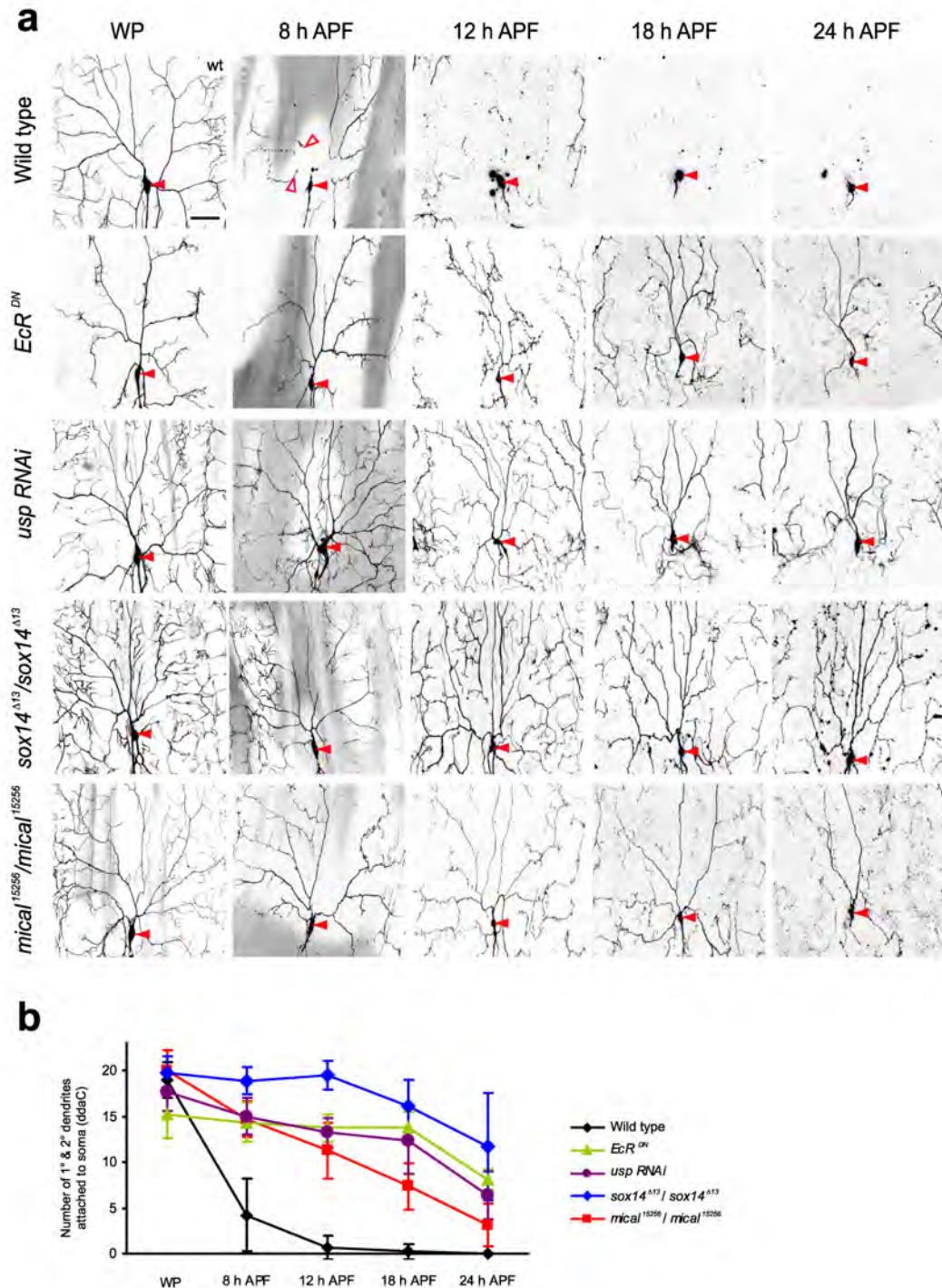


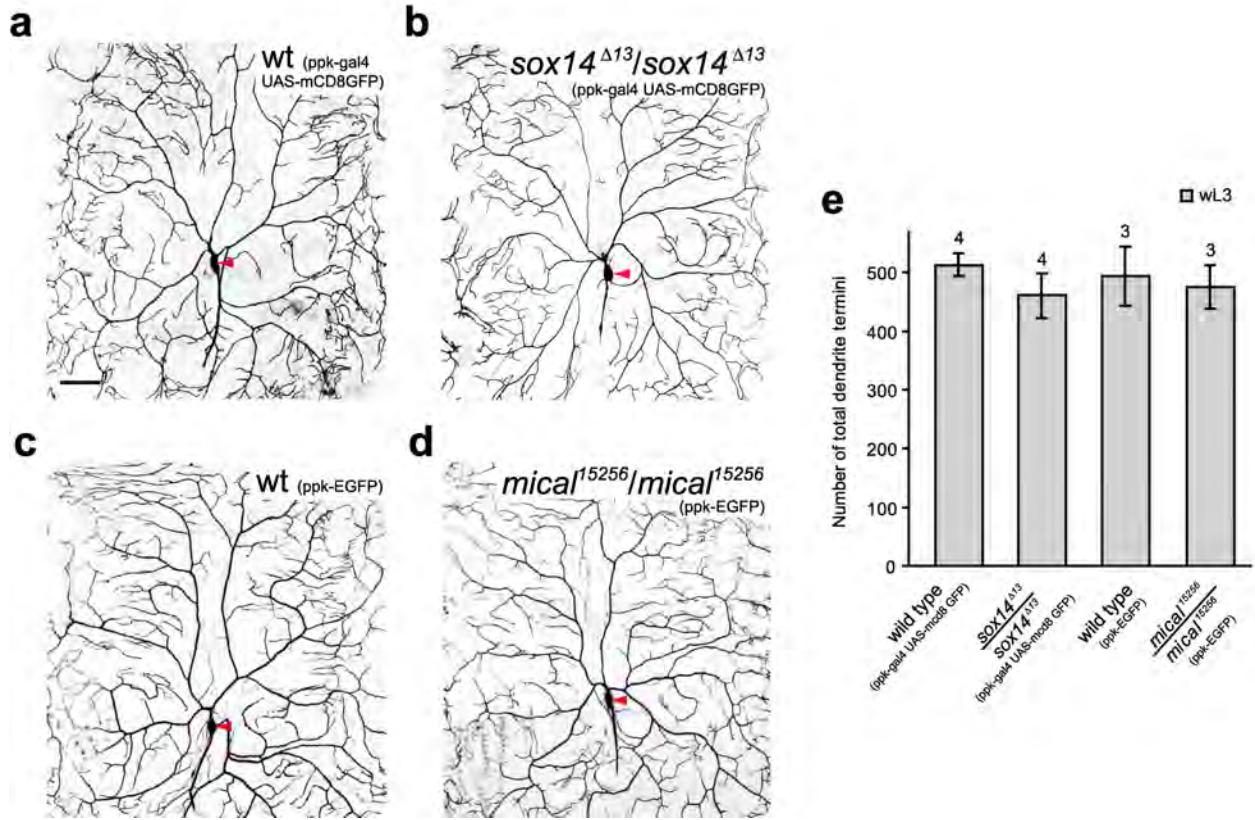
SUPPLEMENTARY INFORMATION

A novel pathway composed of Sox14 and Mical governs severing of dendrites during pruning

