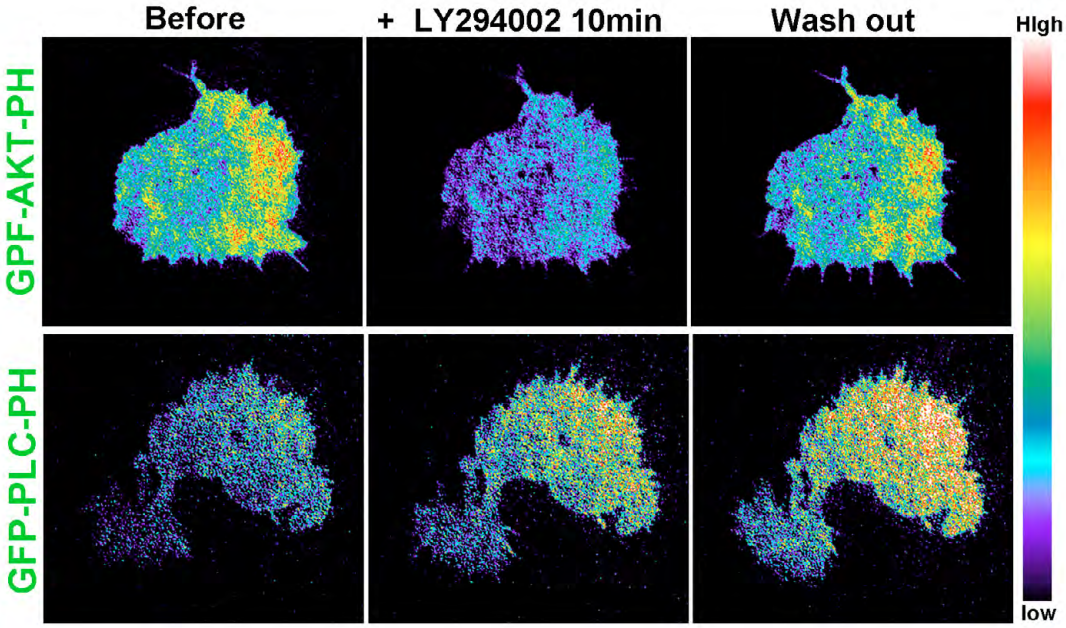


Fig. S1. PI3K inhibitor (LY294002) decreases PIP_3, but increases PIP_2 levels. Pseudocolored images of a stage 1 cortical neurons transfected with GFP-AKT-PH (PIP_3 label) or GFP-PLC-PH (PIP_2 label) before, after 10 minutes of LY294002 (10 μM) treatment and after washout.

