Supplementary Information for

**LGI1–ADAM22–MAGUK configures trans-synaptic nanoalignment for synaptic transmission and epilepsy prevention**

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- Datasets S1 and S2
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Materials and Methods

Antibodies.
The antibodies used here included: rabbit polyclonal antibodies to Arc (#156003, Synaptic Systems), Kv1.1α (#P8607, Sigma-Aldrich), NMDA receptor GluN1 (#GluRz1-Rb-Af720, Frontier Institute), RIM2 (#140103, Synaptic Systems), Stargazin/Cacng2 (#07-577, Millipore) and SynGAP (#3200, Cell Signaling Technology); rabbit monoclonal antibodies to c-Fos (#2250, Cell Signaling Technology, clone 9F6) and HA (#3724, Cell Signaling Technology, clone C29F4); mouse monoclonal antibodies to ADAM22 (#75-105, NeuroMab, N46/30), Bassoon (N) (#ab82958, Abcam), β-catenin (#610153, BD Biosciences), CASK (#75-000, NeuroMab), FLAG (#F3165, Sigma-Aldrich, clone M2), GFP (#M048-3, MBL International), Homer1 (N) (#160011, Synaptic Systems), Kv1.1α (#75-105, NeuroMab for immunohistochemistry), Kvβ1.2 (#75-105, NeuroMab), LGI1 (1), LRRTM4 (#75-261, NeuroMab), NMDA receptor GluN2B (#610416, BD Biosciences), PSD-93 (#75-257, NeuroMab), PSD-95 (#MA1-046, Thermo Scientific), RFP (#M155-3, MBL International) and SAP97 (#75-260, NeuroMab); guinea pig polyclonal antibodies to Bassoon (C) (#141004, Synaptic Systems) and vGluT1 (#AB5905, Millipore); and human recombinant monoclonal antibody to LGI1 (AB060-110) (2) for immunohistochemistry. Rabbit polyclonal antibodies to ADAM22 (mouse, aa 444-526, extracellular) (3), ADAM22 (mouse, aa 858-898, cytoplasmic) (4), ADAM23 (mouse, aa 815-829) (1) and FAH (GGDYKDDDDKGGDTRYIIGGYPYDVPDYAGG; underlining corresponds to FLAG, AU1, and HA epitopes, respectively) (5) were described previously. A rabbit polyclonal antibody to GFP was raised against GST-GFP (aa 1-239) and affinity-purified.

Plasmid constructions for protein expression.
Plasmids for protein expression of LGI1, ADAM22 and PSD-95 were obtained as previously described (3, 4, 6, 7). Indicated mutations of ADAM22 were introduced by site-directed mutagenesis. cDNA of rat Syngap1 (accession number, AF048976) was cloned from rat brain total RNA by RT-PCR with the primers: 5’-GATCAGATCTACCATGCTCTATGCCCCCTTCAGAGA-3’ and 5’-GATCAGATCTTTCACACCGGGTTTGTGGACCCCAGGGGGGAACTGCCTCTCTTGAGCGTCAGCAGCTCTC, and subcloned into the pCAGGS vector. For biolistic expression, Adam22 and Psd-95 were subcloned into the pCAGGS–IRES–GFP vector (5). An Adam22 mutant (W396D) was subcloned into the pCAGGS–IRES–GFP vector using PCR and In-Fusion HD Cloning system (Invitrogen). The construct for crystallography is described later.

Animal experiments.
All animal studies were reviewed and approved by the ethic committees at NINS and were performed according to the institutional guidelines concerning the care and handling of experimental animals, and also conducted according to the Institutional Animal Care and Use Committee guidelines at UCSF. Mouse strains used in this study include: Adam22<sup>Fah/Fah</sup> knock-in mouse (generated in the present study), Adam22<sup>ΔC5/ΔC5</sup> knock-in mouse (generated in the present study), Lgi1 knock-out mouse (4), C57BL/6N mice and
B6D2F1 female mice (Japan SLC). They were bred and maintained in the animal facility of NIPS and UCSF. There was no randomization of mice before analysis, and the mice used in this study were selected based purely on availability including male and female mice (except for female mice to obtain embryonic mice and obtain fertilized eggs).

**Generation of knock-in mice.**

To generate the \(\text{Adam}22^{\text{FAH/FAH}}\) knock-in mouse, the sgRNA target sequence of mouse \(\text{Adam}22\) (HM004095), 5’-TCGATTTCCTCAGGAATGTAG-3’ (sgRNA#1), was subcloned into the pX330 (Addgene 42230) vector, which expresses Cas9 and sgRNA. For a homology-directed repair (HDR) template DNA, the ~1.5 kb homology sequence flanking each side of the target sequence for mouse \(\text{Adam}22\) was isolated by genomic PCR, and then the sequence encoding FAH tag (5’-GGCGGAGACTACAAGGATGACGACAAGGGCGGAGACACATACCGATACATAGGCGGATACCCATACGATGTTCCAGATTACGCTGGCGGA-3’) was inserted after the nucleotide position 696 of the mouse \(\text{Adam}22\) coding sequence. This corresponds to the position between amino acids Val232 and Glu233 of mouse \(\text{ADAM}22\), yielding the protein with an extracellular FAH tag (\(\text{ADAM}22\)-FAH) just after the predicted cleavage site of Pro domain by furin-like protease. The pX330 plasmid harboring sgRNA#1 and the linearized HDR template DNA were co-injected into fertilized eggs, collected from superovulated B6D2F1 female mice (F1 hybrid between C57BL/6 and DBA/2) mated with C57BL/6 male mice.

