

Figure S1. Overall polarity in the comb dendrite of *ddaE*.

A. Example kymographs were generated from dorsal comb-shaped dendrites of *ddaE* neurons expressing EB1-GFP with control RNAi or Trim9 RNAi 1. B. The graph shows quantification of microtubule polarity in dendrites of *ddaE* neurons. Numbers on the graph are numbers of EB1 comets analyzed for each genotype.

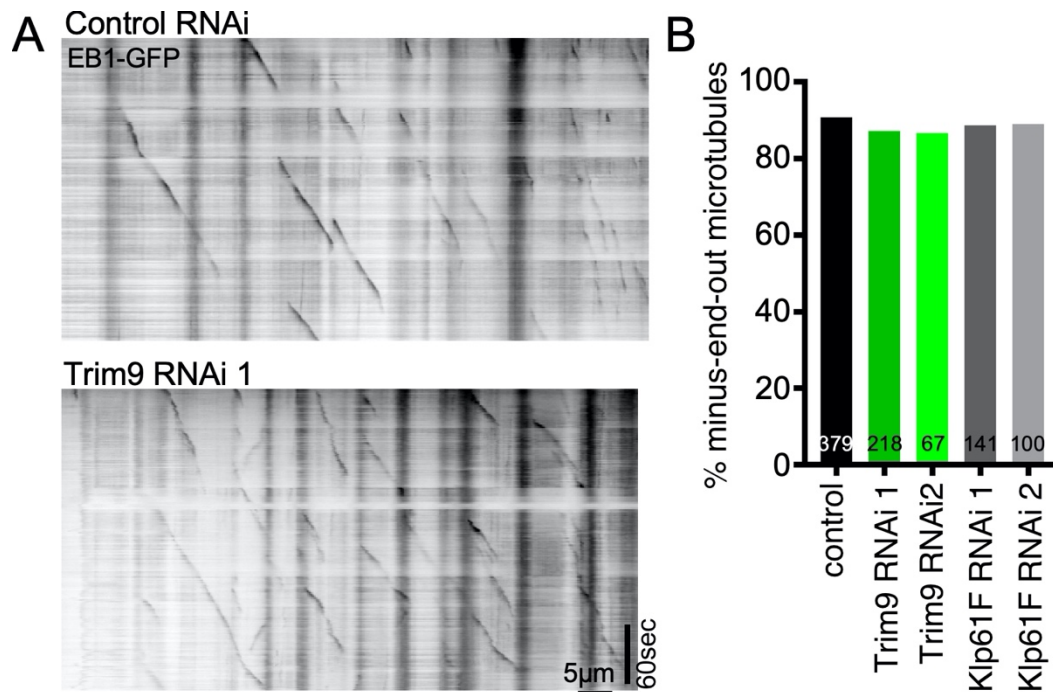


Figure S2. Trim9-RB can associate with microtubules in the presence of EB1-GFP.

S2R⁺ cells were transfected with pAc-Gal4 and pUAST-mNG-Trim9-RB and pUAST-EB1-TagRFP-T. An example of a cell expressing high levels of EB1-TagRFP-T is shown.

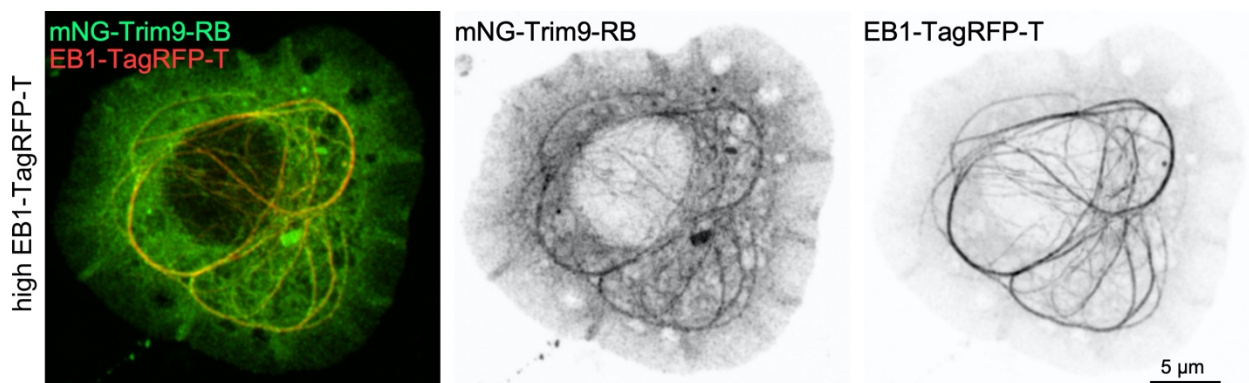


Figure S3. Klp61F-mScarlet accumulates at dendrite branch points with Trim9.

