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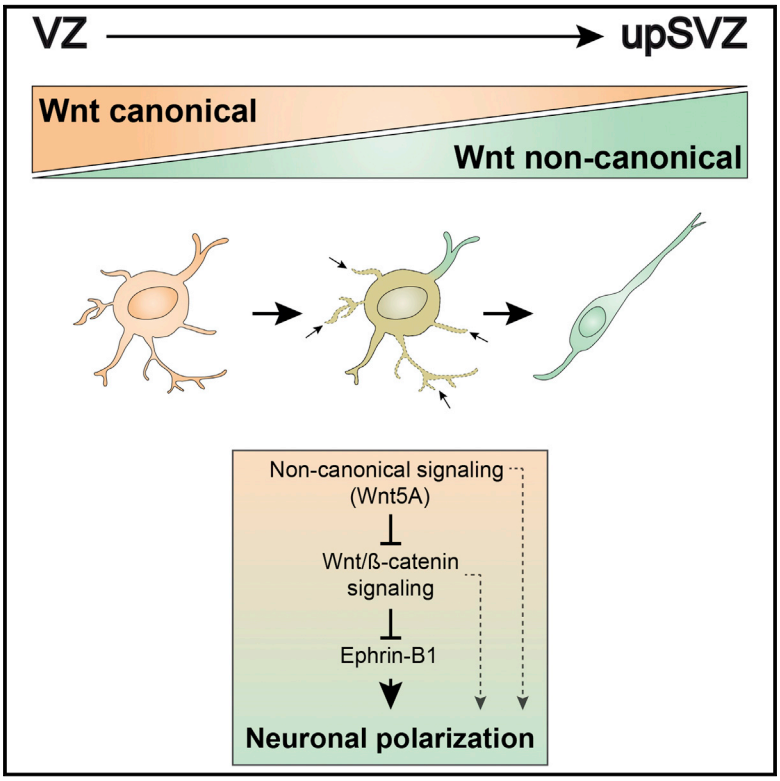
Wnt Signaling Regulates Multipolar-to-Bipolar Transition of Migrating Neurons in the Cerebral Cortex

Graphical Abstract



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In Brief

Boitard et al. demonstrate that transient downregulation of canonical Wnt/β-catenin signaling during the multipolar stage plays a critical permissive role for polarizing and orienting cells for radial migration. This downregulation is initiated cell autonomously by time-dependent expression of Wnt5A and activation of non-canonical signaling enabling ephrin-B1 expression.

Highlights

- Canonical and non-canonical Wnt signaling is active in migrating pyramidal neurons
- Downregulation of canonical Wnt signaling is permissive for neuronal polarization
- Canonical Wnt signaling is reduced by an increase in Wnt5A/non-canonical signaling
- This allows cellular ephrin-B1 expression, thereby enabling neuronal polarization

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Wnt Signaling Regulates Multipolar-to-Bipolar Transition of Migrating Neurons in the Cerebral Cortex

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SUMMARY

The precise timing of pyramidal cell migration from the ventricular germinal zone to the cortical plate is essential for establishing cortical layers, and migration errors can lead to neurodevelopmental disorders underlying psychiatric and neurological diseases. Here, we report that Wnt canonical as well as non-canonical signaling is active in pyramidal precursors during radial migration. We demonstrate using constitutive and conditional genetic strategies that transient downregulation of canonical Wnt/β-catenin signaling during the multipolar stage plays a critical role in polarizing and orienting cells for radial migration. In addition, we show that reduced canonical Wnt signaling is triggered cell autonomously by time-dependent expression of Wnt5A and activation of non-canonical signaling. We identify ephrin-B1 as a canonical Wnt-signaling-regulated target in control of the multipolar-to-bipolar switch. These findings highlight the critical role of Wnt signaling activity in neuronal positioning during cortical development.

INTRODUCTION

The proper development of cortical circuits requires highly orchestrated cell migratory events to establish specific laminar position, orientation, and connectivity of neurons (Kriegstein and Noctor, 2004; Rakic, 2007). Migration errors can lead to neurodevelopmental disorders including lissencephaly, heterotopia, and focal cortical dysgenesis (Sarkisian et al., 2008). Although multiple genes and signaling networks have been implicated in cortical neuron migration, the molecular mechanisms underlying this process are still poorly understood. Wnt signal transduction pathways are integral to cerebral cortical development (Chenn, 2008; Freese et al., 2010). Wnt ligands and components of Wnt signaling pathways are expressed during cortical development, and Wnt signaling has a well-characterized role in embryonic neurogenesis (Chenn, 2008; Rakic et al., 2009). Moreover, Wnt signaling has been implicated in dendritic development and synapse formation of cortical neurons in culture (Dickins and Salinas, 2013). Evidence indicates that Wnt proteins guide cell migration and that multiple Wnt ligands and receptors directly regulate cell polarity and motility in a variety of vertebrate and invertebrate systems (Lyuksyutova et al., 2003; Witze et al., 2008; Mentink et al., 2014). Whether there is a similar direct role of Wnt signaling in regulating neuronal migration in the mammalian cerebral cortex remains largely unexplored.

Wnt proteins are secreted lipid-modified glycoproteins that act via an autocrine or paracrine mode of action to locally activate receptor-mediated signaling pathways (Clevers and Nusse, 2012). The best-characterized Wnt pathway is the canonical Wnt/β-catenin signaling cascade activated via a heterodimeric receptor complex composed of a frizzled receptor and the transmembrane co-receptor LRP5/6. The hallmark of this signaling is the stabilization of β-catenin followed by its translocation to the nucleus and association with TCF/LEF transcription factors, which leads to the transcription of Wnt target genes (Clevers and Nusse, 2012; MacDonald et al., 2009). This pathway mainly controls cell proliferation and differentiation. Wnt ligands also signal through β-catenin-independent (also called non-canonical) mechanisms including at least two distinct signaling pathways, the planar cell polarity (PCP) pathway and the Ca^{2+} pathway (Kikuchi et al., 2012). Non-canonical Wnt ligands activate a receptor complex consisting of a frizzled receptor and co-receptor Ror1, Ror2, or Ryk and regulate cell polarization and cytoskeleton reorganization via the activation of small guanosine-triphosphate-binding proteins and protein kinases (Veeman et al., 2003; Kohn and Moon, 2005; van Amerongen and Nusse, 2009).

The functions of Wnt signaling in cortical development have been extensively studied in proliferating progenitor/stem cells in the ventricular germinal zones (Chenn, 2008). Although Wnt signaling activity seems to be present in postmitotic pyramidal precursor cells (Woodhead et al., 2006) and manipulations of the Wnt canonical signaling could lead to lamination defects in the cortex (Chenn, 2008), the direct impact of this signaling on the migratory behavior of postmitotic neuronal precursors remains unknown. Here, we tested the hypothesis that Wnt signaling contributes to regulate radial migration of pyramidal precursor cells. Radial migration of neurons along glial fibers from the ventricular germinal zone (VZ) to their final positions in

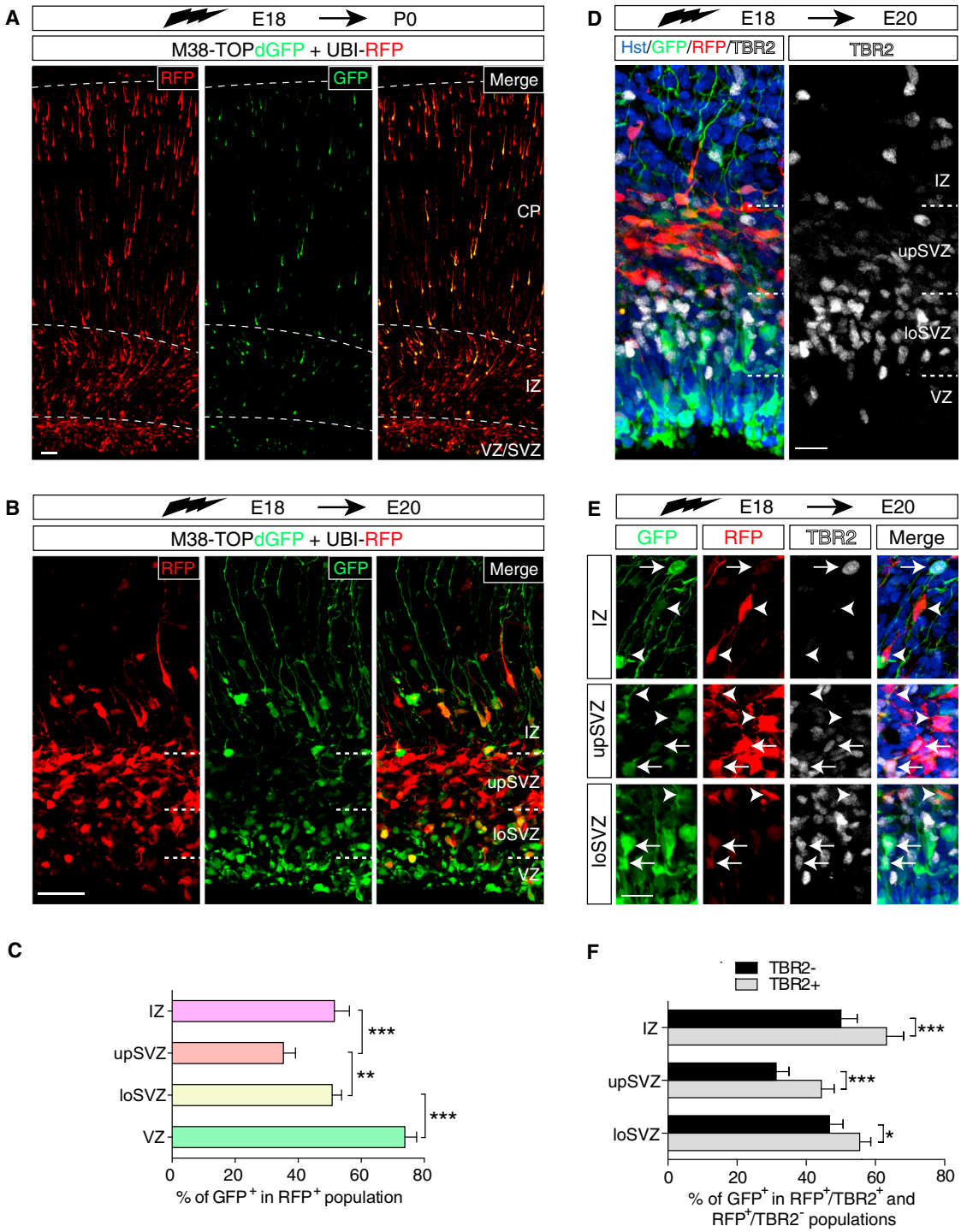

Figure 1. Wnt/β-Catenin Signaling Is Highly Active in Migrating Pyramidal Cells and Dynamically Regulated during Specific Phases of Migration

(A) Coronal slices from P0 brains electroporated with TOPdGFP reporter to monitor canonical Wnt activity. RFP+ migrating cells express variable levels of GFP. VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate.

