Albrecht et al., http://dx.doi.org/10.1083/jcb.201603108

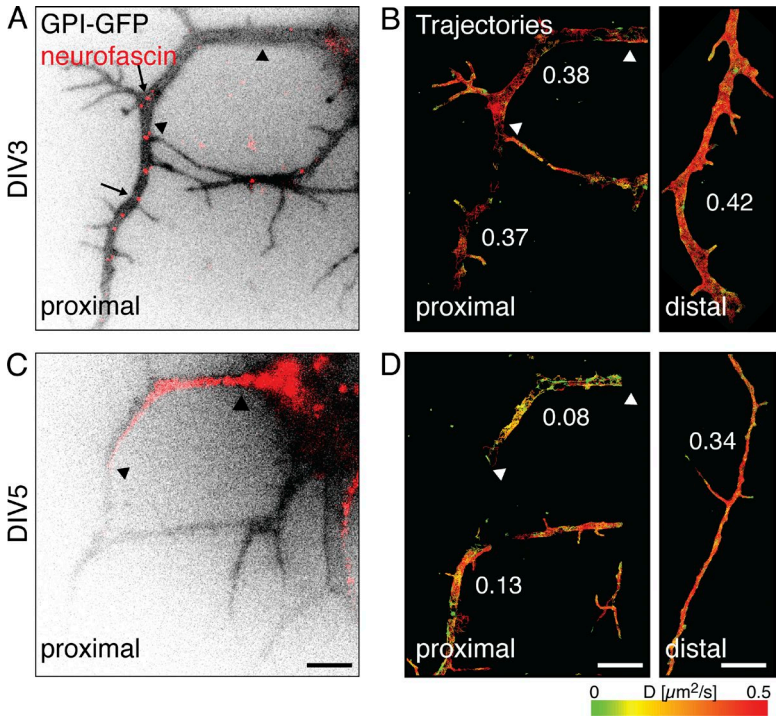


Figure S1. **The reduction of lateral mobility coincides with the accumulation of neurofascin at the AIS between DIV3 and DIV5.** (A) Shown is an area of the proximal axon of a DIV3 neuron expressing GPI-GFP. Live immunolabeling of neurofascin detected several neurofascin molecules along the proximal axon (arrows) but not specifically localized where the AIS later developed (arrowheads). (B) Trajectories from SPT of Atto647N-coupled anti-GFP nanobodies bound to GPI-GFP color-coded for *D* showed no difference along the proximal axon (0.38 vs. 0.37 $\mu m^2/s$, $n = 451$) on DIV3 and little difference compared with the distal axon (0.42 $\mu m^2/s$, $n = 1{,}097$). (C) On DIV5, the AIS was detectable by live neurofascin immunolabeling (arrowheads). (D) Trajectories color-coded for *D* showed that the lateral mobility was reduced in this region (0.08 $\mu m^2/s$, $n = 267$) compared with the adjacent segment of the proximal axon without a clear neurofascin staining (0.13 $\mu m^2/s$, $n = 374$). Particles in both regions exhibited a reduced lateral mobility compared with the distal axon (0.34 $\mu m^2/s$, $n = 1{,}042$), which was slightly reduced compared with DIV3. Bars, 5 µm.

