**Supplementary Figure 1 | DISC1 is a phosphoprotein.**

a, Okadaic acid treatment induced significant mobility shifts of exogenous human DISC1 (hDISC1) signals in COS7 and SH-SY5Y cells, which were reshifted with additional lambda phosphatase in Western blotting. b, Western blotting of mouse brain extracts with or without calf alkaline phosphatase showed that phosphatase treatment caused endogenous DISC1 to migrate faster (asterisk), presumably due to dephosphorylation of the protein. c, Metabolic labeling of exogenous hDISC1 in COS7 cells revealed that Okadaic acid treatment accelerated $^{32}$P incorporation, which was abolished with additional lambda phosphatase.
**Supplementary Figure 2 | DISC1 is phosphorylated at Ser-710 (human Ser-713).**

**a,** Mass spectrometry analysis was performed on tryptic peptides from exogenous human DISC1 proteins in COS7 cells with or without Okadaic acid treatment. Three fragments from samples with Okadaic acid treatment showed spectral changes consistent with phosphorylation.

**b,** *In vitro* kinase study with site-directed mutagenesis revealed that Ser-713 in human C-terminal DISC1 or Ser-710 in mouse C-terminal DISC1 were phosphorylated by CDK5 and PKA.

**c,** HA-tagged wild-type (wt), phospho-dead mutant A710-, or phospho-mimic mutant E710-DISC1 was transfected with or without wt or kinase-dead PKA into HEK293 cells. An antibody generated against a phospho-peptide at S710 of mouse DISC1 (pS710 Ab) reacted with phosphorylated wtDISC1 (red arrowhead, light) and phospho-mimic E710-DISC1 (red arrowhead, right), but not with non-phosphorylated proteins. Asterisk, non-specific signal.

**d,** *In vitro* kinase study with site-directed mutagenesis to alanine (A58) revealed that Ser-58 in human N-terminal DISC1 can be phosphorylated by PKA.

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### Table: Human and Mouse DISC1 Sites

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**b** C-terminal DISC1

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**c** Full length DISC1 (mouse)

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**d** N-terminal DISC1

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**Supplementary Figure 3**  |  Phosphorylation of DISC1 at Ser-710 (S710) selectively increases binding of DISC1 with BBS proteins.

**a.** Original images corresponding to the graph in Fig. 1a.

**b.** Immunoprecipitation of the cell extract transfected with HA-tagged wt, A710-, or E710-DISC1 with GAPDH Ab or normal IgG indicates no DISC1 signal in the immunoprecipitates, supporting specific precipitation of DISC1 by anti-BBS1 antibody in Fig. 1b.

**c.** No difference in the affinity of NDEL1 with wt, A710- and E710-DISC1 detected by co-immunoprecipitation from HT22 cells with HA-tagged DISC1 and myc-NDEL1.

**d.** HA-tagged wild-type (wt) or phospho-dead mutant A58 DISC1 (A58-DISC1) with myc-tagged BBS1 were co-transfected in the HT22 cells with or without Okadaic acid. The binding between DISC1 and BBS1 was examined by co-immunoprecipitation. Okadaic acid treatment increased protein interactions of BBS1/A58-DISC1 and BBS1/wtDISC1 at the equal level. Error bars indicate SEM. *P < 0.05.
**Supplementary Figure 4** | **Phosphorylation of DISC1 at Ser-710 enhances recruitment of BBS1 at the centrosome.**

- **a.** Immunoreactivity of the BBS1 antibody used in this study, which was examined with brains from *Bbs1* knockout and wild-type mice. Specific signal at the expected size of BBS1 protein was obtained in Western blotting (red arrowhead). Specific staining of BBS1 (in green) was observed only in wild-type cells, but not in knockout cells. Scale bar, 10 µm.

- **b.** Negative controls for the immunostaining shown in Fig. 1c. Staining by the exact same condition except lack of each primary antibody (against HA, BBS1, or γ-tubulin) confirmed the specificity of the staining. White circle and while line indicate the centrosome and the whole cell region, respectively. Scale bar, 20 µm.

- **c.** The phosphorylation status of DISC1 does not affect the levels of BBS1 protein in cortical neurons, since no appreciable changes of endogenous BBS1 protein levels was detected among the cells transfected with wt, A710, or E710-DISC1 in cortical neurons.

