Yang et al., http://www.jcb.org/cgi/content/full/jcb.201111052/DC1

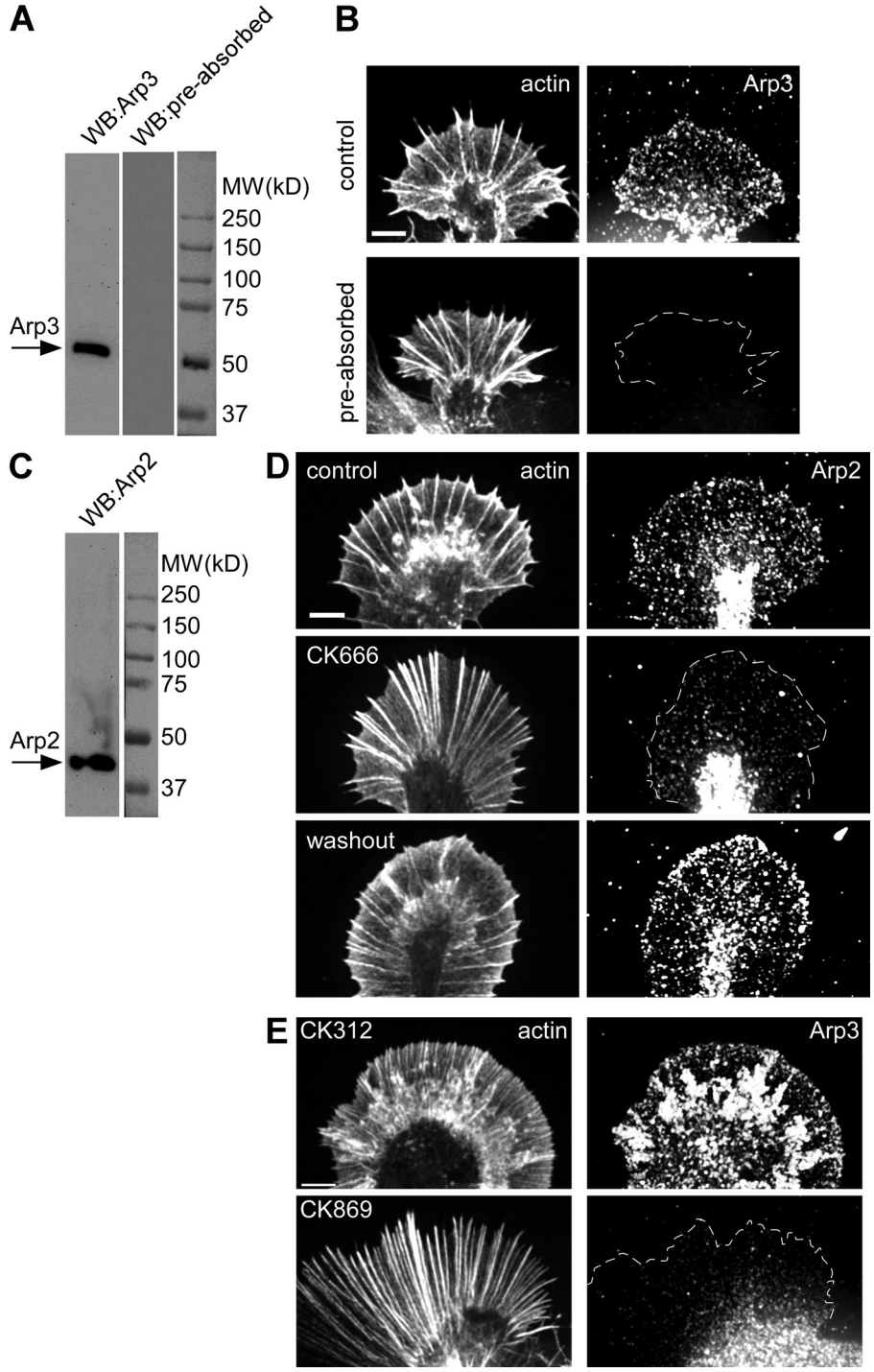


Figure S1. **Verification of Arp2/3 complex localization in neuronal growth cones.** (A) Western blot analysis of *Aplysia* CNS proteins with anti-Arp3 antibody with or without preabsorption using purified bovine Arp2/3 complex. (B) Immunolabeling of *Aplysia* bag