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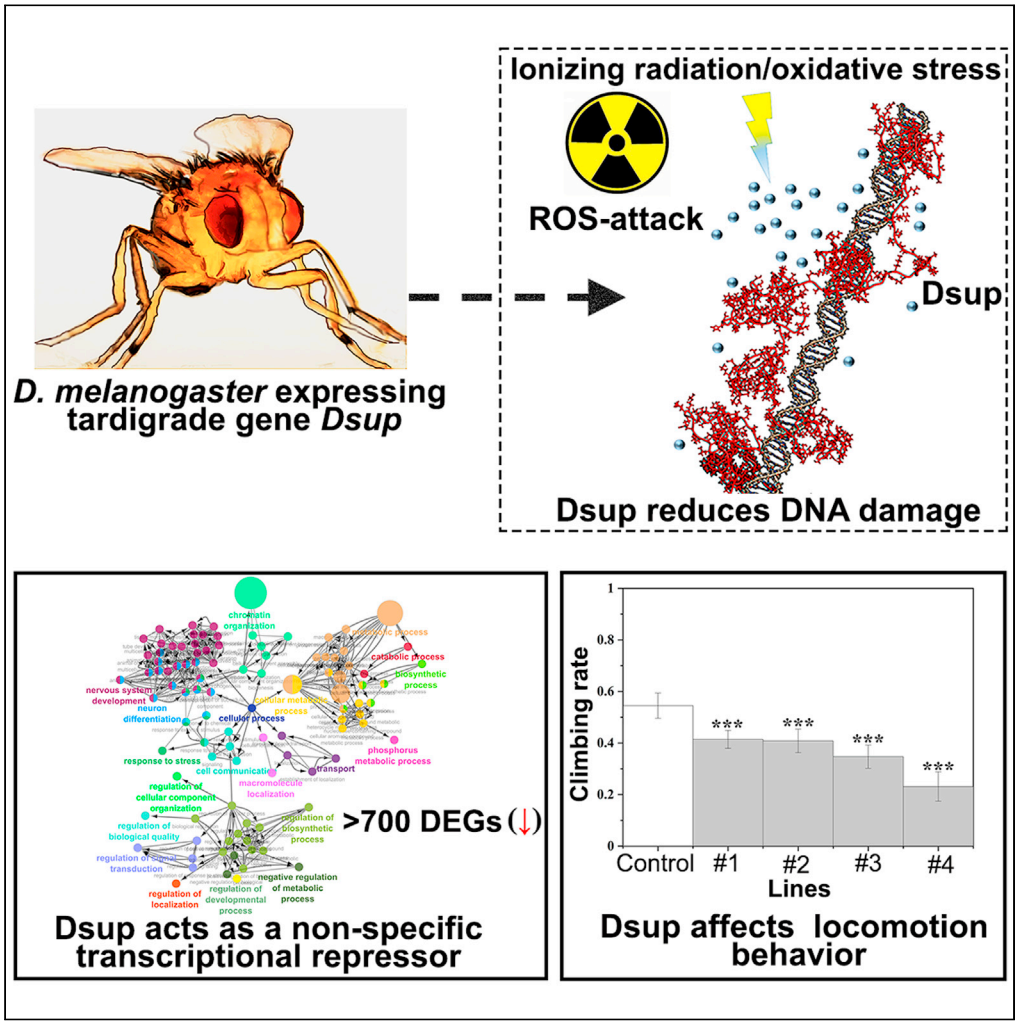

Mikhail Zarubin, Talyana Azorskaya, Olga Kuldoshina, Sergey Alekseev, Semen Mitrofanov, Elena Kravchenko

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Highlights

Dsup enhances radioresistance and tolerance to oxidative stress in *D. melanogaster*

Dsup can bind RNA

Dsup protein acts as an unspecific repressor of transcription

Dsup reduces the level of locomotor activity in *D. melanogaster*

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The tardigrade Dsup protein enhances radioresistance in *Drosophila melanogaster* and acts as an unspecific repressor of transcription

Mikhail Zarubin,[1] Talyana Azorskaya,[1] Olga Kuldoshina,[1] Sergey Alekseev,[2] Semen Mitrofanov,[2] and Elena Kravchenko[1,3,*]

SUMMARY

The tardigrade-unique damage suppressor protein (Dsup) can protect DNA from ionizing radiation and reactive oxygen species (ROS). In this study, we generated *Dsup*-expressing lines of *Drosophila melanogaster* and demonstrated that Dsup increased the survival rate after γ-ray irradiation and hydrogen peroxide treatment in flies too, but reduced the level of their locomotor activity. The transcriptome analyses of *Dsup*-expressing lines revealed a significant number of DEGs, >99% of which were down-regulated. Moreover, Dsup could bind RNA. These findings suggest that Dsup can act not only as a DNA protector but also as a non-specific transcriptional repressor and RNA-binding protein, that may lead to disturbance of a number of biological processes in *D. melanogaster*. The obtained data demonstrate features of the Dsup protein action in non-tardigrade organisms and can be used to understand the impact of other unspecific DNA/RNA-binding proteins on ROS and radiation resistance, gene expression, and epigenetic processes.

INTRODUCTION

One of the most extremotolerant animals on Earth belongs to the phylum Tardigrada. These tiny invertebrates can withstand in the dehydrated state exposure to temperatures from 90°C to −196°C,[1] hydrostatic pressure of 7.5 GPa,[2] treatment with organic solvent,[1,3] and ultraviolet radiation.[4] They can even survive after spending 10 days in outer space in the low-Earth orbit.[5] Remarkably, tardigrades also have unique radioresistance: irradiation with γ-rays at the doses of 0.5 and 1 kGy did not affect the survival rate of tardigrades both in the hydrated and dehydrated states.[6] Median lethal doses 48 h after irradiation for animals in the hydrated state were 5 kGy (gamma rays) and 6.2 kGy (heavy ions).[7] High resistance of tardigrades to multiple stresses has stimulated an extensive research over the past decade, which led to determination of the key factor able to explain such a resistance the abundance of tardigrade-specific intrinsically disordered proteins (TDPs).[8–10]

Recently, the genome of one of the most radiotolerant tardigrade species *Ramazzottius varieornatus* has been sequenced and annotated, which allowed characterizing in the chromatin fraction the damage suppressor (Dsup) protein (UniProt accession number P0DOW4) co-localized with nuclear DNA.[11] This protein is unique for tardigrades, and besides *R. varieornatus*, Dsup-like protein (UniProt accession number A0A1W0XB17) was found only in *Hypsibius exemplaris* that belongs to the same family Hypsibiidae as *R. varieornatus*.[12,13] Dsup (42.8 kDa) is a highly basic 445-residue protein enriched in serine, alanine, glycine, and lysine that decreased DNA damage after ionizing radiation treatment in experiments with Dsup-expressing HEK293 cell culture and tobacco plants.[11,14] Additionally, the Dsup protein suppressed DNA damage induced by reactive oxygen species (ROS) that arise from hydrogen peroxide in a purified biochemical system[12] and in human cell culture.[11] The most important part of Dsup is the C-terminal region that is necessary for sufficient association with DNA and contains the conserved nuclear localization signal.[11,13] Moreover, Chavez et al. found a region within the C-terminal part of Dsup protein that demonstrated sequence similarity to nucleosome-binding domain of high mobility group N (HMGN) proteins of vertebrates.[12]

The real mechanism of DNA protection with the participation of Dsup is an actively debated topic; Hashimoto et al. proposed, based on the Dsup amino acid sequence that Dsup can interact with DNA in a non-specific manner forming a physical barrier that shields DNA from radiation and ROS.[13] The same explanation, put

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forward by Chavez et al., established that Dsup *in vitro* binds to nucleosomes and protects chromatin from hydroxyl radicals in an independent from DNA sequence manner.[12] As a result of computer modeling, it was assumed that the Dsup protein belongs to intrinsically disordered proteins (IDP) with a net charge + 23.[15] *In silico* analyses of Dsup-DNA complex predicted a very tight association between the positively charged Dsup protein and negatively charged DNA due to electrostatic interactions, especially in the area of the C-terminal region.[15] According to the modeling results obtained by Mínguez-Toral et al., intrinsically disordered flexible Dsup can fit to DNA forming a molecular aggregate with the ~4 Å intermolecular distance[15] and provide physical shielding by partial isolation of DNA from ROS that arise during various stress treatments.[12,15]

The study of mechanisms of stress resistance and increasing stress tolerance of various organisms are the most challenging tasks for molecular biology and applied biotechnology. Thus, using some proteins from extremotolerant tardigrades may be useful for the development of new radioprotectors, antioxidants, and stabilizers for freeze/dried conservation, for the generation of strains with enhanced stress resistance, and even for evolving principles of damage prevention from the space-flight exposome during prolonged space missions.[9,11,13,16–20]

Dsup is a prospective candidate protein for new approaches to develop an increased innate radioprotection that does not require any targeted drug delivery in complex multicellular organisms. Some good results in increasing radioresistance with the Dsup protein were obtained for human cultured cells HEK293 and *Nicotiana tabacum*.[11,14] However, not a single experiment has been conducted so far where other effects arising from the induction of the Dsup protein in non-tardigrade organisms were evaluated. This unsolved problem may turn out to be critical since the Dsup protein interacting in a non-specific way with DNA is quite capable of influencing all processes which require an access to DNA: replication, transcription, repair, cell division, chromatin remodeling, regulation of gene expression, etc. Understanding the complete picture of the Dsup effect on the processes occurring in the cell will make it possible not only to draw practical conclusions on the use of Dsup in biotechnology but also to better understand the fundamental mechanisms of action of this unique protein.

