Expanded View Figures

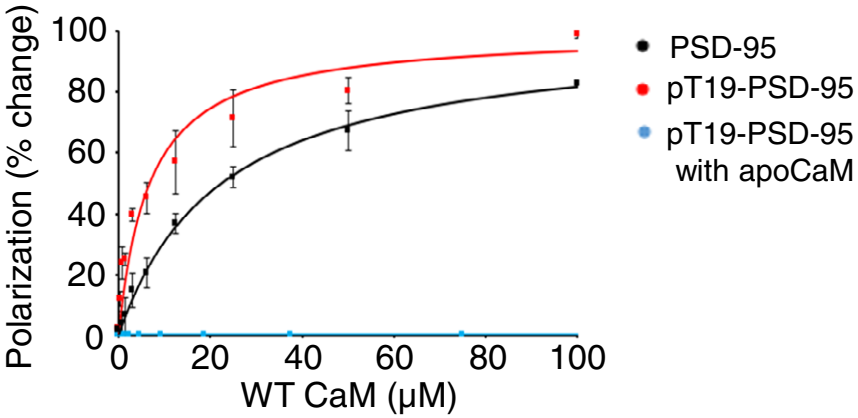

Figure EV1. Titration of PSD-95 peptide with Ca^{2+}/CaM.

Fluorescently labeled PSD-95 peptides (residues 1–21), which were either phosphorylated (red, blue) or unphosphorylated on T19 (black), were titrated with increasing amounts of Ca^{2+}/CaM (black, red) or apoCaM (blue) and fluorescence polarization (FP) monitored. Basal levels of FP obtained in the absence of Ca^{2+}/CaM were determined for each titration and subtracted. Mean values from three independent titrations (± SD) are indicated and fitted to a one-site binding model (see Materials and Methods). Solid lines represent theoretical FP values calculated using a one-site binding model as described in Materials and Methods. Calculated dissociation constants are given in Table 1.

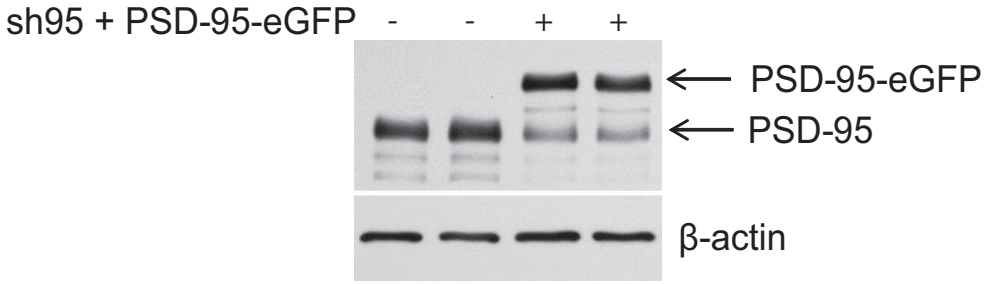

Figure EV2. Molecular replacement of PSD-95.

Cultured cortical neurons were infected at 12 DIV with lentivirus for expression of both shRNA against PSD-95 (sh95) and sh-resistant PSD-95-EGFP. Cultures were extracted at 17 DIV for immunoblotting with an antibody against PSD-95. Endogenous PSD-95 was detected at ~90 kDa and ectopically expressed GFP-tagged PSD-95 ~130 kDa. Probing for β-actin at around 43 kDa showed equal loading.

