

Expanded View Figures

Figure EV1. Mts is essential for dendrite pruning and larval dendrite morphology in ddaC neurons.

- A Live confocal images of ddaC neurons expressing mCD8-GFP driven by *ppk-Gal4* at WP and 16 h APF stages. Dendrites of *mts^{xe2258}* MARCM and *mts^{xe2258}* rescue ddaC neurons at WP and 16 h APF stages. Red arrowheads point to the ddaC somas.
- B, C Live confocal images of ddaC neurons visualized by *ppk-Gal4*-driven mCD8::GFP expression at wL3 stage. ddaC neurons of *mts^{xe2258}* MARCM clone exhibited simplified dendrite arbors at WP stage. The rightmost panel in B is the quantification of number of dendrite terminal and sholl analysis (C) for control and *mts^{xe2258}* MARCM ddaC clones.

Data information: In (B), data are presented as mean \pm SEM from three independent experiments. *** $P < 0.001$ (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 50 μ m and (B) represent 20 μ m. Source data are available online for this figure.

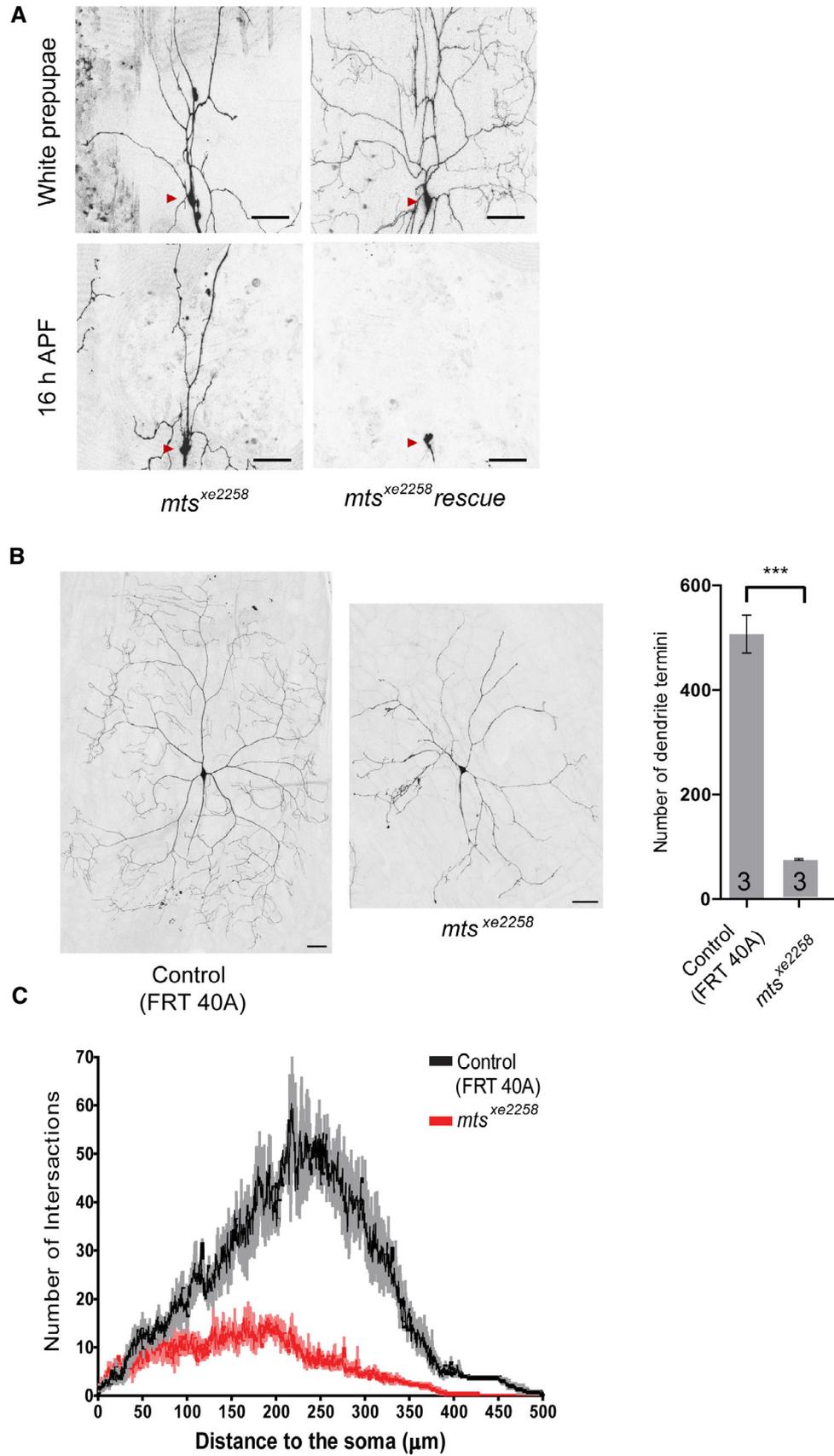


Figure EV1.