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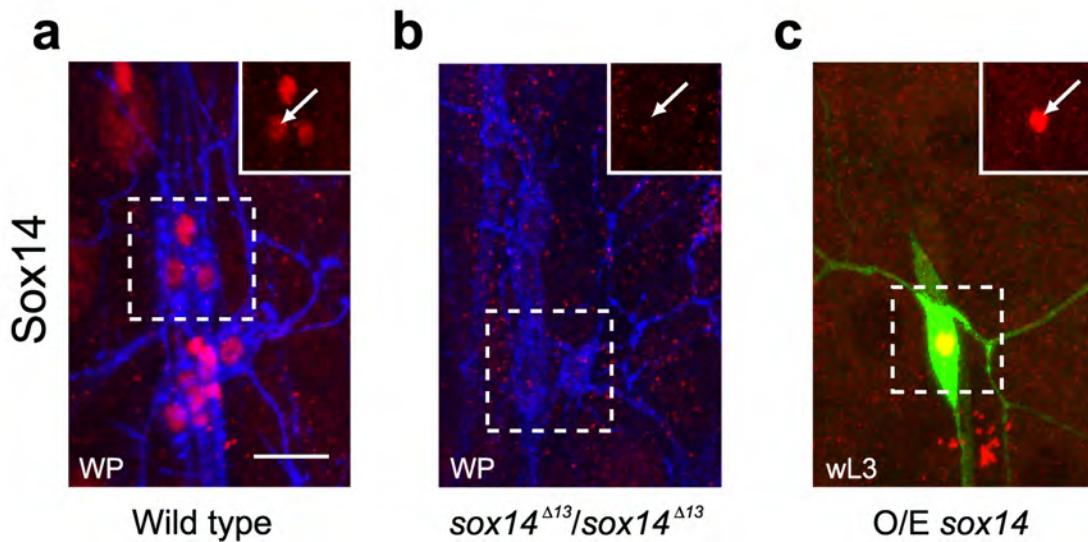


Supplementary Figure 1. Pruning defects of the *mical* and *sox14* mutant ddaC neurons in time course studies. Snapshots and quantification of wt and mutant ddaC dendrite pruning from 0 to 24 h APF. **(a)** The extensive dendritic field of the wt ddaC at the WP stage is gradually removed in the next 18 h. By 8 h APF the majority of dendrites are severed from the soma. By 18 h APF no dendrites or fragments remain, only the soma (red arrowhead) and the axon can be recognized. Overexpression of EcR^{DN} results in attachment of dendrites to the soma even at 24 h APF. Please note the reduced morphology of dendrite branching in the EcR^{DN} WP ddaCs. *usp* RNAi, similar to EcR^{DN}, blocks ecdysone signaling and causes defects in pruning. In *sox14* and *mical* mutants, the severing defects are evident, compared to those in wt. Red arrowheads point to ddaC soma. **(b)** Quantification of the number of the 1° and 2° dendrites attached to the soma of wild type and mutant ddaC neurons during the first 24 h of metamorphosis. In each group at least 8 neurons were quantified. Error bars represent standard deviation. Scale bar is 50 μ m.

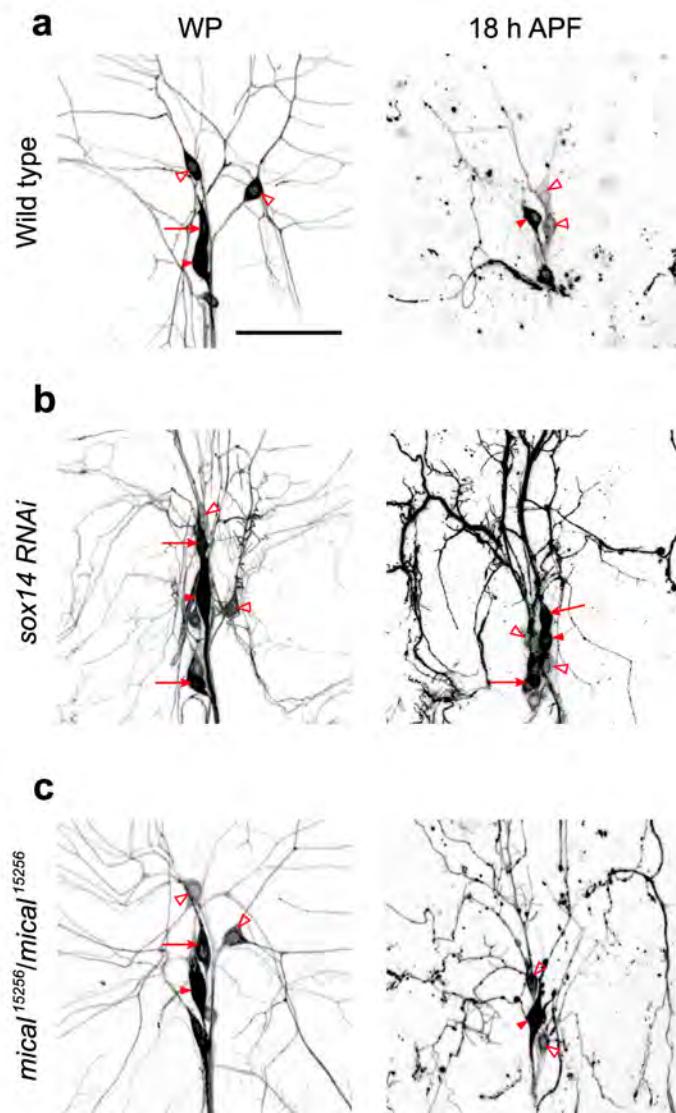


Supplementary Figure 2. Dendrite morphology in *sox14*^{Δ¹³} and *mical*^{¹⁵²⁵⁶} mutant

wL3 larvae appears to be normal. (a-b) Live confocal images of the wL3 larvae with ddaC neurons labeled by the *ppk-Gal4* driven expression of mCD8-GFP. The morphology of *sox14*^{Δ¹³} mutant ddaCs (b) is similar to that of wt ddaCs (a), based on the quantification of dendrite termini (e). (c-d) Live images of the wL3 larvae with ddaC neurons labeled by *ppk-EGFP*. The morphology of ddaCs in *mical*^{¹⁵²⁵⁶} mutant (d) is indistinguishable from that of wt ddaCs (c), based on the quantification of dendrite termini (e). (e) Number of identifiable dendrite termini of the wL3 larvae for each genotype from each individual ddaC is shown. The number of samples (*n*) in the group is above each bar. Error bar represents standard deviation. Scale bar, 50 μ m.

