SUPPLEMENTARY INFORMATION

eIF4E function in somatic cells modulates ageing in *C. elegans*

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Supplementary Information

Supplementary Materials and Methods

Strains and genetics. We followed standard procedures for *C. elegans* strain maintenance, crosses and other genetic manipulations[1]. Nematode rearing temperature was kept at 20°C, unless noted otherwise. Some nematode strains were obtained by the *C. elegans* Knockout consortium (Robert Barstead, Oklahoma Medical Research Foundation, USA) and the *Caenorhabditis* Genetics Center (Theresa Stiernagle, University of Minnesota, Minneapolis, USA). The following strains were used in this study: N2: wild type Bristol isolate, RB579: *ife-2(ok306)X*, KX15: *ife-2(ok306)X*, outcrossed version of RB579, KX17: *ife-4(ok320)X*, TJ1052: *age-1(hx546)II*, CB1370: *daf-2(e1370)III*, GR1307: *daf-16(mgDf50)I*, DR26: *daf-16(m26)I*, KR344: *let-363(h98)dpy-5(e61)unc-13(e450)I;sDp2(I;f)*, MQ130: *clk-1(qm30)III*, DA465: *eat-2(ad465)II*, SS104: *glp-4(bn2ts)I*, TK22: *mev-1(kn1)III*, N2*Ex*[$p_{ife\text{-}2}$IFE-2::GFP, pRF4], *daf-2(e1370)Ex*[$p_{ife\text{-}2}$IFE-2::GFP, pRF4], N2*Ex*[$p_{ife\text{-}2}$GFP, pRF4], KX15*Ex*[$p_{ife\text{-}2}$GFP, pRF4]. *sDp2(I;f)* is unstable during meiosis and is lost in about 30% of KR344 progeny. The presence of *sDp2(I;f)* was monitored by complementation of the mutations in the *let-363* and *dpy-5* loci.

Plasmid constructs and RNA interference. Reporter constructs were generated by fusing PCR-amplified DNA fragments encompassing either the *ife-2* promoter (for $p_{ife\text{-}2}$GFP), or the full-length *ife-2* gene (for $p_{ife\text{-}2}$IFE-2::GFP), with GFP on the plasmid vector pPD95.77[2]. For RNAi experiments, we constructed plasmids that direct the synthesis of dsRNAs corresponding to the genes of interest, in *E. coli* bacteria, which were then fed to animals. Gene-specific fragments were

obtained by PCR amplification directly from *C. elegans* genomic DNA using appropriate primer sets (see Supplementary Information). PCR-generated fragments were sub-cloned into the pL4440 plasmid vector and resulting constructs were transformed into HT115(DE3) *E. coli* bacteria as described previously[3]. In all RNAi experiments, control animal cohorts were grown on HT115 bacteria transformed with empty pL4440 plasmid vector.

PCR oligonucleotides. Gene fragments for RNAi and GFP constructs were amplified directly from genomic DNA, using the oligonucleotide primers incorporating appropriate restriction enzyme sites for subcloning purposes. The following oligonucleotides were used for generating gene-specific RNAi constructs:

For *ife-1*: 5'CGGGATCCATGACTGAAACGGAGCAAAC3'and 5'GCTCTAGACGGCGACTGGAGCATCG3', for *ife-2*: 5'CGGGATCCAGCAAGTAATGTCCGAAG3' and 5'AACTGCAGCATTTCAACAAGTGAAGAAC3', for *ife-3*: 5'CGTCTCGAGACCTGGTATCG3' and 5'GCTCTAGACGCCCTACAAATCATGTG3', for *ife-5*: 5'CGGGATCCATGACTGAATTGACGACAC3' and 5'GCTCTAGACAGAGCCGAGGTGGTCTTC3', for *rskn-1* (T01H8.1; p90S6K): 5'CGGGATCCGATTCATCATCAGAAACCG3' and 5'GCTCTAGAGCTATCGACAGTGGAGAG3', for *rsks-1* (Y47D3A.16; p70S6K; Cep70): 5'AACTGCAGCGACGGGCTCCGACAATG3' and 5'GCTCTAGACGCAGCACCAGCTCTCG3', for *let-363* (CeTOR): 5'AACTGCAGCTCCAACAACACGGAATTAG3' and 5'GCTCTAGACAAGCCATTCAACACC3', for *mnk-1* (R166.5): 5'GCTCTAGACTACTACAAGTTAACGG3' and 5'AACTGCAGCATGGATAGGAAGGATC3', for *daf-2*: 5'GGATCCTGTGCCCACGTGGAGCTT3' and 5'CCGCTCGAGTGAATAGCGTCCGAATCGA3', and for *daf-16*:

