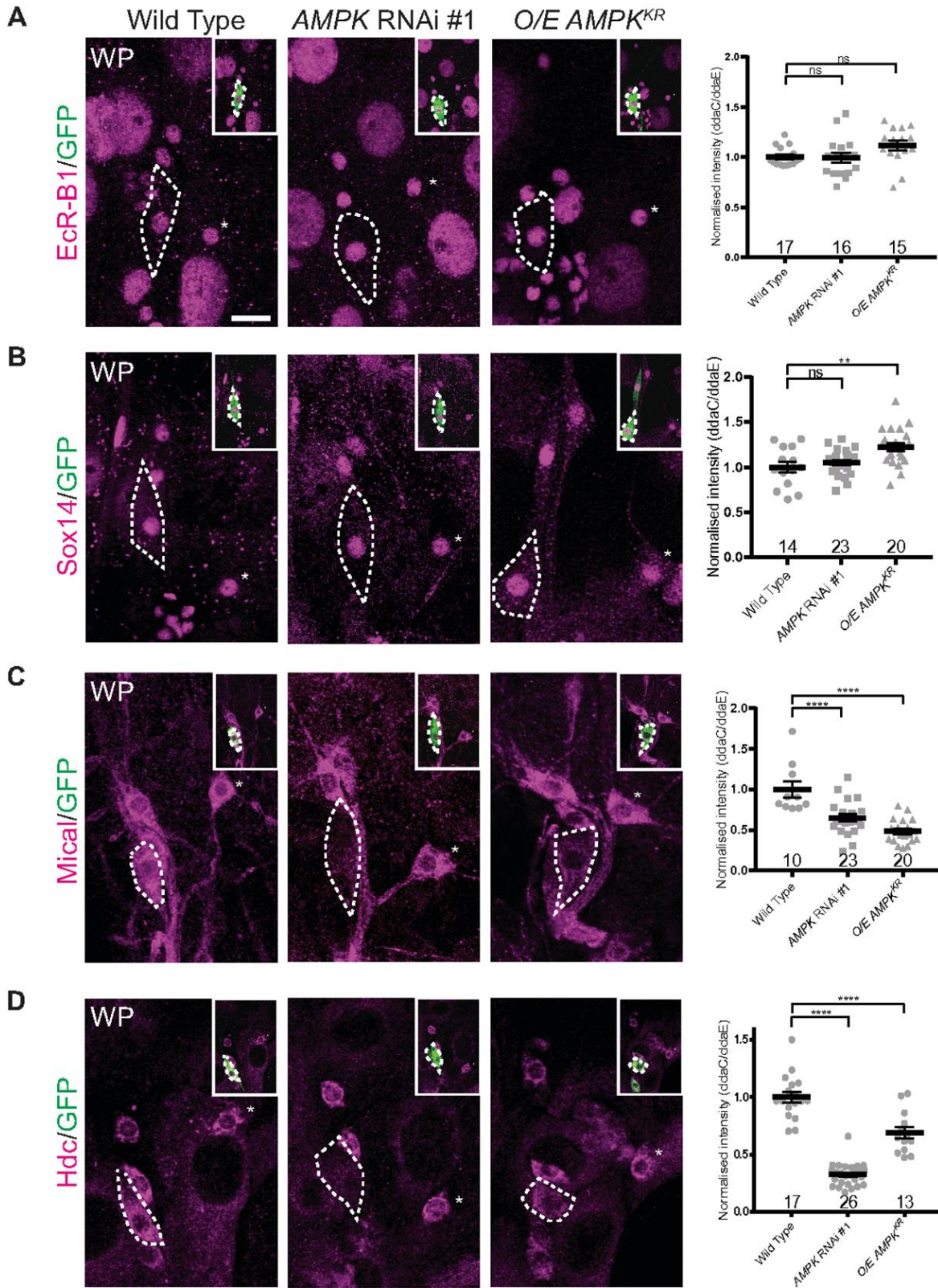


**Fig. S1. Overexpression of AMPK or AMPK<sup>CA</sup> does not affect dendrite pruning at 6 h and 16 h APF.**

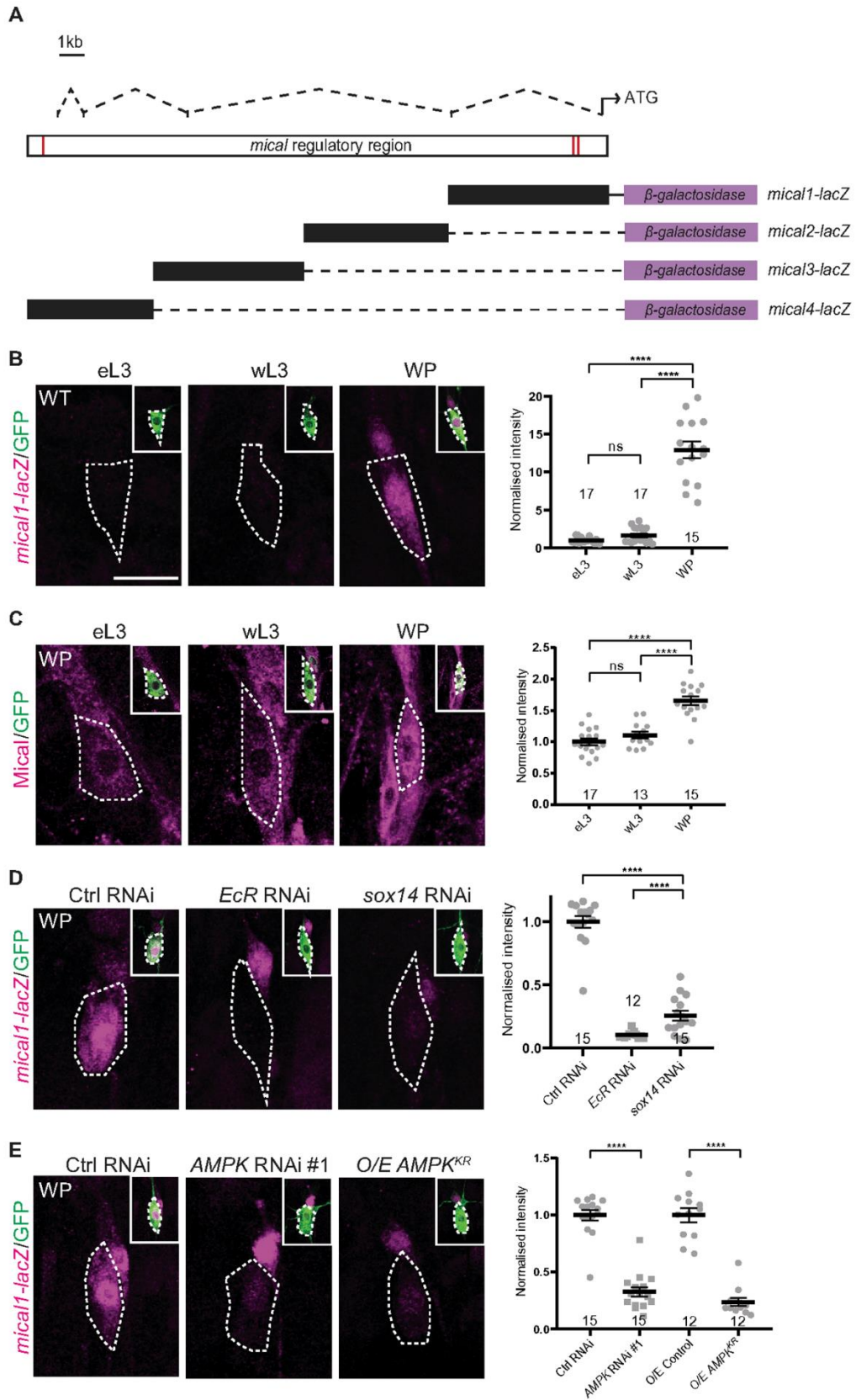
A) Percentages of ddaC neurons showing severing or fragmentation defects at 16 h APF. (B) Dendrites of control, AMPK- or AMPK<sup>CA</sup>-overexpressing ddaC neurons at WP, 6 h APF and 16 h APF stages. Red arrowheads point to the somata of ddaC neurons. The number of neurons (n) examined in each group is shown on the bars. The scale bar in (B) represents 50  $\mu$ m.



