Development 139, 4051-4061 (2012) doi:10.1242/dev.076760

Activated STAT regulates growth and induces competitive interactions independently of Myc, Yorkie, Wingless and ribosome biogenesis

Aloma B. Rodrigues[1], Tamara Zoranovic[1], Aidee Ayala-Camargo[1], Savraj Grewal[2], Tamara Reyes-Robles[1], Michelle Krasny[1], D. Christine Wu[3], Laura A. Johnston[4] and Erika A. Bach[1,*]

SUMMARY

Cell competition is a conserved mechanism that regulates organ size and shares properties with the early stages of cancer. In *Drosophila,* wing cells with increased Myc or with optimum ribosome function become supercompetitors that kill their wild-type neighbors (called losers) up to several cell diameters away. Here, we report that modulating STAT activity levels regulates competitor status. Cells lacking STAT become losers that are killed by neighboring wild-type cells. By contrast, cells with hyper-activated STAT become supercompetitors that kill losers located at a distance in a manner that is dependent on *hid* but independent of Myc, Yorkie, Wingless signaling, and of ribosome biogenesis. These results indicate that STAT, Wingless and Myc are major parallel regulators of cell competition, which may converge on signals that non-autonomously kill losers. As hyper-activated STATs are causal to tumorigenesis and stem cell niche occupancy, our results have therapeutic implications for cancer and regenerative medicine.

KEY WORDS: JAK/STAT, Myc, Cell competition

INTRODUCTION

During development, local interactions between cells influence their growth and their ability to contribute to the adult. The process of cell competition was first reported in studies in the *Drosophila* wing disc of *Minutes* (*M*), dominant mutations in ribosomal protein (*Rp*) genes that are homozygous lethal but when heterozygous yield viable animals that are slow to develop (Morata and Ripoll, 1975; Simpson, 1979; Simpson and Morata, 1981). Despite being viable in a homotypic environment, *M*/+ clones are not recovered in the adult when grown in the presence of wild-type cells. These seminal studies established that wild-type and *M*/+ cells compete for space within a compartment of the wing, that competition between them is local, and that death of *M*/+ cells is associated with proliferation of wild-type cells. However, the final size of the wing remained normal despite the fact that wild-type cells filled large parts of a compartment. Thus, the wild-type cells (termed 'winners') occupy the space of the *M*/+ 'losers', which they have eliminated through cell death. Recent work has established that *M*/+ losers die primarily through apoptosis and most death occurs at the clone boundary with wild-type cells (de la Cova et al., 2004; Moreno and Basler, 2004; Li and Baker, 2007).

This type of 'classical' cell competition has also been documented in the case of Myc, a transcription factor that regulates expression of genes controlling proliferation, cellular growth and ribosome biogenesis (de la Cova et al., 2004; Moreno and Basler, 2004; de la Cova and Johnston, 2006). Animals with hypomorphic mutations in *Myc* [encoded by the *diminutive* (*dm*) gene] are viable but grow more slowly and are smaller than wild-type flies. However, when *dm* mutant cells are placed in apposition to wild-type cells, the *dm* cells are out-competed (Johnston et al., 1999; Moreno and Basler, 2004). Clonal growth assays in the *Drosophila* wing disc have revealed that cells with increased Myc become 'supercompetitors' that can kill losers at a distance – up to 10 cells away (de la Cova et al., 2004; Moreno and Basler, 2004). The fact that Myc supercompetitors lose their ability to out-compete wild-type cells when they are heterozygous for a *Minute* mutation [*M(2)60E*] (Moreno and Basler, 2004) suggests that the ability of Myc to act as a supercompetitor stems from its effects on ribosome function. Pathways that feed into Myc regulation, such as the Hippo pathway, have also been shown to be involved in competition (Tyler et al., 2007; Neto-Silva et al., 2010; Ziosi et al., 2010).

Recently, a Myc-independent mechanism of supercompetition has been reported. Cells lacking Wingless (Wg) signaling are out-competed but survive if they are placed in the context of cells that are growth impaired. Furthermore, cells with elevated Wg signaling eliminate their wild-type neighbors by secreting Notum, a phospholipase that inhibits Wg signaling in surrounding cells and as such become 'supercompetitors' (Vincent et al., 2011). This type of competition does not fit the classical definition as changes in Wg signaling lead to altered patterning of discs and appendages, and Wg loss- or gain-of-function cells cannot give rise to viable adults. However, this work is evidence that competitive events take place without directly involving ribosome function but as a consequence of developmental signaling pathway activation, and that cells are able to compare their 'fitness' to their neighbors by measuring relative signaling activity.

In the past decade, the field of cell competition has exploded, and while it is appreciated that several genes and pathways regulate the process of cell competition, a consensus on definitions for each

[1]Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY 10016, USA. [2]Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta T2N 4N1, Canada. [3]Department of Biological Sciences, Columbia University, New York, NY 10032, USA. [4]Department of Genetics and Development, Columbia University, New York, NY 10032, USA.

*Author for correspondence (erika.bach@nyu.edu)

Accepted 7 August 2012

type of competitive interaction has not yet been achieved. For clarification, we will use the term 'cell competition' to mean the context-specific behavior of cells of a particular genotype: they are out-competed when surrounded by wild-type cells but viable when placed in the context of slower-growing cells (e.g. *M*/+). We will use the term 'supercompetitor' to mean a clone of cells overexpressing a particular factor that causes neighboring wild-type cells to experience a growth disadvantage.

It is not clear what the relationship between growth-promoting pathways and cell competition is. It is known that JAK/STAT signaling is a central regulator of growth/eye size in *Drosophila*, and we asked whether it was involved in competitive interactions as well. Stat92E, the sole STAT transcription factor in *Drosophila*, is a central regulator of eye size. In *Drosophila,* the cytokine Unpaired (Upd; Os – FlyBase) activates the receptor Domeless (Dome), which leads to the activation of the sole Janus kinase Hopscotch (Hop) and Stat92E, which translocates to the nucleus and modulates expression of its target genes (Arbouzova and Zeidler, 2006). Sustained activation of Stat92E causes dramatic overgrowth of the eye, fly leukemia and germ-cell tumors (Hanratty and Dearolf, 1993; Kiger et al., 2001; Bach et al., 2003). Although these gain-of-function phenotypes implicate the JAK/STAT pathway in growth control and possibly in cell competition, the cell-autonomous requirement for Stat92E in these processes has not been elucidated. Here, we show that the JAK/STAT pathway is a crucial regulator of growth during early eye and wing development through competitive interactions: cells lacking *Stat92E* are out-competed by wild-type neighbors and eliminated by apoptosis. Furthermore, cells with sustained activation of Stat92E become winners, manifest supercompetitor characteristics and kill losers located several cell diameters away through non-autonomous induction of apoptosis. Finally, we provide strong evidence supporting the conclusion that JAK/STAT pathway activity does not require Myc, Yorkie (Yki), Wg or Decapentaplegic (Dpp) signaling or de novo ribosome biogenesis for its regulation of growth and competitive interactions between cells.

MATERIALS AND METHODS

Fly stocks

These stocks are described in Flybase: *Stat92E^{85C9}*, *Stat92E^{397}*, *dm^{4}*, *lgl^{4}*, *UAS-P35*, *UAS-hop*, *UAS-hop^{Tum-l}*, *UAS-Myc*, *UAS-upd*, *en-gal4*, *fj-lacZ*, *ex-lacZ*, *hid^{P1642}*, *upd(E132)-gal4*, *dome-gal4*, *10xSTAT-gfp*, *wg^{P} (wg-lacZ)*, *UAS-Arm^{S10}* and *UAS-TCF^{DN}*. *actin5c>stop>lacZ* was obtained from Gary Struhl (Columbia University, New York, NY, USA).

Clonal analysis

Two- to three-hour timed embryo collections were made on yeasted molasses food vials and raised at 25°C. Embryos were processed as described previously (Bach et al., 2007). Clones were induced by heat-shocking at specific times after egg deposition (AED) at 39°C and discs were dissected, fixed and stained at 115±2 hours AED or as noted. The MARCM technique was used to generate GFP-expressing *Stat92E^{85C9}* clones. The sibling clone was detected by CD2 as described (de la Cova et al., 2004). For the *tub>Myc* and null-*Myc* assays, 3-hour timed embryo collections were made, and clones were induced by heat-shocking at 48 hours AED at 37°C for 15 minutes. Wing discs were dissected at 68 hours after clone induction (ACI). For the null-*Myc* assays, wing discs were dissected at 110 hours ACI. *dm^{4} FRT^{19A}/Y; tub>Myc y^{+}>gal4/ UAS-gfp*; *UAS-hop48A/+* animals exhibited strong developmental delays. For the quantitative PCR assays, 2-hour timed embryo collections were made and clones were induced by heat-shocking at 102 hours AED at 39°C for 1 hour, and wing discs were dissected 16 hours ACI. These stocks were used for clonal analyses: *hs-flp^{122}; FRT^{82B} arm-lacZ/TM6c* and *hs-flp^{122}; FRT^{82B} Minute 96(C) ubi-gfp/TM6B*. For the two-clone assay *hs-flp^{122}, tub-gal4,UAS-gfp; FRT^{82B} tub-gal80 hs-CD2 y^{+}/TM6B* and *hs-flp^{122}, tub-gal4,UAS-gfp; FRT^{42D} CD71 tub-gal80/CyO; TM2/TM6B* were used.

Immunofluorescence

We used antibodies specific for: β-galactosidase [1:50; Developmental Studies Hybridoma Bank (DHSB) or 1:2000; Cappel]; cleaved Caspase 3 (1:100; Cell Signaling); rat CD2 (1:500; Serotec); Fibrillarin (1:500; EnCor); Stat92E (1:50); Myc (1:200; a gift from Gines Morata, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain); phospho-JNK (1:100; Promega, #V7938); Vestigial (Vg) (1:2000; a gift from Sean Carroll, University of Wisconsin, Madison, WI, USA); Nubbin (Nub, 1:10); Teashirt (Tsh, 1:500); Engrailed (En, 1:10; DHSB); Wg (1:10; DHSB); Discs large (Dlg, 1:50; DHSB); Crumbs (Crb, 1:50; DHSB); Yorkie (Yki, 1:100); pSmad (1:1250; a gift from Ed Laufer, Columbia University, New York, NY, USA); Distal-less (Dll, 1:500); Sparc (1:300); and Homothorax (Hth, 1:2000). Alexa Fluor 546 Phalloidin was used 1:100 (Molecular Probes). Secondary antibodies (Molecular Probes and Jackson Immunologicals) were used at 1:200. Discs were fixed for 20 minutes in 4% formaldehyde (for β-galactosidase stains) or 4% paraformaldehyde (for all other stains) and were processed as described (Flaherty et al., 2010). Images were captured on an LSM510 Zeiss confocal microscope at 25×, 63× or 100×.

Quantitation of clone areas

The areas of clones were measured by ImageJ. *y*-axis error bars represent s.e.m. in Fig. 2I, s.d. in Fig. 3D, Fig. 4C and Fig. 5C. Statistical significance was determined by the Student's *t*-test.

Quantitative real-time PCR

Quantitative PCR was carried out on 20 wing discs in triplicate carrying clones misexpressing *gfp*, *hop* or *Myc* as described (Grewal et al., 2007). Primer sequences are as follows: *Nop5* (F, AGGAGCTGATGCGCTGTATT; R, AAGTGCCAACCGTACCACTC), *Nop60B* (F, CTCTTGTGACCATGGTGTGG; R, GACATAACCGGTCAGCCACT), *Tif-1A* (F, GTAGCGAAGAACAGCGAAGG; R, AATTGCACATGATGCGTGTT), *dome* (F, CGGACTTTCGGTACTCCATC; R, GATCGATCATCGCCGAGTT) and *chinmo* (F, CAGTGCCAATGAGGCTAATG; R, TCAAGTTCTCCAGCTTCACG).

