

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

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Supplementary Tables

Supplementary Table S1. Age-dependent changes in the efficiency of DNA repair

DNA repair mechanism	Repair efficiency	References
Base excision repair	↓	1, 2
Nucleotide excision repair	↓	3
Mismatch repair	↓	4
Single-strand break repair	↓	5, 6
Homologous recombination	↑	7
Single-strand annealing	↓	7
Non-homologous end joining	↓	8

Supplementary Table S2. Influence of DNA repair genes overexpression on the lifespan

Genotype, +/-RU486	Sex	90%	P(90%)	M	P(M)	n(rep)	n
<i>da-GAL4</i> driver							
<i>da-GAL4>w</i>	M	61		44		4	564
<i>w>UAS-mei-9</i>	M	58		37		1	161
<i>da-GAL4>UAS-mei-9</i>	M	70	3E-05 ^a , 0.0001 ^c	52	1.2E-11 ^a , 0 ^c	1	158
<i>w>UAS-Rrp1</i>	M	59		43		3	438
<i>da-GAL4>UAS-Rrp1</i>	M	63	0.668 ^a , 0.668 ^d	49	0.122 ^a , 0.526 ^d	3	449
<i>w>UAS-Brca2</i>	M	52		33		1	157
<i>da-GAL4>UAS-Brca2</i>	M	55	0.0002 ^a , 2E-05 ^e	42.5	6.86E-06 ^a , 0.0039 ^e	1	154
<i>w>UAS-Ku80</i>	M	51		35		2	297
<i>da-GAL4>UAS-Ku80</i>	M	51	0 ^a , 0.2490 ^f	42	1.96E-10 ^a , 0.0185 ^f	2	266
<i>w>UAS-WRNexo</i>	M	57		43		1	153
<i>da-GAL4>UAS-WRNexo</i>	M	54	0.829 ^a , 0.0004 ^h	26	9.81E-07 ^a , 0.0003 ^h	1	45
<i>da-GAL4>w</i>	F	67		55		4	555
<i>w>UAS-mnk</i>	F	66		54		1	141
<i>da-GAL4>UAS-mnk</i>	F	70	0.009 ^a , 0.723 ^b	58	0.002 ^a , 0.0071 ^b	1	207
<i>w>UAS-mei-9</i>	F	64		58		1	150
<i>da-GAL4>UAS-mei-9</i>	F	74	0 ^a , 0 ^c	64	0 ^a , 0 ^c	1	178
<i>w>UAS-Rrp1</i>	F	66		50.5		3	386
<i>da-GAL4>UAS-Rrp1</i>	F	68	0.578 ^a , 0.0703 ^d	52	0.055 ^a , 0.0269 ^d	3	423
<i>w>UAS-Brca2</i>	F	53		46		1	122
<i>da-GAL4>UAS-Brca2</i>	F	61	0.039 ^a , 1E-05 ^e	48	3.12E-08 ^a , 0.0002 ^e	1	147
<i>w>UAS-spn-B</i>	F	61		59		1	157
<i>da-GAL4>UAS-spn-B</i>	F	72	0.0003 ^a , 0 ^g	62	1.64E-07 ^a , 5E-11 ^g	1	159
<i>w>UAS-WRNexo</i>	F	64		51		1	146
<i>da-GAL4>UAS-WRNexo</i>	F	77	0 ^a , 0 ^h	61	6.15E-07 ^a , 0 ^h	1	123
<i>Act5C-GS</i> driver							
<i>UAS-Hus1/Act5C-GS, -RU486</i>	M	60		42		2	402
<i>UAS-Hus1/Act5C-GS, +RU486</i>	M	24	0 ^a	15	0 ^a	2	433
<i>UAS-mei-9/Act5C-GS, -RU486</i>	M	39		56		2	385
<i>UAS-mei-9/Act5C-GS, +RU486</i>	M	40	0.6825 ^c	56	0.446 ^c	2	411
<i>UAS-mus210/Act5C-GS, -RU486</i>	M	39		59		2	431
<i>UAS-mus210/Act5C-GS, +RU486</i>	M	16	0 ^d	32	0 ^d	2	396
<i>UAS-Brca2/Act5C-GS, -RU486</i>	M	40		63		2	499
<i>UAS-Brca2/Act5C-GS, +RU486</i>	M	12	0 ^f	31	0 ^f	2	473
<i>UAS-WRNexo/Act5C-GS, -RU486</i>	M	43		61		2	422
<i>UAS-WRNexo/Act5C-GS, +RU486</i>	M	14	0 ⁱ	20	0 ⁱ	2	353
<i>UAS-Hus1/Act5C-GS, -RU486</i>	F	64		48		2	409
<i>UAS-Hus1/Act5C-GS, +RU486</i>	F	49	3E-05 ^a	15	0 ^a	2	415
<i>UAS-mei-9/Act5C-GS, -RU486</i>	F	39		59		2	463
<i>UAS-mei-9/Act5C-GS, +RU486</i>	F	18	0 ^c	37	0 ^c	2	439

