Ganguly et al., http://www.jcb.org/cgi/content/full/jcb.201506110/DC1

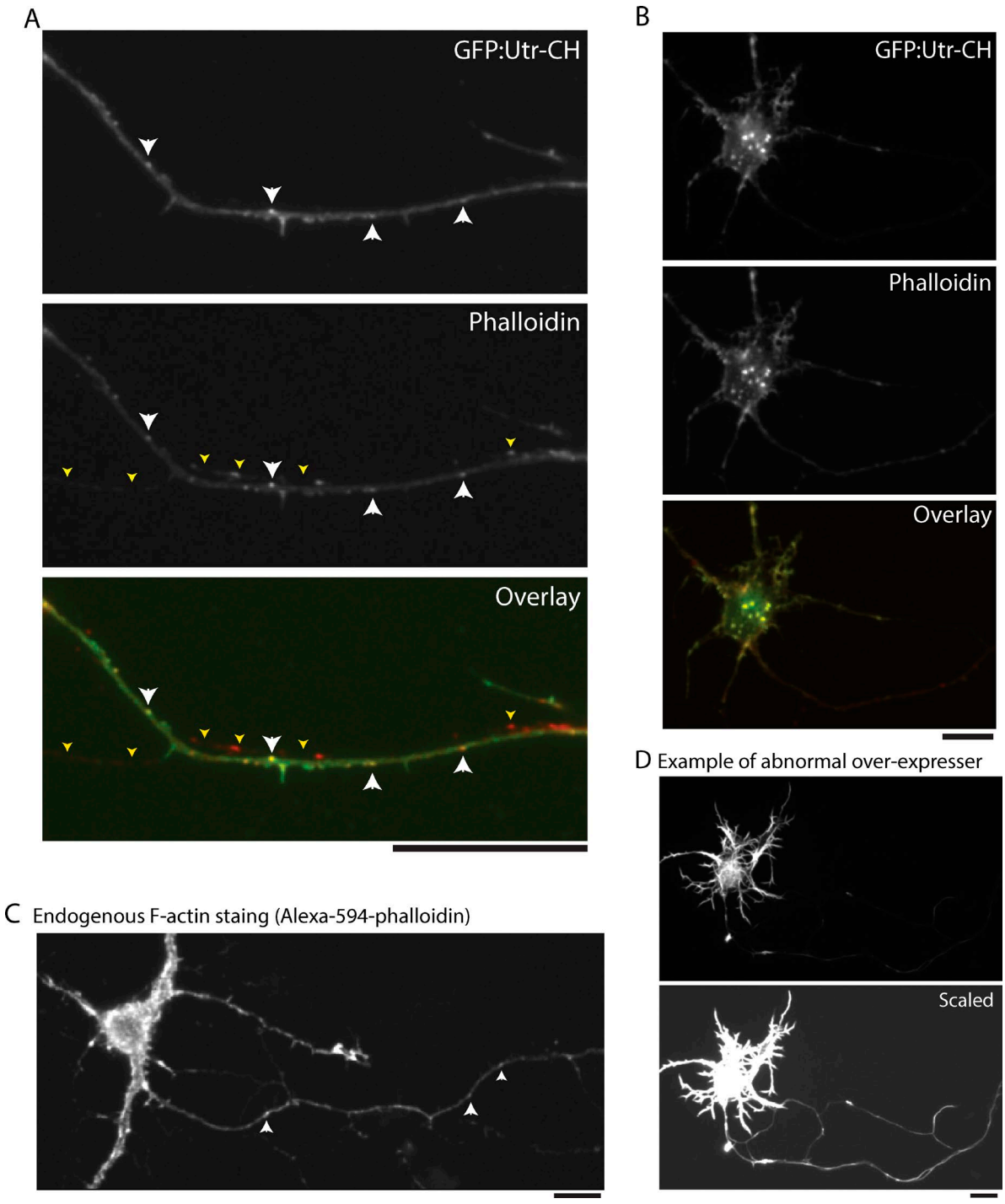
