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Ubiquitous overexpression of the DNA repair factor *dPrp19* reduces DNA damage and extends *Drosophila* life spanKathrin Garschall¹, Hanna Dellago², Martina Gálíková^{3,4}, Markus Schosserer ², Thomas Flatt^{1,3} and Johannes Grillari^{2,5,6}

Mechanisms that ensure and maintain the stability of genetic information are fundamentally important for organismal function and can have a large impact on disease, aging, and life span. While a multi-layered cellular apparatus exists to detect and respond to DNA damage, various insults from environmental and endogenous sources continuously affect DNA integrity. Over time this can lead to the accumulation of somatic mutations, which is thought to be one of the major causes of aging. We have previously found that overexpression of the essential human DNA repair and splicing factor SNEV, also called PRP19 or hPso4, extends replicative life span of cultured human endothelial cells and impedes accumulation of DNA damage. Here, we show that adult-specific overexpression of *dPrp19*, the *D. melanogaster* ortholog of human SNEV/PRP19/hPso4, robustly extends life span in female fruit flies. This increase in life span is accompanied by reduced levels of DNA damage and improved resistance to oxidative and genotoxic stress. Our findings suggest that *dPrp19* plays an evolutionarily conserved role in aging, life span modulation and stress resistance, and support the notion that superior DNA maintenance is key to longevity.

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INTRODUCTION

Aging is characterized by a time-progressive decline of physiological function at the level of cells, tissues, organs, and ultimately affects the whole organism. According to the “disposable soma” hypothesis of aging, this functional decline results from the accumulation of stochastic damage, for example, due to somatic mutations, and is counteracted by investment into somatic maintenance and repair.¹ Accumulation of DNA damage due to decreased repair can accelerate aging, as is observed in segmental progeroid syndromes including the Werner or Hutchinson-Gilford syndromes in humans² and mouse models.³ Similarly, increased exposure to DNA damaging agents, for instance during chemotherapy, can lead to a phenotype of acquired premature progeroid syndrome.⁴ Accelerated accumulation of DNA damage and premature aging phenotypes are typically well correlated, but whether improved DNA damage repair (DDR) can extend organismal life span remains largely unclear.

In the fruit fly (*Drosophila melanogaster*), a well-studied model for dissecting the mechanisms of aging, spontaneous somatic mutations accumulate with age, and defective DNA repair is associated with reduced life span.^{5, 6} However, overexpression of DNA repair factors in the fly seems to have highly variable, sometimes contradictory effects that depend on sex, developmental stage, and the tissue of intervention. For instance, poly (ADP-ribose) polymerase-1 (PARP-1) modifies histones, transcription factors and repair enzymes in response to DNA breaks, and its endogenous activity is well correlated with life span in several mammalian species.⁷ In *Drosophila*, overexpression of PARP-1 prolongs life span in both sexes, yet only when restricted to the adult nervous system.⁸ Similarly, overexpression of Gadd45 (growth arrest and DNA damage 45) (ref. 9), a regulator of DNA

repair and cellular stress responses, in the nervous system increases fly life span but ubiquitous expression is lethal.^{10, 11} Indeed, a recent study by Shaposhnikov *et al.*¹² has found that DNA repair factors can affect *Drosophila* life span and stress resistance either positively or negatively, depending on the sex and on whether overexpression is ubiquitous or limited to the nervous system. Interestingly, all repair factors that were expressed throughout the adult fly body were found to shorten life span. In another study, Barclay and colleagues examined the effects of overexpression of several known *D. melanogaster* homologs of human DNA repair genes on *Drosophila* life span in a genetic model of spinocerebral ataxia (SCA), aiming to identify repair pathways that might be relevant for SCA pathology. They found that an extension of life span and improvement of SCA symptoms could not be attributed to a single repair pathway; instead, each pathway included factors that had either detrimental, beneficial, or no effects on life span.¹³ Yet, these results—obtained in a diseased mutant background—do not necessarily reflect possible life span effects of DNA repair factors in healthy wild-type flies. Thus, to date, the relationship between DNA damage, repair and organismal aging still remains poorly understood.

Here, we examine the role of adult-specific overexpression of the DNA repair factor *Prp19* (pre-messenger RNA (mRNA) processing factor 19) in affecting life span, stress resistance, and DNA damage in *Drosophila*. PRP19 (also called senescence evasion factor, SNEV, or hPso4) was first characterized in a yeast mutant exhibiting increased sensitivity to DNA interstrand crosslinking induced by treatment with psoralen and ultraviolet (UV) radiation.¹⁴ Biochemically, PRP19 acts as an E3 ubiquitin ligase^{15, 16} and interacts with multiple players in the DNA repair pathways, including each of the two core kinases, ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia related).^{17, 18} Apart from

