Developmental Cell *14*

Supplemental Data

The TEAD/TEF Family of Transcription

Factor Scalloped Mediates

Hippo Signaling in Organ Size Control

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Supplemental Experimental Procedures

Transgenes

To construct *UAS-Sd-RNAi* transgenes, genomic DNA fragments corresponding to Sd amino acid (aa) 36-169 (*Sd-RNAi*^{N}) or Sd aa183-440 (*Sd-RNAi*^{N}) were amplified by PCR and subcloned between the *EcoRI* and *Bgl II* sites or *Not I* and *Kpn I* sites of the *pUAST* vector, respectively. The corresponding cDNA fragments were inserted in a reverse orientation between the *XhoI* and *Xba I* sites or *Kpn I* and *Xba I* sites, respectively. To construct *UAS-Yki-RNAi transgenes*, cDNA fragments coding for Yki aa 1–180 (*Yki-RNAi*^{N}) or Yki aa 285–418 (*Yki-RNAi*^{C}) were amplified by PCR. Two copies of the cDNA fragments were subcloned in opposite orientation into the *pUAST* vector. To construct HA-Sd, HA-Sd-N and HA-Sd-C, the corresponding cDNA fragments were amplified by PCR and subcloned in frame with three copies of HA tags in the *pUAST-3HA* vector (Chao and Jiang, 2007). To construct Fg-Yki and Myc-Yki, Yki coding sequence was amplified by PCR and subcloned into the *pUAST-Fg* or *pUSAT-6Myc* vector so that they carry one copy of Flag tag or 6 copies of Myc tag at their N-termini (Chao and Jiang, 2007).

Generating *sd* Deletion Alleles

$sd^{\Delta B}$ was generated using P insertion lines *f04046* and *e02321*. They were placed in trans together with a *hs-FLP* transgene, followed by heat-shock to generate FRT-mediated deletion. PCR primers directed to the remaining P elements (WH5' plus: GACGCATGATTATCTTTTACGTGAC; RB5' minus: GACGCATGATTATCTTTTACGTGAC) were used to identify deletion alleles. $sd^{\Delta C}$ was generated using P insertion lines *d04236* and *e02321*, and deletion alleles were recognized by darker eye color as they carry two copies of w^{+}. Both $sd^{\Delta B}$ and $sd^{\Delta C}$ were further tested for wing phenotypes in transheterozygotes with sd^{1}.

Luciferase Reporter Assay

To generate the *3xSd2-Luc* reporter gene, three copies of a tandem Sd binding sites from the *dSRF* enhancer (dSRF-A2-I; Halder et al., 1998) as well as the HSP70 basal promoter were subcloned into the *XhoI* site and between *BglII* and *HindIII* sites of the *pGL3-Basic* vector, respectively. For luciferase reporter assays, S2 cells were transfected with *3xSd2-Luc* and *copia-renilla* luciferase reporter constructs in 12 well plate together with Yki and/or Sd expressing constructs. Cells were incubated for 48 hr after transfection. The reporter assay were performed using the Dual-Luciferase reporter assay system (Promega). Dual-Luciferase measurements were performed in triplicate using FLUOstar OPTIMA (BMG LABETCH).

ChIP Assay

Formaldehyde-crosslinked and mock-crosslinked chromatin was prepared from eye imaginal discs that express Yki or Sd transgenes with *GMR-Gal4*. Discs were dissected

in serum free SS3 medium (Sigma) and stored on ice before formaldehyde fixation. Groups of 40 discs were fixed 20 min at a time, and immunoprecipitation were performed using the ChIP assay kit (Upstate Biotechnology) according to the manufacture's recommended protocol. One tenth of the DNA from each immunoprecipitation was used in each PCR reaction. Pairs of PCR primers were used for amplification of the following segments of *diap1* enhance element:

E1: 5'-CTCGCTTTGAGCGCTG-3'
5'-GTATACTGATGCAAAAGTATG-3'

E2: 5'-TGAGCCCCAGTTTTAT-3'
5'-ATATGGCCAGAAAAGA-3'

E3: 5'-CATACTTCTGCATACATAAA-3'
5'-GATGGTAGGATGACACAT-3'

E4: 5'-TCCTACCATCAATAGAGAGCT-3'
5'-GCTCAGACTCAGCTCGA-3'

2kb away from the DIAP enhance element
5'-GCTGGCTCCGCTTTCGGCGA-3'
5'-GTATGACTCAATAGGGATAT-3'

PCR scheme: 94^{o}C for 5min, once; 94^{o}C for 30s, 52^{o}C for 30s, 72^{o}C for 1min, 30times (36 cycles for eye disc ChIP); 72^{o}C for 5 min, once. The amplified DNA was separated on 1.5% agarose gel and visualized with ethidium bromide.

