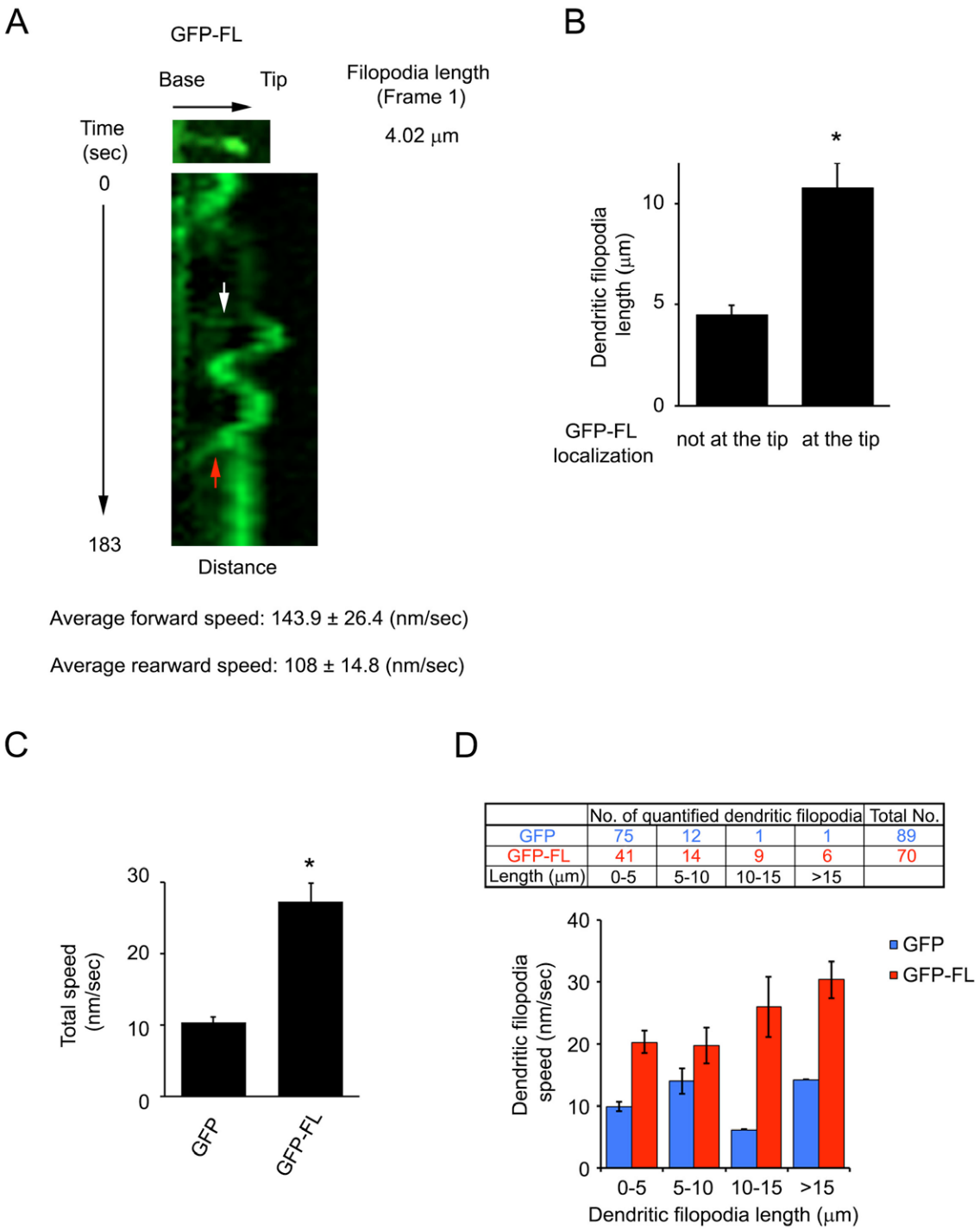


Fig. S1. FL-Myo10 is motile within dendritic filopodia and promotes an increase in length and dynamic movement of these structures. (A) Neurons (DIV5) were co-transfected with GFP-FL-Myo10 (GFP-FL) and a fluorescent filler, mCerulean, and subjected to live-cell imaging the next day (DIV6). Images were collected every 3 sec for 3 min. The dendritic filopodium from the first frame of the movie is shown (top panel). A kymograph was generated from the time-lapse images to show the position of GFP-FL-Myo10 puncta along filopodia (X-axis) over time (Y-axis) (bottom panel). Forward (white arrow) and rearward (red arrow) movement are indicated. 12 and 28 dendritic filopodia from three separate experiments were analyzed for forward and rearward movement, respectively. The speed of movement is shown as the average ± S.E.M. (B) The analysis of filopodia length from Fig. 3B (bottom panel) was further categorized into two groups based on GFP-FL-Myo10 localization. Quantification of filopodia length from GFP-FL-Myo10 expressing neurons based on its localization is shown. Error bars represent S.E.M. for 25-66 filopodia from at least three separate experiments (*$p<0.0001$). (C) Neurons at DIV5 were co-transfected with either GFP-FL-Myo10 or GFP and a fluorescent filler, mCherry, and used for live-cell imaging the next day (DIV6). Quantification of the total speed of dendritic filopodia movement is shown. Images were collected every 10 sec for 10 min. Error bars represent S.E.M. for 68-89 protrusions from three separate experiments (*$p<0.0001$). (D) Total speed of dendritic filopodia movement from panel C was further categorized into four groups based on filopodia length, which was measured from the first time-lapse image. The number of quantified filopodia from GFP-FL-Myo10 (red) and GFP (blue) neurons for each category is shown in the Table. Dendritic filopodia longer than 10 mm were only rarely observed in GFP expressing neurons. Error bars represent S.E.M.