To generate the \(\text{Adam}22^{\text{DC5/DC5}}\) knock-in mouse, the sgRNA target sequence, 5’-AGATTTGCAGTGGTTGCGGG-3’ (sgRNA#2), was used. The ~1.5 kb HDR template DNA has the modified sequences (5’-TGAGAGcaaacaacttgacactgaaactct-3’), instead of the wild type corresponding sequences (5’-TGAGAGACAATGCATCCATTAAgaacaacttgacactgaaactctactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactactact
4°C. After centrifugation at 20,000 g for 1 hour, the pellets (crude plasma-membrane fraction) were resuspended and incubated for 1 hour in extraction buffer [20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 150 mM NaCl, 50 μg/ml PMSF] containing either 1% Triton X-100 or 2% Fos-Choline-14 (FC-14, Anatrace). The lysates were spun at 100,000 g for 30 min and the supernatants were precleared with Protein A Sepharose (GE). The resultant supernatants were incubated with Protein A Dynabeads which were covalently conjugated to anti-FAH antibody (30 μg) using the cross-linker, bis(sulfosuccinimidyl)suberate (BS3). After washing, the beads were suspended in SDS-sampling buffer for SDS-PAGE or in denaturing buffer containing 7 M guanidine hydrochloride for shot-gun LC-MS/MS analysis (in-solution digestion).

**Mass spectrometry.**
For the in-gel digestion, the specific protein bands were excised from a silver-stained SDS-PAGE gel, reduced with dithiothreitol, and alkylated with iodoacetamide. The resultant band slices were digested with trypsin (12 μg/ml) at 37°C overnight. For the in-solution digestion for shot-gun analysis, the proteins on beads suspended in denaturing buffer were reduced with dithiothreitol and alkylated with iodoacetamide. Then proteins were separated from the beads, concentrated and digested with trypsin at 37°C overnight. The obtained peptides were separated via nano-flow liquid chromatography (EASY-nLC1000, Thermo Fisher Scientific) using a reverse-phase C18 column (0.075 × 125 mm; Nikkyo Technos). The liquid chromatography eluent was coupled to a nano ion spray source attached to an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). For protein identification, label-free quantification (LFQ) and volcano plot analyses, we used the Mascot2.6.1 (Matrix Science) and Proteome Discoverer2.2 software (Thermo Fisher Scientific). Two biological replicates (two sets of purifications) were injected twice on the mass spectrometer to obtain four technical replicates. Peptide and protein identifications were calculated with protein false discovery rate (FDR) < 0.01. No filtering was applied and obtained protein lists with a high FDR confidence (< 0.01) from control and Adam22FAH/FAH replicates were used for statistical analysis to compare protein abundances based on peak intensity. To make the volcano plots, proteins generally detected in bead-based affinity purification (8, 9) as well as keratins and trypsin were excluded from the list (protein classes excluded are described in Datasets S1 and S2). Analyses were supported by Functional Genomics Facility, NIBB Core Research Facilities.

**Immunoprecipitation, pull-down assay and Western blotting.**
For SI Appendix, Fig. S4, whole brains from wild-type or Adam22AC5/AC5 mice (littermates) were homogenized and solubilized with 1% Triton X-100 or 2% FC-14 as described for immunoaffinity purification. Precleared lysates (0.5 brains per assay) were immunoprecipitated with 10 μg of the indicated antibodies. Immunoprecipitated proteins were eluted by the addition of SDS-sampling buffer for SDS-PAGE. For Fig. 6I, transfected COS-7 cells were washed with PBS and subsequently lysed with buffer A [20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1.3% Triton X-100 and 50 μg/ml PMSF]. The lysates were cleared by centrifugation at 10,000 g for 5 min at 4°C. PSD-95-FLAG was precipitated with FLAG-M2 agarose (Sigma-Aldrich) for 1 hour, washed with buffer B [20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, 1% Triton X-100 and 50 μg/ml PMSF], and eluted with buffer B containing 0.25 mg/ml FLAG peptide. The eluets were subjected to
SDS-PAGE. For Western blotting, chemical luminescent signals were detected with a cooled CCD camera (the FUSION Solo system, Vilber-Lourmat).

**Blue-native (BN) PAGE.**

BN-PAGE was performed according to the method described previously (10, 11). Mouse brains of the indicated genotypes (littermates) were homogenized in 20 volumes of buffer H. After centrifugation at 1,000 g for 10 min at 4°C, the supernatants were centrifuged at 20,000 g for 1 hour at 4°C. The pellets were homogenized in 20 volumes of extraction buffer [20 mM Tris-HCl (pH8.0), 500 mM 6-aminocaproic acid, 1% Triton X-100, and 50 μg/ml PMSF] and solubilized for 1 hour at 4°C. The lysates were spun at 20,000 g for 20 min. Ten μg of solubilized proteins was mixed in a loading buffer [20 mM Tris-HCl (pH8.0), 500 mM 6-aminocaproic acid, 1% Triton X-100, 0.01% Ponceau S, 5% glycerol, 0.02% Coomassie Brilliant Blue G-250]. Gradient (2-13%) gels were prepared with a gradient maker. The same membrane was used for Western blotting (stripped and reprobed) to detect the indicated proteins.

