TDP-43 and DISC1 Co-Aggregation Disrupts Dendritic Local Translation and Mental Function in Frontotemporal Lobar Degeneration

Supplemental Information

Supplemental Methods and Materials

Immunoprecipitation and Western Blotting

Mouse brains, cultured cortical neurons or human brain samples (temporal lobes) were lysed in immunoprecipitation buffer (50 mM Tris-Cl pH7.4, 150 mM NaCl, 1 mM EDTA, 1 mM $MgCl_2$, 1% Nonidet P-40, 1 mM dithiothreitol (DTT)) supplemented with complete EDTA-free protease inhibitor cocktail (Nacalai tesque) and centrifuged at 1500×*g* for 5 min and the supernatants were collected. Typically, Protein G-dynabeads (Invitrogen) were incubated with an antibody against DISC1 (m317C, m595C or h598C), TDP-43 (Proteintech) or puromycin for 2 hours at 4°C and cell or brain lysates were incubated for additional 2 hours at 4°C. The immunoprecipitates were subjected to western blotting using following antibodies: anti-DISC1 (2B3, HM6-5, M49), anti-TDP-43 (FL4), anti-FMRP, anti-FUS/TLS, anti-GFP (mouse monoclonal) and anti-N-terminal TDP-43 antibodies. Amounts of co-immunoprecipitated proteins were normalized by the immunoprecipitated protein level.

Production of Lentivirus and AAV

Lentiviruses were produced by co-transfection of pCSII-CMV-Venus-TDP220C plasmid or DISC1-HA plasmid with pCAG-HIVgp and pCAG-VSV-G plasmids or expression vectors of scramble RNAi, DISC1 RNAi or DISC1 RNAi with an RNAi resistant form of DISC1 with helper constructs VSV-G and delta8.9 into Lenti-X 293T cells grown in 10 ml of Dubecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal

bovine serum. Infectious lentiviruses were precipitated from cultured medium using Lenti-X concentrator (Clontech) according to manufacturer's protocol. Resulting pellets were resuspended with 250 ml of MACS Neuro Medium (Miltenyi Biotec) supplemented with Neuro Brew-21 (Miltenyi Biotec), 0.5 mM L-glutamine, penicillin and streptomycin (Nacalai). For AAV production, pAAV2 plasmid encoding EGFP, Venus-TDP220C or DISC1-HA was co-transfected with pAAV2-2/1 and pAd delta F6 helper plasmids into AAV-293 cells. AAVs were purified by iodixanol gradient centrifugation as explained elsewhere (1). Virus titer was determined by quantitavie reverse transcription-polymerase chain reaction (RT-qPCR).

Infection of AAV into Mice

AAV encoding EGFP, Venus-TDP220C or DISC1-HA was bilaterally injected into two dorsoventral locations (DV -2.0 and -1.5) from the skull in the frontal cortex by using the following coordinates from Bregma: AP 0, ML +/-2.0, AP +2.0 ML +/-2.0. 2 ml of AAV was injected with the rate of 0.2 ml/min by a microinjector (Narishige). Two weeks after the injection, a range of behavioral tests were performed as described below.

Mouse Behavioral Experiments

The open field test was performed as previously described (2). Briefly, the open field test was performed by placing the mouse in the center of an open field apparatus (50x50x40 cm) illuminated by light emitting diode (LEDs; 70 lx at the center of the field). The mouse was allowed to move freely for 15 min and distance traveled (cm) and time spent (%) in the central area of the field (30% of the field) were adopted as indices and the relevant data were acquired every 1 min. Data were collected and analyzed by using TimeOFCR4 (O'Hara & Co.).

The social behavior test was performed as previously reported (2). Briefly, two

cylindrical wire cages (inner size, 7 cm x 15 cm; outer size, 9 cm x 16.5 cm with 21 of 3 mm vertical stainless wires) were placed in the two adjacent corners of the open field apparatus as described above. The test included 3 sessions and each session continued for 15 min with a 4 min interval in the following order. In the first session, a mouse was placed in the open field apparatus with both empty cages. In the second session, a mouse (6 weeks old male C57BL6/J) that was novel to the test mouse was placed in one of the two cylindrical cages. In the third session, another mouse that was also novel to the test mouse was put in the remaining cylindrical cage. Each session was video-recorded and the time spent (sec) and time entered in the two corner squares containing the cylinders within the 3 x 3 square subdivision (17.7 x 17.7 cm square) were measured with TimeOFCR4 (O'Hara & Co.).

The Morris water maze test was performed as previously described (2) with slight modifications. A circular maze apparatus (1 m diameter, 30 cm depth) was filled with water to a depth of about 20 cm (22 to 23°C). The movements of the mice in the maze were recorded and analyzed with Time MWM (O'Hara & Co.). Mice received 2 sessions (3 trials per session) per day for 4 consecutive days. Each acquisition trial was initiated by placing the mouse into the water facing the outer edge of the maze at one of four starting points quasirandomly. The position of platform remained constant for each mouse throughout the testing. The latency (sec) to reach the platform was measured.

Grip strength test was performed by using a grip strength meter (O'Hara & Co.) according to manufacturer’s instruction. Mice were lifted and held by their tail and were allowed to grasp a wire grid by their forepaws. Mice were then gently pulled backward by tail with their posture parallel to the wire grid until they released the grid. The peak force applied by the forelimbs of mice was recorded (N).

All behavioral experiments were conducted during the light phase. All experiments were blinded; the operators responsible for the experimental procedures were blinded and

unaware of group allocation throughout the experiment.

Primary Cortical Neuron Culture and Viral Infection

Brain cortices from embryonic day 14 to 15 C57BL6/J mice (SLC) were dissected, trypsinized and plated at a density of 2.0×10^5 cells/cm^2 in polyethyleneimine (PEI, Sigma)-coated 6 well plates or at a density of 0.5×10^5 cells/cm^2 in 24 well plates with coverslips in Dubecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum. The medium was then changed to Neuro Medium (MiltyniBiotec) supplemented with 0.5 mM L-glutamine and Neuro Brew-21 (MiltyniBiotec). Typically, for 6 well plates, 80 to 100 ml of lentivirus was infected at day 9 to 10 *in vitro* (DIV). For biochemical assays, neurons were harvested with cold phosphate buffered saline (PBS) by centrifugation at 1000×*g* for 5 min and the cell pellets were stored at -80°C until use.

Neuro2a Cell Culture

Neuro2a cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum. For DISC1 knockdown experiments, neuro2a cells were transfected with scramble RNAi, DISC1 RNAi or DISC1 RNAi with an RNAi resistant form of DISC1 twice on day 1 and day 2. On day 3, cells were split and harvested on day 4. For puromycin treatment, cells were treated with 1 mg/ml puromycin for 10 min and harvested on day 4, followed by western blotting with an anti-puromycin antibody. The relative ratio of nascent polypeptides levels is expressed as the percent of those in the control cells transfected with scramble RNAi.

Immunocytochemistry

Cortical neurons cultured on coverslips were fixed with 4% paraformaldehyde, 4% sucrose in PBS. Cells were permeabilized with 0.1% triton X-100 in PBS for 15 min prior to

blocking with 5% normal goat serum. Then, cells were incubated with primary antibodies against DISC1 (M49), MAP2a/b or puromycin for 12 hours at 4°C followed by incubation with fluorescently labeled secondary antibodies. Fluorescent images were acquired by a Nikon C2 confocal laser microscope. Typically, 25 μm long segments of proximal dendrites were selected as region of interest (ROI) and analyzed.

Preparation of Synaptosomal Fraction

Synaptosomal fraction from mouse brain or cultured cortical neurons were isolated as previously described elsewhere (3-6) with modifications. Isolated synaptosomal fraction was subjected to western blotting with following antibodies: anti-NR1 (mouse monoclonal, NeuroMab, 75-272), anti-NR2B (mouse monoclonal, NeuroMab, 75-028), anti-PSD95 (mouse monoclonal, NeuroMab, 75-028), anti-GluR2 (mouse monoclonal, NeuroMab, 75-002), anti-mGluR1/5 (mouse monoclonal, NeuroMab, 75-116), anti-CaMkIIα (rabbit polyclonal, Sigma), anti-Shank3 (mouse monoclonal, NeuroMab, 75-109) and anti-SV2 (mouse monoclonal, DSHB, SV2) antibodies.