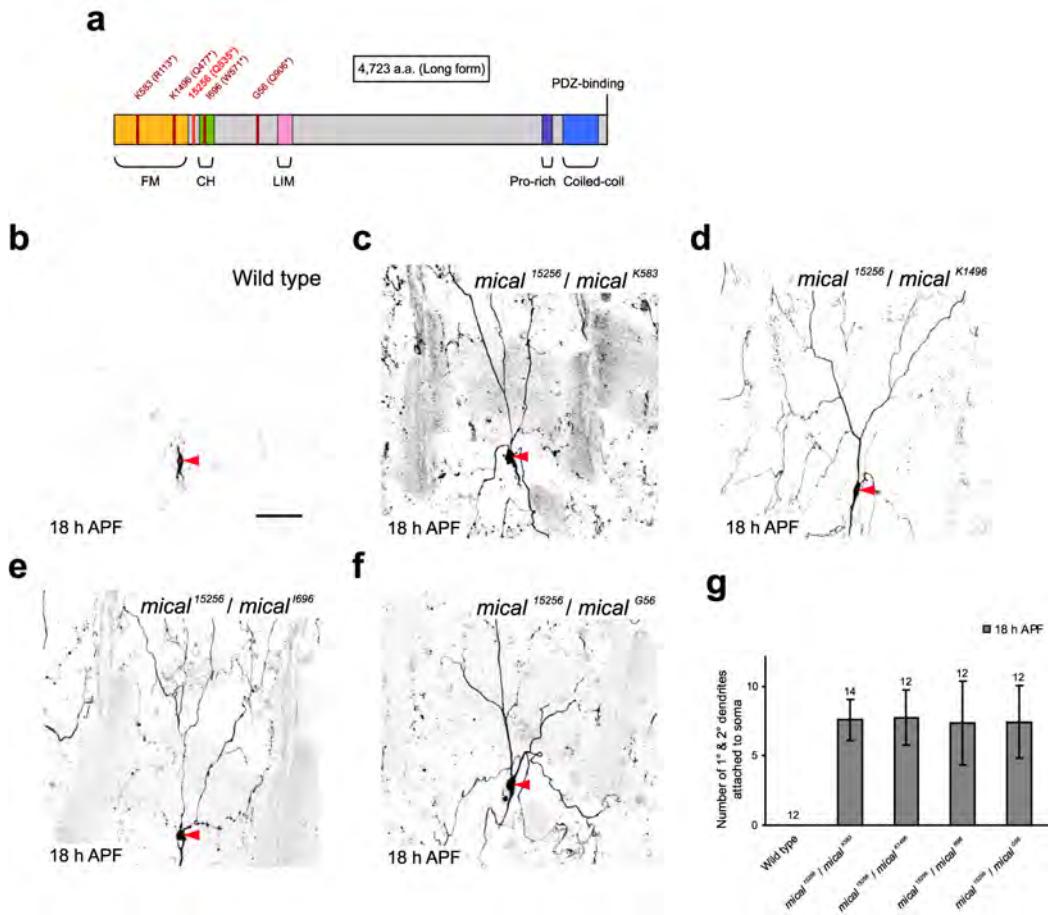


Supplementary Figure 3. The specificity of Sox14 antibody. (a) Sox14 is expressed in the nuclei of all dorsal da neurons at the WP stage. (b) Sox14 immunoreactivity is absent in *sox14*^{Δ13} mutants. (c) Overexpression of *sox14* by the *ppk-gal4* driver causes elevated levels of Sox14 in ddaCs at the wL3 stage when Sox14 is normally not expressed. Coexpression of mCD8-GFP is indicated in green. HRP staining in blue labels all neurons. Insets show the anti-Sox14 staining (in red) in ddaCs from the corresponding dashed rectangles. Arrows point to the nuclei of ddaCs. Scale bar, 20 μ m.

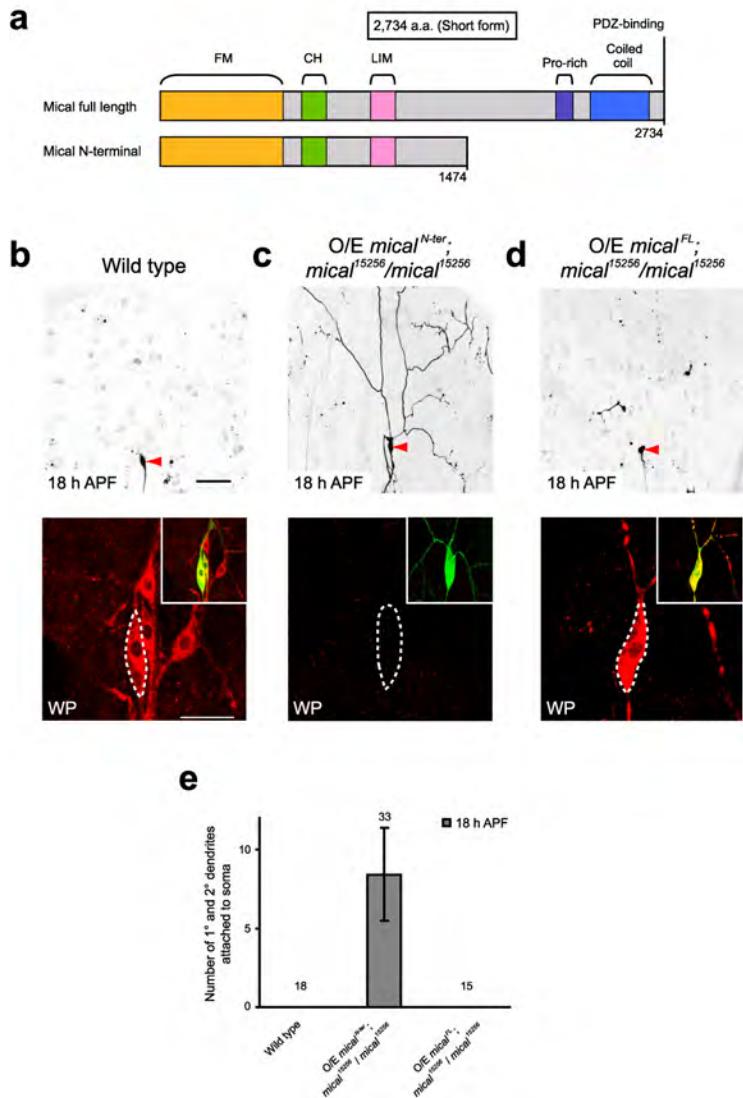


Supplementary Figure 4. Sox14 but not Mical is required for ddaF apoptosis

during metamorphosis. (a-c) Live images of dorsal da neurons labeled by $Gal4^{109(2)80}$ driven mCD8-GFP expression . (a) Among class I (open arrowheads), III (arrows) and IV (filled arrowheads) neurons that are present in the WP stage of wt pupae, only class I (ddAD/E) and IV (ddAC) neurons survive until 18 h APF. Please note that class I ddAD/E neurons show slightly lower GFP signals. (b) From the *sox14* RNAi da neurons at the WP stage, not only class I and IV, but the majority of class III neurons ddaA/F (b, 18 h APF - arrows) also survived until 18 h APF. (c) In *mical*¹⁵²⁵⁶ mutants, class I, III and IV neurons present at the WP stage (c). Similar to those in wt, only class I and IV neurons survive in *mical* mutant until 18 h APF (c). Scale bar, 50 μ m.

