Lifespan and Stress Resistance in *Drosophila* with Overexpressed DNA Repair Genes

Mikhail Shaposhnikov1,2, Ekaterina Proshkina1,2, Lyubov Shilova2, Alex Zhavoronkov3 & Alexey Moskalev1

DNA repair declines with age and correlates with longevity in many animal species. In this study, we investigated the effects of GAL4-induced overexpression of genes implicated in DNA repair on lifespan and resistance to stress factors in *Drosophila melanogaster*. Stress factors included hyperthermia, oxidative stress, and starvation. Overexpression was either constitutive or conditional and either ubiquitous or tissue-specific (nervous system). Overexpressed genes included those involved in recognition of DNA damage (homologs of *HUS1*, *CHK2*), nucleotide and base excision repair (homologs of *XPF*, *XPC* and AP-endonuclease-1), and repair of double-stranded DNA breaks (homologs of *BRCA2*, *XRCC3*, *KU80* and *WRNexo*). The overexpression of different DNA repair genes led to both positive and negative effects on lifespan and stress resistance. Effects were dependent on GAL4 driver, stage of induction, sex, and role of the gene in the DNA repair process. While the constitutive/neuron-specific and conditional/ubiquitous overexpression of DNA repair genes negatively impacted lifespan and stress resistance, the constitutive/ubiquitous and conditional/neuron-specific overexpression of *Hus1*, *mnk*, *mei-9*, *mus210*, and *WRNexo* had beneficial effects. This study demonstrates for the first time the effects of overexpression of these DNA repair genes on both lifespan and stress resistance in *D. melanogaster*.

Aging is a multifactorial process caused by a wide range of physiological phenomena and changes in the functioning of different biological pathways. Over the course of an organism's lifespan, age-dependent mutations accumulate and are assumed to contribute to aging and age-related diseases. While in some organisms, this may not be the case in rodents and humans, many studies have shown that the level of DNA damage increases with age. This damage includes abasic sites, DNA oxidation, DNA alkylation, DNA glycation, DNA cross-linkages, indigenous DNA adducts, and DNA strand breaks (Fig. 1).

The observed age-dependent increase in DNA damage is primarily linked to a decrease in the activity of various DNA repair processes, such as base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), single-strand break (SSB) repair, double-strand break repair (DSBR) by homologous recombination (HR), single strand annealing (SSA), and non-homologous end joining (NHEJ) mechanisms (Supplementary Table S1). Thus, the increase in DNA damage is coupled with a simultaneous reduction in DNA repair, and this is accompanied by an accumulation of somatic mutations in model organisms such as fruit flies (*Drosophila melanogaster*), mice, and humans. The accumulation of mutations leads to carcinogenesis, higher numbers of unfit cells, and aging at the cellular, tissue, and organism levels.

There is a positive relationship between organismal lifespan and efficiency of DNA damage repair. As shown in comparative studies performed on seven mammalian species, species longevity increases with the efficiency of DNA excision repair (ER). The enzymatic activity of poly (ADP-ribose) polymerase...
(PARP1), a sensor of DNA strand breaks, positively correlates with maximum lifespan in 13 species of mammals\textsuperscript{11}. The level of Ku80, a DNA double strand break (DSB) recognition protein, in humans, cows, and mice is also strongly correlated with longevity\textsuperscript{12}.

Species studied for long lifespan, such as naked mole rat Heterocephalus glaber, Brandt's bat Myotis brandtii, and bowhead whale Balaena mysticetus, are characterized by higher numbers of copies or expression of genes controlling DNA repair\textsuperscript{13}. These include positive selection of the gene Apex1 (involved in the control of ER) in Heterocephalus glaber\textsuperscript{14}, amplification of Fb xo31 (involved in the DNA damage response (DDR)) in the genome of Myotis brandtii\textsuperscript{15}, and increased expression of Rpa2 (promotes DNA repair) in Balaena mysticetus.

---

**Figure 1.** The types of DNA damage seen with the age-related increase of DNA damage level and the associated repair mechanisms in human and mammalian cells. The proteins whose Drosophila homologic genes were overexpressed in the present study are highlighted in red.
repair) along with a unique amino acids change in the MMS19 (encoded by Mms19) NER protein in *Balaena mysticetus*16. The bowhead whale also has unique mutations in the ER gene *Erc1* and the PCNA gene, both involved in DNA replication and RAD6-dependent post-replicative DNA repair17.

At present, only limited data are available on the effects of overexpression of the DNA repair genes on longevity, and no studies have addressed the impact of stress, an important longevity factor, on these effects. In mice, a positive effect on longevity is observed with overexpression of human enzyme hMTH1, which eliminates oxidized purine18 and deacetylase *Sirt6*19. Overexpression of *Sirt6* promotes DSB repair by activating PARP1 and facilitating the recruitment of Rad5120 and NBS121 to DNA lesions. In the nervous system of *D. melanogaster*, overexpression of DDR genes GADD45 and PARP1 has a lifespan extending effect22,23. Additionally, introduction of 1–2 extra copies of the gene *mei-41* (homologous to ATR gene in mammals) into the genome of *D. melanogaster* leads to an increase in lifespan compared to wild-type flies24. At the same time, overexpression of the gene O6-methylguanine-DNA-methyltransferase (*hMGMT*) in the tissues of mice does not result in increased longevity25, and widespread ectopic expression of the gene *hPARP1* in mice26 leads to a decrease in survival.

