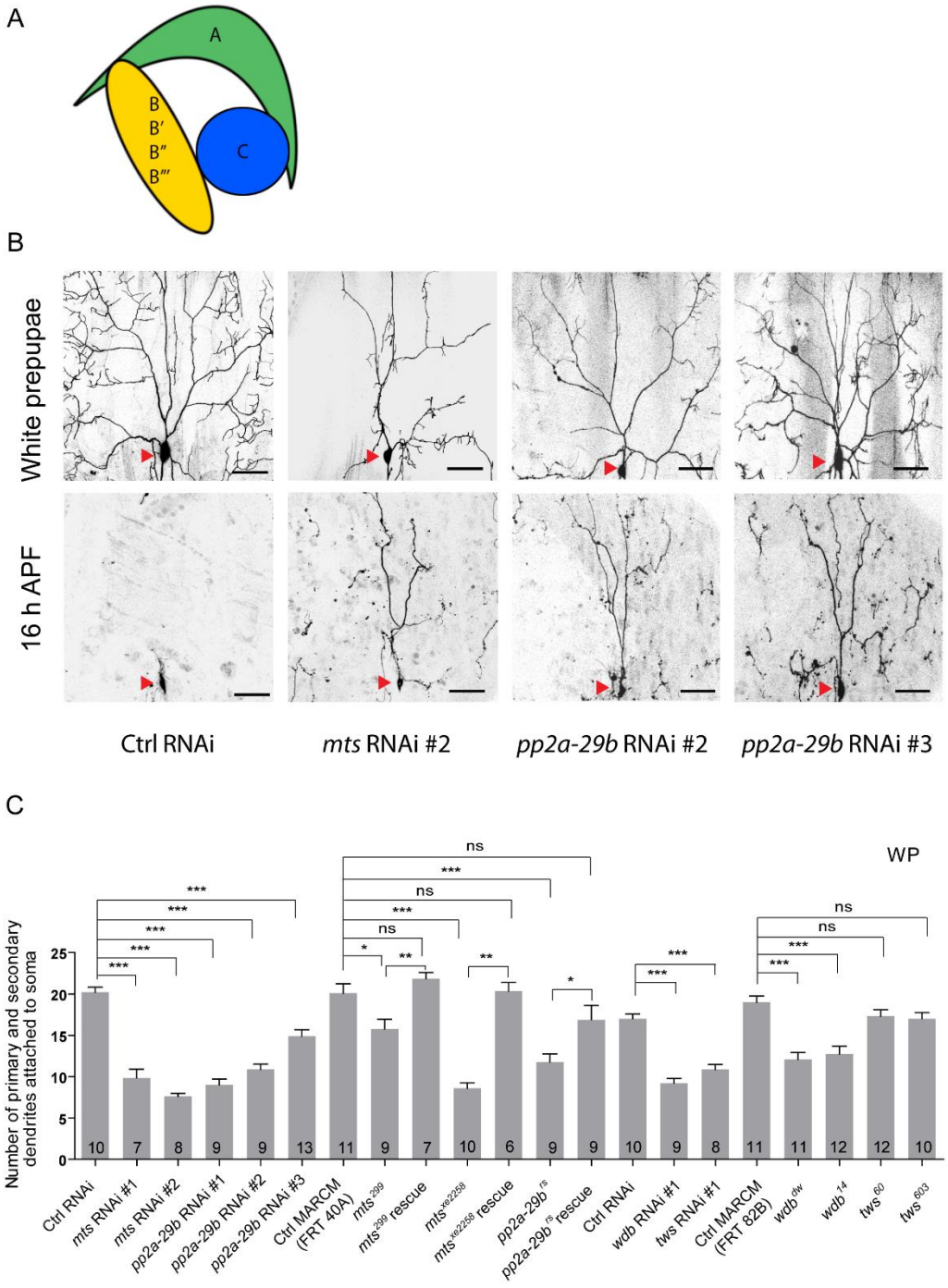
Appendix



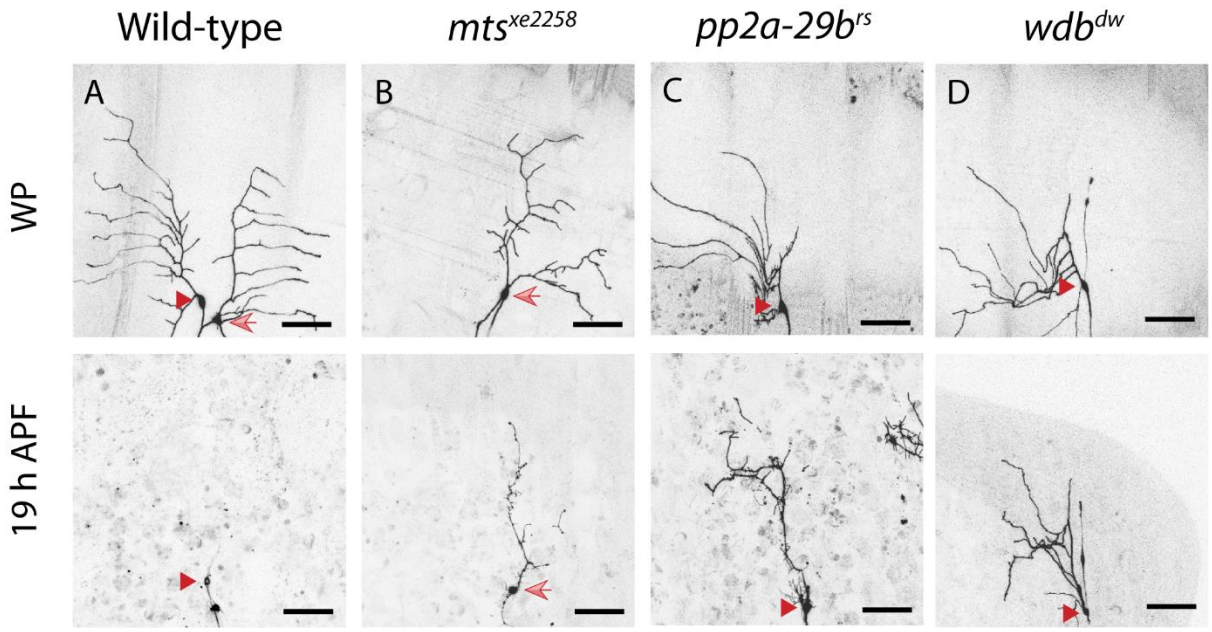
Appendix Figure S1. Mts and PP2A-29B are required for dendrite pruning in ddaC neurons.