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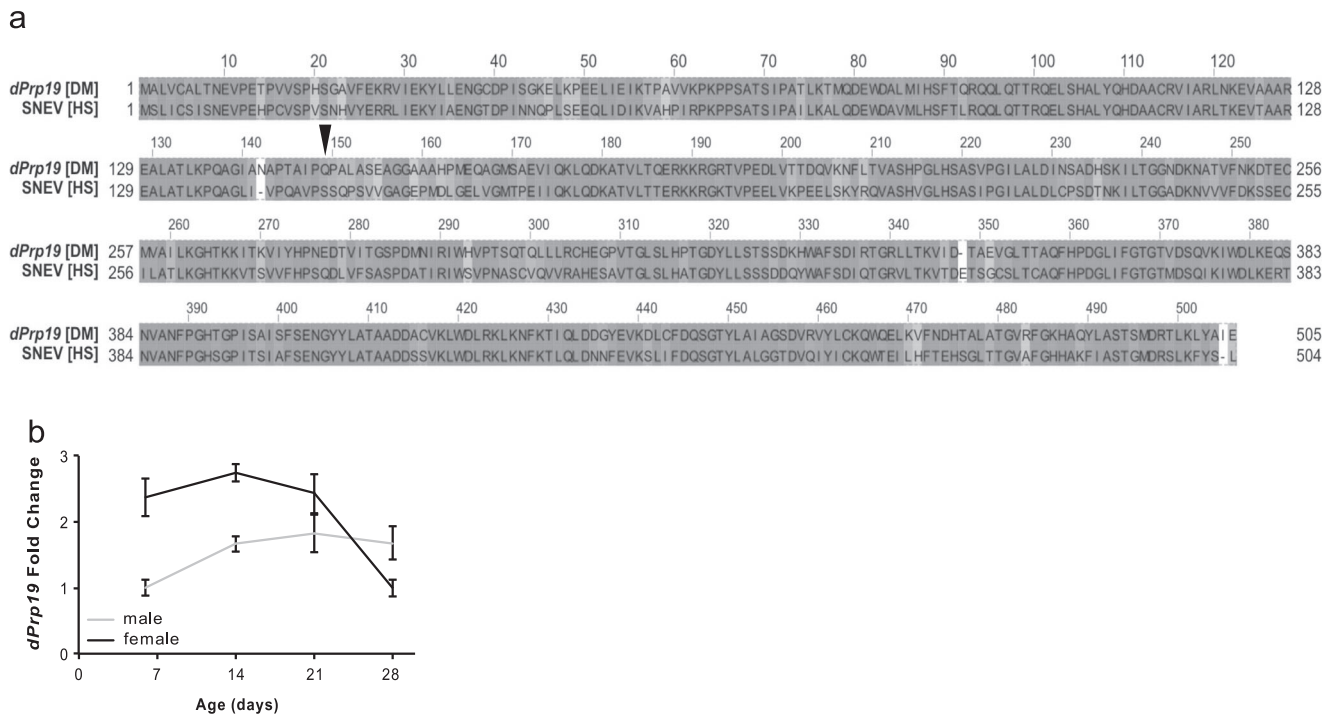


Fig. 1 *dPrp19* is the *Drosophila melanogaster* ortholog of human *PRP19/SNEV/hPso4*. **a** *Prp19* is well-conserved from *D. melanogaster* to humans, as shown in an alignment of the protein sequences of *D. melanogaster dPrp19* and human *PRP19/SNEV/hPso4*. The two orthologs have an amino acid identity of 66% and a sequence similarity of 81%. The human site of ATM phosphorylation, Ser¹⁴⁹, is substituted for Gln in *Drosophila* (indicated by the black arrow). **b** *dPrp19* expression is higher in female *D. melanogaster* and decreases with age. Comparison of *dPrp19* mRNA levels in male (gray) and female (black) wild-type flies at 6, 14, 21, and 28 days of adulthood shows that *dPrp19* levels decrease in females between days 21 and 28. Values represent means across three biological replicates (± 1 standard deviation), normalized to *Gapdh2* and *Tubulin* and averaged

its role in the DNA damage response, an intriguing aspect of PRP19 function is its concomitant and essential involvement in co-transcriptional splicing, where the PRP19 complex regulates the rearrangement of the spliceosome to a catalytically active state through ubiquitination of several factors.^{19–21} The dual role of PRP19 in DNA repair and transcriptional control is further exemplified by its association with transcription-coupled repair, which is activated when DNA damage blocks elongation²² but which has not yet been characterized as a DNA repair mechanism in *Drosophila*.²³

In support of a major role of PRP19/SNEV/hPSO4 in the aging process, it has previously been shown that decreased levels of PRP19 accelerate the induction of cellular senescence in mouse embryonic fibroblasts,²⁴ reduce self renewal of mouse hematopoietic stem cells,²⁵ increase psoralen/UV-A-induced skin aging in mice²⁶ and decrease differentiation of human adipose-derived stromal cells.²⁷ Conversely, increased levels of PRP19 extend the replicative potential and total life span of cultured human endothelial cells.^{17, 28} However, the role of PRP19 in organismal life span is unknown. Here, we show that ubiquitous overexpression of the *Drosophila* ortholog of *PRP19*, *dPrp19* (<http://flybase.org/reports/FBgn0261119.html>), reduces DNA damage and extends organismal life span of adult female flies. Our results suggest that PRP19 plays an evolutionarily conserved role in DDR, aging, and stress resistance.

RESULTS

dPrp19 is the fly ortholog of human *PRP19/SNEV/Pso4* and is regulated in an age-dependent manner

In support of an evolutionarily conserved role of *PRP19/SNEV/Pso4* in aging and DNA repair²¹ in *Drosophila*, we found a high degree of amino acid identity (66%) and similarity (81%) between human

PRP19 and fly *dPrp19* (Fig. 1a). To determine whether *dPrp19* expression is regulated during the fly's life span, we performed quantitative reverse transcription PCR (qRT-PCR) in whole-body samples of adult flies and found that *dPrp19* transcript levels are rather constant in adult males, whereas they decrease with age in adult females (Fig. 1b). Over the course of 28 days, we observed a ~2.5-fold reduction in *dPrp19* mRNA levels in females; in contrast, male expression was lower and largely unaffected by age (Fig. 1b). This observation prompted us to test whether we could—similar to our previous observations in human cells^{17, 28}—extend life span and promote stress resistance in *Drosophila* by overexpressing *dPrp19*.

dPrp19 overexpression extends *Drosophila* female life span and prevents DNA damage accumulation

To examine the life span effects of *dPrp19* in the fly, we drove adult-specific, ubiquitous overexpression of *dPrp19* in three independent transgenic strains carrying a UAS expression cassette (*UAS-dPrp19*) with the inducible GeneSwitch (GS)-Gal4 driver system.²⁹ By supplementing the fly food medium with the antiprogestin compound RU486 (mifepristone), the GeneSwitch (GS) system can be used to induce overexpression in the F1 of a cross between the UAS responder and the Gal4 driver construct. This allows for spatio-temporal control of candidate gene expression and is commonly used in aging studies.^{30, 31} To drive overexpression of *dPrp19* from the UAS lines, we used two different ubiquitously expressing GS-Gal4 driver lines: a *Tubulin*-GeneSwitch-Gal4 (*TubGS-Gal4*) line and a *daughterless*-GeneSwitch-Gal4 (*daGS-Gal4*) line.³² In females, overexpression of *dPrp19* resulted in approximately sevenfold upregulation of mRNA (Fig. 2b, d, and h) and in males in approximately 12- to 22-fold induction (Fig. 2f, j).

