An adhesive signaling axis regulates the establishment of the cortical glial scaffold

Running title: Adhesion signaling in glial scaffold development

Wenny Wong [1,*], Jason A. Estep [2,*], Teresa Ubina [1], Jennifer N. Jahncke [3], Kevin M. Wright [3], Martin M. Riccomagno [1, 2]

[1] Neuroscience Graduate Program,

[2] Cell, Molecular and Developmental Biology Graduate Program, Department of Molecular, Cell & Systems Biology, University of California, Riverside, CA, USA

[3] Neuroscience Graduate Program, Vollum Institute, Oregon Health & Science University, Portland, OR 97239, USA.

* These authors contributed equally to this work

Corresponding Author: Martin M. Riccomagno, martinmr@ucr.edu.

Abstract

The mature mammalian cortex is composed of six architecturally and functionally distinct layers. Two key steps in the assembly of this layered structure are the initial establishment of the glial scaffold and the subsequent migration of post-mitotic neurons to their final position. These processes involve the precise and timely regulation of adhesion and detachment of neural cells from their substrates. Although much is known about the roles of adhesive substrates during neuronal migration and the formation of the glial scaffold, less is understood about how these signals are interpreted and integrated within these neural cells. Here, we provide *in vivo* evidence that Cas proteins, a family of cytoplasmic adaptors, serve a functional and redundant role during cortical lamination. *Cas* triple conditional knock-out (*Cas* TcKO) mice display severe cortical phenotypes that feature cobblestone malformations. Molecular epistasis and genetic experiments suggest that Cas proteins act downstream of transmembrane Dystroglycan and β1-integrin in a radial glial cell-autonomous manner. Overall, these data establish a new and essential role for Cas adaptor proteins during the formation of cortical circuits, and reveal a signaling axis controlling cortical scaffold formation.

Keywords: Radial glial cell; Cortical scaffold; Cortical lamination; Cell adhesion; Cas proteins; cobblestone cortex; Type II lissencephaly

Introduction

The cortex is an exquisitely organized structure and provides an excellent model for understanding the cellular and molecular mechanisms that direct neuronal migration, lamination and stratification (Ramón y Cajal, 1911; Caviness & Takahashi, 1995; Hatten, 1999; Nadarajah et al., 2001; Marín & Rubenstein, 2003; Kriegstein & Noctor, 2004; Ayala et al., 2007; Valiente & Marín, 2010; Kwan et al., 2012). The mammalian neocortex is composed of six architecturally and functionally distinct layers (Rakic, 1972). Layers II to VI of the cortex develop in an inside first-outside last manner, i.e. Layer VI is born first and Layer II is born last (Marin-Padilla, 1978; O'Leary & Koester, 1993; Marín & Rubenstein, 2003; Kriegstein & Noctor, 2004; Molyneaux et al., 2007). Excitatory neurons that form these layers are born in the ventricular zone (VZ) of the dorsal telencephalon and migrate radially towards the pial surface (Angevine & Sidman, 1961; Luskin, 1993; Caviness & Takahashi, 1995; Kriegstein & Noctor, 2004). After an initial phase of glial-independent migration, the majority of these newly-born neurons will switch from a multipolar to a bipolar morphology near the intermediate zone (IZ), and associate with Radial Glial Cell (RGC) processes (Rakic, 1972, 1988; Gadisseux et al., 1989; Noctor et al., 2001; Tabata & Nakajima, 2003; LoTurco & Bai, 2006). The RGC basal process dynamically changes from highly branched to club-like during early development to establish a scaffold for neuronal migration (Rakic, 1972, 2003; Yokota et al., 2010). After this initial remodeling event, RGCs have a bipolar shape with an oval nuclei, a short apical process at the VZ, and a long basal process contacting the cortical surface (Rakic, 2003). Neurons use these glial processes as tracks to migrate into the expanding cortical plate (CP) by glia-guided locomotion (Hatten, 1990, 1999; Nadarajah et al., 2001; Nadarajah & Parnavelas, 2002). Once neurons reach the cortical marginal zone (MZ), they detach from the glial fibers and perform somal translocation to their final location (Miyata et al., 2001; Nadarajah et al., 2001, 2003; Nadarajah & Parnavelas, 2002). Thus, two key steps in the assembly of the cortical layers are the establishment of the glial scaffold and the migration of post-mitotic neurons to their final positions (Marín & Rubenstein, 2003; Rakic, 2003; Noctor et al., 2004).

During these events, neurons and RGCs actively interact with each other and the extracellular matrix (ECM). These processes involve the precise and timely regulation of

adhesion and detachment of neural cells from their substrates (Barros et al., 2011; Kawauchi, 2011, 2012). Newborn excitatory neurons constantly remodel their focal adhesions (FA) as they migrate radially (Rutishauser & Jessell, 1988; Sanes, 1989; Rakic et al., 1994; Anton et al., 1999; Ridley et al., 2003; Kawauchi, 2011, 2012). In addition, these neurons change adhesive preferences during their migratory path when switching between glial-independent translocation and glial-dependent locomotion (Nadarajah et al., 2001; Nadarajah & Parnavelas, 2002; Tabata & Nakajima, 2003). RGCs remodel their points of contacts and actively engage the ECM as the cortex grows (Hatten, 1999; Hartfuss et al., 2003; Rakic, 2003; Haubst et al., 2006; Lu et al., 2011; Lambert de Rouvroit & Goffinet, 2001; Halfter et al., 2002; Marín & Rubenstein, 2003; Franco & Müller, 2012). In support of this, β1-Integrin and Dystroglycan (DG), two important regulators of FA establishment and remodeling, have been shown to be required for cortical histogenesis by acting primarily during the formation and maintenance of the glial scaffold (Georges-Labouesse et al., 1998; Graus-Porta et al., 2001; Schmid & Anton, 2003; Belvindrah et al., 2007; Satz et al., 2010; Myshrall et al., 2012). Furthermore, mutations that disrupt the expression or glycosylation of Dystroglycan cause cobblestone cortex phenotypes in both human and mouse (Endo, 2007; Muntoni et al., 2007; Devisme et al., 2012; Satz et al., 2010; Myshrall et al., 2012).

Although much is known about the roles of adhesive substrates and their cognate receptors during neuronal migration and the formation of the glial scaffold, less is understood about how these signals are interpreted and integrated within these neural cells. The Cas family of cytosolic adaptor proteins are known to participate in focal adhesion turnover, and accumulating evidence suggests that Cas protein phosphorylation and ensuing recruitment of downstream effectors can be modulated by permissive and adhesive cues *in vitro* (Vuori & Ruoslahti, 1995; Harte et al., 1996; Nakamoto et al., 1997). Cas family members have been shown to mediate a variety of biological processes including cell migration and changes in cell morphology *in vitro* (Burnham et al., 1996; Klemke et al., 1998; Cho & Klemke, 2000; Tikhmyanova et al., 2010). Three of the four mammalian *Cas* genes are expressed in the developing rodent CNS, including the dorsal telencephalon (Merrill et al., 2004; Huang et al., 2007; Liu et

al., 2007; Riccomagno et al., 2014; Vahedi-Hunter et al., 2018). Using a conditional gene targeting approach in the mouse to inactivate these three *Cas* genes, we previously uncovered a cell-autonomous role for Cas adaptor proteins acting redundantly to mediate integrin signaling during the organization of the retinal ganglion cell layer (GCL) (Riccomagno et al., 2014). Our findings suggest that inactivation of Cas proteins disrupts normal ganglion cell migration and cell positioning (Riccomagno et al., 2014). Interestingly, recent genetic evidence from our lab demonstrates that Cas proteins also act cell-autonomously to regulate the adhesive preferences of mammalian Dorsal Root Ganglia (DRG) sensory axons (Vahedi-Hunter et al., 2018), and a similar role has also been shown in epidermal cells (Leal et al., 2018). Taken together, these raise the intriguing possibility that Cas proteins could be acting to regulate cortical stratification.

Here, we show that Cas proteins are essential for normal cortical migration and lamination. We also provide evidence suggesting that these adaptor proteins act in a glial-autonomous manner downstream of β1-integrin and Dystroglycan. These data thus uncover an adhesive signaling pathway essential for cortical glial scaffold formation and lamination.

Results

As mentioned above, three *Cas* paralogs are expressed during vertebrate development: *p130Cas/BCar1*, *CasL/Nedd9* and *Sin/Efs* (Merrill et al., 2004; Huang et al., 2007; Liu et al., 2007; Riccomagno et al., 2014; Vahedi-Hunter et al., 2018). A fourth family member, *Cass4*, initially believed to be a pseudogene, appears to only be expressed in the adult (Merrill et al., 2004; Deneka et al., 2015). We started by undertaking expression analysis of *p130Cas*, *CasL* and *Sin* during cortical development using multiplex RNAscope *in situ* hybridization (Wang et al., 2012). Examination of the RNAscope probes in wild-type (WT) mice at Embryonic day (E) 12.5 (**Supplemental Fig. 1A**) indicated *p130Cas* transcripts are strongly concentrated in the developing cortical plate (CP), with weaker expression in other areas of the neocortex. *CasL* transcripts are strongest in the cortical hem (midline) and fainter, but distinctly

expressed on the dorsolateral neocortex. In comparison to *p130Cas*, *CasL* transcripts are less prominent in the CP and are found evenly distributed in the intermediate zone (IZ), subventricular zone (SVZ), and ventricular zone (VZ). Based on transcript expression localization, *p130Cas* puncta are primarily found in postmitotic migrating neurons, while both *p130Cas* and *CasL* are expressed in the proliferative pool of cells in the SVZ and VZ. *Sin* is undetectable at this stage as indicated by the absence of signal on coronal sections of the cerebral hemispheres, even at higher magnification. *Cas* gene expression at E14.5 appears somewhat different from their expression at E12.5 (**Fig. 1A; Supplemental Fig. 1A**). Noticeably, detection of *p130Cas* transcripts at E14.5 is strongest in the IZ instead of the CP. Across the two timepoints, *p130Cas* and *CasL* expression remained consistent in the SVZ and VZ. The previously undetectable mRNA transcripts for *Sin* are now clearly localized to the SVZ and VZ. The overlap of *p130Cas, CasL,* and *Sin* transcripts in the proliferative zone suggest a possible function for these genes during cortical neurogenesis.

We then performed a complementary analysis of Cas protein expression by immunohistochemistry. WT coronal sections at E14.5, E16.5, and Postnatal day (P) 0 were immunostained for p130Cas and CasL (**Supplemental Fig. 1B**). p130Cas and CasL 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. 1A**). Interestingly, p130Cas is abundantly expressed in the subplate (SP) and thalamocortical afferents at E16.5. Both p130Cas and CasL are detected in the upper CP and IZ at this timepoint. Cortical expression of Cas at P0 is very similar to E16.5, where Cas proteins are broadly expressed in axons and the lower CP. Robust Cas protein expression in the CP and proliferative regions indicate the adaptor proteins might be involved in several aspects of cortical development. Taken together, these expression patterns raise the possibility of a role for Cas proteins during cortical neurogenesis, migration, and lamination.

To begin to explore whether Cas adaptor proteins play functional roles during cortical circuit assembly, we generated a cortical-specific *p130Cas* ablation in a $CasL^{-/-};Sin^{-/-}$ double null mutant genetic background (we refer to $p130Cas^{flox/\Delta};CasL^{-/-};Sin^{-/-}$ mice as triple conditional knock-outs: “TcKO”) (Donlin et

al., 2005; Seo et al., 2005; Riccomagno et al., 2014). The highly overlapping expression and redundant roles played by Cas proteins make using the *Cas TcKO* mice of vital importance to understand Cas function (**Fig 1A; Supplemental Fig. 1A-B**) (Riccomagno et al., 2014; Vahedi-Hunter et al., 2018). To drive *Cre* recombinase expression we used the *Emx1Cre* line, which expresses *Cre* in early cortical neural progenitors and radial glial cells (RGCs) in the forebrain (Gorski et al., 2002), resulting in recombination in RGCs themselves and the excitatory neurons in the cortex and hippocampus (**Supplemental Fig. 1C**). RNAscope on E14.5 *Emx1Cre;CasTcKO* coronal sections validated that mutant animals do not produce functional *Cas* transcripts (**Supplemental Fig. 1D**). To test the requirement for *Cas* genes during cortical histogenesis we started by performing an overall examination of *Emx1Cre;CasTcKO* cortical structure using pan-neural markers. When compared to littermate controls (*TcKO*s without *Cre* and *Emx1Cre; p130Cas*$^{+/flox}$*;CasL*$^{-/-}$*; Sin*$^{-/-}$ animals), the *Emx1Cre;CasTcKO* adult cortical phenotype appears dramatically different (**Supplemental Fig. 2A**). The smooth cortical surface, typical for a mouse, is replaced with a bumpy or cobblestone surface. Nissl stain, which highlights the contrast between axons and Nissl bodies, shows empty pockets in the cortical plate (**Supplemental Fig. 2A**). A more in-depth analysis of the cortex with a mature neuronal marker (NeuN) indicated the cortex has similar empty pockets as seen in the Nissl stain (**Supplemental Fig. 2B**). 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 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.

