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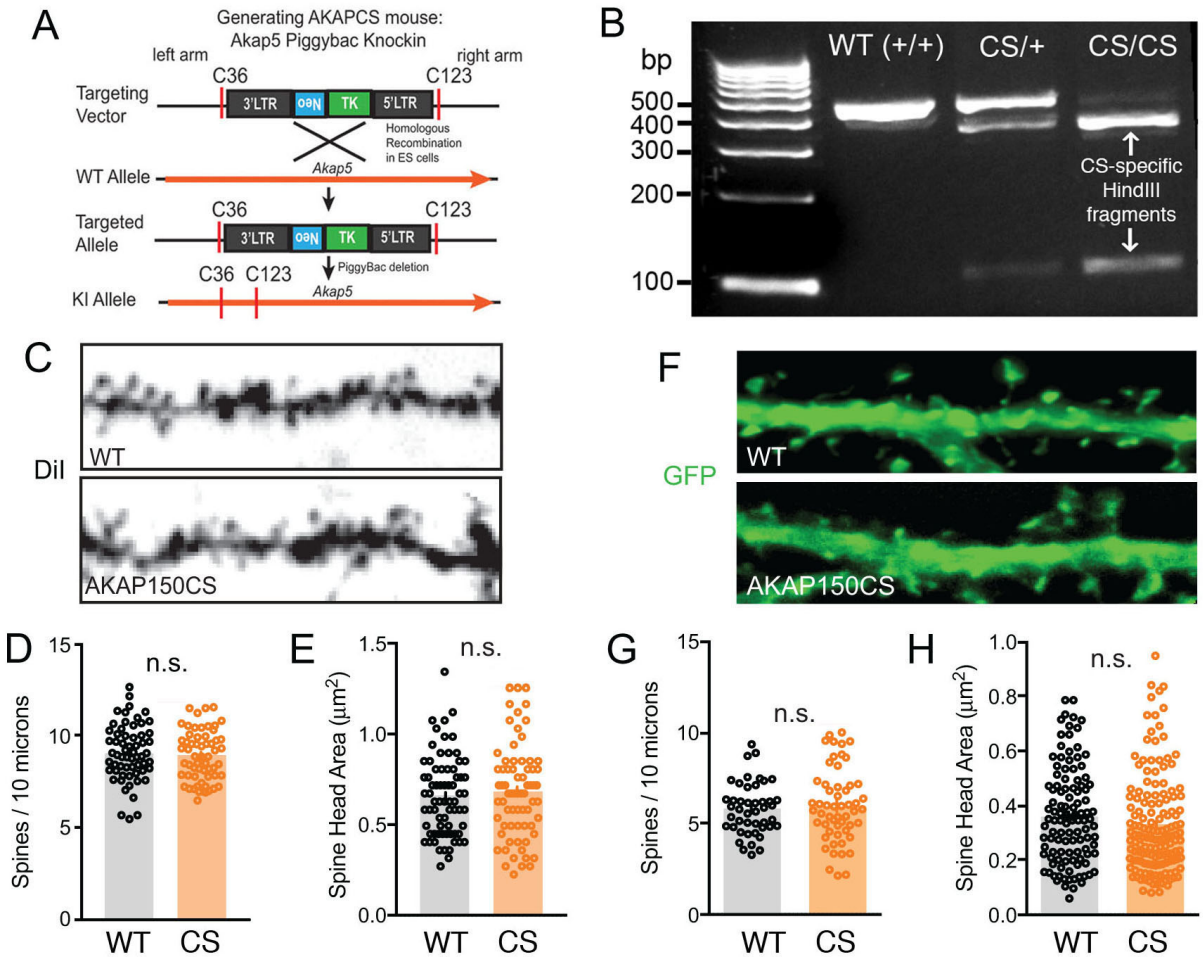
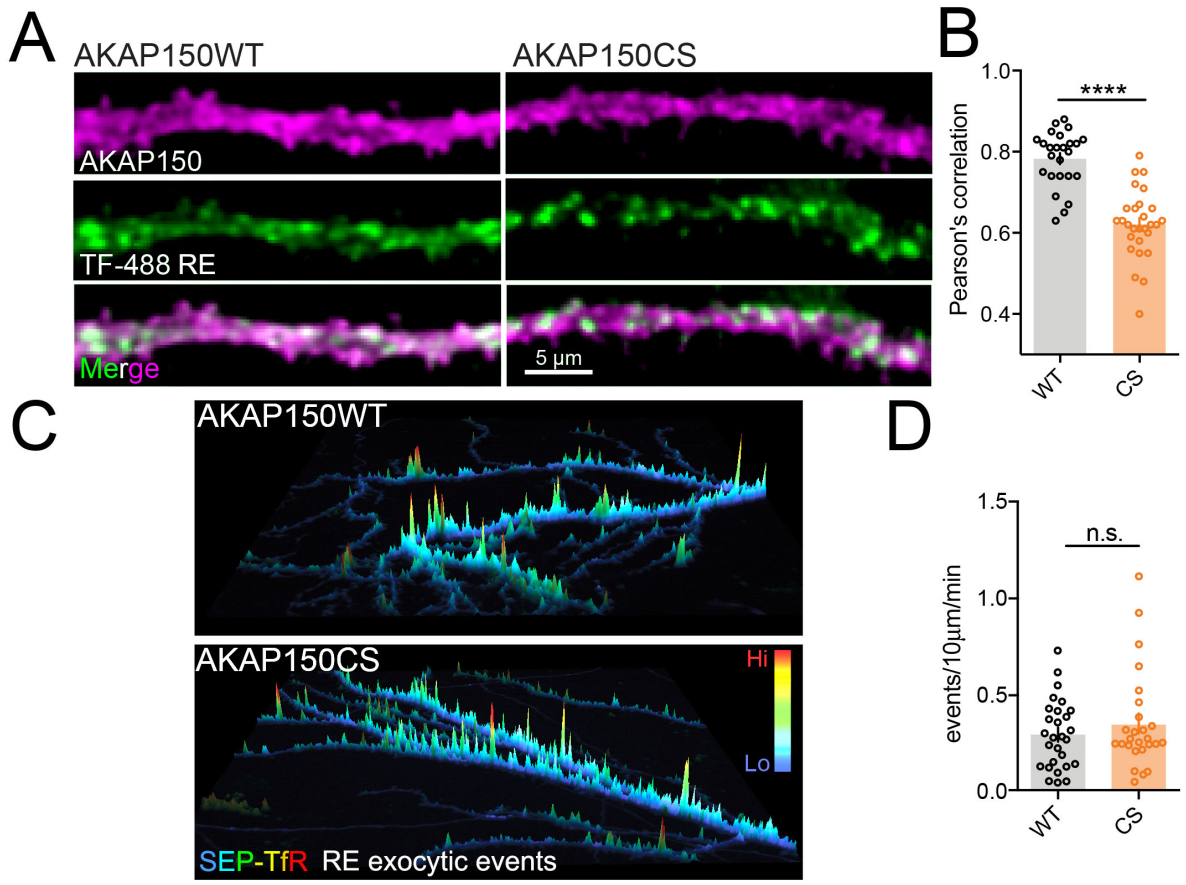
Supplemental Information

AKAP150 Palmitoylation Regulates Synaptic Incorporation of Ca^{2+}-Permeable AMPA Receptors to Control LTP

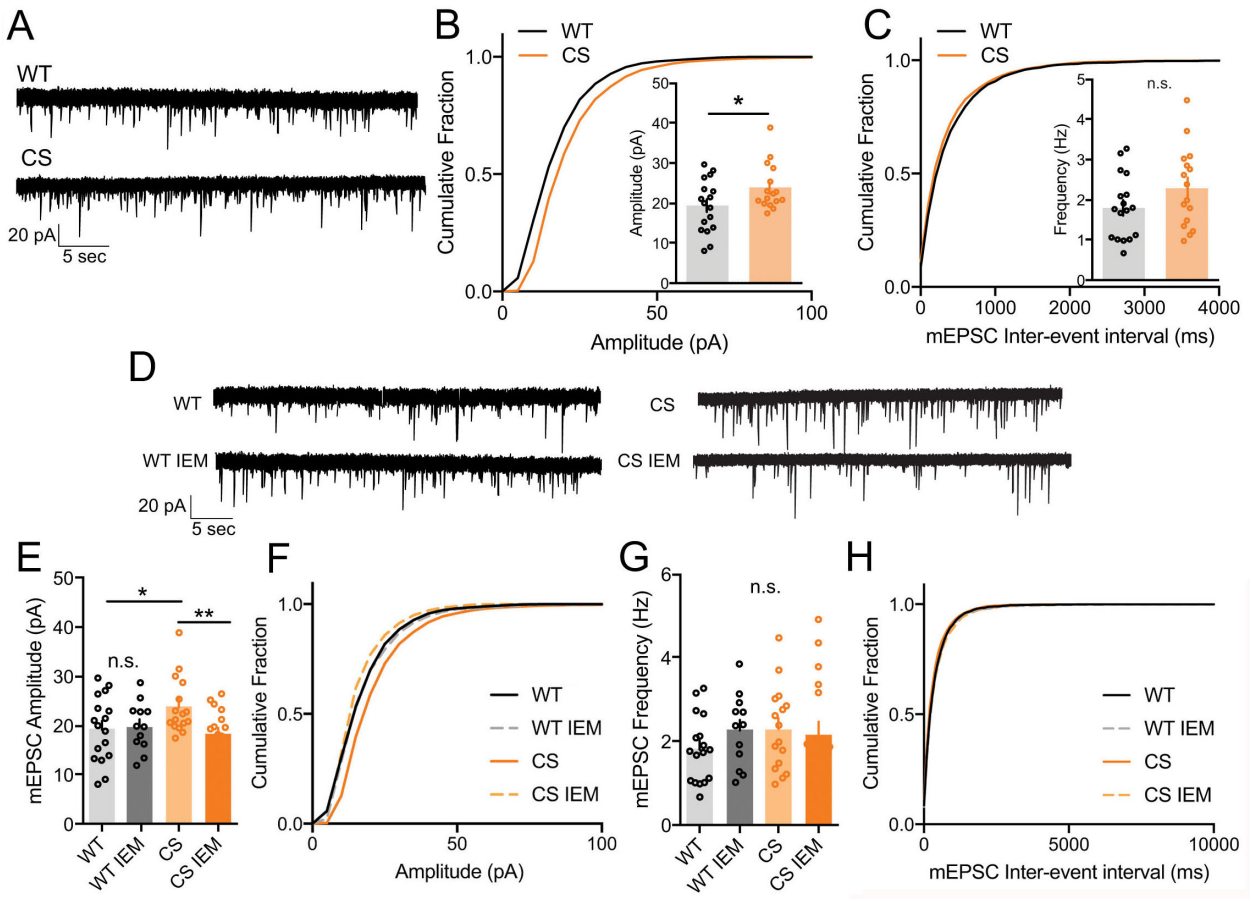
Alicia M. Purkey, Kevin M. Woolfrey, Kevin C. Crosby, Dominik G. Stich, Wallace S. Chick, Jason Aoto, and Mark L. Dell'Acqua



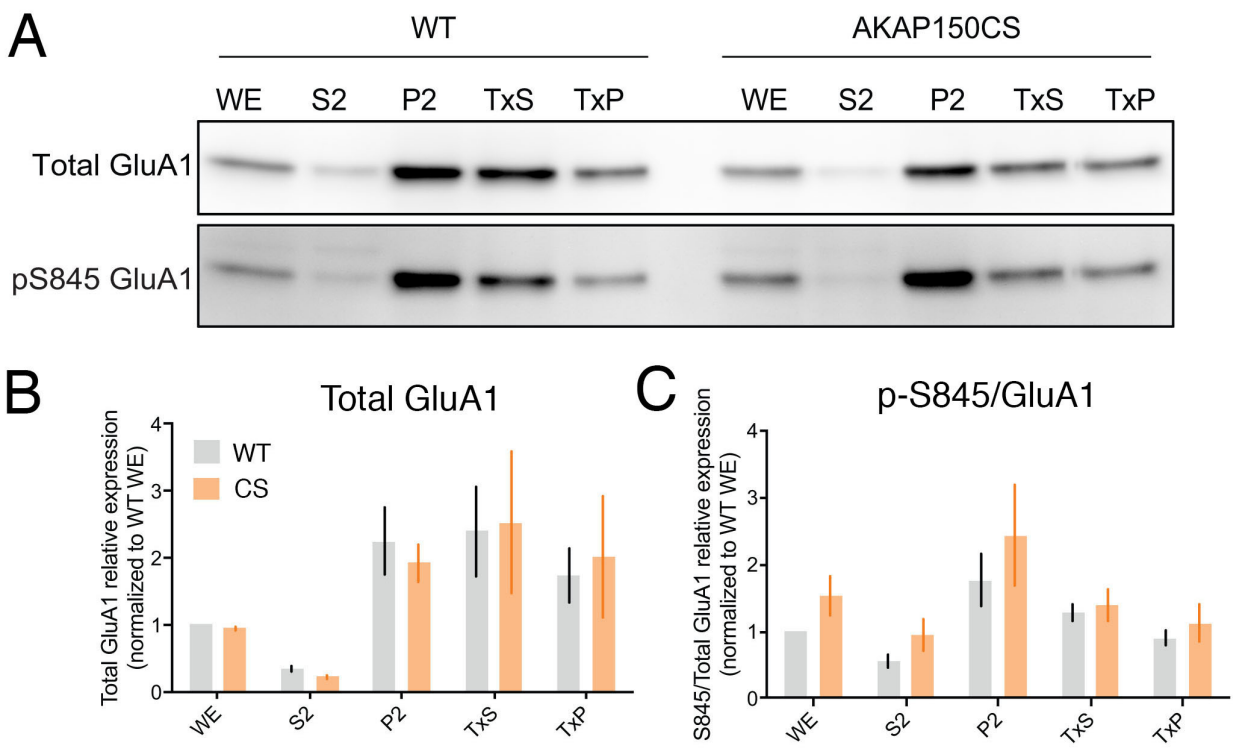
Supplemental Figure 1 Related to Figures 1 and 2: Additional characterization of AKAPCS mice and cultured neurons. (**A**) Generation of AKAPCS mice using the Piggybac method, introducing two point mutations and a *HindIII* site. (**B**) Genotyping by PCR and restriction digestion of AKAP150WT and AKAP150CS heterozygous and