221-Gal4 was used to drive expression of Klp61F-mScarlet with mNG-tagged Trim9 or GFP-tagged EB1. Images of *ddaE* neurons are shown with mNG-Trim9-RA in A, mNG-Trim9-RB in B and EB1-GFP in C.

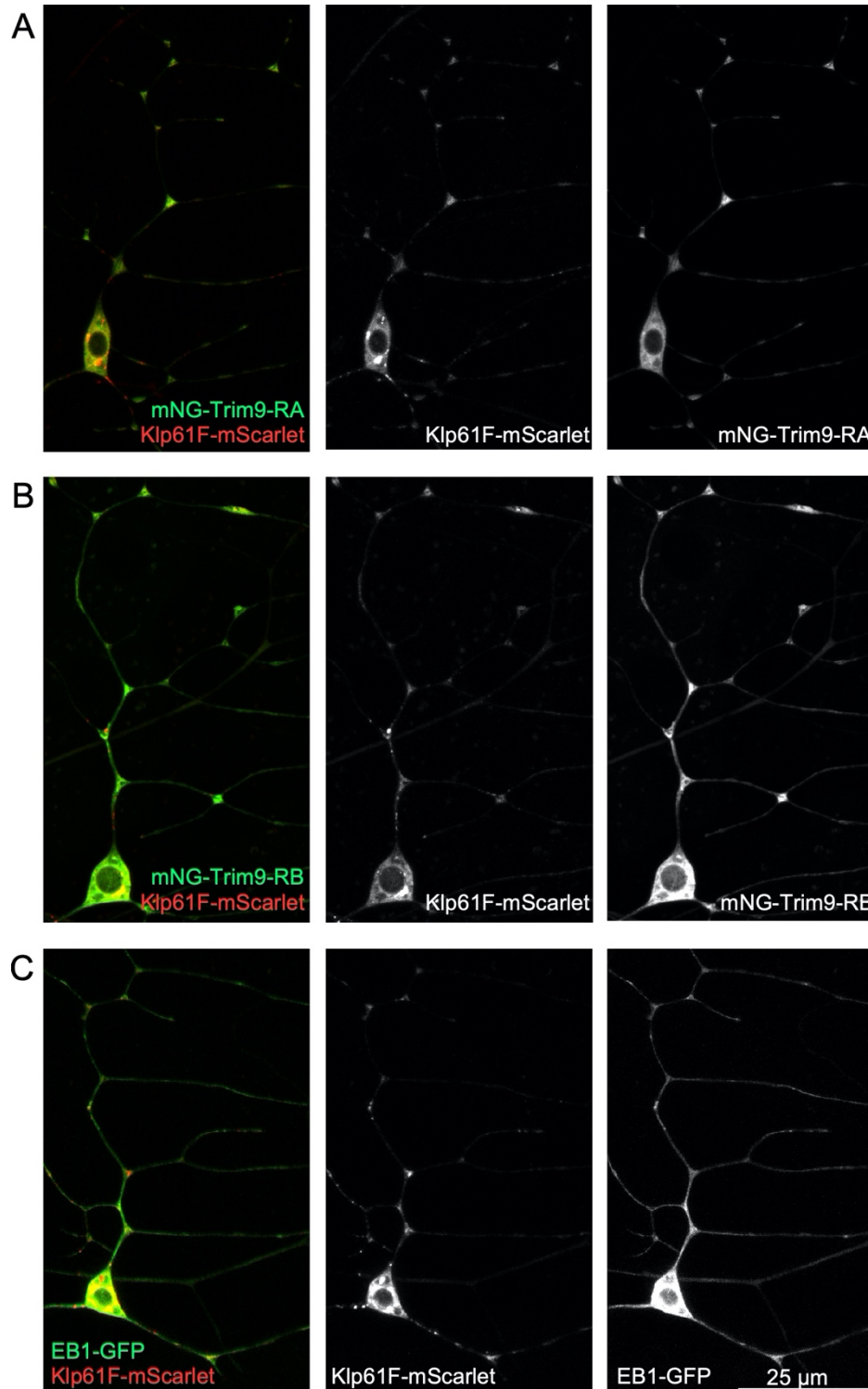
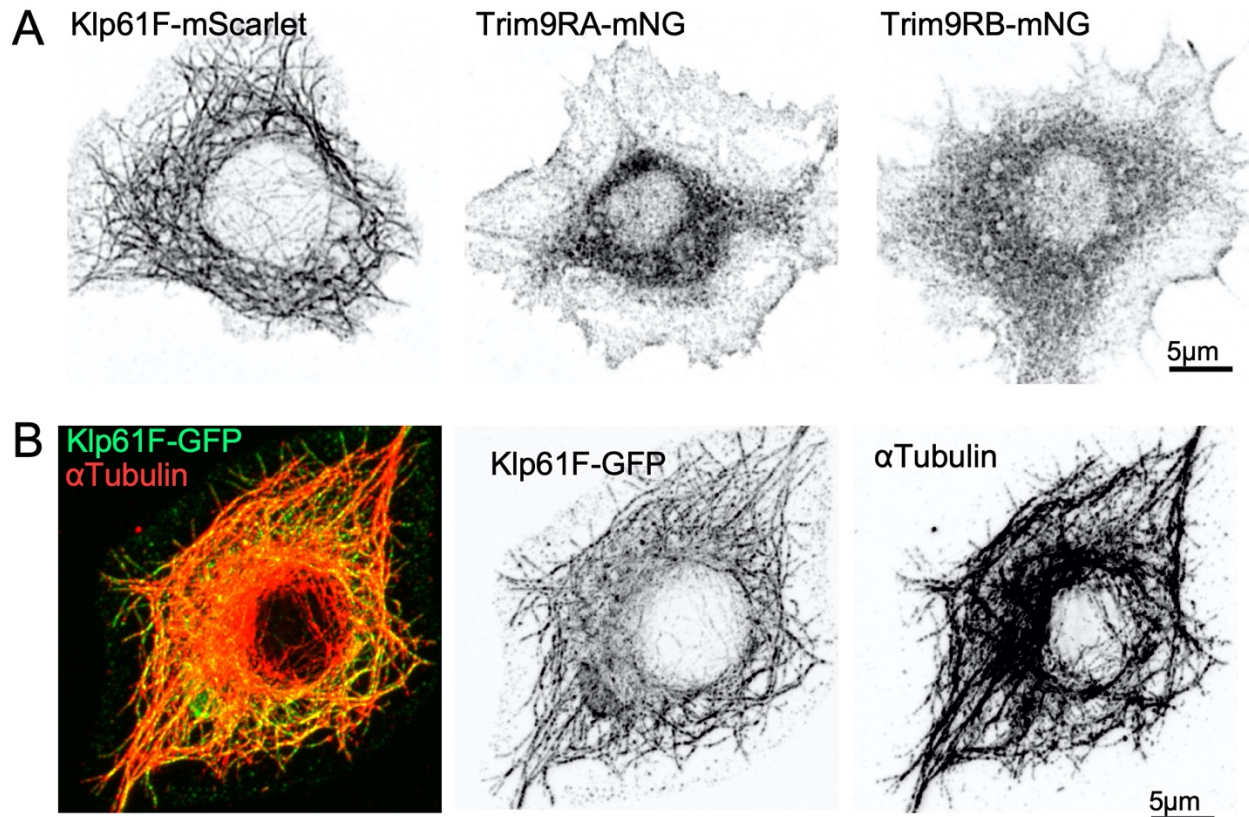
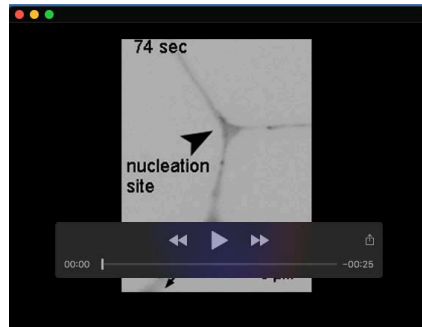


