

Figure S1. Raw is required for dendrite pruning of ddaD/E sensory neurons. (A) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at WP and 16 h APF. The expression of Raw-RFP or GFP-Raw rescued dendrite pruning defects in *raw*^{134.47} clones. Red arrowheads point to the ddaC somas. (B) Live confocal images of ddaD/E neurons expressing mCD8::GFP driven by *Gal4²⁻²¹* at 19 h APF. Dendrites of Ctrl RNAi and *raw* RNAi neurons. Red arrowheads point to the ddaD somas, green arrowheads point to the ddaE somas. Percentages of ddaD/E neurons showing severing defects at 16 h APF (rightest plot). (C) Quantification of number of primary and secondary dendrites at WP stage. (D) Live confocal images of ddaC neurons expressing *ppk-tdGFP* at WP and 16 h APF. The expression of *raw* RNAi driven by *GeneSwitch-Gal4-2295* in the RU-486-induced condition resulted in significant dendrite pruning defects at 16 h APF. Quantitative analysis of percentages of ddaC neurons showing severing defects and unpruned dendrite lengths at 16 h APF (rightest plots). In (C, D), data are presented as mean ± SEM. One-way ANOVA with Bonferroni test and two-tailed Student's T-test were applied to determine statistical significance. **p<0.01; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A, B, D) represent 50 µm.

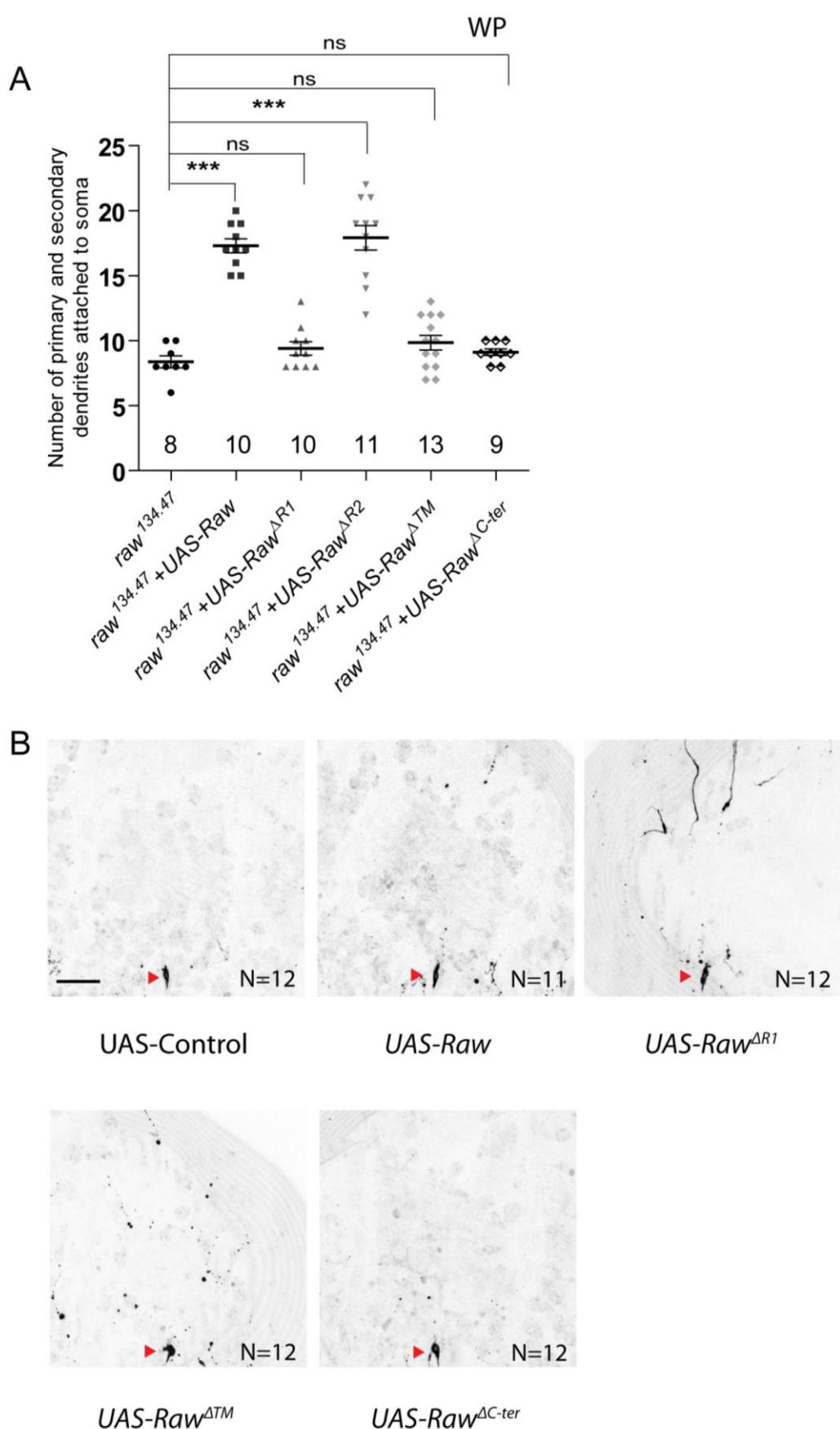


Figure S2. Overexpression of Raw variants did not affect normal dendrite pruning. (A)

Quantification of number of primary and secondary dendrites at WP stage. (B) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at 16 h APF. Dendrites of control, Raw, Raw^{ΔR1}, Raw^{ΔTM}, and Raw^{ΔC-ter}-overexpressing neurons. Red arrowheads point to the ddaC somas. In (A), data are presented as mean ± SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bar in (B) represents 50 μm.

