Figure S1. Additional examples of cells that initiated tip extension from a respecified dendrite. Axons from these cells were severed as in Figure 3, but intermediate time points were not acquired. These examples illustrate the extensive growth that could be initiated by respecified dendrites, and also that these new axons can cross one another.

Figure S2. Neurons that initiate regeneration have a single process that switches to the axonal microtubule polarity. Neuronal shape and microtubule polarity were tracked for four days after axon severing using live imaging of EB1-GFP. Movies of EB1-GFP were acquired at 24, 48 and 96 hours (except for one cell which was only imaged at 24 and 96 hours). Low magnification images were acquired at each time point to map cell shape, and higher magnification movies of the region near the cell body were taken to determine microtubule orientation. If a dendrite extended significantly beyond its normal territory at 96 hr it was scored as having initiated tip growth. The direction of EB1-GFP dot movement was scored manually. Data from 24 and 48 hours was averaged for classification of MT polarity in the figure. If 75% or more comets moved away from the cell body, microtubule polarity was classified as plus-end-out (axonal, green arrow). If 75% or more dots moved towards the cell body, microtubule polarity was classified as minus-end-out (dendritic, red arrow). Numbers in between were classified as mixed (purple double arrow). Microtubule orientation before cutting is not from this data set, it is based on uninjured cells.

Figure S3. Neurons that express hairpin RNAs to target msps are grossly normal. A. Stable microtubules in neurons were visualized by staining with the 22C10 antibody which recognizes a neuronal microtubule-associated protein, futsch (Hummel *et al.*, 2000). Stable microtubules are seen throughout the dendrites of ddaE (cell body marked

with star) in control and msps RNAi neurons, although the futsch staining is reduced in intensity in the dendrite trunk with msps RNAi (right panels). B. An uninjured ddaE neuron expressing EB1-GFP and a hairpin RNA to target msps was imaged over the same time course as for the axon removal experiments. The dendritic tree covers the same region of the body wall as normal, and remains stable over time, increasing in size as the body increases. The only difference in shape of the dendritic tree was an increase in proximal branches (arrow) in some animals.

Figure S4. This is a version of Figure 4 in which only the Apc2-GFP channel is shown as greyscale so that the pattern of Apc2-GFP fluorescence can be seen clearly by itself.

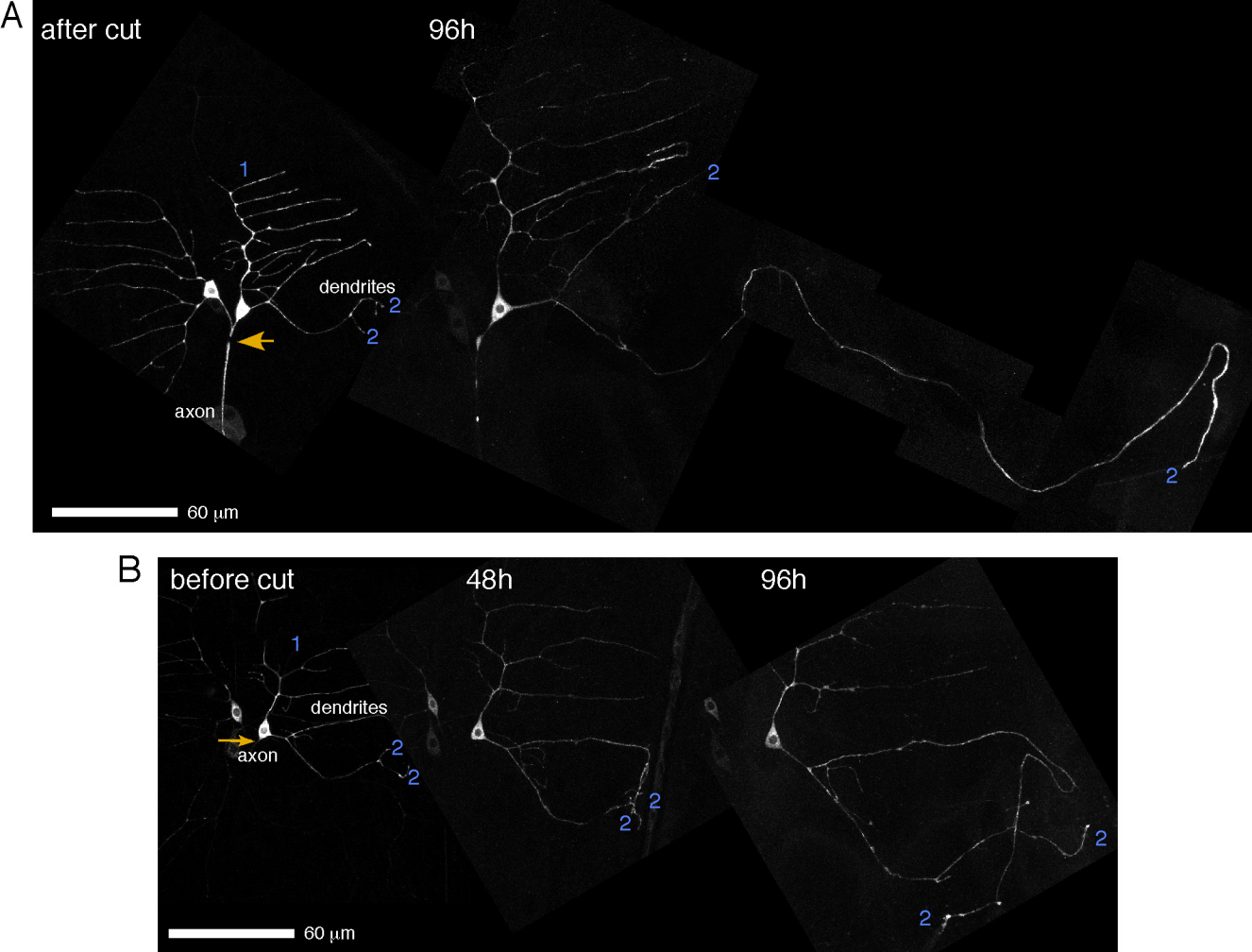
Movie 1. EB1-GFP dynamics in the same cell before and after axon severing. A ddaE cell expressing EB1-GFP was imaged immediately before proximal axotomy. Immediately after this time series was acquired the axon was severed, and after severing the optics were immediately returned to the confocal mode and a new time series was acquired. The larva was then recovered to food and remounted for further imaging after 24 hours.