(B) Coronal slices from E20 brains showing that downregulation of TOPdGFP and, consequently, the reduction of the proportion of RFP+/GFP+ cells occurs at the transition zone between the SVZ and IZ.

(C) Quantification of GFP-labeled cells within RFP+ population in the VZ, SVZ, and IZ. Data represent mean ± SEM of n = 11 brains from two experiments (1,396 IZ RFP+ cells, 1,450 upper SVZ RFP+ cells, 867 lower SVZ RFP+ cells, and 359 VZ RFP+ cells) (one-way ANOVA followed by the Bonferroni post test).

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the cortical plate is crucial for the formation of cortical layers (Rakic et al., 2009). Recent investigations, in particular video time-lapse studies, revealed that radial migration and neuronal positioning is a complex multistep process including multipolar migration, multipolar-to-bipolar transition, radial-glia-guided locomotion, detachment from radial glia, and terminal somal translocation (Kriegstein and Noctor, 2004). The emerging view is that these events may require distinct molecular mechanisms and that disruption of specific signaling pathways impacts cortical lamination in discrete ways (Bielas et al., 2004; LoTurco and Bai, 2006). We focused our investigations on late-generated pyramidal precursors in the neocortex. These neurons are important for intracortical network formation, and their migration is of special interest since they have a relatively long and complicated trajectory before reaching their definitive position in supragranular layers. We found that a shift from canonical to non-canonical Wnt signaling is crucial for polarizing multipolar precursor cells for radial migration. We have also identified ephrin-B1 as a downstream effector for Wnt signaling in this process.

RESULTS

Wnt/β-Catenin Signaling Is Active and Dynamically Regulated in Radially Migrating Neurons

Since canonical Wnt signaling has been shown to regulate neuronal progenitor proliferation and differentiation in the VZ, we first examined if this signaling is also active in radially migrating late-generated pyramidal cells. We took advantage of a Wnt canonical signaling-dependent reporter construct (M38 TOPdGFP) that expresses a destabilized GFP (dGFP) variant under the control of four β-catenin-responsive TCF/LEF-1 consensus sequences (Li et al., 1998; Dorsky et al., 2002; Hurlstone et al., 2003; Munji et al., 2011; Woodhead et al., 2006). Using time-pregnant rats, we introduced TOPdGFP together with a control plasmid coding for red fluorescent protein (RFP) into pyramidal precursors by in utero electroporation at embryonic day 18 (E18).

At postnatal day 0 (P0), we found a widespread distribution of TOPdGFP and RFP double-labeled cells not only in the VZ but also in the subventricular zone (SVZ), intermediate zone (IZ), and cortical plate (CP) (Figure 1A). Thus, canonical signaling is active in migrating pyramidal cells, which is consistent with the expression pattern of Wnt ligands and signaling components in the developing neocortex (Shimogori et al., 2004). A closer inspection at E20 revealed variable levels of TOPdGFP intensity at different stages of the migratory pathway (Figure 1B). Quantitative analysis unveiled that a high percentage of RFP-positive cells in the VZ and lower SVZ (loSVZ) expressed dGFP, while in the upper part of the SVZ (upSVZ), where a large number of multipolar cells are known to acquire a bipolar morphology and initiate radial migration, we systematically observed a reduced activity of the canonical Wnt pathway (Figure 1C). TOPdGFP labeling seemed to be upregulated again in the IZ and CP. To characterize the phenotype of cells displaying reduced Wnt canonical signaling in the transition zone between the SVZ and IZ, we examined the co-localization of TBR2 with TOPdGFP/RFP (Figures 1D and 1E). We found that a significantly higher percentage of TBR2-positive cells displayed TOPdGFP labeling compared to the TBR2-negative population in the SVZ/IZ (Figure 1F). In agreement with previous results (Munji et al., 2011), this observation raised the possibility that reduced Wnt signaling activity coincided predominantly with a stage when intermediate progenitors (IPs) lose TBR2 and differentiate into post-mitotic neurons. This hypothesis was further supported by the co-localization of SatB2 with TOPdGFP/RFP. TBR2 and SatB2 immunoreactivity exhibited a complementary distribution pattern, and TBR2-negative cells appeared to acquire SatB2 immunoreactivity progressively. We found significantly fewer SatB2-negative cells containing TOPdGFP activity compared to SatB2-positive cells in the SVZ/IZ (Figures S1A and S1B). The occurrence of stronger TOPdGFP fluorescence in the IZ was correlated with the apparition of SatB2 staining. It appears thus, that the reduction of Wnt signaling is associated with an initial differentiation stage of neurons occurring between the SVZ and IZ when TBR2 labeling has already disappeared and SatB2 is not yet detectable. We conclude that Wnt canonical signaling is dynamically regulated in migrating pyramidal cells and that different activity levels might be associated with specific stages of migration. In particular, pyramidal cell polarization in the transitional zone between the SVZ and IZ appears to be associated with a reduced level of canonical Wnt signaling activity.

Wnt/β-Catenin Signaling Gain of Function Disrupts the Morphology and Orientation of Pyramidal Precursors and Delays Migration within the Intermediate Zone

The transient reduction of Wnt canonical signaling activity in the transitional zone between the SVZ and IZ raised the possibility that there might be a functional link between the decrease of this signaling and the polarization process. In order to test this hypothesis, we studied the effects of acute canonical Wnt signaling gain of function (GOF) by constitutive overexpression of either Wnt3A, a canonical ligand, or a non-degradable form of β-catenin, Δ45β-catenin (Amit et al., 2002). Wnt3A electroporation at E18 induced striking changes in the cell distribution pattern at P0 (Figure 2A). While control electroporated cells were almost equally distributed throughout the migratory territory, extending from the IZ to the upper portion of the CP, a large number of Wnt3A-overexpressing cells accumulated in the IZ (Figure 2B). Overexpression of Δ45β-catenin resulted in a similar phenotype (Figures 2A and 2B). A closer inspection of individual

(D and E) Co-localization of TBR2 with GFP at E20 after TOPdGFP/UBI-RFP plasmid mix electroporation at E18 (D). Loss of TBR2 immunoreactivity was associated with decreased TOPdGFP levels. Arrows indicate TRB2+ cells and a high canonical Wnt signaling level, whereas arrowheads indicate TBR2− cells and a low level of signaling (E).

(F) Percentage of GFP+ cells in the RFP+/TBR2+ and RFP+/TBR2− population. Data represent mean ± SEM of n = 11 brains (1,396 IZ cells, 1,450 loSVZ cells, and 867 upSVZ cells) from two experiments (paired t test).

Scale bars represent 50 μm (A) and 20 μm (B, D, and E). See also Figure S1.

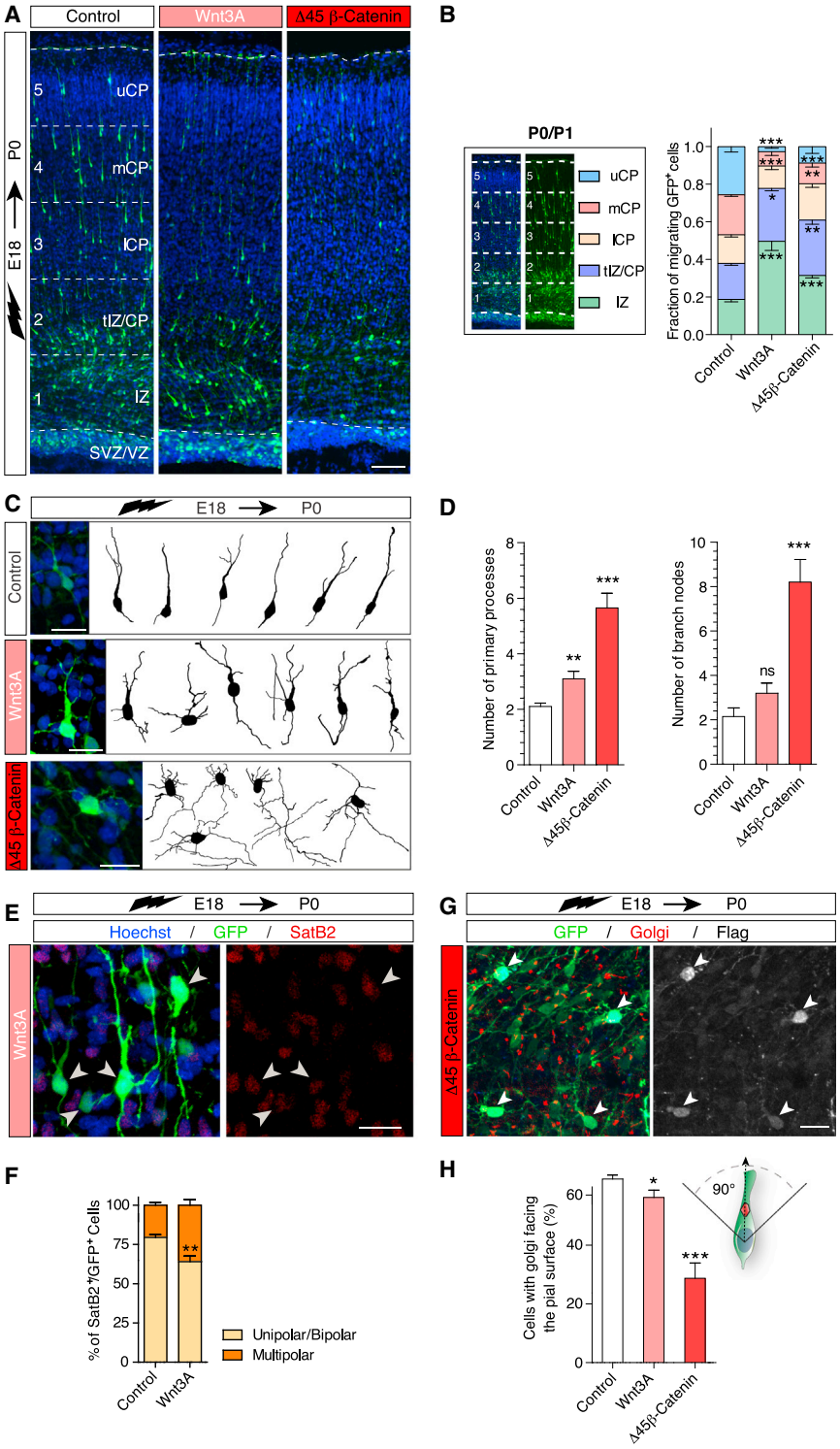


Figure 2. Wnt/β-Catenin Signaling Gain of Function Alters Radial Distribution, Cell Morphology, and Orientation

