Developmental Cell *15*

Supplemental Data

Myosin II Activity Facilitates Microtubule Bundling in the Neuronal Growth Cone Neck

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Figure S1. Individual Microtubules and Actin Filament Bundles Colocalize at the Growth Cone Neck

Original electron micrograph from Fig. 1D before pseudo-coloring. Star denotes actin network, arrowheads denotes the end of microtubules, and arrow denotes actin bundle. Scale bar: 1 μm.

Figure S2. F-Actin Bundles Are Created on the Sides of the Growth Cone Neck

A) Fluorescent phalloidin FSM of the growth cone neck. B) Time lapse montage of the box in (A) showing the movement of an existing actin bundle (closed arrowhead) and the formation of a new bundle (open arrowhead).

Figure S1

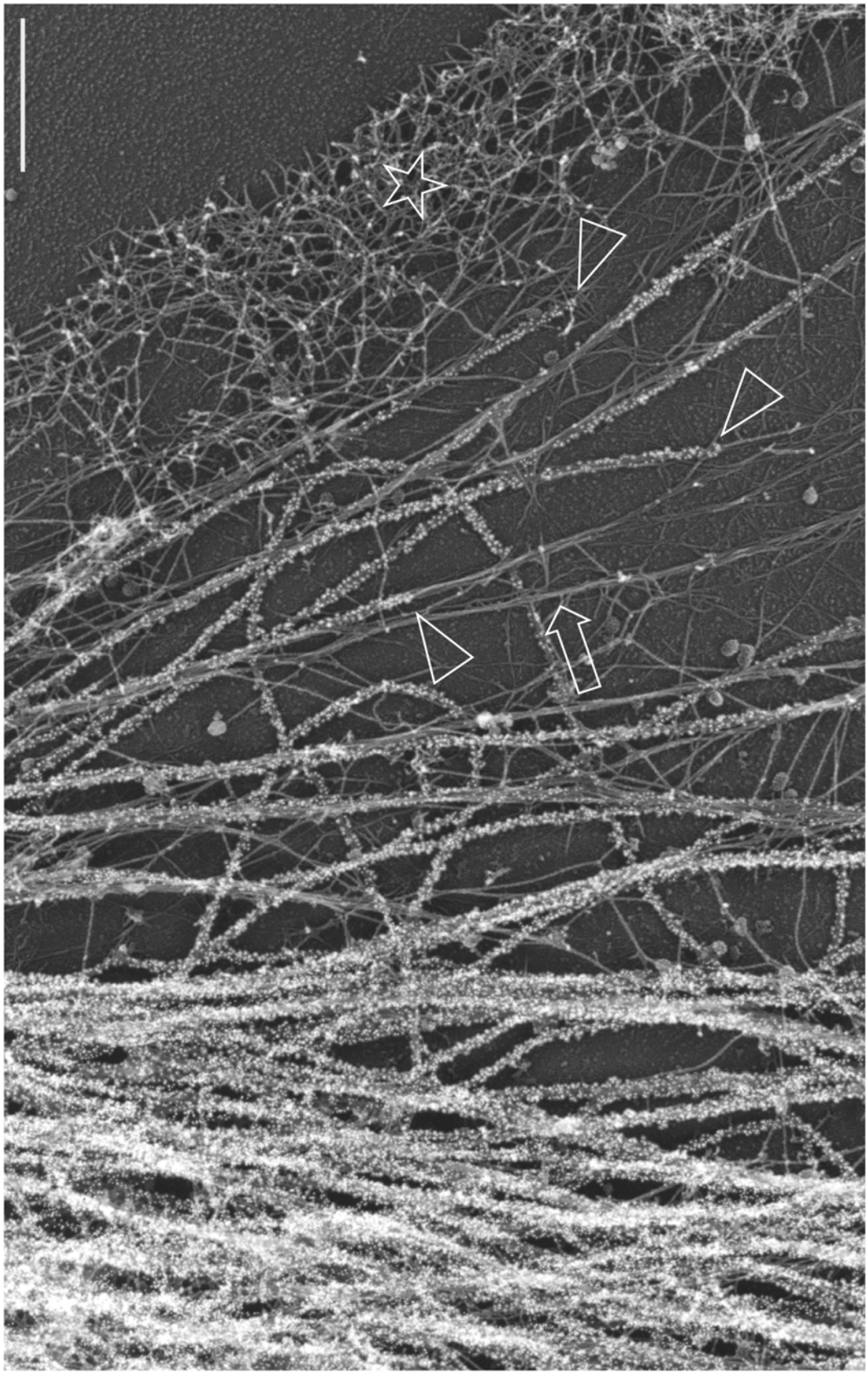

Figure S2

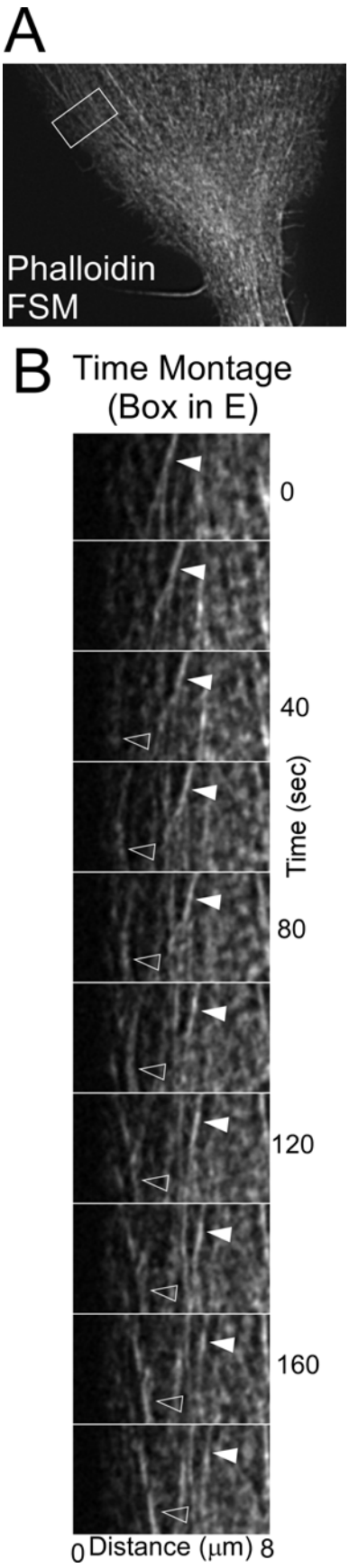
A
Phalloidin
FSM
B Time Montage
(Box in E)
0
40
80
120
160
Time (sec)
0 Distance (μm) 8

