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Supplemental Information

A Balance of Yki/Sd Activator and E2F1/Sd Repressor Complexes Controls Cell Survival and Affects Organ Size

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Supplemental Figures and Legends (S1-S6)

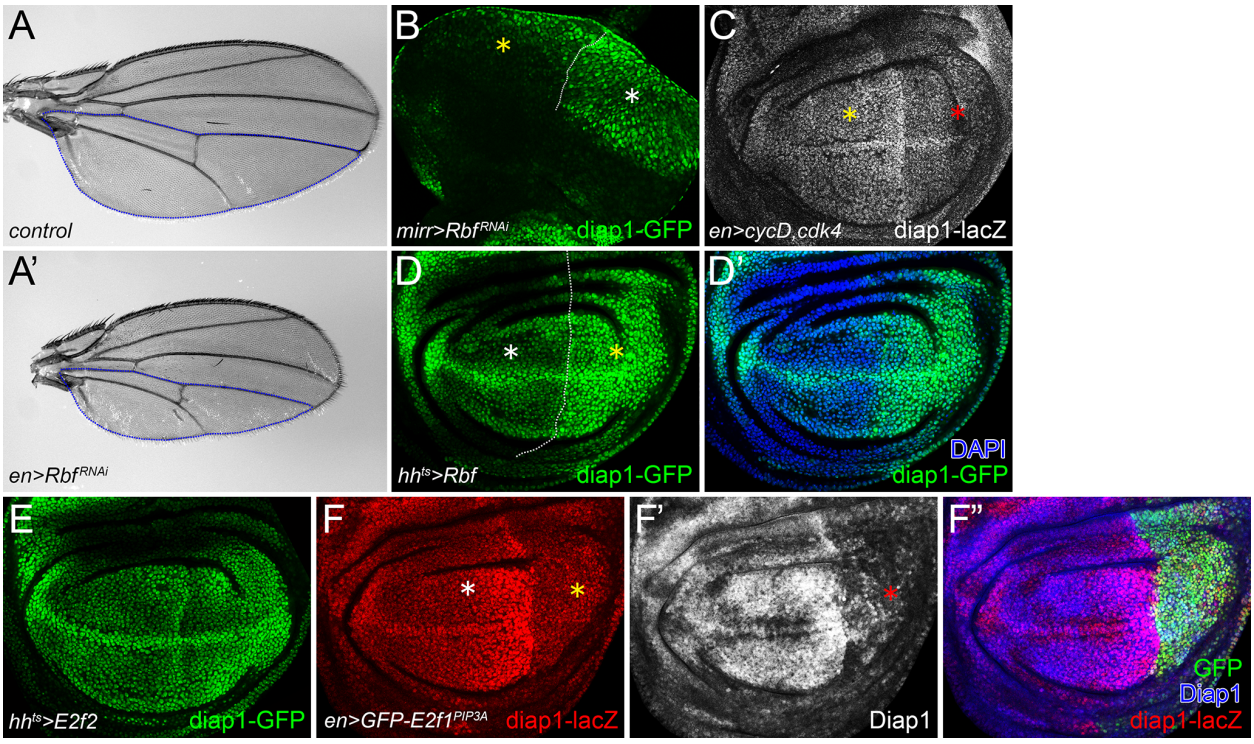

Figure S1. *Drosophila* RBF/E2F1 pathway regulates wing growth and Yki activity, related to Figure 1 and 2.

(A-A') Adult wing size: (A) *enGal4/+* control. (A') *Rbf* knockdown driven by *enGal4*. Blue dotted lines labeled the posterior region of adult wing. (B) The larvae were raised at 18°C. Knockdown of *Rbf* in the dorsal part (indicated by yellow asterisk) of eye disc using *mirrGal4*. (C) *UAS-CycD* and *UAS-CDK4* were co-expressed using *enGal4*. (D-D') Overexpression of *Rbf* was driven by hh^{ts}. The larvae were raised at 18°C, and then shifted to 29°C for 48 hours before dissection in 3rd instar stage. (E) Overexpression of *E2f2* was driven by hh^{ts}. The larvae were raised at 18°C, and then shifted to 29°C for 72 hours before dissection in 3rd instar stage. (F-F") *GFP-E2f1*^{PIP3A} overexpression was driven by *enGal4*. Expression of *diap1* was indicated by *Diap1-lacZ* reporter (F) and Diap1 antibody staining (F'). Nuclei (blue, F") were stained by DAPI.