(A) Position of E18-electroporated cells in P0 coronal sections with either control GFP (left) or Wnt3A-GFP (middle) or Δ45β-catenin-GFP (right) plasmids. The migration path was divided in five equal bins: IZ, intermediate zone; tIZ/CP, transition between intermediate zone and cortical plate; lCP, lower cortical plate; mCP, middle cortical plate; uCP, upper cortical plate.

(B) Fraction of migrating labeled cells in each bin at P0. Data represent mean ± SEM of n = 9 brains from six experiments for the control condition (7,845 cells), n = 7 brains from three experiments for the Δ45β-catenin condition (6,907 cells), and n = 7 brains from two experiments for Wnt3A condition (4,260 cells) (one-way ANOVA followed by the Bonferroni post test).

(C) Neurolucida morphological reconstruction of control, Wnt3A, and Δ45β-catenin cells in the IZ at P0.

(D) Quantitative morphometric analysis in the IZ at P0. Left: number of primary processes. Right: number of branch nodes. Data represent mean ± SEM of n = 20 cells per condition from three experiments (unpaired t test).

(E) Wnt3A electroporated SatB2+ cells often adopt a multipolar morphology in the IZ at P0.

(F) Percentage of SatB2+ electroporated cells displaying either multipolar or unipolar/bipolar morphology. Data represent mean ± SEM of n = 4 brains from two experiments (165 Wnt3A cells and 372 control cells) (unpaired t test).

(G) GM130 staining indicating localization of Golgi apparatus in Δ45β-catenin FLAG+ (arrowheads) cells in the IZ.

(H) Percentage of cells with Golgi apparatus facing the pial surface (±45° from radial glia process) in control, Wnt3A, or Δ45β-catenin FLAG+-overexpressing cells. Data represent mean ± SEM of n = 16 brains for control (2,485 cells), n = 10 brains for Wnt3A (1,348 cells), and n = 6 brains for Δ45β-catenin (905 cells: 218 FLAG+ and 769 FLAG−) obtained from at least three experiments (unpaired t test).

Scale bars represent 100 μm (A) and 20 μm (C, E, and G). See also Figure S2.

cell morphology in the IZ revealed dramatic changes in Wnt canonical GOF cells. While most control cells in this zone exhibited a bipolar shape, forced expression of Wnt3A significantly increased the number of multipolar cells with branched processes (Figures 2C and 2D). It was possible that the increased number of multipolar cells in the IZ was due to a block of the differentiation of IPs into pyramidal precursors. However, Wnt3A overexpression did not change the proportion of SatB2-positive cells within the electroporated population (Figure S2A), indicating that Wnt GOF defects are not due to an altered differentiation of progenitors. Moreover, the shift in multipolar morphology in the Wnt3A group affected the SatB2-positive (Figures 2E and 2F) and mainly TBR2-negative subpopulations (Figures S2B and S2C). This suggests that Wnt canonical GOF mainly impacts postmitotic pyramidal precursors. Since Wnt GOF cells seemed

disoriented, we quantified cell orientation in the IZ, based on the localization of the Golgi apparatus (anti-GM130). In most control cells, the Golgi apparatus was oriented toward the pial surface. However, a large proportion of Wnt3A- and Δ45β-catenin-overexpressing cells displayed a disoriented Golgi apparatus (Figures 2G and 2H).

In order to exclude that the reported alterations in migratory behavior of precursor cells were a consequence of events occurring in proliferative stages, we generated inducible Wnt3A and Δ45β-catenin overexpression constructs to selectively and temporarily activate Wnt pathway in the postmitotic pyramidal precursors. We electroporated these vectors at E18, let the electroporated cells migrate for 72 hr, and activated the constructs by doxycycline at E21, when most of the cells have already exited the proliferative zones. Cell distribution and morphology was then explored at P1. Similar to the effects of constitutive constructs, we found an abnormal accumulation of labeled, multipolar precursors in lower cortical zones (Figures S2D and S2E). We conclude that forced Wnt canonical signaling perturbs polarity and orientation of postmitotic pyramidal precursors in SVZ/IZ and results in a migration deficit.

Wnt/β-Catenin Signaling Downregulation in the Upper SVZ/IZ Is Necessary for Multipolar-to-Bipolar Transition and Persistent Polarization

To explore the dynamic aspects of cell polarization and motility, we first performed real-time confocal imaging of acute cortical slices isolated from electroporated brains at P0. Single-cell imaging directly confirmed that precursor cells exiting the SVZ displayed a decreased TOPdGFP activity (Figure 3A; Movie S1). As expected, many of the control multipolar cells in the SVZ adopted a bipolar morphology and migrated upward into the IZ. In contrast, Wnt3A-overexpressing cells in the SVZ often remained multipolar, displayed dynamic process activity for a longer time period, and failed to move into the IZ (Figures 3B and 3C; Movie S2). These data support the hypothesis that the physiological reduction of canonical signaling activity is required for the multipolar-to-bipolar transition of postmitotic pyramidal neurons at this level.

We next examined cells traversing the IZ using time-lapse imaging. TOPdGFP levels were systematically higher when cells were in a multipolar state, compared to when the same cells displayed a bipolar shape (Figure 4A; Movie S3). Quantitative analysis revealed significant decrease in TOPdGFP detection rate when multipolar and bipolar cells were compared (Figures S3A and S3B).

We also observed that during migration through the IZ, ~20% of control pyramidal neurons with bipolar morphology transiently adopted a multipolar shape before resuming a bipolar morphology and starting migration to the cortical plate (Figure 4B, upper panel, asterisk; Movie S4), suggesting that the bipolar stage of migrating cells in the IZ may be partially unstable. Following the over-activation of canonical Wnt signaling through forced expression of Wnt3A or Δ45β-catenin, a significantly higher proportion of cells displayed multipolar morphology in the IZ (Figures 4B and 4D, middle and lower panels). Moreover, the percentage of tracked time that cells spent in multipolar state was doubled after Wnt3A overexpression compared to control (Figure 4E) and single multipolar phases in the Wnt3A group lasted longer and appeared more frequently (Figures 4F and 4G). In addition, Wnt/β-catenin overexpression almost tripled the proportion of cells, which stopped migration during the 10-hr-long tracking period (Figure S3C). This was paralleled by a decreased migration velocity of mutant cells compared to controls (Figure S3D). We observed no changes during migration in the average duration of internucleokinetic pause and its frequency after Wnt3A overexpression compared to control conditions (Figures S3E and S3F). Thus, the altered migratory behavior of cells with forced Wnt signaling appeared to be due to frequent bipolar-multipolar switches and an increased frequency as well as duration of multipolar phases characterized by a reduced velocity rather than to alterations of the saltatory movement pattern. Together, these results demonstrate that the activity level of Wnt/β-catenin signaling is critical for the multipolar exit and persistent polarization of pyramidal neurons in the SVZ/IZ.

Wnt5A/Non-canonical Signaling Inhibits the Wnt/β-Catenin Pathway in Migrating Pyramidal Cells

Next, we explored how Wnt canonical signaling level is reduced during multipolar-to-bipolar transition. Evidence suggests that non-canonical Wnt signaling, in particular Wnt5A, the best-characterized non-canonical ligand, can directly inhibit canonical signaling in various cell types (Kikuchi et al., 2012; Sato et al., 2010; Topol et al., 2003; Ishitani et al., 1999; Mikels and Nusse, 2006). Such crosstalk has not yet been explored in the developing cortex. To address this question, we first investigated non-canonical signaling activity using an activation transcription factor 2 (ATF2)-based reporter (CHOPdGFP) (Ohkawara and Niehrs, 2011). Most interestingly, and in contrast to the low-level canonical signaling, we found a significantly increased CHOPdGFP labeling in the SVZ compared to the VZ or IZ (Figures 5A and 5B). We then performed in situ hybridization on E20-brain-derived coronal sections to localize Wnt5A as well as Wnt3A coding mRNAs. Whereas Wnt3A seemed to be homogeneously expressed throughout VZ/SVZ regions, the pattern of Wnt5A expression correlated with the observed pattern of non-canonical activity, i.e., an increasing gradient was observed from VZ toward upper SVZ and an absence of labeling occurred in the IZ (Figure 5C). Next, we investigated whether Wnt5A overexpression could inhibit canonical signaling utilizing an in vitro approach in which we co-transfected 293T cells with TOPdGFP together with Wnt3A or Wnt3A+Wnt5A plasmid mix. Western blot analysis of GFP expression in total cell lysates revealed a significant reduction of GFP expression associated with Wnt5A overexpression (Figure S4A). In order to explore whether Wnt5A could inhibit Wnt canonical signaling in the developing cortex, we co-electroporated Wnt5A with TOPdGFP. We observed a significantly reduced TOPdGFP labeling in Wnt5A-overexpressing cells (Figure 5D, upper panel). In contrast, knockdown of Wnt5A by overexpressing Wnt5A small hairpin RNA resulted in a significantly increased TOPdGFP activity in electroporated cells (Figure 5D, lower panel). These results indicate that Wnt5A inhibits canonical Wnt signaling cell autonomously. Moreover, any alteration to Wnt5A expression was sufficient to abolish the decrease of canonical signaling in the upSVZ (Figure 5E).

