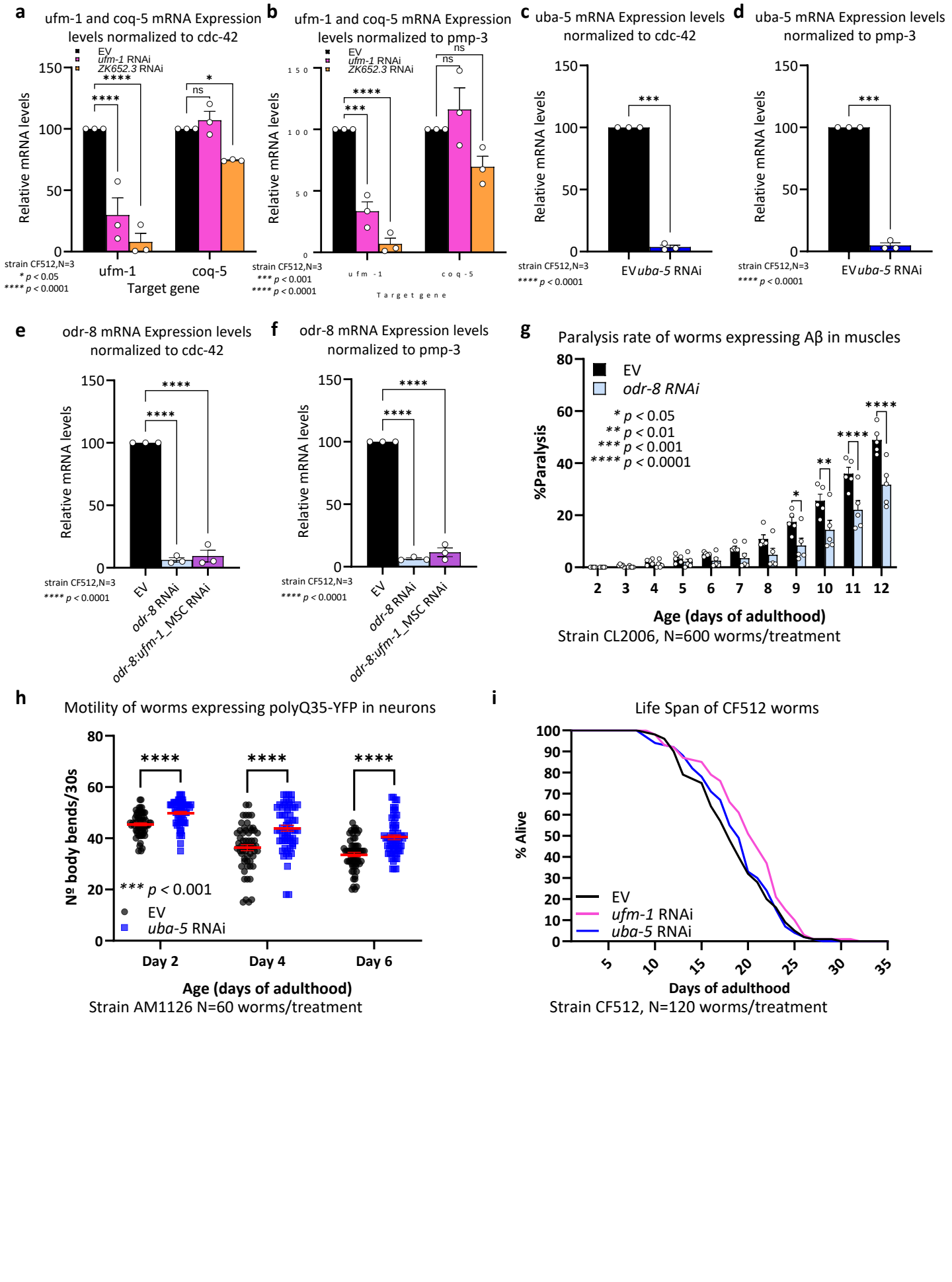
Supplemental Figure 1

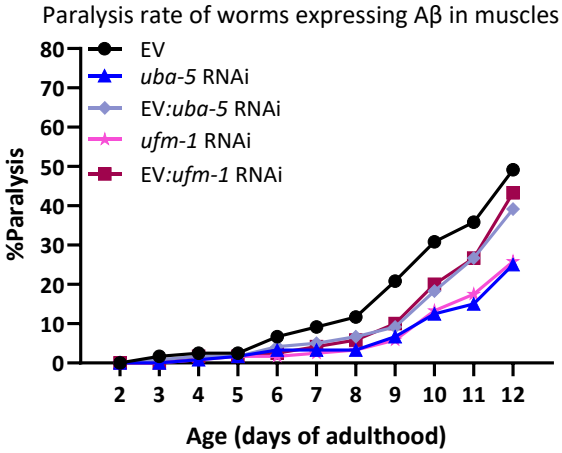


Supplemental table 1: lifespan data

strain	Treatment	# included	Mean LS ± StEr	Life span extension %	T-test
N2	EV	111/120	18.99±4.33		
Fig. 1g	*ufm-1* RNAi	119/120	20.63±5.33	8.6%	0.0056722
	uba-5 RNAi	116/120	19.40±6.122	2.2%	NS 0.2774629
AM140	EV	154/200	12.94±4.25		
Fig. 1h	*ufm-1* RNAi	162/200	14.65±4.54	13.2%	0.0003273
	uba-5 RNAi	183/200	14.68±3.91	13.4%	5.899E-05
CL2006	EV	142/180	15.97±4.47		
Fig. 1i	*ufm-1* RNAi	152/180	17.31±4.74	8.4%	0.0069224
	uba-5 RNAi	151/180	17.66±5.499	10.6%	0.0022128
CF512	EV	116/120	18.45±4.57		
Fig. S1i	*ufm-1* RNAi	110/120	20.20±4.63	9.5%	0.00240229
	uba-5 RNAi	106/120	19.00±4.30	3%	NS 0.17886067