Figure EV2. *Tws* knockdown enhanced the pruning defects in *wdb* RNAi ddaC neurons.

- A Live confocal images of ddaC neurons expressing mCD8-GFP driven by *ppk-Gal4* at 16 h APF stage. Dendrites of ctrl RNAi, *wdb* RNAi #2, *tws* RNAi #2, *wrd* RNAi #1, *wrd* RNAi #2, *wrd* RNAi #3, *pr72* RNAi #1, and *pr72* RNAi #2 ddaC neurons at 16 h APF stage. Red arrowheads point to the ddaC somas. The numbers of neurons (n) examined for *wrd* and *pr72* are shown on the panels.
- B Live confocal images of ddaC neurons expressing mCD8-GFP driven by *ppk-Gal4* at 16 h APF stage. Dendrites of *wdb* RNAi + ctrl RNAi, *tws* RNAi + ctrl RNAi, and *wdb* RNAi + *tws* RNAi ddaC neurons at 16 h APF stage. Red arrowheads point to the ddaC somas. Quantification of number of primary and secondary dendrites attached to soma and percentage of severing defects in these genotypes at 16 h APF (bottom panels).

Data information: In (B), data are presented as mean \pm 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 50 μm .

Source data are available online for this figure.

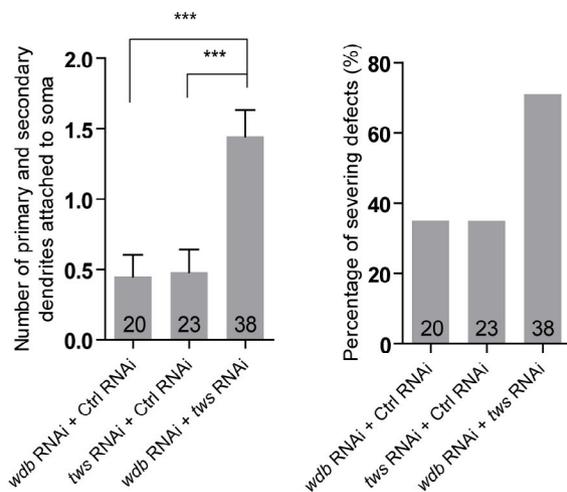
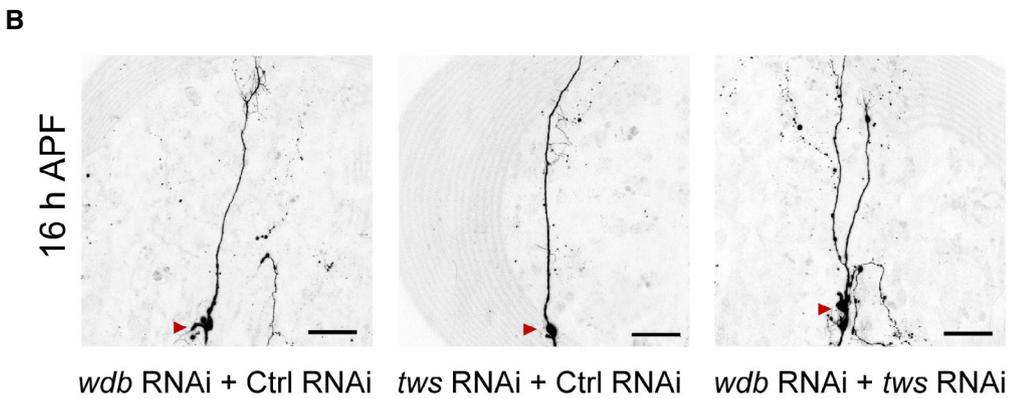
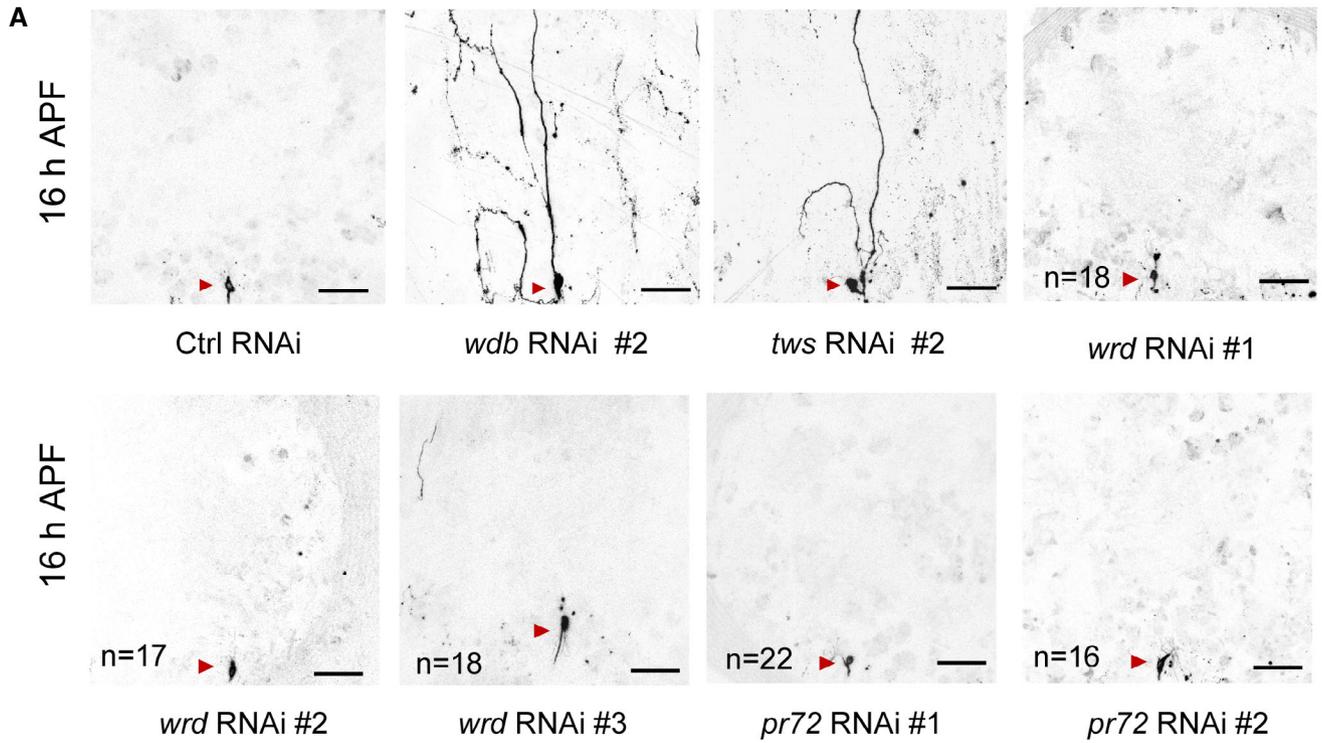


Figure EV2.

