Supplemental Information

Splicing-dependent Trans-synaptic SALM3–LAR-RPTP Interactions Regulate Excitatory Synapse Development and Locomotor

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Supplementary Figure Legends

Figure S1. (Related to Figure 1) Candidate Synaptic Adhesion Molecules Used to Test SALM3 Binding, and Examples of the Cell Aggregation Results.

A group of L cells expressing SALM3 in the pDisplay vector was mixed with another group of L cells expressing candidate cell adhesion molecules or surface membrane proteins, and mixtures were measured for cell aggregation. The interaction between NGL-3 and LAR was used as a positive control. Scale bar, 50 µm.

Figure S2. (Related to Figure 3) The Efficiency of sh-RNA Constructs Targeting LAR-RPTPs.

(A) Knockdown of LAR, PTPσ, or PTPδ in heterologous cells by shRNA. HEK cells were co-transfected with the indicated shRNA vectors plus CFP-tagged LAR or YFP tagged PTPσ or PTPδ constructs for 48 hours. ShRNA was driven by the U6 promoter while another reporter gene (red) was expressed independently in the same vector. Scale bar, 10 µm. (B) Quantification of knockdown of shRNA constructs, measured as the ratio of LAR-RPTP signal to shRNA reporter gene per field. ANOVA p<0.0001 and ***p<0.001 compared with sh-vec or sh-con by Bonferroni posthoc test (n = 5–9 fields). (C) Knockdown of PTPσ in primary rat cortical neurons. pLLsyn-CFP-U6-sh-RNA targeting PTPσ or control were nucleofected into neurons at P0. 20 µg total lysates from transfected neurons at DIV14 was blotted by antibodies against PTPσ or α-tubulin as a loading control.
Figure S3. (Related to Figure 4) Expression Patterns of SALM3 Proteins in the Brain.

SALM3 expression patterns visualized by X-gal staining of Salm3^+/− brain coronal sections at 5 weeks. Note that SALM3 is most strongly expressed in the hippocampus, and fairly strongly in the cortex and striatum. Scale bar, 1 mm.

Figure S4. (Related to Figure 5) Reduced Density of Glutamate-positive PSDs in the Salm3^−/− hippocampus.

(A-E) The Salm3^−/− hippocampal CA1 region (stratum radiatum; 2 weeks) show a reduction in the density of the PSD (arrowheads) apposed to axon terminals immunopositive for glutamate (asterisks, demarked by dashed line) (A and B), whereas it shows normal levels of the perforation, thickness, and length of the PSD (C-E). Scale bar, 200 nm. n = 3 for WT and KO, *p < 0.05, ns, not significant, Student’s t-test.

Figure S5. (Related to Figure 5) Normal mEPSCs in the Salm3^−/− Striatum.

(A-C) Unaltered frequency and amplitude of mEPSCs in Salm3^−/− dorsolateral striatal neurons (P17–19). (n = 13 neurons from 3 mice for WT and KO, ns, not significant, Student’s t-test).

Supplementary Figure Table

Table S1. (Related to Figure 1) Proteins Pulled Down from Brain Crude Synaptosomes with Both PTPδ-Fc and PTPδlg1-3-Fc but Not Neurexin1β-Fc.

Supplementary Experimental Procedures

Cell Aggregation Assays

Cell aggregation assays were performed as described (Kwon et al., 2010). Briefly, one group of L cells was co-transfected with EGFP and SALM3-Ecto-pDis, and another group of L cells was with RFP and LAR-RPTPs-Ecto-pDis. After 24 hrs, L cells were trypsinized and resuspended in opti-MEM, followed by mixing the two groups of L cells and rotation at room temperature for 2 hrs to allow cells to aggregate. Then get images with confocal microscopy (LSM510, Zeiss).