RESULTS

Upd expression and JAK/STAT pathway activity are detected in most cells in a young wing disc

Stat92E activity has been reported in third instar wing discs in the hinge region, but its earlier roles are unclear (Fig. 6I; Fig. 7D) (Mukherjee et al., 2005; Bach et al., 2007). To assess the temporal expression and activity of the JAK/STAT pathway during early stages of wing development, we monitored expression of *upd* using *upd (E132)-gal4*, an insertion of a *gal4* transposon in the *upd* locus (Tsai and Sun, 2004), and the transcriptional activation of Stat92E using the *10xSTAT-gfp* reporter (Bach et al., 2007). In wild-type early stage 15 embryos, cells in the wing disc primordium, marked by Vg, express low levels of *upd* mostly in the anterior domain (Fig. 1A-A‴), and there was little or no expression of *10xSTAT-gfp* at this time (Fig. 1B-B‴). These data indicate that the JAK/STAT pathway is not strongly activated during the earliest stages of wing development. However, in a mid-second instar disc (~60 hours AED), Stat92E activity is detected at higher and equal levels in nearly all cells, including those in the pouch (Fig. 1C,C′, arrow). These results indicate that there is no graded activation of Stat92E at this stage, and suggest that *upd* is expressed broadly and early in wing development. Subsequently, *upd* expression and Stat92E activation become reduced in the pouch and concentrated in the hinge (Fig. 1D′,E′,F′, arrows).

We used lineage tracing to determine the fate of Upd-producing cells, using *upd-gal4*, *UAS-flp* and an

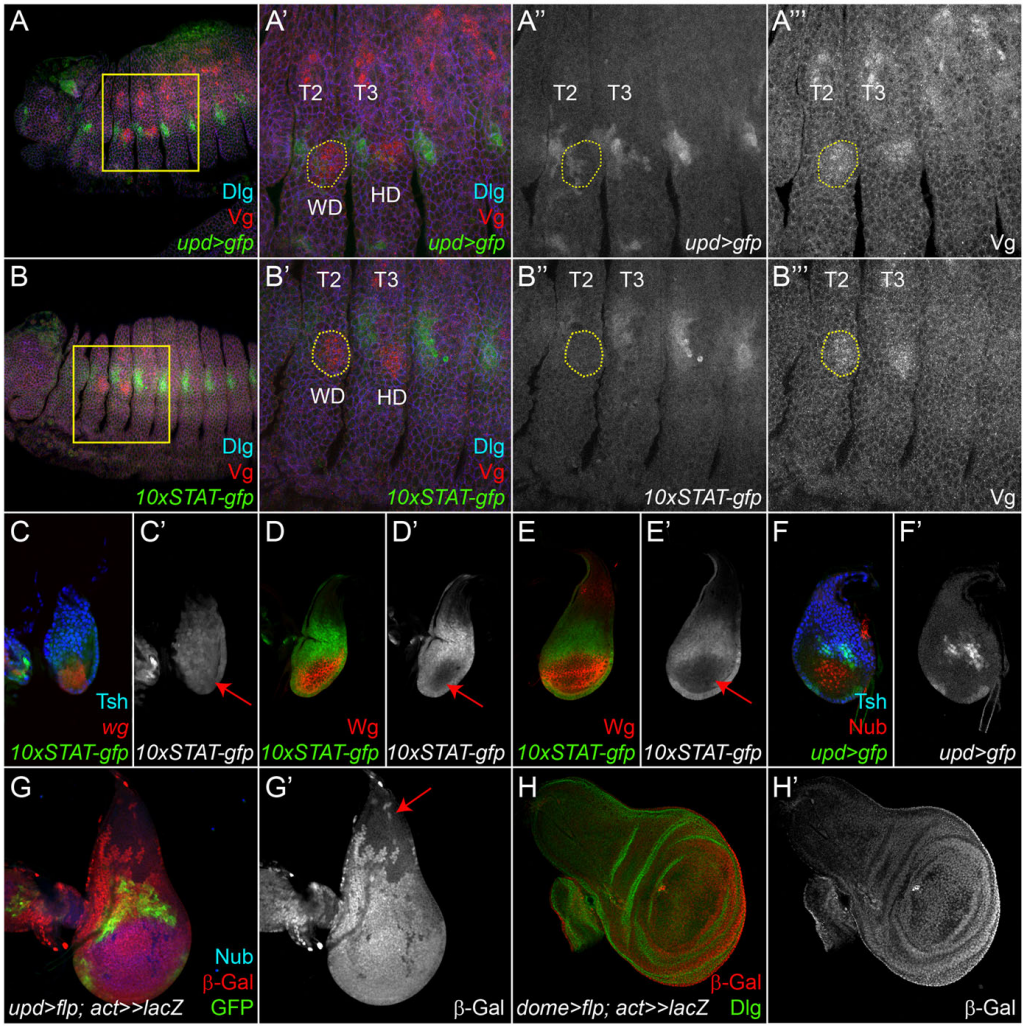


Fig. 1. The JAK/STAT pathway is activated early and broadly during larval wing disc development. (**A-A‴**) A stage 15 *upd-gal4, UAS-gfp* (*upd>gfp*) (green) embryo ~15 hours AED at 22°C at 40× magnification. (A) The *upd* gene is detected at low levels in a few cells in the anterior domain of the wing disc primordium. (**B-B‴**) A stage 15 *10xSTAT-gfp* (green) embryo. (B) Stat92E activity is not detected in the wing disc primordium. Vg is red and Dlg is blue. (A′-A‴,B′-B‴) The regions outlined in yellow in A,B were scanned at 100× magnification. Primordia of the wing (WD) and haltere disc (HD) are labeled, and the WD is outlined by a broken line in A′-A‴,B′-B‴. Thoracic segments 2 and 3 are labeled T2 and T3, respectively. Cells in the central nervous system, located dorsal to the wing and haltere primordia, express Vg (Guss et al., 2008). (**C**,**C′**) Most cells in a mid-second instar wild-type wing disc at ~60 hours AED have equivalent activation of Stat92E as assessed by *10xSTAT-gfp* (green). *wg-lacZ* is red and Tsh is blue. (**D-E′**) Stat92E activation (*10xSTAT-gfp*, green) is reduced in the pouch during mid-to-late second instar but is maintained in the hinge. Wg protein is red. The pouch is marked by an arrowhead in C-E. (**F**,**F′**) *upd* (*upd>GFP*, green) is localized to the hinge during mid-to-late second instar. Nub, which marks the wing pouch, is red. Tsh is blue. (**G**,**G′**) Lineage-tracing experiments with *upd-gal4, UAS-gfp; act5c>stop>lacZ/UAS-flp* indicate that most cells in the larval wing disc expressed the *upd* gene. *upd>gfp* is green, β-Gal is red and Nub is blue. The notum is marked by an arrow in G′. (**H**,**H′**) Lineage-tracing analysis of *dome-gal4, UAS-gfp*; *act5c>stop>lacZ/UAS-flp* indicates that most cells in the disc express *dome*. β-Gal is red and Dlg is green. In all panels, dorsal is upwards and anterior is towards the left.

act5c>stop>lacZ flip-out cassette (Struhl and Basler, 1993). Flp, expressed under the control of the *upd* locus, will excise the 'stop' from the flip-out cassette and allow expression of *lacZ* by the *act5c* promoter. Any cell that produces *upd* will be heritably marked with *lacZ* expression. *upd*-expressing cells, marked by β-galactosidase, populate most parts of the wing disc, including the pouch (Fig. 1G,G′). For *upd*-producing cells to populate large parts of the wing disc, *upd* must be expressed early and broadly during development. This conclusion is supported by lineage-tracing analysis of *dome*, encoding the Upd receptor, which is also a Stat92E target gene (Flaherty et al., 2009). *dome*-expressing cells populate nearly all regions of the disc, including the pouch (Fig. 1H,H′). Very few cells were labeled with β-galactosidase when the *UAS-flp* transgene was excluded from the analysis, indicating that there was no spurious activity of *flp* on the flip-out cassette (supplementary material Fig. S1A,A′ and not shown). By contrast, cells expressing Nub or Tsh, other key transcription factors in wing development, display restricted lineages and populate only the pouch or notum/hinge, respectively (Zirin and Mann, 2007). Taken together, these data indicate that the JAK/STAT pathway is activated and functions broadly and evenly during early wing development (i.e. before 60 hours AED), when exponential growth occurs (Neto-Silva et al., 2009).

Stat92E is required for clonal growth during early disc development

As Upd and JAK/STAT signaling are induced early during eye and wing development (Fig. 1C) (Ekas et al., 2006), we sought to address the cell-autonomous requirement of *Stat92E* in clonal growth. We induced *Stat92E* clones and their wild-type sibling clones by mitotic recombination at 30, 36 and 48 hours AED in timed-collections and measured clone size as a percentage of the total clone area at 115 hours AED in wing and eye discs. As disc cells are epithelial and remain associated after mitosis, differences in clone size reflect differential growth rates (Neufeld et al., 1998). We used $Stat92E^{85C9}$ and $Stat92E^{397}$(not shown), which are functionally amorphic alleles (Ekas et al., 2010), and obtained similar results for both. If *Stat92E* were not required for clonal growth, *Stat92E* clone areas would be ~50% of the total clone area. However, when induced early in wing development (at 30 and 36 hours AED), $Stat92E^{85C9}$ clones displayed severe growth disadvantages when compared with their sibling clones regardless of clone location along the anterior-posterior (AP) or dorsal-ventral (DV) axes (Fig. 2A,B,D,E, arrowheads for *Stat92E* clones, arrows for sibling clones) and comprised only ~5% of the total clone area in the disc (Fig. 2I; supplementary material Fig. S2A). By contrast, control FRT^{82B} wild-type clones and their sibling clones grew to equal sizes and were each ~50% of the total clone area (Fig. 2G-I; supplementary material Fig. S2A). The growth disadvantage of *Stat92E* clones persisted but weakened during development. *Stat92E* clones induced at 48 hours AED were only ~16% of the total clone area (Fig. 2C,F,I; supplementary material Fig. S2A,B). At the 48 hour time-point, *Stat92E* clones located anterior to the morphogenetic furrow or in the wing hinge (Fig. 2F,C) appeared to grow less well than clones located elsewhere in the same disc, but differences in clone sizes were not statistically significant (supplementary material Fig. S2B-D). We also noted that *Stat92E* clones in the notum induced at 48 hours AED tended to be larger than clones residing elsewhere in the disc ($P<0.0073$) (Fig. 2C; supplementary material Fig. S2B-D). Taken together, our results indicate that *Stat92E* has an essential growth requirement in all eye and wing cells in early disc development.

Cells lacking JAK/STAT pathway activity become losers

We found that poorly growing cells in $Stat92E^{85C9}$ clones were apoptotic and basally extruded (Fig. 3A,A′,A‴, green arrowheads; 3B,B′,B‴, yellow arrowheads), in stark contrast to their faster-growing wild-type sibling clones (Fig. 3A,A″, red arrows). Expressing the caspase inhibitor P35 within $Stat92E^{85C9}$ clones rescued their growth such that they grew to equal sizes as their