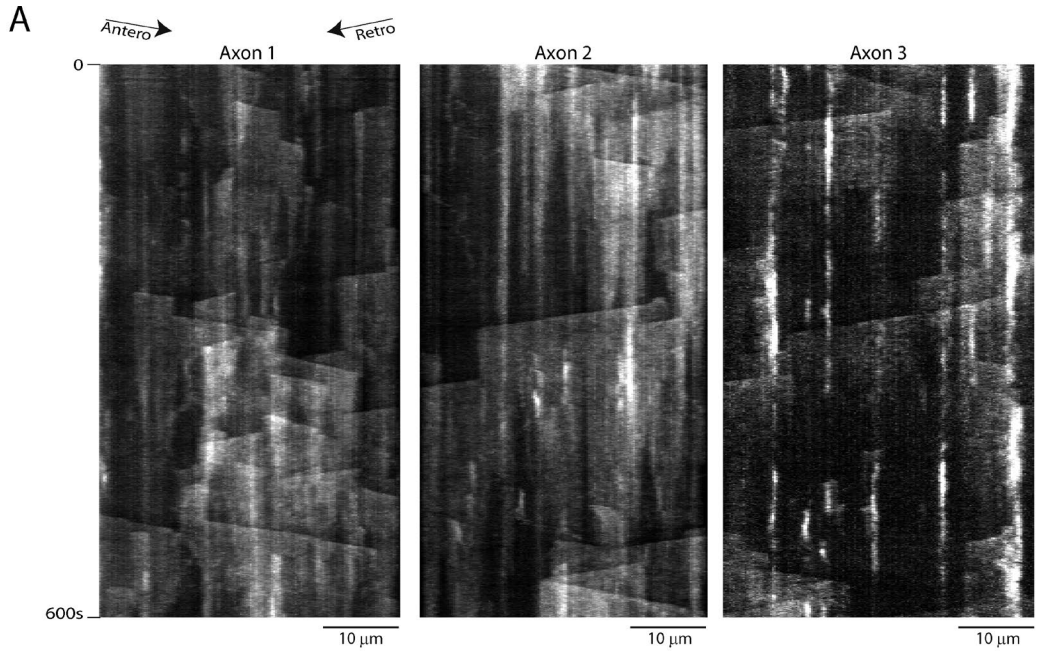
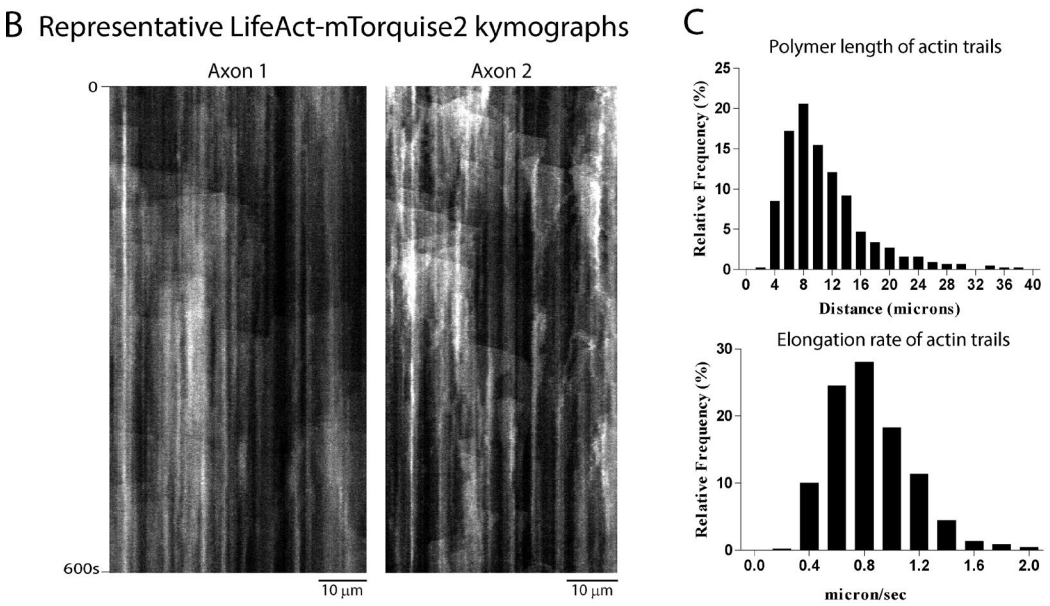


