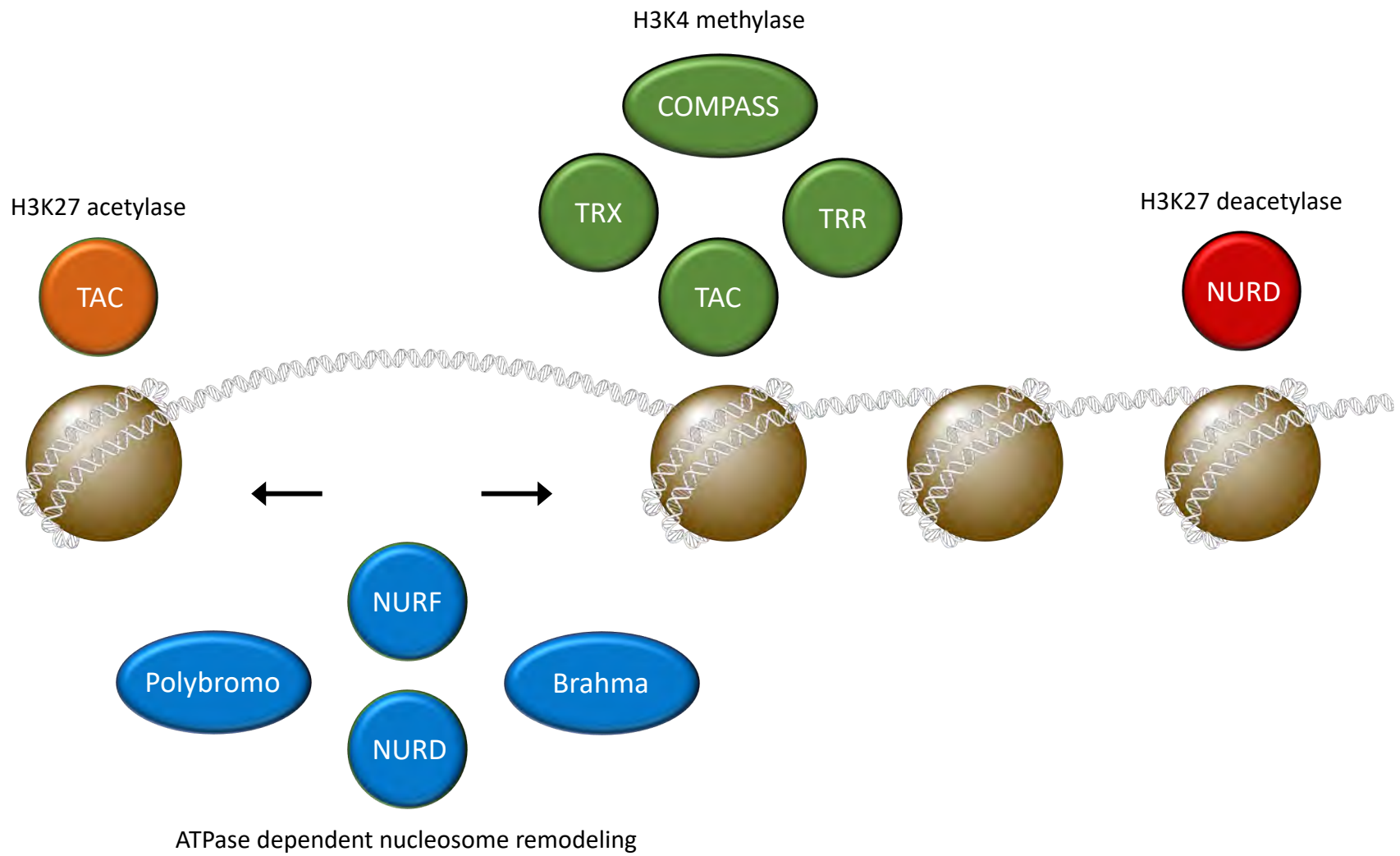
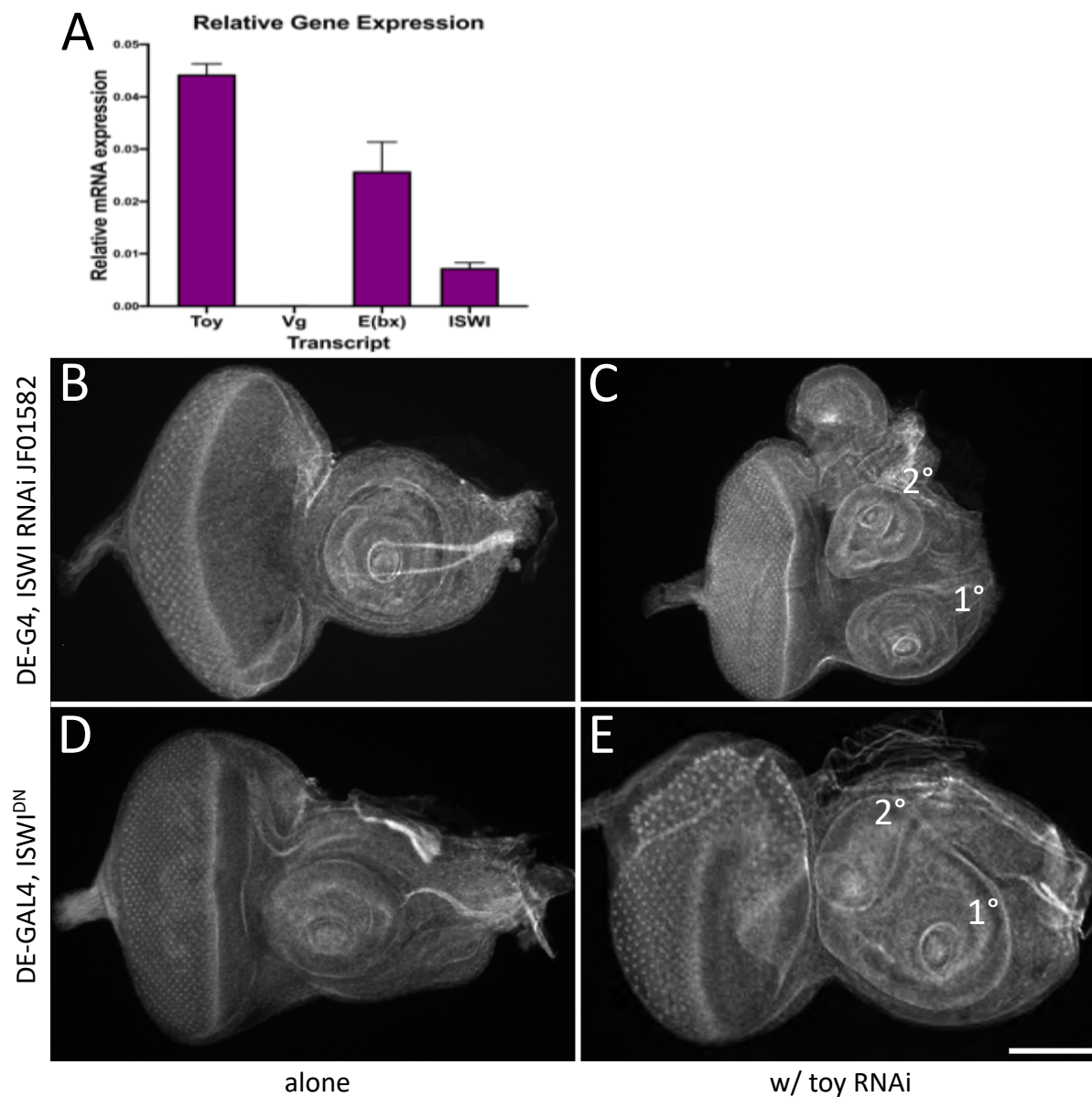


