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Cas Family of Adaptor Proteins are Required in Radial Glial Cells for Cortical Lamination

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Dedication

I dedicate my Dissertation work to my family, friends, and my dog, Lacey. It is with genuine gratitude towards my grandparents (Gen and May), parents, (Jimmy and Doris), sister (Shirley), niece (Madeline), and nephew (Aiden) whose encouragements motivated me over the years. Lacey as my best companion and a reminder of endless support. To my best friend, Philip, who first introduced me to research at the Salk Institute. I am forever grateful to have you as a mentor in in life and your friendship knows no bounds. Thank you to my girlfriends (Chithra, Sami, Marissa, Jill, & Sara) for being the best hiking and camping buddies. I absolutely miss our weekly dinner/chats. Alex, you are one of the kindest human being and are so caring for Lacey. Thomas, as my first friend in Riverside, excellent storyteller, confidant, and a wonderful person in all respects. Christopher as an amazing friend through the years.

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ABSTRACT OF THE DISSERTATION

Cas Family of Adaptor Proteins are Required in Radial Glial Cells for Cortical Lamination

by

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Dr. Martin M. Riccomagno, Chairperson

Laminar organization of the cortex requires neurons to exit the cell cycle, locomote, and assemble into six distinct layers. Neurons encounter multiple permissive and repulsive guidance cues simultaneously during cell migration. The molecular mechanism of how these cues are interpreted by migrating cells is not well understood. To gain insight into the mechanisms of signal transduction that participate during cortical migration and lamination, we studied the three embryonically-expressed Cas signaling adaptor proteins (p130Cas, CasL, and Sin) to uncover the signaling events essential cortical development. We hypothesize that the Cas family of proteins mediate adhesion signal transduction during corticogenesis as they are known to mediate integrin-dependent signals at focal adhesion complexes. Here, we provide *in vivo* genetic evidence that Cas proteins serve a functional and redundant role during cortical lamination. *Cas* triple conditional knock-out (*CasTcKO*) mice display severe cortical

phenotypes that resemble cobblestone lissencephaly. The malformation in *CasTcKO* brains include ectopic clusters of neurons in the marginal zone and meninges. Furthermore, all excitatory neuronal subpopulations are disrupted in *CasTcKO* mutants. Defects in neuronal positioning appear to be non-neuronal autonomous, suggesting that *Cas* genes are required in radial glial cells for proper cortical lamination. Disruption to radial glial interactions with the basement membrane resulted in disarrayed radial glial basal processes and ectopic proliferating cells. Molecular epistasis analysis placed dystroglycan, a known regulator of glial-pial interactions, upstream of Cas phosphorylation. Furthermore, this dystroglycan-dependent recruitment of phosphorylated Cas to radial glial endfeet requires β1 integrin signaling. Overall, our data support an essential role for Cas adaptor proteins during cortical lamination by acting downstream of dystroglycan and β1 integrin in radial glial cells.

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Appendix: **Acronyms and Abbreviations**

β1: Beta 1 subunit of integrin

Cas: Crk-associated substrates

CasTcKO: *Cas* Triple Conditional Knockout

CP: cortical plate

DG: Dystroglycan

DG-FL: pcDNA3.1-myc/his-DG-FL (Full length Dystroglycan)

ECM: extracellular matrix

EdU: Ethynyl deoxyuridine

Empty vector: pcDNA3.1-myc/his

ISH: *in situ* hybridization

IZ: intermediate zone

MZ: marginal zone

PBM: pial basement membrane

PP: preplate

pYCas: phosphorylated Cas

RGC(s): radial glial cell(s)

SP: subplate

SVZ: subventricular zone

VZ: ventricular zone

WT: wild-type

Chapter 1: Introduction to Corticogenesis and Cas Proteins

Developmental Events for Cortical Lamination

Development of the mouse neocortex requires a series of well-orchestrated developmental steps to form functional circuits (Marín & Rubenstein, 2003; Silva et al., 2019). Perturbations to key events during neocortical development, including neuronal proliferation, differentiation, or migration, can result in cortical malformations extending to alterations in circuit assembly and/or functionality (Ayala et al., 2007; Di Bella et al., 2021). Detrimental consequences include limitations in executive functions, sensory misdetection, and epileptiform activity (Ayala et al., 2007; Stouffer et al., 2016).

A pluripotent layer of neuroepithelial cells will mature and give rise to radial glial cells (RGCs) that will eventually divide to generate the neurons and glial cells of the cortex (Bella et al., 2021). The cortex is composed of neurons originating from two different proliferative pools (Rakic 1982; Marín & Rubenstein, 2003). Excitatory neurons derived from the dorsal telencephalon use a radial path to migrate toward the pial surface (Caviness & Takahashi, 1995; Kriegstein & Noctor; 2004). The majority of these excitatory neurons will migrate along radial glial fibers until they reach the cortical marginal zone, where they coalesce to form distinct cellular layers. Seminal experiments showed that the birthdate of neurons in the ventricular zone and migration along radial glial cell processes are key factors in the establishment of the cortical layers (Rakic 1972). Advancement in live imaging studies demonstrated radial migration is complex and adhesion signaling plays an important role in regulating these processes in a crucial

temporal sequence (Marín et al., 2010)**.** Inhibitory neurons migrate tangentially across the plane of the glial fibers from the ventral telencephalon (ganglionic eminences) to the cortex and subsequently use radial glia to migrate to their proper laminar position (Hatten 1999; Nadarajah et al., 2003). Regardless of their origin, migrating neurons need to interpret permissive and repulsive guidance cues to undergo specific morphological changes and selectively choose adhesive substrates to translocate (Ayala et al., 2007; Kawauchi, 2011; Gil-Sanz et al., 2013; Hirota & Kazunori, 2017).

The formation of a cortical plate starts with genesis of the preplate (PP). Asymmetrical division of neuroepithelial cells in the ventricular zone (VZ) to generate neural progenitors that form the PP is indeed one of the crucial steps in forming the cortex (Anthony et al., 2004; Miyata, 2004). Cajal-Retzius cells are thought to be the pioneer neurons in the preplate (Espinosa et al., 2009). The PP will eventually divide to form the subplate (SP) and marginal zone (MZ). Expansion of the cortex generates a cortical plate (CP) situated between these two boundaries. Continuous mitotic divisions generate the six cortical layers in an inside first-outside last manner: neurons born first will populate the deep layers, whereas those born last during corticogenesis will reside in the superficial layers (Marín & Rubenstein, 2003). It is of utmost importance the PP splits properly for the developing CP to form, as malformations may arise if this split does not proceed normally (Olson, 2014).

One of the early steps during migration of newborn neurons is the adoption of a multipolar morphology, and locomotion through the subventricular zone (SVZ)

independently from RGC processes (Tabata and Nakajima, 2003). In this region of the cortex, the neuron is actively seeking guidance cues and selectively interacts with the extracellular matrix or other cells (Guan & Rao, 2003). The ability of a migrating cells to properly respond to the correct guidance cues requires progenitors and migrating cells to be in the right place at the right time (Wong et al., 2002; Guan & Rao, 2003; Cayre et al., 2010). Initial positioning is as important as the choice points the cells encounter during the different modes of migration. If positioned at the wrong location, the cells may miss a guidance cue and respond to a different one (Tessier-Lavigne & Goodman, 1996; Marín et al., 2010). Thus, neural cells that are mitotically active, with the exception of Cajal-Retzius cells that makeup layer I or MZ, are usually localized to the VZ/SVZ (Rice & Curran, 2001). These progenitor cells continue cellular divisions and mature to expand the developing cortex.

As mentioned before, the birth date of a neuron is closely correlated with its laminar fate (Marín & Rubenstein, 2003; Ridley et al., 2003). Cells migrate past the previously born neurons to form the layers of the cortex in an inside-out pattern (Kriegstein & Noctor, 2004). Thus, the earliest wave of neuronal migration forms the deep layers of the CP (layers V & VI) and the last wave corresponds to superficial layer neurons (II/III). Early born neurons that will reside in the deep CP utilizes glial-independent cell migration. Since layer II/III neurons need to travel great distances to integrate into the CP, these neurons require assistance from radial glial cells. The radial glial basal process dynamically changes from highly branched to club-like during

development (Yokota et al., 2010) and functions as a scaffold for neuronal migration of superficial layer neurons from the SVZ/VZ to their target position (Marín et al., 2010). Importantly, the aforementioned multipolar neurons in the SVZ actively seek and interact with extracellular environment (Marín et al., 2010). Once the neuron receives the proper signals, these post-miotic cells will adhere onto the radial glial process and convert to a bipolar morphology with the leading process oriented towards the basal lamina. Proper orientation assists in unidirectional migration into the CP by glial-dependent locomotion (Götz et al., 2002; Yokota et al., 2007). Active remodeling of focal adhesions at the lamellipodia and filopodia of the leading process generates traction force to propel the neuron closer to the pial surface (Nadarajah et al., 2003). At the end of glial-guided locomotion, superficial layer neurons detach from the radial glial scaffold by favoring interactions with the ECM over cell-cell interactions to finish radial migration via somal translocation (Götz et al., 2002).

Fate mapping experiments performed to trace the lineage of clonally-related neurons, indicate some markers that are expressed in the SVZ during neurogenesis, are also expressed in postmitotic neurons (Molyneaux et al., 2007; Franco et al., 2012). This is the easiest and most reliable method to correctly identify changes to laminar organization, rather than sorting each cell based on morphological features and functions that could have been altered. Laminar- and subtype-specific markers can then be used to label the diverse neuronal populations and characterize their positioning in mouse models for neurodevelopmental disorders. This type of approach can be useful

to investigate whether cortical lamination is disrupted and which subpopulations contribute to the malformation.

Migration defects that disrupt cortical lamination and wiring can lead to severe neurodevelopmental disorders. Hallmark characteristics of Cobblestone Lissencephaly (Type II Lissencephaly) include aggregate of cells in the subarachnoid space, breakage in the basal lamina, and cognitive impairments (Devisme et al., 2012). Defects in the signaling interactions between the embryonic pial basement membrane (PBM) and radial glial (RG) processes, rather than neurons are responsible for these pathologies (Hu et al., 2007). Dysregulation of signals that facilitate this pial-glial interaction can cause breaches in the basement membrane and create a permissive environment for overmigration (Myshrall et al., 2012). In regions where PBM-RG interactions are compromised, the discontinuity causes the formation of leptomeningeal heterotopia. This indicates the glial limitans is essential for corticogenesis, and severe cortical malformations can arise from disruptions to this dynamic boundary (Halfter et al., 2002; Hu et al., 2007).

Glycoproteins such as laminin in the ECM are known to bind to dystroglycan and integrin transmembrane receptors expressed on radial glial endfeet (Moore et al., 2002; Haubst et al., 2006). Laminin binds to the alpha subunit of dystroglycan (α-DG) in the ECM to glycosylate the dystrophin glycoprotein complex (Yoshida et al., 2001; Michele et al., 2002; Kim et al., 2004; Schachter et al., 2004). Extensive glycosylation is essential for α-DG signaling, and mutations in the laminin-binding domain are linked to

cobblestone malformations (Hewitt, 2009). Interference in PBM-RG signaling mediated by integrins and DG result in pathology that extends beyond neuronal overmigration and laminar disorganization (Myshrall et al., 2012). A multitude of disruptions occur, including ectopic progenitor proliferation, retraction of the endfeet, radial glial fibers extending into heterotopias, and sporadic distribution of Cajal-Retzius cells in the marginal zone are common defects observed in animals missing different integrin subunits, or with disrupted DG glycosylation (Georges-Labouesse et al., 1998; Halfter et al., 2002; Beggs et al., 2003; Niewmierzycka et al., 2005; Hu et al., 2007).

The complex pial basement membrane-radial glial cells-neuronal interactions involve distinct molecular machineries at choice points to organize the cortex (Halfter et al., 2002; Amin & Borrell, 2020). The adaptor proteins Crk and CrkL (Crk-like) have been shown to be essential for these processes, and function downstream of Reelin/Dab1 during cortical migration (Park and Curran, 2008). They have been implicated in diverse cellular events including cell migration and adhesion (Cho & Klemke, 2000; Feller, 2001; Huang et al., 2015). Conditional deletion of Crk and CrkL (Park and Curran, 2008) indicate the adaptor proteins share the same major anatomical features of failed cell migration, absence of preplate splitting, and disruption to lamination as *Reeler* mutants (Miao et al., 1994; D'Arcangelo et al., 1995). This raises the possibility that ablation of Crk and CrkL binding partners may result in similar morphological defects.

Cas Proteins as Potential Regulators of Cell-ECM and Cell-Cell Adhesion

The Crk-associated substrate (Cas) proteins are a family of cytoplasmic adaptors that interact with Crk, as their name implies. The Cas family of adaptor proteins is of interest due to their broad embryonic expression in the mammalian nervous system (Merrill et al., 2004) and dynamic regulation of their phosphorylation states to mediate adhesion preferences for cell-cell or cell-extracellular matrix (ECM) interactions (Klemke et al., 1998; Chodniewicz et al., 2004). The Cas signaling adapter proteins are evolutionarily conserved across vertebrates and invertebrates (Huang et al., 2007; Riccomagno & Sun et al., 2014; Vahedi-Hunter et al., 2019). A single Cas paralog, DCas, is found in *Drosophila melanogaster* (Huang et al., 2017). In mammals, the Cas paralogs consist of a family of four proteins: three embryonically-expressed proteins include p130Cas/BCAR1, CasL/NEDD9/HEF1, and Sin/EFS and one adult variant, HEPL (HEF1-Efs-p130Cas-like).

p130Cas, CasL, and Sin have evolutionarily conserved structural domains that allow interactions with the same binding partners (Harte, et al., 1996; Bouton & Parsons, 1996; Klemke et al., 1998; Bouton et al., 2001; Birge et al., 2009). The structural domains are relatively conserved across the vertebral paralogs, highlighting the importance of the Src-homology and substrate domains to interact with focal adhesion kinase (FAK) and Src, respectively (Alexandropoulos & Baltimore, 1996; Astier et al., 1997). A fourth member, HEPL is known as a pseudo-member of the Cas family and its function and expression has been briefly explored in adults and was only discovered

through an in silico screen using the *Cas* mRNAs (Singh et al., 2008). Due to the similarity in substrate domains and overlap in embryonic expression during neural development in rodents (Merrill et al., 2004), p130Cas, CasL, and Sin could play redundant roles during neural development (Nasertorabi et al., 2004; Meenderink et al., 2010).

Cas proteins mediate integrin-dependent signals at focal adhesions, where migrating neurons dynamically remodel the lamellipodia found at the leading process by forming and dissolving these complexes (Calalb et al., 1995; O'Neill et al., 2000; Yeo & Song, 2008). Extracellular matrix substrates bind to integrin alpha and beta subunits to transduce Focal Adhesion Kinase (FAK) to phosphorylate proline sites on the Cas SH3 domains and recruit Src to tyrosine phosphorylate residues on the Cas SH2 domains. Once the 15 tyrosine motifs (15 YxxP) are phosphorylated, Cas recruits Crk and DOCK-180 to activate Rac to promote actin cytoskeleton remodeling (Calalb et al., 1995; Klemke et al., 1998; Yeo & Song, 2008; Birge et al., 2009). Importantly, actin polymerization plays a role in cell migration (Di Stefano et al., 2011). In addition to migration, Cas proteins have been shown to regulate attachment-dependent survival signaling and cell motility and invasion in breast cancer models (Cabodi et al., 2006; Kong et al., 2011). These functions reflect the ability of the Cas proteins to interact with multiple binding partners on their substrate domains to act as scaffolding proteins in functional complexes.

p130Cas is known as the canonical Cas family member, given its extensive study in vitro (Burnham et al., 1996; Honda et al., 1999; Cho & Klemke, 2000; Bouton et al.,

2001; Defilippi et al., 2006). Also known as BCAR1 (Breast cancer anti-estrogen resistance protein 1), elevated levels enhanced cell proliferation (Cabodi et al., 2006). Despite the crucial *in vitro* analyses to determine the function of Cas proteins, little is known about their role during neural development *in vivo.* The first suggestion of their relevance for neuronal development came from studies done in chicken and Drosophila melanogaster. p130Cas was proposed to be important for commissural axon guidance in chicken (Liu et al., 2007), whereas *DCas* is required for axon fasciculation and pathfinding of PNS axons (Huang et al., 2007; Liu et al., 2007). In the absence of DCas or p130Cas, axons were led astray, suggesting their inability to interact with signaling cues in the ECM. Often, these guidance cues can be the same molecular players involved in neuronal migration (Tessier-Lavigne & Goodman, 1996; Marín et al., 2010).

More recently, Cas proteins have been shown to be essential in mice for cell positioning in the retina (Riccomagno & Sun et al., 2014) and sensory axon guidance in the spinal cord (Vahedi-Hunter et al., 2019). Compelling *in vivo* evidence from Riccomagno and Sun and colleagues (2014) utilized a novel approach to examine the functional requirement of p130Cas, CasL, and Sin in the developing mouse retina. *p130Cas*$^{-/-}$ mice die early in embryogenesis between E11.5 to E12.5 (Honda et al., 1998), but animals with single knockout of *CasL* (Seo et al., 2005) or *Sin* (Donlin et al., 2005) are viable. To bypass the embryonic lethality from cardiovascular maldevelopment when ablating both p130Cas alleles (Peng et al., 2006), Riccomagno & Sun generated a conditional allele for p130Cas (*p130Cas*^{fl}). The conditional genetic

approach to ablate p130Cas on a double knockout of *CasL* and *Sin* background, resulted in the *Cas* Triple Conditional Knockout (*p130Cas*$^{fl/fl}$; *CasL*$^{-/-}$; *Sin*$^{-/-}$: herein *CasTcKO*) mouse that can be used to remove *Cas* gene function in a tissue and developmental stage-specific manner (Riccomagno & Sun et al., 2014). Using this tool, Riccomagno & Sun et al. showed that Cas adaptor proteins play a cell-autonomous role to mediate integrin signaling during retinal ganglion cell organization. Genetic ablation of these three *Cas* mammalian genes resulted in ectopic cell clusters and abnormal positioning of retinal ganglion cells. Cas proteins appear to act downstream of integrin signaling as ablation of the β1 Integrin led to a decrease in Cas tyrosine phosphorylation. Furthermore, β1 Integrin mutant phenotypes appear similar to *CasTcKOs* (Riccomagno & Sun et al., 2014). Similar to the retina, the neocortex is a laminated structure that requires proper stratification and neuronal positioning to form the six cortical layers and functional neural circuits.

Cas genes were also shown to be required in dorsal root ganglion neurons to regulate axon fasciculation (Vahedi-Hunter et al., 2019). The defects observed in *Cas* KOs stem from axons being unable to distinguish between secreted adhesion molecules in the ECM (i.e. laminin) and cell-cell adhesion molecules (Vahedi-Hunter et al., 2019). The migration of cortical neurons requires the same switch in adhesive preferences as demonstrated in DRG axon fasciculation. Taken together, these findings raise the exciting possibility of Cas proteins playing an essential role during cortical circuit formation. The current study will examine this possibility.

Chapter 2: Materials & Methods

Animals Use

Animal use in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines at the University of California, Riverside (UCR). Mice were housed in a controlled environment (22C, 12 Hrs. light/12 Hrs. dark cycle) and mouse chow and water provided *ad libitum*. Vaginal plug observed is Embryonic day 0.5 (E0.5) and the day of birth is designated as Postnatal day 0 (P0). Weaning age at P21 and breeders were paired after 8 weeks of age. Phenotypic analysis was conducted on at least three different offspring from different litters and breeders, without regard to sex of animals.

Wild-type & Transgenic Mice

ICR (Taconic Biosciences): Wild-type animal

p130Cas-Bac^{GFP} (Riccomagno & Sun et al., 2014): GFP expression under the endogenous promoter for p130Cas using Bacterial Artificial Chromosome (BAC; Gong et al., 2003)

Ai14/R26$^{LSL\text{-}TdTomato}$ (JAX 007914)

β1 integrin^{fl} (Raghavan et al., 2000; JAX 004605)

Emx1-Cre (Gorski et al., 2002; JAX 005628): *Emx1* is weakly expressed in the testes, thus *Cre* is inherited from the female, in the presence of a conditional allele.

Nex-Cre (Goebbels et al., 2006)

Mutant and Control Littermates

Mutant animals for *CasTcKO* are *Emx1Cre;CasTcKO* (*Emx1-Cre; p130Cas$^{fl/\Delta}$; CasL$^{-/-}$; Sin $^{-/-}$*) and *NexCre;CasTcKO* (*Nex-Cre; p130Cas $^{fl/\Delta}$; CasL$^{-/-}$; Sin $^{-/-}$*). Control animals are Cre- and *p130Cas$^{fl/+}$*; *CasL$^{-/-}$*; *Sin$^{-/-}$* littermates. Mutant animals for *β1 integrin* are *Emx1Cre;B1* (*Emx1-Cre;β1 integrin$^{fl/fl}$*) and *Nex-Cre;B1* (*NexCre;β1 integrin$^{fl/fl}$*). Control littermates are *Cre- and β1 integrin$^{fl/fl}$*. Mutant animals for *Dystroglycan* are *Emx1Cre;DG$^{fl/-}$* (*Emx1-Cre;DG*). Control littermates are *Cre+ and DG$^{fl/+}$*. All crosses were maintained on a C57/Bl6J background.

Generation of *CasTcKO* Mice

p130Cas$^{-/-}$ mice die early in embryogenesis (Honda et al., 1998), thus a conditional allele (*p130Cas^{fl}*) was generated by Riccomagno & Sun et al. (2014). *p130Cas* was ablated in a *CasL$^{-/-}$* (Seo et al., 2005); *Sin$^{-/-}$* (Donlin et al., 2005) double null mutant genetic background (*p130Cas$^{fl/\Delta}$*; *CasL$^{-/-}$*; *Sin$^{-/-}$*) to generate the *Cas* Triple Conditional Knockout (*CasTcKO*; Riccomagno & Sun et al., 2014) animal. According to Seo et al. (2005) the C57/Bl6J library was screened with a 300 BP CasL probe that included the SH3 region, where the enhanced GFP (pEGFP; Clontech) was directly combined with the CasL genome within exon 2. Mice deficient in Sin expression using homologous-recombination-mediated gene targeting at first exon, first intron, and a short portion of the second exon (Donlin et al., 2005). *Cre+; p130Cas$^{fl/\Delta}$*; *CasL$^{-/-}$*; *Sin$^{-/-}$* female breeders were crossed to *p130Cas$^{fl/fl}$*; *CasL$^{-/-}$*; *Sin$^{-/-}$* males to generate *Emx1-Cre; p130Cas$^{fl/\Delta}$*;

CasL$^{-/-}$; *Sin*$^{-/-}$ (*Emx1Cre;CasTcKO)* and *Nex-Cre; p130Cas*$^{fl/\Delta}$; *CasL*$^{-/-}$; *Sin*$^{-/-}$ (*NexCre;CasTcKO)* mutant mice and *Cre-; p130Cas*$^{fl/+}$; *CasL*$^{-/-}$; *Sin*$^{-/-}$ littermate controls.

Generation of *β1 integrin* Mice

Cre+;β1 integrin$^{fl/+}$ females mated with *β1 integrin*$^{fl/fl}$ males to generate *Emx1-Cre;β1 integrin*$^{fl/fl}$ (*Emx1Cre;B1*) and *Nex-Cre;β1 integrin*$^{fl/fl}$ *(NexCre;B1)* mutant mice and *Cre-;β1 integrin*$^{fl/fl}$ littermate controls.

Generation of *Dystroglycan* Mice by Kevin Wright (OHSU)

Dystroglycan conditional animal (DG^{fl}; JAX 009652). To generate Dystroglycan conditional knockout animals, *Emx1-Cre+;Dystroglycan*$^{+/-}$ male breeders were crossed to *Dystroglycan*$^{fl/fl}$ females to generate *Emx1-Cre+*;*Dystroglycan*$^{fl/-}$ (*Emx1Cre;DG*) mutants and *Emx1-Cre+;Dystroglycan*$^{fl/+}$control littermates (Lindenmaier et al., 2019).

PCR Primer Sequences:

p130Cas (Riccomagno & Sun et al., 2014):

Loxp5BcarF: 5'- CAA GTT CTA GGA TAG CCA AGG -3'

Loxp5BcarR: 5'- TCA TCT ACT AGG CTG CCA ATG -3'

BcarDeltaR: 5'- CCA CAG GCT TTA TGT TCA CAT C -3'

Delta: 480 BP; Flox: 300 BP; WT: 192 BP

Emx1-Cre (Gorski et al., 2002; JAX 005628) (Primers designed from sequencing results):

F2 (exon 3): 5'-CAT ATC AAC CGG TGG CGC AT-3'

R1 (pGEM): 5'-TCC GGA TCC AAG CTT ATC GA-3'

Cre: 238 BP

Nex-Cre (Goebbels et al., 2006):

Primer No. 4: 5'-GAG TCC TGG AAT CAG TCT TTT TC-3'

Primer No. 5: 5'-AGA ATG TGG AGT AGG GTG AC-3'

Primer No. 6: 5'-CCG CAT AAC CAG TGA AAC AG-3'

WT: 770 BP; Cre: 525 BP

β1 integrin^{fl} (Raghavan et al., 2000; JAX 004605)

olMR1906 F: 5'- CGG CTC AAA GCA GAG TGT CAG TC -3'

olMR1907 R: 5'- CCA CAA CTT TCC CAG TTA GCT CTC -3'

Flox: 280 BP; WT: 160 BP

Cardiac Perfusion & Sample Preservation

Cardiac perfusion with 1 x PBS and 4% paraformaldehyde (PFA), 4% PFA post-fix for 2 Hrs., 4 Hrs., or overnight at 4C, washes with 1 x PBS. Tissue placed in 30% sucrose in 1 x PBS overnight and embedded in OCT for sectioning coronally at 20 um thickness on the

cryostat or stored in 1 x PBS and embedded in 3% agarose for coronal sectioning at 100-150 um thickness on a vibrating microtome (VT1000S; Leica).