cell neuron growth cones using anti-Arp3 antibody with or without preabsorption. Actin was visualized with Alexa 594 phalloidin. Bar, 5 µm. (C) Western blot analysis of *Aplysia* CNS proteins with anti-Arp2 antibody. (D) Immunolabeling of growth cones with Arp2 antibody and Alexa 594 phalloidin after normal fixation. Growth cones were treated with vehicle (DMSO, top), CK666 (100 µM, 20 min, middle), or CK666 (100 µM, 20 min) followed by washout (30 min, bottom). (E) Immunolabeling of growth cones with anti-Arp3 antibody and Alexa 594 phalloidin after treatment with CK869 (100 µM, 20 min) or CK312 (100 µM, 20 min). Bar, 5 µm.

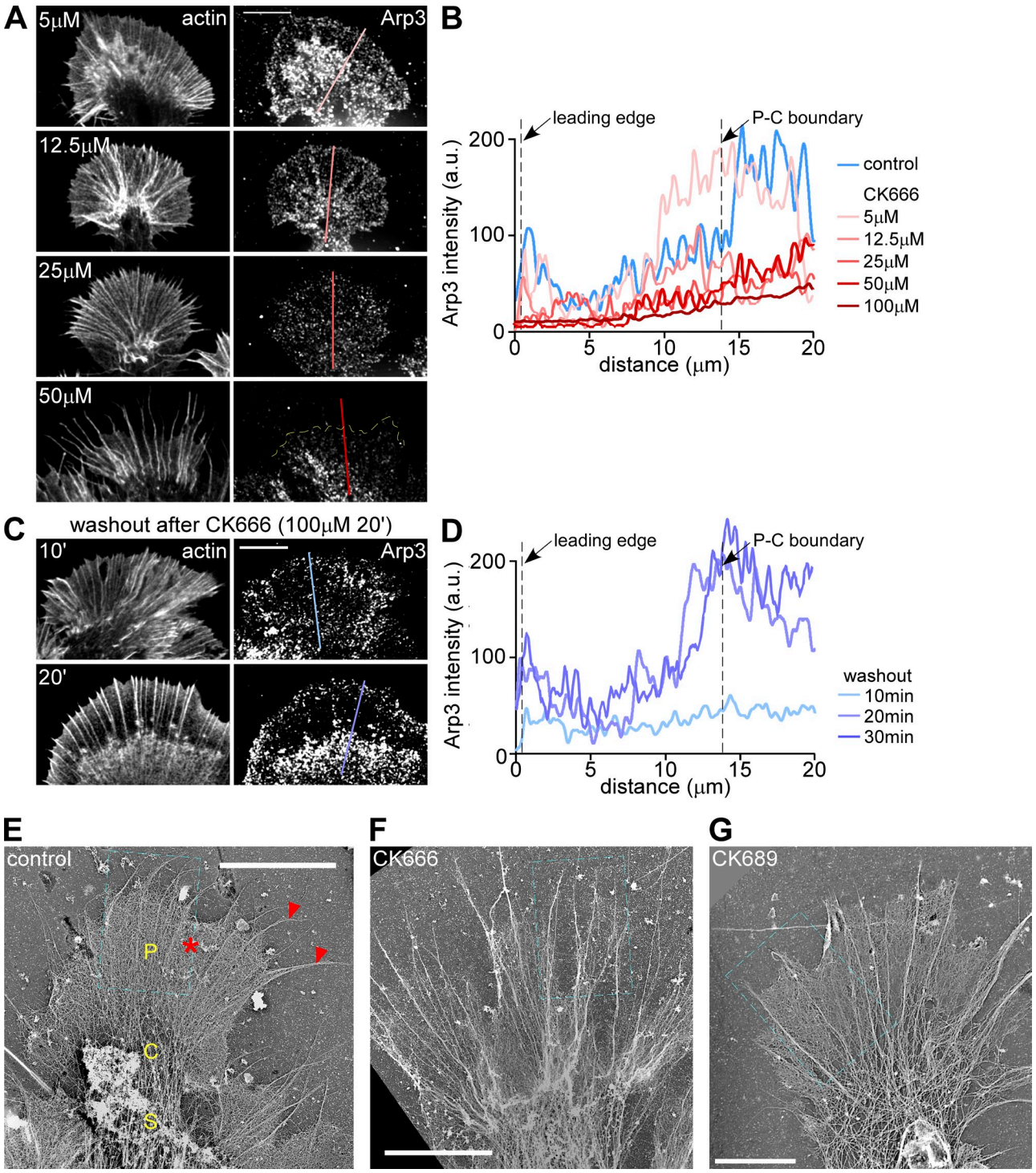
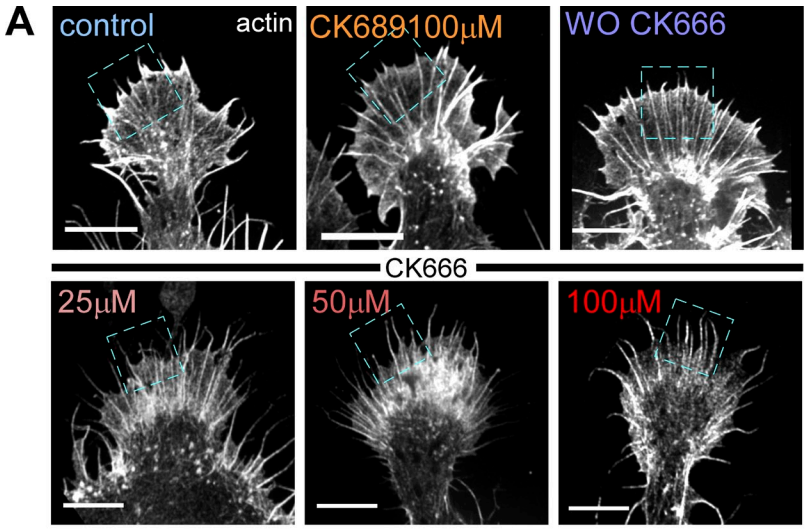
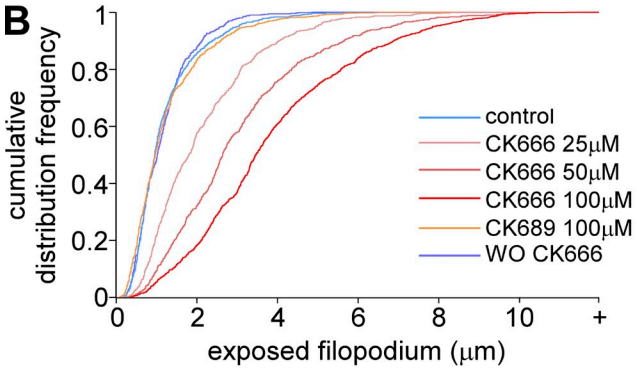