Figure S1. **Characterization of GFP:Utr-CH expression in neurons.** (A) Axon of a neuron transfected with GFP:Utr-CH and stained with rhodamine-phalloidin. Note that the punctate pattern of GFP:Utr-CH fluorescence precisely coincides with rhodamine-phalloidin staining in the same axon (a few puncta are marked with large arrowheads). The smaller arrowheads mark the phalloidin staining in an untransfected axon, and there were no apparent differences between the phalloidin staining in transfected and untransfected neurons. (B) Another neuron transfected with GFP:Utr-CH and stained with rhodamine-phalloidin. Note the low GFP fluorescence in the soma and dendrites, and the precise colocalization of GFP:Utr-CH and rhodamine-phalloidin puncta. (C) A neuron fixed and stained with labeled phalloidin. Note punctate staining of endogenous F-actin (arrowheads). (D) An example of a neuron expressing high levels of GFP:Utr-CH. At high concentrations, Utr-CH stabilizes actin filaments. Note F-actin "cables" distributed throughout the neuron (scaled image on right), distinct from the punctate distribution seen in lower expressers. No movement of F-actin was discernable in such neurons at all. Bars, 10 µM.





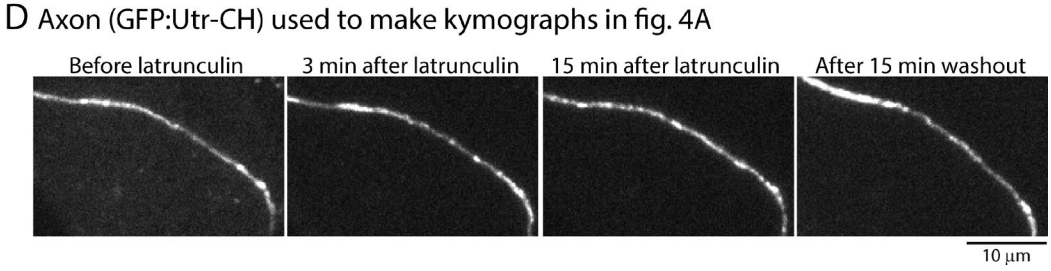


Figure S2. **Kymographs from F-actin imaging in axons with two independent probes.** (A) Kymographs from three independent axons demonstrate consistent GFP:Utr-CH dynamics, across transfected axons. Note the F-actin hotspots and F-actin trails. (B) Representative kymographs from two LifeAct-mTurquoise2–transfected axons. Note that the overall F-actin kinetics are similar to those seen with GFP:Utr-CH, though there is a higher soluble pool, likely caused by the relatively low binding affinity of LifeAct compared with Utr-CH (Field and Lénárt, 2011). (C) Frequency distribution plots of both polymer length (10.89 ± 0.27 µm, 448 events from 19 axons) and elongation rate (0.85 ± 0.01 µm/s, 448 events from 19 axons) for all LifeAct-transfected axons imaged. (D) The axon (transfected with GFP:Utr-CH, first frame of the video) used to make the kymographs shown in Fig. 4 A. Antero, anterograde; Retro, retrograde.

