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

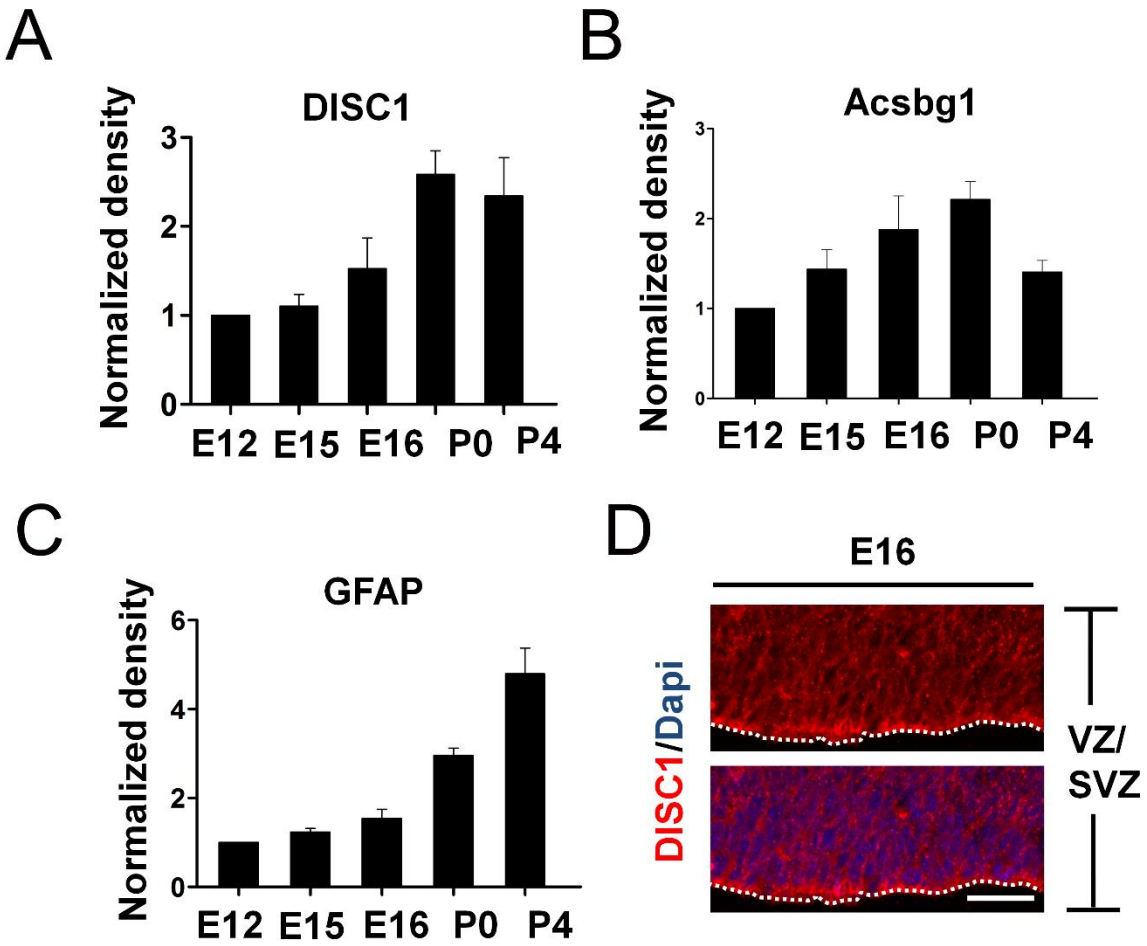
The primary antibodies used were as follows: DISC1 (rabbit, 1:100, NOVUS, NB110-40773) (Mao et al., 2009a), GFP (rat, 1:1000, MBL, D153-3), GFAP (rabbit, 1:3000,Dako, Z033429), GLAST (rabbit, 1:300, Proteintech, 20785-1-AP), FGFR3 (rabbit, 1:100, Bioworld, BS1125), Tuj1 (rabbit, 1:1000, Sigma, T2200), 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).