Microscopes

Laser-scanning Leica SP8 DMI8; Leica Fluorescent Scope

RNAscope

Fluorescent *in situ* hybridization assay for detection of target RNA using specific probes for signal amplification and background suppression for single-molecule visualization (Wang et al., 2012). *BCAR1*, *NEDD9*, and *EFS* probe generated by Acdbio for sequences deleted in *CasTcKO* animals.

***In situ* Hybridization**

In situ hybridization was performed on wholemount embryos at E12.5 using digoxigenin-labeled cRNA probes as previously described (Giger et al., 2000) with cRNA probes generated from cDNA templates using primers from Riccomagno & Sun et al. (2014):

BCAR1

F: 5'- GGG TCT AGA ATC TAC CAA GTT CCT CCA TCT CTG -3'

R: 5'- GGG GAA TTC ACA CCA TCG TCA ACT ACA CTC CC -3'

NEDD9

F: 5'- GGG TCT AGA ACC GCG GTG GAC AAA GTA GAG C -3'

R: 5’- GGG GAA TTC AGA GGG CGT CGA TGG CGT TGA G -3’

EFS

F: 5’- GGG TCT AGA CGT GGC AGA GGT CTA TGA TGT G -3’

R: 5’- GGG GAA TTC GCT GGA TCA TTG GCT ACC TCC C -3’

Immunostains

DAPI (1:500 at 0.5 mg/ml); Nissl (1:200; Invitrogen BFP405)

Immunohistochemistry on cryosections and microtome sections were performed as described (Polleux and Ghosh 2002). Immunocytochemistry on primary neurons and radial glial cells were performed as described (Polleux and Ghosh 2002). Coverslips were blocked in RT in blocking buffer: 0.1% PBST and 10% goat serum. Antibody dilution buffer: 0.1% PBST and 1% goat serum. Primary antibodies incubated at 4C overnight and secondary antibody incubation at RT for 1 Hr. Washes with 1 x PBS with 0.03% NaN3. Mounted with Fluorogel.

Primary Antibodies

Anti-p130Cas c-20 (rabbit 1:250; SCBT sc-17 discontinued); Anti-Nedd9 (rabbit 1:250; HPA038768); Anti-Tyr165 (rabbit 1:250; CST #4015); Anti-Nestin (chicken 1:500; Aves Nes); Anti-Laminin (rabbit 1:500; Sigma L9393); Anti-Cux1/CDP (rabbit 1:500; SCBT sc-13024 discontinued; rabbit 1:250; ProteinTech 11733-1-AP); Anti-Ctip2 (rat 1:500; Abcam ab18465); Anti-Tbr1 (rabbit 1:500; Abcam ab31940); Anti-Rorβ (rabbit 1:250;

Proteintech 17635-1-AP); Anti-Calretinin (rabbit 1:500; Swant 7697); Anti-Calbindin (rabbit 1:500; Swant CB38); Anti-MAP2 (chicken 1:500; Abcam); Anti-CSPG (rabbit 1:1000; Sigma AB5320); Anti-Blbp (rabbit 1:500; Abcam ab32423); Anti-β3-Tubulin (mouse 1:500 CST mAB #4466); Anti-GFAP (rat 1:500; Invitrogen #13-0300); Anti-c-Myc 9E10 (mouse 1:1000; Invitrogen #13-2500)

Secondary Antibodies

Invitrogen Goat anti-chicken IgY, goat anti-rat IgG, goat anti-rabbit IgG, & goat anti-mouse IgG (H+L), Alexa Fluor 488, 546, 647 at 1:1000.

Ethynyl deoxyuridine (EdU) Labeling

500 mg EdU reconstituted in 2 ml of DMSO to 250 mg/ml concentration stock. Stock diluted to 15 mg/ml with ddH20 at time of injection, store in -20C. Intraperitoneal injection at 150 mg/kg. Collection 0.5 Hrs. or 7 days after administration. Postnatal animals were perfused and fixed for 2 Hrs. in 4% PFA, dehydrated in 30% sucrose in 1 x PBS overnight, then cryopreserved with OCT. Cryosections stained according to instructions in Invitrogen EdU Click-It Assay Kit: Alexa Fluor 488. Immunohistochemistry proceeded after EdU Click-It Assay.

Expression Constructs

pcDNA3.1-myc/his, pcDNA 3.1-myc/his-DG-FL

Glass Coverslips for Primary Culture

Circular coverslips for 24-well plates were treated with 12 M HCL overnight and neutralized with deionized water. Acid-treated coverslips were stored in a glass petri dish of 70-100% Ethanol. The coverslips were firepolished into a 24-well plate and coated with laminin and poly-D-lysine/poly-L-lysine (8.3 ug/ml; Polleux & Ghosh 2002) overnight in a tissue culture incubator at 37C in 5% CO2. The coating media was aspirated and air-dried in an airflow cabinet prior to plating cells.

Primary Mixed Radial Glial Cultures

Primary mixed radial glial cultures from timed pregnant mouse at E13.5. Embryos were dissected as previously described (Kim & Magrané, 2011) using Complete Hank's Balanced Salt Solution (Complete HBBS; 10x HBSS, 2.5mM HEPES pH 7.4, 2.5 mM D-glucose, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2.5 mM $NaHCO_3$; Polleux & Ghosh 2002). Radial glial cells were dissociated as described (Kim & Magrané 2011), using 0.25% Trypsin-EDTA, Horse Serum, DNAse I, and Radial Glial Media (Modified DMEM/Eagle's Media, 100x Penicillin/Streptomycin, Glutamax, and D-glucose; modified from Culican et al., 1990). Dissociated cells were plated in a 24-well plate at a density of ~1 x 10^5 cells/well with 500 ul of cell suspension and Radial Glia Media. One-third of the media was replaced daily. Wells were transfected two days in vitro (2DIV) and collected 48 Hrs. after transfection. The coverslips were fixed in 4% PFA for 10 minutes at RT and washed with 1 x PBS with 0.03% NaN3.

Cell Transfection

Transfection agent: Metafectene

Per well in a 24-well plate:

Solution A: 48 ul Opti-MEM + 2ul Metafectene (2 ul transfection agent per 1 ug cDNA)

Solution B: 49 ul Opti-MEM + 0.5 ul cDNA #1 [1 ug/ul] + 0.5 ul cDNA #2 [1 ug/ul]

Mix Solution B into Solution A, rest RT 30 mins., pipette (100 ul/well) droplets onto coverslips while swirling plate, triplicates per condition.

Quantification of Layer Marker/EdU & Statistical Analysis

Graph values are mean ± standard error of mean. n=3 independent samples per group, 3 sections per sample. Cell counts were counted using RapID Cell Counter (Sekar et al., 2021). Ten equal-width bins to sort positioning of cells. For layer markers (Cux1, Ctip2, Rorβ, Tbr1) and EdU, bin 1 is the marginal zone (MZ) and bin 10 is ventral to layer VI. Proportion of cells per bin = number of cells labeled divided by total number of labeled cells. Mann-Whitney *U* test was performed with Bonferroni correction (*p<0.005 two-tailed test).

***Emx1Cre;CasTcKO* Layer Markers Results: Cux1 Graph 1.1** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 $U = 19.5$, p= 0.063; bin 2 $U = 18.5$, p= 0.05; bin 3 $U = 15.5$, p= 0.024; bin 4 $U = 38.5$, p= 0.863; bin 5 $U = 4.5$, p= 0.0; bin 6 $U = 7.5$, p= 0.002; bin 7 $U = 21.5$, p= 0.094; bin 8 $U = 31.5$, p= 0.436; bin 9 $U = 29.5$, p= 0.34; bin 10 $U = 30.5$, p=

0.387). **Ctip2 Graph 1.2** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 *U* = 5, p= 0.001; bin 2 *U* = 8, p= 0.003; bin 3 *U* = 13, p= 0.014; bin 4 *U* = 24, p= 0.161; bin 5 *U* = 3, p= 0.0; bin 6 *U* = 11, p= 0.008; bin 7 *U* = 22, p= 0.113; bin 8 *U* = 28, p= 0.297; bin 9 *U* = 11, p= 0.008; bin 10 *U* = 24, p= 0.161). **Rorβ Graph 1.3** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 *U* = 20, p= 0.077; bin 2 *U* = 2, p= 0.0; bin 3 *U* = 17, p= 0.04; bin 4 *U* = 1, p= 0.0; bin 5 *U* = 31, p= 0.436; bin 6 *U* = 15, p= 0.024; bin 7 *U* = 4, p= 0.0; bin 8 *U* = 4.5, p= 0.0; bin 9 *U* = 40, p= 1; bin 10 *U* = 36, p= 0.736). **Tbr1 Graph 1.4** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 *U* = 0, p= 0.0; bin 2 *U* = 0, p= 0.0; bin 3 *U* = 0, p= 0.0; bin 4 *U* = 6, p= 0.01; bin 5 *U* = 15, p= 0.024; bin 6 *U* = 39, p= 0.931; bin 7 *U* = 22, p= 0.113; bin 8 *U* = 12, p= 0.011; bin 9 *U* = 2, p= 0.8; bin 10 *U* = 20.5, p= 0.077).

Emx1Cre;CasTcKO **EdU Results: EdU E12.5->P0 Graph 2.1** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 *U* = 29, p= 0.34; bin 2 *U* = 10, p= 0.006; bin 3 *U* = 16, p= 0.031; bin 4 *U* = 30.5, p= 0.387; bin 5 *U* = 18, p= 0.05; bin 6 *U* = 38, p= 0.863; bin 7 *U* = 38, p= 0.863; bin 8 *U* = 24, p= 0.190; bin 9 *U* = 33, p= 0.546; bin 10 *U* = 33, p= 0.546). **EdU E15.5->P3 Graph 2.2** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 *U* = 15, p= 0.024; bin 2 *U* = 21, p= 0.094; bin 3 *U* = 30, p= 0.387; bin 4 *U* = 8, p= 0.003; bin 5 *U* = 7, p= 0.002; bin 6 *U* = 10, p= 0.006; bin 7 *U* = 35, p= 0.666; bin 8 *U* = 25, p= 0.190; bin 9 *U* = 15, p= 0.024; bin 10 *U* = 23, p= 0.136).

***NexCre;CasTcKO* Layer Markers Results: Cux1 Graph 3.1** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 $U = 18$, p= 0.05; bin 2 $U = 39$, p= 0.931; bin 3 $U = 33$, p= 0.546; bin 4 $U = 27$, p= 0.258; bin 5 $U = 39$, p= 0.931; bin 6 $U = 30$, p= 0.387; bin 7 $U = 32$, p= 0.489; bin 8 $U = 30$, p= 0.387; bin 9 $U = 40$, p= 1.0; bin 10 $U = 35$, p= 0.666). **Ctip2 Graph 3.2** (Mann-Whitney *U* test; $n_1 = n_2 = 9$; two-tailed test; bin 1 $U = 33$, p= 0.546; bin 2 $U = 22$, p= 0.113; bin 3 $U = 36$, p= 0.73; bin 4 $U = 35$, p= 0.666; bin 5 $U = 31$, p= 0.436; bin 6 $U = 30$, p= 0.387; bin 7 $U = 37.5$, p= 0.796; bin 8 $U = 23.5$, p= 0.136; bin 9 $U = 7$, p= 0.005; bin 10 $U = 9$, p= 0.005).

Mean Fluorescent Intensity & Statistical Analysis

ImageJ to measure mean gray value of proteins expressed in radial glial endfeet. Traced cells using Nestin stain to determine the borders for the radial glial endfeet. ImageJ Analyze -> Set Measurements (Area, Min & max gray value, Integrated density, Mean gray value)-> Measure mean gray value for Nestin, pYCas, and myc channels. n=2 independent samples per condition, 18 cells per sample. Tested dataset for normality using the Shapiro-Wilk test and QQ plot. Two-tailed independent samples t-test, *p<0.025, **p<0.005, ***p<0.0005. Mann-Whitney *U* test was performed on dataset that did not meet the normality criterion. **WT RGC Graph 4.1** (Mann-Whitney *U* test, $U = 86$, $n_1 = n_2 = 18$; p= 0.016 two-tailed test).

Chapter 3: A Role for Cas Adaptor Proteins in Cortical Lamination

The Cas (Crk-associated substrates) family of adaptor proteins includes three known embryonically-expressed proteins and one pseudogene expressed postnatally (Feller 2001; Deneka et al., 2015). In the interest of observing Cas functions in early events, the main focus will examine the requirement for p130Cas/BCAR1, CasL/NEDD9, and Sin/EFS during cortical development. To visualize mRNA expression, we started by performing whole-mount *in-situ* hybridization (ISH) in mouse embryos at E12.5 (embryonic day 12.5). All antisense *Cas* probes show strongest signal in the dorsal telencephalon (Fig. 1A). *BCAR1/p130Cas* transcripts also encompass the ventral telencephalon and parts of the diencephalon. *p130Cas* transcripts are more broadly expressed than *NEDD9/CasL* and *EFS/Sin* in the neocortex. Expression of the *Cas* antisense probes in the developing cortex at this timepoint, suggest a possible role for Cas genes during cortical development.

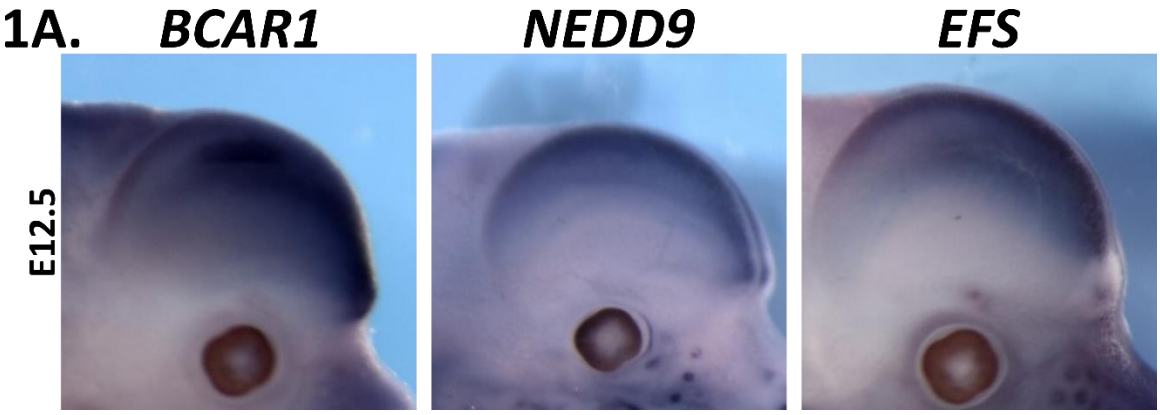


Figure 1A. Whole-mount ISH of Cas probes at E12.5 show BCAR1, NEDD9, and EFS expression in the dorsal telencephalon. BCAR1 is also broadly detected in the diencephalon.

This broad expression of Cas family members in the early telencephalon is intriguing, but examination of their specific localization within the cortex is necessary if we are to understand their potential involvement in cortical developmental processes. To better visualize mRNA expression, we took advantage of a multiplex detection method (RNAscope) to identify multiple target genes on the same histological section (Wang et al., 2012). By observing fluorescent readouts for single RNA molecules, the regional overlap of *Cas* gene expression can be determined. With high sensitivity and specificity, each Z probe amplified *BCAR1*, *NEDD9*, and *EFS* signal with little background noise (Fig. 1B-C). Examination of the RNAscope probes in wild-type (WT) animals at E12.5 (Fig. 1B) indicated *BCAR1* transcripts are strongly concentrated in the developing cortical plate (CP) with weaker expression in other areas of the neocortex. On the same embryonic section, *NEDD9* transcripts are strongest in the cortical hem (midline) and fainter, but distinctly expressed on the dorsolateral neocortex. In comparison to *BCAR1*, *NEDD9* transcripts are less prominent in the CP and are rather found evenly distributed in the intermediate zone (IZ), subventricular zone (SVZ), and ventricular zone (VZ). Based on transcript expression localization, *BCAR1* puncta are primarily found in postmitotic migrating neuron, while both *BCAR1* and *NEDD9* are expressed in the proliferative pool of cells at the SVZ and VZ. *EFS* is undetectable at this stage as indicated by the absence of signal on coronal sections of the cerebral hemispheres, even at higher magnification.

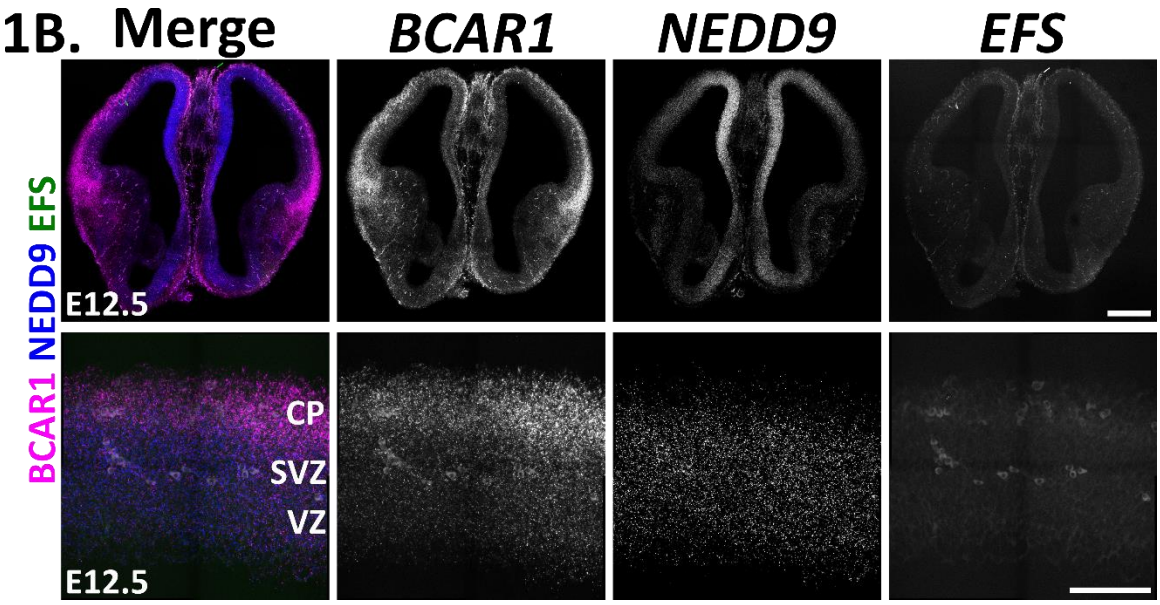


Figure 1B. mRNA transcripts for BCAR1, NEDD9, and EFS at E12.5 using RNAscope. BCAR1 is concentrated in the CP, NEDD9 is evenly distributed in the neocortex, and no signal detected for EFS. Scale bar of whole hemispheres is 500 um and high magnification is 100 um.

Cas gene expression at E14.5 (Fig. 1C) appears somewhat different from their expression at E12.5 (Fig. 1B) in WT animals. Noticeably, *BCAR1* transcripts are now strongest at the IZ, instead of the CP. Across the two timepoints, *BCAR1* and *NEDD9 expression* remained consistent with broad expression in the SVZ and VZ. *NEDD9* transcripts are also found weakly in the IZ. The previously undetectable mRNA transcripts for *EFS* are now localized in the SVZ and VZ. Overlap of *BCAR1, NEDD9,* and *EFS* transcripts in the proliferative zones indicate a possible function for these genes during cortical neurogenesis.

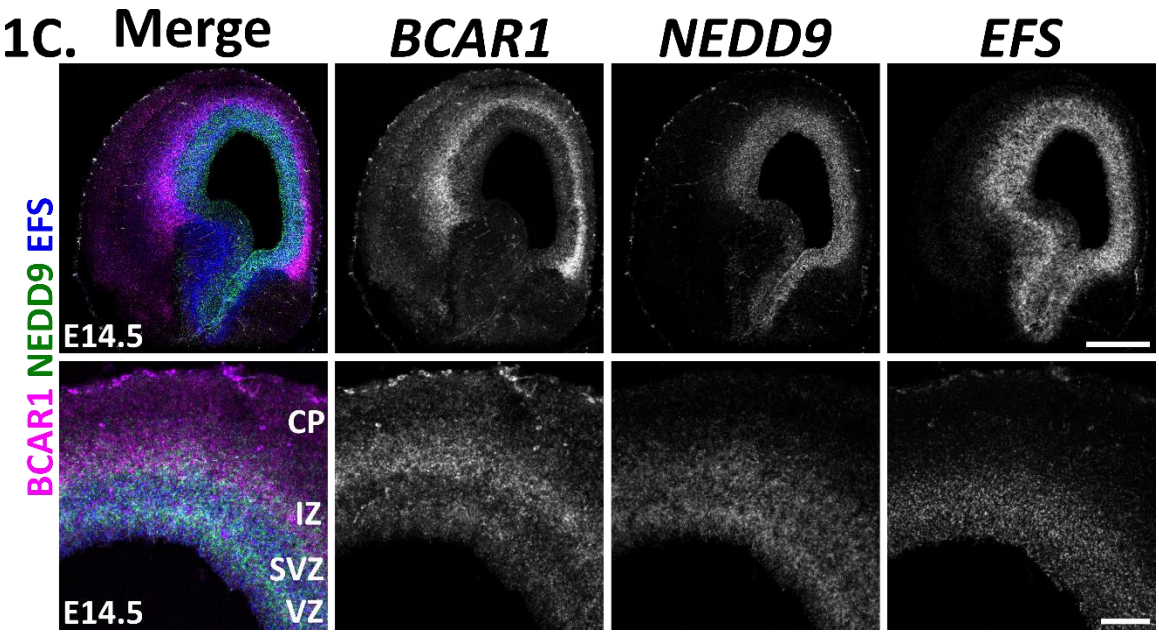


Figure 1C. mRNA transcripts for BCAR1, NEDD9, and EFS at E14.5 using RNAscope. BCAR1 is concentrated in the IZ with some expression in the CP. NEDD9 is also expressed in the IZ. All Cas members are detected in the SVZ and VZ. Scale bar of whole hemispheres is 500 um and high magnification is 100 um.

Given the broad *Cas* mRNA transcript expression in the cortex at E12.5 and E14.5, we performed complementary analysis of protein expression by immunohistochemistry. WT coronal sections at E14.5, E16.5, and P0 (postnatal day 0) were immunostained for BCAR1/p130Cas and Nedd9/CasL (Fig. 1D); there is no commercial antibody available to detect Efs/Sin. p130Cas and NEDD9 protein expression at E14.5 is visible in the CP, IZ, VZ, and pial surface. Protein expression in the CP and IZ is consistent with RNAscope results (Fig. 1C). Interestingly, p130Cas is abundantly expressed in the subplate (SP) and thalamocortical afferents at E16.5. Both p130Cas and NEDD9 are detected in the upper CP and IZ at this timepoint. Cortices are seemingly indistinguishable at P0, where Cas proteins are broadly expressed in axons and the lower CP. p130Cas and Nedd9 proteins are abundant in layer IV, potentially due to

neuronal targets for sensory thalamocortical projections. Robust Cas protein expression in the CP and proliferative regions indicate the adaptor proteins may be involved in many developmental processes.

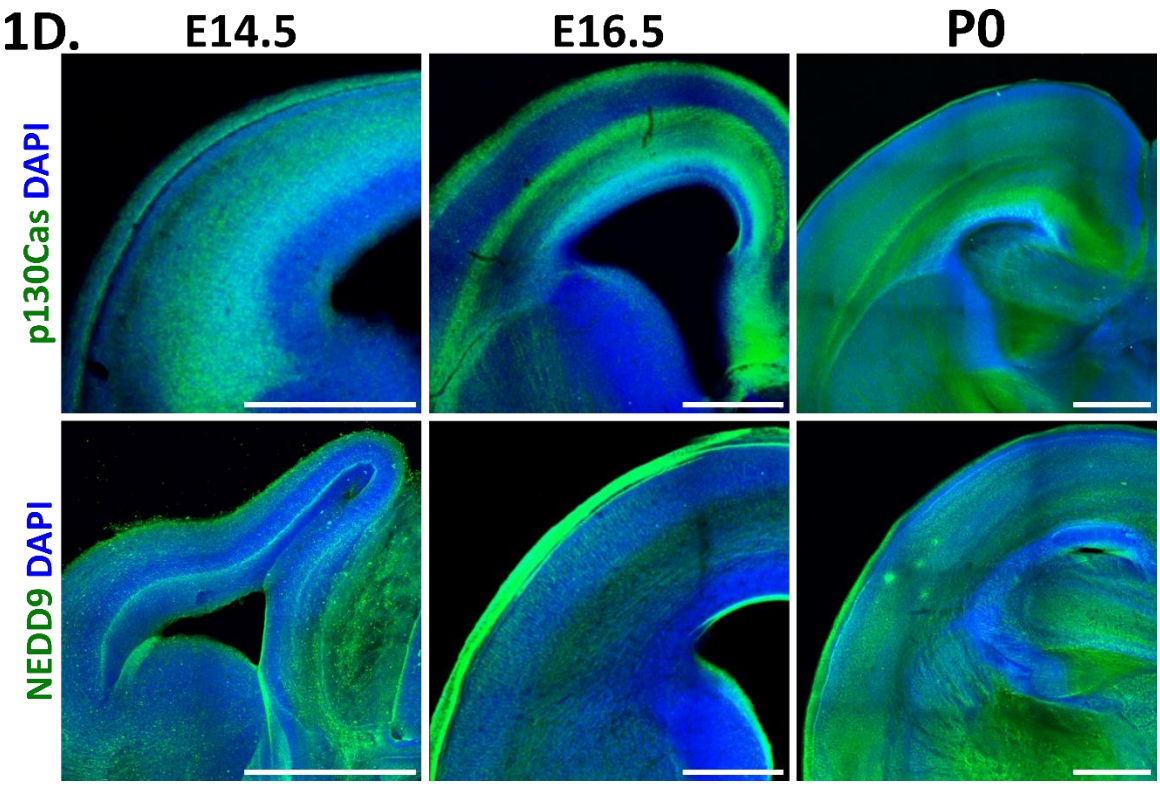


Figure 1D. Analysis of p130Cas and Nedd9 protein expression at E14.5, E16.5, and P0. Expression in the CP and IZ are consistent with RNAscope results. P130Cas is also detected in the subplate and thalamocortical afferents. Scale bar is 500 um.