**Surgery and video-EEG/LFP recordings.**

Eight (7 male and 1 female) Adam22^{ΔCS/ΔC5} mice and one female wild-type littermate were used for simultaneous recordings of behaviors and EEGs or LFPs (video-EEG/LFP recording). Each mouse underwent a surgical operation to implant electrodes for recording EEGs/LFPs at the ages of 83-153 days old. Under anesthesia with ketamine hydrochloride (100 mg/kg body weight, i.p.) and xylazine hydrochloride (5 mg/kg, i.p.), the mouse was placed in a conventional stereotaxic apparatus, the skull was widely exposed, and the periosteum and blood were removed. The exposed skull was covered with bone-adhesive resin (Bisite II; Tokuyama Dental) and transparent acrylic resin (Unifast II; GC Corporation), and then a small polyacetal U-shaped holder was fixed with acrylic resin to its head.

Epidural EEG recordings were conducted in two Adam22^{ΔCS/ΔC5} mice. After drilling the skull, a stainless-steel screw (1.0 mm in diameter) was placed over the sensorimotor cortex, anterior (A) 0.0 mm, lateral (L) 1.5 mm to the bregma, as a recording electrode and was fixed with acrylic resin. LFP recordings in the hippocampus and/or cerebral cortex were also conducted in seven mice (hippocampus alone, three Adam22^{ΔCS/ΔC5} and one wild-type mice; hippocampus and cerebral cortex, three Adam22^{ΔCS/ΔC5} mice). After making a hole in the skull, a glass-coated Elgiloy-alloy electrode (diameter of the shaft, 250 μm; impedance, 100 kΩ) was inserted vertically into the hippocampus (A -1.4 to -2.0; L 0.7 to 1.5; depth from the surface of the dura mater, 1.7 to 2.3 mm) or sensorimotor cortex (A 0.0 to 0.8; L 1.8 to 2.0; depth, 1.0 to 1.1 mm). During insertion, neural activity was recorded from the electrode, amplified, and monitored with an oscilloscope. The tip of the electrode was placed where neural activity with large amplitude was observed, and fixed with acrylic resin. A stainless-steel screw was placed over the cerebellum as an indifferent electrode for both EEG and LFP recordings and was fixed. The screws and Elgiloy-alloy electrodes were wired to connectors that were fixed to the U-shaped holder, and covered with acrylic resin. Antibiotics and analgesics were injected (i.m.) after the surgical operation.

Two or three days after the surgical operation, video-EEG/LFP recordings from free
moving mice were started. The recordings were continuously conducted 11 to 13 hours a day between 6 p.m. and 8 a.m., their active time, and 4-6 times a week. Each mouse was placed inside a transparent acrylic cage (depth 115 mm, width 155 mm, height 200 mm) in a dim room (10 lx). EEG/LFP signals from the mouse were supplied to amplifiers (JB-611J, AB-611J, Nihon Kohden) through an electrical slip ring (TSR6135, Tokyo Tuushin Kizai) that was placed above the acrylic cage. The signals were amplified (x 1,000) and filtered (1.5-1,000 Hz). Behaviors of the mouse were continuously monitored with a digital video camera (HDR-CX680, Sony). Composite video signals from the camera and amplified EEG/LFP signals were connected to video and audio inputs of a conventional video-capture device (GV-USB2, I-O Data), converted to digital signals (video frame rate, 29.97 frames/s; audio sampling rate, 48 kHz; EEG/LFP signals from 4 to 1,000 Hz were successfully digitized), and stored in a computer.

To detect epileptic events, EEG/LFP signals together with video recordings were played back using Premiere Pro software (ver. 14.3, Adobe) and visually inspected off-line. EEG/LFP signals were withdrawn using Audition software (ver. CC 2018, Adobe), down sampled to 4.8 kHz and analyzed using Igor Pro software (ver. 6.37, WaveMetrics). Time-frequency analysis was performed using the Continuous Wavelet Transform (Igor), and power spectral density (PSD) was calculated using Welch’s method with the Hamming window (Igor).

Immunohistochemistry/fluorescence analysis.

Immunohistochemical staining: Adam22ΔC5/ΔC5 (~P140) or Lgi1−/− mouse (P16) and their control wild-type mice were treated with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was removed and immersed in the same fixative for 12 hour at 4°C and then cryoprotected in 20% sucrose in PBS overnight at 4°C. The 50-μm free-floating sections were cut on a cryostat (CM1950, Leica). Sections were blocked for 1 hour in PBS containing 10% normal goat serum and 0.1% Triton X-100 at room temperature (RT), and then incubated in the same buffer containing c-Fos or Arc antibody for 2 hour at RT. Endogenous peroxidase activity was inactivated by incubating brain sections in 1% H2O2 for 30 min. Sections were treated with avidin-biotin complex (ABC Elite, Vector Laboratories) for 1 hour and incubated with DAB substrate for 2-3 min until development of optimum brown color (the same incubation time used for comparison). Images were taken using stereomicroscope equipped with a digital camera (Olympus DP20) with the same exposure time and camera setting.

