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STEM CELLS AND REGENERATION

RESEARCH ARTICLE

DISC1 regulates astrogenesis in the embryonic brain via modulation of RAS/MEK/ERK signaling through RASSF7

Shukun Wang[1,2,*], Qingli Liang[1,3,*], Huimin Qiao[1,3], Hong Li[1], Tianjin Shen[1,3], Fen Ji[1] and Jianwei Jiao[1,‡]

ABSTRACT

Disrupted in schizophrenia 1 (*DISC1*) is known as a high susceptibility gene for schizophrenia. Recent studies have indicated that schizophrenia might be caused by glia defects and dysfunction. However, there is no direct evidence of a link between the schizophrenia gene *DISC1* and gliogenesis defects. Thus, an investigation into the involvement of DISC1 (a ubiquitously expressed brain protein) in astrogenesis during the late stage of mouse embryonic brain development is warranted. Here, we show that suppression of DISC1 expression represses astrogenesis *in vitro* and *in vivo*, and that DISC1 overexpression substantially enhances the process. Furthermore, mouse and human DISC1 overexpression rescued the astrogenesis defects caused by DISC1 knockdown. Mechanistically, DISC1 activates the RAS/MEK/ERK signaling pathway via direct association with RASSF7. Also, the pERK complex undergoes nuclear translocation and influences the expression of genes related to astrogenesis. In summary, our results demonstrate that DISC1 regulates astrogenesis by modulating RAS/MEK/ERK signaling via RASSF7 and provide a framework for understanding how DISC1 dysfunction might lead to neuropsychiatric diseases.

KEY WORDS: DISC1, Astrogenesis, RAS/MEK/ERK signaling, MAP kinase, RASSF7, Cortical development

INTRODUCTION

Disrupted in schizophrenia 1 (*DISC1*) was originally identified in a chromosome translocation (1; 11) (q42.1; q14.3) in a Scottish family with a high susceptibility to schizophrenia and several other psychiatric disorders (Hayashi-Takagi et al., 2010; Millar et al., 2000). It is a crucial element of the microtubule-associated dynein motor complex and is important for maintaining normal microtubular dynamics (Kamiya et al., 2005). Induction of mutant forms of DISC1 in mouse brains led to the manifestation of schizophrenia-like behavioral phenotypes (Li et al., 2007; Pletnikov et al., 2008). Clapcote and colleagues generated two transgenic mouse lines with N-ethyl-N-nitrosourea-induced mutations of DISC1. The Q31L mutant mouse exhibited depressive-like behavior whereas the L100P mutant mouse showed schizophrenic-like behavior (Clapcote et al., 2007). Kuroda et al. showed that *Disc1* mutant mice lacking exons 2 and 3 displayed abnormal behaviors with lower anxiety and higher impulsivity (Kuroda et al., 2011).

Previous studies have revealed that DISC1 is important in embryonic and adult brain development and is involved in the processes of neurogenesis, neuronal migration and axon formation. Kamiya et al. discovered that DISC1 depletion impairs neurite outgrowth (Kamiya et al., 2005). Enomoto et al. reported that DISC1 regulates axonal development by interacting with the protein girdin (CCDC88A; also known as KIAA1212) (Enomoto et al., 2009). Mao et al. provided evidence that DISC1 deficiency decreases the progenitor pool and results in premature neuronal differentiation by regulating GSK3β/β-catenin signaling. DISC1 dysfunction in the dentate gyrus of adult brain suppresses neural progenitor proliferation and causes hyperactive and depressive behaviors (Mao et al., 2009). Kim et al. showed that DISC1 modulates AKT/mTOR signaling, thereby regulating neuronal development (Kim et al., 2009). Most research into DISC1 has been focused on the effects on neurons, but few studies have investigated the role of DISC1 in astrocytes.

Astrocytes, the most abundant type of glial cells in the nervous system, impact the function of surrounding neurons in various ways (López-Hidalgo and Schummers, 2014). During forebrain development, neural stem cells generate neurons first, followed by astrocytes, and finally oligodendrocytes. This temporal sequence of neocortex development is tightly regulated by both intrinsic programs and extracellular signals (Brambilla et al., 2013). Astrocytes originate from progenitor cells in the subventricular zone (SVZ) during late embryonic development and reach a peak level in early postnatal life (Sauvageot, 2002). Glial progenitors, derived from common progenitors, can differentiate into astrocytes (Huse and Holland, 2010).

Astrocytes physically interact with neurons, offer metabolic support, buffer extracellular ions, and modulate information processing and signal transmission by releasing trophic factors and neurotransmitters (Annunziato et al., 2013; Barros, 2013; López-Hidalgo and Schummers, 2014). It has been shown that dysfunctional astrocytes affect neighboring neurons, resulting in neurodegeneration and brain disease (Avila-Muñoz and Arias, 2014). Moreover, it has been reported that in schizophrenic patients a disruption of astrocytes was observed that was associated with a vast increase of extracellular matrix proteins in the brain (Pantazopoulos et al., 2010), and several depressive mouse models show defects in glial cell density (Molofsky et al., 2012). Therefore, we hypothesize that DISC1 dysfunction might affect astrogenesis, resulting in brain defects. Therefore, it is important to understand the molecular mechanism by which DISC1 regulates astrogenesis.

DISC1 is reported to act as a scaffold protein and interact with various partner proteins. In neurogenesis, KIAA1212 has been shown to associate with both C-terminal (CT) and middle domain

[1]The State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. [2]The State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China. [3]University of Chinese Academy of Sciences, Beijing 100049, China.
*These authors contributed equally to this work

‡Author for correspondence (jwjiao@ioz.ac.cn)

J.J., 0000-0002-7893-0721

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(MD) domains of DISC1 when DISC1 regulates new neuron development in the adult brain. In addition, LIS1 (PAFAH1B1), NDE1, NDEL1 (Brandon et al., 2004; Burdick et al., 2008), PDE4B (Fatemi et al., 2008; Pickard et al., 2007) and DIXDC1 (Singh et al., 2010) have been previously reported to interact physically with DISC1. However, the protein involved in the function of DISC1 on astrogenesis remains elusive.

In this study, we focus on the regulation of astrogenesis by DISC1 during the late stages of embryonic brain development and the underlying molecular mechanisms. Using *in utero* electroporation and *in vitro* approaches with DISC1 shRNA or expression plasmids, we found that DISC1 regulates astrogenesis in the embryo brain: DISC1 depletion suppresses astrogenesis and DISC1 overexpression promotes the process. Moreover, human and mouse DISC1 overexpression rescued the astrogenesis defects caused by mouse DISC1 knockdown. A series of experiments demonstrated that DISC1 phosphorylates MEK and ERK downstream of RAS and then upregulates the expression of astrocyte-related genes. Furthermore, we identified RASSF7 as the molecule directly interacting with DISC1: interaction studies revealed that the CT domain of DISC1 was directly associated with the CT domain of RASSF7, a RAS association protein. In summary, these results indicate that DISC1 is required for astrogenesis in the embryonic brain and improve our understanding of the pathophysiology of brain diseases.

RESULTS

DISC1 expression in brain development

To test whether DISC1 regulates astrogenesis at the late stage of embryonic development, we first examined the correlation of DISC1 expression and astrogenesis in brain. Previous studies indicate that DISC1 is crucial to embryogenesis and organ development (Pickard et al., 2007; Singh et al., 2010). We analyzed DISC1 expression in different tissues and different stages of brain development by western blot analysis. The results showed that DISC1 expression was much higher in the brain than in other tissues (Fig. 1A). DISC1 expression increased from embryonic day (E) 12 to postnatal day (P) 0, and was maintained at a high level postnatally. ACSBG1 (related to astrocyte development) and GFAP (astrocyte marker) expression also increased during this period (E12-P0) (Fig. 1B; Fig. S1A-C). To determine DISC1 expression in cell culture, embryonic neural precursor cells (NPCs) were isolated and cultured in differentiation medium for 0, 1 and 3 days. Western blotting showed that the expression of ACSBG1 and GFAP increased along with the increasing expression of DISC1 (Fig. 1C). Immunostaining revealed colocalization of DISC1 and ACSBG1 and of DISC1 and GFAP in differentiated astrocytes (Fig. 1D,E). These results suggest that the initiation of astrogenesis might coincide with the expression of DISC1. In addition, *in vivo* immunostaining of brain slices at E16 showed that DISC1 was highly expressed and restricted to the ventricular zone (VZ)/SVZ (Fig. S1D). These data suggest that DISC1 might be broadly involved in brain development, including astrocyte differentiation.

DISC1 depletion represses astrogenesis *in vitro* and *in vivo*

To investigate further the regulation of astrogenesis by DISC1, a series of DISC1 knockdown experiments were performed *in vitro* and *in vivo*. First, four shRNAs were constructed into a lentiviral vector and the knockdown efficiencies were examined (Fig. S2A). The results showed that shRNA2 and shRNA3 had high knockdown efficiency. Subsequently, shRNA2, which had the highest knockdown efficiency, was used for the most of the knockdown experiments. We also confirmed that the DISC1 level decreased *in vivo* compared with the control after *in utero* electroporation (IUE) of DISC1 shRNA2 (Fig. S2B). Next, we studied the effect of DISC1 on astrocyte differentiation after E15 NPCs were infected with the DISC1 shRNA2 lentivirus. Western blotting and GFAP immunocytochemistry showed that DISC1 shRNA2 inhibited GFAP expression, and the number of NPCs that differentiated into astrocytes dramatically decreased without leukemia inhibitor factor (LIF) treatment (Fig. 2A,B; Fig. S2C). Because astrogenesis initiates and continues during late embryonic development, control or DISC1 shRNA2 constructs were electroporated into the lateral ventricle of embryos at E16 *in vivo*. Brains were then harvested at P2, sectioned and stained

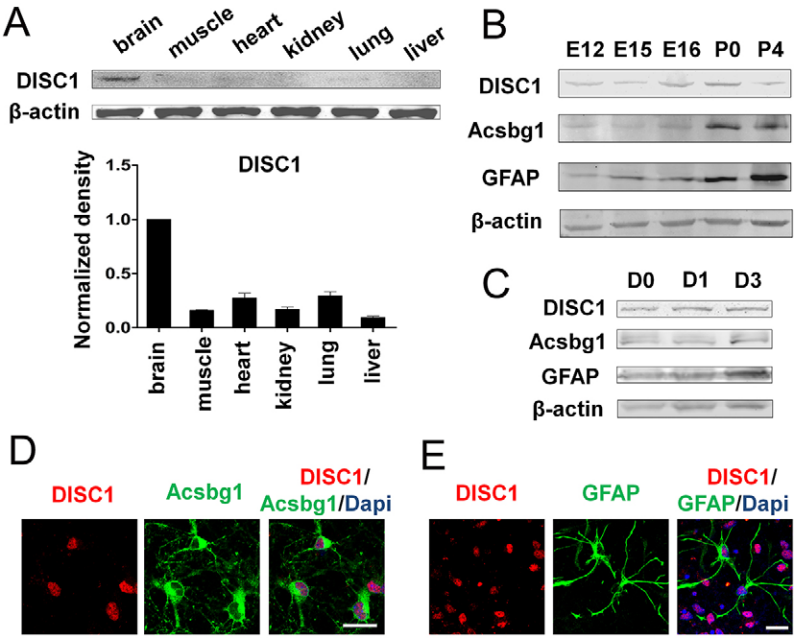

Fig. 1. DISC1 expression in brain development. (A) Western blotting analysis of DISC1 protein in brain, muscle and four other tissues. Quantification of DISC1 expression levels is shown below (normalized to β-actin). Error bars indicate s.e.m. (n=3). (B) Western blot analysis of the level of DISC1, ACSBG1 and GFAP proteins in the brain cortex from E12 to P4. (C) Embryonic neural precursor cells (NPCs) were isolated and cultured in differentiation medium for 0, 1 and 3 days (D0, D1, D3). Western blot analysis of the expression of DISC1, ACSBG1 and GFAP is shown. (D) Colocalization of DISC1 and ACSBG1 in differentiated astrocytes. (E) Colocalization of DISC1 and GFAP in differentiated astrocytes. Scale bars: 10 µm. See also Fig. S1.

