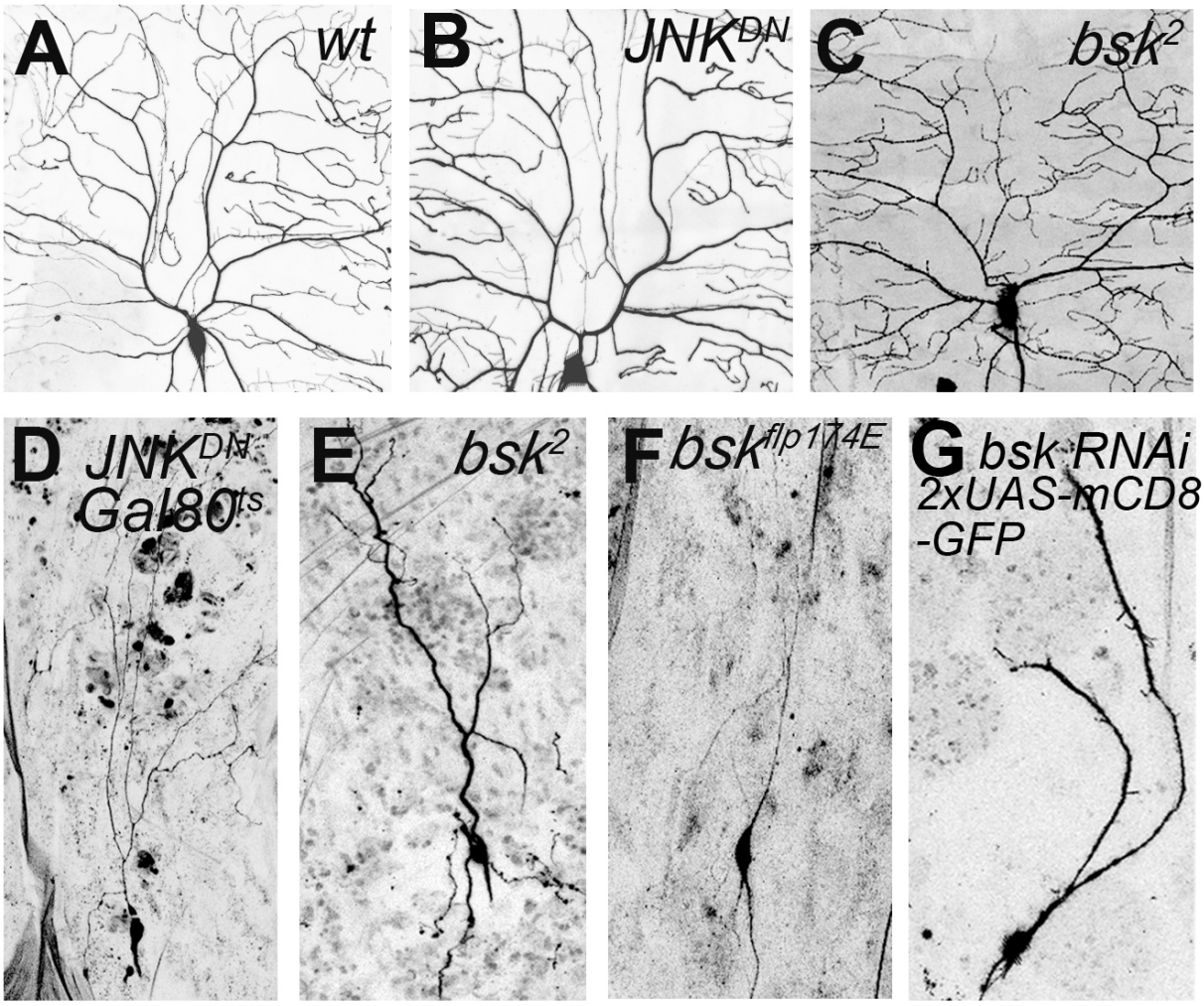
