Tamada et al., http://www.jcb.org/cgi/content/full/jcb.200906043/DC1

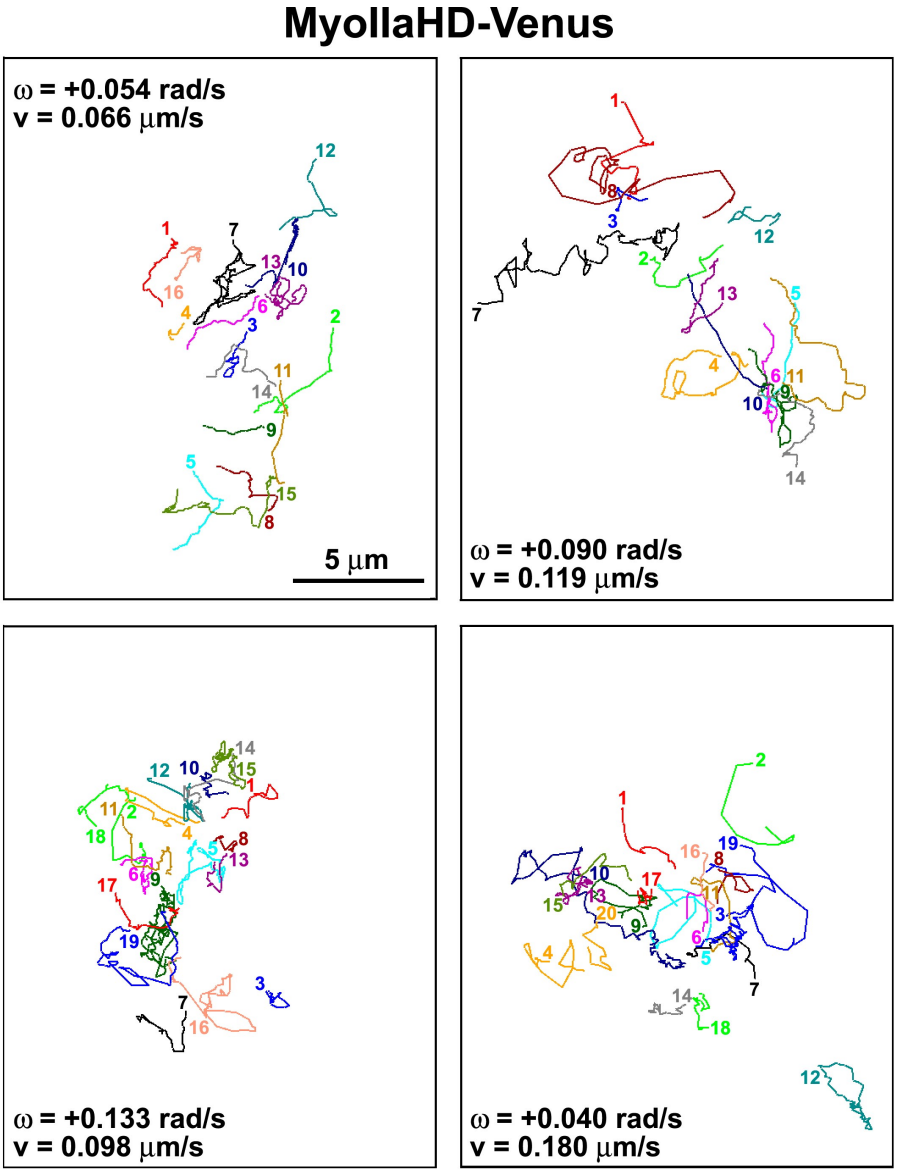


Figure S1. **Trajectories of filopodial tips of four representative growth cones that express the myosin IIa head domain fused to Venus (MyollaHD-Venus).** cDNA for the head domain (aa 1–776) was obtained from myosin IIa cDNA (GenBank/EMBL/DDBJ accession no. AB191263). Each panel shows the tip trajectories of a single growth cone. All of the filopodial tips that appeared in the focal plane for the period of 5-min imaging were included. The numbered end of each line represents the point where the filopodial tip appeared in the focal plane, and the other end of the line is the point at which it moved out of the focal plane. The mean angular velocity (ω) and the mean velocity (v) of filopodial tips for each growth cone are shown. Positive values of the angular velocity indicate right-screw rotation.

