Genes	Expression Vector
LRRTM1	pDisplay (HA-tagged)
LRRTM2	pDisplay (HA-tagged)
LRRTM3	pDisplay (HA-tagged)
LRRTM4	pDisplay (HA-tagged)
BAI1	pCMV5
BAI2	pCMV5
BAI3	pCMV5
CIRL1	pCMV5
CIRL2	pCMV5
CIRL3	pCMV5
AMIGO1	pDisplay (HA-tagged)
AMIGO2	pDisplay (HA-tagged)
AMIGO3	pDisplay (HA-tagged)
FLRT1	pDisplay (HA-tagged)
FLRT2	pDisplay (HA-tagged)
FLRT3	pDisplay (HA-tagged)
LRTM1	pDisplay (HA-tagged)
LRTM2	pDisplay (HA-tagged)
Slitrk1	pDisplay (HA-tagged)
Slitrk2	pDisplay (HA-tagged)
Slitrk3	pDisplay (HA-tagged)
Slitrk4	pDisplay (HA-tagged)
Slitrk5	pDisplay (HA-tagged)
Slitrk6	pDisplay (HA-tagged)
LRRC24	pCMV5
Tpbg	pCMV5
KIAA0644	pCMV5
Lingo2	pDisplay (HA-tagged)
Lingo4	pDisplay (HA-tagged)
LRRN1	pDisplay (HA-tagged)
LRRN3	pDisplay (HA-tagged)
TrkA	pDisplay (HA-tagged)
TrkB	pDisplay (HA-tagged)
TrkC	pDisplay (HA-tagged)
CD97	pDisplay (HA-tagged)
Calsyntenin-1	pDisplay (HA-tagged)
Calsyntenin-2	pDisplay (HA-tagged)
Calsyntenin-3	pDisplay (HA-tagged)

Table S1. Candidate molecules expressed in HEK293T cells that were screened for induction of presynaptic differentiation in heterologous synapse-formation assays.

No.	Accession	Protein Description	Peptide Sequence (Unique)	Peptide Matches	Peptide Delta	Peptide Score	Expect
1	Q99JH7	Calsyntenin-3 OS=Mus musculus GN=Clstn3 PE=1 SV=1	ESLLLDMASLQQR	164	-0.0006	37.17	0.00270
			LHGSGVPFEAVILDK	140	-0.0067	8.61	1.30000
			VNDVNEFAPVFVER	125	-0.0118	49.25	0.00015
			HKPWIEAEYQGIVMENDNTVLLNPPLFALDK	118	0	2.53	5.00000
			IEYAPGAGSLALFPGIR	116	-0.0035	31.77	0.00700
			ADESWQGTVTDTR	102	-0.0074	28.99	0.00470
			FATPGVRPLR	94	-0.0019	8.13	0.95000
			GHQPPPEMAGHSLASSHR	80	-0.0084	18.65	0.11000
			YSSNEFIVEVNVLHSMNR	68	-0.0195	10.94	0.86000
			VAHPSHVLSSQQFLHR	61	-0.0071	0.63	12.00000
			HGAALYAR	58	0.004	41.72	0.00120
			LTTAVK	55	-0.0013	3.40	2.30000
			LQYSGEK	54	-0.0001	21.01	0.08100
			ATGEGLIR	50	-0.005	25.62	0.05600
			EGLDYR	47	-0.0019	18.45	0.12000
			DFESLGK	47	-0.0008	27.25	0.05700
			RIEYAPGAGSLALFPGIR	45	-0.0041	27.33	0.01700
			HKPWIEAEYQGIVMENDNTVLLNPPLFALDKDAPLR	10	-0.0005	35.24	0.00220
			FTVTAYDCGK	1	0.0064	0.53	4.50000
			RVSGTGGPSGASTDPK	1	-0.0101	7.89	5.10000
2	Q9CS84	Neurexin-1-alpha OS=Mus musculus GN=Nrxn1 PE=1 SV=3	LELSR	20	-0.0021	6.79	7.50000
			NIIADPVTFK	10	-0.0048	21.32	0.11000
			SADYVNLALK	9	-0.0093	15.24	1.10000
			ITTQITAGAR	6	0.0091	26.65	0.02600
			NLDLK	4	-0.0005	0.01	19.00000
			EAVLVR	4	-0.0029	23.98	0.04800
			GGGQITYK	1	-0.0008	4.51	13.00000
			IHGVVAFK	1	-0.0016	2.70	3.60000
			DLFIDGQSK	1	0.0077	7.97	3.90000
			YPAGNNDNER	1	0.0018	7.47	1.10000
			VNDGEWYHVDFQR	1	-0.0129	5.20	1.00000
			LEFHNIETGIITER	1	-0.0012	19.28	0.17000
3	Q6P9K9	Neurexin-3-alpha OS=Mus musculus GN=Nrxn3 PE=1 SV=2	DGILVR	2	0.0003	15.01	0.38000
4	Q07310	Neurexin-3-alpha OS=Rattus norvegicus GN=Nrxn3 PE=1 SV=1	DGVLVR	1	0.0058	5.90	7.80000
5	Q63374	Neurexin-2-alpha OS=Rattus norvegicus GN=Nrxn2 PE=1 SV=3	MALGSR	2	0.0023	15.10	0.93000
			SAVLVR	1	0.0005	13.04	1.30000
			LAVGFSTHQR	1	-0.0041	9.02	1.40000

