Supporting Information

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SI Materials and Methods

Recombinant Protein Production. Recombinant NRG-GST proteins (NRG-1β, -2β, and -3) and GST control were a gift from Dr. Cary Lai and were produced as previously described (1). Proteins were expressed in Origami or Origami B cells (Novagen). Protein aliquots were stored at −80 °C until use.

Virus Production and Infection. Lentiviral constructs were generated by subcloning of shRNA sequence and ^{1}H promoter into the Pac1 site of the FUGW lentiviral backbone. Virus was generated as described previously (2), by cotransfection of lentiviral and helper constructs (Δ8.9 and VSVG) into 293FT cells, harvesting of the supernatant fraction after 48–72 h, and filtration and concentration of virus by ultracentrifugation at 25,000 × *g* for 90 min. ShRNA sequences used were as follows: ErbB4, 5′-CCAGACTACCTGCAGGAATAC-3′; ErbB3, 5′- AATCATC-CAGCA CTTGAC-3′; ErbB2, 5′-TTGCAATGATGAATGT-CACAT-3′; Control, scrambled sequence (3).

Subcellular Fractionation. Crude fractionation of mouse brain tissue was carried out as previously described (4). Mice were killed at postnatal day 0 (P0), and their brains removed and dissected on ice to obtain cortical tissue. Tissue was homogenized in TEVP buffer containing 320 mM sucrose (10 mM Tris, 5 mM NaF, 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, 320 mM sucrose, pH 7.4) and centrifuged at 800 × *g* for 10 min to obtain pellet P1. Supernatant was removed and centrifuged at 9,200 × *g* for 15 min to obtain pellet P2. Supernatant was again removed and ultracentrifuged at 165,000 × *g* for 2 h to obtain pellet P3 and supernatant S1. All pellets were resuspended in TEVP buffer and sonicated once.

Immunohistochemistry in Mouse Brains. Staining for DISC1 in NRG1-KO mice with anti-DISC1 antibody mExon3 was performed as previously described (5). Quantification of DISC1 expression was done by densitometric analysis of fluorescent intensity in 25 cells per group, normalized to background intensity. Staining in wild-type and BACE1-KO was performed similarly: mice were perfused with 4% paraformaldehyde, and their brains were dissected and frozen in OCT medium, then sectioned at 20 μm thickness with a cryostat and mounted on poly-L-lysine–coated slides (Electron Microscopy Sciences). Sections were permeabilized (0.05% Triton X-100 in PBS) and blocked (10% normal goat serum) before incubation overnight at 4°C with primary antibody mExon3. Elite Vectastain ABC Rabbit IgG and DAB Peroxidase Substrate kits (Vector Labs) were used to visualize staining according to the manufacturer's protocols. For fluorescent costaining of GFAP and DISC1, sections were incubated in anti-mExon3 DISC1 antibody, or mExon3 and rat anti-GFAP overnight, then washed and incubated with secondary goat anti-mouse Alexa 488 and/or goat anti rabbit Alexa 568. Sections were washed and stained with DAPI before mounting.

1. Ghashghaei HT, et al. (2006) The role of neuregulin-ErbB4 interactions on the proliferation and organization of cells in the subventricular zone. *Proc Natl Acad Sci USA* 103:1930–1935.
2. Sen N, et al. (2009) GOSPEL: A neuroprotective protein that binds to GAPDH upon S-nitrosylation. *Neuron* 63:81–91.
3. Kamiya A, et al. (2005) A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat Cell Biol* 7:1167–1178.
4. Hallett PJ, Collins TL, Standaert DG, Dunah AW (2008) Biochemical fractionation of brain tissue for studies of receptor distribution and trafficking. *Curr Protoc Neurosci* 42:1.16.1-1.16.16.
5. Yokota Y, et al. (2007) Radial glial dependent and independent dynamics of interneuronal migration in the developing cerebral cortex. *PLoS One* 2:e794.

