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Analysis of representative mutants for key DNA repair pathways on healthspan in *Caenorhabditis elegans*

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Abstract

Although the link between DNA damage and aging is well accepted, the role of different DNA repair proteins on functional/physiological aging is not well-defined. Here, using *Caenorhabditis elegans*, we systematically examined the effect of three DNA repair genes involved in key genomic stability pathways. We assayed multiple health proxies including molecular, functional and resilience measures to define healthspan. Mutation in XPF-1/ERCC-1, a protein involved in nucleotide excision repair (NER), homologous recombination (HR) and interstrand crosslink (ICL) repair, showed the highest impairment of functional and stress resilience measures along with a shortened lifespan. *brc-1* mutants, with a well-defined role in HR and ICL are short-lived and highly sensitive to acute stressors, specifically oxidative stress. In contrast, ICL mutants *fcd-2* did not impact lifespan or most healthspan measures. Our efforts also uncover that DNA repair mutants show high sensitivity to oxidative stress with age, suggesting that this measure could act as a primary proxy for healthspan. Together, these data suggest that impairment of multiple DNA repair genes can drive functional/physiological aging. Further studies to examine specific DNA repair genes in a tissue specific manner will help dissect the importance and mechanistic role of these repair systems in biological aging.

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Author Contributions: AUG provided study design. All authors acquired, analysed, or interpreted data. SH performed correlational analysis studies. AUG drafted the manuscript with the help of all co-authors. AUG supervised the study.

Keywords

DNA repair; aging; healthspan; biological aging; stress responses

Introduction

The elderly population around the world is growing at an unprecedented rate, with the World Health Organization (WHO) estimating that by 2050 population aged >60 will total 2 billion [1]. The elderly are highly susceptible to numerous chronic degenerative diseases, including arthritis, obesity, diabetes, as well as, neurodegenerative and cardiovascular disease. The increased demand on primary and long term care means that an aging global population is likely to be one of the most significant challenges for healthcare in the 21st century.

Macromolecular damage is a key driver of aging [2, 3], and evidence suggests that DNA damage and genomic instability contributes to age-related decline [4]. First, a large body of evidence has shown that DNA damage and mutations accumulate naturally with age [5–7]. Additionally, there is evidence that suggests that DNA repair capacity reduces with age [4, 8–11]. Moreover, inherited defects in DNA repair, leading to accumulation of DNA damage, result in several types of progeria (including Werner, XFE progeria, and Cockayne syndromes), which are characterized by *segmental* or *systemic* accelerated aging [12–14]. Finally, cancer patients treated with genotoxic chemotherapeutic agents experience a higher prevalence and earlier onset of several age-related pathologies compared to their siblings [15–17]. This evidence clearly supports the idea that maintaining DNA repair is beneficial to health.

In order to reduce the deleterious effect of DNA damage on health, several sophisticated DNA repair pathways exist. Nucleotide excision repair (NER) senses structural distortions in the double helix, and repairs several types of bulky lesions anywhere in the genome (global GG-NER) or in transcribed regions (transcription coupled TC-NER) [18]. Base excision repair (BER) removes small, non-helix-distorting lesions, whereas Mismatch repair (MMR) fixes insertions, deletions and mis-incorporated bases [19, 20]. Interstrand DNA crosslinks (DNA strands with the covalent bond that prevents separation) can be fixed by factors responsible for Fanconi anemia (FA) along with several other pathways [21]. Double strand breaks (DSBs) are thought to be the most deleterious type of damage due to their ability to cause large chromosomal rearrangements. DSBs are repaired by non-homologous end joining (NHEJ) and by homologous recombination (HR) [22]. While the link between DNA repair and aging is clear, the relative importance of distinct DNA repair genes in maintaining health throughout life has not been well investigated. The aim of this work is to define which representative DNA repair genes in distinct genomic stability pathways impact healthspan, and therefore contribute towards healthy aging.

The nematode *Caenorhabditis elegans* (*C. elegans*) is a model organism which has been used to characterize several well conserved mechanisms regulating longevity. *C. elegans* offered a number of advantages to our study: it is genetically tractable, has a short life cycle (~25 days) and shares basic mechanisms of DNA repair with mammals. XPF-ERCC1 is a DNA repair protein involved in multiple repair pathways, including NER, ICL and HR.

It has been established that mutations causing decreased XPF-ERCC1 expression result in accelerated accumulation of oxidative lesions [23], and accelerated aging in *C. elegans*, mice and humans [14, 24]. However, since loss of XPF-ERCC1 affects several DNA repair pathways it makes it difficult to pinpoint which DNA damage lesions lead to the observed accelerated aging phenotype.

Aging of an individual can be represented by two key measures: lifespan and healthspan. Lifespan is the length of time an individual lives, while healthspan is the length of time the individual lives without major age-related illness. Traditionally, lifespan was assumed to be the ultimate parameter of aging. It was also thought that lifespan and healthspan are highly correlated. For example, several long-lived mutants in *C. elegans* also display a concomitant increase in stress resistance, as well as, better functional parameters such as movement [25, 26]. However, these observations were mostly based off experiments in young animals, thus ignoring the trajectory of healthspan in such long-lived mutants. In contrast, several recent studies indicate that healthspan can be uncoupled from lifespan [24, 27]. A number of long-lived mutants have now been shown to spend a larger fraction of their lifespan in a frail state compared to wild-type animals [28, 29]. Interestingly, we have previously observed that *xpf-1* and *ercc-1* mutants displayed improved healthspan as young adults, however by middle age, they are significantly susceptible to stress, as well as display functional decline [24]. This clearly suggests that assessing health parameters only in young animals may not provide us with the complete picture. Therefore, in this study we systematically tested the role of three representative DNA repair mutants on physiological health and lifespan.

Materials and Methods

Nematode Strains:

N2 Bristol was used as the wild-type strain. All strains (except *brc-1(KO)* gift from Dr. Nicola Silva) were provided by the Caenorhabditis Genetics Centre (CGC)- detailed information in Supp Table 1. All strains were outcrossed at least 5X. Nematode strains were maintained on nematode growth medium (NGM) plates seeded with *E. coli* OP50. All experiments were performed at 20°C unless noted otherwise.

Chemotaxis Assay:

Chemotaxis assay was performed as previously described [30]. Briefly, for each strain and age assessed, about 100–150 worms were collected and washed 3 times in M9 buffer. The worms were put on two NGM plates and the excess liquid was dabbed off with a Kimwipe. The nematodes were placed 5 cm away from a 2μL spot of (chemoattractant): 10% isoamyl alcohol, diluted in 100% ethanol and 1μl of 10% sodium azide. The plates were left at room temperature for 1 hr. The final position of each worm was marked and the distance between each worm and the chemoattractant spot was measured manually in a blinded fashion.

Oxidative stress Assay:

For each experiment a fresh 7.5 mM hydrogen peroxide (H_2O_2) solution was made in S-basal buffer and protected from the light. A total of 40 worms were tested in a blinded set-up; ≥3 biological repeats; 8–12 technical repeats. The worms were added to 1 mL of

H_2O_2 solution in a 24 well plate or 50μl of a 96 well microtiter plate at 20°C. Every hour, the plate was gently tapped and moving/still worms were recorded as described [31].

Measure of Thrashing:

Thrashing assays were performed as previously described [24]. Briefly, a single worm was placed in a 500μL drop of M9 buffer in a 12-well plate. They were allowed to acclimate for 1 min. The number of movements of each worm was counted twice, for 30 sec, and results were averaged and represented per min. 10–15 worms were assayed per genotype and age in a blinded manner (technical repeats); ≥3 biological repeats.

Autofluorescence measurement:

On day 1, 4, 7, 11, 15 and 20 of adulthood, autofluorescence was measured in DNA repair mutants and wild-type worms. About 5–8 worms were anesthetized in 2mM levamisole and mounted on a slide with a 2% agarose pad. Photos were then taken at 300-ms exposure under a GFP filter and 1000-ms for TRITC using an Axioskop 2 Plus microscope. The same exposure time was used for each fluorescence channel across the full experiment. The fluorescence was calculated using a custom-build pipeline in cell profiler [32]. Briefly, in order to clearly distinguish each worm, the intensity of GFP image was increased by 10,000-fold using ‘image math’ module to obtain the worm outline. The outlined worms were then identified using ‘identify primary object’. The worm outline was then overlayed on the original GFP and TRITC images and the total intensity was measured for each worm.

Embryonic Lethality and Incidence of males:

Twenty day 1 adults were placed on OP50 seeded NGM plates. After a two hour egg lay, 100 eggs were transferred with a platinum pick to a new plate; 3 technical repeats. The worms were grown at 20°C for 36 h. ≥3 biological repeats.

Number of unhatched eggs versus total number of eggs was recorded (blinded to genotype) as % embryonic lethality post 36 hrs of egg lay.

Number of males versus total number of worms was recorded (blinded to genotype) as % incidence of males post 72 hrs of egg lay.

