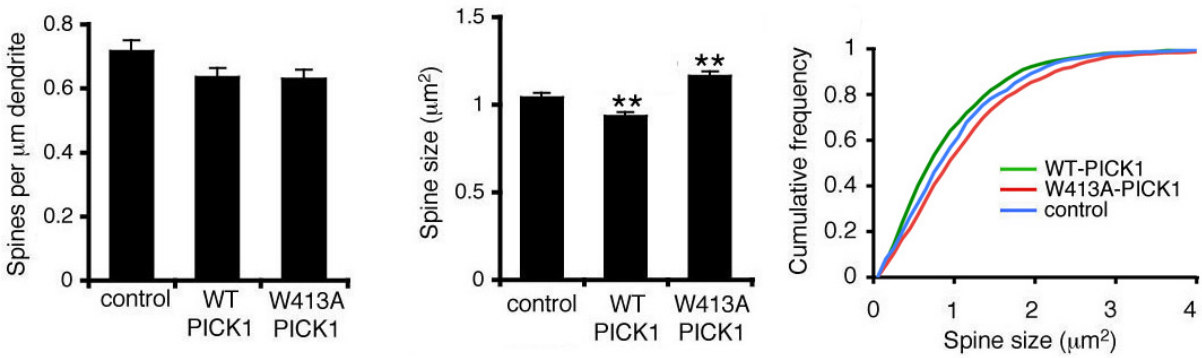
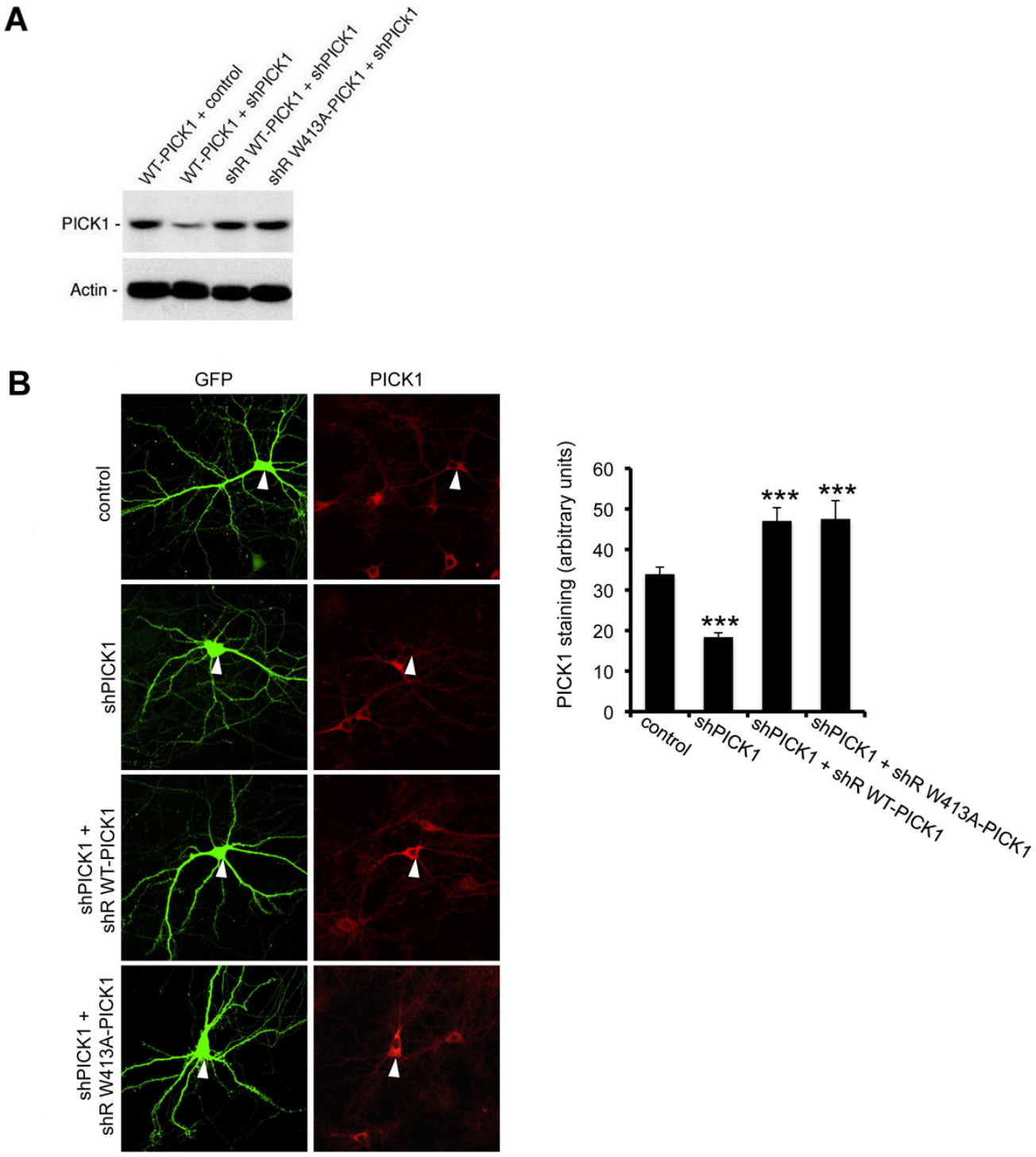


