent any thawing. Without allowing the tissue to thaw, 350 μ L RLT Buffer (RNeasy Micro Kit, Qiagen) containing 1% β -Mercaptoethanol was added to the tissue and triturated. The lysate was then homogenized with a 28½ gauge 1 cc insulin syringe (Becton Dickinson). After centrifuging for 3 minutes at 13,000 rpm, the supernatant was transferred to an RNase free tube and stored at -80°C

until the remainder of the RNA purification protocol could be completed using the RNeasy Micro Kit. DNase I digestion was not performed in the column. RNA was quantified using a NanoDrop spectrophotometer and then stored at -80°C.

To produce cDNA, 2 µg of RNA was first treated with Applied Biosystems' DNA-free™ Kit to remove any residual genomic DNA contamination. The treated RNA template was then combined with 300 ng of random primers (Invitrogen) and allowed to anneal for 10 minutes at 65°C followed by 10 minutes on ice. Superscript Reverse Transcriptase (RT) III and associated reagents (Invitrogen) were placed in a reaction mixture according to manufacturer specification along with 1mM dNTP and the annealed RNA mixture. Elongation was completed by incubating the reaction at room temperature for 10 minutes, 42°C for 1 hour, and 95°C for 5 min to inactivate the RT enzyme. A no RT control was done for each experiment to ensure there was no DNA contamination.

The protocol for optimizing primer conditions and the reaction conditions can be found in section 2.7. The Foxg1 R9 primer set was used to measure total Foxg1 and an L32 primer set was used as a loading control. Results were calculated as a fold change compared to wild type using the Pfaffl method (Pfaffl, 2001).

L32 Primers

L32-F 5'-GTTTCATCAGGCACCA-3'

L32-R 5'-CTGGCCCTTGAACCT-3'

2.9 Semi-Quantitative PCR

All solutions were made using distilled H₂O treated with 0.1% diethylpyrocarbonate (DEPC), allowing it to hydrolyze overnight followed by autoclaving to remove any excess. Filtered tips were used to transfer solutions and plasticware was RNase-free.

To extract RNA E15.5 forebrain tissue (from two embryos), that had been flash-frozen on liquid nitrogen and stored at -80°C, was homogenized in 200 µL of TRIzol[®] Reagent (Invitrogen) using an RNase-Free Pellet Pestle (Kontes). The volume of TRIzol[®] Reagent was then brought up to 1 mL and samples were allowed to sit at ambient temperature for 5 minutes to ensure complete dissociation. 200 µL of chloroform was added followed by vigorous mixing by hand for 15 seconds. To separate the mixture into phases, samples were centrifuged at 14,000 rpm for 15 minutes at 4 °C. The upper aqueous phase was placed into a clean Eppendorf tube. To precipitate the RNA, 5 µg glycogen (Ambion) and 500 µL isopropanol were added and incubated for 10 minutes at room temperature. Centrifugation at 14,000 rpm for 10 minutes at 4°C formed an RNA pellet. The supernatant was removed and the RNA pellet was washed in 1 mL of 75% ethanol. The wash was removed after a centrifugation step of 8,500 rpm for 5 minutes at 4 °C and the RNA pellet was allowed to air dry. The RNA was resuspended in 15 µL of DEPC H₂O and incubated at 55°C for 10 minutes. The concentration of each sample was measured by taking the absorbance of a 1:50 dilution at 260 nm with a BioPhotometer spectrophotometer (Eppendorf).

To produce cDNA, 2 µg of RNA was first treated with Applied Biosystems' DNA-*free*[™] Kit to remove any residual genomic DNA contamination. The treated RNA template was then combined with 300 ng of random primers (Invitrogen) and allowed to anneal for 10 minutes at 65°C followed by 10 minutes on ice. Superscript Reverse Transcriptase (RT) III and associated reagents (Invitrogen) were placed in a reaction mixture according to manufacturer specification along with 1mM dNTP and the annealed RNA mixture. Elongation was completed by incubating the reaction at room temperature for 10 minutes,

42°C for 1 hour, and 95°C for 5 min to inactivate the RT enzyme. A no RT control was done for each experiment to ensure there was no DNA contamination.

To determine the relative levels of class1 and 2 Foxg1 transcripts (Fig. 19A) primer sets were used that would amplify each specifically. To look at overall changes in total Foxg1 expression the Foxg1 R9 primer set was used and an L32 primer set was used as a loading control (see both sequences above).

Class 1 Foxg1 mRNA

Foxg1 Class1-F 5'-TTGAGGGGTGGTTGCAGCTTTTGC-3'

Foxg1 Class1-R 5'-GGTGGTGATGATGATGGTGA-3'

Class 2 Foxg1 mRNA

Foxg1 R7-F 5'-GCTGAAGAGGAGGTGGAGTG-3'

Foxg1 R7-R 5'-AGCACAGACCCAAACAGTCC-3'

The reaction contents for a single reaction were 1X PCR Buffer (Invitrogen), 1.5 mM magnesium chloride (Invitrogen), 25 nM dNTP (Invitrogen), 0.2 µM Primer DNA, 1 µL Sample cDNA and 0.5 µL Taq Polymerase (prepared in the lab, concentration unknown) in a total of 50 µL. The reactions were run on an Eppendorf Mastercycler thermocycler under the following conditions: one cycle at 94°C for 2 min and 27 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min. The reaction for all primers sets was previously determined to be in the linear phase after 27 cycles. The reaction products were run on a 1% agarose gel and were imaged using a Gene Genius Bio Imaging System (Syngene) running GeneSnap software (Syngene). Gel images were exported as uncompressed bitmap photos and were inverted using Adobe Photoshop. The inverted images were analyzed with Image J software (National Institutes of Health, <http://rsbweb.nih.gov/ij>). The values obtained for peak area

were first normalized to an L32 loading control and used to calculate values relative to wildtype samples.

2.10 BrdU-Birthdating

Pregnant mares received intraperitoneal (I.P.) injections of 300 μ L of 16 mg/mL Bromodeoxyuridine (BrdU, Sigma) dissolved in DMEM (Gibco) at either gestational day 12.5 or 15.5. Two injections were given two hours apart. Mares were sacrificed and embryos were removed as described above at gestational day 18.5. To allow for complete fixation, brains were dissected from the embryos.

2.11 Calculation of Cell Kinetics Using IdU/BrdU Double Labelling

300 μ L of 1 mg/mL Iododeoxyuridine (IdU, Sigma) was injected I.P. into pregnant mares at gestational days E13.5, 15.5 and 17.5. This was followed, an hour and a half (T_i) later, by a similar injection of 300 μ L of 1 mg/mL BrdU. The female was euthanized and the embryos removed 30 minutes after the second injection. These two separate analogs are differentially detected by specific antibodies. Those cells that have taken up IdU but not BrdU have exited S phase (L_c) but those that incorporate both analogs were still replicating their DNA at the time of sacrifice (S_c) [Figure 6]. These numbers as well as the number of proliferating cells in the population (P_c) allow for the calculation of both the length of S phase (T_s) and the cell cycle (T_c) in the ventricular zone and subventricular zone using the following calculations (Martynoga et al., 2005):

$$T_s = T_i \times S_c / L_c \qquad T_c = T_s \times P_c / S_c$$

2.12 Cryosectioning

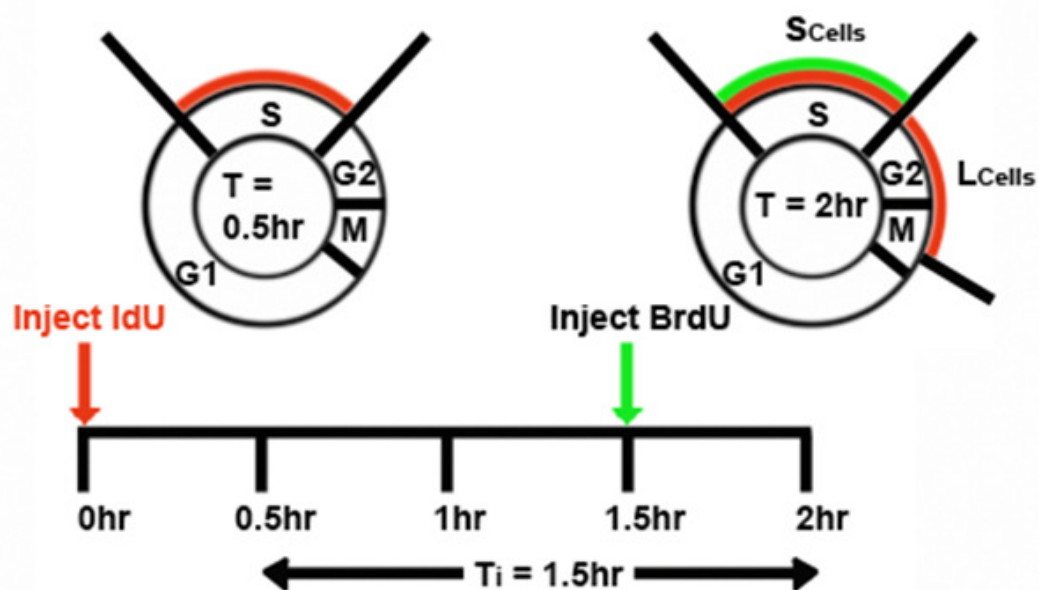
To prepare tissue for histology, E13.5 to E17.5 embryos were decapitated and E18.5 embryos and P7 pups had their brain dissected. Heads or brains were then submerged in 4% PFA overnight at 4°C to be fixed, as an exception IdU/BrdU double labeled tissues were only

Figure 6. Double-thymidine analog labeling of neural precursors.

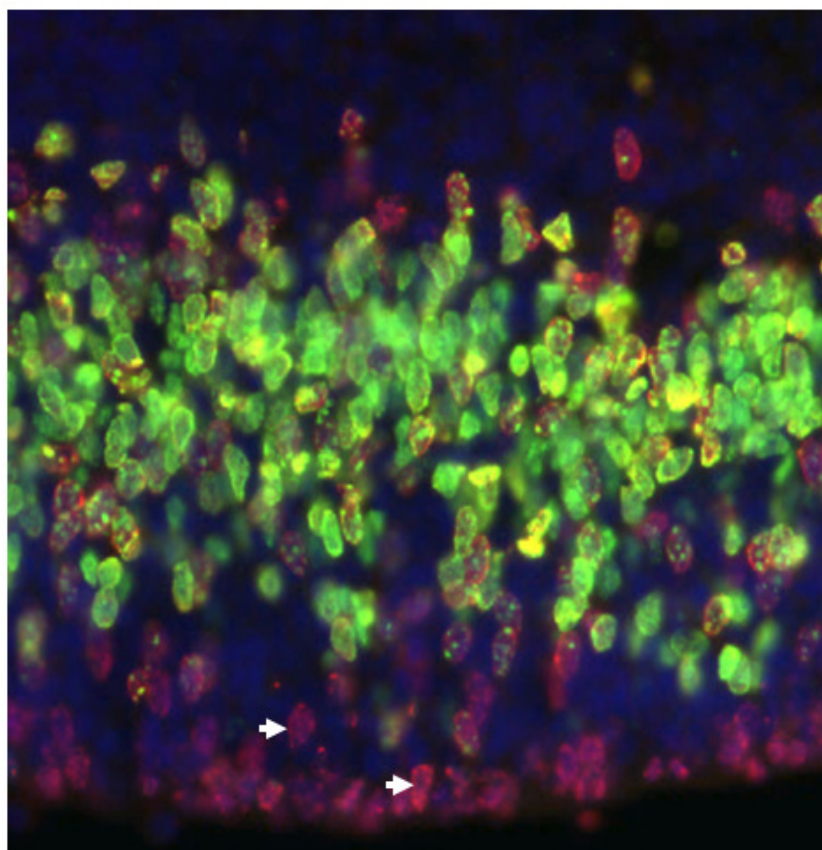
(A) Schematic of double-labeling methodology. Tissue is pulsed with iododeoxyuridine (IdU), followed 1.5 hours later with bromodeoxyuridine (BrdU), and finally fixation after another 30 min. Only cells in S-phase at the time of the pulse will incorporate an analog into their replicating DNA. These incorporated analogs can then be differentially detected using immunofluorescence. Single-labelled cells represent those that complete replication before the BrdU pulse and are termed the leaving cells (L_{cells}). Cells that remain in S-phase (S_{cells}) are double-labelled. The ratio of these two populations as well as the interval between label incorporation (T_i) can be used to calculate S-phase length. Figure adapted from Martynoga et al., 2005.

(B) An example of E13.5 ventricular zone/subventricular zone tissue that has undergone double-thymidine analog labeling. L_{cells} can be seen as nuclei that are labeled only in red (white arrowheads).

A



B



fixed 3-5 hours to prevent the antigens from being obscured. The tissue was then rinsed in 1X PBS and placed in 30% sucrose/PBS solution for cryopreservation. The tissue was equilibrated in 30% sucrose at 4°C until it sunk to the bottom of the tube. Tissues were embedded in labeled molds in a 1:1 mixture of 30% sucrose and OCT (Tissue-Tek) and frozen on liquid nitrogen. Coronal sections of forebrain 10 µm thick were cut using a Leica CM1850 cryostat. Sections were placed on Superfrost Plus coated slides (Fisher) and allowed to dry for 2 hours. Tissue sections were stored at either -20°C or -80°C in a container containing anhydrous calcium sulfate desiccant (Drierite).

2.13 Cortical Thickness Measurements

Coronal brain sections of P7 WT and Ex6DEL mice were Nissl stained. Sections were first rehydrated by passing through the following baths: 95% ethanol for 10 minutes, 70% ethanol for 1 minute, 50% ethanol for 1 min and twice in distilled H₂O for 5 minutes. To stain, sections were incubated in a solution of 0.25% Cresyl Violet and 1% acetic acid for 4 minutes. Washing was completed in distilled H₂O for several 5 minute intervals. To dehydrate the stained tissue another set of baths was used: 50% ethanol for 2 minutes, 70% ethanol with 1% acetic acid for 30 seconds, 95% ethanol for 2 minutes and xylene for 5 minutes. Once the slides were removed from the xylene they were allowed to dry completely before using Permount[®] to permanently mount a coverslip (VWR) overtop of the tissue.

Images of the stained tissue were taken on an Axio Skop 2 Zeiss microscope fitted with an AxioCam Camera (Zeiss) at 50x magnification. Measurements were performed using Image J software from pictures of the rostral and caudal cortex taken at medial and medial-lateral positions. The width of the cortex was measured from the boundary of layers I and II to the underlying white matter.

2.14 Immunohistochemistry

Frozen tissue sections were allowed to equilibrate at room temperature to avoid condensation formation. Slides were fixed in 70% ethanol for 5 minutes and then rehydrated in 1X PBS for 10 minutes. Antigen retrieval was performed by microwaving the sections in 1X Sodium Citrate (10 mM Sodium Citrate pH 6, Sigma) for 10 minutes at power level 6. Any tissue that was to be stained for BrdU also underwent HCl treatment by incubating in 2 N hydrochloric acid (Fisher) for 30 minutes at 37°C. After rinsing in 1X PBS, sections were blocked in Blocking Solution, 20% fetal bovine serum (FBS, PAA) and 0.3% TritonX-100 diluted in TBLS [50 mM Tris-Cl pH 7.4, 0.1% sodium azide (Fisher), 1% bovine serum albumin, 0.6 M L-Lysine (Sigma), 0.15 M sodium chloride], for an hour at room temperature. Sections were then incubated in primary antibody, diluted in Blocking Solution, overnight at 4°C. Refer to Table 4 for the details of all the primary antibodies used. Slides were washed 3 times for 10 minutes in 1X PBS before being incubated with secondary antibody diluted 1:1,000 in TBLS for 1 hour at room temperature. Secondary antibodies used were anti rabbit AlexaFluor555 conjugate, anti rabbit AlexaFluor488 conjugate, anti rabbit AlexaFluor594 conjugate, anti mouse AlexaFluor488 conjugate and anti rat AlexaFluor647 conjugate (Invitrogen). 1 mg/mL DAPI was used to counterstain all nuclei of the tissue sections by incubating with a 1:10,000 dilution for 5 minutes. Slides were covered by coverslips (VWR) mounted on DAKO Fluorescence Protector (Denmark). Slides were imaged using a Zeiss Axio Imager M.1 microscope and Axiovision software. Images were processed in Adobe Photoshop.

Table 4. Primary antibodies used for IHC

Antibody	Source	Dilution
Mouse Anti-BrdU/IdU	BD Biosciences	1:100

Rat Anti-BrdU/CldU	Abcam	1:100
Rat Anti-Ctip2	Abcam	1:250
Rabbit Anti-Tbr2	Abcam	1:500
Rabbit Anti-Ki67	Abcam	1:250
Rabbit Anti-Nurr1	Santa Cruz	1:500
Rabbit Anti-Brn2 (H-60)	Santa Cruz	1:200

Table 4. The primary antibodies used for immunohistochemistry are listed along with their commercial source and the working dilution.

2.15 *In Situ* Hybridization

In Situ Hybridization was carried out as previously described with some modification (Jensen and Wallace, 1997). All control and Ex6DEL tissue that was used for comparison was placed on the same slide for consistency in the staining. Tissue sections were incubated with digoxigenin (DIG)-labelled riboprobes for ER81, Tbr1 and Foxg1 (Obendorf et al. 2007) diluted 1:1,000 in Hybridization Buffer (1X Salt Buffer, 50% formamide, 10% dextran sulfate, 1 mg/mL rRNA, 1X Denhardt's Solution in DEPC H₂O) overnight at 65°C in a humidified chamber to allow hybridization. The slides were washed at 65°C in Wash Buffer (1X SSC, 50% formamide, 0.1% Tween-20 in DEPC H₂O) for 15, 30, and 30 minutes followed by four 20 minute washes in 1X MABT (100 mM Maleic Acid, 150 mM sodium chloride, 0.1% Tween-20) at room temperature. Sections were incubated with Blocking Solution [20% sheep serum, 2% Blocking Reagent (Roche), 1X MABT] for 1 hour at room temperature prior to adding Anti-DIG-Alkaline Phosphatase, Fab Fragment (Roche) diluted 1:1,500 in Blocking Solution. The antibody was then allowed to bind overnight at 4°C. Four 20 minute washes were completed in 1X MABT followed by two 20 minute washes in Prestaining Buffer (100 mM sodium chloride, 50 mM magnesium chloride, 100 mM Tris-Cl pH 9, 0.1% Tween-20), all at room temperature. The colourimetric reaction was developed in a Staining Buffer [100 mM NaCl, 50 mM MgCl₂, 100 mM Tris-Cl pH 9, 10% polyvinyl alcohol, 0.1% Tween-20, 4.5 µl/mL 4-Nitro blue tetrazolium chloride (Roche) and 3.5 µl

/mL 5-bromo-4-chloro-3-indolyl-phosphate (Roche)] for 2 hours to overnight. Slides were washed in 1X PBS to stop the reaction and then mounted with DAKO Fluorescence Protector. An Axio Skop 2 Zeiss microscope fitted with an AxioCam Camera (Zeiss) was used to image the slides. Image processing was then completed using Photoshop (Adobe).

2.16 TUNEL Staining

Coronal sections of E13.5, 15.5 and 17.5 cortex were used for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Gavrieli et al., 1992). First, slides were rehydrated in distilled H₂O for 2 minutes. Sections are then fixed for 10 minutes 1% PFA at room temperature followed by two 5 minute washes in 1X PBS. Tissue was then incubated in precooled 2:1 Ethanol/Acetic Acid at -20°C for 5 minutes to permeabilize the cells. To generate a positive control, one slide was incubated with DN Buffer (30 mM Tris pH 7.2, 4 mM MgCl₂, 0.1 mM DTT) for 5 minutes and then with 25 µg/mL DNase I diluted in DN Buffer for 10 minutes, both at room temperature. To stop the reaction the slides were rinsed four times for 3 minutes in distilled H₂O. All slides were then incubated for 10 minutes with TdT Reaction Buffer (pH 6.6, 24 mM Tris-Cl, 192 mM Sodium cacodylate trihydrate, 0.024% BSA, 1 mM Cobalt chloride hexahydrate). Terminal deoxynucleotidyl transferase (TdT) enzyme (0.44%, Roche) and DIG-11-dUTP (0.35%, Roche) were then applied in TdT Reaction Buffer for 1 hour at 37°C. Tissue was washed in Stop Buffer (300 mM NaCl, 30 mM Sodium Citrate) for 5 minutes at room temperature to stop the TdT reaction. For storage until the protocol was continued, slides were kept in a Coplin jar containing 70% Ethanol overnight at -20°C. The slides were washed in 1X PBS and then blocked in 1% BSA in 1X PBS for 1 hour at room temperature. Anti-DIG CY3 conjugate (Roche) was added to the slides diluted 1:20 in 1% BSA in 1X PBS containing 0.03% TritonX-100 and allowed to bind for 30 minutes at room temperature. Slides were then

washed in 1X PBS and counterstained with DAPI as was mentioned previously. The slides were mounted using DAKO Fluorescence Protector and imaged using a Zeiss Axio Imager M.1 microscope and Axiovision software. Images were processed in Photoshop.

2.17 Neural Stem Cell Culture Generation

At gestational day 12.5 just before embryo removal 70% ethanol was sprayed onto a pregnant mare to reduce the possibility of bacterial contamination. The embryos were removed as described above and transferred into a clean 10cm² Petri dish containing Hank's Balanced Salt Solution. Using a dissection microscope, the embryo was cradled in a supine position between the tines of sterile curved forceps so the forebrain was accessible. The uppermost layer of skin is punctured directly between the eyes using sterile #5 forceps and grasping the skin layer posterior to the cortex the layer is gently peeled back to expose the cortex. Each individual hemisphere of the cortex was then removed using the #5 forceps. Forebrain tissue was then placed into Eppendorf tubes stored on ice. Tissue was taken into a level II biosafety cabinet (Forma Scientific) and was triturated into single cell suspensions in Proliferation Media (10% NeuroCult[®] NSC Proliferation Supplement, 20 ng/mL hEGF and 1% anti-biotic/anti-micotic in NeuroCult[®] NSC Basal Media). Reagents for the media were obtained from Stem Cell Technologies, Gibco and Invitrogen (Biosource). The single cell suspension was transferred into a T-25 culture flask (Corning) containing 6.5 mL of Proliferation Media. Cultures were incubated at 37°C and 5% CO₂ for 8 days in a Sanyo CO₂ incubator before passaging using the NeuroCult[®] Chemical Dissociation Kit (Stem Cell Technologies). Neurosphere pellets were collected at 16 days in vitro and stored at -80°C.

2.18 Western Blot

Neurosphere cell pellets were resuspended in 150 µL of RIPA Buffer (10 mM Sodium Phosphate Buffer pH 7, 150 mM sodium chloride, 1% NP-40, 0.1% SDS, 1%

sodium deoxycholate, 10 mM sodium fluoride, 2 mM EDTA) containing protease inhibitor cocktail (Roche). Samples were homogenized with a Tissue Tearor homogenizer (Biospec Products) and then incubated on ice for 1 hour to ensure complete lysis. Samples were cleared by centrifugation at 10,000 x g for 10 minutes at 4°C. The protein concentration of the supernatant was quantified using Bradford reagent and a BioPhotometer Spectrophotometer (Eppendorf).

35 µg of protein from each neurosphere extract along with a Precision Plus marker (Bio-Rad) was run on a precast NuPAGE® 3-8% Tris-Acetate gradient gel (Invitrogen) in an XCell SureLock™ Mini-Cell system (Invitrogen). An overnight wet transfer at 10v was used to transfer the gel contents onto a Hybond-C Extra nitrocellulose membrane (Amersham Biosciences). The transfer efficacy was verified by incubating the membrane with Ponceau S stain (0.1% Ponceau S, 5% acetic acid) for 5 minutes and then rinsing it clean with distilled H₂O. The blot was then blocked for 2 hours in 5% milk (Carnation) in TBS-t (10 mM Tris-Cl pH8, 150 mM NaCl, 0.05% Tween-20). Rabbit anti-Foxg1 (Abcam) was diluted 1:500 in 5% BSA in TBS-t and incubated with the blot overnight at 4°C. The blot was washed three times for 5 minutes and twice for 10 minutes in TBS-t and then incubated with goat anti rabbit Horse Radish Peroxidase (HRP) conjugate (Sigma) diluted 1:5,000 in %5 milk in TBS-t for 1 hour at room temperature. Non-specific signal was washed off with TBS-t three times for 10 minutes. The blot was developed with an ECL Plus Western Blotting Detection kit (GE Healthcare) and exposed on a CL-Xposure film (Fisher). The film was developed using a SRX-101A developer (Konica Minolta). The blot was then stripped using Restore Western Blot Stripping Reagent (Thermo Scientific) and reprobed with mouse anti-βActin (1:1,000) as a loading control.

3.0 Results

3.1 Characterization of Changes in Foxg1 Expression

Microarray and RT-PCR have shown an upregulation in Foxg1 expression (Yip et al., 2012). To look at the upregulation in a tissue-specific context, *in situ* hybridization was performed on coronal embryonic day (E) 15.5 cortical sections. Sections of WT and Ex6DEL tissue were placed on the same slide so staining conditions were identical for comparison. This was repeated for three separate sets of tissue. The expression of Foxg1 was confined to the telencephalon and was observed in both germinal zones and the cortical plate, consistent with documented patterns (Hatini et al., 1994; Siegenthaler et al., 2008). While Foxg1 levels show heterogeneity between cells, there was an obvious increase in overall staining intensity of Ex6DEL embryos from all tissue sets with a slightly denser staining pattern in the ventricular zone. [Figure 7, compare panels A to B, C to D, and E to F]

Protein expression does not always correlate with mRNA levels (Gry et al., 2009). Foxg1 is upregulated throughout the Ex6DEL cortex at the level of mRNA but to confirm that Foxg1 is upregulated at the protein level Western blot was used. Since achieving a consistent protein extraction from whole cortical tissue proved to be difficult, neurosphere cultures from E12.5 forebrain were generated and grown in culture for 16 days under proliferative conditions. This method is akin to examining the expression of Foxg1 in cortical progenitor cells since the E12.5 cortex primarily consists of progenitors and these are the cells capable of forming neurosphere cultures. Ex6DEL neurosphere cultures show an increase in Foxg1 levels compared to WT cultures [Figure 8A, compare lanes 1, 2, 5, 6 to 3, 7]. Consistent with RT-PCR which demonstrated a 1.4-fold increase in Foxg1 mRNA expression, we observed a similar increase in the protein level (1.65 ± 0.5) as determined by

Figure 7. Increased Foxg1 expression in the Ex6DEL cerebral cortex.

In situ hybridization was used to examine Foxg1 mRNA expression coronal sections of E15.5 cortex using a probe specific for Foxg1. Ex6DEL (**A, C, E**) cortex has increased levels of Foxg1 throughout the tissue, with particularly dense staining in the ventricular zone as compared to WT (**B, D, F**) controls. CP, cortical plate; IZ, intermediate zone; VZ/SVZ, ventricular zone/subventricular zone; LV, lateral ventricle; Scale bar = 200 μ m.

