Supporting Information

Ancona Esselmann et al. 10.1073/pnas.1716022114

SI Methods

Experimental Constructs. All AMPAR constructs and Cre:mCherry were cloned into the pFUGW expression plasmid by PCR and the In-Fusion HD Cloning System (Invitrogen). pFUGW-GluA2 constructs were coexpressed with GFP behind an internal ribosomal entry site. Knockdown constructs targeting GluA2 (shGluA2; target sequence: 5′ GGAGCACTCCTTAGCTTGA 3′) and GluA1 (shGluA1; target sequence: 5′ GGAATCCGAAAGATTGGT 3′) were expressed from an H1 promoter in pFHUGW along with mCherry expressed from the ubiquitin promoter to mark the transfected cells. AMPAR subunit truncations, chimeras, and mutation constructs were generated by overlap extension PCR. GluA2ΔCTD ended in amino acid 838, with the last four amino acids being FCYK. GluA2Δ847 ended with the sequence KRMK. GluA2 A841S contained the proximal C-tail sequence EFCYKSR-**S**EAKRMK, GluA2 A843S contained the proximal C-tail sequence EFCYKSRAE**S**KRMK, and GluA2 A841S, A843S contained the proximal C-tail sequence EFCYKSR**S**E**S**KRMK. Chimeric AMPAR constructs were made by swapping C-tail sequences of GluA1 and GluA2.

Neuronal Transfection. In brief, 50–100 μg total of mixed plasmid DNA was coated on 1-μm-diameter gold particles in 0.5 mM spermidine, precipitated with 0.1 mM $CaCl_2$, and then washed four times in pure ethanol. The gold particles were coated onto PVC tubing, dried using ultra-pure N_2 gas, and stored at 4 °C in desiccant. DNA-coated bullets were shot with a Helios Gene Gun (Bio-Rad). Cre expression was confirmed by mCherry epifluorescence, and replacement AMPAR subunits were confirmed by GFP epifluorescence.

For in utero electroporation, ∼E15.5 pregnant *GRIA1–3*$^{fl/fl}$ mice were anesthetized with 2.5% isoflurane in O_2 and injected with buprenorphine for analgesia. Embryos within the uterus were temporarily removed from the abdomen and injected with 2 μL of mixed plasmid DNA into the left ventricle via a beveled micropipette. Each embryo was electroporated with five 50-ms, 35-V pulses. The positive electrode was placed in the lower right hemisphere, and the negative electrode was placed in the upper left hemisphere. Electroporation has been described in more detail previously (33). Following electroporation, the embryos were sutured into the abdomen, and killed on postnatal day 6–9 for organotypic slice cultures.

Electrophysiology in slice cultures. Cultures were either untreated or chronically incubated for 48 h with 1 μM TTX, and recordings were made after TTX was washed off in the recording bath with artificial cerebrospinal fluid (ACSF) for >5 min unless indicated otherwise. For asynchronous EPSC recordings, a bipolar stimulation electrode (FHC) was placed in the stratum radiatum, and stimulation was increased from 0.2 Hz to 2 Hz to increase the frequency of Sr^{2+}-evoked responses (34). Sr^{2+}-evoked aEPSCs were analyzed off-line with custom software (IGOR Pro), with at least 100 quantal events used in all cases. (Scale bars for aEPSC sample traces: 5 pA and 20 ms unless indicated otherwise.) Responses were collected with a Multiclamp 700A amplifier (Axon Instruments), filtered at 2 kHz, and digitized at 10 kHz.

To examine AMPAR rectification, 0.1 mM D-AP5 was washed in to block NMDA receptors. Rectification was calculated as the ratio of the slopes of the lines connecting AMPA EPSC amplitude from 0 to +40 mV and from −70 mV to 0 mV. This calculation can be expressed as follows: $RI = 7(I_{40} - I_0)/4(I_0 - I_{70})$ where I_x represents EPSC amplitude at x mV.

Outside-out patches. Outside-out patches were taken from CA1 cells by obtaining whole-cell access to CA1 pyramidal neurons at −70 mV with a 4- to 5-MΩ patch pipette, then slowly pulling the pipette away from the soma until a high-resistance seal reformed. Hepes-ACSF containing 150 mM NaCl, 2.5 mM KCl, 10 mM Hepes, 10 mM glucose, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM D-AP5, 0.1 mM picrotoxin, 0.1 mM cyclothiazide, and 0.5 μM TTX was then perfused over the tip of the pipette. Glutamate currents were evoked by perfusing Hepes-ACSF containing 1 mM L-glutamic acid. A ValveLink 8 (AutoMate Scientific) was used for fast perfusion of control and glutamate containing Hepes-ACSF. Voltage ramps from −70 mV to +40 mV were generated in the presence and absence of glutamate. The glutamate-activated current was obtained by subtraction. Rectification was calculated as in synaptic experiments.

