Supplemental Information

Noelin1 Affects Lateral Mobility of Synaptic AMPA Receptors

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Supplemental Experimental Procedures

Preparation of subcellular fractions
Tissue from adult C57/Bl6J (Charles River) was homogenized in homogenization buffer (0.32 M Sucrose, 5 mM HEPES, pH 7.4; protease inhibitor cocktail (Roche)), to obtain the homogenate fraction. The homogenate was then centrifuged at 1000x g for 10 minutes to obtain the supernatant. To obtain P2+microsome (P2+M) pellets, the supernatant was then centrifuged at 100,000x g for 2 h at 4 °C. Pellets were resuspended in sample suspension buffer (25 mM HEPES, 150 mM NaCl, pH 7.4 and a protease inhibitor cocktail) and stored at −80 °C. For subcellular fractionation of the separate P2 and microsome fractions, the supernatant was centrifuged at 18,000x g for 20 minutes to obtain the P2 fraction. The microsome fraction was subsequently obtained by centrifuging the supernatant at 100,000x g for 20 minutes. To obtain the postsynaptic density fraction, the supernatant was separately loaded onto a 0.85 M/1.2 M sucrose gradient, centrifuged for 2 h at 100,000x g to first obtain the synaptosome fraction at the 0.85/1.2 M interface. The synaptosome fraction was either given an osmotic shock in 5 mM HEPES, pH 7.4 for 30 minutes and then loaded on a 0.85/1.2 M sucrose gradient to obtain the synaptic membrane fraction, or extracted twice with 1% Triton X-100 for 30 minutes, then loaded on a 1.5/2.0 M sucrose gradient to obtain the postsynaptic density fraction. Of each sub-fraction 5 µg protein was mixed with 5x SDS-containing loading buffer loaded on a SDS-PAGE gel, transferred overnight on PVDF membranes and stained for the protein of interest.

Immunoblot analysis
Samples containing SDS loading buffer were run on 5–12% Criterion TGX Stain-Free Precast Gels (Bio-Rad) and transferred on a PVDF membrane overnight. The membranes were then blocked using 5% non-fat milk in TBS with Tween-20 followed by an overnight incubation with the primary antibody at 4 °C. The blots were washed three times in TBS with Tween-20 after the primary antibody incubation followed by a 1-h incubation with a HRP conjugated secondary antibody in 3% non-fat milk. After three more washes, the blots were incubated with SuperSignal West Femto Chemiluminescent Substrate (Pierce) and scanned on an Odyssey Fc scanner (Licor Biosciences).

DNA constructs
Full length cDNA constructs for Noelin1-1 (pN1_Noelin1-1-GFP-Kanamycin) [NM_019498.2], Noelin1-4 (pcDNA 3.1_Noelin1-4_Ampicillin_CMV) [NM_001038614.1] and Noelin1-3 (pDEST_Noelin1-3_IRES2_mCherry_Ampicillin_CMV) [NM_001038613.1] were used for expression in HEK293T cells. For GluA2, full length GluA2 (flip isoform) [NM_001039195] was inserted in pTRCGW construct to give pTRCGW_GluA2_Ampicillin_CMV. The superecliptic pHluorin (SEP) constructs of GluA1 and GluA2 (SEP::GluA1, SEP::GluA2) were used as described previously (Klueva et al., 2014).

HEK293T cell transfections
HEK293T cells were transfected with either Noelin1 constructs alone or in combination with GluA2 constructs using polyethylenimine (PEI 25000).

**Determination of Noelin1-3 concentration in HEKT293 cell medium**

Medium (10 µL) was mixed with 5x SDS sample buffer and run on an SDS-PAGE gel. Gel slices at approximately 75 kDa, corresponding to Noelin1-3 bands, were cut out followed by in gel digestion. Half of the samples were analyzed in triplicates as described before on Orbitrap mass spectrometer. To determine the amount of protein, peptide LTGISDPVTVK, which is a tryptic product of Noelin1-3, was run in a concentration of 100, 200, 1000 and 2000 fmol to make a standard curve using the total peak area of the peptide (m/z=565.38) using skyline v2.5 (Bateman et al., 2014). The Noelin1-3 concentration in the sample was calculated 720 fmolµL⁻¹.