5'CCGCTCGAGCACTGATCTTTCAAGCCG3' and

5'CCCAAGCTTCTTGTGCAAGAGTTAACCG3'.

The following oligonucleotides were used for generating GFP reporter constructs: For the p_{ife-2}GFP reporter fusion: 5'ACATGCATGCCTAAATTTTAAGGGTACAAG3' and 5'CGGGATCCCATTACTTGCTGAAAAGAAAAC3', and for the p_{ife-2}IFE-2::GFP fusion: 5'CACCGGTGCTGCAGCCAC3' and 5'CGGGATCCAGTGGCTGGTGTGGCAGG3'.

Heat-shock resistance assays. Resistance to heat shock was assayed as described previously[4]. Briefly, synchronized nematode populations were reared at 20°C and shifted to 35°C during early adulthood. Survival at high temperature was assessed approximately every three hours.

Oxidative stress resistance assays. Paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) and sodium azide (NaN_3) were used to induce oxidative stress. Paraquat is readily reduced to radical ions, which generate superoxide radicals inducing oxidative stress in worms[5]. NaN_3 binds to the heme cofactor of cytochrome c oxidase, similarly to carbon monoxide, and inhibits the mitochondrial electron transport chain complex IV, resulting in increased H_2O_2 production and acute oxidative stress[6]. For paraquat resistance assays, L4 larvae were exposed to 2 mM paraquat (Aldrich, Munich Germany) on agar plates at 20˚C and survival was scored on day 5 of exposure. For NaN_3 resistance, nematodes at the L4 stage of development were selected, washed with M9 and incubated for 30 min at 20˚C with 0.5 M freshly made NaN_3 (Sigma, Munich Germany) in M9. Worms were washed with M9 and placed into NGM plates to recover. The percentage of living worms was calculated after 24 hours of recovery (adapted from ref. 7).

Fluorescence recovery after photobleaching (FRAP) and quantification of GFP emission. For FRAP measurements, transgenic animals expressing GFP were photographed at the young adult stage as described below, on an Axioskop 2 Plus, epifluorescence microscope (Carl Zeiss, Jena,

Germany), and subsequently illuminated with a mercury lamp via a 485/20 nm band-pass filter and a 10x objective lens (numerical aperture 0.5). The illumination power on the specimens was 32mW. Fluorescence was bleached for 15 min to about 30% of initial emission intensity. Animals were allowed to recover and were photographed at 1-hour intervals. Experiments were performed at 25^{o}C, with photography exposure time kept identical for each animal. We performed experiments with GFP expressed under various promoters (both tissue specific and generally expressing), with similar results. We present data with GFP expressed under the *ife-2* promoter in all somatic cells that also express the *ife-2* gene (Fig. 1c). For photography and quantification of GFP emission, transgenic animals were either scanned with a 488 nm laser beam, under a confocal microscope or photographed using a 480±10 nm band-pass excitation filter on an epifluorescence microscope. Images were acquired using a 515±15 nm band-pass emission filter. Emission intensity was measured on greyscale images with a pixel depth of 8 bit (256 shades of grey). We calculated the mean and maximum pixel intensity for each animal in these images using the ImageJ software (http://rsb.info.nih.gov/ij/). For each transgenic line, we processed at least 250 images over at least 3 independent trials. Statistical analysis was carried out using the Microsoft Office 2003 Excel software package (Microsoft Corporation, Redmond Washington USA).