Here we report our study of the Dsup protein contribution to the prevention of radiation and ROS damages and also to other biological processes at transcriptome and organism levels in *Dsup*-expressing lines of *Drosophila melanogaster*. The fruit fly is a widely used model for studying diseases, aging, complex behavior, neuro-developmental processes, the effect of ionizing radiation, responses to different types of stress (including spaceflight), etc. And a large amount of experimental data obtained for *D. melanogaster* makes it possible to accurately identify the changes that occur in flies during their response to various types of stress.[21–28] Thus, this study reveals the role of the Dsup protein in a complicated network of interactions between biological processes in a non-tardigrade organism and assesses the possibility of using Dsup to increase resistance to ionizing radiation and ROS in the complex multicellular model organism *D. melanogaster*.

RESULTS

D. melanogaster Dsup-expressing lines

To test the properties of Dsup protein action in *D. melanogaster*, we created lines with the genomic insertion of an optimized sequence coding the Dsup protein under control of *Act5C* gene constitutive strong promoter. During P-element-based transgenic integration, we obtained four independent lines with a single insertion in different positions in the *D. melanogaster* genome on the first (X) (line #2), the second (lines #1, #3), and the third (line #4) chromosomes. Lines #1 and #2 are nonlethal in homozygous (or hemizygous) and were used in experiments in the homozygous (or hemizygous) state whereas lines #3 and #4 are lethal in homozygous condition, therefore, all experiments for lines #3 and #4 were carried out with flies in the heterozygous (Dsup/+) state.

The quantification of *Dsup* transcript levels by RT-qPCR in male adult flies of *Dsup*-expressing lines revealed a significant level of *Dsup* expression in all lines compared to the original y^1w^{1118} line (Figure 1). The maximum level of *Dsup* expression was observed for line #2. Lines #1, #3, and #4 demonstrated slightly lower levels of expression similar to each other. The presence of Dsup protein was also confirmed by the results of Western blotting analysis (Figure S1).

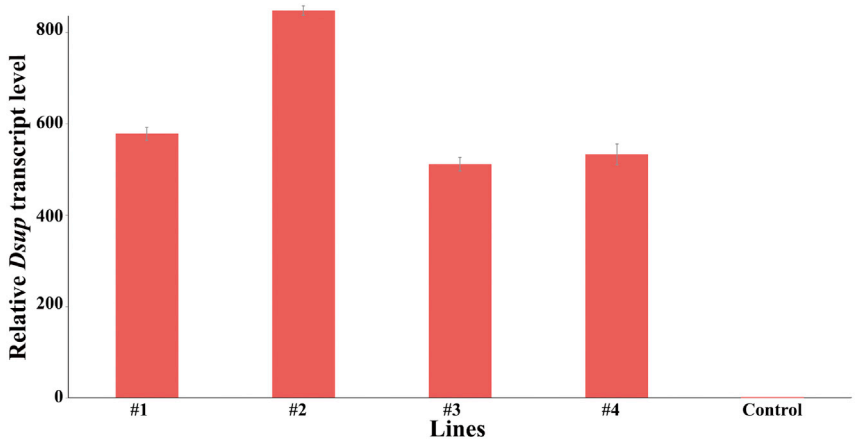

Figure 1. Quantification of Dsup transcript levels by RT-qPCR in *Dsup*-expressing lines (#1–4) and initial y^1w^{1118} line (control)

Dsup did not affect the lifespan of *Dsup*-expressing flies, but caused the increase of the medium lifespan of *Dsup*-expressing females

Dsup did not statistically have a significant effect on the lifespan of *Dsup*-expressing line #1 males and females in comparison with control flies ($p > 0.05$) (Figure 2). However, the increase in median lifespan of ~7.5% in *Dsup*-expressing females was registered ($p < 0.01$). Thus, the influence of Dsup protein on medium lifespan is modified by sex.

Level of locomotion activity was decreased in *Dsup*-expressing *D. melanogaster* lines

Negative gravitaxis climbing test is among the most reproducible and simple assays for the determination of the physiological state in fruit flies.[29,30] We performed climbing tests with different parameters: in the first type of test, we estimated the number of flies that ran 10 cm in 10 s, in the second type of test, we estimated the number of flies that overcame 12 cm in 8 s. In the first type of test, *Dsup*-expressing lines #2–4 demonstrated the significant reduction of mean passing rate compared to the control line (Figure 3A), and with the complication of the test, the significant reduction of mean passing rate for all *Dsup*-expressing

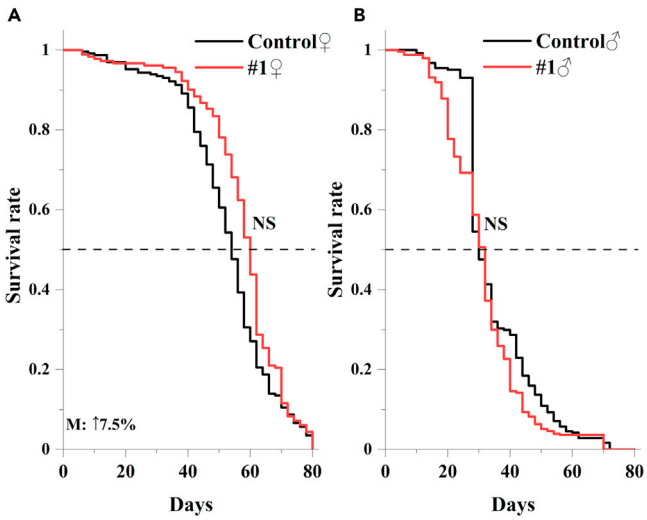

Figure 2. Dsup did not affect the lifespan of *Dsup*-expressing flies, but increased the medium lifespan of *Dsup*-expressing females

Survival curves of control and *Dsup*-expressing line #1 males (A) and females (B). M—median lifespan. NS—not significant; log rank test.

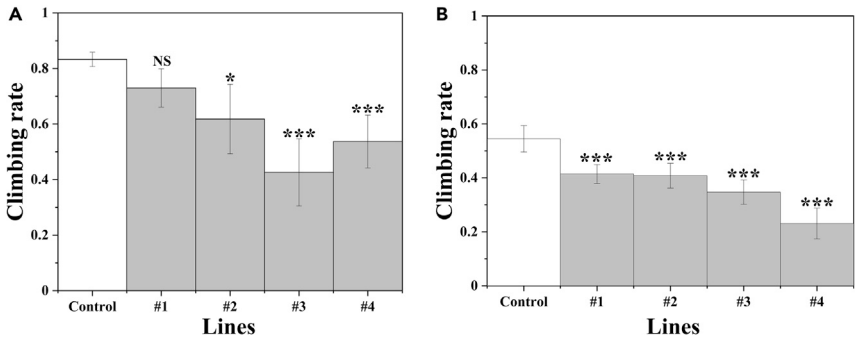

Figure 3. Analysis of climbing test results revealed decreasing of locomotor activity in *Dsup*-expressing lines

(A) - flies ran 10 cm in 10 s. (B) - flies ran 12 cm in 8 s. NS—not significant. *, $p < 0.05$; ***, $p < 0.001$, Dunnett's test. Data are represented as mean ± SEM.

lines (#1–4) was registered (Figures 3B and S2). Thus, we observed the decrease in locomotor activity in *Dsup*-expressing lines that indicated changes in locomotor behavior in the presence of Dsup protein.

Dsup increased survival rate in *D. melanogaster* after hydrogen peroxide treatment in depending on the level of *Dsup* expression

First of all, we examined the effect of the Dsup protein which, as shown in previous studies, protected DNA from ROS[11,12,14] in the experiments where ROS were generated at hydrogen peroxide treatment. Exposure to hydrogen peroxide induces DNA damage (DNA strand breaks, oxidized bases, abasic sites, etc.) by the same mechanisms as in the course of action of ROS generated by ionizing radiation treatment during water radiolysis (indirect effects of irradiation).[31] Female and male flies of control and *Dsup*-expressing lines #1–4 did not show a significant difference ($p > 0.05$, Dunnett's Test) in the food intake of the medium containing 9% hydrogen peroxide (Figure S3). At hydrogen peroxide treatment, we observed the difference in survival parameters between all *Dsup*-expressing and control *D. melanogaster* lines: *Dsup*-expressing lines demonstrated a higher survival rate and an increase of the median lifespan by 43–90% in males and by 23–49% in females (Figure 4, Table 1). This indicates that *Dsup*-expressing *D. melanogaster* lines exhibited much less damage caused by hydrogen peroxide than the control line, and Dsup can protect fruit flies from ROS.

To examine the effect of the *Dsup* expression level, we performed the same treatment with hydrogen peroxide on heterozygous and homozygous flies of the *Dsup*-expressing line #1 and the control line (Figure 5). Surprisingly, heterozygous Dsup/+ flies demonstrated a survival rate very close to that of the control line, in other words, the lower amount of Dsup protein in heterozygotes does not provide significant protection against oxidative stress.

Dsup decreased hydrogen peroxide induced DNA fragmentation in *D. melanogaster*

After hydrogen peroxide treatment, during alkaline comet assay, we observed a decrease in comet head size and an increase in DNA tail size in nuclei of hemocytes of control drosophila larvae and more intact nuclei (~18% less damage) in hemocytes of *Dsup*-expressing larvae of line #1 (Figure 6). These data suggest

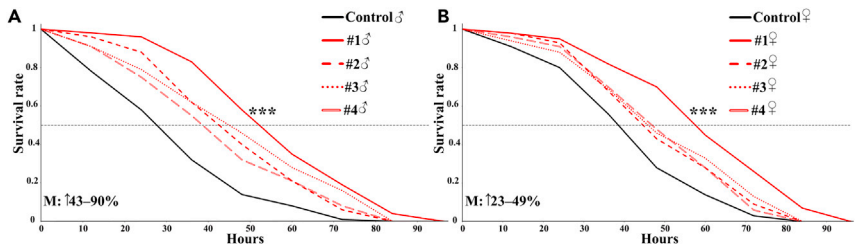

Figure 4. Dsup increased survival rate in *D. melanogaster* during hydrogen peroxide treatment

Survival curves of control *Dsup*-expressing (#1–4) males (A) and females (B) during hydrogen peroxide treatment. M—median lifespan. ***, $p < 0.001$, log rank test.

