Supporting Information

Ma et al. 10.1073/pnas.1415020112

SI Materials and Methods

Clonal Analysis. *wts* mutant clones were generated by crossing *hs-FLP; ubi*-GFP, FRT82B/TM6B with wts^{X1} FRT82B/TM6B. Flip-out ectopic expression clones were generated by crossing *UAS*-transgenes with *y w hs-FLP;act* > y^{+}>Gal4 *UAS*-GFP. Clones were induced at the second instar: heat shock for 30 min at 37 °C 48–72 h after egg laying (AEL); dissection was performed 36 h or 72 h after clone induction.

Clone size measurements were done as follows: we randomly picked up four clones from each wing disk, excluding clones along the dorsal/ventral boundary (low Yki activity due to reduced tension), and four wing discs were examined for each genotype. In total, 16 clones of indicated genotype were picked in double-blind experiments, and the pixels of each clone were measured using Photoshop software (Adobe), and the graphs were generated using GraphPad Prism 5.

qRT-PCR. Total RNA was extracted from wing discs of indicated genotype or culture S2 cells using PureLink RNA Mini kit (Ambion); cDNA was synthesized using PrimeScript RT Master mix (TaKaRa); and quantitative PCR was performed using SYBR Premix Ex Taq II(TaKaRa) on a Stratagene MX3000P system (Stratagene). The following primer sequences were used for RT-PCR:

tublin-FW: GCTGTTCCACCCCGAGCAGCTGATC

tublin-RV: GGCGAACTCCAGCTTGGACTTCTTGC

Rho1-FW: GTGGATGGCAAACAGGTGGAGC

Rho1-RV: GCGAATCGGGTGAATCCACTGAG.

Luciferase Reporter Assay. The *luciferase* reporters were generated through cloning the dissected elements of Rho1 first intron (E1, E2, and E3 in Fig. 4*I*) into KpnI/XhoI sites of *pGL3-Basic* Vector containing the hsp70 basal promoter. For luciferase assays, S2 cells were transfected with the above luciferase reporters and *copia-renilla* reporter in 12-well plates together with yki–HA-expressing pAc5.1/V5-HisB expressing plasmids. Cells were incubated for 36 h after transfection. Luciferase assays were performed using the Dual-Luciferase Reporter Assay system (Promega). Dual-luciferase measurements were performed using FlexStation 3 (Molecular Devices).

Western Blotting and ChIP. For Western blot analysis, 20 third instar wing discs were lysed in 1× SDS/PAGE sample loading buffer (Beyotime) and proceeded by the standard Western blot protocol. Proteins were probed with the following primary antibodies: anti-Rho1 (1:1,000; DSHB) and β-tubulin (1:3,000; CMC Scientific). After incubation with secondary antibodies (1:3,000; CST), signals were visualized by enhanced chemiluminescence (Beyotime). Chromatin immunoprecipitation was carried out as described in the protocol of the Magna ChIP G Tissue kit (Millipore). Transient transfections were performed with equal amounts of DNA (2.5 μg per plasmids) using Effectene transfection reagent (Qiagen) for S2 cells in 100 mm × 20 mm. HA-Tag (CMCTAG), Sd (a kind gift from Kirsten A. Guss, Dickinson College, Carlisle, PA), and mouse IgG (CST) were used for immunoprecipitation of ChIP assay.

