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Gene Deletion Mutants Reveal a Role for Semaphorin Receptors of the Plexin-B Family in Mechanisms Underlying Corticogenesis▽

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Semaphorins and their receptors, plexins, are emerging as key regulators of various aspects of neural and nonneural development. Semaphorin 4D (Sema4D) and B-type plexins demonstrate distinct expression patterns over critical time windows during the development of the murine neocortex. Here, analysis of mice genetically lacking plexin-B1 or plexin-B2 revealed the significance of Sema4D–plexin-B signaling in cortical development. Deficiency of plexin-B2 resulted in abnormal cortical layering and defective migration and differentiation of several subtypes of cortical neurons, including Cajal-Retzius cells, GABAergic interneurons, and principal cells *in vivo*. In contrast, a lack of plexin-B1 did not impact on cortical development *in vivo*. In various *ex vivo* assays on embryonic forebrain, Sema4D enhanced the radial and tangential migration of developing neurons in a plexin-B2-dependent manner. These results suggest that Sema4D–plexin-B2 interactions regulate mechanisms underlying cell specification, differentiation, and migration during corticogenesis.

Semaphorins were originally identified as a large family of axon guidance cues (13). Several recent studies have implicated them in distinct processes underlying the proliferation, differentiation, and migration of several cell types both inside and outside of the nervous system (13, 43). Cellular functions of semaphorins are mediated by cell surface receptors called plexins and their coreceptors called neuropilins (42). A large part of our current knowledge on the role of semaphorin-plexin interactions in neural development stems from studies on plexin-A family proteins and their class III semaphorin ligands (13, 21). In contrast, the role of B-type plexins and their ligands in the establishment of neural circuits is less well understood. Among the three B-type plexins, plexin-B1 and plexin-B2 are known to be expressed in neurons whereas plexin-B3 is expressed in oligodendrocytes (46). Semaphorin 4D (Sema4D), a class IV semaphorin which exists in a transmembrane form as well as a soluble protein, binds plexin-B1 with high affinity and plexin-B2 with low affinity (28, 42). In contrast, Sema4C has been reported to bind plexin-B2 with high affinity (12). Recently, we generated mice genetically lacking either plexin-B1 or plexin-B2 in a constitutive manner and observed a critical requirement for plexin-B signaling in neural tube closure and migration of cerebellar granule cell precursors (12). Although Sema4D, plexin-B1, and plexin-B2 are also highly expressed in the developing neocortex over embryonic stages (46), their functional roles in cortical development have not been characterized so far.

Precise regulation of the cell cycle and temporal coordination of the characteristic programs governing cell migration and differentiation are very essential for the development of the neocortex (9, 19). During corticogenesis, neuroblasts undergo active mitosis in the ventricular germinal zone between embryonic day 10 (E10) and E17. Neurons born subsequently migrate radially and form cortical laminae II to VI between the lowermost subplate (SP) layer and the uppermost marginal zone (MZ) in an "inside-out" pattern, where neurons born early occupy deeper layers, whereas superficial layers are populated with cells born late (9). It is generally believed that excitatory projection neurons in the cortical plate (CP) are born locally, whereas GABAergic inhibitory neurons born elsewhere invade the neocortex via tangential migration (35).

In the past years, several transcription factors which govern cell specification and migration in the developing neocortex have been described (9, 18, 35). In addition to such cell-autonomous programs, cell-derived extracellular cues have been proposed to come into play in regulating and orchestrating distinct steps in migratory and differentiation programs. Several such cues, e.g., slit proteins, brain-derived neurotrophic factor (BDNF), stromal cell-derived factor 1α (SDF-1α), neuregulin, and reelin, among others, have been identified in previous studies (2, 24, 26). Recently, class III semaphorins such as Sema3A and Sema3F have been proposed to regulate the migration of cortical neurons (8, 27, 44).

Based upon our findings obtained by expression analyses in the developing neocortex, we hypothesized that plexin-B family proteins and their semaphorin ligands may participate in the guidance of developing neurons during the development of the CP. Using genetic mouse mutants and a variety of *ex vivo* functional assays, we observed that not only migration but also the differentiation and generation of specific subtypes of cortical cells involve plexin-B2 signaling. This study suggests that semaphorin–plexin-B interactions are involved in the regulation of several distinct processes underlying neocortical development.

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MATERIALS AND METHODS

Genetically modified animals and nomenclature. The generation of global, constitutive plexin-B1 knockout (*plxnb1*$^{-/-}$ mutant) mice lacking exons 13 to 16 of the murine *plxnb1* gene and plexin-B2 knockout (*plxnb2*$^{-/-}$ mutant) mice lacking exons 19 to 23 of the mouse *plxnb2* gene has been described in detail previously (12). Here, the mRNAs and proteins corresponding to the *Sema4d*/*Sema4c* genes are referred to as Sema4d/Sema4c and Sema4D/Sema4C, respectively. Furthermore, the mRNAs and proteins corresponding to the *plxnb1*/*plxnb2* genes are referred to as plexin-B1/plexin-B2 and plexin-B1/plexin-B2, respectively (46).

Preparation of recombinant Sema4D. The soluble, N-terminal fragment of recombinant Sema4D was generated as described in detail previously (12, 22). Briefly, the calcium phosphate method was applied to transfect HEK293T cells with a plasmid expressing alkaline phosphatase (AP)-tagged Sema4D (Sema4D-AP) or the empty AP expression vector (mock transfection) and cell supernatants were centrifuged and concentrated 60-fold using Amicon-Millipore Centricon Plus-20 concentrators with a cutoff of 100 kDa. An AP activity assay was used to determine the activity of Sema4D preparations (12), and the working concentration of different preparations was standardized to 150 mU/ml of medium.

Explant cultures. Microexplants were prepared from vibratome-cut 250-μm slices of mouse brains at E14.5. The neocortical ventricular and subventricular zones (VZ/SVZ) were isolated, and 200- to 300-μm explants were cultured on poly-D-lysine-coated coverslips with 50 μl of culture medium. Three hours later, the medium was changed to Dulbecco's minimal essential medium (Gibco) containing B-27 supplement (Invitrogen), 100 μg/ml penicillin and streptomycin, and 1 mM glutamine. Explants were exposed to 100 ng/ml BDNF or Sema3A (1 μg/ml; R&D), Sema4D-AP medium or control medium, prepared as described above, was added, and explants were cultured for 48 h at 37°C in a humidified 5% CO_2 atmosphere. Thereafter, explants were fixed with 4% paraformaldehyde for 20 min, blocked for 30 min with goat serum, incubated with the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI) and a mouse anti-β-III-tubulin antibody (1:100; Chemicon) for 1 h at room temperature, blocked, and incubated with a secondary antibody (anti-rabbit antibody–fluorescein isothiocyanate at 1:200; Dianova) using standard immunohistochemistry protocols.

Preparation of cell aggregates. Cell aggregates were prepared as described previously (36). Briefly, HEK293 cells were transfected to express Sema4D by the calcium phosphate method, trypsinated after 48 h, washed with Eagle's medium and 10% calf serum, and resuspended in 0.4 ml of medium. HEK cell aggregates were formed overnight in a hanging-drop culture. Clusters of cells were placed in close proximity to VZ/SVZ explants on poly-D-lysine-coated coverslips with 500 μl of culture medium, and the culture was incubated at 37°C.

Organotypic slice cultures. Brains obtained from embryos at E14.5 were removed and rapidly placed in cold artificial corticospinal fluid containing 5% CO_2, 95% O_2, 124 mM NaCl, 3 mM KCl, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 10 mM glucose, and 2 mM $CaCl_2$. Brains were embedded in 5% agar and sliced coronally (500 μm) on a vibrating microtome. Slices were placed onto Millicell-CM membranes (Millipore, Bedford, MA) and covered with rat tail collagen (Roche) in 35-mm petri dishes containing 1 ml of a medium (Life Technologies, Cergy Pontoise, France) consisting of 50% basal medium with Eagle's salts, 25% horse normal serum, 25% Hanks' balanced salt solution, 4.5 mg/ml D-glucose, and 0.1 mM L-glutamine. Sema4D medium or mock medium was added to the collagen matrix. In some experiments, HEK cell aggregates expressing soluble AP-tagged Sema4D or control vector were coincubated at the ventral aspect of the organotypic slices. Slices were incubated in 5% CO_2 at 35°C for 1.5 to 4.5 days. To observe cortical migration, organotypic slices were fixed at defined time points *in vitro*, sectioned at a 20-μm thickness on a cryostat, and processed for immunohistochemistry.

***In vivo* BrdU incorporation assay.** Pregnant females were injected intraperitoneally with 300 mg/kg 5-bromo-2-deoxyuridine (BrdU) on E14.5. Embryos were harvested 2 h later and processed for immunohistochemistry with an anti-BrdU antibody (1:500; Sigma).

Immunohistochemistry analysis. At least four embryos were analyzed per genotype in immunohistochemical stainings. The antibodies and reagents used were mouse anti-β-III-tubulin (1:100; Chemicon), mouse anti-neuron-specific nuclear protein (NeuN; 1:2,000; Chemicon), rabbit anticalretinin (1:600; Chemicon), mouse antireelin (1:500; Chemicon), rabbit anti-RC2 (1:200; Hybridoma Bank), mouse antinestin (1:40; Hybridoma Bank), mouse anti-GAD67 (1:1500; Chemicon), rabbit anti-glial fibrillary acidic protein (GFAP; 1:500; Dako, High Wycombe, United Kingdom), rat anti-Ki-67 (1:25; Dako), mouse anti-BrdU (1:500; Sigma), and mouse anti-GABA (1:500; Sigma) and anti-MAP2 (1:100; Sigma-Aldrich). Immunohistochemistry analysis was performed on cryosections (20 μm) or vibratome sections (50 μm) using standard reagents and protocols (Vector Laboratories). Apoptotic cell death was detected with paraformaldehyde-fixed cryosections using the In Situ Cell Death Detection kit (Roche). For counting of immunolabeled cells, a rectangular region of interest was outlined in each microscopic image where cells were manually counted. At least four sections were counted per antibody per embryo. At least four embryos were analyzed per genotype.

RESULTS

Expression of Sema4d and plexin-B mRNAs during early neocortical development. *In situ* mRNA hybridization revealed that in mice, the plexin-B1 and plexin-B2 mRNAs are prominently expressed in an overlapping manner in the forebrain during the induction and formation of the CP (E12.5 to E17.5; Fig. 1). At E13.5, both plexin-B1 and plexin-B2 were found to be expressed throughout the telencephalic ventricular and subventricular zones (VZ and SVZ, respectively; Fig. 1A and B), the origin of radially migrating excitatory cortical neurons (9). Sema4d mRNA, in contrast, was found to be selectively expressed in the cortical preplate over early periods of cortical development, directly juxtaposed to the plexin-B1/B2-expressing germinal layers (Fig. 1C). Magnified views of the reciprocal expression of plexin-B1/B2-expressing germinal epithelium and Sema4d-expressing CP are shown in Fig. 1D. Furthermore, the neuroepithelium of the medial and lateral ganglionic eminences (Fig. 1E and F), from where neuronal progenitors migrate tangentially toward the developing CP (44), also expressed both the plexin-B1 and plexin-B2 mRNAs. At E15.5 and E17.5, expression of the plexin-B1 and plexin-B2 mRNAs was also evident in the CP (46), suggesting that neurons migrating from the VZ and ganglionic eminence to occupy the developing cortical layers continue to express these plexins. In contrast to the Sema4d mRNA, we could not clearly detect expression of the Sema4c mRNA in the CP. However, the choroid plexus in the adjoining ventricles clearly showed expression of Sema4c, as well as plexin-B2, mRNA (Fig. 1G and H).

Normal neocortical development in mice lacking plexin-B1. Although the complementary expression patterns of Sema4d and plexin-B1/B2 suggested a regulatory role for Sema4D–plexin-B interactions in cortical development, mice lacking plexin-B1 (*plxnb1*$^{-/-}$) showed normal development of the cortex over the embryonic and postnatal stages (Fig. 2). Upon the assessment of typical marker proteins, no differences were found in the morphology, number, and positioning of neurons (NeuN positive), specific populations of interneurons (calretenin or parvalbumin positive), Cajal-Retzius cells (reelin positive), and glia (GFAP positive) in the cortices of *plxnb1*$^{-/-}$ mutant mice (examples of analysis at postnatal day 5 are shown in Fig. 2).

Aberrant development of the CP in mice lacking plexin-B2. In contrast to plexin-B1 mutants, mice lacking plexin-B2 show severe anatomical malformations in the nervous system, including neural tube defects, exencephaly with an inversion of the entire forebrain, and perinatal lethality, with a penetrance of more than 95% (12, 15). To exclude the primary influences of exencephaly on cortical development, we therefore studied the very small proportion of *plxnb2*$^{-/-}$ mutants which bypassed exencephaly and developed a normally oriented forebrain.