Movie 2. The number of growing microtubules is upregulated by axon, but not dendrite, severing. Time series of EB1-GFP in the ddaE neuron 24 hours after axon or dendrite severing. Images from some of these movies are shown in Figure 1B- scale bars can be found there.

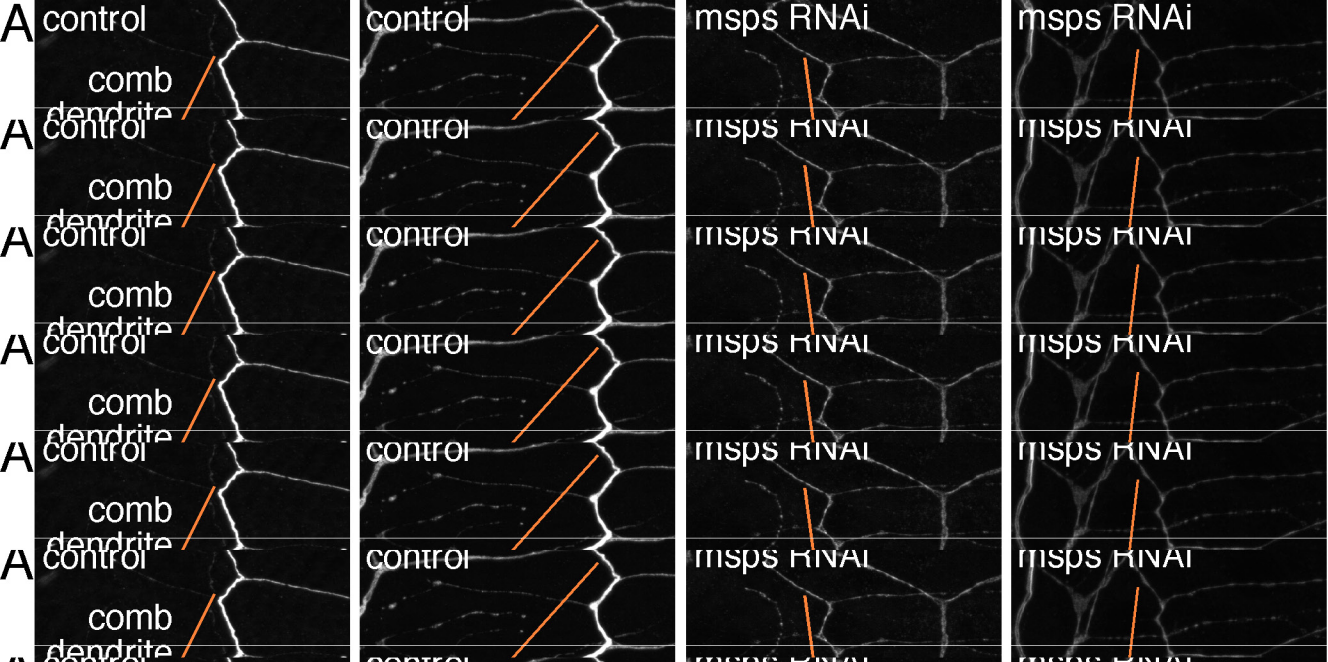
Movie 3. Microtubule orientation switches as a dendrite is respecified into an axon. EB1-GFP movies of the same ddaE neuron before axon severing and 24, 48 and 72 hours after axon severing. The exact orientation of the cell is slightly different at the different time points. Dendrite 2 is respecified to an axon, it is at the right or lower right in all movies, and direction of EB1-GFP movements in this dendrite is indicated with arrows during the movie. This is a movie of the cell shown in Figure 2A.