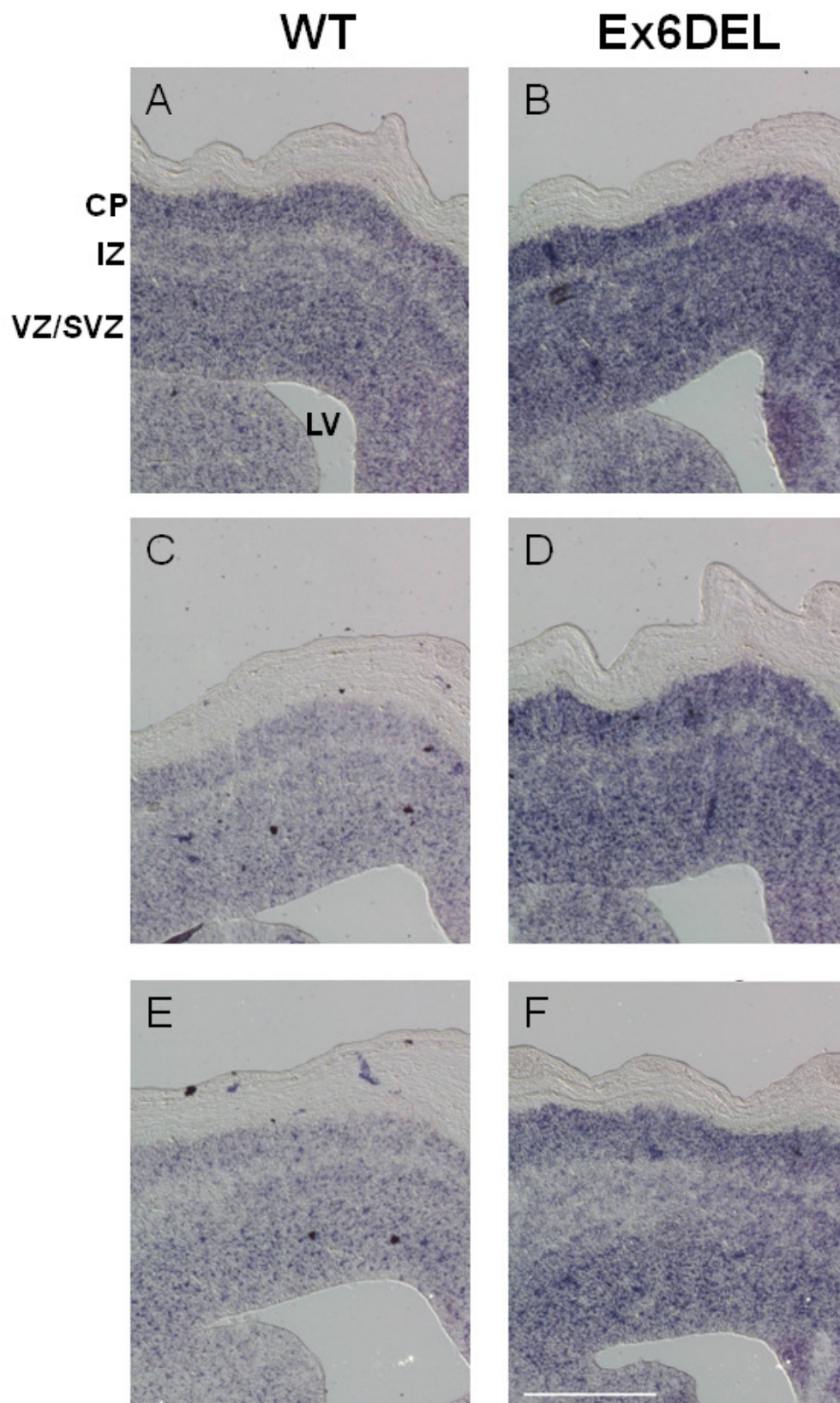
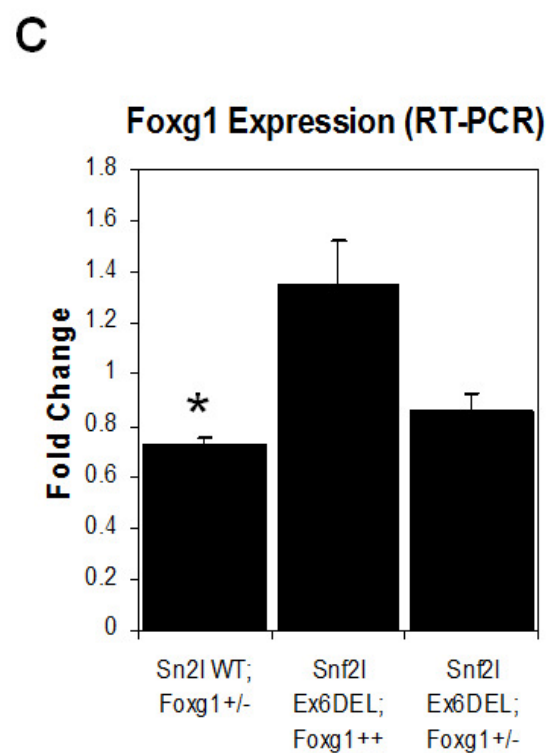
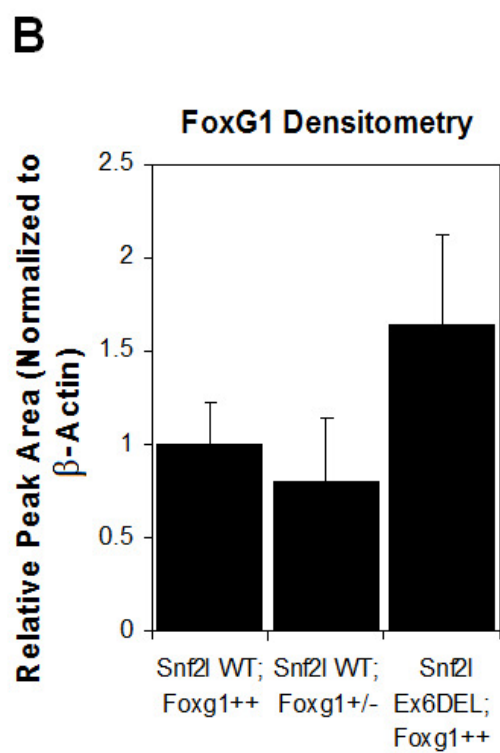
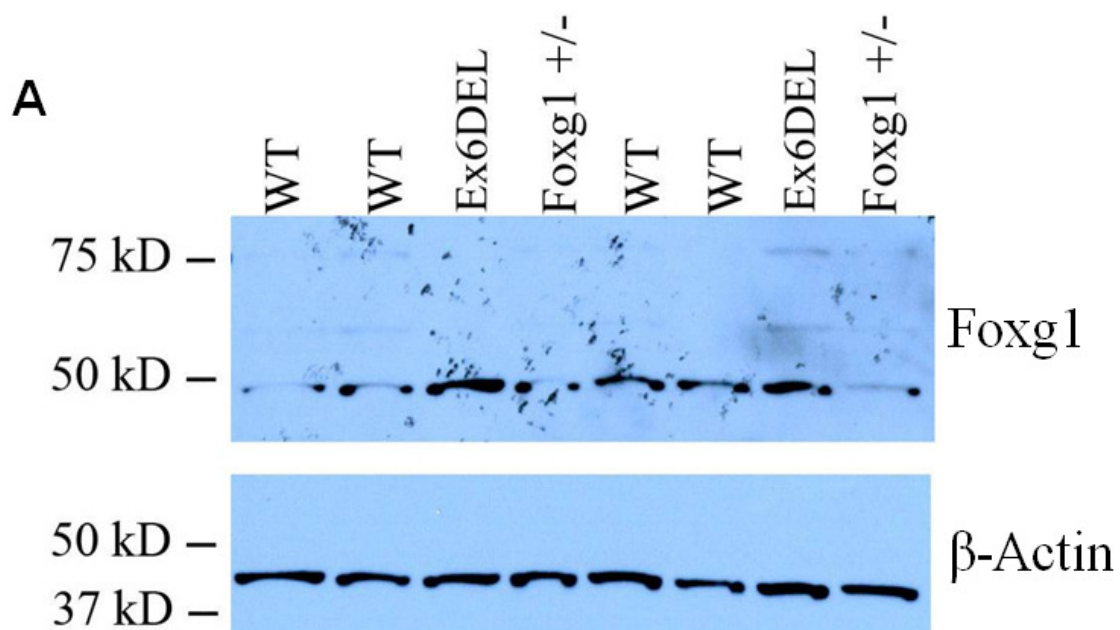


Figure 8. Genetic rescue of Foxg1 restores expression levels.

(A) Western blot for Foxg1 showing upregulation in Snf2l Ex6DEL neurospheres. Lysate was obtained from proliferating neurosphere cultures, isolated from E12.5 forebrain, of the indicated genotype. A decrease in Foxg1 expression is present in Foxg1 heterozygous cultures demonstrating the sensitivity of the antibody. β -Actin is used as a loading control.

(B) Densitometry analysis of the western blot signals from (A) using Image J.

(C) Expression of Foxg1 relative to wildtype at E15.5 as measured by qRT-PCR. * $p < 0.01$; $n = 3$; Error bars represent SEM.



densitometry [Figure 8B]. This increase was not statistically significant due to low sample size and sample variability. Nonetheless, Foxg1 heterozygous cultures show a decrease in expression demonstrating the sensitivity of the western blot (0.8 ± 0.3) [Figure 8A, B]. From this analysis we conclude that Snf2l inactivation leads to a modest increase in Foxg1 protein expression in cortical progenitor cells.

Previous work demonstrated that the Ex6DEL phenotype could be rescued by removing one copy of Foxg1 (Yip et al., 2012). RT-PCR was used to assess whether this also resulted in reduction of Foxg1 levels. The tissue needed to study genetic rescue was obtained by crossing Snf2l heterozygous females with males that are Foxg1 heterozygous and dissecting out the cortex at E15.5. This cross generates all four genotypes required for comparison: WT, Snf2l WT; Foxg1^{+/-}, Snf2l Ex6DEL, and Snf2l Ex6DEL; Foxg1^{+/-}. An RT-PCR primer whose amplicon lies within the coding sequence of Foxg1 showed a 1.35 ± 0.18 -fold Foxg1 upregulation in Ex6DEL cortex, consistent with previous results [Figure 8C]. This was not significant due to one replicate out of three that was not upregulated and introduced a source of variability. Levels in the Foxg1 heterozygous cortex were significantly reduced ($p = 0.005$, student t-test). A 0.73 ± 0.02 -fold Foxg1 expression level in the heterozygous cortex suggests that the single gene copy is being induced to compensate for the loss the other allele [Figure 8C]. The Ex6DEL; Foxg1^{+/-} cortex expression levels were restored to wildtype levels (0.86 ± 0.07 -Fold, $p = 0.14$) showing effective rescue of the upregulation present in Ex6DEL cortex [Figure 8C].

3.2 Effects of Snf2l Ablation on the Neural Progenitor Population

Foxg1 is involved in maintaining the proliferation of telencephalic progenitors and determining the differentiation timing of cortical neurons (Xuan et al., 1995). Ex6DEL mice have increased cell density in the cortex postnatally but since most of the neurons that make

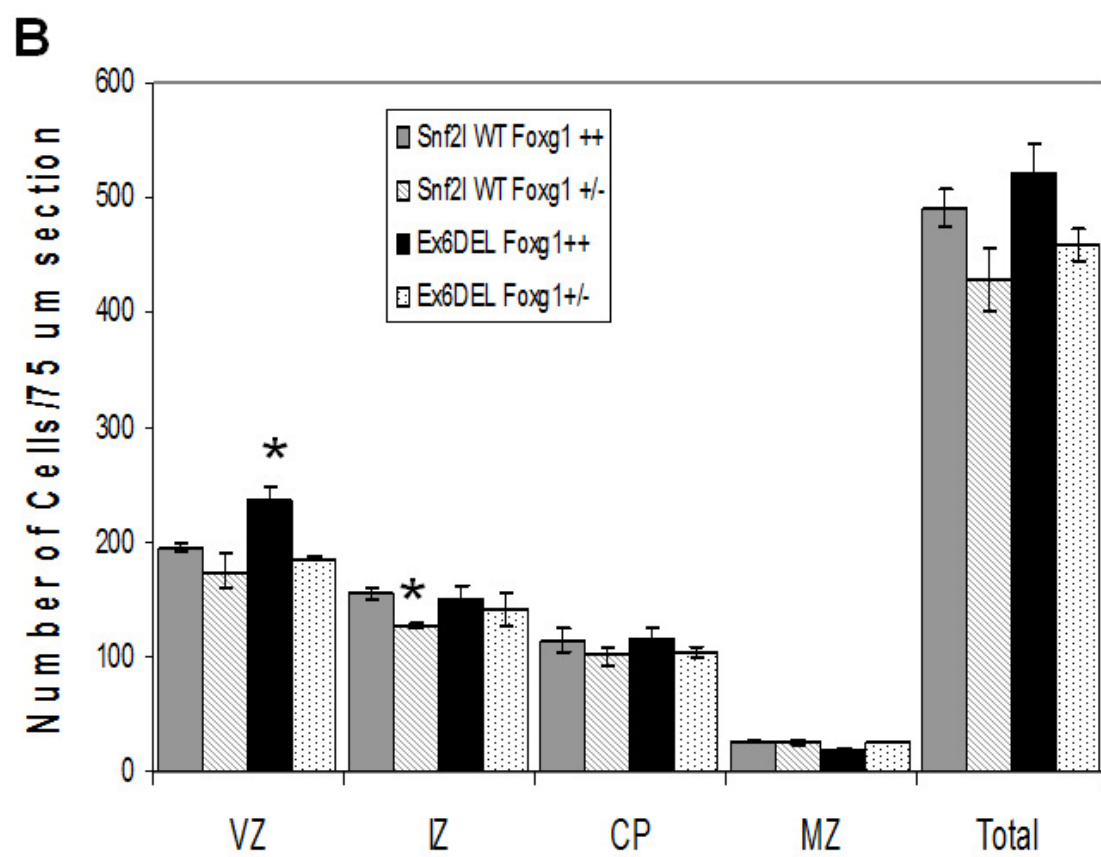
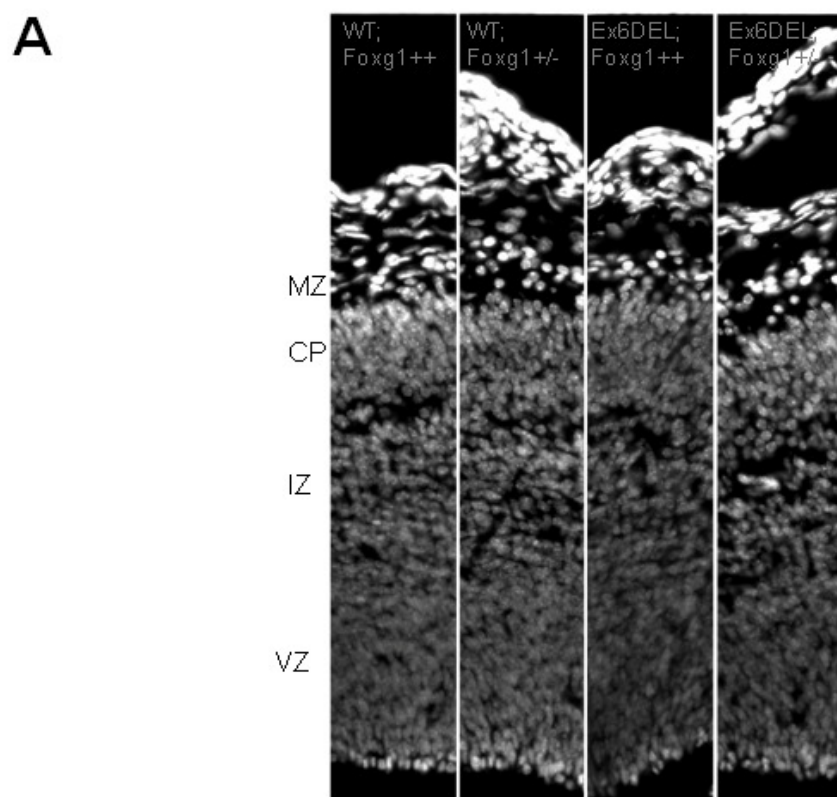
up the cortical plate have been generated by birth, we asked whether populations are affected during their development. The onset of *Foxg1* expression is prior to the beginning of corticogenesis and reaches peak expression at mid-corticogenesis (E15.5) (Shimamura et al., 1995). Therefore E15.5 is a viable timepoint for observing developmental phenotypic changes. To examine the cell population of E15.5 cerebral cortex, coronal sections were stained with DAPI, a fluorescent DNA stain, to identify all cell nuclei. The overall cell organization of the cortex was the same for each genotype but Ex6DEL cortical sections often appeared to have greater cell density in some areas [Figure 9A]. A 75 μ m width of dorsal cortex was used to count the cell numbers in the ventricular zone/subventricular zone (VZ), intermediate zone (IZ), cortical plate (CP) and marginal zone (MZ) separately and as part of a total cell count. *Snf2l* WT; *Foxg1*^{+/-} embryos showed a decreasing trend in cell number in the VZ, IZ and total numbers but was only significant in the IZ ($p = 0.01$) [Figure 9B]. For the Ex6DEL samples there was a trend towards increasing cell number in all areas but the MZ. The average total cell number per 75 μ m width of cortex for WT was 491 ± 16 cells and was increased to 521 ± 26 cells in the Ex6DEL cortex ($p = 0.37$) [Figure 9B]. This increase is primarily attributed to the VZ since it is the only region with a significant increase in the number of cells (WT: 195 ± 3 cells, Ex6DEL: 236 ± 12 cells, $p = 0.03$). These cell population changes indicate that early increases in cell number during development are in the progenitor population and have not yet been propagated to mature neurons in the cortical plate. *Snf2l* Ex6DEL; *Foxg1*^{+/-} cell counts were not significantly different from WT in any cortical region [Figure 9B]. Full rescue of the cell number phenotype in the *Snf2l* Ex6DEL; *Foxg1*^{+/-} indicates that it is a *Foxg1*-mediated effect.

In the Ex6DEL cortex, increased *Foxg1* expression affects the self-renewal/differentiation balance of cortical progenitors. At E15.5 there is a significant

Figure 9. Increased ventricular zone in Ex6DEL embryonic cortex.

(A) 75 μ m-wide coronal sections of DAPI-stained E15.5 cortex.

(B) Counts of the number of cells in the ventricular zone/subventricular zone (VZ), intermediate zone (IZ), cortical plate (CP) and marginal zone (MZ) per 75 μ m width of developing cortex. Total cell number was calculated as well. Snf2l Ex6DEL cortex had increased numbers of cells in the VZ and a trend towards greater total cell number (not statistically significant). The increase in cell numbers was rescued by genetically decreasing the expression of Foxg1. *, $p < 0.05$, comparison with WT; $n = 3$; error bars represent SEM.



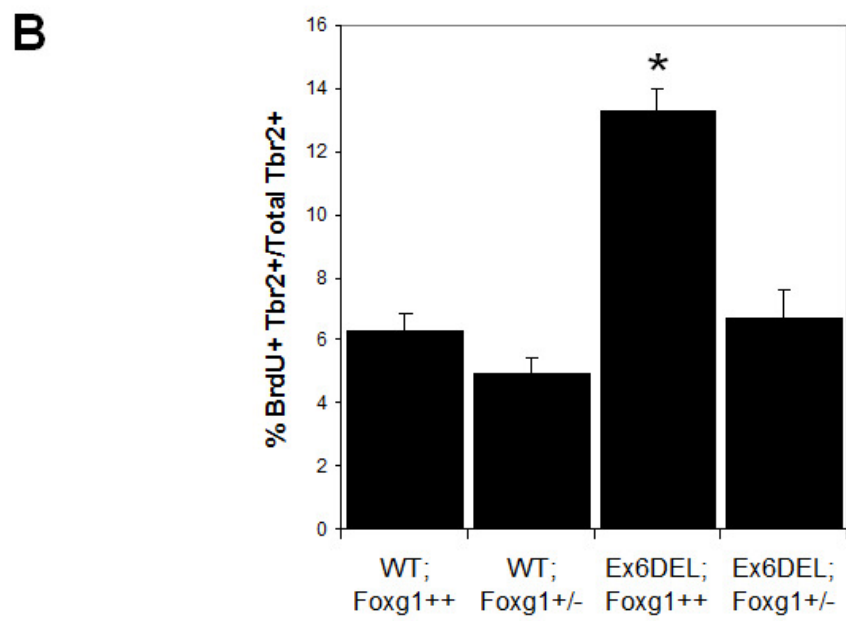
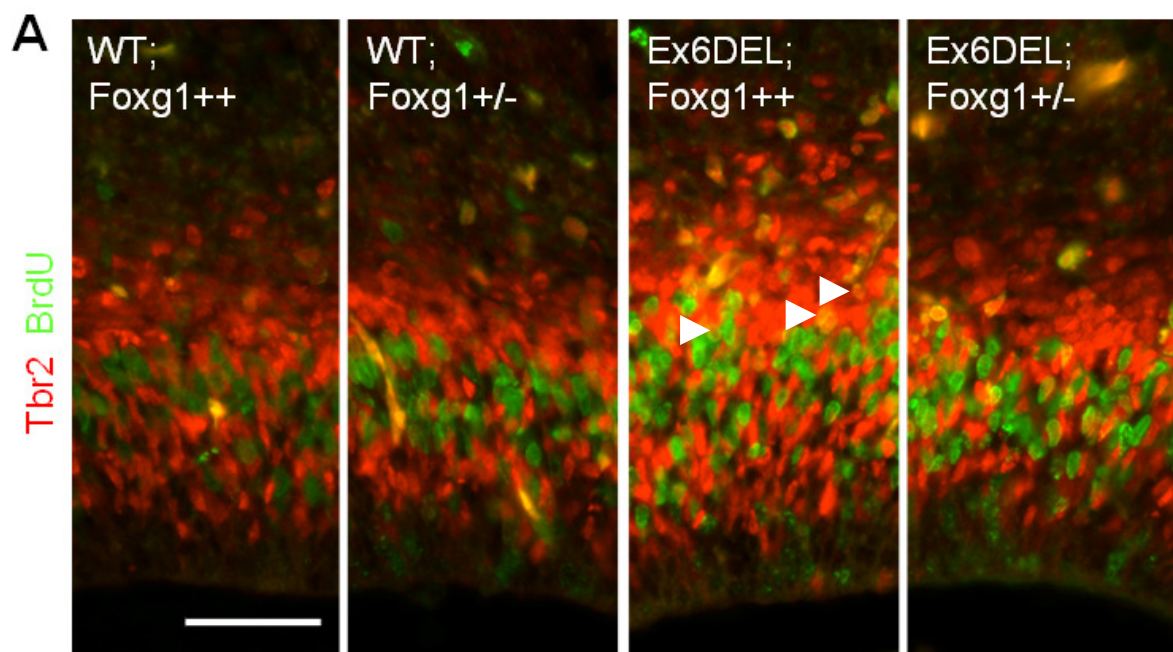
increase in the number of VZ/SVZ cells, a region that contains both apical progenitor and intermediate progenitor cells. Since it is known that Foxg1 regulates IPC expansion and that Foxg1 is upregulated in the Ex6DEL cortex we explored this population (Siegenthaler et al., 2008; Yip et al., 2012). To look specifically at proliferation of IPC population, Tbr2-positive progenitors were co-labeled with BrdU after a 2 hour pulse at E15.5. The Tbr2 staining was more prominent in the Ex6DEL cortex with some dorsal expansion evident [Figure 10A]. There also appeared to be more Tbr2/BrdU double-positive cells visible especially on the superficial side of the SVZ [Figure 10A]. Quantification of Tbr2/BrdU double-positive cells as a percentage of the total number of Tbr2-positive cells showed that the Ex6DEL cortex has more than double the number of proliferative IPCs (WT: $6.3 \pm 0.6\%$, Ex6DEL: $13.3 \pm 0.7\%$, $p = 0.001$) [Figure 10B]. Removing one copy of Foxg1 resulted in decreased proliferation of IPCs on both Snf2l WT and Ex6DEL backgrounds consistent with data published by Siegenthaler *et al* showing decreased IPC number and production in Foxg1 heterozygous mice (Snf2l WT; Foxg1^{+/-}: $5 \pm 0.5\%$, Snf2l Ex6DEL; Foxg1^{+/-}: $6.7 \pm 0.9\%$, $p > 0.1$) [Figure 10B]. The Snf2l Ex6DEL; Foxg1^{+/-} IPC proliferation is identical to WT levels indicating that increased Foxg1 expression is responsible for increased IPC proliferation. It can also be concluded that intermediate progenitor cells in the cortex are sensitive to dosage effects of Foxg1.

The cell cycle lengthens as differentiation proceeds and, conversely, modulating cell cycle length affects progenitor differentiation. Mice with reduced levels of Foxg1 have a premature lengthening of the cell cycle and precocious differentiation (Martynoga et al., 2005; Siegenthaler et al., 2008; Xuan et al., 1995). These effects are associated with increased expression of the CKI p21^{Cip1} in the murine forebrain (Siegenthaler et al., 2008).

Figure 10. Increased intermediate progenitor cell proliferation.

(A) Sections of E15.5 cortex, that had undergone a two hour thymidine analog pulse labelling, were stained with primary antibodies for BrdU (Green) and Tbr2 (Red).

(B) The Ex6DEL cortex has a much greater percentage of BrdU labelled cells indicating increased proliferation of intermediate progenitors. The majority of the co-labelled cells are found in the subventricular zone (arrowheads). $n = 3$; *, $p < 0.001$, comparison with WT; error bars represent SEM; Scale bar = 50 μm .



Since Ex6DEL embryos have increased Foxg1 and decreased CKI expression in the cerebral cortex we wanted to determine if a shortened cell cycle was a contributing factor in the phenotype. A double-thymidine analog labeling technique, previously used to study Foxg1 mutants, was used to measure cell cycle parameters at E13.5, E15.5 and E17.5 (Martynoga et al., 2005; Siegenthaler et al., 2008). The experimental technique involved injecting IdU followed by BrdU an hour and a half later into a timed-pregnant female. The female was sacrificed and the embryos removed 30 minutes after the second injection. These two separate analogs were then differentially detected by specific antibodies: one which detects both analogs and the other which detects BrdU but not IdU. Cells that were in S-phase at the time of the first injection and incorporated IdU but subsequently exited will be labeled by only one fluorophore whereas those that remained in S-phase at the time of the second injection with BrdU will have incorporated both analogs and be double-labelled.