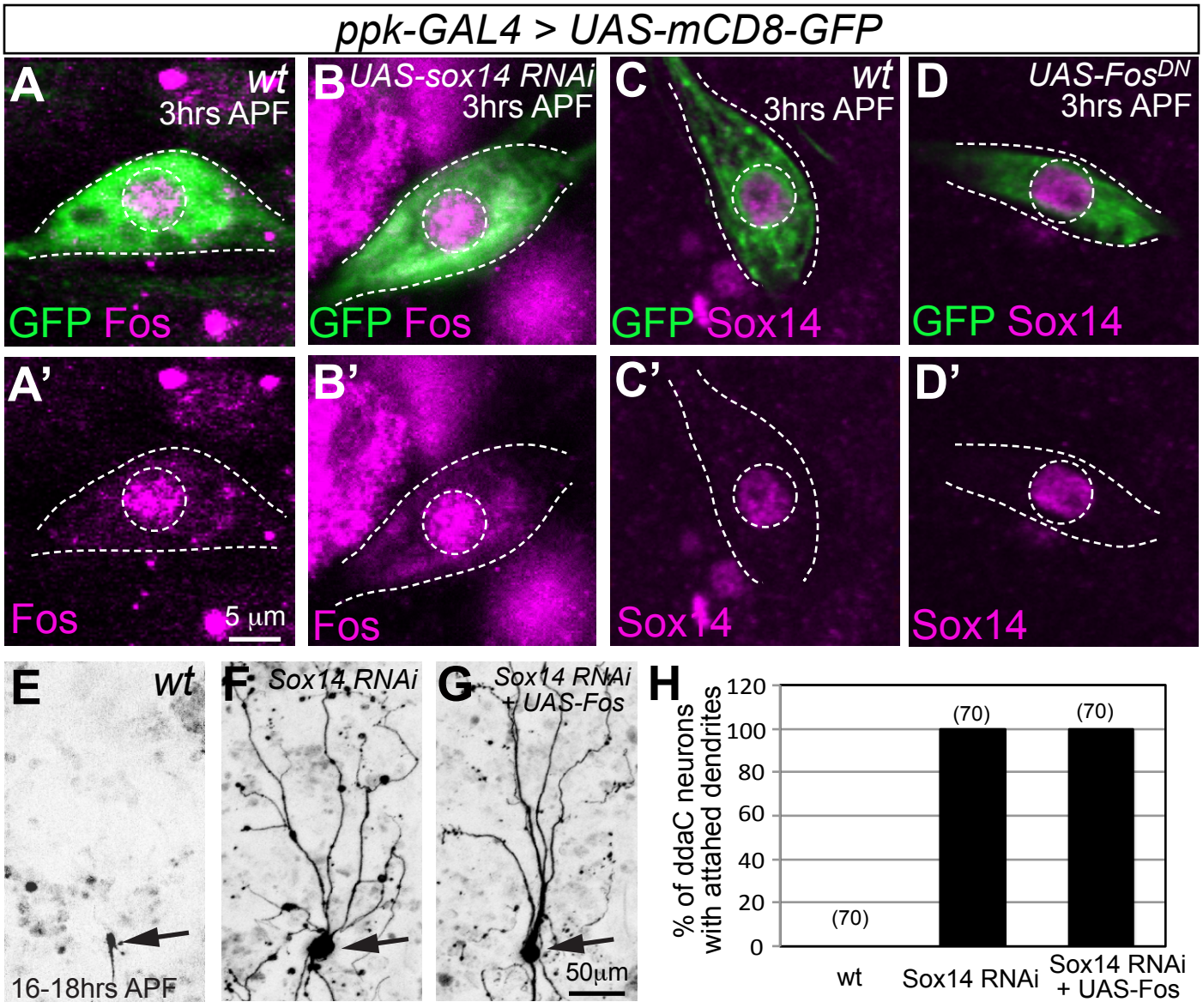