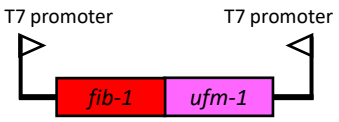
Supplemental Figure 2

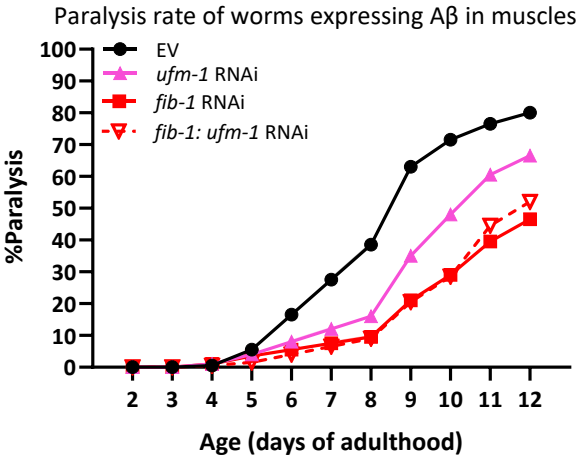
a



Strain CL2006,N=120 worms/ treatment

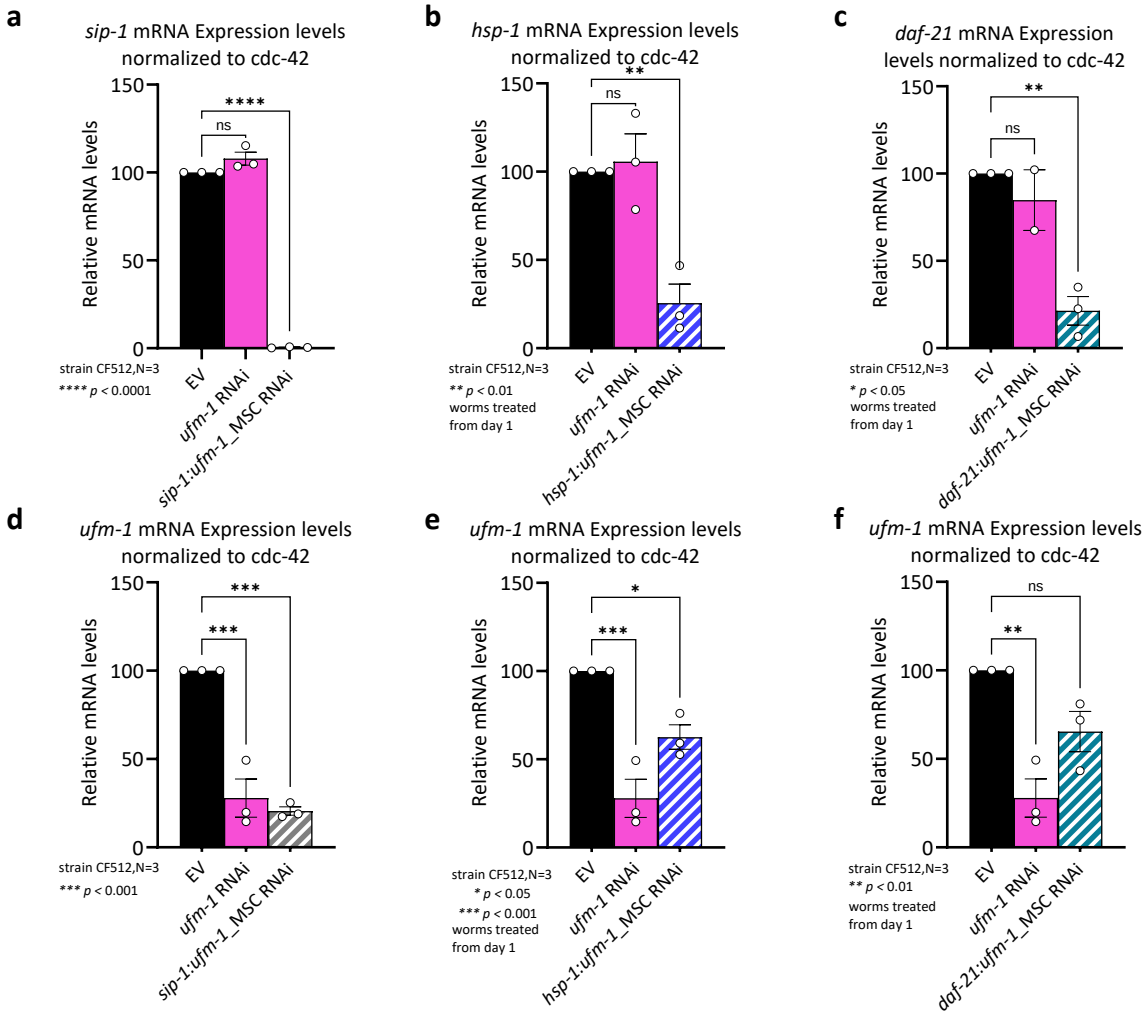
b

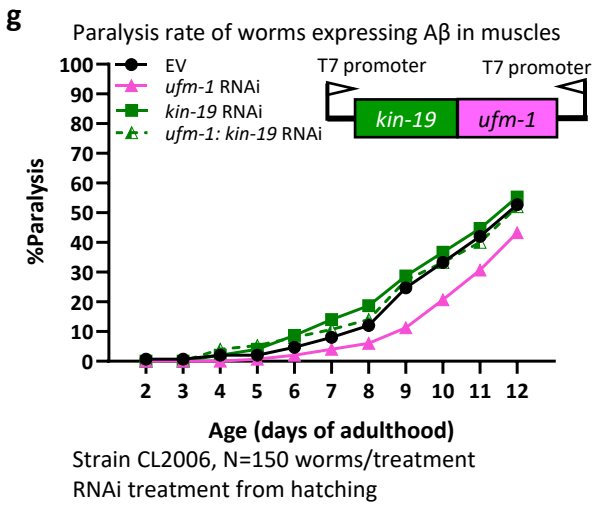


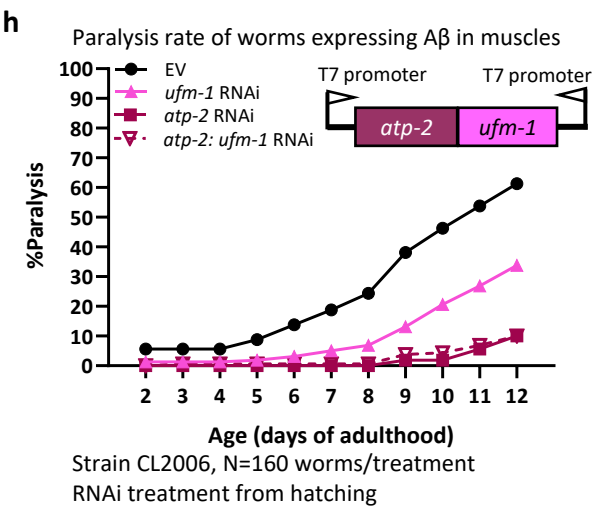


