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Supplementary Materials for

The Hippo pathway coactivator Yorkie can reprogram cell fates and create compartment-boundary–like interactions at clone margins

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This PDF file includes:

Supplementary Materials

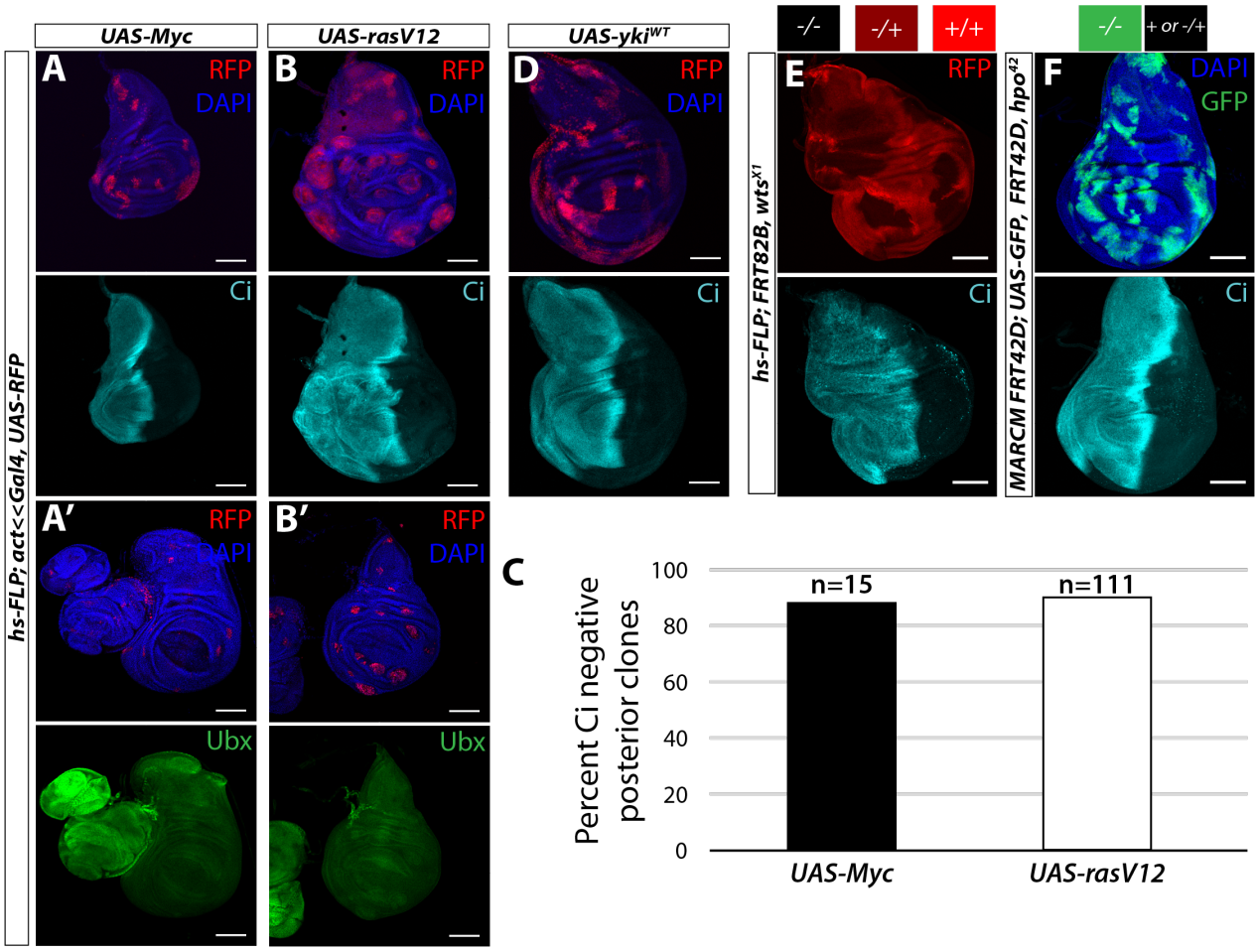

Figure S1: Ectopic Ci activation requires high Yki activity. (**A-B**) Overexpression of two other oncogenes, Myc (**A**) and RasV12 (**B**) did not elicit ectopic Ci, nor ectopic Ubx (**A'-B'**). (**C**) Quantification of percent of Ci-positive posterior clones overexpressing Myc or RasV12. No clones expressed a high level of ectopic Ci, but some potentially expressed low-level ectopic Ci. (**D**) Clones expressing UAS-Yki (wild type) do not induce ectopic Ci expression. (**E-F**) Clones homozygous for loss of function alleles of *wts* (**E**, wts^{X1}) or *hpo* (**F**, hpo^{42}) overgrew but did not express Ci ectopically. In (**E**) clones without RFP are *wts* null. In (**F**), GFP-positive clones are *hpo* null.

