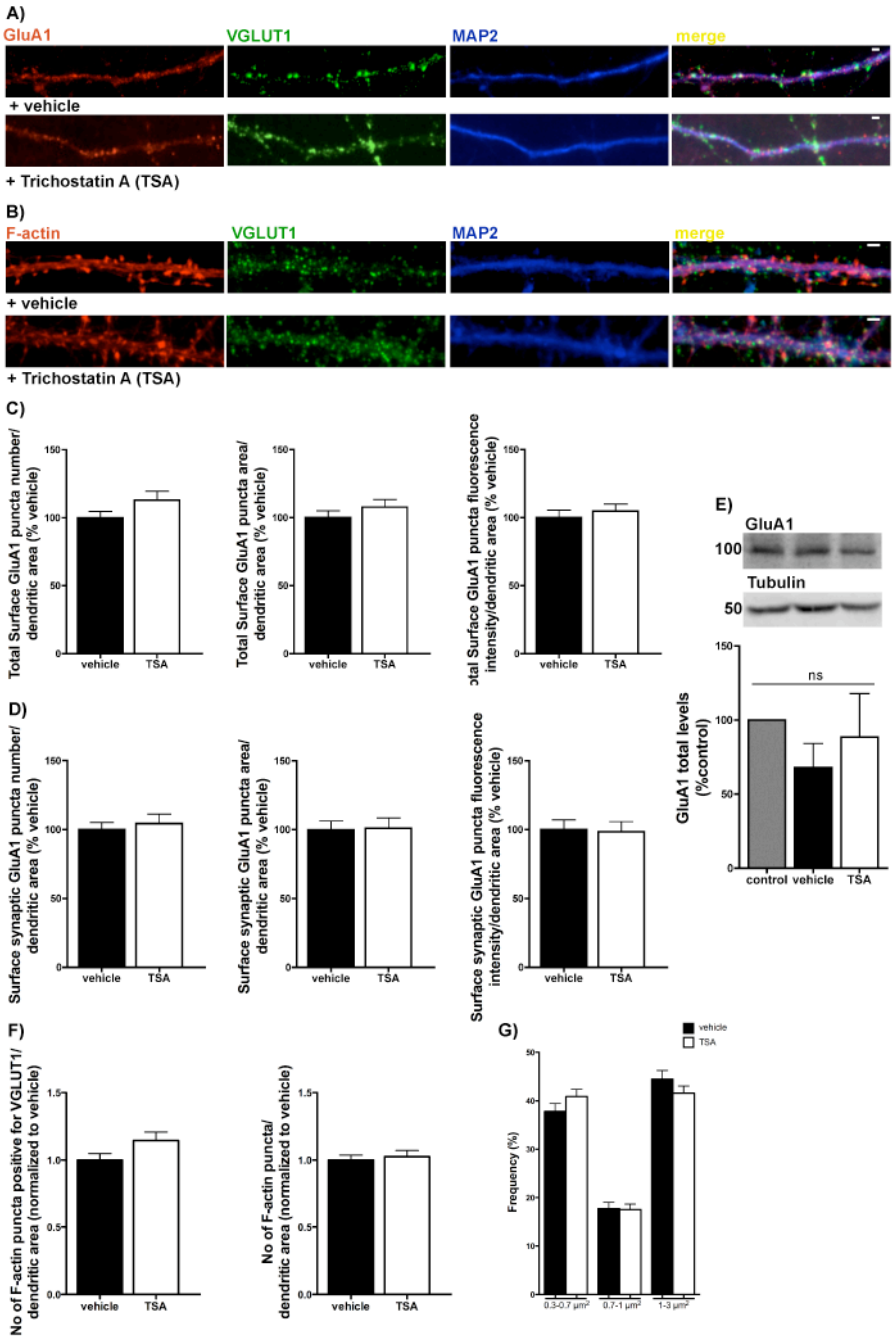

Fig. S1 – TSA treatment of hippocampal neurons does not change the number, area or intensity of surface GluA1 clusters or the number or size of dendritic F-actin clusters. Hippocampal neurons were treated with vehicle or TSA (400ng/ml) for 12hr. Neurons were live-stained for surface GluA1 (A) and, after fixation, for VGLUT1 and MAP2. Polymerized F-actin was detected in dendrites with Acti-stain 555 phalloidin (B) (Scale bar: 2µm). Neurons were analysed for total (C) and synaptic (D) surface GluA1 cluster fluorescence intensity, area and number, per dendritic area. Synaptic GluA1 is defined as GluA1 signal that overlaps with VGLUT1. (E) TSA treatment does not change GluA1 expression levels. Hippocampal neurons at 15 DIV were treated with vehicle or TSA for 12hr. Western blot was performed using an anti-GluA1