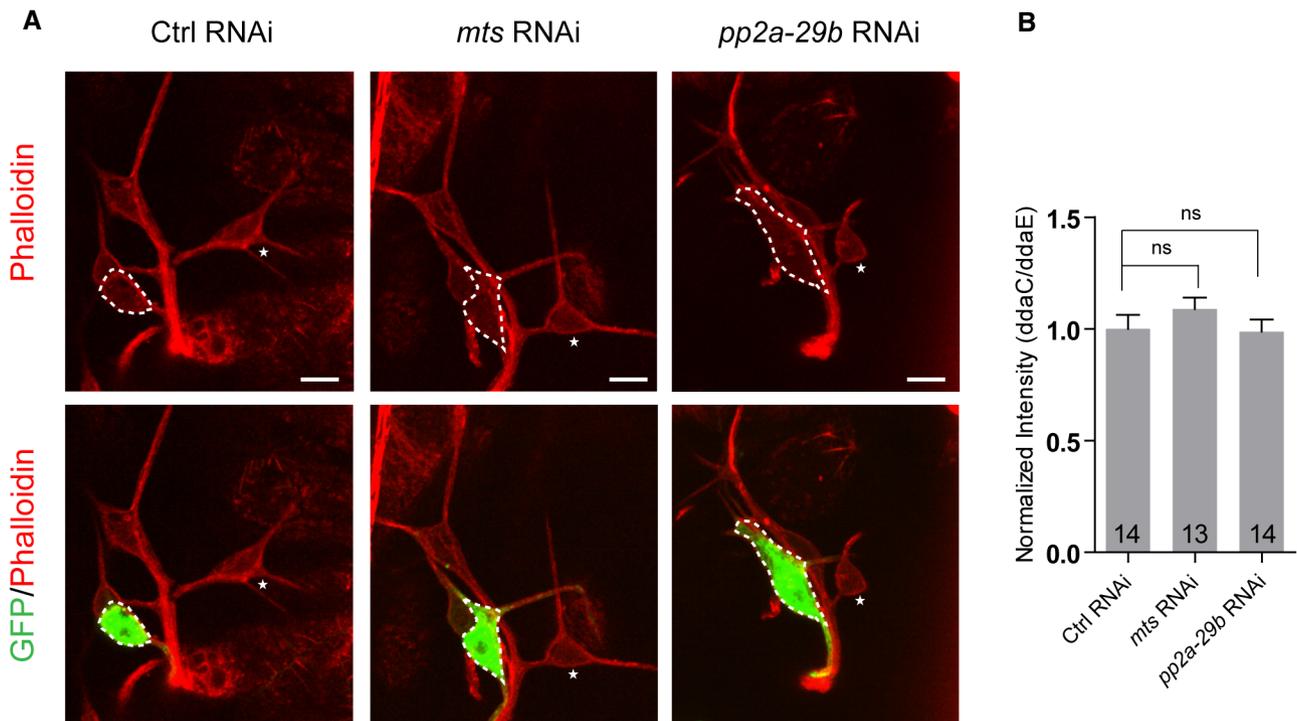


Figure EV3. The distribution of F-actin appeared to be largely normal between wild-type and *mts/pp2a-29b* RNAi *ddaC* neurons at wL3.

A Confocal images of ctrl RNAi, *mts* RNAi, and *pp2a-29b* RNAi *ddaC* neurons immunostained for phalloidin at wL3 stage. *ddaC* somas are labeled by dashed lines. *ddaC* neurons were identified by *ppk-Gal4*-driven *mCD8::GFP* (green channel) expression. *ddaC* somas are labeled by dashed lines, and *ddaE* somas are marked by asterisks. B Quantitative analysis of normalized phalloidin fluorescence intensities of *ddaC* somas.

Data information: In (B), data are presented as mean \pm SEM from three independent experiments. ns, not significant (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 μ m. Source data are available online for this figure.

Figure EV4. PP2A is required to suppress the levels of Klp10A in class I *ddaD/E* neurons.

A–H Confocal images of *ddaC* neurons of (A) ctrl RNAi, *mts* RNAi + control RNAi and *mts* RNAi + *klp10a* RNAi; (C) ctrl RNAi, *mts* RNAi and *pp2a-29b* RNAi; (E) ctrl MARCM and *wdb^{dww}* MARCM; and (G) ctrl RNAi and *mical* RNAi. All were immunostained for Klp10A at wL3 stage. *ddaC* (A, E, G) and *ddaD/E* (C) somas are labeled by dashed lines, and *ddaE* somas (A, E, G) are marked by asterisks. *ddaC* (A, E, G) and *ddaD/E* (C) neurons were identified by the *mCD8::GFP* expression driven by *ppk-Gal4* and *Gal4²⁻²¹*-driven, respectively. (B, D, F, H) Quantitative analysis of normalized Klp10A fluorescence intensities of *ddaC* or *ddaD* somas.

Data information: In (B, D, F, H), data are presented as mean \pm SEM from three independent experiments. ns, not significant; *** $P < 0.001$ (B, D, one-way ANOVA with Bonferroni test; F, H, two-tailed Student's *t*-test). The number of neurons (n) examined in each group is shown on the bars. Scale bars in (A, C, E, G) represent 10 μ m. Source data are available online for this figure.