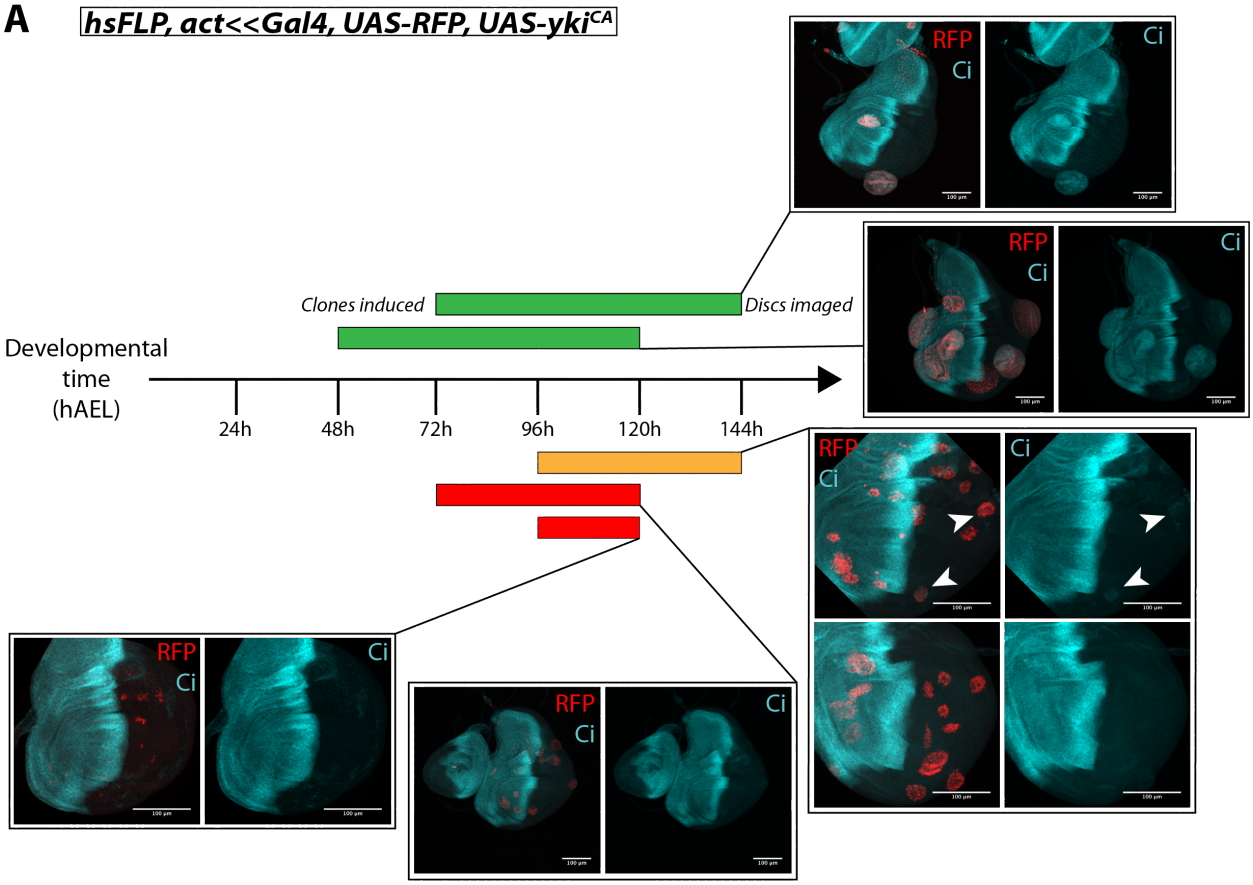

Figure S2: *yki*^{CA} clones disrupt patterning gene expression late in clone development. (**A**) FLP-out clones expressing *yki*^{CA} were induced at different developmental time points and allowed to grow for different durations before dissection. Start point of colored boxes indicates when clones were induced, and end point of colored boxes indicates when discs were dissected and imaged. Length of box corresponds to the amount of time between clone induction and disc imaging, or the duration of clone growth. Red-filled boxes indicate that no ectopic Ci was seen under that clone induction/imaging condition, green-filled boxes indicate ectopic Ci was seen consistently, and yellow filled boxes indicate ectopic Ci was sometimes seen. All larvae were raised at 25° C and heat shocked at 37° C.