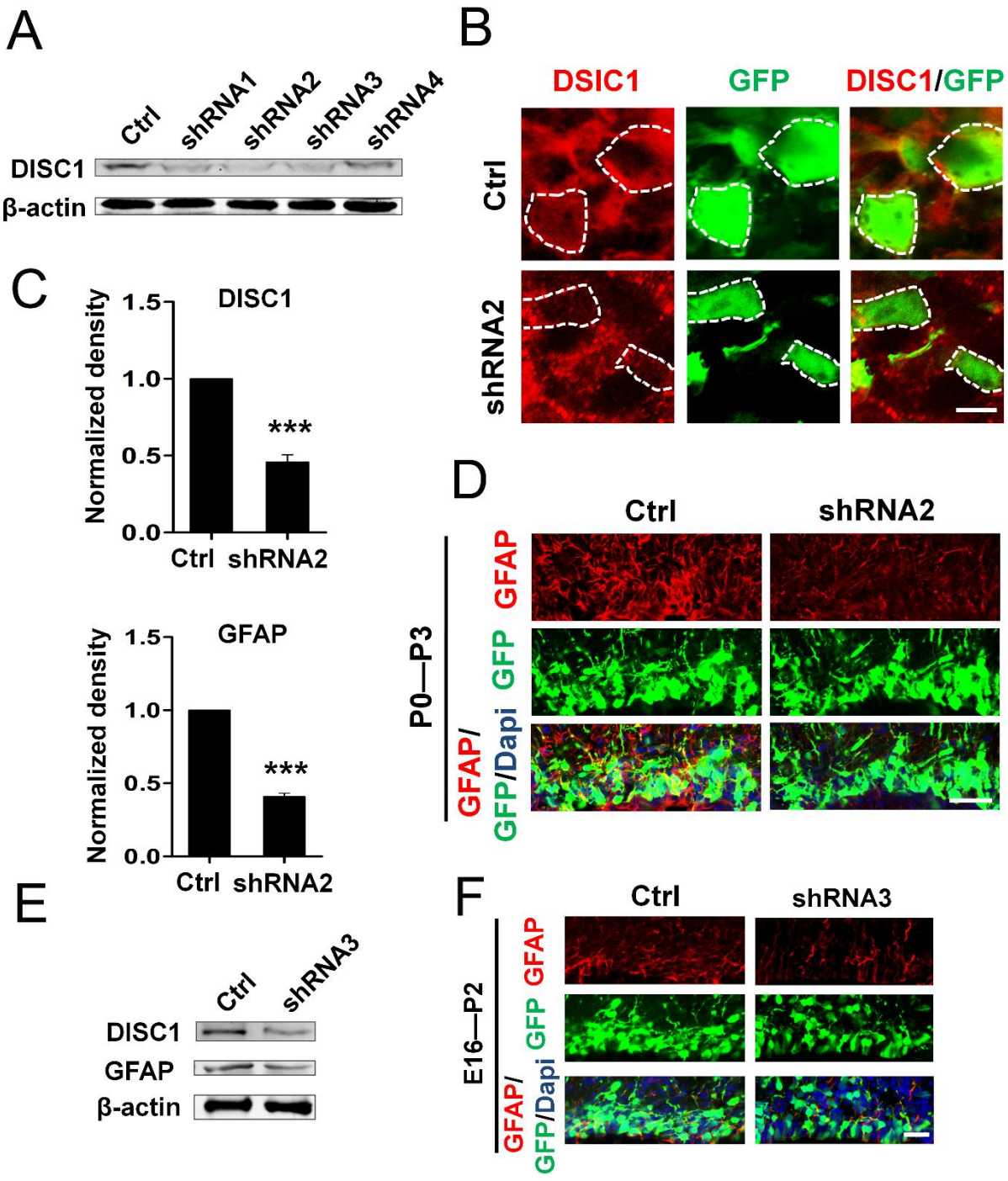
Supplementary figures



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

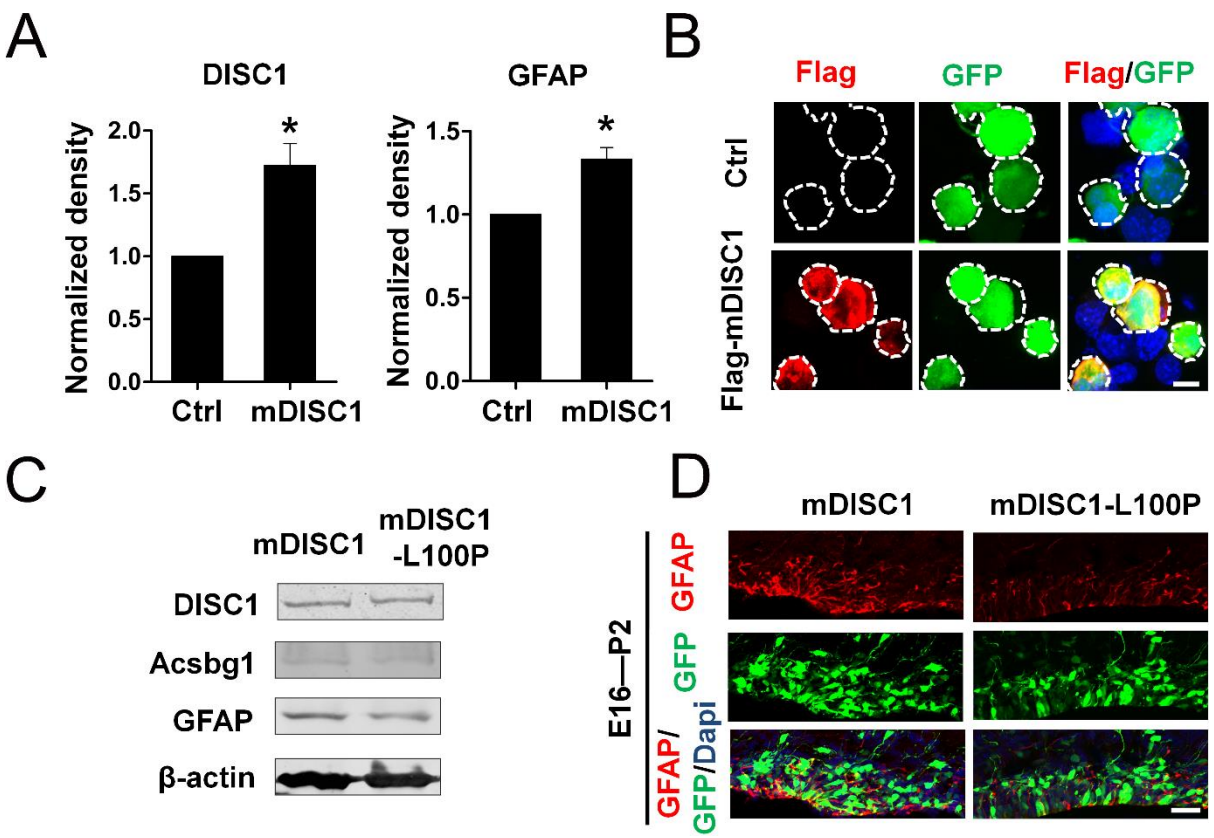
DISC1 shRNA *in vivo*. The embryos were electroporated in utero with DISC1 shRNA2 or control plasmids at E16 and developed until P2. Scale bar: 5 μm.

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.