The six-layered cortical plate contains distinct populations of projection neurons that can be distinguished based on cell morphology, connectivity, or expression of specific transcription factors (Hevner, 2007; Molyneaux et al., 2007; Kwan et al., 2012). To test whether the laminar structure of the cortex is affected in *Emx1Cre;CasTcKO* animals, we performed immunohistochemistry with a variety of cortical layer markers. Cux1, Ctip2, Rorβ, and Tbr1 were chosen from a 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 and IV) are identifiable with Cux1 (Ferrere et al., 2006), while Rorβ is one of the few markers that will exclusively label layer IV neurons (Nakagawa & O'Leary, 2003). The deep layer neurons can be identified with Ctip2 (high expression in layer V & low in layer VI) (Leid et al., 2010) and Tbr1 (layer VI) (Hevner et al., 2001; Bedogni et al., 2010). To precisely quantify any cortical stratification defects, the lamination patterns of control and *Emx1Cre;CasTcKO* animals were analyzed by binning the cortex into ten regions of equal-width as previously described (Rosário et al., 2012; Franzoni et al., 2015; Tonchev et al., 2016; Umemura et al., 2021; Sokpor et al., 2021). Histological analyses of *Emx1Cre;CasTcKO* animals at P7 (**Fig. 1B; Supplemental Fig. 2C-D**) indicated notable lamination defects for all of the different populations examined in comparison to control animals. Significant differences were observed in the locations of Cux1+, Ctip2+, Rorβ+, and Tbr1+ between *Emx1Cre;CasTcKO* and control animals (**Fig. 1B; Supplemental Fig. 2C-D;** Mann-Whitney *U* test with Bonferroni correction, *$p<0.005$ two-tailed test). Cortical dysplasia includes Ctip2+/Tbr1+ cell clusters at the upper cortical plate and Cux1+ neurons at the lower cortical plate (**Fig. 1B; Supplemental Fig. 2D**). Furthermore, cortices missing *Cas* genes have a wave-like appearance for layer IV, with Rorβ+ cells invading the superficial cortical plate and meninges (**Supplemental Fig. 2C**). This shows that abnormal positioning of both deep- and superficial-layer neurons contribute to the cobblestone phenotype. These data revealed that *Cas* genes are required for cortical lamination and suggest a possible role for these genes in radial migration.

The misplacement of layer-specific markers observed in *Emx1Cre;CasTcKO* animals could be due to defects in migration/neural positioning, or defects in neuronal fate specification (Guillemot, 2007; Pramparo et al., 2010; Wang et al., 2011; Kwan et al., 2012). 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 the cell cycle (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. 1C**) or E15.5 (**Fig. 1D**) 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 these populations of excitatory neurons to finish radial migration (Frantz & McConnell, 1996; Hevner et al., 2001; Lambert de Rouvroit & Goffinet, 2001; Nadarajah et al., 2003; Ayala et al., 2007; Kwan et al., 2008, 2012). *Emx1Cre;CasTcKO* animals show ectopic EdU+ cells positioned outside of the compromised basal lamina (stained with laminin). In addition, sporadic columns of EdU+ cells are a distinct feature of *Emx1Cre;CasTcKO* animals compared to controls. Quantification of the position of E12.5 pulsed cells shows a clear trend for EdU+ cells to locate closer to the pial surface (Bin 1) in *Emx1Cre;CasTcKO* cortices than in controls, where they tend to settle in layer VI (**Fig. 1C;** Mann-Whitney *U* test with false discovery rate<0.1 and p<0.05 for Bin 2).). More noticeable differences are observed when animals are pulsed at E15.5 and collected at P3: prospective superficial neurons that are EdU+ are spread across the ten bins in *Emx1Cre;CasTcKO* animals, whereas in control mice these EdU+ cells are primarily positioned closer to the pial surface (**Fig. 1D;** Mann-Whitney *U* test with false discovery rate<0.1 and p<0.05 for Bins 1, 2, 4, and 5). This misplacement of cells appears to be caused, at least in part, by columns of ectopic EdU cells in the CP. These data suggest that the cortical dysplasia observed in *Emx1Cre;CasTcKO* animals is primarily due to a migration defect and not due to cell fate specification errors.

One possible contributing factor for the migration defects observed in animals is the improper splitting of the preplate (Sheppard & Pearlman, 1997; Magdaleno et al., 2002; Park & Curran, 2008). This involves the establishment of layer VI within a plexus of pioneer neurons (Kostovic & Rakic, 1990; Nichols & Olson, 2010; Olson, 2014). Layer VI splits the preplate (PP) to form the marginal zone (MZ) on the surface of the cortex and the subplate (SP) as the ventral boundary. Successive waves of neuronal migration and lamination of cortical neurons form the CP in between these two boundaries. Thus, disruption of this early developmental event can change laminar organization (Takahashi et al., 1999; Hevner et al., 2003; Molyneaux et al., 2007). To better understand when and how the migration defects observed *Emx1Cre;CasTcKO* animals arise, preplate splitting was examined in these mutants and control mice. The use of selected subplate and marginal zone markers at E15.5 indicated a defective preplate split in *Emx1Cre;CasTcKO* cortices compared to age-matched littermate

controls (**Supplemental Fig. 2E**). Immunostaining for Microtubule Associated Protein 2 (MAP2), which labels differentiating neurons and the cell bodies of subplate neurons (Hevner et al., 2001; Magdaleno et al., 2002), revealed disorganized subplate cells underneath the CP. MAP2+ subplate neurons also form ectopic clusters that invade the breached MZ (**Supplemental Fig. 2E**). Staining for Chondroitin sulfate proteoglycan (CSPG), which labels the SP (Bicknese et al., 1994), also demonstrated an abnormal distribution of SP neurons, with columns of CSPG+ cells extending dorsally from the SP to contact the pial surface in *Emx1Cre;CasTcKO* cortices. To better characterize preplate split phenotypes, we then stained for calcium binding proteins Calretinin and Calbindin (**Supplemental Fig. 2E**). Calretinin is normally expressed by Cajal-Retzius (CR) cells in the MZ and thalamocortical projections in the IZ (del Río et al., 1995). Calretinin+ cells in the MZ appear disorganized, forming aggregates on the surface of the cortex, and thalamocortical afferents appear to invade the cortical plate prematurely in *Emx1Cre;CasTcKO* mice compared to controls. This early disorganization of the SP, CP, IZ and MZ was confirmed using Calbindin. Calbindin also labels the MZ and migrating interneurons (Meyer et al., 1998; Ang et al., 2003). Calbindin staining shows a disorganized tangential stream of interneurons originating from the medial ganglionic eminence and abnormal positioning of CR cells in the MZ of *Emx1Cre;CasTcKO* cortices. Overall, these suggest that the splitting of the preplate and the subsequent organization of the MZ and SP are severely affected in *Emx1Cre;CasTcKO* cortices.

The use of the *Emx1Cre* mice precludes us from determining whether *Cas* genes act in a neuronal-autonomous or non-neuronal-autonomous manner during cortical migration and lamination: recombination in these mice occurs as early as E10 in RGCs, the neural progenitors that generate all excitatory neurons and macro glial cells in the dorsal telencephalon (Gorski et al., 2002; **Supplemental Fig. 1C**). To tease apart the neuronal-autonomous and non-neuronal-autonomous requirements for *Cas* genes, and their relative contributions to the cobblestone phenotypes observed in *Emx1Cre;CasTcKO* animals, we generated *NexCre;CasTcKO* mice*. NexCre* is expressed only in pyramidal cells, but not in RGCs, targeting early postmitotic, premigratory excitatory neurons (Wu et al., 2005; Goebbels et al., 2006; Supplemental Fig 3A). This pattern of Cre activity was confirmed by crossing the *NexCre* mice to the

Cre reporter line *Ai14* (Madisen et al., 2010). Analysis of *NexCre;Ai14* cortices at E13.5 revealed that tdTomato expression was indeed found in the IZ and CP (**Supplemental Fig. 3A**) and E15.5 included the SVZ (Wu et al., 2005; Goebbels et al., 2006).

To elucidate the neuronal-autonomous requirement for *Cas* genes during radial migration and cortical stratification, the laminar organization of *NexCre;CasTcKO* cortices was examined (**Fig. 2A; Supplemental Fig. 3B-C**). *NexCre;CasTcKO* mutants and control littermates were immunostained with the same layer-specific markers as *Emx1Cre;CasTcKO* animals (**Fig. 1B; Supplemental Fig. 2C-D**). 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 migration and lamination. 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. 2A; Supplemental Fig. 3B-C**). 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 spatially distinct. No significant differences in cell positioning were observed for any of the markers examined in Control and *NexCre;CasTcKO* animals (Mann-Whitney *U* test with Bonferroni correction). These results strongly suggest that Cas adaptor proteins are not required in a neuronal-autonomous manner during cortical migration.

To further confirm the lack of migration defects in *NexCre;CasTcKO*, we performed the same unbiased pulse-chase experiment to track migration of early post-mitotic cells that we used on *Emx1Cre;CasTcKO* animals (**Fig. 1C-D**). EdU administration into *NexCre;CasTcKO* animals at E12.5 (**Fig. 2B**) or at E15.5 (**Fig. 2C**) resulted in labeling that was indistinguishable from that of control littermates: both deep and superficial layer neurons exhibit a uniform band of EdU+ cells in the cortical plate. Thus, the migration defects observed in *Emx1Cre;CasTcKO* animals are not recapitulated in *NexCre;CasTcKO* mutants. This further demonstrates that *Cas* genes act in a non-neuronal autonomous manner during cortical migration, and that they are likely functioning in RGCs to direct cortical lamination.

As the cortex expands in size, radial glial cells create a scaffold for neurons to migrate (Rakic, 1972; 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 (Halfter et al., 2002; Rakic, 2003). The absence of the cobblestone appearance and intact basement membrane in *NexCre;CasTcKO* cortices (**Fig. 2A; Supplemental Fig. 3B-C**), 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* in RGCs. Strikingly, tyrosine phosphorylated p130Cas (pY165Cas) is concentrated in RGC (Nestin+) endfeet and cell bodies in the VZ in WT animal at E13.5 (**Fig. 3A**). This indicates that post-translational modification of Cas is present at sites where the radial glial endfeet actively interact with the basement membrane.

To test whether *Cas* genes are required for the embryonic pial basement membrane and radial glial endfeet integrity, we performed histological assessment of the cortical scaffold of *Emx1Cre;CasTcKO* at E15.5 (**Fig. 3B**). 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 sites, where basal processes extend into the subarachnoid space. These results suggest that *Cas* function is required for the maintenance of the embryonic pial basement membrane-RGC interactions.