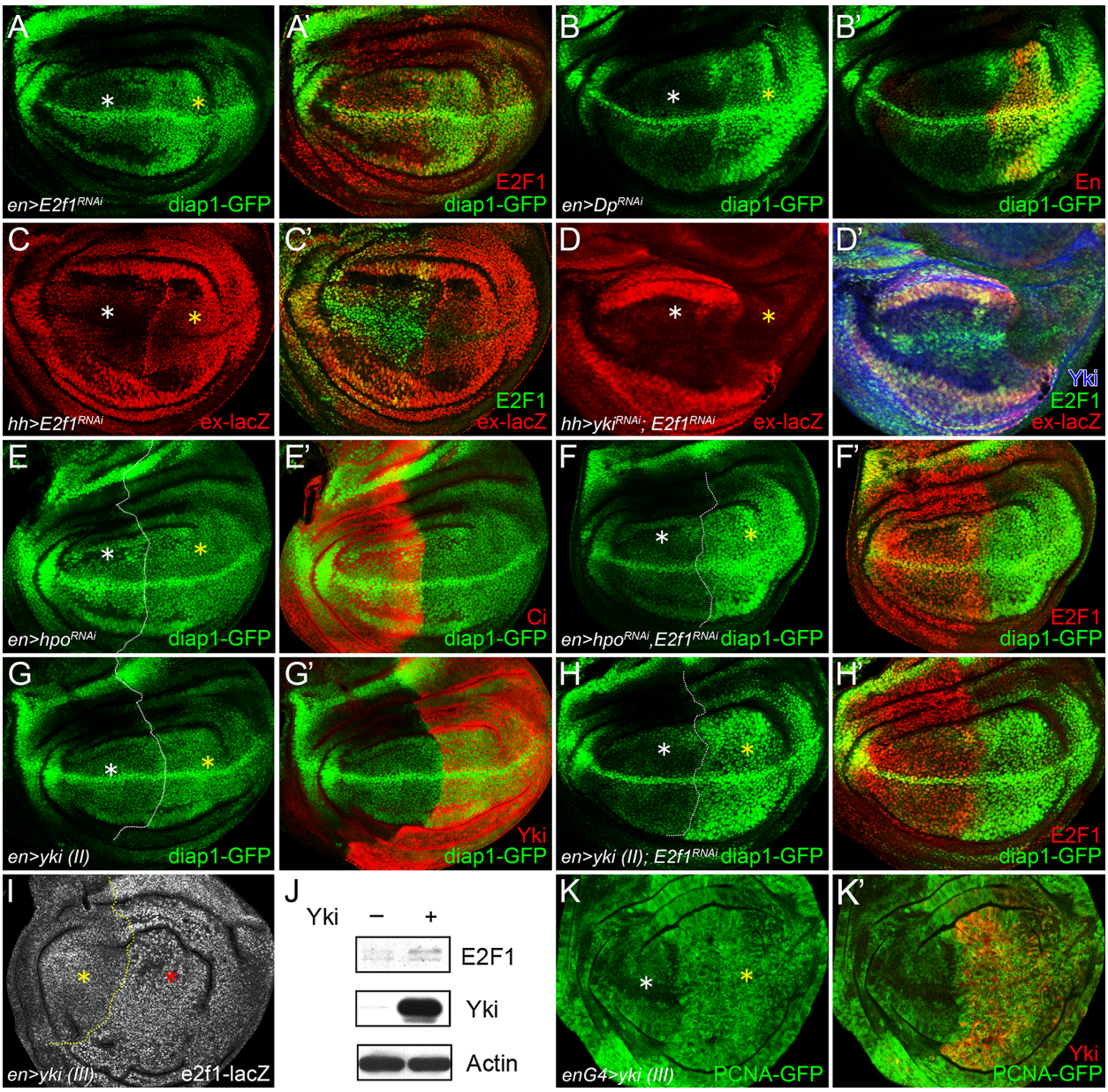

Figure S2. Incoherent regulations between Yki and E2F1, related to Figure 3.