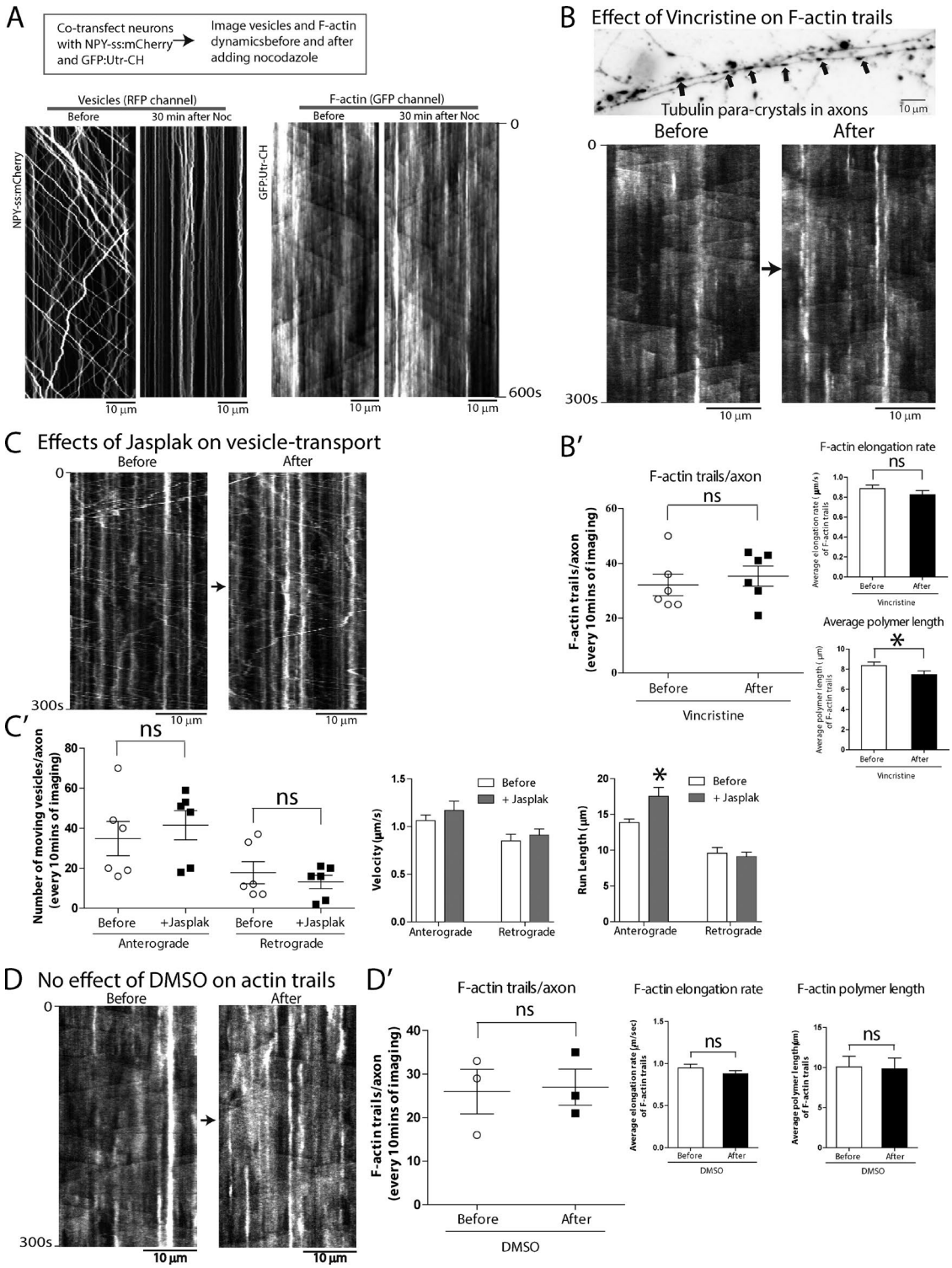


Figure S3. **Microtubule disruption does not alter F-actin dynamics.** (A) Neurons were cotransfected with a vesicle-marker (NPYss:mCherry) and GFP:Utr-CH. GFP and mCherry dynamics were evaluated by live imaging before and after adding 10 µg/ml nocodazole. Note that the 30-min incubation with nocodazole (Noc) almost completely blocked vesicle transport (left) but had no obvious effect on F-actin kinetics (right). (B) The microtubule-disrupting drug vincristine (5 µM), when incubated with neurons for 60 min results in the formation of tubulin paracrystals, seen after staining for Tubulin-βIII in axons (top). Note that the formation of tubulin paracrystals does not alter GFP:Utr-CH dynamics. Bottom shows representative kymographs from an axon, before and after vincristine treatment. (B′) Quantification of GFP:Utr-CH dynamics before and after 5 µM vincristine treatment (n = 6 axons imaged, for detailed statistics see Table S1). (C) Neurons were transfected with synaptophysin:dsRed, a vesicle marker and incubated with jasplakinolide. Representative kymographs from an axon before and after 100 nM jasplakinolide (Jasplak) treatment for 15 min show no effect on vesicle movement. Note that a similar treatment completely obliterates F-actin trails (Fig. 4 B). (C′) Quantification of synaptophysin-dsRed vesicle dynamics before and after 100 nM jasplakinolide treatment. Note that there is no attenuation of vesicle transport (n = 6 axons imaged, for detailed statistics see Table S2). (D and D′) DMSO, a solvent used to dissolve some reagents in this study did not have any effects on GFP:Utr-CH dynamics. Representative kymograph from an axon and summary statistics in D′ (n = 3 axons imaged, for detailed statistics see Table S1). All values represent means ± SEM. Paired t test. *, $P < 0.05$.

