HHS Public Access

Author manuscript

*Nat Genet*. Author manuscript; available in PMC 2010 April 01.

Published in final edited form as:

*Nat Genet*. 2009 October ; 41(10): 1150–1155. doi:10.1038/ng.445.

# A tumor suppressive activity of Drosophila Polycomb genes mediated by JAK/STAT signaling

**Anne-Kathrin Classen**[1], **Brandon D. Bunker**[1], **Kieran F. Harvey**[2,3], **Thomas Vaccari**[4], and **David Bilder**[1]

[1]Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720, USA

[2]Peter MacCallum Cancer Centre, 7 St Andrews Place, East Melbourne, Victoria, Australia, 3002

[3]Department of Pathology, University of Melbourne, Parkville, Victoria, Australia, 3010

[4]Istituto FIRC di Oncologia Molecolare 20139 Milano, Italy

## Abstract

A prevailing paradigm posits that Polycomb Group (PcG) proteins maintain stem cell identity by repressing differentiation genes, and abundant evidence points to an oncogenic role for PcG in human cancer 1,2. Here we demonstrate using Drosophila that a conventional PcG complex can also have a potent tumor suppressive activity. Mutations in all core PRC1 components cause dramatic hyperproliferation of eye imaginal tissue, accompanied by deregulation of epithelial architecture. The mitogenic JAK/STAT pathway is strongly and specifically activated in mutant tissue; activation is driven by transcriptional upregulation of Unpaired (Upd) family ligands. We show that *upd* genes are direct targets of PcG-mediated repression in imaginal discs. Ectopic JAK/STAT activity is sufficient to induce overproliferation, while reduction of JAK/STAT activity suppresses the *PRC1* mutant tumor phenotype. These findings show that PcG proteins can restrict growth directly by silencing mitogenic signaling pathways, shedding light onto an epigenetic mechanism underlying tumor suppression.

### Keywords

Polycomb; tumor suppressor genes; Unpaired; JAK/STAT

The allele *P3C* was identified as a Drosophila tumor suppressor mutation with unusual properties 3. Mutant clones generated in genetically mosaic eye imaginal discs do not survive well nor persist through metamorphosis, but cause non-autonomous overgrowth of surrounding wild type tissue (Fig.S1a,b). Since certain tumor suppressor mutations manifest their full phenotypes only when cell competition is eliminated 4,5, we utilized the *FLP/cell*



Corresponding author: Prof. David Bilder Department of Molecular & Cell Biology University of California Berkeley 142 LSA #3200 Berkeley, CA 94720-3200 Phone: (510) 642-8605 Fax: (510) 643-7448 bilder@berkeley.edu.

**Author Contribution Statement** A.K.C and D.B conceived the study and wrote the paper. A.K.C performed the experiments and analyzed the data. B.B performed the molecular and T.V the genetic mapping of the P3C allele, which was isolated and initially characterized by K.H.

*lethal* system 6 to generate eye and wing discs consisting predominantly of *P3C* mutant cells. Such *P3C* imaginal discs are dramatically overgrown (Fig.1a,b; S2h) and larvae that contain these discs become `giant larvae' and die in pupation. Mutant tissue fails to undergo terminal differentiation (Fig.1c,d) and exhibits a range of architectural defects (Fig.S3a–d). These epithelial defects occur in the context of upregulation of F-actin (Fig.1e,f), loss of E-cadherin (Fig.1g,h) and ectopic expression of Matrix Metalloprotease 1 (Fig.1i,j). Overgrowth, differentiation defects and disrupted epithelial architecture are phenotypes reminiscent of previously described neoplastic tumor suppressor mutations 5.

Genetic and molecular mapping of *P3C* reveals that it is a small deletion removing the two neighboring homologous genes *Posterior Sex Combs (Psc)* and *Suppressor of Zeste 2 (Su(z)2)* (Fig.S1g) 7. A related but more complex phenotype was obtained with the previously studied deficiency *Psc-Su(z)2*$^{1b8}$, which deletes seven additional genes (Fig.S1g) 8,9. However, eye mosaic clones for null alleles of *Psc* or *Su(z)2* alone did not exhibit a proliferation phenotype (Fig.S1c–f), suggesting that the genes are functionally redundant for growth control.