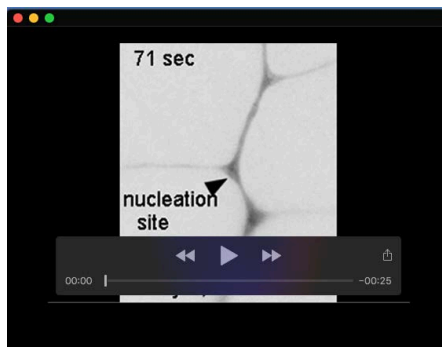
Figure S4. Klp61F localizes to microtubules in S2 cells.

S2R+ cells were transfected with pAc-Gal4 and either UAS-Klp61F-mScarlet, UAS-mNG-Trim9-RA or UAS-mNG-Trim9-RB or UAS-Klp61F-GFP. A. Cells were extracted with cold methanol and visualized directly. B. Cells were processed for immunostaining with α Tubulin as in Figure 5.





Movie 1. A segment of dendrite of *ddaE* neuron expressing EB1-GFP. Multiple EB1 comets emerged at the dendrite branch points.



Movie 2. Nucleation events at the dendrite branch point of a *ddaE* neuron. Microtubule growth at the plus end was marked with EB1-GFP comets. Microtubule plus ends were growing towards the wrong direction.



Movie 3. Microtubule in vitro growth with purified tubulin dimers. Adding chimeric Klp61F protein promoted persistent microtubule polymerization.

Table S1. A list of plasmids, Drosophila stocks and other reagents used in the manuscript is provided.

[Click here to download Table S1](#)