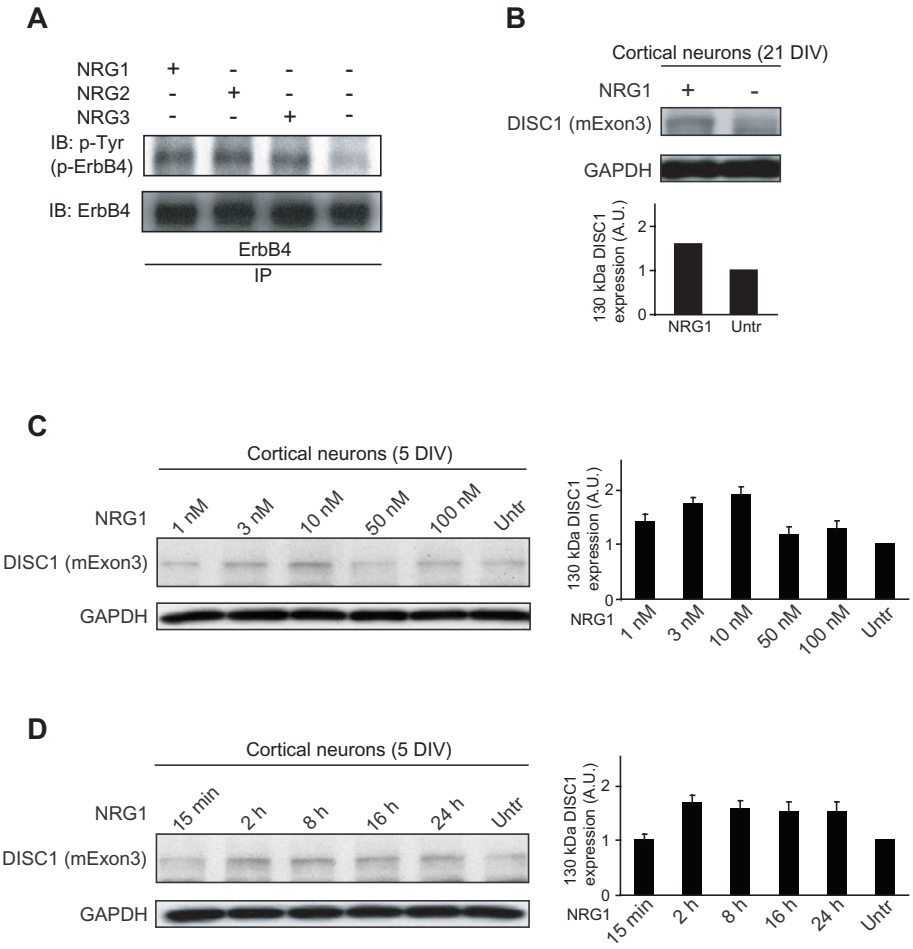

Fig. S1. Treatment with recombinant NRG proteins increases 130 kDa DISC1 expression. (*A*) Confirmation of bioactivity of recombinant NRG proteins. HEK-293FT cells overexpressing ErbB4 were treated with recombinant NRG proteins at 10 nM concentration for 15 min. ErbB4 was immunoprecipitated and Western blotting was performed for phospho-tyrosine, showing increased activation in lysates of cells treated with NRG1, NRG2, or NRG3. (*B*) NRG1 treatment induces 130 kDa DISC1 expression in mature neurons. Primary cortical neurons at 21 DIV were treated with recombinant NRG1 at 3 nM concentration for 2 h, and expression of 130 kDa DISC1 assessed by Western blotting with antibody mExon3. Increased expression of this isoform was observed. (*C*) Dose–response of induction of 130 kDa DISC1 to NRG1 treatment. Immature primary neurons were treated with recombinant NRG1 protein at 1 nM, 3 nM, 10 nM, 50 nM, or 100 nM concentrations, and DISC1 expression was assayed by Western blotting with mExon3. The 130 kDa DISC1 isoform was strongly induced by 3 nM NRG1 (quantification done by densitometric analysis, $n = 3$). (*D*) Time course of induction of 130-kDa DISC1 by NRG1. Immature primary neurons were treated with recombinant NRG1 protein at 3 nM concentration for 15 min, 2 h, 8 h, 16 h, and 24 h, and DISC1 expression was assayed by Western blotting with mExon3. The 130-kDa DISC1 isoform was maximally induced at 2 h and continued to be elevated up to 24 h after addition of NRG1 (quantification done by densitometric analysis, $n = 3$).