**Affinity purification of protein complexes by immunoprecipitation**

Methods for affinity purification was as previously described (Chen et al., 2014). In short, for a single IP experiment, 2 mg of whole homogenate fractions was extracted twice in 1 mL extraction buffer (25 mM HEPES, 150 mM NaCl, 1% DDM (Sigma Aldrich), pH 7.4 and a protease Inhibitor cocktail) for 1 h each at 4 °C on a rotor at 10 rpm. After each extraction, the samples were centrifuged at 20,000x g for 20 minutes. The supernatant was pooled after each extraction. Of each antibody 10 µg was added to the pooled supernatant and incubated overnight at 4 °C on a rotor at 10 rpm. Per IP, 50 µL of protein A/G beads (Santa Cruz) were added, and incubated for 1 h at 4 °C. After incubation with the beads, the samples were centrifuged at 1000x g for 1 minute and the supernatant was discarded. The beads were washed four times with 1 mL washing buffer (25 mM HEPES, 150 mM NaCl, 1% DDM, pH 7.4), supernatants were discarded and the final pellet was dissolved in 2% SDS buffer (pH 8.8) for separation on SDS-PAGE for immunoblotting experiments or trypsinized peptides were extracted using the filter aided sample preparation protocol (Wiśniewski et al., 2009). As negative controls, IgG or peptide blocking control (Li et al., 2012). For peptide blocking controls, 10 µg antibody was incubated with 50 µg peptide for 30 minutes on ice, prior to addition to the supernatant; samples were treated similarly thereafter.

**Immunocytochemistry and image acquisition.**

Primary hippocampal neurons were obtained from E18 pups as described previously (Frischknecht et al., 2009). Briefly, 18,000 cells were grown on a glial feeder layer in neurobasal medium supplemented with B27 on poly D-Lysine coated coverslips. For surface staining of Brevican and GluA2, coverslips were first incubated with the primary antibody dissolved in cell culture media for 5 minutes at 37 °C in the incubator. Next, coverslips were fixed with ice-cold methanol for 10 minutes, followed by three washes in sterile H₂O and PBS. After blocking and permeabilization (1% FCS, 0.1% Triton X-100 in PBS) for 1 h, neurons were incubated with primary antibodies in blocking buffer overnight at 4 °C, and with respective secondary antibodies. After washing and fixing on glass slides (Superfrost Plus, Thermo) using Moviol, confocal images were taken using an LSM Meta 510 confocal microscope using 63x oil immersion lens (N.A. 1.4) and analyzed using ImageJ.
Immunostainings of for fluroscence microscopy were acquired on a Zeiss Axio Imager A2 microscope using a 63x oil immersion lens (N.A. 1.4) with Cool Snap EZ camera (Visitron Systems) controlled by VisiView (Visitron Systems GmbH) software. All coverslips compared in one experiments were processed in parallel using identical antibodies solutions and other reagents.

Dual color STED data was obtained (Ivanova et al., 2015) and line scan analysis was performed (Frischknecht et al., 2009) as previously described.

Quantification of (peri)synaptic GluA2, Brevican and Noelin1 immunoreactivity before and after hyaluronidase treatment was done using OpenView software (Tsuriel et al., 2006) using Homer1 staining to identify synaptic puncta. In Fig. 4d–h Noelin1 and Brevican immunoreactivity was measured in a 0.36-µm and 1.44-µm box around the center of the Homer1+ puncta, respectively.

Chemical LTP induction was performed as previously described (Ivanova et al., 2015) with minor modifications. Briefly, DIV21 neurons were treated with 1 mM 4-AP and 25 μM bicuculline for 15 minutes followed by recovery for 1 h. After GluA2 surface labeling (see above), the cells received a methanol fixation followed by Noelin1 and Homer1 staining.

**Electrophysiology**

All electrophysiological recordings in HEK293T cells were made as previously described (Klaassen et al., 2016) with minor modifications. HEK293T cells were gently lifted from the cover slip and placed in front of a Piëzo-driven theta-barrel electrode (TGC 200; Harvard Apparatus), filled with standard aCSF on one side and standard aCSF supplemented with 1 mM L-glutamate Hydrochloride (Sigma Aldrich) on the other side. Exchange rate and response time were all well below 0.5 ms. HEK293T cells were perfused with standard artificial cerebrospinal fluid (room temperature). Control cells were bathed in either control medium or Noelin1-expressing medium for at least 1 h prior to measurement and as well during the course of the measurements.

Whole-cell recordings were carried out at room temperature (22–24 °C). Dissociated hippocampal neurons at DIV18 were visualized by a 63x water immersion objective (Zeiss, Jena, Germany) using a fixed-stage microscope (Zeiss Axio Examiner; Zeiss, Jena, Germany) equipped with Dodt contrast. For the recording, an EPC-10 patch-clamp amplifier was used (Heka Elektronik, Lambrecht, Germany). Currents were low-pass filtered at 2.9 kHz using a built-in Bessel filter, and digitized at 10 kHz with Patchmaster software (Heka Elektronik, Lambrecht, Germany). Patch electrodes were pulled from borosilicate glass (Sutter Instruments, Novato, CA) to a resistance of 5–7 MΩ. The extracellular solution contained in mM: 116 NaCl, 22.6 NaHCO₃, 1.25 NaH₂PO₄, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, 5 HEPES (pH 7.4). Intracellular solution contained (in mM): 136.6 Cs-glucanote, 5 EGTA, 13 TeaCl, 4 Mg-ATP, 0.4 GTP, 15 HEPES (pH 7.2). Electrical pulses (1 – 5 V, 1 ms) were applied with an isolated pulse stimulator (A-M Systems, Model 2100, USA) through a platinum-iridium bipolar stimulation electrode (Science Products, Hofheim, Germany) for electrical stimulation. The stimulation electrode was placed on the coverslip to the position that elicited postsynaptic responses in the recorded cell. Very strong electrical stimulation was avoided not to cause electrolysis. Data were analyzed with Fitmaster (Heka Elektronik, Lambrecht, Germany) and Igor Pro 6.1
Data are reported as mean ± SEM, and n indicates the number of cells measured.

