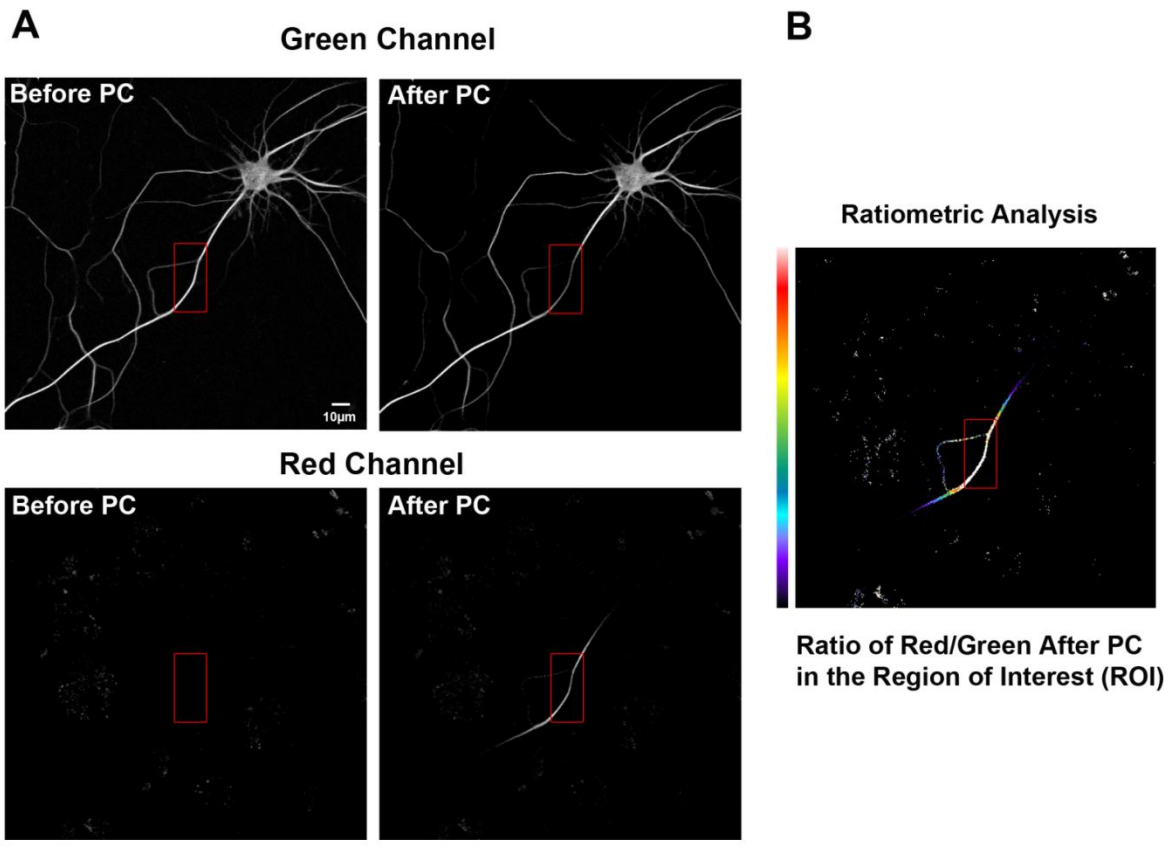
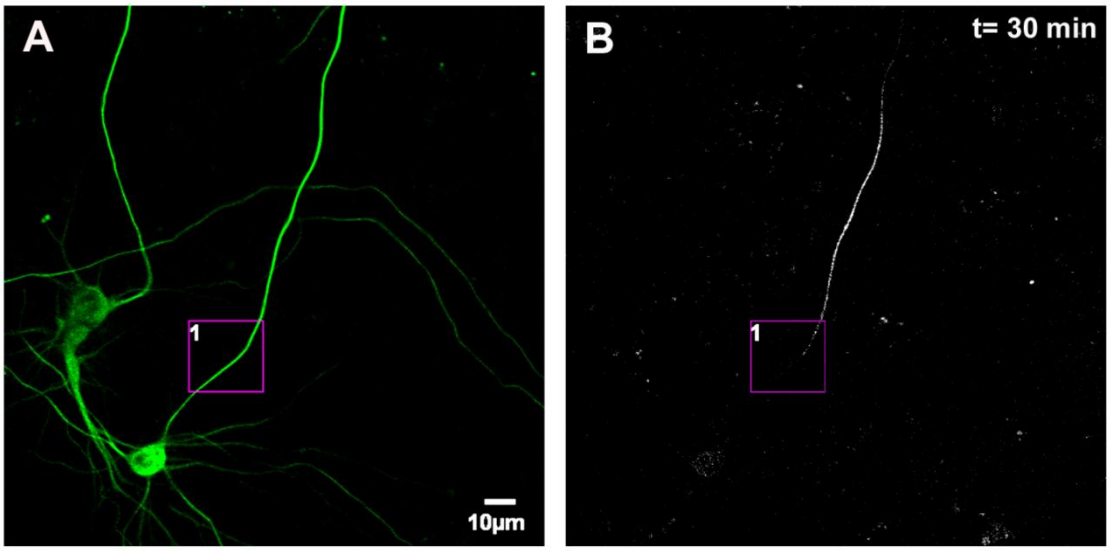
