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Supplementary Information for

The amino-terminal domain of GluA1 mediates LTP maintenance via interaction with neuroplastin-65

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Dataset S1

SI Materials and Methods

DNA constructs. Rat cDNAs encoding full-length GluA1 and GluA2 were cloned into a pCAGGS vector. GluA1ΔATD and GluA2(Q)ΔATD were created by excising the coding sequences of GluA1 ATD (residues 19-393) and GluA2(Q) ATD (residues 22-402) after the signal sequences, respectively. GluA1$^{A2\text{-}ATD}$ was produced by replacing the ATD of GluA1 (residues 1-393) with that of GluA2 (residues 1-402). For exogenous expression experiments, an HA epitope (YPYDVPDYA) was fused to the C-terminus of AMPAR subunits GluA1, GluA2 and GluA3. GluA1 ATD was produced with a stop codon inserted at the end of the coding sequences (residues 1-393). The coding sequences of rat Np65 and Np55 and mouse Pdgfrα were cloned with a FLAG epitope (DYKDDDDK) inserted at the C-terminus. Np65ΔCTD was created by deleting the intracellular sequences (residues 360-393) of Np65, Np65-Ex was generated by replacing the extracellular domain of Pdgfrα (residues 1-528) with that of Np65 (residues 1-338).

sgRNAs were identified using the online tool (http://crispor.tefor.net). For knockout constructs, the sgRNAs together with Cas9-T2A-EGFP were cloned into a pCAGGS vector. For lentivirus production, sgRNAs were cloned into a pFugW vector. The primers used to design the specific sgRNAs targets were: *Gria1* forward (5' to 3') ACCG cggttttctaggtgcggttg; *Gria1* reverse (3' to 5') AAAC caaccgcacctagaaaaccg; *Gria2* forward (5' to 3') ACCG ctaacagcatacagataggt; *Gria2* reverse (3' to 5') AAAC acctatctgtatgctgttag; *Gria3* forward (5' to 3') ACCG cgtagaccacttggattcct; *Gria3* reverse (3' to 5') AAAC aggaatccaagtggtctacg; *Nptn* forward (5' to 3') ACCG ctagctgggtttgtcaagtc; *Nptn* reverse (3' to 5') AAAC gacttgacaaacccagctag. *Gria1* sgRNA targets part of the coding region of exon 1, therefore, we changed the sgRNA-targeting and PAM sequences in accordance with the rules of codon degeneracy to exclude the recognition of the GluA1 construct. GluA2 sgRNA targets a region between exon 1 and intron 1, allowing replacements with wide-type constructs. *Nptn* sgRNA targets a region between intron 1 and exon 2, thus allowing replacements with wild-type constructs. All constructs in this study were cloned using the In-Fusing HD Cloning Kit (Clontech) .

Antibodies. Rabbit anti-GluA1 (cat.no. ab31232; Abcam), mouse anti-GluA1 (cat.no. MAB2263; Millipore), rabbit anti-GluA2/3 (cat.no. AB1506; Millipore), rabbit anti-Tubulin beta (cat.no. BS1482; Bioworld Technology), rabbit anti-Flag (cat.no. 2368; Cell Signaling Technology), mouse anti-Flag (cat.no. F1804; Sigma-Aldrich), rabbit anti-HA (cat.no. 3724; Cell Signaling Technology), mouse anti-HA (cat.no. 2367; Cell Signaling Technology), goat anti-Neuroplastin 65 (cat.no. AF5360; R & D Systems), mouse anti-GAPDH (cat.no. MB001H; Bioworld Technology), rabbit anti-PSD-95 (cat.no. MA1-045; Invitrogen), rabbit anti-GluN2B (cat.no. 14544S; Cell Signaling Technology) and chicken anti-GFP(cat.no. ab13970; Abcam).

Lentivirus production. Four 10 cm dish of rapidly dividing HEK293T cells (ATCC) were transfected with 36 μg pFugW construct, plus helper plasmids pVSVG (24 μg) and psPAX2 (36 μg) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Culture supernatants were collected 48 h posttransfection, then filtered, and concentrated by centrifugation at 80,000×*g* for 2 h. The resulting pellets were resuspended in Opti-MEM, divided into aliquots, flash-frozen and stored at -80°C until use.

Primary neural culture and transfection. Hippocampal neurons were derived from newborn mouse pups of both sexes. Hippocampi were quickly dissected and digested in 0.25% trypsin (Invitrogen) at 37°C for 10 min. The tissues were triturated, and the cells were plated on poly-D-lysine-coated coverslips or dishes (600,000 cells/well for 6-well dishes; 100,000 cells/well for 24-well dishes). Cultures were maintained in Neurobasal medium supplemented with 2% B-27 and 1% Glutamax (all from Invitrogen) in a humidified incubator at 37°C with 5% CO_2; half of the medium was changed every three days. For CRISPR/Cas9-mediated gene deletion, neurons derived from Cas9-knock-in pups were infected with concentrated lentivirus at 3 days *in vitro* (DIV3). For neural immunostaining, wild-type neurons were transfected with the indicated plasmids using Lipofectamine 2000 (Invitrogen) at DIV11.

***In utero* electroporation.** *In utero* electroporation was performed as previously described (1). E14.5 pregnant wild-type ICR mice were anesthetized with a mixture of ketamine (100 mg/kg body weight) and xylazine (5 mg/kg body weight) via intraperitoneal injection; then the mice were subjected to surgical procedures to expose the uterus. Each embryo was injected with about 2 μL of plasmid DNA mixed with Fast-Green into the lateral ventricle via a beveled glass micropipette. The embryos were then electroporated with five 42-V pulses of 50 ms, delivered at 1 Hz, using platinum tweezertrodes in a square-wave pulse generator (BTX, Harvard Apparatus). Following electroporation, the embryos were placed back into the abdominal cavity, and the muscle and skin were sutured. Mice were injected i.p. with carprofen (5mg/kg body weight) and monitored until fully awake. All of the surgical procedures were performed in accordance with the established guidelines of the IACUC of Nanjing University.