Table S2. Detailed list of peptides indicating that neurexin-α proteins as the specific binding proteins of CST-3.

Extended Experimental Procedures

Construction of Expression Vectors. HA-CSTs encode full-length mouse CSTs containing an inserted HA-epitope. The signal peptide sequence of CST-1 (amino acids [aa] 1-28) was initially PCR-amplified, digested with *Hin*dIII/*Bgl*II, and cloned into the GW1 vector (British Biotechnology). The remaining of CST-1 sequences (aa 29-979) were PCR-amplified, digested with *Bgl*II and *Eco*RI, and cloned into the GW1 vector. Full-length CST-2 and CST-3, excluding the corresponding signal peptide sequences (CST-2, aa 21-966; CST-3, aa 20-956), were PCR-amplified, digested with *Xma*I and *Sac*II, and subcloned into the pDisplay vector (Invitrogen). L-313 HA-CSTs were constructed by amplifying CST-1 (aa 29-979), CST-2 (aa 21-966) and CST-3 (aa 20-956) by PCR, digesting with *Nhe*I and *BsrG*I, and subcloning into the L-313 lentiviral vector. pDis-CSTs encode the entire extracellular regions of CSTs (CST-1, aa 30-861; CST-2, aa 20-838; CST-3, aa 20-846), cloned into *Xma*I and *Sac*II sites. pDis-CST-3 deletion variants encode the indicated CST-3 fragment (Cad+LNS, aa 36-541; LNS+linker, aa 335-846; Cad repeats, aa 20-257; LNS, aa 335-541; Cad repeat 1, aa 36-158; and Cad repeat 2, aa 152-259) cloned into the pDisplay vector at *Xma*I and *Sac*II sites. pDis-Nrxs encode the indicated Nrx-1α, Nrx-1β, Nrx-2α, or Nrx-3α fragment (Nrx1α-1, aa 15-1361; Nrx-1α-18, aa 15-207; Nrx-1α-21, aa 298-506; Nrx-1α-24, aa 298-506; Nrx-1α-25, aa 298-506; Nrx-1α-31, aa 920-1361; Nrx-1α-32, aa 920-1361; Nrx-1α-37, aa 738-931; Nrx-1α-38, aa 738-931; Nrx-1α-39, aa 486-931; Nrx-1α-40, aa 486-931; Nrx-1α-41, aa 15-1361; Nrx-1β-1, aa 15-299; Nrx-2α, aa 29-1628; and Nrx-3α, aa 28-1513) cloned into the pDisplay vector at *Xma*I and *Sac*II sites (Nrx1α-1, -31, -32, -37, -38, -39, -40, -41, and Nrx-3α), *Bgl*II and *Sal*I sites (Nrx-1α-18, -21, -24, -25, Nrx-1β-1, and Nrx-2α). To construct shRNA lentiviral expression vectors, oligonucleotides targeting mouse CST-1, CST-2, or CST-3 were annealed, phosphorylated, and cloned into the *Xho*I and *Xba*I sites of a single KD vector (L-309 vector; see **Figure 4A** for a schematic diagram of vectors) immediately downstream of the human H1 promoter. For CST-TKD, oligonucleotides targeting CST-1 (J73), CST-2 (J76), and CST-3 (J81) were subcloned respectively into the

