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Supplemental information

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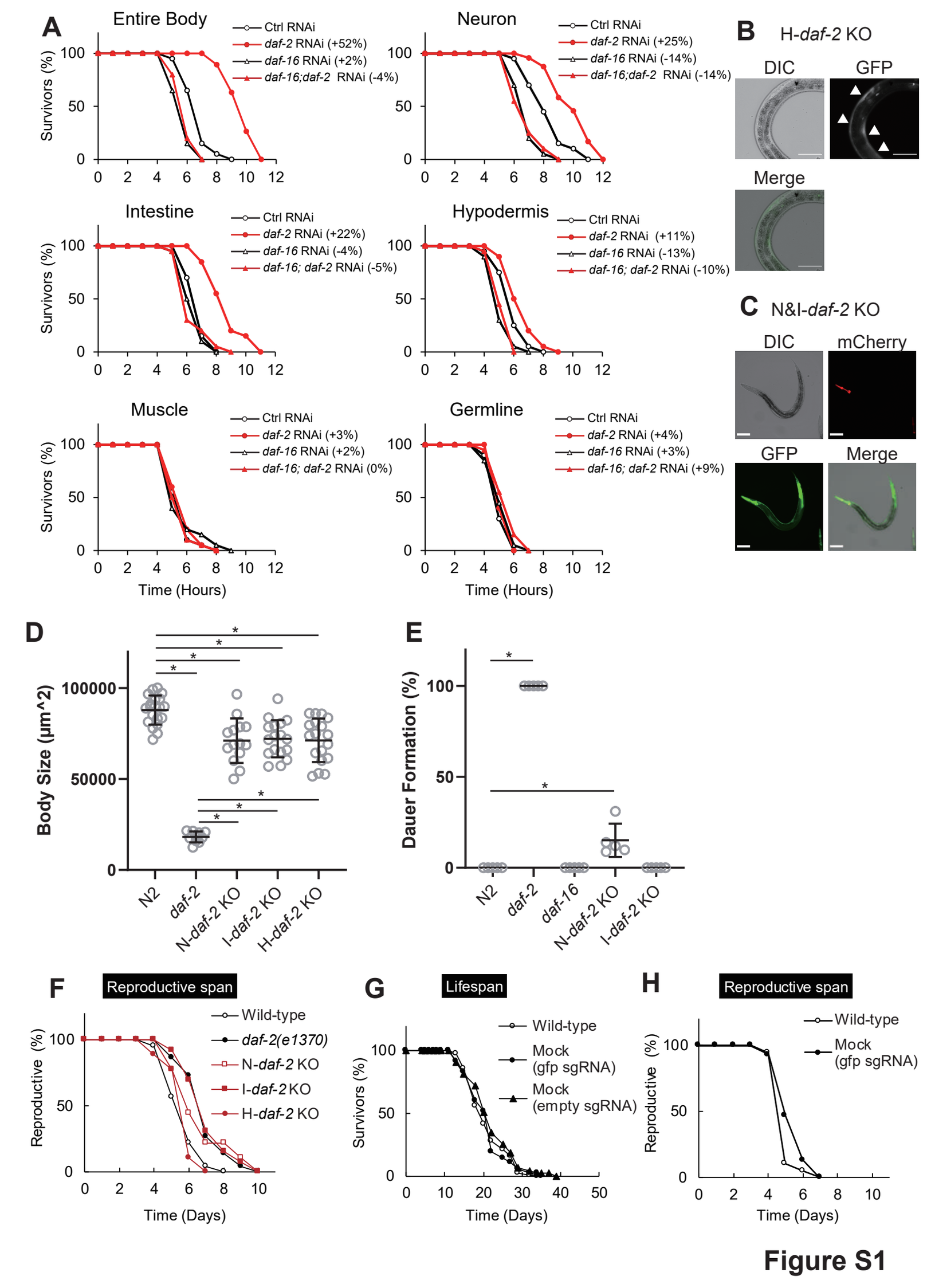

A
Entire Body
Ctrl RNAi
daf-2 RNAi (+52%)
daf-16 RNAi (+2%)
daf-16;daf-2 RNAi (-4%)
Neuron
Ctrl RNAi
daf-2 RNAi (+25%)
daf-16 RNAi (-14%)
daf-16;daf-2 RNAi (-14%)
Intestine
Ctrl RNAi
daf-2 RNAi (+22%)
daf-16 RNAi (-4%)
daf-16; daf-2 RNAi (-5%)
Hypodermis
Ctrl RNAi
daf-2 RNAi (+11%)
daf-16 RNAi (-13%)
daf-16; daf-2 RNAi (-10%)
Muscle
Ctrl RNAi
daf-2 RNAi (+3%)
daf-16 RNAi (+2%)
daf-16; daf-2 RNAi (0%)
Germline
Ctrl RNAi
daf-2 RNAi (+4%)
daf-16 RNAi (+3%)
daf-16; daf-2 RNAi (+9%)
Survivors (%)
Time (Hours)
B
H-daf-2 KO
DIC
GFP
Merge
C
N&I-daf-2 KO
DIC
mCherry
GFP
Merge
D
Body Size (μm^2)
N2
daf-2
N-daf-2 KO
I-daf-2 KO
H-daf-2 KO
E
Dauer Formation (%)
N2
daf-2
daf-16
N-daf-2 KO
I-daf-2 KO
F
Reproductive span
Wild-type
daf-2(e1370)
N-daf-2 KO
I-daf-2 KO
H-daf-2 KO
Reproductive (%)
Time (Days)
G
Lifespan
Wild-type
Mock (gfp sgRNA)
Mock (empty sgRNA)
Survivors (%)
Time (Days)
H
Reproductive span
Wild-type
Mock (gfp sgRNA)
Reproductive (%)
Time (Days)

Figure S1

Figure S1 *daf-2* Knockdown in the Neuron, Intestine, and Hypodermis Increases Oxidative Stress Resistance in a Cell Autonomous DAF-16-Dependent Manner. Related to Figure 1.

(A) Oxidative stress resistance in worms treated with *daf-2* and/or *daf-16* RNAi in the entire body (wild type N2, upper left), neuron (TU3401, upper middle), intestine (VP303, upper right), hypodermis (NR222, lower left), muscle (NR350, lower middle), or germline cells (NL2098, lower right). Representative data from two or three independent experiments are shown.