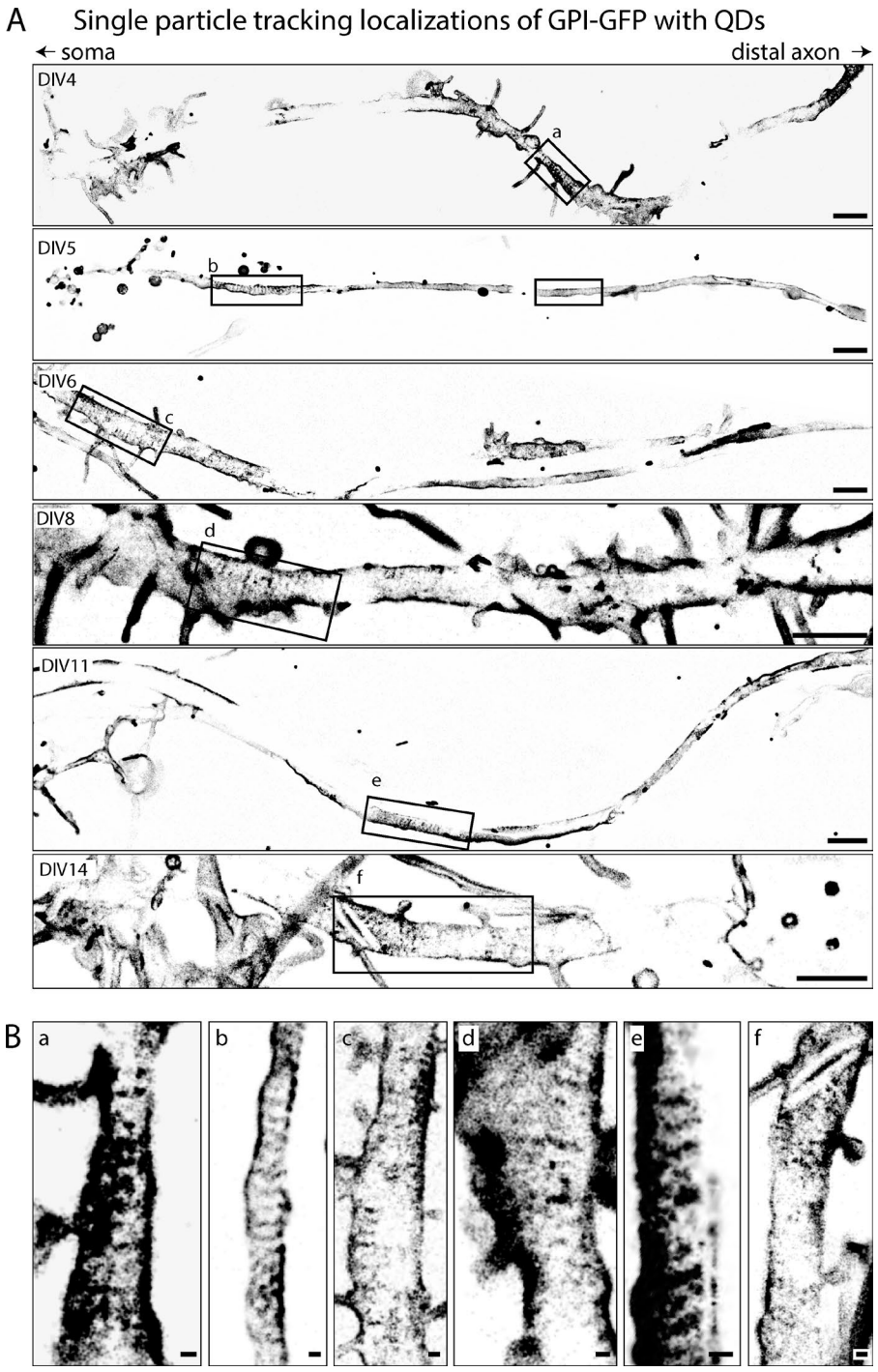


Figure S2. **Examples of the periodic pattern of localizations from SPT on the proximal axon over the time course of neuronal development.** (A) Neurons at DIV4 to DIV14 expressing GPI-GFP tracked with QDs on the proximal axon where the AIS develops. A minimum of 25,000 frames were acquired. (B) Selected regions (a–f) with periodic stripes of localizations are enlarged. Notably, the pattern was not always observed continuously along the entire proximal axon but rather in segments with higher localization densities. Sometimes, the pattern was not entirely perpendicular toward axon propagation (e). Bars: (A) 2 µm; (B) 200 nm.

