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Supplementary Materials for

Genetic and pharmacological interventions in the aging motor nervous system slow motor aging and extend life span in *C. elegans*

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This PDF file includes:

Figure S1

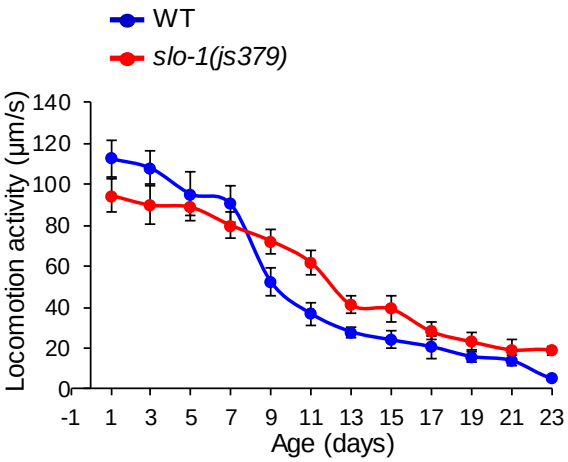

Fig. S1. *slo-1* mutant worms exhibit slower locomotor activity decline throughout life span. The experiments were done as described in Figure 1 except that worms were analyzed for locomotion throughout the entire lifespan. Error bars: SEM. n≥20.

Figure S2

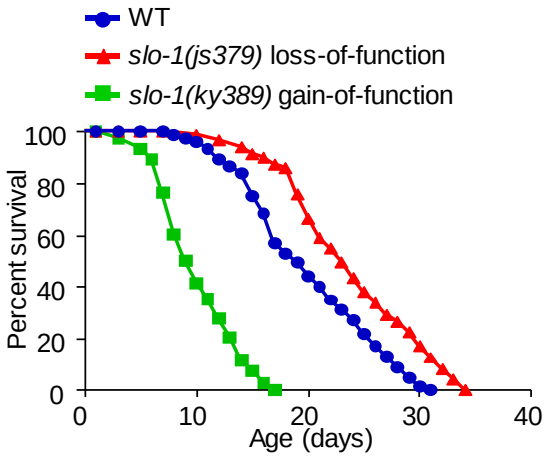

Fig. S2. *slo-1* gain-of-function mutation greatly shortens life span. *slo-1(ky389)* mutation greatly shortens lifespan ($p<0.0001$, Log Rank test).