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. 3C-D**). However, collection is 0.5 Hrs. following injection at E12.5 (**Fig. 3C**) or E15.5 (**Fig. 3D**). This short EdU pulse will label the proliferating cells during neurogenesis of deep layer and superficial layer neurons, respectively. At E12.5, EdU+ proliferating neural progenitors are positioned in the SVZ and VZ (**Fig. 3C**). At this stage, a few proliferative cells are also observed in the MZ of both controls and *Emx1Cre;CasTcKO* animals (**Fig. 3C)**. At E15.5, proliferative cells are restricted to the SVZ and VZ of control animals, but in *Emx1Cre;CasTcKO* animals EdU+ cells are also observed in the MZ and upper CP in regions where the basement

membrane is ruptured (**Fig. 3D**). This indicates that the breakage in the basement membrane and disruption of radial glial endfeet observed in *Emx1Cre;CasTcKO* cortices likely result in ectopic progenitor cells, which could contribute to the cobblestone phenotype.

Dystroglycan (DG) 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 DG is responsible for maintaining the pial basement membrane integrity (Satz et al., 2010; Myshrall et al., 2012). Previous analysis of *NestinCre;DG* $^{flox/flox}$ animals at E14.5 to P0.5 showed a heterotopic distribution of cortical neurons (Myshrall et al., 2012), and an intriguing resemblance to the *Emx1Cre;CasTcKO* cortical phenotype. We thus re-examined these cortical phenotypes at P7 using the same layer markers and the same cortical Cre driver (*Emx1Cre*) that we used to study the *CasTcKO* mice. Genetic ablation of *DG* using *Emx1Cre* (*Emx1Cre;DG*$^{flox/-}$) indeed caused cobblestone malformations, as suggested by previous studies using a pan-neural Cre (Satz et al., 2010; Myshrall et al., 2012) (**Fig. 4A; Supplemental Fig. 4A**). Mispositioning of the different neuronal subpopulations in *Emx1Cre;DG*$^{flox/-}$ mice is strikingly similar to that of *Emx1Cre;CasTcKO* animals, where superficial layer neurons undermigrate and deep layer neurons overmigrate (**Fig 4A; Supplemental Fig. 4A**). Interestingly, these phenotypes are also likely RGC-autonomous, as deletion of *DG* in postmitotic neurons using *NexCre* does not disrupt cortical lamination (Satz et al., 2010; Miller & Wright, 2021). In addition to supporting the reproducibility of previous reports, this provides definitive evidence that the *Emx1Cre;CasTcKO* cortical organization phenocopies multiple aspects of the defects observed when *DG* is conditionally ablated in early cortical progenitors.

Several studies using cell-based assays have shown that DG is required for the initial clustering of laminin-1 on cells (Cohen et al., 1997; Durbeej et al., 1998; Montanaro et al., 1998; Henry et al., 2001). Integrins subsequently bind to clustered laminin through a distinct interaction site, as well as other ECM proteins to transduce outside-in signaling (Colognato & Yurchenco, 2000; Henry et al., 2001; Cabodi et al., 2010). A previous study showed that Cas phosphorylation in the retina is highly

dependent on β1-integrin function (Riccomagno et al., 2014). Interestingly, pan-neural *β1-integrin* ablation (Graus-Porta et al., 2001) leads to heterotopias similar to the ones observed in *Emx1Cre;DG*$^{flox/-}$ mice and *Emx1Cre;CasTcKO*. These phenotypes also appear to be RGC-autonomous, since *NexCre*-driven ablation of *β1-integrin* does not cause these types of defects (Belvindrah et al., 2007). The laminar organization of *NestinCre;β1-integrin* mice has not been fully characterized (Graus-Porta et al., 2001; Belvindrah et al., 2007). To begin to probe whether β1-integrin might be acting together with *Cas* genes and *DG* during cortical scaffold formation, we revisited the cortical phenotypes of *β1-Integrin* knock-outs using layer-specific markers and *Emx1Cre* as a driver. Staining for layer-specific transcription factors confirmed a distinct cobblestone phenotype in *Emx1Cre;β1-Integrin*$^{flox/flox}$ cortices, as compared to control littermates (*Emx1Cre;β1-Integrin*$^{flox/+}$) (**Fig. 4B; Supplemental Fig. 4B-C**). Cux1, Ctip2, Rorβ, and Tbr1 labeling revealed that cortical layer misplacement occurred for all the examined subpopulations in *Emx1Cre;β1-Integrin*$^{flox/flox}$ animals. On the other hand, *NexCre;β1-Integrin*$^{flox/flox}$ and control P7 cortices are indistinguishable from each other: cortical layers are uniform and defined, as previously shown (Belvindrah et al., 2007; **Supplemental Fig. 4D-F**). This reinforces that *β1-Integrin* acts in an RGC-autonomous manner similar to *DG* and *Cas* genes to control cortical lamination.

Based on the robust phospho-Tyrosine Cas (pY165Cas) expression at sites where radial glial endfeet interact with the basement membrane, and the close resemblance between the *Emx1Cre;CasTcKO, Emx1Cre;DG*$^{flox/-}$, and *Emx1Cre;β1-Integrin*$^{flox/flox}$ cortical phenotypes, we hypothesized that DG, β1-Integrin, and Cas proteins might act in the same signaling pathway to maintain the pial basement membrane integrity. To assess this possibility, we first tested whether DG expression can modulate Cas tyrosine phosphorylation in WT RGCs. Mixed primary cultures containing radial glial cells and neurons were transfected at two days *in vitro* with either a myc-tagged empty vector or myc-tagged full-length Dystroglycan (myc-DG). The presence of radial glial cells was confirmed using previously validated developmental markers (Malatesta et al., 2003; Markó et al., 2011; **Supplemental Fig. 3D**). Subsequently, RGCs were identified by co-labeling with Nestin. Nestin^{+} WT RGCs transfected with an empty vector (pcDNA3.1-myc/his) showed basal endogenous

pY165Cas, where puncta are distributed broadly in cell bodies, with enhanced localization at adhesion points between cells (**Fig 4C).** In Nestin^{+} myc-DG-transfected WT cells, we observed a significant accumulation of pY165Cas in the glial endfeet that colocalizes with the expression of myc-DG when compared to control transfected WT cells (**Fig. 4C-D**, Mann-Whitney *U* test, ***p<0.0001 two-tailed test). This finding suggests that DG can modulate p130Cas phosphorylation, and may be acting in the same pathway to regulate RGC endfeet interaction with the pial basement membrane.

To test whether DG-dependent phosphorylation of Cas proteins requires β1-Integrin as a signal transducing receptor, *Emx1Cre;β1-Integrin*$^{flox/flox}$ cultures were transfected with the empty vector or full-length Dystroglycan (**Fig. 4C**). Analysis using the Kruskal-Wallis test provided very strong evidence of a difference (p=0.002) between the mean ranks of at least one pair of treatments, where a series of Mann-Whitney *U* tests indicated a significant difference between WT RGCs transfected with myc-DG and all of the other groups (**Fig. 4D**; ***p<0.001 two-tailed test). Importantly, *Emx1Cre;β1-Integrin*$^{flox/flox}$ cells overexpressing myc-DG yielded weak pY165Cas expression in radial glial endfeet, in comparison to WT cultures overexpressing myc-DG (**Fig. 4C-D,** Mann-Whitney *U* test, ***p<0.001 two-tailed test). Furthermore, no significant difference was observed between *Emx1Cre;β1-Integrin*$^{flox/flox}$ RGCs transfected with the control vector or myc-DG (**Fig. 4C-D)**. These data indicate that DG-dependent phosphorylation or recruitment of pYCas to the endfeet requires β1-integrin function.

Discussion

Lamination and stratification of neuronal cell bodies are widespread early developmental events that organize many regions of the CNS (Rakic, 1988; O'Leary & Koester, 1993; Monuki & Walsh, 2001; Marín & Rubenstein, 2003; Rash & Grove, 2006; Molyneaux et al., 2007). These foundational processes are thought to provide a structure upon which neural circuits can form (Huang & Zeng, 2013; Motta et al., 2019; Sanes & Zipursky, 2020). During many of these stratification events, migrating neurons utilize RGCs to reach the proper layer (Rakic, 1971, 1972; Campbell & Gotz, 2002; Marín et al., 2010). In this study we describe a molecular mechanism by which the glial

scaffold is assembled to facilitate cortical migration and lamination. Our data identify an adhesion signaling axis that acts in RGCs to maintain glial endfeet-pial interactions, and establish a novel and essential role for Cas proteins in these developmental processes.

Assembly and maintenance of the glial scaffold is particularly important during neocortical lamination (Rakic, 1972; Haubst et al., 2006; Ferent et al., 2020). The formation of this scaffold requires proper attachment of the glial endfeet to the pial basement membrane. Disruption of these interactions in mouse and human results in severe lamination defects like cortical dysplasia and cobblestone cortex (Devisme et al., 2012; Satz et al., 2010; Myshrall et al., 2012; Subramanian et al., 2020). Why is constant engagement of the basal lamina by RGCs so critical? While the cortical scaffold is traditionally portrayed as a somewhat rigid structure, the cortex expands vastly during neurogenesis (Rakic, 2009; Molnár et al., 2006; Cadwell et al., 2019). RGC processes must grow and remodel to accommodate this rapid expansion of the cortical plate as new layers are born and incorporated (Nowakowski et al., 2016; Ferent et al., 2020). RGC endfeet must actively adhere to the basement membrane to maintain the structure of the scaffold (Halfter et al., 2002; Yurcheno et al., 2011; Ferent et al., 2020). Thus, dynamic regulation of adhesion signaling and cytoskeletal remodeling are essential for these glial-pial interactions.

Our data support a model whereby Cas adaptor proteins act as signal transductors downstream of the β1-integrin and Dystroglycan transmembrane proteins to regulate RGC-basement membrane interactions and maintain the integrity of the glial scaffold, allowing for normal neuronal migration and lamination (**Fig. 4A-D; Supplemental Fig. 4A-F**). Our *in vitro* data further suggests that the DG-dependent phosphorylation of Cas proteins is mediated by β1-integrin. This signaling axis is likely modulated by other transducers of integrin signaling known to act in non-neuronal-autonomous manner to establish or maintain the RG scaffold (Beggs et al., 2003; Niewmierzycka et al., 2005; Valiente et al., 2011). While there is a clear disruption of the glial scaffold in the *Emx1Cre;CasTcKO* animals, the alterations to the dorsal and ventral boundaries of the CP, the misplacement of proliferating cells, and clustering of Cajal-Retzius cells observed in these animals also likely contribute to the lamination defects (Meyer et al., 1998; Hartfuss et al., 2003; Hevner et al., 2003; Voss et al., 2008;

Gil-Sanz et al., 2013), similar to what occurs in *DG* mutant cortices (Satz et al., 2010; Myshrall et al., 2012; Nakagawa et al., 2015; Booler et al., 2016; 2017).

The findings add to a growing list of studies in a variety of animal models that have demonstrated the evolutionarily conserved requirement for Cas adaptor proteins during cell migration and axon pathfinding (Huang et al., 2007; Liu et al., 2007; Riccomagno et al., 2014; Vahedi-Hunter et al., 2018). In the cortex, the aforementioned Integrin-Cas axis appears to act primarily in a glial-autonomous manner (Graus-Porta et al., 2001; Belvindrah et al., 2007), yet strong genetic evidence suggest a neuronal-autonomous function for a similar signaling pathway in mouse and fly peripheral axon pathfinding, and mammalian retina development (Huang et al., 2007; Riccomagno et al., 2014; Vahedi-Hunter et al., 2018). Interestingly, during retina lamination and stratification, neurons initiate migration without the aid of radial glia and the laminin-rich basement membrane provides an instructive cue that defines the position where the single-cell ganglion cell layer will form (Zolessi et al., 2006; Edwards et al., 2010; Pinzon-Duarte et al., 2010; Sanes & Zipursky, 2010; Randlett et al., 2011; Riccomagno et al., 2014). In the eye, the Integrin-Cas signaling axis acts in migrating retinal ganglion cells to sense the inner limiting membrane and is necessary for the reorganization of the ganglion cell layer (Riccomagno et al., 2014). Of note, even though Crk-Associated Substrates (Cas) are essential for RGC process and endfeet organization, histological and biochemical evidence support the idea that the Crk proteins themselves function in a neuronal-autonomous manner downstream of reelin signaling to regulate cortical lamination (Huang et al., 2004; Park & Curran, 2008; Franco et al., 2011).

