Supplementary Figure Legends.

Fig S1. Characterization of PIPP antibodies. (A) SDS-PAGE with Coomassie Blue staining showing whole cell lysate expressing GST-PIPP and purified GST-PIPP following affinity purification on glutathione Sepharose. This purified protein was used as an immunogen to raise PIPP-AB2, AB3 and AB4 antibodies. (B) COS1 cells transiently transfected with constructs encoding HA-PIPP, HA-72 kDa 5-phosphatase, HA-SKIP, HA-SHIP1, HA-SHIP2, HA-OCRL or HA vector alone were immunoblotted with affinity-purified PIPP antibodies (PIPP-AB2) (left panel) or HA antibodies (right panel). Molecular weight markers are indicated. (C) Cell lysates prepared from 5 DIV cultured hippocampal neurons were immunoblotted with affinity-purified PIPP antibodies (PIPP-AB1). Cell lysates from mouse brain or rat PC12 cells were immunoblotted with affinity-purified PIPP antibodies (PIPP-AB2). Molecular weight markers (kDa) are indicated. (D) Hippocampal neurons were transfected with PIPP or scram siRNA and costained with PIPP antibodies (PIPP-AB1) and phalloidin. Confocal images were taken in the optical section of maximum fluorescence intensity in cell bodies. Bar = 40 μm. (E) Hippocampal neurons at 2 DIV costained with phalloidin and either PIPP-AB1, or PIPP-AB1 antibodies preabsorbed with free-peptide to which the antibody was raised (Pep. deplete). Bar=20 μm.

Fig S2. PIPP exhibits a nonpolarized distribution in primary hippocampal neurons. Cultured hippocampal neurons were fixed, permeabilized and costained with PIPP-specific antibodies (PIPP-AB2) and antibodies against either MAP2 (dendrites; upper panels) or Tau-1 (axons; lower panels). Merged images are shown on the right. Arrowheads indicate PIPP co-localization with MAP2 and Tau-1 in dendrites and axons respectively. Scale bars represent 20 μm.

Fig S3. GSK3β(S9A) phosphorylates CRMP2. Lysates from COS1 cells expressing FLAG-CRMP2 in the presence or absence of myc-GSK3β(S9A) were immunoblotted with FLAG (upper panel) or myc antibodies (lower panel). The migration of non-phosphorylated and phosphorylated FLAG-CRMP2 is indicated on the right.