Supplemental Figure 1



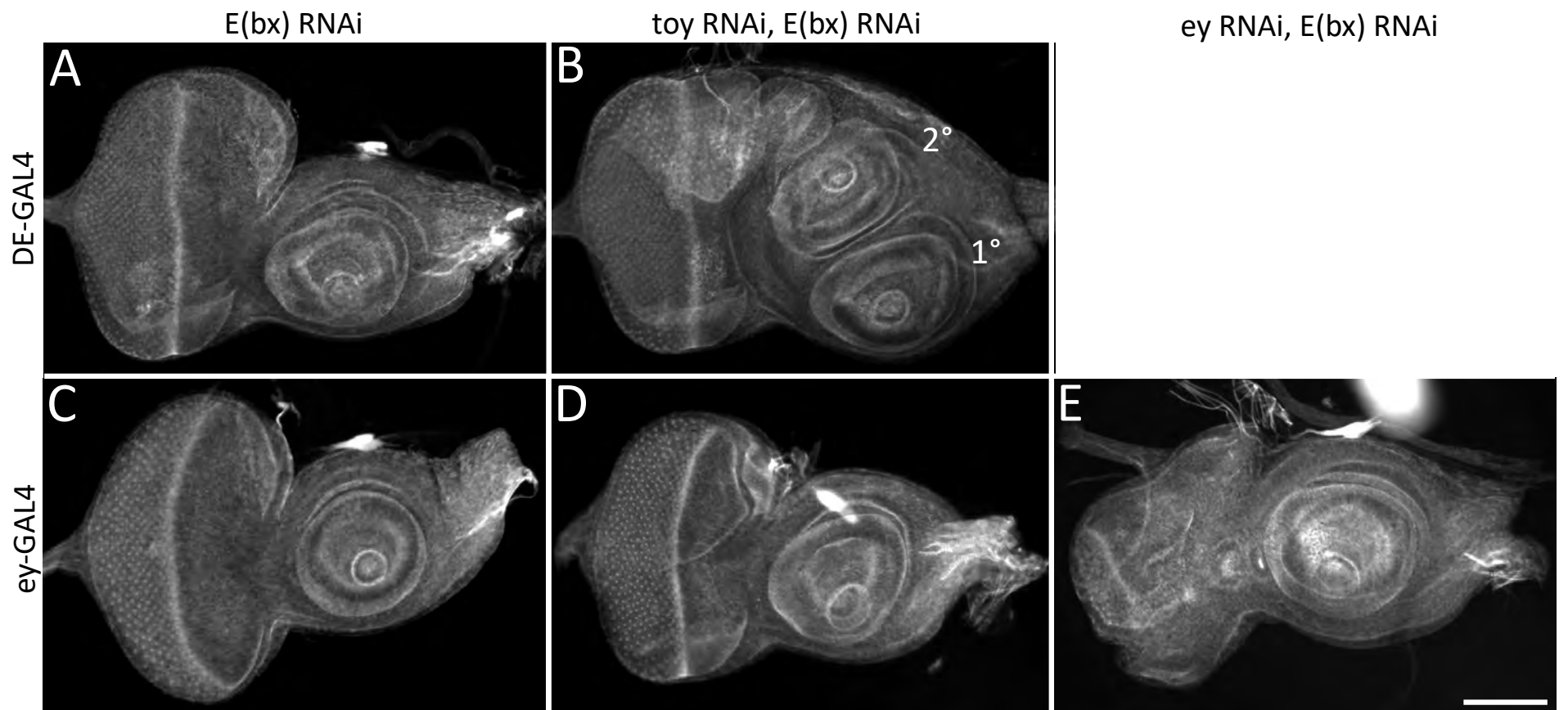
**Fig. S1. Schematic drawing depicting the role that chromatin modifying proteins play in the activation of transcription.** Chromatin modifying proteins are generally divided into three broad classes. Several are involved in addition/subtraction of epigenetic marks on histones. Examples include the methylation, acetylation, and deacetylation of histones. Other complexes are involved in the remodeling of nucleosomes at promoters and enhancers. NURF, which is the focus of this paper, is one such complex.

## Supplemental Figure 2



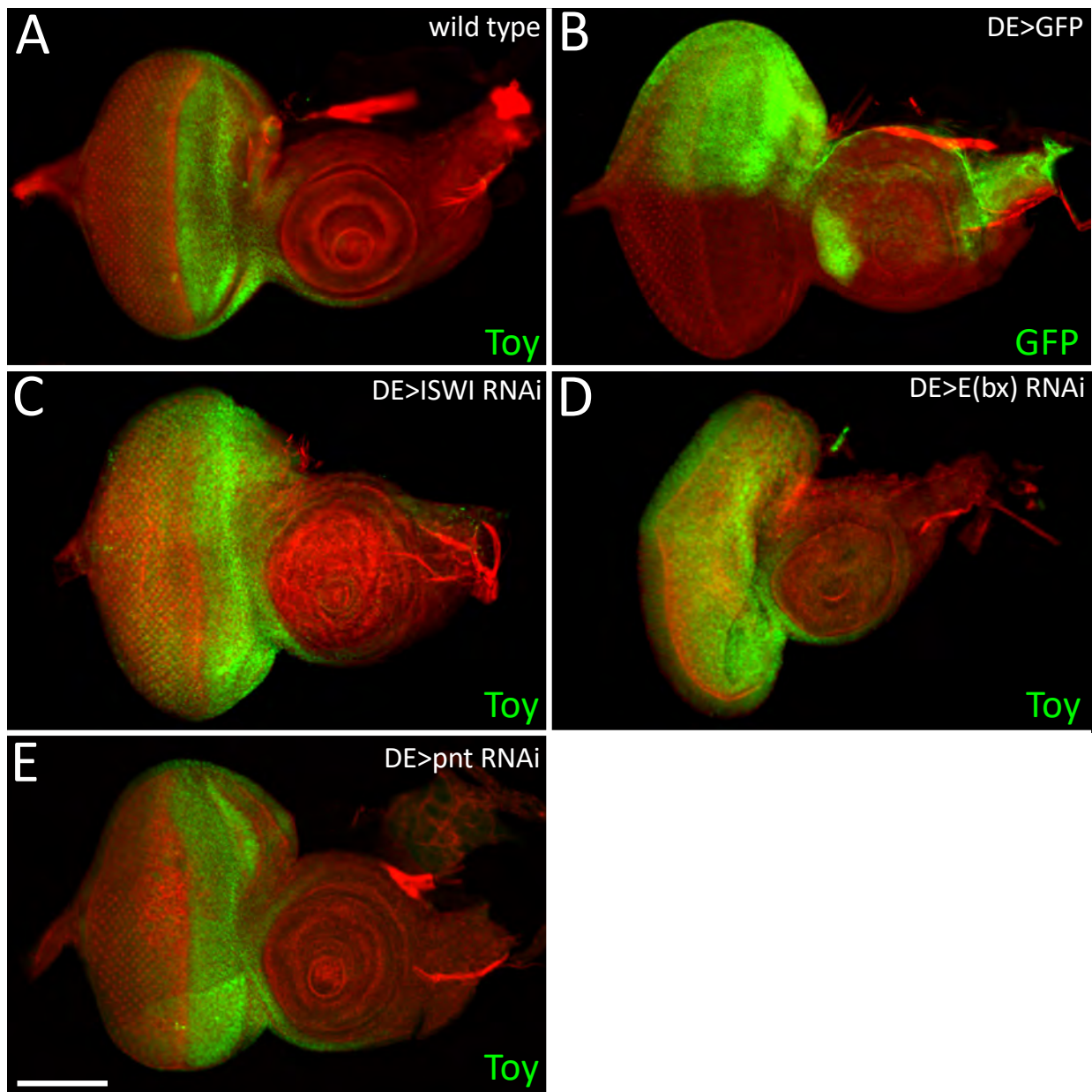
**Fig. S2. Combining reductions in *toy* expression with either reductions in either ISW expression activity induces the formation of a second antenna.** (A) qRT-PCR analysis of ISWI and E(bx) expression in the eye-antennal disc. These are compared to the expression levels of *toy* and *vestigial* (*vg*), a wing selector gene. (B,C) A second RNAi line that targets ISWI (JF01582) was used to complement the HMS00628 line that was used in the main text. Expression of this line on its own does not affect the number of antennal segments. However, if it is combined with the Toy RNAi line, then a second antenna forms. (D,E) We expressed a dominant negative ISWI protein as a mechanism to disrupt ISWI activity. On its own, expression of the ISWI<sup>DN</sup> protein also does not affect antennal numbers. However, a second antenna does form when Toy levels are also reduced. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