Cobblestone or type II lissencephaly is a debilitating neurodevelopmental disorder caused by breaks in the basement membrane (BM) at the pial surface (Olson & Walsh, 2002; Muntoni & Voit, 2004; Devisme et al., 2012; LoTurco & Booker, 2013). These defects have been associated with RGC end-feet that are not well attached to the ECM (Nickolls & Bonnemann, 2018; Ferent et al., 2020). The BM breakages result in the over-migration of neurons and a dramatic loss of normal cortical lamination (Haubst et al., 2006; Hu et al., 2007; Satz et al., 2010; Myshrall et al., 2012; Nakagawa et al., 2015). The genetic causes of cobblestone lissencephaly have largely been linked

to autosomal recessive mutations in genes that encode for proteins that participate in the post-translational modification of alpha-Dystroglycan: POMT1, POMT2, POMGNT1, FCMD, FKRP, TMEM5/ RXYLT1, ISPD and LARGE (Moore et al., 2002; Manzini et al., 2008; Satz et al., 2010; Devisme et al., 2012; Vuillaumier-Barrot et al., 2012). Mutations in one of these genes are found in 32–66% of patients with Type II lissencephaly (Godfrey et al., 2007; Mercuri et al., 2009; Devisme et al., 2012; Vuillaumier-Barrot, 2012). However, despite intensive research in this area, more than one-third of the cases have an unknown genetic etiology (Devisme et al., 2012). The striking resemblance of the phenotypes observed in cortical *CasTcKOs* and patients with cobblestone lissencephaly, in addition to the molecular epistasis experiments suggesting that Cas proteins act downstream of DG and β1-Integrin in radial glial cells to maintain basement membrane integrity, pinpoint cytoplasmic effectors of adhesion signaling as possible contributing factors to the etiology of these neurodevelopmental disorders. These findings open new and intriguing avenues in the study of the genetic causes of cobblestone lissencephaly.

Materials & Methods

Animals

The day of vaginal plug observation was designated as embryonic day 0.5 (E0.5) and the day of birth was postnatal day 0 (P0). The ICR mouse strain was purchased from Taconic. The following animals were purchased from The Jackson Laboratory: *Ai14/R26*$^{LSL\text{-}TdTomato}$ (stock 007914), *β1-Integrin*^{flox} (stock 004605), *Emx1Cre* (stock 005628), and DG^{flox} (stock 009652). *NexCre* mice were kindly provided by Drs. Nave and Goebbels (Wu et al., 2005; Goebbels et al., 2006). Generation of the *p130Cas*$^{flox/flox}$, *CasL*$^{-/-}$ and *Sin*$^{-/-}$ transgenic mouse lines has been described previously (Seo et al., 2005; Donlin et al., 2005; Riccomagno et al., 2014). All animal procedures were performed according to the University of California, Riverside's Institutional Animal Care and Use Committee (IACUC) guidelines.

Immunofluorescence

Embryonic brains were dissected in cold PBS and postnatal mice were perfused then dissected. The brain samples were post-fixed in 4% PFA for 2 Hrs., 4 Hrs., or overnight at 4°C and washed with PBS the next day. The samples were either kept in PBS or sunk in 30% sucrose overnight at 4°C prior to cryopreservation with Optimum Cutting Temperature (Tissue-Tek). Brains were either embedded in 3% agarose for coronal sectioning at 150 µm thickness on a vibrating microtome (VT1000S; Leica) or cryoprotected samples and sectioned coronally at 20 µm on a Leica CM3050 cryostat. Cryosections were blocked for 1 hour in RT in blocking buffer (0.1% PBST and 10% goat serum) in a humidified chamber and free- floating slices were blocked in permeabilization solution overnight at 4°C as previously described (Polleux & Ghosh, 2002). Primary antibodies were diluted in antibody dilution buffer (cryosections; 0.1% PBST, 1% goat serum) or permeabilization solution (free-floating slices) with the following antibodies: rabbit anti-p130Cas c-20 (1:250, SCBT sc-17), rabbit anti-Nedd9 (1:250, Sigma# HPA038768), rabbit anti-Cux1 (1:250, ProteinTech# 11733-1-AP), rat anti-Ctip2 (1:500, Abcam# ab18465), rabbit anti-Tbr1 (1:500, Abcam# ab31940), rabbit anti-Rorβ (1:250, Proteintech# 17635-1-AP), rabbit anti-Calretinin (1:500, Swant# 7697), rabbit anti-Calbindin (1:500, Swant# CB38), chicken anti-MAP2 (1:500, Aves MAP), and rabbit anti-CSPG (1:1000; Sigma# AB5320). The samples incubated in the primary antibody mix overnight at 4°C. Cryosections were washed with PBS 15 mins x 4 in a slide mailer and incubated in secondary antibody (goat anti-chicken 647, Themo Fisher Scientific Cat# A-21449 and goat anti-rabbit 546, Thermo Fisher Scientific Cat# A-11035) with DAPI (1 µg/ml) and antibody dilution buffer at RT for 1 hour in a humidified chamber. Slides were washed with PBS 15 mins x 4 in a slide mailer and sealed with fluorogel. Free floating sections were washed with PBS 15 mins x 8 and incubated in secondary antibody at 1:1000 dilution in permeabilization solution overnight at 4°C. Sections were washed with PBS 15 mins x 4 and sealed with fluorogel. Samples were imaged with a confocal microscope.

Fluorescent *in situ* Hybridization

Fluorescent *in situ* hybridization was performed on 20 µm E12.5 and E14.5 cryosections using the RNAScope LS Multiplex Assay (Advanced Cell Diagnostics). RNAscope was

performed as described in manufacturer's instructions (Wang et al., 2012). Target RNAs are hybridized to DNA "z-probes" complementary to the RNA of interest, a spacer sequence, and a tail region. Oligos bind to the tail region of the z-probe, which are then bound to amplifiers labeled with horse radish peroxidase (HRP) and fluorophores. *p130Cas/BCAR1*, *CasL/NEDD9*, and *Sin/EFS* probes generated by Advanced Cell Diagnostics were uniquely amplified with a dye (Opal 520, Opal 570, and Opal 620) and stained with DAPI (1 µg/ml) in PBS. Slides were sealed with fluorogel and imaged with a confocal microscope.

Ethynyl deoxyuridine (EdU) Labeling

EdU was dissolved in DMSO and diluted with sterile water to 15 mg/ml at the time of injection. EdU was injected into the intraperitoneal cavity of pregnant mice at a dosage of 150 mg/kg. Animals were collected 30 minutes or 7 days after administration. Embryonic brains were dissected in cold PBS and postnatal animals were perfused then dissected. The brains were fixed for 2 hours in 4% PFA, dehydrated in 30% sucrose overnight, then cryopreserved with Optimum Cutting Temperature (Tissue-Tek). Cryosections were stained according to the instructions in the Click-iT EdU Cell Proliferation Kit for Imaging, Alexa Fluor 488 dye, Invitrogen Cat# C10337. Slides were incubated with 3% BSA for 30 mins at RT, then blocked in 0.5% PBST at RT for 1 hour, washed with 3% BSA in PBS x 2, and incubated in Click-iT Reaction Cocktail (1x Click-iT Reaction Buffer, $CuSO_4$, Alexa Fluor Azide 488, and Reaction Buffer Additive) for 1 hour at RT. Cryosections were washed with 3% BSA in PBS x 4 and immunohistochemistry proceeded as described (Polleux & Ghosh, 2002). Primary antibodies chicken anti-Nestin (1:500, Aves Nes) and rabbit anti-Laminin (1:500, Sigma# L9393) were diluted in antibody dilution buffer (0.1% PBST, 1% goat serum). Slides incubated in the primary antibody mix in a humidified chamber at 4°C overnight. Slides were washed with PBS 15 mins x 4 in a slide mailer and incubated in secondary antibody at 1:1000 dilution (goat anti-chicken 647, Themo Fisher Scientific Cat# A-21449 and goat anti-rabbit 546, Thermo Fisher Scientific Cat# A-11035) at RT for 1 hour in a humidified chamber. Slides were washed with PBS 15 mins x 4 in a slide

mailer. Fluorogel was applied to the slide and sealed with a coverslip. Samples were imaged with a confocal microscope.

Mixed Primary Cell Culture

Primary mixed radial glial cells and cortical neurons were isolated from E13.5 embryos essentially as described in Kim & Magrané (2011) using Complete Hank's Balanced Salt Solution (HBSS, 2.5 mM HEPES pH 7.4, 2.5 mM D-glucose, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2.5 mM $NaHCO_3$; from Polleux & Ghosh, 2002). Cells were dissociated as described (Kim & Magrané, 2011) and grown in Radial Glia Media modified from Culican et al. (1990) (Modified DMEM/Eagle's Media, 1x Penicillin/Streptomycin, 0.5 mM Glutamax, 20 mM D-glucose, and 5% horse serum). 12 mm circular coverslips were treated with 12 M HCL overnight, neutralized with deionized water, and stored in a glass petri dish with 70-100% ethanol. The coverslips were fire-polished into a 24-well plate and coated with laminin (8.3 µg/ml; Polleux & Ghosh, 2002) and poly-D-lysine/poly-L-lysine (83.3 µg/ml; Polleux & Ghosh, 2002) in sterile water overnight in a tissue culture incubator at 37°C in 5% CO_2. The coating media was aspirated and the coverslips were air-dried in an airflow cabinet prior to plating cells. Dissociated cells were plated in a 24-well plate at a density of 1 x 10^5 cells/well with 500 µl of cell suspension and Radial Glia Media. One-third of the media was replaced daily and wells were transfected two days *in vitro* (2DIV) with Metafectene PRO (Biontex Cat# T040-1.0), according to the manufacturer's suggested protocol of 1 µg cDNA to 2 µl Metafectene ratio. Cells were transfected with either pcDNA3.1-myc/his (control vector) or pcDNA3.1-myc/his-DG-FL (myc-DG) plasmids previously described in Lindenmaier et al. (2019). At 48 hours after transfection (4DIV), the coverslips were fixed in 4% PFA for 10 minutes at RT and washed with PBS with 0.03% NaN_3. Cells incubated in blocking buffer (0.1% PBST, 10% goat serum) for 30 minutes at RT. Un-transfected cells were stained with the following primary antibodies: rabbit anti-Blbp (1:500, Abcam# ab32423), mouse anti-β3-Tubulin (1:500, CST# 4466), rat anti-GFAP (1:500, Invitrogen# 13-0300), rabbit anti-Tbr2 (1:500, Abcam# ab23345), and chicken anti-Nestin (1:500, Aves Nes) in antibody dilution buffer (0.1% PBST, 1% goat serum) overnight at 4°C. Transfected cells incubated in primary antibodies: mouse anti-c-myc 9E10 (1:1000, Invitrogen Cat#

13-2500), rabbit anti-Tyr165 (1:250, CST Cat# 4015), and chicken anti-Nestin (1:500, Aves Nes) in antibody dilution buffer overnight at 4°C. Cells were then washed with PBS with 0.03% NaN_3 for 15 mins RT x 4 and incubated in secondary antibodies at 1:1000 dilution (goat anti-mouse 488, Thermo Fisher Scientific Cat# A-28175, goat anti-rabbit 647, Thermo Fisher Scientific Cat# A-27040, goat anti-chicken 546, Thermo Fisher Scientific Cat# A-11040, goat anti-rat 647, Thermo Fisher Scientific Cat# A-21247) and DAPI (1 µg/ml) in antibody dilution buffer at RT for 2 hours. Cells were washed with PBS with 0.03% NaN_3 for 15 mins x 4 and coverslips were mounted on microscope slides with fluorogel and imaged with a Leica confocal microscope.

Quantification of Layer Markers/EdU & Statistical Analysis

Cells were counted using the RapID Cell Counter (Sekar et al., 2021). The thickness of the cortex was divided into 10 bins of equal width and the number of cells labeled with the layer markers (Cux1, Ctip2, Rorβ, Tbr1) and/or EdU were counted for each bin. Bin 1 is the marginal zone (MZ) and bin 10 is ventral to layer VI. The proportion of cells in each bin= number of cells labeled in bin divided by total number of labeled cells. The graph values are mean ± standard error of mean, n=3 independent samples per group, and 3 sections per sample. The dataset was tested for normality using the Shapiro-Wilk test and QQ plot. The Mann-Whitney *U* test was performed with Bonferroni correction or Benjamini-Hochberg with false discovery rate<0.1 and $p<0.05$ (Benjamini et al., 2001).