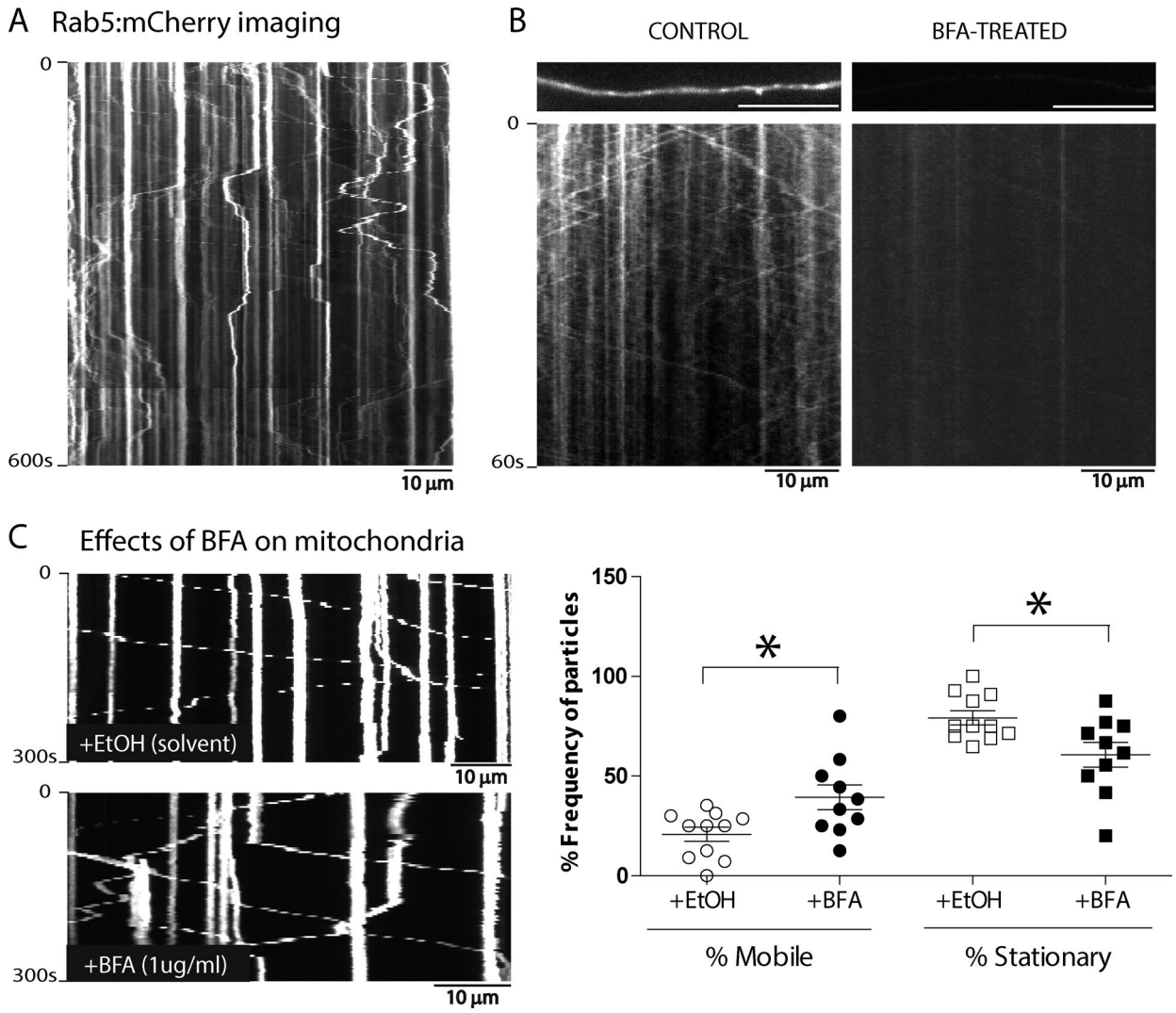


Figure S4. **BFA treatment leads to depletion of Golgi-derived vesicle in axons.** (A) An exemplary kymograph showing kinetics of Rab5:mCherry in axons. Note that a large number of endosomes are largely stationary along axons, at regularly spaced intervals. (B, top) Representative axonal segments from synaptophysin-dsRed–transfected neurons treated either with 1 µg/ml BFA for 12–16 h or ethanol (solvent control). Note depletion of vesicles in the BFA-treated axon. (bottom) Kymographs for the respective axonal segments. (C) 1 µg/ml BFA treatment for 12–16 h has no deleterious effect on mitochondrial dynamics, though there was a slight increase in the mobile population. Representative kymographs (left) and the quantified data (right, for detailed statistics, see Table S2). For BFA treatment, n = 10 axons were imaged and for ethanol treatment, n = 11 axons were imaged. All values represent means ± SEM. Unpaired t test. *, $P < 0.05$.