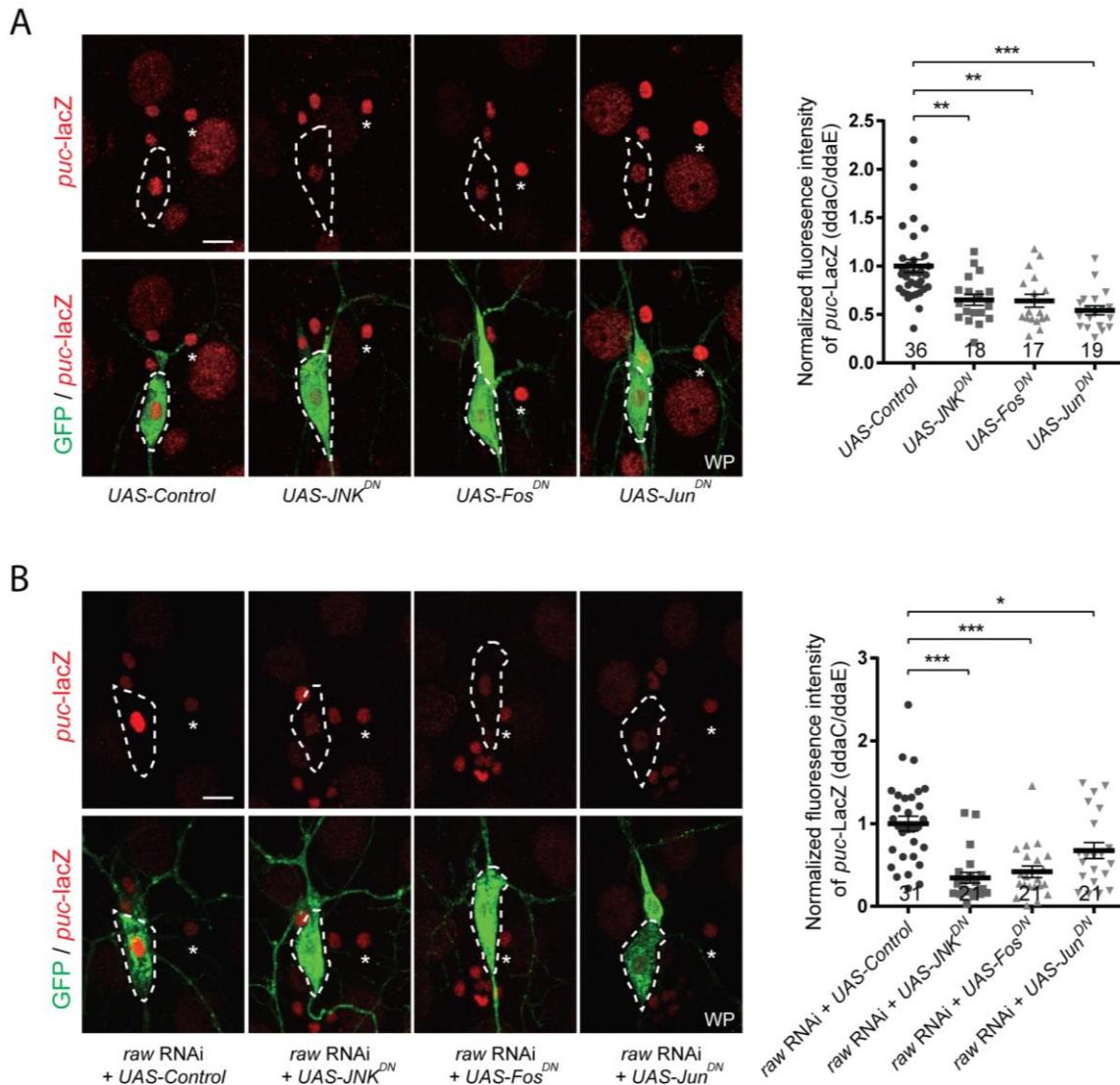


Figure S3. JNK^{DN}, Fos^{DN}, and Jun^{DN} expression attenuates JNK signaling in wild-type and raw RNAi ddaC neurons. (A) Confocal images of ddaC neurons expressing *UAS*-Control, *UAS-JNK*^{DN}, *UAS-Fos*^{DN} and *UAS-Jun*^{DN} that were immunostained for β -galactosidase at WP stage. ddaC somas are labeled by dashed lines. Quantitative analyses of normalized *puc-lacZ* fluorescence intensity (rightest plot). (B) Confocal images of ddaC neurons expressing *raw RNAi* + *UAS*-Control, *raw RNAi* + *UAS-JNK*^{DN}, *raw RNAi* + *UAS-Fos*^{DN} and *raw RNAi* + *UAS-Jun*^{DN} that were immunostained for β -galactosidase at WP stage. ddaC somas are labeled by dashed lines. Quantitative analyses of normalized *puc-lacZ* fluorescence intensity (rightest plot). In (A, B), data are presented as mean \pm SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. *p<0.05; **p<0.01; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A, B) represent 10 μ m.

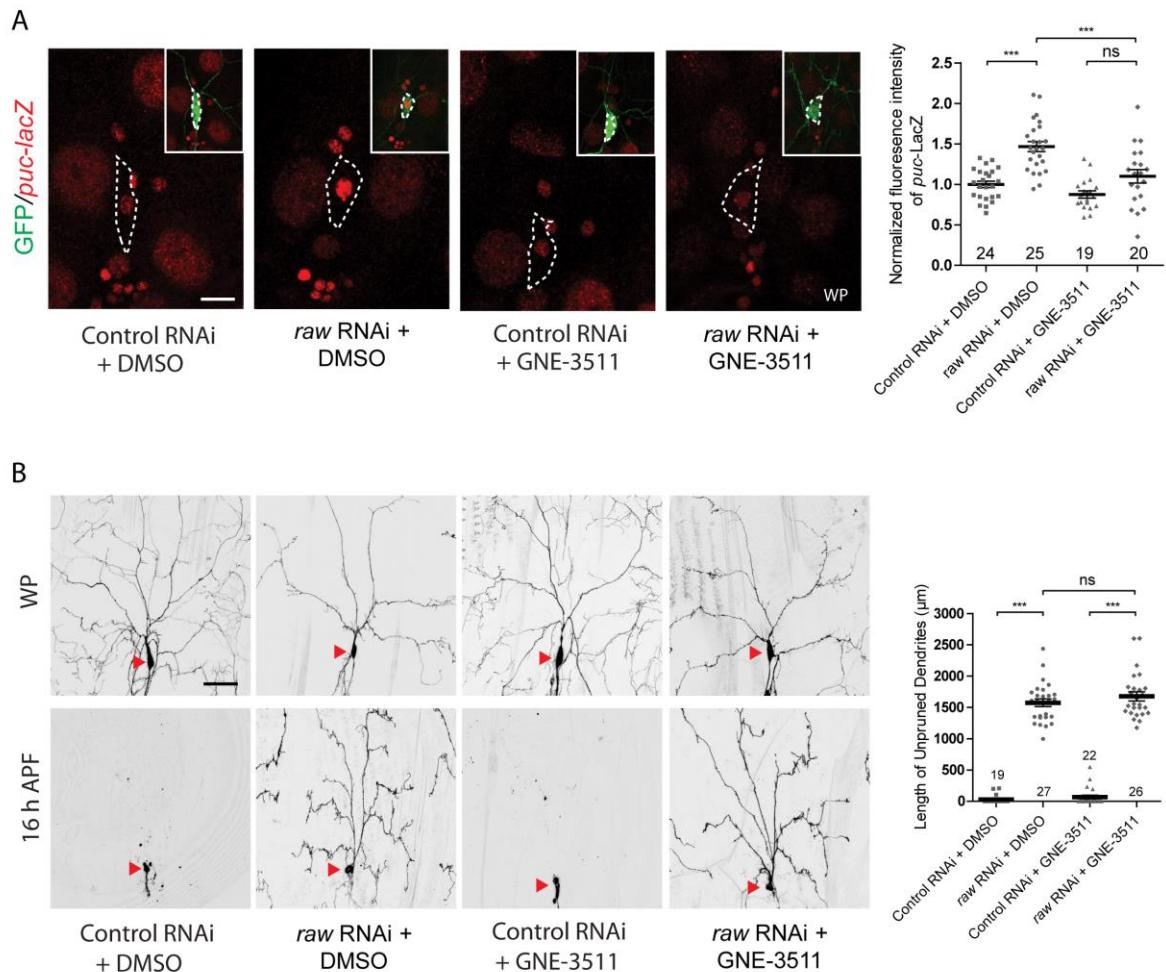


Figure S4. Raw regulates dendrite pruning independently of JNK signalling. (A) Confocal images of ddaC neurons of control RNAi + DMSO, *raw* RNAi + DMSO, control RNAi + GNE-3511 and *raw* RNAi + GNE-3511 that were immunostained for β -galactosidase at WP stage after 1-day GNE-3511 treatment. ddaC somas are labeled by dashed lines. Quantitative analyses of normalized *puc-lacZ* fluorescence (rightest plot). (B) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at WP and 16 h APF. Dendrites of control RNAi + DMSO, *raw* RNAi + DMSO, control RNAi + GNE-3511 and *raw* RNAi + GNE-3511. Red arrowheads point to the ddaC somas. Quantitative analysis of unpruned dendrite lengths at 16 h APF (rightest plot). In (A-B), data are presented as mean \pm SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant; *** $p<0.001$. The number of neurons (n) examined in each group is shown on the bars. Scale bars represent 10 μ m in (A) and 50 μ m in (B).