The inside-out gradient of cell occupancy is believed to be

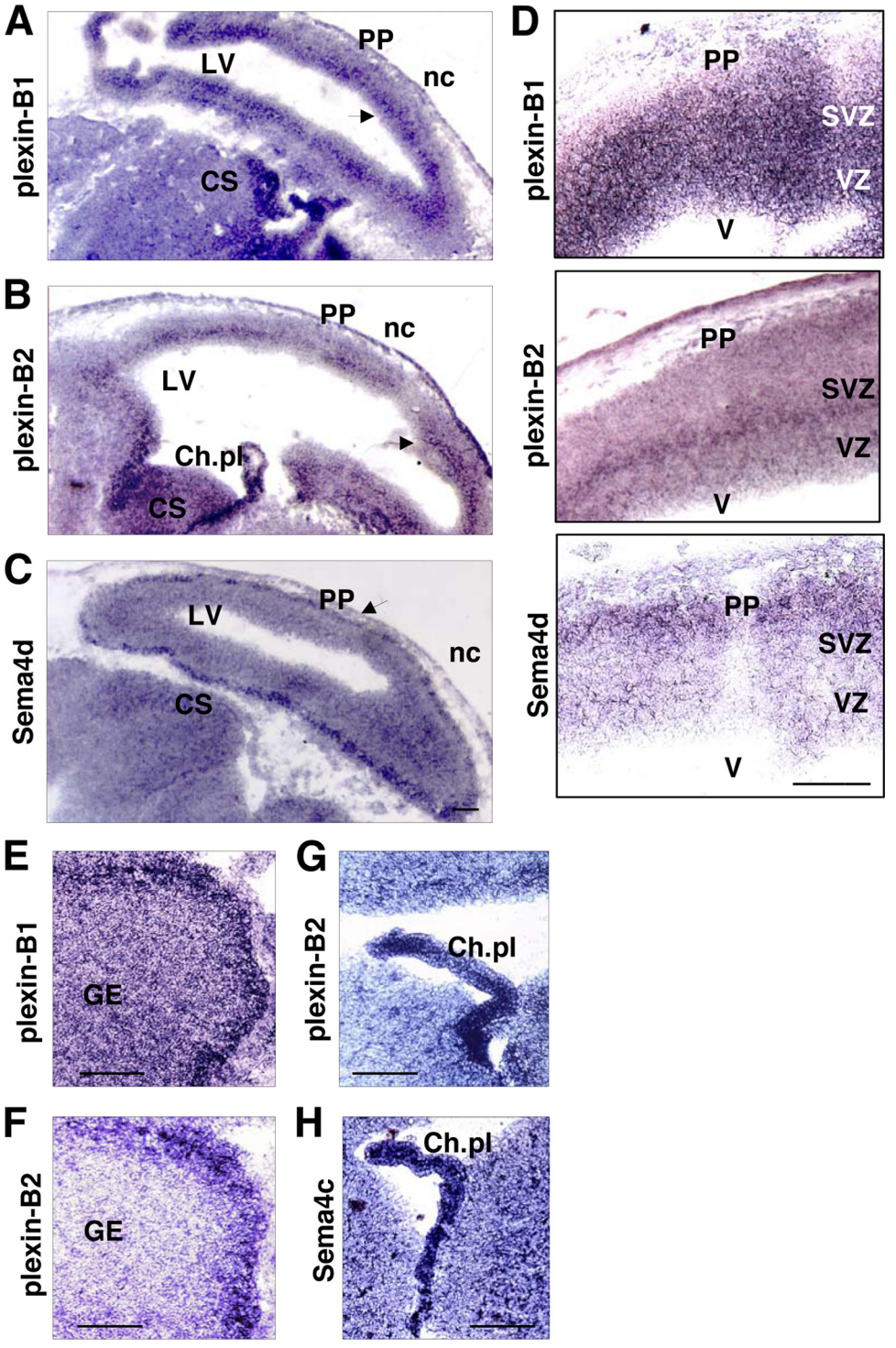

FIG. 1. Reciprocal expression of plexin-B1/B2 and Sema4d mRNAs, assessed via *in situ* hybridization during early stages of murine cortical development. Boxed areas are magnified in the inset. At E13.5, plexin-B1 mRNA (A) and plexin-B2 mRNA (B) are strongly expressed in the neuroepithelium (VZ and SVZ) whereas Sema4d is primarily found in the developing CP (C). Magnified views of expression in the developing neocortex are shown in panel D. At E13.5, the plexin-B1 and plexin-B2 mRNAs are strongly expressed in the neuroepithelium of the ganglionic eminence (GE) (E, F). Plexin-B2 and Sema4c mRNAs are expressed in the choroid plexus (G, H). Scale bars, 50 μm in all panels. Abbreviations: Ch.Pl, choroid plexus; CS, corpus striatum; NC, neocortex; LV, lateral ventricle; PP, preplate.

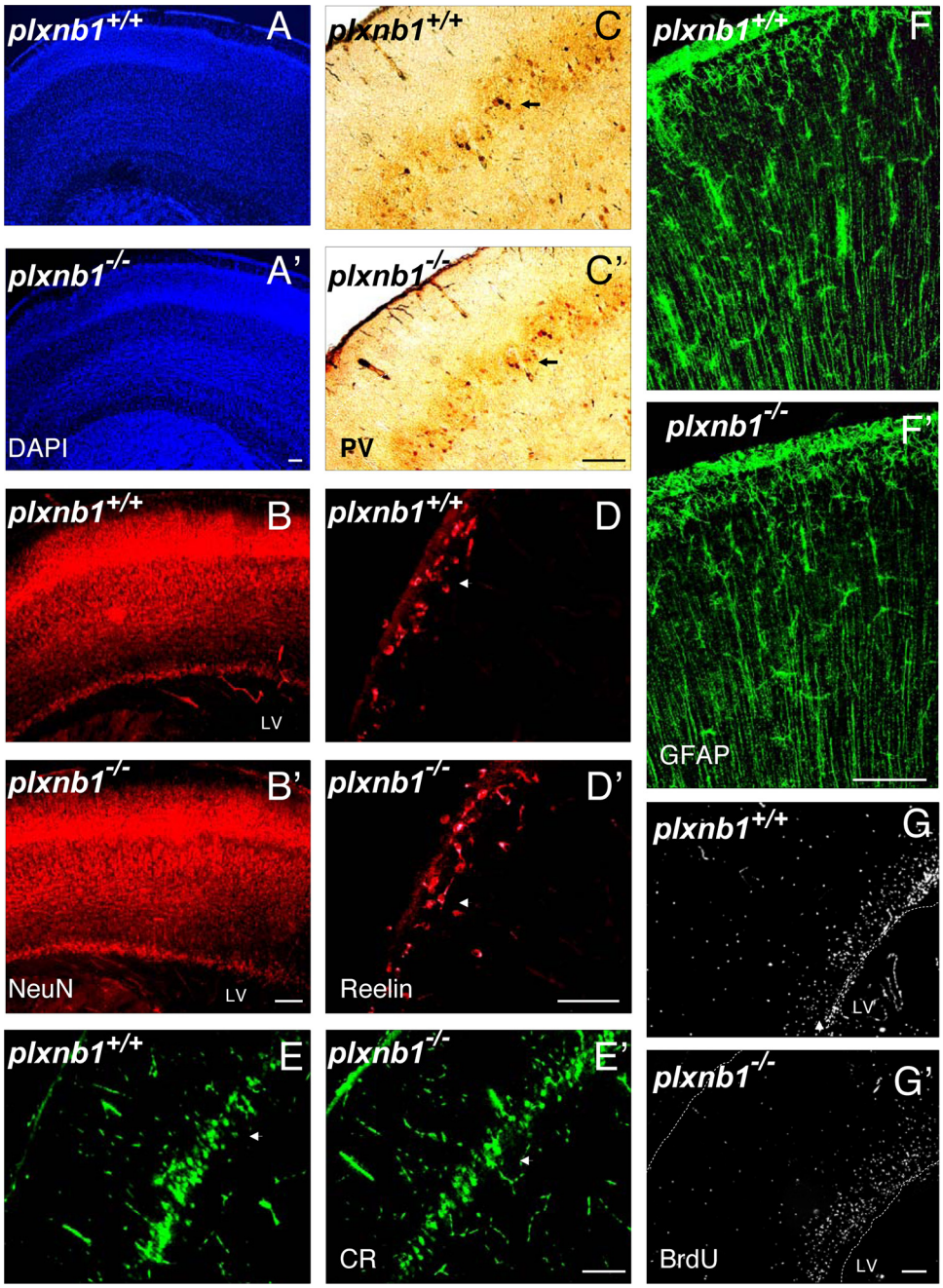

FIG. 2. The architecture of the developing neocortex in mice lacking plexin-B1 (*plxnb1*$^{-/-}$) is similar to that in their wild-type littermates (*plxnb1*$^{+/+}$). Shown are representative images, derived on postnatal day 5, of immunoreactivity for various marker proteins in diverse populations of neurons and other cell types in the developing cortex, as well as BrdU birth-dating experiments. The nuclear stain DAPI (A, A′), the panneuronal marker NeuN (B, B′), cortical interneurons labeled via paralbumin (PV) (C, C′), Cajal-Retzius cells identified via antireelin immunohistochemistry analysis (D, D′), calretinin (CR) (E, E′), and development of astrocytes labeled via anti-GFAP immunoreactivity (F, F′) are similar in plexin-B1 deletion mutants (panels B′ to F′) and their wild-type littermates (B to F). (G, G′) Incorporation of BrdU in organotypic slice cultures of the developing cortex at E14.5 is comparable across plexin-B1 deletion mutants and wild-type embryos. LV, lateral ventricle. Scale bars, 50 μm in all panels.

established by the end of the embryonic period in mice (9), which enables analysis of both modes of cortical migration at E18 (birth). We observed that, in comparison with wild-type embryos, *plxnb2*$^{-/-}$ mutants showed lower numbers of Tuj1-positive cells (Fig. 3A) and γ-aminobutyric acid (GABA)-positive cells (Fig. 3B and C; also confirmed via analysis of GAD67-positive cells) in the CP. This was also reflected in the analysis of cells in the CP staining for diagnostic markers for mature neurons, such as MAP2 and NeuN (Fig. 4A and B). Whereas wild-type littermate embryos showed abundant MAP2-positive cells throughout the CP, these cells were reduced in numbers and mostly localized in deeper zones in

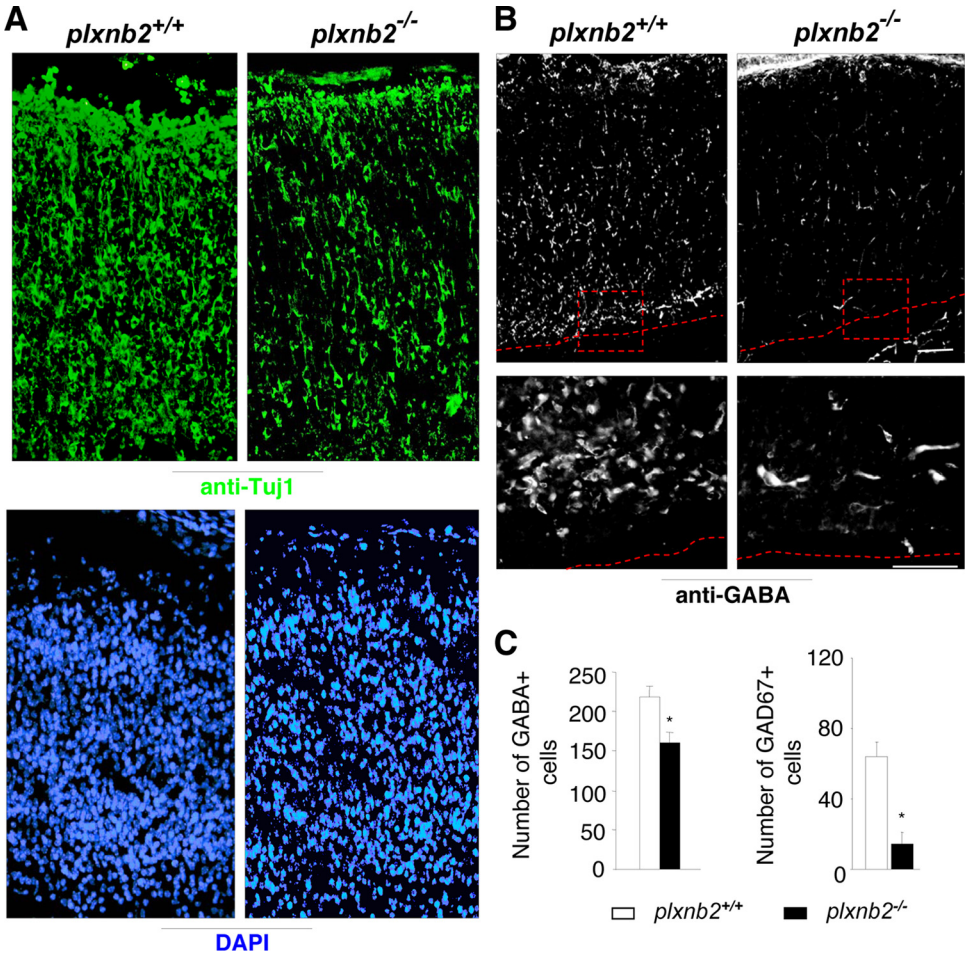

FIG. 3. Mice lacking plexin-B2 demonstrate a marked reduction in the density of neuronal populations in the developing CP at E18. (A) Decreased incidence of Tuj1-positive neurons in the CP of mice lacking plexin-B2 (*plxnb2*$^{-/-}$) in comparison with their wild-type littermates (*plxnb2*$^{+/+}$) at E18.5. Lower panels show staining of corresponding sections with the nuclear stain DAPI. (B and C) Typical examples (B) and quantification (C) of GABA-positive and GAD67-positive cells showing reduced numbers of GABAergic interneurons in the CP of mice lacking plexin-B2 at E18.5 in comparison with their wild-type littermates. Boxed areas are provided at a higher magnification in lower panels. *, $P < 0.05$ (Student's *t* test). Four embryos per genotype were tested. Scale bars, 25 μm.