(B, C) Representative expression patterns of wGFP reconstituted from wGxxFP as observed in hypodermis *daf-2* knockout (H-*daf-2* KO, arrow heads indicate hypodermis) (B) or neuron and intestinal *daf-2* knockout (N&I-*daf-2* KO) (C) transgenic worms. Scale bar indicates 50 or 100 μm in B or C, respectively.

(D) Scatter plots of the body sizes of worms (N2, *daf-2(e1370)*, and neuron-, intestine-, and hypodermis-*daf-2* KO). Representative data from three independent experiments are shown. Statistical significance was calculated by one-way ANOVA with a post hoc Tukey's test. **P*<0.05. Error bars represent the mean ± s.d. Data represent size of individual animals in a representative experiment.

(E) Scatter plots of the dauer formation ratio from five independent experiments are shown. Error bars represent the mean ± s.d. of five independent experiments. Statistical significance was calculated by one-way ANOVA with a post hoc Dunn's test. **P*<0.05.

(F) Reproductive spans of worms with the knockout *daf-2* in the neuron (N-*daf-2*), intestine (I-*daf-2*), or hypodermis (H-*daf-2*). Representative data from three independent experiments are shown.

(G) Survival curves of worms expressing CRISPR/Cas9 and gfp sgRNA or empty sgRNA. Representative data from three independent experiments are shown.

(H) Reproductive spans of worms expressing CRISPR/Cas9 and gfp sgRNA. Representative

data from two independent experiments are shown.

Statistics are presented in Tables S3, 6, and 7.

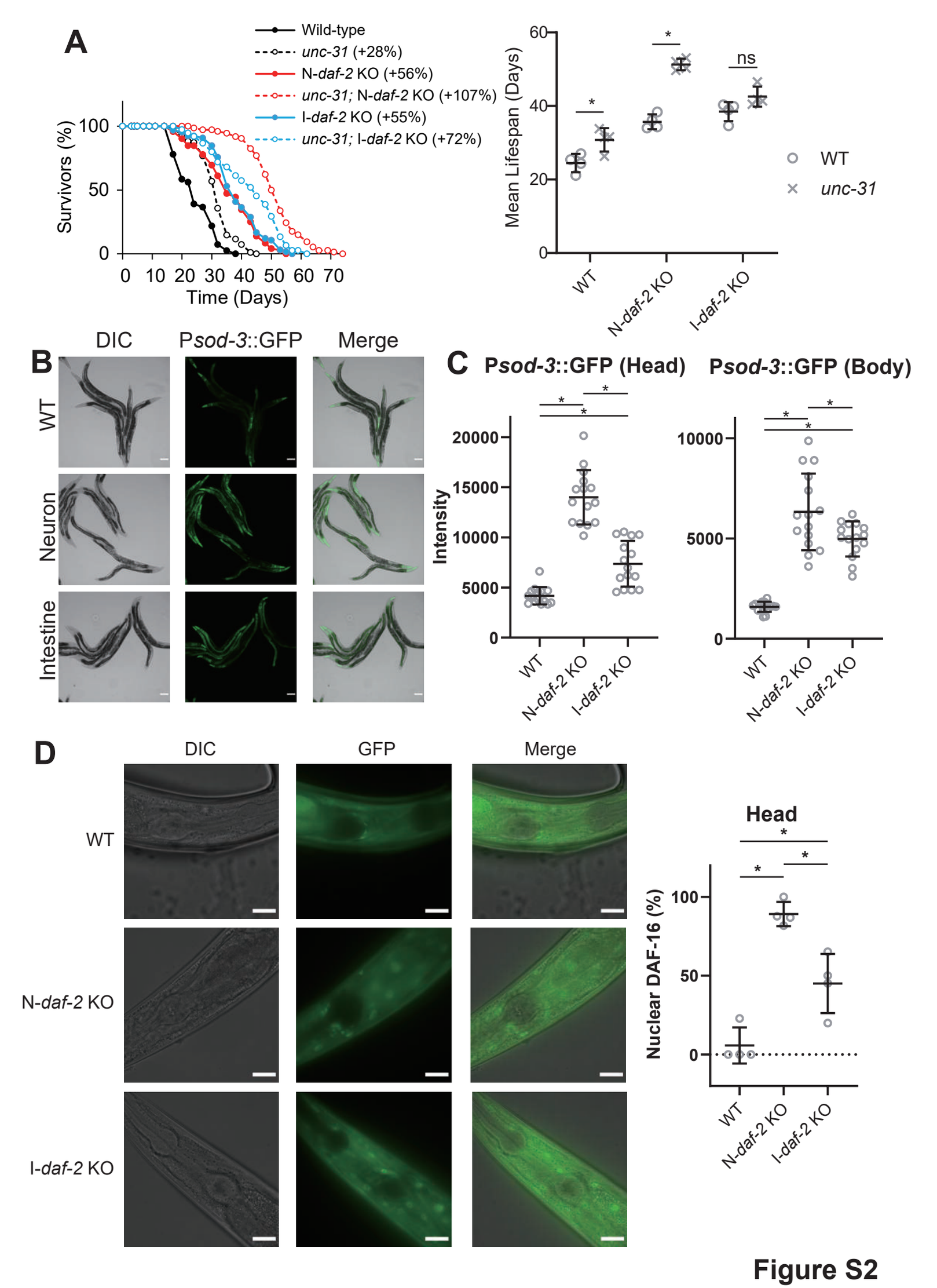

A
Wild-type
unc-31 (+28%)
N-daf-2 KO (+56%)
unc-31; N-daf-2 KO (+107%)
I-daf-2 KO (+55%)
unc-31; I-daf-2 KO (+72%)
Survivors (%)
Time (Days)
Mean Lifespan (Days)
WT
unc-31
ns
N-daf-2 KO
I-daf-2 KO
B
DIC
Psod-3::GFP
Merge
WT
Neuron
Intestine
C
Psod-3::GFP (Head)
Psod-3::GFP (Body)
Intensity
D
DIC
GFP
Merge
WT
N-daf-2 KO
I-daf-2 KO
Head
Nuclear DAF-16 (%)

Figure S2

Figure S2 *daf-2* Knockout in the Neuron Extends Lifespan in a UNC-31-Independent Manner. Related to Figure 2.