Strain CL2006, N=200 worms/treatment
RNAi treatment from day 1

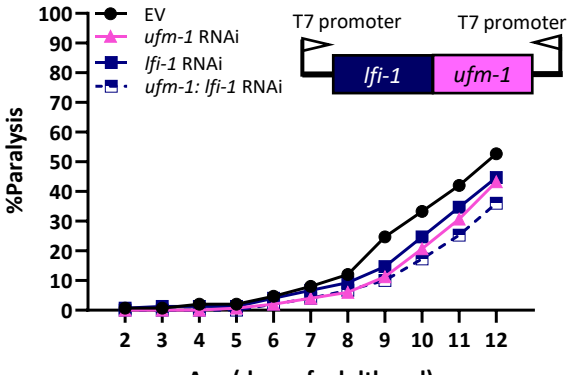
Supplemental Figure 3





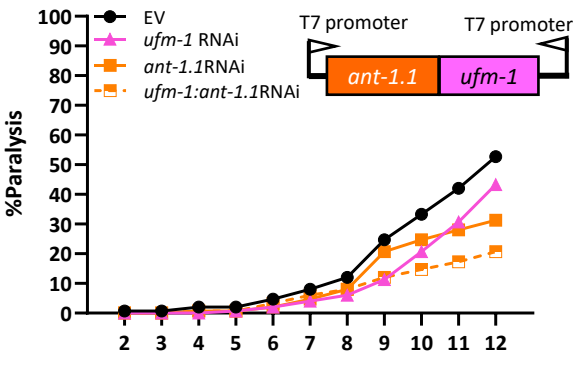


i



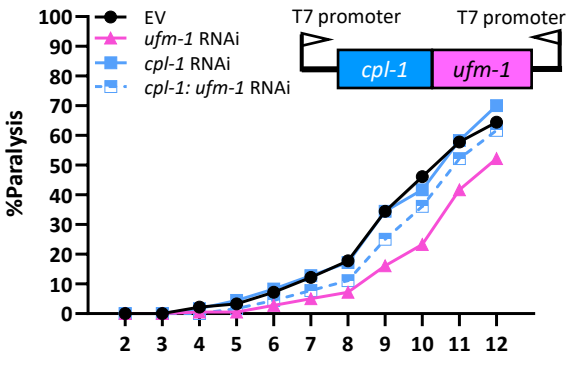
Strain CL2006, N=150 worms/treatment