Figure S3

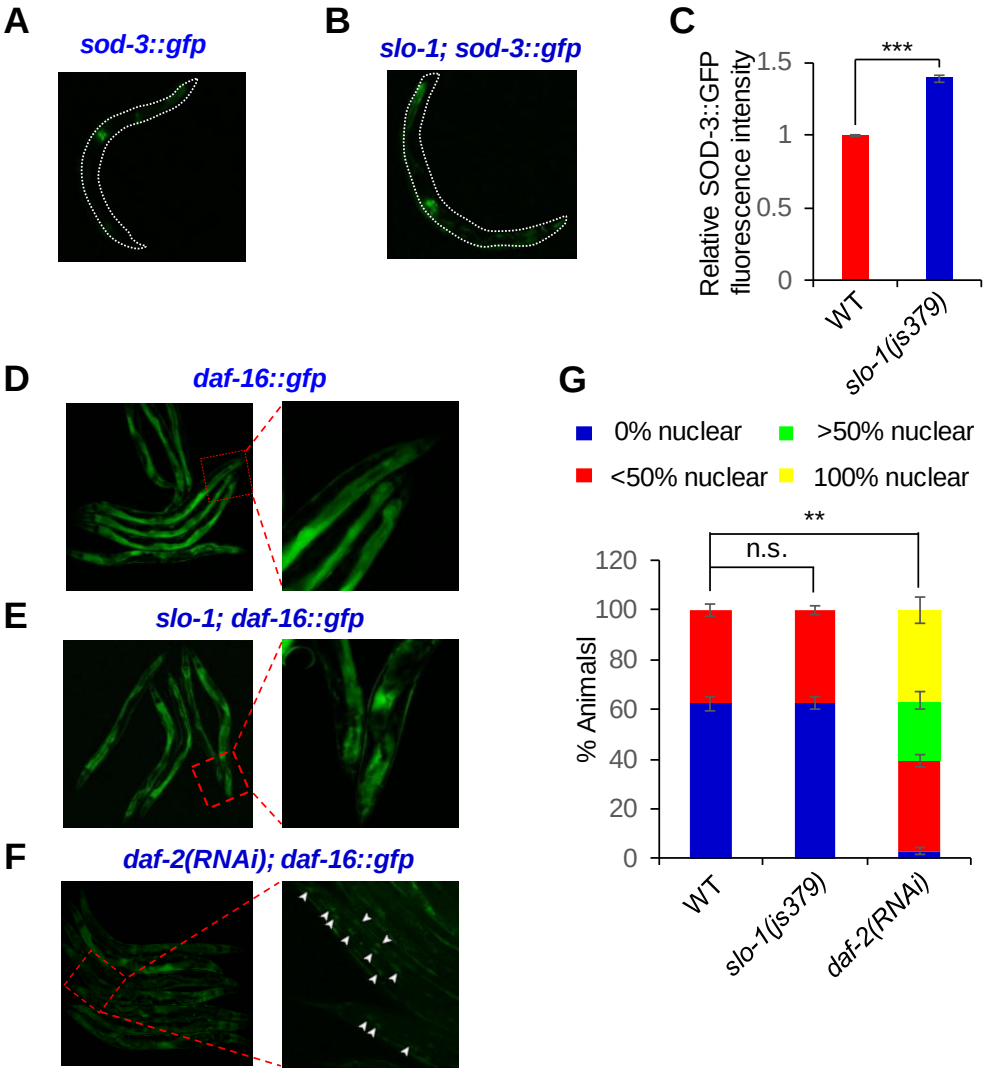

Fig. S3. DAF-16 target gene expression and DAF-16 nuclear translocation in *slo-1* mutant worms.

(A-C) the DAF-16 target gene *sod-3* is upregulated in *slo-1(js379)* mutant worms. Shown in (A) and (B) are representative images of worms expressing a transgene expressing SOD-3::GFP. The dotted lines in white drawn around the worm denote the boundary of the worm. Data in (A) and (B) are summarized in (C). Error bars: SEM. ***p<0.00001 (t test). n≥20

(D-G) DAF-16 subcellular localization is not affected in *slo-1* mutant worms. Shown in (D-F) are sample images of worms carrying a *daf-16::gfp* transgene. Shown to the right are zoom-in images. (G) bar graph summarizing the data in (D-F). No notable difference was observed between WT and *slo-1(js379)* mutant worms in DAF-16::GFP nuclear localization pattern. *daf-2(RNAi)* serves as a control. Arrow heads in (F) point to nuclei. Error bars: SEM. **p<0.001. n≥25.