(A-B') Knockdown of *E2f1* or *Dp* was driven by *enGal4*. En staining (B') was used to label the A/P boundary. (C-D') *E2f1* knockdown (C-C') or *yki/E2f1* double knockdown (D-D') was driven by *hhGal4*. (E-F') *hpo* knockdown (E-E') or *yki* overexpression (F-F') was driven by *enGal4*, respectively. Ci staining in (E') was used to label the anterior compartment. RNAi-mediated depletion of *E2f1* in conjunction with *hpo* knockdown (G-G') or *yki* overexpression (H-H') was

driven by *enGal4*, respectively. E2F1 staining in (A'), (F') and (H') were used to indicate the knockdown efficiency of *UAS-E2f1*^{RNAi}. (I) *yki* overexpression was driven by *enGal4*. The transcription levels of *E2f1* were indicated by *E2f1-lacZ*. (J) The control (*nubGal4/+)* and *yki* overexpression (*nubGal4>UAS-yki*) wing discs were dissected in 3rd instar stage and lysed for western blot. Lysates were immunoblotted with E2F1 antibody. The amount of E2F1 in cell lysates was increased upon *yki* overexpression. (K-K') *yki* overexpression was driven by *enGal4*. The transcriptional activity of *E2f1* was indicated by the expression of *PCNA-GFP*.

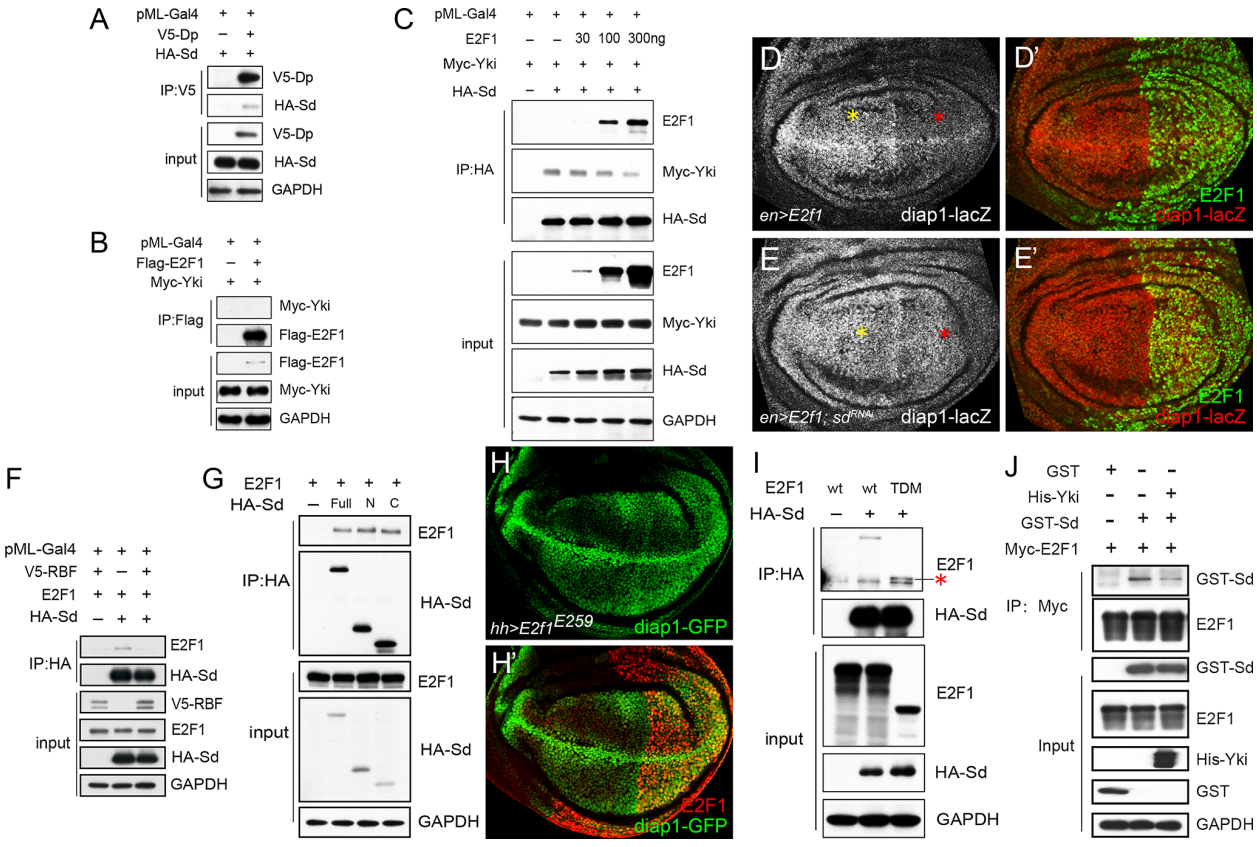

Figure S3. E2F1 competes with Yki for Sd interaction, related to Figure 4.