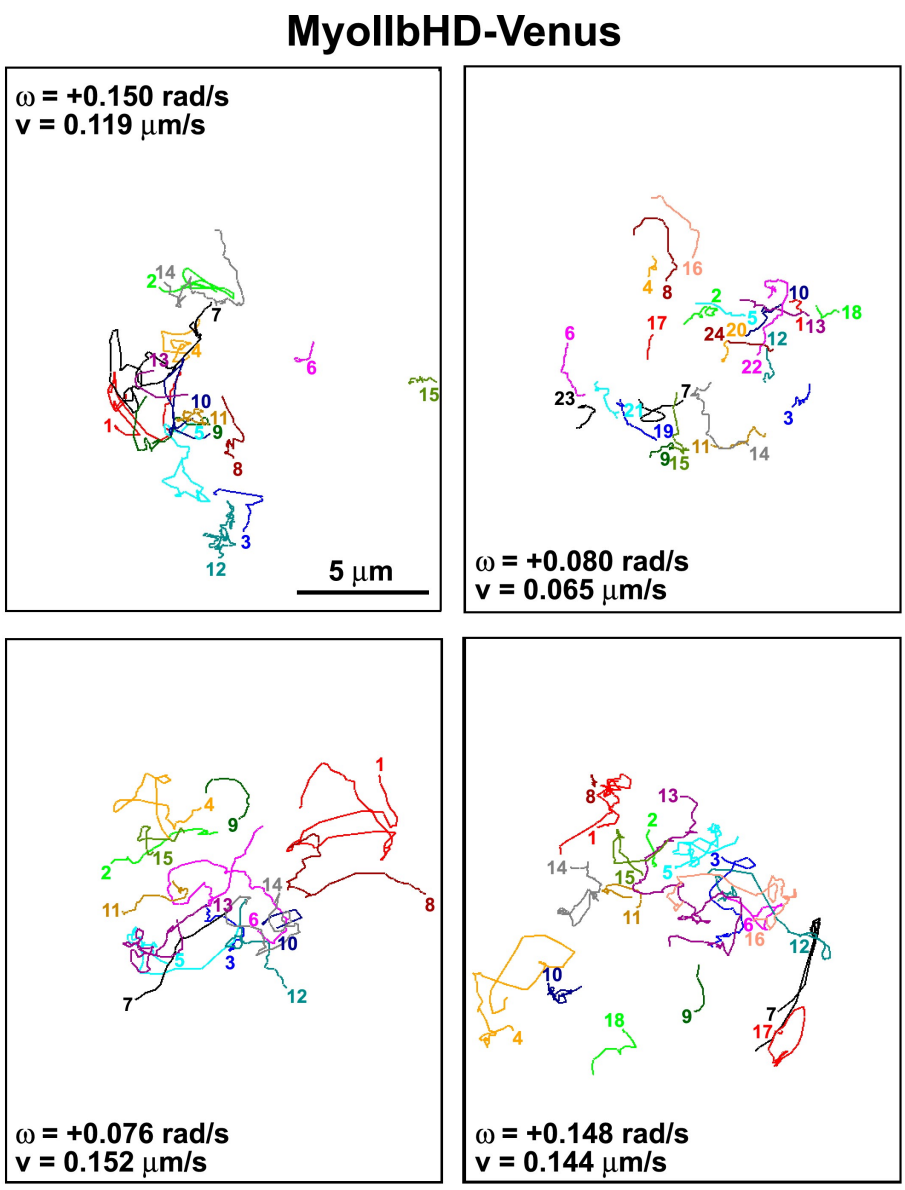


Figure S2. **Trajectories of filopodial tips of four representative growth cones that express the myosin IIb head domain fused to Venus (MyoIIbHD-Venus).** cDNA for the head domain (aa 1–783) was obtained from myosin IIb cDNA (GenBank/EMBL/DDBJ accession no. AK029236). The data have been obtained and presented here as described in the legend for Fig. S1.