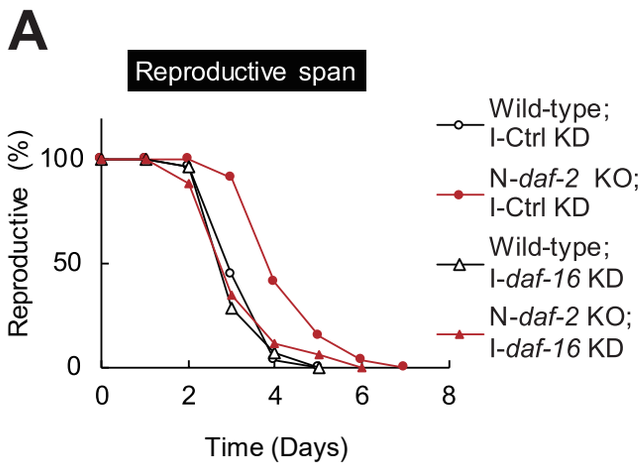
(A) Survival curves of *unc-31(e298)* worms with the knockout of *daf-2* in the neuron or intestine (left). Mean lifespan from four technical experiments of the representative data are shown (right). To examine the functional interaction between neuron-*daf-2* KO and *unc-31* mutation, we conducted two-way ANOVA analysis with post hoc Sidak's test. (right). Statistical significance was calculated by two-way ANOVA with a post hoc Sidak's test. **P*<0.05. Representative data from two independent experiments are shown.

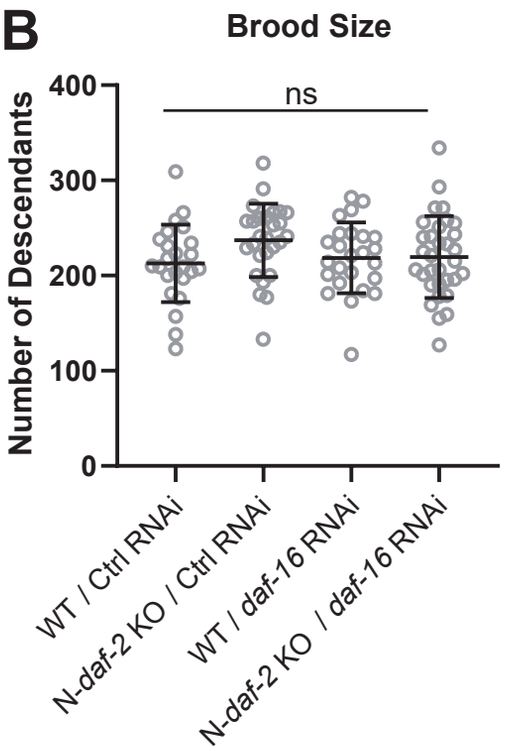
(B) Representative images showing the expression patterns of GFP driven by the promoter of *sod-3* in wildtype (upper) and transgenic worms with knockout of the *daf-2* gene in the intestine (N-*daf-2* KO, middle) or neuron (I-*daf-2* KO, lower).

(C) Quantification of GFP fluorescence intensity of head (left) or body (right) region of animals. Representative data from two independent experiments are shown. Data represent mean ± s.d. from 15 individual animals. Statistical significance was calculated by one-way ANOVA with a post hoc Tukey's test. **P*<0.05.

(D) Localization pattern of DAF-16::GFP in the head region of wildtype (left-upper) and that of transgenic worms with knockout of the *daf-2* gene in the neuron (N-*daf-2* KO, left-middle) or intestine (I-*daf-2* KO, left-lower). Scale bar indicates 20 μm. Ratio of the animals with DAF-16::GFP nuclear accumulation in the head neuron (right). Value represents mean ± s.d. from 4 independent experiments. Statistical significance was calculated by one-way ANOVA with a post hoc Tukey's test. **P*<0.05.

Statistics are presented in Table S5**.**


A
Reproductive span
Reproductive (%)
100
50
0
0
2
4
6
8
Time (Days)
Wild-type; I-Ctrl KD
N-daf-2 KO; I-Ctrl KD
Wild-type; I-daf-16 KD
N-daf-2 KO; I-daf-16 KD


B
Brood Size
Number of Descendants
400
300
200
100
0
ns
WT / Ctrl RNAi
N-daf-2 KO / Ctrl RNAi
WT / daf-16 RNAi
N-daf-2 KO / daf-16 RNAi

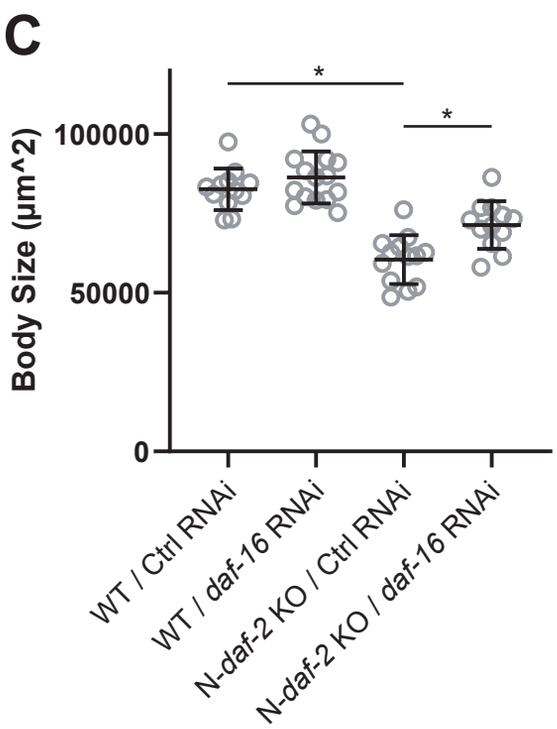

C
Body Size (µm^2)
100000
50000
0
*
*
WT / Ctrl RNAi
WT / daf-16 RNAi
N-daf-2 KO / Ctrl RNAi
N-daf-2 KO / daf-16 RNAi

Figure S3

Figure S3 *daf-2* knockout in the Neuron Slows Development in an Intestinal DAF-16-Dependent Manner. Related to Figure 3.

(A) Reproductive spans of worms with deficiency in *daf-2* in the neuron and /or *daf-16* in the intestine. Representative data from two independent experiments are shown. Data represent mean ± s.d. from 4 technical replicates. Statistical significance was calculated by two-way ANOVA with a post hoc Sidak's test. **P*<0.05.

(B) Scatter plots of the brood size of individual worm with deficiency of *daf-2* in the neuron and/or *daf-16* in the intestine. Statistical significance was calculated by one-way ANOVA with a post hoc Tukey's test. **P*<0.05.