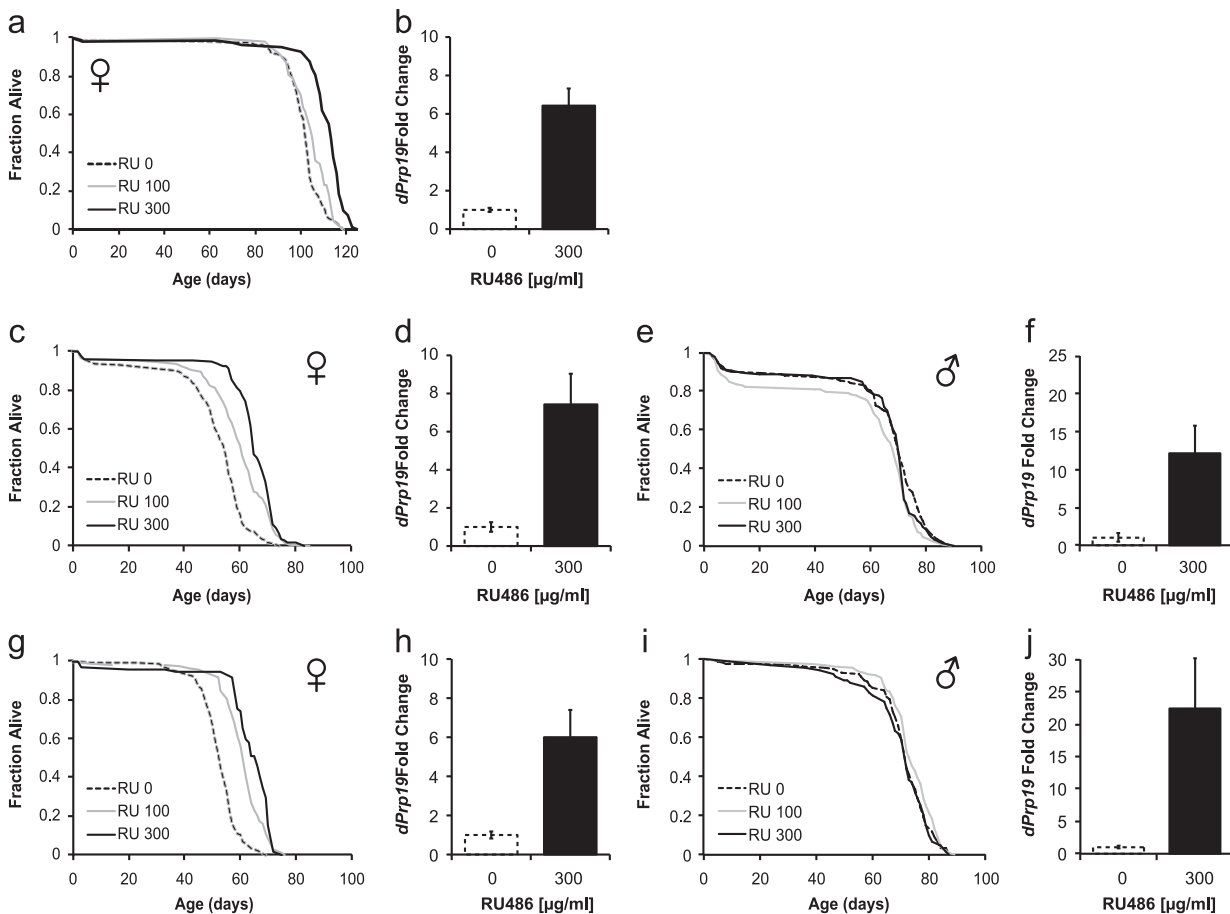


Fig. 2 Overexpression of *dPrp19* leads to dose-dependent extension of female but not male life span. Effects of the induction of the *dPrp19* UAS cassette in three independent chromosomal insertions of the same transgenic construct on adult survival and *dPrp19* mRNA levels: **a, b** *TubGS-Gal4 > UAS-dPrp19-1* (females only), **c–f** *daGS-Gal4 > UAS-dPrp19-2* (females and males), and **g–j** *daGS-Gal4 > UAS-dPrp19-4* (females and males). **a, c, e, g,** and **i** show survival curves of experimental flies at three concentrations of the inducer drug RU486; **b, d, f, h,** and **j** show quantification of *dPrp19* expression levels relative to the *Rp49* control after 72 h of exposure to 300 µg/ml RU486. For all three overexpression constructs, we find a significant dose-dependent extension of female life span. Overall, we did not find any life span extension in males (for *daGS-Gal4 > UAS-dPrp19-2* males at 100 µg/ml RU486 we observed a slight reduction in survival, possibly due to inadvertent “setup mortality” that might have occurred when the assay was set up). For details of life span statistics see Supplementary Table 1 (females) and Supplementary Table 2 (males); for experimental details see Materials and Methods

Across independent experiments in our laboratories in Vienna and Lausanne, three different UAS responder constructs, and two different Gal4 drivers we observed significant and robust dose-dependent extension of female life span (Fig. 2, Supplementary Fig. 1, Supplementary Table 1). At the highest level of induction (food supplemented with 300 µg/ml RU486) median female life span was increased by between 9.6 and 25%. In one of these assays, *dPrp19* overexpression was carried out on food medium containing 2% yeast (Fig. 2a, b), whereas all other assays were performed on a diet containing 5% yeast (Fig. 2c–j). Although mean and maximum life span of controls were strongly affected by the two dietary conditions, *dPrp19* overexpression robustly extended female life span in all cases (Fig. 2a, c, and g, Supplementary Table 1). In contrast, male life span was not consistently affected by *dPrp19* overexpression in any of our assays (Fig. 2e, i, Supplementary Table 2), despite higher levels of *dPrp19* mRNA than in females.