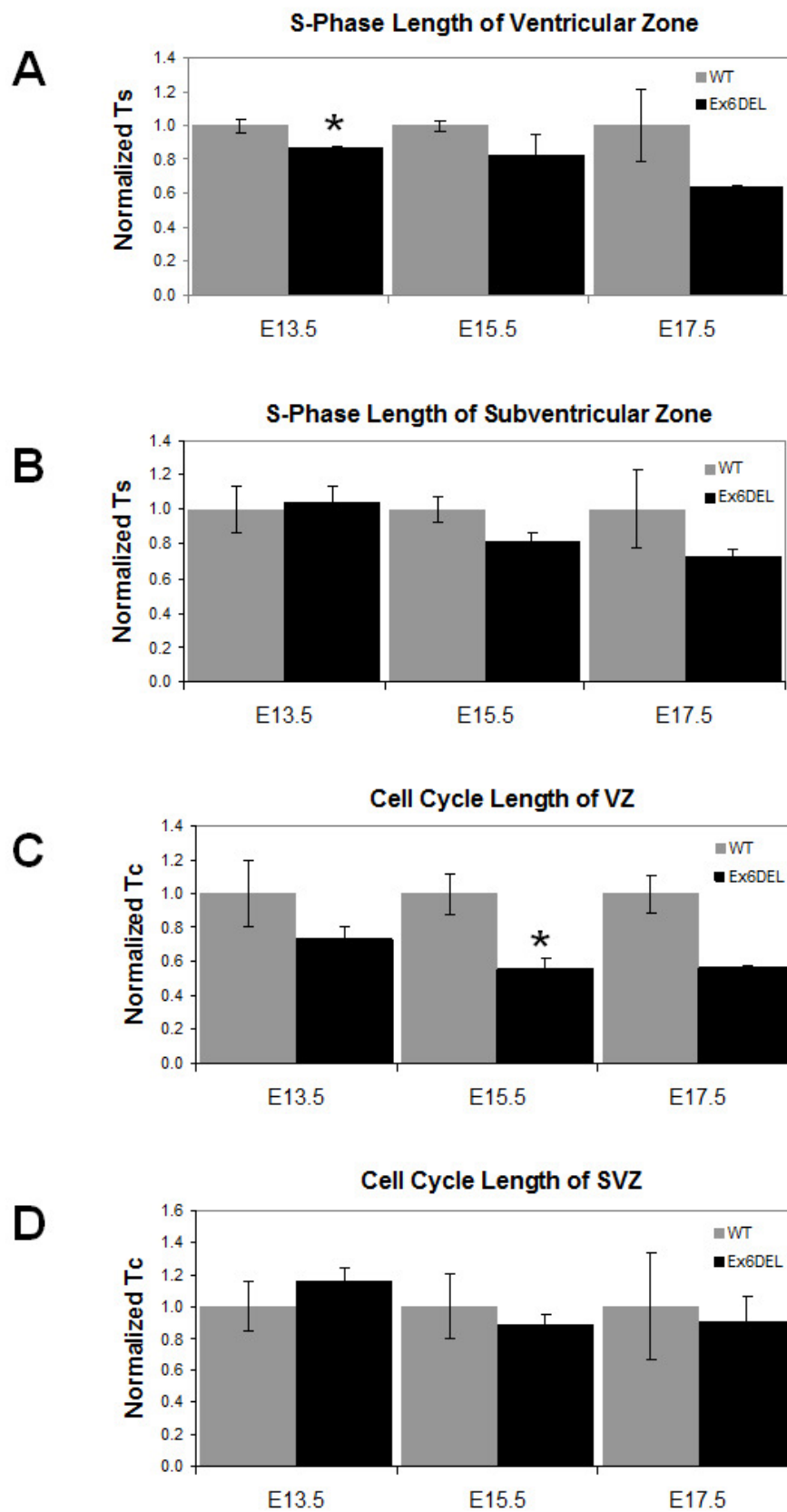
Quantification of these two populations of cells was used to calculate both S-phase length (T_s) and overall cell cycle length (T_c) [Figure 6A]. Since differentiation timing follows a rostrocaudal gradient and differences between genotypes can vary by region (Martynoga et al., 2005), sections from four different anatomical points spanning the rostrocaudal axis of the forebrain were stained and quantified to achieve an overall estimate of cell cycle parameters. Observation of stained cortical sections revealed that the vast majority of cells that had incorporated thymidine analogs were found in the germinal areas of the cortex and, among those, single-labeled cells were primarily found at the apical membrane of the lateral ventricle consistent with interkinetic movement of apical progenitors [Figure 6B].

Quantification was done using 100 μm -width areas of dorsal cortex which were further subdivided in VZ and SVZ. The S-phase and cell cycle lengths calculated had a tendency of being longer than published values. It should be noted that the literature values can vary

between publications but define a range of accepted values. For example, Ts at E14 has been calculated as a range of values between 2.4 and 5 hours (Estivill-Torrus et al., 2002; Martynoga et al., 2005; Siegenthaler et al., 2008; Takahashi et al., 1996; Yuasa et al., 2002). At E13.5, the average S-phase length of WT progenitors measured 4.6 ± 0.2 hours which falls within the range identified in the literature but cell cycle length measured 17.3 ± 3.4 hours falls outside the usual lengths of 10-12 hours (Martynoga et al., 2005; Siegenthaler et al., 2008; Takahashi et al., 1996). This increase becomes much more prominent at later timepoints where at E17.5 the Ts and Tc values of 10.2 ± 6 and 44.3 ± 3 hours, respectively, are approximately double those in the literature (Martynoga et al., 2005; Siegenthaler et al., 2008; Takahashi et al., 1996). Despite the discrepancies between these experimental results and those published previously the overall trend of the cell cycle increasing through development is still present. The experimental parameters were consistent throughout these studies and the visual differences observed between genotypes at each timepoint translated into differences in cell cycle length when quantified. As such, the data was normalized to wildtype values to better analyze the differences between genotypes. Relative S-phase lengths of Ex6DEL progenitors in the ventricular zone as development proceeds are 0.87 ± 0.01 (E13.5, $p = 0.03$), 0.83 ± 0.13 (E15.5, $p = 0.26$), and 0.64 (E17.5, $p = 0.23$) [Figure 11A]. The shorter Ts times relative to controls become more pronounced through development. This is also observed in the subventricular zone but differences only become apparent at E15.5 when this area becomes more prominent in the developing cortex (E13.5: 1.04 ± 0.09 , $p = 0.8$, E15.5: 0.82 ± 0.04 , $p = 0.1$, E17.5: 0.72 ± 0.05 , $p = 0.35$) [Figure 11B]. Cell cycle length is significantly shortened, almost 50%, in ventricular zone progenitors of the Ex6DEL cortex compared to WT controls (E13.5: 0.73 ± 0.07 , $p = 0.28$, E15.5: 0.55 ± 0.06 , $p = 0.03$, E17.5: 0.56 , $p = 0.06$) but not in the SVZ (E13.5: 1.16 ± 0.08 , $p = 0.42$,

Figure 11. Ex6DEL embryos show dysregulation of cell cycle timing in cortical progenitors.

Embryos were pulsed with IdU followed by BrdU at E13.5, E15.5 and E17.5 as described by Martynoga et al., 2005. Length of S-phase (T_s) normalized to wildtype was calculated for both ventricular zone (A) and subventricular zone (B), as well as normalized cell cycle length (T_c) (C and D). A relative decrease was seen in both T_s and T_c in Ex6DEL embryos that increased with age, although not significant for all values. *, $p < 0.05$; $n = 3$; error bars represent SEM.



E15.5: 0.89 ± 0.05 , $p = 0.63$, E17.5: 0.91 ± 0.15 , $p = 0.83$) [Figure 11C, D]. This data shows there is dysregulation of cell cycle kinetics in the Ex6DEL cortex. In the Ex6DEL cortex progenitors are proliferating more rapidly and this continues throughout corticogenesis. To examine the role of Foxg1 in shortening the cell cycle of progenitor cells, the Ts and Tc of Ex6DEL; Foxg1^{+/-} progenitors were calculated. The Ts of the double transgenic cortical progenitors is rescued from the start of corticogenesis, at E13.5 the relative length is 1.10 ± 0.02 ($p = 0.07$) and this remains fairly constant at E15.5 and E17.5 with values of 0.95 ± 0.02 ($p = 0.17$) and 0.93 ± 0.01 ($p = 0.36$) [Figure 12A]. Unexpectedly though, rescuing the amount of Foxg1 does not fully rescue the defect in Tc. At E13.5, the cell cycle length is equivalent between Ex6DEL and Ex6DEL; Foxg1^{+/-} progenitors (0.79 ± 0.07 vs. 0.79 ± 0.05) [Figure 12B] By E15.5 the cell cycle has begun to lengthen in the Ex6DEL; Foxg1^{+/-} (0.68 ± 0.11 , $p = 0.13$) compared to the Ex6DEL progenitors (0.60 ± 0.05 , $p = 0.04$) but has still not reached wild type length by E17.5 (Ex6DEL; Foxg1^{+/-}: 0.85 ± 0.02 , $p = 0.17$, Ex6DEL: 0.69 ± 0.03 , $p = 0.05$) [Figure 12B]. The rescue of the Ex6DEL hypercellularity phenotype despite a decreased cell cycle length indicates that other cues signaling neural progenitors to arrest are intact in the double transgenic animals.

To be sure that the Foxg1 rescue was not due to enhanced apoptosis, cortical tissue was TUNEL-stained to detect any apoptotic cells. Few apoptotic cells were seen in the cerebral cortex at either E15.5 or E17.5 in any of the genotypes. At E13.5, the cortical tissue that was heterozygous for Foxg1 displayed more TUNEL-positive cells than its Foxg1^{+/+} counterparts [Figure 13A]. However, when quantified, the numbers of TUNEL-positive cells was the same on both the Snf2l wildtype and Ex6DEL backgrounds [Figure 13B]. The fact that Foxg1^{+/-} neural progenitors do not have large deficits in the assays above suggests cell death is not responsible for the rescue of the Ex6DEL phenotype.

Figure 12. Genetic rescue of Foxg1 incompletely rescues dysregulation of cell cycle timing in cortical progenitors.

WT, Ex6DEL, and Ex6DEL;Foxg1+/- Embryos were pulsed with IdU followed by BrdU at E13.5, E15.5 and E17.5 as described by Martynoga et al., 2005. Length of S phase (**A**) and cell cycle (**B**) normalized to wildtype was calculated for cortical progenitors. Genetically reducing Foxg1 levels completely rescues Ts but not Tc. Ts, S-phase length; Tc, Cell cycle length; *, $p < 0.05$; $n = 3$; error bars represent SEM.

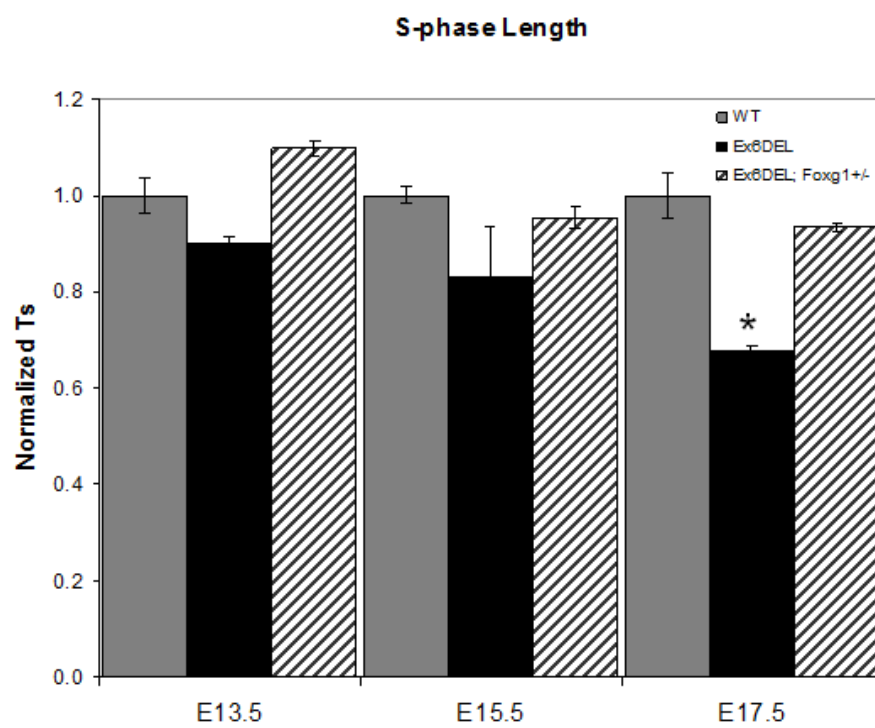
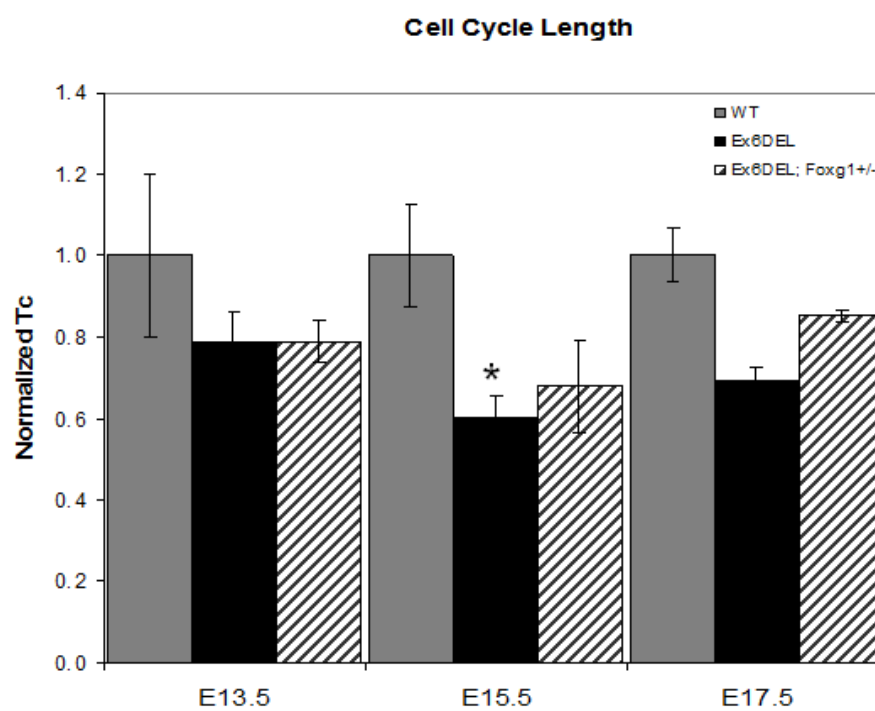
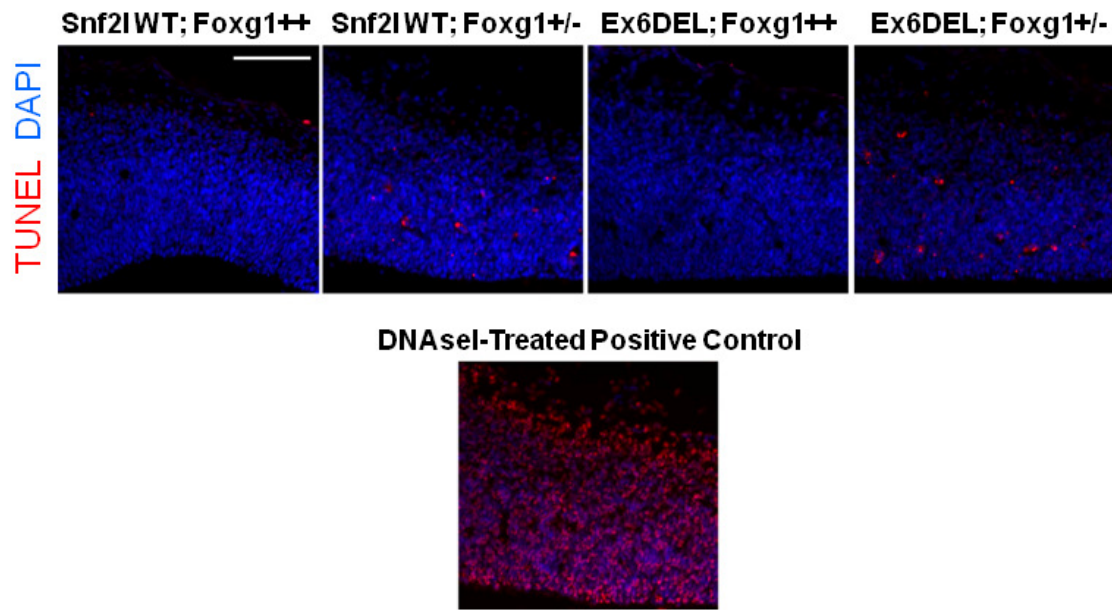
A**B**

Figure 13. Rescue of progenitor cell phenotypes are not due to apoptosis.

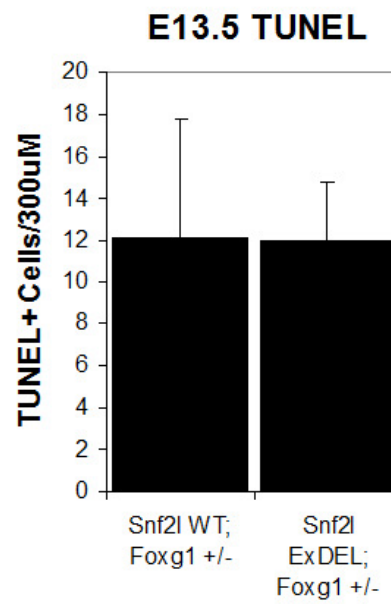
(A) E13.5 cortical tissue was TUNEL stained (red) to detect the presence of apoptotic cells and counterstained with DAPI (blue). Staining revealed some increase in cell death levels in Foxg1 heterozygous embryos. Positive control was obtained by treating tissue with DNaseI. Scale bar = 100 μ m.

(B) TUNEL+ cells in Foxg1 heterozygous samples were quantified on both the wildtype and Ex6DEL backgrounds. n = 3; error bars represent SEM.

A



B



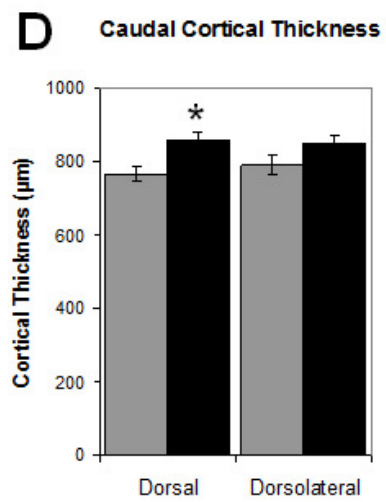
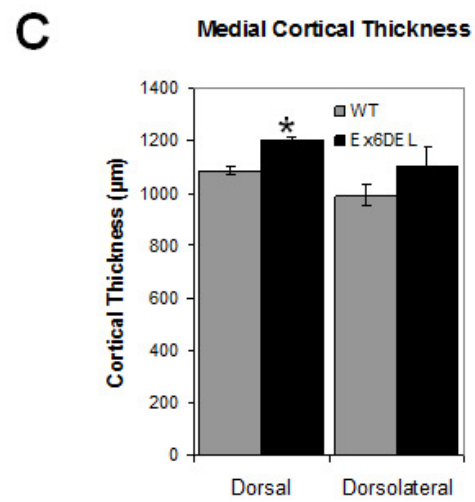
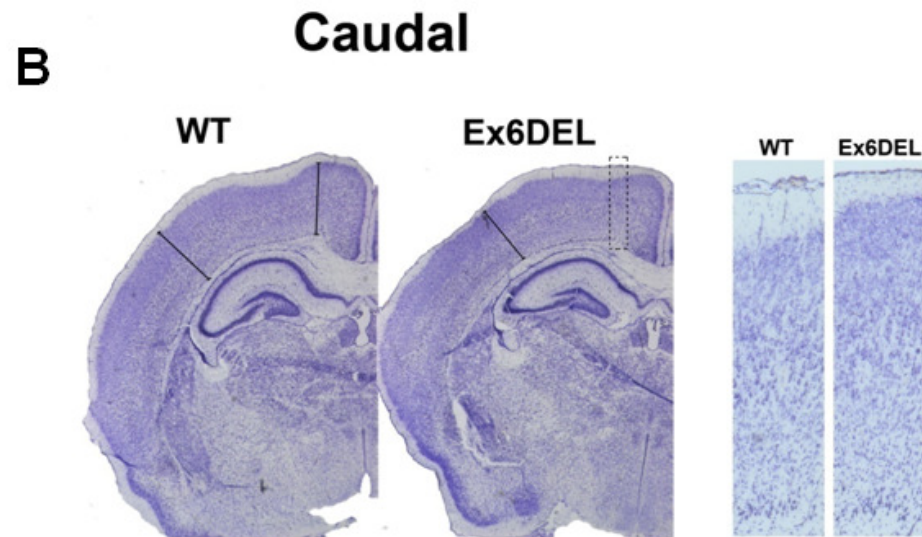
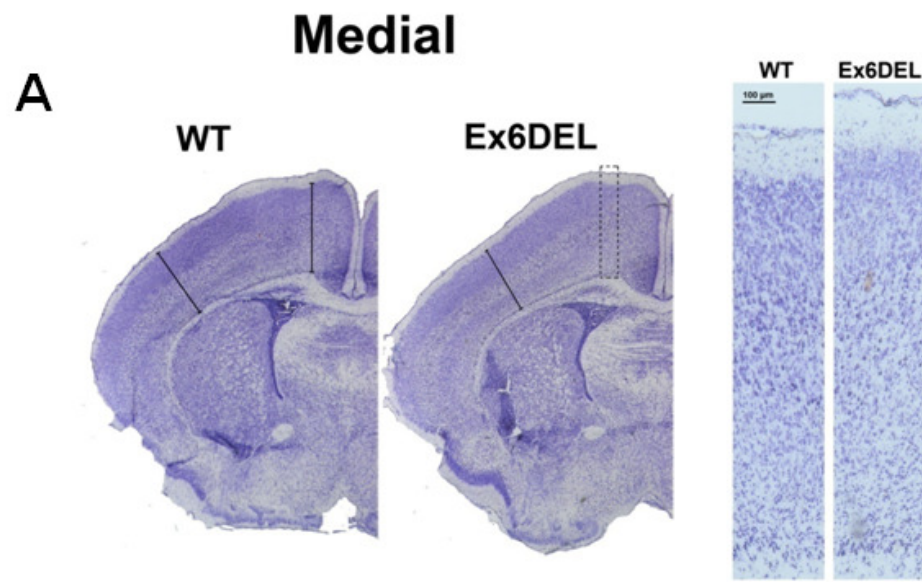
3.3 Altered Generation and Timing of Cortical Layers

The long term consequence of excess proliferation is hypercellularity and increased cell density in the cerebral cortex. Measurement of the cortical thickness of P7 animals was used to determine if the increased cell numbers also affect cortical size. The width of the cortex was measured from the boundary of layers I and II to the underlying white matter at dorsal and dorsolateral positions in medial and caudal coronal cortical sections stained with Nissl [Figure 14A, B - black lines in whole brain sections indicate where measurements were taken]. The corpus callosum along with the choroid plexus were evident in the medial sections and caudal sections were taken at the level of the hippocampus [Figure 14A, B]. The laminar and cellular organization of the cortex observed in the magnified strips of dorsal cortex was normal in Ex6DEL cortex but were slightly thicker [Figure 14A, B – sections on the right]. Measurements of dorsal cortical thickness revealed significant differences of 116 μm and 91 μm in medial and caudal cortex, respectively (Medial: $1089 \pm 13 \mu\text{m}$ vs. $1203 \pm 13 \mu\text{m}$, $p = 0.003$; Caudal: $766 \pm 19 \mu\text{m}$ vs. $857 \pm 23 \mu\text{m}$, $p = 0.04$) [Figure 14C, D]. Dorsolateral cortex showed a trend towards increased cortical thickness with medial and caudal differences of 111 μm and 62 μm , respectively but the differences were not statistically significant (Medial: $990 \pm 42 \mu\text{m}$ vs. $1101 \pm 72 \mu\text{m}$, $p = 0.25$; Caudal: $790 \pm 26 \mu\text{m}$ vs. $851 \pm 20 \mu\text{m}$, $p = 0.14$) [Figure 14C, D]. The hypercellularity of the Ex6DEL cortex manifests as both increased cell density and an increase in cortical thickness (Yip et al., 2012).

Since laminar organization appeared normal at E15 and P7 in the Ex6DEL cortex despite increased progenitor cell self-renewal and cell density, we sought to investigate the Ex6DEL phenotype by looking at specific cortical layer markers. In situ hybridization was used to look at the expression of neuronal layer markers Er81 (layer V) and Tbr1 (Layer VI)

Figure 14. Ex6DEL mice have increased cortical thickness.

Nissl-stained coronal sections of post-natal day 7 brain from wildtype (WT) and Ex6DEL mice were used to determine the thickness of the cortical plate. Measurements were taken at dorsal and dorso-lateral position from medial (**A**) and caudal (**B**) cortex by taking the distance from the top of the white matter to the bottom of the marginal zone as indicated by the solid black line. Images on the right represent a higher magnification of the region indicated by boxes on the left-hand images. Scalebar = 100 μ m. (**C and D**) Average cortical thickness (μ m) measured as described above. n = 3; *, P<0.05; error bars represent SEM.



in three sets (two are represented here) of E18.5 cortex. The staining confirmed that layers V and VI are laminated normally [Figures 15, *Er81*: compare A to B, C to D, *Tbr1*: compare E to F, G to H]. Nonetheless, the increased intensity of the staining on E18.5 cortex suggests increased numbers of both subtypes of neurons that are positioned correctly.

Increased rates of proliferation and cell cycle re-entry in the Ex6DEL cortex lead to a greater progenitor cell population and eventually hypercellularity, increased cortical thickness, and altered cell fate of the cortical plate (Yip et al., 2012). This and the knowledge that *Foxg1* mutants have precocious neural differentiation prompted us to look at the timing of neuronal differentiation. For this experiment, BrdU was injected at various timepoints during corticogenesis for incorporation into the DNA of proliferating progenitors. Those cells that exit the cell cycle shortly after the injection will retain the BrdU as they differentiate and migrate into the appropriate layer of the cortical plate. First, embryos were injected with BrdU at E12.5 to label cells destined for layer VI. Brains were harvested at E18.5 and then the cortex was co-stained for BrdU and *Nurr1*, a layer VI neuronal marker. The *Nurr1*-positive cells appeared more prominent and to be densely packed in many Ex6DEL tissue sections observed [Figure 16A]. This was corroborated by cell counts showing a non-significant increase in *Nurr1*-positive cells in the Ex6DEL cortex within an 85 μm section (WT: 71 ± 3 cells, Ex6DEL: 92 ± 7 cells, $p = 0.06$) [Figure 16B]. When the differentiation timing of *Nurr1*-positive neurons was examined by looking at the percentage that were BrdU-labeled there was a significant decrease in the Ex6DEL cortex (WT: $30.8 \pm 3.9\%$, Ex6DEL: $22.4 \pm 0.9\%$, $p = 0.02$) [Figure 16C]. The decreased percentage of the *Nurr1* population that co-label with BrdU suggests a delay in the birthdate of Ex6DEL Layer VI neurons. To further explore the extent of the delay, the same experimental procedure was

Figure 15. Differences in laminar marker expression in Ex6DEL cortex.

In situ hybridization of E18.5 coronal brain sections with probes for Er81 (**A-D**) and Tbr2 (**E-H**). Er81 and Tbr2 label cortical layers V and I/VI, respectively. The Ex6DEL (**A, C, E, G**) tissue has more intense staining. Scale bar = 100 μ m.

