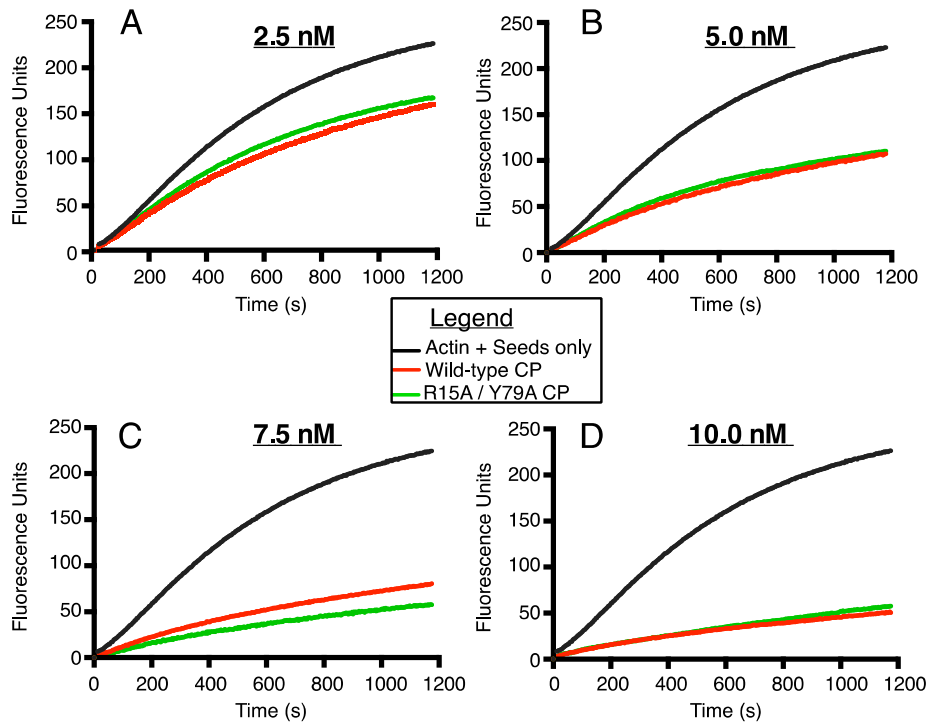
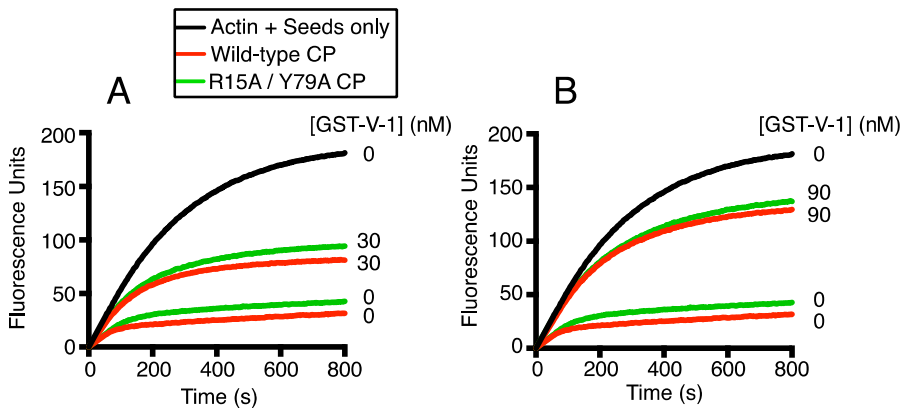


## Supplementary Information

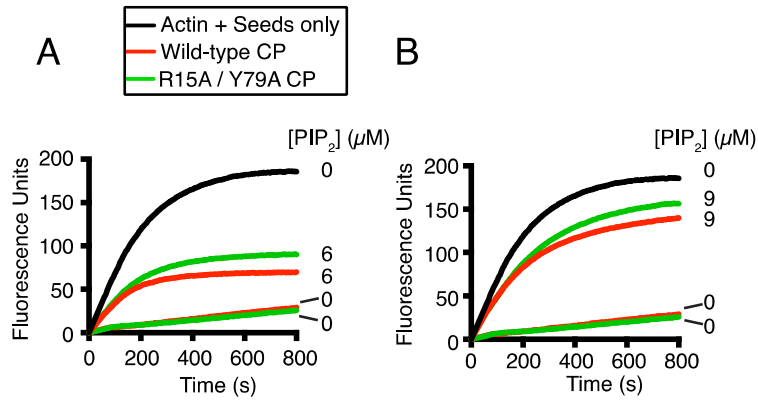


**Supplementary Figure 1. CP R15A/Y79A mutant Caps Actin Normally.** Actin capping activity is not affected by the CP mutations. Actin polymerization from barbed ends, with pyrene-actin fluorescence plotted vs. time. Experiments were performed with the indicated concentrations of wild-type CP (red curves) and R15A/Y79A mutant (green curves). CP concentrations are indicated on the panels.