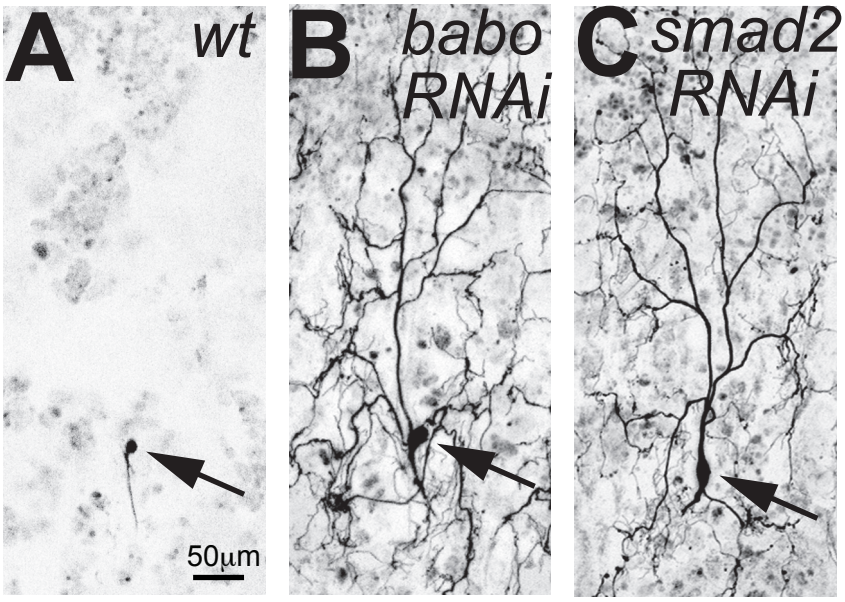

Supplementary Figure S1

**Figure S1.** Dendrite pruning defects resulting from the loss of JNK are unlikely due to dendrite morphogenesis defects in c4da neurons. (A) A wild type ddaC neuron at the 3rd instar larval stage. (B-C) No obvious dendrite morphogenesis defects are observed in ddaC neurons expressing *UAS-JNK$^{DN}$* (B) or mutant for *bsk$^{2}$* (C) at the 3rd instar larval stage. (D) Restricting the expression of *UAS-JNK$^{DN}$* to 1 day before puparium formation with *tub-GAL80$^{ts}$* still leads to dendrite severing defects at 11-13 hrs APF. Animals were shifted from 18°C to 29°C at 1 day before puparium formation to inactivate the GAL80$^{ts}$. (E-F) *bsk$^{2}$* (E) or *bsk$^{flp174E}$* (F) mutant ddaC clones retain unsevered dendrites at 16-18hrs APF. (G) Bsk RNAi knockdown at 29°C with 2 copies of *UAS-mCD8-GFP* still leads to dendrite severing defects in ddaC neurons at 11-13 hrs APF.