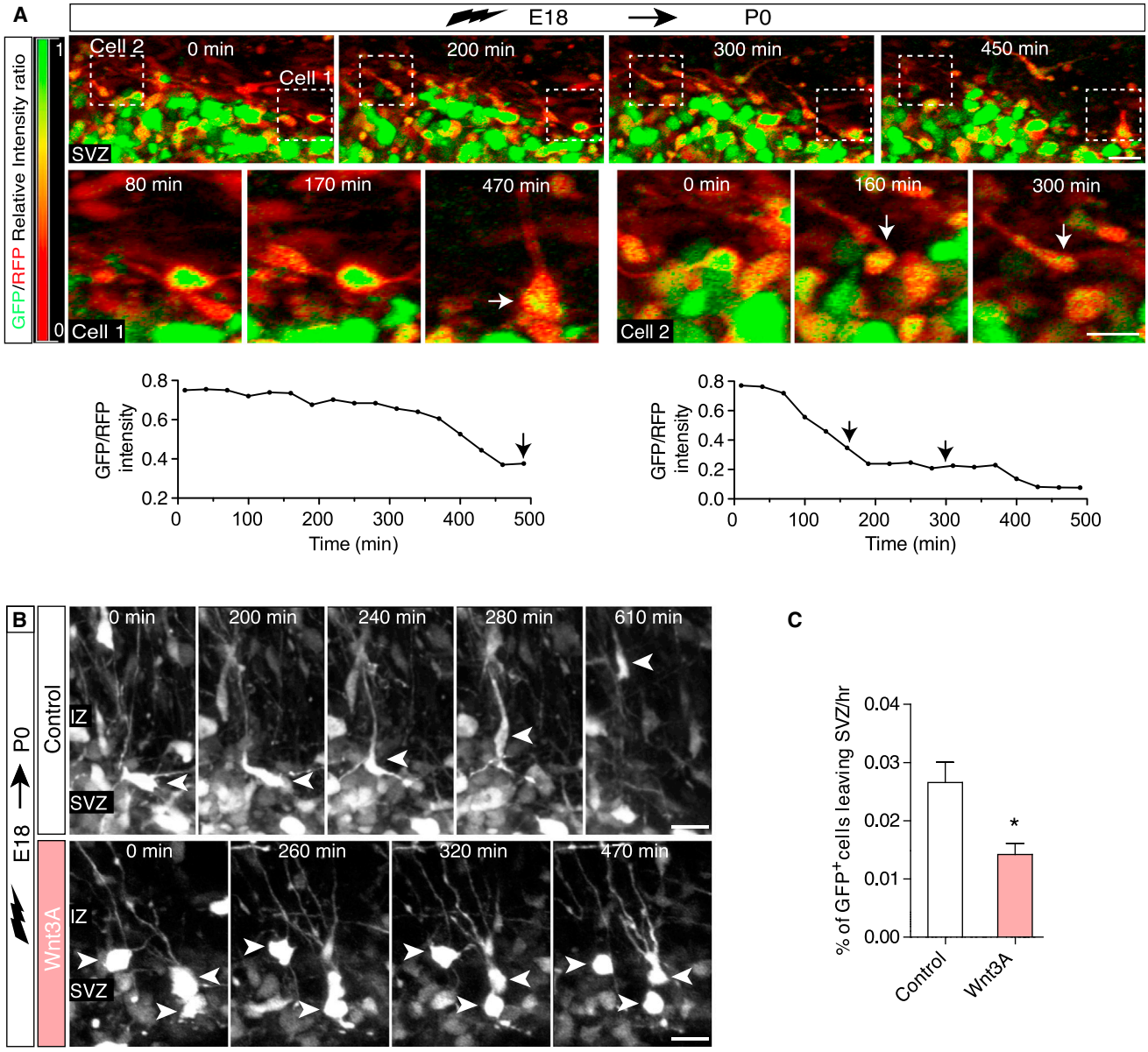


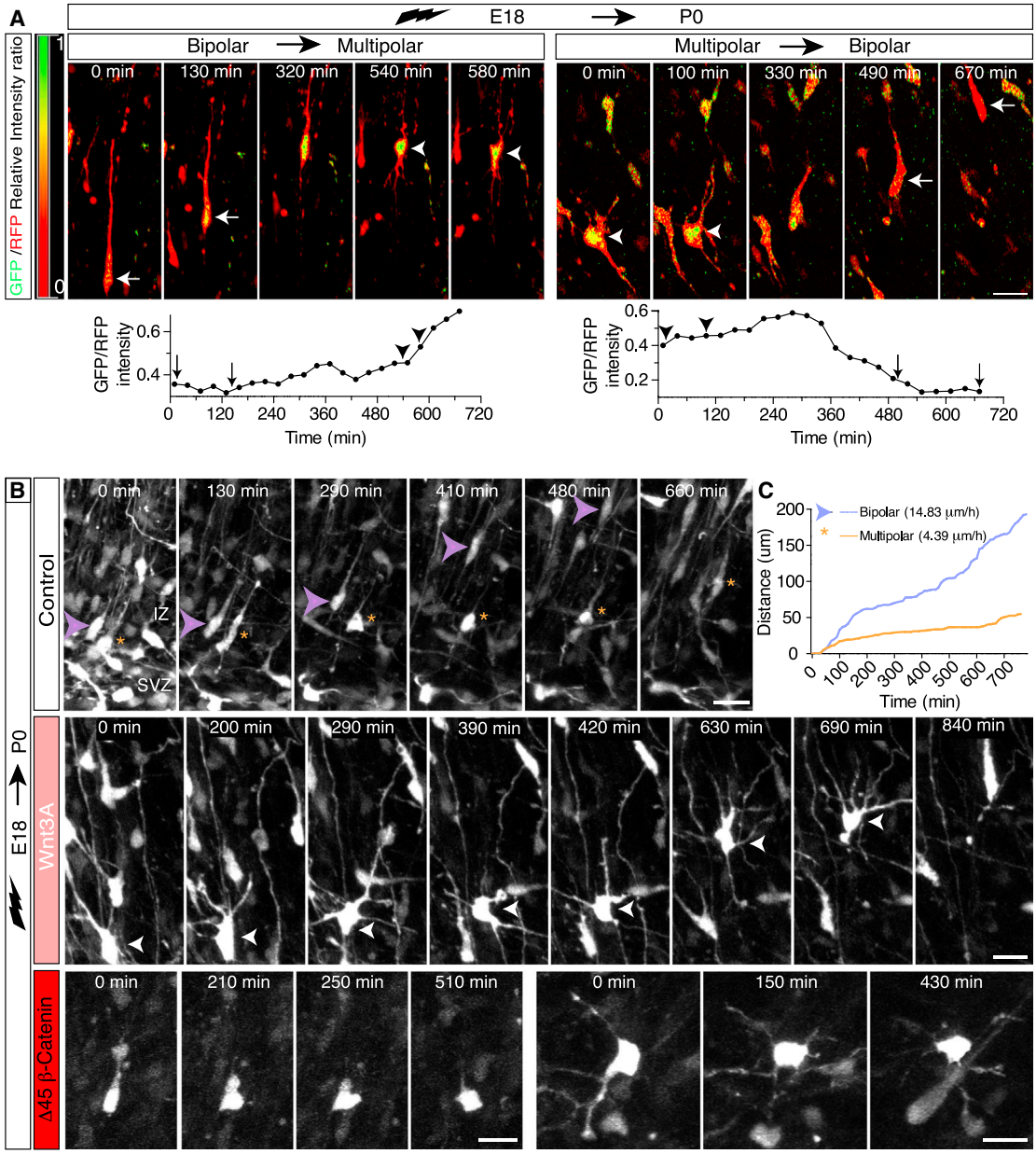
Figure 3. Physiological Reduction of Wnt/β-Catenin Signaling Is Necessary for the SVZ/IZ Transition

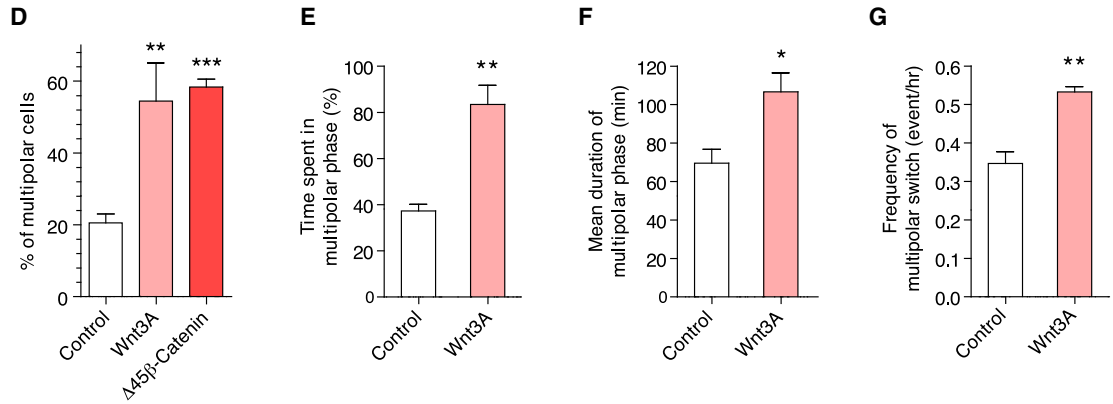
(A) Single-cell, confocal time-lapse imaging of TOPdGFP/UBI-RFP-electroporated cells exiting the SVZ. The intensity ratio of TOPdGFP/UBI-RFP is color-coded (red corresponds to low-level canonical Wnt activity, whereas green is high canonical Wnt activation). Insets show two examples of cells progressively orienting and transiting from the SVZ to the IZ (cell 1 and cell 2). This transition was correlated with a decreased ratio of GFP/RFP (arrows), as illustrated by the corresponding intensity curves. See also Movie S1.

