Xrp1 is a transcription factor required for cell competition-driven elimination of loser cells

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The elimination of unfit cells from a tissue is a process known in Drosophila and mammals as cell competition. In a well-studied paradigm “loser” cells that are heterozygous mutant for a haploinsufficient ribosomal protein gene are eliminated from developing tissues via apoptosis when surrounded by fitter wild-type cells, referred to as “winner” cells. However, the mechanisms underlying the induction of this phenomenon are not fully understood. Here we report that a CCAAT-Enhancer-Binding Protein (C/EBP), Xrp1, which is known to help maintaining genomic stability after genotoxic stress, is necessary for the elimination of loser clones in cell competition. In loser cells, Xrp1 is transcriptionally upregulated by an autoregulatory loop and is able to trigger apoptosis - driving cell elimination. We further show that Xrp1 acts in the nucleus to regulate the transcription of several genes that have been previously involved in cell competition. We therefore speculate that Xrp1 might play a fundamental role as a molecular caretaker of the genomic integrity of tissues.
to the removal of one copy of a haploinsufficient ribosomal protein gene, where, similarly to C/EBP homologs, it regulates its own expression via a positive autoregulatory loop, the expression of pro-apoptotic genes and that of other genes that were previously implicated in cell competition.

In order to identify genes whose function is necessary for the elimination of RPG heterozygous mutant loser cells, we performed a forward genetic screen using ethyl methanesulfonate (EMS) in *Drosophila melanogaster* cells, we performed a forward genetic screen using ethyl methanesulfonate (EMS) in Drosophila melanogaster.

Figure 1. *Xrp1* mutations suppress cell competition driven elimination of loser cells in an EMS-based screen. Schematic of the genetics used to generate RpL19<sup>+/−</sup> loser clones in a wild-type background using the FLP/FRT system. Lines represent chromosomes, numbers at the end of each line indicate the chromosome number and triangles represent FRTs on the right arm of chromosome 3. Site-directed recombination between FRTs occurs when the expression of FLP is induced via heat shock. The yellow asterisk marks the chromosome to be tested (A). Representative examples of living larvae displaying GFP clones in the pouch of the wing imaginal discs. SalE drives Gal4 expression in the wing pouch. (Left) Positive control for clone induction using the FRT82 RpL19<sup>+</sup> chromosome. Recombination generates RpL19<sup>+/+</sup> cells that are not eliminated. (Middle) Negative control for clone induction using the isogenized FRT82 chromosome. Recombination produces RpL19<sup>+/−</sup> cells that are efficiently eliminated. (Right) Suppressor *Xrp1*<sup>08</sup> rescues the elimination of RpL19<sup>+</sup>-<sup>−</sup> cells (B). List of suppressive mutations retrieved with the EMS screen. Intronic mutations *Xrp1*<sup>08</sup> and *Xrp1*<sup>29</sup> are strong suppressors (C). Different *Xrp1* mRNA isoforms (from A to G). Blue color indicates the coding regions and light blue the untranslated regions. The red lines indicate the position of the three *Xrp1* alleles retrieved from the EMS screen (*Xrp1*<sup>08</sup>, *Xrp1*<sup>29</sup> and *Xrp1*<sup>20</sup>) (D).
Figure 2. Xrp1 is required for the elimination of RpL19\(^{+/−}\) loser cells. Schematic representation of the twin spot MARCM system used to generate RpL19\(^{+/−}\) loser clones (red) and their respective twin spot clones (bright green), two copies of GFP) in a background of wild-type GFP positive cells (dark green) (A). RpL19\(^{+/−}\) loser cells are eliminated (B). RpL19\(^{+/−}\) cells are eliminated when Xrp1 mutations are rescued with the re-introduction of one copy of Xrp1 (B'). Loser cells elimination is rescued via either intronic Xrp1\(^{108−/−}\) mutations retrieved from the EMS screen (B') or, even more efficiently, via Xrp1\(^{108−/−}\) mutations in the coding sequence of Xrp1 (B''). Quantification of the mean ratio between mCherry Area and GFP Area (mChe/GFP). Minute loser cells, labeled with mCherry, are eliminated and the mChe/GFP ratio is close to 0. Xrp1 mutants rescue the elimination of loser cells (ratio close to 1). ***P < 0.001, Kruskal-Wallis test. Bars represent SEM. n = 52, 47, 45, 48. Additional significance was calculated via assessing distribution normality (D’Agostino & Pearson normality test). Xrp1\(^{108−/−}\) and Xrp1\(^{61−/−}\) follow a normal distribution (P < 0.001) (C).