plxnb2$^{-/-}$ mutants, suggesting migratory defects (Fig. 4A). Consistent with the above, the density of NeuN-positive cells was lower in the CP of *plxnb2*$^{-/-}$ mutants than in that of wild-type embryos (Fig. 4B). Taken together, these results show that development of the CP is hampered *in vivo* upon a loss of plexin-B2.

Multiple defects in cellular functions in the mutant neocortex. Abnormalities in several cellular processes could underlie the complex phenotype observed in *plxnb2*$^{-/-}$ mutants. Based upon our results described above, we hypothesized that these may include, but are not limited to, (i) defective differentiation of neuronal precursors into neurons, (ii) abnormal migration into the CP owing to glial defects, (iii) abnormal migration into the CP owing to lack of promigratory signals, and (iv) defects in proliferation in the VZ. We endeavored to address these possibilities in an effort to clarify the primary functions of plexin-B2 in cortical development.

The first hypothesis appeared plausible because immunohistochemical studies revealed some indications of abnormal differentiation in plexin-B2 mutants. We observed several DAPI-positive cells in the CP of *plxnb2*$^{-/-}$ mutants which lacked expression of neuronal markers such as Tuj1, GABA, and MAP2 (e.g., Fig. 3A and B and 4A). We observed that expression of nestin, a pan marker for neuronal and glial progenitors (14), was increased in the CP of *plxnb2*$^{-/-}$ mutants over that in wild-type embryos at E18.5 (Fig. 5A). These results suggest that in *plxnb2*$^{-/-}$ mutant mice, the differentiation of nestin-positive precursor cells is impaired or retarded.

The second hypothesis was based upon the known critical role of the radial glia system in supporting neuronal migration during embryonic brain development (9). In order to determine if early defects in abnormal glial architecture might contribute to the delay in cortical migration in *plxnb2*$^{-/-}$ mutant mice, we labeled cortical radial fibers at E15.5, a time point representing the peak of ongoing radial migration, with RC2, a marker for radial glia (25, 30). We only found sporadic changes in a few areas of the cortex in *plxnb2*$^{-/-}$ mutant mice, where the radial alignment of RC2-positive glial fibers was severely disorganized, accompanied by an abnormal morphology of glial cells (typical examples are shown in Fig. 5B). However,

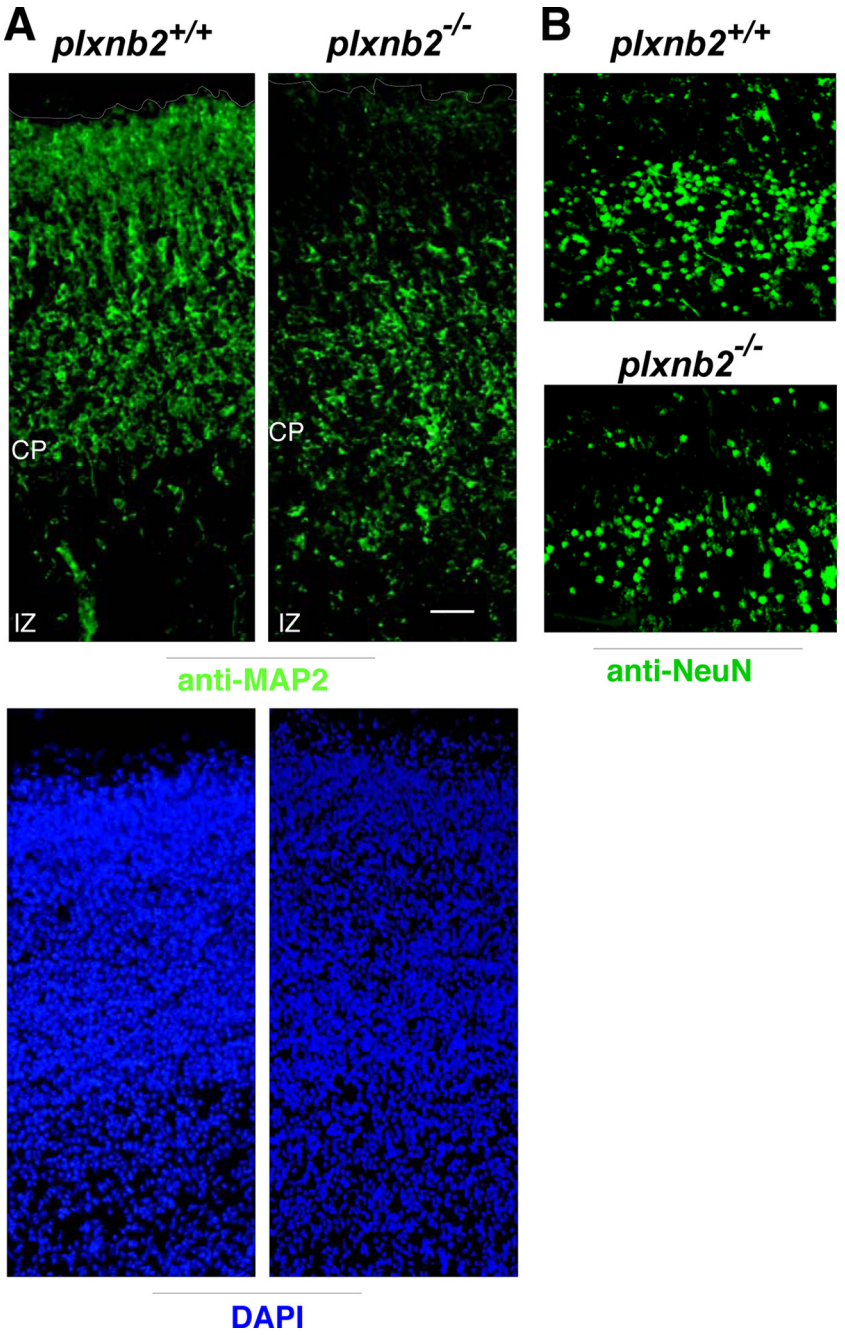

FIG. 4. Mice lacking plexin-B2 show reduced numbers of mature neurons in the developing CP at E18. (A, B) Abnormal distribution and reduced density of mature neurons identified via immunoreactivity to MAP2 and NeuN in the CP of mice lacking plexin-B2 in comparison with wild-type littermates at E18. Four embryos per genotype were tested.

because these abnormalities were only found over a few areas in the CP, it is unclear whether they hold significance in the context of the phenotype in *plxnb2*$^{-/-}$ mutant mice. Whereas wild-type embryos at E18.5 showed a normal organization of GFAP-positive end feet of the radial glia at the basal membrane (37), several glial fibers from *plxnb2*$^{-/-}$ mutant mice terminated randomly within the MZ (Fig. 5C).

Another possibility in this context is that aberrant migration results from defects in the differentiation programs of early cortical pioneer cells such as Cajal-Retzius cells, which provide guidance cues for the correct migration and patterning of successive cohorts of cortical cells migrating in an inside-out fashion. Indeed, we observed that the number of reelin-positive Cajal-Retzius cells was markedly lower in the MZ of *plxnb2*$^{-/-}$ mutant embryos than in that of their wild-type littermates (Fig. 6A and B). This was also confirmed by staining for expression of calretinin, another marker for Cajal-Retzius cells, which was also remarkably reduced in the MZ of *plxnb2*$^{-/-}$ mutant em-

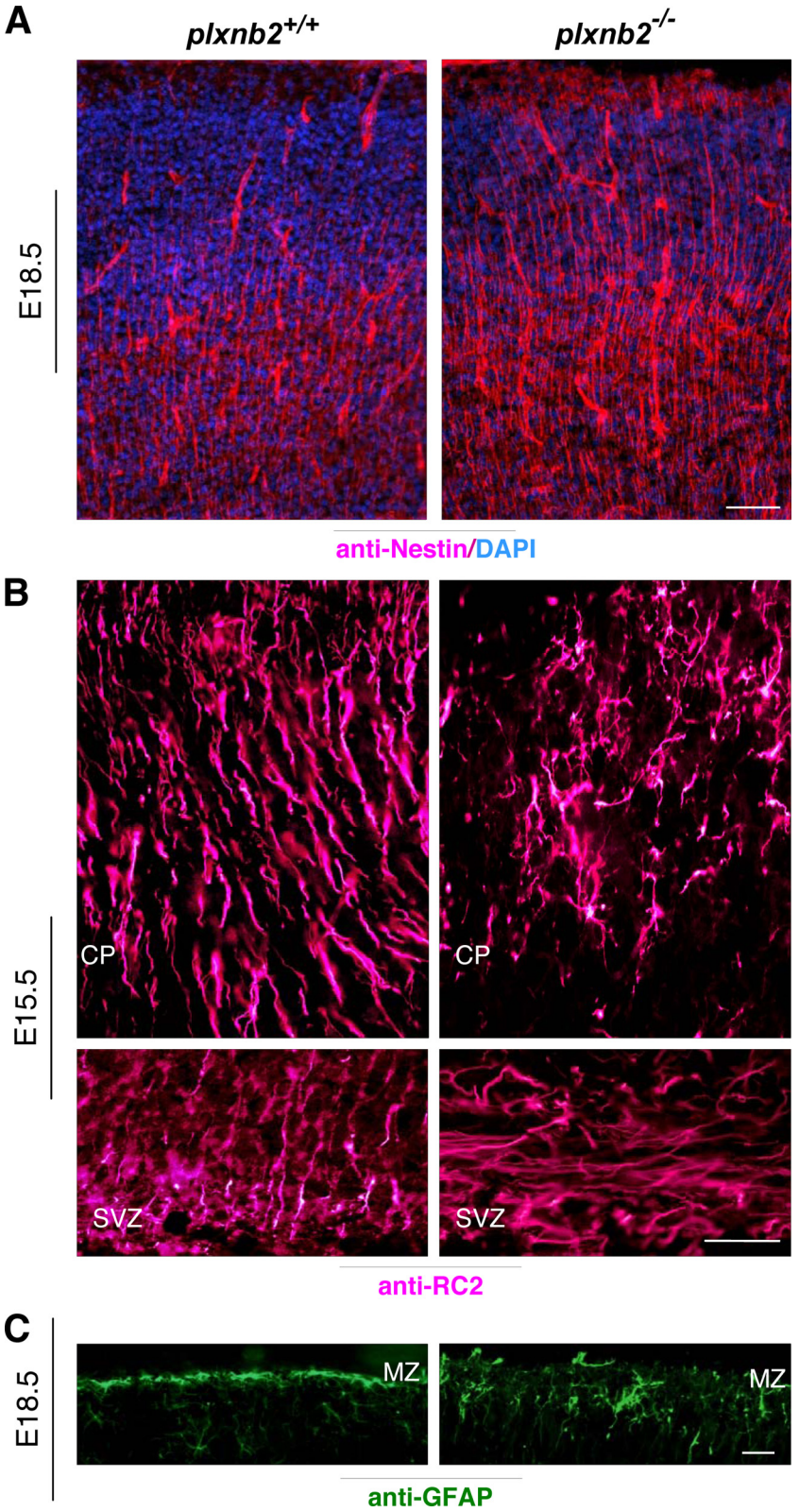

FIG. 5. Abnormal patterning and density of radial glia and progenitor cells, respectively, in the developing CP of mice lacking plexin-B2 (*plxnb2*$^{-/-}$) in comparison with their wild-type littermate embryos (*plxnb2*$^{+/+}$) at E18.5. (A) Nestin-positive neuronal and glial progenitors are found at a higher density in the developing CP of mice lacking plexin-B2 than in that of wild-type embryos at E18.5. (B) Radial glia, identified via RC2 immunoreactivity in the CP, are misformed and misaligned in mice lacking plexin-B2 at E15.5. Patterning of radial glia in the SVZ is shown in the lower panel. (C) Glial end feet identified via GFAP immunoreactivity at the basal laminae in the MZ are sporadically disrupted in mice lacking plexin-B2 at E18.5. Scale bars, 25 μm.