**Fig. S2. AMPK is required for the expression of Mical/Hdc.**

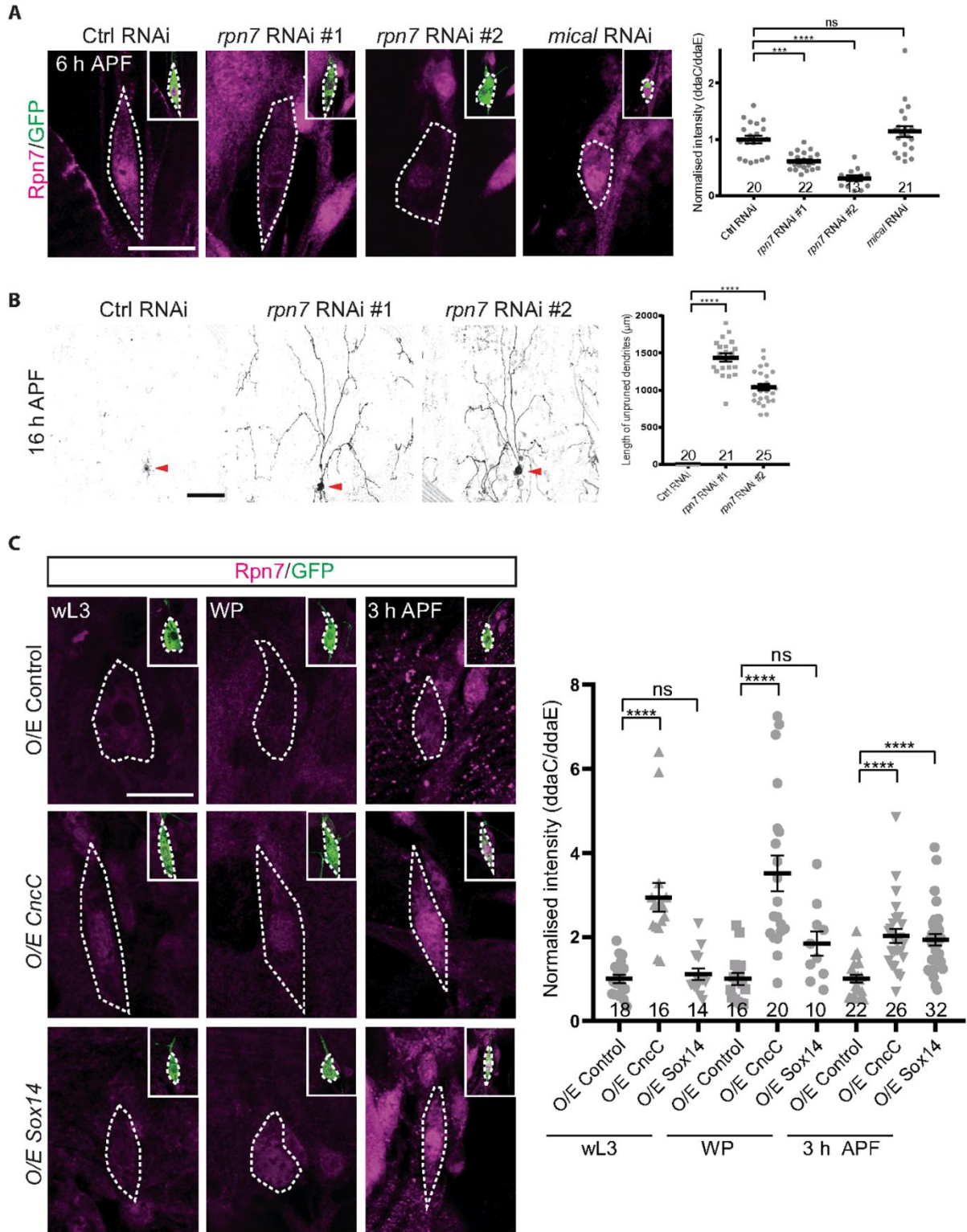
(A-D) Expression of EcR-B1 (A), Sox14 (B), Mical (C) and Hdc (D) in wild-type, *AMPK* RNAi #1 and *AMPK<sup>KR</sup>* ddaC neurons at WP stage. ddaC somata are marked by dashed lines. Quantitative analyses of normalized EcR-B1, Sox14, Mical and Hdc fluorescence are shown in the rightmost panels. Error bars represent  $\pm$  SEM. One-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant, \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ . The number of neurons (n) examined in each group is shown on the bars. The scale bar in (A) represents 10  $\mu\text{m}$ .





**Fig. S3. AMPK induces Mical expression.**

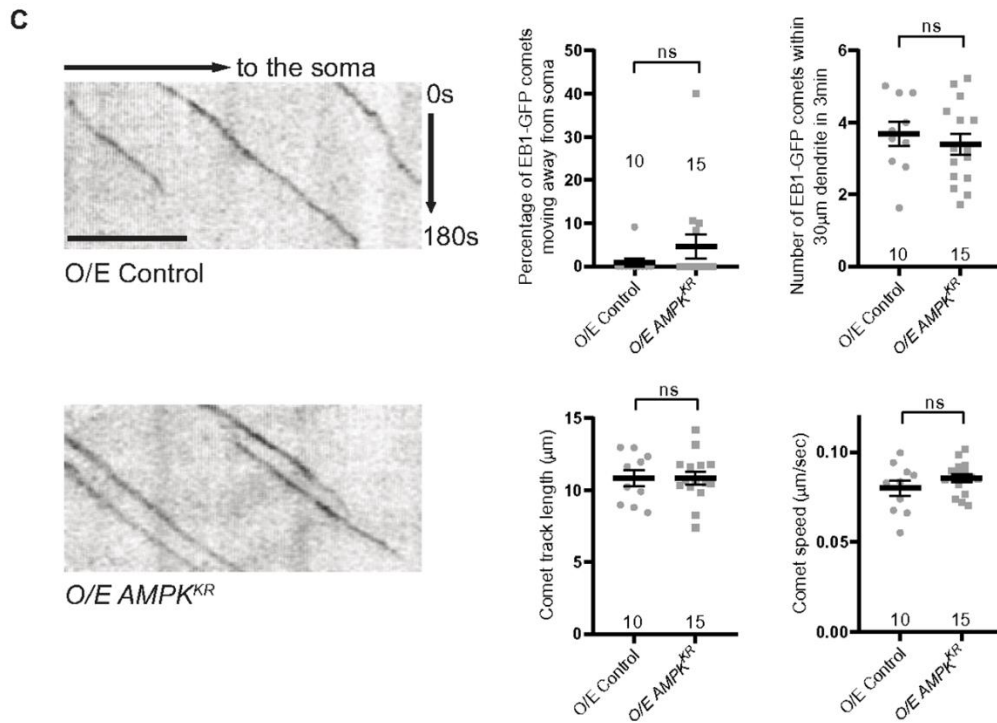
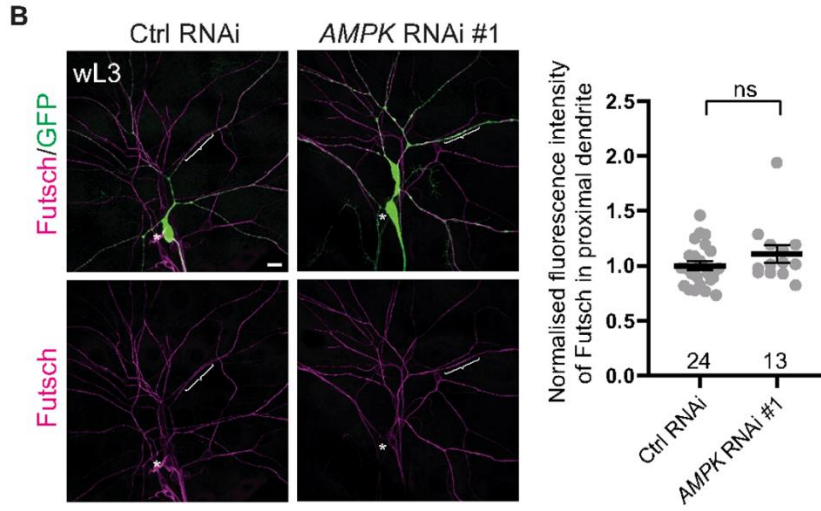
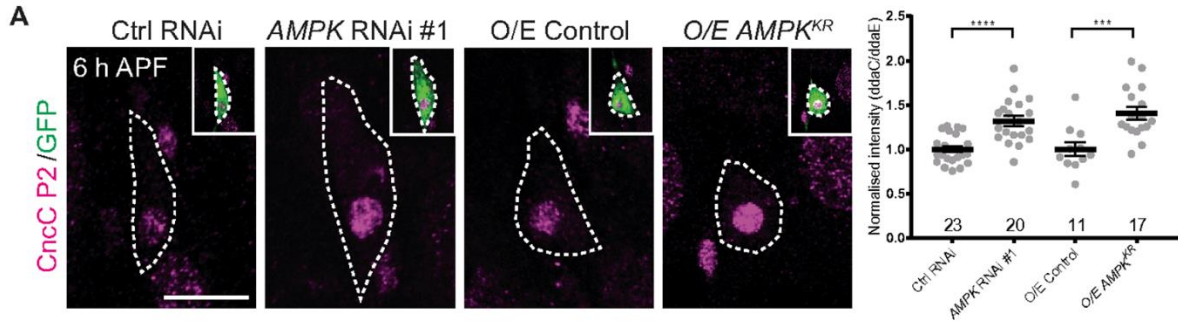
(A) The regulatory region of *mical* gene. Four *lacZ* reporter lines under the control of *mical* enhancers were generated. Red lines indicate three putative ecdysone-response elements. (B-C) Expression of *mical1-lacZ* (B) and Mical (C) in wild-type *ddaC* neurons at eL3, wL3 and WP stages. (D-E) Expression of *mical1-lacZ* in control RNAi, *EcR* RNAi and *sox14* RNAi *ddaC* neurons (D), control RNAi, *AMPK* RNAi #1 and *AMPK*<sup>KR</sup>-overexpressing neurons (E) at WP stage. *ddaC* somata are marked by dashed lines. Quantitative analyses of normalized *mical1-lacZ* and Mical fluorescence are shown in the rightmost panels. Error bars represent  $\pm$  SEM. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison, whereas one-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant, \*\*\*\*p<0.0001. The number of neurons (n) examined in each group is shown on the bars. The scale bar in (B) represents 10  $\mu$ m.