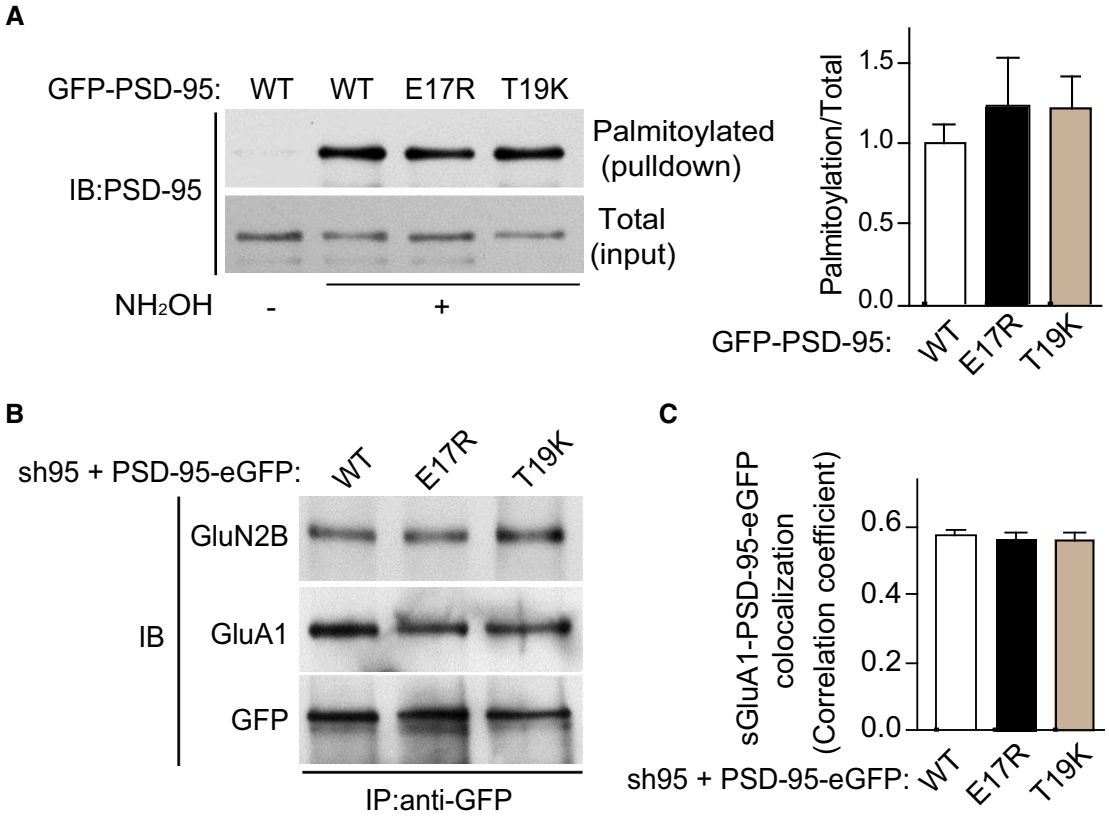

Figure EV3. Palmitoylation and association of PSD-95 with AMPARs and NMDARs are not affected by the E17R and T19K mutations.

A HEK293 cells were transfected with PSD-95-EGFP cDNA plasmids. Cultures were extracted after 24 h for analysis of palmitoylation by the biotin switch method and pull down with NeutrAvidin-agarose beads. Left panels show representative immunoblots (IB) of pull-down samples (top panel) and total lysate (bottom panel) for PSD-95. Omission of NH_2OH before biotinylation resulted in minimal NeutrAvidin pull down as negative control for non-specific pull down. Right panel shows quantification of PSD-95 palmitoylation (mean ± SEM, n = 3–4 per condition from three independent experiments).

B Cultured cortical neurons were infected at 12 DIV with lentivirus for expression of both shRNA against PSD-95 (sh95) and sh-resistant PSD-95-EGFP WT, E17R, or T19K. Cultures were extracted at 17 DIV for immunoprecipitation (IP) and subsequent immunoblotting (IB) with antibodies against GluA1 (~100 kDa) and GluN2B (~180 kDa), showing comparable co-immunoprecipitations of GluA1 and GluN2B. Probing for GFP showed comparable loading for PSD-95-EGFP WT, E17R, and T19K. Shown are representative IBs. Similar results were obtained in three other cultures.

