Supporting Information

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SI Materials and Methods

Electrophysiology. ***Slice preparation.*** Acute coronal brain slices (350 μm) were prepared from postnatal day 13 (P13) to P16 C57/Bl6 WT or $\beta 2m^{-/-}TAP^{-/-}$ mice. Animals were anesthetized with halothane or isoflurane inhalation and decapitated, and brains were quickly removed. Slices were cut with a Vibratome into ice-cold cutting solution (in mM: 85 NaCl, 75 sucrose, 2.5 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, and 2.25 ascorbate) equilibrated with 95% O_2/5% CO_2. After cutting, slices were incubated 30 min at 30 °C in oxygenated artificial cerebrospinal fluid (ACSF) (in mM: for whole-cell recording experiments, 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 18 glucose; for field recording experiments, 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1.3$MgSO_4$, 2.5$CaCl_2$, 26 $NaHCO_3$, and 10 glucose) and maintained at room temperature at least 1 h before being transferred to a submerged recording chamber.

Whole-cell recordings. Visualized whole-cell patch-clamp recordings of evoked excitatory postsynaptic currents (EPSCs) from individual CA1 pyramidal neurons were conducted at room temperature (~25 °C) by using standard methods. Picrotoxin (50 μM) was added to the ACSF to block $GABA_A$ receptor-mediated inhibition, and connections between CA3 and CA1 were cut to reduce epileptiform activity. Patch electrodes (3–5 MΩ) were filled with intrapipette solution [in mM: 108 cesium gluconate, 20 Hepes, 0.4 EGTA, 2.8 NaCl, 5 TEACl, 4 MgATP, 0.3 NaGTP, and 10 phosphocreatine, adjusted to pH 7.2 with CsOH (290 mosM)]. CA1 pyramidal cells were voltage-clamped at −70 mV, and EPSCs were evoked by stimulating Schaffer collateral presynaptic fibers at 0.033 Hz with stainless-steel monopolar electrodes. A −5-mV, 300-ms hyperpolarizing voltage step was delivered 200 ms before each stimulus pulse to monitor the series and input resistance throughout the experiment, and recordings were terminated if the series or input resistance changed >30%. After break-in, cells were held at −70 mV for a 10-min baseline. The AMPA/NMDA ratio was determined by holding the cell at −80 mV and +40 mV to detect AMPAR-mediated EPSCs and mixed AMPAR + NMDAR-mediated EPSCs, respectively. AMPA EPSC amplitudes for each recording were determined by averaging the peak amplitude of 12 EPSCs recorded at −80 mV. NMDA EPSC amplitudes for each recording were determined by averaging 12 EPSCs recorded at +40 mV. For NMDA EPSCs, the amplitude was measured 100 ms after the stimulation artifact, a time when the 6,7-dinitroquinoxaline-2,3-dione (DNQX)-sensitive (10 μM) AMPA component is attenuated and the remaining current is completely blocked by the NMDAR blocker D-(−)-2-Amino-5-phosphonopentanoic acid (D-APV) (50 μM) (Fig. S1; ref. 1).

Coefficient of variation (CV). Presynaptic CA3/Schaffer collateral fibers were stimulated at 0.2 Hz, and epochs of 100 consecutive EPSCs were collected at −80 mV and at +40 mV. The stimulation intensity was set so that the peak amplitude of the EPSCs was between 50 and 100 pA when recorded at −80 mV. For each epoch, the variance of the EPSC peak amplitude was measured, and the CV was calculated as the variance divided by the mean EPSC amplitude for each epoch.

NR2B. To isolate NMDAR currents mediated by non-NR2B-containing NMDARs in P14 acute slices, AMPAR-mediated currents were blocked with 10 μM DNQX, and the holding potential was switched to +40 mV to relieve the Mg^{2+} block of NMDARs. After recording the total NMDAR-mediated current, 3 μM ifenprodil was bath-applied for 40 min to block NR2B-containing NMDARs. NMDA EPSC amplitudes for each recording were determined by averaging 12 EPSCs recorded at +40 mV in DNQX before and 30 min after introduction of ifenprodil. The percentage inhibition by ifenprodil was calculated by normalizing the mean NMDA EPSC peak amplitude 30 min after ifenprodil to the NMDA EPSC peak amplitude before ifenprodil.