Statistical analysis of lifespan data. The log-rank (Mantel-Cox) test was used to evaluate differences between survivals and determine *P* values. We used the Prism software package (GraphPad Software Inc., San Diego, USA), to carry out statistical analysis and to determine lifespan values. Worms that died due to internally hatched eggs, an extruded gonad or desiccation due to crawling on the edge of the plates, were censored and incorporated into the data set as described[8].

Supplementary References

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7. Scott, B.A., Avidan, M.S. & Crowder, C.M. Regulation of hypoxic death in C. elegans by the insulin/IGF receptor homolog DAF-2. *Science* **296**, 2388-91 (2002).
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Supplementary Table 1. Adult lifespan values

Strain	Mean±s.e.m. days	Max* days	Number of animals that died/total†	*P* value
N2	18.2±0.5	29.4	2171/2420 (27)	
RB579	24.6±1.2	41.6	436/450 (4)	<0.0001‡
KX15	28.8±0.8	39.8	1338/1550 (19)	<0.0001‡
ife-1(RNAi)	17.0±1.0	31.0	520/595 (7)	ns‡
ife-2(RNAi)	22.4±1.1	40.4	895/952 (17)	<0.0001‡
ife-3(RNAi)	19.0±1.3	27.8	211/265 (3)	ns‡
ife-4(ok320)	20.0±0.9	29.8	572/825 (10)	0.21‡
ife-5(RNAi)	17.1±1.1	30.1	326/413 (5)	ns‡
ife-4(ok320);ife-2(RNAi)	23.3±1.2	41.5	71/187 (3)	ns,<0.0001§§
age-1(hx546)	22.8±0.8	46.2	307/349 (3)	
age-1(hx546);ife-2(RNAi)	27.5±1.1	58.6	243/299 (3)	<0.0001§
daf-2(e1370)	40.8±0.5	62.3	191/256 (3)	
daf-2(e1370);ife-2(RNAi)	46.6±0.8	72.9	196/222 (3)	<0.0001§
daf-16(mgDf50)	17.2±0.3	27.9	254/305 (3)	
daf-16(mgDf50);ife-2(RNAi)	22.7±0.4	39.1	254/286 (3)	<0.0001§
daf-16(m26)	12.0±0.7	17.8	652/925 (11)	
daf-16(m26);ife-2(RNAi)	15.0±0.8	24.4	507/720 (11)	<0.0001§
clk-1(qm30)	20.9±0.4	36.7	210/268 (3)	
clk-1(qm30);ife-2(RNAi)	29.7±0.4	47.5	212/259 (3)	<0.0001§
eat-2(ad465)	24.3±0.2	31.8	97/125 (3)	
eat-2(ad465);ife-2(RNAi)	27.2±0.3	42.6	105/135 (3)	<0.0001§
KR344(*-sDp2*)¶	18.0±0.7	29.2	148/162 (3)	
KR344(*-sDp2*);*ife-2(RNAi)*¶	21.3±1.0	35.8	163/189 (3)	<0.0001§
KR344#	12.1±0.5	25.0	109/210 (3)	
KR344;*ife-2(RNAi)*#	15.7±0.5	31.2	123/208 (3)	<0.0001§
rsks-1(RNAi)	19.1±0.5	29.2	230/255 (3)	ns‡
rskn-1(RNAi)	19.8±0.5	29.3	306/325 (4)	ns‡
rsks-1(RNAi);KX15	27.6±0.6	36.9	92/120 (3)	ns††
rskn-1(RNAi);KX15	26.9±0.5	39.8	116/132 (3)	ns††
mnk-1(RNAi)	13.9±0.6	25.5	191/405 (4)	<0.0001‡
mnk-1(RNAi);KX15	25.2±0.6	35.9	169/325 (4)	<0.0001‡‡
mev-1(kn1)	7.6±0.8	13.9	94/125 (2)	<0.0001‡
mev-1(kn1);ife-2(RNAi)	16.1±1.3	24.0	91/127 (2)	<0.0001§
N2**	11.6±0.7	21.7	1747/1902 (22)	
*ife-2(RNAi)***	16.0±0.7	29.9	588/730 (9)	<0.0001‡
glp-4(bn2ts)	10.0±0.5	22.8	955/1100 (13)	
*glp-4(bn2ts);ife-2(RNAi)***	14.3±1.0	28.1	677/720 (8)	<0.0001§
*glp-4(bn2ts);mnk-1(RNAi)***	10.9±0.7	22.0	205/295 (3)	ns§
KX15**	17.1±0.4	26.0	563/853 (8)	0.0001‡
*let-363(RNAi)***	13.4±0.9	25.2	863/923 (11)	0.038‡
let-363(RNAi);KX15**	19.2±0.8	30.1	587/829 (7)	<0.001§