**Fig. S4. Overexpression of CncC or Sox14 leads to elevated Rpn7 expression.**

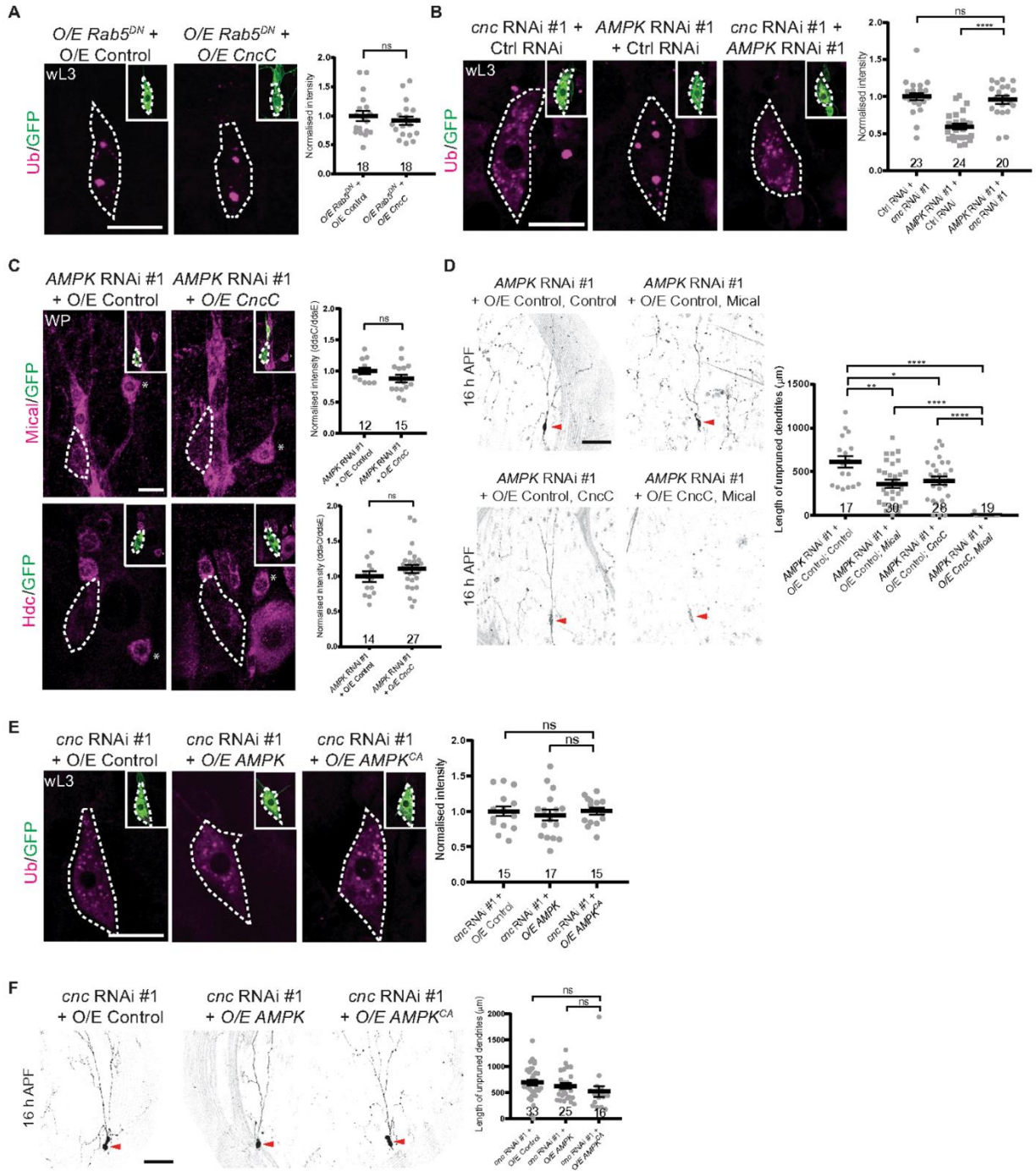
(A, C) Expression of Rpn7 in control RNAi, *rpn7* RNAi #1/#2, and *mical* RNAi (A), control, CncC-overexpressing and Sox14-overexpressing ddaC neurons at 6 h APF (A) or wL3, WP and 3 h APF stages (C). ddaC somata are marked by dashed lines. Quantitative analyses of normalized Rpn7 fluorescence are shown in the rightest panels. (B) Dendrites of control RNAi, *rpn7* RNAi #1, or #2 at 16 h APF stages. Quantitative analysis of unpruned dendrite length at 16 h APF. Red arrowheads point to the somata of ddaC neurons. Error bars represent  $\pm$  SEM. One-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant, \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A, C) and (B) represent 10  $\mu$ m and 50  $\mu$ m, respectively.





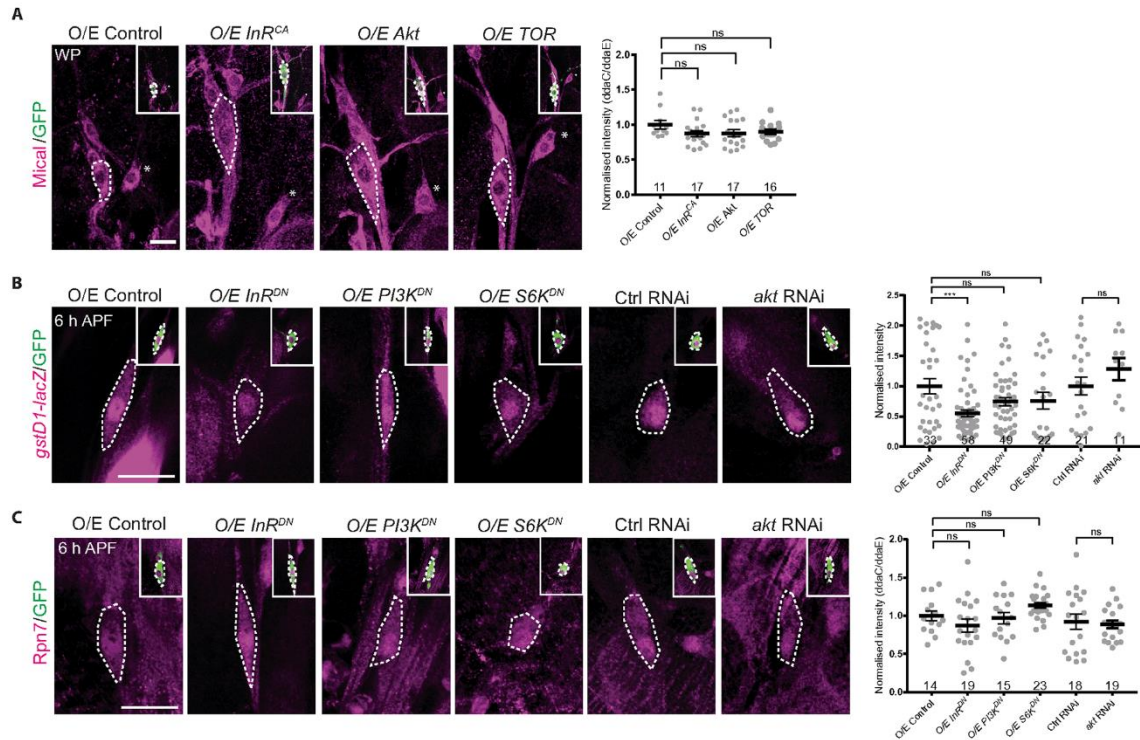
**Fig. S5. AMPK does not affect microtubule mass or polarity in ddaC dendrites.**