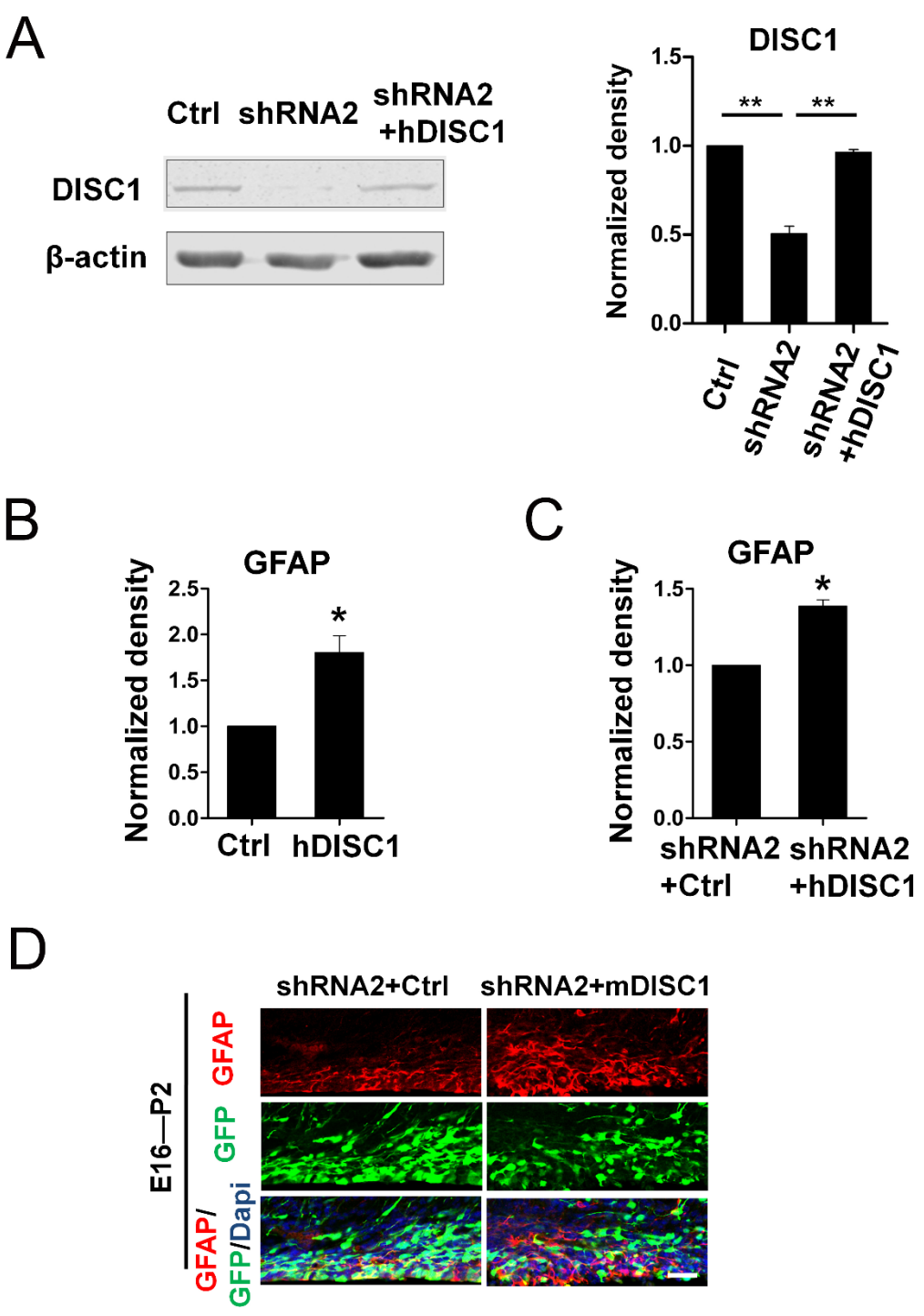
Supplementary Figure S3 DISC1 overexpression enhances gliogenesis (Related to Fig. 3).

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.