revealed an embedded motif of these nucleotides in conserved sequence motifs (Fig. S1). Of particular interest are the polypyrimidine motifs containing the nucleotide mutations in Xrp1\(^{20}\) and Xrp1\(^{61}\). These motifs flank the alternative first exon and are potential splice regulators. The CTCTCT motif in proximity of the 5′ splice site of Xrp1 has been identified as a putative intronic splicing enhancer (ISE) predicted to serve as binding site for the polypyrimidine tract binding protein (PTB) splicing regulator\(^{27}\). The presence of these motifs prompted us to investigate the consequences of the Xrp1\(^{108}\) allele on exonic junctions. The most prominent effect of this allele is a strong and consistent reduction in the expression of two similar Xrp1 transcripts, RC and RC (Fig. S1), which only differ in the composition of their 5′ UTRs. They share the transcriptional start site and contain the same long open reading frame that codes for the short isoform of Xrp1 (Fig. S1).

We then checked the behavior of RpL19\(^{+/−}\) clones in the presence and absence of Xrp1 function. To this end we used the twin spot MARCM system, which enables us to differently mark twin clones generated by the same recombination event. In our genetic set up, mCherry expression marks loser clones whereas two copies of GFP mark wild-type twin clones (Fig. 2A). As expected, RpL19\(^{+/−}\) loser clones are eliminated from the tissue (Fig. 2B). Elimination is also observed when RpL19\(^{+/−}\) cells within these clones are additionally mutant for Xrp1\(^{108}\) but contain a transgene comprising the genomic region of Xrp1 (Fig. 2B'). Importantly, when Xrp1 mutations are not rescued cell competition-driven elimination of RpL19\(^{+/−}\) losers no longer occurs. In particular, we show that the Xrp1\(^{108}\) intronic mutation retrieved from the EMS screen is able to prevent loser cell elimination (Fig. 2B''), and that a similar result is obtained with a newly generated complete loss-of-function allele, Xrp1\(^{108}\) (Fig. 2B'''), as well as with Xrp1\(^{61}\) (Fig. S4). Xrp1\(^{61}\) contains a frame shift mutation upstream of the Xrp1 basic region-leucine zipper domain (b-ZIP), and is considered a null allele. Like other Xrp1 alleles analyzed it is homozygous viable and does not impair the development of mutant animals. To confirm that Xrp1 function is of general importance for the elimination of hRPG\(^{+/−}\) cells, and not limited to RpL19\(^{+/−}\) loser cells, we tested the effect of Xrp1 mutations on RpL14\(^{+/−}\) loser clones (Fig. S2). Similarly to RpL19\(^{+/−}\) cells, RpL14\(^{+/−}\) cells are normally eliminated from wing discs during larval development. No elimination occurs if these cells express RpL14 from a transgene, or when Xrp1 is mutated (Xrp1\(^{61}\)) (Fig. S2).

Since Xrp1 is transcriptionally induced in response to various forms of stress\(^ {19−22}\), and since Xrp1 has been found to be upregulated in RpS3\(^{+/−}\) wing discs when compared to WT discs\(^{25,28}\), we hypothesized that its expression is induced in loser clones as a result of the loss of a haploinsufficient ribosomal protein gene. We therefore used a transcriptional reporter for Xrp1 - Xrp1\(^{P[Gal4]29}\) containing a lacZ P-element\(^{28} -\) and found that Xrp1 expression is indeed upregulated in RpL19\(^{+/−}\) cells, indicating that the upregulation of Xrp1 might play a crucial early role in the elimination of loser cells (Fig. 3A-A). In line with the recent report by Lee et al.\(^{29}\), we found that Xrp1 is upregulated in wing discs that are lacking one copy of a ribosomal protein gene, indicating that Xrp1’s role in cell competition does not depend on clonality (Fig. S3). In order to gain insights into this function we conditionally forced the expression of Xrp1 in the posterior half of the wing discs and observed a massive induction of apoptosis, as revealed by anti-cleaved caspase 3 staining (Fig. 3B,C).