Figure S4

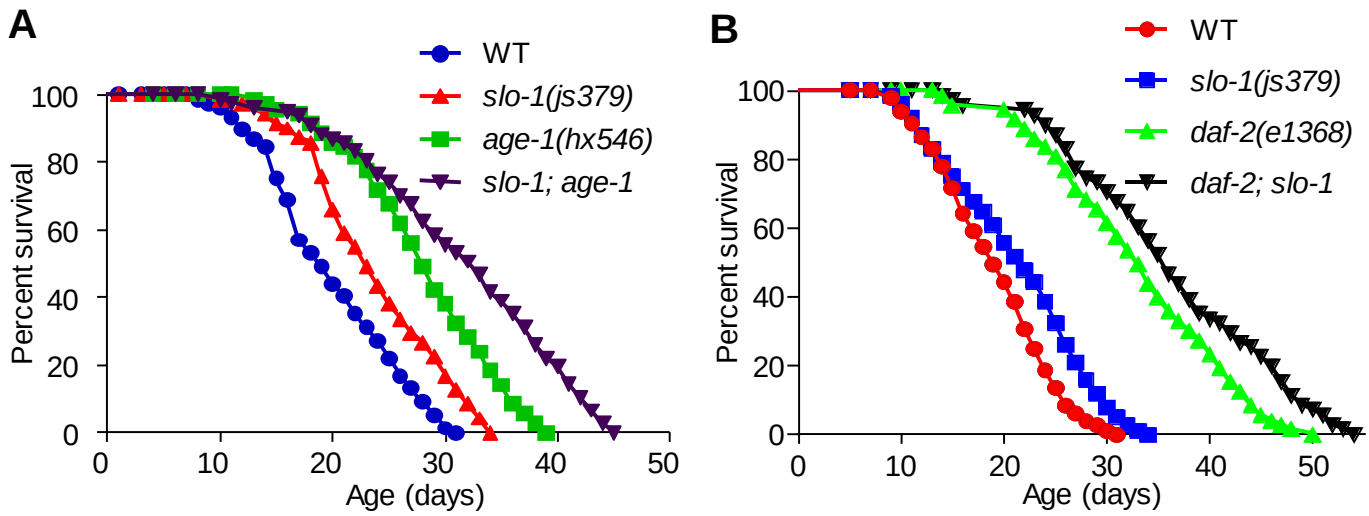

Fig. S4. *slo-1* and IIS act in parallel to regulate life span. *slo-1* mutation can further extend the lifespan of mutants defective in insulin/IGF-1signaling, such as *age-1(hx546)* (A) ($p<0.001$, Log Rank test), and *daf-2(e1368)* mutants (B) ($p<0.005$, Long Rank test). Fig. S4A and Fig. S2 were performed at the same time and share the same WT and *slo-1* lifespan curves.

Figure S5

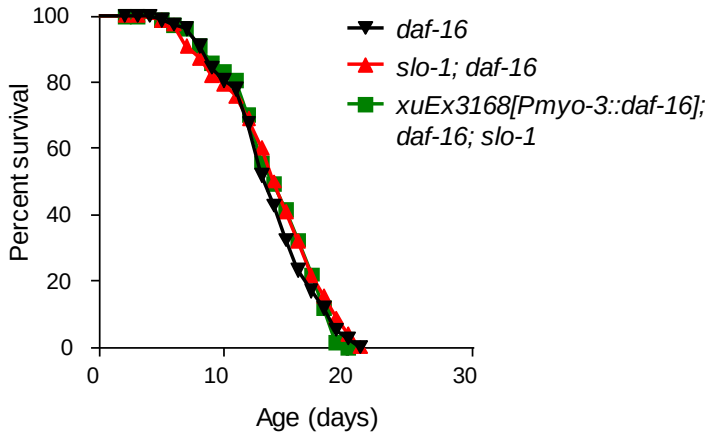

Fig. S5. Expression of *daf-16* cDNA in the muscle fails to rescue the *daf-16* mutant phenotype. Loss of *daf-16* suppressed the long-lived phenotype of *slo-1* worms (see Figure 4A-B). Expression of *daf-16* cDNA in the muscle using *myo-3* promoter cannot rescue the *daf-16* phenotype (p=0.843, Log Rank test).