<i>UAS-Rrp1/Act5C-GS, -RU486</i>	F	44		64		2	365
<i>UAS-Rrp1/Act5C-GS, +RU486</i>	F	13	0 ^e	49	0 ^e	2	522
<i>UAS-Brc2/Act5C-GS, -RU486</i>	F	49		63		2	552
<i>UAS-Brc2/Act5C-GS, +RU486</i>	F	16	0 ^f	29	0 ^f	2	449
<i>UAS-Ku80/Act5C-GS, -RU486</i>	F	58		71		2	349
<i>UAS-Ku80/Act5C-GS, +RU486</i>	F	24	0 ^h	36	0 ^h	2	588
<i>UAS-WRNexo/Act5C-GS, -RU486</i>	F	46		59		2	410
<i>UAS-WRNexo/Act5C-GS, +RU486</i>	F	19	0 ⁱ	33	0 ⁱ	2	296
1407-GAL4 driver							
<i>1407-GAL4>w^a</i>	M	74		53		2	189
<i>1407-GAL4> w^b</i>	M	61		48		2	283
<i>1407-GAL4> w^c</i>	M	72		55		2	245
<i>1407-GAL4> w^d</i>	M	63		49		2	175
<i>1407-GAL4>w^e</i>	M	67		50		2	145
<i>w>UAS-Hus1</i>	M	50		39		2	191
<i>1407-GAL4>UAS-Hus1</i>	M	63	0.005 ^a , 0 ^f	51	0.023 ^a , 0 ^f	2	246
<i>w>UAS-mnk</i>	M	53		33		2	215
<i>1407-GAL4>UAS-mnk</i>	M	64	0.061 ^a , 0 ^g	43	2.71E-05 ^a , 1.15E-09 ^g	2	182
<i>w>UAS-mei-9</i>	M	53		51		2	280
<i>1407-GAL4>UAS-mei-9</i>	M	58	0 ^c , 0 ^h	52	0 ^c , 1.31E-09 ^h	2	227
<i>w>UAS-mus210</i>	M	46		40		2	268
<i>1407-GAL4>UAS-mus210</i>	M	55	0.0002 ^b , 0 ⁱ	43	7.36E-08 ^b , 0 ⁱ	2	250
<i>w>UAS-Rrp1</i>	M	51		36		1	159
<i>1407-GAL4>UAS-Rrp1</i>	M	50	0 ^c , 0.4331 ^j	29	0 ^c , 0.0984 ^j	2	160
<i>w>UAS-Brc2</i>	M	57		46		2	202
<i>1407-GAL4>UAS-Brc2</i>	M	48	0 ^a , 0.0007 ^k	19	0 ^a , 0 ^k	2	146
<i>w>UAS-spn-B</i>	M	55		43		2	209
<i>1407-GAL4>UAS-spn-B</i>	M	69	0.093 ^a , 0 ^l	50	0.13 ^a , 4E-12 ^l	2	257
<i>w>UAS-WRNexo</i>	M	61		37		2	254
<i>1407-GAL4>UAS-WRNexo</i>	M	32	0 ^d , 0 ⁿ	18	0 ^d , 0 ⁿ	2	197
<i>1407-GAL4>w^a</i>	F	80		62		2	243
<i>1407-GAL4> w^b</i>	F	76		63		2	241
<i>1407-GAL4> w^c</i>	F	73		58.5		2	158
<i>1407-GAL4> w^d</i>	F	82		69		2	280
<i>1407-GAL4>w^e</i>	F	64		53		2	147
<i>w>UAS-Hus1</i>	F	78		55		2	174
<i>1407-GAL4>UAS-Hus1</i>	F	49	0 ^a , 0 ^f	41	0 ^a , 0 ^f	2	227
<i>w>UAS-mnk</i>	F	62		47		2	226
<i>1407-GAL4>UAS-mnk</i>	F	57	0 ^a , 0 ^g	48	0 ^a , 0.0014 ^g	2	132
<i>w>UAS-mei-9</i>	F	81		64		2	245
<i>1407-GAL4>UAS-mei-9</i>	F	70	0.7756 ^c , 0 ^h	60	0.849 ^c , 0 ^h	2	264
<i>w>UAS-mus210</i>	F	71		57		2	248

