

# Supplemental Materials

*Molecular Biology of the Cell*

Sinnar et al.

## Capping Protein in Filopodia

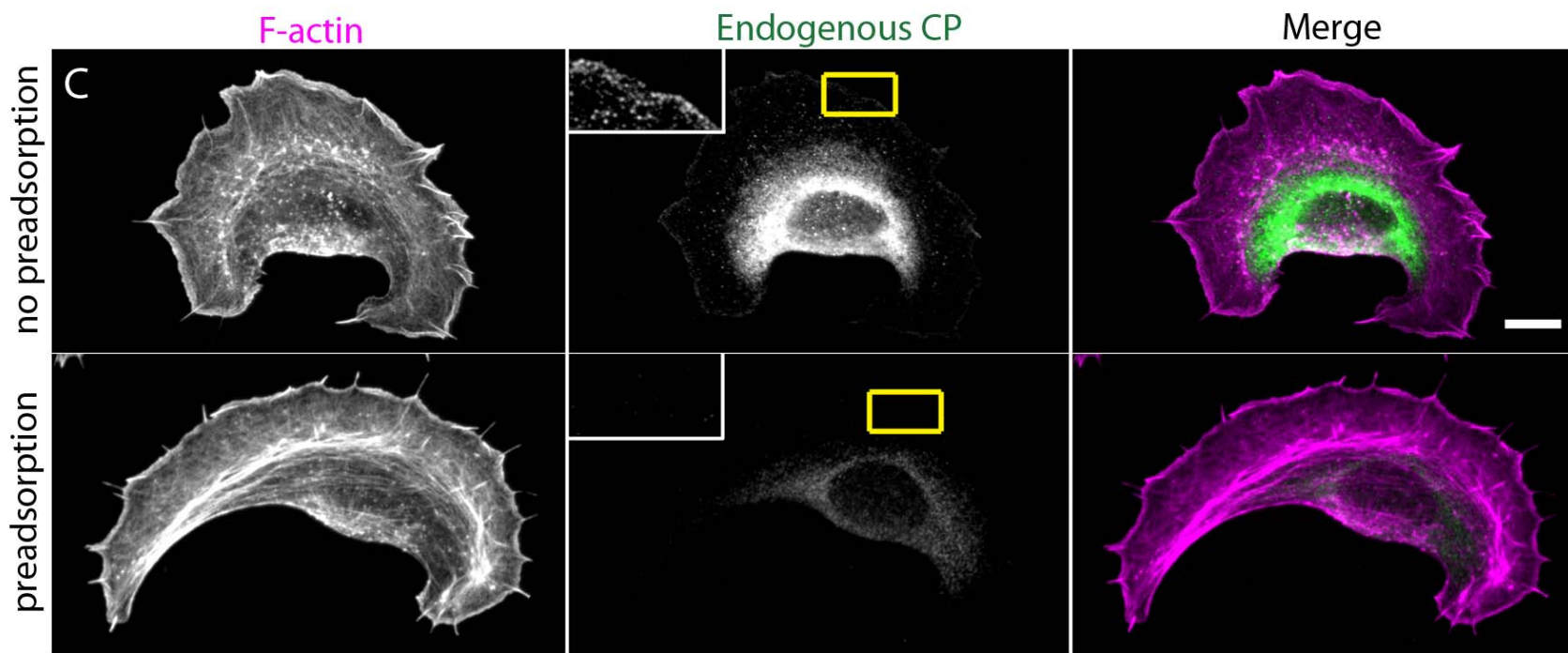
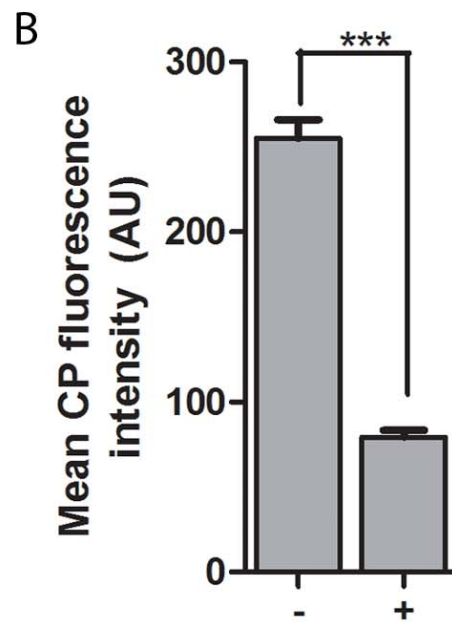
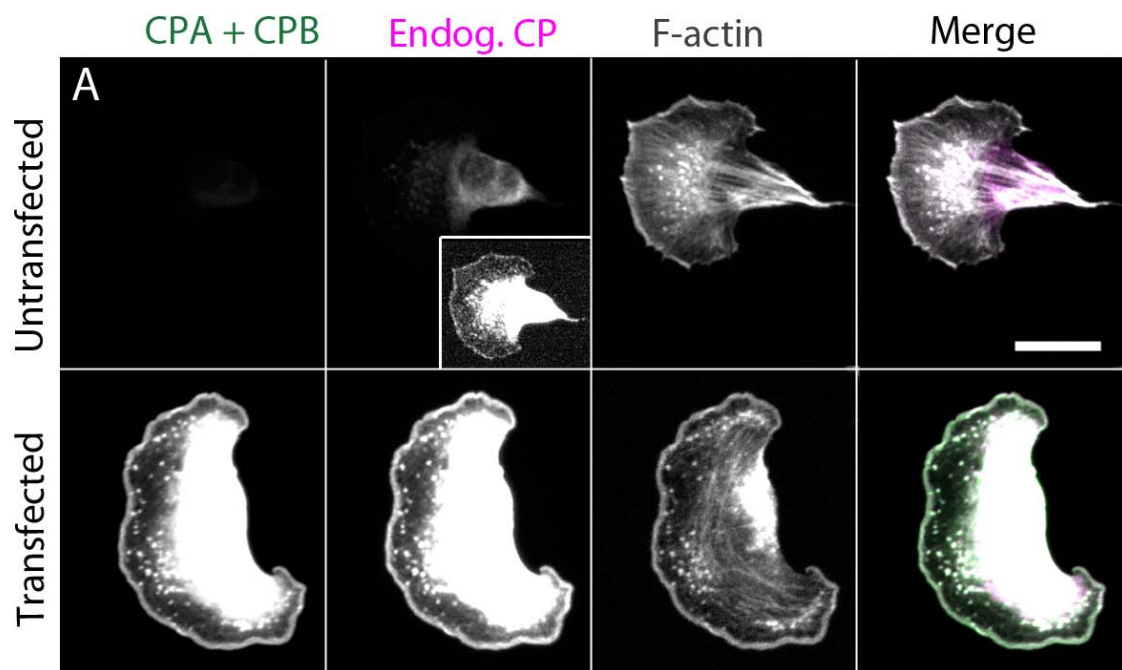
**Supplemental Figure 1.** Millipore AB6017 polyclonal antibody is specific for CP. (A) Immunoreactivity increases with overexpression of eGFP-tagged CP  $\alpha$ 2 and  $\beta$ 2 subunits in B16F10 melanoma cells (compare top row, untransfected, with bottom row, transfected). Images of endogenous CP ir in the untransfected and transfected cells are shown with identical display settings. Inset in the top row is a contrast-enhanced image that shows that CP ir is indeed present in the expected distribution in a non-transfected cell. (B) Mean CP ir decreases with preadsorption of antibody with holoprotein. -, no preadsorption; +, plus preadsorption. Thirty cells were analyzed in each category;  $p < 0.001$ . Scale bar, 20  $\mu$ m. (C) An example of cells stained with CP antibody without (top) and with (bottom) prior preadsorption of antibody with holoprotein. Note absence of signal at leading edge (boxed regions and insets) and greatly diminished signal in cell interior of preadsorbed cell. Zoomed images of the leading edge (insets) are shown with identically enhanced display settings. Scale bar, 10  $\mu$ m.

