Development/Plasticity/Repair

Coronin 2B Regulates Neuronal Migration via Rac1-Dependent Multipolar–Bipolar Transition

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In the developing cortex, excitatory neurons migrate along the radial fibers to their final destinations and build up synaptic connection with each other to form functional circuitry. The shaping of neuronal morphologies by actin cytoskeleton dynamics is crucial for neuronal migration. However, it is largely unknown how the distribution and assembly of the F-actin cytoskeleton are coordinated. In the present study, we found that an actin regulatory protein, coronin 2B, is indispensable for the transition from a multipolar to bipolar morphology during neuronal migration in ICR mice of either sex. Loss of coronin 2B led to heterotopic accumulation of migrating neurons in the intermediate zone along with reduced dendritic complexity and aberrant neuronal activity in the cortical plate. This was accompanied by increased seizure susceptibility, suggesting the malfunction of cortical development in coronin 2B-deficient brains. Coronin 2B knockdown disrupted the distribution of the F-actin cytoskeleton at the leading processes, while the migration defect in coronin 2B-deficient neurons was partially rescued by overexpression of Rac1 and its downstream actin-severing protein, cofilin. Our results collectively reveal the physiological function of coronin 2B during neuronal migration whereby it maintains the proper distribution of activated Rac1 and the F-actin cytoskeleton.

Key words: **actin cytoskeleton; coronin 2B; cortical development; multipolar–bipolar transition; neuronal migration**

Significance Statement

Deficits in neuronal migration during cortical development result in various neurodevelopmental disorders (e.g., focal cortical dysplasia, periventricular heterotopia, epilepsy, etc.). Most signaling pathways that control neuronal migration process converge to regulate actin cytoskeleton dynamics. Therefore, it is important to understand how actin dynamics is coordinated in the critical processes of neuronal migration. Herein, we report that coronin 2B is a key protein that regulates neuronal migration through its ability to control the distribution of the actin cytoskeleton and its regulatory signaling protein Rac1 during the multipolar–bipolar transition in the intermediate zone, providing insights into the molecular machinery that drives the migration process of newborn neurons.

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Introduction

During cortical development, neuronal migration directs newborn projection neurons navigating along radial fibers to their proper destinations, which is a critical step for the formation of the 6-layered cortical architecture and functional circuitry (Ayala et al., 2007; Hippenmeyer, 2014). Defects in neuronal migration during development lead to cortical malformations and are often accompanied by neural developmental disorders, such as focal cortical dysplasia, periventricular heterotopia, intellectual disability, epilepsy, etc. (Stouffer et al., 2016; Francis and Cappello, 2021).

The neuronal migration process in the cortex is tightly controlled by both extrinsic stimuli and intrinsic signaling, most of

which converge toward cytoskeleton remodeling to shape the morphology and function of migrating neurons, such as the multipolar–bipolar transition, locomotion, and terminal translocation (Evsyukova et al., 2013; Hippenmeyer, 2014). Of note, the accumulation of F-actin at the dendritic tips determines the direction of neuronal migration. Previous studies show that the selective concentration of the actin cytoskeleton at specific neurites toward the apical side provides a dense filament belt to support tight adhesive junctions, which directs the migrating neurons to their destination (Lian and Sheen, 2015).

Actin regulatory proteins (e.g., Rac1, cofilin, and filamin A) regulate the actin cytoskeleton at the leading process and thereby facilitate the multipolar–bipolar transition in the intermediate zone (IZ), enabling neurons to continue their journey to the cortical plate (CP) (Konno et al., 2005; Bellenchi et al., 2007; Zhang et al., 2013). Both the activity and subcellular location of these actin regulatory proteins are well orchestrated during the migration process. For example, Rac1, a member of Rho family of small GTPases, bridges upstream signals with cytoskeletal rearrangement to establish neuronal polarity and adjust cell morphology (Govek et al., 2011; Xu et al., 2019). Interfering with Rac1 activity through the overexpression of Rac1-G12V (the constitutively active form of Rac1) or Rac1-T17N (the dominant-negative form of Rac1) stalls the migrating neurons at the IZ; meanwhile, perturbation of the localization of activated Rac1 through knockdown of the Rac1-interacting protein POSH (Plenty of SH3 Domains) also affects neuronal migration (Konno et al., 2005; Yang et al., 2012).

On the other hand, the formation of the leading process, which involves proximal cytoplasmic dilation, is a critical step that facilitates neuronal migration (Reiner and Sapir, 2009; Evsyukova et al., 2013). Both the actin and microtubule cytoskeleton are essential for the formation and stabilization of leading processes. In particular, a stable and polarized actin cytoskeleton provides an instructive scaffold for the establishment of a bipolar structure, while the disruption of actin cytoskeleton tends to result in the disappearance of leading processes (Zhang et al., 2013; Chai et al., 2016; Govek et al., 2018). Thus, precise actin cytoskeleton arrangement, which predominantly accumulates in the proximal cytoplasmic dilation, helps the multipolar–bipolar transition, provides contractile force, and coordinates the alignment of the Golgi apparatus, centrosome, and nucleus to determine the direction of neuronal migration (Yanagida et al., 2012; Minegishi and Inagaki, 2020; Nakazawa and Kengaku, 2020). Although the accumulation of F-actin and its regulatory proteins at the tips of potential leading processes is likely critical, it is incompletely understood how these actin regulatory proteins are coordinated to form F-actin belts and subsequently generate a leading process to guide the migrating neurons toward the pial surface.

Coronins are a family of WD-repeat domain-containing proteins widely expressed in eukaryotes and participate in diverse cellular processes (Appleton et al., 2006; Chan et al., 2011). Coronins modulate the dynamics of the F-actin cytoskeleton either through direct binding with F-actin or through interactions with other actin regulatory proteins, such as actin-related protein 2/3 complex (Arp2/3 complex) and cofilin (Cai et al., 2007; Chan et al., 2011). In particular, some coronins contain a highly conserved Cdc42/Rac-interactive binding motif, which can interact with Rac1 and in turn regulate their distribution within cells (Xavier et al., 2008; Swaminathan et al., 2014; Williamson et al., 2014). We previously reported that coronin 2B, a neuronally enriched coronin, is important for early dendrite outgrowth in cultured neurons, suggesting that coronin 2B plays a role in shaping neuronal polarity (Chen et al., 2020).

In the present study, we demonstrate that coronin 2B is required for neuronal migration during cortical development. Delivery of shRNA against coronin 2B by *in utero* electroporation in mice impaired neuronal migration and increased epilepsy susceptibility. Coronin 2B-deficient neurons failed to accomplish the multipolar–bipolar transition and accumulated in the IZ. This defect could be partially rescued by overexpression of Rac1. Thus, our findings suggest that coronin 2B plays a critical role in the multipolar–bipolar transition during neuronal migration by modulating actin cytoskeleton reorganization via Rac1-dependent signaling cascades.