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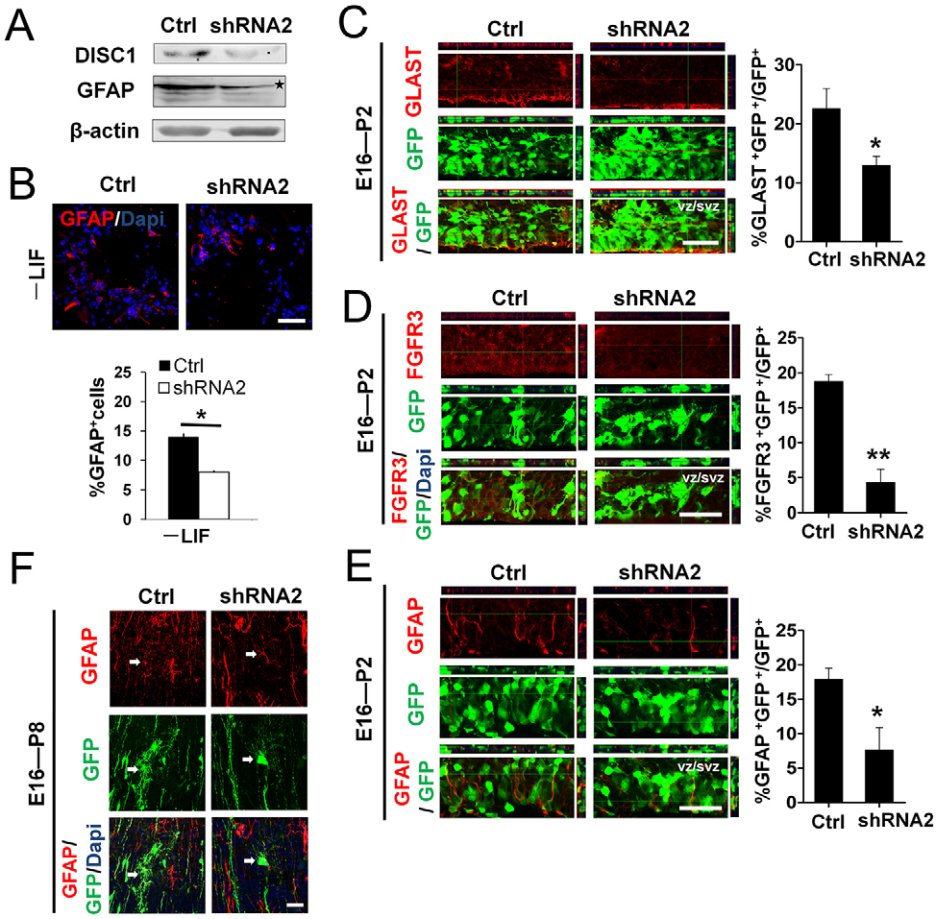

Fig. 2. DISC1 knockdown results in astrogenesis defects *in vitro* and *in vivo*. (A) DISC1 knockdown decreases the levels of DISC1 and GFAP in embryonic E15 NPCs infected with lentivirus and cultured in differentiation medium for 3 days. (B) DISC1 deficiency induces fewer astrocytes to differentiate from embryonic NPCs treated in glial cell differentiation medium without LIF. Top: staining for GFAP reveals astrocyte differentiation. Bottom: the percentage of GFAP-positive astrocytes was measured (*t*-test, **P*=0.047). Error bars indicate s.e.m. (*n*=4). (C) DISC1 knockdown decreases the number of glial progenitor cells. Left: GLAST immunostaining of P2 cortices electroporated with control or DISC1 knockdown plasmid at E16. Right: proportion of $GLAST^+GFP^+$ glial progenitor cells (*t*-test, **P*=0.040). Error bars indicate s.e.m. (*n*=4). (D) DISC1 knockdown reduces the number of FGFR3-labeled astrocyte progenitor cells. Confocal images of FGFR3 immunostaining are shown on the left, and the percentage of double-labeled $FGFR3^+GFP^+$ cells relative to GFP^+ cells is shown on the right (*t*-test, ***P*=0.009). Error bars indicate s.e.m. (*n*=3). (E) DISC1 knockdown decreases the number of GFAP-labeled astrocytes, as shown in the images of immunostaining. The graph shows percentage of $GFAP^+GFP^+$ astrocytes relative to GFP^+ cells (*t*-test, **P*=0.036). Error bars indicate s.e.m. (*n*=4). (F) Images of astrocyte morphology in P8 brains. The morphology of GFP-labeled mature astrocytes in the brains of mice electroporated with DISC1 shRNA2 plasmid at E16 was not typical compared with that of control brains. Arrows indicate $GFAP^+$ GFP^+ cells. Scale bars: 50 µm (B-E); 20 µm (F). See also Fig. S2.

for markers of different glial subtypes at different stages of astrogenesis. According to previous studies, common progenitor cells give rise to glial progenitor cells, astrocyte progenitor cells and astrocytes during astrogenesis. Here, we used GLAST (SLC1A3), FGFR3 and GFAP to label glial progenitor cells, astrocyte progenitor cells and astrocytes, respectively (Li et al., 2012; Molofsky et al., 2012; Pringle, 2003). The immunostaining results revealed that the numbers of GLAST-positive glial progenitor cells, FGFR3-labeled astrocyte progenitor cells and GFAP-labeled astrocytes were reduced by 42.4%, 67.8% and 57.5%, respectively, in response to DISC1 knockdown (Fig. 2C-E), and a concomitant decrease of astrocytes by DISC1 knockdown could be seen in postnatal mouse brains after electroporation at P0, at which time neurogenesis is almost finished (Fig. S2D). Moreover, rare morphologically mature astrocytes were observed in brains electroporated with DISC1 shRNA2 at the later stage (Fig. 2F). To exclude off-target effects, another plasmid (DISC1 shRNA3) was used to examine astrocyte differentiation *in vitro* and *in vivo*. Consistent with shRNA2, shRNA3 knockdown decreased GFAP expression and the number of GFAP-GFP-positive cells (Fig. S2E,F). Collectively, these data suggest that DISC1 is necessary for astrocyte differentiation.

DISC1 overexpression enhances astrogenesis

The astrogenesis defect caused by DISC1 knockdown prompted us to perform reciprocal DISC1 gain-of-function experiments. To analyze further the role of DISC1 during astrogenesis, we created a mouse DISC1 (mDISC1) overexpression plasmid to increase efficiently the DISC1 expression level *in vitro* (Fig. 3A; Fig. S3A); immunostaining of Flag showed that the Flag-mDISC1 had high overexpression efficiency (Fig. S3B). Moreover, the results obtained from the immunocytochemistry and western blot analyses showed that mDISC1 significantly promoted astrogenesis and increased the expression of GFAP *in vitro* (Fig. 3A,B). The amount of GFAP-positive cells was increased by 55.2% (Fig. 3B). Additionally, mDISC1 overexpression *in vivo* by electroporation increased the number of GLAST-positive radial progenitor cells, FGFR3-positive astrocyte precursor cells and GFAP-positive

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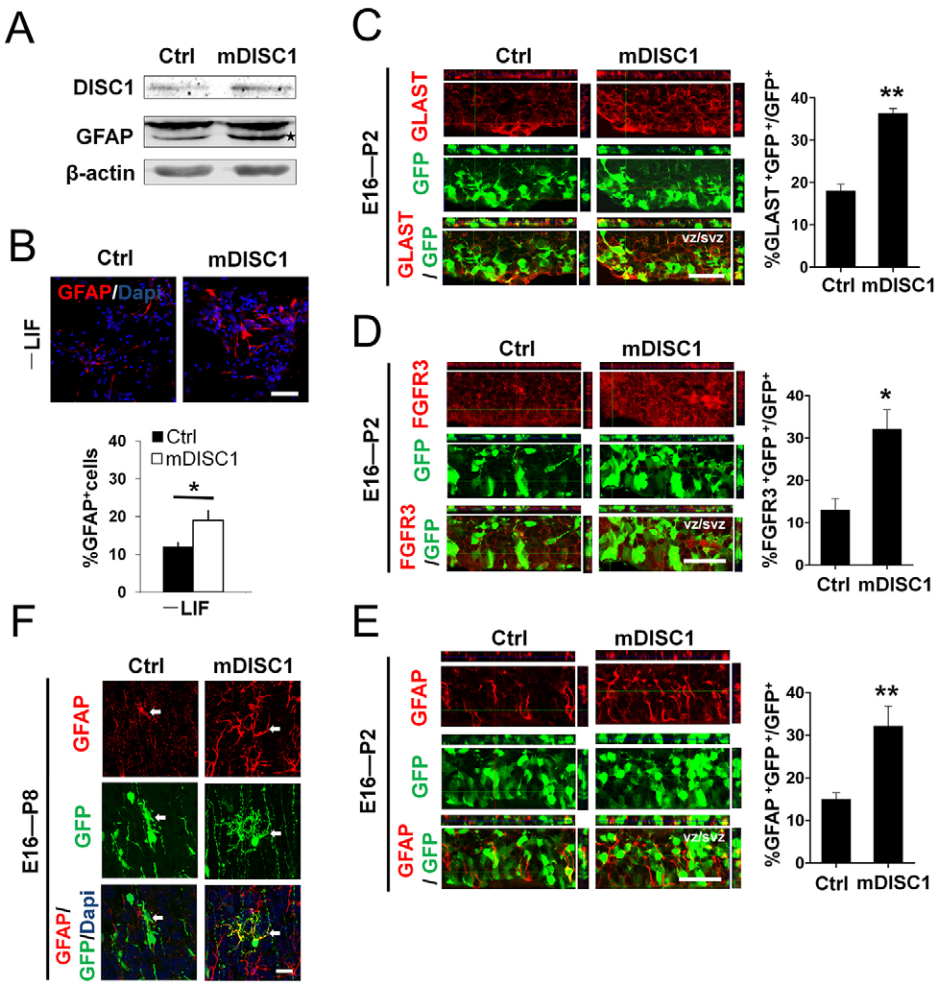

Fig. 3. DISC1 overexpression enhances astrogenesis. (A) E15 NPCs were infected with lentivirus and cultured in differentiation medium for 3 days. Western blotting showed increased GFAP level with DISC1 overexpression. β-actin was used as an internal control for immunoblotting. (B) DISC1 overexpression induces more astrocyte differentiation from E15 NPCs treated in differentiation medium without with LIF. Top: staining for GFAP reveals astrocyte differentiation. Bottom: the percentage of GFAP-positive astrocytes was measured (*t*-test, **P*=0.022). Error bars indicate s.e.m. (*n*=4). (C-E) DISC1 overexpression enhances astrogenesis *in vivo*. Embryonic brains were electroporated with control and DISC1 overexpression plasmids at E16 and analyzed at P2. Images in C-E show immunostaining for GLAST, FGFR3 and GFAP, respectively. The proportion of GFP^+ cells that are also $GLAST^+$ ($GLAST^+GFP^+$ glial progenitor cells; *t*-test, ***P*=0.006), $FGFR3^+$ ($FGFR3^+GFP^+$ cells; *t*-test, **P*=0.048) and $GFAP^+$ ($GFAP^+GFP^+$ astrocytes; *t*-test, ***P*=0.003) is shown in the graphs in C-E, respectively. Error bars indicate s.e.m. (*n*=4). (F) Images of astrocyte morphology in P8 brains. The morphology of GFP-labeled astrocytes in the brains electroporated with DISC1 overexpression plasmid at E16 was more mature than that in control brains. Scale bars: 50 µm (B-E); 20 µm (F). See also Fig. S3.

astrocytes (Fig. 3C-E). Moreover, the morphology of astrocytes electroporated with the DISC1 overexpression plasmid was typically more mature and complex *in vivo* (Fig. 3F). Interestingly, expression of GFAP and ACSBG1 was decreased and the number of $GFAP^+$ astrocytes was reduced when the mutant DISC1 (DISC1-L100P) was overexpressed (Fig. S3C,D). Taken together, these results indicate that DISC1 overexpression facilitates astrogenesis both *in vitro* and *in vivo*.

DISC1 overexpression rescues astrogenesis defects caused by DISC1 depletion

To further examine DISC1 as a key regulator in astrogenesis, we generated a human DISC1 (hDISC1) overexpression plasmid and performed rescue experiments. First, the DISC1 expression level in the experiments was examined (Fig. S4A). Then, western blotting and immunostaining were used to examine the effect of hDISC1 on astrogenesis. The results showed a higher level of GFAP expression and an increased number of GFAP-labeled astrocytes when hDISC1 was overexpressed (Fig. 4A,B; Fig. S4B). After IUE *in vivo*, we observed that GFAP expression and the astrocyte number were increased when hDISC1 and DISC1 shRNA were co-expressed (Fig. 4C,D; Fig. S4C). Additionally, mDISC1 could also rescue the astrocyte differentiation defect caused by DISC1 knockdown (Fig. S4D). These data suggest that mDISC1 or hDISC1 overexpression could restore the astrogenesis defects caused by mDISC1 knockdown.