*Psc* and *Su(z)2* encode members of the Polycomb Group (PcG) of epigenetic silencers, and can functionally substitute for each other in Polycomb Repressive Complex 1 (PRC1) 10. The PRC1 core component Polycomb (Pc) mediates recognition and binding to trimethylated Lysine 27 of Histone H3 (H3K27me3), an epigenetic mark whose placement is catalyzed by Polycomb Repressive Complex 2 (PRC2). Binding of PRC1 to trimethylated target loci is thought to mediate transcriptional repression 11–13. A growth regulatory effect in wing discs was previously described for Psc-Su(z)2 and Polyhomeotic-distal and -proximal (Ph) but not other PcG members 8,14. To distinguish whether control of eye disc growth is a function only of Psc-Su(z)2 or instead a function of general PcG activity, we tested null or strong mutations in PRC1 members. Strikingly, eye discs mutant for PRC1 components *Polycomb (Pc)*, *polyhomeotic-distal and -proximal (ph)*, or *Sex combs extra (Sce)* all strongly overgrow (Fig.1u) and cause pupal lethality. *PRC1* mutant phenotypes are not fully identical: *Psc-Su(z)2* and *ph* show more severe epithelial organization and differentiation defects than *Pc* and *Sce* (Fig.1k–t; S3) and the former cause overgrowth of both eye and wing imaginal disc tissue whereas growth affects of the latter are seen predominantly in the eye (Fig.1u; S2). Additionally, survival of *Psc-Su(z)2* clones in mosaic tissue is impaired compared to other *PRC1* mutant clones (Fig.S2a–f). We also tested the PRC2 components *Enhancer of Zeste (E(z))* (Fig.S4g'–i') and *Suppressor of Zeste 12 (Su(z)12)* (data not shown) and found consistent but mild overgrowth in mutant discs, paralleling the relatively limited requirement of *E(z)* function in imaginal target gene repression 8. Nevertheless, from the common overgrowth mutant phenotype, we conclude that the canonical activity of PRC1 proteins, mediated by their cooperative function, is required to restrict imaginal disc growth.

The best-known PcG targets are Hox genes and other transcription factors, and the role of PcG in differentiation has been intensively studied 8,15–18. Several cell cycle regulators have also been identified as PcG targets 8,14,19,20, but a role for PcG in controlling cell proliferation is poorly understood. To identify growth-regulatory targets of PcG in Drosophila discs, we used a battery of signaling reporters to test whether known mitogenic

pathways are upregulated in *PRC1* mutant eye discs (Table 1). The results show that potent growth regulatory pathways involving Myc 21, Ras 22, and Dpp 23 are not consistently upregulated in *PRC1* mutant tissue (Table 1, Fig.2a–d). Spatial activation of Notch 24 and Hippo/Warts 25 pathways appears abnormal in *PRC1* mutant mosaic clones, but again our assays did not detect pathway hyperactivation within mutant cells of all genotypes (Fig.2e–h, S5a).

By contrast, JAK/STAT signaling, assessed by the 10XSTAT92E>GFP reporter26, is robustly hyperactivated in *PRC1* mutant tissue. 10XSTAT92E>GFP is expressed at very low levels in wild type L3 eye discs, but in similarly staged discs lacking PRC1 components, strong and consistent expression is seen (Fig.2i–l; S4a–e). Mild 10XSTAT92E>GFP upregulation can also be seen in *E(z)* mutant tissue, correlating with the mild degree of overgrowth (Fig.S4g–j). JAK/STAT pathway activation is not secondary to epithelial defects (Fig.S4e) and is not a consequence of generally disrupting epigenetic modifications or cell identity (Fig.S4f). Altogether, these results suggest that repression of JAK/STAT signaling is a key function of PcG activity in imaginal discs.

To determine how PcG normally restrains JAK/STAT activity, we considered components of the pathway whose derepression might enhance signaling. Because the pathway ligand Upd is rate-limiting for signaling activation, we assayed *upd* expression using quantitative real-time PCR. The data show that *upd* and its paralogs *upd2* and *upd3* are dramatically upregulated in *PcG* mutants. Specifically, *upd* transcription is at least more than 5-fold higher in *PRC1* mutant eye discs than in wild type (Fig.3a; S6a); it is also elevated in *E(z)* mutant tissue (Fig.S6a). In contrast, transcription of genes encoding other JAK/STAT pathway components including the receptor Domeless, the Janus kinase Hopscotch and the downstream transcription factor Stat92E are not strongly elevated in *PRC1* mutant tissue (Fig.3a; S6a). Notably, transcripts encoding rate-limiting components of other oncogenic growth pathways such as Notch/Delta, Myc, Akt, InR, Wingless or Dpp are not consistently nor strongly upregulated in all *PRC1* mutants (Fig.S5). These data indicate that, amongst many signaling components, *upd* is particularly sensitive to PcG regulation.