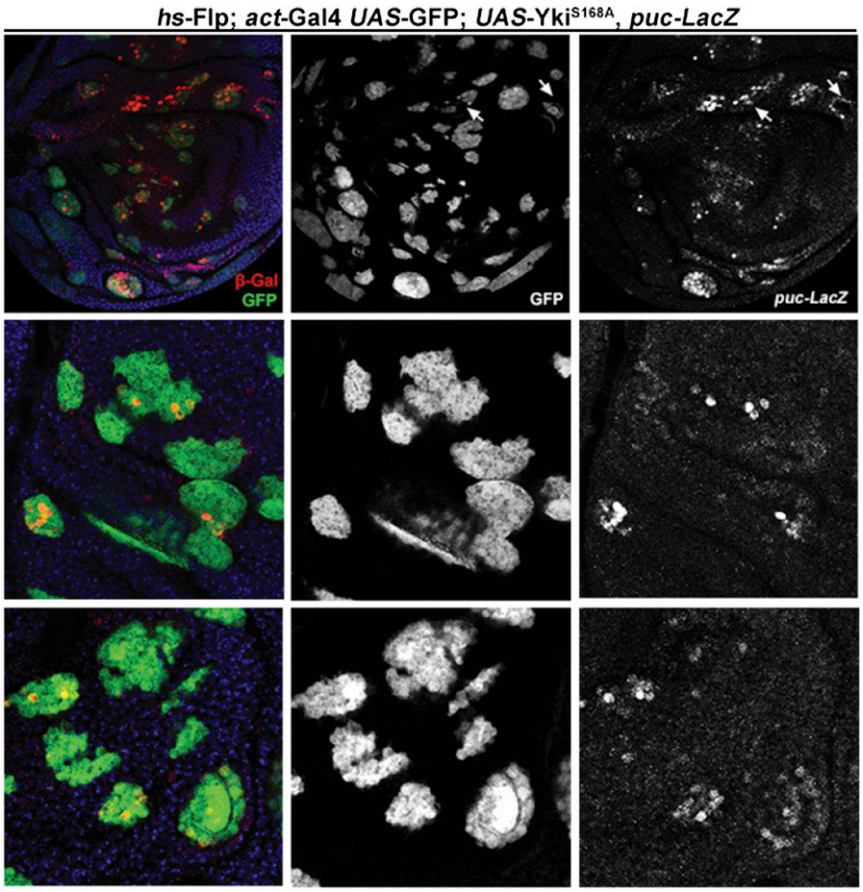

Fig. S1. Yki-expressing clones induces JNK activation throughout the wing disk. Yki^{S168A}-expressing Flp-out clones up-regulate *puc* transcription throughout the wing disk in an autonomous manner, although occasionally, nonautonomous JNK activation is observed in surrounding cells (first row, indicated by white arrow).

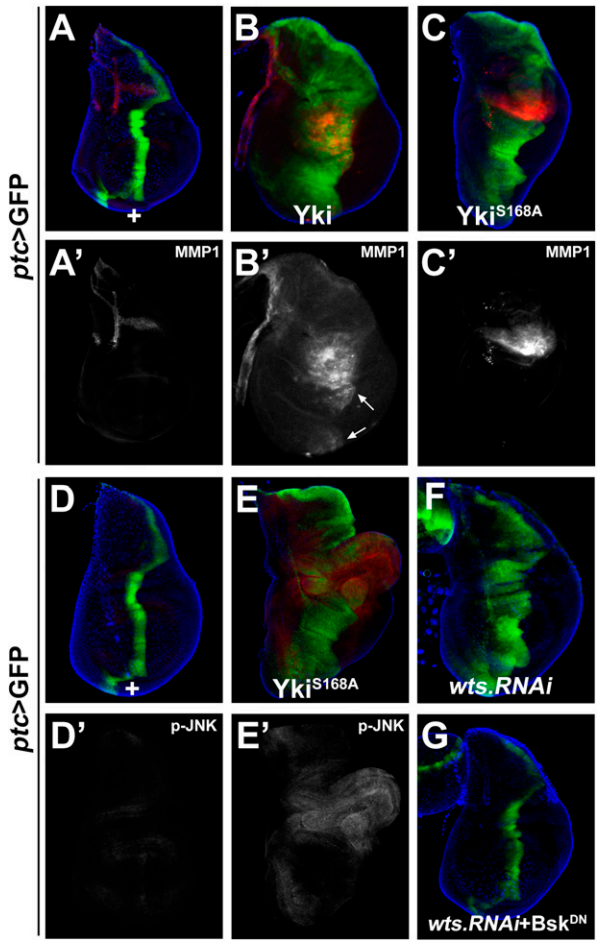

Fig. S2. Ectopic Yki expression induces JNK activation. (*A*–*E*) Ectopic Yki or Yki^{S168A} expression driven by *ptc*–Gal4 induces MMP1 expression (*A*–C) and JNK phosphorylation (*D* and *E*). (*F* and *G*) Blocking JNK signal via expressing Bsk^{DN} suppresses loss of *wts*-induced overgrowth phenotype.