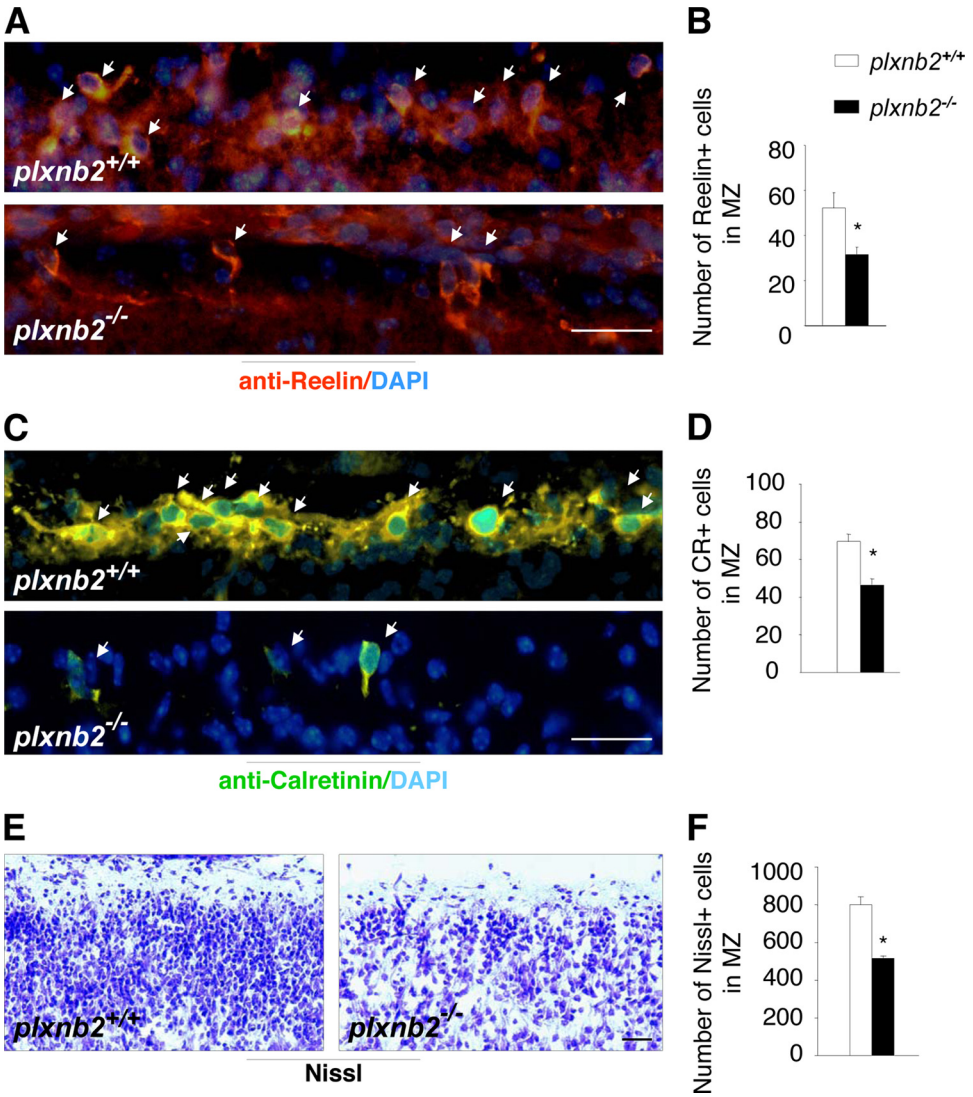

FIG. 6. Analysis of Cajal-Retzius cells and the MZ in mice lacking plexin-B2 (*plxnb2*$^{-/-}$) in comparison with their wild-type littermate embryos (*plxnb2*$^{+/+}$) at E18.5. (A to D) Cajal-Retzius cells in the MZ, identified via reelin immunohistochemistry (A) or via expression of calretinin (C), are markedly reduced in the MZ of mice lacking plexin-B2 in comparison with their wild-type littermates. A quantitative summary is provided in panels B and D. (E) Nissl staining reveals that the packing density of cells in the MZ is markedly reduced in *plxnb2*$^{-/-}$ mutant mice compared with that of wild-type embryos. A quantitative summary is provided in panel F. *, $P < 0.05$ (Student's *t* test). Four embryos per genotype were tested. Scale bars, 25 μm.

bryos (typical examples are shown in Fig. 6C, and a quantitative summary is shown in Fig. 6D). Consistent with a paucity of pioneer cells, Nissl staining revealed a reduced cell density, as well as mispatterning of cells in the MZ of *plxnb2*$^{-/-}$ mutants (Fig. 6E and 4F).

Finally, although we found indications for specific losses of particular cortical cell types (described above), it is plausible that an overall reduction in the proliferative capacity of germinal zone cells contributes to the neocortical abnormalities observed in mice lacking plexin-B2. Consistent with the results described above, the germinal neuroepithelial zone is hypoplasic in *plxnb2*$^{-/-}$ mutants in comparison with that of wild-type littermate embryos (Fig. 7A) (12). Furthermore, counting of Nissl-stained cells in randomly chosen regions of the cerebral wall (dotted rectangles in Fig. 7A) revealed a marked reduction in the number cells per square millimeter in *plxnb2*$^{-/-}$ mutants compared to that in wild-type controls at E18.5 (a quantitative summary is shown in Fig. 7B; $P < 0.05$ [Student's *t* test]). Our previous observation that cell proliferation is reduced in the pallial germinal zone of plexin-B2 mutants at E15.5 may account for the cortical hypoplasia we observed in these mice at E17 to E18.

To observe whether ongoing proliferation is still reduced at E17 to E18, we performed immunohistochemistry analysis for Ki-67, a cell cycle marker (17), as well as PCNA, which labels dividing cells in the S phase (23). The number of cells expressing Ki-67 or PCNA was markedly lower in the pallial germinal zone of *plxnb2*$^{-/-}$ mutant embryos than in wild-type controls (Fig. 7C).

Finally, consistent with an overall decrease in the cell pack-

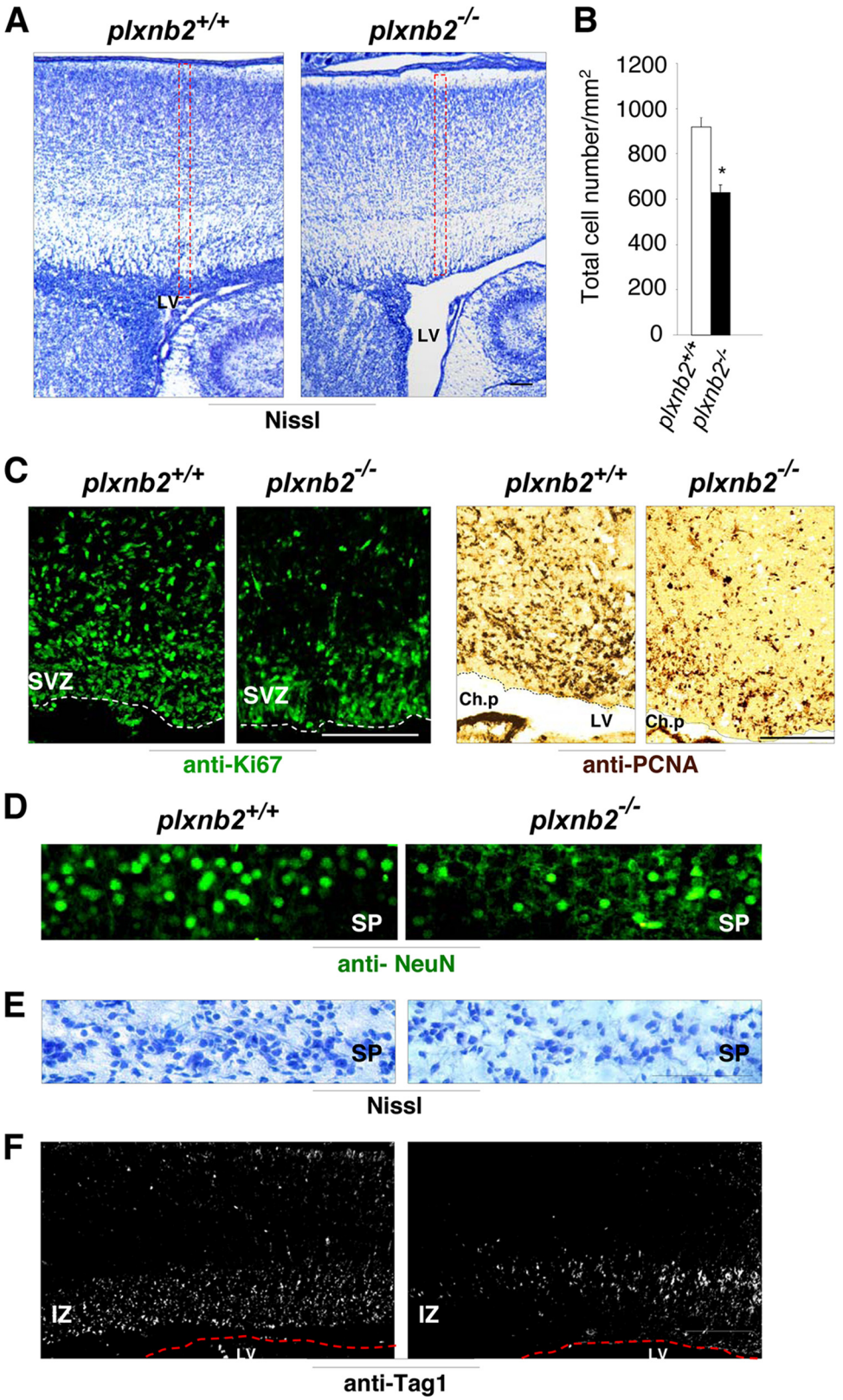

FIG. 7. Proliferation defects in the developing CP of mice lacking plexin-B2 (*plxnb2*$^{-/-}$) in comparison to their wild-type littermate embryos (*plxnb2*$^{+/+}$) at E18. (A, B) Nissl staining shows a marked hypoplasia of the CP, as well as the VZ, in plexin-B2 deletion mutants. A quantitative summary of the cell numbers/mm^2 analyzed over the boxed areas is shown in panel B. (C) Mitogenic index of the germinal cells in the VZ, as identified via immunoreactivity for Ki-67 or PCNA, is reduced in plexin-B2 deletion mutants in comparison to that of their wild-type littermates. *, $P < 0.05$ (Student's t test). (D) Reduced density of anti-NeuN-immunoreactive neurons and (E) Nissl-stained cells in the SP of mice lacking plexin-B2. (F) Occurrence of anti-TAG1-immunoreactive afferents is reduced in the SP of plexin-B2 mutant mice compared to that in wild-type embryos. Scale bars, 75 μm and 50 μm in panels A to D, E, and F, respectively. LV, lateral ventricle.

ing density of the cortical wall of plexin-B2 mutants, we also observed that the numbers of mature NeuN-positive neurons (Fig. 7D) and Nissl-positive cells (Fig. 7E) appeared to be lower in the SP of plexin-B2 mutants than in that of wild-type embryos at E18. Furthermore, anti-TAG1 immunoreactivity in the intermediate zone was reduced in mice lacking plexin-B2 (Fig. 7F), which may reflect a decrease in the density of thalamocortical and corticofugal afferents, which are known to express TAG1 (16).