Is JAK/STAT signaling controlled by PcG in discs because *upd* is a *bona fide* target of PcG mediated repression? To investigate this hypothesis, we performed chromatin immunoprecipitation (ChIP) on wild type L3 imaginal discs using antibodies against H3K27me3 and Pc, the PRC1 component that binds to H3K27me3. ChIP-quantification by real-time PCR shows that the *upd* and neighboring *upd2* gene regions contain high (8–12 fold) enrichment of H3K27me3 and Pc binding as compared to a previously described non-target control region (Fig.3b; S6b) 27. Levels of H3K27me3 and Pc binding at *upd* loci are similar to those at a well-characterized direct PcG target gene, the Hox gene *Abdominal-B*. These results were confirmed by H3K27me3 ChIP-Seq analysis, which also revealed high levels of H3K27me3 across the *upd3* gene region (Fig.S6c). This suggests that *upd* genes are indeed direct targets of PcG-mediated repression in imaginal discs.

As an additional test for direct regulation of *upd* genes by PcG activity *in vivo*, we assayed transcriptional silencing of the *updLacZ* transposon, which inserts the *white* gene required for eye pigmentation into *upd* regulatory elements. Previous experiments have shown that

*white* in this insertion is silenced by mechanisms unrelated to PEV-sensitive heterochromatin modifications 28. To test whether the regulatory silencer is instead responsive to PcG activity, we looked for derepression of *updLacZ*-associated pigmentation in eyes heterozygous for PcG components. Loss of one copy of *Pc* or *Psc-Su(z)2* causes an increase in pigmentation in *updLacZ* flies, while PcG heterozygosity has no effect on pigmentation caused by an unrelated transposon insertion (Fig.3c,d; S6d,e). These data are consistent with the presence of a PcG-responsive silencer upstream of *upd*.

To assess the functional significance of PRC1-mediated regulation of Upd ligands in growth control, we asked whether the JAK/STAT pathway was involved in PcG mutant tumor formation. We first compared the effects of ectopic JAK/STAT pathway activation to loss of PcG function. Previous studies have demonstrated a strong growth promoting function for Upd in the eye disc 29–31. Similarly, overexpression of Upd, or of constitutively activated Hopscotch, in the wing disc causes a striking expansion of the epithelial field (Fig.4a,b; Fig.S7a,b). These data indicate that ectopic Upd expression is sufficient to drive overgrowth generally in imaginal discs.

To determine whether Upd-mediated signaling is required for *PRC1* mutant imaginal overproliferation, we tested whether reducing JAK/STAT activity in *Psc-Su(z)2*, *Pc* or *Sce* eye discs would suppress tumor growth. We first examined animals heterozygous for genes encoding JAK/STAT components, which causes no change in WT eye growth. Interestingly, heterozygosity for *Stat92E*, *upd*, or a deletion removing all three *upd* genes partially rescues the pupal lethality induced by the presence of *Psc-Su(z)2* eye tumors (Fig.4o) 5,32,33, and causes a mild but significant reduction in tumor size (Fig.4c,d,m). To more potently inhibit the JAK/STAT pathway, we co-expressed a dominant negative version of the receptor Domeless 34 or the endogenous STAT inhibitor SOCS36E 29,35 in *Pc* or *Sce* mutant eye disc cells (Fig.4e–l). Interference with Domeless function slightly decreases WT disc growth (Fig.4f) but dramatically and consistently reduces *Pc* and *Sce* tumor growth (Fig.4i,l). Striking suppression is also seen when SOCS36E is expressed: overgrowth is strongly perturbed in *Sce* discs, which approach the size of WT discs (Fig.4j,n), while the analogous manipulation has almost no effect on growth of WT discs (Fig.4g). These experiments confirm that interfering with JAK/STAT signaling can ameliorate overgrowth in *PRC1* mutants, and together reinforce the conclusion that hyperactivation of the JAK/STAT pathway via derepression of Upd ligands underlies overgrowth of PcG mutant discs.