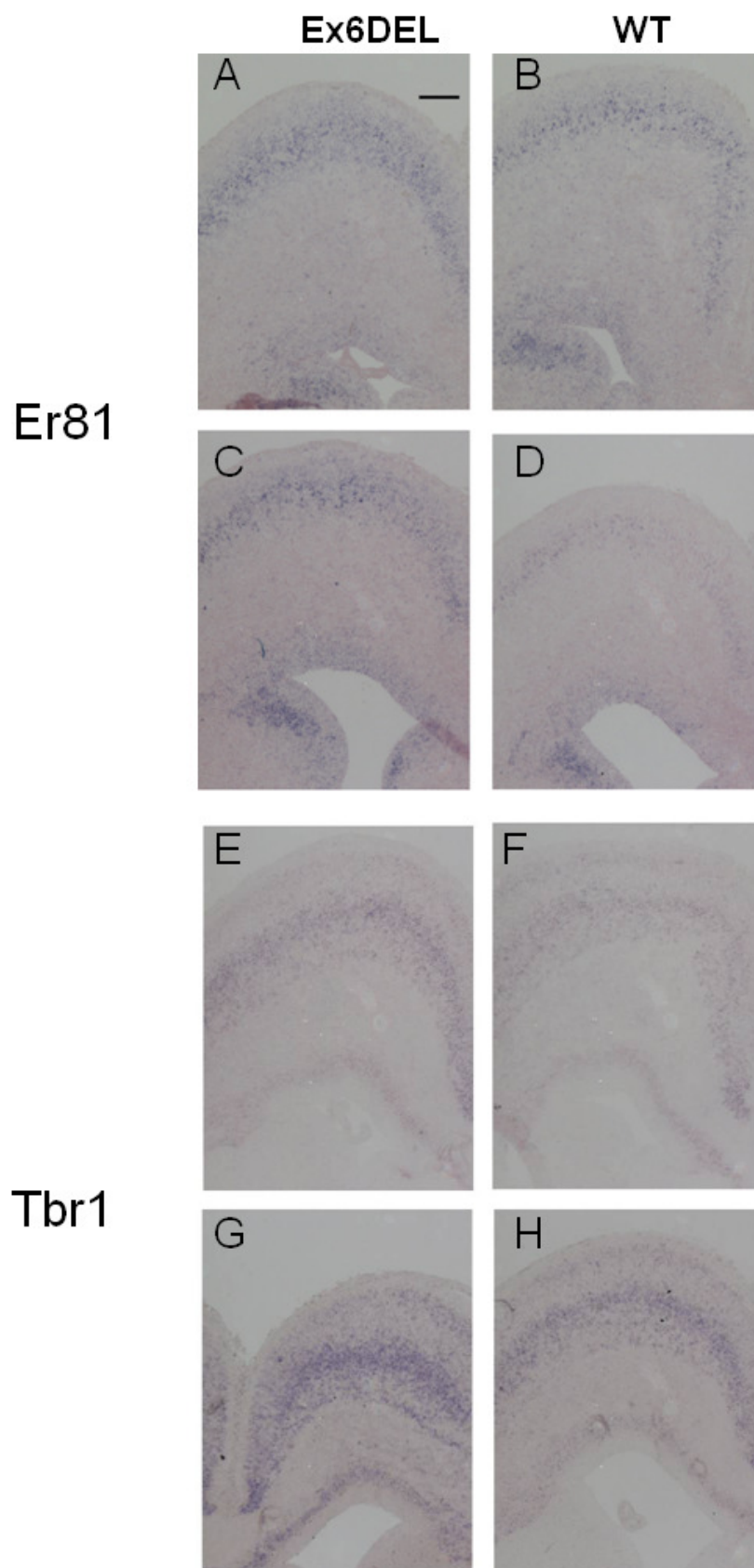
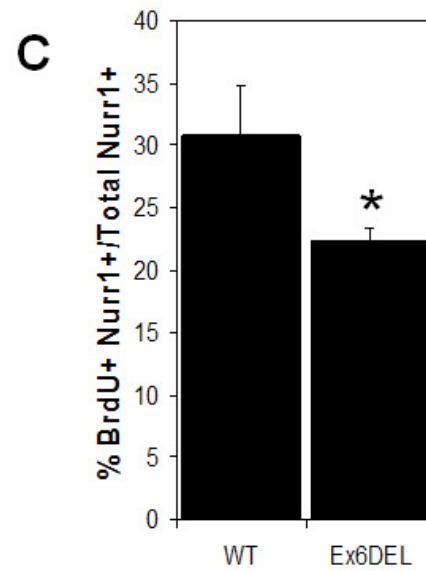
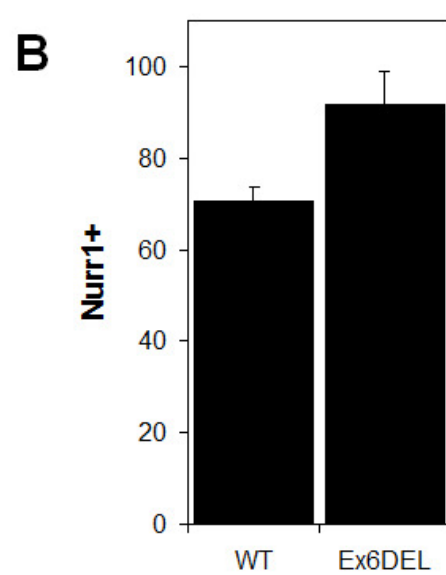
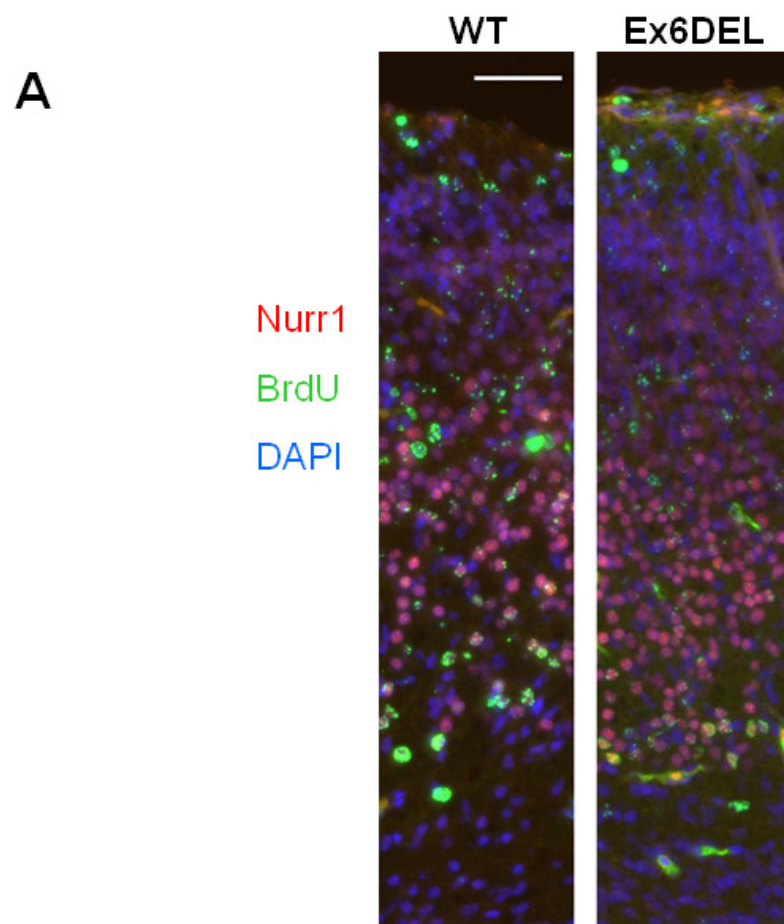


Figure 16. Delayed neurogenesis of Layer VI.

Layer VI cells in the Ex6DEL cortex have a delayed migration into the cortical plate compared to WT counterparts. Embryos were pulsed with BrdU at E12.5 and then sacrificed at E18.5. **(A)** Sections of the cortex were stained for BrdU (green) and Nurr1 (red), a layer VI marker, and counterstained with DAPI (blue). More BrdU-positive cells can be seen spread throughout the Nurr1 layer in the WT cortex whereas more of the labeling in the Ex6DEL cortex is concentrated near the apical boundary of the Nurr1 layer. Scale bar = 50 μm **(B)** Ex6DEL cortex has a non-significant increase in Nurr1 positive cells but has a significant decrease in the number of BrdU/Nurr1 co-labeled cells suggesting an altered timing of differentiation of layer VI neurons. *, $p < 0.03$; $n = 3$ embryos; error bars represent SEM.



performed with a BrdU injection at E15.5 followed by co-staining with Brn2, a marker of layers II, III and V (the layer II/III neurons are born at ~E15.5) at E18.5. In the WT cortex $14 \pm 4.9\%$ of Brn2-positive cells in layers II/III were co-labeled with BrdU compared to $23 \pm 2.7\%$ of cells in the mutants ($p = 0.05$) [Figure 17D]. The delay in neuron generation is recapitulated in layer II/III neurons. There is no significant increase in the number of Brn2-positive neurons in the Ex6DEL cortex (WT: 49 ± 9 cells/150 μm , Ex6DEL: 56 ± 6 cells/150 μm , $p = 0.5$) yet apparent at E18.5 but BrdU staining patterns indicate that migration of new neurons is not complete [Figure 17A, C]. Work by others in the lab has shown that other layer II/III neurons are increased when examined at post-natal timepoints suggesting that later examination of Brn2 might yield increased Brn2-positive neurons later in development. The timing of corticogenesis is altered when Snf2l activity is ablated. To determine what effect genetic rescue has on differentiation delay, embryos were injected with BrdU at E13.5 and then co-labeled with Ctip2, a layer V marker, at E18.5. A shift in the staining pattern of BrdU-labeled cells from predominantly later-born layers in the wildtype cortex to earlier-born layers in Ex6DEL samples was observed [Figure 18A]. When the numbers of Ctip2/BrdU co-labeled cells were quantified there was a significant increase in the Ex6DEL cortex (WT: $22.1 \pm 5.8\%$, Ex6DEL: $40.9 \pm 0.7\%$, $p = 0.02$) [Figure 18B]. This is in contrast to the decrease in double-labeled cells observed with the Nurr1 birthdating experiment. The reason for the opposing trends is due to the timing of the BrdU incorporation relative to the differentiation of the neurons labeled. At the time of BrdU injection in the E12.5 WT cortex, layer VI Nurr1-positive neurons are differentiating. Differentiation of the Nurr1-positive cells in the Ex6DEL cortex lags behind and many of the BrdU-labeled cells are found at the apical edge of the Nurr1-positive population [Figure 16A]. In contrast, cells born at the time

Figure 17. Delayed neurogenesis of Layer II/III.

Layer II/III cells in the Ex6DEL cortex have a delayed differentiation compared to WT counterparts. Embryos were pulsed with BrdU at E15.5 and then sacrificed at E18.5.

(A) Sections of the cortex were stained for BrdU (green) and Brn2 (red), a layer II, III and V marker. Scale bar = 100 μ m.

(B) Magnified images of layer II/III cells in the region outlined by the boxes in (A). Scale bar = 25 μ m.

(C) Quantification shows no difference in the number of Brn2 positive cells but Ex6DEL embryos have a significant increase in the number of BrdU/Brn2 co-labeled cells. *, $p < 0.03$; $n = 3$; error bars represent SEM.

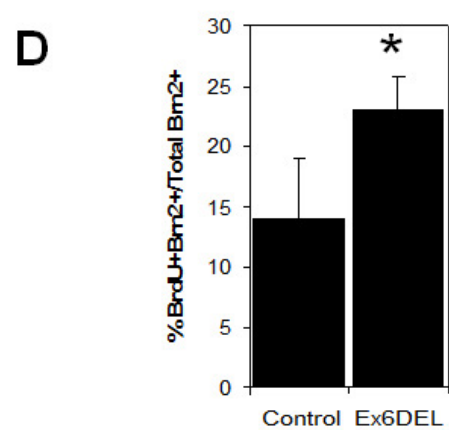
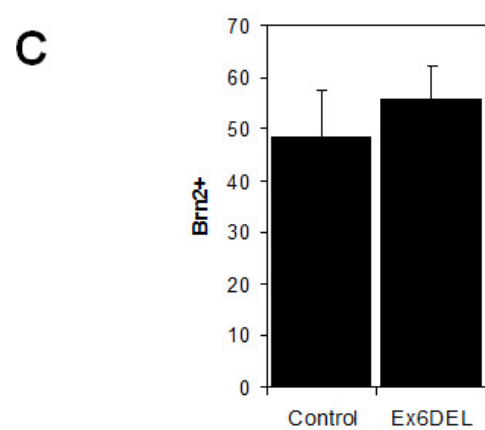
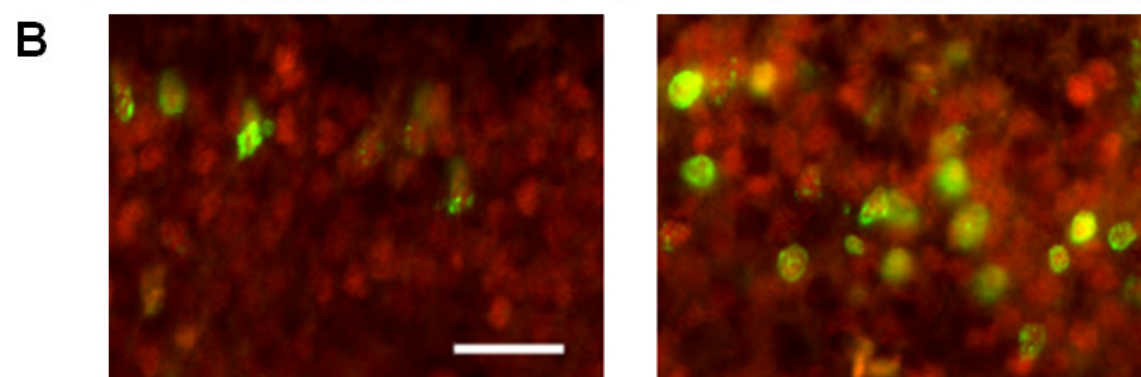
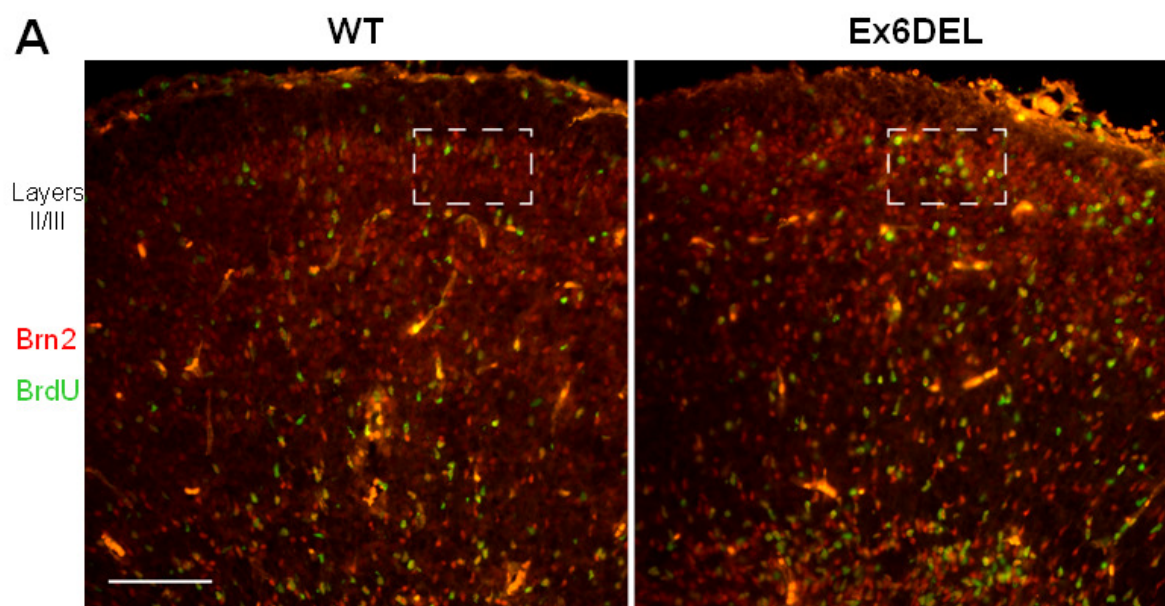
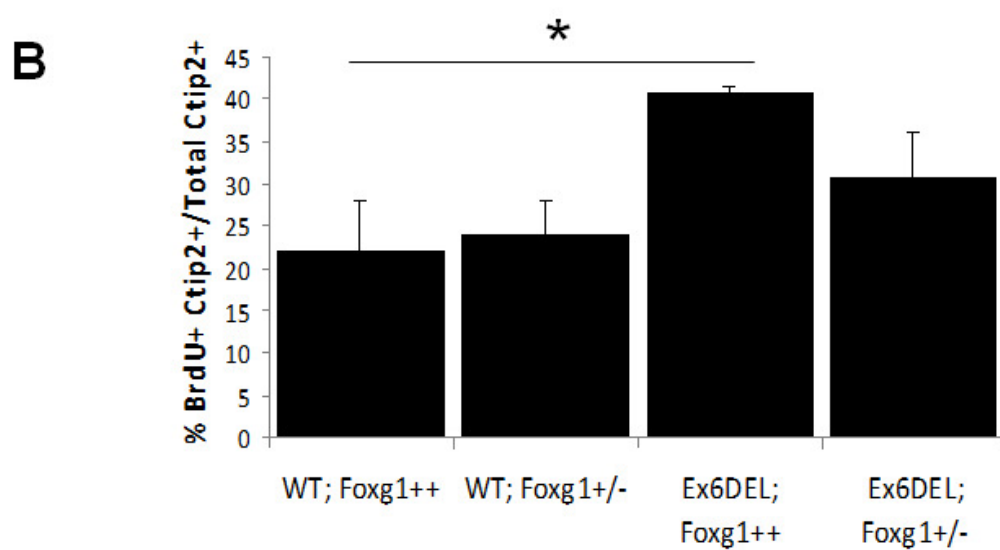
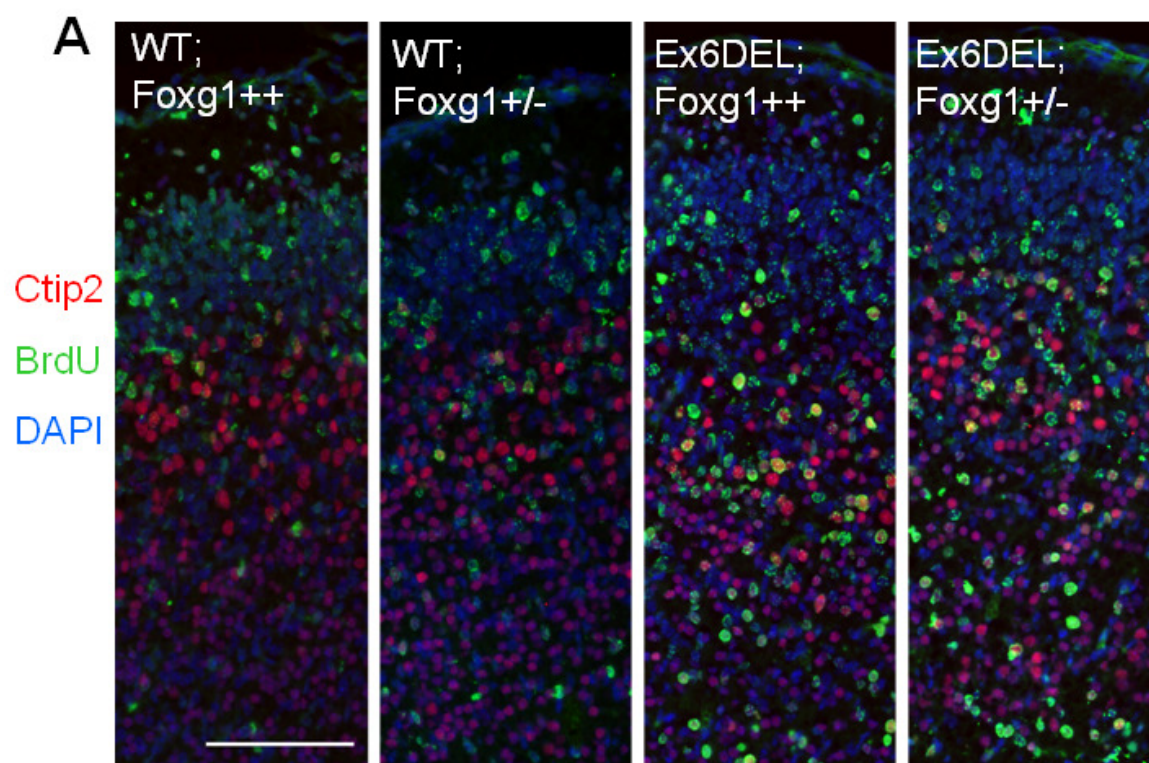


Figure 18. Differentiation timing of Layer V neurons is rescued by a decrease in Foxg1 expression.

WT, Foxg1^{+/-}, Ex6DEL, and Ex6DEL; Foxg1^{+/-} embryos were pulsed with BrdU at E13.5 and then sacrificed at E18.5.

(A) Sections of the cortex (10 μ m) were stained for BrdU (green) and Ctip2 (red), a layer V marker, and counterstained with DAPI (blue). Scale bar = 100 μ m.

(B) Quantification of the Ctip2⁺/BrdU⁺ population shows the delay in differentiation of layer V neurons is rescued by genetic decrease of Foxg1. n = 4; *, p<0.05; error bars represent SEM.



of the E13.5 BrdU injection in the WT cortex are finishing the formation of the Ctip2-positive population but the cells in the Ex6DEL cortex are still actively forming Ctip2-positive neurons [Figure 18A]. All of the birthdating experiments together show that differentiation delay occurs in at least three separate neuronal populations in the Ex6DEL cortex. The percentage of double-labeled cells was $30.6 \pm 5.5\%$ in the Ex6DEL; Foxg1^{+/-} cortex which falls between the percentages obtained for WT and Ex6DEL samples and is statistically significant from neither (WT: $p = 0.3$, Ex6DEL: $p = 0.1$) [Figure 18B]. The delay in differentiation was partially rescued by a decrease in Foxg1 expression. The Ex6DEL phenotype continues to affect neuronal differentiation throughout the whole process of corticogenesis.

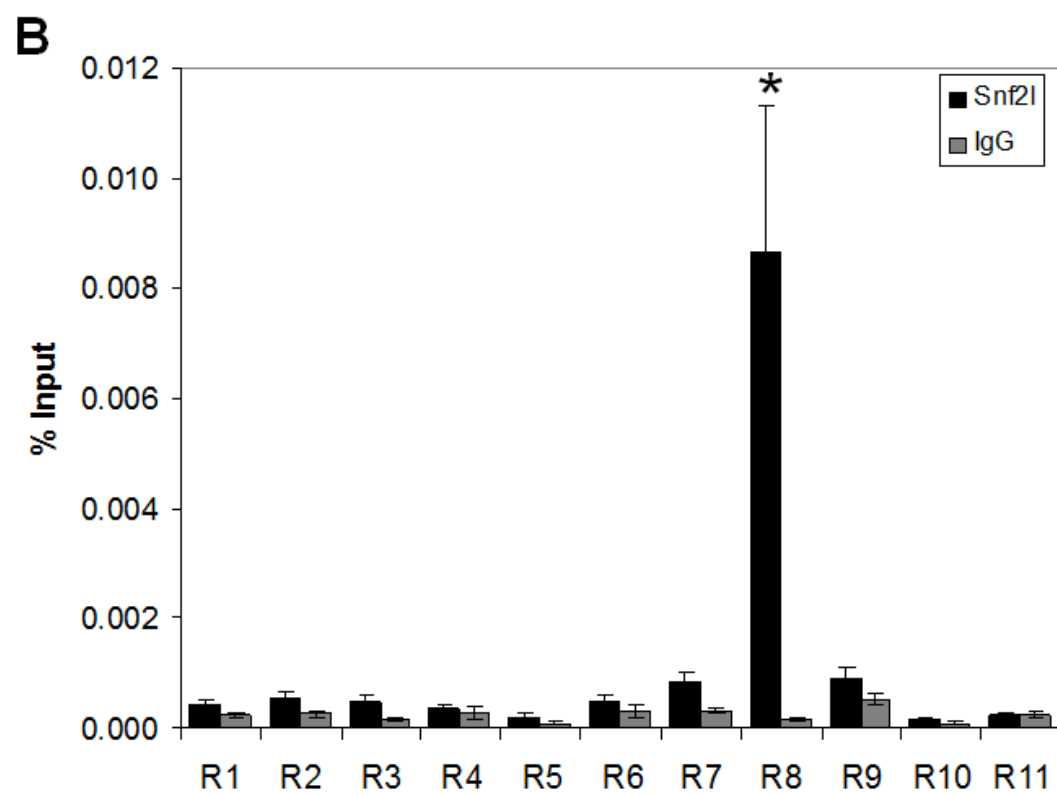
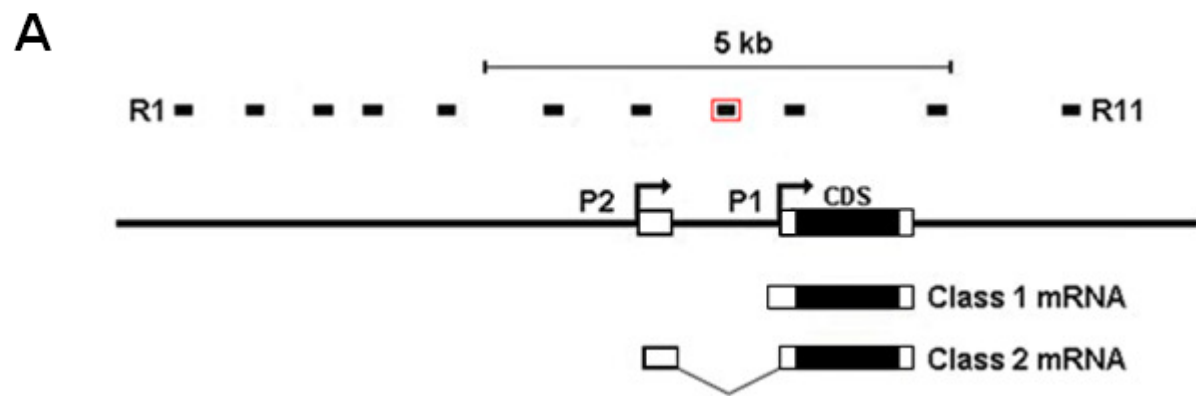
3.4 Snf2l Binding to the Foxg1 Locus

The increase in Foxg1 expression in the absence of active Snf2l suggests a role for Snf2l in regulating gene expression. To confirm that Snf2l is able to directly interact with the Foxg1 locus, chromatin immunoprecipitation (ChIP) experiments were performed on E15.5 forebrain. Eleven sets of primers (R1-R11) were designed to span the conserved regions both upstream and downstream of the Foxg1 coding sequence as determined by the mammalian conservation function in UCSC genome browser (<http://genome.ucsc.edu/>) [Figure 19A]. This approach was used with the aim of covering all regulatory regions, such as enhancers and proximal promoters, where Snf2l might bind and exert influence over Foxg1 expression. The primer sets were spaced 500 bp apart on average, exceptions can be seen where there were difficulties in amplifying a region or primers had to be redesigned, and had amplicons of 100 to 244 bp (average = 160 bp) in length. The reaction conditions were optimized to give r^2 values greater than 0.99 using the linear amplifying portion of a serial dilution curve and between 90-100% efficiency values by varying primer concentration. Each ChIP

Figure 19. Snf2l binds directly to a Foxg1 promoter region.

(A) Schematic representation of the Foxg1 locus showing two different promoter regions (P1 and P2) which transcribe two different mRNA classes. Both classes contain the same protein coding sequence (CDS, light gray region). The locations of all amplicons used to identify binding are shown in relation to the Foxg1 genomic sequence. Adapted from Li et al., 1996.