## Supplemental Figure 3



**Fig. S3. The role for the NURF complex in head epidermis formation is revealed by the requirement for E(bx).** While ISWI is found in several nucleosome remodeling complexes, E(bx) is only found within the NURF complex. (A,C) Knockdown of E(bx) individually does not alter the number of antennal segments. (B,D) Its role in maintaining the fate of the head epidermis is revealed when *toy* expression is simultaneously reduced. Please note that a second antenna is produced when E(bx) RNAi is expressed using the DE-GAL4 driver but not the ey-GAL4 driver. It is possible that the ey-GAL4 driver is expressed at lower levels than DE-GAL4. If this were to be the case then the lack of a second antenna might be due to insufficient knockdown of E(bx). (E) Reductions in *ey* expression (in combination with E(bx)) does not produce a second antenna. This suggests that the requirement for Pax6 is specific to Toy. All discs are treated with phalloidin which binds to F-actin. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

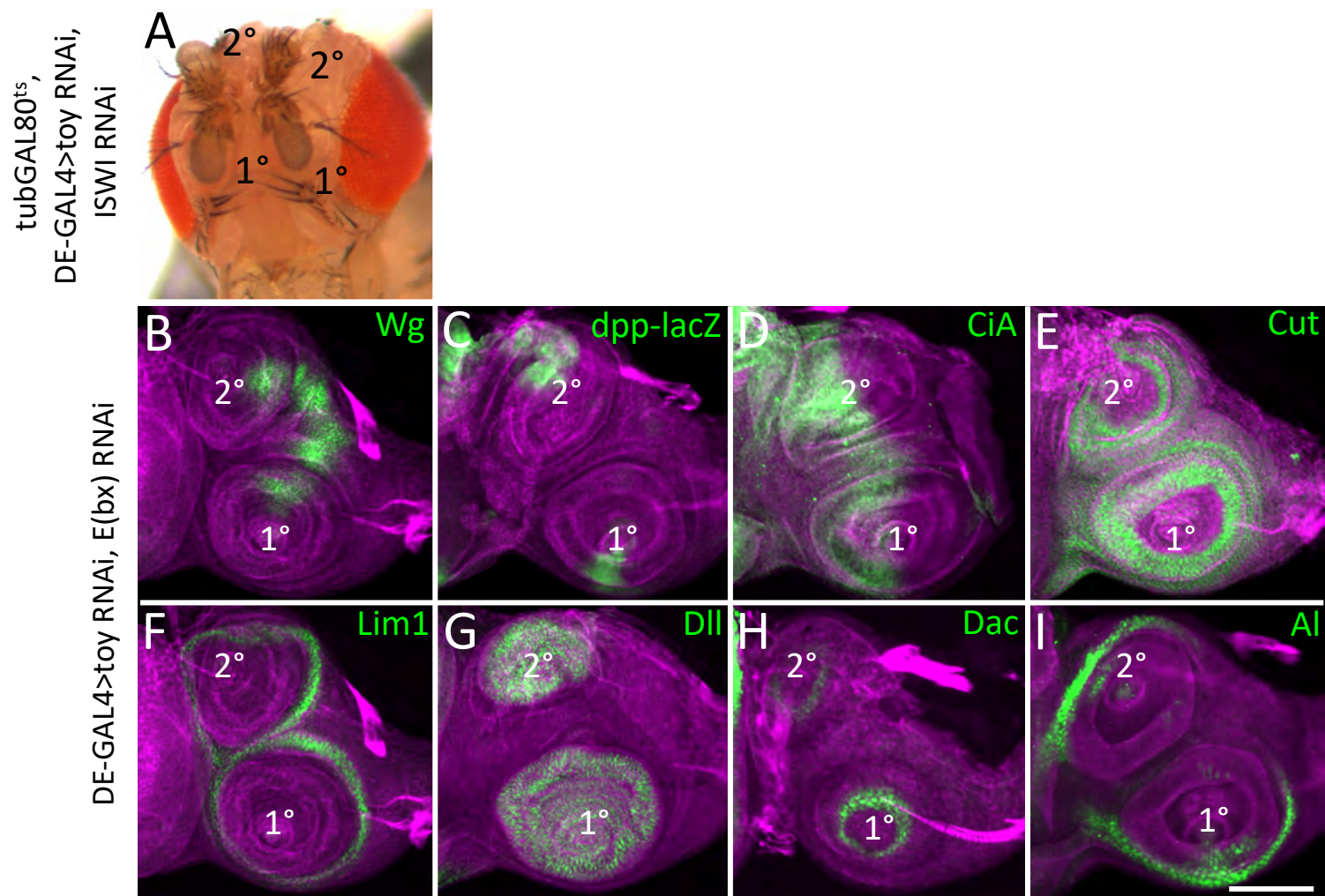
## Supplemental Figure 4



**Fig. S4. *toy* expression is not regulated either NURF or Pnt.** (A) Expression of *toy* in a wild type eye-antennal disc. (B) The DE-GAL4 driver directs expression to the dorsal half of the eye, the ocellar domain, and a portion of the dorsal head epidermis. (C-E) Expression of *toy* within eye-antennal discs in which ISWI (C), E(bx) (D), and Pnt (E) are knocked down individually. These data suggest that NURF and Pnt do not regulate *toy* expression. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