Studies of PcG activity in cell proliferation have focused on a role in repressing transcription factors that drive differentiation, thereby maintaining a stem cell-like identity 2,36,37. In stem cells and several cancers, PcG activity promotes sustained cell division. Here we show that in the Drosophila imaginal disc, a favored model system for understanding organ growth, PcG activity is instead required to restrain proliferation. This finding, which provides a clear counterexample to the general paradigm that PcG activity maintains a proliferative state, should inspire renewed attention to contexts in which mammalian PcG proteins seem to act as negative, rather than positive, regulators of cell proliferation. For instance, recent studies describe an antiproliferative activity for PcG in transiently amplifying cells of mammalian hematopoetic progenitor pools 1,38–40. The proliferative potential, partially differentiated state, and developmental plasticity of transiently

amplifying populations are traits similar to those ascribed to early imaginal disc tissue in Drosophila 41, which lacks characterized stem cells. Distinct PcG activities in undifferentiated stem cells and partially differentiated proliferative populations could reconcile data regarding oncogenic and tumor-suppressive functions in different contexts.

In this study, we find that in Drosophila discs, PcG proteins directly regulate a mitogenic signaling pathway by repressing expression of the pathway ligand. Currently, the best-known targets of PcG are transcription factors involved in cell fate and differentiation. Recent studies have suggested that cell cycle regulators are PcG targets as well, but none of those identified are sufficient to drive excess tissue growth 14,19,20. It is intriguing that the growth regulatory pathway targeted by PcG repression is the JAK/STAT pathway, which is oncogenic in both mammalian 42 and Drosophila tissues 29,30, and regulates fly stem cell populations 43. Interestingly, genes encoding the mammalian JAK/STAT pathway ligands Interferon-γ and Interleukins 4 and 13 become H3K27 trimethylated and silenced in TH1 and TH2 helper cells, respectively, as they undergo maturation from naïve T-cells 44,45. This implies that regulation of JAK/STAT ligands by PcG's may be evolutionary conserved. Similarly, our data indicate that during imaginal disc development, the increasingly restricted pattern of Upd expression (Fig. S7c–f) 46 requires PcG silencing, which perhaps serves as an epigenetic `brake' on organ growth. JAK/STAT activity is required in early discs for full growth 31,46 and as discs enter a slower growth phase expression of *upd* decreases 47; whether PcG activity participates in the control of disc size by switching tissue `growth states' via silencing *upd* remains to be investigated.

Why would organ growth be negatively regulated by epigenetic mechanisms such as PcG activity? One reason is that epigenetic modifications can act as flexible but heritable switches for gene expression. The switches may be especially suited for proliferating cells as they rapidly turn over epigenetic marks during cell divisions, a provision lost upon terminal differentiation. A second reason is the ability of PcG to control broad gene networks to regulate developmental states in response to changing signaling environments. Indeed, during Drosophila disc regeneration, downregulation of PcG activity has been shown to promote cell fate plasticity 48–50; our results suggest that it may do so for proliferative potential as well.

We define here a new and distinct class of Drosophila TSGs that encode chromatin-modifying proteins of the PcG family. We further show a major role for one set of their targets - the *upd* genes - in the control of imaginal growth. However, the complex as well as differing phenotypes of PRC1 mutant discs suggest that other targets are also involved in PcG tumor suppressive activity during development. Regulators of signaling and patterning (such as Notch and Dpp pathways, see Table1, Fig.S5), the cell cycle and of epithelial polarity are likely to play additional roles. Future genome-wide analyses will reveal how PcG activity coordinates growth, architecture and differentiation during Drosophila organogenesis.

## Materials and Methods

### Genetics

The *P3C* allele was generated on a *FRT42D* chromosome by using EMS mutagenesis; *Psc-Su(z)2* discs in the text were created using this allele. The strong or null mutants used in this study are: Pc [XT109]; Su(z)2 [1b8]; Su(z)2 [1b7];; ph [505]; Sce[1]; E(z) [731]; Su(z)12 [4]; trx [E2]; Stat92E [85c9]; Upd [YM55]; os [1A]; scrib[1]. Mosaic imaginal discs were generated as described 52 using *eyflp* or *hsflp* to induce recombination. Discs consisting predominantly of mutant cells (referred to in the text as mutant discs) were generated using the *FLP/cell-lethal* system as described 6 utilizing *eyflp* for eye and *ubxflp* for wing discs. Other fly strains are: *unpairedLacZ (PD); E(spl)m*β-LacZ; exLacZ (ex[e1]); 10x STAT GFP; UAS Upd; UAS Hop[TumL]; upd GAL4, UAS GFP (E132); MS1096 GAL4; UAS Dome Δ*Cyt; UAS SOCS36E; act>CD2>GAL4, UAS GFP*. Wild type controls were outcrosses to *white* or isogenized *FRT42* and *FRT82* chromosomes. Crosses were reared at 22°C. Detailed genotypes are listed in Supplementary Table 1.