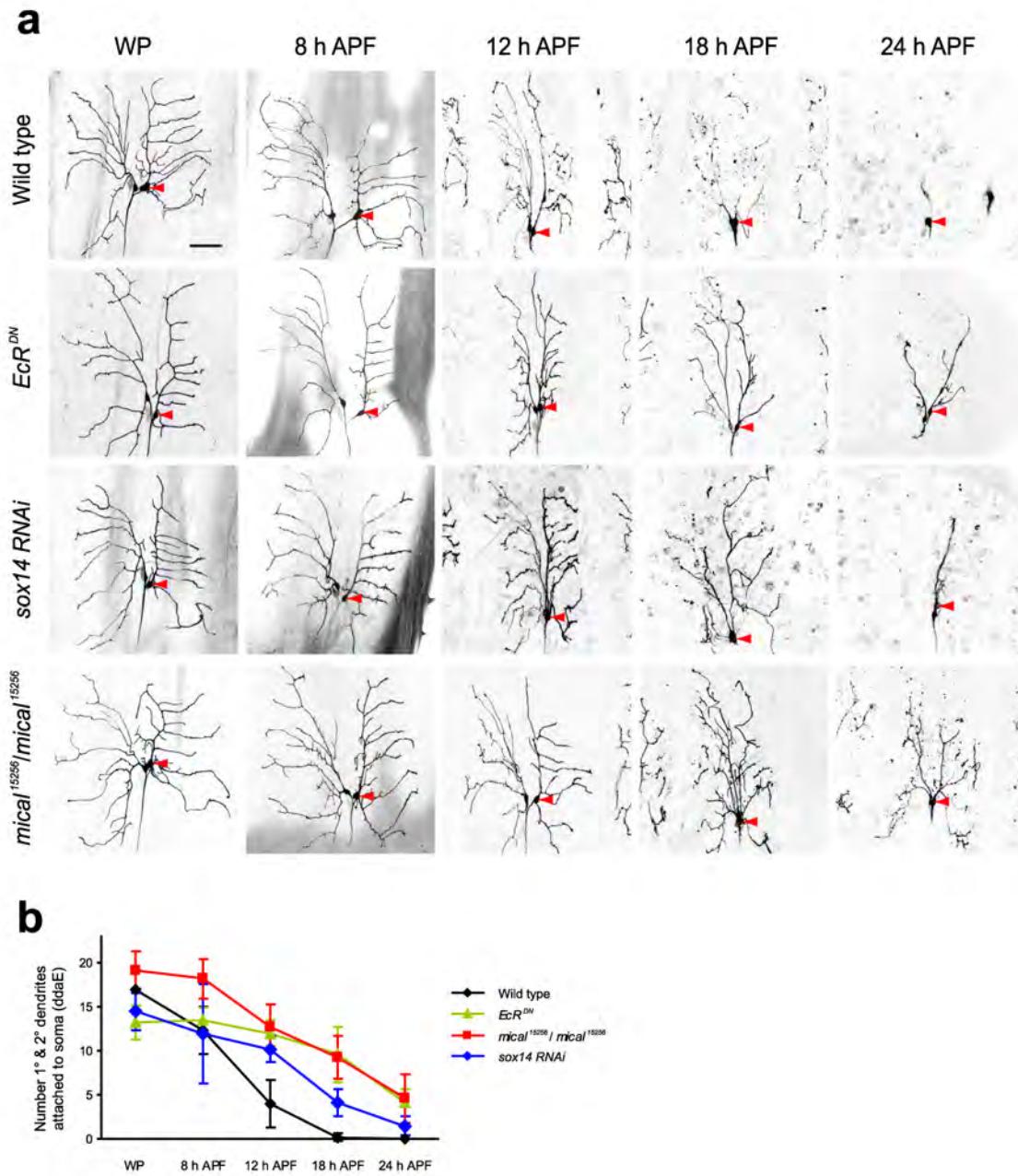


Supplementary Figure 5. Dendrite pruning defects in various *mical* mutants. (a) A diagram showing the known domains of the long isoform Mical protein and the mutation sites for previously identified *mical* alleles (brown bars) and *mical*¹⁵²⁵⁶ that we identified from this study (red bar). (b-f) Live confocal images of ddaCs from wt and *mical* mutant pupae at 18 h APF. While in wt (a) dendrite pruning is complete, in various *mical* allelic combinations (c-f) proximal dendrites are still attached to the soma of ddaCs at 18 h APF. Red arrowheads point to soma of ddaCs. (g) Quantitative analysis of the number of 1° and 2° dendrites attached to the soma of wild type and *mical* mutant ddaCs at 18 h APF. Different *mical* allelic combinations show similar strong phenotype in dendrite pruning. The number of samples (*n*) in the group is above each bar. Error bars represent standard deviation. Scale bar, 50 μm.



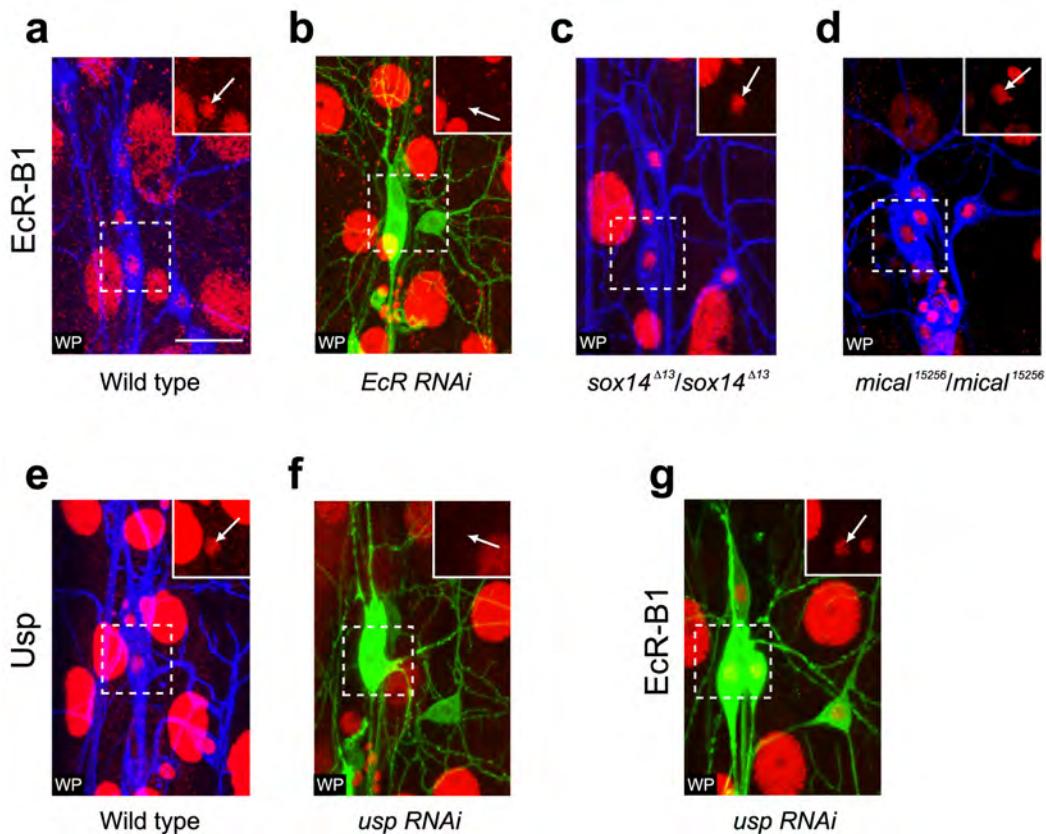
Supplementary Figure 6. Overexpression of full-length Mical but not N-terminal Mical rescues the defects in *mical* mutant. (a) The short isoform (2734 amino acids), full-length Mical (Mical^{FL}), was used to generate the rescue construct. The truncated Mical construct ($\text{Mical}^{\text{N-ter}}$), which contains the first 1474 amino acids of the short isoform Mical, was used as a control. (b-d) Live images of wt (b) and mical^{l15256} ddaCs overexpressing Mical $^{\text{N-ter}}$ (c) or Mical $^{\text{FL}}$ (d) by the *ppk-Gal4* driver and marked by mCD8-GFP expression. Red arrowheads point to ddaC soma. (b-d) WP fillets stained with anti-Mical antibody (red) in wt (b) and mical^{l15256} pupae overexpressing Mical $^{\text{N-ter}}$ (c) or Mical $^{\text{FL}}$ (d) by the *ppk-Gal4* driver. ddaCs, identified by GFP expression, are

marked by the dashed lines. Red channel is separated below for better visualization. (e) Quantitative analysis of the number of 1° and 2° dendrites attached to the soma of wt and *mical* mutant ddaCs with different rescue constructs at 18 h APF. The number of samples (*n*) in the group is above each bar. Error bar represents standard deviation. Scale bars in b are 50 μ m and 20 μ m.