antibody. Staining for tubulin was used for normalization of GluA1 values. Data are presented as average ± S.E.M. of three experiments performed in independent preparations, and are expressed as a percentage of GluA1 expression levels in control conditions. Data were statistically analysed using One-way ANOVA. $p>0.05$. Neurons were analysed for number (F) and size (G) of F-actin clusters, and synaptic F-actin is defined as F-actin signal that overlaps with VGLUT1. Results are presented as % of vehicle control cells, and are averaged from three independent experiments ($n\geq47$ cells). Errorbars, ± S.E.M. (unpaired student t-test).

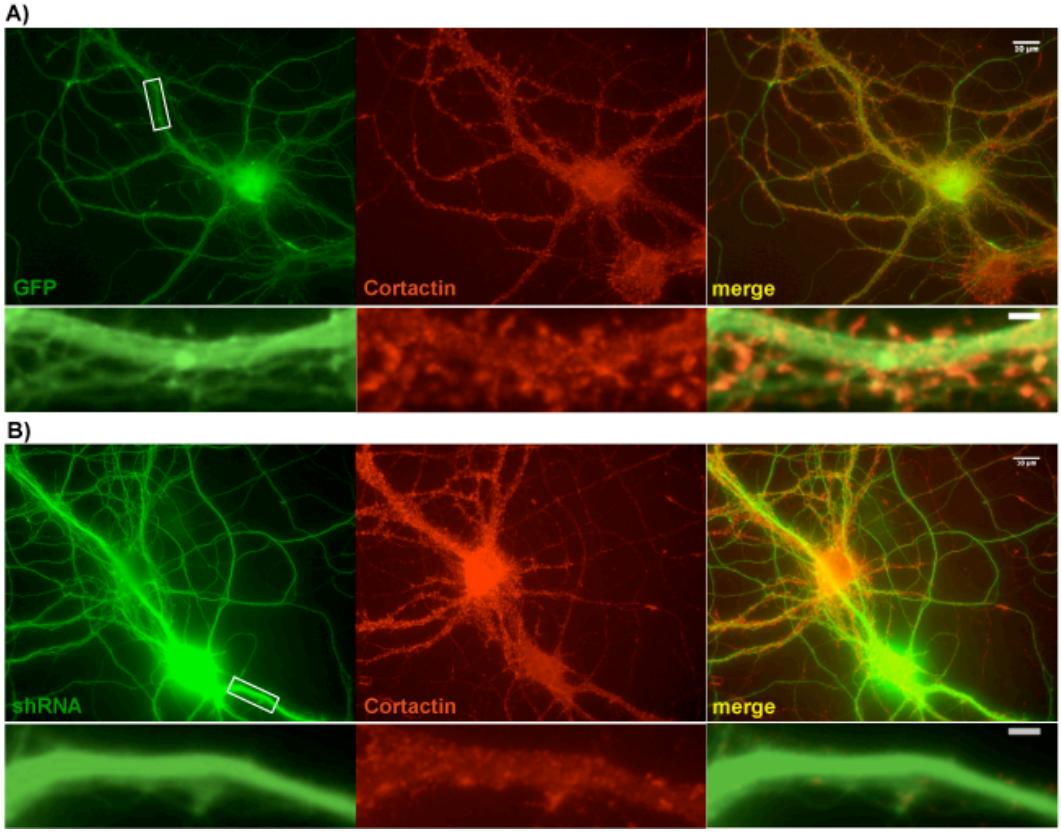

Fig. S2 – shRNA inhibition of cortactin. Hippocampal neurons at 15 DIV, transfected with control or cortactin-shRNA, were stained with an antibody for cortactin (A). Hippocampal neurons transfected with cortactin-shRNA showed a strong decrease in the fluorescence intensity, area and number of dendritic cortactin clusters (B). Scalebar: 10 μm, insert:2 μm

