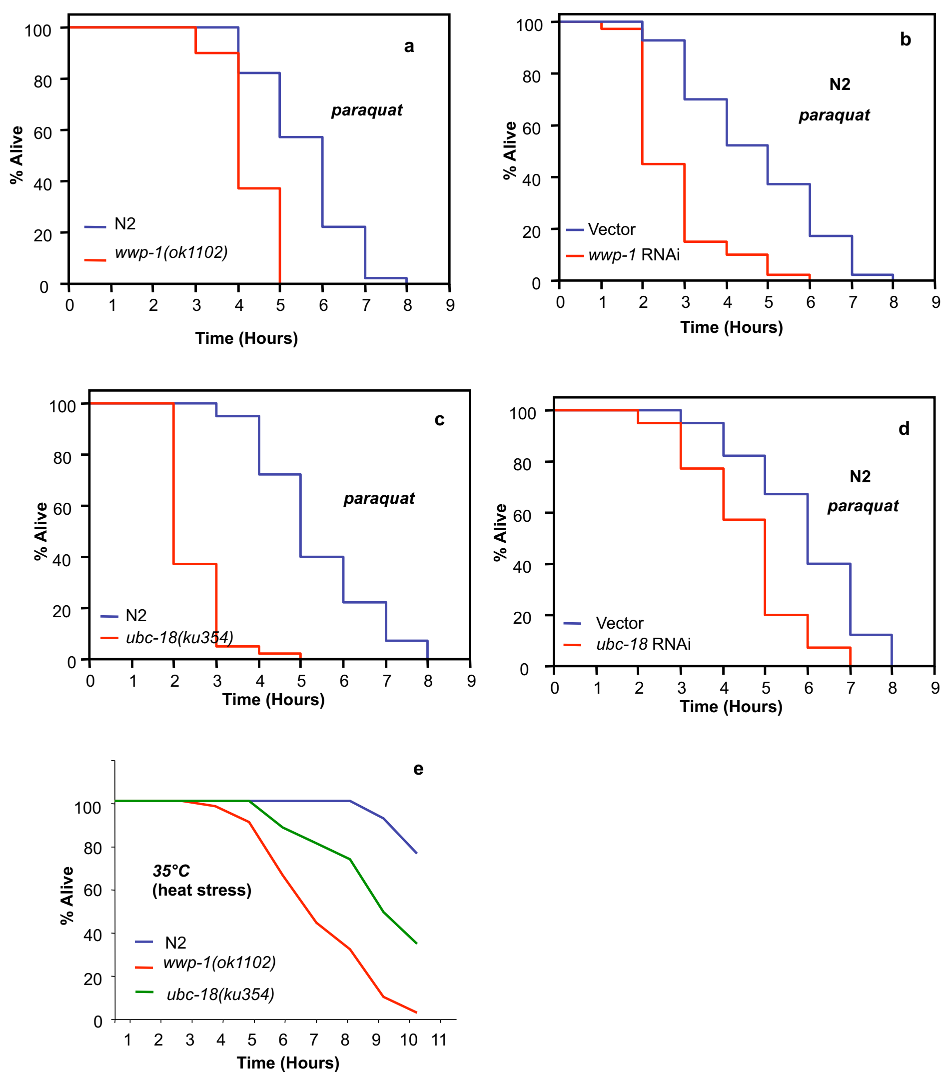
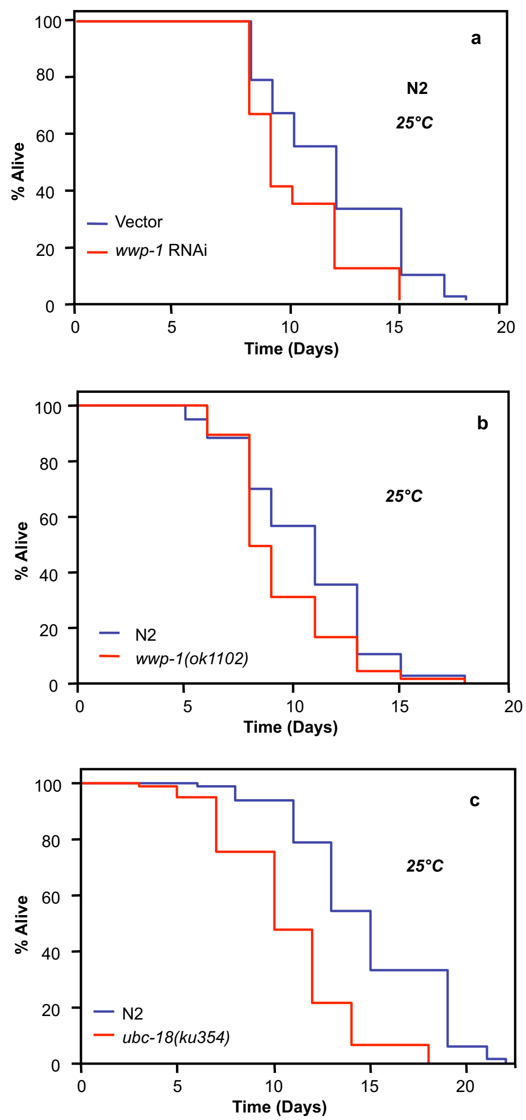
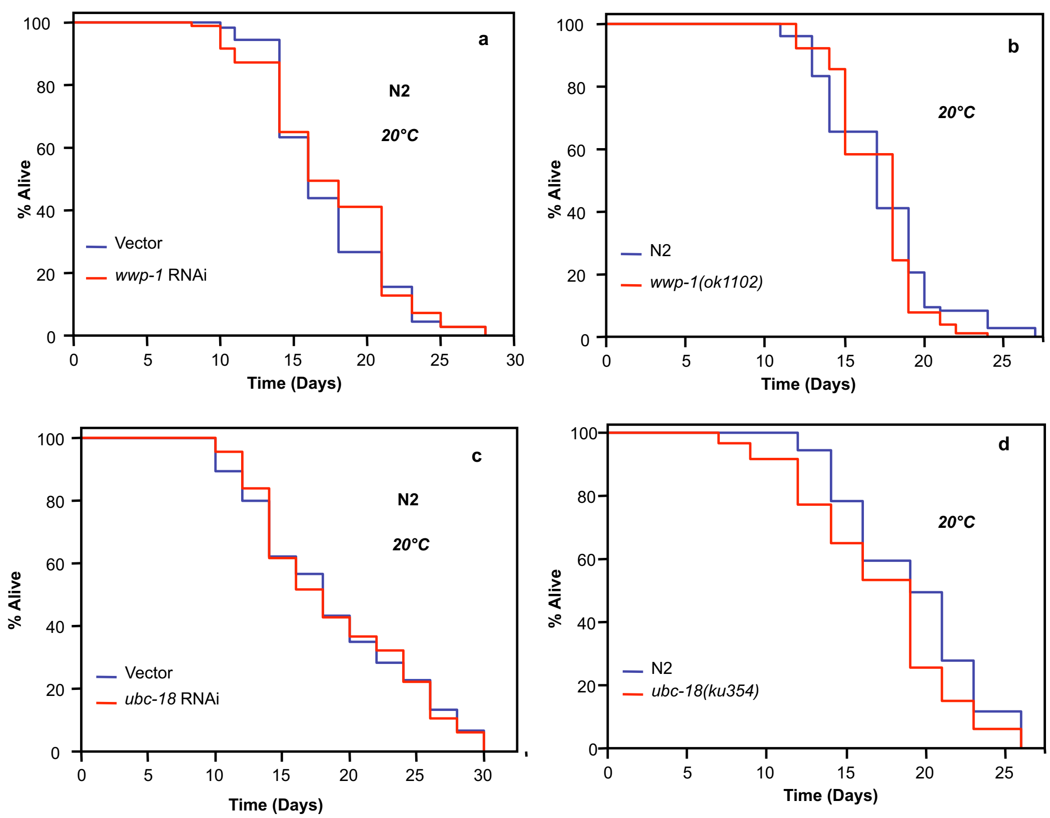


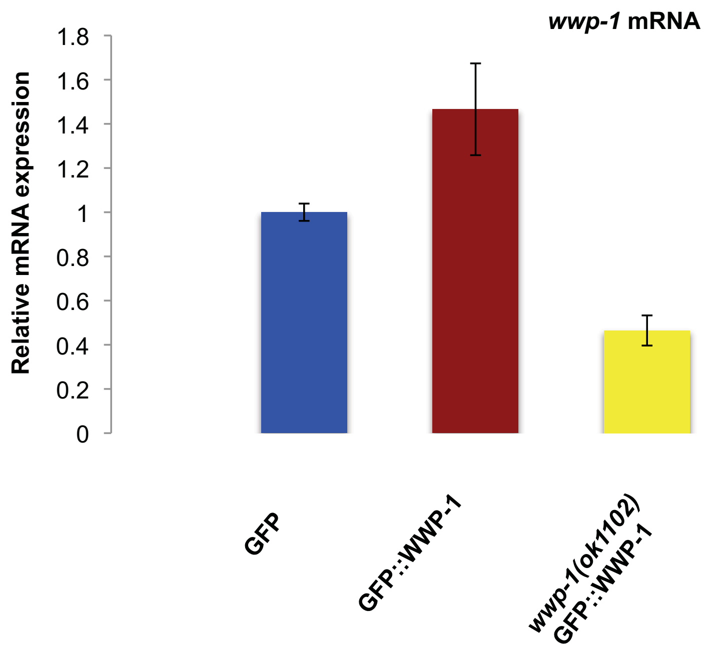
Supplementary Figure 1. ***wwp-1* and *ubc-18* regulate stress resistance**. **a**,**b**, *wwp-1(ok1102)* mutant animals (**a**) and *wwp-1* dsRNA fed N2 animals (**b**) are more sensitive to paraquat (300 mM) compared to control worms. **c**,**d**, *ubc-18(ku354)* mutant animals (**c**) and *ubc-18* dsRNA fed N2 animals (**d**) are more sensitive to paraquat compared to controls. The percentage of animals remaining alive is plotted against time incubated with paraquat. Statistical values are given in Supplementary Table 3. **e**, *wwp-1(ok1102)* and *ubc-18(ku354)* mutant animals are more sensitive to heat stress (35°C) (n = 40).