*Xho*I-*Xba*I (J73), *Bst*EII-*Bsi*WI (J76), and *Sbf*I-*Bst*BI (J81) sites of a TKD vector (L-313 vector) containing two human H1 promoters and two human U6 promoters. The following nucleotide sequences were targeted: CST-1 [J73], 5'-CTG TGG ACA AAG ACG GTT ATA-3'; CST-2 [J76], 5'-CGG AGT CAT AAC TGA GAA CAA-3'; and CST-3 [J81], 5'-CCA AGG TCT TAC TGT CTC TAT-3'). For the rescue vectors, full-length human CST-1, CST-2 ,or CST-3 was subcloned into the L-313 vector at the *Nhe*I and *Bsr*GI sites. The following constructs were previously described: pCMV5-NL1-mVenus (Lee et al., 2013); pCMV5-NL2-mVenus (Lee et al., 2013); pCMV5-IgC-NL2 (Lee et al., 2013); L315-LRRTM-DKD (Ko et al., 2011); L-315-Nrx-TKD (Zhang et al., 2010); pDis-HA-Slitrk1 (Yim et al., 2013); pCMV-IgC-Nrx-1α-1 and pCMV-IgC-Nrx-1β-1 (Boucard et al., 2005). pcDNA3.1-CST-1, pcDNA3.1-CST-2, and pcDNA3.1-CST-3 were gifts from Peter Sonderegger (Univ. of Zurich, Switzerland (Hintsch et al., 2002)).

Antibodies. CST-3 peptides (JK001; CSDERRIIESPPHRY) were synthesized and conjugated to keyhole limpet hemocyanin through a cysteine added to the N-terminus of the peptide. Glutathione-S-transferase (GST) fusion proteins encoding mouse CST-2 (JK010; aa 818-912) were produced in BL21 *E. coli* and purified on a glutathione-Sepharose column (GE Healthcare). Following the immunization of rabbits with these immunogens, the specific antibodies were affinity-purified using a Sulfolink column (Pierce). The following antibodies were obtained commercially: rabbit polyclonal anti-synapsin I (Millipore), guinea pig polyclonal anti-VGLUT1 (Millipore), mouse monoclonal anti-GAD67 (clone 1G10.2; Millipore), mouse monoclonal anti-PSD-95 (clone 7E3-1B8; Thermo Scientific), mouse monoclonal anti-HA (clone HA-7; Covance), goat polyclonal anti-EGFP (Rockland), mouse monoclonal anti-gephyrin (clone mAb7a; Synaptic Systems), mouse monoclonal anti-synaptophysin (clone SVP-38; Sigma), rabbit polyclonal anti-MAP2 (Sigma), mouse monoclonal anti-MAP2 (clone HM-2; Sigma), and mouse monoclonal anti-α-tubulin (clone

DM1A; Hybridoma Bank). Rabbit polyclonal anti-Homer1 (1133; a gift from Eunjoon Kim) was previously described (Sala et al., 2001).

Heterologous Synapse-formation Assays. Heterologous synapse-formation assays were performed using HEK293T cells as described previously (Yim et al., 2013). Briefly, HEK293T cells were transfected with EGFP (negative control), pCMV-5-NL1-mVenus (positive control), or various CST expression vectors as indicated (described in **"Construction of Expression Vectors"**) using FuGene6 (Roche). After 48 h, the transfected HEK293T cells were trypsinized, seeded onto hippocampal neuron cultures at DIV8-10, cocultured for an additional 72 h, and double-immunostained at DIV11-13 with antibodies against GFP or HA (to detect various CST expression vectors containing an HA epitope) and synapsin I, VGLUT1, GAD67, or PSD-95, as previously described (Ko et al., 2009). All images were acquired using a confocal microscope. For quantification, the contours of the transfected HEK293T cells were chosen as the region of interest. The fluorescence intensity of synapsin puncta normalized to each HEK293T cell area was quantified for both red and green channels using MetaMorph Software (Molecular Devices).