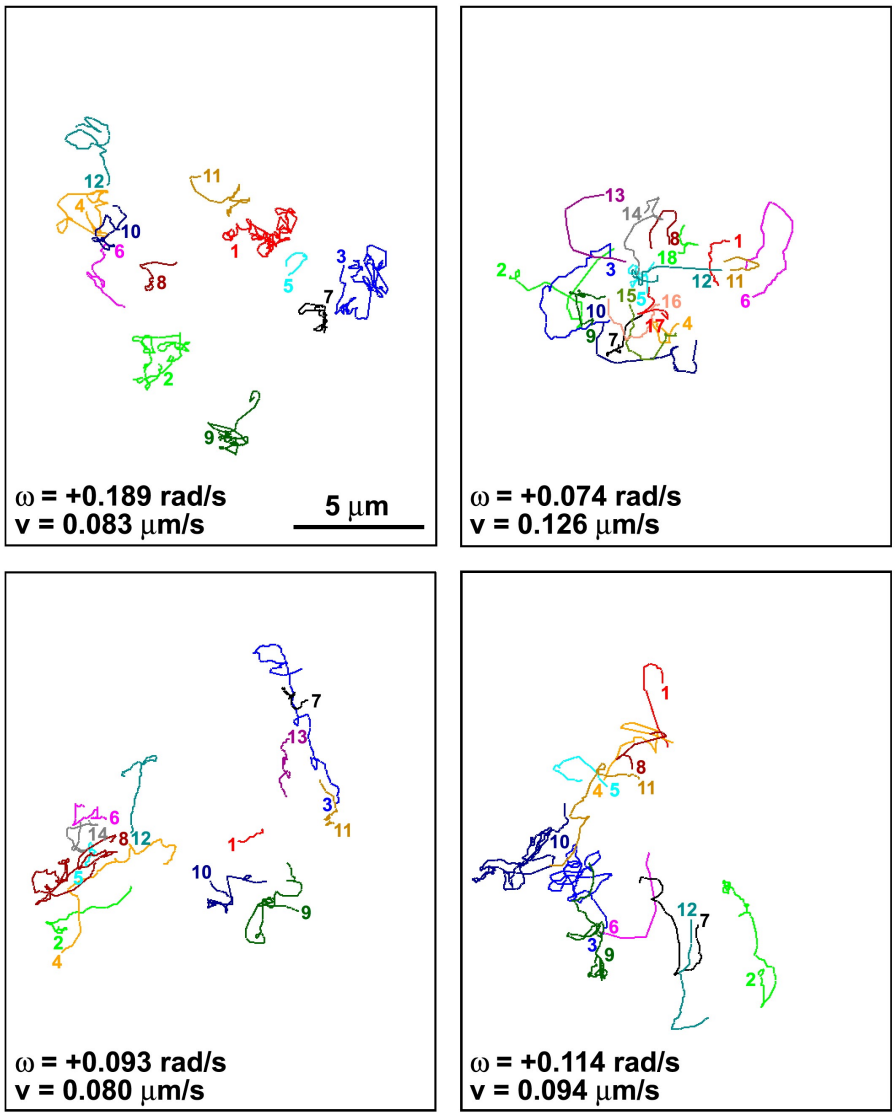


Figure S3. **Trajectories of filopodial tips of four representative growth cones that express the myosin IIc head domain fused to Venus (MyoIIcHD-Venus).** cDNA for the head domain (aa 1–800) was obtained from myosin IIc cDNA (GenBank/EMBL/DDBJ accession no. AK165122). The data have been obtained and presented here as described in the legend for Fig. S1.