Supplementary Figure 2. Mild heat stress shortens lifespan with loss of *wwp-1* and *ubc-18*. The percentage of animals remaining alive is plotted against animal age. Lifespan analysis of N2 worms fed *wwp-1* dsRNA (**a**) or control vector at 25°C. Lifespan analysis of N2, *wwp-1(ok1102)* (**b**) and *ubc-18(ku354)* (**c**) mutant worms at 25°C. Lifespan values are given in Supplementary Table 5.

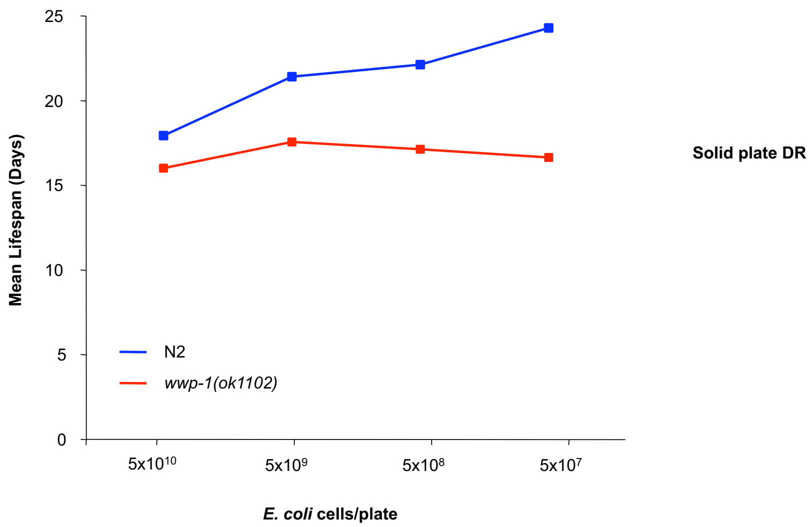


Supplementary Figure 3. Loss of *wwp-1* and *ubc-18* has no/minimal affect on lifespan at 20°C. Lifespan analysis of N2 worms fed bacteria expressing *wwp-1* dsRNA (**a**), *ubc-18* dsRNA (**c**) or control vector and *wwp-1(ok1102)* mutant worms (**b**) at 20°C. **d**, *ubc-18(ku354)* mutant animals have a slightly shorter lifespan at 20°C. Lifespan values are given in Supplementary Table 5.

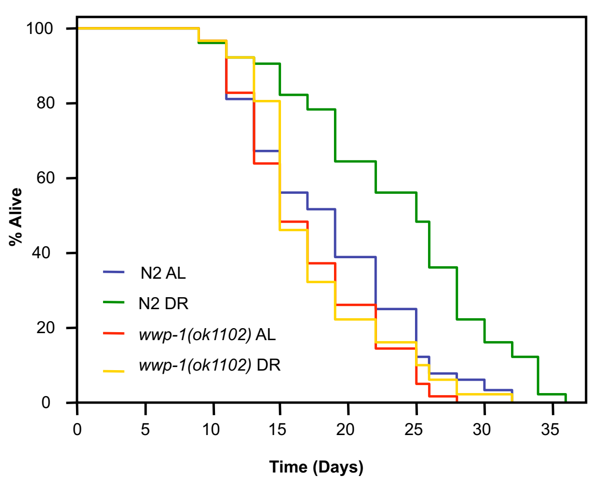


Supplementary Figure 4. Quantitative real time PCR analysis of *wwp-1* overexpression transgenic lines. qPCR of *wwp-1* mRNA in transgenic lines that express an N-terminal GFP-WWP-1 fusion protein in N2 animals (GFP::WWP-1) or *wwp-1(ok1102)* mutant animals [*wwp-1(ok1102)*GFP::WWP-1] compared to N2 animals that express *gfp* under the same promoter (GFP). Samples were normalized to control levels of *pmp-3* cDNA. Error bars represent standard error of the mean (SEM). An average of three experiments is presented. Similar results were found when samples were normalized to *act-1* cDNA.

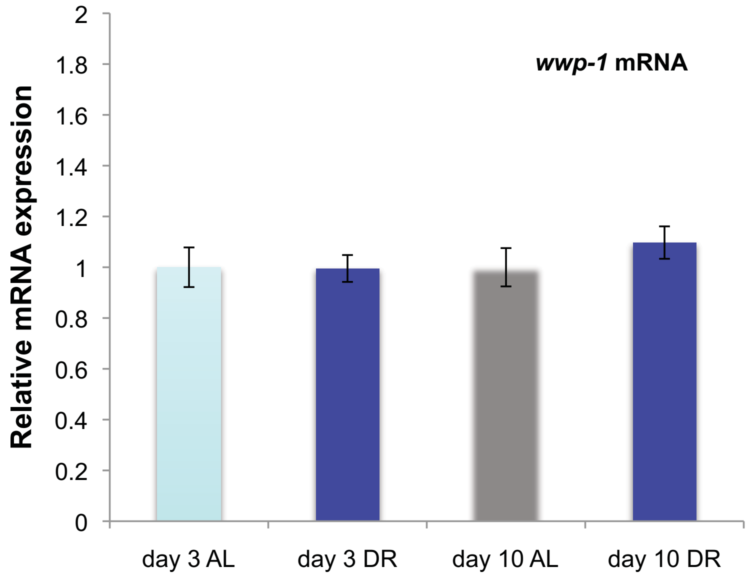
a



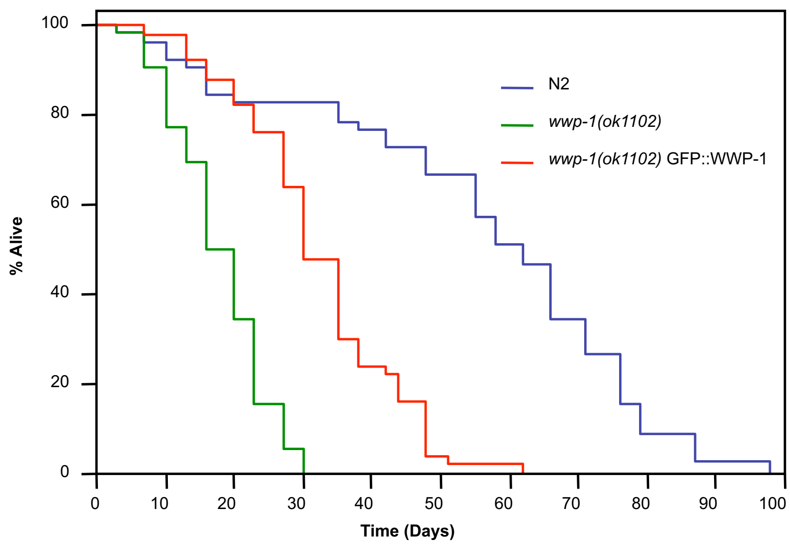
b



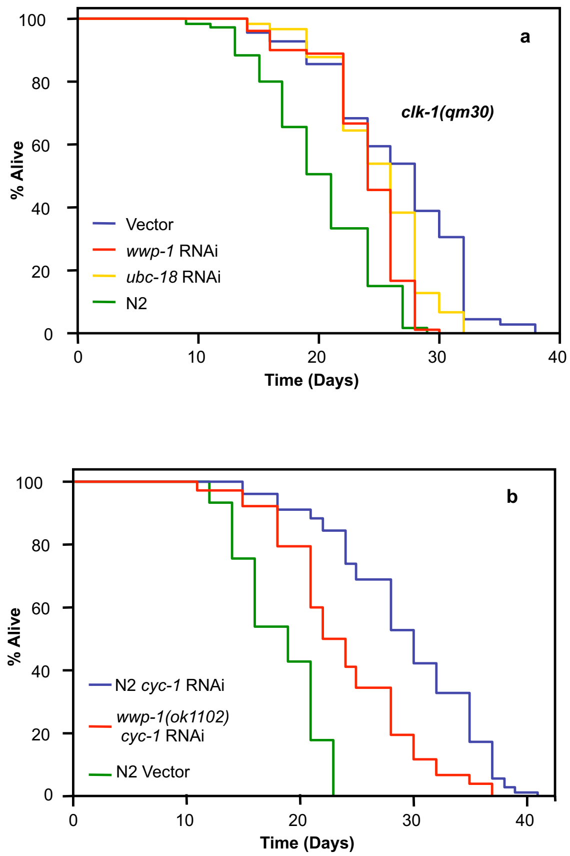
Supplementary Figure 5. *wwp-1* is required for solid plate DR. a, Lifespans of N2 and *wwp-1(ok1102)* mutant animals grown on NG plates with different *E. coli* concentrations. **b**, Lifespan analysis of N2 and *wwp-1(ok1102)* mutant worms grown in DR (5 x 10^7) or AL (5 x 10^{10}) *E. coli* concentrations. Lifespan values are given in Supplementary Table 6.



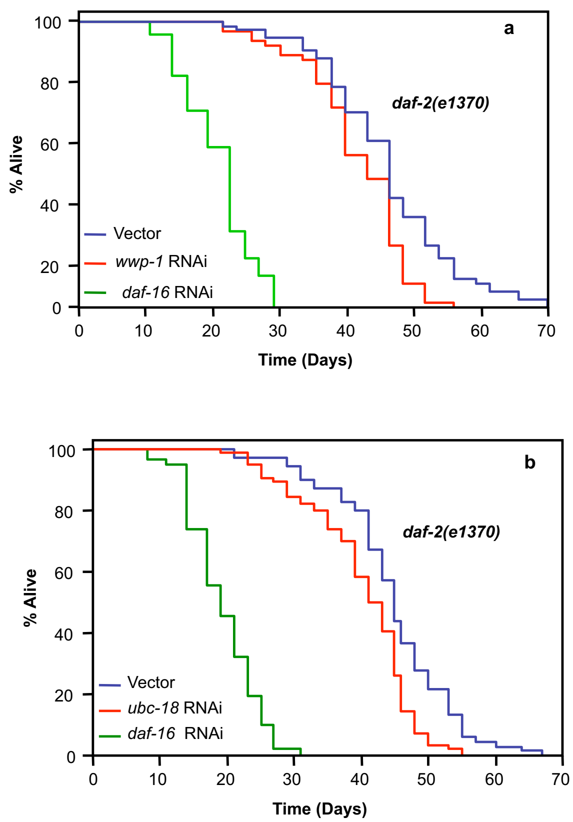
Supplementary Figure 6. Dietary restriction does not affect *wwp-1* mRNA expression. qPCR of endogenous *wwp-1* in N2 animals at Day 3 and Day 10 adults grown in DR (7.5 x 10^7 cells/ml) or AL (7.5 x 10^8 cell/ml) *E. coli* concentrations by bacterial dilution at 20°C. Samples were normalized to control levels of *pmp-3* cDNA. Error bars represent standard error of the mean (SEM). Similar results were found when samples were normalized to *act-1* cDNA.