C Quantification of colocalization of endogenous surface GluA1 and the indicated PSD-95-EGFP variant in hippocampal neurons. Bar graph shows correlation coefficients for the colocalization indicating comparable colocalization of GluA1 with each of the PSD-95-EGFP variants. Mean ± SEM, three dendritic segments from each of 32–34 neurons from three independent cultures were analyzed per condition.

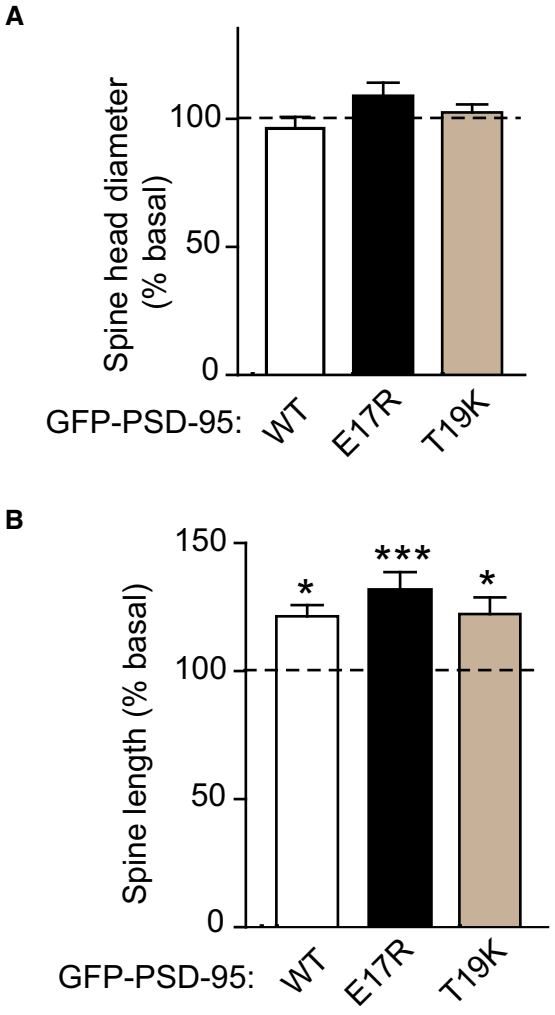

Figure EV4. Chronic bicuculline treatment increases length of dendritic spines.

A, B Cultured hippocampal neurons were co-transfected with mCherry and EGFP-tagged PSD-95 wild type (WT), E17R, or T19K at 10-11 DIV and treated with BIC (50 μM) or left untreated at 17 DIV for 24–48 h before fixation. Spine head diameter (A) and length (B) were comparable for neurons expressing WT, E17R, or T19K PSD-95 under basal conditions (see Fig 6H and I). BIC treatment did not affect spine head diameter but increased spine length for all PSD-95-EGFP variants. Mean ± SEM are shown. Statistical analysis was performed on n = 166–202 spines from 10 to 16 neurons per condition from three independent sets of cultures (*$P < 0.05$, ***$P < 0.001$; one-way ANOVA followed by Bonferroni's *post hoc* test; values for spine head diameter and length for basal conditions were those graphed in Fig 6H and I, respectively).