Detection of Extracellular Glutamate Receptors

Cultured cortical neurons were washed with PBS and treated with 0.5 mg/ml EZ-Link-Sulfo-NHS-LC-Biotin (Thermo Scientific) for 30 min at 4°C and the reaction was quenched by washing neurons with PBS containing 50 mM glycine. The neurons were harvested, lysed in the immunoprecipitation buffer with brief sonication and centrifuged at 1000×*g* for 5 min. Streptavidin-conjugated beads (Invitrogen) were added to the supernatants and incubated for 4 hours at 4°C. Proteins pulled down by the Streptavidin-conjugated beads were used for western blotting with an antibody against NR2B, GluR2, PSD95 and αTubulin. Surface-localized NR2B and GluR2 protein levels are expressed as the percent of those of control neurons.

Neuronal Stimulation and Puromycin Treatment

For KCl stimulation, cultured cortical neurons were incubated with either 5 mM KCl containing Tyrode's buffer (25 mM Hepes pH 7.4, 124 mM NaCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 30 mM glucose) or 55 mM KCl containing Tyrode's buffer (25 mM Hepes pH7.4, 74 mM NaCl 2 mM $MgCl_2$, 2 mM $CaCl_2$, 30 mM glucose) for 4 hours. For BDNF stimulation, cultured cortical neurons were incubated with or without 20 ng/ml BDNF in Neuro Medium for 2 hours. Subsequently, the neurons were treated with 10 μg/ml puromycin for 30 min and harvested, followed by western blotting with an anti-puromycin antibody. The relative ratio of nascent polypeptides levels is expressed as the percent of those in the control neurons treated with 5 mM KCl.

Polysome Gradient Analysis

Cultured cortical neurons, mouse cerebral cortex, mouse cerebellum or neuro2a cells were homogenized with the polysome gradient buffer (10 mM Hepes pH7.4, 150 mM KCl, 5 mM $MgCl^2$, 1 mM DTT, 50 μg/ml cyclohexamide) supplemented with a complete EDTA-free protease inhibitor cocktail. Homogenates were passed through a 26-gauge needle 10 times and centrifuged at 1000×*g* for 10 min. The supernatant (S1) was recovered and NP-40 was added to the final concentration of 0.5% and incubated on ice for 5 min. The S1 fraction was then centrifuged at 14,000×*g* for 10 min and the supernatant (S2) fraction was recovered. The S2 fraction was incubated with or without 30 mM EDTA or 190 U RNase T1 and 19 μg/ml RNase A for 10 min at room temperature. The S2 fraction was loaded onto 20-50% of the linear sucrose density gradient. The gradients were centrifuged at 40,000 rpm for 2 hours at 4°C in a Beckman Instruments with the SW41 rotor. Continuous fractions of 0.57 ml volume were collected using Gradient station (BIOCOMP) and Micro Collector (ATTA) monitoring absorbance at 254 nm with Bio-Mini-UV-Monitor. Each fraction was precipitated by addition of 100% ethanol and

incubated at -30°C for overnight. Each fraction was then centrifuged at 20,000x*g* for 30 min and the resulting pellets were used for western blotting with following antibodies: anti-DISC1 (M49), anti-TDP-43 (FL4), anti-FMRP (2F5-1-S), anti-RS6 and anti-αTubulin antibodies.

Ribosomal Run-off Assay

Cultured cortical neurons were treated with or without 2 mM hippuristanol for 45 min. A supernatant was prepared and used for polysome gradient analysis as described above.

mRNA Migration Assay

Free mRNAs/mRNPs, 40S ribosomes, 60S ribosomes, monosomes and polysomes fractions from neuro2a cells were obtained by sucrose gradient assay as described above. Total RNA was isolated from each fraction with Sepasol-RNA I Super G (Nacalai Tesque) according to manufacturer's protocol. Isolated RNAs were treated with DNase I, reverse transcribed to single-stranded cDNAs using Rever Tra Ace qPCR RT Kit (Toyobo) and relative mRNA levels were measured by quantitative RT-PCR using a SYBR green-based detection system on an Applied Biosystems Step One Plus Real Time PCR System (Applied Biosystems).

***In vitro* Transcription and Translation**

DNA template containing phage T7 promoter for *in vitro* transcription was produced by PCR utilizing pCMV-Gluc plasmid. *In vitro* transcription was performed using mMESSAGE mMACHINE T7 Ultra Kit (Ambion), according to manufacturer's protocol and RNA product was purified with RNeasy mini kit (Qiagen). N2a cells were lysed with brief sonication followed by passing through a 27-gauge needle 25 times in *in vitro* translation lysis buffer (10 mM Hepes pH7.4, 10 mM KAc, 0.5 mM $MgCl^2$, 5 mM DTT)

supplemented with a complete EDTA-free protease inhibitor cocktail and centrifuged at 1000xg for 3 min. The cell extracts were then treated with 15 U/ml micrococcal nuclease in the presence of 0.75 mM $CaCl^2$ at room temperature for 7 min and the reaction was terminated by adding EGDA to the final concentration of 3 mM. 10 x energy regenerating buffer (100 mM creatine phosphate, 1 mg/ml creatine kinase, 100 mM spermidine, 100 mM amino acids, 1 mM ATP, 1 mM GTP, 500 mM KAc, 25 mM MgAc), RNase inhibitor (Invitrogen) and 1.0 mg of mRNA were added to the cell extracts and incubated for 5 hours at 32°C. Luminescent signals were measured immediately after 20 mM coelenterazine addition and monitored for 10 sec using a luminometer.

Measurement of Total mRNA Levels

Total RNA from synaptosomal fraction was isolated using Sepasol (NacalaiTesque) and mRNAs were further purified using Oligotex-dt super mRNA purification kit (Takara) according to manufacturer's protocol. Total mRNA levels in the synaptosomal fraction were measured by a fluorometer Qubit 2.0 (Invitrogen) and normalized to total RNA levels.

Immunohistochemistry for Human Brain Sections

Frozen blocks of human tissue samples were embedded in Tissue-Tek O.C.T Compound (Shirai Kogyo) and cryostatically sectioned at 10 mm thickness. The sections were fixed with 4% paraformaldehyde for 20 min, permeabilized and blocked with 3% normal goat serum, 0.3% triton X-100 in PBS for 45 min. The sections were incubated with a primary antibody against DISC1 (h598C, HM6-5), TDP43 (mouse monoclonal, Abnova) for overnight at 4°C, followed by incubation with fluorescently labeled secondary antibodies. The sections were then treated with 10 mM $CuSO_4$ for 1 hour and neuclei were stained with 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI). Fluorescent images were acquired as described above.

Detection of Detergent-Resistant Insoluble Aggregates

Human brain samples or cultured cortical neurons were homogenized in the buffer (10 mM Tris-Cl pH7.5, 10% sucrose, 0.5M NaCl, 1mM EDTA) supplemented with a complete EDTA-free protease inhibitor cocktail. The homogenates were briefly sonicated and centrifuged at 150×*g* for 3 min and supernatants were recovered. Sarkosyl was added to the supernatants to the final concentration of 2% and incubated at 4°C for spin down assay or at 37°C for filter trap assay for 30 min. For the spin down assay, the samples were centrifuged at 100,000×*g* for 30 min and the resulting pellets were used for western blotting using anti-TDP-43 (FL4), anti-DISC1 (h598C) and anti-phosho-Ser409/410 antibodies. For the filter trap assay, brain samples (125 mg) were placed onto the 0.22 mm cellulose acetate membrane using a Bio-dot apparatus (Bio-Rad). The membrane was washed twice with 2% sarkosyl and trapped proteins were detected by western blotting with following antibodies: anti-TDP43 (FL4), anti-DISC1 (h598C), anti-phospho-Ser409/410, anti-Tau, anti-a synuclein and anti-GFP (mouse monoclonal) antibodies. The relative ratio of detergent-resistant insoluble protein levels was expressed as the percent of those of control neurons or brains.