Field recordings. Field recordings from populations of CA1 pyramidal cells were conducted at room temperature by using standard methods. Picrotoxin (100 μM) was added to the ACSF to block $GABA_A$ receptor-mediated inhibition and connections between CA3 and CA1 were cut to reduce epileptiform activity. Stainless-steel bipolar electrodes were used to stimulate Schaffer collateral fibers (0.05 Hz), and field excitatory postsynaptic potentials (fEPSPs) were recorded from stratum radiatum by using extracellular glass microelectrodes (3–5 MΩ) filled with ACSF. Input–output (I/O) relationships were determined by measuring the peak fiber volley (input) and fEPSP (output) amplitudes after isolating AMPAR- or NMDAR-mediated synaptic transmission in the presence of D-APV (50 μM) or 0 Mg^{2+} and DNQX (10 μM), respectively.

For both whole-cell and field recordings, signals were measured with a Multiclamp 700B amplifier and digitized with a Digidata 1322A (both from Axon Instruments). Traces were digitized at 10 or 20 kHz, low-pass filtered at 2 kHz with Clampex 9.2 and analyzed offline with Clampfit 9.2. Picrotoxin, DNQX, ifenprodil, and D-(−)-2-amino-5-phosphonopentanoic acid (D-APV) were obtained from Tocris; all of the other chemicals were from Sigma.

Hippocampal cultures. Pups were decapitated, and brains were quickly removed and placed in ice-cold dissecting solution composed of HBSS supplemented with sodium bicarbonate (4 mM), Hepes (10 mM), and a mix of penicillin and streptomycin. Hippocampi were dissected out and dissociated, first enzymatically, with 0.25% trypsin (for 15 min at 37 °C), and then mechanically, by gentle trituration with fire-polished Pasteur pipettes of decreasing diameters. After counting, neurons were plated at a density of ~12,000 cells/cm^2 onto glass cover slips precoated with poly-L-lysine (1 mg/mL) in plating medium consisting of MEM supplemented with horse serum (10%), glucose (0.6%), GlutaMAX (2 mM), and sodium pyruvate (1 mM). After allowing the cells to adhere for 3 h, the plating medium was replaced by a defined growing media consisting of Neurobasal A media supplemented with B27 and GlutaMAX (2 mM). Seven days after plating, arabinofuranosylcytosine (Ara-C; 5 μM) was added to limit proliferation of nonneuronal cells.

Glutamate receptor immunocytochemistry. Surface labeling. Mature hippocampal neurons [16–20 d in vitro (DIV)] were live labeled for 15 min at 37 °C with 10 μg/mL anti-GluR1, 10 μg/mL anti-GluR2, 10 μg/mL anti-NR1, or 20 μg/mL anti-NR2B diluted in conditioned media. After two washes in ice-cold PBS, neurons were processed for antibody detection under nonpermeabilizing conditions. Neurons were fixed 20 min at room temperature in 4% paraformaldehyde (PFA) and 4% sucrose in PBS, and excess PFA was quenched 10 min at room temperature in PBS/100 mM glycine. After a 45-min incubation in blocking buffer (PBS supplemented with 0.2% BSA) at 37 °C, primary antibodies were detected by a 45-min incubation at room temperature with appropriate fluorophore-conjugated secondary antibodies (4 μg/mL of Alexa Fluor 488 or 568 anti-rabbit or anti-mouse IgG) diluted in blocking buffer, followed by four washes in blocking buffer and one final wash in PBS. In pilot experiments, a 3-min incubation in ice-cold acidic media (200 mM acetic acid, 500 mM

NaCl, pH 2.8) was included before fixation to selectively remove cell-surface but not internalized antibodies. The complete loss of labeling after acid treatment confirmed that surface-expressed receptors were selectively labeled by using this method (Fig. S2). When specified, a 3-min preincubation with 40 μM NMDA diluted in conditioned media was included before live labeling.

Double-label immunostaining. Hippocampal neurons were double-labeled with anti-NR1 (rabbit anti-NR1, Millipore) and anti–PSD-95 (mouse anti–PSD-95 clone K28/43, NeuroMab), or anti-SV2 (mouse anti-SV2 clone SP2/0, Developmental Studies Hybridoma Bank). In brief, mature hippocampal neurons (16–20 DIV) were processed for antibody detection under permeabilizing conditions following the same protocol as described above with the addition of 0.05% saponin to the blocking buffer.

Image Quantification. ***Surface labeling.*** For each image, the background was determined by measuring the mean fluorescence intensity in a portion of the image containing no cells or neurites. Threshold for positive staining in each set of concurrent experiments was set as a two-pixel or larger area that was a fixed factor above background. For each neuron, three proximal dendrites were digitally straightened, and the mean intensity of positive labeling and number of positive objects were measured in a fixed-area region of interest in each dendrite by using National Institutes of Health ImageJ software.