(B) Time-lapse sequences from freshly isolated slices of control and Wnt3A-electroporated cells in the upper SVZ. Example of a control multipolar cell (arrowhead in the top panel) that polarizes and enters into the IZ. In contrast, Wnt3A multipolar cells remain multipolar and stationary (arrowheads in the low panel). See also Movie S2.

(C) SVZ exit rate of control and Wnt3A-electroporated cells. Data represent mean ± SEM of n = 4 brains for control (533 cells) and n = 3 brains for Wnt3A (614 cells) obtained from at least three experiments (unpaired t test). Scale bars, 20 μm.

To investigate if Wnt5A expression is required for the multipolar-to-bipolar transition, we performed confocal time-lapse imaging of acute coronal slices isolated from shWnt5A-electroporated P0 brains. We found that shWnt5A cells displayed a polarization deficit and reduced SVZ exit rate similar to what we obtained after canonical GOF (Figures S4B and S4C; Movie S5). Moreover, an increase in the proportion of multipolar cells was also observed in the SVZ and IZ compared to the control condition (Figure S4D). All of the above support the hypothesis that an increase in Wnt5A-related non-canonical signaling activity is necessary to induce a downregulation of Wnt/β-catenin signaling in the SVZ. To test whether Wnt5A overexpression





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could rescue the Wnt canonical GOF phenotype, we performed rescue experiments by electroporating Wnt3A together with Wnt5A at E18. We observed that at P0, while a large number of Wnt3A-overexpressing cells accumulated in the IZ, Wnt3A+Wnt5A double-electroporated cells were almost equally distributed throughout the migratory territory (IZ and CP), similarly to the control condition (Figures 5F and 5G). Moreover, Wnt5A overexpression was sufficient to rescue the increase in the proportion of multipolar cells in both the SVZ and the IZ (Figure 5H). Together, these data are consistent with the hypothesis that a precursor-intrinsic activation of Wnt5A/non-canonical signaling reduces Wnt canonical signaling, thereby allowing the multipolar-bipolar switch of precursor cells.

Wnt Canonical Signaling Regulates Polarization of Pyramidal Precursors through Ephrin-B1 Expression

Next, we investigated potential downstream molecular mechanisms that could mediate the effects of canonical Wnt signaling on the multipolar-to-bipolar transition. The guidance molecule ephrin-B1 is expressed in a graded manner in the developing cortex and has been shown to play a critical role in regulating process dynamics and tangential spreading of pyramidal precursors during the multipolar phase (Dimidschstein et al., 2013). It has also been demonstrated that ephrin-B1 expression is inversely regulated by the genetic program driven by Wnt/β-catenin/TCF-4 in colorectal cancer cells (Batlle et al., 2002). To explore the possibility that canonical signaling may also regulate ephrin-B1 expression in migrating neuronal progenitors, we evaluated ephrin-B1 mRNA levels using in situ hybridization in control and Wnt3A-electroporated animals. We confirmed previous observations that ephrin-B1 is expressed in the VZ/SVZ of the normal cortex at E20 (Figure 6A, upper panel). In addition, we observed that Wnt3A overexpression induced a significant reduction in the in situ hybridization signal in electroporated regions compared to control animals or the adjacent non-electroporated regions (Figure 6A, middle panel). Real-time qPCR measurements on mRNA from electroporated VZ/SVZ regions micro-dissected at E20 further confirmed this observation (Figure 6B). Most importantly, co-electroporation of Wnt5A along with Wnt3A restituted normal levels of ephrin-B1 mRNA (Figures 6A and 6B, lower panel). These results indicate that similar to colon cancer cells, canonical Wnt signaling inversely regulates ephrin-B1 expression in the developing cortex. We then tested the hypothesis that the reduced polarization of pyramidal precursors after Wnt canonical GOF is due to a decreased ephrin-B1 expression. We observed that ephrin-B1 overexpression rescued the polarization defect that we can see after Wnt3A overexpression (Figures 6C–6E). We conclude that reduced activity level of canonical Wnt signaling is required to maintain an adequate level of ephrin-B1 expression allowing the polarization of multipolar cells.

DISCUSSION

In the present study, we investigated the role of Wnt signaling in regulating radial migration of pyramidal precursors. Our principal findings can be summarized as follows: (1) Wnt/β-catenin as well as non-canonical Wnt signaling is active and dynamically regulated in radially migrating pyramidal neurons, (2) transient downregulation of canonical Wnt/β-catenin signaling activity is required for the proper transition of pyramidal precursors from multipolar into bipolar state, (3) reduced canonical Wnt/β-catenin signaling is initiated by increased Wnt5A/non-canonical signaling, and (4) downregulation of canonical Wnt signaling by Wnt5A allows cells to express ephrin-B1 thereby enabling polarization and initiation of glia-guided locomotion. Together, these results support the hypothesis that a crosstalk between the Wnt5A/non-canonical and Wnt/β-catenin signaling pathways takes part of a core signaling that regulates pyramidal cell migration via multipolar-to-bipolar transition. This is an unexpected finding given the classical role of Wnt pathways in cortical progenitor proliferation and differentiation, extending the range of functions previously attributed to this signaling in cortical development.

The multipolar-to-bipolar transition is a critical event of the radial migration process (Tabata and Nakajima, 2003; Kriegstein and Noctor, 2004; LoTurco and Bai, 2006) that is regulated by a multitude of signaling molecules (Jossin and Cooper, 2011; Chen et al., 2008; Miyoshi and Fishell, 2012; Xie et al., 2013; Nagano et al., 2004; Ohshima et al., 2007; Westerlund et al., 2011; Heng et al., 2008; Bai et al., 2003; Ohtaka-Maruyama et al., 2013). The large majority of late-generated pyramidal precursors are generated from unpolarized intermediate progenitors (TBR2 positive cells) and inherit multipolar morphologies with highly dynamic processes (Kriegstein and Alvarez-Buylla, 2009). In order to start radial migration toward the pial surface, multipolar cells in the upper SVZ/lower IZ need to go through a polarization process and adopt a bipolar state. We showed that a low level of

Figure 4. Physiological Reduction of Wnt/β-Catenin Signaling Is Necessary for the Multipolar/Bipolar Transition

(A) Single-cell, confocal time-lapse sequences of TOPdGFP/UBI-RFP-electroporated cells migrating in the IZ and switching between a bipolar and multipolar state. The intensity ratio of TOPdGFP/UBI-RFP is color-coded (red corresponds to a low level of canonical Wnt activity, whereas green is high canonical Wnt activation). Decreased signaling level was associated with bipolar state as shown by the corresponding intensity curves (arrows indicate the bipolar state, whereas arrowheads show the multipolar state). See also Movie S3.

(B) Time-lapse sequences from freshly isolated slices of control, Wnt3A-, and Δ45β-catenin-electroporated cells in the IZ. Examples of control pyramidal neurons (upper panel) assuming stable bipolar morphology (arrowhead) or displaying a bipolar-multipolar-bipolar switch (asterisk) are shown. Cells overexpressing Wnt3A (mid panel) or Δ45β-catenin (lower panel) often stalled in a multipolar and stationary stage. See also Movie S4.

(C) Graph showing the typical migratory distances traveled by the bipolar and the "multipolar switching" cells shown in (B) (upper panel).

(D) Proportion of tracked cells that transited into the multipolar stage at least once during tracking time.

(E) Proportion of total tracking time that cells spent in the multipolar stage.

(F) Mean duration of a multipolar phase.

(G) Frequency of multipolar switch. Data represent mean ± SEM of n = 6 brains for control (180 cells), n = 3 brains for Wnt3A (90 cells), and n = 4 brains for Δ45β-catenin (240 cells) from at least three experiments (unpaired t test).

Scale bars, 20 μm. See also Figure S3.