Figure S3

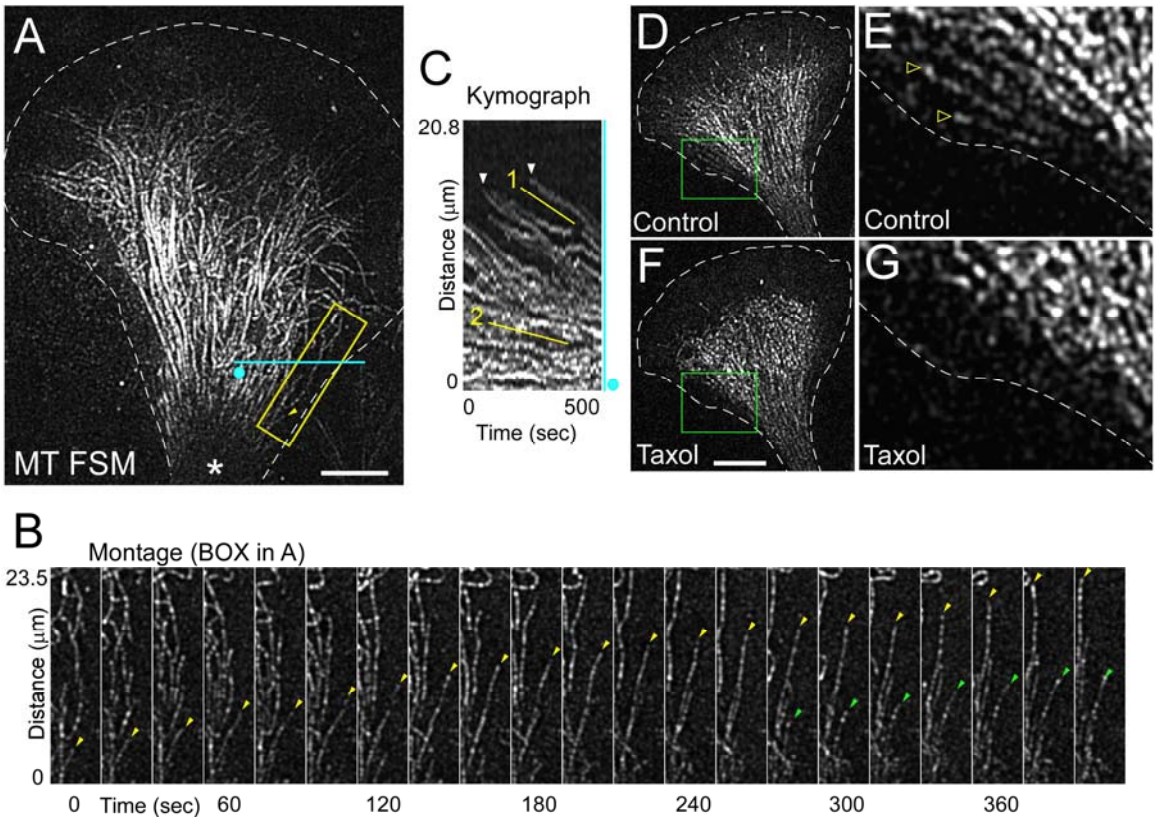

Figure S3. Polymerization Plays a Key Role in Microtubule Advance along the Sides of the Growth Cone

A) Microtubule FSM in a control growth cone. Dotted line denotes leading edge of growth cone. B) Microtubule FSM Montage of the box in (A) showing microtubule growth. Arrowhead in (A) is the same as the arrowhead in the first frame of the montage in (B); arrowheads track microtubule tip growth. C) Microtubule kymograph through the green line in (A). Line 1 denotes fast lateral microtubule movement into the C-domain and line 2 denotes slower movement in more central region. Green dots in A and B show kymograph orientation. Microtubules entering the kymograph sampling region appear as new traces (arrowheads). D-G) To investigate the role polymerization plays in microtubule

entry into the neck region, microtubule behavior was assessed before (D-E) and after (F-G) treatment with 100 nM taxol to damp microtubule dynamics (Suter et al., 2004). Individual microtubules are clearly present in the control (arrowheads in E) and are absent after a 5 min in taxol (G.) These results suggest polymerization is necessary for net microtubule advance along the sides of the growth cone, however, they do not rule out or address participation of polymerization independent processes. High microtubule density in the C-domain precluded analysis of microtubule dynamics in this region. Scale bars, 10μm.

Figure S4

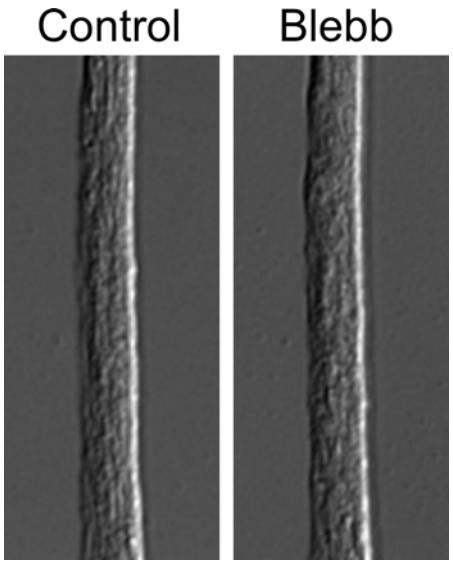

Figure S4. Neurite Shaft Region Does Not Spread after Blebbistatin Treatment

Representative neurite shaft before and 20 mins after treatment with 70μM blebbistatin.

Table S1. Microtubule Dynamic Parameters at the Growth Cone Neck

Conditions	Growth velocity (μm/min)	% time growing	% time paused	% time shortening
Control	3.9 +/- 0.2	62.6	35.5	1.9*
Blebbistatin	3.5 +/- 0.2	55.9	43.5	0.6*

Plus-end periphery microtubules: control (n=13 microtubules) and 5-10 mins blebbistatin. (n=15 microtubules). * denotes 1 microtubule that briefly shortened and then continued to grow.