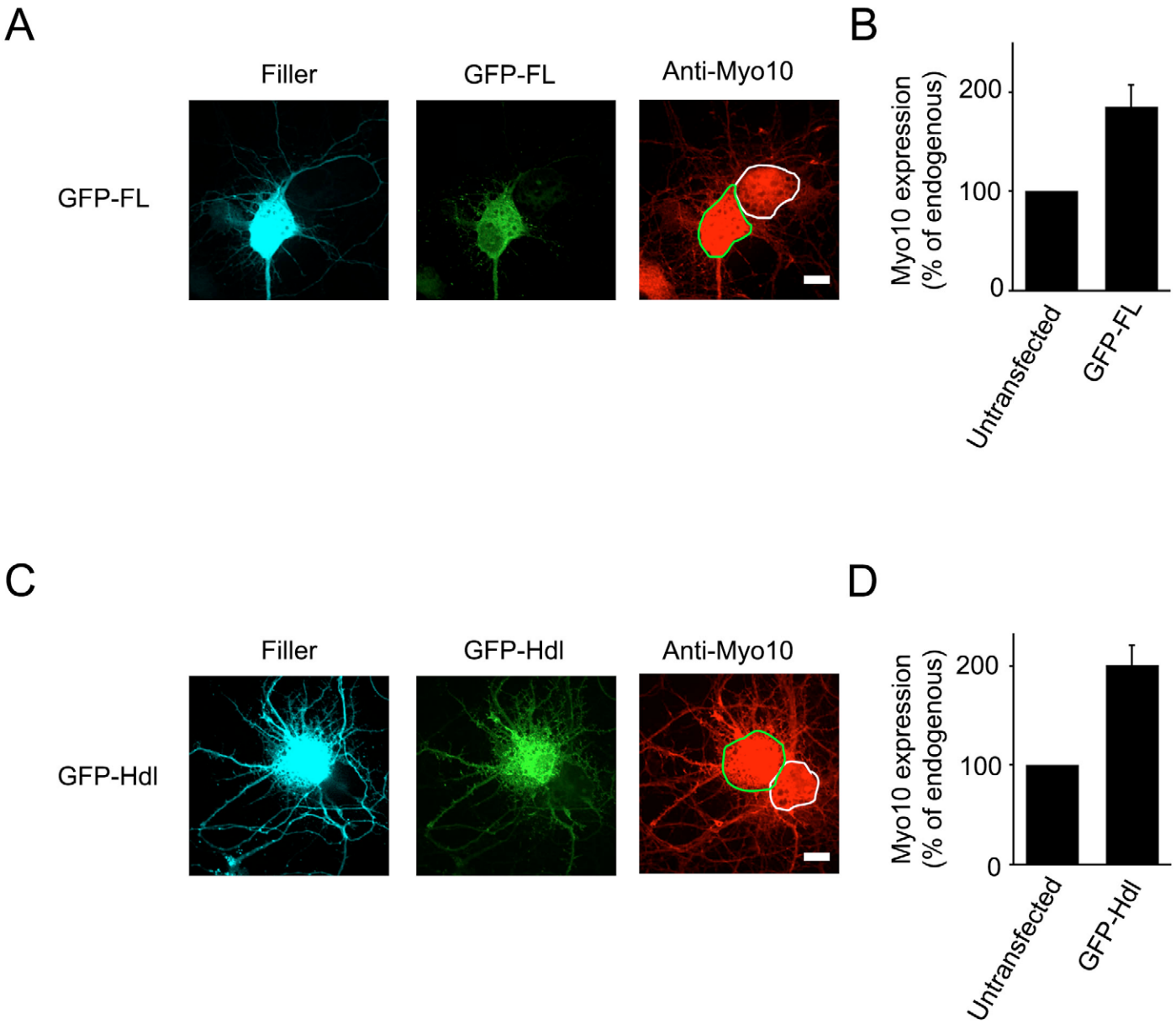


Fig. S2. Expression levels of GFP-FL-Myo10 and GFP-Hdl-Myo10. (A) Neurons were co-transfected with GFP-FL-Myo10 (GFP-FL, middle panel) and a fluorescent filler, mCerulean, (filler, left panel) at DIV6 and immunostained for Myo10 (anti-Myo10, right panel) at DIV7. The cell body of a transfected (green outline) neuron and a neighboring untransfected neuron (white outline) are traced (right panel). Scale bar: 10 mm. (B) The amount of Myo10 was quantified by measuring the fluorescence intensity in the cell body of untransfected neurons and neurons expressing GFP-FL-Myo10. Error bars represent S.E.M. for 49 pairs of cells from four separate experiments. (C) Neurons were co-transfected with GFP-Hdl-Myo10 (GFP-Hdl, middle panel) and a fluorescent filler, mCerulean (filler, left panel) at DIV6 and stained for Myo10 (anti-Myo10, right panel) at DIV12. The cell body of a transfected (green outline) neuron and a neighboring untransfected neuron (white outline) are traced (right panel). Scale bar: 10 mm. (D) Myo10 levels were quantified by measuring the fluorescence intensity of the cell body of untransfected neurons and neurons expressing GFP-Hdl-Myo10. Error bars represent S.E.M. for 46 pairs of cells from five separate experiments.