Mechanisms underlying cortical defects in mice lacking plexin-B2: the role of Sema4D in cortical migration. In an effort to understand ligand-receptor interactions underlying the cortical defects we observed in mice lacking plexin-B2, we addressed the effects of recombinant Sema4D on developmental parameters in cortical neuroblasts. The choice of Sema4D was guided by the *in vivo* availability of Sema4D, but not Sema4C, as a plausible promigratory ligand for plexin-B1/B2, as suggested by our expression analyses (described above). The most obvious effect of the application of soluble Sema4D microexplants of the pallial VZ/SVZ from mouse embryos at E14.5 was an increase in the area of migration of precursors out of the explants, compared to mock treatment. Typical examples of migrating cells, visualized by staining of cell nuclei with DAPI, which express Tuj1 are shown in Fig. 8A, and a quantitative summary is shown in Fig. 8B. When we applied Sema4D to neuronal precursors dissociated out of the pallial VZ, we did not see an increase in their mitotic index, as assessed via measurement of BrdU uptake in culture (Fig. 8C), in contrast to epidermal growth factor (EGF, 50 nM), a classical mitogen. This suggests that Sema4D does not stimulate proliferation of neuronal precursors in the VZ.

To follow the effects of Sema4D on neuronal precursors more closely, we determined the positioning of neurons that became postmitotic at different times by using the BrdU "birth-dating" method and using a more physiologically relevant preparation, namely, organotypic slice cultures of embryonic mouse brain. We applied a single pulse of BrdU to mouse embryos at E14.5, generated organotypic slice cultures 2 h later, and thereafter traced BrdU-labeled cells at 1.5, 3, and 4.5 days *in vitro* (DIV) in the presence of either Sema4D or mock medium. The total number of BrdU-labeled cells was comparable across slices treated with Sema4D or mock medium (Fig. 8D; $P > 0.05$), again showing that Sema4D does not function as a mitogen on VZ precursors. Importantly, at 1.5 and 3 DIV, Sema4D-treated slices showed higher numbers of BrdU-labeled cells in the intermediate zone, as well as the superficial telencephalic CP, compared to mock-treated slices (a typical example is shown in Fig. 8E, and a quantitative summary is shown in Fig. 8F; $P < 0.05$ by analysis of variance [ANOVA] followed by Fisher's post-hoc test). Sema4D-treated slices showed a corresponding decrease in the number of BrdU-labeled cells in the pallial SVZ (region I in Fig. 8F), again suggesting that BrdU-labeled cells migrated faster from the SVZ toward the developing CP upon stimulation with Sema4D. Taken together, these results show that Sema4D enhances cell migration during the development of the neocortex.

Sema4D-induced stimulation of migration of plexin-B1/B2-expressing neuronal precursors was also evident upon analysis of the migratory patterns of neurons expressing GABA, a marker for inhibitory neurons, in organotypic slice cultures of the mouse brain at E14.5 (schematic in Fig. 9A). We studied their presence in the site of their origin in the lateral ganglionic eminence (region IV in the schematic in Fig. 9A) and their migration into the dorsal (region I), lateral (region II), and ventral (piriform, region III) aspects of the developing CP. Application of recombinant Sema4D to the collagen matrix surrounding the organotypic slice led to a significant increase in the density of GABA-expressing cells in regions I to III, suggesting enhanced migration of GABAergic neurons from the ganglionic eminence toward their correct destination zones in the CP (examples are shown in Fig. 9B, and a summary is shown in Fig. 9C). A continued increase in the presence of GABA-positive cells in the CP was observed until the end of the experiment at 4.5 DIV.

Interestingly, upon interruption of the directionality of endogenous Sema4D, which is presumably released by cells in the CP, via application of a Sema4D-secreting source (transfected HEK293 aggregate) at the ventral aspect of the slice also led to a patterning of GABA-positive cells similar to that produced by application of soluble Sema4D in the culture matrix (Fig. 10A; compare results to those in Fig. 9). Similar results were obtained upon the culturing of SVZ explants with HEK cell aggregates expressing soluble Sema4D—neuronal precursors did not migrate preferentially in the direction of the Sema4D-expressing HEK aggregate; rather, Sema4D from HEK aggregates uniformly increased the number of precursors emerging from the explants (Fig. 10B) and showed a promigratory capacity similar to that shown by Sema4D added to the culture matrix (Fig. 8B). These results were further confirmed by following the migration of Tuj1-expressing excitatory neuron precursors in the developing CP in organotypic slices (Fig. 11). Sema4D increased the density of TuJ1 immunoreactivity in the dorsal layers of the CP compared to that in mock-treated samples, irrespective of whether soluble Sema4D was added to the culture matrix (Fig. 11A) or secreted by a HEK cell aggregate placed at the ventral aspect of the slice (Fig. 11B). Thus, several independent experiments suggest that Sema4D does not constitute a directional chemoattractive cue but rather functions as a motogen for neuronal precursors and newly born neurons.

In an effort to understand the potential functions of Sema4D in the modulation of cortical migration, we had based all of the analyses described above upon the exogenous application of Sema4D. In order to address the contribution of endogenously expressed Sema4D, we cultured organotypic slices of the developing forebrain with a rabbit anti-Sema4D neutralizing antibody, which has been reported to attenuate Sema4D-induced signaling previously (28).

The anti-Sema4D antibody attenuated migration of GABA-immunoreactive developing inhibitory interneurons into the CP, compared to a control antiserum (rabbit immunoglobulin G [IgG]; Fig. 12A; $P < 0.05$ [ANOVA]). Similar results were obtained upon analysis of the migration of TuJ1-positive neuronal precursors in the dorsal CP in organotypic slice cultures (Fig. 11C). These results indicate that endogenous Sema4D stimulates the migration of neuronal precursors and developing neurons in the embryonic forebrain.

Finally, we addressed the role of the Rho GTPase RhoA, which is a key modulator of migration in various cell types, including developing neurons, in the promigratory effects of

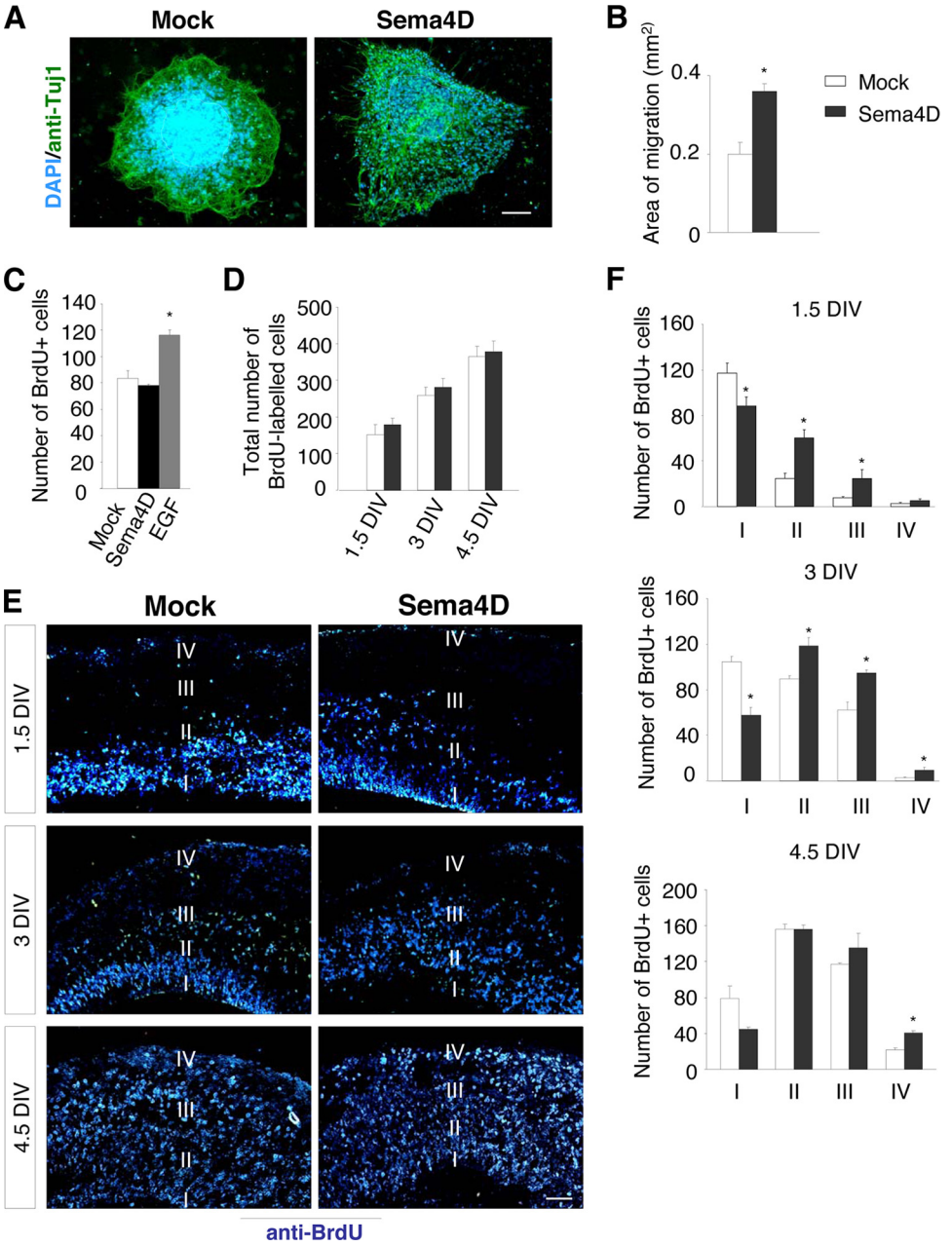

FIG. 8. Soluble Sema4D modulates migration, but not proliferation, of cells in VZ/SVZ explants and organotypic brain slice cultures derived from mice at E14.5. (A, B) Immunofluorescence images (A) and areas of migration (B) of Tuj1-positive cells out of VZ/SVZ explants cultured in the presence of either mock medium or Sema4D medium (130 explants per group). *, $P < 0.05$ (Student's t test). (C) Quantitative estimation of BrdU uptake in dissociated VZ/SVZ cells treated with mock medium, Sema4D medium, or EGF (50 nM). (D) Quantitative estimation of the total number of BrdU-labeled cells in the telencephalon in organotypic slices cultured in the presence of mock medium or Sema4D medium at 1.5, 3, or 4.5 DIV. (E) Typical examples of organotypic slice cultures treated with mock medium or Sema4D showing migration of BrdU-labeled precursors at various depths in the CP in the areas designated I to IV. (F) Quantitative analysis of numbers of BrdU-labeled cells in the areas designated I to IV in slices treated with mock medium or Sema4D, corresponding to the examples shown in panel E. Sema4D-treated cultures show an enhanced incidence of BrdU-labeled cells in the upper cortical layers, in contrast to the VZ (region I) (at least five or six slice cultures per treatment per DIV). *, $P < 0.05$ (ANOVA followed by Fisher's post hoc test). Scale bars, 30 μm.

Sema4D. RhoA is a key effector of plexin-B-mediated signaling in axonal growth cones (39) and is known to mediate promigratory functions of Sema4D/plexin-B1 in endothelial cells via activation of the RhoA-dependent kinase (ROCK) (6). We therefore analyzed the tangential migration of developing neurons in organotypic slices of the developing forebrain in the presence or absence of the ROCK inhibitor Y27632 (3 μM). Inhibition of ROCK attenuated the tangential migration of GABAergic neurons in mock-treated slices to a small yet significant extent ($P < 0.001$ [ANOVA]) but did not completely hinder the ability of cells to migrate (approximately 19% mean inhibition). This inhibition could also stem from