(C) Scatter plots of the body sizes of worms with deficiency of *daf-2* in the neuron and/or *daf-16* in the intestine. Representative data from three independent experiments are shown. Statistical significance was calculated by one-way ANOVA with a post hoc Tukey's test. **P*<0.05.

Statistics are presented in Table S11 and S12.

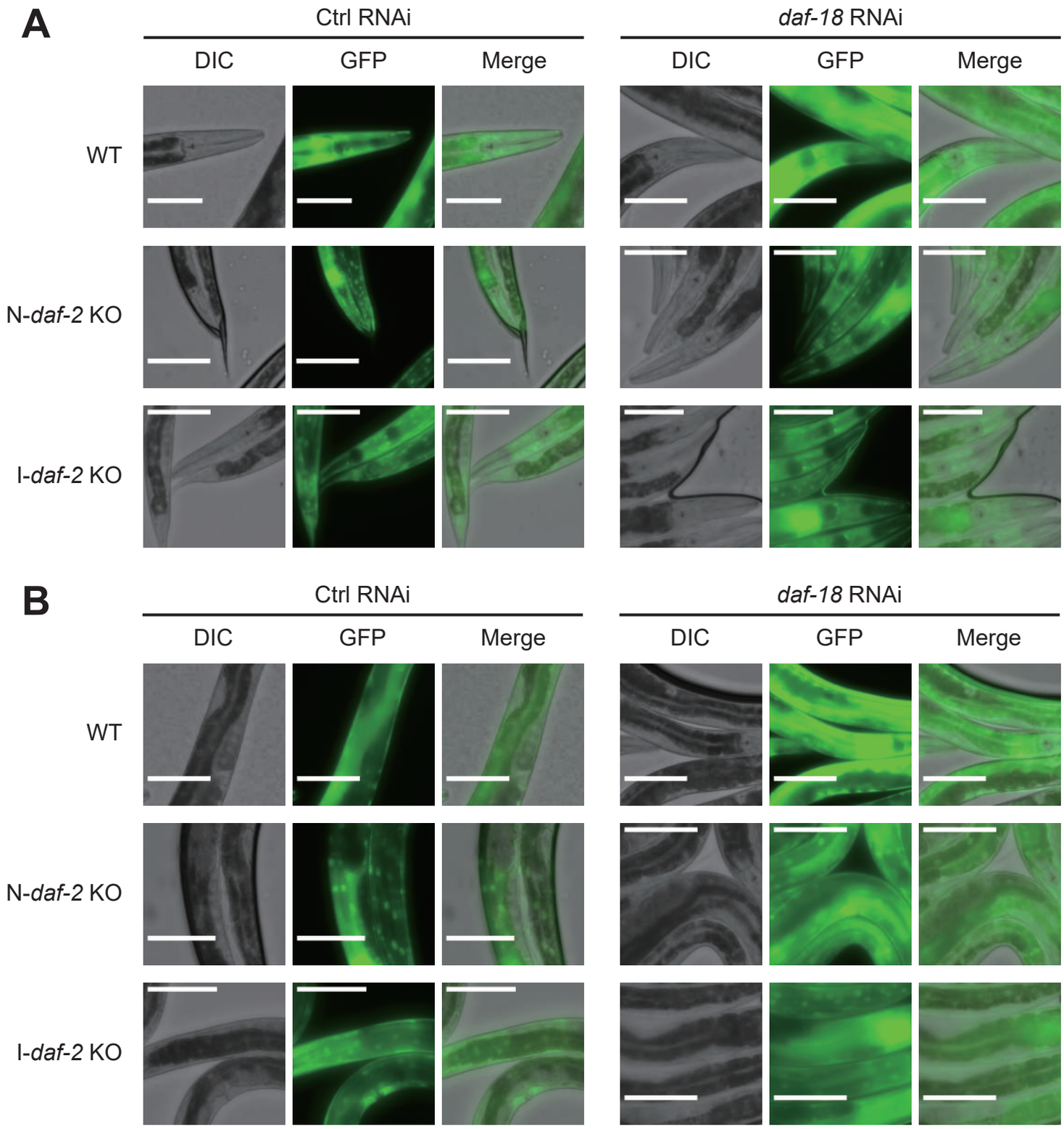

A
Ctrl RNAi
daf-18 RNAi
DIC
GFP
Merge
DIC
GFP
Merge
WT
N-daf-2 KO
I-daf-2 KO
B
Ctrl RNAi
daf-18 RNAi
DIC
GFP
Merge
DIC
GFP
Merge
WT
N-daf-2 KO
I-daf-2 KO

Figure S4

Figure S4 DAF-16 Nuclear Accumulation in the Intestine Is Retarded by *daf-18* RNAi. Related to Figure 5.

(A) Expression patterns of DAF-16::GFP in the head region of wildtype (left-upper) and that of transgenic worms with knockout of the *daf-2* gene in the neuron (N-*daf-2* KO, left-middle) or intestine (I-*daf-2* KO, left-lower). Each strain was treated with control (left) or *daf-18* (right) RNAi. Scale bar indicates 100 μm.

(B) Expression patterns of DAF-16::GFP in the body region of wildtype (left-upper) and that of transgenic worms with knockout of the *daf-2* gene in the intestine (N-*daf-2* KO, left-middle) or neuron (I-*daf-2* KO, left-lower). Each strain was treated with control (left) or *daf-18* (right) RNAi. Scale bar indicates 100 μm.

Statistics are presented in Table S13.