The purpose of this study was to determine whether overexpression of genes involved in the control of various DNA repair pathways would result in an increased lifespan and stress resistance in *D. melanogaster*. We studied the effects of overexpression of genes encoding for enzymes coordinating the recognition of DNA damage (homologs of *HU51, CHK2*), NER and BER (homologs of *XPF, XPC* and *AP-endonuclease-1*), and DSB repair (homologs of *B RCA2, XRCC3, KU80* and *WRNexo*) on lifespan and resistance to stress factors (hyperthermia, oxidative stress and starvation). Most of the UAS-bearing transgenic flies for overexpression of DNA repair genes, including *UAS-Ku80* (*Ku80* homologue), *UAS-mei-9* (*XPF* homologue), *UAS-mus210* (*XPC* homologue), *UAS-Rrp1* (orthologue of *APE1*), and *UAS-W RNexo* (orthologue of *WRN 3′–5′ exonuclease domain*), were produced for the first time for this study. Because a variety of types of age-related accumulation of DNA damage exist, we used DNA repair genes that control most of the known mechanisms of DNA repair (Fig. 1).

**Results**

We investigated the effects of overexpression of DNA repair genes on *D. melanogaster* lifespan and stress resistance. To activate the expression of DNA repair genes, we used the GAL4-UAS binary regulatory system27. We crossed transgenic flies (holding extra copies of the gene of interest under control of the UAS promoter) with flies with GAL4 drivers. We then assessed lifespan in the offspring.

Because somatic mutations accumulate with age in a tissue-specific manner4, we activated the overexpression of DNA repair genes both globally and tissue-specifically. Tissue-specific overexpression was activated in the nervous system. This system was selected for several reasons. First, nerve cells are chronically exposed to oxidative stress and thus vulnerable to accumulating DNA damage28. Secondly, aging of the brain leads to onset and progression of neurological diseases, which accelerate and aggravate the aging process29. And finally, many transgenes have been identified that can increase lifespan when specifically expressed in the nervous system or ubiquitously in all tissues (*da-GAL4* and *Act5C-GS*)31,32.

**Lifespan effects.** *Constitutive/ubiquitous.* The expression level of DNA repair genes under the control of a ubiquitous constitutive driver *da-GAL4* increased by 1.2–3.5 fold (Supplementary Fig. S1). This resulted in an increase of the median lifespan in males by 7–40% (with overexpression of *mnk, mei-9, and spn-B*) and in females by 10–30% (with overexpression of *mnk, mei-9, spn-B*, and *WRNexo*: Fig. 2A and Supplementary Table S2). Notably, the positive effect of the *da-GAL4*-driven overexpression of *B ra2* and *Ku80* (males only) was only observed in comparison with short-lived UAS control flies. Also, the large increase in expression of *WRNexo* (8.7 fold) in males (Supplementary Fig. S1) actually decreased median lifespan by 40% (Fig. 2A and Supplementary Table S2).

*Conditional/Ubiquitous.* Under the control of the conditional ubiquitous driver *Act5C-GS*, the expression level of *Hus1, mei-9, mus210, Rrp1, Brca2, Ku80*, and *WRNexo* increased by 1.5–15.6 fold in males and 1.2–5.7 fold in females (Supplementary Fig. S1). Conditional ubiquitous activation of DNA repair genes resulted in a reduction of the median lifespan in male and female flies by 49–72% (Fig. 2B and Supplementary Table S2).

*Constitutive/neurospecific.* Under the control of constitutive neurospecific driver *1407-GAL4*, the expression level of *Hus1, mnk, mus210, Rrp1, spn-B, Brca2*, and *Ku80* increased by 1.5–5.5 fold in males and 1.5–4 fold in females (Supplementary Fig. S1). The relative expression level of *WRNexo* and *mei-9* increased 36.5–50.5 fold in males and 11.5–30.5 fold in females, respectively (Supplementary Fig. S1). This resulted in a reduction of the median lifespan of males by 4–64%. In females, the overexpression of genes *spn-B, Brca2, Hus1, mnk, mus210* and *WRNexo* reduced lifespan by 5–56%, while overexpression of *Ku80* and *Rrp1* led to an increase of 3–9% (Fig. 2C and Supplementary Table S2). Notably, the positive
effect of the 1407-GAL4-driven overexpression of Hus1, mnk, mus210, mei-9 and spn-B (in males) was observed only in the background of short-lived UAS controls.

Conditional/neurospecific. The relative expression levels of DNA repair genes under the control of conditional neurospecific driver Elav-GS increased 1.3–8.2 fold in the nervous tissue of imago males and 1.4–7 fold in female flies (Supplementary Fig. S1). Meanwhile, the overexpression of WRNexo in females increased 16.7 fold. The Elav-GS-driven overexpression resulted in increased median lifespan in males overexpressing Hus1 (4%), mnk (3%), mei-9 (28%), mus210 (8%), WRNexo (48%) and slightly in females overexpressing genes Hus1 (1.5%) and mei-9 (1.5%) (Fig. 2D and Supplementary Table S2). The median lifespan was reduced in males with conditional gene overexpression of spn-B (14%) and Ku80 (2%), as well as in females with the overexpressed gene Brca2 (21%; Fig. 2D and Supplementary Table S2).

Thus, the most significant lifespan-extending effect was found in flies with constitutive ubiquitous overexpression of mnk, spn-B, and WRNexo (in females) and mei-9 (in both sexes), genes under control of da-GAL4 driver.