Interestingly, unlike loss of Xrp1, blocking apoptosis by means of overexpression of dIAP1 or p35, or by abrogating the function of Dronc or Hid, does not fully suppress RpL19\(^{+/−}\) cell elimination, suggesting that Xrp1 does more than merely induce apoptosis. Only the co-overexpression of CycE, which promotes cell cycle entry,
together with \(dIAP1\), which suppresses apoptosis, lead to a degree of suppression of \(Rpl19^{+/−}\) cell elimination comparable to that obtained with \(Xrp1\) loss-of-function mutations (Fig. S4). This indicates that the combined effects of blocking cell cycle progression and promoting apoptosis are critical for the elimination of \(RPG^{+/−}\) cells.

Given the strength of the effect of \(Xrp1\) mutations, \(Xrp1\) may therefore additionally hinder cells to progress through the cell cycle. This is in line with Akdemir et al. who found that \(Xrp1\) expression induces cell cycle arrest in cultured \(Drosophila\) cells. Since \(Xrp1\) possesses a sequence-specific DNA binding domain (Fig. S5), either one or both of these cellular functions might be directly regulated at the transcriptional level.

To further explore this notion we set out to identify direct genomic targets of \(Xrp1\) by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) on wing imaginal discs. In order to do this, we induced \(Xrp1\) expression in wing discs. The top targets revealed by ChIP-seq comprise a number of genes that are already implicated in cell competition, cell cycle regulation and apoptosis. Figure 4A shows a list of the most interesting genes that are bound by \(Xrp1\). Among these we identified \(Xrp1\) itself, suggesting the existence of a potential autoregulatory loop. To test this notion we overexpressed \(Xrp1\) in the posterior compartment of the wing disc and checked the transcriptional behavior of \(Xrp1\) with the aforementioned \(Xrp1\)-lacZ reporter. We observed the upregulation of lacZ expression in response to \(Xrp1\) overexpression, indicating that \(Xrp1\) can boosts its own expression in a positive autoregulatory loop (Fig. 4B-B'). We confirmed these observations by measuring mRNA levels of \(Xrp1\) upon forced \(Xrp1\) expression (Fig. 4C). With a similar strategy we also checked the response of other putative transcriptional targets from our ChIP-seq experiment. We could show that \(Xrp1\) promotes the transcription of \(Dif\) (Fig. 4D-D'), a \(Drosophila\) NFkB homolog gene that has previously been implicated in the cell competition-dependent induction of apoptosis via the induction of \(rpr\) transcription. We also tested \(puc\), \(Upd3\), \(Nedd4\) and \(rad50\): all of these genes were upregulated upon induction of \(Xrp1\) expression (Fig. 4F). \(puc\), \(Upd3\) and \(Nedd4\) are involved in the JAK/STAT and Hippo signaling pathways, both of which have previously been implicated in cell competition.

The most prominent sequence motif of \(Xrp1\) derived from ChIP-seq data shows a strong similarity with the \(b\)-ZIP binding motif of the human C/EBP protein family. We therefore checked whether \(Xrp1\) shows homology to C/EBP transcription factors, being itself a bona fide transcription factor. We found that \(Xrp1\) shares a 40% identity with the human C/EBPs (PSI-BLAST). Phylogenetic reconstruction allowed us to recognize three \(Drosophila\) C/EBP homologs, one of which is \(Xrp1\) (Fig. S5). Interestingly, human C/EBP-alpha is retained in the nucleolus and binds to ribosomal DNA, a feature that may be evolutionarily conserved since \(Xrp1\) binds rDNA loci with high affinity (Fig. 4A). The encoded RNA is found in the nucleoli.

We therefore propose a working model in which \(Xrp1\), under normal conditions, sits on rDNA in the nucleolus. In the presence of genotoxic stress or of a ribosomal imbalance, as in the context of \(Minute\) cell competition, \(Xrp1\) acts nuclearly as a C/EBP transcription factor that stimulates its own transcription and the expression of...
Xrp1 regulates its own expression, the expression of pro-apoptotic genes and of genes previously linked to cell competition. ChIP-seq on Xrp1 OE wing discs reveals targets of Xrp1, including rDNA. Xrp1 itself, pro-apoptotic genes such as hid and rpr and several other genes that have been linked to either cell competition or cell proliferation (A). Using a transcriptional reporter for Xrp1 and via the overexpression of Xrp1 in the posterior compartment (en-Gal4), immunostaining reveals a positive feedback loop by which Xrp1 regulates its own transcription. Xrp1 (B–B’). The observation is confirmed by qPCR, Xrp1 is overexpressed in the wing disc 24 hours before analysis. We used primers that recognize all forms of Xrp1 including the overexpressed isoform and confirmed that the Xrp1 overexpression construct is functional (Xrp1 all). With primers that detect only the endogenous forms of Xrp1 we observe Xrp1-dependent induction of Xrp1 expression (Xrp1 vlong, Xrp1 3′UTR) (C). A representative example showing upregulation of the Dif protein upon overexpression of Xrp1 in the posterior compartment (D–D’). Effects on putative Xrp1 target genes are confirmed via qPCRs. The pro-apoptotic gene rpr is upregulated in response to Xrp1 OE in wing discs. Xrp1 OE is induced 24 hours before analysis (E). Other putative target genes are also upregulated under the same conditions, respectively Dif, puc, Upd3, Ned4 and rad50 (F). t-test was applied. *P < 0.05; **P < 0.01; ***P < 0.001.