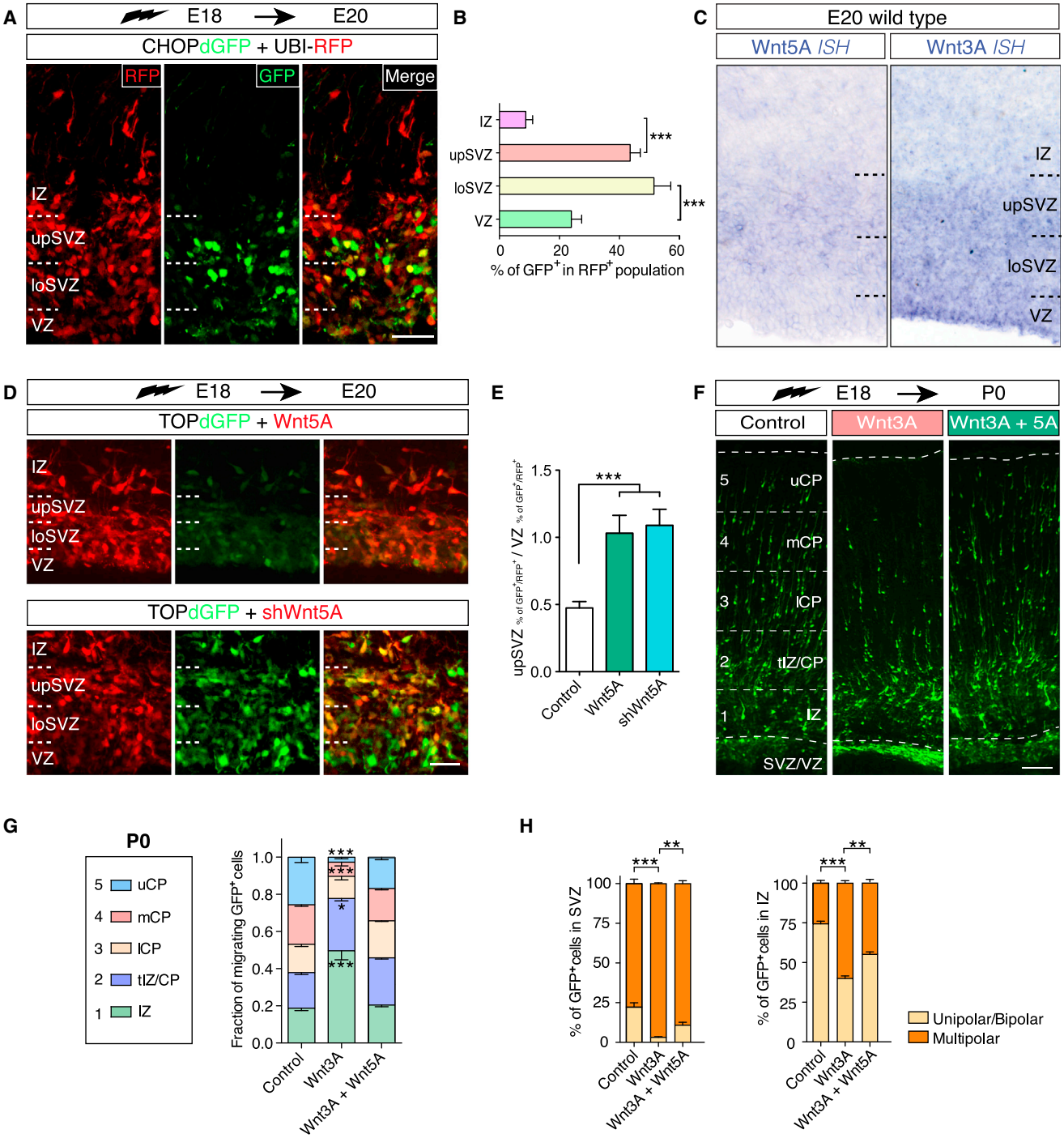

Figure 5. Cell-Autonomous Wnt5A/Non-canonical Signaling Inhibits the Wnt/β-Catenin Pathway in Migrating Pyramidal Cells in the upSVZ

(A) E20 brains electroplated at E18 with CHOPdGFP reporter to monitor non-canonical Wnt PCP activity showing an increase in the proportion of RFP+/GFP+ cells in the SVZ.

(B) Quantification GFP-labeled cells within RFP+ population. Data represent mean ± SEM of n = 8 brains from three experiments (1,121 IZ RFP+ cells, 1,112 upper SVZ RFP+ cells, 789 lower SVZ RFP+ cells, and 312 VZ RFP+ cells) (one-way ANOVA followed by the Bonferroni post test).

(C) In situ hybridization on wild-type E20 brains for Wnt5A and Wnt3A. Wnt5A transcript displays a gradient from VZ toward upper SVZ and is absent in the IZ, while Wnt3A mRNA is homogeneously expressed in the VZ/SVZ regions.

(D) E20 brains electroporated with TOPdGFP together with Wnt5A or shWnt5A showing that percentages of GFP+ cells along VZ to IZ regions do not change.

(E) Ratio of the percentage of RFP+ cells displaying GFP fluorescence between the upSVZ and VZ. Data represent mean ± SEM of at least n = 6 brains (unpaired t test).

(legend continued on next page)

Wnt canonical activity occurs precisely during this specific time window. Our single-cell imaging revealed that canonical Wnt activity is high during the multipolar phase of pyramidal precursors and then significantly reduced at the level of the upper SVZ, where the transformation from a multipolar to bipolar state occurs. Subsequently, bipolar cells in the IZ upregulate canonical Wnt signaling activity that will persist throughout the CP. Moreover, we observed in agreement with a previous study (Munji et al., 2011) that reduced Wnt signaling activity occurs mainly at a stage, when cells become TBR2 negative prior to the apparition of SatB2 immunoreactivity. During the multipolar-to-bipolar transition, precursor cells reorient their centrosomes and Golgi toward the cortical plate, initiate a leading process, extend an axon and suppress excess processes (LoTurco and Bai, 2006; Namba et al., 2014; Kriegstein and Noctor, 2004). Our real-time imaging shows that failure to downregulate Wnt canonical signaling results in increased proportion of randomly moving cells with numerous highly dynamic processes. Moreover, we provide direct evidence for the reduced exit of cells from the SVZ upon Wnt canonical GOF. The significantly delayed migration we observed under this condition is consistent with the notion that multipolar-to-bipolar transition is a critical checkpoint for radial migration determining the number of cells exiting and moving up to the cortical plate (LoTurco and Bai, 2006). Video time-lapse observations in this study also demonstrate that under normal conditions, a number of control multipolar cells in the IZ may transiently adopt a bipolar shape and subsequently revert back to a multipolar form. This is consistent with the idea that cells here enter into a non-stable, transitional state from which they can be polarized for radial migration or switch back to a multipolar state. We found that a significantly higher proportion of precursors with excess Wnt signaling revert back to the multipolar state after being polarized transiently. Thus, a reduced level of Wnt canonical signaling is required for persistent polarity of precursor cells.

An important new observation of the present study is that Wnt5A/non-canonical signaling activity is critical for the transient downregulation of canonical Wnt signaling during the multipolar stage. Consistent with previous published reports (Endo et al., 2012), we found that the non-canonical ligand,Wnt5A is expressed in pyramidal precursor cells. Furthermore, we demonstrate that the expression level of Wnt5A is relatively low in the VZ but progressively increased as cells pass through the SVZ. The increased level of Wnt5A is required for the transient reduction of canonical signaling during the multipolar state, as demonstrated by our knockdown experiments using shWnt5A. Moreover, we found that overexpressing Wnt5A leads to decreased canonical signaling levels and restitute a normal polarization phenotype in the upper SVZ/IZ after canonical GOF. These observations support the hypothesis that time-dependent and pyramidal precursor-intrinsic regulation of Wnt5A/non-canonical signaling inhibits the canonical pathway, thereby allowing proper cell polarization. This hypothesis is consistent with previous observations that pyramidal precursors in the developing cortex express not only Wnt5A but also the non-canonical receptors Ror 1 and 2 (Endo et al., 2012). Intriguingly, a similar cell-intrinsic modulation of Wnt canonical as well as non-canonical signaling has been implicated in the control of neuroblast migration in *C. elegans* (Mentink et al., 2014), indicating that this regulatory mechanism, similarly to guidance by Wnt morphogenic gradient, is evolutionarily conserved. How Wnt5A expression is regulated remains to be determined. It is important to emphasize that non-canonical signaling has a well-established role in regulating cytoskeleton dynamics and cell polarity, which are critical for cell migration (Witze et al., 2008). Whereas in the current studies we focused our attention on the potential crosstalk with the canonical signaling, Wnt5A/non-canonical signaling could regulate multipolar-to-bipolar transition via alternative pathways. Finally, we cannot exclude the possibility that additional canonical as well as non-canonical Wnt ligands may contribute to the regulation of this process.

We have identified ephrin-B1 signaling as a potential downstream effector of the Wnt canonical pathway in regulating the multipolar-to-bipolar transition. Ephrin/Eph signaling plays a key role in short-distance cell-cell signaling, typically through contact repulsion (Klein and Kania, 2014; Wilkinson, 2001). Ephrin-mediated contact repulsion is critical for the generation and maintenance of cell segregation into domains during development (Wilkinson, 2001), and it has also been implicated in regressive events including axonal and dendritic spine pruning and neuronal death (Vanderhaeghen and Cheng, 2010). Interestingly, members of both class A and B ephrins appear to play an important role in regulating lateral dispersion of pyramidal precursors during the multipolar stage (Torii et al., 2009; Dimidschstein et al., 2013). In particular, ephrin-B1 reverse signaling was shown to activate Rac3 through the P-Rex1 guanylate exchange factor and to reduce neurite dynamics, thereby restricting tangential migration of pyramidal precursors (Dimidschstein et al., 2013). On the other hand, ephrin-B1 loss of function resulted in increased lateral dispersion of cells, most likely as a consequence of increased neurite dynamics and exploratory behavior (Dimidschstein et al., 2013; Torii et al., 2009). We show here that a similar, "multiple protrusion" phenotype after Wnt canonical gain-of-function is due, at least in part, to a reduced expression level of ephrin-B1, hence confirming the previously identified inverse relationship between Wnt canonical signaling and ephrin-B1 expression

(F) Position of electroporated cells in coronal sections at P0 in control GFP (left), Wnt3A (middle), or Wnt3A+Wnt5A (right) plasmids. The migration path was divided into five equal bins as shown in Figure 2. While a large number of Wnt3A-overexpressing cells accumulated in the IZ, Wnt3A+Wnt5A double-electroporated cells were almost equally distributed throughout the migratory territory (IZ and CP), similarly to the control condition.

(G) Fraction of migrating electroporated cells in each bin at P0. Data represent mean ± SEM of n = 9 brains from six experiments for the control condition (7,845 cells), n = 7 brains from two experiments for the Wnt3A condition (4,260 cells), and n = 8 brains from three experiments for the Wnt3A+Wnt5A condition (5,987 cells) (one-way ANOVA followed by the Bonferroni post test).

(H) Percentage of GFP+-electroporated cells displaying either multipolar or unipolar/bipolar morphology in the SVZ (left) and IZ (right). Data represent mean ± SEM of n = 5 brains from at least two experiments (SVZ: 358 control cells, 201 Wnt3A cells, and 254 Wnt3A+Wnt5A cells; IZ: 420 control cells, 254 Wnt3A cells, and 248 Wnt3A+Wnt5A cells) (unpaired t test).

Scale bars represent 100 μm (F) and 20 μm (A and D). See also Figure S4.

