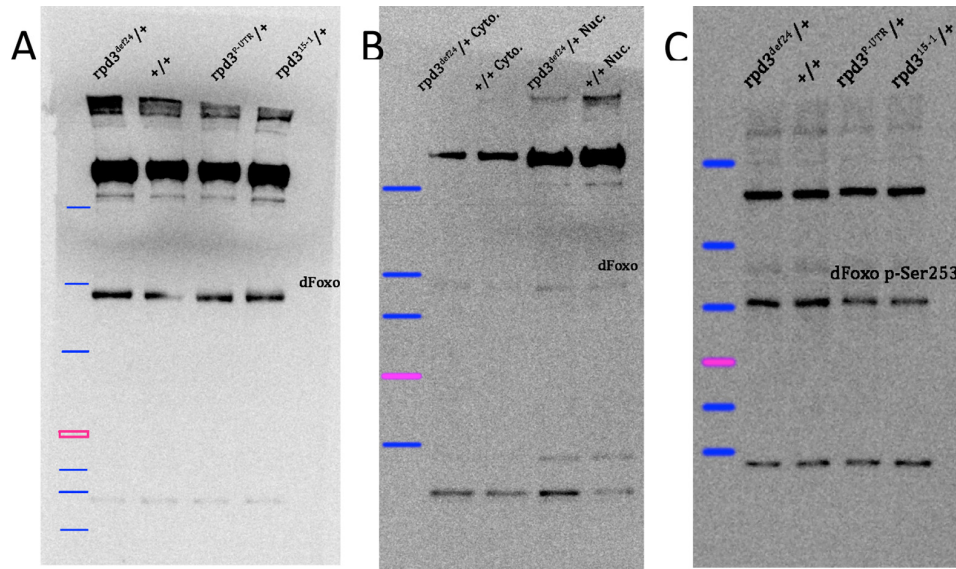


SUPPLEMENTAL MATERIAL



Supplemental Figure 1. (A) Western blot staining for dFOXO in 40 day old *rpd3^{def24}/+*, control (+/+), *rpd3^{P-UTR}/CS* and *rpd3^{P-1.8}/CS* male flies. (B) Western blot staining for dFOXO in the cytoplasmic and nuclear fractions of 40 day old *rpd3^{def24}/+* and control (+/+) male flies. (C) Western blot staining for phosphorylated Serine 253 dFOXO in 40 day old *rpd3^{def24}/+*, control (+/+), *rpd3^{P-UTR}/CS*, and *rpd3^{P-1.8}/CS* (control) male flies.

Supplemental Table 1A. Starvation resistance of *rpd3^{def}/+* heterozygous flies and genetic controls.

Gender	Genotype	N	Age	Mean LS (% change)	Median LS	χ^2	p	Maximal life span (% change)
M	<i>rpd3^{def}/+</i>	39	10	32 (38)	30	54.82	<0.0001*	44 (40)
M	<i>Control</i>	31	10	20	20			26
F	<i>rpd3^{def}/+</i>	60	10	74 (28)	53	24.880	<0.0001*	92 (2)
F	<i>Controls</i>	75	10	54	72			90
M	<i>rpd3^{def}/+</i>	69	40	27 (44)	30	20.9381	<0.0001*	37 (11)
M	<i>Control</i>	17	40	18	17			34
F	<i>rpd3^{def}/+</i>	71	40	64 (108)	61	70.1045	<0.0001*	96 (54)
F	<i>Controls</i>	46	40	31	30			63

The mean, median, and maximal lifespan of *rpd3^{def}/+* and genetic control (+/+) heterozygous male (M) and female (F) flies after exposure to starvation at 10 or 40 days of age. Control values are compared to either male or female *rpd3^{def}/+* groups to determine the percent change in mean, median, and maximal lifespan. Mean, median, and maximal lifespan are in hours. Log-rank analyses were performed using the JMP 12 program. M = Males, F = Females, N = number of flies in the experiment.