Mean Fluorescent Intensity & Statistical Analysis

ImageJ (NIH) was used to measure the fluorescent intensity of anti-Nestin and anti-Tyr165 in radial glial endfeet. The Nestin channel was used to trace the borders for the radial glial endfeet. The following selections were used to determine the fluorescent intensity of Nestin and pY165Cas channels using ImageJ: Analyze -> Set Measurements (Area, Min & max gray value, Integrated density, Mean gray value)-> Measure mean gray value. Relative fluorescent intensity is mean fluorescent intensity of pY165Cas divided by mean fluorescent intensity of Nestin. The graph values are mean ± standard error of mean, n=6-7 independent samples per group, and 9-18 cells per sample. The dataset was tested for normality using the Shapiro-Wilk test and QQ plot.

The Kruskal-Wallis test was performed for multiple-comparisons, followed by a series of Mann-Whitney *U* tests to examine which pairs are significantly different.

Acknowledgements

We would like to thank Drs. Sachiko Haga-Yamanaka and Edward Zagha for critically reading the manuscript and providing helpful comments. We would like to thank Drs. Nave and Goebbels for the *NexCre* mice. We would also like to thank Drs. Sachiko Seo and Mineo Kurokawa, and Dr. Konstantina Alexandropoulos for sharing the *CasL*$^{-/-}$ and *Sin*$^{-/-}$ mouse lines, respectively.

Competing interests

The authors declare no competing financial interests.

Availability of data and materials

All data analyzed during this study are included in this article.

Authors Contributions

WW designed and performed experiments, and wrote the manuscript. JAE, TU, JNJ and KMW designed and performed experiments. JAE, JNJ and KMW also provided comments and suggestions for the manuscript. MMR conceived the project, designed experiments, and wrote the manuscript.

Ethics approval

All animal procedures presented here were performed according to the University of California, Riverside's Institutional Animal Care and Use Committee (IACUC)-approved guidelines, under protocol A-20200021

Figure Legends:

Fig. 1. *Cas* genes are required for cortical lamination and neuronal migration. (**A**) Expression analysis of *p130Cas* (magenta), *CasL* (green), and *Sin* (blue) mRNA on coronal sections of E14.5 WT cortex using RNAscope. All *Cas* members are detected in the SVZ and VZ. (**B**) Upper panels: coronal sections of P7 control and *Emx1Cre;CasTcKO* cortices stained for the layer markers Cux1 (green, layer II/III) and Ctip2 (magenta, layer V & VI), counterstained with DAPI (blue). Lower panels: quantification of the proportion of Cux1+ and Ctip2+ cells per bin. Values provided are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney *U* test with Bonferroni correction, *p<0.005 two-tailed test. (**C-D**) Detection of prospective deep layer-(**C**) and superficial layer-neurons (**D**) labeled by EdU (green) at E12.5 and E15.5, respectively, on coronal sections of control and *Emx1Cre;CasTcKO* cortices. Sections were also stained for laminin (magenta) counterstained with DAPI (blue). Ectopic deep layer cells are found in the upper CP and outside of the meninges (**C,** white arrows), while EdU revealed distinct columns of cells extending from the superficial layer into the subarachnoid space (**D,** white arrows) of *Emx1Cre;CasTcKO* animals. Right panels: quantification of proportion of EdU+ cells per bin. Values provided are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney *U* test with false discovery rate<0.1 at p<0.05 is significant for (**C**) Bin 2 and (**D**) Bins 1, 2, 4, and 5. Bin 1 is the MZ and Bin 10 is ventral to layer VI. CP: cortical plate; IZ: intermediate zone; SVZ: subventricular zone; VZ: ventricular zone. Scale bars for lower magnification panels in A, B,C, D: 500 µm; higher magnification panels in A, C, D: 100 µm.

Fig. 2. Cas adaptor proteins are required in a non-neuronal-autonomous manner for cortical lamination. (**A**) Coronal cortical sections of P7 control and *NexCre;CasTcKO* mice stained for the layer markers Cux1 (green) and Ctip2 (magenta), counterstained with DAPI (blue). Normal positioning of Cux1+ superficial layer- and Ctip2+ deep layer-neurons. Bottom: quantification of proportion of Cux1+ and Ctip2+ cells per bin. (**B**) Detection of deep layer neurons born at E12.5 and stained for EdU (green; pulsed at E12.5 and collected at P0) in coronal sections of control and *NexCre;CasTcKO* cortices. (**C**) EdU labeling (green) of superficial layer neurons in

coronal sections of control and *NexCre;CasTcKO* cortices (pulsed at E15.5 and collected at P3). (**B-C**) Sections were also stained for laminin (magenta) and DAPI (blue). Right panel: quantification of proportion of EdU+ cells per bin. Migration does not seem to be affected in *NexCre;CasTcKO* mice. Values given are Mean ± S.E.M., n=3 independent samples per group, 3 sections per sample, Mann-Whitney *U* test with Bonferroni correction or with false discovery rate<0.1 and p<0.05, no significant differences. Bin 1 is the MZ and Bin 10 is ventral to layer VI. MZ: marginal zone; CP: cortical plate; IZ: intermediate zone; VZ: ventricular zone. Scale bar for A, lower mag images in B, C: 500 µm; high magnification panels in B, C: 100 µm.

Fig. 3. A requirement for Cas adaptors during glial scaffold formation. (**A**) Expression of tyrosine phosphorylated Cas (pY165Cas; magenta) is detected in Nestin+ (green) radial glial cell bodies located at the VZ and in basal processes contacting the MZ at E13.5. (**B**) Laminin (green) and Nestin (magenta) antibody staining on E15.5 control and *Emx1Cre;CasTcKO* coronal sections. Radial glial endfeet do not make proper contact with the basal lamina in *Emx1Cre;CasTcKO* animals at exposed laminin sites (arrows). (**C-D**) Detection of proliferative cells using EdU (green) at E12.5 (**C**) and E15.5 (**D**). Proliferating cells are ectopically observed within the CP of *Emx1Cre;CasTcKO* animals (white arrows). Scale bar in lower magnification panels for B, C, D: 500 µm; A, and higher mag panels in B, C, D: 100 µm.

Fig. 4. DG and integrin act upstream of Cas phosphorylation to control RGC endfeet adhesion. (**A**) Coronal cortical sections of P7 control and *Emx1Cre;DG*$^{flox/-}$ animals stained for the layer markers Cux1 (green) and Ctip2 (magenta), counterstained with DAPI (blue). *Emx1Cre;DG*$^{flox/-}$ mice display a similar cortical phenotype to *Emx1Cre;CasTcKO* mutants with disorganized distribution of superficial and deep layer neurons. (**B**) Ctip2 (magenta) and Cux1 (green) immunostaining on coronal sections of P7 control and *Emx1Cre;β1-Integrin*$^{flox/flox}$ cortices. Similar laminar disorganization and cobblestone malformation were observed in *Emx1Cre;CasTcKO* animals. (**C**) Immunofluorescence for pY165Cas (magenta), c-Myc (green) and Nestin (blue) of WT and *Emx1Cre;β1-Integrin*$^{flox/flox}$ mixed cortical cultures transfected with a

control vector (myc) or a construct overexpressing myc-tagged full-length Dystroglycan (myc-DG). DG recruited pY165Cas to the radial glial endfeet. In the absence of *β1-integrin*, overexpression of full-length DG does not result in an increase in p130Cas phosphorylation. **(D)** Quantification of the relative fluorescent intensity of pY165Cas at RGC endfeet. Values given are Mean ± S.E.M., n=6-7 independent samples per group; 9-18 cells per sample, Mann-Whitney *U* test, ***p=0.0001 two-tailed test). Scale bars for A: 70 μm; B: 500 μm; C: 1 μm.

References

Ang, E. S., Jr, Haydar, T. F., Gluncic, V., & Rakic, P. (2003). Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(13), 5805–5815. https://doi.org/10.1523/JNEUROSCI.23-13-05805.2003

Angevine, J. B., Jr, & Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*, *192*, 766–768. https://doi.org/10.1038/192766b0

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

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

Barros, C. S., Franco, S. J., & Müller, U. (2011). Extracellular matrix: functions in the nervous system. *Cold Spring Harbor perspectives in biology*, *3*(1), a005108. https://doi.org/10.1101/cshperspect.a005108

Bedogni, F., Hodge, R. D., Elsen, G. E., Nelson, B. R., Daza, R. A., Beyer, R. P., Bammler, T. K., Rubenstein, J. L., & Hevner, R. F. (2010). Tbr1 regulates regional and laminar identity of postmitotic neurons in developing neocortex. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 13129–13134. https://doi.org/10.1073/pnas.1002285107

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

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behavioural brain research*, *125*(1-2), 279–284. https://doi.org/10.1016/s0166-4328(01)00297-2

Bicknese, A. R., Sheppard, A. M., O'Leary, D. D., & Pearlman, A. L. (1994). Thalamocortical axons extend along a chondroitin sulfate proteoglycan-enriched pathway coincident with the neocortical subplate and distinct from the efferent path. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *14*(6), 3500–3510. https://doi.org/10.1523/JNEUROSCI.14-06-03500.1994

Booler, H. S., Pagalday-Vergara, V., Williams, J. L., Hopkinson, M., & Brown, S. C. (2017). Evidence of early defects in Cajal-Retzius cell localization during brain development in a mouse model of dystroglycanopathy. *Neuropathology and applied neurobiology*, *43*(4), 330–345. https://doi.org/10.1111/nan.12376

Booler, H. S., Williams, J. L., Hopkinson, M., & Brown, S. C. (2016). Degree of Cajal-Retzius Cell Mislocalization Correlates with the Severity of Structural Brain Defects in Mouse Models of Dystroglycanopathy. *Brain pathology (Zurich, Switzerland)*, *26*(4), 465–478. https://doi.org/10.1111/bpa.12306

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., del Pilar Camacho-Leal, M., Di Stefano, P., & Defilippi, P. (2010). Integrin signalling adaptors: not only figurants in the cancer story. *Nature reviews. Cancer*, *10*(12), 858–870. https://doi.org/10.1038/nrc2967

Cadwell, C. R., Bhaduri, A., Mostajo-Radji, M. A., Keefe, M. G., & Nowakowski, T. J. (2019). Development and Arealization of the Cerebral Cortex. *Neuron*, *103*(6), 980–1004. https://doi.org/10.1016/j.neuron.2019.07.009

Camacho Leal, M., Costamagna, A., Tassone, B., Saoncella, S., Simoni, M., Natalini, D., Dadone, A., Sciortino, M., Turco, E., Defilippi, P., Calautti, E., & Cabodi, S. (2018). Conditional ablation of p130Cas/BCAR1 adaptor protein impairs epidermal homeostasis by altering cell adhesion and differentiation. *Cell communication and signaling : CCS*, *16*(1), 73. https://doi.org/10.1186/s12964-018-0289-z

Campbell, K., & Götz, M. (2002). Radial glia: multi-purpose cells for vertebrate brain development. *Trends in neurosciences*, *25*(5), 235–238. https://doi.org/10.1016/s0166-2236(02)02156-2

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

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

Cohen, M. W., Jacobson, C., Yurchenco, P. D., Morris, G. E., & Carbonetto, S. (1997). Laminin-induced clustering of dystroglycan on embryonic muscle cells: comparison with agrin-induced clustering. *The Journal of cell biology*, *136*(5), 1047–1058. https://doi.org/10.1083/jcb.136.5.1047

Colognato, H., & Yurchenco, P. D. (2000). Form and function: the laminin family of heterotrimers. *Developmental dynamics : an official publication of the American Association of Anatomists*, *218*(2), 213–234. https://doi.org/10.1002/(SICI)1097-0177(200006)218:2<213::AID-DVDY1>3.0.CO;2-R

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

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.