A. A schematic representation of the components of PP2A holoenzyme. It consists of a catalytic C subunit, a structural A subunit and four types of regulatory B subunits.

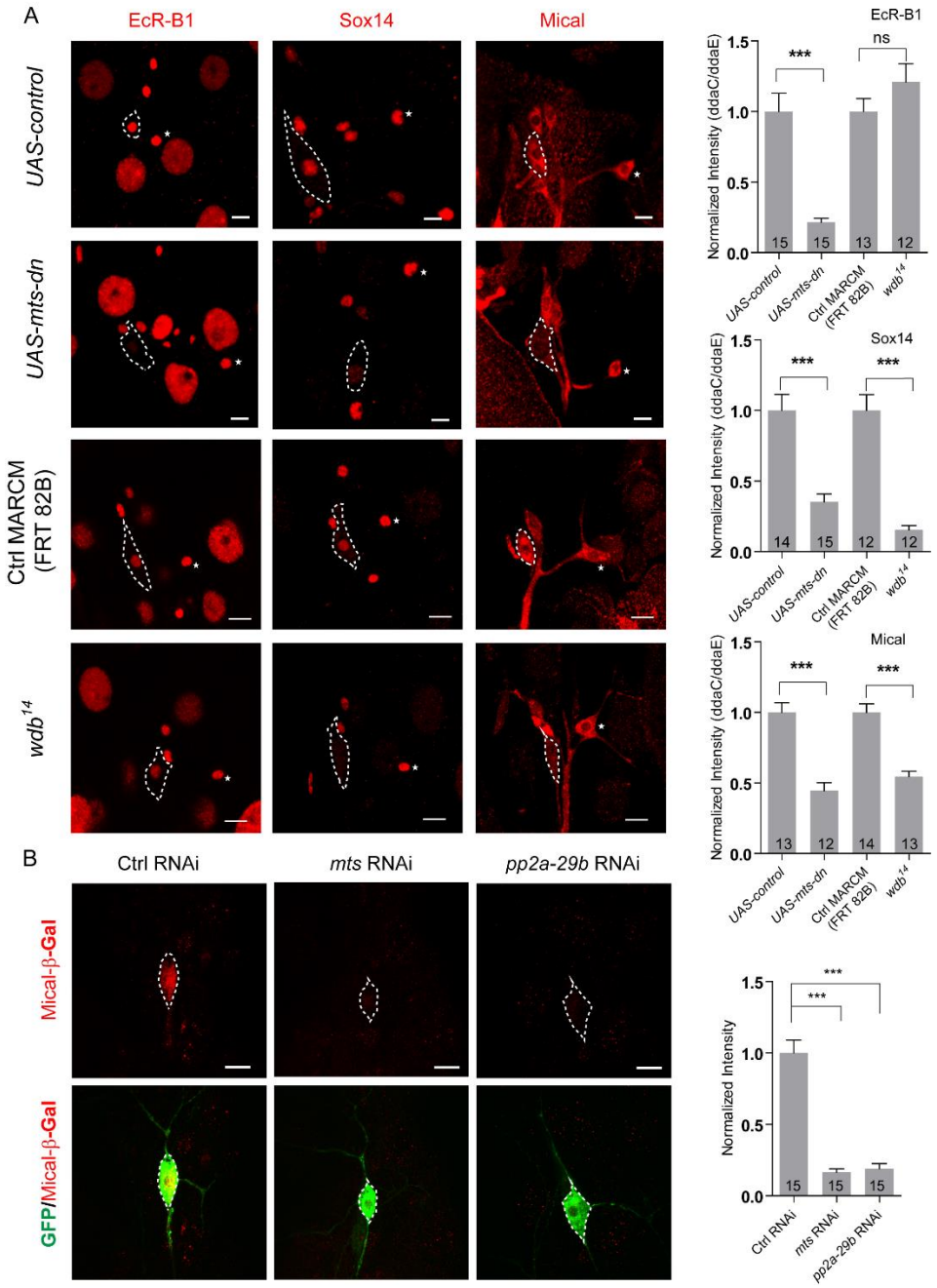
B. Live confocal images of ddaC neurons expressing mCD8-GFP driven by *ppk-Gal4* at WP and 16 h APF stages. Dendrites of ctrl RNAi, *mts* RNAi #2, *pp2a-29b* RNAi #2, and *pp2a-29b* RNAi #3 at WP and 16 h APF stages. Red arrowheads point to the ddaC somas.

C. Quantification of number of primary and secondary dendrites attached to soma at WP stage.

Data information: In (C), data are presented as mean ± SEM from three independent experiments. ns, not significant; *p<0.05; **p<0.01; ***p<0.001 (one-way ANOVA with Bonferroni test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (B) represent 50 μm. Source data are available online for this figure.