(A) Expression of CncC in control RNAi, *AMPK* RNAi #1, control and *AMPK<sup>KR</sup>*-overexpressing ddaC neurons at 6 h APF. ddaC somata are marked by dashed lines. (B) Expression of Futsch in control RNAi and *AMPK* RNAi #1 ddaC neurons at wL3 stage. Quantitative analysis of normalized Futsch fluorescence in proximal dendrites is shown in the rightest panel. The ddaC somata are labelled by asterisks and the dendrites by brackets. (C) Representative kymographs depicting the movement patterns of EB1 comets in the proximal dendrites of control and *AMPK<sup>KR</sup>*-overexpressing ddaC neurons at 96 h AEL. Quantitative analyses of the percentages of anterograde EB1 comets in each neuron, the average numbers of EB1-GFP comets within 30  $\mu$ m dendrite in 3 min, the average comet track length, and the average comet speed are shown in the right panels. Error bars represent  $\pm$  SEM. ns, not significant. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison. ns, not significant, \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A-C) represent 10  $\mu$ m.



**Fig. S6. Overexpression of AMPK<sup>CA</sup> does not rescue the *cncC* RNAi defects in terms of ubiquitinated protein aggregation and dendrite pruning.**

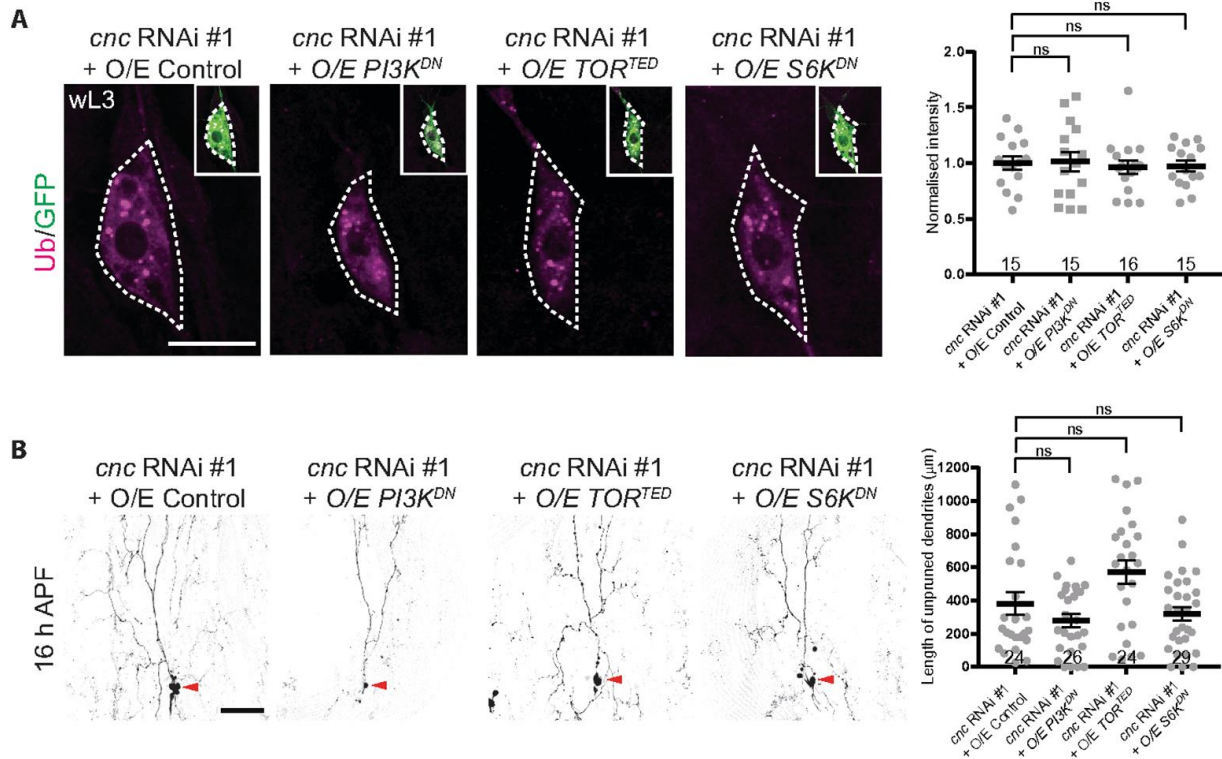
(A-B) Levels of ubiquitinated protein aggregates in *Rab5<sup>DN</sup>* neurons co-overexpressing control or CncC (A), *cncC* RNAi #1 neurons overexpressing control RNAi or *AMPK* RNAi as well as *AMPK* RNAi #1 overexpressing control RNAi at wL3 stage (B). (C) Expression of Mical and Hdc in *AMPK* RNAi #1 neurons overexpressing control or CncC at WP stage. (D) Dendrites of *AMPK* RNAi #1 neurons overexpressing control + control, control + Mical, control + CncC, and CncC + Mical at 16 h APF. (E) Levels of ubiquitinated protein aggregates in *cncC* RNAi #1 neurons overexpressing control, *AMPK* or *AMPK<sup>CA</sup>* at wL3 stage. *ddaC* somata are marked by dashed lines. (F) Dendrites of *cncC* RNAi #1 neurons overexpressing control, *AMPK* or *AMPK<sup>CA</sup>* at 16 h APF. Red arrowheads point to the somata of *ddaC* neurons. Quantitative analyses of normalized Mical, Hdc and ubiquitinated protein aggregates fluorescence are shown in the rightest panels (A-C, E). Quantitative analysis of unpruned dendrite length at 16 h APF are shown at the right panels (D, F). Error bars represent  $\pm$  SEM. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison, whereas one-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ . The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A-C, E) and (D, F) represent 10  $\mu\text{m}$  and 50  $\mu\text{m}$ , respectively.



**Fig. S7. Inhibition of insulin-TOR pathway is not sufficient for the activation of Nrf2-Keap1 pathway in ddaC neurons.**