Immunoblotting. HEK293T cells were maintained and transfected as described previously (35). Cells were washed in PBS, resuspended directly in SDS/PAGE sample buffer, and subjected to Western blot analysis. Membranes were blocked with blotting grade buffer (Bio-Rad, catalog no. 170–6404). Antibodies used in the study were GluA1 (Rb; Synaptic Systems; catalog no. 182-103) and actin (ABM; catalog no. G043).

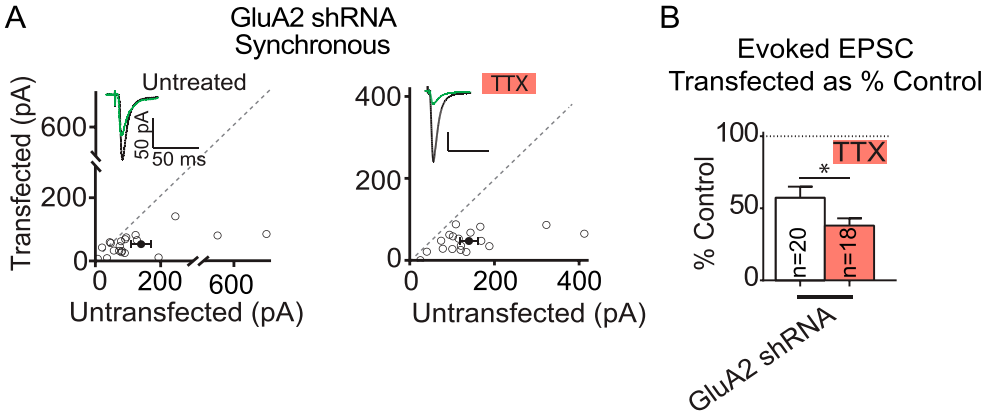

Fig. S1. (*A*) Paired synchronous recordings without and with preceding chronic TTX treatment in control neurons and neighboring neurons transfected with GluA2 shRNA. (*B*) Summary graph of percent control (data from *A*). Amplitude deficit in EPSCs of GluA2-lacking neurons was exacerbated, not preserved, suggesting an inability to scale up. $*P < 0.05$.

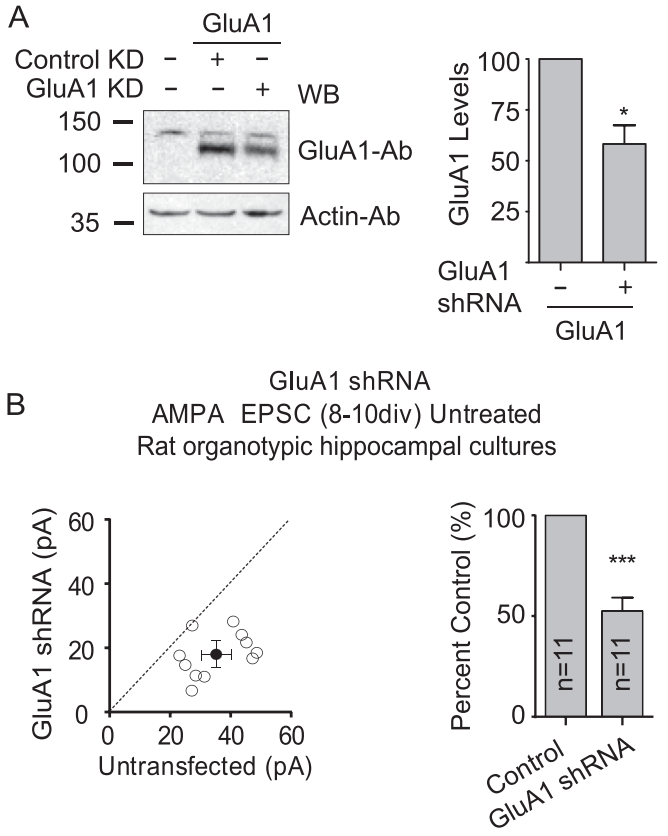

Fig. S2. (*A*) Immunoblot analysis of GluA1 transfected in HEK293T cells. Total GluA1 lysate levels (mean ± SEM) normalized to control ($n = 3$). Immunoblots were probed with indicated antibodies. Ab, antibody; WB, Western blot. (*B*) Paired EPSC recordings of GluA1 shRNA-transfected and neighboring untransfected neurons confirms the expected EPSC phenotype for pyramidal neurons in the hippocampus completely lacking the GluA1 subunit. The remaining EPSC is from GluA2/3. $*P < 0.05$; $***P < 0.001$.