Lifespan analysis:

Lifespan analysis was performed as described [33] with a few changes. Briefly, 40–60 animals were used per conditions and scored every other day in a blinded manner. Day 1 adults were transferred to no FUDR (supplemental data) or 2.5 μg/ml FUDR containing NGM plates and were maintained on FUDR for the rest of the lifespan analysis. All lifespan experiments were performed at 20°C in a blinded fashion.

Nucleoli imaging and quantification:

DIC microscopy was used to perform all the nucleolar imaging. Hypodermal and germ cell nucleoli of age-matched day 1 adults were imaged using 100X magnification (Plan Apo VC 100x Oil DIC N2) with Nikon Ti microscope and 63x Oil Leica TCS SP8. Nucleolar area was quantified manually with the freehand tool using Fiji software.

Thermorecovery:

Animals were raised on OP50 bacteria until they reached desired age of adulthood. The worms were then transferred to fresh OP50 plates, placed at 36°C for 3h. Plates were transferred back to 20°C overnight and survival was scored by gentle prodding with a platinum-wire pick.

Statistics:

All statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). Survival analyses were performed using the Kaplan-Meier method and the significance of differences between survival curves calculated using the log rank (Mantel-Cox) test. Differences between groups were considered to be significant at a P value of <0.05. One way ANOVA was performed with multiple comparisons for most assays. Detailed information is in Figure Legends. Spearman rank-order correlation coefficients (ρ) were computed with the '*cor*' function of the R language to show the associations between the strains. For all scatter plots a linear regression analysis was performed. The linear regression estimated the effect of health parameters (as indicated on y-axis) on average lifespan.

Results

Mutations that result in a severe reduction of XPF-ERCC1, a structure-specific endonuclease, result in accelerated aging, in humans, mice and *C. elegans* [14, 24]. Although the role of XPF-ERCC1 in nucleotide excision repair (NER) is well established, recent evidence suggests that the 5' endonuclease also plays a role in interstrand crosslink repair (ICL) [34, 35] and repair of some types of double strand breaks (DSB) [36–39]. ICLs formed due to endogenous damage, such as those from lipid peroxidation, link the DNA covalently and impede replication, as well as transcription [40, 41]. The crosslink is unhooked by endonucleases (one of which is XPF-ERCC1), and then repaired by the Fanconi Anemia (FA) pathway [42–44]. Central to the function and regulation of the FA pathway is FANCD2, which is monoubiquitinated, localized at the site of damage and is crucial for proper recruitment of downstream repair proteins [45]. Similarly, recent evidence suggests that XPF-ERCC1 is important for completion of homologous recombination (HR), removal of 3′ non-homologous termini, or processing of looped-out heteroduplex intermediates [46]. BRCA1 is a key protein involved in DSB repair and plays a distinct role in HR [47]. Although there is evidence that some types of DNA damage lead to accelerated aging, the role of different types of DNA damage in healthspan is not yet well understood.

We chose to compare and contrast three distinct DNA repair mutants; XPF-ERCC1, BRCA1 and FANCD2 (Fig 1A). These DNA repair genes were picked based on three important criteria: (1) they are the essential or rate-limiting enzymes in distinct DNA repair pathways [47–51] (2) Mutations associated with these genes drive age-related pathologies in mammals [14, 52–54] (3) they have well-conserved DNA repair function in *C. elegans* (Supp Table 1). To begin characterizing DNA repair-deficient mutants, we first examined incidence of males, and unhatched eggs. *C. elegans* are usually hermaphrodites (XX) and males can arise from loss of one X chromosome due to a meiotic non-disjunction event during hermaphrodite

(XX) meiosis. Consistent with previous findings we observed *xpf-1* mutant worms showed a 5% increased incidence of males at 20°C [24]. In contrast, *brc-1* and *fcd-2* did not have any discernible difference compared to wild-type (N2) worms (Fig 1B). *C. elegans* DNA repair-deficient mutants also display a high level of embryonic lethality, likely due to defects in the replication and segregation of the damaged genome during the rapid early cell cycles (in contrast to dividing germ cells, early embryos do not undergo cell cycle arrest following DNA damage) [55–62]. *xpf-1* (~29%), *brc-1* (~18%) and *fcd-2* (~5%) mutant worms showed higher percentage of unhatched eggs than N2 (Fig 1C). Next, we examined lifespan of these DNA repair-deficient mutants at 20°C. Interestingly, *brc-1* and *xpf-1* mutants exhibited a shortened lifespan compared to N2, whereas *fcd-2* did not show any significant difference in lifespan (Fig 1D). To characterize the role of different types of DNA damage in healthspan, we examined (1) Molecular markers (2) Functional markers and (3) Resilience response of each of these DNA repair-deficient mutants with age.

Molecular markers of healthspan in DNA repair-deficient mutants

To understand the effect of DNA damage on molecular markers of healthspan we examined nucleolar size and autofluorescence with age. Nucleolar size has recently emerged as a predictive marker for longevity [63, 64]. Studies in *C. elegans*, mice and humans display a trend of larger nucleolar size with premature aging (shortened lifespan). To address whether DNA repair mutants with differential effect on lifespan display any changes in nucleolar size, we measured the nucleolar size of gonadal cells and superficial hypodermal cells on the first day (D1) of adulthood. Interestingly, nucleolar size was enlarged in gonadal cells in both *brc-1* and *fcd-2* mutants (Fig 2A), but only increased in hypodermal cells in *fcd-2* mutant animals (Fig 2B). Although, both *xpf-1* and *brc-1* display reduced lifespan compared to N2, only *brc-1* had increased gonadal nucleolar size. Thus, comparing lifespan with average nucleolar size (Fig 2C) suggests that this may not be a clear marker of lifespan at least in DNA repair mutants.

Autofluorescence due to accumulation of age-related pigments or lipofuscin granules in the lysosomes has been adopted as another molecular marker of aging in *C. elegans*. To examine whether autofluorescence was increased in DNA repair-deficient mutants with age, we measured GFP [480/20nm(ex), 510/20nm(em)] and TRITC [544nm(ex), 570nm(em)] autofluorescence throughout adulthood. GFP, as well as, TRITC channel fluorescence increased with age in N2 and all DNA repair-deficient mutants with age (Fig 2 D–E). Surprisingly, *brc-1* GFP autofluorescence was lower at D1 of adulthood, whereas *xpf-1*, *brc-1* and *fcd-2* had significantly lower TRITC autofluorescence on D1. However, autofluorescence increased with time for all strains. Moreover, values in DNA repair-deficient mutants were never greater than wild-type. *fcd-2* mutants consistently displayed lower GFP autofluorescence until Day 11 of adulthood, but then showed no significant difference at Day 15. In order to see how DNA damage mutants influenced autofluorescence in these strains we performed spearman correlation analysis between autofluorescence of each of the DNA repair mutants, as well as, wild-type. The analysis will provide us the relationship between the accumulation of autofluorescence in the DNA repair mutants over their lifetime. Green autofluorescence of *xpf-1* and *brc-1* mutants showed strong correlation with wild-type, whereas *fcd-2* showed moderate correlation with wild-type autofluorescence

(Fig 2D–H). However, the association between the DNA repair mutants further weakened with red autofluorescence, suggesting that these DNA repair mutants have limited effect on autofluorescence (Fig 2F–H). Our data suggests that although autofluorescence changes with age, the increased levels do not significantly correlate with lifespan of DNA repair mutants.

Functional healthspan in DNA repair-deficient mutants

Sarcopenia, the progressive loss of skeletal muscle mass and strength, is a natural part of aging in humans [65]. Reduced mobility and gait speed are well known characteristics of sarcopenia. As in humans, sarcopenia has also been observed in nematodes with age, affecting their mobility [66]. Therefore, to define functional health, we examined the changes in the movement capacity of DNA repair-deficient animals with age.

We assessed movement ability of wild-type and mutant animals with age in liquid media (also known as thrashing) by recording the number of body bends for an individual animal per minute. Since we were interested in early changes that could be used as markers of healthspan, we characterized nematodes from Day 1– 11 of adulthood (prior to any major death events). As expected, thrashing progressively decreased with age, from Day 1 (D1) to Day 11 (Fig 3A–C), in wild-type, as well as, all mutants. Interestingly, thrashing was compromised in *brc-1* mutants at D1 of adulthood compared to N2 by ~20%. However, no significant differences were observed between *xpf-1* or *fcd-2* compared to N2 at D1 (Fig 3A). Although thrashing was compromised in all mutants with age, *fcd-2* and *brc-1* did not show any significant difference at day 7 and 11 compared to N2. In contrast, thrashing was significantly reduced in *xpf-1* with age (~30% at D7 and ~45% at D11) (Fig 3A).