Immunohistofluorescence staining: Freshly dissected brains of Adam22ΔC5/ΔC5 or Lgi1−/− mice and their littermate controls were frozen in n-hexane cooled on dry ice and embedded in OCT compound. Then, the frozen sections (7 μm in thickness) were cut on a cryostat (CM1950, Leica) and fixed with acetone for 20 min on ice (on glass slides). Fixed sections were rehydrated and blocked for 1 hour at RT in PBS containing 10% normal goat or donkey serum, and were incubated in the mixture of indicated primary antibodies for 1 hour at RT, followed by the mixture of Alexa Fluor 488, Cy3 and Alexa Fluor 647-conjugated secondary antibodies. Fluorescent images were acquired with a confocal laser scanning microscopy system (TCS SP5 II, Leica) equipped with an HCX PL APO 63x/1.40
oil immersion objective lens or a microscopy system (TCS SP8, Leica) equipped with an HC PL APO 63x/1.20 water immersion lens. Microscope control and all image analyses were performed with Leica LAS AF or LAS X software, and images were acquired with the same laser power and detector settings for comparison. To measure the fluorescence intensity, 2-3 fields for each hippocampal subregion (CA1, CA3 or DG) were imaged with 5x zoom in each animal. The mean fluorescence intensities of PSD-95, LGI1, VGluT1 and Kv1 signals in ROIs covering a whole area of each image (except for the accidentally damaged area) were quantified. For SI Appendix, Fig. S3F, fixed brain sections (50 μm in thickness) from Adam22:6C5:4C5 (~P140) or control wild-type mice were incubated with 10 μg/ml Hoechst33342. Fluorescent images were acquired with a confocal laser scanning microscopy system (LSM5 Exciter, Carl Zeiss) using a Plan-Apochromat 5x/0.16 objective lens. For figure images, the same adjustments of brightness and contrast were applied among samples to be compared.

Immunocytofluorescence analysis of mouse hippocampal neuron culture.
Hippocampal neurons were dissociated from brains of E16 Adam22:4C5:4C5 and wild-type C57BL/6N mice and seeded onto 0.1% polyethylenimine and 50 μg/ml collagen I-coated 12-mm coverslips (24-well plate) in Neurobasal medium with 2 mM GlutaMax, B-27 and 10% fetal bovine serum. After 3-hour incubation, the media was changed to Neurobasal medium supplemented with 2 mM GlutaMax and B-27. Dissociated mouse hippocampal neurons were co-transfected with plasmids encoding PSD-95-GFP and mCherry by Lipofectamine 2000 [13 days in vitro (DIV)]. To quantify the spine size, neurons were fixed with 4% paraformaldehyde for 10 min at RT and permeabilized with 0.1% Triton X-100 for 10 min on ice. Neurons were then blocked with PBS containing 10 mg/ml BSA for 10 min on ice and incubated with anti-GFP, mCherry, and VGluT1 antibodies. Signals were visualized with Alexa Fluor 488, Cy3, and Alexa Fluor 647-conjugated secondary antibodies. Each image was reconstructed by a z-series maximum intensity projection of 5-8 images, taken at 0.5 μm-depth intervals (TCS SP5 II, Leica). The spine size was defined as the full width at half maximum [FWHM] across the longest axis of the mCherry signal (co-transfected fill-in marker) in the spine (LAS X software). The FWHM of all the detectable PSD-95-GFP clusters opposed to VGluT1 was measured. To quantify the number of cell-surface LGI1 clusters associated with PSD-95, live transfected neurons (~30 DIV) were incubated with human anti-LGI1 antibody for 1 hour at RT. The neurons were then fixed and blocked as above. The bound human IgG was visualized using Cy3-conjugated secondary antibody. After the permeabilization, the neurons were subsequently incubated with anti-GFP antibody and visualized by Alexa Fluor 488-conjugated secondary antibody. Z-stacked images were acquired with an HC PL APO 100x/1.40 Oil STED WHITE objective lens (the confocal imaging mode, TCS SP8, Leica) and processed with Lightning deconvolution (Leica) to obtain the maximum resolution, ~120 nm. The number of LGI1 clusters overlapped/associated with a single PSD-95-GFP cluster was counted.

Cell-surface staining of COS-7 cells.
For Fig. 6D, transfected COS-7 cells were washed with DMEM and surface-expressed ADAM22 and surface-bound LGI1-FLAG were live-labeled for 30 min at 37°C with antibodies against the extracellular epitope of ADAM22 and FLAG, respectively. Cells were subsequently fixed with 2% paraformaldehyde/120 mM sucrose/100 mM HEPES (pH
ADAM22 was performed to detect cell
Live and experiment. Images of more than three fields of CA1 (scale images (the same settings of thresholding and range were applied for comparison) nanodomains without setting a threshold (measured by setting circular ROIs with 130
f
synapses in face view) or > 250 detect apposed synaptic objects (Fig. 3
samples, the brightness of images from intensities of PSD
and the peak distance was determined using LAS
highest peaks of apposed RIM2 and PSD
between RIM2 and PSD, respectively
Antibodies against the C
was determined using LAS
FAH and target proteins,
and time
accomplished with a
were excited at 4
1,024 x 1,024 pixel format; 200 Hz scan speed; 2 line
acquired with
Research Center on Life and Living Systems
Fisher Scientific
For human anti
Fluor 488
described above. For
immersion objective lens
confocal microscopy (LSM5 Exciter, Carl Zeiss) using a Plan-Apochromat 63x/1.40 oil immersion objective lens.

