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Hippo pathway and Bonus control developmental cell fate decisions in the *Drosophila* eye

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Summary

The canonical function of the Hippo signaling pathway is the regulation of organ growth. How this pathway controls cell fate determination is less well understood. Here, we identify a function of the Hippo pathway in cell fate decisions in the developing *Drosophila* eye, exerted through the interaction of Yorkie (Yki) with the transcriptional regulator Bonus (Bon), an ortholog of mammalian Transcriptional Intermediary Factor 1/tripartite motif (TIF1/TRIM) family proteins. Instead of controlling tissue growth, Yki and Bon promote epidermal and antennal fates at the expense of the eye fate. Proteomic, transcriptomic, and genetic analyses reveal that Yki and Bon control these cell fate decisions by recruiting transcriptional and post-transcriptional co-regulators, and by repressing Notch target genes and activating epidermal differentiation genes. Our work expands the range of functions and regulatory mechanisms under Hippo pathway control.

eTOC Blurb:

Zhao et al. question how different cell fates are specified during *Drosophila* development and show that the Hippo pathway, previously mainly implicated in growth regulation, controls major cell fate decisions in the eye. This function engages a pathway member, Bonus, and involves a non-canonical transcriptional program.

Graphical Abstract

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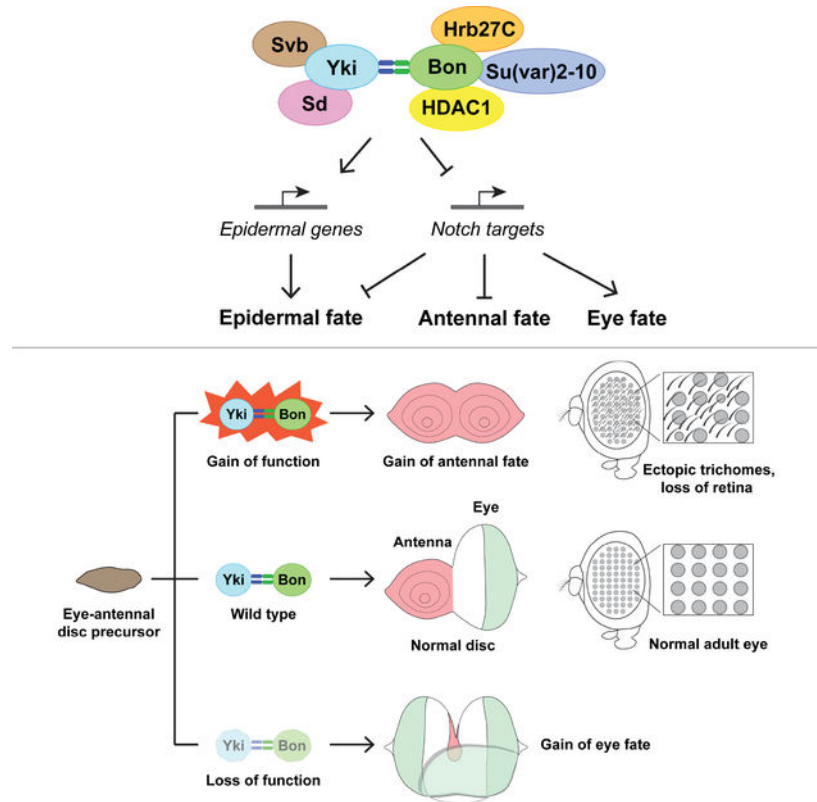
Author contributions

Conceptualization, H.Z., K.H.M., and A.V.; Methodology, H.Z., K.H.M., and A.V.; Formal analysis, H.Z.; Investigation, H.Z.; Resources, K.H.M. and A.V.; Data Curation, H.Z.; Writing – Original Draft, H.Z.; Writing – Review & Editing, H.Z., K.H.M., and A.V.; Visualization, H.Z.; Supervision, A.V.; Funding Acquisition, K.H.M. and A.V.

Declaration of interests

The authors declare no competing interests.

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Keywords

Yki; Bon; cell fate; eye specification; Notch; E(spl)-C; HDAC1; Su(var)2-10; transcriptional regulation; protein interaction

Introduction

Tissue growth and cell fate determination are critical developmental processes controlled by multiple signaling pathways, including the evolutionarily conserved Hippo pathway, whose dysregulation leads to developmental abnormalities and diseases^{1,2}. The core Hippo (Hpo, MST1/2 in mammals)/Warts (Wts, LATS1/2 in mammals) kinase cascade inhibits the activity of the transcriptional coactivator Yorkie (Yki, YAP/TAZ in mammals) by phosphorylation and cytoplasmic retention, whereas unphosphorylated nuclear Yki associates with DNA-binding proteins such as Scalloped (Sd, TEAD1–4 in mammals) to activate gene expression^{3,4}. The canonical transcriptional targets of the Yki-Sd complex in *Drosophila* include *Cyclin E* (*CycE*), *Death-associated inhibitor of apoptosis 1* (*Diap1*), *bantam* microRNA (*mir-ban*), and *expanded* (*ex*), which promote proliferation, inhibit apoptosis, and enable negative feedback regulation⁵. Although increasing evidence supports an essential role of the Hippo pathway in cell fate determination^{5,6}, the cellular mechanisms remain poorly understood.

The *Drosophila* eye is an excellent model to study gene regulatory networks controlling cell fate determination⁷. Most of the *Drosophila* adult head structures develop from the

larval eye-antennal disc, with the compound eye and ocelli originating from the eye disc compartment, the antenna and maxillary palp from the antennal compartment, and the head epidermis from the tissues surrounding the two compartments⁸. Segregation of the mutually antagonistic eye, antennal, and head epidermal fates, which begins at the second instar larval stage (L2), is regulated by several signaling pathways including Notch, EGFR, Wingless, and Hedgehog, and retinal determination genes such as *eyeless* (*ey*) and *homothorax* (*hth*)^{9,10}. Alteration of these regulatory inputs can cause a switch from one fate to another, leading to partial or in some cases complete homeotic transformations of the affected structures^{11–14}. Key patterning events in the eye are linked to a wave of differentiation called the morphogenetic furrow (MF) that starts in the early third instar larval stage (L3) and proceeds from the posterior to the anterior of the eye disc, resulting in the differentiation of an array of optical units called ommatidia, each consisting of photoreceptor cells, cone cells, primary pigment cells, interommatidial bristles, and secondary and tertiary pigment cells (also called interommatidial cells)^{15,16}.

Previous studies of the Hippo pathway in *Drosophila* eye differentiation focused on MF progression, terminal differentiation of photoreceptor cells, and formation of peripodial epithelium^{17–20}. Mutant analyses of the Hippo pathway components *ex*, *Merlin* (*Mer*) and *mob as tumor suppressor* (*mats*) have suggested an earlier and broader impact of the Hippo pathway in eye specification^{21–24}. However, the involvement of the Hippo pathway in controlling major cell fate decisions among the eye, antenna, and head epidermis remains elusive, and the underlying transcriptional mechanisms are unknown.

We reasoned that the Hippo pathway may function in controlling the eye-antenna-epidermis fate determination through yet unknown interactors that regulate the transcriptional output of the Yki-Sd complex. To identify such interactors, we performed proteomic analyses and identified a Yki-interacting protein, Bonus (Bon). Bon is the only *Drosophila* ortholog of mammalian TIF1 family proteins TIF1 α (TRIM24), TIF1 β (TRIM28/KAP1), TIF1 γ (TRIM33), and TIF1 δ (TRIM66)^{25,26}. TIF1/Bon proteins are chromatin-associated factors that activate or repress transcription by binding to co-regulators and controlling chromatin state^{25–28}. TIF1 proteins play various roles during vertebrate development and are implicated in cancer^{29–31}. *Drosophila* Bon is essential for nervous system development, embryo patterning, metamorphosis, and cell survival^{25,28,32–34}.

Here, we present evidence that Bon and the Hippo pathway co-regulate major cell fate decisions during the development of the *Drosophila* eye. Yki and Bon bind via WW domain-PPxY motif interactions and cooperate to produce epidermal cells in the eye at the expense of ommatidial cells, while loss of *bon* induces ectopic eye markers, suggesting that the Hippo pathway and Bon control the choice between the eye and epidermal fates. The Hippo pathway and Bon also regulate the eye-antennal specification, with Yki and Bon inhibiting the eye fate and promoting the antennal fate. Through the analysis of Bon and Yki protein interactors, we have identified multiple transcriptional and post-transcriptional regulators that are necessary for their control of cell fate decisions. Transcriptomic and genetic analyses have revealed that Bon and Yki exert their functions by jointly activating epidermal differentiation genes and, unexpectedly, repressing Notch target genes. Overall, we have identified a function of the Hippo pathway in the eye/antenna/epidermis cell fate

decisions during *Drosophila* eye development. This function requires the interaction of Yki with Bon, their recruitment of co-regulators, and the joint transcriptional control of a non-canonical set of target genes.

Results

Bon is a Yki interactor

To identify regulators of the Hippo pathway, we performed affinity purification-mass spectrometry (AP-MS) using *Drosophila* embryos expressing Yki-EGFP with a ubiquitous driver *da-GAL4*⁵, as well as cultured *Drosophila* S2 cells expressing streptavidin-binding peptide (SBP)-tagged Yki (see Method Details). We identified the core Hippo pathway components, accessory regulators, and several putative Yki interactors, including Bon (Figure 1A, Table S1)^{5,36,37}. We confirmed the interaction between Bon and Yki by co-immunoprecipitation (co-IP) of tagged Bon and Yki proteins in S2 cells (Figures 1C–D). We hypothesized that the Yki-Bon interaction may be mediated by the two PPxY motifs in Bon (PPLY⁵⁰⁷ and PPSY⁵⁸⁵) binding to the WW domains in Yki (Figure 1B), similar to other known Yki interactors^{37,38}. Mutating the key tyrosine residue to alanine³⁹ in either single or both WW domains in Yki strongly reduced its binding to Bon, with the first WW domain (WW1) showing a stronger reduction in binding upon mutation (Figure 1C). Similarly, substituting tyrosine with alanine in one or both PPxY motifs in Bon also largely reduced its binding to Yki, with a stronger reduction by the mutation in the second PPxY motif (PPxY2) (Figure 1D). These results indicate that Bon interacts with Yki through PPxY motifs in Bon and WW domains in Yki.

Activation of Bon or Yki induces epidermal trichomes in adult eyes

We next sought to investigate whether Bon is involved in Yki-mediated growth regulation. RNAi knockdown of *bon* did not affect the overgrowth or cell proliferation induced by Yki overexpression in adult eyes, larval eye discs, and wing discs (Figures S1A–D). In addition, RNAi knockdown of *bon* in wing discs did not affect the reporters of canonical Yki target genes *Diap1* and *ex* (Figure S1E). Loss-of-function *bon* mutants exhibit ectopic abdominal peripheral neurons in the embryo^{25,32}. Null mutants of *wts* (*wts*^{X1})⁴⁰ and *yki* (*yki*^{B5})⁴¹ did not show loss or gain of peripheral neurons and did not affect the ectopic neuron phenotype in null (*bon*^{21B}) or hypomorphic (*bon*⁴⁸⁷) *bon* mutant embryos (Figure S1F). Collectively, these data argue that Yki and Bon have independent functions in growth regulation and embryonic peripheral nervous system (PNS) development.

Interestingly, overexpression of Bon with *GMR-GAL4*, which drives expression in all cells after the MF and persists through pupal eye development^{42–44}, resulted in the formation of trichomes on the surface of adult eyes and disruption of the ommatidial array (Figures 2A–B and S2A), which is consistent with a previous report⁴⁵. Trichomes are actin-rich, non-neuronal apical extensions, which are present on *Drosophila* epidermal cells but not retinal cells^{46,47}. This trichome induction suggests that Bon promotes epidermal fate in the eye.

To examine whether the Hippo pathway is involved in Bon trichome induction, we overexpressed or knocked down key components of the Hippo pathway in the Bon overexpression background and quantified trichome numbers. Yki overexpression or *wts* knockdown increased Bon-induced trichomes (Figures 2C–D and 2H). In contrast, knockdown of *yki* or *sd*⁴⁸ strongly suppressed Bon-induced trichomes, with only mild eye roughness observed in single knockdowns (Figures 2E–F, 2H, S2B, and S2R–T). Thus, the core Hippo pathway and Yki transcriptional activity are essential for Bon-induced trichomes in adult eyes. Furthermore, overexpression of Bon-PPxY, a Bon mutant with both PPxY motifs mutated to PPxY that cannot bind to Yki (see Figure 1D), resulted in significantly fewer trichomes and milder ommatidial defects (Figures 2G–H), indicating that Bon must bind Yki to efficiently induce eye trichomes.

We then investigated whether Yki activation is sufficient to induce trichomes in adult eyes. Indeed, Yki overexpression by *GMR-GAL4* resulted in trichome formation in the eye (Figures 2I and S2C–D). Overexpression of constitutively active Yki-S168A, a Yki mutant insensitive to Wts phosphorylation and inhibition⁴⁹, induced even more trichomes (Figures 2J, 2O, and S2G). Knockdown of *wts* also induced more trichomes than Yki overexpression (Figures 2K, 2O, and S2K). Therefore, Yki activation promotes trichome formation, with trichome number positively correlated with Yki activity. Knockdown of *bon* strongly suppressed trichome formation induced by wild-type Yki, Yki-S168A, and *wts*-RNAi, with only mild roughness when knocked down alone (Figures 2L–N, 2P–R, and S2D–Q), suggesting that Bon is necessary for trichome formation induced by activated Yki. Together, these results show that the Hippo pathway and Bon jointly regulate the ectopic trichome formation in the eye.

Hippo pathway and Bon control the decision between eye and epidermal cell fates

Next, we investigated cell fate changes underlying ectopic trichome induction in the retina. The establishment of trichomes occurs at 30–36 hrs after puparium formation (APF) in the wing and 38–39 hrs APF in the notum^{47,50}. F-actin and cell boundary (Dlg) staining of pupal eyes revealed that Bon-induced trichomes are initiated at 40 hrs APF (Figures S3A–C) and are easily visible at 44 hrs APF (Figure 3A). Bon overexpression resulted in severe disruption of the ommatidia and produced extra interommatidial-like cells from which the trichomes derive (Figure 3A). Importantly, the excess interommatidial-like cells were generated without alteration in DNA synthesis (Figure S1D) or overall apical cell number (Figure S3D), suggesting a failure of differentiation rather than aberrant proliferation or survival. Interommatidial cells are believed to be the default state in retinal differentiation^{15,51}. Trichomes on these cells indicate that they may be reprogrammed into epidermal-like cells. Knockdown of *yki* or *sd* strongly suppressed the initiation of Bon-induced trichomes in the eye and partially restored the ommatidia (Figure 3A). Therefore, Bon induces epidermal cell fate at the expense of the eye fate in a Yki and Sd-dependent manner.

To determine more precisely which retinal cells were affected, we immunostained pupal eyes at 44 hrs APF for cone cell marker Ct, primary pigment cell marker BarH1, and photoreceptor cell and bristle group marker Elav¹⁵. Bon overexpression with *GMR-GAL4*

strongly inhibited the formation of cone cells and primary pigment cells, and affected the patterning of photoreceptor cells and bristle groups (Figures 3A–B and S3E). *yki* or *sd* knockdown in this background partially rescued the loss of cone cells and the mispatterned photoreceptor cells and bristle groups (Figures 3A–B and S3E), but did not rescue the loss of primary pigment cells (Figure S3E), likely because the differentiation of primary pigment cells requires successful differentiation of cone cells⁵². These results demonstrate that Bon, Yki, and Sd jointly suppress eye fate.

wts knockdown by RNAi also induced trichomes on interommatidial cells at the mid-pupal stage, suggesting that these cells were reprogrammed into the epidermal fate (Figure 3C). Although the trichomes induced by *wts* RNAi were shorter and thicker than Bon-induced trichomes (Figures 2B and 2K), they were clearly distinguishable from the bristle shafts of the bristle groups which have sockets (Figure 3C, Dlg staining) and neuronal input (Figure S3F, Elav staining)¹⁵. *bon* knockdown strongly suppressed *wts* RNAi-induced trichome initiation, but not the excess interommatidial cells and bristles which result from overproliferation^{41,53}, suggesting that Bon is specifically necessary for cell fate reprogramming (Figure 3C). Consistent with previous studies, *wts* RNAi reduced the number of cone cells^{17,54}, which was rescued by knockdown of *bon* (Figures 3C–D). Moreover, *bon* knockdown alone resulted in gain of cone cells, especially at the periphery of the retina (Figures S3H–I). Loss of *wts* and *bon* had a mild effect on photoreceptor patterning and showed a mixed outcome for primary pigment cells (either gain or loss per ommatidium) (Figure S3G). Together, these results show that either Bon activation or Wts inactivation behind the MF can induce epidermal fate at the expense of the eye fate, as represented by trichome formation and inhibition of cone cells.

We further investigated endogenous control of cell fate determination using loss of function clones of *wts* and *bon* alleles. Trichomes were apparent in *wts^{XI}* eye clones at 46 hrs APF, and the trichome-making cells strongly expressed Hth, which normally marks the head epidermis at this stage (Figures 3E–F)^{55,56}. *wts^{XI}* clones also suppressed eye fate, as evidenced by loss of ommatidia (Dlg in Figure 3F) and photoreceptors (Elav in Figure 3G). In adult eyes, *wts^{XI}* clones produced outgrowths of epidermal tissue covered with trichomes (Figure 3H). Interestingly, *bon^{21B}* clones led to gain of neuronal eye marker Elav at the basal side of the larval eye disc, without affecting the normal apical patterns of Elav, suggesting an induction of ectopic eye fate (Figure 3I). Altogether, our results using both loss and gain of function analyses suggest that Bon and Yki suppress eye fate and promote epidermal fate.

Hippo pathway and Bon regulate the choice between eye and antennal cell fates

Since eye fate is antagonistic to epidermal and antennal fates¹⁰, we asked whether Hippo and Bon also regulate the balance of eye-antennal fates. To explore this hypothesis, we used the early eye driver *ey-GAL4* which is expressed in the entire eye disc beginning at the L2 stage, and examined L3 eye-antennal discs using Hth and Ct as antennal markers, and Elav as the differentiated neuron/eye marker^{12,14,57,58}. *wts* knockdown or Yki-S168A overexpression by *ey-GAL4* significantly suppressed the eye field, with the respective penetrance of 56% (*wts*-RNAi), 100% (*yki-S168A-1*), and 85% (*yki-S168A-2*), and even occasionally led to a complete eye-to-antenna transformation, with the respective

penetrance of 11%, 5%, and 6% (Figures 4A–C', 4I, S4A–C', S4E–E', S4I–K' and Table S5). Remarkably, *wts* knockdown or Yki-S168A overexpression also led to clear eye-to-antenna transformations in some adult flies (Figures 4E–G). Wildtype Yki overexpression with *ey-GAL4* suppressed the eye field and resulted in extra epidermal bristles (vibrissae) and occasional epidermal outgrowths, but without eye-to-antenna transformation (Figures S4G–H).

bon knockdown significantly rescued the smaller eye field resulting from Yki-S168A overexpression (from 100% to 3%, and 85% to 43%, with two lines) and suppressed the formation of double antennae (from 5% to 0%, and 6% to 2.5%, with two lines) (Figures 4D–D', 4I, S4D–D', S4F–F', and Table S5). In adults, *bon* knockdown also largely rescued Yki-S168A-induced eye loss (Figure 4H). Bon overexpression using *ey-GAL4* suppressed the differentiated eye field in eye-antennal discs with a penetrance of 20% (Figures 4J–J', S4O–O', and Table S5), and led to a complete or partial loss of the eye with a penetrance of 18% and frequent epidermal outgrowths in adults (Figure 4K and Table S5), but without eye-to-antenna transformation. In contrast, Bon-PPxA overexpression led to largely normal eyes (Figure 4L and Table S5). Altogether, these results show that early and strong activation of Yki across the eye field can switch the eye fate to antennal fate in a Bon-dependent manner, while weaker activation of Yki or Bon transforms the eye to the epidermis.

To determine whether the eye vs. antennal fate choice is under the control of endogenous *yki* and *bon*, we tested the effects of *yki/bon* loss in the antenna using the *hth-GAL4* driver^{14,55}. Since eye and antennal fates are mutually antagonistic and reciprocally transformable¹⁴, we hypothesized that loss of *yki* or *bon* in the antenna would promote eye fate. Indeed, *yki* knockdown alone resulted in variable degrees of antenna loss with 100% penetrance, and moderate antenna-to-eye transformations (13% penetrance) (Figures 4M–N', 4P, S4L–N', and Table S5). This partial antenna-to-eye transformation exhibited loss of Ct and gain of Elav in the antennal field, suggesting a suppression of the antennal fate and differentiation of ectopic eye tissue (Figure 4N). Strikingly, a double knockdown of *yki* and *bon* resulted in a severe antenna-to-eye transformation with a 26% penetrance, in which the antennal field marked by Ct was restricted to the center of the eye-antennal disc, and the ectopic Elav pattern resembled a mirror-image duplication of the endogenous eye field (Figures 4O–P). These results demonstrate that endogenous Yki and Bon are necessary for the specification of the antennal fate and the suppression of the eye fate. Altogether, our results support a model of eye specification regulated by the Hippo pathway and Bon, where Yki and Bon promote the epidermal and antennal fates and suppress the eye fate (Figure 4Q).