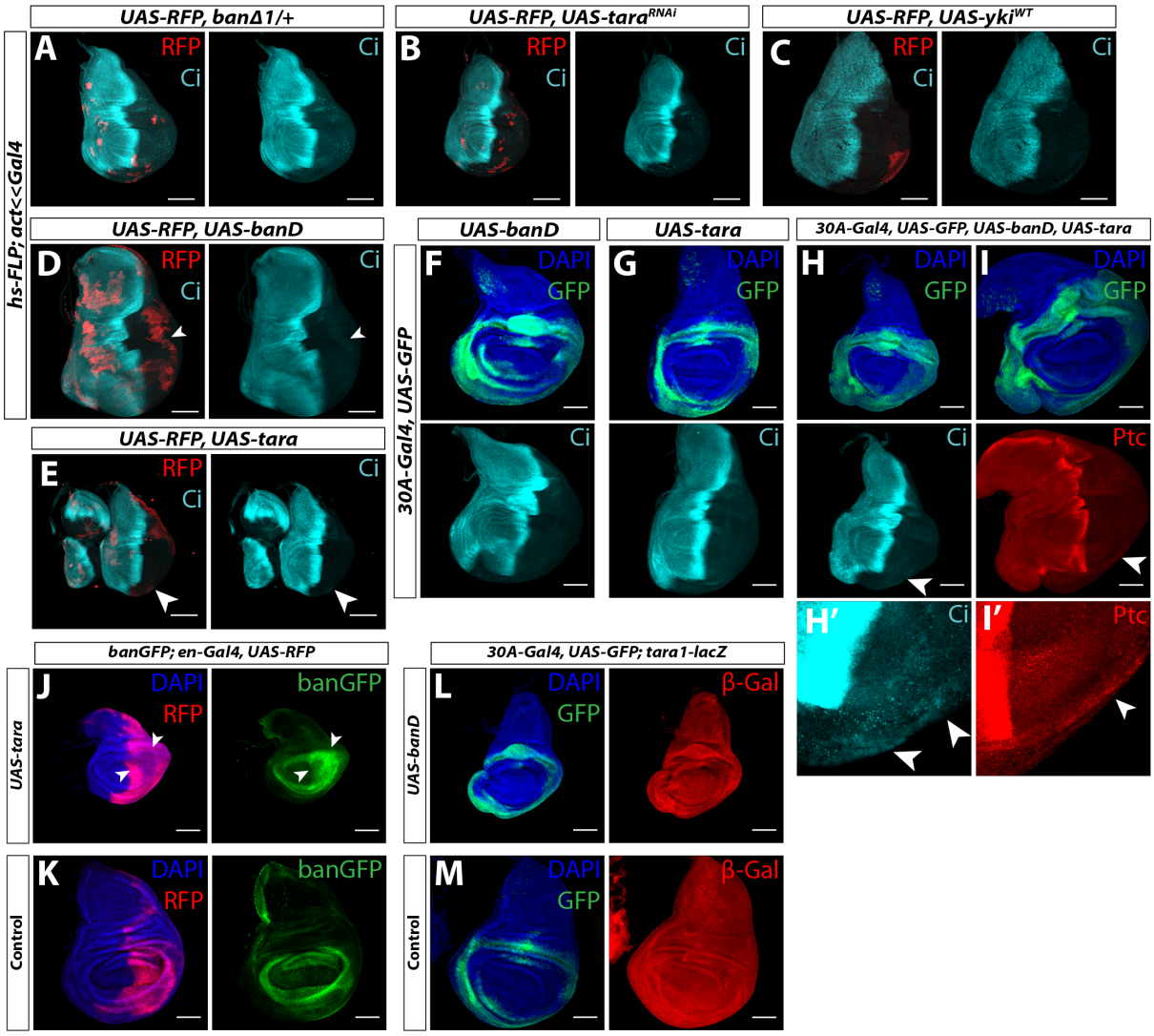

Figure S3: Manipulating *ban* or *tara* alone in wild-type cells does not alter Ci levels. (**A**) RFP-marked neutral clones induced in a $ban^{\Delta 1}$ /+ disc are small and do not express ectopic Ci. (**B**) Clones expressing $tara^{RNAi}$ alone do not express ectopic Ci. (**C**) Yki^{WT} expression alone also did not induce ectopic Ci, or did so at a very low level. (**D**) Clones expressing *banD,* did not express ectopic Ci, or did so at a very low level. (**E**) Overexpression of *tara* alone causes a low level of ectopic Ci expression on its own. This is especially evident in the hinge (white arrows). (**F**) *ban* overexpression in the hinge using *30A-Gal4* caused overgrowth, but not upregulation of Ci in the posterior. (**G**) Overexpression of Tara in the hinge using *30A-Gal4* did not cause overgrowth or ectopic Ci in the posterior. (**H-I**) Overexpression of both *ban* and *tara* together in the hinge using *30A-Gal4* caused a marked increase in overgrowth, as well as subtle but consistent upregulation of Ci (**H'**) and Ptc in the posterior hinge (**I'**). (**J-K**) Overexpression of *tara* with *en-Gal4* causes upregulation of *ban-GFP* in the posterior compartment (white

arrows). (**L-M**) Overexpression of *banD* in the hinge with *30A-Gal4* does not cause obvious upregulation of *tara-lacZ*.