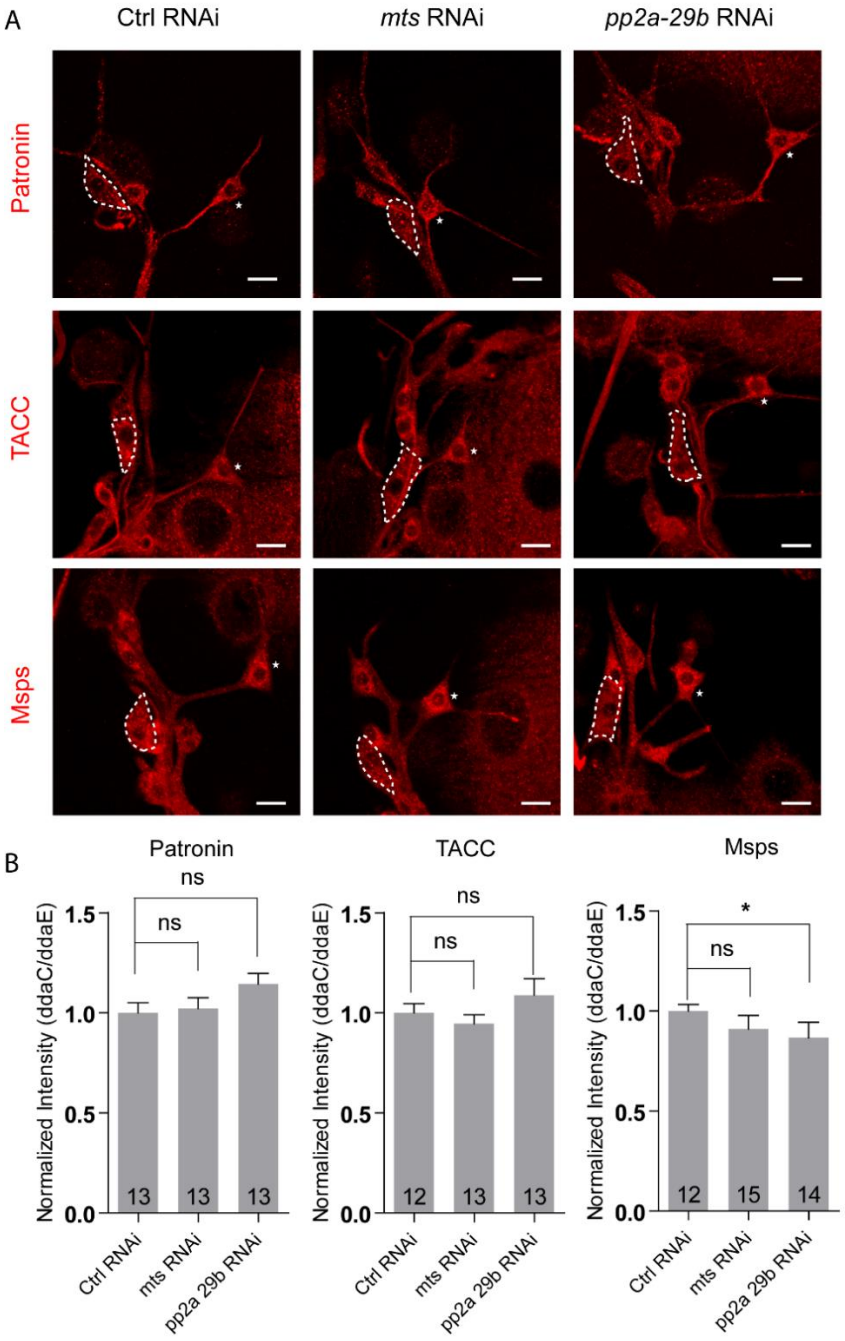


Appendix Figure S2. PP2A is required for dendrite pruning of class I ddaD/E neurons.
A–D. Live confocal images of ddaD/E neurons expressing mCD8-GFP driven by *Gal4*$^{2\text{-}21}$ at WP and 19 h APF. While the wild-type neurons pruned all the dendrites (A), ddaD/E neurons derived from *mts*^{xe2258} (B), *pp2a-29b*^{rs} (C), and *wdb*^{dw} MARCM clones (D) showed simple arbors at WP stage and dendrite pruning defects at 19 h APF. Red arrowheads point to the ddaC somas. Scale bar in **(**A-D**)** represents 50 μm.



Appendix Figure S3. PP2A regulates Sox14/Mical expression and also *mical* transcription.

A. Confocal images of ddaC neurons of *UAS-contol*, *UAS-mts-dn*, ctrl MARCM and *wdb*^{14} MARCM clones that were immunostained for EcR-B1/Sox14/Mical at WP stage. Quantitative analyses of normalized EcR-B1, Sox14 and Mical fluorescence intensities in ddaC neurons of these genotypes (rightest panels). ddaC somas are labeled by dashed lines, ddaE somas are marked by asterisks.
B. Confocal images of ddaC neurons at w3L stage immunostained for anti-β-galactosidase in ctrl RNAi, *mts* RNAi, and *pp2a-29b* RNAi ddaC neurons. Mical-β-gal signals were significantly reduced in *mts* RNAi and *pp2a-29b* RNAi ddaC somas. Quantitative analyses of normalized β-galactosidase intensity in ddaC somas (rightest panels). ddaC somas are labeled by dashed lines.
Data information: In (A-B), data are presented as mean ± SEM from three independent experiments. ns, not significant; ***p<0.001 (A, two-tailed Student's t-test; B, one-way ANOVA with Bonferroni test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-B) represent 10 μm. Source data are available online for this figure.