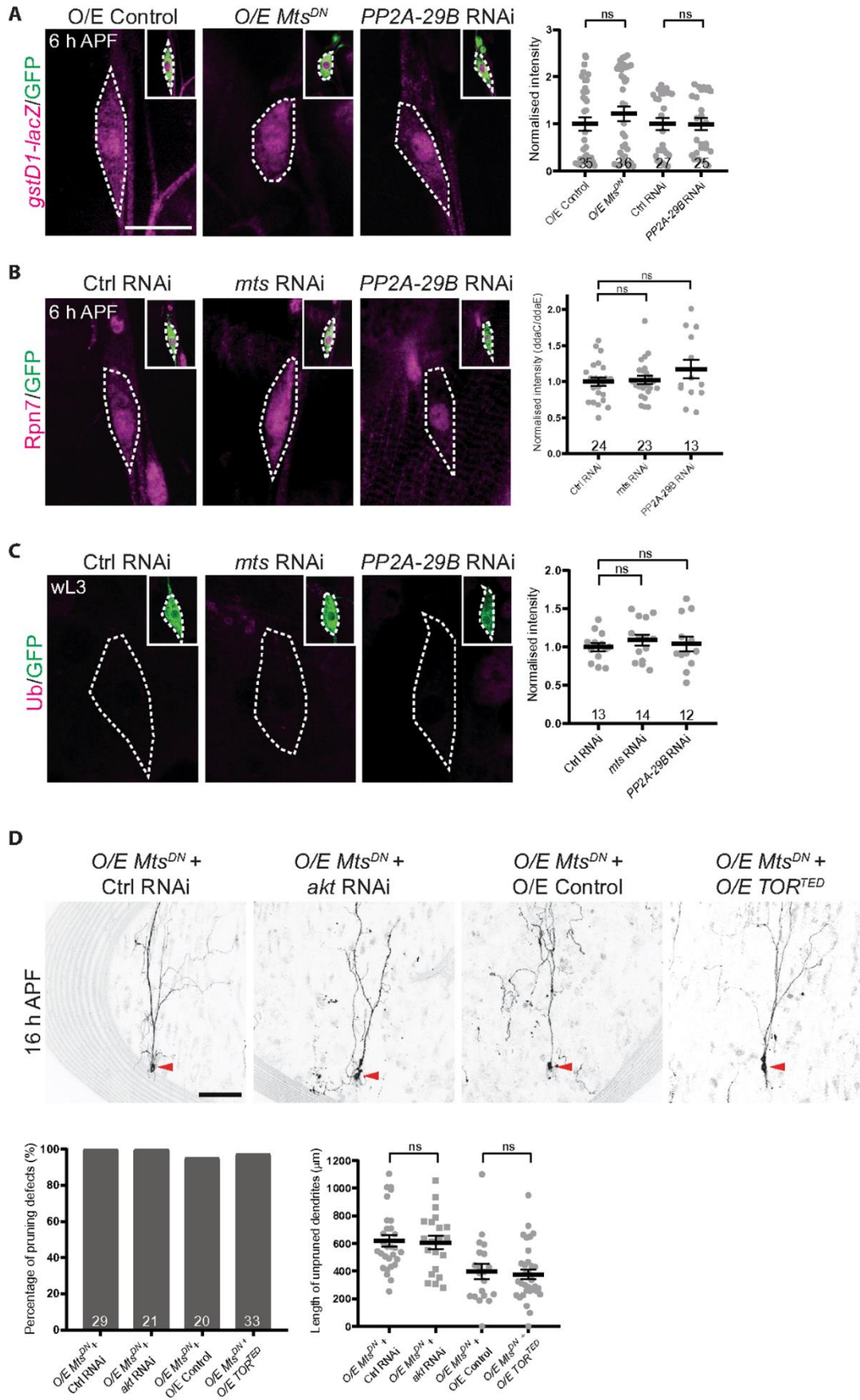
(A) Expression of Mical in *InR<sup>CA</sup>*, Akt or TOR-overexpressing ddaC neurons at WP stage. (B) Expression of *gstD1-lacZ* in control, *InR<sup>DN</sup>*-overexpressing, *PI3K<sup>DN</sup>*-overexpressing, *S6K<sup>DN</sup>*-overexpressing, control RNAi and *akt* RNAi ddaC neurons at 6 h APF. (C) Expression of Rpn7 in control, *InR<sup>DN</sup>*-overexpressing, *PI3K<sup>DN</sup>*-overexpressing, *S6K<sup>DN</sup>*-overexpressing, control RNAi and *akt* RNAi ddaC neurons at 6 h APF. ddaC somata are marked by dashed lines. Quantitative analyses of normalized Mical, *gstD1-lacZ* and Rpn7 fluorescence are shown in the rightest panels (A-C). Error bars represent  $\pm$  SEM. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison, whereas one-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant. The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A-C) represent 10  $\mu$ m.





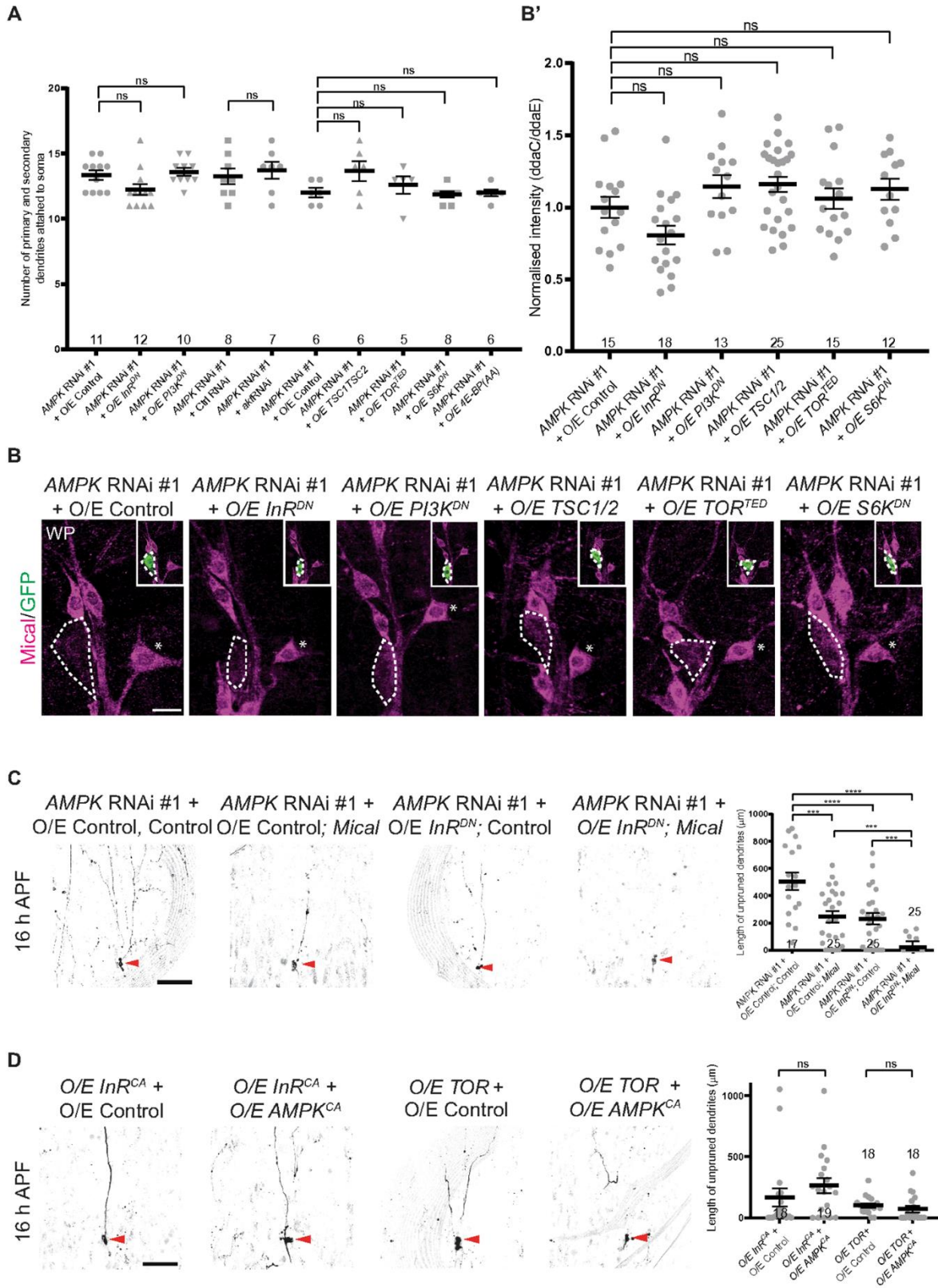
**Fig. S8. The *cncC* RNAi defects are not rescued by attenuation of insulin-TOR pathway.**

(A) Levels of ubiquitinated protein aggregates in *cncC* RNAi #1 *ddaC* neurons overexpressing control, *PI3K*<sup>DN</sup>, *TOR*<sup>TED</sup> or *S6K*<sup>DN</sup>. *ddaC* somata are marked by dashed lines. (B) Dendrites of *cnc* RNAi #1 *ddaC* neurons overexpressing control, *PI3K*<sup>DN</sup>, *TOR*<sup>TED</sup> or *S6K*<sup>DN</sup> at 16 h APF. Red arrowheads point to the somata of *ddaC* neurons. Quantitative analyses of normalized ubiquitinated protein aggregates fluorescence are shown in the rightest panels (A). Quantitative analysis of unpruned dendrite length at 16 h APF is shown in the rightest panel (B). Error bars represent  $\pm$  SEM. One-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant. The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A) and (B) represent 10  $\mu$ m and 50  $\mu$ m, respectively.