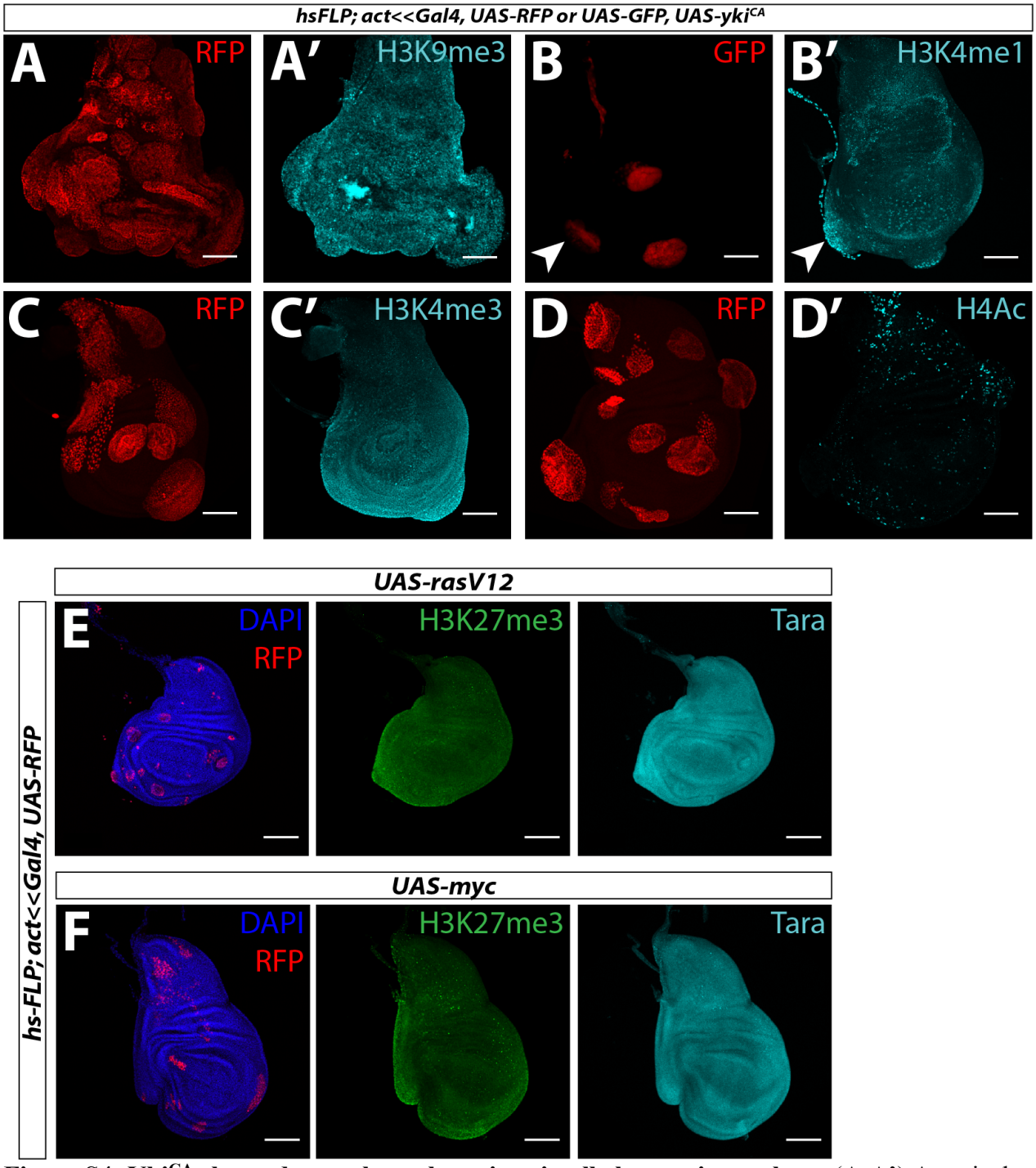

Figure S4: Yki^{CA} clones do not show alterations in all chromatin markers. (**A-A'**) A typical marker of heterochromatin, H3K9 trimethylation, is not altered in *yki^{CA}* clones. (**B-B'**) *yki^{CA}* clones (visualized with GFP) show increased H4K3 monomethylation, a mark of active enhancers, in the hinge, though not less than H3K27me3. (**C-D**) Two markers of active genes, H3K4 trimethylation and H4 acetylation, are not altered in *yki^{CA}* clones. H3K4 trimethylation in *yki^{CA}* clones is the same as surrounding wild type tissue (**C-C'**), and H4 acetylation is largely

absent throughout the disc (**D-D'**). (**E-F**) Overexpression of either *rasV12* or *myc* does not change expression of H3K27me3 or Tara.