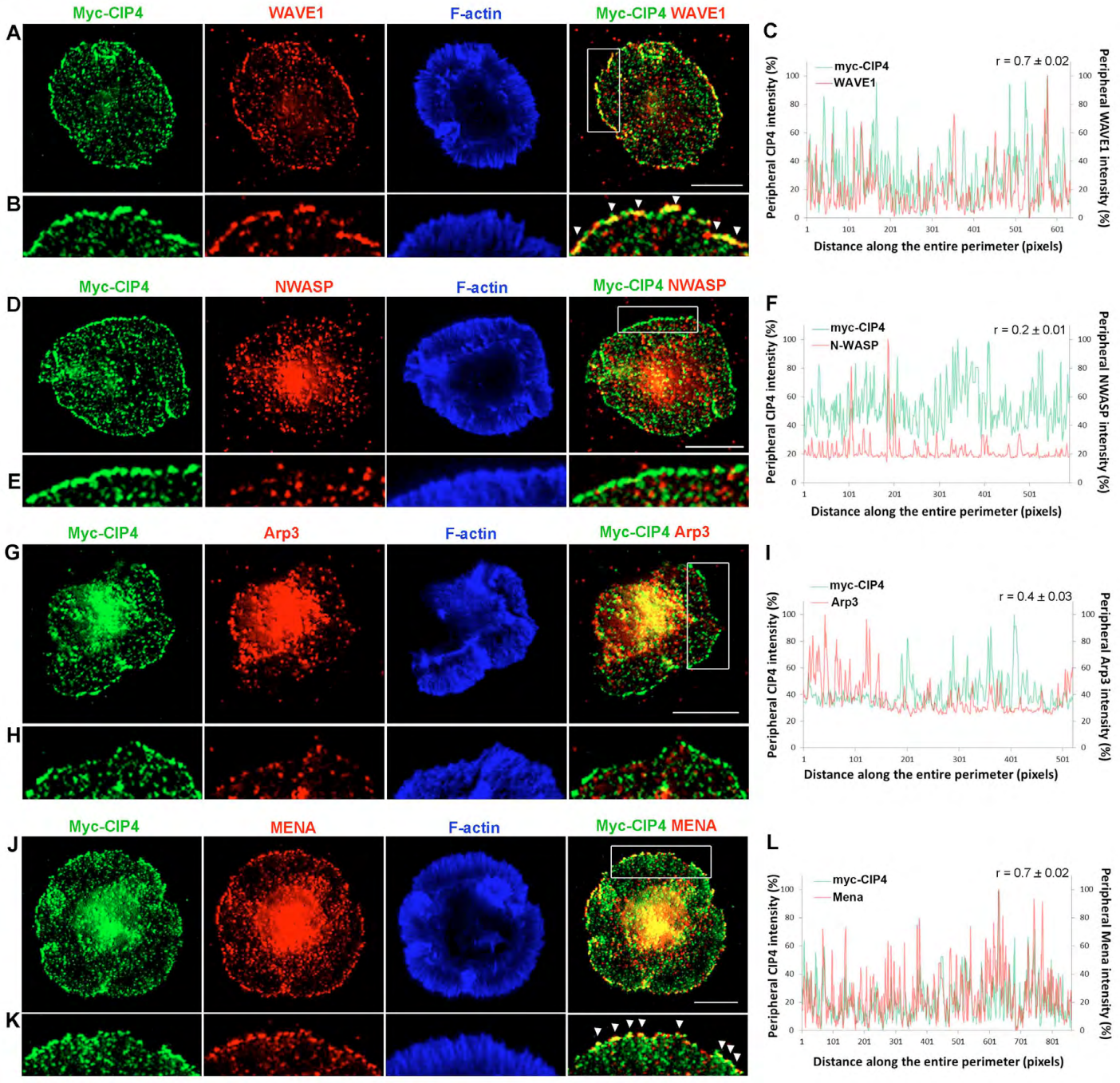


Fig. S2. CIP4 co-localizes with endogenous WAVE1 and Mena but not N-WASP or Arp3 in cortical neurons. (A, D, G, J) Images of stage 1 cortical neurons transfected with myc-CIP4, fixed after 1DIV and stained with anti-myc antibody (green), and anti-WAVE1 (**A**), anti-N-WASP (**D**), anti-Arp3 (**G**) or anti-Mena antibody (**J**) (red) and phalloidin 670 (blue). (**B, E, H, K**) Images magnified from boxes in **A**, **D, G** and **J** showing that CIP4 co-localizes with endogenous WAVE1 (**B**) and Mena (**K**) but not with endogenous N-WASP (**E**) and Arp3 (**H**) at the peripheral membrane. (**C, F, I, L**) Representative graphs showing the normalized intensity of CIP4 (red) and WAVE1 (**C**), N-WASP (**F**), Arp3 (**I**) or Mena (**L**) (all green) at the peripheral membrane of neurons shown in **A, D, G** and **J**. White arrowheads indicate co-localization of CIP4 and WAVE1(**B**) or Mena (**K**). Numbers above the graphs indicate the average Pearson's correlation coefficient (n = 19, 6, 7 and 9 neurons for **C, F, I,** and **L**, respectively). Scale bars, 10 μm.