DISC1 regulates astrogenesis by modulating pMEK and pERK levels

Multiple signaling pathways are reportedly involved in gliogenesis, including the JAK/STAT, MEK/ERK, and NOTCH pathways (Bonni et al., 1997; Li et al., 2012; Zhou et al., 2010). Li et al. found that the RAS/MEK/ERK signaling pathway plays a crucial role in activating the expression of astrocyte-specific genes (Li et al., 2012). Furthermore, the activation of RAS, MEK and ERK are three major nodes in the pathway. To test whether DISC1 regulates astrogenesis via this pathway, we checked the level of phosphorylated and total ERK after treatment with LIF. The data showed that LIF activated ERK (Fig. S5A). Western blot analysis showed that the levels of phosphorylated (p)MEK and pERK decreased due to DISC1 knockdown and increased due to DISC1 overexpression (Fig. 5A,B; Fig. S5B). We further observed that overexpression of ERK could rescue the deficits of astrocytes caused by DISC knockdown *in vivo* (Fig. 5C). The active form of ERK (pERK) is reported to function following translocation into the nucleus (Clark et al., 2004). Thus, we detected the location of pERK treated with LIF for different time intervals after DISC1 was knocked down or overexpressed. The results showed that pERK was primarily located in the cytoplasm in the absence of treatment, transported to the nucleus after 10 min of short-term treatment with LIF, and increasingly re-localized to the cytoplasm after 2 h (Fig. S5C). Collectively, these results indicate that DISC1 regulates astrogenesis by modulation of pMEK and pERK levels.

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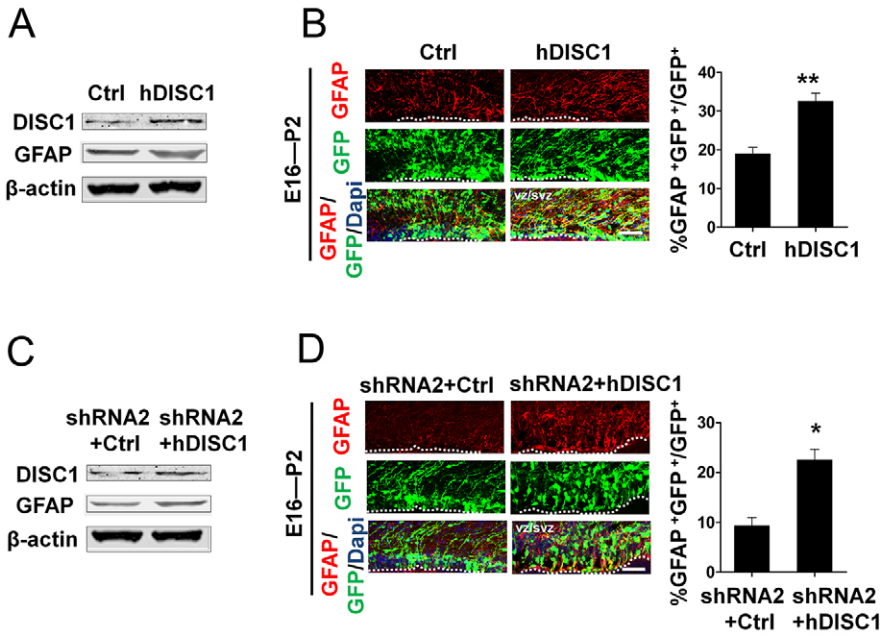

Fig. 4. DISC1 overexpression rescues astrogenesis defects induced by mouse DISC1 depletion. (A,C) Reduced GFAP expression due to mouse DISC1 (mDISC1) knockdown was rescued by human DISC1 (hDISC1) overexpression. Embryonic NPCs were infected with different combinations of lentivirus and cultured for 4 days. (B) Overexpression of hDISC1 leads to more astrocytes *in vivo*. Left: increased numbers of GFAP-positive cells in P2 cortices of embryos electroporated at E16. Right: the proportion of $GFAP^+GFP^+$ astrocytes (*t*-test, $^{**}P$=0.0094) relative to total GFP^+ cells. Error bars indicate s.e.m. (n=4). (D) The decrease in astrocyte number caused by mDISC1 knockdown is rescued by a gain of hDISC1 *in vivo*. Left: confocal images of GFAP immunostaining. Right: the percentage of double-labeled $GFAP^+GFP^+$ cells relative to GFP^+ cells (*t*-test, $^*P$=0.023). Error bars indicate s.e.m. (n=3). Scale bars: 50 µm. See also Fig. S4.

DISC1 regulates the RAS/MEK/ERK signaling pathway by direct interaction with RASSF7

Co-immunoprecipitation (co-IP) analysis showed that DISC1 does not directly interact with ERK, MEK or c-RAF (RAF1) (Fig. S5D). Therefore, we hypothesize that DISC1 may directly interact with an upstream component of the RAS pathway. Human RASSF7, a member of the RAS-association domain family, was previously reported as a potential binding partner of human DISC1 based on yeast two-hybrid screens (Morris et al., 2003). Furthermore, the similarities of human and mouse DISC1 and of human and mouse RASSF7 are 54.76% and 73.08%, respectively. First, we investigated whether there was direct interaction between RASSF7 and RAS and the effect of RASSF7 on the phosphorylation of MEK and ERK. Our co-IP analysis results showed that RASSF7 does indeed interact with RAS (Fig. 6A). We next investigated whether there was an interaction between DISC1 and RASSF7 and whether this interaction is involved in astrogenesis. To address this possibility, we generated recombinant Flag-tagged DISC1, HA-tagged DISC1, Flag-tagged RASSF7 and HA-tagged RASSF7. The results of co-IP showed that DISC1 and RASSF7 directly interact with each other (Fig. 6B,C). This interaction between DISC1 and RASSF7 was further verified by the co-IP of the purified tagged proteins (Fig. S6A). Moreover, colocalization of DISC1 and RASSF7 was observed in cells co-transfected with Flag-tagged DISC1 and HA-tagged RASSF7 (Fig. S6B), and the binding complex of DISC1-RASSF7 gradually increased as the levels of RASSF7 increased (Fig. S6C). Furthermore, *in utero* electroporation demonstrated that RASSF7 overexpression enhanced astrocyte differentiation *in vivo* (Fig. 6D).

How does DISC1 interact with RASSF7? DISC1 contains multiple interaction domains: a globular domain localized in the N-terminus, two leucine zipper domains localized in the middle and C-terminus, and several coiled-coil domains along the full length (Singh et al., 2010). To map the region(s) of DISC1 involved in the association with RASSF7, we generated a series of Flag-tagged fragments of DISC1 and co-expressed them with HA-tagged RASSF7. The co-IP results revealed that the CT domain of DISC1 was associated with RASSF7 in the protein complex. In addition, the MD domain of DISC1 also directly bound to RASSF7 (Fig. 6E). RASSF7, originally named HRC1, contains a RAS-association domain located at its N terminus (Sherwood et al., 2008). To map the RASSF7 domain(s) involved in RASSF7-DISC1 association, we also generated a series of Flag-tagged fragments of RASSF7 and co-expressed them with HA-tagged DISC1 in progenitor cells. The

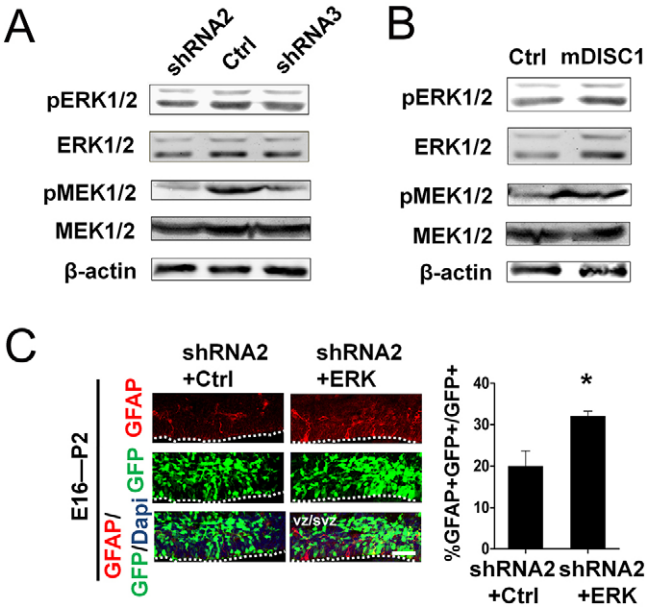

Fig. 5. DISC1 regulates astrogenesis by modulation of pMEK and pERK levels. (A) Western blot analysis of MEK and ERK. NPCs were infected with DISC1 shRNA2 or shRNA3 lentivirus and cultured in differentiation medium for 3 days. Following serum starvation overnight, cells were treated with LIF (50 ng/µl) for 10 min. The cell lysates were subjected to western blot analysis for phosphorylated and total MEK, and phosphorylated and total ERK. The levels of pMEK and pERK decreased due to DISC1 knockdown. (B) Western blotting of NPCs infected with the DISC1 overexpression lentivirus showed increased levels of MEK and ERK phosphorylation. (C) Left: the decrease in astrocytes in response to DISC1 shRNA2 was rescued by ERK overexpression *in vivo*, as demonstrated by GFAP staining. Right: the percentages of $GFAP^+$ GFP^+ cells relative to GFP^+ cell numbers (*t*-test, $^*P$=0.033). Error bars indicate s.e.m. (n=3). Scale bar: 50 µm. See also Fig. S5.

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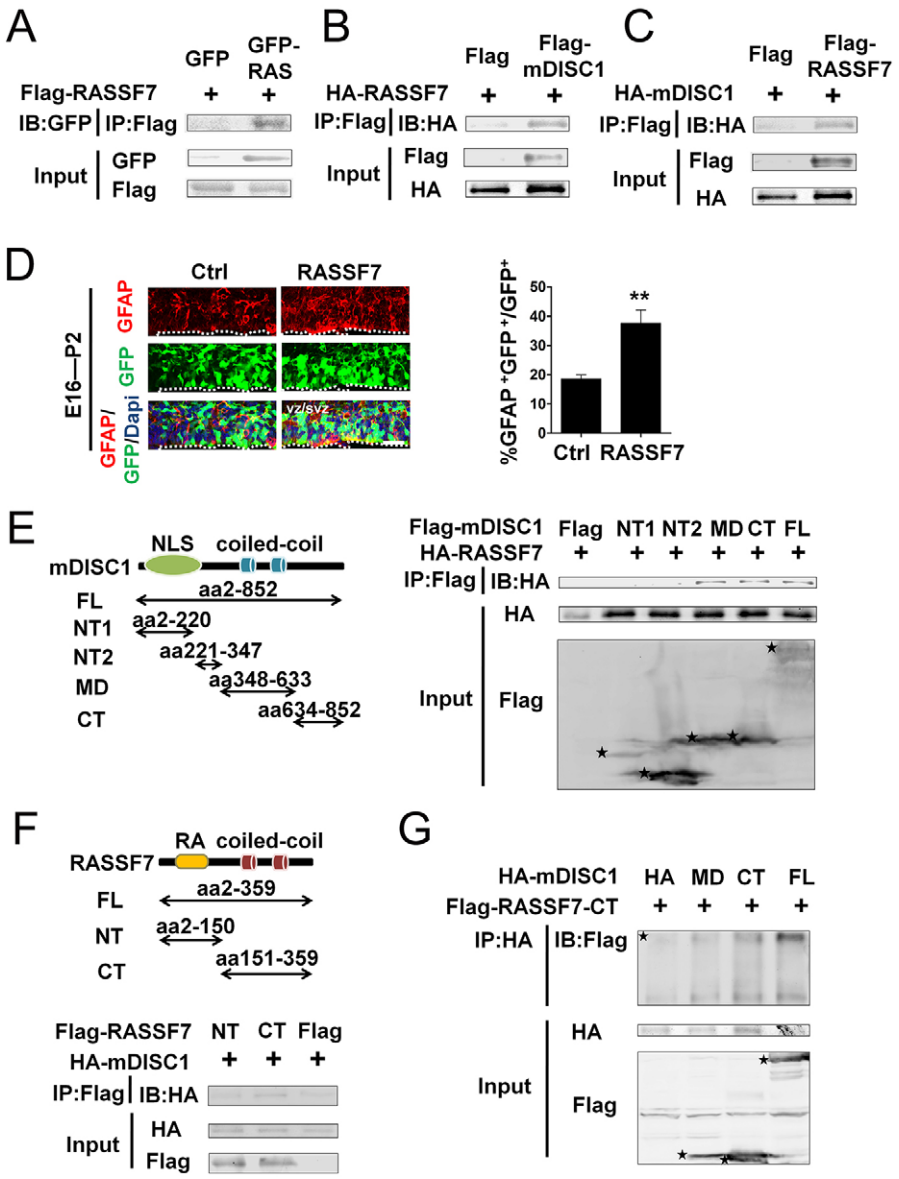