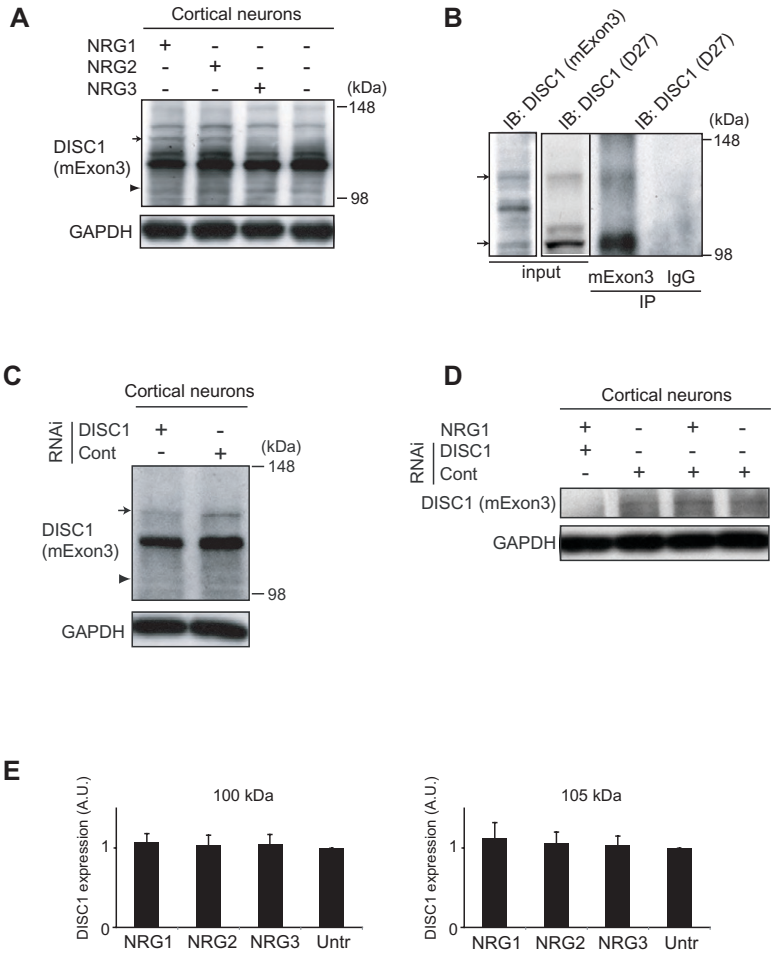

Fig. S2. Further characterization of 130-kDa DISC1 induced by NRG1, in contrast to 100- and 105-kDa DISC1. (*A*) Induction of 130-kDa expression detected by mExon3. Immature primary cortical neurons were treated with recombinant NRG1, NRG2, or NRG3 at 3 nM concentration for 2 h, and Western blotting was done for DISC1 with antibody mExon3. A selective increase in intensity of a band at 130 kDa was seen, similar to results obtained with antibody D27. (*B*) Validation of 130-kDa band as DISC1. Primary neuron cultures lysates were immunoprecipitated with mExon3, and Western blotting was performed with D27. A 130-kDa band is consistently observed in cell lysates as well as immunoprecipitated samples, indicating that this band represents a form of DISC1. (*C*) Effect of DISC1 RNAi on 130 kDa DISC1. Immature primary neurons were infected with lentivirus expressing an established shRNA to DISC1 (3) or its scrambled control sequence. Cells were harvested 7 days after infection and Western blotting was performed using DISC1 antibody mExon3. Expression of DISC1 shRNA resulted in knockdown of the 130 kDa isoform compared with control shRNA, implying that this band represents DISC1. (*D*) DISC1 RNAi prevents induction of 130 kDa band by NRG1. Immature primary neurons were infected with lentivirus expressing DISC1 shRNA or scrambled control shRNA, then treated with NRG1 at 3 nM concentration for 2 h and harvested 7 days later. Western blotting was performed using DISC1 antibody mExon3. No induction of a band at 130 kDa was observed in NRG1-treated DISC1 RNAi-infected neurons, indicating that this band represents an isoform of DISC1. (*E*) Treatment with recombinant NRG proteins does not affect 100 kDa or 105 kDa DISC1 expression. Immature primary cortical neurons were treated with recombinant NRG1, NRG2, or NRG3, at 3 nM concentration for 2 h and Western blotting was done for DISC1 with antibody D27. Expression of 100 and 105 kDa DISC1 was quantified by densitometric analysis. No change was observed in expression levels of this isoform following NRG treatment.