heterozygotes; *HindIII* digestion results in AKAP150CS specific fragments. (**C**) Dendritic segments from neurons in region CA1 of the hippocampus from WT or AKAPCS DiI stained slices, showing no significant difference in (**D**) spine number or (**E**) spine head area. (**F**) DIV14-16 hippocampal cultures transfected with GFP, show no significant difference in (**G**) spine number or (**H**) spine head area.



Supplementary Figure 2 Related to Figures 1 and 2: **AKAP150CS endosome localization and RE exocytosis.** (**A**) Maximum projection images of confocal z-stacks for DIV14 WT or AKAP150CS mouse neurons immunostained to visualize AKAP150 and labeled with Alexa488-transferrin (TF-488) to mark REs. (**B**) AKAPCS neurons show a significant decrease in AKAP co-localization with TF-488 (Pearson's Correlation WT 0.78±0.02, n=25 cells; CS 0.62±0.02, n=28 cells, ****p<0.0001 by t-test) (**C**) Time composite (5 min, 0.2 Hz) images of DIV15-17 hippocampal neurons from WT or AKAPCS mice showing RE exocytic events in dendrites imaged with SEP-TfR (integrated intensity plotted on the z-axis in pseudocolor: blue, low to red, high). (**D**) No significant difference between genotypes was detected in the number of exocytic events (defined as 2.5-fold the median intensity and calculated as events/10 µm/min).



Supplemental Figure 3 Related to Figure 3 and 4: Electrophysiological characterization of AKAPCS cultures. (**A-C**) Whole-cell recordings from DIV13-14 hippocampal neuron cultures from WT and CS mice. AKAPCS cultures show an enhancement in (**B**) mEPSC amplitude (WT=19.34 ± 1.599 n=17 cells, CS=23.86 ± 1.434 n=16, unpaired t-test p=0.0443) and no change in (**C**) mEPSC frequency. (**D-H**) AKAPCS neuron cultures also exhibit enhancement in CP-AMPAR sensitivity, showing a significant decrease in mEPSC amplitude with IEM blockade of CP-AMPARs (CS=23.86 ± 1.434 pA n=16 cells, CS IEM=18.42 ± 1.107 pA n=17 cells, unpaired t-test p=0.0050). *p<0.05, **p<0.01; unpaired t-test.



Supplemental Figure 4 Related to Figures 1 and 4: Analysis of GluA1 Ser845 phosphorylation. (**A**) Blots from fractionation of WT and CS hippocampal lysates probed for pS845 GluA1 and GluA1 and (**B,C**) quantification of these blots (n=3 animals) showing no significant difference in total GluA1 protein or S845 phosphorylation although trends toward increased S845 phosphorylation are seen for CS across all fractions.