<i>1407-GAL4>UAS-mus210</i>	F	74	1E-04 ^b , 0.0043 ⁱ	60	0.0004 ^b , 0.237 ⁱ	2	228
<i>w>UAS-Rrp1</i>	F	63		46		1	145
<i>1407-GAL4>UAS-Rrp1</i>	F	65	0.0323 ^c , 0.0605 ^j	57	0.0012 ^e , 3.12E-06 ^j	2	147
<i>w>UAS- Brca2</i>	F	74		62.5		2	260
<i>1407-GAL4>UAS-Brca2</i>	F	53	1E-05 ^a , 0.0155 ^k	27	0 ^a , 0 ^k	2	125
<i>w>UAS- spn-B</i>	F	73		60		2	257
<i>1407-GAL4>UAS-spn-B</i>	F	72	0 ^a , 0.481 ^l	55	9.19E-09 ^a , 0.17 ^l	2	231
<i>w>UAS-Ku80</i>	F	65		50		1	141
<i>1407-GAL4>UAS-Ku80</i>	F	75	2E-05 ^e , 0 ^m	58	1.37E-09 ^e , 1.8E-08 ^m	2	122
<i>w>UAS-WRNexo</i>	F	74		55		2	258
<i>1407-GAL4>UAS-WRNexo</i>	F	50	0 ^d , 0 ⁿ	37	0 ^d , 0 ⁿ	2	245
<i>Elav-GS driver</i>							
<i>Elav-GS>UAS-Hus1, -RU486</i>	M	76		64		2	324
<i>Elav-GS>UAS-Hus1, +RU486</i>	M	81	0.0354 ^a	66.5	2.41E-07 ^a	2	339
<i>Elav-GS>UAS-mnk, -RU486</i>	M	71		62		2	342
<i>Elav-GS>UAS-mnk, +RU486</i>	M	82	0 ^b	64	3E-12 ^b	2	371
<i>Elav-GS>UAS-me1-9, -RU486</i>	M	65		50		1	108
<i>Elav-GS>UAS-me1-9, +RU486</i>	M	72	2E-05 ^c	64	0 ^c	1	125
<i>Elav-GS>UAS-mus210, -RU486</i>	M	64		50		1	140
<i>Elav-GS>UAS-mus210, +RU486</i>	M	68	5E-05 ^d	54	8.25E-05 ^d	1	123
<i>Elav-GS>UAS-Brca2, -RU486</i>	M	65		54		2	337
<i>Elav-GS>UAS-Brca2, +RU486</i>	M	67	0.4472 ^e	51	0.196 ^f	2	270
<i>Elav-GS>UAS-spn-B, -RU486</i>	M	64		56		2	270
<i>Elav-GS>UAS-spn-B, +RU486</i>	M	61	1E-05 ^f	48	4.5E-11 ^g	2	322
<i>Elav-GS>UAS-WRNexo, -RU486</i>	M	64		44		1	137
<i>Elav-GS>UAS-WRNexo, +RU486</i>	M	72	3E-04 ^g	65	0 ⁱ	1	140
<i>Elav-GS>UAS-Hus1, -RU486</i>	F	84		68		2	328
<i>Elav-GS>UAS-Hus1, +RU486</i>	F	88	0.0096 ^a	69	0.0012 ^a	2	338
<i>Elav-GS>UAS-me1-9, -RU486</i>	F	77		70		1	149
<i>Elav-GS>UAS-me1-9, +RU486</i>	F	78	0.012 ^c	71	0.0131 ^c	1	112
<i>Elav-GS>UAS-mus210, -RU486</i>	F	78		68		1	162
<i>Elav-GS>UAS-mus210, +RU486</i>	F	78	0.0031 ^d	65	0.187 ^d	1	150
<i>Elav-GS>UAS-Brca2, -RU486</i>	F	75		64		2	360
<i>Elav-GS>UAS-Brca2, +RU486</i>	F	71	1E-05 ^e	50	0 ^f	2	379
<i>Elav-GS>UAS-spn-B, -RU486</i>	F	76		59		2	281
<i>Elav-GS>UAS-spn-B, +RU486</i>	F	74	0.003 ^f	61	0.867 ^g	2	278
<i>Elav-GS>UAS-WRNexo, -RU486</i>	F	74		64		1	142
<i>Elav-GS>UAS-WRNexo, +RU486</i>	F	73	0.079 ^g	64	0.288 ⁱ	1	116