Table 1. Impact of Dsup on lifespan of *D. melanogaster* exposed to different stress factors

	Line	Median Lifespan	Δ Median Lifespan, %	Age of 90%-Mortality	Δ Age of 90%-Mortality, %
Chronic hydrogen peroxide treatment		Hours			
	Control♂	27.34		54.56	
	#1♂	52.05 ***	90	78.28 **	43
	#2♂	41.29 ***	51	68 *	25
	#3♂	44.5 ***	63	73.81 **	35
	#4♂	39.02 ***	43	70.75 *	30
	Control	38.55		63.15	
	#1♀	57.5 ***	49	80 *	27
	#2♀	43.33 **	12	70.79	12
	#3♀	46.14 ***	20	73.33	16
	#4♀	47 ***	22	70.5	12
1000 Gy γ-ray irradiation		Days			
	Control♂	1.6		9.57	
	#1♂	5.5	343	8.95	−7
	Control♀	9		13.4	
	#1♀	10.36	16	14.02	5
500 Gy γ-ray irradiation					
	Control♂	12.43		15	
	#1♂	17.45 ***	40	19.89 *	33
	Control♀	26.2		31.33	
	#1♀	32.7 ***	25	36.67 **	17

For median lifespan and differences in the age of 90% mortality Fisher exact test's results: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

that Dsup protects DNA from ROS, resulting in a reduction in hydrogen peroxide-induced DNA fragmentation in *D. melanogaster*.

Dsup increased survival rate in *D. melanogaster* after γ-ray treatment

For experiments with γ-ray treatment, we choose *Dsup*-expressing line #1 because it demonstrated the best protection from hydrogen peroxide. In order to make sure that the insertion of the *Dsup* gene did not disrupt the functioning of any other gene in the drosophila genome, we identified the place of insertion: it was the noncoding 5′ flanking region of the *peste* gene, and the transgene insertion did not change the *peste* gene expression level (Figure S4). After irradiation at a dose of 500 Gy, the *Dsup*-expressing line demonstrated better characteristics of many survival parameters in relation to the control line both for males and females: survival function ($p < 0.001$), median lifespan ($p < 0.001$), maximum lifespan ($p < 0.01$), and age of 90% mortality ($p < 0.05$) (Figure 7, Table 1). After irradiation at a dose of 1000 Gy, which is close to LD_{50} for *D. melanogaster*,[32] *Dsup*-expressing line demonstrated a better survival rate for females ($p < 0.001$) and an increased median lifespan both for female and male fruit flies ($p < 0.01$) (Figure 7 and Table 1). In addition, no difference in survival parameters between the control line and the *Dsup*-expressing line was observed after irradiation at a dose of 1500 Gy (Figure S5). Considering the obtained results, we can conclude that the Dsup protein is able to reliably enhance the radiotolerance of the complex model animal *D. melanogaster*, however, with increasing dose, the radioprotective effect of Dsup decreased.

Transcriptome analysis of *Dsup*-expressing and control lines revealed DEGs involved in nucleosome assembly, chromatin assembly or disassembly, transcription, neural system organization and functioning

To find out whether the appearance of the Dsup protein in drosophila cells affects any biological processes, we carried out the transcriptome analysis of males representing *Dsup*-expressing and control lines. To

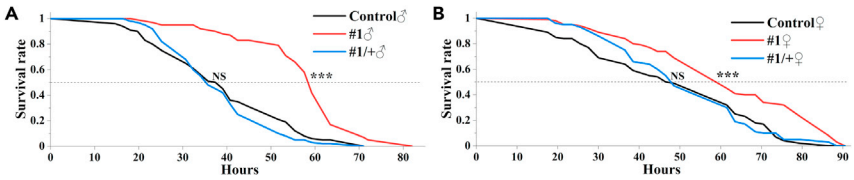

Figure 5. Dsup increased survival rate of homozygous (#1/#1) but not heterozygous (#1/+) males (A) and females (B) of *Dsup*-expressing line #1 during hydrogen peroxide treatment

***, $p < 0.001$; NS—not significant; log rank test

exclude the potential transgenic insertion effect on the activity of other genes not related to Dsup protein action, all *Dsup*-expressing lines with different genomic locations were analyzed in one group as replicates (#1–#4) and compared with the control line.

During the transcriptome analysis, it was revealed that the expression of 735 genes was significantly different (more than 2-fold, $p < 0.01$) between *Dsup*-expressing and control lines (Table S1). Remarkably, 99.73% of DEGs in *Dsup*-expressing lines were down-regulated. It is interesting that the fold change for all down-regulated DEGs in *Dsup*-expressing lines did not exceed four. To identify enriched biological process categories, the direct DAVID (the Database for Annotation, Visualization and Integrated Discovery) GO (Gene Ontology) analysis based on the list of obtained DEGs was performed, and enriched biological processes categories were identified (Table 2). The DAVID analysis for functional domains of proteins coded by DEGs indicated a significant enrichment associated with histone-fold, histone H3 and H4, TATA box binding protein associated factor (TAF), zinc finger, BTB/POZ (broad complex Tramtrack bric-a-brac/Pox virus and zinc finger) fold, BTB/POZ-like, PDZ domain, kinesin, immunoglobulin, and several other protein domains (Table S1). The Reactome pathway analysis suggested that the DEGs were significantly involved ($p < 0.05$) in PRC2 methylation of histones and DNA (R-DME-212300), HATs acetylation of histones (R-DME-3214847), PKMTs histone lysines methylation (R-DME-3214841), RMTs histone arginines methylation (R-DME-3214858), recruitment and ATM-mediated phosphorylation of repair and signaling proteins at DNA double-strand breaks (R-DME-5693565), chromatin modifying enzymes (R-DME-3247509), factors involved in megakaryocyte development and platelet production (R-DME-983231), HuR (ELAVL1) binding and stabilizing of mRNA (R-DME-450520).

To validate the microarray results, we used RT-qPCR for eight differentially expressed genes involved in different important processes, such as *mod(mdg4)* (regulation of transcription, chromatin condensation), *gogo* (axon-axon and axon-target interactions), mthl2 (G protein-coupled receptor signaling pathway), etc.

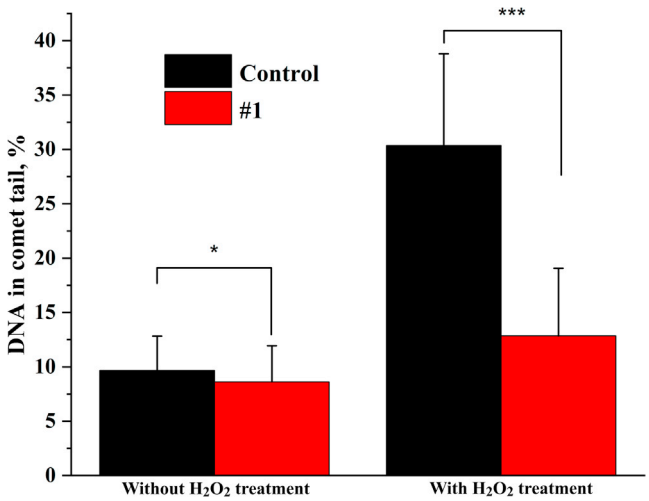

Figure 6. Dsup decreased DNA damage in *Dsup*-expressing flies

Alkaline comet assays results for hemocytes of *Dsup*-expressing line #1 and control line. Estimated % of DNA in the comet's tail (TriTek CometScore software v2.0.0.38). *, $p < 0.05$; ***, $p < 0.001$; t-test. Data are represented as mean ± SEM.

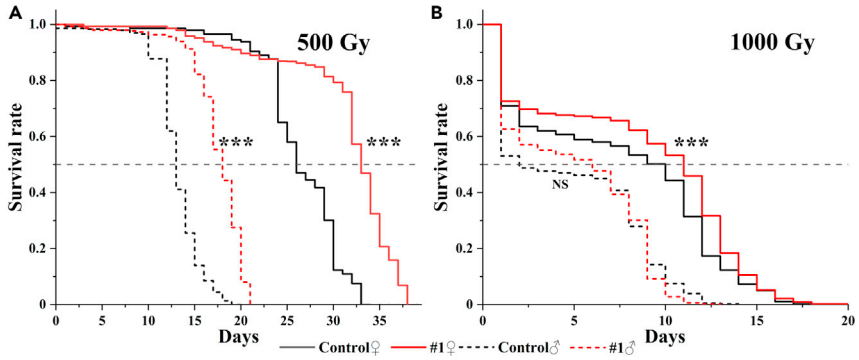

Figure 7. Influence of γ-rays treatment on the survival of *D. mealanogaster Dsup*-expressing line #1 and control line

Dsup increased survival rate in *D. mealanogaster Dsup*-expressing line #1 after γ-rays treatment at doses of 500 Gy (A) and 1000 Gy (B). The data of replicates were combined. M—median lifespan. ***, $p < 0.001$; NS—not significant; log rank test.

The results obtained by microarrays agreed with the results obtained by RT-qPCR, which demonstrates the reproducibility of the difference in gene expression between the control and *Dsup*-expressing lines (Figure S5).

Transcriptome analysis of *Dsup*-expressing and control lines after ionizing radiation treatment revealed DEGs involved in DNA replication, DNA reparation, DNA recombination and neurogenesis

To study the effect of the Dsup protein during irradiation, we carried out the transcriptome analysis of males from *Dsup*-expressing and control lines after γ-ray treatment at a dose of 1000 Gy. A total of 435 significantly differentially expressed genes (more than 2-fold, $p < 0.01$) were obtained with 0.69% of up-regulated and 99.31% of down-regulated genes (Table 3). Based on the GO enrichment in the category BP, DEGs were enriched in 32 BP GO terms associated with DNA replication, recombination and repair, protein folding and refolding, neurogenesis, immune response, and several metabolic processes (Table 3).