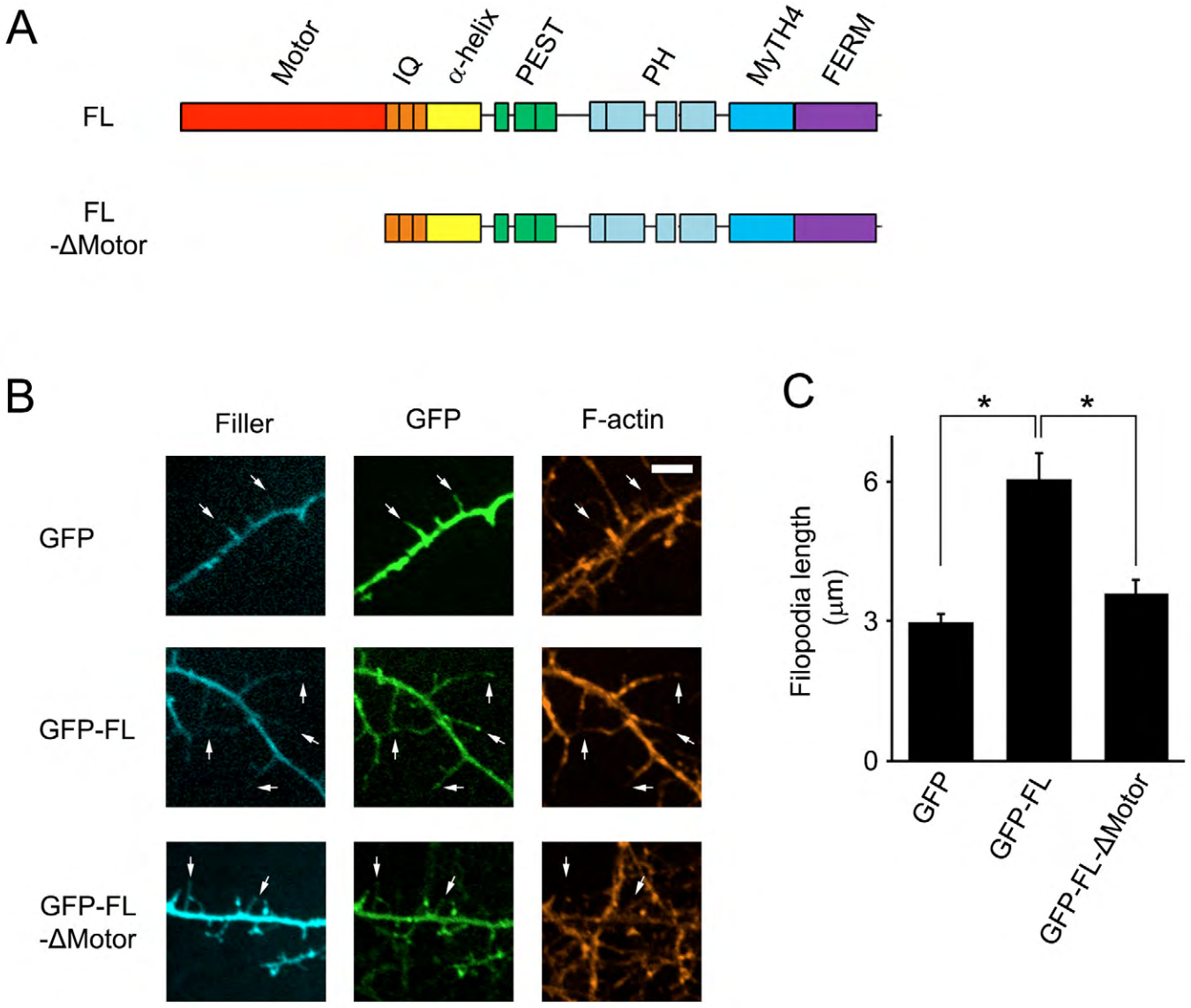


Fig. S3. The motor domain of FL-Myo10 is required for the increased filopodia length. (A) A schematic of FL-Myo10 (FL) and a FL-Myo10 motor domain deletion mutant (FL-ΔMotor) is shown. (B) Neurons were co-transfected with a fluorescent filler, mCerulean, and either GFP, GFP-FL-Myo10 (GFP-FL) or GFP-FL-Myo10-ΔMotor (GFP-FL-ΔMotor) at DIV5, fixed, and stained for F-actin at DIV7-8. Dendritic filopodia are indicated (arrows). Scale bar: 5 mm. (C) Quantification of the length of dendritic filopodia from neurons expressing the indicated constructs is shown. Error bars represent S.E.M. for 97-150 filopodia from at least three separate experiments (*$p<0.0001$).