Cap Purification Assay

Cultured cortical neurons were lysed in cap purification buffer (50 mM Tris-Cl pH7.4, 150 mM NaCl, 1 mM EDTA, 1 mM $MgCl_2$, 1% Nonidet P-40, 1 mM dithiothreitol (DTT)) supplemented with complete EDTA-free protease inhibitor cocktail (Nacalai tesque) and centrifuged at 1500×*g* for 5 min and the supernatants were collected. 7-Methyl GTP (m7GTP)-Sepharose beads (Jena Biosciences) were added to the supernatants and incubated for 2 hours at 4°C. Proteins pulled down by the m7GTP-sepharose beads were subjected to western blotting using following antibodies: anti-eIF4A, anti-eIF4E and anti-eIF4G antibodies.

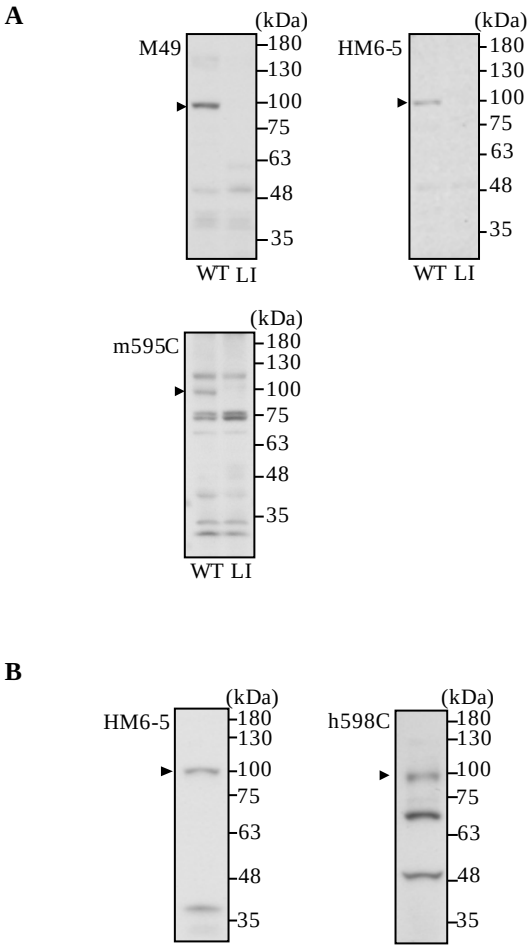

Figure S1. Validation of DISC1 antibodies used in this study. Monoclonal or polyclonal anti-DISC1 antibodies were validated by western blotting. DISC1 in **(A)** WT and DISC1 Locus Impairment (LI) mouse (7,8) cerebral cortex or **(B)** in human cerebral cortex from a healthy individual was detected with indicated antibodies. Arrowheads indicate full-length DISC1.

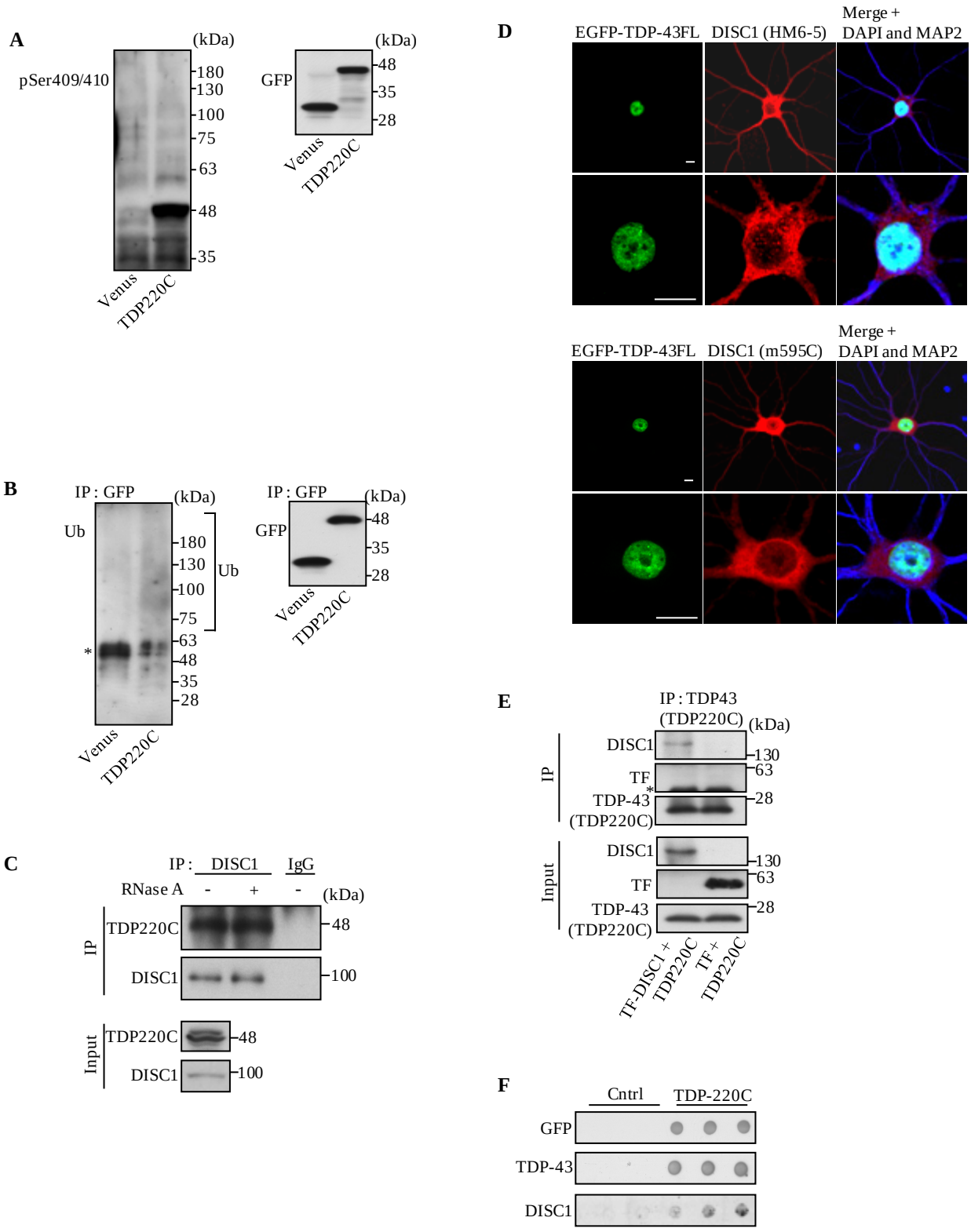

Figure S2. Characterization of TDP-220C aggregates in cultured cortical neurons. **(A-C)** Cultured cortical neurons were infected with lentivirus encoding Venus or Venus-TDP-220C. **(A)** Phosphorylation of TDP-220C aggregates at serine 409/410 was detected by western blotting. **(B)** TDP-220C aggregates are ubiquitinated. Venus or

Venus-TDP-220C was immunoprecipitated (IP) using an anti-GFP antibody, followed by western blotting with an anti-ubiquitin antibody. An asterisk shows an IgG control. **(C)** Neuronal lysate infected with lentivirus encoding Venus-TDP220C were treated with or without 200 μg/ml RNase A, followed by immunoprecipitation with an anti-DISC1 antibody (m595C) and western blotting (M49 for DISC1). **(D)** Cultured cortical neurons were infected with a lentivirus expression vector encoding EGFP-TDP-43FL (green), and endogenous DISC1 was immunostained with an anti-DISC1 antibody (HM6-5 or m595C) (red). Nuclei and dendrites were stained with DAPI (blue) and an anti-MAP2 antibody (blue), respectively. Representative images of a whole neuron (top) and a soma (bottom) are shown. Scale bar represents 5 μm. **(E)** Recombinant trigger factor (TF)-tagged full-length DISC1 or TF was incubated with seeds (amyloid fibrils) of a C-terminal fragment of TDP-43 (TDP220-C). TDP220-C was immunoprecipitated with an anti-TDP-43 antibody, followed by western blotting with an antibody against DISC1 (h598C), TDP-43 or TF. An asterisk shows an IgG band. **(F)** TDP-220C aggregates are detergent-resistant and DISC1 is sequestered into TDP-220C aggregates in cultured cortical neurons expressing TDP-220C. 60 μg of cell lysates were incubated with 2% sarkosyl, followed by filter-trap assay. Detergent-resistant insoluble proteins were detected by western blotting with indicated antibodies (M49 for DISC1).