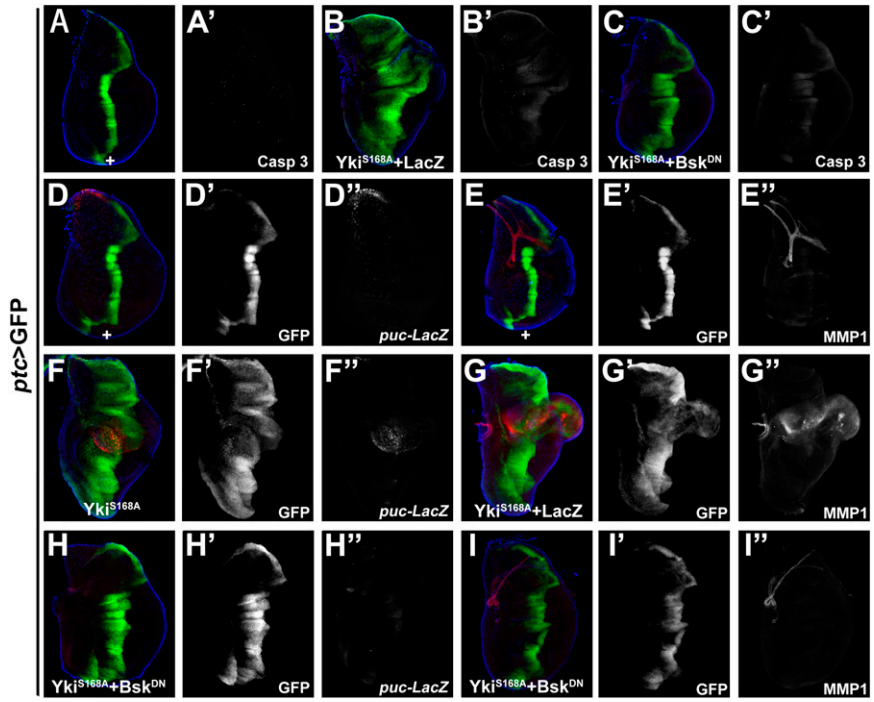

Fig. S3. Yki expression induces JNK-dependent target gene expression. (*A*–*C*) Third instar wing discs stained with antiactive caspase 3 antibody. Blocking JNK signaling fails to suppress Yki^{S168A}-induced apoptosis. (*D*–*I*) Third instar wing discs stained with antibodies against β-Gal (*D*, *F*, and *H*) or MMP1 (*E*, *G*, and *I*). Yki^{S168A}-induced *puc*–LacZ and MMP1 expression (*F* and *G*) were both suppressed by Bsk^{DN} (*H* and *I*).