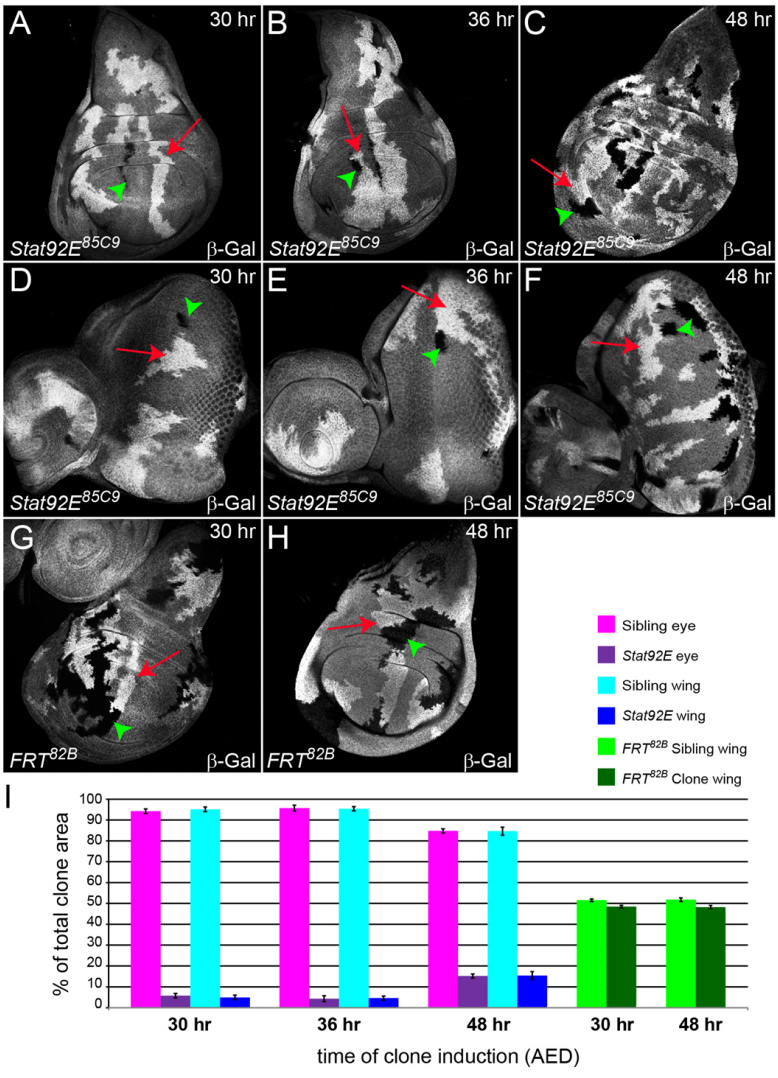


Fig. 2. *Stat92E* is required for clone survival. (**A-F**) $Stat92E^{85C9}$ clones (arrowheads) exhibit strong growth defects compared with their sibling clones (arrows). $Stat92E^{85C9}$ clones were induced at 30 (A,D), 36 (B,E) or 48 (C,F) hours AED and analyzed in wing discs (A-C) and eye disc (D-F) at 115 hours AED. (**G**,**H**) Wild-type FRT^{82B} control clones (arrowheads) and sibling clones (arrows), induced at 30 (G) or 48 (H) hours and analyzed in wing discs at 115 hours AED, grow to similar sizes. (A-H) Clones lack β-Gal and appear black, whereas sibling clones have two copies of β-Gal and appear white. (**I**) The area of $Stat92E^{85C9}$ or FRT^{82B} control clones expressed as a percentage of the total clone area (clone + sibling) per disc. Clones were induced at the times indicated and analyzed in eye and wing discs (for *Stat92E* clones) and in wing discs (for FRT^{82B} controls). There were greater than 50 sibling-clone pairs for each time point. The entire sibling clone (+/+) and *Stat92E* or FRT^{82B} (–/–) clone areas were measured in each disc and the standard error was calculated. Percentage area of sibling clones in eye discs (magenta bars) or in wing discs (light-blue bars). Percentage area of *Stat92E* clones in eye discs (dark-purple bars) or in wing discs (dark-blue bars). Percentage area of FRT^{82B} sibling clones (light-green bars) or FRT^{82B} control clones (dark-green bars) in wing discs.

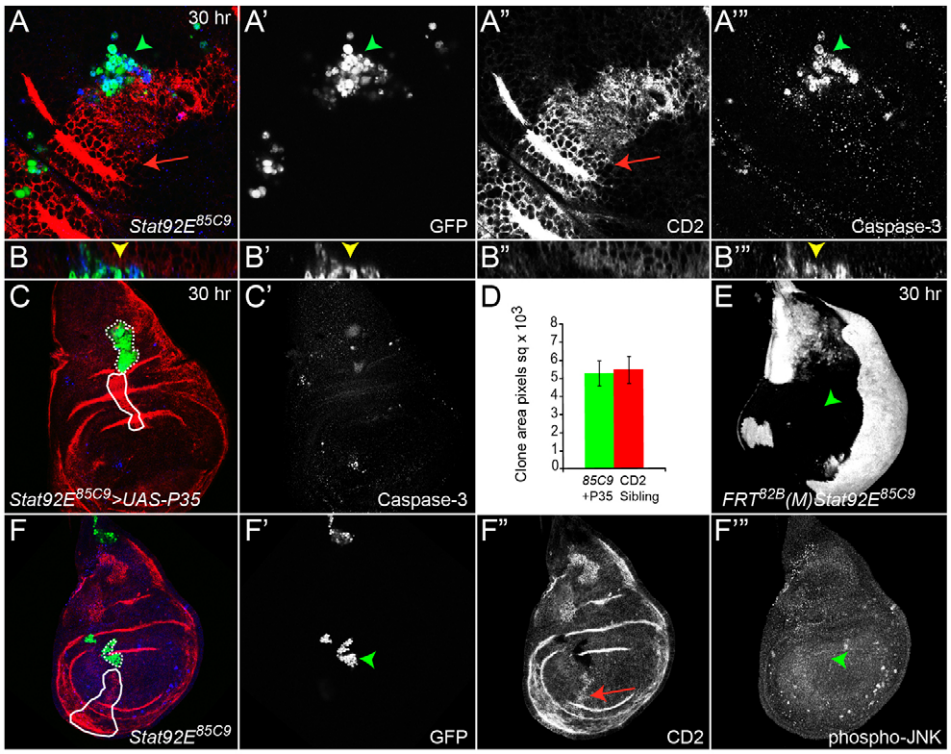


Fig. 3. Cells lacking *Stat92E* become losers and are killed by induction of apoptosis. (**A-A‴**) A GFP$^+$ *Stat92E*^{85C9} MARCM clone (A,A′,A‴, arrowheads) grows poorly compared with its CD2$^+$ (red) sibling clone (A,A″, arrows). Cells in the *Stat92E*^{85C9} clone have activated Caspase 3 (blue) (A,A′,A‴, arrowheads). (**B-B‴**) *x-z* sections of images in A-A‴ reveal that *Stat92E*^{85C9} clones are basally extruded (arrowheads). (**C**,**C′**) Inhibiting cell death by expression of P35 prevents the death of *Stat92E*^{85C9} clones in green (broken line), which now grow to similar sizes as sibling clones in red (solid line). Cells in the *Stat92E*^{85C9} + P35 clone do not have activated Caspase 3 (blue). (**D**) The areas of 19 *Stat92E*^{85C9} MARCM clones expressing P35 (green bar) and their CD2-positive sibling clones (red bar). The average area of a *Stat92E* + P35 clone was 5276±708 pixels^2, whereas that of the CD2-positive sibling clone was 5484±744 pixels^2. (**E**) *Stat92E*^{85C9} clones (arrowhead) induced at 30 hours AED in a *Minute* background grow to large sizes. *Stat92E* clones lack GFP and appear black, whereas heterozygous *Minute*/+ clones have one copy of GFP and appear white. (**F-F‴**) A GFP$^+$ *Stat92E*^{85C9} clone (broken line in F) does not have phospho-JNK (F′,F‴, arrowheads). The CD2$^+$ sibling clone (solid line in F) is marked by an arrow (F″).

siblings, confirming that the reduced growth of *Stat92E*^{85C9} clones was due to cell death (Fig. 3C,C′,D). The cell death in *Stat92E* clones could be due to an autonomous requirement for *Stat92E* in cell viability or a competitive disadvantage in cells lacking *Stat92E*. To distinguish between these possibilities, we provided *Stat92E* clones with a growth advantage by inducing them in a *Minute* background. *Stat92E*^{85C9} clones induced at 30 hours AED in a *Minute* background grew well and filled large parts of the compartment in which they arose (Fig. 3E, arrowhead). We note that we cannot exclude the possibility that *Stat92E*$^{-/-}$ cells competed against *M*/+ cells. Nevertheless, *Stat92E*-deficient cells exhibit the same context-specific behavior (i.e. that they are eliminated by apoptosis in normal tissue but are able to contribute to tissue growth when placed with poorly growing cells) that was observed for cell interactions induced by differences in Wg signaling (Vincent et al., 2011).

To date, few molecular markers have been reported for cell competition. In some instances, JNK-dependent death and Sparc expression have been described in loser cells (Moreno et al., 2002; Portela et al., 2010). We found that activated JNK is not detected in *Stat92E*^{85C9} clones (Fig. 3F-F‴), suggesting that they are eliminated through a JNK-independent pathway. By contrast, phospho-JNK was readily detected in *lgl*^{4} clones, consistent with a prior report (supplementary material Fig. S3A-B‴) (Tamori et al., 2010). Sparc was reported to be a marker for a broad range of loser cells, including low-Myc cells and *lgl* clones (Portela et al., 2010). We did not observe Sparc in *Stat92E*^{85C9} clones (supplementary material Fig. S3C-C‴). However, we also did not observe Sparc in low-Myc cells or *lgl* clones (supplementary material Fig. S3D-E‴). These results suggest that Sparc is not a general marker for losers. Nevertheless, the fact that the growth of *Stat92E* clones is rescued by expression of P35, as is that of other loser cells (Li and Baker, 2007), suggests that cells lacking *Stat92E* are out-competed through the induction of apoptosis.

Cells with hyper-activated Stat92E become supercompetitors

To determine whether the cell-autonomous growth-promoting effects of Stat92E could lead to competition with neighboring cells, we used a clonal growth assay that serves as a direct measurement of cell competition and is predicated on the fact that wild-type cells neighboring supercompetitors will be at a growth disadvantage compared with the wild-type neighbors of control cells (de la Cova et al., 2004; Wu and Johnston, 2010). The two-clone assay was used to generate two different daughter cells from a single parent cell: a ‘Gal4 clone’ expressing a gene under the control of a *UAS* promoter and *UAS-gfp*, and a ‘sibling clone’ marked with CD2. We compared the growth rates of Gal4 clones, which overexpress only

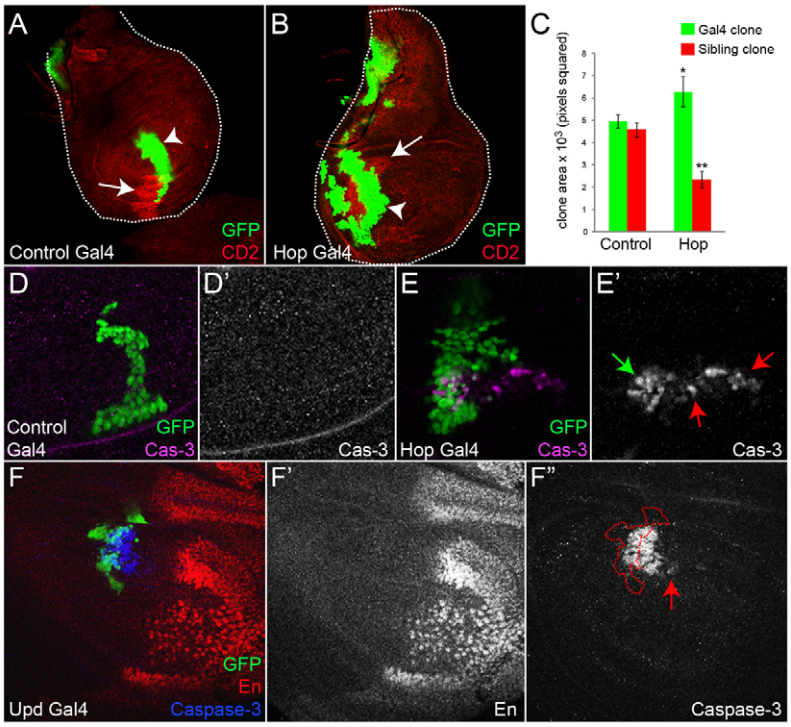


Fig. 4. Cells with hyper-activated Stat92E become supercompetitors. (**A**) Control Gal4 clones marked by GFP (arrowhead) grow to equal sizes as Control Sibling clones, marked by CD2 in red (arrow). (**B**) GFP$^+$ Hop Gal4 clones (arrowhead) grow to larger sizes than Hop Sibling clones in red (arrow). (**C**) Median area of Gal4 clones (green bars) and of sibling clones (red bars). The median areas in pixels^2 were: 4951±294 (Control Gal4) and 4578±317 (Control Sibling), *n*=39 clone pairs; 6276±676 (Hop Gal4) and 2339±369 (Hop Sibling), *n*=33 clone pairs. Hop Gal4 clones are significantly larger than Control Gal4 clones (*P<0.037). Hop Sibling clones are significantly smaller than Control Sibling clones (**P<10^{-6}). (**D**,**D′**) Neither cells in Control Gal4 clone (green cells) nor neighboring wild-type cells contain activated Caspase 3 (Cas-3, magenta). (**E**,**E′**) A Hop Gal4 clone (green cells) induces cell death (activated Caspase 3, magenta) in surrounding wild-type cells (E′, red arrows). Mis-expression of Hop also induces apoptosis within the Hop Gal4 clone itself (E′, green arrow). (**F-F″**) A Upd Gal4 clone (green cells, outlined in F″) induces cell death (activated Caspase 3, blue) in surrounding wild-type cells (F′,arrow). Mis-expression of Upd also induces apoptosis within the Upd Gal4 clone itself (i.e. within the broken red line in F″). Engrailed (En, red) marks posterior cells.