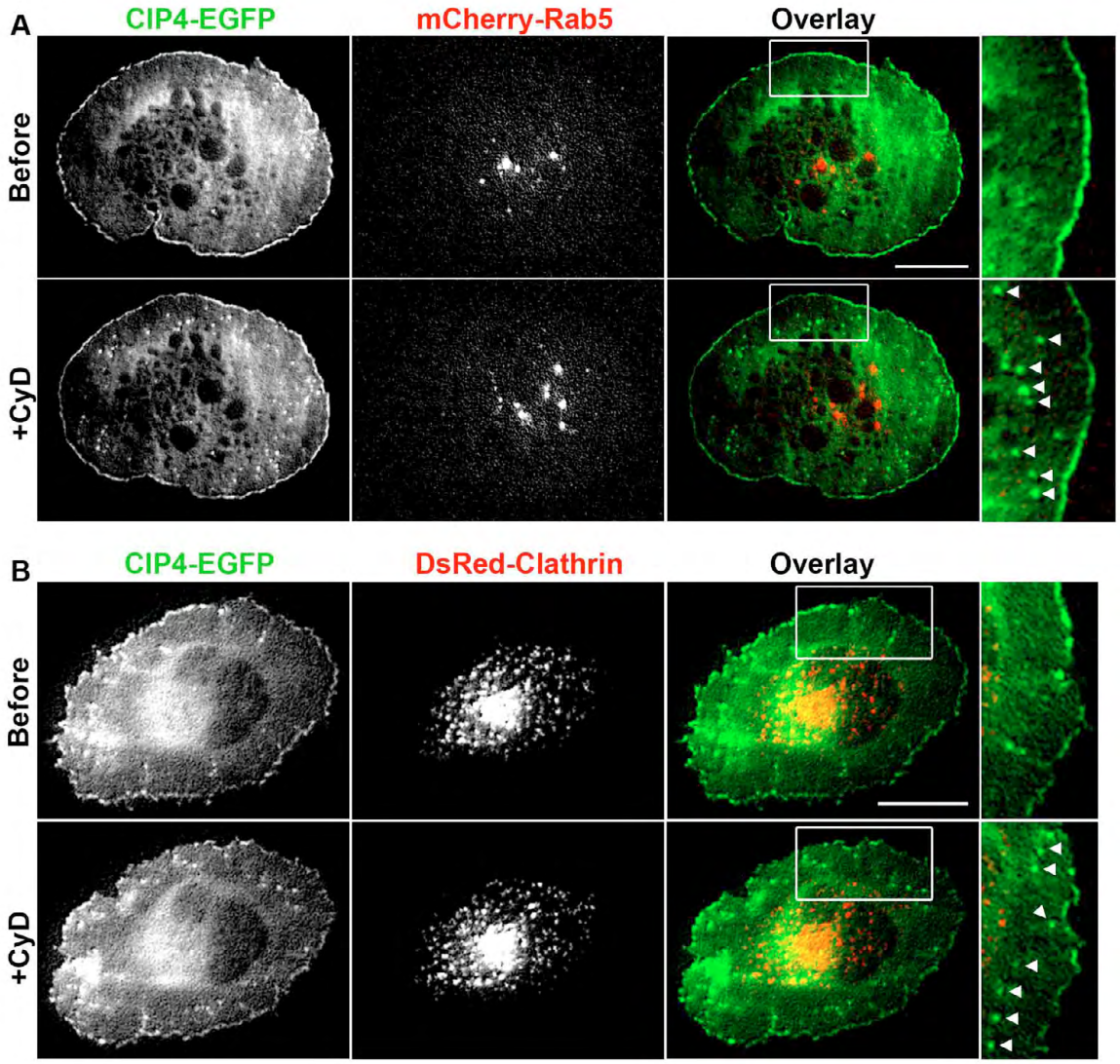


Fig. S3. CIP4 puncta that developed after cytochalasin D treatment do not overlap with Rab5 or Clathrin. (**A, B**) Images of a living stage 1 cortical neuron co-transfected with CIP4-EGFP and mCherry-Rab5 (**A**) or DsRed-Clathrin (**B**) before and after Cytochalasin D (CyD) treatment (100nM). White arrowheads indicate CIP4 puncta moving retrogradely, away from the membrane edge. Scale bars, 10 μm.