**STED superresolution imaging and image analysis.**
Immunohistofluorescence staining of fresh-frozen mouse brain sections was performed as described above. For two color (2C)-STED imaging with 660-nm depletion laser, Alexa Fluor 488- and 555-conjugated secondary antibodies (Thermo Fisher Scientific) were used. For human anti-LGI1 antibody, DyLight 488-conjugated secondary antibody was used (Jackson Immunoresearch). Specimens were mounted in ProLong Diamond (Thermo Fisher Scientific). Gated STED imaging was performed using Leica TCS SP8 gated STED superresolution system combined with Leica HyD detectors (supported by Exploratory Research Center on Life and Living Systems, ExCELLS). Single optical slices were acquired with an HC PL APO 100x/1.40 Oil STED WHITE objective lens (5-10x zoom; 1.024 x 1.024 pixel format; 200 Hz scan speed; 2 line-averaging). Alexa Fluor-488 and 555 were excited at 488 and 554-nm white-light laser, respectively. Depletion was accomplished with a CW 660-nm laser (set at 65% of laser power, 10% for z-depletion; and time-gating of HyD detectors at 0.5-3 ns). To measure the distance between ADAM22-FAH and target proteins, the peak distance between closely apposed/overlapped clusters was determined using LAS X software (Leica) and no exclusion of data was applied. Antibodies against the C-terminal part of Bassoon and PSD-95 were used to label presynaptic active zone and PSD, respectively. To measure the nearest neighbor distance between RIM2 and PSD-95 or Homer1, intensity line profiles were drawn across the highest peaks of apposed RIM2 and PSD-95/Homer1 clusters within synapses in side view, and the peak distance was determined using LAS X software (Leica). Because the signal intensities of PSD-95 clusters were lower in Adam22AC5/AC5 samples than in wild-type samples, the brightness of images from Adam22AC5/AC5 samples was enhanced to clearly detect apposed synaptic objects (Fig. 3E). Data with < 50-nm distance (corresponding to synapses in face view) or > 250-nm (no synaptic apposition) (12) were excluded. Mean fluorescence intensities of PSD-95 nanodomains apposed to presynaptic RIM2 were measured by setting circular ROIs with 130-nm diameter on the individual nanodomains without setting a threshold (LAS X software). The size and number of PSD-95 nanodomains were measured using ImageJ1.51s particle analysis using the original grayscale images (the same settings of thresholding and range were applied for comparison). Images of more than three fields of CA1 (10x zoom) were quantified and averaged for each experiment. Multiple nanodomains in single synapses were often not completely separated and thus counted/measured en bloc.

Live-cell labeling of COS-7 cells transfected with plasmids encoding PSD-95-GFP and ADAM22 was performed to detect cell-surface expressed ADAM22, and fixed cells were
then permeabilized and stained with anti-GFP antibody. Cells were imaged with Leica TCS SP5II STED CW system equipped with an HCX PL APO 100x/1.40 NA oil immersion objective lens, combined with the Leica HyD detectors (5.2x zoom; 1,024 x 1,024 pixel format; 200 Hz scan speed; 2 line-averaging). ATTO425 (Rockland)-labeled ADAM22 and Alexa Flou 488-labeled GFP were excited at 458 and 514 nm, respectively. Depletion was accomplished with a CW 592-nm laser (set at 100%). Obtained STED images were further deconvoluted with the built-in deconvolution algorithms of the LAS-AF software (13).

**Slice culture preparation and transfection.**
Hippocampal slice cultures were prepared from 7- to 10-day old mice as previously described (14). At 4 DIV, for overexpression experiments, slice cultures were transfected using a Helios Gene Gun (BioRad). For biolistic transfection, 50 μg total of each construct was coated on 1-μm diameter gold particles, which were then coated onto PVC tubing and stored at 4°C. For experiments in which bullets were coated with two different constructs, coexpression was visually confirmed by using different fluorophores for each construct.

**Electrophysiology.**
All datasets include recordings from at least seven hippocampal slices from three different animals. Recordings were made at DIV8-10, using 3- to 4-MΩ glass electrodes filled with an internal solution consisting (in mM): 130 CsMeSO₃, 8 NaCl, 10 Hepes, 4 Mg-ATP, 0.3 Na-GTP, 10 HEPES, 5 QX314-Cl, and 0.1 spermine, pH 7.2 with CsOH. External perfusion medium consisted of 140 NaCl, 2.4 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 10 glucose, 4 CaCl₂, 4 MgSO₄, and 100 μM picrotoxin, saturated with 95% (vol/vol) O₂ and 5% (vol/vol) CO₂. 4-10 μM 2-Chloroadenosine was also used to suppress epileptic activity. Transfected pyramidal cells were identified using fluorescence microscopy. In all paired experiments, transfected and neighboring control neurons were recorded simultaneously. A bipolar stimulating electrode was placed in stratum radiatum of CA1 pyramidal cells. After gaining whole-cell access, cells were held at −70 mV and stimulated for 5 min to allow for response stabilization. After this period, 20 trials were obtained at 0.2 Hz while holding the cells at −70 mV, followed by 20 trials at +40 mV. AMPA EPSCs were measured as the peak amplitude of the averaged traces recorded at −70 mV, and the NMDA EPSC was measured at +40 mV as the average amplitude of the current 100 ms after stimulation, at which time the AMPA receptor-mediated EPSC had completely decayed. Series resistances typically ranged from 10 to 20 MΩ; a cell pair was discarded if the series resistance of either increased to > 30 MΩ. For two-way statistical comparisons Student’s t-test was used.

For LTP recording, 300-μm transverse acute slices were cut from P18-26 mice with a Leica vibratome in chilled high sucrose cutting solution containing (in mM): 2.5 KCl, 7 MgSO₄, 1.25 NaH₂PO₄, 25 NaHCO₃, 7 glucose, 210 sucrose and 1.3 ascorbic acid. The slices were then incubated for 30 min at 37°C in aCSF containing (in mM): 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 11 glucose, 2.5 CaCl₂ and 1.3 MgSO₄. The slices were then transferred to the recording chamber and 0.1mM picrotoxin was added to the aCSF for recordings. LTP was induced by stimulating Schaffer collateral axons at 2 Hz for 90 s while
clamping the cell at 0 mV, after recording at least 3 min baseline. Mann-Whitney U test was used for comparing two different LTP group.