Recordings for mEPSC from dissociated cortical neurons (DIV21) were done as described above in the presence of TTX (1 μM).

**LC-MS/MS analysis**

Peptides were analyzed by nano-LC MS/MS using an Ultimate 3000 LC system (Dionex, Thermo Scientific) coupled to the TripleTOF 5600 mass spectrometer (Sciex).

Peptides were trapped on a 5 mm Pepmap 100 C18 column (300 μm i.d., 5μm particle size, from Dionex) and fractionated on a 200 mm Alltima C18 column (100 μm i.d., 3 μm particle size). The acetonitrile concentration in the mobile phase was increased from 5 to 30% in 90 min, to 40% in 5 min, and to 90% in another 5 min, at a flow rate of 500 nL/min. The eluted peptides were electro-sprayed into the TripleTOF MS. The nano-spray needle voltage was set to 2500V. The mass spectrometer was operated in a data-dependent mode with a single MS full scan (m/z 350–1200, 250 msec) followed by a top 25 MS/MS (85 msec per MS/MS, precursor ion > 90 counts/s, charge state from +2 to +5) with an exclusion time of 16 sec once the peptide was fragmented. Ions were fragmented in the collision cell using rolling collision energy, and a spread energy of 10eV.

The MS raw data were imported into MaxQuant (Cox and Mann, 2008) (version 1.5.2.8, and searched against the UniProt mouse proteome (SwissProt December 2015 release) with MMTS as the fixed modification and Methionine oxidation and N-terminal acetylation as variable modifications. Match between runs was activated. All other parameters were set to default.

**HEK293T cell transfections**

HEK293T cells were transfected with either Noelin1 constructs alone or in combination with GluA2 constructs using polyethylenimine (PEI 25000). Briefly, 2.2 x 10⁶ cells were grown in 10 cm dishes in Dulbecco's modified eagle medium + 10% fetal calf serum until 70% confluence was reached. The cells were transfected with 2.5 µg DNA, 35 µL PEI, 250 µL PBS mix. The cells were harvested 48 h post transfection for IP experiments.

For preparation of HEK293T cell pellets for SPR biosensor analysis, 1.8x10⁷ cells were plated on a 245 mm by 245 mm plate (Corning) and allowed to reach 70% confluence. The cells were then transfected with 50 µg of GluA2 or Noelin1-3 DNA or without DNA (control) along with 350 µL PEI and 2500 µL PBS. Cells were washed 48 h post-transfection for three times with ice-cold PBS and scraped cells (GluA2 vs. control) were collected in falcon tubes and stored at –80 °C, and used to prepare the SPR surface (Fig. S2).

For collection of Noelin1-3 medium or control medium, HEK293T cells grown on 245 mm by 245 mm plates were transfected at 70% confluence with Noelin1-3 construct (Noelin1 enriched medium) or mock transfected (control medium) respectively. The medium was replaced with low serum OptiMEM (Gibco, Life Technologies), 24 h post-transfection (transfected cells). This cell medium was collected 48 h after cell medium change, centrifuged at 800 rpm for 5 minutes to remove cell debris and concentrated 100 times
using a 10 kDa cutoff filter (Vivaspin 10, Sartorius) and stored at –80 °C, and used as conditioned medium (Noelin1-3) vs. control medium.

**Single nanoparticle tracking (QD) for surface diffusion of AMPAR**

For AMPAR-QD tracking, hippocampal neurons transfected with GluA1::pHluorin or GluA2::pHluorin along with Homer1::dsRed were used as previously described (Klueva et al., 2014). Briefly, neurons were incubated with a monoclonal antibody directed to GluA1::pHluorin or GluA2::pHluorin subunit for 10 minutes followed by a 5-minute incubation with QDs 655 Goat F(ab')2 anti-mouse IgG (Invitrogen). QDs were detected by using a mercury lamp and appropriate excitation/emission filters. Images were obtained with an interval of 30 ms and up to 1000 consecutive frames. QD recording sessions were processed with the Metamorph software (Universal Imaging Corp.). The instantaneous diffusion coefficient ($D_{inst}$), was calculated for each trajectory, from linear fits of the first 4 points of the mean square displacement versus time function using $MSD(t) = \langle r^2(t) \rangle = 4D t$. The two-dimensional trajectories of single molecules in the plane of focus were constructed by correlation analysis between consecutive images using a Vogel algorithm. Average QD traces and Homer1::dsRed co-localizing spots were used to define synaptic and extra-synaptic structures (Frischknecht et al., 2009). Immobile receptors were defined as having $D_{inst} < 0.001 \mu m^2 s^{-1}$.