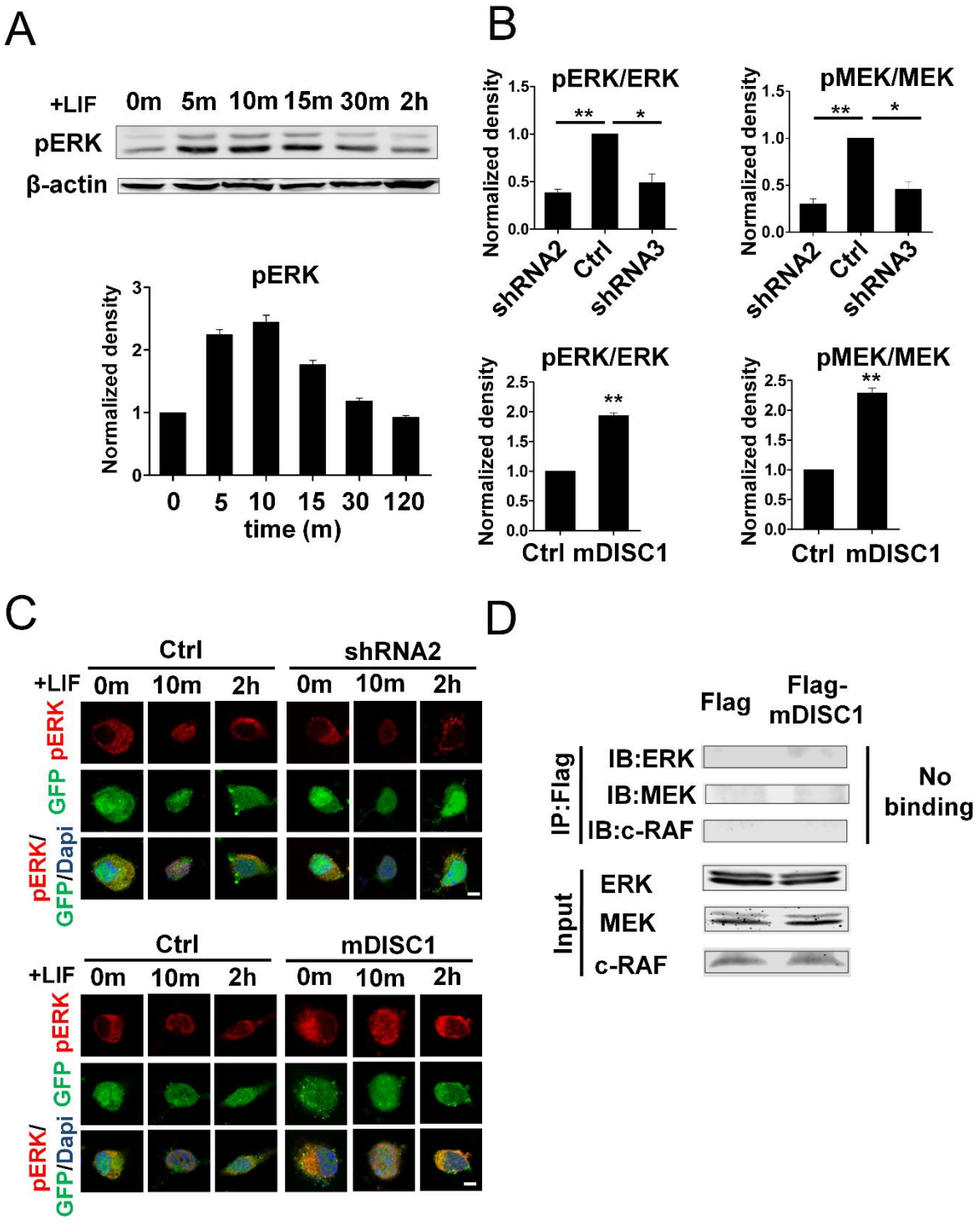
Supplementary Figure S4 DISC1 overexpression rescues astrogenesis defects caused by DISC1 depletion (Related to Fig. 4).