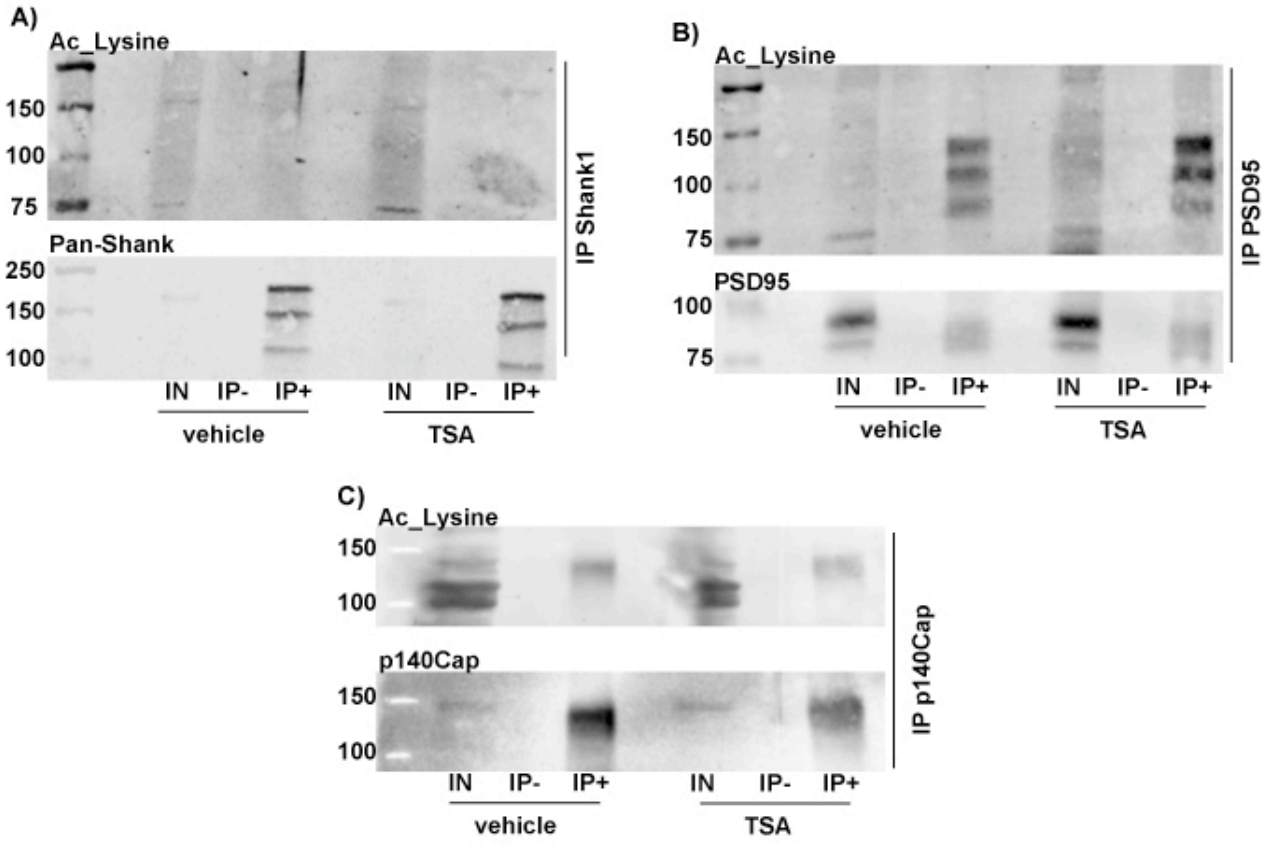

Fig. S3 – Acetylation of synaptic proteins in cultured hippocampal neurons. Control hippocampal neurons (vehicle) or TSA-treated neurons were used to immunoprecipitate (A) Shank, (B) PSD95 or (C) p140Cap. The efficiency of the immunoprecipitation was confirmed in each case, and the acetylation of the immunoprecipitated proteins or their co-immunoprecipitated binding partners was tested using an anti-acetyl-lysine antibody. IN: input samples used for immunoprecipitation; IP-: immunoprecipitation control using non-immuno immunoglobulins; IP+: immunoprecipitation with the indicated antibody.