HEK293T (A-C, F-H) cells were transfected with indicated plasmids and subjected to IP. (A) V5-Dp co-IPed with Sd. (B) Myc-Yki cannot be pulled down by Flag-E2F1. (C) Increasing

concentrations (does 30-300ng) of E2F1 reduced the amount of Myc-Yki pulled down by HA-Sd. Yki was normalized to the same expression levels. (D-E') *E2f1* overexpression (D-D') or *E2f1* overexpression in conjunction with *sd* knockdown (E-E') was driven by *enGal4*, respectively. Knockdown of *sd* restored the reduction of *diap1-lacZ* caused by *E2f1* overexpression. (F) Overexpression of *V5-RBF* reduced the amount of E2F1 pulled down by HA-Sd. (G) Physical association between E2F1 and Sd. E2F1 was detected in HA-IP from HEK293T cells co-expressing HA-Sd (full length), HA-Sd^{N} (N-terminal half of Sd) and HA-Sd^{C} (C-terminal half of Sd). (H) A truncation mutant of the transactivation domain of E2F1, $E2F1^{TDM}$ (the Q525 was mutated to a stop codon), showed similar capability with wild type E2F1 to bind to HA-Sd (upper band, indicated by red asterisk). (I-I') Overexpression of $E2f1^{TDM}$ this mutant using *hhGal4* significantly suppressed the expression of *diap1-GFP*. (J) In vitro binding assay: Myc-E2F1 was expressed in HEK293T cells and IPed by anti-Myc beads. Purified Myc-E2F1 was incubated with beads bound to GST or GST-Sd (10ug) for 1 hour. The pre-incubated beads-Myc-E2F1-GST-Sd complex was then incubated with or without 2ug purified His-Yki for another 1 hour. Then, all samples were subjected to IP and western blots using indicated antibodies. His-Yki reduced the amount of GST-Sd pulled down by Myc-E2F1.

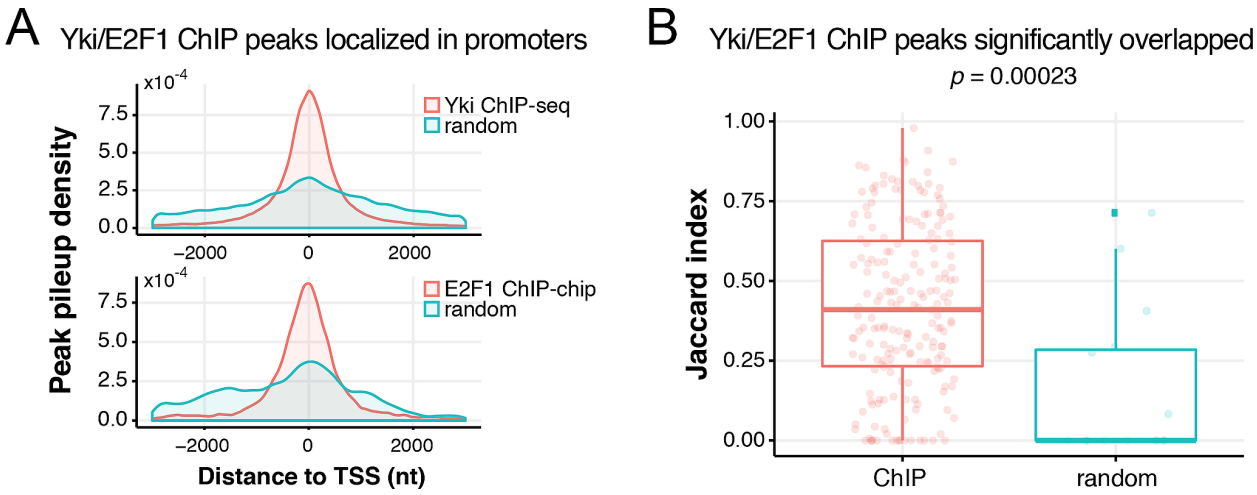

Figure S4. Genome-wide interactions between Yki and E2F1 on target gene promoters, related to Figures 4 and 6.

