Roles of C/EBP class bZip proteins in the growth and cell competition of Rp (‘Minute’) mutants in Drosophila

Jorge Blanco1†, Jacob C Cooper2, Nicholas E Baker1*

1Department of Genetics, Albert Einstein College of Medicine, New York, United States; 2School of Biological Sciences, University of Utah, Salt Lake City, United States

Abstract Reduced copy number of ribosomal protein (Rp) genes adversely affects both flies and mammals. Xrp1 encodes a reportedly Drosophila-specific AT-hook, bZIP protein responsible for many of the effects including the elimination of Rp mutant cells by competition with wild type cells. Irbp18, an evolutionarily conserved bZIP gene, heterodimerizes with Xrp1 and with another bZip protein, dATF4. We show that Irbp18 is required for the effects of Xrp1, whereas dATF4 does not share the same phenotype, indicating that Xrp1/Irbp18 is the complex active in Rp mutant cells, independently of other complexes that share Irbp18. Xrp1 and Irbp18 transcripts and proteins are upregulated in Rp mutant cells by auto-regulatory expression that depends on the Xrp1 DNA binding domains and is necessary for cell competition. We show that Xrp1 is conserved beyond Drosophila, although under positive selection for rapid evolution, and that at least one human bZip protein can similarly affect Drosophila development.

Introduction

Organ structure and function can be affected by the presence of abnormal cells and there is increasing evidence that they can be removed by both cell intrinsic and cell-nonautonomous mechanisms. A cell intrinsic mechanism is exemplified by P53, which activates a transcriptional program that leads either to cell cycle arrest or to the elimination of the damaged cells through apoptosis (Kastenhuber and Lowe, 2017). Abnormal cells can also be removed by cell interactions in the process of cell competition, which recognizes and eliminate cells that are different from their neighbors. The classic example of cell competition is that of the Minute (M) mutations in Drosophila, which are loss of function mutations in ribosomal protein (Rp) genes (Marygold et al., 2007). Heterozygous Rp+/− animals are viable, although they generally display a slower cell proliferation rate and developmental delay (Bridges and Morgan, 1923; Morata and Ripoll, 1975) but Rp+/− cells undergo apoptosis when surrounded by wild-type Rp+/+ cells (Morata and Ripoll, 1975; Simpson and Morata, 1981; Moreno et al., 2002; Li and Baker, 2007). Such non-autonomous cell competition also affects a number of other genotypes of cells in both Drosophila and in mammals (Amoyel and Bach, 2014; Clavería and Torres, 2016; Di Gregorio et al., 2016; Merino et al., 2016; Baker, 2017; Maruyama and Fujita, 2017; Nagata and Igaki, 2018). Interestingly, P53 is important for some examples of cell competition in mammals, but dispensable for the elimination of Rp+/− cells in Drosophila (Baker et al., 2019). Although the potential roles of cell competition in development and in disease such as cancer are of considerable interest, little is yet known about molecular mechanisms of cell competition.

We, and others, identified Xrp1 as a key factor in the cell competition of Rp+/− cells (Lee et al., 2016; Baillon et al., 2018; Lee et al., 2018). Xrp1 loss-of-function mutations allow Rp+/− cells to survive when surrounded by wild-type (Rp+/+) cells, preventing their elimination by cell
competition (Balloon et al., 2018; Lee et al., 2018). Remarkably, Xrp1 is also required for the slow growth, reduced translation, and most of the gene expression changes shown by Rp\(^{+/+}\) cells, showing that Xrp1 is a central mediator of these effects of Rp gene mutations, none of which seems to depend simply on a reduced number of ribosomes (Lee et al., 2018). Xrp1 encodes a Basic region Leucine-Zipper (bZIP) protein that also has an AT-hook domain, and was known earlier as a p53-target that is also implicated in P element transposition (Brodsky et al., 2004; Akdemir et al., 2007; Francis et al., 2016). Recently it has also been implicated in coordination of organ growth following local growth retardation (Boulan et al., 2019). bZIP proteins typically bind DNA as homo- or hetero- dimers and many are evolutionarily conserved (Amoutzias et al., 2007; Reinke et al., 2013). Dimerization of Drosophila bZIP proteins has been analyzed in silico and in vitro (Fassler et al., 2002; Reinke et al., 2013). The bZIP protein encoded by the CG6272 gene was the only heterodimer partner of Xrp1 identified by in vitro FRET assays (Reinke et al., 2013). This heterodimer is also the sequence-specific DNA-binding component of a multiprotein complex that binds to the P-element Terminal Inverted Repeats leading to the naming of CG6272 as Inverted Repeat Binding Protein 18 (IRBP18) (Francis et al., 2016). Unusually, Xrp1 has been described as specific to the genus Drosophila (Akdemir et al., 2007), whereas irbp18 is well-conserved and belongs to the CAAT/Enhancer Binding Protein (C/EBP) superfamily of transcription factors, being most similar to human C/EBP\(\beta\) (Ramji and Foka, 2002; Francis et al., 2016). IRBP18 can also heterodimerize with a second bZIP protein, dATF4 (Reinke et al., 2013). dATF4, encoded by the cryptocephal (crc) gene, controls molting and metamorphosis as a cofactor for the ecdysone receptor (Gauthier et al., 2012). Ecdysone is an important signal for imaginal disc growth (Delanoue et al., 2010; Jaszczak and Halme, 2016). In addition, dATF4 is neuroprotective in a model of Parkinsons Disease (Celardo et al., 2017), and regulates 4E-BP transcription downstream of GCN2 during nutrient stress (Kang et al., 2017; Malzer et al., 2018). dATF4 can heterodimerize with a further C/EBP protein, encoded by slow border cells (slbo) (Reinke et al., 2013). Thus, Xrp1 is potentially connected to a network of more conserved C/EBP-class bZIP proteins, through IRBP18 (Figure 1A).

In the present study, we have analyzed the role of other bZip proteins connected to Xrp1 in Rp\(^{+/+}\) mutant cell competition. Out of the many possibilities, we were particularly interested in whether Xrp1 acts positively in cell competition as a heterodimer with IRBP18, potentially a Drosophila-specific complex, or behaves as a competitive inhibitor of the conserved stress response protein dATF4/Crc by sequestering IRBP18 away from it. Our results show that Xrp1 and irbp18 are both necessary for the slow-growth and cell competition of Rp\(^{+/+}\) cells, apparently independently of other IRBP18 partners, consistent with the first hypothesis. Additionally, we find that the upregulation of both Xrp1 and irbp18 in Rp\(^{+/+}\) cells depends on one another's function, and that the DNA binding and dimerization domains are important for the function and regulation of these genes, further evidence of an auto-regulatory loop based on the Xrp1/IRBP18 heterodimer and necessary for cell competition. Finally, we identify rapidly-evolving Xrp1 homologs beyond Drosophila and suggest that divergent mammalian proteins might resemble Xrp1 functionally.

**Results**

**Irbp18 is required for cell competition**

Cell competition eliminates Rp\(^{+/+}\) cells from the developing wing mosaic in an Xrp1-dependent manner (Lee et al., 2016; Balloon et al., 2018; Lee et al., 2018). Two distinct assays were used here to analyze the requirement for irbp18 in cell competition, making use of the irbp18\(^{G500C}\) mutation, a PiggyBac insertion within the irbp18 open reading frame that eliminates IRBP18 protein production (Francis et al., 2016). In the first assay, mitotic recombination in wild-type (RpL36\(^{+/+}\)) progenitor cells generates clones of RpL36\(^{+/+}\) cells, which are normally eliminated by cell competition (Figure 1B) (Tyler et al., 2007). By contrast, in the irbp18\(^{G500C}\) background the RpL36\(^{+/+}\) clones were always recovered, indicating a failure of cell competition when irbp18 was mutated (Figure 1C). In the second assay, mitotic recombination in RpS18\(^{+/+}\) progenitor cells generated wild-type clones (RpS18\(^{+/+}\)) that progressively eliminated their slower-growing neighbors and eventually occupied almost the entire wing imaginal disc (86% on average, Figure 1D, F). By contrast, mutating irbp18 prevented the RpS18\(^{+/+}\) cells from taking over the RpS18\(^{+/+}\) wing imaginal disc, where they occupied a much smaller fraction (25%, on average) (Figure 1E, F). Remarkably, RpS18\(^{+/+}\) cells,
Figure 1. Xrp1 and related bZip proteins in cell competition. (A) Known dimers of Drosophila C/EBP -class bZip proteins and their potential functions. (B,C) Mitotic recombination in RpL36+/+ wing discs (grey) generates clones of RpL36+/++ cells (light grey) and reciprocal clones of RpL36+/− cells (black, lacking beta-Gal labeling). RpL36+/− clones that did not survive in the irbp18+/− background (B) always survived in the irbp18+/− background (C). (D,E) Mitotic recombination in RpS18+/− wing discs (green) generates clones of RpS18+/− cells (black, lacking GFP expression). In the irbp18+/− background (D) these have a growth and competitive advantage and come to dominate wing disc territory, eliminating remaining RpS18+/− cells by cell death (anti-active caspase DCP1 labeling in magenta). RpS18+/− cells had less advantage in the irbp18+− background (E). In addition, reciprocally recombinant RpS18+/− cells survived as small clones (bright green, eg arrows in E). (F) Quantitative comparison of RpS18+/− clone growth in irbp18+/− and irbp18+/− backgrounds. Wing pouch areas were the same in irbp18+/− and irbp18+/− backgrounds (p=0.191, two-tailed t-test), but the RpS18+/− fractions were not (p<0.0001, two-tailed t-test). Data derived from measurements of 4 irbp18+/− discs and five irbp18+/− discs. (G,H) Mitotic recombination of the irbp18 locus in the RpS18+/− and RpS18+/− backgrounds. Reciprocal clones of RpS18+/− irbp18+/− and RpS18+/− irbp18+/− cells grew comparably (G) whereas clones of RpS18+/− irbp18+/− cells expanded at the expense of RpS18+/− irbp18+/− cells and RpS18+/− irbp18+/− cells (H). (I) Quantification of growth in RpS18+/− and RpS18+/− wing discs (eg panels G,H). irbp18+/− clone area was compared to reciprocal irbp18+/− controls. Probability that clone areas are the same in the RpS18+/− and RpS18+/− backgrounds = 1.4 x 10−9 (2-tailed t-test with unequal variances). Data derived from measurements of 7 RpS18+/− discs and 9 RpS18+/− discs. (J) irbp18+/− clones (pigmented) contributing to the adult eye. (K) irbp18+/− clones (pigmented) occupy nearly all the RpS18+/− eye. Genotypes B) hsFlp/M(1)Bld, P[RpL36+/+; irbp18+/−] arm-LacZ FRT80B/FRT80B; C) hsFlp/M(1)Bld, P[RpL36+/+; irbp18+/−] arm-LacZ FRT80B/irbp1805006 FRT80B. D) y w hs-FLP; FRT42D ubi-GFP M(1)C56f/FRT42D. E) y w hs-FLP; FRT42D ubi-GFP M(1)C56f/FRT42D; irbp1805006/irbp1805006. G) y w hs-FLP, +/+; irbp1805006 FRT80B/arm LacZ FRT80B. H) y w hs-FLP; FRT42D ubi-GFP M(1)56f+/+; irbp1805006 FRT80B/arm LacZ FRT80B. J) y w ey-FLP/Y; FRT42D ubi-GFP/+; irbp1805006 FRT80B/FRT80B K) y w ey-FLP/Y; FRT42D ubi-GFP/+; irbp1805006 FRT80B/FRT80B. Note that the irbp1805006 allele includes a w+ element that is responsible for most of the eye pigmentation.