Electrophysiology. Transverse 300 μm slices were cut using a Leica vibratome in chilled high-sucrose cutting solution containing (in mM): 2.5 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 7 glucose, 210 sucrose, 0.5 $CaCl_2$ and 7 $MgSO_4$. Slices were then incubated for 30 min at 34°C in artificial cerebrospinal fluid (aCSF) bubbled with 95% O2/5% CO2 containing (in mM): 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 11 glucose, 2.5 $CaCl_2$ and 1.3 $MgSO_4$. After a 45- to 60-min recovery at room temperature, the slices were transferred to a submersion chamber on an upright Olympus microscope, and perfused in aCSF supplemented with 0.1 mM picrotoxin and 0.01 mM bicuculline, saturated with 95% O_2/5% CO_2. Neuronal synaptic responses were recorded by 4- to 6-MΩ borosilicate glass pipettes filled with intracellular solution consisted of (in mM): 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.3 EGTA, 5 QX-314, 4 Mg-ATP, 0.3 Na-GTP and 0.1 spermine, following stimulation of Schaffer collaterals with a bipolar stimulation electrode placed in stratum radiatum at the CA1 region. The input resistance and pipette series resistance were monitored throughout the recording; recordings in which series resistance increased to >30 MΩ or varied by >50% between neurons were discarded. In LTP recordings, cells in which series resistance varied by 25% during a recording session were discarded. Data were collected with a MultiClamp 700B amplifier (Axon Instruments), filtered at 2 kHz, and digitized at 10 kHz.

Hippocampal CA1 pyramid neurons were visualized via infrared differential interference contrast microscope. GFP- or mCherry-positive neurons were identified by epifluorescence microscope. Paired whole-cell recordings were achieved through the simultaneous recording of a GFP- or mCherry-positive neuron and a neighboring untransfected neuron. AMPAR-EPSCs were isolated at a holding potential of -70 mV, whereas NMDAR-mediated responses were recorded at +40 mV and measured 100 ms after the stimulus to avoid contamination of AMPAR currents. The paired-pulse ratio was measured by applying two pulses at an interval of 50 ms and then calculating the ratio of the peak amplitudes. The rectification index was calculated as the ratio of the slopes of the lines connecting AMPAR-EPSC values from 0 mV to 40 mV and from -70 mV to 0 mV in the presence of 100 μM D-AP5 to block NMDARs. The calculation was as follows:

$$RI = \frac{7(I_{40} - I_0)}{4(I_0 - I_{-70})}$$

where I_x represents the EPSCs recorded at a holding potential of x mV. mEPSCs were acquired in the presence of 1 μM tetrodotoxin and conducted through a template-based search in Clampfit (Molecular Devices).

LTP was induced by a pairing protocol in which Schaffer collateral axons were stimulated at 2 Hz for 90 s, while neurons were depolarized to 0 mV, after 3- to 5-min steady baseline recording, but no more than 7 min when achieved whole-cell configuration to avoid "wash-out" of LTP. To induce LTD, a standard low-frequency stimulation (LSF) protocol (1 Hz, 900 s) was applied, with neurons clamped at -40 mV, after 5- to 10-min of stable baseline recording.

Out-side out patches were pulled from the soma of CA1 neurons after obtaining the whole-cell approach, and recorded at -70 mV using 3- to 5-MΩ glass pipettes containing intracellular solution consisting of (in mM): 95 CsF, 45 CsCl, 10 HEPES, 2 NaCl, 10 EGTA, 1 $CaCl_2$, 2 Mg-ATP, 5 TEA-Cl, 1 QX-314 and 0.1 spermine. Patches were perfused in HEPES-based aCSF containing (in mM): 150 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 2 $CaCl_2$ and 1 $MgCl_2$, supplemented with 1 μM tetrodotoxin, 100 μM picrotoxin, 100 μM D-AP5, 1 mM trichlormethiazide and 500 μM L-glutamate to isolate AMPAR-mediated currents. Voltage ramps from -100 mV to +100 mV over 700 ms were applied in the absence and in the presence of 500 μM L-glutamate.

Rectification index values were measured using the subtracted currents at -70 mV, 0 mV and +40 mV and calculated as described above.

Neural immunostaining and fluorescence microscopy. At DIV17, hippocampal cultures on coverslips were incubated with anti-N-terminal antibodies for 20 min and then fixed with 4% paraformaldehyde/4% sucrose for 10 min at RT. After blocking with a mixture of 10% donkey serum and 5% BSA for 1 h at RT, the coverslips were incubated with Alexa Fluor-conjugated secondary antibodies followed by mounting. To visualize intracellular molecules, cultures were permeabilized in 0.1% Triton X-100 in PBS for 10 min at RT and subsequently subjected to primary antibodies incubation. All images of the primary cultures were acquired with a Zeiss LSM880 confocal microscope using a 63× or 40× oil immersion lens. Neural surface protein colocalization ratio was quantified using ImageJ.

Cell surface biotinylation. Cultured cells were washed twice with ice-cold PBS containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS^{2+}) and then incubated in PBS^{2+} with 1 mg/mL EZ-link Sulfo-NHS-LC-biotin (Thermo Fisher Scientific) for 30 min at 4°C with gentle shaking. The biotinylation reaction was quenched and cells were washed with 100 mM glycine diluted in PBS^{2+} to remove unreacted biotin. Then, the cells were lysed in lysis buffer (150 mM NaCl, 50 mM Tris-Cl, 10 mM HEPES pH 7.4, 1 mM EDTA, 1% Np-40, 0.1% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 1 mM Na_3VO_4 and protease inhibitor cocktail). The supernatants were collected after centrifugation at 12,000 ×*g* for 10 min at 4°C, and incubated with streptavidin agarose resins (Thermo Fisher Scientific) overnight at 4°C. The resins were washed four times with lysis buffer, and the biotinylated proteins were eluted with 2× Laemmli sample buffer and analyzed by SDS-PAGE and immunoblotting.

Protein affinity purification. Soluble recombinant GluA1-ATD-Fc was prepared as described previously (2). Briefly, HEK293T cells were transfected with GluA1-ATD-Fc constructs using polyethylenimine (Polysciences, USA.) reagents. Forty-eight hours after transfection, the cell medium was collected and filtered through a 0.45 μm filter with low protein binding. The medium was then adjusted to 1 mM EDTA, 10 mM HEPES-NaOH (pH 7.4), 1 mM PMSF and proteinase inhibitor cocktail, and incubated with protein A-Sepharose beads (Amersham Pharmacia Biotech) for 12 h at 4°C. After incubation, the beads were washed three times with PBS.