Table S1. Statistics of the Independent Lifespan Experiments 1. Related to Figure 1

Trial	Strain Trial	RNAi	Average life (Days)	Std. error	Worms (n)	Bonferoni P-value		
						Ctrl vs *daf-2*	Ctrl vs *daf-16*	*daf-2* vs *daf-2;daf-16*
#1	N2	Ctrl RNAi	19.79	0.59	26/40	<0.0001	0.0053	<0.0001
		daf-2 RNAi	38.5	0.82	58/72			
		daf-16 RNAi	17.36	0.41	46/80			
		daf-16;daf-2 RNAi	18.61	0.64	39/80			
#2	N2	Ctrl RNAi	19.91	0.35	75/80	<0.0001	<0.0001	<0.0001
		daf-2 RNAi	36.47	0.72	49/60			
		daf-16 RNAi	16.47	0.38	65/80			
		daf-16;daf-2 RNAi	17.73	0.47	77/80			
#3	N2	Ctrl RNAi	26.52	0.48	69/80	<0.0001	0.0001	<0.0001
		daf-2 RNAi	39.41	0.49	56/80			
		daf-16 RNAi	24.02	0.47	71/80			
		daf-16;daf-2 RNAi	23.54	0.51	70/80			
#1	TU3401	Ctrl RNAi	20.19	0.58	58/60	<0.0001	0.0013	<0.0001
		daf-2 RNAi	32.49	0.6	55/80			
		daf-16 RNAi	17.56	0.53	48/60			
		daf-16;daf-2 RNAi	18.41	0.61	29/40			
#2	TU3401	Ctrl RNAi	21.24	0.41	59/60	<0.0001	<0.0001	<0.0001
		daf-2 RNAi	34.08	0.68	40/40			
		daf-16 RNAi	18.46	0.37	37/40			
		daf-16;daf-2 RNAi	18.22	0.48	56/60			
#3	TU3401	Ctrl RNAi	27.86	0.58	53/60	<0.0001	<0.0001	<0.0001
		daf-2 RNAi	36.37	0.44	54/60			
		daf-16 RNAi	23.79	0.58	55/60			
		daf-16;daf-2 RNAi	24.46	0.55	49/60			
#1	VP303	Ctrl RNAi	20.95	1	19/40	<0.0001	0.0183	<0.0001
		daf-2 RNAi	29.21	0.9	61/80			
		daf-16 RNAi	17.95	0.54	31/80			
		daf-16;daf-2 RNAi	20.14	0.59	32/80			
#2	VP303	Ctrl RNAi	22.57	0.52	35/40	<0.0001	<0.0001	<0.0001
		daf-2 RNAi	32.22	1.09	18/40			
		daf-16 RNAi	19.59	0.34	54/60			
		daf-16;daf-2 RNAi	20.33	0.54	55/60			
#3	VP303	Ctrl RNAi	26.53	0.57	47/60	<0.0001	0.0025	<0.0001
		daf-2 RNAi	31.66	0.65	41/60			
		daf-16 RNAi	24.55	0.52	31/40			
		daf-16;daf-2 RNAi	24.46	0.48	45/60			
#1	NR222	Ctrl RNAi	19.44	0.42	68/80	<0.0001	0.0566	<0.0001
		daf-2 RNAi	23.17	0.74	37/40			
		daf-16 RNAi	17.85	0.45	58/80			
		daf-16;daf-2 RNAi	18.46	0.45	68/80			
#2	NR222	Ctrl RNAi	19.89	0.57	39/60	<0.0001	0.0122	<0.0001
		daf-2 RNAi	24.74	0.57	57/60			
		daf-16 RNAi	17.33	0.55	51/60			
		daf-16;daf-2 RNAi	17.79	0.67	53/60			
#3	NR222	Ctrl RNAi	25.9	0.84	38/60	0.0012	0.0065	<0.0001
		daf-2 RNAi	29.8	0.66	50/60			
		daf-16 RNAi	22.9	0.73	45/60			
		daf-16;daf-2 RNAi	23.15	0.83	47/60			
#1	DCL569	Ctrl RNAi	15.62	0.2	76/80	<0.0001	1	<0.0001
		daf-2 RNAi	18.5	0.36	72/81			
		daf-16 RNAi	15.31	0.24	73/80			
		daf-16;daf-2 RNAi	15.63	0.22	78/80			
#2	DCL569	Ctrl RNAi	15.96	0.2	63/78	<0.0001	<0.0001	<0.0001
		daf-2 RNAi	18.68	0.38	72/80			
		daf-16 RNAi	14.32	0.22	69/80			
		daf-16;daf-2 RNAi	15.53	0.25	78/80			
#1	NR350	Ctrl RNAi	17.56	0.55	27/40	1	1	1
		daf-2 RNAi	17.41	0.57	25/40			
		daf-16 RNAi	17.23	0.35	45/80			
		daf-16;daf-2 RNAi	17	0.48	32/40			
#2	NR350	Ctrl RNAi	17.62	0.35	52/60	0.2851	1	0.0039
		daf-2 RNAi	18.85	0.56	26/31			
		daf-16 RNAi	17.29	0.83	14/20			
		daf-16;daf-2 RNAi	16.58	0.35	40/60			

Table S2. Statistics of the Independent Oxidative Sress Experiments 1. Related to Figure S1