Figure S6

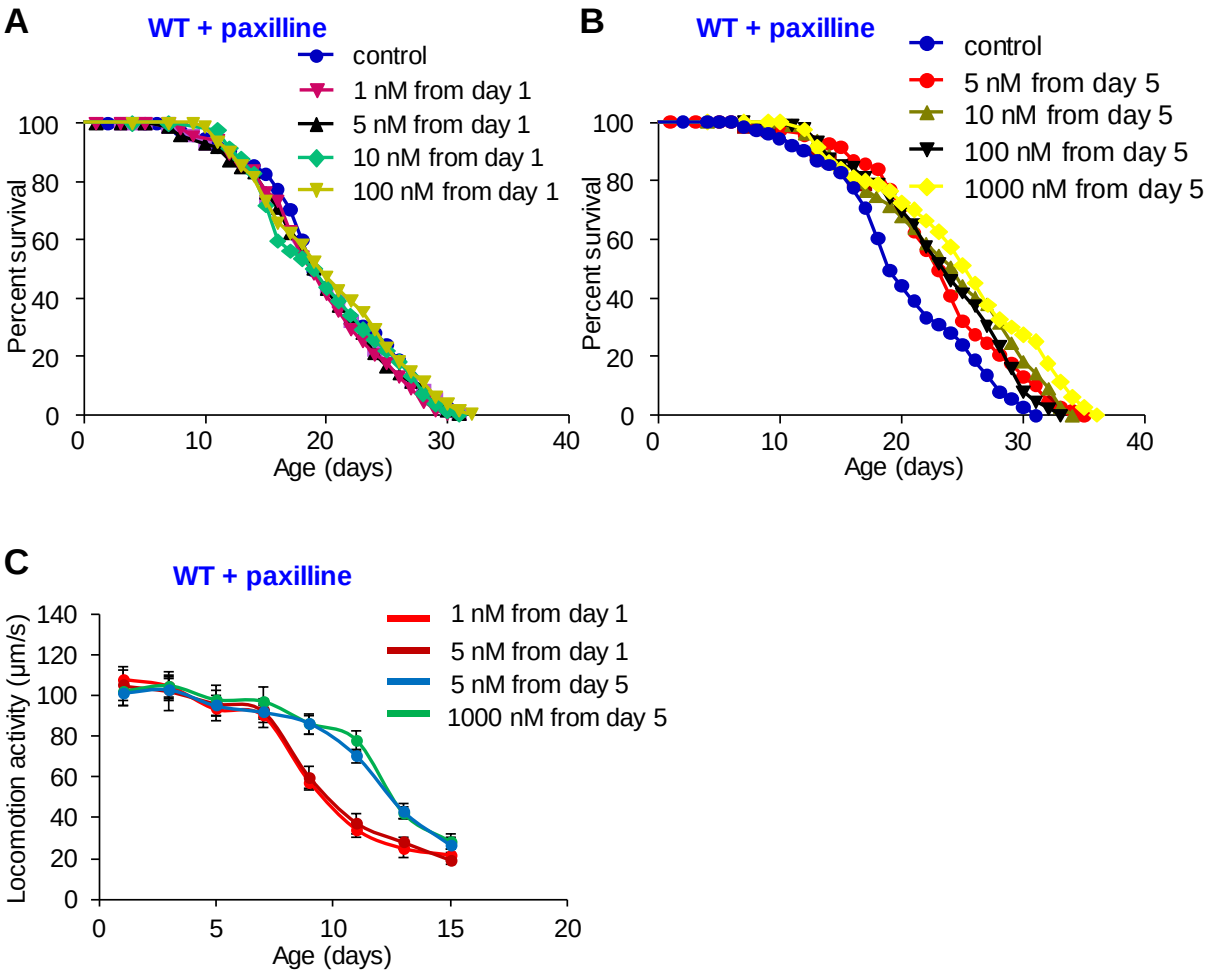

Fig. S6. The effects of different concentrations of paxilline on life span and motor aging. (A) paxilline, when applied to worms in early life (day 1), do not extend lifespan even at concentrations as low as 1 nM ($p=0.664$, Log Rank test). **(B)** paxilline, when applied to worms at a later age (day 5) can extend lifespan even at concentrations as high as 1000 nM ($p<0.003$, Log Rank test). (A) and (B) were performed at the same time and share the same control curve. **(C)** paxilline does not slow motor activity decline with age when applied to worms in early life (day 1) even at concentrations as low as 1 nM; by contrast, paxilline slows motor activity decline with age when applied to worms at a later age (day 5) even at concentrations as high as 1000 nM. Error bars: SEM. $n \geq 20$.