The E2F1 ChIP-chip data (Korenjak et al., 2012) and Yki ChIP-seq data (Oh et al., 2013) were processed using custom Rs script (R 3.4.1). The nearest promoter for each peak was determined according to FlyBase gene annotation. Peak regions were piled up in the flanking region of annotated transcription start sites, and then pileup density was calculated and compared to randomized peaks. Target genes of Yki and E2F1 were defined as ones with Yki and E2F1 peaks appearing within +/-1Kb region surrounding their transcription start sites, respectively. In genes targeted by both Yki and E2F1, the extent of peak overlaps was measured by the Jaccard index. The Jaccard index was calculated as the size of intersection peak region over the size of union region. Its value ranges from 0 and 1. Higher Jaccard index values indicate the larger overlaps between peak regions; if two regions are entirely overlapped, the Jaccard index is 1, and if two regions are not overlapped at all, the index is 0. Randomized peaks were generated by randomly re-distributing Yki and E2F1 peaks coordinates across the whole genome, using bedtools (v2.26.0) the shuffle function. The same procedure that applied to ChIP peaks was executed to random peaks as well, but there were a fewer number of genes targeted by random peaks originated from Yki

and E2F1 binding peaks simultaneously. To assess the significance of overlapping between Yki and E2F1 binding peaks, the Jaccard index of random peaks was also calculated. (A) Peak pileup density of Yki ChIP-seq data (up) and E2F1 ChIP-chip data (bottom). Comparing to randomized peaks, both proteins showed an enrichment to target gene promoters. (B) Boxplots showing the distribution of Jaccard index of co-targeted Yki and E2F1 binding peaks, indicating the overlapping extent between Yki and E2F1 binding peaks. Comparing to random peaks, the overlapping between Yki and E2F1 binding peaks was significant.

