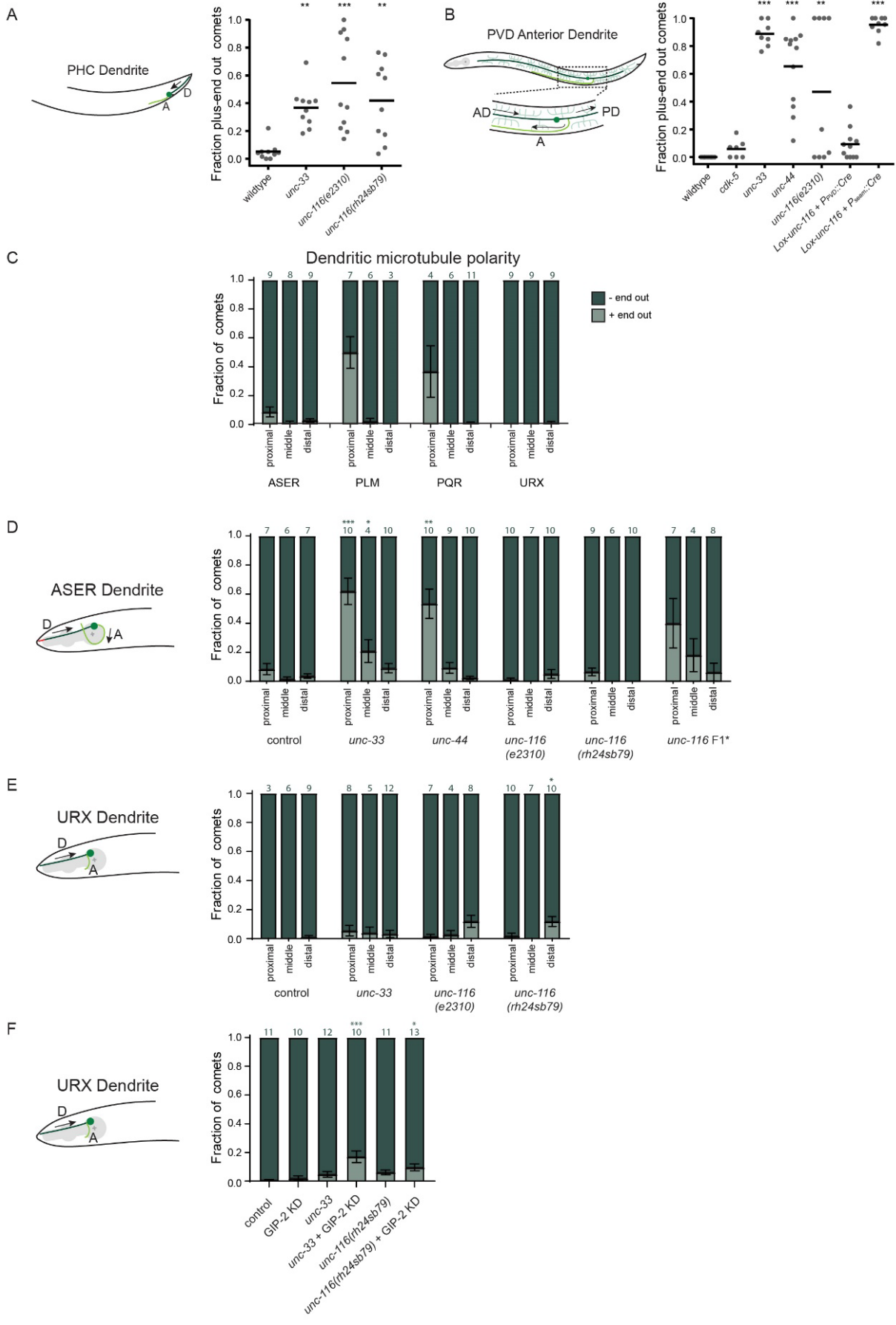


**Figure S1. Expression of the MAPH-1 microtubule marker and the *osm-6* reporter**

(A) Microtubule distribution in the PVD, URX and PQR neurons using the MAPH-1 microtubule marker. Arrowheads mark the sensory dendrite tip.