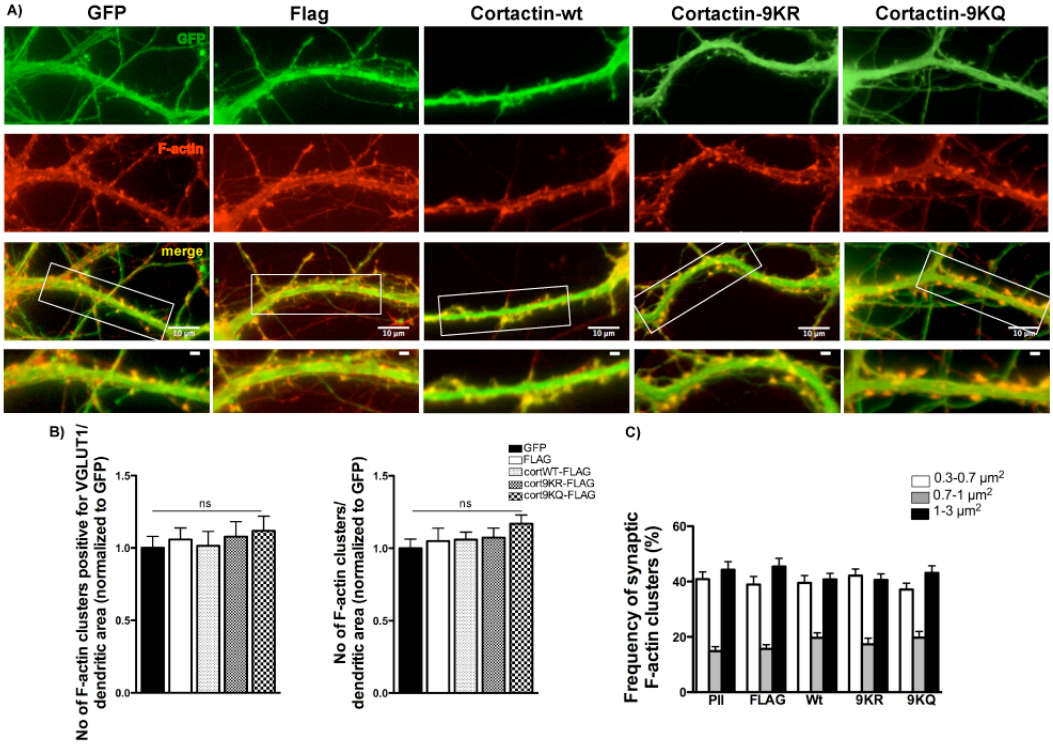

Fig. S4 – Acetylation of cortactin does not change the number or size of dendritic F-actin clusters in hippocampal neurons (A) Images of 15 DIV hippocampal neurons expressing GFP alone, or GFP along with different cortactin constructs. Hippocampal neurons were transfected at 7 DIV with GFP alone, or GFP along with FLAG, cortactinWT-FLAG, cortactin9KR-FLAG or cortactin9KQ-FLAG. Dendritic spine morphology was observed with GFP fluorescence (green). Polymerized F-actin was detected in dendrites with Acti-stain 555 phalloidin (red). (B) Transfected neurons, identified by GFP fluorescence, were analysed for F-actin cluster number. Data represent the average number of F-actin clusters per dendritic area. Results are presented as % of GFP-transfected control cells, and are averaged from three independent experiments (n≥32 cells). (C) Analysis of size distribution for dendritic F-actin clusters. Errorbars, ± S.E.M. (One-way ANOVA $p>0.05$). (scalebar: 10 µm, insert: 2µm).