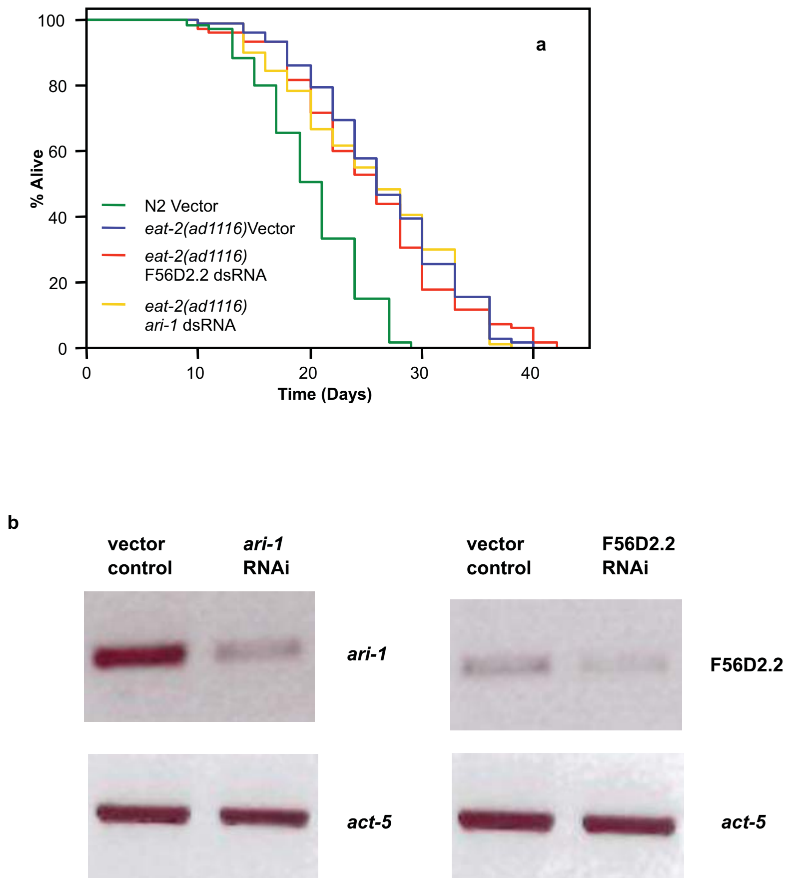
Supplementary Figure 7. Expression of *wwp-1* in the *wwp-1(ok1102*) mutant strain can partially rescue the DR longevity suppression. Lifespan analysis of N2, *wwp-1(ok1102)* and *wwp-1(ok1102)* mutant worms that express an N-terminal GFP-WWP-1 fusion protein grown in DR *E. coli* concentrations. Lifespan values are given in Supplementary Table 5.



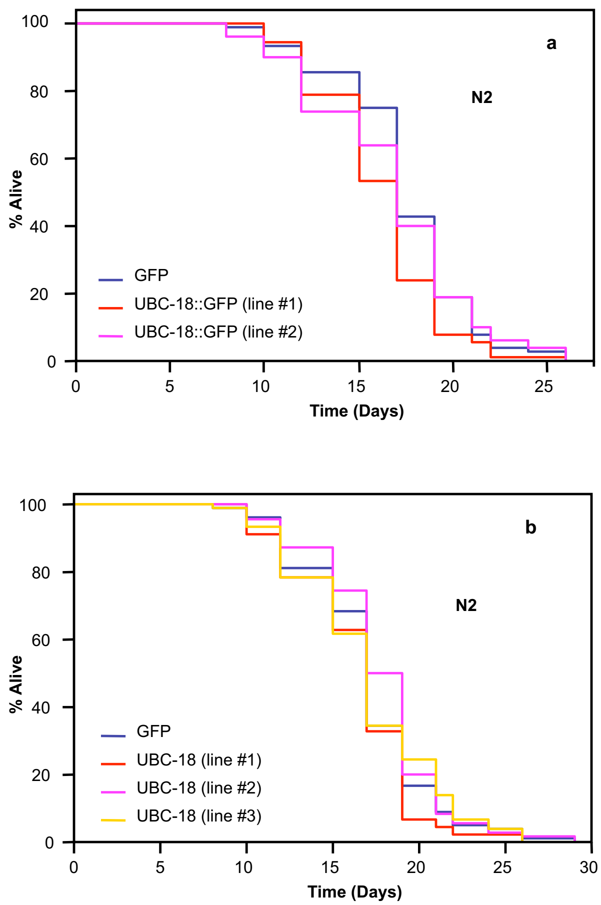
Supplementary Figure 8. Loss of *wwp-1* and *ubc-18* cannot fully suppress the extended lifespan of animals with reduced mitochondrial function. **a**, Lifespan analysis of *clk-1(qm30)* mutant worms fed bacteria expressing *wwp-1* dsRNA, *ubc-18* dsRNA or control vector. **b**, Lifespan analysis of N2 and *wwp-1(ok1102)* mutant worms fed bacteria expressing *cyc-1* dsRNA. Lifespan values are given in Supplementary Table 5. Two-way ANOVA analysis is presented in Supplementary Table 7.



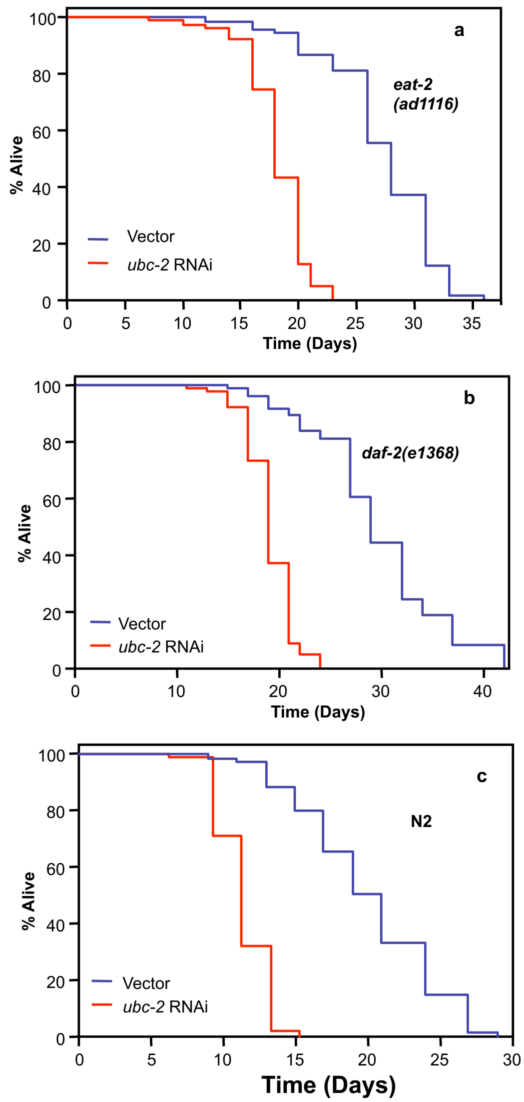
Supplementary Figure 9. Loss of *wwp-1* or *ubc-18* partially suppresses the increased longevity of *daf-2(e1370)* mutants. Lifespan analysis of *daf-2(e1370)* mutant worms fed bacteria expressing *wwp-1* dsRNA (**a**), *ubc-18* dsRNA (**b**) or *daf-16* dsRNA. The reduction of the mean lifespan of *daf-2(e1370)* mutant animals may be explained by the fact that the two classes of *daf-2* alleles can act very differently: *daf-2(e1370)* mutant animals exhibit a slight eat mutant phenotype, whereas *daf-2(e1368)* mutant animals do not. Lifespan values are given in Supplementary Table 5 and two-way ANOVA analysis is given in Supplementary Table 7.