To complement this expression analysis, we took advantage of a GENSAT bacterial artificial chromosome (BAC) transgenic mouse line that expresses stable EGFP under the native regulatory sequences for *BCAR1* (Gong et al., 2003; Heintz, 2004), and allows for detection of cells expressing p130Cas (Fig. 1E). The transgenic EGFP expression is consistent with endogenous p130Cas expression using immunohistochemistry in WT animals (Fig. 1D). Rostral to caudal coronal sections

indicate EGFP is detected throughout the cortical plate at E16.5 and display broad expression in the cortex. EGFP is visible strongly in the IZ where axons reside and throughout the neocortex, in areas important for cell proliferation and migration. *p130Cas-Bac*^{GFP} further emphasizes *Cas* is expressed in cortical areas important for cortical development. The different approaches indicated Cas exhibit specific expression patterns in cells important for cortical development.

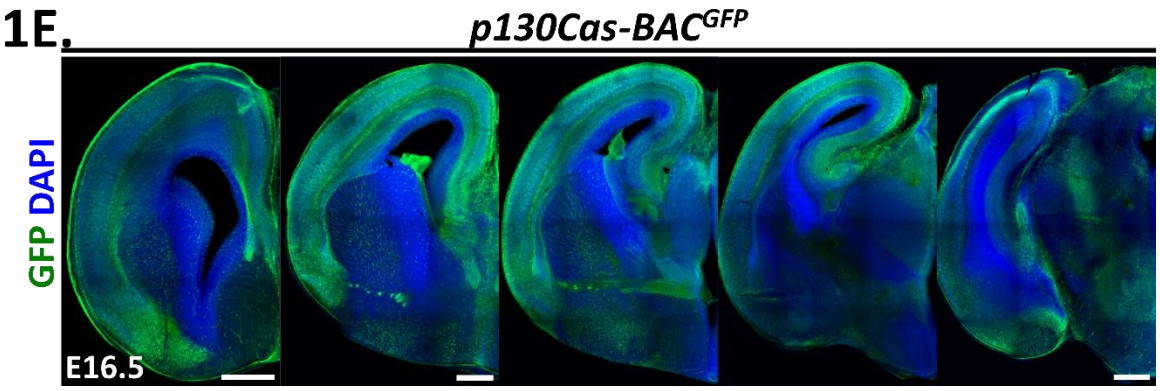


Figure 1E. Rostral to caudal sections of p130Cas-BAC^{GFP} *cortex at E16.5. EGFP is under the regulatory promoter for BCAR1 and shows expression is consistent WT endogenous p130Cas: throughout neocortex and in axons. Scale bar is 500 um.*

The *Cas* gene and protein expression profile suggest important functions during development; thus, we generated a mouse model to test the requirement of the Cas family of adaptor proteins during murine cortical development. Based on the overlapping patterns of expression and previously established redundancy in other systems, we generated triple conditional knock-out (*CasTcKO*) animals for the three embryonically expressed Cas family members. A conditional knockout approach was used to bypass the early lethality observed in *p130Cas*-null animals caused by cardiovascular maldevelopment (Honda et al., 1998; Peng et al., 2006). Single knockout

of *CasL* (Seo et al., 2005) and *Sin* (Donlin et al., 2005) did not result in embryonic mortality, but their roles in the cortex are unknown, thus the deletion of the three Cas members will ensure there are no overlapping compensatory functions. To investigate the role of *Cas* genes during cortical lamination and migration, we started by crossing *CasTcKO* animals to *Emx1-Cre* mice (Gorski et al., 2002). *Emx1-Cre* drives the expression of Cre recombinase in early cortical neural progenitors and radial glial cells (RGCs), resulting in recombination in RGCs themselves and all of the excitatory neurons in the cortex and hippocampus (Fig. 1F; Gorski et al., 2002). Thus, in the context of the *CasTcKOs*, *Cas* is ablated in neural progenitors responsible for excitatory neuron population and does not directly affect interneurons. RNAscope was performed on *Emx1Cre;CasTcKO* coronal sections to validate that mutant animals do not produce functional *Cas* transcripts (Fig. 1G). In comparison to WT at E14.5 (Fig. 1C), no *BCAR1* or *EFS* mRNA transcripts are found in the neocortex. *NEDD9* has a few puncta in the SVZ and VZ, but Seo et al. (2005) has demonstrated *NEDD9-/-* mice do not produce the functional protein. The sparse single molecules may represent where EGFP directly combined with the *NEDD9/CasL* genome within exon 2 at the initial library screen to generate the transgenic animal.

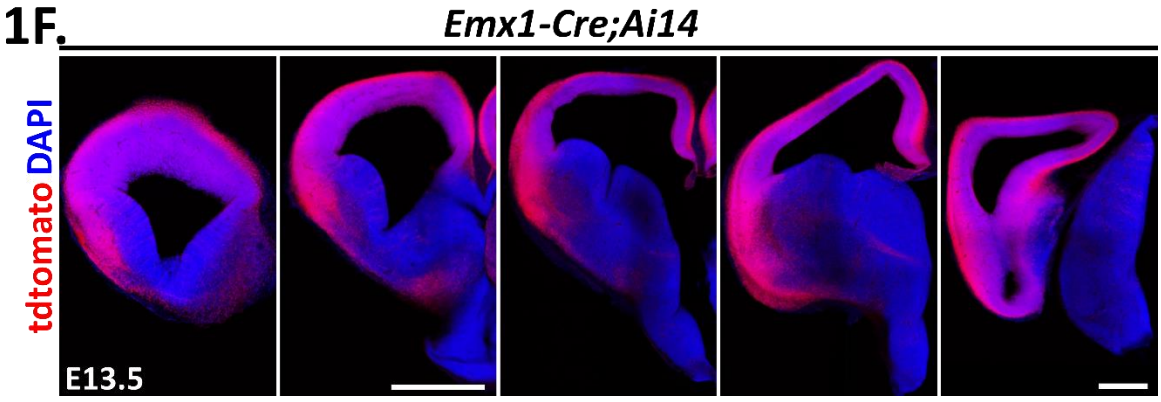


Figure 1F. Emx1-Cre will drive Cre recombinase in cortical neural progenitors and radial glial cells that generate excitatory neurons in the forebrain and hippocampus. Scale bar is 100 um.

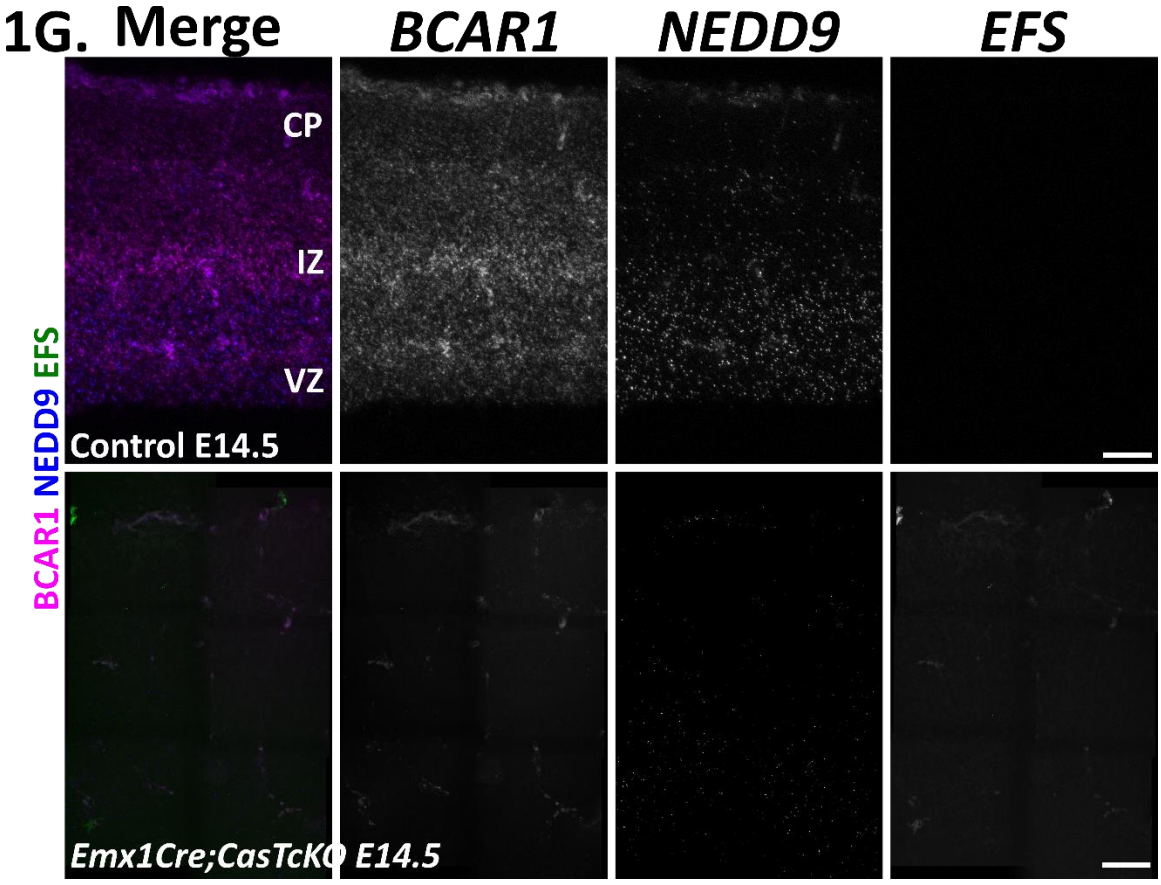


Figure 1G. RNAscope at E14.5 indicate Emx1Cre;CasTcKO do not produce functional BCAR1, NEDD9, and EFS transcripts. Control littermates express BCAR1 transcripts strongly in the IZ and broadly throughout the neocortex. The NEDD9 single molecules do not make functional proteins as demonstrated by Seo et al. (2005) and could be due to the probe detecting the EGFP recombining with NEDD9 exon 2. Scale bar is 100 um.

Mutant animals hereby known as *Emx1Cre;CasTcKO* (*Emx1-Cre+;p130Cas*$^{fl/\Delta}$*;CasL*$^{-/-}$*;Sin*$^{-/-}$) and the Control littermates (*Emx1-Cre-;p130Cas*$^{fl/+}$*;CasL*$^{-/-}$*;Sin*$^{-/-}$) were assessed to test the requirement of *Cas* during cortical development. Starting with an overall examination of *Emx1Cre;CasTcKO* cortices using pan-neuronal markers, the adult phenotype appears dramatically different from Control littermates (Fig. 1H). The smooth cortical surface, typical for a mouse, is replaced with a bumpy or cobblestone surface. Nissl staining, which highlights the contrast between axons and Nissl bodies, shows empty pockets in the cortical plate. A more in-depth analysis of the cortex with a mature neuron marker (NeuN) indicated the cortex has similar empty pockets as seen in the Nissl stain (Fig. 1H). Interestingly, NeuN+ cells appear to organize in a wave-like pattern in *Emx1Cre;CasTcKO* animals . In some areas of these cortices, there are cell clusters forming outside of the pial surface and are infiltrating into the subarachnoid space of the meninges. This displacement of neurons in the cortex and the cobblestone appearance suggest a possible disruption in the laminar organization of the cortical plate.

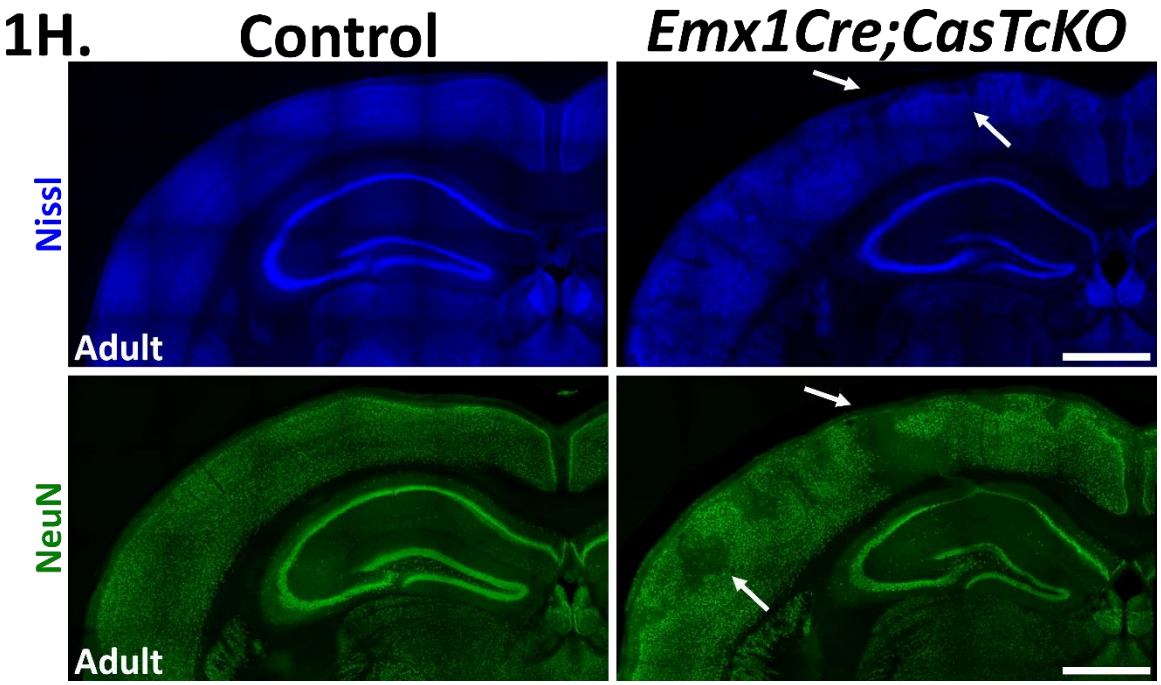


Figure 1H. Pan-neuronal markers using Nissl and NeuN (mature neurons) in adult cortices indicate a disruption in laminar organization in Emx1Cre;CasTcKO animals. Scale bar is 1 mm.

The six-layered cortical plate contains distinct populations of projection neurons. Each neuronal subtype can be distinguished based on cell morphology, connectivity, or expression of specific transcription factors (Molyneaux et al., 2007; Kwan et al., 2012). To test whether the laminar structure of the cortex was affected in *Emx1Cre;CasTcKO* animals, we performed immunohistochemistry with a variety of cortical markers. Cux1, Ctip2, Rorβ, and Tbr1 were chosen from the selected number of transcription factors that maintained their cell lineage identity throughout development (Molyneaux et al., 2007; Kwan et al., 2012). Superficial layer neurons (II/III) are identifiable with Cux1, while Rorβ is one of the few markers that will exclusively label layer IV neurons. The deep layer neurons can be identified with Ctip2 (layers V & VI) or Tbr1 (layer VI). Histological analyses of *Emx1Cre;CasTcKO* animals at P7 (Fig. 2) indicated the presence

of neuronal mispositioning of different populations of cortical neurons, compared to control animals. Quantified the position of labeled cells by binning the cortex into ten equal-width bins, where bin 1 corresponds to the MZ and bin 10 is ventral to layer VI. The proportion of cells in each bin is the number of cells labeled by a specific marker divided by the total number of labeled cells. A Mann-Whitney *U* test was performed for each group and the proportion of cells in each bin for all of the layer markers (Cux1, Ctip2, Rorβ, and Tbr1) and Bonferroni correction (*$p<0.005$ two-tailed test). The full statistical results are in Chapter 2: Materials & Methods, subheading Quantification of Layer Marker/EdU & Statistical Analysis. We saw significant differences in Cux1+, Ctip2+, Rorβ+, and Tbr1+ cell position between *Emx1Cre;CasTcKO* and control animals in many of the bins for each of the layer markers (Graph 1.1-1.4; Mann-Whitney *U* & Bonferroni correction, *$p<0.005$ two-tailed test). The abnormal positioning of both deep- and superficial-layer neurons contributed to the cobblestone phenotype. Cortical dysplasia includes Ctip2+/Tbr1+ cell clusters at the upper cortical plate and Cux1+ neurons at the lower cortical plate. Furthermore, cortices missing *Cas* genes have a wave-like appearance for layer IV, with Rorβ+ cells invading the superficial cortical plate and meninges. These data revealed that *Cas* is required for cortical lamination and suggest a possible role for these genes in radial migration.

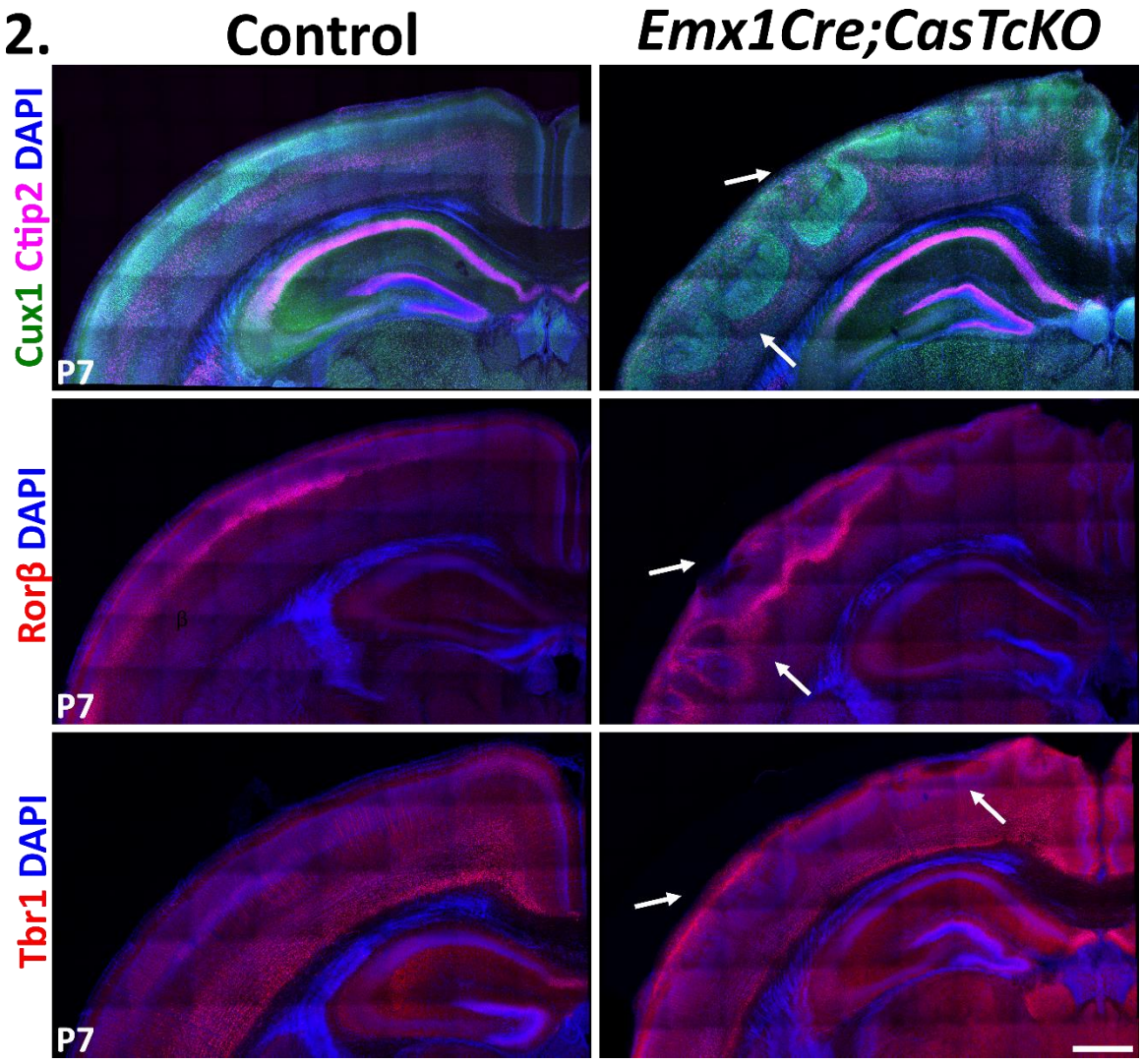
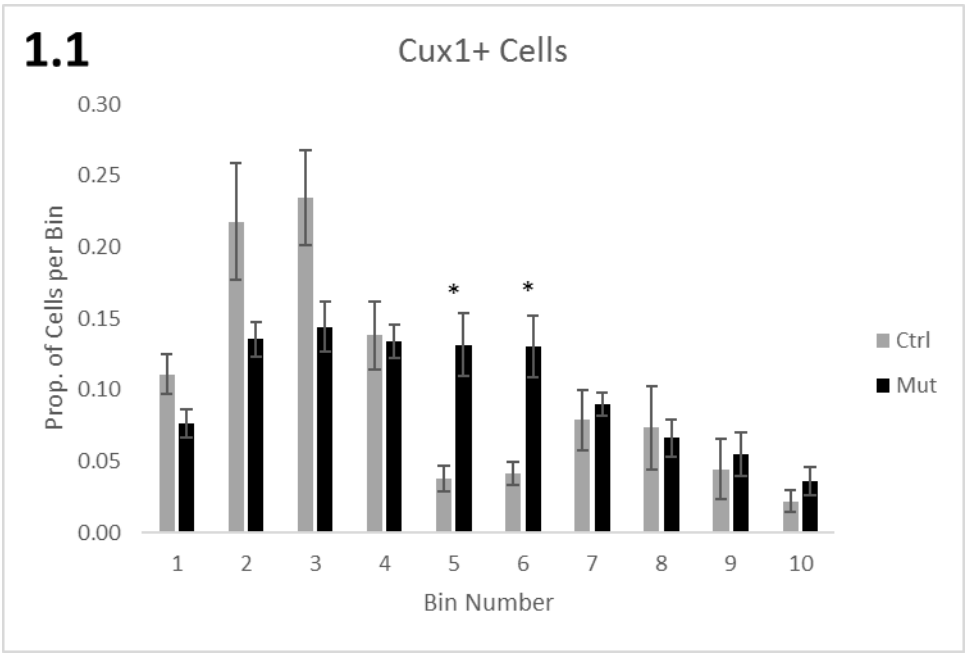
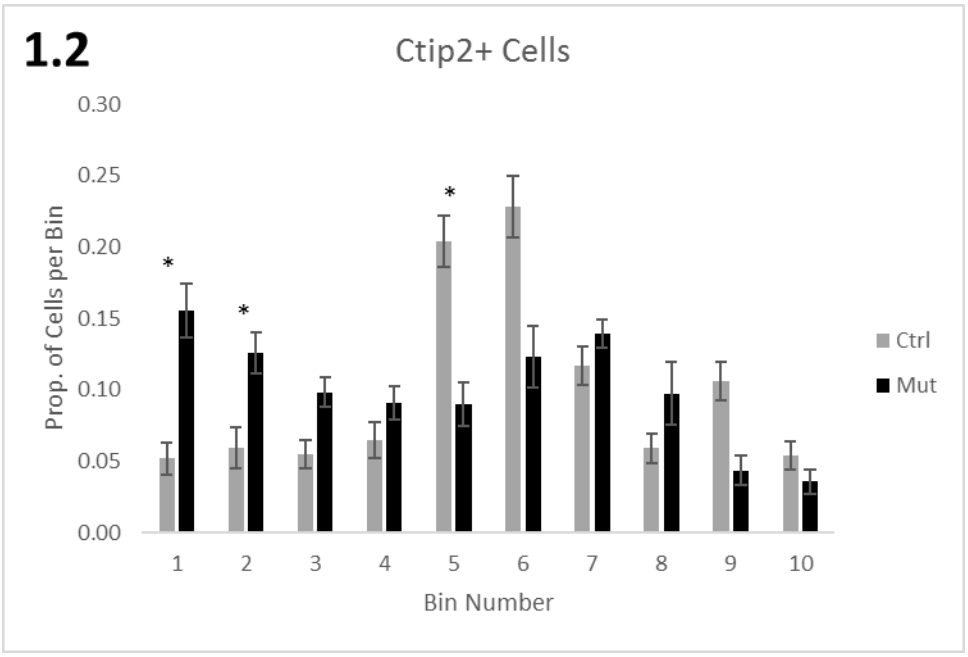


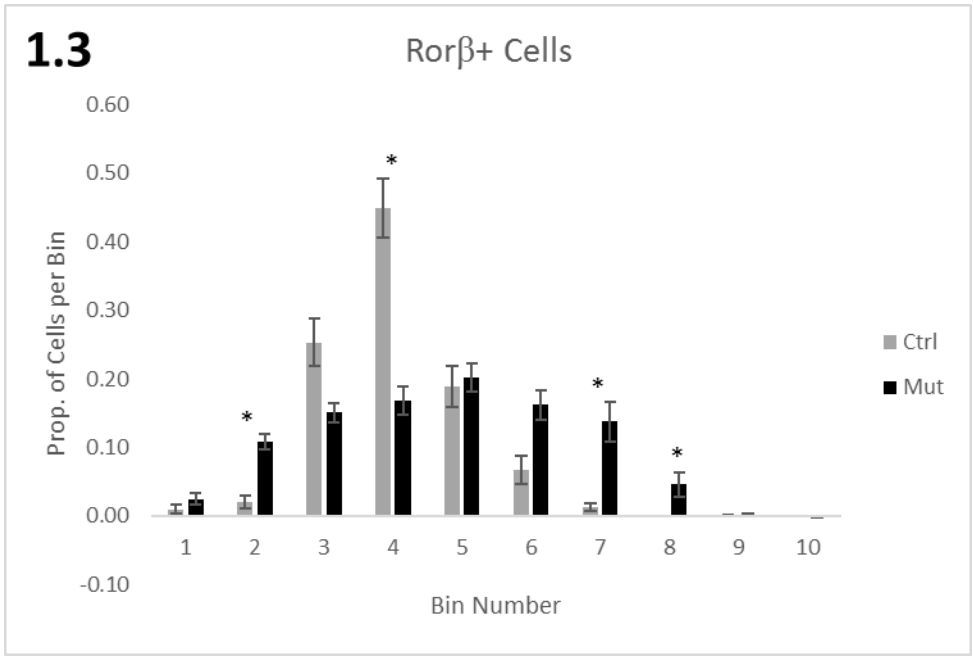
Figure 2. Layer markers to identify the disorganized neuronal subpopulations at P7. Cux1 (layer II/III), Rorβ (layer IV), Ctip2 (layer V & VI), and Tbr1 (layer VI) neurons disorganization contributed to cortical delamination and cobblestone phenotype. Scale bar is 500 um.