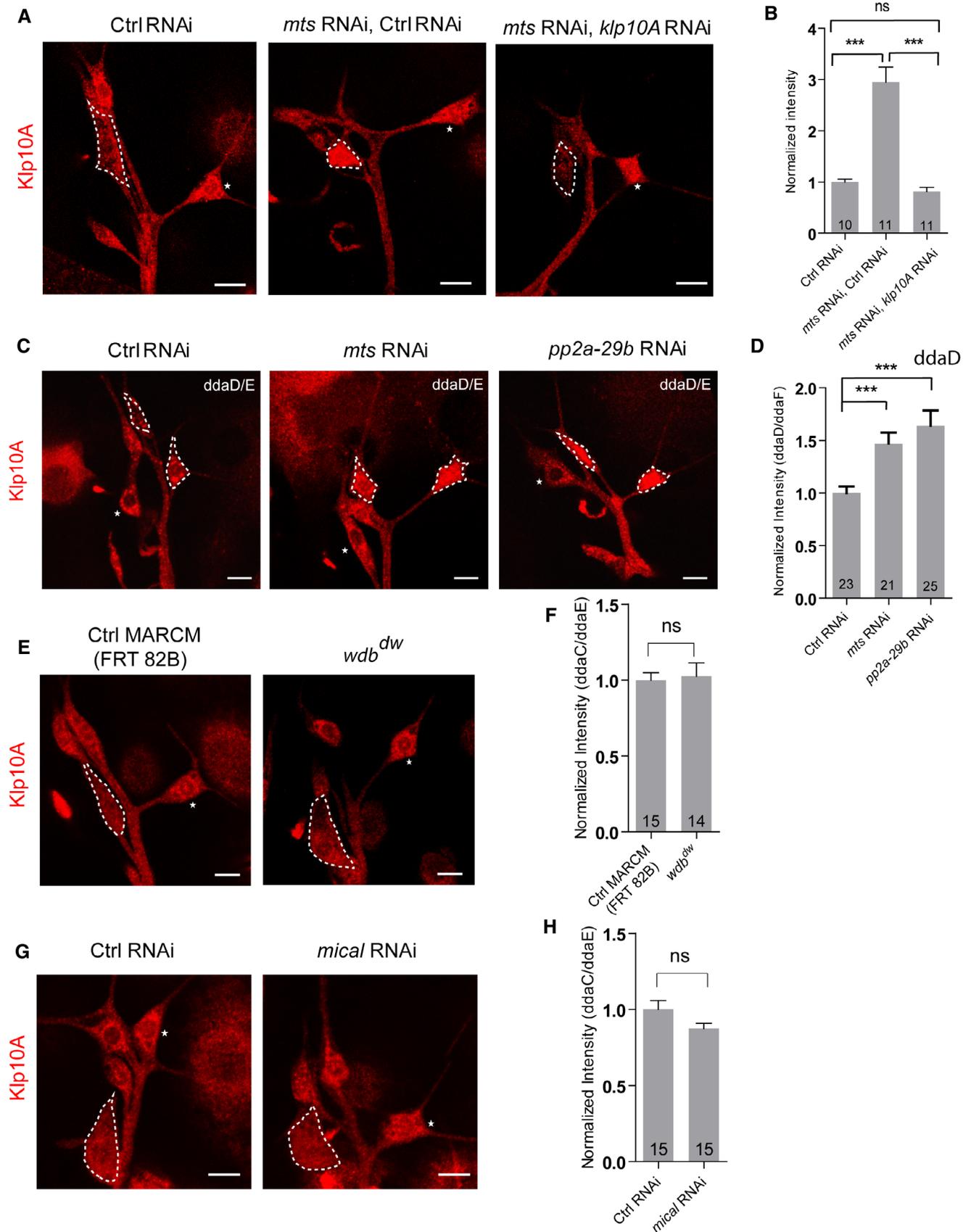


Figure EV4.

Figure EV5. PP2A regulates dendrite pruning partially through suppressing the Klp10A level.

- A Live confocal images of ddaC neurons expressing mCD8-GFP driven by *ppk-Gal4* at 16 h APF stage. Dendrites of *mts* RNAi + Ctrl RNAi, *mts* RNAi + *klp10a* RNAi, *pp2a-29b* RNAi + Ctrl RNAi, and *pp2a-29b* RNAi + *klp10a* RNAi ddaC neurons at 16 h APF stage. Red arrowheads point to the ddaC somas. Quantification of number of primary and secondary dendrites attached to soma and percentage of severing defects at 16 h APF (rightest panels).
- B Confocal images of ddaC neurons of wild-type, *mts* RNAi + Ctrl RNAi, *mts* RNAi + *klp10a* RNAi, wild-type, *pp2a-29b* RNAi + Ctrl RNAi, and *pp2a-29b* RNAi + *klp10a* RNAi that were immunostained for Mical at WP stage. Quantitative analyses of normalized Mical fluorescence (rightest panels). ddaC somas are labeled by dashed lines, and ddaE somas are marked by asterisks.