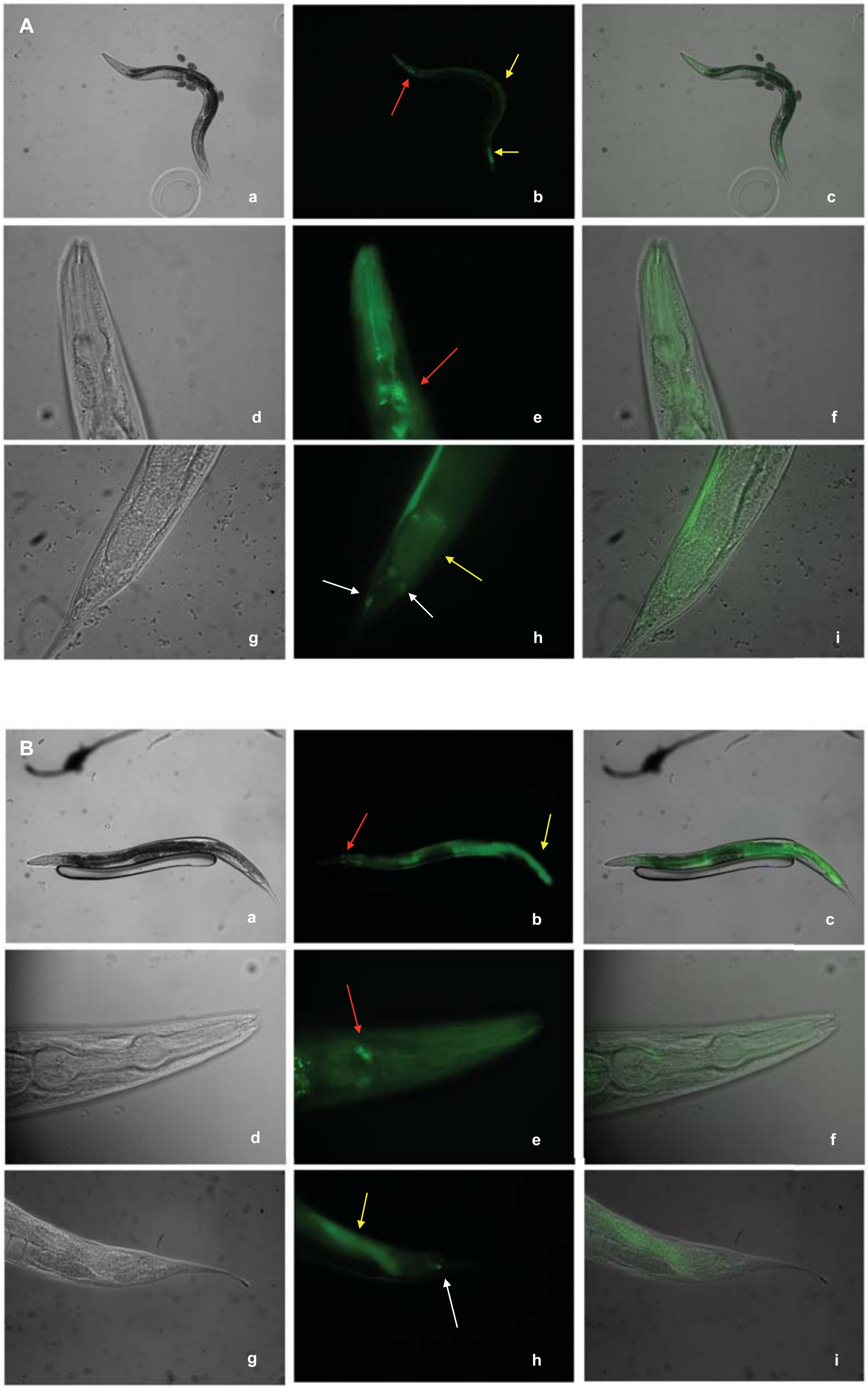
Supplementary Figure 10. Depletion of other ligases that interact with UBC-18 does not affect the extended lifespan of *eat-2* mutant animals. a, Lifespan analysis of *eat-2(ad1116)* mutant worms fed bacteria expressing F56D2.2, *ari-1* or vector control. RNAi was initiated at day 1 of adulthood. Lifespan values are given in Supplementary Table 5. **b**, RT-PCR analysis of worms fed bacteria expressing *ari-1* and F56D2.2 dsRNA or vector control (top panels). *act-5* serves as a control (bottom panels).



Supplementary Figure 11. Overexpression of *ubc-18* does not extend lifespan. Two independent strains expressing UBC-18-GFP fusion protein (UBC-18::GFP) under the control of the endogenous promoter (**a**) or three independent strains expressing non-tagged UBC-18 (UBC-18) under the control of the endogenous promoter (**b**) cannot extend longevity compared to control worms expressing *gfp* under the control of the same promoter. Lifespan values are given in Supplementary Table 5.



Supplementary Figure 12. Loss of *ubc-2* shortens lifespan via sickness. Lifespan analysis of *eat-2(ad1116)* (**a**), *daf-2(e1368)* (**b**) mutant or N2 animals (**c**) fed bacteria expressing *ubc-2* dsRNA. Lifespan values are given in Supplementary Table 5.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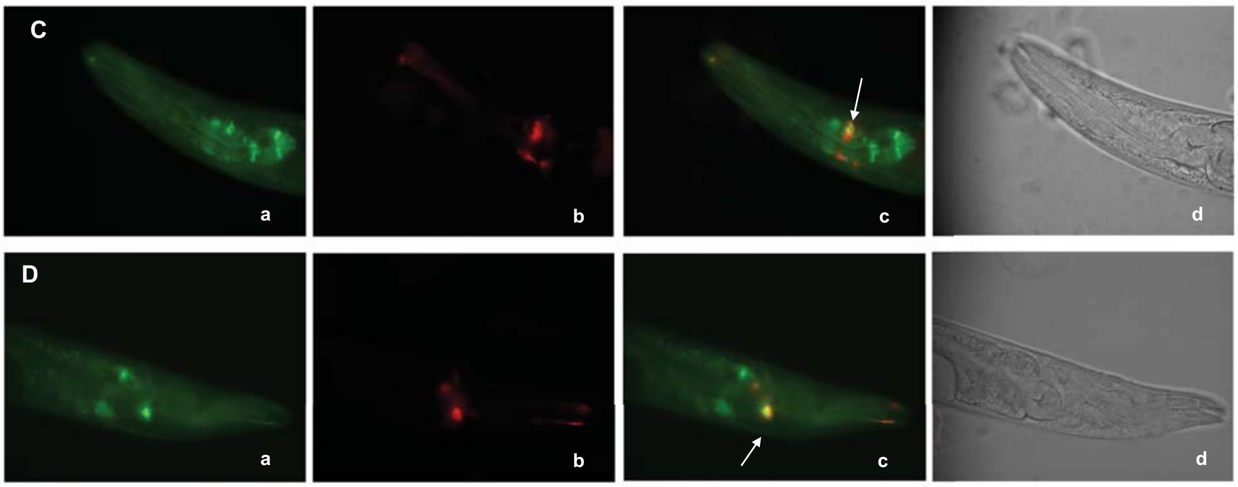
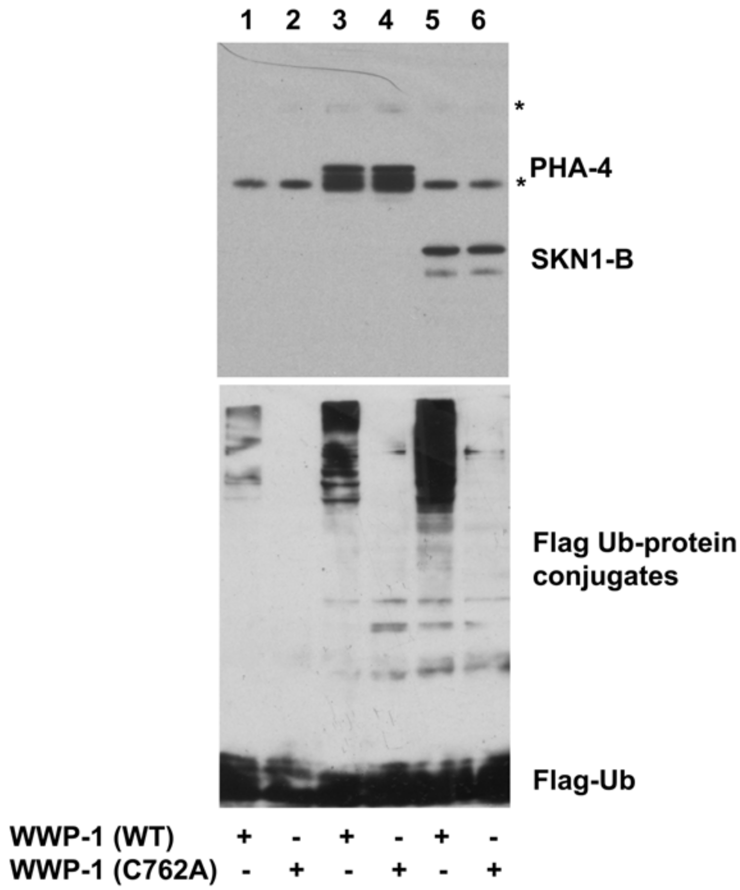
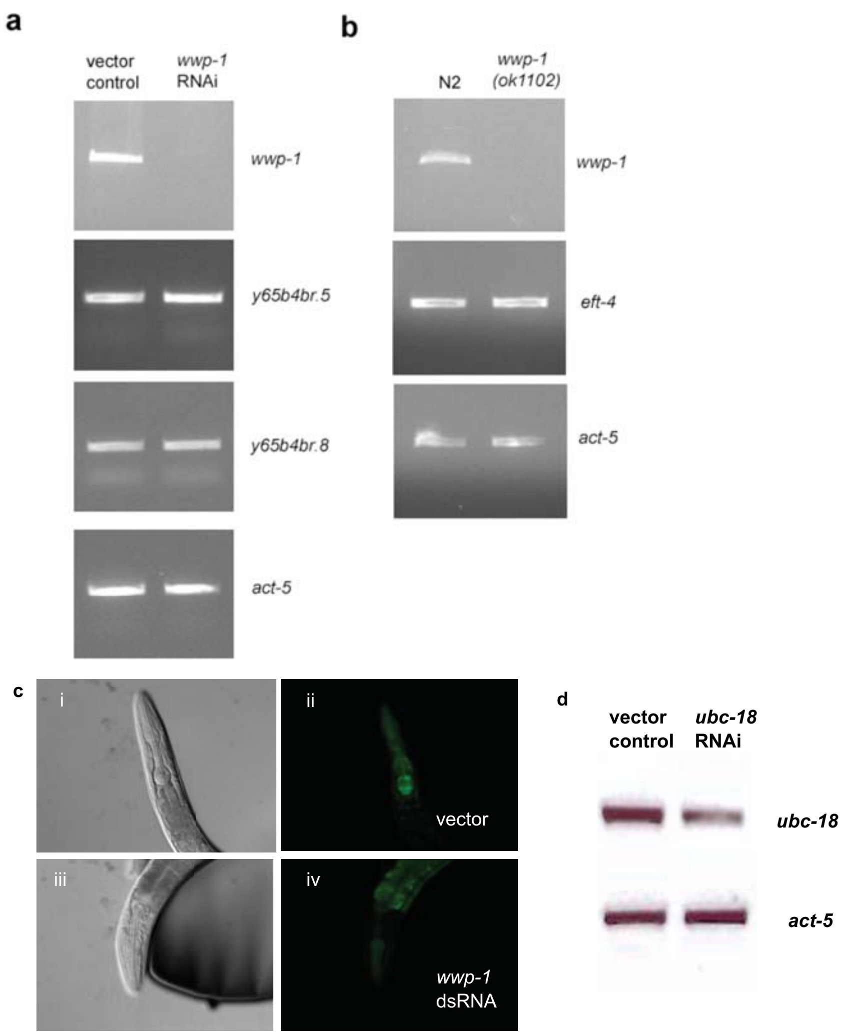


Supplementary Figure 13. WWP-1 and UBC-18 are expressed in similar tissues in *C. elegans.* Using a N-terminal GFP-tagged WWP-1 under the control of the endogenous promoter expressed in *wwp-1(ok1102)* adult worms (**A**) and a C-terminal GFP-tagged UBC-18 under the control of its endogenous promoter in adult N2 worms (**B**), GFP fluorescence is detected in head neurons (b and e, red arrows), intestines (b and h, yellow arrows) and tail neurons (h, white arrows). DIC images (a,d and g) and fluorescent images (b, e and h) are shown followed by composite DIC/fluorescent images (c, f and i). Similar GFP expression was seen with *wwp-1* overexpression in N2 worms (data not shown). Intensity of GFP expression in the intestines varied slightly in overexpressing lines. **C** and **D**, WWP-1 and UBC-18 are expressed in sensory head neurons. *wwp-1* (**C**) and *ubc-18* (**D**) overexpressing worms were exposed to DiI and expression in sensory neurons is shown (c, white arrows). Fluorescent images for GFP (a) and DiI (b) are shown followed by composite fluorescent images (c) and DIC images (d).