Next to determine whether the DNA repair mutant worms display neuromuscular issues, we tested their locomotion in response to a chemoattractant. Chemotaxis measures neuronal function by testing the ability of worms to sense the chemoattractant, as well as, motor function by testing the ability to crawl towards the chemoattractant [67, 68]. Chemotaxis was measured by putting worms on a plate, 5cm away from a spot of isoamyl alcohol (IA), a chemical known to be a chemoattractant for *C. elegans* [69] and sodium azide, which acts as an anaesthetic. After 1 hour, distance was measured between each worm and the chemoattractant spot, the distance being inversely proportional to the chemotaxis ability. At Day 1 of adulthood, the DNA repair mutant worms were able to reach the IA spot with the same efficiency as wild-type worms. However, as the *xpf-1 and brc-1* worms grew older, at day 4 of adulthood they became increasingly incapable of accomplishing this task. Interestingly, *fcd-2* mutants did not show compromised chemotaxis until Day 11 of adulthood. At Day 11, all genotypes tested lost their ability to sense and/or migrate to the chemoattractant, including the wild-type worms. Further experimentation will be required to determine whether the inability to move in *xpf-1* and *brc-1* mutants is caused by dysfunction of the muscle cells themselves, or the motor neurons that innervate them. To understand how each DNA repair pathway affects functional healthspan, we performed spearman's correlation analysis between each of the DNA repair mutants. The results suggested that the different DNA repair genes did not influence movement ability (Fig 3C). However, as seen in Fig 3D, the *xpf-1* worms behaved differently with regard to their chemotaxis ability than the wildtype worms and *brc-1* or *fcd-2*. Additionally, despite the apparent decrease in

functional health with age between DNA repair mutants, no clear association was noted with lifespans (Fig 3E and Fig 3F).

Resilience healthspan in DNA repair-deficient mutants

A key hallmark of aging is the ability to respond to stress. To test the resilience of DNA repair mutants, we exposed them to heat and oxidative stress. Although, it is well accepted that increased thermotolerance corresponds to longevity, in recent years evidence has emerged to the contrary [24, 27, 70]. For thermorecovery, worms grown and maintained at 20°C were transferred to 36°C for 3 h and then allowed to recover at 20°C overnight. As mentioned earlier, we only characterized nematodes from Day 1– 11 of adulthood (prior to any major death observed). Thermorecovery decreased with age in all strains tested (Fig 4A). Consistent with previous reports, *xpf-1* was resistant to heat stress on D1, but was significantly sensitive starting at D7 of adulthood. In contrast, *brc-1* mutants were sensitive to heat stress as D1 and D11 adults, but did not show any increased sensitivity at D4 or D7. Interestingly, *fcd-2* mutants were sensitive to heat stress only as D1 adults, but did not show any significant changes after. The strain-specific reduction was greatest for *brc-1*, which was ~88%, followed by ~82% for *xpf-1* on Day 11 (Fig 4A).

Next, we tested the ability of DNA repair-deficient mutants to respond to oxidative stress. Here, wild-type worms, as well as mutants, were distributed to individual wells of 24-well plate and exposed to 7.5-mM hydrogen peroxide (H_2O_2) in S-basal buffer. Interestingly, *brc-1* mutants were highly sensitive to oxidative stress starting from D1 and stayed sensitive through age (Fig 4B). Again, consistent with previous data, initially *xpf-1* mutants were resistant to oxidative stress (p <0.0001) compared with N2 at both D1 and D4 of adulthood [24] (Fig 4B). *fcd-2* mutants were also resistant to oxidative stress as young D1 adults, but lost this resistance starting at D4. With age, all of the strains were sensitive to oxidative stress (Fig 4B). Spearman correlation analysis of resilience between each DNA repair mutant revealed that these resilience healthspan parameters were not compromised by defective DNA repair pathways (Fig 4C and Fig 4D). Next, we measured the average survival time in H_2O_2 for D4 and D7 and calculated the change in average survival time for each assay timepoint. The rate of change in response to oxidative stress between day 4 and day 7 significantly correlates with lifespan, suggesting that oxidative stress could be one of the major factors in determining the lifespan in both wild-type and DNA repair-deficient worms (Fig 4E).

To further confirm the role of the three key DNA repair proteins, we analysed *ercc-1* (DNA repair partner of *xpf-1*; heterodimeric complex) [24] and additional alleles of *brc-1*(*KO*) [71] and *fcd-2(tm1298)* (Supp Fig 1). We tested the DNA repair mutants for one key healthspan feature in each of the categories tested: (1) Molecular: Nucleolar size (2) Functional: Thrashing and (3) Resilience. Interestingly, although *ercc-1* mutants were short-lived [24] (Supp Fig 1A), they had a smaller gonadal nucleolar size (Supp Fig 1B). As seen in Fig 2A, gonadal nucleolar size was enlarged in *brc-1(KO)* and *fcd-2(tm1298)* mutants (Supp Fig 1B); whereas no changes in hypodermal nucleolar size was observed in any of the DNA repair mutants (Supp Fig 1C). Of note, *fcd-2(ok1145)* mutants had increased hypodermal nucleolar size, but this was not observed in *fcd-2(tm1298)*. Next we tested functional

healthspan by measuring thrashing in liquid. As with *xpf-1* mutants, *ercc-1* did not show any significant changes in thrashing at day 1 (Supp Fig 1D). We further measured thrashing of *ercc-1* mutants at day 4 and did not observe any significant decline compared to N2. However, consistent with *xpf-1* mutants, *ercc-1* had significant functional decline by day 7 of adulthood (Supp Fig 1D). Of note, although the *brc-1(KO)* mutants displayed reduced movement in liquid at day 1 and day 4, the rate of decline with age was not as significant as for N2 and the other DNA repair mutants (Supp Fig 1D). *fcd-2(tm1298)* mutants did not show any significant changes in thrashing, consistent with the *fcd-2(ok1145)* mutant. To further confirm our results, that DNA repair mutants are sensitive to oxidative stress, we analysed *ercc-1, brc-1(KO)* and *fcd-2(tm1298)* at D1, 4, 7 and 11 (Supp Fig 1E). Consistent with *xpf-1*, *ercc-1* mutants were resistant to oxidative stress in young adulthood, but with age displayed high sensitivity. *brc-1(KO)* mutants were highly sensitive to H2O2 starting at D1, whereas *fcd-2(tm1298)* had increased sensitivity to oxidative stress with age. Lastly, we performed spearman's correlation analysis between the DNA repair mutants and the wild-type using all the measured parameters (Fig 4F). In all, age-associated decline seen in DNA repair mutants was quite similar to that of wild-type and not altered in any specific manner.

Discussion

Although it is controversial whether lifespan can be significantly extended in humans, there is growing consensus that improving healthspan (rather than lifespan) is an important objective in order to increase quality of life for the elderly and alleviate the effects of an aging population. Therefore, it is critical that we tease apart the molecular mechanisms which contribute to healthspan. Using the model *C. elegans*, we aimed to identify the importance of three major DNA repair genes in maintenance of health. Damage to DNA can take place due to both endogenous (e.g. increased oxidative stress), as well as, exogenous sources (e.g. ultraviolet radiation). DNA damage includes bulky adducts that can block replication/transcription, as well as abasic sites, single strand or double strand DNA breaks, and interstrand crosslinks. Such accumulated damage is known to underlie disease and contribute to age-related pathology. Herein, we determined the effect of three key DNA repair genes on embryonic survival, male incidence, tissue functionality and lifespan during adulthood.

In this study we broadly defined healthspan as three major categories that are frequently analysed in *C. elegans* as markers of aging. They include molecular markers that comprise of nucleolar size and accumulation of autofluorescence pigments, functional markers such as neuromuscular integrity and muscle function, and resilience to acute stress events. In addition, we looked at the correlation of each of these healthspan measures between the DNA repair mutants and the wild-type. To the best of our knowledge, our study is the first attempt to compare the contribution of key DNA repair genes to multiple measures of healthspan.

XPF-ERCC1 is a heterodimeric 5' endonuclease involved in several DNA repair pathways. Traditionally, its role in removal of bulky lesions on DNA during NER has been the best characterized. XPF-ERCC1 excises the damage as a single-stranded oligonucleotide,

protects the gap and allows for repair using the non-damaged strand as a template. More recently, the role of XPF-ERCC1 in homologous recombination for repair of double strand breaks (DSB) has been recognized. Upon resection of a DSB, 5′- to 3′-degradation results in a single stranded overhang. ERCC1-XPF promotes the cleavage of 3' overhangs and has been reported to play a role in removing potentially problematic branched DNA structures that arise as a result of such DSB [37, 72, 73]. Additionally, interstrand crosslink repair (ICL) is responsible for correcting lesions that arise from mutagens such as cisplatin and carcinogens from smoking, as well as due to lipid peroxidation. ICL is a highly coordinated process that is regulated by the Fanconi anemia (FA) network. The FA network coordinates major proteins also involved in NER and HR- such as XPF-ERCC1. XPF-ERCC1 is responsible for the incision of DNA flanking the damage site, creating a DSB and unhooking the cross-link at the stalled replication fork. In humans, loss of functional XPF/ERCC1 leads to systemic progeroid symptoms or the Cerebro-oculo-facio-skeletal Syndrome (COFS) syndrome. XFE progeria is characterized by multi-tissue accelerated aging pathology including neurodegeneration, sarcopenia, vision loss etc. Recently, two patients with pathogenic mutations in XPF were identified with adult-onset neurodegeneration involving chorea, ataxia, hearing loss, cognitive deficits, profound brain atrophy, and a history of skin photosensitivity, skin freckling, and/or skin neoplasms [74].