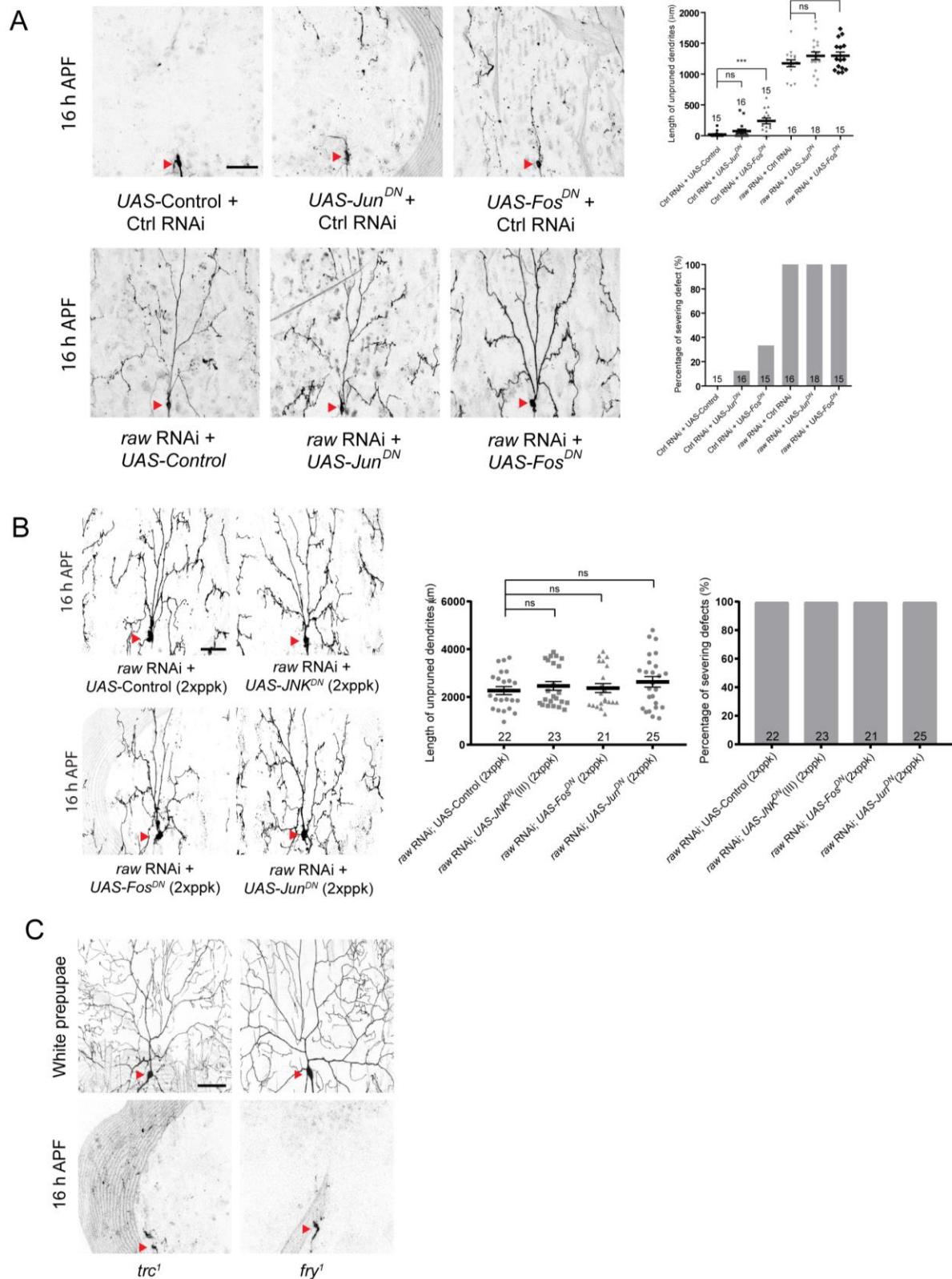


Figure S5. Raw regulates dendrite pruning independently of JNK signalling. (A) Live confocal images of ddaC neurons expressing mCD8::GFP driven by one copy of *ppk-Gal4* at 16 h APF. Dendrites of *UAS-Control* + Ctrl RNAi, *UAS-Jun^{DN}* + Ctrl RNAi, *UAS-Fos^{DN}* + Ctrl RNAi, *raw* RNAi + UAS-Control, *raw* RNAi + *UAS-Jun^{DN}*, and *raw* RNAi + *UAS-Fos^{DN}*. Red arrowheads point to the ddaC somas. Quantitative analysis of unpruned dendrite lengths and percentages of ddaC neurons showing severing defects at 16 h APF (rightest plots). (B) Live confocal images of ddaC neurons expressing mCD8::GFP driven by two copies of *ppk-Gal4* at 16 h APF. Dendrites of *raw* RNAi + UAS-Control, *raw RNAi + UAS-JNK^{DN}*, *raw RNAi + UAS-Fos^{DN}*, and *raw RNAi + UAS-Jun^{DN}*. Red arrowheads point to the ddaC somas. Quantitative analysis of unpruned dendrite lengths and percentages of ddaC neurons showing severing defects at 16 h APF (rightest plots). (C) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at WP and 16 h APF. Dendrites of *trc^l* and *fry^l* MARCM ddaC clones. Red arrowheads point to the ddaC somas. In (A, B), data are presented as mean ± SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-C) represent 50 μm.

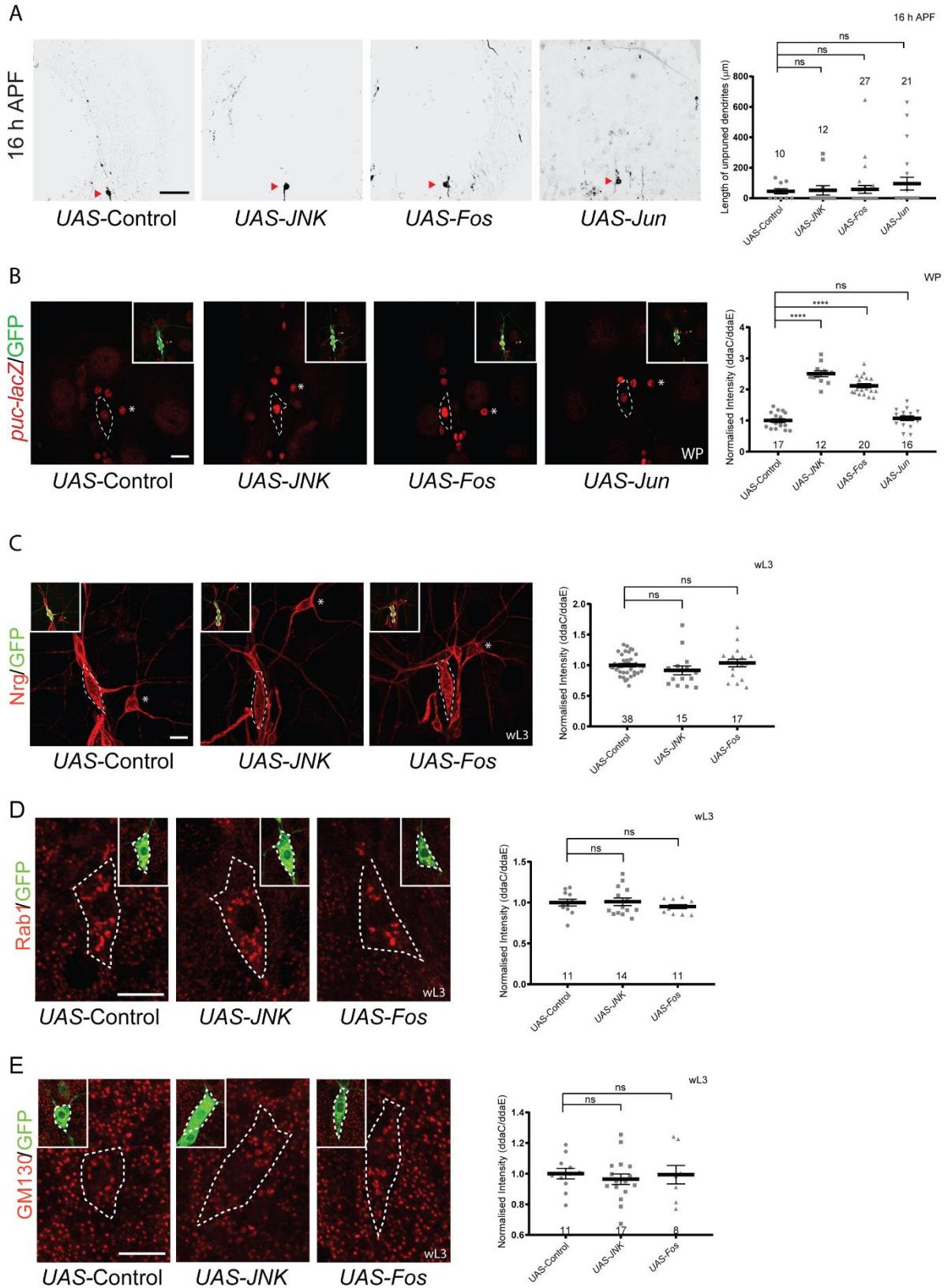


Figure S6. Hyperactivated JNK signaling does not affect dendrite pruning, Nrg level and Rab1/GM130 expression. (A) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at 16 h APF. Dendrites of *UAS-Control*, *UAS-JNK*, *UAS-Fos* and *UAS-Jun*. Red arrowheads point to the ddaC somas. Quantitative analysis of unpruned dendrite lengths at 16 h APF (rightest plot). (B) Confocal images of *UAS-Control*, *UAS-JNK*, *UAS-Fos* and *UAS-Jun* ddaC neurons of that were immunostained for β -galactosidase at WP stage. ddaC somas are labeled by dashed lines, ddaE by asterisks. ddaC neurons were identified by *ppk-Gal4*-driven mCD8::GFP (green channel) expression. Quantitative analysis of normalized *puc-lacZ* fluorescence (rightest plot). (C) Confocal images of *UAS-Control*, *UAS-JNK* and *UAS-Fos* ddaC neurons immunostained for Nrg at wL3 stage. ddaC somas are labeled by dashed lines, ddaE by asterisks. ddaC neurons were identified by *ppk-Gal4*-driven mCD8::GFP (green channel) expression. Quantitative analysis of normalized Nrg fluorescence (rightest plot). (D-E) Confocal images of *UAS-Control*, *UAS-JNK* and *UAS-Fos* ddaC neurons immunostained for Rab1 (D) and GM130 (E) at wL3 stage. ddaC somas are labeled by dashed lines. ddaC neurons were identified by *ppk-Gal4*-driven mCD8::GFP (green channel) expression. Quantitative analysis of normalized fluorescence (rightest plots). In (A-E), data are presented as mean \pm SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant; ***p<0.0001. The number of neurons (n) examined in each group is shown on the bars. Scale bars represent 50 μ m in (A) and 10 μ m in (B-E).