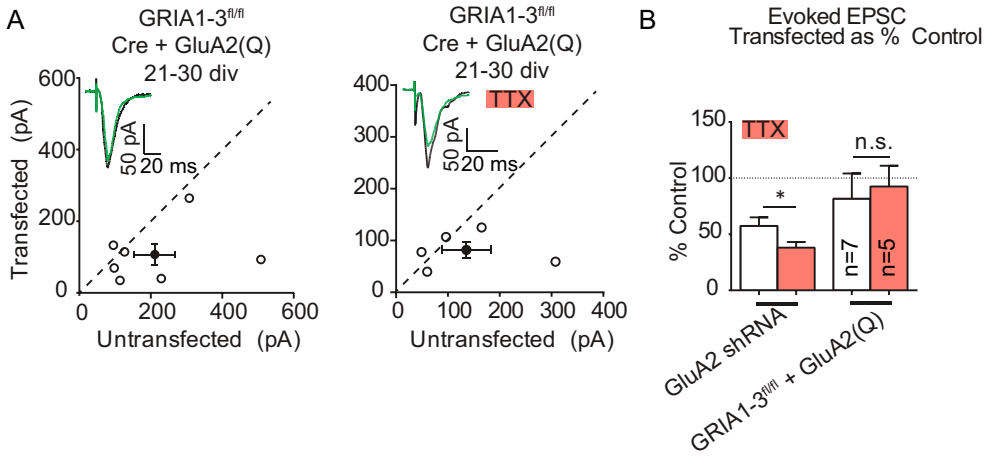

Fig. S3. (*A*) Similar to Fig. S1. Paired synchronous recordings without and with preceding chronic TTX treatment in control *GRIA1–3*$^{fl/fl}$ neurons and neighboring *GRIA1–3*$^{fl/fl}$ neurons transfected with Cre + GluA2(Q) cDNA. In control conditions, GluA2(Q) rescues 70% of the AMPAR EPSCs. If GluA2(Q) receptors are unable to scale in TTX, we would expect the rescue to be <70%, because the control cells would scale, increasing the difference between the two cells. We found that the relative EPSC size was preserved when these experiments were repeated with TTX. (*B*) Summary graph of percent control (data from *A*). Amplitude deficit in EPSCs of GluA2-replacement neurons was preserved by TTX, suggesting a preservation of scaling up. GluA2 shRNA % control data are included for reference. *$P < 0.05$; n.s., not significant.

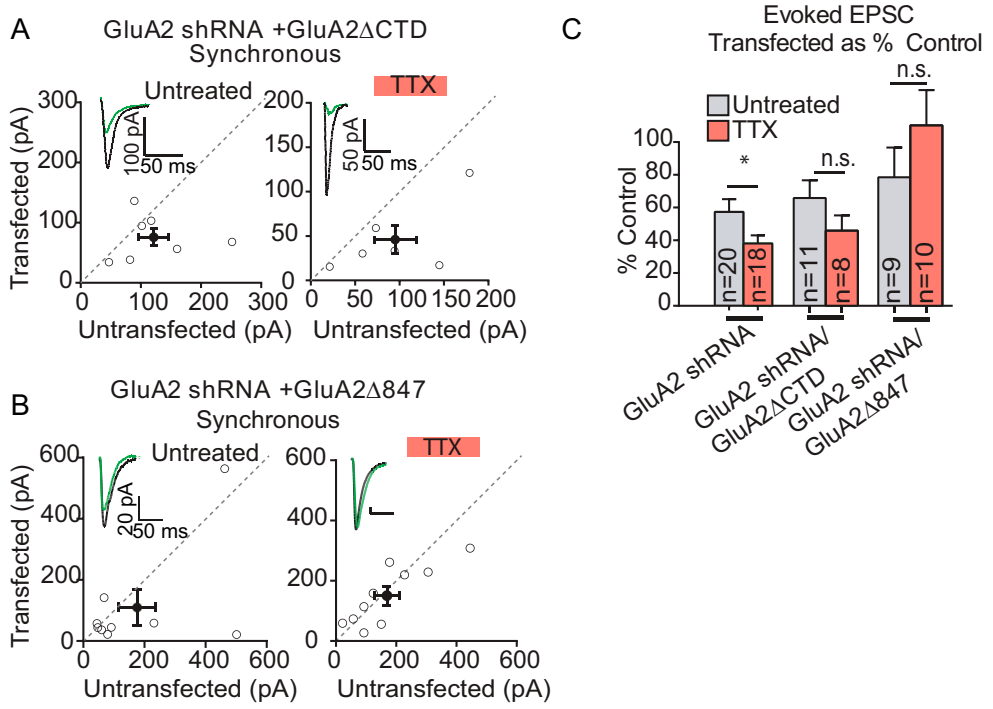

Fig. S4. (*A*) Similar to Figs. S1*A* and S3*A*. Paired synchronous recordings without and with preceding chronic TTX treatment in control neurons and neighboring neurons transfected with the GluA2 shRNA and GluA2ΔCTD insensitive to the shRNA. (*B*) Paired synchronous recordings without and with preceding chronic TTX treatment in control neurons and neighboring neurons transfected with the GluA2 shRNA and GluA2Δ847 insensitive to the shRNA. (*C*) Summary graph of % control (data from *A* and *B*). GluA2 shRNA % control data are included for reference. *$P < 0.05$; n.s., not significant.