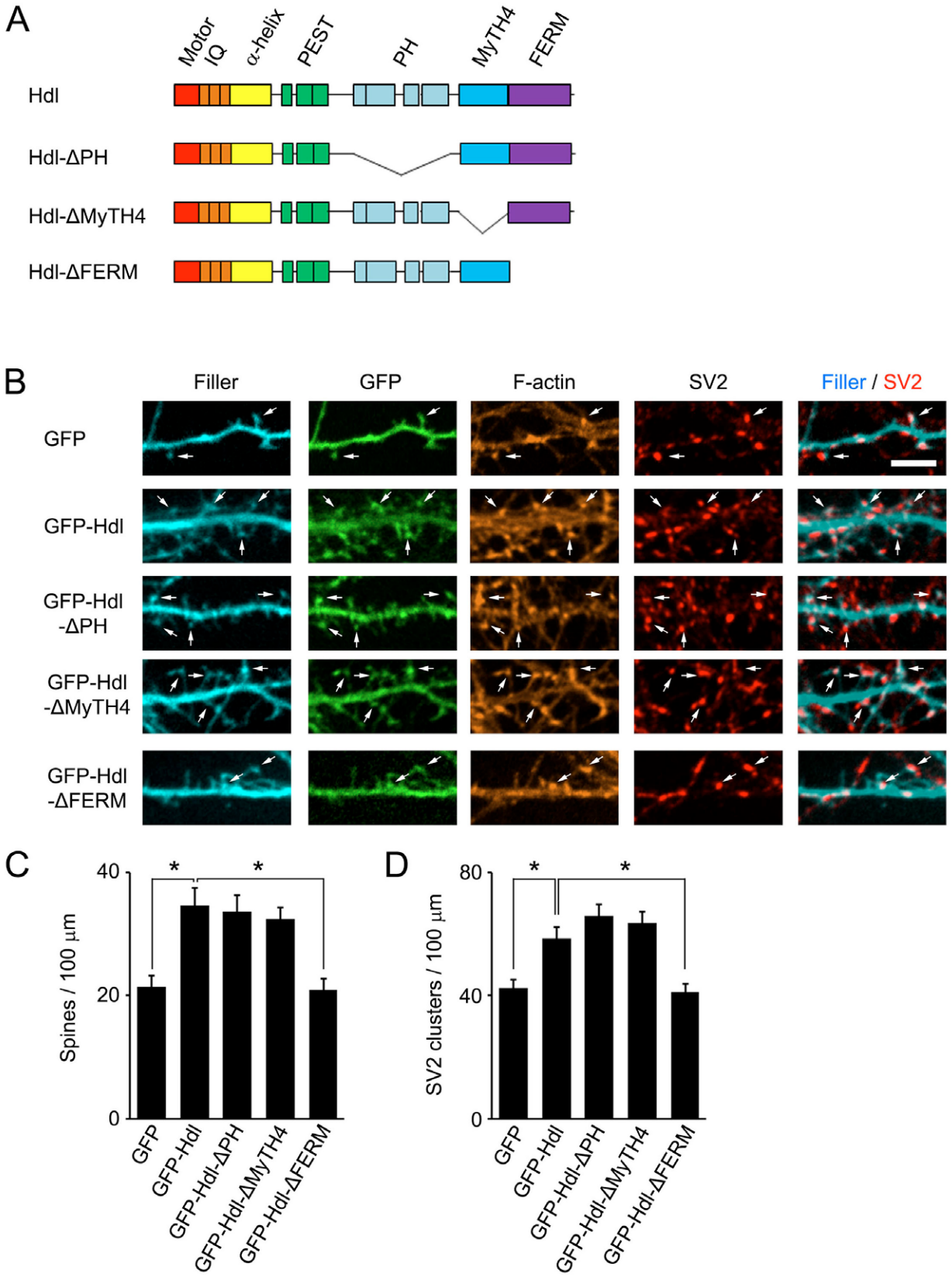


Fig. S4. The Hdl-Myo10 FERM domain is necessary for its function. (A) A schematic of Hdl-Myo10 (Hdl) and Hdl-Myo10 deletion mutants (ΔPH, ΔMyTH4, and ΔFERM) is shown. (B) Neurons (DIV5-6) were co-transfected with a fluorescent filler, mCerulean, and either GFP or the indicated GFP-Hdl-Myo10 constructs, fixed, and stained for F-actin and a synaptic marker (SV2) at DIV11-12. Dendritic spines are indicated (arrows). Scale bar: 5 mm. (C,D) Quantification of the dendritic spine (C) and synaptic density (SV2 clusters, D) from neurons transfected with the indicated constructs is shown. Error bars represent S.E.M. for 26-30 dendrites from three separate experiments (*$p<0.001$).