Cell-surface Labeling Assays. Fc-fusion proteins of CST-3, various IgNrx-1α and IgNrx-1β deletions and NL2 were produced from HEK293T cells. Soluble Fc-fused proteins were purified using protein A-Sepharose beads (GE Healthcare). The bound proteins were eluted with 0.1 M glycine (pH 2.5) and immediately neutralized with 1 M Tris-HCl (pH 8.0). Transfected HEK293T cells expressing pDisplay-Nrx variants were incubated with 0.2 μM Fc or CST-3-Fc as indicated in **Figure 7**. The images were acquired using a confocal microscope (LSM510; Zeiss).

Cell-adhesion Assays. Cell-adhesion assays were performed using L cells, as previously described (Ko et al., 2009). Briefly, L cells were transfected with individual expression

vectors, as indicated. After 48 h, the cells were detached, mixed, and incubated at room temperature with gentle agitation. The extent of cell aggregation was measured at 2 h, spotting cell aliquots onto four-well culture slides, and imaging with confocal microscopy. For quantitation of the cell number per cluster, the cell clusters were defined as cell aggregates containing four or more cells that included at least one green (EGFP) and one red (DsRed) cell. Frames with no detectable cell clusters were counted as zero.

Affinity Chromatography. The brains from ten 6-week-old rats were homogenized in buffer [50 mM HEPES (pH 7.4), 50 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and protease inhibitors], extracted with 1% Triton X-100 for 1 h at 4°C, and centrifuged at 32,000 x g for 1 h at 4°C to pellet insoluble materials. Protein A-Sepharose beads (GE Healthcare), prebound to 100 μg of IgC-CST-3 or IgC protein, were added to the supernatant and rotated overnight at 4°C. The beads were packed into Poly-prep chromatography columns (BioRad) and washed first with washing buffer A [50 mM HEPES (pH 7.4), 300 mM NaCl, 0.1 mM $CaCl_2$, 5% (w/v) glycerol, and protease inhibitors], followed by washing with buffer B [50 mM HEPES (pH 7.4), 150 mM NaCl, 0.1 mM $CaCl_2$, 5% (w/v) glycerol, and protease inhibitors]. The bound proteins were eluted from the beads through sequential incubation with elution buffer I [50 mM HEPES (pH 7.4), 500 mM NaCl, 0.1 mM $CaCl_2$, and 5% (w/v) glycerol], elution buffer II [50 mM HEPES (pH 7.4), 1 M NaCl, 0.1 mM $CaCl_2$, and 5% (w/v) glycerol] and elution buffer III [50 mM HEPES (pH 7.4), 1 M NaCl, 0.5 mM EGTA, 0.1 mM $CaCl_2$, and 5% (w/v) glycerol], and subsequently precipitated with trichloroacetic acid. The precipitate was resuspended in 8 M urea containing ProteaseMAX (Promega) according to the manufacturer's instructions. The proteins were digested with sequencing grade, modified trypsin (Promega) for mass spectroscopy analysis (see below).

Mass Spectrometry Analysis. The tryptic-dried samples were analyzed using the Agilent HPLC-Chip/TOF MS system equipped with an Agilent 1260 nano-LC system, HPLC Chip-

