Ubiquitous overexpression of the DNA repair factor dPrp19 reduces DNA damage and extends Drosophila life span

Kathrin Garschall, Hanna Dellago, Martina Gáliková, Markus Schosserer, Thomas Flatt and Johannes Grillari

Mechanisms that ensure and maintain the stability of genetic information are fundamentally important for organisational 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.

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. 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 humans and mouse models. Similarly, increased exposure to DNA damaging agents, for instance during chemotherapy, can lead to a phenotype of acquired premature progeroid syndrome. 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. 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. In Drosophila, overexpression of PARP-1 prolongs life span in both sexes, yet only when restricted to the adult nervous system. 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. Indeed, a recent study by Shaposhnikov et al. 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 beneficial, or no effects on life span. 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. Biochemically, Prp19 acts as an E3 ubiquitin ligase 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). Apart from...
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. 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 elongation, but which has not yet been characterized as a DNA repair mechanism in *Drosophila.*

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, reduce self renewal of mouse hematopoietic stem cells, increase psoralen/UV-A-induced skin aging in stromal cells. Conversely, increased levels of PRP19 extend the replicative potential and total life span of cultured human endothelial cells. However, the role of PRP19 in organismal life span is unknown. Here, we show that ubiquitous over-expression 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/hPSO4 and is regulated in an age-dependent manner in *Drosophila* melanogaster, 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 Glu49 in *Drosophila* (indicated by the black arrow).

In *Drosophila* (Fig. 1a), ubiquitous overexpression of *dPrp19* extends organismal life span of adult female flies between days 21 and 28. Values represent means across three biological replicates (±1 standard deviation), normalized to Gapdh2 and Tubulin and averaged.

![Fig. 1](image)

**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 Glu49 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.
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 γH2Av, a well-established marker for DNA damage. Upon dPrp19 overexpression in females, we observed a clear-cut decrease in the levels of γH2Av (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 γH2Av 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 and conveys resistance to genotoxic and cytotoxic stress, we next asked whether dPrp19 overexpression increases resistance against two DNA damaging agents, paraquat (methyl viologen), a herbicide known to induce reactive oxygen species, and the genotoxic compound cisplatin, which causes crosslinks between adjacent nucleosomes and which has previously been used to study DNA damage in Drosophila. 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).
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. 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 γH2Av, the fly homolog of...
Certain aging interventions have been widely observed, indicating that the genetic architecture of life span is very different in female and male Drosophila
cannot become the source of mutation and DNA breaks double strand
encountering a site of DNA damage, and the transcript folds back
when the transcription machinery is slowed down, for instance by
γ
-irradiation had the largest effect on life span of a mutant model of SCA,
and the overexpression we have
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. Similarities and identities were
determined from a BLAST 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 (Finzymes, 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 5x HOT FIREPol EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) and the following primer pairs:dPrp19: 5′-GCTACGTTCCCTCTT-3′, and 5′-TGAATCTCAGTCCTCG-3′; Gapdh2: 5′-CCCCGTTAGGTTGGTGAGAC-3′, and 5′-TGAGAGGCGAAAACTGATC-3′; Tubulin: 5′-CCTGTCTTCTAGACAGCCTTGGAAA-3′, and 5′-GACAGCCGACCACTATGGGA-3′; Rps4: 5′-CCACCGGATACAAAGTGT-3′, and 5′-AATGTGGATTTCGCCAGCATT-3′. Since Rps4 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; Betaphar, 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 Rps4.
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 2x SDS loading dye (1 M Tris-Cl 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 a 12% Bis/Tris gel buffer at 200 V. Electrophoresis and blotting to polyvinylidene fluoride membrane (Roche, Mannheim, Germany) were performed using the BioRad TGX Gel + Trans Turbo 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
Paraquat resistance assay
Paraquat resistance assays were performed as previously described with minor modifications. Briefly, newly eclosed flies (TubGS-Gal4 & dPrp19-1) were kept 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 between 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 overexpression 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.

Cisplatin resistance assays
Resistance to cisplatin was assayed on F1 cohorts of experimental flies lacking the driver (UAS-Gal4) as well as dPrp19 lines. 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 3.

References
1. Kirkwood, T. B. L. & Melov, S. On the programmed/non-programmed nature of ageing within the life history. Curr. Biol. 21, R701–R707 (2011).
2. Martin, G. M. & Oshima, J. Lessons from human progeroid syndromes. Nature 408, 263–266 (2000).
3. Schumacher, B., Hoeijmakers, J. H. & Garinis, G. A. Sealing the gap between nuclear DNA damage and longevity. Mol. Cell Endocrinol. 299, 112–117 (2009).
4. 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).
5. Garcia, A. M. et al. Age- and temperature-dependent somatic mutation accumulation in Drosophila melanogaster. PLoS Genet. 6, e1000950 (2010).
6. Whitehead, I. & Grigliatti, T. A. A correlation between DNA repair capacity and longevity in adult Drosophila melanogaster. J. Gerontol. 48, B124–B132 (1993).
7. Bürkle, A., Brabec, 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).
8. Shaposhnikov, M. V., Moskalev, A. A. & Plyusnina, E. N. Effect of PARP-1 overexpression and pharmacological inhibition of NF-kB on the lifespan of Drosophila melanogaster. Adv. Gerontol. 24, 405–419 (2011).
9. Moskalev, A. A. et al. The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance. Cell Cycle 11, 4222–4241 (2012).
10. 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).
11. Peretz, G., Bakhrt, 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).
12. 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).
13. 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).
14. Henríquez Pérez, J. A., JoséVicente, E., Correa Leandro da Silva, K. V. & Guerini Schenberg, A. C. PS04: a novel gene involved in error-prone repair in Saccharomyces cerevisiae. Mutat. Res. Repair 218, 111–124 (1989).
15. Lösch, 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. Ubiquitin 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 SNEVhPprp19/Pso4 is involved in extending cellular life span and suppression of apoptosis. *Aging (Albany NY)* 4, 290–304 (2012).
18. Maréchal, A. et al. PPRP19 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. Fortscheberger, 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. SNEVprrp19/Pso4 deficiency increases PUVA-induced senescence in mouse skin. *Exp. Dermatol.* 25, 212–217 (2016).
27. Khan, A. et al. SNEVhPprp19/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. U.S.A.* 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. Paraguay: 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 yH2AX 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. & García-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. Minoiu, N. et al. Spermidine promotes stress resistance in Drosophila melanogaster through autophagy-dependent and -independent pathways. *Cell Death Dis.* 3, e401 (2012).

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit [http://creativecommons.org/licenses/by-nc-sa/4.0/](http://creativecommons.org/licenses/by-nc-sa/4.0/)