Supplemental Reference

Tong, C., and Jiang, J. (2007). Using Immunoprecipitation to study Protein-Protein interaction in the Hedgehog Signaling Pathway. Methods Mol. Biol. 397, 215–230.

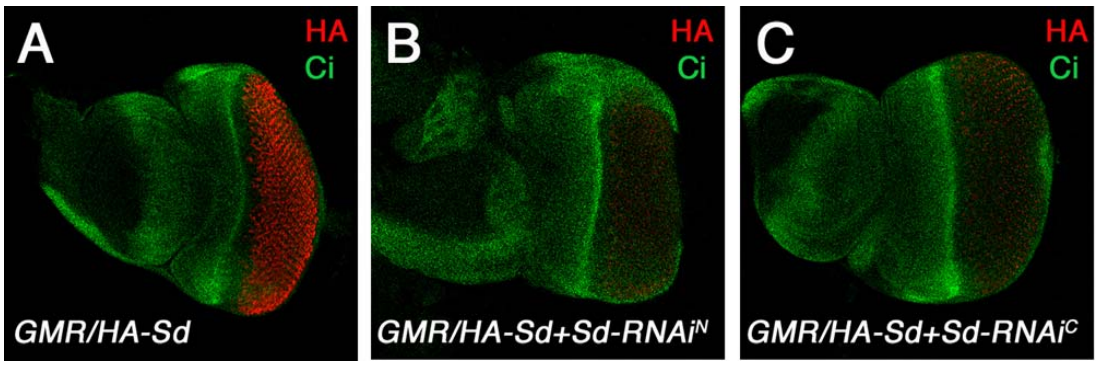

Figure S1. Sd RNAi Effectively Knocks Down Coexpressed HA-Sd
(A-C) eye discs expressing *GMR-Gal4/UAS-HA-Sd* (A), *GMR-Gal4/UAS-HA-Sd* plus *UAS-Sd-RNAi*^{N} (B), or *GMR-Gal4/UAS-HA-Sd* plus *UAS-Sd-RNAi*^{C} (C) were immunostained with anti-Ci (green) and anti-HA (red) antibodies. Both *Sd-RNAi*^{N} and *Sd-RNAi*^{C} dramatically reduced the levels of coexpressed HA-Sd.

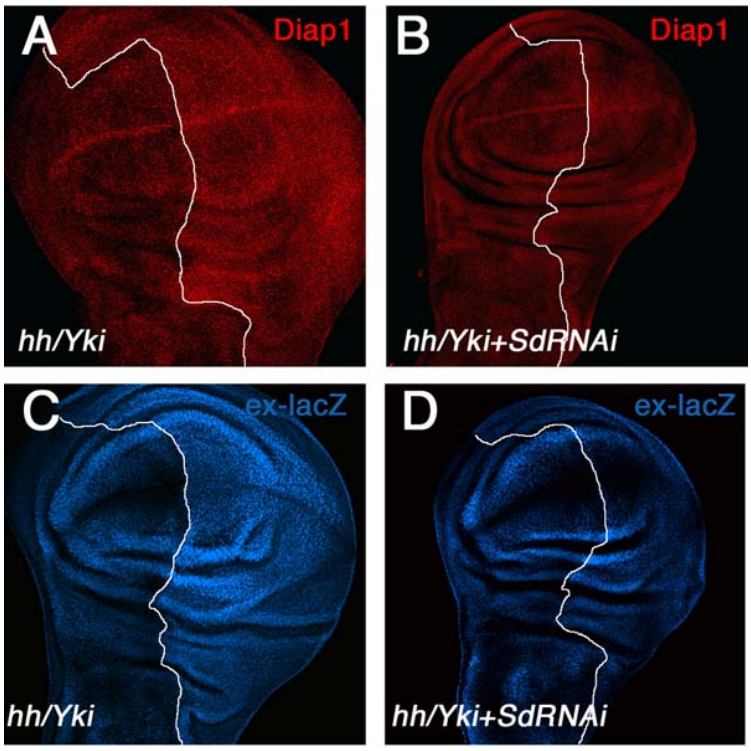

Figure S2. Sd RNAi Suppresses the Phenotype Caused by Yki Overexpression
(A-D) Wing discs expressing Yki (A, C) or Yki plus Sd RNAi transgenes (B, D) with *hh-Gal4* were immunostained to show the expression of Diap1 (A, B) or *ex-lacZ* (C, D). The A/P compartment border is outlined in each panel by a white line based on the expression pattern of coexpressed GFP (not shown). Overexpression of Yki in the P-compartment resulted in elevated expression of *diap1* and *ex-lacZ* and increased compartment size (A, C), which were suppressed by Sd RNAi (B, D).