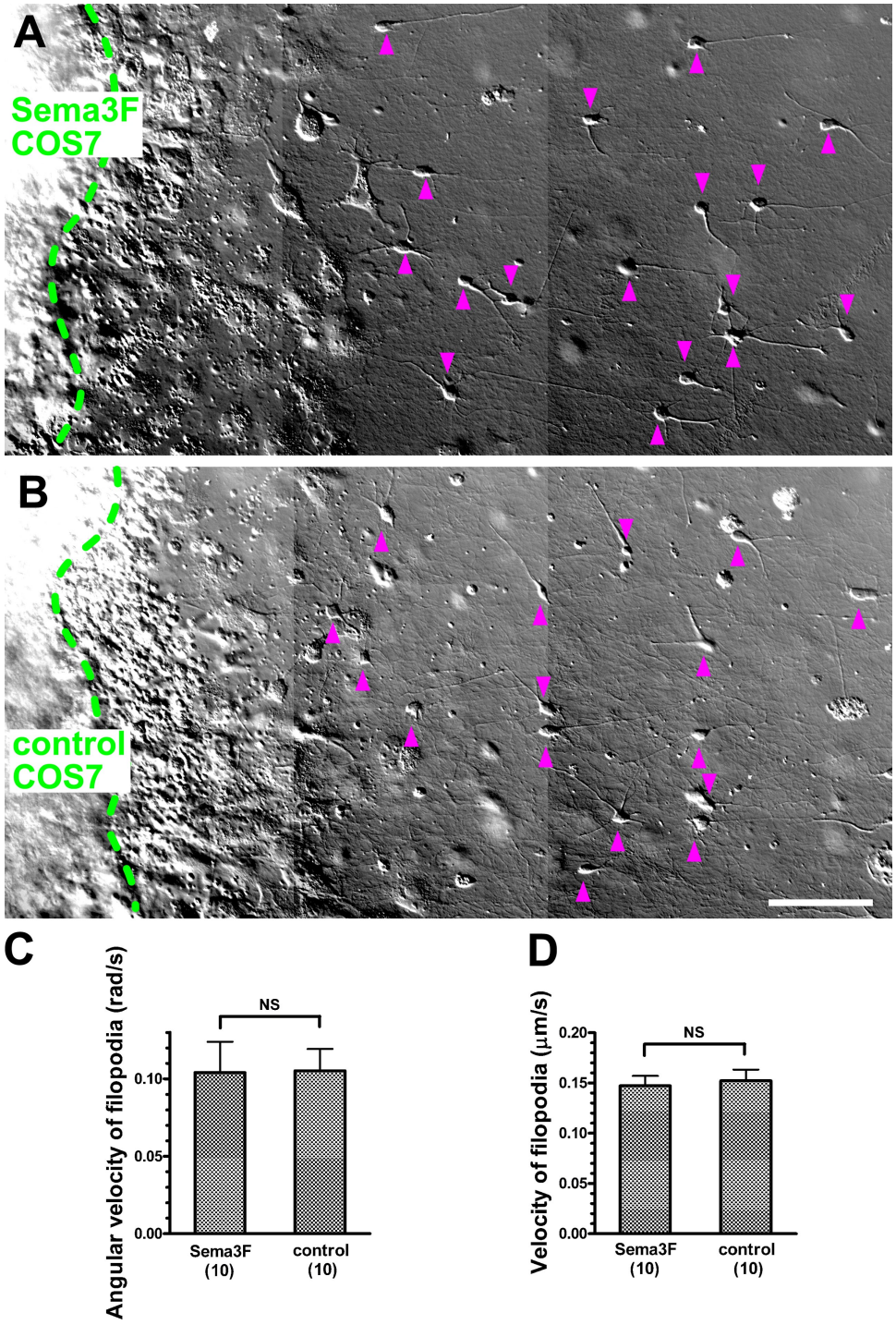
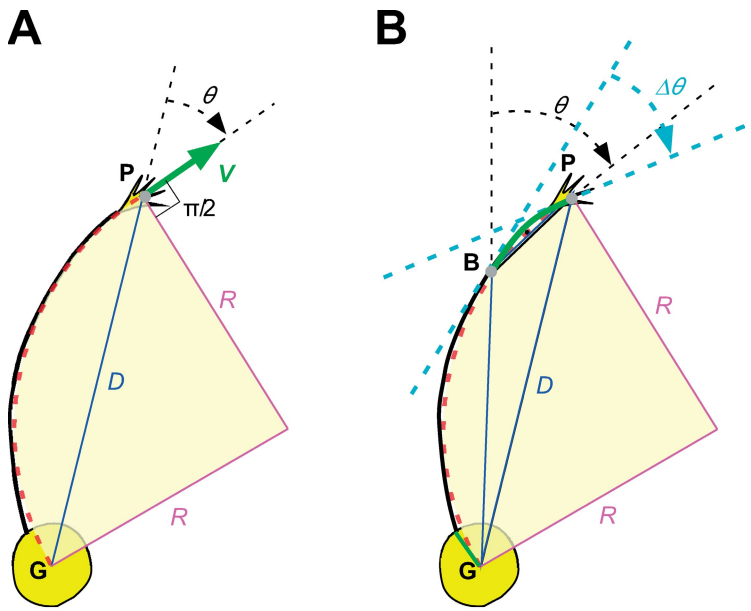