Supplementary Figure 14. PHA-4 and SKN-1B are not targets for ubiquitination by the UBC-18/WWP-1 complex. *In vitro* ubiquitination assay for PHA-4 (top panel lanes 3-4) and SKN-1B (top panel lanes 5-6) using a purified system with addition of UBC-18 and WT WWP-1 (lanes 1, 3 and 5) or mutant WWP-1 (C762A) (lanes 2, 4 and 6). * are background bands from the addition of recombinant His-E1. Bottom panel is a western blot using anti-Flag antibody showing there is active ubiquitin ligase activity with the addition of WT WWP-1.



Supplementary Figure 15. ***wwp-1* and *ubc-18* RNA-mediated interference specifically interferes with its gene expression.** See next page for full figure legend.

Supplementary Figure 15. *wwp-1* and *ubc-18* RNA-mediated interference specifically interferes with its gene expression. Approximately 15% of *C. elegans* genes are encoded in operons. Operons contain co-transcribed genes that make a polycistronic pre-mRNA that are subsequently separated into single-gene mRNA by trans-splicing (reviewed in [1]). *wwp-1* has been predicted to be organized in an operon downstream of two uncharacterized genes Y65B4BR.5 and Y65B4BR.8 [2]. *wwp-1* contains a putative SL2 site, an internal trans-splice site found in polycistronic pre-mRNAs, upstream of its coding region. **a**, Worms fed bacteria expressing *wwp-1* dsRNA had significant loss of *wwp-1* mRNA expression but not Y65B4BR.5 or Y65B4BR.8 mRNA expression even after dsRNA treatment over multiple generations. RT-PCR analysis of worms fed bacteria expressing *wwp-1* dsRNA or vector control. *wwp-1* RNAi is able to knock down expression of *wwp-1* (top panel) but not Y65B4BR.5 and Y65B4BR.8 (middle panels). *act-5* serves as a control (bottom panel). **b**, To confirm the results obtained using *wwp-1* dsRNA, we have performed parallel experiments with the small fraction of *wwp-1(ok1102)* null worms that survive to adulthood. RT-PCR analysis of N2 and *wwp-1(ok1102)* worms indicating loss of *wwp-1* mRNA in mutant worms (top panel). *eft-4* and *act-5* serve as controls (bottom panels). **c**, GFP signal is reduced upon *wwp-1* dsRNA treatment in GFP-tagged WWP-1 overexpressing worms. The GFP signal was reduced upon treatment with *wwp-1* dsRNA, confirming that WWP-1 was specifically expressed and that our *wwp-1* RNAi construct targeted *wwp-1*. DIC images (i, iii) or fluorescent images (ii, iv) of day 3 adult transgenic worms fed bacteria expressing *wwp-1* dsRNA (iii, iv) or control vector (i, ii) since L1 stage. Intestinal auto-fluorescence was observed with longer exposure in adults. **d**, RT-PCR analysis of worms fed bacteria expressing *ubc-18* dsRNA or vector control (top panel). *act-5* serves as a control (bottom panel).

1. Blumenthal, T. & Spieth J. Gene Structure and organization in Caenorhabditis elegans. *Curr Opin Genet Dev* **6**, 692-8 (1996).
2. Blumenthal, T. et al. A global analysis of Caenorhabditis elegans operons. *Nature* **417**, 851-4 (2002).

Supplementary Table 1. Lifespan data for main figures.

Figure	Strain	Treatment	Mean Lifespan +/- SEM (Days)	p Value	75th Percentile	Total Animals Died/Total
1a	N2 GFP[a]		16.6 ± 0.7		22	54/90
	N2 GFP::WWP-1[b,c]		20.0 ± 0.8	0.0047[d]	23	48/72
	N2 GFP::WWP-1[b.e]		20.4 ± 0.8	0.0003[d]	25	65/90
(exp. 2)	N2 GFP[a]		17.3 ± 0.7		22	66/100
	N2 GFP::WWP-1 [b,c]		20.1 ± 0.7	0.0061[d]	24	71/100
	N2 GFP::WWP-1 [b,e]		19.7 ± 0.7	0.0091[d]	24	71/100
1b	*eat-2(ad1116)*	Vector	28.3 ± 1.0		35	66/80
	eat-2(ad1116)	*wwp-1* dsRNA	17.1 ± 0.6	<0.0001[f]	18	73/80
	N2	Vector	17.2 ± 0.6	<0.0001 [f], 0.8882 [g]	21	71/80
(exp. 2)	*eat-2(ad1116)*	Vector	26.9 ± 1.0		33	69/81
	eat-2(ad1116)	*wwp-1* dsRNA	20.9 ± 0.7	<0.0001[f]	26	70/80
(exp. 3)	*eat-2(ad1116)*	Vector	26.5 ± 0.9		31	72/80
	eat-2(ad1116)	*wwp-1* dsRNA	18.5 ± 0.8	<0.0001[f]	22	78/80
1d	N2	AL[h]	27.0 ± 1.2		36	73/91
	N2	DR[i]	51.9 ± 1.8	<0.0001[j]	64	74/90
	wwp-1(ok1102)	AL[h]	20.3 ± 0.9		22	71/90
	wwp-1(ok1102)	DR[i]	18.9 ± 1.0	<0.0001[j], 0.3865[k]	25	89/90
(exp. 2)	N2	AL[h]	21.8 ± 1.6		28	46/61
	N2	DR[i]	48.8 ± 3.0	<0.0001[j]	62	44/61
	wwp-1(ok1102)	AL[h]	18.8 ± 0.9		24	46/60
	wwp-1(ok1102)	DR[i]	22.9 ± 1.6	<0.0001[j], 0.2786 [k]	28	44/59
1e	N2 GFP[a]	Vector	18.7 ± 0.5		21	72/80
	N2 GFP[a]	*pha-4* dsRNA	16.7 ± 0.5	0.0100[l]	21	68/80
	N2 GFP::WWP-1[b,c]	Vector	23.1 ± 0.5	<0.0001[l]	27	70/80
	N2 GFP::WWP-1[b,c]	*pha-4* dsRNA	17.5 ± 0.5	0.0164[l], 0.6058[m]	21	66/80
(exp. 2)	N2 GFP[a]	Vector	17.3 ± 0.5		20	81/96
	N2 GFP[a]	*pha-4* dsRNA	15.6 ± 0.5	0.0112[l]	18	73/96
	N2 GFP::WWP-1[b,c]	Vector	21.1 ± 0.5	<0.0001[l]	24	68/96
	N2 GFP::WWP-1[b,c]	*pha-4* dsRNA	16.4 ± 0.6	0.9867[l], 0.0330[m]	20	85/96
(exp. 3)	N2 GFP[a]	Vector	19.4 ± 0.7		24	54/67
	N2 GFP[a]	*pha-4* dsRNA	16.3 ± 1.0	0.0602[l]	22	24/77
	N2 GFP::WWP-1[b,c]	Vector	21.5 ± 0.8	0.0384[l]	24	63/80
	N2 GFP::WWP-1[b,c]	*pha-4* dsRNA	17.1 ± 0.7	0.0643[l], 0.6322[m]	22	44/80
1f	N2 GFP[a]	Vector	18.1 ± 0.7		21	58/80
	N2 GFP[a]	*daf-16* dsRNA	17.2 ± 0.6	0.2682[l]	21	64/80
	N2 GFP::WWP-1[b,c]	Vector	22.5 ± 0.7	<0.0001[l]	25	65/80
	N2 GFP::WWP-1[b,c]	*daf-16* dsRNA	21.0 ± 0.7	0.2474[n], 0.0001[o]	23	62/80
(exp. 2)	N2 GFP[a]	Vector	19.4 ± 0.7		24	54/67
	N2 GFP[a]	*daf-16* dsRNA	18.6 ± 0.8	0.5228[l]	24	49/57
	N2 GFP::WWP-1[b,c]	Vector	21.5 ± 0.8	0.0384[l]	24	63/80
	N2 GFP::WWP-1[b,c]	*daf-16* dsRNA	21.1 ± 0.7	0.9212[n], 0.0091[o]	24	72/80