Figure S2. **CK666 treatment dose-dependently and reversibly delocalizes Arp2/3 complex from the leading edge and induces changes in growth cone ultrastructure.** (A) Fluorescent images of growth cones treated with various concentrations of CK666 for 20 min, fixed and labeled with Arp3 antibody (right) and Alexa 594 phalloidin (left). Yellow dotted line demarcates the leading edge. (B) Arp3 distribution profile sampled from the designated lines in A. Control and CK666 100 μM lines from Fig. 1 C are shown for comparison. (C) Arp3 and actin labeling of growth cones treated with CK666 (100 μM) for 20 min followed by 10 or 20 min recovery in control medium. (D) Arp3 distribution profile sampled from the designated lines in C. Washout 30-min line from Fig. 1 C is shown for comparison. Bars, 10 μm. (E–G) Electron micrographs of growth cones treated with vehicle (E, DMSO), CK666 (F, 100 μM), or CK689 (G, 100 μM) for 20 min. P, peripheral domain; C, central domain; S, neurite shaft. Arrowheads, filopodia; asterisk, veil. Areas indicated by blue boxes are presented in high magnification in Fig. 2. Bars, 10 μm.





C

		control	CK666 25μM	CK666 50μM	CK666 100μM	CK689 100μM	WO after CK666
KS test against control	D		0.351	0.549	0.677	0.066	0.060
	p		2.9×10^{-39}	2.3×10^{-89}	2.7×10^{-179}	0.06	0.15
n	filopodia	766	675	596	1060	850	655
	GC	22	22	24	41	37	27

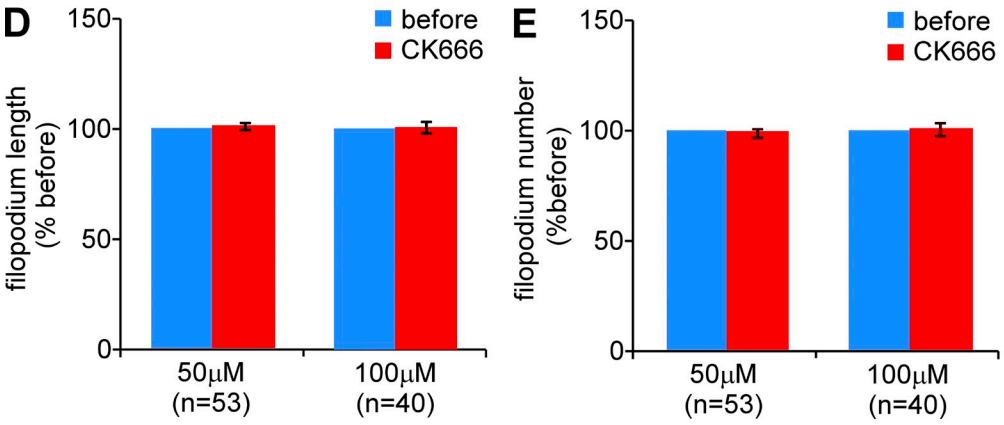


Figure S3. **Arp2/3 complex inhibitor CK666 reversibly retracts actin veil but does not affect filipodium length or number.** (A) Fluorescent labeling of growth cones with Alexa 594 phalloidin after normal fixation. Growth cones were treated with vehicle (DMSO), CK689 (100 μM), or various concentrations of CK666 for 20 min, or treated with CK666 (100 μM) for 20 min followed by washout in regular medium for 30 min. Regions marked by blue boxes are shown in Fig. 2 D. (B) Cumulative distribution frequency plots of data from histograms in Fig. 2 E. (C) Significance assessment by Kolmogorov-Smirnov test with control data as the reference. D, maximum vertical deviation between two curves; p, significance level. (D) Average filopodium length in growth cones before and after treatment with CK666 (50 or 100 μM, 15–30 min). (E) Filopodium number in growth cones before and after treatment with CK666 (50 or 100 μM, 15–30 min). Data are normalized to the values before CK666 addition for each growth cone. Two-tailed paired *t* test showed no significant difference. n, number of growth cones measured.