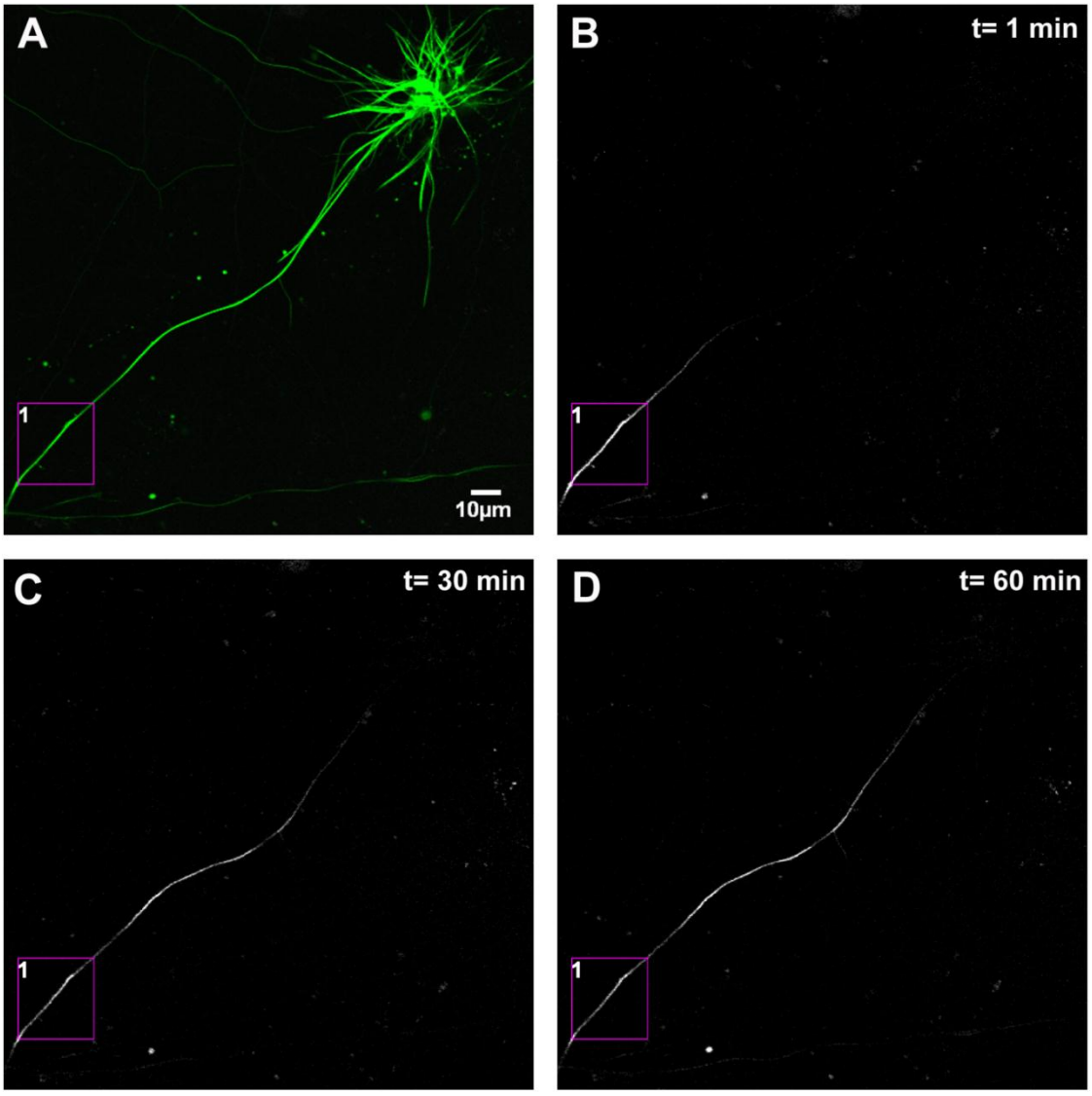
# Supplementary Figure 1