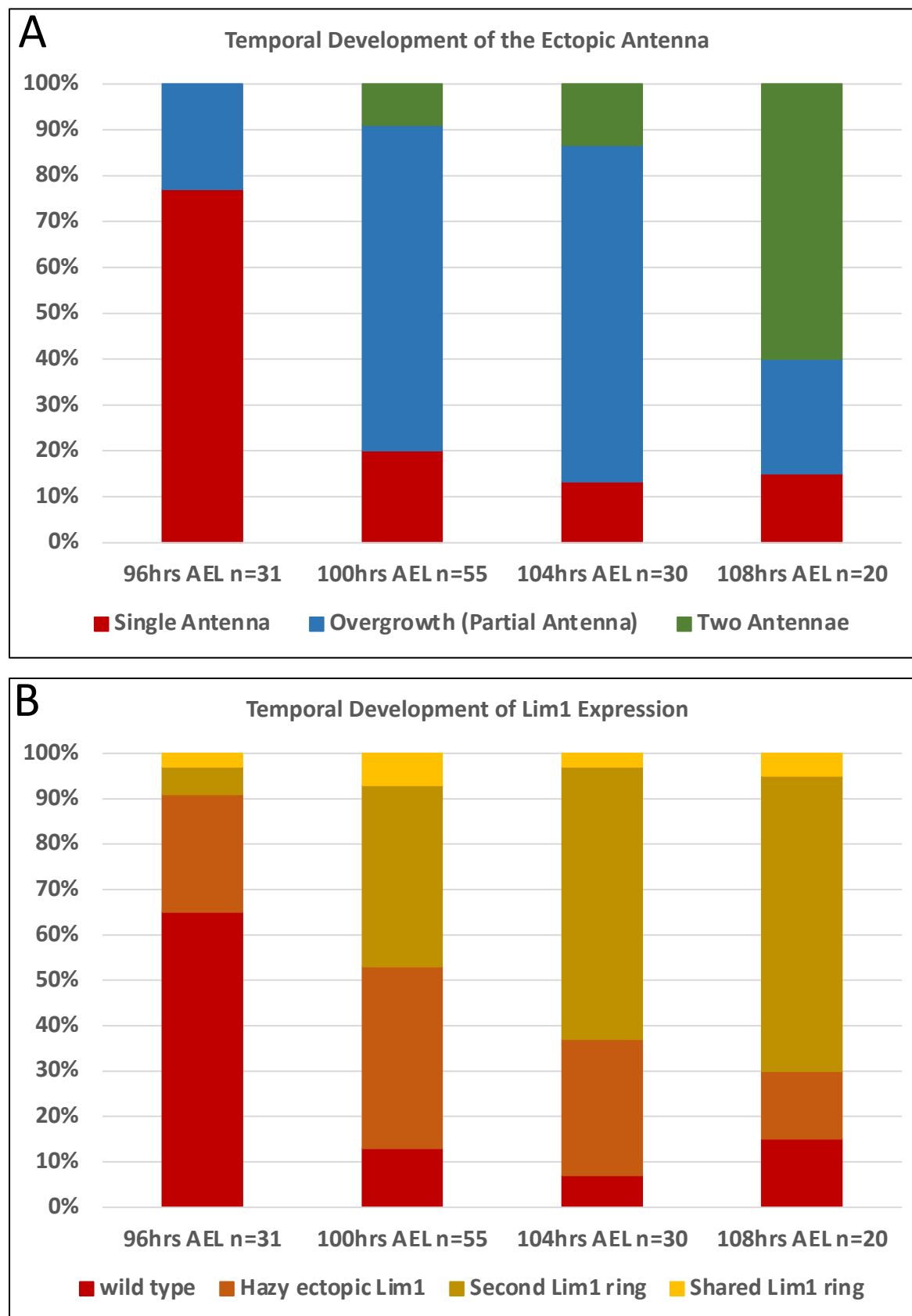


## Supplemental Figure 5



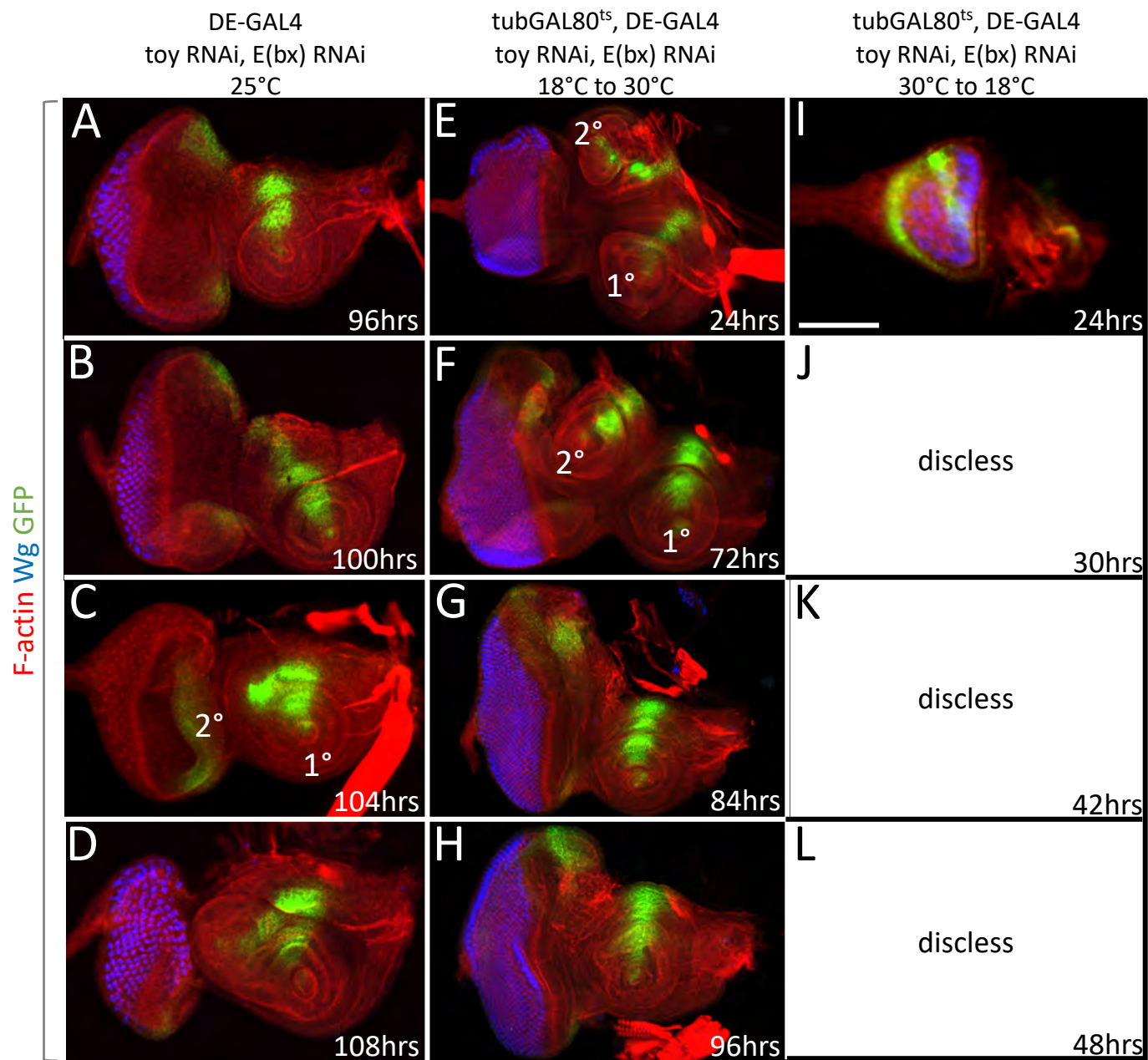
**Fig. S5. Ectopic antennae that result from the loss of Toy/NURF are normally patterned.** (A) Light microscope image of an adult head showing the complete duplication of antennal segments. This fly has four fully formed antennae instead of its normal two. (B-D) The distribution patterns of Wg, Dpp, and the active version of Ci indicate that the dorsal-ventral and anterior-posterior axes develop normally in Toy/E(bx) double knockdown discs. (E-I) Expression patterns of *cut*, *Lim1*, *Dll*, *Dac*, and *al* also suggest that the individual segments of the antenna and as such the proximal-distal axis forms correctly. Scale bar: 50µm. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

Supplemental Figure 6



**Fig. S6. Time course of Lim1 and ectopic antennal development.** (A) Quantification of ectopic antennal development during development. (B) Quantification of Lim1 expression within the ectopic antenna during development.