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).

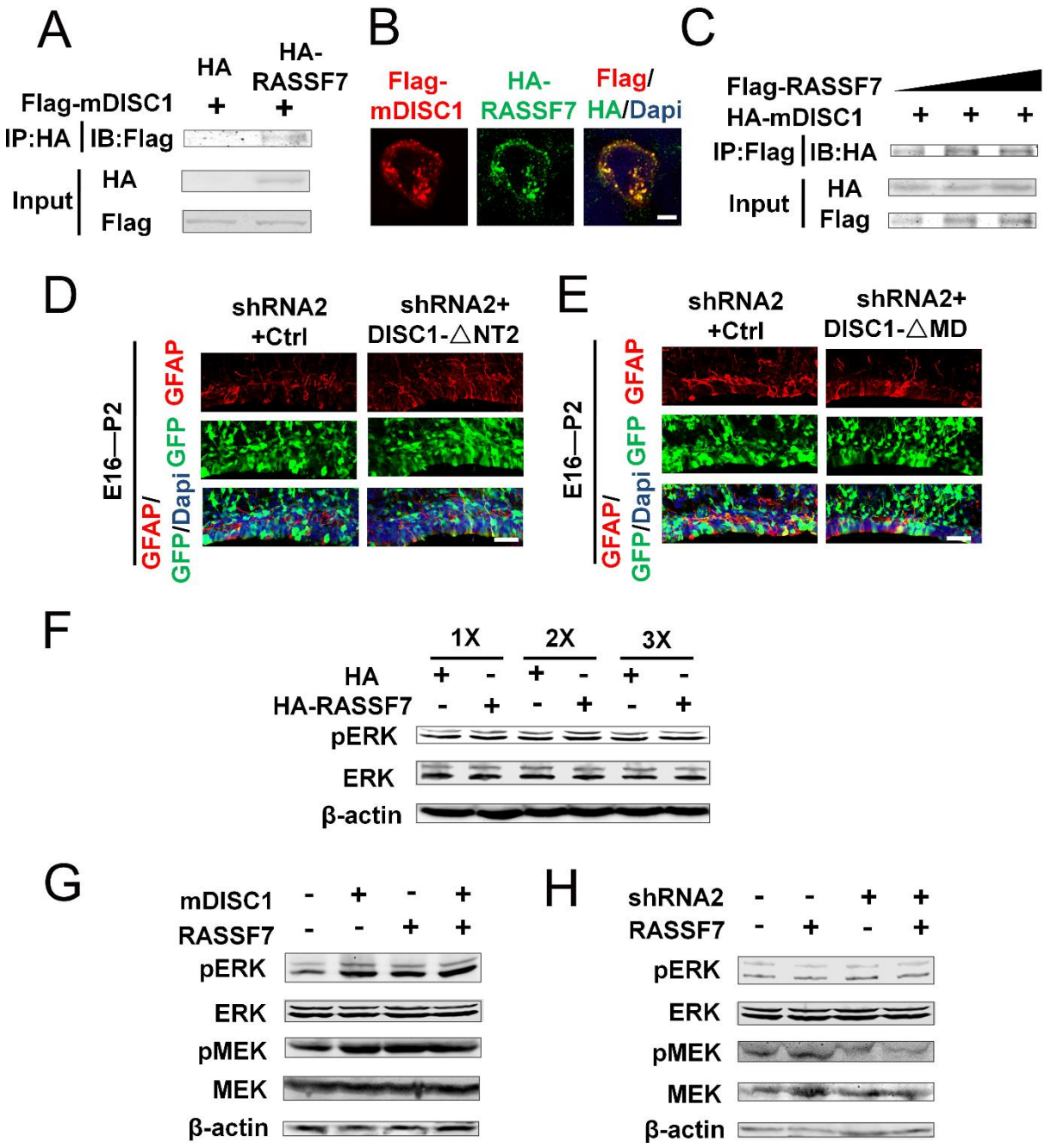
A Western blots for the expression of pERK protein in NPCs that were serum starved