**Surface plasmon resonance biosensor based interaction analysis**

GluA2-containing AMPA receptors were extracted from HEK293T cell pellets using 25 mM HEPES, 150 mM NaCl, 1% DDM (w/v) (Affymetrix), pH 7.4 and one protease inhibitor tablet per 50 mL buffer (buffer S). Buffers were chilled prior to usage and solubilization was performed on ice or at 4 °C. Buffer S (2.5 mL, GluA2; 5 mL, control) was added to the frozen cell pellets. Thawed pellets were homogenized by 10 strokes using a glass homogenizer (Kontes Glass Co). Solubilization was performed on a rocker shaker for 1 h, followed by centrifugation (20,000x g, 30 minutes) in a Beckman L7-55 ultracentrifuge (Beckman Coulter) using a Ti-50 rotor. Aliquots were stored at –80 °C.

SPR-based biosensor studies were performed using a Biacore S51 instrument and CM5 sensor chips (GE Healthcare). The GluA2 antibody was covalently immobilized on two spots of the sensor surface by standard amine coupling chemistry at a temperature of 25 °C. The antibody, in 10 mM Na-acetate, pH 4.5, was injected for 2 min (10 µLmin$^{-1}$) at a concentration of 50 µgµL$^{-1}$. The running buffer consisted of 10 mM HEPES, 150 mM NaCl, and 0.05% Tween-20 (v/v), pH 7.4. After amine coupling, the temperature was reduced to 10 °C. Solubilized membranes, containing the GluA2 AMPAR were injected for 75 minutes at a flow rate of 2 µLminute$^{-1}$, generating GluA2 receptor surfaces. Control surfaces were generated by injecting solubilized membranes from non-transfected cells (Fig. S2). The running buffer consisted of 25 mM HEPES, 150 mM NaCl, 0.1% DDM (w/v), pH 7.4 (buffer A).

Interaction analyses were performed at 10 °C using buffer A as the running buffer. Noelin1-3 conditioned medium (or control medium), diluted into buffer A, was injected in 2-fold dilution series (7.2 nM – 0.45 nM) in ascending order for 120 s (30 µLmin$^{-1}$) and allowed to dissociate for 180 s. Buffer injections served as
blank samples. Analyses were repeated on freshly prepared biosensor surfaces. For experiments under reducing conditions, Noelin1-3 was diluted into Buffer A supplemented with 1 mM DTT (Fig. S2) and incubated for 30 minutes at room temperature on a rocker shaker (10 rpm). Biacore BIAevaluation Software 4.1 (GE Healthcare) was used for data analysis. Sensorgrams from GluA2 receptor surfaces were double-referenced by subtracting the signals from control surfaces (Fig. S2) and the average signals from the blank injections. The kinetic rate constants were ($k_{on}$ and $k_{off}$) was determined by global non-linear regression analysis using a reversible 1-step interaction model (Fig. 2b).

**Cross-linking analysis**

A synaptosomal fraction of C57BL6 mouse hippocampi was enriched as previously described (Pandya et al., 2017), and the proteins were crosslinked using disuccinimidyl sulfoxide (DSSO) (Liu et al., 2015). Cross-linked peptides were analyzed with an Orbitrap Fusion mass spectrometer and identified with XlinkX v2.0, as previously described (Liu et al., 2017).

**AMPAR-Noelin1 modeling**

Explorative modeling of the interaction between AMPA receptor and pancortin was performed using homodimeric Noelin1/Olfactomedin1 (PDB 5AMO) and homotetrameric GluA2 (PDB 5KBS) structures. The accessible interaction space (i.e., the space containing all possible protein complex conformations) was calculated with DisVis web server (van Zundert et al., 2017), assuming a maximal Ca-Ca distance of 23.4 Å between K283 and K229 of GluA2 and Noelin1, respectively (based on unpublished data). Occupancy analysis was performed, which yielded the preferred occupied regions of space by Noelin1.