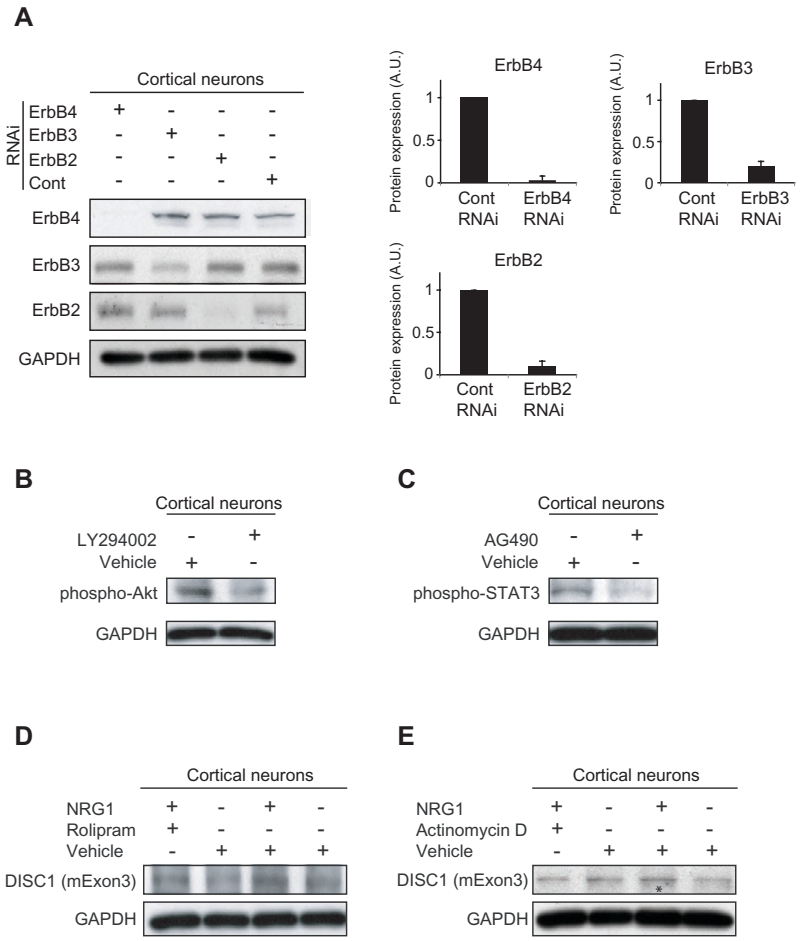

Fig. S3. Knockdown of ErbB receptors by RNAi and secondary signaling pathways by pharmacological agents. (*A*) Specific knockdown of ErbB receptors by RNAi. Western blotting for ErbB4, ErbB3, and ErbB2 was performed in lysates from primary neurons infected with lentivirus expressing shRNA to each receptor, confirming specific knockdown of each individual protein and absence of off-target effects. Knockdown of each receptor was quantified by densitometric analysis ($n = 3$). For ErbB4, 97 ± 5% knockdown; for ErbB3, 80 ± 8% knockdown; and for ErbB2, 90 ± 6% knockdown. (*B*) Blockade of PI3K/Akt signaling by LY294002. Immature primary neurons were treated with PI3K/Akt signaling blocker LY294002 or vehicle (DMSO) under the described conditions (*Materials and Methods*), and Western blotting was done to measure levels of phospho-Akt. Phosphorylation of Akt was reduced by treatment with LY294002. (*C*) Blockade of JAK2/STAT3 signaling by AG490. Immature primary neurons were treated with JAK2/STAT3 signaling blocker AG490 or vehicle (DMSO) under the described conditions (*Materials and Methods*), and Western blotting done to measure levels of phospho-STAT3. Phosphorylation of STAT3 was reduced by treatment with AG490. (*D*) No contribution of PDE4 signaling to induction of DISC1 by NRG1. Immature primary neurons were treated with NRG1 with or without cotreatment with PDE4 inhibitor rolipram, and DISC1 expression assayed by Western blotting with mExon3. Rolipram cotreatment did not affect induction of 130-kDa DISC1 by NRG1 treatment. (*E*) Involvement of transcription-associated mechanism in the induction of DISC1 by NRG1. Immature primary neurons were treated with NRG1 with or without cotreatment with transcription inhibitor actinomycin D, and DISC1 expression assayed by Western blotting with mExon3. Cotreatment of actinomycin D with NRG1 prevented induction of 130-kDa DISC1.