For brain affinity chromatography experiments, one rat brain was homogenized in 5 ml of homogenate buffer (PBS, 0.1 mM EDTA, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM PMSF, proteinase inhibitor cocktail, 1% Triton X-100) and rotated at 4°C for 2 h. The insoluble fractions were removed by centrifugation at 100,000 ×*g* for 1 h. For pull-down experiments, the GluA1-ATD-Fc-bound protein A-Sepharose beads and the control Fc-bound protein A-Sepharose beads (1.5 nM) were added to 5 mL of brain extracts, and rotated at 4°C for 12 h. After incubation, the beads were washed six times with PBS containing 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The binding proteins were eluted with sample buffer and subjected to SDS-PAGE and Coomassie staining. Subsequent mass spectrometry analysis was performed on an Applied Biosystem QSTAR XL mass spectrometer.

Mass spectrometry. For identification of GluA1-ATD-interacting proteins, the SDS-PAGE gel containing the protein sample was cut into pieces and destained with 25 mM ammonium bicarbonate/50% acetonitrile. The gels were reduced with 10 mM DTT at 37°C for 1 h, and then alkylated with 25 mM iodoacetamide at RT for 1 h before digestion with trypsin. The digested peptides were extracted by sonication with 5% trifluoroacetic acid and 50% acetonitirile. After drying by SpeedVac, the peptides were resolubilized in 0.1% formic acid and filtered with 0.45 μm centrifugal filters before subjecting to the mass spectrometer. Proteins were identified by searching the MS/MS spectra against the *Rattus norvegicus* Swiss-Prot database using ProteinPilot.

Co-immunoprecipitation. HEK293T cells transfected with the indicated plasmids were washed with ice-cold PBS and lysed in nondenatured lysis buffer (BioTeke). Then, 0.5-2 μg of specific antibody was added to the cell lysates and incubated overnight at 4°C. Next, the samples were

supplemented with protein A/G agarose beads (Santa Cruz Biotechnology) and incubated at 4°C for 3 h with mild rotation. Beads were washed four times with PBS and eluted with 2× Laemmli sample buffer followed by SDS-PAGE and immunoblotting. For endogenous immunoprecipitation, the hippocampi of 10 week-old wild-type mice were homogenized in 10 volumes of ice-cold lysis buffer (BioTeke) and centrifuged at 12,000 ×*g* for 15 min at 4°C. The collected supernatants were mixed with 0.5-2 μg specific antibody and incubated overnight at 4°C. Samples were then treated with protein A/G agarose beads, washed, eluted and analyzed as described above.

Immunoblotting. Protein lysates were mixed with 2× Laemmli sample buffer and boiled at 98°C for 5 min. Then, proteins were subjected to electrophoresis in 7.5% - 10% gradient tris-glycine gels and transferred to PVDF membranes (Millipore). The membranes were blocked with 5% nonfat milk diluted in TBS-T and incubated with primary antibodies overnight at 4°C with mild shaking. The samples were then washed with TBS-T and incubated with HRP-conjugated secondary antibodies. Signals were detected in high-sig ECL western blot substrate (Tanon) and quantified using ImageJ.

Statistical analysis. Data analysis was carried out in Excel (Microsoft) and GraphPad Prism (GraphPad Software). Normalization was performed by dividing both the control and experimental conditions by the average value of the control. The paired whole-cell data were analyzed using the two-tailed Wilcoxon signed rank test. LTP data were obtained from pairs of transfected and untransfected (control) neurons; however, some patches were lost during the recording, resulting in a mix of interleaved and paired data; thus, comparisons were made using the Mann-Whitney test. All summarized data were presented as the mean ± standard error of the mean (SEM). Statistical significance values were set as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