Next, to examine whether ubiquitously increased expression of *dPrp19* affects DDR in the fly, we analyzed the abundance of the histone variant γ H2Av, a well-established marker for DNA damage.³³ Upon *dPrp19* overexpression in females, we observed a clear-cut decrease in the levels of γ H2Av (Fig. 3a), suggesting a general reduction in the number of DNA double-strand breaks under basal conditions when *dPrp19* is upregulated. Interestingly,

we did not observe any reduction of γ H2Av signal in males (Fig. 3b), indicating that in contrast to females *dPrp19* overexpression does not improve DNA repair capacity and probably thereby does not extend life span.

dPrp19 overexpression increases resistance to DNA damaging compounds

Since human PRP19 is induced by various DNA damaging agents^{17, 34} and conveys resistance to genotoxic and cytotoxic stress,^{17, 26–28, 34, 35} we next asked whether *dPrp19* overexpression increases resistance against two DNA damaging agents, paraquat (methyl viologen), a herbicide known to induce reactive oxygen species,³⁶ and the genotoxic compound cisplatin, which causes crosslinks between adjacent nucleosomes and which has previously been used to study DNA damage in *Drosophila*.³⁷ Indeed, overexpression of *dPrp19* significantly improved the adult survival of females exposed to both paraquat (Fig. 4a; Supplementary Table 3) and cisplatin (Fig. 4b; Supplementary Table 4).

Since in the above experiments *dPrp19* overexpression was induced by adding RU486 to the food medium before-but not during-cisplatin exposure, it is possible that transgenic induction of *dPrp19* might have decreased during treatment with cisplatin. To rule out this possibility, we examined the adult survival of F1

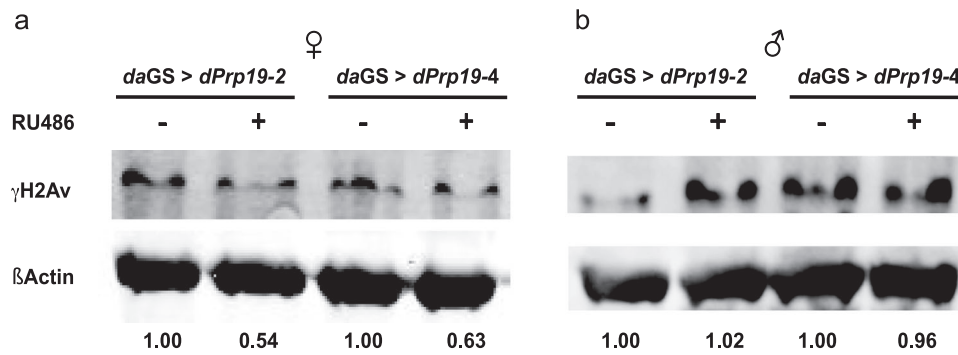


Fig. 3 Overexpression of *dPrp19* decreases γ H2Av as marker for DNA double-strand breaks in females but not males. Overexpression of *dPrp19* (*daGS-Gal4 > UAS-dPrp19-2/4*) induced by 300 μ g/ml RU486 decreases the accumulation of γ H2Av by approximately 40–50% in female flies **a** as compared to the uninduced controls. In males **b**, we did not observe an effect of *dPrp19* induction on DNA damage levels. Numbers represent protein signals of γ H2Av normalized to β Actin. The blot shown is representative; qualitatively identical results were obtained with at least two biological and two technical replicates

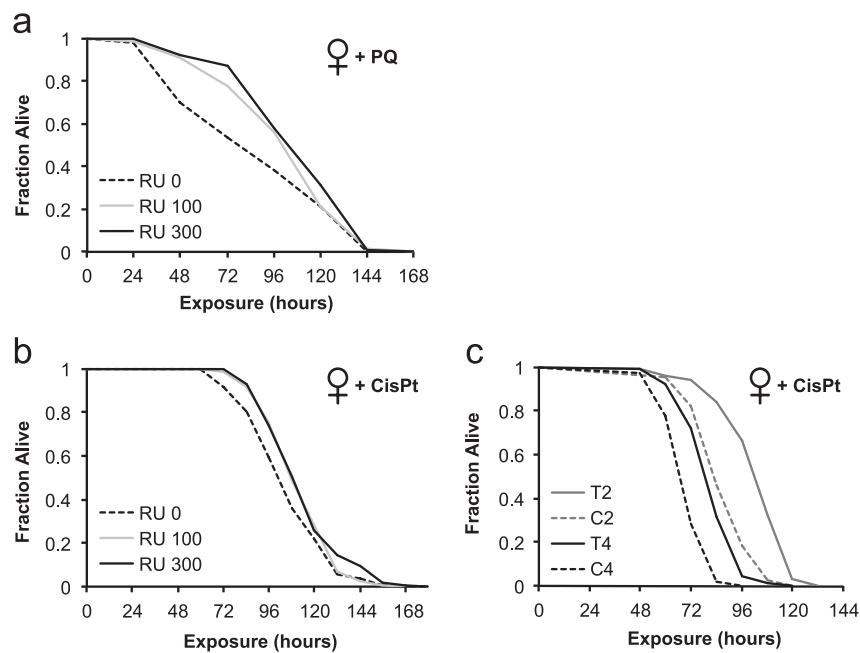


Fig. 4 Overexpression of *dPrp19* promotes resistance to genotoxic agents in female flies. **a** Induction of the *dPrp19* cassette with either 100 (gray line) or 300 μ g/ml RU486 (black, solid line) significantly increases resistance to 20 mM paraquat (methyl viologen) in females (*TubGS-Gal4 > UAS-dPrp19-1*) as compared to the uninduced control (black, dashed line; see Supplementary Table 3 for statistics). **b** Resistance of females (*daGS-Gal4 > UAS-dPrp19-2*) to genotoxic stress induced by treatment with 400 μ g/ml cisplatin was improved significantly by *dPrp19* induction at 300 μ g/ml RU486; the intermediate induction level of 100 μ g/ml RU486 was not significant after Bonferroni correction (see Supplementary Table 4 for statistics). **c** Females with constitutively active *dPrp19* expression (T2: *Tub-Gal4 > UAS-dPrp19-2* in gray; T4: *Tub-Gal4 > UAS-dPrp19-4* in black) showed significantly improved resistance to cisplatin relative to controls (C2: TM65b; *UAS-dPrp19-2*, in dashed gray; C4: TM65b; *UAS-dPrp19-4*, in dashed black line; see Supplementary Table 4 for statistics)

flies from a cross between a constitutively active (non-inducible) *Tub-Gal4* driver and the UAS overexpression lines. Transgenic F1 offspring carrying both the driver and the overexpression cassette had strongly improved survival over sibling F1 controls lacking the driver construct, thus clearly confirming the protective effects of *dPrp19* upon exposure to genotoxic stress (Fig. 4c; Supplementary Table 4).