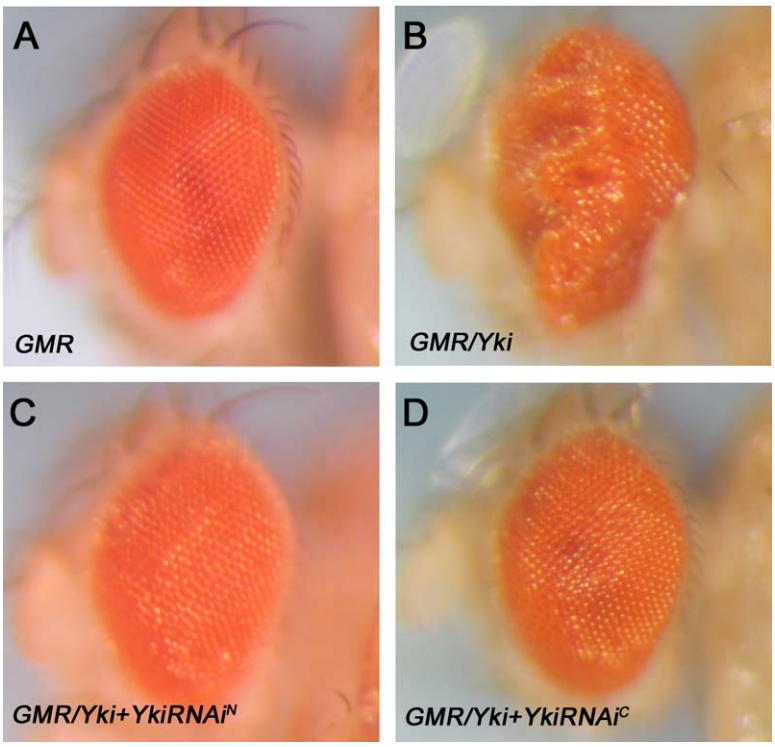

Figure S3. Yki RNAi Suppresses the Overgrowth Phenotype Caused by Yki Overexpression

(A-D) Wild type adult eye (A) or adult eyes derived from eye discs expressing *UAS-Yki* (B), *UAS-Yki* plus *UAS-Yki-RNAi*^{N} (C), or *UAS-Yki* plus *UAS-Yki-RNAi*^{C} (D) with *GMR-Gal4*. Expressing *GMR-Yki* resulted in overgrown eyes (B). Expression of either *Yki-RNAi*^{N} or *Yki-RNAi*^{C} suppressed the overgrowth phenotype caused by Yki overexpression.