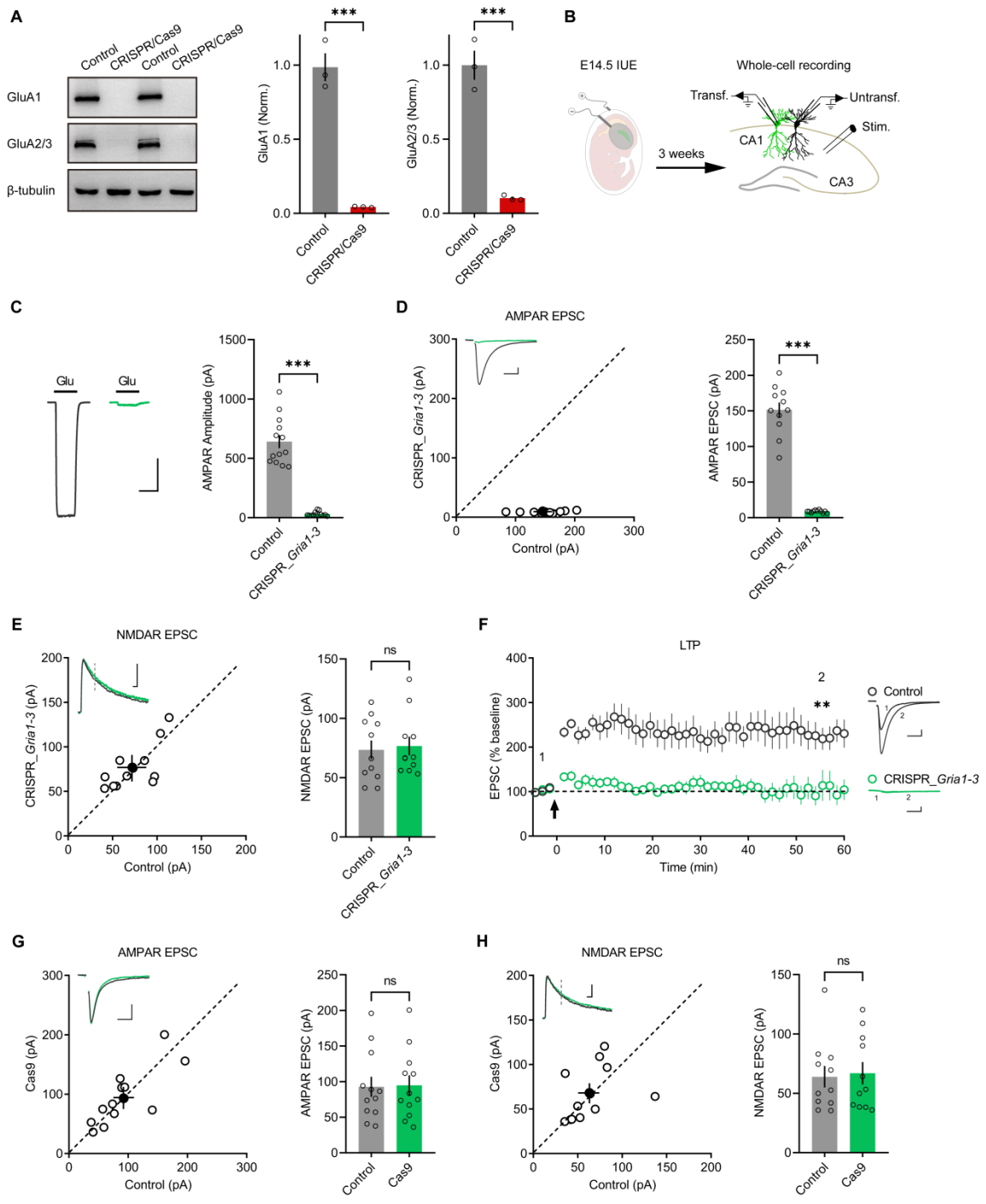

Fig. S1. CRISPR/Cas9 efficiently depletes endogenous AMPAR subunits. (*A*) Western blots for GluA1 and GluA2/3 from primary neuronal cultures infected with lentivirus (n = 3 replicates). (*B*) Schematic of *in utero* electroporation and paired whole-cell recording approach. (*C*) Representative traces of AMPAR-mediated currents from neurons transfected with CRISPR_*Gria1-3* (green) and control (grey) obtained with outside-out patches. Scale bar: 200 pA, 1 s. Summary bar graph shows mean ± SEM amplitude (Control: 641 ± 55.4, n = 13; CRISPR_*Gria1-3*: 31.4 ± 5.64, n = 12; 3 mice; *** $p < 0.001$). (*D* and *E*) Scatterplots show amplitudes of AMPAR-EPSCs (*D*) and NMDAR-EPSCs (*E*) for single pairs (open circles) of

control and CRISPR_*Gria1-3*-transfected cells; filled circle indicates mean amplitude ± SEM. Insets show representative traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean ± SEM of AMPAR-EPSC (*D*) (Control: 151.4 ± 10.35; CRISPR_*Gria1-3*: 8.7 ± 0.53; n = 11 pairs; 3 mice; *** $p < 0.001$) and NMDAR-EPSCs amplitudes (*E*) (Control: 73.4 ± 7.96; CRISPR_*Gria1-3*: 76.7 ± 7.82; n = 11 pairs; 3 mice; $p = 0.4131$; ns: not significant). Scale bars: 25 pA, 25 ms. (*F*) Plots show the mean ± SEM AMPAR-EPSC amplitude of control (grey) and CRISPR_*Gria1-3*-transfected (green) neurons normalized to the mean AMPAR-EPSC amplitude before LTP induction (arrow) (Control: n = 9; CRISPR_*Gria1-3*: n = 8; 3 mice; ** $p < 0.01$ at 55.5 min). Representative AMPAR-EPSC traces from control (grey) and transfected (green) neurons before and after LTP are shown to the right of the graph (scale bars: 25 pA, 25 ms). (*G* and *H*) Scatterplots show amplitudes of AMPAR-EPSCs (*G*) and NMDAR-EPSCs (*H*) for single pairs (open circles) of control and Cas9-transfected cells; filled circle indicates mean amplitude ± SEM. Insets show representative traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean ± SEM of AMPAR-EPSC (*G*) (Control: 92.67 ± 14.16; Cas9: 94.77 ± 14.03; n = 12 pairs; 3 mice; $p = 0.52$; ns: not significant) and NMDAR-EPSCs amplitudes (*H*) (Control: 63.93 ± 9.02; Cas9: 66.95 ± 9.46; n = 11 pairs; 3 mice; $p = 0.8311$; ns: not significant). Scale bars: 25 pA, 25 ms.