Figure S7

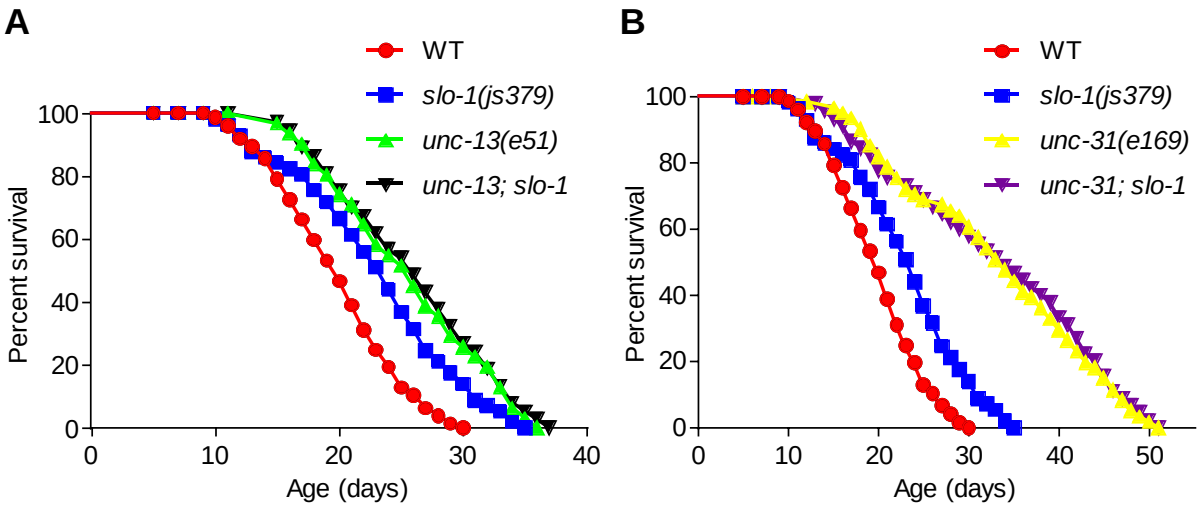

Fig. S7. Loss of *slo-1* does not extend the life span of mutants defective in neurotransmission. *slo-1* null mutation (*js379*) cannot further extend the lifespan of mutants defective in neurotransmission, such as *unc-13(e51)* (A) (p=0.712, Log Rank test), and *unc-31(e169)* mutants (B) (p=0.830, Log Rank test). WT and *slo-1* curves in (A) and (B) are identical and shown for convenience of comparison.

Table S1. Summary of life-span data.

Strain	Temp(°C)	Mean Lifespan (days)	SEM	SD	Median Lifespan (days)	75% Lifespan (days)	n (assay/ total)	P Value against	Figure
N2	20	18.4	0.6	5.4	19	23	72/112	—	1C
slo-*1(js379)*	20	22	0.6	5.1	22	26	58/80	<0.005	1C
N2	20	20.5	0.6	4.7	21	24	72/112	—	Exp.2
slo-*1(js379)*	20	23	0.7	5.7	23	27	58/80	<0.0005	Exp.2
N2	20	18.9	0.6	4.8	19	23	65/98	—	2A
slo-*1(js379)*	20	22.6	0.8	6.5	23	28	60/92	<0.0001	2A
slo-1; Prgef-1::slo-1	20	20	0.7	5.6	20	24	62/90	0.1033	2A
slo-1; Pmyo-3::slo-1	20	23.1	0.6	5.2	24	27	87/110	<0.005	2A
slo-1; Posm-6::slo-1	20	22.7	0.7	6.2	24	27	69/103	<0.0001	2A
slo-1; Pacr-2::slo-1+Punc-25::slo-1	20	20.3	0.3	5.1	20	24	60/104	0.1086	2A
N2	20	20.1	0.7	5.7	20	25	67/99	—	Exp.2
slo-*1(js379)*	20	23.5	0.9	6.8	24	28	63/97	<0.005	Exp.2
slo-1; Prgef-1::slo-1	20	19.7	0.9	5.7	20	24	61/93	0.812	Exp.2
slo-1; Pmyo-3::slo-1	20	22.5	0.9	7.6	23	28	71/100	<0.005	Exp.2
slo-1; Posm-6::slo-1	20	22.9	0.8	6.6	24	28	64/98	<0.005	Exp.2
slo-1; Pacr-2::slo-1+Punc-25::slo-1	20	20.3	0.6	4.3	21	24	88/101	0.978	Exp.2
L4440 control	20	15.7	0.3	3.3	15	18	110/120	—	3E
***slo-1* RNAi from day 1**	20	15.3	0.4	3.7	15	18	68/88	0.5827	3E
***slo-1* RNAi from day 3**	20	15.9	0.3	3.3	15	18	92/102	0.8497	3E
L4440 control	20	15.7	0.3	3.3	15	18	110/120	—	3F