Loss of *xpf-1/ercc-1* in *C. elegans*, shortened lifespan, increased embryonic lethality, impaired thrashing, chemotaxis and reduced resilience with age. The lifespan of *xpf-1/ercc-1* mutants has been controversial. Although a group has reported no effect on lifespan [75], several others have reported a small but significant reduction in lifespan [24, 56, 76] consistent with mice and humans that harbour loss of functional ERCC1-XPF [14]. Our result is also consistent with previous findings where we reported a shortened lifespan in *xpf-1* and *ercc-1* mutants in the presence and absence of FUDR, as well as in in the homozygous mutant progeny from a genetically balanced *ercc-1* strain [24]. The functional healthspan measures are affected early on (by day 4 of adulthood) in *xpf-1* and *ercc-1* mutants. Since both thrashing in liquid and chemotaxis is impaired in *xpf-1* mutants, these functional phenotypes arise most likely due to an inability to move, and not an inability to sense the chemoattractant. However, further studies are required to determine whether the motor issues are mainly due to dysfunctional muscle cells or the motor neurons that innervate the muscle. It is important to note that a recent report suggested that although XPF/ERCC1 is expressed in germline, muscle, intestine and neurons, expression of *xpf-1* in neurons could rescue sensitivity to UV [77]. This suggests that *xpf-1/ercc-1* may have an important role in maintaining neuronal health with age. Consistent with our previous findings, we did find *xpf-1/ercc-1* mutants are stress resistant when young (day 1 of adulthood), but this resilience is lost in an accelerated manner with age. We have previously shown that this early stress resistance is due to DAF-16/FOXO3A activity which is lost with age [24]. Although an increase in nucleolar size is correlated with a shortened lifespan in other studies, the *xpf-1* mutants did not display any significant changes in nucleolar size compared to wild-type. Since we measured nucleolar size only in day 1 adults, one possibility is that we did not capture changes with age. This suggests that nucleolar size may not be a consistent marker for lifespan, at least in mutants that are short-lived.

The second *C. elegans* DNA repair mutant we analysed is *brc-1*. The role of Breast cancer type 1 susceptibility protein (BRCA1) has been well characterized in homologous recombination (HR). BRCA1 is also known to play complex roles in DNA repair, replication fork protection, as well as in transcription. BRCA1 recruits the recombinase, RAD51, to double strand break foci which is a crucial early event in HR. Additionally BRCA1 plays a role in DSB end resection thus favouring HR over non-homologous end joining repair of DSBs. BRCA1 is also important for fork stabilization, repair and restart of replication forks and for protection of stressed forks from other nucleases. Recent data indicates that BRCA1 plays a key role in ICL repair too. BRCA1 facilitates the displacement of the stalled replicative helicase during a dual-fork convergence, thus activating the Fanconi anemia (FA) pathway. BRCA1 mutations is known to cause familial breast and ovarian cancers and are linked to a variety of sporadic cancers [78]. In *C. elegans*, *brc-1* plays a role in both ICL and HR, as in the mammalian counterparts [76, 79, 80]. In contrast to a previous report [81], backcrossed *brc-1* mutants in our hands did display a shortened lifespan. We do not know the origin of this discrepancy as both studies use the same allele (as well as, an additional allele tested in our study), maintenance temperature of 20°C and FUDR conditions (Supp Fig 1). Our data shows that *brc-1* mutants, have increased embryonic lethality (consistent with [81]), gonadal nucleolar size and are highly sensitive to acute stress, especially oxidative stress. Several reports suggest that BRCA1 mutant tumours are sensitive to oxidative stress, and BRCA1 is important for regulation of oxidative stress, partially through induction of antioxidant genes [82, 83]. *brc-1* mutants are also sensitive to heat stress, suggesting a general failure of stress responses in an accelerated manner. Although loss of *brc-1* has some impact on functional healthspan, it does not seem to be as significant as observed in *xpf-1* mutants.

We also assessed the importance of FANCD2 in healthspan and lifespan. A major pathway involved in repair of ICLs is the FA network. FA dysfunction in humans leads to bone marrow failure, cancer predisposition and profound sensitivity to crosslinking agents [84]. *C. elegans* have a number of conserved FA gene homologs, including FCD-2 (FANCD2) [76]. The *fcd-2* mutant did not display increased embryonic lethality, shortened lifespan, impaired functional healthspan or increased sensitivity to heat stress, suggesting that loss of *fcd-2* is similar to wild-type. However, the *fcd-2* mutants exhibit an increased nucleolar size in the gonad and an increased sensitive to oxidative stress. Intriguingly, recent reports suggest that FA proteins, including FANCD2 associates with components of nucleoli and nuclear speckles and are possibly involved in liquid–liquid phase transitions [85, 86]. This could possibly explain the increased nucleolar size observed in *fcd-2* mutants. Impaired ROS detoxifying machinery has been observed upon loss of FA pathway [87]. Another study also showed an association of FANCD2 with FOXO3a (a master regulator of oxidative stress), suggesting that FANCD2 may play a role in ROS homeostasis [88], suggesting that this may be the primary reason for increased susceptibility to oxidative stress in *fcd-2* mutants. It is possible that although *fcd-2* mutants are sensitive to DNA cross-linking agents, the role of *fcd-2* in resolving ICL in somatic cells in *C. elegans* may be minimal or compensated by other FA proteins. It will be important to test healthspan and lifespan parameters in *fcd-2* mutants after exposure to DNA cross-linking agents in the future to fully resolve this challenge.

In our study, we focused on health at early timepoints so as to eliminate the effects from lifespan. Therefore, it is possible that we missed the period of gerospan in these DNA repair mutants. Overall, our data suggests the decline in individual healthspan measures is minimally accelerated by the lack of these DNA repair genes. However, these small effects on multiple parameters, may have a substantial impact on health. Interestingly, oxidative stress appears to be one of the key players in determining lifespan in DNA repair mutants as suggested by the spearman correlation analysis. Our observations demonstrate that the loss of *xpf-1/ercc-1* significantly impairs a number of healthspan measures, followed by *brc-1* and least affected by *fcd-2*. Several possibilities could explain the phenotypes observed in the *xpf-1* mutant compared to other DNA repair mutants. (A) XPF-ERCC1 plays a role in a number of DNA repair pathways suggesting that accumulation of a number of different types of DNA damage impacts health greatly OR (B) NER could be the major DNA repair pathway affecting both lifespan and healthspan. NER is responsible for repair of several different types of lesions including cyclopurine adducts arising from oxidative stress. Future studies with other NER mutants such as XPG could help us delve into this deeper. (C) XPF-1 is expressed in multiple tissues, including the gonad, hypodermis, intestine, neurons, pharynx and muscle [77]. Indeed, a recent study did show that XPF-ERCC1 function was required in neurons to maintain genome stability [77]. In contrast, *brc-1* is expressed mainly in the germline and in some somatic tissues like muscle and hypodermis, whereas *fcd-2* is mostly limited to the germline. This data suggests that loss of *xpf-1/ercc-1* could impact health and homeostasis much more significantly because of the multiple tissues that rely on it. Future studies on the role of DNA repair pathways in a tissue specific manner will be of utmost importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Nations, U., World Population Ageing 2019, D.o.E.a.S. Affairs, Editor. 2019.

[2]. Lopez-Otin C, et al. , The hallmarks of aging. Cell, 2013. 153(6): p. 1194–217. [PubMed: 23746838]

[3]. Kennedy BK, et al. , Geroscience: linking aging to chronic disease. Cell, 2014. 159(4): p. 709–13. [PubMed: 25417146]

[4]. Niedernhofer LJ, et al. , Nuclear Genomic Instability and Aging. Annu Rev Biochem, 2018. 87: p. 295–322. [PubMed: 29925262]

[5]. Brazhnik K, et al. , Single-cell analysis reveals different age-related somatic mutation profiles between stem and differentiated cells in human liver. Sci Adv, 2020. 6(5): p. eaax2659.