Durbeej, M., Henry, M. D., Ferletta, M., Campbell, K. P., & Ekblom, P. (1998). Distribution of dystroglycan in normal adult mouse tissues. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, *46*(4), 449–457. https://doi.org/10.1177/002215549804600404

Edwards, M. M., Mammadova-Bach, E., Alpy, F., Klein, A., Hicks, W. L., Roux, M., Simon-Assmann, P., Smith, R. S., Orend, G., Wu, J., Peachey, N. S., Naggert, J. K., Lefebvre, O., & Nishina, P. M. (2010). Mutations in Lama1 disrupt retinal vascular development and inner limiting membrane formation. *The Journal of biological chemistry*, *285*(10), 7697–7711. https://doi.org/10.1074/jbc.M109.069575

Endo T. (2007). Dystroglycan glycosylation and its role in alpha-dystroglycanopathies. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology*, *26*(3), 165–170.

Ferent, J., Zaidi, D., & Francis, F. (2020). Extracellular Control of Radial Glia Proliferation and Scaffolding During Cortical Development and Pathology. *Frontiers in cell and developmental biology*, *8*, 578341. https://doi.org/10.3389/fcell.2020.578341

Ferrere, A., Vitalis, T., Gingras, H., Gaspar, P., & Cases, O. (2006). Expression of Cux-1 and Cux-2 in the developing somatosensory cortex of normal and barrel-defective mice. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology*, *288*(2), 158–165. https://doi.org/10.1002/ar.a.20284

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

Franco, S. J., & Müller, U. (2011). Extracellular matrix functions during neuronal migration and lamination in the mammalian central nervous system. Developmental neurobiology, 71(11), 889–900. https://doi.org/10.1002/dneu.20946

Frantz, G. D., & McConnell, S. K. (1996). Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron*, *17*(1), 55–61. https://doi.org/10.1016/s0896-6273(00)80280-9

Franzoni, E., Booker, S. A., Parthasarathy, S., Rehfeld, F., Grosser, S., Srivatsa, S., Fuchs, H. R., Tarabykin, V., Vida, I., & Wulczyn, F. G. (2015). miR-128 regulates neuronal migration, outgrowth and intrinsic excitability via the intellectual disability gene Phf6. *eLife*, *4*, e04263. https://doi.org/10.7554/eLife.04263

Gadisseux, J. F., Evrard, P., Misson, J. P., & Caviness, V. S. (1989). Dynamic structure of the radial glial fiber system of the developing murine cerebral wall. An immunocytochemical analysis. *Brain research. Developmental brain research*, *50*(1), 55–67. https://doi.org/10.1016/0165-3806(89)90126-0

Georges-Labouesse, E., Mark, M., Messaddeq, N., & Gansmüller, A. (1998). Essential role of alpha 6 integrins in cortical and retinal lamination. *Current biology : CB*, *8*(17), 983–986. https://doi.org/10.1016/s0960-9822(98)70402-6

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

Godfrey, C., Clement, E., Mein, R., Brockington, M., Smith, J., Talim, B., Straub, V., Robb, S., Quinlivan, R., Feng, L., Jimenez-Mallebrera, C., Mercuri, E., Manzur, A. Y., Kinali, M., Torelli, S., Brown, S. C., Sewry, C. A., Bushby, K., Topaloglu, H., North, K., … Muntoni, F. (2007). Refining genotype phenotype correlations in muscular dystrophies with defective glycosylation of dystroglycan. *Brain : a journal of neurology*, *130*(Pt 10), 2725–2735. https://doi.org/10.1093/brain/awm212

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

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

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

Guillemot F. (2007). Cell fate specification in the mammalian telencephalon. *Progress in neurobiology*, *83*(1), 37–52. https://doi.org/10.1016/j.pneurobio.2007.02.009

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

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. *The Journal of biological chemistry*, *271*(23), 13649–13655. 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. (1990). Riding the glial monorail: A common mechanism for glialguided neuronal migration in different regions of the developing mammalian brain. *Trends in neuroscience*, *13*(5), 179-184. https://doi.org/10.1016/0166-2236(90)90044-B

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., & Go□tz, 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

Henry, M. D., Cohen, M. B., & Campbell, K. P. (2001). Reduced expression of dystroglycan in breast and prostate cancer. *Human pathology*, *32*(8), 791–795. https://doi.org/10.1053/hupa.2001.26468

Hevner R. F. (2007). Layer-specific markers as probes for neuron type identity in human neocortex and malformations of cortical development. *Journal of neuropathology and experimental neurology*, *66*(2), 101–109. https://doi.org/10.1097/nen.0b013e3180301c06

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., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T., & Rubenstein, J. L. (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

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, Y., Magdaleno, S., Hopkins, R., Slaughter, C., Curran, T., & Keshvara, L. (2004). Tyrosine phosphorylated Disabled 1 recruits Crk family adapter proteins. *Biochemical and biophysical research communications*, *318*(1), 204–212. https://doi.org/10.1016/j.bbrc.2004.04.023

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

Huang, Z. J., & Zeng, H. (2013). Genetic approaches to neural circuits in the mouse. *Annual review of neuroscience*, *36*, 183–215. https://doi.org/10.1146/annurev-neuro-062012-170307

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

Kawauchi T. (2012). Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. International journal of molecular sciences, 13(4), 4564–4590. https://doi.org/10.3390/ijms13044564

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

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

Kostovic, I., & Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *The Journal of comparative neurology*, *297*(3), 441–470. https://doi.org/10.1002/cne.902970309

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., Lam, M. M., Krsnik, Z., Kawasawa, Y. I., Lefebvre, V., & Sestan, N. (2008). SOX5 postmitotically regulates migration, postmigratory differentiation, and projections of subplate and deep-layer neocortical neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(41), 16021–16026. https://doi.org/10.1073/pnas.0806791105

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

Lambert de Rouvroit, C., & Goffinet, A. M. (2001). Neuronal migration. *Mechanisms of development*, *105*(1-2), 47–56. https://doi.org/10.1016/s0925-4773(01)00396-3

Leid, M., Ishmael, J. E., Avram, D., Shepherd, D., Fraulob, V., & Dollé, P. (2004). CTIP1 and CTIP2 are differentially expressed during mouse embryogenesis. *Gene expression patterns : GEP*, *4*(6), 733–739. https://doi.org/10.1016/j.modgep.2004.03.009

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

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

LoTurco, J. J., & Bai, J. (2006). The multipolar stage and disruptions in neuronal migration. *Trends in neurosciences*, *29*(7), 407–413. https://doi.org/10.1016/j.tins.2006.05.006

LoTurco, J. J. & Booker, A. B. (2013). Neuronal migration disorders. In J. L. R. Rubenstein & P. Rakic (Ed.). *Cellular migration and formation of neuronal connections* (481-494). San Diego, CA: Academic Press.

Lu, P., Takai, K., Weaver, V. M., & Werb, Z. (2011). Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harbor perspectives in biology*, *3*(12), a005058. https://doi.org/10.1101/cshperspect.a005058

Luskin M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron*, *11*(1), 173–189. https://doi.org/10.1016/0896-6273(93)90281-u

Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zariwala, H. A., Gu, H., Ng, L. L., Palmiter, R. D., Hawrylycz, M. J., Jones, A. R., Lein, E. S., & Zeng, H. (2010). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nature neuroscience*, *13*(1), 133–140. https://doi.org/10.1038/nn.2467

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

Marin-Padilla M. (1978). Dual origin of the mammalian neocortex and evolution of the cortical plate. *Anatomy and embryology*, *152*(2), 109–126. https://doi.org/10.1007/BF00315920

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

Mercuri, E., Messina, S., Bruno, C., Mora, M., Pegoraro, E., Comi, G. P., D'Amico, A., Aiello, C., Biancheri, R., Berardinelli, A., Boffi, P., Cassandrini, D., Laverda, A., Moggio, M., Morandi, L., Moroni, I., Pane, M., Pezzani, R., Pichiecchio, A., Pini, A., … Bertini, E. (2009). Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. *Neurology*, *72*(21), 1802–1809. https://doi.org/10.1212/01.wnl.0000346518.68110.60

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.

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

Molnár, Z., Métin, C., Stoykova, A., Tarabykin, V., Price, D. J., Francis, F., Meyer, G., Dehay, C., & Kennedy, H. (2006). Comparative aspects of cerebral cortical development. *The European journal of neuroscience*, *23*(4), 921–934. https://doi.org/10.1111/j.1460-9568.2006.04611.x

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

Montanaro, F., Gee, S. H., Jacobson, C., Lindenbaum, M. H., Froehner, S. C., & Carbonetto, S. (1998). Laminin and alpha-dystroglycan mediate acetylcholine receptor aggregation via a MuSK-independent pathway. *The Journal of neuroscience : the*

official journal of the Society for Neuroscience, *18*(4), 1250–1260. https://doi.org/10.1523/JNEUROSCI.18-04-01250.1998

Monuki, E. S., & Walsh, C. A. (2001). Mechanisms of cerebral cortical patterning in mice and humans. Nature neuroscience, 4 Suppl, 1199–1206. https://doi.org/10.1038/nn752

Motta, A., Berning, M., Boergens, K. M., Staffler, B., Beining, M., Loomba, S., Hennig, P., Wissler, H., & Helmstaedter, M. (2019). Dense connectomic reconstruction in layer 4 of the somatosensory cortex. *Science (New York, N.Y.)*, *366*(6469), eaay3134. https://doi.org/10.1126/science.aay3134

Muntoni, F., Brockington, M., Godfrey, C., Ackroyd, M., Robb, S., Manzur, A., Kinali, M., Mercuri, E., Kaluarachchi, M., Feng, L., Jimenez-Mallebrera, C., Clement, E., Torelli, S., Sewry, C. A., & Brown, S. C. (2007). Muscular dystrophies due to defective glycosylation of dystroglycan. *Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology*, *26*(3), 129–135.

Muntoni, F., & Voit, T. (2004). The congenital muscular dystrophies in 2004: a century of exciting progress. *Neuromuscular disorders : NMD*, *14*(10), 635–649. https://doi.org/10.1016/j.nmd.2004.06.009

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., Brunstrom, J. E., Grutzendler, J., Wong, R. O., & Pearlman, A. L. (2001). Two modes of radial migration in early development of the cerebral cortex. Nature neuroscience, 4(2), 143–150. https://doi.org/10.1038/83967

Nadarajah, B., & Parnavelas, J. (2002) Modes of neuronal migration in the developing cerebral cortex. *Nat Rev Neurosci* 3, 423–432. https://doi.org/10.1038/nrn845

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. *et al.* 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 (2015). https://doi.org/10.1038/srep11163

Nakagawa, Y., & O'Leary, D. D. (2003). Dynamic patterned expression of orphan nuclear receptor genes RORalpha and RORbeta in developing mouse forebrain. *Developmental neuroscience*, *25*(2-4), 234–244. https://doi.org/10.1159/000072271

Nakamoto, T., Sakai, R., Honda, H., Ogawa, S., Ueno, H., Suzuki, T., Aizawa, S., Yazaki, Y., & Hirai, H. (1997). Requirements for localization of p130cas to focal adhesions. *Molecular and cellular biology*, *17*(7), 3884–3897. https://doi.org/10.1128/MCB.17.7.3884

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*, *25*(30), 7022–7031. https://doi.org/10.1523/JNEUROSCI.1695-05.2005

Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S., & Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature*, *409*(6821), 714–720. https://doi.org/10.1038/35055553

Noctor, S. C., Martínez-Cerdeño, V., Ivic, L., & Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature neuroscience*, *7*(2), 136–144. https://doi.org/10.1038/nn1172

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'Leary, D. D., & Koester, S. E. (1993). Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. *Neuron*, *10*(6), 991–1006. https://doi.org/10.1016/0896-6273(93)90049-w

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

Pinzón-Duarte, G., Daly, G., Yong, L., Hunter, D. D., Koch, M., & Brunken, W. J. (2010). Defective Formation of the Inner Limiting Membrane in Laminin β2- and γ3-null Mice Produces Retinal Dysplasia. *Invest. Ophthalmol. Vis. Sci.* 51, 1773–1782. https://doi.org/10.1016/j.exer.2012.01.007

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

Pramparo, T., Youn, Y. H., Yingling, J., Hirotsune, S., & Wynshaw-Boris, A. (2010). Novel embryonic neuronal migration and proliferation defects in Dcx mutant mice are exacerbated by Lis1 reduction. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *30*(8), 3002–3012. https://doi.org/10.1523/JNEUROSCI.4851-09.2010

Rakic P. (1971). Guidance of neurons migrating to the fetal monkey neocortex. *Brain research*, *33*(2), 471–476. https://doi.org/10.1016/0006-8993(71)90119-3

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

Rakic P. (1988). Specification of cerebral cortical areas. *Science (New York, N.Y.)*, *241*(4862), 170–176. https://doi.org/10.1126/science.3291116

Rakic P. (2003). Developmental and evolutionary adaptations of cortical radial glia. *Cerebral cortex (New York, N.Y. : 1991)*, *13*(6), 541–549. https://doi.org/10.1093/cercor/13.6.541

Rakic P. (2009). Evolution of the neocortex: a perspective from developmental biology. *Nature reviews. Neuroscience*, *10*(10), 724–735. https://doi.org/10.1038/nrn2719

Rakic, R. S., Cameron, H., & Komuro, H. (1994). Recognition, adhesion, transmembrane signaling, and cell motility in guided neuronal migration. *Curr. Opin. Neurobiol*, 4, 63-69. https://doi.org/10.1016/0959-4388(94)90033-7

Ramón y Cajal S. (1911). Histologie du Système Nerveux de l'Homme et des Vertébrés, Vol. 2 Ed. Paris: Maloine.