Figure S4. **Analysis of filopodial rotation in Sema3F-guided growth cones.** (A and B) Hippocampal neurons (magenta arrowheads) co-cultured for 2 d in a collagen gel with a reaggregate of COS-7 cells transfected with Sema3F (A) or control (B) expression vectors. Each panel is a composite of three DIC images acquired under the same conditions. Green broken lines delineate the COS-7 reaggregates. (C and D) The y axis represents the filopodial angular velocity (C) or the filopodial velocity (D) in hippocampal neurons co-cultured for 1 d with Sema3F-secreting or control reaggregates. Numbers in parentheses indicate the total number of growth cones examined. Data represent mean ± SEM. P = 0.96 (C); P = 0.74 (D; unpaired *t* test). Bar, 100 μm.

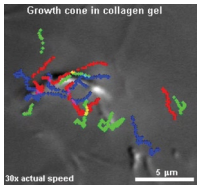


$$\kappa = \frac{1}{R} = \frac{2\sin\theta}{D}$$

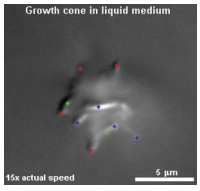
$$\kappa = \frac{\Delta\theta}{\Delta S}$$

$$L = \frac{\theta}{\sin\theta} D - \sqrt{\frac{A_{agr}}{\pi}}$$

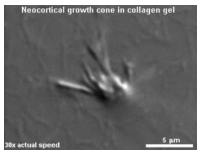
Figure S5. **Neurite curvature and length in explant/reaggregate cultures.** (A) Determination of the neurite curvature in explant cultures (Fig. 1 H). The curvature (κ) was calculated as described in detail in Materials and methods. (B) Determination of the neurite curvature and length in reaggregate cultures (Fig. 6). The curvature (κ) in the distal 100-μm arc of a neurite was calculated. It was assumed that the neurite length (L) is the arc length between the periphery of reaggregate and the neurite tip. See Materials and methods for details.



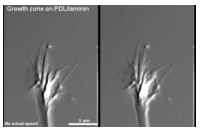
Video 1. **Time-lapse DIC video of filopodial dynamics in a hippocampal neuronal growth cone.** This growth cone (Fig. 2 A) was migrating in a 3D collagen gel toward the observer. Note collagen fibers that are stationary in the video. The filopodia could be easily distinguished from collagen fibers by moving the focal plane in the z direction toward the growth cone body (not depicted). Frames were acquired every 1 s for 10 min with an ORCA-ER CCD camera on a BX61WI upright microscope and are displayed at 30 frames/s. Color-coded diamond traces indicate trajectories of all of the filopodial tips in the focal plane. Each diamond represents the position of the tip at 1-s intervals. Each diamond remains on the video for 4 s (2 min in actual speed).