Production of Soluble Fc-fusion Proteins

Fc fusion proteins were custom generated. To make a stable cell line for the effective production of Fc-fusion proteins, we made several cloning vectors: pc4-sp-PTPδ A*B^+-Fc (Peptide 21-762), pc4-sp- PTPδ (lg1-3)A'B^-Fc, pc4-sp- PTPδ (lg1-3)A'B^+-Fc, and pc4-sp-SALM3-Fc (peptide 17aa-518 aa). To make stable cell lines that produce soluble
fused Fc protein, HEK-293 cells were transfected with FuGENE 6 (Roche) with all the constructs above respectively, and cultured in DMEM (medium containing 10% FBS and 0.5 mg/ml Zeocin (Invitrogen)). After a 14 day-long selection with Zeocin, medium was replaced with serum-free AIM V synthetic medium (Invitrogen). The conditioned medium was collected every 2-3 days for two weeks, and frozen at -80°C. For purification, about 500 ml of conditioned medium was concentrated using Centricon Plus-70 ultrafiltration units (30 kDa cutoff; Millipore). Concentrated proteins were purified using 500 l protein G–Sepharose (GE Healthcare) and eluted with 5 ml glycine 0.1M pH 2.7. Fractions of 1 ml were collected into tubes containing 100 l Tris 1M, pH 8.6, to neutralize. Glycine was further dialysis out. Purified recombinant proteins were estimated by resolving in SDS-PAGE and Sypro Ruby gel stain (Invitrogen).

**Pull-down of PTPδ-Fc and Mass Spectrometry**

25 fresh rat brains were homogenized in 200 ml cold homogenization buffer (320 mM sucrose, 4 mM Hepes-NaOH pH7.3) at 900 rpm. Then crude synaptosomal fraction P2' from the total homogenized brains was dissolved in extraction solution (50 mM HEPES-NaOH pH7.4, 100 mM NaCl and protease inhibitors, 1% CHAPS, 2 mM MgCl₂, 2 mM CaCl₂). 80 µg PTPδ-hFc (Group 1), or mixture of Ig1-3 A B⁺ and Ig1-3A B⁻ of PTPδ-Fc (1:1, Group 2), or neurexin1β-hFc (Group 3) were bound to protein G beads (150 ul slurry). Different hFc bound protein G beads (Group1, 2, and 3) were further mixed with brain lysates in extraction solution overnight at 4 degree. After extensive wash, bound proteins were eluted first by 50 mM HEPES-NaOH pH7.4, 1.0 M NaCl, 5 mM EGTA with 1% CHAPS, followed by the second elution with glycine pH 2.4, (neutralized by Tris-HCl pH 8.6). Eluted proteins are precipitated with 20% TCA overnight at 4 degree. The pellets were washed twice with cold acetic acid, and were re-suspended with 50 µl TE buffer for mass spectrometry analysis in Proteomic Core Facility (PCF) in the University of British Columbia.

**Surface Binding Assay**

COS7 cells were plated at 60,000/well onto glass cover slips in a 12-well plate. The next day, 1 ug plasmid was transfected into each well by the TransIT®-LT1 Reagent (Mirus Bio LLC) according manufacturer’s instruction. 24 hours later, coverslips were washed with cold EBG buffer (168 mM NaCl, 2.6mM KCl, 10 mM HEPS pH7.4, 2 mM CaCl₂, 2mM MgCl₂, 5 mM Glucose, 0.1% BSA) twice, then incubated with 100 nM purified PTPδ-Fc Ig1-3 hFc or SALM3-hFc proteins for 1 hour at 4 degree. All coverslips were washed by cold EBG for three times before the fixation with 4% paraformaldehyde for 12 min at room temperature. Bound hFc proteins were immunostained by goat anti-human Fc-594 (1:500).

**RT-PCR and Sequencing Analysis of LAR-RPTP Splice Variants**

mRNAs from rat hippocampi (P12; n = 2) were subjected to RT-PCR and DNA sequencing of 50 randomly chosen clones for each gene. Primers for the RT-PCR were as follows: LAR, GCTTCAAGCAACCGGTCGTAT-3’ (forward), 5’ GGGTAACACTGGGTCAGTT-3’ (reverse); PTPσ, 5’- TTCAAGGACTTCTGCTTG-
3' (forward), 5'-ACATCCGTGAGTTCGAGGAC-3' (reverse); PTPδ, 5'-
CACAATTGAAGGTGGTGGAA-3' (forward), 5'-ACACTTCCACCTGGCATGAT-3'
(reverse). Both of the MeA and MeB splice inserts were amplified and sequenced by a
single set of primers.