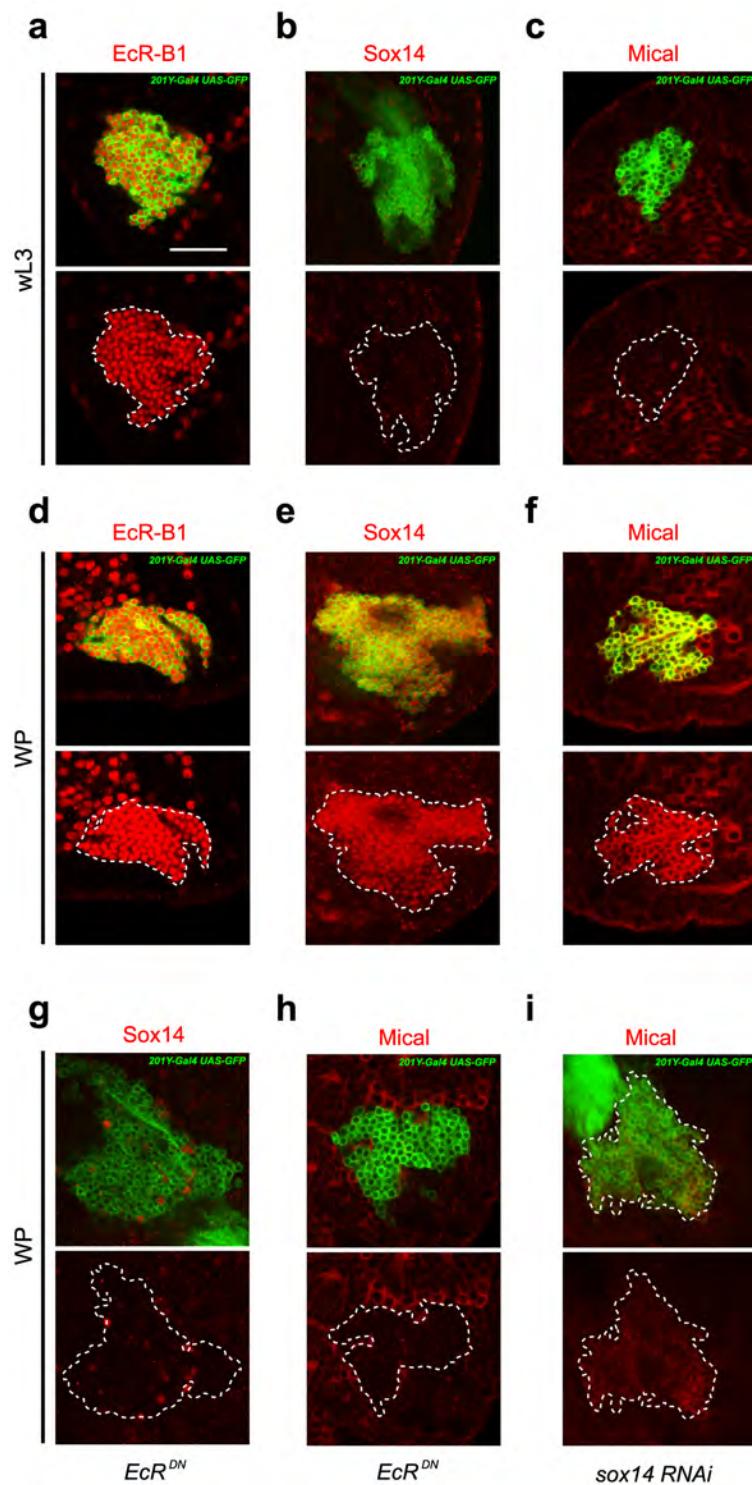


Supplementary Figure 7. Sox14 and Mical regulates dendrite pruning in class I neurons ddAD/E. (a) Live confocal images of class I neurons were taken at various time points in the early pupae using the expression of mCD8-GFP by the *Gal4²⁻²¹* driver. Dorsal class I neurons (ddAD and ddaE) sever their dendrites at both distal and proximal sites slightly later than ddaCs. At 12 h APF there are still proximal dendrites attached to the wt soma, but by 18 h APF the majority of them are severed and

degraded. Red arrowheads point to the class I ddaE soma. EcR^{DN} , *sox14* RNAi, or *mical* mutants showed similar pruning defects in class I neurons. **(b)** Quantification of the number of the 1° and 2° dendrites attached to the soma of wild type and mutant ddaE neurons during the first 24 h of metamorphosis. In each group at least 8 neurons were quantified. Error bars represent standard deviation. Scale bar, 50 μ m.

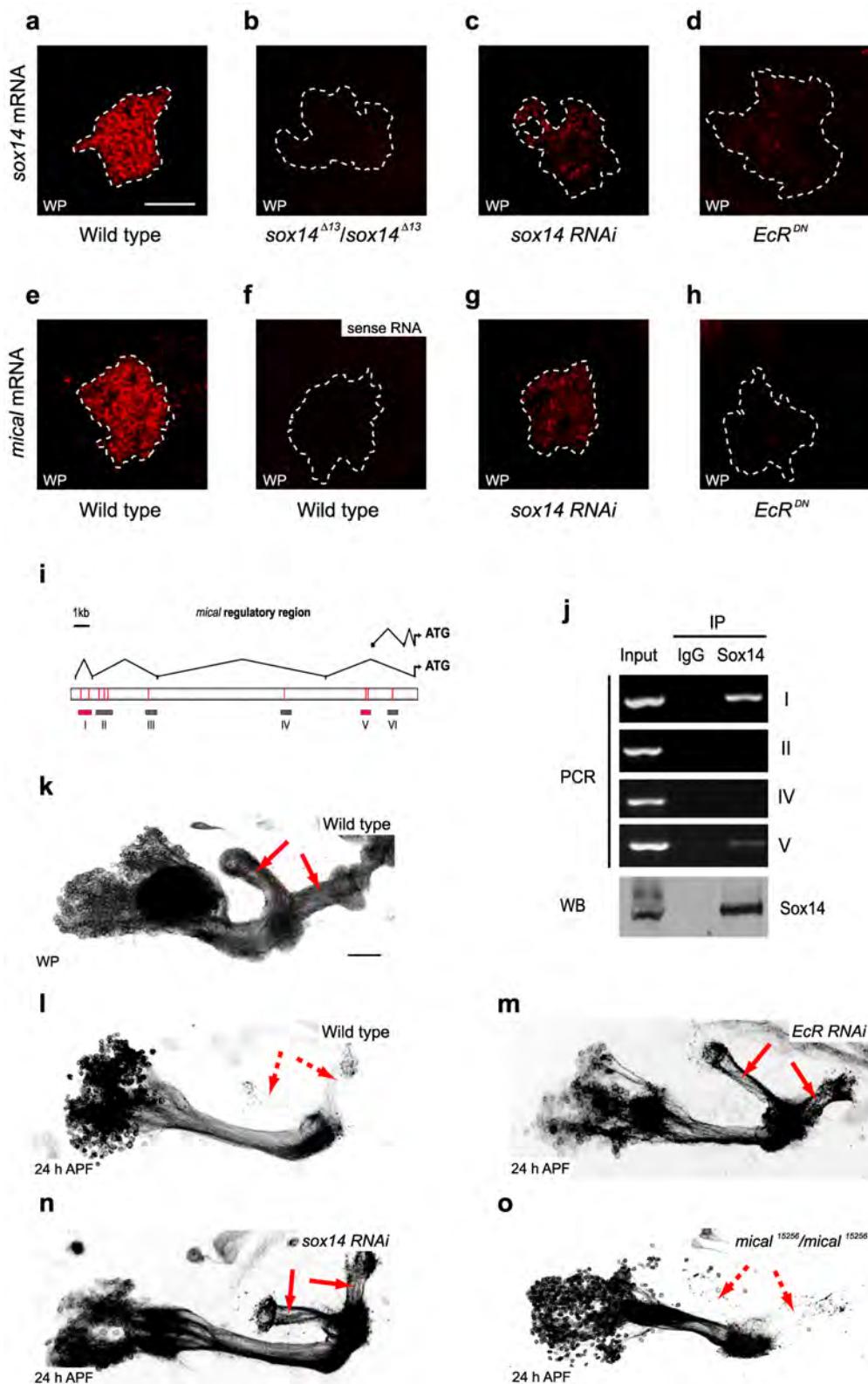


Supplementary Figure 8. EcR-B1 levels are not affected in *sox14^{Δ13}* or *mical¹⁵²⁵⁶* mutants. (a-d) EcR-B1 expression at the WP stage in wt dorsal da neurons (a) is absent from all da neurons by *EcR* RNAi using the *Gal4¹⁰⁹⁽²⁾⁸⁰* driver (b). EcR-B1 expression is unaffected in *sox14^{Δ13}* (c) and *mical¹⁵²⁵⁶* (d) mutants. Usp is present in all dorsal da neurons (e), but absent in those *usp* RNAi da neurons driven by the *Gal4¹⁰⁹⁽²⁾⁸⁰* driver, at the WP stage (f). *usp* RNAi does not affect EcR-B1 levels (g). HRP staining in blue labels all neurons. mCD8-GFP in green. Insets show the red channel of the corresponding dashed rectangles to emphasize the antibody staining for EcR-B1 (a-d, g) or Usp (e,f) in ddaCs. Arrows point to the nuclei of ddaCs. Scale bar, 20 μm.