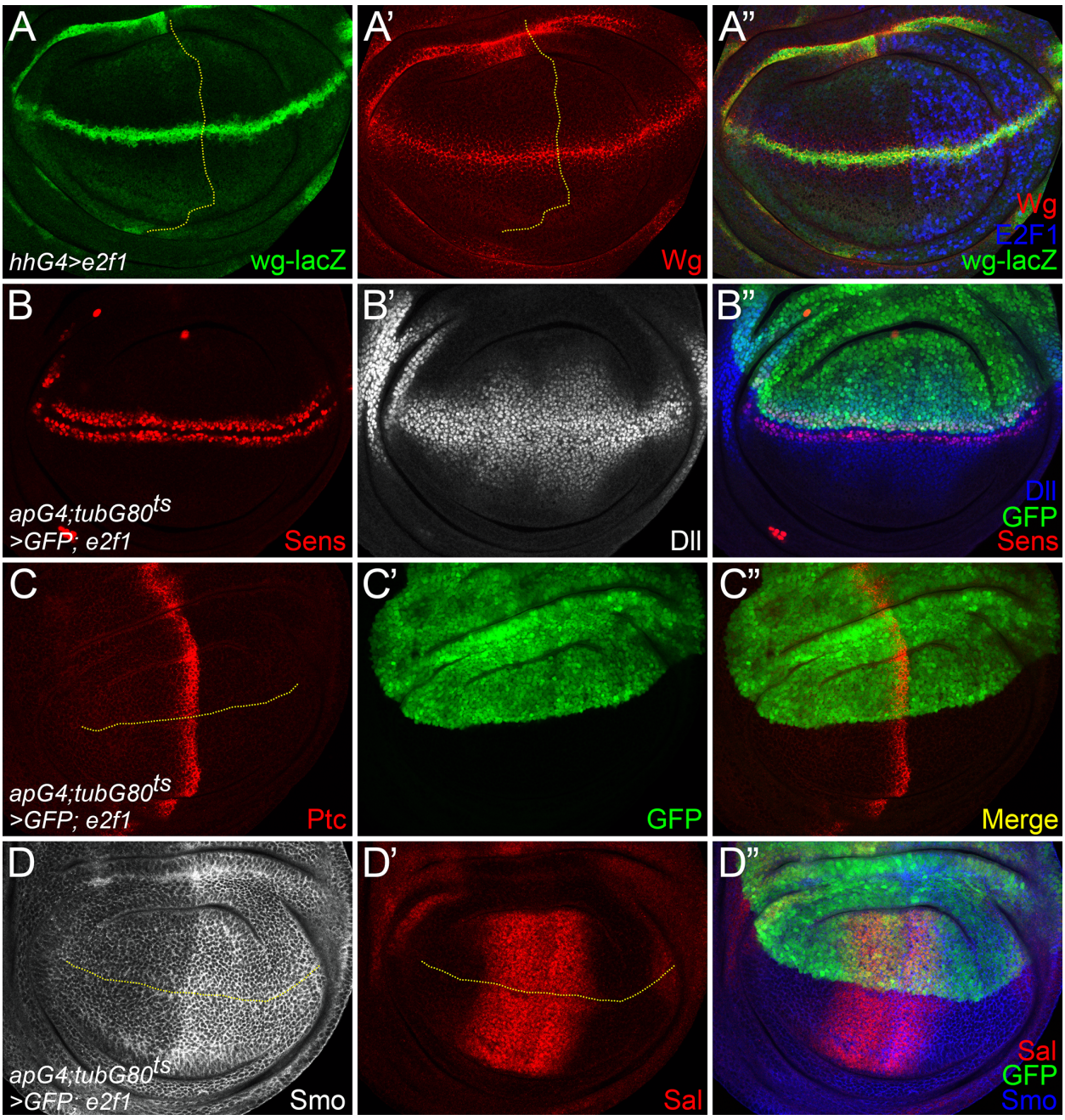

Figure S5. E2F1 has no effects on Notch, Wg, Hh and Dpp signaling pathways, related to Figure 2.

(A-A'') The larvae were raised at 18°C. *UAS-E2f1* was overexpressed using *hhGal4*. Neither *wingless-lacZ* (*wg-lacZ*) nor Wg protein levels were decreased in posterior compartment. As *wg* transcription in wing discs is controlled by Notch signaling, we suggest that E2F1 didn't repress

Notch signaling. (B-D'') *E2f1* was overexpressed in the dorsal compartment of wing disc using *apGal4, tubGal80^{ts}*. Larvae were raised at 18°C, and then shifted to 29°C for 24 hours before dissection in 3rd instar stage. (B-B'') The Wg signaling downstream target genes, Senseless (Sens, B) and Distal-less (Dll, B'), showed no obvious change upon *E2f1* overexpression, indicating that E2F1 has no effect on Wg signaling transduction. (C-C'') Patched (Ptc), the Hedgehog (Hh) signaling receptor, was normal in *E2f1*-overexpressing cells. (D-D'') Neither Smoothened (Smo), another component of Hh signaling, nor Spalt (Sal), the downstream targets of Decapentaplegic (Dpp) signaling, were repressed by *E2f1* overexpression. The data shown in C-D'' indicate that E2F1 doesn't repress both Hh and Dpp pathways in wing discs.