### Genetic interaction tests

Larvae were raised at 50 animals per vial from 4 hour-staged collections at 25°C. Tumors for size analysis were dissected 96 hours or 120 hours after hatching, stained with phalloidin and scored in double-blind tests. A Student T-test was used to calculate P-values. Adult escapers were counted at eclosion. Adult fly heads were imaged using a Z16 APO microscope (Leica) fitted with a DFC300 FX camera. *UpdLacZ* eye color modification was scored in double-blind tests on male flies 24 hours after eclosion.

### Immunohistochemistry

Imaginal disc tissues were fixed in 4% formaldehyde and stained under standard conditions with TRITC-phalloidin (SIGMA) and TOPRO-3 (Invitrogen) and primary antibodies against the following antigens: Notch (NECD), Elav, DEcad, Arm, Wg (all obtained from Developmental Studies Hybridoma Bank), β-Gal (Capell), Capicua (kindly provided I. Hariharan), Fibrillarin (MCA-38F3, EnCor Biotech.) and Phospho-SMAD (kindly provided by T. Tabata). Secondary antibodies were obtained from Invitrogen.

Mutant and wild-type discs were processed in the same tubes, and confocal settings were adjusted to maintain a linear intensity range for signals in different genotypes. Images are single confocal cross sections collected on a Leica TCS microscope. All scalebars are 100μm.

### Quantitative Real-time PCR

cDNA libraries of *FLP/cell-lethal* eye imaginal discs were generated using standard procedures. Real-time PCR was carried out using SYBR GreenER qPCR Supermix for ABI PRISM (Invitrogen) on a StepOnePlus ABI machine. The standard curve and ΔΔCt method was used and expression levels were normalized to at least two endogenous cDNA controls (CG12703 and GAPDH). Fold induction relative to WT expression levels are shown for one representative biological replicate. Primer sequences are listed in Supplemental Table 2. Detailed protocols are available on request.

### Chromatin Immunoprecipitation

ChIP was carried out as previously described 53 on imaginal tissue from 50 third-instar larvae for H3K27me3 ChIP and 200 third-instar larvae for Pc ChIP. Fixed and sheared chromatin was precipitated using an anti-Histone3 trimethylK27 mouse mAb (Lake Placid, # AM-0174) or Polycomb rabbit Ab (kindly provided by V. Pirrotta) and ProteinA-coupled Dynabeads (Invitrogen). Chromatin precipitated in Polycomb ChIP was preamplified using PCR as previously described 54. A negative control lacking Ab yielded less than 0.2% of specific pull-down observed with Ab, ChIP carried out with non-specific mouse IgG failed to enrich for sequences tested. Quantification was carried out using real-time PCR on a StepOnePlus ABI machine. Primer sequences and amplified regions are listed in Supplemental Table 2. Detailed protocols are available on request.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

We thank Paula Lueras for assistance with genetic screening, Crystal Marconett for help with genetic mapping, the Vance and Barton labs for assistance with quantitative PCR, and G. Cavalli and A.-M. Martinez for communication prior to publication. We further thank the Bloomington Stock Center and the Developmental Studies Hybridoma Bank, as well as J. Treisman, R. Emmons, I. Hariharan, J. Simon, J. Mueller, E. Bach, J. Parrish, R. Mann, G. Halder, N. Perrimon, D. Harrison and B. Mathey-Prevot for kindly providing flies and reagents. The authors are grateful to anonymous reviewers and members of the Bilder, Hariharan, Karpen, Speed and Biggin labs for their invaluable input and help. This work was supported by grants from the NIH (R01 GM068675) and The Burroughs Welcome Trust to D.B. A.K.C was supported by a fellowship from Jane Coffin Childs Memorial Foundation. K.F.H holds Career Development Awards from the International Human Frontier Science Program Organization and the National Health and Medical Research Council of Australia. T.V was supported by a fellowship from the American Heart Association.