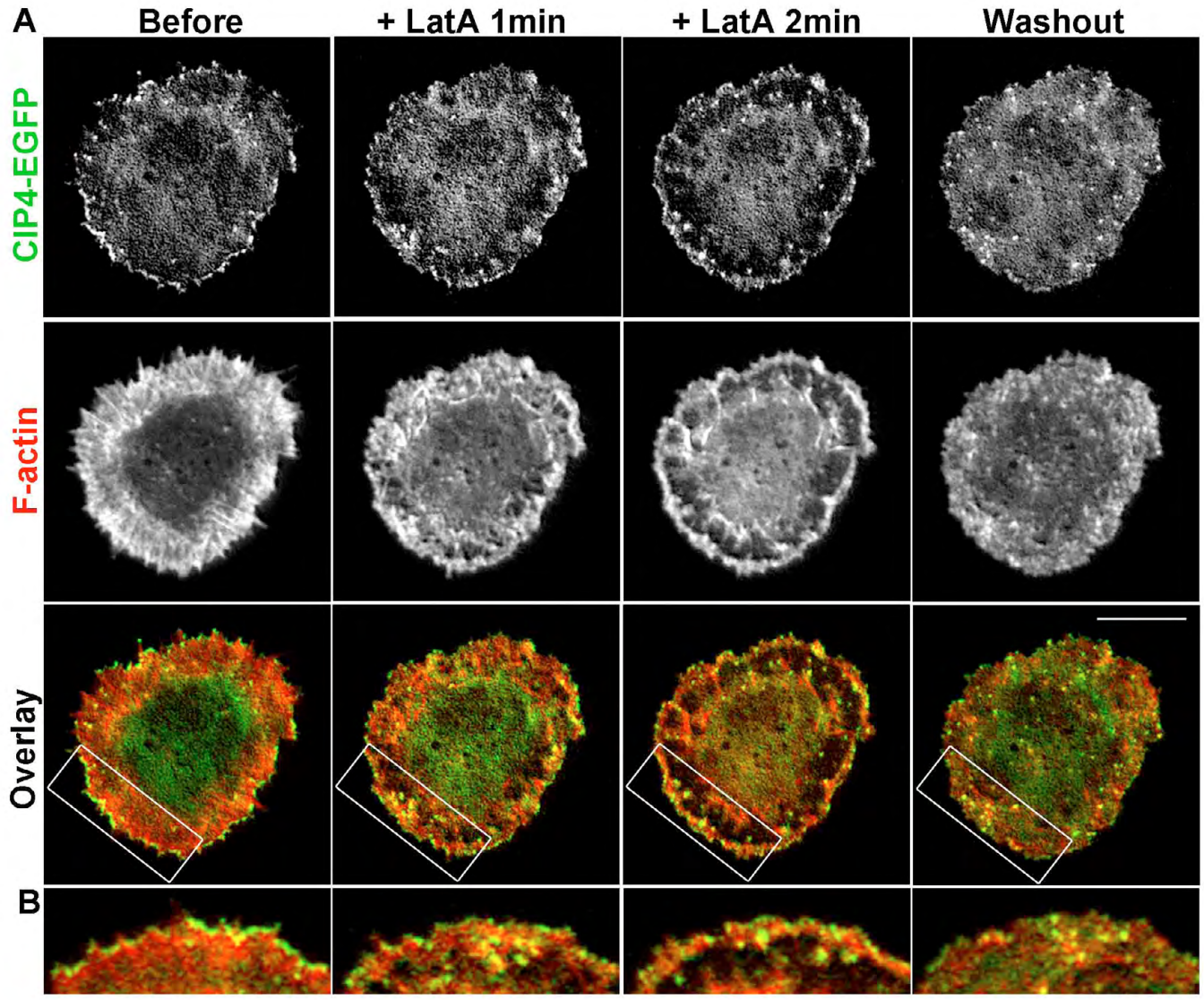


Fig. S4. Latranculin A does not induce membrane tubulation in CIP4 expressing neurons. (**A**) Image of a stage 1 cortical neuron transfected with CIP4-EGFP and tdTomato-F-tractin before Latranculin A (LatA) treatment (1μM), one and two minutes after LatA treatment and after washout. (**B**) Images magnified from boxes in **A** showing CIP4 at the peripheral area. Scale bars, 10 μm.