Stress resistance. Stress can have a variety of detrimental effects on an organism. To reveal the role of overexpression of DNA repair genes in organismal stress resistance, we analyzed fly survival under constant conditions of hyperthermia, oxidative stress (paraquat), and starvation. Treatment of flies with paraquat and high temperature may cause somatic mutations to accumulate, while nutrient deprivation may impair DNA repair processes. Stress resistance results are presented in Table 1.

Hyperthermia. Constitutive overexpression of DNA repair genes had an overall positive effect on resistance to hyperthermia. Resistance to hyperthermia increased in males with constitutive ubiquitous overexpression of all DNA repair genes (da-GAL4 driver) and in flies of both sexes with constitutive neurospecific overexpression of all genes except spn-B in females (1407-GAL4 driver; Supplementary Fig. S2). Conditional overexpression, on the other hand, had mixed results. The conditional ubiquitous overexpression (Act5C-GS driver) of WRNexo in both sexes and Hus1 in females led to an increase in resistance to hyperthermia (Supplementary Fig. S2), but the conditional ubiquitous overexpression of Hus1, mei-9, mus210, Brca2 in males and Rrp1 and Ku80 in females led to a decrease (Supplementary Fig. S2). Similarly, the conditional neurospecific overexpression of mnk and WRNexo in males and mei-9 and Hus1 in females (Elav-GS driver) led to an increase in hyperthermia resistance (Supplementary Fig. S2), but the conditional neurospecific overexpression of mus210, Brca2, and spn-B in both sexes and Hus1 in males led to a decrease (Supplementary Fig. S2).
**Table 1. Effect of overexpression of DNA repair genes on median lifespan and stress resistance.** ML – median lifespan; HT – hyperthermia; OS – oxidative stress; ST – Starvation; M – males; F – females; ↑ – increase; ↓ – decrease; — – no statistically significant effects; empty cell—data are not analyzed due to the lack of statistically significant differences in the level of gene expression.

Thus, constitutive overexpression of DNA repair genes under control of da-GAL4 and 1407-GAL4 drivers, respectively, whether throughout the body or confined to the nervous system, demonstrated a predominantly positive effect on thermotolerance. Conversely, conditional ubiquitous or neurospecific overexpression under control of Act5C-GS and Elav-GS drivers, respectively, either increased or decreased resistance to higher temperature depending on the gene studied, with decreased resistance predominating.

**Oxidative stress.** Resistance to oxidative stress decreased after activation of overexpression of all DNA repair genes in all experimental conditions (Supplementary Fig. S2), except males with constitutive ubiquitous overexpression (da-GAL4 driver) of mei-9, Rrp1, and Ku80 and females with conditional ubiquitous overexpression (Act5C-GS driver) of Hus1 or conditional neurospecific expression (Elav-GS driver) of mei-9 and Brca2 (Supplementary Fig. S2).

Thus, constitutive ubiquitous overexpression under control of da-GAL4 in males had the most positive effect on resistance to oxidative stress, but the overall effect of the DNA repair genes’ overexpression, with a few exceptions, was decreased oxidative stress resistance.

**Starvation.** Ubiquitous overexpression of DNA repair genes had both positive and negative effects on starvation resistance whether constitutive or conditional, depending on the genes overexpressed. For example, the constitutive ubiquitous overexpression of Rrp1 in males (da-GAL4 driver) increased resistance to starvation (Supplementary Fig. S2); however, resistance to starvation decreased after activation of constitutive ubiquitous overexpression of Brca2 and WRNexo in males and Rrp1 and WRNexo in females (Supplementary Fig. S2). Likewise, the conditional ubiquitous expression of Brca2 and WRNexo in males and Ku80 in females (Act5C-GS driver) increased resistance to starvation (Supplementary Fig. S2), but the conditional ubiquitous expression of mei-9 and mus210 in males and Hus1 and Brca2 in females decreased resistance to starvation (Supplementary Fig. S2). Neurospecific overexpression of DNA repair genes, whether constitutive or conditional, had a negative effect on starvation resistance. Constitutive neurospecific overexpression (1407-GAL4 driver) decreased resistance to starvation after activation of all DNA repair genes in males and in females (Supplementary Fig. S2), and conditional neurospecific expression (Elav-GS driver) of spn-B and Brca2 in males and mei-9 in females also decreased resistance to starvation (Supplementary Fig. S2).
Thus, resistance to starvation was most positively affected by overexpression of DNA repair genes with conditional ubiquitous expression, under the control of Act5C-GS driver; however, both positive and negative effects of ubiquitous expression, whether constitutive or conditional, occurred and were dependent on gene and sex. Any effects of neurospecific expression on starvation resistance were negative.

Generally, these data demonstrate that the positive effects of the overexpression of DNA repair genes on resistance to different stressors are more evident in males. The constitutive ubiquitous overexpression of mei-9, Rrp1, Brca2, Ku80, and WRNexo genes, under control of da-GAL4 driver, are the most beneficial.

Discussion

Here, we have shown that increasing the expression level of DNA repair genes in Drosophila melanogaster has both positive and negative effects on lifespan and stress resistance depending on the type of GAL4 driver used, the genes overexpressed, and, in some cases, sex of the organism (Table 1).

Increased lifespan with constitutive ubiquitous and conditional neuronal overexpression. The constitutive ubiquitous overexpression of most DNA repair genes tested resulted in increased lifespan. This effect was seen in all experimental conditions, except when Ku80 and WRNexo were overexpressed in males and Brca2 in females. Conditional neuronal activation of expression only at the adult stage also increased lifespan, with the exception of Brca2 when overexpressed in females, and spn-B and Ku80 when overexpressed in males.