Fig. 6. DISC1 regulates the RAS/MEK/ERK signaling pathway by direct interaction with RASSF7. (A) Direct interaction between RAS and RASSF7. Lysates from NPCs infected with Flag-RASSF7 and GFP-vector or GFP-RAS lentivirus and differentiated for 3 days were immunoprecipitated with a GFP antibody and immunoblotted with a Flag antibody. (B,C) Direct interaction between DISC1 and RASSF7. (B) NPCs infected with full-length HA-tagged RASSF7 lentivirus together with full-length Flag-tagged DISC1 lentivirus or Flag-vector lentivirus were cultured in differentiation medium for 3 days. Cell lysates were subjected to co-IP using antibodies against Flag and immunoblotted for HA-RASSF7. (C) co-IP of lysates from cells infected with full-length HA-tagged mDISC1 lentivirus together with full-length Flag-tagged RASSF7 lentivirus or Flag-vector lentivirus. (D) RASSF7 overexpression increases the number of astrocytes *in vivo*. The images show GFAP-labeled astrocytes and the graph shows the percentage of $GFAP^+GFP^+$ astrocytes relative to the number of GFP^+ cells (*t*-test, $^{**}P$=0.0041). Error bars indicate s.e.m. (n=3). Scale bar: 50 µm. (E) Left: schematic of the Flag-tagged DISC1 recombinant fragments used for domain mapping. Right: RASSF7 binds to the CT region of DISC1 between residues 634 and 852. Lysates from NPCs co-infected with HA-RASSF7 lentivirus and each of the Flag-tagged DISC1 domains were immunoprecipitated with an antibody against Flag and immunoblotted with an antibody against HA. (F) Top: schematic showing the different domains and regions of RASSF7 used to generate Flag-tagged fragments. Bottom: western blot of the *in vitro* co-IP of recombinant HA-tagged DISC1 with different recombinant Flag-tagged fragments shows that DISC1 preferentially binds to the C terminus of RASSF7 (CT region). (G) Direct interaction between the fragments of DISC1 and RASSF7. Lysates from NPCs cells co-infected with the Flag-tagged RASSF7-CT fragment lentivirus together with full-length HA-tagged DISC1 lentivirus, HA-tagged DISC1-CT fragment lentivirus, or Flag-vector lentivirus were subjected to co-IP using antibodies against HA and immunoblotted for Flag-RASSF7-CT. Stars indicate the target bands. CT, C terminus; FL, full length; MD, middle domain; NLS, nuclear localization sequence; NT, N terminus; RA, RAS-association domain. See also Fig. S6.

co-IP results demonstrated that the CT domain of RASSF7 was involved in the association with DISC1 in the complex (Fig. 6F). Furthermore, the co-IP results demonstrated that the CT domain of DISC1 was sufficient to associate with the CT domain of RASSF7 (Fig. 6G). We then constructed DISC1-△NT2 or DISC1-△MD plasmids and performed rescue experiments to test whether DISC1 mutants could rescue the mDISC1 shRNA effects *in vivo*. Immunostaining of GFAP showed that the number of astrocytes was increased when DISC1-ΔNT2 and mDISC1 shRNA2 were co-expressed (Fig. S6D). However, the number of $GFAP^+$ GFP^+ cells was not significantly affected when DISC1-ΔMD and mDISC1 shRNA2 were co-expressed (Fig. S6E). These data suggest that the

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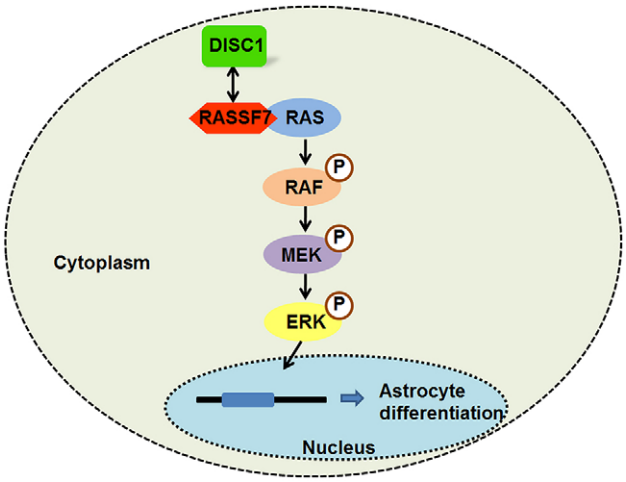

Fig. 7. A model of DISC1 function in astrogenesis during cortical development. DISC1 activates the RAS/MEK/ERK signaling pathway by direct association with RASSF7 and modulates astrogenesis in the embryonic brain.

MD domain, not the NT2 domain, is important for the function of DISC1. Taken together, these results indicate a direct and complex interaction between DISC1 and RASSF7 via multiple domains.

Because RASSF7 belongs to the RAS family, we then investigated whether RAS/MEK/ERK signaling is involved in the process. First, we examined pERK and found that the levels of pERK were increased in the presence of RASSF7 in a dose-dependent manner (Fig. S6F). Moreover, the results showed that the overexpression of RASSF7 or DISC1 alone increased the levels of pMEK and pERK in the presence of LIF; and RASSF7 and DISC1 overexpression resulted in higher levels of pMEK and pERK (Fig. S6G). Interestingly, the overexpression of RASSF7 restored the pMEK and pERK levels caused by the reduction of DISC1 shRNA (Fig. S6H). These data suggest that RASSF7 is required for the DISC1-dependent modulation of MEK/ERK signaling (Fig. 7).

DISCUSSION

DISC1 is one of only a few single genes definitively associated with psychiatric disease, such as schizophrenia, bipolar disorder and unipolar depression (Bradshaw and Porteous, 2012). However, it is unclear how DISC1 dysfunction results in a series of mental disorders. Recently, the important role of DISC1 in the processes of neuronal development has been demonstrated (Mao et al., 2009; Niwa et al., 2010), but its role in astrogenesis is not clear. Furthermore, the etiology of schizophrenia remains to be comprehensively characterized. Thus, we set out to describe the role of DISC1 in astrocyte progenitor differentiation during the late stage of brain development by using *in utero* electroporation approaches of gene manipulation.

In this study, we found that DISC1 depletion reduces the expression of ACSBG1 and GFAP *in vitro* and decreases astrocyte number *in vivo*, whereas DISC1 overexpression substantially enhances the process. Moreover, a missense mutation of DISC1 (DISC1-L100P) constructed according to a previous paper (Clapcote et al., 2007) causes abnormal gliogenesis. In order to test whether the decrease in astrocyte number is the result of changes in proliferation, we performed BrdU-labeling experiments. The results showed that there was no significant difference in BrdU labeling of $GFAP^+$ GFP^+ cells by DISC1 knockdown and DISC1 overexpression (Fig. S7A,B). Furthermore, brains electroporated with DISC1 overexpression plasmid at E16 were analyzed at P10 and P16 to examine the longer-term effect of DISC1. The results showed the GFP-labeled cells were slightly increased when DISC1 was overexpressed and mainly migrate to the cortical plate. Compared with the control group, the morphology of GFP-labeled astrocytes in the DISC1 overexpression group was more mature (Fig. S8A,B). In addition, we also checked neural stem cell proliferation and neuron differentiation. E16 embryonic brains electroporated with control and DISC1 knockdown plasmids were analyzed at E18 or P0 (Fig. S8C,D). The results showed that DISC1 knockdown cells mainly located at the VZ/SVZ and a small proportion of cells migrated to the intermediate zone. When brains were electroporated at E16 and analyzed at P0, we found that the number of nestin-positive cells was slightly decreased with DISC1 knockdown or slightly increased with DISC1 overexpression (Fig. S9A,B). These data suggest that DISC1 knockdown may not affect nestin-positive progenitor cell proliferation at late stages of development. We also examined neuron differentiation at a late stage. The results showed that the number of neurons differentiated from isolated P0 NPCs and TUJ1 $(TUBB3)^+$ cells were not significantly affected by DISC1 knockdown or overexpression (Fig. S9C-F).

Gliogenesis occurs during the late stages of embryogenesis after neurogenesis, when NPCs generate more astrocytes and fewer neurons. Specific genes related to astrocyte development are temporally and spatially expressed during late embryonic brain development following the activation of different relevant intrinsic signaling pathways. Among these pathways, the JAK-STAT pathway is important for controlling the onset of astrogenesis (Fan et al., 2005). In addition, the RAS/MEK/ERK signaling pathway has been shown to regulate gliogenesis (Li et al., 2012). Our data indicate that DISC1 regulates astrogenesis by modulating pMEK and pERK levels. Furthermore, consistent with a previous study (Clark et al., 2004), we confirmed that the active form of ERK translocated into the nucleus. Interestingly, DISC1 participates in this pathway by interaction with RASSF7, a member of the RAS-association domain family. A previous study used yeast two-hybrid screening to identify that hRASSF7 interacts with hDISC1. We compared the similarities of protein sequences and found that human DISC1 and mouse DISC1, as well as human and mouse RASSF7, were highly homologous. Based on this information, co-immunostaining was performed and the result showed that mouse DISC1 interacts with RASSF7.

To date, various lines of evidence have revealed multiple signaling pathways that correlate with the intrinsic pathogenesis of schizophrenia (Kyosseva, 2004). Recently, DISC1 was proven to be involved in GSK3β/β-catenin signaling (Mao et al., 2009), AKT/mTOR signaling (Kim et al., 2009) and GABA signaling. Given the variety of proteins that bind DISC1, it evidently plays many roles in the central nervous system via different signaling pathways. Here, we add that DISC1 regulates the RAS/MEK/ERK pathway, which is important for brain astrocyte development and function. The influence of DISC1 on the development of neurons and oligodendrocytes has been investigated (Hattori et al., 2014; Mao et al., 2009). Here, we further investigate the effects of DISC1 on astrocyte development. In summary, our results provide a framework for understanding the effect of DISC1 on astrogenesis, which may contribute to brain development and the etiology of psychiatric disorders.

MATERIALS AND METHODS

Mice

ICR female mice were used for *in utero* and postnatal electroporation experiments. For timed breedings, the day after detection of a vaginal plug was considered to be E1. Mice were maintained and bred in the Experiment

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Animal Center of Institute of Zoology, Chinese Academy of Sciences. All animal experiments and protocols were approved by the Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Sciences.

Plasmid constructs

Lentivirus vectors were used for cloning of plasmid constructs (Duan et al., 2007). See supplementary Materials and Methods for further details.

Cell culture

Lentivirus was produced by 293FT and the collected virus used to infect NPCs. NPCs used for immunofluorescence and western blotting were isolated from E15 mouse embryonic brains. See supplementary Materials and Methods for culture conditions.

Western blotting and co-immunoprecipitation

Proteins from cortices or cultured primary NPCs were prepared in the RIPA buffer containing 1% protease inhibitor cocktail and 1% phenylmethylsulfonyl fluoride and ultrasonicated on ice. After centrifugation at 12,000 rpm (13,523 ***g***) for 10 min (4°C), protein concentrations were measured using the Pierce BCA Protein Assay Kit (Thermo Scientific). Lysates were size-separated by 12% SDS-PAGE, blotted onto nitrocellulose membrane (Millipore) by the semi-dry electrophoretic transfer according to standard protocols (Bio-Rad) (Lv et al., 2014). For co-IP analysis, fresh samples were immunoprecipitated with anti-Flag or anti-GFP antibodies that had been incubated with Dynabeads Protein A (Life Technology) overnight at 4°C, and then subjected to western blotting according to the manufacturer's protocols (Life Technology). See supplementary Materials and Methods for details of antibodies used.

In utero and postnatal electroporation

Every embryo was electroporated with five 50 ms pulses at 50 V with 950 intervals, using 5 mm paddle electrodes. The embryos were returned to the abdominal cavity 30 min after electroporation (Wang et al., 2014). Neonatal pups were electroporated with four 50 ms pulses at 90 V with a 950 ms interval. See supplementary Materials and Methods for further details.