Materials and Methods

Reagents and constructs. The antibodies and their corresponding dilution factors used for immunofluorescence staining are as follows: mouse anti-GFP (1:2000, A11120) and rabbit anti-GFP (1:2000, A11122) were purchased from Thermo Fisher Scientific; chicken anti-GFP (1:2000, ab13970), rabbit anti-GM130 (1:200, ab52649), rabbit anti-mCherry (1:200, ab167453), rat anti-Ctip2 (1:200, ab18465), and rabbit anti-NeuN (1:200, ab177487) were from Abcam; rabbit anti-Cux1 (1:100, sc-13 024) was from Santa Cruz Biotechnology; and mouse anti-CS-56 (1:200, C8035), rabbit anti-coronin 2B (1:50, HPA017960), and mouse anti-Tuj1 (1:1000, T8578) were from Merck.

The shRNA sequences against mouse/rat coronin 2B (shCoro2B) were as follows: shCoro2B #1, 5′-GAGGATCTGTCCATGCCAA-3′; shCoro2B #2, 5′-GGACCTATCTACACAGGAACC-3′. Their corresponding scramble controls were as follows: Scramble #1, 5′-GCAGTTAGTGAACGCA GTC-3′; Scramble #2, 5′-GCCACAGCGATATCCGTAACA-3′. All shRNAs were cloned into pSUPER vector. Expression constructs of cofilin, cofilin-S3A, cofilin-S3D, Rac1, Rac-G12V, Rac1-T17N, lifeact-GFP, lifeact-mCherry, and the EYFP-linked p21-binding domain (PBD) of PAK1 (PBD-EYFP) were cloned into pCAGIG vector by using the In-Fusion HD Cloning Kit EC (Clontech).

Animals. Animals (female ICR mice and female Sprague–Dawley rats) were purchased from Zhejiang Vital River Laboratory Animal Technology. All the animal experiments were performed by strictly following the guidelines approved by the Institutional Animal Care And Use Committee at the Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences.

In utero electroporation. In utero electroporation on female ICR mice was conducted as described previously (Ip et al., 2011). For knockdown experiments, pCAGIG plasmid expressing GFP protein together with shCoro2B or its corresponding scramble control (scramble) at a 1:1 ratio was injected into ventricles at embryonic day 14.5 (E14.5). Mice of either sex were perfused with 4% PFA (w/v) in PBS on E17.5, postnatal day 2 (P2), and P5. The brains were collected and postfixed in 4% PFA for 2 h, followed by dehydration in 30% sucrose solution (w/v) overnight at 4°C. After embedded in Optimal Cutting Temperature compound, the fixed brains were sliced into coronal sections (20 μm in thickness) using a Cryostar NX70 (Thermo Scientific).

Immunofluorescence staining, image acquisition, and analysis. After washing 3 times with PBS for 5 min, the brain sections were then permeabilized and incubated in blocking buffer (4% goat serum [v/v], 1% BSA [wt/vol], and 0.4% Triton X-100 [v/v] in PBS) at room temperature for 20 min. The sections were then immersed with primary antibodies solution overnight at 4°C, incubated with fluorescence-conjugated secondary antibodies for 1 h at room temperature, and then mounted in Hydromount (National Diagnostics). Representative confocal images of cortical sections were acquired using LSM900 confocal microscope (Zeiss).

The morphology of dendritic trees of transfected neurons was analyzed as described previously (Yuan et al., 2021). Briefly, the images were processed with IMARIS software (version 9.6) to reconstruct the neurons and their dendrites using the Filament Tracer module. The dendrite length was measured using the Filament Dendrite Length module, and

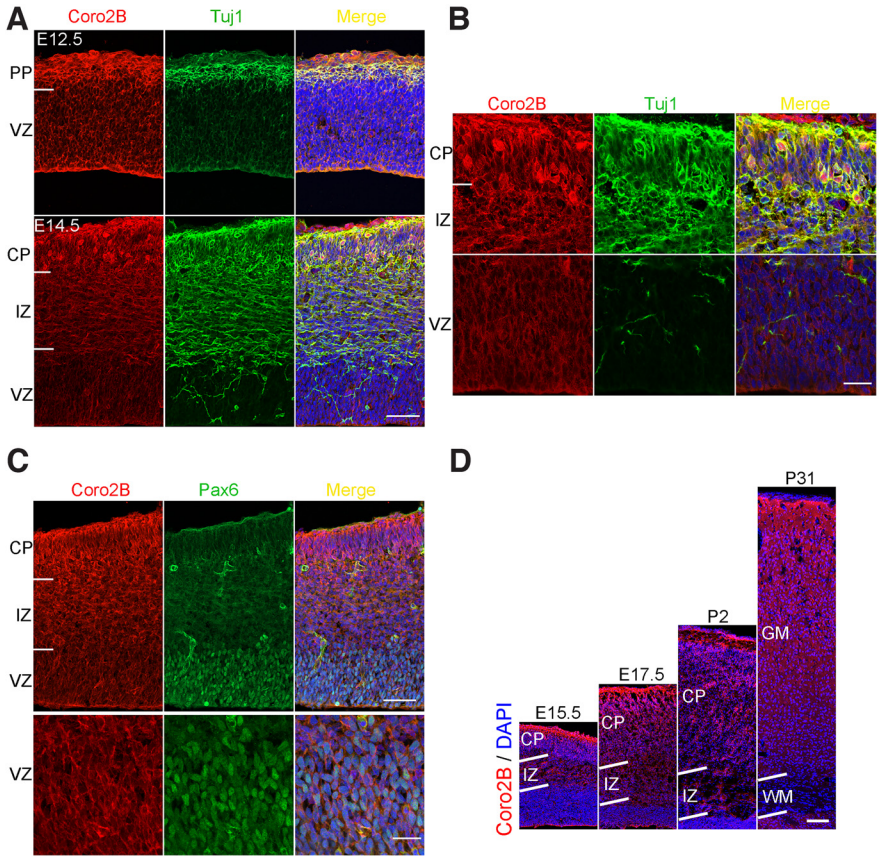

Figure 1. Spatiotemporal expression of coronin 2B in the developing cortex. ***A***, Coronin 2B expression in the cortex at embryonic day 12.5 (E12.5) and E14.5. PP, pre-plate. Scale bar, 100 μm. ***B***, Enlarged images of E14.5 cortex in ***A***. Scale bar, 20 μm. ***C***, Costaining of coronin 2B (red) with Pax6 (green) in the VZ at E14.5. Scale bars: top, 50 μm; bottom, 20 μm. ***D***, Coronin 2B expression in the cortex from E15.5 to postnatal day 31 (P31). GM, gray matter; WM, white matter. Scale bar, 100 μm.

the dendrite number was calculated using Filament No. Dendrite Terminal Pts module according to the manufacturer's instructions. Fluorescence intensity of indicated neurons was calculated by ImageJ (Fiji) with plot profile.

For c-Fos staining and quantification, cortical sections at P30 of either sex were stained with c-Fos antibody (1:500, Cell Signaling Technology, 2250S). The number of c-Fos-positive neurons within 100 μm^2 area containing the GFP-positive cells was quantified as described previously (Cho et al., 2017).