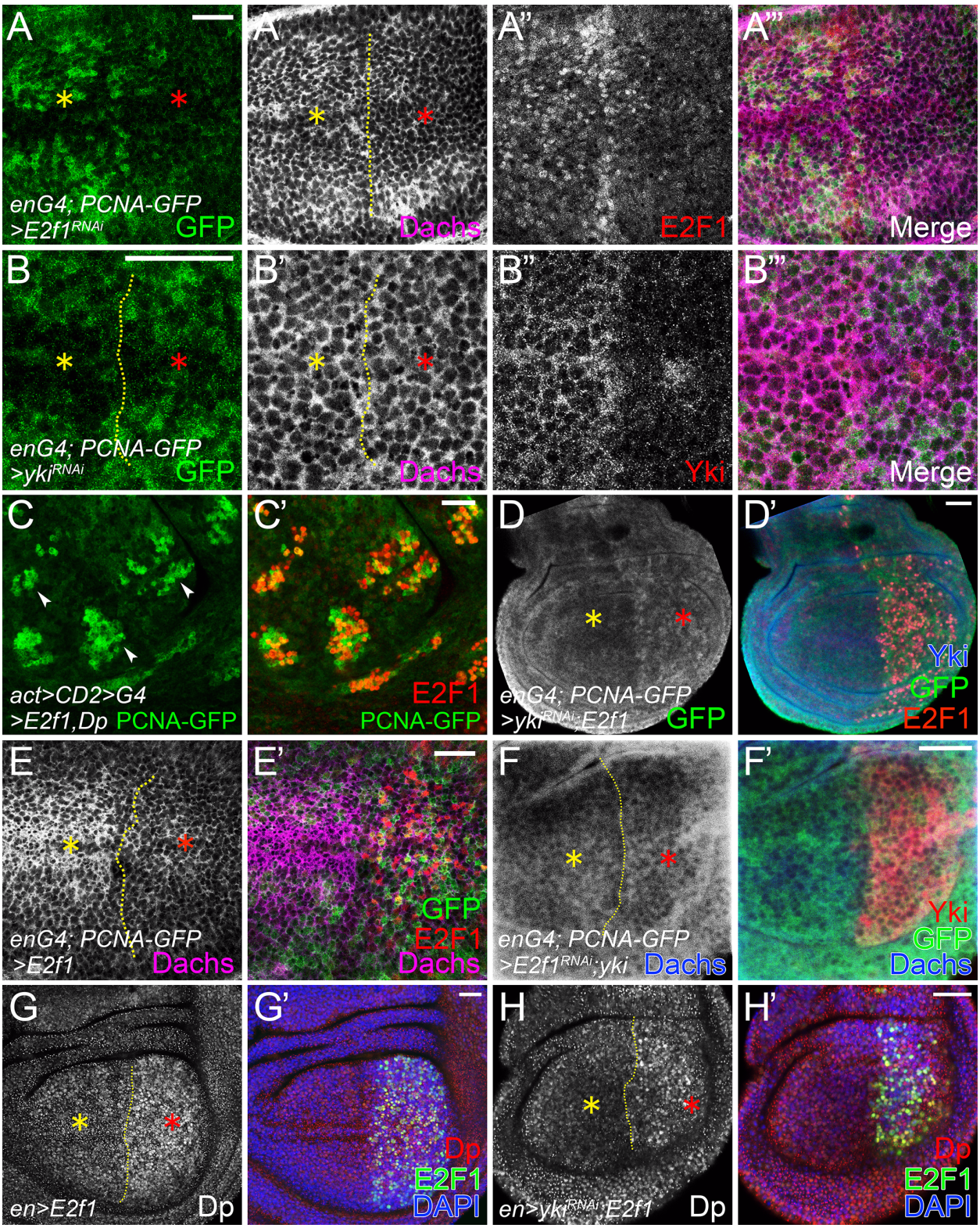

Figure S6. Yki and E2F1 show inconsistent regulations on *Dachs*, *PCNA*, and *Dp*, related to Figure 6.

(A-A”’) *UAS-E2f1*^{RNAi} was overexpressed using *enGal4*. The levels of PCNA-GFP were decreased in posterior compartment (red asterisk in A). However, Dachs showed no obvious alterations (red asterisk in A’). (B-B”’) *UAS-yki*^{RNAi} was overexpressed using *enGal4*. Neither PCNA-GFP (B) nor Dachs (B’) was regulated by *yki* knockdown. (C-C’) PCNA-GFP levels were detected in flip-out clones overexpressing *E2f1+Dp*. Arrowheads in (C) showed the upregulation of PCNA-GFP. (D-D’) *UAS-yki*^{RNAi} and *UAS-E2f1* were co-expressed using *enGal4*. PCNA-GFP showed obvious increasing in posterior compartment (red asterisk in E). (E-E’) Overexpression of *E2f1* was driven by *enGal4*. The levels of Dachs were decreased in posterior part (red asterisk in E). (F-F’) *UAS-yki*^{RNAi} and *UAS-E2f1* were co-expressed using *enGal4*. Dachs showed no obvious alterations (red asterisk in F). (G-H’) *UAS-E2f1* (G-G’) and *UAS-E2f1+UAS-yki*^{RNAi} (H-H’) were driven by *enGal4*, respectively. The levels of Dp were upregulated in *E2f1* overexpressing cells (red asterisk in G). The upregulation of Dp caused by E2F1 overexpression was not repressed by *yki* depletion.