(B) Chromatin immunoprecipitation (ChIP) of Snf2l shows direct binding at the Foxg1 locus in promoter region P1 within Intron 1 (see red rectangle in A). ChIP was performed on Snf2l^{flox/flox} E15.5 forebrain, with each biological replicate incorporating tissue from approx. 10 embryos. The results above incorporate % Input values from 3 biological replicates, each having the Q-PCR reaction run in duplicate. *, $p = 0.012$; error bars represent the SEM.



experiment combined input tissue from approx. 10 embryos (4×10^7 cells) and the ChIP was repeated independently three times. Only primer set R8, lying within intron 1 of Foxg1, showed a significant increase in % Input value (Snf2l: $0.009 \pm 0.003\%$, IgG: $1.7 \times 10^{-4} \pm 3 \times 10^{-5}\%$, $p < 0.01$) when compared to the IgG control. [Figure 19B] We conclude from this experiment that a Snf2l complex binds directly to a very specific point on the Foxg1 locus.

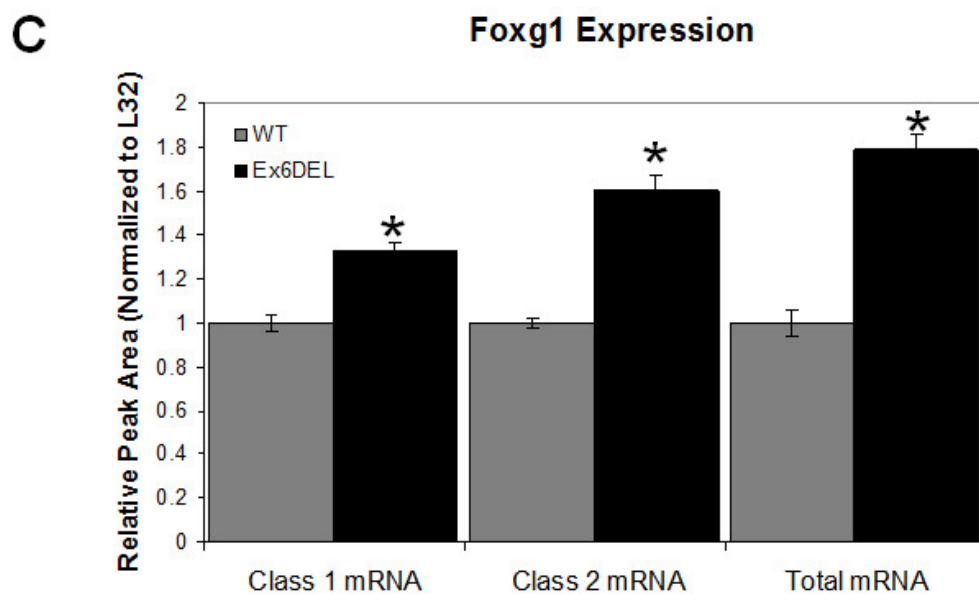
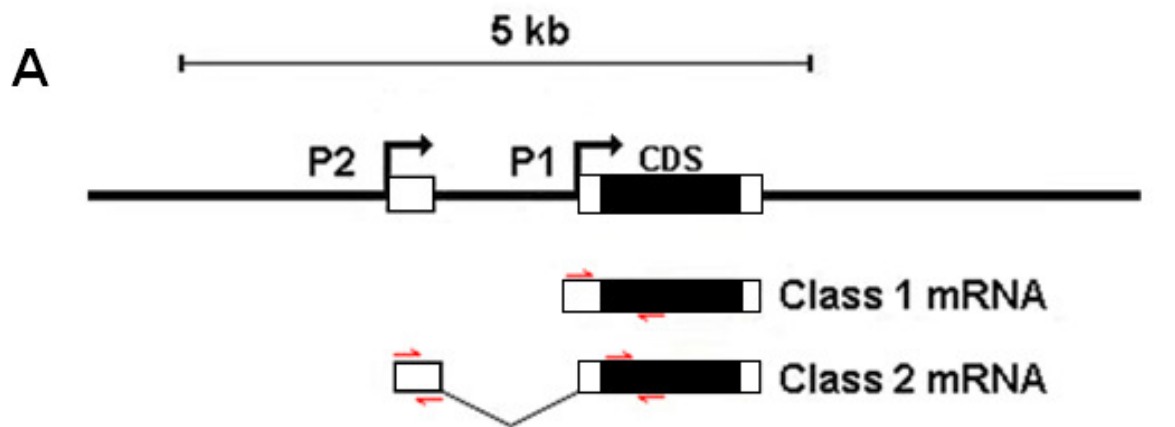
Two alternative classes of mRNA transcripts exist for Foxg1 in the mouse, which are transcribed from two distinct transcriptional start sites (Li et al., 1996). The most abundant transcript is derived from promoter region P1 which is found within intron 1 and corresponds to the site of Snf2l binding [Figure 20A]. To determine whether Snf2l's repression of Foxg1 is based on a mechanism localized to the P1 promoter or has a more generalized effect semi-quantitative PCR was used to examine the relative expression of both Foxg1 mRNA classes. Primer sets specific to each class of transcript along with one that captures all Foxg1 transcripts [Figure 20A] were used to amplify cDNA from three different control and Ex6DEL E15.5 forebrains. A primer pair for L32 was used as a loading control and a no-reverse transcriptase control was amplified to detect possible DNA contamination. The semi-quantitative PCR products were quantified by running them on an agarose gel [Figure 20B] and performing densitometry analysis. First of all, the overall levels of Foxg1 transcript were upregulated 1.79 ± 0.07 -fold ($p = 0.001$) in this experiment compared to wildtype. Class 1 Foxg1 mRNA, which is transcribed from the P1 promoter, was upregulated 1.32 ± 0.04 -fold in Ex6DEL forebrain ($p = 0.004$). Class 2 Foxg1 mRNA, whose promoter (P2) is found upstream of Exon 1, had a relative upregulation of 1.6 ± 0.14 ($p = 0.015$) as determined by semi-quantitative PCR and had been confirmed with qRT-PCR (Relative fold change = 1.3 ± 0.2) [Figure 20C]. Both classes of transcript are upregulated in Ex6DEL cortex providing

Figure 20. Snf2l regulates expression of both Foxg1 Transcripts

(A) Schematic representation of the Foxg1 locus showing two different promoter regions (P1 and P2) which transcribe two different mRNA classes. Both classes contain the same protein coding sequence (CDS, light gray region). The red arrows indicate the location of primers designed to amplify class 1 and 2 mRNA, specifically, as well as a pair that amplifies all Foxg1 mRNA. Adapted from Li et al., 1996.

(B) Agarose gel of semi-quantitative PCR products amplified from E15.5 cortical cDNA. The primers shown in (A) were used to measure the relative abundance of class 1, class 2 and total Foxg1 transcript levels in wild type (WT) and Ex6DEL samples. An L32 primer set was used as a loading control and a No RT control was used to ensure there was no genomic DNA contamination.

(C) The signal from each band in (B) was quantified using Image J software and normalized to the corresponding L32 signal. The values on the graph represent the relative increase in expression of each transcript normalized to WT levels. Both class 1 and 2 Foxg1 mRNA are elevated in Ex6DEL cortex. *, $p < 0.05$; error bars represent the SEM.



evidence that Snf2l's activity on the Foxg1 locus is not localized to a specific transcriptional start site but may also be affecting enhancer elements in its binding region.

4.0 Discussion

4.1 Altered Cell Cycle and Progenitor Cell Kinetics

Cell cycle length is deregulated in Ex6DEL neural progenitors. Both the cell cycle length and S-phase length are shortened compared to controls with the relative differences increasing through development. These cell cycle changes can lead to increased proliferation and altered cell fate decisions. Cells make fate decisions during the G1 phase of the cell cycle. They must decide based on intrinsic networks and extrinsic cues whether they will enter quiescence, senescence, differentiate or die by apoptosis before they reach the restriction point in late G1. The restriction point acts as a 'point of no return' and once it has been passed a cell is committed to undergo DNA replication and mitosis (Blomen and Boonstra, 2007; Pardee, 1974). The primary factors identified as being involved in regulating the restriction point are the pRb-E2F pathway and cyclin E-Cdk2:p27 ratio (Conradie et al., 2010; Hitomi et al., 2006; Yao et al., 2008). pRb is phosphorylated by cyclin D-Cdk4/6 and cyclin E-Cdk2 during G1. Hypophosphorylated pRb is able to bind E2F transcription factors but as phosphorylation marks are added by the cyclin-Cdk complexes E2Fs are freed. The released E2Fs then recruit activating complexes to several genes responsible for cell cycle progression (Singh et al., 2010). The activity of G1 cyclin-Cdks, which phosphorylates pRB, is inhibited by Cdk inhibitors (CKIs), particularly p21 and p27 (Lee, 2009). The levels of p27 in a cell during G1 are sufficient to determine whether it will activate Cdk2 and pass the restriction point or remain in G1 (Hitomi et al., 2006). Manipulation of any of the reactions involved in restriction point regulation predicts its advance or delay and can cause cell cycle

arrest (Conradie et al., 2010). In models where activity of these genes is altered the outcome is an upset of the proliferation/differentiation balance and altered growth of the cerebral cortex (Ferguson et al., 2002; Fero et al., 1996; Goto et al., 2004; MacPherson et al., 2003; Mitsuhashi and Takahashi, 2009; Pilaz et al., 2009; Tarui et al., 2005).

Foxg1 has been shown to suppress p21 and p27 in mice and *Xenopus*, respectively (Hardcastle and Papalopulu, 2000; Siegenthaler et al., 2008). In the Ex6DEL cortex, where Foxg1 expression is elevated, CKIs p16, p21 and p27 are significantly downregulated [Figure 5A]. Concordant with these gene expression changes, the cell cycle in Ex6DEL progenitor cells tends to be shorter than in its WT counterparts. In ventricular zone progenitors, S-phase and cell cycle length are both shorter in early and late periods of neurogenesis whereas subventricular zone progenitors begin to show distinct differences in cell cycle length at E15.5 when intermediate progenitor cells become prominent. The cell cycle lengthens faster in WT than in Ex6DEL progenitors accentuating the difference between the two at later timepoints [Figure 11]. Differences in the length of G1 are most likely responsible for the shortened cell cycle length in these progenitors for a number of reasons: G1 has been observed as the source of variability in *in vitro* cultures, G1 normally lengthens over the course of normal corticogenesis, the CKIs downregulated in the Ex6DEL cortex are mainly responsible for inhibiting G1 cyclin-Cdks, and the differences observed in S phase length are not sufficient to cover the differences in overall cell cycle length (Sherr and Roberts, 1999; Shields, 1977; Takahashi et al., 1995). The shortening of G1 and the possible advance of the restriction due to reduced p27 expression are increasing the proportion of cells in the germinal zones of the cortex that decide to undergo replication. This is consistent with previous experiments that show increased cell cycle re-entry in the Ex6DEL cortex. The rate of re-entry slows in WT but not Ex6DEL tissue between E13.5 and

E17.5 [Figure 4G, I]. The increased cycle rate also translates into an increased proliferation rate when Ex6DEL tissue is BrdU-pulsed [Figure 9]. Interestingly, despite the kinetic changes to the cell cycle in the ventricular zone the Pax6-positive progenitor population does not appear to be significantly increased (Yip et al., 2012). The Tbr2-positive population is expanded suggesting that the additional proliferation of apical progenitors is directly contributing to an increase in IPCs.

The increased proliferation and hypercellularity of the Ex6DEL cortex is restored to WT parameters by rescue of Foxg1 levels. While cell cycle length increases faster in Ex6DEL; Foxg1^{+/-} progenitors than Ex6DEL ones, they remain faster than WT cell cycle times [Figure 12B]. This discord between rescue of other observed phenotypes and incomplete rescue of cell cycle length suggests that a certain threshold of restriction point alteration is needed to affect cell fate decisions. Both p21 and p27 are known to be downstream of Foxg1 and the level of p27 in a cell is especially important for deciding between proliferation and cell cycle exit (Hardcastle and Papalopulu, 2000; Hitomi et al., 2006; Siegenthaler et al., 2008). If the genes downstream of Foxg1 are also rescued in Ex6DEL; Foxg1^{+/-} tissue then normal restriction point function could be restored. 256 genes have a greater than 2-fold alteration in their expression (both increased and decreased) in the Ex6DEL cortex at E15.5. Many of those genes are transcription factors and could have downstream effects on cell cycle regulation. Not all of these genes are dependent on Foxg1 regulation including the CKI, p16, that is downregulated. Foxg1 independent gene expression changes may cause persistent cell cycle changes that are not sufficient for restriction point delay allowing for recovery of cell fate decisions.

Unlike cell cycle length, the shorter S-phase length of Ex6DEL is completely rescued in the Ex6DEL; Foxg1^{+/-} cortex. The determinants of S-phase timing are independent from

those that regulate G1 length allowing both to be separately affected in the Ex6DEL phenotype. S-phase length varies developmentally and is heavily influenced by chromatin state (Ding and MacAlpine, 2011; Shermoen et al., 2010). So far, only Snf2h, not Snf2l, containing chromatin remodeling complexes have been implicated in DNA replication. ACF is required for replication through pericentric heterochromatin and WICH is targeted to the DNA clamp proliferating cell nuclear antigen (PCNA) to maintain the chromatin structure of newly replicated chromatin (Collins et al., 2002; Poot et al., 2004). Recently a role for Snf2h in the loading of the minichromosome maintenance (MCM) proteins to form the pre-replication complex required to ‘license’ origins of replication (Sugimoto et al., 2011). Snf2h silencing was able to slightly delay S-phase but the effects of Snf2l were not examined since the authors used HeLa cells that do not express Snf2l for their experiments (Sugimoto et al., 2011). However, it is unlikely that changes in chromatin dynamics are the cause of a shortened S-phase in the Ex6DEL neural progenitors since this would be independent of Foxg1 expression. Ts might be influenced by developmental changes instead. As cortical progenitors make a commitment to the neurogenic lineage they significantly reduce the length of their S-phase, from 8.3 to 1.8 hours in Pax6-positive apical progenitors and from 6.4 to 2.8 hours in Tbr2-positive basal progenitors (Arai et al., 2011). Increased Foxg1 expression not only increases the neural progenitor population but also promotes neurogenesis rendering it a possibility that a greater proportion of Ex6DEL progenitors are undergoing neurogenic divisions (Brancaccio et al., 2010). Since neurogenic progenitors have drastically reduced Ts, having a greater proportion of them making up the progenitor pool would reduce the population’s average S-phase length. This phenotype is Foxg1 dependant and is rescued in the Ex6DEL; Foxg1^{+/-} neural progenitors.

Ex6DEL progenitor cells display increased proliferation rates and cell cycle re-entry mediated by altered cell cycle parameters. Despite this phenotype, the cues that induce differentiation remain intact as evidenced by a lack of exencephaly and grossly normal lamination patterns in the Ex6DEL cortex.

4.2 Differentiation Timing and Cell Fate Determination

Ex6DEL mice have normal lamination patterns in their cerebral cortex but exhibit a delay in neuronal differentiation accompanied by increased neuronal output throughout corticogenesis. Subtle changes in cortical progenitor pools can have an effect on how cortical plate neurons are generated. At the onset of corticogenesis neural progenitors are multipotent but become progressively restricted to later-born fates independent of environmental cues throughout cortical development (Desai and McConnell, 2000; Frantz and McConnell, 1996; McConnell, 1988; Shen et al., 2006b). This temporal regulation of projection neuron fate is maintained even in transgenic models that interrupt the balance between progenitor self-renewal and differentiation. Genetic changes that favour differentiation result in premature cell cycle exit of progenitors producing primarily early-born neuronal cell types before the progenitor population is depleted. Examples of this are a forebrain-specific knockout of *Ezh2*, knockout of *tailless (Tlx)* and overexpression of *COUP-TFI* (Faedo et al., 2008; Pereira et al., 2010; Roy et al., 2002). In the study examining *Ezh2*, these mice begin to produce neurons before controls and at early stages of corticogenesis have a thicker cortex. By the end of the neurogenic interval their cortex is thinner and they have normal numbers of early-born layer markers but a deficit in *Brn2*⁺ neurons (Pereira et al., 2010). Knock-out of *Foxg1* constitutes a severe example of this phenomenon where the progenitor pool is exhausted very early through precocious differentiation of neurons (Xuan et al., 1995). On the other hand, increased self-renewal of the progenitor population, that does not result in

major developmental abnormalities, can result in a shift towards the production of late-born neurons. Loss of Geminin in the cortex results in expanded progenitor populations and 17% fewer early-born neurons produced by progenitors and a similar increase in the number of late-born neurons (Spella et al., 2011). Other mouse phenotypes describe an increase in progenitor self-renewal but do not examine whether a change in ratio of deep to superficial neurons is also present (Dugani et al., 2010; Jung et al., 2010). Birthdating studies were conducted to see if the increased cell cycle re-entry of Ex6DEL progenitor cells affected the differentiation timing of their progeny. Comparing BrdU-labeled cells that were pulsed on either E12.5 or E15.5 and co-labeled with layer markers Nurr1 (layer VI) or Brn2 (Layers II/III), respectively, showed a delay in the differentiation of both early and late-born neurons [Figures 16, 17]. Cells are remaining in a proliferative progenitor state longer before terminally differentiating in the Ex6DEL cortex. Contrary to what might be expected with increased progenitor self-renewal, most layers of the cerebral cortex are increased in the Ex6DEL model (Figure 15; Yip et al., 2012). The number of layer II/III Brn2-positive cells is unchanged at E18.5 but there is more BrdU staining in the SVZ and intermediate zone of the Ex6DEL sections suggesting there is more differentiation and migration occurring still and this population might be greater at post-natal timepoints. In fact, analysis of late-born layer markers Cux1 and Satb2 staining at P0 showed increased neuronal output in this model (Yip et al., 2012). The gene expression changes in the Ex6DEL cortex are affecting the signals that initiate neuronal differentiation and shift the program of corticogenesis to a later gestational age for both deep and superficial layers of projection neurons. The reason for global increases in neuron production is the consistency of decreased cell cycle length, increased progenitor cell proliferation and increased population number of neuronal progenitor cells from the beginning to the end of corticogenesis. The delay in production of

Ctip2-positive (layer V) neurons is partially rescued in the Ex6DEL; Foxg1^{+/-} cortex demonstrating its dependence on aberrant Foxg1 activity in the Ex6DEL cortex [Figure 18]. Maintenance of increased Foxg1 expression increases the progenitor pool and delays neurogenesis by approximately 1 day which results in increased neuronal output in all layers. This demonstrates the importance of the Snf2l-Foxg1 pathway in corticogenesis.

4.3 Snf2l Directly Affects Foxg1 Transcription

Snf2l is bound to the Foxg1 gene locus during development, a gene that is derepressed when Snf2l is absent. Snf2l is known to play a role in transcriptional regulation and can slide nucleosomes along DNA in cis but its mechanism of action *in vivo* has not been fully characterized (Barak et al., 2003; Hamiche et al., 1999; Landry et al., 2011; Lazzaro et al., 2006). Nucleosomes can pose an impediment to transcription by preventing the binding of activators and RNA Polymerase II to the DNA and possibly blocking transcription elongation (reviewed in Bai and Morozov, 2010). In a study of human promoters, it was found that their nucleosome occupancy is negatively correlated with the corresponding mRNA abundance (Tirosh and Barkai, 2008). This type of transcriptional regulation has been best studied in the yeast, *Saccharomyces cerevisiae*. By taking into account data for nucleosome occupancy at promoters Liu and colleagues were able to predict that Leu3 transcription factors would bind to accessible binding sites *in vivo* (Liu et al., 2006). The link between nucleosome occupancy and transcriptional activation has been well characterized at the yeast PHO5 promoter. The promoter region contains two binding sequences for the transcription factor Pho4, one that is accessible and the other blocked by nucleosome occupancy under repressive conditions. When phosphate levels in the cell drop requiring the activation of PHO5, Pho4 binds to the accessible site and precipitates the eviction of nucleosomes expanding the nucleosome free region from 80 to 600 bp in size. The eviction

also removes nucleosome repression of the promoter's TATA box allowing maximal transcription of the PHO5 gene. Chromatin remodeling factors are required to open the chromatin in response to PHO5 gene activation (Mao et al., 2011; Musladin and Barbarić, 2010; Svaren and Hörz, 1997). ISWI can remodel the nucleosome landscape to affect transcription. ISWI activity was studied using *in vitro* assays where nucleosomes were reconstituted on DNA containing GAL4-binding sites upstream of the adenovirus E4 TATA box and a minimal core promoter (Kang et al., 2002). The ISWI complex NURF is able to translocate the nucleosomes along the DNA but does not uncover all the GAL4-binding sites. When the GAL4 DNA binding domain is introduced into the reaction nucleosome sliding is extended to free all binding sites. The GAL4 DNA binding domain does not induce nucleosome repositioning on its own indicating the requirement for NURF (Kang et al., 2002). *In vivo*, the yeast Isw2 ISWI complex cooperates with the histone deacetylase complex Sin3-Rpd3 to repress multiple genes. In the genes dependent on Isw2 function it forms a DNase I-inaccessible chromatin structure (Fazzio et al., 2001). In *Drosophila*, in the absence of ISWI both upregulation and downregulation of gene expression is observed (Corona et al., 2007). However, 75% of genes whose expression is changed greater than 2-fold are expressed at greater levels possibly due to changes in global chromatin compaction and reduced association of the linker histone H1 (Corona et al., 2007). *Drosophila* ISWI is bound to chromatin in the vicinity of transcriptional start sites (TSS) with the peak binding density occurring at 300 base pairs (bp) downstream (Sala et al., 2011). When comparing the nucleosome positioning of wildtype versus ISWI-mutant salivary gland chromatin it was observed that genes where ISWI is bound had a delocalization of the nucleosomes downstream of the TSS and sometimes at the 3' end of the gene (Sala et al., 2011). How consistent ISWI's mechanism of transcriptional regulation is between lower eukaryotes and

mammals has not been investigated. Nucleosome mapping has not been completed in mice and only recently has the activity of mammalian ISWI orthologs Snf2h and Snf2l begun to be studied *in vivo*.

In murine forebrain Snf2l is bound to the Foxg1 locus in intron 1 between the two distinct TSSs for Foxg1's two transcripts [Figure 19]. It is bound approximately 800 bp downstream of the TSS for class 2 mRNA and 350 bp upstream of the class 1 mRNA TSS. This area was identified as the promoter region for class 1 Foxg1 mRNA (Li et al., 1996). The specific binding location would suggest a model where Snf2l is acting locally at the promoter of the prominent Foxg1 transcript to control expression but RT-PCR analysis shows this is not the case. In the absence of Snf2l both Foxg1 transcripts are upregulated [Figure 20]. Luciferase assays point to the importance of the region between 79 and 1179 bp upstream of the class 1 mRNA TSS in activating Foxg1 expression (Li et al., 1996; Yip et al., 2012). The requirement of the Snf2l-binding region for Foxg1 activation independent of chromatin state and the ability of Snf2l to repress both Foxg1 transcripts suggest a model where chromatin state (such as nucleosome occupancy) is altered by Snf2l to block DNA access. This could be accomplished by either blocking activating factor(s) access to an important regulatory sequence that controls the output of both class 1 and 2 Foxg1 transcripts or controls access to an activating factor important for expression of class 1 mRNA and blocks the progression of RNA polymerase II through class 2 Foxg1 mRNA. Future experiments studying chromatin state (nucleosome position, histone modifications) at the Foxg1 locus, both in the presence and absence of Snf2l, will provide more insight into the specific mechanism of transcriptional control employed by Snf2l.

4.4 Snf2l and its Cofactors in Transcriptional Regulation

Snf2l is associated with two complexes, NURF and CERF, where it interacts with other factors. These cofactors are important for recruiting and modulating the chromatin remodeling activity of Snf2l. Nucleosome remodeling factor (NURF) was first identified as a complex capable of facilitating GAGA factor-induced nucleosome remodeling at the Hsp70 promoter and was found to contain Snf2l, BPTF and RbAP46/48 in mammals (Barak et al., 2003; Tsukiyama and Wu, 1995). Studies of NURF activity in *Drosophila* and mammalian culture models implicate it in both gene activation and repression with NURF upregulating engrailed, heat shock genes, Wingless target genes (via recruitment by Armadillo), and Ultrabithorax (Badenhorst et al., 2002; Barak et al., 2003; Schuettengruber et al., 2007; Song et al., 2009). NURF represses the JAK-STAT pathway in *Drosophila* and in BPTF-null thymocytes 152 genes show lower expression and 154 genes show higher activity (Kwon et al., 2008; Landry et al., 2011). The recruitment mechanisms of NURF are diverse and include sites of histone variant H2A.Z incorporation, histone modifications H3K4me3 and H4H16Ac, recruitment by specific transcription factors, and its association with heterochromatin protein 2 (Goldman et al., 2010; Kwon et al., 2009; Wysocka et al., 2006; transcription factor summary in Alkhatib and Landry, 2011; Stephens et al., 2006).

Less is known about the biological function of the CERF complex. CERF is composed of the transcription factor Cecr2 and Snf2l (Banting et al., 2005). Although the chromatin binding properties of Cecr2 have not been characterized, microarray analysis of Cecr2 genetrap mutants shows both up and downregulation of gene expression with no tendency towards one or the other (Fairbridge et al., 2010). Foxg1 is not present in the microarray but this could be due to the early developmental stage of the tissue analyzed. None of the data published in *Drosophila* ISWI studies shows effects on Foxg1 either

leaving speculation as to which complex is responsible for its regulation. Both BPTF and Ccér2 are expressed in the brain and capable of mediating gene repression so both are potential binding partners to Snf2l at the FoxG1 locus (Banting et al., 2005; Barak et al., 2003; Chen et al., 2010; Jones et al., 2000). Further immunoprecipitation experiments with complex-specific subunits of both NURF and CERF would determine whether one of these two known complexes or a novel one are responsible for the repression of Foxg1 *in vivo*.

Mutation of either Ccér2 or BPTF in mice produces more severe phenotypes than that of the Snf2l Ex6DEL mice. Genetrap Ccér2 mutants have up to a 74% penetrance of exencephaly that is strain-dependent (Banting et al., 2005). Generation of a null mouse line resulted in a greater penetrance of exencephaly as well as the presence of midline facial clefts and forebrain encephalocele. The null mutation is also lethal perinatally, with any pups born without exencephaly dying within a couple days of an unknown cause (Fairbridge et al., 2010). BPTF mutations are even more catastrophic where BPTF^{-/-} embryos are reabsorbed by E8.5 (Goller et al., 2008; Landry et al., 2008). BPTF is required for embryonic stem cell differentiation and the proper formation of extraembryonic support tissue, mesoderm, endoderm and more differentiated ectoderm lineages (Goller et al., 2008; Landry et al., 2008). The mild Ex6DEL phenotype indicates that both Ccér2 and BPTF have functions independent of their association with Snf2l or there is some functional compensation by the other mammalian ISWI ortholog, Snf2h, that lessens the phenotype. An example of this type of compensation relationship is seen in the SWI-SNF family chromatin remodeling proteins Brg1 and Brm. Brg1 mutations are embryonic lethal and cannot be compensated for by Brm (Bultman et al., 2000). Brm-null mice only have a mild phenotype of increased mass and cell proliferation but an increase in Brg1 protein levels suggests functional compensation (Reyes et al., 1998). No increase in Snf2h expression has been detected in Ex6DEL mice but such an

increase might not be necessary for a compensatory mechanism considering the normal abundance of Snf2h (Erdel et al., 2010; Yip, 2009). The observation that both ISWI subunits can be incorporated into certain complexes, sometimes in a context-specific manner, lends support to the notion that Snf2h could be compensating for Snf2l when it is depleted (Poot et al., 2000; Thompson et al., 2012). While the molecular mechanism and associations of Snf2l in the brain remain to be completely elucidated, it can be concluded that Snf2l is an important regulator of neural progenitor cells in the forebrain via its repression of Foxg1 transcription.