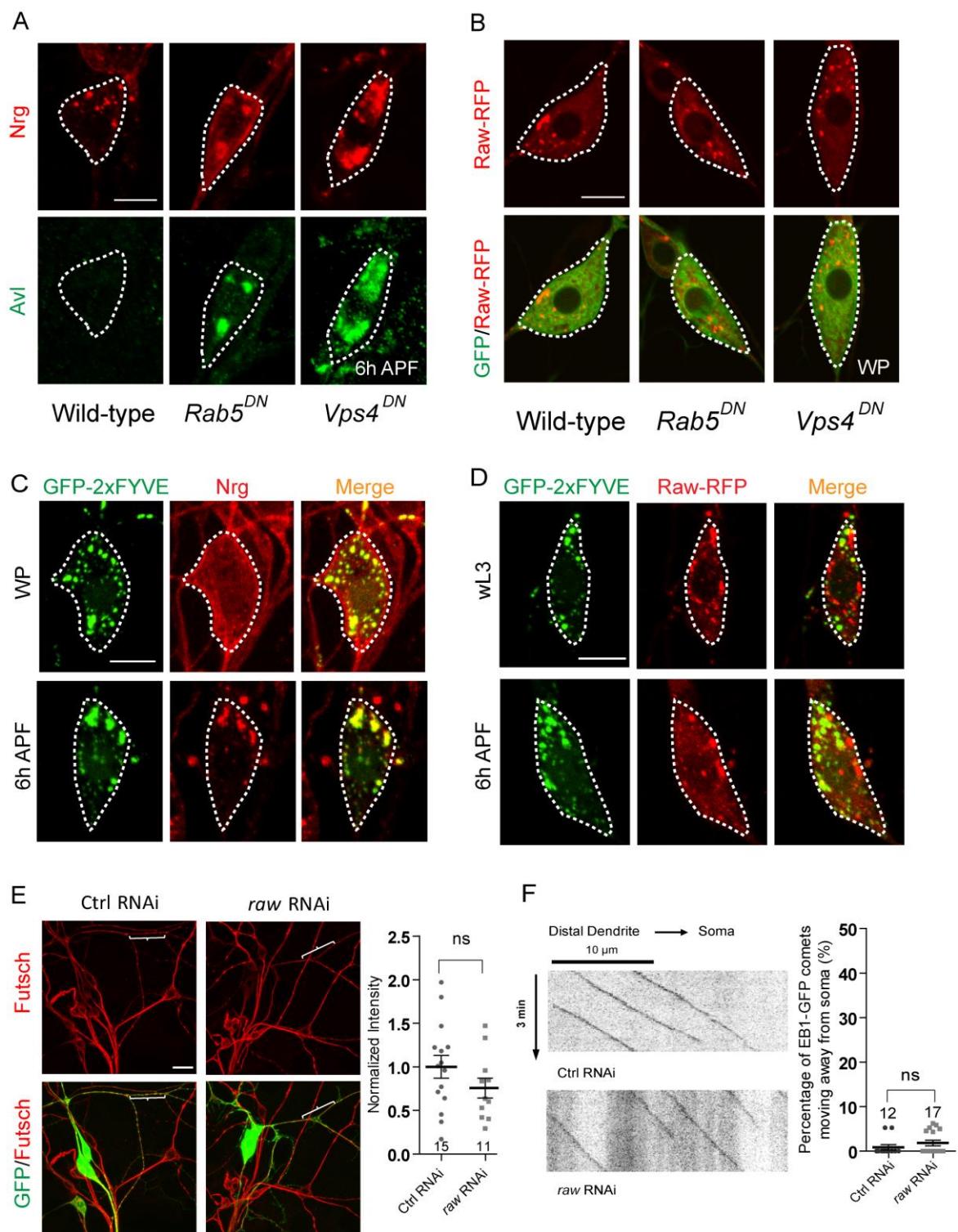


Figure S7. Raw is not internalized nor degraded via the endolysosomal degradation prior to dendrite pruning, and it is dispensable for dendritic microtubule levels and orientation.

(A) Confocal images of ddaC neurons of control, $Rab5^{DN}$ or $Vps4^{DN}$ -overexpressing neurons

that were labeled by Nrg and Avl at 6 h APF stages. ddaC somas are labeled by dashed lines.

(B) Confocal images of ddaC neurons of control, Rab5^{DN} or Vps4^{DN}-overexpressing neurons that were labeled by Raw-RFP at WP stage. ddaC somas are labeled by dashed lines. (C) Confocal images of ddaC neurons that were labeled by GFP-2xFYVE and Nrg at WP and 6 h APF stages. ddaC somas are labeled by dashed lines. (D) Confocal images of ddaC neurons that were labeled by GFP-2xFYVE and Raw-RFP at wL3 and 6 h APF stages. ddaC somas are labeled by dashed lines. (E) Confocal images of ddaC neurons of Ctrl RNAi and *raw* RNAi that were immunostained for Futsch at wL3 stage. (F) Representative kymographs depicting the movement patterns of EB1 comets in the proximal dendrites of ddaC neurons of Ctrl RNAi and *raw* RNAi at 96 h AEL. Quantitative analyses of the percentages of anterograde EB1 comets in each neuron imaged (right panel). In (E-F), data are presented as mean \pm SEM. Two-tailed Student's T-test was applied to determine statistical significance. ns, not significant. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-E) represent 10 μ m.

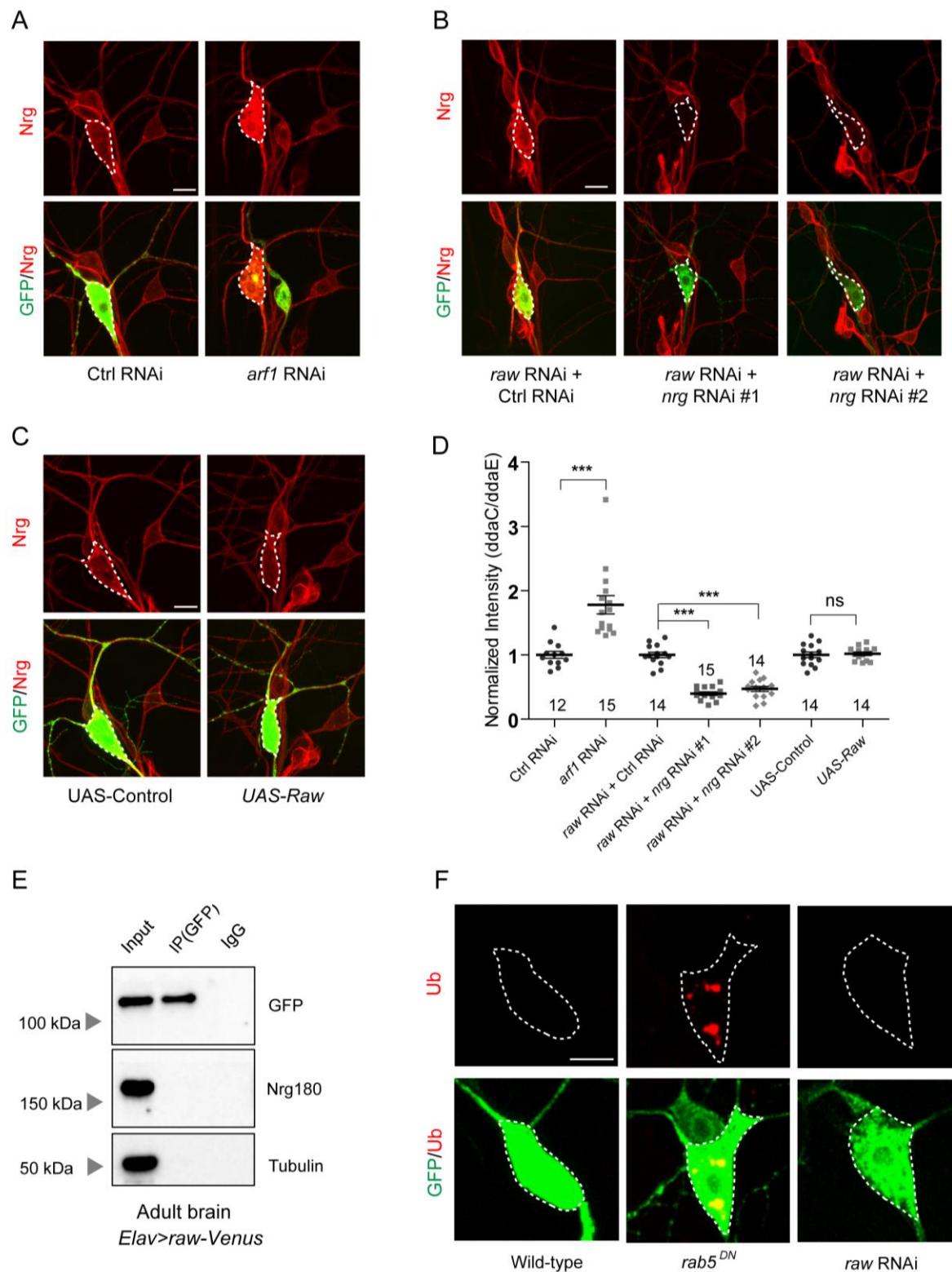


Figure S8. Raw does not form a protein complex with Nrg. (A) Confocal image of ddaC neurons of Ctrl RNAi and *arf1* RNAi that were immunostained for Nrg at wL3 stages. ddaC somas are labeled by dashed lines. (B) Confocal image of ddaC neurons of *raw* RNAi + Ctrl RNAi + *nrg* RNAi #1, *raw* RNAi + *nrg* RNAi #2, and UAS-Raw. (C) Confocal image of ddaC neurons of UAS-Control and UAS-Raw. (D) Dot plot showing Normalized Intensity (ddaC/ddaE) for various genotypes. The y-axis ranges from 0 to 4. Sample sizes (n) are indicated below each bar. Statistical significance relative to Ctrl RNAi is shown: *** p < 0.001, ns = not significant. (E) Western blot analysis of adult brain lysates from *Elav>raw-Venus* flies. Lanes include Input, IP(GFP), and IgG. Probes are GFP, Nrg180, and Tubulin. Molecular weight markers (100, 150, 50 kDa) are indicated on the left. (F) Confocal images of ddaC neurons in Wild-type, *rab5* DN, and raw RNAi flies. Top row shows Ub staining (red). Bottom row shows GFP/Ub double-staining. Dashed lines outline the neuron somas.