***slo-1* RNAi from day 5**	20	18.5	0.4	4.0	19	21	88/108	<0.0001	3F
***slo-1* RNAi from day 7**	20	18.6	0.4	4.0	19	22	98/109	<0.0001	3F
L4440 control	20	21.1	0.6	5.4	21	26	84/95	—	Exp.2
***slo-1* RNAi from day 1**	20	21.1	0.5	7.3	21	27	106/116	0.3813	Exp.2
***slo-1* RNAi from day 3**	20	21.7	0.6	6.1	22	27	113/123	0.2368	Exp.2
L4440 control	20	21.1	0.6	5.4	21	26	84/95	—	Exp.2
***slo-1* RNAi from day 5**	20	23.1	0.7	6.9	23	30	106/117	<0.05	Exp.2
***slo-1* RNAi from day 7**	20	23.2	0.7	6.9	23	29	97/110	<0.005	Exp.2
N2	20	18.1	0.3	4.2	18	22	67/84	—	4A,B
slo-1(js379)	20	21.1	0.9	8.3	22	25	69/84	<0.0001	4A,B
daf-16 (mgDF47)	20	13.3	0.7	6.4	14	17	75/84	<0.0001	4A,B
slo-1(js379); daf-16 (mgDF47)	20	14	0.2	3.2	14	18	66/84	<0.005	4A,B
Pges-1::DAF-16; *slo-1(js379); daf-16(mgDF47)*	20	19.2	0.2	3.2	19	22	72/84	0.3433	4A,B
Prgef-1::DAF-16; *slo-1(js379); daf-16(mgDF47)*	20	22.1	0.3	4.1	22	26	71/84	<0.005	4A,B
N2	20	18.6	0.2	3.1	18	22	64/83	—	Exp.2
slo-1(js379)	20	22.1	0.3	3.3	22	25	72/84	<0.005	Exp.2
daf-16 (mgDF47)	20	13.2	0.2	3.3	13	18	75/84	<0.005	Exp.2
slo-1(js379); daf-16 (mgDF47)	20	14.1	0.3	4.1	14	18	69/86	<0.005	Exp.2
Pges-1::DAF-16; *slo-1(js379); daf-16(mgDF47)*	20	18	0.4	5.1	18	22	67/84	0.2236	Exp.2
Prgef-1::DAF-16; *slo-1(js379); daf-	20	22.1	0.4	5.3	22	25	69/87	<0.005	Exp.2

16(mgDF47)									
Control	20	18.1	0.3	4.1	18	21	80/100	—	5A
day 1 + Paxiline	20	18.4	0.3	3.6	18	21	82/100	0.2641	5A
day 3 + Paxiline	20	18.5	0.3	4.1	18	22	83/100	0.3754	5A
Control	20	18.4	0.1	1.7	18	21	79/100	—	5B
day 5 + Paxiline	20	20.5	0.5	6.2	21	23	78/100	<0.001	5B
day 7 + Paxiline	20	23.3	0.5	6.4	23	26	74/100	<0.0001	5B
slo-1(js379)	20	22.1	0.3	4.2	22	25	82/100	—	5C
slo-1(js379)**; day 5 + Paxiline**	20	22.2	0.4	3.7	22	25	80/100	0.2244	5C
slo-1(js379)**; day 7 + Paxiline**	20	21.1	0.4	4.1	21	24	77/101	0.3901	5C
daf-16 (mgDF47)	20	14.3	0.3	4.3	14	18	75/84	—	
***daf-16 (mgDF47); day* 5 + Paxiline**	20	14.4	0.6	7.1	14	18	72/84	0.5542	
***daf-16 (mgDF47);* day 7 + Paxiline**	20	14.1	0.3	4.3	14	18	74/84	0.4371	
Control	20	18.2	0.5	6.2	18	21	67/100	—	Exp.2
day 1 + Paxiline	20	17.6	0.7	7.4	18	21	66/100	0.4351	Exp.2
day 3 + Paxiline	20	18.2	0.6	7.3	18	22	69/100	0.4455	Exp.2
Control	20	18.3	0.4	5.2	18	21	70/100	—	Exp.2
day 5 + Paxiline	20	19.8	0.6	7.1	20	23	77/100	<0.001	Exp.2
day 7 + Paxiline	20	22.4	0.5	6.2	22	25	87/100	<0.005	Exp.2

slo-1(js379)	20	22.3	0.6	6.6	22	25	74/100	—	Exp.2
***slo-1(js379)*;** **day 5 + Paxiline**	20	22.4	0.5	6.3	22	25	71/100	0.5332	Exp.2
***slo-1(js379)*;** **day 7 + Paxiline**	20	23.3	0.4	5.2	23	25	67/100	0.5134	Exp.2
daf-16 (mgDF47)	20	14.2	0.4	4.7	14	18	76/84	—	Exp.2
***daf-16 (mgDF47); day* 5 + Paxiline**	20	14.5	0.2	3.3	14	18	73/84	0.5001	Exp.2
***daf-16 (mgDF47); day* 7 + Paxiline**	20	14.2	0.2	3.3	14	18	74/84	0.6054	Exp.2

Note: two sets of lifespan data are listed for each experiment. The first set is graphed in the figures. The second set (replicates) is shown here as "Exp. 2". Figure 3E and Fig. 3F share the same control curve.
The Log Rank (Mantel-Cox) test was used for statistical analysis.