**Knockdown of LAR-RPTPs Family in Co-culture**

The sh-RNA sequence against PTPδ was described in our earlier work (Takahashi et al.,
2012). LAR sh-RNA sequence was 5’-GGCCTACATAGCTACACAG-3’, which was
validated before (Mander et al., 2005). The sh-RNA sequence for PTPc is 5’-
GGCATCATGGGTAGTGATT-3’, which targeted a region between the transmembrane
part and D1 domain. All sh-RNA sequence was cloned under a U6 promoter in the
pLL3.7-syn-CFP vector which also independently expresses CFP driven by a synapsin
promoter. 3.3 µg of sh-RNA construct was used in the individual knockdown groups. For
the triple knockdown, 3.3 µg of each construct (9.9 µg in total) were mixed. For the
control group, 9.9 µg sh-RNA construct targeting MorB, which had no influence on
synaptogenesis (Takahashi et al., 2012), was used.

sh-RNA constructs were delivered into 1 M primary hippocampal neurons using
the AMAXA nucleofector system (Kit, VPG-1003; Program, O-003; Lonza), and plated
to coverslips in a glia dish. At DIV 10, neurons were co-cultured with transfected COS7
cells expressing HA-SALM3 or HA-neuroligin2 for 24 hours in a glia dish. Coverslips
were fixed by 4% paraformaldehyde for 12 min at room temperature. After three wash
with PBS, coverslips were blocked in a block buffer (3% BSA, 1% Goat serum in PBS)
first, and then incubated with anti-HA (3F10, 1:1000, Roche) for 1 hour. After three
washes with PBS, coverslips were permeabilized by PBS containing 0.2 Triton X-100
for 5 min. Coverslips were re-blocked by the block buffer, followed by primary antibodies
in the block buffer over night at 4 degree. Secondary antibodies were incubated with
coverslips for 45 minutes at 37 degree. To label presynaptic cluster, we used rabbit
 polyclonal antibody against synapsin-1 (1:2000; Millipore; AB1543P). For labeling
dendrites, we used antibody to MAP2 (chicken polyclonal IgY, 1:8,000, Abcam, ab5392).
We used highly cross-adsorbed, Alexa dye–conjugated secondary antibodies generated
in goat toward the appropriate species and monoclonal isotype (1:500, Invitrogen,
Alexa-488, Alexa-568 and Alexa-647). AMCA-conjugated antibody to chicken
IgY(donkey IgG,1:200, Jackson ImmunoResearch, 703-155-155) was used for
visualizing dendrites.

Images were collected blindly based on the similar HA surface intensity on
COS7 cells and analyzed by ImageJ 1.48. The intensity of synapsin-1 was normalized
to the area of CFP-positive and COS7-contacting axons across all groups. The data
was analyzed by ANOVA with Bonferroni posthoc test in Graphpad Prism5.

**Generation of Salm3<sup>−/−</sup> Mice**

*Salm3<sup>−/−</sup>* sperms in the genetic background of C57BL/6NTac were purchased from
KOMP (VG10115) were in vitro fertilized with oocytes in the C57BL/6N background.
Resulting heterozygous mice were interbred to generate homozygous Salm3-deficient
(Salm3−/−) mice. Mice in mixed genetic backgrounds of C57BL/6N and C57BL/6J were used for all the assays. Mice were bred and maintained according to the Requirements of Animal Research at KAIST, and all procedures were approved by the Committee of Animal Research at KAIST (KA2012-19). Mice were fed ad libitum and housed under 12-hour light and dark cycles. For behavioral experiments, we used hetero x hetero breeding and strict sex-matched controls.