Supplementary Figure 1

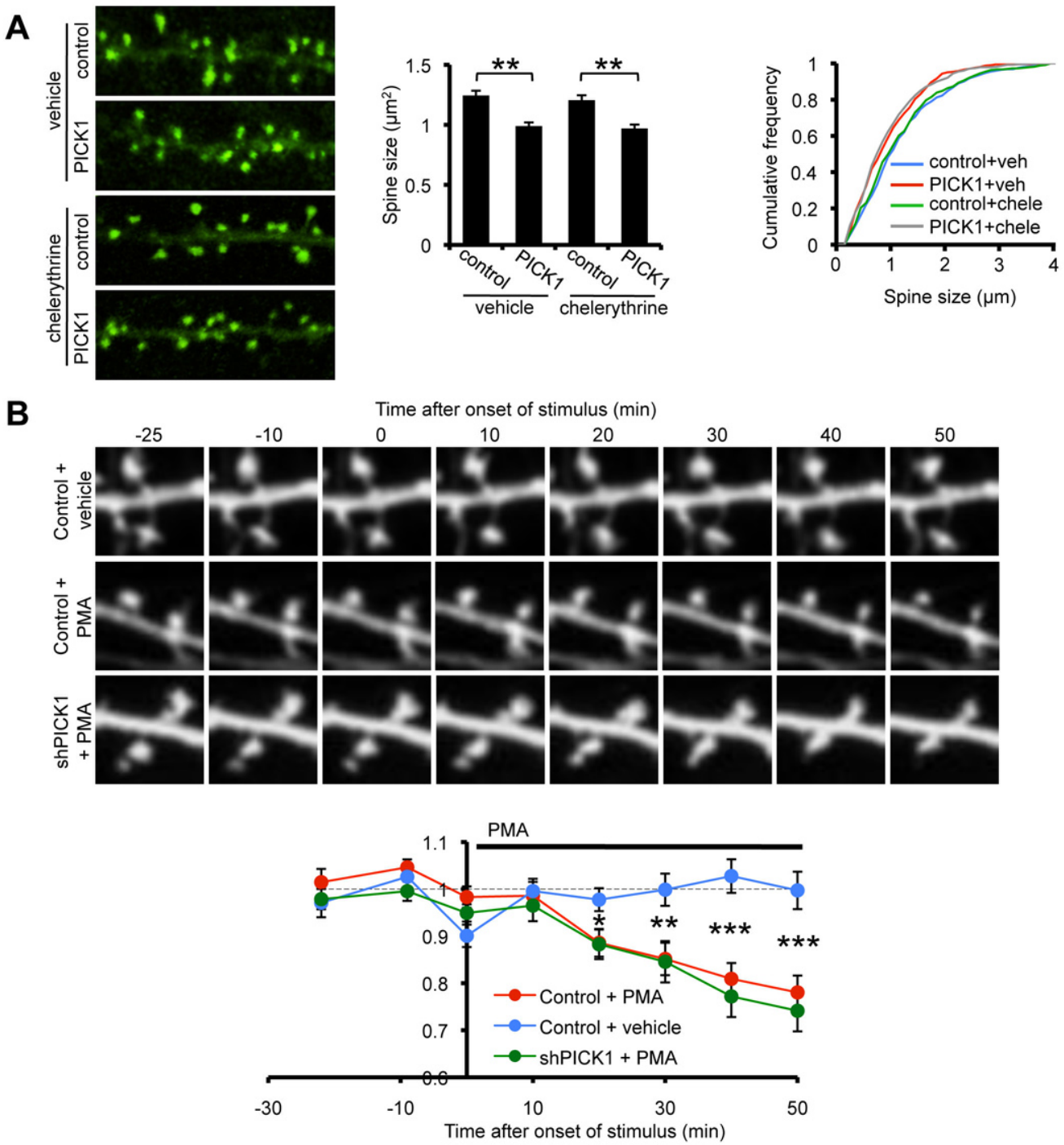
In neurons using EGFP as a morphological marker, overexpression of PICK1 results in reduced spine size via its interaction with Arp2/3.
Cultures were transfected with control-IRES-EGFP, WT-PICK1-IRES-EGFP, or W413A-PICK1-IRES-EGFP. Graphs show quantification of linear spine densities and spine area. **p<0.01, compared to control, K-S test.



Supplementary Figure 2

PICK1 shRNA reduces PICK1 expression.

A, COS cells were transfected with either PICK1 or sh-resistant (shR) PICK1 constructs as well as either control or PICK1 shRNA constructs. Lysates were prepared and proteins analysed by western blotting for PICK1. Actin is shown as a loading control.
B, Cultured hippocampal neurons were transfected with plasmids expressing either PICK1 shRNA-GFP (shPICK1) or control-GFP and sh-resistant (shR) WT-PICK1-IRES-EGFP, shR W413A-PICK1-IRES-EGFP, or control-IRES-EGFP. Cultures were prepared for immunocytochemistry and stained for PICK1 (red channel). Graph shows PICK1 staining per cell area. n=15 cells, ***p<0.001 compared to control.



Supplementary Figure 3

PKC and PICK1 do not functionally interact in the regulation of dendritic spine size.
A, PKC inhibition does not affect spine shrinkage induced by PICK1 overexpression. Dissociated hippocampal cultures transfected with either control-IRES-actin^{EGFP}, or WT-PICK1-IRES-actin^{EGFP} were treated with 5 μM chelerythrine or vehicle control for 1 h prior to fixation. Left panels show representative images of dendrites from neurons treated as above. Graphs show quantification of spine area. **, p<0.005 compared to control, K-S test. B, PICK1 knockdown does not affect spine shrinkage induced by PKC activation. Top panels show representative images at timepoints shown relative to start of drug (see methods) or vehicle treatment of neurons transfected with either mCherry PICK1 shRNA or mCherry control. Graph shows pooled data for spine size plotted against time. Spine size is normalised to the baseline value for each recording. Data are from 6-8 cells, 58-79 spines per condition *p<0.05 **p<0.01 ***p<0.0001