-RU486, no mifepristone (no overexpression); +RU486, mifepristone (overexpression); M, Male; F, Female; 90%, 90th percentile; P(90), P-value (Wang-Allison test); P(M), P-value (Mantel-Cox test): NS, not significant; n(rep), number of experiments; n, number of flies.

Controls for *da-GAL4>UAS*: ^a*da-GAL4>w* and ^b*UAS-mnk*; ^c*UAS-me1-9*; ^d*UAS-Rrp1*; ^e*UAS-Brca2*; ^f*UAS-Ku80*; ^g*UAS-spn-B*; ^h*UAS-WRNexo*.

Controls for *Act5C-GS>UAS*: ^a*Act5C-GS>UAS-Hus1*, -RU486; ^b*Act5C-GS>UAS-mnk*, -RU486; ^c*Act5C-GS>UAS-me1-9*, -RU486; ^d*Act5C-GS>UAS-mus210*, -RU486; ^e*Act5C-GS>UAS-Rrp1*, -RU486; ^f*Act5C-GS>UAS-Brca2*, -RU486; ^g*Act5C-GS>UAS-spn-B*, -RU486; ^h*Act5C-GS>UAS-Ku80*, -RU486; ⁱ*Act5C-GS>UAS-WRNexo*, -RU486.

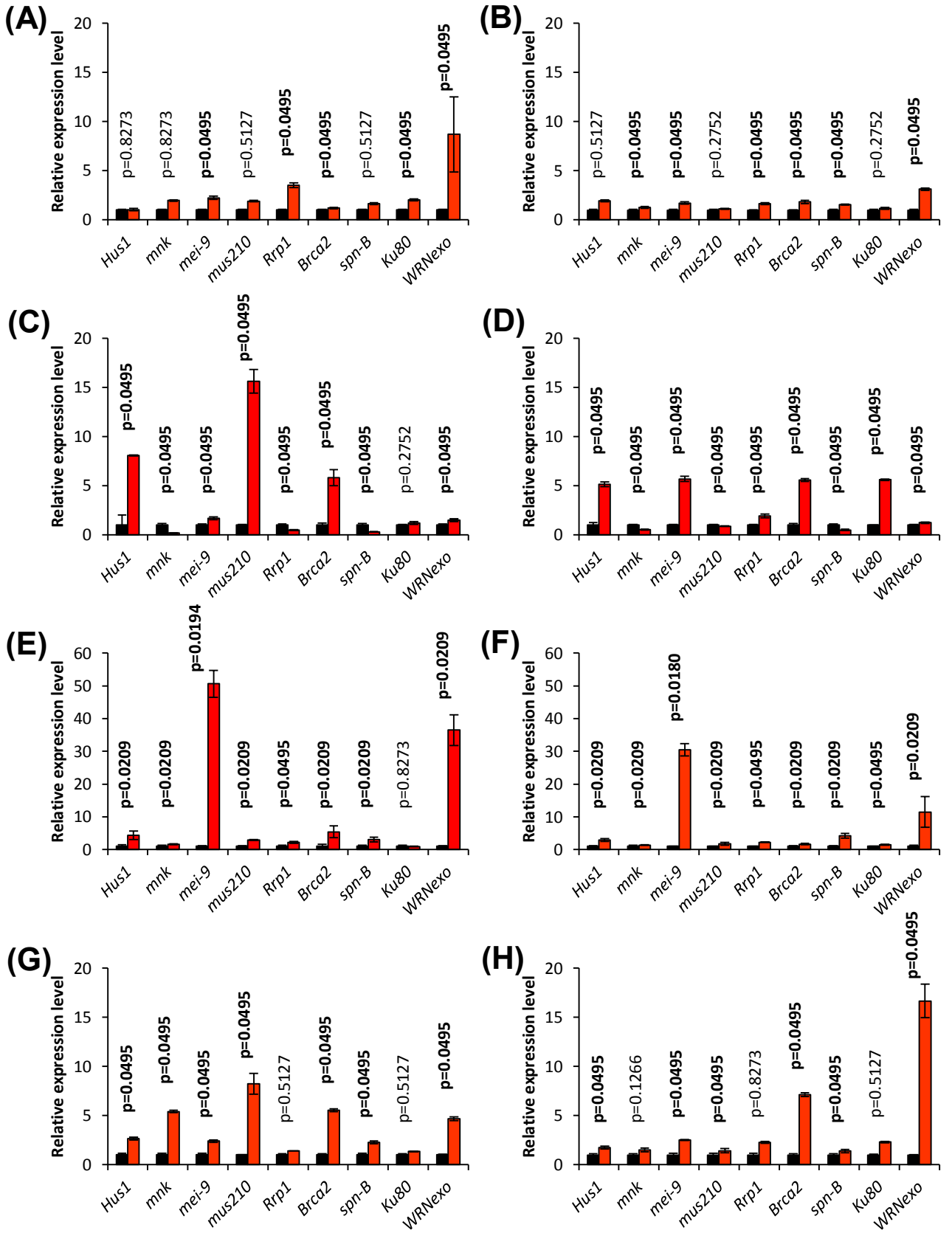
Controls for *1407-GAL4>UAS*: ^a*1407-GAL4>w^a*; ^b*1407-GAL4>w^b*; ^c*1407-GAL4>w^c*; ^d*1407-GAL4>w^d*; ^e*1407-GAL4>w^e*; ^f*w>UAS-Hus1*; ^g*w>UAS-mnk*; ^h*w>UAS-me1-9*; ⁱ*w>UAS-mus210*; ^j*w>UAS-Rrp1*; ^k*w>UAS-Brca2*; ^l*w>UAS-spn-B*; ^m*w>UAS-Ku80*; ⁿ*w>UAS-WRNexo*.

Controls for *Elav-GS>UAS*: ^a*Elav-GS>UAS-Hus1*, -RU486; ^b*Elav-GS>UAS-mnk*, -RU486; ^c*Elav-GS>UAS-me1-9*, -RU486; ^d*Elav-GS>UAS-mus210*, -RU486; ^e*Elav-GS>UAS-Brca2*, -RU486; ^f*Elav-GS>UAS-spn-B*, -RU486; ^g*Elav-GS>UAS-WRNexo*, -RU486.