Cell culture, transfection, and Western blotting. Cortical neurons were dissociated from Sprague–Dawley rat embryos of either sex at E18.5, transfected with shRNAs or scramble control by nucleofection using a Lonza Amaxa RAT Neuron Nucleofector Kit (Lonza) as described previously (Chen et al., 2020). Transfected neurons were seeded on poly-L-lysine (Sigma-Aldrich) coated 60 mm plates and cultured in Neurobasal medium (Invitrogen) containing 2% (v/v) B27 supplement (Fisher Scientific) at 37°C in a humidified atmosphere with 5% CO_2. Three days after transfection, neurons were lysed in RIPA buffer and subjected to immunoblotting for measuring the knockdown efficiency.

Seizure induction by pentylenetetrazol (PTZ). The plasmids containing shCoro2B or its corresponding scramble control were delivered into the embryonic brain of E14.5 ICR mice of either sex by *in utero* electroporation. PTZ-induced seizure was performed at P30. Mice of either sex were intraperitoneally injected with PTZ (25 mg/kg) every 10 min until generalized seizures occurred. After each injection, the mice were carefully transferred to a new cage for recording seizure behaviors according to Racine's scale (Van Erum et al., 2019). At the end of experiment, the mice were immediately killed to confirm the effect of electroporation by visualizing the GFP signals in brain.

Quantification and statistical analysis. The quantification data from at least three independent experiments were analyzed using GraphPad Prism 8 and are presented as mean ± SEM. Student's *t* test were performed to compare the difference between two groups. Differences between three groups or more independent groups were analyzed by one-way ANOVA. *p* values <0.05 were considered statistically significant.

Results

Coronin 2B is required for radial migration in the developing cerebral cortex

We and others previously reported that coronin 2B is abundantly expressed in the nervous system, especially in the developing cortex (Nakamura et al., 1999; Rogg et al., 2017; Chen et al., 2020). To investigate the role of coronin 2B during cortical development, we first examined the spatiotemporal expression profile of coronin 2B in the mouse brain from E12.5 to P31. Coronin 2B was highly expressed in the pre-plate at E12.5, and in the IZ and CP at E14.5, whereas its expression in the ventricular zone (VZ) was relatively low. Coronin 2B was mainly expressed in neurons as revealed by its colocalization with neuronal marker β-tubulin Type III (i.e., $Tuj1^+$ neurons) (Fig. 1*A*, *B*). Costaining of coronin 2B with Pax6, a VZ marker (Duan et al., 2013), revealed that coronin 2B is also present in $Pax6^+$ neurons (Fig. 1*C*), suggesting that coronin 2B is expressed in neuronal progenitors during development. When neurons finished their migration at P31, coronin 2B was mainly expressed in the gray matter (Fig. 1*D*).

To decipher the function of coronin 2B during cortical development, we designed 2 shRNAs against coronin 2B (shCoro2B #1 and shCoro2B #2) and their corresponding scramble controls (Scramble #1 and Scramble #2). The shRNA knockdown efficiency was confirmed by nucleofection into cultured primary cortical neurons. Western blot analysis showed that both shRNAs significantly reduced the expression of endogenous coronin 2B (Fig. 2*A*,*B*). *In utero* electroporation of the shRNAs into the embryonic cortex at E14.5 caused obvious migration defects, with ~60% of coronin 2B-knockdown neurons having been stalled in the IZ and ~20% arrested in the VZ. Less than 20% of the coronin 2B-deficient neurons finally arrived at the CP at E17.5, while >40% of the control neurons successfully migrated into the CP, suggesting that the loss of coronin 2B led to migration defects in cortical projection neurons (Fig. 2*C*,*D*). At P2, when ~90% control neurons completed the migration process and stayed in layers II-IV (i.e., $Cux1^+$ layers), approximately half of the coronin 2B-deficient neurons remained in the white matter (i.e., former IZ), indicating that the migration defect observed in coronin 2B-knockdown neurons is not because of delayed neuronal migration (Fig. 2*E*–*G*). The effect of coronin 2B knockdown on neuronal identity was analyzed by immunofluorescence staining of Cux1 and Ctip2, which are

markers of neuronal identity in layers II-IV and V-VI, respectively. The arrested neurons in the shCoro2B groups were neither Cux1$^+$ nor Ctip2$^+$ at P2, whereas control neurons were positive for Cux1 in the upper layers (i.e., II-IV) of the cortex, indicating that coronin 2B-deficient neurons failed to develop into mature pyramidal neurons (Fig. 2*E–H*). These results suggest that coronin 2B is required for radial migration and subsequent neuronal maturation during cortical development.

Coronin 2B knockdown increases PTZ-induced seizure susceptibility

In coronin 2B-knockdown brains, the GFP$^+$ neurons that accumulated in the IZ were positive for NeuN but failed to achieve the typical morphology of pyramidal neurons (Fig. 3*A*, *B*). The few coronin 2B-knockdown neurons that migrated to layers II-IV of CP exhibited significantly reduced dendritic complexity as revealed by a decreased length and number of dendrites (Fig. 3*C–E*). To determine whether the observed defects are associated with functional abnormalities, we examined the seizure susceptibility of young adult mice at P30 or P31 by PTZ injection. Minimal motor seizures with the occurrence of abnormal facial and neck jerks were observed after 2 or 3 PTZ injections for almost all coronin 2B-knockdown and control mice. In addition, most mice exhibited other seizure behaviors on PTZ injection, ranging from behavioral arrest to clonic and tonic seizures. However, compared with control mice, coronin 2B-knockdown mice exhibited tonic–clonic seizures with a significantly lower dose of PTZ (Fig. 3*F*). In addition, the time to the induction of tonic–clonic seizures was significantly shortened after coronin 2B knockdown (Fig. 3*G*). These results suggest that coronin 2B-knockdown mice are more susceptible to PTZ-induced seizures. Notably, the interval time between minimal and tonic–clonic seizures was shorter in coronin 2B-knockdown mice than the controls (Fig. 3*H*), indicating much more severe seizure phenotypes in these mice. Seizures are often caused by an imbalance of excitatory and inhibitory activity in the brain (Ziburkus et al., 2013). Therefore, we performed c-Fos staining to examine the neuronal activity in the CP, which revealed that the number of c-Fos$^+$ neurons was significantly greater in the coronin 2B-knockdown mice, suggesting aberrant neuronal activity in the presence of coronin 2B-knockdown neurons (Fig. 3*I*,*J*).