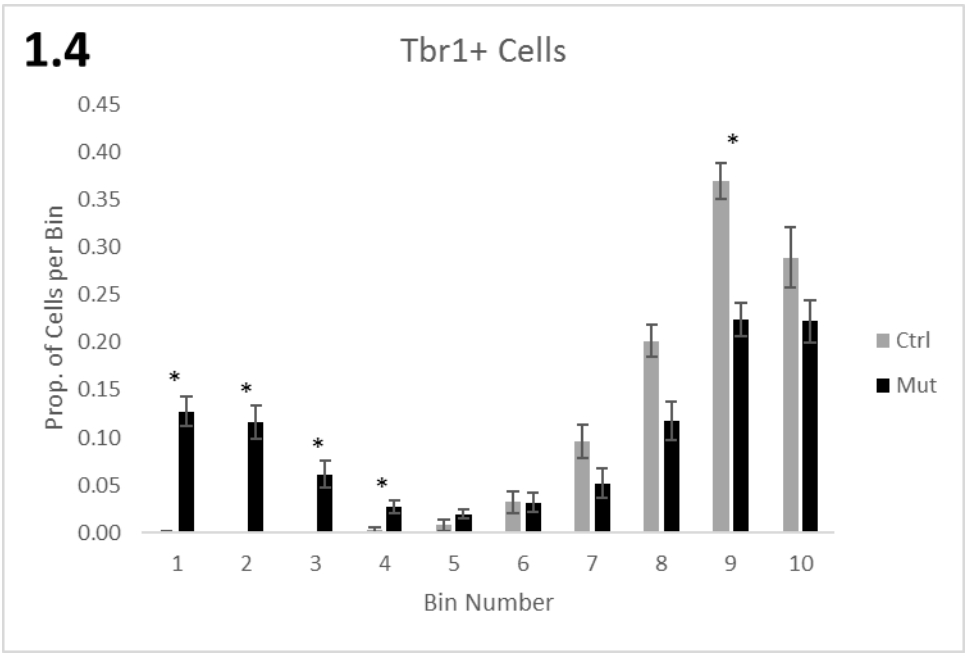
*Graph 1.1. Quantification of the position of Cux1+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the MZ, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*



*Graph 1.2. Quantification of the position of Ctip2+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the MZ, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*



*Graph 1.3. Quantification of the position of Rorβ+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the MZ, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*



*Graph 1.4. Quantification of the position of Tbr1+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the MZ, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*

The mispositioning of layer-specific markers observed in *Emx1Cre;CasTcKO* animals could be due to defects in migration or defects in neuronal fate specification. To distinguish between these possibilities, newly born cells were tracked during the course of radial migration by performing Ethynyl deoxyuridine (EdU) pulse-chase experiments. This thymidine analog is incorporated in cells undergoing the S-phase of mitosis (Salic & Mitchison, 2008) and can be used to observe the final position of cells born at a specific timepoint. EdU was administered to pregnant dams at E12.5 (Fig. 3A) or E15.5 (Fig. 3B) to label newly born deep and superficial layer neurons, respectively. Collection at seven days post-intraperitoneal injection for each timepoint (E12.5->P0, E15.5->P3) is sufficient for deep layer and superficial layer neurons to finish radial migration (Nadarajah et al., 2003). The long EdU+ pulse chase ensures proliferating cells undergoing DNA replication in the VZ/SVZ are now mostly localized in either the lower or upper CP as seen in Control animals. Distinctly, *Emx1Cre;CasTcKO* animals have ectopic EdU+ cells positioned outside of the compromised basal lamina (laminin+) and the CP has sporadic labeling and columns of EdU+ cells. Quantification of the position of E12.5->P0 EdU+ cells show a trend of more EdU+ cells located closer to the pial surface (Bin 1) than towards the bottom of layer VI (Bin 10) (Graph 2.1). Full statistical results for Mann-Whitney *U* test in Chapter 2: Materials & Methods, subheading Quantification of Layer Marker/EdU & Statistical Analysis. There was no significant difference after Bonferroni correction, but trending for bins 1 and 2. More noticeable differences are observed when pulsed at E15.5 and collected at P3 (Graph 2.2); EdU+ cells for superficial

layers are spread across the first five bins in *Emx1Cre;CasTcKO* than Control EdU+ cells positioned closer to the pial surface (bin 1). Mann-Whitney *U* test and Bonferroni correction indicated bins 4 and 5 are significant at $p<0.005$ two-tailed test. These data suggest that the cortical dysplasia observed in *Emx1Cre;CasTcKO* animals is due to a migration defect and not due to cell fate specification errors.

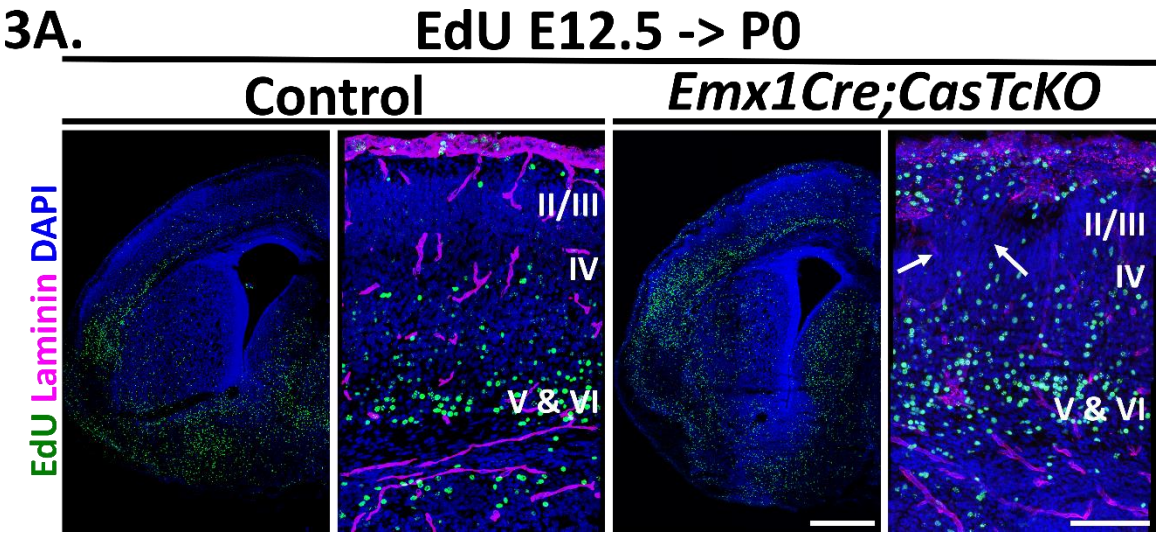
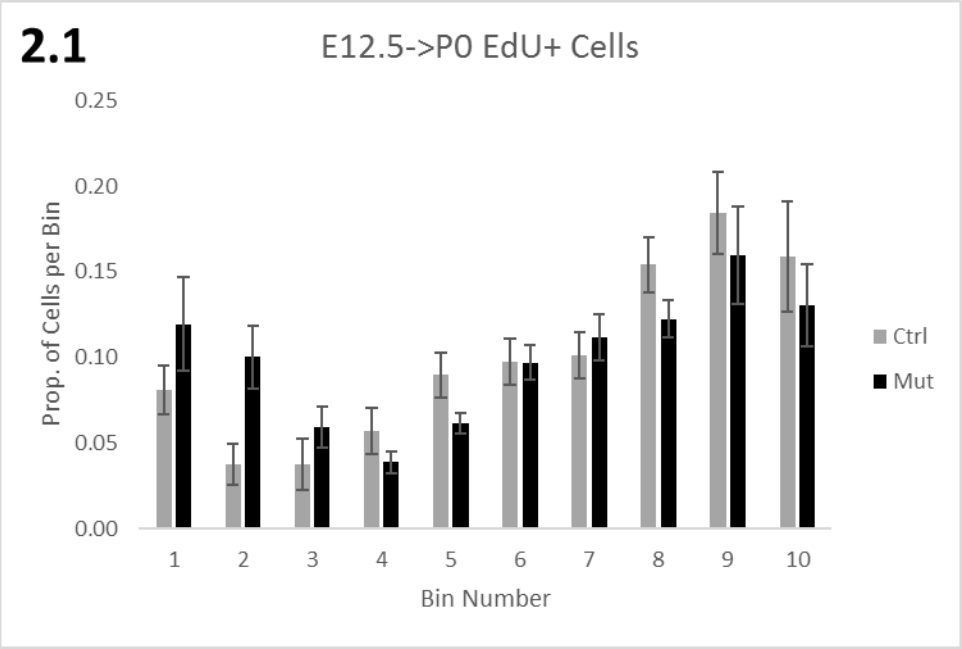


Figure 3A. Deep layer neurons labeled with EdU at E12.5 and collected at P0. Ectopic EdU+ cells are found in the upper CP and outside of the meninges (white arrows) in Emx1Cre;CasTcKO, indicating the phenotype is a migration defect and not cell misspecification. Scale bar is 500 um for half hemisphere and 100 um at high magnification.



*Graph 2.1. Quantification of the position of E12.5->P0 EdU+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the MZ, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test. No sig. differences noted.*

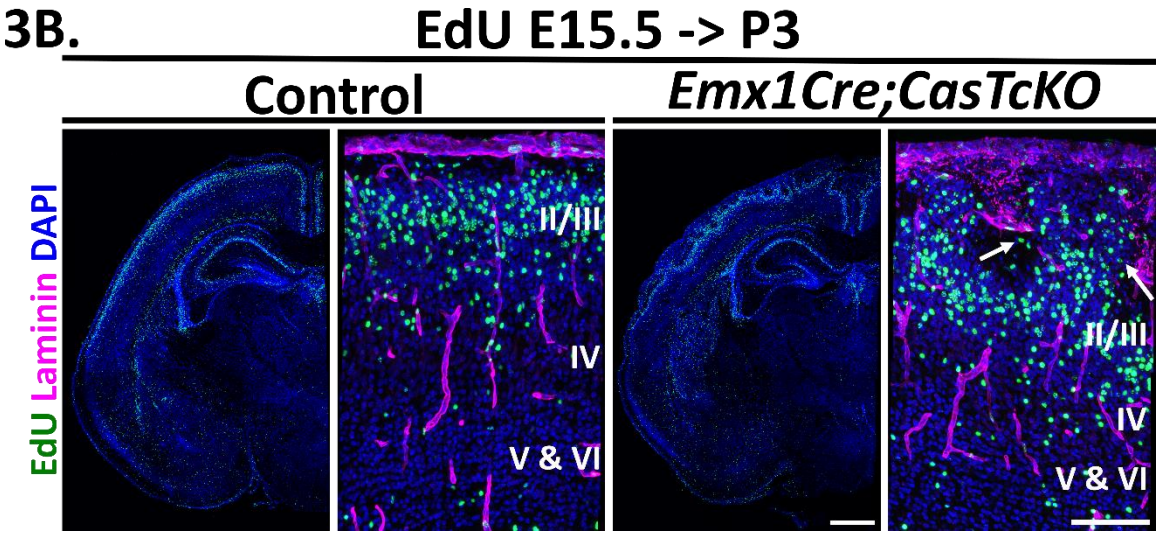
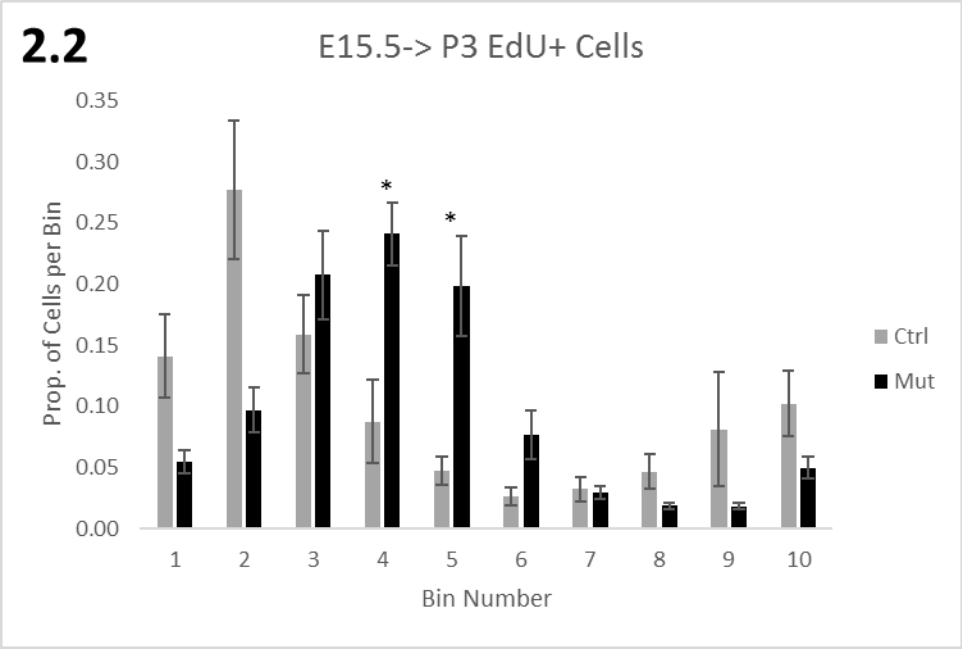


Figure 3B. Superficial layer neurons labeled with EdU at E15.5 and collected at P3. EdU+ cells are found forming columns in the CP and ectopically positioned outside of the meninges (white arrows) in Emx1Cre;CasTcKO. The phenotype is a migration defect and not cell misspecification. Scale bar is 500 um for half hemisphere and 100 um at high magnification.



*Graph 2.2 Quantification of the position of E15.5->P3 EdU+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the pial surface, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*

One possible cause for migration defect is the improper splitting of the preplate (Magdaleno et al., 2002). This involves the establishment of layer VI within a plexus of pioneer neurons (Nichols & Olson, 2010). Layer VI splits the preplate (PP) to form the marginal zone (MZ) found at the superficial cortex and the subplate (SP) ventral boundary. Successive neuronal migration and lamination of cortical layers form the CP in between these two boundaries. Disruption during this early developmental event can change laminar organization (Takahashi et al., 1999; Hevner et al., 2003; Molyneaux et al., 2007) and examining the preplate split is important to help understand when and how migration defects arise in *Emx1Cre;CasTcKO* animals. The use of select subplate and marginal zone markers at E15.5 indicated irregularities during the preplate split in *Emx1Cre;CasTcKO* cortices (Fig. 3C). Calretinin, expressed by Cajal-Retzius cells in the MZ

(del Río et al., 1995), reveal thalamocortical afferents in the IZ deviate to innervate these calcium-binding ectopias. Clustering of calretinin+ Cajal Retzius cells can affect radial glial cell morphology and neuronal responses to adhesion signaling during migration (Meyer et al., 1998; Hartfuss et al., 2003; Gil-Sanz et al., 2013). Although the SP is not distinctly labeled, calbindin shows a disorganized tangential stream of interneurons originating from the medial ganglionic eminence. In addition to interneurons, Cajal-Retzius cells in the MZ also express calbindin, where labeling revealed exposures in the pial basement membrane. Markers used to identify the subplate include microtubule associated protein 2 (MAP2) and chondroitin sulfate proteoglycan (CSPG). MAP2 shows a discontinuous SP, where subplate cells are pushed upwards as a cluster into the breached MZ. Remarkably, a column of CSPG+ cells extend from the SP to contact the pial surface. The diverse SP markers identified abnormal cell clusters outside of the MZ and breaches in the pial basement membrane. Alterations to the dorsal and ventral boundaries seen in *Emx1Cre;CasTcKO* embryos will actively affect how neurons integrate and form the CP. The findings demonstrate the migration defects seen in *Emx1Cre;CasTcKO* appear to involve molecular players to establish the neuroepithelial boundaries and a disruption in radial migration may be a subsequent consequence.

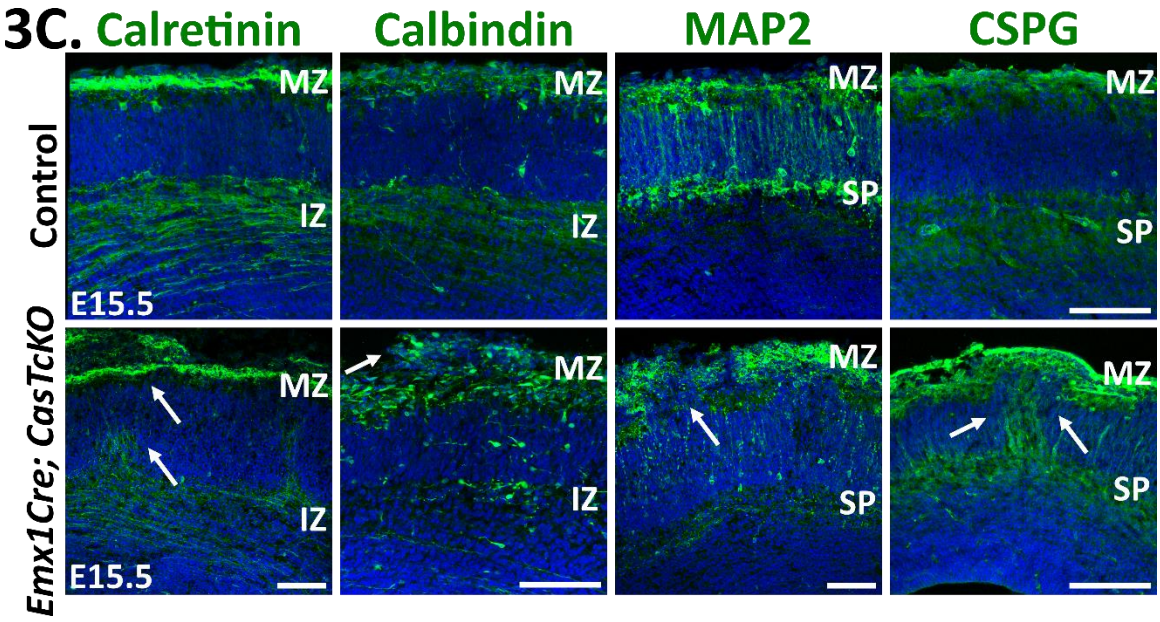


Figure 3C. MZ and subplate markers at E15.5 to examine the preplate split in Emx1Cre;CasTcKO. Calretinin, Calbindin, MAP2, and CSPG show clustering of cells in the MZ. Thalamocortical afferents innervate the Calretinin+ ectopias. CSPG+ cells form a column connecting the SP with the MZ. Scale bar is 100 um.

This chapter demonstrated the Cas family of adaptor proteins are expressed in areas of the cortex important during corticogenesis. The absence of *Cas* genes resulted in a disruption in cortical lamination, where superficial and deep layer neurons are mispositioned. The disrupted migration pattern was confirmed with EdU labeling and the phenotype observed is not a change in cell fate specification. Upon further investigation, improper preplate split disrupted the positioning of cells into the cortical plate. Disruptions in the local environment affected radial migration and the current data cannot distinguish whether Cas proteins are required in neurons for proper migration. The next chapter will focus on the cell-autonomous roles for *Cas* during cortical lamination.

Chapter 4: An Adhesion Signaling Pathway in Radial Glia is Essential for Cortical Scaffold Formation

The ability to remove *Cas* functions early in cortical development is important to understand their role during cortical development, however *Emx1-Cre* (Gorski et al., 2002) removes *Cas* function in the dorsal telencephalon, specifically in neural progenitor cells starting at E10 (Fig. 1F). Due to the pluripotency of neural progenitors, *Emx1Cre;CasTcKO* cortical phenotypes can be attributed to a functional requirement in radial glial cells and/or neurons. Thus, ablating *Cas* function in neural progenitors precludes from distinguishing neuronal-autonomous from glial-autonomous functions of *Cas* genes during these developmental processes. To delineate the cell-autonomous role of *Cas* during neuronal migration, *Nex-Cre* mice (Goebbels et al., 2006) were mated to *CasTcKO* animals. This transgenic line will drive Cre recombinase expression at E11.5 to target postmitotic, premigratory excitatory neurons (Wu et al., 2005; Goebbels et al., 2006). Prior to proceeding with this genetic experiment, the pattern of Cre activity was confirmed by crossing the *Nex-Cre* mice to the Cre reporter line *Ai14.* Analysis of *Nex-Cre;Ai14* cortices at E13.5 revealed that tdtomato expression was found only at the CP in rostral to caudal coronal sections (Fig. 4A).

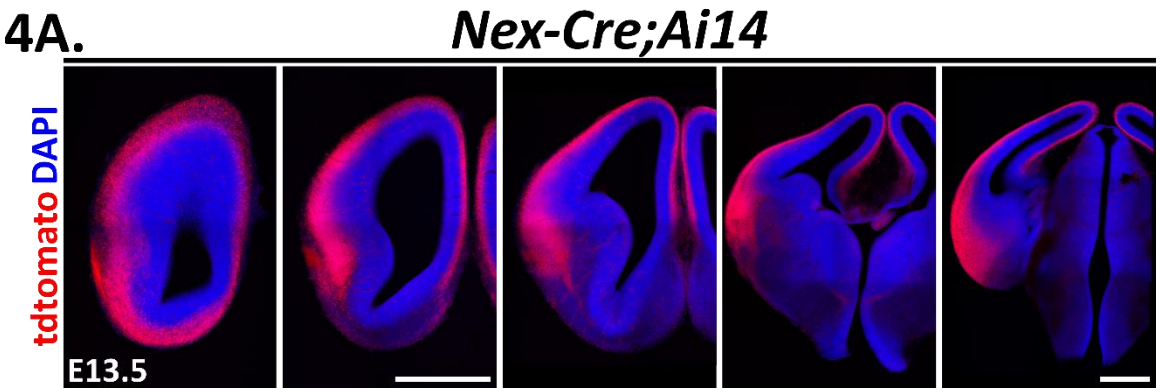


Figure 4A. Nex-Cre will target postmitotic, premigratory neurons in the neocortex. Scale bar is 100 um.

To elucidate *Cas* requirement in neurons during radial migration, the laminar organization of *NexCre;CasTcKO* cortices was examined (Fig. 4B). *NexCre;CasTcKO* mutants and control littermates were immunostained with the same layer-specific markers as *Emx1Cre;CasTcKO* animals (Fig. 2). A prediction is that, if subpopulations of cortical excitatory neurons are mispositioned in *NexCre;CasTcKO* mice, this will lend evidence to a neuronal-autonomous requirement for *Cas* genes during cortical radial migration. If the cortical pattern appears similar to control, mismigration would be an indirect consequence of a disruption of *Cas* normal function in radial glial cells. There are no apparent differences in lamination between control and *NexCre;CasTcKO* cortices when these are immunostained with a battery of cortical layer markers (Fig. 4B). The transcription factors are expressed in the appropriate laminar layer at P7, where superficial layer (Cux1+), layer IV (Rorβ+), and deep layer (Ctip2+/Tbr1+) subpopulations are distinctly separate. Quantification of the position of Cux1+ (Graph 3.1) and Ctip2+ (Graph 3.2) cells in the cortex with the binning method from previous analysis, indicated no differences in cell positioning between Control and *NexCre;CasTcKO* animals (Mann-

Whitney *U* & Bonferroni correction, *p<0.005 two-tailed test). Full statistical results for Mann-Whitney *U* test in Chapter 2: Materials & Methods, subheading Quantification of Layer Marker/EdU & Statistical Analysis. These results strongly suggest that *Cas* is not required in a neuronal-autonomous manner during cortical migration or for proper laminar organization.

