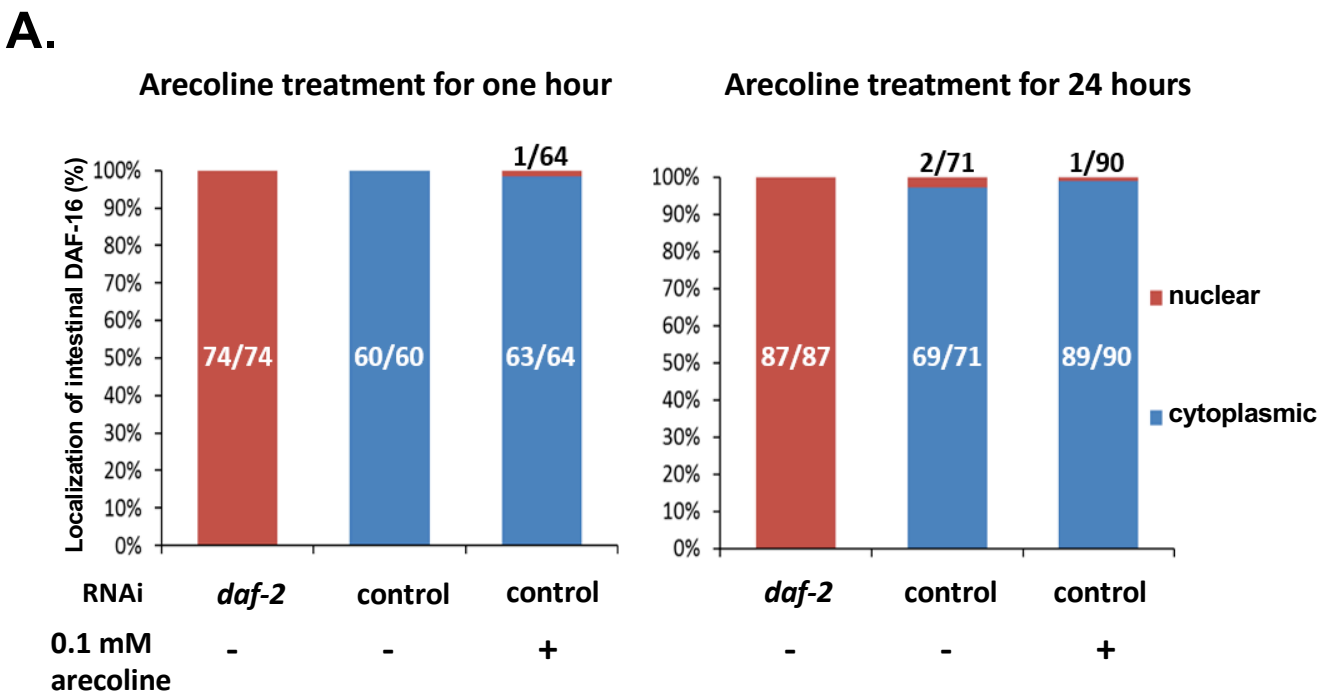
Supplementary Figures and Table



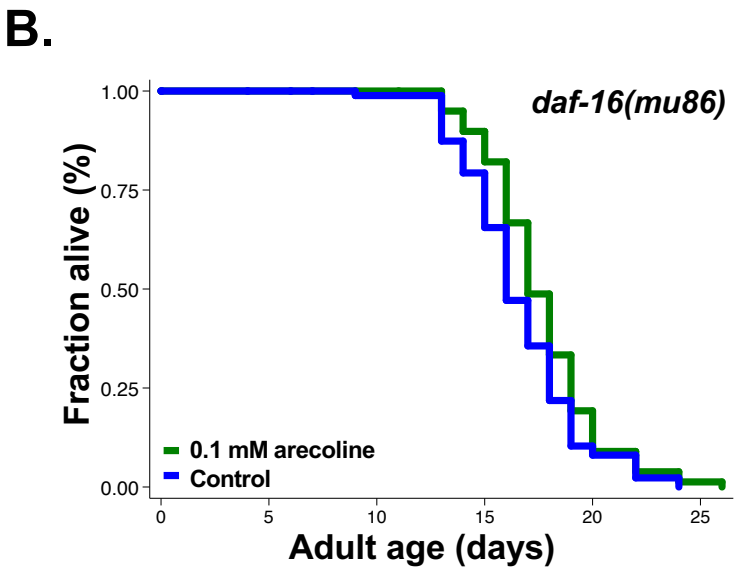


Figure S1. Arecoline-induced longevity is partially dependent on DAF-16

A. DAF-16::GFP subcellular localization assay. Day 1 transgenic worms expressing DAF-16::GFP grown on bacteria containing empty vector were treated with or without 0.1 mM arecoline for one and 12 hours. Worms fed with *daf-2* RNA bacteria were served as positive controls. Results show the percentage of worms showing cytosolic (blue) and nuclear (red) localization of DAF-16. n=60–90 worms per condition.