Interactors of Bon and Yki are required for the eye-epidermal fate choice

We sought to further investigate the mechanism through which Yki and Bon regulate the eye-epidermal fate choice. One potential mechanism is that Bon enhances Yki activity by competing Yki away from Wts, given that both Bon and Wts bind to Yki through the PPxY-WW interaction. However, co-IP in S2 cells showed no competition between Bon and Wts for binding to Yki (Figure S5A). This result suggests that rather than competing with Wts, Bon may employ another mechanism, such as engaging a special set of interactors to mediate the eye-epidermal cell fate decision together with Yki.

To identify Bon cofactors, we analyzed the Bon interactome by AP-MS using Bon-SBP expressed in S2 cells (Figure 5A and Table S2). Yki was identified as one of the top Bon interactors, further validating their interaction (Figure 5A). In addition, we identified Histone deacetylase 1 (HDAC1), Suppressor of variegation 2–10 (Su(var)2–10), and Heterogeneous nuclear ribonucleoprotein at 27C (Hrb27C) as top Bon interactors (Figure 5A). HDAC1 and its mammalian orthologs are key components of several transcriptional corepressor complexes, such as NuRD, Sin3, and CoRest⁵⁹. Su(var)2–10 belongs to the PIAS family of SUMO ligases and controls gene transcription as well as *Drosophila* eye specification^{60–62}. Hrb27C is an abundant hnRNP that is involved in various aspects of post-transcriptional mRNA regulation and is implicated in the modulation of Yki activity^{63–65}. We tested whether these three Bon interactors are involved in trichome formation in adult eyes. Knockdown of *HDAC1* strongly suppressed, whereas its overexpression enhanced, both Bon- and Yki-S168A-induced trichomes (Figures 5B–C, 5G–H, 5L, and S5B–C), and knockdown of *Su(var)2–10* or *Hrb27C* suppressed these trichomes (Figures 5D–E, 5I–J, 5L, and S5B–C). Therefore, HDAC1, Su(var)2–10, and Hrb27C promote Bon- or Yki-induced epidermal fate in the eye.

The transcription factor Shavenbaby/Ovo (Svb/Ovo) plays a key role in the formation and patterning of epidermal trichomes^{47,66}. Also, Svb interacts with Yki and regulates apoptosis through *Diap1*⁶⁷. We tested if Svb/Ovo was involved in the regulation of Bon- and Yki-induced trichomes in adult eyes. Overexpression of the constitutive activator OvoB enhanced Bon- and Yki-S168A-induced trichomes, while overexpression of the constitutive repressor OvoA, or knockdown of somatic *svb*, strongly suppressed Bon- and Yki-S168A-induced trichomes (Figures 5F, 5K–L, and S5B–C)^{47,68}. These results suggest that Svb/Ovo is required for ectopic trichome generation in the eye induced by Bon or Yki.

We then asked if these interactors are involved in Bon and Yki regulated suppression of eye fate. In pupal eyes, knockdown of *HDAC1* or *svb* with *GMR-GAL4* suppressed Bon-induced trichomes but did not rescue the loss of cone cells (Figures 5M–N). In contrast, knockdown of *Su(var)2–10* or *Hrb27C* not only suppressed the trichomes, but also largely rescued the number of cone cells (Figures 5M–N). Interestingly, *Su(var)2–10* RNAi even partially restored the loss of primary pigment cells and mispatterned ommatidial array (Figure S5D). These results suggest that Su(var)2–10 and Hrb27C contribute to both eye fate suppression and epidermal fate promotion in the pupal eye, while HDAC1 and Svb/Ovo are only involved in the latter function at this stage. In L3 eye-antennal discs, Bon overexpression with the *bi-GAL4* driver, which drives expression at the dorsal and ventral margins of the eye disc from L2 stage⁶⁹, inhibited Elav expression (Figures 5O–Q), similar to Yki overexpression in a previous report¹⁷. Knockdown of *Su(var)2–10* or *HDAC1* suppressed the loss of Elav due to Bon overexpression (Figures 5R–S and 5O). Furthermore, knockdown of *HDAC1* under *ey-GAL4* suppressed the loss of eye field due to Yki-S168A overexpression (Figures S5E–G). Collectively, these results suggest that Bon, Yki, Hrb27C, Su(var)2–10, and HDAC1 work together to suppress eye fate and promote epidermal fate, with the involvement of Svb/Ovo in the latter.

Bon and Yki control cell fate decisions in the eye through transcriptional regulation of joint target genes

Since Bon, Yki, and interactors described above are all transcriptional or post-transcriptional regulators, we hypothesized that they mediate cell fate choices in the eye by regulating a unique set of transcriptional targets. To identify these target genes, we performed RNA sequencing (RNA-seq) using pupal eyes at 40–41 hrs APF (when the trichomes initiate) that overexpressed Bon, with or without a simultaneous RNAi knockdown of *yki*. Through differential gene expression analysis, we identified 216 genes as Bon-activated/Yki-dependent genes, and 119 genes as Bon-repressed/Yki-dependent genes (Figures 6A, S6A–B, and Table S3). Correlation coefficient analysis revealed the significantly concordant regulation of gene expression by Bon and Yki (Figure 6B). Also, *ex* and *Rhodopsin 5* (*Rh5*), which are known targets of Yki in the pupal eye^{18,70}, were among Bon-activated/Yki-dependent genes (Figures 6A and S6A–B). These transcriptomic data suggest that Bon and Yki work together to regulate gene expression in pupal eyes, consistent with our genetic results.

We evaluated genes jointly regulated by Bon and Yki by Gene Ontology analysis of Biological Process (GO_BP, Table S4). Bon-activated/Yki-dependent genes were enriched for GO terms “epidermal cell differentiation”, “cuticle development”, and “sensory perception of taste”, while Bon-repressed/Yki-dependent genes were enriched for GO terms “compound eye development” and “Notch signaling pathway”, consistent with the genetic function of Bon and Yki in promoting epidermal/antennal fates and suppressing the eye fate (Figure 6C). Bon-activated/Yki-dependent epidermal genes, *shavenoid* (*sha*), *forked* (*f*), *nyobe* (*nyo*), and *neyo* (*neo*), are known Svb/Ovo target genes that are essential for the formation and patterning of epidermal trichomes, suggesting that they may mediate Bon- and Yki-induced trichome formation in the eye (Figures 6A and 6C)^{66,71}. Bon-repressed/Yki-dependent Notch pathway genes, *E(spl)mdelta-HLH*, *E(spl)m3-HLH*, and *E(spl)m2-BFM*, are members of the Enhancer of split gene complex (*E(spl)-C*), which is a major transcriptional target of Notch (Figures 6A and 6C)^{72,73}. Two other Notch target genes, *ct* and *Transcription factor AP-2* (*TfAP-2*), were also identified among Bon-repressed/Yki-dependent genes (Figure 6A)^{74,75}. Previous work showed that Notch signaling promotes eye fate and suppresses antennal fate, and that Notch, *E(spl)-C*, *Ct*, and *TfAP-2* are all critical for cell fate establishment in the eye^{12,14,52,76–79}. These observations raised an intriguing possibility that downregulation of Notch targets by Yki and Bon mediates suppression of eye fate and promotion of antennal fate. Finally, Bon-activated/Yki-dependent genes included sensory perception genes such as antenna-specific odorant receptor *Or47b*, as well as a group of gustatory receptors (*Gr64a-f*) that are expressed in multiple sensory organs including the antenna, indicating induction of antennal identity at the molecular level (Figures 6A, 6C, and S6A–B)^{80–83}.

We validated the transcriptional regulation of *sha*, *f*, *E(spl)m3-HLH*, *E(spl)m2-BFM*, and *E(spl)mdelta-HLH* by Bon and Yki using target-specific qPCR, which generally confirmed the RNA-seq findings (Figure 6D). To determine whether the transcriptional targets of Yki and Bon are under their direct control, we analyzed published ChIP-seq datasets for Yki and Bon, and the DamID-seq dataset for Sd^{84–86}. Pairwise overlaps of binding sites were

significant, with 75 loci shared among all three proteins, including the *E(spl)-C* region, suggesting a general co-localization of Yki, Bon, and Sd on chromosomes and their direct control of *E(spl)-C* (Figure 6E and S6C). Yki and Sd also bound to *ct* and *TfAP-2*, raising the possibility of a direct regulation (Figure S6C). Yki, Sd, and Bon did not associate with the epidermal differentiation genes and antennal sensory receptor genes, except for Yki binding to *neo*, suggesting indirect activation of these genes by Bon and Yki (Figure S6C). To validate the binding of Bon to the *E(spl)mdelta-HLH* genomic locus, we performed ChIP-qPCR using L3 eye-antennal discs expressing Bon-mCherry with *GMR-GAL4*. We chose two DNA segments (P1 and P2) that overlapped the Yki and Sd binding regions (Figure 6F). Bon bound to P2 but did not significantly associate with P1 (Figure 6G). Together, these results suggest that Bon and Yki jointly and directly repress Notch targets and indirectly activate epidermal and antennal genes.

Identification of genes that are repressed by Yki and Bon was unexpected and suggests that binding by Bon may shift Yki activity towards repression. We further evaluated the repression of *E(spl)-C* by Bon and Yki using a BAC transgene reporter expressing GFP-tagged E(spl)mdelta-HLH (*GFPmδ*) and a transcriptional reporter of *E(spl)m3-HLH* (*m3-GFP*)⁸⁷. In the L3 eye disc, both reporters are expressed within and posterior to the MF (Figures 6H and 6J). *wts^{X1}* clones showed strongly reduced expression of *GFPmδ* and *m3-GFP*, and moderately reduced Elav (Figures 6H and S6D). In pupal eyes at 25 hrs APF, *GFPmδ* is mainly expressed in primary pigment cells (Figure 6I). *bon^{21B}* clones resulted in a gain of *GFPmδ*-positive cells, suggesting that Bon suppresses *E(spl)mdelta-HLH* expression and inhibits the primary pigment cell fate (Figure 6I). *wts* knockdown with the *DE-GAL4* driver, which is expressed in the dorsal eye in both columnar cells of the disc proper (DP) and squamous peripodial epithelium (PE) (Figure S6E)⁸⁸, reduced *GFPmδ*, *m3-GFP*, and Elav (Figures 6J and S6F). Knockdown of *yki* or *sd* expanded *GFPmδ*, *m3-GFP*, and Elav into the PE (Figures 6K and S6G–I), reminiscent of a PE to DP transformation previously reported for loss of *yki* and *sd*¹⁹. *bon* knockdown also led to an occasional expansion of *m3-GFP* and Elav into the PE, with a penetrance of 8.3% and 12.5% for two RNAi lines (Figures 6L, S6J, and Table S5). Together, these results show that Bon, Yki, and Sd repress, while Wts promotes, the expression of *E(spl)mdelta-HLH* and *E(spl)m3-HLH* during eye development.

We then asked whether the target genes we identified are involved in the eye-antenna-epidermis fate determination controlled by Bon and Yki. *sha* overexpression enhanced, whereas *f* knockdown suppressed, trichomes induced by Bon and Yki-S168A in adult eyes (Figures 7A–C, 7E–G, 7I, and S6K–L). Therefore, *sha* and *f* promote Bon/Yki-induced epidermal fate in the eye, consistent with their transcriptional activation by Bon and Yki. In contrast, overexpression of E(spl)mdelta-HLH and E(spl)m3-HLH suppressed the trichomes induced by Bon and Yki-S168A, suggesting that they inhibit the epidermal fate in the eye, in agreement with their transcriptional repression by Bon and Yki (Figures 7D, 7H, 7I, and S6M–N). Overexpression of E(spl)m3-HLH significantly rescued the loss of eye field and completely suppressed the formation of double antennae due to Yki-S168A overexpression, suggesting that E(spl)-C promotes eye fate and suppresses antennal fate (Figures 7J–K, S5G, and Table S5). Therefore, Bon and Yki control cell fate decisions in the eye through

transcriptional regulation of a set of non-canonical target genes: activation of *sha* and *f*, and repression of *E(spl)-C* genes.

Discussion

We have revealed a previously unappreciated and profound role of the Hippo pathway in controlling cell fate determination in the *Drosophila* eye (Figure 7L). This function depends on a regulatory program in which Yki and Bon interact, and likely function within larger multiprotein complexes that include other transcriptional and post-transcriptional regulators. Instead of mediating the previously described independent functions of Yki (growth control) and Bon (PNS differentiation), the Yki-Bon module regulates proper segregation of the eye, epidermal, and antennal fates in the developing eye. This function involves promotion of the epidermal and antennal fates, and suppression of the eye fate, via transcriptional regulation of a distinct set of target genes (Figure 7L). Our study thus provides a molecular mechanism for the biological function of the Hippo pathway and Bon in cell fate determination during eye development.

Hippo pathway and Bon control eye-antenna-epidermis cell fate decisions at two levels

Our results suggest that the Hippo pathway and Bon regulate the developmental cell fate decisions in the eye at two levels (Figure S7). First, the Yki-Bon complex promotes antennal and epidermal fates and suppresses the eye fate during the early eye field specification, before the L3 larval stage. This is supported by the phenotypes observed under various genetic manipulations of Bon, Wts, Yki, and Sd during the L1 and L2 larval stages, including the reciprocal transformations of eye and antenna, epidermal outgrowths in the eye, and ectopic eye fate (Figures 3, 4, 6, S4, and S6). The Yki-Bon module is thus an essential component of the extensive gene regulatory network that controls these cell fate decisions in early eye development^{9,12}. Previous studies showed that *ex*, *Mer*, and *mats* mutants exhibit eye-to-epidermal transformation and an occasional eye-to-antenna transformation (in an *ex* mutant combination), suggesting that the upstream Hippo pathway may also regulate the Yki-Bon module in fate determination at this stage^{21–24}.

Second, after the segregation of the eye/antenna/epidermis fields and the start of MF in L3, the Yki-Bon complex promotes the epidermal cell fate while suppressing ommatidia, whereas Wts counteracts this activity. This is evidenced by the formation of epidermal trichomes in the retina and the suppression of ommatidial cell types, especially cone cells, upon knockdown of *wts* or overexpression of Bon or Yki with the late eye driver *GMR-GAL4* (Figures 2, 3, and S3). Furthermore, our RNA-seq data also revealed cell fate regulation at the molecular level: the Yki-Bon complex activates epidermal differentiation genes (*sha*, *f*, *nyo*, and *neo*), and represses Notch targets (*E(spl)-C*, *ct*, and *TfAP-2*) that are required for eye fate establishment and are expressed in ommatidial cells (e.g., *ct* in cone cells and *E(spl)mdelta-HLH* in primary pigment cells) (Figure 6). Although activation of the Yki-Bon complex at this stage did not exhibit an eye-to-antenna transformation phenotype, certain antennal genes (*Gr64s* and *Or47b*) were upregulated, suggesting transformation at the level of gene expression.

The eye-antenna-epidermis fate determination was previously studied during the early stages of eye-antennal disc development¹⁰. Our work shows that these fates are not completely defined during the early stages, as the retina could still transform into epidermal tissue and express epidermal and even antennal genes when the Hippo pathway and Bon were modulated after MF formation. Notably, conditional knockout of *eya* after the MF results in suppression of ommatidia and formation of trichomes in the eye⁸⁹. This suggests that the retinal determination genes are also involved in eye-epidermal fate decisions during later stages of eye development, and that trichome induction may be a general biological outcome of interference with the eye vs. epidermis specification after the start of MF. Thus, we conclude that the eye is still developmentally plastic at late stages, with a latent epidermal fate that is normally inhibited. Interestingly, this fate is revealed in the insect order Strepsiptera, whose compound eyes are comprised of optical units that are separated by epidermal tissue bearing trichome-like extensions⁹⁰.

Given Bon's role in promoting the epidermal fate in the eye, we asked if it is involved in epidermal differentiation in other tissues. Knockdown of *bon* by RNAi in the wing with the *C5-GAL4* driver⁹¹ did not affect the number of trichomes, but trichome morphology was abnormal, with *bon*-RNAi wing cells growing thinner trichomes (Figures S3J–K). *bon*^{21B} mutant sensory bristles on the notum showed a similar thinning effect, although the *Sb* clonal marker precluded genotyping surrounding epidermal cells (Figures S3L–M"). These results suggest that Bon may contribute to epidermal differentiation in other contexts, in addition to its role in the eye.

Yki and Bon control cell fate decisions in the eye by recruiting cofactors and regulating a distinct set of target genes

We have identified an unexpected layer of control over eye specification exerted by Yki and Bon at the level of Notch target genes. The Hippo pathway has been reported to control cell fate determination in other biological contexts through regulation of the Notch receptor or ligands^{92–94}. Although we have identified several Notch targets that are repressed by Bon and Yki, Notch and its ligands, Serrate and Delta, were not jointly regulated or found in high-confidence Yki or Bon protein interactome (Tables S1–S3). Therefore, we propose that during cell fate determination in the eye, Bon and Yki repress Notch targets (such as *E(spl)-C* genes) independently from upstream Notch signaling. We note that not all *E(spl)-C* genes are under Bon and Yki control, implying context-dependent regulation and functional divergence of *E(spl)-C* genes, as suggested by previous studies (Table S3)^{87,95}. Both Hippo and Notch contribute to cell proliferation and growth of the eye^{8,96}. Our data suggest that Bon is not required for the growth-controlling function of the Hippo pathway (Figure S1). Instead, the Bon-Yki complex directs the acquisition of appropriate cell fates in the eye through the regulation of Notch targets. We speculate that Bon may function as a switch that redirects some of Hippo pathway activities from growth regulation to cell fate determination.

So far, *Drosophila* Yki has only been implicated in transcriptional activation⁵. However, studies in mammalian systems have shown that the YAP/TAZ-TEAD complex can also function as a transcriptional repressor on non-canonical target genes^{97–99}. The repression of Notch targets reported here suggests that *Drosophila* Yki can also function in transcriptional

repression, likely via recruitment of corepressors mediated by Bon. HDAC1 and its associated corepressor complexes repress gene transcription, including Notch targets^{59,100}. We identified HDAC1 and its corepressor, CoRest, in the Bon interactome (Figure 5A), raising the possibility that Bon and Yki repress Notch target genes in part via recruiting this repressor complex. The involvement of epigenetic regulators is further exemplified by Su(var)2–10, which has a role in chromatin SUMOylation and piRNA target silencing⁶². Interestingly, Su(var)2–10 can suppress eye fate and even induce antennal fate in a sensitized background⁶¹. Due to the strong genetic interaction between Su(var)2–10 and the Bon-Yki complex (Figures 5 and S5) and the identification of the *Drosophila* SUMO (smt3) in the Bon interactome (Figure 5A), chromatin SUMOylation may be involved in gene repression by Bon and Yki. Future studies of chromatin status and epigenetic marks may reveal the mechanistic details of gene repression by Bon and Yki.

The Hippo pathway and TIF1 family proteins are conserved and broadly expressed in higher eukaryotes^{5,26}, raising the possibility that they may also function together in other species and developmental processes, such as retinogenesis and hematopoiesis^{101–104}. Thus, the biological functions controlled by the Hippo pathway and Bon and the underlying molecular mechanisms we report here may be evolutionarily conserved.

Limitations of the Study

RNA-seq was performed using pupal eyes when trichomes initiate; however, the pupal eye patterning defects were detectable before trichome initiation (Figures S3A–C). Thus, there might be additional differentially expressed genes at earlier stages of cell fate determination that we have missed. Cells mutant for a null allele of *bon* tend to be eliminated²⁵, potentially masking additional cell differentiation defects in *bon* mutant clones. Although our study has largely focused on eye development, it is possible that Bon and Yki interaction has additional functions in other tissues. Further studies are needed to analyze the precise composition of multiprotein complexes involving Yki and Bon, as well as their effects on the target genes we identified here.

STAR Methods

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Alexey Veraksa (alexey.veraksa@umb.edu).

Materials availability—Plasmids, cell lines, and transgenic *Drosophila* strains generated in this study are available upon request.

Data and code availability—The RNA-seq dataset generated in this study has been deposited in NCBI's Gene Expression Omnibus¹⁰⁹ and is accessible through GEO Series accession number GSE181299. Mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE¹¹⁰ partner repository with the dataset identifier PXD027934. Raw images of Western blots have been deposited to Mendeley Data.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines—*Drosophila* S2 cells were cultured in Schneider's *Drosophila* Medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 100 unit/ml of penicillin/streptomycin at 25°C. Stable cell lines with recombinant DNA were selected by culturing the cells in the medium supplemented with 300 µg/ml Hygromycin.