Trial	Strain Trial	RNAi	Average life (Hours)	Std. error	Worms (n)	Bonferoni P-value		
						Ctrl vs *daf-2*	Ctrl vs *daf-16*	*daf-2* vs *daf-2;daf-16*
1	N2	Ctrl RNAi	4.25	0.10	20/20	1.6e-9	1	0
		daf-2 RNAi	6.45	0.22	20/20			
		daf-16 RNAi	4.35	0.11	20/20			
		daf-16;daf-2 RNAi	4.1	0.07	20/20			
2	N2	Ctrl RNAi	6.5	0.21	20/20	5.5e-6	1	9.5e-8
		daf-2 RNAi	8.55	0.25	20/20			
		daf-16 RNAi	6.3	0.26	20/20			
		daf-16;daf-2 RNAi	6	0.19	20/20			
3	N2	Ctrl RNAi	6.8	0.19	20/20	7.8e-9	0.0013	0
		daf-2 RNAi	9.79	0.22	20/20			
		daf-16 RNAi	5.8	0.15	20/20			
		daf-16;daf-2 RNAi	6	0.14	20/20			
1	TU3401	Ctrl RNAi	7	0.41	20/20	0.019	1	0.0002
		daf-2 RNAi	8.85	0.28	20/20			
		daf-16 RNAi	6.85	0.22	20/20			
		daf-16;daf-2 RNAi	7.35	0.19	20/20			
2	TU3401	Ctrl RNAi	5.25	0.10	20/20	0	0.07	4.9e-8
		daf-2 RNAi	6.95	0.05	20/20			
		daf-16 RNAi	5.7	0.16	20/20			
		daf-16;daf-2 RNAi	5.4	0.15	20/20			
3	TU3401	Ctrl RNAi	8.06	0.24	20/20	1.4e-6	0.0042	0
		daf-2 RNAi	10.08	0.22	20/20			
		daf-16 RNAi	6.95	0.18	20/20			
		daf-16;daf-2 RNAi	6.9	0.22	20/20			
1	VP303	Ctrl RNAi	6.2	0.11	20/20	2.3e-5	0.004	0.0041
		daf-2 RNAi	7.6	0.23	20/20			
		daf-16 RNAi	7	0.18	20/20			
		daf-16;daf-2 RNAi	6.25	0.25	20/20			
2	VP303	Ctrl RNAi	7.35	0.19	20/20	3.5e-8	0.0005	0
		daf-2 RNAi	9.55	0.11	20/20			
		daf-16 RNAi	8.55	0.13	20/20			
		daf-16;daf-2 RNAi	7.25	0.12	20/20			
3	VP303	Ctrl RNAi	6.85	0.15	20/20	3.5e-8	0.0005	0
		daf-2 RNAi	8.35	0.20	17/17			
		daf-16 RNAi	6.55	0.17	20/20			
		daf-16;daf-2 RNAi	6.5	0.22	20/20			
1	NR222	Ctrl RNAi	6	0.20	20/20	0.14	0.017	0.0001
		daf-2 RNAi	6.65	0.23	20/20			
		daf-16 RNAi	5.25	0.16	20/20			
		daf-16;daf-2 RNAi	5.4	0.13	20/20			
2	NR222	Ctrl RNAi	6.3	0.14	20/20	0.017	0.17	3.5e-5
		daf-2 RNAi	7	0.16	20/20			
		daf-16 RNAi	6.7	0.16	20/20			
		daf-16;daf-2 RNAi	6.1	0.07	20/20			
3	NR222	Ctrl RNAi	6.9	0.19	20/20	0.24	1.80E-05	2.2e-6
		daf-2 RNAi	7.45	0.26	20/20			
		daf-16 RNAi	5.45	0.17	20/20			
		daf-16;daf-2 RNAi	5.6	0.13	20/20			
1	NL2098	Ctrl RNAi	6.5	0.15	20/20	1	0.16	0.88
		daf-2 RNAi	6.35	0.11	20/20			
		daf-16 RNAi	7	0.19	20/20			
		daf-16;daf-2 RNAi	6.2	0.09	20/20			
2	NL2098	Ctrl RNAi	5.2	0.13	20/20	0.98	1	0.52
		daf-2 RNAi	5.4	0.11	20/20			
		daf-16 RNAi	5.35	0.18	20/20			
		daf-16;daf-2 RNAi	5.65	0.18	20/20			
1	NR350	Ctrl RNAi	4.35	0.16	20/20	1	1	0.39
		daf-2 RNAi	4.2	0.09	20/20			
		daf-16 RNAi	4.3	0.12	20/20			
		daf-16;daf-2 RNAi	4.45	0.13	20/20			
2	NR350	Ctrl RNAi	5.7	0.17	20/20	1	1	1
		daf-2 RNAi	5.85	0.19	20/20			
		daf-16 RNAi	5.8	0.27	20/20			
		daf-16;daf-2 RNAi	5.65	0.18	20/20			

Table S3. (A) Statistics of the Independent Dauer Assay. Related to Figure S1

	N2		*daf-2*		*daf-16*		N-*daf-2* KO		I-*daf-2* KO		N2	*daf-2*	*daf-16*	N-*daf-2* KO	I-*daf-2* KO
	normal	**dauer**	**normal**	**dauer**	**normal**	**dauer**	**normal**	**dauer**	**normal**	**dauer**					
Exp #1	196	0	0	78	158	0	60	27	88	0	0	100	0	31.0	0
Exp #2	70	0	0	31	73	0	54	8	35	0	0	100	0	12.9	0
	62	0	0	29	63	0	47	4	46	0	0	100	0	7.8	0
	67	0	0	28	66	0	45	8	40	0	0	100	0	15.1	0
Exp #3	108	0	0	25	83	0	91	12	105	0	0	100	0	11.7	0
	114	0	0	28	90	0	105	7	105	0	0	100	0	6.3	0
Exp #4	132	0	0	32	130	0	80	15	222	0	0	100	0	15.8	0
	129	0	0	50	133	0	75	10	163	0	0	100	0	11.8	0
Exp #5	83	0	0	30	121	0	97	11	142	0	0	100	0	10.2	0
	82	0	0	49	103	0	60	6	133	0	0	100	0	9.1	0
	n = 1043		n = 380		n = 1020		n = 822		n = 1079		0 ± 0	100 ± 0	0 ± 0	13 ± 7.0	0 ± 0

Table S3. (B) Statistics of the Independent Dauer Assay. Related to Figure S1

ANOVA summary	
F	571.3
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.(	Yes
R square	0.9913

Multiple comparison (Dunn's multiple comparisons test)				
Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value
N2 vs. *daf-2*	-30	Yes	****	<0.0001
N2 vs. *daf-16*	0	No	ns	>0.9999
N2 vs. N-*daf-2* KO	-20	Yes	**	0.005
N2 vs. I-*daf-2* KO	0	No	ns	>0.9999
daf-2 vs. *daf-16*	30	Yes	****	<0.0001
daf-2 vs. N-*daf-2* KO	10	No	ns	0.8168
daf-2 vs. I-*daf-2* KO	30	Yes	****	<0.0001
daf-16 vs. N-*daf-2* KO	-20	Yes	**	0.005
daf-16 vs. I-*daf-2* KO	0	No	ns	>0.9999
N-*daf-2* KO vs. I-*daf-2* KO	20	Yes	**	0.005

Table S4. Statistics of the Independent Lifespan Experiments 2. Related to Figure 1

Trial	Strain	Mean lifespan (Days)	Std. error	Worms (n)	Bonferroni P-value				
					vs WT	vs *daf-2(e1370)*	vs neuronal *daf-2* KO	vs intestinal *daf-2* KO	vs hypodermal *daf-2* KO
# 1	**WT**	21.51	0.46	61 / 101	-	-	< 0.0001	< 0.0001	0.0218
	Neuronal *daf-2* KO	36.47	2.32	26 / 80			-	0.0848	< 0.0001
	Intestinal *daf-2* KO	32.32	1.16	55 / 80				-	< 0.0001
	Hypodermal *daf-2* KO	23.32	1.07	34 / 80					-
# 2	**WT**	22.05	0.36	75 / 100	-	< 0.0001	< 0.0001	< 0.0001	0.2221
	daf-2(e1370)	43.27	2.15	54 / 100		-	0.0001	0.0001	< 0.0001
	Neuronal *daf-2 KO*	33.58	1.59	47 / 80			-	0.5565	< 0.0001
	Intestinal *daf-2* KO	34.83	1.44	61 / 81				-	< 0.0001
	Hypodermal *daf-2* KO	22.63	0.67	48 / 80					-
# 3	**WT**	20.94	0.54	62 / 100	-	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	daf-2(e1370)	42.92	2.48	39 / 80		-	1	0.9405	0.003
	Neuronal *daf-2 KO*	47.26	2.27	45 / 80			-	0.0278	< 0.0001
	Intestinal *daf-2* KO	40.24	1.44	52 / 80				-	0.0029
	Hypodermal *daf-2* KO	31.64	1.91	21 / 80					-