[6]. Zhang L, et al. , Single-cell whole-genome sequencing reveals the functional landscape of somatic mutations in B lymphocytes across the human lifespan. Proc Natl Acad Sci U S A, 2019. 116(18): p. 9014–9019. [PubMed: 30992375]

[7]. Lodato MA, et al. , Aging and neurodegeneration are associated with increased mutations in single human neurons. Science, 2018. 359(6375): p. 555–559. [PubMed: 29217584]

[8]. Vaidya A, et al. , Knock-in reporter mice demonstrate that DNA repair by non-homologous end joining declines with age. PLoS Genet, 2014. 10(7): p. e1004511. [PubMed: 25033455]

[9]. Goukassian D, et al. , Mechanisms and implications of the age-associated decrease in DNA repair capacity. FASEB J, 2000. 14(10): p. 1325–34. [PubMed: 10877825]

[10]. Imam SZ, et al. , Mitochondrial and nuclear DNA-repair capacity of various brain regions in mouse is altered in an age-dependent manner. Neurobiol Aging, 2006. 27(8): p. 1129–36. [PubMed: 16005114]

[11]. Boyd WA, et al. , Nucleotide excision repair genes are expressed at low levels and are not detectably inducible in Caenorhabditis elegans somatic tissues, but their function is required for normal adult life after UVC exposure. Mutat Res, 2010. 683(1–2): p. 57–67. [PubMed: 19879883]

[12]. Karikkineth AC, et al. , Cockayne syndrome: Clinical features, model systems and pathways. Ageing Res Rev, 2017. 33: p. 3–17. [PubMed: 27507608]

[13]. Gray MD, et al. , The Werner syndrome protein is a DNA helicase. Nat Genet, 1997. 17(1): p. 100–3. [PubMed: 9288107]

[14]. Niedernhofer LJ, et al. , A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature, 2006. 444(7122): p. 1038–43. [PubMed: 17183314]

[15]. Armstrong GT, et al. , Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. J Clin Oncol, 2014. 32(12): p. 1218–27. [PubMed: 24638000]

[16]. Armstrong GT, et al. , Long-term outcomes among adult survivors of childhood central nervous system malignancies in the Childhood Cancer Survivor Study. J Natl Cancer Inst, 2009. 101(13): p. 946–58. [PubMed: 19535780]

[17]. Ness KK, et al. , Physiologic frailty as a sign of accelerated aging among adult survivors of childhood cancer: a report from the St Jude Lifetime cohort study. J Clin Oncol, 2013. 31(36): p. 4496–503. [PubMed: 24248696]

[18]. Marteijn JA, et al. , Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol, 2014. 15(7): p. 465–81. [PubMed: 24954209]

[19]. Beard WA, et al. , Eukaryotic Base Excision Repair: New Approaches Shine Light on Mechanism. Annu Rev Biochem, 2019. 88: p. 137–162. [PubMed: 31220977]

[20]. Kunkel TA and Erie DA, Eukaryotic Mismatch Repair in Relation to DNA Replication. Annu Rev Genet, 2015. 49: p. 291–313. [PubMed: 26436461]

[21]. Ceccaldi R, Sarangi P, and D'Andrea AD, The Fanconi anaemia pathway: new players and new functions. Nat Rev Mol Cell Biol, 2016. 17(6): p. 337–49. [PubMed: 27145721]

[22]. Vitor AC, et al. , Studying DNA Double-Strand Break Repair: An Ever-Growing Toolbox. Front Mol Biosci, 2020. 7: p. 24. [PubMed: 32154266]

[23]. Wang J, et al. , The oxidative DNA lesions 8,5'-cyclopurines accumulate with aging in a tissue-specific manner. Aging Cell, 2012. 11(4): p. 714–6. [PubMed: 22530741]

[24]. Gurkar AU, et al. , Dysregulation of DAF-16/FOXO3A-mediated stress responses accelerates oxidative DNA damage induced aging. Redox Biol, 2018. 18: p. 191–199. [PubMed: 30031267]

[25]. Kapahi P, Boulton ME, and Kirkwood TB, Positive correlation between mammalian life span and cellular resistance to stress. Free Radic Biol Med, 1999. 26(5–6): p. 495–500. [PubMed: 10218637]

[26]. Lithgow GJ, et al. , Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc Natl Acad Sci U S A, 1995. 92(16): p. 7540–4. [PubMed: 7638227]

[27]. Dues DJ, et al. , Resistance to Stress Can Be Experimentally Dissociated From Longevity. J Gerontol A Biol Sci Med Sci, 2019. 74(8): p. 1206–1214. [PubMed: 30247515]

[28]. Bansal A, et al. , Uncoupling lifespan and healthspan in Caenorhabditis elegans longevity mutants. Proc Natl Acad Sci U S A, 2015. 112(3): p. E277–86. [PubMed: 25561524]

[29]. Podshivalova K, Kerr RA, and Kenyon C, How a Mutation that Slows Aging Can Also Disproportionately Extend End-of-Life Decrepitude. Cell Rep, 2017. 19(3): p. 441–450. [PubMed: 28423308]

[30]. Haroon S, et al. , Multiple Molecular Mechanisms Rescue mtDNA Disease in C. elegans. Cell Rep, 2018. 22(12): p. 3115–3125. [PubMed: 29562168]

[31]. Possik E and Pause A, Measuring oxidative stress resistance of Caenorhabditis elegans in 96-well microtiter plates. J Vis Exp, 2015(99): p. e52746. [PubMed: 25993260]

[32]. McQuin C, et al. , CellProfiler 3.0: Next-generation image processing for biology. PLoS Biol, 2018. 16(7): p. e2005970. [PubMed: 29969450]

[33]. Lucanic M, et al. , N-acylethanolamine signalling mediates the effect of diet on lifespan in Caenorhabditis elegans. Nature, 2011. 473(7346): p. 226–9. [PubMed: 21562563]

[34]. De Silva IU, et al. , Defects in interstrand cross-link uncoupling do not account for the extreme sensitivity of ERCC1 and XPF cells to cisplatin. Nucleic Acids Res, 2002. 30(17): p. 3848–56. [PubMed: 12202770]

[35]. Zhang N, et al. , Differential processing of UV mimetic and interstrand crosslink damage by XPF cell extracts. Nucleic Acids Res, 2000. 28(23): p. 4800–4. [PubMed: 11095693]

[36]. Adair GM, et al. , Role of ERCC1 in removal of long non-homologous tails during targeted homologous recombination. EMBO J, 2000. 19(20): p. 5552–61. [PubMed: 11032822]

[37]. Al-Minawi AZ, Saleh-Gohari N, and Helleday T, The ERCC1/XPF endonuclease is required for efficient single-strand annealing and gene conversion in mammalian cells. Nucleic Acids Res, 2008. 36(1): p. 1–9. [PubMed: 17962301]

[38]. Niedernhofer LJ, et al. , The structure-specific endonuclease Ercc1-Xpf is required for targeted gene replacement in embryonic stem cells. EMBO J, 2001. 20(22): p. 6540–9. [PubMed: 11707424]

[39]. Sargent RG, et al. , Role of the nucleotide excision repair gene ERCC1 in formation of recombination-dependent rearrangements in mammalian cells. Nucleic Acids Res, 2000. 28(19): p. 3771–8. [PubMed: 11000269]

[40]. Zhu G, Song L, and Lippard SJ, Visualizing inhibition of nucleosome mobility and transcription by cisplatin-DNA interstrand crosslinks in live mammalian cells. Cancer Res, 2013. 73(14): p. 4451–60. [PubMed: 23695549]

[41]. Hanada K, et al. , The structure-specific endonuclease Mus81-Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. EMBO J, 2006. 25(20): p. 4921–32. [PubMed: 17036055]

[42]. De Silva IU, et al. , Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol Cell Biol, 2000. 20(21): p. 7980–90. [PubMed: 11027268]

[43]. Klein Douwel D, et al. , XPF-ERCC1 acts in Unhooking DNA interstrand crosslinks in cooperation with FANCD2 and FANCP/SLX4. Mol Cell, 2014. 54(3): p. 460–71. [PubMed: 24726325]

[44]. Manandhar M, Boulware KS, and Wood RD, The ERCC1 and ERCC4 (XPF) genes and gene products. Gene, 2015. 569(2): p. 153–61. [PubMed: 26074087]

[45]. Langevin F, et al. , Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. Nature, 2011. 475(7354): p. 53–8. [PubMed: 21734703]

[46]. Anderson KD and Reiner A, Striatonigral projection neurons: a retrograde labeling study of the percentages that contain substance P or enkephalin in pigeons. J Comp Neurol, 1991. 303(4): p. 658–73. [PubMed: 1707424]

[47]. Zhao W, et al. , The BRCA Tumor Suppressor Network in Chromosome Damage Repair by Homologous Recombination. Annu Rev Biochem, 2019. 88: p. 221–245. [PubMed: 30917004]

[48]. Li S, et al. , ERCC1/XPF Is Important for Repair of DNA Double-Strand Breaks Containing Secondary Structures. iScience, 2019. 16: p. 63–78. [PubMed: 31153042]

[49]. Usanova S, et al. , Cisplatin sensitivity of testis tumour cells is due to deficiency in interstrand-crosslink repair and low ERCC1-XPF expression. Mol Cancer, 2010. 9: p. 248. [PubMed: 20846399]

[50]. Gentile F, et al. , Computer-aided drug design of small molecule inhibitors of the ERCC1-XPF protein-protein interaction. Chem Biol Drug Des, 2020. 95(4): p. 460–471. [PubMed: 31891209]

[51]. Kim H and D'Andrea AD, Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev, 2012. 26(13): p. 1393–408. [PubMed: 22751496]

[52]. Akbari MR, et al. , Mutations in Fanconi anemia genes and the risk of esophageal cancer. Hum Genet, 2011. 129(5): p. 573–82. [PubMed: 21279724]

[53]. Mantere T, et al. , Case-control analysis of truncating mutations in DNA damage response genes connects TEX15 and FANCD2 with hereditary breast cancer susceptibility. Sci Rep, 2017. 7(1): p. 681. [PubMed: 28386063]

[54]. Prakash R, et al. , Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol, 2015. 7(4): p. a016600. [PubMed: 25833843]

[55]. Craig AL, et al. , Methods for studying the DNA damage response in the Caenorhabdatis elegans germ line. Methods Cell Biol, 2012. 107: p. 321–52. [PubMed: 22226529]