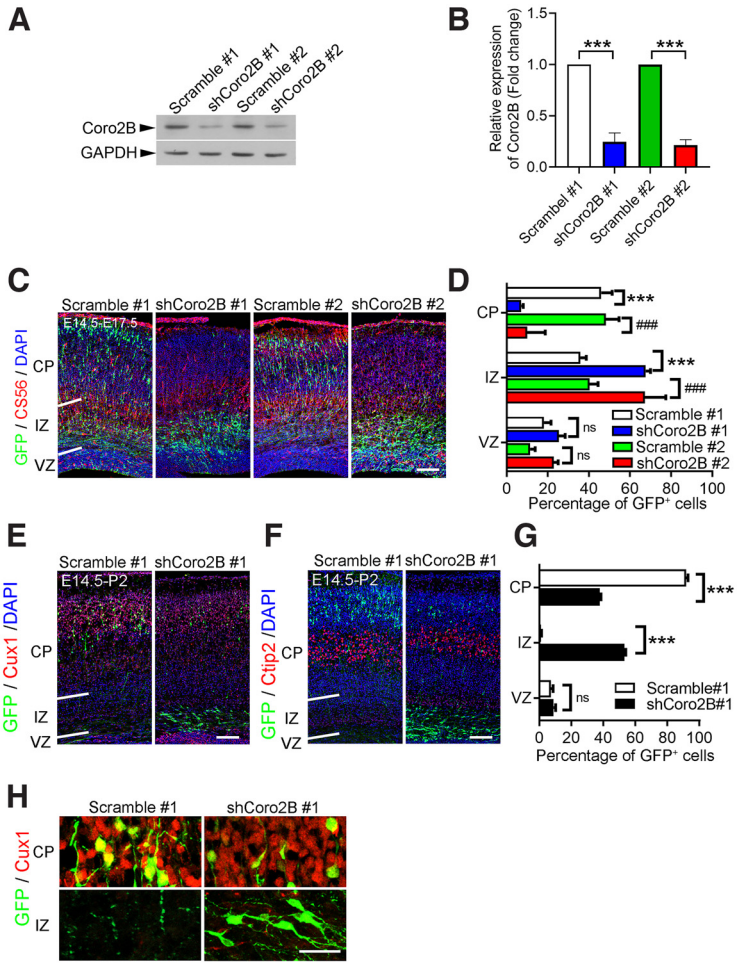

Figure 2. Coronin 2B is essential for neuronal migration. ***A***, ***B***, The knockdown efficiency of shRNAs against coronin 2B (shCoro2B #1 and #2) was validated by Western blotting of cultural cortical neurons (4 independent experiments). $^{***}p < 0.001$ (Student's *t* test). ***C***, Coronin 2B knockdown impaired neuronal migration in the embryonic stage (embryonic day 14.5 [E14.5] to E17.5). Scale bar, 100 μm. ***D***, Quantification of the distribution of GFP$^+$ neurons in ***C*** (3 independent experiments). $^{***}p < 0.001$ versus Scramble #1 (one-way ANOVA). $^{\#\#\#}p < 0.001$ versus Scramble #2 (one-way ANOVA). More than 500 GFP$^+$ neurons were analyzed in each group. ***E***, ***F***, Coronin 2B knockdown resulted in neurons arrested in the IZ during the postnatal stage (E14.5 to postnatal day 2 [P2]). Scale bar, 100 μm. ***G***, Quantification of the distribution of GFP$^+$ neurons in ***E*** (3 independent experiments). $^{***}p < 0.001$ versus Scramble #1 (one-way ANOVA). More than 400 GFP$^+$ neurons were analyzed in each group. ***H***, Enlarged images represent that coronin 2B-knockdown neurons in the IZ were Cux1$^-$. Scale bar, 20 μm. Data are mean ± SEM.

Coronin 2B is essential for the multipolar–bipolar transition

Given that multipolar–bipolar transition is the fundamental biological event during neuronal migration and is highly dependent on actin dynamics (Cooper, 2014), we subsequently examined whether coronin 2B is required to shape neuronal polarity during the multipolar–bipolar transition. *In utero* electroporation was performed to knock down coronin 2B at E14.5, and brains were collected at E17.5. The percentage of bipolar neurons among the total GFP$^+$ cells in the IZ was analyzed. In the coronin 2B-knockdown group, ~80% of neurons maintained a multipolar morphology, whereas >50% neurons in the control groups had acquired a bipolar morphology with an obvious leading process toward the pial surface (Fig. 4*A*,*B*), suggesting that coronin 2B is essential for the multipolar–bipolar transition during neuronal migration.

During neuronal migration, the Golgi apparatus translocate toward the leading process to direct the neuronal polarity and subsequent nucleokinesis (Yanagida et al., 2012). To further understand the roles of coronin 2B in the establishment of neuronal polarity, we visualized the Golgi apparatus by immunostaining with anti-GM130 antibody, a *cis*-Golgi matrix protein (Nakamura et al., 1995). In control neurons in the IZ, the Golgi apparatus was localized near the leading process, and its orientation was altered after coronin 2B knockdown (Fig. 4*C*, *D*). Quantitative analysis showed that the percentage of migrating neurons with the Golgi apparatus facing the pial surface was significantly lower in the coronin 2B-knockdown groups than in the control group (Fig. 4*E*). These results