Reduced lifespan with conditional ubiquitous or constitutive neuronal overexpression. Conversely, when overexpression of DNA repair genes occurred throughout the body but was limited to adulthood, lifespan was reduced. At first sight, this may suggest that RU486 (mifepristone) negatively impacts longevity. However, according to a recent study by Landis et al., RU486 does not affect the lifespan of males and virgin females and actually may increase the lifespan of mating females up to 68%35. In addition, RU486 increases lifespan observed using the Elav-GS driver. Thus, while RU486 remains a possible factor, the available evidence does not support this. We also observed shorter lifespan in flies with constitutive overexpression of DNA repair genes in the nervous system in all experimental conditions, with the exception of Rrp1 and Ku80 in females. One possible explanation for this is that ectopic overexpression of DNA repair genes under control of Act5C-GS and 1407-GAL4 drivers may disturb cell energy metabolism and intracellular signaling pathways, decreasing organismal viability.

Thus, overexpression of DNA repair genes throughout development leads to opposite effects on lifespan when compared to adult-specific overexpression, and the direction of this dichotomy depends on whether the overexpression was ubiquitous or limited to the nervous system. It is difficult to explain these effects on the basis of the available experimental or published data, but it is possible that transcriptome analysis carried out at different stages of development could be informative.

In addition, there were opposing effects on the two sexes, depending on driver. Increased lifespan driven by da-GAL4 was more pronounced in females, but the same driven by Elav-GS was observed specifically in males, with the exception of Hus1. Sex-specific effects of transgenes that can increase lifespan when overexpressed are well known39.

Identification of candidate genes for future studies of life extension via DNA repair. The ambiguous effect of constitutive versus adult-specific overexpression may be also related to the different functions of genes and different levels of their activity. In accordance with our data, the most positive effects on lifespan were observed in flies with constitutive ubiquitous overexpression of mnk (in both sexes), mei-9, spn-B, and WRNexo (in females) and in flies with conditional neurospecific overexpression of Hus1 and mei-9 (in both sexes), mnk, mus210, and WRNexo (in males). These genes are involved in various DNA damage recognition and repair mechanisms.

Drosophila mei-9 is essential for several DNA repair and recombination pathways, including NER, interstrand crosslink repair, and meiotic recombination36. In the mammalian 9-1-1 complex, Hus1 forms a DNA damage sensor clamp37. In Drosophila, the Hus1 homologue plays a critical role in the regulation of the S-phase meiotic DNA damage checkpoint and DSB repair during meiotic recombination37,38. Mnk (also known as Chk2) is involved in regulating the activity of the DNA damage sensors Ku70 and Ku8039; the overexpression of Ku80 is characterized by a positive effect on the lifespan. Mus210 (also known as XPC) may act as a general sensor of damaged DNA40. These data are supported by reports of a positive correlation between the activity of DNA damage-sensing enzymes such as Ku8012 and PARP1 and longevity of different species of animals11 and reports that increased gene expression of DNA damage sensors mei-41 (throughout the body) and PARP1 (in the nervous system) also leads to increased longevity in Drosophila melanogaster23,24. Thus, the ability of enzymatic systems to recognize DNA damage may influence longevity.

Moderate expression of WRNexo in female flies increased longevity, while high expression in males substantially reduced lifespan. The reasons for this remain unclear, but several possibilities exist. First, WRNexo is known to be an orthologue of human WRN 3′–5′ exonuclease domain41 and it is known that excess nuclease activity, e.g. XPF (ortholog of mei-9)42, leads to DNA damage and genomic instability.
Secondly, because the DNA repair process is ATP-dependent, high levels of WRN may lead to the depletion of energy and cell death. *Drosophila* WRNexo shows conservation of structural motifs and catalytic residues with human protein, but lacks a helicase domain. WRNexo is required for response to replicative stress, restraining of mitotic DNA recombination, and maintenance of genome stability. In *Drosophila* cells lacking WRNexo, collapsed replication forks persist and promote Holliday junction formation and HR. Additionally, WRNexo degrades SSD, duplex DNA substrates, and bubble structures, but has no effect on blunt ended DNA duplexes. Taken together, these findings point to its possible involvement in DNA excision repair and DSB repair.

Overexpression of *spn-B* and Brca2, both globally and in the nervous system, had a predominantly negative effect on lifespan. This may be related to the fact that *spn-B* and Brca2 both control the processes of HR. These processes are of paramount importance during mitosis and meiosis but do not play a significant role in the post-mitotic cells of the adult organism. The gene *spn-B* is required for the progression of the meiotic cell cycle. Rad51-related proteins spn-B and spn-D physically interact and promote HR during meiotic prophase with accompanied suppression of the NHEJ repair pathway. Mutations in DNA repair genes such as *spn-B* lead to persistence of DSBs in the germline, which activates an ATR-Chk2-dependent checkpoint.

It is also important that DNA repair is carried out by large multienzyme complexes, as imbalance of one component may lead to its inefficiency. For example, supplementation of Rad51, Rad51C, Rad52, and NBS1 proteins in human fibroblasts, either individually or in combination, did not rescue the senescence-related decline of homologous recombination without overexpression of deacetilase SIRT6.

In this regard, it would be useful to assess the effects on lifespan of overexpression of several proteins from a single DNA repair pathway.