References

- Abrajano, J.J., Qureshi, I.A., Gokhan, S., Molero, A.E., Zheng, D., Bergman, A., and Mehler, M.F. (2010). Corepressor for element-1-silencing transcription factor preferentially mediates gene networks underlying neural stem cell fate decisions. *Proceedings of the National Academy of Sciences of the United States of America* 107, 16685-16690.
- Abrajano, J.J., Qureshi, I.A., Gokhan, S., Zheng, D., Bergman, A., and Mehler, M.F. (2009). REST and CoREST modulate neuronal subtype specification, maturation and maintenance. *PloS one* 4.
- Adesina, A.M., Nguyen, Y., Guanaratne, P., Pulliam, J., Lopez-Terrada, D., Margolin, J., and Finegold, M. (2007a). FOXG1 is overexpressed in hepatoblastoma. *Human pathology* 38, 400-409.
- Adesina, A.M., Nguyen, Y., Mehta, V., Takei, H., Stangeby, P., Crabtree, S., Chintagumpala, M., and Gumerlock, M.K. (2007b). FOXG1 dysregulation is a frequent event in medulloblastoma. *Journal of neuro-oncology* 85, 111-122.
- Ahlgren, S., Vogt, P., and Bronner-Fraser, M. (2003). Excess FoxG1 Causes Overgrowth of the Neural Tube. *Journal of neurobiology* 57, 337-349.
- Aihara, T., Miyoshi, Y., Koyama, K., Suzuki, M., Takahashi, E., Monden, M., and Nakamura, Y. (1998). Cloning and mapping of SMARCA5 encoding hSNF2H, a novel human homologue of Drosophila ISWI. *Cytogenetics and cell genetics* 81, 191-193.
- Alkhatib, S.G., and Landry, J.W. (2011). The nucleosome remodeling factor. *FEBS letters* 585, 3197-3207.
- Alter, M., Liebo, J., Desnick, S.O., and Strommer, B. (1968). The behavior of the reeler neurological mutant mouse. *Neurology* 18, 289.
- Amir, R.E., Van Den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl- CpG-binding protein 2. *Nature genetics* 23, 185-188.
- Angevine Jr, J.B., and Sidman, R.L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192, 766-768.
- Arai, Y., Pulvers, J.N., Haffner, C., Schilling, B., Nüsslein, I., Calegari, F., and Huttner, W.B. (2011). Neural stem and progenitor cells shorten S-phase on commitment to neuron production. *Nature Communications* 2.

- Ariani, F., Hayek, G., Rondinella, D., Artuso, R., Mencarelli, M.A., Spanhol-Rosseto, A., Pollazzon, M., Buoni, S., Spiga, O., Ricciardi, S., *et al.* (2008). FOXG1 Is Responsible for the Congenital Variant of Rett Syndrome. *American Journal of Human Genetics* 83, 89-93.
- Awad, S., and Hassan, A.H. (2008). The Swi2/Snf2 bromodomain is important for the full binding and remodeling activity of the SWI/SNF complex on H3- and H4-acetylated nucleosomes. *Annals of the New York Academy of Sciences* 1138, 366-375.
- Ayers, K.L., and Théron, P.P. (2010). Evaluating Smoothened as a G-protein-coupled receptor for Hedgehog signalling. *Trends in cell biology* 20, 287-298.
- Bérubé, N.G., Mangelsdorf, M., Jagla, M., Vanderluit, J., Garrick, D., Gibbons, R.J., Higgs, D.R., Slack, R.S., and Picketts, D.J. (2005). The chromatin-remodeling protein ATRX is critical for neuronal survival during corticogenesis. *Journal of Clinical Investigation* 115, 258-267.
- Badenhorst, P., Voas, M., Rebay, I., and Wu, C. (2002). Biological functions of the ISWI chromatin remodeling complex NURF. *Genes and Development* 16, 3186-3198.
- Bai, L., and Morozov, A.V. (2010). Gene regulation by nucleosome positioning. *Trends in Genetics* 26, 476-483.
- Ballas, N., Grunseich, C., Lu, D.D., Speh, J.C., and Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 121, 645-657.
- Balordi, F., and Fishell, G. (2007). Hedgehog signaling in the subventricular zone is required for both the maintenance of stem cells and the migration of newborn neurons. *Journal of Neuroscience* 27, 5936-5947.
- Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410, 120-124.
- Banting, G.S., Barak, O., Ames, T.M., Burnham, A.C., Kardel, M.D., Cooch, N.S., Davidson, C.E., Godbout, R., McDermid, H.E., and Shiekhatar, R. (2005). CECR2, a protein involved in neurulation, forms a novel chromatin remodeling complex with SNF2L. *Human molecular genetics* 14, 513-524.
- Barak, O., Lazzaro, M.A., Cooch, N.S., Picketts, D.J., and Shiekhatar, R. (2004). A tissue-specific, naturally occurring human SNF2L variant inactivates chromatin remodeling. *The Journal of biological chemistry* 279, 45130-45138.

- Barak, O., Lazzaro, M.A., Lane, W.S., Speicher, D.W., Picketts, D.J., and Shiekhhattar, R. (2003). Isolation of human NURF: A regulator of Engrailed gene expression. *EMBO Journal* 22, 6089-6100.
- Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007). High-Resolution Profiling of Histone Methylations in the Human Genome. *Cell* 129, 823-837.
- Bayer, S.A., and Altman, J. (1990). Development of layer I and the subplate in the rat neocortex. *Experimental neurology* 107, 48-62.
- Bayer, S.A., Altman, J., Russo, R.J., Dai, X., and Simmons, J.A. (1991). Cell migration in the rat embryonic neocortex. *Journal of Comparative Neurology* 307, 499-516.
- Belichenko, P.V., Wright, E.E., Belichenko, N.P., Masliah, E., Li, H.H., Mobley, W.C., and Francke, U. (2009). Widespread changes in dendritic and axonal morphology in Mecp2-mutant mouse models of Rett syndrome: Evidence for disruption of neuronal networks. *Journal of Comparative Neurology* 514, 240-258.
- Benes, F.M., and Berretta, S. (2001). GABAergic interneurons: Implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 25, 1-27.
- Blaschke, A.J., Weiner, J.A., and Chun, J. (1998). Programmed cell death is a universal feature of embryonic and postnatal neuroproliferative regions throughout the central nervous system. *Journal of Comparative Neurology* 396, 39-50.
- Blomen, V.A., and Boonstra, J. (2007). Cell fate determination during G1 phase progression. *Cellular and Molecular Life Sciences* 64, 3084-3104.
- Botuyan, M.V., Lee, J., Ward, I.M., Kim, J.E., Thompson, J.R., Chen, J., and Mer, G. (2006). Structural Basis for the Methylation State-Specific Recognition of Histone H4-K20 by 53BP1 and Crb2 in DNA Repair. *Cell* 127, 1361-1373.
- Bourguignon, C., Li, J., and Papalopulu, N. (1998). XBF-1, a winged helix transcription factor with dual activity, has a role in positioning neurogenesis in *Xenopus* competent ectoderm. *Development* 125, 4889-4900.
- Boyer, L.A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L.A., Lee, T.I., Levine, S.S., Wernig, M., Tajonar, A., Ray, M.K., *et al.* (2006). Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 441, 349-353.

- Bozhenok, L., Wade, P.A., and Varga-Weisz, P. (2002). WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO Journal* 21, 2231-2241.
- Brancaccio, M., Pivetta, C., Granzotto, M., Filippis, C., and Mallamaci, A. (2010). *Emx2* and *Foxg1* inhibit gliogenesis and promote neuronogenesis. *Stem cells* 28, 1206-1218.
- Breidenkamp, N., Seoighe, C., and Illing, N. (2007). Comparative evolutionary analysis of the *FoxG1* transcription factor from diverse vertebrates identifies conserved recognition sites for microRNA regulation. *Development genes and evolution* 217, 227-233.
- Brown, C.R., Mao, C., Falkovskaia, E., Law, J.K., and Boeger, H. (2011). In Vivo role for the chromatin-remodeling enzyme SWI/SNF in the removal of promoter nucleosomes by disassembly rather than sliding. *Journal of Biological Chemistry* 286, 40556-40565.
- Brunetti-Pierri, N., Paciorkowski, A.R., Ciccone, R., Mina, E.D., Bonaglia, M.C., Borgatti, R., Schaaf, C.P., Sutton, V.R., Xia, Z., Jelluma, N., *et al.* (2011). Duplications of *FOXG1* in 14q12 are associated with developmental epilepsy, mental retardation, and severe speech impairment. *European Journal of Human Genetics* 19, 102-107.
- Bultman, S., Gebuhr, T., Yee, D., La Mantia, C., Nicholson, J., Gilliam, A., Randazzo, F., Metzger, D., Chambon, P., Crabtree, G., *et al.* (2000). A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Molecular cell* 6, 1287-1295.
- Buss, R.R., and Oppenheim, R.W. (2004). Role of programmed cell death in normal neuronal development and function. *Anatomical Science International* 79, 191-197.
- Calegari, F., and Huttner, W.B. (2003). An inhibition of cyclin-dependent kinases that lengthens, but does not arrest, neuroepithelial cell cycle induces premature neurogenesis. *Journal of cell science* 116, 4947-4955.
- Campos, E.I., and Reinberg, D. (2009). Histones: Annotating chromatin. In *Annual Review of Genetics*, pp. 559-599.
- Caviness Jr, V.S. (1982). Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. *Brain Research* 256, 293-302.
- Caviness Jr, V.S., Goto, T., Tarui, T., Takahashi, T., Bhide, P.G., and Nowakowski, R.S. (2003). Cell output, cell cycle duration and neuronal specification: A model of integrated mechanisms of the neocortical proliferative process. *Cerebral Cortex* 13, 592-598.

- Caviness Jr, V.S., and Sidman, R.L. (1973). Time of origin or corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: an autoradiographic analysis. *Journal of Comparative Neurology* 148, 141-151.
- Cayuso, J., Ulloa, F., Cox, B., Briscoe, J., and Marti, E. (2006). The Sonic hedgehog pathway independently controls the patterning, proliferation and survival of neuroepithelial cells by regulating Gli activity. *Development* 133, 517-528.
- Cecconi, F., Alvarez-Bolado, G., Meyer, B.I., Roth, K.A., and Gruss, P. (1998). Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* 94, 727-737.
- Chai, X., Förster, E., Zhao, S., Bock, H.H., and Frotscher, M. (2009). Reelin stabilizes the actin cytoskeleton of neuronal processes by inducing n-cofilin phosphorylation at serine. *Journal of Neuroscience* 29, 288-299.
- Chameau, P., Inta, D., Vitalis, T., Monyer, H., Wadman, W.J., and Van Hooft, J.A. (2009). The N-terminal region of reelin regulates postnatal dendritic maturation of cortical pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America* 106, 7227-7232.
- Chan, D.W., Liu, V.W.S., To, R.M.Y., Chiu, P.M., Lee, W.Y.W., Yao, K.M., Cheung, A.N.Y., and Ngan, H.Y.S. (2009). Overexpression of FOXG1 contributes to TGF-B resistance through inhibition of p21WAF1/CIP1 expression in ovarian cancer. *British journal of cancer*.
- Chapleau, C.A., Calfa, G.D., Lane, M.C., Albertson, A.J., Larimore, J.L., Kudo, S., Armstrong, D.L., Percy, A.K., and Pozzo-Miller, L. (2009). Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. *Neurobiology of disease* 35, 219-233.
- Chen, J., Morosan-Puopolo, G., Dai, F., Wang, J., and Brand-Saberi, B. (2010). Molecular cloning of chicken Ccr2 and its expression during chicken embryo development. *International Journal of Developmental Biology* 54, 925-929.
- Chen, R.Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nature genetics* 27, 327-331.
- Chen, Z.F., Paquette, A.J., and Anderson, D.J. (1998). NRSF/REST is required in vivo for repression of multiple neuronal target genes during embryogenesis. *Nature genetics* 20, 136-142.

- Chenn, A., and Walsh, C.A. (2002). Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 297, 365-369.
- Chenn, A., and Walsh, C.A. (2003). Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in B-catenin overexpressing transgenic mice. *Cerebral Cortex* 13, 599-606.
- Chesnutt, C., Burrus, L.W., Brown, A.M.C., and Niswander, L. (2004). Coordinate regulation of neural tube patterning and proliferation by TGF-B and WNT activity. *Developmental biology* 274, 334-347.
- Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H., and Beachy, P.A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* 383, 407-413.
- Chiba, H., Muramatsu, M., Nomoto, A., and Kato, H. (1994). Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila* brhma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic acids research* 22, 1815-1820.
- Clapier, C.R., Nightingale, K.P., and Becker, P.B. (2002). A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI. *Nucleic acids research* 30, 649-655.
- Cohen Jr, M.M. (2010). Hedgehog signaling update. *American Journal of Medical Genetics, Part A* 152, 1875-1914.
- Collins, N., Poot, R.A., Kukimoto, I., García-Jiménez, C., Dellaire, G., and Varga-Weisz, P.D. (2002). An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. *Nature genetics* 32, 627-632.
- Conradie, R., Bruggeman, F.J., Ciliberto, A., Csikász-Nagy, A., Novák, B., Westerhoff, H.V., and Snoep, J.L. (2010). Restriction point control of the mammalian cell cycle via the cyclin E/Cdk2:p27 complex. *FEBS Journal* 277, 357-367.
- Corbin, J.G., and Butt, S.J.B. (2011). Developmental mechanisms for the generation of telencephalic interneurons. *Developmental Neurobiology* 71, 710-732.
- Corona, D.F., Siriaco, G., Armstrong, J.A., Snarskaya, N., McClymont, S.A., Scott, M.P., and Tamkun, J.W. (2007). ISWI regulates higher-order chromatin structure and histone H1 assembly in vivo. *PLoS biology* 5.
- Culican, S.M., Baumrind, N.L., Yamamoto, M., and Pearlman, A.L. (1990). Cortical radial glia: Identification in tissue culture and evidence for their transformation to astrocytes. *Journal of Neuroscience* 10, 684-692.

- Cunningham, J.J., and Roussel, M.F. (2001). Cyclin-dependent kinase inhibitors in the development of the central nervous system. *Cell Growth and Differentiation* 12, 387-396.
- Dahmane, N., Sánchez, P., Gitton, Y., Palma, V., Sun, T., Beyna, M., Weiner, H., and Ruiz i Altaba, A. (2001). The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. *Development* 128, 5201-5212.
- Danesin, C., Peres, J.N., Johansson, M., Snowden, V., Cording, A., Papalopulu, N., and Houart, C. (2009). Integration of Telencephalic Wnt and Hedgehog Signaling Center Activities by Foxg1. *Developmental Cell* 16, 576-587.
- Dang, W., and Bartholomew, B. (2007). Domain architecture of the catalytic subunit in the ISW2-nucleosome complex. *Molecular and cellular biology* 27, 8306-8317.
- Dang, W., Kagalwala, M.N., and Bartholomew, B. (2006). Regulation of ISW2 by concerted action of histone H4 tail and extranucleosomal DNA. *Molecular and cellular biology* 26, 7388-7396.
- Dastidar, S.G., Landrieu, P.M.Z., and D'Mello, S.R. (2011). FoxG1 promotes the survival of postmitotic neurons. *Journal of Neuroscience* 31, 402-413.
- De Carlos, J.A., and O'Leary, D.D.M. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *Journal of Neuroscience* 12, 1194-1211.
- De Santis, P., Rizzo, R. and Savino, M. (1973). Conformational Studies on Deoxyribonucleic Acid-Polylysine Complexes. *Macromolecules* 6, 520-522.
- De Zio, D., Giunta, L., Corvaro, M., Ferraro, E., and Cecconi, F. (2005). Expanding roles of programmed cell death in mammalian neurodevelopment. *Seminars in Cell and Developmental Biology* 16, 281-294.
- Del Rìo, J.A., Martínez, A., Auladell, C., and Soriano, E. (2000). Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. *Cerebral Cortex* 10, 784-801.
- Desai, A.R., and McConnell, S.K. (2000). Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development* 127, 2863-2872.
- Deuring, R., Fanti, L., Armstrong, J.A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S.L., Berloco, M., *et al.* (2000). The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Molecular cell* 5, 355-365.

- Diamond, I.T., and Hall, W.C. (1969). Evolution of neocortex. *Science* 164, 251-262.
- Ding, Q., and MacAlpine, D.M. (2011). Defining the replication program through the chromatin landscape. *Critical reviews in biochemistry and molecular biology* 46, 165-179.
- Dirscherl, S.S., Henry, J.J., and Krebs, J.E. (2005). Neural and eye-specific defects associated with loss of the Imitation Switch (ISWI) chromatin remodeler in *Xenopus laevis*. *Mechanisms of development* 122, 1157-1170.
- Dirscherl, S.S., and Krebs, J.E. (2004). Functional diversity of ISWI complexes. *Biochemistry and Cell Biology* 82, 482-489.
- Dou, C., Lee, J., Liu, B., Liu, F., Massagué, J., Xuan, S., and Lai, E. (2000). BF-1 interferes with transforming growth factor β signaling by associating with Smad partners. *Molecular and cellular biology* 20, 6201-6211.
- Dou, C.L., Li, S., and Lai, E. (1999). Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cerebral Cortex* 9, 543-550.
- Dugani, C.B., Paquin, A., Kaplan, D.R., and Miller, F.D. (2010). Coffin-Lowry syndrome: A role for RSK2 in mammalian neurogenesis. *Developmental biology* 347, 348-359.
- Duggan, C.D., DeMaria, S., Baudhuin, A., Stafford, D., and Ngai, J. (2008). Foxg1 is required for development of the vertebrate olfactory system. *Journal of Neuroscience* 28, 5229-5239.
- Eagleson, K.L., Schlueter McFadyen-Ketchum, L.J., Ahrens, E.T., Mills, P.H., Does, M.D., Nickols, J., and Levitt, P. (2007). Disruption of Foxg1 expression by knock-in of Cre recombinase: Effects on the development of the mouse telencephalon. *Neuroscience* 148, 385-399.
- Edwards, M.A., Yamamoto, M., and Caviness Jr, V.S. (1990). Organization of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker. *Neuroscience* 36, 121-144.
- Elfring, L.K., Deuring, R., McCallum, C.M., Peterson, C.L., and Tamkun, J.W. (1994). Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. *Molecular and cellular biology* 14, 2225-2234.
- Englund, C., Fink, A., Lau, C., Pham, D., Daza, R.A.M., Bulfone, A., Kowalczyk, T., and Hevner, R.F. (2005). Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *Journal of Neuroscience* 25, 247-251.

- Erdel, F., and Rippe, K. (2011a). Binding kinetics of human ISWI chromatin-remodelers to DNA repair sites elucidate their target location mechanism. *Nucleus* 2.
- Erdel, F., and Rippe, K. (2011b). Chromatin remodelling in mammalian cells by ISWI-type complexes - Where, when and why? *FEBS Journal* 278, 3608-3618.
- Erdel, F., Schubert, T., Marth, C., Längst, G., and Rippe, K. (2010). Human ISWI chromatin-remodeling complexes sample nucleosomes via transient binding reactions and become immobilized at active sites. *Proceedings of the National Academy of Sciences of the United States of America* 107, 19873-19878.
- Estivill-Torrus, G., Pearson, H., van Heyningen, V., Price, D.J., and Rashbass, P. (2002). Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* 129, 455-466.
- Faedo, A., Tomassy, G.S., Ruan, Y., Teichmann, H., Krauss, S., Pleasure, S.J., Tsai, S.Y., Tsai, M.J., Studer, M., and Rubenstein, J.L.R. (2008). COUP-TFI coordinates cortical patterning, neurogenesis, and laminar fate and modulates MAPK/ERK, AKT, and β -catenin signaling. *Cerebral Cortex* 18, 2117-2131.
- Fairbridge, N.A., Dawe, C.E., Niri, F.H., Kooistra, M.K., King-Jones, K., and McDermid, H.E. (2010). Ccnc mutations causing exencephaly trigger misregulation of mesenchymal/ectodermal transcription factors. *Birth Defects Research Part A - Clinical and Molecular Teratology* 88, 619-625.
- Falconer, D.S. (1951). Two new mutants, 'trembler' and 'reeler', with neurological actions in the house mouse (*Mus musculus* L.). *Journal of Genetics* 50, 192-205.
- Falk, S., Wurdak, H., Ittner, L.M., Ille, F., Sumara, G., Schmid, M.T., Draganova, K., Lang, K.S., Paratore, C., Leveen, P., *et al.* (2008). Brain Area-Specific Effect of TGF- β Signaling on Wnt-Dependent Neural Stem Cell Expansion. *Cell Stem Cell* 2, 472-483.
- Fan, G., Beard, C., Chen, R.Z., Csankovszki, G., Sun, Y., Siniaia, M., Biniszkiewicz, D., Bates, B., Lee, P.P., Kohn, R., *et al.* (2001). DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *Journal of Neuroscience* 21, 788-797.
- Fan, H.Y., Trotter, K.W., Archer, T.K., and Kingston, R.E. (2005). Swapping function of two chromatin remodeling complexes. *Molecular cell* 17, 805-815.

- Fasano, C.A., Dimos, J.T., Ivanova, N.B., Lowry, N., Lemischka, I.R., and Temple, S. (2007). shRNA Knockdown of Bmi-1 Reveals a Critical Role for p21-Rb Pathway in NSC Self-Renewal during Development. *Cell Stem Cell* 1, 87-99.
- Fasano, C.A., Phoenix, T.N., Kokovay, E., Lowry, N., Elkabetz, Y., Dimos, J.T., Lemischka, I.R., Studer, L., and Temple, S. (2009). Bmi-1 cooperates with Foxg1 to maintain neural stem cell self-renewal in the forebrain. *Genes and Development* 23, 561-574.
- Fazzio, T.G., Kooperberg, C., Goldmark, J.P., Neal, C., Basom, R., Delrow, J., and Tsukiyama, T. (2001). Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Molecular and cellular biology* 21, 6450-6460.
- Feng, J., Zhou, Y., Campbell, S.L., Le, T., Li, E., Sweatt, J.D., Silva, A.J., and Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nature neuroscience* 13, 423-430.
- Ferguson, K.L., Vanderluit, J.L., Hébert, J.M., McIntosh, W.C., Tibbo, E., MacLaurin, J.G., Park, D.S., Wallace, V.A., Vooijs, M., McConnell, S.K., *et al.* (2002). Telencephalon-specific Rb knockouts reveal enhanced neurogenesis, survival and abnormal cortical development. *EMBO Journal* 21, 3337-3346.
- Fero, M.L., Rivkin, M., Tasch, M., Porter, P., Carow, C.E., Firpo, E., Polyak, K., Tsai, L.H., Broudy, V., Perlmutter, R.M., *et al.* (1996). A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27Kip1-deficient Mice. *Cell* 85, 733-744.
- Ferrer, I., Tortosa, A., Blanco, R., Martí, F., Serrano, T., Planas, A., and Macaya, A. (1994). Naturally occurring cell death in the developing cerebral cortex of the rat. Evidence of apoptosis-associated internucleosomal DNA fragmentation. *Neuroscience letters* 182, 77-79.
- Flaus, A., and Owen-Hughes, T. (2011). Mechanisms for ATP-dependent chromatin remodelling: The means to the end. *FEBS Journal* 278, 3579-3595.
- Frantz, G.D., and McConnell, S.K. (1996). Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron* 17, 55-61.
- Freese, J.L., Pino, D., and Pleasure, S.J. (2010). Wnt signaling in development and disease. *Neurobiology of disease* 38, 148-153.
- Fuccillo, M., Rallu, M., McMahon, A.P., and Fishell, G. (2004). Temporal requirement for hedgehog signaling in ventral telencephalic patterning. *Development* 131, 5031-5040.

- Götz, M., Stoykova, A., and Gruss, P. (1998). Pax6 controls radial glia differentiation in the cerebral cortex. *Neuron* 21, 1031-1044.
- Gangaraju, V.K., Prasad, P., Srour, A., Kagalwala, M.N., and Bartholomew, B. (2009). Conformational Changes Associated with Template Commitment in ATP-Dependent Chromatin Remodeling by ISW2. *Molecular cell* 35, 58-69.
- García-Moreno, F., López-Mascaraque, L., and De Carlos, J.A. (2007). Origins and migratory routes of murine Cajal-Retzius cells. *Journal of Comparative Neurology* 500, 419-432.
- Gavrieli, Y., Sherman, Y., and Ben-Sasson, S.A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *Journal of Cell Biology* 119, 493-501.
- Geng, X., and Oliver, G. (2009). Pathogenesis of holoprosencephaly. *Journal of Clinical Investigation* 119, 1403-1413.
- Ghosh, A., and Shatz, C.J. (1993). A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* 117, 1031-1047.
- Gibbons, R.J., Picketts, D.J., Villard, L., and Higgs, D.R. (1995). Mutations in a putative global transcriptional regulator cause X-linked mental retardation with $\hat{\pm}$ -thalassemia (ATR-X syndrome). *Cell* 80, 837-845.
- Glickstein, S.B., Alexander, S., and Ross, M.E. (2007). Differences in cyclin D2 and D1 protein expression distinguish forebrain progenitor subsets. *Cerebral Cortex* 17, 632-642.
- Glickstein, S.B., Monaghan, J.A., Koeller, H.B., Jones, T.K., and Ross, M.E. (2009). Cyclin D2 is critical for intermediate progenitor cell proliferation in the embryonic cortex. *Journal of Neuroscience* 29, 9614-9624.
- Goldman, J.A., Garlick, J.D., and Kingston, R.E. (2010). Chromatin remodeling by imitation switch (ISWI) class ATP-dependent remodelers is stimulated by histone variant H2A.Z. *Journal of Biological Chemistry* 285, 4645-4651.
- Goller, T., Vauti, F., Ramasamy, S., and Arnold, H.H. (2008). Transcriptional regulator BPTF/FAC1 is essential for trophoblast differentiation during early mouse development. *Molecular and cellular biology* 28, 6819-6827.
- Goto, T., Mitsuhashi, T., and Takahashi, T. (2004). Altered patterns of neuron production in the p27Kip1 knockout mouse. *Developmental neuroscience* 26, 208-217.