Table S5. (A) Statistics of the Independent Lifespan Experiments 3_1 (log-rank test with Bonferroni correction). Related to Figure 1 and S2

Trial	Strain	Mean lifespan (Days)	Std. error	Worms (n)	Bonferroni P-value							
					vs WT	vs *daf-2(e1370)*	vs Neuronal *daf-2* KO	vs Intestinal *daf-2* KO	vs Neuronal & Intestinal *daf-2* KO	vs *unc-31(e298)*	vs *unc-31(e298);* neuronal *daf-2* KO	vs *unc-31(e298);* intestinal *daf-2* KO
# 1	**WT**	24.73	0.97	41 / 100	-	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	daf-2(e1370)	49.79	1.5	67 / 78		-	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1	0.0002
	Neuronal *daf-2* KO	38.68	1.15	72 / 80			-	1	0.0176	< 0.0001	< 0.0001	0.0187
	Intestinal *daf-2* KO	38.38	1.09	66 / 80				-	0.0384	< 0.0001	< 0.0001	0.0106
	Neuronal & intestinal *daf-2* KO	33.25	1.17	62 / 78					-	0.3257	< 0.0001	< 0.0001
	unc-31(e298)	31.63	0.61	95 / 100						-	< 0.0001	< 0.0001
	***unc-31(e298);* neuronal *daf-2* KO**	51.23	1.03	74 / 80							-	0.0001
	***unc-31(e298);* intestinal *daf-2* KO**	42.53	1.29	75 / 81								-
# 2	**WT**	22.97	0.87	37 /102	-	< 0.0001	< 0.0001	< 0.0001	0.0072	< 0.0001	0.2221	0.1035
	daf-2(e1370)	55.27	1.46	48 / 62		-	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.2564	0.0001
	Neuronal *daf-2 KO*	37.71	1.08	68 / 81			-	0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001
	Intestinal *daf-2* KO	40.96	1.02	57 / 80				-	< 0.0001	0.5565	< 0.0001	< 0.0001
	Neuronal & intestinal *daf-2* KO	27.62	0.96	66 / 78					-	0.2035	< 0.0001	< 0.0001
	unc-31(e298)	30.82	0.67	98 / 102						-	< 0.0001	< 0.0001
	***unc-31(e298);* neuronal *daf-2* KO**	51.85	1.07	75 / 80							-	0.4515
	***unc-31(e298);* intestinal *daf-2* KO**	45.27	1.29	71 / 81								-
# 3	**WT**	27.8	0.87	50 / 101	-	< 0.0001	< 0.0001	< 0.0001	< 0.0001			
	daf-2(e1370)	48.9	2.17	33 / 78		-	0.0747	0.0712	1			
	Neuronal *daf-2* KO	42.82	1.28	53 / 80			-	1	0.0027			
	Intestinal *daf-2* KO	41.1	1.43	10 / 79				-	0.0011			
	Neuronal & Intestinal *daf-2 KO*	49.44	1.34	40 / 74					-			

Table S5. (B) Statistics of the Independent Lifespan Experiments 3_2 (2-way ANOVA post hoc Sidak's test). Related to Figure 1 and S2

Trial	Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
#1	**WT vs *unc-31***	-6.325	-10.94 to -1.713	Yes	**	0.006
	N-*daf-2* KO vs *unc-31;* N-*daf-2* KO	-15.59	-20.20 to -10.98	Yes	****	<0.0001
	I-*daf-2* KO vs *unc-31;* I-*daf-2* KO	-4.1	-8.712 to 0.5120	No	ns	0.0903
#2	**WT vs *unc-31***	-7.818	-11.60 to -4.036	Yes	***	0.0001
	N-*daf-2* KO vs *unc-31;* N-*daf-2* KO	-14.21	-17.99 to -10.42	Yes	****	<0.0001
	I-*daf-2* KO vs *unc-31;* I-*daf-2* KO	-3.218	-6.999 to 0.5644	No	ns	0.1099

Mean lifespan of replicates	#1	**WT**				***unc-31***			
	WT	25.41 (n=25)	24.35 (n=25)	21.13 (n=25)	27 (n=25)	33.81 (n=25)	31.45 (n=25)	31.6 (n=25)	26.33 (n=25)
	N-*daf-2* KO	36.1 (n=20)	34.32 (n=20)	34 (n=20)	38.37 (n=20)	49.68 (n=20)	53.06 (n=20)	52.12 (n=20)	50.3 (n=20)
	I-*daf-2* KO	40.33 (n=20)	38.97 (n=20)	39.88 (n=20)	34.65 (n=20)	41.8 (n=20)	46.56 (n=20)	40.47 (n=21)	41.4 (n=20)

Mean lifespan of replicates	#2	**WT**				***unc-31***			
	WT	24.91 (n=26)	24.33 (n=26)	23.27 (n=25)	19 (n=25)	29.05 (n=25)	31.95 (n=26)	30.89 (n=26)	30.89 (n=25)
	N-*daf-2* KO	39.39 (n=21)	35.2 (n=20)	37.67 (n=20)	38.44 (n=20)	50.16 (n=20)	53.94 (n=20)	50.68 (n=20)	52.74 (n=20)
	I-*daf-2* KO	40.89 (n=20)	42.77 (n=21)	40.83 (n=20)	39.38 (n=20)	48.28 (n=21)	42.1 (n=20)	43.74 (n=20)	42.62 (n=20)

Table S6. Statistics of the Independent Reproductive Span Experiments 1. Related to Figure S1