Double-label immunostaining. Degree of colocalization in confocal images was analyzed in single 0.20-μM-thick optical sections by using National Institutes of Health ImageJ software. Images were thresholded by using constant settings within experiment as described above, and the number of NR1-positive puncta colocalizing with PSD-95 or SV2 labeling was measured. Briefly, the degree of colocalization between NR1 and either PSD-95 or SV2 was estimated for each pair of images by creating a mask of NR1-positive puncta that was applied to the corresponding PSD-95 or SV2 image. The degree of colocalization between NR1 and the synaptic markers PSD-95 or SV2 was calculated by dividing the number of synaptic marker puncta that overlapped with the NR1 mask over the total number of puncta positive for that marker that were present in the image.

Subcellular Fractionation. ***Synaptic fractions.*** Briefly, animals were anesthetized with halothane or isoflurane inhalation and decapitated, brains were quickly removed, and hippocampi were dissected out and homogenized in 10 vol of Hepes sucrose buffer (0.32 M sucrose and 4 mM Hepes, pH 7.4) supplemented with protease inhibitor mixture (Complete, Roche) using a glass-Teflon homogenizer at 900 rpm (12 strokes). The homogenate was centrifuged for 10 min at 1,000 × *g*, and the pellet (P1) containing the nuclei was discarded. The supernatant (S1) was centrifuged for 20 min at 10,000 × *g*, resulting in a crude synaptosomal pellet (P2). P2 was resuspended in 10 vol of Hepes sucrose buffer and centrifuged for 10 min at 20,000 × *g*, resulting in a washed crude synaptosomal pellet (P2′). P2′ was lysed by hypo-osmotic shock in water and adjusted to 4 mM Hepes. After 30 min of gentle rotation at 4 °C, P2′ was centrifuged for 20 min at 25,000 × *g*, resulting in a crude synaptosomal membrane fraction (P3) and a crude synaptic vesicle fraction (S3). Aliquots were collected at multiple steps of the fractionation and solubilized in Triton X-100 (1%) for Western blot analysis.

PSD and synaptosomal fractions: PSD-enriched fractions were prepared as previously described (2). Briefly, the forebrains of young mice (P18) were dissected and homogenized in ice-cold homogenization buffer [4 mM Hepes-NaOH (pH 7.4), 0.32 M sucrose, protease inhibitor mixture, and phosphatase inhibitors (Complete, Roche)]. Supernatant (S1) was collected after centrifugation of homogenates (1,000 × *g* for 10 min at 4 °C). S1 was diluted with an equal volume of 10% Percoll (GE Healthcare Bio-Sciences) and then laid on top of a 10–20% discontinuous Percoll gradient. After centrifugation (33,000 × *g* for 5 min at 4 °C), the interface layer between 10% and 20% Percoll was collected and diluted with PSD buffer [40 mM Hepes-NaOH (pH 8.1), protease inhibitor mixture, and phosphatase inhibitors]. After centrifugation at 20,000 × *g* for 20 min at 4 °C, the pellet [synaptosomal fraction (SS)] was resuspended in PSD buffer (200 μl was saved for analysis of synaptosomal fractions). Next, 0.5% Triton X-100 was added to SS lysates, stirred for 15 min at 4 °C, and centrifuged for 40 min (40,000 × *g* at 4 °C). The pellet (PSD) was resuspended in 100 μl of PSD buffer and stored at −80 °C. Protein concentrations were determined by using a BCA protein assay kit (Thermo Scientific). SS or PSD fractions (30 mg of either) were analyzed by Western blot.

Surface Biotinylation. Hippocampal neurons in culture (16 DIV; WT or $\beta 2m^{-/-}TAP^{-/-}$) were rinsed three times in progressively cooler ACSF (in mM: 145 NaCl, 10 Hepes, 2.5 KCl, 10 glucose, 1 $MgCl_2$, and 2 $CaCl_2$) and then incubated for 20 min at 4 °C with 1 mg/mL Sulfo-NHS-LC-Biotin (Thermo Scientific) diluted in ACSF. To quench unreacted biotin, neurons were rinsed four times in ACSF containing 100 mM glycine. Subsequently, neurons were lysed in modified RIPA buffer [150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris (pH 7.5), and 1mM EDTA] supplemented with protease inhibitor mixture (Complete, Roche) for 20 min at 4 °C. Insoluble material was removed by centrifugating the homogenate at 8,000 × *g* for 10 min, and protein quantification was performed on the supernatant (total lysate) with a BCA protein assay kit (Pierce) according to manufacturer's instructions. Total lysates (250 μg) was incubated with NeutrAvidin agarose beads (Thermo Scientific) for 3 h at 4 °C. Beads were washed five times in modified RIPA buffer, and bound biotinylated proteins were eluted in 50 μL of sample buffer containing DTT at 85 °C. When specified, neurons were incubated at 37 °C in conditioned media containing 40 μM NMDA for 3 min and allowed to recover for 15 min at 37 °C in conditioned media before being processed for surface biotinylation.