**Crystallography of PSD-95 PDZ3–ADAM22C.**

The gene encoding rat PSD-95 PDZ3 (residues 309–422; NP_062567.1) C-terminally fused with a Gly-Ser-Ser-Gly linker and the C-terminal 15 residues of human ADAM22 (ADAM22C; residues 892–906; NP_068369.1) was subcloned into the pCold I vector (Takara Bio) using the Ndel and XhoI sites. The protein (containing an N-terminal His6 tag) was expressed in *Escherichia coli* Rosetta (DE3) cells (Merck Millipore). The cells were suspended in 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl and 1% Triton X-100, and disrupted by sonication. The cleared lysate was applied onto a Ni-NTA (Qiagen) column. After washing with 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl and 50 mM imidazole, the protein was eluted with 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl and 500 mM imidazole. The eluted protein was further purified by size exclusion chromatography using a HiLoad 16/60 Superdex 200 (GE Healthcare) column with 20 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl. The fractions abundant in the protein were collected and concentrated to 24 g/L using an Amicon Ultra centrifugal filter (MWCO 10,000; Merck Millipore). Initial crystallization screening was performed using the sitting drop vapor diffusion method at 20°C with a Mosquito liquid-handling robot (TTP Lab Tech). About 1,000 conditions were tested with crystallization reagent kits supplied by Hampton Research, Molecular Dimensions, and Qiagen. The condition D5 in MemGold2 (Molecular Dimensions; 0.2 M choline chloride, 0.1 M Tris-HCl (pH 7.5), 14% (w/v) PEG 2000 MME) yielded diffraction-quality crystals of the protein. The crystals were soaked in the reservoir solution supplemented with 30% polyethylene glycol, and then flash-frozen in liquid N2.

Diffraction data sets were collected at 100 K at BL41XU in SPring-8 and processed with HKL2000 (15) and the CCP4 program suite (16). The PSD-95 PDZ3–ADAM22C structure was determined by molecular replacement with the program MOLREP (17) using the structure of PSD-95 PDZ3 in complex with KKETPV peptide ligand (PDB 1TP3 [https://doi.org/10.2210/pdb1TP3/pdb]) as the search model. The atomic model of ADAM22C was manually built using the program Coot (18). The PSD-95 PDZ3–ADAM22C structure was refined using the program Refmac5 (19). Iterative model building and refinement improved the model with good stereochemistry [98.17% and 0.00% of residues in Ramachandran favored and outliers, respectively; validated by MolProbity (20)]. Data collection and refinement statistics are summarized in SI Appendix, Table S1. All structure figures were prepared using the program PyMOL (Schrödinger, LLC; https://pymol.org/2/).

**Statistical analysis.**

Statistical details of individual experiments are described in figure legends or corresponding method sections. To perform statistical analysis, at least 3 independent tissue samples from at least 3 animal pairs were included in the analyses (except for Fig. 1F). We analyzed the normality of the datasets using Shapiro-Wilk test and the variances between groups using F tests. Survival curves were analyzed by Kaplan-Meier survival estimate using a log-rank test for curve comparisons. For paired sample comparisons
(fluorescence intensity), paired $t$-test was used. To compare unpaired two samples, Student’s $t$-test or non-parametric Kolmogorov-Smirnov or Mann-Whitney $U$ test was used. For multiple test subjects, Kruskal-Wallis with post-hoc Scheffe test or two-way repeated ANOVA test was used. Box-and-whisker plots are shown to identify the median, 25th, 75th percentiles and 1.5 x interquartile range (IQR). Results are shown as means ± SEM or medians (IQR). Statistical analysis was performed with Ekuseru-Toukei 2012 software (BellCurve) and Igor Pro (WaveMetrics).

**Data availability.**
The coordinates and structure factors of PSD-95-PDZ3–ADAM22 complex have been deposited in the Protein Data Bank under the accession codes of 7CQF. Other data are available from the corresponding authors upon reasonable request.
Fig. S1. ADAM22 participates in trans-synaptic protein networks involving MAGUKs.

(A) Schematic presentation of ADAM22 tagged with FLAG-AU1-HA (ADAM22-FAH). The tandem tag sequence (31 aa) is inserted after the furin-like proteases-cleavage site in the extracellular region of ADAM22. Pro, prodomain; MP, inactive metalloprotease domain; DI, disintegrin domain; CR, cysteine-rich domain; EGF, EGF-like domain; TM, transmembrane domain. Antibodies used in the study to detect the ADAM22-FAH protein are shown. (B) Specific detection of ADAM22-FAH by ADAM22 and epitope-tag antibodies. Whole brain extracts (20 μg) from the wild-type mouse (+/+) and Adam22-FAH knock-in mouse (heterozygous, FAH+/+) were probed with anti-ADAM22, -FAH and -HA antibodies. Western blotting with anti-ADAM22 antibody shows that the expression level of ADAM22-FAH (asterisk) is equivalent to that of ADAM22 (lower band) in Adam22-FAH knock-in mouse. (C) ADAM22-associated protein network under the low stringency condition (1% Triton X-100). Sixty proteins were significantly enriched in the ADAM22-FAH purification. All the protein data (161 proteins) for the plot is shown in Dataset S1. (D) The identified specific proteins under the low stringency condition are lined up based on the protein functions and Mascot scores. Error bars show ± SEM (n = 4).
Fig. S2. ADAM22 colocalizes with pre- and postsynaptic MAGUKs and Kv1 channels.