cube MS interface and 6530 QTOF single quadrupole-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA). The dried peptide samples were re-suspended in 2% ACN/0.1% FA and concentrated on a Large-capacity HPLC Chip (Agilent Technologies). The HPLC chip was incorporated onto an enrichment column (9 mm, 75 µm I.D., 160 nl) and reverse-phase column (15 cm, 75 µm I.D., packed with Zorbax 300SB-C18 5 µm resins). The peptide separation was performed using a 110min gradient of 3-45% buffer B (buffer A containing 0.1% FA and buffer B containing 90% ACN/0.1% FA) at a flow rate of 300 nl/min. The MS and MS/MS data were acquired in the positive ion mode, and the data were stored in the centroid mode. The chip spray voltage was set at 1850 V and carried with chip conditions. The drying gas temperature was set at 325 with a flow rate of 3.5 L/min. A medium isolation (4 m/z) window was used for precursor isolation. Collision energy with slope of 3.7 V/100Da and offset of 2.5 V was used for fragmentation. The MS data were acquired over a mass range of 300-3000 m/z, and the MS/MS data were acquired over a mass range of 50-2500 m/z. The reference mass correction was activated using a reference mass of 922. The precursors were set in an exclusion list for 0.5 min after two MS/MS spectra. The MS/MS spectra were extracted using the MassHunterQualitative Analysis B.05.00 software (Agilent Technologies, Santa Clara, CA) with default parameters, and the spectra were interpreted using Mascot v2.3 (Matrix Science, London, UK) to search against the Uniprot/Swiss-Prot database (10/5/2012). Individual ion scores >23 indicated identity or extensive homology (*p value* < 0.05). The database searches were performed with a peptide mass tolerance of 10 ppm, a MS/MS tolerance of 0.5 Da, and a strict tryptic specificity that allowed one missed cleavage site. The carbamidomethylation of Cys was set as a fixed modification, whereas oxidation (M) was considered a variable modification.

In Situ Hybridization. In situ hybridization probes were constructed into pGEM7zf (Promega) vectors containing mouse CST-1 (nucleotides [nt] 2921-3236 [GenBank accession number: NM_023051]), CST-2 (nt 2844-3343 [GenBank accession number:

NM_022319], and CST-3 (nt 2931-3442 [GenBank accession number: NM_153508]). In situ hybridization was performed as previously described (Ko et al., 2006; Lee et al., 2013). Briefly, riboprobes were transcribed in the presence of ^{35}S-UTP (Amersham), and subsequently the labeled probes were hybridized with mouse brain sections (as indicated in **Figure 3A**) overnight at 52°C. The next day, the mouse brain sections were treated with RNase A (Boehringer-Manheim) for 30 min at 37°C, washed, dehydrated with ethanol, and air-dried. The hybridized radioactivity for CSTs was visualized through exposure to Biomax film (Kodak).

Preparation of the Postsynaptic Density Fraction. The PSD fraction was prepared as previously described (Dosemeci et al., 2006). Briefly, the mouse brains were homogenized in 0.32 M sucrose/1 mM $MgCl_2$ solution containing protease inhibitor cocktail (Thermo Scientific) using a Teflon/glass homogenizer. The homogenates were transferred to microfuge tubes and centrifuged at 470 x g for 2 min using a fixed-angle rotor. The resultant supernatant was decanted into a fresh microfuge tube and centrifuged at 10,000 x g for 10 min using the same rotor to obtain a mitochondria- and synaptosome-enriched pellet (P2). The (P2) pellet was resuspended in 0.32 M sucrose, and the suspension was layered onto 750 μl of 0.8 M sucrose in a microfuge tube. The samples were centrifuged 9100 x g for 15 min using a swinging bucket rotor. Following centrifugation, the myelin/light membrane layer at the 0.32/0.8 M sucrose interface was removed, and the 0.8 M sucrose layer, and most of the loose pellet containing the synaptosomes were collected, leaving the harder mitochondrial pellet. In subsequent experiments, the centrifugation step was shortened to 10 min to minimize the pelleting of synaptosomes. The collected synaptosomes in 0.8 M sucrose were diluted with 20 mM HEPES (pH 7) to obtain a final sucrose concentration of 0.4 M. An equal volume of 2% Triton X-100/150 mM KCl solution was added, and the samples were rotated in the cold for 15 min, followed by centrifugation at 20,800 x g for 45 min using a fixed-angle rotor. The pellets were resuspended in a 1% Triton X-100/75 mM

KCl solution using a mini dounce homogenizer and centrifuged at 20,800 x g for 30 min using the same rotor. The pellets were resuspended in 20 mM HEPES and centrifuged as described above. For immunoblotting, the nitrocellulose membranes were incubated with antibodies against CST-2 (JK010; 1:1000), CST-3 (JK001; 1:1000), PSD-93 (NeuroMab; 1:1000), and synaptophysin (Sigma; 1:3000).