**DNA repair and the prevention of neurodegeneration in aging.** Evidence suggests that the nervous system plays a critical role in longevity and the aging process. The results of the current study lend support for this view by demonstrating that lifespan in *Drosophila* can be increased by overexpression of DNA repair genes in the adult nervous system alone (under the control of the neurospecific driver Elav-GS). We have also previously demonstrated that Elav-GS-specific overexpression of DNA repair genes such as *PARP1* and *D-GADD45* in the *Drosophila* nervous system is sufficient to increase the lifespan of the whole organism. While the mechanisms underlying these lifespan effects are not immediately apparent, one possibility is that neurospecific overexpression of DNA repair genes may prevent the development of age-dependent neurodegeneration. In line with this is the reverse scenario, in which DNA damage causes neurodegeneration. Indeed, the loss of heterochromatin and subsequent accumulation of DNA damage in the *Drosophila* brain have been shown to promote neurodegeneration. Moreover, other experiments involving overexpression of *Kn70* and *D-GADD45* confirm that DNA repair genes are important for maintaining the normal functions of neurons and the prevention of age-related neurodegeneration.

Alternatively, it is also possible that the longer lifespan observed using the Elav-GS driver could include effects of mifepristone, or, since the process of DNA repair is also closely linked with aging-related mechanisms such as cell cycle regulation, apoptosis, autophagy, and IGF-1 signaling, lifespan may have been extended via alterations of aging-related cell signaling pathways. Finally, it is important to consider that nervous system-specific overexpression of DNA repair genes actually decreased life span when the overexpression was constitutive instead of conditional to adulthood. Thus, the lifespan effects are influenced by driver or stage of development.

**Stress Resistance.** Lifespan and stress resistance are interrelated and DNA repair can affect both. The three stressors selected for this study (hyperthermia, paraquat, and starvation) each have specific detrimental effects. Hyperthermia causes nuclear protein aggregation and stalling of DNA replication forks and leads to the induction of DNA damage, including DSB. Paraquat induces reactive oxygen species-mediated DNA damage. Starvation may impair DNA repair processes, as many steps in DNA repair are ATP dependent.

While overexpression of DNA repair genes in the absence of stressors had a more pronounced effect in females, the beneficial effects of overexpression of these genes on resistance to stress was more pronounced in males. The effects of overexpression of DNA repair genes on different types of stress resistance were varied. Constitutive ubiquitous overexpression of the majority of the studied DNA repair genes led not only to increased lifespan in males, but also improved resistance to hyperthermia and oxidative stress (Table 1), whereas conditional ubiquitous overexpression under the control of the Act5C-GS driver in imagos resulted in reduced lifespan but increases in resistance to hyperthermia, oxidative stress and starvation in male and female flies (Table 1). Constitutive neurospecific overexpression of DNA repair genes, under the control of 1407-GAL4 driver, increased the resistance to hyperthermia, but reduced lifespan and resistance to oxidative stress and starvation. The correlation between stress resistance and lifespan were most closely correlated in the cases of conditional neurospecific overexpression of the
spn-B (reduction), mnk, Rrp1 and WRNexo (increase) in males, and Brca2 (reduction), Hus1 and mei-9 (increase) in females, under the control of driver Elav-GS.

Different stress factors may induce DNA damage via the generation of free radicals. The observed increases in stress resistance may reflect elevated efficiency of DNA repair. However, the involvement of alternative mechanisms affecting such stress-resistance mechanisms as cell cycle regulation, apoptosis, autophagy, and IGF-1 signaling are also possible. Thus, our results are consistent with stress resistance being necessary, but not sufficient, for longevity.

Conclusions

Aging is a complex process that is far from being fully understood. Of the many factors that contribute to aging and the multiple changes on many levels that take place, one in need of further study at this time is the role of DNA repair. Because DNA damage does accumulate with age and appears to be associated with some of the detrimental aspects of aging, including neurodegeneration, boosting DNA repair mechanisms may be one approach to intervention. Here, we investigated the potential life-extending effects of increasing the expression of genes known to be involved in DNA repair in Drosophila. We compared the overexpression of these genes throughout the body versus in the nervous system alone and throughout the lifespan versus in adulthood alone. We also included three known stressors. We found both positive and negative effects on lifespan, with many important variables, including gene, sex, stress exposure, extent of overexpression, and type of GAL4 driver used, which determined developmental stage and distribution of overexpression in the body. The most pronounced effects of overexpression on lifespan occurred with Hus1, mnk, mei-9, mus210, spn-B, and WRNexo, which control the processes of DNA damage recognition and repair. Lifespan and stress resistance were interrelated, moreso in males than females, in that increased lifespan was associated with increased resistance to hyperthermia and oxidative stress, while decreased lifespan was associated with decreased resistance to all three stressors tested. Aging research is still in need of basic studies to address a wide variety of unanswered questions. This study presents a valuable set of preliminary data on the role of DNA repair in aging and points to a promising set of DNA repair genes and experimental conditions to pursue in greater detail in future studies that incorporate both transcription-level and protein-level effects on a wider variety of lifespan-and aging-related parameters.

Materials and Methods

Drosophila strains. In order to match the genetic background of UAS and GAL4 strains utilized in this study, flies all were backcrossed into w1118 (#3605, Bloomington Drosophila Stock Center) background for 6–8 times.