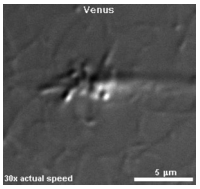
Video 2. **Time-lapse DIC video of filopodial dynamics in a hippocampal growth cone.** This growth cone (Fig. 2 C) has protruded from a collagen gel into a liquid medium. Color dots indicate the position of all of the filopodial tips in the focal plane. Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 15 frames/s.



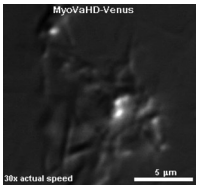
Video 3. **Time-lapse DIC video of filopodial dynamics of an E19 rat neocortical neuron in a collagen gel.** The growth cone was migrating in a 3D substrate toward the observer. Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 30 frames/s.



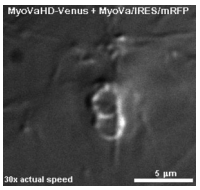
Video 4. **Autostereoscopic video of filopodial dynamics in a hippocampal growth cone on a 2D PDL/laminin substrate.** With an ORCA-ER CCD camera on a BX61WI microscope, DIC images of the growth cone were acquired at three different focal planes (each separated by 1 µm in the z direction), and each set of the three images was acquired every 3 s for a total of 60 min. Using MetaMorph software, the original images were converted to stereo images that have been presented for divergent wall-eyed viewing and are displayed at 10 frames/s. Confirm that top and bottom are closest to and furthest from the observer, respectively. The filopodia, which have been detached from the substrate, often exhibit right-screw rotation while retracting.



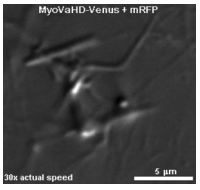
Video 5. **Time-lapse DIC video of filopodial dynamics in the hippocampal growth cone that had been transfected with Venus cDNA.** This growth cone (Fig. 3 A) was migrating in a 3D substrate toward the observer. The filopodia could be easily distinguished from collagen fibers by moving the focal plane in the z direction toward the growth cone body or by observing Venus fluorescence (not depicted). Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 30 frames/s.



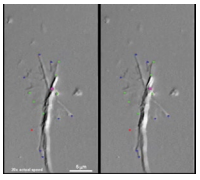
Video 6. **Time-lapse DIC video of filopodial dynamics in the hippocampal growth cone that had been transfected with MyoVaHD-Venus cDNA.** This growth cone (Fig. 3 B) was migrating in a 3D substrate toward the observer. Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 30 frames/s.



Video 7. **Time-lapse DIC video of filopodial dynamics in the hippocampal growth cone that had been transfected with MyoVaHD-Venus cDNA plus MyoVa/IRES/mRFP cDNA.** This growth cone (Fig. 4 A) was migrating in a 3D substrate toward the observer. Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 30 frames/s.



Video 8. **Time-lapse DIC video of filopodial dynamics in the hippocampal growth cone that had been transfected with MyoVaHD-Venus cDNA plus mRFP cDNA.** This growth cone (Fig. 4 D) was migrating in a 3D substrate toward the observer. Frames were acquired every 1 s for 5 min with an ORCA-ER CCD camera on a BX61WI microscope and are displayed at 30 frames/s.



Video 9. **Autostereoscopic video of a growth cone.** DIC images of a hippocampal growth cone (Fig. 9) on a 2D PDL/laminin substrate were acquired at three different focal planes (each separated by 1 µm in the z direction) with an ORCA-ER CCD camera on a BX61WI microscope. The bottom plane was kept focused on the glass surface, and the top plane was closest to the observer. Each set of the three images was acquired every 4 s for a total of 60 min. Using MetaMorph software, the original images were converted to stereo images that have been presented for divergent wall-eyed viewing and are displayed at 5 frames/s. Red, blue, and green dots represent the filopodial tips that are present in the bottom, middle, and top planes, respectively. A larger magenta circle represents the midpoint of the distal edge of the growth cone central domain. Note that the filopodia unattached to the substrate rotate in the right-screw direction from the viewpoint of the growth cone body.