***Drosophila* strains**—*Drosophila melanogaster* stocks were maintained at 18°C or room temperature (RT, 22°C) on standard medium containing cornmeal, molasses, yeast and agar. Genetic crosses were carried out at 25°C unless otherwise indicated. Both male and female animals were used unless otherwise noted.

METHOD DETAILS

Plasmid construction—pMK33-yki-SBP, pUASTattB-yki-EGFP and pMT-yki-HA were generated by cloning *yki-RG* isoform from pMT-yki-V5³⁹ into pMK33-SBP-C vector¹¹¹, pUASTattB vector¹¹², and pMT-V5-His vector (Invitrogen, Cat# V412020), respectively. The numbering of the key tyrosine residues for Yki corresponds to the first reported *yki* sequence (*yki-RH*) which has a stretch of additional 23 amino acids at the N-terminus⁴¹. pMT-bon-V5, pMK33-bon-SBP, and pUASTattB-bon-mCherry were generated by cloning *bon-RB* isoform from pFlc-1-bon (DGRC, RE48191). All tags for Yki and Bon were added to the C-terminus. Mutagenesis in WW domains of Yki and PPxY motifs of Bon was done by Q5 Site-Directed Mutagenesis Kit (NEB, Cat# E0554S). pMT-Myc-wts was generated by cloning the coding sequence of *wts* from pFlag-wts plasmid kindly offered by Maxim Frolov Lab. Myc tag for Wts was added to the N-terminus.

Stable cell lines—S2 cells were transfected with pMK33-yki-SBP and pMK33-bon-SBP constructs using the Effectene transfection reagent (Qiagen, Cat# 301427). Stable cell lines were selected with medium containing 300 µg/ml of Hygromycin B (MilliporeSigma, Cat# H3274). Yki-SBP and Bon-SBP stable cells were induced overnight with 0.07 mM CuSO₄, and protein expression was verified by western blot using anti-SBP antibody (1:1000, Santa Cruz, Cat# sc-101595).

Transgenic fly lines—pUASTattB-yki-EGFP plasmid was injected into the attP2 site in *D. melanogaster* embryos (Genetic Services, Inc). pUASTattB-bon-mCherry and pUASTattB-bon-Y507A Y585A-mCherry plasmids were injected into the attP40 site in *D. melanogaster* embryos (Rainbow Transgenic Flies, Inc). *yw*, Chr. 2 and Chr. 3 balancer stocks were used in standard crossing schemes to establish the homozygous transgenic lines.

Genetic crosses—To score the trichome phenotype, *UAS-bon-mCherry*, *UAS-yki-EGFP*, *UAS-yki-S168A-1* (*UAS-yki.S168A.GFP.HA*, BDSC: 28816) and *UAS-wts-RNAi* (VDRC: 111002) were combined with the eye driver *GMR-GAL4* to generate *UAS-bon-mCherry/CyO wg-lacZ*; *GMR-GAL4/TM6B*, *GMR-GAL4 UAS-yki-EGFP/TM6B*, *GMR-GAL4 UAS-yki-S168A-GFP-1/TM6B* and *UAS-wts-RNAi/CyO wg-lacZ*; *GMR-GAL4/*

TM6B fly lines, respectively. *GFP*, *w*-RNAi-1, or *w*-RNAi-2 were coexpressed for transgene dosage compensation.

To achieve higher GAL4 activity, all crosses with *GMR-GAL4 UAS-yki-EGFP*, *GMR-GAL4 UAS-yki-S168A-GFP-1*, and *UAS-wts*-RNAi *GMR-GAL4* were set up at 25°C and shifted to 29°C after the emergence of first instar larvae. All the crosses with *UAS-bon-mCherry*; *GMR-GAL4* were carried out at 25°C.

All the crosses using *ey-GAL4* and *ey-GAL4-2* were carried out at 29°C except for the cross of *ey-GAL4* and *UAS-wts*-RNAi which was carried out at 25°C. The cross of *da-GAL4* and *UAS-yki-EGFP* for embryo collection and affinity purification was carried out at RT. All other crosses were carried out at 25°C.

Mosaic clones with hsFLP/FRT were generated by heat-shocking first instar larvae with indicated time (figure legends) after egg deposition (AED) at 37°C for 1 hr. Genotypes used for clones are as follows:

3E: *hsFLP*::*FRT82B/FRT82B Ubi-GFP*

3F-G: *hsFLP*::*FRT82B wts^{X1}/FRT82B Ubi-GFP*

3H: *hsFLP*::*FRT82B wts^{X1}/FRT82B Sb^{63b}*

3I ctrl: *eyFLP*::*FRT82B/FRT82B Ubi-GFP*

3I *bon^{21B}*: *eyFLP*::*FRT82B bon^{21B}/FRT82B Ubi-GFP*

S3L: *hsFLP*::*FRT82B/FRT82B Sb^{63b}*

S3M: *hsFLP*::*FRT82B bon^{21B}/FRT82B Sb^{63b}*

6H ctrl: *eyFLP*::*GFPmδ/+; FRT82B/FRT82B Ubi-mRFP*

6H *wts^{X1}*: *eyFLP*::*GFPmδ/+; FRT82B wts^{X1}/FRT82B Ubi-mRFP*

6I *bon^{21B}*: *eyFLP*::*GFPmδ/+; FRT82B bon^{21B}/FRT82B Ubi-mRFP*

S6D ctrl: *eyFLP*::*m3-GFP/+; FRT82B/FRT82B Ubi-mRFP*

S6D *wts^{X1}*: *eyFLP*::*m3-GFP/+; FRT82B wts^{X1}/FRT82B Ubi-mRFP*

Affinity Purification

Affinity purification from S2 cells.: Blank S2 cells (control) and stable cell lines with 50 ml dense culture were induced overnight (~16 hrs) with 0.07 mM CuSO₄ at 25°C. Cells were collected and lysed on ice for 20 min using 1 ml of ice-cold Default Lysis Buffer (DLB) (50 mM Tris pH 7.5, 125 mM NaCl, 5% (v/v) glycerol, 0.2% (v/v) IGEPAL, 1.5 mM MgCl₂, 25 mM NaF, 1 mM Na₃VO₄, 1 mM DTT, and 2x cComplete Protease Inhibitor (MilliporeSigma, Cat# 11836145001, 1 tablet per 25 ml lysis buffer)). The lysate was centrifuged at 16,000 rcf for 20 min at 4°C, and the supernatant was incubated with

50 μ l of packed Streptavidin beads (Thermo Fisher Scientific, Cat# 53117) for 2 hrs at 4°C with rotation. The beads were then washed 5 times with 1 ml DLB. Proteins on the beads were eluted with 300 μ l DLB containing 2 mM biotin and precipitated by adding 1/10 volume of 8.74 M Trichloroacetic acid solution (TCA, Fisher Scientific, Cat# BP555–500). Precipitated proteins were washed once with 500 μ l of 0.874 M TCA and four times with ice-cold acetone. The protein pellet was dried overnight at RT and incubated in 40 μ l of 2x SDS sample buffer at 95°C for 5 min. Samples were assessed by silver staining using NuPAGE 4–12% Bis-Tris Protein Gel (Thermo Fisher Scientific, Cat# NP0335) and SilverQuest Silver Staining Kit (Thermo Fisher Scientific, Cat# LC6070). The protein samples for mass spectrometry were separated on a short SDS-PAGE gel (8% Tris-Glycine) followed by Coomassie blue staining. Two gel pieces (>75 kDa and < 75 kDa) for each sample were submitted to Taplin Mass Spectrometry Facility at Harvard Medical School for mass spectrometry analysis.

Affinity purification from embryos.: 5 L fly cages with apple juice plates¹¹³ were set up for *yw* (control) and the cross *da-GAL4* x *UAS-yki-EGFP*. Flies were allowed to lay eggs for 15 hrs at RT, then the apple juice plates containing the embryos were incubated for 3 hrs at 25°C. Approximately 1 g embryos were dechorionated with 50% (v/v) Clorox bleach and washed with water. Collected embryos were then mixed with 6 ml of ice-cold DLB containing 0.5% (v/v) IGEPAL and 10 μ M MG132 in a glass homogenizer and lysed on ice with 20–30 strokes using a tight pestle. The lysate was kept on ice for 20 min and centrifuged at 16,000 rcf for 20 min at 4°C. The supernatant was filtered with pre-chilled 0.45 μ m filter and incubated with 50 μ l of packed Pierce Control Agarose Resin (Thermo Fisher Scientific, Cat# 26150) for 30 min at 4°C with rotation to preclear unspecific binding. The lysate was then incubated with 20 μ l of packed GFP-Trap Agarose (Bulldog Bio, Cat# GTA020) for 2 hrs at 4°C with rotation. After binding, the GFP beads were washed 5 times with 1 ml DLB containing 0.5% (v/v) IGEPAL and 10 μ M MG132, followed by addition of 40 μ l of 4x SDS sample buffer and heating at 95°C for 6 min. The samples were then assessed by silver staining and the protein samples for mass spectrometry were prepared and submitted as above.

Mass Spectrometry and data analysis—In-gel trypsin digestion and liquid chromatography/tandem mass spectrometry (nanoLC-MS/MS) were performed by Taplin Mass Spectrometry Facility at Harvard Medical School. Mass spectrometry results from two gel pieces for each sample were combined, and the results from the experimental and control samples were analyzed with Significance Analysis of INteractome (SAINT) (v2.5.0) using the total peptides identified for each protein¹⁰⁵. Any protein with the SAINT score (average probability) \geq 0.8 was considered as a high-confidence interactor and was included in the interactome. SAINT analysis included the following numbers of samples: Yki-SBP, four experiments and four controls; Yki-EGFP, three experiments and five controls; Bon-SBP, two experiments and three controls.

Yki protein interactome.: Yki protein interactors with SAINT score \geq 0.8 either in S2 cells or in embryos were included in the Yki protein interactome. The Yki protein interactome was further populated with the interactions incorporated from the STRING

database (v11.0) and FlyBase (vFB2020_04)^{106,107}. All proteins in the Yki interactome from the current study were imported into the STRING database and analyzed with default settings: full network, medium confidence 0.4 and all active interaction sources (Textmining, Experiments, Databases, Co-expression, Neighborhood, Gene Fusion and Co-occurrence). The summary of physical interactions of Yki in FlyBase was selected to show neighbor-neighbor interactions, and the summary of genetic interactions was selected to show both suppression and enhancement. The interaction data from FlyBase were exported through esyN¹¹⁴, while only the interactions between the proteins that were identified as components of the Yki protein interactome from the current study were incorporated into the Yki protein interactome. The Yki protein interactome was visualized with Cytoscape¹¹⁵. Nodes represent Yki and Yki interactors identified in the current study. Edges represent the interactions incorporated from STRING and FlyBase. Clustering was done manually based on FlyBase annotations and publications¹⁰⁷. Gene symbols were updated to FlyBase version FB2020_04, released Aug 18, 2020.

Bon protein interactome. Bon protein interactors with SAINT score > 0.8 from S2 cells were included in the Bon protein interactome which was visualized with Cytoscape. The nodes and edges represent the interactors and interactions identified in the current study, respectively. Gene symbols were updated to FlyBase version FB2020_04, released Aug 18, 2020.

Co-Immunoprecipitation—S2 cells were transfected with indicated plasmids or blank pMT-V5-His vector using the Effectene transfection reagent. 24 hrs after transfection, cells were induced with 0.35 mM CuSO₄ overnight at 25°C, collected and lysed on ice for 20 min with 600 µl of ice-cold DLB. The lysate was centrifuged at 16,000 rcf for 20 min at 4°C. 40 µl supernatant was mixed with 20 µl of 4x SDS sample buffer and heated at 95°C for 6 min to generate lysate samples. The rest of the lysate was incubated with 20 µl of packed anti-V5 beads (MilliporeSigma, Cat# A7345) or anti-HA beads (MilliporeSigma, Cat# E6779) for 2 hrs at 4°C. The beads were then washed 3 times (or 4 times for co-IP with Myc-wts) with 1 ml DLB, mixed with 40 µl of 4x SDS sample buffer, and heated at 95°C for 6 min to generate IP samples. The lysate samples and IP samples were subjected to SDS-PAGE followed by western blot using Odyssey Blocking Buffer (PBS) (LI-COR Biosciences, Cat# 927-40003) as blocking buffer, mouse anti-V5 antibody (1:1000, MilliporeSigma, Cat# V8012), Rabbit anti-HA antibody (1:1000, MilliporeSigma, Cat# H6908) and Mouse anti-Myc antibody (1:1000, Cell Signaling, Cat# 2276S) as primary antibody, and Goat anti-Mouse IgG (1:20,000, LI-COR Biosciences, Cat# 926-68070) and Donkey anti-Rabbit IgG (1:20,000, LI-COR Biosciences, Cat# 926-32213) as secondary antibodies. Blotting membranes were scanned with LI-COR Odyssey CLx Imaging Systems.

EdU incorporation assay—EdU incorporation assay was performed using Click-iT[®] EdU Imaging Kit (Thermo Fisher Scientific, C10086) with adapted procedures from¹¹⁶. Eye discs were dissected in unsupplemented Schneider's *Drosophila* Medium and incubated in 100 µM EdU for 20 min in the dark at RT. Discs were then washed three times with 1x PBS, fixed in 3.7% (v/v) Formaldehyde (MilliporeSigma, Cat# F8775) in 1x PBS for 15 min in the dark at RT, followed by three washes with 1x PBS and permeabilization in PB-Triton

(1x PBS containing 0.3% (v/v) Triton X-100) for 20 min at RT. Discs were then washed twice with 3% BSA (m/v, VWR, Cat# RLBSA50) in 1x PBS and incubated in Click-iT reaction cocktail containing Alexa Fluor 488 (for *bon-mChery*) or 594 (for *yki-S168A-GFP*) for 30 min in the dark at RT. After washing once with 3% BSA in 1x PBS and once with 1x PBS, discs were mounted in ProLong Gold Antifade Mountant with DAPI (Thermo Fisher Scientific, Cat# P36931). All incubation and washing were carried out on a platform shaker with mild shaking.

Tissue staining

Antibodies and phalloidin.: All antibodies and phalloidin reagents used in this study are listed in the Key Resources Table. All primary antibodies from DSHB were used at 1:50 (v/v) dilution. All primary and secondary antibodies and phalloidin from Thermo Fisher Scientific were used at 1:500 (v/v) dilution. Mouse anti- β -Gal antibody (Promega, Cat# Z3783) was used at 1:100 (v/v) dilution. Guinea Pig anti-Bon antibody was used at 1: 5000 (v/v) dilution²⁵. Guinea Pig anti-Hth antibody was used at 1:1000 (v/v) dilution¹¹⁷. Rabbit anti-BarH1 was used at 1:500 (v/v) dilution¹¹⁸. Phalloidin 405 (Biotium, Cat# 00034-T) was used at 1:100 (v/v) dilution.

Embryo staining.: Stage 16 embryos were collected and dechorionated with 50% (v/v) Clorox bleach, rinsed with water, and fixed in 20 ml of fixative containing 4% (v/v) paraformaldehyde (Electron Microscopy Sciences, Cat# 15710), 25% (v/v) Heptane and 1x PBS for 20 min at RT. 8 ml of methanol was added to remove the vitelline membrane. Fixed and devitellinized embryos were washed three times with 1.4 ml methanol, four times with 1.4 ml ethanol and twice with PBT (1x PBS containing 0.1% (v/v) Tween 20). Embryos were subsequently incubated in 1 ml of blocking solution (1:1 (v/v) of Western Blocking Reagent (Roche, Cat#11921681001) and PBT) for 2 hrs at RT and incubated overnight in 500 μ l of blocking solution containing primary antibody at 4°C. Embryos were then washed five times with 0.1% BSA (m/v in PBT) and incubated in 1 ml of blocking solution for 1 hr at RT. The embryos were then incubated in secondary antibody diluted in blocking solution for 2 hrs in the dark at RT, washed sequentially in 0.1% BSA, PBT and 1x PBS, and mounted with ProLong Gold Antifade Mountant with DAPI. All incubation and washing were carried out on a nutator.

L3 imaginal disc staining.: 3rd instar larval wing discs and eye-antennal discs were dissected in ice-cold 1x PBS and were fixed in 3.7% (v/v) formaldehyde (MilliporeSigma, Cat# F8775) for 15–20 min at RT. The discs were washed three times with 1x PBS, permeabilized with PB-Triton for 20 min at RT and were incubated in primary antibodies diluted in blocking solution (1:1 (v/v) of Western Blocking Reagent and PBT) overnight at RT. The discs were then washed four times with PBT, incubated in secondary antibodies diluted in blocking solution for 2–3 hrs in the dark at RT, washed four times with PBT, and mounted with ProLong Gold Antifade Mountant with DAPI. All incubation and washing were carried out on a platform shaker with mild shaking.

Pupal eye staining.: Pupal eye staging, dissection and staining procedures were adapted from^{119,120}. Newly formed white pupae were circled on the fly vial. 32–46 hrs later, pupal

eyes were dissected with forceps in ice-cold 1x PBS, transferred to 3.7% (v/v) formaldehyde (MilliporeSigma, Cat# F8775) and fixed for 15–20 min at RT. Fixative was replaced with 1x PBS and washed three times followed by permeabilizing with PB-Triton for 30 min. The pupal eyes were then incubated in primary antibodies diluted in PB-Triton overnight at RT, washed four times with PB-Triton, and incubated in secondary antibody or Phalloidin diluted in PB-Triton for 3 hrs in the dark at RT. The pupal eyes were then washed four times with PB-Triton and mounted with ProLong Gold Antifade Mountant (Thermo Fisher Scientific, Cat# P10144). All incubation and washing were carried out on a platform shaker with mild shaking.

Fluorescence and bright-field microscopy—Fluorescent images were acquired using Zeiss LSM 880 Confocal Microscope. For embryos, 20x objective and 1 Airy Unit (AU) pinhole were used. For L3 wing discs, 20x objective was used to take a z stack, and maximum intensity projection of the entire disc proper was performed to generate final images. For L3 eye-antennal discs, 20x or 63x objective was used to take z stacks with optimal step size, and maximum intensity projection was performed for the entire disc proper, except for the anti-Ct staining which was focused on the disc proper while minimizing the inclusion of the cone cell focal plane, or for the orthogonal sections and their corresponding 2D views which are indicated in the figures and legends. For pupal eyes, 63x objective and z stack were used with focal plane set to the corresponding cell type, except for the final images of *GFPm δ* and anti-Elav staining which were generated by maximum intensity projection of the entire depth of pupal eye unless indicated otherwise. In mispatterned pupal eyes overexpressing Bon, Ct-positive cone cells and BarH1-positive primary pigment cells were distinguished from bristle groups which have both Ct and BarH1 expression in the basal nuclei by not having both signals^{15,121,122}. Bright-field images of adult eyes were taken under Zeiss Stemi 2000-C Microscope with an attached camera at 50x magnification. Adult wings were separated, immersed briefly in isopropanol, mounted with 3:1 (v/v) CMCP-10 (Polysciences, Inc., Cat# 16300–250) and lactic acid, and imaged with Olympus BX60 Microscope under 4x and 40x objectives for the entire wing and wing trichomes, respectively. Due to the difference in size, only images from female adult flies were analyzed. All images were processed with Fiji¹²³.

Scanning electron microscopy (SEM)—Preparation of adult flies for SEM was adapted from^{124,125}. 1–2 days old adult flies were sequentially dehydrated in 1 ml of 25%, 50%, 75%, 100%, 100% and 100% ethanol (v/v in water) for 8–16 hrs per step at RT (overnight for the first step). The flies were then chemically dried in 500 μ l of 25%, 50%, 75%, 100%, 100% and 100% Hexamethyldisilazane (v/v in ethanol) (HMDS, Electron Microscopy Sciences, Cat# 16700) for 30 min per step at RT. Most of the HMDS was then removed and the remaining HMDS was allowed to be dried under vacuum in a desiccator containing Indicating Drierite (W.A. Hammond, Cat# 23005) overnight at RT. The samples were then mounted on Aluminum Mount using Carbon Adhesive Tape, and stored in Mount Holder (Electron Microscopy Sciences, Cat# 75220, 77816 and 76510). The samples were subsequently coated with gold at 50 mAmps for 150 secs with Denton Vacuum Desk V sputter coater for most samples or coated with platinum for 8 nm with Denton Vacuum 502-B for samples in Figures S2O–T. Most images were acquired with JEOL JSM-6010LA

IntouchScope Scanning Electron Microscope using 20 kV voltage, 10 mm working distance, 40–50 spot size, and 150x, 300x, 500x, 1000x, and 3000x magnifications as needed. Images in Figures S2O–T were acquired with FEI Quanta 200 FEG MK II Scanning Electron Microscope using 10 kV voltage, 10 mm working distance, 2.5 spot size, and 150x and 1000x magnifications as needed. Due to the difference in size, only images from female adult flies were analyzed. Images were processed with Fiji. The numbers of trichomes were counted with Fiji in an area of $1306 \mu\text{m}^2$ ($41.73 \mu\text{m} \times 31.30 \mu\text{m}$) per image for all genotypes.