RNAi treatment from hatching

j



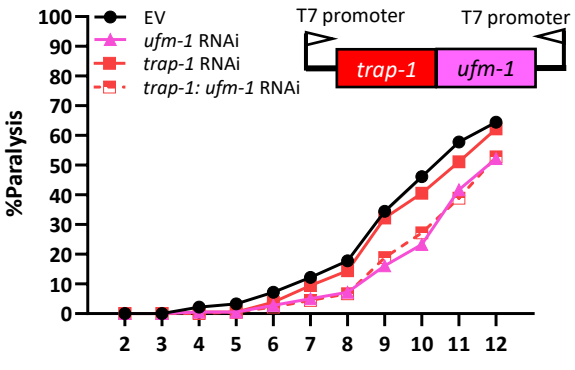
Strain CL2006, N=150 worms/treatment
RNAi treatment from hatching

k



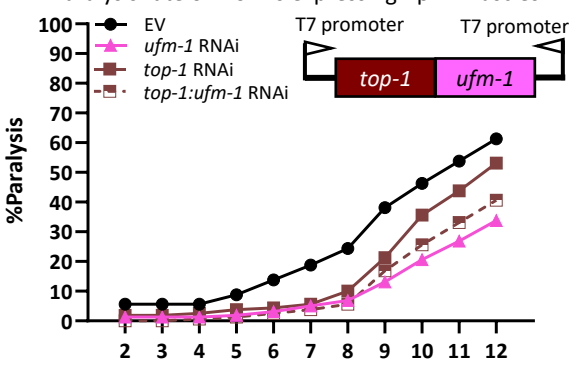
Strain CL2006, N=180 worms/treatment
RNAi treatment from hatching