The online version of this article includes the following source data for figure 1:

Source data 1. Measurements of clone and wing pouch sizes corresponding to Figure 1F.
Source data 2. Clone size data corresponding to Figure 1I.

generated as reciprocal recombinants in the RpS18+/− wing discs, survived as small clones in the absence of irbp18, whereas they normally disappear very quickly in irbp18+/− backgrounds (Figure 1E). It is already known that Xrp1 mutations similarly protect RpS18+/− clones (Lee et al., 2018). These similar effects of irbp18 and Xrp1 mutations are expected if it is the Irbp18/Xrp1 heterodimer that promotes both the competitive elimination of Rp+/− cells and also the prompt apoptosis of Rp−/− cells.

To confirm that Irbp18 acts in Rp+/− cells like Xrp1 does, irbp1805006 homozygous clones were induced in both wild-type and Rp+/− genetic backgrounds. In the wild-type, Rp+/− background,
irbp18^{f05006} homozygous clones were the same size as their irbp18^{+/+} controls (Figure 1G,I). By contrast, RpS18^{+/+}/irbp18^{+/−} clones were significantly larger compared to their RpS18^{+/+}/irbp18^{+/+} controls, indicating that Irbp18 acts in RpS18^{+/−} cells to slow their growth, and may also make them less competitive (Figure 1H,I). RpS18^{+/+}/irbp18^{+/−} clones were disadvantaged in eye development as well as in the wing disc (Figure 1J,K). These results indicate that whereas Irbp18 had no effect on growth of cells in wild-type wing or eye discs, it reduced the growth and competitiveness of Rp^{+/−} cells, like Xrp1 does.

### Role of Crc/dATF4 in Minute cells

Irbp18 can also heterodimerize with Crc/dATF4, a protein that plays several roles in growth (Gauthier et al., 2012; Kang et al., 2017; Malzer et al., 2018) (Figure 1A). We did not succeed in recombinating crc/dATF4 mutations with FRT42D, to which it is very closely linked, and so used the TIE-DYE system for an RNAi approach rather than mitotic recombination of crc mutations. In TIE-DYE, FLP-mediated excision of transcriptional stop sequences independently enables transcription of GFP, b-galactosidase, and Gal4 proteins, which thereafter mark the independent lineages descended from the recombined cells (Worley et al., 2013) (Figure 2A). We used TIE-DYE in an RpS18^{+/−} background to express dsRNA specific for Crc/dATF4, Xrp1, or w along with UAS-His2A::mRFP in recombinant cell clones, and compared their contributions to control clones in the same wing discs. Xrp1 knockdown cell-autonomously enhanced growth of RpS18^{+/−} cells (Figure 2B, E), consistent with previous observations of Xrp1 mutant alleles (Lee et al., 2018), but Crc/dATF4 knockdown had the opposite effect, severely restricting the growth of RpS18^{+/−} cells (Figure 2C,E). The small remaining RpS18^{+/−} dsRNA_{Crc/dATF4} clones were often fragmented, suggesting they were in the process of apoptosis and/or extrusion from the wing disc (Figure 2D). Knock down of neither Xrp1 nor dATF4 significantly affected clone size in the wild type (RpS18^{+/+}) background (Figure 2F), although interestingly some RpS18^{+/+} dsRNA_{Crc/dATF4} clones showed signs of fragmentation (Figure 2G,H). These findings indicated that Crc/dATF4 had the opposite effects on growth of RpS18^{+/−} cells than did Xrp1 and Irbp18.

### Irbp18 is upregulated in Rp^{+/−} cells

Since irbp18 was required in Rp^{+/−} cells for their cell competition, and Xrp1 expression is higher in Rp^{+/−} cells, we compared Irbp18 expression in wild-type and Rp^{+/−} cells. Indeed, like Xrp1 (Figure 3A,B), Irbp18 protein was elevated in RpS18^{+/−} cells in mosaic wing discs (Figure 3C,D). We also found that Irbp18 upregulation in Rp^{+/−} cells was Xrp1-dependent, since clones of RpS18^{+/−}/Xrp1^{−/−} cells had lower Irbp18 levels (Figure 3E,F). Interestingly, clones of RpS18^{+/−}/irbp^{−/−} cells similarly failed to upregulate Xrp1 protein (Figure 3G,H). Consistent with these protein findings, mRNA-Seq data (Lee et al., 2018) show irbp18 transcripts elevated more than three-fold in non-mosaic RpS3^{+/−} and RpS17^{+/−} wing discs, in an Xrp1-dependent manner, much as was also seen for Xrp1 mRNA itself (Figure 3I,J). This cross-regulation between Xrp1 and irbp18 is simply explained if the Xrp1/Irbp18 heterodimer stimulates transcription of Xrp1 and/or irbp18. Consistent with this, transcription of a LacZ enhancer trap construct inserted onto the Xrp1 locus was detected upon ectopic expression of Xrp1 (Figure 3K,L). Transcriptional cross-regulation does not rule out additional effects of Xrp1 and Irbp18 proteins on one another’s stability, which would also affect protein levels.

### Xrp1 DNA-binding domains necessary for cell competition and auto-regulation

To assess the contribution of the Xrp1 DNA binding domains in loss-of-function studies, we modified the endogenous Xrp1 locus to generate new HA-tagged alleles lacking individual domains. This was achieved using homologous recombination to first generate an insertion of ptvcherry into the last intron of the Xrp1 locus (Figure 4A). The insertion contains an attP sequence and a mini-white gene flanked by LoxP sites. This insertion behaves as a loss of function allele of Xrp1 (Xrp1^{attP flox}, Figure 4-figure supplement 1A-D). Cre-mediated excision of the mini-white gene and other sequences leaves only the attP site and a single LoxP; this allele (Xrp1^{attP loxP}) behaves as a wild type allele of Xrp1 (Figure 4A, Figure 4-figure supplement 1E,F). Subsequent PhiC31-mediated insertion into the attP site of Xrp1^{attP loxP} inserted recombinant exons, leading to synthesis of recombinant Xrp1 proteins encoded at the endogenous locus. An HA-tagged final exon with otherwise wild type
dATF4/crc requirement in wild type and RpS18−/− wing discs. (A) In the TIE-DYE method, independent recombination events generate parallel clones expressing GFP (green), Gal4 (detected through UAS-RFP expression in red), and β-Gal (blue). These parallel clones grow equivalently in RpS18−/− wing discs. In this control, dsRNA targeting transcripts from the white gene was co-expressed with RFP under Gal4 control. (B) Co-expression of dsRNA targeting Xrp1 increased the contribution of RFP-positive clones in RpS18−/− wing discs. (C) Co-expression of dsRNA targeting dATF4/crc decreased the contribution of RFP-positive clones in RpS18−/− wing discs. (D) Enlarged portion of the wing disc from panel C to show the fragmentation of RFP-positive, dATF4/crc knock-down RpS18−/− cells (eg arrows). (E) Quantification of growth for various dsRNA-expressing RFP-positive clones in RpS18−/− wing discs. Shown is the mean ratio of RFP-positive to GFP-positive areas for each wing disc. Error bars represent ± 1 SEM. Probabilities that clone sizes are the same as for the w control (2-tailed t-tests): Xrp1 - 3.4 × 10^{-5}; 2934–3.1 × 10^{-12}; 2935–2.2 × 10^{-11}. VDRC2934 and VDRC2935 encode Blanco et al. eLife 2020;9:e50535. DOI: https://doi.org/10.7554/eLife.50535

Figure 2 continued on next page
sequence was inserted using this strategy (Figure 4A). This Xrp1HA allele behaved as a wt allele during competition targets. That is, Rps18+/− cells were normally outcompeted by wild type cells in the homozygous Xrp1HA background, occupying only the minority of the wing disc and exhibiting competitive cell death at boundaries with Rps18+/+ cells (Figure 4B). As is seen for the endogenous protein, expression of the Xrp1HA protein was elevated in Rps18+/− cells and could be detected with either anti-Xrp1 antibody or with anti-HA (Figure 4C,D). In contrast to Xrp1HA alleles encoding Xrp1 proteins lacking either the AT hook or the basic sequence (Xrp1AT-HA and Xrp1BS-HA, respectively) behaved as loss-of-function alleles, allowing Rps18+/− cells to occupy most of mosaic discs without experiencing competitive cell death (Figure 4E,H). Significantly, both Xrp1AT-HA and Xrp1BS-HA also prolonged the survival of homozygous Rps18+/− clones, which is a feature of Xrp1+/− genotypes (Figure 4E,H). Both Xrp1AT-HA and Xrp1BS-HA also impaired the upregulation of Xrp1 in Rps18+/− cells, although Xrp1 protein levels were still elevated in Rps18+/− clones (Figure 4F,G,I,J). These results indicate that DNA-binding domains are essential components of Xrp1 function and auto-regulation in Rps18+/− cells. Xrp1 appears to be elevated in Rps18+/− clones by a mechanism independent of auto-regulation, although Xrp1 function to eliminate Rps18+/− cells still depends on the DNA-binding domains.