Total RNA preparation—Total RNA preparation method was adapted from³⁹. Pupal eyes were dissected from 40–41 hrs APF pupae (newly formed white pupae were staged for 40 hrs at 25°C and dissected within 1 hr at RT) in ice cold RNase-free 1x PBS diluted from RNase-free 10x PBS (Fisher Scientific, Cat# BP3994) with RNase-free water (Thermo Fisher Scientific, Cat# 10977015). Dissected pupal eyes were immediately transferred to 100 μl of TRIzol (Thermo Fisher Scientific, Cat# 15596026) and kept at RT for 5 min to lyse. Samples in TRIzol were stored at -80°C until 60 pupal eyes for each genotype were collected. All TRIzol samples for each genotype were then thawed and combined, and the volume was brought up to 500 μl per sample. Total RNA was extracted twice by adding 100 μl of chloroform, vigorously shaking for 15 secs, incubating at RT for 3 min, centrifuging at 11,000 rcf at 4°C for 15 min, and collecting the aqueous phase. The total RNA was then precipitated by adding 1 volume of 70% ethanol (v/v in RNase-free water), and purified with RNeasy Mini Kit (QIAGEN, Cat# 74104, 79254).

RNA-sequencing, data analysis, and qPCR validation—The RNA-seq libraries were prepared from 100 ng of total RNA per genotype with NEBNext Ultra II RNA Library Prep Kit mRNA Isolation Module (NEB, Cat# E7775S, E7490S, E7500S). Quality control and sequencing of the RNA-seq libraries were performed by GENEWIZ, Inc. using Agilent TapeStation and Illumina HiSeq 4000 with a 2 \times 150 paired-end configuration.

The adapter sequences were trimmed from raw reads, and reads shorter than 18 nt were removed by Cutadapt (v2.9)¹²⁶. The reads mapped to tRNAs, rRNA, snRNA and snoRNA were removed by mapping through Bowtie 2 (v2.3.5.1) with very sensitive setting and maximum fragment size of 1000 in addition to default setting¹²⁷. The tRNA, rRNA, snRNA and snoRNA reference sequences (dmel-all-tRNA-r6.33.fasta and dmel-all-miscRNA-r6.33.fasta) were obtained from FlyBase¹⁰⁷. The remaining reads were mapped to *D. melanogaster* genome Ensembl_BDGP6¹²⁸ by STAR (v2.7.0e)¹²⁹ with default parameters. Gene counting was achieved by featureCounts (Subread v1.6.2) with overlapping or polycistronic genes counted as a fraction¹³⁰. Differential gene expression analysis was performed with DESeq2 (v1.30.1) through DEBrowser (v1.16.3) with default settings^{108,131}, and genes with maximum count fewer than 10 were filtered out. Normalization method used in DESeq2 was Median Ratio Normalization (MRN)¹³². The Pearson Correlation Coefficient analysis was performed by ggpubr R package. Plots were generated using ggplot2¹³³. The Gene Ontology (GO) analysis was performed with DAVID Bioinformatics Resources (v6.8)¹³⁴.

cDNA was generated from total RNA using SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific, Cat# 18080051). qPCR was performed with biological and technical triplicates using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Cat# A25742) on QuantStudio 3 Real-Time PCR System. Gene expression was normalized to ribosomal protein *rp49*. P values were calculated using the values of $-Cq$ (Cq (gene of interest) - Cq (*rp49*))¹³⁵. The sequences of the primers are listed in the Key Resources Table.

DNA binding analysis and ChIP-qPCR—Overlap in the DNA binding locus for Yki, Bon, and Sd was analyzed and visualized by ChIPpeakAnno¹³⁶ using published ChIP-seq datasets of Yki (GSE38594)⁸⁴ and Bon (GSE25921)⁸⁵ after converting to dm6 coordinates, and DamID-seq dataset of Sd (GSE120731)⁸⁶. The p values between every two datasets were determined by the hypergeometric test using totalTest number for number of potential peaks¹³⁶. The totalTest number here (89144) was calculated by dividing the *Drosophila* genome (143.7 Mb)¹³⁷ with the largest mean peak width from three datasets (1612 bp for Sd DamID), assuming that these three proteins can potentially bind anywhere in the genome. The presence of ChIP peaks and DamID peaks at Bon-Yki jointly regulated genes was analyzed using IGV¹³⁸.

ChIP assay was performed using Chromatin Immunoprecipitation (ChIP) Assay Kit (MilliporeSigma, Cat# 17–295) with adaptations based on the application notes on GFP- and RFP-Trap by ChromoTek. 100 L3 eye-antennal discs for each replicate were dissected in ice-cold 1x PBS. Protein and DNA were crosslinked by incubating the discs in 1.8% (v/v) formaldehyde (Thermo Fisher Scientific, Cat# 28906) in 1x PBS for 15 min at RT and quenched with 225 mM Glycine for 5 min at RT⁸⁴. Discs were washed three times with 1x PBS, transferred to 200 μ l SDS lysis buffer containing 2.5 x EDTA-free mini cOmplete Protease Inhibitor (MilliporeSigma, Cat# 11836170001, 1 tablet per 4 ml SDS lysis buffer), homogenized with Pellet Pestle for 30 secs on ice, and incubated for 10 min on ice. The lysate was sonicated using Bioruptor sonicator for 15 min (30 secs on and 30 secs rest at high power) in 4°C water bath to shear the DNA. Samples were centrifuged at 16,000 rcf for 10 min at 4°C, and the supernatant was diluted 10-fold in ChIP Dilution Buffer containing 2.5 x mini cOmplete inhibitor. Samples were pre-cleared with 75 μ l (50% slurry) of Pierce Control Agarose Resin for 1 hr at 4°C with rotation, and after a brief centrifugation, 5% sample was saved as input sample. The supernatant was then incubated with 25 μ l (50% slurry) of RFP-Trap agarose overnight at 4°C with rotation. The agarose beads were washed as indicated in the kit. The protein-DNA complex was eluted twice with 250 μ l elution buffer for 15 min, first at RT on a nutator and second on a 65°C shaker. Input samples and elutes were reverse cross-linked with 0.2 M NaCl at 65°C overnight, digested with RNase A (Thermo Fisher Scientific, Cat# EN0531) for 30 min at 37°C and Proteinase K (Thermo Fisher Scientific, Cat# 26160) as indicated in the kit. DNA was recovered by phenol/chloroform extraction and ethanol precipitation.

qPCR was performed with biological triplicates and technical duplicates using PowerUp SYBR Green Master Mix on QuantStudio 3 Real-Time PCR System. Ratio to the input was presented. P values were calculated using the values of $-Cq$ (Cq (ChIPped) - Cq (input)). The sequences of the primers are listed in the Key Resources Table.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical significance of categorical variables was determined by Fisher's exact test with two-sided p value calculation using GraphPad Prism (v9.1.0). Unless indicated otherwise, in all other statistical analyses, data were presented as mean \pm SD of at least three replicates, and the significance was determined by unpaired t-test with Welch's correction using GraphPad Prism (v9.1.0). The numbers (n) and values of each sample used per experiment are provided in Table S5. The p values are presented as follows: ns ($p > 0.05$, not significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kulkarni A, Chang MT, Vissers JHA, Dey A, and Harvey KF (2020). The Hippo Pathway as a Driver of Select Human Cancers. *Trends Cancer* 6, 781–796. 10.1016/j.trecan.2020.04.004. [PubMed: 32446746]
2. Ma S, Meng Z, Chen R, and Guan KL (2019). The Hippo Pathway: Biology and Pathophysiology. *Annu Rev Biochem* 88, 577–604. 10.1146/annurev-biochem-013118-111829. [PubMed: 30566373]
3. Misra JR, and Irvine KD (2018). The Hippo Signaling Network and Its Biological Functions. *Annu Rev Genet* 52, 65–87. 10.1146/annurev-genet-120417-031621. [PubMed: 30183404]
4. Manning SA, Kroeger B, and Harvey KF (2020). The regulation of Yorkie, YAP and TAZ: new insights into the Hippo pathway. *Development* 147. 10.1242/dev.179069.
5. Zheng Y, and Pan D (2019). The Hippo Signaling Pathway in Development and Disease. *Dev Cell* 50, 264–282. 10.1016/j.devcel.2019.06.003. [PubMed: 31386861]
6. Davis JR, and Tapon N (2019). Hippo signalling during development. *Development* 146. 10.1242/dev.167106.
7. Weasner BP, and Kumar JP (2022). The early history of the eye-antennal disc of *Drosophila melanogaster*. *Genetics*. 10.1093/genetics/iyac041.
8. Dominguez M, and Casares F (2005). Organ specification-growth control connection: new in-sights from the *Drosophila* eye-antennal disc. *Dev Dyn* 232, 673–684. 10.1002/dvdy.20311. [PubMed: 15704149]
9. Kumar JP (2010). Retinal determination the beginning of eye development. *Curr Top Dev Biol* 93, 1–28. 10.1016/B978-0-12-385044-7.00001-1. [PubMed: 20959161]
10. Kumar JP (2018). The fly eye: Through the looking glass. *Dev Dyn* 247, 111–123. 10.1002/dvdy.24585. [PubMed: 28856763]
11. Ordway AJ, Teeters GM, Weasner BM, Weasner BP, Policastro R, and Kumar JP (2021). A multi-gene knockdown approach reveals a new role for Pax6 in controlling organ number in *Drosophila*. *Development* 148. 10.1242/dev.198796.

12. Kumar JP, and Moses K (2001). EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell* 104, 687–697. 10.1016/s0092-8674(01)00265-3. [PubMed: 11257223]
13. Weasner BM, and Kumar JP (2013). Competition among gene regulatory networks imposes order within the eye-antennal disc of *Drosophila*. *Development* 140, 205–215. 10.1242/dev.085423. [PubMed: 23222441]
14. Wang CW, and Sun YH (2012). Segregation of eye and antenna fates maintained by mutual antagonism in *Drosophila*. *Development* 139, 3413–3421. 10.1242/dev.078857. [PubMed: 22912416]
15. Charlton-Perkins M, and Cook TA (2010). Building a fly eye: terminal differentiation events of the retina, corneal lens, and pigmented epithelia. *Curr Top Dev Biol* 93, 129–173. 10.1016/B978-0-12-385044-7.00005-9. [PubMed: 20959165]
16. Cagan RL, and Ready DF (1989). The emergence of order in the *Drosophila* pupal retina. *Dev Biol* 136, 346–362. 10.1016/0012-1606(89)90261-3. [PubMed: 2511048]
17. Wittkorn E, Sarkar A, Garcia K, Kango-Singh M, and Singh A (2015). The Hippo pathway effector Yki downregulates Wg signaling to promote retinal differentiation in the *Drosophila* eye. *Development* 142, 2002–2013. 10.1242/dev.117358. [PubMed: 25977365]
18. Jukam D, Xie B, Rister J, Terrell D, Charlton-Perkins M, Pistillo D, Gebelein B, Desplan C, and Cook T (2013). Opposite feedbacks in the Hippo pathway for growth control and neural fate. *Science* 342, 1238016. 10.1126/science.1238016. [PubMed: 23989952]
19. Zhang T, Zhou Q, and Pignoni F (2011). Yki/YAP, Sd/TEAD and Hth/MEIS control tissue specification in the *Drosophila* eye disc epithelium. *PLoS One* 6, e22278. 10.1371/journal.pone.0022278. [PubMed: 21811580]
20. Pojer JM, Manning SA, Kroeger B, Kondo S, and Harvey KF (2021). The Hippo pathway uses different machinery to control cell fate and organ size. *iScience* 24, 102830. 10.1016/j.isci.2021.102830. [PubMed: 34355153]
21. Boedigheimer M, and Laughon A (1993). Expanded: a gene involved in the control of cell proliferation in imaginal discs. *Development* 118, 1291–1301. [PubMed: 8269855]
22. Lai ZC, Wei X, Shimizu T, Ramos E, Rohrbach M, Nikolaidis N, Ho LL, and Li Y (2005). Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. *Cell* 120, 675–685. 10.1016/j.cell.2004.12.036. [PubMed: 15766530]
23. McCartney BM, Kulikauskas RM, LaJeunesse DR, and Fehon RG (2000). The neurofibromatosis-2 homologue, Merlin, and the tumor suppressor expanded function together in *Drosophila* to regulate cell proliferation and differentiation. *Development* 127, 1315–1324. [PubMed: 10683183]
24. Pellock BJ, Buff E, White K, and Hariharan IK (2007). The *Drosophila* tumor suppressors Expanded and Merlin differentially regulate cell cycle exit, apoptosis, and Wingless signaling. *Dev Biol* 304, 102–115. 10.1016/j.ydbio.2006.12.021. [PubMed: 17258190]
25. Beckstead R, Ortiz JA, Sanchez C, Prokopenko SN, Chambon P, Losson R, and Bellen HJ (2001). Bonus, a *Drosophila* homolog of TIF1 proteins, interacts with nuclear receptors and can inhibit betaFTZ-F1-dependent transcription. *Mol Cell* 7, 753–765. [PubMed: 11336699]
26. Cammas F, Khetchoumian K, Chambon P, and Losson R (2012). TRIM involvement in transcriptional regulation. *Adv Exp Med Biol* 770, 59–76. 10.1007/978-1-4614-5398-7_5. [PubMed: 23631000]
27. Beckstead RB, Ner SS, Hales KG, Grigliatti TA, Baker BS, and Bellen HJ (2005). Bonus, a *Drosophila* TIF1 homolog, is a chromatin-associated protein that acts as a modifier of position-effect variegation. *Genetics* 169, 783–794. 10.1534/genetics.104.037085. [PubMed: 15545640]
28. Ito H, Sato K, Koganezawa M, Ote M, Matsumoto K, Hama C, and Yamamoto D (2012). Fruitless recruits two antagonistic chromatin factors to establish single-neuron sexual dimorphism. *Cell* 149, 1327–1338. 10.1016/j.cell.2012.04.025. [PubMed: 22682252]
29. Petrera F, and Meroni G (2012). TRIM proteins in development. *Adv Exp Med Biol* 770, 131–141. 10.1007/978-1-4614-5398-7_10. [PubMed: 23631005]

30. Cambiaghi V, Giuliani V, Lombardi S, Marinelli C, Toffalorio F, and Pelicci PG (2012). TRIM proteins in cancer. *Adv Exp Med Biol* 770, 77–91. 10.1007/978-1-4614-5398-7_6. [PubMed: 23631001]
31. McAvera RM, and Crawford LJ (2020). TIF1 Proteins in Genome Stability and Cancer. *Cancers (Basel)* 12. 10.3390/cancers12082094.
32. Salzberg A, Prokopenko SN, He Y, Tsai P, Pal M, Maroy P, Glover DM, Deak P, and Bellen HJ (1997). P-element insertion alleles of essential genes on the third chromosome of *Drosophila melanogaster*: mutations affecting embryonic PNS development. *Genetics* 147, 1723–1741. [PubMed: 9409832]
33. Allton K, Jain AK, Herz HM, Tsai WW, Jung SY, Qin J, Bergmann A, Johnson RL, and Barton MC (2009). Trim24 targets endogenous p53 for degradation. *Proc Natl Acad Sci U S A* 106, 11612–11616. 10.1073/pnas.0813177106. [PubMed: 19556538]
34. Wisotzkey RG, Quijano JC, Stinchfield MJ, and Newfeld SJ (2014). New gene evolution in the bonus-TIF1-gamma/TRIM33 family impacted the architecture of the vertebrate dorsal-ventral patterning network. *Mol Biol Evol* 31, 2309–2321. 10.1093/molbev/msu175. [PubMed: 24881051]
35. Wodarz A, Hinz U, Engelbert M, and Knust E (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* 82, 67–76. 10.1016/0092-8674(95)90053-5. [PubMed: 7606787]
36. Kwon Y, Vinayagam A, Sun X, Dephore N, Gygi SP, Hong P, and Perrimon N (2013). The Hippo signaling pathway interactome. *Science* 342, 737–740. 10.1126/science.1243971. [PubMed: 24114784]
37. Oh H, Slattery M, Ma L, White KP, Mann RS, and Irvine KD (2014). Yorkie promotes transcription by recruiting a histone methyltransferase complex. *Cell Rep* 8, 449–459. 10.1016/j.celrep.2014.06.017. [PubMed: 25017066]
38. Oh H, Reddy BV, and Irvine KD (2009). Phosphorylation-independent repression of Yorkie in Fat-Hippo signaling. *Dev Biol* 335, 188–197. 10.1016/j.ydbio.2009.08.026. [PubMed: 19733165]
39. Zhang C, Robinson BS, Xu W, Yang L, Yao B, Zhao H, Byun PK, Jin P, Veraksa A, and Moberg KH (2015). The ecdysone receptor coactivator Taiman links Yorkie to transcriptional control of germline stem cell factors in somatic tissue. *Dev Cell* 34, 168–180. 10.1016/j.devcel.2015.05.010. [PubMed: 26143992]
40. Xu T, Wang W, Zhang S, Stewart RA, and Yu W (1995). Identifying tumor suppressors in genetic mosaics: the *Drosophila* *lats* gene encodes a putative protein kinase. *Development* 121, 1053–1063. [PubMed: 7743921]
41. Huang J, Wu S, Barrera J, Matthews K, and Pan D (2005). The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the *Drosophila* Homolog of YAP. *Cell* 122, 421–434. 10.1016/j.cell.2005.06.007. [PubMed: 16096061]
42. Hay BA, Wolff T, and Rubin GM (1994). Expression of baculovirus P35 prevents cell death in *Drosophila*. *Development* 120, 2121–2129. [PubMed: 7925015]
43. Ellis MC, O'Neill EM, and Rubin GM (1993). Expression of *Drosophila* glass protein and evidence for negative regulation of its activity in non-neuronal cells by another DNA-binding protein. *Development* 119, 855–865. [PubMed: 8187644]
44. Freeman M (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. *Cell* 87, 651–660. 10.1016/s0092-8674(00)81385-9. [PubMed: 8929534]
45. Tardi NJ, Cook ME, and Edwards KA (2012). Rapid phenotypic analysis of uncoated *Drosophila* samples with low-vacuum scanning electron microscopy. *Fly (Austin)* 6, 184–192. 10.4161/fly.20525. [PubMed: 22722327]
46. Arif S, Kittelmann S, and McGregor AP (2015). From shavenbaby to the naked valley: trichome formation as a model for evolutionary developmental biology. *Evol Dev* 17, 120–126. 10.1111/ede.12113. [PubMed: 25627718]
47. Delon I, Chanut-Delalande H, and Payre F (2003). The Ovo/Shavenbaby transcription factor specifies actin remodelling during epidermal differentiation in *Drosophila*. *Mech Dev* 120, 747–758. 10.1016/s0925-4773(03)00081-9. [PubMed: 12915226]