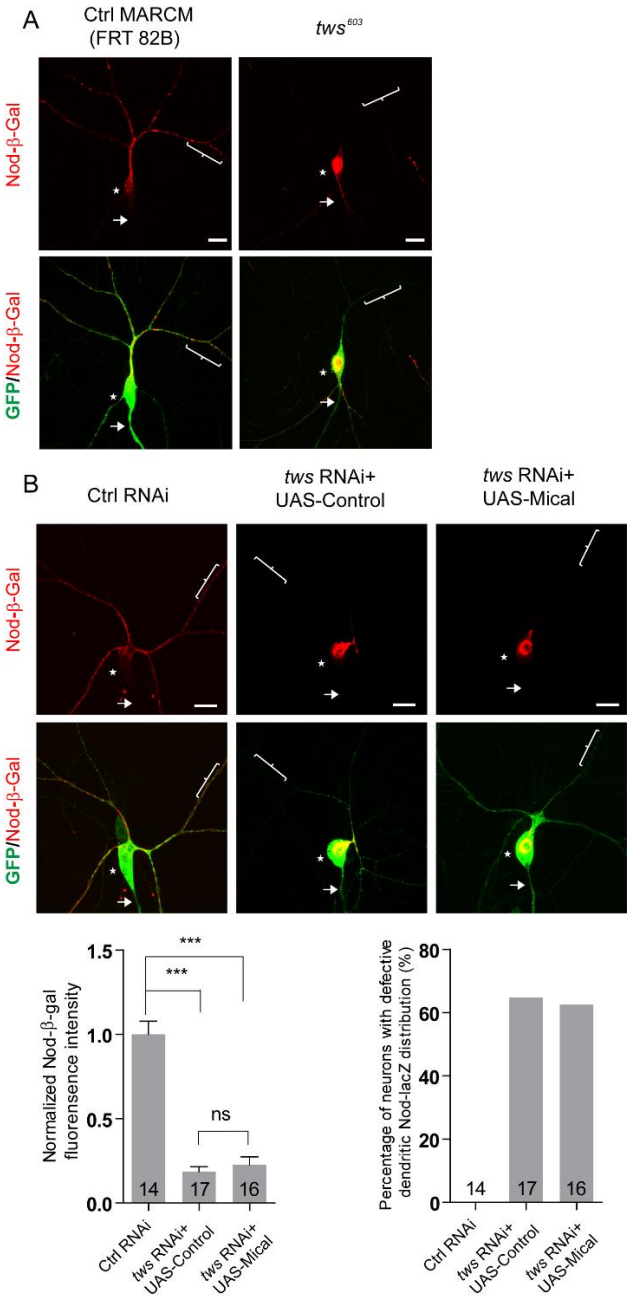


Appendix Figure S4. PP2A is not required for the overall levels of Patronin, TACC and Msps in ddaC neurons.

A. Confocal images of ddaC neurons of ctrl RNAi, *mts* RNAi and *pp2a-29b* RNAi that were immunostained for Patronin, TACC and Msps at wL3 stage. ddaC somas are labeled by dashed lines, ddaE somas are marked by asterisks.

B. Quantitative analyses of normalized Patronin, TACC and Msps fluorescence intensities in ddaC neurons.

Data information: In (B), data are presented as mean ± SEM from three independent experiments. ns, not significant; *p<0.05 (one-way ANOVA with Bonferroni test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A) represent 10 μm. Source data are available online for this figure.