(B) The transcriptional reporter using the promoter region of the cilia specific gene *osm-6* (*Posm-6*) (Collet et al., 1998) is expressed in both the PQR as well as the URX neuron (arrows) marked by BFP expression using the *gcy-36* promoter (*Pgcy-36*). Scale, 10  $\mu$ m.



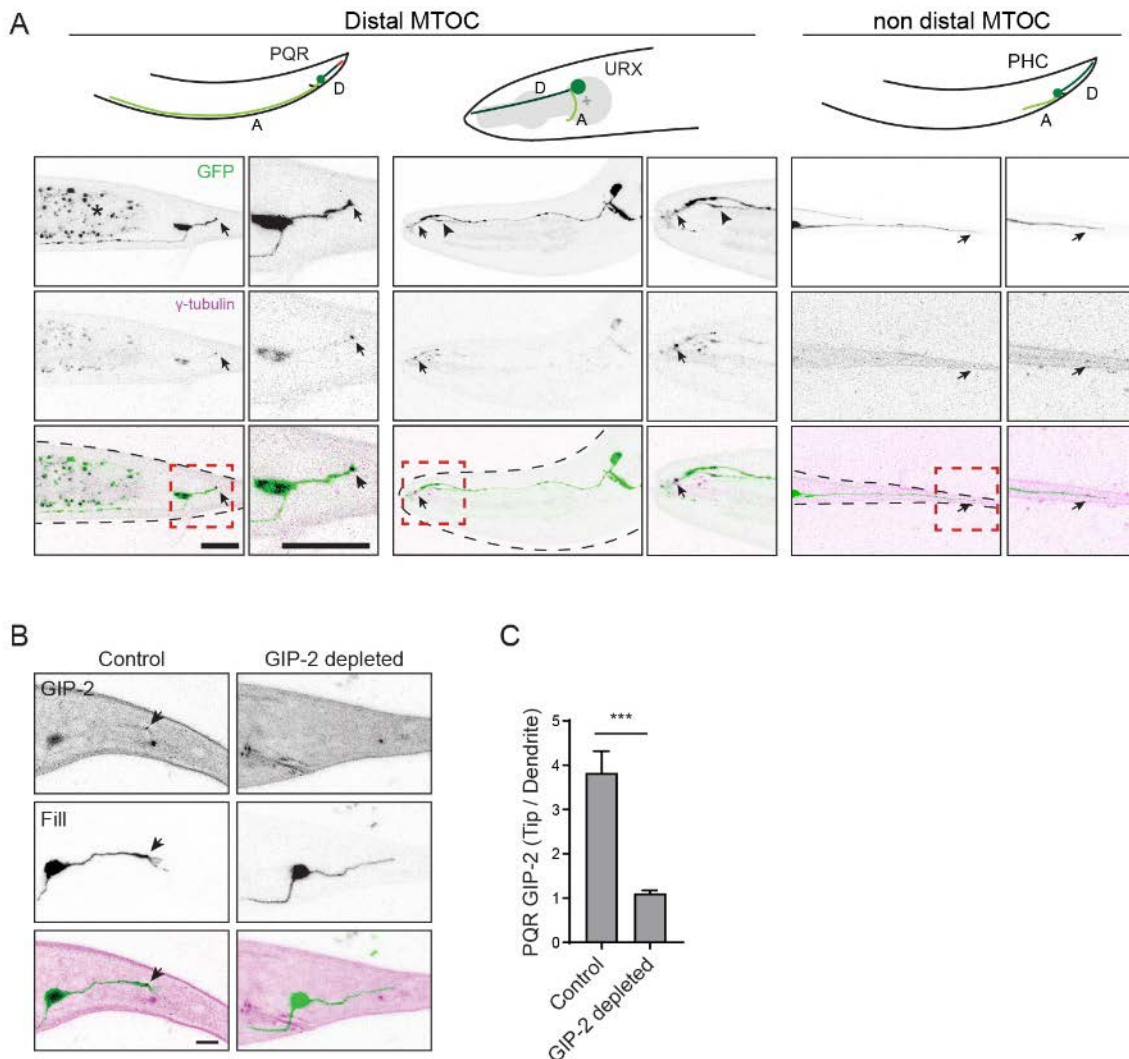
## **Figure S2. Quantification of microtubule polarity along dendrites and in diverse backgrounds**