**Synaptotagmin1 uptake assay**

The pre-labeled lumenal domain synaptotagmin1 antibody (105 103C3; Oyster@550 1:200; Synaptic Systems) was added to the culture medium of dissociated cortical neurons (DIV21) in presence of 1 µm TTX. After 20 minutes cells were fixed in PBS containing 4% PFA for 5 minutes and subsequently blocked for 1 h in blocking solution containing PBS, 10% FBS and 0.1% Triton X-100. Cells were then stained with guinea pig anti-Shank2 antibody (162 204; 1:1000; Synaptic System) for 1 h in PBS, 3 % FCS. Subsequently cells were washed 3x 5 minutes with PBS and incubated for 45 minutes with secondary antibody (goat anti-guinea pig Alexa 488) for 45 minutes. Cells were washed 3x with PBS and mounted onto slides using Aquamount (Polysciences, Inc.).
Supplemental figures

Figure S1 | Noelin1 isoforms are expressed throughout the brain; related to Figure 1. 

a) Schematic representation of the four Noelin1 (Olfactomedin1) protein isoform that arise from alternative splicing according to (Danielson et al., 1994). All forms share the common M-region (yellow). In this region the antigen is indicated to which the Noelin1 antibody was generated (orange box). Cysteine residues (black lines) in the M-region are involved in oligomerization (C73, C75, C85). Noelin1-1 and -2 isoforms contain the A1-region (also called B-region; dark red), and Noelin1-3 and -4 isoforms contain the A2-region (also called A-region; dark blue) at their N terminus. Noelin1-1 and -3 isoforms contain the C1-region (also called Z-region; green) at the C-terminus, which contains the Olfactomedin domain. The M-region of Noelin1-2 and 1-4 only has a short C-terminal region (C2- or Y-region; brown). Red empty boxes show the peptides for Noelin1 obtained by LC-MS/MS analysis of GluA2/3 immunoprecipitation from hippocampus.

b) Allen brain atlas in situ hybridization of Noelin1 (Olfm1; probe RP_040324_01_F03) in adult mouse brain shows Noelin1 expression in hippocampus, cortex and cerebellum.

c) Immunoblotting for Noelin1 on P2+M isolated from hippocampus (HC), cortex (Cx), cerebellum (Cb) shows that the antibody recognizes all four isoforms. The expression in cerebellum is lower than in hippocampus and cortex. Full blots are given in Fig. S8.
Figure S2 | Tetrameric Noelin1 interacts with AMPARs; related to Figure 1 & 2. a) Noelin1-1, Noelin1-3 and Noelin1-4, individually expressed with GluA1 subunit, show immunoreactivity in lysate. Each Noelin1 isoform is specifically enriched in GluA1 IP. b) Standard curve for the peptide (LTGISDPVTVK) used to estimate Noelin1-3 levels in medium of HEK293T cells. Red circle shows the peak area for the Noelin1-3 sample used for SPR measurements (below). c,d) Cell medium from HEK293T cells expressing Noelin1-3 shows immunoreactivity at 75 kDa, but control medium does not (c). Additional immunoreactivity is observed above 250 kDa when HEK293T cell medium was run under non-reducing conditions (i.e., without DTT), (d). Full blots are given in Fig. S8. e) Schematic representation of the sensor surfaces used for SPR-biosensor based interaction analysis and representative sensorgrams obtained during surface preparation. The dextran matrix of two sensor surfaces was coated with an anti-GluA2 antibody. Detergent-solubilized GluA2 receptor (from GluA2 transfected HEK293T cells) was immobilized by affinity capture on ‘receptor surfaces’ at surface densities of approximately 1400 RU (left). ‘Control surfaces’ were prepared by instead injecting non-transfected HEK293T cell medium over the antibody surface to approximately 400 RU (right). The control surface was used to estimate unspecific binding of Noelin1-3 to the sensor surface. f) Representative sensorgrams obtained during interaction analysis of Noelin1-3 on GluA2 ‘receptor surface’ and ‘control surface’. Gray sensorgrams are corrected for the response generated from injection of buffer (blank sample). The final sensorgram of Noelin1-3 binding to GluA2 (blue) used for kinetic analysis is also corrected for non-specific interactions with the control surface. It was obtained by subtraction of the blank-corrected response on the control surface from the blank-corrected response on the GluA2 receptor surface. g,h) Schematic representations of the receptor surface (top) and SPR sensorgrams for evaluating the effect of non-reducing and reducing conditions (bottom). Sensorgrams were corrected by subtraction of signals from blank injections and with the control surface. Noelin1-3 was only seen to interact with GluA2 in the absence of DTT (g, blue), binding was not detected in the presence of 1 mM DTT (h, red).
Figure S3 | **Noelin1 does not affect homomeric GluA1 AMPAR currents; related to Figure 3.**

**a)** Peak-scaled example traces of whole-cell recording from HEK293T cells expressing homomeric GluA1-containing AMPAR channels in the absence (gray) or presence (red) of Noelin1. Currents were evoked by direct application of 1 mM glutamate during 1 ms.