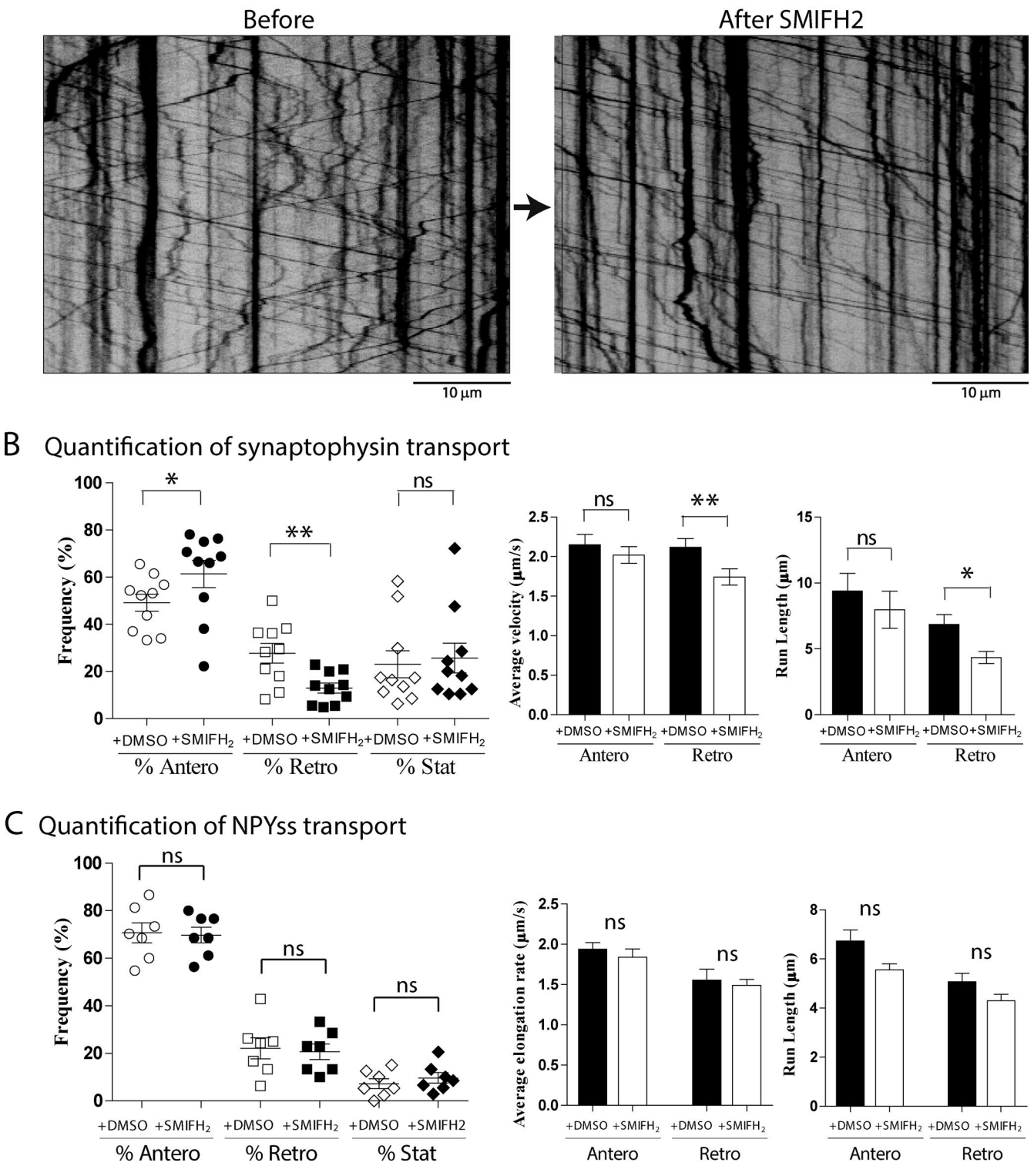


Figure S5. **Formin inhibitor (SMIFH2) has minimal effect on vesicle dynamics in axons.** (A) Representative kymographs from a synaptophysin-dsRed (vesicle marker) transfected axon, before and after 30 µM SMIFH2 for 30 min. Note SMIFH2 has no obvious effect on vesicle trafficking. (B and C) Quantification of SMIFH2 on synaptophysin-dsRed and NPY-ss:mCherry vesicle dynamics reveals minimal changes upon drug treatment (for detailed statistics, see Table S2). For SMIFH2 treatment on synaptophysin-dsRed vesicles, $n = 10$ axons were imaged and for SMIFH2 treatment on NPY-ss:mCherry vesicles, $n = 7$ axons were imaged. All values represent means ± SEM. Paired t test. **, $P < 0.01$; *, $P < 0.05$. Antero, anterograde; Retro, retrograde; Stat, stationary.