DISCUSSION

Here, we have shown that ubiquitous, adult-specific overexpression of *dPrp19*, an evolutionarily conserved DNA repair factor, significantly and robustly extends female life span in *Drosophila*.

Overexpression of *dPrp19* further reduces DNA damage levels and enhances survival upon oxidative and genotoxic stress. The effects we observed in our experiments are independent of the details of chromosomal insertion position of the *UAS-dPrp19* cassette, the *Gal4* driver system, and laboratory (food) conditions.

Our data clearly support previous results from cultured human endothelial cells, where PRP19/SNEV/hPso4 overexpression strongly extends replicative life span, lowers basal levels of DNA double-strand breaks, and increases resistance to pro-oxidants as well as to the DNA crosslinker cisplatin.^{17, 28} In line with these findings, female flies overexpressing *dPrp19* exhibited decreased accumulation of DNA double-strand breaks, as indicated by reduced levels of the histone variant γ H2Av, the fly homolog of

γ H2AX. During *Drosophila* aging increased levels of γ H2Av are associated with age-dependent degeneration, for example, in tissues such as the intestine³³ and muscle.³⁸

Interestingly, the benefits of *dPrp19* overexpression we have observed in terms of increased life span and reduced DNA damage seem to be restricted to females. Sex-specific effects of aging interventions have been widely observed,^{12, 39} and quantitative genetic analyses indicate that the genetic architecture of life span is very different in female and male *Drosophila*⁴⁰; yet, the reasons for this sex-specificity remain unknown. Further work will be required to understand why males do not benefit from overexpression of *dPrp19*.

Mechanistically, there are several possible explanations for how *dPrp19* might improve DNA repair in females and thereby promote stress survival and longer life span: *dPrp19* might either directly recruit other DNA repair factors in order to promote fast and efficient repair, or it might act via the regulation of pre-mRNA processing. Human PRP19, for example, is known to interact with proteins involved in DNA repair such as Werner syndrome helicase (WRN),⁴¹ Metnase,⁴² ATR¹⁸ and ATM.¹⁷ In response to DNA damage, ATM phosphorylates human PRP19, thereby possibly contributing to an arrest of the cell cycle in order to allow for repair. However, signaling from ATM to PRP19 is not solely responsible for this effect since overexpression of a non-phosphorylatable PRP19 protein still extends replicative potential in human cells, albeit to a lesser extent.¹⁷ Since the ATM target phosphorylation site is not conserved in the fly homolog *dPrp19*, extension of female life span under *dPrp19* overexpression could be a consequence of its essential role in pre-mRNA splicing.^{4, 15, 21}

In a large short interfering RNA screen for factors affecting γ H2AX levels in human HeLa cells, Paulsen et al.⁴³ found strong evidence for the importance of mRNA processing factors, such as PRP19, in maintaining genome stability. They observed that the γ H2AX signal for double strand breaks was strongly increased upon knockdown of RNA-processing and attributed this at least partly to an increased formation of R-loops. R-loops occur when the transcription machinery is slowed down, for instance by encountering a site of DNA damage, and the transcript folds back on the template duplex DNA strand.⁴⁴ The resulting DNA–RNA hybrid structure activates non-canonical ATM signaling⁴⁵ and can become the source of mutation and DNA breaks double strand itself.⁴⁴

Interestingly, PRP19 has also been described as being a sensor of DNA damage during replication stress: It binds and ubiquitinates replication protein A, a key factor in the response DNA damage which protectively binds single-stranded DNA, which causes recruitment of ATR and thus initiates activation of further DNA repair factors.¹⁸ In a *Drosophila* mutant model of SCA, overexpression of *Drosophila* RpA had the largest effect on life span of all assayed DNA repair factors,¹³ indicating a possible joint role in promoting longevity.

In conclusion, overexpression of *dPrp19*, a protein that functionally interacts with many important DNA repair factors, robustly extends life span of female fruit flies. Importantly, our results demonstrate that this female-specific life span extension is accompanied by decreased DNA damage and by significantly increased resistance to damage caused by paraquat and the DNA crosslinking agent cisplatin. Our data therefore strongly suggest that improved DNA repair capacity can extend the life span of a healthy organism, a view that has so far been almost exclusively based on the study of premature aging phenotypes induced by impaired DNA repair.

MATERIALS AND METHODS

Drosophila stocks and maintenance

A *D. melanogaster* complementary DNA (cDNA) clone of *dPrp19* in pBluescript (clone LD02793, stock 13414) was obtained from the

Drosophila Genome Resource Center (DGRC) at Indiana University (Bloomington, IN, USA). The cDNA was cloned into pUAST-C5 and verified by sequencing. We received three independent UAS-*dPrp19* insertions on the second chromosome in a *w¹¹¹⁸* background (one balanced, two homozygous viable lines) from Genetic Services Inc. (Cambridge, MA, USA). To drive inducible, ubiquitous expression of the UAS constructs we used two different Gal4 constructs: a *Tubulin*–GeneSwitch-Gal4 (*TubGS*-Gal4) (courtesy of Scott Pletcher, University of Michigan, USA) and a *daughterless*-GeneSwitch-GAL4 (*daGS*-Gal4) line.³² F1 progeny of crosses between the UAS-*dPrp19* and either the *daGS*-Gal4 or the *TubGS*-Gal4 line were used as experimental flies in stress resistance and life span assays. As controls for potential confounding effects of RU486 we used flies from crosses between *daGS*-Gal4 with *w¹¹¹⁸*; these controls were exposed to the same treatment with RU486 as the experimental flies. Constitutive overexpression (for the purpose of continuous *dPrp19* expression during cisplatin treatment) was achieved by crossing the UAS-*dPrp19* lines to a constitutive balanced *Tubulin*-Gal4 (*Tub*-Gal4) driver obtained from the Bloomington *Drosophila* Stock Center (BDSC, stock #5138). We used the F1 offspring carrying both the driver and *dPrp19* expression cassette (*Tub*-Gal4 > UAS-*dPrp19*) as experimental flies and F1 flies carrying the balancer and one copy of *dPrp19* cassette (UAS-*dPrp19*; TM65b) as controls. Experiments involving the UAS line *dPrp19-1* were performed in Vienna on a cornmeal-agar diet containing 2% inactivated yeast (a food level corresponding to life span extending dietary restriction); experiments using the lines UAS-*dPrp19-2* and UAS-*dPrp19-4* were performed in Lausanne on a cornmeal-sucrose-agar medium with 5% yeast. In both laboratories, flies were maintained and assayed at 25 °C on a 12 h:12 h light:dark cycle at approx. 60–70% humidity.