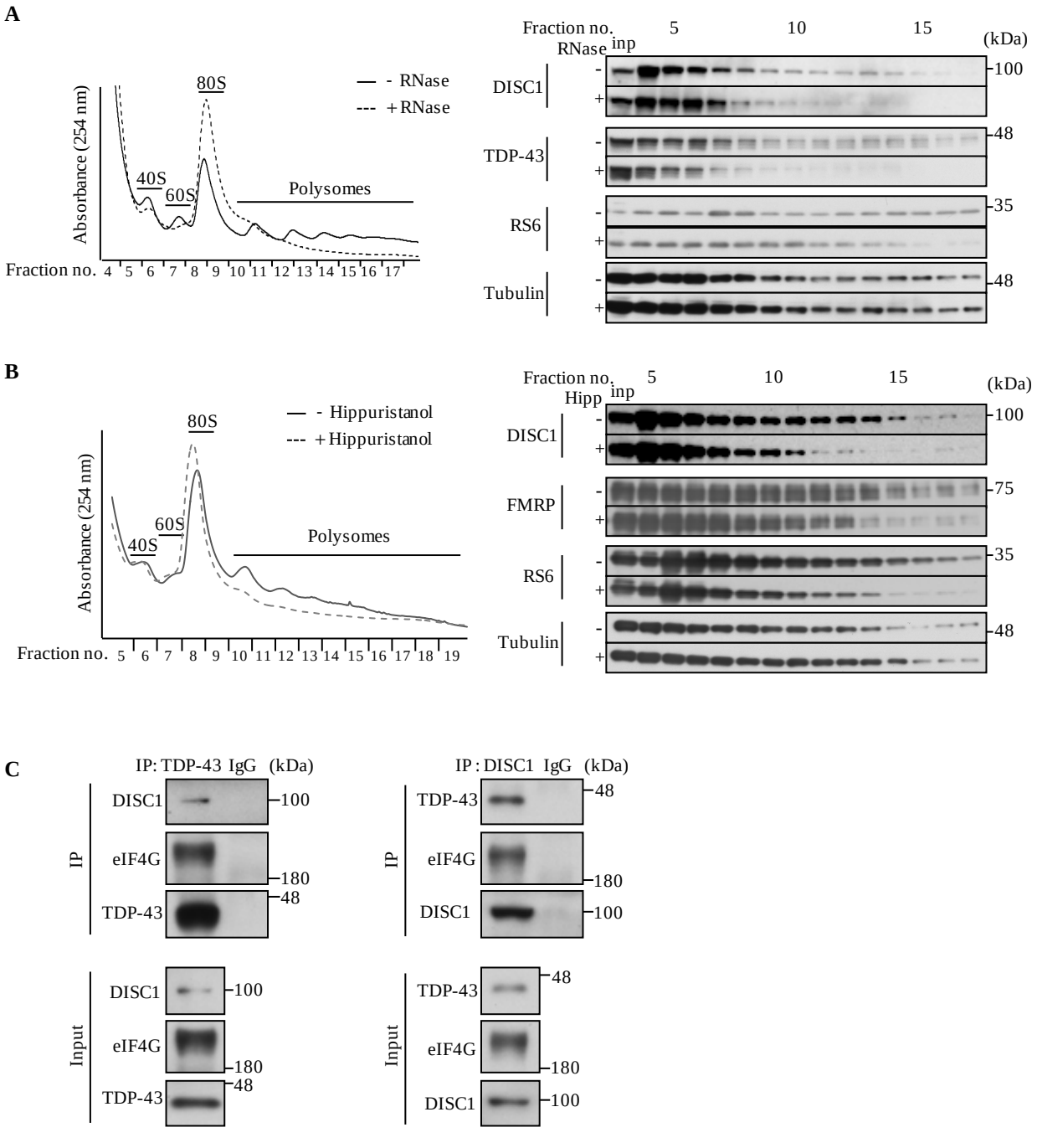

Figure S3. DISC1 is associated with translating polyribosomes but not with stalled ribosomes and forms a complex with TDP-43. **(A)** The polysome gradient analysis using extracts of cultured cortical neurons treated with or without RNase prior to centrifugation. The top panel shows the absorption profile of sucrose gradient at 254 nm. The collected fractions were analyzed by western blotting with indicated antibodies (M49 for DISC1) (bottom). DISC1 was detected in the polysome fractions and migrated to the monosome fractions by the RNase treatment. "inp" denotes input. **(B)** The polysome gradient analysis using extracts of cultured cortical neurons treated with or without 2 mg/ml hippuristanol for 45 min to induce ribosome run-off while leaving stalled ribosomes on mRNAs. The

collected fractions were analyzed by western blotting with indicated antibodies (M49 for DISC1) (bottom). The DISC1 levels in the polysome fractions were significantly decreased (72% reduction) by the hippuristanol treatment whereas a large amount of FMRP remained (33% reduction) in the polysome fractions. “inp” denotes input. **(C)** DISC1 or TDP-43 in 40S ribosome fractions purified from wild-type mouse cerebral cortex was immunoprecipitated with an anti-DISC1 antibody (m595C) or anti TDP-43 antibody, followed by western blotting with indicated antibodies (HM6-5 for DISC1).