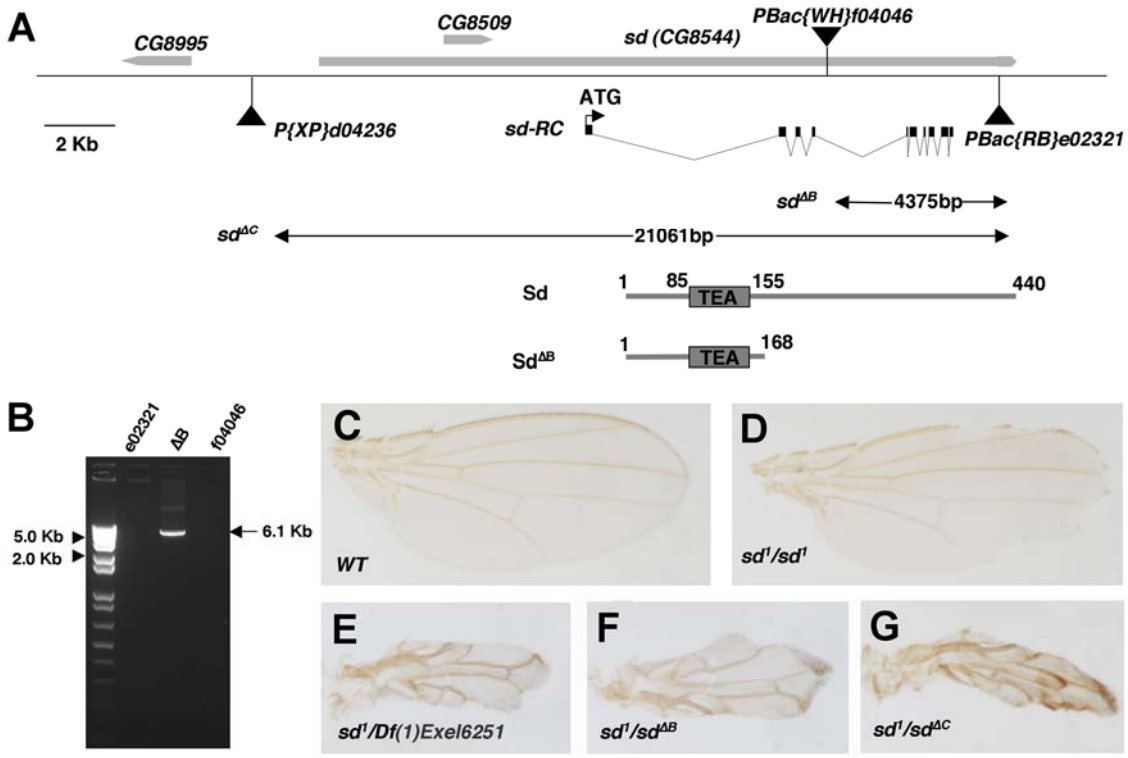

Figure S4. Generation of *sd* Null Alleles by FLP/FRT Mediated Deletion
(A) Diagram of the *sd* locus. The insertion sites for the three P-elements *P{XP}d04236*, *PBac{WH}f04046*, and *PBac{RB}e02321* used to generate *sd* deletion alleles are indicated. The genomic regions deleted in $sd^{\Delta B}$ and $sd^{\Delta C}$ are demarcated. $sd^{\Delta C}$ deletes the entire *sd* coding region plus a neighboring gene *CG8509*. $sd^{\Delta B}$ deletes the C-terminal coding region, resulting in a truncated protein that contains aa 1-168. (B) PCR products using a pair of primers complement to the sequences in the P-elements (see Experimental Procedures) and genomic DNA from indicated flies as templates. No PCR fragments were generated using the genomic DNA from the parental flies *PBac{WH}f04046* and *PBac{RB}e02321* as templates whereas a 6.1 kb fragment was amplified using the genomic DNA from the recombinant ($sd^{\Delta B}$) as template. (C-G) Adult wings of the indicated genotypes. Transheterozygotes of sd^{1} with the *sd* deficiency *Df(1)Exel6251* (E), $sd^{\Delta B}$ (F), or $sd^{\Delta C}$ (G) produced similar rudimentary wings.