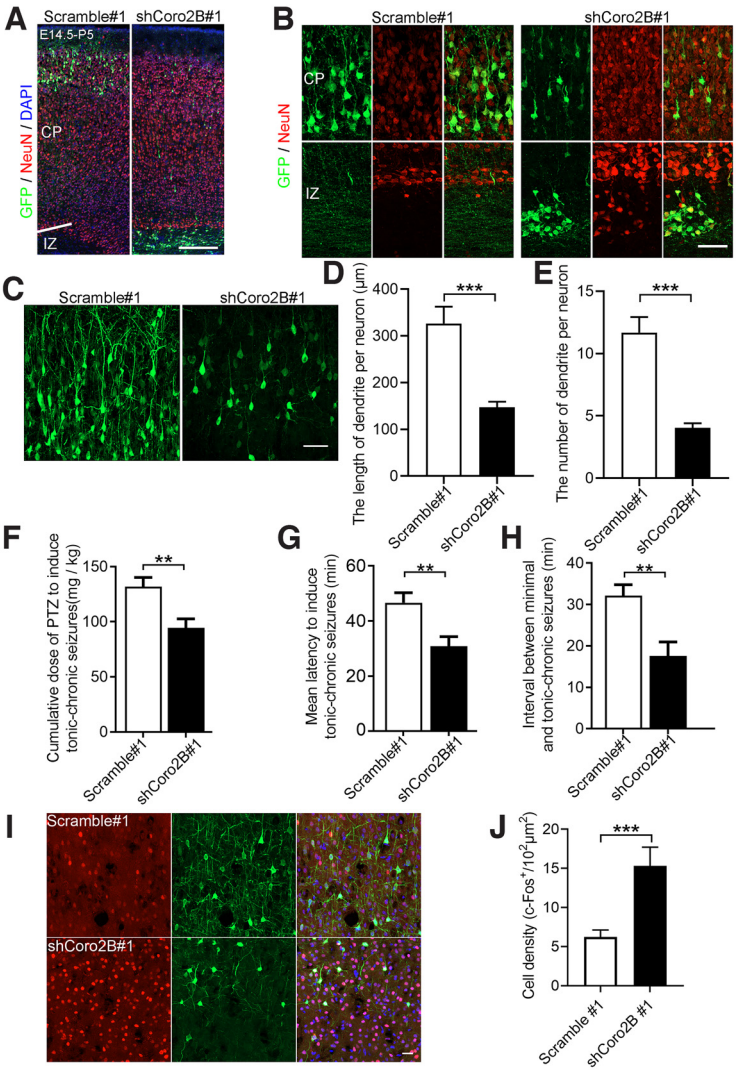

Figure 3. Coronin 2B knockdown in mice increases PTZ-induced seizure susceptibility. ***A***, Coronin 2B knockdown led to ectopic accumulation of neurons in the white matter at postnatal day 5 (P5). Scale bar, 200 μm. ***B***, Enlarged images represent that coronin 2B-knockdown neurons in the IZ were NeuN^{+}. Scale bar, 50 μm. ***C***, Dendritic morphology of transfected neurons in the CP. Scale bar, 50 μm. ***D***, ***E***, Quantification of dendrite length and number ($n = 30$; 3 independent experiments). ***$p < 0.001$. ***F–H***, PTZ-induced seizure behaviors were recorded using Racine's scale. Cumulative PTZ doses (***F***), the mean latency to induce generalized tonic–clonic seizures (***G***), and the interval between minimal and generalized seizures (***H***) were measured (3 independent experiments; at least 9 mice were analyzed in each group). **$p < 0.01$ (Student's t test). ***I***, Increase of c-Fos^{+} neurons in coronin 2B-knockdown cortices. Scale bar, 20 μm. ***J***, Quantification of the number of c-Fos^{+} neurons per 100 μm^2 of filed in the CP region containing the transfected cells (labeled by GFP signal; $n = 6$-12; from 3 independent experiments). ***$p < 0.001$ (Student's t test).

indicate that coronin 2B knockdown impairs neuronal polarity and migration direction.

Coronin 2B knockdown disrupts F-actin and active Rac1 distribution at the leading process

The formation and stabilization of the leading processes of migrating neurons rely on the precise regulation of actin dynamics and are essential for the establishment of neuronal polarity (Evsyukova et al., 2013; Lian and Sheen, 2015; Dogterom and Koenderink, 2019). Therefore, we investigated whether actin cytoskeleton remodeling is disrupted in coronin 2B-knockdown neurons. To visualize the F-actin cytoskeleton, lifeact-GFP was transfected into embryos at E14.5, and the embryos were examined at E17.5. The subcellular distribution of actin in migrating neurons was evaluated by GFP signals. The percentage of fluorescence signals in each quadrant (indicated by dashed lines; quadrant I points to the pial surface; Fig. 5*A–C*) was calculated for cells in the IZ. In control neurons, there was an obvious trend of the GFP signal toward quadrant I (~60%), which is concordant with the roles of actin in dilation orientation and force generation in leading processes. However, coronin 2B knockdown resulted in the loss of the trend of migrating neurons toward quadrant I in the IZ (Fig. 5*A–C*). Although most coronin 2B-knockdown neurons had a multipolar morphology, a few of them acquired a bipolar morphology with a leading process and no specific direction (Fig. 5*B*). In these neurons, actin accumulated in the cell body rather than being localized to the leading process, indicating a failure of proper actin distribution in the leading process. These findings collectively suggest that coronin 2B knockdown interferes with the precise regulation of actin dynamics and the subsequent formation of the leading process.

Rac1 is one of the major signaling molecules that promote actin assembly via the inhibition of the activity of cofilin, an F-actin–severing protein (Yang et al., 1998). Active Rac1 accumulates at the leading process and determines neuronal polarity (Tahirovic et al., 2010). Considering the presence of the Cdc42/Rac-interactive binding motif in coronins, we subsequently examined the distribution of active Rac1 in coronin 2B-deficient neurons during neuronal migration using PBD-EYFP to visualize active Rac1. In control neurons, active Rac1 distribution exhibited a similar trend toward quadrant I, whereas coronin 2B knockdown significantly reduced the signal of active Rac1 in quadrant I, indicating aberrant Rac1 distribution and leading process impairment (Fig. 5*D–F*). The neurons were also cotransfected with lifeact-mCherry, which recognizes F-actin in live cells, together with PBD-EYFP by *in utero* electroporation. In control neurons, the signals for lifeact-mCherry and PBD were highly colocalized and restricted to leading processes. However, in coronin 2B-knockdown neurons, PBD signal was ectopically aggregated in the cell body and not enriched in lifeact-mCherry-labeled F-actin cytoskeleton (Fig. 5*G*). This indicates that coronin 2B knockdown disrupts the localization of active Rac1 on F-actin cytoskeleton in leading processes.

Rac1 overexpression partially restores the defective migration of coronin 2B-knockdown neurons

We subsequently investigated whether Rac1 is downstream of coronin 2B in the signaling cascade that regulates neuronal migration. Overexpression of WT Rac1 in coronin 2B-knockdown neurons partially rescued the migration defect, with few neurons being stalled in the IZ and more neurons in the CP (Fig. 6*A,B*). Although most coronin 2B-knockdown neurons in the IZ maintained a multipolar morphology, Rac1 overexpression significantly increased the proportion of bipolar

neurons (Fig. 6*C*,*D*). However, overexpression of the constitutively active form of Rac1 in coronin 2B-knockdown neurons produced no rescue effect (Fig. 6*A*–*D*), suggesting that Rac1 activity is precisely controlled to establish neuronal polarity.

Cofilin, a critical downstream molecule of Rac1 signaling, acts as an actin-severing factor to regulate actin dynamics and is implicated in neuronal migration (Bellenchi et al., 2007; Frotscher et al., 2017). Interestingly, overexpression of the dominant-negative form of cofilin (i.e., cofilin-S3D) rather than the constitutively active form (i.e., cofilin-S3A) or WT form partially rescued the migration defect caused by coronin 2B knockdown (Fig. 6*E*–*H*). Overexpression of cofilin-S3D in coronin 2B-knockdown neurons reduced the proportion of neurons arrested in the IZ (Fig. 6*E*,*F*) with a concomitantly greater percentage of bipolar cells in the IZ (Fig. 6*G*,*H*). Together, our results collectively suggest that coronin 2B regulates neuronal migration by maintaining proper F-actin cytoskeleton dynamics and active Rac1 signaling at the leading process.