Sequence alignment

Protein sequences of *PRP19/SNEV/Pso4* from *Homo sapiens* [GenBank ID: NP_055317.1] and of *dPrp19* from *D. melanogaster* [GenBank ID: NP_523783.1] were aligned with T-Coffee.⁴⁶ We plotted the alignment with the help of Jalview (version 2.7).⁴⁷ Similarities and identities were derived from a pBLAST alignment of both sequences.

Quantitative reverse transcription-PCR

Ten flies per treatment were suspended in TRI reagent (Sigma-Aldrich, St Louis, USA) and mechanically homogenized by using a pellet pestle (Sigma-Aldrich, St Louis, USA). RNA extraction was performed following the manufacturer's instructions and reverse-transcribed into cDNA using the DyNAmo cDNA Synthesis Kit (Finnzymes, Vantaa, Finland). We assayed mRNA levels of target genes relative to housekeeping control genes were assayed with SYBR Green-based qRT-PCR on a Rotorgene Q thermal cycler (Qiagen, Hilden, Germany) using the 5× HOT FIREPol EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) and the following primer pairs:(*dPrp19*: 5'-GTCATACCGGTCCCATTT-3', and 5'-TGAATACCTTCAGTTCCTGC-3';

Gapdh2: 5'-GCGGTAGAATGGGGTGGAGAC-3', and 5'-TGAAGAGCGAAAACAGTAGC-3'; *Tubulin*: 5'-CGTCTCTGAGTCAGACCTCGAAA-3', and 5'-GACACCAGCCTGACCAACATGGA-3'; *Rp49*: 5'-CCCACCGGATTCAAGAAGTT-3', and 5'-AATGTGTATTCCGACCACGTTAC-3'). Since *Rp49* levels, as compared to the two other "housekeeping" genes, changed with age (data not shown), *Gapdh2*, and *Tubulin* were used for normalization of samples from flies of different ages. To test for transgene induction by RU486 (mifepristone; Beta Pharma, Princeton, NJ, USA), we collected a 24 h cohort of flies and kept them on food containing 0, 100, or 300 µg/ml RU486 for 3 days. For each sample, we harvested 10 female or male flies and processed them as described above; data were normalized to *Rp49*.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting

For SDS-PAGE, we used young (3–4 days old) flies that had been exposed to 300 µg/ml RU486 for 3 days. For each sample, we homogenized 5 female or 8 male flies in 150 µl 2× SDS loading dye (1 M Tris-Clat pH 6.8, 4% SDS, 20% glycerol, 0.025% bromophenol blue, 2.5% β -mercapto-ethanol), heated to 95 °C for 5 min, cooled on ice and spun down. We separated 30 µl of supernatant, corresponding to the protein content of one fly, on a NuPAGE 4–12% Bis/Tris polyacrylamide gel (Invitrogen, Carlsbad, CA, USA) in MOPS buffer at 200 V. Electrophoresis and blotting to polyvinylidene fluoride membrane (Roth, Karlsruhe, Germany) were performed using the Biorad TGX Gel + TransTurbo Blotting system (Biorad, Hercules, CA, USA) in accordance with the manufacturer's protocol. After incubation with blocking buffer (3% skim milk powder in phosphate-buffered saline

(PBS) with 0.1% Tween-20) on an orbital shaker for 1 h at room temperature, membranes were incubated overnight with a mix of both primary antibodies diluted in blocking buffer, followed by 1 h incubation with a mix of the secondary antibodies. Antibody incubations were followed by three washes with PBS with 0.1% Tween-20. Membranes were scanned and signal intensity was quantified using the Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA). We used primary antibodies against Histone H2AvD p137 (rabbit, diluted 1:1000; Rockland, Limerick, PA, USA) and β Actin (mouse, diluted 1:2500; Abcam, Cambridge, UK). Secondary antibodies were anti-Rabbit-IR-Dye 800 and anti-Mouse-Alexa 680 (LI-COR, Lincoln, NE, USA), both diluted 1:10,000. Each sample was blotted in at least two biological (lysates from a different set of flies) and two technical (same lysates loaded, blotted and analyzed independently) replicates.

Life span assays

Adult survival was determined using standard methods. For experiments in Vienna we collected 24-h cohorts of F1 of reciprocal crosses between *TubGS-Gal4* and *dPrp19-1*. In Lausanne, we collected the F1 of reciprocal crosses between *daGS-Gal4* and the *dPrp19-2* and *dPrp19-4* lines as well as *w¹¹¹⁸* within 24 h of eclosion. Following sorting of genotypes under mild CO₂ anesthesia, we initiated two replicate cages per RU486 concentration (0, 100, or 300 μ g/ml food) with 75 females and 75 males for each genotype. Flies were kept in incubators at 25 °C, 60% humidity and a 12:12 light/dark cycle; dead flies were scored every 24 h, blind with respect to genotype identity. We censored flies that escaped or got stuck in the food medium and replaced food vials every second day with fresh ones. To assess pairwise differences in survivorship within each genotype and across RU486 treatments, we used log-rank tests implemented in JMPv.10.0 (SAS Institute Inc., Cary, NC, USA). Effects of *dPrp19* over-expression on survival were considered significant if *P*-values passed a Bonferroni-corrected threshold of *P* < 0.0167. For details of cohort (sample) sizes see Supplementary Tables 1 and 2.