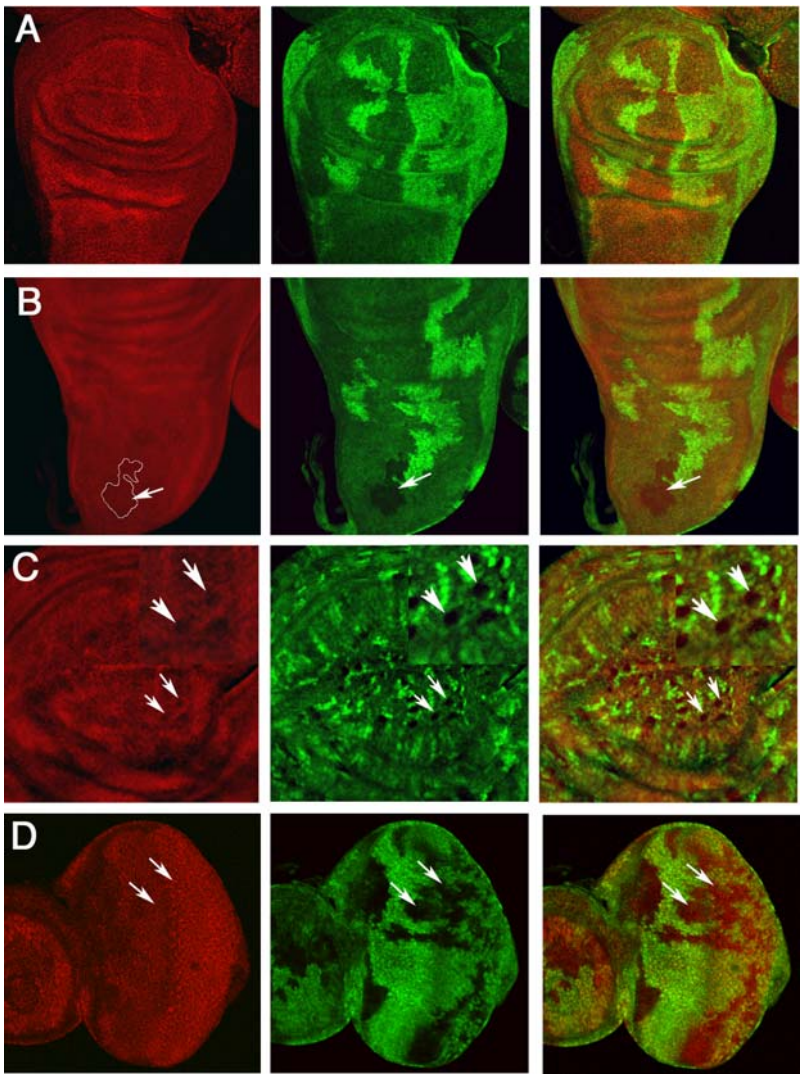

Figure S5. Phenotypes Associated with *sd* Null Clones
(A-D) Larvae of the genotype, $sd^{\Delta B}$ *FRT19/hs-HA-GFP-HA FRT19; hs-FLP*, were heat shocked at 48-72 hrs AEL (A, B, D) or 72-96 hrs AEL (C) to induce FLP/FRT mediated mitotic recombination. Imaginal discs were dissected out from late third instar larvae and immunostained to show the expression of Diap1 (red) and GFP (green). $sd^{\Delta B}$ clones were marked by the lack of GFP expression whereas the twin spots were marked by high levels of GFP expression due to the presence of two copies of *hs-HA-GFP-HA*. Early-induced $sd^{\Delta B}$ clones did not survive to late third instar in the wing pouch region (A) but did survive in the notal region of wing discs as well as in eye discs (arrows in B and D). Late-induced clones were recovered in the wing pouch region and exhibited diminished *diap1* expression (arrows in C). Enlarged views are shown in the insets (C).