UAS-gfp (called Control Gal4), with that of their sibling clones (called Control Sibling) and the growth rates of Hop Gal4 clones, which express a constitutively activated form of Hop *UAS-hop^{Tum-l}* and *UAS-gfp*, with their sibling clones (called Hop Sibling). The growth rates of Control Gal4 clones and Control Sibling clones were similar (Fig. 4A,C). By contrast, Hop Gal4 clones grew significantly faster and were ~25% larger than Control Gal4 clones (P<0.037) (Fig. 4B,C), consistent with a role for activated Stat92E in cell-autonomous growth. The increase in Hop Gal4 clone size is not due to an increase in cellular volume since cells overexpressing Hop were approximately the same size as cells overexpressing GFP (supplementary material Fig. S4A,B,E), a result that is in stark contrast to Myc, which increases cell size when misexpressed (not shown) (Johnston et al., 1999). These data suggest that hyper-activation of the JAK/STAT pathway increases clone size by augmenting both cellular growth and mitosis, a conclusion supported by cell cycle analysis. Posterior cells with sustained Stat92E activation (*en>hop*) had the same cell cycle distribution as posterior cells expressing only GFP (*en>gfp*) (supplementary material Fig. S4F). By contrast, mis-expressing Myc (*en>Myc*) led to an increased percentage of cells in G2, as previously reported (supplementary material Fig. S4F) (Johnston et al., 1999). These observations indicate that Stat92E is a balanced growth regulator that jointly accelerates cellular growth and proliferation, consistent with our previous findings in eye disc (Bach et al., 2003).

We predicted that if Hop Gal4 clones induce cell competition, the area of their sibling clones (called Hop Sibling) would be smaller than that of the siblings of Control Gal4 clones (i.e. Control Sibling clones). Indeed, we found that Hop Sibling clones grew poorly and were ~50% smaller (P<10^{-6}) than Control Sibling clones (Fig. 4A-C), indicating that sibling clones neighboring Hop Gal4 clones suffer a severe growth disadvantage. To examine whether Hop Sibling clones were eliminated by apoptosis, we monitored activation of Caspase 3. Activated Caspase 3 was observed in wild-type cells located several cell diameters away from the boundary with Hop Gal4 clones (Fig. 4E′, red arrows), a distinguishing feature of cells undergoing competitive stress within the same compartment (de la Cova et al., 2004; Moreno and Basler, 2004). We did observe varying degrees of death in wild-type cells in the vicinity of Hop Gal4 clones (*n*=30). In ~50% of these discs, we observe a cluster of wild-type cells undergoing programmed cell death as in Fig. 4E. In about 30%, we see only a few Caspase 3-positive wild-type cells; in 20% of discs we observed little or no death in nearby wild-type cells (not shown). The variation in death of wild-type cells neighboring Hop Gal4 supercompetitors is consistent with Caspase 3 activation being a snapshot of a long process of elimination of losers from the epithelium. Apoptosis was also seen within Hop Gal4 clones (Fig. 4E′, green arrow), a result that has been reported for other growth regulators and for *axin* mutant supercompetitor clones (de la Cova et al., 2004; Vincent et al., 2011). By contrast, Caspase 3 activation was not observed in Control Gal4 clones or in the cells surrounding them (Fig. 4D′). Additionally, clonal overexpression of the JAK/STAT ligand Upd, a noted mitogen, resulted in autonomous (Fig. 4F″, inside red line) and non-autonomous (Fig. 4F″, arrow) cell death, further validating that hyper-activation of the JAK/STAT pathway results in competitive interactions leading to death of neighboring wild-type cells.

To prove that the non-autonomous cell death observed in Hop Sibling clones is specifically due to the competitive stress exerted by neighboring Hop Gal4 clones, we mis-expressed Hop throughout the posterior compartment of the wing disc (*en>hop*) and monitored apoptosis in anterior cells. Anterior cells in *en>hop* discs should not be subjected to competition by posterior cells that have higher levels of Hop because compartment boundaries insulate cells from competition (Simpson and Morata, 1981; de la Cova et al., 2004). Consistent with these predictions, we did not detect activated Caspase 3 in anterior cells in *en>hop* discs (supplementary material Fig. S4C,D). Some apoptosis was observed within posterior cells in these discs, similar to apoptosis seen within Hop Gal4 clones (supplementary material Fig. S4D′,

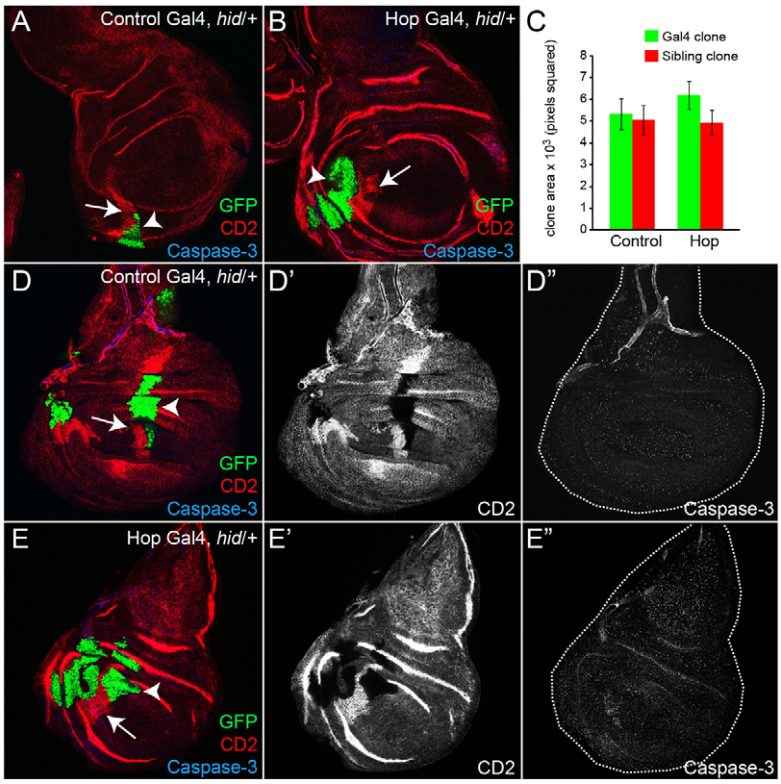


Fig. 5. Activated Stat92E requires the pro-apoptotic gene *hid* to become a supercompetitor. (**A**) Control Gal4 clones marked by GFP (arrowhead) in a hid^{P1642}/+ background and sibling clones marked by CD2 in red (arrow) in a hid^{P1642}/+ background. (**B**) Hop Gal4 clones marked by GFP (arrowheads) in a hid^{P1642}/+ background and sibling clones marked by CD2 in red (arrow) in a hid^{P1642}/+ background. (**C**) Median areas of Gal4 clones (green bars) and sibling clones (red bars). The median areas in pixels^2 were: 5326±716 (Control Gal4) and 5056±666 (Control Sibling), n=23 clone pairs; 6192±650 (Hop Gal4) and 4943±564 (Hop Sibling), n=33 clone pairs. Hop Gal4 clones are no longer significantly larger than Control Gal4 clones (P<0.187). Hop Sibling clones are now similar in size to Control Sibling clones (P<0.338). (**D-D″**) Neither Control Gal4 clones (green cells, arrowhead) nor their CD2$^+$ sibling clones (red cells, arrows) contain activated Caspase 3 (blue) in a hid^{P1642}/+ background. (**E-E″**) Hop Gal4 clones (green cells, arrowhead) in a hid^{P1642}/+ background do not induce cell death (activated Caspase 3, blue) in their sibling clones (arrowhead) or in surrounding wild-type cells.

arrow). These data demonstrate that wild-type cells adjacent to Hop Gal4 clones become losers and are eliminated from the epithelium. The increased size of Hop Gal4 clones when compared with Control Gal4 clones, and the reduced size of Hop Sibling clones when compared with Control Sibling clones, suggests that Hop Gal4 clones acquire winner status and Hop Sibling clones become losers and undergo apoptosis, features that are hallmarks of cell competition (de la Cova et al., 2004; Neto-Silva et al., 2010; Wu and Johnston, 2010). Our results demonstrate that cells within a growing tissue that acquire increased levels of activated Stat92E become supercompetitors and kill their wild-type neighbors. Furthermore, they respect compartment boundaries, another hallmark of competition.

Activated Stat92E supercompetitors require the pro-apoptotic gene *hid* to kill their neighbors

To investigate the mechanism by which activated Stat92E induces death in its neighbors, we performed the two-clone cell competition assay in a background in which one copy of the pro-apoptotic gene *hid* was inactivated, which severely impedes Myc-dependent cell competition (de la Cova et al., 2004). In a *hid*/+ background, Hop Gal4 clones were no longer significantly larger than Control Gal4 clones (P<0.187) (Fig. 5A-C). Importantly, Hop Sibling clones were now similar in size to Control Sibling clones (Fig. 5A-C). Moreover, in contrast to Hop Sibling clones in a wild-type background, no apoptosis was observed in Hop Sibling clones in a *hid*/+ background (n=18 discs, compare Fig. 5E-E″ and Fig. 4E-E″). Thus, when *hid* levels are reduced, cells neighboring Hop Gal4 winners are no longer at a growth disadvantage. Furthermore, our findings demonstrate that cells with hyper-activated Stat92E achieve winner status through non-autonomous, *hid*-dependent induction of apoptosis in losers, similar to cells with high Myc.

Activated Stat92E rescues null-Myc cells from competition

Given the similarities between our findings and those reported for the competitive growth regulatory functions of Myc, we investigated potential epistasis between Stat92E and Myc. We used the *tubulin(tub)>Myc* competition assay to assess whether activated Stat92E could rescue wild-type cells that would normally be eliminated by neighbors expressing higher levels of Myc (Fig. 6A,B) (de la Cova et al., 2004; Moreno and Basler, 2004). Clones of wild-type cells, called ‘low-Myc’, have lower levels of Myc than the surrounding *tub>Myc*-expressing cells and are eliminated from the wing disc due to cell competition imposed by their neighbors (Fig. 6E). By contrast, low-Myc clones expressing Hop were frequently recovered (Fig. 6F), indicating that if these losers have hyper-active Stat92E they suffer less competitive stress and/or have increased survival.