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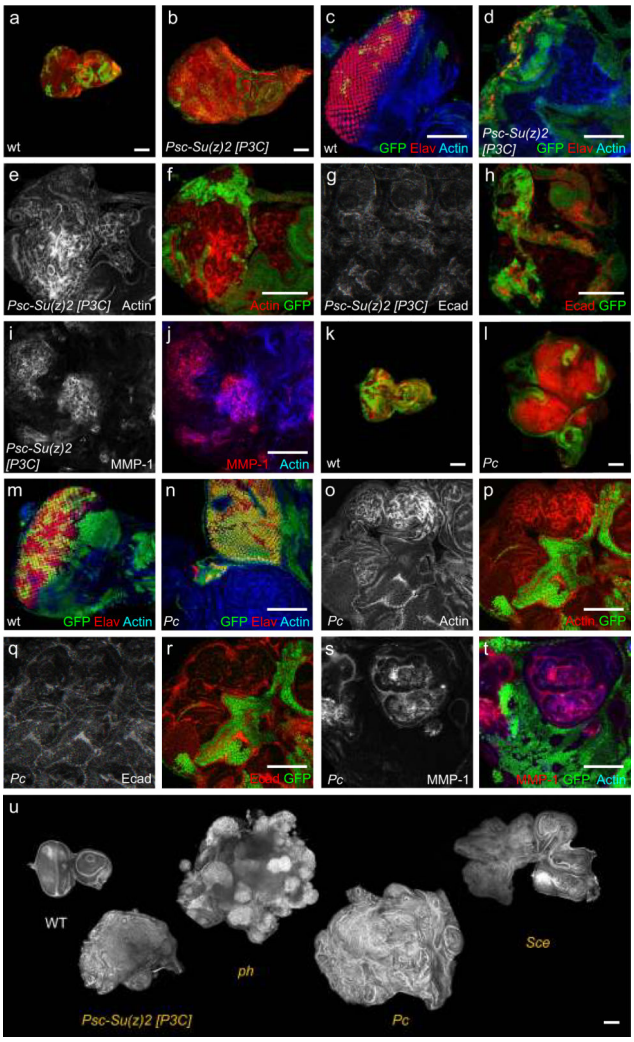


**Figure 1. PRC1 components are fly tumor suppressors**

**(a–j).** Phenotype of *Psc-Su(z)2* mutant eye imaginal discs generated with the *eyFlp/cell lethal* system. Heterozygous tissue is marked by expression of GFP (green). WT **(a)** and *Psc-Su(z)2* discs **(b)** stained for Actin to reveal size difference. **(c)** WT and **(d)** *Psc-Su(z)2* discs stained for Elav (red) and Actin (blue) showing impaired differentiation. *Psc-Su(z)2* mutant eye imaginal discs stained for Actin (**e**, red in **f**) and E-cadherin (**g**, red in **h**) revealing defective epithelial organization. *Psc-Su(z)2* mutant eye imaginal discs stained for MMP1 (**i,** red in **j**) and Actin (blue in **j**). **(k–t)** Phenotype of *Pc* mutant mosaic eye imaginal discs generated with *eyFLP*. WT or heterozygous tissue is marked by expression of GFP (green). **(k)** Wild type and **(l)** *Pc* mosaic discs stained for Actin to reveal size difference. **(m)** Wild type and **(n)** *Pc* mosaic discs stained for Elav (red) and Actin (blue) showing impaired differentiation. *Pc* mosaic discs stained for Actin (**o**, red in **p**) and E-cadherin (**q**, red in **r**) revealing defective epithelial organization. *Pc* mosaic discs stained for MMP-1 (**s**, red in **t**) and Actin (blue in **t**). **(u)** Eye discs mutant for core members of PRC1 (*Psc-Su(z)2, ph, Pc and Sce*) stained for Actin; strong overgrowth is seen in all genotypes. See Supplementary Table 1 for detailed genotypes.