Supplemental Figure 7

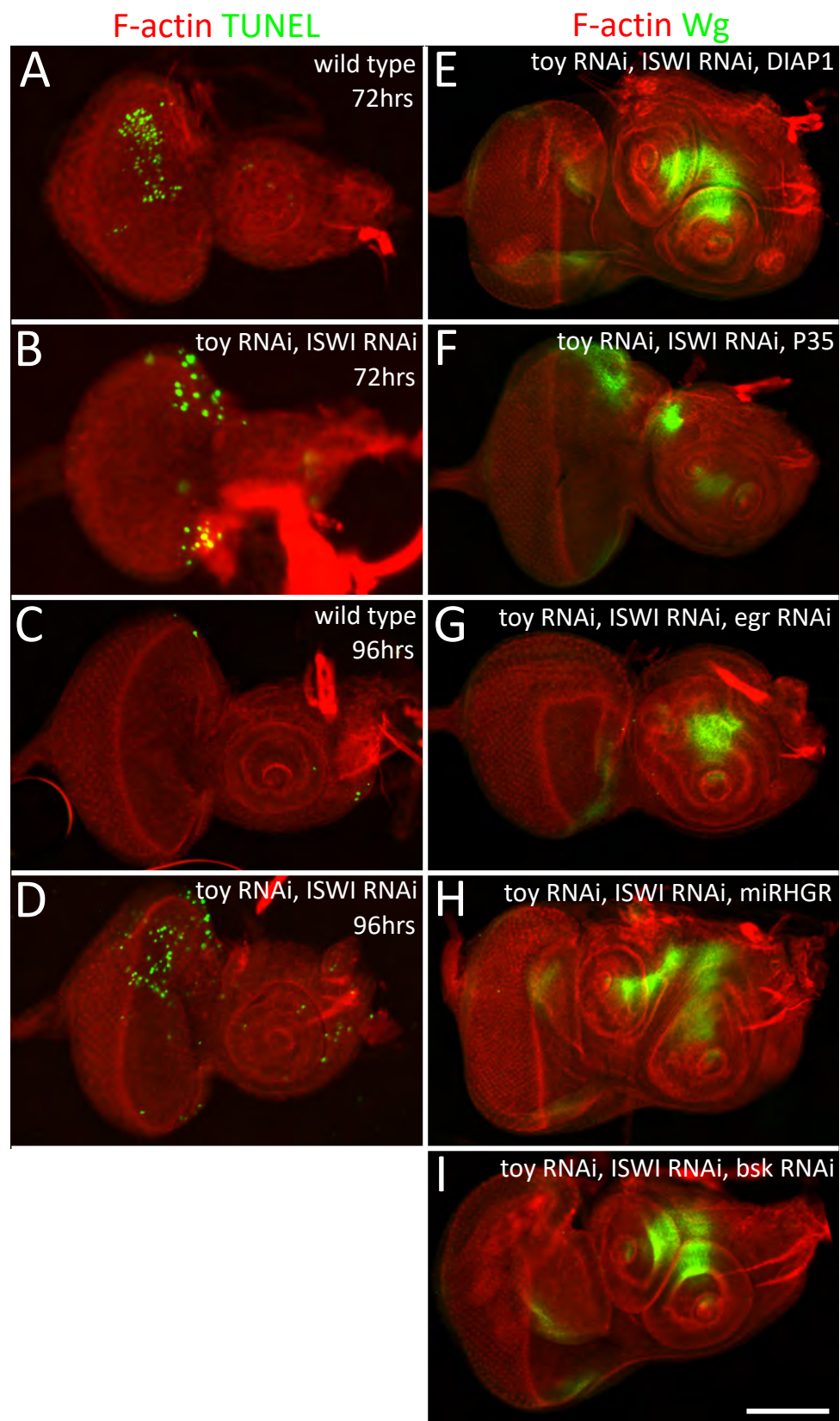


**Fig. S7. Toy/E(bx) are required during the second larval instar to suppress formation of the second antenna.**

(A-D) The second antenna begins to develop at 96hrs AEL and is complete by 108hrs AEL. (E-L) Controlling the timing of RNAi expression indicates that Toy/E(bx) suppresses formation of the second antenna during the second larval instar. Scale bar: 50µm. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.



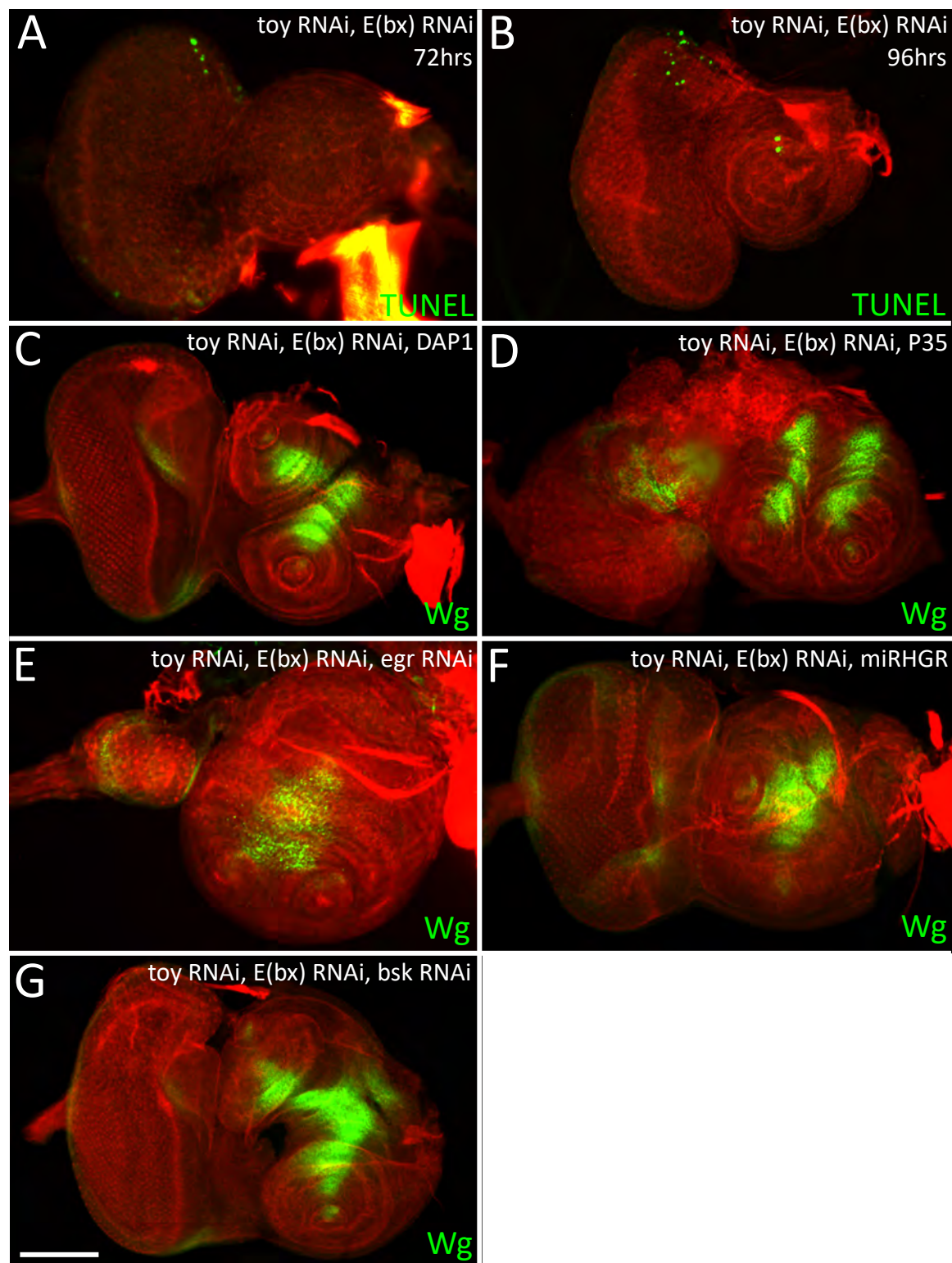
## Supplemental Figure 8



**Fig. S8. Cell death and regeneration do not play a significant role in the generation of the second antenna in Toy/ISWI knockdown discs.** (A-B) At 72hrs AEL wild type and Toy/ISWI double knockdown discs have similar levels of apoptosis in the ocellar region of the eye-antennal disc. (C,D) While cell death is absent from this region of a 96hr AEL wild type disc, a low level of apoptosis persists in Toy/ISWI discs. (E-I) Blocking cell death via expression of DIAP1 (E), P35 (F), egr RNAi (G), miRHGR (H), and bsk RNAi (I) does not suppress the formation of the second antenna. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