48. Zhang L, Ren F, Zhang Q, Chen Y, Wang B, and Jiang J (2008). The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. *Dev Cell* 14, 377–387. 10.1016/j.devcel.2008.01.006. [PubMed: 18258485]
49. Oh H, and Irvine KD (2008). In vivo regulation of Yorkie phosphorylation and localization. *Development* 135, 1081–1088. 10.1242/dev.015255. [PubMed: 18256197]
50. Chanut-Delalande H, Hashimoto Y, Pelissier-Monier A, Spokony R, Dib A, Kondo T, Bohere J, Niimi K, Latapie Y, Inagaki S, et al. (2014). Pri peptides are mediators of ecdysone for the temporal control of development. *Nat Cell Biol* 16, 1035–1044. 10.1038/ncb3052. [PubMed: 25344753]
51. Kumar JP (2012). Building an ommatidium one cell at a time. *Developmental Dynamics* 241, 136–149. 10.1002/dvdy.23707. [PubMed: 22174084]
52. Nagaraj R, and Banerjee U (2007). Combinatorial signaling in the specification of primary pigment cells in the *Drosophila* eye. *Development* 134, 825–831. 10.1242/dev.02788. [PubMed: 17251265]
53. Udan RS, Kango-Singh M, Nolo R, Tao C, and Halder G (2003). Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. *Nat Cell Biol* 5, 914–920. 10.1038/ncb1050. [PubMed: 14502294]
54. Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, Tao C, Jafar-Nejad H, and Halder G (2006). The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. *Nat Cell Biol* 8, 27–36. 10.1038/ncb1339. [PubMed: 16341207]
55. Wernet MF, Labhart T, Baumann F, Mazzoni EO, Pichaud F, and Desplan C (2003). Homothorax switches function of *Drosophila* photoreceptors from color to polarized light sensors. *Cell* 115, 267–279. 10.1016/s0092-8674(03)00848-1. [PubMed: 14636555]
56. Lim HY, and Tomlinson A (2006). Organization of the peripheral fly eye: the roles of Snail family transcription factors in peripheral retinal apoptosis. *Development* 133, 3529–3537. 10.1242/dev.02524. [PubMed: 16914498]
57. Pai CY, Kuo TS, Jaw TJ, Kurant E, Chen CT, Bessarab DA, Salzberg A, and Sun YH (1998). The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, extradenticle, and suppresses eye development in *Drosophila*. *Genes Dev* 12, 435–446. 10.1101/gad.12.3.435. [PubMed: 9450936]
58. Blochlinger K, Jan LY, and Jan YN (1993). Postembryonic patterns of expression of cut, a locus regulating sensory organ identity in *Drosophila*. *Development* 117, 441–450. 10.1242/dev.117.2.441. [PubMed: 8330519]
59. Seto E, and Yoshida M (2014). Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb Perspect Biol* 6, a018713. 10.1101/cshperspect.a018713. [PubMed: 24691964]
60. Hari KL, Cook KR, and Karpen GH (2001). The *Drosophila* Su(var)2–10 locus regulates chromosome structure and function and encodes a member of the PIAS protein family. *Genes Dev* 15, 1334–1348. 10.1101/gad.877901. [PubMed: 11390354]
61. Betz A, Lampen N, Martinek S, Young MW, and Darnell JE Jr. (2001). A *Drosophila* PIAS homologue negatively regulates stat92E. *Proc Natl Acad Sci U S A* 98, 9563–9568. 10.1073/pnas.171302098. [PubMed: 11504941]
62. Ninova M, Chen YA, Godneeva B, Rogers AK, Luo Y, Fejes Toth K, and Aravin AA (2020). Su(var)2–10 and the SUMO Pathway Link piRNA-Guided Target Recognition to Chromatin Silencing. *Mol Cell* 77, 556–570 e556. 10.1016/j.molcel.2019.11.012. [PubMed: 31901446]
63. Goodrich JS, Clouse KN, and Schupbach T (2004). Hrb27C, Sqd and Otu cooperatively regulate gurken RNA localization and mediate nurse cell chromosome dispersion in *Drosophila* oogenesis. *Development* 131, 1949–1958. 10.1242/dev.01078. [PubMed: 15056611]
64. Mach J, Atkins M, Gajewski KM, Mottier-Pavie V, Sansores-Garcia L, Xie J, Mills RA, Kowalczyk W, Van Huffel L, Mills GB, and Halder G (2018). Modulation of the Hippo pathway and organ growth by RNA processing proteins. *Proc Natl Acad Sci U S A* 115, 10684–10689. 10.1073/pnas.1807325115. [PubMed: 30257938]
65. Yano T, Lopez de Quinto S, Matsui Y, Shevchenko A, Shevchenko A, and Ephrussi A (2004). Hrp48, a *Drosophila* hnRNPA/B homolog, binds and regulates translation of oskar mRNA. *Dev Cell* 6, 637–648. 10.1016/s1534-5807(04)00132-7. [PubMed: 15130489]

66. Chanut-Delalande H, Fernandes I, Roch F, Payre F, and Plaza S (2006). Shavenbaby couples patterning to epidermal cell shape control. *PLoS Biol* 4, e290. 10.1371/journal.pbio.0040290. [PubMed: 16933974]
67. Bohere J, Mancheno-Ferris A, Al Hayek S, Zanet J, Valenti P, Akino K, Yamabe Y, Inagaki S, Chanut-Delalande H, Plaza S, et al. (2018). Shavenbaby and Yorkie mediate Hippo signaling to protect adult stem cells from apoptosis. *Nat Commun* 9, 5123. 10.1038/s41467-018-07569-0. [PubMed: 30504772]
68. Andrews J, Garcia-Estefania D, Delon I, Lu J, Mevel-Ninio M, Spierer A, Payre F, Pauli D, and Oliver B (2000). OVO transcription factors function antagonistically in the *Drosophila* female germline. *Development* 127, 881–892. [PubMed: 10648246]
69. Tare M, Puli OR, Moran MT, Kango-Singh M, and Singh A (2013). Domain specific genetic mosaic system in the *Drosophila* eye. *Genesis* 51, 68–74. 10.1002/dvg.22355. [PubMed: 23109378]
70. Deng H, Yang L, Wen P, Lei H, Blount P, and Pan D (2020). Spectrin couples cell shape, cortical tension, and Hippo signaling in retinal epithelial morphogenesis. *J Cell Biol* 219. 10.1083/jcb.201907018.
71. Fernandes I, Chanut-Delalande H, Ferrer P, Latapie Y, Waltzer L, Affolter M, Payre F, and Plaza S (2010). Zona pellucida domain proteins remodel the apical compartment for localized cell shape changes. *Dev Cell* 18, 64–76. 10.1016/j.devcel.2009.11.009. [PubMed: 20152178]
72. Lai EC, Bodner R, and Posakony JW (2000). The enhancer of split complex of *Drosophila* includes four Notch-regulated members of the bearded gene family. *Development* 127, 3441–3455. [PubMed: 10903170]
73. Knust E, Schrons H, Grawe F, and Campos-Ortega JA (1992). Seven genes of the Enhancer of split complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* 132, 505–518. [PubMed: 1427040]
74. Kerber B, Monge I, Mueller M, Mitchell PJ, and Cohen SM (2001). The AP-2 transcription factor is required for joint formation and cell survival in *Drosophila* leg development. *Development* 128, 1231–1238. [PubMed: 11262225]
75. Micchelli CA, Rulifson EJ, and Blair SS (1997). The function and regulation of cut expression on the wing margin of *Drosophila*: Notch, Wingless and a dominant negative role for Delta and Serrate. *Development* 124, 1485–1495. [PubMed: 9108365]
76. Lai EC, and Rubin GM (2001). Neuralized is essential for a subset of Notch pathway-dependent cell fate decisions during *Drosophila* eye development. *Proc Natl Acad Sci U S A* 98, 5637–5642. 10.1073/pnas.101135498. [PubMed: 11344304]
77. Monge I, Krishnamurthy R, Sims D, Hirth F, Spengler M, Kammermeier L, Reichert H, and Mitchell PJ (2001). *Drosophila* transcription factor AP-2 in proboscis, leg and brain central complex development. *Development* 128, 1239–1252. [PubMed: 11262226]
78. Cagan RL, and Ready DF (1989). Notch is required for successive cell decisions in the developing *Drosophila* retina. *Genes Dev* 3, 1099–1112. 10.1101/gad.3.8.1099. [PubMed: 2792755]
79. Kurata S, Go MJ, Artavanis-Tsakonas S, and Gehring WJ (2000). Notch signaling and the determination of appendage identity. *Proc Natl Acad Sci U S A* 97, 2117–2122. 10.1073/pnas.040556497. [PubMed: 10681430]
80. Vosshall LB (2001). The molecular logic of olfaction in *Drosophila*. *Chem Senses* 26, 207–213. 10.1093/chemse/26.2.207. [PubMed: 11238253]
81. Barish S, Li Q, Pan JW, Soeder C, Jones C, and Volkan PC (2017). Transcriptional profiling of olfactory system development identifies distal antenna as a regulator of subset of neuronal fates. *Sci Rep* 7, 40873. 10.1038/srep40873. [PubMed: 28102318]
82. Fujii S, Yavuz A, Slone J, Jagge C, Song X, and Amrein H (2015). *Drosophila* sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. *Curr Biol* 25, 621–627. 10.1016/j.cub.2014.12.058. [PubMed: 25702577]
83. Menuz K, Larter NK, Park J, and Carlson JR (2014). An RNA-seq screen of the *Drosophila* antenna identifies a transporter necessary for ammonia detection. *PLoS Genet* 10, e1004810. 10.1371/journal.pgen.1004810. [PubMed: 25412082]

84. Oh H, Slattery M, Ma L, Crofts A, White KP, Mann RS, and Irvine KD (2013). Genome-wide association of Yorkie with chromatin and chromatin-remodeling complexes. *Cell Rep* 3, 309–318. 10.1016/j.celrep.2013.01.008. [PubMed: 23395637]
85. Negre N, Brown CD, Ma L, Bristow CA, Miller SW, Wagner U, Kheradpour P, Eaton ML, Loriaux P, Sealfon R, et al. (2011). A cis-regulatory map of the *Drosophila* genome. *Nature* 471, 527–531. 10.1038/nature09990. [PubMed: 21430782]
86. Vissers JHA, Froidi F, Schroder J, Papenfuss AT, Cheng LY, and Harvey KF (2018). The Scalloped and Nerfin-1 Transcription Factors Cooperate to Maintain Neuronal Cell Fate. *Cell Rep* 25, 1561–1576 e1567. 10.1016/j.celrep.2018.10.038. [PubMed: 30404010]
87. Couturier L, Mazouni K, Corson F, and Schweisguth F (2019). Regulation of Notch output dynamics via specific E(spl)-HLH factors during bristle patterning in *Drosophila*. *Nat Commun* 10, 3486. 10.1038/s41467-019-11477-2. [PubMed: 31375669]
88. Morrison CM, and Halder G (2010). Characterization of a dorsal-eye Gal4 Line in *Drosophila*. *Genesis* 48, 3–7. 10.1002/dvg.20571. [PubMed: 19882738]
89. Jin M, Eblimit A, Pulikkathara M, Corr S, Chen R, and Mardon G (2016). Conditional knockout of retinal determination genes in differentiating cells in *Drosophila*. *FEBS J* 283, 2754–2766. 10.1111/febs.13772. [PubMed: 27257739]
90. Buschbeck EK (2005). The compound lens eye of Strepsiptera: morphological development of larvae and pupae. *Arthropod Structure & Development* 34, 315–326. 10.1016/j.asd.2005.04.002.
91. Yeh E, Gustafson K, and Boulianne GL (1995). Green fluorescent protein as a vital marker and reporter of gene expression in *Drosophila*. *Proc Natl Acad Sci U S A* 92, 7036–7040. 10.1073/pnas.92.15.7036. [PubMed: 7624365]
92. Reddy BV, Rauskolb C, and Irvine KD (2010). Influence of fat-hippo and notch signaling on the proliferation and differentiation of *Drosophila* optic neuroepithelia. *Development* 137, 2397–2408. 10.1242/dev.050013. [PubMed: 20570939]
93. Totaro A, Castellan M, Battilana G, Zanconato F, Azzolin L, Giulitti S, Cordenonsi M, and Piccolo S (2017). YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. *Nat Commun* 8, 15206. 10.1038/ncomms15206. [PubMed: 28513598]
94. Yimlamai D, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ, and Camargo FD (2014). Hippo pathway activity influences liver cell fate. *Cell* 157, 1324–1338. 10.1016/j.cell.2014.03.060. [PubMed: 24906150]
95. Ligoxygakis P, Bray SJ, Apidianakis Y, and Delidakis C (1999). Ectopic expression of individual E(spl) genes has differential effects on different cell fate decisions and underscores the biphasic requirement for notch activity in wing margin establishment in *Drosophila*. *Development* 126, 2205–2214. [PubMed: 10207145]
96. Peng HW, Slattery M, and Mann RS (2009). Transcription factor choice in the Hippo signaling pathway: homothorax and yorkie regulation of the microRNA bantam in the progenitor domain of the *Drosophila* eye imaginal disc. *Genes Dev* 23, 2307–2319. 10.1101/gad.1820009. [PubMed: 19762509]
97. Beyer TA, Weiss A, Khomchuk Y, Huang K, Ogunjimi AA, Varelas X, and Wrana JL (2013). Switch enhancers interpret TGF-beta and Hippo signaling to control cell fate in human embryonic stem cells. *Cell Rep* 5, 1611–1624. 10.1016/j.celrep.2013.11.021. [PubMed: 24332857]
98. Kim M, Kim T, Johnson RL, and Lim DS (2015). Transcriptional co-repressor function of the hippo pathway transducers YAP and TAZ. *Cell Rep* 11, 270–282. 10.1016/j.celrep.2015.03.015. [PubMed: 25843714]
99. He L, Yuan L, Yu W, Sun Y, Jiang D, Wang X, Feng X, Wang Z, Xu J, Yang R, et al. (2020). A Regulation Loop between YAP and NR4A1 Balances Cell Proliferation and Apoptosis. *Cell Rep* 33, 108284. 10.1016/j.celrep.2020.108284. [PubMed: 33086070]
100. Borggreffe T, and Oswald F (2009). The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci* 66, 1631–1646. 10.1007/s00018-009-8668-7. [PubMed: 19165418]
101. Rossmann MP, Hoi K, Chan V, Abraham BJ, Yang S, Mullahoo J, Papanastasiou M, Wang Y, Elia I, Perlin JR, et al. (2021). Cell-specific transcriptional control of mitochondrial metabolism by

- TIF1gamma drives erythropoiesis. *Science* 372, 716–721. 10.1126/science.aaz2740. [PubMed: 33986176]
102. Milton CC, Grusche FA, Degoutin JL, Yu E, Dai Q, Lai EC, and Harvey KF (2014). The Hippo pathway regulates hematopoiesis in *Drosophila melanogaster*. *Curr Biol* 24, 2673–2680. 10.1016/j.cub.2014.10.031. [PubMed: 25454587]
 103. Lundin V, Sugden WW, Theodore LN, Sousa PM, Han A, Chou S, Wrighton PJ, Cox AG, Ingber DE, Goessling W, et al. (2020). YAP Regulates Hematopoietic Stem Cell Formation in Response to the Biomechanical Forces of Blood Flow. *Dev Cell* 52, 446–460 e445. 10.1016/j.devcel.2020.01.006. [PubMed: 32032546]
 104. Lee M, Goraya N, Kim S, and Cho SH (2018). Hippo-yap signaling in ocular development and disease. *Dev Dyn* 247, 794–806. 10.1002/dvdy.24628. [PubMed: 29532607]
 105. Choi H, Larsen B, Lin ZY, Breikreutz A, Mellacheruvu D, Fermin D, Qin ZS, Tyers M, Gingras AC, and Nesvizhskii AI (2011). SAINT: probabilistic scoring of affinity purification-mass spectrometry data. *Nat Methods* 8, 70–73. 10.1038/nmeth.1541. [PubMed: 21131968]
 106. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 47, D607–D613. 10.1093/nar/gky1131. [PubMed: 30476243]
 107. Thurmond J, Goodman JL, Strelts VB, Attrill H, Gramates LS, Marygold SJ, Matthews BB, Millburn G, Antonazzo G, Trovisco V, et al. (2019). FlyBase 2.0: the next generation. *Nucleic Acids Res* 47, D759–D765. 10.1093/nar/gky1003. [PubMed: 30364959]
 108. Love MI, Huber W, and Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550. 10.1186/s13059-014-0550-8. [PubMed: 25516281]
 109. Edgar R, Domrachev M, and Lash AE (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 30, 207–210. 10.1093/nar/30.1.207. [PubMed: 11752295]
 110. Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, Inuganti A, Griss J, Mayer G, Eisenacher M, et al. (2019). The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res* 47, D442–D450. 10.1093/nar/gky1106. [PubMed: 30395289]
 111. Yang L, and Veraksa A (2017). Single-Step Affinity Purification of ERK Signaling Complexes Using the Streptavidin-Binding Peptide (SBP) Tag. *Methods Mol Biol* 1487, 113–126. 10.1007/978-1-4939-6424-6_8. [PubMed: 27924562]
 112. Bischof J, Maeda RK, Hediger M, Karch F, and Basler K (2007). An optimized transgenesis system for *Drosophila* using germ-line-specific phiC31 integrases. *Proc Natl Acad Sci U S A* 104, 3312–3317. 10.1073/pnas.0611511104. [PubMed: 17360644]
 113. Yang L, Paul S, DuBois-Coyne S, Kyriakakis P, and Veraksa A (2017). Medium-scale Preparation of *Drosophila* Embryo Extracts for Proteomic Experiments. *J Vis Exp*. 10.3791/55804.
 114. Bean DM, Heimbach J, Ficorella L, Micklem G, Oliver SG, and Favrin G (2014). esyN: network building, sharing and publishing. *PLoS One* 9, e106035. 10.1371/journal.pone.0106035. [PubMed: 25181461]
 115. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, and Ideker T (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13, 2498–2504. 10.1101/gr.1239303. [PubMed: 14597658]
 116. Gouge CA, and Christensen TW (2010). *Drosophila* Sld5 is essential for normal cell cycle progression and maintenance of genomic integrity. *Biochem Biophys Res Commun* 400, 145–150. 10.1016/j.bbrc.2010.08.033. [PubMed: 20709026]
 117. Ozel MN, Simon F, Jafari S, Holguera I, Chen YC, Benhra N, El-Danaf RN, Kapuralin K, Malin JA, Konstantinides N, and Desplan C (2021). Neuronal diversity and convergence in a visual system developmental atlas. *Nature* 589, 88–95. 10.1038/s41586-020-2879-3. [PubMed: 33149298]

118. Kang J, Yeom E, Lim J, and Choi KW (2014). Bar represses dPax2 and decapentaplegic to regulate cell fate and morphogenetic cell death in *Drosophila* eye. *PLoS One* 9, e88171. 10.1371/journal.pone.0088171. [PubMed: 24505414]
119. Hsiao HY, Johnston RJ Jr., Jukam D, Vasiliauskas D, Desplan C, and Rister J (2012). Dissection and immunohistochemistry of larval, pupal and adult *Drosophila* retinas. *J Vis Exp*, 4347. 10.3791/4347.
120. Walther RF, and Pichaud F (2006). Immunofluorescent staining and imaging of the pupal and adult *Drosophila* visual system. *Nat Protoc* 1, 2635–2642. 10.1038/nprot.2006.379. [PubMed: 17406519]
121. Beam CK, and Moberg K (2010). The gang of four gene regulates growth and patterning of the developing *Drosophila* eye. *Fly (Austin)* 4, 104–116. 10.4161/fly.4.2.11890. [PubMed: 20473027]
122. Cadigan KM, and Nusse R (1996). wingless signaling in the *Drosophila* eye and embryonic epidermis. *Development* 122, 2801–2812. 10.1242/dev.122.9.2801. [PubMed: 8787754]
123. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9, 676–682. 10.1038/nmeth.2019. [PubMed: 22743772]
124. Pepple KL, Anderson AE, Frankfort BJ, and Mardon G (2007). A genetic screen in *Drosophila* for genes interacting with senseless during neuronal development identifies the importin moleskin. *Genetics* 175, 125–141. 10.1534/genetics.106.065680. [PubMed: 17110483]
125. Wolff T (2011). Preparation of *Drosophila* eye specimens for scanning electron microscopy. *Cold Spring Harb Protoc* 2011, 1383–1385. 10.1101/pdb.prot066506. [PubMed: 22046032]
126. Martin M (2011). Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads. *EMBnet.journal* 17, 10–12. 10.14806/ej.17.1.200.
127. Langmead B, and Salzberg SL (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9, 357–359. 10.1038/nmeth.1923. [PubMed: 22388286]
128. Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, et al. (2014). Ensembl 2014. *Nucleic Acids Res* 42, D749–755. 10.1093/nar/gkt1196. [PubMed: 24316576]
129. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, and Gingeras TR (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. 10.1093/bioinformatics/bts635. [PubMed: 23104886]
130. Liao Y, Smyth GK, and Shi W (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. 10.1093/bioinformatics/btt656. [PubMed: 24227677]
131. Kucukural A, Yukselen O, Ozata DM, Moore MJ, and Garber M (2019). DEBrowser: interactive differential expression analysis and visualization tool for count data. *Bmc Genomics* 20, 6. 10.1186/s12864-018-5362-x. [PubMed: 30611200]
132. Maza E, Frasse P, Senin P, Bouzayen M, and Zouine M (2013). Comparison of normalization methods for differential gene expression analysis in RNA-Seq experiments: A matter of relative size of studied transcriptomes. *Commun Integr Biol* 6, e25849. 10.4161/cib.25849. [PubMed: 26442135]
133. Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
134. Huang da W, Sherman BT, and Lempicki RA (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4, 44–57. 10.1038/nprot.2008.211. [PubMed: 19131956]
135. Yuan JS, Reed A, Chen F, and Stewart CN Jr. (2006). Statistical analysis of real-time PCR data. *BMC Bioinformatics* 7, 85. 10.1186/1471-2105-7-85. [PubMed: 16504059]
136. Zhu LJ, Gazin C, Lawson ND, Pages H, Lin SM, Lapointe DS, and Green MR (2010). ChIPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. *BMC Bioinformatics* 11, 237. 10.1186/1471-2105-11-237. [PubMed: 20459804]
137. dos Santos G, Schroeder AJ, Goodman JL, Strelets VB, Crosby MA, Thurmond J, Emmert DB, Gelbart WM, and FlyBase C (2015). FlyBase: introduction of the *Drosophila melanogaster*