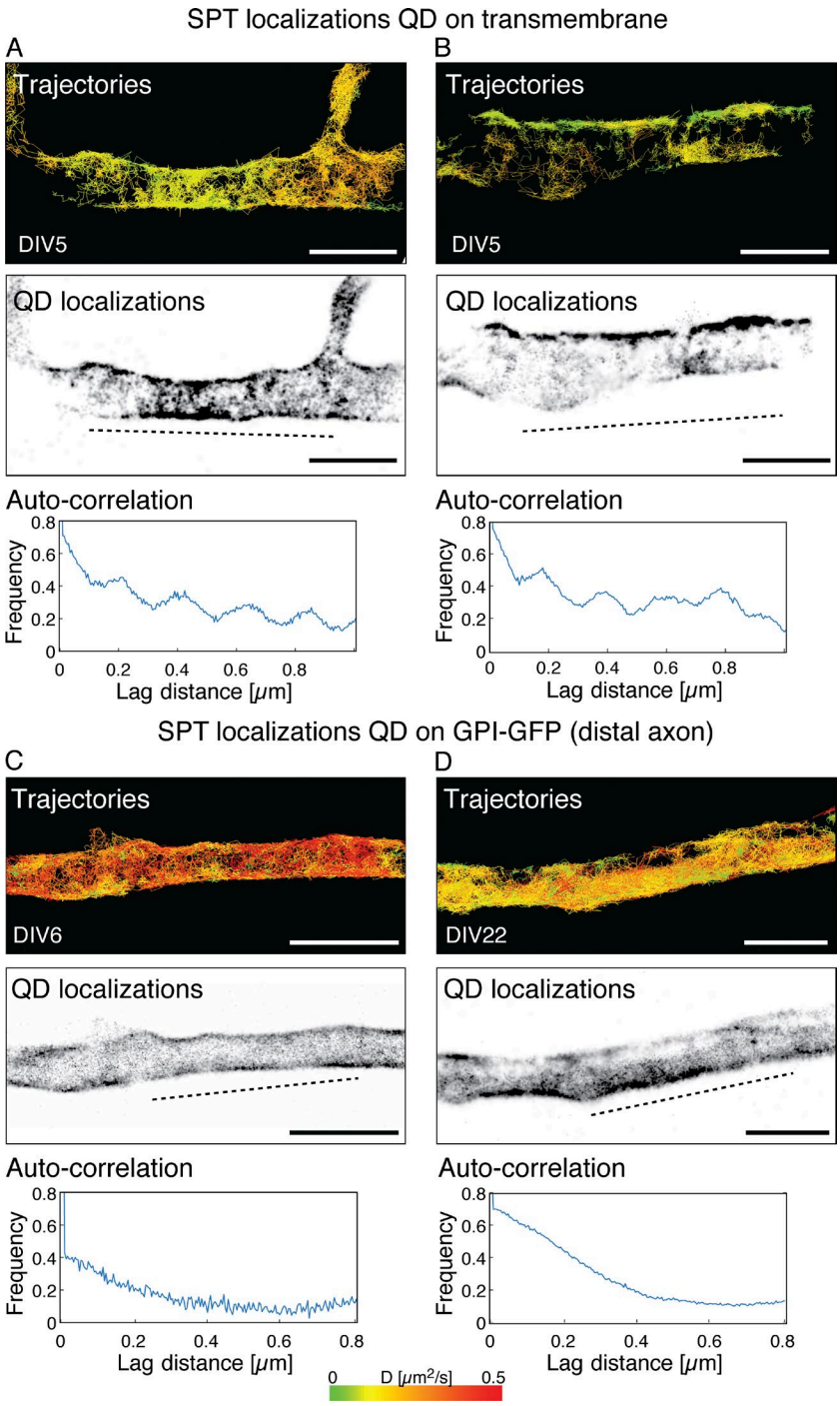
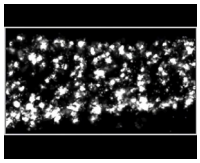
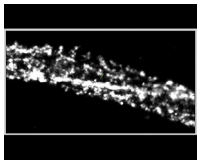


Figure S3. **SPT experiments of a transmembrane protein in the proximal and of GPI-GFP in the distal axon.** (A and B) The transmembrane probe L-YFP-GT-mHoneydew-46 was expressed in neurons and tracked at DIV5 with QDs on the proximal axon for at least 25,000 frames. Shown are trajectories of individual molecules (top), reconstructions of accumulated single-molecule localizations (middle) and autocorrelations along the axon (bottom and dashed lines in the middle row) confirming that SPT localizations are arranged periodically at ~190-nm spacing. (C and D) GPI-GFP was expressed in neurons and tracked at with QDs on the proximal axon for at least 25,000 frames at DIV6 (left) and DIV22 (right). Shown are trajectories of individual molecules (top), reconstructions of accumulated single-molecule localizations (middle), and autocorrelations along the axon (bottom and dashed lines in the middle row), which failed to detect any periodical spacing. Bars, 1 μm.



Video 1. **βII-spectrin superresolution micrograph overlaid with trajectories of QDs on GPI-GFP in the AIS at DIV5.** Shown is a movie assembled from 3,652 frames with trajectories from connecting localizations in subsequent frames. Trajectories are projected in real time but sequentially and in random order on the superresolution micrograph of βII-spectrin. SPT data are correlated with the superresolution micrograph based on fiduciary marker. Movie size is 2.25 × 7.5 µm. Axes show pixel numbers. Time resolution is 12.1 ms. Playback speed is set to 83 frames per second (real time).



Video 2. **Actin superresolution micrograph overlaid with trajectories of QDs on GPI-GFP in the AIS at DIV4.** Shown is a movie assembled from 7,680 frames with trajectories from connecting localizations in subsequent frames. Trajectories are projected in real time but sequentially and in random order on the superresolution micrograph of actin. SPT data are correlated with the superresolution micrograph based on fiduciary marker. Movie size is 2.05 × 5.9 µm. Axes show pixel numbers. Time resolution is 5.4 ms. Playback speed is set to 192 frames per second (real time).