**Electron Microscopy**

Wild-type and Salm3−/− mice (2 weeks) were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and were intracardially perfused with 10 ml of heparinized normal saline, followed by 50 ml of a freshly prepared fixative of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.4). Hippocampi were removed from the whole brain, postfixed in the same fixative for 2 hours and stored in PB (0.1M, pH 7.4). Sections were cut transversely on a vibratome at 60 μm thickness. The sections were osmicated with 1% osmium tetroxide (in 0.1 M PB) for 1 hour, dehydrated in graded alcohols, flat embedded in Durcupan ACM (Fluka), and cured for 48 hours at 60°C. Small pieces containing CA1 stratum radiatum (∼150 μm from CA1 pyramidal cell body layer) were cut out of the wafers and glued onto the plastic block by cyanoacrylate. Ultrathin sections were cut and mounted on Formvar-coated single slot grids. For quantification of excitatory synapse sections were stained with uranyl acetate and lead citrate, and examined with an electron microscope (Hitachi H-7500; Hitachi) at 80 kV accelerating voltage. For quantification of excitatory synapse, sections were also immunogold stained for glutamate.

**Postembedding Immunogold Staining for Glutamate**

Sections were immunostained for glutamate by the postembedding immunogold method, as previously described (Paik et al., 2012a; Paik et al., 2012b), with some modifications. Briefly, grids were treated for 6 min in 1 %periodic acid, to etch the resin, and for 10 min in 9 % sodium periodate to remove the osmium tetroxide, washed in distilled water, and transferred to Tris-buffered saline containing 0.1 % Triton X-100 (TBST; pH 7.4) for 10 min. After incubation in 2 % human serum albumin (HSA) in TBST for 10 min, the grids were incubated with rabbit antiserum against glutamate (Glut 607, 1:1,100) in TBST containing 2 % HSA for 2 hours at room temperature. The antiserum (a kind gift from professor O. P. Ottersen at the Center for Molecular Biology and Neuroscience, University of Oslo) was raised against glutamate conjugated to bovine serum albumin (BSA) by glutaraldehyde and formaldehyde and characterized extensively (Ottersen, 1989). To eliminate cross-reactivity, the diluted antiserum was preabsorbed overnight with glutaraldehyde (G)-conjugated amino acids (300 μM glutamine-G, 100 μM aspartate-G, and 200 μM b-alanine-G) (Ottersen et al., 1986). After extensive rinsing in TBST, grids were incubated for 3 hours in goat anti-rabbit IgG coupled to 15 nm gold particles (1:25 in TBST containing 0.05 % polyethylene glycol; BioCell Co., Cardiff, UK). After washing in distilled water, the grids were counterstained with uranyl acetate and lead citrate, and examined on a Hitachi H-7500 electron microscope (Hitachi, Tokyo, Japan) at 80 kV accelerating voltage. To assess
immunoreactivity for glutamate, gold particle density (number of gold particles per μm²) for glutamate over each axon terminal was compared with the mean tissue density indicating metabolic glutamate level. Mean tissue density was determined by counting the number of gold particles in 15 randomly selected fields of 1.5 μm² in each section; boutons with gold particle density >Mean + 2.576 SD of the mean tissue density (99% confidence level) were considered glutamate immunopositive (Paik et al., 2012a; Paik et al., 2012b).

Quantitative Analysis of PSD Density and Morphology

For quantification of PSD density and morphology, thirty micrographs representing 461.1 μm² neuropil regions in each mouse were photomicrographed at a 40,000×. The density and morphology (proportion of perforation, length, and thickness) of the postsynaptic densities (PSDs) apposed to axon terminals from three WT and Salm3−/− mice were quantified by using image J software (v.1.47; NIH, Bethesda, MD). For the density and morphology of PSDs apposed to glutamate-positive axon terminals, electron micrographs were taken from sections performed with postembedding immunogold staining for glutamate: thirty micrographs representing 461.1 μm² neuropil regions in each mouse were taken at a 40,000× from the sections. The number of glutamate-immunopositive terminals apposed to clear PSDs from each of three WT and SALM3−/− mice were quantified by using ImageJ software. The measurements were all performed by an experimenter blind to the genotype. Digital images were captured with GATAN DigitalMicrograph software driving a CCD camera (SC1000 Orius; Gatan) and saved as TIFF files. Brightness and contrast of the images were adjusted in Adobe Photoshop 7.0 (Adobe Systems).