Supplemental Table 1B. Starvation resistance of *rpd3*^{P-UTR} heterozygous flies and genetic controls *rpd3*^{P-1.8}/+ flies.

Gender	Genotype	N	Age	Mean LS (% change)	Median LS	X ²	p	Maximal life span (% change)
M	<i>rpd3</i> ^{P-UTR} /+	80	10	32 (9)	31	3.6315	0.0567	38
M	<i>rpd3</i> ^{P-1.8} /+	40	10	29	31			37
F	<i>rpd3</i> ^{P-UTR} /+	78	10	96 (40)	99	81.6546	<0.0001*	139 (36)
F	<i>rpd3</i> ^{P-1.8} /+	32	10	57	55			89

The mean, median, and maximal lifespan of *rpd3*^{P-UTR}/+ and their genetic control (*rpd3*^{P-1.8}/+) heterozygous male (M) and female (F) flies after exposure to starvation at 10 days of age. Control values are compared to either male or female *rpd3*^{P-UTR}/+ to determine the percent change in mean and maximal lifespan. Mean and median lifespan are in hours. N = number of flies in the experiment. Log-rank analyses were performed using the JMP 12 program.

Supplemental Table 2. *dfoxo* is required for longevity extension in *rpd3*^{def} mutant flies

Gender	Genotype	N	Mean (% change)	Median LS	X ²	p	Maximal life span (% change)
M	<i>rpd3</i> ^{def} /+	180	77.7	82			101.1
M	<i>rpd3</i> ^{def} / <i>dfoxo</i> ^{c01841}	225	65.6 (-16)	68	65.99	<0.0001*	91.5 (-9.5)
M	<i>dfoxo</i> ^{c0184} /+	123	50 (36)	51	176.40	<0.0001*	76.7 (-24)
F	<i>rpd3</i> ^{def} /+	200	68.6	70.5			93.7
F	<i>rpd3</i> ^{def} / <i>dfoxo</i> ^{c0184}	238	78.8 (-15)	82	27.868	<0.0001*	101.2 (8)
F	<i>dfoxo</i> ^{c0184} /+	132	67 (2)	72	2.474	0.1158	91.4 (3)

The mean, median, and maximal lifespans of *rpd3*^{def}/+, *rpd3*^{def}/*dfoxo*^{c01841}, and *dfoxo*^{x01841}/+ heterozygous male (M) and female (F) flies. *dfoxo*^{x01841} flies are hypomorphic for *dfoxo*. All values are compared to either male or female *rpd3*^{def}/+ groups to determine the percent change in median and maximal lifespan. Data are censored for 0-10 days. N: number of flies used in the experiment. Median and maximal lifespan are in days. Log-rank analyses were performed using the JMP 12 program.

Supplemental Table 3. Heterozygous flies with reduced *rpd3* mRNA levels have increased resistance to H₂O₂ compared to the genetic control flies at 40 days.

Gender	Genotype	N	Age	Mean (% change)	Median LS	χ^2	p	Maximal life span (% change)
M	<i>rpd3^{def}/+</i>	65	10	34 (-2.5)	34	0.4934	0.4824	43 (-8.5)
M	<i>Control</i>	30	10	35	34			49
F	<i>rpd3^{def}/+</i>	73	10	55 (4.9)	55	1.3942	0.2377	80 (2.7)
F	<i>Controls</i>	79	10	52	55			78
M	<i>rpd3^{def}/+</i>	53	40	25 (155.8)	24	31.3716	0.0001*	31 (17.8)
M	<i>Control</i>	31	40	15	12			26
F	<i>rpd3^{def}/+</i>	55	40	39 (39.5)	36	5.7003	0.0170*	56 (-7.9)
F	<i>Controls</i>	39	40	28	31			60
M	<i>rpd3^{P-UTR}/+</i>	58	10	39 (-3)	38	1.4847	0.2230	48 (-60)
M	<i>rpd3^{P-1.8}/+</i>	56	10	40	40			51
F	<i>rpd3^{P-UTR}/+</i>	52	10	97 (30)	92	55.2690	<0.0001*	116 (20)
F	<i>rpd3^{P-1.8}/+</i>	57	10	68	64			94