RNAi, *raw* RNAi + *nrg* RNAi#1 and *raw* RNAi + *nrg* RNAi#2 that were immunostained for Nrg at wL3 stages. ddaC somas are labeled by dashed lines. (C) Confocal image of control, Raw-overexpressing ddaC neurons immunostained for Nrg at wL3 stages. ddaC somas are labeled by dashed lines. (D) Quantitative analyses of normalized Nrg fluorescence. (E) Pan-neuronal driver *elav-Gal4* was used to express Raw-Venus in postmitotic neurons. Nrg was not pulled down by Raw-Venus. α -tubulin was used as a loading and probing control. (F) Confocal images of ddaC neurons of control, Rab5^{DN} or *raw* RNAi neurons that were labeled by Ubiquitin at wL3 stage. ddaC somas are labeled by dashed lines. In (D), data are presented as mean \pm SEM. One-way ANOVA with Bonferroni test and two-tailed Student's T-test were applied to determine statistical significance. ns, not significant; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-C, F) represent 10 μ m.

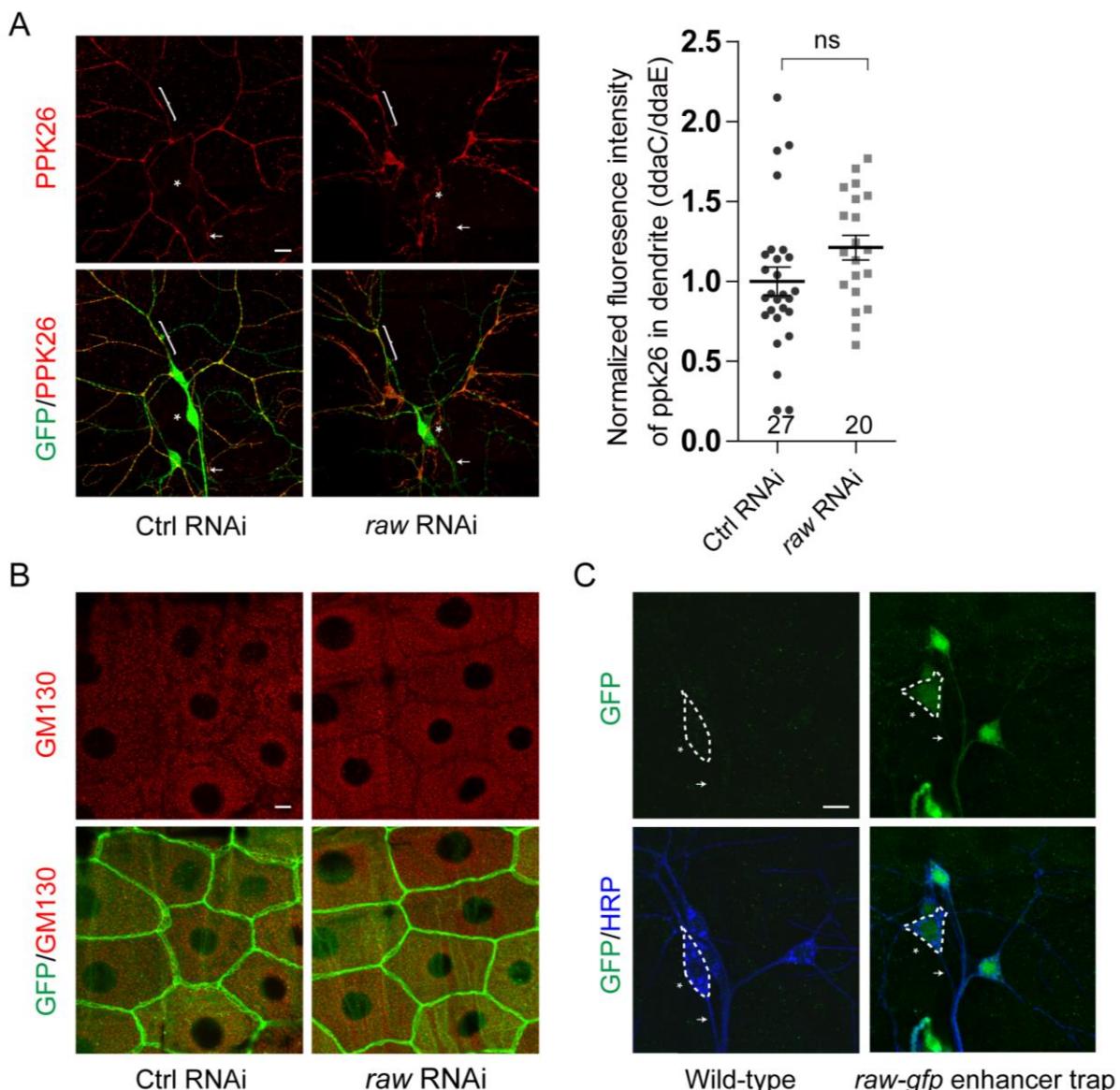


Figure S9. Raw is predominantly expressed in sensory neurons. (A) Confocal images of ddaC neurons expressing Ctrl RNAi and *raw* RNAi that were immunostained for Ppk26 at wL3 stage. Curly brackets mark the dendritic regions where fluorescence intensity was measured. ddaC somas are labelled by asterisks, axons by arrows. Quantitative analyses of normalized ppk26 fluorescence intensity in dendrites (rightest plot). (B) Confocal images of epidermal cells expressing Ctrl RNAi ($n=6$) and *raw* RNAi ($n=7$) driven by *A58-Gal4*. The epidermal cells were labelled by mCD8::GFP and immunostained for GM130 at wL3 stage. (C) *raw-GFP* enhancer trap *raw*^{MI07292} was predominantly expressed in sensory neurons ($n=15$), compared to the wild type ($n=12$). ddaC somas are labeled by dashed lines, axons by arrows. In (A), data are presented as mean \pm SEM. Two-tailed Student's T-test were applied to determine statistical significance. ns, not significant. Scale bars in (A, B, C) represent 10 μ m.