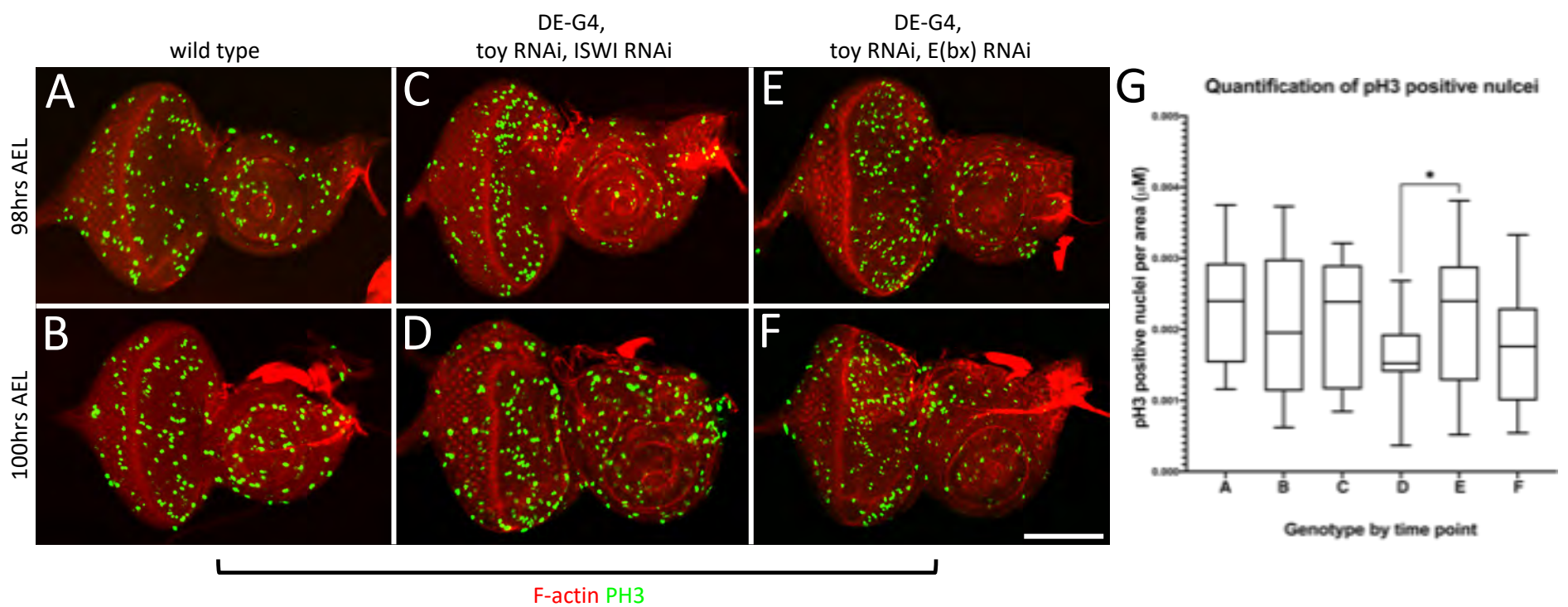


## Supplemental Figure 9



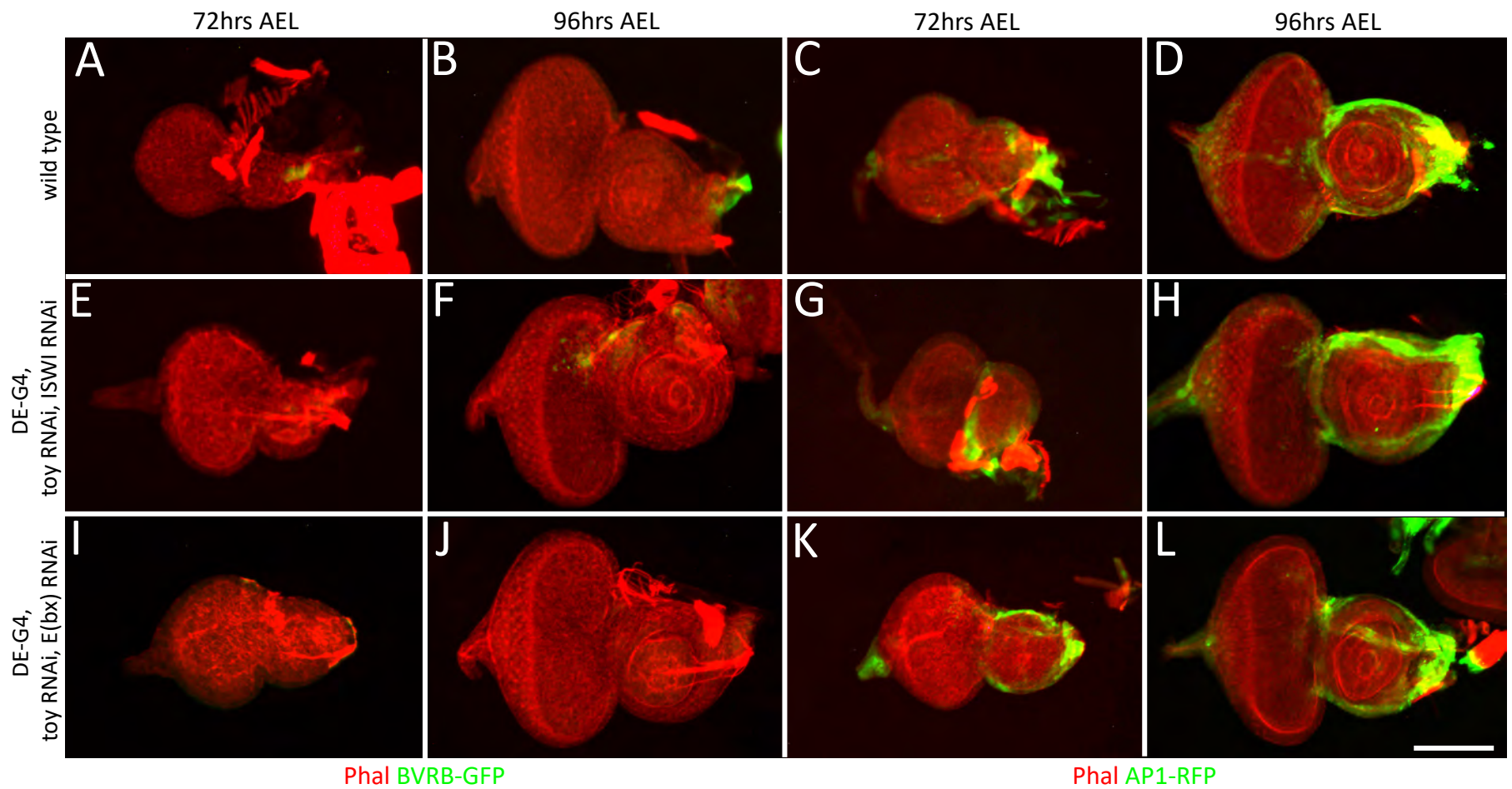
**Fig. S9. Cell death does not play a significant role in the generation of the second antenna in Toy/E(bx) knockdown discs.** (A,B) Similar to Toy/ISWI discs, a low level of apoptosis is present in Toy/E(bx) discs at both 72hrs and 96hrs AEL. Likewise, blocking cell death through the expression of DIAP1 (C), P35 (D), egr RNAi (E) egr RNAi, and miRGHR (F) fails to suppress the formation of the second antenna. We tried to express a bsk RNAi but the combination of GAL4 drivers and responders was lethal. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