The mean, median, and maximal lifespan of *rpd3^{def}/+* and genetic control (+/+), *rpd3^{P-UTR}/+*, and genetic control (*rpd3^{P-1.8}/+*) heterozygous male (M) and female (F) flies after exposure to H₂O₂ at 10 or 40 days of age. Control values are compared to either male or female *rpd3^{def}/+* groups to determine the percent change in mean and maximal lifespan. Mean and median lifespan are in hours. N = number of flies in the experiment. Log-rank analyses were performed using the JMP 12 program.

Supplemental Table 4. *dfoxo* is required for increased starvation resistance in *rpd3^{def}* heterozygous flies at 40 days.

Gender	Genotype	N	Mean (% change)	Median LS	χ^2	p
M	<i>rpd3^{def}/yw</i>	102	29	29		
M	<i>dfoxo^{c01841}/yw</i>	101	20 (-31)	20	82.3659	<0.0001
M	<i>rpd3^{def}/yw</i>	102	29	29		
M	<i>rpd3^{def}/dfoxo^{c01841}</i>	60	24 (-17)	23	16.1367	<0.0001
F	<i>rpd3^{def}/yw</i>	83	55	47		
F	<i>dfoxo^{c01841}/yw</i>	94	53 (-4)	51	1.7	0.18
F	<i>rpd3^{def}/yw</i>	83	55	47		
F	<i>rpd3^{def}/dfoxo^{c01841}</i>	90	44 (20)	45	16.3721	<0.0001*

The mean and median lifespans of *rpd3^{def}/+*, *rpd3^{def}/dfoxo^{c01841}*, and *dfoxo^{c01841}/+* heterozygous male (M) and female (F) flies. *dfoxo^{c01841}* flies are hypomorphic for *dfoxo*. All values are compared to either male or female *rpd3^{def}/+* groups to determine the percent change in mean and median lifespan. Mean and median lifespan are in hours. The similarity in stress resistance between *rpd3^{def}/dfoxo^{c01841}* and *dfoxo^{c01841}/+* male and female flies was also determined. N: number of flies used in the experiment. Three independent experiments with similar results were combined. Mean and Median lifespans are in hours. Log-rank analyses were performed using the JMP 12 program.

Supplemental Table 5. *dfoxo* is required for increased H₂O₂ resistance in *rpd3^{def}* heterozygous flies.

Gender	Genotype	N	Mean (% change)	Median LS	χ^2	p	Maximal life span (% change)
M	<i>rpd3^{def}/+</i>	166	30	32			42
M	<i>rpd3^{def}/dfoxo^{c01841}</i>	82	17 (43)	18	139.6062	<0.0001*	24 (42)
M	<i>dfoxo^{c01841}/+</i>	88	21 (30)	23	82.7128	<0.0001*	32 (22)
F	<i>rpd3^{def}/+</i>	166	41	40			57
F	<i>rpd3^{def}/dfoxo^{c01841}</i>	128	48 (-17)	47	26.7491	<0.0001*	71 (-20)
F	<i>dfoxo^{c01841}/+</i>	80	30 (27)	32	89.8302	<0.0001*	43 (25)

The mean and median lifespans of *rpd3^{def}/+*, *rpd3^{def}/dfoxo^{c01841}*, and *dfoxo^{c01841}/+* heterozygous male (M) and female (F) flies after exposure to H₂O₂ at 40 days of age. *Dfoxo^{c01841}* flies are hypomorphic for *dfoxo*. All values are compared to either male or female *rpd3^{def}/+* groups to determine the percent change in mean or median lifespan. N: number of flies used in the experiment. Three independent experiments with similar results were combined. Mean and median lifespan are in hours. Log-rank analyses were performed using the JMP 12 program.