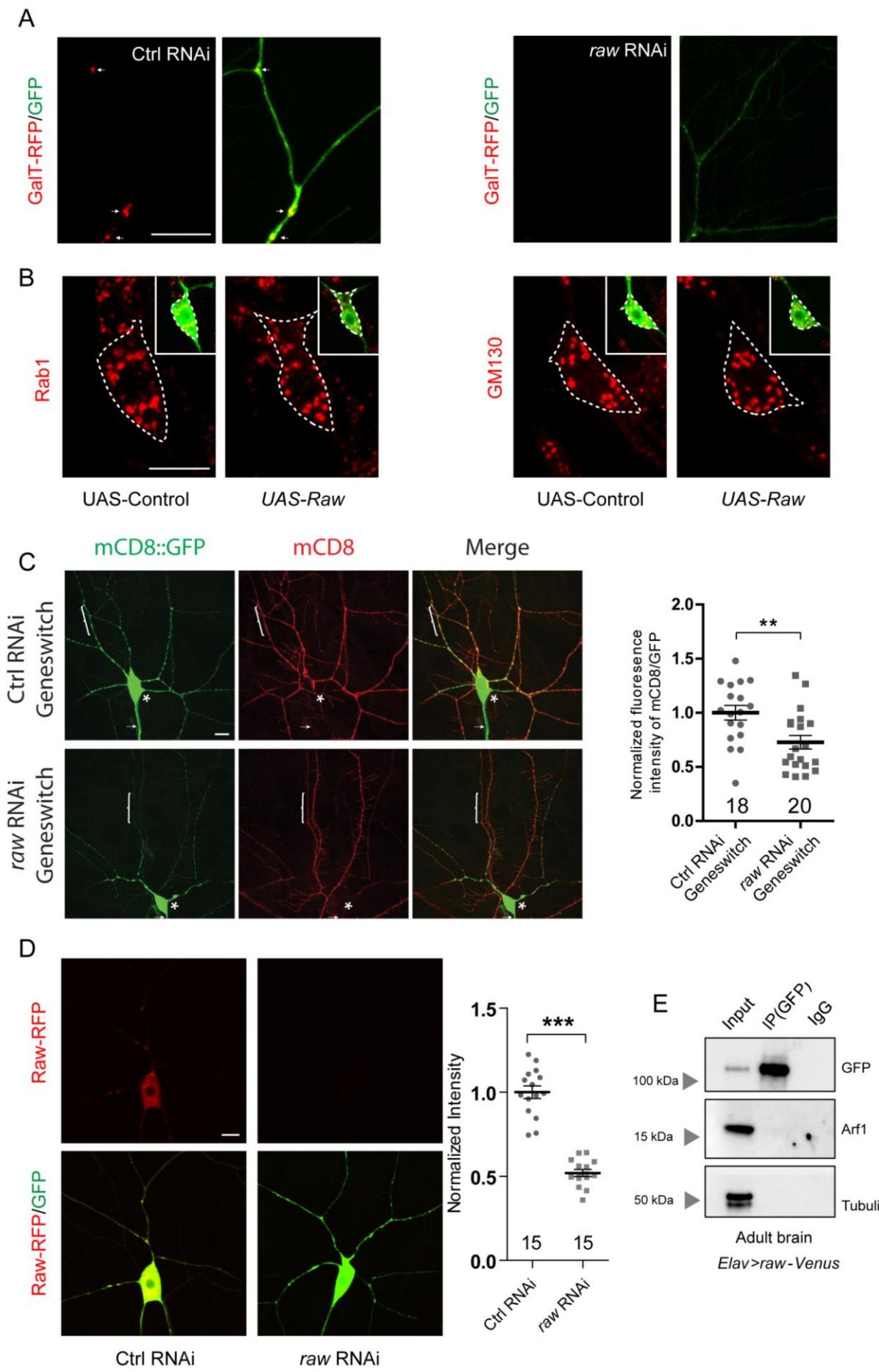


Figure S10. Raw regulates protein secretion in ddaC neurons. (A) Confocal images of Ctrl RNAi and *raw* RNAi ddaC dendrites that were labelled for GalT-RFP at wL3 stage. (B) Confocal image of control and Raw-overexpressing ddaC neurons that were immunostained for Rab1 and GM130 at wL3 stage. ddaC somas are labeled by dashed lines. ddaC neurons were identified by *ppk-Gal4*-driven mCD8::GFP (green channel) expression. (C) Confocal image of control and *raw* RNAi ddaC neurons that were immunostained for Rab1 and GM130 at wL3 stage. mCD8 signals on the surface of the dendrites in wild-type ddaC neurons were detected by the antibody against its extracellular epitope in the detergent-free condition. The mCD8 signals were significantly reduced in *raw* RNAi ddaC neurons. (D) Confocal live images of Ctrl RNAi and *raw* RNAi ddaC neurons that were labelled for Raw-GFP at wL3 stage. (E) Pan-neuronal driver *elav-Gal4* was used to express Raw-Venus in adult brain neurons. Arf1 was not co-immunoprecipitated by Raw-Venus. Tubulin was used as a loading and probing control. In (C, D), data are presented as mean \pm SEM. Two-tailed Student's T-test were applied to determine statistical significance. ** $p < 0.01$, *** $p < 0.001$. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-D) represent 10 μm .

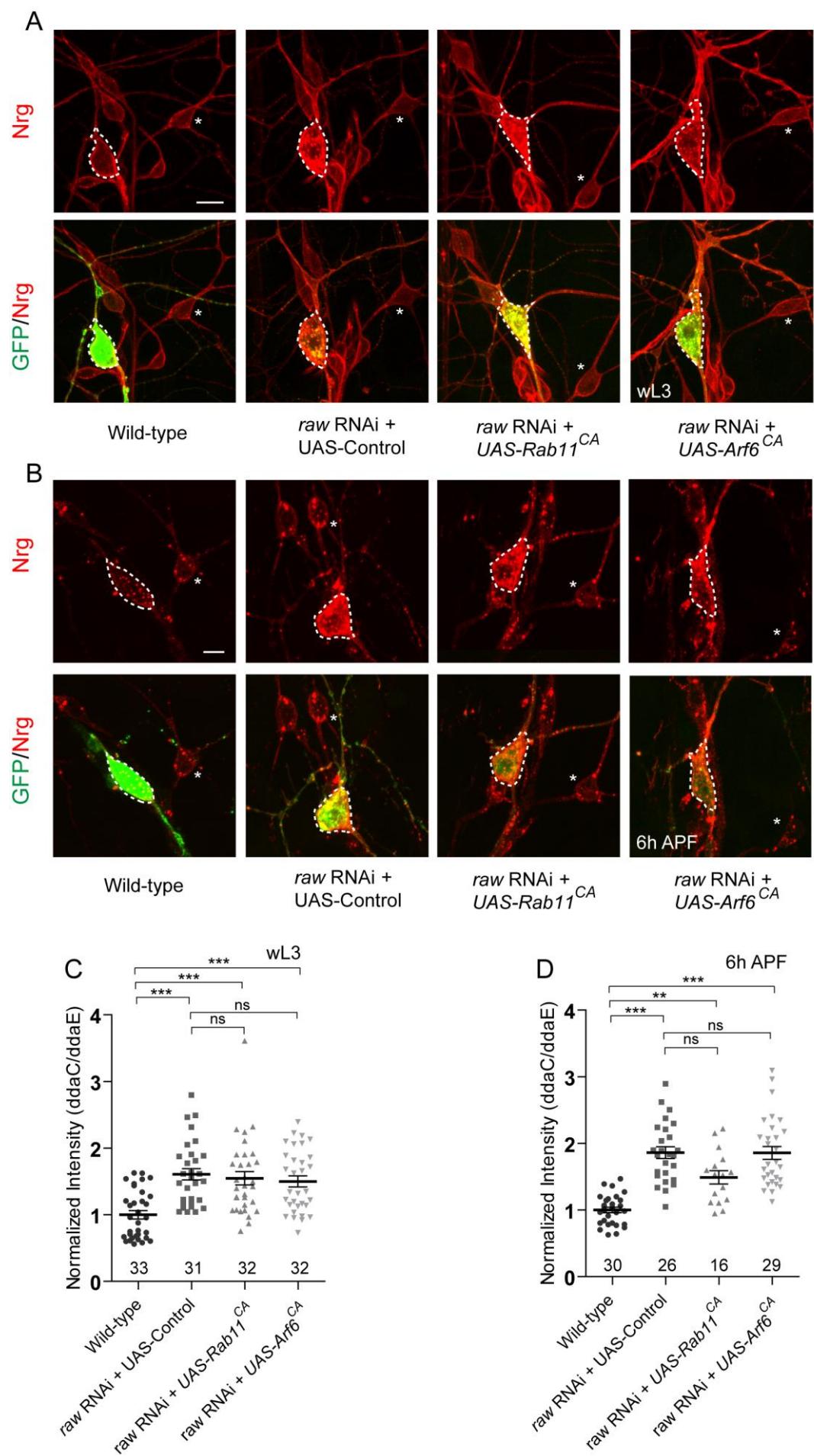


Figure S11. The Nrg degradation defect in *raw* RNAi neurons is not rescued by Rab11^{CA}

or Arf6^{CA} overexpression. (A-B) Confocal images of wild-type, *raw* RNAi + *UAS-Control*, *raw* RNAi + *UAS-Rab11^{CA}*, and *raw* RNAi + *UAS-Arf6^{CA}* ddaC neurons that were immunostained for Nrg at wL3 and 6 h APF stages. ddaC somas are labeled by dashed lines, ddaE by asterisks. ddaC neurons were identified by *ppk-Gal4*-driven mCD8::GFP (green channel) expression. (C-D) Quantitative analysis of normalized Nrg fluorescence intensities in ddaC somas at wL3 and 6h APF stage. In (C-D), data are presented as mean ± SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant; **p<0.01; ***p<0.001. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-B) represent 10 μm.