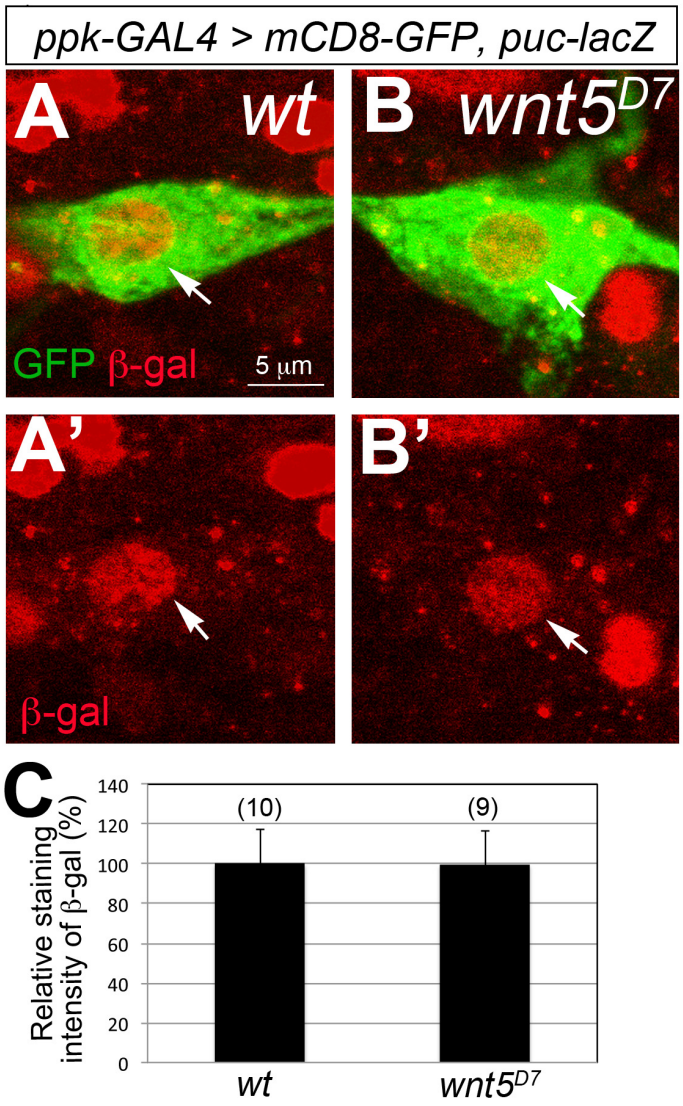

Supplementary Figure S2

**Figure S2**. dFos and Sox14 may function independently in regulating dendrite pruning of c4da neurons. (A-B') d-Fos staining in a wild type (A-A') and a Sox14 knockdown (B-B') ddaC neurons at 3 hrs APF. (C-D') Sox14 staining in a wild type (C-C') or *UAS-dFos$^{DN}$* expressing (D-D') ddaC neurons at 3 hrs APF. Dashed lines outline the soma of ddaC neurons and dashed circles outline the nuclei. (E) Dendrites are all pruned in a wild type ddaC neuron at 16-18hrs APF. (F) Knocking down Sox14 leads to retention of attached primary dendrites at 16-18hrs APF in a ddaC neuron. (G) Expressing *UAS-Fos* does not rescue the dendrite severing defects in a ddaC neurons resulting from Sox14 knockdown. (H) Quantifications of ddaC neurons with unsevered dendrites at 16-18hrs APF in wild type ddaC neurons or ddaCs expressing *UAS-Sox14 RNAi* alone or together with *UAS-Fos*.