Supplementary Figure 9. Mical expression depends on EcR-B1 and Sox14 in MB γ neurons. Images are single confocal sections of GFP-labeled (green) MB γ neurons from the wL3 larvae (a-c) and WP (d-i). Soma of MB γ neurons are marked by dashed lines on the red channel based on the boundary of GFP expression. In the wL3 larvae,

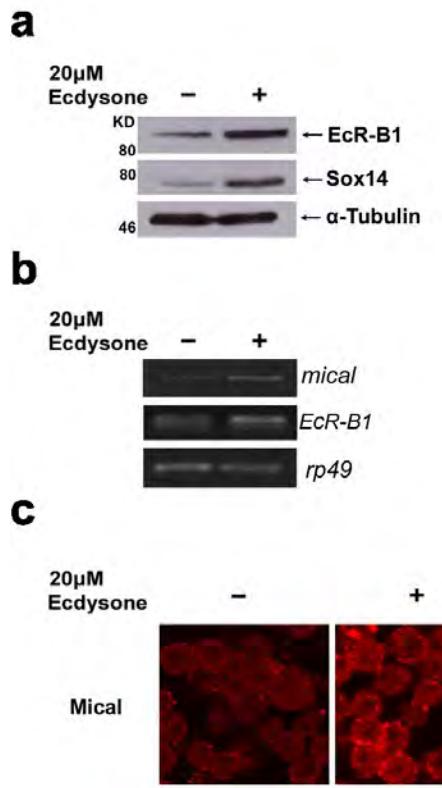
only EcR-B1 is present (**a**), while Sox14 (**b**) or Mical (**c**) expression is undetectable or very low at this stage. In the WP, EcR-B1 expression is maintained at the high levels (**d**); Sox14 (**e**) and Mical (**f**) expression is drastically upregulated. Blocking ecdysone signaling by EcR^{DN} expression in MB γ neurons prevents the expression of Sox14 (**g**) and Mical (**h**). (**i**) The attenuation of Sox14 expression by *sox14* RNAi also reduces the levels of Mical in MB γ neurons. Scale bar, 50 μ m.



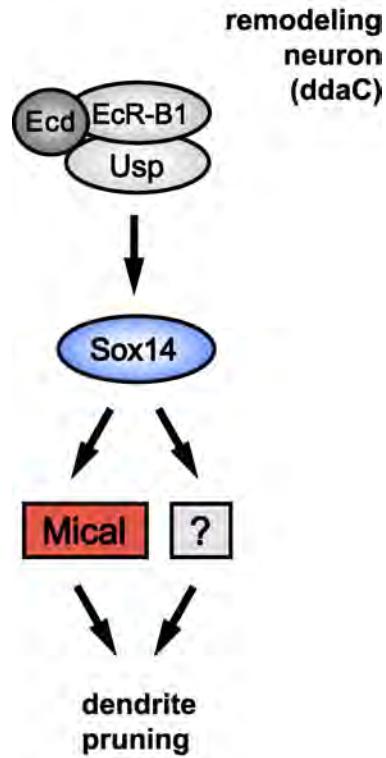
Supplementary Figure 10. *mical* is a direct transcriptional target of Sox14. (a-h)

Single confocal images of fluorescent in situ labeled MB γ neurons from WP brains.

sox14 mRNA is labeled in wild-type (**a**), *sox14* zygotic mutant (**b**), *sox14* RNAi (**c**) and *EcR*^{DN} (**d**) MB γ neurons. *mical* mRNA is labeled in wild-type (**e**), *sox14* RNAi (**g**) and *EcR*^{DN} (**h**), respectively. (**f**) A sense probe of *mical* shows no signal, serving as a control. The area of MB γ neurons is marked by dashed lines determined by the expression of *201Y-Gal4* driven mCD8-GFP. (**i-j**) Sox14 directly binds to the *mical* promoter regions. (**i**) Schematic representation of the regulatory region and possible transcripts of the *mical* gene. The arrows indicate the start codon of the *mical* ORFs. Red bars indicate the potential Sox14-binding sites. (**j**) ChIP analysis of Sox14 on binding to the *mical* regulatory region. In a ChIP experiment, I and V fragments are associated with Sox14 while the rest of the fragments display no affinity with Sox14. The specific immunoprecipitation of Sox14 was also confirmed by the western blotting with the anti-Sox14 antibody (bottom panel). (**k-o**) Sox14 but not Mical is required for axon pruning in MB γ neurons. MB γ neurons of wt and mutant pupae are labeled by the *201Y-Gal4* driven mCD8-GFP expression. (**k**) At the WP stage, MB γ neurons project a dorsal and a medial axonal branch (arrows) which are pruned (dashed arrows) at 24 h APF (**l**). *EcR* RNAi (**m**) or *sox14* RNAi (**n**) blocks the pruning of axons (arrows), while *mical* mutants show normal axon pruning (**o**, dashed arrows). For each RNA probe, fluorescence in situ experiments were done in the same tube and images were taken at the same gain and processed in parallel. Scale bars, 50 μ m.



Supplementary Figure 11. The expression of EcR-B1, Sox14 and Mical in S2 cells is upregulated by the treatment with ecdysone. (a) Immunoblotting analysis of EcR-B1 and Sox14 expression after ecdysone stimulation. S2 cells were treated with ecdysone or vehicle methanol for 8 h, lysed and subjected to immunoblotting with anti EcR-B1, anti-Sox14 and anti- α -Tubulin. (b) RT-PCR analysis of *mical* and EcR expression upon ecdysone treatment. rp49 was used as internal control. (c) Immunofluorescence of Mical in ecdysone or vehicle treated S2 cells. Upon treatment with ecdysone, S2 cells exhibits elevated levels of Mical expression with punctate staining near the plasma membrane.



Supplementary Figure 12. A novel genetic pathway governs severing of dendrites during pruning of ddaC neurons. Upon binding Ecdysone (Ecd), the EcR/Usp nuclear receptor heterodimer induces the expression of Sox14 transcription factor. Sox14 acts as a key regulator of ddaC dendrite pruning and activates the transcription of its downstream target genes including *mical*. Mical is an important target of Sox14 that acts together with other yet-to-be-identified targets to promote dendrite severing in remodeling ddaC neurons.