**Supplemental Figure 2.** Endogenous CP is detected in filopodia of hippocampal growth cones. (A and B) Two different examples of growth cones from primary hippocampal neurons illustrating immunopositivity for CP. (B) CP-ir detectable along the shaft (arrowheads) and at the presumptive tip (arrow) of individual filopodia. Scale bar, 5  $\mu$ m.

**Supplemental Figure 3.** Silencing of CP using a different targeting sequence results in a similar filopodial phenotype. B16F10 cells were transfected with either a mismatch control sequence, T2\* (*CTL*) or a sequence directed against the CP  $\beta$  subunit, T2 (*KD*). After 5d, cells were fixed and stained with phalloidin to visualize F-actin. Right panels show magnifications of boxed regions. *CTL* and *KD* images are shown with identical display settings. Arrowheads point to cattail filopodia. Scale bar, 5  $\mu$ m.

**Supplemental Video 1.** This sequence shows filopodial dynamics from a control cell co-transfected with RFP-LifeAct. This is a 2 minute sequence and frames are 5 sec apart. Playback: 7 frames/sec.

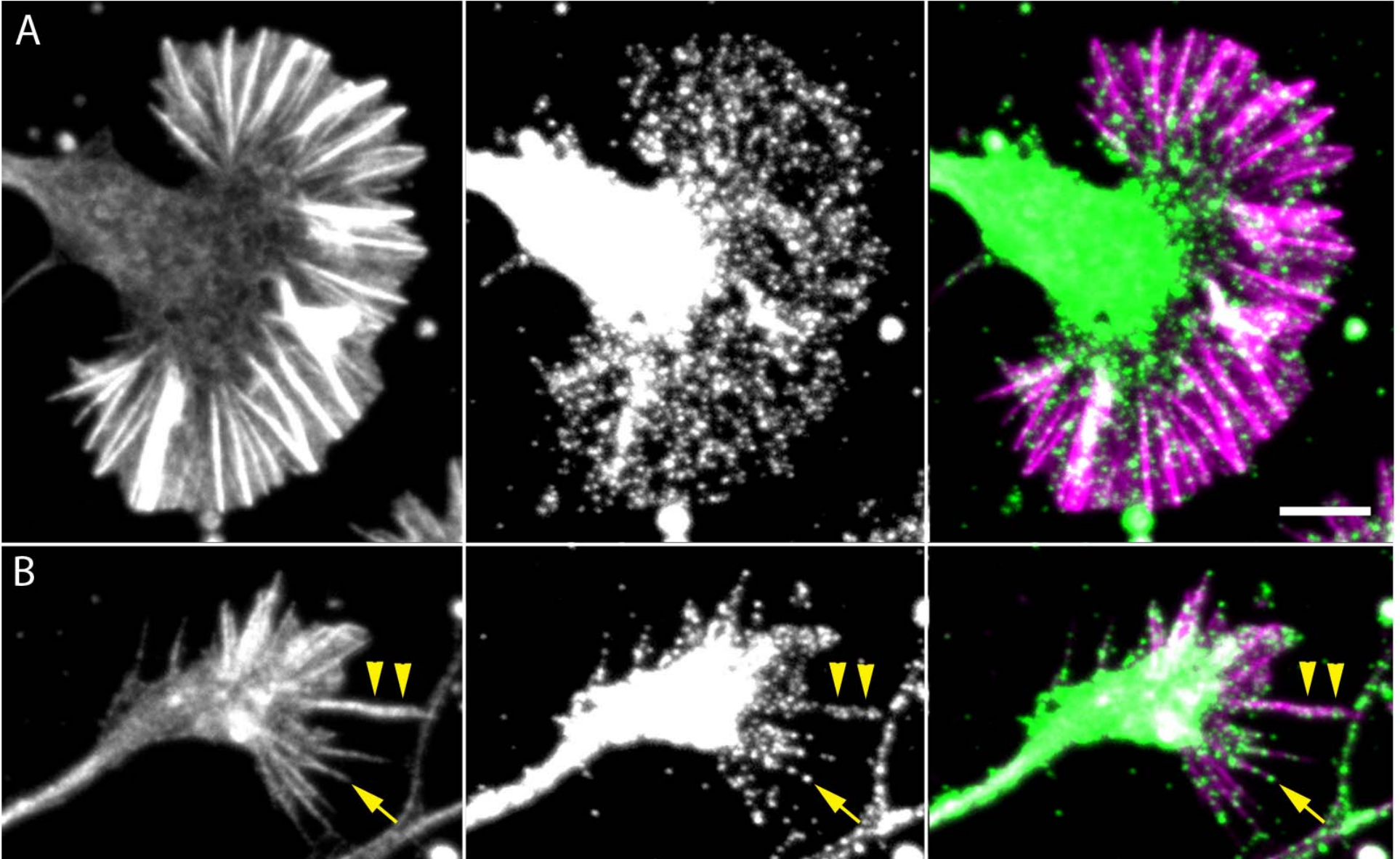
**Supplemental Video 2.** This sequence shows filopodial dynamics from a knockdown cell co-transfected with RFP-LifeAct. This is a 2 minute sequence and frames are 5 sec apart. Playback: 7 frames/sec.