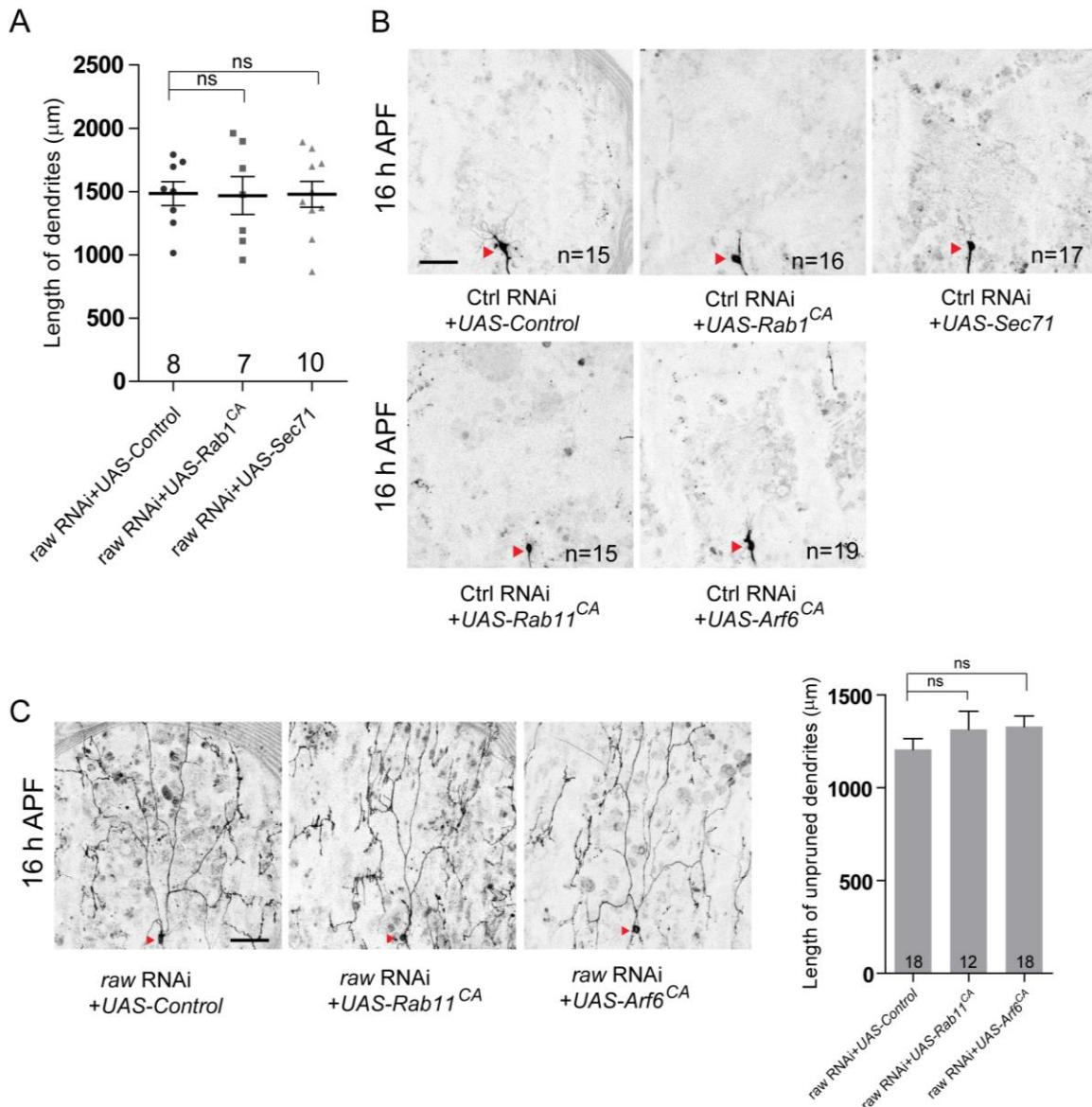


Figure S12. The dendrite pruning defects in *raw* RNAi neurons were not rescued by Rab11^{CA} or Arf6^{CA} overexpression. (A) Quantification of dendrite length at WP stage. (B) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at 16 h APF. Dendrites of Ctrl RNAi + *UAS-Control*, Ctrl RNAi + *UAS-Rab1^{CA}*, Ctrl RNAi + *UAS-Sec71*, Ctrl RNAi + *UAS-Rab11^{CA}*, and Ctrl RNAi + *UAS-Arf6^{CA}* ddaC neurons. Red arrowheads point to the ddaC somas. (C) Live confocal images of ddaC neurons expressing mCD8::GFP driven by *ppk-Gal4* at 16 h APF. Dendrites of *raw* RNAi + *UAS-Control*, *raw* RNAi + *UAS-Rab11^{CA}*, and *raw* RNAi + *UAS-Arf6^{CA}* ddaC neurons. Red arrowheads point to ddaC somas. Quantitative analysis of unpruned dendrite lengths at 16 h APF. In (A, C), data are presented as mean ± SEM. One-way ANOVA with Bonferroni test was applied to determine statistical significance. ns, not significant. The number of neurons (n) examined in each group is shown on the bars. Scale bars in (B, C) represent 50 μm.

Supplementary Materials and methods

Figure 1: (B) *w**; *UAS-control RNAi* / *UAS-control RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (C) *w**; *UAS-raw RNAi* / *UAS-raw RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (D) *w**; *FRT40A* / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (E) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (F) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw-Venus*. (G) *w**; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (H) *w**; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw-Venus*.

Figure 2: (B) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (C) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw*. (D) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw^{AR1}*. (E) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw^{AR2}*. (F) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw^{ATM}*. (G) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-Raw^{AC-ter}*.

Figure 3: (A) *w**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-control RNAi*; *puc-lacZ*/. (B) *w**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-raw RNAi*; *puc-lacZ*/. (C) *w**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-control RNAi*; *puc-lacZ*/. (D) *w**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-raw RNAi*; *puc-lacZ*/. (E) *w**; *UAS-raw RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Mical^{NT}*. (F) *w**; *UAS-raw RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-JNK^{DN}*. (H) +/*UAS-JNK^{DN}*; *UAS-raw RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* /. (I) *w**; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (J) *w**; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / *UAS-JNK^{DN}*. (K) +/*UAS-JNK^{DN}*; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +.

Figure 4: (A, C) *w**; *UAS-control RNAi* / *UAS-control RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (B, D) *w**; *UAS-raw RNAi* / *UAS-raw RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (G) *w**; *FRT40A* / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (H) *w**; *FRT40A*, *raw*^{134.47} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (I) *w**; *FRT40A*, *raw*^{155.27} / *FRT 40A*, *tubP-Gal80*; *ppk-Gal4*, *UAS-mCD8GFP*, *SOP-flp* / +. (K) *w**; *UAS-control RNAi* / *UAS-control RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (L) *w**; *UAS-raw RNAi* / *UAS-raw RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (M) *w**; *ppk-Gal4* / *UAS-Rab5^{DN}*; *ppk-Gal4*, *UAS-mCD8GFP* / +.

Figure 5: (A) ***Ctrl RNAi*:** *w**; *UAS-control RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Sec31-mCherry*. ***raw RNAi*:** *w**; *UAS-raw RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Sec31-mCherry*. (B, C) ***Ctrl RNAi*:** *w**; *UAS-control RNAi* / *UAS-control RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. ***raw RNAi*:** *w**; *UAS-raw RNAi* / *UAS-raw RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (D) ***Ctrl RNAi*:** *w**; *UAS-control RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-GalT-RFP*. ***raw RNAi*:** *w**; *UAS-raw RNAi* / +; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-GalT-RFP*. (E, F) ***Ctrl RNAi*:** *w**; *UAS-control RNAi* / *UAS-control RNAi*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-*

Dcr2. raw RNAi: *w**; UAS-raw RNAi / UAS-raw RNAi; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2. (G) *w**; *ppk-Gal4/+ ;UAS-Raw-RFP/UAS-KDEL-GFP*. (H) *w**; *ppk-Gal4/+ ;UAS-Raw-RFP/ UAS-GalT-GFP*.

Figure 6: (A, F) *w**; UAS-control RNAi / UAS-control RNAi; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2. (B, G) *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical^{NT}. (C, H) *w**; UAS-raw RNAi / UAS-Rab1^{CA}; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / +. (D, I) *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-Sec71.

Figure 7: (A) *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical^{NT}. (B) *w**; UAS-raw RNAi / UAS-Rab1^{CA}; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / +. (C) *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-Sec71. (E) *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-control RNAi. (F) *w**; UAS-raw RNAi / UAS-nrg RNAi#1; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / +. (G) *w**; UAS-raw RNAi / UAS-nrg RNAi#2; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / +.

Figure S1: (A) **Ctrl MARCM:** *w**; FRT40A / FRT 40A, tubP-Gal80; *ppk-Gal4*, UAS-mCD8-GFP, SOP-flp / +. **raw^{134.47}+ UAS-Raw-RFP:** *w**; FRT40A, *raw^{134.47}* / FRT 40A, tubP-Gal80; *ppk-Gal4*, UAS-mCD8GFP, SOP-flp / UAS-Raw-RFP. **raw^{134.47}+ UAS-GFP-Raw:** *w**; FRT40A, *raw^{134.47}* / FRT 40A, tubP-Gal80; *ppk-Gal4*, UAS-mCD8GFP, SOP-flp / UAS-GFP-Raw. (B) **Ctrl RNAi:** *w**; UAS-control RNAi / +; *Gal4²⁻²¹*, UAS-mCD8GFP, UAS-Dcr2 / +. **raw RNAi:** *w**; UAS-raw RNAi / +; *Gal4²⁻²¹*, UAS-mCD8GFP, UAS-Dcr2 / +. (D) *w**; GSG2295, *ppk-tdGFP* / *raw RNAi*; UAS-Dcr2 / +.

Figure S2: (B) **UAS-Control:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Mical^{NT}. **UAS-Raw:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Raw. **UAS-Raw^{4R1}:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Raw^{4R1}. **UAS-Raw^{4R2}:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Raw^{4R2}. **UAS-Raw^{4TM}:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Raw^{4TM}. **UAS-Raw^{4C-ter}:** *w**; *ppk-Gal4/+ ;ppk-Gal4*, UAS-mCD8GFP/ UAS-Raw^{4C-ter}.

Figure S3: (A) **UAS-Control:** *w**; *ppk-Gal4*, UAS-mCD8GFP / +; *puc-lacZ* / UAS-Mical^{NT}. **UAS-JNK^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP / +; *puc-lacZ* / UAS-JNK^{DN}. **UAS-Fos^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP / +; *puc-lacZ* / UAS-Fos^{DN}. **UAS-Jun^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP / +; *puc-lacZ* / UAS-Jun^{DN}. (B) **raw RNAi + UAS-Control:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *raw RNAi*; *puc-lacZ* / UAS-Mical^{NT}. **raw RNAi + UAS-JNK^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *raw RNAi*; *puc-lacZ* / UAS-JNK^{DN}. **raw RNAi + UAS-Fos^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *raw RNAi*; *puc-lacZ* / UAS-Fos^{DN}. **raw RNAi + UAS-Jun^{DN}:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / *raw RNAi*; *puc-lacZ* / UAS-Jun^{DN}.