## Supplemental Figure 10



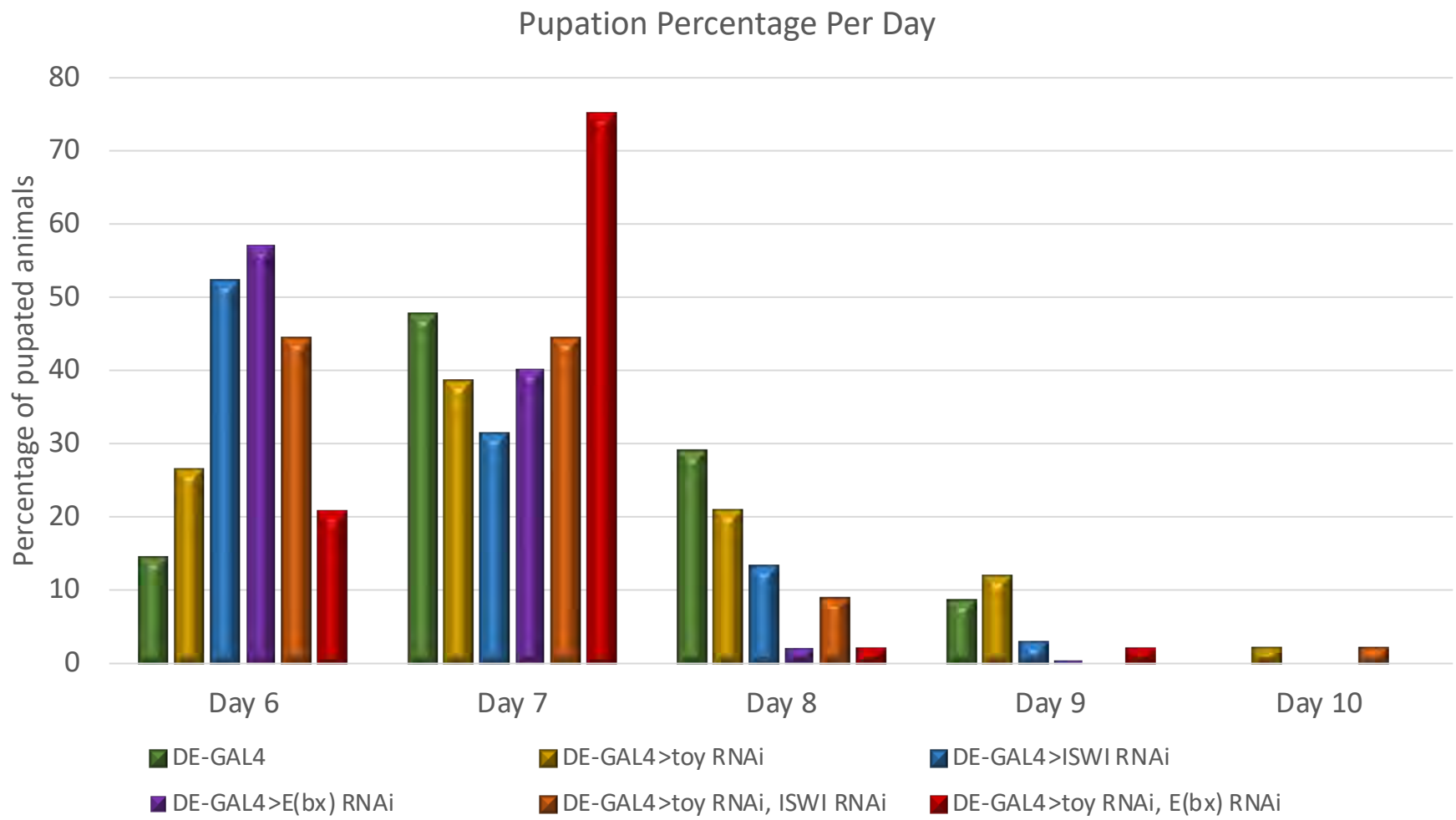
**Fig. S10. Regeneration does not play a significant role in the generation of the second antenna in Toy/NURF knockdown discs.** (A-F) Visual inspection of Toy/ISWI and Toy/E(bx) knockdown discs at 98hrs and 100hrs AEL. Indicates that cell proliferation levels (assayed by visualizing mitotically active cells) appear similar to wild type discs at the same time points. (G) Quantification of cell proliferation levels in wild type and knockdown discs confirms that there is no statistical difference in the number of mitotically active cells. The genotypes in panel G are as follows: (A) DE-GAL4 98hrs, (B) DE-GAL4, toy RNAi ISWI RNAi 98hrs, (C) DE-GAL4, toy RNAi, E(bx) RNAi 98hrs, (D) DE-GAL4 100hrs, (E) DE-GAL4, toy RNAi, ISWI RNAi 100hrs, (F) DE-GAL4, toy RNAi, E(bx) RNAi 100hrs. Scale bar: 50µm. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

## Supplemental Figure 11



**Fig. S11. Blastema formation is not part of the developmental process that leads to the formation of the second antenna.** (A-D) wild type (E-H) Toy/ISWI RNAi. (I-L) Toy/E(bx) RNAi. (A,B,E,F,I,J) The BVRB-GFP blastema reporter remains silent in Toy/ISWI and Toy/E(bx) knockdown discs. The only exception is a relatively small number of cells in Toy/ISWI discs at 96hr AEL. (C,D,G,H,K,L) The AP1-RFP blastema marker is not hyperactivated beyond its normal expression pattern or levels. Scale bar: 50µm. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

Supplemental Figure 12

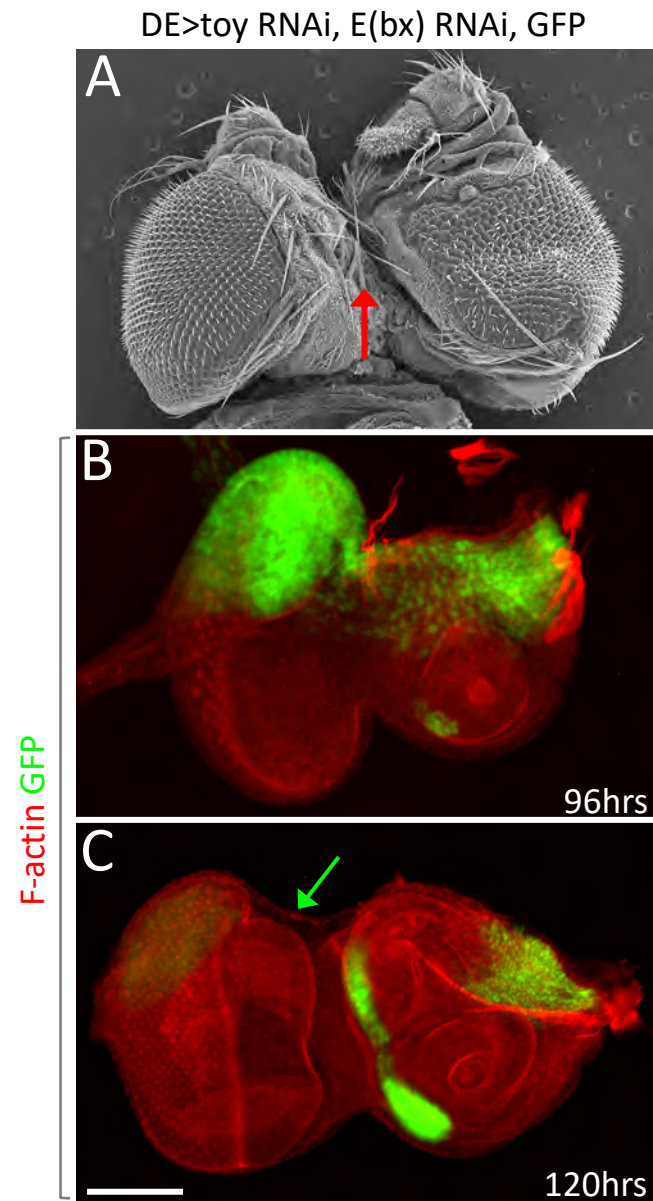


**Fig. S12. The transformation of the head epidermis into an antenna does not trigger a developmental delay.**

Graph demonstrating that the pupation rate of Toy single, NURF single, or Toy/NURF double knockdowns does not differ from the GAL4 driver control.