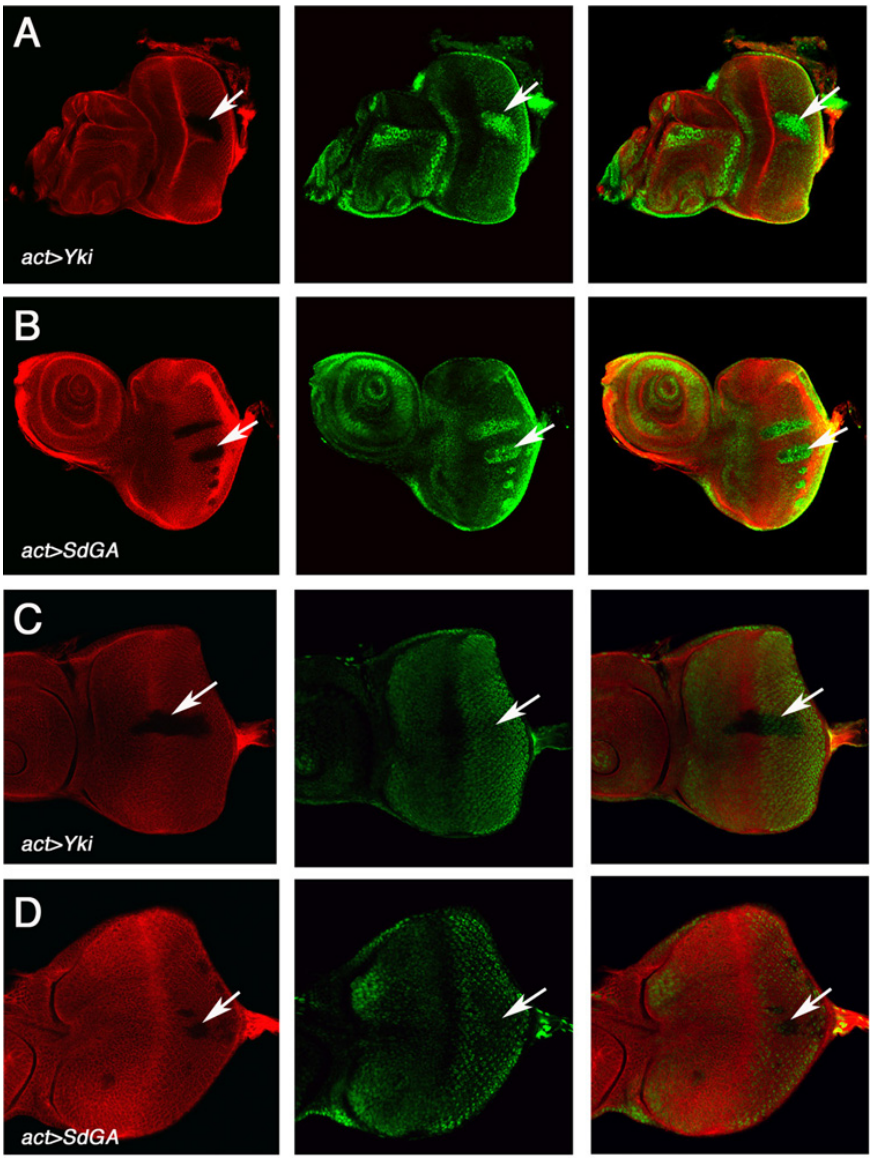

Figure S6. *diap1-GFP3.5* but *diap1-GFP1.8* Responds to Yki and Sd-GA
(A-D) Eye discs carrying *diap1-GFP3.5* (A-B) or *diap1-GFP1.8* (C-D) and flip-out clones expressing *act>CD2>Gal4/UAS-Yki* (A, C) or *act>CD2>Gal4/UAS-Sd-GA* (B, D) were immunostained to show the expression of CD2 (red) and GFP (green). Yki or Sd-GA expressing clones were marked by the lack of CD2 expression. Expression of Yki or Sd-GA enhanced the GFP expression from *diap1-GFP3.5* (arrows in A, B) but not from *diap1-GFP1.8* (arrows in C, D).

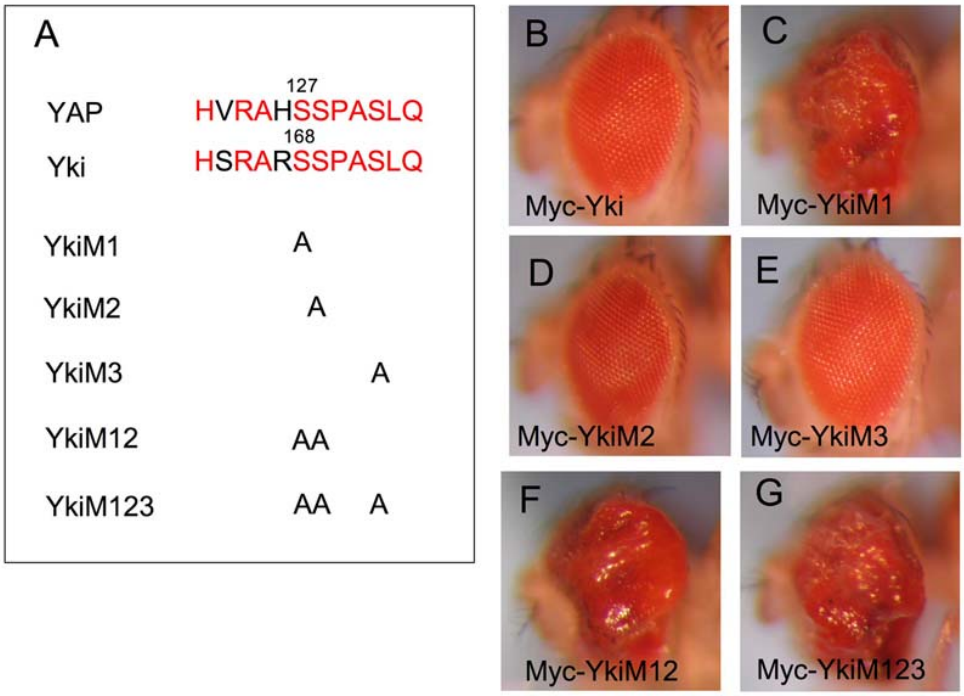

Figure S7. Comparison of *In Vivo* Activity of Myc-Tagged Wild-Type Yki and Yki Variants

(A) Alignment of the sequences surrounding YAP S127/Yki S168. Individual Yki variants with indicated S to A substitutions are listed. (B-G) Adult eyes derived from eye discs expressing *UAS-Myc-Yki* (B), *UAS-Myc-YkiM1*(C), *UAS-Myc-YkiM2* (D), *UAS-Myc-YkiM3* (E), *UAS-Myc-YkiM12* (F), or *UAS-Myc-YkiM123* (G) with *GMR-Gal4*.