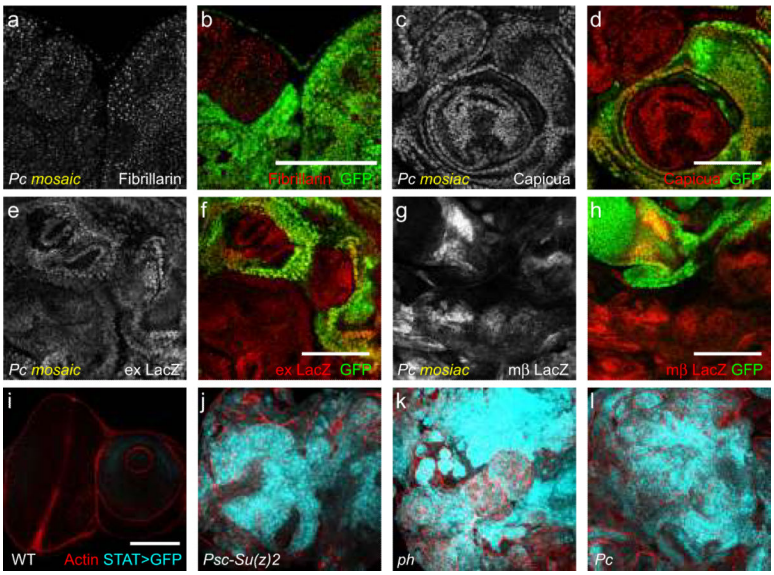


**Figure 2. JAK/STAT signaling is ectopically activated in *PRC1* mutant tissue**

**(a–h)** Immunofluorescent stains for reporters in *Pc* eye mosaic clones; WT cells are marked by GFP (green in b,d,f,h). Fibrillarin (**a**, red in **b**), Capicua (**c**, red in **d**). *expanded-LacZ* (**e**, red in **f**) and *m*β*-LacZ* (**g**, red in **h**) are not consistently upregulated in mutant cells. **(i–l)** Expression of a JAK/STAT reporter (cyan) in WT or PRC1 mutant eye imaginal discs; actin staining is red. Compared to WT (**i**), the reporter is strongly and consistently elevated in *Psc-Su(z)2* (**j**), *ph* (**k**) and *Pc* (**l**) mutant discs.

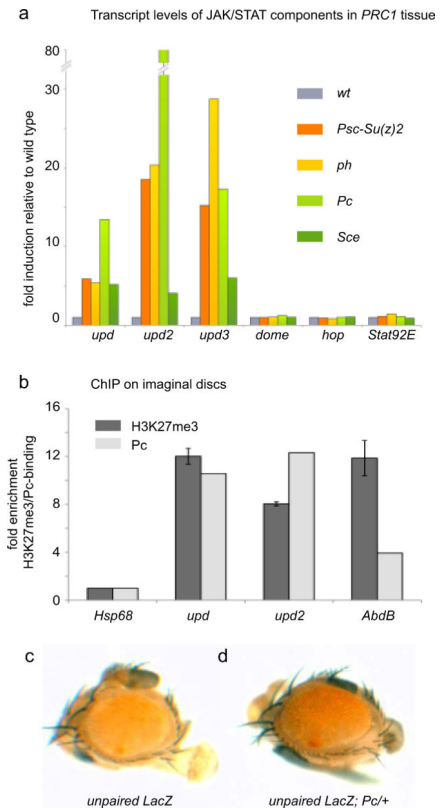


**Figure 3. *Unpaired* is a direct target of PcG-mediated silencing in imaginal discs**

**(a)** Real-time PCR analysis measuring transcription of JAK/STAT pathway components in *Psc-Su(z)2* (orange), *ph* (yellow), *Pc* (light green) and *Sce* (dark green) mutant eye discs. Ligand-encoding *upd* transcripts are upregulated while other pathway components are relatively unchanged. **(b)** ChIP quantifying H3K27me3 enrichment (SD, n=3) and Pc-binding at *upd*, *upd2* and *AbdB* relative to the unmethylated control *hsp68* in WT imaginal discs. **(c)** Eye color induced by a *mini-white* transgene inserted in the *upd* regulatory region. **(d)** Darker eye color indicates reduction of silencing of the *mini-white* gene in *Pc* heterozygous males.