Field Potential Recording

Transverse hippocampal slices (400 μm) were prepared from 3- to 4-week-old male littermates. The brain was rapidly isolated and placed to ice-cold, oxygenated (95% O2 and 5% CO2) dissection buffer containing (mM) 212.7 sucrose, 5 KCl, 1.23 NaH2PO4, 0.5 CaCl2, 10 MgCl2, 26 NaHCO3 and 10 glucose. Hippocampal slices were prepared by cutting with a Leica VT1000P vibratome (Leica) and transferred for recovery to a holding chamber containing oxygenated ACSF consisted of (mM) 124 NaCl, 5 KCl, 1.23 NaH2PO4, 2.5 CaCl2, 1.5 MgCl2, 26 NaHCO3 and 10 glucose at 28-30 °C for at least 1 hr before recording. After recovery, slices were transferred to a recording chamber where they were perfused continuously with oxygenated ACSF (27-28 °C) at a flow rate of 2 ml/min. Hippocampal CA1 field excitatory postsynaptic potential (fEPSP) was evoked by Schaffer collateral stimulation (0.2 msec current pulses) with concentric bipolar electrode. Synaptic responses were recorded with ACSF-filled microelectrodes (1-2 MΩ) and were quantified as the initial slope of fEPSP in CA1.

LTP was induced by four episodes of theta-burst stimulation (TBS) with 10 s interval. TBS consisted of ten stimulus trains delivered at 5 Hz, each train consisted of four pulses at 100 Hz. LTD was induced by single-pulse low-frequency stimulation (SP-LFS, 1 Hz for 900 s). Data from slices with stable recordings (< 5% change over the
baseline period) were included in the analysis. For late-LTP experiments, slices were preincubated for at least 2 hours before recording the baseline (Huang and Kandel, 1995). Baseline responses were recorded for 30 min before LTP induction. Late-LTP was induced by 3 stimulus trains of high-frequency tetanus at 100 Hz, with an inter-train interval of 10 min (O'Carroll and Morris, 2004). All data are presented as mean ± SEM normalized to the preconditioning baseline (at least 20 min of stable responses). The experiments were blind to mouse genotypes. Recordings were performed using an AM-1800 Microelectrode amplifier (A-M systems) and IGOR software (Wavemetrics) was used for digitizing and analyzing the responses.

**Patch Analysis**

For whole-cell patch-clamp analysis, sagittal hippocampal slices (300 μm thick) of Salm3−/− mice and their wild-type littermates of 18-25 postnatal days were prepared using a vibratome (Leica VT1200) in ice-cold dissection buffer containing (in mM) 212 sucrose, 25 NaHCO₃, 5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 3.5 MgSO₄, 10 D-glucose, 1.25 L-ascorbic acid, and 2 Na-pyruvate bubbled with 95% O₂/5% CO₂. The slices were recovered at 32 °C for 30 min in normal artificial CSF (ACSF) (in mM: 124 NaCl, 2.5 kCl, 1 NaH₂PO₄, 25 NaHCO₃, 10 glucose, 2 CaCl₂, 2 MgSO₄ oxygenated with 95% O₂/5% CO₂). For the recording, a single slice was moved to and maintained in submerged-chamber at 28 °C, continuously perfused with ACSF (2 mL/min) saturated with 95% O₂/5% CO₂. Stimulation and recording pipettes were pulled from borosilicate glass capillaries (Harvard Apparatus) using a micropipette electrode puller (Narishege).

Whole-cell patch-clamp recordings of hippocampal CA1 pyramidal neurons were made using a MultiClamp 700B amplifier (Molecular Devices) and Digidata 1440A (Molecular Devices) under visual control with differential interference contrast illumination in an upright microscope. Signals were filtered at 2 kHz and digitized at 10 kHz.

