Table 6: Effect of *lin-23(ot1)* on the extension of lifespan induced by *daf-2* reduction-of-function mutations and RNAi

Genotype*	Temperature=	Trial	Mean LS ± SEM (days)	Events /Obs‡	P value vs. control§	% change in lifespan
Table 6.A: Effect of *lin-23(ot1)* mutation on the extended lifespan of *daf-2* mutants						
daf-2(e1368)	20°C whole life	1	33.4 ± 1.0	67/94		
lin-23(ot1); daf-2(e1368) line # 15			25.0 ± 0.8	64/85	<0.0001	-25.1
lin-23(ot1); daf-2(e1368) line # 28			24.3 ± 0.8	70/88	<0.0001	-27.2
daf-2(e1368)	20°C whole life	2¶	36.3 ± 1.1	43/72		
lin-23(ot1); daf-2(e1368) line # 15			21.6 ± 0.7	46/68	<0.0001	-40.4
lin-23(ot1); daf-2(e1368) line # 28			23.7 ± 0.7	56/86	<0.0001	-34.7
daf-2(e1370) ¶	20°C whole life	1¶	55.2 ± 2.0	58/86		
lin-23(ot1); daf-2(e1370) line # 12.2¶			37.4 ± 2.2	41/79	<0.0001	-32.2
lin-23(ot1); daf-2(e1370) line # 27.1¶			38.8 ± 1.6	57/90	<0.0001	-29.7
lin-23(ot1); daf-2(e1370) line # 29.1			37.3 ± 2.1	44/73	<0.0001	-32.4
lin-23(ot1); daf-2(e1370) line # 11.1			42.2 ± 1.9	47/87	<0.0001	-23.5
lin-23(ot1); daf-2(e1370) line # 13.2			36.0 ± 2.2	47/75	<0.0001	-34.7
lin-23(ot1); daf-2(e1370) line # 37.1			39.6 ± 1.7	63/79	<0.0001	-28.2
daf-2(e1370)	20°C whole life	2	51.1 ± 1.1	102/111		
lin-23(ot1); daf-2(e1370) line # 12.2			35.3 ± 1.5	96/107	<0.0001	-30.9
lin-23(ot1); daf-2(e1370) line # 27.1			34.6 ± 1.5	83/105	<0.0001	-32.2
lin-23(ot1); daf-2(e1370) line # 29.1			32.0 ± 1.8	86/102	<0.0001	-37.3
lin-23(ot1); daf-2(e1370) line # 11.1			38.1 ± 1.9	65/109	<0.0001	-25.4
lin-23(ot1); daf-2(e1370) line # 13.2			36.8 ± 1.7	92/110	<0.0001	-27.9
lin-23(ot1); daf-2(e1370) line # 37.1			39.6 ± 1.6	80/101	<0.0001	-22.5
daf-2(e1368)	25°C Adult-only	1	34.1 ± 1.1	84/95		
lin-23(ot1); daf-2(e1368) line # 15			21.2 ± 0.7	82/84	<0.0001	-37.8
lin-23(ot1); daf-2(e1368) line # 28			22.9 ± 0.8	75/85	<0.0001	-32.8

Genotype[*]	Temperature[=]	Trial	Mean LS ± SEM (days)	Events/ Obs[‡]	P value vs. control[§]	% change in lifespan
daf-2(e1370)	25°C Adult-only[‡‡]	1	40.7 ± 1.7	89/113		
lin-23(ot1); daf-2(e1370) line # 12.2			42.7 ± 1.4	90/111	0.7	+4.9
lin-23(ot1); daf-2(e1370) line # 27.1			44.8 ± 1.9	91/93	0.03	+10.0
lin-23(ot1); daf-2(e1370) line # 29.1			46.6 ± 1.5	96/99	0.04	+14.4
lin-23(ot1); daf-2(e1370) line # 11.1			52.1 ± 1.1	107/109	<0.0001	+28.0
lin-23(ot1); daf-2(e1370) line # 13.2			51.3 ± 1.6	106/107	<0.0001	+26.0
lin-23(ot1); daf-2(e1370) line # 37.1			55.4 ± 1.2	112/112	<0.0001	+36.1