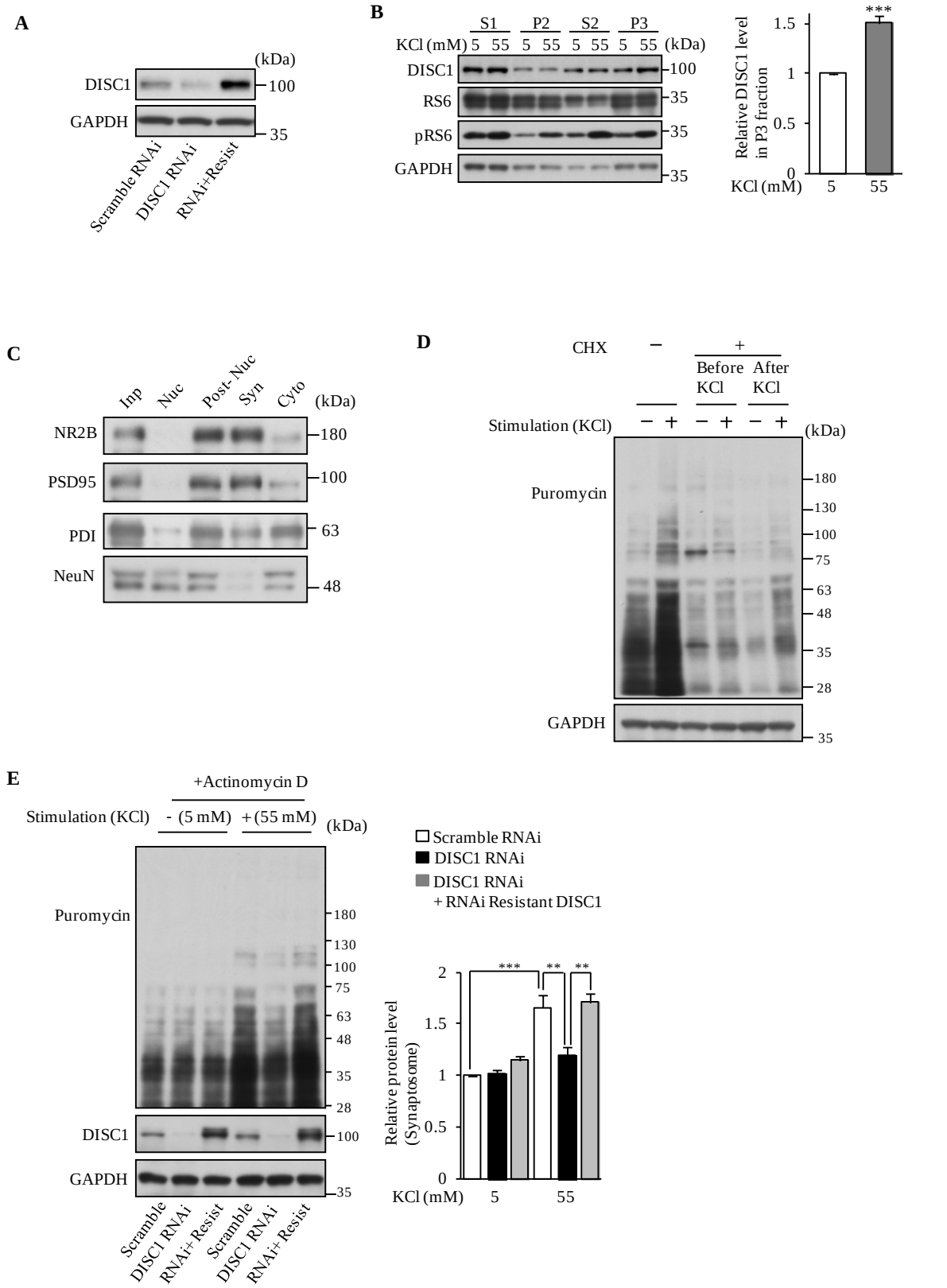

Figure S4. DISC1 amounts in ribosomes are increased by neuronal stimulation. **(A)** Cultured cortical neurons were infected with indicated lentivirus and DISC1 was detected by western blotting with an anti-DISC1 antibody (2B3). **(B)** KCl stimulation increased

DISC1 level in ribosomes-enriched fraction (P3) in neurons. Ribosome-enriched fraction (P3) was isolated from cultured cortical neurons, followed by western blotting with indicated antibodies (M49 for DISC1). The levels of DISC1 in P3 fraction is expressed as the relative ratio to 5 mM KCl (right). (n=3, t=7.416, ***P=0.0003, unpaired two-tailed t-test). **(C)** Isolation of synaptosomal fraction. A synaptosomal fraction was isolated from cultured cortical neurons. The western blotting showed that NR2B and PSD95 are enriched in synaptosomal (Syn) fraction while the nuclear protein NeuN and ER protein PDI are not. Nuc, Post-Nuc and Cyto denote nuclear, post-nuclear and Cytosol fraction respectively. **(D and E)** The increase in newly synthesized protein levels by neuronal stimulation is dependent on translation. **(D)** Cultured cortical neurons were treated with cyclohexamide (CHX) either 15 min prior to and during the KCl stimulation (Before KCl) or after the KCl treatment but 20 min prior to the puromycinylation (After KCl). The puromycin-labeled proteins in the isolated synaptosomal fraction were detected by western blotting with an anti-puromycin antibody. **(E)** Cultured cortical neurons were treated with 5 μg/ml actinomycin D prior to and during KCl stimulation to inhibit transcription, followed by puromycinylation of newly synthesized proteins. The puromycin-labeled proteins in the isolated synaptosomal fraction were detected by western blotting with an anti-puromycin antibody. (n=3, F(5,12)=18.85, P<0.0001, one-way ANOVA; **P<0.01, ***P<0.001, Bonferroni's multiple comparison test *post hoc*). Throughout the figures, error bars represent S.E.M.

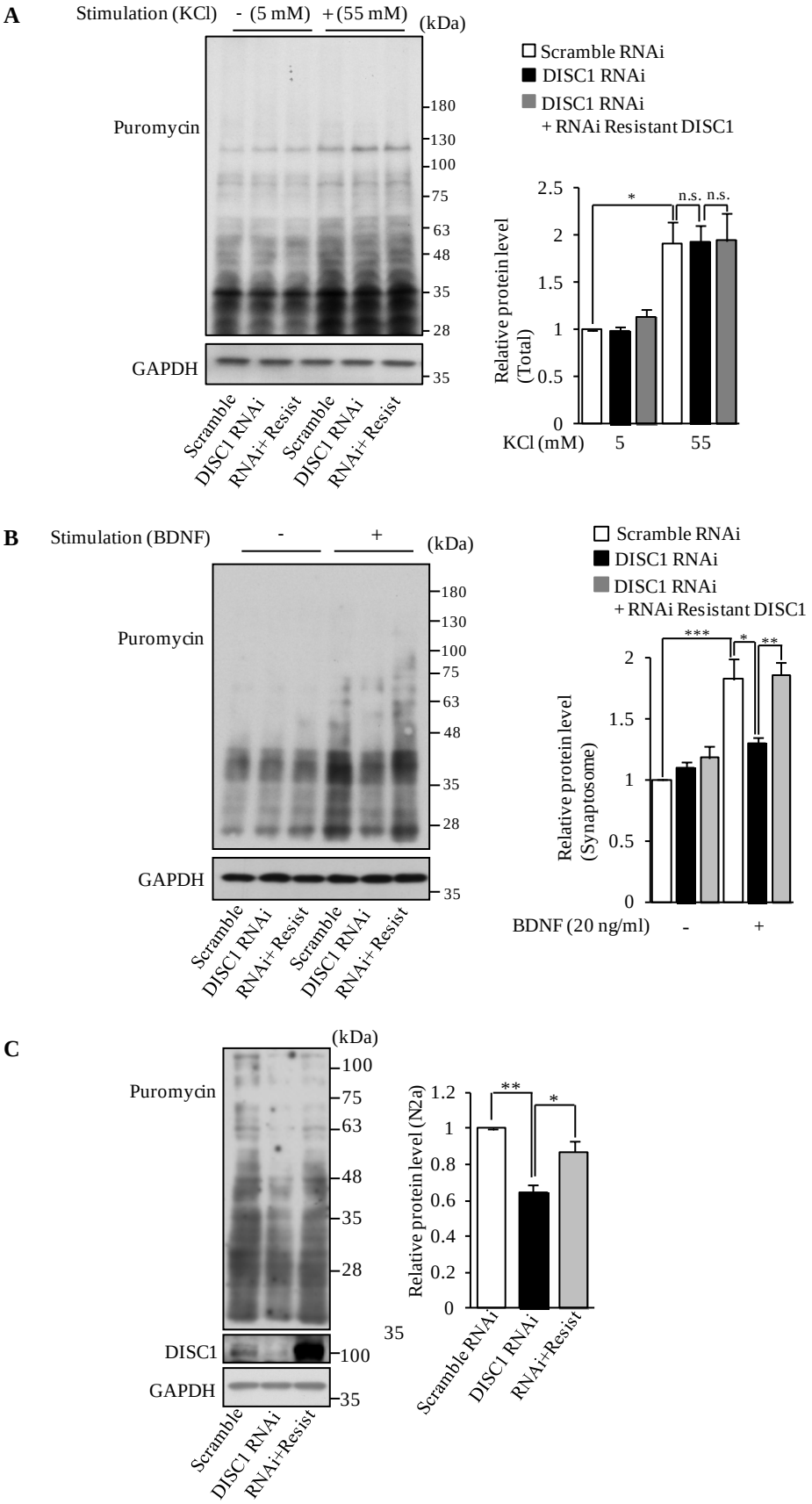

Figure S5. DISC1 regulates activity-dependent local translation in dendrites. **(A)** The

puromycin-labeled proteins in the total fraction were detected by western blotting with an anti-puromycin antibody. The signal intensities of puromycin-incorporated polypeptides were normalized to those of GAPDH and then expressed as a relative ratio of control neurons (right) (n=3, F(5,12)=8.482, P=0.0012, one-way ANOVA; *P<0.05, Bonferroni's multiple comparison test *post hoc*). **(B)** Cultured cortical neurons were incubated with or without 20 ng/ml BDNF prior to puromycin treatment. The isolated synaptosomal fraction was subjected to western blotting with an anti-puromycin antibody for detection of puromycin-incorporated newly synthesized proteins. The signal intensities of puromycin-labeled polypeptides were normalized to those of GAPDH and then expressed as the relative ratio of control neurons (right) (n=4, F(5,18)=15.81, P<0.0001, one-way ANOVA; *P<0.05, **P<0.01, ***P<0.001, Bonferroni's multiple comparison test *post hoc*). **(C)** DISC1-knockdown N2a cells were treated with puromycin, and puromycin-incorporated newly synthesized proteins were detected by western blotting (M49 for DISC1). The signal intensities of puromycin-labeled polypeptides were normalized to those of GAPDH and then expressed as a relative ratio of control N2a cells (right) (n=3, F(2,6)=17.09, P=0.0033, one-way ANOVA; *P<0.05, **P<0.01, Bonferroni's multiple comparison test *post hoc*). Throughout the figures, error bars represent S.E.M.