Irbp18 is required for ectopic Xrp1 effects in wings and eyes

The requirement for DNA binding domains is consistent with transcriptional regulation of cell competition targets. It might have been possible, however, that the Xrp1 and Irbp18 genes themselves were the only significant transcriptional targets, and that Xrp1 and Irbp18 caused cell competition through non-transcriptional mechanisms after reaching sufficient expression levels. To explore this possibility, we examined ectopic Xrp1 phenotypes in which Xrp1 transcription was driven independently of its own regulatory sequences. First we tested whether effects of ectopic Xrp1 were also dependent on Irbp18, as expected if Xrp1 functions as a heterodimer with Irbp18 when ectopically expressed.

Xrp1 over-expression has a strong effect on cell survival in the normal wing imaginal disc, causing the elimination of Xrp1-expressing cell clones within 72 hr (Figure 5A–B) (Tsursui-Nishimura et al., 2013). By contrast, MARCM clones overexpressing Xrp1 and also homozygous mutant for Irbp18 (CG627205056) survived, proliferated and were present in the wing epithelium 72 hr after clone induction (Figure 5C,D). Therefore the negative effect of Xrp1 on growth and survival of wing disc clones depended on Irbp18. In contrast to Xrp1, ectopic expression of Irbp18 had no effect on clone survival (Figure 5E,F).

During eye development, ectopic expression of Xrp1 using the GMR-gal4 driver at 18˚C, leads to a small, glossy eye (Figure 6A). Like the effect on Xrp1 in the wing, the eye phenotype of ectopic Xrp1 was almost completely suppressed in the absence of irbp18, so that irbp18+/− GMR >Xrp1 eyes were close to wild type in size (Figure 6B). This indicates that Irbp18 is required for ectopic Xrp1 activity in the eye also.

Results were slightly different at a higher temperature. At 25˚C, GMR > Xrp1 reduces the eye to only a small remnant (Figure 6C) (Tsursui-Nishimura et al., 2013). Although eye size was rescued
considerably in the \textit{irbp18} mutant background, the eyes were not normal, instead having a glossy appearance (Figure 6D). This phenotype was definitely due to ectopic Xrp1, because both eye size and appearance were rescued by co-expressing dsRNA for Xrp1 along with ectopic Xrp1 (dsRNA for Xrp1 had no effect on eye development by itself) (Figure 6E). The restoration of eye size definitely reflected a requirement for Irbp18, because a less complete rescue was seen when co-expressing dsRNA for Irbp18 (Figure 6F), and because restoring Irbp18 expression to the \textit{irbp18} mutant restored the full GMR > Xrp1 size reduction (Figure 6G). Therefore, Xrp1 function at higher temperature remains substantially dependent on Irbp18 but also exhibited an Irbp18-independent component. Ectopic expression of Irbp18 had no effect on the eye (Figure 6H), although we confirmed by in situ hybridization that GMR-gal4 drove strong expression in the eye imaginal disc (Figure 6I).
Figure 4. Modifications of the endogenous Xrp1 locus and their consequences for cell competition. (A) Sequential modifications of the Xrp1 locus in the 18925–18926 kb region of chromosome arm 3R by homologous recombination, Cre recombination and PhiC31 integration introduced modified and HA-tagged exon seven sequences into intron 6. (B–D) Cell competition in the homozygous Xrp1 HA background. RpS18+/+ Xrp1 HA/HA clones (unlabeled in B) grow to occupy most of the wing disc at the expense of RpS18+/− Xrp1 HA/HA cells (green in B), similar to what is seen in the Xrp1+/− background (see Figure 3A and C). (C) Xrp1 protein is elevated in RpS18+/− Xrp1 HA/HA cells, as was seen for RpS18+/− Xrp1+/* cells (see Figure 3B). (D) HA-tagged protein is detected only in the RpS18+/− Xrp1 HA/HA cells. (E–G) Cell competition in the homozygous Xrp1ΔAT-HA background. RpS18+/+ Xrp1ΔAT-HA/ΔAT-HA clones (unlabeled in E) occupy less of the wing disc than RpS18+/− Xrp1ΔAT-HA/ΔAT-HA cells (green in E), similar to what is seen in the Xrp1+/− or irbp18−/− backgrounds (see Figure 1E). Note the survival of small clones of RpS18+/−Xrp1ΔAT-HA/ΔAT-HA (brighter green in E, eg arrows). Survival of Rp−/− genotypes is a feature of Xrp1

Figure 4 continued on next page

Blanco et al. eLife 2020;9:e50535. DOI: https://doi.org/10.7554/eLife.50535
Figure 4 continued

The eye imaginal disc from GMR > Xrp1 exhibited apoptosis soon after Xrp1 was expressed (Figure 6J,K). This apoptosis was dependent on irbp18 (Figure 6L). This eye disc region overlaps the second mitotic wave of dividing cells, and cell divisions are reduced in GMR > Xrp1 eye imaginal discs (Figure 6—figure supplement 1A-D) (Tsurui-Nishimura et al., 2013). Co-overexpression of either anti-apoptotic proteins P35 or diap1 (Figure 6M,N), or depletion of caspases and pro-apoptotic proteins only rescued the GMR > Xrp1 eye phenotype modestly (Figure 6—figure supplement 1E-J). Whereas over-expression of UAS-Rheb enlarged wild type eyes (Figure 6O) it had no effect on GMR-Xrp1 eyes (Figure 6P). By contrast over-expression of UAS-yki both enlarged wild type eyes (Figure 6Q) and partially rescued the GMR > Xrp1 eye phenotype (Figure 6R). Co-expression of Yki was also able to rescue the viability of wild-type MARCM clones overexpressing Xrp1 in

Figure 5. Ectopic Xrp1 requires IRBP18 to affect clone survival. (A) Wing imaginal discs containing clones of control cells lacking b-galactosidase (magenta) and reciprocal clones expressing GFP (green). In panels B-D) Xrp1 is co-expressed with GFP. Xrp1 led to complete elimination of GFP-positive lineages in the irbp18+/+ background (B), but not when irbp18 was mutated (C). Panel D shows that the GFP-marked clones nevertheless highly over-expressed Xrp1 protein in the irbp18−/− background. (E,F) co-expression of Irbp18 with GFP had no effect on clone survival, even though Irbp18 protein was highly over-expressed (F). Genotypes A) y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/+; FRT82B arm-LacZ/FRT82B tubP-GAL80. B) y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/UAS-Xrp1; FRT82B arm-LacZ/FRT82B tubP-GAL80. C–D) y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/UAS-Xrp1; irbp18f05006 arm-LacZ FRT80/tubP-GAL80 FRT80. E,F) y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/UAS-irbp18; arm-LacZ FRT80/tubP-GAL80 FRT80.
Thus, as noted previously (Akdemir et al., 2007; Tsurui-Nishimura et al., 2013), ectopic Xrp1 affects proliferation and survival, but Yki is able to modulate the effects.

These studies show that Irbp18 remains positively required for Xrp1 function when Xrp1 is transcribed from a transgene, independently of transcriptional auto-regulation. The reason ectopic expression of Irbp18 alone had no effect may be that insufficient Xrp1 is available in normal eye and wing discs to heterodimerize with excess Irbp18.
Xrp1 domains necessary for ectopic function

To assess the role of domains required for dimerization and DNA binding, we used UAS-transgenes encoding the Xrp1 protein tagged with HA. We compared full length Xrp1 protein with proteins deleted for the conserved AT-hook, the basic region, or the Leucine Zipper (Figure 8C). As a negative control, we expressed an Xrp1 protein truncated after Tyr546, lacking all of these domains. UAS-Xrp1 is lethal in combination with drivers such as en-Gal4, because ectopic Xrp1 eliminates imaginal disc cells very efficiently, so these analyses were performed using late-acting and tissue-restricted drivers.

These proteins were expressed ectopically in the developing wing using nub-Gal. Xrp11-546 expression had almost no effect on the adult wing or wing imaginal disc (Figure 7A–D). At 18°C, the nub-Gal4 UAS-Xrp11HA genotype died before emergence so that adult wings could not be examined (Figure 7E). The third instar wing discs exhibited massive cell death throughout the wing pouch, and complete absence of the developing wing margin structures labeled by the neural transcription factor Senseless (Figure 7F). Expression of each of the other deletion proteins were intermediate. They permitted survival, but reduced wing size compared to wild-type controls, showing that they each lacked full Xrp1 function but retained partial function. The weakest phenotype, reflecting least remaining function, was shown by Xrp11BR-HA, whose expression only reduced adult wing size modestly, and only in males (Figure 7G). Cell death in the developing wing pouch was elevated above background but wing margin structures expressing Senseless were still present (Figure 7H). Expression of Xrp11LZ-HA protein led to a larger reduction in wing size, particularly in males, associated with significant cell death in the wing pouch and abnormal differentiation of the wing margin (Figure 7I, J). The strongest phenotype, indicating greatest retention of function, was shown by expression of Xrp11SAT-HA, which led to almost no adults; a single nub-Gal4 UAS-Xrp11SAT-HA male had underdeveloped, unexpanded wings (Figure 7K). Cell death was elevated compared to controls but not to the degree seen after expression of full-length Xrp1 (Figure 7L). The phenotypic differences did not reflect differences in protein stability or localization, because similar levels of anti-HA labeling were observed for all the mutant proteins, which was nuclear in all cases (Figure 7F, H, J, L).