[56]. Mueller MM, et al. , DAF-16/FOXO and EGL-27/GATA promote developmental growth in response to persistent somatic DNA damage. Nat Cell Biol, 2014. 16(12): p. 1168–79. [PubMed: 25419847]

[57]. Saito TT, et al. , Caenorhabditis elegans HIM-18/SLX-4 interacts with SLX-1 and XPF-1 and maintains genomic integrity in the germline by processing recombination intermediates. PLoS Genet, 2009. 5(11): p. e1000735. [PubMed: 19936019]

[58]. Lans H, et al. , Involvement of global genome repair, transcription coupled repair, and chromatin remodeling in UV DNA damage response changes during development. PLoS Genet, 2010. 6(5): p. e1000941. [PubMed: 20463888]

[59]. Agostinho A, et al. , Combinatorial regulation of meiotic holliday junction resolution in C. elegans by HIM-6 (BLM) helicase, SLX-4, and the SLX-1, MUS-81 and XPF-1 nucleases. PLoS Genet, 2013. 9(7): p. e1003591. [PubMed: 23901331]

[60]. O'Neil NJ, et al. , Joint molecule resolution requires the redundant activities of MUS-81 and XPF-1 during Caenorhabditis elegans meiosis. PLoS Genet, 2013. 9(7): p. e1003582. [PubMed: 23874209]

[61]. Saito TT, et al. , Interplay between structure-specific endonucleases for crossover control during Caenorhabditis elegans meiosis. PLoS Genet, 2013. 9(7): p. e1003586. [PubMed: 23874210]

[62]. Ermolaeva MA, et al. , DNA damage in germ cells induces an innate immune response that triggers systemic stress resistance. Nature, 2013. 501(7467): p. 416–20. [PubMed: 23975097]

[63]. Tiku V, et al. , Small nucleoli are a cellular hallmark of longevity. Nat Commun, 2017. 8: p. 16083. [PubMed: 28853436]

[64]. Buchwalter A and Hetzer MW, Nucleolar expansion and elevated protein translation in premature aging. Nat Commun, 2017. 8(1): p. 328. [PubMed: 28855503]

[65]. Lexell J, Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci, 1995. 50 Spec No: p. 11–6. [PubMed: 7493202]

[66]. Fisher AL, Of worms and women: sarcopenia and its role in disability and mortality. J Am Geriatr Soc, 2004. 52(7): p. 1185–90. [PubMed: 15209660]

[67]. Wes PD and Bargmann CI, C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature, 2001. 410(6829): p. 698–701. [PubMed: 11287957]

[68]. Kashyap L, Perera S, and Fisher AL, Identification of novel genes involved in sarcopenia through RNAi screening in Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci, 2012. 67(1): p. 56–65. [PubMed: 21593014]

[69]. Bargmann CI, Hartwieg E, and Horvitz HR, Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell, 1993. 74(3): p. 515–27. [PubMed: 8348618]

[70]. Rollins JA, et al. , Assessing Health Span in Caenorhabditis elegans: Lessons From Short-Lived Mutants. J Gerontol A Biol Sci Med Sci, 2017. 72(4): p. 473–480. [PubMed: 28158466]

[71]. Janisiw E, et al. , BRCA1-BARD1 associate with the synaptonemal complex and pro-crossover factors and influence RAD-51 dynamics during Caenorhabditis elegans meiosis. PLoS Genet, 2018. 14(11): p. e1007653. [PubMed: 30383754]

[72]. Bennardo N, et al. , Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet, 2008. 4(6): p. e1000110. [PubMed: 18584027]

[73]. Motycka TA, et al. , Physical and functional interaction between the XPF/ERCC1 endonuclease and hRad52. J Biol Chem, 2004. 279(14): p. 13634–9. [PubMed: 14734547]

[74]. Faridounnia M, Folkers GE, and Boelens R, Function and Interactions of ERCC1-XPF in DNA Damage Response. Molecules, 2018. 23(12).

[75]. Lans H, et al. , DNA damage leads to progressive replicative decline but extends the life span of long-lived mutant animals. Cell Death Differ, 2013. 20(12): p. 1709–18. [PubMed: 24013725]

[76]. Wilson DM 3rd, et al. , Systematic analysis of DNA crosslink repair pathways during development and aging in Caenorhabditis elegans. Nucleic Acids Res, 2017. 45(16): p. 9467–9480. [PubMed: 28934497]

[77]. Sabatella M, et al. , Tissue-Specific DNA Repair Activity of ERCC-1/XPF-1. Cell Rep, 2021. 34(2): p. 108608. [PubMed: 33440146]

[78]. Symington LS and Gautier J, Double-strand break end resection and repair pathway choice. Annu Rev Genet, 2011. 45: p. 247–71. [PubMed: 21910633]

[79]. Ward JD, et al. , Replication blocking lesions present a unique substrate for homologous recombination. EMBO J, 2007. 26(14): p. 3384–96. [PubMed: 17611606]

[80]. Park S, Choi S, and Ahn B, DNA Strand Breaks in Mitotic Germ Cells of Caenorhabditis elegans Evaluated by Comet Assay. Mol Cells, 2016. 39(3): p. 204–10. [PubMed: 26903030]

[81]. Adamo A, et al. , BRC-1 acts in the inter-sister pathway of meiotic double-strand break repair. EMBO Rep, 2008. 9(3): p. 287–92. [PubMed: 18219312]

[82]. Bae I, et al. , BRCA1 induces antioxidant gene expression and resistance to oxidative stress. Cancer Res, 2004. 64(21): p. 7893–909. [PubMed: 15520196]

[83]. Gorrini C, et al. , BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival. J Exp Med, 2013. 210(8): p. 1529–44. [PubMed: 23857982]

[84]. Bogliolo M, et al. , Mutations in ERCC4, encoding the DNA-repair endonuclease XPF, cause Fanconi anemia. Am J Hum Genet, 2013. 92(5): p. 800–6. [PubMed: 23623386]

[85]. Moriel-Carretero M, et al. , Fanconi anemia FANCD2 and FANCI proteins regulate the nuclear dynamics of splicing factors. J Cell Biol, 2017. 216(12): p. 4007–4026. [PubMed: 29030393]

[86]. Gueiderikh A, et al. , Fanconi anemia A protein participates in nucleolar homeostasis maintenance and ribosome biogenesis. Sci Adv, 2021. 7(1).

[87]. Kumari U, et al. , Evidence of mitochondrial dysfunction and impaired ROS detoxifying machinery in Fanconi anemia cells. Oncogene, 2014. 33(2): p. 165–72. [PubMed: 23318445]

[88]. Li X, et al. , Fancd2 is required for nuclear retention of Foxo3a in hematopoietic stem cell maintenance. J Biol Chem, 2015. 290(5): p. 2715–27 [PubMed: 25505262]

Highlights

- DNA damage and genome instability are hallmarks of aging.
- We assessed the effect of three key DNA repair genes on physiological health-'healthspan'.
- Oxidative stress resilience can act as a primary proxy for healthspan in DNA repair mutants.
- Chemotaxis is also compromised in DNA repair mutants.
- Average nucleolar size, an emerging biomarker of longevity, does not corelate with lifespan at least in DNA repair mutants.