Table S1. **Quantification of F-actin dynamics**

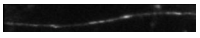
Parameters	Frequency (mean ± SEM)	Elongation rate (mean ± SEM)	Polymer length (mean ± SEM)	Hotspot duration (mean ± SEM)	Axons imaged	Figure in paper	Experiment repeats
		μm/s	*μm*	*s*			
GFP-Utr:CH (F-actin) dynamics							
Before Latrunculin A	26.38 ± 3.65	0.92 ± 0.04	9.2 ± 0.53	72.94 ± 7.42	8	Fig. 4, D–G	3
After 100 nM Latrunculin A (30 min)	13.88 ± 2.13[a]	0.14 ± 0.01[b]	5.27 ± 0.39[b]	153.1 ± 41.86	8	Fig. 4, D–G	3
Before Cytochalasin-D	37.25 ± 3.15	0.85 ± 0.02	8.38 ± 0.33	79.19 ± 8.11	8	Fig. 4, D–G	3
After 1 μM Cytochalasin-D (30 min)	5.75 ± 0.79[b]	0.52 ± 0.03[b]	4.13 ± 0.27[b]	273.4 ± 27.41[b]	8	Fig. 4, D–G	3
Before Jasplakinolide	26.83 ± 4.93	0.89 ± 0.04	8.29 ± 0.63	140.2 ± 15.48	6	Fig. 4, D–G	3
After 100 nM Jasplakinolide (10 min)	0[a]	0	0	506.1 ± 30.44[b]	6	Fig. 4, D–G	3
Before Nocodazole	21.63 ± 1.90	0.79 ± 0.01	8.90 ± 0.53	101.8 ± 8.10	8	Fig. 4, D–G	4
After 10 μg/ml Nocodazole (60 min)	20.38 ± 3.22	0.74 ± 0.02	8.06 ± 0.41	128.8 ± 18.48	8	Fig. 4, D–G	4
Before Vincristine	32.17 ± 3.94	0.88 ± 0.03	8.36 ± 0.36	81.43 ± 8.90	6	Fig. S3 B′	2
After 5 μM Vincristine (30 min)	35.33 ± 3.68	0.82 ± 0.04	7.45 ± 0.38[c]	84.54 ± 9.96	6	Fig. S3 B′	2
Before SMIFH2	31.56 ± 3.41	0.92 ± 0.03	8.69 ± 0.50	79.39 ± 5.86	9	Fig. 6, C–F	4
After 30 μM SMIFH2 (30 min)	8.55 ± 0.86[b]	0.66 ± 0.06[b]	6.76 ± 0.48[c]	177.6 ± 25.58[a]	9	Fig. 6, C–F	4
Before CK666	24.33 ± 3.01	0.84 ± 0.06	7.90 ± 0.72	87.39 ± 8.99	6	Fig. 6, C–F	3
After 50 μM CK666 (30 min)	28.0 ± 3.24	0.92 ± 0.06	8.19 ± 0.60	71.62 ± 5.94[c]	6	Fig. 6, C–F	3
Before DMSO	26.00 ± 5.13	0.95 ± 0.04	10.07 ± 1.33	85.17 ± 2.33	3	Fig. S3 D′	2
After DMSO	27.0 ± 4.16	0.87 ± 0.04	9.83 ± 1.34	69.58 ± 7.43	3	Fig. S3 D′	2
Utr-GFP:CH (+EtOH; solvent for BFA)	34.47 ± 3.05	0.89 ± 0.01	7.73 ± 0.20	59.58 ± 3.02	17	Fig. 5 E	3
Utr-GFP:CH (+1 μg/ml BFA for 12–16 h)	10.85 ± 1.80[d]	0.65 ± 0.01[d]	5.75 ± 0.28[d]	146.6 ± 6.58[d]	27	Fig. 5 E	3
Utr-GFP:CH (+1 μg/ml BFA) after washout	33.57 ± 7.54	0.90 ± 0.02	7.34 ± 0.32	78.34 ± 6.88	7	Fig. 5 E	3
Lifeact-mTorquise2 dynamics							
Lifeact-mTorquise2 (no treatment)	23.63 ± 2.99	0.85 ± 0.01	10.89 ± 0.27	155.3 ± 9.27	19	Fig. S2 C	3

Frequency refers to number of F-actin trails/10 min of imaging.
[a]Paired *t* test. $P < 0.01$.
[b]Paired *t* test. $P < 0.001$.
[c]Paired *t* test. $P < 0.05$.
[d]One-way analysis of variance. $P < 0.001$.

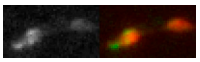
Table S2. **Quantification of vesicle and mitochondrial transport**