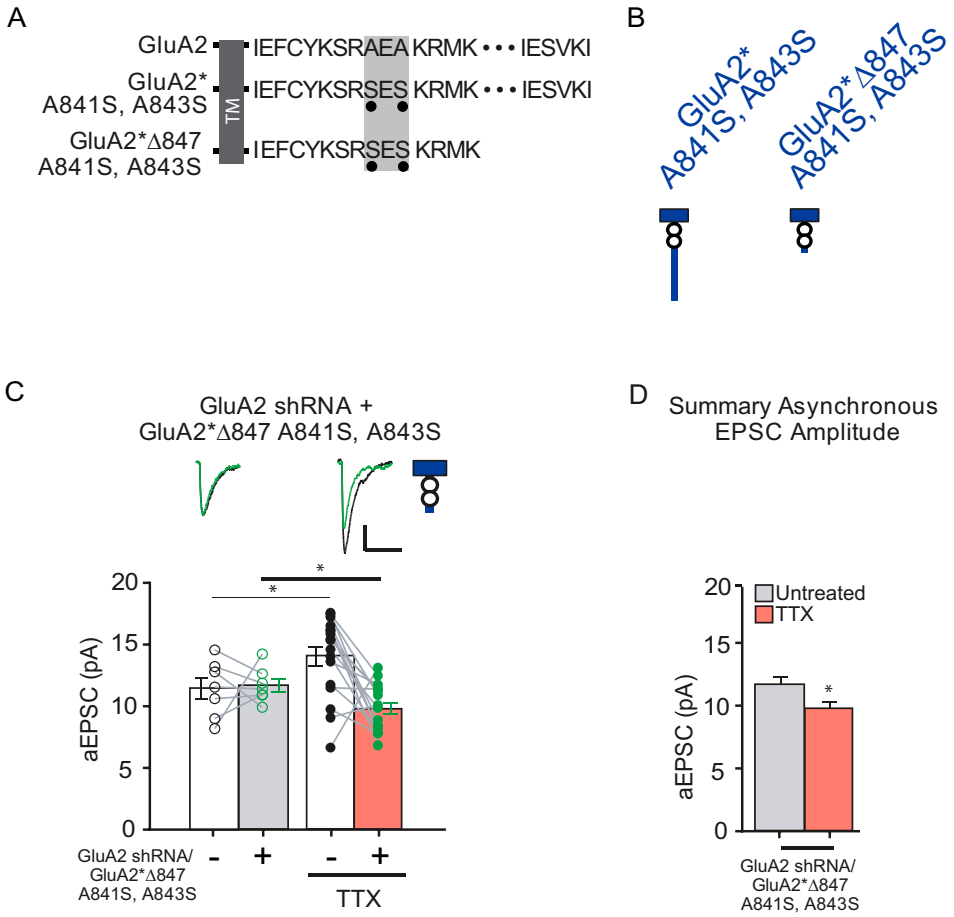

Fig. S5. GluA2*Δ847 A841S, A843S does not rescue scaling-up. (*A*) Endogenous GluA2 C-tail amino acid sequence as well as full length GluA2 C-tail with point mutations (GluA2*A841S, A843S) and truncated GluA2 C-tail with the same point mutations (GluA2*Δ847 A841S, A843S). (*B*) Schematic diagram of GluA2*A841S, A843S, and GluA2*Δ847 A841S, A843S AMPAR subunits. Boxes indicate amino terminal domains (ATDs) and transmembrane (TM) regions of AMPARs, while vertical lines indicate intracellular C-tails. (*C*) Paired asynchronous recordings without and with preceding chronic TTX treatment in control neurons and neighboring neurons transfected with GluA2 shRNA and shRNA-insensitive AMPAR subunit called "GluA2*Δ847 A841S, A843S." (*D*) Summary bar graph indicating unpaired scaling data, within the same transfection conditions. Significance measured across treatment conditions. All scale bars for aEPSC sample traces represent 5 pA and 20 ms unless otherwise noted. *$P < 0.05$.