Immunohistochemistry and immunocytochemistry

Brains of mice were fixed in 4% paraformaldehyde (PFA) for 24 h at 4°C, gradually dehydrated in 5%, 10%, 15%, 25%, 30% sucrose for 8 h in sequence, and then embedded in OCT overnight at −20°C. Coronal sections (15 µm) were cut using Leica CM1950 cryostat. For IHC, sections were fixed in 4% PFA for 30 min, washed twice with PBS, blocked with 5% bovine serum albumin in PBS containing 0.1% Triton X-100 for 1 h, and then incubated in primary antibodies overnight at 4°C. The next day, brain slices were washed three times with PBS, and incubated with secondary antibody for 2 h at room temperature. After three washes in PBS, sections were counterstained with DAPI (2 µg/ml). The steps for immunocytochemistry and BrdU labeling were similar to the above protocol (Zhang et al., 2014). See supplementary Materials and Methods for details of antibodies used.

Statistical analysis

Statistical analysis was performed using Student's *t*-test or one-way ANOVA. All bar graphs are plotted as mean±s.e.m. *P*-values of <0.05 were considered to be significant (**P*<0.05; ***P*<0.01; ****P*<0.001). For details of quantification methods (Barnabé-Heider et al., 2005; Xie et al., 2007), see the supplementary Materials and Methods.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.W., Q.L. and J.J. designed the study, analyzed the date, wrote the paper; S.W. and Q.L. performed all the experiments; H.Q. and T.S. did several of the experiments; H.L. and F.J. helped to conceive the study.

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Supplementary information

Supplementary information available online at
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Supplementary Materials and Methods

Plasmid constructs

Full-length mouse DISC1 (transcript variant 1, NM_174854.2), Full-length human DISC1 (transcript L, NM_018662.2), mouse RASSF7 (NM_025886.3) were amplified and cloned into lentiviral expression vector pCDH-GFP (System Biosciences) to generate destination constructs. The oligonucleotides targeting DISC1 were subcloned into the other lentiviral vector pSicoR-GFP (Addgene) to construct DISC1 shRNA plasmids. And the sequences recognizes mouse DISC1 are as follows: shRNA1, 5'-GGCAAACACTGTGAAGTGC-3' (Mao et al., 2009); shRNA2, 5'-GGCTACATGAGAAGCACAG-3'; shRNA3, 5'-GGTCACTTCCTTAATTTTA-3'; shRNA4, 5'-GGACTACCTACTGAGCAAC-3' (Duan et al., 2007).

pEGFP-ERK, and pEGFP-RAS were constructed by our lab. Full-length mouse DISC1 (amino acid 2-852), NT1 (amino acid 2-220), NT2 (amino acid 221-347), MD (amino acid 348-633), CT (amino acid 634-852) were cloned into pCDH-3Flag lentivirus vector for the Flag-tag or pCDH-3HA lentivirus vector for the HA-Tag. Full-length mouse RASSF7 (amino acid 2-359), NT (amino acid 2-150), and CT (amino acid 151-359) were cloned into Flag-fusion lentivirus vector pCDH-3Flag and HA-fusion lentivirus vector pCDH-3HA. The mutation DISC1 (DISC1-L100P) was constructed into lentivirus vector pCDH-3Flag (Clapcote et al., 2007).

Cell culture

Lentivirus was produced by 293FT and the collected virus infected neuronal progenitor cells (NPCs). NPCs used in immunofluorescence and western blot were isolated from E15 mouse embryonic brains and cultured as monolayer on plates which were pre-coated with Poly-D-ornithine (10μg/ml, Sigma, P3655) and Laminin (10μg/ml, Invitrogen, 23017015) in

Neural Stem Cell Basal Medium (Millipore, SM008) supplemented with bFGF (5ng/ml, Invitrogen, PHG0026), EGF (5ng/ml, Invitrogen, PHG0311), and 1% penicillin/streptomycin. NPCs were infected with lentivirus for 8 h by adding 2 μg/ml polybrene to improve the infection efficiency. 12 h later, proliferation medium were changed into glial differentiation medium with DMEM medium supplemented with 1%FBS and 1 x B27. LIF (20ng/ml, Millipore, ESG1107) was added in some experiments (Barnabe-Heider et al., 2005). Proteins were harvested and processed for western blot assay after 3 days. For LIF stimulation experiments, cells were serum starved (DMEM) overnight and treated with 50ng/ml LIF for 10min to analyze the levels of pMEK and pERK.

Western blotting and co-immunoprecipitation

The following primary antibodies were used for WB: DISC1 (rabbit, 1:200, Santa Cruz, sc-47990), GFAP (mouse, 1:1000, Sigma, G6171), p-p44/42 MAPK (Thr202/Tyr204) (rabbit, 1:1000, CST, #4370), p44/42 MAPK (137F5) (rabbit, 1:1000, CST, #4695), p-MEK1/2 (Ser217/221) (41G9) (rabbit, 1:1000, CST, #9154), MEK1/2 (L38C12) (mouse, 1:1000, CST, #4694) (Li et al., 2012), β-actin (mouse, 1/2000, Proteintech, 60008-1), Flag (rabbit, 1:2000, Sigma, F7452), HA (26D11) (mouse, 1:5000, Abmart, #M20003), and GFP (mouse, 1:1000, MBL, M048-3). The seconodary antibodies were 800CW Donkey-anti-Rabbit IgG, 800CW Donkey-anti-Mouse IgG, 680CW Donkey-anti-Rabbit IgG, and 680CW Donkey-anti-Mouse IgG (1:10000, Odyssey, 926-32213, 926-32212, 926-68072, 926-68072) (Lv et al., 2014; Wang et al., 2014; Zhang et al., 2014). Membranes were scanned with the Odyssey Infrared Imaging Systems.

In utero and postnatal electroporation

Using 0.7% pentobarbital sodium intraperitoneally (70mg/kg,0.7g/kg), the pregnant mice were anesthetized and embryos were gently exposed, while the P0 mice were cryo anesthetized. 0.03% fast green was added into the DNA solution (about 2ug) containing the target recombinant plasmid (1500ng/ul) and a GFP-expressing plasmid in a molar ratio of 3:1. Then the mixture was injected into the lateral ventricle of embryos or neonatal pups. Every embryo was electroporated with five 50 ms pulses at 50V with 950 intervals (BTX electroporator), using 5 mm paddle electrodes. This manipulation was finished in 30 min before the embryos were returned to the abdominal cavity (Wang et al., 2014). For neonatal pups, they were electroporated with four 50 ms pulses at 90V with a 950 ms interval.

Immunohistochemistry and immunocytochemistry

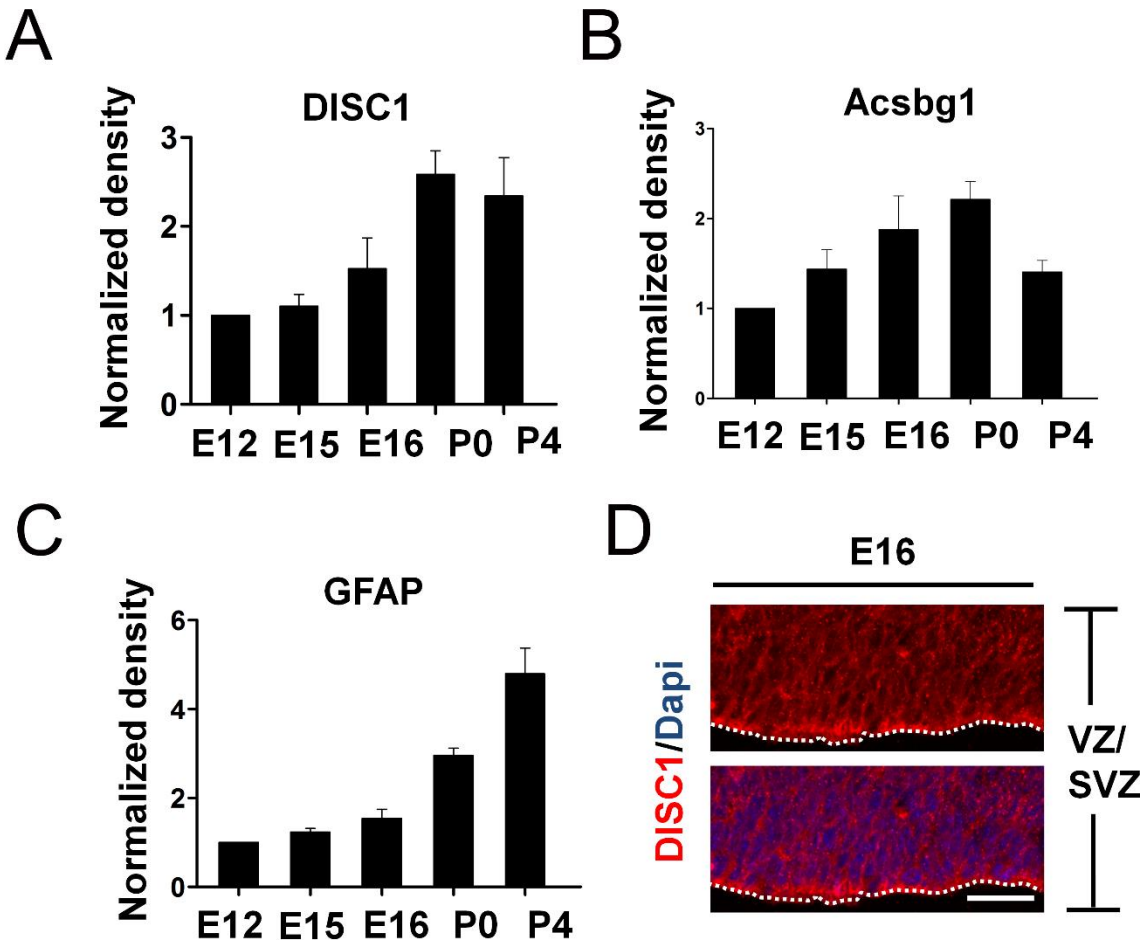
The primary antibodies used were as follows: DISC1 (rabbit, 1:100, NOVUS, NB110-40773) (Mao et al., 2009a), GFP (rat, 1:1000, MBL, D153-3), GFAP (rabbit, 1:3000,Dako, Z033429), GLAST (rabbit, 1:300, Proteintech, 20785-1-AP), FGFR3 (rabbit, 1:100, Bioworld, BS1125), Tuj1 (rabbit, 1:1000, Sigma, T2200), p-p38/42 MAPK (Thr202/Tyr204) (rabbit, 1:100, CST, #4370), HA (rabbit, 1:800, CST, #3724), and Flag (rabbit, 1:1000, CST, #814), Nestin (mouse, 1:200; Millipore, MAB353). The secondary antibodies used were: Cy3 Donkey-Anti-Mouse IgG, Cy3 Donkey-Anti-Rabbit IgG, (1:1000, Jackson ImmunoResearch, 715-165-150, 711-165-152,), and Alexa488 Donkey-Anti-Rat IgG (Life Technologies, A21208) (Lv et al., 2014; Wang et al., 2014; Zhang et al., 2014).

Statistical analysis

For brain section analysis, all images were acquired with Zeiss confocal LSM780 microscope. Three to five random fields of defined regions on sections were captured and

analyzed the percentage of double-labelled cells of GFP positive cells (Barnabe-Heider et al., 2005; Xie et al., 2007). Images were further analyzed by Adobe Photoshop and the "Ipwin 32" was used for cell counting. Quantification of bands in WB was performed using the software (Odyssey V3.0).