**Fig. S9. PP2A is dispensable for activation of Nrf2-Keap1 pathway.**

(A-C) Expression levels of *gstD1-lacZ* (A), Rpn7 (B) and ubiquitinated protein aggregates (C) in control, *Mts<sup>DN</sup>* or *mts* RNAi, *PP2A-29B* RNAi ddaC neurons at 6 h APF (A-B) or wL3 (C) stage. ddaC somata are marked by dashed lines. (D) Dendrites of *Mts<sup>DN</sup>* neurons expressing control RNAi, *akt* RNAi, control or *TOR<sup>TEO</sup>* at 16 h APF. Red arrowheads point to the somata of ddaC neurons. Quantitative analyses of normalized *gstD1-lacZ*, Rpn7 and ubiquitinated protein aggregates fluorescence are shown in the rightest panels (A-C). Quantitative analyses of percentage of pruning defects and unpruned dendrite length at 16 h APF are shown in the bottom panels (D). Error bars represent  $\pm$  SEM. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison, whereas one-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant. The number of neurons (n) examined in each group is shown on the bars. The scale bars in (A) and (D) represent 10  $\mu$ m and 50  $\mu$ m, respectively.



**Fig. S10. Attenuation of insulin-TOR pathway does not restore Mical expression in AMPK RNAi #1 neurons.**

(A) Quantitative analysis of the number of primary and secondary dendrites in ddaC neurons at WP stage. (B) Expression of Mical in AMPK RNAi #1 ddaC neurons expressing control, InR<sup>DN</sup>, PI3K<sup>DN</sup>, TSC1/2, TOR<sup>TED</sup>, and S6K<sup>DN</sup> at WP stage. ddaC somata are marked by dashed lines. (C) Dendrites of AMPK RNAi #1 neurons overexpressing control + control, control + Mical, control + InR<sup>DN</sup>, and Mical + InR<sup>DN</sup> at 16 h APF. (D) Dendrites of control or AMPK<sup>CA</sup> neurons co-expressing InR<sup>CA</sup> or TOR at 16 h APF. Red arrowheads point to the somata of ddaC neurons. Quantitative analyses of normalized Mical fluorescence are shown in the panel (B'). Quantitative analysis of unpruned dendrite length at 16 h APF are shown in the rightest panel (C-D). Error bars represent  $\pm$  SEM. Two-tailed Student's T-test was used to determine statistical significance for pairwise comparison, whereas one-way ANOVA with Bonferroni test was applied to determine significance for multiple-group comparison. ns, not significant, \*\*\*p<0.001, \*\*\*\*p<0.0001. The number of neurons (n) examined in each group is shown on the bars. The scale bars in (B) and (C-D) represent 10  $\mu$ m and 50  $\mu$ m, respectively.



## Supplementary Materials and Methods

### Genotypes of fly strains

**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-AMPK RNAi #1 / UAS-AMPK RNAi #1; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*. (D) *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*; UAS-AMPK RNAi #2 / UAS-AMPK RNAi #2. (E) *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-AMPK<sup>KR</sup> / UAS-AMPK<sup>KR</sup>. (F) *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-AMPK<sup>KR</sup> / UAS-*Mical*<sup>NT</sup>. (G) *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-AMPK; UAS-AMPK<sup>KR</sup> / *ppk-Gal4*, UAS-*mCD8GFP*. (H) **Control (FRT19A):** *FRT19A* / *FRT19A*, *tubP-Gal80*, *hs-FLP*, *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, *SOP-flp* / +. **ampk<sup>D2</sup>:** *FRT19A*, *ampk<sup>D2</sup>* / *FRT19A*, *tubP-Gal80*, *hs-FLP*, *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, *SOP-flp* / +. (I) *FRT19A*, *ampk<sup>D2</sup>* / *FRT19A*, *tubP-Gal80*, *hs-FLP*, *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, *SOP-flp* / UAS-AMPK.

**Figure 2:** (A) *w\**; ; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. (B) **Ctrl RNAi:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*; UAS-Control RNAi / UAS-Control RNAi. **cnc RNAi #1:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2*; UAS-*cnc* RNAi #1 / UAS-*cnc* RNAi #1. **cnc RNAi #2:** *w\**; UAS-*cnc* RNAi #2 / UAS-*cnc* RNAi #2; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. (C) **Ctrl RNAi:** *w\**; UAS-*Dcr2* / +; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-Control RNAi. **EcR RNAi:** *w\**; UAS-*Dcr2* / +; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*EcR* RNAi. **sox14 RNAi:** *w\**; UAS-*Dcr2* / +; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*sox14* RNAi. (D) **O/E Control:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-*Mical*<sup>NT</sup> / UAS-*Mical*<sup>NT</sup>. **O/E CncC:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-*CncC* / UAS-*CncC*. **O/E Sox14:** *w\**; *ppk-Gal4* / +; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*Sox14*

**Figure 3:** (A) **Ctrl RNAi:** *w\**; UAS-Control RNAi / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*. **AMPK RNAi #1:** *w\**; UAS-AMPK RNAi #1 / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*. **AMPK RNAi #2:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *gstD1-lacZ*; UAS-AMPK RNAi #2 / *ppk-Gal4*, UAS-*mCD8GFP*. (B-C) **Ctrl RNAi:** *w\**; UAS-Control RNAi / UAS-Control RNAi; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / *ppk-Gal4*, UAS-*mCD8GFP*,

UAS-Dcr2. **AMPK RNAi #1:** *w\**; UAS-AMPK RNAi #1 / UAS-AMPK RNAi #1; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2. **AMPK RNAi #2:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2; UAS-AMPK RNAi #2 / UAS-AMPK RNAi #2. **(D-E) O/E Control:** *w\**; UAS-Dcr2 / UAS-Dcr2; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E AMPK:** *w\**; UAS-AMPK / UAS-AMPK; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E AMPK<sup>CA</sup>:** *w\**; UAS-AMPK<sup>CA</sup> / UAS-AMPK<sup>CA</sup>; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*.

**Figure 4: (A) AMPK RNAi #1 + O/E Control:** *w\**; UAS-AMPK RNAi #1 / *ppk-Gal4*, *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #1 + O/E CncC:** *w\**; UAS-AMPK RNAi #1 / *ppk-Gal4*, *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / UAS-*CncC*. **AMPK RNAi #2 + O/E Control:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, *gstD1-lacZ*; UAS-AMPK RNAi #2 / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #2 + O/E CncC:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, *gstD1-lacZ*; UAS-AMPK RNAi #2 / UAS-*CncC*. **(B-D) AMPK RNAi #1 + O/E Control:** *w\**; UAS-AMPK RNAi #1 / *ppk-Gal4*, UAS-*mCD8GFP*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #1 + O/E CncC:** *w\**; UAS-AMPK RNAi #1 / *ppk-Gal4*, UAS-*mCD8GFP*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / UAS-*CncC*. **AMPK RNAi #2 + O/E Control:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-AMPK RNAi #2 / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #2 + O/E CncC:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-Dcr2 / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-AMPK RNAi #2 / UAS-*CncC*.

**Figure 5: (A) O/E Control:** *w\**; UAS-Dcr2 / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E InR<sup>CA</sup>:** *w\**; UAS-*InR<sup>CA</sup>* / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E Akt:** *w\**; UAS-*Akt* / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E S6K<sup>CA</sup>:** *w\**; UAS-*S6K<sup>CA</sup>* / *gstD1-lacZ*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **(B-C) O/E Control:** *w\**; UAS-Dcr2 / UAS-Dcr2; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E InR<sup>CA</sup>:** *w\**; UAS-*InR<sup>CA</sup>* / UAS-*InR<sup>CA</sup>*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E Akt:** *w\**; UAS-*Akt* / UAS-*Akt*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E Tor:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*; UAS-*Tor* / UAS-*Tor*. **(D) O/E InR<sup>CA</sup> + O/E Control:**

*w\**; *UAS-InR<sup>CA</sup> / ppk-Gal4, gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / UAS-Mical<sup>NT</sup>. O/E InR<sup>CA</sup> + O/E CncC*: *w\**; *UAS-InR<sup>CA</sup> / ppk-Gal4, gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / UAS-CncC. O/E S6K<sup>CA</sup> + O/E Control*: *w\**; *UAS-S6K<sup>CA</sup> / ppk-Gal4, gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / UAS-Mical<sup>NT</sup>. O/E S6K<sup>CA</sup> + O/E CncC*: *w\**; *UAS-S6K<sup>CA</sup> / ppk-Gal4, gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / UAS-CncC. (E) O/E InR<sup>CA</sup> + O/E Control*: *w\**; *UAS-InR<sup>CA</sup> / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP / UAS-Mical<sup>NT</sup>. O/E InR<sup>CA</sup> + O/E CncC*: *w\**; *UAS-InR<sup>CA</sup> / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP / UAS-CncC. O/E S6K<sup>CA</sup> + O/E Control*: *w\**; *UAS-S6K<sup>CA</sup> / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP / UAS-Mical<sup>NT</sup>. O/E S6K<sup>CA</sup> + O/E CncC*: *w\**; *UAS-S6K<sup>CA</sup> / ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP / UAS-CncC.*