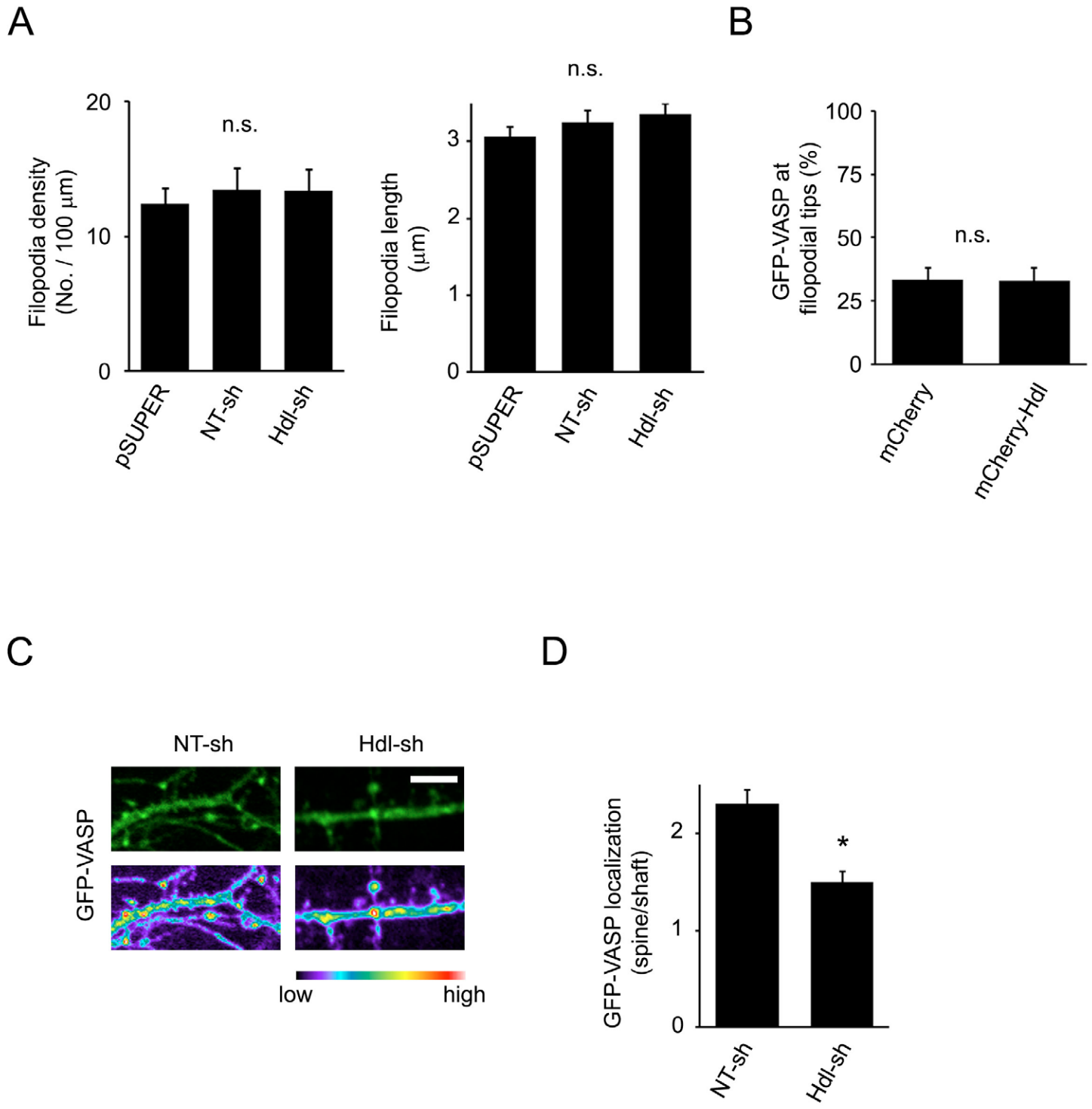
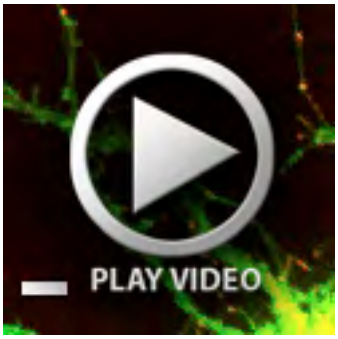