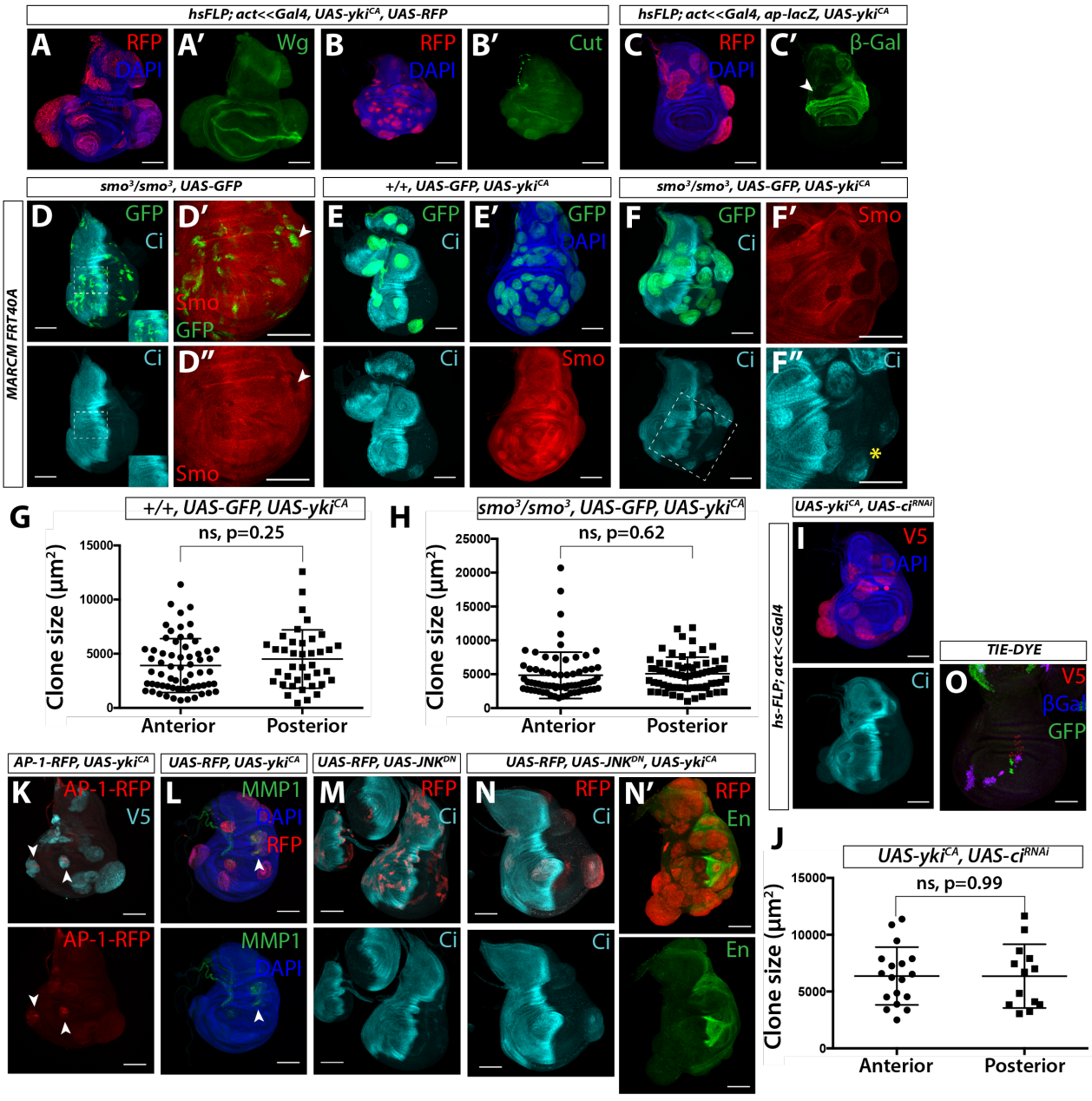

Fig S5: Overgrowth and ectopic Ci expression in *yki*^{CA} clones is not dependent on Hh signaling or the JNK pathway.

Figure S5: Overgrowth and ectopic Ci expression in *yki*^{CA} clones is not dependent on Hh signaling or the JNK pathway. (**A-C**) Clones expressing *UAS-yki*^{CA} do not ectopically express the dorsoventral boundary marker Wg (**A-A'**), but do infrequently express the dorsoventral boundary marker Cut in the ventral compartment (**B-B'**). Clones do not ectopically express the dorsal compartment marker Ap (**C-C'**). 43% (13/30) of dorsal *yki*^{CA} clones had reduced or absent ap-lacZ (white arrow in B'). (**D-F**) Clones made by mitotic recombination using the MARCM system such that clones lacking Gal80 express UAS-GFP and/or *UAS-yki*^{CA}. (**D**) MARCM *smo*^{3}*/smo*^{3} clones expressing GFP are small and irregular. (**E**) MARCM +/+ clones expressing *UAS-yki*^{CA} and *UAS-GFP* are overgrown with smooth boundaries. Ectopic Ci is observed in posterior ventral hinge clones. (**F**) MARCM *smo*^{3}*/smo*^{3} clones expressing *UAS-yki*^{CA} and *UAS-GFP* are also overgrown and have smooth edges. Although Smo protein is absent in clones (**F'**), ectopic Ci protein is still observed (**F''**). Thus, Ci expression per