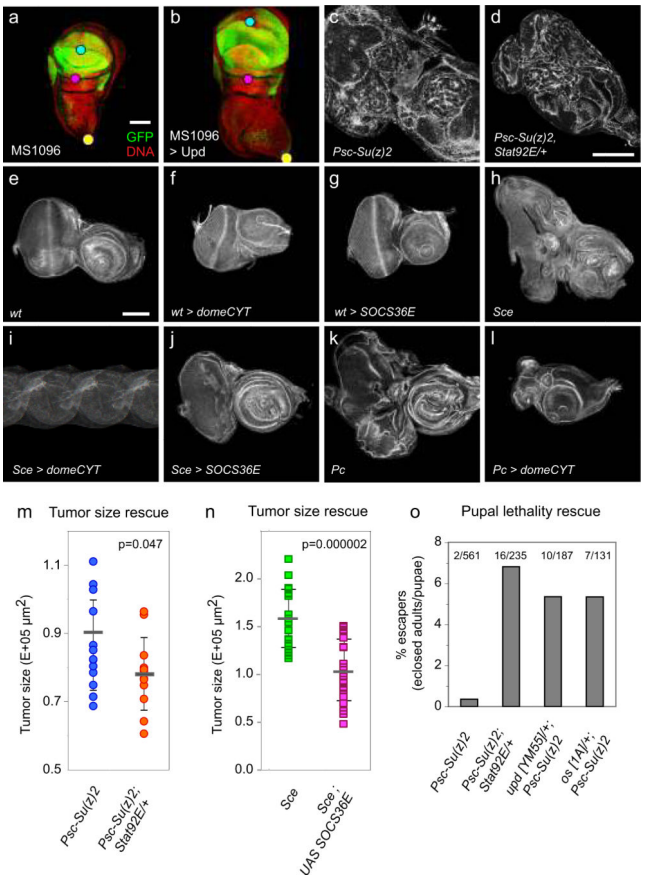


**Figure 4. JAK/STAT signaling drives *PRC1* mutant overgrowth**

**(a,b)** Wing imaginal discs expressing GFP (green) under the control of *MS1096GAL4* stained for DNA (red). Characteristic landmarks of wing discs are indicated by colored dots: cyan – dorsal-ventral boundary; magenta – second fold; yellow – peripodial stalk. Compared to discs expressing GFP alone (**a**), discs coexpressing Unpaired (**b**) show expansion of the disc epithelium along with increased epithelial folding. **(c–l)** Phenotype of PRC1 mutant eye imaginal discs with reduced JAK/STAT activity, stained for Actin. Compared to *Psc-Su(z)2* mutant eye discs (**c**), *Psc-Su(z)2* mutant discs heterozygous for *Stat92E* (**d**) show reduced size. Control imaginal discs expressing no transgene (**e**), DomeΔCyt (**f**) or SOCS36E (**g**) under the control of *eyFLP FLPout-GAL4*. Expression in *Sce* (**h**) or *Pc* (**k**) mutant discs of DomeΔCyt (**i, l**) or SOCS36E (**j**) reduces overgrowth of mutant eyes. **(m–n)** Quantitation of disc size: individual discs (circles/squares), average (grey bar) and SD are shown with P values (Student's T-test). (**m**) *Psc-Su(z)2* disc size (0.87 +/− 0.13 $\times 10^5$ µm$^2$, n=12) is reduced when heterozygous for *Stat92E* (0.78 +/− 0.12 $\times 10^5$ µm$^2$, n=12). **(n)** *Sce* disc size (1.59 +/− 0.32 $\times 10^5$ µm$^2$, n=20) is reduced when discs express SOCS36E (1.05 +/− 0.33 $\times 10^5$ µm$^2$, n=22). **(o)** Rate of eclosion in animals carrying *Psc-Su(z)2* mutant eye discs alone, heterozygous for *Stat92E*, *upd*, or the *os[1A]* deficiency deleting *upd*, *upd2*, and *upd3*. > 15-fold increase in eclosion rate is seen.

**Table 1**

**Growth pathway signaling in *PRC1* mutant imaginal discs**

Summary of signaling reporter assays for mitogenic pathways tested in *PRC1* mutant eye discs. Immunofluorescence microscopy assays on *eyFLP* clones and quantitative realtime PCR assays of downstream signaling targets in *eyFLP/cell-lethal* discs were used to analyze growth pathway signaling. Note that *eyFLP/cell-lethal* eye imaginal disc contain a proportion of wild type tissue.

| **Pathway** | **JAK/STAT** | **Notch** | **Hippo/Warts** | **Myc** | **EGF/ras** | **Dpp** |
|---|---|---|---|---|---|---|
| **Reporter Assay** | **STAT-{GFP}** | ***mβ {LacZ}*[1]*/mβ,eyg* QPCR[2]** | ***ex {LacZ}*[1]*/mer,ex,Diap* QPCR[2]** | **Fibrillarin[3]** | **Capicua** | **P-SMAD** |
| *Psc-Su(z)2* | ectopic activation | variably elevated[1]/not elevated[2] | nd[1]/not elevated[2] | some ectopic activation | some ectopic activation | nd |
| *ph* | ectopic activation | nd[1]/not elevated[2] | nd[1]/not elevated[2] | unchanged | some ectopic activation | some ectopic activation |
| *Pc* | ectopic activation | altered pattern[1]/not elevated[2] | altered pattern[1]/not elevated[2] | unchanged | unchanged | nd |
| *Sce* | ectopic activation | altered pattern[1]/not elevated[2] | nd[1]/not elevated[2] | unchanged | unchanged | unchanged |
| | Ectopic activation | Abnormal spatial pattern | Abnormal spatial pattern | No consistent upregulation | No consistent upregulation | No consistent upregulation |

**nd -** not determined.

[1] Assayed by LacZ reporter immunofluorescence in tissue.

[2] Assayed by QPCR of downstream pathway targets (see Fig.S5A).

[3] Assay determines nuclear/nucleolar size ratio.