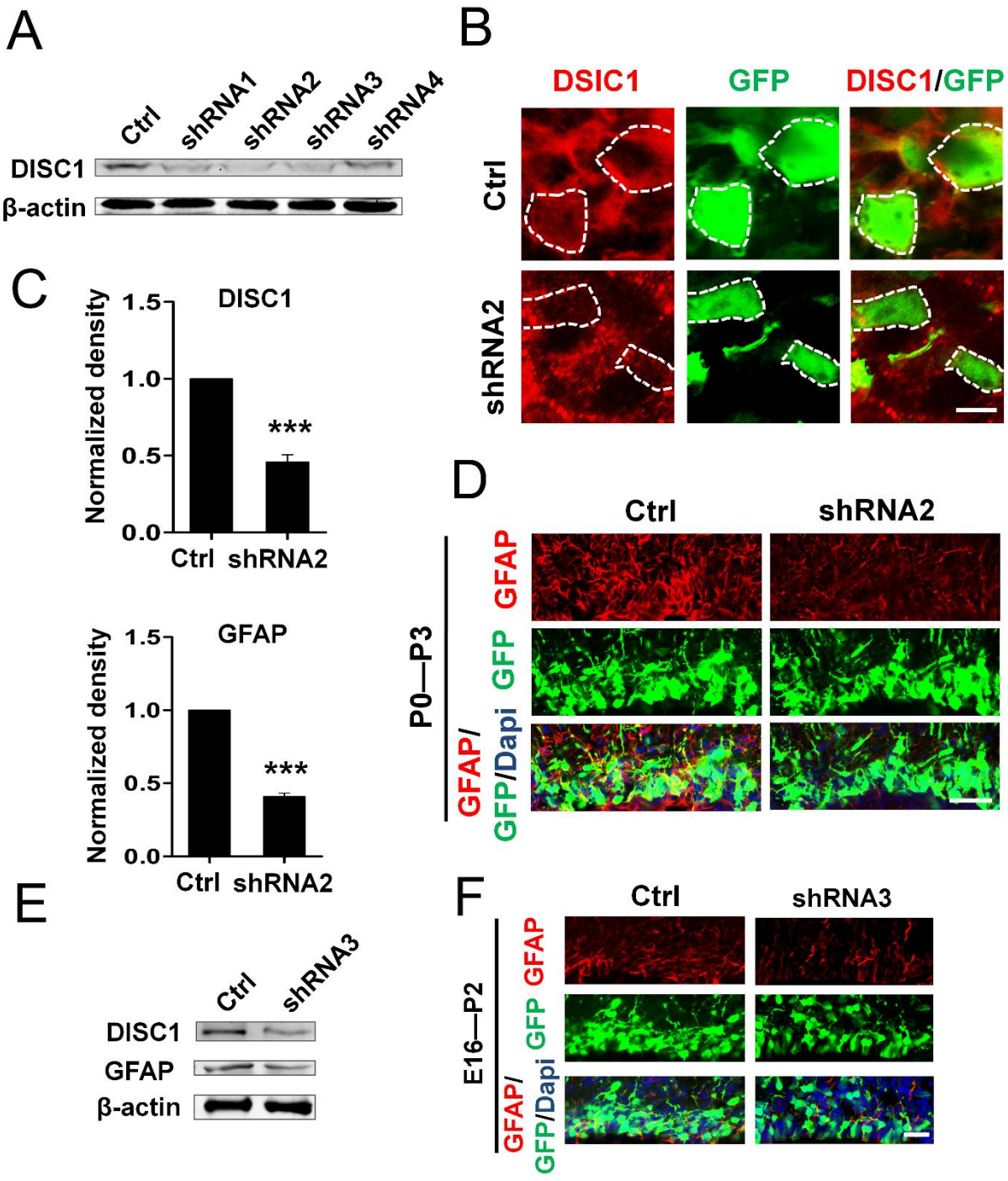
Supplementary figures



Supplementary Figure S1 DISC1 expression in brain development (Related to Fig. 1).

A-C The graphs demonstrating the quantification of the level of DISC1 (A), Acsbg1 (B) and GFAP (C) protein in the brain cortex from E12 to P4 during embryonic cortical development. β-actin was used as an internal control for immunoblotting. Error bars indicate s.e.m. (n=4).

D Immunostaining of DISC1 in the subventricular zone (SVZ) of brain at E16. The dotted line indicates the edge of the neocortical SVZ. Scale bar: 50μm.



Supplementary Figure S2 DISC1 depletion results in gliogenesis defects (Related to Fig. 2).

A Western blots for the knockdown efficiency of four DISC1 shRNAs in NPCs infected with lentiviruses *in vitro*.

B Immunostaining for DISC1 in coronal sections displaying the knockdown efficiency of

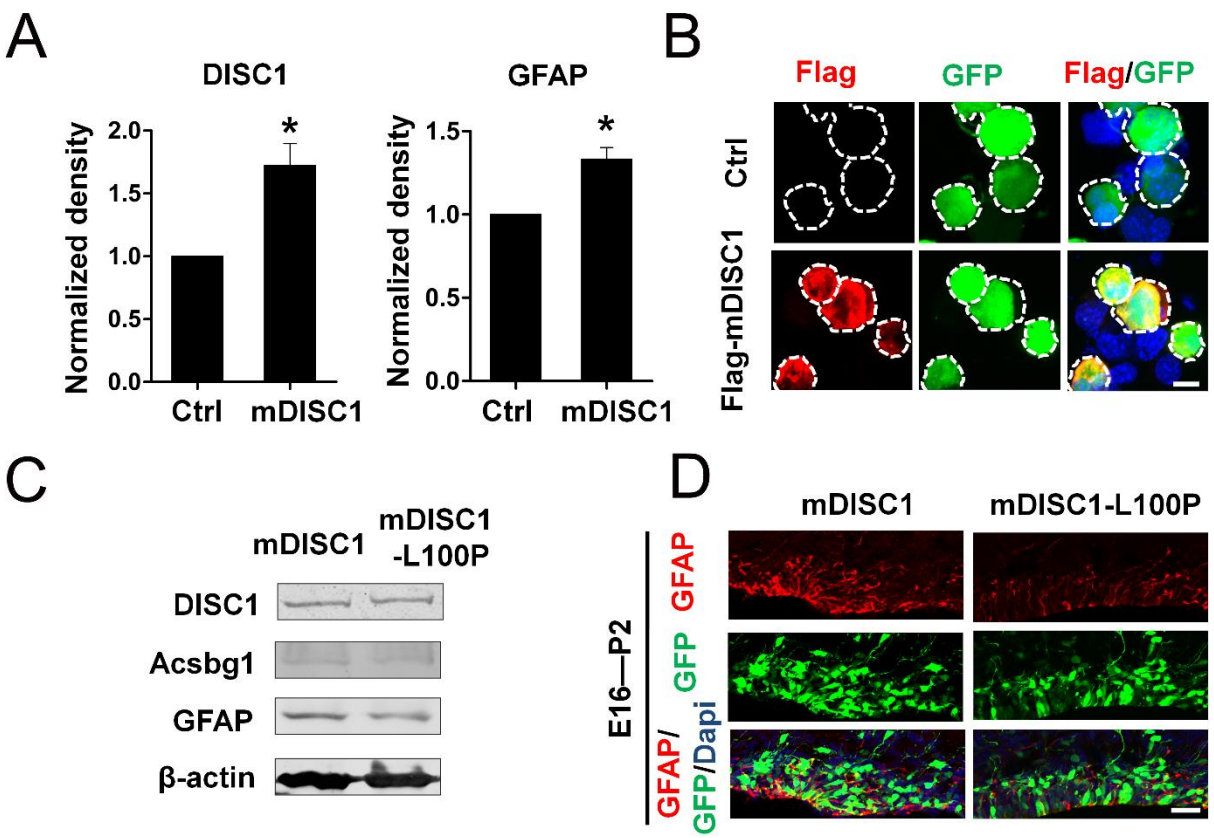
DISC1 shRNA *in vivo*. The embryos were electroporated in utero with DISC1 shRNA2 or control plasmids at E16 and developed until P2. Scale bar: 5 μm.

C Top, the graph demonstrated the efficiency of DISC1 knockdown in NPCs infected with lentiviruses (*t*-test, ***p=0.0009). Bottom, quantification of GFAP expression in NPCs that were infected with control or DISC1 knockdown lentiviruses (*t*-test, ***p=0.0006). Error bars indicate s.e.m. (n=4).

D Suppression of DISC1 expression results in a decrease of astrocyte number in P3 mouse brains harvested after control or DISC1 shRNA2 plasmids were electroporated at P0. Scale bar: 50μm.

E DISC1 knockdown decreases the levels of DISC1, GFAP in embryonic E15 NPCs infected with lentivirus DISC1 shRNA3 and cultured in differentiation medium for three days.

F DISC1 shRNA3 decreased the number of GFAP-labeled astrocytes in vivo, as shown in the images of immunostaining. Scale bar: 50μm.



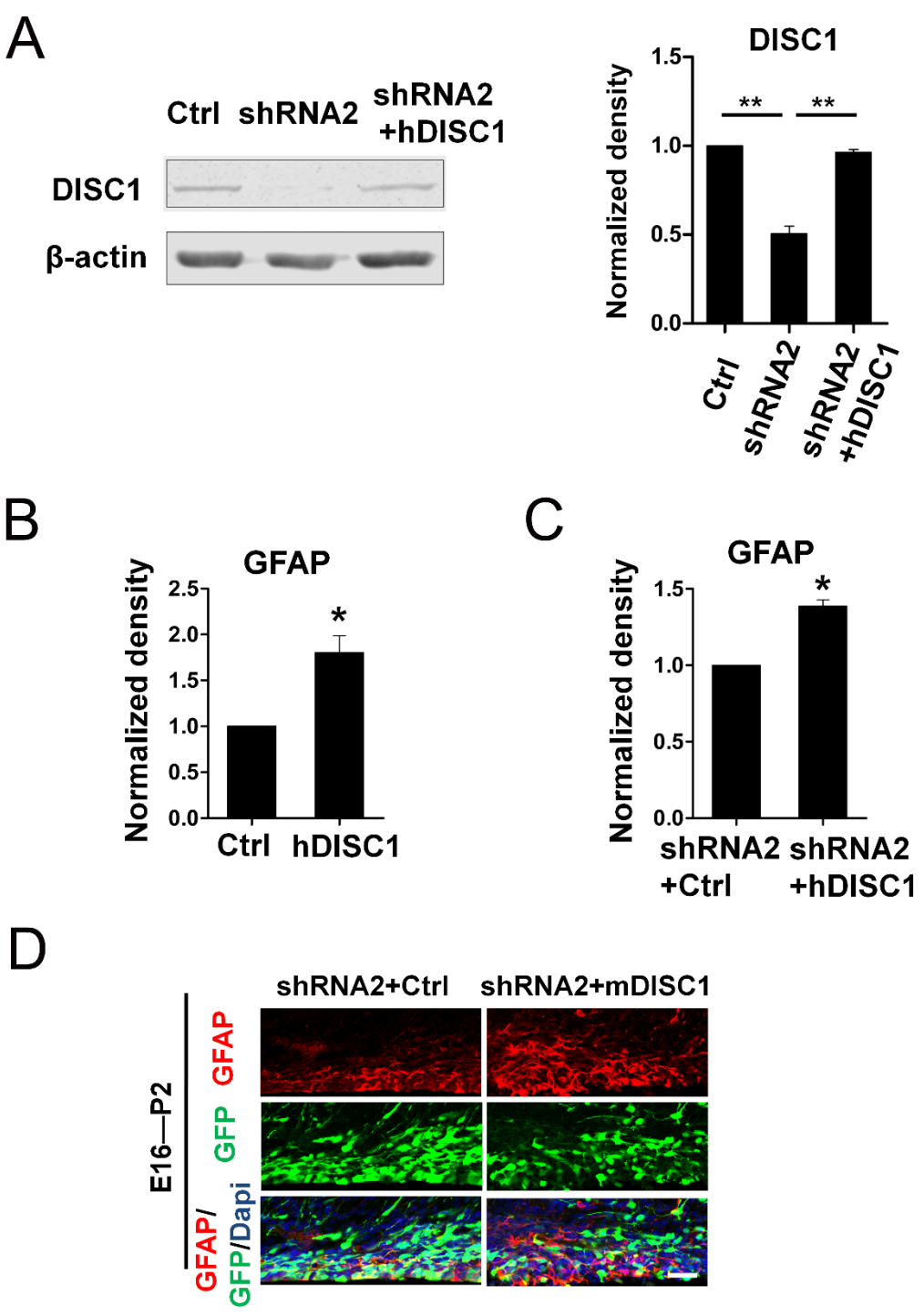
Supplementary Figure S3 DISC1 overexpression enhances gliogenesis (Related to Fig. 3).

A Left, the graph demonstrated the efficiency of DISC1 overexpression in NPCs infected with lentiviruses (*t*-test, **p*=0.041). Right, quantification of GFAP expression in NPCs that were infected with control or DISC1 overexpression lentiviruses (*t*-test: **p*=0.037). Error bars indicate s.e.m. (n=4).

B Immunostaining for Flag in cells displaying the overexpression of Flag-mDISC1 *in vitro*. Scale bar: 10 μm.

C E15 NPCs were infected with lentivirus and cultured in differentiation medium for three days. Western blotting showed the levels of DISC1, Acsbg1, GFAP with missense mutant DISC1 (mDISC1-L100P) overexpression. β-actin was used as an internal control for immunoblotting.