B. Survival curves of *daf-16(mu86)* mutants with vehicle (blue) or 0.1 mM arecoline (green) from adult Day 4 to Day 6. $p = 0.0351$. *P*-values are derived from log-rank test. The graphs are representative of three independent experiments.

Table S1. Statistical data for lifespan experiments, Related to Figures 1 and S1.

	Strain	Treatment	Mean Lifespan ±SEM	75% Percentile (Days)	n	*P* value	Extension (%)
*	N2	0 mM	22.05±0.95	27	65/72		
*	N2	0.2 mM whole life	7.60±0.54	9	61/72	<0.00001	-65.5
*	N2	0.2 mM D1-D3	19.54±0.80	25	67/72	0.1173	-11.4
	N2	0 mM	19.16±0.81	23	55/69		
	N2	0.2 mM whole life	7.38±0.50	9	57/72	<0.00001	-61.5
	N2	0 mM	14.81±0.50	18	65/75		
	N2	0.2 mM D1-D3	11.78±0.74	16	59/79	0.0741	-20.5
	N2	0.2 mM D4-D6	20.01±0.76	24	54/82	<0.00001	35.1
*	N2	0 mM	26.68±0.83	29	60/72		
*	N2	0.2 mM D3-D5	28.19±0.85	33	60/72	0.1389	5.7
*	N2	0.2 mM D4-D6	30.31±0.52	35	64/72	0.0002	13.6
	N2	0.2 mM D5-D7	30.25±0.78	35	61/72	0.0004	13.4
	N2	0 mM	24.19±0.65	28	46/68		
	N2	0.2 mM D6-D8	23.75±0.92	28	56/77	0.8875	
	N2	0 mM	25.96±2.04	31	49/73		
	N2	0.2 mM D4-D6	27.64±2.51	33	57/73	0.071	6.5
	N2	0.2 mM D5-D7	29.14±1.84	35	55/73	0.0044	12.3
	N2	0.2 mM D6-D8	25.41±1.04	31	56/75	0.5953	
	N2	0 mM	26.02±0.72	29	67/72		
	N2	0.1 mM D4-D6	28.14±0.83	33	69/72	0.0272	8.2
	N2	0.2 mM D4-D6	28.67±0.86	33	65/72	0.0061	10.2
	N2	0 mM	27.31±0.52	31	58/72		
	N2	0.004 mM D4-D6	29.19±0.85	33	62/76	0.0647	6.9
	N2	0.02 mM D4-D6	28.84±0.46	33	51/65	0.1309	5.6
	N2	0.1 mM D4-D6	30.24±1.00	33	56/72	0.0066	10.6
	N2	0 mM	25.46±1.33	29	73/84		
	N2	0.05 mM D4-D6	27.02±1.53	31	73/84	0.1863	6.1
	N2	0.1 mM D4-D6	28.01±1.52	33	71/84	0.0423	10
*	N2	0 mM	25.71±0.95	29	72/83		
	N2	0.05 mM D4-D6	28.42±0.82	33	70/83	0.0053	9.5
*	N2	0.1 mM D4-D6	29.01±1.00	33	65/83	0.0007	12.8
*	*gar-1(ok755)*	0 mM	26.54±0.68	31	68/85		
*	*gar-1(ok755)*	0.1 mM D4-D6	29.78±0.81	33	66/84	0.0009	12.2
	gar-1(ok755)	0 mM	25.33±1.03	30	82/84		
	gar-1(ok755)	0.1 mM D4-D6	27.31±1.00	32	77/84	0.0391	7.8
*	*gar-2(ok520)*	0 mM	24.89±1.47	30	68/84		
*	*gar-2(ok520)*	0.1 mM D4-D6	24.47±1.33	30	69/84	0.897	
	gar-2(ok520)	0 mM	25.83±1.09	31	64/84		
	gar-2(ok520)	0.1 mM D4-D6	25.97±0.95	31	51/84	0.9372	
	gar-2(ok520)	0 mM	25.15±1.33	29	74/84		
	gar-2(ok520)	0.1 mM D4-D6	25.95±1.81	33	67/84	0.7197	
*	*gar-3(gk305)*	0 mM	24.54±1.30	27	62/84		
*	*gar-3(gk305)*	0.1 mM D4-D6	27.61±1.15	33	57/84	0.0227	12.5
	gar-3(gk305)	0 mM	28.29±0.87	34	78/84		
	gar-3(gk305)	0.1 mM D4-D6	30.46±1.15	36	68/84	0.0566	7.7
	gar-3(gk305)	0 mM	30.40±0.63	35	60/84		
	gar-3(gk305)	0.1 mM D4-D6	32.42±0.68	35	69/84	0.0417	6.6
	egl-8(n488)	0 mM	39.36±1.08	46	99/120		
	egl-8(n488)	0.1 mM D4-D6	39.53±0.78	44	86/120	0.8892	
	egl-8(n488)	0 mM	35.90±1.73	42	96/120		
	egl-8(n488)	0.1 mM D4-D6	35.28±0.86	42	101/120	0.7125	
	egl-8(n488)	0 mM	37.99±0.69	43	100/120		
	egl-8(n488)	0.1 mM D6-D8	37.37±0.75	41	95/120	0.4018	
*	N2		21.55±0.59	23	53/84		
*	*gar-2(ok520)*		22.43±0.44	25	64/84		
*	*gar-2(ok520); myo-3p::gar-2*		21.69±0.68	23	57/83	0.4667 (vs *gar-2(ok520)*)	-3.3
*	*gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2*		25.94±0.99	31	66/83	0.0004 (vs *gar-2(ok520)*)	15.7
	N2		24.85±0.78	27	61/84		
	gar-2(ok520)		23.06±0.66	25	57/84		
	gar-2(ok520); myo-3p::gar-2		22.58±0.38	25	74/84	0.6749 (vs *gar-2(ok520)*)	-2.1
	gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2		26.42±0.53	29	76/84	<0.00001(vs *gar-2(ok520)*)	14.6
*	*gar-2(ok520)*	0 mM	24.89±1.47	30	68/84		
*	*gar-2(ok520)*	0.1 mM D4-D6	24.47±1.33	30	69/84	0.6837	
*	*gar-2(ok520); myo-3p::gar-2*	0 mM	21.52±0.55	23	67/84		
*	*gar-2(ok520); myo-3p::gar-2*	0.1 mM D4-D6	20.82±0.67	23	66/84	0.2625	
	gar-2(ok520); myo-3p::gar-2	0 mM	20.86±0.43	23	61/84		
	gar-2(ok520); myo-3p::gar-2	0.1 mM D4-D6	20.14±0.31	21	66/84	0.1097	
*	*gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2*	0 mM	22.54±0.42	25	78/84		
*	*gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2*	0.1 mM D4-D6	24.21±0.52	26	75/84	0.0302	7.4
	gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2	0 mM	23.00±0.55	25	65/84		
	gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2	0.1 mM D4-D6	24.45±0.44	25	62/84	0.0523	6.3
	gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2	0 mM	24.31±0.53	27	70/84		
	gar-2(ok520); acr-2p::gar-2 +unc-25p::gar-2	0.1 mM D4-D6	25.76±0.46	29	71/84	0.0541	6
	daf-16(mu86)	0 mM	17.34±0.25	19	66/84		
	daf-16(mu86)	0.1 mM D4-D6	18.40±0.37	20	66/84	0.0311	6.1
	daf-16(mu86)	0 mM	16.64±0.36	18	87/96		
	daf-16(mu86)	0.1 mM D4-D6	17.63±0.34	19	78/96	0.0112	6