- Grüne, T., Brzeski, J., Eberharder, A., Clapier, C.R., Corona, D.F.V., Becker, P.B., and Müller, C.W. (2003). Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Molecular cell* 12, 449-460.
- Gry, M., Rimini, R., Strömberg, S., Asplund, A., Pontén, F., Uhlén, M., and Nilsson, P. (2009). Correlations between RNA and protein expression profiles in 23 human cell lines. *BMC Genomics* 10.
- Guillemot, F. (2007). Spatial and temporal specification of neural fates by transcription factor codes. *Development* 134, 3771-3780.
- Guo, X., and Wang, X.F. (2009). Signaling cross-talk between TGF-B/BMP and other pathways. *Cell research* 19, 71-88.
- Gupta, A., Tsai, L.H., and Wynshaw-Boris, A. (2002). Life is a journey: a genetic look at neocortical development. *Nature reviews Genetics* 3, 342-355.
- Gutiérrez, J., Paredes, R., Cruzat, F., Hill, D.A., Van Wijnen, A.J., Lian, J.B., Stein, G.S., Stein, J.L., Imbalzano, A.N., and Montecino, M. (2007). Chromatin remodeling by SWI/SNF results in nucleosome mobilization to preferential positions in the rat osteocalcin gene promoter. *Journal of Biological Chemistry* 282, 9445-9457.
- Hébert, J.M., and McConnell, S.K. (2000). Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. *Developmental biology* 222, 296-306.
- Hébert, J.M., Mishina, Y., and McConnell, S.K. (2002). BMP signaling is required locally to pattern the dorsal telencephalic midline. *Neuron* 35, 1029-1041.
- Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., and Wu, C. (1999). ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* 97, 833-842.
- Hanashima, C., Li, S.C., Shen, L., Lai, E., and Fishell, G. (2004). Foxg1 Suppresses Early Cortical Cell Fate. *Science* 303, 56-59.
- Hanashima, C., Shen, L., Li, S.C., and Lai, E. (2002). Brain factor-1 controls the proliferation and differentiation of neocortical progenitor cells through independent mechanisms. *Journal of Neuroscience* 22, 6526-6536.
- Happel, N., and Doenecke, D. (2009). Histone H1 and its isoforms: Contribution to chromatin structure and function. *Gene* 431, 1-12.
- Hardcastle, Z., and Papalopulu, N. (2000). Distinct effects of XBF-1 in regulating the cell cycle inhibitor p27(XIC1) and imparting a neural fate. *Development* 127, 1303-1314.

- Hatini, V., Tao, W., and Lai, E. (1994). Expression of winged helix genes, BF-1 and BF-2, define adjacent domains within the developing forebrain and retina. *Journal of neurobiology* 25, 1293-1309.
- Haydar, T.F., Kuan, C.Y., Flavell, R.A., and Rakic, P. (1999). The role of cell death in regulating the size and shape of the mammalian forebrain. *Cerebral Cortex* 9, 621-626.
- He, W., Ingraham, C., Rising, L., Goderie, S., and Temple, S. (2001). Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis. *Journal of Neuroscience* 21, 8854-8862.
- He, X., Fan, H.Y., Narlikar, G.J., and Kingston, R.E. (2006). Human ACF1 alters the remodeling strategy of SNF2h. *Journal of Biological Chemistry* 281, 28636-28647.
- Heintzman, N.D., Stuart, R.K., Hon, G., Fu, Y., Ching, C.W., Hawkins, R.D., Barrera, L.O., Van Calcar, S., Qu, C., Ching, K.A., *et al.* (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nature genetics* 39, 311-318.
- Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience* 6, 877-888.
- Hevner, R.F. (2006). From radial glia to pyramidal-projection neuron: transcription factor cascades in cerebral cortex development. *Molecular neurobiology* 33, 33-50.
- Hirabayashi, Y., Itoh, Y., Tabata, H., Nakajima, K., Akiyama, T., Masuyama, N., and Gotoh, Y. (2004). The Wnt/B-catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development* 131, 2791-2801.
- Hitomi, M., Yang, K., Guo, Y., Fretthold, J., Harwalkar, J., and Stacey, D.W. (2006). p27Kip1 and cyclin dependent kinase 2 regulate passage through the restriction point. *Cell Cycle* 5, 2281-2289.
- Holliday, R. (1990). Mechanisms for the control of gene activity during development. *Biological reviews of the Cambridge Philosophical Society* 65, 431-471.
- Huang, X., Litington, Y., and Chiang, C. (2007). Ectopic sonic hedgehog signaling impairs telencephalic dorsal midline development: Implication for human holoprosencephaly. *Human molecular genetics* 16, 1454-1468.
- Huh, S., Hatini, V., Marcus, R.C., Li, S.C., and Lai, E. (1999). Dorsal-ventral patterning defects in the eye of BF-1-deficient mice associated with a restricted loss of shh expression. *Developmental biology* 211, 53-63.

- Hutnick, L.K., Golshani, P., Namiyara, M., Xue, Z., Matynia, A., Yang, X.W., Silva, A.J., Schweizer, F.E., and Fan, G. (2009). DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. *Human molecular genetics* 18, 2875-2888.
- Ingham, P.W., Nakano, Y., and Seger, C. (2011). Mechanisms and functions of Hedgehog signalling across the metazoa. *Nature Reviews Genetics* 12, 393-406.
- Ito, T., Bulger, M., Pazin, M.J., Kobayashi, R., and Kadonaga, J.T. (1997). ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* 90, 145-155.
- Jensen, A.M., and Wallace, V.A. (1997). Expression of Sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina. *Development* 124, 363-371.
- Jones-Davis, D., Calcagnotto, M.E., and Sebe, J.Y. (2009). Interneuron loss as a cause of seizures: Lessons from interneuron-deficient mice. In *Neuromethods*, pp. 121-139.
- Jones, M.H., Hamana, N., and Shimane, M. (2000). Identification and characterization of BPTF, a novel bromodomain transcription factor. *Genomics* 63, 35-39.
- Jung, S., Park, R.H., Kim, S., Jeon, Y.J., Ham, D.S., Jung, M.Y., Kim, S.S., Lee, Y.D., Park, C.H., and Suh-Kim, H. (2010). Id proteins facilitate self-renewal and proliferation of neural stem cells. *Stem Cells and Development* 19, 831-841.
- Kang, J.G., Hamiche, A., and Wu, C. (2002). GAL4 directs nucleosome sliding induced by NURF. *EMBO Journal* 21, 1406-1413.
- Kassabov, S.R., Henry, N.M., Zofall, M., Tsukiyama, T., and Bartholomew, B. (2002). High-resolution mapping of changes in histone-DNA contacts of nucleosomes remodeled by ISW2. *Molecular and cellular biology* 22, 7524-7534.
- Kawauchi, S., Santos, R., Kim, J., Hollenbeck, P.L.W., Murray, R.C., and Calof, A.L. (2009). The role of Foxg1 in the development of neural stem cells of the olfactory epithelium. In *Annals of the New York Academy of Sciences*, pp. 21-27.
- Khavari, P.A., Peterson, C.L., Tamkun, J.W., Mendel, D.B., and Crabtree, G.R. (1993). BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature* 366, 170-174.
- Klose, R.J., and Bird, A.P. (2006). Genomic DNA methylation: The mark and its mediators. *Trends in biochemical sciences* 31, 89-97.

- Koinuma, S., Umesono, Y., Watanabe, K., and Agata, K. (2003). The expression of planarian brain factor homologs, DjFoxG and DjFoxD. *Gene Expression Patterns* 3, 21-27.
- Kolasinska-Zwierz, P., Down, T., Latorre, I., Liu, T., Liu, X.S., and Ahringer, J. (2009). Differential chromatin marking of introns and expressed exons by H3K36me3. *Nature genetics* 41, 376-381.
- Komada, M., Saitsu, H., Kinboshi, M., Miura, T., Shiota, K., and Ishibashi, M. (2008). Hedgehog signaling is involved in development of the neocortex. *Development* 135, 2717-2727.
- Kornberg, R.D. (1974). Chromatin structure: a repeating unit of histones and DNA. *Science* 184, 868-871.
- Kortüm, F., Das, S., Flindt, M., Morris-Rosendahl, D.J., Stefanova, I., Goldstein, A., Horn, D., Klopocki, E., Kluger, G., Martin, P., *et al.* (2011). The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *Journal of medical genetics* 48, 396-406.
- Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *Journal of Comparative Neurology* 297, 441-470.
- Kowalczyk, T., Pontious, A., Englund, C., Daza, R.A.M., Bedogni, F., Hodge, R., Attardo, A., Bell, C., Huttner, W.B., and Hevner, R.F. (2009). Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cerebral Cortex* 19, 2439-2450.
- Kuida, K., Haydar, T.F., Kuan, C.Y., Gu, Y., Taya, C., Karasuyama, H., Su, M.S.S., Rakic, P., and Flavell, R.A. (1998). Reduced apoptosis and cytochrome C-mediated caspase activation in mice lacking Caspase 9. *Cell* 94, 325-337.
- Kuida, K., Zheng, T.S., Na, S., Kuan, C.Y., Yang, D., Karasuyama, H., Rakic, P., and Flavell, R.A. (1996). Decreased apoptosis in the brain and premature lethality in CPP32- deficient mice. *Nature* 384, 368-372.
- Kwon, S.Y., Xiao, H., Glover, B.P., Tjian, R., Wu, C., and Badenhorst, P. (2008). The nucleosome remodeling factor (NURF) regulates genes involved in Drosophila innate immunity. *Developmental biology* 316, 538-547.
- Kwon, S.Y., Xiao, H., Wu, C., and Badenhorst, P. (2009). Alternative splicing of NURF301 generates distinct NURF chromatin remodeling complexes with altered modified histone binding specificities. *PLoS Genetics* 5.

- Längst, G., and Becker, P.B. (2001a). ISWI induces nucleosome sliding on nicked DNA. *Molecular cell* 8, 1085-1092.
- Längst, G., and Becker, P.B. (2001b). Nucleosome mobilization and positioning by ISWI-containing chromatin-remodeling factors. *Journal of cell science* 114, 2561-2568.
- Längst, G., and Becker, P.B. (2004). Nucleosome remodeling: One mechanism, many phenomena? *Biochimica et Biophysica Acta - Gene Structure and Expression* 1677, 58-63.
- Ladd-Acosta, C., Pevsner, J., Sabunciyan, S., Yolken, R.H., Webster, M.J., Dinkins, T., Callinan, P.A., Fan, J.B., Potash, J.B., and Feinberg, A.P. (2007). DNA methylation signatures within the human brain. *American Journal of Human Genetics* 81, 1304-1315.
- Lagali, P.S., Corcoran, C.P., and Picketts, D.J. (2010). Hippocampus development and function: Role of epigenetic factors and implications for cognitive disease. *Clinical genetics* 78, 321-333.
- Lan, L., Ui, A., Nakajima, S., Hatakeyama, K., Hoshi, M., Watanabe, R., Janicki, S.M., Ogiwara, H., Kohno, T., Kanno, S.I., *et al.* (2010). The ACF1 Complex Is Required for DNA Double-Strand Break Repair in Human Cells. *Molecular cell* 40, 976-987.
- Landry, J., Sharov, A.A., Piao, Y., Sharova, L.V., Xiao, H., Southon, E., Matta, J., Tessarollo, L., Zhang, Y.E., Ko, M.S.H., *et al.* (2008). Essential role of chromatin remodeling protein bptf in early mouse embryos and embryonic stem cells. *PLoS Genetics* 4.
- Landry, J.W., Banerjee, S., Taylor, B., Aplan, P.D., Singer, A., and Wu, C. (2011). Chromatin remodeling complex NURF regulates thymocyte maturation. *Genes and Development* 25, 275-286.
- Lange, C., Huttner, W.B., and Calegari, F. (2009). Cdk4/CyclinD1 Overexpression in Neural Stem Cells Shortens G1, Delays Neurogenesis, and Promotes the Generation and Expansion of Basal Progenitors. *Cell Stem Cell* 5, 320-331.
- Lazzaro, M.A., Pépin, D., Pescador, N., Murphy, B.D., Vanderhyden, B.C., and Picketts, D.J. (2006). The imitation switch protein SNF2L regulates steroidogenic acute regulatory protein expression during terminal differentiation of ovarian granulosa cells. *Molecular Endocrinology* 20, 2406-2417.

- Lazzaro, M.A., and Picketts, D.J. (2001). Cloning and characterization of the murine Imitation Switch (ISWI) genes: Differential expression patterns suggest distinct developmental roles for Snf2h and Snf2l. *Journal of neurochemistry* 77, 1145-1156.
- Lee, J. (2009). Regulation of CIP/KIP cell cycle inhibitors and their biological implications. *Current Trends in Biotechnology and Pharmacy* 3, 128-137.
- Lee, S., and Lee, S.K. (2010). Crucial roles of histone-modifying enzymes in mediating neural cell-type specification. *Current opinion in neurobiology* 20, 29-36.
- Lee, T.I., Jenner, R.G., Boyer, L.A., Guenther, M.G., Levine, S.S., Kumar, R.M., Chevalier, B., Johnstone, S.E., Cole, M.F., Isono, K.i., *et al.* (2006). Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells. *Cell* 125, 301-313.
- LeRoy, G., Orphanides, G., and Lane, W.S. (1998). Requirement of RSF and FACT for transcription of chromatin templates in vitro. *Science* 282, 1900-1904.
- LeRoy, G., Rickards, B., and Flint, S.J. (2008). The Double Bromodomain Proteins Brd2 and Brd3 Couple Histone Acetylation to Transcription. *Molecular cell* 30, 51-60.
- Lessard, J., Wu, J.I., Ranish, J.A., Wan, M., Winslow, M.M., Staahl, B.T., Wu, H., Aebersold, R., Graef, I.A., and Crabtree, G.R. (2007). An Essential Switch in Subunit Composition of a Chromatin Remodeling Complex during Neural Development. *Neuron* 55, 201-215.
- Li, H., Tao, W., and Lai, E. (1996). Characterization of the structure and function of the gene for transcription factor BF-1, an essential regulator of forebrain development. *Molecular Brain Research* 37, 96-104.
- Li, J., Hwai Wen, C., Lai, E., Parker, E.J., and Vogt, P.K. (1995). The oncogene qin codes for a transcriptional repressor. *Cancer research* 55, 5540-5544.
- Lin, Y.W., Chen, H.M., and Fang, J.Y. (2011). Gene silencing by the polycomb group proteins and associations with cancer. *Cancer investigation* 29, 187-195.
- Lindsten, T., Golden, J.A., Zong, W.X., Minarcik, J., Harris, M.H., and Thompson, C.B. (2003). The Proapoptotic Activities of Bax and Bak Limit the Size of the Neural Stem Cell Pool. *Journal of Neuroscience* 23, 11112-11119.
- Lindsten, T., Ross, A.J., King, A., Zong, W.X., Rathmell, J.C., Shiels, H.A., Ulrich, E., Waymire, K.G., Mahar, P., Frauwirth, K., *et al.* (2000). The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. *Molecular cell* 6, 1389-1399.

- Liu, X., Lee, C.K., Granek, J.A., Clarke, N.D., and Lieb, J.D. (2006). Whole-genome comparison of Leu3 binding in vitro and in vivo reveals the importance of nucleosome occupancy in target site selection. *Genome research* 16, 1517-1528.
- Liu, Y.I., Chang, M.V., Li, H.E., Barolo, S., Chang, J.L., Blauwkamp, T.A., and Cadigan, K.M. (2008). The chromatin remodelers ISWI and ACF1 directly repress Wingless transcriptional targets. *Developmental biology* 323, 41-52.
- Lodato, S., Rouaux, C., Quast, K., Jantrachotechatchawan, C., Studer, M., Hensch, T., and Arlotta, P. (2011). Excitatory Projection Neuron Subtypes Control the Distribution of Local Inhibitory Interneurons in the Cerebral Cortex. *Neuron* 69, 763-779.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251-260.
- Luskin, M.B., and Shatz, C.J. (1985). Studies of the earliest generated cells of the cat's visual cortex: Cogeneration of subplate and marginal zones. *Journal of Neuroscience* 5, 1062-1075.
- Mabie, P.C., Mehler, M.F., and Kessler, J.A. (1999). Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. *Journal of Neuroscience* 19, 7077-7088.
- MacDonald, B.T., Tamai, K., and He, X. (2009). Wnt/ β -Catenin Signaling: Components, Mechanisms, and Diseases. *Developmental Cell* 17, 9-26.
- Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritli-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., *et al.* (2003). Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron* 39, 937-950.
- MacPherson, D., Sage, J., Crowley, D., Trumpp, A., Bronson, R.T., and Jacks, T. (2003). Conditional mutation of Rb causes cell cycle defects without apoptosis in the central nervous system. *Molecular and cellular biology* 23, 1044-1053.
- Malumbres, M., and Barbacid, M. (2009). Cell cycle, CDKs and cancer: A changing paradigm. *Nature Reviews Cancer* 9, 153-166.
- Mandel, G., Fiondella, C.G., Covey, M.V., Lu, D.D., LoTurco, J.J., and Ballas, N. (2011). Repressor element 1 silencing transcription factor (REST) controls radial migration and temporal neuronal specification during neocortical development. *Proceedings of the National Academy of Sciences of the United States of America* 108, 16789-16794.

- Manuel, M., Martynoga, B., Yu, T., West, J.D., Mason, J.O., and Price, D.J. (2010). The transcription factor Foxg1 regulates the competence of telencephalic cells to adopt subpallial fates in mice. *Development* 137, 487-497.
- Manuel, M.N., Martynoga, B., Molinek, M.D., Quinn, J.C., Kroemmer, C., Mason, J.O., and Price, D.J. (2011). The transcription factor Foxg1 regulates telencephalic progenitor proliferation cell autonomously, in part by controlling Pax6 expression levels. *Neural Development* 6.
- Mao, C., Brown, C.R., Griesenbeck, J., and Boeger, H. (2011). Occlusion of regulatory sequences by promoter nucleosomes In Vivo. *PLoS ONE* 6.
- Marçal, N., Patel, H., Dong, Z., Belanger-Jasmin, S., Hoffman, B., Helgason, C.D., Dang, J., and Stifani, S. (2005). Antagonistic effects of Grg6 and Groucho/TLE on the transcription repression activity of brain factor 1/FoxG1 and cortical neuron differentiation. *Molecular and cellular biology* 25, 10916-10929.
- Martín Caballero, I., Hansen, J., Leaford, D., Pollard, S., and Hendrich, B.D. (2009). The methyl-CpG binding proteins Mecp2, Mbd2 and Kaiso are dispensable for mouse embryogenesis, but play a redundant function in neural differentiation. *PLoS ONE* 4.
- Martínez-Cerdeño, V., Noctor, S.C., and Kriegstein, A.R. (2006). The role of intermediate progenitor cells in the evolutionary expansion of the cerebral cortex. *Cerebral cortex* (New York, NY: 1991) 16 Suppl 1.
- Martynoga, B., Morrison, H., Price, D.J., and Mason, J.O. (2005). Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. *Developmental biology* 283, 113-127.
- Matsumoto, S., Banine, F., Struve, J., Xing, R., Adams, C., Liu, Y., Metzger, D., Chambon, P., Rao, M.S., and Sherman, L.S. (2006). Brg1 is required for murine neural stem cell maintenance and gliogenesis. *Developmental biology* 289, 372-383.
- McConnell, S.K. (1988). Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. *Journal of Neuroscience* 8, 945-974.
- McConnell, S.K., Ghosh, A., and Shatz, C.J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* 245, 978-982.
- Mencarelli, M.A., Kleefstra, T., Katzaki, E., Papa, F.T., Cohen, M., Pfundt, R., Ariani, F., Meloni, I., Mari, F., and Renieri, A. (2009). 14q12 Microdeletion syndrome and congenital variant of Rett syndrome. *European Journal of Medical Genetics* 52, 148-152.

- Mikoshiba, K., Kohsaka, S., and Takamatsu, K. (1980). Morphological and biochemical studies on the cerebral cortex from reeler mutant mice: Development of cortical layers and metabolic mapping by the deoxyglucose method. *Journal of neurochemistry* 34, 835-844.
- Miller, M.W. (2003). Expression of transforming growth factor-B in developing rat cerebral cortex: Effects of prenatal exposure to ethanol. *Journal of Comparative Neurology* 460, 410-424.
- Mitsuhashi, T., and Takahashi, T. (2009). Genetic regulation of proliferation/differentiation characteristics of neural progenitor cells in the developing neocortex. *Brain and Development* 31, 553-557.
- Mohrmann, L., Langenberg, K., Krijgsveld, J., Kal, A.J., Heck, A.J.R., and Verrijzer, C.P. (2004). Differential Targeting of Two Distinct SWI/SNF-Related Drosophila Chromatin-Remodeling Complexes. *Molecular and cellular biology* 24, 3077-3088.
- Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., and Morrison, S.J. (2003). Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* 425, 962-967.
- Molyneaux, B.J., Arlotta, P., Menezes, J.R.L., and Macklis, J.D. (2007). Neuronal subtype specification in the cerebral cortex. *Nature Reviews Neuroscience* 8, 427-437.
- Mondal, S., Ivanchuk, S.M., Rutka, J.T., and Boulianne, G.L. (2007). Sloppy paired 1/2 regulate glial cell fates by inhibiting Gcm function. *Glia* 55, 282-293.
- Montel, F., Fontaine, E., St-Jean, P., Castelnovo, M., and Faivre-Moskalenko, C. (2007). Atomic force microscopy imaging of SWI/SNF action: Mapping the nucleosome remodeling and sliding. *Biophysical journal* 93, 566-578.
- Munji, R.N., Choe, Y., Li, G., Siegenthaler, J.A., and Pleasure, S.J. (2011). Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. *Journal of Neuroscience* 31, 1676-1687.
- Murphy, D.B., Wiese, S., Burfeind, P., Schmundt, D., Mattei, M.G., Schulz-Schaeffer, W., and Thies, U. (1994). Human brain factor 1, a new member of the fork head gene family. *Genomics* 21, 551-557.
- Musladin, S., and Barbarić, S. (2010). Yeast PHO genes: An excellent model for elucidation of chromatin-remodelling mechanisms. *Food Technology and Biotechnology* 48, 308-316.

- Muzio, L., and Mallamaci, A. (2005). Foxg1 confines Cajal-Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25, 4435-4441.
- Nguyen, L., Besson, A., Heng, J.I.T., Schuurmans, C., Teboul, L., Parras, C., Philpott, A., Roberts, J.M., and Guillemot, F. (2006). p27kip1 independently promotes neuronal differentiation and migration in the cerebral cortex. *Genes and Development* 20, 1511-1524.
- Noctor, S.C., Flint, A.C., Weissman, T.A., Wong, W.S., Clinton, B.K., and Kriegstein, A.R. (2002). Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *Journal of Neuroscience* 22, 3161-3173.
- Noctor, S.C., Martínez-Cerdeño, V., and Kriegstein, A.R. (2008). Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *Journal of Comparative Neurology* 508, 28-44.
- Obendorf, M., Meyer, R., Henning, K., Mitev, Y.A., Schröder, J., Patchev, V.K., and Wolf, S.S. (2007). FoxG1, a member of the forkhead family, is a corepressor of the androgen receptor. *Journal of Steroid Biochemistry and Molecular Biology* 104, 195-207.
- Ogawa, M., Miyata, T., Nakajima, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H., and Mikoshiba, K. (1995). The reeler gene-associated antigen on cajal-retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* 14, 899-912.
- Okabe, I., Bailey, L.C., Attree, O., Srinivasan, S., Perkel, J.M., Laurent, B.C., Carlson, M., Nelson, D.L., and Nussbaum, R.L. (1992). Cloning of human and bovine homologs of SNF2/SW12: A global activator of transcription in yeast *S.cerevisiae*. *Nucleic acids research* 20, 4649-4655.
- Ortega, J.A., and Alcántara, S. (2010). BDNF/MAPK/ERK-induced BMP7 expression in the developing cerebral cortex induces premature radial glia differentiation and impairs neuronal migration. *Cerebral Cortex* 20, 2132-2144.
- Palmer, D.K., O'Day, K., Hai Le, T., Charbonneau, H., and Margolis, R.L. (1991). Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone. *Proceedings of the National Academy of Sciences of the United States of America* 88, 3734-3738.
- Panchision, D.M., Pickel, J.M., Studer, L., Lee, S.H., Turner, P.A., Hazel, T.G., and McKay, R.D.G. (2001). Sequential actions of BMP receptors control neural precursor cell production and fate. *Genes and Development* 15, 2094-2110.

- Pandya, D.N., and Seltzer, B. (1982). Association areas of the cerebral cortex. *Trends in neurosciences* 5, 386-390.
- Papa, F.T., Mencarelli, M.A., Caselli, R., Katzaki, E., Sampieri, K., Meloni, I., Ariani, F., Longo, I., Maggio, A., Balestri, P., *et al.* (2008). A 3 Mb deletion in 14q12 causes severe mental retardation, mild facial dysmorphisms and rett-like features. *American Journal of Medical Genetics, Part A* 146, 1994-1998.
- Pardee, A.B. (1974). A restriction point for control of normal animal cell proliferation. *Proceedings of the National Academy of Sciences of the United States of America* 71, 1286-1290.
- Parnavelas, J.G. (1999). Glial cell lineages in the rat cerebral cortex. *Experimental neurology* 156, 418-429.
- Pauley, S., Lai, E., and Fritzsche, B. (2006). Foxg1 is required for morphogenesis and histogenesis of the mammalian inner ear. *Developmental Dynamics* 235, 2470-2482.
- Pereira, J.D., Sansom, S.N., Smith, J., Dobenecker, M.W., Tarakhovsky, A., and Livesey, F.J. (2010). Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* 107, 15957-15962.
- Perpelescu, M., Nozaki, N., Obuse, C., Yang, H., and Yoda, K. (2009). Active establishment of centromeric cenp-a chromatin by rsf complex. *Journal of Cell Biology* 185, 397-407.
- Peterson, C.L., Dingwall, A., and Scott, M.P. (1994). Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proceedings of the National Academy of Sciences of the United States of America* 91, 2905-2908.
- Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research* 29.
- Pienta, K.J., and Coffey, D.S. (1984). A structural analysis of the role of the nuclear matrix and DNA loops in the organization of the nucleus and chromosome. *Journal of cell science SUPPL.* 1, 123-135.
- Pilaz, L.J., Patti, D., Marcy, G., Ollier, E., Pfister, S., Douglas, R.J., Betizeau, M., Gautier, E., Cortay, V., Doerflinger, N., *et al.* (2009). Forced G1-phase reduction alters mode of division, neuron number, and laminar phenotype in the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America* 106, 21924-21929.

- Poot, R.A., Bozhenok, L., van den Berg, D.L.C., Steffensen, S., Ferreira, F., Grimaldi, M., Gilbert, N., Ferreira, J., and Varga-Weisz, P.D. (2004). The Williams syndrome transcription factor interacts with PCNA to target chromatic remodelling by ISWI to replication foci. *Nature cell biology* 6, 1236-1244.
- Poot, R.A., Dellaire, G., Hülsmann, B.B., Grimaldi, M.A., Corona, D.F.V., Becker, P.B., Bickmore, W.A., and Varga-Weisz, P.D. (2000). HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. *EMBO Journal* 19, 3377-3387.
- Pratt, T., Tian, N.M.M.L., Simpson, T.I., Mason, J.O., and Price, D.J. (2004). The winged helix transcription factor Foxg1 facilitates retinal ganglion cell axon crossing of the ventral midline in the mouse. *Development* 131, 3773-3784.
- Price, D.J., and Willshaw, D.J. (2000). Early Development of the Telencephalon. In *Mechanisms of Cortical Development* (Oxford University Press), pp. 9-48.
- Qureshi, I.A., Gokhan, S., and Mehler, M.F. (2010). REST and CoREST are transcriptional and epigenetic regulators of seminal neural fate decisions. *Cell Cycle* 9, 4477-4486.
- Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *Journal of Comparative Neurology* 145, 61-83.
- Randazzo, F.M., Khavari, P., Crabtree, G., Tamkun, J., and Rossant, J. (1994). Brg1: A putative murine homologue of the Drosophila brahma gene, a homeotic gene regulator. *Developmental biology* 161, 229-242.
- Regad, T., Roth, M., Bredenkamp, N., Illing, N., and Papalopulu, N. (2007). The neural progenitor-specifying activity of FoxG1 is antagonistically regulated by CKI and FGF. *Nature cell biology* 9, 531-540.
- Reyes, J.C., Barra, J., Muchardt, C., Camus, A., Babinet, C., and Yaniv, M. (1998). Altered control of cellular proliferation in the absence of mammalian brahma (SNF2 α). *EMBO Journal* 17, 6979-6991.
- Rippe, K., Schrader, A., Riede, P., Strohnner, R., Lehmann, E., and Längst, G. (2007). DNA sequence- and conformation-directed positioning of nucleosomes by chromatin-remodeling complexes. *Proceedings of the National Academy of Sciences of the United States of America* 104, 15635-15640.
- Robertson, R.T., Annis, C.M., Baratta, J., Haraldson, S., Ingeman, J., Kageyama, G.H.; Kimm, E., Yu, J. (2000). Do subplate neurons comprise a transient population of cells in developing neocortex of rats? *Journal of Comparative Neurology* 426, 632-650.