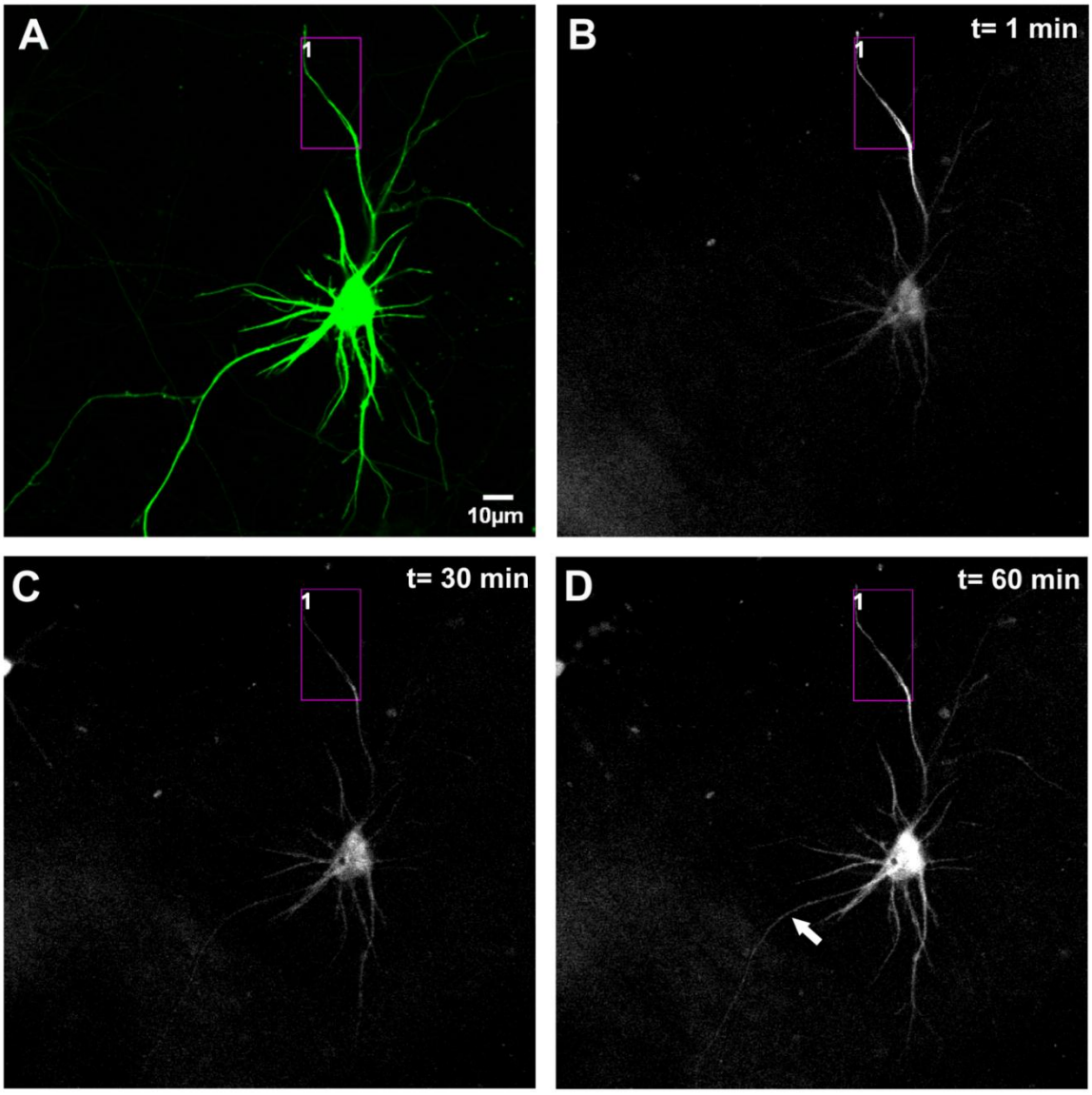
# Supplementary Figure 2



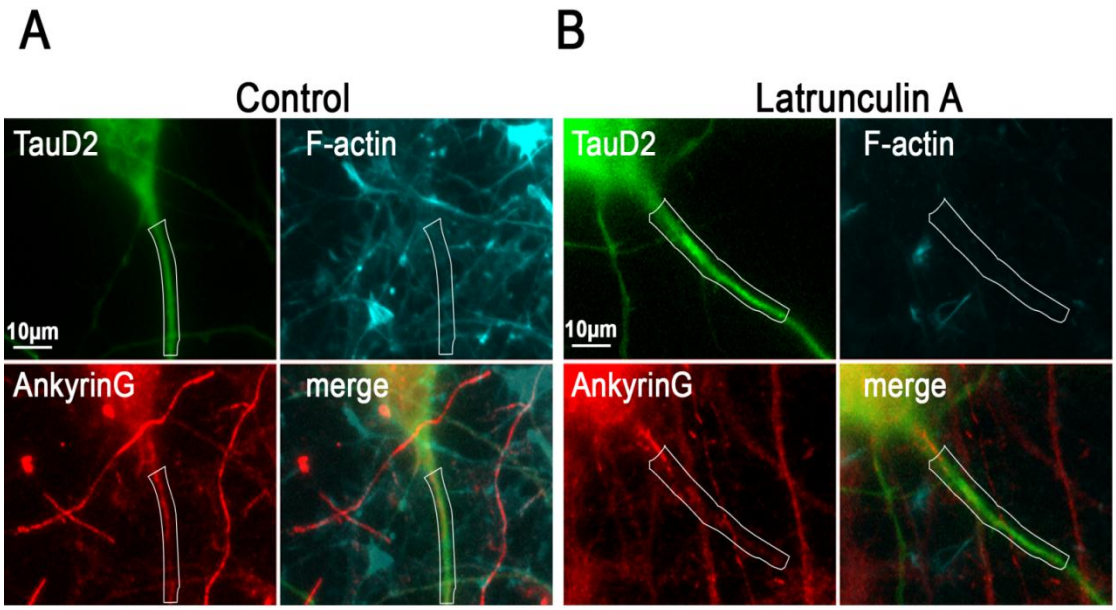
# Supplementary Figure 3



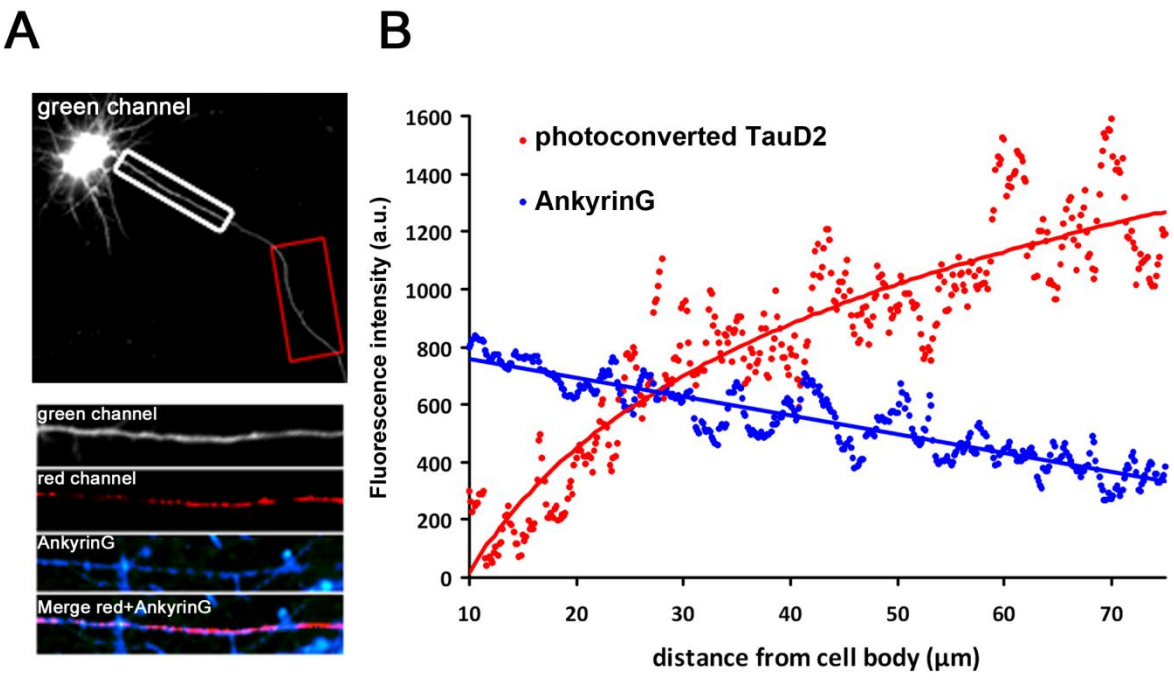
# Supplementary Figure 4



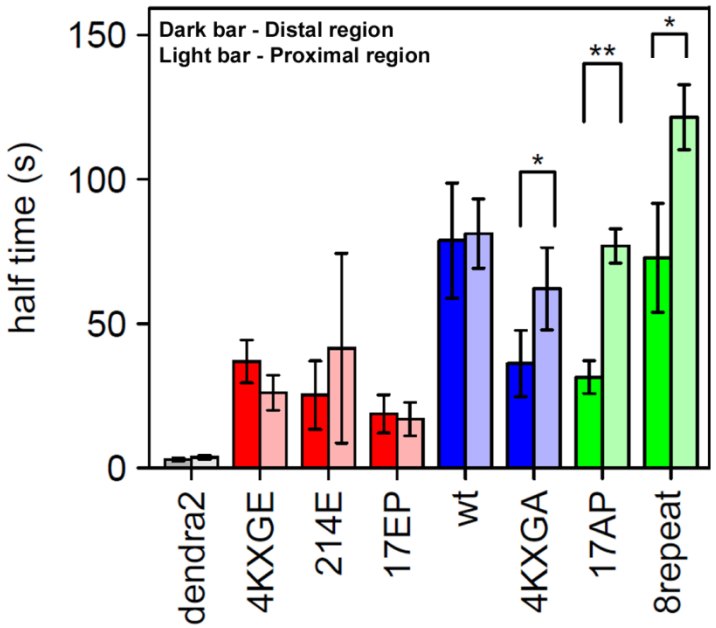
# Supplementary Figure 5



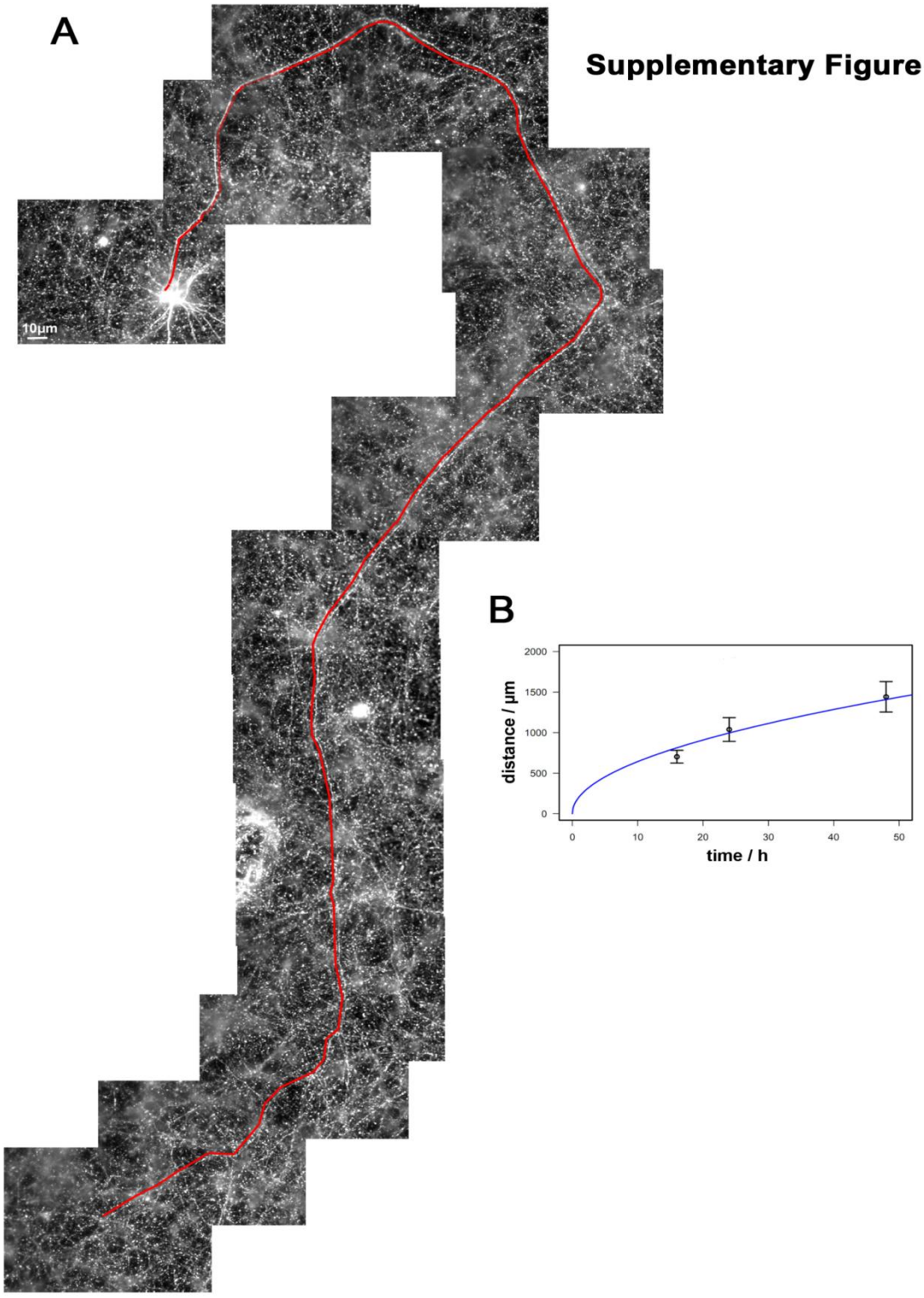
# Supplementary Figure 6



# Supplementary Figure 7



**Supplementary Figure 8**



**Supplementary Figure Legends**

**Figure S1: Ratiometric analysis of photoconversion of hTau40$^{D2}$ (A)** Raw images of green and red fluorescent channels of a neuron (10DIV) expressing hTau40$^{D2}$ are shown before and after photoconversion in the region of