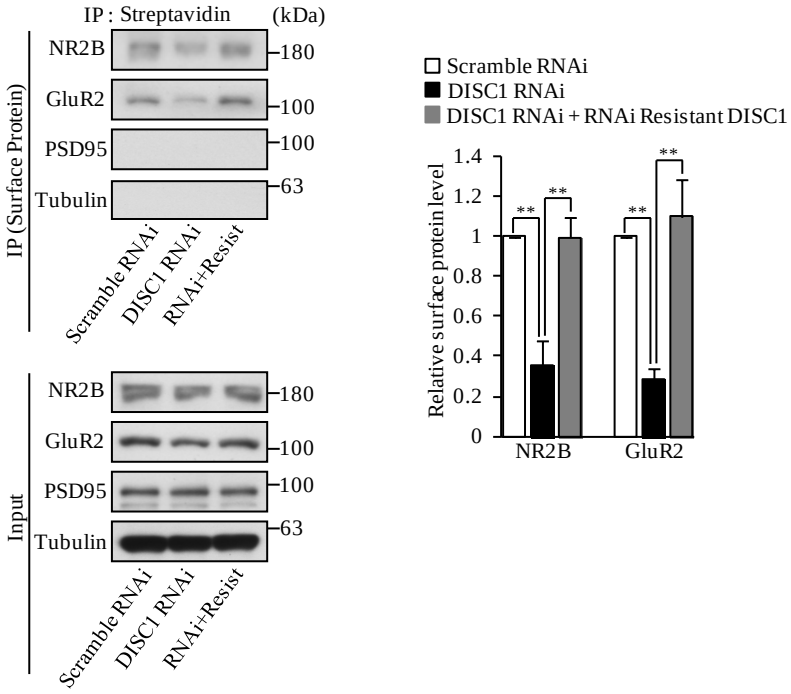

Figure S6. DISC1 regulates protein levels of surface-localized neuronal receptors. Surface-localized NR2B and GluR2 protein levels are decreased by DISC1 knockdown. Extracellular proteins of cultured cortical neurons infected with indicated lentivirus were biotinylated and the surface biotinylated proteins were pulled down by streptavidin-conjugated beads, followed by western blotting. PSD95 and tubulin, which are not expressed at the surface of synaptic membrane, were used as negative controls. The relative binding affinity is shown (right) (n=4, NR2B: $F(2,9)=16.47$, $P=0.001$; GluR2: $F(2,9)=15.34$, $P=0.0013$, one-way ANOVA; $^{**}P<0.01$, Bonferroni's multiple comparison test *post hoc*). Throughout the figures, error bars represent S.E.M.

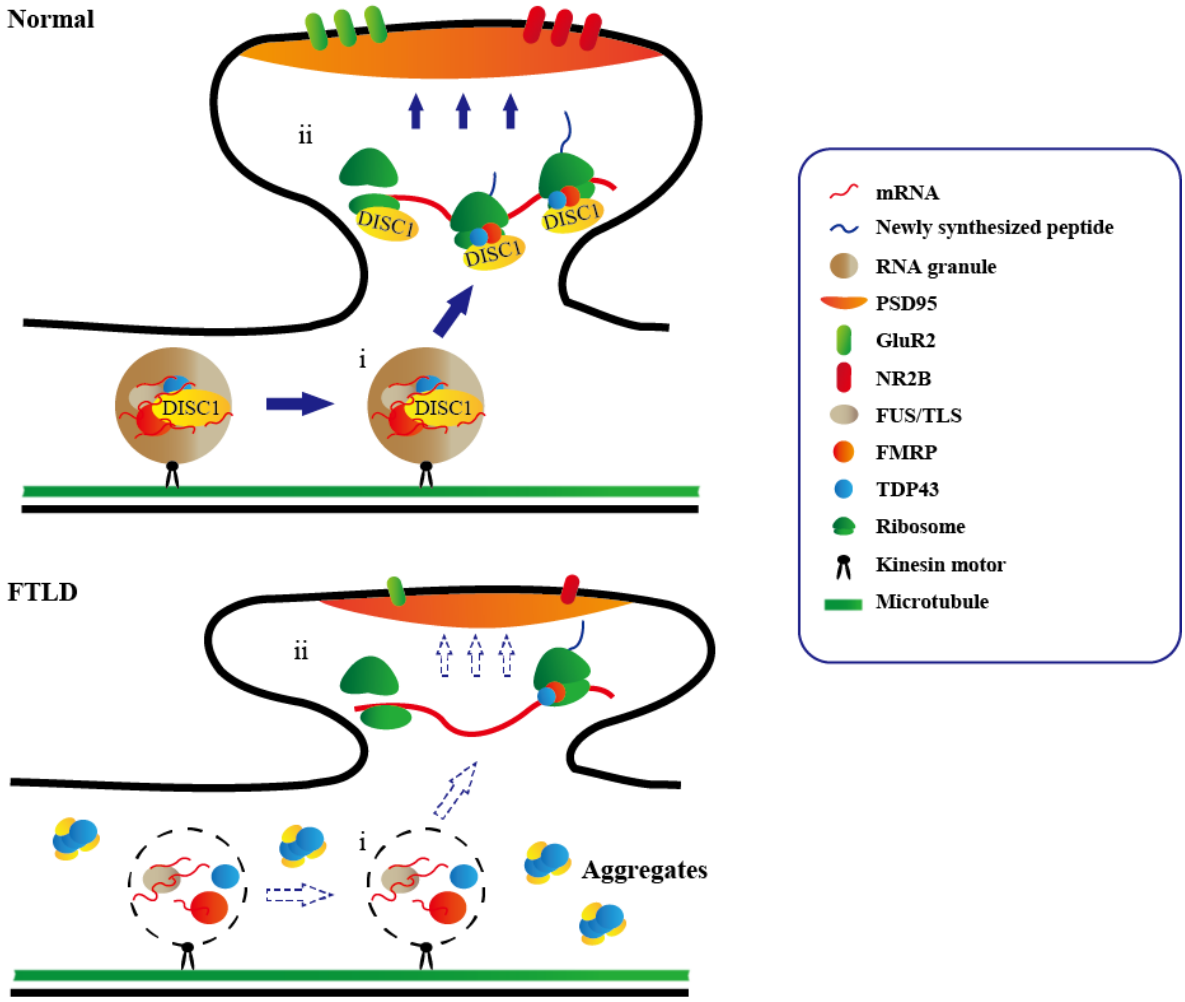

Figure S7. A model for DISC1-mediated impaired local translation in dendrites of FTLD neurons. In normal neurons, DISC1 regulates local translation of synaptic mRNAs, including NR2B and PSD95, in dendrites in a neuronal activity-dependent manner. In FTLD neurons, DISC1 forms co-aggregates with TDP-43. The depletion of functional DISC1 impairs local translation in dendrites, which could elicit psychiatric symptoms.