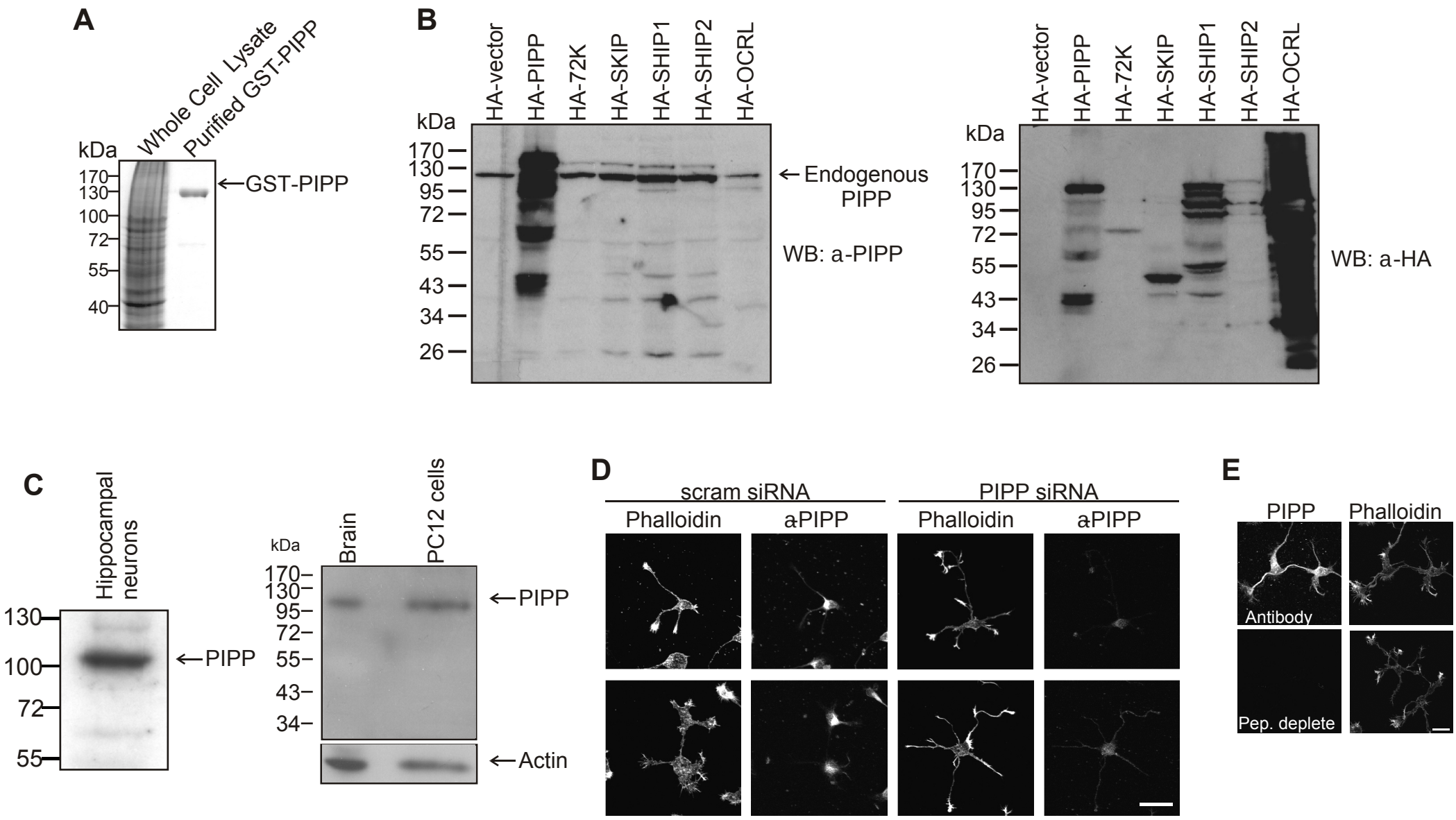
Fig S4. CRMP2 binding has no effect on PIPP PtdIns(3,4,5)P_3 5-phosphatase activity. (A) GST-PIPP purified from COS1 cells was incubated with GST or increasing amounts of GST-CRMP2 purified from *E. coli* and assayed for PtdIns([^{32}P]3,4,5)P_3 5-phosphatase activity. Lipid products were analyzed by thin layer chromatography (top panel). The migration of PtdIns([^{32}P]3,4)P_2 and PtdIns([^{32}P]3,4,5)P_3 are indicated on the right. The relative amount of GST or GST-CRMP2 added to each reaction was determined by SDS-PAGE and Western blotting with GST antibodies (lower panel). (B) GST-PIPP purified from COS1 cells was incubated with FLAG immunoprecipitations from COS1 cells either mock transfected or expressing FLAG-CRMP2 and assayed for PtdIns([^{32}P]3,4,5)P_3 5-phosphatase activity as in (A). The relative amount of FLAG-CRMP2 added to the reactions was determined by immunoblotting immunoprecipitations with FLAG antibodies (right panel). A Coomassie Blue stain of the original preparation of GST-PIPP is shown in Fig S1B.

Fig S5. PIPP and CRMP2 siRNA do not affect actin polymerization or growth cone size. PC12 cells expressing control, PIPP or CRMP2 Cy3-labeled siRNA were differentiated for 3 days and stained with phalloidin to outline differentiated neurites. (A) Fluorescence intensity of phalloidin in the growth cone relative to the neurite shaft of transfected cells. Bars = mean ± s.e.m., control is designated 1, (10 cells, n=4). (B) Growth cone area was measured. Bars = mean ± s.e.m., control is designated 1, (10 cells, n=4).