Fig. S5. Hdl-Myo10 does not affect filopodia density, length, or VASP localization, but is crucial for VASP distribution to spines. (A) Neurons (DIV3-4) were co-transfected with GFP and either empty pSUPER vector, NT shRNA (NT-sh), or Hdl-Myo10 shRNA (Hdl-sh), fixed, and stained for F-actin using Alexa Fluor® 546 phalloidin at DIV6-7. Quantification of the dendritic filopodia density (left panel) and length (right panel) in neurons transfected with indicated constructs is shown. Error bars represent S.E.M. for 40-48 dendrites (left panel) and 91-101 dendritic filopodia (right panel) from three separate experiments. (B) Neurons were co-transfected with GFP-VASP, a fluorescent filler, mCerulean, and either mCherry or mCherry-Hdl-Myo10 (mCherry-Hdl) at DIV5-6 and fixed the following day. Quantification of the percentage of GFP-VASP puncta localizing to filopodia tips for mCherry and mCherry-Hdl-Myo10 expressing neurons is shown. Error bars represent S.E.M. for 28 cells from three separate experiments. (C) Neurons were co-transfected with GFP-VASP, a fluorescent filler, mCherry or mCerulean, and either NT shRNA or Hdl-Myo10 shRNA and fixed at DIV11. GFP-VASP is shown in pseudo-color coding to indicate the range of fluorescence intensities to the assigned color. Bar: 5 mm. (D) Quantification of GFP-VASP localization to spines from neurons transfected with the indicated constructs is shown. Error bars represent S.E.M. for 84 spines from three separate experiments (*$p<0.0001$). For panels A and B, Уn.s.Φ denotes no statistically significant difference.

Movie 1. Dendritic filopodia in GFP expressing neurons are not very motile. Neurons were co-transfected with GFP and mCherry at DIV5 and subjected to time-lapse microscopy the next day (DIV6). Images were acquired every 3 sec for 3 min. GFP and mCherry images were superimposed in the movie. The frame rate for the movie is 5 frames per sec. Scale bar: 5 mm.

Movie 2. Dendritic filopodia in neurons expressing GFP-FL-Myo10 are highly dynamic. Neurons were co-transfected with GFP-FL-Myo10 and mCherry at DIV5 and subjected to time-lapse imaging the next day (DIV6). Images were acquired every 3 sec for 3 min. GFP-FL-Myo10 and mCherry images were superimposed in the movie. The frame rate for the movie is 5 frames per sec. Scale bar: 5 mm.

Movie 3. Dendritic filopodia in GFP-Hdl-Myo10 neurons are not very dynamic. Neurons were co-transfected with GFP-Hdl-Myo10 and mCherry at DIV5 and subjected to time-lapse imaging the next day (DIV6). Images were acquired every 3 sec for 3 min. GFP-Hdl-Myo10 and mCherry images were superimposed in the movie. The frame rate for the movie is 5 frames per sec. Scale bar: 5 mm.



Movie 4. mCherry-FL-Myo10 and GFP-VASP traffic together along dendritic filopodia. Neurons at DIV5 were co-transfected with mCherry-FL-Myo10, GFP-VASP, and a fluorescent filler, mCerulean, and subjected to live-cell imaging two days later (DIV7). Images were collected every 5 sec for 2 min. mCherry-FL-Myo10 and GFP-VASP images were superimposed in the movie. The frame rate for the movie is 5 frames per sec. Scale bar: 5 mm.

Movie 5. mCherry-FL-Myo10 and GFP-VASP co-trafficking is shown within a single dendritic filopodium. Neurons at DIV5 were co-transfected with mCherry-FL-Myo10, GFP-VASP, and a fluorescent filler, mCerulean, and subjected to live-cell imaging two days later (DIV7). Images were collected every 5 sec for 20 sec. mCherry-FL-Myo10 and GFP-VASP images were superimposed in the movie. The frame rate for the movie is 1 frame per sec. Scale bar: 1 mm