F-actin

CP

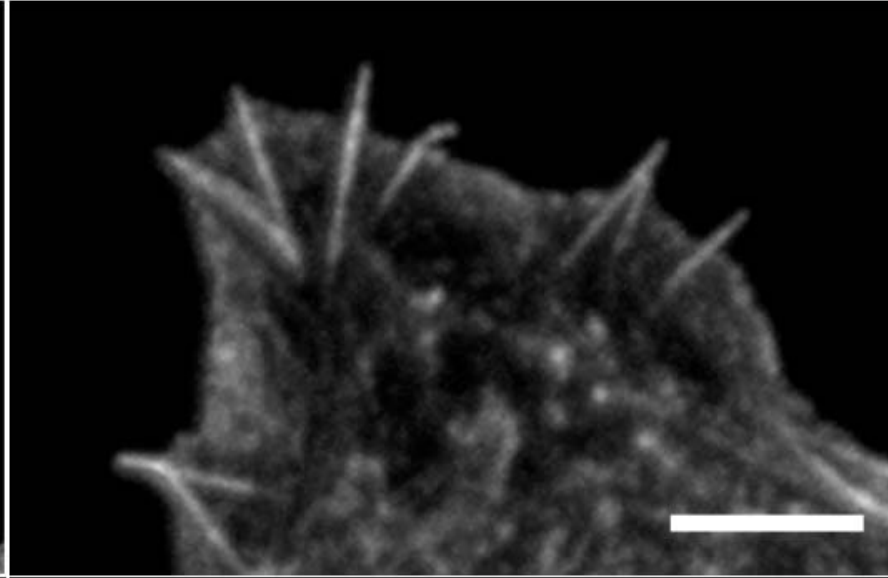
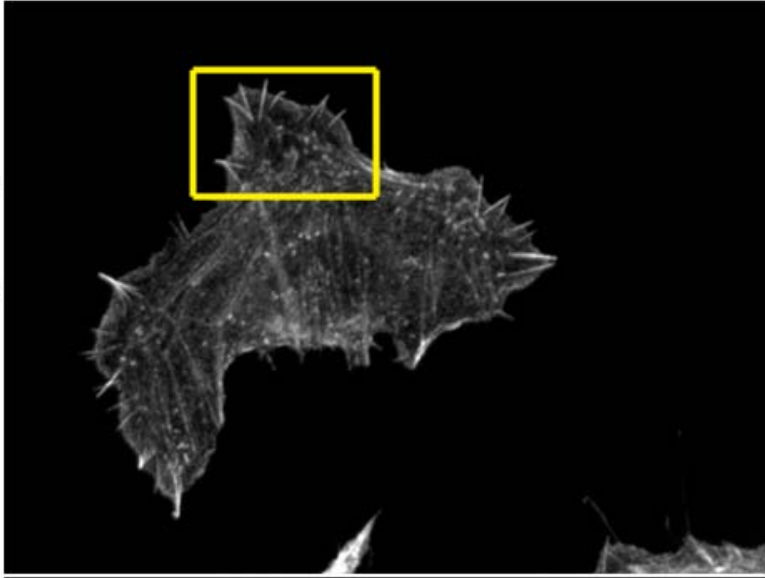
merge



hippocampal growth cones

F-actin

CTL (T2\*)



KD (T2)

