**Supporting Information**

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**SI Materials and Methods**

**Caenorhabditis elegans and Culture Methods.** Worm cultures were maintained as described (1). The mutant alleles used in this study were unc-73 (rh40) I; max-2 (cy2) II; ced-10 (n1993) IV, en-1 (mg360) IV; max-1 (ju39) V; and dds12[Punc-47::GFP, lin-15 (+)] X, pak-1 (ok448) X, mig-2 (mu28) X. For RNAi experiments, dsRNA was microinjected into the gonads of young adult animals as described (2). All RNAi experiments were performed in ori-1 genetic background. For the rolipram experiments, L4 worms were incubated in M9 solution with or without rolipram. During the incubation, *Escherichia coli* OP50 strain was added to keep animals in a well-fed state. The axon guidance phenotypes of their progeny were scored when they reached L4 or young adult stage.

Mouse DISC1 (mDISC1) and the unc-25 promoter were inserted into vector pPD95.75. Strain N2 was injected with punc-25::GFP::mDISC1 constructs (100 ng/μL) and punc-25::mRFP (5 ng/μL; a gift from Ken-Ichi Ogura, Yokohama City University Graduate School of Medicine, Yokohama, Japan) (3) to produce worms expressing mDISC1 and monomeric red fluorescent protein (mRFP). Transgenes were integrated by exposing animals to 4.5',8-trimethylpsoralen combined with UV light, and animals were out-crossed five times.

**Molecular Biology.** Cloning of DNA and generation of transgenes were accomplished by standard techniques and confirmed by sequencing. The full-length mDISC1 cDNA was provided by Hongjun Song, Johns Hopkins University School of Medicine, Baltimore, MD (4). Different fragments of mDISC1 were amplified by PCR and subcloned to generate His-tagged constructs or punc-25::GFP constructs. The cDNAs corresponding to TRIO subdomains were digested from pEGFP-TRIO1–3038 (a gift from Anne Debant, Centre de Recherche de Biochimie Macromoléculaire–Centre National de la Recherche Scientifique, Montpellier, France) (5). Human RAC1 (hRAC1) cDNA was cloned from human lymphocyte cDNA. For RAC1 activity assay, pGEXTK-Pak1 70–117 plasmid was purchased from Addgene. All of the rac(gf), rac, and unc-73 cDNA constructs for genetic studies were gifts from Ken-Ichi Ogura (3) and Erik Lundquist, University of Kansas, Lawrence, KS (6). The rac dominant-negative constructs were generated by site-directed mutagenesis.

**In Vitro Binding Assay.** For GST pull-down experiments, GST fusion constructs and His fusion constructs were introduced into *E. coli* BL21 (DE3) pLysS, and the fusion proteins were induced with 0.1–1 mM isopropyl-β-D-thiogalactopyranoside for 3–6 h. For binding assays, 5 μg of GST-tagged proteins was incubated with approximately the same amount of His-tagged proteins in binding buffer containing 0.2% Triton X-100, 50 mM Tris-Cl (pH 7.5), 100 mM NaCl, 15 mM EGTA, 1 mM DTT, and 1 mM PMSF. Protein complex was pulled down with glutathione-Sepharose beads (Thermo Scientific), washed four times with PBS, then with PBS containing 0.5% Triton X-100, 50 mM Tris-Cl (pH 7.5), 100 mM NaCl, 15 mM EGTA, 1 mM DTT, and 1 mM PMSF, and then subjected to Western blot analysis. For RAC1 binding experiments, GST-TRIO was incubated with His-TRIO or His-DISC1 first. After incubation for 4 h and washes, 5 μg of RAC1 proteins was added and incubated for another 4 h. Beads were then washed four times and subjected to Western blot analysis. For quantification, the densitometry measurement of bands (using Image J) was first normalized to the amount of GST fusion protein pulled down, and signal intensities were then normalized to control (GST-TRIO and RAC1 only) samples. The following antibodies were used: anti-GST (1:1,000; Santa Cruz), anti-6xHis (1:1,000; Clontech), and anti-hRAC1 (1:1,000, clone 23A8; Millipore).

**Cell Culture, Transfection, and Communoprecipitation.** COS cells were cultured at 37 °C in the presence of 5% CO₂ in DMEM containing 10% FBS. For transfection, cells were transfected with the indicated DNA using Lipofectamine Plus Reagent (Invitrogen). For communoprecipitation, total proteins from transfected COS cells were prepared in the lysis buffer containing 50 mM Hepes (pH 6.9), 10% glycerol, 0.2% Nonidet P-40, 5 mM EGTA, 1 mM MgCl₂, and 1× complete protease inhibitor mixture (Roche). Insoluble material was removed by centrifugation at 4 °C for 15 min at 10,000 × g. The recovered supernatant was immunoprecipitated by noted antibodies overnight at 4 °C. The immune complexes were immobilized on protein G– or protein A–Sepharose beads (Roche), washed six times with lysis buffer, and then subjected to Western blot analysis.