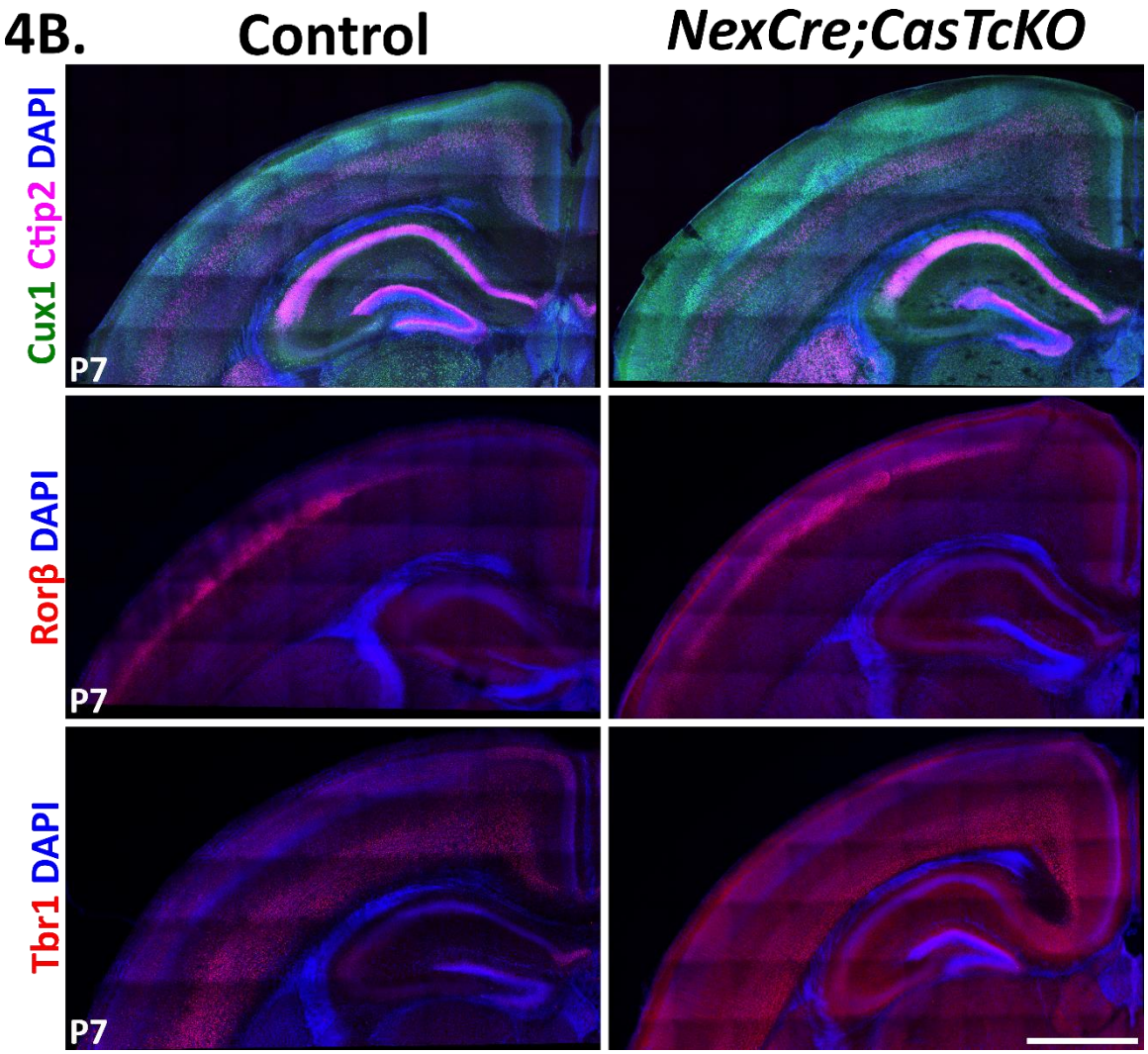
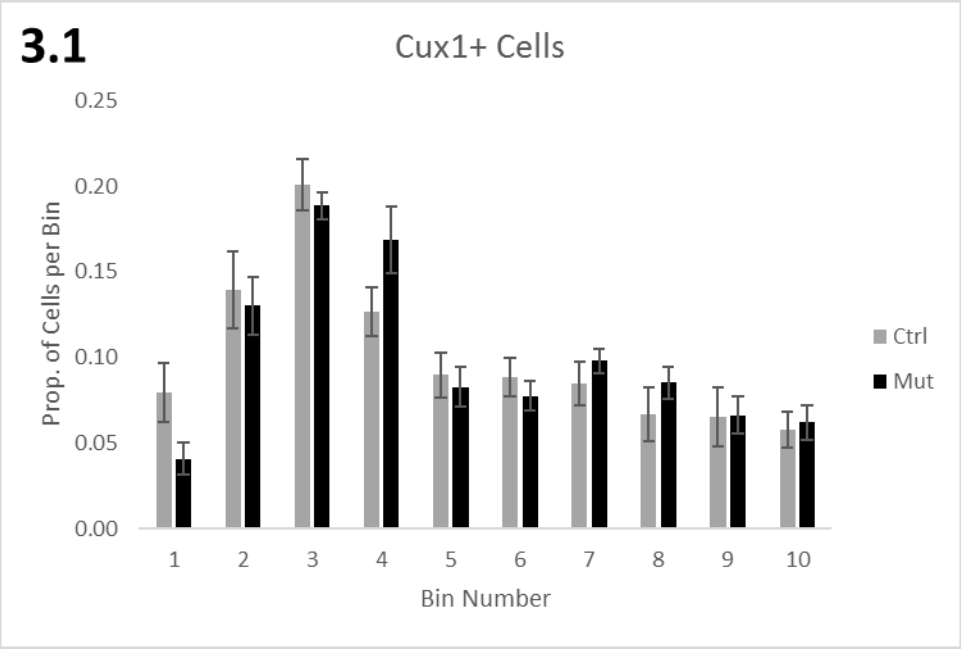
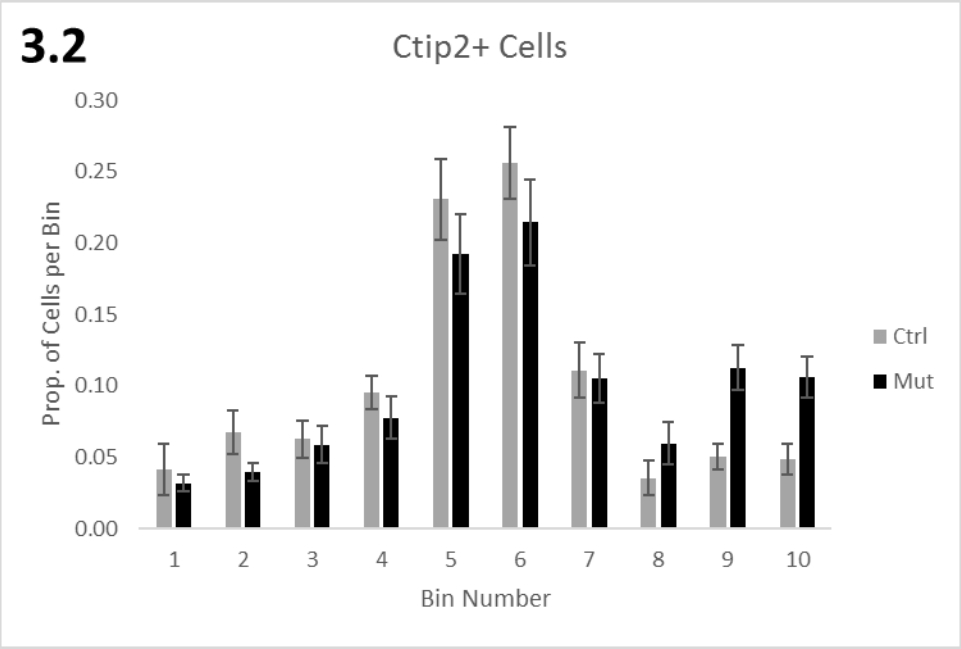


Figure 4B. Layer markers in NexCre;CasTcKO at P7 show normal positioning of superficial layer (Cux1+), layer IV (Rorβ+), and deep layer (Ctip2+/Tbr1+) neurons. Scale bar is 500 um.



*Graph 3.1 Quantification of the position of Cux1+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the pial surface, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*



*Graph 3.2 Quantification of the position of Ctip2+ cells in the cortex by binning the cortical plate into ten equal bins. Bin 1 is the pial surface, bin 10 is ventral to layer VI. Values given are Mean ± S.E.M., n=3 independent samples per group, Mann-Whitney U test & Bonferroni correction, *p<0.005 two-tailed test.*

To ensure there are no migration defects in *NexCre;CasTcKO*, the unbiased method to track migration of progenitor cells previously analyzed in *Emx1Cre;CasTcKO* animals (Fig. 3A-B), was performed on *NexCre;CasTcKO* (Fig. 4C-D). EdU administration at E12.5 and collection at P0 (Fig. 4C) or intraperitoneal injection at E15.5 and collection at P3 (Fig. 4D), again demonstrated migration defects in *Emx1Cre;CasTcKO* animals are not recapitulated in *NexCre;CasTcKO* mutants. Both deep (pulse at E12.5) and superficial (pulse at E15.5) layer neurons exhibit a uniform band of EdU+ cells in the cortical plate. This further demonstrates that *Cas* genes act in a non-neuronal autonomous manner during cortical lamination, and that they are likely functioning in radial glial cells to direct neuronal migration.

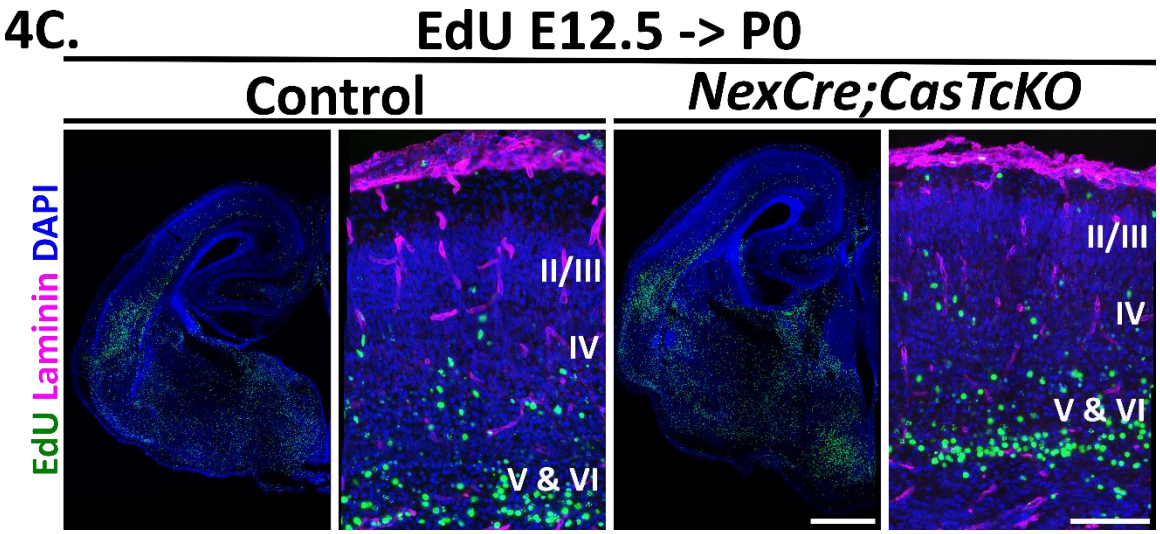


Figure 4C. EdU to label migration of deep layer neurons (pulsed at E12.5 and collected at P0). Migration is not affected in NexCre;CasTcKO. Scale bar is 500 um for half hemisphere and 100 um at high magnification.

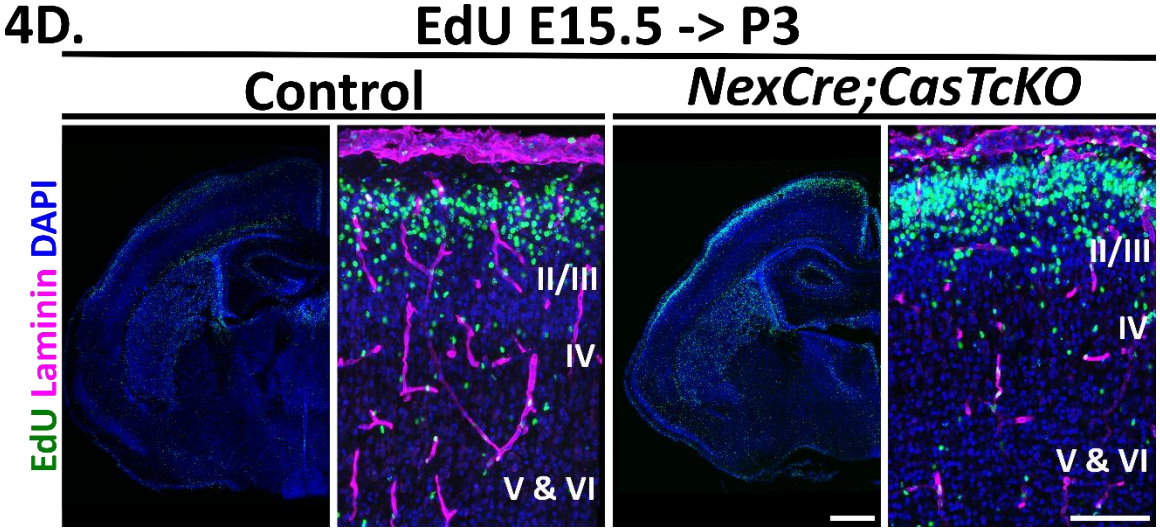


Figure 4D. EdU to label migration of superficial layer neurons (pulsed at E15.5 and collected at P3). Migration is not affected in NexCre;CasTcKO. Scale bar is 500 um for half hemisphere and 100 um at high magnification.

Basement Membrane-Radial Glial Cell Interaction for Proper Neuronal Migration

As the cortex expands in size, radial glial cells create a scaffold for neurons to migrate (Kwan et al., 2012; Nowakowski et al., 2016). Interactions between the embryonic pial basement membrane and radial glial endfeet are essential for development of the cortex (Kawaguchi, 2021). The absence of the cobblestone appearance and intact basement membrane in *NexCre;CasTcKO* cortices (Fig. 4B), suggest that the major laminar disruptions observed in the *Emx1Cre;CasTcKO* cortices might be a result of radial glial cell dysfunction. Based on this information, we examined the expression of the tyrosine-phosphorylated "active" form of *Cas*. Strikingly, tyrosine phosphorylated Cas (pYCas165) is concentrated in radial glial (Nestin+) endfeet and cell bodies in the VZ (Fig. 5A) in WT animal at E13.5. This indicates that this post-

translational modification of Cas is present at sites where radial glial endfeet actively interact with the basement membrane.

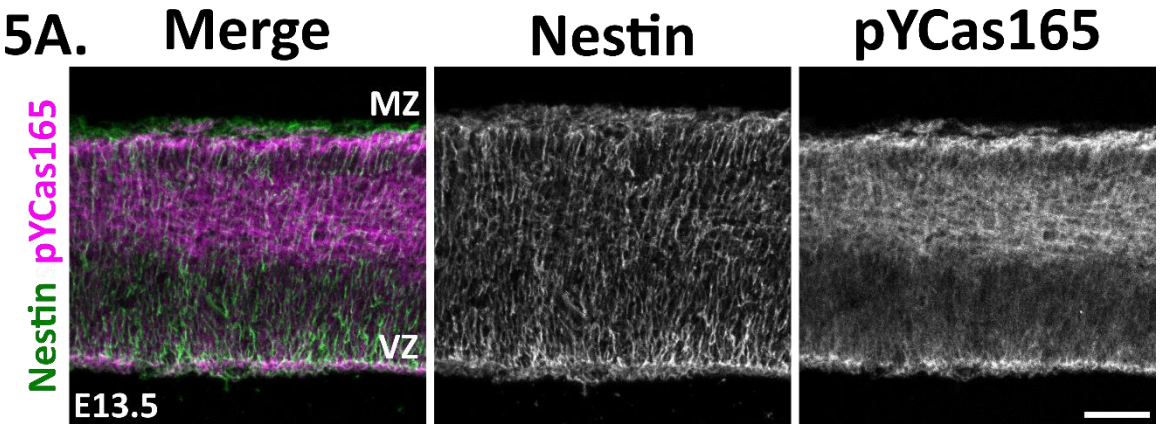


Figure 5A. Tyrosine phosphorylated Cas is detected in Nestin+ radial glial cell bodies located at the VZ and in basal processes contacting the MZ at E13.5. Scale bar is 100 um.

To test whether Cas is required for the embryonic pial basement membrane and radial glial endfeet integrity, we performed histological assessment of *Emx1Cre;CasTcKO* at E15.5 (Fig. 5B). While no obvious defects were observed in control animals, there are several disruptions to the glial-pial interface in *Emx1Cre;CasTcKO* cortices. There is widespread rupture of the basal lamina as indicated by regions with breached Laminin staining. The Nestin+ radial glial cells do not make proper adhesion contact with the basal lamina at the exposed Laminin, where basal processes extend into the subarachnoid space. The exciting results imply *Cas* is required for the maintenance of the embryonic pial basement membrane-radial glial cells interaction and perturbation to Cas-dependent signaling can disrupt laminar organization.

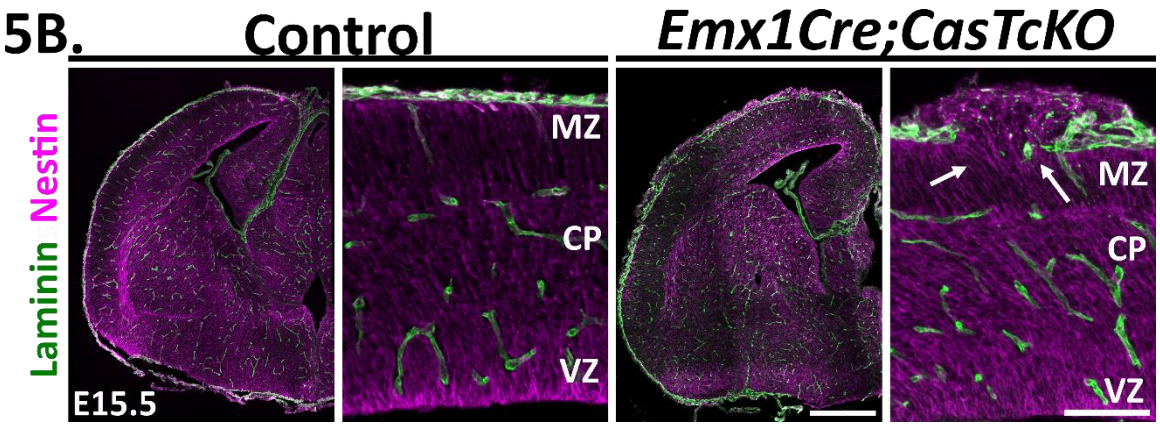


Figure 5B. Disruption to Laminin shows radial glial endfeet (Nestin) do not make proper contact with the basal lamina in Emx1Cre;CasTcKO. Scale bar is 500 um for half hemisphere and 100 um at high magnification.

To determine whether the disrupted basement membrane-radial glial cell interaction caused mispositioning of proliferating progenitor cells, EdU was again administered to *Emx1Cre;CasTcKO* animals (Fig. 5C-D). However, collection is 0.5 Hrs. following injection at E12.5 (Fig. 5C) or E15.5 (Fig. 5D). The short EdU pulse will label the proliferating cells committed to become deep layer and superficial layer neurons, respectively. Regardless of timepoint, the short window ensures proliferating neural progenitors are positioned in the SVZ and VZ as noted in control animals. EdU+ cells are observed at the MZ and upper CP, specifically in regions where the basement membrane is ruptured in *Emx1Cre;CasTcKO*. Careful examination of the cortex indicates the breakage in the basement membrane and mispositioning of radial glial endfeet likely contribute to ectopic progenitors and the cobblestone phenotype.

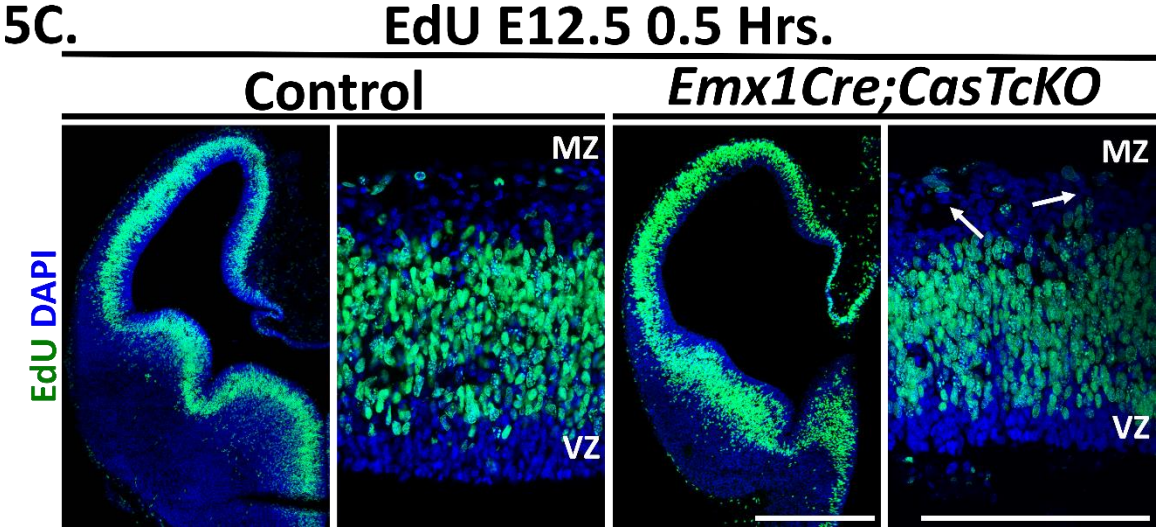


Figure 5C. EdU at E12.5 label cells committed to become part of the deep layer, show ectopic proliferating cells clustering in the MZ. Scale bar is 500 um for half hemisphere and 100 um for high magnification.

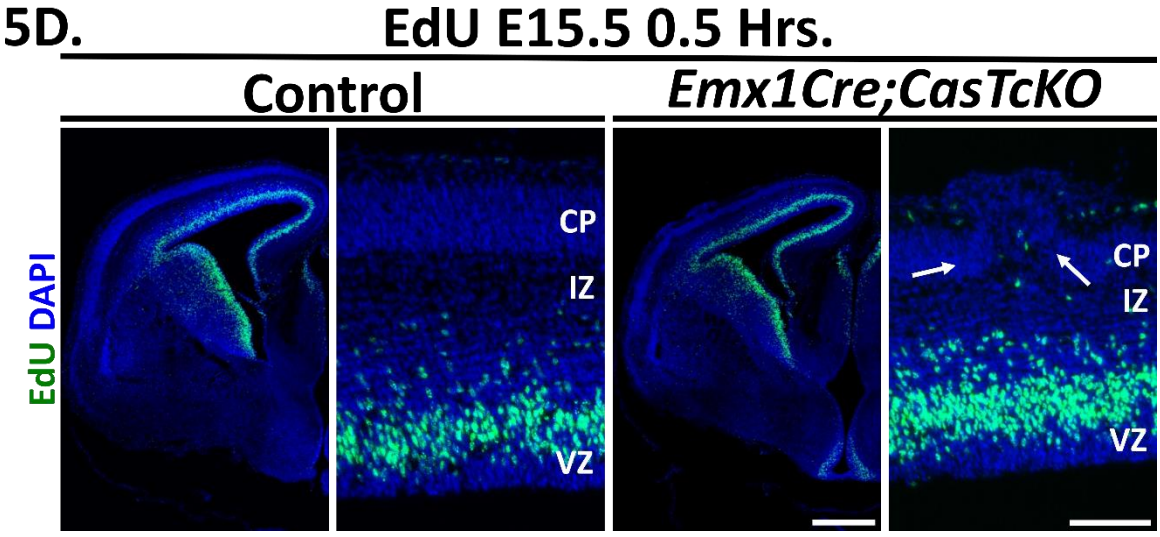


Figure 5D. EdU at E15.5 labels cells committed to become part of the superficial layer and shows ectopic proliferating cells clustering in the MZ (white arrow). Scale bar is 500 um for half hemisphere and 100 um at high magnification.

Dystroglycan Recruits Phosphorylated Cas to Radial Glial Endfeet

The dystroglycan complex provides a structural link between the basement membrane and components that remodel the actin cytoskeleton (Satz et al., 2010; Waite et al., 2012). Glycosylation of the extracellular alpha subunit of dystroglycan is

responsible for maintaining the pial basement membrane integrity (Myshrall et al., 2012). Similar to pYCas165 expression (Fig. 5A), DG protein is expressed at the surface of the cortex on radial glial endfeet and *Dag1* mRNA encoding the protein is expressed in the nuclei at the VZ (Williamson et al., 1997). *Dag1* mutants resulted in a discontinuity of the basement membrane that allowed fibers, cortical neurons, and progenitor cells to bypass this boundary to form a leptomeningeal heterotopia (Nickolls & Bönnemann, 2018). Previous analyses of $DG^{-/-}$ animals at E14.5 to P0.5 show a heterotopic distribution of cortical neurons (Myshrall et al., 2012), and an intriguing resemblance to the *CasTcKO* cortical phenotype. We thus examined this phenotype at P7, using the same layer markers that we used to study the *CasTcKO* mice. Genetic ablation of *Dystroglycan* using the *Emx1-Cre* driver (*Emx1Cre;DG*) at P7 indeed caused cobblestone malformations (Fig. 6A), as suggested by previous studies (Myshrall et al., 2012; Riemersma et al., 2015). *Emx1-Cre+;*$DG^{fl/+}$ Control animals display a distinct separation between Cux1+ and Ctip2+ neurons. Furthermore, layer VI Tbr1+ population is distinguishable. Positioning of the different neuronal subpopulations in *Emx1Cre;DG* (*Emx1-Cre+;*$DG^{fl/-}$) is strikingly similar to *Emx1Cre;CasTcKO* mice (Fig. 2), where superficial layer neurons undermigrate and deep layer neurons overmigrate. Interestingly, these phenotypes are likely RGC-autonomous, as deletion of DG in postmitotic neurons does not disrupt cortical lamination (Miller & Wright, 2021). Taken together, this provides definitive evidence that the *Emx1Cre;CasTcKO* cortical delamination phenocopies multiple aspects of the defects observed in $DG^{-/-}$ cortices.

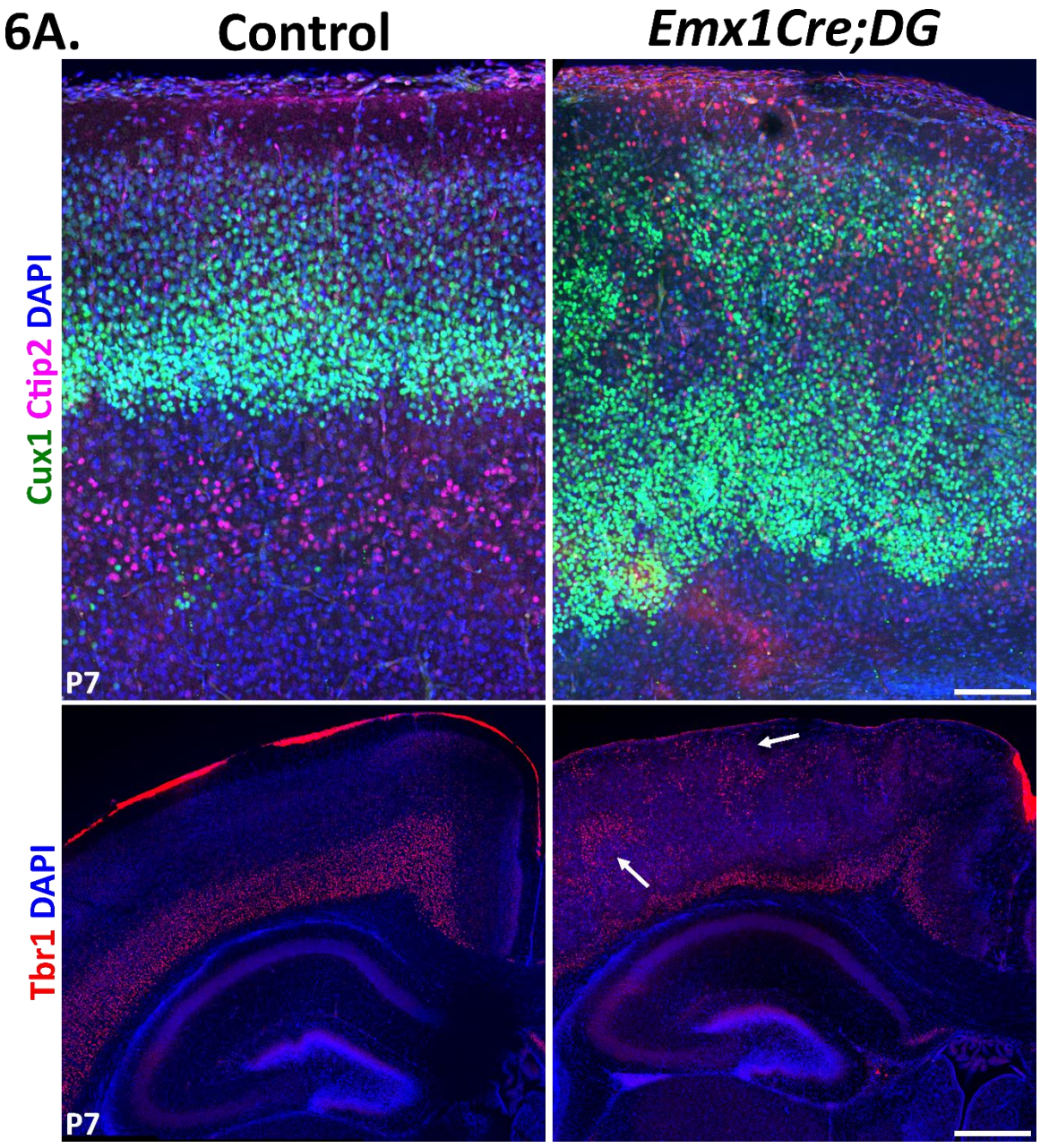


Figure 6A. Emx1Cre;DG cortices are similar to Emx1Cre;CasTcKO mutants with disorganized distribution of superficial and deep layer neurons. Scale bar is 70 um at high magnification and 500 um in hippocampal section.