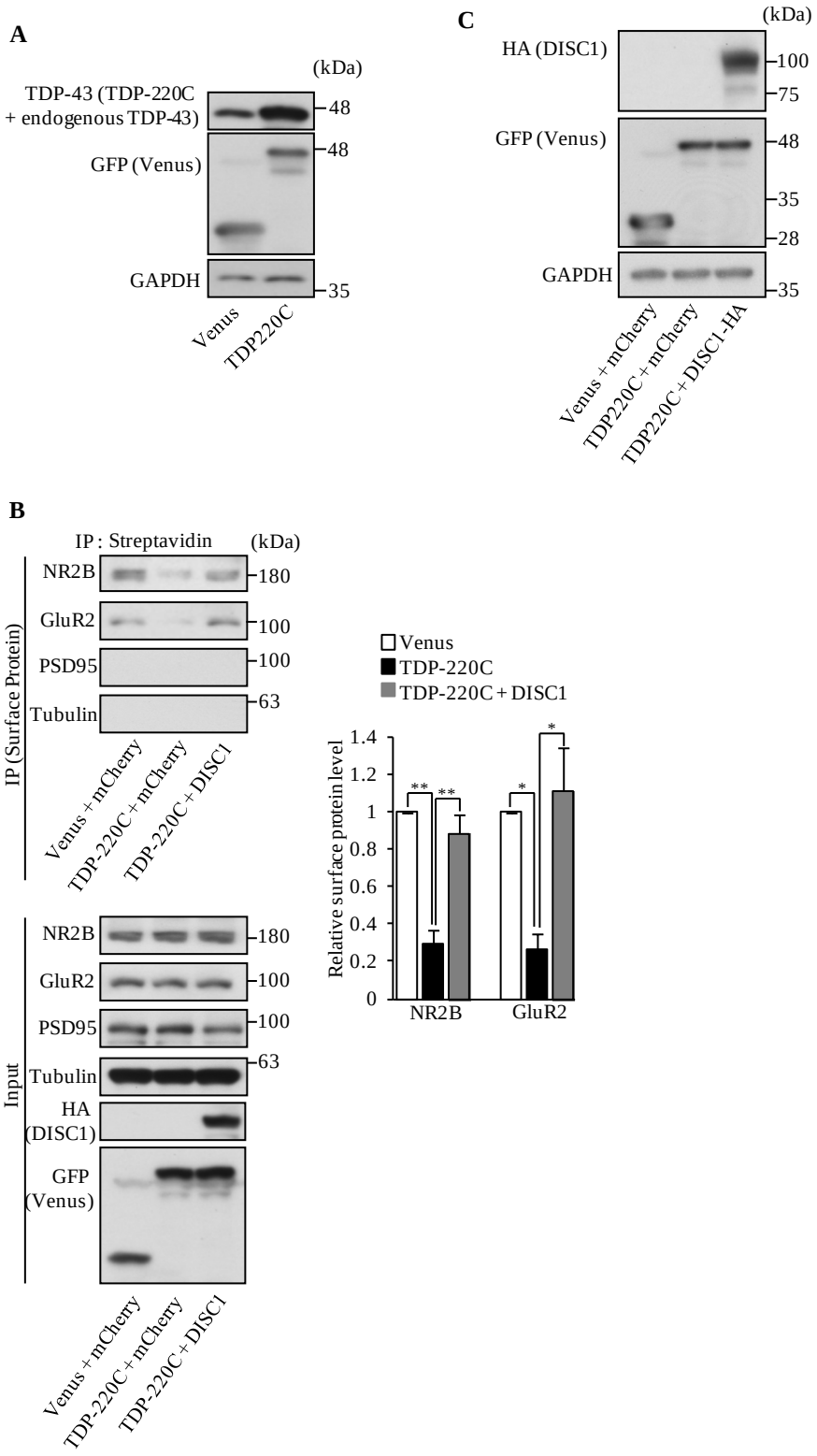

Figure S8. Aggregation of TDP-220C decreases levels of surface glutamate receptors, which can be rescued by DISC1 expression. **(A)** Cultured cortical neurons were infected with indicated lentiviruses and proteins levels were detected by western blotting with an anti-TDP-43 antibody for endogeneous TDP-43 and TDP-220C and an anti-GFP antibody for Venus and TDP-220C. **(B)** Surface-localized NR2B and GluR2 protein levels were

analyzed by biotinylated surface proteins in cultured cortical neurons infected with indicated lentivirus. The relative binding affinity is also shown (right) (*n*=3, NR2B: *F*(2,6)=28.92, *P*=0.0008; GluR2:*F*(2,6)=10.32, *P*=0.0114, one-way ANOVA; * *P*<0.05, ***P*<0.01, Bonferroni's multiple comparison test *post hoc*). PSD95 and tubulin, which are not expressed at the surface of synaptic membrane, were used as negative controls. The error bars represent S.E.M. **(C)** Cultured cortical neurons were infected with indicated lentiviruses and proteins levels in the isolated synaptosomal fraction were analyzed by western blotting with an anti-GFP antibody for Venus and TDP-220C and an anti-HA antibody for DISC1.

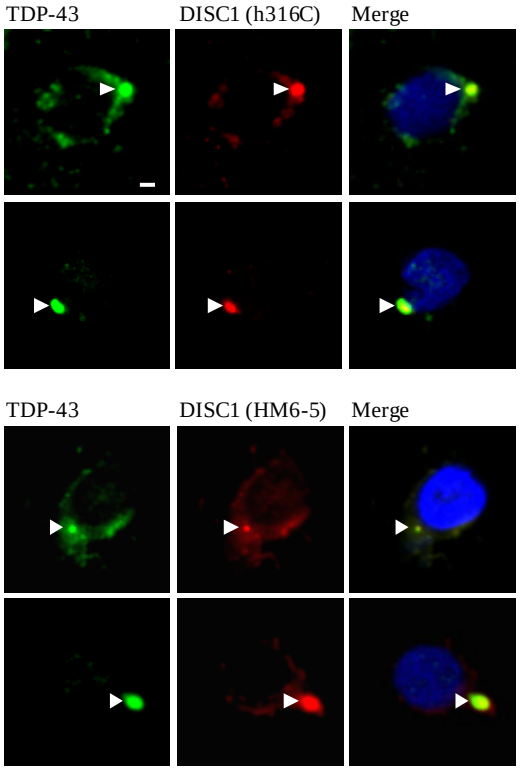

Figure S9. DISC1 and TDP-43 in FTLD patient brains were immunostained by an anti-DISC1 (h316C or HM6-5) (Red) and anti-TDP43 (Green) antibodies, respectively. Scale bar represents 5 ㎜.