Similar experiments performed using GMR-Gal4 to express proteins in the eye also showed that, while deleting the C-terminal region of Xrp1 abolished function (Figure 7M–P), deletion of each individual domain reduced function but did not eliminate it (Figure 7Q–X). The deleted proteins significantly restored eye size compared to GMR-Gal4 UAS-Xrp1, but still had abnormal, glossy eyes. Whereas none of the Xrp1 constructs much affected initial ommatidial patterning, revealed by Senseless labeling, cell death rates were elevated posterior to the morphogenetic furrow, particularly for the full-length Xrp1 protein and the AT hook deletion. As noted previously, eye phenotypes were stronger at 25°C, and at this temperature even Xrp11-546 expression modestly affected the eye (Figure 7—figure supplement 1).

These data indicate that the C-terminal portion of the Xrp1 protein is necessary overall, and each of the LZ, BR and AT domains contributes to Xrp1 function, even when Xrp1 transcription is under Gal4/UAS control.

Conservation of Xrp1 beyond Drosophila

The Xrp1 gene has only been reported from the genus Drosophila (Akdemir et al., 2007). This restricted evolutionary distribution seems surprising if Xrp1 functions with Irbp18, since C/EBP proteins are conserved throughout eukaryotes. This lack of conservation could be due either to lack of selection to retain Xrp1 sequences, leading to evolutionary drift and loss, or to recurrent positive selection of Xrp1, causing it to diversify rapidly in different lineages over evolutionary time. To test for a signature of recurrent positive selection in the in Xrp1, we analyzed sequences of Xrp1 from 13 species in the Melanogaster group of Drosophila using Phylogenetic Analysis by Maximum Likelihood (PAML) (Yang, 2007). We discovered evidence of diversification under positive selection in these 13 species, with 68 amino acid positions changing rapidly in Xrp1 (Figure 8A). This result suggested that Xrp1 was under strong positive selection for evolutionary change.

Using BLAST searches we have now identified apparent Xrp1 orthologs in other Dipteran insects (Figure 8B, Figure 8—figure supplement 1). These homologs are the most similar genes to Xrp1 in their respective genomes, and correspondingly Xrp1 is their most similar Drosophila homolog. All share the AT-hook and bZIP domains (Figure 8C), and a separated Xrp1-homology domain more
Figure 7. Ectopic expression of mutated Xrp1 proteins. (A–L) Over-expression of Xrp1 proteins during wing development using nub-Gal4 at 18°C. A,C,E,G,I,K show wings from males misexpressing the indicated proteins. Results were similar from females although the male wings were affected more. (B,D,F,H,J,L) show third instar wing imaginal discs labeled for Senseless (red) to reveal the neural differentiation pattern along the future wing margin, anti-active caspase Dcp1 (green) to reveal cell death, and anti-HA (blue) to detect expression of mutated Xrp1 proteins. The Xrp1\(^{1-546}\) protein,
Figure 7 continued

which had little or no effect on wing development, was not detected by anti-HA because it was not tagged (D) Notably, each of the other deletion proteins was expressed more highly than the wild type (compare blue signal in panels H, J, L with F) although it can’t be excluded this reflects apoptosis of many cells expressing wild type Xrp1 (F) No adults survived expression of full-length Xrp1 in wings (E). (M–X) Over-expression of Xrp1 proteins during eye development using GMR-Gal4 at 18°C. M, O, Q, S, U, W show eyes from males misexpressing the indicated proteins. Results were similar from females. N, P, R, T, V, X show third instar eye imaginal discs labeled for Senseless (red) to reveal the retinal differentiation pattern posterior to the morphogenetic furrow, anti-active caspase Dcp1 (green) to reveal cell death, and anti-HA (blue) to detect expression of mutated Xrp1 proteins. Genotypes A, B) nub-Gal4/+; C, D) nub-Gal4/+; UAS-Xrp11–546; E, F) nub-Gal4/+; UAS-Xrp11–546; G, H) nub-Gal4/+; UAS-Xrp1ABR-HA; I, J) nub-Gal4/+; UAS-Xrp11–546; K, L) nub-Gal4/+; UAS-Xrp1AT-HA; M, N) w1118; O, P) GMR-Gal4/+; UAS-Xrp11–546; Q, R) GMR-Gal4/+; UAS-Xrp111–546; (S–T) GMR-Gal4/+; UAS-Xrp1ABR-HA; (U–V) GMR-Gal4/+; UAS-Xrp11–546; (W–X) GMR-Gal4/+; UAS-Xrp1AT-HA

The online version of this article includes the following figure supplement(s) for figure 7:

**Figure supplement 1.** Xrp11–546 expression at higher temperature.

It is possible that rapid divergence under positive selection obscures Xrp1 homologs beyond Dipteran insects. Accordingly, we reviewed human bZIP proteins that in vitro interact with C/EBPβ, the human protein most resembling Irbp18 (Reinke et al., 2013; Francis et al., 2016). The DNA Damage Induced Transcript 3 (DDIT3) encodes a protein that shares some properties with Xrp1. Like Xrp1, DDIT3 is induced in response to DNA damage, regulates apoptosis, and heterodimerizes with C/EBPβ. DDIT3, also known as CHOP, lacks any recognizable ortholog in Drosophila. Interestingly, phylogenetic bootstrapping analysis has suggested that DDIT3 might be the most similar human bZIP domain to that of Drosophila Xrp1 (Baillon et al., 2018). We used GMRgal4 to express hDDIT3 ectopically during Drosophila eye development. Interestingly, the ectopic expression of hDDIT3 not only reduced the eye size like Xrp1 does (Figure 8E), but this phenotype was ameliorated by the simultaneous expression of RNAi against Irbp18 (Figure 8F) or Xrp1 (Figure 8G).

**Discussion**

Heterozygous mutation of ribosomal protein genes lead to cell-autonomous, deleterious phenotypes in both flies and mammals and provide the classic example of a genotype that is eliminated from mosaics by competition (Morata and Ripoll, 1975; Oliver et al., 2004; Clavería and Torres, 2016). There is increasing interest in the potential roles of cell competition in mammalian development, cancer development, and in regenerative medicine (Clavería and Torres, 2016; Di Gregorio et al., 2016; Merino et al., 2016; Baker, 2017; Moruyama and Fujita, 2017; Ellis et al., 2019). A remarkable recent finding from Drosophila is that many of the phenotypic effects of mutating ribosomal proteins genes are mediated by a putative transcription factor, Xrp1, rather than as a direct consequence of altered ribosome number (Lee et al., 2018). Accordingly, Xrp1 plays a key role in the elimination of Rp mutant cells by cell competition (Lee et al., 2016; Baillon et al., 2018; Lee et al., 2018). Xrp1 transcription and protein expression are elevated in Rp mutant cells, restricting translation, cellular growth rate, and the rate of organismal development, and enabling cell competition with nearby wild type cells (Lee et al., 2018). Xrp1 had previously been implicated in the DNA damage response downstream of p53 and in the transposition of P elements, and contributes to the pathology of a Drosophila model of Amyotrophic Lateral Sclerosis, as well as to the coordination of organ growth in flies with Rp gene knockdowns (Brodsky et al., 2004; Akdemir et al., 2007; Francis et al., 2016; Mallik et al., 2018; Boulan et al., 2019).

Xrp1 has been reported not to have homologs in other eukaryotes (Akdemir et al., 2007). This seems surprising given the highly conserved and fundamental roles of ribosomal proteins, and is a barrier to investigating the potential conservation of cell competition mechanisms and the roles of cell competition in mammals, for example in the development of cancer. Xrp1 binds to DNA as a heterodimer with Irbp18, the Drosophila homolog of the C/EBP protein family, which is a conserved protein (Akdemir et al., 2007; Francis et al., 2016) and co-purifies with it in cultured cells (Francis et al., 2016; Mallik et al., 2018). Irbp18 in turn heterodimerizes with the conserved protein dATF4, encoded by the crc gene in Drosophila (Reinke et al., 2013). This led us to...
Figure 8. Xrp1 gene conservation. (A) PAML results for 12 Drosophila species showing the location of sites under strong positive selection in the Xrp1 protein. (B) The conservation (% amino-acid identity to Xrp1 from Drosophila melanogaster) is plotted for Drosophila virilis and for predicted proteins from five other Dipterans. The C-terminal DNA-binding domain region (corresponding to amino acids 565–668 from the long form of D. melanogaster Xrp1) is highlighted in Cyan, and the more amino-terminal conserved sequence (corresponding to amino acids 189–234 from the long form of D. melanogaster) in green. The locations of the alternative amino-terminus of Xrp1 short isoforms in D. melanogaster, and the position of intron six whose modification was described in Figure 6A are indicated. (C) Clustal Omega alignment of the C-terminal DNA-binding domain region of insect Xrp1 sequences. The core consensus sequences defining the AT-hook domain, basic region of bZip domains, and the Leucine Zipper, are overlined in magenta. The deletions made in this study for structure-function analysis of D. melanogaster Xrp1 are underlined in green. (D) Clustal Omega alignment of the Xrp1 Homology domain in the amino-terminal portion of the insect Xrp1 sequences. The Bactrocera dorsalis sequence is perhaps not optimally aligned by this program. (E) Expression of hDDIT3 under GMR-Gal4 control (at 25˚C) reduces eye size (compare the effects of Xrp1 overexpression, Figure 6A,C). (F) Eye size reduction by hDDIT3 depended in part on the Drosophila irbp18 gene. (G) Eye size reduction by hDDIT3 depended in part on the Drosophila Xrp1 gene.