l



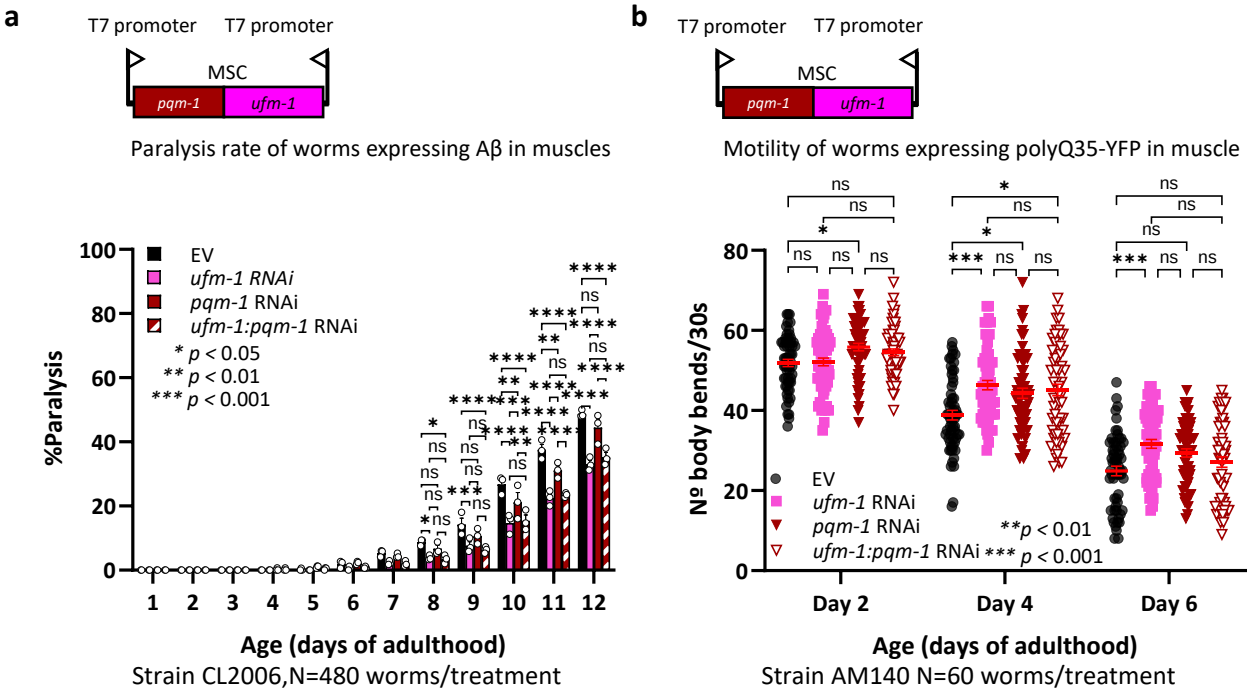
Strain CL2006, N=180 worms/treatment
RNAi treatment from hatching

m

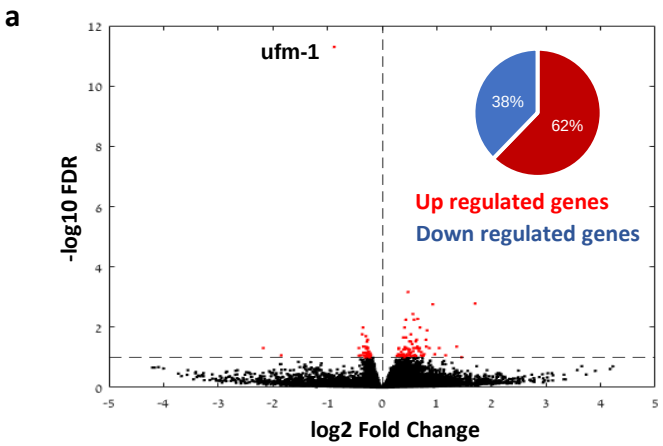


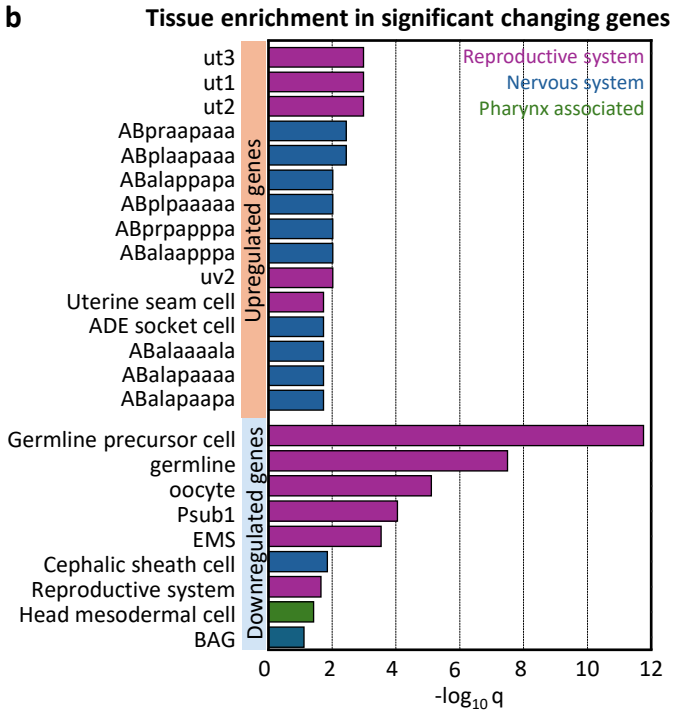
Strain CL2006, N=160 worms/ treatment
RNAi treatment from hatching