Randlett, O., Poggi, L., Zolessi, F. R., & Harris, W. A. (2011). The oriented emergence of axons from retinal ganglion cells is directed by laminin contact in vivo. *Neuron*, *70*(2), 266–280. https://doi.org/10.1016/j.neuron.2011.03.013

Rash, B. G., & Grove, E. A. (2006). Area and layer patterning in the developing cerebral cortex. *Current opinion in neurobiology*, *16*(1), 25–34. https://doi.org/10.1016/j.conb.2006.01.004

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

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

Rosário, M., Schuster, S., Jüttner, R., Parthasarathy, S., Tarabykin, V., & Birchmeier, W. (2012). Neocortical dendritic complexity is controlled during development by NOMA-

GAP-dependent inhibition of Cdc42 and activation of cofilin. *Genes & development*, *26*(15), 1743–1757. https://doi.org/10.1101/gad.191593.112

Rutishauser, U., & Jessell, T. M. (1988). Cell adhesion molecules in vertebrate neural development. *Physiological reviews*, *68*(3), 819–857. https://doi.org/10.1152/physrev.1988.68.3.819

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

Sanes J. R. (1989). Extracellular matrix molecules that influence neural development. *Annual review of neuroscience*, *12*, 491–516. https://doi.org/10.1146/annurev.ne.12.030189.002423

Sanes, J. R., & Zipursky, S. L. (2010). Design principles of insect and vertebrate visual systems. *Neuron*, *66*(1), 15–36. https://doi.org/10.1016/j.neuron.2010.01.018

Sanes, J. R., & Zipursky, S. L. (2020). Synaptic Specificity, Recognition Molecules, and Assembly of Neural Circuits. *Cell*, *181*(3), 536–556. https://doi.org/10.1016/j.cell.2020.04.008

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

Schmid, R. S., & Anton, E. S. (2003). Role of Integrins in the Development of the Cerebral Cortex. *Cerebral Cortex*. *13*(3), 219–224. https://doi.org/10.1093/cercor/13.3.219

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

Sheppard, A. M., & Pearlman, A. L. (1997). Abnormal reorganization of preplate neurons and their associated extracellular matrix: an early manifestation of altered neocortical development in the reeler mutant mouse. *The Journal of comparative neurology*, *378*(2), 173–179. https://doi.org/10.1002/(sici)1096-9861(19970210)378:2<173::aid-cne2>3.0.co;2-0

Sokpor, G., Kerimoglu, C., Nguyen, H., Pham, L., Rosenbusch, J., Wagener, R., Nguyen, H. P., Fischer, A., Staiger, J. F., & Tuoc, T. (2021). Loss of BAF Complex in Developing Cortex Perturbs Radial Neuronal Migration in a WNT Signaling-Dependent Manner. *Frontiers in molecular neuroscience*, *14*, 687581. https://doi.org/10.3389/fnmol.2021.687581

Subramanian, L., Calcagnotto, M. E., & Paredes, M. F. (2020). Cortical Malformations: Lessons in Human Brain Development. *Frontiers in cellular neuroscience*, *13*, 576. https://doi.org/10.3389/fncel.2019.00576

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

Tikhmyanova, N., Little, J. L., & Golemis, E. A. (2010). CAS proteins in normal and pathological cell growth control. *Cellular and molecular life sciences : CMLS*, *67*(7), 1025–1048. https://doi.org/10.1007/s00018-009-0213-1

Tonchev, A.B., Tuoc, T.C., Rosenthal, E.H. *et al. Zbtb20* modulates the sequential generation of neuronal layers in developing cortex. *Mol Brain* 9, 65 (2016). https://doi.org/10.1186/s13041-016-0242-2

Umemura, M., Kaneko, Y., Tanabe, R. *et al.* ATF5 deficiency causes abnormal cortical development. *Sci Rep* 11, 7295 (2021). https://doi.org/10.1038/s41598-021-86442-5

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

Valiente, M., & Marín, O. (2010). Neuronal migration mechanisms in development and disease. Current opinion in neurobiology, 20(1), 68–78. https://doi.org/10.1016/j.conb.2009.12.003

Voss, A. K., Britto, J. M., Dixon, M. P., Sheikh, B. N., Collin, C., Tan, S. S., & Thomas, T. (2008). C3G regulates cortical neuron migration, preplate splitting and radial glial cell attachment. *Development (Cambridge, England)*, *135*(12), 2139–2149. https://doi.org/10.1242/dev.016725

Vuillaumier-Barrot, S., Bouchet-Séraphin, C., Chelbi, M., Devisme, L., Quentin, S., Gazal, S., Laquerrière, A., Fallet-Bianco, C., Loget, P., Odent, S., Carles, D., Bazin, A., Aziza, J., Clemenson, A., Guimiot, F., Bonnière, M., Monnot, S., Bole-Feysot, C., Bernard, J. P., Loeuillet, L., … Seta, N. (2012). Identification of mutations in TMEM5 and ISPD as a cause of severe cobblestone lissencephaly. *American journal of human genetics*, *91*(6), 1135–1143. https://doi.org/10.1016/j.ajhg.2012.10.009

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

Wang, H., Ge, G., Uchida, Y., Luu, B., & Ahn, S. (2011). Gli3 is required for maintenance and fate specification of cortical progenitors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *31*(17), 6440–6448. https://doi.org/10.1523/JNEUROSCI.4892-10.2011

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

Yokota, Y., Eom, T. Y., Stanco, A., Kim, W. Y., Rao, S., Snider, W. D., & Anton, E. S. (2010). Cdc42 and Gsk3 modulate the dynamics of radial glial growth, inter-radial glial interactions and polarity in the developing cerebral cortex. *Development (Cambridge, England)*, *137*(23), 4101–4110. https://doi.org/10.1242/dev.048637

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

Zolessi, F. R., Poggi, L., Wilkinson, C. J., Chien, C. B., & Harris, W. A. (2006). Polarization and orientation of retinal ganglion cells in vivo. *Neural development*, *1*, 2. https://doi.org/10.1186/1749-8104-1-2

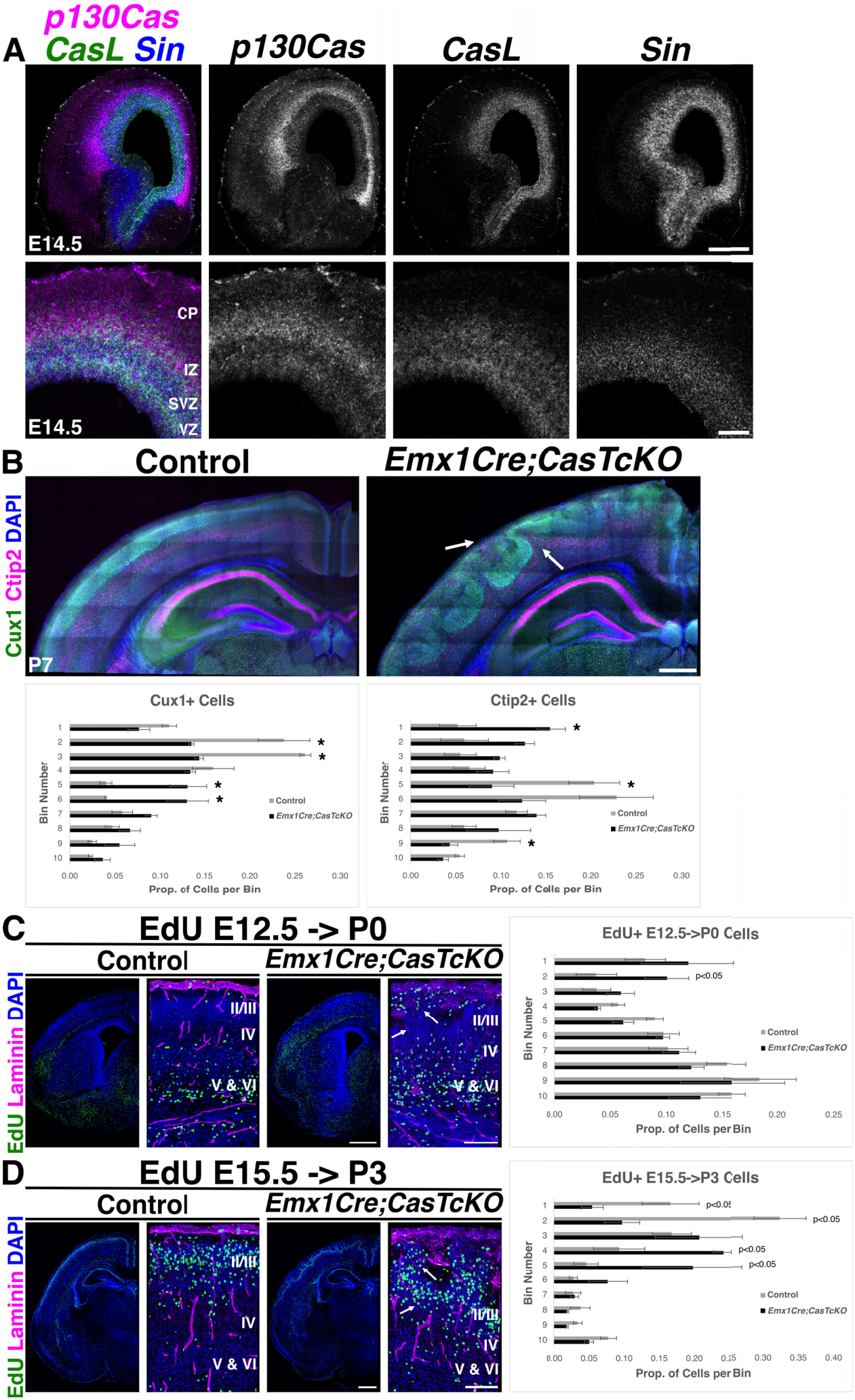
A
p130Cas
CasL Sin
p130Cas
CasL
Sin
E14.5
CP
IZ
SVZ
VZ
E14.5
B
Control
Emx1Cre;CasTcKO
Cux1 Ctip2 DAPI
P7
Cux1+ Cells
Ctip2+ Cells
Bin Number
Prop. of Cells per Bin
Control
Emx1Cre;CasTcKO
C
EdU E12.5 -> P0
Control
Emx1Cre;CasTcKO
EdU Laminin DAPI
II/III
IV
V & VI
EdU+ E12.5->P0 Cells
p<0.05
D
EdU E15.5 -> P3
EdU+ E15.5->P3 Cells
p<0.05