For the validation of the microarray results, we used RT-qPCR for five genes that can be considered as radiation-induced stress markers—*Ku80, Irbp, Hsp26, RfC4*, and *Pa1*. The microarray results are consistent with the RT-qPCR results (Figure S5).

Dsup binds to RNA

Previously, it has been shown that Dsup has a certain affinity to free DNA and nucleosomes in *in vitro* experiments.[11,12] We examined in the same way whether the Dsup protein could bind to RNA. The bacterially expressed Dsup protein was purified and incubated with total *D. melanogaster* RNA at various wt:wt ratios, and after gel mobility shift analysis, we observed a significantly slowed-down RNA migration depending on the Dsup dose (Figure 8), which indicated the binding of Dsup to RNA.

DISCUSSION

In this study, we have found that the tardigrade Dsup protein can protect *D. melanogaster* from γ-ray irradiation and ROS-related stress. In *Dsup*-expressing lines, we detected an increase in the survival rate, median lifespan, maximum lifespan, and age of 90% mortality after irradiation with γ-rays and hydrogen peroxide treatment. Therefore, our data confirm the results obtained for Dsup in previous *in vivo* experiments with human cultured cells HEK293 and tobacco plants.[11,14,33] Apparently, the efficiency of Dsup-mediated protection depends on the *Dsup* expression level, which must exceed a certain point when the amount of produced Dsup protein is sufficient to provide an effective physical shielding of DNA.

Using the fruit fly as a complex model organism made it possible to study not only the viability but also a number of important physiological parameters. The locomotor activity was decreased in *Dsup*-expressing *D. melanogaster* lines that indicated the certain level of stress at the organism level. In addition, we

Table 2. Top 25 enriched BP GO terms

BP GO Identifier	BP GO term	Number of genes	P-value
GO:0006334	nucleosome assembly	42	<0.001
GO:0016321	female meiosis chromosome segregation	24	<0.001
GO:0006352	DNA-templated transcription, initiation	19	<0.001
GO:0006333	chromatin assembly or disassembly	19	<0.001
GO:0051298	centrosome duplication	21	<0.001
GO:0050808	synapse organization	13	<0.001
GO:0002121	inter-male aggressive behavior	10	0.001
GO:0048065	male courtship behavior, veined wing extension	3	0.006
GO:0006355	regulation of transcription, DNA-templated	31	0.007
GO:0042067	establishment of ommatidial planar polarity	6	0.011
GO:0007411	axon guidance	17	0.012
GO:0007601	visual perception	8	0.014
GO:0007218	neuropeptide signaling pathway	8	0.017
GO:0035023	regulation of Rho protein signal transduction	4	0.020
GO:0035317	imaginal disc-derived wing hair organization	5	0.023
GO:0019722	calcium-mediated signaling	4	0.025
GO:0007367	segment polarity determination	6	0.027
GO:0006351	transcription, DNA-templated	27	0.027
GO:0007042	lysosomal lumen acidification	3	0.028
GO:0007186	G-protein coupled receptor signaling pathway	13	0.034
GO:0001738	morphogenesis of a polarized epithelium	4	0.035
GO:0007464	R3/R4 cell fate commitment	4	0.035
GO:0007157	heterophilic cell-cell adhesion	5	0.043
GO:0016318	ommatidial rotation	5	0.043
GO:0007476	imaginal disc-derived wing morphogenesis	17	0.045

observed an increase in the median lifespan of *Dsup*-expressing females, possibly due to the influence of Dsup protein on the expression of some sex-limited genes.

At the transcriptome level, we observed that *Dsup*-expressing and control *D. melanogaster* lines exhibited different transcription patterns of 735 genes—2 of them (0.27%) were up-regulated and 733 of them (99.73%) were down-regulated (FC more than 2, $p < 0.01$). Interestingly, when we decreased the threshold of the fold change to 1.5 ($p < 0.01$), the number of DEGs increased to 8434 (8430 [99.95%] down-regulated and 4 [0.05%] up-regulated), which is 44.48% of the total number of transcripts analyzed with GeneChip™ Drosophila Genome 2.0 Arrays. Such a significant number of DEGs indicates the pronounced effect of the Dsup protein, and since the vast majority of the DEGs with different functions were down-regulated, we can suggest that Dsup induces a slowdown in a number of cellular processes.

Down-regulated genes (more than 2-fold, $p < 0.01$) were enriched in biological processes categories (Table 2) that can be roughly combined into five main groups: DNA-templated transcription-related activities, neural system functioning, nucleosome-chromatin-chromosome assembling/disassembling-related processes, cellular metabolic processes, and the last one responds to the drosophila development and morphogenesis (Figure 9).

The expression spectrum of *Dsup*-expressing lines indicated the decreased activity of genes associated with spatial DNA organization at all levels (nucleosome-chromatin-chromosome) including *His3* and *His4* genes (coding core histones H3 and H4), *Art8* (coding histone methyltransferase), *mod(mdg4)*

Table 3. Top 20 enriched BP GO terms in DEGs after 1000 Gy γ-rays treatment

BP GO Identifier	BP GO term	Number of genes	P-Value
GO:0006260	DNA replication	12	<0.001
GO:0006749	glutathione metabolic process	11	<0.001
GO:0006310	DNA recombination	8	<0.001
GO:0022008	neurogenesis	33	<0.001
GO:0042026	protein refolding	5	<0.001
GO:0061077	chaperone-mediated protein folding	5	0.001
GO:0006388	tRNA splicing, via endonucleolytic cleavage and ligation	4	0.001
GO:0009408	response to heat	8	0.003
GO:0000723	telomere maintenance	5	0.003
GO:0071897	DNA biosynthetic process	3	0.004
GO:0032508	DNA duplex unwinding	4	0.010
GO:0006281	DNA repair	8	0.011
GO:0006303	double-strand break repair via non-homologous end-joining	3	0.014
GO:0009631	cold acclimation	3	0.019
GO:0006013	mannose metabolic process	3	0.024
GO:0044719	regulation of imaginal disc-derived wing size	4	0.027
GO:0045824	negative regulation of innate immune response	4	0.040
GO:0006400	tRNA modification	3	0.041
GO:0009263	deoxyribonucleotide biosynthetic process	2	0.054
GO:0008065	establishment of blood-nerve barrier	2	0.054

(participating in the regulation of chromatin organization), *RecQ5* (coding helicase involved in many nuclear DNA metabolic processes), *neb, Mis12, Nnf1a* (involved in chromosome segregation), and others (Table S1). These data can be explained by Dsup protein competitions for binding to DNA or nucleosomes with host proteins involved in chromatin organization and remodeling which leads to their excess and a decrease in the expression of the corresponding genes.

Highly likely for the same reason, there was a decrease in the expression level of many genes involved in DNA-templated transcription and its regulation, for instance, *bigmax* (helix-loop-helix-leucine zipper transcription factor), *cas* (transcription factor), *bap* (homeodomain transcription factor), *can* (TBP-associated factor), *ash1* (histone methyltransferase involved in transcription regulation), *mirr* (iroquois homeobox transcription factor), *TfAP-2* (transcription factor that is critical for leg, proboscis, and central nervous system development), *pdm3* (POU domain transcription factor), *Myb* (transcription factor), and others (Table S1). Interestingly, Ricci et al. observed a similar effect of Dsup in human cell culture—in cells expressing *Dsup*, a number of transcription factors were down-regulated compared to untransfected HEK293.[33] In addition to the direct competition of proteins involved in DNA-templated transcription with the Dsup protein for binding sites on DNA, a decrease in the activity of genes participating in DNA-templated transcription may be associated with reduced chromatin dynamics caused by Dsup-nucleosome contacts at the level of chromatin remodeling that decreases the accessibility of the DNA to transcription factors, RNA polymerase II, etc. We suppose that the Dsup protein can be considered from this point of view as a non-specific transcriptional repressor.

It is important to note that between DEGs involved in DNA-templated transcription and its regulation, there is a significant number of genes coding transcription factors expressed in the nervous system (*FoxP, pdm3, TfAP-2, mirr, lola, scro, sbb, Hey, CG32105,* and *cas*) (Table S1). Highly likely, the decrease in expression of these genes leads to the decline in the activity of a large group of DEGs belonging to the enriched BP categories, such as synapse organization, inter-male aggressive behavior, male courtship behavior, axon

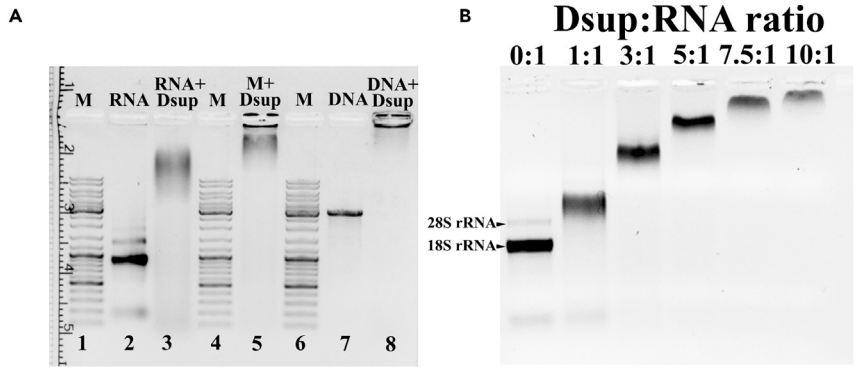

Figure 8. Dsup shifts mobility of RNA and DNA

(A) M—GeneRuler DNA Ladder Mix (Thermo Fisher); 1 - total RNA *D. melanogaster* (1 μg); 2 - total RNA (1 μg) *D. melanogaster* + Dsup (2 μg); 3 - GeneRuler DNA Ladder Mix (Thermo Fisher) (1 μg); 4 - GeneRuler DNA Ladder Mix (Thermo Fisher) (1 μg) + Dsup (2 μg); 5 - GeneRuler DNA Ladder Mix (Thermo Fisher), 6 - linearized pGEM7Zf (0.25 μg), linearized pGEM7Zf (0.25 μg) + Dsup (0.75 μg).