- **d.** Specific immunoreactivities (red arrow heads) of anti-DISC1 (hExon2) and -γ-tubulin (commercial antibody from Sigma) antibodies used in experiments of Supplementary Fig. 4e.

- **e.** Accumulation of BBS1 into γ-tubulin-enriched fractions by phospho-mimic mutant E710E-DISC1, but not by phospho-dead mutant A710-DISC1. Fractions obtained in HEK293 cells by a discontinuous sucrose gradient were subjected to Western blotting. The sample with phospho-mimic mutant E710-DISC1 showed concentrated BBS1 protein in the γ-tubulin-enriched fractions. Of note, in order to show all the lanes, we have combined Western data from more than one film into one image.
Supplementary Figure 5 | Phosphorylated DISC1 at S710 (pS710 DISC1) localizes to the centrosome and is enriched in γ-tubulin-enriched fractions.

a, Immunoreactivity of pS710 antibody in cell extracts from E18 embryonic brain. Specific signal at the expected size of full-length DISC1 was observed (red arrowhead). Preabsorption was performed with the phospho-peptide antigen.

b, Localization of pS710 DISC1 stained with the pS710 Ab in both cortical neurons and in PC12 cells. In each case, endogenous pS710 DISC1 (red) colocalized with γ-tubulin (green). Blue indicates the nucleus. Scale bar, 20 µm.
Supplementary Figure 6 | A710-DISC1 and E710-DISC1 bind preferentially with GSK3β and BBS1, respectively.

Purified MBP-tagged wild-type (wt) DISC1 interacted directly with purified GST-GSK3β and GST-BBS1 in vitro. A710-DISC1 bound preferentially with GSK3β, whereas E710-DISC1 associated preferentially with BBS1. Error bars indicate SEM. *P < 0.05.
Supplementary Figure 7 | Immunoreactivity of pS710 antibody in the embryonic brain. High magnification images of Fig. 3b. Scale bar, 10 μm.
Supplementary Figure 8 | pS710-DISC1 dependent switch of DISC1-GSK3β to DISC1-BBS1 binding in mitotic progenitors and postmitotic neurons in vivo.

a, Representative FACS plots for Nestin-KOr (left) and DCX-GFP (right) cells, respectively.
b, Progenitor cells and post-mitotic neural cells were purified by FACS from the brains of transgenic mice expressing Nestin promoter-driven Kusabira-Orange and DCX promoter-driven EGFP, respectively. DISC1-GSK3β binding was enhanced in mitotic progenitors, while DISC1-BBS1 binding was augmented in post-mitotic neurons. pS710-DISC1-BBS1 binding was significantly greater in post-mitotic neurons compared to that in mitotic progenitors, whereas there was almost negligible binding of pS710-DISC1 with GSK3β.
Supplementary Figure 9 | Aberrant radial migration in BBS1 KO mice.

a, BrdU birth-dating analysis: Significant migration defect was observed in Bbs1 knockout mice compared with wild-type littermates, when BrdU was injected at E15 and the final positioning of the late-born superficial layer neurons was analyzed at P0. Scale bar, 50 μm. Error bars indicate SEM. *P < 0.001.

b, GFP was injected at E15 via in utero gene transfer and the final positioning of neurons was analyzed at E19. Bbs1 knockout mice showed delayed migration compared to wild-type littermates. Scale bar, 50 μm. Error bars indicate SEM. *P < 0.001.
**Supplementary Figure 10** | Co-transfection efficiency of *in utero* gene transfer and definition of the ventricular zone (VZ), subventricular zone (SVZ), and intermediate zone (IZ) in E15 brain.

**a.** Two constructs (the first construct expressed shRNA to DISC1 and GFP and the second construct expressed HA-tagged wt, A710-, or E710-DISC1) were injected to the embryonic brain at E13, followed by the analysis at E15. We found almost identical overlap between GFP-positive and HA-positive cells. Scale bar, 10 µm. GFP-positive and -negative cells were purified by FACS from *in utero* electroporated brains, and subjected to Western blotting with HA antibody. Consistent with immunostaining, HA signals (red asterisk) were observed in GFP-positive, but not in GFP-negative, cells.