- Robinson, P.J.J., Fairall, L., Huynh, V.A.T., and Rhodes, D. (2006). EM measurements define the dimensions of the "30-nm" chromatin fiber: Evidence for a compact, interdigitated structure. *Proceedings of the National Academy of Sciences of the United States of America* 103, 6506-6511.
- Rodriguez, C., Huang, L.J.S., Son, J.K., McKee, A., Xiao, Z., and Lodish, H.F. (2001). Functional Cloning of the Proto-oncogene Brain Factor-1 (BF-1) As a Smad-binding Antagonist of Transforming Growth Factor- β Signaling. *Journal of Biological Chemistry* 276, 30224-30230.
- Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *Journal of Biological Chemistry* 273, 5858-5868.
- Roh, T.Y., Cuddapah, S., Cui, K., and Zhao, K. (2006). The genomic landscape of histone modifications in human T cells. *Proceedings of the National Academy of Sciences of the United States of America* 103, 15782-15787.
- Roth, M., Bonev, B., Lindsay, J., Lea, R., Panagiotaki, N., Houart, C., and Papalopulu, N. (2010). FoxG1 and TLE2 act cooperatively to regulate ventral telencephalon formation. *Development* 137, 1553-1562.
- Routh, A., Sandin, S., and Rhodes, D. (2008). Nucleosome repeat length and linker histone stoichiometry determine chromatin fiber structure. *Proceedings of the National Academy of Sciences of the United States of America* 105, 8872-8877.
- Roy, K., Thiels, E., and Monaghan, A.P. (2002). Loss of the tailless gene affects forebrain development and emotional behavior. *Physiology and Behavior* 77, 595-600.
- Sánchez, I., and Dynlacht, B.D. (2005). New insights into cyclins, CDKs, and cell cycle control. *Seminars in Cell and Developmental Biology* 16, 311-321.
- Sala, A., Toto, M., Pinello, L., Gabriele, A., Di Benedetto, V., Ingrassia, A.M.R., Lo Bosco, G., Di Gesà, V., Giancarlo, R., and Corona, D.F.V. (2011). Genome-wide characterization of chromatin binding and nucleosome spacing activity of the nucleosome remodelling ATPase ISWI. *EMBO Journal* 30, 1766-1777.
- Sauer, F.C. (1935). Mitosis in the Neural Tube. *The Journal of comparative neurology* 62, 377-405.
- Saxonov, S., Berg, P., and Brutlag, D.L. (2006). A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proceedings of the National Academy of Sciences of the United States of America* 103, 1412-1417.

- Schalch, T., Duda, S., Sargent, D.F., and Richmond, T.J. (2005). X-ray structure of a tetranucleosome and its implications for the chromatin fibre. *Nature* 436, 138-141.
- Schmechel, D.E., and Rakic, P. (1979). A Golgi study of radial glial cells in developing monkey telencephalon: Morphogenesis and transformation into astrocytes. *Anatomy and Embryology* 156, 115-152.
- Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B., and Cavalli, G. (2007). Genome Regulation by Polycomb and Trithorax Proteins. *Cell* 128, 735-745.
- Schwanbeck, R., Xiao, H., and Wu, C. (2004). Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *Journal of Biological Chemistry* 279, 39933-39941.
- Schwartz, S., Meshorer, E., and Ast, G. (2009). Chromatin organization marks exon-intron structure. *Nature Structural and Molecular Biology* 16, 990-995.
- Seah, C., Levy, M.A., Jiang, Y., Mokhtarzada, S., Higgs, D.R., Gibbons, R.J., and Bérubé, N.G. (2008). Neuronal death resulting from targeted disruption of the Snf2 protein ATRX is mediated by p53. *Journal of Neuroscience* 28, 12570-12580.
- Seo, S., Richardson, G.A., and Kroll, K.L. (2005). The SWI/SNF chromatin remodeling protein Brg1 is required for vertebrate neurogenesis and mediates transactivation of Ngn and NeuroD. *Development* 132, 105-115.
- Seoane, J., Le, H.V., Shen, L., Anderson, S.A., and Massagué, J. (2004). Integration of smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell* 117, 211-223.
- Shen, L., Nam, H.S., Song, P., Moore, H., and Anderson, S.A. (2006a). FoxG1 haploinsufficiency results in impaired neurogenesis in the postnatal hippocampus and contextual memory deficits. *Hippocampus* 16, 875-890.
- Shen, Q., Wang, Y., Dimos, J.T., Fasano, C.A., Phoenix, T.N., Lemischka, I.R., Ivanova, N.B., Stifani, S., Morrissey, E.E., and Temple, S. (2006b). The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nature neuroscience* 9, 743-751.
- Sheppard, A.M., and Pearlman, A.L. (1997). Abnormal reorganization of preplate neurons and their associated extracellular matrix: An early manifestation of altered neocortical development in the reeler mutant mouse. *Journal of Comparative Neurology* 378, 173-179.
- Shermoen, A.W., McClelland, M.L., and O'Farrell, P.H. (2010). Developmental control of late replication and s phase length. *Current Biology* 20, 2067-2077.

- Sherr, C.J., and Roberts, J.M. (1999). CDK inhibitors: Positive and negative regulators of G1-phase progression. *Genes and Development* 13, 1501-1512.
- Shibata, M., Kurokawa, D., Nakao, H., Ohmura, T., and Aizawa, S. (2008). MicroRNA-9 modulates Cajal-Retzius cell differentiation by suppressing Foxg1 expression in mouse medial pallium. *Journal of Neuroscience* 28, 10415-10421.
- Shibata, M., Nakao, H., Kiyonari, H., Abe, T., and Aizawa, S. (2011). MicroRNA-9 regulates neurogenesis in mouse telencephalon by targeting multiple transcription factors. *Journal of Neuroscience* 31, 3407-3422.
- Shields, R. (1977). Transition probability and the origin of variation in the cell cycle. *Nature* 267, 704-707.
- Shikata, Y., Okada, T., Hashimoto, M., Ellis, T., Matsumaru, D., Shiroishi, T., Ogawa, M., Wainwright, B., and Motoyama, J. (2011). Ptch1-mediated dosage-dependent action of Shh signaling regulates neural progenitor development at late gestational stages. *Developmental biology* 349, 147-159.
- Shimamura, K., Hartigan, D.J., Martinez, S., Puellas, L., and Rubenstein, J.L.R. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* 121, 3923-3933.
- Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L. (2006). Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844-847.
- Shoichet, S.A., Kunde, S.A., Viertel, P., Schell-Apacik, C., von Voss, H., Tommerup, N., Ropers, H.H., and Kalscheuer, V.M. (2005). Haploinsufficiency of novel FOXP1B variants in a patient with severe mental retardation, brain malformations and microcephaly. *Human genetics* 117, 536-544.
- Siegenthaler, J.A., and Miller, M.W. (2005). Transforming growth factor B1 promotes cell cycle exit through the cyclin-dependent kinase inhibitor p21 in the developing cerebral cortex. *Journal of Neuroscience* 25, 8627-8636.
- Siegenthaler, J.A., Tremper-Wells, B.A., and Miller, M.W. (2008). Foxg1 haploinsufficiency reduces the population of cortical intermediate progenitor cells: effect of increased p21 expression. *Cerebral Cortex* 18, 1865-1875.
- Siegmund, K.D., Connor, C.M., Campan, M., Long, T.L., Weisenberger, D.J., Biniszkiwicz, D., Jaenisch, R., Laird, P.W., and Akbarian, S. (2007). DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. *PLoS ONE* 2.

- Singh, S., Johnson, J., and Chellappan, S. (2010). Small molecule regulators of Rb-E2F pathway as modulators of transcription. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* 1799, 788-794.
- Siriaco, G., Deuring, R., Chioda, M., Becker, P.B., and Tamkun, J.W. (2009). *Drosophila* ISWI regulates the association of histone H1 with interphase chromosomes in vivo. *Genetics* 182, 661-669.
- Slack, R.S., El-Bizri, H., Wong, J., Belliveau, D.J., and Miller, F.D. (1998). A critical temporal requirement for the retinoblastoma protein family during neuronal determination. *Journal of Cell Biology* 140, 1497-1509.
- Smrt, R.D., Eaves-Egenes, J., Barkho, B.Z., Santistevan, N.J., Zhao, C., Aimone, J.B., Gage, F.H., and Zhao, X. (2007). Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons. *Neurobiology of disease* 27, 77-89.
- Sockanathan, S., and Gaiano, N. (2009). Meninges Play a RADical Role in Embryonic Neural Stem Cell Regulation. *Cell Stem Cell* 5, 455-456.
- Song, H., Spichiger-Hausermann, C., and Basler, K. (2009). The ISWI-containing NURF complex regulates the output of the canonical Wingless pathway. *EMBO reports*.
- Spella, M., Kyrousi, C., Kritikou, E., Stathopoulou, A., Guillemot, F., Kioussis, D., Pachnis, V., Lygerou, Z., and Taraviras, S. (2011). Geminin regulates cortical progenitor proliferation and differentiation. *Stem cells* 29, 1269-1282.
- Stephens, G.E., Xiao, H., Lankenau, D.H., Wu, C., and Elgin, S.C.R. (2006). Heterochromatin protein 2 interacts with Nap-1 and NURF: A link between heterochromatin-induced gene silencing and the chromatin remodeling machinery in *Drosophila*. *Biochemistry* 45, 14990-14999.
- Stewart, G.R., and Pearlman, A.L. (1987). Fibronectin-like immunoreactivity in the developing cerebral cortex. *Journal of Neuroscience* 7, 3325-3333.
- Stipursky, J., and Gomes, F.C.A. (2007). TGF-B1/SMAD signaling induces astrocyte fate commitment in vitro: Implications for radial glia development. *Glia* 55, 1023-1033.
- Stopka, T., and Skoultchi, A.I. (2003). The ISWI ATPase Snf2h is required for early mouse development. *Proceedings of the National Academy of Sciences of the United States of America* 100, 14097-14102.
- Striano, P., Paravidino, R., Sicca, F., Chiurazzi, P., Gimelli, S., Coppola, A., Robbiano, A., Traverso, M., Pintaudi, M., Giovannini, S., *et al.* (2011). West

syndrome associated with 14q12 duplications harboring FOXG1. *Neurology* 76, 1600-1602.

- Strohner, R., Nemeth, A., Jansa, P., Hofmann-Rohrer, U., Santoro, R., Längst, G., and Grummt, I. (2001). NoRC - A novel member of mammalian ISWI-containing chromatin remodeling machines. *EMBO Journal* 20, 4892-4900.
- Sugimoto, N., Yugawa, T., Iizuka, M., Kiyono, T., and Fujita, M. (2011). Chromatin remodeler sucrose nonfermenting 2 homolog (SNF2H) is recruited onto DNA replication origins through interaction with Cdc10 protein-dependent transcript 1 (Cdt1) and promotes pre-replication complex formation. *Journal of Biological Chemistry* 286, 39200-39210.
- Sun, Y., Hu, J., Zhou, L., Pollard, S.M., and Smith, A. (2011). Interplay between FGF2 and BMP controls the self-renewal, dormancy and differentiation of rat neural stem cells. *Journal of cell science* 124, 1867-1877.
- Supèr, H., Del Río, J.A., Martínez, A., Pérez-Sust, P., and Soriano, E. (2000). Disruption of neuronal migration and radial glia in the developing cerebral cortex following ablation of Cajal-Retzius cells. *Cerebral Cortex* 10, 602-613.
- Svaren, J., and Hörz, W. (1997). Transcription factors vs nucleosomes: Regulation of the PHO5 promoter in yeast. *Trends in biochemical sciences* 22, 93-97.
- Takahashi, T., Nowakowski, R.S., and Caviness Jr, V.S. (1995). The cell cycle of the pseudostratified ventricular epithelium of the embryonic murine cerebral wall. *Journal of Neuroscience* 15, 6046-6057.
- Takahashi, T., Nowakowski, R.S., and Caviness Jr, V.S. (1996). The leaving or Q fraction of the murine cerebral proliferative epithelium: A general model of neocortical neuronogenesis. *Journal of Neuroscience* 16, 6183-6196.
- Tao, W., and Lai, E. (1992). Telencephalon-restricted expression of BF-1, a new member of the HNF- 3/fork head gene family, in the developing rat brain. *Neuron* 8, 957-966.
- Tarabykin, V., Stoykova, A., Usman, N., and Gruss, P. (2001). Cortical upper layer neurons derive from the subventricular zone as indicated by Svet1 gene expression. *Development* 128, 1983-1993.
- Tarui, T., Takahashi, T., Nowakowski, R.S., Hayes, N.L., Bhide, P.G., and Caviness, V.S. (2005). Overexpression of p27Kip1, probability of cell cycle exit, and laminar destination of neocortical neurons. *Cerebral Cortex* 15, 1343-1355.

- Thomaidou, D., Mione, M.C., Cavanagh, J.F.R., and Parnavelas, J.G. (1997). Apoptosis and its relation to the cell cycle in the developing cerebral cortex. *Journal of Neuroscience* 17, 1075-1085.
- Thompson, P.J., Norton, K.A., Niri, F.H., Dawe, C.E., and McDermid, H.E. (2012). CECR2 Is Involved in Spermatogenesis and Forms a Complex with SNF2H in the Testis. *Journal of Molecular Biology* 415, 793-806.
- Tirosh, I., and Barkai, N. (2008). Two strategies for gene regulation by promoter nucleosomes. *Genome research* 18, 1084-1091.
- Tohyama, J., Yamamoto, T., Hosoki, K., Nagasaki, K., Akasaka, N., Ohashi, T., Kobayashi, Y., and Saitoh, S. (2011). West syndrome associated with mosaic duplication of FOXP1 in a patient with maternal uniparental disomy of chromosome 14. *American Journal of Medical Genetics, Part A* 155, 2584-2588.
- Tomoda, T., Shirasawa, T., Yahagi, Y.I., Ishii, K., Takagi, H., Furiya, Y., Arai, K.I., Mori, H., and Muramatsu, M.A. (1996). Transforming growth factor- β is a survival factor for neonate cortical neurons: Coincident expression of type I receptors in developing cerebral cortices. *Developmental biology* 179, 79-90.
- Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., and Warman, M.L. (2000). Preparation of PCR-quality mouse genomic dna with hot sodium hydroxide and tris (HotSHOT). *BioTechniques* 29, 52-54.
- Tsukiyama, T., and Wu, C. (1995). Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* 83, 1011-1020.
- Tury, A., Mairet-Coello, G., and Diccio-Bloom, E. (2011). The cyclin-dependent kinase inhibitor p57Kip2 regulates cell cycle exit, differentiation, and migration of embryonic cerebral cortical precursors. *Cerebral Cortex* 21, 1840-1856.
- Ulloa, F., Itasaki, N., and Briscoe, J. (2007). Inhibitory Gli3 Activity Negatively Regulates Wnt/ β -Catenin Signaling. *Current Biology* 17, 545-550.
- Ulloa, F., and Martí, E. (2010). Wnt won the war: Antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube. *Developmental Dynamics* 239, 69-76.
- Van Holde, K.E., and Isenberg, I. (1975). Histone interactions and chromatin structure. *Accounts of Chemical Research* 8, 327-335.
- Varga-Weisz, P.D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P.B. (1997). Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature* 388, 598-602.

- Vermeulen, M., Mulder, K.W., Denissov, S., Pijnappel, W.W.M.P., van Schaik, F.M.A., Varier, R.A., Baltissen, M.P.A., Stunnenberg, H.G., Mann, M., and Timmers, H.T.M. (2007). Selective Anchoring of TFIID to Nucleosomes by Trimethylation of Histone H3 Lysine 4. *Cell* 131, 58-69.
- Vernon, A.E., Devine, C., and Philpott, A. (2003). The cdk inhibitor p27Xic1 is required for differentiation of primary neurones in *Xenopus*. *Development* 130, 85-92.
- Vincent, J.A., Kwong, T.J., and Tsukiyama, T. (2008). ATP-dependent chromatin remodeling shapes the DNA replication landscape. *Nature structural & molecular biology* 15, 477-484.
- Vogel, T., Ahrens, S., Büttner, N., and Krieglstein, K. (2010). Transforming growth factor β promotes neuronal cell fate of mouse cortical and hippocampal progenitors in vitro and in vivo: Identification of nedd9 as an essential signaling component. *Cerebral Cortex* 20, 661-671.
- Wang, W. (2003). The SWI/SNF family of ATP-dependent chromatin remodelers: Similar mechanisms for diverse functions. In *Current Topics in Microbiology and Immunology*, pp. 143-169.
- Wang, W., Bu, B., Xie, M., Zhang, M., Yu, Z., and Tao, D. (2009). Neural cell cycle dysregulation and central nervous system diseases. *Progress in neurobiology* 89, 1-17.
- Wang, Y., Guan, Y., Wang, F., Huang, A., Wang, S., and Zhang, Y.A. (2010). Bmi-1 regulates self-renewal, proliferation and senescence of human fetal neural stem cells in vitro. *Neuroscience letters* 476, 74-78.
- Whitehouse, I., Flaus, A., Cairns, B.R., White, M.F., Workman, J.L., and Owen-Hughes, T. (1999). Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* 400, 784-787.
- Wiese, S., Murphy, D.B., Schlung, A., Burfeind, P., Schmundt, D., Schnülle, V., Mattei, M.G., and Thies, U. (1995). The genes for human brain factor 1 and 2, members of the fork head gene family, are clustered on chromosome 14q. *Biochimica et Biophysica Acta - Gene Structure and Expression* 1262, 105-112.
- Wijchers, P.J.E.C., Burbach, J.P.H., and Smidt, M.P. (2006). In control of biology: Of mice, men and foxes. *Biochemical Journal* 397, 233-246.
- Wonders, C., and Anderson, S.A. (2005). Cortical interneurons and their origins. *Neuroscientist* 11, 199-205.

- Woodhead, G.J., Mutch, C.A., Olson, E.C., and Chenn, A. (2006). Cell-autonomous β -catenin signaling regulates cortical precursor proliferation. *Journal of Neuroscience* 26, 12620-12630.
- Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhorst, P., *et al.* (2006). A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442, 86-90.
- Xi, R., and Xie, T. (2005). Developmental biology: Stem cell self-renewal controlled by chromatin remodeling factors. *Science* 310, 1487-1489.
- Xia, Y., Jiang, B., Zou, Y., Gao, G., Shang, L., Chen, B., Liu, Q., and Gong, Y. (2008). Sp1 and CREB regulate basal transcription of the human SNF2L gene. *Biochemical and biophysical research communications* 368, 438-444.
- Xiao, A., Li, H., Shechter, D., Ahn, S.H., Fabrizio, L.A., Erdjument-Bromage, H., Ishibe-Murakami, S., Wang, B., Tempst, P., Hofmann, K., *et al.* (2009). WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature* 457, 57-62.
- Xu, G.L., Bestor, T.H., Bourc'His, D., Hsieh, C.L., Tommerup, N., Bugge, M., Hulten, M., Qu, X., Russo, J.J., and Viegas-Péquignot, E. (1999). Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* 402, 187-191.
- Xu, Q., Wonders, C.P., and Anderson, S.A. (2005). Sonic hedgehog maintains the identity of cortical interneuron progenitors in the ventral telencephalon. *Development* 132, 4987-4998.
- Xuan, S., Baptista, C.A., Balas, G., Tao, W., Soares, V.C., and Lai, E. (1995). Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. *Neuron* 14, 1141-1152.
- Yao, G., Lee, T.J., Mori, S., Nevins, J.R., and You, L. (2008). A bistable Rb-E2F switch underlies the restriction point. *Nature cell biology* 10, 476-482.
- Yao, J., Lai, E., and Stifani, S. (2001). The winged-helix protein brain factor 1 interacts with Groucho and Hes proteins to repress transcription. *Molecular and cellular biology* 21, 1962-1972.
- Ye, W., Mairet-Coello, G., Pasoreck, E., and DiCicco-Bloom, E. (2009). Patterns of p57Kip2 expression in embryonic rat brain suggest roles in progenitor cell cycle exit and neuronal differentiation. *Developmental Neurobiology* 69, 1-21.

- Yip, D.J. (2009). The chromatin remodelling protein SNF2L regulates cell number in the developing brain (University of Ottawa, Department of Biochemistry, Microbiology and Immunology).
- Yip, D.J., Corcoran, C.P., Alvarez-Saavedra, M., Demaria, A., Rennick, S., Mears, A.J., Rudnicki, M.A., Messier, C., and Picketts, D.J. (2012). *Snf2l* regulates *foxg1*-dependent progenitor cell expansion in the developing brain. *Developmental cell* 22, 871-878.
- Yokoyama, H., Rybina, S., Santarella-Mellwig, R., Mattaj, I.W., and Karsenti, E. (2009). ISWI is a RanGTP-dependent MAP required for chromosome segregation. *Journal of Cell Biology* 187, 813-829.
- Yoshida, H., Kong, Y.Y., Yoshida, R., Elia, A.J., Hakem, A., Hakem, R., Penninger, J.M., and Mak, T.W. (1998). *Apaf1* is required for mitochondrial pathways of apoptosis and brain development. *Cell* 94, 739-750.
- Yuasa, S., Nakajima, M., Aizawa, H., Sahara, N., Koizumi, K.I., Sakai, T., Usami, M., Kobayashi, S.I., Kuroyanagi, H., Mori, H., *et al.* (2002). Impaired cell cycle control of neuronal precursor cells in the neocortical primordium of presenilin-1-deficient mice. *Journal of neuroscience research* 70, 501-513.
- Zecevic, N., Milosevic, A., Rakic, S., and Marin-Padilla, M. (1999). Early development and composition of the human primordial plexiform layer: An immunohistochemical study. *Journal of Comparative Neurology* 412, 241-254.
- Zecevic, N., and Rakic, P. (2001). Development of layer I neurons in the primate cerebral cortex. *Journal of Neuroscience* 21, 5607-5619.
- Zencak, D., Lingbeek, M., Kostic, C., Tekaya, M., Tanger, E., Hornfeld, D., Jaquet, M., Munier, F.L., Schorderet, D.F., Van Lohuizen, M., *et al.* (2005). *Bmi1* loss produces an increase in astroglial cells and a decrease in neural stem cell population and proliferation. *Journal of Neuroscience* 25, 5774-5783.
- Zeng, S., Chen, J., and Shen, H. (2010). Controlling of bone morphogenetic protein signaling. *Cellular signalling* 22, 888-893.
- Zhao, X.F., Suh, C.S., Prat, C.R., Ellingsen, S., and Fjose, A. (2009). Distinct expression of two *foxg1* paralogues in zebrafish. *Gene Expression Patterns* 9, 266-272.
- Zhou, Q.P., Le, T.N., Qiu, X., Spencer, V., de Melo, J., Du, G., Plews, M., Fonseca, M., Sun, J.M., Davie, J.R., *et al.* (2004). Identification of a direct *Dlx* homeodomain target in the developing mouse forebrain and retina by optimization of chromatin immunoprecipitation. *Nucleic acids research* 32, 884-892.

- Zhou, Y., and Grummt, I. (2005). The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. *Current Biology* 15, 1434-1438.

Curriculum Vitae

Qualifications Summary

- Completing M.Sc. Biochemistry at the University of Ottawa
- Honours B.Sc. Specialization in Biochemistry from the University of Ottawa
- Over a year of cumulative work experience in the scientific field
- Proficient in English, intermediate French
- Working knowledge of Microsoft Office Suite (Word, Excel, Powerpoint), Adobe Photoshop and Axiovision software
- Can self-direct on a problem or collaborate with a team to achieve a common research goal

Education

M.Sc. Biochemistry – Human Molecular Genetics 2008-Current
University of Ottawa, Ottawa, Ontario

- Expected Date of Completion – Spring 2012
- Thesis Supervisor – Dr. David Picketts
- Thesis - Snf2l Regulates Foxg1 Expression to Control Cortical Progenitor Cell Proliferation and Differentiation

Honours. B.Sc. Specialization in Biochemistry 2008
University of Ottawa, Ottawa, Ontario

- *Magna Cum Laude*
- Thesis Supervisor – Dr. Catherine Tsilfidis
- Honours Thesis - Expression Pattern of TAT Fusion Proteins in Retinal Explants

Publications

Yip, D. J., C. P. Corcoran, M. Alvarez-Saavedra, A. DeMaria, S. Rennick, M. A. Rudnicki, C. Messier, and D. J. Picketts, Snf2l regulates Foxg1-dependent progenitor cell expansion in the developing brain. *Developmental Cell*, 2012. 22(4): p. 871-878.

Lagali, P. S., C. P. Corcoran, and D. J. Picketts, Hippocampus development and function: Role of epigenetic factors and implications for cognitive disease. *Clinical Genetics*, 2010. 78(4): p. 321-333.

Work Experience

Co-op Student Jan - Apr 2007
GeminX Biotechnologies Inc., Montreal, Québec

- Used yeast and siRNA screening methods to search for the target of an anti-cancer therapeutic.
- Presented my results to the biology department via multimedia presentations.

Student Researcher

May - Aug 2006, May - Aug 2007

University of Ottawa Eye Institute, Ottawa, Ontario

- Optimized experimental protocols in collaboration with colleagues.
- Analyzed results and evaluated their reproducibility.

Assistant Regulatory Officer

Sep - Dec 2005

Health Canada (Pest Management Regulatory Agency), Ottawa, Ontario

- Screened chemical specification forms of pest control products for errors, missing information, or inconsistencies.
- Communicated with the registrants of products to resolve any issues discovered during the screening through diverse means of communication such as e-mail, fax and telephone.
- Participated in section meetings concerning formulants policy.

Sales Assistant

Sep 2004 - Dec 2006

Mark's Work Wearhouse, Cumberland, Ontario

- Provided resolutions to customer needs by recommending available services and products.
- Maintained the store's flow of transfers and consolidations as well as keeping debits and customer service items up to date during administrative shifts.

Academic Awards**University of Toronto National Biology Competition Scholar**

2003

Admission Scholarship, Faculty of Science, University of Ottawa

2003

Dean's Honour List, Faculty of Science, University of Ottawa

2004-2008

Dr. Goodman Cohen Summer Student Award,

Ottawa Hospital Research Institute

2007

Admission Scholarship, Faculty of Graduate and Postdoctoral Studies,

University of Ottawa

2008

Ontario Graduate Scholarship, Province of Ontario

2009

1st Place Poster Presentation in the M.Sc. Category at OHRI Research Day,

Ottawa Hospital Research Institute

2011