(A-B) Quantification of microtubule polarity in the PHC dendrite (A) and the PVD anterior dendrite (B). Same data as Fig. 3E and 3F, here each neurite is separately shown as well as the average.

(C-E) Quantification of microtubule polarity based on EBP-2::GFP along the dendrites. Dendrites were divided in 3 equal parts: proximal, middle and distal to the cell body. Only segments with  $\geq 5$  growth events per worm were used (n is indicated on top).

(F) Quantification of microtubule polarity upon specific depletion of GIP-2::GFP in the URX neuron, in wildtype or indicated mutants.

Statistics for A-B,D-F comparing the mutant dendrite or dendrite segment to the wildtype, one-way ANOVA followed by Dunnett's multiple comparisons test, \*\*\* $p < 0.005$ , \*\* $p < 0.01$ , \* $p < 0.05$ ; A, Axon (light green); D, dendrite (dark green); AD, anterior dendrite; PD, posterior dendrite.

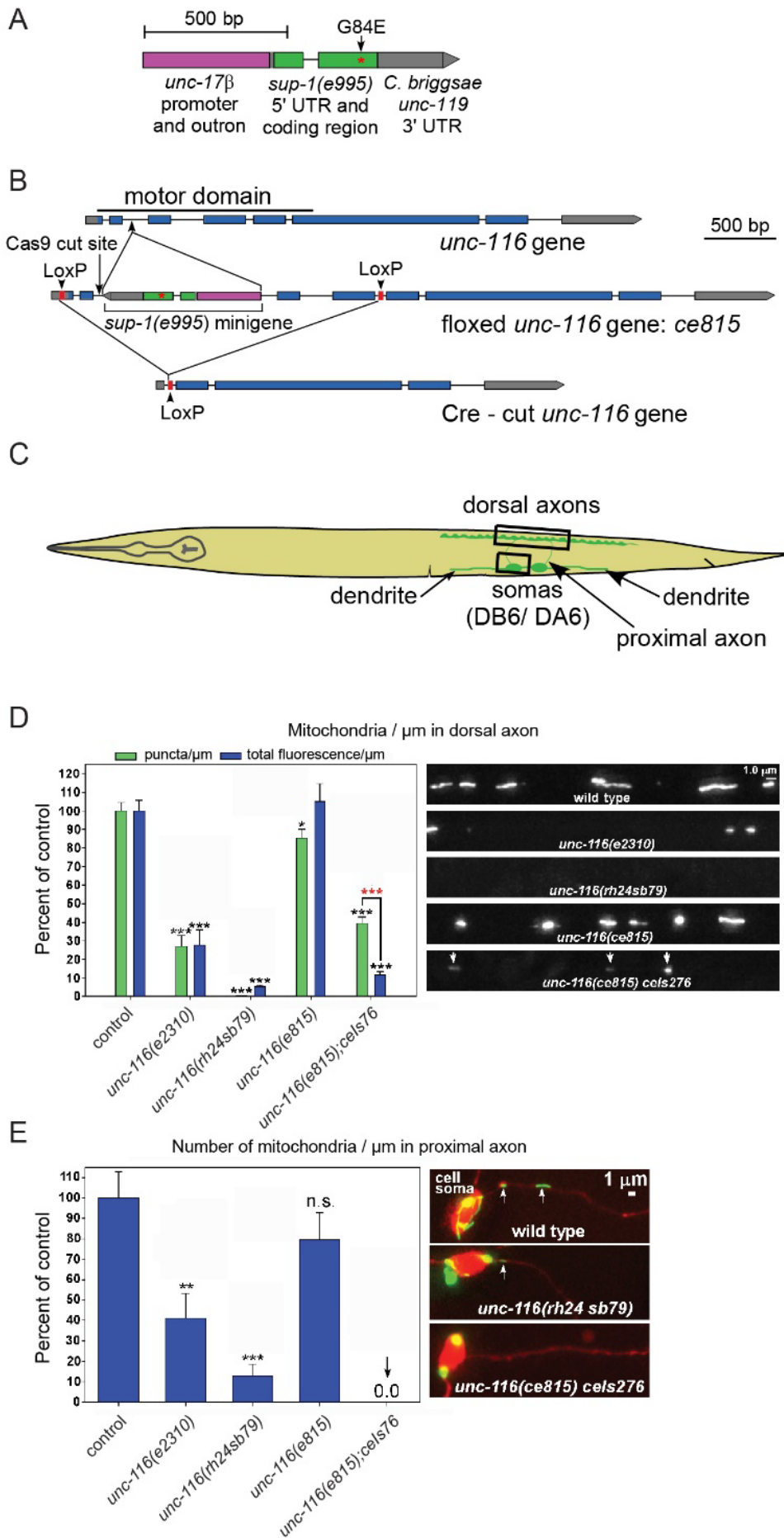


**Figure S3. TBG-1/ $\gamma$ -tubulin accumulates at the tip of the PQR and URX neurons**

(A) TBG-1/ $\gamma$ -tubulin expression accumulates at the tip of the ciliated PQR and URX dendrites, whereas in the non-ciliated PHC dendrite it is hardly detected at the dendrite tip. The arrowhead marks a part of another neuron, the asterisk marks autofluorescent gut granules. Scale, 20 $\mu$ m.

(B) anti-GFP nanobody::ZIF-1 expression in the PQR leads to loss of GIP-2::GFP in the distal dendrite. note that GIP-2 depletion occasionally lead to loss of dendrite formation in the PQR neuron (not used for quantification), but not the URX. Scale, 10  $\mu$ m.

(C) Quantification of the distal GIP-2::GFP over the mid-dendrite ( $n \geq 10$ ). Student's t-test, \*\*\* $p < 0.005$ .