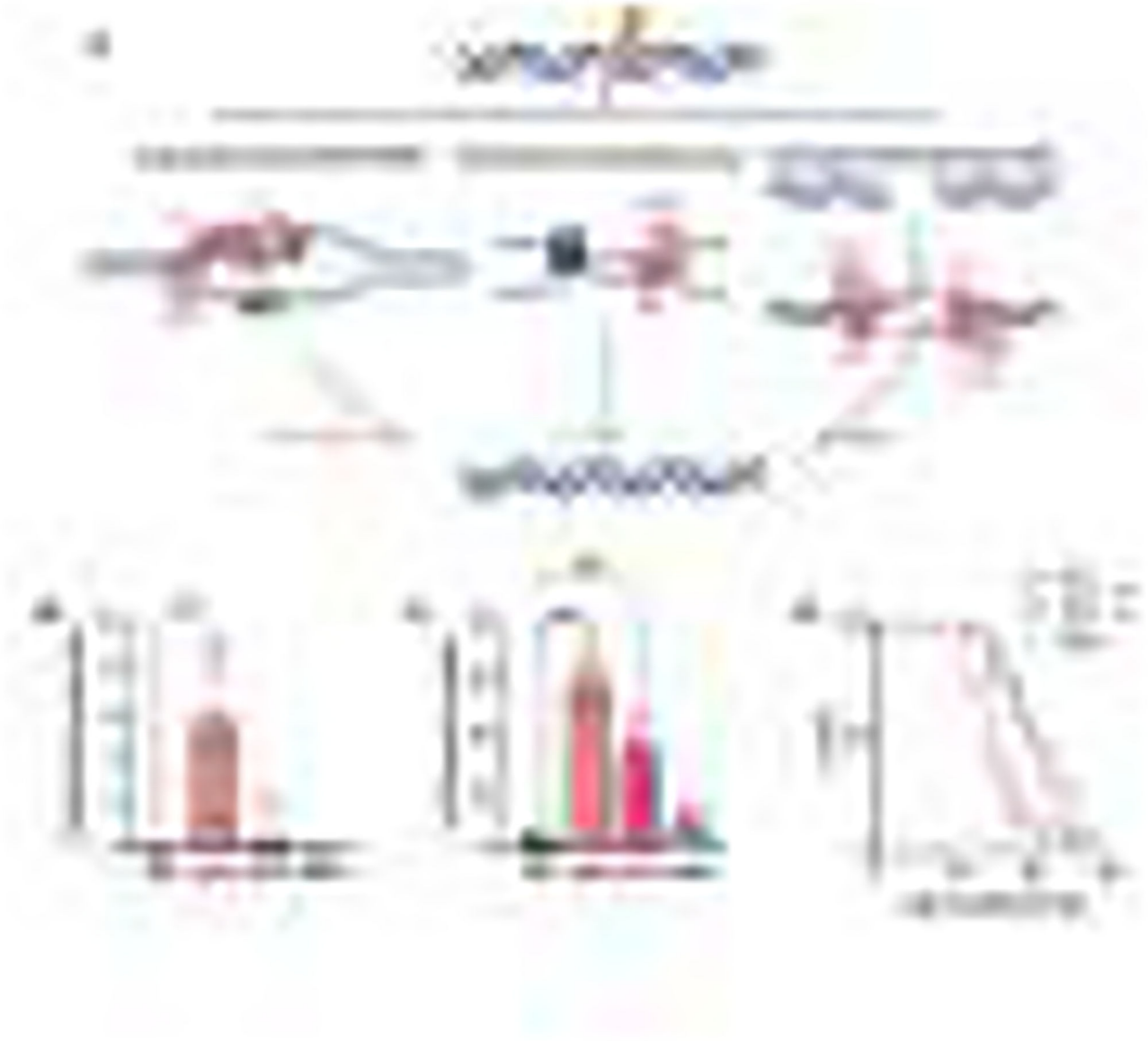

Figure 1:

(A) Role of XPF-ERCC1 in DNA repair: XPF-ERCC1 complex plays a role in Nucleotide excision repair (NER) both in global genome NER (GG-NER) and transcription coupled (TC-NER). Additionally, XPF-ERCC1 complex also plays a role in crosslink repair, as well as, repair of double strand breaks. To compare and contrast the role of three DNA repair genes we analysed healthspan measures *of xpf-1, brc-1*: involved in Homologous recombination (HR) and *fcd-2*: involved in crosslink repair (ICL). **(B)** *xpf-1(tm2842)* mutant worms have an increased incidence of males, but not *brc-1(ok1261)* and *fcd-2(ok1145)*: mutants. Plotted bar graph represents mean ± s.e.m. Each experiment consists of 100 eggs laid by 20 worms/ genotype. Experiment performed in triplicate at 20°C. One-way ANOVA with multiple comparisons. **(C)** Embryonic lethality (% unhatched eggs/total) was assessed for wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals. Data

shown is mean ± s.e.m. Three independent experiments performed (biological repeats):, Experiment performed in triplicate at 20°C (technical repeats). One-way ANOVA with multiple comparisons. **(D)** Representative survival curves of wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals. Survival curves were compared by Mentel-Cox log rank test. All replicates for survival data is shown in Supp Table 2A. For all statistics: **P*<0.05, ***P*<0.01 and ****P*<0.001.

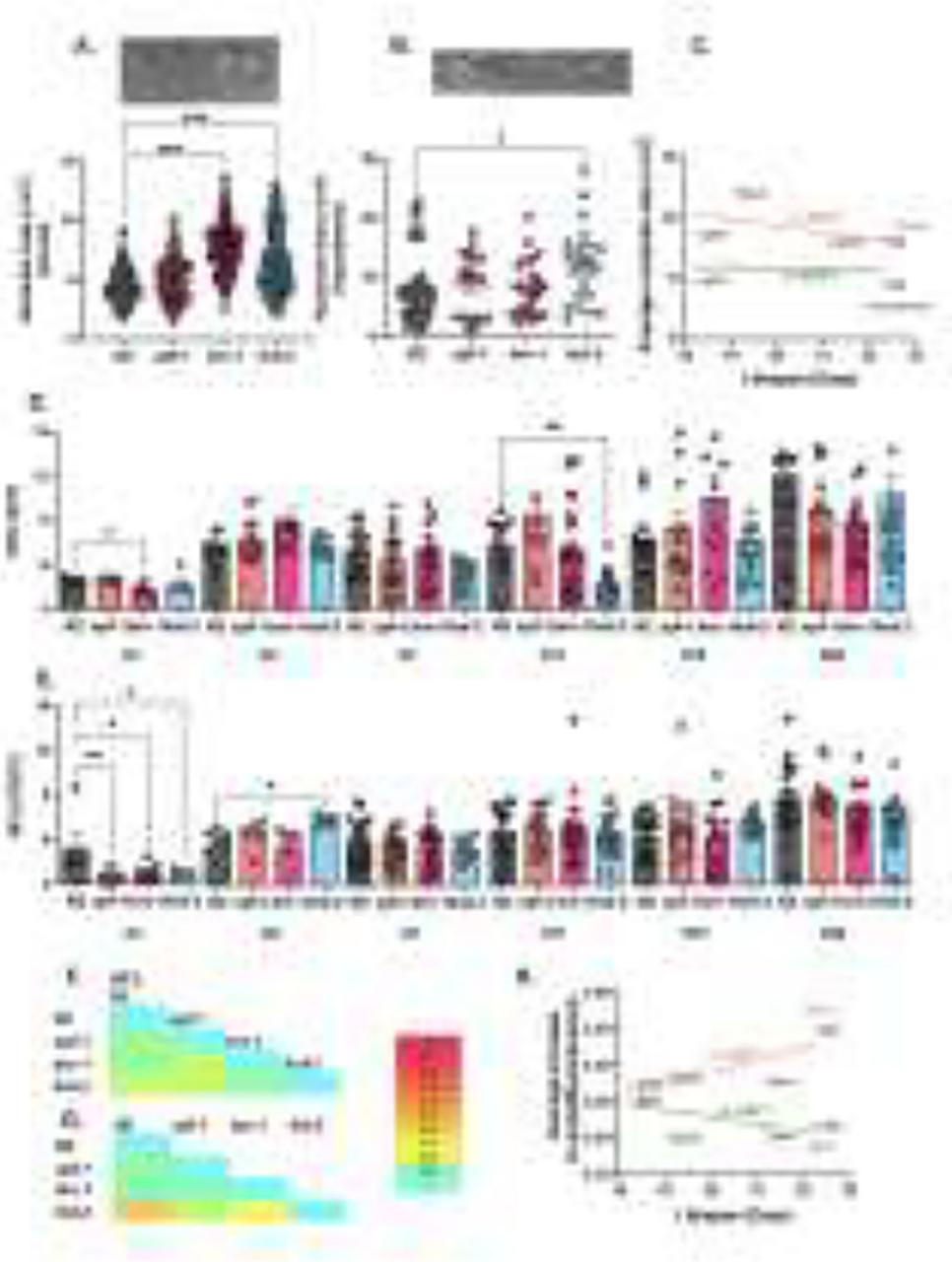

Figure 2: Molecular healthspan measures:

Nucleolar size measured in wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and *fcd-2(ok1145)* animals on day 1 of adulthood in gonad (**A**) and hypodermis (**B**). Data shown is mean ± s.e.m. **P*<0.05 and ****P*<0.001 by one-way ANOVA. (15–20 worms imaged per strain). Data were pooled from 3 independent experiments. All replicates for nucleolar size is shown in Supp Table 3A and 4A. (**C**) Scatter plot showing the relationship between lifespan of each strain with average nucleolar size of gonad and hypodermis. Autofluorescence measured in (**D**) GFP channel (**E**) TRITC channel throughout lifespan (D1- D20). Data shown is mean ± s.e.m. ns: **P*<0.05 and ***P*<0.01 by one-way ANOVA.

(**F-G**) Heat map showing spearman's rank correlation between green (**E**) and red (**F**) autofluorescence (AFL) of wildtype (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* worms. (**H**) Scatter plot showing the relationship between lifespan of each strain with average increase in autofluorescence.