Materials and Methods
Drosophila strains and cultures. Flies were grown on a standard cornmeal medium at 25°C unless otherwise specified. The salE-GAL4 (2nd), the P[EP]dIAP1 and the en-GAL4, UAS-mCD8::GFP, tubP-GAL80 lines were generated in our laboratory. The UAS-Xrp1.ORF.3xHA (ZH-86Fb) line was obtained from FlyORF. The UAS-E2F, UAS-DP (2nd), Xrp1-LacZ lines were obtained from the Bloomington Drosophila Stock Center. The UAS-CycEg
The genomic DNA region was identified in 5 of them. After the causative mutation was balanced. A mutation in the Xrp1.

**Cloning and transgenesis.** The RpL19 3.08 kbp genomic rescue (2R:24967017..24970096 Dmel_r6.08) was amplified from a genomic DNA template. After sequence confirmation it was cloned within the NotI restriction site of the pUASTattB and inserted into the attP landing site ZH-attP-68E (3L tester line) and ZH-attP-68E (3R tester line)47. The Xrp1 15.88 kbp BamHI-BglII genomic rescue (3R:18911505..18927381 Dmel_r6.08) was digested from CH321-38O16 of the P{acman} BAC Libraries48. After sequence confirmation it was cloned into the pattB vector49 and inserted into the attP landing site ZH-attP-68E. The Xrp1 mutated genomic rescue was generated by inserting 5bp (C > GATCC) at the beginning of the second coding exon in the wild-type genomic fragment, which shifts the frame of all Xrp1 isoforms. Transgenesis was performed according to standard germ-line transformation procedures.

**Mutagenesis and screen.** EMS screens were performed according to standard procedure50. y w hs-FLP; M{3xP3-RFP,attP}ZH-36B; FRT82B starter line was first isogenized for the 3R cell competition screen. Isogenized males were fed with a 25 mM, 1% sucrose solution and crossed to tester virgin females. RpL19+/− clones were induced in the resulting progeny. A total of 20,000 F1 larvae were screened for the persistence of RpL19+/− GFP positive clones at the end of the third instar larval stage. 182 larvae showed persistence of GFP clones clearly above background noise. 125 of them gave rise to fertile adults and were further rescreened. 12 heritable suppressors were doubly balanced. For the Xrp1 coding sequence directed mutagenesis y w; Xrp1{GSV6}14 was obtained from Wei Du51. Xrp1{GSV6}14 (#200976) was obtained from the DGRC Kyoto stock center. The UAS-p35 (2nd)52, the UAS-Rab5 (2nd)53, the UAS-puckered (2nd)54, the sau55 were additionally used. Act5C > GAL4 was obtained by flipping out the y+ FRT cassette of Act5C > y + > GAL4. Bac3-dsRNA-GD9924 (2nd) was obtained from VDRC.

**Mapping the mutations.** We initially mapped cell competition suppressors through meiotic recombinations coupled with DHPLC (Denaturing High-Performance Liquid Chromatography) for PCR amplion analysis. The interval containing the suppressors Xrp108 and Xrp129 was narrowed down to a 106.5 Kb interval (3R:18872668..18979166 Dmel_r6.08). Sanger sequencing of the coding regions in this interval did not reveal the presence of any mutation. We then performed whole-genome sequencing on Xrp108, Xrp120 and Xrp128 with the Illumina’s Genome analyser Ix (Genomics Platform of the University of Geneva). Mutations were identified by visual inspection of the sequences in this interval: Xrp108 (T > A 3R:18921364 Dmel_r6.08), Xrp120 (C > T...
3R:18920194 Dmel_r6.08), Xrpl29(G > A 3R:18921450 Dmel_r6.08). Other suppressors were roughly mapped to the second chromosome or to one of the arms of the third chromosome as indicated in the test complementation table. Minute mutants were identified on the basis of their characteristic bristle phenotype and developmental delay. warts and salvador mutants were identified on the basis of their clonal overgrown phenotypes and failure to complement independent loss of function alleles (warts268 and sav4). Note that for sup85 the suppressive mutation is the Minute on the second chromosome and not the mutation in the salvador gene. Xrpl2 suppressors isolated from the coding sequence directed mutagenesis were identified by direct sequencing of PCR amplicons: Xrpl102 (G > A 3R:18926271 Dmel_r6.08), Xrpl26 (C > T 3R:18926088 Dmel_r6.08), Xrpl13 (C > T 3R:18926394 Dmel_r6.08), Xrpl19 (C > T 3R:18925431 Dmel_r6.08), Xrpl26 (TC > ACA 3R:18925609..18925610 Dmel_r6.08).