To test potential cooperativity between Stat92E and Myc in cell competition, we examined the ability of activated Stat92E to rescue null-Myc cells (Fig. 6C,D). Null-Myc cells (i.e. dm^4 mutant cells) surrounded by ‘high-Myc’ cells expressing *tub>Myc* were not recovered (not shown) (Wu and Johnston, 2010). Remarkably, null-Myc clones overexpressing Hop survived even in the absence of the endogenous *Myc* gene, suggesting a role for activated Stat92E in growth and cell competition independent of Myc (Fig. 6G,G′). Consistent with this, we found that Myc levels were not elevated in Hop-expressing clones, which have hyper-activated Stat92E (Fig. 6H,H′), but were dramatically increased in Myc-expressing clones (supplementary material Fig. S5B,B′, arrow). Conversely, Stat92E was not upregulated in Myc-expressing clones (Fig. 6I,I′) but was autonomously activated in Hop-expressing clones (supplementary material Fig. S5A,A′, arrowhead). Moreover, *Myc* transcripts were not elevated in posterior cells in *en>hop* wing discs (Fig. 6K). By contrast, *Myc* transcripts were dramatically

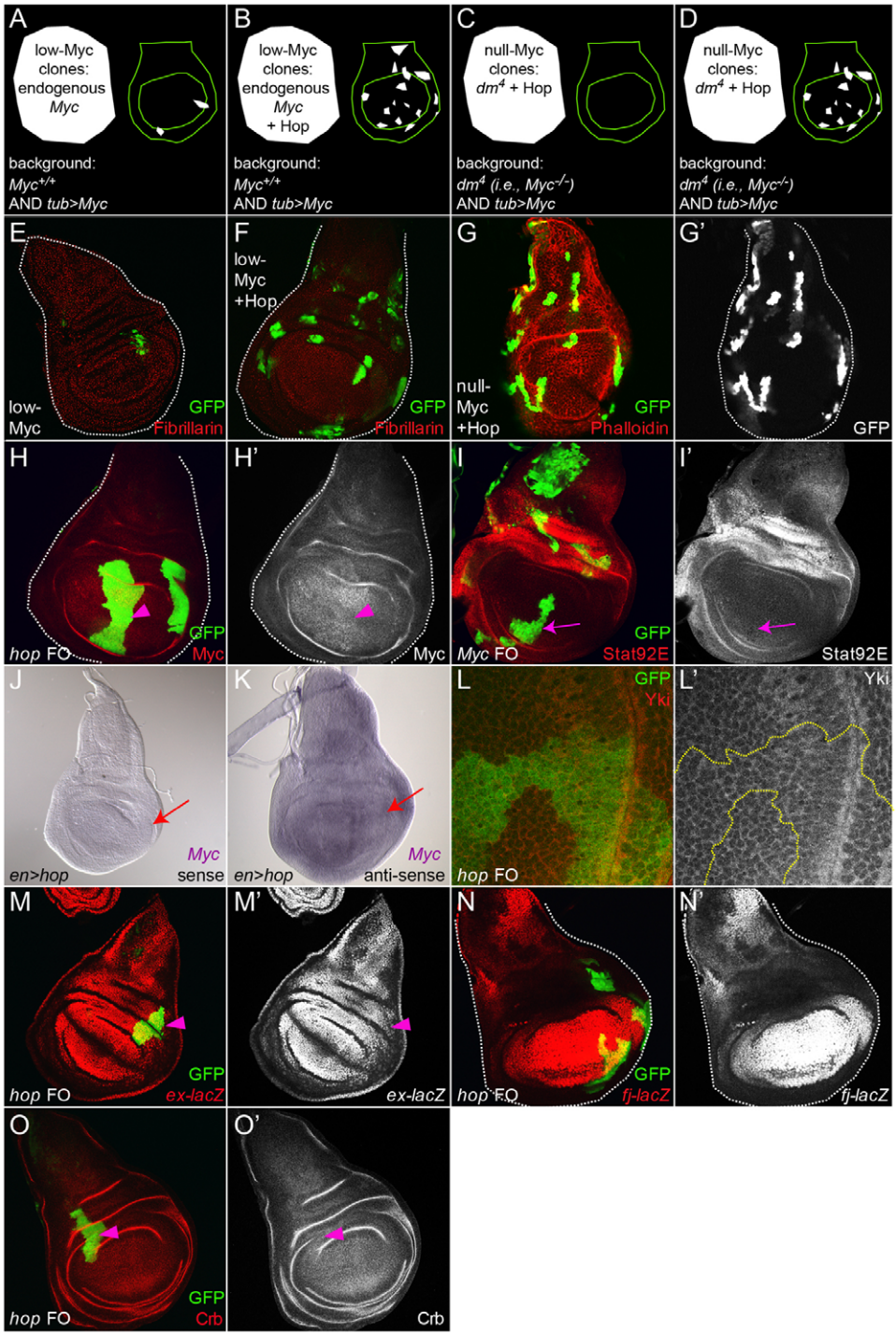


Fig. 6. Activated Stat92E rescues null-Myc cells but does not upregulate Myc or activate Yki or its target genes. (**A**,**B**) The *tub>Myc* assay. The ubiquitous *tubulin* (*tub*) promoter drives expression of the *Myc* cDNA, which is flanked by FRT sites. FLP causes excision of the *Myc* cassette, resulting in constitutive expression of Gal4 and *UAS-gfp*. (A) As these 'low-Myc' cells (white) express *Myc* from only the endogenous gene, they have lower Myc levels than their neighbors (which have the endogenous gene and *tub>Myc* transgene) and will be eliminated. (B) We overexpressed Hop within low-Myc cells. (**C**,**D**) We generated low-Myc cells that overexpressed Hop in the background of a *Myc* null allele dm^4. Low-Myc cells in a dm^4 background are null for *Myc* and so we refer to them as 'null-Myc'. If JAK/STAT signaling acts through *Myc*, null-Myc cells will be eliminated even with ectopic Stat92E activation (C). If JAK/STAT signaling acts independently of *Myc*, null-Myc+Hop cells should survive (D). (**E**) Low-Myc cells (green) generated in a *tub>Myc* background are largely eliminated. (**F**) Low-Myc cells that mis-express Hop (green), which autonomously activates Stat92E, are partially protected from cell competition. (**G**,**G'**) Null-Myc cells (green in G and white in G′) that mis-express Hop survive. These results were observed in 10 independent wing discs. (**H**,**H'**) Myc protein (red in H, white in H′) was not elevated in Hop-expressing clones (arrowheads). (**I**,**I'**) Stat92E protein (red in I, white in I′) was not upregulated in Myc-expressing clones (arrows). Stat92E protein is stabilized in the hinge in its endogenous pattern of activation. (**J**,**K**) *Myc* transcripts were not elevated in posterior cells in *en>hop* wing discs (K). No specific staining was observed with the *Myc* sense riboprobe (J). Arrows mark the posterior compartment. (**L**,**L'**) Yki (red in L, white in L′) was localized to the cytoplasm in Hop-expressing clones (green and outlined) in the wing disc. (**M-O'**) Expression of *ex-lacZ* (red in M), *fj-lacZ* (red in N) or Crb (red in O) is not altered in Hop flip-out clones (green). Single channels are in M′,N′,O′. Arrowheads indicate Hop-expressing clones.

increased in posterior cells in *en>Myc* wing discs (supplementary material Fig. S5D). The sense probe had low background in both genotypes (Fig. 6J; supplementary material Fig. S5C). Taken together, these data indicate that activated Stat92E does not affect *Myc* at the level of the protein or the gene. These results also indicate that *Stat92E* and *Myc* are not epistatic and that Stat92E probably acts independently of Myc in cell competition.

Activated Stat92E does not upregulate Yki, Wg or Dpp signaling or ribosome biogenesis genes

To test whether activated Stat92E induces targets of the Hippo pathway, we examined subcellular localization of Yki – the functional effector of the pathway – and expression of established target genes, *expanded* (*ex*) and *four-jointed* (*fj*) (Reddy and Irvine, 2008; Badouel et al., 2009), when the JAK/STAT pathway was ectopically activated. Inactive Yki is cytoplasmic, whereas activated Yki is nuclear (Oh and Irvine, 2008). In Hop-expressing clones, Yki was cytoplasmic (Fig. 6L,L′), suggesting that Yki is inactive despite ectopic activation of the JAK/STAT pathway. In addition, we observed no alteration in *ex-lacZ* or *fj-lacZ* in Hop-expressing clones (Fig. 6M-N′). Furthermore, we found no change in the expression of Crb (Fig. 6O,O′), an upstream regulator of the Hippo pathway (Grusche et al., 2010) that is a target of Stat92E in embryonic posterior

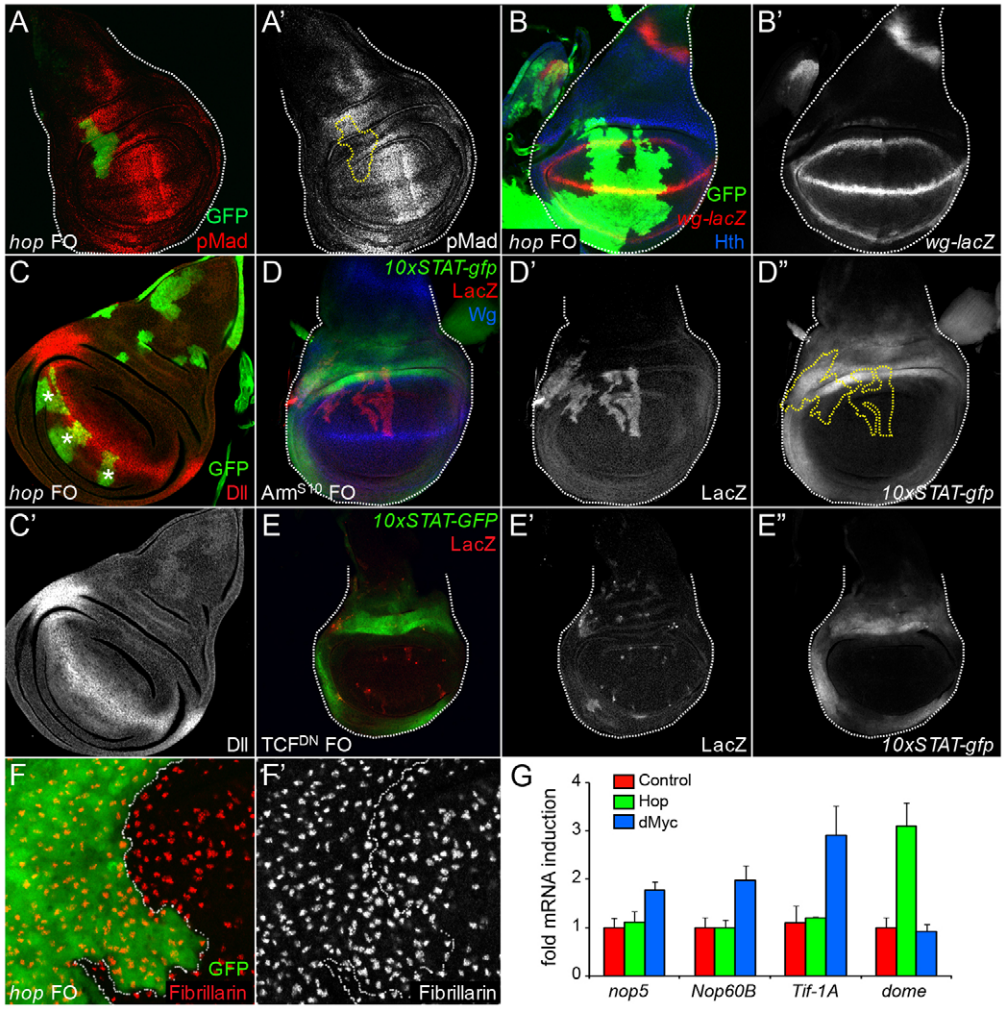


Fig. 7. Activated Stat92E does not induce Dpp or Wg signaling or ribosome biogenesis genes, and Wg signaling does not alter JAK/STAT activity. (**A**,**A′**) Phospho-Mad (red in A, white in A′) was not increased in Hop-expressing clones (green in A and outlined in A′). (**B**,**B′**) Expression of *wg-lacZ* (red, white in B′) at the DV boundary is not altered in Hop flip-out clones (green). Hth is blue. (**C**,**C′**) Dll (red in C, white in C′) is not altered in Hop-expressing clones (green, asterisks). (**D-D″**) STAT activation (*10xSTAT-gfp*, green in D, white in D″) is not altered in clones (outlined in D″) mis-expressing dominant-activate Armadillo (Arm^{S10}, red in D, white in D′), which dominantly activates Wg signaling. Wg is blue. (**E-E″**) STAT activation (*10xSTAT-gfp*, green in E, white in E″) is not altered in clones misexpressing dominant-negative TCF (TCF^{DN}, red in E, white in E′), which dominantly blocks Wg signaling. Clones expressing TCF^{DN} grew poorly. (**F**,**F′**) Fibrillarin (red in F, white in F′), a nucleolar marker, is not increased in clones overexpressing Hop (green). (**G**) mRNAs for ribosome biogenesis genes (*nop5*, *Nop60B*, *Tif-1A*) are upregulated in wing discs overexpressing Myc (blue bars) but not in those overexpressing Hop (green) or in controls overexpressing only GFP (red). mRNA of the Stat92E target *dome* is increased in Hop samples, but not in Myc or controls.

spiracles (Lovegrove et al., 2006). These data strongly suggest that JAK/STAT signaling does not lead to activation of Yki.