Supplementary Results and Discussion

Pruning defects of the *sox14* and *mical* remodeling neurons in time course studies

To compare *sox14* and *mical* phenotypes with those observed in *EcR* and *usp* mutants at various time points during early pupal development, we expressed the dominant negative form of EcR-B1 (*EcR*^{DN})¹ or conducted *usp* RNAi experiments to efficiently block ecdysone signaling in ddaC neurons (Fig. S8f, compared to Fig. S8e in wt). Overexpression of *EcR*^{DN} led to formation of simplified dendritic arbors in ddaC neurons², compared to those in the wt ddaC neurons (Fig. S1a). However, the morphology of larval dendritic arbors in either *sox14* or *mical* mutant ddaC neurons was unaffected at the wL3 stage, as determined by the number of dendrite termini (Fig. S2b, d; compared to those in wt, Fig. S2a,c). The majority of wt ddaC neurons exhibited severed proximal dendrites by 8 h APF (open arrowheads; Fig. S1). In contrast, both *sox14* and *mical* mutant ddaCs failed to sever proximal dendrites by 24 h APF, similar to dendrites observed in *EcR*^{DN} or *usp* RNAi ddaC neurons (Fig. S1).

We next asked whether Sox14 and Mical are required for severing of the class I da neurons ddaD/E. Similar to *EcR*^{DN} or *usp* RNAi mutant neurons, *sox14* RNAi and *mical* mutant ddaD/E neurons failed to prune their dendritic processes by 18 h APF (Fig. S7). Thus, Sox14 and Mical mediates dendrite pruning in both class I and class IV da sensory neurons during early metamorphosis.

mical is a direct transcriptional target of Sox14

Neuronal remodeling of MB γ neurons shares some of the molecular mechanisms employed in ddaC neuron remodeling. MB γ neurons can be specifically labeled by the expression of mCD8-GFP with a MB-specific driver *201Y-Gal4*. In MB γ neurons, EcR-B1 was undetectable at the eL3 stage (data not shown) and was

upregulated at the wL3 and WP stages (**Fig. S9a, d**), whereas Sox14 and Mical expression were either undetectable or very low in all brain neurons at the eL3 and wL3 stages (Sox14: **Fig. S9b**; Mical: **Fig. S9c**). Sox14 and Mical proteins accumulated to high levels in MB γ neurons at the WP and 5 h APF stages, in contrast to their low level expression in the majority of neighboring neurons (**Fig. S9e, f**, and data not shown). Therefore, the temporal regulation of *EcR-B1*, *sox14* and *mical* expression in MB γ neurons is highly similar to that we observe in da sensory neurons. The upregulation of Sox14 and Mical was not confined to MB γ neurons but also occurred in the ventral nerve cord, where a number of neurons expressed elevated levels of EcR-B1 (data not shown). Ecdysone signaling is also required for Sox14 and Mical expression in MB γ neurons, since overexpression of EcR^{DN} eliminated the expression of both Sox14 (**Fig. S9g**) and Mical (**Fig. S9h**). *sox14* RNAi caused a decrease in Mical levels in MB γ neurons (**Fig. S9i**).

To further examine whether ecdysone signaling may activate the expression of Sox14 and Mical at the transcriptional level, we carried out fluorescent RNA in situ hybridization assays in the brain, where the densely packed group of MB γ neurons offers a favorable context for detecting *sox14* and *mical* RNA. High levels of *sox14* and *mical* transcripts could be detected in MB γ neurons at the WP stage (**Fig. S10a, e**) but not at the wL3 stage (data not shown), similar to their protein expression.

Overexpression of EcR^{DN} abolished the presence of both *sox14* and *mical* mRNAs at the WP stage (**Fig. S10d, S10h**). Consistent with this observation, genome-wide microarray analyses of ecdysone-responsive genes in salivary glands or MB γ neurons indicated that *sox14* mRNA levels are potentially induced by ecdysone signaling³. Interestingly, *sox14* RNAi, which strongly reduced *sox14* mRNA levels (**Fig. S10c**), caused downregulation of *mical* RNA in MB γ neurons (**Fig. S10g**). This suggests that

Sox14 might regulate *mical* transcription during early metamorphosis and prompted us to investigate whether Sox14 directly binds to the regulatory region of the *mical* gene.

Sox group proteins activate gene transcription via the bending of DNA to form enhanceosomes. Sox-mediated transcription is achieved by its binding to a consensus DNA motif (A/G)ACAA(A/T)(G/A). We identified at least ten potential Sox14 binding sites covered by the I-VI fragments in the regulatory region of the *mical* gene (**Fig. S10i**). To examine whether Sox14 regulates *mical* transcription via its direct binding to these sites, we performed chromatin immunoprecipitation (ChIP) assays using extracts of S2 cells that were treated with ecdysone. Upon treatment, EcR-B1, Sox14 and Mical were upregulated, as judged by western blot, RT-PCR and immunofluorescence analysis (**Fig. S11**). Sox14 was immunoprecipitated by the Sox14 antibody but not a control antibody (**Fig. S10j**, bottom panel). Interestingly, the ChIP analysis indicated that the sites in the I and V fragments, but not the other predicted sites of the *mical* promoter region, associated with Sox14 (**Fig. S10j**; for the III and VI fragments, data not shown). Thus, Sox14 may activate *mical* gene expression in vivo via direct binding to the *mical* promoter region.

We further assessed the effects of *sox14* and *mical* in axon pruning of MB γ neurons. In wt MB γ neurons, the medial and dorsal axonal branches that formed during the larval stages (n=13; **Fig. S10k**) were pruned by 24 h APF (n=15; **Fig. S10l**). This axon pruning was inhibited in *EcR* RNAi γ neurons, resulting in the persistence of larval branches at 24 h APF (n=8; **Fig. S10m**). *sox14* RNAi mutants exhibited severe axon pruning defects in MB γ neurons at 24 h APF as well (n=13 ; **Fig. S10n**). Interestingly, the axon branches were pruned normally by 24 h APF in homozygous *mical* mutant (n=18; **Fig. S10o**) and hemizygous *mical*¹⁵²⁵⁶/*Df(3R) swp2^{MICAL}* fly brains (n=20, data not shown).

Therefore, the expression of Mical is regulated by EcR-B1 and Sox14 in remodeling neurons of both da sensory neurons and MB γ neurons, presumably via transcriptional regulation. Sox14 affects neuronal remodeling in both types of remodeling neurons whereas Mical is only required for dendrite pruning in ddaC neurons.

Conservation of Sox14 and Mical in mammals

Drosophila sox14 belongs to the *sox* group C genes and has three mammalian homologs, *sox4*, *sox11* and *sox12*⁴. All these genes are expressed at high levels in neuronal tissues⁴ and are necessary for neuronal maturation⁵. Interestingly, similar to fly *sox14*, mammalian *sox4* is also a steroid-regulated gene in cancer cells⁶. The treatment with progestin steroid hormone upregulates the expression of Sox4, leading to a marked increase in Sox-mediated transcriptional activity⁶. Sox-mediated transcription is achieved by its binding to a consensus DNA sequence, DNA bending in the target gene loci, and thereby the formation of enhanceosomes^{7,8}.

Similar to *sox* group genes, *Drosophila mical* is also highly conserved and there are three homologues (*mical-1/2/3*) in mammals. It was reported that the C-terminal portion of Mical interacts with the C2 domains in the cytoplasmic region of Plexin A receptor to control Semaphorin 1a-mediated axonal repulsion during axon guidance. During repulsive axon guidance, Mical likely acts as a downstream effector of Semaphorin/Plexin signaling to rearrange the axonal cytoskeleton components destined for degradation or dismantlement during repulsive guidance. Currently, it is unknown whether dendrite pruning requires Plexin/Semaphorin-mediated signaling in *Drosophila* ddaC neurons. Interestingly, the interaction between Mical and Plexin A appears to be conserved, as the Plexin-interacting domains of mammalian Mical interact with the C2 domains of mammalian Plexins. Mammalian Micals are enriched in the postnatal and

adult nervous systems including hippocampus and cerebellum, where neuronal remodeling and synapse plasticity occurs extensively and continuously⁹. It was reported that Semaphorins and their Plexin receptors can also function as retraction inducers to trigger stereotyped axon pruning of postnatal hippocampal neurons in the mammalian brain¹⁰. Therefore, it will be interesting to investigate whether Mical homologues play similar roles in neuronal remodeling/plasticity in mammals.