1. Clark KA, Collingridge GL (1995) Synaptic potentiation of dual-component excitatory postsynaptic currents in the rat hippocampus. *J Physiol* 482:39–52.

2. Zhou Y, et al. (2007) Interactions between the NR2B receptor and CaMKII modulate synaptic plasticity and spatial learning. *J Neurosci* 27:13843–13853.

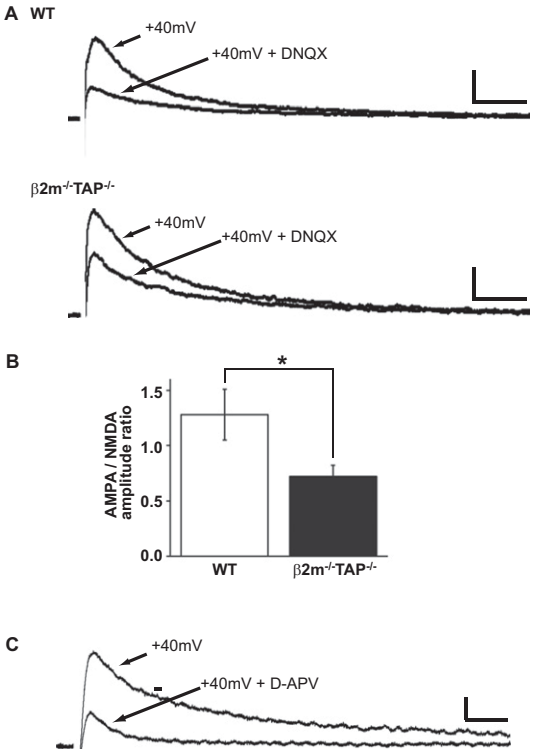

Fig. S1. Pharmacologically isolated AMPA/NMDA ratio is decreased in $\beta 2m^{-/-}TAP^{-/-}$ CA1 pyramidal neurons. (*A*) Representative EPSCs recorded from individual WT (*Upper*) or $\beta 2m^{-/-}TAP^{-/-}$ (*Lower*) CA1 pyramidal neurons voltage-clamped at +40 mV in presence or absence of 10 mM DNQX. (Scale bar: 50 pA/100 ms.) (*B*) Mean AMPA/NMDA ratio calculated from recordings in which NMDAR-mediated currents were isolated with DNQX as in *A* above. The AMPA/NMDA ratio is significantly decreased in $\beta 2m^{-/-}TAP^{-/-}$ neurons (WT 1.3 $\pm$ 0.2, n = 7 cells; $\beta 2m^{-/-}TAP^{-/-}$ 0.7 $\pm$ 0.1, n = 7 cells; $^*P < 0.05$, two-tailed unpaired t test). (*C*) Representative EPSCs recorded from an individual WT CA1 pyramidal neuron voltage-clamped at +40 mV or +40 mV in presence of D-APV. NMDAR-mediated currents were measured at the time marked with horizontal bar. (Scale bar: 20 pA/50 ms.)