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.

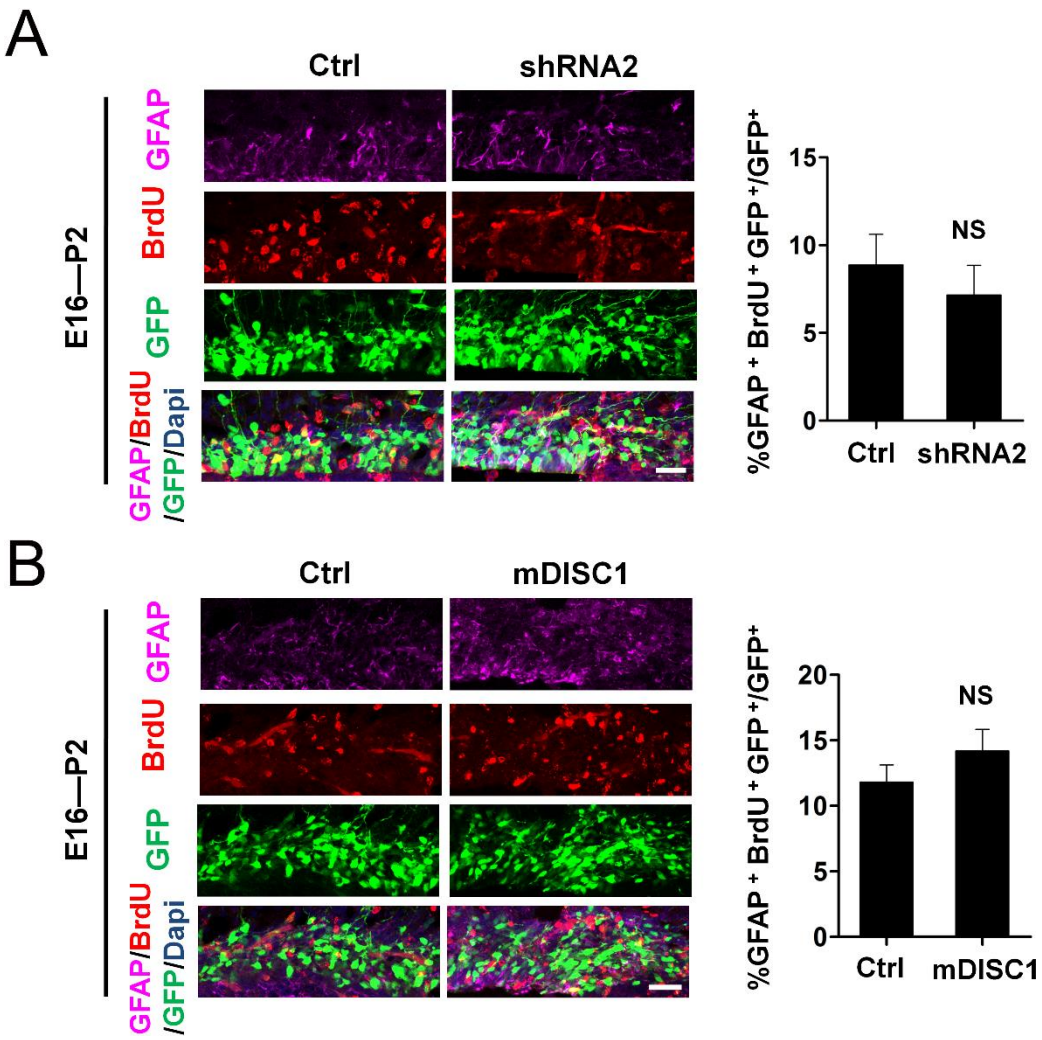
D The decrease of astrocyte number caused by mDISC1 shRNA is rescued by DISC1-△NT2 overexpression *in vivo*. GFAP immunostaining showed the astrocyte number in the E16 embryonic brains electroporated with plasmids were analyzed at P2. Scale bar: 50μm.