Supplementary Materials and Methods

Fluorescent mRNA *in situ* hybridization.

White pupal brains were dissected and fixed in 4% formaldehyde for 15 minutes, followed by five washes using PBS +0.1% Tween 20. Samples were prehybridized in the hybridization solution (50% formamide, 5xSSC, 100 mg/ml boiled salmon sperm DNA, 50 mg/ml heparin, 0.1% Tween 20) at 55°C. The DIG labeled *sox14* or *mical* probes were added for hybridization overnight. After the samples were washed and blocked, primary antibodies (HRP-conjugated anti-Digoxigenin-POD IgG (Roche) (1:1000 dilution) and rabbit anti-GFP (Molecular Probes) were incubated, followed by the secondary antibody against GFP (FITC-conjugated goat anti-rabbit 1:200 (Molecular Probes). Subsequent amplification with the Cy3 tyramide reagent (Perkin Elmer Life Sciences) was performed to detect mRNA. The following primers were used for in vitro transcription:

5'-CCGCTTCGAAACGATTG-3' and 5'-TTAATACGACTCACTA TAGGGAGAT CGTGTGC GGCGTAGTT-3' (*sox14* anti-sense probe); 5'-TGAGC CGCCAACAC-3' and 5'-TTAATACGACTCACTATAAGGGAGAAGT GCGTC TCG TCCT-3' (*mical* anti-sense probe); 5'-TTAATACGACTCACTATAAGGGAGAATGA GCCGCCAAC AC-3' and 5'-AGT GCGTCTCGTCCT-3' (*mical* sense probe)

S2 cell culture, Ecdysone treatment and Immunoblotting

S2 cells were grown in the Express Five serum free medium (SFM) at 25°C as previously described¹¹. Where necessary, 20μM 20-OH ecdysone was added and incubated for 8h before cells were harvested and subjected to immunoblotting or chromatin immunoprecipitation (ChIP) assay.

For immunoblotting, protein extracts were prepared by lysing the S2 cells in RIPA buffer (50mM Tris-HCl, pH8.0, 150mM NaCl, 10mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and boiled in the SDS sample buffer for 5 min. Proteins samples were resolved in 10% SDS-PAGE gels, transferred onto nitrocellulose membranes and blocked for 1h in 10% skim milk. Membranes were incubated with primary antibody anti-EcR-B1 AD4.4 (DSHB, 1:250 dilution), anti Sox14 (1:500 dilution) or anti α -Tubulin DM1A (Sigma-Aldrich, 1:5000 dilution) overnight at 4°C and then further incubated with HRP conjugated secondary antibody for 1h. Signals were detected using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology).

RT-PCR

S2 cells were seeded into 6 cm dishes and treated with or without 20 μM 20-OH ecdysone for 8 h. Total RNA was isolated with RNeasy plus mini kit (Qiagen) and reverse-transcribed with oligo-dT primers and High Expand reverse transcriptase (Roche) according to the manufacturer's instructions. To analyze the transcriptional regulation of *mical*, primers were designed against the intronic region common to *mical* or *EcR* pre-mRNA forms: For *mical*: 5'GGGACATCAGTGCCTATAAC3'; 5'ATCTCAGCAACGTGGCACCC3'; for *EcR*: 5'TCTCAGGCGTATAATGGTG3'; 5'CAGGCCTAACGTTGATGGG3'. *rp49* was used as an internal control and the

primers for RT-PCR were 5'CTGCCACCAGGATTCAAG3' and 5'CGATCTGCCGCACAGTAAAC3'.

ChIP assay

ChIP assay was performed by using Upstate Biotechnology Chromatin Immunoprecipitation kit with some modifications. Briefly, 2×10^7 Ecdysone stimulated cells were cross-linked by 1% formaldehyde for 10 min at room temperature. Fixed cells were lysed with RIPA buffer supplemented with protease inhibitor (Roche). The sample was then equally divided into two parts and incubated with anti-Sox14 or a control anti-LacZ antibody respectively overnight at 4°C. For the PCR reaction, we searched potential Sox14 binding motifs among the *mical* promoter region upstream of *mical* coding region. We then designed the primers 300-500bp flanking these predicted binding regions as below. 1μL of 30μL DNA extraction was used for PCR reaction. I: 5'GTGCGGT TATATAATGCGGATATAG3', 5'AGCAG AGGAAGAGAAGAAGAATAAG3'; II: 5'TTCTTTACTTGAGATTGGTATATG3', 5'GCGACTCTCTGGTAATTCCATAG3'; III: 5'AATTGAAAT CATCCAAA CAAATTGC3', 5'AGAAAACATTGTGTTGGGAAGGCG3'; IV: 5'CATCGGGT GGGCCCGTATCTTCAG3', 5'TGAG ATACGGCATCGTTCGAGCTG3'; V: 5'CTGCAAT TTCAACAATTTCGCTCG3', 5'AGATGACAAAGATAACAATGA GTCCG3'. VI: 5'TGCGTGACCCACCGCCTTTCTG3', 5'CGGCTTGTTTGCTCGGCCAGGG3'

Supplementary References

1. Cherbas, L., Hu, X., Zhimulev, I., Belyaeva, E. & Cherbas, P. EcR isoforms in Drosophila: testing tissue-specific requirements by targeted blockade and rescue. *Development* **130**, 271-84 (2003).

2. Ou, Y., Chwalla, B., Landgraf, M. & van Meyel, D.J. Identification of genes influencing dendrite morphogenesis in developing peripheral sensory and central motor neurons. *Neural Dev* **3**, 16 (2008).
3. Hoopfer, E.D., Penton, A., Watts, R.J. & Luo, L. Genomic analysis of Drosophila neuronal remodeling: a role for the RNA-binding protein Boule as a negative regulator of axon pruning. *J Neurosci* **28**, 6092-103 (2008).
4. Dy, P. et al. The three SoxC proteins--Sox4, Sox11 and Sox12--exhibit overlapping expression patterns and molecular properties. *Nucleic Acids Res* **36**, 3101-17 (2008).
5. Bergsland, M., Werme, M., Malewicz, M., Perlmann, T. & Muhr, J. The establishment of neuronal properties is controlled by Sox4 and Sox11. *Genes Dev* **20**, 3475-86 (2006).
6. Graham, J.D., Hunt, S.M., Tran, N. & Clarke, C.L. Regulation of the expression and activity by progestins of a member of the SOX gene family of transcriptional modulators. *J Mol Endocrinol* **22**, 295-304 (1999).
7. Liu, F., Chau, K.Y., Arlotta, P. & Ono, S.J. The HMG I proteins: dynamic roles in gene activation, development, and tumorigenesis. *Immunol Res* **24**, 13-29 (2001).
8. Koopman, P. SRY and DNA-bending proteins. in *Encyclopedia of Life Sciences* (John Wiley & Sons, Ltd: Chichester, 2001).
9. Pasterkamp, R.J. et al. MICAL flavoprotein monooxygenases: expression during neural development and following spinal cord injuries in the rat. *Mol Cell Neurosci* **31**, 52-69 (2006).
10. Bagri, A., Cheng, H.J., Yaron, A., Pleasure, S.J. & Tessier-Lavigne, M. Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. *Cell* **113**, 285-99 (2003).

11. Varghese, J. & Cohen, S.M. microRNA miR-14 acts to modulate a positive autoregulatory loop controlling steroid hormone signaling in *Drosophila*. *Genes Dev* **21**, 2277-82 (2007).