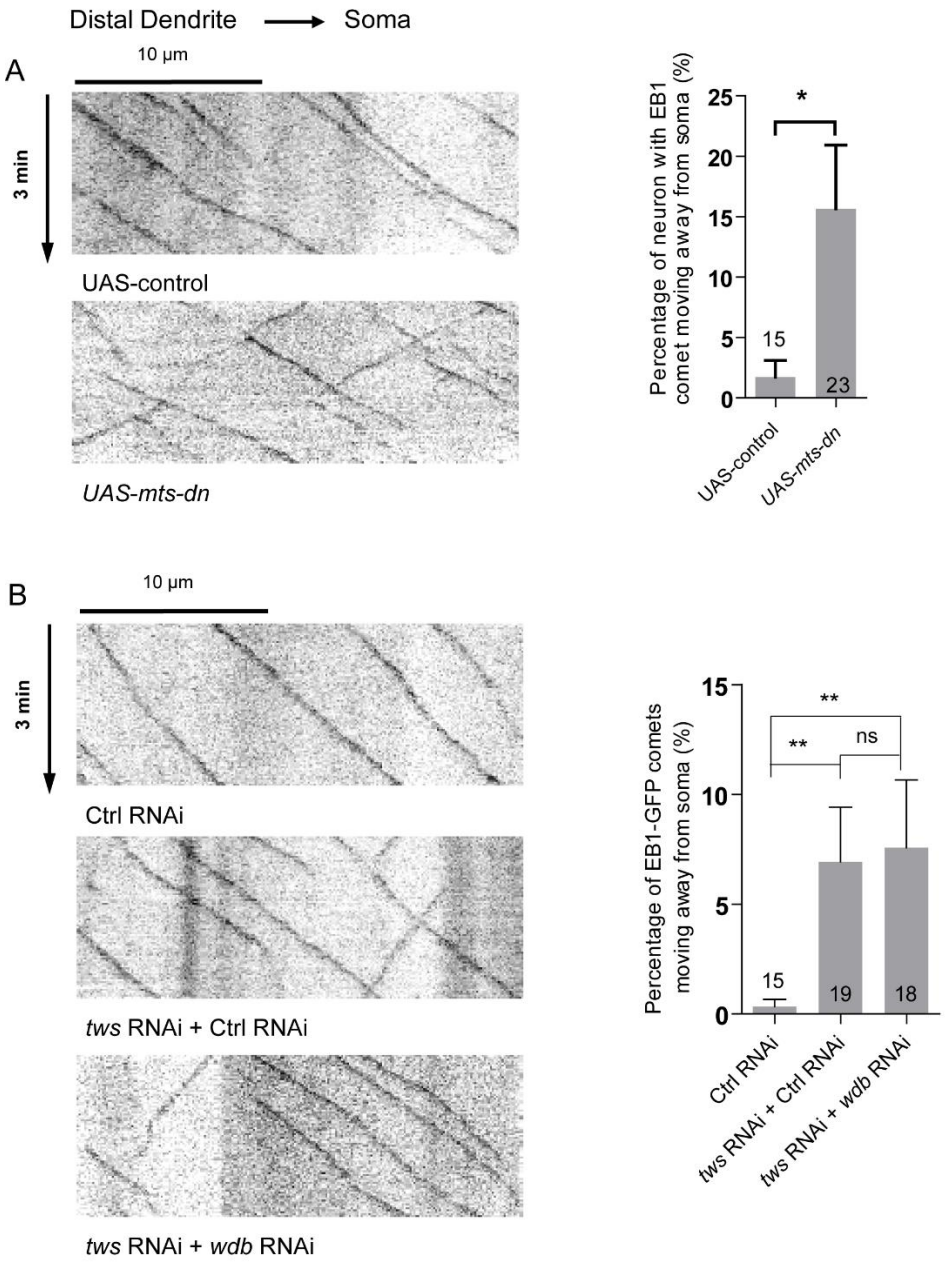


Appendix Figure S5. Tws regulates proper distribution of Nod-β-gal independently of Mical.

A. Confocal images of ddaC neurons at w3L stage immunostained for anti-β-galactosidase. Nod-β-gal signals were localized in the dendrites of the ctrl MARCM, however, Nod-β-gal levels were strongly reduced in the dendrites and accumulated in the somas in *tws*^{603} MARCM clones. ddaC somas are marked by asterisks, axons by arrows and dendrites used for analysing by curly brackets.

B. Nod-β-gal signals were localized in the dendrites of the ctrl RNAi, however, Nod-β-gal levels were strongly reduced in the dendrites and accumulated in the somas of *tws* RNAi neurons, overexpression of Mical in *tws* RNAi neurons did not rescue the Nod-β-gal mis-localization defect. Quantification of normalized Nod-β-gal fluorescence intensity and percentage of neurons with defective Nod-β-gal distribution in these genotypes (bottom panels).

Data information: In (B), data are presented as mean ± SEM from three independent experiments. ***p<0.001 (one-way ANOVA with Bonferroni test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A-B) represent 10 μm. Source data are available online for this figure.