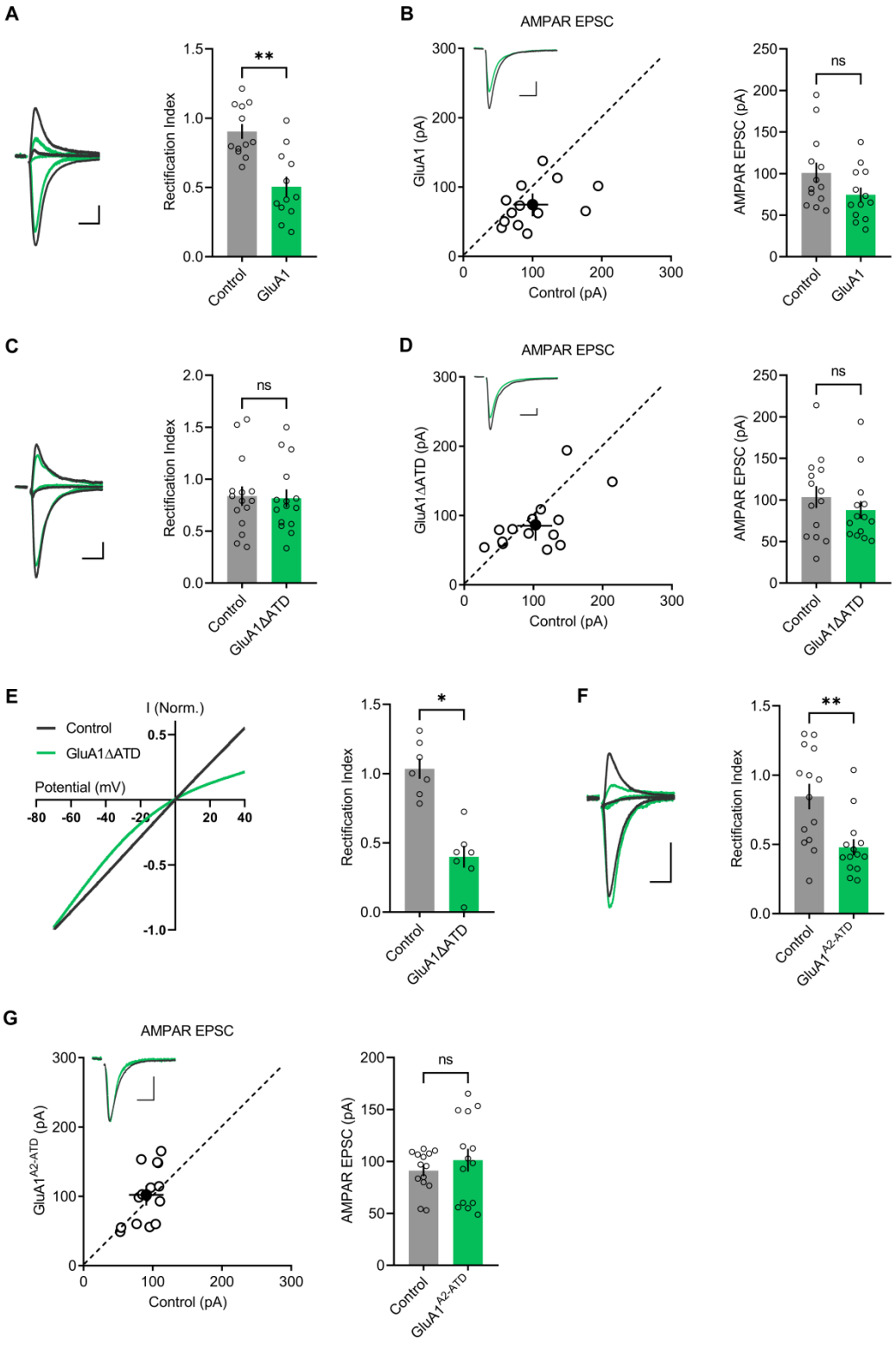

Fig. S2. The ATD is required for the synaptic function of GluA1. (*A*, *C* and *F*) Bar graphs show the mean ± SEM rectification index of control and transfected neurons ((*A*) Control: 0.9 ± 0.05; GluA1: 0.5 ± 0.07; n = 12 pairs; 3 mice; ** $p < 0.01$); (*C*) Control: 0.8 ± 0.09; GluA1ΔATD: 0.8 ± 0.09; n = 15 pairs; 3 mice; $p = 0.804$; ns: not significant); (*F*) Control: 0.8 ± 0.09; GluA1$^{A2\text{-}ATD}$: 0.5

± 0.06; n = 14 pairs; 3 mice; ** $p < 0.01$). Representative traces of control (grey) and transfected (green) neurons are shown on the left. Scale bars: 25 pA, 25 ms. (*B*, *D* and *G*) Scatterplots show the AMPAR-EPSC amplitudes of single pairs (open circles) of control and (*B*) GluA1, (*D*) GluA1ΔATD, or (*G*) $GluA1^{A2\text{-}ATD}$-transfected neurons; the filled circle represents the mean ± SEM amplitude. Insets show sample traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean amplitude ± SEM of AMPAR-EPSC ((*B*) (Control: 101 ± 12.3; GluA1: 74.5 ± 8.67; n = 13 pairs; 3 mice; $p = 0.0681$; ns: not significant); (*D*) Control: 103 ± 13.2; GluA1ΔATD: 87.9 ± 10.7; n = 14 pairs; 3 mice; $p = 0.3258$; ns: not significant); (*G*) (Control: 91.1 ± 5.30; $GluA1^{A2\text{-}ATD}$: 101 ± 11.0; n = 14 pairs; 3 mice; $p = 0.2676$; ns: not significant). Scale bars: 25 pA, 25 ms. (*E*) Normalized I-V curves recorded from out-side out patches of control (grey) and GluA1ΔATD-transfected (green) CA1 pyramid neurons. The bar graph shows evident inward rectification in cells expressing GluA1ΔATD (Control: 1 ± 0.07, n = 7; GluA1ΔATD: 0.4 ± 0.08, n = 7; 2 mice; *** $p < 0.001$).

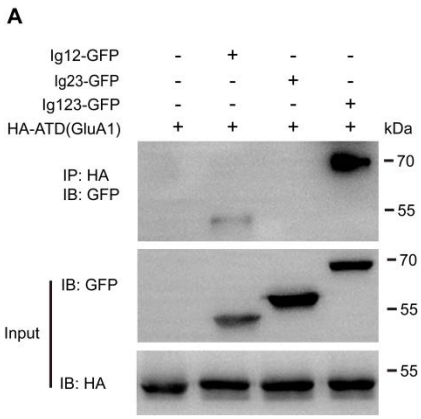

Fig. S3. The extracellular domain of Np65 mediated the interaction with GluA1 ATD. (*A*) Coimmunoprecipitation of HA-tagged GluA1 ATD with GFP-tagged Ig12, Ig23, Ig123 domains of neuroplastins expressed in HEK293T cells (n = 3 replicates).

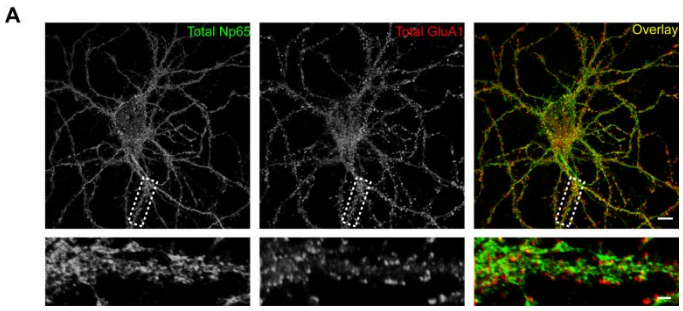

Fig. S4. Expression properties of Np65 and GluA1 in hippocampal neurons. (*A*) Cultured hippocampal neurons (DIV17) derived from wild-type P0 mice were co-stained with Np65 and GluA1 antibodies. Bottom panels are enlarged areas from the top panels as indicated (n = 27 neurons from three independent cultures). Scale bars, 10 μm in top panels and 2 μm in bottom panels.