We also examined the activity of other pathways reported to regulate cell competition. Dpp signaling has been associated with winner status (Moreno et al., 2002; Moreno and Basler, 2004), but its activity, as assessed by phospho-Mad (pMad), was not upregulated in Hop-expressing clones (Fig. 7A,A′; supplementary material Fig. S5E for the control). Clonal differences in Wg signaling have been shown to trigger cell competition in the wing disc (Vincent et al., 2011). *wg* expressed at the DV boundary of the wing disc is not altered by ectopic activation of the JAK/STAT pathway, indicating activated Stat92E does not regulate Wg expression in the wing pouch (Fig. 7B,B′). Furthermore, Wg signal transduction, as assessed by its target Dll (Zecca et al., 1996; Neumann and Cohen, 1997), is unaffected in Hop-expressing clones (Fig. 6C,C′). Finally, we found that neither ectopic clonal activation (Arm^{S10}) nor inhibition (TCF^{DN}) of Wg signaling alters JAK/STAT pathway activity (Fig. 7D-E″). These striking results support the conclusion that activated Stat92E confers supercompetitor status without upregulation of Yki, Dpp or Wg pathway activity.

Finally, we monitored aspects of ribosome activity in wing cells with hyper-active Stat92E. Nucleolar size, which serves as an index for ribosomal activity, was not increased by hyper-active Stat92E (Fig. 7F,F′). Furthermore, quantitative PCR analysis revealed that activated Stat92E increased expression of known targets *dome* (Fig. 7G) and *chinmo* (not shown) (Flaherty et al., 2010). However, activated Stat92E did not alter the expression of genes required for ribosome biogenesis that are known targets of Myc: *nop5*, *Nop60B* and *Tif-1A* (Fig. 7G) (Grewal et al., 2005). These data show that activated Stat92E does not increase at least some ribosome biogenesis genes at late larval stages.

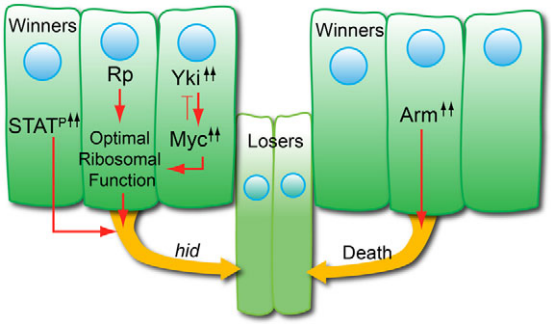


Fig. 8. Model for JAK/STAT signaling in cell competition. See text for details.

DISCUSSION

This study establishes a role for JAK/STAT signaling in cell competition between somatic cells that contribute to the adult organism. We show that wing disc cells lacking *Stat92E* activity suffer from competitive stress exerted by their wild-type neighbors and undergo apoptosis. However, when these same cells are placed with growth-disadvantaged cells (i.e. *M*/+), they are viable. This context-dependent behavior of cells (i.e. viable when homotypic but disadvantaged when in apposition to more robust cells) is a hallmark of cell competition (Johnston, 2009). Interestingly, the growth of *Stat92E* clones can be rescued by inhibition of apoptosis (Fig. 3C,D). By contrast clones lacking *Myc* or ribosomal genes such as *Rpl135* cannot grow even when death is inhibited (Johnston et al., 1999; Grewal et al., 2005). This may represent an important difference between activated Stat92E and Myc function in losers.

Despite these differences, activated Stat92E does in fact share distinguishing features of cell competition with Myc: winners with activated Stat92E (1) induce programmed cell death in losers at a distance and (2) require *hid* to do so (this study) (de la Cova et al., 2004). In addition, our observation that cells null for *Myc* can be at least partially rescued by autonomous activation of Stat92E is noteworthy because autonomous expression of Yki does not rescue these cells (Neto-Silva et al., 2010). We propose that the partial rescue of null *Myc* cells by activated Stat92E is probably not due to an increase in ribosome activity or in expression of Myc target genes that drive ribosome assembly as activated Stat92E does not induce a subset of ribosomal genes during third instar (Fig. 7G).

Stat92E is required early in development for growth of the wing disc

We demonstrate that the JAK/STAT pathway plays an obligate role in growth of all cells in the young wing disc (30-48 hours AED). During this period of exponential growth, we show that imaginal cells lacking *Stat92E* are less competitive and are subjected to stress imposed by their wild-type neighbors and they are ultimately killed by *hid*-dependent apoptosis. We did not observe any regional effects of Stat92E at early time points: *Stat92E* clones grew poorly regardless of their position on the AP and DV axes when induced at 30 or 36 hours AED. The results from the *Stat92E* clonal analyses presented here are consistent with but stronger than those published by another group (Mukherjee et al., 2005). This discrepancy may be due to their use of a weaker allele $Stat92E^{06346}$ (Ekas et al., 2010).

Sustained Stat92E activation and supercompetitor status

We demonstrate that cells with activated Stat92E also achieve supercompetitor status and induce death of their wild-type neighbors up to several cell diameters away, which is similar to the non-autonomous death of wild-type cells induced by Myc or Wg supercompetitors. These results strongly suggest that non-autonomous cell death is a key feature of cell competition in response to local cellular differences in either STAT activity, Wg signaling or Myc. Moreover, we demonstrate that, like Myc (de la Cova et al., 2004), cells with activated Stat92E require the pro-apoptotic gene *hid* to kill surrounding neighbors and achieve supercompetitor status. Although these results suggest a link between Stat92E and Myc, to our surprise we found no link between JAK/STAT signaling and *Myc* mRNA or Myc protein or in targets of the Hippo pathway. Furthermore, we found no regulation of Wg signaling by activated STAT and no effect of Wg on STAT activity. Taken together, our results strongly suggest that activated STAT functions in parallel to Yki, Myc and Wg in growth and cell competition (Fig. 8).

Differences in ribosome activity between winners and losers appear to be crucial to Myc- and *Minute*-induced cell competition and may also be required by Myc for its supercompetitor activity. We find that activated STAT does not increase expression of an important set of ribosome biogenesis genes during late larval stages (Fig. 7G). We acknowledge that it is conceivable that JAK/STAT signaling might affect other ribosomal aspects not tested in this study. Assuming a similar relationship exists at earlier larval stages – when *Stat92E* is required for clonal growth – we favor the model that STAT-dependent cell competition is largely independent of de novo ribosome biogenesis. This would represent an important difference between JAK/STAT and Myc- or *Minute*-dependent cell competition (Fig. 8). Regardless, our results at the very least suggest the presence of multiple sensors of competitive situations and indicate that the way cells compare their fitness with one another is more complex than previously believed. Indeed, Myc- and ribosome-independent supercompetition appears to be a newly emerging paradigm in the field (this study) (Vincent et al., 2011). In conclusion, we find that differences in Stat92E activity reveal differences in cellular fitness that are in large part unrelated to Myc, ribosome biogenesis, Hippo, Wg or Dpp signaling activity. Moreover, given the conservation between the components of the *Drosophila* and mammalian JAK/STAT signaling pathway, our findings lead the way for further investigation of cell competition in mammals.

Acknowledgements

We thank our colleagues for flies and antibodies and Marc Amoyel and anonymous reviewers for helpful comments.

Funding

This work was supported by Canadian Institutes of Health Research [MOP86622 to S.G.] and by the National Institutes of Health [R01-GM078464 to L.A.J. and R01-GM085075 to E.A.B.]. Deposited in PMC for release after 12 months.

Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.076760/-/DC1

References

Arbouzova, N. I. and Zeidler, M. P. (2006). JAK/STAT signalling in Drosophila: insights into conserved regulatory and cellular functions. *Development* **133**, 2605-2616.

Bach, E. A., Vincent, S., Zeidler, M. P. and Perrimon, N. (2003). A sensitized genetic screen to identify novel regulators and components of the Drosophila janus kinase/signal transducer and activator of transcription pathway. *Genetics* **165**, 1149-1166.
Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G. H. (2007). GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. *Gene Expr. Patterns* **7**, 323-331.
Badouel, C., Garg, A. and McNeill, H. (2009). Herding Hippos: regulating growth in flies and man. *Curr. Opin. Cell Biol.* **21**, 837-843.
de la Cova, C. and Johnston, L. A. (2006). Myc in model organisms: a view from the flyroom. *Semin. Cancer Biol.* **16**, 303-312.
de la Cova, C., Abril, M., Bellosta, P., Gallant, P. and Johnston, L. A. (2004). Drosophila myc regulates organ size by inducing cell competition. *Cell* **117**, 107-116.
Ekas, L. A., Baeg, G. H., Flaherty, M. S., Ayala-Camargo, A. and Bach, E. A. (2006). JAK/STAT signaling promotes regional specification by negatively regulating wingless expression in Drosophila. *Development* **133**, 4721-4729.
Ekas, L. A., Cardozo, T. J., Flaherty, M. S., McMillan, E. A., Gonsalves, F. C. and Bach, E. A. (2010). Characterization of a dominant-active STAT that promotes tumorigenesis in Drosophila. *Dev. Biol.* **344**, 621-636.
Flaherty, M. S., Zavadil, J., Ekas, L. A. and Bach, E. A. (2009). Genome-wide expression profiling in the Drosophila eye reveals unexpected repression of notch signaling by the JAK/STAT pathway. *Dev. Dyn.* **238**, 2235-2253.
Flaherty, M. S., Salis, P., Evans, C. J., Ekas, L. A., Marouf, A., Zavadil, J., Banerjee, U. and Bach, E. A. (2010). Chinmo is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in Drosophila. *Dev. Cell* **18**, 556-568.
Grewal, S. S., Li, L., Orian, A., Eisenman, R. N. and Edgar, B. A. (2005). Myc-dependent regulation of ribosomal RNA synthesis during Drosophila development. *Nat. Cell Biol.* **7**, 295-302.
Grewal, S. S., Evans, J. R. and Edgar, B. A. (2007). Drosophila TIF-IA is required for ribosome synthesis and cell growth and is regulated by the Tor pathway. *J. Cell Biol.* **179**, 1105-1113.
Grusche, F. A., Richardson, H. E. and Harvey, K. F. (2010). Upstream regulation of the hippo size control pathway. *Curr. Biol.* **20**, R574-R582.
Guss, K. A., Mistry, H. and Skeath, J. B. (2008). Vestigial expression in the Drosophila embryonic central nervous system. *Dev. Dyn.* **237**, 2483-2489.
Hanratty, W. P. and Dearolf, C. R. (1993). The Drosophila Tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. *Mol. Gen. Genet.* **238**, 33-37.
Holz, A., Meise, M. and Janning, W. (1997). Adepithelial cells in Drosophila melanogaster: origin and cell lineage. *Mech. Dev.* **62**, 93-101.
Johnston, L. A. (2009). Competitive interactions between cells: death, growth, and geography. *Science* **324**, 1679-1682.
Johnston, L. A., Prober, D. A., Edgar, B. A., Eisenman, R. N. and Gallant, P. (1999). Drosophila myc regulates cellular growth during development. *Cell* **98**, 779-790.
Kiger, A. A., Jones, D. L., Schulz, C., Rogers, M. B. and Fuller, M. T. (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* **294**, 2542-2545.
Li, W. and Baker, N. E. (2007). Engulfment is required for cell competition. *Cell* **129**, 1215-1225.
Lovegrove, B., Simões, S., Rivas, M. L., Sotillos, S., Johnson, K., Knust, E., Jacinto, A. and Hombría, J. C. (2006). Coordinated control of cell adhesion, polarity, and cytoskeleton underlies Hox-induced organogenesis in Drosophila. *Curr. Biol.* **16**, 2206-2216.
Morata, G. and Ripoll, P. (1975). Minutes: mutants of drosophila autonomously affecting cell division rate. *Dev. Biol.* **42**, 211-221.
Moreno, E. and Basler, K. (2004). dMyc transforms cells into super-competitors. *Cell* **117**, 117-129.
Moreno, E., Basler, K. and Morata, G. (2002). Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. *Nature* **416**, 755-759.
Mukherjee, T., Hombría, J. C. and Zeidler, M. P. (2005). Opposing roles for Drosophila JAK/STAT signalling during cellular proliferation. *Oncogene* **24**, 2503-2511.
Neto-Silva, R. M., Wells, B. S. and Johnston, L. A. (2009). Mechanisms of growth and homeostasis in the Drosophila wing. *Annu. Rev. Cell Dev. Biol.* **25**, 197-220.
Neto-Silva, R. M., de Beco, S. and Johnston, L. A. (2010). Evidence for a growth-stabilizing regulatory feedback mechanism between Myc and Yorkie, the Drosophila homolog of Yap. *Dev. Cell* **19**, 507-520.
Neufeld, T. P., de la Cruz, A. F., Johnston, L. A. and Edgar, B. A. (1998). Coordination of growth and cell division in the Drosophila wing. *Cell* **93**, 1183-1193.
Neumann, C. J. and Cohen, S. M. (1997). Long-range action of Wingless organizes the dorsal-ventral axis of the Drosophila wing. *Development* **124**, 871-880.
Oh, H. and Irvine, K. D. (2008). In vivo regulation of Yorkie phosphorylation and localization. *Development* **135**, 1081-1088.
Portela, M., Casas-Tinto, S., Rhiner, C., López-Gay, J. M., Domínguez, O., Soldini, D. and Moreno, E. (2010). Drosophila SPARC is a self-protective signal expressed by loser cells during cell competition. *Dev. Cell* **19**, 562-573.
Reddy, B. V. and Irvine, K. D. (2008). The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. *Development* **135**, 2827-2838.
Simpson, P. (1979). Parameters of cell competition in the compartments of the wing disc of Drosophila. *Dev. Biol.* **69**, 182-193.
Simpson, P. and Morata, G. (1981). Differential mitotic rates and patterns of growth in compartments in the Drosophila wing. *Dev. Biol.* **85**, 299-308.
Struhl, G. and Basler, K. (1993). Organizing activity of wingless protein in Drosophila. *Cell* **72**, 527-540.
Tamori, Y., Bialucha, C. U., Tian, A. G., Kajita, M., Huang, Y. C., Norman, M., Harrison, N., Poulton, J., Ivanovitch, K., Disch, L. et al. (2010). Involvement of Lgl and Mahjong/VprBP in cell competition. *PLoS Biol.* **8**, e1000422.
Tsai, Y. C. and Sun, Y. H. (2004). Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in Drosophila eye development. *Genesis* **39**, 141-153.
Tyler, D. M., Li, W., Zhuo, N., Pellock, B. and Baker, N. E. (2007). Genes affecting cell competition in Drosophila. *Genetics* **175**, 643-657.
Vincent, J. P., Kolahgar, G., Gagliardi, M. and Piddini, E. (2011). Steep differences in wingless signaling trigger Myc-independent competitive cell interactions. *Dev. Cell* **21**, 366-374.
Wu, D. C. and Johnston, L. A. (2010). Control of wing size and proportions by Drosophila myc. *Genetics* **184**, 199-211.
Zecca, M., Basler, K. and Struhl, G. (1996). Direct and long-range action of a wingless morphogen gradient. *Cell* **87**, 833-844.
Ziosi, M., Baena-López, L. A., Grifoni, D., Froldi, F., Pession, A., Garoia, F., Trotta, V., Bellosta, P., Cavicchi, S. and Pession, A. (2010). dMyc functions downstream of Yorkie to promote the supercompetitive behavior of hippo pathway mutant cells. *PLoS Genet.* **6**, e1001140.
Zirin, J. D. and Mann, R. S. (2007). Nubbin and Teashirt mark barriers to clonal growth along the proximal-distal axis of the Drosophila wing. *Dev. Biol.* **304**, 745-758.