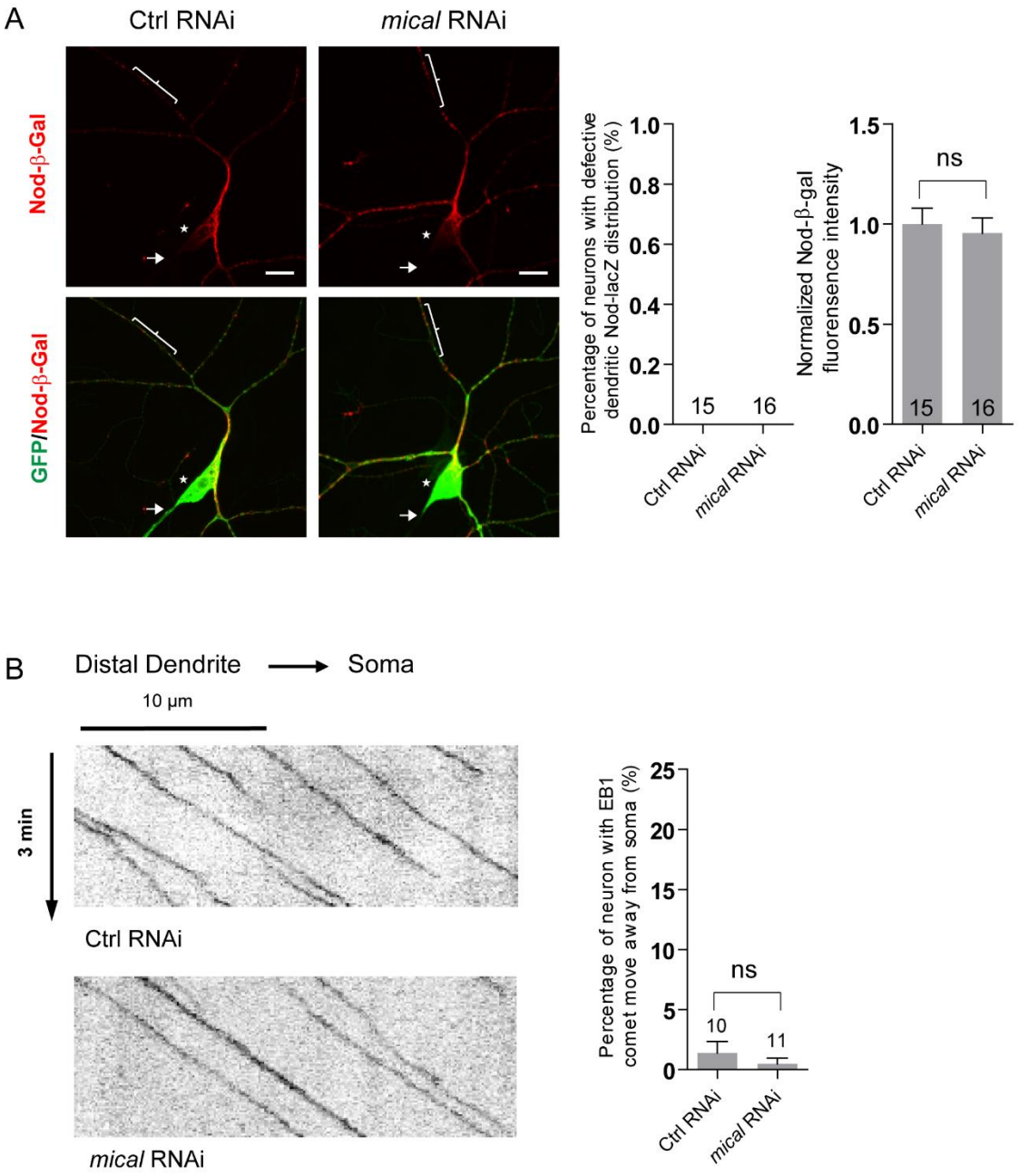


Appendix Figure S6. Knockdown of *wdb* in the *tws* RNAi background did not enhance the dendritic MT orientation phenotype.

A. Representative kymographs depicting the movement patterns of EB1 comets in the proximal dendrites of ddaC neurons at 96 h AEL. In *UAS-control* dendrites, EB1-GFP comets predominantly moved toward the somas (retrograde). However, in *mts-dn*-overexpressing ddaC dendrite branches, some EB1-GFP comets moved away from the somas (anterograde). Quantitative analyses of the percentages of anterograde EB1 comets in ddaC neurons (right panel).

B. Representative kymographs depicting the movement patterns of EB1 comets in the proximal dendrites of ddaC neurons at 96 h AEL. Double knockdown of *tws* and *wdb* did not significantly elevate the percentage of anterograde EB1 comets. Quantitative analyses of the percentages of anterograde EB1 comets in ddaC neurons (right panel).

Data information: In (A-B), data are presented as mean ± SEM from three independent experiments. ns, not significant; *p<0.05; **p<0.01 (A, two-tailed Student's t-test; B, one-way ANOVA with Bonferroni test). The number of neurons (n) examined in each group is shown on the bars. Horizontal arrow indicates the direction towards the somas, and vertical arrow indicates that each movie was taken for 3 minutes. Scale bar in represents 10 μm. Source data are available online for this figure.



Appendix Figure S7. Mical is dispensable for dendritic MT orientation.

A. Confocal images of ddaC neurons at wL3 stage immunostained for anti-β-galactosidase. Nod-β-gal signals were localized in the dendrites of the ctrl RNAi and *mical* RNAi. Right panels show the quantification of normalized Nod-β-gal fluorescence intensity and percentage of neurons with defective Nod-β-gal distribution. ddaC somas are marked by asterisks, axons by arrows and dendrites used for analysing by curly brackets.

B. Representative kymographs depicting the movement patterns of EB1 comets in the proximal dendrites of ddaC neurons at 96 h AEL. In both ctrl RNAi and *mical* RNAi dendrites, EB1-GFP comets predominantly moved toward the somas (retrograde). Quantitative analyses of the percentages of anterograde EB1 comets (right panel).

Data information: In (A-B), data are presented as mean ± SEM from three independent experiments. ns, not significant (two-tailed Student's t-test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A) represent 10 μm. Source data are available online for this figure.

Genotypes of fly strains

Figure 1: (B) *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. (C) *w*; ppk-Gal4, UAS-mCD8GFP* / *UAS-mts* RNAi #1*; UAS-Dcr2* / +. (D) *w*; FRT40A* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (E) *w*; FRT40A, mts^{299}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (F) *w*; FRT40A, mts^{299}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / *UAS-mts.* (G) *w\;* GSG2295-*Gal4, ppk-CD4-tdGFP / +; UAS-mts-dn / +***.** (H) *w\;* GSG2295-*Gal4, ppk-CD4-tdGFP / +; UAS-mts-dn / +***.**

Figure 2: (A) *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. (B) *w*; ppk-Gal4, UAS-mCD8GFP /+; UAS-Dcr2* / *UAS-pp2a-29b* RNAi #1. (C) *w*; FRT40A* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (D) *w*; FRT40A, pp2a-29b^{rs}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (E) *w*; FRT40A, pp2a-29b^{rs}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / *UAS-pp2a-29b.*

Figure 3: (A) *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. (B) *w*; UAS-wdb* RNAi #1/ *UAS-wdb* RNAi #1*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2* / *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2*. (C) *w*; ppk-Gal4, UAS-mCD8GFP* / *UAS-tws* RNAi #1*; UAS-Dcr2* / +. (D) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B/ FRT82B, tubP-Gal80.* (E) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb^{dw} / FRT82B, tubP-Gal80.* (F) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb^{14} / FRT82B, tubP-Gal80.* (G) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, tws^{60} / FRT82B, tubP-Gal80.* (H) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, tws^{603} / FRT82B, tubP-Gal80.*

Figure 4: (A-C) *w*; FRT40A, mts^{xe2258} / FRT40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (D-F) *w*; FRT40A, mts^{xe2258} / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (G-I) *w*; FRT40A, pp2a-29b^{rs} / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (J-L) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb^{dw} / FRT82B, tubP-Gal80.* (M-O) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, tws^{60} / FRT82B, tubP-Gal80.*

Figure 5: (A) *w*; FRT40A* / *FRT40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (B) *w*; FRT40A, mts^{299}* / *FRT40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (C) *w*; FRT40A, mts^{299}* / *FRT40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / *UAS-Mical.* (D) *w*; FRT40A* / *FRT40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (E) *w*; FRT40A, pp2a-29b^{rs}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / +. (F) *w*; FRT40A, pp2a-29b^{rs}* / *FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp* / *UAS-Mical.* (G) *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. (H) *w*; UAS-wdb* RNAi / *ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2* / *UAS-Mical^{NT}*. (I) *w*; UAS-wdb* RNAi / *ppk-Gal4, UAS-mCD8GFP; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2* / *UAS-Mical*.