Paraquat resistance assay

Paraquat resistance assays were performed as previously described⁴⁸ with minor modifications. Briefly, newly eclosed flies (*TubGS-Gal4* > *UAS-dPrp19-1*) were allowed to mate in bottles containing 100 μ g/ml, 300 μ g/ml, or no RU486. After 3 days of mating, flies were sexed and 20 females were flipped to vials containing a solution consisting of 2% agar, 5% glucose, and 20 mM paraquat (Sigma-Aldrich, St Louis, USA). Vials were checked for dead flies every 24 h until the last fly was dead. Significant differences in survival between the different RU486 treatments were tested using log-rank tests implemented in JMPv.10.0 (SAS Institute Inc., Cary, NC, USA). For details of cohort (sample) size see Supplementary Table 3.

Cisplatin resistance assays

Resistance to cisplatin was assayed on F1 cohorts of experimental (*daGS-Gal4* > *UAS-dPrp19-2*) and (no construct) control flies from the same cross (*daGS-Gal4*; *w¹¹¹⁸*), collected within 24 h of eclosion on standard food. We selected and counted female flies under mild CO₂ exposure and housed them on medium supplemented with different concentrations of RU486 (0, 100, or 300 μ g/ml food) for 3 days. For each genotype, we used 20 females per vial (10 replicate vials per genotype-RU486 combination), the vials containing 2 ml of an agar (0.5%)-sucrose (5%) solution supplemented with 400 μ g/ml cisplatin (P4394, Sigma-Aldrich, St Louis, USA). Since the cisplatin medium did not contain any RU486, we expected induction of *dPrp19* expression to decrease over time. To rule out this possibility, we also included flies from a cross between a constitutively active *Tub-Gal4* driver and the overexpression lines to compare experimental flies with an activated *dPrp19* cassette (*Tub-Gal4* > *UAS-dPrp19-2* / *UAS-dPrp19-4*) to control flies lacking the driver (*UAS-dPrp19-2* / *UAS-dPrp19-4*; TM65b). Scoring was performed every 12 h until the last fly died. We tested for pairwise significant differences in survival between different RU486 concentrations or between pairs of experimental flies and their respective controls with log-rank tests in JMPv.10.0 (SAS Institute Inc., Cary, NC, USA). For details of cohort (sample) sizes see Supplementary Table 4.

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AUTHOR CONTRIBUTIONS

K.G., H.D., M.S., T.F., and J.G. designed experiments; K.G., H.D., M.S., and M.G. performed experiments; K.G., H.D., and M.S. designed figures and wrote the manuscript; T.F. and J.G. supervised the writing of the manuscript. All authors read, edited, and approved the final manuscript.

ADDITIONAL INFORMATION

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REFERENCES

- Kirkwood, T. B. L. & Melov, S. On the programmed/non-programmed nature of ageing within the life history. *Curr. Biol.* **21**, R701–R707 (2011).
- Martin, G. M. & Oshima, J. Lessons from human progeroid syndromes. *Nature* **408**, 263–266 (2000).
- Schumacher, B., Hoesjmakers, J. H. & Garinis, G. A. Sealing the gap between nuclear DNA damage and longevity. *Mol. Cell Endocrinol.* **299**, 112–117 (2009).
- Grillari, J., Katinger, H. & Voglauer, R. Contributions of DNA interstrand cross-links to aging of cells and organisms. *Nucleic Acids Res.* **35**, 7566–7576 (2007).
- Garcia, A. M. et al. Age- and temperature-dependent somatic mutation accumulation in *Drosophila melanogaster*. *PLoS Genet.* **6**, e1000950 (2010).
- Whitehead, I. & Grigliatti, T. A. A correlation between DNA repair capacity and longevity in adult *Drosophila melanogaster*. *J. Gerontol.* **48**, B124–B132 (1993).
- Bürkle, A., Brabeck, C., Diefenbach, J. & Beneke, S. The emerging role of poly(ADP-ribose) polymerase-1 in longevity. *Int. J. Biochem. Cell Biol.* **37**, 1043–1053 (2005).
- Shaposhnikov, M. V., Moskalev, A. A. & Plyusnina, E. N. Effect of PARP-1 over-expression and pharmacological inhibition of NF- κ B on the lifespan of *Drosophila melanogaster*. *Adv. Gerontol.* **24**, 405–419 (2011).
- Moskalev, A. et al. The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance. *Cell Cycle* **11**, 4222–4241 (2012).
- Plyusnina, E. N., Shaposhnikov, M. V. & Moskalev, A. A. Increase of *Drosophila melanogaster* lifespan due to D-GADD45 overexpression in the nervous system. *Biogerontology* **12**, 211–226 (2011).
- Peretz, G., Bakhrat, A. & Abdu, U. Expression of the *Drosophila melanogaster* GADD45 homolog (CG11086) affects egg asymmetric development that is mediated by the c-Jun N-terminal kinase pathway. *Genetics* **177**, 1691–1702 (2007).
- Shaposhnikov, M., Proshkina, E., Shilova, L., Zhavoronkov, A. & Moskalev, A. Lifespan and stress resistance in *Drosophila* with overexpressed DNA repair genes. *Sci. Rep.* **5**, 15299 (2015).
- Barclay, S. S. et al. Systems biology analysis of *Drosophila* in vivo screen data elucidates core networks for DNA damage repair in SCA1. *Hum. Mol. Genet.* **23**, 1345–1364 (2014).
- Henriques Pêgas, J. A., JoséVicente, E., Correia Leandro da Silva, K. V. & Guerrini Schenberg, A. C. PSO4: a novel gene involved in error-prone repair in *Saccharomyces cerevisiae*. *Mutat. Res. Repair* **218**, 111–124 (1989).
- Löscher, M. et al. Interaction of U-box E3 ligase SNEV with PSMB4, the beta7 subunit of the 20 S proteasome. *Biochem. J.* **388**, 593–603 (2005).