UAS strains. Hus1 (genotype: w1118, UAS-Hus1)—Carries an additional copy of gene Hus1 under the UAS promoter's control on chromosome 2. Hus1 is a protein from the PCNA-like complex 9-1-1 that is required for the activation of an S phase checkpoint and DSB repair during meiotic recombination. Kindly provided by Dr. Schupbach (Princeton University, Princeton, USA).

mnk (genotype: w1118, UAS-mnk)—Carries an additional copy of an ortholog of the mammalian DNA damage sensor gene chk2 under the control of promoter UAS on chromosome 2. Kindly provided by Dr. Schupbach (Princeton University, Princeton, USA).

mei-9 (genotype: w1118, UAS-mei-9)—Carries an additional copy of an ortholog of the mammalian excision DNA repair gene XPF under the control of promoter UAS on chromosome 2. Ordered from GenetiVision (GenetiVision Houston, USA), with authorship transfer.

mus210 (genotype: w1118, UAS-mus210)—Carries an additional copy of an ortholog of the mammalian excision DNA repair gene XPC under the control of promoter UAS on chromosome 2. Ordered from GenetiVision (GenetiVision Houston, USA), with authorship transfer.

Rrp1 (genotype: w1118, UAS-Rrp1)—Carries an additional copy of ortholog of the mammalian excision DNA repair gene APE1 under the control of promoter UAS on chromosome 2. Ordered from GenetiVision (GenetiVision Houston, USA), with authorship transfer.

Brca2 (genotype: w1118, UAS-Brca2)—Carries an additional copy of Drosophila ortholog of mammalian Brca2 gene under the UAS promoter's control on chromosome 2. Brca2 is involved in DSB repair. Kindly provided by Dr. Schupbach (Princeton University, Princeton, USA).

spn-B (genotype: w1118, UAS-spn-B)—Carries an additional copy of an ortholog of the mammalian DSB repair gene XRCC3 under the control of promoter UAS on chromosome 2. Kindly provided by Dr. Schupbach (Princeton University, Princeton, USA).

Ku80 (genotype: w1118, UAS-Ku80)—Carries an additional copy of gene Ku80 under the control of UAS promoter on chromosome 3. Ku80 is involved in the DSB repair by NHEJ. Ordered from GenetiVision (GenetiVision Houston, USA), with authorship transfer.

WRNexo (genotype: w1118, UAS-WRNexo)—Carries an additional copy of gene WRNexo under the control of promoter UAS on chromosome 3. WRNexo is the orthologue of human WRN 3′-5′ exonuclease domain involved in DSB repair. Drosophila WRNexo shows conservation of structural motifs and catalytic residues with human protein, but lacks a helicase domain. Ordered from GenetiVision (GenetiVision Houston, USA), with authorship transfer.
Driver GAL4 strains. *da-GAL4* (genotype: w1118; P* [da-GAL4.w-]*)—Expresses GAL4 ubiquitously and strongly under the control of *daughterless* 25. This driver expresses throughout development and in most adult tissues 31. Kindly provided by Dr. Seroude, (Queen’s University, Kingston, Canada).

*Act5c-GS* (genotype: P* [Act5c(-FRT)]GAL4.Switch.PR3/TM6B, Tb1*)—Expresses mifepristone-inducible GAL4 in all cells. Provided by Drosophila Stock Center (#9431, Bloomington, USA).

*1407-GAL4* (genotype: w*; P* [GawB]insc Mz1407*)—Driver line containing GAL4 selectively expressed in nervous system cells throughout the life cycle: during embryonic 68 and larval 69 stages and imagos 70. Provided by Drosophila Stock Center (#8751, Bloomington, USA).

*Elav-GS* (genotype: P* [ELAV- GeneSwitch]*)—Expresses mifepristone-inducible GAL4 in nervous system cells 31. Kindly provided by Dr. Keshishian (Yale University, New Haven, USA).

Activation of overexpression. The GAL4-UAS system were used to activate the expression of DNA repair genes 27. We assessed the lifespan in the offspring obtained by mating the transgenic flies with extra copies of the studied gene under UAS promoter and flies with GAL4 drivers. We used constitutively active (1407-GAL4 and da-GAL4) and conditional (Elav-GS and Act5c-GS) drivers of GAL4 that activate the gene overexpression in neurons and throughout the body, respectively.

To activate the overexpression under the control of conditional drivers, adult flies were fed on yeast paste containing mifepristone (RU486, Sigma, USA) at a concentration of 200 μM 32. Mifepristone was administered in the diet of flies throughout their lifespan. Control animals were fed with yeast paste without mifepristone. To prepare 100 ml of the paste, 50 g of dried yeast and 60 ml of water were used. To exclude the probability of absorption of the active substance by live yeast, the paste was pre-boiled in a water bath for 30 minutes. Five days after placing the flies on the yeast paste containing mifepristone, their stress-resistance and the relative expression levels of genes of interest were evaluated.

Quantitative Real Time PCR (qRT-PCR). To confirm overexpression of studied genes in the whole body or nervous system ten imagos or 50 heads were used in every variant of the experiment. Gene expression levels were analyzed in flies at the age of 2–5 days after imago hatching, separately for males and females. Experiments were performed in 3–4 replicates. Whole flies or heads were homogenized with the Silent Crusher-S homogenizer (Heidolph, Germany) in TRIzol Reagent (Invitrogen, USA). RNA was separated using BCP (Invitrogen, USA), in accordance with the manufacturer’s protocol. To test that RNA samples were DNA-free, control PCR experiments without the reverse transcription step were performed with primers for the β-Tubulin.

All target genes and -Tubulin were amplified in separate PCR tubes. Quantitative real-time PCR (qRT-PCR) assays were performed using SYBRGreen PCR Master Mix (Applied Biosystems, USA). The list of primers is presented in Table S3. All reactions were performed using a CFX96 real-time PCR detection system (Bio-Rad Laboratories, USA). The thermal cycle conditions were: initial denaturation step at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s (denaturation), 60°C for 30 s (annealing) and 60°C for 30 s (elongation). Expression levels were normalized against the housekeeping gene β-Tubulin. All target genes and β-Tubulin were amplified in separate PCR tubes. Four measurements were performed for each version of the experiment.