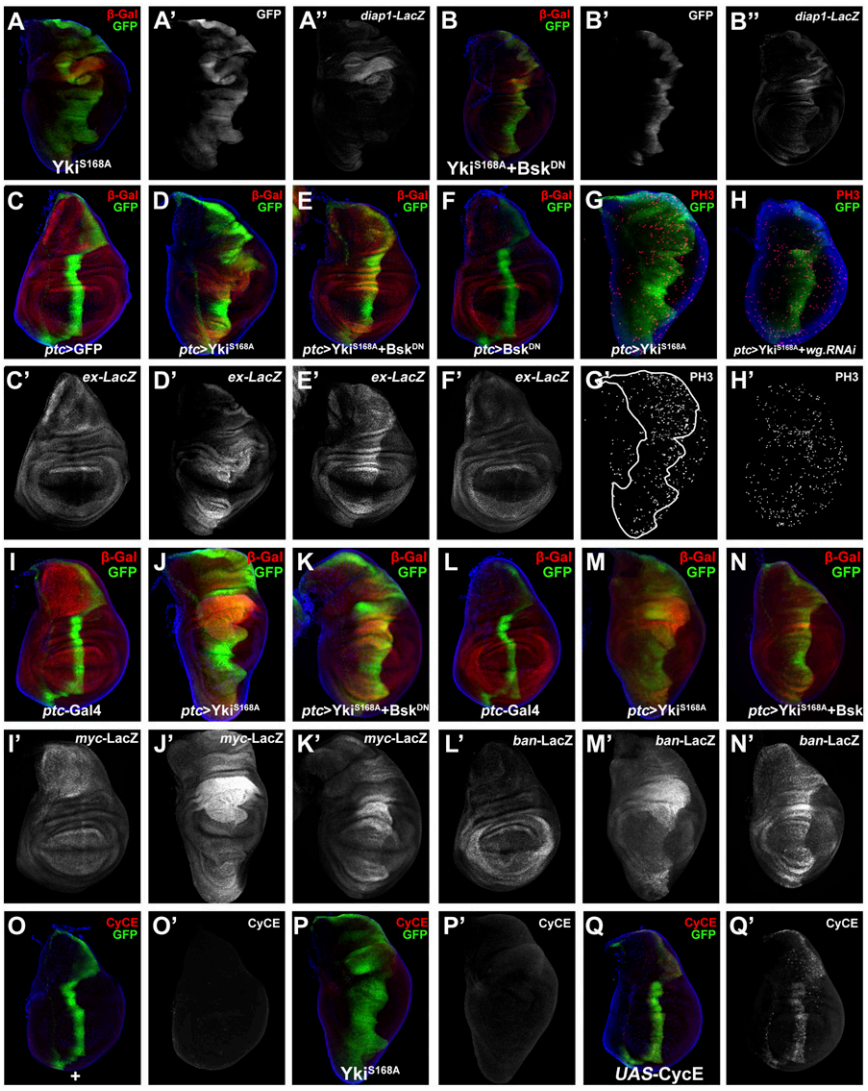

Fig. S4. JNK is dispensable for Yki-induced *diap1*, *ex*, *dmyc*, and *ban* expression. Third instar wing discs stained with antibodies against β-Gal (*A*–*F* and *I*–*N*), PH3 (*G* and *H*), or CycE (*O*–*Q*). (*A*–*F*) Yki^{S168A}-induced *diap1* and *ex* transcription (*A* and *D*) remained unchanged by coexpressing Bsk^{DN} (*B* and *E*). (*G* and *H*) Coexpression of a *wg* RNAi dramatically suppressed Yki-induced overgrowth and proliferation, as indicated by PH3 staining. (*I*–*N*) Blocking JNK activity cannot impede Yki-induced *dmyc* (*I*–*K*) and *ban* (*L*–*N*) transcription. (*O*–*Q*) Compared with control (*O*), Yki^{S168A} expression does not induce CycE activation (*P*). As a positive control, CycE is up-regulated in *ptc*–Gal4 *UAS*–CycE wing discs (*Q*).

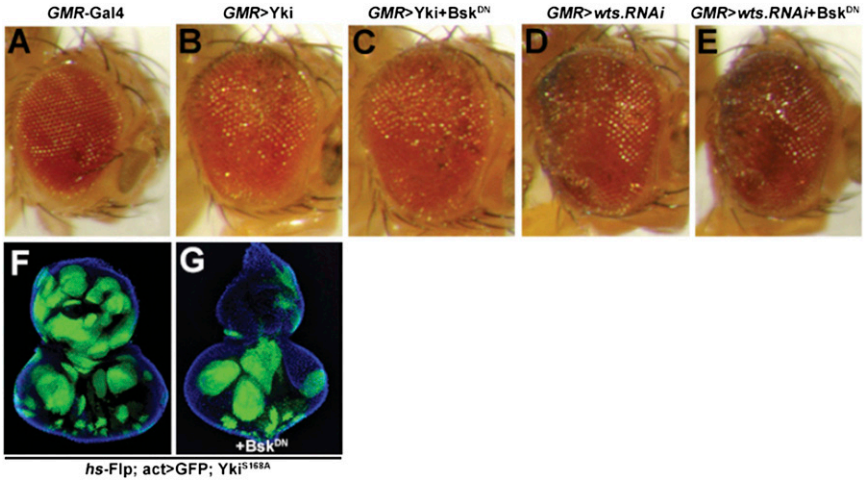

Fig. S5. JNK signaling is dispensable for Yki-induced overgrowth in the developing eye. (*A*–*E*) Light micrographs of *Drosophila* adult eyes are shown. Compared with control (*A*), overexpression of Yki or knock-down *wts* under *GMR* promoter results in overgrowth of adult eye (*B* and *D*), both of which remains unaffected by corepression of Bsk^{DN} (*C* and *E*). (*F* and *G*) Third instar eye-antennal discs are shown. Blocking JNK activity does not suppress Yki^{S168A}-induced clone overgrowth.