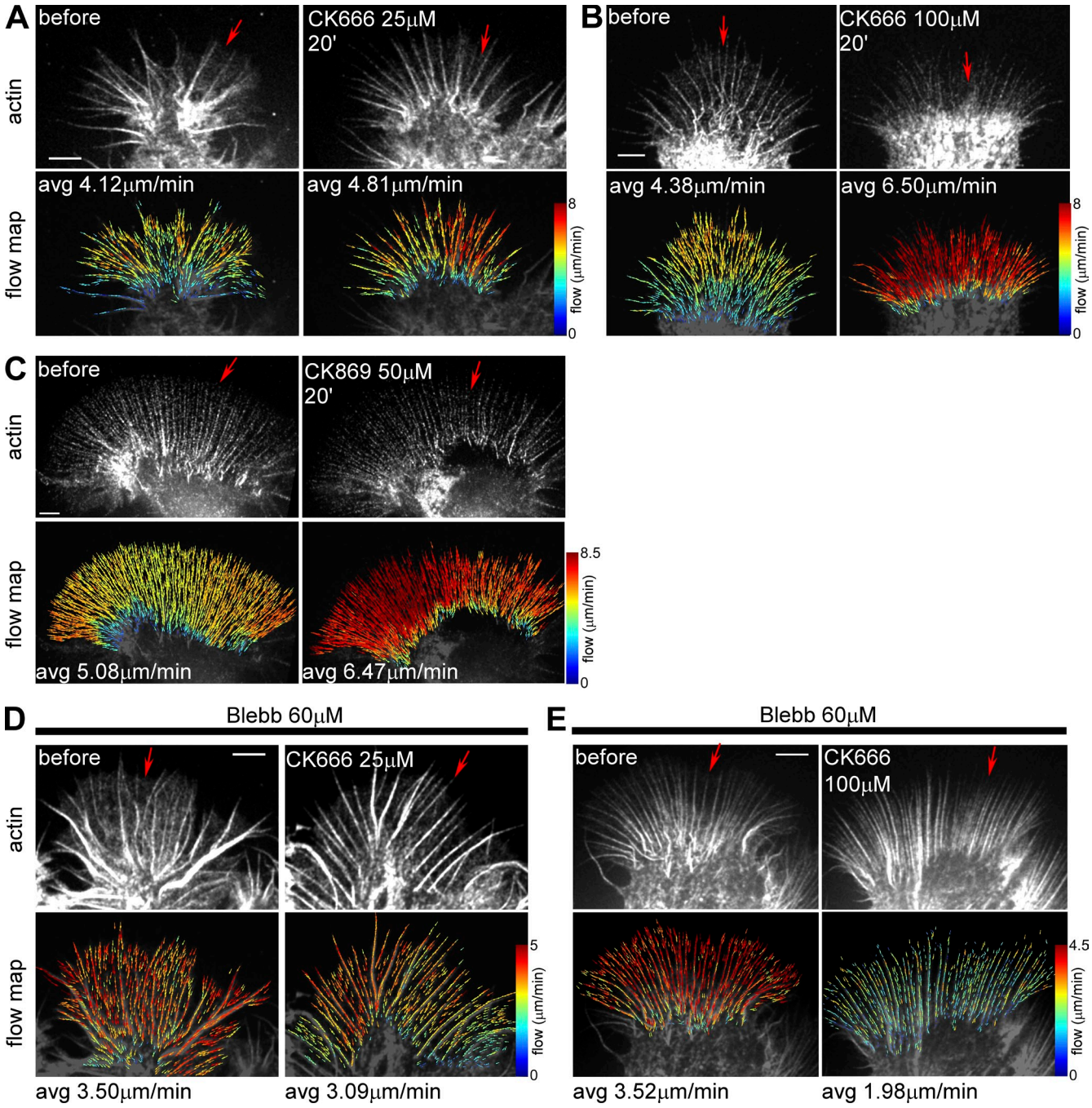
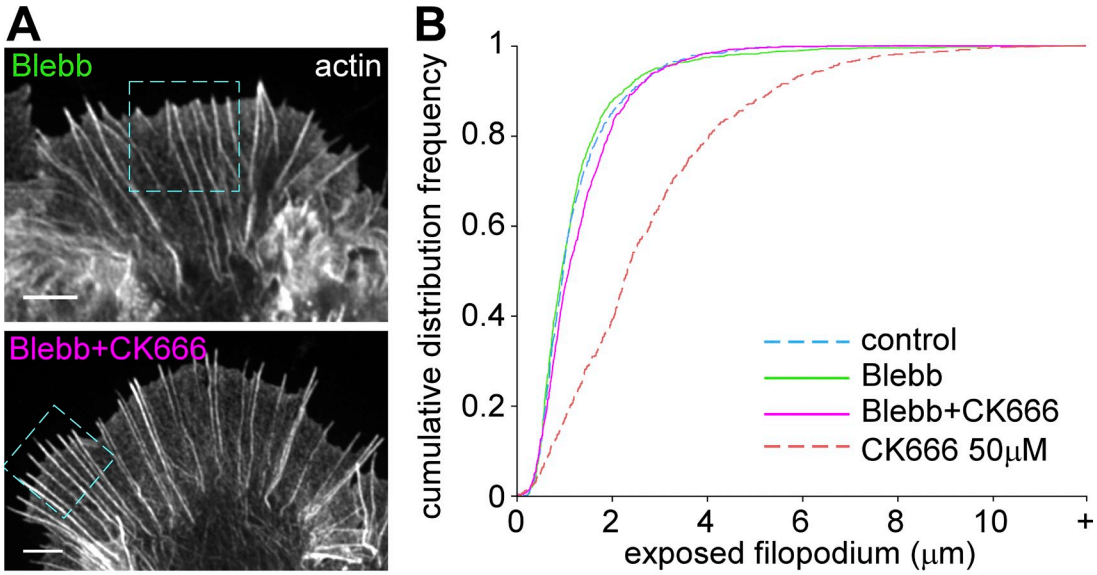