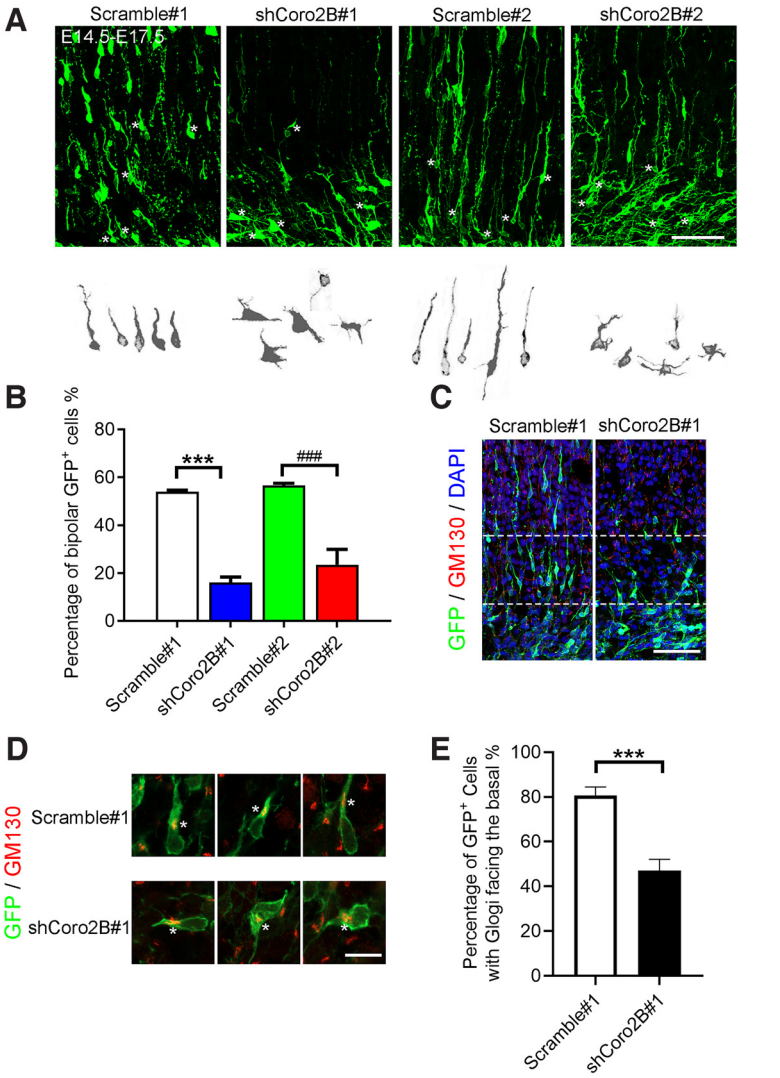

Figure 4. Coronin 2B knockdown abolishes the neuronal multipolar–bipolar transition. ***A***, Morphologic defects of migrating neurons in the IZ after coronin 2B knockdown. Bottom, Drawings of representative neurons denoted by asterisks. Scale bar, 50 μm. ***B***, Quantification of the percentages of neurons with a multipolar or bipolar morphology in the IZ in ***A*** (3 independent experiments). $^{***}p < 0.001$ versus Scramble #1 (one-way ANOVA). $^{\#\#\#}p < 0.001$ versus Scramble #2 (one-way ANOVA). More than 200 GFP^+ neurons in the IZ were analyzed in each group. ***C–E***, Coronin 2B knockdown impaired neuronal polarization in the IZ. ***C***, Staining of the Golgi apparatuses of migrating neurons. Scale bar, 50 μm. ***D***, Direction of the Golgi apparatuses in migrating neurons located in upper the IZ (between the 2 white lines in ***C***). Scale bar, 10 μm. ***E***, Quantification of the proportion of cells with their Golgi apparatus facing the pial surface (3 independent experiments). $^{***}p < 0.001$ (Student's *t* test). More than 150 GFP^+ neurons in the upper IZ were analyzed in each group. Data are mean ± SEM.

Discussion

The precise molecular mechanisms underlying the control of actin dynamics in neurons undergoing dramatic morphologic changes during radial migration remain unclear. In the present work, we found that coronin 2B, a member of coronin family that is abundantly expressed in the brain, is essential for the multipolar–bipolar transition in migrating neurons through the regulation of F-actin and Rac1 at the leading process, thereby directing radial migration. In addition, coronin 2B-knockdown neurons were stalled in the IZ, and the malfunction of cortical development in coronin 2B-knockdown animals increased susceptibility to PTZ-induced seizure. These findings collectively suggest that coronin 2B is a key cytoskeleton regulator that controls the morphologic transition for the radial migration of projection neurons.

During cortical development, the actin cytoskeleton attains polarity to promote the radial migration of neurons toward the pial surface (Evsyukova et al., 2013). This is consistent with our observations in the upper IZ, where the actin cytoskeleton was concentrated at the leading process and exhibited a polar distribution toward the pial surface. The precise spatiotemporal regulation of actin distribution highly depends on the dynamic actin assembly and disassembly; it not only provides contractile force, but also coordinates Golgi apparatus/centrosomes movement and nucleokinesis for neuronal migration (Solecki et al., 2009; Yanagida et al., 2012; Minegishi and Inagaki, 2020; Nakazawa and Kengaku, 2020). Actin instability caused by mutations of *Acta1*, *Actb*, and other genes that encode actin-associated proteins results in significantly impaired neuronal polarization and migration (Evsyukova et al., 2013). In present work, we observed that coronin 2B is expressed in different layers of the developing cortex. In particular, coronin 2B knockdown predominantly disturbed the actin cytoskeleton at the leading process and not the rear of the cell body, which subsequently developed into axons. This is corroborated by our previous observation that in primary cultured neurons, coronin 2B is concentrated in developing dendrites but not axons (Chen et al., 2020). In the present work, we also found that, although a small proportion of coronin 2B-deficient neurons still migrated into the CP region, they were unable to develop into functional neurons with mature dendritic arborization. One explanation is that the knockdown efficiency varied among individual neurons; therefore, a few neurons with low coronin 2B knockdown efficiency were still able to leave the IZ and finish the migration process but were unable to develop into normal functioning neurons in the CP. Alternatively, cortical