(B) Total *D. melanogaster* RNA incubated with Dsup in different wt:wt ratio: 1 μg of total RNA and 0, 1, 3, 5, 7.5, 10 μg of Dsup.

guidance, and visual perception. Moreover, various neurodegenerative disorders were observed in organisms with overexpressed genes coding several other DNA-binding intrinsically disorder proteins, such as HMGN, that has sequence similarity with Dsup,[12] FUS, and TDP43.[34–38] Apparently, Dsup-induced misbalance in neural and neuromuscular communications caused deterioration of the climbing test results in *Dsup*-expressing *D. melanogaster* lines.

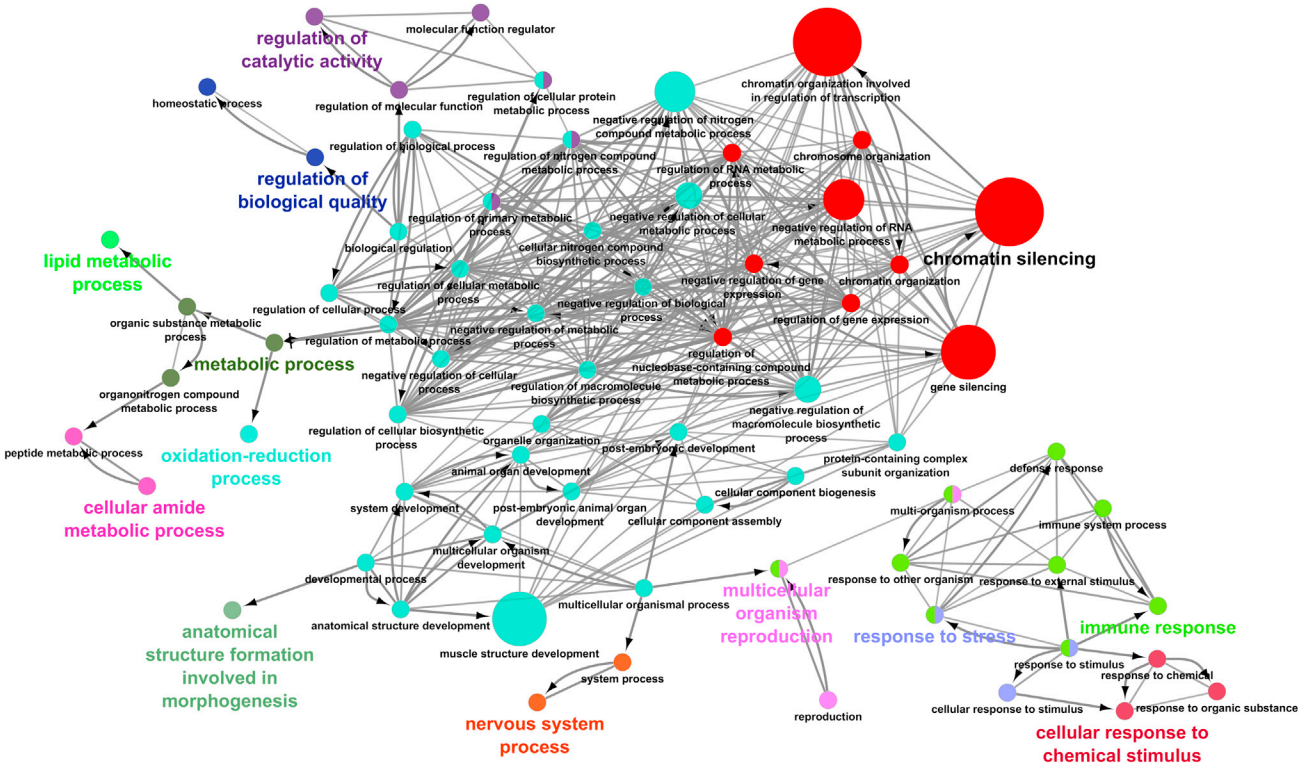

Figure 9. Clustering of biological processes enriched in DEGs in *Dsup*-expressing *D. melanogaster* lines

performed with Cytoscape 3.7.1. ClueGO 2.5.6 plot.

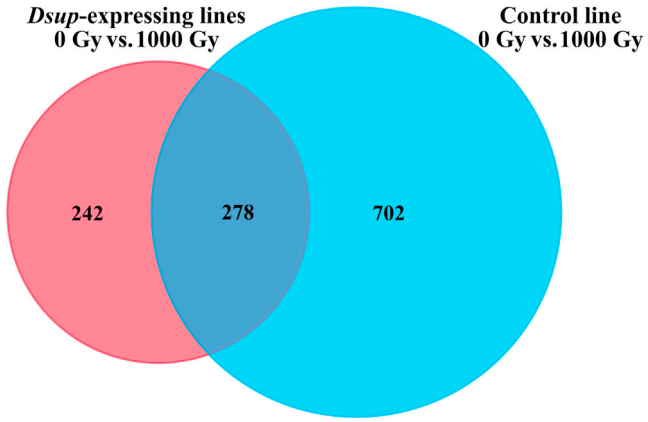

Figure 10. The comparison of the amount of DEGs involved in the response to irradiation (γ, 1000 Gy) between males of *Dsup*-expressing lines and control line revealed a reduced response to irradiation in *Dsup*-expressing lines

In addition, we detected in our gel shift assay that Dsup could bind to RNA (Figures 8 and S6). Thus Dsup as an RNA-binding protein (RBP) should compete with cell RBPs which, in turn, may influence the transport, maturation, and stability of RNA in the cell and consequently affect the gene expression.[39]

It can be assumed that the concentration of the DNA/RNA-binding Dsup protein can reach only a certain level in cells, and any excess of this level leads to significant hindrances for DNA/RNA-related processes and subsequent disturbances in cellular metabolism. Indeed, it was previously shown that high concentrations of bacterial DNA-binding proteins induced DNA aggregation and cell death,[40,41] and the C-terminal region of Dsup may also cause DNA condensation in human cell culture.[11] It is interesting that two of four generated *Dsup*-expressing lines exist only in the heterozygous state. Therefore, a high lethal rate between *Dsup*-expressing homozygous lines can be explained not only by a possible transgenic insertion at important points in the genome but also by the critical expression level of Dsup in certain homozygotes.

Transcriptome profiling of *Dsup*-expressing and control lines irradiated with γ-rays at a dose of 1000 Gy revealed a decrease in the expression level for 432 genes (99.3% of total DEGs) in *Dsup*-expressing lines that were enriched in BP categories related to DNA repair, neurogenesis, and proteostasis (Tables 3 and S1). Comparison of DEGs between non-irradiated group and irradiated group in the presence or absence of Dsup revealed a reduced response to irradiation in *Dsup*-expressing lines (980 DEGs in control lines versus 520 DEGs in *Dsup*-expressing lines) (Figure 10). These data leave open the question of what caused the decrease in expression of genes involved in radiation-induced stress in *Dsup*-expressing lines - reduction of DNA radiation damage due to Dsup shielding,[11,12] or unspecific repression of transcription by Dsup. Highly likely, there was a combination of both of these processes.

It is interesting how tardigrades *R. varieornatus* and *H. exemplaris* containing Dsup and Dsup-like proteins[11,12] overcome the possible side effects associated with the presence of these proteins. Moreover, in *R. varieornatus*, the highest level of *Dsup* expression was observed at the early embryonic stage, when DNA is rapidly replicating.[11] *R. varieornatus* and *H. exemplaris*, potentially, might develop some compensatory mechanisms, such as more effective systems of chromatin remodeling, and ensure the concentration of Dsup and Dsup-like proteins in the cell at the minimum required level. In addition, taking into account that *Dsup* is possibly expressed in a replication-dependent manner and binds to DNA and nucleosomes, we can also assume that Dsup protein may be a common and even necessary participant of chromosomal architecture formation processes in tardigrades. For instance, it can perform functions similar to histone H1 or HMGN proteins.

In conclusion, in this study we revealed that *Dsup*-expressing *D. melanogaster* demonstrated improved radiotolerance and resistance to oxidative stress. However, the presence of the DNA/RNA-binding Dsup protein in the cell also caused adverse effects, which are based on the ability of the Dsup protein to create hindrances for the normal cell processes associated with the organization of DNA, chromatin, and

chromosomes and to act as an unspecific transcriptional repressor. This, apparently, may limit the use of Dsup protein in non-tardigrade organisms in some cases, but certainly provides a new direction for the development of effective reagents for DNA/RNA protection and methods for regulation of gene expression and epigenetic processes in animals.

Limitations of the study

In this study, we investigated the effect of overexpression of the tardigrade Dsup protein in the complex model organism *D. melanogaster*. We demonstrated increasing survival in *Dsup*-expressing flies after γ-ray irradiation and hydrogen peroxide treatment, decreasing expression of a number of genes, and reduction of the level of locomotor activity. The main limitation of this study is the expression of *Dsup* under the control of a strong constitutive *Act5C* gene promoter, which probably does not reflect the pattern of expression of this gene in tardigrades. Thus, the observed Dsup effects in flies may be related to some extent to significant amounts of this protein above some normal physiological level in cells, and more research is needed to understand the general picture of Dsup action in *D. melanogaster*.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106998.

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AUTHOR CONTRIBUTIONS

M.Z.: Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. T. A.: Investigation. O.K.: Investigation. S.A.: Resources. S.M.: Resources. E.K.: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Horikawa, D.D., Kunieda, T., Abe, W., Watanabe, M., Nakahara, Y., Yukuhiro, F., Sakashita, T., Hamada, N., Wada, S., Funayama, T., et al. (2008). Establishment of a rearing system of the extremotolerant tardigrade Ramazzottius varieornatus: a new model animal for astrobiology. Astrobiology *8*, 549–556. https://doi.org/10.1089/ast.2007.0139.