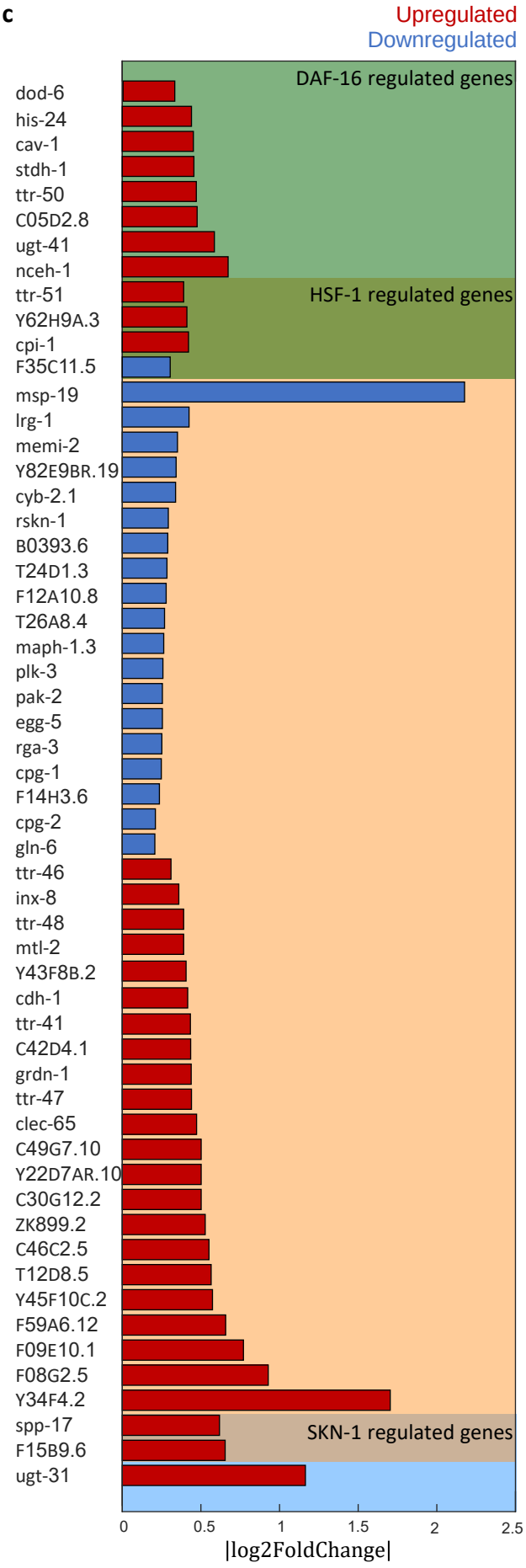
Supplemental Figure 4



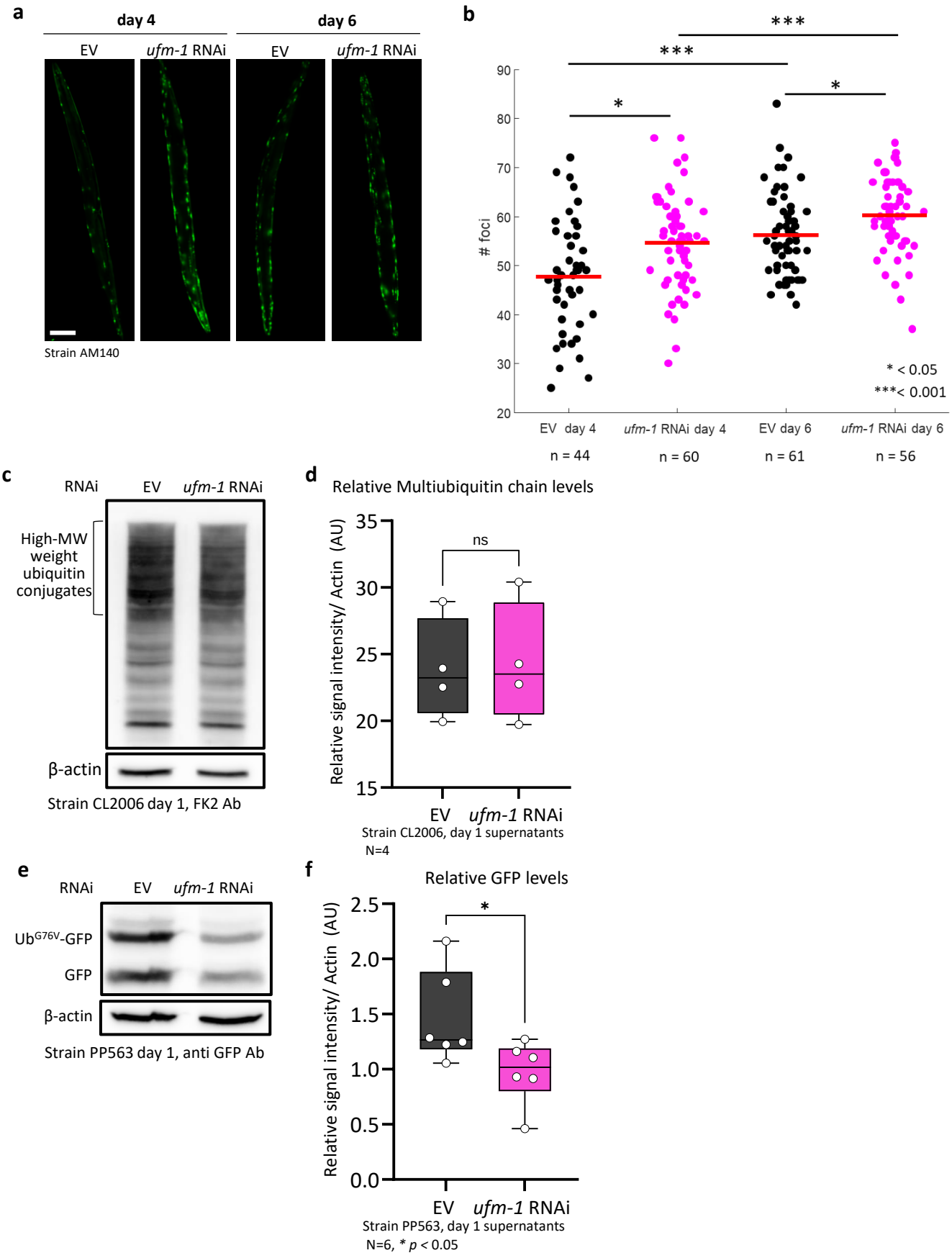
Supplemental Figure 5





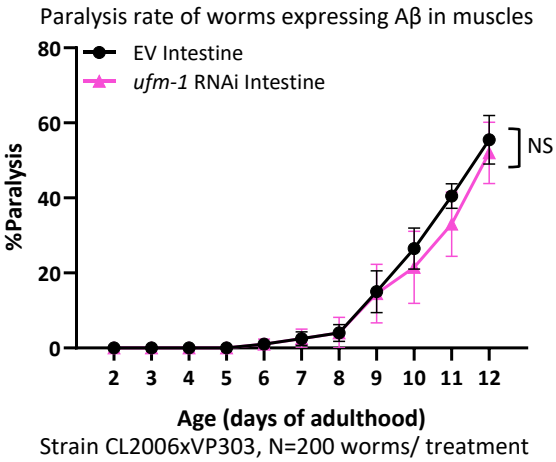


Supplemental Figure 6



Supplemental Figure 7

a



Strain CL2006xVP303, N=200 worms/ treatment

Supplemental Figure 1|

(a-f) Quantitative real-time PCR confirms the specific and efficient knockdown of *ufm-1* (a-b), *uba-5* (c-d) or *odr-8* the UFSP-1/2 homologue in *C. elegans* (e-f) using the RNAi's created for this study. Bars represent average expression levels of the indicated genes in three independent repeats ± SEM. Statistical test used: Statistical test used: Two-way ANOVA followed by Holm-Sidak correction for multiple comparisons, *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. (g) Paralysis assays of CL2006 worms that were treated with *odr-8* RNAi or left untreated (EV), showing reduced paralysis rate upon *odr-8* knockdown. N=5 independent experiments, Bars represent average daily rates of paralysis of the population± SEM. Statistical test used: Two-way ANOVA followed by Holm-Sidak correction for multiple comparisons, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. (h) *uba-5* RNAi treatment protects AM1126 worms from neuronal polyQ35-YFP-mediated proteotoxicity. N=3 independent experiments. Bars represent average thrashing rate within the population ± SEM. Statistical test used: Two-way ANOVA followed by Holm-Sidak correction for multiple comparisons, ****$p < 0.0001$. (i) Lifespan analysis of CF512 worms treated with *ufm-1* or *uba-5* RNAi, showing modest, but significant, lifespan extension upon *ufm-1* knockdown.