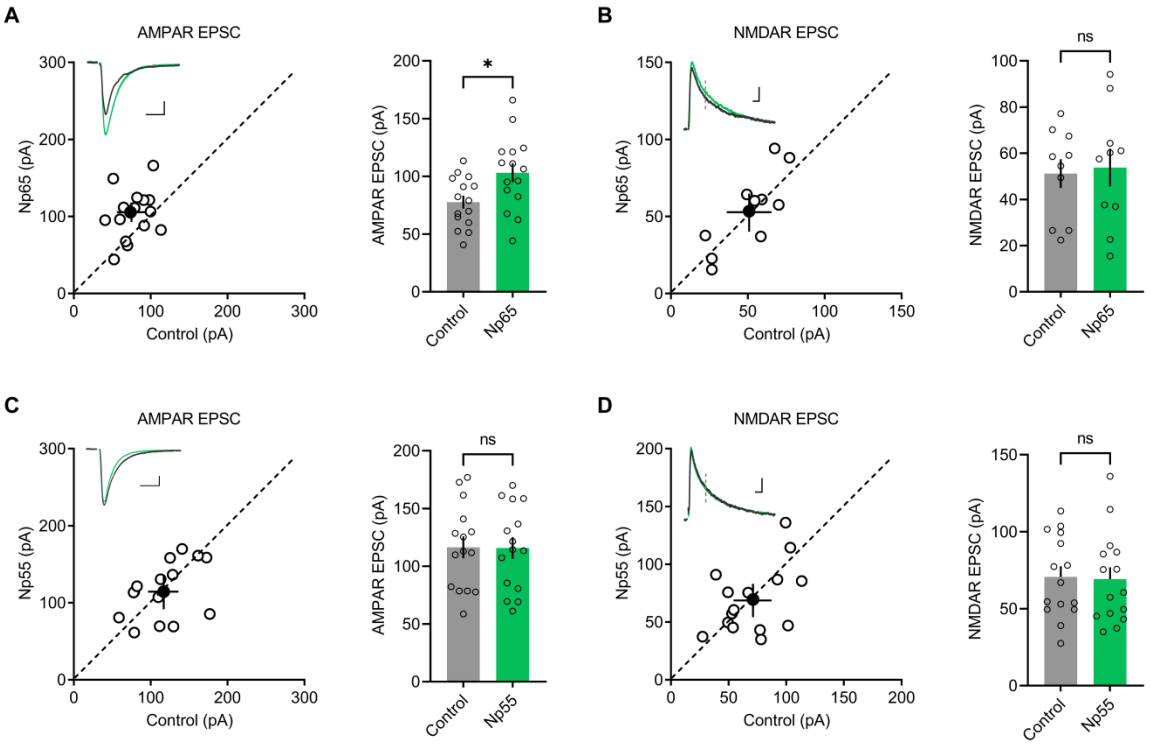

Fig. S5. Overexpression of Np65 increases AMPAR-EPSC amplitude but not NMDAR-EPSC amplitude. (*A* and *C*) Scatterplots show the AMPAR-EPSC amplitudes of single pairs (open circles) of control and (*A*) Np65 and (*C*) Np55-transfected neurons; the filled circle represents the mean ± SEM amplitude. Insets show sample traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean AMPAR-EPSC amplitude ± SEM: ((*A*) Control: 77.7 ± 5.60; Np65: 103 ± 8.31; n = 15 pairs; 3 mice; * $p < 0.05$); (*C*) Control: 116 ± 9.46; Np55: 116 ± 9.47; n = 15 pairs; 3 mice; $p = 0.7615$; ns: not significant). Scale bars: 25 pA, 25 ms. (*B* and *D*) Scatterplots show the NMDAR-EPSC amplitudes of single pairs (open circles) of control and (*B*) Np65 and (*D*) Np55-transfected neurons; the filled circle represents the mean ± SEM amplitude. Insets show sample traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean NMDAR-EPSC amplitude ± SEM: ((*B*) Control: 51.2 ± 6.21; Np65: 53.8 ± 8.17; n = 10 pairs; 3 mice; $p = 0.625$; ns: not significant); (*D*) Control: 70.6 ± 6.87; Np55: 69.3 ± 7.61; n = 15 pairs; 3 mice; $p = 0.9341$; ns: not significant). Scale bars: 25 pA, 25 ms.