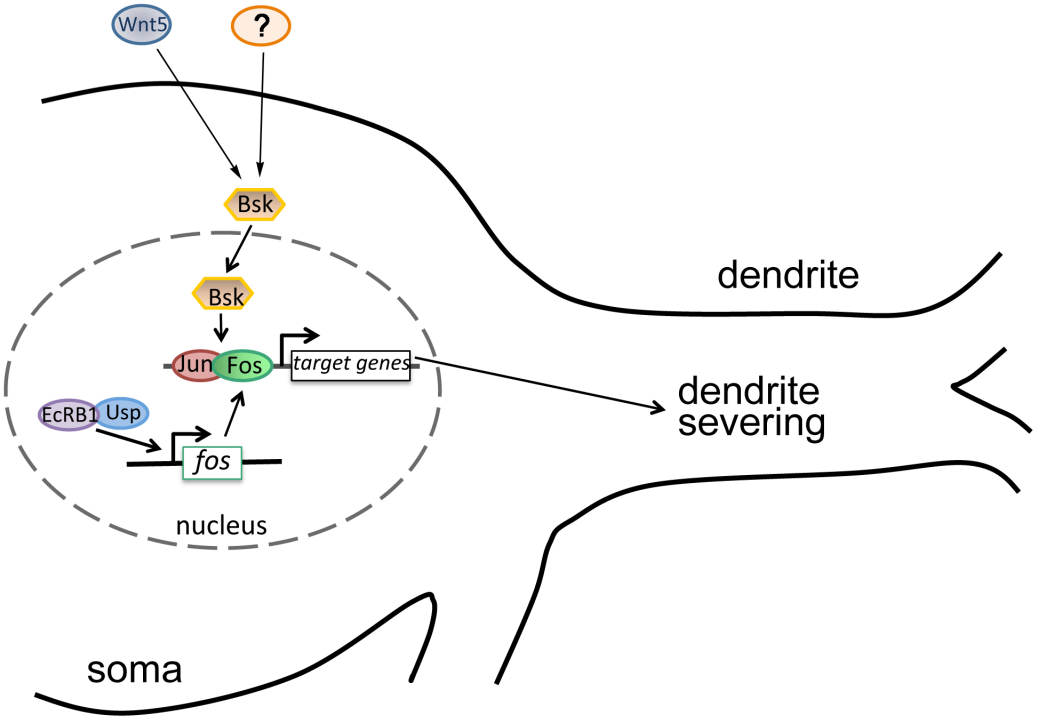

Supplementary Figure S3

**Figure S3.** Babo and dSmad2 are required for dendrite pruning. (A) A wild type ddaC neuron has no remaining dendrites at 16-18hrs APF. (B-C) Dendrites fail to be pruned in ddaC neurons at 16-18hrs APF when Babo (B) or dSmad2 are knocked down. Arrows point to the soma of ddaC neurons.




Supplementary Figure S4

**Figure S4.** *puc-lacZ* does not show obvious changes in *wnt5* mutants. (A-B) *puc-lacZ* expression in wild type (A-A') and *wnt5*$^{D7}$ hemizygous mutant (B-B') ddaC neurons at 0 hrs APF. (C) Quantifications of relative β-gal staining intensities in wild type and *wnt5*$^{D7}$ hemizygous mutant ddaC neurons. The staining intensities of d-Fos in the nuclei were measured with Adobe Photoshop. The mean staining intensity of β-gal in the wild type is set as 100%. Data are mean ± s.d. Sample sizes are indicated on top of each bar.




**Supplementary Figure S5**

**Figure S5**. A working model of coordinated regulation of dendrite pruning by JNK signaling and ecdysone signaling in c4da neurons. Wnt5 and other unknown signals possibly activate JNK (Bsk) in c4da neurons throughout development, whereas EcRB1 together with its binding partner Ultraspiracle (Usp) activates the expression of the JNK substrate d-Fos specifically at the early pupal stage. JNK then phosphorylates and activates the AP-1 complex composed of d-Jun and d-Fos, which in turn activates the expression of target genes that are required for dendrite pruning.

**Table S1.** RNAi knockdown or mutant phenotypic analyses of candidate JNK activators in dendrite pruning of c4da neurons

| **RNAi knockdown analyses** | | |
|---|---|---|
| genes | UAS-RNAi lines | dendrite pruning |
| *wengen* | BL #58994 | normal |
| *VEGFR* | VDRC #43461 | normal |
| | VDRC #13502 | normal |
| | VDRC #977 | normal |
| *EGFR* | VDRC #43267 | normal |
| | VDRC #43268 | normal |
| *babo* | VDRC #853 | defective |
| *dSmad2* | NIG #2262R-1 | defective |
| **Mutant analyses** | | |
| genes | mutants | dendrite pruning |
| *eiger* | $egr^{1}$ | normal |
| | $egr^{3}$ | normal |
| *traf6* | $Traf6^{ex1}$ | normal |
| *traf4* | $Traf4^{ex1}$ | normal |
| *wnt5* | $wnt5^{400}$ | defective |
| | $wnt5^{D7}$ | defective |