**Microscopy.** Animals were mounted on 2% agarose pads with 5 mM sodium azide. Animals were scored by examination with microscopy at either 40× or 630× on a Zeiss Axiosoplan II. Confocal images were captured with a Zeiss LSM 510 META laser-scanning confocal microscope. Images were analyzed by using Zeiss META software version 3.2 SPZ.

**C. elegans Embryonic Cell Culture.** Primary cell cultures of embryonic *C. elegans* neurons were performed as described (7). Briefly, gravid hermaphrodites were lysed with 0.5 M NaOH and 0.5% NaOCl to release embryos, which were digested with 5 mg/mL chitinase (Sigma) and 5 mg/mL chymotrypsin (ICN Pharmaceuticals) to dissolve the eggshell. Cells were separated by digestion with trypsin (Gibco) and trituration. The mixture was filtered through a 5-μm Durapore syringe filter (Millipore) and then transferred to slides (MatTek) coated with peanut lectin (Sigma). Cells were cultured in L-15 cell culture medium (Invitrogen) and maintained in a humidified container at 20 °C for 3 d.

**RAC1 Activity Assay.** GST fusion of PAK1 70–117 was immobilized on glutathione-Sepharose beads (Thermo Scientific) in the cell lysis buffer containing 25 mM Hepes (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 10 mM MgCl₂, 5% glycerol, and 1× complete protease inhibitor mixture (Roche). Transfected COS cells were washed once with PBS containing 1 mM MgCl₂ and 1 mM CaCl₂ and lysed in ice-cold cell lysis buffer. Samples were centrifuged for 10 min at 15,000 × g, and 500 μg of cell lysates from the supernatant was incubated with GST-PAK1 70–117 beads and rotated at 4 °C for 30 min. Beads were collected by centrifugation, washed twice with the cell lysis buffer, and then subjected to Western blot analysis. The following antibodies were used: anti-HA (1:5,000, clone HA-7; Sigma), anti-GFP (1:1,000; Invitrogen), and anti-hRAC1 (1:1,000, clone 23A8; Millipore).


Fig. S1. Subcellular localization of mDISC1 in C. elegans motor commissural axons. (A) GFP::mDISC1 (green) is expressed in the migrating growth cone (red) of VD neurons during L1/L2 stage. Note that, at this stage, the VD neuron’s growth cone assumes a characteristic anvil shape with some protruding filopodia. (B) At young adult stage, GFP::mDISC1 (green) is localized to the distal tip of mature DD or VD axons (red). For A and B, anterior is to the left and dorsal is up. (C) GFP::mDISC1 (green) is accumulated in the growth cone (red) of a C. elegans dissociated neuron cultured from mDISC1 transgenic animals. (D) Schematic diagram showing various constructs of mDISC1 used to generate GFP-tagged fusion proteins. The numbers indicate the beginning and ending amino acids for each mDISC1 construct. Their localizations at young adult stage animals are marked as positive if the GFP signal (green) is highly concentrated at the axon tip (red). (Scale bars: 2.5 μm.)

Fig. S2. pde-4 is expressed in C. elegans ventral motor neurons. The expression pattern of pde-4 from the GFP transcriptional reporter is shown. The promoter element containing approximately 3.0 kb between primers (5’ primer: 5’-CTCGTCTTACACGTTACAACAAGATT-3’; 3’ primer: 5’-GGCTCTTCTCCTGAAATTCAATAGTGAT-3’) was cloned from N2 genomic DNA and then fused to the GFP::unc-54 (3’ UTR) from pPD95.75 (Fire Vector Kit) for studying the expression pattern of pde-4. At L1 stage, the RFP driven by unc-25 promoter is expressed in DD neurons, which are coexpressed with pde-4::GFP (yellow color in overlay). The lower three images are details from the boxed area in the top image. Dashed lines delineate the outlines of the animal. (Scale bars: 5 μm.) Anterior is to the left and dorsal is up.
Fig. S3. Mapping the interacting domains of mDISC1 and hTRIO. (A) The hTRIO-binding site is mapped to the N-terminal globular head domain of mDISC1. (B) hTRIO has two DISC1-binding sites: the amino half of spectrin repeats (Spn) and the GEF2 domain. The inputs in A and B represent 5% of total purified His-tagged proteins. The various constructs of mDISC1 and hTRIO are the same as depicted in Fig. 4.

Fig. S4. RAC1 does not bind to DISC1. In vitro GST pull-down assays show that none of the various His-tagged mDISC1 fragments are pulled down by GST-RAC1. His-tagged hTRIO-GEF1 was used as a positive control. The inputs are 5% of total purified His-tagged proteins. The various constructs of mDISC1 are the same as depicted in Fig. 4.