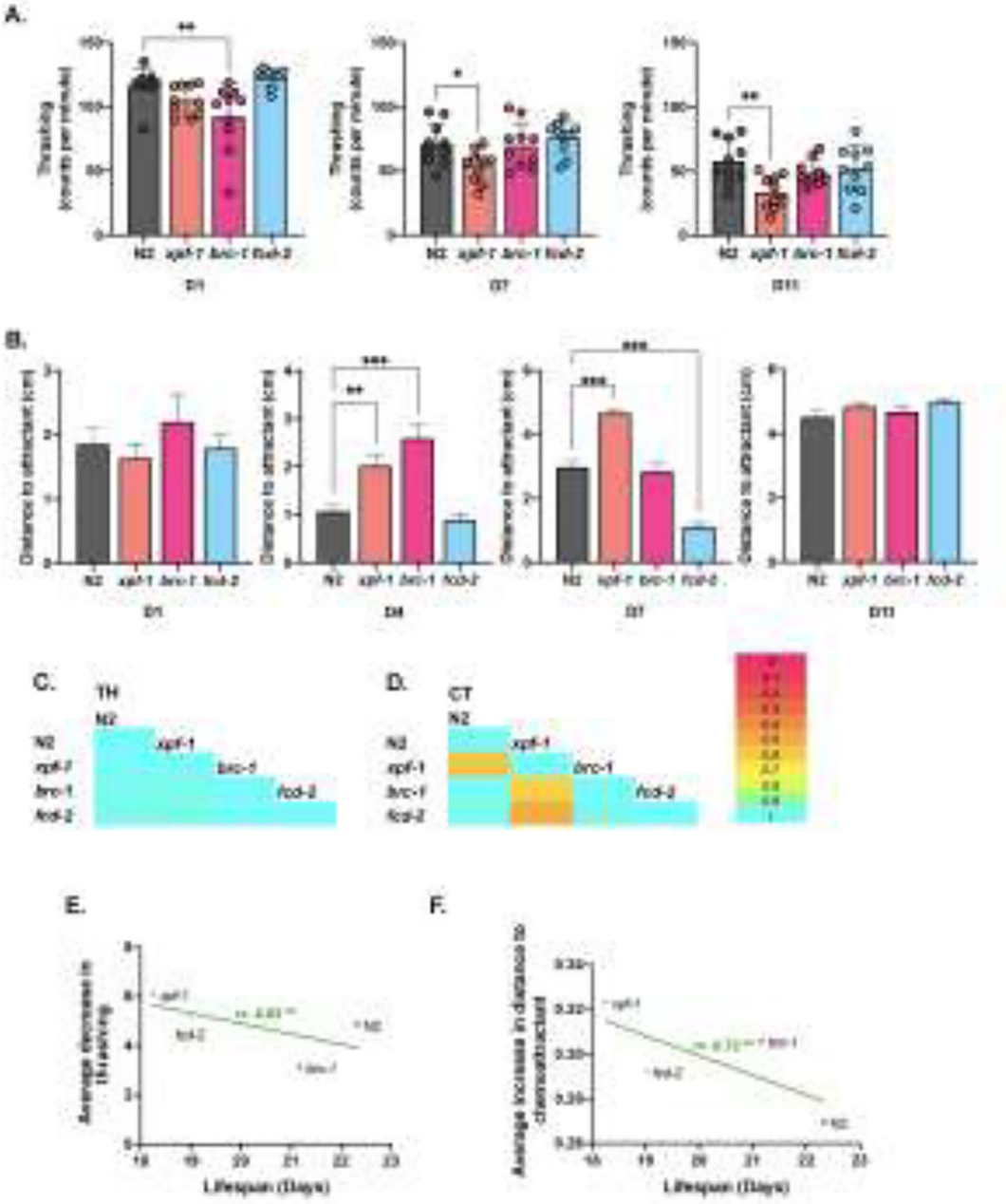

Figure 3: Functional healthspan measures:

(A) Bar graph represents average thrashing per minute for wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals on day 1 (D1), day 7 (D7) day 11 (D11) of adulthood. n = 10–15/ strain. Error bars indicate s.e.m **P*<0.05 and ***P*<0.005 by one-way ANOVA with multiple comparisons to N2. Three independent replicates for thrashing data is shown in Supp Table 5A, 6A, 7A. (**B**) Neuromuscular function assessed by chemotaxis for wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals on D1, 4, 7 and D11 of adulthood. Error bars indicate s.e.m. n = 30–50/ strain/ technical replicate (2 technical replicates). Three independent experiments performed. ***P*<0.01 and ****P*<0.001

by one-way ANOVA. **(C-D)** Heat map showing spearman's rank correlation between average thrashing (counts per minute) (**C**) and chemotaxis distance (**D**) for wildtype (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* worms. Scatter plot showing correlation between changes in average thrashing (**E**), chemotaxis distance (**F**) and lifespan of wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* worms.

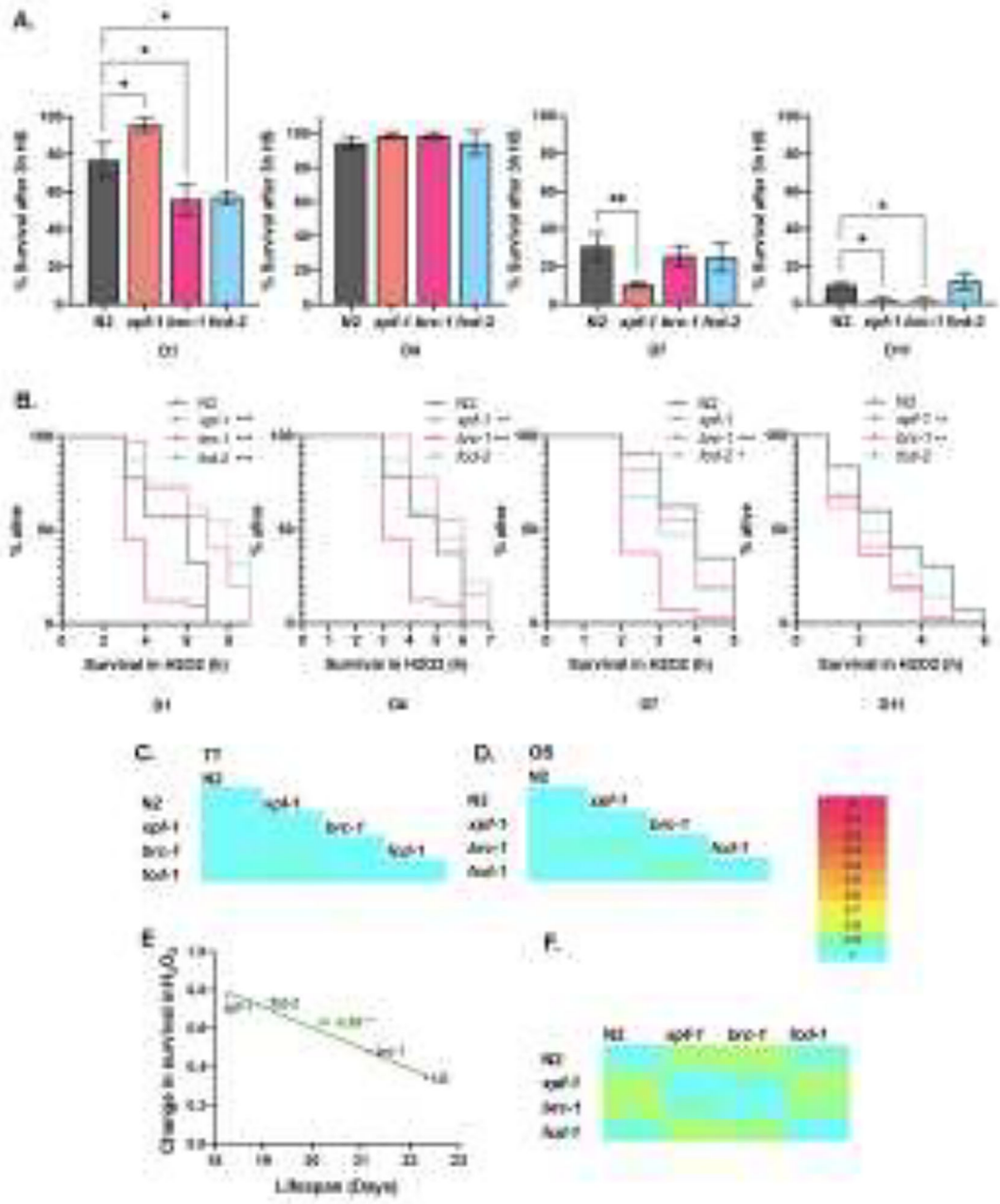

Figure 4: Resilience healthspan measures

(**A**) Survival of wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals incubated for 3h at 36°C on D1, 4, 7 and D11 of adulthood (2–3 technical replicates). Recovery for 16 h. Three independent experiments, n = 89–95/ strain. Error bars indicate s.d. **P*<0.05 and ***P*<0.01 by one-way ANOVA. All replicates for survival data is shown in Supp Table 9–12. (**B**) Survival curve of wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* animals incubated in 7.5μM hydrogen peroxide at D1, 4, 7 and D11 of adulthood (*n*= 4 wells). Three independent experiments, Each experiment: n = 35–40/ strain. **P*<0.05, ***P*<0.01 and ****P*<0.001 by Log-rank test. All replicates for survival data

is shown in Supp Table 13–16. Heat map showing spearman's rank correlation between survival after heat-shock (**C**) and treatment with hydrogen peroxide (**D**) for wildtype (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* worms. (**E**) Scatter plot showing the relationship between rate of change in resistance to oxidative stress (between day 7 and day 4) and lifespan. (**F**) Heat map representing spearman's rank correlation among wild-type (N2), *xpf-1(tm2842), brc-1*(*ok1261) and fcd-2(ok1145)* worms based on molecular, functional and resilience healthspan markers.