Supplementary Table S3. Primers for quantitative RT-PCR

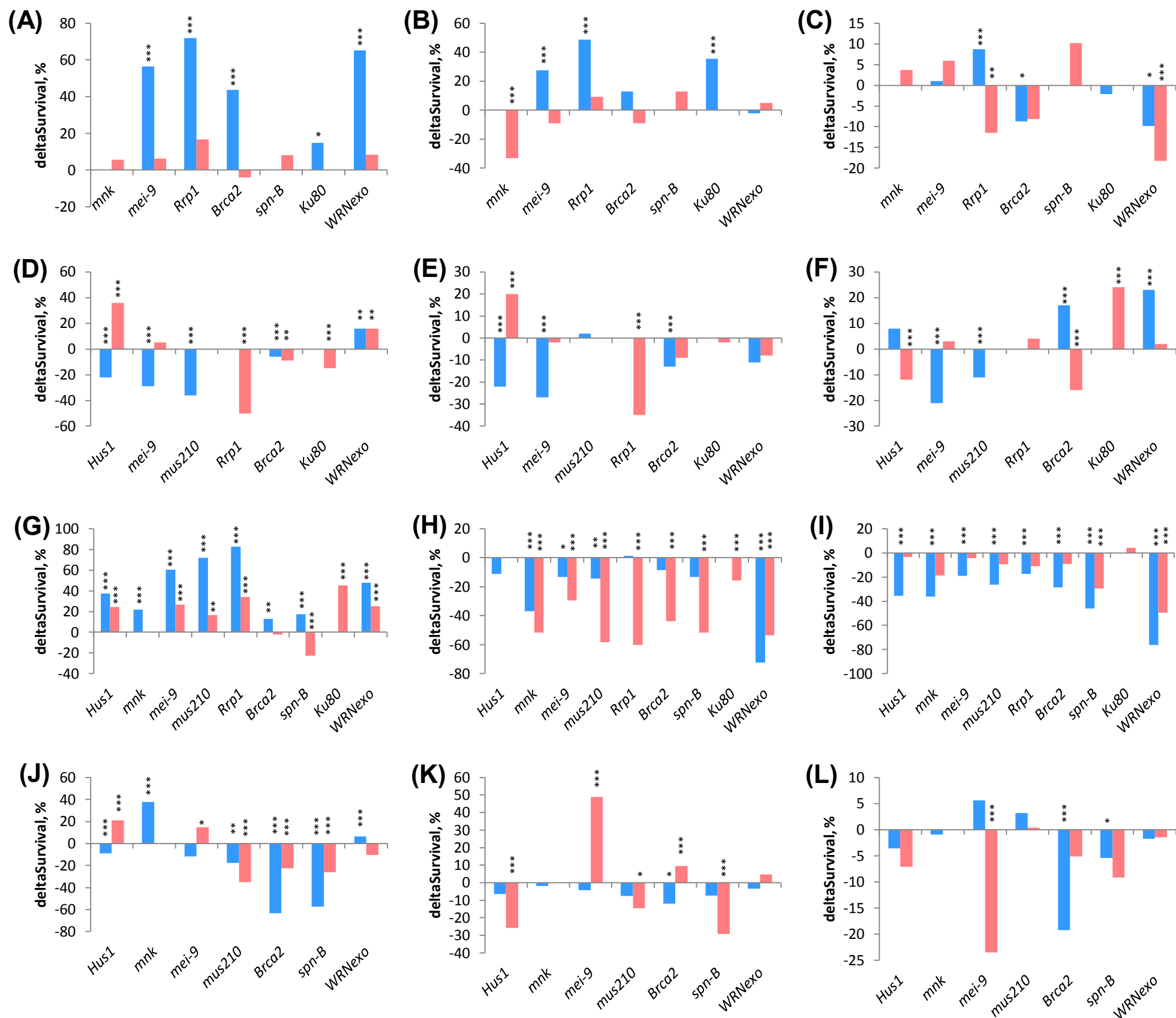
Gene	Forward	Reverse
<i>β-Tubulin</i>	5'-GCAACTCCACTGCCATCC-3'	5'-CCTGCTCCTCCTCGAACT-3'
<i>Hus1</i>	5'-TGATGCAGGATCCGCTGTACATGA-3'	5'-TCCTCAGCTGTAATTCCTGCCCAA-3'
<i>mnk</i>	5'-ATGTGCCATGCCGTCAAGTACCTA-3'	5'-TCCTCGTCATTGGTCTCCAGCAA-3'
<i>mei-9</i>	5'-TCCTCAAGGCCTACAGCGATTC-3'	5'-TCCAGATAAACGCGCTCTCTTTC-3'
<i>mus210</i>	5'-AGAAGACGGTGCATTTGAGATTGC-3'	5'-CCTCGCAAACAATGAAGCCATCG-3'
<i>Rrp1</i>	5'-AGGATGGTCTGCAGTTGATTGAC-3'	5'-GTTTGCGCACTTGGTTTCCTG-3'
<i>Brca2</i>	5'-TCGTCGCCGTGGAGGATCTTATTT-3'	5'-TCTGCGTATGTTGGAGACGAGCAA-3'
<i>spn-B</i>	5'-AGATTGCTGCAGATGAGCAAAGCC -3'	5'-TTTATAACGCACGCCAGGAGAGGT-3'
<i>Ku80</i>	5'-GAGCTTCAGAATGTCGCAACTACC-3'	5'-GGAAAGTCGTTGAAATCGAAGAGC -3'
<i>WRNexo</i>	5'-TGGTGGCCCTTATCAATCATCCC-3'	5'-GTGCCAGCTTTCGGAAATCGTTC-3'