**b)** Bar graphs (mean±SEM) summarize the absence of effect of co-expression (GluA1_Noelin1-3; light red) or addition or addition (GluA1 + Noelin1-3, dark red) of Noelin1 on rise time and decay time of homomeric GluA1 AMPAR currents. P-values for rise time were GluA1 (n=24) vs. GluA1_Noelin1-3 (n=9) \( P=0.970 \) (unpaired t-test), GluA1 vs. GluA1+Noelin1-3 (n=9) \( P=0.861 \) (unpaired t-test), and P-values for decay time were GluA1 vs. GluA1_Noelin1-3, \( P=0.961 \) (unpaired t-test), GluA1 vs. GluA1+Noelin1-3, \( P=0.207 \) (Mann Whitney U-test). Number of cells used are indicated.

**c)** Both co-expression and addition of Noelin1 shows normal rectification of homomeric GluA1 AMPARs.
Figure S4 | Noelin1 is enriched at extrasynaptic and postsynaptic sites of hippocampal neurons, where it colocalizes with Brevican; related to Figure 4. a) STED imaging in cultured hippocampal neurons at DIV21 showing an overview or zoom-ins of dendrites (right) or spines (right, inset). Permeabilized staining for Noelin1 (green) and Homer1 (blue, synaptic marker), as well as surface staining for Brevican (red) are shown. Merged picture shows color-overlay images of the three channels. Scale bars are indicated. Inset (right) shows a 2-fold enlargement. Line scans over dendrite (1, 2) are displayed in panel b. b) Numbered dashed white lines on the overlay image (in a) bottom right) indicate locations of line scans across the three channels (x-axis: distance (µm); y-axis: intensity (arbitrary units)). Graphs illustrate the co-enrichment of immunofluorescence intensities of Noelin1, Brevican and Homer1 at dendrites and spines, with the latter showing extrasynaptic and synaptic sites.
Figure S5 | Noelin1 regulates GluA1 AMPAR mobility in HEK293T cells; related to Figure 6. a) Schematic representation of HEK293T cell expression of SEP-tagged constructs of GluA1 and GluA2 that are still able to bind to Noelin1. b) Noelin1-3 (75 kDa) individually expressed with GluA1 or GluA2 (100 kDa, triangle), or SEP::GluA1 and SEP::GluA2 subunit (~130 kDa, triangle) in HEK293T cells. Noelin1 is enriched in the GluA IPs showing that the SEP-tag does not influence Noelin1-AMPAR interaction. Molecular weights are indicated. Full blots are given in Fig. S8. b) Frequency distribution histogram of all particles for SEP::GluA2 receptors in HEK293T cells with either coexpression of GFP or Noelin1 (GluA2_GFP, GluA2_Noelin1 respectively), and with either addition of control medium or Noelin1-conditioned medium (GluA2+control, GluA2+Noelin1, respectively) shows that the SEP::GluA2 AMPARs move slower. c) Frequency distribution histogram of all particles for SEP::GluA1 receptors in HEK293T cells with either coexpression of GFP or Noelin1 (GluA1_GFP, GluA1_Noelin1, respectively) and with addition of Noelin1-conditioned medium (GluA1+Noelin1) shows that the SEP::GluA1 AMPARs move slower. d) Box plots indicate a significant effect on the diffusion coefficient ($D_{\text{inst}}$) for SEP::GluA1 AMPARs...
upon coexpression of Noelin1 (GluA1 (n=31) vs. GluA1_Noelin1 (n=26); \(P=0.034\) (unpaired t-test), and a trend for addition of Noelin1 (GluA1 vs. GluA1+Noelin1 (n=24); \(P=0.100\) (unpaired t-test). e) Quantification of the immobile fraction obtained from panel b shows that immobility of GluA1 AMPARs is significantly increased upon addition of Noelin1 (GluA1 vs. GluA1+Noelin1, \(P=0.0242\) (unpaired t-test)), and shows a trend for co-expression of Noelin1 (GluA1 vs. GluA1_Noelin1; \(P=0.094\) (Mann-Whitney U-test)).
Figure S6 | Noelin1 regulates AMPAR mobility in neurons; related to Figure 6. a-c) Mobility of extrasynaptic SEP::GluA1 AMPARs in young neurons (DIV11-13). a) Frequency distribution histogram of GluA1 AMPAR after 1-h incubation of control medium or Noelin1-conditioned medium. b) Box plots indicate a trend for the diffusion coefficient of SEP::GluA1 AMPARs without and with Noelin1 incubation (GluA1+control (n=15) vs. GluA1+Noelin1 (n=11); P=0.059 (Mann Whitney U-test)). c) Quantification of the immobile fraction obtained (panel a) shows a significant increase in the immobile fraction (P=0.020; Mann Whitney U-test). d-f) Mobility of synaptic SEP::GluA2 AMPARs in young neurons (DIV11–13). d) Frequency distribution histogram of GluA2 AMPARs after 1-h incubation of control medium or Noelin1-conditioned medium. e) Box plots indicate no effect for the diffusion coefficient of SEP::GluA2 AMPARs without and
with Noelin1 incubation (GluA2+control (n=17) vs. GluA2+Noelin1 (n=17); P=0.304 (unpaired t-test)). f) Quantification of the immobile fraction obtained (panel d) shows no effect either (P=0.111; unpaired t-test).