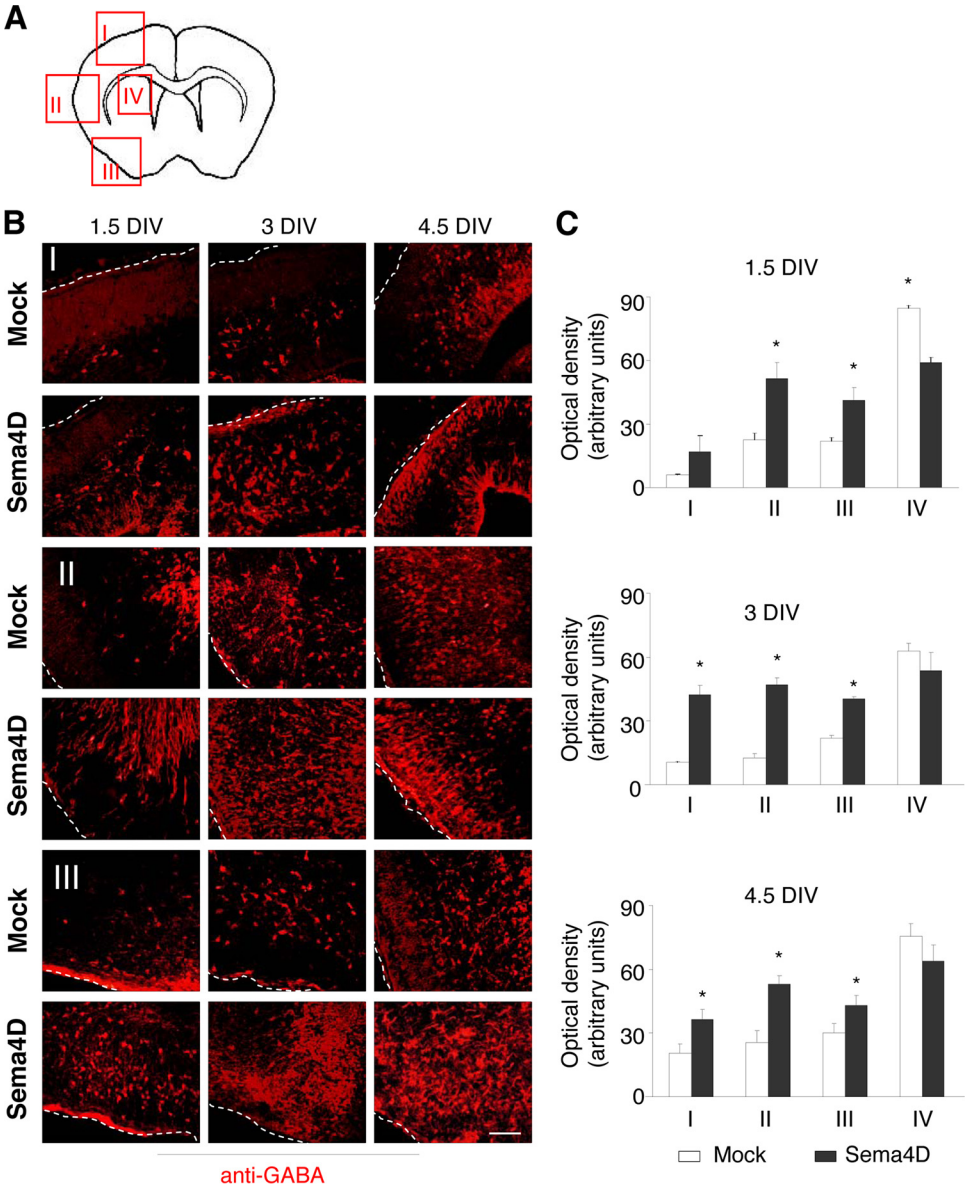

FIG. 9. Analysis of migration of newly born GABAergic neurons in organotypic slice cultures from mouse brain at E14.5 in the presence of mock medium or medium containing soluble Sema4D. (A) Schematic representation of an organotypic slice culture showing the regions designated I to IV in which GABA expression was studied. (B) Typical examples of immunofluorescence for GABA in the regions designated I, II, and III at 1.5, 3, and 4.5 DIV in the presence of mock medium or Sema4D. (C) Quantitative analysis of optical densities of GABA immunofluorescence in arbitrary units in the areas designated I to IV in the presence of mock medium or Sema4D (eight slice cultures per treatment per DIV). *, $P < 0.05$ (ANOVA followed by Fisher's post-hoc test). Scale bar in panel B, 25 μm.

blockade of signaling of endogenous Sema4D in the CP, as suggested by the results described above (Fig. 12A). Importantly, blockade of ROCK completely abrogated the stimulation of tangential migration elicited by exogenously applied Sema4D (Fig. 12B; $P < 0.05$ [ANOVA]) and reduced it to levels of migration similar to those of the mock-treated group. These results suggest that the RhoA-ROCK pathway plays an important role in molecular processes underlying Sema4D-induced migration in developing neurons of the forebrain.

The differential contribution of plexin-B1 and plexin-B2 to Sema4D-induced effects during neocortical development. In microexplants derived from the pallial VZ/SVZ of *plxnb1*$^{-/-}$ mice, Sema4D still enhanced the migration of Tuj1-positive cells (Fig. 13A). Furthermore, the migration of GABA-positive cells into the CP was stimulated by recombinant Sema4D to a similar extent in organotypic slice cultures derived from *plxnb1*$^{-/-}$ mutant embryos and their wild-type littermate controls (Fig. 13B). Finally, in BrdU-labeling and birth-dating experiments, Sema4D stimulated the migration of labeled neuronal progenitors to similar extents in *plxnb1*$^{-/-}$ mutant embryos and wild-type controls, showing thereby that plexin-B1 alone does not account for these functions of Sema4D (Fig. 13C).

Because plexin-B1 and plexin-B2 demonstrate overlapping expression in the VZ and ganglionic eminence (Fig. 1) (46) and

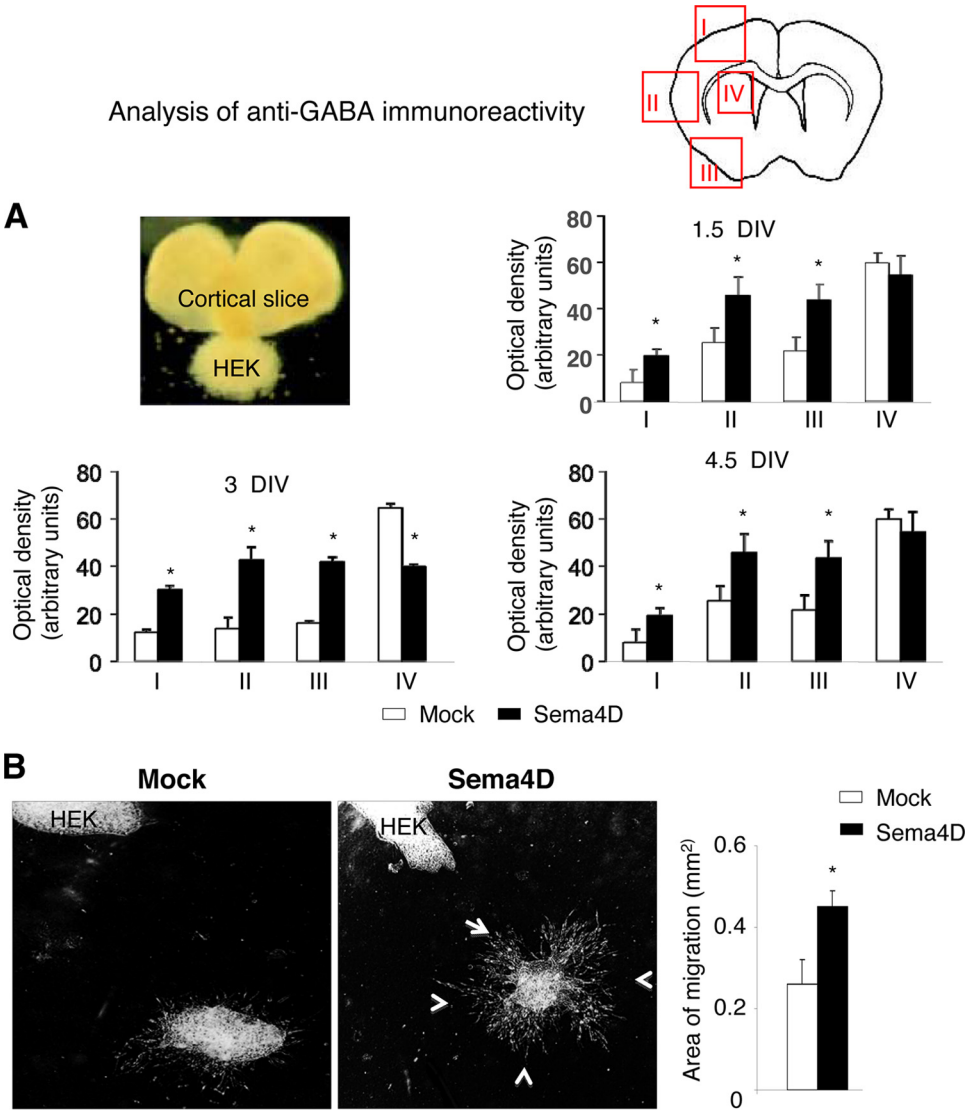

FIG. 10. Analysis of directionality of Sema4D effects on the migration of newly born GABAergic neurons in organotypic slice cultures from mouse brain at E14.5. The schematic represents an organotypic slice culture showing the regions designated I to IV in which GABA expression was studied. (A) HEK293 cell aggregates expressing soluble Sema4D were cocultured at the ventral aspect of organotypic slices, and the bar graphs represent optical densities of GABA immunofluorescence in the areas designated I to IV in slices cultured in the presence of mock medium or Sema4D. *, $P < 0.05$ (ANOVA followed by Fisher's post hoc test; five slices per group). (B) Sema4D released by HEK293 cell aggregates increases the migratory capacity of newly born neurons out of cocultured VZ/SVZ explants not only in the quadrant adjacent to the HEK aggregate (arrow) but also in other quadrants of the VZ/SVZ explants (arrowheads), thereby showing a lack of directional guidance. The panel on the right represents the mean area of migration out of the VZ explant. *, $P < 0.05$ (Student's t test; 30 explants per group).

Sema4D can serve as a low-affinity ligand for plexin-B2 (28), we tested whether plexin-B2 mediates Sema4D-induced effects and could functionally compensate for the lack of plexin-B1 signaling in mutant mice. Owing to the severely distorted forebrain in a large population of *plxnb2*$^{-/-}$ mutant mice, we found that it is technically not feasible to generate organotypic slice cultures for further characterization of migratory defects. In pallial VZ explants derived from *plxnb2*$^{-/-}$ mutant mice, Sema4D failed to produce an increase in the migratory capacity of neuroblasts, in contrast to wild-type embryos from the same litters (typical examples and a quantitative summary are shown in Fig. 13D). In contrast, unrelated motogens such as BDNF (100 ng/ml) and Sema3A (1 μg/ml) promoted the migration of neuroblasts from VZ/SVZ explants derived from *plxnb2*$^{-/-}$ mutant mice to a similar extent as in explants derived from control mice (Fig. 13D; $P = 0.823$ and 0.912 for comparisons of *plxnb2*$^{-/-}$ mutant mice and control mice for BDNF and Sema3A), respectively; $P < 0.05$ in both genotypes for BDNF and Sema3A, compared to control-treated cultures [ANOVA, Fisher's post-hoc test]). These results show that developing neurons in *plxnb2*$^{-/-}$ mutant mice do not unspecifically show a lack of migratory capacity. Taken together, our data suggest that plexin-B2, not plexin-B1, mediates the promigratory functions of Sema4D in neuronal precursors in the developing cortex.