**Figure 6:** (A) *w\**; *UAS-AMPK RNAi #1 / UAS-Dcr2; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP. (B) w\**; *UAS-AMPK RNAi #1 / UAS-InR<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP. (C) w\**; *UAS-AMPK RNAi #1 / UAS-PI3K<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP. (D) w\**; *UAS-AMPK RNAi #1 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Control RNAi. (E) w\**; *UAS-AMPK RNAi #1 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-akt RNAi. (F) w\**; *UAS-AMPK RNAi #1 / +; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical<sup>NT</sup>. (G) w\**; *UAS-AMPK RNAi #1 / UAS-TSC1,TSC2; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +. (H) w\**; *UAS-AMPK RNAi #1 / UAS-Tor<sup>TED</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +. (I) w\**; *UAS-AMPK RNAi #1 / UAS-S6K<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +. (J) w\**; *UAS-AMPK RNAi #1 / +; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-4E-BP(AA).*

**Figure 7:** (A) *O/E Sox14 + Ctrl RNAi*: *w\**; *ppk-Gal4, gstD1-lacZ / UAS-Control RNAi ; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. O/E Sox14 + AMPK RNAi #1*: *w\**; *ppk-Gal4, gstD1-lacZ / UAS-AMPK RNAi #1; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. O/E Sox14 + O/E Control*: *w\**; *ppk-Gal4, gstD1-lacZ / UAS-Dcr2; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP. O/E Sox14 + O/E InR<sup>CA</sup>*: *w\**; *ppk-Gal4, gstD1-lacZ / UAS-InR<sup>CA</sup>; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP. O/E Sox14 + O/E Akt*: *w\**; *ppk-Gal4, gstD1-lacZ / UAS-Akt; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP. (B-C) O/E Sox14 + Ctrl*

**RNAi:** *w\**; *ppk-Gal4, UAS-mCD8GFP / UAS-Control RNAi; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. O/E Sox14 + AMPK RNAi #1:* *w\**; *ppk-Gal4, UAS-mCD8GFP / UAS-AMPK RNAi #1, UAS-Dcr2; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. O/E Sox14 + O/E Control:* *w\**; *ppk-Gal4, UAS-mCD8GFP / UAS-Dcr2; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP. O/E Sox14 + O/E InR<sup>CA</sup>:* *w\**; *ppk-Gal4, UAS-mCD8GFP / UAS-InR<sup>CA</sup>; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP. O/E Sox14 + O/E Akt:* *w\**; *ppk-Gal4, UAS-mCD8GFP / UAS-Akt; UAS-Sox14 / ppk-Gal4, UAS-mCD8GFP.*

**Figure S1: (B) O/E Control:** *w\**; *ppk-Gal4 / +; UAS-Mical<sup>NT</sup> / ppk-Gal4, UAS-mCD8GFP. O/E AMPK:* *w\**; *UAS-AMPK / ppk-Gal4; + / ppk-Gal4, UAS-mCD8GFP. O/E AMPK<sup>CA</sup>:* *w\**; *UAS-AMPK<sup>CA</sup> / ppk-Gal4; + / ppk-Gal4, UAS-mCD8GFP.*

**Figure S2: (A-D) WT:** *w\**; *ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. AMPK RNAi #1:* *w\**; *UAS-AMPK RNAi #1 / UAS-AMPK RNAi #1; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2. O/E AMPK<sup>KR</sup>:* *w\**; *ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP; UAS-AMPK<sup>KR</sup> / UAS-AMPK<sup>KR</sup>.*

**Figure S3: (B)** *w\**; *ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP; mical1-lacZ / mical1-lacZ. (C)* *w\**; *ppk-Gal4 / ppk-Gal4; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. (D) Ctrl RNAi:* *w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Control RNAi / mical1-lacZ. EcR RNAi:* *w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-EcR RNAi / mical1-lacZ. sox14 RNAi:* *w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-sox14 RNAi / mical1-lacZ. (E) Ctrl RNAi:* *w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Control RNAi / mical1-lacZ. AMPK RNAi #1:* *w\**; *UAS-AMPK RNAi #1 / +; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / mical1-lacZ. O/E AMPK<sup>KR</sup>:* *w\**; *ppk-Gal4, UAS-mCD8GFP / +; UAS-AMPK<sup>KR</sup> / mical1-lacZ.*

**Figure S4: (A) Ctrl RNAi:** *w\**; *UAS-Dcr2 / +; ppk-Gal4, UAS-mCD8GFP / UAS-Control RNAi. rpn7 RNAi #1:* *w\**; *UAS-Dcr2 / +; ppk-Gal4, UAS-mCD8GFP / UAS-rpn7 RNAi #1. rpn7 RNAi #2:* *w\**; *UAS-Dcr2 / UAS-rpn7 RNAi #2; ppk-Gal4, UAS-mCD8GFP / +. mical RNAi:* *w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; UAS-mical RNAi / UAS-mical RNAi. (B) Ctrl RNAi:* *w\**; *UAS-Dcr2 / +; ppk-Gal4, UAS-mCD8GFP / UAS-Control RNAi. rpn7 RNAi #1:* *w\**; *UAS-Dcr2 / +;*

*ppk-Gal4*, *UAS-mCD8GFP* / *UAS-rpn7* RNAi #1. **rpn7 RNAi #2:** *w\**; *UAS-Dcr2* / *UAS-rpn7* RNAi #2; *ppk-Gal4*, *UAS-mCD8GFP* / +. (C) **O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*; *UAS-Mical<sup>NT</sup>* / *UAS-Mical<sup>NT</sup>*. **O/E CncC:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*; *UAS-CncC* / *UAS-CncC*. **O/E Sox14:** *w\**; *ppk-Gal4* / +; *ppk-Gal4*, *UAS-mCD8GFP* / *UAS-Sox14*.

**Figure S5: (A) Ctrl RNAi:** *w\**; *UAS-Control* RNAi / *UAS-Control* RNAi; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. **AMPK RNAi #1:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-AMPK* RNAi #1; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. **O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*; *UAS-Mical<sup>NT</sup>* / *UAS-Mical<sup>NT</sup>*. **O/E AMPK<sup>KR</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*; *UAS-AMPK<sup>KR</sup>* / *UAS-AMPK<sup>KR</sup>*. (B) **Ctrl RNAi:** *w\**; *UAS-Control* RNAi / *UAS-Control* RNAi; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. **AMPK RNAi #1:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-AMPK* RNAi #1; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (C) **O/E Control:** *w\**; *Gal4<sup>4-77</sup>*, *UAS-EB1-GFP* / +; + / *UAS-Mical<sup>NT</sup>*. **O/E AMPK<sup>KR</sup>:** *w\**; *Gal4<sup>4-77</sup>*, *UAS-EB1-GFP* / +; + / *UAS-AMPK<sup>KR</sup>*.