Figure 6: (A) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Nod-lacZ / UAS-*control RNAi. (B) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-mts* RNAi #2*; UAS-Nod-lacZ / +*. (C) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Nod-lacZ / UAS-pp2a-29b* RNAi #1. (D) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-tws* RNAi #1*; UAS-Nod-lacZ / +*. (E) *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb^{dw}, UAS-Nod-lacZ / FRT82B, tubP-Gal80.* (F) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+; UAS-Kin-lacZ / UAS-*control RNAi. (G) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-mts* RNAi #2*; UAS-Kin-lacZ /+*.(H) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+; UAS-Kin-lacZ / UAS-pp2a-29b* RNAi #1. (I) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-tws* RNAi #1*; UAS-Kin-lacZ / +*. (J) *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Kin-lacZ, wdb^{dw} / wdb^{14}*.

Figure 7: (A) *w*; Gal4$^{4-77}$, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-*control RNAi. (B) *w*; Gal4$^{4-77}$, UAS-EB1-GFP* / *UAS-mts* RNAi #2*; UAS-Dcr2 /+*. (C) *w*; Gal4$^{4-77}$, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1. (D) *w*; Gal4$^{4-77}$, UAS-EB1-GFP* / *UAS-tws* RNAi #1*; UAS-Dcr2 /+*. (I) *w*; Gal4$^{4-77}$, UAS-EB1-GFP / +; + / +*. (J) *w*; Gal4$^{4-77}$, UAS-EB1-GFP / +; wdb^{dw} / wdb^{14}*.

Figure 8: (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP* / *UAS-mts* RNAi #2*; UAS-Dcr2* / +. ***pp2a-29b RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1. (C) ***mts RNAi, Ctrl RNAi:*** *w*; Gal4$^{4-77}$, UAS-EB1-GFP* / *UAS-mts* RNAi #2*; UAS-Dcr2* / *UAS-*control RNAi. ***mts RNAi, klp10a RNAi:*** *w*; Gal4$^{4-77}$, UAS-EB1-GFP* / *UAS-mts* RNAi #2*; UAS-Dcr2* / *UAS-klp10a* RNAi #2. (E) ***pp2a-29b RNAi, Ctrl RNAi:*** *w*; Gal4$^{4-77}$, UAS-EB1-GFP* / *+; UAS-Dcr2* / *UAS-pp2a-29b* RNAi #1, *UAS-*control RNAi.

pp2a-29b RNAi, klp10a RNAi: *w*; Gal4$^{4-77}$, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1, *UAS-klp10a* RNAi #1. (G) ***tws RNAi, Ctrl RNAi:*** *w*; Gal4$^{4-77}$, UAS-EB1-GFP / UAS-tws* RNAi #1*; UAS-Dcr2 / UAS-*control RNAi. ***tws RNAi, klp10a RNAi:*** *w*; Gal4$^{4-77}$, UAS-EB1-GFP / UAS-tws* RNAi #1*; UAS-Dcr2 / UAS-klp10a* RNAi #2.

Figure EV1. (A) ***mts^{xe2258}:*** *w*; FRT40A, mts^{xe2258} / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +.* ***mts^{xe2258} rescue:*** *w*; FRT40A, mts^{xe2258} / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8-GFP, SOP-flp / UAS-mts.* (B) **Control (FRT 40A):** *w*; FRT40A / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +.* ***mts$^{xe-2258}$:*** *w*; FRT40A, mts^{xe2258} / FRT 40A, tubP-Gal80; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +.*

Figure EV2. (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***wdb RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP /+; UAS-Dcr2 / UAS-wdb* RNAi #2. ***tws RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-tws* RNAi #2*; UAS-Dcr2 /+.* ***wrd RNAi #1:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-wrd* RNAi #1*; UAS-Dcr2 /+.* ***wrd RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP /+; UAS-Dcr2 / UAS-wrd* RNAi #2. ***wrd RNAi #3:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-wrd* RNAi #3*; UAS-Dcr2 /+.* ***pr72 RNAi #1:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-pr72* RNAi #1*; UAS-Dcr2 /+.* ***pr72 RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-pr72* RNAi #2*; UAS-Dcr2 /+.* (B) ***wdb RNAi #1 + Ctrl RNAi :*** *w*; UAS-wdb* RNAi #1/*UAS-*control RNAi; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2.* ***tws RNAi #1 + Ctrl RNAi :*** *w*; UAS-tws* RNAi #1/*UAS-*control RNAi; *ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2.* ***wdb RNAi #1 + tws RNAi #1:*** *w*; UAS-wdb* RNAi #1/ *UAS-tws* RNAi #1*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2.*

Figure EV3. (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2 / +.* ***pp2a-29b RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1.