(A) Fluorescence signals of HA antibody in Adam22^FAH/FAH mice are specific, as no signals were observed in wild-type mice (+/+) . ML, molecular layer; GL, granular cell layer; PL, polymorphic cell layer. Boxed areas in left panels are magnified (DG, right panels; CA3 in Fig. 1E). Bars: (left) 250 μm; (right) 20 μm. (B) ADAM22 (green) and LGI1 (blue) colocalize with MAGUKs, postsynaptic PSD-95 (red, upper) and presynaptic CASK (red, lower) in the hippocampal CA1 region. Py, stratum pyramidale; SR, stratum radiatum. Bar: 20 μm. (C and D) Colocalization of ADAM22 and Kv1,2 at the synapse of the molecular layer of DG (C) and at the cerebellar pinceau and axon initial segments (AIS) (D). Boxed areas in left panels are magnified. Bars: 20 μm; 5 μm (magnified). ML, molecular layer; GL, granular cell layer; PL, polymorphic cell layer; PCL, Purkinje cell layer.
Fig. S3. Targeting construct and characterization of Adam22\(\Delta C5/\Delta C5\) knock-in mice.

(A) Targeting strategy for Adam22\(\Delta C5/\Delta C5\) knock-in allele. The PAM sequence (red) and the sgRNA target sequence (blue) in exon 31 of the Adam22 gene are indicated. The ~1.5 kb homology-directed repair (HDR) template DNA has a nonsense mutation (a red asterisk) and lacks 72 base pairs (gray), leading to C-terminal five amino-acid deletion. (B) PCR genotyping. Wild-type (WT, +) and knock-in (ΔC5) alleles give 300- and 228-bp PCR products, respectively. (C) Expression levels of ADAM22 and ADAM22-associated proteins are not changed in Adam22\(\Delta C5/\Delta C5\) mice. (D and E) Immunohistochemical analysis of Arc (D) and c-Fos (E), neuronal immediate early genes. Arc expression is upregulated in the dentate granule cells and dentate molecular layer of Adam22\(\Delta C5/\Delta C5\) mouse (P131) at 30 min after the first generalized seizure event (D). c-Fos expression is increased in epileptic Lgi1\(^{-/-}\) mouse (P16) (E). Bars: 0.5 mm (D and lower in E); 1 mm (upper in E). (F) No major cell death and hippocampal disorganization are evident in the Hoechst 33342-stained section of Adam22\(\Delta C5/\Delta C5\) mouse with a seizure attack. Bar: 0.5 mm.
Fig. S4. Supramolecular complex of ADAM22–LGI1 is disrupted in Adam22ΔC5/ΔC5 mice. (A) ADAM22ΔC5 interacts with LGI1 in Adam22ΔC5/ΔC5 mice. IP, immunoprecipitation; WB, Western blotting. (B) ADAM22ΔC5 does not interact with MAGUK family proteins in Adam22ΔC5/ΔC5 mice. (C) Association of ADAM22 with PDZ-binding proteins (Kv1.2, GluNs, Stargazin and LRRTM4) is robustly reduced in Adam22ΔC5/ΔC5 mice. Low or high stringency conditions was used for protein solubilization. (D) BN-PAGE and Western blotting of brain extracts from wild-type (+/+), Lgi1−/− and Adam22ΔC5/ΔC5. About 1.2 MDa and 1.0 MDa supramolecular complexes (arrows) that contain ADAM22, LGI1 and Kv1.2 are disrupted in Lgi1−/− and Adam22ΔC5/ΔC5 mice. The same membrane was used (stripped and reprobed) to detect ADAM22, LGI1 and Kv1.2. (E) PSD-95-associated protein complex isolated from wild-type (+/+ ) and Adam22ΔC5/ΔC5 mice. Western blotting shows that co-immunoprecipitations of LGI1 and ADAM22 with PSD-95 were completely lost and that of Kv1 was greatly reduced in Adam22ΔC5/ΔC5 mice. An asterisk indicates mouse IgG heavy chain.
**Fig. S5.** Delocalization of PSD-95 and dissociation of LGI1 from PSD-95 in the *Adam22ΔC3/ΔC5* hippocampus.

(A–C) PSD-95 fluorescence intensity is significantly reduced in the hippocampal regions in adult *Adam22ΔC3/ΔC5* mice. Boxed areas (A) are magnified in (B). DG, dentate gyrus; ML, molecular layer; GL, granular cell layer; PL, polymorphic cell layer; IML, inner molecular layer. P-values were determined by paired t tests; n = 5 independent experiments for CA1/CA3; n = 3 for DG. Bars: (A) 250 μm; (B, left) 10 μm; (B, middle, right) 50 μm. (D) LGI1 is dissociated from PSD-95 in the hippocampus of *Adam22ΔC3/ΔC5* mice. Insets, merged images. Bar: 1 μm. (E and F) LGI1 fluorescence intensity, but not VGluT1, is moderately reduced in the hippocampal regions of *Adam22ΔC3/ΔC5* mice. P-values were determined by paired t tests. n = 3 independent experiments (E). n = 5 for CA1/CA3 and n = 3 for DG (F). (G) PSD-95 is delocalized from the cerebellar pinceau and juxtaparanodes in *Adam22ΔC3/ΔC5* mice. Bars: (left) 50 μm; (middle) 10 μm; (right) 2.5 μm. Cb, cerebellum; PCL, Purkinje cell layer; JXP, juxtaparanode.
Fig. S6. Reduced levels of PSD-95 in the *Lgi1*−/− hippocampus.