The online version of this article includes the following figure supplement(s) for figure 8:

Figure supplement 1. Clustal O alignment of protein sequences from the Dipteran insects Drosophila melanogaster, Drosophila virilis, Musca domestica, Stomoxys calcitrans, Lucilia cuprina, Bactrocera dorsalis, and Glossina morsitans.
investigate whether it is the Xrp1 heterodimer with the conserved Irbp18 protein that functions in Rp<sup>+/-</sup> cells, and if so whether Xrp1/Irbp18 acts positively; alternatively, Xrp1 could act as a competitive inhibitor of Irbp18 function with its other partner, dAtf4/Crc, in which case Xrp1 could represent a Drosophila-specific regulator of a more conserved pathway.

Our data provide overwhelming genetic evidence that Xrp1 does function along with Irbp18. Like Xrp1 mutations, irbp18 mutation suppressed multiple effects of Rp mutations, including the elimination of Rp<sup>+/</sup> cells by competitive apoptosis in the proximity of wild type cells and the reduced growth of Rp<sup>+/</sup> cells. Like Xrp1, irbp18 was also required for the prompt disappearance of Rp<sup>-/-</sup> cell clones, which survived in the irbp18 mutant background. All these data were consistent with the model that Xrp1/Irbp18 heterodimers are the active species in Rp mutant cells and were inconsistent with the idea that Xrp1 might act as a competitive inhibitor of other Irbp18-containing species, as this would have predicted that Irbp18 mutations would have had phenotypes opposite to those of Xrp1.

The phenotype of Crc knockdown is different from those of Xrp1 and irbp18 mutations. Whereas Xrp1 and irbp18 mutations enhance the growth and competitiveness of Rp<sup>-/-</sup> cells, crc knockdown greatly diminished growth and survival of Rp<sup>-/-</sup> cells. If Xrp1 was a Drosophila-specific competitive inhibitor of a conserved Crc/Irbp18 heterodimer that was required for growth, both irbp18 and crc mutants would show reduced growth, similar to Rp<sup>-/-</sup> genotypes. In contrast to this, irbp18 mutants have little phenotype except in Rp<sup>-/-</sup> genotypes, where their effects closely resemble those of Xrp1 mutants. Also, whereas crc knockdown strongly and cell-autonomously affected the growth of Rp<sup>-/-</sup> cells, it had less effect on Rp<sup>+/</sup> cells.

In addition to these findings in loss-of-function experiments, we also found that Xrp1 over-expression phenotypes depended on IRBP18, as would be expected if these proteins function together. We also found that Xrp1 over-expression at higher temperatures resulted in a still stronger eye phenotype where simultaneous irbp18 mutation restored normal eye size but did not completely restore eye morphology. This is consistent with some IRBP18-independent component to ectopic Xrp1 function that is either cold-sensitive or only apparent at the highest ectopic expression levels. There is not yet any evidence whether these over-expression effects are physiologically relevant.

Taken together, our findings suggest that the Xrp1/IRBP18 and Crc/IRBP18 heterodimers have independent and perhaps unrelated functions. Consistent with this, ectopic expression of IRBP18 had no phenotypic effect, suggesting that IRBP18 is normally made in excess, so that it is Xrp1 that is limiting for the growth inhibiting activities of the Xrp1/IRBP18 heterodimers, which do not impact IRBP18 availability sufficiently to affect Crc/irbp18 functions.

As expected if Xrp1 functions in a heterodimer, the basic and Leucine Zipper domains were important for Xrp1 function, as was the AT hook. In over-expression assays only, there could be reduced activity of proteins deleted for any of these domains individually, but not of a truncation that precedes them all. When encoded from the endogenous locus, basic and AT-hook domains appeared absolutely required.

Mutual auto-regulation may be a significant feature of Xrp1 and IRBP18 function. As noted previously, the elevated Xrp1 and irbp18 trancription observed in Rp<sup>-/-</sup> wing discs is dependent on Xrp1 function (Baillon et al., 2018; Lee et al., 2018)(Figure 3Q–R). Here we show that IRBP18 protein levels are also elevated in Rp<sup>+/</sup> cells in an Xrp1-dependent fashion, and that irbp18 is also required for the autoregulation. In principle, autoregulation could have been the major or indeed the only transcriptional function of the Xrp1/IRBP18 heterodimer, ie perhaps these proteins could control cell competition through other mechanisms once levels were sufficient. This cannot be completely correct, however, because Xrp1 was still substantially dependent on the irbp18 gene and on the Leucine Zipper and DNA binding domains when expressed using GAL4-driven transgenes that are independent of auto-regulation, so by-passing the requirement of auto-regulation does not relieve the requirements for heterodimerization and DNA binding domains. It is also worth noting that Xrp1 and irbp18 are both required to promptly eliminate Rp<sup>-/-</sup> cells, where their expression does not require auto-regulation. Although implicating other transcriptional targets of Xrp1/IRBP18 in Rp<sup>+/</sup> and Rp<sup>-/-</sup> cells, these studies do not rule out other functions besides transcription.

Previously it was thought that Xrp1 was restricted to the genus Drosophila, a surprising finding for a protein that has an important cellular function. We found, however, that Xrp1 genes have been under strong positive selection for rapid evolutionary change. Recurrent positive selection is often the sign of an evolutionary arms race, such as are often driven by host-pathogen interactions, sexual
competition, or intra-genomic conflict (Valen, 1973; Sawyer et al. (2005); Clark et al. (2006); Elde and Malik (2009); Levine and Malik (2013); Enard et al., 2016). Possibly pathogens target Xrp1 to promote growth and survival of infected cells. It is interesting that Xrp1 is already documented to interact with one transposable element, the P element (Francis et al., 2016). However, none of these scenarios for positive selection, or indeed additional possibilities, can yet be ruled out.

Rapid divergence makes homology difficult to detect, and accordingly we now identify divergent Xrp1 homologs in other Dipteran insects that have not previously been annotated because their sequence similarities are restricted to the key DNA-binding portion of the protein, and to a more amino-terminal Xrp1-homology domain. The failure to identify still more distant homologs might be genuine, or might reflect further divergence beyond our ability to recognize homology. Mammals do contain other members of the C/EBP protein family without identified Drosophila homologs, and we show that DDIT3 (aka CHOP and C/EBP-Z) can generate a similar phenotype to Xrp1 when expressed in Drosophila. Interestingly C/EBP-α, one of the mammalian proteins more related to Irbp18, has been implicated in a cell competition-like phenomenon, the elimination of cells from the multipotent hematopoietic stem cell niche following irradiation (Fleenor et al., 2015).

Materials and methods

Fly strains and clonal analysis

Flies were reared on standard medium at 25°C. The genetic strains used are described in the Key Resources Table (Supplementary file 1).

Mitotic recombination was induced 24–72 hr after egg laying by a 1 hr heat shock at 37°C and the larvae dissected 72 or 96 hr later. Genotypes of the analyzed larvae were as follows:

- **wt MARCM clones overexpressing Xrp1:** y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/UAS-Xrp1; FRT82B arm-LacZ/FRT82B tubP-GAL80.
- **wt MARCM clones overexpressing CG6272:** y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/UAS-CG6272; arm-LacZ FRT80/tubP-GAL80 FRT80.
- **CG6272<sup>05006</sup> MARCM clones:** y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/+; CG6272<sup>05006</sup> arm-LacZ FRT80/tubP-GAL80 FRT80. (UAS-Xrp1 instead of + for the ectopic expression of Xrp1 in CG6272<sup>05006</sup> clones).
- **Xrp<sup>1M2-73</sup> MARCM clones in M/+ background:** y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/FRT42 ubi-GFP M(2)56F; FRT82B Xrp<sup>1M2-73</sup>/FRT82B tubP-GAL80.
- **CG6272<sup>05006</sup> MARCM clones in M/+ background:** y w hs-FLP; tubP-GAL4 UAS-mCD8::GFP/FRT42 ubi-GFP M(2)56F; CG6272<sup>05006</sup> FRT80/tubP-GAL80 FRT80.
- **M/+ clones in wt background:** w M(1)Bld hs FL3P; F[RpL36+ w+] arm-LacZ FRT80/FRT80* (CG6272<sup>05006</sup> instead of + for the generation of M/+ clones homozygous for CG6272<sup>05006</sup>.
- **wt clones in M/+ background:** y w hs-FLP; FRT42 ubi-GFP M(2)S6F/FRT42; +/+ (instead of + the following alleles were used to study their effect on cell competition: CG6272<sup>05006</sup>, Xrp<sup>1HA</sup>, Xrp<sup>1A<sub>4.7</sub>HA</sup>, and Xrp<sup>1A<sub>5.4</sub>HA</sup>.
- **CG6272<sup>05006</sup> clones in wt and M/+ backgrounds:** y w hs-FLP; FRT42 ubi-GFP M(2)S6F*/+; CG6272<sup>05006</sup> FRT80/arm LacZ FRT80 (+ instead of + for the wt background).

Replicates and power analysis

We did not perform a prior power analysis for the quantitative comparisons shown in Figure 1B,C, Figure 1G,H, and Figure 2A–D because previous studies of comparable Xrp1 mutant genotypes had established that statistically-significant results could be obtained from ~5 preparations (Lee et al., 2018). For other experiments where statistical analysis was not reported at least two biological replicates were generally performed, ie the complete immunostaining and imaging was performed twice independently. For the over-expression experiments shown in Figure 7, dozens of adult flies were examined for each genotype and two technical replicates for each recorded by confocal microscopy after immunohistochemistry.
Immunohistochemistry

Antibody staining on discs and most antibodies were described previously (Baker et al., 2014). Antibodies and their dilutions are described in the Key Resources Table (Supplementary file 1). Fluorescent images were captured with Leica TCS SP5 AND SP8 scanning confocal microscopes and processed using Image J64 and Adobe Photoshop CS5.

Phylogenetic analysis

To analyze the evolutionary history of Xrp1, we compared sequences from 13 species of Drosophila ranging from D. melanogaster to D. annanase. We chose these species based on strong homology hits in tBLASTn to the D. melanogaster Xrp1 protein coding sequence from the publicly available Drosophila genomes. To test for recurrent positive selection across these species of flies, we used Phylogenetic Analysis by Maximum Likelihood (PAML) v4.9 [1]. We used three NSsites models in our analysis – Model 7, Model 8, and Model 8a – to discriminate between purifying, positive, and neutral selection respectively. We used a log-likelihood ratio test to determine if there was a significant difference in the fit of the models to our data.

DNA constructs and transgenic flies

The Xrp1<sup>HA</sup>, Xrp1<sup>ΔAT-HA</sup> and Xrp1<sup>ΔBS-HA</sup> alleles were obtained from the strain Xrp1<sup>attPFlox</sup> following the method described (Baena-Lopez et al., 2013).

**Generation of the Xrp1<sup>attPlox</sup> allele**

5' and 3' homology arms (HA) were amplified by PCR using as a template genomic DNA from D. melanogaster yw flies and the following primer combinations

1) 5'HA, Length 1088
   F-GAATTCTGCACAATACAGGGTGTTCT (EcoRI)
   R-CATATGAAAGAGCTGACAATTTTTCGGTAC (Ndel)

2) 3'HA, Length 1174
   F-ACTAGTTGAAGTCGAAATATCCAGCACAGCC (SpeI)
   R-CCTAGGAAGAACGTTATAGAACCCATTCAAA (AvrII)

The 5'HA and 3'HA DNA fragments were cloned, respectively, into the 5' and 3' MCS (Multiple Cloning Site) of the pTV<sup>Cherry</sup> vector and the resulting construct (pTV-Xrp1) was used for germline transformation of yw flies (Rainbow transgenics). Transgenic male flies harbouring the pTV-Xrp1 construct were crossed to females containing the Flippase and I-SceI genes under the control of the heatshock promoter (Bloomington stock #25679). Larvae from this cross were heat-shocked to induce the excision and linearization of the 5'HA-attP-mini White-3'HA cassette and adults with motted red eyes were crossed to ubi-gal4 flies to eliminate false positives (see Baena-Lopez et al., 2013). Red eye flies from this cross were considered candidates and further analysed by PCR and Sanger sequencing to confirm the reintegration of the cassette into the Xrp1 locus by homologous recombination.

The Xrp1<sup>attPlox</sup> allele behaves during cell competition as a LOF allele (Suppl. Fig. 1).

**Generation of Xrp1<sup>attPloxP</sup>**

The 5'HA-attP-mini White-3'HA cassette integrated onto the Xrp1 locus contains LoxP sites flanking the mini-White gene. By crossing red eyes Xrp1<sup>attPlox</sup> flies with hs-Cre flies (Bloomington #851), white eyes Xrp1<sup>attPloxP</sup> flies lacking the mini-White gene were obtained. The Xrp1<sup>attPloxP</sup> allele behaves as a wt allele during cell competition (Suppl. Fig. 1).

| Xrp1   | CGGTTAGATCTGTAAAGAGCTGACAATTTTTCGGTAC |
|--------|---------------------------------------|
| Xrp1<sup>attPloxP</sup> | CGGTTAGATCTGTAAAGAGCTGACAATTTTTCGGTAC |
| Xrp1   | ***************************************** |
| Xrp1<sup>attPloxP</sup> | TATGCAAGTACCCTAGGCGCCTACGTTCTCAGGGGCGGTT |

Continued on next page
The end of exon 6 and beginning of exon 7 are shown in black bold letters.

Introduced restriction sites: NdeI: CATATG and SpeI: ACTAGT
Significant sequences: attP and LoxP sites.

**Generation of the Xrp1^HA, Xrp1^AT-HA, Xrp1^LZ-HA and Xrp1^BS-HA**

The attP site present in the Xrp1^attPloxP allele was used as a platform to introduce modifications in the last exon of the Xrp1 gene using PhiC31-mediated recombination. For this purpose, flies having a source of the PhiC31 integrase gene on the X chromosome under the control of a germline-specific promoter (Bloomington #40161), were crossed to Xrp1^attPloxP flies to generate the recipient strain y^1 M(vas-int.Dm)ZH2A; Xrp1^attPloxP. This strain was used in germline transformation experiments (Bestgene) using pRIVW-based plasmids that contain the attB site upstream of the modified last exon of Xrp1. The pRIVW vector derives from the reintegration vector RIVFRT MCS FRT MCS3 (Baena-Lopez et al., 2013) by cloning the mini-White gene between the PstI and HpaI restriction sites. The modifications introduced in the last exon of the Xrp1 gene were generated by PCR using proper primer combinations. Each modified version of the Xrp1 last exon was generated using 2 PCR products (5’ and 3’ products) that share a common restriction site (Shared Restriction Site) at the place where the modification was introduced. The 5’ products (SpeI-SRS) were cloned into the pRIVW vector digested with XbaI (partial digestion) and SRS. The 3’ products (SRS-AvrII) were subsequently cloned using the SRS and AvrII restriction sites. By cloning the PCR products between XbaI and AvrII restriction sites, 2 FRT sites present in the pRIVW vector are eliminated. The final constructs were injected into y^1 M(vas-int.Dm)ZH2A; Xrp1^attPloxP flies and the candidate red eye transformants analysed by PCR.

1) Introduction of a HA-tag at the C-terminal end of Xrp1
- 5’PCR product
  F: ACTAGTTGAAGTCGAAATATCCAGCACACGCC (SpeI)
  R: CGAATTTCAGTCGCCATAGAATGAAATTTGGCG (EcoRI)
- 3’PCR
  F: GGAATTCGATTCTCGAGCATGAAAGGATTTGCGG (EcoRI)
  R: CCTAGGAAGAAGCTTTAGAAACCGATTC (AvrII)
Relevant sequences of the resulting pRIVW-Xrp1-HA vector: **attB site** in **bold letters**, **HA sequence**, **Xrp1 Exon7** is underlined.

```
ATAATTTGGCGAGTACGCAAAGCTATGCATGTAATGAATTGCCGCGGTGGCGG
GTGCCAGGGCGT
GCCCTTGGGCTCCCCGGGCGCG
TACTCCCGTACGACGAAGTTCCTATTCCGAAGTTCCTATTC
TCTAGTTGAAGTCGAAATATCCAGCACAGCCTAATTTACTTAATCTCTCCTATTTGCAG
TTCG
AGGCTCCCACCACAAGTACCATACAGCAAGCAACACCAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
AGATGCGGTCCTCCACCGTCTATTTGCTATGAAATGCTGTTGG
GCCCTTCGCCAGCCAGCGGAATCACGCGGAAAGCGGCGGGTGCGCCCTGCCAGAACTGATG
GCCGACGGTCCCGATCCCGTGCTCATGTCAAGCATGAAAAGCGAGGAGGAGAGGAAGGCCTACCG
GACAGACTCAAGACAGAACAGGCGTATCGCGCCGGAAGACGAAGGTGCGCGAAG
AGGAGGAGAAGCGC
GCCGAGGACACGCTATTGGCCGAGAATCTGCGACTGCGTGCCCGGGCCG
ACGAAGTGGCCTCCCGGGAACGGAAGTTCAAGAAGTACCTGATGGAGCGCCAGCGG
CAGAAGA
GCACTTATTTGAGAAGGTACCTAACCGGATCAGGCTCTATTTTGAATCTG
AGTCTGAGCCATAGAATAGAAATTGCGGAGCATAAGCCTGTCATAGTACCTATCGTCGTCA
TCCAACTTATTTAAGGTTAGTTAAGGTTGCTGGAGAAGTCGAAATATTGGAAGAAGTGAAGAAAC
TTGCTATTGTTAAGAACEATTGAAATGTATATTTTACAGATATCAACTTATATAACCGTAATTG
TTTGAATAAAAAGCATGTTCAAATAAACGTGACAAAATCAACTAACTTTGTATATTTGAATGG
GTTCTATAACGTTCCTT
```

The STOP codon is shown in bold black underlined letters. The DNA binding domains and the dimerization domain are shown in **bold letters**: **AT Hook**, **Basic domain** (**italicized**) and **Leucine zipper**. Introduced restriction site **EcoRI** is italicized: **GAATTC**.

The 5’ cloning site of the 5’PCR product is a **XbaI/SpeI** fusion site, shown italicized: **TCTAGT**. The pRIVW-Xrp1-HA vector was used as a template to generate the deletions using the following primer combinations:

2) Deletion in the AT-Hook domain

- 5’PCR product
  F: AGCTAGGTGAAAGTCGACAATCCACGACAGGCC (SpeI)
  R: AGATCTGCGCTGATTGCCGCTGG (SRS: BglII)

- 3’PCR product
  F: AGATCTCCCGATCCCGTGCTCAGCATGCA (SRS: BglII)
  R: CAGTATTTTTGAGAAGGTACCTAACCGGATCAGGCTCTATTTTGAATCTG

Exon7 alignment

Xrp1
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1 ΔAT-HA
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1 ΔAT-HA
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1 ΔAT-HA
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1 ΔAT-HA
```
TTGGGCTTGCCACCAAGTAGTACCATCAGCAAGCAACACTCTTCTTGCCGAGGCGC
ACACACCAGACACACCTACTGCACAGCGACGGTGCGCGGGGGCGAAGCTGCGCGAACGCTGCGCG
```

Xrp1
```
AGTACGTCGCTGTTAGCGACGCTTCATTGCACCTGCGCGCTGTTATTGCACATCAAGCC
```

Xrp1 ΔAT-HA
```
AGTACGTCGCTGTTAGCGACGCTTCATTGCACCTGCGCGCTGTTATTGCACATCAAGCC
```

Xrp1
```
CSGCTTGGGCTGTCCTGCGCTGTTAGCGACGCTTCATTGCACCTGCGCGCTGTTATTGCACATCAAGCC
```

Continued on next page
The STOP codon is shown in bold black letters. Introduced restriction sites \textit{BglII}: AGATCT and \textit{EcoRI}: GAATTC. Significant sequences: AT-hook domain, \textit{basic domain} (italicized in bold) and \textit{HA tag sequence}.

3) Deletion in the \textit{Basic domain} - 5’PCR product

\textbf{F.} ACTAGTTGAAGTCGAAATATCCAGCACAGCC (SpeI)

Blanco et al. eLife 2020;9:e50535. DOI: https://doi.org/10.7554/eLife.50535
R- GCTAGCCTGGTAGGCCCTTCCTCCTCTC (SRS: NheI) 
-3’PCR product
F- GCTAGCGTGCGCGAAGAGGAGGAGAAG (SRS: NheI) 
R- CCTAGGAAGAAGCTTTATAGAACCGGACCATTCAA (AvrII) 
Exon7 alignment

Continued on next page
The STOP codon is shown in bold black letters. Introduced restriction sites italicized in bold NdeI: GCTAGC and EcoRI: GAATTC. Significant sequences: AT-hook domain, basic domain and HA sequence.

3) Deletion in the Leucine Zipper domain

-5'PCR product
F: ACTAGTGAAGTCGAAATATCCAGCACAGCC (SpeI)
R: ACTAGTCGTTCCTCCGCGCTTCT (SRS: SpeI)

-3'PCR product
F: ACTAGTATGGAGCGCCAGCGCGAGAA (SRS: SpeI)
R: CCTAGGAAGAAGCTTATAGAACCCATTCAAAA (AvrII)

Exon7 alignment

**Xrp1**

|     | TGGCAGGCTCCACCCACCAAGTACCAGTCGGAGCCAACATTTTCTCTTCATCGACAGCGAC |
|-----|------------------------------------------------------------------|
| Xrp1 | TGGCAGGCTCCACCCACCAAGTACCAGTCGGAGCCAACATTTTCTCTTCATCGACAGCGAC |

**Xrp1**

|     | CTTCCACGGCTCAGTTGAGCCCTCCATCTCCGCCCTATACAGCAAGCGGAAGCGCGGT |
|-----|--------------------------------------------------------------|
| Xrp1 | CTTCCACGGCTCAGTTGAGCCCTCCATCTCCGCCCTATACAGCAAGCGGAAGCGCGGT |

**Xrp1**

|     | GACAGACTCAAGAACAAGCGAGCCGCGTATCG |
|-----|----------------------------------|
| Xrp1 | GACAGACTCAAGAACAAGCGAGCCGCGTATCG |

Continued on next page
The STOP codon is shown in bold black letters. Introduced restriction sites SpeI: **ACTAGT** and EcoRI: **GAATTC** are italicized in bold. Significant sequences: **AT-hook domain**, **Basic domain** (italicised), **Leucine zipper** and **HA sequence**.

Alignment of the C-terminal part of Xrp1 in several Drosophila species, showing the **AT hook domain** (with the crucial GRP tripeptide underlined) the **Basic domain** and the **Leucine zipper**. Additional underlined sequences in D.melanogaster indicate the extent of the deletions.

D.vir  
**GFGFITAPVSPAFSTASQFSVTSITGNSSKRPGP**AKEHAGPDPELM**AQTG**

D.pseudo  
**GGGGFTAPASPVASTQFSATT**NSN-APKRPG**PK**EKEHAGPDPEVM**RMDDE**

Continued on next page
D.erec  GFGGFLTAPQSPAYSTSAFSPSPAG-G\text{SGKRKRPAKDHGPDPELMSGM}TSE
D.mel  GYGGLTAPTSPAYSTSTSFSPAS-G\text{SGKRKRPAKDHGPDVLMSM}KSE
D.sim  GYGGLTAPSSAYSTSAFSPSPAG-G\text{SGKRKRPAKDHGPDVLMSMK}SE

*:::*;*** **:**:**: **: **;: :*: **;: **: ;:*:**;:* ;: : ;: ***: **:**;:*;: * : : : : : : : : : : ;: ;: : : : : ;: : **:**:**:**;:*:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:**:*
NIH SIG 1S10 OD023591. DNA sequencing was performed by the Genomics Core of Albert Einstein College of Medicine.

### Additional information

#### Funding

| Funder                                   | Grant reference number | Author             |
|------------------------------------------|------------------------|--------------------|
| National Eye Institute                   | EY026720               | Nicholas E Baker   |
| National Institute of General Medical Sciences | GM120451               | Nicholas E Baker   |
| Eunice Kennedy Shriver National Institute of Child Health and Human Development | ST32 HD0741 | Jacob C Cooper |

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

#### Author contributions

Jorge Blanco, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review and editing; Jacob C Cooper, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—review and editing; Nicholas E Baker, Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Writing—review and editing

#### Author ORCIDs

Nicholas E Baker [https://orcid.org/0000-0002-4250-3488](https://orcid.org/0000-0002-4250-3488)

#### Decision letter and Author response

Decision letter [https://doi.org/10.7554/eLife.50535.sa1](https://doi.org/10.7554/eLife.50535.sa1)
Author response [https://doi.org/10.7554/eLife.50535.sa2](https://doi.org/10.7554/eLife.50535.sa2)

### Additional files

#### Supplementary files
- Supplementary file 1. Key Resources.
- Transparent reporting form

#### Data availability

All data generated or analysed during this study are included in the manuscript and supporting files.

The following previously published dataset was used:

| Author(s) | Year | Dataset title | Dataset URL | Database and Identifier |
|-----------|------|---------------|-------------|-------------------------|
| Chang-Hyun Lee, Marianne Kiparaki, Jorge Blanco, Virginia Folgado, Zhejun Ji, Amit Kumar, Gerard Rimesso, Nicholas E Baker | 2018 | RNA-seq analysis to assess transcriptional effects of Rp mutations in wing imaginal discs and their dependence on Xrp1 | [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse112864](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse112864) | GEO, GSE112864 |

### References

Akdemir F, Christich A, Sogame N, Chapo J, Abrams JM. 2007. p53 directs focused genomic responses in Drosophila. Oncogene 26:5184–5193. DOI: [https://doi.org/10.1038/sj.onc.1210328](https://doi.org/10.1038/sj.onc.1210328), PMID: 17310982
Amoutzias GD, Veron AS, Weiner J, Robinson-Rechavi M, Bornberg-Bauer E, Oliver SG, Robertson DL. 2007. One billion years of bZIP transcription factor evolution: conservation and change in dimerization and DNA-binding site specificity. Molecular Biology and Evolution 24:827–835. DOI: https://doi.org/10.1093/molbev/msl211, PMID: 17194801

Amoyel M, Bach EA. 2014. Cell competition: how to eliminate your neighbours. Development 141:988–1000. DOI: https://doi.org/10.1242/dev.079129, PMID: 24550108

Baena-Lopez LA, Alexandre C, Mitchell A, Pasakaris L, Vincent J-P. 2013. Accelerated homologous recombination and subsequent genome modification in Drosophila. Development 140:4818–4825. DOI: https://doi.org/10.1242/dev.100933

Baillon L, Germani F, Rockel C, Hilchenbach J, Basler K. 2018. Xrp1 is a transcription factor required for cell competition-driven elimination of loser cells. Scientific Reports 8:17712. DOI: https://doi.org/10.1038/s41598-018-36277-4, PMID: 30531963

Baker NE, Li K, Quiquand M, Ruggiero R, Wang LH. 2014. Eye development. Methods 68:252–259. DOI: https://doi.org/10.1016/j.jymeth.2014.04.007, PMID: 24784530

Baker NE. 2017. Mechanisms of cell competition emerging from Drosophila studies. Current Opinion in Cell Biology 48:40–46. DOI: https://doi.org/10.1016/jceb.2017.05.002, PMID: 28609679

Baker NE, Kiparaki M, Khan C. 2019. A potential link between p53, cell competition and ribosomopathy in mammals and in Drosophila. Developmental Biology 446:17–19. DOI: https://doi.org/10.1016/j.ydbio.2018.11.018, PMID: 30513308

Boulan L, Andersen D, Colombani J, Boone E, Léopold P. 2019. Inter-Organ growth coordination is mediated by the Xrp1-Dilp8 Axis in Drosophila. Developmental Cell 49:811–818. DOI: https://doi.org/10.1016/j.devcel.2019.03.016, PMID: 31006647

Bridges CB, Morgan TH. 1923. The Third-Chromosome Group of Mutant Characters of Drosophila Melanogaster. 327 Carnegie Institute Publication.