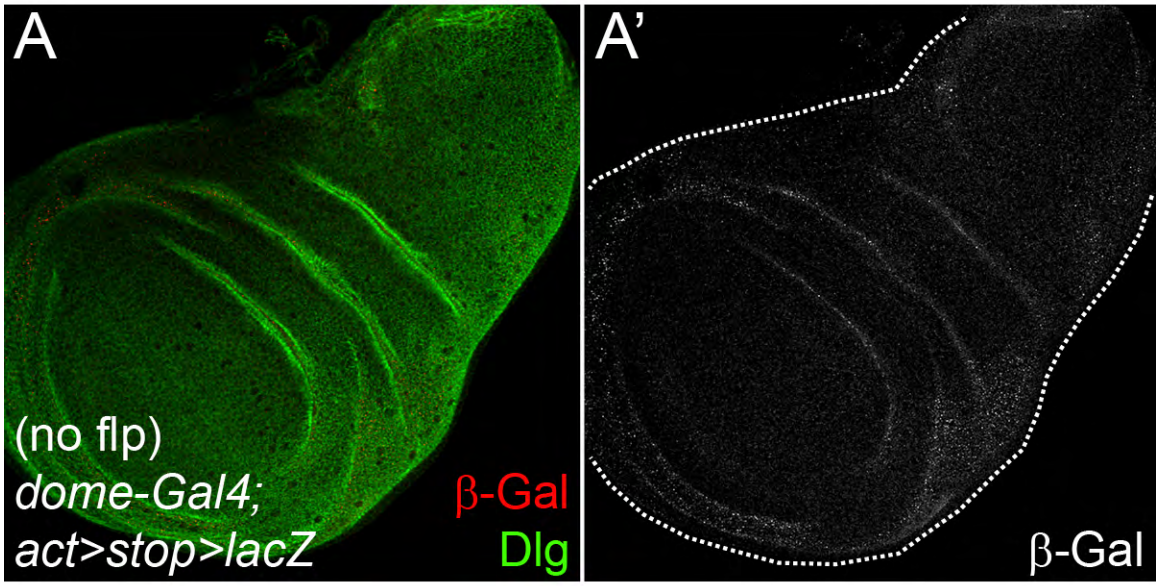


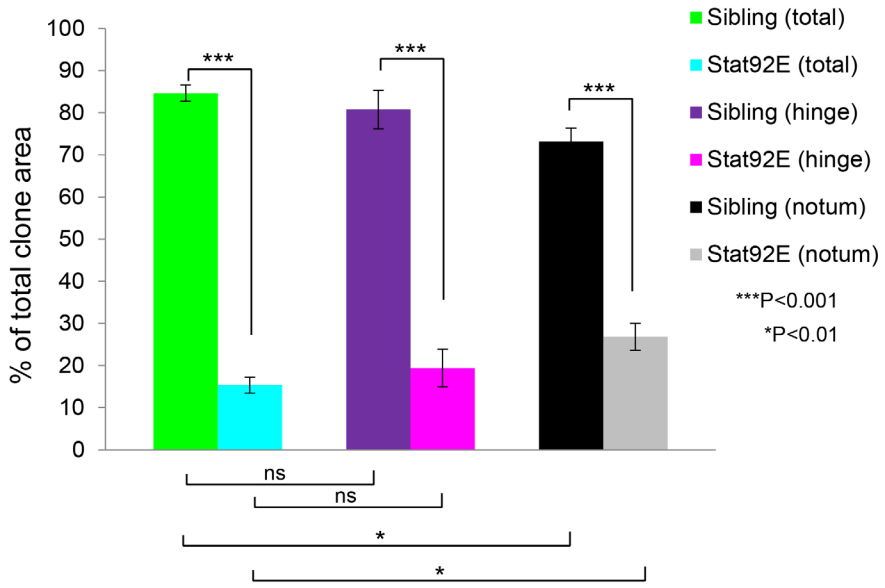
Fig. S1. The *act5c>stop>lacZ* flip-out cassette is not spuriously excised. (**A**,**A′**) Genotype: *dome-gal4*; *act5c>stop>lacZ*. In the absence of *UAS-flp*, there is no expression of the *act5c>stop>lacZ* flip-out cassette. β-Gal is red and Dlg is green.

A

Clone induction	Sibling (eye)	*Stat92E* (eye)	P value	Sibling (wing)	*Stat92E* (wing)	P value
30	94.22 +/- 1.06	5.77 +/- 1.06	6×10^{-10}	94.22 +/- 1.06	4.89 +/- 1.10	1.03×10^{-18}
36	95.67 +/- 1.40	4.32 +/- 1.40	4.18×10^{-10}	95.40 +/- 0.97	4.59 +/- 0.97	4.20×10^{-17}
48	84.79 +/- 2.92	15.21 +/- 2.92	1×10^{-4}	84.65 +/- 1.91	15.35 +/- 1.91	4.73×10^{-6}

Clone induction	FRT^{82B} Sibling clone area (wing)	FRT^{82B} control clone area (wing)	P value
30	51.52 +/- 0.62	48.47 +/- 0.62	ND
48	51.79 +/- 0.83	48.21 +/- 0.83	ND

B



C

Region	Sibling clone area	*Stat92E* clone area	P value
total wing	84.65 +/- 1.91	15.35 +/- 1.91	4.73×10^{-6}
hinge	80.76 +/- 4.55	19.40 +/- 4.46	5.2×10^{-4}
notum	73.17 +/- 3.19	26.83 +/- 3.19	3.87×10^{-4}

D

P values: Difference between total wing and hinge	P values: Difference between total wing and notum
Sibling: 0.229	Sibling: 0.00732
Stat92E: 0.216	*Stat92E*: 0.00732

Fig. S2. *Stat92E* clones grow poorly. (**A**) *Stat92E* and FRT^{82B} control clones were induced at the indicated times in hours AED and analyzed at 115 hours AED in eye and wing discs. Areas of siblings and clones were measured by Image J and then calculated as a percentage of the total clone area in the disc (e.g. sibling clone area + clone area). More than 50 pairs of sibling and clone were measured at each time point. Values are expressed as percentage±s.e. of clone size averaged across discs. Clone induction values are in hours AED. *P* values represent probability that differences in clone areas and sibling clone areas are statistically significant. (**B**) *Stat92E* clones induced at 48 hours AED and analyzed at 115 hours AED in wing discs. 'Total' represents the areas of sibling or *Stat92E* clones in the entire disc. Hinge or Notum represents sibling and *Stat92E* clones residing in the hinge or notum only, respectively. ***$P<0.001$; *$P<0.01$. ns, not statistically significant. (**C**) Area of sibling and *Stat92E* clones in the total wing, the hinge and the notum expressed as percentage±s.d. of clone size averaged across discs. The *P* values for the differences between sibling and *Stat92E* clones in each domain are shown in the rightmost column. (**D**) Probability (*P* values) that the difference between sibling clones located in the total wing versus the hinge or total wing versus the notum (2nd row) or between *Stat92E* clones located in the total wing versus the hinge or total wing versus the notum (3rd row) are statistically significant. Only comparisons of clone areas (both sibling and *Stat92E*) in the total wing versus the notum are statistically significant (3rd column).