Release 6 reference genome assembly and large-scale migration of genome annotations. *Nucleic Acids Res* 43, D690–697. 10.1093/nar/gku1099. [PubMed: 25398896]

138. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, and Mesirov JP (2011). Integrative genomics viewer. *Nat Biotechnol* 29, 24–26. 10.1038/nbt.1754. [PubMed: 21221095]

Highlights:

1. In *Drosophila*, Bon interacts with Yki through the PPxY motif-WW domain interaction
2. The Yki-Bon complex promotes epidermal and antennal fates and suppresses eye fate
3. Yki and Bon recruit transcriptional co-regulators for cell fate control
4. Yki and Bon control cell fates via repressing Notch targets

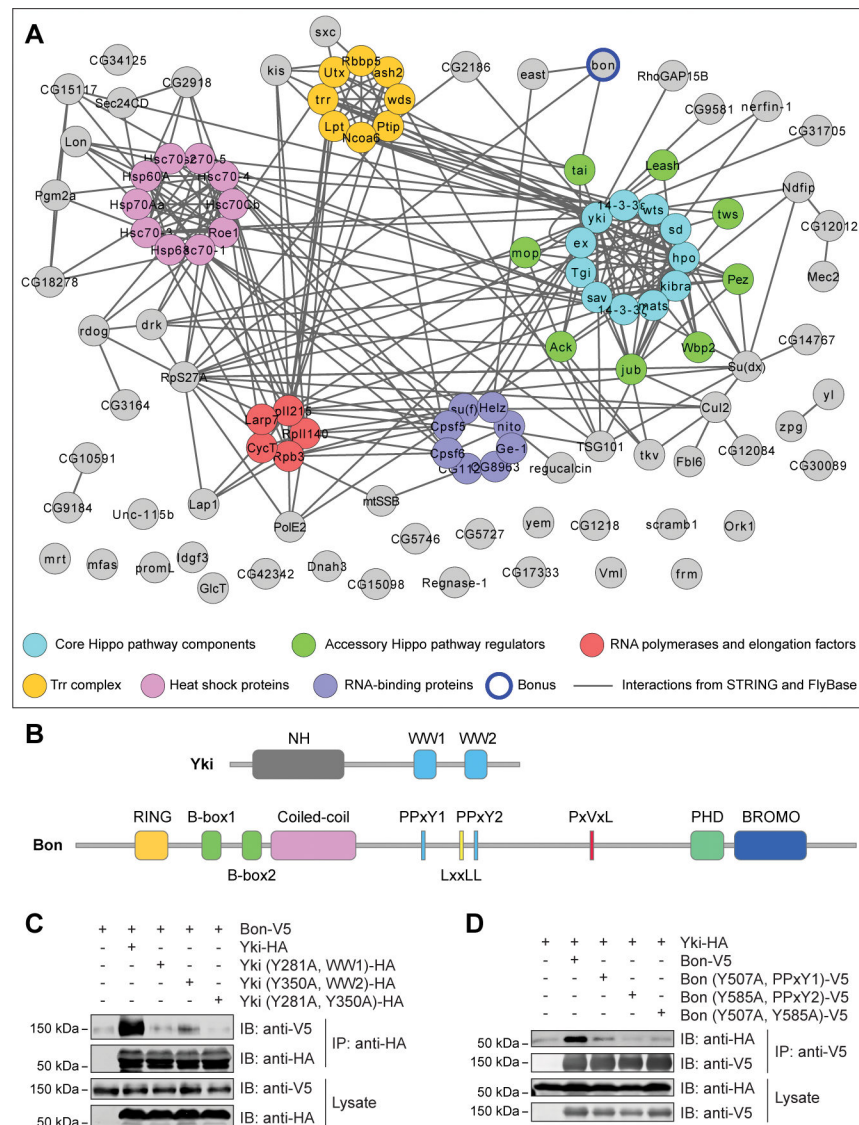


Figure 1. Identification of Bonus as a Yorkie interactor

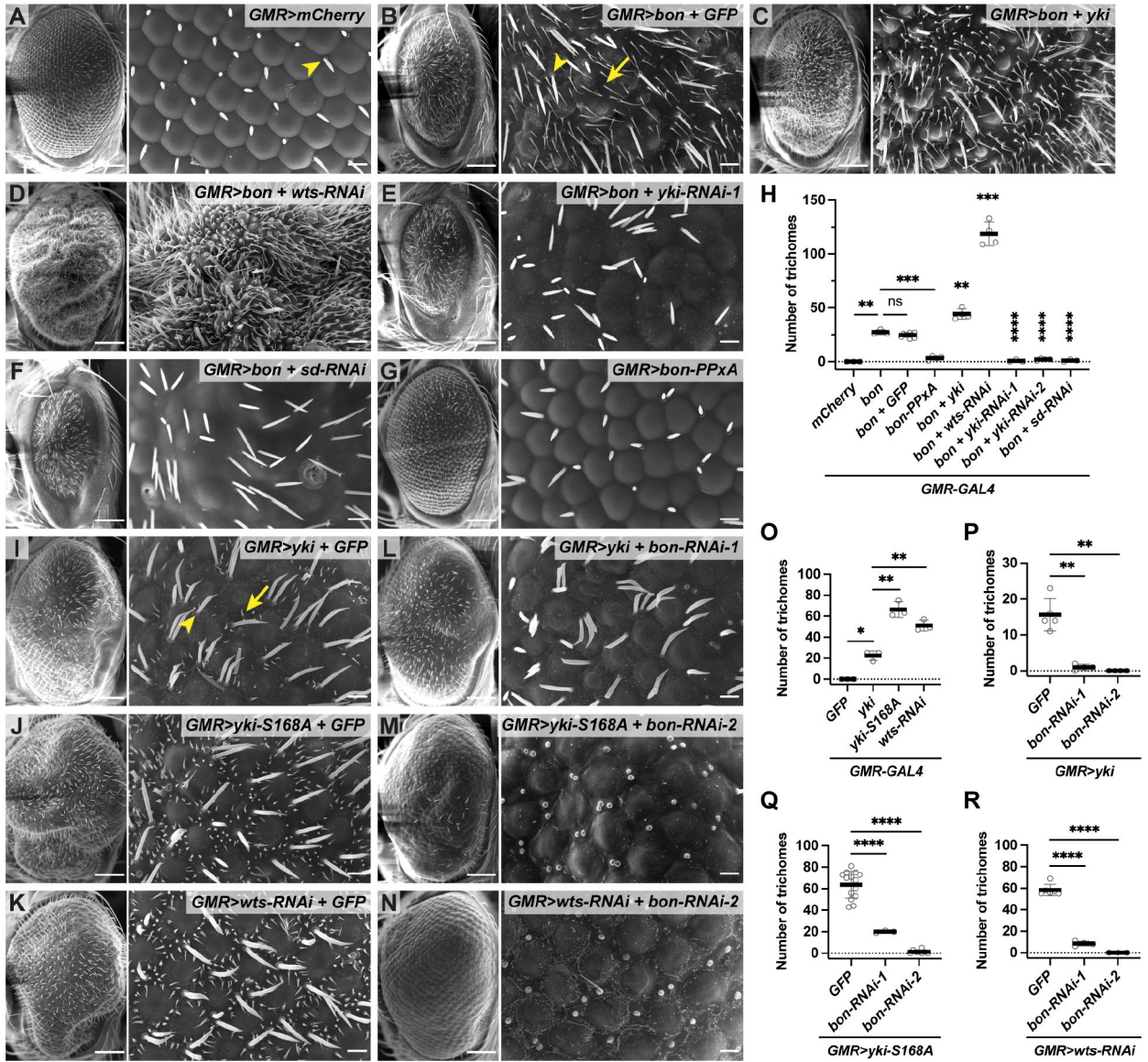
(A) The Yki protein interaction network. AP-MS results of Yki-SBP from *Drosophila* S2 cells and Yki-EGFP from embryos were analyzed with Significance Analysis of INteractome (SAINT)¹⁰⁵. Number of biological replicates: Yki-SBP, 4; blank S2, 4; Yki-EGFP, 3; *yw*, 5. All the interactors (nodes) in this network were identified as significant Yki interactors (SAINT score ≥ 0.8); these interactions (edges) are not shown to avoid clutter. The shown edges were incorporated from the STRING database and FlyBase, and include various types of evidence^{106,107}. See also Method Details.

(B) Schematic diagram showing the major domains and motifs in Yki and Bon. Note two WW domains in Yki and two PPxY motifs in Bon.

(C) Co-IP of Bon-V5 and wild-type or mutant Yki-HA expressed in S2 cells.

(D) Co-IP of Yki-HA and wild-type or mutant Bon-V5 expressed in S2 cells.

See also Table S1.



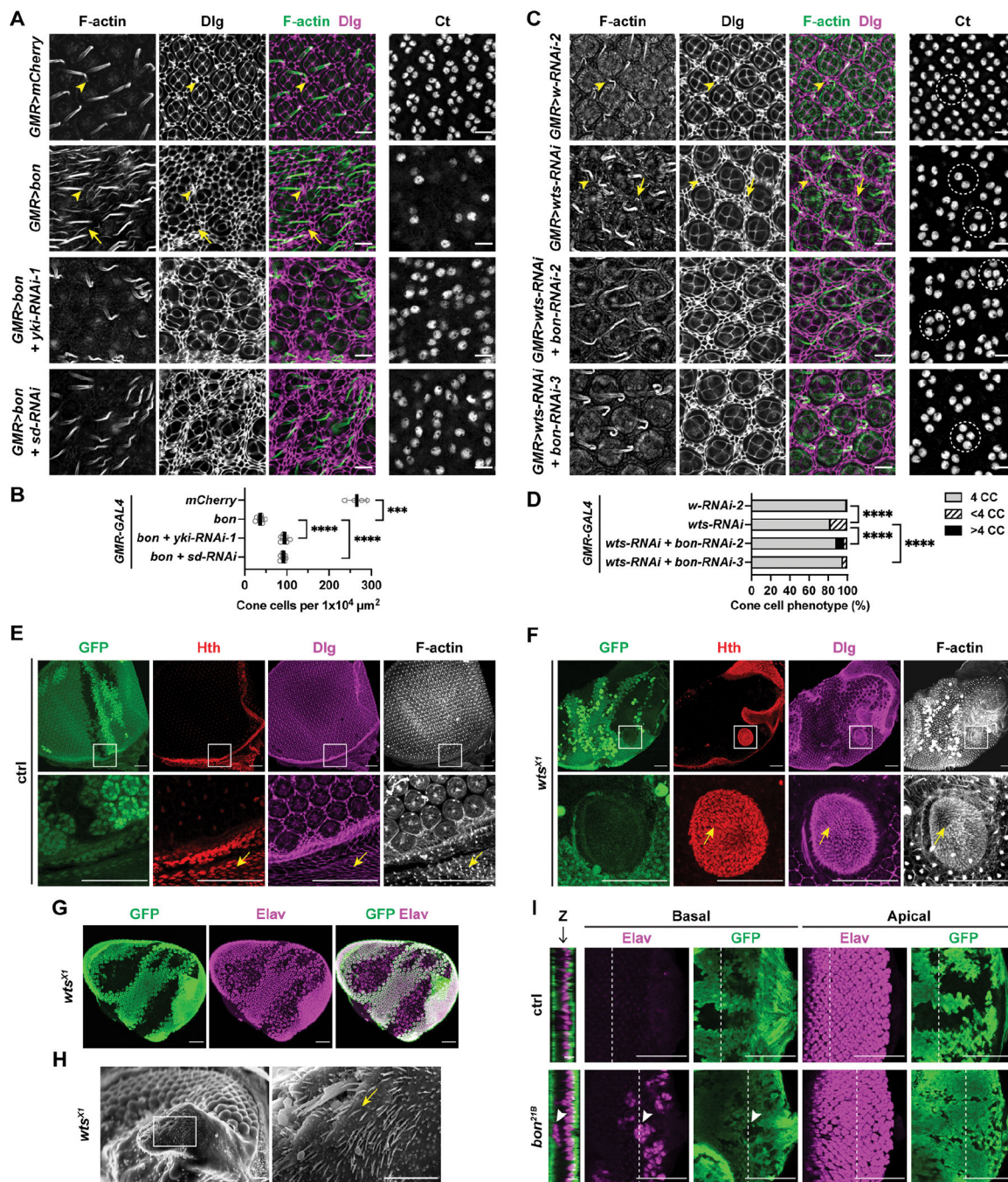


Figure 3. Yki and Bon promote the epidermal fate at the expense of the eye fate

(A, C) Pupal eyes expressing the indicated transgenes with *GMR-GAL4* were stained with phalloidin for F-actin and anti-disc large (Dlg) antibody for cell boundaries, or anti-Cut (Ct) antibody for cone cells. (A) 44 hrs APF grown at 25°C, (C) 40 hrs APF grown at 29°C (equivalent to 48 hrs APF at 25°C). Arrowheads: interommatidial bristles and sockets, arrows: trichomes and the corresponding cells, dashed circles in C: individual cone cell clusters per ommatidium. Scale bars: 10 μm .

(B, D) Quantification of cone cell numbers per area ($1 \times 10^4 \mu\text{m}^2$) due to a severe phenotype (B) or per ommatidium (D) for genotypes in A and C, respectively. Data in B shown as mean \pm SD of 4 pupal eyes. *p* values in D were determined by Fisher's exact test for ommatidia with 4 cone cells (CC) and those with <4 CC per ommatidium. Number of cone cell clusters quantified in D: $n = 300$ from 4 pupal eyes. See also Table S5 for details. (E-F) Pupal eyes at 46 hrs APF with mosaic clones generated by heat shock at 39 hrs after egg deposition (AED) and marked by absence of GFP were stained with anti-Hth antibody for head epidermis, anti-Dlg antibody for cell boundaries, and phalloidin for F-actin. Enlarged views of the boxed regions are shown in the bottom panels. Arrows: trichomes and the corresponding cells in wild-type head cuticle (E) or ectopic epidermis in the eye (F). Scale bars: 50 μm .

(G) Pupal eye at 46 hrs APF with mosaic clones of *wts^{XI}* induced by heat shock at 42 hrs AED was immunostained with anti-GFP antibody to negatively mark the clones and anti-Elav antibody for neuronal eye fate. Scale bars: 50 μm .

(H) A representative adult eye with mosaic clones of *wts^{XI}* generated with heat shock at 39 hrs AED. Right panel: enlarged view of the boxed region. Arrow: trichome. Scale bars: 20 μm .

(I) L3 eye discs with mosaic clones generated with *eyFLP* were immunostained with anti-GFP and anti-Elav antibodies. Z: orthogonal sections at the dashed lines of basal/apical views. Arrows: ectopic expression of the eye marker (Elav) and the corresponding clone (loss of GFP). The orthogonal sections and their scale bars were scaled 2x along the z axis for easier visualization.

Scale bars: 5 μm in orthogonal views and 50 μm in basal/apical views.

Detailed genotypes of controls and mutants are given in Method Details. See also Figure S3 and Table S5.

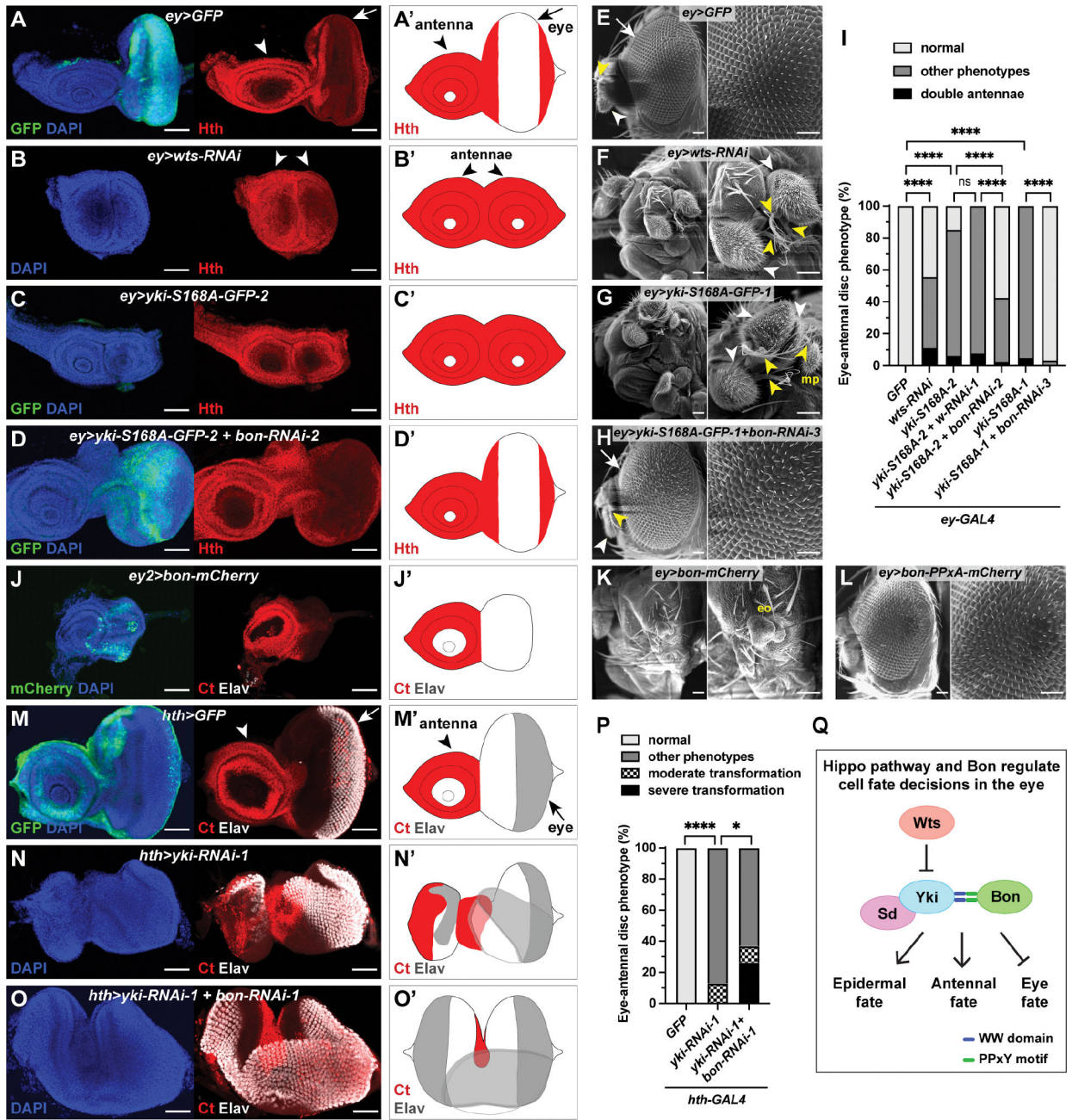


Figure 4. Yki and Bon promote antennal fate and suppress eye fate (A-D') L3 eye-antennal discs expressing indicated transgenes with *ey-GAL4* were immunostained with anti-Hth antibody for the antennal field. (A'-D') Schematic illustrations for A-D. (E-H) Representative images of adult heads with indicated genotypes. *ey>wts-RNAi* had double antennae with three aristae (F). *ey>yki-S168A* had double or triple antennae with one arista each and an ectopic maxillary palp (mp) (G). Right panels: enlarged views of the phenotypes, arrows: eyes, white arrowheads: antennal a3 segments, yellow arrowheads: aristae.

(I) Quantification of the phenotypes in eye-antennal discs shown in A-D, S4A-F and S4I-K. *p* values were determined by Fisher's exact test for normal and abnormal eye discs. Number of discs quantified: n = 18. See also Table S5 for details.

(J-J') L3 eye-antennal disc expressing *bon-mCherry* with *ey-GAL4-2* was immunostained with anti-Ct antibody for the antennal field and anti-Elav antibody for the neuronal eye fate. mCherry and Ct signals were colored green and red, respectively. (J') Schematic illustration of J. Control with GFP expressed under the same driver is shown in S4O-O'. Penetrance: 20% (n=15).

(K-L) Representative images of adult heads with indicated genotypes. Right panels: enlarged views of the phenotypes, eo: epidermal outgrowth. Loss of eye penetrance: 18%, n=56 (K); 0%, n=35 (L).

(M-O') L3 eye-antennal discs expressing indicated transgenes with *hth-GAL4* were immunostained with anti-Ct and anti-Elav antibodies. Representative moderate (N) and severe (O) antenna-to-eye transformations are shown. (M'-O') Schematic illustrations of M-O.

(P) Quantification of the phenotypes in eye-antennal discs shown in M-O and S4L-N. *p* values were determined by Fisher's exact test for normal and abnormal antennal discs (*GFP* vs. *yki-RNAi-1*) or for severe transformation and non-severe phenotypes (*yki-RNAi-1 + bon-RNAi-1* vs. *yki-RNAi-1*). Number of discs quantified: n = 19.