D Immunostaining for GFAP in embryonic brains electroporated at E16 with control and the DISC1 mutant plasmids. Scale bar: 50μm.



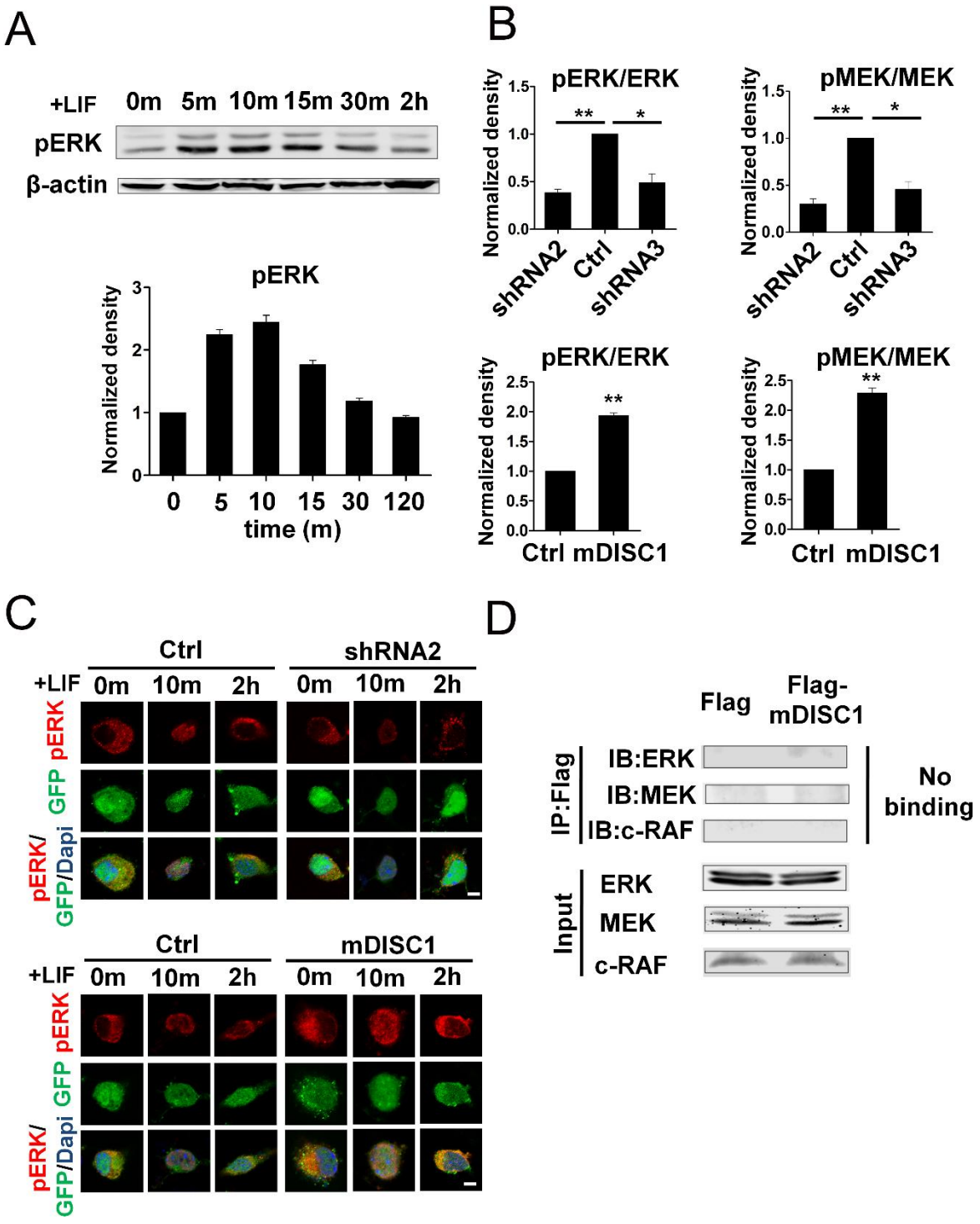
Supplementary Figure S4 DISC1 overexpression rescues astrogenesis defects caused by DISC1 depletion (Related to Fig. 4).

A Western blots for the DISC1 expression level in NPCs that were infected with control, DISC1 shRNA2 or hDISC1 overexpression lentiviruses. Right, quantification of DISC1 expression in the rescue experiment (*t*-test, ***p*=0.007, **p*=0.011). Error bars indicate s.e.m.

(n=3).

B-C The quantification of GFAP protein expression in embryonic NPCs infected with different combinations of lentivirus and cultured for four days (t-test: B, **p*=0.047; C, **p*=0.012). Error bars indicate s.e.m. (n=4).

D GFAP immunostaining showed that the decrease of astrocyte number caused by DISC1 shRNA was rescued by overexpression of mDISC1 *in vivo*. Scale bar: 50μm.



Supplementary Figure S5 DISC1 regulates astrogenesis by modulating pMEK and pERK levels (Related to Fig. 5).

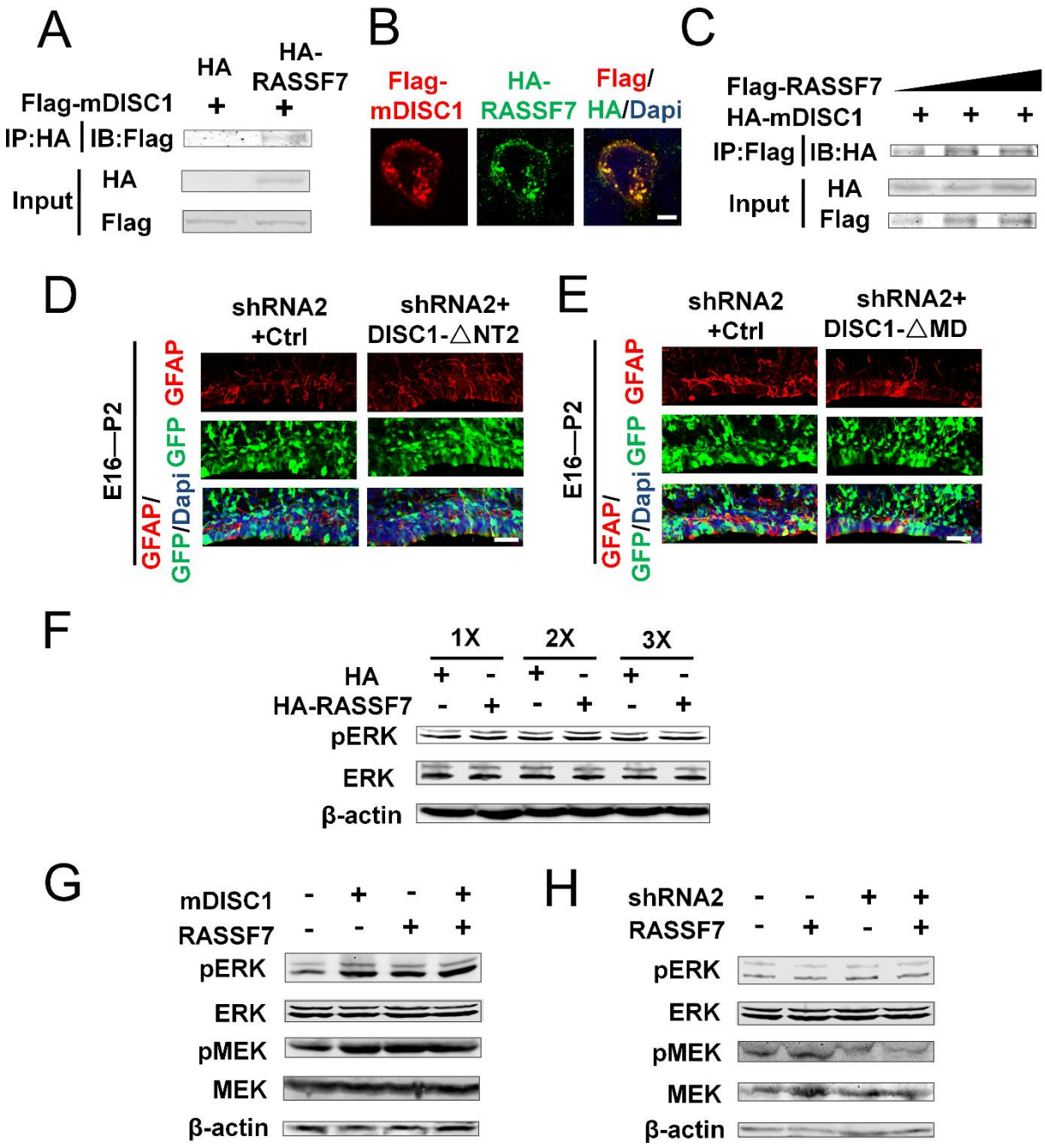
A Western blots for the expression of pERK protein in NPCs that were serum starved

overnight and then treated with LIF (50 ng/μl) or control vehicle for 5 min,10 min,15 min,30 min, and 2 h. The levels of pERK were calculated. Error bars indicate s.e.m. (n=3).

B The quantification for the levels of pERK (*t*-test, ***p*=0.008 in the shRNA2 group, **p*=0.024 in the shRNA3 group, ***p*=0.004 in the mDISC1 group) and pMEK (*t*-test, ***p*=0.006 in the shRNA2 group, **p*=0.041 in the shRNA3 group, ***p*=0.009 in the mDISC1 group) in NPCs infected with control or DISC1 shRNA lentiviruses, differentiated for three days, starved overnight and treated with LIF for 10 min. Error bars indicate s.e.m. (n=4).

C DISC1 activates the function of pERK via translocating from the cytoplasm into the nucleus in NPCs treated with LIF for several intervals (0 min, 10 min, and 2 h). Scale bar: 5 μm.

D DISC1 does not directly interact with c-RAF, ERK or MEK. Lysates from cells transfected with Flag-vector or full-length Flag-tagged were subjected to co-IP using antibodies against Flag and immunoblotted for c-RAF, ERK and MEK.



Supplementary Figure S6 DISC1 regulates RAS/MEK/ERK signaling pathway by direct interaction with RASSF7 (Related to Fig. 6).

A The co-IP of the interaction between the purified protein Flag-DISC1 and HA-RASSF7.

B The co-localization of DISC1 and RASSF7 in cells co-transfected with Flag-tagged DISC1 and HA-tagged RASSF7. Scale bar: 5 μm.

C The association between DISC1 and RASSF7 gradually increases with an increasing amount of RASSF7. Lysates from NPCs coinfected with HA-mDISC1 lentivirus and increasing concentrations of Flag-RASSF7 lentivirus were immunoprecipitated with a Flag antibody and immunobloted with a HA antibody.