Supplementary Table 1. Footnote

*Maximum lifespan shown is the median lifespan of the longest-lived 10% of the animals assayed.

†The total number of animals included in lifespan assays equals the number of animals that died plus the number of animals that were censored (see Materials and Methods). The number of independent lifespan assays for each strain is shown in parentheses. The least number of animals followed for any strain tested was 120.

¶Arrested L3, Dumpy and Uncoordinated (DpyUnc) *let-363(h98)dpy-5(e61)unc-13(e450)* larvae that have lost the duplication *sDp2* were assayed.

#Uncoordinated (Unc) *let-363(h98)dpy-5(e61)unc-13(e450);sDp2*, (KR344) isogenic adults, carrying the duplication *sDp2*, which balances the *let-363* and *dpy-5* loci were assayed.

**Assays were carried out at 25^{o}C.

‡Compared with wild type control animals, assayed at the same temperature.

§Compared with the corresponding mutant subjected to control RNAi (see Materials and Methods).

††Compared with KX15 control animals, assayed at the same temperature.

‡‡Compared with both wild type and KX15 control animals, assayed at the same temperature.

§§Compared with *ife-2(RNAi)* and *ife-4(ok320)*, respectively.

ns: no significant difference compared to control (P>0.5).

Supplementary Table 2. Phenotypic analysis of *ife-2* mutants. All assays were performed at 20°C unless noted otherwise.

	Pharyngeal pumping*	Defecation†	Sinusoidal locomotion¶	Body size#	Fecundity‡	Embryonic lethality§	Developmental timing**	Dauer formation††
wild type	219±16	44±8	0.70±0.1	1.2±0.3	296±12 (204±17)	4.1±1.1 (7.9±1.6)	51±7	16±7
ife-2(ok306)	208±23	49±11	0.76±0.1	1.1±0.2	284±19 (176±21)	5.9±1.4 (91.8±4.3)	54±9	18±9

*Pumps per minute

†Duration of defecation cycle in seconds

¶Wavelength of sinusoidal track in mm

#Adult body length in millimetres

‡Number of eggs laid per worm (the number of eggs laid at 25°C per worm are shown in parentheses; animals were moved to 25°C at the L1 larva stage)

§Percentage of eggs that failed to hatch (the percentage of eggs that failed to hatch at 25°C are shown in parentheses; animals were moved to 25°C at the L1 larva stage)