Somatic whole-cell recordings of miniature EPSCs (mEPSC) were obtained at a holding potential of -70 mV using patch electrode (3.5-4.0 MΩ) filled with a solution containing the following (in mM): 100 CsMeSO₄, 10 TEA-Cl, 8 NaCl, 10 HEPES, 5 QX-314-Cl, 2 Mg-ATP, 0.3 Na-GTP, 10 EGTA, with pH 7.25, 295 mOsm. TTX (0.5 μM) and picrotoxin (100 μM) were included to ACSF to inhibit spontaneous action potential-mediated synaptic currents and IPSCs, respectively. To measure miniature IPSCs (mIPSC), CA1 pyramidal cells were also held at -70 mV, and pipette internal solution was used containing following (in mM): 115 CsCl, 10 TEA-Cl, 8 NaCl, 10 HEPES, 5 QX-314-Cl, 4 Mg-ATP, 0.3 Na-GTP, 10 EGTA, with pH 7.35, 295 mOsm. To inhibit excitatory synaptic currents, TTX (0.5 μM), D-AP5 (25 μM), and NBQX (10 μM) were added to ACSF.

For NMDA/AMPA ratio experiments, CA1 pyramidal neurons were voltage-clamped at -70 mV, and EPSCs were evoked at every 15 sec. AMPAR-mediated EPSCs were recorded at -70 mV, and 30 consecutive responses were recorded after stable baseline. After recording AMPAR-mediated EPSCs, holding potential was changed to +40 mV to record NMDA receptor-mediated EPSCs. NMDA component was measured at 60 ms after the stimulation. The NMDA/AMPA ratio was determined by...
dividing the mean value of 30 NMDA components of EPSCs by the mean value of 30 AMPAR-mediated EPSC peak amplitudes. Data were acquired by Clampex 10.2 (Molecular Devices) and analyzed by Igor (mEPSC and mIPSC; Wavemetrics) and by Clampfit 10 (NMDA/AMPA ratio; Molecular Devices). Drugs were purchased from Tocris (TTX, NBQX and D-AP5), and Sigma (picrotoxin).

**Animal Behavioral Tests**

Mice used in all behavioral tests were 2~5 months of age. All assays used littersmates or age-matched animals.

**Open Field Test**

Mice were put into a white 40 x 40 x 40 cm open field box. Mice were allowed to explore freely in the box for an hour, which is recorded by a video camera and analyzed using the EthoVision 3.1 program (Noldus).

**Home-cage Activity**

Mice were placed in their ordinary cages, and movements were recorded for 24 hrs with 12h light-dark cycles. Movements were analyzed using the EthoVision 3.1 program (Noldus).

**Elevated Plus Maze**

The apparatus consisted of 2 open arms, 2 closed arms, and a center area, elevated to a height of 50cm above the floor. Mice were placed on the center area faced to the closed arm and allowed to freely explore the space for 5min.

**Rotarod**

For this assay, rotating speed of rod was gradually increased from 4 rpm to 40 rpm over 5 min. Mice were placed on the rotating rod for 20s, followed by the start of rod rotation. The assay was performed for 5 consecutive days, while measuring the latencies of mice falling from the rod.

**Repetitive Behavior**

Grooming and digging tests were performed in mice home cages. For grooming test, mice were individually placed into a home cage without bedding for 20 min. For digging test, home cages were filled with 2 cm-deep beddings, where mice were placed for 20 min, of which the activities during the last 10 min were measured and analyzed.

**T maze: Spontaneous Alteration**

T maze tests were performed as previously described (Deacon and Rawlins, 2006). This task was performed in a T maze that mice never visited before. Mice were placed in the start arm and allowed to make a choice to visit the right or left arm, which was
repeated 10 times. After all four paws of a mouse entered the arm, the arm was closed, the mouse was allowed to explore the arm for 5 s.

**T maze: Rewarded Alteration**

Mice were fed with a small amount of food (2 g/mouse) to have them reach the body weight of 85% of normal free-feeding body weights until the task is completed. Before the test, mice were allowed to freely explore the T maze for 5 min for two consecutive days. 70 μl of 70% of milk was used as a reward. During the test phase, mice were trained with 4 trails per day for 12 consecutive days, and each trial consisted of one forced choice and one free choice.