(Q) Model showing that the Yki-Bon complex promotes epidermal and antennal fates, and suppresses eye fate.

Scale bars: 50 μ m. See also Figure S4 and Table S5.

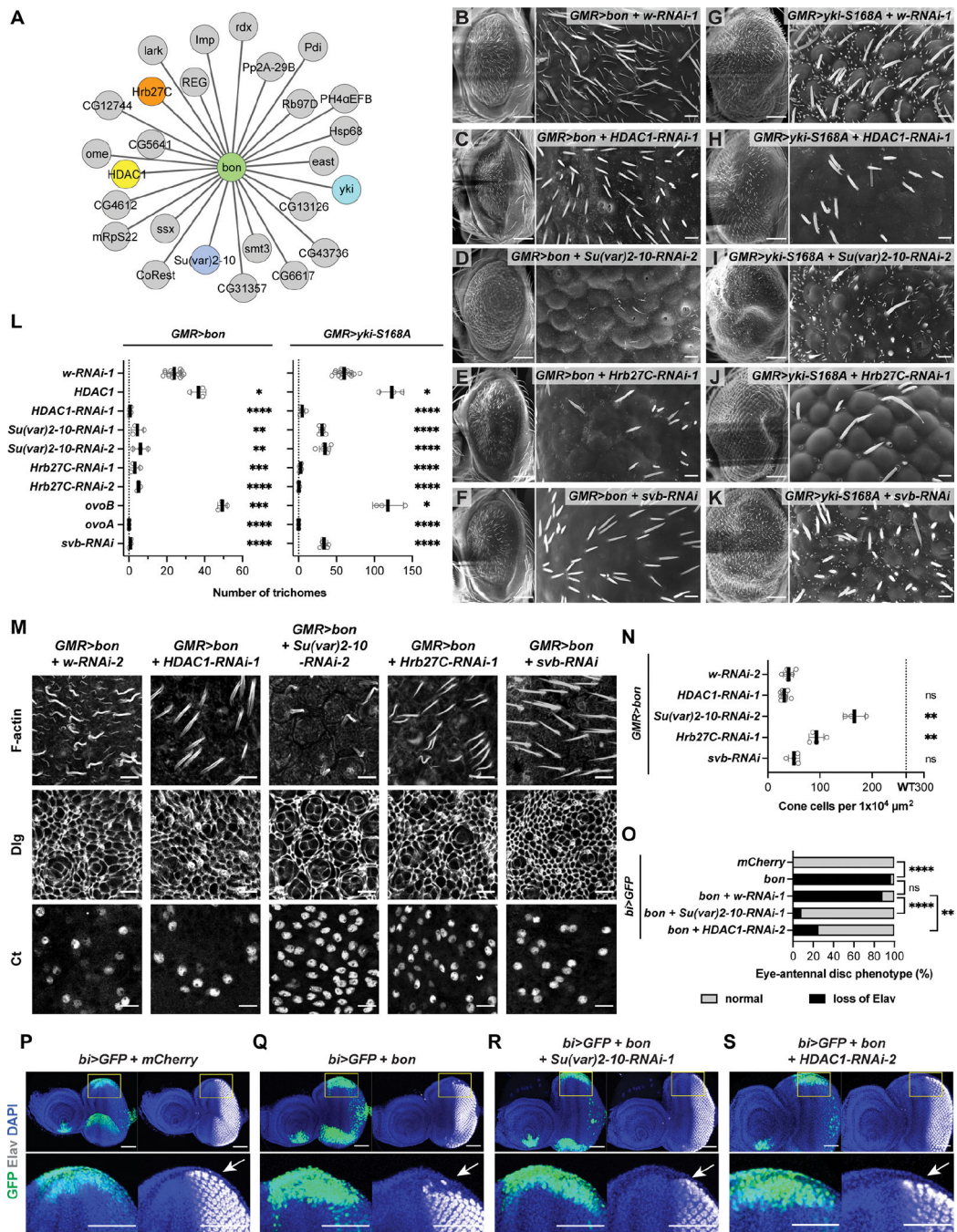


Figure 5. Interactors of Bon and Yki are required for the eye-epidermis fate decision

(A) Bon protein interactome showing significant interactors (SAINT score > 0.8) identified from AP-MS of Bon-SBP in S2 cells. Highlighted interactors were tested in genetic experiments. Number of biological replicates: Bon-SBP, 2; blank S2, 3.

(B-K) SEM images of adult eyes with indicated genotypes. Scales bars: left panels, 100 μm ; enlarged views, 10 μm .

(L) Quantification of the trichome numbers for the indicated genotypes in B-K and S5B-C. Data shown as mean \pm SD of 3 adult eyes. See also Table S5 for details.

(M) Pupal eyes at 44 hrs APF with indicated genotypes were stained with phalloidin for F-actin and anti-Dlg antibody for cell boundaries, or anti-Ct antibody for cone cells. Scale bars: 10 μm .

(N) Quantification of the cone cell numbers per $1 \times 10^4 \mu\text{m}^2$ area in M. Dashed line indicates the mean cone cell number in WT (*GMR>mCherry*) shown in Figure 3B. Data shown as mean \pm SD of 3 pupal eyes.

(O) Quantification of the phenotypes in eye-antennal discs shown in P-S. *p* values were determined by Fisher's exact test for normal and loss-of-Elav populations. Number of discs quantified: n = 8.

(P-S) L3 eye-antennal discs expressing indicated transgenes with the *bi-GAL4* driver were immunostained with anti-Elav antibody. Bottom panels: enlarged views of the dorsal margins (boxed). Arrows: Elav expression at the dorsal margins. Scale bars: 50 μm .

See also Figure S5, Table S2, and Table S5.

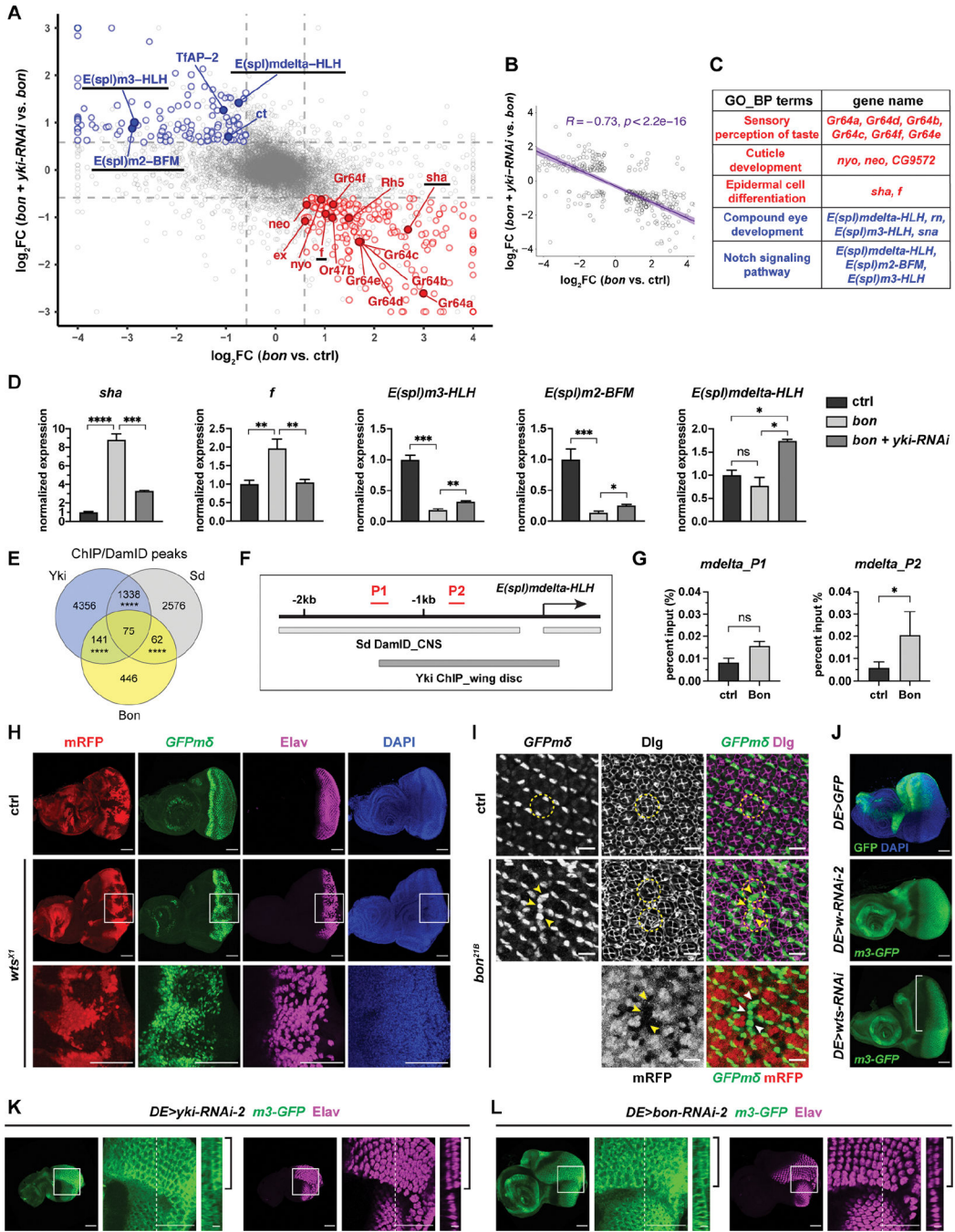


Figure 6. Bon and Yki jointly regulate a set of non-canonical transcriptional target genes
 (A) Differential gene expression analysis of RNA-seq data from 40–41 hrs pupal eyes. Output from DESeq2¹⁰⁸ from biological triplicates for each condition was plotted using log₂ fold changes (FC) for *GMR>bon-mCherry + yki-RNAi-1* vs. *GMR>bon-mCherry* (y axis) and that for *GMR>bon-mCherry* vs. *GMR>mCherry* (ctrl) (x axis). Dashed lines: FC = 1.5 in both directions. Red circles: Bon-activated/Yki-dependent genes. Blue circles: Bon-repressed/Yki-dependent genes. All colored circles fulfill *p* adj. 0.05. Solid circles with labels: genes of interest. Labels underlined: genes validated with qRT-PCR.

(B) Pearson correlation coefficient analysis of genes jointly regulated by Bon and Yki. Axes are the same as in A. All genes with FC > 1.5 in both directions and p adj. < 0.05 were included in the analysis.

(C) Terms of interest from GO_BP analysis of Bon-activated/Yki-dependent (red) and Bon-repressed/Yki-dependent (blue) genes. Complete GO_BP analysis is in Table S4.

(D) qRT-PCR validation for the genes of interest using 40–41 hrs APF pupal eyes with the genotypes *GMR>mCherry* (ctrl), *GMR>bon-mCherry*, and *GMR>bon-mCherry + yki-RNAi-1*. Data shown as mean \pm SD of biological triplicates.

(E) Venn diagram showing the overlaps in chromatin binding sites among Yki, Bon, and Sd analyzed using published datasets: Yki ChIP-seq from wing discs⁸⁴, Bon ChIP-seq from embryos⁸⁵, and Sd DamID-seq from larval CNS⁸⁶.

(F) Schematic diagram showing the genomic region around the transcription start site (arrow) of *E(spl)mdelta-HLH* (–2.2 kb to +0.5 kb). Yki and Sd binding regions from previous publications are indicated^{84,86}. P1 and P2: amplicons for ChIP-qPCR.

(G) ChIP-qPCR analysis for the binding of Bon to P1 and P2 shown in F, using L3 eye-antennal discs from *GMR>mCherry* (ctrl) and *GMR>bon-mCherry*. Data shown as mean \pm SD of biological triplicates.

(H) L3 eye-antennal discs with mosaic clones generated by *eyFLP* and negatively marked by mRFP were immunostained with anti-GFP antibody for the *GFPm δ* reporter and anti-Elav antibody. Bottom panels for *wts^{X1}*: enlarged views of the boxed area. Scale bars: 50 μ m.

(I) Pupal eyes at 25 hrs APF of control (*DE>w-RNAi*) and *bon^{21B}* mosaic clones were immunostained with anti-GFP antibody for the *GFPm δ* reporter and anti-Dlg antibody for cell boundaries. *bon^{21B}* clones were generated with *eyFLP* and marked by the absence of mRFP. Dashed circles: individual ommatidium, arrowheads: ectopic expression of *GFPm δ* in *bon^{21B}* clones. Scale bars: 10 μ m.

(J) L3 eye-antennal discs expressing indicated transgenes with *DE-GAL4*. The expression region of the driver is shown with GFP in the top panel here and in Figure S6E. Signal from the *m3-GFP* reporter was amplified by immunostaining with anti-GFP antibody. Bracket: loss of *m3-GFP* in the dorsal compartment of *DE>wts-RNAi*. Scale bars: 50 μ m.

(K-L) L3 eye-antennal discs expressing indicated transgenes with *DE-GAL4* were immunostained with anti-GFP antibody for the *m3-GFP* reporter and anti-Elav antibody. Penetrance of L: 8.3% (n=12). Left and middle panels are focused on the PE layer. Middle panels: enlarged views of the boxed regions, right panels: orthogonal sections at the dashed lines, brackets: gain of *m3-GFP* and Elav in the PE layer of the dorsal compartment. The orthogonal sections and their scale bars were scaled 2x along the z axis for easier visualization. Scale bars: 5 μ m in orthogonal views, 50 μ m in others.

Detailed genotypes of controls and mutants are given in Method Details. See also Figure S6 and Tables S3–S5.

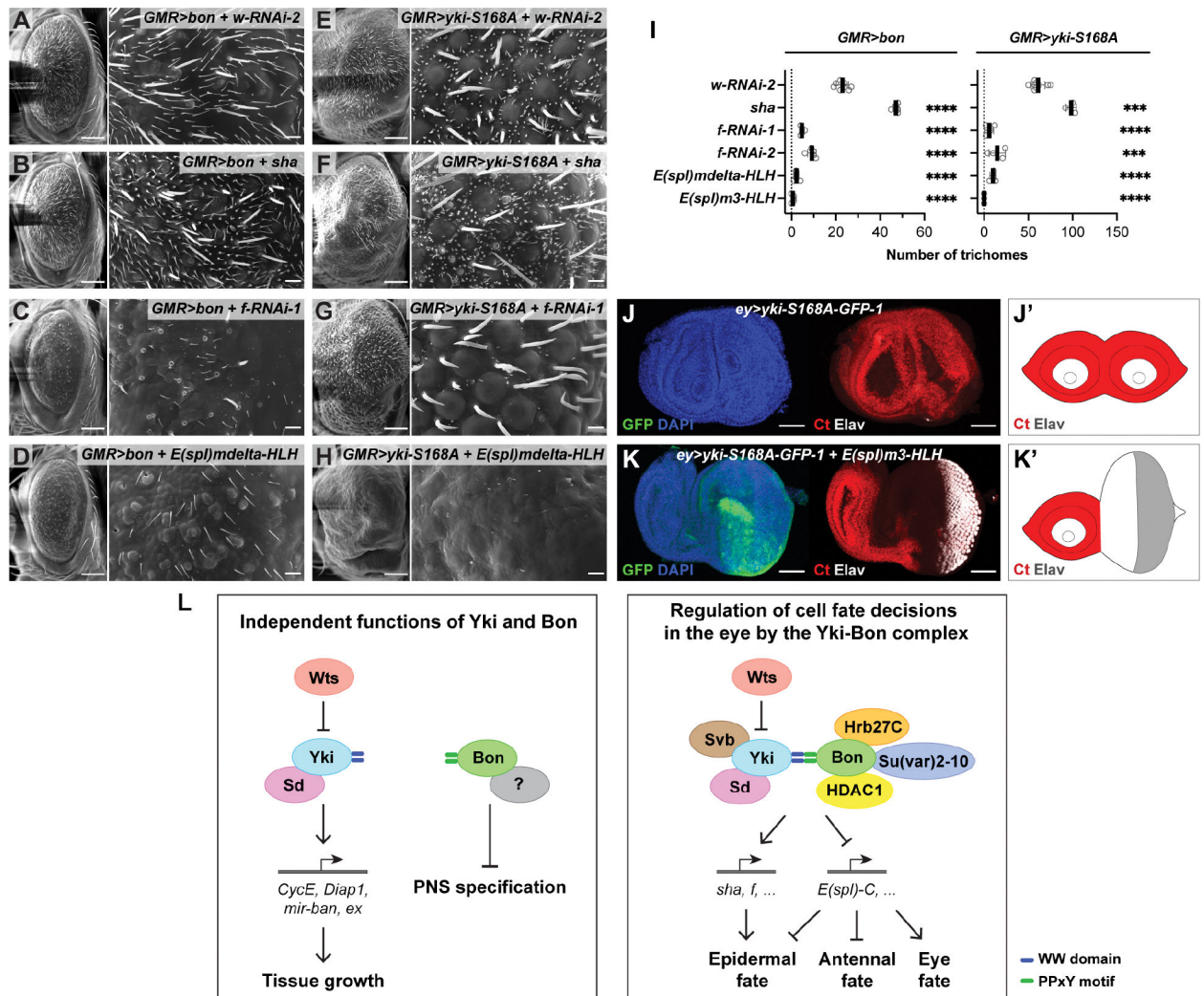


Figure 7. Bon and Yki control the eye-antenna-epidermis fate determination through their joint transcriptional targets.

(A-H) SEM images of adult eyes with indicated genotypes. Scales bars: left panels, 100 μ m; enlarged views, 10 μ m.

(I) Quantification of trichome numbers for the indicated genotypes in A-H and S6K-N. Data shown as mean \pm SD of 3 adult eyes. See also Table S5 for details.

(J-K') L3 eye-antennal discs with indicated genotypes were immunostained with anti-Ct and anti-Elav antibodies. (J'-K') Schematic illustrations of J-K. Scale bars: 50 μ m.

Quantification of the phenotypes is shown in S5G and detailed in Table S5; number of discs quantified for each was 36.

(L) Left: independent functions of Yki and Bon in growth regulation and embryonic PNS specification. Right: Yki and Bon jointly regulate cell fate decisions in the eye through recruitment of transcriptional and post-transcriptional regulators and transcriptional control of a set of non-canonical target genes.

See also Figures S5–S7 and Table S5.