**Duration of postembryonic development (hours from egg hatching to L4 moult)

††Percentage of animals that become dauer larvae at 27°C

Legends to supplementary figures

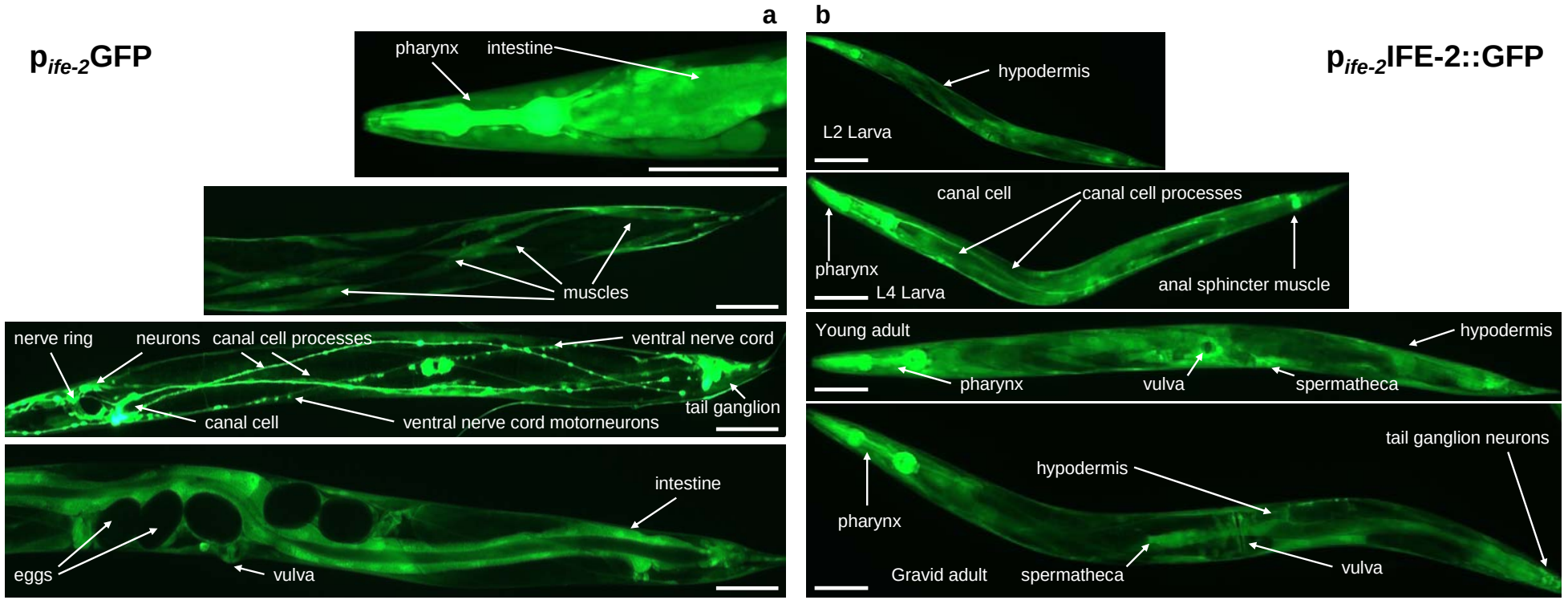
Supplementary Figure 1. Details of spatiotemporal *ife-2* expression in *C. elegans*. **a,** Images of transgenic animals carrying p_{ife-2}GFP. **b,** Images of transgenic animals carrying p_{ife-2}IFE-2::GFP. Various cells and organs are indicated by arrows. White bars denote 100 microns.

Supplementary Figure 2. Heat shock resistance of *ife-2(ok306)* deletion mutants vs. wild type animals. The percentage of young adult animals that survive exposure to high temperature (35^{o}C) is plotted over time.

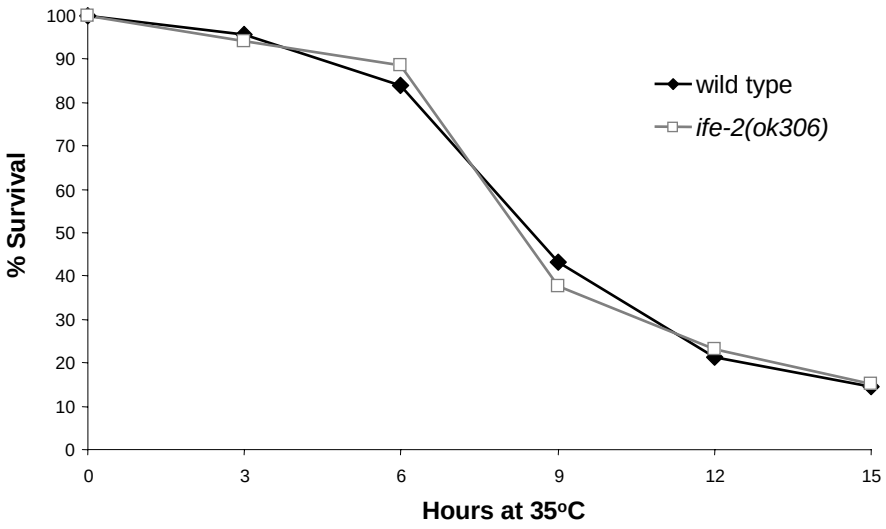
Supplementary Figure 3. RNAi with *ife-2*, which extents animal lifespan, does not eliminate expression of *ife-2* in the nervous system. **a,** Images of transgenic animals carrying a full-length p_{ife-2}IFE-2::GFP reporter fusion, reared on plates with *E. coli* bacteria producing dsRNA derived from *ife-2*. Expression is quenched in all somatic cells except neurons. **b,** Images of control transgenic animals carrying p_{ife-2}IFE-2::GFP, grown on bacteria that do not produce dsRNA derived from *ife-2*. Neurons and neuronal processes are shown by arrows. White bars denote 100 microns.