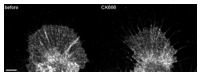
Figure S4. **Arp2/3 complex inhibition increased peripheral F-actin retrograde flow rate in a myosin II–dependent manner.** (A–E) Representative examples of FSM images (top) and corresponding flow maps (bottom) of growth cones injected with Alexa 594 phalloidin. (A and B) Before and after treatment for 20 min with (A) 25 µM CK666 or (B) 100 µM CK666. (C) Before and after treatment for 20 min with 50 µM CK869. (D and E) Before and after treatment for 20 min with (D) 25 µM CK666 or (E) 100 µM CK666 in 60 µM blebbistatin (10 min pretreatment). Arrows in FSM images mark the edge of the actin veil. On flow maps, colors encode speed (see color bar) and vectors indicate flow direction. Bar, 5 µm.



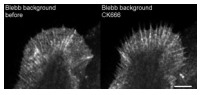
C

		control	Blebb	Blebb +CK666	CK666 50μM
KS test against control	D		0.057		0.549
	p		0.10		2.3×10^{-89}
KS test against Blebb	D			0.135	
	p			4.5×10^{-9}	
n	filopodia	766	1097	1060	596
	GC	22	40	38	24

Figure S5. **Myosin II inhibition attenuates veil retraction evoked by Arp2/3 complex inhibition.** (A) Fluorescent labeling of growth cones with Alexa 594 phalloidin after normal fixation. Growth cones were treated with blebbistatin (60 μM, 20 min, top) or pretreated with blebbistatin (60 μM, 10 min) followed by blebbistatin and CK666 (100 μM, 20 min, bottom). Blue boxed areas are shown in Fig. 8 C. Bar, 5 μm. (B) Cumulative distribution frequency plots of data from histograms in Fig. 8 D. Dotted curves from Fig. S3 B are shown for comparison. (C) Significance assessment by Kolmogorov-Smirnov test with control or blebbistatin data as the reference. D, maximum vertical deviation between two curves; p, significance level.



Video 1. **CK666 (50 μM, 20 min) increases the peripheral retrograde F-actin flow rate.** FSM time lapse of F-actin dynamics before and after 20 min in CK666 recorded from an *Aplysia* bag cell neuron growth cone in a cell injected with trace levels of Alexa 568 G-actin. Images were acquired with a spinning disk system (Revolution XD; Andor) using a confocal head (CSU-X1; Yokogawa) coupled to an inverted microscope (TE 2000E; Nikon) equipped with Perfect Focus. Images were recorded with an EMCCD camera (iXonEM+ 888; Andor). Bar, 5 μm. 5-s interval; elapsed time, 2 min; playback, 8 frames per second. This movie is from the growth cone in Fig. 4 A.



Video 2. **CK666 (50 μM, 20 min) decreases the peripheral retrograde F-actin flow rate in blebbistatin (60 μM, 10 min pretreatment and present throughout) backgrounds.** FSM time lapse of F-actin dynamics before and after 20 min in CK666 recorded from an *Aplysia* bag cell neuron growth cone in a cell injected with trace levels of Alexa 568 G-actin. Images were acquired with a spinning disk system (Revolution XD; Andor) using a confocal head (CSU-X1; Yokogawa) coupled to an inverted microscope (TE 2000E; Nikon) equipped with Perfect Focus. Images were recorded with an EMCCD camera (iXonEM+ 888; Andor). Bar, 5 μm. 5-s interval; elapsed time, 2 min; playback, 8 frames per second. This movie is from the growth cone in Fig. 5 A.