16. Hatakeyama, S., Yada, M., Matsumoto, M., Ishida, N. & Nakayama, K. I. U box proteins as a new family of ubiquitin-protein ligases. *J. Biol. Chem.* **276**, 33111–33120 (2001).
17. Dellago, H. *et al.* ATM-dependent phosphorylation of SNEVhPrp19/hPso4 is involved in extending cellular life span and suppression of apoptosis. *Aging (Albany NY)* **4**, 290–304 (2012).
18. Maréchal, A. *et al.* PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR Activation via a ubiquitin-mediated circuitry. *Mol. Cell.* **53**, 235–246 (2014).
19. Song, E. J. *et al.* The Prp19 complex and the Usp4Sart3 deubiquitinating enzyme control reversible ubiquitination at the spliceosome. *Genes Dev.* **24**, 1434–1447 (2010).
20. Chanarat, S., Seizl, M. & Strässer, K. The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes. *Genes Dev.* **25**, 1147–1158 (2011).
21. Grillari, J. *et al.* SNEV is an evolutionarily conserved splicing factor whose oligomerization is necessary for spliceosome assembly. *Nucleic Acids Res.* **33**, 6868–6883 (2005).
22. Kuraoka, I. *et al.* Isolation of XAB2 complex involved in pre-mRNA splicing, transcription, and transcription-coupled repair. *J. Biol. Chem.* **283**, 940–950 (2008).
23. Sekelsky, J. J., Brodsky, M. H. & Burtis, K. C. DNA repair in *Drosophila*: Insights from the *Drosophila* genome sequence. *J. Cell Biol.* **150**, 31–36 (2000).
24. Fortschegger, K. *et al.* Early embryonic lethality of mice lacking the essential protein SNEV. *Mol. Cell Biol.* **27**, 3123–3130 (2007).
25. Schraml, E. *et al.* Haploinsufficiency of SNEV causes defects of hematopoietic stem cells functions. *Stem Cells Dev.* **17**, 355–366 (2008).
26. Monteforte, R. *et al.* SNEVPrp19/PSO4 deficiency increases PUVA-induced senescence in mouse skin. *Exp. Dermatol.* **25**, 212–217 (2016).
27. Khan, A. *et al.* SNEVhPrp19/hPso4 regulates adipogenesis of human adipose stromal cells. *Stem Cell Rep.* (2016). doi:10.1016/j.stemcr.2016.12.001
28. Voglauer, R. *et al.* SNEV overexpression extends the life span of human endothelial cells. *Exp. Cell Res.* **312**, 746–759 (2006).
29. Osterwalder, T., Yoon, K. S., White, B. H. & Keshishian, H. A conditional tissue-specific transgene expression system using inducible GAL4. *Proc. Natl. Acad. Sci.* **98**, 12596–12601 (2001).
30. Slack, C., Giannakou, M. E., Foley, A., Goss, M. & Partridge, L. dFOXO-independent effects of reduced insulin-like signaling in *Drosophila*. *Aging. Cell.* **10**, 735–748 (2011).
31. Ford, D. *et al.* Alteration of *Drosophila* life span using conditional, tissue-specific expression of transgenes triggered by doxycycline or RU486/Mifepristone. *Exp. Gerontol.* **42**, 483–497 (2007).
32. Tricoire, H. *et al.* The steroid hormone receptor EcR finely modulates *Drosophila* lifespan during adulthood in a sex-specific manner. *Mech. Ageing Dev.* **130**, 547–552 (2009).
33. Park, J.-S. *et al.* Age- and oxidative stress-induced DNA damage in *Drosophila* intestinal stem cells as marked by Gamma-H2AX. *Exp. Gerontol.* **47**, 401–405 (2012).
34. Mahajan, K. N. & Mitchell, B. S. Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase. *Proc. Natl Acad. Sci. U.S.A.* **100**, 10746–10751 (2003).
35. Lu, X. & Legerski, R. J. The Prp19/Pso4 core complex undergoes ubiquitylation and structural alterations in response to DNA damage. *Biochem. Biophys. Res. Commun.* **354**, 968–974 (2007).
36. Bus, J. S. & Gibson, J. E. Paraquat: model for oxidant-initiated toxicity. *Environ. Health Perspect.* **55**, 37–46 (1984).
37. García Sar, D. *et al.* Relationships between cisplatin-induced adducts and DNA strand-breaks, mutation and recombination in vivo in somatic cells of *Drosophila melanogaster*, under different conditions of nucleotide excision repair. *Mutat. Res.* **741**, 81–88 (2012).
38. Jeon, H.-J. *et al.* Age-related change in γH2AX of *Drosophila* muscle: its significance as a marker for muscle damage and longevity. *Biogerontology* **16**, 503–516 (2015).
39. Burger, J. M. S. & Promislow, D. E. L. Sex-specific effects of interventions that extend fly life span. *Sci. Aging Knowl. Environ.* **2004**, pe30 (2004).
40. Lehtovaara, A., Schielzeth, H., Flis, I. & Friberg, U. Heritability of life span is largely sex limited in *Drosophila*. *Am. Nat.* **182**, 653–665 (2013).
41. Zhang, N. X. *et al.* The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *J. Biol. Chem.* **280**, 40559–40567 (2005).
42. Beck, B. D., Lee, S. S., Hromas, R. & Lee, S.-H. Regulation of Metnase's TIR binding activity by its binding partner, Pso4. *Arch. Biochem. Biophys.* **498**, 89–94 (2010).
43. Paulsen, R. D. *et al.* A Genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability. *Mol. Cell* **35**, 228–239 (2009).
44. Aguilera, A. & Garcia-Muse, T. R Loops: from transcription byproducts to threats to genome stability. *Mol. Cell* **46**, 115–124 (2012).
45. Tresini, M. *et al.* The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* **523**, 53–58 (2015).
46. Di Tommaso, P. *et al.* T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. *Nucleic Acids Res.* **39**, W13–W17 (2011).
47. Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M. & Barton, G. J. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189–1191 (2009).
48. Minois, N. *et al.* Spermidine promotes stress resistance in *Drosophila melanogaster* through autophagy-dependent and -independent pathways. *Cell Death Dis.* **3**, e401 (2012).



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