**qRT-PCR.** qRT-PCR was performed according to standard protocol. RNA was extracted with TRIzol Reagent and genomic DNA was digested with the Ambion DNase kit. RNA was isolated from third instar wing imaginal discs with the exception of the reaction to evaluate the expression of the different splicing variants in WT and Xrpl26 mutant conditions. In this experiment we used the following primers (primer sequences are oriented 5′ to 3′).

Pr_1: GCGTAGAGAAAGAGAAGTGA; Pr_2: CGACACAAGTTCCTCCTAAAC; Pr_3: TCATTGTTCTCTTCAAGGTCA; Pr_4: GGTGTCGTGTTTTGATTTCTG; Pr_5: CCTACTGCAACAGTTGAAGAGATAAG; Pr_6: GACGTTACGTGACTTGTGGTG.

Pr_1 and Pr_2 were used for Xrpl1_GCA; Pr_3 and Pr_3 were used for Xrpl1_BGA; Pr_1 and Pr_6 were used for Xrpl1_E; Pr_5 and Pr_6 were used for Xrpl1_BGA; Pr_7 and Pr_8 were used for Xrpl1_all. For measuring Xrpl target genes in loser cells large clones overexpressing Xrpl1 were induced. To achieve this, UAS-xrp1:HA line were crossed with female virgins of the y w hs-FLP, Act > CD2 > Gal4, UAS-GFP line. Heat shock was induced for 45′ at 37°C, 4 days AED to induce recombination in most of the cells of the wing disc. The following primers were used.

Act5C_fw: GCGCTTGAATTGTGTAACCT; Act5C_rev: ACAGGCCAGAAGGGAG; puc_rev: TTGGGATAGTCCTTCTGATTGG; Upd3-fw: CCCAGCCAACGATTTTTATG; Upd3_rev: TGTTGGAGCAAT; Xrp1_156_rev: ATGTCTGCATGGGTGCTG; Xrp1_VL-fw: CGGGATGTGAGCCTCAGTGA; Xrp1_3UTR_rev: TAAACACTCCTCGCGCACTA; Xrp1_GCA-fw: GCG

**ChIP-seq preparation and analysis.** Wing imaginal discs expressing HA-tagged Xrpl1 (FlyORF-F000655) were mass isolated and sorted, chromatin was immunoprecipitated and DNA libraries were prepared according to standard protocol. Rabbit anti-HA ChIP grade antibody (ab9110, Abcam) was used. Libraries were mass isolated and sorted, chromatin was immunoprecipitated and DNA libraries were prepared according to standard protocol29. Rabbit anti-HA ChIP grade antibody (ab9110, Abcam) was used. Libraries were mass isolated and sorted, chromatin was immunoprecipitated and DNA libraries were prepared according to standard protocol29.

**Clone induction, immunostaining and imaging.** Rpl19+/-: loser clones for dissections: males of the appropriated genotype were crossed to the “3R” or “3L” tester virgin females. “3R” tester virgin females: y w hs-FLP; Act5C > GAL4-w, UAS-mCherry-CAAX, Df(2R)M60E; FRT82B, ubi-GFP-nls, tubP-GAL80, M[Rpl19 genomic]ZH-86Bf/SMSa-TM6B. “3L” tester virgin females: y w, hs-FLP; Act5C > GAL4-w, UAS-mCherry-CAAX, Df(2R)M60E, ubi-GFP-D, tubP-GAL80, M[Rpl19 genomic]ZH-68E, FRT80Bt/1 SMSa-TM6B. Parents were allowed to lay eggs for 24 hours and Rpl19+/- loser clones were heat-shock induced for 30 minutes at 37°C, 24–48 hours AED. Progeny were screened at the end of the third instar larval stage when larvae stop feeding and move away from the food. No water was added nor was heat-shock applied to force the remaining larvae out of the food as it is routinely done.