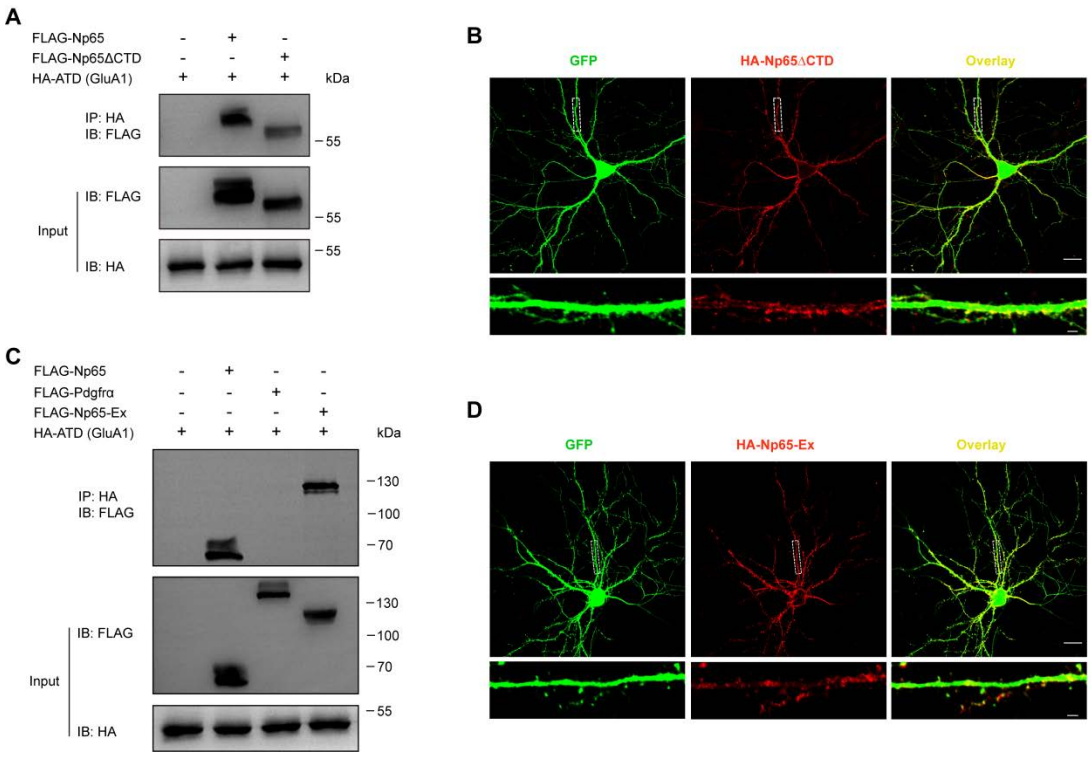

Fig. S6. The Np65 extracellular domain, but not the CTD, is required to interact with GluA1 and for synaptic trafficking. (*A*) Coimmunoprecipitation of HA-tagged GluA1 ATD with FLAG-tagged Np65ΔCTD expressed in HEK293T cells (n = 3 replicates). (*B* and *D*) Cultured hippocampal neurons (DIV17) derived from wild-type P0 mice were cotransfected with GFP and HA-tagged Np65ΔCTD (*B*) or Np65-Ex (*D*) at DIV11. An HA antibody was applied to detect the surface expression of HA-Np65ΔCTD (*B*) or HA-Np65-Ex (*D*). Bottom panels are enlarged areas from the top panels as indicated (n = 30 neurons from three independent cultures). Scale bars, 20 μm in top panels and 2 μm in bottom panels. (*C*) Coimmunoprecipitation of HA-tagged GluA1 ATD with FLAG-tagged Pdgfrα and Np65-Ex expressed in HEK293T cells (n = 3 replicates).

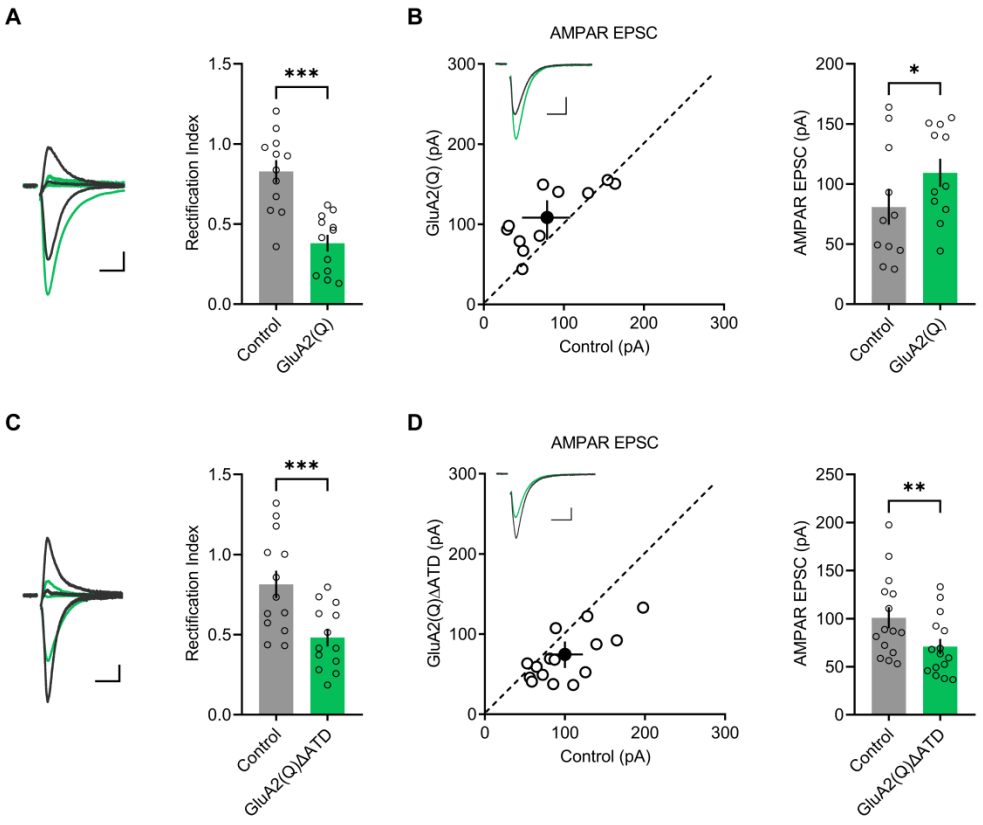

Fig. S7. GluA2 ATD is not required for synaptic targeting. (*A* and *C*) Bar graphs show the mean ± SEM rectification index of control and transfected neurons ((*A*) Control: 0.83 ± 0.07; GluA2(Q): 0.38 ± 0.05; n = 12 pairs; 3 mice; *** $p < 0.001$); (*C*) Control: 0.81 ± 0.09; GluA2(Q)ΔATD: 0.48 ± 0.06; n = 13 pairs; 3 mice; *** $p < 0.001$). Representative traces of control (grey) and transfected (green) neurons are shown on the left of the bar graphs. Scale bars: 25 pA, 25 ms. (*B* and *D*) Scatterplots show the AMPAR-EPSC amplitudes of single pairs (open circles) of control and (*B*) GluA2(Q) or (*D*) GluA2(Q)ΔATD-transfected neurons; the filled circle represents the mean ± SEM amplitude. Insets show sample traces from control (grey) and transfected cells (green). The bar graphs to the right of the scatterplots show the mean amplitude ± SEM of AMPAR-EPSC ((*B*) (Control: 80.75 ± 14.66; GluA2(Q): 109.3 ± 11.75; n = 11 pairs; 3 mice; * $p < 0.05$); (*D*) Control: 100.9 ± 11.02; GluA2(Q)ΔATD: 70.99 ± 7.99; n = 15 pairs; 3 mice; ** $p < 0.01$). Scale bars: 25 pA, 25 ms.