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Monoclonal anti-SBP Tag (SB19-C4)	Santa Cruz Biotechnology	Cat# sc-101595, RRID: AB_1128239
Mouse Monoclonal anti-V5	MilliporeSigma	Cat# V8012, RRID: AB_261888
Rabbit Polyclonal anti-HA	MilliporeSigma	Cat# H6908, RRID: AB_260070
Mouse Anti-Myc-Tag Monoclonal Antibody	Cell Signaling Technology	Cat# 2276, RRID: AB_331783
Mouse Anti- β -Galactosidase mAb	Promega	Cat# Z3783, RRID: AB_430878
Mouse Monoclonal anti-futsch (22C10) supernatant	DSHB	Cat# 22c10, RRID: AB_528403
Mouse Monoclonal anti-discs large (Dlg) supernatant	DSHB	DSHB Cat# 4F3 anti-discs large, RRID: AB_528203
Mouse Monoclonal anti-Cut homeobox supernatant	DSHB	Cat# 2b10, RRID: AB_528186
Rat Monoclonal anti-Elav supernatant	DSHB	Cat# Rat-Elav-7E8A10 anti-elav, RRID: AB_528218
Rat Monoclonal anti-E-caderin supernatant	DSHB	Cat# DCAD2, RRID: AB_528120
Rabbit anti-GFP Polyclonal Antibody	Thermo Fisher Scientific	Cat# A-11122, RRID: AB_221569
Guinea Pig anti-Bon	Gift from Hugo Bellen: Beckstead et al. ²⁵	N/A
Guinea Pig anti-Hth	Gift from Claude Desplan: Ozel et al. ¹¹⁷	N/A
Rabbit anti-BarH1	Gift from Kwang-Wook Choi: Kang et al. ¹¹⁸	N/A
Goat anti-Mouse IgG, IRDye 680RD	LI-COR Biosciences	Cat# 926-68070, RRID: AB_10956588
Donkey anti-Rabbit IgG, IRDye 800CW	LI-COR Biosciences	Cat# 926-32213, RRID: AB_621848
Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-11073, RRID: AB_2534117
Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Thermo Fisher Scientific	Cat# A-21435, RRID: AB_2535856
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat# A-31571, RRID: AB_162542
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Thermo Fisher Scientific	Cat# A-21422, RRID: AB_2535844
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21202, RRID: AB_141607
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat# A-21247, RRID: AB_141778
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Thermo Fisher Scientific	Cat# A-21434, RRID: AB_2535855
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-11006, RRID: AB_2534074
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647	Thermo Fisher Scientific	Cat# A32795, RRID: AB_2762835
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21206, RRID: AB_2535792

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Pierce Streptavidin Plus UltraLink Resin	Thermo Fisher Scientific	Cat# 53117
Pierce Control Agarose Resin	Thermo Fisher Scientific	Cat# 26150
GFP-Trap Agarose	Bulldog Bio	Cat# GTA020
RFP-Trap Agarose	Bulldog Bio	Cat# RTA020
Anti-V5 Agarose Affinity Gel	MilliporeSigma	Cat# A7345
EZview Red Anti-HA Affinity Gel	MilliporeSigma	Cat# E6779
Odyssey Blocking Buffer (PBS)	LI-COR Biosciences	Cat# 927-40003
Western Blocking Reagent, Solution	MilliporeSigma (Roche)	Cat# 11921681001
cComplete Protease Inhibitor Cocktail	MilliporeSigma (Roche)	Cat# 11836145001
cComplete, Mini, EDTA-free Protease Inhibitor Cocktail	MilliporeSigma (Roche)	Cat# 11836170001
Hexamethyldisilazane (HMDS)	Electron Microscopy Sciences	Cat# 16700
Formaldehyde solution	MilliporeSigma	Cat# F8775
16% Paraformaldehyde (formaldehyde) aqueous solution	Electron Microscopy Sciences	Cat# 15710
Pierce 16% Formaldehyde (w/v), Methanol-free	Thermo Fisher Scientific	Cat# 28906
ProLong Gold Antifade Mountant with DAPI	Thermo Fisher Scientific	Cat# P36931
ProLong Gold Antifade Mountant	Thermo Fisher Scientific	Cat# P10144
Gibco Schneider's <i>Drosophila</i> Medium	Thermo Fisher Scientific	Cat# 21720001
Gibco Fetal Bovine Serum, US certified, heat inactivated	Thermo Fisher Scientific	Cat# 10082147
Gibco Penicillin-Streptomycin (5,000 U/mL)	Thermo Fisher Scientific	Cat# 15070063
Hygromycin B	MilliporeSigma	Cat# H3274
IGEPAL CA-630	MilliporeSigma	Cat# I8896
Bovine Serum Albumin (BSA)	VWR	Cat# RLBSA50
Phalloidin, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A12379
Phalloidin, Alexa Fluor 594	Thermo Fisher Scientific	Cat# A12381
Phalloidin, CF405M	Biotium	Cat# 00034-T
Invitrogen TRIzol Reagent	Thermo Fisher Scientific	Cat# 15596026
Trichloroacetic Acid (Flakes or Crystals)	Fisher Scientific	Cat# BP555-500
MG-132 - CAS 133407-82-6 - Calbiochem	MilliporeSigma	Cat# 474790
Indicating Drierite	W.A. Hammond	Cat# 23005
10x PBS	Fisher Scientific	Cat# BP3994
UltraPure DNase/RNase-Free Distilled Water	Thermo Fisher Scientific	Cat# 10977015
RNase A, DNase and protease-free	Thermo Fisher Scientific	Cat# EN0531
Proteinase K Solution, ChIP grade	Thermo Fisher Scientific	Cat# 26160
CMCP-10 High viscosity mountant	Polysciences, Inc.	Cat# 16300-250
Critical commercial assays		
Q5 Site-Directed Mutagenesis Kit	NEB	Cat# E0554S
NEBuilder HiFi DNA Assembly Cloning Kit	NEB	Cat# E5520S
Effectene transfection reagent	QIAGEN	Cat# 301427

REAGENT or RESOURCE	SOURCE	IDENTIFIER
NuPAGE 4–12% Bis-Tris Protein Gel	Thermo Fisher Scientific	Cat# NP0335
SilverQuest Silver Staining Kit	Thermo Fisher Scientific	Cat# LC6070
Click-iT™ Edu Imaging Kit with Alexa Fluor™ 488, 594, and 647 Azides	Thermo Fisher Scientific	Cat# C10086
RNeasy Mini Kit	QIAGEN	Cat# 74104
RNase-Free DNase Set	QIAGEN	Cat# 79254
SuperScript III First-Strand Synthesis System	Thermo Fisher Scientific	Cat# 18080051
PowerUp SYBR Green Master Mix	Thermo Fisher Scientific	Cat# A25742
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	NEB	Cat# E7775S
NEBNext Poly(A) mRNA Magnetic Isolation Module	NEB	Cat# E7490S
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	NEB	Cat# E7500S
Chromatin Immunoprecipitation (ChIP) Assay Kit	MilliporeSigma	Cat# 17-295
Aluminum Mount, Carbon Adhesive Tape, and Mount Holder	Electron Microscopy Sciences	Cat# 75220, 77816 and 76510
Deposited data		
RNA-seq	Gene Expression Omnibus (GEO)	GSE181299
Proteomics	Proteomics Identification Database (PRIDE)	PXD027934
Mendeley Data	Mendeley Data	doi: 10.17632/v2v9n9tbfp.1
Experimental models: Cell lines		
<i>D. melanogaster</i> S2 cell line	Gift from Spyros Artavanis-Tsakonas (Harvard Medical School)	N/A
<i>D. melanogaster</i> S2- <i>yki-SBP</i>	This study	N/A
<i>D. melanogaster</i> S2- <i>bon-SBP</i>	This study	N/A
Experimental models: Organisms/strains		
<i>D. melanogaster</i> : <i>w*</i> ; <i>UAS-yki-EGFP attP2</i>	This study	N/A
<i>D. melanogaster</i> : <i>w</i> ; <i>UAS-bon-mCherry attP40</i>	This study	N/A
<i>D. melanogaster</i> : <i>w</i> ; <i>UAS-bon-PPxA-mCherry attP40</i>	This study	N/A
<i>D. melanogaster</i> : <i>y[1], w[*]</i>	Gift from Alexei Tulin (University of North Dakota)	N/A
<i>D. melanogaster</i> : <i>Oregon R</i>	Bloomington Drosophila Stock Center	BDSC: 25211
<i>D. melanogaster</i> : <i>UAS-GFP</i>	Bloomington Drosophila Stock Center	BDSC: 5430
<i>D. melanogaster</i> : <i>da-GAL4</i>	Wodarz et al. ³⁵	N/A
<i>D. melanogaster</i> : <i>GMR-Gal4, UAS-yki^{S168A}:YFP/TM6B, Tb, Hu</i> (used in Figure S1A only)	Gift from Duojia Pan (UT Southwestern Medical Center)	N/A
<i>D. melanogaster</i> : <i>en-GAL4, UAS-GFP/Cyo, tub-GAL80; UAS-Yki/TM6B, Tb</i> (used in Figure S1C only)	Gift from Duojia Pan	N/A
<i>D. melanogaster</i> : <i>GMR-GAL4</i>	Freeman ⁴⁴ and Hay et al. ⁴²	N/A
<i>D. melanogaster</i> : <i>en-GAL4</i>	Bloomington Drosophila Stock Center	BDSC: 30564
<i>D. melanogaster</i> : <i>en-GAL4, ex-lacZ/Cyo, twi>GFP</i>	Derivative of BDSC: 44248	BDSC: 44248
<i>D. melanogaster</i> : <i>en-GAL4/Cyo, Dfd-GFP; Diap1-lacZ/TM6B</i>	Derivative of BDSC: 12093	BDSC: 12093

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>D. melanogaster</i> : UAS-bon-RNAi-1 w ¹¹¹⁸ ; P{GD1388}v44283	Vienna Drosophila Resource Center	VDRC: 44283
<i>D. melanogaster</i> : UAS-bon-RNAi-2 w ¹¹¹⁸ ; P{GD1388}v44284	Vienna Drosophila Resource Center	VDRC: 44284
<i>D. melanogaster</i> : UAS-bon-RNAi-3 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS01657}attP40	Bloomington Drosophila Stock Center	BDSC: 37515
<i>D. melanogaster</i> : UAS-bon-RNAi-4 y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02373}attP2	Bloomington Drosophila Stock Center	BDSC: 27047
<i>D. melanogaster</i> : bon ^{21B} null allele P{ry[+t7.2]=hsFLP}22, y[1] w[*]; P{ry[+t7.2]=neoFRT}82B bon[21B]/TM3, Sb[1]	Bloomington Drosophila Stock Center	BDSC: 43660
<i>D. melanogaster</i> : bon ⁴⁸⁷ hypomorphic allele y[1] w[67c23]; P{w[+mC]=lacW}bon[S048706]/TM3, Sb[1] Ser[1]	Bloomington Drosophila Stock Center	BDSC: 4543s
<i>D. melanogaster</i> : wts ^{X1} null allele w[*]; wts[x1] P{ry[+t7.2]=neoFRT}82B/TM6B, Tb[1]	Bloomington Drosophila Stock Center	BDSC: 44251
<i>D. melanogaster</i> : yki ^{B5} null allele yki ^{B5} /CyO: twi>GFP	Gift from Duoqia Pan: Huang et al. ⁴¹	N/A
<i>D. melanogaster</i> : UAS-w-RNAi-1 y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00004}attP2/TM3, Sb[1]	Bloomington Drosophila Stock Center	BDSC: 33613
<i>D. melanogaster</i> : UAS-w-RNAi-2 y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02387}attP2	Bloomington Drosophila Stock Center	BDSC: 33762
<i>D. melanogaster</i> : UAS-yki-RNAi-1 y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00041}attP2	Bloomington Drosophila Stock Center	BDSC: 34067
<i>D. melanogaster</i> : UAS-yki-RNAi-2 P{KK109756}VIE-260B (@40D and 30B)	Vienna Drosophila Resource Center	VDRC: 104523
<i>D. melanogaster</i> : UAS-sd-RNAi	Gift from Jin Jiang: Zhang et al. ⁴⁸	N/A
<i>D. melanogaster</i> : UAS-wts-RNAi P{KK101055}VIE-260B (inserted at 30B only)	Vienna Drosophila Resource Center	VDRC: 111002
<i>D. melanogaster</i> : UAS-yki-S168A-1 y[1] w[*]; P{w[+mC]=UAS-yki.S168A.GFP.HA}10-7-Y	Bloomington Drosophila Stock Center	BDSC: 28816
<i>D. melanogaster</i> : UAS-yki-S168A-2 y[1] w[*]; P{w[+mC]=UAS-yki.S168A.GFP.HA}10-12-1	Bloomington Drosophila Stock Center	BDSC: 28836
<i>D. melanogaster</i> : UAS-mCherry y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=UAS-mCherry.VALIUM10}attP2	Bloomington Drosophila Stock Center	BDSC: 35787
<i>D. melanogaster</i> : w; FRT82B	Gift from Spyros Artavanis-Tsakonas	N/A
<i>D. melanogaster</i> : y w hsFLP122; FRT82B Ubi-GFP	Gift from Spyros Artavanis-Tsakonas	N/A
<i>D. melanogaster</i> : y w eyFLP; FRT82B Ubi-GFP/TM6B	Gift from Iswar Hariharan (UC Berkeley)	N/A
<i>D. melanogaster</i> : Ubx-FLP, y w; FRT40A/CyO; FRT82B Sb[63b]/TM3, Ser	Bloomington Drosophila Stock Center	BDSC: 43337
<i>D. melanogaster</i> : eyFLP	Bloomington Drosophila Stock Center	BDSC: 5580
<i>D. melanogaster</i> : y w hsFLP122; TM3Sb/ TM6B	Gift from Naoto Ito	N/A
<i>D. melanogaster</i> : w; FRT82B Ubi-mRFP	Bloomington Drosophila Stock Center	BDSC: 30555
<i>D. melanogaster</i> : UAS-ovoA w[*]; P{w[+mC]=UAS-ovo.A}1M	Bloomington Drosophila Stock Center	BDSC: 38428
<i>D. melanogaster</i> : UAS-ovoB w[*]; P{w[+mC]=UASp-ovo.B}Cmm	Bloomington Drosophila Stock Center	BDSC: 38429
<i>D. melanogaster</i> : UAS-svb-RNAi w ¹¹¹⁸ ; P{GD9026}v41584	Vienna Drosophila Resource Center	VDRC: 41584

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>D. melanogaster</i> : UAS-HDAC1 y[1] w[*]; P{w[+mC]=UAS-HDAC1. V5}A2a	Bloomington Drosophila Stock Center	BDSC: 32242
<i>D. melanogaster</i> : UAS-HDAC1-RNAi-1 w ¹¹¹⁸ ; P{GD4513}v30600	Vienna Drosophila Resource Center	VDRC: 30600
<i>D. melanogaster</i> : UAS-HDAC1-RNAi-2 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00164}attP2	Bloomington Drosophila Stock Center	BDSC: 34846
<i>D. melanogaster</i> : UAS-Su(var)2-10-RNAi-1 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00705}attP2/TM3, Sb[1]	Bloomington Drosophila Stock Center	BDSC: 32915
<i>D. melanogaster</i> : UAS-Su(var)2-10-RNAi-2 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00750}attP2	Bloomington Drosophila Stock Center	BDSC: 32956
<i>D. melanogaster</i> : UAS-Hrb27C-RNAi-1 w ¹¹¹⁸ ; P{GD6964}v16040	Vienna Drosophila Resource Center	VDRC: 16040
<i>D. melanogaster</i> : UAS-Hrb27C-RNAi-2 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00597}attP2	Bloomington Drosophila Stock Center	BDSC: 33716
<i>D. melanogaster</i> : UAS-sha w[*]; P{w[+mC]=UAS-sha.GFP}3	Bloomington Drosophila Stock Center	BDSC: 32096
<i>D. melanogaster</i> : UAS-f-RNAi-1 w ¹¹¹⁸ ; P{GD1443}v33200	Vienna Drosophila Resource Center	VDRC: 33200
<i>D. melanogaster</i> : UAS-f-RNAi-2 y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS02251}attP2	Bloomington Drosophila Stock Center	BDSC: 41687
<i>D. melanogaster</i> : UAS-E(spl)mdelta-HLH y[1] w[*]; P{w[+mC]=UA S-mdelta}h 8	Bloomington Drosophila Stock Center	BDSC: 26677
<i>D. melanogaster</i> : UAS-E(spl)m3-HLH	Gift from Eric Lai (Sloan Kettering Institute)	N/A
<i>D. melanogaster</i> : (y) w; M[p3xP3-RFP floxed out), E(spl)-C GFP-mdelta]51D/Cyo	Gift from François Schweisguth: Couturier et al. ⁸⁷	N/A
<i>D. melanogaster</i> : w ¹¹¹⁸ ; M[p3xP3-RFP floxed out), E(spl)-C m3-sGFP]51D/Cyo	Gift from François Schweisguth: Couturier et al. ⁸⁷	N/A
<i>D. melanogaster</i> : y; DE-GAL4/TM6B	Derivative of BDSC: 29650	BDSC: 29650
<i>D. melanogaster</i> : ey-GAL4 w[*]; P{w[+m*]= GAL4-ey.H}3-8	Bloomington Drosophila Stock Center	BDSC: 5534
<i>D. melanogaster</i> : ey-GAL4-2 w[*]; P{w[+m*]= GAL4-ey.H}4-8/CyO	Bloomington Drosophila Stock Center	BDSC: 5535
<i>D. melanogaster</i> : Hth-GAL4/TM3Sb	Gift from Claude Desplan: Wernet et al. ⁵⁵	N/A
<i>D. melanogaster</i> : UAS-GFP, bi-GAL4	Bloomington Drosophila Stock Center	BDSC: 58815
<i>D. melanogaster</i> : C5-GAL4	Yeh et al. ⁹¹	N/A
Oligonucleotides		
RT-qPCR primers for <i>rp49</i> (normalizing control): Fwd: CGGATCGATATGCTAAGCTGT; Rev: GCGCTTGTTCGATCCGTA	Zhang et al. ³⁹	N/A
RT-qPCR primers for <i>sha</i> : Fwd: TCGCTGTGAAATCGAACAAG; Rev: GCCGCCATAGTGACAACTT	This study	N/A
RT-qPCR primers for <i>f</i> : Fwd: GAAGGTACCGAAGCCCTACC; Rev: CTTCTTGATGCCGGTATGT	This study	N/A
RT-qPCR primers for <i>E(spl)m3-HLH</i> : Fwd: CAGGGAGTAGTGGCTGGTGT; Rev: GGTAATCTGATCGGCAGCAT	This study	N/A

REAGENT or RESOURCE	SOURCE	IDENTIFIER
RT-qPCR primers for <i>E(spl)m2-BFM</i> : Fwd: CATGCGTAACGTGTGGAAAC; Rev: TCAATGAGCAACTCTGCTG	This study	N/A
RT-qPCR primers for <i>E(spl)mdelta-HLH</i> : Fwd: ACTCAGCATTACCGCAAGGT; Rev: CTTTCTCCAGCTTGCTGACC	This study	N/A
ChIP-qPCR primers for <i>E(spl)mdelta-HLH-P1</i> : Fwd: ATCCCCGAATACCCAATCTC; Rev: GCATGTGCATCGTGAGAAAG	This study	N/A
ChIP-qPCR primers for <i>E(spl)mdelta-HLH-P2</i> : Fwd: TCTTTTCTCGAGGGAAGTGG; Rev: AAGAGTCGGAGCAATCAACC	This study	N/A
Recombinant DNA		
pMT-yki-V5	Zhang et al. ³⁹	N/A
pMK33-SBP-C	Yang and Veraksa ¹¹¹	N/A
pUASTattB	Bischof et al. ¹¹²	N/A
pMT-V5-His	Invitrogen	Cat# V412020
pMK33-yki-SBP	This study	N/A
pUASTattB-yki-EGFP	This study	N/A
pMT-yki-HA	This study	N/A
pMT-yki-Y281A-HA	This study	N/A
pMT-yki-Y350A-HA	This study	N/A
pMT-yki-Y281A Y350A-HA	This study	N/A
pFlc-1-bon	Drosophila Genomics Resource Center	DGRC: RE48191
pMT-bon-V5	This study	N/A
pMT-bon-Y507A-V5	This study	N/A
pMT-bon-Y585A-V5	This study	N/A
pMT-bon-Y507A Y585A-V5	This study	N/A
pMK33-bon-SBP	This study	N/A
pUASTattB-bon-mCherry	This study	N/A
pUASTattB-bon-Y507A Y585A-mCherry	This study	N/A
pFlag-wts	Gift from Maxim Frolov (University of Illinois at Chicago)	N/A
pMT-Myc-wts	This study	N/A
Software and algorithms		
Significance Analysis of INteractome (SAINT) (v2.5.0)	Choi et al. ¹⁰⁵	http://saint-apms.sourceforge.net/Main.html
Cytoscape (v3.8.0)	Shannon et al. ¹¹⁵	https://cytoscape.org/ ; RRID: SCR_003032
STRING (v11.0)	Szkarczyk et al. ¹⁰⁶	https://string-db.org/
Fiji (v2.1.0)	Schindelin et al. ¹²³	https://imagej.net/Fiji/ ; RRID:SCR_002285
GraphPad Prism (v9.1.0)	GraphPad	https://www.graphpad.com/ ; RRID:SCR_002798

REAGENT or RESOURCE	SOURCE	IDENTIFIER
R (v4.0.0)	R Core Team	https://www.r-project.org/ ; RRID:SCR_001905
RStudio (v1.2.5042)	RStudio, Inc.	https://www.rstudio.com/ ; RRID: SCR_000432
ggplot2 (v3.3.2)	Wickham ¹³³	https://ggplot2.tidyverse.org/
ggpubr (v0.4.0)	Alboukadel Kassambara	https://rpkgs.datanovia.com/ ggpubr/
Cutadapt (v2.9)	Martin ¹²⁶	https://cutadapt.readthedocs.io/en/ stable/ ; RRID:SCR_011841
Bowtie 2 (v2.3.5.1)	Langmead and Salzberg ¹²⁷	http://bowtie-bio.sourceforge.net/ bowtie2/index.shtml ; RRID:SCR_016368
STAR (v2.7.0e)	Dobin et al. ¹²⁹	https://github.com/alexdobin/ STAR ; RRID:SCR_004463
featureCounts (Subread v1.6.2)	Liao et al. ¹³⁰	http://bioinf.wehi.edu.au/ featureCounts/ ; RRID:SCR_012919
Deseq2 (v1.30.1)	Love et al. ¹⁰⁸	https://bioconductor.org/packages/ release/bioc/html/DESeq2.html ; RRID:SCR_015687
DEBrowser (v1.16.3)	Kucukural et al. ¹³¹	https://www.bioconductor.org/ packages/release/bioc/html/ debrowser.html
DAVID Bioinformatics Resources (v6.8)	Huang da et al. ¹³⁴	https://david.abcc.ncifcrf.gov/ home.jsp ; RRID:SCR_001881
ChIPpeakAnno (v3.30.1)	Zhu et al. ¹³⁶	https://bioconductor.org/ packages/release/bioc/html/ ChIPpeakAnno.html ; RRID: SCR_012828
Integrative Genomics Viewer (IGV) (v2.8.2)	Robinson et al. ¹³⁸	https:// software.broadinstitute.org/ software/igv/ ; RRID:SCR_011793