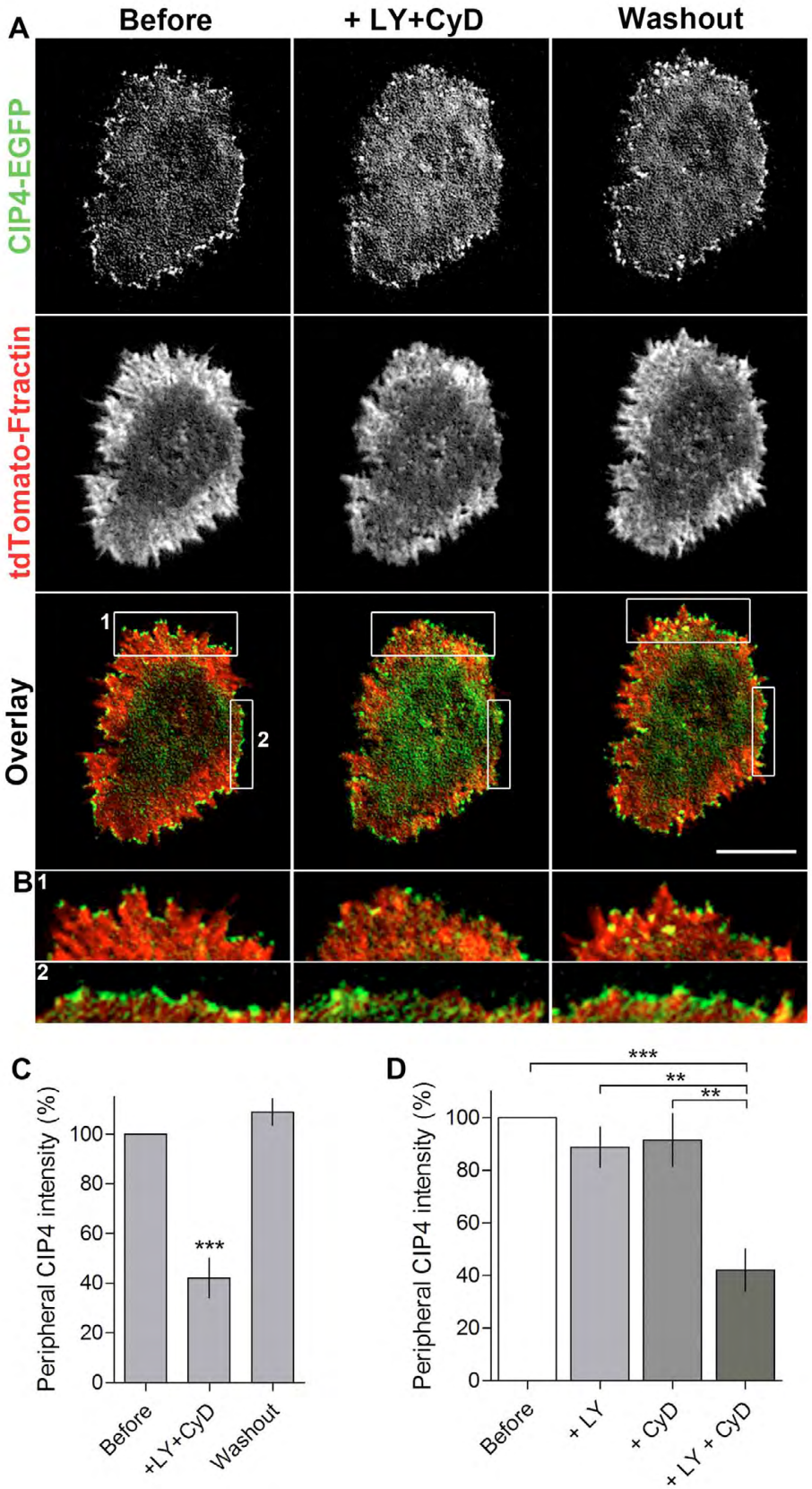
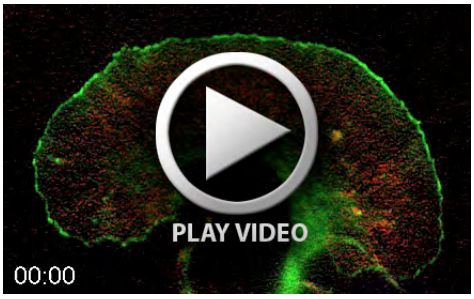