Production of Recombinant Lentiviruses. To generate the recombinant lentiviruses, human embryonic kidney 293T cells were transfected with four plasmids—L-313 vectors (L-313-Nrx-TKD or L-313 alone), pRRE, pVSVg, and pREV—using FuGENE-6 (Roche), as previously described (Ko et al., 2011).

Primary Neuronal Culture, Transfections, Immunocytochemistry, Image Acquisition and Analyses. E18 embryos were used to prepare hippocampal or cortical cultures, as previously described (Ko et al., 2003; Ko et al., 2011). For immunocytochemistry, the cultured neurons were fixed with 4% paraformaldehyde/4% sucrose for 10 min at room temperature, permeabilized with 0.2% Triton X-100 in phosphate-buffered saline (PBS) for 5 min at 4°C, blocked with 3% horse serum/0.1% crystalline grade BSA in PBS for 30 min at room temperature, and incubated with the indicated primary and secondary antibodies in blocking solution for 1 h at room temperature. For overexpression of CST-3, hippocampal neurons were co-transfected with untagged full-length CST-3 expression vectors (pcDNA3.1-CST-3) and/or pEGFP-N1 at DIV8 and immunostained at DIV14. For neuronal infections with either CST expression vectors or shRNA vectors, hippocampal neurons were infected with the lentiviruses as indicated at DIV3-4 and immunostained at DIV14, as shown in the figure legends. The transfected or infected neurons were randomly chosen and images were acquired using a confocal microscope (LSM710; Carl Zeiss) with a 63× objective lenses; all image settings were kept constant. The Z-stack images obtained through confocal microscopy were converted to maximal projection and analyzed to obtain

the size and density of the presynaptic terminals or postsynaptic densities using MetaMorph software (Molecular Devices). Out of focus and nondiscrete regions of staining (i.e., puncta less than 0.1 microns squared) were excluded for the analyses. For the analysis of spine density, spines were defined as dendritic protrusions of 0.5-3 μm in length. The length of each spine was measured by manually drawing a vertical line from the base of the neck to the furthest point on the spine head, and spine width was measured by drawing a maximal line perpendicular to the length line in the spine head. All analyses were performed in a blind manner.

In Utero **Electroporation.** Pregnant ICR mice at 15 days post coitum (d.p.c) were anesthetized with an intraperitoneal injection of pentobarbital sodium (64.8 mg/kg), and the uterine horns were exposed through a longitudinal incision (~2-cm) in the abdomen. Approximately 1 μl of DNA solution containing 1 mg/ml pCAGGS-EGFP, 1.5 mg/ml L-315-CST-TKD shRNA, and 0.01% Fast Green in PBS or 1 mg/ml pCAGGS-EGFP, 1.5 mg/ml L-315 control shRNA, and 0.01% Fast Green in PBS, was injected into the lateral ventricle of each embryo through a glass capillary electrode. The head of each embryo was placed between tweezer-type electrodes (CUY650P5; NEPA Gene). An electrode was placed on the injection side for the transfection of layer 2/3 pyramidal neurons in the somatosensory cortex, whereas an anode was placed at this position for the transfection of hippocampal CA1 pyramidal neurons. Square electric pulses (35 V, 50 ms) were administered four times at 1 Hz using an electroporator (CUY21; NEPA Gene). The uteri were returned to the peritoneal cavity, and the incisions were sutured. The operated mice were returned to their home cages and subsequently allowed to deliver naturally. The transfected pups were identified at P_0 by visualizing the GFP signals through the scalp using an LED penlight (Handy Blue; Reryon).