Figure 1

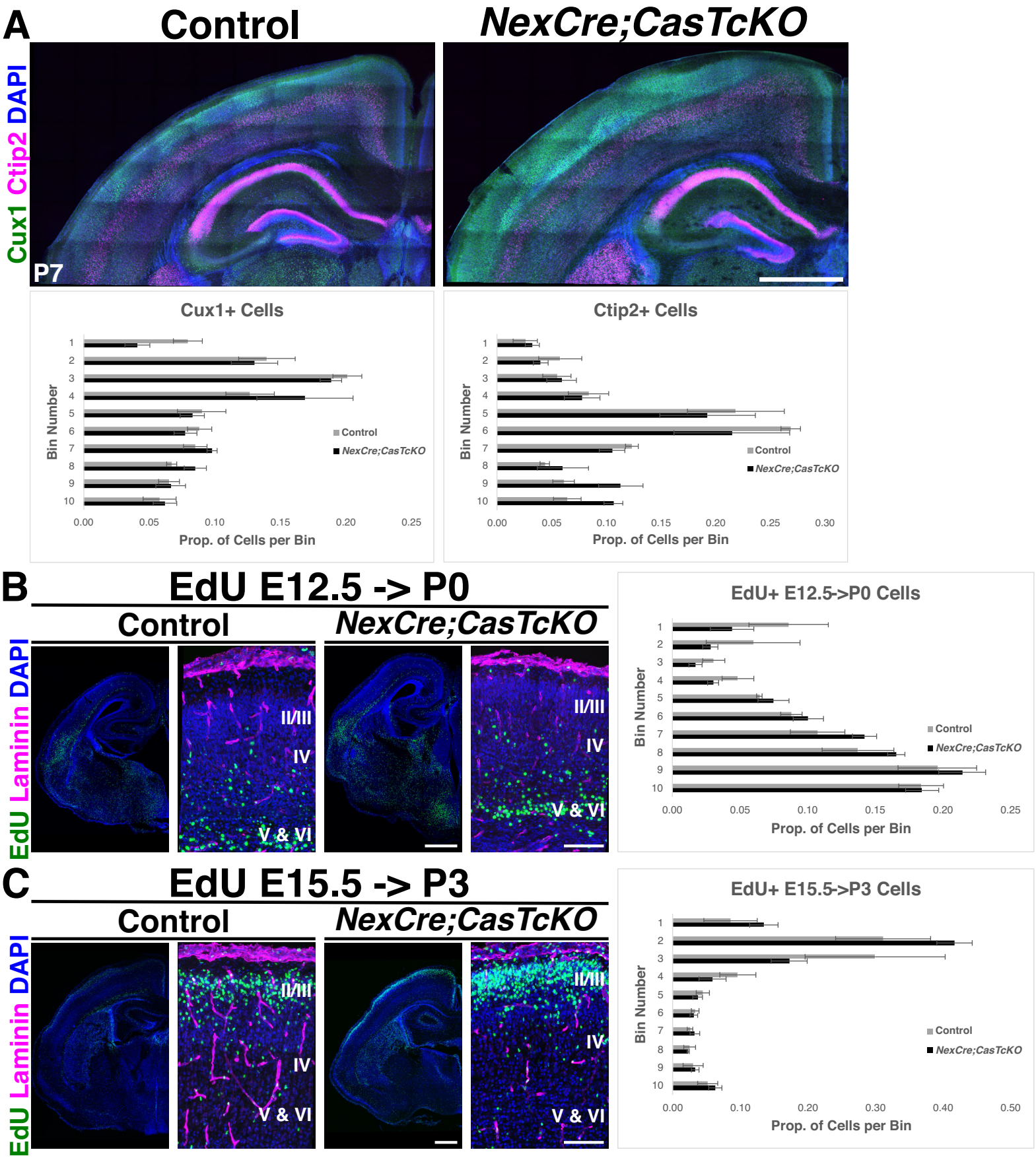
A
Control
NexCre;CasTcKO
Cux1 Ctip2 DAPI
P7
Cux1+ Cells
Bin Number
Prop. of Cells per Bin
Control
NexCre;CasTcKO
Ctip2+ Cells
B
EdU E12.5 -> P0
EdU Laminin DAPI
II/III
IV
V & VI
EdU+ E12.5->P0 Cells
C
EdU E15.5 -> P3
EdU+ E15.5->P3 Cells

Figure 2

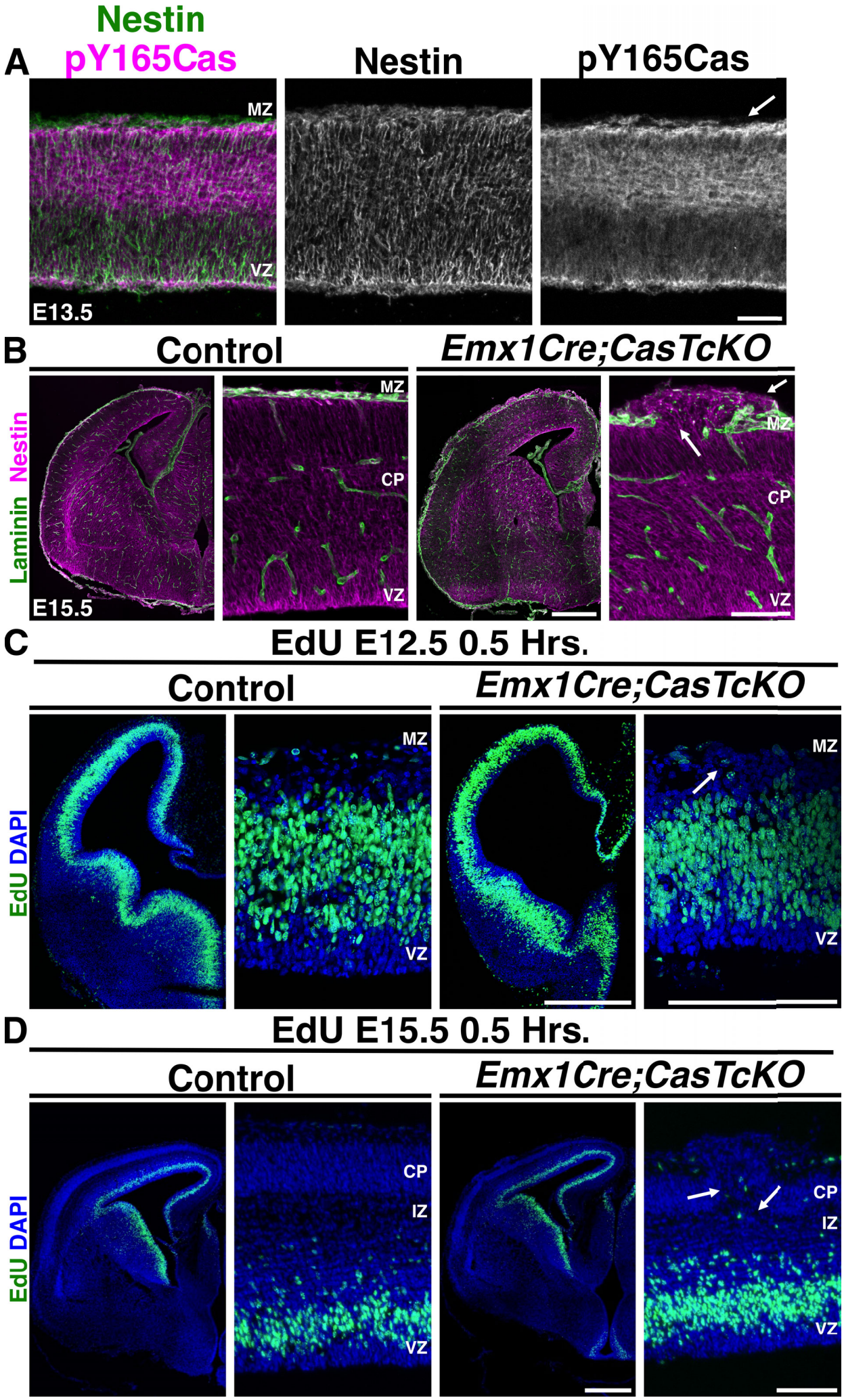
A
Nestin
pY165Cas
Nestin
pY165Cas
MZ
VZ
E13.5
B
Control
Emx1Cre;CasTcKO
Laminin
Nestin
MZ
CP
VZ
E15.5
C
EdU E12.5 0.5 Hrs.
Control
Emx1Cre;CasTcKO
EdU
DAPI
MZ
VZ
D
EdU E15.5 0.5 Hrs.
Control
Emx1Cre;CasTcKO
EdU
DAPI
CP
IZ
VZ

Figure 3

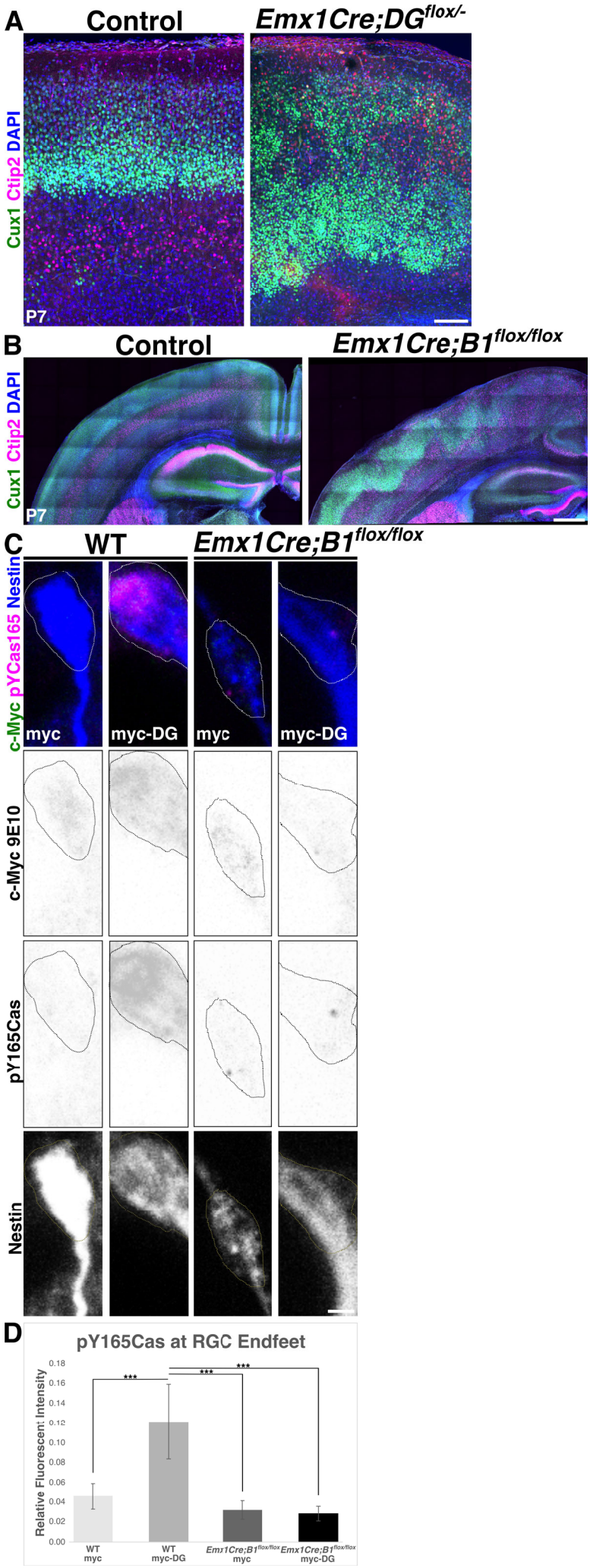
A
Control
Emx1Cre;DG flox/-
Cux1 Ctip2 DAPI
P7
B
Control
Emx1Cre;B1 flox/flox
Cux1 Ctip2 DAPI
P7
C
WT
Emx1Cre;B1 flox/flox
c-Myc pYCas165 Nestin
myc
myc-DG
myc
myc-DG
c-Myc 9E10
pY165Cas
Nestin
D
pY165Cas at RGC Endfeet
Relative Fluorescent Intensity
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
***
***
***
WT myc
WT myc-DG
Emx1Cre;B1 flox/flox myc
Emx1Cre;B1 flox/flox myc-DG

Figure 4