### Figure S4. Design and validation of the floxed *unc-116* allele

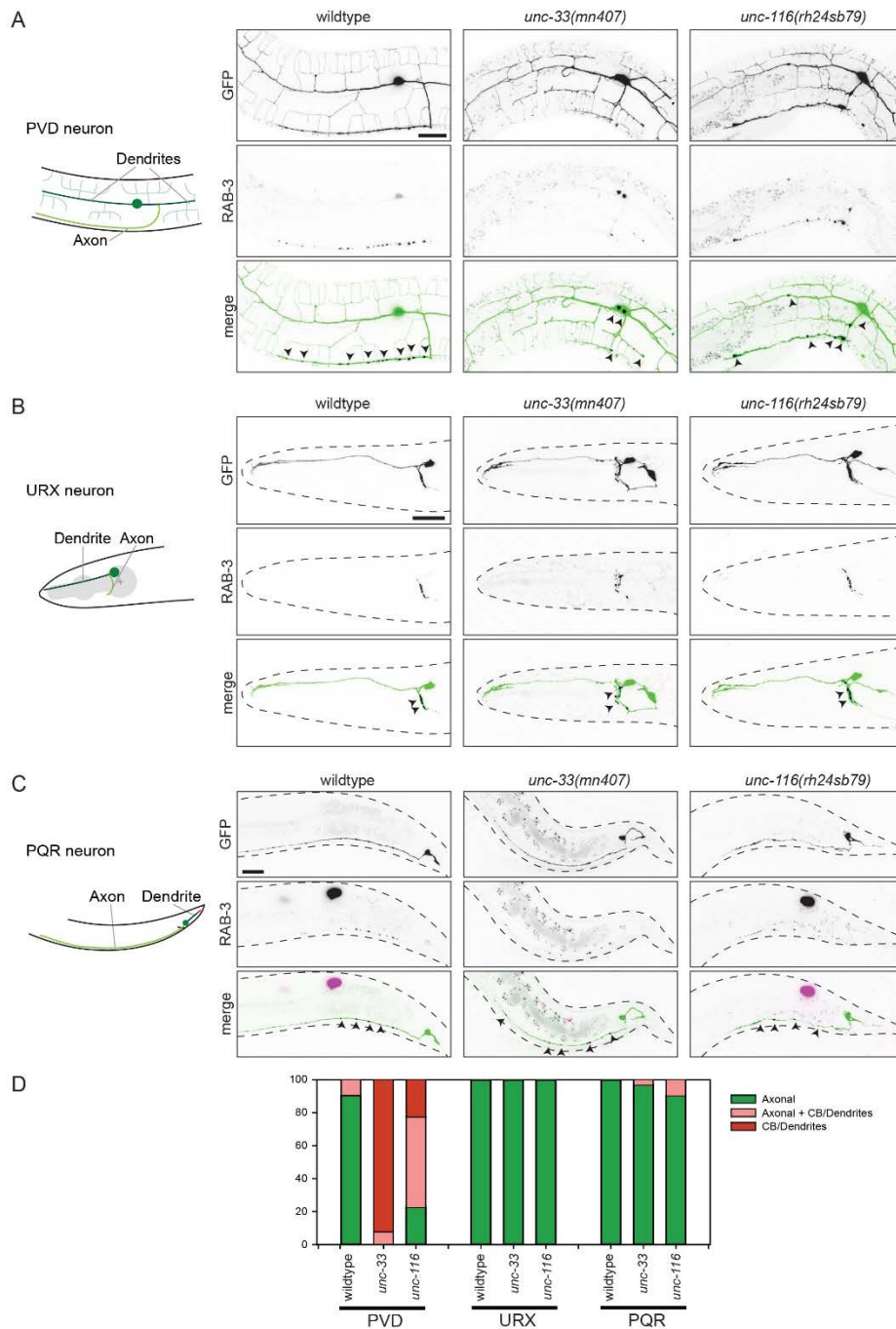
Generation and validation of the *sup-1* minigene and floxed *unc-116* (kinesin-1) approach, which is further detailed in the supplementary data.

(A) Construction of the *sup-1* minigene.

(B) Overview of the *unc-116* targeting with the *sup-1* minigene (generating the floxed *unc-116(e815)* transgene) and Cre mediated excision.

(C) Schematic representation of one of the motor neuron of the DB and DA classes. Boxes indicate the imaged regions in D and E.

(D-E) Quantification of the mitochondria number (puncta) and fluorescence in the dorsal axons of the motor neurons (D) or the mitochondria numbers in proximal axons of the motor neurons (E). Here the classic *unc-116* mutants (*e2310* and *rh24sb79*) are compared to the floxed *unc-116(e815)* supplemented with neuron specific Cre expression (*ceIs76*). Mitochondria were visualized using an integrated TOMM-20-Venus reporter (Rawson et al., 2014).



**Figure S5. RAB-3 localization is unaffected in neurons with distal microtubule nucleation**

mKate2::RAB-3 localization (A-C) and quantification (D) in the non-ciliated PVD neuron and the ciliated URX and PQR neurons in wildtype and the *unc-33* and *unc-116* mutants. The neurons were visualized with a cytosolic GFP fill. Graph represents the location with the strongest RAB-3 signal, Axon / cell body (CB) / dendrites, visually scored as absent, weak, moderate or strong for  $\geq 40$  animals. Scale, 20  $\mu\text{m}$ .