Data information: In (A, B), data are presented as mean \pm SEM from three independent experiments. ns, not significant; ** $P < 0.01$ (two-tailed Student's *t*-test); *** $P < 0.001$ (one-way ANOVA with Bonferroni test). The number of neurons (*n*) examined in each group is shown on the bars. Scale bars in (A) and (B) represent 50 and 10 μ m, respectively.

Source data are available online for this figure.

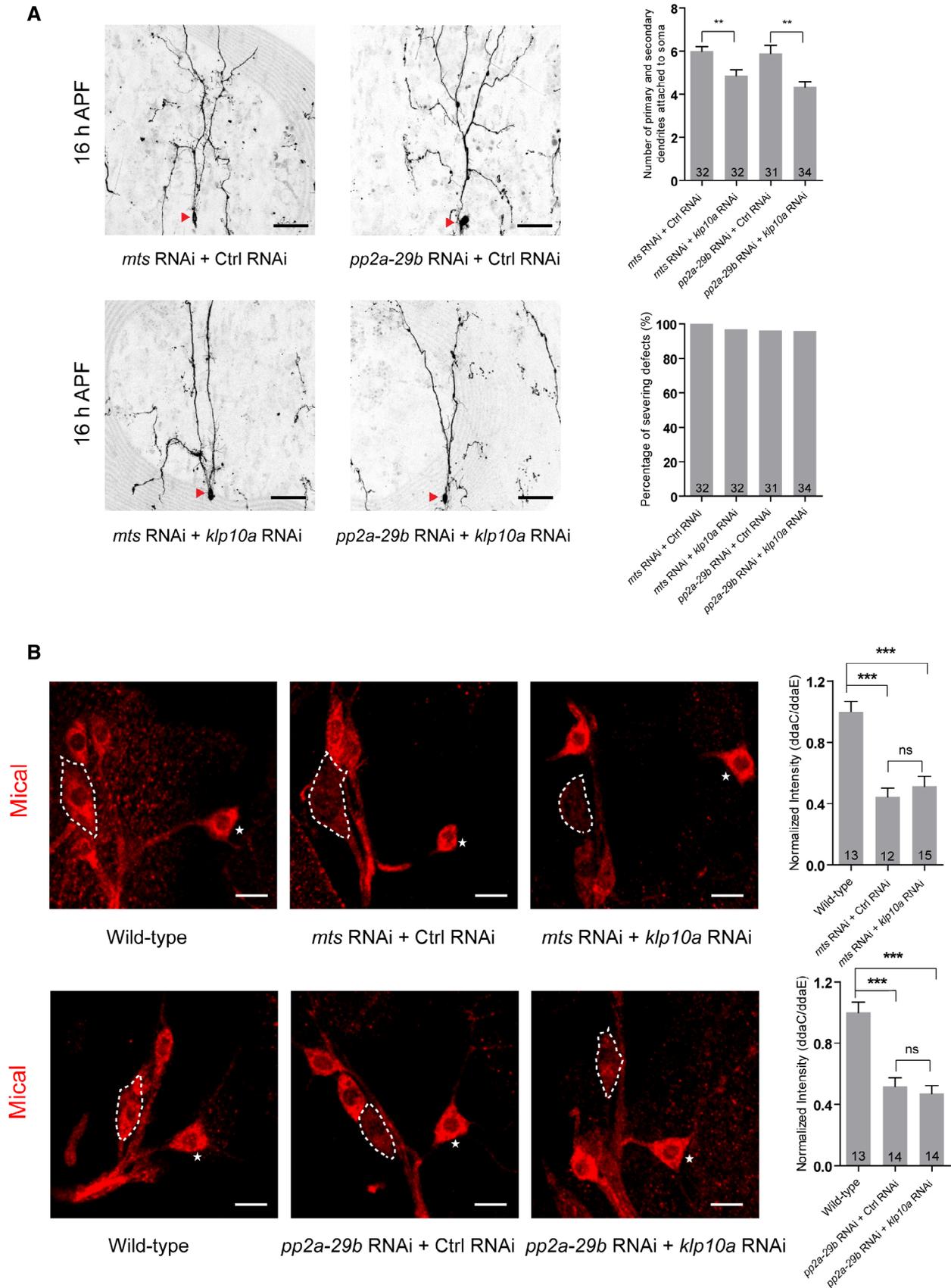


Figure EV5.