Electrophysiology. Cell culture electrophysiology was performed as previously described (Maximov et al., 2007; Xu et al., 2009). Briefly, recordings were performed using DIV14-16 hippocampal cultured neurons. For recording mEPSC, the neurons were patched with the following internal solution (in mM): 90 Cs-gluconate, 10 CsCl, 10 HEPES, 10 EGTA, 5 NaCl, 4 Mg-ATP, and 0.3 Na-GTP. For mIPSC, the internal solution was (in mM) 100 CsCl, 5 NaCl, 10 EGTA, 10 HEPES, 4 Mg-ATP, and 0.3 Na-GTP. The recordings were performed in artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 100 NaCl, 4 KCl, 1 NaH_2PO_4, 20 HEPES, 30 glucose, 2 $CaCl_2$, and 1 $MgCl_2$. Internal solutions were saturated with 95% O_2/5% CO_2. mEPSC and mIPSC were recorded at a holding potential of -70 mV in the presence of 1 µM tetrodotoxin (TTX; Ascent) and 100 µM picrotoxin (for mEPSCs) or 1 µM TTX and 10 µM 6-cyano- 7-nitroquinoxaline-2,3-dione (CNQX; for mIPSC). The experimenter was blinded to the identity of the constructs throughout data collection and analysis. The slice electrophysiological recordings were performed as previously described (Etherton et al., 2009; Sugita et al., 2001). Briefly, P14-P19 mouse brains were removed and immediately immersed for 1 min in ice-cold ACSF (in mM: 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2.5 $CaCl_2$, and 1.3 $MgCl_2$) saturated with 95% O_2/5% CO_2. The chilled brain was dissected to remove the olfactory bulb and cerebellum, and subsequently bonded onto a metal platform and placed in the chamber of a vibratome (Campden 7000 smz) filled with ice-cold slicing solution (in mM: 238 sucrose, 10 glucose, 26 $NaHCO_3$, 1 NaH_2PO_4, 2.5 KCl, 3.3 $MgCl_2$, and 0.5 $CaCl_2$). Coronal sections (0.35 mm thick) were sliced and transferred to a chamber containing ACSF for recovery, with an initial incubation at 34°C for 30 min, followed by incubation at room temperature for 1 h. All recordings were performed with ACSF at 28-30°C. For whole-cell voltage-clamp recordings in acute cortical and hippocampal slices, patch pipettes (2–4 MΩ) were filled with excitatory-specific (in mM: 120 Cs-gluconate, 10 CsCl, 10 HEPES, 10 EGTA, 5 NaCl, 4 Mg-ATP, 0.3 Na-GTP, and 5 QX-314 [pH 7.2]) or inhibitory-specific (in mM: 130 CsCl, 5 NaCl, 10 EGTA, 10 HEPES, 4 Mg-ATP, 0.3 Na-GTP, and 5 QX-314 [pH 7.2]) internal solutions. Picrotoxin (100 µM) was

included in ACSF for all excitatory recordings and CNQX (10 μM; Tocris) and AP5 (25 μM; Tocris) were included for all inhibitory recordings. mEPSC and mIPSC recordings were performed with voltage clamped at -70 mV in the presence of 1 μM TTX to block action potential-evoked responses. To monitor the membrane statistics in each experiment, three 1-min traces were collected with a -2 mV voltage step between each trace. A glass capillary electrode filled with ACSF was used during stimulation to record the evoked responses. Paired-pulse facilitation experiments were performed at a holding potential of -70 mV with 100 ms inter-stimulus intervals. The amplitude of the second EPSC was measured relative to the amplitude of the first EPSC. For all whole-cell recordings, the membrane statistics were monitored after each trace. The following whole-cell recording criteria were used: R_a was < 25 MΩ and cells were rejected if R_a or R_m changed 20% over the course of the experiment. All recordings were digitized at 10 kHz and filtered at 2 kHz. Recordings were monitored with EPC10 double USB (HEKA) and analyzed offline using Mini Analysis Program (Synaptosoft). An experimenter blinded to the identity of expression constructs conducted all recordings and analyses.

Statistics. All data are expressed as the means ± SEM. All experiments were performed with at least three independent cultures and statistically evaluated using Student's t-test and one-way ANOVA, with cell numbers as the basis for 'n'.

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