Supplementary Figure 4. Expression of *ife-2* is unaffected by DAF-2 deficiency. Knockdown of *daf-2* by mutation or RNAi does not affect expression of a full-length IFE-2::GFP driven by the *ife-2* promoter (p_{ife-2}IFE-2::GFP). Representative images of *daf-2(e1360)* mutant animals bearing the p_{ife-2}IFE-2::GFP transgene, or animals subjected to *daf-2* RNAi vs. wild type controls. Quantification of animal fluorescence is also shown (error bars denote standard deviation; P>0.5, unpaired t test).

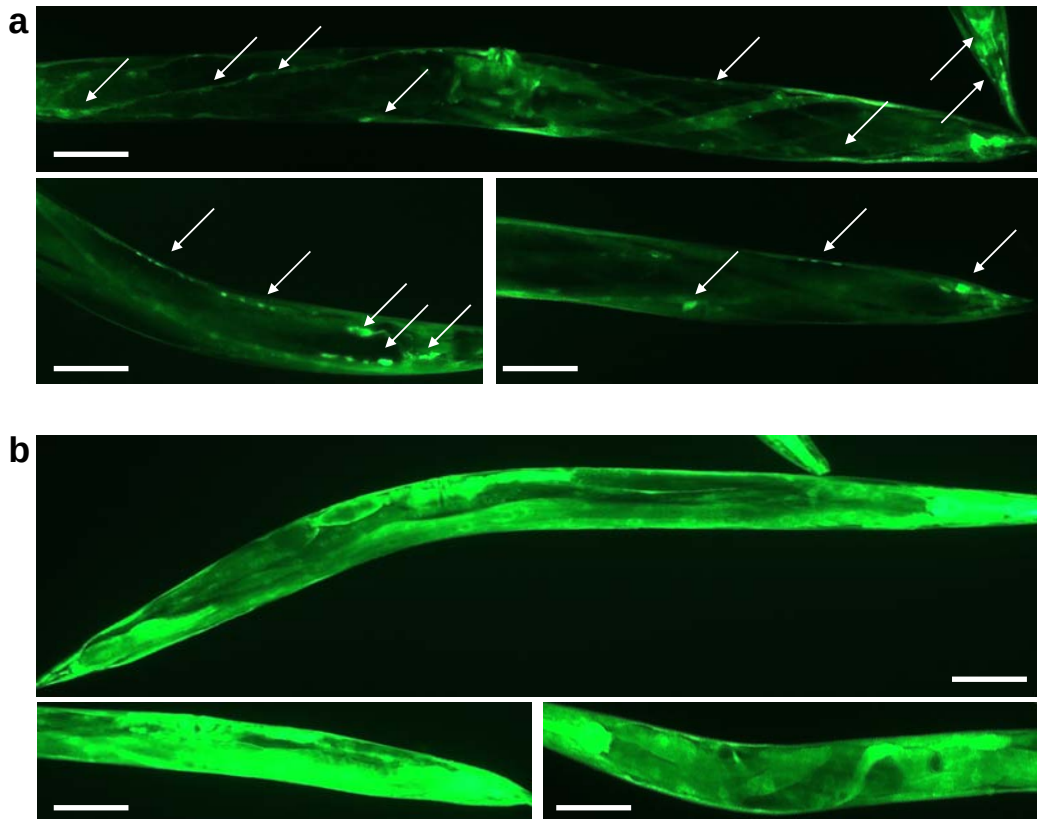
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4