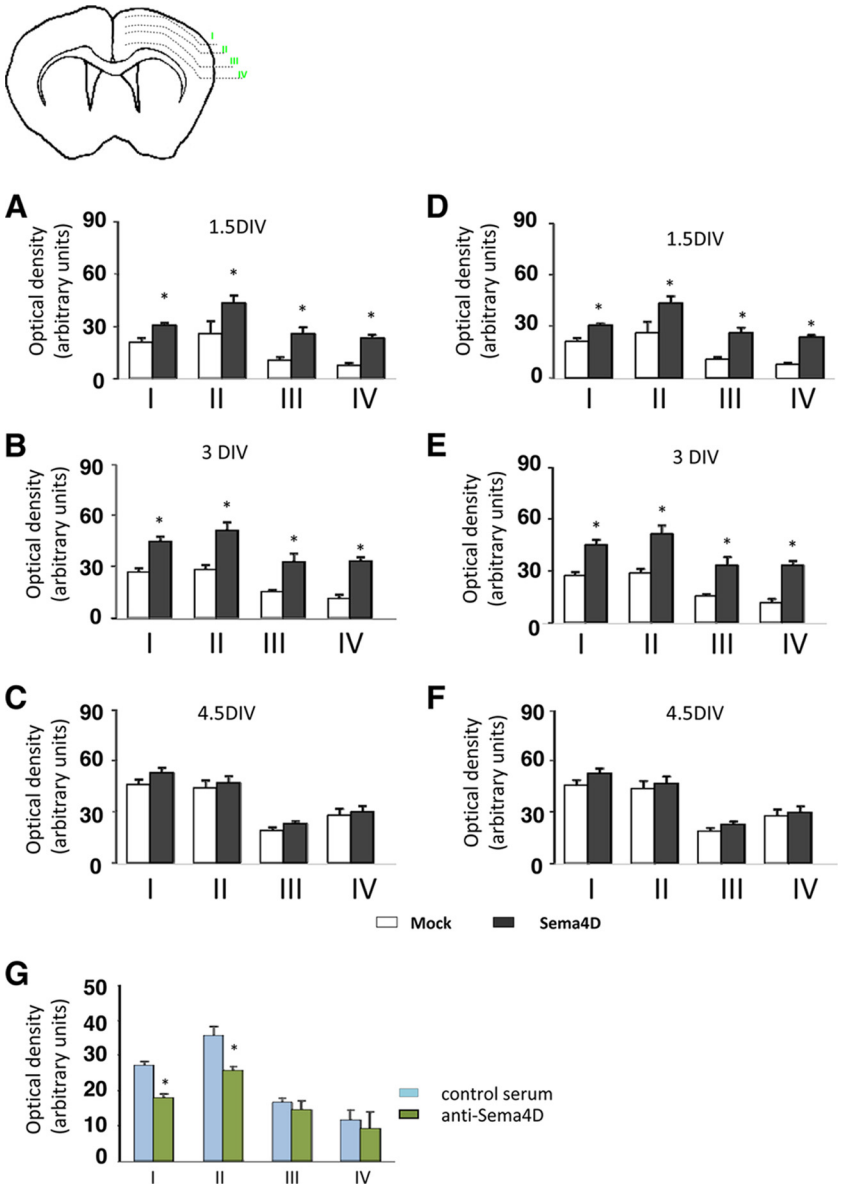

FIG. 11. Exogenously added, as well as endogenous, Sema4D promotes migration of newly born Tuj1-positive neurons in organotypic slice cultures from mouse brain at E14.5. The schematic represents an organotypic slice culture showing the regions designated I to IV in which Tuj1 immunoreactivity was studied. (A and B) Exogenous soluble Sema4D was either added to the culture matrix or released by HEK293 cell aggregates, which were cocultured at the ventral aspect of organotypic slices. The bar graphs represent the optical densities of Tuj1 immunofluorescence in the areas designated I to IV in slices cultured in the presence of mock medium or Sema4D (eight slice cultures per treatment). (C) Quantitative analysis of optical densities of Tuj1 immunofluorescence in the areas designated I to IV in the presence of control IgG or an antibody neutralizing endogenous Sema4D (six slices per treatment). *, $P < 0.05$ (ANOVA followed by post hoc Fisher test).

DISCUSSION

In recent years, it has become clear that, in addition to cell-intrinsic programs, secreted and contact-based cues play a prominent role in determining neocortical architecture (2, 20, 21, 26). The findings described here ascribe a novel role to class IV semaphorins and their receptors from the plexin-B family in the formation of topographic maps in the neocortex.

Here, we observed that the class IV semaphorin Sema4D positively regulates radial and tangential neuronal migration during cortical development. This is remarkably different from the repulsive functions which are generally attributed to class III semaphorins; akin to their repulsive activity during axonal navigation, Sema3A and Sema3F mediate repulsive interactions between migrating cortical neurons and the striatum (27), which is an important step in the sorting of distinct neuronal populations into different brain structures via the establishment of boundaries. However, a recent study has also linked Sema3A to positive modulation of radial migration in the developing cortex (8). Because Sema4D enhanced migration

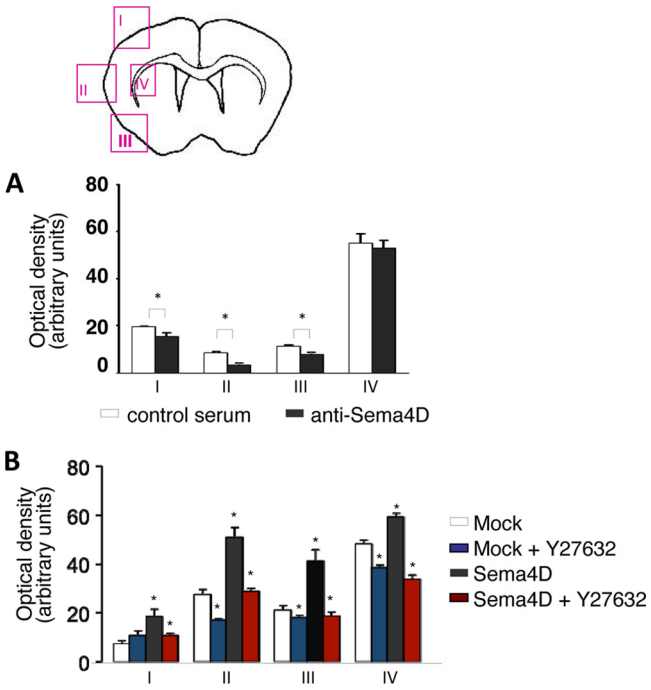

FIG. 12. Role of endogenous Sema4D and ROCK activation in the migration of newly born GABAergic neurons in organotypic slice cultures from mouse brain at E14.5. The schematic represents an organotypic slice culture showing the regions designated I to IV in which GABA expression was studied. (A) Quantitative analysis of optical densities of GABA immunofluorescence in the areas designated I to IV in the presence of control IgG or an antibody neutralizing endogenous Sema4D (six slices per treatment). (B) Effects of treatment with the ROCK inhibitor Y27632 (3 μM) on GABAergic migration in organotypic slices cultured in the presence of exogenously added mock medium or Sema4D medium (eight slice cultures per treatment). *, $P < 0.05$ (ANOVA followed by Fisher's post hoc test).

irrespective of the directionality of application, we infer that it does not function as a layer-specific chemotactic cue to specifically attract cells in a directional manner but rather acts as a general motogen to stimulate migratory activity in precursors and developing neurons. It is interesting that Sema4D–plexin-B interactions have been implicated in both positive and negative modulation of cell polarization and mobilization in diverse cell types outside of the nervous system (5, 18, 41), emphasizing the context dependence of semaphorin-plexin signaling.

Because Sema4D can act on multiple molecular targets which are expressed in the nervous system (31, 33, 46), the receptors and cellular signaling events underlying its functions in cortical development are of arch significance. Surprisingly, we observed that the absence of the classical Sema4D receptor plexin-B1 neither attenuated Sema4D-induced effects on VZ/SVZ cells nor led to abnormalities in cortical architecture *in vivo*. Instead, mice lacking plexin-B2 showed marked cortical patterning defects *in vivo*. Moreover, Sema4D-induced facilitation of migration of developing neurons was abrogated in plexin-B2 deletion mutant mice. B-type plexins can trigger multiple signaling events in parallel by modulating the activity of GEFs and GAPs, which govern the activation status of Rho and Ras family GTPases such as RhoA, Rac, and R-Ras, among others, and several cytosolic kinases, including Pyk2, Src, FAK, PI3K, and Akt (4, 5, 18, 20, 32, 34, 39, 40, 45). Several of these mediators play a role in cell polarity and directional cell migration (20), although their functions in cortical development have not been established in detail so far. Importantly, activation of B-type plexins by semaphorins also leads to an indirect activation of key receptor tyrosine kinases (RTKs) such as Met and ErbB-2. In cancer cell lines, Sema4D inhibits cell migration if Met is recruited to the plexin-B signaling complex (41). In developing neurons, however, a clear role for Met in the stimulation of neuronal migration, particularly that of tangentially migrating GABAergic interneurons, has been established (35). Furthermore, ErbB-2 has been directly implicated in migration (3). Indeed, there are many similarities between our observations on the effects of Sema4D and those reported for neurotrophins (7) and the classical Met ligand hepatocyte growth factor (35) in cortical patterning, suggesting that RTK signaling plays a central role in migratory processes during cortical development.

We found that mice lacking plexin-B2 display cortical abnormalities independently of an exencephaly phenotype which render it likely that these reflect deficits in primary functions of plexin-B2. Nevertheless, the phenotype we observed is suggestive of a complex mixture of defects in proliferation, differentiation, and migration. We found a striking reduction in the mitotic index of germinal cells in plexin-B2 mutants, hypoplasia of the germinative neuroepithelium, and reduced expression of cell cycle markers such as Ki-67 and PCNA (23, 38). However, several observations suggest that it is unlikely that a proliferation defect alone underlies the reduced density of newly born and mature neurons which we observed in the cortices of mice lacking plexin-B2. Rather, we found indications for defects in the differentiation of nestin-positive progenitors in the CP of plexin-B2 mutants.

These defects in proliferation and differentiation may also contribute to the abnormal migration of cortical precursors and newly born neurons. It is known that in addition to cell-intrinsic cues, successful migration requires support structures such as radial glia, which form scaffolds via anchorage of their end feet on the basal lamina on the pial surface. Here we observed that radial glia showed aberrant morphology and failure of attachment of end feet on the basal lamina in some regions of the cortex in mice lacking plexin-B2. Another key requirement for cortical migration is the presence of directional migratory cues secreted by pioneer neurons such as Cajal-Retzius cells. Reduced secretion of reelin by Cajal-Retzius cells is associated with a disruption of the cortical layering and radial glia scaffold disorganization (10). Importantly, we observed that although Cajal-Retzius cells were still capable of synthesizing reelin in plexin-B2 mutants, their density in the MZ was significantly reduced, a phenomenon which may be associated with defective proliferation, as well as differentiation owing to a loss of plexin-B2. It is plausible that these defects impede the radial glial mode of migration, which is a characteristic of newly generated cortical projection neurons (29), in mice lacking plexin-B2.

On the other hand, defective migration of GABAergic interneurons into the CP of plexin-B2 mutant mice may result from defects in proliferation and differentiation of SP neurons. SP cells are known to be essential for the navigation of TAG1-

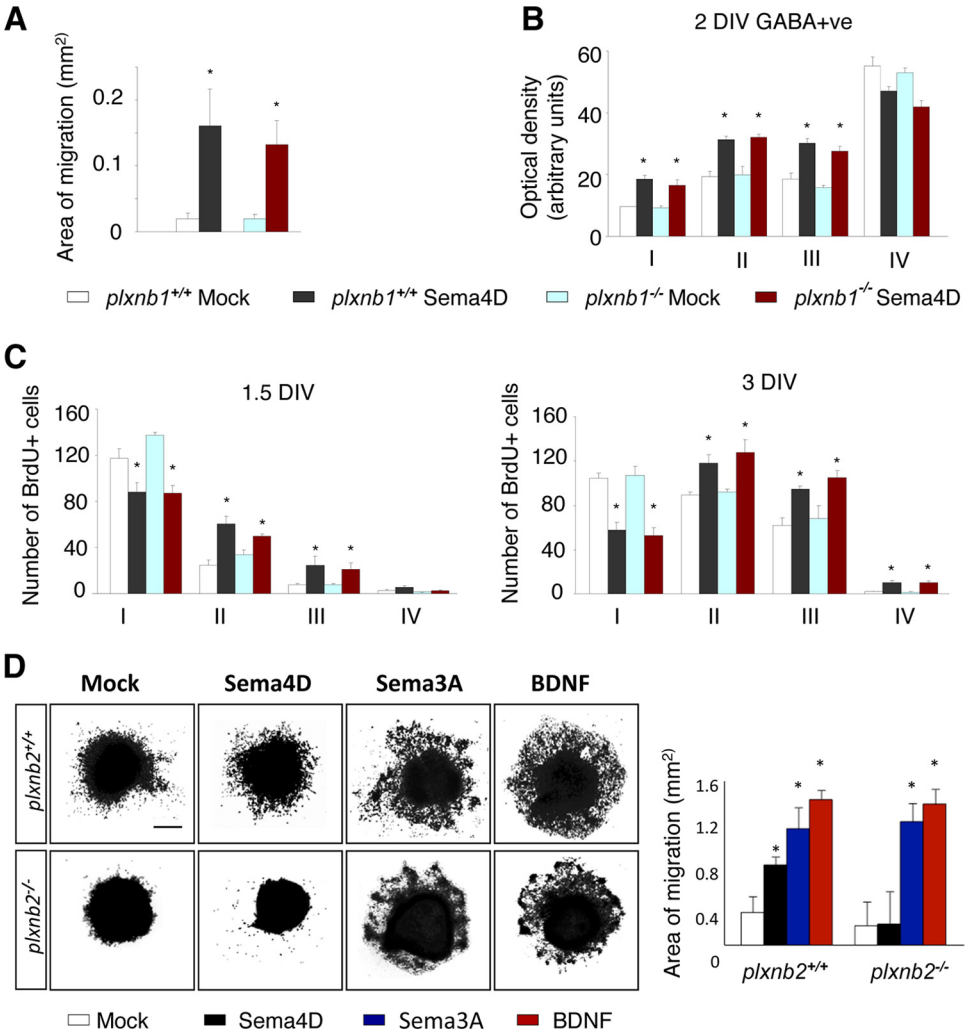

FIG. 13. The differential contribution of plexin-B1 and plexin-B2 to Sema4D-induced effects on migration in assays of VZ/SVZ explants and organotypic slice cultures of mouse embryonic cortex at E14.5. (A) In VZ/SVZ explants, Sema4D increases the migration of β-III-tubulin (Tuj1)-stained newly born neurons in mice lacking plexin-B1 ($plxnb1^{-/-}$), as well as wild-type littermate controls ($plxnb1^{+/+}$) (50 explants per treatment). (B) Sema4D-induced increase in the density of GABA-positive cells in various parts of the cortex (regions I to III) is maintained in mice lacking plexin-B1 (four or five slices per treatment per DIV). (C) Sema4D-induced increase in the migratory distance of BrdU-labeled cortical precursors in the upper cortical walls (regions II and III) is maintained in mice lacking plexin-B1 (four or five slices per treatment per DIV). (D) Sema4D-induced increase in the migratory capacity of DAPI+ cells out of VZ explants is lost in mice lacking plexin-B2 ($plxnb2^{-/-}$). In contrast, promigratory effects of BDNF and Sema3A are preserved in explants derived from $plxnb2^{-/-}$ mutant mice (about 50 to 60 explants per treatment per genotype) *, $P < 0.05$ in all cases (ANOVA followed by Fisher's post hoc test). Scale bar in panel D, 50 μm.

expressing thalamocortical and corticofugal afferents (1), which, in turn, guide migrating GABAergic interneurons from the ganglionic eminences into the CP (1, 11). Interestingly, we observed that plexin-B2 mutant mice show a reduced density of SP neurons, which is accompanied by a reduction in the occurrence of TAG1 immunoreactivity on the SP, which may contribute to defects in the migration of GABAergic neurons in plexin-B2 mutant mice. Indeed, blocking of TAG1 function has been shown to result in a reduction of GABA-positive cells in the developing neocortex (11), in a manner similar to our observations in mice lacking plexin-B2.

Thus, although independent roles for plexin-B2 in proliferation, differentiation, and migration are plausible, given that these key functions are closely interrelated, as discussed above, it is also possible that one phenotype leads to another. A strict delineation of these functions is not feasible in the context of constitutive, global deletion of plexin-B2, and future studies with conditional, inducible mouse mutants are required to fully understand the precise mechanisms via which plexin-B2 signaling regulates the complex developmental programs in developing neurons.

In summary, this study extends the functional repertoire of the plexin-B family of semaphorin receptors to modulation of cell migration and specification during corticogenesis and identifies Sema4D as a novel positive regulator of migration and differentiation during cortical development.

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REFERENCES

1. **Allendoerfer, K. L., and C. J. Shatz.** 1994. The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu. Rev. Neurosci. **17:**185–218.
2. **Andrews, W. D., M. Barber, and J. G. Parnavelas.** 2007. Slit-Robo interactions during cortical development. J. Anat. **211:**188–198.
3. **Anton, E. S., M. A. Marchionni, K. F. Lee, and P. Rakic.** 1997. Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. Development **124:**3501–3510.
4. **Aurandt, J., H. G. Vikis, J. S. Gutkind, N. Ahn, and K. L. Guan.** 2002. The semaphorin receptor plexin-B1 signals through a direct interaction with the Rho-specific nucleotide exchange factor, LARG. Proc. Natl. Acad. Sci. U. S. A. **99:**12085–12090.
5. **Basile, J. R., T. Afkhami, and J. S. Gutkind.** 2005. Semaphorin 4D/plexin-B1 induces endothelial cell migration through the activation of PYK2, Src, and the phosphatidylinositol 3-kinase-Akt pathway. Mol. Cell. Biol. **25:**6889–6898.
6. **Basile, J. R., A. Barac, T. Zhu, K. L. Guan, and J. S. Gutkind.** 2004. Class IV semaphorins promote angiogenesis by stimulating Rho-initiated pathways through plexin-B. Cancer Res. **64:**5212–5224.
7. **Behar, T. N., M. M. Dugich-Djordjevic, Y. X. Li, W. Ma, R. Somogyi, X. Wen, E. Brown, C. Scott, R. D. McKay, and J. L. Barker.** 1997. Neurotrophins stimulate chemotaxis of embryonic cortical neurons. Eur. J. Neurosci. **9:**2561–2570.
8. **Chen, G., J. Sima, M. Jin, K. Y. Wang, X. J. Xue, W. Zheng, Y. Q. Ding, and X. B. Yuan.** 2008. Semaphorin-3A guides radial migration of cortical neurons during development. Nat. Neurosci. **11:**36–44.
9. **Cooper, J. A.** 2008. A mechanism for inside-out lamination in the neocortex. Trends Neurosci. **31:**113–119.
10. **D'Arcangelo, G., G. G. Miao, S. C. Chen, H. D. Soares, J. I. Morgan, and T. Curran.** 1995. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature **374:**719–723.
11. **Denaxa, M., C. H. Chan, M. Schachner, J. G. Parnavelas, and D. Karagogeos.** 2001. The adhesion molecule TAG1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system. Development **128:**4635–4644.
12. **Deng, S., A. Hirschberg, T. Worzfeld, J. Y. Penachioni, A. Korostylev, J. M. Swiercz, P. Vodrazka, O. Mauti, E. T. Stoeckli, L. Tamagnone, S. Offermanns, and R. Kuner.** 2007. Plexin-B2, but not plexin-B1, critically modulates neuronal migration and patterning of the developing nervous system in vivo. J. Neurosci. **27:**6333–6347.
13. **Fiore, R., and A. W. Püschel.** 2003. The function of semaphorins during nervous system development. Front. Biosci. **8:**s484–s499.
14. **Frederiksen, K., and R. D. McKay.** 1988. Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. J. Neurosci. **8:**1144–1151.
15. **Friedel, R. H., G. Kerjan, H. Rayburn, U. Schuller, C. Sotelo, M. Tessier-Lavigne, and A. Chedotal.** 2007. Plexin-B2 controls the development of cerebellar granule cells. J. Neurosci. **27:**3921–3932.
16. **Fukuda, T., H. Kawano, K. Ohyama, H. P. Li, Y. Takeda, A. Oohira, and K. Kawamura.** 1997. Immunohistochemical localization of neurocan and L1 in the formation of thalamocortical pathway of developing rats. J. Comp. Neurol. **382:**141–152.
17. **Gerdes, J., H. Lemke, H. Baisch, H. H. Wacker, U. Schwab, and H. Stein.** 1984. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J. Immunol. **133:**1710–1715.
18. **Giordano, S., S. Corso, P. Conrotto, S. Artigiani, G. Gilestro, D. Barberis, L. Tamagnone, and P. M. Comoglio.** 2002. The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat. Cell Biol. **4:**720–724.
19. **Götz, M., and Y. A. Barde.** 2005. Radial glial cells defined and major intermediates between embryonic stem cells and CNS neurons. Neuron **46:**369–372.
20. **Govek, E. E., S. E. Newey, and A. L. Van.** 2005. The role of the Rho GTPases in neuronal development. Genes Dev. **19:**1–49.
21. **Kolodkin, A. L.** 1998. Semaphorin-mediated neuronal growth cone guidance. Prog. Brain Res. **117:**115–132.
22. **Korostylev, A., T. Worzfeld, S. Deng, R. H. Friedel, J. M. Swiercz, P. Vodrazka, V. Maier, A. Hirschberg, Y. Ohoka, S. Inagaki, S. Offermanns, and R. Kuner.** 2008. A functional role for semaphorin 4D/plexin B1 interactions in epithelial branching morphogenesis during organogenesis. Development **135:**3333–3343.
23. **Landberg, G., and G. Roos.** 1991. Antibodies to proliferating cell nuclear antigen as S-phase probes in flow cytometric cell cycle analysis. Cancer Res. **51:**4570–4574.
24. **Lazarini, F., T. N. Tham, P. Casanova, F. Arenzana-Seisdedos, and M. Dubois-Dalcq.** 2003. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia **42:**139–148.
25. **Malatesta, P., E. Hartfuss, and M. Götz.** 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development **127:**5253–5263.
26. **Marín, O., and J. L. Rubenstein.** 2003. Cell migration in the forebrain. Annu. Rev. Neurosci. **26:**441–483.
27. **Marín, O., A. Yaron, A. Bagri, M. Tessier-Lavigne, and J. L. Rubenstein.** 2001. Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. Science **293:**872–875.
28. **Masuda, K., T. Furuyama, M. Takahara, S. Fujioka, H. Kurinami, and S. Inagaki.** 2004. Sema4D stimulates axonal outgrowth of embryonic DRG sensory neurones. Genes Cells **9:**821–829.
29. **Misson, J. P., C. P. Austin, T. Takahashi, C. L. Cepko, and V. S. Caviness, Jr.** 1991. The alignment of migrating neural cells in relation to the murine neopallial radial glial fiber system. Cereb. Cortex **1:**221–229.
30. **Misson, J. P., M. A. Edwards, M. Yamamoto, and V. S. Caviness, Jr.** 1988. Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker. Brain Res. Dev. Brain Res. **44:**95–108.
31. **Moreau-Fauvarque, C., A. Kumanogoh, E. Camand, C. Jaillard, G. Barbin, I. Boquet, C. Love, E. Y. Jones, H. Kikutani, C. Lubetzki, I. Dusart, and A. Chedotal.** 2003. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J. Neurosci. **23:**9229–9239.
32. **Oinuma, I., Y. Ishikawa, H. Katoh, and M. Negishi.** 2004. The Semaphorin 4D receptor plexin-B1 is a GTPase activating protein for R-Ras. Science **305:**862–865.
33. **Perälä, N. M., T. Immonen, and H. Sariola.** 2005. The expression of plexins during mouse embryogenesis. Gene Expr. Patterns **5:**355–362.
34. **Perrot, V., J. Vazquez-Prado, and J. S. Gutkind.** 2002. Plexin B regulates Rho through the guanine nucleotide exchange factors leukemia-associated Rho GEF (LARG) and PDZ-RhoGEF. J. Biol. Chem. **277:**43115–43120.
35. **Powell, E. M., W. M. Mars, and P. Levitt.** 2001. Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. Neuron **30:**79–89.
36. **Püschel, A. W., R. H. Adams, and H. Betz.** 1995. Murine semaphorin D/collapsin is a member of a diverse gene family and creates domains inhibitory for axonal extension. Neuron **14:**941–948.
37. **Sancho-Tello, M., S. Valles, C. Montoliu, J. Renau-Piqueras, and C. Guerri.** 1995. Developmental pattern of GFAP and vimentin gene expression in rat brain and in radial glial cultures. Glia **15:**157–166.
38. **Starborg, M., K. Gell, E. Brundell, and C. Hoog.** 1996. The murine Ki-67 cell proliferation antigen accumulates in the nucleolar and heterochromatic regions of interphase cells and at the periphery of the mitotic chromosomes in a process essential for cell cycle progression. J. Cell Sci. **109:**143–153.
39. **Swiercz, J. M., R. Kuner, J. Behrens, and S. Offermanns.** 2002. Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. Neuron **35:**51–63.
40. **Swiercz, J. M., R. Kuner, and S. Offermanns.** 2004. Plexin-B1/RhoGEF-mediated RhoA activation involves the receptor tyrosine kinase ErbB-2. J. Cell Biol. **165:**869–880.
41. **Swiercz, J. M., T. Worzfeld, and S. Offermanns.** 2008. ErbB-2 and met reciprocally regulate cellular signaling via plexin-B1. J. Biol. Chem. **283:**1893–1901.
42. **Tamagnone, L., S. Artigiani, H. Chen, Z. He, G. I. Ming, H. Song, A. Chedotal, M. L. Winberg, C. S. Goodman, M. Poo, M. Tessier-Lavigne, and P. M. Comoglio.** 1999. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell **99:**71–80.
43. **Tamagnone, L., and P. M. Comoglio.** 2004. To move or not to move? Semaphorin signalling in cell migration. EMBO Rep. **5:**356–361.
44. **Tamamaki, N., K. Fujimori, Y. Nojyo, T. Kaneko, and R. Takauji.** 2003. Evidence that Sema3A and Sema3F regulate the migration of GABAergic neurons in the developing neocortex. J. Comp. Neurol. **455:**238–248.
45. **Vikis, H. G., W. Li, Z. He, and K. L. Guan.** 2000. The semaphorin receptor plexin-B1 specifically interacts with active Rac in a ligand-dependent manner. Proc. Natl. Acad. Sci. U. S. A. **97:**12457–12462.
46. **Worzfeld, T., A. W. Püschel, S. Offermanns, and R. Kuner.** 2004. Plexin-B family members demonstrate nonredundant expression patterns in the developing mouse nervous system: an anatomical basis for morphogenetic effects of Sema4D during development. Eur. J. Neurosci. **19:**2622–2632.