**Morris Water Maze**

This assay was performed in a plastic tank (100 cm diameter) with a hidden platform (10 cm diameter). The tank was filled with tap water at temperature of 20~22 °C made opaque with a white paint. Mice were trained to find the platform with 3 trials per day with an inter-trial interval of 30 min for 5 days. Mice that did not find the platform within 60 sec were guided to the platform and allowed to rest on the platform for 15 s. On day 6, a probe test was performed for 1 min in the absence of the platform. Mice were retrained to find the hidden platform in the tank after the probe test to avoid extinction. To test long-term memory, probe tests were given to the mice 7 days after the training. The number of exact crossing over the platform region, quadrant occupancy, and swimming speed were analyzed using the EthoVision3.1 software (Noldus).

**Object Recognition**

This test was performed in an open field test apparatus. Mice were habituated in the apparatus for 30 min a day before training. For novel object test, mice were allowed to explore two same objects for 10min on the training day. 24hr after training, one of the two objects was replaced with a new one. For displaced object recognition test, after exploring two same objects for 10min, mice were put back to homecage for 5min. One of the two objects was translocated to an opposite position in the same apparatus. Mice were allowed to explore the objects for 10min. Exploration time for the two objects was measured. Object exploration was defined by the amount of time spent for each object, with the nose of mice touched or faced to the objects within 2 cm from them.

**Fear Conditioning and Extinction**

On the training day, mice were placed in the fear chamber and allowed to freely explore the chamber for 2 min. Three unsigned foot shocks were given to the mice (2 sec, 0.5 mA, 1 min apart). Mice were remained in the chamber for another 1 min and returned to their home cages (total 5 min). To measure fear conditioning or extinction, mice were returned to the same context 24 hrs and 7 days after training and allowed to explore for 5 min. Freezing was defined as the absence of mouse movement longer than a consecutive 1 sec. For fear extinction, mice were returned to the chamber and allowed to explore for 5 min for 5 consecutive days.
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Figure S1. Related to Figure 1

|                | NGL-3 | LAR | PTP α_1 B | PTP β_1 B | SynCAM1 | NGL-1 | NGL-2 |
|----------------|-------|-----|-----------|-----------|---------|-------|-------|
| SALM3          |       |     |           |           |         |       |       |
| NGL-3          |       |     |           |           |         |       |       |
| LRRM1          |       |     |           |           |         |       |       |
| SALM3          |       |     |           |           |         |       |       |
| Neurexin-1α    |       |     |           |           |         |       |       |
| SALM3          |       |     |           |           |         |       |       |
| GluN1          |       |     |           |           |         |       |       |
| SALM3          |       |     |           |           |         |       |       |
| ALCAM          |       |     |           |           |         |       |       |
| SALM3          |       |     |           |           |         |       |       |
| Synxin-2       |       |     |           |           |         |       |       |
Figure S2. Related to Figure 3

A

Sh-control

Target shRNA

LAR-CFP

PTPσ-YFP

PTPδ-YFP

B

LAR-RPTPs/shRNA

Sh-control  Sh-LAR  Sh-control  Sh-PTPσ  Sh-controll  Sh-PTPδ

C

Sh-control  Sh-PTPσ

α-tubulin

-170
-130
-100
-70
-55
Figure S4. Related to Figure 5

A

WT

KO

B

Glutamate-positive of PSD density / 1000 μm²

C

Glutamate-positive of perforated synapse (%)

D

PSD thickness (μm)

E

PSD length (μm)

* ns
Figure S5. Related to Figure 5

A

WT

KO

B

mEPSC frequency (Hz)

WT  KO

C

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Table S1. Proteins Pulled Down from Brain Crude Synaptosomes with Both PTPδ-Fc and PTPδlg1-3-Fc but Not Neurexin1β-Fc