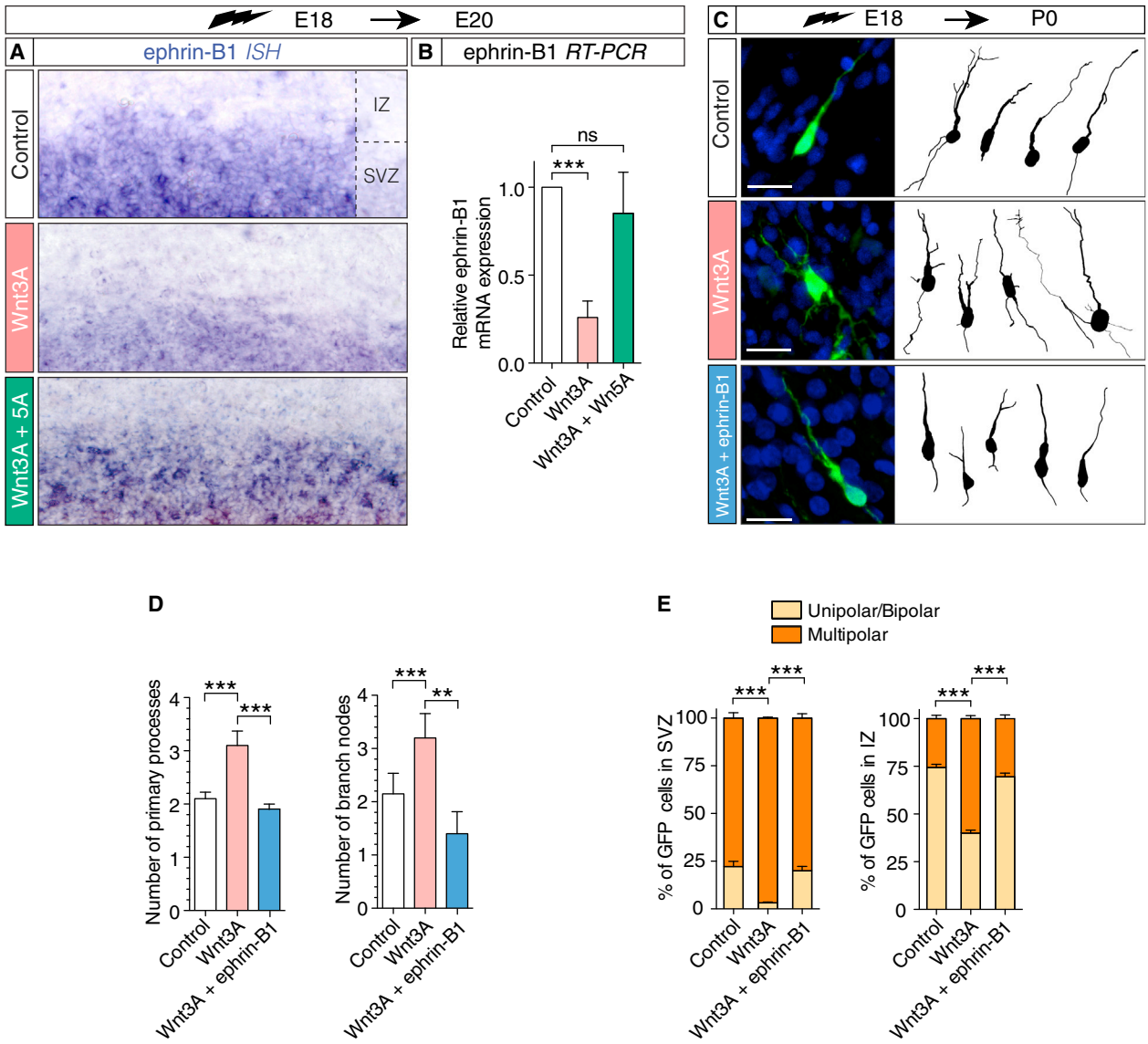

Figure 6. Reduction of Canonical Wnt Signaling in SVZ Permits Neuronal Polarization through Ephrin-B1 Expression

(A) In situ hybridization on control, Wnt3A-, or Wnt3A+Wnt5A-electroporated E20 brains for ephrin-B1.

(B) Relative ephrin-B1 mRNA transcript quantified by RT-PCR on VZ/SVZ micro-dissected cells at E20 from control, Wnt3A-, and Wnt3A+Wnt5A-electroporated brains. Data represent mean ± SEM of n = 3 experiments (unpaired t test).

(C) Neurolucida morphological reconstruction of control, Wnt3A, and Wnt3A+ephrin-B1 cells in the IZ at P0. Scale bars, 20 μm.

(D) Quantitative morphometric analysis in IZ at P0. Left panel: number of primary processes. Right panel: number of branch nodes. Data represent mean ± SEM of n = 20 cells per condition from three experiments (unpaired t test).

(E) Percentage of GFP+ electroporated cells displaying either multipolar or unipolar/bipolar morphology in the SVZ (left) and IZ (right). Data represent mean ± SEM of n = 5 brains from at least two experiments (SVZ: 358 control cells, 201 Wnt3A cells, and 214 Wnt3A+ephrin-B1 cells; IZ: 420 control cells, 254 Wnt3A cells, and 265 Wnt3A+ephrin-B1 cells) (unpaired t test).

(Batlle et al., 2002). Our studies lend strong support to this notion by demonstrating the same relationship in migrating neuronal precursor cells. Thus, a decreased signal intensity of canonical Wnt signaling during the multipolar phase appears necessary for allowing adequate expression levels of ephrin-B1. Ephrin-B1 signaling in turn may limit neurite dynamics and permit pruning of excess cellular processes through contact repulsion. This effect not only limits the exploratory behavior and lateral dispersion of multipolar cells (Dimidschstein et al., 2013) but also permits the proper polarization rate of cells for radial migration.

EXPERIMENTAL PROCEDURES

Animals

The experimental procedures described here were conducted in accordance with the Swiss laws, previously approved by the Geneva Cantonal Veterinary

Authority. Wistar rats were provided by Charles River Laboratories. Where indicated, doxycycline (Sigma) was administered (1 mg/ml) in drinking water at E21 in order to activate transgene expression from inducible constructs.

Plasmids

The different expression constructs were generated using the Gateway recombination cloning technology on lentiviral destination vectors (Giry-Laterrière et al., 2011a, 2011b). In a first type of vector, a single expression unit is composed of one promoter driving one gene. This is the case of UBI-GFP, UBI-tdTomato, M38 TOPdGFP (Addgene: clone number 17114), and ATF2 CHOPdGFP (GenBank AY880949; Ohkawara and Niehrs, 2011). In a second type of vector, a dual constitutive expression vector is composed of one unit with the human ubiquitin promoter driving the gene of interest (Δ45-β-catenin, Wnt3A, Wnt5A, or shWnt5A), followed by one unit with the human PGK promoter driving either GFP or TurboRFP. pCIG-ephrin-B1 plasmid was a kind gift from Dr. P. Vanderhaeghen (Dimidschstein et al., 2013). Further details on plasmids and cloning procedures used in this study can be obtained at http://lentilab.unige.ch/.

In Utero Electroporation

In utero electroporation was performed at embryonic day 18 (E18) as previously described (Saito and Nakatsuji, 2001), with some modifications (see Supplemental Experimental Procedures). In order to obtain internal control, bilateral electroporation was developed. For this purpose, after classical electroporation of one hemisphere with a control plasmid, the contralateral hemisphere was injected and electroporated with an experimental plasmid.

Tissue Processing, Immunofluorescence, and Image Acquisition

For post hoc in vivo studies, 20-μm-thick, fixed cryostat sections of rat brains were obtained at three different time points: E20, P0, and P1. Immunofluorescence was carried out based on previously described protocols (Zgraggen et al., 2012). Images were acquired with a Nikon Eclipse 80i epifluorescence microscope. For analyses at high-power magnifications, a Nikon A1r confocal laser scanning microscope with a 40× Plan Fluor oil differential interference contrast H N2 objective and laser illumination diode at 405 nm, 488 nm, 561 nm, and 640 nm was used.

In Situ Hybridization

40-μm-thick frozen sections were melted on SuperFrost Ultra Plus slides (Menzel-Glaser), fixed in 4% paraformaldehyde (Sigma), digested with 2 μg/ml Proteinase K (Roche), and treated with 2 mg/ml glycine (Biosolve). Following prehybridization for 1 hr at 60°C, hybridization was carried out in a humidified chamber at 60°C overnight (ON) using digoxigenin-labeled cRNA probes generated by in vitro transcription (DIG RNA labeling Kit, Roche) of PCR-amplified rat cDNA using primers for Wnt3A, Wnt5A, and ephrin-B1. Hybridized probes were incubated with an anti-digoxigenin antibody conjugated to alkaline phosphatase (1:2,000, Roche) ON at 4°C. Alkaline phosphatase was detected using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution (Roche) with reaction times ranging from 5 hr to 3 days.

Quantitative Real-Time PCR Analysis

Total RNAs was isolated from micro-dissected VZ/SVZ cells at E20 using the RNeasy kit (QIAGEN). The cDNAs were reverse transcribed using SuperScript II Synthesis System according to the kit protocol (Invitrogen Life Technologies). FastStart Universal SYBR Green Master (Roche) was used for the real-time PCR. Sense and antisense primers of ephrin-B1 and cyclophilin were used.

Cortical Slice Preparation and Time-Lapse Confocal Imaging

Coronal slices (300 μm thick) were obtained at P0 (constitutive plasmids) or P1 (inducible plasmids) and placed in a chamber maintained at 37°C with 5% CO_2 during acquisition with a Nikon A1r Resonant Scanner Upright Microscope.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and five movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.01.061.

AUTHOR CONTRIBUTIONS

M.B. and R.B. conceived, designed, and performed experiments, analyzed data, and wrote the paper; V.P. improved the electroporation technique; R.B. and V.P. performed in utero electroporation; K.E. helped with the in situ hybridization; P.S., E.Z., B.V., and S.G. helped to clone different constructs; and J.Z.K. conceived the project, designed experiments, and wrote the paper.

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REFERENCES

Amit, S., Hatzubai, A., Birman, Y., Andersen, J.S., Ben-Shushan, E., Mann, M., Ben-Neriah, Y., and Alkalay, I. (2002). Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev. *16*, 1066–1076.

Bai, J., Ramos, R.L., Ackman, J.B., Thomas, A.M., Lee, R.V., and LoTurco, J.J. (2003). RNAi reveals doublecortin is required for radial migration in rat neocortex. Nat. Neurosci. *6*, 1277–1283.

Batlle, E., Henderson, J.T., Beghtel, H., van den Born, M.M., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T., and Clevers, H. (2002). Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell *111*, 251–263.

Bielas, S., Higginbotham, H., Koizumi, H., Tanaka, T., and Gleeson, J.G. (2004). Cortical neuronal migration mutants suggest separate but intersecting pathways. Annu. Rev. Cell Dev. Biol. *20*, 593–618.