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.

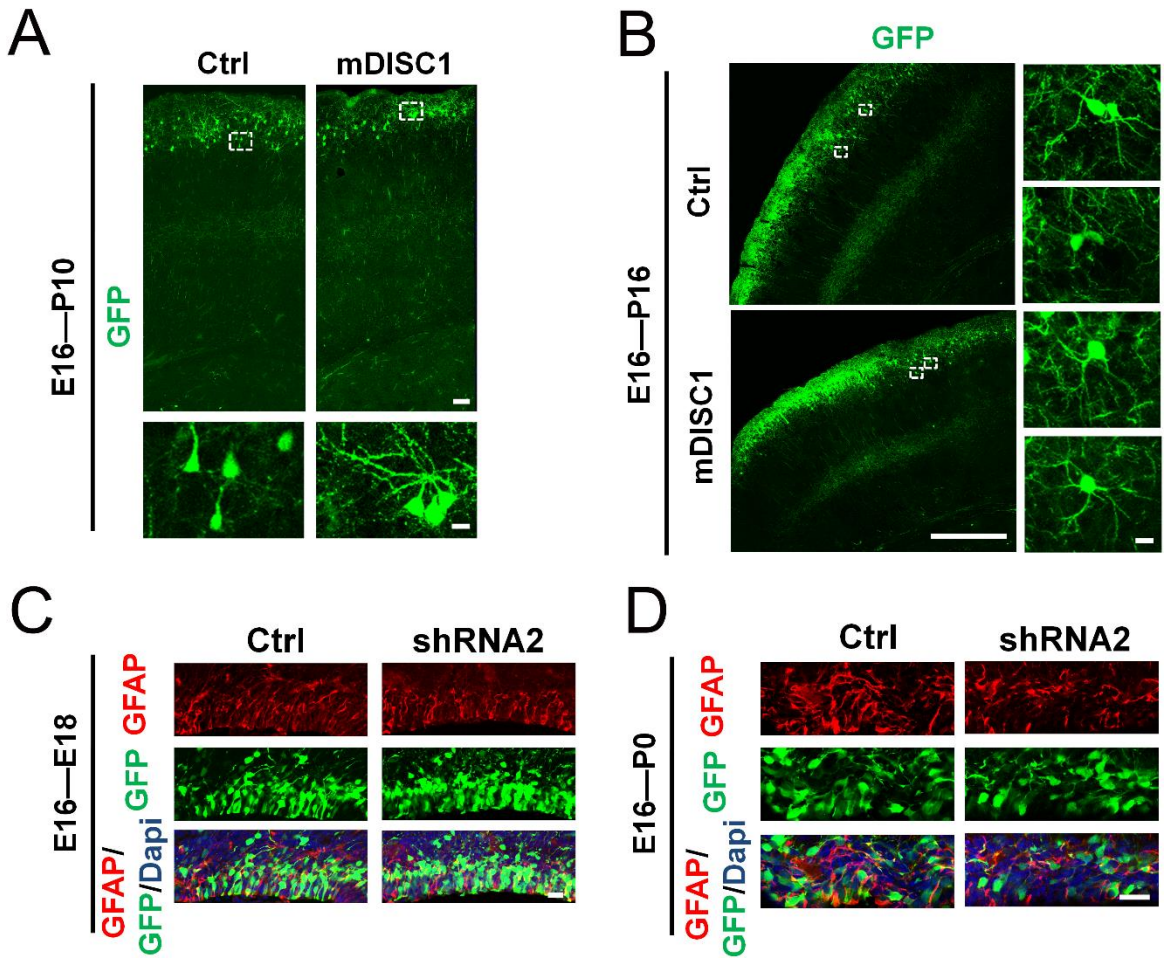
H Western blot analysis of MEK/ERK signaling. NPCs infected with DISC1 shRNA2, RASSF7 or both, were serum starved overnight and then treated with LIF (50 ng/μl) for 10 min. The cell lysates were subjected to western blot analysis for phosphorylated and total MEK, ERK.