1g	*isp-1(qm150)*	Vector	26.2 ± 1.1		32	67/96
	isp-1(qm150)	*wwp-1* dsRNA	28.1 ± 0.9	0.6438[p]	32	49/80
	N2	Vector	18.4 ± 0.6	<0.0001[p], <0.0001[q]	21	64/80
(exp. 2)	*isp-1(qm150)*	Vector	23.5 ± 1.0		29	59/80
	isp-1(qm150)	*wwp-1* dsRNA	27.7 ± 0.7	0.0044[p]	31	71/80
1h	*daf-2(e1368)*	Vector	33.5 ± 0.8		39	57/80
	daf-2(e1368)	*wwp-1* dsRNA	32.3 ± 0.8	0.5147[r]	39	72/80
	daf-2(e1368)	*daf-16* dsRNA	18.3 ± 0.6	<0.0001[r]	22	63/80
(exp. 2)	*daf-2(e1368)*	Vector	32.7 ± 0.8		39	56/80
	daf-2(e1368)	*wwp-1* dsRNA	32.3 ± 0.8	0.5301[r]	37	69/80
	daf-2(e1368)	*daf-16* dsRNA	18.7 ± 0.6	<0.0001[r]	21	74/79
2b	*eat-2(ad116)* GFP[a]		23.4 ± 0.9		28	65/80
	eat-2(ad1116) GFP::WWP-1(C762A)[s,c]		17.9 ± 0.7	<0.0001[d]	22	73/80
	eat-2(ad1116) GFP::WWP-1(C762A)[s,e]		17.0 ± 0.7	<0.0001[d]	22	71/80
	N2		18.8 ± 0.7	<0.0001[d], 0.2519 [t,c], 0.0381 [t,e]	24	74,80
(exp. 2)	*eat-2(ad116)* GFP[a]		26.1 ± 1.0		33	62/80
	eat-2(ad1116) GFP::WWP-1(C762A) [s,c]		19.1 ± 0.8	<0.0001[d]	24	55/80
(exp. 3)	*eat-2(ad116)* GFP[a]		23.8 ± 2.0		31	20/23
	eat-2(ad1116) GFP::WWP-1(C762A) [s,e]		18.8 ± 1.3	0.0171[d]	25	28/46
4a	*eat-2(ad1116)*	Vector	28.2 ± 0.9		35	74/80
	eat-2(ad1116)	*ubc-18* dsRNA L1[u]	20.5 ± 0.6	<0.0001[f]	25	78/80
	eat-2(ad1116)	*ubc-18* dsRNA D1[v]	21.1 ± 0.7	<0.0001[f]	27	69/80
	N2	Vector	21.7 ± 0.7	<0.0001[f], 0.4080[w],0.6069[x]	26	67/80
(exp. 2)	*eat-2(ad1116)*	Vector	26.1 ± 0.7		30	70/80
	eat-2(ad1116)	*ubc-18* dsRNA[u]	19.9 ± 0.5	<0.0001[f]	24	77/80
(exp. 3)	*eat-2(ad1116)*	Vector	26.3 ± 0.8		30	68/80
	eat-2(ad1116)	*ubc-18* dsRNA[u]	17.4 ± 0.6	<0.0001[f]	20	63/80
4b	*isp-1(qm150)*	Vector	32.4 ± 1.2		38	66/80
	isp-1(qm150)	*ubc-18* dsRNA	31.0 ± 1.4	0.3882[p]	38	42/80
	N2	Vector	21.7 ± 0.6	<0.0001 [p], <0.0001 [y]	26	67/80
(exp. 2)	*isp-1(qm150)*	Vector	26.2 ± 1.1		32	67/80
	isp-1(qm150)	*ubc-18* dsRNA	28.7 ± 1.2	0.3341[p]	35	49/95
4c	*daf-2(e1368)*	Vector	33.5 ± 0.8		39	57/80
	daf-2(e1368)	*ubc-18* dsRNA	34.9 ± 1.4	0.0977[r]	41	34/80
	daf-2(e1368)	*daf-16* dsRNA	18.7 ± 0.5	<0.0001[r]	21	75/80
(exp. 2)	*daf-2(e1368)*	Vector	32.7 ± 1.0		39	56/80
	daf-2(e1368)	*ubc-18* dsRNA	32.6 ± 1.3	0.9855[r]	39	34/121
	daf-2(e1368)	*daf-16* dsRNA	18.7 ± 0.6	<0.0001[r]	21	74/79
4d	*eat-2(ad1116)*	Vector	26.2 ± 0.7		30	90/96
	eat-2(ad1116)	*wwp-1* dsRNA[z]	20.6 ± 0.5	<0.0001[f]	17	91/96
	eat-2(ad1116)	*ubc-18* dsRNA[aa]	19.1 ± 0.5	<0.0001[f]	15	89/96
	eat-2(ad1116)	*wwp-1+ubc-18* dsRNA[bb]	19.2 ± 0.5	<0.0001[f], 0.8732[cc]	17	92/96
(exp. 2)	*eat-2(ad1116)*	Vector	25.2 ± 0.8		29	72/80
	eat-2(ad1116)	*wwp-1* dsRNA[z]	18.6 ± 0.7	<0.0001[f]	21	74/80
	eat-2(ad1116)	*ubc-18* dsRNA[aa]	19.2 ± 0.6	<0.0001[f]	21	72/80
	eat-2(ad1116)	*wwp-1+ubc-18* dsRNA[bb]	18.6 ± 0.6	<0.0001[f], 0.3431[cc]	21	71/80

4e	N2 GFP[a]	Vector	18.7 ± 0.5		21	72/80
	N2 GFP[a]	*ubc-18* dsRNA	18.9 ± 0.5	0.6335[l]	23	70/80
	N2 GFP::WWP-1[b,c]	Vector	23.1 ± 0.5	<0.0001[l]	27	70/80
	N2 GFP::WWP-1[b,c]	*ubc-18* dsRNA	19.1 ± 0.5	0.6772[l], 0.9020[dd]	21	70/80
(exp. 2)	N2 GFP[a]	Vector	18.6 ± 0.6		24	68/80
	N2 GFP[a]	*ubc-18* dsRNA	18.3 ± 0.7	0.7387[l]	24	60/80
	N2 GFP::WWP-1[b,c]	Vector	21.1 ± 0.7	0.0018[l]	21	56/80
	N2 GFP::WWP-1[b,c]	*ubc-18* dsRNA	17.9 ± 0.7	0.5228[l], 0.4006[dd]	26	71/80

p values were calculated for individual experiments, each consisting of control and experimental animals examined at the same time. The 75^{th} percentile is the age when the fraction of animals alive reaches 0.25. The total number of observations equals the number of animals that died plus the number censored. Animals that crawled off the plate, exploded, or bagged were censored at the time of event. The log-rank (Mantel-Cox) test was used for statistical analysis.

[a] Transgenic worms expressing GFP under the control of the *wwp-1* promoter.

[b] Transgenic worms expressing GFP-WWP-1 fusion protein under the control of the *wwp-1* promoter.

[c] Line #1.

[d] Compared to transgenic worms expressing GFP under the control of the *wwp-1* promoter.

[e] Line #2.

[f] Compared to *eat-2(ad1116)* worms grown on HT115 bacteria harboring the RNAi plasmid vector.

[g] Compared to *eat-2(ad1116)* worms grown on *wwp-1* dsRNA expressing bacteria.

[h] Worms fed ad libitum (AL) where the *E. coli* (OP50) concentration was 7.5×10^8 cells/ml.

[i] Worms fed a restricted diet (DR) where the *E. coli* (OP50) concentration was 7.5×10^7 cells/ml.

[j] Compared to N2 worms fed AL diet.

[k] Compared to *wwp-1(ok1102)* worms fed AL diet.

[l] Compared to transgenic worms expressing GFP under the control of the *wwp-1* promoter grown on HT115 bacteria harboring the RNAi plasmid vector.

[m] Compared to transgenic worms expressing GFP under the control of the *wwp-1* promoter grown on *pha-4* dsRNA expressing bacteria.

[n] Compared to transgenic worms expressing GFP-WWP-1 fusion protein under the control of the *wwp-1* promoter grown on HT115 bacteria harboring the RNAi plasmid vector.

[o] Compared to transgenic worms expressing GFP under the control of the *wwp-1* promoter grown on *daf-16* dsRNA expressing bacteria.

[p] Compared to *isp-1(qm150)* grown on HT115 bacteria harboring the RNAi plasmid vector.

[q] Compared to *isp-1(qm150)* grown on *wwp-1* dsRNA expressing bacteria.

[r] Compared to *daf-2(e1368)* grown on HT115 bacteria harboring the RNAi plasmid vector.

[s] Transgenic worms expressing GFP-WWP-1(C762A) fusion protein under the control of the *wwp-1* promoter.

[t] Compared to transgenic worms expressing GFP::WWP-1(C762A) fusion.

[u] Grown on *ubc-18* dsRNA expressing bacteria from hatching of eggs (L1).

[v] Grown on HT115 bacteria harboring the RNAi plasmid vector from hatching of eggs. At Day1 adulthood worms were switched to plates with bacteria expressing *ubc-18* dsRNA.

[w] Compared to *eat-2(ad1116)* worms grown on *ubc-18* dsRNA expressing bacteria from hatching (L1).

[x] Compared to *eat-2(ad1116)* worms grown on *ubc-18* dsRNA expressing bacteria only during adulthood.

[y] Compared to *isp-1(qm150)* grown on *ubc-18* dsRNA expressing bacteria.
[z] Worms grown on HT115 bacteria harboring the RNAi plasmid vector and *wwp-1* RNAi vector.
[aa] Worms grown on HT115 bacteria harboring the RNAi plasmid vector and *ubc-18* RNAi vector.
[bb] Worms grown on HT115 bacteria harboring the *wwp-1* and *ubc-18* RNAi vectors.
[cc] Compared to worms grown on bacteria harboring the RNAi plasmid vector and *ubc-18* RNAi vector.
[dd] Compared to transgenic worms expressing GFP under the control of the *wwp-1* promoter grown on *ubc-18* dsRNA expressing bacteria.

Supplementary Table 2. Effects of dietary restriction on lifespan.