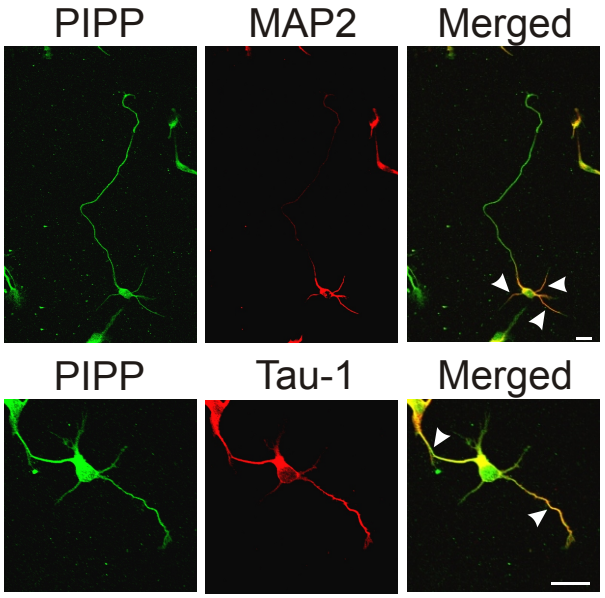
Fig S6. PIPP does not affect CRMP2 phosphorylation. (A) PC12 cells expressing control, PIPP or CRMP2 siRNA were immunoblotted with phospho-Thr509/Thr514-CRMP2 antibodies, then re-probed with β-tubulin antibodies. Relative phospho-CRMP2 expression was determined by densitometry and expressed relative to control, designated 1. Bars = mean

± s.e.m. (n=3, *p<0.05). (B) Cells transfected with control or PIPP siRNA were differentiated for 3 days and costained with phospho-Thr509/Thr514-CRMP2 antibodies (green) and imaged by confocal microscopy at the same laser attenuation. Bar=20 μm. Transfected cells are indicated by Cy3 siRNA label (red) and growth cones are indicated by arrowheads. Bars = mean fluorescence intensity of phospho-CRMP2 in the growth cone relative to the neurite shaft ± s.e.m., where the control is designated 1 (≥ 10 cells, n=3)