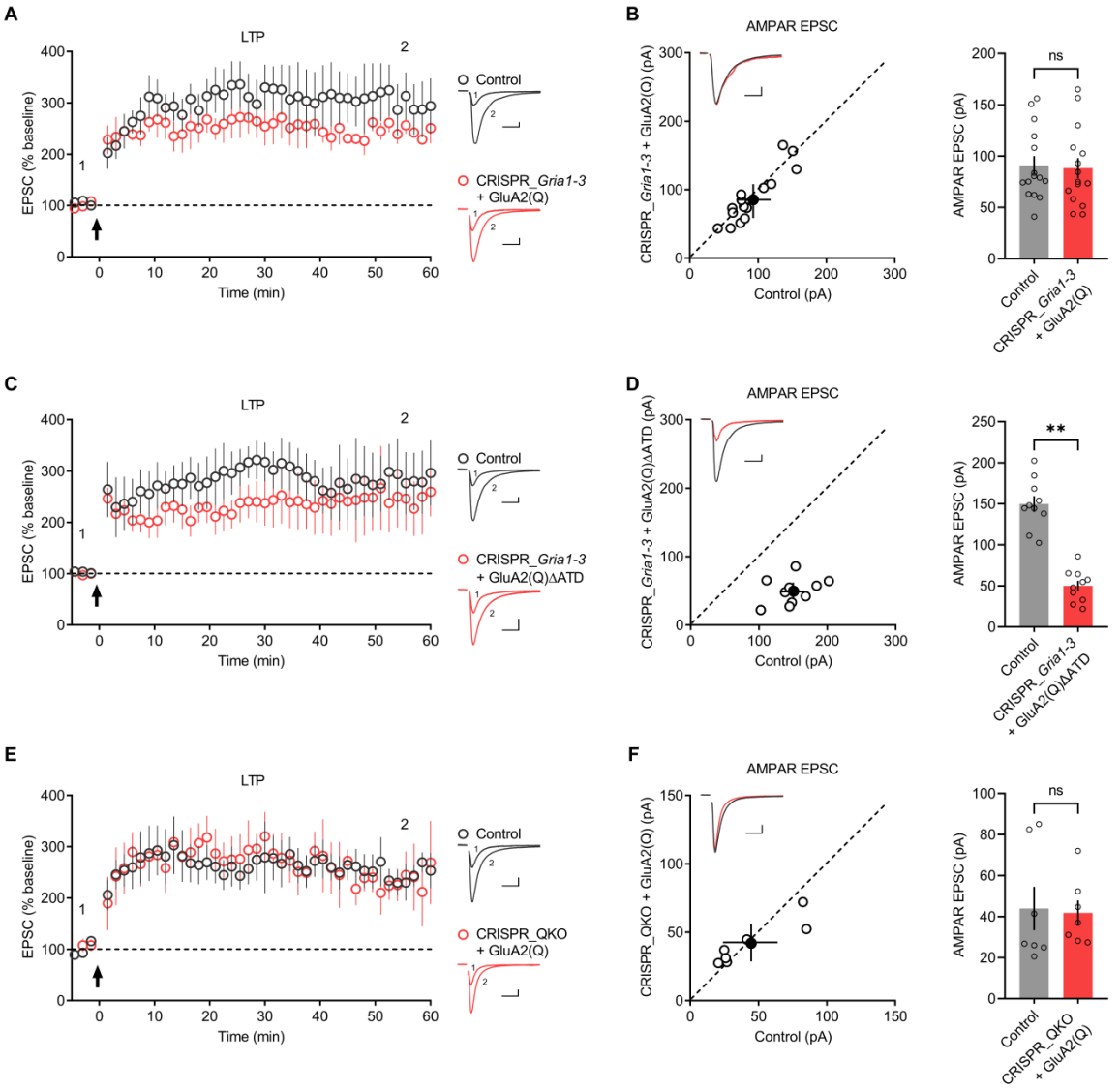

Fig. S8. GluA2-mediated LTP is independent of the ATD and Np65. (*A, C* and *E*) Plots show the mean ± SEM AMPAR-EPSC amplitude normalized to the mean AMPAR-EPSC amplitude before LTP induction (arrow) in control (grey) and transfected (red) neurons (*A*) (Control: n = 8; CRISPR_*Gria1-3* + GluA2(Q): n = 8; 4 mice; p = 0.3095 at 55.5 min); (*C*) (Control: n = 7; CRISPR_*Gria1-3* + GluA2(Q)ΔATD: n = 7; 4 mice; p = 0.6623 at 55.5 min); (*E*) (Control: n = 8; CRISPR_QKO + GluA2(Q): n = 7; 4 mice; p = 0.9048 at 55.5 min). Representative AMPAR-EPSC current traces from control (grey) and transfected (red) neurons before and after LTP are shown to the right of the graphs (scale bars: 25 pA, 25 ms). (*B*, *D* and *F*) Scatterplots show the AMPAR-EPSC amplitudes of single pairs (open circles) of control and transfected neurons; filled circle indicates mean ± SEM amplitude. Insets show sample traces from control (grey) and transfected cells (red). The bar graphs to the right of the scatter plots show mean ± SEM amplitude of AMPAR-EPSC of control and transfected neurons (*B*) (Control: 90.99 ± 9.03; CRISPR_*Gria1-3* + GluA2(Q): 88.24 ± 9.85; n = 15 pairs; 4 mice; p = 0.5245; ns: not significant); (*D*) (Control: 149.8 ± 9.58; CRISPR_*Gria1-3* + GluA2(Q)ΔATD: 49.93 ± 6.21; n = 10 pairs; 3 mice; ** $p < 0.01$); (F) (Control: 43.9 ± 10.6; CRISPR_QKO + GluA2(Q): 41.8 ± 6.09; n = 7 pairs; 3 mice; p = 0.8125; ns: not significant). Scale bars: 25 pA, 25 ms.

Dataset S1 (separate file). Mass spectrometry results of GluA1-ATD-Fc bounded proteomes, related to Figure 2*A*. (Tab 1) Mass spectrometry analysis of GluA1-ATD-Fc bounded proteomes near the bait (about 80-110 kDa) from the immunoprecipitation of GluA1-ATD-Fc from adult rat brain lysates. (Tab 2) Mass spectrometry analysis of GluA1-ATD-Fc bounded proteomes which the bait area was excluded from the immunoprecipitation of GluA1-ATD-Fc from adult rat brain lysates. (Tab 3) Mass spectrometry analysis of Fc bounded proteomes near the bait (about 40-60 kDa) from the immunoprecipitation of Fc from adult rat brain lysates. (Tab 4) Mass spectrometry analysis of Fc bounded proteomes which the bait area was excluded from the immunoprecipitation of Fc from adult rat brain lysates.

SI References

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