(A–C) PSD-95 fluorescence intensity, but not VGluT1, is reduced in the hippocampal regions in *Lgi1*−/− mice (P16). Note that PSD-95 signals, but not VGluT1 (insets, in blue), are almost lost in the inner molecular layer (IML, indicated as asterisks) of DG in *Lgi1*−/− mouse. SR, stratum radiatum; SL, stratum lucidum; Py, stratum pyramidale; ML, molecular layer; IML, inner molecular layer; GL, granular cell layer; PL, polymorphic cell layer. P-values were determined by paired t tests. n = 5 independent experiments for CA1/CA3 and n = 3 for DG (B). n = 4 for CA1/CA3 and n = 3 for DG (C). Bars: (left) 10 μm; (middle, right) 50 μm.
Fig. S7. Delocalization of presynaptic Kv1 channels in the Adam22^{ΔC5/ΔC5} hippocampus.
Fluorescence signals of presynaptic Kv1 channel were reduced in the CA3 stratum lucida (Kv1.1) and dentate molecular layer (Kv1.2) of Adam22^{ΔC5/ΔC5} mice. Bars: 20 μm. P-values were determined by paired t tests; n = 3 mouse pairs.
Fig. S8. ADAM22-PDZ binding is not required for spine enlargement by PSD-95 overexpression.

(A) Expression of ADAM22 W396D, lacking the LGI1 binding activity, in Adam22ΔC5/ΔC5 neurons does not increase AMPAR- and NMDAR-mediated synaptic transmission. (B and C) Overexpression of PSD-95 does not affect NMDAR-mediated EPSCs in wild-type (B) and Adam22ΔC5/ΔC5 neurons (C). Scatterplot of NMDAR EPSC amplitudes simultaneously recorded from non-transfected control (black) and PSD-95-overexpressing neurons (green). Representative traces of dual recordings are shown as insets. Bars: 50 ms and 50 pA. p = 0.6, n = 8 (B); p = 0.23, n = 11 (C). n.s., not significant. (D) Overexpressed PSD-95-GFP (green) is targeted
to dendritic spines and causes spine enlargement in cultured neurons derived from wild-type (+/+) and $\text{Adam22}^\text{DC5/DC5}$ mouse. No significant (n.s.) differences in the spine enlargement were observed between genotypes (P-values were determined by two-way ANOVA; n = 3 independent experiments). Red line indicates the average for each data set. Number of spines analyzed are indicated. FWHM, full-width at half maximum. Blue, VGluT1; Red, mCherry (a volume marker). Bar: 1 μm. (E) Cell-surface clusters of LGI1 (red) associated with PSD-95-GFP clusters (blue, pseudocolor) are significantly reduced in $\text{Adam22}^\text{DC5/DC5}$ neurons. P-value was determined by paired $t$ tests; n = 3 independent experiments. Red circle indicates the average for each data set. Number of PSD-95-GFP clusters analyzed are indicated. Bar: 1 μm.
Fig. S9. Model for an essential role for trans-synaptic LGI1–ADAM22–MAGUK in precise synaptic transmission and epilepsy prevention.
Table S1. Data collection and refinement statistics.

|                       | PSD-95 PDZ3–ADAM22-C (PDB 7CQF) |
|-----------------------|----------------------------------|
| **Data collection**   |                                  |
| Beamline              | SPring-8 BL41XU                  |
| Space group           | $P6_1$                           |
| Cell dimensions       |                                   |
| $a, b, c$ (Å)         | 64.0, 64.0, 48.8                 |
| $\alpha, \beta, \gamma$ (°) | 90.0, 90.0, 120.0 |
| Resolution (Å)        | 50–1.8 (1.83–1.80)               |
| $R_{sym}$             | 0.130 (0.745)                    |
| $\|\|I\|/\sigma I$    | 26.2 (1.4)                       |
| Completeness (%)      | 100 (100)                        |
| Redundancy            | 16.1 (8.7)                       |
| **Refinement**        |                                  |
| Resolution (Å)        | 1.8                              |
| No. reflections       | 10,052                           |
| $R_{work} / R_{free}$ | 0.167 / 0.205                    |
| No. atoms             |                                   |
| Protein               | 877                              |
| Ligand/Ion            | 24                               |
| Water                 | 46                               |
| $B$ factors (Å$^2$)   |                                   |
| Protein               | 33.7                             |
| Ligand/Ion            | 42.3                             |
| Water                 | 35.8                             |
| R.m.s. deviations     |                                   |
| Bond lengths (Å)      | 0.0108                           |
| Bond angles (°)       | 1.6593                           |

Data were collected from a single crystal. Highest-resolution shell is in parentheses.
Dataset S1. Proteomic data for volcano plot under the low stringency condition. (separate file)

Dataset S2. Proteomic data for volcano plot under the high stringency condition. (separate file)

Movie S1. Epileptic phenotypes of Adam22<sup>ΔC3/ΔC3</sup> mice. (separate file)
Behaviors of two Adam22<sup>ΔC3/ΔC3</sup> mice (P150, left; P135, right) and epidural electroencephalography (EEG) simultaneously recorded from the left mouse with a spontaneous seizure event are shown. The left mouse behaved normally at first. During small spikes in the EEG, the animal rotated its head to the right, then its trunk. As the large spike waves began in the EEG, the animal started convulsion, and finally ran and jumped. When the EEG was flattened, the animal became atonic. On the other hand, the right mouse kept sleeping. The left mouse died on P167. The right mouse showed similar seizures later starting on P161 and died on P182.
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