2. Ono, F., Saigusa, M., Uozumi, T., Matsushima, Y., Ikeda, H., Saini, N.L., and Yamashita, M. (2008). Effect of high hydrostatic pressure on to life of the tiny animal tardigrade. J. Phys. Chem. Solids *69*, 2297–2300. https://doi.org/10.1016/j.jpcs.2008.04.019.

3. Ramløv, H., and Westh, P. (2001). Cryptobiosis in the Eutardigrade Adorybiotus (Richtersius) coronifer: tolerance to alcohols, temperature and de novo protein synthesis. Zool. Anz. *240*, 517–523. https://doi.org/10.1078/0044-5231-00062.

4. Altiero, T., Guidetti, R., Caselli, V., Cesari, M., and Rebecchi, L. (2011). Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. J. Zool. Syst. Evol. Res. *49*, 104–110. https://doi.org/10.1111/j.1439-0469.2010.00607.x.

5. Jönsson, K.I., Rabbow, E., Schill, R.O., Harms-Ringdahl, M., and Rettberg, P. (2008). Tardigrades survive exposure to space in low Earth orbit. Curr. Biol. *18*, R729–R731. https://doi.org/10.1016/j.cub.2008.06.048.

6. Jönsson, K.I., Harms-Ringdahl, M., and Torudd, J. (2005). Radiation tolerance in the eutardigrade Richtersius coronifer. Int. J. Radiat. Biol. *81*, 649–656. https://doi.org/10.1080/09553000500368453.

7. Horikawa, D.D., Sakashita, T., Katagiri, C., Watanabe, M., Kikawada, T., Nakahara, Y., Hamada, N., Wada, S., Funayama, T., Higashi, S., et al. (2006). Radiation tolerance in the tardigrade Milnesium tardigradum. Int. J. Radiat. Biol. *82*, 843–848. https://doi.org/10.1080/09553000600972956.

8. Kamilari, M., Jørgensen, A., Schiøtt, M., and Møbjerg, N. (2019). Comparative transcriptomics suggest unique molecular adaptations within tardigrade lineages. BMC Genom. *20*, 607. https://doi.org/10.1186/s12864-019-5912-x.

9. Hesgrove, C., and Boothby, T.C. (2020). The biology of tardigrade disordered proteins in extreme stress tolerance. Cell Commun. Signal. *18*, 178. https://doi.org/10.1186/s12964-020-00670-2.

10. Chakrabortee, S., Tripathi, R., Watson, M., Schierle, G.S.K., Kurniawan, D.P., Kaminski, C.F., Wise, M.J., and Tunnacliffe, A. (2012). Intrinsically disordered proteins as molecular shields. Mol. Biosyst. *8*, 210–219. https://doi.org/10.1039/c1mb05263b.

11. Hashimoto, T., Horikawa, D.D., Saito, Y., Kuwahara, H., Kozuka-Hata, H., Shin-I, T., Minakuchi, Y., Ohishi, K., Motoyama, A., Aizu, T., et al. (2016). Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat. Commun. *7*, 12808. https://doi.org/10.1038/ncomms12808.

12. Chavez, C., Cruz-Becerra, G., Fei, J., Kassavetis, G.A., and Kadonaga, J.T. (2019). The tardigrade damage suppressor protein binds to nucleosomes and protects dna from hydroxyl radicals. Elife *8*, e47682. https://doi.org/10.7554/eLife.47682.

13. Hashimoto, T., and Kunieda, T. (2017). DNA protection protein, a novel mechanism of radiation tolerance: lessons from tardigrades. Life *7*, 26. https://doi.org/10.3390/life7020026.

14. Kirke, J., Jin, X.L., and Zhang, X.H. (2020). Expression of a tardigrade Dsup gene enhances genome protection in plants. Mol. Biotechnol. *62*, 563–571. https://doi.org/10.1007/s12033-020-00273-9.

15. Mínguez-Toral, M., Cuevas-Zuviría, B., Garrido-Arandia, M., and Pacios, L.F. (2020). A computational structural study on the DNA-protecting role of the tardigrade-unique Dsup protein. Sci. Rep. *10*, 1–18. https://doi.org/10.1038/s41598-020-70431-1.

16. Afshinnekoo, E., Scott, R.T., MacKay, M.J., Pariset, E., Cekanaviciute, E., Barker, R., Gilroy, S., Hassane, D., Smith, S.M., Zwart, S.R., et al. (2020). Fundamental biological features of spaceflight: advancing the field to enable deep-space exploration. Cell *183*, 1162–1184. https://doi.org/10.1016/j.cell.2020.10.050.

17. Boothby, T.C., Tapia, H., Brozena, A.H., Piszkiewicz, S., Smith, A.E., Giovannini, I., Rebecchi, L., Pielak, G.J., Koshland, D., and Goldstein, B. (2017). Tardigrades use intrinsically disordered proteins to survive desiccation. Mol. Cell *65*, 975–984.e5. https://doi.org/10.1016/j.molcel.2017.02.018.

18. Furukawa, S., Nagamatsu, A., Nenoi, M., Fujimori, A., Kakinuma, S., Katsube, T., Wang, B., Tsuruoka, C., Shirai, T., Nakamura, A.J., et al. (2020). Space radiation biology for "living in space". BioMed Res. Int. *2020*, 4703286. https://doi.org/10.1155/2020/4703286.

19. Jönsson, K.I. (2019). Radiation tolerance in tardigrades: current knowledge and potential applications in medicine. Cancers *11*, 1333. https://doi.org/10.3390/cancers11091333.

20. Patel, Z.S., Brunstetter, T.J., Tarver, W.J., Whitmire, A.M., Zwart, S.R., Smith, S.M., and Huff, J.L. (2020). Red risks for a journey to the red planet: the highest priority human health risks for a mission to Mars. npj Microgravity *6*, 33. https://doi.org/10.1038/S41526-020-00124-6.

21. Chow, C.Y., and Reiter, L.T. (2017). Etiology of human genetic disease on the fly. Trends Genet. *33*, 391–398. https://doi.org/10.1016/j.tig.2017.03.007.

22. Gilbert, R., Torres, M., Clemens, R., Hateley, S., Hosamani, R., Wade, W., and Bhattacharya, S. (2020). Spaceflight and simulated microgravity conditions increase virulence of Serratia marcescens in the Drosophila melanogaster infection model. npj Microgravity *6*, 4–9. https://doi.org/10.1038/s41526-019-0091-2.

23. Marcu, O., Lera, M.P., Sanchez, M.E., Levic, E., Higgins, L.A., Shmygelska, A., Fahlen, T.F., Nichol, H., and Bhattacharya, S. (2011). Innate immune responses of Drosophila melanogaster are altered by spaceflight. PLoS One *6*, e15361. https://doi.org/10.1371/journal.pone.0015361.

24. Moskalev, A.A., Shaposhnikov, M.V., Zemskaya, N.V., Koval, L.A., Schegoleva, E.V., Guvatova, Z.G., Krasnov, G.S., Solovev, I.A., Sheptyakov, M.A., Zhavoronkov, A., and Kudryavtseva, A.V. (2019). Transcriptome analysis of long-lived Drosophila melanogaster E(z) mutants sheds light on the molecular mechanisms of longevity. Sci. Rep. *9*, 9151. https://doi.org/10.1038/s41598-019-45714-x.

25. Moskalev, A., Zhikrivetskaya, S., Krasnov, G., Shaposhnikov, M., Proshkina, E., Borisoglebsky, D., Danilov, A., Peregudova, D., Sharapova, I., Dobrovolskaya, E., et al. (2015). A comparison of the transcriptome of Drosophila melanogaster in response to entomopathogenic fungus, ionizing radiation, starvation and cold shock. BMC Genom. *16*, S8–S18. https://doi.org/10.1186/1471-2164-16-S13-S8.

26. Zarubin, M., Gangapshev, A., Gavriljuk, Y., Kazalov, V., and Kravchenko, E. (2021). First transcriptome profiling of *D. melanogaster* after development in a deep underground low radiation background laboratory. PLoS One *16*, e0255066. https://doi.org/10.1371/journal.pone.0255066.

27. Karam, C.S., Coie, L.A., and Javitch, J.A. (2020). Small flies meet big data: Genetic convergence of neurodevelopmental disorders modeled in drosophila. Am. J. Psychiatry *177*, 482–484. https://doi.org/10.1176/appi.ajp.2020.20040412.

28. Dissel, S. (2020). Drosophila as a model to study the relationship between sleep,

plasticity, and memory. Front. Physiol. *11*, 533. https://doi.org/10.3389/fphys.2020.00533.

29. Gargano, J.W., Martin, I., Bhandari, P., and Grotewiel, M.S. (2005). Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. Exp. Gerontol. *40*, 386–395. https://doi.org/10.1016/j.exger.2005.02.005.

30. Zarubin, M., Yakhnenko, A., and Kravchenko, E. (2020). Transcriptome analysis of Drosophila melanogaster laboratory strains of different geographical origin after long-term laboratory maintenance. Ecol. Evol. *10*, 7082–7093. https://doi.org/10.1002/ece3.6410.

31. Ward, J.F., Evans, J.W., Limoli, C.L., and Calabro-Jones, P.M. (1987). Radiation and hydrogen peroxide induced free radical damage to DNA. Br. J. Cancer *8*, 105–112.

32. Parashar, V., Frankel, S., Lurie, A.G., and Rogina, B. (2008). The effects of age on radiation resistance and oxidative stress in adult Drosophila melanogaster. Radiat. Res. *169*, 707–711. https://doi.org/10.1667/RR1225.1.

33. Ricci, C., Riolo, G., Marzocchi, C., Brunetti, J., Pini, A., and Cantara, S. (2021). The tardigrade damage suppressor protein modulates transcription factor and dna repair genes in human cells treated with hydroxyl radicals and uv-c. Biology *10*, 970. https://doi.org/10.3390/biology10100970.