Genotype[*]	RNAi Treatment[§§]	Temperature[=]	Mean LS ± SEM (days)	Events /Obs[‡]	P value (control vs. *daf-2* RNAi)	P value (N2 vs. *lin-23*)	% change in lifespan
Table 6.B: Response of *lin-23(ot1)* mutants to *daf-2* RNAi							
N2	Control[¶]	20°C whole life	20.7 ± 0.6	58/111			
	daf-2[¶]		39.7 ± 1.4	74/106	<0.0001		+91.7
lin-23(ot1) Line #1	Control[¶]		19.1 ± 0.5	62/106		0.09	-7.7 (vs. N2)[¶¶]
	daf-2[¶]		24.3 ± 1.5	68/105	<0.0001		+27.2
lin-23(ot1) Line #2	Control		17.2 ± 0.4	62/103		<0.0001	-16.9 (vs. N2)[¶¶]
	daf-2		21.8 ± 1.3	58/99	<0.0001		+26.7
N2	Control	25°C whole life	16.3 ± 0.3	72/98			
	daf-2		26.5 ± 1.0	75/100	<0.0001		+62.5
lin-23(ot1) Line #1	Control		13.0 ± 0.2	89/94		<0.0001	-20.2 (vs. N2)[¶¶]
	daf-2		16.6 ± 0.7	81/94	<0.0001		+27.6
lin-23(ot1) Line #2	Control		13.2 ± 0.2	84/93		<0.0001	-19.0 (vs. N2)[¶¶]
	daf-2		13.5 ± 0.6	91/100	0.11		+2.2
N2	Control	25°C Adult-only	16.2 ± 0.4	82/105			
	daf-2		28.9 ± 0.9	88/105	<0.0001		+78.3
lin-23(ot1) Line #1	Control		14.7 ± 0.3	49/85		<0.009	-9.2 (vs. N2)[¶¶]
	daf-2		17.0 ± 0.8	78/91	<0.0083		+15.6
lin-23(ot1) Line #2	Control		13.4 ± 0.3	81/108		<0.0001	-12.3 (vs. N2)[¶¶]
	daf-2		14.2 ± 0.6	95/101	0.02		+5.9

* See below for details of strains.

= Worms were grown from hatching onwards for their entire life at 20°C (20°C whole life) or 25°C (25°C whole life). In temperature transfer experiments, worms were grown at 20°C till L4 or the first day of adulthood, and then transferred to 25°C (25°C adult only). In all double mutant experiments, worms were grown on normal food (OP50) throughout their lifespan.

§§ For all RNAi experiments, animals were fed normal food till L4 or the first day of adulthood and then transferred to bacteria expressing dsRNA for empty vector (control) or *daf-2* (*daf-2* RNAi).

‡ Some animals were censored as described in Materials and Methods.

§ Control refers to worms exposed to empty vector plasmid without an RNAi insert (in RNAi experiments, 6.B), or the relevant *daf-2* single mutant (in double mutant lifespans, 6.A).

¶ Lifespans shown in Fig. 6.

¶¶ In all cases tested, the *lin-23(ot1)* reduces the lifespan of wild-type worms (empty vector control) to a smaller degree, as compared to the effect on the lifespan extension induced by *daf-2* RNAi.

‡‡ *lin-23(ot1)* extended the lifespan of *daf-2(e1370)* mutants at 25°C. The reason underlying this temperature-dependent lifespan effect is unclear, but the phenomenon is reminiscent of the lifespan extension produced by the *daf-2(e1370)*

mutation in combination with other longevity mutations such as *sir-2.1(ok434)* (1) and *daf-12(m20) (2) .* Similar to *lin-23, sir-2.1* and *daf-12* are longevity-promoting genes in other contexts (3, 4).

***lin-23(ot1) mutant strains**

Since all other known *lin-23* mutants have cell-cycle defects they could not be used to address the role of this gene in lifespan regulation.

The *lin-23(ot1)* mutant (OH1476 *lin-23(ot1) II; oxIs12 [Punc-47::gfp] X*) was outcrossed thrice to our laboratory N2 stock (N2A) to generate the strain CF2279 *lin-23(ot1) II; oxIs12 [Punc-47::gfp] X*. Two independent isolates [CF2279a (Line #7.4, designated Line#1 above in 6.B) and CF2279b (Line #11.7, designated Line #2 above in 6.B)] were used in this study. CF2279a was crossed to CF1041 *daf-2(e1370)* and DR1572 *daf-2(e1368)* to generate double mutants strains with the following genotypes: *lin-23(ot1) II; daf-2(e1370) III*; *oxIs12 [Punc-47::gfp] X;* and *lin-23(ot1) II; daf-2(e1370) III*. In each case, three independent lines CF2272a (Line # 12.2, designated Line#1 in Fig. 5), CF2272b (Line #27.1, designated Line #2 in Fig. 5), CF2272c (Line #29.1) and CF2273a (Line #11.1), b (Line #13.2), c (line #37.1), respectively, were tested in lifespan assays. Expectedly, we found no effect of the *oxIs12* construct on lifespan. CF2279a was crossed to DR1572 *daf-2(e1368)* to generate the strains CF2274a (Line #15, designated Line #1 in Fig. 5) and CF2274b (Line #28, designated

Line #2 in Fig. 5) with the following genotype: *lin-23(ot1) II; daf-2(e1368) III; oxIs12 [Punc-47::gfp] X.* These strains were used in the experiments described in this study.

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