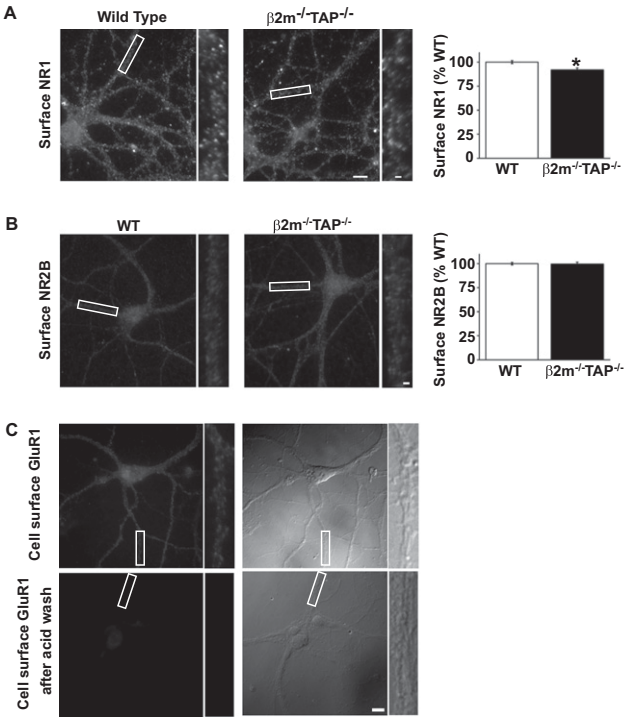

Fig. S2. Cell-surface levels of NR1 and NR2B are not increased in $\beta 2m^{-/-}TAP^{-/-}$ hippocampal neurons. (*A Left*) Representative cell-surface NR1 immunostaining in WT and $\beta 2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture. (Scale bar: 10 μm; high magnification: 1 μm.) (*Right*) Quantification of dendritic cell-surface expression of NR1, averaged over four separate experiments. Surface NR1 levels are decreased in $\beta 2m^{-/-}TAP^{-/-}$ neurons (WT 100.0 ± 1.4%, n = 54 cells; $\beta 2m^{-/-}TAP^{-/-}$ 91.9 ± 2.0%, n = 50 cells; $^*P < 0.01$, two-tailed unpaired t test). (*B Left*) Representative labeling of endogenous cell-surface NR2B in WT and $\beta 2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture. (Scale bar: 10 μm; high magnification: 1 μm.) (*Right*) Quantification of dendritic cell-surface expression of NR2B averaged over four separate experiments (WT, n = 50 cells; $\beta 2m^{-/-}TAP^{-/-}$, n = 52 cells). (*C*) Representative cell-surface GluR1 immunostaining and corresponding phase contrast in WT hippocampal neurons in culture before and after a 3-min acid wash. (Scale bar: 10 μm; high magnification: 1 μm.)

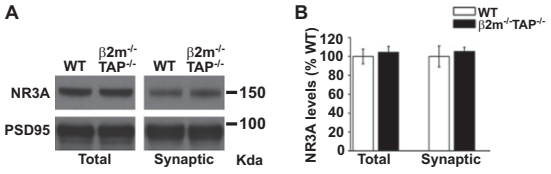

Fig. S3. Total and synaptic NR3A levels are normal in $\beta 2m^{-/-}TAP^{-/-}$ hippocampi. (*A*) Representative Western blot of total (S1) and synaptic plasma membrane–enriched (P3) fractions from WT and $\beta 2m^{-/-}TAP^{-/-}$ mouse hippocampi probed for NR3A and PSD-95. (*B*) Total and synaptic levels of NR3A in samples from four WT and four $\beta 2m^{-/-}TAP^{-/-}$ animals, normalized to PSD-95 and represented as percentage of WT.

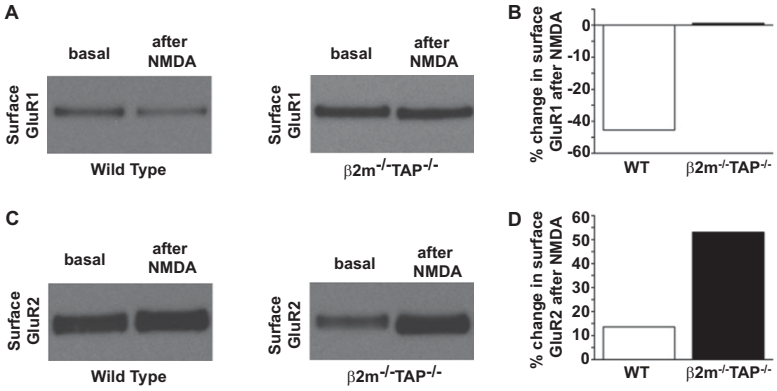

Fig. S4. NMDA application increases cell-surface GluR1 and GluR2 in $\beta 2m^{-/-}TAP^{-/-}$ neurons. (*A*) Western blots of biotinylated cell-surface GluR1 at rest or after NMDA treatment in WT and $\beta 2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture. (*B*) Quantification of the change in cell-surface GluR1 after NMDA treatment as measured by densitometry. (*C*) Western blots of biotinylated cell-surface GluR2 at rest or after NMDA treatment in WT and $\beta 2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture. (*D*) Quantification of the change in cell-surface GluR2 after NMDA treatment as measured by densitometry.