Based on the robust pYCas165 expression at sites where radial glial endfeet interact with the basement membrane (Fig. 5A) and the close resemblance between *Cas* and *DG* mutant phenotypes (Fig. 2; Fig. 6A; Myshrall et al., 2012), we hypothesize there

may be a requirement for both *DG* and *Cas* genes to maintain the glial-pial membrane interaction and cortical scaffold integrity in a RGC-autonomous manner. To examine whether DG and Cas proteins act in the same signaling pathway to maintain the pial basement membrane integrity, we established an *in vitro* paradigm of a mixed primary culture containing radial glial cells and neurons (Fig. 6B). The primary cultures cannot be radial glial exclusive, as RGCs need structural and chemical support from other cells to display the morphological features of a long process and a highly branched or club-like endfeet (Culican et al., 1990; Yokota et al., 2010). The presence of radial glial cells was confirmed using previously validated developmental markers (Malatesta et al., 2003; Markó et al., 2011). E13.5 RGCs cultured for four days (4DIV) express Blbp or Nestin and have low expression of the astrocytic marker GFAP (Fig. 6B). RGC-like, Nestin+ cells are not co-labeled with the intermediate progenitor marker, Tbr2 or neuronal marker, Tuj1. This *in vitro* approach can provide high resolution of subcellular protein localization in individual cells to determine whether DG and Cas are the same signaling pathway in radial glial cells.

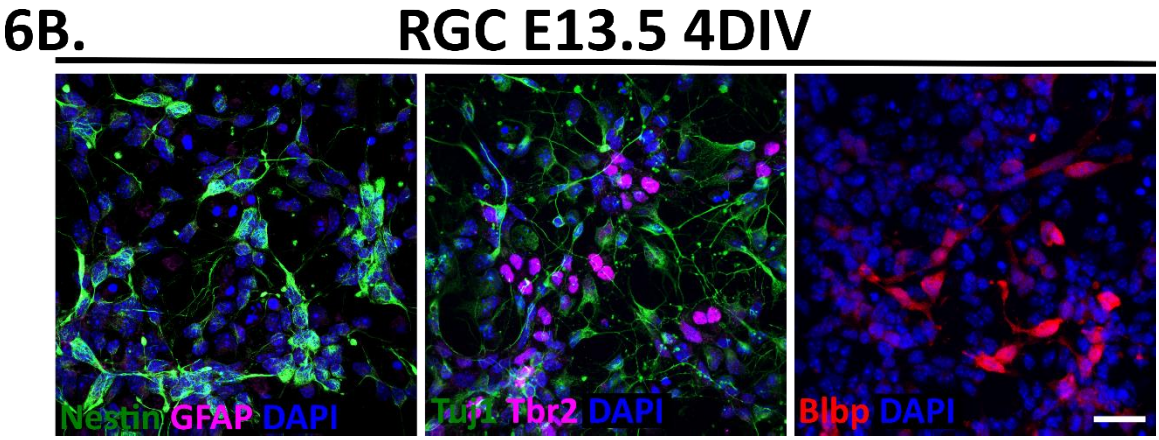


Figure 6B. Specific markers to identify cell types in E13.5 4DIV mixed primary cultures. Nestin (neuroepithelial and radial glial cells), GFAP (astrocytes), Tuj1 (mature neurons), Tbr2 (immature neurons), and Blbp (radial glial cells). Scale bar is 25 um.

To test whether DG expression can modulate Cas tyrosine phosphorylation in RGCs, mixed cultures were transfected at 2DIV with either a myc-tagged empty vector or myc-tagged full length Dystroglycan (DG-FL). The overexpression constructs were given two additional days *in vitro*, and RGCs were immunostained at 4DIV (Fig. 6C). Tyrosine phosphorylated Cas (pYCas165) levels are examined in transfected cells that are myc-labeled and co-positive for Nestin (radial glial cell-like). Glial-neuronal cultures transfected with an empty vector (pcDNA3.1-myc/his) showed basal and endogenous pYCas165, where puncta are distributed in cell bodies and localized between cells at adhesion points. Upon initial examination of cells overexpressing the myc-tagged DG-FL under the pcDNA3.1 promoter (pcDNA3.1-myc/his-DG-FL), the radial glial cell morphology is strikingly different. Cells have numerous small processes protruding from the cell body, in addition to the stereotypical long process and prominent club endfeet. pYCas165 is present in the cell bodies as seen in control cultures and appears

unremarkable in cells transfected with DG-FL. The main feature in Nestin+ DG-FL transfected cells is the upregulated accumulation of pYCas165 at the endfeet that coincides with the dense localization of DG. Mean fluorescent intensity of phosphorylated Cas in radial glial endfeet shows DG localized significantly increased pYCas. The dataset did not pass the Shapiro-Wilk test for normality; thus a Mann-Whitney *U* test was performed. (Graph 4.1; $U = 86$, $n_1 = n_2 = 18$; *p= 0.016 two-tailed test). The novel findings demonstrate DG and Cas may be acting in the same pathway to stabilize radial glial endfeet with the pial basement membrane to maintain laminar organization.

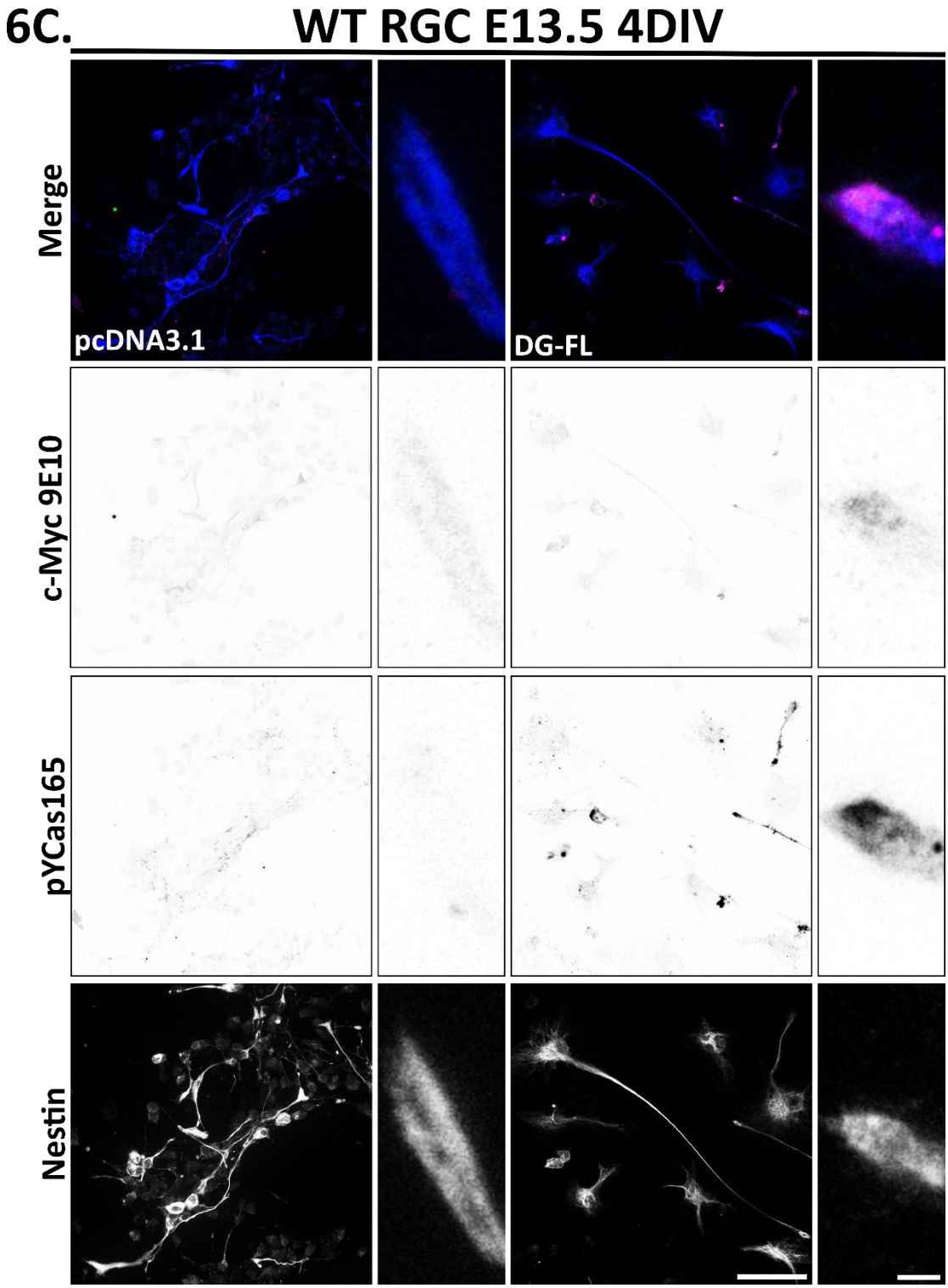
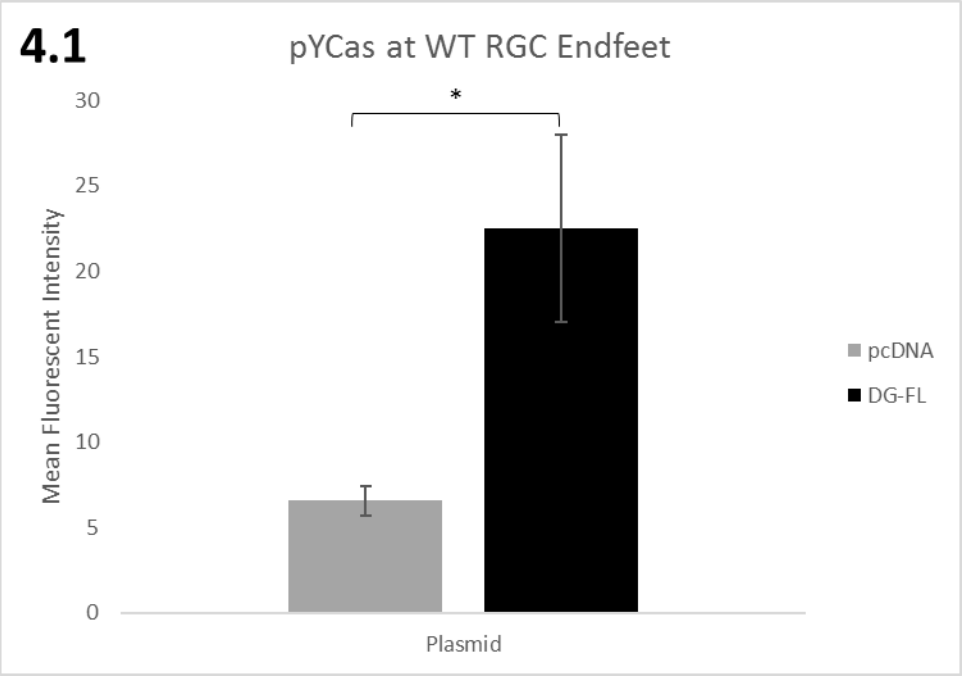


Figure 6C. WT RGCs cultured at E13.5 were transfected with an empty vector (pcDNA3.1) or a construct overexpressing full length Dystroglycan (DG-FL). DG recruited pYCas165 to the radial glial endfeet. Zoomed in example of a radial glial endfeet is to the right of each image. Scale bar is 25 um for wide view and 1 um for zoomed image.



Graph 4.1 Quantification of the mean fluorescent intensity of pYCas165 at WT RGC endfeet. WT cells transfected with DG-FL significantly recruited more pYCas to the basal process than when transfected with an empty vector (pcDNA3.1). Values given are Mean ± S.E.M., n=2 independent samples per group, Mann-Whitney U test U = 86, n_1 = n_2 *= 18; *p= 0.016 two-tailed test.*

B1 Integrins Regulate Cas Phosphorylation at RGC Endfeet

Glycoproteins in the ECM (i.e. laminin) are known to bind to d57ystroglycan and integrin transmembrane receptors on radial glial endfeet (Henry et al., 2001). Fig. 6C demonstrated Dystroglycan overexpression promotes pYCas165 accumulation in radial glial endfeet. Perhaps β1 integrin also mediates Cas phosphorylation in radial glial cells. Previous evidence from Riccomagno & Sun et al. (2014) showed Cas phosphorylation in the retina is highly dependent on β1 integrin signaling. Graus-Porta et al. (2001) identified β1 integrin ablation led to laminar disorganization similar to the ones observed in *Emx1Cre;CasTcKO*. The phenotypes also appear to be RGC-autonomous (Belvindrah et al., 2007). We started by revisiting the phenotypes in pan-cortical vs.

neuronal specific β1 integrin Kos using layer-specific markers. Staining for the layer-specific transcription factors confirmed the cobblestone phenotype in *Emx1Cre;B1* (*Emx1-Cre+;β1$^{fl/fl}$*) P7 cortices in comparison to Control littermates (*Emx1-Cre-;β1$^{fl/fl}$*) (Fig. 7A). Cux1, Ctip2, Rorβ, and Tbr1 labeling indicated cortical layer misplacement occurred for all subpopulations in *Emx1Cre;B1* animals. This included an invasion of deep layer neurons in the dorsal cortex and superficial layer neurons in the lower CP. *NexCre;B1* (*Nex-Cre+;β1$^{fl/fl}$*) and Control (*Nex-Cre-;β1$^{fl/fl}$*) P7 cortices (Fig. 7B) are indistinguishable from each other and appear similar to *NexCre;CasTcKO* animals (Fig. 4B). Cortical layers are uniform and defined, further highlighting that the cobblestone malformations seen in *Emx1Cre;CasTcKO* (Fig. 2), *Emx1Cre;DG* (Fig. 6A), and *Emx1Cre;B1* (Fig. 7A) animals are due to disruptions in ECM and radial glial cell signaling. This further validates and reinforces previous observations suggesting that integrin acts in an RGC-autonomous manner to control cortical lamination.

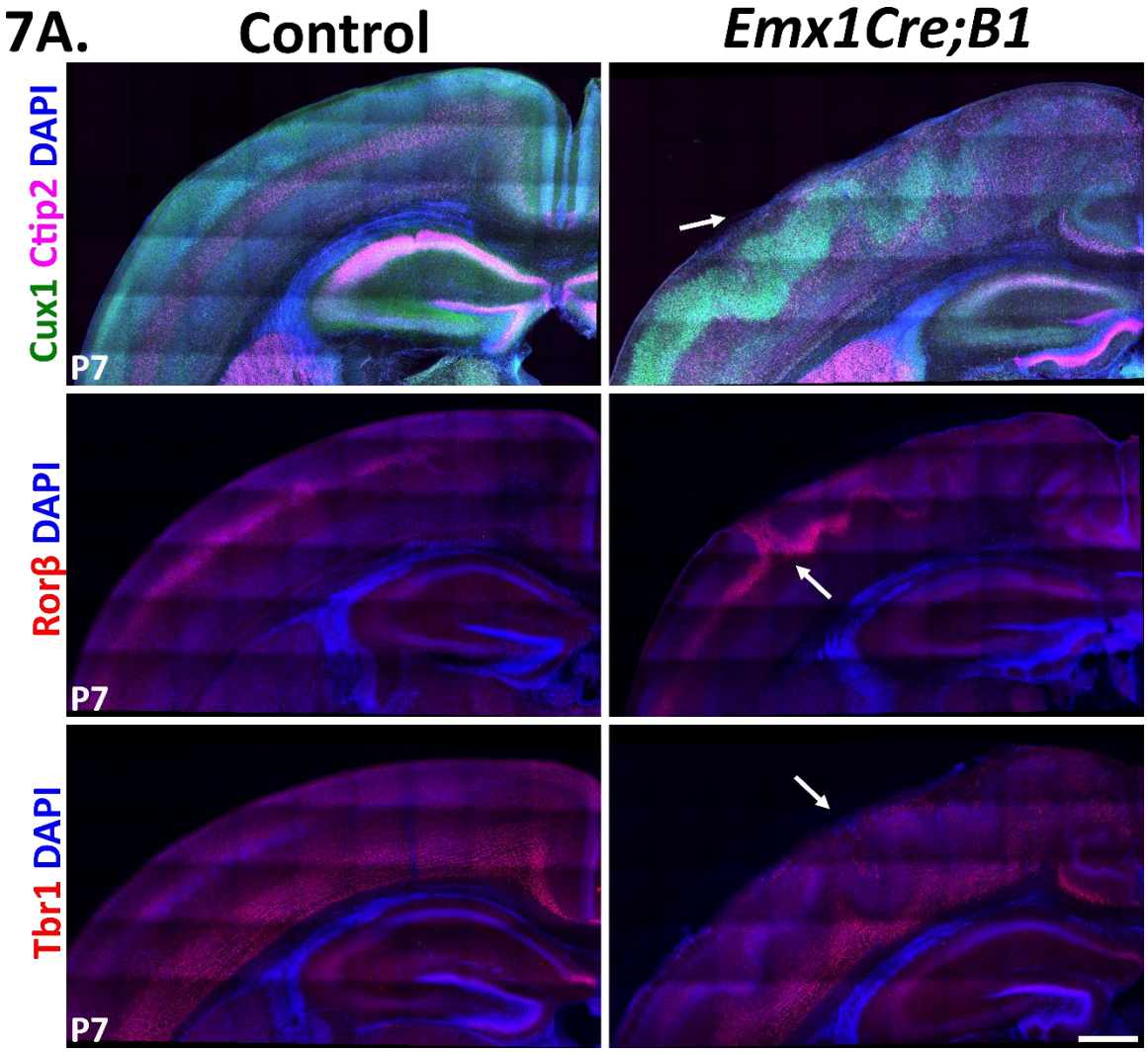


Figure 7A. Layer markers for Emx1Cre;B1 at P7 show similar laminar disorganization and cobblestone malformation observed in Emx1Cre;CasTcKO and Emx1Cre;DG mutants. Scale bar is 500 um.

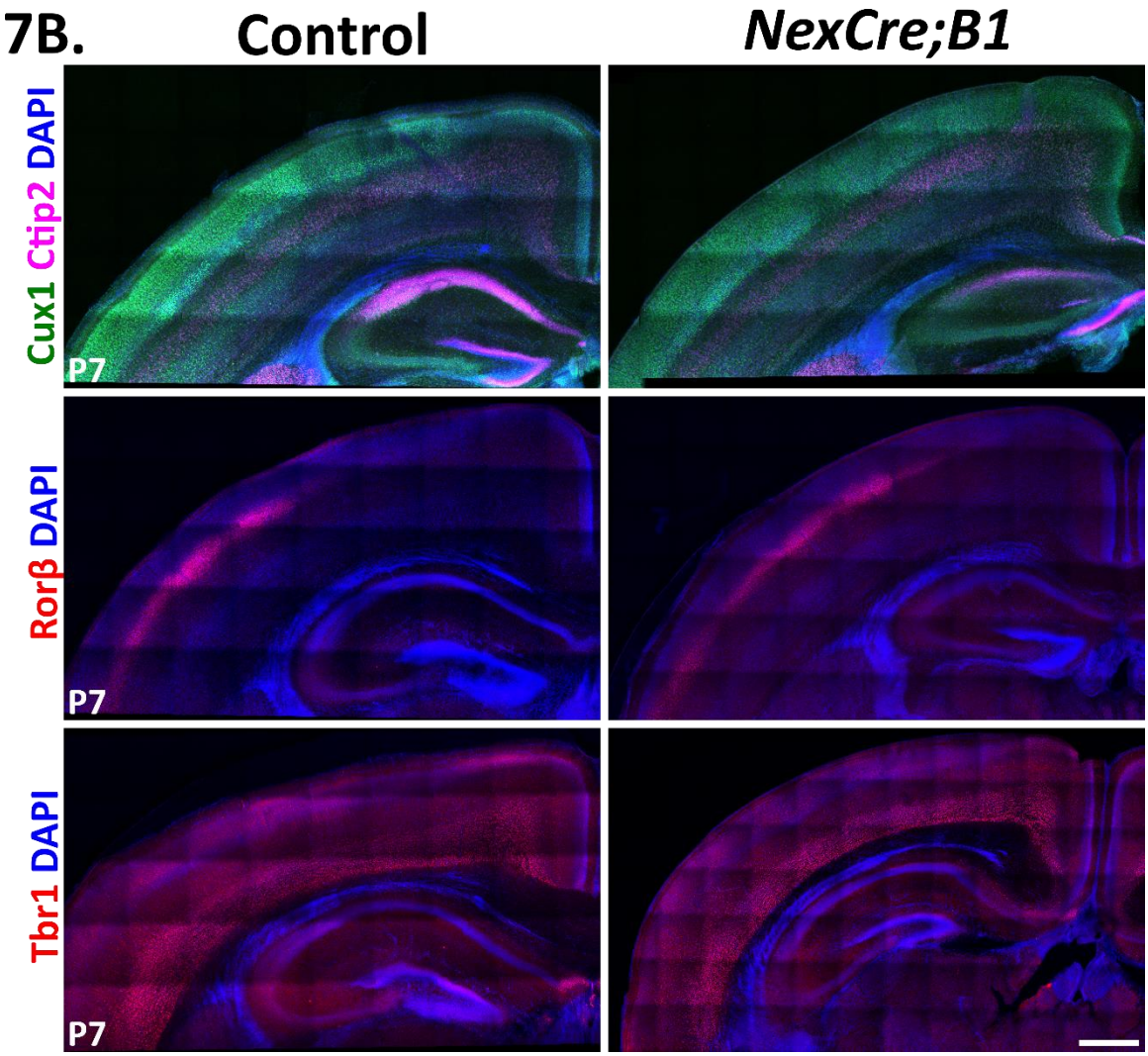


Figure 7B. Layer markers for NexCre;B1 at P7 show normal laminar organization as observed in NexCre;CasTcKO. This indicates Cas and integrin signaling are non-neuronal autonomous for cortical lamination. Scale bar is 500 um.

We used the same RGC mixed culture setup to test whether DG-dependent phosphorylation of Cas requires integrin as a signal transducing receptor. *Emx1Cre;B1* cultures transfected with the empty vector (pcDNA3.1-myc/his) or overexpressing DG-FL (pcDNA3.1-myc/his-DG-FL) have similar levels of pYCas165 throughout the radial glial cell (Fig. 7C). The mean fluorescent intensity of pYCas165 at the radial glial endfeet in *Emx1Cre;B1* cells passed the Shapiro-Wilk test for normality and a two-tailed

independent samples t-test indicated there was no difference in phosphorylated Cas in RGC endfeet when transfected with either construct (Graph 4.2). Noticeably, *Emx1Cre;B1* cells overexpressing DG-FL in now yielded weak pYCas165 expression in radial glial endfeet, in comparison to WT cultures overexpressing DG (Fig. 6C). The key findings indicate DG-dependent phosphorylation or recruitment of pYCas to the endfeet require β1 integrin function.

The cobblestone malformation observed in *Emx1Cre;CasTcKO*, *Emx1Cre;DG*, and *Emx1Cre;B1* is due to a radial glial cell dysfunction. There is a non-neuronal autonomous requirement for these proteins during cortical lamination. Dystroglycan successfully recruited phosphorylated Cas to the radial glial endfeet and this adhesion signaling required β1 integrin. Both β1 integrin and DG are transmembrane receptors on radial glial endfeet to dynamically interact with the basement membrane and disruption of this signal upstream of Cas appears to alter cortical lamination.