Supplementary Figures



Supplementary Figure S1. The relative level of expression of DNA repair genes in control flies (black) and flies with overexpression (red).

Overexpression under control of *da-GAL4* (A, males and B, females), *Act5C-GS* (C, males and D, females), *1407-GAL4* (E, males and F, females), and *Elav-GS* (G, males and H, females). P-values were calculated by Mann-Whitney U-test. The error bars represent standard error of the mean.



Supplementary Figure S2. The influence of overexpression of DNA repair genes on the stress resistance of males (blue) and females (red).

Overexpression under control of *da-GAL4* (A, B, C), *Act5C-GS* (D, E, F), *1407-GAL4* (G, H, I), and *Elav-GS* (J, K, L). Survival after hyperthermia (A, D, G, J), oxidative stress (B, E, H, K), and starvation (C, F, I, L). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Fisher's exact test.

Supplementary References

- 1 Atamna, H., Cheung, I. & Ames, B. N. A method for detecting abasic sites in living cells: Age-dependent changes in base excision repair. *Proc. Natl. Acad. Sci. USA* **97**, 686-691 (2000).
- 2 Rao, K. S., Annapurna, V. V., Raji, N. S. & Harikrishna, T. Loss of base excision repair in aging rat neurons and its restoration by DNA polymerase beta. *Mol. Brain Res.* **85**, 251-259 (2000).
- 3 Vijg, J., Mullaart, E., Lohman, P. H. & Knook, D. L. UV-induced unscheduled DNA synthesis in fibroblasts of aging inbred rats. *Mutat. Res.* **146**, 197-204 (1985).
- 4 Toyota, M. *et al.* CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA* **96**, 8681-8686 (1999).
- 5 Newton, R. K., Ducore, J. M. & Sohal, R. S. Effect of age on endogenous DNA single-strand breakage, strand break induction and repair in the adult housefly, *Musca domestica*. *Mutat. Res.* **219**, 113-120 (1989).
- 6 Singh, N. P., Danner, D. B., Tice, R. R., Brant, L. & Schneider, E. L. DNA damage and repair with age in individual human lymphocytes. *Mutat. Res.* **237**, 123-130 (1990).
- 7 Preston, C. R., Flores, C. & Engels, W. R. Age-dependent usage of double-strand-break repair pathways. *Curr. Biol.* **16**, 2009-2015, doi:10.1016/j.cub.2006.08.058 (2006).
- 8 Frasca, D. *et al.* Effect of age on DNA binding of the ku protein in irradiated human peripheral blood mononuclear cells (PBMC). *Exp. Gerontol.* **34**, 645-658 (1999).