Figure S4: (A, B) **Ctrl RNAi + DMSO:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-control RNAi; *puc-lacZ/+*. **raw RNAi + DMSO:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-raw RNAi; *puc-lacZ/+*. **Ctrl RNAi + GNE-3511:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-control RNAi; *puc-lacZ/+*. **raw RNAi + GNE-3511:** *w**; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-raw RNAi; *puc-lacZ/+*.

Figure S5: (A) **UAS-Control + Ctrl RNAi:** *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical^{NT}. **UAS-JUN^{DN} + Ctrl RNAi:** *w**; UAS-control RNAi / +;

ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-JUN^{DN}. UAS-FOS^{DN} + Ctrl RNAi: w; UAS-control RNAi /+; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-FOS^{DN}. raw RNAi + UAS-Control: w*; UAS-raw RNAi /+; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical^{NT}. raw RNAi + UAS-JUN^{DN}: w*; UAS-raw RNAi /+; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-JUN^{DN}. raw RNAi + UAS-FOS^{DN}: w*; UAS-raw RNAi /+; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-FOS^{DN}. (B) raw RNAi + UAS-Control (2xppk): w*; UAS-raw RNAi / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical^{NT}. raw RNAi + UAS-JNK^{DN} (2xppk): w*; UAS-raw RNAi / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-JNK^{DN}. raw RNAi + UAS-Fos^{DN} (2xppk): w*; UAS-raw RNAi / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Fos^{DN}. raw RNAi + UAS-Jun^{DN} (2xppk): w*; UAS-raw RNAi / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Jun^{DN}. (C) trc¹: w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp; FRT80B, trc¹ / FRT 80B, tubP-Gal80. fry¹: w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp; FRT80B, fry¹ / FRT 80B, tubP-Gal80.*

Figure S6: (A) **UAS-Control:** w*; ppk-Gal4 /+; UAS-Mical^{NT} / ppk-Gal4, UAS-mCD8GFP. **UAS-JNK:** w*; ppk-Gal4 / UAS-JNK; +/ppk-Gal4, UAS-mCD8GFP. **UAS-Fos:** w*; ppk-Gal4 / UAS-Fos; +/ppk-Gal4, UAS-mCD8GFP. **UAS-Jun:** w*; ppk-Gal4 /+; UAS-Jun / ppk-Gal4, UAS-mCD8GFP. (B) **UAS-Control:** w*; ppk-Gal4, UAS-mCD8GFP /+; puc-lacZ / UAS-Mical^{NT}. **UAS-JNK:** w*; ppk-Gal4, UAS-mCD8GFP / UAS-JNK; puc-lacZ/+ . **UAS-Fos:** w*; ppk-Gal4, UAS-mCD8GFP / UAS-Fos; puc-lacZ/+ . **UAS-Jun:** w*; ppk-Gal4, UAS-mCD8GFP /+; puc-lacZ / UAS-Jun. (C-E) **UAS-Control:** w*; ppk-Gal4 /+; UAS-Mical^{NT} / ppk-Gal4, UAS-mCD8GFP. **UAS-JNK:** w*; ppk-Gal4 / UAS-JNK; +/ppk-Gal4, UAS-mCD8GFP. **UAS-Fos:** w*; ppk-Gal4 / UAS-Fos; +/ppk-Gal4, UAS-mCD8GFP.

Figure S7: (A) **Wild-type:** w*; UAS-control RNAi / UAS-control RNAi; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. **Rab5^{DN}:** w*; ppk-Gal4 / UAS-Rab5^{DN}; ppk-Gal4, UAS-mCD8GFP /+. **Vps4^{DN}:** w*; ppk-Gal4 / UAS-Vps4^{DN}; ppk-Gal4, UAS-mCD8GFP /+. (B) **Wild-type:** w*; ppk-Gal4 /+; ppk-Gal4, UAS-mCD8GFP / UAS-Raw-RFP. **Rab5^{DN}:** w*; ppk-Gal4 / UAS-Rab5^{DN}; / ppk-Gal4, UAS-mCD8GFP / UAS-Raw-RFP. **Vps4^{DN}:** w*; ppk-Gal4 / UAS-Vps4^{DN}; ppk-Gal4, UAS-mCD8GFP / UAS-Raw-RFP. (C) w*; ppk-Gal4, FYVE-GFP /+;+/ UAS-Mical^{NT}. (D) w*; ppk-Gal4, FYVE-GFP /+;+/ UAS-Raw-RFP. (E) **Ctrl RNAi:** w*; UAS-control RNAi / UAS-control RNAi; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. (F) **Ctrl RNAi:** w*; Gal4⁴⁻⁷⁷, UAS-EB1-GFP /+; UAS-Dcr2 / UAS-control RNAi. **raw RNAi:** w*; Gal4⁴⁻⁷⁷, UAS-EB1-GFP / UAS-raw RNAi; UAS-Dcr2 /+.

Figure S8: (A) **Ctrl RNAi:** w*; ppk-Gal4, UAS-mCD8GFP /+; UAS-Dcr2 / UAS-control RNAi. **arf1 RNAi:** w*; ppk-Gal4, UAS-mCD8GFP /+; UAS-Dcr2 / UAS-arf1. (B) **raw RNAi + Ctrl RNAi:** w*; UAS-raw RNAi /+; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-control RNAi. **raw RNAi + nrg RNAi #1:** w*; UAS-raw RNAi / UAS-nrg RNAi#1; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+. **raw RNAi + nrg RNAi #2:** w*; UAS-raw RNAi / UAS-nrg RNAi#2; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+. (C) **UAS-Control:** w*; ppk-Gal4 /+; ppk-Gal4, UAS-mCD8GFP / UAS-Mical^{NT}. **UAS-Raw:** w*; ppk-Gal4 /+; ppk-Gal4, UAS-mCD8GFP / UAS-Raw. (E) **elav-Gal4 /+;** UAS-Raw-Venus /+. (F) **Ctrl RNAi:** w*; UAS-control RNAi / UAS-control RNAi; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. **Rab5^{DN}:** w*; ppk-Gal4 / UAS-Rab5^{DN}; ppk-Gal4, UAS-mCD8GFP /+. **raw RNAi:** w*; UAS-raw RNAi / UAS-raw RNAi; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2.

Figure S9: (A) ***Ctrl RNAi***: *w**; UAS-control RNAi / UAS-control RNAi; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*. ***raw RNAi***: *w**; UAS-raw RNAi / UAS-raw RNAi; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*. (B) ***Ctrl RNAi***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *A58-Gal4*. ***raw RNAi***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *A58-Gal4*. (C) **wild-type**: *w*¹¹¹⁸. ***raw-GFP enhancer trap***: *raw*^{M107292}.

Figure S10: (A) ***Ctrl RNAi***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*GalT-RFP*. ***raw RNAi***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*GalT-RFP*. (B) ***UAS-Control***: *w**; *ppk-Gal4*/+; *ppk-Gal4*, UAS-*mCD8GFP*/UAS-*Mical*^{NT}. ***UAS-Raw***: *w**; *ppk-Gal4*/+; *ppk-Gal4*, UAS-*mCD8GFP*/UAS-*Raw*. (C) ***Ctrl RNAi***: *w**; GSG2295/+; UAS-Dicer2/ UAS-*mCD8GFP*. ***raw RNAi***: *w**; GSG2295/raw RNAi; UAS-Dicer2/ UAS-*mCD8GFP*. (D) ***Ctrl RNAi***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Raw-RFP*. ***raw RNAi***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Raw-RFP*. (E) ***elav-Gal4*** / + ; UAS-*Raw-Venus* / +.

Figure S11: (A, B) ***Wild-type***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*GalT-RFP*. ***raw RNAi + UAS-Control***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*^{NT}. ***raw RNAi + UAS-Rab11*^{CA}**: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Rab11*^{CA}. ***raw RNAi + UAS-Arf6*^{CA}**: *w**; UAS-raw RNAi / UAS-*Arf6*^{CA}; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +.

Figure S12: (A) ***raw RNAi + UAS-Control***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*^{NT}. ***raw RNAi + UAS-Rab1*^{CA}**: *w**; UAS-raw RNAi / UAS-*Rab1*^{CA}; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +. ***raw RNAi + UAS-Sec71***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Sec71*. (B) ***Ctrl RNAi + UAS-Control***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*^{NT}. ***Ctrl RNAi + UAS-Rab1*^{CA}**: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +. ***Ctrl RNAi + UAS-Sec71***: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Sec71*. ***Ctrl RNAi + UAS-Rab11*^{CA}**: *w**; UAS-control RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Rab11*^{CA}. ***Ctrl RNAi + UAS-Arf6*^{CA}**: *w**; UAS-control RNAi / UAS-*Arf6*^{CA}; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +. (C) ***raw RNAi + UAS-Control***: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*^{NT}. ***raw RNAi + UAS-Rab11*^{CA}**: *w**; UAS-raw RNAi / +; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Rab11*^{CA}. ***raw RNAi + UAS-Arf6*^{CA}**: *w**; UAS-raw RNAi / UAS-*Arf6*^{CA}; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +.