1 Cell competition removes segmental aneuploid cells from *Drosophila* imaginal
2 disc-derived tissues based on ribosomal protein gene dose

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ABSTRACT

Aneuploidy causes birth defects and miscarriages, occurs in nearly all cancers, and is a hallmark of aging. Individual aneuploid cells can be eliminated from developing tissues by unknown mechanisms. Cells with ribosomal protein (*Rp*) gene mutations are also eliminated, by cell competition with normal cells. Because *Rp* genes are spread across the genome, their copy number is a marker for chromosome aberrations. Elimination of imaginal disc cells with irradiation-induced genome damage often required cell competition genes. When defined chromosome regions were deleted, segmentally-aneuploid cells were eliminated by the RpS12-Xrp1 cell competition pathway in an apoptosis-dependent manner when they differed from neighboring cells in *Rp* gene dose. Cells with normal doses of the *Rp* (and *eIF2γ*) genes survived and differentiated adult tissues. Thus, cell competition, triggered by differences in *Rp* gene dose between cells, is a significant mechanism for the elimination of aneuploid somatic cells, likely to contribute to preventing cancer.

INTRODUCTION

Aneuploidy (gain or loss of whole chromosomes resulting in an abnormal karyotype) is a hallmark of spontaneous abortions and birth defects and observed in virtually every human tumor(Hassold & Hunt, 2001; Hanahan & Weinberg, 2011; Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013). It was suggested over 100 years ago that aneuploidy contributes to cancer development(Boveri, 1914). Aneuploidy can change the copy number of important oncogenes and tumor suppressors, cause stress due to gene expression imbalance, and promote further genetic instability(Naylor & van Deursen, 2016; Rutledge & Cimini, 2016; Chunduri & Storchova, 2019; Ben-David & Amon, 2020)(Zhu, Tsai, Gordon, & Li, 2018). Mouse models of chromosome instability that result in aneuploidy are oncogenic(Foijer, Draviam, & Sorger, 2008; Baker, Jin, Jeganathan, & van Deursen, 2009; Mukherjee et al., 2014). *Drosophila* cells with chromosome instability undergo a p53-independent death, but can form tumors if their apoptosis is prevented(Dekanty, Barrio, Muzzopappa, Auer, & Milan, 2012; Gerlach & Herranz, 2020).(Moraes da Silva, Moutinho-Santos, & Sunkel, 2013)

Because aneuploidy is thought to be detrimental to normal cells, aneuploid cells arising sporadically in vivo should, as a rule, grow poorly(Sheltzer & Amon, 2011). Studies of yeast carrying extra chromosomes reveal a stress response in these cells, thought to result from the cumulative mismatch in levels of many proteins that interact in the cell, which resembles hypo-osmotic stress, and inhibits growth(Torres et al., 2007; Zhu et al., 2018; Tsai et al., 2019).

Increasing evidence points to the capacity of normal tissues to recognize and eliminate aneuploid cells(Hook, 1981; van Echten-Arends et al., 2011; Bazrgar, Gourabi, Valojerdi, Yazdi, & Baharvand, 2013; Pfau, Silberman, Knouse, & Amon, 2016; Santaguida et al., 2017). Array Comparative Genome Hybridization detects mosaic aneuploidy in as many as 60% of normal human embryos, which can nonetheless develop into healthy babies without birth defects or evidence of aneuploid cells, suggesting their elimination(Greco,

Minasi, & Fiorentino, 2015). In mice, chimeric embryos can be constructed using both normal diploid cells and cells with a high rate of aneuploidy due to treatment with reversine, an inhibitor of the spindle assembly checkpoint. The reversine-treated cells are actively eliminated from the chimeric embryos, which can develop into morphologically normal adult mice from which reversine-treated cells have been eliminated (Bolton et al., 2016). Other observations point to the loss of aneuploid cells in other biological processes. For example, the cortex of normal mouse embryos contains as many as 30% aneuploid cells, but only ~1% are detected by 4 months post partum, suggesting selective loss of the aneuploid fraction (Andriani et al., 2016).

The mechanisms of recognition and removal of aneuploid cells are still poorly understood. In mouse tissues, cells with complex karyotypes may be recognized by the immune system (Santaguida et al., 2017). In *Drosophila*, clones of segmentally aneuploid cells (cells with loss or gain of chromosome segments) can survive development and differentiate in the adult abdomen, but their representation decreases as more genetic material is lost, whereas cells carrying extra genetic material are less affected (Ripoll, 1980). Clonal loss of heterozygosity is also tolerated in the abdomens of DNA repair pathway mutants, and studies with genetic markers indicate that this frequently represents loss of substantial chromosome segments (Baker, Carpenter, & Ripoll, 1978).

The *Drosophila* adult abdomen derives from larval histoblasts. In the head and thorax, which develop instead from the larval imaginal discs, there is evidence that aneuploid cells undergo apoptosis. DNA damage following ionizing irradiation rapidly leads to apoptosis, but is followed by a smaller amount of delayed apoptosis that is independent of p53 and Chk2 and therefore unlikely to reflect unrepaired DNA damage (Brodsky et al., 2004; Wichmann, Jaklevic, & Su, 2006). A similar biphasic response is seen after mitotic breakage of dicentric chromosomes, and in this case the delayed, p53-independent cell death only occurs in genotypes likely to lead to aneuploid products (Titen & Golic, 2008). Accordingly, it is suggested that post-irradiation apoptosis independent from p53 also represents removal of aneuploid cells that arise following DNA repair, and

that ‘cell competition’ may provide the p53-independent mechanism (McNamee & Brodsky, 2009).

The term ‘cell competition’ was originally coined to describe the elimination of *Drosophila* cells heterozygous for mutant alleles of ribosomal protein genes (*Rp* genes)(Morata & Ripoll, 1975). Most *Rp*’s are essential, even to the individual cell, so that homozygosity for *Rp*⁻ mutations is rapidly lethal, whereas *Rp*^{+/-} heterozygotes are viable and fertile, although slow growing with minor morphological defects such as thin adult bristles(Marygold et al., 2007). By contrast to their whole animal viability, individual *Rp*^{+/-} heterozygous cells or clones are actively eliminated from mosaic *Drosophila* tissues(Morata & Ripoll, 1975; Simpson, 1979). This involves apoptosis specific to *Rp*^{+/-} heterozygous cells near to *Rp*^{+/+} cells (Morata & Ripoll, 1975; Simpson, 1979; Moreno, Basler, & Morata, 2002; Li & Baker, 2007). The defining feature of cell competition is therefore the elimination of cells based on their difference from other neighboring cells rather than based on their intrinsic properties(Morata & Ripoll, 1975; Baker, 2020).

The 80 eukaryotic *Rp*’s are mostly encoded by single copy genes transcribed by RNA polymerase II, and are dispersed throughout the genome in both humans and in *Drosophila*(Uechi, Tanaka, & Kenmochi, 2001; Marygold et al., 2007). Accordingly, aneuploidy and other large-scale genetic changes will usually affect *Rp* gene dose. Since *Rp* proteins are required stoichiometrically for ribosome assembly, which generally stalls when any one *Rp* is limiting, imbalanced *Rp* gene dose can perturb ribosome biogenesis(de la Cruz, Karbstein, & Woolford, 2015). This provides an almost perfectly suited mechanism to serve as an indicator of unbalanced chromosome content(McNamee & Brodsky, 2009). Cell competition may thus have evolved to recognize and remove cells with large-scale genetic changes such as aneuploidy, recognized on the basis of their mis-matched Ribosomal protein (*Rp*) gene complements. In this view, cells heterozygous for point mutations in *Rp* genes are eliminated because they mimic larger genetic changes.

A stress response pathway that is activated by *Rp* mutations in *Drosophila* has recently been described, and is required for *Rp* point mutated cells to undergo cell competition (Baillon, Germani, Rockel, Hilchenbach, & Basler, 2018; Kale et al., 2018; Lee et al., 2018; Ji et al., 2019; Blanco, Cooper, & Baker, 2020). In *Rp*^{+/-} genotypes, RpS12, an essential, eukaryote-specific component of the ribosomal Small Subunit, is required to activate expression of Xrp1, a rapidly evolving AT-hook, bZip domain transcription factor (Fig 1C, Fig 2A,B) (Lee et al., 2018; Ji et al., 2019). Although *rpS12* null mutations are homozygously lethal, a particular point mutation, *rpS12*^{G97D}, appears defective only for the cell competition aspect of RpS12 function. Homozygotes for the *rpS12*^{G97D} mutation are viable, showing only minor effects on morphology and longevity, yet *rpS12*^{G97D} prevents elimination of *Rp*^{+/-} cells by cell competition (Kale et al., 2018; Ji et al., 2019). A key target of RpS12 appears to be the putative transcription factor Xrp1, because Xrp1 protein is barely detected in wild type cells but significantly elevated in *Rp*^{+/-} wing discs. Xrp1 controls most of the phenotype of *Rp*^{+/-} cells, including their reduced translation (Lee et al., 2018; Ji et al., 2019; Blanco et al., 2020). *Xrp1* mutants have negligible effect in wild type backgrounds, and normal lifespan (Baillon et al., 2018; Lee et al., 2018; Mallik et al., 2018). In response to RpS12 and Xrp1 activities, *Rp*^{+/-} cells both grow more slowly than surrounding *Rp*^{+/+} cells and are also actively eliminated by apoptosis that occurs where *Rp*^{+/-} cells and *Rp*^{+/+} cells meet (Baillon et al., 2018; Lee et al., 2018; Ji et al., 2019). Why apoptosis occurs at these interfaces in particular is not certain. A role for innate immune pathway components has been proposed (Meyer et al., 2014), and different levels of oxidative stress response in *Rp*^{+/-} cells and *Rp*^{+/+} cells (Kucinski, Dinan, Kolahgar, & Piddini, 2017) or local induction of autophagy have also been suggested (Nagata, Nakamura, Sanaki, & Igaki, 2019).

Here we test the hypothesis that cell competition specifically removes cells with aneuploidies that result in loss of *Rp* genes in *Drosophila* imaginal discs. We show that, as hypothesized previously (McNamee & Brodsky, 2009), most of the p53-independent cell death that follows irradiation resembles cell

competition genetically. We then use a targeted recombination method to investigate the fate of somatic cells that acquire large-scale genetic changes directly, and confirm that it is cell competition that removes cells heterozygous for large deletions, when they include ribosomal protein genes. By contrast, when ribosomal protein genes are unaffected, or when the cell competition pathway is inactivated genetically, cells carrying large deletions remain largely un-competed, proliferate, and contribute to adult structures. Thus, cell competition is a highly significant mechanism for elimination of aneuploid somatic cells. We discuss how cell competition to remove aneuploid cells could play a role preventing tumor development in humans.

RESULTS

Potential competition of irradiated cells

The idea that cell competition removes aneuploid cells was suggested by studies of p53-independent cell death following chromosome breakage or ionizing irradiation (Titen & Golic, 2008; McNamee & Brodsky, 2009). Importantly, in *Drosophila* cell competition of *Rp*^{+/-} cells does not depend on p53 (Kale, Li, Lee, & Baker, 2015). If the model was correct, it would be expected that the p53-independent apoptosis that follows irradiation would depend on the genes recently discovered to be required for cell competition.

We first confirmed the previous findings (Wichmann et al., 2006; McNamee & Brodsky, 2009). Irradiating third-instar larvae resulted in rapid induction of cell death in the wing imaginal disc that was largely p53-dependent (Figure 1A,C,D,E,G,H). The p53-dependent cell death, attributable to the DNA-damage response, was not much affected by the *rpS12*^{G97D} mutation that interferes with cell competition (Figure 1B,F,H). While total cell death tailed off with time, p53-independent cell death increased around 18-24 h post-irradiation, as reported previously (Wichmann et al., 2006; McNamee & Brodsky, 2009) (Figure 1I-L). As expected for cell competition, 24h after irradiation, p53-independent cell death was reduced by 66% in the *rpS12*^{G97D} p53 double mutant compared to the p53

mutant alone(Figure 1K-M). To exclude the possibility that other genetic background differences were responsible, *rpS12* function was restored to the *rpS12^{G97D}* *p53* double mutant strain using a P element transgene encoding the wild type *rpS12* gene, and this restored *p53*-independent cell death(Figure 1M). Notably, a genomic transgene encoding the *rpS12^{G97D}* cell competition-defective allele did not, leading to 86% less *p53*-independent cell death than the wild type *rpS12* transgene(Figure 1M). Thus, from 66-86% of the *p53*-independent apoptosis that was *RpS12*-dependent might represent cell competition.

Nuclear Xrp1 protein, which is only at low levels in control wing imaginal discs, was detected in scattered cells throughout *p53* mutant wing discs 24h after irradiation, similar to the distribution of dying cells (Figure 1N). Strikingly, most (58%) of this Xrp1 expression was *rpS12*-dependent, similar to the *rpS12*-dependency of *p53*-independent cell death itself(Figure 1O-P). This is also as expected for cell competition, which is mediated through the induction of Xrp1 expression(Baillon et al., 2018; Lee et al., 2018; Ji et al., 2019).

Radiation-damaged cells that have reduced *Rp* gene dose would be expected to differentiate short, thin bristles in adults, as reported previously in studies of DNA repair mutants(Baker et al., 1978), in studies of ionizing radiation(McNamee & Brodsky, 2009), and when aneuploidy is induced by mutation of spindle assembly checkpoint genes(Dekanty et al., 2012). We found *Rp^{+/-}*-like thoracic bristles at a frequency of ~1/300 following irradiation of either wild type or *p53* mutant larvae(Figure 1Q,R). Their frequency increased ~3-4 fold in *rpS12^{G97D}* mutants or *p53 rpS12^{G97D}* double mutants (Figure 1Q). In the absence of irradiation, only 2 *Rp^{+/-}*-like bristles were observed from 500 unirradiated *rpS12^{G97D}* mutant flies. Since 16 macrochaetae were examined on each fly thorax, this indicated a frequency of ~1/8,000 macrochaetae progenitor cells was *Rp^{+/-}*-like. We found none in 1000 unirradiated wild type flies (16,000 macrochaetae examined).

While the actual nature of radiation-induced genetic changes in cells forming Minute-like bristles is not directly demonstrated, previous studies of DNA-repair mutants including *mei-41*, the *Drosophila* ATR homolog,

demonstrated using multiply-marked chromosomes that the majority of Minute-like bristles reflect loss of heterozygosity for large, contiguous chromosome regions (Baker et al., 1978). In a small-scale experiment to compare γ -irradiation to the DNA repair defects studied previously (Baker et al., 1978), $y^{+/-} rpS12^{G97D}$ larvae were irradiated (1000 Rad) and 2178 adult flies examined for phenotypically y thoracic bristles representing cells where the y^{+} allele had been mutated or deleted. Six times more y bristles were recovered in females than in males. Since the y locus is X-linked, this is most easily explained if y bristles generally result from deletions including essential genes linked to y that could not survive in males. In females, 37.5% of y bristles were also phenotypically Minute, consistent with loss of chromosome regions extending at least from the y locus to the $RpL36$ gene 0.3 Mb more centromere-proximal that is the nearest Rp locus (Figure 9S). Although this study was small scale, these findings support the conclusion from DNA repair mutant studies that Minute-like bristles seen following irradiation most commonly reflect loss of substantial chromosome segments including Rp genes, (Baker et al., 1978). Accordingly, many Minute-like bristles removed by cell competition genes following irradiation could represent such segmentally-aneuploid cells.

Taken together, these bristle results are also consistent with the notion that cell competition removes $\sim 3/4$ of the cells with genetic changes that encompass dose-sensitive Rp loci that arise after irradiation.

FLP-*FRT* recombination to generate segmental aneuploidy

Having confirmed that cell competition could potentially be important for removing cells following irradiation, we sought to assess the fate of sporadic cells that lose chromosome regions, using an assay where the cell genotypes would be definitively known and the dependence on competition with normal cells could be established. We used the FLP-*FRT* site-specific recombination system (Golic & Lindquist, 1989) to achieve this, exploiting large collections of transgenic flies that contain *FRT* sequence insertions at distinct chromosomal locations (Thibault et al., 2004). FLP recombination between pairs of *FRT* elements linked in cis

excises intervening sequences to make defined deletions with a single *FRT* remaining at the recombination site(Figure 2A,B). Insertion elements of the Exelixis collection exist in several configurations, and FLP-mediated excision from paired *FRT* strains in the *FRT* w^+ ... w^+ *FRT* configuration removes both the associated w^+ genes, so that affected cells can be identified in the adult eye by loss of pigmentation(Figure 2A,B). Accordingly, we assembled a collection of genetic strains containing linked pairs of appropriate *FRT* w^+ elements, each flanking a distinct genomic region (Supplementary File 1).

It was first necessary to verify FLP recombination between *FRT* sequences, and investigate any cell-autonomous effects of the resulting segmental-monosomies. We used the eyFlp transgene, which confers continuous FLP expression to the eye and head primordia during larval life, so that FLP-*FRT* recombination is expected to approach completion(Newsome, Asling, & Dickson, 2000). Excision should result in white adult eyes, and also reveal any cell-autonomous effect of the resulting heterozygous deletion genotype on growth or differentiation of cells contributing to the adult eye. If the recombined genotype was autonomously cell-lethal, we would expect the developing animal to lack head structures and be unable to emerge from the pupa. If FLP-*FRT* recombination did not occur (or occurred inefficiently), we would expect adult eyes expressing the parental eye color (or with only scattered white spots that recombine late in development as cell number increases).

We identified 17 paired *FRT* strains that were efficient FLP targets in this assay. These 17 strains were completely or substantially white-eyed in the presence of eyFlp, indicating excision between *FRT* sites in most or all eye cells. We also identified paired *FRT* strains that were poor substrates for Flp (Supplementary File 1; Figure 2C-T). These either retained the parental eye color in the presence of eyFlp or produce a salt and pepper pattern of very small clones (Supplementary File 1; Figure 2U-Z). No genotype tested was inviable in the presence of eyFlp, so there was no evidence that haploinsufficiency of any of the chromosome segments tested was incompatible with cell viability or severely impacted head development. Instead, most of the 17 genotypes that recombined

differentiated heads of remarkably normal external appearance and morphology (Figure 2C-T).

Although we did not measure head size, we noticed 3 genotypes in which eyes and heads were obviously smaller, consistent with a reduced growth rate of the recombined genotypes. The three small eye regions were 48B2-50C1, 565F16-59B1, and 87B8-89E5 (Figure 2E,H,Q). Since the *Drosophila* genome is divided cytologically into 102 band intervals, each with lettered and numbered subdivisions, in this paper we refer to the chromosome $P\{XP\}^{d09761}$ $pBAC\{WH\}^{f00157}$, for example, by the cytological locations of the *FRT* sequences present in the P element and PiggyBac element insertions, which are at 48B2 and 50C1 respectively in this case (Supplementary File 1). This nomenclature quickly communicates the genome location under study, whether it overlaps or is distinct from that affected in another strain, and also indicates that in this case the *FRT* elements are likely separated by ~2% of the genome. The full description of each insertion strain is given in Supplementary File 1. The three excisions that substantially reduce eye size could delete a copy of one or more haploinsufficient genes important for growth during eye development, but there also could be a dominant effect of the novel junction generated by *FLP/FRT* recombination. Notably, excision between chromosome bands 87B8-93A2 led to eyes of normal size, although this excises all the sequences between 87B8-89E5 which led to reduced eyes. *FLP* recombination results in a different junctions in 87B8-89E5 and 87B8-93A2, however (Figures 2R).

Overall, these results showed that 17 segmentally-aneuploid genotypes were cell-viable, and able to grow and differentiate in the *Drosophila* eye, although a minority might have an effect on growth in this tissue. Recombination in these 17 strains each deleted from 1.4 Mb – 8.5 Mb of autosomal DNA, representing from 1-6% of the sequenced genome (Supplementary File 1, Figure 3A). Together these deletions encompass 25.3 Mb of DNA, corresponding to 21.1% of the *Drosophila* euchromatin and 17.7% of the sequenced genome. 11 of these 17 genotypes deleted one or more *Rp* loci (*RpS11*, *RpS13*, *RpS16*, *RpS20*, *RpS24*, *RpS30*, *RpL14*, *RpL18*, *RpL28* or *RpL36A*), whereas 6 affected

no *Rp* gene (Supplementary File 1, Figure 3A). In this paper, we use the symbol *Rp* to indicate a mutation affecting any of the 66 ribosomal protein genes that are dominant through haploinsufficiency, in distinction to 13 *Rp* encoded by *Drosophila* loci where heterozygous mutations have no phenotype.

Rp and *eIF2 γ* genes determine survival and growth of segmentally-aneuploid cells in mosaics

The seventeen FRT pair strains that were efficient FLP targets were each exposed to a single burst of FLP expression using the heat-shock FLP transgene, intended to stimulate excision in a fraction of cells during early larval life (see Methods). This led to mosaic eyes where segmentally-aneuploid cells and diploid cells would be in competition. Clones of excised cells appeared only after heat-shock, confirming strict FLP-dependence.

In contrast to eyFlp recombination, mosaic eyes containing sporadic clones of excised cells were only recovered at high frequencies for 4 segmental aneuploid genotypes, none of which affected *Rp* loci (Figure 3). This confirmed that most segmentally aneuploid genotypes were selected against in mosaic eyes where diploid cells were also present, because all had been shown to be intrinsically viable when competing diploid cells were absent (Figure 2). Importantly, no deletion that included an *Rp* locus showed more than minimal survival of sporadic clones induced with hsFlp, suggesting *Rp* loci could be the determinants of cell competition between segmental aneuploid and wild type cells (Figure 3B-D). To test this in a specific case, 5.6 kb of genomic DNA encompassing the *RpL28* locus was introduced onto the second chromosome using PhiC31-mediated transgenesis. This transgene proved completely sufficient to rescue the survival of cell clones heterozygous for Df(3L)63A3-65A9, a deletion of 3.2 Mb including the *RpL28* locus (Figure 4A-C). Clones of Df(3L)63A3-65A9 heterozygous cells barely survived alone, with a median contribution of 2% to the eyes of males and 0% to females (Figure 4H). In the presence of the *RpL28*⁺ transgene, however, Df(3L)63A3-65A9/+ clones survived in 49 out of 50 eyes, with median contributions of 37% of the eye in males and

53% in females, not statistically different from clones heterozygous for Df(3L)63C1-65A9, an overlapping deletion of 3.0 Mb excluding the *RpL28* locus(Figure 4H). Thus in this case, *RpL28* gene dose alone determined whether a segmentally-aneuploid genotype affecting hundreds of genes would be eliminated in competition with diploid cells.

Most of the other 10 segmental aneuploid genotypes that deleted one or more *Rp* loci contributed to adult eyes to a very significantly lower degree than overlapping segmental aneuploidies that spared *Rp* loci (Figure 3). This reflected the fact that, in contrast to genotypes affecting *Rp* genes, 4/6 segmental aneuploidies that spared *Rp* loci survived in eye clones at high frequencies and large sizes (Figure 3A-C). The two exceptions were Df(3R)87B8-89B16/+ and Df(3R)87B8-89E5/+, for which little eye tissue was recovered(Figure 3A-C). Although neither affected any *Rp* gene, both deleted a locus mapping to 88E5-6 encoding the translation factor eIF2 γ . Since independent studies in our laboratory already identified a role for the eIF2 α protein in cell competition (Kiparaki, Khan, Chuen and Baker, in preparation), we tested the possible role of eIF2 γ by restoring eIF2 γ diploidy to Df87B8-89B16/+ or Df87B8-89E5/+ cells using a 11.5 kb genomic transgene including the eIF2 γ locus(Tschiersch et al., 1994). This completely rescued the growth and differentiation of these cells to the levels typical for aneuploidies not affecting *Rp* genes(Figure 4D-G, I). Thus, the locus encoding the translation factor eIF2 γ behaved similarly to an *Rp* gene in triggering competitive elimination of heterozygous cells.

If these studies, which tested a significant fraction of the *Drosophila* genome, are representative, they indicate that the normal diploid complement of *Rp* loci is important for sporadic segmentally-aneuploid cells to evade cell competition, and that few other genes are comparably important. The one example of such another gene uncovered in our analysis encoded eIF2 γ , another protein affecting translation.

Apoptotic genes contribute to removing segmentally-aneuploid cells

If the segmentally-aneuploid cells were competed by virtue of their $Rp^{+/-}$ genotypes, the genetic pathways should be similar. Elimination of $Rp^{+/-}$ point-mutant cells by competition depends on apoptosis and is suppressed by a genetic deletion, *Df(3L)H99*, that removes three pro-apoptotic genes *reaper* (*rpr*), *grim*, and *head-involution defective* (*hid*) (Moreno et al., 2002; Kale et al., 2015). These genes are also required for the p53-independent cell death that follows irradiation, much of which resembles cell competition (Figure 1) (McNamee & Brodsky, 2009). Using the *Df(3L)63A3-65A9*, where clone loss was demonstrably due to heterozygosity for the *RpL28* gene (Figure 4H), we found that recovery of *Df(3L)63A3-65A9/+* cell clones was enhanced by genetic suppression of apoptosis in the *Df(3L)H99/+* background that experiences loss of heterozygosity for *rpr*, *grim*, and *hid* (Figure 5A,D). A similar rescue was obtained with the *Df(3L)63C1-65F5*, which deletes the *RpL18* locus (Figure 5A,D). The recoveries of *Df(3L)63A3-65A9/Df(3L)H99* clones and *Df(3L)63C1-65F5/Df(3L)H99* clones approached that of the overlapping genotype *Df(3L)63C1-65A5/Df(3L)H99*, in which no *Rp* genes were affected (Figure 5A,D). Recovery was quantitatively inferior to that seen for *Df(3L)63A3-65A9/+ p{RpL28⁺}* clones (Figure 4H), but it is to be noted that the *Df(3L)H99/+* background unexpectedly reduced recovery of the control *Df(3L)63C1-65A5/+* cells (Figure 5A). Regardless of whether this reflects the recently-described role for basal caspase activity in promoting imaginal disc growth in the wild type (Shinoda, Hanawa, Chihara, Koto, & Miura, 2019), or some other genetic interaction, it complicates assessment of whether H99 heterozygosity and *RpL28⁺* transgenesis rescue *Df(3L)63A3-65A9/+* clones equally. We attempted to prevent apoptosis more completely using the genetic background *hid^{WRX1}/Df(3L)H99* in which *hid* is homozygously affected in addition to heterozygosity for *rpr* and *grim*. Although *hid^{WRX1}/Df(3L)H99* adult animals were recovered in the absence of other mutations, they became exceptionally rare in heat-shocked combinations with the dual *FRT* chromosomes: as a result, insufficient data could be obtained to address this question. In any case, it is clear from our results that

pro-apoptotic genes contribute significantly to eliminating segmentally-aneuploid cells (Figure 5A).

Xrp1 and RpS12 participate in removing segmentally-aneuploid cells

Mutations in the *rpS12* and *Xrp1* genes are more specific for cell competition than mutations in cell death genes. The *rpS12* and *Xrp1* mutations prevent the elimination of *Rp*^{+/-} point mutant cells from mosaics, but otherwise lead to seemingly normal flies, and do not affect other cell death processes (Kale et al., 2018; Lee et al., 2018). These genes should be required if segmental aneuploid cells are competed due to reduced *Rp* gene dose. Our results strongly support this conclusion in nearly all cases. Because the results are too extensive to present together in a single figure, they are presented in groups according to chromosome region (Figures 5-8).

Beginning with the Df(3L)63A3-65A9/+ genotype where clone loss was demonstrably due to heterozygosity for the *RpL28* gene (Figure 4H), we found that heterozygosity for an *Xrp1* mutation greatly restored contribution of Df(3L)63A3-65A9/+ clones (*RpL28*^{+/-}) to the eye (Figure 5B,D). Similar results were seen for the Df(3L)63C1-65F5/+ genotype that is heterozygous for *RpL18*, but *Xrp1* heterozygosity did not affect recovery of clones of the overlapping Df(3L)63C1-65A9/+ that is *Rp*^{+/+} (Figure 5B,D). The *Xrp1* mutation even improved the survival of larger segmental-aneuploidies where combinations of the *RpL18*, *RpL28*, and *RpL14* genes were affected (Figure 6A-C).

Because the *rpS12*^{G97D} mutation that affects cell competition recessively maps to the third chromosome, it was simpler to examine in combination with segmental aneuploidies affecting chromosome 2. These aneuploid eye clones, which like those discussed above were also recovered in the presence of an *Xrp1* mutation, included Df(2L)26A1-29F8/+, heterozygous for *RpL36A* and *RpS13*, and Df(2R)56F16-59B1/+, heterozygous for *RpS16* and *RpS24* (Figure 7A,B,D). Clones of the Df(2L)26A1-28C3/+ or Df(2R)56F16-58E2/+ cells that did not affect any *Rp* loci were recovered at high rates, independently of *Xrp1* genotype (Figure 7A,B,D). As expected, *rpS12*^{G97D} homozygosity also led to

significant recovery of Df(2R)56F16-59B1/+ clones that were heterozygous for *RpS16* and *RpS24*, although to a quantitatively lesser degree than *Xrp1* (Figure 7C,D). The contribution of Df(2R)56F16-58E2/+ cells, where no *Rp* gene is affected, was unaltered by the *rpS12^{G97D}* mutation (Figure 7C,D).

Xrp1 also affected other segmentally-aneuploid regions. *Xrp1* mutations had a minor but statistically significant effect on clones of Df(2R)48B2-50C1/+ cells, heterozygous for *RpS11* (Figure 8A,D), a genotype that also had an autonomous effect on eye growth (Figure 2E). *Xrp1* mutations enhanced the contributions of Df(3R)87B8-89B16/+ and Df(3R)87B8-89E5/+ cells that were heterozygous for the *eIF2 γ* gene (Figure 8B-D). Crossing to *Xrp1* only slightly improved survival of Df(3R)87B8-93A2/+ clones that were heterozygous for the *eIF2 γ* , *RpS20* and *RpS30* genes, and did little to enhance recovery of Df(3R)89B13-93A2/+ genotypes that were heterozygous for *RpS20* and *RpS30* alone. As these deficiencies already delete the *Xrp1* locus itself (within 91D3-5 region), introducing an *Xrp1* mutation in trans leads to *Xrp1^{-/-}* genotypes (Figure 8B-D). Heterozygous mutation of *Xrp1* is already sufficient to suppress competition of *Rp^{+/-}* point mutant cells (Lee et al., 2018), probably explaining why *Xrp1* homozygosity had little further effect.

Surrounding *Rp^{+/+}* cells are necessary to eliminate segmentally-aneuploid cells

Further evidence that segmentally-aneuploid cells are eliminated by cell competition due to their *Rp*/+ genotypes came from studies in homotypic *Rp* mutant backgrounds (Figure 9). It is known from previous work that cells heterozygous at two *Rp* loci do not suffer more severe competition than cells heterozygous for only one *Rp* mutation, and therefore that cells heterozygous for two *Rp* loci generally cannot be eliminated by cells heterozygous at only one *Rp* locus (Simpson & Morata, 1981). Accordingly, clones of segmental aneuploid cells affecting the *RpS11*, *RpS13*, *RpS16*, *RpS20*, *RpS24*, *RpS30*, *RpL14*, *RpL18*, *RpL28* or *RpL36A* genes were all recovered significantly better in an *RpS3* point mutant background, ie *RpS3^{+/-}* Df(*Rp*)/+ clones were not eliminated from *RpS3^{+/-}* tissues (Figure 9A,B,D). This applied to segmental aneuploid clones

heterozygous for *eIF2 γ* as well (Figure 9C,D). On the other hand, the *RpS3* point mutant background usually had no effect on the survival of clones of genotypes that did not delete other *Rp* loci (Figure 9C). One exception was *Df(2L)26A1-28C3/+*, for which the *RpS3*^{+/-} background generated significantly larger clones (Figure 9C,D). Notably, *Df(2L)26A1-28C3/+* cells had previously been recovered less than the other non-*Rp* segmental aneuploidies (Figure 3B,C; Figure 9C). Although this could also have reflected a lower rate of FLP-recombination between the 26A1 and 28C3 *FRT* sites, in the *RpS3*^{+/-} background the recovery of *Df(2L)26A1-28C3/+* clones was similar to that of *Df(2R)48F6-50C1/+*, *Df(2R)56F16-58E2/+* or *Df(3L)63C1-65A9/+*, suggesting instead that *Df(2L)26A1-28C3/+* might be subject to a mild cell competition that can be rescued in the *RpS3*^{+/-} background (Figure 9C).

Although suppression of apoptosis, mutation of cell competition genes, or a germline-inherited *RpS3* background all restored the growth and survival of cells hemizygous for *Rp* loci, they may not have done so equally. Suppressing apoptosis was least effective at expanding the contribution of aneuploid cells in the rescued eyes (Figure 5A). *Xrp1*, *rpS12*^{G97D}, and the *RpS3*^{+/-} background suppressed cell competition to similar extents, although the general *RpS3* background often had the greatest effect, comparable to those of *RpL28*⁺ and *eIF2 γ* ⁺ transgenes (see statistical comparisons for the 63A3-65F5 and 56F16-59B1 regions in Figures 5,6 legends). Although several explanations could justify these differences, it is worth noting that the results correlate with the effects of these genetic backgrounds on translation and growth. Thus *Df(3L)H99*, which suppresses apoptosis with no known increase translation or cellular growth, is expected to suppress the competition of *Rp*^{+/-} cells but not restore their translation. As a consequence, clones of *Rp*^{+/-} *Df(H99)/+* cells, although surviving, are not expected to grow as rapidly or contribute as much to the eye as clones of *Rp*^{+/+} cells. By contrast the *rpS12*^{G97D} and *Xrp1* mutations restore the general translation rate of *Rp*^{+/-} cells, with *Xrp1* mutation also restoring more normal rates of cellular and organismal growth (Lee et al., 2018; Ji et al., 2019). A background mutation in *RpS3* is not expected to restore

translation or growth to segmentally aneuploid cells, but by equally impairing the unrecombined cells, and systemically delaying the growth and developmental rate of the organism as a whole, it equalizes the contributions of aneuploid and control genotypes.

In summary, our results strongly support the conclusion that the growth and survival of most segmentally-aneuploid regions is determined by cell competition according to *Rp* gene copy number, and show that the *RpS12/Xrp1*-dependent process that eliminates *Rp*^{+/-} point mutated cells also acts on cells with large losses of genetic material that include *Rp* genes.

Competition of segmentally-aneuploid cells in the thorax

Experiments using the hsFLP transgene should stimulate recombination and segmental aneuploidy in all tissues, not only in the eye where excision causes loss of pigmentation. To test this we looked for cells with deletions encompassing *Rp* loci in the thorax, where *Rp* haploinsufficiency leads to small, thin thoracic bristles (Marygold et al., 2007). This was explored using *Df(2R)56F16-59B1*, which deletes the *RpS16* and *RpS24* loci. Minute-like bristles were not observed on the thoraces of heat-shocked flies carrying *Df(2R)56F16-58E2* heterozygous clones, which affect no *Rp* locus, or on the thoraces of heat-shocked *56F16-59B1* flies lacking *rpS12* or *Xrp1* mutations, but they represented 0.5% of the thoracic bristles in the *56F16-59B1/+ rpS12^{G97D}* flies and 0.25% of the thoracic bristles in the *56F16-59B1/+ Xrp1^{m2-73/+}* background (Figure 10A,B). These findings indicate that *Df(2R)56F16-59B1/+* cells also survive to adulthood in the thorax if cell competition is suppressed, albeit at lower frequency than observed in the eye.

Discussion

We sought to test the hypothesis that cell competition is a mechanism that can target aneuploid cells based on their altered *Rp* gene dose (McNamee & Brodsky, 2009). It was already known that cells carrying point mutations at *Rp*

loci are eliminated from developing imaginal discs by cell competition (Morata & Ripoll, 1975; Simpson, 1979; Baker, 2020). Here we tested whether cells with more extensive genetic defects that reduce *Rp* gene dose also experience cell competition, and if so how significant this is for the removal of cells with damaged genomes.

The idea that cell competition eliminates aneuploid cells developed from studies of cellular responses to DNA damage, where a delayed, p53-independent process follows after the acute, p53-dependent DNA damage response (Wichmann et al., 2006; Titen & Golic, 2008; McNamee & Brodsky, 2009). We found that a substantial proportion of p53-independent cell death shared genetic requirements with cell competition, consistent with cell competition being responsible (Figure 1). Accordingly, when the cell competition pathway was inhibited, more Minute-like bristles were recovered on the irradiated flies, an indication that cell competition could be removing cells that experience substantial losses of genetic material (Figure 1R). A proportion of both the p53-independent cell death and Minute-like bristles were independent of *rpS12*, however, suggesting that cell competition might not be the only process at work.

To measure the role of cell competition on defined genotypes, where the role of surrounding wild type cells could also be assessed, we then used site-specific recombination to excise chromosome segments from isolated cells during imaginal disc development. As expected, segmental aneuploidy prevented cells contributing clones to the adult eye whenever *Rp* gene dose was reduced (Figure 11A). More significantly, cell competition appears to be the primary mechanism limiting the contribution of segmental aneuploidies in the tested size ranges to adult tissues, because segmental aneuploid cells easily survived and contributed large fractions of the adult tissue when they did not affect *Rp* loci, when diploidy for *Rp* loci was restored with a transgene, or when the cell competition pathway that depends on *RpS12* and *Xrp1* function was mutated.

The segmental-aneuploid genotypes examined here were able to form entire heads of aneuploid cells when *eyFlp* was used to drive recombination in all

the cells (Figure 2). The removal of sporadic aneuploid cells therefore depended on competition with diploid cells. In fact in the cases of Df(2R)56F16-59B1/+, heterozygous for the *RpS16* and *RpS24* genes, and Df(3L)65A5-65A9/+, heterozygous for the *RpL28* gene, we bred flies that received heat-shock recombination, and recovered non-mosaic, entirely segmentally aneuploid flies in the next generation, derived from FLP-*FRT* recombination in the germlines of the parents. Thus, these segmentally-aneuploid genotypes, which rarely survived in sporadic clones, were viable in all tissues when competing wild type cells were not present.

The most effective suppression of *Rp*^{+/-} segmental aneuploid clones was generally seen when the whole animal was heterozygous for a point mutation in *RpS3* (Figure 9). This is further, compelling evidence that cell competition due to reduced *Rp* gene dose is the main mechanism eliminating segmentally-aneuploid because it shows that the feature of euploid cells that enables them to eliminate aneuploid cells is their *Rp*^{+/+} genotype.

In contrast to these results, segmental aneuploidy leaving *Rp* loci unaffected was compatible with clonal growth and differentiation for four of the five genomic regions tested (Figure 3). In the exception we identified *eIF2γ* as the locus responsible for loss Df(3R)87B8-89B16/+ clones and Df(3R)87B8-89E5/+ clones (Figure 4I). No point mutant alleles of the *eIF2γ* gene are known and the locus is believed to be haplo-lethal to *Drosophila* (Marygold et al., 2007). It is cell competition that eliminates *eIF2γ*^{+/-} aneuploid cells from the eye, however, since they could form apparently normal adult heads when no diploid competitor cells were present (Figure 2P-R). Moreover, clones of the *eIF2γ*^{+/-} genotypes Df(3R)87B8-89B16/+ and Df(3R)87B8-89E5/+ were restored by both the *Xrp1* mutant and by the *RpS3*^{+/-} mutant background, as expected for cell competition (Figure 8). It is possible that the 26A1-28C3 region might also contain a non-*Rp* gene whose deletion leads to a cell competition, although much less severe.

Out of 63 other translation factor genes examined in a systematic study of whole body, non-mosaic phenotypes, *eIF2α* and *eIF2γ* were the only

haploinsufficient loci found(Marygold et al., 2007). Notably, the *eIF2 α* gene is the only other locus known where point mutants lead to the developmental delay and thin bristle phenotype that is otherwise typical of heterozygous *Rp* mutants(Marygold et al., 2007), suggesting a functional relationship between *Rp* mutants and the eIF2 complex. Independent studies in our laboratory already indicate that eIF2 α is regulated by Xrp1 and contributes directly to the cell competition mechanism (Kiparaki, Khan, Cheun and Baker, in preparation).

Previous studies suggested that cells with whole chromosome aneuploidies experience a stress associated with mismatched dose of many proteins, either related to the unfolded protein response or to hypo-osmotic stress(Torres et al., 2007; Zhu et al., 2018; Tsai et al., 2019). We cannot measure how such stresses reduced clonal growth of segmental aneuploid cells in our experiments, but the effect must be small compared to cell competition, since without cell competition, segmental aneuploid cells easily contributed half or more of the eye, whereas cell competition drastically reduces this contribution. It seems unlikely that all five independent genomic regions examined here, comprising 21.1% of the euchromatic genome, represent exceptional cases. It is plausible, however, that additional stresses increase in importance with greater chromosome loss, eg clones heterozygous for a 6.3Mb deletion removing the *RpL14*, *RpL18*, and *RpL28* loci were recovered less well than smaller deletions (Figure 6), as if larger monosomies experience other stresses in addition to cell competition. Since extra copies of at least one *Rp* gene do not trigger cell removal (Kale et al., 2018), other mechanisms would also be required to eliminate cells with triploidies, or act in tissues that lack cell competition(Ripoll, 1980). Finally, preventing apoptosis of *Drosophila* cells that have chromosome instability leads to invasive tumor growth that can be propagated after transplantation(Dekanty et al., 2012; Benhra, Barrio, Muzzopappa, & Milan, 2018). We did not observe invasive growth after blocking cell death of segmentally-aneuploid cells, suggesting that chromosome instability may lead to different classes of aneuploidy, or to other additional effects.

If cell competition is the main mechanism eliminating cells with segmental monosomies, at least up to a certain size, how important is this? The segmental aneuploidies we studied were comparable in genetic content to some whole chromosome monosomies in humans. For example, cells heterozygous for Df(2R)56F16-58E2 were hemizygous for a 2.2Mb region including 1.5% of the genome, about as large a region as can be expected to lack any *Rp* gene, encoding 333 protein coding genes and 55 non-coding RNAs. Human chromosome 21, which also contains 1.5% of the genome that lacks any *Rp* gene, encodes 234 protein coding genes and 404 non-coding RNAs(Uechi et al., 2001). The similarity is not coincidental, because *Rp* number is conserved and the total gene number is also comparable, so genome segments that lack *Rp* loci are expected to be similar when measured by gene number or fraction of the genome. Thus, Df(2R)56F16-58E2 is comparable in genetic terms to loss of a small human chromosome. Some of the segmental aneuploidies we studied in *Drosophila* were several-fold larger than Df(2R)56F16-58E2 (Supplementary File 1; Figure 3A). Thus, our studies may best model aneuploidies affecting one or a few human chromosomes. Because ~80% of the *Drosophila* genome is carried on two autosomes, whole-chromosome aneuploidies in *Drosophila*, by contrast, better mimic complex karyotypes seen in tumors or in cells with chromosome instability, which affect many chromosomes.

Little is known about what aneuploidies arise spontaneously in normal development. Ionizing radiation generates many kinds of chromosome aberration, so if more than half the p53-independent cell death following irradiation resembles cell competition(Figure 1), this suggests cell competition could be significant for removing many, although not all, the damaged cells that arise.

Could cell competition be important in humans? As in *Drosophila*, the 80 *Rp* gene loci are distributed seemingly randomly around the 24 pairs of human chromosomes(Uechi et al., 2001). At least 21 human *Rp* genes have so far been found to be haploinsufficient and are responsible for the dominant syndrome Diamond Blackfan Anemia (DBA)(Ulirsch et al., 2018). Thus *Rp* genes could be

sensors for aneuploidy in humans. The retention of aneuploid cells in *Drosophila* that inherit an *Rp* mutation from the germline(Figure 9) resembles the situation in human DBA patients, the majority of whom are constitutively heterozygous for a *Rp* gene mutation or deletion(Ulirsch et al., 2018). DBA patients experience 4.8x higher lifetime incidence of multiple cancers, not limited to the hematopoietic system(Vlachos et al., 2018). Current hypotheses for this cancer predisposition include specific alterations to the spectrum of translation due to defective ribosome biogenesis, a loss of translational fidelity due to selection of second-site suppressor mutations, selective pressure for p53 mutations due to the chronic p53 activity in such genotypes, and oxidative stress or metabolic reprogramming in *Rp*^{+/−} cells(Sulima, Kampen, & De Keersmaecker, 2019). To these we can now add the possibility that DBA patients experience a diminished capacity to recognize and eliminate aneuploid cells, because their euploid cells are not *Rp*^{+/+}(Figure 11B). The nearly five-fold increase in tumor incidence suggests that if this was correct, cell competition might remove as many as 80% of pre-neoplastic cells from normal individuals due to their aneuploidy, This seems comparable to our findings that cell competition removes from 58-86%, depending on the assay, of the cells with radiation-damaged genomes in *Drosophila*(Figure 1M,P,R).

MATERIALS AND METHODS

Fly strains and FLP-*FRT* methods

Flies were reared on standard medium at 25°C unless otherwise noted. The genetic strains used are described in the Key Resources Table.

Strains carrying pairs of *FRT* transgenic elements in cis were obtained after meiotic recombination using appropriate genetic crosses, monitoring recombination frequency to confirm the expected transgene locations. FLP expression was induced by 37°C heat shock for 30 or 60 min at 36±12h after egg laying. Adult flies were aged ~ 1 week to allow eye color to darken fully, then stored at -20°C for later measurement and photography. The fraction of each adult eye populated by unpigmented cells was estimated manually under a dissecting microscope by an independent investigator. Samples were blinded for genotype before scoring. Although we don't think these measurements are subject to large errors, it is worth mentioning that the Mann Whitney procedure used to compare results between genotypes compares relative sizes of clones, not absolute.

Many of the genetic backgrounds in which mosaics were generated carried other, distant *FRT* sites as part of the *FRT82B Xrp1^{m2-73}*, *FRT82B RpS3*, *rpS12^{G97D}*, *FRT80B*, *Df(3L)H99 FRT80B* chromosomes. Accordingly, the control backgrounds in these experiments always included *FRT82B* or *FRT80B*, as appropriate, and as described in the figure legends.

The *RpL28* rescue transgene was obtained by inserting genomic sequences 3L: 3220152-3225729 (*Drosophila* genome Release 6) into pTL780, which uses DsRed expression as a transgenic marker (Blanco-Redondo & Langenhan, 2018). The genomic DNA was amplified from the *Drosophila* genomic reference strain (Adams & al., 2000). The resulting pTL780(RpL28+) plasmid was used for integration at the VK37 landing site on chromosome 2 (Venken et al., 2009).

For irradiation, food vials containing larvae were exposed to 500, 1000 or 4000 rad from a γ -ray source 84±12 h after egg laying. Dissection, fixation, and

immuno-labeling of wing imaginal discs with anti-active Dcp1 and anti-Xrp1 was performed as described previously (Baker, Li, Quiquand, Ruggiero, & Wang, 2014; Lee et al., 2018).

Statistics. Frequencies of cell death and of Xrp1 expression were compared pairwise by t-tests (Figure 1). For multiple comparisons, 1-way ANOVA was used with the Holm correction for multiple testing (Figures 1,10). Previous studies indicated that significant results could be obtained from 5 biological replicates, where a biological replicate is an imaginal disc preparation labeled, imaged, and quantified (McNamee & Brodsky, 2009). N for each experiment is reported in the figure legends. The extent of white tissue in mosaic eyes was compared using pairwise Mann-Whitney tests with the Benjamini-Hochberg (BH) correction for multiple testing, using $FDR \leq 0.05$. There are 109 pairwise Mann-Whitney comparisons made in the main text of this paper, their P-values and the BH corrections are summarized in Supplementary File 2. Where the extent of white tissue in mosaic eyes was compared between multiple genotypes simultaneously, the Kruskal-Wallis test was used with post-hoc follow-up tests using the method of Conover with BH correction using $FDR \leq 0.05$. No explicit power analysis was used. All flies obtained were scored in initial experiments, sometimes leading to unequal sample sizes, subsequently we considered 20 eyes of each sex generally sufficient for significant results (while the number of flies that can be obtained is rarely limiting, blinding and scoring clone sizes is time-consuming). N is given in the figures for each experiment. All the figures show experimental and control data obtained from simultaneous parallel experiments in each case, for which all the data scored were included. Some of the genotypes have been generated on multiple occasions with similar results, not all included in the figures.

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FIGURES

Figure 1 Role of cell competition after irradiation

Panels A-G and I-L show third instar wing imaginal discs labeled to detect the activated Dcp1 caspase in apoptotic cells at the indicated times post-irradiation. A) extensive cell death follows within 4h after gamma-irradiation (4000 Rads). B) Little change is seen in the *rpS12^{G97D}* mutant. C) Most of the acute cell death is

760 p53-dependent, consistent with a DNA damage response. D) A small amount of
761 cell death persists in the *rpS12^{G97D} p53⁻* double mutant. E) Cell death is reduced
762 to more quantifiable levels 4h after a lower radiation dose (500 Rad). F) This
763 DNA damage-induced cell death is not significantly affected by the *rpS12^{G97D}*
764 mutation. G) Most cell death 4h after irradiation is p53-dependent. H)
765 Quantification of cell death (numbers of cells per wing pouch) 4h following
766 irradiation with 500 Rad. ns – difference not statistically significant ($p>0.05$). ** -
767 difference highly significant ($p<0.01$). N = 6 for each genotype. I) Cell death
768 24h after irradiation (4000 Rad). J) Comparable levels of cell death in *rpS12^{G97}*
769 mutants. K) Although cell death appears reduced in *p53* mutant wing discs 24h
770 after irradiation compared to the wild type control (4000 Rad), this p53-
771 independent cell death is substantially increased compared to 4h after irradiation
772 (compare panel C). L) p53-independent cell death is reduced in the *rpS12^{G97D}*
773 *p53⁻* double mutant compared to the *p53⁻* mutant. M) Cell death quantification in
774 *p53* and *rpS12^{G97D} p53⁻* wing discs, and in wing discs from *rpS12^{G97D} p53⁻* larvae
775 carrying genomic transgenes encoding either wild type *rpS12* or *rpS12^{G97D}*. The
776 results show that between 66-86% of p53-independent cell death was *RpS12*-
777 dependent. We did not quantify cell death in wild type and *rpS12^{G97D}* wing discs
778 because of the large number and aggregation of dead cells. ns – difference not
779 statistically significant ($p>0.05$). ** - difference highly significant ($p<0.01$). N: 10
780 (*p53⁻*); 13 (*rpS12^{G97D} p53⁻*); 9 (*P{rpS12⁺} rpS12^{G97D} p53⁻*); 8 (*P{rpS12^{G97D}}*
781 *rpS12^{G97D} p53⁻*). N) 24h after irradiation, *p53* mutant wing discs exhibit cells
782 expressing nuclear Xrp1 in a pattern similar to that of dying cells. We were
783 unable to double-label with anti-Xrp1 and anti-active Dcp1 simultaneously
784 because both are rabbit antisera. n: 33 (*p53⁻*); 26 (*rpS12^{G97D} p53⁻*). O) Fewer
785 Xrp1-expressing cells were seen in *rpS12^{G97D} p53⁻* double mutant wing discs. P)
786 Quantification of Xrp1 expression. Most (58%) of the p53-independent Xrp1
787 expression 24h post-irradiation was *RpS12*-dependent. ** - difference highly
788 significant ($p<0.01$). Q) Irradiated adult flies with a short, thin scutellar bristle
789 (white arrow, compare normal contralateral bristle – black arrow) like those
790 typical of *Rp^{+/-}* mutant flies. R) The frequency of sporadic *Rp*-like bristles

increases 3.33x and 4x on the thoraces of *rpS12^{G97D}* and *rpS12^{G97D} p53* flies (respectively) where cell competition is inhibited. ns – difference not statistically significant ($p > 0.05$). ** - difference highly significant ($p < 0.01$). N=3 sets of 100 flies for each genotype. S) Frequencies of *y* bristles found on the thoraces of ~2000 *y/+* ; *rpS12^{G97D}* female and *+/Y* ; *rpS12^{G97D}* male flies following irradiation (1000 rad) in the mid-third larval instar. The preponderance of *y* bristles in females suggests that induced *y* mutations typically affect other genes including essential genes. The occurrence of phenotypically *y M* bristles in females is consistent with deletions extending at least from the *y* locus to the nearest *Rp* locus, *RpL36*.

Statistics: Significance was assessed using t-test (panel P) or 1-way ANOVA with the Holm procedure for multiple comparisons (panels H,M,R). ** = $p < 0.01$. ns = not significant. Genotypes: A, E, I) *w¹¹⁻¹⁸* B, F, J) *rpS12^{G97D}* C,G,K,N) *p53^{5A-1-4}* D, L, O) *rpS12^{G97D} p53^{5A-1-4}*.

Figure 2 Generating segmental aneuploidy with FLP-FRT recombination

A). Segmental aneuploidy can be generated in a mosaic fashion using FLP-FRT. At left is a cell carrying two transposable elements encoding *w*⁺ and *FRT*, arranged in cis on one chromosome arm, in this case at chromosome bands

811 63C1 and 65A9, respectively. B) FLP-mediated recombination between *FRT*
812 sites excising the intervening sequences. In this example where the w^+ genes lie
813 between the *FRT* sites, both are excised resulting in a loss of eye pigmentation.
814 The *RpL28* gene at chromosome band 63B14 lies outside the deletion and is
815 unaffected. B) A comparable recombination between elements at chromosome
816 bands 63A3-65A9 also deletes the *RpL28* locus, so that the resulting
817 segmentally-aneuploid cells are heterozygously deleted for this gene. These
818 cartoons show recombination in G1-phase of the cell cycle. In the G2-phase
819 configuration, recombination between non-homologous *FRT* sites on the
820 chromatids can occur leading to a deleted chromatid and a chromatid bearing 3
821 *FRT* insertion elements and a duplication of the intervening region. Such
822 genotypes are substrates for further FLP recombination to the parental or deleted
823 state, but sometimes we see them persist in adults and an example is shown in
824 Figure 3D. Panels C-Z show adult eyes in the presence of *eyFlp*, which drives
825 recombination close to completion in the eye and head, with the chromosome
826 positions of parental w^+ *FRT* insertions indicated. Panels C-T show genotypes
827 where *eyFlp* recombined most eye cells, Panels U-Z illustrate genotypes where it
828 did not. Strains that were poor substrates for *Flp*, either retained the parental
829 eye color in the presence of *eyFlp*, or produce a salt and pepper pattern of very
830 small clones that is indicative of excision occurring only late in development once
831 large cell numbers are present. See Table 1 for more details and further
832 genotypic information. C) $y w eyFlp; P\{XP\}^{d08241} PBac\{WH\}^{f04888}/+$; D) $y w$
833 $eyFlp; P\{XP\}^{d08241} PBac\{WH\}^{f00857}/+$. This recombination deletes the *RpL36A*
834 and *RpS13* genes; E) $y w eyFlp; P\{XP\}^{d09761} PBac\{WH\}^{f00157}/+$. This
835 recombination deletes the *RpS11* gene. Note the particularly small size of the
836 recombinant heads; F) $y w eyFlp; P\{XP\}^{d09417} PBac\{WH\}^{f00157}/+$. G) $y w eyFlp;$
837 $P\{XP\}^{d02302} PBac\{WH\}^{f04349}/+$; H) $y w eyFlp; P\{XP\}^{d02302} PBac\{WH\}^{f00464}/+$. This
838 recombination deletes the *RpS16* and *RpS24* genes. Note the particularly small
839 size of the recombinant heads; I) $y w eyFlp; P\{XP\}^{d02302} PBac\{WH\}^{f00464}/Xrp1^{m2-}$
840 ⁷³. Eye size is partially rescued by heterozygosity for *Xrp1*. J) $y w eyF$;
841 $PBac\{WH\}^{f01922} P\{XP\}^{d02570} /+$. This recombination deletes the *RpL28* gene. K)

842 *y w eyF* ; *PBac{WH}^{f01922} P{XP}^{d02813} /+*. This recombination deletes the *RpL28*
843 and *RpL18* genes; L) *y w eyF* ; *PBac{WH}^{f01922} P{XP}^{d07256} /+*. This
844 recombination deletes the *RpL28*, *RpL18*, and *RpL14* genes; Note the small eye
845 size. M) *y w eyF* ; *PBac{WH}^{f05041} P{XP}^{d02570} /+*; N) *y w eyF* ; *PBac{WH}^{f05041}*
846 *P{XP}^{d02813} /+*. This recombination deletes the *RpL18* gene; O) *y w eyF* ;
847 *PBac{WH}^{f05041} P{XP}^{d07256} /+*. This recombination deletes the *RpL18* and *RpL14*
848 genes; P) *y w eyF* ; *P{XP}^{d06796} PBac{WH}^{f04937} /+*. This recombination deletes
849 the *eIF2 γ* gene. Q) *y w eyF* ; *P{XP}^{d06796} PBac{WH}^{f00971} /+*. This recombination
850 deletes the *eIF2 γ* gene. Note the particularly small size of the recombinant
851 heads, which also retain an unusual amount of unrecombined cells; R) *y w*
852 *eyF* ; *P{XP}^{d06796} PBac{WH}^{f03502} /+*. This recombination deletes the *eIF2 γ* , *Xrp1*,
853 *RpS20* and *RpS30* genes. S) *y w eyF* ; *P{XP}^{d06928} PBac{WH}^{f03502} /+*. This
854 recombination deletes the *Xrp1*, *RpS20* and *RpS30* genes. T) *y w eyF* ;
855 *P{XP}^{d06928} PBac{WH}^{f01700} /+*. This recombination deletes the *Xrp1*, *RpS20* and
856 *RpS30* genes. U) *y w eyF* ; *PBac{WH}^{f04180} P{XP}^{d07944} /+*. The whole eye
857 resembles the parental genotype lacking *eyFlp*; V) *y w eyF* ; *P{XP}^{d06796}*
858 *PBac{RB}^{e03186} /+*. The whole eye resembles the parental genotype lacking
859 *eyFlp*; W) *y w eyF* ; *P{XP}^{d06796} PBac{RB}^{e03144} /+*. The whole eye resembles
860 the parental genotype lacking *eyFlp*. X) *y w eyF* ; *P{XP}^{d08241} PBac{RB}^{e02272} /+*.
861 The eye has a mottled appearance indicative of small recombinant clones
862 generated late in development; Y) *y w eyF* ; *P{XP}^{d08241} PBac{RB}^{e03937} /+*. The
863 eye has a mottled appearance indicative of small recombinant clones generated
864 late in development; Z) *y w eyF* ; *P{XP}^{d06928} PBac{RB}^{e03144} /+*. The eye has a
865 mottled appearance indicative of small recombinant clones generated late in
866 development.

876 B) Graphs show the % of the male adult eye comprising *w*, segmentally-
877 aneuploid cells for 17 chromosome regions. Each data-point represents the
878 extent of *w* territory of an individual eye, median values shown as black bars, N
879 as indicated for each genotype. Geneotypes from overlapping genomic regions
880 are shown in a common color. Yellow background indicates deletions that
881 encompass one or more *Rp* loci. Deletion of *Rp* loci reduces contribution to the
882 adult eye for all regions except the 87-93 region (brown). C) As for panel A
883 except data from females is shown. D) Examples of typical eyes for each of the
884 genotypes analyzed (males shown). Arrow on the Df(3R)89B13-93A2.2/+ eye
885 indicates a small clone of darker cells reflecting four *w*⁺ elements associated with
886 tandem duplication of the 89B13-93A2 region due to a FLP-*FRT* recombination
887 event in G2 that was not resolved by further recombination (see text). Statistics.
888 Pairwise comparisons using the Mann-Whitney procedure with the Benjamini-
889 Hochberg correction for multiple testing (see Table 2). ns – difference not
890 statistically significant (p>0.05). * - difference significant (p<0.05). ** -
891 difference highly significant (p<0.01). Genotypes: For 26A1-28C3, y w hsF ;
892 *P{XP}*^{d08241} *PBac{WH}*^{f04888}/+; FRT82B/+. For 26A1-29F8, y w hsF ; *P{XP}*^{d08241}
893 *PBac{WH}*^{f00857}/+ ; FRT82B/+. For 48B2-50C1, y w hsF ; *P{XP}*^{d09761}
894 *PBac{WH}*^{f00157}/+ ; FRT82B/+. For 48F6-50C1, y w hsF ; *P{XP}*^{d09761}
895 *PBac{WH}*^{f00157}/+ ; FRT82B/+. For 56F16-58E2, y w hsF ; *P{XP}*^{d02302}
896 *PBac{WH}*^{f04349}/+ ; FRT82B/+. For 56F16-59B1, y w hsF ; *P{XP}*^{d02302}
897 *PBac{WH}*^{f00464}/+ ; FRT82B/+. For 63A3-65A9, y w hsF ; *PBac{WH}*^{f01922}
898 *P{XP}*^{d02570} /FRT82B. For 63A3-65F5, y w hsF ; *PBac{WH}*^{f01922} *P{XP}*^{d02813}
899 /FRT82B. For 63A3-67B2, y w hsF ; *PBac{WH}*^{f01922} *P{XP}*^{d07256} /FRT82B. For
900 63C1-65A9, y w hsF ; *PBac{WH}*^{f05041} *P{XP}*^{d02570} /FRT82B. For 63C1-65F5, y w
901 hsF ; *PBac{WH}*^{f05041} *P{XP}*^{d02813} /FRT82B. For 63C1-67B2, y w hsF ;
902 *PBac{WH}*^{f05041} *P{XP}*^{d07256} /FRT82B. For 87B8-89B16, y w hsF ; *P{XP}*^{d06796}
903 *PBac{WH}*^{f04937} /FRT82B. For 87B8-89E5, y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f00971}
904 /FRT82B. For 87B8-93A2, y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f03502} /FRT82B. For
905 89B13-93A2a, y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f03502} / FRT82B. For 89B13-
906 93A2b, y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f01700} /FRT82B.

Figure 4 Rescue of segmental aneuploidy by single transgenes

A) Df63C1-65A9/+ cells contribute ~50% of the adult eye. B) Very few cells heterozygous for the overlapping Df63A3-65A9 were recovered. C) Df63A3-65A9/+ cells contribute ~50% of the adult eye in the presence of the *RpL28*⁺ transgene. D). Very few cells heterozygous for Df87B8-89B16 contribute to the adult eye. E). Df87B8-89B16/+ cells contribute ~50% of the adult eye in the presence of the *eIF2 γ* ⁺ genomic transgene. F). Very few cells heterozygous for Df87B8-89E5 contribute to the adult eye. G). Df87B8-89E5/+ cells contribute ~20% of the adult eye in the presence of the *eIF2 γ* ⁺ genomic transgene. H) Quantification of results for the *RpL28* region shown in panels A-C. Data for Df63C1-65A9 is the same as that already shown in Figure 2A,B. I) Quantification of results for the *eIF2 γ* region shown in panels D-G. Statistics. Pairwise comparisons used the Mann-Whitney procedure with the Benjamini-Hochberg correction for multiple testing (see Table 2). N as indicated for each genotype. ns – difference not statistically significant ($p > 0.05$). ** - difference

924 highly significant ($p < 0.01$). Genotypes: For 63C1-65A9, y w hsF ;
 925 $PBac\{WH\}^{f05041} P\{XP\}^{d02570} / FRT82B$. For 63A3-65A9, y w hsF ; $PBac\{WH\}^{f01922}$
 926 $P\{XP\}^{d02570} / FRT82B$ and y w hsF ; $\{RpL28^+P3-DsRed\}/+$; $PBac\{WH\}^{f01922}$
 927 $P\{XP\}^{d02570} /+$; FRT82B/+. For 87B8-89B16, y w hsF/+ ; $P\{XP\}^{d06796}$
 928 $PBac\{WH\}^{f04937} / FRT82B$ and y w hsF/+ ; $P\{ry^+ Su(var3-9^+) eIF2\gamma^+\}/+$;
 929 $P\{XP\}^{d06796} PBac\{WH\}^{f04937} /+$. For 87B8-89E5, y w hsF/+ ; $P\{XP\}^{d06796}$
 930 $PBac\{WH\}^{f00971} / FRT82B$ and y w hsF/+ ; $P\{ry^+ Su(var3-9^+) eIF2\gamma^+\}/+$;
 931 $P\{XP\}^{d06796} PBac\{WH\}^{f00971} /+$.
 932

Figure 5 Contributions of apoptosis and Xrp1 to elimination of segmentally-aneuploid cells

Panels A-B show the contribution of *w* segmentally-aneuploid cells to adult eyes of indicated genotypes, N as indicated for each genotype. A) Heterozygosity for Df(3L)H99, which deletes the proapoptotic genes *rpr*, *hid*, and *grim* ('RHG') significantly increases contribution of Df63A3-65A9/+ and Df63C1-65F5/+ cells to adult eyes. These genotypes approach the contribution level of Df63C1-65A9/Df(3L)H99 cells which do not affect any *Rp* locus (the results for 63A3-65A9/Df(3L)H99, 63C1-65A9/Df(3L)H99, and 63C1-65F5/Df(3L)H99 cannot be distinguished in males: KruskalWallis test, $p=0.18$; in females, this hypothesis is rejected and post-hoc testing identified the 63C1-65A9/Df(3L)H99 data as different from the other genotypes ($p=0.018$ for the comparison to 63A3-

65A9/H99, $p=0.027$ for the comparison to 63C1-65F5/H99). B) Heterozygosity for *Xrp1*, which is required for the slow growth and cell competition of *Rp*^{+/-} point-mutant cells, significantly increases contribution of Df63A3-65A9/+ and Df63C1-65F5/+ cells to adult eyes. *Xrp1* did not affect the contribution of Df63C1-65A9/+ cells that do not affect any *Rp* locus. We also tested the hypothesis that Df(3L)H99, *Xrp1* mutation, and an *RpS3*/+ genetic background (see Figure 8) suppressed cell competition to an equal degree. This hypothesis was rejected for both males and females of Df63A3-65A9/+ and Df(3L)63C1-65F5/+ (Kruskal Wallis test $p=2.9 \times 10^{-17}$, 9.7×10^{-21} , 2.1×10^{-5} , and 1.8×10^{-7} respectively). For each deletion, all the genotypes were individually significantly different except Df(3L)63C1-65F5/H99 and Df(3L)63C1-65F5/*Xrp1* males, which were not significantly different ($p=0.06$, Conover post-hoc test with Holm correction). C) Representative examples of these genotypes. Statistics. Pairwise comparisons using the Mann-Whitney procedure with the Benjamini-Hochberg correction for multiple testing (see Table 2). ns – difference not statistically significant ($p>0.05$). ** - difference highly significant ($p<0.01$). Multiple comparisons using the Kruskal Wallis test as described for panel B. Genotypes. For 63A3-65A9 in panel A: *y w hsF* ; *PBac{WH}^{f01922} P{XP}^{d02570} /FRT80B* and *y w hsF* ; *PBac{WH}^{f01922} P{XP}^{d02570} /Df(3L)H99 FRT80B*. For 63C1-65A9 in panel A: *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02570} /FRT80B* and *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02570} /Df(3L)H99 FRT80B*. For 63C1-65F5 in panel A: *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02813} /FRT80B* and *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02813} /Df(3L)H99 FRT80B*. For 63A3-65A9 in panel B: *y w hsF* ; *PBac{WH}^{f01922} P{XP}^{d02570} /FRT82B* and *y w hsF* ; *PBac{WH}^{f01922} P{XP}^{d02570} /FRT82B *Xrp1*^{m2-73}*. For 63C1-65A9 in panel B: *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02570} /FRT82B* and *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02570} /FRT82B *Xrp1*^{m2-73}*. For 63C1-65F5 in panel B: *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02813} /FRT82B* and *y w hsF* ; *PBac{WH}^{f05041} P{XP}^{d02813} /FRT82B *Xrp1*^{m2-73}*.

Figure 6 Contributions of Xrp1 to competition of larger segmental aneuploidies

A) Heterozygosity for Xrp1 has progressively less effect on the contributions of larger segmental-aneuploid genotypes in males, although still significant statistically. B) In females, the effect of Xrp1 on Df(3L)63A3-67B2/+ cells, haploinsufficient for *RpL14*, *RpL18*, and *RpL28*, is no longer significant

981 statistically. *Xrp1* heterozygosity does affect Df(3L)63C1-67B2 females, although
 982 many zero values are superimposed so that this is hard to appreciate on the
 983 Df(3L)63C1-67B2/+ *Xrp1*^{+/+} graph. C). Representative examples of these
 984 phenotypes (males are shown). Statistics. Pairwise comparisons used the
 985 Mann-Whitney procedure with the Benjamini-Hochberg correction for multiple
 986 testing (see Table 2). N as indicated for each genotype. ns – difference not
 987 statistically significant (p>0.05). * - difference significant (p<0.05). ** -
 988 difference highly significant (p<0.01). Genotypes. For 63A3-65F5: y w hsF ;
 989 *PBac{WH}*^{f01922} *P{XP}*^{d02813} /FRT82B and y w hsF ; *PBac{WH}*^{f01922} *P{XP}*^{d02813}
 990 /FRT82B *Xrp1*^{m2-73}. For 63C1-67B2: y w hsF ; *PBac{WH}*^{f05041} *P{XP}*^{d07256}
 991 /FRT82B and y w hsF ; *PBac{WH}*^{f05041} *P{XP}*^{d07256} /FRT82B *Xrp1*^{m2-73}. For
 992 63A3-67B2: y w hsF ; *PBac{WH}*^{f01922} *P{XP}*^{d07256} /FRT82B and y w hsF ;
 993 *PBac{WH}*^{f01922} *P{XP}*^{d07256} /FRT82B *Xrp1*^{m2-73}.
 994

1003 improved the eye contribution of Df(56F16-59B1)/+, heterozygous for *RpS16* and
1004 *RpS24*, but had no effect on the contribution of Df(56F16-58E2)/+ cells that affect
1005 no *Rp* loci. D) Representative examples of these phenotypes (males shown).
1006 Statistics. Pairwise comparisons used the Mann-Whitney procedure with the
1007 Benjamini-Hochberg correction for multiple testing (see Table 2). N as indicated
1008 for each genotype. ns – difference not statistically significant ($p > 0.05$). ** -
1009 difference highly significant ($p < 0.01$). Genotypes. For 26A1-28C3: y w hsF ;
1010 $P\{XP\}^{d08241} PBac\{WH\}^{f04888}/+$; FRT82B/+ and y w hsF ; $P\{XP\}^{d08241}$
1011 $PBac\{WH\}^{f04888}/+$; FRT82B *Xrp1*^{m2-73}/+. For 26A1-29F8: y w hsF ; $P\{XP\}^{d08241}$
1012 $PBac\{WH\}^{f00857}/+$; FRT82B/+ and y w hsF ; $P\{XP\}^{d08241} PBac\{WH\}^{f00857}/+$;
1013 FRT82B *Xrp1*^{m2-73}/+. For 56F16-58E2 in panel B: y w hsF ; $P\{XP\}^{d02302}$
1014 $PBac\{WH\}^{f04349}/+$; FRT82B/+ and y w hsF ; $P\{XP\}^{d02302} PBac\{WH\}^{f04349}/+$;
1015 FRT82B *Xrp1*^{m2-73}/+. For 56F16-59B1 in panel B: y w hsF ; $P\{XP\}^{d02302}$
1016 $PBac\{WH\}^{f00464}/+$; FRT82B/+ and y w hsF ; $P\{XP\}^{d02302} PBac\{WH\}^{f00464}/+$;
1017 *Xrp1*^{m2-73}/+. For 56F16-58E2 in panel C: y w hsF ; $P\{XP\}^{d02302}$
1018 $PBac\{WH\}^{f04349}/+$; FRT80B/FRT80B and y w hsF ; $P\{XP\}^{d02302}$
1019 $PBac\{WH\}^{f04349}/+$; *rpS12*^{G97D} FRT80B/*rpS12*^{G97D} FRT80B. For 56F16-59B1 in
1020 panel B: y w hsF ; $P\{XP\}^{d02302} PBac\{WH\}^{f00464}/+$; FRT80B/FRT80B and y w
1021 hsF ; $P\{XP\}^{d02302} PBac\{WH\}^{f00464}/+$; *rpS12*^{G97D} FRT80B/*rpS12*^{G97D} FRT80B.
1022

Figure 8 Contribution of Xrp1 to competition of further genotypes

A) *Xrp1* mutation made a small but significant improvement to the contribution of *Df(48B2-50C1)/+* cells, heterozygous for *RpS11*, but its effect on *Df(48F6-50C1)/+* cells, which have no affected *Rp* genes, was only significant in males.

B,C) *Xrp1* mutation significantly rescued the contribution of all segmental aneuploid genotypes affecting the *eIF2γ* locus. There was little effect of mutating the second *Xrp1* copy on *Df(89B13-93A2.1)/+* and *Df(89B13-93A2.2)/+* cells,

both of which are haplo-insufficient for *RpS20* and *RpS30* as well as *Xrp1*. D) Representative examples of these genotypes (males shown). For panels A-C, the control data (*Xrp1*^{+/+} for A and *Xrp1*^{+/-} for B,C) were also shown in Figure 2). Statistics. Pairwise comparisons used the Mann-Whitney procedure with the Benjamini-Hochberg correction for multiple testing (see Table 2). N as indicated for each genotype. ns – difference not statistically significant (p>0.05). * - difference significant (p<0.05). ** - difference highly significant (p<0.01). Genotypes. For 48B2-50C1: y w hsF ; *P{XP}*^{d09761} *PBac{WH}*^{f00157/+} ; FRT82B/+ and y w hsF ; *P{XP}*^{d09761} *PBac{WH}*^{f00157/+} ; FRT82B *Xrp1*^{m2-73/+}. For 48F6-50C1: y w hsF ; *P{XP}*^{d09761} *PBac{WH}*^{f00157/+} ; FRT82B/+ and y w hsF ; *P{XP}*^{d09761} *PBac{WH}*^{f00157/+} ; FRT82B *Xrp1*^{m2-73/+}. For 87B8-89B16: y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f04937} /FRT82B and y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f04937} /FRT82B *Xrp1*^{m2-73}. For 87B8-89E5: y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f00971} /FRT82B and y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f00971} /FRT82B *Xrp1*^{m2-73}. For 87B8-93A2: y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f03502} /FRT82B and y w hsF ; *P{XP}*^{d06796} *PBac{WH}*^{f03502} /FRT82B *Xrp1*^{m2-73}. For 89B13-93A2.1: y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f03502} / FRT82B and y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f03502} /FRT82B *Xrp1*^{m2-73}. For 89B13-93A2.2: y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f01700} /FRT82B and y w hsF ; *P{XP}*^{d06928} *PBac{WH}*^{f01700} /FRT82B *Xrp1*^{m2-73}.

Eye contributions of clones of segmentally-aneuploid genotypes deleting the indicated *Rp* loci. in *RpS3^{+/+}* and *RpS3^{+/-}* genetic backgrounds. Magenta bars show the median contributions, N as indicated for each genotype. A) In males, the *RpS3^{+/-}* genetic background (black datapoints) allows for significantly greater contribution in all cases except *Df(48B2-50C1)/+*. No *Df(3R)89B13-93A2.2/RpS3* flies were obtained, this genotype is lethal due to an unidentified shared lethal outside the 89B13-93A2.2 region. B) Comparable data from females. The *RpS3^{+/-}* genetic background (black datapoints) always allows for significantly greater contribution. C) Eye contributions of clones of segmentally-aneuploid cells where no *Rp* loci are affected. The *RpS3^{+/-}* genetic background (black datapoints) had no significant effect on many such genotypes, but did enhance the contribution of *Df(26A1-28C3)/+* clones and of the *Df(3R)87B8-89B16/+* and *Df(3R)87B8-89E5/+* clones that were haploinsufficient for *eIF2 γ ⁺*. No *Df(3R)87B8-89B16/RpS3* males were obtained. D) Representative examples of these *RpS3^{+/-}* genotypes (males shown). The *RpS3^{+/+}* control data were shown previously in Figure 2, except for 87B8-89B16 females. Statistics. Pairwise comparisons using the Mann-Whitney procedure with the Benjamini-Hochberg correction for multiple testing (see Table 2). ns – difference not statistically significant ($p > 0.05$). * - difference significant ($p < 0.05$). ** - difference highly significant ($p < 0.01$). Genotypes: Same as for Figure 3, with an *FRT82B RpS3* chromosome substituting for *FRT82B* where indicated.

Figure 10 Segmentally-Aneuploid cells in the thorax

A) White arrow indicates a 'Minute' (*Rp*^{+/-})-like short thin scutellar bristles on a fly containing clones of 56F16-59B1 cells. Compare the normal contralateral scutellar bristles (black arrow). B) Frequency of affected bristles in genotypes as indicated. Data represent averages from 3 sets of 100 adults of each genotype. Minute-like bristles were not seen with the 56F16-58E2 deletion that does not affect any *Rp* locus. This provides as a baseline for any spontaneous loss of heterozygosity for *Rp* gene that might occur unrelated to the FLP-*FRT* excision, and which is evidently rare. Minute-like bristles were also not seen on cell competition-competent flies where 56F16-59B1 excisions would create heterozygosity for *RpS16* and *RpS24*. These bristles only appeared in the *rpS12* and *Xrp1* mutant backgrounds where cell competition was compromised. Statistics. 3 sets of 100 flies analyzed for each genotype. 1-way ANOVA rejects the hypothesis that the six datasets are indistinguishable ($p=4.28 \times 10^{-7}$). The Holm procedure for multiple comparisons showed that results for 56F16-59B1 in the *rpS12* and *Xrp1* backgrounds were different from all others and from one another (adjusted $p < 0.05$). For simplicity, significance is only indicated for 56F16-59B1 genotypes. ns – difference not statistically significant ($p > 0.05$). * - difference significant ($p < 0.05$). ** - difference highly significant ($p < 0.01$).

Figure 11 Models

A. Model for the elimination of segmentally-aneuploid cells from imaginal discs. Cells that lose part of one chromosome may become haploinsufficient for one or more *Rp* loci, affecting ribosome assembly and triggering *RpS12* and *Xrp1* activities that lead to cell elimination by competition with unaffected neighboring cells. B. Our studies show that segmentally-aneuploid cells proliferate and contribute to adult tissues in animals where all cells are heterozygous for an *Rp* mutation, reducing the difference between aneuploid and diploid cells. A similar situation might apply in Diamond-Blackfan patients, many of whom are haploinsufficient for *Rp* loci. If *Rp* loci are also indicators of chromosome rearrangements in mammalian cells, such patients might face a greater accumulation of aneuploid cells.

1112 SOURCE DATA FILES

1113

1114 Figure 1 Source Data File 1

1115 Source Data for Figure 1H,M,P,R

1116

1117 Figure 3 Source Data File 1

1118 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1119 duplication were also observed in this specimen. Gaps between measurements
1120 separate replicate data

1121

1122 Figure 4 Source Data File 1

1123 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1124 duplication were also observed in this specimen. Gaps between measurements
1125 separate replicate data

1126

1127 Figure 5 Source Data File 1

1128 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1129 duplication were also observed in this specimen. Gaps between measurements
1130 separate replicate data

1131

1132 Figure 6 Source Data File 1

1133 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1134 duplication were also observed in this specimen. Gaps between measurements
1135 separate replicate data

1136

1137 Figure 7 Source Data File 1

1138 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1139 duplication were also observed in this specimen. Gaps between measurements
1140 separate replicate data

1141

1142 Figure 8 Source Data File 1

1143 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1144 duplication were also observed in this specimen. Gaps between measurements
1145 separate replicate data

1146

1147 Figure 9 Source Data File 1

1148 % eye white data. * indicates that 4x w+ cells indicative of FLP-mediated
1149 duplication were also observed in this specimen. Gaps between measurements
1150 separate replicate data

1151

1152 Figure 10 Source Data File 1

1153 Source data for Figure 10B

1154

SUPPLEMENTARY FILES

insertion	e00462	f04180	d08241	d09761	d09417	d02302
location	2L:206,074	2L:473,071	2L:5,923,740	2R:11,646,10	2R:12,306,45	2R:20,306,586
cytolog	21B4	21D1	26A1	48B2	48F6	56F16
d07944	2L:2,670,580	23A2	RpLP1			
d06074	2L:3,580,955	24A1	RpS21			
f04888	2L:7,780,729	28C3				
e02272	2L:8,211,772	28F3	RpL36A			
e03937	2L:8,400,900	29C1	RpL36A, RpS13			
f00857	2L:9,025,734	29F8	RpL36A, RpS13			
f00157	2R:13,667,20	50C1		RpS11		
e00535	2R:14,157,50	50E1		RpS11		
e03998	2R:14,514,50	50E1		RpS11, RpS2	RpS23	
f04349	2R:22,466,60	58E2				
f00464	2R:22,791,80	59B1				RpS16, RpS24
insertion	f01922	f05041	d06455	d05983	d06796	d06928
location	3L:3,042,58	3L:3,239,72	3R:10,889,30	3R:11,200,30	3R:12,372,80	3R:16,351,726
cytolog	63A3	63C1	86C7	86D7	87B8	89B13
d02570	3L:6,218,125	65A9	RpL28			
d02813	3L:7,334,980	65F5	RpL28, RpL			
d07256	3L:9,358,902	67B2	RpL28, RpL18, RpL14			
f04937	3R:16,442,90	89B16			eIF2γ	
e03186	3R:16,485,30	89B18			eIF2γ	
f00971	3R:16,977,70	89E5			eIF2γ	
e03728	3R:18,648,20	91B8			eIF2γ	
e03144	3R:20,553,50	92F1	RpS25, eIF2γ	RpS25, eIF2γ	eIF2γ	
f03502	3R:20,864,10	93A2			eIF2γ	RpS20, RpS30
f01700	3R:20,873,00				eIF2γ	RpS20, RpS30

Supplementary File 1 FRT insertions used in this study and their combinations.

Columns indicate the specific insertion, genome location, and cytological position of the elements used as the left FRT site. Rows indicate the same information

for the right FRT site. Insertions in the “d” family are of the P{XP} element, the “e” family the PBac{RB} element, and “f” family PBac{WH}(Thibault et al., 2004). For clarity, in the main text we refer to genetic strains by the cytological insertion point eg “63A3-65A9” is the shorthand descriptive name for the PBac{WH}^{f01922} P{XP}^{d02570} chromosome. Which *Rp* genes are included between FRT sites is shown, as is *eIF2γ*. Some FRT combinations were poor substrates for Flp, either retained the parental eye color in the presence of eyFlp, or produce a salt and pepper pattern of very small clones that is indicative of excision occurring only late in development once large cell numbers are present (Table 1; Figure 1U-Z). These results are summarized by shading FRT combinations tested as follows: Green – eyFlp recombination in essentially all cells; Blue – eyFlp recombination in most cells, associated with small eye size (≤ 0.5 linear dimensions); magenta – eyFlp recombination not detected; Orange – eyFlp recombination only late in development gives a mottled eye. The interpretation that recombination is reduced or absent is preferred to the alternative possibility that excision results in a cell-lethal genotype that later disappears, in part because results correlated with individual FRT elements and not with the genetic material between them. For example, recombination between 26A1 and 28F3 or 29C1, revealed only small, late recombination, but the 26A1-29F8 recombination that deletes all the same sequences was completely excised from EyFlp eyes and developed normal eye size with entirely Df(2L)26A1-29F8/+ cells (Table 1, Figure 1D). Other examples of recombinations that could not readily be obtained were 87B8-89B18, 87B8-91B8, 87B8-92F1 and 89B13-92F1, although the larger 89B8-93A2 and 89B13-93A2 recombinations were readily obtained (Table 1, Figures 1R-T). In contrast to the lack of correlation with deleted chromosome regions, when an element was not recombined by eyFLP this was the case with all the partner elements tested, so each FRT element could be designated as green or orange/magenta without ambiguity (for the 21-23/4 region elements there is insufficient information to identify the particular non-recombining elements). These data suggest that some *FRT*-containing Exelixis elements are poor substrates for cis-recombination in the head. Interestingly, all 7 insertions of

1192 the PBac(Adams & Cory) element tested belong in this category, although this
1193 element has previously been recombined successfully in the germline(Parks et
1194 al., 2004).

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Rank	ID	Original P value	Critical Value	Benjamini-Hochberg Adjusted P value	Significant using an FDR of 0.05?
1	63A3-65A9m-63A3-65A9Mf	5.83E-28	0.000458716	6.35E-26	Yes
2	56F16-58B1m-56F16-58B1e12m	3.43E-27	0.000917431	1.87E-25	Yes
3	63A3-65A9m-63A3-65A9Mm	6.55E-25	0.01376147	3.47E-23	Yes
4	63C1-65A9m-63C1-65F5f	1.85E-24	0.001834882	5.04E-23	Yes
5	63C1-65A9m-63C1-67B2f	3.64E-23	0.002293578	7.94E-22	Yes
6	63C1-65A9m-63C1-67B2m	1.59E-21	0.002752284	2.87E-20	Yes
7	63C1-65A9m-63C1-65F5m	2.61E-20	0.003211009	4.06E-19	Yes
8	63C1-65A9m-63A3-65A9f	5.20E-18	0.003669725	7.09E-17	Yes
9	63C1-65F5f-63C1-65F5Xf	2.04E-17	0.00412844	2.47E-16	Yes
10	63C1-65F5m-63C1-65F5Xm	1.33E-16	0.004587156	1.45E-15	Yes
11	26A1-29F8f-26A1-29F8Mf	1.59E-16	0.005045872	1.58E-15	Yes
12	63C1-65A9m-63A3-67B2f	1.71E-16	0.00504587	1.55E-15	Yes
13	26A1-29F8m-26A1-29F8Mm	1.97E-16	0.005963303	1.65E-15	Yes
14	63C1-65A9m-63A3-65A9m	8.23E-16	0.006422018	6.41E-15	Yes
15	63C1-65F5f-63C1-65F5Mf	1.65E-15	0.006880734	1.20E-14	Yes
16	63A3-65A9m-63A3-65A9Xm	3.93E-15	0.00733945	2.68E-14	Yes
17	26A1-28C3m-26A1-28C3Mm	9.07E-15	0.007788165	5.62E-14	Yes
18	63C1-65F5m-63C1-65F5Mm	9.15E-15	0.008256881	5.54E-14	Yes
19	87B8-89E5m-87B8-89E5Xm	1.95E-14	0.008715596	1.12E-13	Yes
20	63C1-65A9m-63A3-65F5f	3.61E-14	0.009174312	1.97E-13	Yes
21	26A1-28C3f-26A1-28C3Mf	4.21E-14	0.009633026	2.18E-13	Yes
22	63C1-67B2f-63C1-67B2Mf	4.48E-14	0.010091743	2.22E-13	Yes
23	48B2-50C1f-48B2-50C1Mtotf	5.90E-14	0.010550459	2.80E-13	Yes
24	56F16-58E2m-56F16-58B1m	1.44E-13	0.011009174	6.54E-13	Yes
25	63A3-65A9m-63A3-65A9Xf	2.09E-13	0.01146780	9.11E-13	Yes
26	87B8-89E5m-87B13-93A2.2m	5.05E-13	0.011926606	2.12E-12	Yes
27	63A3-65A9m-63A3-65A9+RplL28+H	6.61E-13	0.012383321	2.67E-12	Yes
28	56F16-58E2f-56F16-58B1f	7.31E-13	0.01284037	3.32E-12	Yes
29	87B8-89E5f-87B8-89E5Xf	4.13E-11	0.013302752	1.55E-10	Yes
30	63C1-65A9m-63A3-65F5m	5.33E-11	0.013761468	1.94E-10	Yes
31	87B8-89E5m-87B13-93A2.1m	6.53E-11	0.014220183	2.30E-10	Yes
32	87B8-89E5f-87B13-93A2.1f	9.78E-11	0.014678696	3.32E-10	Yes
33	87B8-89E5f-87B8-89E5f+eIF2g+	1.86E-10	0.015137615	6.14E-10	Yes
34	56F16-58B1f-56F16-58B1e12f	2.17E-10	0.01559683	6.96E-10	Yes
35	87B8-89E5f-87B13-93A2.2f	2.84E-10	0.016055046	8.84E-10	Yes
36	63A3-65F5f-63A3-65F5Mf	1.67E-09	0.016513761	5.06E-09	Yes
37	48B2-40C2f-48F6-50C1f	1.88E-09	0.016972477	5.54E-09	Yes
38	63A3-67B2m-63A3-67B2Mm	3.18E-09	0.017431193	9.12E-09	Yes
39	48B2-40C2m-48F6-50C1m	5.02E-09	0.017869608	1.40E-08	Yes
40	63A3-65F5m-63A3-65F5Mm	8.00E-09	0.018348624	2.18E-08	Yes
41	63A3-65A9m-63A3-65A9+RplL28+m	8.78E-09	0.018807339	2.33E-08	Yes
42	26A1-28C3f-26A1-29F8f	1.47E-08	0.019260355	3.82E-08	Yes
43	87B8-93A2f-87B8-93A2Mf	1.65E-08	0.01924771	4.66E-08	Yes
44	56F16-58B1m-56F16-58B1Xm	2.26E-07	0.020183486	5.60E-07	Yes
45	63C1-67B2m-63C1-67B2Mm	3.79E-07	0.020642202	9.18E-07	Yes
46	89B13-93A2.1f-89B13-93A2.1Mf	4.20E-07	0.021100917	9.95E-07	Yes
47	87B8-89E5m-87B8-89E5Mm	1.00E-06	0.02159633	2.32E-06	Yes
48	87B8-87B16m-87B8-87B16Xm: Nov	3.00E-06	0.022018349	6.81E-06	Yes
49	87B8-87B16f-87B8-87B16Mf: Nov	3.00E-06	0.022477064	6.67E-06	Yes
50	56F16-58B1f-56F16-58B1Mf	6.01E-06	0.02263378	1.31E-05	Yes
51	87B8-89B16m-87B13-93A2.2m	1.10E-05	0.023394495	2.35E-05	Yes
52	26A1-29F8f-26A1-29F8Xf	1.50E-05	0.02383211	3.14E-05	Yes
53	87B8-89E5f-87B8-89E5Mf	2.10E-05	0.024311927	4.32E-05	Yes
54	87B8-89B16f-87B8-89B16f+eIF2g+	3.60E-05	0.024770642	7.27E-05	Yes
55	63A3-65F5f-63A3-65F5Xf	4.10E-05	0.025229358	8.13E-05	Yes
56	87B8-93A2m-87B8-93A2Mm	4.10E-05	0.025688073	7.98E-05	Yes
57	63C1-67B2m-63A3-67B2Xm	4.10E-05	0.026146789	7.84E-05	Yes
58	63C1-65A9m-63A3-67B2m	6.80E-05	0.026605505	0.000127793	Yes
59	87B8-87B16f-87B8-87B16Xf: Nov	0.000102	0.02706422	0.000188441	Yes
60	63A3-65A9m-63A3-65A9H9m	0.000143	0.027522936	0.000259783	Yes
61	63C1-65F5m-63C1-65F5H9m	0.000163	0.027981651	0.000291262	Yes
62	56F16-58B1m-56F16-58B1Mm	0.000187	0.028440367	0.000328758	Yes
63	56F16-58B1f-56F16-58B1Xf	0.000313	0.028899083	0.00054154	Yes
64	48B2-50C1f-48B2-50C1Xtotf	0.000341	0.029327738	0.000580766	Yes
65	87B8-89B16f-87B8-93A2f	0.000439	0.029816514	0.000736169	Yes
66	63C1-65A9m-63C1-65A9H9f	0.000612	0.030275229	0.001010727	Yes
67	63C1-65F5f-63C1-65F5H9f	0.001509	0.030733945	0.00245484	Yes
68	87B8-93A2f-87B8-93A2Xf	0.002556	0.031162861	0.004097118	Yes
69	87B8-89B16f-87B13-93A2.1f	0.002646	0.031651376	0.004179913	Yes
70	63A3-65F5m-63A3-65F5Xm	0.002725	0.032110092	0.004243214	Yes
71	63C1-65A9m-63C1-65A9H9m	0.003178	0.032658807	0.004678901	Yes
72	63A3-67B2m-63A3-67B2Xm	0.003634	0.033027523	0.005501472	Yes
73	63A3-67B2m-63A3-67B2Mm	0.004058	0.033486239	0.006059205	Yes
74	87B8-89B16m-87B13-93A2.1m	0.004636	0.033944954	0.006828703	Yes
75	87B8-89B16f-87B13-93A2.1f	0.007291	0.03440367	0.010596253	Yes
76	63C1-67B2f-63C1-67B2Xf	0.011063	0.034862385	0.015866671	Yes
77	89B13-93A2.1m-89B13-93A2.1Mm	0.014367	0.035321101	0.020337701	Yes
78	48F6-50C1m-48F6-50C1Xm	0.015118	0.035779817	0.021126436	Yes
79	48B2-50C1m-48B2-50C1Mtotm	0.019613	0.036236532	0.027060975	Yes
80	87B8-89E5m-87B8-93A2m	0.019625	0.036697248	0.026739063	Yes
81	87B8-93A2m-87B8-93A2Xm	0.021765	0.037155963	0.029288704	Yes
82	48F6-50C1f-48F6-50C1Mf	0.023889	0.037614679	0.033175469	Yes
83	48B2-50C1m-48B2-50C1Xtotm	0.024827	0.038073394	0.032604133	Yes
84	89B13-93A2.1m-89B13-93A2.1Xm	0.036348	0.03853211	0.047165857	Yes
85	26A1-29F8m-26A1-29F8Xm	0.041284	0.038990826	0.052940659	No
86	48F6-50C1m-48F6-50C1Mm	0.057212	0.039449541	0.072512884	No
87	63C1-65A9m-63C1-65A9Mm	0.071887	0.039908257	0.090065322	No
88	89B13-93A2.2f-89B13-93A2.2Xf	0.075925	0.040366972	0.094043466	No
89	26A1-28C3m-26A1-28C3Xm	0.080791	0.040825688	0.106294586	No
90	56F16-58E2m-56F16-58E2e12m	0.1006	0.041284404	0.121837778	No
91	26A1-28C3f-26A1-28C3Xf	0.136783	0.041743119	0.163838978	No
92	26A1-28C3m-26A1-29F8m	0.14865	0.042201835	0.176117935	No
93	63C1-65A9m-63C1-65A9Mf	0.155343	0.04266055	0.182638677	No
94	56F16-58E2f-56F16-58E2Xf	0.168677	0.043119266	0.195593543	No
95	87B8-89B16m-87B8-93A2m	0.179244	0.043577982	0.205658905	No
96	87B8-89E5f-87B8-93A2f	0.228638	0.044056697	0.259593936	No
97	63A3-67B2m-63A3-67B2Xm	0.240953	0.044465413	0.27073553	No
98	63A3-65A9m-63A3-65A9H9f	0.26499	0.044954128	0.294733776	No
99	56F16-58E2f-56F16-58E2e12f	0.275596	0.045412844	0.30343398	No
100	63C1-65A9m-63A3-65A9+RplL28m	0.294581	0.045871556	0.32109329	No
101	48F6-50C1f-48F6-50C1Xf	0.310526	0.046330275	0.335122119	No
102	89B13-93A2.2m-89B13-93A2.2Xm	0.329238	0.046788991	0.351832765	No
103	89B13-93A2.1f-89B13-93A2.1Xf	0.384984	0.047247706	0.407410252	No
104	56F16-58E2m-56F16-58E2Xm	0.70665	0.047706422	0.740823558	No
105	63C1-65A9m-63C1-65A9Xm	0.719199	0.048165138	0.746597057	No
106	63C1-65A9m-63A3-65A9+RplL28m	0.941325	0.048623853	0.967966274	No
107	56F16-58E2m-56F16-58E2Mm	0.952979	0.049082569	0.970791682	No
108	63C1-65A9m-63C1-65A9Xm	0.956944	0.049541284	0.965804583	No
109	56F16-58E2m-56F16-58E2Mm	1	0.05	1	No