**Figure S6: (A) O/E Rab5<sup>DN</sup> + O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-Rab5<sup>DN</sup>*; *ppk-Gal4*, *UAS-mCD8GFP* / *UAS-Mical<sup>NT</sup>*. **O/E Rab5<sup>DN</sup> + O/E CncC:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-Rab5<sup>DN</sup>*; *ppk-Gal4*, *UAS-mCD8GFP* / *UAS-CncC*. (B) **cnc RNAi #1 + Ctrl RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Control* RNAi; *UAS-cnc* RNAi #1 / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. **AMPK RNAi #1 + Ctrl RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-AMPK* RNAi #1; *UAS-Control* RNAi / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. **AMPK RNAi #1 + cnc RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-AMPK* RNAi #1; *UAS-cnc* RNAi #1 / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*. (C) **AMPK RNAi #1 + O/E Control:** *w\**; *UAS-AMPK* RNAi #1 / *ppk-Gal4*, *UAS-mCD8GFP*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Mical<sup>NT</sup>*. **AMPK RNAi #1 + O/E CncC:** *w\**; *UAS-AMPK* RNAi #1 / *ppk-Gal4*, *UAS-mCD8GFP*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-CncC*. (D) **AMPK RNAi #1 + O/E Control; Control:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-Mical<sup>NT</sup>*; *ppk-*



*Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical<sup>NT</sup>. AMPK RNAi #1 + O/E Control; Mical: w\**; *UAS-AMPK RNAi #1 / UAS-Mical<sup>NT</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Mical. AMPK RNAi #1 + O/E Control; CncC : w\**; *UAS-AMPK RNAi #1 / UAS-Mical<sup>NT</sup>; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-CncC. AMPK RNAi #1 + O/E CncC, Mical: w\**; *UAS-AMPK RNAi #1 / +; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-CncC, UAS-Mical. (E-F) cnc RNAi #1 + O/E Control: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-Dcr2; UAS-cnc RNAi #1/ ppk-Gal4, UAS-mCD8GFP. cnc RNAi #1 + O/E AMPK: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-AMPK; UAS-cnc RNAi #1/ ppk-Gal4, UAS-mCD8GFP. cnc RNAi #1 + O/E AMPK<sup>CA</sup>: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-AMPK<sup>CA</sup>; UAS-cnc RNAi #1/ ppk-Gal4, UAS-mCD8GFP.*

**Figure S7:** (A) **O/E Control:** *w\**; *UAS-Dcr2 / UAS-Dcr2; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E InR<sup>CA</sup>: w\**; *UAS-InR<sup>CA</sup> / UAS-InR<sup>CA</sup>; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E Akt: w\**; *UAS-Akt / UAS-Akt; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E TOR: w\**; *ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP; UAS-Tor / UAS-TOR. (B) O/E Control: w\**; *UAS-Dcr2 / gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E InR<sup>DN</sup>: w\**; *UAS-InR<sup>DN</sup> / gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E PI3K<sup>DN</sup>: w\**; *UAS-PI3K<sup>DN</sup> / gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E S6K<sup>DN</sup>: w\**; *UAS-S6K<sup>DN</sup> / gstD1-lacZ; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. Ctrl RNAi: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / gstD1-lacZ; UAS-control RNAi / ppk-Gal4, UAS-mCD8GFP. akt RNAi: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / gstD1-lacZ; UAS-akt RNAi / ppk-Gal4, UAS-mCD8GFP. (C) O/E Control: w\**; *UAS-Dcr2 / UAS-Dcr2; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E InR<sup>DN</sup>: w\**; *UAS-InR<sup>DN</sup> / UAS-InR<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E PI3K<sup>DN</sup>: w\**; *UAS-PI3K<sup>DN</sup> / UAS-PI3K<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. O/E S6K<sup>DN</sup>: w\**; *UAS-S6K<sup>DN</sup> / UAS-S6K<sup>DN</sup>; ppk-Gal4, UAS-mCD8GFP / ppk-Gal4, UAS-mCD8GFP. Ctrl RNAi: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; UAS-control RNAi / UAS-control RNAi. akt RNAi: w\**; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2; UAS-akt RNAi / UAS-akt RNAi.*

**Figure S8: (A-B) *cnc* RNAi #1 + O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-Dcr2*; *UAS-cnc* RNAi #1/ *ppk-Gal4*, *UAS-mCD8GFP*. ***cnc* RNAi #1 + O/E *PI3K*<sup>DN</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-PI3K*<sup>DN</sup>; *UAS-cnc* RNAi #1/ *ppk-Gal4*, *UAS-mCD8GFP*. ***cnc* RNAi #1 + O/E *TOR*<sup>TED</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-TOR*<sup>TED</sup>; *UAS-cnc* RNAi #1/ *ppk-Gal4*, *UAS-mCD8GFP*. ***cnc* RNAi #1 + O/E *S6K*<sup>DN</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *UAS-S6K*<sup>DN</sup>; *UAS-cnc* RNAi #1/ *ppk-Gal4*, *UAS-mCD8GFP*.

**Figure S9: (A) O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *gstD1-lacZ*; *UAS-Mical*<sup>NT</sup> / *ppk-Gal4*, *UAS-mCD8GFP*. **O/E *Mts*<sup>DN</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *gstD1-lacZ*; *UAS-Mts*<sup>DN</sup> / *ppk-Gal4*, *UAS-mCD8GFP*. **Ctrl RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *gstD1-lacZ*; *UAS-control* RNAi / *ppk-Gal4*, *UAS-mCD8GFP*. **PP2A-29B RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *gstD1-lacZ*; *UAS-PP2A-29B* RNAi / *ppk-Gal4*, *UAS-mCD8GFP*. **(B-C) Ctrl RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*; *UAS-Control* RNAi / *UAS-Control* RNAi. ***mts* RNAi:** *w\**; *UAS-mts* RNAi / *UAS-Dcr2*; *ppk-Gal4*, *UAS-mCD8GFP* / +. **PP2A-29B RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*; *UAS-PP2A-29B* RNAi / *UAS-PP2A-29B* RNAi. **(D) O/E *Mts*<sup>DN</sup> + Ctrl RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*; *UAS-Mts*<sup>DN</sup> / *UAS-Control* RNAi. **O/E *Mts*<sup>DN</sup> + *akt* RNAi:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2*; *UAS-Mts*<sup>DN</sup> / *UAS-akt* RNAi. **O/E *Mts*<sup>DN</sup> + O/E Control:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *ppk-Gal4*, *UAS-mCD8GFP*; *UAS-Mts*<sup>DN</sup> / *UAS-Mical*<sup>NT</sup>. **O/E *Mts*<sup>DN</sup> + O/E *Tor*<sup>TED</sup>:** *w\**; *ppk-Gal4*, *UAS-mCD8GFP* / *UAS-Tor*<sup>TED</sup>; *UAS-Mts*<sup>DN</sup> / *ppk-Gal4*, *UAS-mCD8GFP*.

**Figure S10 (B) AMPK RNAi #1 + O/E Control:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-Dcr2*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*. **AMPK RNAi #1 + O/E *InR*<sup>DN</sup>:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-InR*<sup>DN</sup>; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*. **AMPK RNAi #1 + O/E *PI3K*<sup>DN</sup>:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-PI3K*<sup>DN</sup>; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / *ppk-Gal4*, *UAS-mCD8GFP*. **AMPK RNAi #1 + O/E *TSC1/2*:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-TSC1/2*; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / +. **AMPK RNAi #1 + O/E *TOR*<sup>TED</sup>:** *w\**; *UAS-AMPK* RNAi #1 / *UAS-TOR*<sup>TED</sup>; *ppk-Gal4*, *UAS-mCD8GFP*, *UAS-Dcr2* / +. **AMPK RNAi #1 + O/E *S6K*<sup>DN</sup>:** *w\**; *UAS-AMPK*

RNAi #1 / UAS- *S6K<sup>DN</sup>*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / +. (C) **AMPK RNAi #1 + O/E Control; Control:** *w\**; UAS-AMPK RNAi #1 / UAS-*Mical<sup>NT</sup>*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #1 + O/E Control; Mical:** *w\**; UAS-AMPK RNAi #1 / UAS-*Mical<sup>NT</sup>*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*. **AMPK RNAi #1 + O/E InR<sup>DN</sup>; Control:** *w\**; UAS-AMPK RNAi #1 / UAS-*InR<sup>DN</sup>*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical<sup>NT</sup>*. **AMPK RNAi #1 + O/E InR<sup>DN</sup>; Mical:** *w\**; UAS-AMPK RNAi #1 / UAS-*InR<sup>DN</sup>*; *ppk-Gal4*, UAS-*mCD8GFP*, UAS-*Dcr2* / UAS-*Mical*. (D) **O/E InR<sup>CA</sup> + O/E Control:** *w\**; UAS-*InR<sup>CA</sup>* / UAS-*Dcr2*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E InR<sup>CA</sup> + O/E AMPK<sup>CA</sup>:** *w\**; UAS-*InR<sup>CA</sup>* / UAS- *AMPK<sup>CA</sup>*; *ppk-Gal4*, UAS-*mCD8GFP* / *ppk-Gal4*, UAS-*mCD8GFP*. **O/E TOR + O/E Control:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*Dcr2*; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*TOR*. **O/E TOR + O/E AMPK<sup>CA</sup>:** *w\**; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*AMPK<sup>CA</sup>*; *ppk-Gal4*, UAS-*mCD8GFP* / UAS-*TOR*.

### Table S1. Source data for all the figures

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