Supplemental Figure 2|

(a) Paralysis assay using CL2006 worms, treated with *uba-5* or *ufm-1* RNAi, or with mixtures of 50% *uba-5* RNAi and EV or the mixture of 50% *ufm-1* RNAi, indicates that the dilution of either *uba-5* RNAi or *ufm-1* RNAi lowers the efficiency of its counter-proteotoxic effect. N=120 worms/treatment. (b) Paralysis assay of CL2006 worms grown on control bacteria (EV), *ufm-1* RNAi, *fib-1* RNAi or *ufm-1/fib-1* dual silencing cassette, indicates that the concurrent knockdown of *fib-1* and *ufm-1* does not further reduce the rate of paralysis below the level which is mediated by a sole knockdown of *fib-1* RNAi. N=200 worms/treatment.

Supplemental Figure 3|

(a-c) Quantitative real-time PCR confirms the efficiency and specificity of knockdown of *sip-1* (a), *hsp-1* (b) or *daf-21* (c) by the dual silencing cassettes which target either one of these genes together with *ufm-1* RNAi. (D-F) Quantitative real-time PCR confirms the knockdown specificity of *ufm-1* in the dual silencing cassettes that concurrently knockdown *ufm-1* and *sip-1* (d), *hsp-1* (e) or *daf-21* (f). In a-f: bars represent average expression levels in three independent repeats ± SEM. Statistical test

used: ordinary one-way ANOVA mean of treatment compared to mean of EV control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (g) Paralysis assay using CL2006 worms grown on EV, *ufm-1* RNAi, *kin-19* RNAi or *ufm-1/kin-19* dual silencing cassette, shows that the knockdown of *kin-19,* prevents *ufm-1* RNAi from alleviating the paralysis phenotype. N=150 worms/treatment. (h) Paralysis assay of CL2006 worms grown on EV, *ufm-1* RNAi, *atp-2* RNAi or *ufm-1/atp-2* dual silencing cassette, shows that the knockdown of *atp-2,* alleviates the paralysis phenotype regardless of whether *ufm-1* has been knocked down or not. N=160 worms/treatment (I-M) The concurrent knockdown of *ufm-1* and either one of the other five genes *lfi-1* (i), *ant-1.1* (j), *cpl-1* (k), *trap-1* (l) and *top*-1 (m), did not prevent *ufm-1* RNAi from mitigating proteotoxicity as was shown by paralysis assays employing CL2006 worms. N>=150 worms/treatment as indicated in each panel.

Supplemental Figure 4|

(a-b) Paralysis assay of CL2006 worms (a) and thrashing assay of AM140 worms grown on EV, *ufm-1* RNAi, *pqm-1* RNAi or *ufm-1/pqm-1* dual silencing cassette. The results show that a concurrent knockdown of *ufm-1* and *pqm-1* does not significantly change the counter proteotoxic effect that stems from the knockdown of *ufm-1*. N=3 independent experiments, bars represent average daily rates of paralysis of CL2006 population± SEM (a). Statistical test used: Two-way ANOVA followed by Holm-Sidak correction for multiple comparisons, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Supplemental Figure 5|

(a) Volcano plot of genes that exhibited modulated expression levels in RNA sequencing data, showing that 62% of the significantly affected genes are upregulated and 38% are down regulated. (b) Clustering of the genes that show significantly modulated expression levels, shows enrichment of genes that are known to be expressed in the neuronal and reproductive systems. (c) Targets of DAF-16 and SKN-1 were relatively abundant among genes that exhibited modulated expression level upon the knockdown of *ufm-1*.

Supplemental Figure 6|

(a-b) The knockdown of *ufm-1* enhances the number of polyQ35-YFP containing foci, visualized by a fluorescent microscope (a, scale bar 100µm), and measured by foci quantification (b). Bars represent average number of foci. Statistical test used: One-way ANOVA, followed by Tukey-Kramer

correction for multiple comparisons. (c-d) Western blot analysis of highly ubiquitinated proteins shows no change in ubiquitin conjugate levels in homogenates of day 1 old CL2006 animals regardless of whether they were treated with *ufm-1* RNAi or left untreated, Bars represent average signal intensity of 4 replicates ± SEM. Statistical test used: unpaired, two-tailed Student's *t*-test. (e-f) Western blot analysis of ubiquitinated GFP (strain PP563) shows that *ufm-1* RNAi treatment reduces GFP intensity (e). This is indicative of significant enhancement of proteasome activity (f). Bars represent average signal intensity of 6 replicates ± SEM. Statistical test used: unpaired, two-tailed Student's *t*-test, $*p < 0.05$.

Supplemental Figure 7|

(a) Intestine-specific knockdown of *ufm-1* does not affect the toxicity of Aβ in muscles as measured by the paralysis assay. Statistical test used within 10 plates/treatment of the experiment: two-way ANOVA followed by Holm-Sidak correction for multiple comparisons.