Chen, G., Sima, J., Jin, M., Wang, K.-Y., Xue, X.-J., Zheng, W., Ding, Y.-Q., and Yuan, X.-B. (2008). Semaphorin-3A guides radial migration of cortical neurons during development. Nat. Neurosci. *11*, 36–44.

Chenn, A. (2008). Wnt/beta-catenin signaling in cerebral cortical development. Organogenesis *4*, 76–80.

Clevers, H., and Nusse, R. (2012). Wnt/β-catenin signaling and disease. Cell *149*, 1192–1205.

Dickins, E.M., and Salinas, P.C. (2013). Wnts in action: from synapse formation to synaptic maintenance. Front. Cell. Neurosci. *7*, 162.

Dimidschstein, J., Passante, L., Dufour, A., van den Ameele, J., Tiberi, L., Hrechdakian, T., Adams, R., Klein, R., Lie, D.C., Jossin, Y., and Vanderhaeghen, P. (2013). Ephrin-B1 controls the columnar distribution of cortical pyramidal neurons by restricting their tangential migration. Neuron *79*, 1123–1135.

Dorsky, R.I., Sheldahl, L.C., and Moon, R.T. (2002). A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. Dev. Biol. *241*, 229–237.

Endo, M., Doi, R., Nishita, M., and Minami, Y. (2012). Ror family receptor tyrosine kinases regulate the maintenance of neural progenitor cells in the developing neocortex. J. Cell Sci. *125*, 2017–2029.

Freese, J.L., Pino, D., and Pleasure, S.J. (2010). Wnt signaling in development and disease. Neurobiol. Dis. *38*, 148–153.

Giry-Laterrière, M., Cherpin, O., Kim, Y.-S., Jensen, J., and Salmon, P. (2011a). Polyswitch lentivectors: "all-in-one" lentiviral vectors for drug-inducible gene expression, live selection, and recombination cloning. Hum. Gene Ther. *22*, 1255–1267.

Giry-Laterrière, M., Verhoeyen, E., and Salmon, P. (2011b). Lentiviral vectors. Methods Mol. Biol. *737*, 183–209.

Heng, J.I.-T., Nguyen, L., Castro, D.S., Zimmer, C., Wildner, H., Armant, O., Skowronska-Krawczyk, D., Bedogni, F., Matter, J.-M., Hevner, R., and Guillemot, F. (2008). Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. Nature *455*, 114–118.

Hurlstone, A.F.L., Haramis, A.P.G., Wienholds, E., Begthel, H., Korving, J., Van Eeden, F., Cuppen, E., Zivkovic, D., Plasterk, R.H., and Clevers, H. (2003). The Wnt/β-catenin pathway regulates cardiac valve formation. Nature *425*, 633–637.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. Nature *399*, 798–802.

Jossin, Y., and Cooper, J.A. (2011). Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex. Nat. Neurosci. *14*, 697–703.

Kikuchi, A., Yamamoto, H., Sato, A., and Matsumoto, S. (2012). Wnt5a: its signalling, functions and implication in diseases. Acta Physiol. (Oxf.) *204*, 17–33.

Klein, R., and Kania, A. (2014). Ephrin signalling in the developing nervous system. Curr. Opin. Neurobiol. *27*, 16–24.

Kohn, A.D., and Moon, R.T. (2005). Wnt and calcium signaling: beta-catenin-independent pathways. Cell Calcium *38*, 439–446.

Kriegstein, A., and Alvarez-Buylla, A. (2009). The glial nature of embryonic and adult neural stem cells. Annu. Rev. Neurosci. *32*, 149–184.

Kriegstein, A.R., and Noctor, S.C. (2004). Patterns of neuronal migration in the embryonic cortex. Trends Neurosci. *27*, 392–399.

Li, X., Zhao, X., Fang, Y., Jiang, X., Duong, T., Fan, C., Huang, C.C., and Kain, S.R. (1998). Generation of destabilized green fluorescent protein as a transcription reporter. J. Biol. Chem. *273*, 34970–34975.

LoTurco, J.J., and Bai, J. (2006). The multipolar stage and disruptions in neuronal migration. Trends Neurosci. *29*, 407–413.

Lyuksyutova, A.I., Lu, C.C., Milanesio, N., King, L.A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M., and Zou, Y. (2003). Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. Science *302*, 1984–1988.

MacDonald, B.T., Tamai, K., and He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev. Cell *17*, 9–26.

Mentink, R.A., Middelkoop, T.C., Rella, L., Ji, N., Tang, C.Y., Betist, M.C., van Oudenaarden, A., and Korswagen, H.C. (2014). Cell intrinsic modulation of Wnt signaling controls neuroblast migration in C. elegans. Dev. Cell *31*, 188–201.

Mikels, A.J., and Nusse, R. (2006). Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. PLoS Biol. *4*, e115.

Miyoshi, G., and Fishell, G. (2012). Dynamic FoxG1 expression coordinates the integration of multipolar pyramidal neuron precursors into the cortical plate. Neuron *74*, 1045–1058.

Munji, R.N., Choe, Y., Li, G., Siegenthaler, J.A., and Pleasure, S.J. (2011). Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. J. Neurosci. *31*, 1676–1687.

Nagano, T., Morikubo, S., and Sato, M. (2004). Filamin A and FILIP (Filamin A-Interacting Protein) regulate cell polarity and motility in neocortical subventricular and intermediate zones during radial migration. The Journal of neuroscience *24*, 9648–9657.

Namba, T., Kibe, Y., Funahashi, Y., Nakamuta, S., Takano, T., Ueno, T., Shimada, A., Kozawa, S., Okamoto, M., Shimoda, Y., et al. (2014). Pioneering axons regulate neuronal polarization in the developing cerebral cortex. Neuron *81*, 814–829.

Ohkawara, B., and Niehrs, C. (2011). An ATF2-based luciferase reporter to monitor non-canonical Wnt signaling in Xenopus embryos. Dev. Dyn. *240*, 188–194.

Ohshima, T., Hirasawa, M., Tabata, H., Mutoh, T., Adachi, T., Suzuki, H., Saruta, K., Iwasato, T., Itohara, S., Hashimoto, M., et al. (2007). Cdk5 is required for multipolar-to-bipolar transition during radial neuronal migration and proper dendrite development of pyramidal neurons in the cerebral cortex. Development *134*, 2273–2282.

Ohtaka-Maruyama, C., Hirai, S., Miwa, A., Heng, J.I., Shitara, H., Ishii, R., Taya, C., Kawano, H., Kasai, M., Nakajima, K., and Okado, H. (2013). RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex. Cell Rep. *3*, 458–471.

Rakic, P. (2007). The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. Brain Res. Brain Res. Rev. *55*, 204–219.

Rakic, P., Ayoub, A.E., Breunig, J.J., and Dominguez, M.H. (2009). Decision by division: making cortical maps. Trends Neurosci. *32*, 291–301.

Saito, T., and Nakatsuji, N. (2001). Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. Dev. Biol. *240*, 237–246.

Sarkisian, M.R., Bartley, C.M., and Rakic, P. (2008). Trouble making the first move: interpreting arrested neuronal migration in the cerebral cortex. Trends Neurosci. *31*, 54–61.

Sato, A., Yamamoto, H., Sakane, H., Koyama, H., and Kikuchi, A. (2010). Wnt5a regulates distinct signalling pathways by binding to Frizzled2. EMBO J. *29*, 41–54.

Shimogori, T., VanSant, J., Paik, E., and Grove, E.A. (2004). Members of the Wnt, Fz, and Frp gene families expressed in postnatal mouse cerebral cortex. J. Comp. Neurol. *473*, 496–510.

Tabata, H., and Nakajima, K. (2003). Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. J. Neurosci. *23*, 9996–10001.

Topol, L., Jiang, X., Choi, H., Garrett-Beal, L., Carolan, P.J., and Yang, Y. (2003). Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. J. Cell Biol. *162*, 899–908.

Torii, M., Hashimoto-Torii, K., Levitt, P., and Rakic, P. (2009). Integration of neuronal clones in the radial cortical columns by EphA and ephrin-A signalling. Nature *461*, 524–528.

van Amerongen, R., and Nusse, R. (2009). Towards an integrated view of Wnt signaling in development. Development *136*, 3205–3214.

Vanderhaeghen, P., and Cheng, H.J. (2010). Guidance molecules in axon pruning and cell death. Cold Spring Harb. Perspect. Biol. *2*, a001859.

Veeman, M.T., Axelrod, J.D., and Moon, R.T. (2003). A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Dev. Cell *5*, 367–377.

Westerlund, N., Zdrojewska, J., Padzik, A., Komulainen, E., Björkblom, B., Rannikko, E., Tararuk, T., Garcia-Frigola, C., Sandholm, J., Nguyen, L., et al. (2011). Phosphorylation of SCG10/stathmin-2 determines multipolar stage exit and neuronal migration rate. Nat. Neurosci. *14*, 305–313.

Wilkinson, D.G. (2001). Multiple roles of EPH receptors and ephrins in neural development. Nat. Rev. Neurosci. *2*, 155–164.

Witze, E.S., Litman, E.S., Argast, G.M., Moon, R.T., and Ahn, N.G. (2008). Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors. Science *320*, 365–369.

Woodhead, G.J., Mutch, C. a., Olson, E.C., and Chenn, A. (2006). Cell-autonomous beta-catenin signaling regulates cortical precursor proliferation. The Journal of neuroscience *26*, 12620–12630.

Xie, M.-J., Yagi, H., Kuroda, K., Wang, C.-C., Komada, M., Zhao, H., Sakakibara, A., Miyata, T., Nagata, K.-I., Oka, Y., Iguchi, T., and Sato, M. (2013). WAVE2-Abi2 complex controls growth cone activity and regulates the multipolar-bipolar transition as well as the initiation of glia-guided migration. Cerebral cortex (New York, N.Y.: 1991) *23*, 1410–1423.

Zgraggen, E., Boitard, M., Roman, I., Kanemitsu, M., Potter, G., Salmon, P., Vutskits, L., Dayer, A.G., and Kiss, J.Z. (2012). Early postnatal migration and development of layer II pyramidal neurons in the rodent cingulate/retrosplenial cortex. Cereb. Cortex *22*, 144–157.