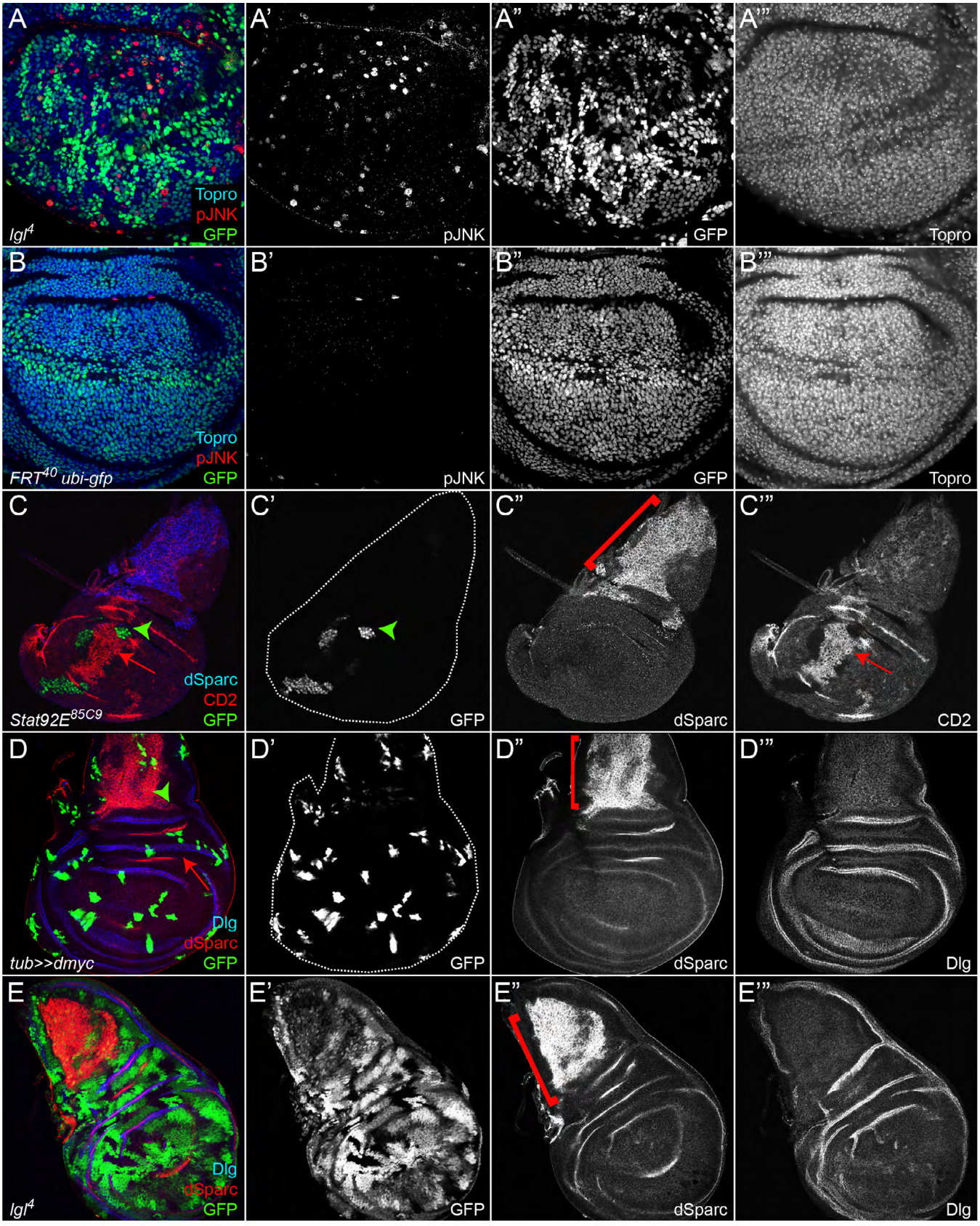
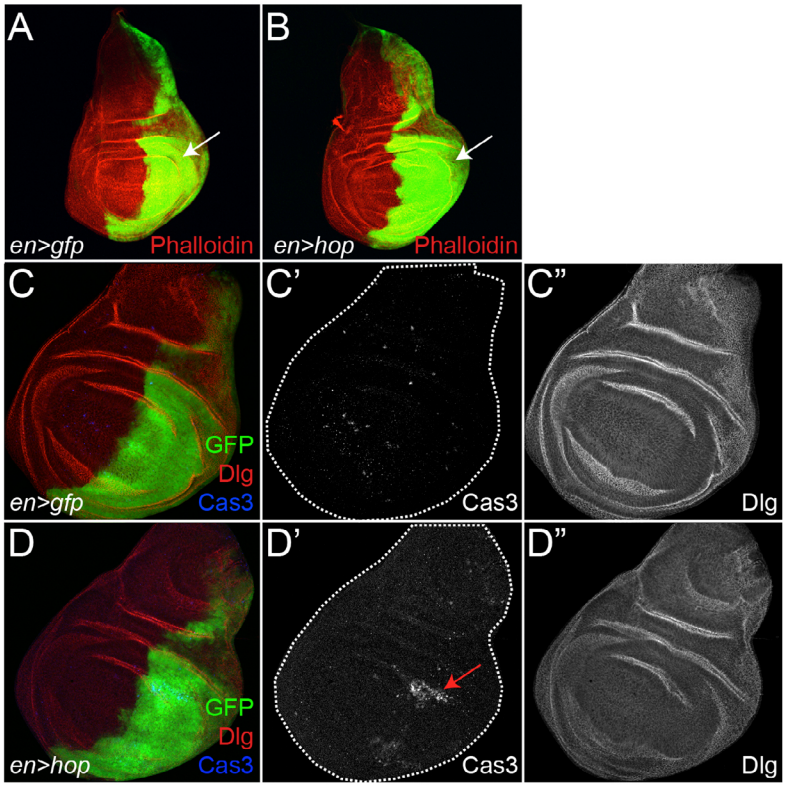
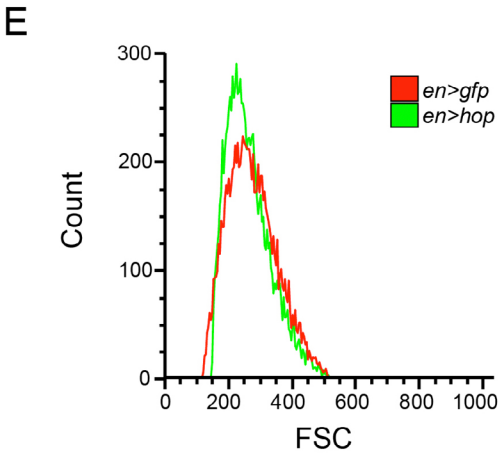
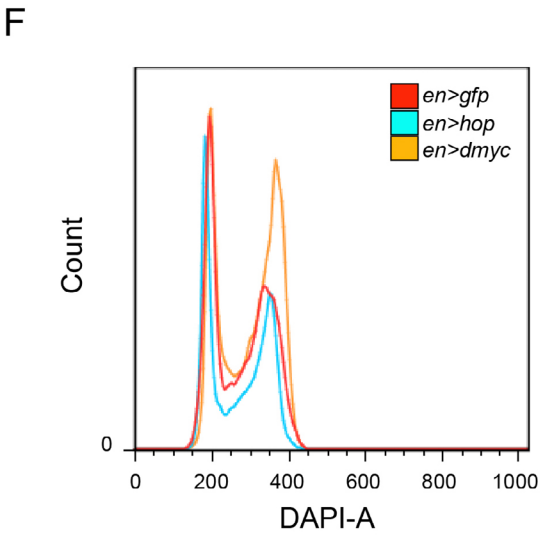


Fig. S3. pJNK is detected in *lgl* clones but Sparc is not expressed in losers. (**A**) Phospho-JNK (pJNK, red) is detected in lgl^4 clones. Clones lack GFP. Torpo, which marks nuclei, is blue. (**B**) pJNK (red) is detected at very low levels in a control FRT^{40} *ubi-gfp* disc. Torpo, which marks nuclei, is blue. (**C**) Sparc (blue) is not expressed in MARCM $Stat92E^{85C9}$ clones. Clones are marked by GFP. The sibling clone is marked by CD2 (red). (**D**) Sparc (red) is not expressed in low-Myc cells (*tub>>Myc*), which are GFP^+. Dlg is blue. (**E**) Sparc (red) is not expressed lgl^4 clones, which lack GFP. Dlg is blue. In B-D, the bracket marks the endogenous expression of Sparc in adepithelial cells in the wing disc (Holz et al., 1997), a pattern that we have observed in all genotypes examined.







	en>gfp	*en>hop*	*en>dmyc*
G1	40.0	45.0	30.9
S	11.3	10.2	10.9
G2/M	48.7	44.8	58.2

Fig. S4. Activation of Hop in posterior cells does not induce death in anterior cells, increase cell size or alter cell cycle profiles. (**A**,**B**) Arrows mark the posterior compartment of *en-gal4/UAS-gfp* (*en>gfp*) (A) or *en-gal4/UAS-hop; UAS-gfp/+* (*en>hop*) (B) wing discs. Phalloidin (red) marks F-actin. (**C**) Very low levels of Caspase 3 activation (blue) are detected in *en>gfp* discs. Dlg (red) marks cell membranes in C,D. GFP marks cells in the posterior compartment of the wing disc. (**D**) Caspase 3 activation (blue) is observed within the posterior compartment in *en>hop* discs but none is detected in the anterior compartment. (**E**) FSC plots of control *en-gal4/UAS-gfp* (*en>gfp*) (red histogram) and *en-gal4/UAS-hop; UAS-gfp/+* (*en>hop*) (green histogram). Only GFP$^+$ cells in the live gate were analyzed. Overexpression of Hop does not increase cell size as the mean FSC for *en>hop* was 261 (*n*=10,803 events) as compared with 272 (*n*=10,968 events) for *en>gfp*. Similar results were observed in ten independent experiments. (**F**) FACS plot of control *en-gal4/UAS-gfp* (*en>gfp*) (red histogram), *en-gal4/UAS-hop; UAS-gfp/+* (*en>hop*) (blue histogram) or *en-gal4/UAS-Myc; UAS-gfp/+* (orange histogram). Only GFP$^+$ cells in the live gate were analyzed. Table shows the percentage of cells in G1, S or G2/M. Overexpression of Hop does not alter cell cycle phasing as the percentages of cells in G1, S or G2/M phase in *en>hop* were the same as *en>gfp*. By contrast, *en>Myc* samples showed fewer cells in G1 but more cells in G2/M, consistent with a previous report and with the ability of Myc to promote progression through G1 but not G2/M (Johnston et al., 1999). Similar results were observed in five independent experiments. *en>gfp* (*n*=4599); *en>hop* (*n*=4172); *en>Myc* (*n*=5465).

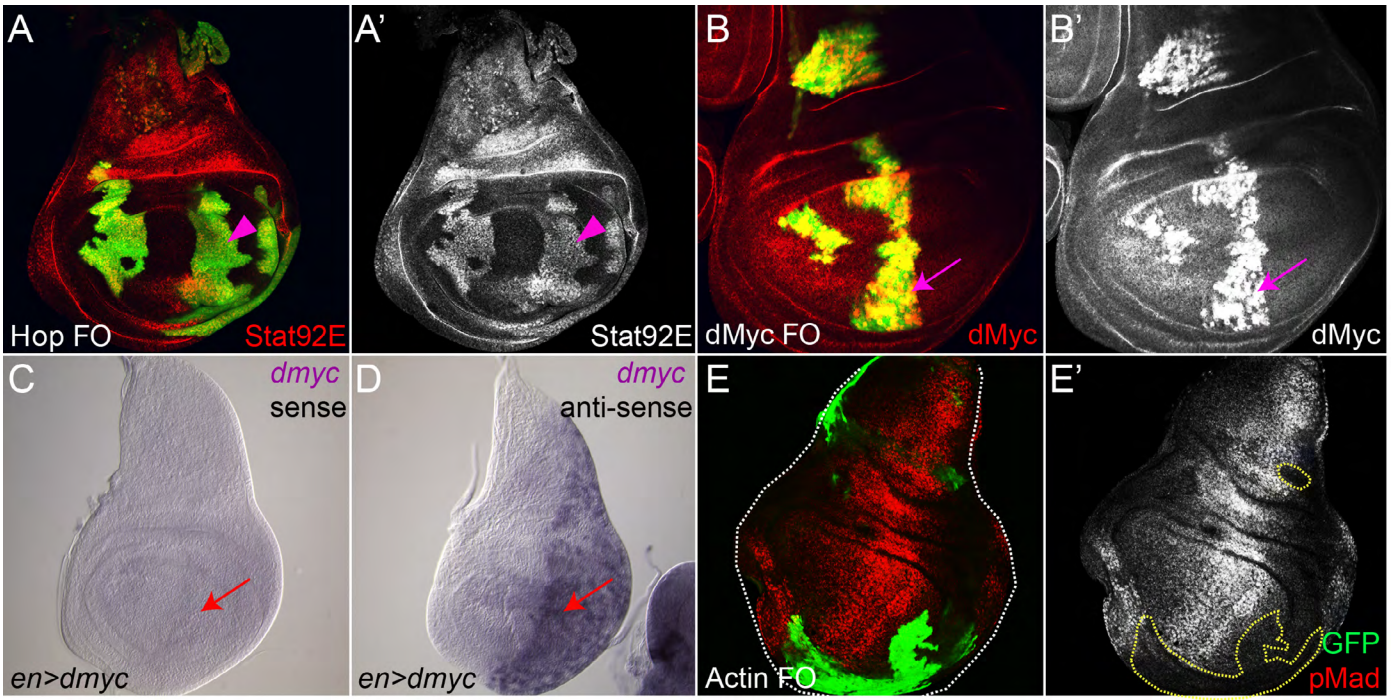


Fig. S5. Controls for Figs 6 and 7. (**A**,**A′**) Stat92E protein was stabilized in Hop-expressing clones (arrowheads). Note in A that Stat92E protein is stabilized in the hinge in its endogenous pattern of activation (Bach et al., 2007). Flip-out clones express GFP in A,B. (**B**,**B′**) Myc protein (red) was increased in Myc-expressing clones (arrowhead). (**C**) No specific staining was observed with the *Myc* sense riboprobe in *en>Myc* wing discs. Arrow marks the posterior compartment. (**D**) *Myc* transcripts were elevated in posterior cells in *en>Myc* samples. Arrow marks the posterior compartment. (**E**,**E′**) Levels of phospho-Mad (red) were not elevated in control-expressing clones. Clones are outlined in yellow in E′.