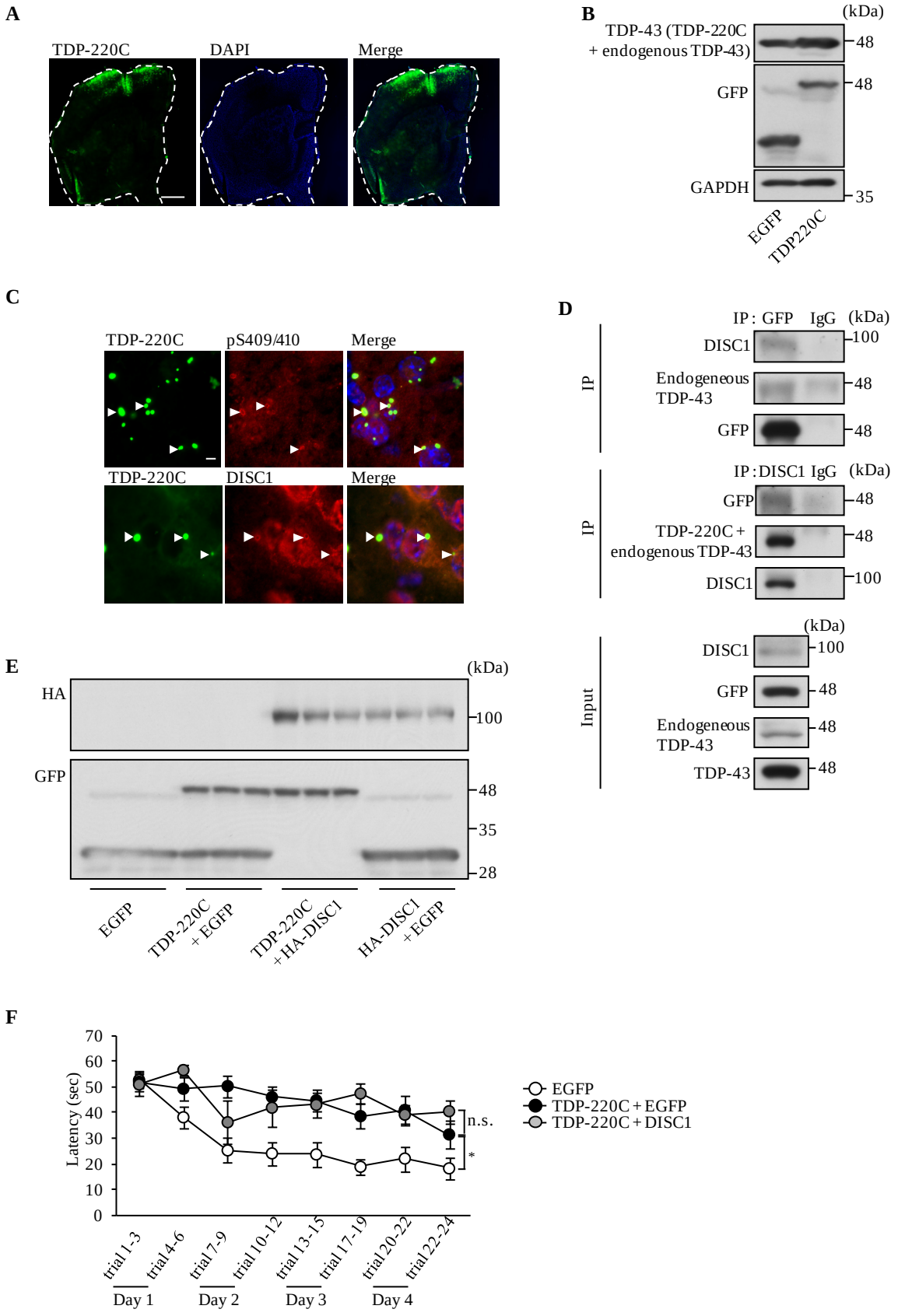

Figure S10. TDP-220C forms co-aggregates with DISC1 *in vivo*. **(A-D)** AAV encoding Venus-TDP-220C was stereotaxically injected into mouse prefrontal cortex.

(A) Fluorescent image of AAV-injected brain section. **(B)** Western blotting with an anti-TDP-43 antibody for endogeneous TDP-43 and TDP-220C and an anti-GFP antibody for EGFP and TDP-220C. **(C)** Immunostaining with antibodies against phosphorylated Ser409/410 (top) or DISC1 (HM6) (bottom) showed that TDP-220C aggregates are highly phosphorylated at Ser409/410 and endogenous DISC1 is sequestered into TDP-220C aggregates. Scale bar represents 5 μm. **(D)** Overexpressed TDP-220C binds to endogenous DISC1 and full-length TDP-43. Venus-TDP-220C or DISC1 were immunoprecipitated with an anti-GFP or anti-DISC1 antibody (m595C), followed by western blotting with indicated antibodies. Endogenous TDP-43 was detected by the antibody specific to an N-terminal region of endogenous TDP-43. **(E and F)** AAV encoding an indicated gene was stereotaxically injected into mouse prefrontal cortex. **(E)** Overexpressed EGFP and Venus-TDP-220C were detected by an anti-GFP antibody while overexpressed DISC1-HA was observed by an anti-HA antibody. **(F)** The Morris water maze memory acquisition test (3 trials per block, 6 trials per day) was performed. The latency to reach the target was significantly longer in TDP-220C mice than that in control EGFP mice. The defect of memory acquisition in TDP-220C mice was not rescued by DISC1 co-expression. n=12, 11, 12, for EGFP (white), TDP-220C+EGFP (black), TDP 220C+DISC1 (light gray), mice, respectively ($F(2,31)=6.567$, $P=0.0042$, one-way ANOVA; $^{*}P<0.05$, Bonferroni's multiple comparison test *post hoc*). n.s., not significant. The error bars represent S.E.M.

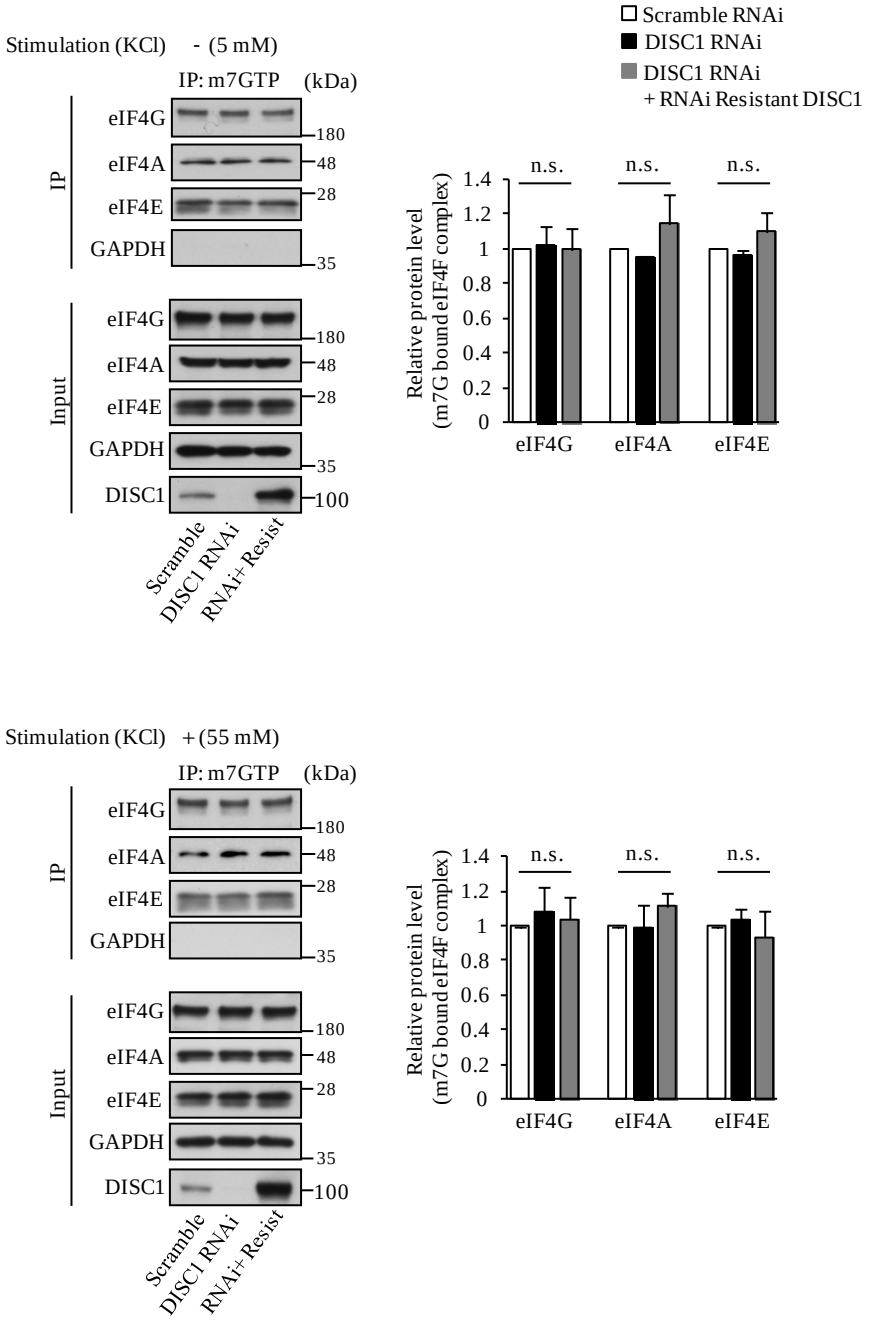

Figure S11. The eIF4F complex formation is not affected by DISC1 depletion. The eIF4F complex was pulled down from either non-stimulated (5 mM KCl, top) or KCl-stimulated neurons (55 mM KCl, bottom) by m7GTP-sepharose beads followed by western blotting with indicated antibodies (HM6-5 for DISC1). (n=3. 5 mM KCl, eIF4G: $F(2,9)=0.0298$, $P=0.9708$; eIF4A: $F(2,6)=1.126$, $P=0.3844$; eIF4E: $F(2.9)=1.024$, $P=0.3974$. 55 mM KCl, eIF4G: $F(2,6)=0.1324$, $P=0.8785$; eIF4A: $F(2,6)=1.248$, $P=0.3523$; eIF4E: $F(2.6)=0.3231$, $P=0.7358$, one-way ANOVA; Bonferroni's multiple comparison test *post hoc*). n.s., not significant. The error bars represent S.E.M.

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