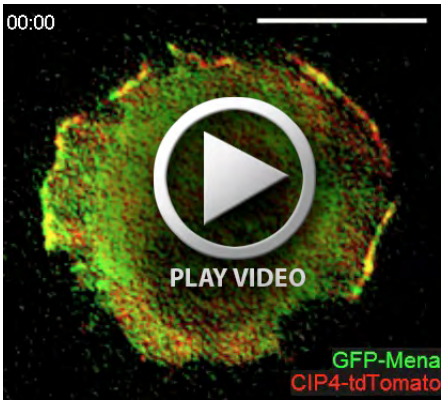
Fig. S5. PI3K inhibitor and CyD synergistically reduce CIP4 from the protruding edge. (**A**) Image of a stage 1 cortical neuron transfected with CIP4-EGFP and tdTomato-F-tractin before and after LY294002 (LY) + Cytochalasin D (CyD) treatment. (**B**) Images magnified from boxes in **A** showing CIP4 at the peripheral area. (**C**) Bar graph of average intensity of CIP4 at the peripheral membrane of the cells in CIP4 transfected neurons before, after 1minute of LY294002 (LY) + Cytochalasin D (CyD) treatment and after washout. (**D**) Bar graph shows comparison of the effect of LY alone, CyD alone and LY+CyD on CIP4 intensity at the peripheral membrane after 1 minute treatment (n =6, 5 and 4 cells, respectively from three independent experiments). Data are expressed as mean ± SEM. **, P <0.01 and ***, P <0.001 compared with before treatment (One-way ANOVA with Dunnett's (**C**) or Bonferroni (**D**) post-test comparison) (n =4 cells from three independent experiments). Scale bars, 10 µm.

Movie 1. CIP4 co-localizes with WAVE1 in neurons. A movie of a stage1 cortical neuron transfected with GFP-WAVE1 (green) and CIP4-tdTomato (red) (related to Fig. 3) and imaged in TIRFM. Images captured every 2 seconds. Scale bar, 10 μm.



Movie 2. Cytochalasin D affects CIP4 distribution in neurons. A movie of a lamellipodial growth cone from a cortical neuron transfected with CIP4-EGFP (green) and mRuby-lifeact (red) (related to Fig. 5) and imaged in TIRFM. Note that upon cytochalasin D treatment CIP4 redistributes from the protruding edge to internal puncta that move retrogradely in actin flow. Those puncta then form an actin comet tail after the cytochalasin D washout. Images captured every 6 seconds. Scale bar, 10 μm.

Movie 3. CIP4 co-localizes with DAAM1 in neurons. A movie of a stage1 cortical neuron transfected with GFP-DAAM1 (green) and CIP4-tdTomato (red) (related to Fig. 6A-C) and imaged in TIRFM. Images captured every 2 seconds. Scale bar, 10 μm.



Movie 4. CIP4 co-localizes with Mena in neurons. A movie of a stage1 cortical neuron transfected with GFP-Mena (green) and CIP4-tdTomato (red) (related to Fig. 6G-I) and imaged in TIRFM. Images captured every 2 seconds. Scale bar, 10 μm.