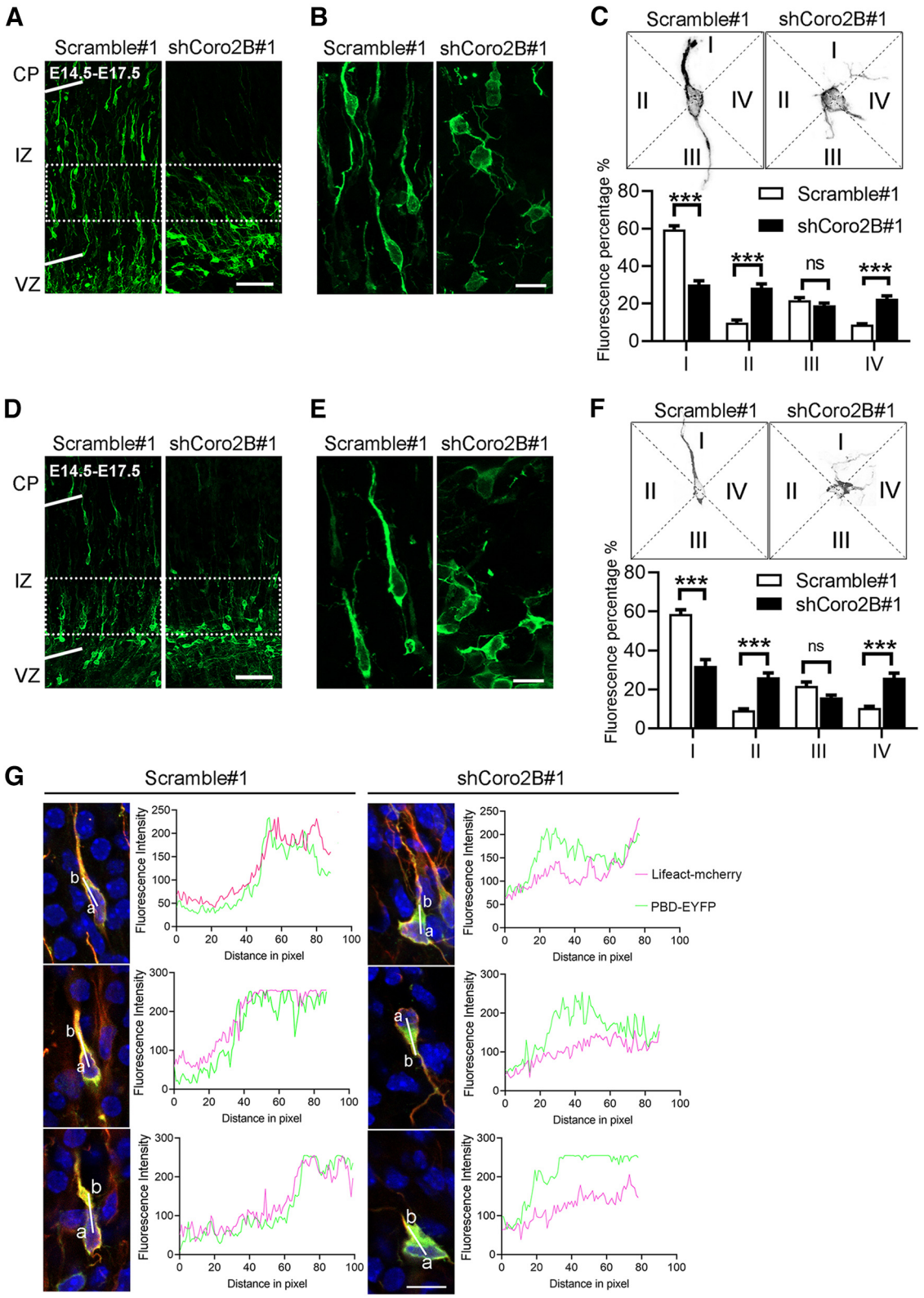

Figure 5. Coronin 2B knockdown impairs actin cytoskeleton distribution in the leading processes of migrating neurons. ***A***, The actin cytoskeleton in the developing cortex was labeled with lifeact-GFP. Scale bar, 50 μm. ***B***, Enlarged images represent the actin cytoskeleton in lifeact-GFP labeled neurons in the upper IZ (between the white lines indicated in ***A***). Scale bar, 20 μm. ***C***, Quantification of the cumulative fluorescence percentage in each quadrant (dashed lines; 3 independent experiments). ***$p < 0.001$ (one-way ANOVA). More than 30 lifeact-labeled cells were analyzed in each group. ***D***, Active Rac1 in the developing cortex was labeled with PBD-EYFP. Scale bar, 50 μm. ***E***, Enlarged images represent active Rac1 distribution in PBD-labeled neurons in IZ (between the white lines in ***D***). Scale bar, 20 μm. ***F***, Quantification of the cumulative fluorescence percentage in each quadrant (dashed lines; 3 independent experiments). ***$p < 0.001$ (one-way ANOVA). More than 25 PBD-labeled cells were analyzed in each group. ***G***, Disruption of active Rac1 localization at the leading process in coronin 2B-knockdown neurons. PBD-EYFP and lifeact-mCherry were used for visualizing active Rac1 and F-actin cytoskeleton, respectively. Quantification of the fluorescence intensity of PBD-EYFP (green) and lifeact-mCherry (red) from center of neuron (a) to leading process (b). More than 10 cells from each condition were quantified and 3 representative cells in each condition together with the quantification of fluorescence signal were presented. Scale bar, 10 μm.

progenitors and their postmitotic neurons are highly molecularly heterogeneous in terms of layer identity and expression of specific proteins (Soriano et al., 1995; Ferreira et al., 2022); therefore, it is also possible that coronin 2B-knockdown neurons are heterogeneous and behave differently during the migration process (e.g., being able to migrate into the CP), although we did not observe any obvious differences in coronin 2B expression between the Cux1-positive and Ctip2-positive neurons.

F-action assembly and disassembly in neurons occur in response to both intrinsic and extracellular signaling. Among them, Rac1, a member of the small Rho GTPase family, is considered one of the most prominent signals for F-actin assembly (Chung et al., 2000), as activated Rac1 promotes local actin polymerization (Faix and Weber, 2013). Thus, the distribution of active Rac1 at neurite tips facilitates F-actin assembly and then promotes neurite outgrowth. Active Rac1 is enriched at the leading processes of neurons (Yang et al., 2012), and the roles of Rac1 in radial migration have been investigated (Kawauchi et al., 2003; Konno et al., 2005; Yang et al., 2012). Importantly, balanced Rac1 activity is essential for the critical multipolar–bipolar transition during neuronal migration; overexpression of either the constitutively active or dominant-negative form of Rac1 leads to ectopic accumulation of multipolar neurons in the IZ (Kawauchi et al., 2003; Konno et al., 2005). Therefore, it is critical to identify the factors that regulate the activity of Rac1 at the leading process to understand the molecular mechanisms underlying neuronal migration. In this study, we characterized the role of coronin 2B in the regulation of the distribution of active Rac1 in migrating neurons. The finding that the overexpression of WT Rac1 and not its constitutively active form alleviates the defect in the multipolar–bipolar transition in coronin 2B-knockdown neurons suggests that this process requires the precise regulation and balancing of Rac1. Coronin 2B may help coordinate the activity and distribution of Rac1 in the leading process to generate the contractile force and determine the direction of neuronal migration.