Figure EV4. (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi, Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2, UAS-*control RNAi */+.* ***mts RNAi, klp10a RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2, UAS-klp10a* RNAi #2 */+.* (C) ***Ctrl RNAi:*** *w*; Gal4$^{2-21}$, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi:*** *w*; Gal4$^{2-21}$, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2 /+.* ***pp2a-29b RNAi:*** *w*; Gal4$^{2-21}$, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1. (E) ***Ctrl MARCM (FRT 82B):*** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B/ FRT82B, tubP-Gal80.* ***wdb^{dw}:*** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb^{dw} / FRT82B, tubP-Gal80.* (G) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mical RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*mical RNAi.

Figure EV5. (A) ***mts RNAi + Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP,UAS-Dcr2 / UAS-mts* RNAi #2*; UAS-*control RNAi /+. ***mts RNAi + klp10a RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-mts* RNAi #2*; klp10a* RNAi #2 /+. ***pp2a-29b RNAi + Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+; UAS-*control RNAi */ UAS-pp2a-29b* RNAi #1. ***pp2a-29b RNAi + klp10a RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+; klp10a* RNAi #2 */ UAS-pp2a-29b* RNAi #1. (B) ***Wild-type:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-*control RNAi /+. ***mts RNAi + Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-mts* RNAi #2*; UAS-*control RNAi /+. ***mts RNAi + klp10a RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-mts* RNAi #2*; klp10a* RNAi #2 /+. ***Wild-type:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-*control RNAi /+. ***pp2a-29b RNAi + Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 /+; UAS-*control RNAi */ UAS-pp2a-29b* RNAi #1. ***pp2a-29b RNAi + klp10a RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; klp10a* RNAi #2 */ UAS-pp2a-29b* RNAi #1.

Appendix Figure S1: (B) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2 / +.* ***pp2a-29b RNAi #2:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #2. ***pp2a-29b RNAi #3:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #3.

Appendix Figure S2: (A) *w*;; Gal4$^{2-21}$, UAS-mCD8-GFP / Gal4$^{2-21}$, UAS-mCD8-GFP.* (B) *elav-Gal4, UAS-mCD8GFP, hs-FLP, w* / Gal4$^{5-40}$, UAS-Venus:pm, SOP-flp #42, w*; FRT40A, mts$^{xe-2258}$ / FRT 40A, tubP-Gal80.* (C) *elav-Gal4, UAS-mCD8GFP, hs-FLP, w* / Gal4$^{5-40}$, UAS-Venus:pm, SOP-flp #42, w*; FRT40A, pp2a-29b^{rs} / FRT 40A, tubP-Gal80.* (D) *elav-Gal4, UAS-mCD8GFP, hs-FLP, w* / Gal4$^{5-40}$, UAS-Venus:pm, SOP-flp #42, w*;; FRT82B, wdb^{dw} / FRT82B, tubP-Gal80.*

Appendix Figure S3: (A) ***UAS-control:*** *w*; ppk-Gal4* / *+ ; ppk-Gal4, UAS-mCD8GFP* / *UAS-mical*^{NT}. ***UAS-mts-dn:*** *w*; ppk-Gal4* / *UAS-mts-dn; ppk-Gal4, UAS-mCD8GFP* /+. ***Ctrl MARCM (FRT 82B):*** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B/ FRT82B, tubP-Gal80.* ***Wdb*^{14}:** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, wdb*^{dw} */ FRT82B, tubP-Gal80.* (B) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2, UAS-*control RNAi */Mical-lacZ.* ***mts RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / UAS-mts* RNAi #2*; UAS-Dcr2 /Mical-lacZ.* ***pp2a-29b RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2, UAS-pp2a-29b* RNAi #1*/Mical-lacZ.*

Appendix Figure S4: (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mts RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP* / *UAS-mts* RNAi #2*; UAS-Dcr2* / *+*. ***Pp2a-29b RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP / +; UAS-Dcr2 / UAS-pp2a-29b* RNAi #1.

Appendix Figure S5: (A) ***Ctrl MARCM (FRT 82B):*** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, UAS-Nod-lacZ / FRT82B, tubP-Gal80.* ***Tws*^{603}:** *w*; ppk-Gal4, UAS-mCD8GFP, SOP-flp / +; FRT82B, tws*^{603}*, UAS-Nod-lacZ / FRT82B, tubP-Gal80.* (B) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Nod-lacZ / UAS-*control RNAi. ***tws RNAi + UAS-control:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-tws* RNAi #1*; UAS-Nod-lacZ / UAS-mical*^{NT}. ***Tws RNAi + UAS-Mical:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / UAS-tws* RNAi #1*; UAS-Nod-lacZ / UAS-mical.*

Appendix Figure S6: (A) ***UAS-control*****:** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP* / *+; +* / *UAS-Mical*^{NT}. ***UAS-mts-dn:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP* /*+; UAS-mts-dn* /+. (B) ***Ctrl RNAi:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***tws RNAi + Ctrl RNAi:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP / UAS-tws* RNAi #1*; UAS-Dcr2 / UAS-*control RNAi. ***tws RNAi + wdb RNAi:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP/ UAS-wdb* RNAi #1, *UAS-tws* RNAi #1*; UAS-Dcr2 /* +.

Appendix Figure S7: (A) ***Ctrl RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Nod-lacZ / UAS-*control RNAi. ***mical RNAi:*** *w*; ppk-Gal4, UAS-mCD8GFP, UAS-Dcr2 / +; UAS-Nod-lacZ / UAS-mical* RNAi. (B) ***Ctrl RNAi:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-*control RNAi. ***mical RNAi:*** *w*; Gal4*$^{4-77}$*, UAS-EB1-GFP / +; UAS-Dcr2 / UAS-mical* RNAi.