Brodsky MH, Weinert BT, Tsang G, Rong YS, McGinnis NM, Golic KG, Rio DC, Rubin GM. 2004. Drosophila Melanogaster MNK/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. Moleular and Cellular Biology 24:1219–1231. DOI: https://doi.org/10.1128/MCB.24.3.1219-1231.2004, PMID: 14729967

Celardo I, Lehmann S, Costa AC, Loh SH, Miguel Martins L. 2017. dATF4 regulation of mitochondrial folate-mediated one-carbon metabolism is neuroprotective. Cell Death & Differentiation 24:638–648. DOI: https://doi.org/10.1038/cdd.2016.158, PMID: 28211874

Clark NL, Aagaard JE, Swanson WJ. 2006. Evolution of reproductive proteins from animals and plants. Reproduction 131:11–22. DOI: https://doi.org/10.1530/repp.1.00357, PMID: 16380004

Claveria C, Torres M. 2016. Cell competition: mechanisms and physiological roles. Annual Review of Cell and Developmental Biology 32:411–439. DOI: https://doi.org/10.1146/annurev-cellbio-111315-125142, PMID: 27501445

Delanoë R, Slidina M, Léopold P. 2010. The steroid hormone ecdysone controls systemic growth by repressing dMyc function in Drosophila fat cells. Developmental Cell 18:1012–1021. DOI: https://doi.org/10.1016/j.devcel.2010.05.007, PMID: 20627082

Di Gregorio A, Bowling S, Rodriguez TA. 2016. Cell competition and its role in the regulation of cell fitness from development to Cancer. Developmental Cell 38:621–634. DOI: https://doi.org/10.1016/j.devcel.2016.08.012, PMID: 27676435

Elde NC, Malik HS. 2009. The evolutionary conundrum of pathogen mimicry. Nature Reviews Microbiology 7:787–797. DOI: https://doi.org/10.1038/nrmicro2222, PMID: 19806153

Ellis SJ, Gomez NC, Levorse J, Mertz AF, Ge Y, Fuchs E. 2019. Distinct modes of cell competition shape mammalian tissue morphogenesis. Nature 569:497–502. DOI: https://doi.org/10.1038/s41586-019-1199-y, PMID: 31092920

Enard D, Cai L, Gwennap C, Petrov DA. 2016. Viruses are a dominant driver of protein adaptation in mammals. eLife 5:e12469. DOI: https://doi.org/10.7554/eLife.12469, PMID: 27187613

Fassler J, Landsman D, Acharya A, Moll JR, Bonovich M, Vinson C. 2002. B-ZIP proteins encoded by the Drosophila genome: evaluation of potential dimerization partners. Genome Research 12:1190–1200. DOI: https://doi.org/10.1101/gr.67902, PMID: 12176927

Fleenor CJ, Rozhok AI, Zaberezhnyy V, Mathew D, Kim J, Tan AC, Leopold P. 2015. Contrast roles for C/EBPα and notch in irradiation-induced multipotent hematopoietic progenitor cell defects. Stem Cells 33:1345–1358. DOI: https://doi.org/10.1002/stem.1936, PMID: 25546133

Francis MJ, Roche S, Cho MJ, Beall E, Min B, Panganiban RP, Rio DC. 2016. Drosophila IRBP bZIP heterodimer binds p-element DNA and affects hybrid dysgenesis. PNAS 113:13003–13008. DOI: https://doi.org/10.1073/pnas.1613508113, PMID: 27799520

Gauthier SA, VanHaften E, Cherbas L, Cherbas P, Hewes RS. 2012. Cryptopogenic, the Drosophila Melanogaster ATF4, is a specific coactivator for ecdysone receptor isoform B2. PLOS Genetics 8:e1002883. DOI: https://doi.org/10.1371/journal.pgen.1002883, PMID: 22912598

Jaszczak JS, Halme A. 2016. Arrested development: coordinating regeneration with development and growth in Drosophila Melanogaster. Current Opinion in Genetics & Development 40:87–94. DOI: https://doi.org/10.1016/j.cbg.2016.06.008, PMID: 27394031

Kang MJ, Vasudevan D, Kang K, Kim K, Park JE, Zhang N, Zeng X, Neubert TA, Marr MT, Ryoo HD. 2017. 4E-BP1 is a target of the GCN2-ATF4 pathway during Drosophila development and aging. The Journal of Cell Biology 216:115–129. DOI: https://doi.org/10.1083/jcb.201511073, PMID: 27979906
Kastenhuber ER, Lowe SW. 2017. Putting p53 in context. Cell 170:1062–1078. DOI: https://doi.org/10.1016/j.cell.2017.08.028, PMID: 28886379

Lee C-H, Rimesso G, Reynolds DM, Cai J, Baker NE. 2016. Whole-Genome sequencing and iPLEX MassARRAY genotyping map an EMS-Induced mutation affecting Cell competition in Drosophila Melanogaster. G3: Genes, Genomes, Genetics 6:3207–3217. DOI: https://doi.org/10.1534/g3.116.029241

Lee CH, Kiparaki M, Blanco J, Folgado V, Ji Z, Kumar A, Rimesso G, Baker NE. 2018. A regulatory response to ribosomal protein mutations controls translation, growth, and cell competition. Developmental Cell 46:456–469. DOI: https://doi.org/10.1016/j.devcel.2018.07.003, PMID: 30078730

Levine MT, Malik HS. 2013. A rapidly evolving genomic toolkit for Drosophila heterochromatin. Fly 7:137–141. DOI: https://doi.org/10.1042/fly20130116, PMID: 23519206

Li W, Baker NE. 2007. Engagement is required for cell competition. Cell 129:1215–1225. DOI: https://doi.org/10.1016/j.cell.2007.03.054, PMID: 17574031

Mallick M, Catinozzi M, Hug CB, Zhang L, Wagner M, Bussmann J, Bittern J, Mersmann S, Klämbt C, Drexler HCA, Huynen MA, Vaquerizas JM, Storkebaum E. 2018. Xrp1 genetically interacts with the ALS-associated FUS orthologue caz and mediates its toxicity. The Journal of Cell Biology 217:3947–3964. DOI: https://doi.org/10.1083/jcb.201802151, PMID: 30209068

Malz E, Dominicus CS, Chambers JE, Dickens JA, Mookerjee S, Marciniak SJ. 2018. The integrated stress response regulates BMP signalling through effects on translation. BMC Biology 16:34. DOI: https://doi.org/10.1186/s12915-018-0503-x, PMID: 29609607

Maruyama T, Fujita Y. 2017. Cell competition in mammals - novel homeostatic machinery for embryonic development and Cancer prevention. Current Opinion in Cell Biology 48:106–112. DOI: https://doi.org/10.1016/jceb.2017.06.007, PMID: 28718966

Marygold SJ, Roote J, Reuter G, Lambertsson A, Ashburner M, Millburn GH, Harrison PM, Yu Z, Kenmochi N, Kaufman TC, Leevers SJ, Cook KR. 2007. The ribosomal protein genes and minute loci of Drosophila Melanogaster. Genome Biology 8:R216. DOI: https://doi.org/10.1186/gb-2007-8-10-r216, PMID: 17927810

Merino MM, Levayer R, Moreno E. 2016. Survival of the fittest: essential roles of cell competition in development, aging, and Cancer. Trends in Cell Biology 26:776–788. DOI: https://doi.org/10.1016/j.tcb.2016.05.009, PMID: 27319281

Morata G, Ripoll P. 1975. Minutes: mutants of Drosophila autonomously affecting cell division rate. Developmental Biology 42:211–221. DOI: https://doi.org/10.1016/0012-1606(75)90330-9, PMID: 1116643

Moreno E, Basler K, Morata G. 2002. Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. Nature 416:755–759. DOI: https://doi.org/10.1038/416755a, PMID: 11961558

Nagata R, Igaki T. 2018. Cell competition: emerging mechanisms to eliminate neighbors. Development, Growth & Differentiation 60:522–530. DOI: https://doi.org/10.1011/dgd.12575, PMID: 30443922

Oliver ER, Saunders TL, Tarlé SA, Glaser T. 2004. Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse minute. Development 131:3907–3920. DOI: https://doi.org/10.1242/dev.01268, PMID: 15289434

Ramji DP, Foka P. 2002. CCAAT/enhancer-binding proteins: structure, function and regulation. Biochemical Journal 365:561–575. DOI: https://doi.org/10.1042/bj200202050, PMID: 12006130

Reinke AW, Baek J, Ashenberg O, Keating AE. 2013. Networks of bZIP protein-protein interactions diversified over a billion years of evolution. Science 340:730–734. DOI: https://doi.org/10.1126/science.1233465, PMID: 23661758

Sawyer SL, Wu JJ, Emerman M, Malik HS. 2005. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. PNAS 102:2832–2837. DOI: https://doi.org/10.1073/pnas.0409853102, PMID: 15689398

Simpson P, Morata G. 1981. Differential mitotic rates and patterns of growth in compartments in the Drosophila wing. Developmental Biology 85:299–308. DOI: https://doi.org/10.1016/0012-1606(81)90261-X, PMID: 7262460

Tsurui-Nishimura N, Nguyen TQ, Katsuwaya T, Minami T, Furushashi H, Oshima Y, Kurata S. 2013. Ectopic antenna induction by overexpression of CG17836/Xrp1 encoding an AT-hook DNA binding motif protein in Drosophila. Bioscience, Biotechnology, and Biochemistry 77:339–344. DOI: https://doi.org/10.1271/bbb.120756, PMID: 23391928

Tyler DM, Li W, Zhuo N, Pellock B, Baker NE. 2007. Genes affecting cell competition in Drosophila. Genetics 175:643–657. DOI: https://doi.org/10.1534/genetics.106.061929, PMID: 17110495

Valen V. 2013. A new evolutionary law. Evolution Theory 1:1–30.

Worley MI, Setiawan L, Hariharan IK. 2013. TIE-DYE: a combinatorial marking system to visualize and genetically manipulate clones during development in Drosophila Melanogaster. Development 140:3275–3284. DOI: https://doi.org/10.1242/dev.096057, PMID: 23785055

Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24:1586–1591. DOI: https://doi.org/10.1093/molbev/msm088, PMID: 17483113