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.

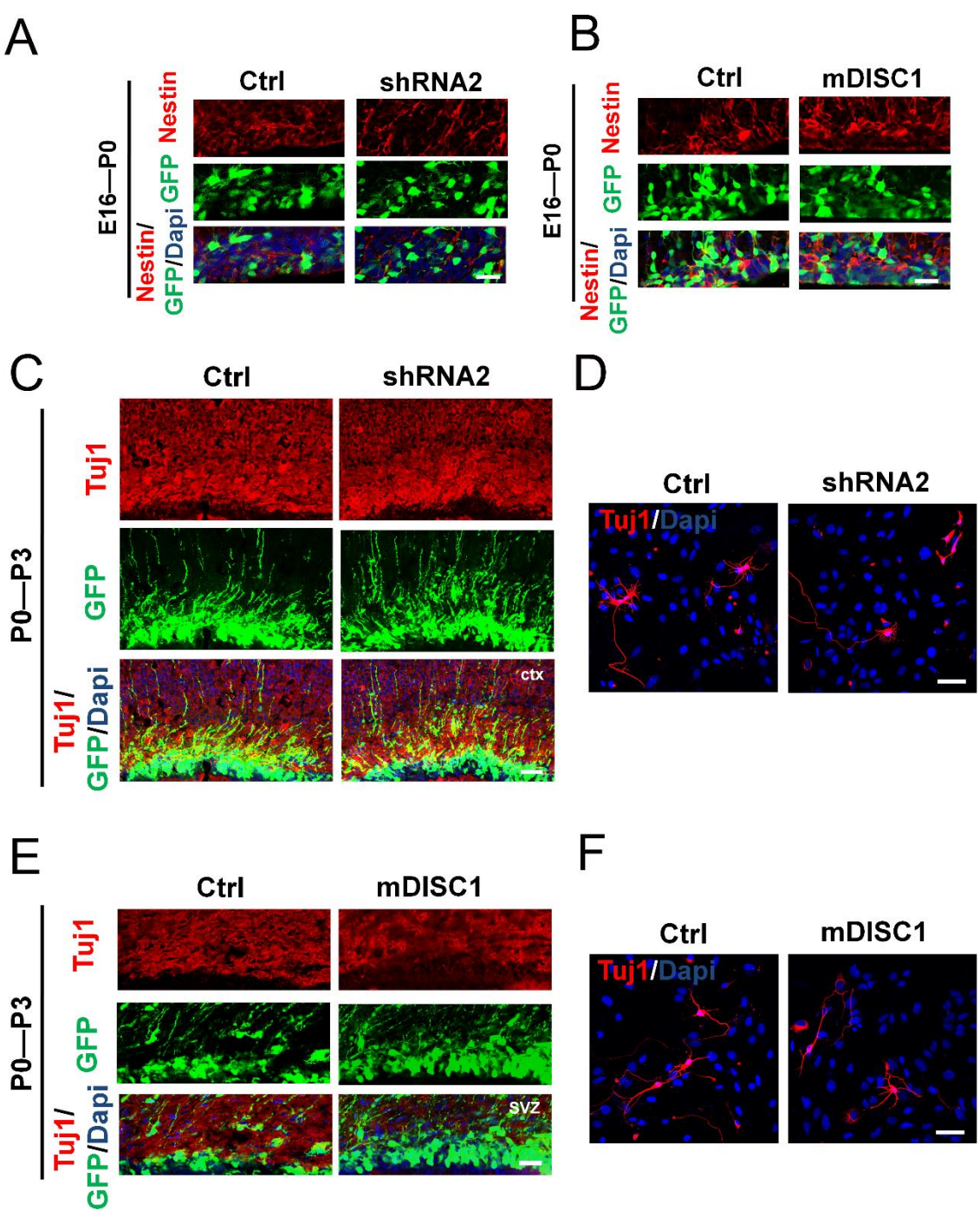


Supplementary Figure S8 DISC1 regulates astrogenesis *in vivo*.

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