Lifespan assay. We used flies with statistically significant overexpression for the lifespan assay. Control and experimental flies were collected during 24h after imago hatching, and divided into males and non-virgin females and maintained in a constant climate chamber Binder KBF720-ICh, 720 l-(Binder, Germany) on a yeast medium at 25°C and 60% humidity in a 12:12 h light-dark cycle. Thirty flies of the same sex and age were maintained in a *Drosophila* vial. Five vials were used in each experiment (a total of 150 males and 150 females). Experiments were performed in several replicates. Flies were transferred to a fresh medium twice a week. Lifespan was analyzed daily, separately for males and females. The median lifespan and the age of 90% mortality were calculated. The data are presented in the form of histograms reflecting the percentage of changes in median lifespan between experimental and control variants.

Estimation of stress resistance. We used flies with statistically significant overexpression for the stress-resistance estimation. Evaluation of stress-resistance (to hyperthermia, oxidative stress and starvation) was performed in the flies at the age of 5 days. To induce hyperthermia, the flies were kept at 35°C. To trigger oxidative stress, the flies were kept on filter paper moistened with 5% sucrose solution with the addition of paraquat at 20 mM concentration. Starved flies were kept on filter paper moistened with distilled water. Flies with overexpression of DNA repair genes and without overexpression lived under stress conditions until the whole experimental group died. The survival was evaluated every 24 hours. The results obtained are presented in the form of histograms reflecting the percentage of dead flies after 24–96 hours.

Statistics. To compare the statistical differences in median lifespan between control and experimental groups, the Mantel-Cox test was used 72. A Wang-Allison test was used to estimate the differences in the age of 90% mortality 73. To assess the statistical significance of differences in resistance to stress factors,
ΔΔCt was calculated according to equation \( \Delta \Delta Ct = \Delta Ct (\text{experiment}) - \Delta Ct (\text{control}) \), where \( \Delta Ct = Ct (\text{target gene}) - Ct (\beta\text{-Tubulin}) \). Statistical analyses of the data were performed using STATISTICA software, the Fisher's exact test was used. Relative levels of expression were calculated using 2^\(-\Delta\Delta Ct\) method. Statistical significance of expression differences was estimated using Mann-Whitney U-test. Statistical analyses of the data were performed using STATISTICA software, version 6.1 (StatSoft, USA), R, version 2.15.1, and OASIS: Online Application for the Survival Analysis of Lifespan Assays.
37. Abdu, U., Klovstad, M., Butin-Israeli, V., Bakhrat, A. & Schupbach, T. An essential role for Drosophila hsl1 in somatic and meiotic DNA damage responses. *J. Cell. Sci.* **120**, 1042–1049, doi:10.1242/jcs.034414 (2007).

38. Peretz, G., Arie, L. G., Bakhrat, A. & Abdu, U. The Drosophila hsl1 gene is required for homologous recombination repair during meiosis. *Mech. Dev.* **126**, 677–686, doi:10.1016/j.mod.2009.05.004 (2009).

39. Brodsky, M. H. *et al.* Drosophila melanogaster MKI67 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. *Mol. Cell. Biol.* **24**, 1219–1231 (2004).

40. Shell, S. M. *et al.* Xeroderma pigmentosum complementation group C protein (XPC) serves as a general sensor of damaged DNA. *DNA Repair* **11**, 947–953, doi:10.1016/j.dnarep.2013.08.013 (2013).

41. Boubriak, I. *et al.* DmWRNexo is a 3′-5′ exonuclease: phenotypic and biochemical characterization of mutants of the Drosophila orthologue of human WRN exonuclease. *Biogerontology* **10**, 267–277, doi:10.1007/s10522-008-9181-3 (2009).

42. Sollier, J. *et al.* Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability. *Mol. Cell* **56**, 777–785, doi:10.1016/j.molcel.2014.10.020 (2014).

43. Saunders, R. D., Boubriak, I., Clancy, D. J. & Cox, L. S. Identification and characterization of a Drosophila orthologue of WRN exonuclease that is required to maintain genome integrity. *Aging Cell* **7**, 418–425, doi:10.1111/j.1474-9726.2008.00388.x (2008).

44. Bolterstein, E., Rivero, R., Marquez, M. & McVey, M. The Drosophila Werner exonuclease participates in an exonuclease-independent response to replication stress. *Genetics* **197**, 643–652, doi:10.1534/genetics.114.164228 (2014).

45. Mason, P. A. *et al.* The Drosophila orthologue of progeroid human WRN exonuclease, DmWRNexo, cleaves replication substrates but is inhibited by uracil or abasic sites: analysis of DmWRNexo activity in vitro. *Age* **35**, 793–806, doi:10.1016/j.s scientist.2013.012-9411-0 (2013).

46. Klovstad, M., Abdu, U. & Schupbach, T. Drosophila brca2 is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. *PloS Genet.* **4**, e31, doi:10.1371/journal.pgen.0040031 (2008).

47. Gaboriau, A., Ray, R. P. & Schupbach, T. okra and spindle-B encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in Drosophila oogenesis. *Genes & development* **12**, 2711–2723 (1998).

48. Joyce, E. E., Paul, A., Chen, K. E., Tanneti, N. & McKim, K. S. Multiple barriers to nonhomologous DNA end joining during meiosis in *Drosophila*. *Genetics* **191**, 739–746, doi:10.1534/genetics.112.140906 (2012).