Parameters	Frequency of particles	Velocity (mean ± SEM)	Run length (mean ± SEM)	Axons imaged	Figure in manuscript	Experiment repeats
	%	μm/s	μm			
Synaptophysin (p38-dsRed) dynamics						
Before SMIFH2 (anterograde)	49.19 ± 3.63	2.15 ± 0.13	9.38 ± 1.34	10	Fig. S5 B	3
Before SMIFH2 (retrograde)	27.73 ± 4.14	2.12 ± 0.11	6.85 ± 0.75	10	Fig. S5 B	3
Before SMIFH2 (stationary)	23.08 ± 5.72	NA	NA	10	Fig. S5 B	3
After 30 μM SMIFH2 (anterograde)	61.34 ± 5.82[a]	2.02 ± 0.10	7.97 ± 1.40	10	Fig. S5 B	3
After 30 μM SMIFH2 (retrograde)	12.98 ± 2.12[b]	1.74 ± 0.10[b]	4.34 ± 0.46[b]	10	Fig. S5B	3
After 30 μM SMIFH2 (stationary)	25.68 ± 6.28	NA	NA	10	Fig. S5 B	3
Before Jasplakinolide (anterograde)	34.83 ± 8.51	1.06 ± 0.05	13.86 ± 0.48	6	Fig. S3 C′	2
Before Jasplakinolide (retrograde)	17.83 ± 5.52	0.84 ± 0.07	9.55 ± 0.81	6	Fig. S3 C′	2
Before Jasplakinolide (stationary)	4.83 ± 0.54	NA	NA	6	Fig. S3 C′	2
After 100 nM Jasplakinolide (anterograde)	41.5 ± 7.27	1.16 ± 0.09	17.53 ± 1.22[a]	6	Fig. S3 C′	2
After 100 nM Jasplakinolide (retrograde)	13.17 ± 3.33	0.91 ± 0.04	9.11 ± 0.06	6	Fig. S3 C′	2
After 100 nM Jasplakinolide (stationary)	7.5 ± 0.67	NA	NA	6	Fig. S3 C′	2
Mitochondria (Mito-dsRed) dynamics						
Control (+EtOH); mobile particles	20.80 ± 3.49	Anterograde 0.56 ± 0.04; retrograde 0.72 ± 0.07	Anterograde 12.10 ± 1.49; retrograde 11.33 ± 1.99 μm	11	Fig. S4 C	2
Control (+EtOH); stationary particles	79.20 ± 3.49	NA	NA	11	Fig. S4 C	2
After 1 μg/ml BFA (12–16 h); mobile particles	39.37 ± 6.23[a]	Anterograde 0.46 ± 0.03; retrograde 0.55 ± 0.05	Anterograde 9.81 ± 1.03 μm; retrograde 11.63 ± 1.47	10	Fig. S4 C	2
After 1 μg/ml BFA (12–16 h); stationary particles	60.63 ± 6.23[a]	NA	NA	10	Fig. S4 C	2
Golgi-derived vesicles (NPYss-mCherry) dynamics						
Before SMIFH2 (anterograde)	70.67 ± 4.21	1.93 ± 0.08	6.73 ± 0.44	7	Fig. S5 C	2
Before SMIFH2 (retrograde)	22.11 ± 4.44	1.55 ± 0.13	5.06 ± 0.34	7	Fig. S5 C	2
Before SMIFH2 (stationary)	7.22 ± 2.06	NA	NA	7	Fig. S5 C	2
After 30 μM SMIFH2 (anterograde)	69.71 ± 3.29	1.84 ± 0.09	5.59 ± 0.23	7	Fig. S5 C	2
After 30 μM SMIFH2 (retrograde)	20.64 ± 3.29	1.49 ± 0.07	4.30 ± 0.25	7	Fig. S5 C	2
After 30 μM SMIFH2 (stationary)	9.64 ± 2.20	NA	NA	7	Fig. S5 C	2

NA, not available.
[a]Unpaired *t* test. $P < 0.05$.
[b]Unpaired *t* test. $P < 0.01$.



Video 1. **Video of GFP:Utr-CH kinetics in an axon.** The kymograph in Fig. 1 B was generated from this video. Frame rate was 1 frame/s and playback rate is 10 frames/s. Total time of the movie is 600 s. Note: (a) rapidly moving fluorescent F-actin trails (few marked with arrowheads) and (b) hotpots of F-actin dispersed along the axon.



Video 2. **Video of GFP:Utr-CH kinetics in an en passant synaptic bouton.** Left bouton corresponding to Fig. 7 A. Greyscale frames of GFP:Utr-CH on left and corresponding overlaid frames of GFP:Utr-CH (green)/synaptophysin-mRFP (red) on right. Frame rate was 1 frame/s and playback rate is 10 frames/s. Total time of the movie is 600 s. Note transient patches of F-actin circumferentially surrounding the synaptic vesicle cluster (labeled by synaptophysin).

Reference

Field, C.M., and P. Lénárt. 2011. Bulk cytoplasmic actin and its functions in meiosis and mitosis. *Curr. Biol.* 21:R825–R830. http://dx.doi.org/10.1016/j.cub.2011.07.043