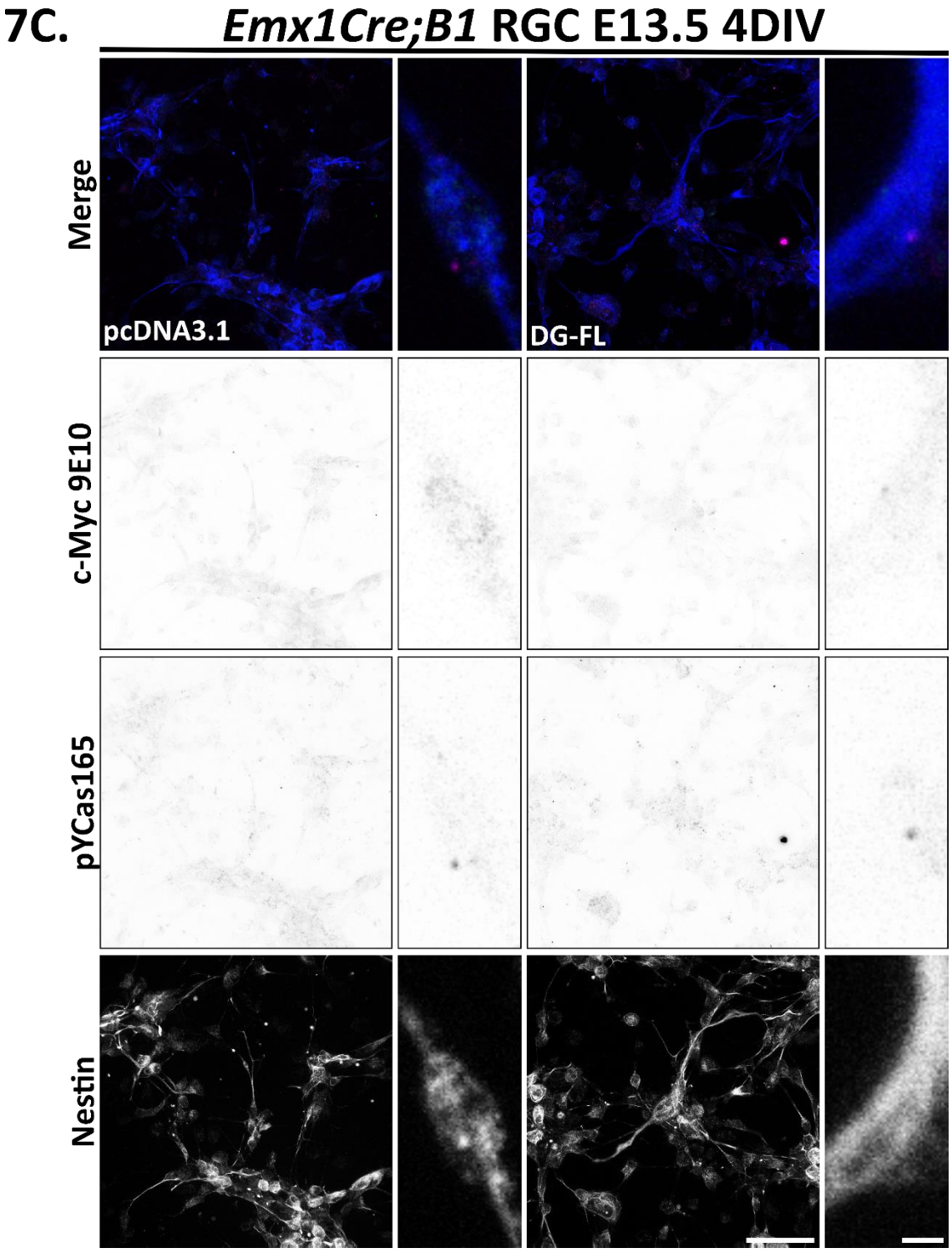
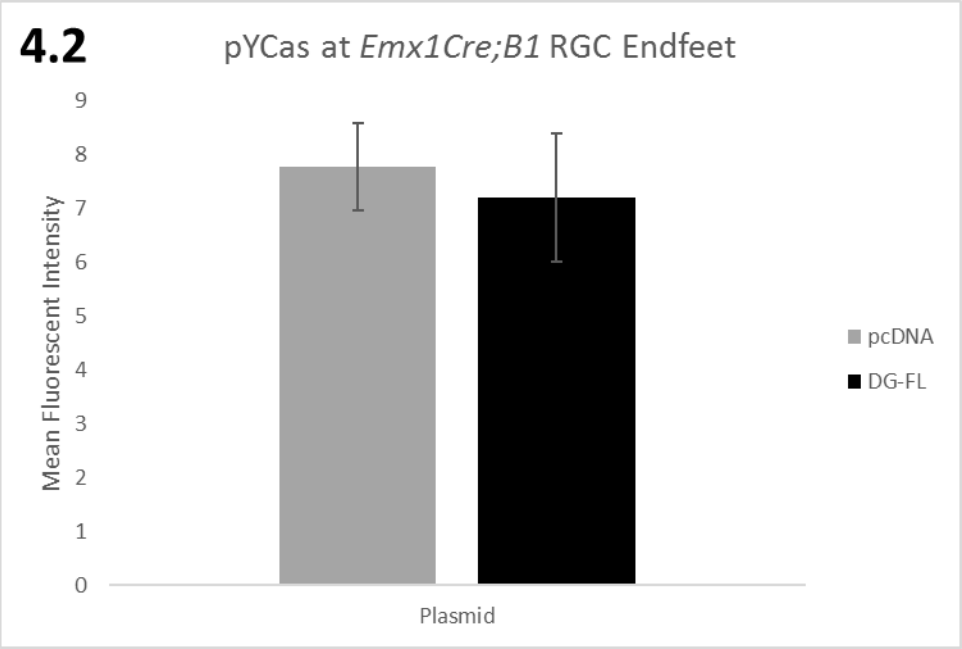


Figure 7C. Emx1Cre;B1 radial glial cells transfected with the empty vector or overexpressing DG does not affect Cas phosphorylation. Zoomed in example of a radial glial endfeet is to the right of each image. Scale bar is 25 um for wide view and 1 um for zoomed image.



Graph 4.2 Mean fluorescent intensity of pYCas165 at Emx1Cre;B1 RGC endfeet. Cells missing β1 integrin were transfected with either an empty vector (pcDNA3.1) or Dystroglycan (DG-FL). There is no difference in pYCas165 expression. The previously seen increase in pYCas when DG is localized to the endfeet in WT RGCs is diminished. DG-dependent recruitment of pYCas may require β1 integrin signaling. Values given are Mean ± S.E.M., n=2 independent samples per group (two-tailed independent samples t-test). No significant difference observed.

Chapter 5: Discussion

The developmental events that lead to the formation of the six-layered cortical plate provide a great example of how adhesion signaling plays a role in cortical lamination. These complex processes involve proliferation of progenitor cells in the dorsal telencephalon, radial migration of post-mitotic neurons from the subventricular zone (SVZ) and ventricular zone (VZ) to the cortical plate (CP), integration of neurons according to birthdate and establishment of connections (Molyneaux et al., 2007; Kwan et al., 2012). Disruptions to normal corticogenesis cause severe developmental disorders with debilitating consequences. Cas signaling adaptor proteins are critical for cell-ECM adhesion signaling in other systems (Vuori & Ruoslahti, 1995; Vuori et al., 1996; Garcia-Guzman et al., 1999), making them good candidates to study how cells interact and interpret adhesion to the ECM during these important developmental events. Different models demonstrate the evolutionarily conserved requirement for Cas adaptor proteins in cell migration and axon pathfinding: mammalian retina organization (Riccomagno & Sun et al., 2014), *Dcas* in Drosophila development (Huang et al., 2007), and Cas for PNS axon guidance and fasciculation in mice (Vahedi-Hunter et al., 2019). Given the ability of Cas to interpret guidance cues and transduce signals in the ECM for cell migration, we hypothesized that these adaptor proteins may be involved in laminar organization of the cortex.

Cas mRNA transcripts are expressed in the dorsal telencephalon during the neurogenic period and more specifically, Cas proteins are found in regions of the cortex

important for cell proliferation and neuronal migration (Fig. 1). The absence of *Cas* genes resulted in a cobblestone cortex, ectopic proliferation, and a discontinuation of the basal lamina (Fig. 2). Neurons migrated into wrong target layers and into the meninges. The cortical layers are not uniform and generated a wave-like pattern. Fate-mapping studies demonstrated the disorganization is not from cell misspecification, as EdU+ ectopias were found outside of the basal lamina, in regions where the MZ is exposed (Fig. 3). Based on all these data we concluded that *Cas* is required for proper neuronal migration to laminate the cortex.

To explore the how *Cas* plays a role in neuronal migration, early cortical events such as the preplate (PP) split were investigated (Fig. 3). Improper splitting of the preplate can contribute to errors in migration (Hevner et al., 2001). The subplate is one of the boundaries to ensure neurons migrate into the CP, whereas the marginal zone (MZ) ensures neurons stop migration (Kanold & Luhmann, 2010). Calretinin and calbindin clustered in the MZ can alter radial glial cell morphology and neuronal responses (Götz et al., 2002; Hartfuss et al., 2003; Radakovits et al., 2009). Subplate markers demonstrated a discontinuity in the ventral boundary, with MAP2+ and CSPG+ cells positioned in the upper CP and MZ. This highly suggests that improper preplate splitting contributed to the migration defect in *Cas* mutants.

Evidence suggests that the defects observed in cortical lamination may not be due to a cell-autonomous role for *Cas* in migrating neurons, but is rather an indirect consequence. To determine whether *Cas* is required in neurons to migrate properly, we

performed cell-autonomous studies by comparing the cortical phenotype of two different Cre lines that remove *Cas* functions in different cell types. Disorganization of projection neurons in the cortex and breaches in the basement membrane characteristic of the cobblestone malformation observed in *Emx1Cre;CasTcKO* mutants (Fig. 2) are not observed in cortices where *Cas* is ablated in early post-miotic, premigratory neurons (*NexCre;CasTcKO*; Fig. 4). The lack of an obvert cortical lamination phenotype in *NexCre;CasTcKO* animals suggest that *Cas* genes act in a non-neuronal autonomous manner. This crucial finding indicates that the cobblestone phenotype observed is likely a consequence of radial glial cell dysfunction and not neurons.

Radial glial cells (RGCs) are multipotent progenitors that can generate numerous cell subtypes, while also providing a scaffold for migrating neurons. Disruption to the direct interface between radial glial cells and the pial basement membrane (PBM) provide a basis for understanding the pathogenesis of neuronal migration disorders. The basal process connects radial glial cells to the basement, where it can act as a scaffold for neurons during glial-dependent migration. We can examine the molecular mechanisms regulating adhesion of the radial glial basal process to understand how the disconnection alters cortical lamination. Ligand-receptor pairs, such as laminin binding to 66dystroglycan and integrin transmembrane receptors are important to maintain the basement membrane (Raghavan et al., 2000; Graus-Porta et al., 2001; Henry et al., 2001; Satz et al., 2010; Devisme et al., 2012; Myshrall et al., 2012). However, little was known about how these signals are interpreted inside RGCs.

Examination of the role of *Cas* in radial glial cells during corticogenesis provided valuable insight into how RGCs transduce these signals, and how their dysfunction alters normal cortical development. Phosphorylated Cas is expressed where the RGC endfeet contact the basement membrane to establish the glia limitans and in cell bodies localized to the VZ (Fig. 5). Morphological changes to *Emx1Cre;CasTcKO* RGCs include disarrayed basal processes and truncated fibers at sites where the basement membrane is breached. This likely contributes to the overmigration of cells into the meninges. More importantly, short pulse chase experiments demonstrated the presence of ectopic proliferating cells in the upper CP and invading heterotopias outside of the MZ of *Emx1Cre;CasTcKO* cortices, something that is never observed in controls (Fig. 5). EdU+/proliferating cells in the upper CP (outside of the SVZ or VZ) can change how cells respond to cues, and can lead to additional problems (Guan & Rao, 2003; Marín et al., 2010; Nakagawa et al., 2015). These major disruptions show that *Cas* genes are required in radial glial cells to regulate neuronal migration, and that the defects in cortical lamination observed in *Emx1Cre;CasTcKO* animals are a subsequent consequence to defects in radial glial cells.

The pathogenesis of cobblestone malformations studied in several mouse models with targeted mutation of genes show PBM-RG interactions are mediated by integrins and DG. Human cobblestone disorders demonstrate ECM receptor signaling is attenuated as a result of deficient glycosylation of 67dystroglycan (Devisme et al., 2012). Dystroglycan mediates PBM-RGC signaling interaction through extracellular

glycoproteins such as laminin binding to the alpha subunit of DG in the dystrophin glycoprotein complex (Henry & Campbell, 1998; Yurchenco, 2010).

Dysfunction of radial glial processes in Dystroglycan mutants (*Emx1Cre;DG*) altered the interaction between the basal endfeet and pial basement membrane (Fig. 6; Myshrall et al., 2012). The pial basement membrane disintegration in DG KO resulted in the formation of meningeal heterotopia. However, the ectopic distribution and migration of progenitors and neurons did not affect the layer-specific differentiation of projection neurons (Myshrall et al., 2012). The cobblestone malformation observed in *CasTcKO* mutants mirrors dystroglycanopathies (Devisme et al., 2012). Embryonic events including a breakage in the basal lamina, ectopic proliferating cells and neuronal clusters in the meninges, and cortical delamination observed in *CasTcKO* mice match the phenotype characterized in DG mutants (Myshrall et al., 2012). Investigating *DG* and *Cas* signaling can provide mechanistic insight into how the pial basement membrane-radial glial interaction is important for cortical lamination.

To visualize the fine basal processes on radial glial cells, a primary culture approach was taken to explore whether Cas adaptor proteins may be a cytoplasmic effector of DG (Fig. 6). Dystroglycan expression in radial glial cells are localized to the cell body and endfeet, consistent with previous data (Myshrall et al., 2012). Endogenous phosphorylated Cas puncta are visible at adhesion sites between cells. Overexpression of Dystroglycan prominently recruited phosphorylated Cas to the club-like radial glial endfeet. This excitingly shows Cas phosphorylation at radial glial endfeet, is downstream

of DG signaling. DG-Cas signaling may be an important molecular mechanism to maintain the basement membrane integrity to allow proper neuronal migration.

Of interest, Cas phosphorylation and function during retinal ganglion cell layer organization (Riccomagno & Sun et al., 2014) is mainly regulated by β1 integrin. DG-Cas interact at the basal endfeet and perhaps, Integrin-Cas signaling may also be crucial in PBM-RG interactions. Integrins function to mediate cell-extracellular matrix and cell-cell interactions (Anton et al., 1999; Henry et al., 2001; Chodniewicz & Klemke, 2004). Upon removal of the β1 subunit of integrins (Graus-Porta et al., 2001), the cortex appears very similar to *Cas* mutants. The cobblestone phenotype of highly disorganized neuronal subpopulations in the cortical plate is similarly distributed in *NestinCre;B1* (Graus-Porta et al., 2001), *Emx1Cre;B1* (Fig. 7.), and *Emx1Cre;CasTcKO* animals. *NexCre;B1* and *NexCre;CasTcKO* do not have lamination defects, indicating β1 integrin and Cas functions are essential in radial glia and not neurons for proper neuronal migration. The very similar phenotype of their respective null mutants and molecular epistasis results in other models, suggest β1 integrin may also act as the primary regulator of Cas function in the neocortex.

The RGC/neuronal mixed culture experiments determined that dystroglycan can modulate Cas phosphorylation (pYCas165). Interestingly, genetic removal of β1 integrin significantly reduced the effect of DG overexpression on pYCas165 levels at the endfeet (Fig. 7). Overall, these data provide a direct link between Cas phosphorylation and both

70dystroglycan and integrin, and suggest that DG and integrin act upstream of Cas proteins in radial glia to regulate cortical lamination.

Migration is a complex interplay of receptor-ligand interactions mediated by membrane proteins (Huttenlocher et al., 1996; Henry et al., 2001; Hansen & Hippenmeyer et al., 2020). The cortical phenotype in *CasTcKO* mutants appear very similar to DG and β1 integrin mutants. Cas proteins are not required in neurons for proper radial migration, but are rather essential in radial glial cells for cortical lamination. Our data support a model whereby Cas adaptor proteins act as signal transductors downstream of the β1 integrin and dystroglycan transmembrane protein in a non-neuronal autonomous manner to maintain the integrity of the basement membrane and allow for normal neocortical development. The absence of DG or β1 integrin in radial glial cells affected how the endfeet interacts the basement membrane and affected downstream signaling of Cas to remodel the actin cytoskeleton. This adhesion signaling pathway can be used as a basis to understand Cobblestone Lissencephaly (Fig. 8). Furthermore, results from current findings can be used to explore whether DG and β1 integrin can also act as primary regulators of *Cas* in other model systems and in the *DCas* variant.

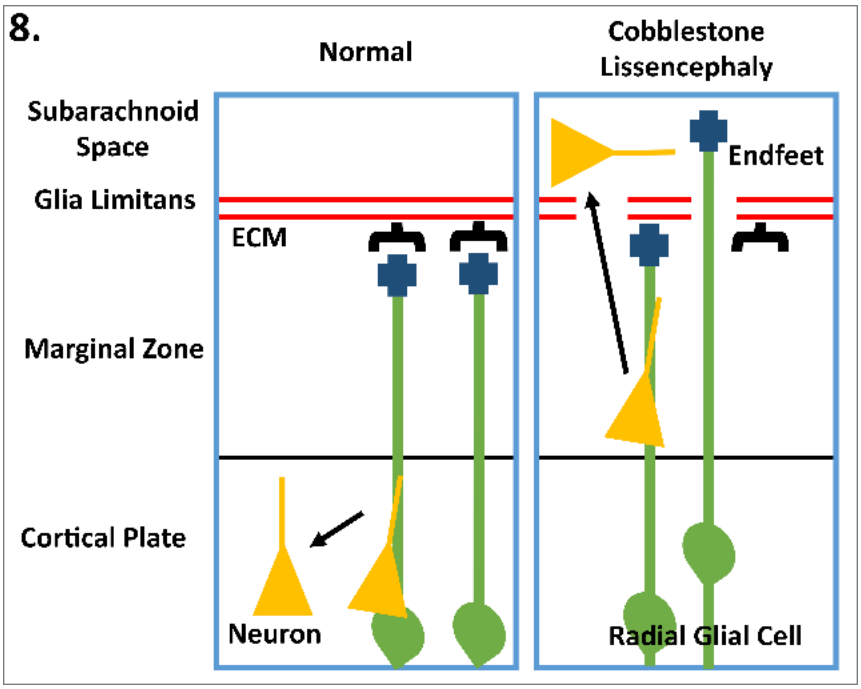


Figure 8. Basement membrane and radial glial endfeet interactions are important to maintain a boundary for migrating neurons. A disruption to the basal lamina resulted in basal processes overextending and neurons overmigrating to generate the Cobblestone Lissencephaly phenotype.

References

Alexandropoulos, K., & Baltimore, D. (1996). Coordinate activation of c-Src by SH3-and SH2-binding sites on a novel, p130(Cas)-related protein, Sin. *Genes and Development*, *10*(11), 1341–1355. https://doi.org/10.1101/gad.10.11.1341

Amin, S., & Borrell, V. (2020). The Extracellular Matrix in the Evolution of Cortical Development and Folding. *Frontiers in cell and developmental biology*, *8*, 604448 https://doi.org/10.3389/fcell.2020.604448.

Anthony, T. E., Klein, C., Fishell, G., & Heintz, N. (2004). Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron*, *41*(6), 881–890. https://doi.org/10.1016/S0896-6273(04)00140-0

Anton, E. S., Kreidberg, J. A., & Rakic, P. (1999). Distinct functions of α3and α(v) integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron*, *22*(2), 277–289. https://doi.org/10.1016/S0896-6273(00)81089-2

Astier, A., Avraham, H., Manie, S. N., Groopman, J., Canty, T., Avraham, S., & Freedman, A. S. (1997). The related adhesion focal tyrosine kinase is tyrosine-phosphorylated after beta1-integrin stimulation in B cells and binds to p130cas. *The Journal of biological chemistry*, *272*(1), 228–232. https://doi.org/10.1074/jbc.272.1.228

Ayala, R., Shu, T., & Tsai, L. H. (2007). Trekking across the Brain: The Journey of Neuronal Migration. *Cell*. https://doi.org/10.1016/j.cell.2006.12.021

Beggs, H. E., Schahin-Reed, D., Zang, K., Goebbels, S., Nave, K. A., Gorski, J., Jones, K. R., Sretavan, D., & Reichardt, L. F. (2003). FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron*, *40*(3), 501–514. https://doi.org/10.1016/S0896-6273(03)00666-4

Belvindrah, R., Graus-Porta, D., Goebbels, S., Nave, K.-A., & Muller, U. (2007). B1 Integrins in Radial Glia But Not in Migrating Neurons Are Essential for the Formation of Cell Layers in the Cerebral Cortex. *Journal of Neuroscience*, *27*(50), 13854–13865. https://doi.org/10.1523/JNEUROSCI.4494-07.2007

Birge, R. B., Kalodimos, C., Inagaki, F., & Tanaka, S. (2009). Crk and CrkL adaptor proteins: Networks for physiological and pathological signaling. *Cell Communication and Signaling*. https://doi.org/10.1186/1478-811X-7-13

Bouton, A. H., Riggins, R. B., & Bruce-Staskal, P. J. (2001). Functions of the adapter protein Cas: Signal convergence and the determination of cellular responses. *Oncogene*, *20*(44), 6448–6458. https://doi.org/10.1038/sj.onc.1204785

Burnham, M. R., Harte, M. T., Richardson, A., Parsons, J. T., & Bouton, A. H. (1996). The identification of p130cas-binding proteins and their role in cellular transformation. *Oncogene*, *12*(11), 2467–2472.

Cabodi, S., Tinnirello, A., Di Stefano, P., Bisarò, B., Ambrosino, E., Castellano, I., Sapino, A., Arisio, R., Cavallo, F., Forni, G., Glukhova, M., Silengo, L., Altruda, F., Turco, E., Tarone, G., & Defilippi, P. (2006). p130Cas as a new regulator of mammary epithelial cell proliferation, survival, and HER2-Neu oncogene- dependent breast tumorigenesis. *Cancer Research*, *66*(9), 4672–4680. https://doi.org/10.1158/0008-5472.CAN-05-2909

Calalb, M. B., Polte, T. R., & Hanks, S. K. (1995). Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Molecular and cellular biology*, *15*(2), 954–963. https://doi.org/10.1128/MCB.15.2.954

Caviness, V. S., & Takahashi, T. (1995). Proliferative events in the cerebral ventricular zone. *Brain and Development*, *17*(3), 159–163. https://doi.org/10.1016/0387-7604(95)00029-B

Cayre, M., Canoll, P., & Goldman, J. E. (2009). Cell migration in the normal and pathological postnatal mammalian brain. *Progress in neurobiology*, *88*(1), 41–63. https://doi.org/10.1016/j.pneurobio.2009.02.001

Cho, S. Y., & Klemke, R. L. (2000). Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration and suppress apoptosis during invasion of the extracellular matrix. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.149.1.223

Chodniewicz, D., & Klemke, R. L. (2004). Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. *Biochimica et Biophysica Acta - Molecular Cell Research*. https://doi.org/10.1016/j.bbamcr.2004.03.006

Culican, S. M., Baumrind, N. L., Yamamoto, M., & Pearlman, A. L. (1990). Cortical radial glia: identification in tissue culture and evidence for their transformation to astrocytes. *Journal of Neuroscience, 10*(2), 684-692. https://doi.org/10.1523/JNEUROSCI.10-02-00684.1990

D'Arcangelo, G., G. Miao, G., Chen, SC. Scares, H. D., Morgan, J. I., & Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant *reeler*. *Nature* 374, 719–723. https://doi.org/10.1038/374719a0

Defilippi, P., Di Stefano, P., & Cabodi, S. (2006). p130Cas: a versatile scaffold in signaling networks. *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2006.03.003

del Río, J. A., Martínez, A., Fonseca, M., Auladell, C., & Soriano, E. (1995). Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. *Cerebral cortex (New York, N.Y. : 1991)*, *5*(1), 13–21. https://doi.org/10.1093/cercor/5.1.13

Deneka, A., Korobeynikov, V., & Golemis, E. A. (2015). Embryonal Fyn-associated substrate (EFS) and CASS4: The lesser-known CAS protein family members. *Gene*, *570*(1), 25–35. https://doi.org/10.1016/j.gene.2015.06.062

Devisme, L., Bouchet, C., Gonzals, M., Alanio, E., Bazin, A., Bessires, B., Bigi, N., Blanchet, P., Bonneau, D., Bonnieres, M., Bucourt, M., Carles, D., Clarisse, B., Delahaye, S., Fallet-Bianco, C., Figarella-Branger, D., Gaillar, D., Gasser, B., Delezoide, A., … & Encha-Razavi, F. (2012). Cobblestone lissencephaly: Neuropathological subtypes and correlations with genes of dystroglycanopathies. *Brain*. https://doi.org/10.1093/brain/awr357

Di Bella, D. J., Habibi, E., Stickels, R. R., Scalia, G., Brown, J., Yadollahpour, P., Yang, S. M., Abbate, C., Biancalani, T., Macosko, E. Z., Chen, F., Regev, A., & Arlotta, P. (2021). Molecular logic of cellular diversification in the mouse cerebral cortex. *Nature*, *595*(7868), 554–559. https://doi.org/10.1038/s41586-021-03670-5

Di Stefano, P., Leal, M. P., Tornillo, G., Bisaro, B., Repetto, D., Pincini, A., Santopietro, E., Sharma, N., Turco, E., Cabodi, S., & Defilippi, P. (2011). The adaptor proteins p140CAP and p130CAS as molecular hubs in cell migration and invasion of cancer cells. *American journal of cancer research*, *1*(5), 663–673.

Donlin, L. T., Danzl, N. M., Wanjalla, C., & Alexandropoulos, K. (2005). Deficiency in Expression of the Signaling Protein Sin/Efs Leads to T-Lymphocyte Activation and Mucosal Inflammation. *Molecular and Cellular Biology* https://doi.org/10.1128/MCB.25.24.11035-11046.2005.