g-i) Mobility of synaptic SEP::GluA1 AMPARs in young neurons (DIV11–13). g) Frequency distribution of GluA1 AMPAR after 1-h incubation of control medium or Noelin1-conditioned medium. h) Box plots indicate a significant effect for the diffusion coefficient of SEP::GluA1 AMPARs without and with Noelin1 incubation (GluA1+control (n=15) vs. GluA1+Noelin1 (n=11); P=0.016 (unpaired t-test with Welch's correction)). i) Quantification of the immobile fraction obtained (panel g) shows a trend (P=0.055; unpaired t-test with Welch's correction).
Figure S7 | Noelin1 effects on AMPAR mobility are postsynaptic; related to Figure 7. a) Recordings of mEPSC in dissociated primary cultures at DIV21 were measured under control conditions (Con) or after treatment with hyaluronidase (Hya), in which either HEK cell medium (+control) or Noelin1-conditioned medium (+Noelin1) was added. After outlier analysis, 1 cell was removed from the Hya+Noelin1 group). The one-way ANOVA (Welch test) analysis showed no significant difference for amplitude (F(3,26.4)=2.30, \(P=0.10\)), or frequency (F(3,25.8)=0.73, \(P=0.542\)), and no group differences were detected by Duncan’s post-hoc test that is stringent for type II errors (\(P=0.053; P=0.154\)). b) Synaptotagmin1 uptake assay in presence of tetrodotoxin (TTX) to avoid network activity was performed in order to assess basal presynaptic function (Kraszewski et al., 1995). Pre-labeled synaptotagmin1 antibodies (Oyster 550) were added to culture medium for 20 minutes prior to fixation and subsequent staining with shank2 antibody, a reliable postsynaptic marker for excitatory synapses. Shank2 labeling was used to identify synapses and synaptotagmin1 fluorescence intensity, which correlates with synaptic activity, was measured. In line with the mEPSC measurements, we found no difference in synaptotagmin1 uptake (one-way ANOVA (Welch test) analysis, F(3,51.4)=1.80, \(P=0.158\); Duncan’s post-hoc test, \(P=0.066\)). Scale bar indicates 5 µm. Number of cells measured are indicated.
Figure S8 | Full immunoblots; related to Figures 1, 2, S1, S2, S5. For all immunoblots in the main and supplemental figures, full blots are given. The molecular weight and antibody used (if applicable) are indicated, as well as the part given in the figure (red box).
Table S1 Mass spectrometric analysis of native hippocampal Noelin1 complexes reveals association with AMPAR and established AMPAR interactors, related to Figure 1. Analysis of native Noelin1 complexes, immunoprecipitated from hippocampi of WT animals (crude synaptic membranes; n=4 IPs), identified AMPAR subunits and Neuritin, an established AMPAR interactor (Schwenk et al., 2012), as most prominent parts of the Noelin1 complex compared with the IP using an Ig-G antibody (negative control). For reference, 2 IPs are listed for GluA2/3 and GluA2, respectively. IP-ed proteins: Noelin1 (dark blue), AMPAR subunits (light blue), established AMPAR interacting proteins (yellow). Values (iBAQ) obtained from MaxQuant search of IP experiments are indicated for each IP.