interest (rectangle in red). Uniform background signals were subtracted in both green and red channels using Metamorph Software. **(B)** Ratiometric image depicting the ratio of red/green channels after photoconversion in the region of interest (rectangle in red).

**Figure S2: Photoconversion of hTau40$^{D2}$ at AIS in transfected neurons. (A)** Cortical neuron (10DIV) transfected with hTau40$^{D2}$ for 2 days, showing the green fluorescence before photoconversion. **(B)** Time-lapse image taken after 30 min depicts propagation of photoconverted hTau40$^{D2}$ from the AIS towards the distal axon.

**Figure S3: Photoconversion of hTau40$^{D2}$ in the distal axon in transfected neurons. (A)** Cortical neuron (10DIV) transfected with hTau40$^{D2}$ for 2 days, showing the green fluorescence before photoconversion. **(B-C)** Time-lapse images trace the propagation of photoconverted hTau40$^{D2}$ from the distal axon to the proximal axon but not beyond the AIS even after a second photoconversion (t= 33min) (**D**).

**Figure S4: Photoconversion of hTau40$^{D2}$ in distal dendrites in transfected neurons. (A)** Cortical neuron (10DIV) transfected with hTau40$^{D2}$ for 2 days, showing the green fluorescence before photoconversion. **(B-D)** Time-lapse images trace the propagation of photoconverted hTau40$^{D2}$ from the distal dendrites to the cell body, other dendrites and the axon (after second photoconversion, t= 33min). Arrow indicates movement of photoconverted hTau40$^{D2}$ into the axon.

**Figure S5: Latrunculin disassembles F-actin in the AIS.** Cortical neurons (7DIV) were transfected with hTau40$^{D2}$; cells were treated with latrunculin A (2.5µM, 1hr) or vehicle (0.025% DMSO), fixed, and stained with an AnkyrinG antibody and phalloidin to visualize F-actin. **(A)** Control cells show broad F-actin stain, but not particularly strong, in the AIS of transfected cells. **(B)** After treatment with latrunculin A, F-actin staining globally disappears, including in the AIS.