**Immunostaining on wing discs** were performed according to standard protocols. The following antibodies were used: rabbit anti-Cleaved-Caspase-3 (Asp175, Cell Signaling), mouse anti-β-Galactosidase (Z3781, Promega), mouse anti-Fibrillarin (38F3; Santa Cruz). The rabbit anti-Dif antibody was obtained from Ylva Engström, the monoclonal mouse anti-Hid antibody was obtained from Hermann Steller. The following secondary antibodies were used: goat anti-mouse Alexa Fluor 647, goat anti-rabbit Alexa Fluor 647 and goat anti-rat Alexa Fluor 647 (Molecular Probes).

Wing discs were imaged using a Leica LSM710 confocal microscope.
Quantification and statistics. Statistical analyses were performed in Graphpad Prism 7 or Microsoft Excel. Depending on the distribution of data, t-test or Mann-Whitney test were used, unless differently indicated. Regarding RpL19−/− loser clones for dissections and clone size quantification, we undertook a stringent comparative analysis based on the ratios between the areas of loser (mCherry) and winner (GFP+) clones. Areas were quantified with FIJI. We applied standardized statistical tests (Mann-Whitney test). In addition, we reasoned that a genuine suppressor of RPG mutant cell elimination should not only increase the mean size of RPG mutant clones but also restore a normal distribution of RPG mutant clones (in this case statistical analysis was performed by using the D’Agostino & Pearson normality test). For RpL14+/− loser clones, GFP area was measured with FIJI and Mann-Whitney test was applied.

Signal intensity calculation for Xrp1 targets was performed in FIJI with the mean gray intensity measurement tool. Statistical significance was calculated with a paired-ratio t-test.

Generation of imaginal wing discs with RpL19+/− and RpL19+/+ compartments. y w hs-FLP; Act5C > GAL4-w, UAS-mCherry-CAAX, Df(2R)M60E/ RpL19+/−, FRT82B, ubiqui-GFP-nls, tubP-GAL80, M[RpL19 genomic/ZH-86Fb/ FRT82B larvae were heat-shocked 15 min at 37 °C during L1. Wing discs were dissected at the end of the third instar larval stage, fixed, stained and imaged.

Identification of Xrp1 homologs. In a heuristic approach, two iterations of PSI-BLAST52 were performed using the bZIP domain of Csm as a query. The COBALT constraint-based multiple protein alignment tool provided on the BLAST interface53 was used to align all Drosophila Xrp1 protein sequences with the human CEBPs family members identified with the PSI-Blast search. In a non-heuristic approach, BZip containing proteins from human and D. melanogaster were searched, aligned and trimmed according to the bZIP_2 motif from Pfam (PF07716) using probabilistic hmmer profiles54 (hmmer.org). The resulting alignment was visualized with CLC Main Workbench and then used for phylogenetic reconstruction using the PhyML algorithm55 with LG substitution models56, SPR topological rearrangements57 and 100 bootstrap replicates. Phylogenetic tree was then mid-point rooted and displayed with the iTOL online tool58.

Drosophila RPGs map/gene density. Gene coordinates for each chromosome arm were retrieved using the cytosearch tool of Flybase. Gene positions were considered as the middle point between the start and the end of each gene. Gene density was calculated for 40 kb bins and the final map was visualized using the radar chart type of Excel. The percentage of intragenic sequences was calculated as the complement of the total size of the genome minus the sum of the intergenic sequences downloaded from Flybase (Genome, FTP, r6.1).

Monte-carlo simulation for RPGs as caretakers of genomic integrity. The computational model was realized with Phyton (detailed code is provided with the supplementary material). The Monte-carlo simulation was designed to determine the probability that a certain number of different genes is disturbed (both alleles are mutated) when a certain number of random mutations occur. It is assumed that each allele has the same probability to be hit by a mutation and that each mutation hits an allele.

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Author Contributions
L.B. performed most of the experiments and wrote an earlier version of the manuscript. L.B., F.G. and C.R. analyzed data. F.G. wrote the current version of the manuscript and performed the RpL14+/− cell competition experiments. C.R. performed the ChIP-seq experiment. J.H. performed qRT-PCR experiments. L.B. and K.B. conceived and planned experiments. K.B. supervised the project.

Additional Information
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