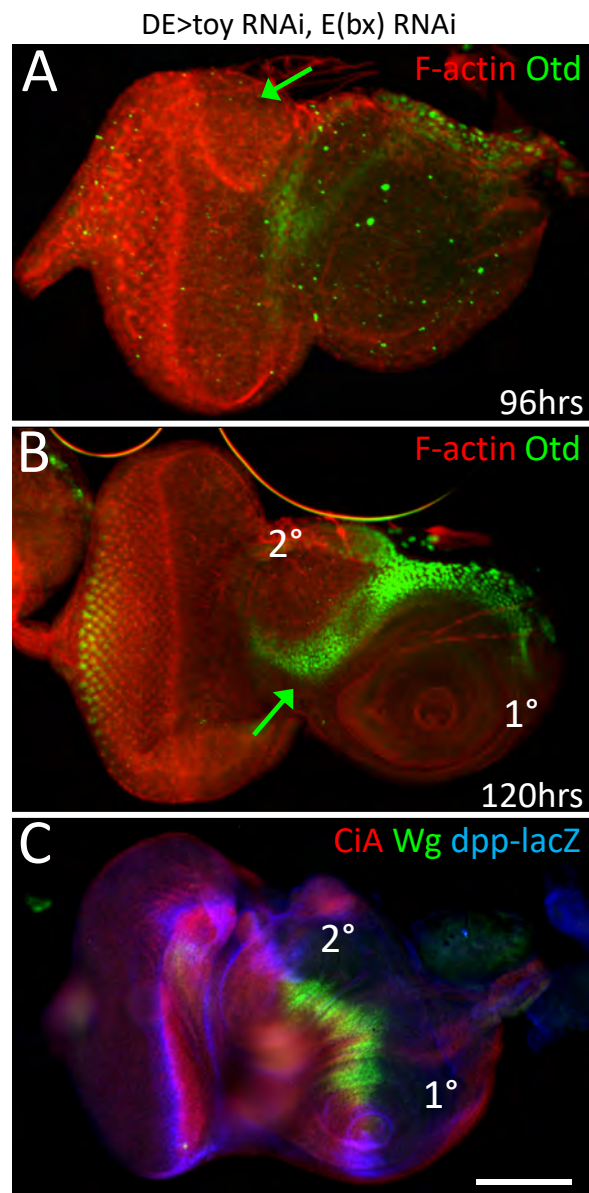


## Supplemental Figure 13



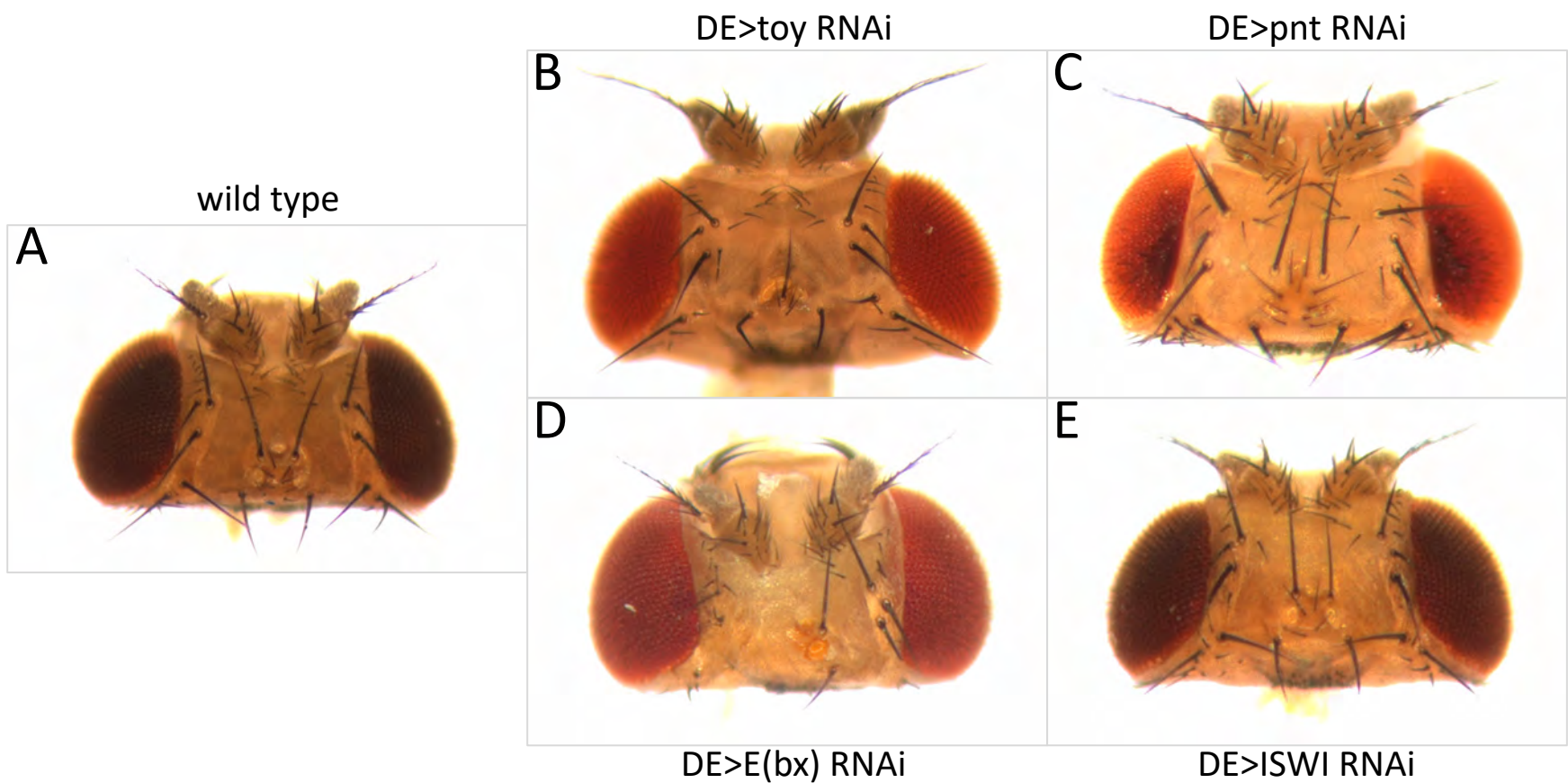
**Fig. S13. The loss of Toy/E(bx) alters the fate of the head epidermis. (A) SEM image of an adult head.** The dorsal head epidermis is lost in Toy/E(bx) mutants – this is seen as a fissure between the two compound eyes (red arrow). (B,C) Light microscope images of third instar imaginal discs. (B) At 96hrs, the continued expression of DE>GFP indicates that the dorsal head epidermis has retained its fate at this stage. (C) By 120hrs, expression of DE>GFP is eliminated within the dorsal head epidermis (green arrow) indicating that its fate has been altered. Scale bar: 50 $\mu$ m. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

## Supplemental Figure 14



**Fig. S14. The dorsal head epidermis loses its fate in Toy/E(bx) knockdowns.** (A-C) Light microscope images of third instar imaginal discs. (A) Otd, a marker of dorsal epidermal tissue is lost at 96hrs (green arrow). (B) By 120hrs, Otd expression is reactivated between the two antennae indicating the re-specification of those cells as head epidermis. (C) Ectopic dpp-lacZ expression in the dorsal head epidermis abuts Wg expression. This likely leads to the formation of the ectopic antenna. Scale bar: 50µm. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

Supplemental Figure 15



**Fig. S15. Individual knockdown of Toy, Pnt, ISWI, E(bx) inhibit ocellar and head epidermis specification.** (A) Wild type flies have three ocelli that sit atop the vertex of the fly head. (B-E) Ocellar and head epidermis development is inhibited by the individual knockdown of Toy (B,) Pnt (C), E(bx) (D), and ISWI (E). The head epidermis to antenna transformation is never seen in individual knockdowns. This transdetermination event is only seen when Toy RNAi lines are combined with RNAi lines with one of three other genes. See Supplemental Table 4 for a complete listing of all full genotypes for each panel.

Table S1. RNAi lines used to target chromatin modifying enzymes

[Click here to download Table S1](#)

Table S2. Phenotypes associated with the loss of chromatin modifying proteins within the eye-antennal disc

[Click here to download Table S2](#)

Table S3. Screen of Annotated Head Capsule Genes

[Click here to download Table S3](#)

Table S4. Genotypes for main and supplemental figure panels

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