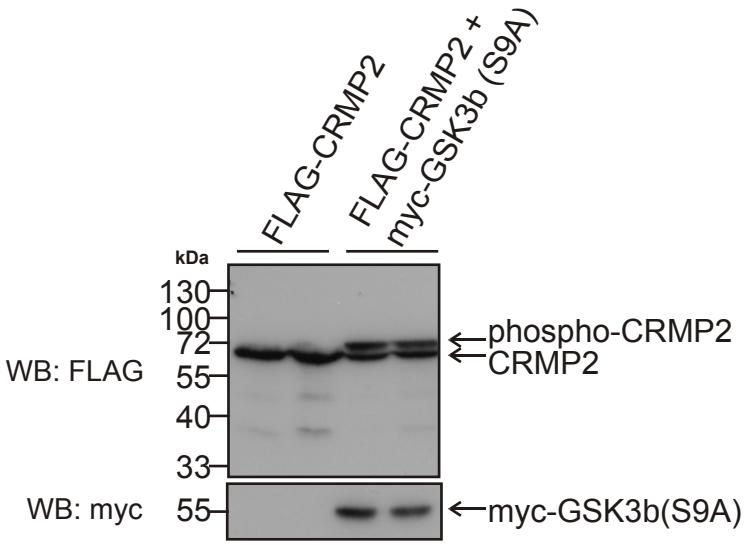
Supplementary Figure 1



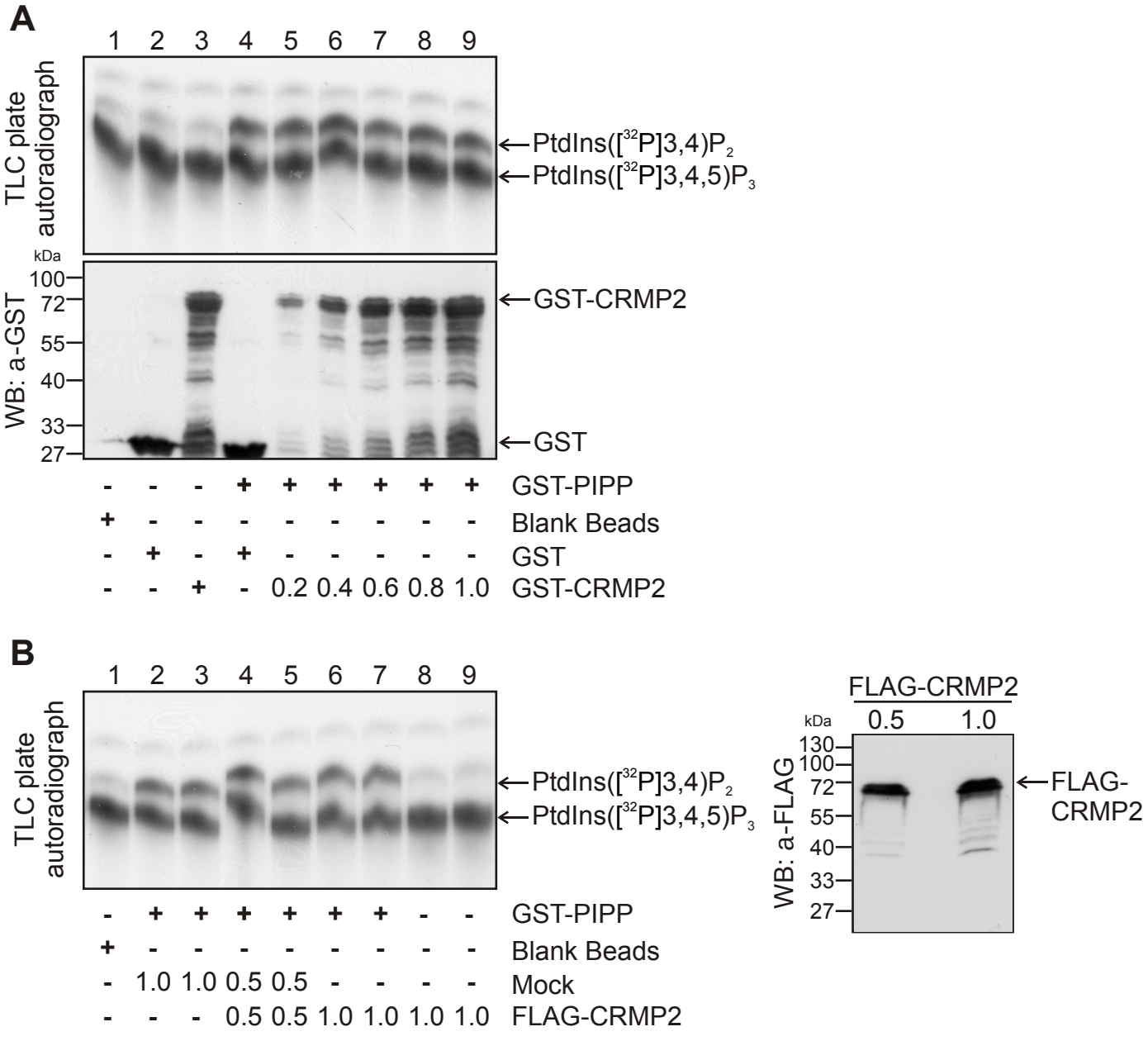
Supplementary Figure 2



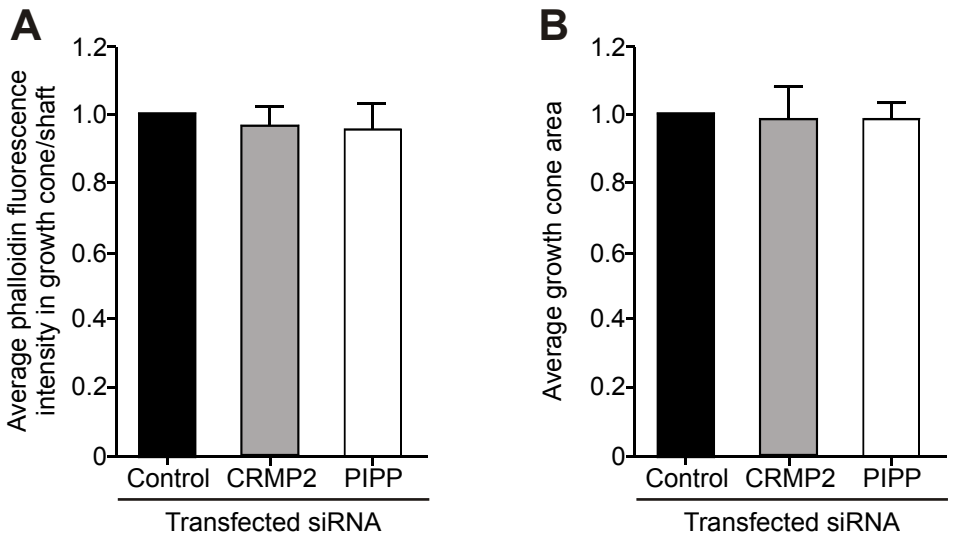
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6