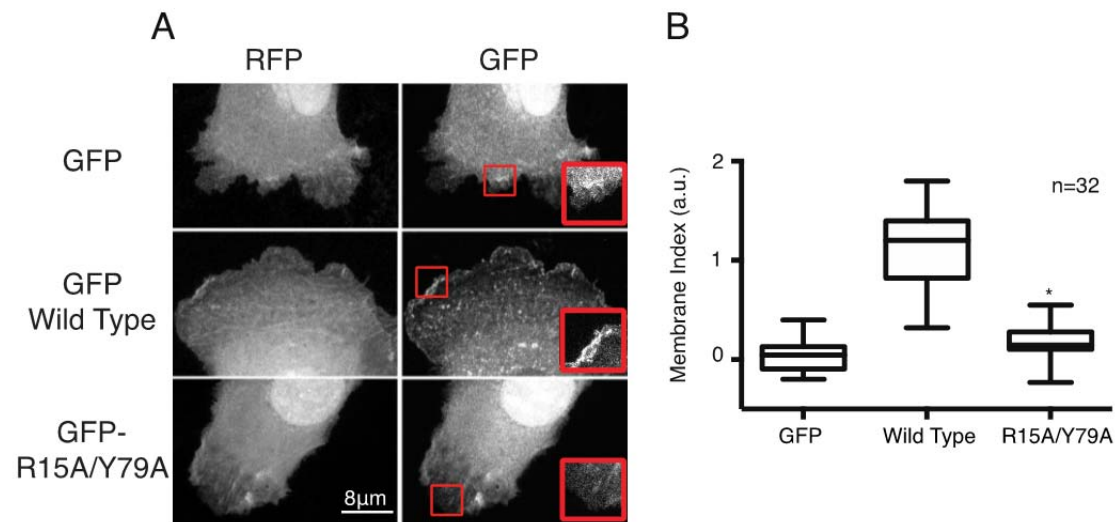


**Supplementary Figure 2. Inhibition of CP by V-1 in Actin Capping Assays.** V-1 inhibits wild-type CP and R15A/Y79A mutant CP to very similar, nearly identical,

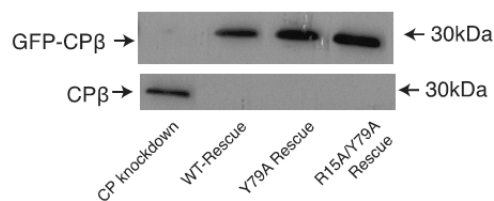
degrees. Actin polymerization from barbed ends, measured by pyrene-actin fluorescence, is plotted vs. time. GST V-1 was added at the beginning of the assays at the concentrations indicated.



**Supplementary Figure 3. Inhibition of CP by PIP<sub>2</sub> in Actin Capping Assays.** PIP<sub>2</sub> inhibits wild-type CP and R15A/Y79A mutant CP to very similar, nearly identical, degrees. Actin polymerization from barbed ends, measured by pyrene-actin fluorescence, is plotted vs. time. PIP<sub>2</sub> was added at the beginning of the assays at the concentrations indicated.



**Supplementary Figure 4. CP Plasma Membrane Localization Depends on the Ability to Bind CPI Proteins.** A) Confocal fluorescence images of HT-1080 cells co-expressing RFP and either GFP, GFP- CP $\beta$ 2 or GFP- CP $\beta$ 2R15A/Y79A. Boxed regions are inset in the bottom right corner of each GFP panel. B) Quantifications of the PM index of each construct. Box-and-whisker plots indicate median, quartiles and 5th / 95th percentiles. n=32. The difference between R15A/Y79A and wild-type is statistically significant ( $p < 0.01$ ); the difference between R15A/Y79A and GFP is not.



**Supplementary Figure 5. Knockdown and Rescue of CP $\beta$ 2.** Anti- CP $\beta$ 2 immunoblot documenting CP knockdown and the expression levels of wild type, single and double mutant rescue constructs