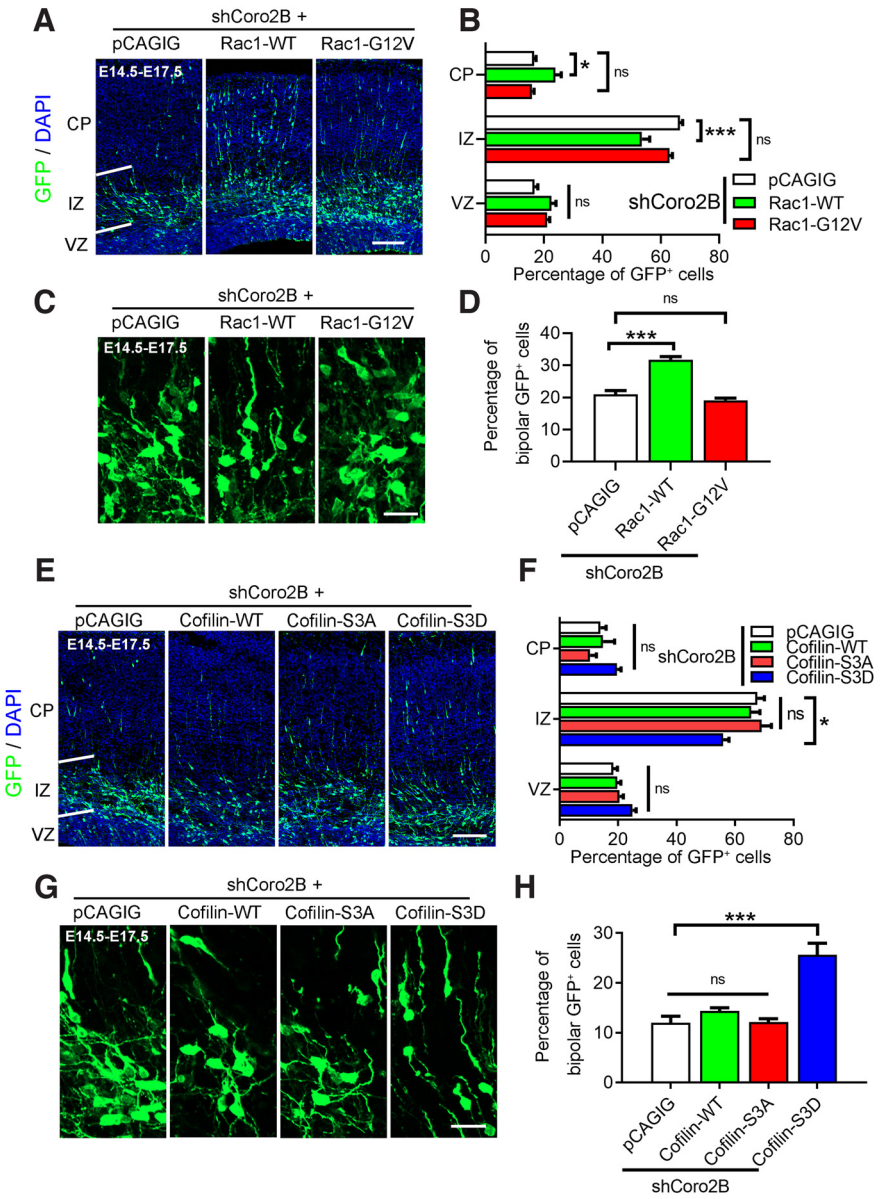

Figure 6. Coronin 2B regulates neuronal migration via the Rac1/cofilin pathway. ***A***, The defective migration caused by coronin 2B knockdown was partially rescued by Rac1 overexpression. Scale bar, 100 μm. ***B***, Quantification of the distribution of GFP$^+$ neurons in ***A*** (3 independent experiments). *$p < 0.05$ (one-way ANOVA). ***$p < 0.001$ (one-way ANOVA). More than 1000 GFP$^+$ neurons were analyzed in each group. ***C***, Enlarged images represent the neuronal morphology in the IZ. Scale bar, 20 μm. ***D***, Quantification of the percentage of bipolar neurons in the IZ (6 independent experiments). ***$p<0.001$ (one-way ANOVA). More than 500 GFP$^+$ neurons from were analyzed in each group. ***E***, Overexpression of the dominant-negative form of cofilin (cofilin-S3D) partially rescued the migration defect caused by coronin 2B knockdown. Scale bar, 100 μm. ***F***, Quantification of the distribution of GFP$^+$ neurons in ***E*** (3 independent experiments). *$p < 0.05$ (one-way ANOVA). More than 300 GFP$^+$ neurons were analyzed in each group. ***G***, Enlarged images represent the neuronal morphology in the IZ. Scale bar, 20 μm. ***H***, Quantification of the bipolar neurons of GFP$^+$ neurons in the IZ in ***G*** (3 independent experiments). ***$p < 0.001$ (one-way ANOVA). More than 130 GFP$^+$ neurons in the IZ were analyzed in each group. Data are mean ± SEM.

Cofilin, an actin-severing protein, plays an essential role in neuronal migration, and its activity is tightly controlled to ensure the appropriate balance between F-actin assembly and disassembly (Bellenchi et al., 2007; Galkin et al., 2011). Destabilization of the actin cytoskeleton caused by cofilin hyperactivation leads to neuronal migration defects. For example, loss of reelin, a strong regulator of neuronal migration, causes cofilin overactivation and actin cytoskeleton destabilization (Frotscher et al., 2017). Given that overexpression of the dominant-negative form of cofilin (i.e., cofilin-S3D) rather than the WT or constitutively active form (i.e., cofilin-S3A) partially rescued the migration defect caused by coronin 2B knockdown, coronin 2B likely promotes actin cytoskeleton stabilization through the inhibition of cofilin activity at the leading process. Of note, the upstream signaling molecules that regulate cofilin activity, such as its protein kinase LIMK and Rac1/Cdc42, are also key regulators of

neuronal migration (Konno et al., 2005; Xie et al., 2017). Therefore, coronin 2B might directly or indirectly regulate cofilin activity by modulating its upstream signaling molecules.

Newborn postmitotic neurons and intermediate progenitor neurons acquire a bipolar or pin-like morphology and exit the VZ. In the subventricular zone (SVZ) and IZ, neurons, which have a multipolar morphology, extend their neurites to explore the new environment. Unlike the niches in the VZ, the SVZ/IZ contains much more informative cues to guide neuronal migration toward the pia. At this stage, in response to environmental cues, multipolar neurons will undergo dramatic cytoskeleton reorganization to finish the multipolar–bipolar transition before moving out of the SVZ/IZ to the CP (Bressan and Saghatelyan, 2020). Herein, we provide critical evidence that coronin 2B is essential for the multipolar–bipolar transition at this stage, during which it regulates the molecular machinery to control the navigation of the leading process to explore environmental cues and determine the direction for neuronal migration. Nevertheless, whether coronin 2B also plays a critical role in the VZ for newborn neurons to migrate out of the VZ awaits further investigation. Furthermore, in this study, we observed increased seizure susceptibility in coronin 2B-knockdown animals, which indicates excitatory/inhibitory imbalance in the cortex. This may not necessarily be generated directly by the ectopic neurons in the white matter of the coronin 2B-knockdown brain. Previous studies from us and another group showed that heterotopic neurons accumulated in the white matter region do not enhance sEPSC frequency and amplitude (Ip et al., 2011; Petit et al., 2014), whereas synchronous bursts of sEPSCs were observed in the layer II-III of the brains with migration defects, suggesting that disruption of excitatory/inhibitory balance in the CP may increase the susceptibility to PTZ-induced seizure. Interestingly, we observed some coronin 2B-knockdown neurons distributed in the CP with reduced dendritic complexity, accompanied by increased c-Fos activation in this area. Therefore, we hypothesize that these neurons might disrupt the excitation/inhibition balance in the brain and cause an epileptic phenotype. Nevertheless, further study is required to decipher the underlying circuit and mechanisms.

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