Figure	Strain	bacterial conc. (10^8 cells/ml)	Mean Lifespan +/- SEM (Days)	p Value[a]	Risk Ratio, p Value[b]	75th Percentile	Total Animals Died/Total
1c	N2	0.05	43.7 ± 1.9	<0.0001		53	36/40
	N2	0.25	59.5 ± 1.9	<0.0001		69	39/40
	N2	0.75	55.2 ± 3.0	<0.0001		66	35/40
	N2	1.5	34.3 ± 2.3	<0.0001		46	38/40
	N2	7.5	18.2 ± 1.4			25	38/41
	N2	15	14.9 ± 1.0	0.0394		22	39/40
	wwp-1(ok1102)	0.05	15.7 ± 1.1	0.1059	33.0, <0.0001	22	39/40
	wwp-1(ok1102)	0.25	19.6 ± 1.3	0.6916	112.3, <0.0001	25	37/40
	wwp-1(ok1102)	0.75	20.9 ± 1.6	0.1236	15.1, <0.0001	29	40/40
	wwp-1(ok1102)	1.5	18.8 ± 1.7	0.6061	3.4, <0.0001	25	31/40
	wwp-1(ok1102)	7.5	13.9 ± 0.9	0.0072	1.7, 0.0235	18	38/40
	wwp-1(ok1102)	15	16.5 ± 0.6	0.1203	0.9, 0.7410	18	39/40
(exp. 2)	N2	0.05	41.0 ± 1.8	<0.0001		53	53/61
	N2	0.25	41.7 ± 1.9	<0.0001		53	49/60
	N2	0.75	39.2 ± 1.7	<0.0001		46	48/60
	N2	1.5	41.9 ± 1.9	<0.0001		53	51/60
	N2	7.5	21.0 ± 1.1			25	51/60
	N2	15	19.0 ± 1.0	0.2729		25	43/60
	wwp-1(ok1102)	0.05	15.5 ± 0.9	0.0007	13.5, <0.0001	21	52/61
	wwp-1(ok1102)	0.25	12.5 ± 0.9	<0.0001	28.6, <0.0001	14	42/60
	wwp-1(ok1102)	0.75	16.3 ± 1.0	0.0073	11.7, <0.0001	25	52/60
	wwp-1(ok1102)	1.5	19.1 ± 1.1	0.5174	12.5, <0.0001	25	49/60
	wwp-1(ok1102)	7.5	18.6 ± 1.0	0.0678	1.4, 0.1171	21	40/60
	wwp-1(ok1102)	15	15.9 ± 0.6	<0.0001	1.7, 0.0164	18	45/60
(exp. 3)	N2	0.05	39.8 ± 1.8	<0.0001		46	56/60
	N2	0.25	42.1 ± 1.6	<0.0001		53	59/65
	N2	0.75	42.5 ± 1.5	<0.0001		53	52/61
	N2	1.5	37.4 ± 1.3	<0.0001		46	56/60
	N2	7.5	21.0 ± 1.0			25	46/57
	N2	15	19.7 ± 1.6	0.4635		25	35/60
	wwp-1(ok1102)	0.05	19.6 ± 1.4	0.6303	4.9, <0.0001	29	58/60
	wwp-1(ok1102)	0.25	18.3 ± 1.0	0.4571	10.8, <0.0001	26	60/60
	wwp-1(ok1102)	0.75	22.2 ± 1.2	0.0746	6.8, <0.0001	29	56/60
	wwp-1(ok1102)	1.5	22.9 ± 1.1	0.0198	3.9, <0.0001	29	60/60
	wwp-1(ok1102)	7.5	13.9 ± 0.6	<0.0001	2.7, <0.0001	15	55/60
	wwp-1(ok1102)	15	14.0 ± 0.7	<0.0001	1.7, 0.0306	15	50/60

[a] Compared to N2 worms fed AL (7.5 x 10^8 cells/ml).
[b] Risk ratio [N2 to *wwp-1(ok1102)* grown in the same bacterial dilution concentrations] and p value determined from Cox-proportional Hazard.

Supplementary Table 3. Effects of paraquat.

Figure	Strain	Treatment	Mean Survival after treatment +/- SEM (Hours)	p Value	Total Animals Died/Total
S1a	N2		5.7 ± 0.2		40/40
	wwp-1(ok1102)		4.3 ± 0.1	<0.0001[a]	40/40
S1b	N2	Vector	4.7 ± 0.3		40/40
	N2	*wwp-1* dsRNA	2.7 ± 0.2	<0.0001[b]	40/40
S1c	N2		5.4 ± 0.2		40/40
	ubc-18(ku354)		2.5 ± 0.1	<0.0001[a]	40/40
S1d	N2	Vector	6.0 ± 0.2		40/40
	N2	*ubc-18* dsRNA	4.6 ± 0.2	<0.0001[b]	40/40

[a] Compared to similarly treated N2 worms.
[b] Compared to N2 worms grown on HT115 bacteria harboring the RNAi plasmid vector.

Supplementary Table 4. Pumping rates.

Strain	Treatment	Pumps/min (±SD)
N2	Vector	205.2 ± 15.2
N2	*wwp-1* RNAi	202.2 ± 21.1
N2	*ubc-18* RNAi	200.6 ± 13.2
eat-2(ad1116)	Vector	50.3 ± 11.9
eat-2(ad1116)	*wwp-1* RNAi	53.3 ± 10.1
eat-2(ad1116)	*ubc-18* RNAi	49.1 ± 8.3

Supplementary Table 5. Lifespan data for supplementary figures.

Figure	Strain	Treatment	Mean Lifespan +/- SEM (Days)	p Value	75th Percentile	Total Animals Died/Total
S2a	N2 (25°C)	Vector	11.8 ± 0.4		15	76/80
	N2 (25°C)	*wwp-1* dsRNA	10.1 ± 0.3	0.0002[a]	12	78/80
(exp. 2)	N2 (25°C)	Vector	13.3 ± 0.4		16	60/80
	N2 (25°C)	*wwp-1* dsRNA	12.1 ± 0.3	0.0038[a]	14	76/80
S2b	N2	25°C	10.6 ± 0.4		13	75/80
	wwp-1(ok1102)	25°C	9.4 ± 0.3	0.0063[b]	11	72/80
(exp. 2)	N2	25°C	11.6 ± 0.4		14	70/80
	wwp-1(ok1102)	25°C	10.0 ± 0.3	0.0251[b]	12	77/80
S2c	N2	25°C	14.9 ± 0.5		19	66/80
	ubc-18(ku354)	25°C	10.8 ± 0.3	<0.0001[b]	12	94/120
(exp. 2)	N2	25°C	12.2 ± 0.4		15	62/80
	ubc-18(ku354)	25°C	10.1 ± 0.4	0.0006[b]	12	72/80
S3a	N2	Vector	17.2 ± 0.5		21	71/80
	N2	*wwp-1* dsRNA	17.5 ± 0.5	0.5789[a]	21	71/80
(exp. 2)	N2	Vector	19.1 ± 0.5		23	71/80
	N2	*wwp-1* dsRNA	18.9 ± 0.5	0.9706[a]	23	64/80
(exp. 3)	N2	Vector	17.1 ± 0.5		23	97/100
	N2	*wwp-1* dsRNA	17.9 ± 0.6	0.0164[a]	21	93/100
S3b	N2		17.2 ± 0.4		19	76/80
	wwp-1(ok1102)		16.9 ± 0.3	0.9725[b]	18	77/80
(exp. 2)	N2		18.6 ± 0.4		22	73/80
	wwp-1(ok1102)		18.6 ± 0.3	0.3602[b]	20	76/80
(exp. 3)	N2		18.8 ± 0.4		22	66/80
	wwp-1(ok1102)		19.5 ± 0.3	0.4558[b]	22	72/80
S3c	N2	Vector	18.8 ± 0.7		24	74/80
	N2	*ubc-18* dsRNA	18.9 ± 0.7	0.9469[a]	24	68/80
(exp. 2)	N2	Vector	21.7 ± 0.7		26	67/80
	N2	*ubc-18* dsRNA	19.8 ± 0.8	0.2268[a]	26	61/80
S3d	N2		19.1 ± 0.5		23	69/80
	ubc-18(ku354)		17.1 ± 0.6	0.0237[b]	21	52/80
(exp. 2)	N2		16.6 ± 0.5		20	75/80
	ubc-18(ku354)		14.0 ± 0.8	0.0058[b]	18	27/64
S7	N2	DR	55.7 ± 3.4		76	48/53
	wwp-1(ok1102)	DR	17.9 ± 1.0	<0.0001[c]	23	52/53
	*wwp-1(ok1102)*GFP::WWP-1	DR	32.3 ± 1.0	<0.0001[c], <0.0001[d]	38	50/51
(exp. 2)	N2	DR	53.4 ± 2.8		68	39/40
	wwp-1(ok1102)	DR	22.4 ± 1.4	<0.0001 [c]	48	39/40
	*wwp-1(ok1102)*GFP::WWP-1	DR	35.4 ± 2.6	<0.0001 [c], <0.0001 [d]	28	40/40
(exp. 3)	N2	DR	49.0 ± 2.7		62	40/40
	wwp-1(ok1102)	DR	19.3 ± 1.1	<0.0001 [c]	25	40/40
	*wwp-1(ok1102)*GFP::WWP-1	DR	38.0 ± 2.3	<0.0001 [c], <0.0001 [d]	49	38/40
S8a	*clk-1(qm30)*	Vector	26.5 ± 0.7		32	69/80
	clk-1(qm30)	*wwp-1* dsRNA	23.9 ± 0.4	<0.0001[e]	26	72/80
	clk-1(qm30)	*ubc-18* dsRNA	25.0 ± 0.5	0.0034[e]	28	64/80
	N2	Vector	20.1 ± 0.6	<0.0001[e],	24	68/80