se is not dependent on Hh signaling. Hh signaling could potentially affect the relative amounts of the activator and repressor form of Ci. (**G, H**) Anterior and posterior clones are not significantly different in size for MARCM +/+ clones expressing *UAS-yki*^{CA} (**G**) or MARCM *smo*^{3}*/smo*^{3} clones expressing *UAS-yki*^{CA} (**H**). (**I**) Ci expression is not required for Yki^{CA}-induced overgrowth. Clones expressing *UAS-yki*^{CA} and *UAS-ci*^{RNAi} lack Ci expression in clones (including ectopic Ci in posterior clones) but still overgrow. (**J**) Anterior and posterior clones expressing *UAS-yki*^{CA} and *UAS-ci*^{RNAi} are not significantly different in size. (**K**) Some Yki^{CA}-expressing clones (marked with V5) express the AP-1 reporter, AP-1-RFP (arrows), but most clones express a low level or no RFP. (**L**) A minority of *yki*^{CA} clones (marked with RFP) express MMP1 (arrows), a target of JNK signaling, but most clones do not express MMP1. (**M, N**) Clones expressing Yki^{CA} and Jnk^{DN} overgrow and express ectopic Ci, similar to Yki^{CA} alone. (**M**) Expression of JNK^{DN} in wild-type clones does not alter Ci expression. (**N**) Clones expressing Yki^{CA} and Jnk^{DN} express ectopic Ci and also downregulate En (**N'**). **(O)** TIE-DYE control disc.