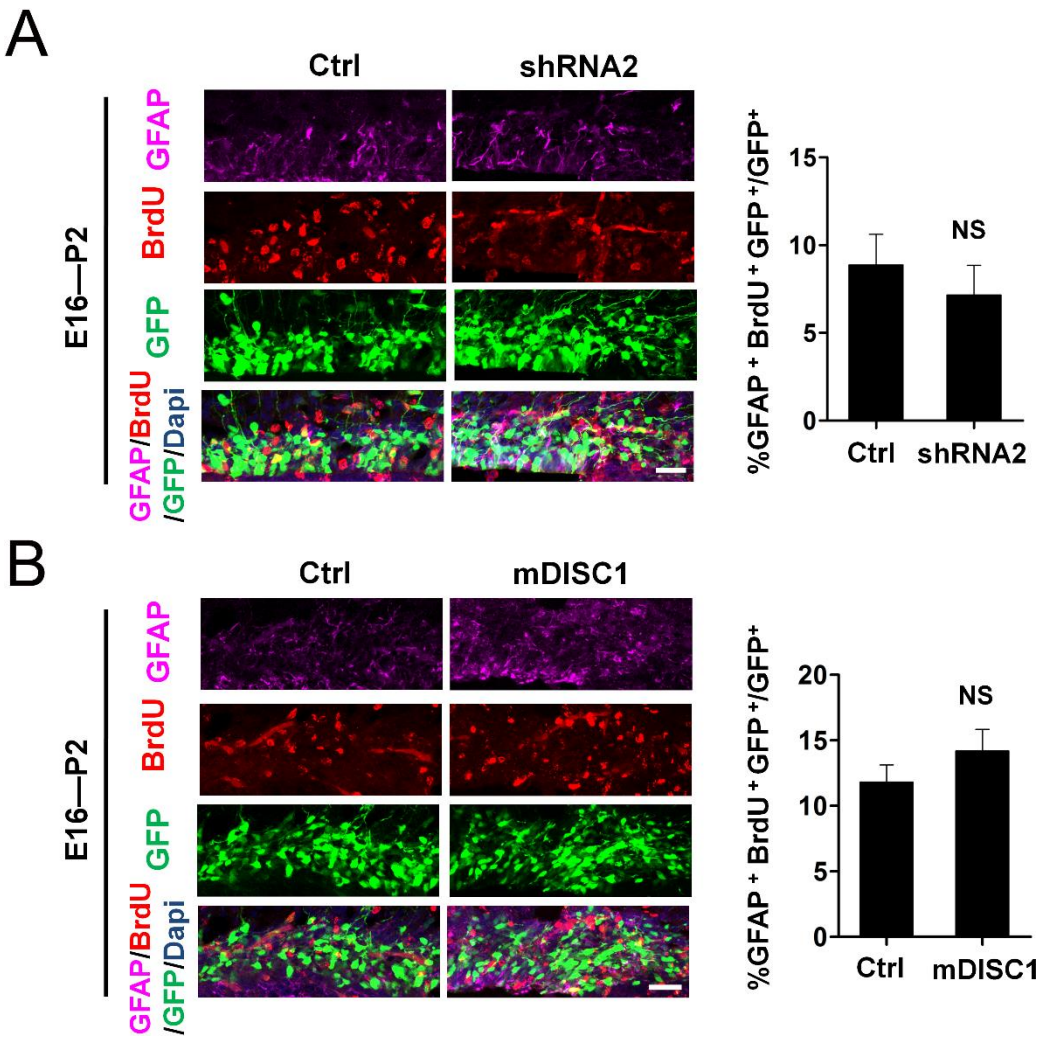
D The decrease of astrocyte number caused by mDISC1 shRNA is rescued by DISC1-△NT2 overexpression *in vivo*. GFAP immunostaining showed the astrocyte number in the E16 embryonic brains electroporated with plasmids were analyzed at P2. Scale bar: 50μm.

E After IUE *in vivo*, GFAP immunostaining were performed when DISC1-△MD and mDISC1 shRNA were co-expressed *in vivo*. Scale bar: 50μm.

F pERK expression level after RASSF7 overexpression. Lysates from NPCs infected with increasing concentration of HA-vector or full-length HA-RASSF7 lentivirus respectively were immunoblotted for pERK, ERK.

G Western blot analysis of MEK/ERK signaling. NPCs infected with DISC1, RASSF7, or both were serum starved overnight and then treated with LIF (50 ng/μl) for 10 min. The cell lysates were subjected to western blot analysis for phosphorylated and total MEK, ERK.

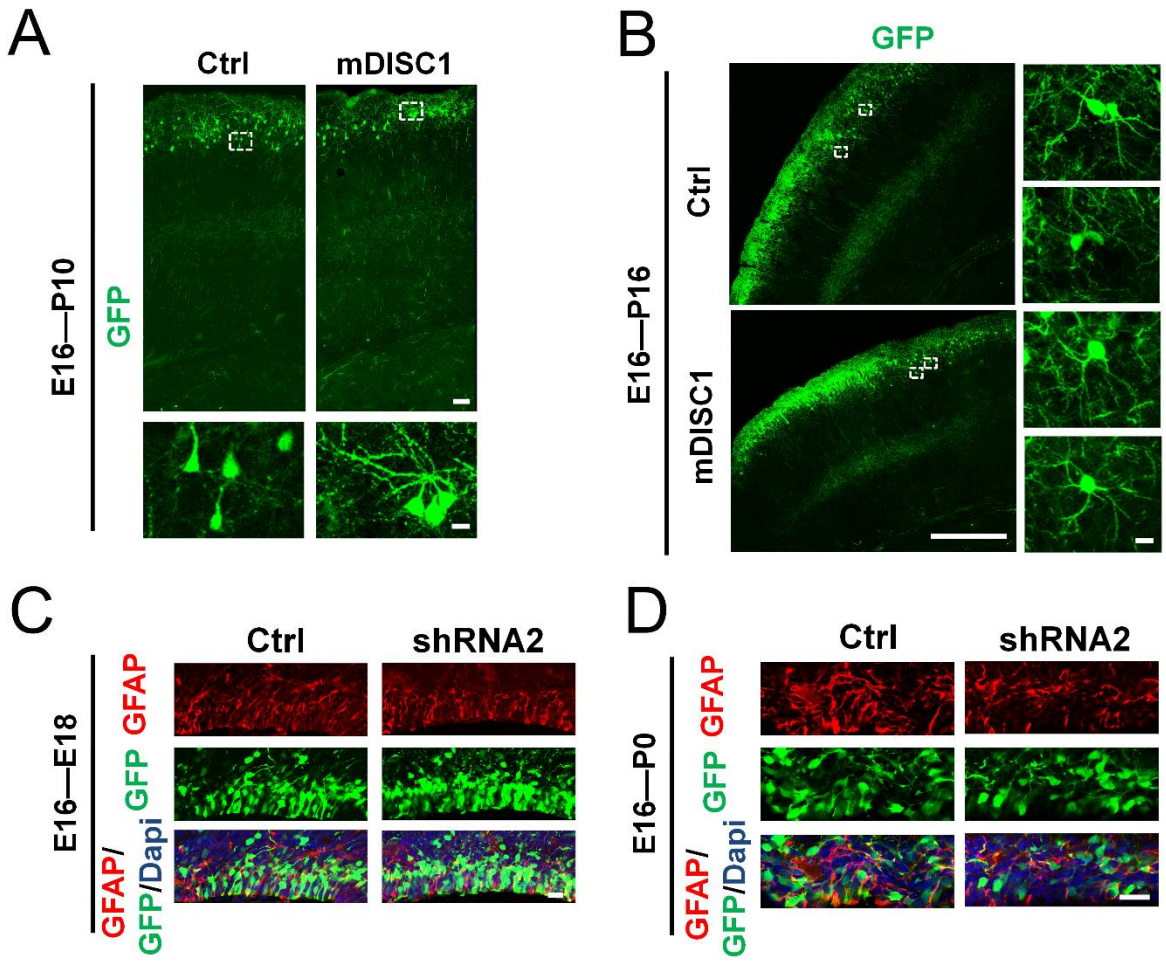
H Western blot analysis of MEK/ERK signaling. NPCs infected with DISC1 shRNA2, RASSF7 or both, were serum starved overnight and then treated with LIF (50 ng/μl) for 10 min. The cell lysates were subjected to western blot analysis for phosphorylated and total MEK, ERK.



Supplementary Figure S7 The effects of DISC1 on cell proliferation.

A E16 embryonic brains electroporated with control and DISC1 knockdown plasmids were analyzed at P2. Left, the immunostaining images of GFAP+ GFP+ BrdU+ cells showed the effect of DISC1 knockdown on the change in proliferation. Right, the percentages of $GFAP^+$ $BrdU^+$ GFP^+ cells were measured (*t*-test, p=0.437). Error bars indicate s.e.m. (n=3). Scale bar: 50μm.

B Left, the immunostaining images of GFAP+ GFP+ BrdU+ cells in E16 embryonic brains electroporated with control and DISC1 overexpression plasmids. Right, the graph showing the proportion of $GFAP^+$ $BrdU^+$ GFP^+ cells (*t*-test, p=0.243). Error bars indicate s.e.m. (n=3). Scale bar: 50μm.

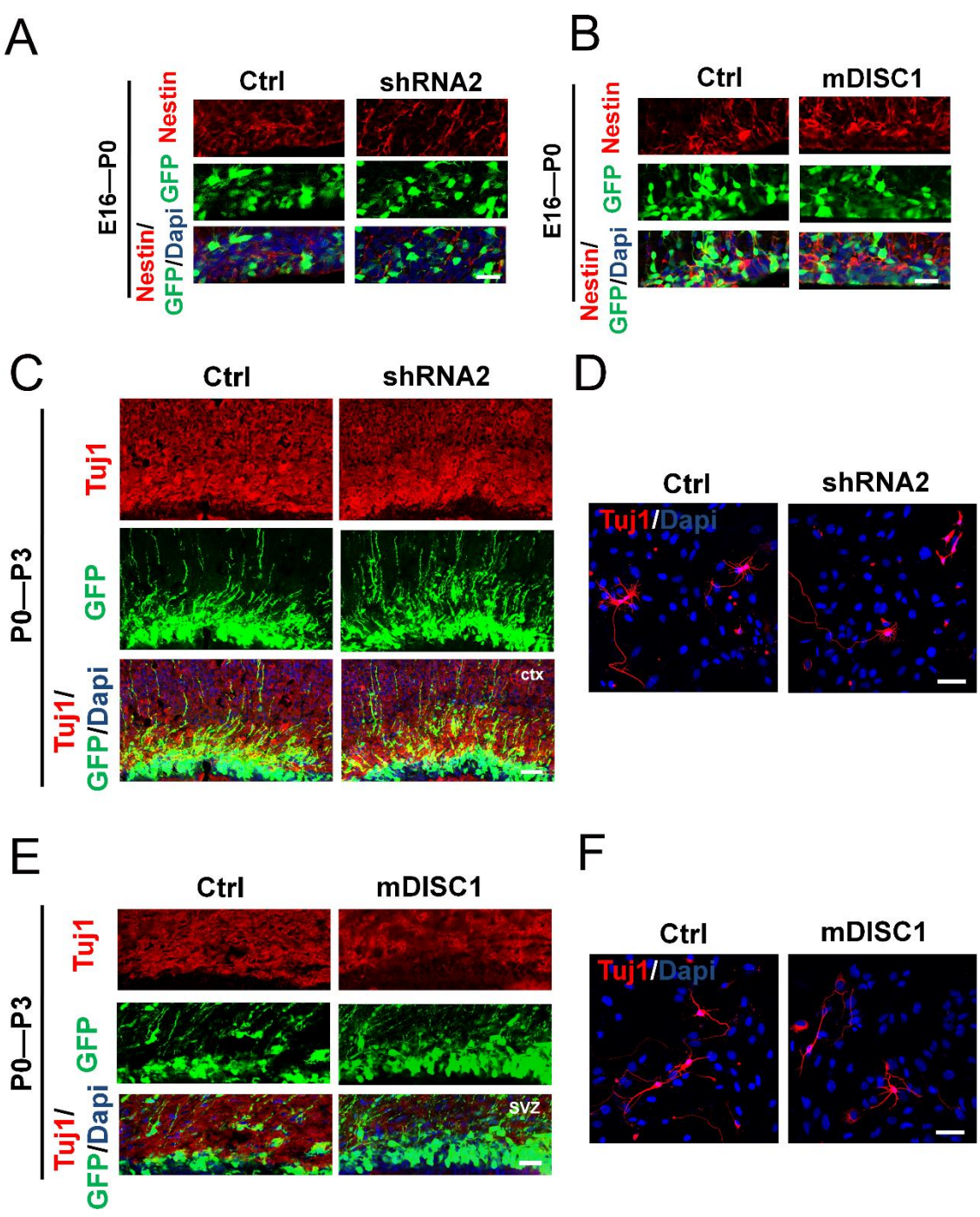


Supplementary Figure S8 DISC1 regulates astrogenesis *in vivo*.

A E16 embryonic brains electroporated with control and DISC1 overexpression plasmids were analyzed at P10. Top, images of the morphology of GFP-labeled astrocytes. Scale bar: 100μm.. Bottom, the higher-magnified view of the boxed region. Scale bar: 5μm

B E16 embryonic brains electroporated with control and DISC1 overexpression plasmids were analyzed at P16. Left, images of the morphology of GFP-labeled astrocytes. Scale bar: 100μm. Right, the higher-magnified view of the boxed region. Scale bar: 5μm.

C-D E16 embryonic brains electroporated with control and DISC1 knockdown plasmids were analyzed at intermediate times (E18 and P0). The images of GFAP immunostaining showed the effect of DISC1 knockdown on astrocyte number at different stage. Scale bar: 50μm.



Supplementary Figure S9. The effects of DISC1 on Nestin and Tuj1 positive cells.

A-B Nestin immunostaining showed the effect of DISC1 knockdown or DISC1 overexpression on the number of Nestin positive cells. Scale bar: 50μm.

C-D The effect of DISC1 on Tuj1-positive cells in P3 mouse brains electroporated with control or DISC1 shRNA2 plasmids at P0. Scale bar: 50μm.

D-F The number of neurons differentiated from P0 NPCs was not significantly affected in vitro when DISC1 was knocked down or overexpressed. Scale bar: 50μm