**b.** The VZ/SVZ boundary was defined by the segregation of Pax6- and Tbr2-positive cells. IZ was determined as Tbr2-negative and DCX-positive area. In addition, morphological characteristics were used as indicators of VZ/SVZ and SVZ/IZ boundaries in this study: VZ and SVZ were separated by existence of multipolar cells (arrowhead), and SVZ and IZ were divided by cell density detected by DAPI staining. Scale bar, 20 µm.
Supplementary Figure 11 | Suppression of DISC1 leads to defects in cell proliferation. The results shown in Fig. 4 were reproduced by DISC1 knockdown with another shRNA to DISC1 (DISC1 RNAi#2). Scale bar, 20 μm. Error bars indicate SEM. *P < 0.05, †P < 0.01.
**Supplementary Figure 12 | Progenitor proliferation-related phenotypes caused by knockdown of DISC1.**

**a,** BrdU incorporation assay. Cells incorporating BrdU were reduced in the VZ at E15, which were rescued by wt and phospho-dead A710-DISC1, but not by phospho-mimic E710-DISC1. Green, GFP (cells with DISC1 knockdown); red, BrdU; Yellow, merge. Scale bar, 20 μm. Error bars indicate SEM. *P < 0.05, †P < 0.01.

**b,** Cell cycle exit assay. Cells exiting the cell cycle were GFP-positive, BrdU-positive, and Ki67-negative (arrowheads). DISC1 knockdown at E13 increased the premature cell cycle exit (the percentage of GFP-positive/BrdU-positive/Ki67-negative cells to GFP-positive/BrdU-positive cells) assayed at E15. This increase was rescued by wt and A710-DISC1, but not by E710-DISC1. White arrows, GFP-positive/BrdU-positive/Ki67-negative cells. Scale bar, 20 μm. Error bars indicate SEM. *P < 0.05.

**c,** Cell fate assay. DISC1 knockdown at E13 increased the percentage of GFP/DCX double-positive cells (white arrows) to total GFP-positive cells in VZ/SVZ, which was rescued by wt and A710-DISC1 but not by E710-DISC1. This result is consistent with that from cell cycle exit assay. Scale bar, 20 μm. Error bars indicate SEM. *P < 0.05, †P < 0.01.

**d,** Knockdown of DISC1 at E13 leads to no significant change in N-cadherin at E15. Scale bar, 20 μm.
Supplementary Figure 13 | Suppression of DISC1 leads to defects in migration.

The results shown in Fig. 4 were reproduced by DISC1 knockdown with another shRNA to DISC1 (DISC1 RNAi#2). Scale bar, 50 μm. Error bars indicate SEM. *P < 0.001.
Injection E13, Analysis E15

**Supplementary Figure 14**  |  Knockdown of DISC1 at E13 leads to no significant change in the radial fiber elongation at E15.

Radial glia labeling with radial-glial-specific RC2 antibody at E15. Radial glial fiber elongation was indistinguishable between brains electroporated with control RNAi and those electroporated with DISC1 RNAi at E13. Scale bar, 20 μm.
**Supplementary Figure 15**  Migration phenotype in BBS1 knockout mice is not normalized by wt DISC1.

*In utero* gene transfer at E15 with wt DISC1 was analyzed at E19 in BBS1 knockout mouse brains. Migration phenotype in BBS1 knockout mice was not affected by knockdown or overexpression of DISC1. Scale bar, 50 µm.
Injection E15, Analysis E19

Control RNAi DISC1 RNAi#2 + DCX-wt DISC1 + DCX-A710-DISC1 + DCX-E710-DISC1

Migrated Cells

Supplementary Figure 16  DCX promoter-driven wt and E710-DISC1 (DCX-wt DISC1 and DCX-E710-DISC1) rescue migration defects induced by DISC1 knockdown in the developing cortex. 

In utero gene transfer at E15 with DCX-wt, A710-, or E710-DISC1 was analyzed in at E19. Overexpression of wt and E710-DISC1 under DCX promoter resulted in phenotypes similar to those obtained from overexpression of these protein under the control of the CAG promoter (expression in both mitotic and postmitotic cells), suggesting that DISC1 mediates migration that is distinct from its effect on progenitor proliferation. Scale bar, 50 µm. Error bars indicate SEM. *P < 0.001.