| Gene name | Swiss prot ID | Uniprot ID | Uniprot recommended name | iBAQ Noelin1-iP, Mean (n = 4) | iBAQ IgG | iBAQ GluA2/3 IP | iBAQ GluA2 |
|-----------|--------------|------------|--------------------------|--------------------------------|---------|----------------|-----------|
| Olfm1     | O88998       | NOE1_MOUSE | Noelin1                  | 112485                         | NA      | 6361           | 1579      |
| Gria1     | P23818       | GRIA1_MOUSE| Glutamate receptor 1     | 722                            | NA      | 467530         | 282930    |
| Gria2     | P23819       | GRIA2_MOUSE| Glutamate receptor 2     | 1324                           | 146     | 865900         | 364860    |
| Gria3     | Q9Z2W9       | GRIA3_MOUSE| Glutamate receptor 3     | 140                            | NA      | 169820         | 124330    |
| Gria4     | Q9Z2W8       | GRIA4_MOUSE| Glutamate receptor 4     | NA                             | NA      | 7164           | 3793      |
| Cacng2    | O88602       | CCG2_MOUSE | Voltage-dependent calcium channel gamma-2 subunit | NA | NA | 43951 | 34055 |
| Cacng3    | Q9JJV5       | CCG3_MOUSE | Voltage-dependent calcium channel gamma-3 subunit | NA | NA | 7811 | 2736 |
| Cacng8    | Q8VHW2       | CCG8_MOUSE | Voltage-dependent calcium channel gamma-8 subunit | 299 | NA | 85992 | 47545 |
| Olfm2     | Q8BM13       | NOE2_MOUSE | Noelin-2                 | 777                             | NA      | 2579           | 634       |
| Olf3      | P63056       | NOE3_MOUSE | Noelin-3                 | 598                             | NA      | 805            | 500       |
| Nrn1      | Q8CFV4       | NRN1_MOUSE | Neuritin                 | NA                             | NA      | 1109           | NA        |
| Cnih2     | O35089       | CNIH2_MOUSE | Protein cornichon homolog 2 | NA | NA | 48179 | 24448 |
| Cnih3     | Q6ZWS4       | CNIH3_MOUSE | Protein cornichon homolog 3 | NA | NA | 37045 | 19424 |
| Cpt1c     | Q8BGD5       | CPT1C_MOUSE | Carnitine O-palmitoyltransferase 1, brain isoform | NA | NA | 1231 | 604 |
| Frrs1l    | B1AXV0       | FRS1L_MOUSE | DOMON domain-containing protein FRRS1L | NA | NA | 24613 | 13372 |
| Prtt1     | O35449       | PRRT1_MOUSE | Proline-rich transmembrane protein 1; SynDIG4 | 313 | NA | 203370 | 97991 |
| Prtt2     | E9PUL5       | PRRT2_MOUSE | Proline-rich transmembrane protein 2 | NA | NA | 2348 | NA |
| Abhd12    | Q99LR1       | ABD12_MOUSE | Monoacylglycerol lipase ABHD12 | 387 | NA | 650 | 754 |
Table S2. **Antibodies used; related to Figures 1,2,4–7 and Figures S1,2,4–7.** All antibodies used for the different applications are listed. *Antigen: QNFATYKEGNYVESVKI.*

| Purpose                  | Protein          | Vendor              | Name / Clone / Catalog # | Host       | Dilution          |
|--------------------------|------------------|---------------------|--------------------------|------------|-------------------|
| **Primary antibodies**   |                  |                     |                          |            |                   |
| IP                       | GluA2/3*         | Genscript           | -                        | Rabbit     | 10 µg/IP          |
| IP                       | GluA2            | Neuromab            | L21/L32                  | Mouse      | 10 µg/IP          |
| Blotting                 | GluA2            | Neuromab            | L21/L32                  | Mouse      | 1:1000            |
| Immunocytochemistry       | GluA2            | Synaptic systems    | 182 103                  | Rabbit     | 1:250             |
| SPR                      | GluA2            | Neuromab            | L21/L32                  | Mouse      | 50 µg/µL          |
| Immunocytochemistry       | GluA2-N          | Millipore           | 6C4                      | Mouse      | 1:250             |
| IP & blotting            | GluA1            | Abcam               | ab109450                 | Rabbit     | 10 µg/IP; 1:20000 |
| IP & blotting            | Noelin1          | Neuromab            | Clone K96/7              | Mouse      | 1:500             |
| Immunocytochemistry       | Noelin1          | Neuromab            | Clone K96/7              | Mouse      | 1:500             |
| IP & blotting            | PSD-95           | Neuromab            | Clone K28/43             | Mouse      | 1:5000            |
| IP & blotting            | Synaptophysin    | SantaCruz           | sc-9116                  | Mouse      | 1:500             |
| Immunocytochemistry       | Homer1           | Synaptic Systems    | 160 004                  | Guinea pig | 1:1000            |
| Immunocytochemistry       | Brevican         | Home-made           | -                        | Rabbit     | 1:1000            |
| Quantum dots              |                  |                     |                          |            |                   |
| Synaptotagmin1 uptake     |                  |                     |                          |            |                   |
| assay                    | Syt1             | Synaptic systems    | 105 103C3                | Mouse      | 1:200             |
|                          | Shank2           | Synaptic systems    | 162 204                  | Guinea pig | 1:1000            |
| **Secondary antibodies**  |                  |                     |                          |            |                   |
| Immunocytochemistry       | Anti-mouse Alexa 488 | Molecular probes | R37120                  | Goat       | 1:1000            |
| Immunocytochemistry       | Anti-rabbit Alexa 568 | Molecular probes | A-11011                 | Goat       | 1:1000            |
| Immunocytochemistry       | Anti-guinea pig Alexa 647 | Molecular probes | A-21450                | Goat       | 1:1000            |
| STED                     | anti-rabbit Abberior STAR 580 | Abberior GmbH | 41367                  | Goat       | 1:250             |
| STED                     | Anti-mouse Atto 647N | Atto-Tec GmbH      | 50185                   | Goat       | 1:250             |
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