49. Li, W., Klovstad, M. & Schupbach, T. Repression of Gurken translation by a meiotic checkpoint in *Drosophila* oogenesis is suppressed by a reduction in the dose of ef1A. *Development* **141**, 3910–3921, doi:10.1242/dev.109306 (2014).

50. Drouh, R. *et al.* Functional analysis of Drosophila Garken mutants. *Drosophila DNA repair* **7**, 10–19, doi:10.1016/j.danrep.2007.07.013 (2008).

51. Batastelesi, V. *et al.* Gadd45 expression correlates with age dependent neurodegeneration in *Drosophila melanogaster*. *Biogerontology*, doi:10.1007/s10522-014-9533-0 (2014).

52. Frost, B., Hemberg, M., Lewis, I. & Feany, M. B. Tau promotes neurodegeneration through global chromatin relaxation. *Nat. Neurosci.* **17**, 357–366, doi:10.1038/nneurosci.2013.3639 (2014).

53. Tamura, T. *et al.* Ku70 alleviates neurodegeneration in *Drosophila* models of Huntington’s disease. *PLoS ONE* **6**, e27408, doi:10.1371/journal.pone.0027408 (2011).

54. Czarny, P., Pawłowka, E., Bialkowska-Warzecha, J., Kaarniranta, K. & Blasiak, J. Autophagy in DNA damage response. *International Journal of molecular sciences* **16**, 2641–2662, doi:10.3390/ijms16022641 (2015).

55. Hinkal, G. & Donehower, L. A. How does suppression of IGF-1 signaling by DNA damage affect aging and longevity? *Mech. Aging Dev.* **129**, 243–253, doi:10.1016/j.mad.2008.02.005 (2008).

56. Hyun, M. *et al.* Longevity and resistance to stress correlate with DNA repair capacity in *Caenorhabditis elegans*. *Nucleic Acids Res.* **36**, 1380–1389, doi:10.1093/nar/gkm1161 (2008).

57. Roti, R. *et al.* 3′-Termini cellular responses to hyperthermia (40–46 degrees C): cell killing and molecular events. *Int J Hyperthermia* **24**, 3–15, doi:10.1080/02656730701679841 (2008).

58. Mehdi, S. H. & Qamar, A. Paraquavit ultrastructural changes and DNA damage in the nervous system is mediated via oxidative-stress-induced cytotoxicity in *Drosophila melanogaster*. *Toxicol. Sci.* **134**, 355–365, doi:10.1093/toxsci/kfl116 (2014).

59. Velchko, A. K., Petrova, N. V., Kantidze, O. L. & Razin, S. V. Dual effect of hexavalent chromium induced DNA double strand breaks and oxidative stress-induced cytotoxicity in *Drosophila*. *Toxicol. Sci.* **355**, 365–376, doi:10.1083/jour2013.04.005 (2013).

60. Radford, S. J., Goley, E., Baxter, K., McManus, S. & Sekelsky, J. *Drosophila* ERC1 is required for a subset of MEI-9-dependent meiotic crossovers. *Genetics* **170**, 1737–1745 (2005).

61. Henning, K. A., Peterson, C., Legerius, R. & Friedrich, E. C. Cloning the Drosophila homolog of the xeroderma pigmentosum complementation group C gene reveals homology between the predicted human and Drosophila polypeptides and that encoded by the yeast RAD4 gene. *Nucleic Acids Res.* **22**, 257–261 (1994).

62. Sander, M. & Huang, S. M. Characterization of the nuclease activity of Drosophila Rrp1 on phosphoglycolate- and phosphate-modified DNA 3′-termini. *Biochemistry* **34**, 1267–1274 (1995).

63. Ravi, D. *et al.* A network of conserved damage survival pathways revealed by a genomic RNAi screen. *PLoS Genet.* **5**, e1000527, doi:10.1371/journal.pgen.1000527 (2009).

64. Wang, C., Li, Q., Redden, D. T., Weinrich, R. & Allison, D. B. Statistical methods for testing effects on “maximum lifespan” in *Drosophila melanogaster*. *Mol. Cell. Biol.* **163**, 126–136, doi:10.1016/j.molcl.2004.07.003 (2004).
74. Yang, J.-S. et al. OASIS: Online Application for the Survival Analysis of Lifespan Assays Performed in Aging Research. PLoS ONE 6, e23525, doi: 10.1371/journal.pone.0023525 (2011).
75. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408, doi: 10.1006/meth.2001.1262 (2001).
76. Mann, H. B. & Whitney, D. R. On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. The Annals of Mathematical Statistics 18, 50–60 (1947).

Acknowledgments
This work was supported by the Russian Science Foundation grant N 14-50-00060. The authors would like to thank Dr. Leslie C. Jellen from the University of Tennessee for her help with editing the manuscript and valuable comments. We thank the Institute of Biology of Komi Science Center of Ural Branch of RAS for the unique collection of Drosophila transgenic lines and in Insilico Medicine, Inc. for their assistance in data analysis.

Author Contributions
A.M. coordinated the study. M.S., E.P., L.S. and A.M. designed the study and analyzed the data. M.S., A.Z. and A.M. wrote the manuscript. M.S., E.P. and L.S. collected data and performed experiments.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Shaposhnikov, M. et al. Lifespan and Stress Resistance in Drosophila with Overexpressed DNA Repair Genes. Sci. Rep. 5, 15299; doi: 10.1038/srep15299 (2015).

This work is licensed under a Creative Commons Attribution 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/4.0/