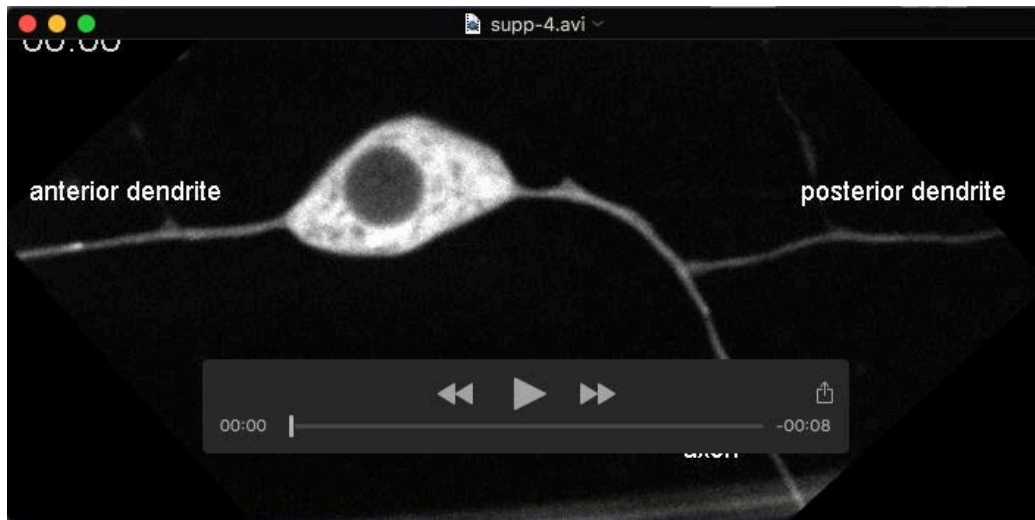
**Table S1.** List of strains used in the study

[Click here to Download Table S1](#)

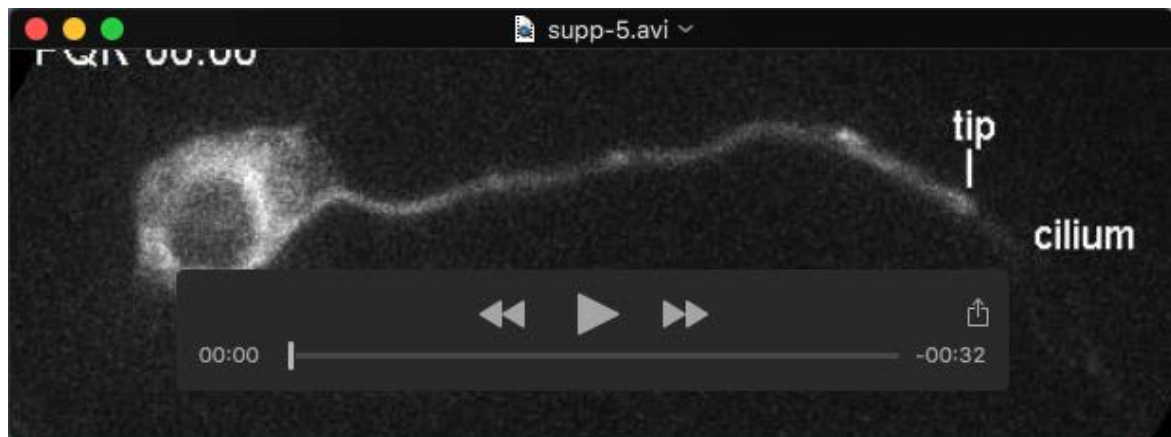




**Movie 1.** EBP-2::GFP expression in the PQR neuron (this video corresponds to Fig. 1A). Total time: 2 minutes. Acquired with 1 seconds between frames. (AVI, 2.4 Mb)



**Movie 2.** EBP-2::GFP expression in the PVD neuron (this video corresponds to Fig. 1A). Total time: 2 minutes. Acquired with 1 seconds between frames. (AVI, 2.3 Mb)



**Movie 3.** Imaging of EBP-2::GFP expression in the distal dendrite in the ciliated PQR and ASER neuron and in the PHC and PLM neuron (this video corresponds to Fig. 3C-D). The ASER neuron was left-right flipped to have the same dendrite orientation. Total time: 2 minutes each. Acquired with 1 seconds between frames. (AVI, 5.6 Mb)