**Figure S6: Tau diffusion barrier and classical AIS overlap.** Cortical neurons (7DIV) were transfected with hTau40$^{D2}$, and several cells were photoactivated. After 30min cells were fixed and stained with an AnkyrinG antibody. **(A) Upper panel:** Example of a transfected cell. Red rectangle indicates the area of photoconversion, white box indicates the area of maginification depicted in lower panels. **Lower panels:** Magnification of the region around the classical AIS and the Tau diffusion barrier of the transfected cell after photoconversion. Green channel shows the homogenous presence of unconverted hTau40$^{D2}$, red channel shows the typical intensity distribution of photoconverted hTau40$^{D2}$ (minimal intensity close to the cell body, left side, maximal intensity in the distal region closer to the photactivation area, right side). Blue channel shows antibody staining against AnkyrinG, which is more intense close to the cell body. **(B)** Measurements of the fluorescence intensities of the photoactivated hTau40$^{D2}$ (red) and AnkyrinG (blue) near the diffusion barrier. The Tau diffusion barrier and the AnkyrinG defined AIS show minimal overlap.

**Figure S7: Anterograde and retrograde diffusion rates of various hTau40$^{D2}$ mutants.** In order to carry out a comparative analysis of diffusion rates for the proximal and distal regions to the region of photoconversion, an equivalent region was chosen on either side (dark bars for distal and light bars for proximal), and kept at same distance (at 10 µm) from region of photoconversion. Tau variants such as 4KXGA, 17AP and 8-repeat-tau, which have high affinity for microtubules, showed slower diffusion rates into the direction of the proximal region whereas Tau and phospho-mimicking mutants (4KXGE, 214E, 17EP) did not differ on either side. **p<0.01 and *p<0.05.

**Figure S8: Tau propagates slowly into the axon.** Cortical neurons (7DIV) were transfected with hTau40$^{D2}$, and cells were fixed after different expression periods and stained with an antibody against Dendra2 to enhance the signal. Axons of Dendra2 positive cells were traced until the fluorescence front. **(A)** An example of a neuron that expressed hTau40$^{D2}$ for 24h. The axon is highlighted with a red tracer line. **(B)** Quantification of the propagation of the fluorescence front (n=4-6 cells per timepoint; error bars: SE). Data is consistent with a partially diffusion based mechanism of Tau movement (Konzack et al., 2007).

**Movie Legends**

**Movie S1: Photoconversion of Dendra2 by UV illumination of the soma.** Cortical neurons transfected with Dendra2 for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest 1 (ROI 1). The movie depicts the rapid propagation of photoconverted Dendra2 from the soma (ROI 1) into the dendrites and the axon. Images were acquired every 20 sec for 45 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.

**Movie S2: Photoconversion of hTau40$^{D2}$ by UV illumination of the soma.** Cortical neurons transfected with hTau40$^{D2}$ for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest 1 (ROI 1). The movie depicts the much slower propagation of photoconverted hTau40$^{D2}$ from the soma (ROI 1) into the dendrites and the axon. Images were acquired every 20 sec for 80 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.

**Movie S3: Photoconversion of Dendra2 by UV illumination of the axon.** Cortical neurons transfected with Dendra2 for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest 1 (ROI 1). The movie depicts the rapid propagation of photoconverted Dendra2 from the axon (ROI 1) into the soma. Images were acquired every 20 sec for 30 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.

**Movie S4: Photoconversion of hTau40$^{D2}$ by UV illumination of the axon.** Cortical neurons transfected with hTau40$^{D2}$ for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest 1 (ROI 1). The movie depicts the presence of a diffusion barrier at the AIS for photoconverted hTau40$^{D2}$; inhibiting propagation from the axon (ROI 1) into the soma. Images were acquired every 20 sec for 60 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.

**Movie S5: Photoconversion of hTau40$^{D2}$ by UV illumination of the distal dendrite.** Cortical neurons transfected with hTau40$^{D2}$ for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest 1 (ROI 1). The movie depicts the rapid propagation of photoconverted hTau40$^{D2}$ from the distal dendrite (ROI 1) into the soma and the axon. Images were acquired every 20 sec for 40 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.

**Movie S6: Nocodazol treatment disrupts the diffusion barrier**. Cortical neurons transfected with hTau40$^{D2}$ for 2 days were imaged by time-lapse video microscopy after photoconversion in the region of interest. After addition of nocodazol (**t = 30 min**), hTau40$^{D2}$ is able to pass the diffusion barrier of the initial axon segment/AIS, and thus gets missorted into the somatodendritic compartment (dashed circle). Images were acquired every 20 sec for 50 min and played at 10 frames per second. The movie starts recording 90 seconds before photoconversion. Bar, 10 µm.