				<0.0001[f], <0.0001[g]		
(exp. 2)	*clk-1(qm30)*	Vector	20.9 ± 0.8		29	65/79
	clk-1(qm30)	*wwp-1* dsRNA	21.7 ± 0.7	<0.7478[e]	27	76/80
S8b	N2	*cyc-1* dsRNA	29.3 ± 0.7		35	76/80
	wwp-1(ok1102)	*cyc-1* dsRNA	24.0 ± 0.7	<0.0001[h]	28	78/80
	N2	Vector	18.2 ± 0.5	<0.0001[h], <0.0001[i]	21	61/80
(exp. 2)	N2	*cyc-1* dsRNA	26.7 ± 0.7		31	76/80
	wwp-1(ok1102)	*cyc-1* dsRNA	22.4 ± 0.6	<0.0001[h]	26	70/80
(exp. 3)	N2	*cyc-1* dsRNA	28.8 ± 0.8		32	72/80
	wwp-1(ok1102)	*cyc-1* dsRNA	22.0 ± 0.8	<0.0001[h]	25	79/80
S9a	*daf-2(e1370)*	Vector	42.8 ± 1.1		48	70/80
	daf-2(e1370)	*wwp-1* dsRNA	38.6 ± 0.9	0.0011[j]	43	60/72
	daf-2(e1370)	*daf-16* dsRNA	19.3 ± 0.6	<0.0001[j]	23	64/80
(exp. 2)	*daf-2(e1370)*	Vector	41.9 ± 1.3		49	77/102
	daf-2(e1370)	*wwp-1* dsRNA	35.6 ± 1.0	<0.0001[j]	42	86/100
S9b	*daf-2(e1370)*	Vector	44.7 ± 1.0		50	69/80
	daf-2(e1370)	*ubc-18* dsRNA	40.1 ± 0.9	0.0002[j]	46	84/120
	daf-2(e1370)	*daf-16* dsRNA	19.3 ± 0.6	<0.0001[j]	23	58/80
(exp. 2)	*daf-2(e1370)*	Vector	43.4 ± 1.5		53	70/80
	daf-2(e1370)	*ubc-18* dsRNA	37.7 ± 1.7	0.0047[j]	47	40/80
(exp. 3)	*daf-2(e1370)*	Vector	45.8 ± 1.5		55	65/80
	daf-2(e1370)	*ubc-18* dsRNA	37.9 ± 1.6	0.0002[j]	45	44/100
	daf-2(e1370)	*daf-16* dsRNA	20.2 ± 0.7	<0.0001[j]	25	64/80
S10	*eat-2(ad1116)*	Vector	26.6 ± 0.8		33	71/80
	eat-2(ad1116)	F56D2.2 dsRNA	25.9 ± 0.9	0.7535[k]	33	77/80
	eat-2(ad1116)	*ari-1* dsRNA	25.6 ± 0.9	0.6258[k]	30	69/80
	N2	Vector	20.1 ± 0.7	<0.0001[k], <0.0001[l], <0.0001[m]	24	68/80
(exp. 2)	*eat-2(ad1116)*	Vector	26.2 ± 0.7		30	72/80
	eat-2(ad1116)	F562D.2 dsRNA	26.9 ± 0.9	0.0594[k]	34	67/80
	eat-2(ad1116)	*ari-1* dsRNA	26.8 ± 0.9	0.1214[k]	32	73/80
S11a	GFP[n]		17.3 ± 0.4		19	75/96
	UBC-18::GFP[o,p]		16.1 ± 0.4	0.0115[q]	17	75/96
	UBC-18::GFP[o,r]		16.7 ± 0.5	0.6828[q]	19	80/96
(exp. 2)	GFP[n]		18.2 ± 0.6		21	69/80
	UBC-18::GFP[o,p]		19.1 ± 0.6	0.2830[q]	21	66/80
	UBC-18::GFP[o,r]		18.1 ± 0.6	0.9655[q]	21	59/80
S11b	GFP[n]		17.0 ± 0.4		19	79/96
	UBC-18[s,p]		16.3 ± 0.5	0.3160[q]	19	46/76
	UBC-18[s,q]		17.7 ± 0.5	0.2868[q]	19	70/96
	UBC-18[s,t]		17.0 ± 0.2	0.9075[q]	19	73/96
(exp. 2)	GFP[g]		17.6 ± 0.8		19	33/40
	UBC-18[s,p]		17.5 ± 0.7	0.7505[q]	19	41/62
	UBC-18[s,q]		17.7 ± 0.9	0.8319[q]	19	23/38
	UBC-18[s,t]		15.7 ± 0.6	0.0336[q]	18	41/67
S12a	*eat-2(ad1116)*	Vector	27.3 ± 0.6		31	67/80
	eat-2(ad1116)	*ubc-2* dsRNA	18.3 ± 0.3	<0.0001[k]	20	78/80
(exp. 2)	*eat-2(ad1116)*	Vector	26.1 ± 0.7		30	70/80
	eat-2(ad1116)	*ubc-2* dsRNA	11.2 ± 0.2	<0.0001[k]	12	80/80

S12b	*daf-2(e1368)*	Vector	29.7 ± 0.7		32	74/80
	daf-2(e1368)	*ubc-2* dsRNA	19.2 ± 0.3	<0.0001[u]	21	78/80
S12c	N2	Vector	20.1 ± 0.6		24	68/80
	N2	*ubc-2* dsRNA	11.1 ± 0.2	<0.0001[a]	13	80/80
(exp. 2)	N2	Vector	21.4 ± 0.5		27	71/80
	N2	*ubc-2* dsRNA	16.7 ± 0.2	<0.0001[a]	17	73/80

[a] Compared to N2 worms grown on HT115 bacteria harboring the RNAi plasmid vector.
[b] Compared to N2 worms grown on OP50 bacteria.
[c] Compared to N2 worms fed a restricted diet (DR) where the *E. coli* concentration was 7.5 x 10^7 cells/ml.
[d] Compared to *wwp-1(ok1102)* worms fed a restricted diet (DR) where the *E. coli* concentration was 7.5 x 10^7 cells/ml.
[e] Compared to *clk-1(qm30)* worms grown on HT115 bacteria harboring the RNAi plasmid vector.
[f] Compared to *clk-1(qm30)* worms grown *wwp-1* dsRNA expressing bacteria.
[g] Compared to *clk-1(qm30)* worms grown *ubc-18* dsRNA expressing bacteria.
[h] Compared to N2 worms grown on *cyc-1* dsRNA expressing bacteria.
[i] Compared to *wwp-1(ok1102)* worms grown on *cyc-1* dsRNA expressing bacteria.
[j] Compared to *daf-2(e1370)* worms grown on HT115 bacteria harboring the RNAi plasmid vector.
[k] Compared to *eat-2(ad1116)* worms grown on HT115 bacteria harboring the RNAi plasmid vector.
[l] Compared to *eat-2(ad1116)* worms grown on *ari-1* dsRNA expressing bacteria.
[m] Compared to *eat-2(ad1116)* worms grown on F56D2.2 dsRNA expressing bacteria.
[n] Transgenic worms expressing GFP under the control of the *ubc-18* promoter.
[o] Transgenic worms expressing GFP-UBC-18 fusion protein under the control of the *ubc-18* promoter.
[p] Line #1.
[q] Compared to transgenic worms expressing GFP under the control of the *ubc-18* promoter.
[r] Line #2
[s] Transgenic worms expressing UBC-18 under the control of the *ubc-18* promoter.
[t] Line #3.
[u] Compared to *daf-2(e1368)* worms grown on HT115 bacteria harboring the RNAi plasmid vector.

Supplementary Table 6. Effects of solid plate dietary restriction on lifespan.

Figure	Strain	bacterial conc. (cells/ml)	Mean Lifespan +/- SEM (Days)	p Value[a]	Risk Ratio, p Value[b]	75th Percentile	Total Animals Died/Total
S4	N2	5 x 10^{10}	18.5 ± 0.8			25	64/65
	N2	5 x 10^{9}	21.3 ± 0.8	0.0134		26	59/65
	N2	5 x 10^{8}	21.9 ± 0.8	0.0063		26	60/66
	N2	5 x 10^{7}	23.6 ± 1.0	<0.0001		28	64/65
	wwp-1(ok1102)	5 x 10^{10}	16.9 ± 0.6	0.0590	1.3, 0.1035	22	63/66
	wwp-1(ok1102)	5 x 10^{9}	18.1 ± 0.6	0.4110	1.9, 0.0018	22	59/65
	wwp-1(ok1102)	5 x 10^{8}	17.8 ± 0.6	0.2875	2.0, 0.0006	19	58/68
	wwp-1(ok1102)	5 x 10^{7}	17.4 ± 0.7	0.3283	2.6, <0.0001	19	50/65
(exp. 2)	N2	5 x 10^{10}	18.2 ± 0.6			20	86/90
	N2	5 x 10^{9}	19.7 ± 0.5	0.1182		22	85/90
	N2	5 x 10^{8}	18.9 ± 0.7	0.2985		25	78/90
	N2	5 x 10^{7}	22.3 ± 0.6	<0.0001		25	82/80
	wwp-1(ok1102)	5 x 10^{10}	18.6 ± 0.5	0.8659	1.0, 0.8910	20	86/90
	wwp-1(ok1102)	5 x 10^{9}	18.3 ± 0.5	0.6954	1.3, 0.0774	20	88/90
	wwp-1(ok1102)	5 x 10^{8}	18.3 ± 0.5	0.6687	1.2, 0.1849	20	81/90
	wwp-1(ok1102)	5 x 10^{7}	18.9 ± 0.5	0.5924	1.8, 0.0003	22	82/90

[a] Compared to N2 worms fed AL (5 x 10^{10} cells/ml).

[b] Risk ratio [N2 to *wwp-1(ok1102)* grown in the same bacteria concentrations] and p value determined from Cox-proportional Hazard.

Supplementary Table 7. Two-way ANOVA analysis.

Figure	Strain	RNAi treatment	Strain (F, p Value)	RNAi treatment (F, p Value)	Interaction (F, p Value)
1b	N2, *eat-2(ad1116)*	Vector, *wwp-1* RNAi	181.2, <0.0001	128.81, <0.0001	117.1, <0.0001
1g	N2, *isp-1(qm150)*	Vector, *wwp-1* RNAi	353.14, <0.0001	9.36, =0.0023	12.22, =0.0005
1h	N2, *daf-2 (e1368)*	Vector, *wwp-1* RNAi	1183.72, <0.0001	1.65, =0.1999	0.67, =0.4145
4a	N2, *eat-2(ad1116)*	Vector, *ubc-18* RNAi	10,459.16, <0.0001	16,446.38, <0.0001	10,459.16, <0.0001
4b	N2, *isp-1(qm150)*	Vector, *ubc-18* RNAi	154.92, <0.0001	0.02, =0.8936	0.88, =0.3492
4c	N2, *daf-2 (e1368)*	Vector, *ubc-18* RNAi	365.31, <0.0001	0.02, =0.8849	1.03, =0.3111
S8a	N2, *clk-1(qm30)*	Vector, *wwp-1* RNAi	187.25, <0.0001	1.98, =0.1599	0.80, =0.3709
S8a	N2, *clk-1(qm30)*	Vector, *ubc-18* RNAi	89.9, <0.0001	3.86, =0.05	0.36, =0.5502
S8b	N2, *wwp-1(ok1102)*	Vector, *cyc-1* RNAi	72.9, <0.0001	532.9, <0.0001	78.4, <0.0001
S9a	N2, *daf-2 (e1370)*	Vector, *wwp-1* RNAi	1592.56, <0.0001	25.93, <0.0001	22.3, <0.0001
S9b	N2, *daf-2 (e1370)*	Vector, *ubc-18* RNAi	851.39, <0.0001	19.36, <0.0001	11.0, =0.0009