34. Hazelett, D.J., Chang, J.C., Lakeland, D.L., and Morton, D.B. (2012). Comparison of parallel high-throughput RNA sequencing between knockout of TDP-43 and its overexpression reveals primarily nonreciprocal and nonoverlapping gene expression changes in the central nervous system of drosophila. G3 *2*, 789–802. https://doi.org/10.1534/g3.112.002998.

35. Nagao, M., Lanjakornsiripan, D., Itoh, Y., Kishi, Y., Ogata, T., and Gotoh, Y. (2014). High mobility group nucleosome-binding family proteins promote astrocyte differentiation of neural precursor cells. Stem Cell. *32*, 2983–2997. https://doi.org/10.1002/stem.1787.

36. Rochman, M., Taher, L., Kurahashi, T., Cherukuri, S., Uversky, V.N., Landsman, D., Ovcharenko, I., and Bustin, M. (2011). Effects of HMGN variants on the cellular transcription profile. Nucleic Acids Res. *39*, 4076–4087. https://doi.org/10.1093/nar/gkq1343.

37. Nakaya, T., Alexiou, P., Maragkakis, M., Chang, A., and Mourelatos, Z. (2013). FUS regulates genes coding for RNA-binding proteins in neurons by binding to their highly conserved introns. RNA *19*, 498–509. https://doi.org/10.1261/rna.037804.112.

38. Uversky, V.N. (2015). Intrinsically disordered proteins and their (disordered) proteomes in neurodegenerative disorders. Front. Aging Neurosci. *7*, 18. https://doi.org/10.3389/fnagi.2015.00018.

39. Keene, J.D. (2007). RNA regulons: coordination of post-transcriptional events. Nat. Rev. Genet. *8*, 533–543. https://doi.org/10.1038/nrg2111.

40. Spurio, R., Dürrenberger, M., Falconi, M., La Teana, A., Pon, C.L., and Gualerzi, C.O. (1992). Lethal overproduction of the Escherichia coli nucleoid protein H-NS: ultramicroscopic and molecular autopsy. Mol. Gen. Genet. *231*, 201–211. https://doi.org/10.1007/BF00279792.

41. Setlow, B., Hand, A.R., and Setlow, P. (1991). Synthesis of a Bacillus subtilis small, acid-soluble spore protein in Escherichia coli causes cell DNA to assume some characteristics of spore DNA. J. Bacteriol. *173*, 1642–1653. https://doi.org/10.1128/jb.173.5.1642-1653.1991.

42. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press).

43. Rubin, G.M., and Spradling, A.C. (1982). Genetic transformation of Drosophila with transposable element vectors. Science *218*, 348–353. https://doi.org/10.1126/science.6289436.

44. Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., and Chang, W. (2022). DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. *50*, W216–W221. https://doi.org/10.1093/nar/gkac194.

45. Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W.H., Pagès, F., Trajanoski, Z., and Galon, J. (2009). ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics *25*, 1091–1093. https://doi.org/10.1093/bioinformatics/btp101.

46. Shannon, P., Markiel, A., Owen, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models. Genome Res. *13*, 426. https://doi.org/10.1101/gr.1239303.metabolite.

47. Madabattula, S.T., Strautman, J.C., Bysice, A.M., O'Sullivan, J.A., Androschuk, A., Rosenfelt, C., Doucet, K., Rouleau, G., and Bolduc, F. (2015). Quantitative analysis of climbing defects in a drosophila model of neurodegenerative disorders. J. Vis. Exp. *100*, e52741. https://doi.org/10.3791/52741.

48. Jünger, M.A., Rintelen, F., Stocker, H., Wasserman, J.D., Végh, M., Radimerski, T., Greenberg, M.E., and Hafen, E. (2003). The Drosophila Forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. *2*, 20. https://doi.org/10.1186/1475-4924-2-20.

49. Irving, P., Ubeda, J.M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J.A., Hetru, C., and Meister, M. (2005). New insights into Drosophila larval haemocyte functions through genome-wide analysis. Cell Microbiol. *7*, 335–350. https://doi.org/10.1111/j.1462-5822.2004.00462.x.

50. Siddique, H.R., Chowdhuri, D.K., Saxena, D.K., and Dhawan, A. (2005). Validation of Drosophila melanogaster as an in vivo model for genotoxicity assessment using modified alkaline Comet assay. Mutagenesis *20*, 285–290. https://doi.org/10.1093/mutage/gei032.

51. Han, S.K., Lee, D., Lee, H., Kim, D., Son, H.G., Yang, J.S., Lee, S.J.V., and Kim, S. (2016). Oasis 2: online application for survival analysis 2 with features for the analysis of maximal lifespan and healthspan in aging research. Oncotarget *7*, 56147–56152. https://doi.org/10.18632/oncotarget.11269.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-Dsup antibodies	This paper	N/A
Bacterial and virus strains		
E. coli BL21(DE3)	Evrogen	Cat#CC002
Chemicals, peptides, and recombinant proteins		
Coomassie Brilliant Blue R-250	Merck	Cat#1125530025
XbaI	Thermo Fisher Scientific	Cat#ER0681
BamHI	Thermo Fisher Scientific	Cat#ER0051
EcoRI	Thermo Fisher Scientific	Cat#ER0271
BsrGI	New England Biolabs	Cat#R0575
T4 DNA Ligase	New England Biolabs	Cat#M0202
BglII	Thermo Fisher Scientific	Cat#ER0081
DNaseI	Thermo Fisher Scientific	Cat#18047019
Critical commercial assays		
Ni-NTA Fast Start Kit	QIAGEN	Cat#30600
GeneRuler DNA Ladder Mix	Thermo Fisher Scientific	Cat# SM0331
Qubit dsDNA BR Assay Kit	Thermo Fisher Scientific	Cat#Q32850
Qubit RNA BR Assay Kit	Thermo Fisher Scientific	Cat#Q10210
Qubit Protein Assay Kit	Thermo Fisher Scientific	Cat#Q33211
TRIzol™ Reagent	Thermo Fisher Scientific	Cat#15596026
GeneChip™ Drosophila Genome 2.0 Array	Thermo Fisher Scientific	Cat#900533
Phusion Hot Start II High-Fidelity PCR Master Mix	Thermo Fisher Scientific	Cat#F565S
BigDye Terminator v3.1 Cycle Sequencing Kit	Thermo Fisher Scientific	Cat#4337458
Maxima™ H Minus cDNA Synthesis Master Mix, with dsDNase	Thermo Fisher Scientific	Cat#M1681
iTaq Universal SYBR Green Supermix	BioRad	Cat#1725122
GeneChip® 3′ IVT PLUS Reagent	Thermo Fisher Scientific	Cat#902416
cOmplete™ Protease Inhibitor Cocktail	Roche	Cat#11873580001
SuperSignal™ West Pico Rabbit IgG Detection Kit	Thermo Fisher Scientific	Cat#34083
Deposited data		
Raw and analyzed data	This paper	GEO: GSE181453
Experimental models: Organisms/strains		
D. melanogaster: y^1w^{1118} line	Bloomington Drosophila Stock Center	BDSC:6598 FlyBase: FBal0018186
D. melanogaster: *Dsup*-expressing lines #1-4	This paper	N/A
Oligonucleotides		
Primers for RT-qPCR, see Table S1	This paper	N/A
Primers for iPCR : tgactgtgcgttaggtcctg, ggagttttcaccaaggctgc	This paper	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Primers for Act5C gene promoter: gtgaattctagagtacactcttcatggcg, tgtggaggatccgtctctggattagacg	This paper	N/A
Primers for *Dsup* coding sequences : primers gtcgatgctgtgaagaacgc, ctccgccacagtcgatgcagc, tgcagatctatggcatccacacac, tcaagatctttcctcttccgacctccag	This paper	N/A
Recombinant DNA		
DNA fragment coding Dsup protein with codon optimization for *D. melanogaster*	Evrogen (Russia)	N/A
pAL2 plasmid	Evrogen (Russia)	TA002
pCaSpeR4 plasmid	Drosophila Genomics Resource Center	DGRC:1213
pCold-I-Dsup plasmid	Hashimoto et al.[1]	Addgene plasmid #90021
Software and algorithms		
TriTek CometScore software v2.0.0.38	Rex Hoover	http://rexhoover.com/index.php?id=cometscore
Transcriptome analysis console software (TAC) version 4.0.2.	Thermo Fisher	https://www.thermofisher.com/
DAVID version 6.8	Sherman B. et al.[2]	https://david.ncifcrf.gov/
OASIS 2.0.	Han et al.[3]	https://sbi.postech.ac.kr/oasis2/
Cytoscape version 3.7.1.	Shannon P. et al.[4]	https://cytoscape.org/release_notes_3_7_1.html
ClueGO version 2.5.6	Bindea et al.[5]	https://apps.cytoscape.org/apps/cluego

RESOURCE AVAILABILITY

Lead contact

Inquiries and requests for resources should be directed to and will be fulfilled by the lead contact, Dr. Elena Kravchenko (elenakravchenko@jinr.ru).

Materials availability

All constructs and *Drosophila* lines generated in this study will be made available on request to the lead contact; however, requestor will cover shipping costs. This study did not generate new unique reagents.

Data and code availability

Transcriptomic data have been deposited at GEO and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. The published article includes all datasets generated or analyzed during this study. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

This paper does not report original code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

D. melanogaster lines, maintenance and genetic crosses

D. melanogaster recipient strain y^1w^{1118} (Bloomington Drosophila Stock Center #6598) and transgene *Dsup*-expressing lines were maintained at 25°C, 60% humidity and 12 h light-dark cycle on a standard yeast medium. Obtained after germline transformation flies were crossed with y^1w^{1118} flies and transgenic individuals were selected by their eye color. The copy number of transgenes in obtained transgenic lines was

estimated by Southern blot hybridization.[42] Chromosome localizations of the transgene insertions were determined using common balancer lines. Lethal in the homozygous state lines are maintained as heterozygotes Dsup/+ with the selection of red-eyed flies in each generation.