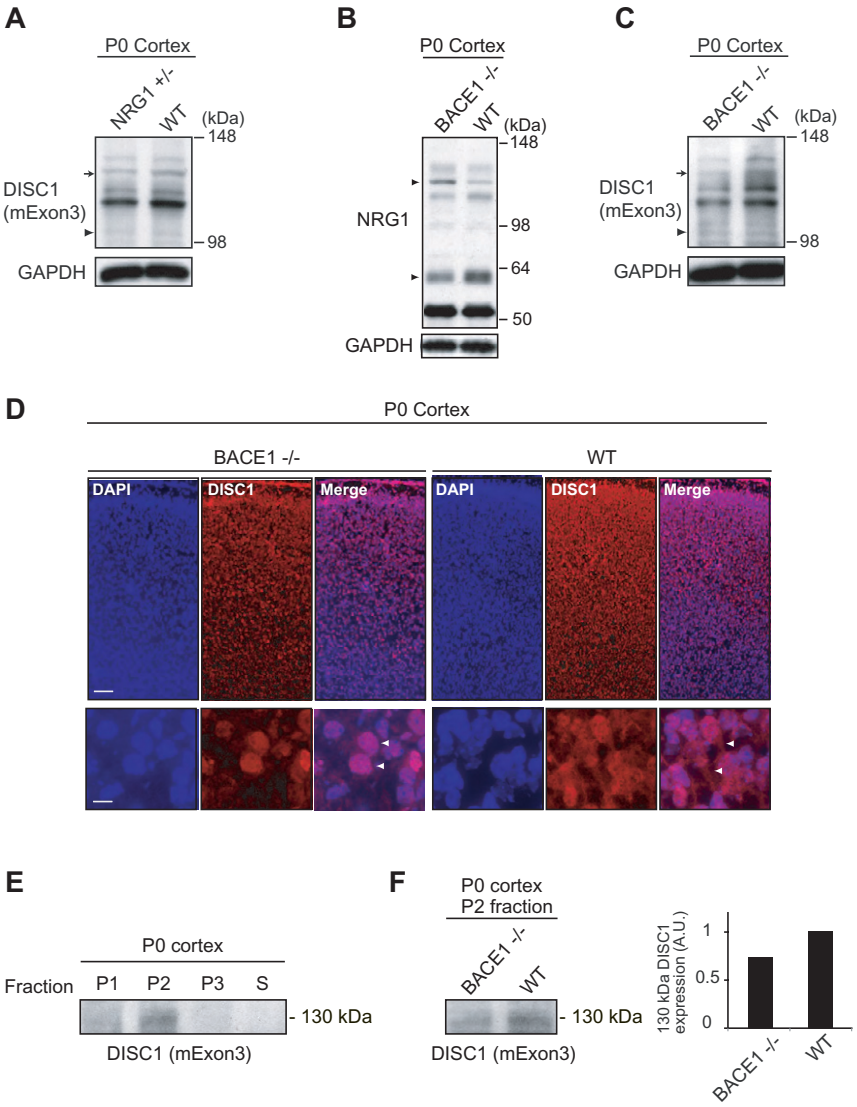

Fig. S4. Expression change and subcellular localization of 130-kDa DISC1 isoform in NRG1- and BACE1-KO mice. (*A*) Reduced 130-kDa DISC1 expression in NRG1-KO mice. Expression of 130-kDa DISC1 in cortical tissue samples from heterozygous NRG1-KO mice at P0 was assessed by Western blotting for DISC1 using antibody mExon3. (*B*) Impaired NRG1 processing in BACE1-KO mice. Western blotting was done for NRG1 with C-terminal directed antibody sc-348 in cortical lysates of BACE1-KO mice at postnatal day 0 (P0), showing reduction in cleaved NRG1 (~60 kDa) and accumulation of full-length, uncleaved NRG1 (130 kDa) in homozygous knockout mice. (*C*) Reduced 130-kDa DISC1 expression in BACE1-KO mice. Expression of 130-kDa DISC1 in cortical tissue samples from homozygous BACE1-KO mice at P0 was assessed by Western blotting for DISC1 using antibody mExon3. (*D*) Neurite-specific reduction in DISC1 expression in BACE1-KO mice. Immunohistochemistry was done for DISC1 with antibody mExon3 in homozygous BACE1-KO mice and wild-type littermates at P0, showing reduced DISC1 expression in the cortex. Expression appeared to be lost selectively in neurites (arrowheads). Magnification, 200× and 630×. (Scale bar, 50 μm and 10 μm.) (*E*) Localization of 130-kDa DISC1 to the P2 fraction in the mouse cortex. Western blotting for DISC1 using the mExon3 antibody showed localization of 130-kDa DISC1 to the P2. (*F*) Reduced 130-kDa DISC1 expression in the P2 fraction of BACE1-KO mice. Western blotting for DISC1 using mExon3 was performed in cortical tissue from homozygous BACE1-KO mice at P0 and their wild-type littermates, and 130-kDa DISC1 expression was found to be reduced in the P2 fraction (26% reduction, $n = 3$ mice pooled per sample).

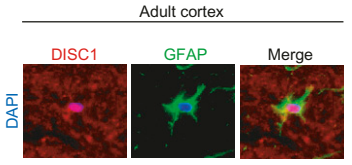

Fig. S5. Expression of DISC1 in astrocytes in vivo. Immunohistochemistry for DISC1 using antibody mExon3 and astrocyte marker GFAP was performed in brain tissue sections from adult mice, showing coexpression of DISC1 and GFAP in cortical astrocytes.