| ID | Accession   | Description                                                                 | OS     | GN   | PE  | SV |
|----|-------------|-----------------------------------------------------------------------------|--------|------|-----|----|
| 1  | sp|Q01853|TERA_MOUSE | Transitional endoplasmic reticulum ATPase | OS=Mus musculus | GN=Vcp | PE=1 | SV=4 |
| 2  | sp|Q8VDD5|MYH9_MOUSE | Myosin-9 | OS=Mus musculus | GN=Myh9 | PE=1 | SV=4 |
| 3  | tr|Q3UH59|Q3UH59_MOUSE | Myosin, heavy polypeptide 10, non-muscle | OS=Mus musculus | GN=Myh10 | PE=2 | SV=1 |
| 4  | sp|Q8JZW4|CPNE5_MOUSE | Copine-5 | OS=Mus musculus | GN=Cpne5 | PE=2 | SV=1 |
| 5  | tr|Q3UYN2|Q3UYN2_MOUSE | Copine-6 | OS=Mus musculus | GN=Cpne6 | PE=2 | SV=1 |
| 6  | sp|P59108|CPNE2 MOUSE | Copine-2 OS=Mus musculus | GN=Cpne2 | PE=2 | SV=1 |
| 7  | sp|P59108|CPNE2 MOUSE | Protein Sec24c OS=Mus musculus | GN=Sec24c | PE=4 | SV=1 |
| 8  | tr|Q3U3J1|Q3U3J1_MOUSE | 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial | OS=Mus musculus | GN=Bckdha | PE=2 | SV=1 |
| 9  | sp|P29341|PABP1_MOUSE | Polyadenylate-binding protein 1 | OS=Mus musculus | GN=Pabpc1 | PE=1 | SV=2 |
| 10 | sp|Q60605-2|MYL6_MOUSE | Isoform Smooth muscle of Myosin light polypeptide 6 | OS=Mus musculus | GN=Myl6 | |
| 11 | sp|Q8K310|MATR3_MOUSE | Matrin-3 | OS=Mus musculus | GN=Matr3 | PE=1 | SV=1 |
| 12 | sp|Q8BX10-2|PGAM5_MOUSE | Isoform 2 of Serine/threonine-protein phosphatase PGAM5, mitochondrial | OS=Mus musculus | GN=Pgam5 | |
| 13 | tr|A3KFU5|A3KFU5_MOUSE | Poly A binding protein, cytoplasmic 4 | OS=Mus musculus | GN=Pabpc4 | PE=4 | SV=1 |
| 14 | sp|Q8BT60|CPNE3 MOUSE | Copine-3 OS=Mus musculus | GN=Cpne3 | PE=1 | SV=2 |
| 15 | sp|Q6A0A9|F120A_MOUSE | Constitutive coactivator of PPAR-gamma-like protein 1 | OS=Mus musculus | GN=FAM120A | PE=1 | SV=2 |
| 16 | sp|P38647|GRP75_MOUSE | Stress-70 protein, mitochondrial | OS=Mus musculus | GN=Hspa9 | PE=1 | SV=3 |
| 17 | tr|Q8C788|Q8C788_MOUSE | Sorting nexin 18 | OS=Mus musculus | GN=Snx18 | PE=2 | SV=1 |
| 18 | sp|Q80XU8|LRFN4_MOUSE | Leucine-rich repeat and fibronectin type-III domain-containing protein 4 | OS=Mus musculus | GN=Lrfn4 | PE=1 | SV=1 |
| 19 | tr|A2BEE9|A2BEE9_MOUSE | Discs, large homolog 3 (Drosophila) | OS=Mus musculus | GN=Dlg3 | PE=4 | SV=1 |
Table S1. Proteins Pulled Down from Brain Crude Synaptosomes with Both PTPδ-Fc and PTPδig1-3-Fc but Not Neurexin1β-Fc

| # | ID          | Description                                      | Organism   | Gene   | Peptide Extent | Spectra Count |
|---|-------------|--------------------------------------------------|------------|--------|---------------|---------------|
| 20 | tr|F6RT34|F6RT34_MOUSE | Myelin basic protein (Fragment) OS=Mus musculus GN=Mbp PE=4 SV=1 |
| 21 | sp|P12815|PDCD6_MOUSE | Programmed cell death protein 6 OS=Mus musculus GN=Pdcd6 PE=1 SV=2 |
| 22 | tr|A2ALV1|A2ALV1_MOUSE | Endophilin-A1 OS=Mus musculus GN=Sh3gl2 PE=4 SV=1 |
| 23 | tr|F6QJV5|F6QJV5_MOUSE | Protein Tfg (Fragment) OS=Mus musculus GN=Tfg PE=4 SV=1 |