	daf-16(mu86)	0 mM	16.35±0.35	18	62/84		
	daf-16(mu86)	0.1 mM D4-D6	17.47±0.47	19	64/84	0.0321	6.9
	RB756: *gar2(ok520)*		22.25±0.63	25	76/84		
	EQ1023: *gar-2(ok520) 6X*		21.56±0.48	25	70/84	0.2603	
	***tom-1* RNAi treatment**						
	N2 on EV		19.93±0.67	25	91/110		
	N2 on *tom-1* RNAi (eggs)		18.89±0.79	23	76/99	0.2952	
*	N2 on EV		18.49±1.30	23	72/111		
*	N2 on *tom-1* RNAi (from Day1)		15.57±0.79	19	78/105	0.0005	-15.8
*	N2 on EV		19.22±0.75	22	50/68		
*	N2 on *tom-1* RNAi (from Day5)		21.27±0.77	25	59/66	0.0434	10.4
	N2 on EV		15.63±0.90	18	54/72		
	N2 on *tom-1* RNAi (eggs)		15.57±1.00	18	57/72	0.8269	
	N2 on *tom-1* RNAi (from Day1)		15.72±1.04	18	59/72	0.8358	
	N2 on *tom-1* RNAi (from Day5)		17.17±0.76	20	59/72	0.0287	9.9

Adult mean lifespan, in days, observed in lifespan analysis. Lifespan experiments were carried out at 20 °C , unless noted otherwise. '75%' is the mean lifespan of the 75th percentile. 'n' shows number of observed deaths relative to total number of animals started. The difference between these numbers represents the number of animals censored during the experiment, and includes animals that exploded, bagged, or crawled off the plates. '% LS change' is the percentage of lifespan extension compared to the control. *P* values were calculated by pair-wise comparisons to the control, each consisting of control and experimental animals examined at the same time. We used Stata 12 software for statistical analysis and to determine means and percentiles. The logrank or Wilcoxon test was used to test the hypothesis that the survival functions among groups were equal. Repetitions of the same experiments are listed in order. '*' indicates the sets of experiments plotted and shown in Figures.