1197

1198 Supplementary File 2 Statistical comparisons of segmental-aneuploid cell

1199 contribution to adult eyes.

1200 109 pairwise comparison between segmental aneuploid genotypes were

1201 performed in this study, which requires multiple testing correction. The table

1202 shows each comparison ranked according to raw p-value (Mann-Whitney),
1203 Benjamini-Hochberg critical value for $FDR \leq 0.05$, Adjusted p-value, and
1204 significance.
1205
1206

Appendix 1. Key Resources Table

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Gene (<i>Drosophila melanogaster</i>)	RpL28		Flybase: FBgn0035422	
Gene (<i>Drosophila melanogaster</i>)	eIF2 γ		Flybase: FBgn0263740	
genetic reagent (<i>D. melanogaster</i>)	w ¹¹⁻¹⁸	(Hazelrigg, Levis, & Rubin, 1984; Lee, Rimesso, Reynolds, Cai, & Baker, 2016)	FLYBASE: FBal0018186	Bloomington Drosophila Stock Center #3605
genetic reagent (<i>D. melanogaster</i>)	Xrp1 ^{m2-73}	(Lee et al., 2016)	FLYBASE:FBal0346068	Bloomington Drosophila Stock Center #81270
genetic reagent (<i>D. melanogaster</i>)	rpS12 ^{G97D}	(Tyler, Li, Zhuo, Pellock, & Baker, 2007)	FLYBASE:FBal0193403	
genetic reagent (<i>D. melanogaster</i>)	Df(3L)H99	(Abbott & Lengyel, 1991)	FLYBASE:FBal0022359	Bloomington Drosophila Stock Center #1576
genetic reagent (<i>D. melanogaster</i>)	M(3R)w ¹²⁴ aka RpS3 ²	(Ferrus, 1975; Abbott & Lengyel, 1991)	FLYBASE: FBal0011951	

genetic reagent (<i>D. melanogaster</i>)	hs-FLP	(Struhl & Basler, 1993)	FLYBASE:FBt p0001101	
genetic reagent (<i>D. melanogaster</i>)	ey-FLP	(Newsome et al., 2000)	FLYBASE:FB al0098303	
genetic reagent (<i>D. melanogaster</i>)	P{neoFRT} 80B	(Xu & Rubin, 1993)	FLYBASE:FBt i0002073	Bloomington Drosophila Stock Center #1988
genetic reagent (<i>D. melanogaster</i>)	P{neoFRT} 82B	(Xu & Rubin, 1993)	FLYBASE:FBt i0002074	Bloomington Drosophila Stock Center #2050, 2051
genetic reagent (<i>D. melanogaster</i>)	P{rpS12+8 kb}	(Xu & Rubin, 1993; Kale et al., 2018)	FLYBASE:FBal0 337985	
genetic reagent (<i>D. melanogaster</i>)	P{rpS12-G97D8kb}	(Xu & Rubin, 1993; Kale et al., 2018)	FLYBASE:FBal0 337986	
genetic reagent (<i>D. melanogaster</i>)	P{ry ⁺ Su(var)3-9 ⁺ eIF2 γ ⁺ }	(Xu & Rubin, 1993; Tschiersch et al., 1994; Kale et al., 2018)		
genetic reagent (<i>D. melanogaster</i>)	P{DsRed; RpL28 ⁺ }	This study		See Materials and Methods; Dr. Nicholas Baker's lab.
genetic reagent (<i>D. melanogaster</i>)	P53 ^{5A-1-4}	(Xie & Golic, 2004)	FLYBASE:FBal0 138188	Bloomington Drosophila Stock Center #6815
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}C G11617^{e00462}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0 162546	Bloomington Drosophila Stock Center #17859

genetic reagent (<i>D. melanogaster</i>)	PBac{WH}M ED15 ^{f04180}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBti0042319	Bloomington Drosophila Stock Center #18739
genetic reagent (<i>D. melanogaster</i>)	P{XP}CG9016^{d08241}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160858	Bloomington Drosophila Stock Center #19290
genetic reagent (<i>D. melanogaster</i>)	P{XP}CG9003^{d09761}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160860	Bloomington Drosophila Stock Center #19321
genetic reagent (<i>D. melanogaster</i>)	P{XP}salto^{d09417}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159854	Bloomington Drosophila Stock Center #19315
genetic reagent (<i>D. melanogaster</i>)	P{XP}CG11200^{d02302}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162606	Bloomington Drosophila Stock Center #19173
genetic reagent (<i>D. melanogaster</i>)	P{XP}sob^{d06074}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0158622	Bloomington Drosophila Stock Center #19230
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}Uro ^{f04888}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159557	Bloomington Drosophila Stock Center #18814
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}Ssb-c31a^{e02272}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159728	Bloomington Drosophila Stock Center #18032
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}CG31898^{e03937}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0161732	Bloomington Drosophila Stock Center #18211
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}CG9582^{f00857}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160790	Bloomington Drosophila Stock Center #18378

genetic reagent (<i>D. melanogaster</i>)	PBac{WH}te^{f00157}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159247	Bloomington Drosophila Stock Center #18299
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}C^{G13018}e00535	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162408	Bloomington Drosophila Stock Center #17863
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}Cp^{r51A}e03998	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162723	Bloomington Drosophila Stock Center #18221
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}C^{G10384}f04349	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162694	Bloomington Drosophila Stock Center #18762
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}C^{G42260}f00464	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0225307	
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}Ja^{frac2}f01922	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160273	Bloomington Drosophila Stock Center #18489
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}C^{G17746}f05041	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162020	Bloomington Drosophila Stock Center #18834
genetic reagent (<i>D. melanogaster</i>)	P{XP}Leash^d₀₆₄₅₅	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0161383	Bloomington Drosophila Stock Center #19240
genetic reagent (<i>D. melanogaster</i>)	P{XP}cu^{d0598}₃	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0158886	Bloomington Drosophila Stock Center #19225
genetic reagent (<i>D. melanogaster</i>)	w¹¹¹⁸, P{XP}d06796/TM6B, Tb¹	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBti0042862	Bloomington Drosophila Stock Center #19250

genetic reagent (<i>D. melanogaster</i>)	P{XP}CG10311^{d06928}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162706	Bloomington Drosophila Stock Center #19255
genetic reagent (<i>D. melanogaster</i>)	P{XP}d02570	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBti0054904	
genetic reagent (<i>D. melanogaster</i>)	P{XP}wrm1^{d02813}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160902	Bloomington Drosophila Stock Center #19182
genetic reagent (<i>D. melanogaster</i>)	P{XP}UGP^{d07256}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159573	Bloomington Drosophila Stock Center #19267
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}CG14894^{f04937}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162215	Bloomington Drosophila Stock Center #18821
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}Ca^{d89D^{e03186}}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0160726	Bloomington Drosophila Stock Center #18129
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}A^{ctn3^{f00971}}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162831	Bloomington Drosophila Stock Center #18397
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}qi^{n^{e03728}}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0162275	Bloomington Drosophila Stock Center #18186
genetic reagent (<i>D. melanogaster</i>)	PBac{RB}D^{PCoAC^{e03144}}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0175762	Bloomington Drosophila Stock Center #18121
genetic reagent (<i>D. melanogaster</i>)	PBac{WH}K^{aiRID^{f03502}}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0161451	Bloomington Drosophila Stock Center #18663

genetic reagent (<i>D. melanogaster</i>)	PBac{WH}T otC^{f01700}	(Xu & Rubin, 1993; Thibault et al., 2004)	FLYBASE:FBal0159656	Bloomington Drosophila Stock Center #18460
Antibody	polyclonal Rabbit anti-XRP1(short)	(Francis et al., 2016)		(1:200) dilution
Antibody	Polyclonal Rabbit anti-active-Dcp1	Cell Signalling Technology	Cat #9578	(1:50) dilution
Antibody	Polyclonal Donkey anti-Rabbit IgG, Cy3 conjugate	Jackson ImmunoResearch	Cat # 711-165-152	(1:200) dilution
recombinant DNA reagent	P{DsRed; RpL28*}	This study		See Materials and Methods; Dr. Nicholas Baker's lab.
recombinant DNA reagent	pGE-attBTT-loxP-DsRed (pTL780)	(Blanco-Redondo & Langenhan, 2018)		Addgene Plasmid #115160

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