METHOD DETAILS

Plasmids

DNA fragment coding Dsup protein with codon optimization for *D. melanogaster* was synthesized in Evrogen (Russia) and introduced in pAL2 plasmid Evrogen (Russia). *D. melanogaster Act5C* gene promoter was PCR amplified with the primers gtgaattctagagtacactcttcatggcg and tgtggaggatccgtctctggattagacg from *D. melanogaster* Oregon-R strain genomic DNA, digested with XbaI and BamHI (Thermo Fisher Scientific) and cloned into the pCaSpeR4 plasmid (Drosophila Genomics Resource Center, Bloomington USA, stock #1213) digested with XbaI and BamHI (Thermo Fisher Scientific). The BglII – EcoRI fragment containing *Dsup* gene from pAL2-Dsup plasmid was cloned into the pCaSpeR4-Act5Cpromoter plasmid digested with BamHI and EcoRI (Thermo Fisher Scientific). Resulted pCaSpeR4-Act5Cpromoter-Dsup plasmid was used for P-element mediated germline transformation.[43]

Inverse PCR and sequencing

D. melanogaster total DNA was isolated using standard phenol-chlorophorm method, digested with BsrGI (New England Biolabs) and ligated overnight at 14°C with T4 DNA Ligase (New England Biolabs). Resulted mix was used as a template for PCR with Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific), tgactgtgcgttaggtcctg and ggagttttcaccaaggctgc primers. Obtained PCR-product was sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific) and SeqStudio Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific).

RNA extraction and RT-qPCR

Total RNA was extracted from twenty 3 days adult flies using TRIzol Reagent according to the manufacturer's protocol. The integrity of RNA was verified using a QIAxcel Advanced System (QIAGEN). RNA extraction from irradiated flies was performed after 1 h after radiation treatment. cDNA was obtained using Maxima™ H Minus cDNA Synthesis Master Mix, with dsDNase (Thermo Fisher Scientific) and the resulting cDNA was used as an template in real-time qPCR with iTaq Universal SYBR Green Supermix (BioRad) on a CFX96 Touch Real-Time PCR Detection System (BioRad). RT-qPCR data were normalized with reference gene *RpL32* and analyzed using ΔΔCt method. For each set of primers three biological replicates were performed. The sequences of primers used in this study are listed in the Table S3.

RNA electrophoretic mobility shift assay

Recombinant Dsup was produced using pCold-I-Dsup (a gift from Takekazu Kunieda (Addgene plasmid #90021; http://n2t.net/addgene:90021; RRID:Addgene_90021)[11]) and purified in native conditions with Ni-NTA Fast Start Kit (QIAGEN) (Figure S2) and NGC Quest 10 Plus Chromatography System (BioRad). As a control DNA probe we used plasmid pGEM7Zf linearized by EcoRI. Total *D. melanogaster* RNA or control DNA probe were incubated with Dsup in 1xGibco™ PBS buffer pH-7.4 at 25°C for 15 min in different wt:wt ratio: 250 ng of control DNA and 750 ng of Dsup; 1 μg of total RNA and 0, 1, 3, 5, 7.5, 10 μg of Dsup. Protein and nucleic acid concentrations were determined using Qubit dsDNA BR Assay Kit, Qubit RNA BR Assay Kit and Qubit Protein Assay Kit (Thermo Fisher Scientific). The samples were electrophoresed in a 1% agarose gel and stained with ethidium bromide.

Microarray analysis

cRNA was prepared with GeneChip® 3′ IVT PLUS Reagent Kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's protocol. The GeneChip Drosophila Genome 2.0 Arrays (Applied Biosystems, Thermo Fisher Scientific Inc.) were hybridized, stained, and washed according to the manufacturer's protocol and scanned with Affymetrix 3000 7G scanner. The first data set contains the scans of eight GeneChip™ Drosophila Genome 2.0 Arrays, representing of recipient strain y^1w^{1118} (four replicates) and *Dsup*-expressing lines (lines #1-4). Another data set contains the scans of recipient strain y^1w^{1118} (three replicates) and *Dsup*-expressing lines #1-4 (four replicates) exposed to ionizing radiation (γ-rays, 1000 Gy). All data is stored in NCBI GEO database under accession number GSE181453. CEL files obtained after scanning were studied using the Transcriptom analysis console software 4.0.2. Background correction and normalization were made by RMA algorithm using default parameters. A probe was selected to be

differentially expressed if its P-value was <0.01 and its mean fold change value across replicates was more than twofold. Gene ontology analysis for enrichment of biological processes was done by DAVID version 6.8 (database of all annotated genes were used as a background list, enrichment cutoff $p < 0.1$).[44] All lists of DEGs with annotations and ontological data were deposited in Tab. S. Biological network was created with Cytoscape 3.7.1. ClueGO 2.5.6.[45,46]

Climbing assay

Climbing assay was performed as described in Madabatulla et al.[47] Briefly, the group of 10 males and 10 females (2-3 days old) was placed in an empty 50 mL glass graduated cylinder with a black threshold line drawn at 8 cm from the bottom. Flies tapped down to the cylinder bottom had 10 s to climb. The number of flies that crossed the 8 cm threshold line in 10 s was registered as a percentage of flies above the threshold line. Each group climbed 20 times. The same experiment was done to estimate the number of flies that overcame 12 cm in 8 s. Five biological replicates were performed for each *Dsup*-expressing line (#1-4) and control y^1w^{1118} line in the same ambient light and temperature.

Longevity test

Fifty 1-2 days males or females were placed in vials with standard medium. Flies were transferred to new vials with standard medium every 2 days, at which point all dead flies were counted. Lifespan experiments were performed in three biological replicates, at least 50 flies in each.

Hydrogen peroxide stress treatment

For hydrogen peroxide stress treatment, 2-3 days old flies were transferred to vials containing a gel of phosphate-buffered saline (PBS), 10% sucrose, 0.8% low-melt agarose and 9% hydrogen peroxide (added to medium cooled to 40°C).[48] We measured the survival rate under 9% hydrogen peroxide with mortality counts every 12 h using 50 males and females for *Dsup*-expressing and control y^1w^{1118} lines. For all lines, hydrogen peroxide stress treatment experiments were performed at least five times.

Treatment by ionizing radiation

Fifty 2-3 days males or females were placed in vials with standard medium and irradiated with gamma rays from microtron MT-25 of Flerov Laboratory of Nuclear Reactions (JINR, Dubna, Russia). Irradiation dose rate was controlled with ionization chamber SNC600c (Sun Nuclear Corporation, USA) and was 300 mGy s^{-1}. The obtained absorbed dose for *Dsup*-expressing line #1 and control y^1w^{1118} was 500, 1000 and 1500 Gy. The experiments performed for each dose were repeated at least three times, 50 flies each. After radiation treatment, flies were transferred to new vials with standard medium every 2 days, at which point all dead flies were counted.

Comet assay

The third-instar larvae (72 ± 2 h) of *Dsup*-expressing line #1 and control y^1w^{1118} were allowed to feed on standard *Drosophila* yeast medium and medium containing 9% H_2O_2 for 18 h. Than 50 larvae were washed in PBS, dried and disrupted in the latero-posterior region. The haemolymph with circulating haemocytes was directly collected in cold PBS and centrifuged at 300 g for 1 min. The supernatant was removed and the pellet containing haemocytes collected from 50 larvae was used for Comet assay.[49] Cell samples were resuspended in 40 µL of cold PBS and mixed with 40 µL of 1.5% LMA. The mixture was layered on slides pre-coated with 1% agarose with a normal melting point, and after solidification lysed for 2 h at 4°C in a freshly prepared solution containing 2.5 M of NaCl, 100 mM of EDTA, 10 mM of Tris, pH 10 and 1% Triton X-100, pH 10. Than the slides were transferred to an electrophoresis unit (BioRad) filled with cold electrophoresis solution (1 mM of Na_2 EDTA and 300 mM of NaOH, pH >13) and after 10 min of incubation electrophoresis was performed for 15 min at 4°C (0.7 V/cm (300 mA/25 V)). After electrophoresis, the slides were immediately neutralized with 0.4 M of Tris buffer (pH 7.5) for 5 min, the neutralizing process was repeated three times.[50] The slides were stained with DAPI (1 µg/mL) and examined within 3 h, using EVOS® FL Cell Imaging System (Thermo Fisher Scientific). The images were analyzed with TriTek CometScore software v2.0.0.38. At least 100 randomly selected nuclei obtained from three experimental replicates were analyzed per treatment and control conditions (Figure S7).

Western blot analysis

Anti-Dsup antibodies was raised in rabbits against the bacterially expressed Dsup protein using pCold-I-Dsup (a gift from Takekazu Kunieda (Addgene plasmid #90021; http://n2t.net/addgene:90021; RRID:Addgene_90021)) and purified with Ni-NTA Fast Start Kit (QIAGEN) (Figure S8) and NGC Quest 10 Plus Chromatography System (BioRad). 30 adult males and females of control y^1w^{1118} and *Dsup*-expressing lines were homogenized in a buffer containing 20 mM Tris HCl (pH 8.0), 137 mM NaCl, 1% NP-40, 2 mM EDTA and cOmplete™ Protease Inhibitor Cocktail (Roche). Western blotting was done using standard protocols. Protein bands were identified with SuperSignal™ West Pico Rabbit IgG Detection Kit (Thermo Fisher Scientific).

QUANTIFICATION AND STATISTICAL ANALYSIS

Survival curves were compared by the log-rank test; the analysis of specific time points, 50th (median lifespan) and 90th (maximum lifespan) percentiles of lifespan curves was made by the exact Fisher's test (OASIS 2.0. (http://sbi.postech.ac.kr/oasis2)[51]). Dunnett's multiple comparison test was implemented to assess the statistical significance of differences in locomotion data results. Comet assay results were compared by Student t-test. Quantitative data are presented as mean ± standard deviation. (SD). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS - not significant.