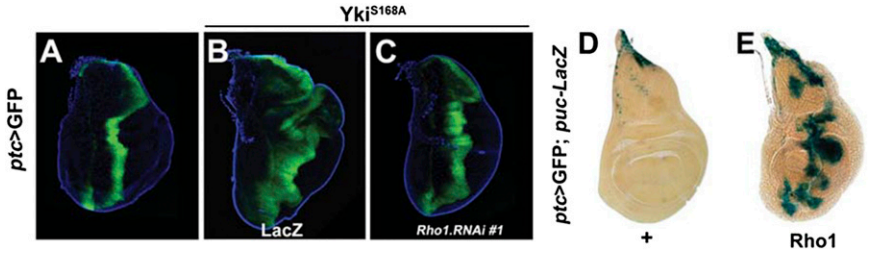

Fig. S6. Rho1 is required for Yki-induced overgrowth. (*A*–*C*) *ptc* > Yki^{S168A}-induced expansion of GFP^{+} region (*B*) was suppressed by another independent *Rho1* RNAi line (*C*). (*D* and *E*) X-Gal staining of third instar wing discs. Compared with the controls (*D*), *ptc* > Rho1 induces strong *puc* transcription along the A-P boundary (*E*).

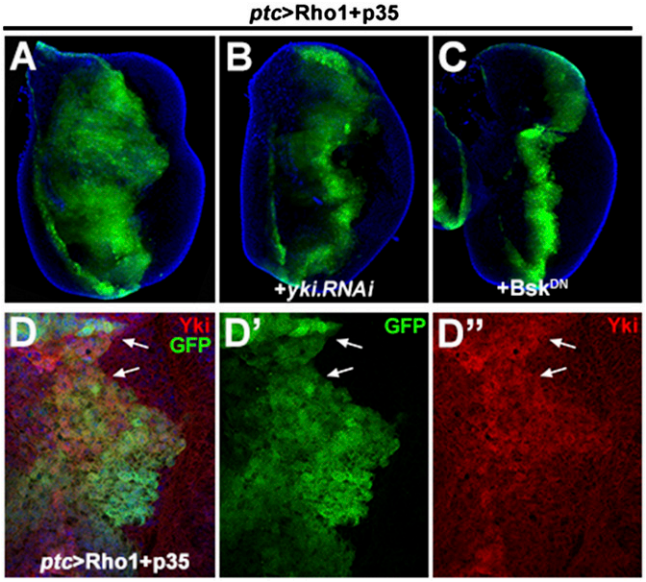

Fig. S7. Both Yki and JNK signaling are required for Rho1-induced growth. Third instar wing disk are shown. (*A*–*C*) Reducing Yki (*B*) or JNK (*C*) activity dramatically suppresses Rho1-induced overgrowth when cell death is compromised. (*D*) Increased Yki nuclear accumulation was observed in Rho1-expressing cells when cell death is inhibited.

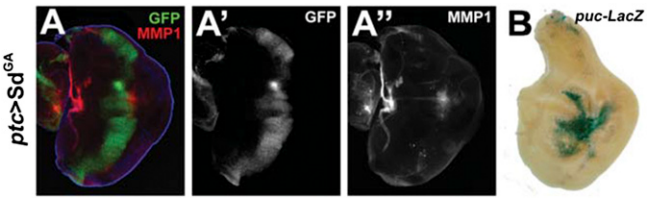

Fig. S8. Sd^{GA} overexpression induces JNK activation. Third instar wing discs stained with MMP1 (*A*) or X-Gal (*B*). Expression of an activated form of Sd (Sd^{GA}) up-regulates MMP1 (*A*) and *puc*–LacZ expression (*B*).

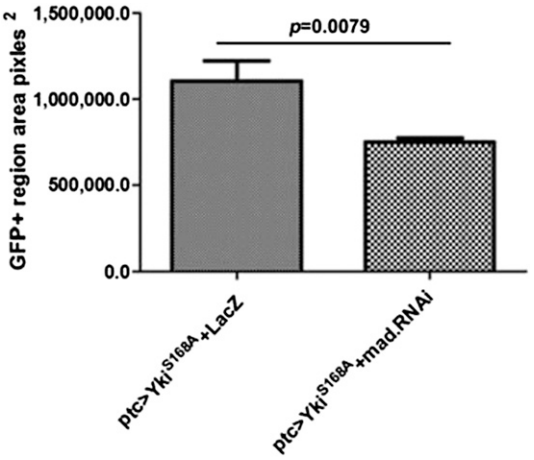

Fig. S9. *mad* is required for Yki-induced overgrowth. Statistical analysis of GFP^{+} regions shown in Fig. 4 *A* and *C*. Error bars are +SD; two-tailed, unpaired Student's *t* test.