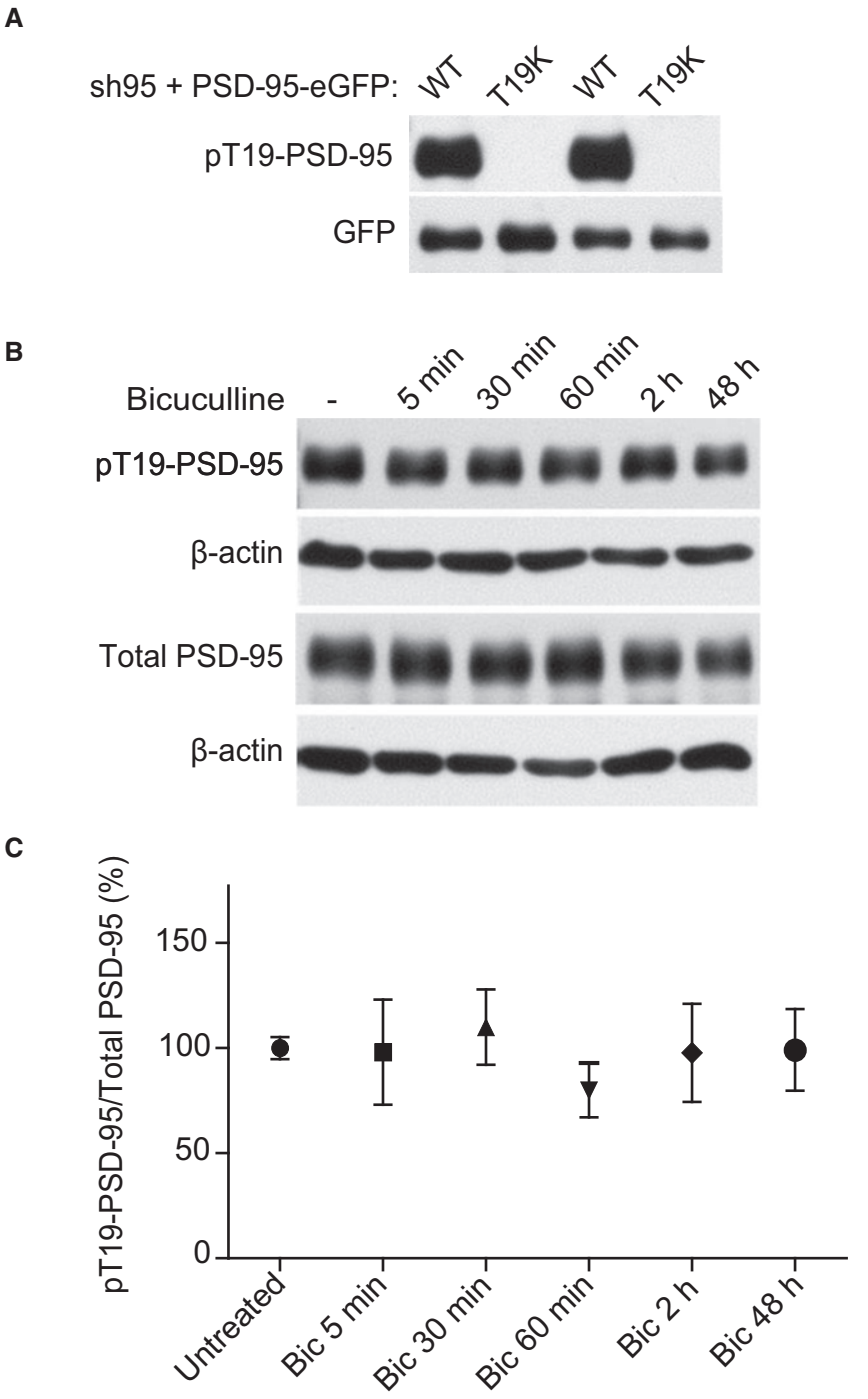

Figure EV5. Bicuculline treatment does not alter PSD-95 phosphorylation at T19.

A Cultured cortical neurons were infected at 12 DIV with lentivirus for expression of both shRNA against PSD-95 (sh95) and sh-resistant PSD-95-EGFP WT or T19K. Whole-cell lysates were prepared at 17 DIV and analyzed for pT19-PSD-95 levels by immunoblotting. Probing for GFP showed comparable loading for PSD-95-EGFP WT and T19K. No detection of pT19-PSD-95 signal corresponding to PSD-95-EGFP in T19K samples showed specificity of the antibody.

B Cultured cortical neurons (17 DIV) were either untreated or treated with BIC for the indicated time periods, and whole-cell lysates were analyzed for endogenous pT19-PSD95 and total PSD-95 levels by immunoblotting. Probing for β-actin showed comparable loading. Representative immunoblots are shown.

C Quantification of relative pT19-PSD-95 levels normalized to total PSD-95 (mean ± SEM, n = 4–5 per condition from three independent sets of cultures).