Espinosa, A., Gil-Sanz, C., Yanagawa, Y., & Fairén, A. (2009). Two separate subtypes of early non-subplate projection neurons in the developing cerebral cortex of rodents. *Frontiers in neuroanatomy*, *3*, 27. https://doi.org/10.3389/neuro.05.027.2009

Feller, S. M. (2001). CrK family adaptors-signalling complex formation and biological roles. *Oncogene*. https://doi.org/10.1038/sj.onc.1204779

Franco, S. J., Gil-Sanz, C., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S. R., Ramos, C., & Müller, U. (2012). Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science (New York, N.Y.)*, *337*(6095), 746–749. https://doi.org/10.1126/science.1223616

Garcia-Guzman, M., Dolfi, F., Russello, M., & Vuori, K. (1999). Cell adhesion regulates the interaction between the docking protein p130(Cas) and the 14-3-3 proteins. *J Biol Chem*, 274:5762-5768. https://doi.org/10.1074/jbc.274.9.5762

Georges-Labouesse, E., Mark, M., Messaddeq, N., & Gansmüller, A. (1998). *Essential role of α6 integrins in cortical and retinal lamination*. *Current Biology* (Vol. 8). https://doi.org/10.1016/s0960-9822(98)70402-6

Giger, R. J., Cloutier, J. F., Sahay, A., Prinjha, R. K., Levengood, D. V., Moore, S. E., Pickering, S., Simmons, D., Rastan, S., Walsh, F. S., Kolodkin, A. L., Ginty, D. D., & Geppert, M. (2000). Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron 25, 29–41. https://doi.org/10.1016/S0896-6273(00)80869-7

Gil-Sanz, C., Franco, S. J., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S., & Müller, U. (2013). Cajal-Retzius cells instruct neuronal migration by coincidence signaling between secreted and contact-dependent guidance cues. *Neuron*. https://doi.org/10.1016/j.neuron.2013.06.040

Goebbels, S., Bormuth, I., Bode, U., Hermanson, O., Schwab, M. H., & Nave, K. A. (2006). Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. *Genesis*, *44*(12), 611–621. https://doi.org/10.1002/dvg.20256

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., & Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature*. https://doi.org/10.1038/nature02033

Gorski, J. A., Talley, T., Qiu, M., Puelles, L., Rubenstein, J. L. R., & Jones, K. R. (2002). Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(15), 6309–6314. https://doi.org/20026564

Götz, M., Hartfuss, E., & Malatesta, P. (2002). Radial glial cells as neuronal precursors: A new perspective on the correlation of morphology and lineage restriction in the developing cerebral cortex of mice. *Brain Research Bulletin*, Vol. 57, 777–788. https://doi.org/10.1016/S0361-9230(01)00777-8

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., Orban, P., Klein, R., Schittny, J. C., & Müller, U. (2001). Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron*, *31*(3), 367–379. https://doi.org/10.1016/s0896-6273(01)00374-9

Guan, K. L., & Rao, Y. (2003). Signalling mechanisms mediating neuronal responses to guidance cues. *Nat Rev Neurosci* 4, 941–956. https://doi.org/10.1038/nrn1254

Halfter, W., Dong, S., Yip, Y. P., Willem, M., & Mayer, U. (2002). A critical function of the pial basement membrane in cortical histogenesis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(14), 6029–6040. https://doi.org/10.1523/JNEUROSCI.22-14-06029.2002

Hansen, A. H., & Hippenmeyer, S. (2020). Non-Cell-Autonomous Mechanisms in Radial Projection Neuron Migration in the Developing Cerebral Cortex. *Frontiers in Cell and Developmental Biology*, *8*, 1–13. https://doi.org/10.3389/fcell.2020.574382

Harte, M. T., Hildebrand, J. D., Burnham, M. R., Bouton, A. H., & Parsons, J. T. (1996). P130Cas, a substrate associated with v-Src and v-Crk, localizes to focal adhesions and binds to focal adhesion kinase. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.271.23.13649

Hartfuss, E., Förster, E., Bock, H. H., Hack, M. A., Leprince, P., Lugue, J. M., Herz, J., Frotscher, M., & Götz, M. (2003). Reelin signaling directly affects radial glia morphology and biochemical maturation. *Development*, *130*(19), 4597–4609. https://doi.org/10.1242/dev.00654

Hatten M. E. (1999). Central nervous system neuronal migration. *Annual review of neuroscience*, *22*, 511–539. https://doi.org/10.1146/annurev.neuro.22.1.511

Haubst, N., Georges-Labouesse, E., Arcangelis, A. D., Mayer, U., & Götz, M. (2006). Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. *Development*, 133 (16): 3245–3254. https://doi.org/10.1242/dev.02486

Heintz, N. (2004). Gene Expression Nervous System Atlas (GENSAT). *Nature Neuroscience*. https://doi.org/10.1038/nn0504-483

Henry, M. D., & Campbell, K. P. (1998). A role for dystroglycan in basement membrane assembly. *Cell*, *95*(6), 859–870. https://doi.org/10.1016/s0092-8674(00)81708-0

Henry, M. D., Satz, J. S., Brakebusch, C., Costell, M., Gustafsson, E., Fässler, R., & Campbell, K. P. (2001). Distinct roles for dystroglycan, beta1 integrin and perlecan in cell surface laminin organization. *Journal of cell science*, *114*(Pt 6), 1137–1144. https://doi.org/10.1242/jcs.114.6.1137

Hevner, R. F., Daza, R. A., Rubenstein, J. L., Stunnenberg, H., Olavarria, J. F., & Englund, C. (2003). Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons. *Developmental neuroscience*, *25*(2-4), 139–151. https://doi.org/10.1159/000072263

Hevner, R. F., Shi, L., Justice, N., Hsueh, Y. P., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T., & Rubenstein, J. L. R. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron*, *29*(2), 353–366. https://doi.org/10.1016/S0896-6273(01)00211-2

Hewitt, J. E. (2009). Abnormal glycosylation of dystroglycan in human genetic disease. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1792*(9), 853–861. https://doi.org/10.1016/j.bbadis.2009.06.003

Hirota, Y., & Kazunori, N. (2017). Control of neuronal migration and aggregation by reelin signlaing in the developing cerebral cortex. *Frontiers in Cell and Developmental Biology, 5*(40). https://doi.org/10.3389/fcell.2017.00040

Honda, H., Nakamoto, T., Sakai, R., & Hirai, H. (1999). P130(Cas), an assembling molecule of actin filaments, promotes cell movement, cell migration, and cell spreading in fibroblasts. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1006/bbrc.1999.1162

Honda, H., Oda, H., Nakamoto, T., Honda, Z. I., Sakai, R., Suzuki, T., Saito, T., Nakamura, K., Nakao, K., Ishikawa, t., Katsuki, M., Yazaki, Y., & Hirai, H. (1998). Cardiovascular

anomaly, impaired actin bundling and resistance to Src- induced transformation in mice lacking p130(Cas). *Nature Genetics*. https://doi.org/10.1038/1246

Hu, H., Yang, Y., Eade, A., Xiong, Y., & Qi, Y. (2007). Breaches of the pial basement membrane and disappearance of the glia limitans during development underlie the cortical lamination defect in the mouse model of muscle-eye-brain disease. *The Journal of comparative neurology*, *501*(1), 168–183. https://doi.org/10.1002/cne.21238

Huang, Z., Yazdani, U., Thompson-Peer, K. L., Kolodkin, A. L., & Terman, J. R. (2007). Crk-associated substrate (Cas) signaling protein functions with integrins to specify axon guidance during development. *Development (Cambridge, England)*, *134*(12), 2337–2347. https://doi.org/10.1242/dev.004242

Huttenlocher, A., Ginsberg, M. H., & Horwitz, A. F. (1996). Modulation of cell migration by integrin-mediated cytoskeletal linkages and ligand-binding affinity. *The Journal of cell biology*, *134*(6), 1551–1562. https://doi.org/10.1083/jcb.134.6.1551

Kanold, P. O., & Luhmann, H. J. (2010). The subplate and early cortical circuits. *Annual review of neuroscience*, *33*, 23–48. https://doi.org/10.1146/annurev-neuro-060909-153244

Kawaguchi, A. (2021). Neuronal Delamination and Outer Radial Glia Generation in Neocortical Development. *Frontiers in Cell and Developmental Biology*, *8*, 1–10. https://doi.org/10.3389/fcell.2020.623573

Kawauchi T. (2011). Regulation of cell adhesion and migration in cortical neurons: Not only Rho but also Rab family small GTPases. *Small GTPases*, *2*(1), 36–40. https://doi.org/10.4161/sgtp.2.1.15001

Kim, H. J., & Magrané, J. (2011). Isolation and culture of neurons and astrocytes from the mouse brain cortex. *Methods in molecular biology*, *793*, 63–75. https://doi.org/10.1007/978-1-61779-328-8_4

Kim, D. S., Hayashi, Y. K., Matsumoto, H., Ogawa, M., Noguchi, S., Murakami, N., Sakuta, R., Mochizuki, M., Michele, D. E., Campbell, K. P., Nonaka, I., & Nishino, I. (2004). POMT1 mutation results in defective glycosylation and loss of laminin-binding activity in alpha-DG. *Neurology*, *62*(6), 1009–1011. https://doi.org/10.1212/01.wnl.0000115386.28769.65

Klemke, R. L., Leng, J., Molander, R., Brooks, P. C., Vuori, K., & Cheresh, D. A. (1998). CAS/Crk coupling serves as a “molecular switch” for induction of cell migration. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.140.4.961

Kriegstein, A. R., & Noctor, S. C. (2004). Patterns of neuronal migration in the embryonic cortex. *Trends in neurosciences*, *27*(7), 392–399. https://doi.org/10.1016/j.tins.2004.05.001

Kwan, K. Y., Sestan, N., & Anton, E. S. (2012). Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development*. https://doi.org/10.1242/dev.069963

Kong, C., Wang, C., Wang, L., Ma, M., Niu, C., Sun, X., Du, J., Dong, Z., Zhu, S., Lu, J., & Huang, B. (2011). NEDD9 is a positive regulator of epithelial-mesenchymal transition and promotes invasion in aggressive breast cancer. *PloS one*, *6*(7), e22666. https://doi.org/10.1371/journal.pone.0022666

Liu, G., Li, W., Gao, X., Li, X., Jürgensen, C., Park, H. T., Shin, N. Y., Yu, J., He, M. L., Hanks, S. K., Wu, J. Y., Guan, K. L., & Rao, Y. (2007). p130CAS is required for netrin signaling and commissural axon guidance. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(4), 957–968. https://doi.org/10.1523/JNEUROSCI.4616-06.2007

Lindenmaier, L. B., Parmentier, N., Guo, C., Tissir, F., & Wright, K. M. (2019). Dystroglycan is a scaffold for extracellular axon guidance decisions. *ELife*, *8*, 1–26. https://doi.org/10.7554/eLife.42143

Magdaleno, S., Keshvara, L., & Curran, T. (2002). Rescue of ataxia and preplate splitting by ectopic expression of Reelin in reeler mice. *Neuron*, *33*(4), 573–586. https://doi.org/10.1016/s0896-6273(02)00582-2

Malatesta, P., Hack, M. A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., & Götz, M. (2003). Neuronal or glial progeny: regional differences in radial glia fate. *Neuron*, *37*(5), 751–764. https://doi.org/10.1016/s0896-6273(03)00116-8.

Marín, O., & Rubenstein, J. L. R. (2003). Cell migration in the forebrain. *Annual Review of Neuroscience*, *26*, 441–483. https://doi.org/10.1146/annurev.neuro.26.041002.131058

Marín, O., Valiente, M., Ge, X., & Tsai, L. H. (2010). Guiding neuronal cell migrations. *Cold Spring Harbor perspectives in biology*, *2*(2), a001834. https://doi.org/10.1101/cshperspect.a001834

Markó, K., Köhidi, T., Hádinger, N., Jelitai, M., Mezö, G., & Madarász, E. (2011). Isolation of radial glia-like neural stem cells from fetal and adult mouse forebrain via selective adhesion to a novel adhesive peptide-conjugate. *PLoS ONE*, *6*(12). https://doi.org/10.1371/journal.pone.0028538

Meenderink, L. M., Ryzhova, L. M., Donato, D. M., Gochberg, D. F., Kaverina, I., & Hanks, S. K. (2010). P130Cas Src-binding and substrate domains have distinct roles in sustaining focal adhesion disassembly and promoting cell migration. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0013412

Merrill, R. A., See, A. W. M., Wertheim, M. L., & Clagett-Dame, M. (2004). Crk-associated substrate (Cas) family member, NEDD9, is regulated in human neuroblastoma cells and in the embryonic hindbrain by all-trans retinoic acid. *Developmental Dynamics*. https://doi.org/10.1002/dvdy.20159

Meyer, G., Soria, J. M., Martínez-Galán, J. R., Martín-Clemente, B., & Fairén, A. (1998). Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *The Journal of comparative neurology*, *397*(4), 493–518. https://doi.org/10.1002/(SICI)1096-9861(19980810)397:4<493::AID-CNE4>3.0.CO;2-X

Miao, G. G., Smeyne, R. J., D'Arcangelo, G., Copeland, N. G., Jenkins, N. A., Morgan, J. I., & Curran, T. (1994). Isolation of an allele of reeler by insertional mutagenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(23), 11050–11054. https://doi.org/10.1073/pnas.91.23.11050

Michele, D. E., Barresi, R., Kanagawa, M., Saito, F., Cohn, R. D., Satz, J. S., Dollar, J., Nishino, I., Kelley, R. I., Somer, H., Straub, V., Mathews, K. D., Moore, S. A., & Campbell, K. P. (2002). Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature*, *418*(6896), 417–422. https://doi.org/10.1038/nature00837

Miller, D. S., & Wright, K. M. (2021). Neuronal Dystroglycan regulates postnatal development of CCK/cannabinoid receptor-1 interneurons. *Neural development*, *16*(1), 4. https://doi.org/10.1186/s13064-021-00153-1

Miyata, T. (2004). Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development*. https://doi.org/10.1242/dev.01173

Molyneaux, B. J., Arlotta, P., Menezes, J. R. L., & Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2151

Moore, S. A., Saito, F., Chen, J., Michele, D. E., Henry, M. D., Messing, A., Cohn, R. D., Ross-Barta, S. E., Westra, S., Williamson, R. A., Hoshi, T., & Campbell, K. P. (2002). Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. *Nature*, *418*(6896), 422–425. https://doi.org/10.1038/nature00838

Myshrall, T. D., Moore, S. A., Ostendorf, A. P., Satz, J. S., Kowalczyk, T., Nguyen, H., Daza, R. A., Lau, C., Campbell, K. P., & Hevner, R. F. (2012). Dystroglycan on radial glia end feet is required for pial basement membrane integrity and columnar organization of the developing cerebral cortex. *Journal of neuropathology and experimental neurology*, *71*(12), 1047–1063. https://doi.org/10.1097/NEN.0b013e318274a128

Nadarajah, B., Alifragis, P., Wong, R. O. L., & Parnavelas, J. G. (2003). Neuronal migration in the developing cerebral cortex: Observations based on real-time imaging. *Cerebral Cortex*. https://doi.org/10.1093/cercor/13.6.607

Nakagawa, N., Yagi, H., Kato, K., Takematsu, H., & Oka, S. (2015). Ectopic clustering of Cajal–Retzius and subplate cells is an initial pathological feature in *Pomgnt2*-knockout mice, a model of dystroglycanopathy. *Sci Rep* **5,** 11163. https://doi.org/10.1038/srep11163

Nasertorabi, F., Garcia-Guzman, M., Briknarová, K., Larsen, E., Havert, M. L., Vuori, K., & Ely, K. R. (2004). Organization of functional domains in the docking protein p130Cas. *Biochemical and biophysical research communications*, *324*(3), 993–998. https://doi.org/10.1016/j.bbrc.2004.09.148

Nichols, A. J., & Olson, E. C. (2010). Reelin promotes neuronal orientation and dendritogenesis during preplate splitting. *Cerebral cortex (New York, N.Y. : 1991)*, *20*(9), 2213–2223. https://doi.org/10.1093/cercor/bhp303

Nickolls, A.R., & Bönnemann. C. G. (2018). The roles of dystroglycan in the nervous system: insights from animal models of muscular dystrophy. *Dis Model Mech*, 11 (12): dmm035931. https://doi.org/10.1242/dmm.035931

Niewmierzycka, A., Mills, J., St-Arnaud, R., Dedhar, S., & Reichardt, L. F. (2005). Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*. https://doi.org/10.1523/JNEUROSCI.1695-05.2005

Nowakowski, T. J., Pollen, A. A., Sandoval-Espinosa, C., & Kriegstein, A. R. (2016). Transformation of the Radial Glia Scaffold Demarcates Two Stages of Human Cerebral Cortex Development. *Neuron*, *91*(6), 1219–1227. https://doi.org/10.1016/j.neuron.2016.09.005

O'Neill, G. M., Fashena, S. J., & Golemis, E. A. (2000). Integrin signalling: A new Cas(t) of characters enters the stage. *Trends in Cell Biology*. https://doi.org/10.1016/S0962-8924(99)01714-6

Olson, E. C. (2014). Analysis of preplate splitting and early cortical development illuminates the biology of neurological disease. *Frontiers in Pediatrics*, *2*(NOV), 1–9. https://doi.org/10.3389/fped.2014.00121

Park, T.-J., & Curran, T. (2008). Crk and Crk-Like Play Essential Overlapping Roles Downstream of Disabled-1 in the Reelin Pathway. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.4323-08.2008

Peng, X., Kraus, M. S., Wei, H., Shen, T. L., Pariaut, R., Alcaraz, A., Ji, G., Cheng, L., Yang, Q., Kotlikoff, M. I., Chen, J., Chien, K., Gu, H., & Guan, J. L. (2006). Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *The Journal of clinical investigation*, *116*(1), 217–227. https://doi.org/10.1172/JCI24497

Polleux, F., & Ghosh, A. (2002). The Slice Overlay Assay: A Versatile Tool to Study the Influence of Extracellular Signals on Neuronal Development. *Science Signaling*. https://doi.org/10.1126/stke.2002.136.pl9

Radakovits, R., Barros, C. S., Belvindrah, R., Patton, B., & Müller, U. (2009). Regulation of radial glial survival by signals from the meninges. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(24), 7694–7705. https://doi.org/10.1523/JNEUROSCI.5537-08.2009

Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *Journal of Comparative Neurology*, *145*(1), 61–83. https://doi.org/10.1002/cne.901450105

Rakic P. (1982). Early developmental events: cell lineages, acquisition of neuronal positions, and areal and laminar development. *Neurosciences Research Program bulletin*, *20*(4), 439–451.

Raghavan, S., Bauer, C., Mundschau, G., Li, Q., & Fuchs, E. (2000). Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. *The Journal of cell biology*, *150*(5), 1149–1160. https://doi.org/10.1083/jcb.150.5.1149

Riccomagno, M. M., Sun, L. O., Brady, C. M., Alexandropoulos, K., Seo, S., Kurokawa, M., & Kolodkin, A. L. (2014). Cas adaptor proteins organize the retinal ganglion cell layer downstream of integrin signaling. *Neuron*. https://doi.org/10.1016/j.neuron.2014.01.036

Rice, D. S., & Curran, T. (2001). Role of the reelin signaling pathway in central nervous system development. *Annual review of neuroscience*, *24*, 1005–1039. https://doi.org/10.1146/annurev.neuro.24.1.1005

Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parson, J. T., & Horwitz, A. R. (2003). Cell Migration: Integrating Signals from Front to Back. *Science*. https://doi.org/10.1126/science.1092053

Riemersma, M., Mandel, H., van Beusekom, E., Gazzoli, I., Roscioli, T., Eran, A., Gershoni-Baruch, R., Gershoni, M., Pietrokovski, S., Vissers, L. E., Lefeber, D. J., Willemsen, M. A., Wevers, R. A., & van Bokhoven, H. (2015). Absence of α- and β-dystroglycan is associated with Walker-Warburg syndrome. *Neurology*, *84*(21), 2177–2182. https://doi.org/10.1212/WNL.0000000000001615

Salic, A., & Mitchison, T. J. (2008). A chemical method for fast and sensitive detection of DNA synthesis *in vivo. Proceedings of the National Academy of Sciences*, *105*(7), 2415-2420. https://doi.org/10.1073/pnas.0712168105

Satz, J. S., Ostendorf, A. P., Hou, S., Turner, A., Kusano, H., Lee, J. C., Turk, R., Nguyen, H., Ross-Barta, S. E., Westra, S., Hoshi, T., Moore, S. A., & Campbell, K. P. (2010). Distinct functions of glial and neuronal dystroglycan in the developing and adult mouse brain. *Journal of Neuroscience*, *30*(43), 14560–14572. https://doi.org/10.1523/JNEUROSCI.3247-10.2010

Schachter, H., Vajsar, J., & Zhang, W. (2004). The role of defective glycosylation in congenital muscular dystrophy. *Glycoconjugate journal*, *20*(5), 291–300. https://doi.org/10.1023/B:GLYC.0000033626.65127.e4

Sekar, A., Sanches, T. M., Hino, K., Kumar, M., Wang, J., Ha., E, Durbin-Johnson, B., Simo, S., & Dennis, M. Y. (2021). RapID Cell Counter : Semi-automated and mid-throughput estimation of cell density within diverse cortical layers. *Eneuro*, ENEURO.0185–21.2021. https://doi.org/10.1523/ENEURO.0185-21.2021

Seo, S., Asai, T., Saito, T., Suzuki, T., Morishita, Y., Nakamoto, T., Ichikawa, M., Yamamoto, G., Kawazu, M., Yamagata, T., Sakai, R., Mitani, K., Ogawa, S., Kurokawa, M., Chiba, S., & Hirai, H. (2005). Crk-associated substrate lymphocyte type is required for lymphocyte trafficking and marginal zone B cell maintenance. *J. Immunol*. 175 (6): 3492–501. https:/doi.org/10.4049/jimmunol.175.6.3492

Silva, C. G., Peyre, E., & Nguyen, L. (2019). Cell migration promotes dynamic cellular interactions to control cerebral cortex morphogenesis. *Nature Reviews Neuroscience*, *20*(6), 318–329. https://doi.org/10.1038/s41583-019-0148-y

Singh, M. K., Dadke, D., Nicolas, E., Serebriiskii, I. G., Apostolou, S., Canutescu, A., Egleston, B. L., & Golemis, E. A. (2008). A novel Cas family member, HEPL, regulates FAK and cell spreading. *Molecular biology of the cell*, *19*(4), 1627–1636. https://doi.org/10.1091/mbc.e07-09-0953

Stouffer, M. A., Golden, J. A., & Francis, F. (2016). Neuronal migration disorders: Focus on the cytoskeleton and epilepsy. *Neurobiology of disease*, *92*(Pt A), 18–45. https://doi.org/10.1016/j.nbd.2015.08.003

Tabata, H., & Nakajima, K. (2003). Multipolar Migration: The Third Mode of Radial Neuronal Migration in the Developing Cerebral Cortex. *Journal of Neuroscience, 23*(31)*,* 9996-10001. https://doi.org/10.1523/JNEUROSCI.23-31-09996.2003

Takahashi, T., Goto, T., Miyama, S., Nowakowski, R. S., & Caviness, V. S., Jr (1999). Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *19*(23), 10357–10371. https://doi.org/10.1523/JNEUROSCI.19-23-10357.1999

Tessier-Lavigne, M., & Goodman, C. S. (1996). The molecular biology of axon guidance. *Science (New York, N.Y.)*, *274*(5290), 1123–1133. https://doi.org/10.1126/science.274.5290.1123

Vahedi-Hunter, T. A., Estep, J. A., Rosette, K. A., Rutlin, M. L., Wright, K. M., & Riccomagno, M. M. (2018). Cas Adaptor Proteins Coordinate Sensory Axon Fasciculation. *Scientific Reports*. https://doi.org/10.1038/s41598-018-24261-x

Vuori, K., Hirai, H., Aizawa, S., & Ruoslahti, E. (1996). Induction of p130Cas Signaling Complex Formation upon Integrin-Mediated Cell Adhesion: a Role for Src Family Kinases. *Molecular and Cellular Biology*, *16*(6), 2606–2613. https://doi.org/10.1128/MCB.16.6.2606

Vuori, K., & Ruoslahti, E. (1995). Tyrosine phosphorylation of p130Cas and cortactin accompanies integrin-mediated cell adhesion to extracellular matrix. *The Journal of biological chemistry*, *270*(38), 22259–22262. https://doi.org/10.1074/jbc.270.38.22259

Waite, A., Brown, S. C., & Blake, D. J. (2012). The dystrophin-glycoprotein complex in brain development and disease. *Trends in neurosciences*, *35*(8), 487–496. https://doi.org/10.1016/j.tins.2012.04.004

Wang, F., Flanagan, J., Su, N., Wang, L. C., Bui, S., Nielson, A., Wu, X., Vo, H. T., Ma, X. J., & Luo, Y. (2012). RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *The Journal of molecular diagnostics : JMD*, *14*(1), 22–29. https://doi.org/10.1016/j.jmoldx.2011.08.002

Williamson, R. A., Henry, M. D., Daniels, K. J., Hrstka, R. F., Lee, J. C., Sunada, Y., Ibraghimov-Beskrovnaya, O., & Campbell, K. P. (1997). Dystroglycan is essential for early embryonic development: Disruption of Reichert's membrane in Dag1-null mice. *Human Molecular Genetics*, *6*(6), 831–841. https://doi.org/10.1093/hmg/6.6.831

Wong, K., Park, H. T., Wu, J. Y. & Rao, Y. Slit proteins: molecular guidance cues for cells ranging from neurons to leukocytes. *Curr. Opin. Genet. Dev.* **12**, 583–591 (2002). https://doi.org/10.1016/s0959-437x(02)00343-x

Wu, S. X., Goebbels, S., Nakamura, K., Nakamura, K., Kometani, K., Minato, N., Kaneko, T., Nave, K-A., & Tamamaki, N. (2005). Pyramidal neurons of upper cortical layers generated by NEX-positive progenitor cells in the subventricular zone. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(47), 17172–17177. https://doi.org/10.1073/pnas.0508560102

Yeo, M. G., & Song, W. K. (2008). v-Crk regulates membrane dynamics and Rac activation. *Cell adhesion & migration*, *2*(3), 174–176. https://doi.org/10.4161/cam.2.3.6578

Yokota, Y., Gashghaei, H. T., Han, C., Watson, H., Campbell, K. J., & Anton, E. S. (2007). Radial glial dependent and independent dynamics of interneuronal migration in the developing cerebral cortex. *PloS one*, *2*(8), e794. https://doi.org/10.1371/journal.pone.0000794

Yoshida, A., Kobayashi, K., Manya, H., Taniguchi, K., Kano, H., Mizuno, M., Inazu, T., Mitsuhashi, H., Takahashi, S., Takeuchi, M., Herrmann, R., Straub, V., Talim, B., Voit, T., Topaloglu, H., Toda, T., & Endo, T. (2001). Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Developmental cell*, *1*(5), 717–724. https://doi.org/10.1016/s1534-5807(01)00070-3

Yurchenco P. D. (2011). Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harbor perspectives in biology*, *3*(2), a004911. https://doi.org/10.1101/cshperspect.a004911