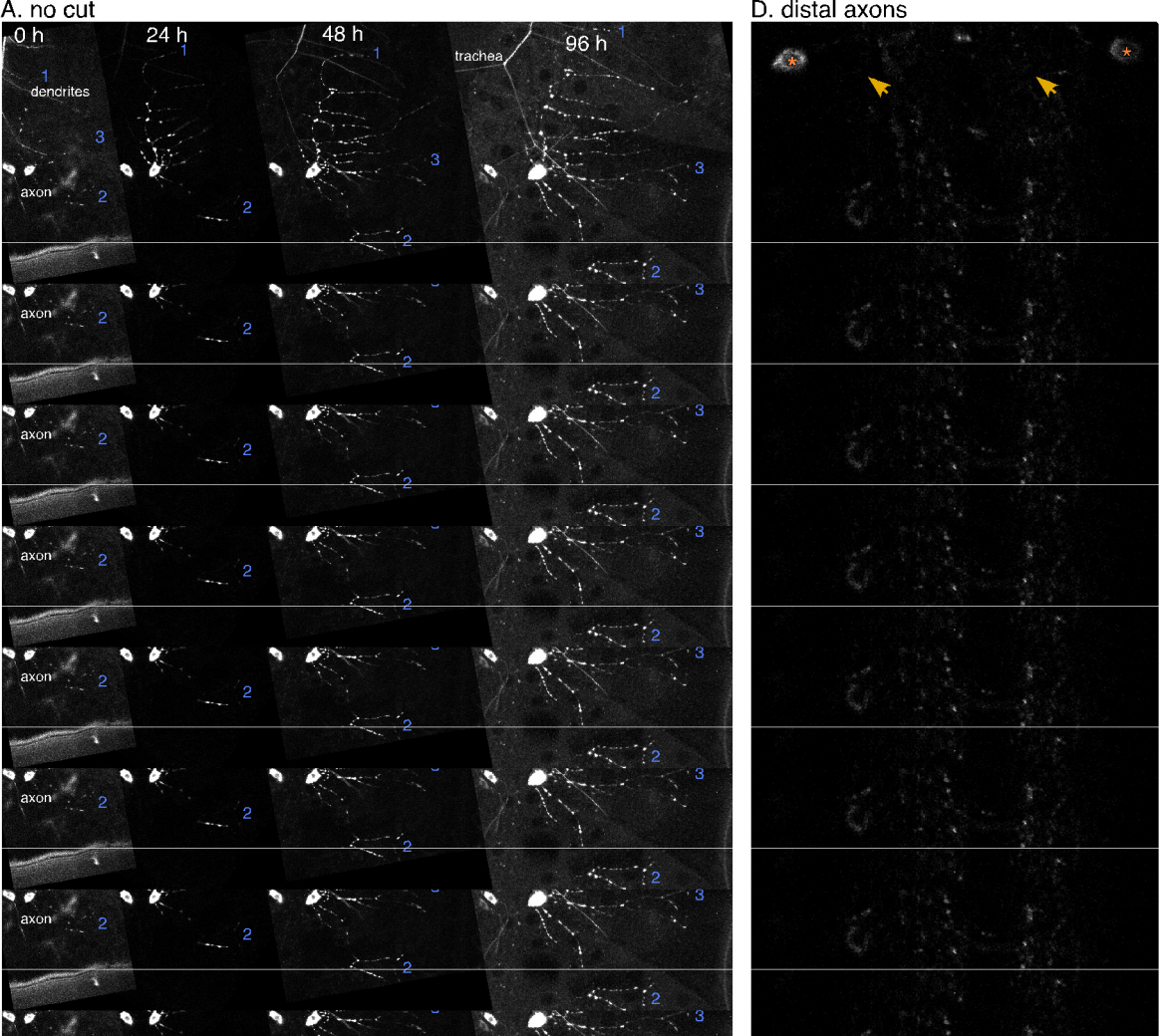
Movie 4. EB1-GFP comets are extremely rare in neurons expressing a hairpin RNA that targets msps. A ddaE neuron expressing EB1-GFP, dicer2 and a hairpin RNA targeting msps is shown. No distinct comets are visible. For comparison see Movie 5.

Movie 5. Microtubule dynamics in animals expressing a control (rtnl2) hairpin RNA. Movies of the ddaE neurons were acquired in larvae expressing EB1-GFP, dicer2 and a control RNA hairpin (Rtnl2) that does not show any neuronal phenotypes. EB1-GFP comets are seen moving towards the cell body in the main trunk of the dendrites.


A
after cut
96h
1
2
dendrites
2
2
axon
60 μm
B
before cut
48h
96h
1
dendrites
axon
2
2
2
2
2
2
2
60 μm

before
24-48h
96h
number of neurons
regeneration
5
1
no regeneration
1
1
1


A
control
comb dendrite
control
msps RNAi
msps RNAi


A. no cut
0 h
24 h
48 h
96 h
trachea
dendrites
axon
D. distal axons