Trial	Strain	Mean reproductive lifespan (Days)	Std. error	Worms	Bonferroni P-value						
					vs WT	vs *daf-2(e1370)*	vs Neuronal *daf-2* KO	vs Intestinal *daf-2* KO	vs Hypodermal *daf-2* KO	vs Somatic gonadal *daf-2* KO	vs mock (gfp sgRNA)
# 1	**WT**	5.11	0.13	19 / 20	-	0.0001	0.0001	0.0052	1		
	daf-2(e1370)	6.53	0.25	15 / 20		-	0.1538	1	0.0201		
	Neuronal *daf-2* KO	7.86	0.47	7 / 20			-	1	0.0047		
	Intestinal *daf-2* KO	7	0.53	6 / 20				-	0.0756		
	Hypodermal *daf-2* KO	5.25	0.27	12 / 20					-		
# 2	**WT**	5.32	0.09	24 / 30	-	<0.0001	<0.0001	<0.0001	0.0596	0.1728	0.075
	daf-2(e1370)	7.44	0.33	21 / 30		-	0.9872	1	0.0604	0.0274	1
	Neuronal *daf-2* KO	8.29	0.43	9 / 30			-	1	0.0047	0.0018	0.0912
	Intestinal *daf-2* KO	8.75	0.88	10 / 30				-	0.0168	0.0032	0.1272
	Hypodermal *daf-2* KO	6.17	0.29	11 / 30					-	1	1
	Somatic gonadal *daf-2* KO	6.16	0.29	22 / 30						-	1
	mock (gfp sgRNA)	6.67	0.45	17 / 30							-
# 3	**WT**	5.81	0.19	23/ 30	-	0.0001	0.0001	0.0003	0.4365	0.6741	1
	daf-2(e1370)	7.41	0.28	22 / 30		-	1	1	0.2731	0.4672	0.0001
	Neuronal *daf-2* KO	8.19	0.47	17 / 30			-	1	0.3932	0.0683	0.0003
	Intestinal *daf-2* KO	7.72	0.38	9 / 30				-	0.3013	0.2511	0.0002
	Hypodermal *daf-2* KO	6.23	0.2	13 / 30					-	1	0.369
	Somatic gonadal *daf-2* KO	6.33	0.31	9 / 30						-	0.7356
	mock (gfp sgRNA)	5.83	0.16	22 / 29							-

Table S7. Statistics of the Independent Lifespan Experiments 4. Related to Figure S1

Trial	Strain	Mean lifespan (Days)	Std. error	Worms (n)	Bonferroni P-value		
					vs WT	vs mock (empty sgRNA)	vs mock (gfp sgRNA)
# 1	**WT**	21.39	0.57	76 / 100	-	0.797	1
	mock (empty sgRNA)	22.11	0.9	43 / 100		-	0.808
	mock (gfp sgRNA)	20.99	0.85	35 / 100			-
# 2	**WT**	23.22	0.59	82 / 100	-	0.235	0.937
	mock (empty sgRNA)	25.11	0.86	38 / 100		-	0.050
	mock (gfp sgRNA)	22.88	0.54	63 / 100			-
# 3	**WT**	24.35	0.72	77 / 100	-	0.502	1
	mock (empty sgRNA)	26.05	1.11	28 / 60		-	0.374
	mock (gfp sgRNA)	23.68	0.88	56 / 100			-

Table S8. (A) Statistics of the Independent DAF-16::GFP Nuclear Accumulation Experiments 1. Figure 3 and S2

	WT				**N-*daf-2* KO**				**I-*daf-2* KO**			
	Head		**Intestine**		**Head**		**Intestine**		**Head**		**Intestine**	
	Nuclear	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**
Exp. 1	0	51	0	51	36	8	41	4	2	8	8	2
Exp. 2	0	31	0	59	40	6	66	5	16	16	47	3
Exp. 3	0	49	0	98	43	6	80	8	14	17	41	5
Exp. 4	8	27	2	59	21	0	26	3	15	8	33	2

Table S8. (B) Statistics of the Independent DAF-16::GFP Nuclear Accumulation Experiments 1. Figure 3 and S2

Head

ANOVA summary	
F	38.49
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8953

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
WT vs. N-*daf-2* KO	-83.42	-110.0 to -56.86	Yes	****	<0.0001
WT vs. I-*daf-2* KO	-39.38	-65.94 to -12.82	Yes	**	0.0064
N-*daf-2* KO vs. I-*daf-2* KO	44.04	17.48 to 70.60	Yes	**	0.0032

Body

ANOVA summary	
F	686.6
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9935

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
WT vs. N-*daf-2* KO	-90.74	-98.56 to -82.92	Yes	****	<0.0001
WT vs. I-*daf-2* KO	-88.94	-96.75 to -81.12	Yes	****	<0.0001
N-*daf-2* KO vs. I-*daf-2* KO	1.804	-6.013 to 9.621	No	ns	0.8

Table S9. Statistics of the Independent DAF-16::GFP Nuclear Accumulation Experiments 2. Related to Figure 3

ANOVA summary

F	280.6
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9791

Dunnett's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Summary**	**Adjusted P Value**	
Wild-type vs. N-*daf-2* KO	-7.36	-8.193 to -6.527	Yes	****	<0.0001	vs N-*daf-2* KO
Wild-type vs. I-*daf-2* KO	-1.24	-2.073 to -0.4075	Yes	**	0.0054	vs I-*daf-2* KO

	Wild-type	**N-daf-2 KO**	**I-daf-2 KO**
Exp. #1	0	5.9	0.9
Exp. #2	0.2	7.7	1.4
Exp. #3	0	7.5	1.4
Exp. #4	0	8	1.5
Exp. #5	0.1	8	1.3

Table S10. Statistics of the Independent Lifespan Experiments 5. Related to Figure 4

Trial	Strain	Mean lifespan (Days)	Std. error	Worms (n)	Bonferroni P-value			
					vs intestine ctrl RNAi	vs intestine *daf-16* RNAi	vs neuronal *daf-2* KO; intestine ctrl RNAi	vs neuronal *daf-2* KO; intestine *daf-16* RNAi
# 1	**Intestine ctrl RNAi**	17.68	0.2	37 / 80	-	0.0001	<0.0001	0.0009
	Intestine *daf-16* RNAi	15.73	0.27	55 / 80		-	<0.0001	<0.0001
	Neuronal *daf-2* KO; intestine ctrl RNAi	24.41	1.11	32 / 80			-	0.0001
	Neuronal *daf-2* KO; intestine *daf-16* RNAi	19.79	0.51	43 / 80				-
# 2	**intestine ctrl RNAi**	18.3	0.31	57 / 100	-	0.0007	<0.0001	0.0888
	intestine *daf-16* RNAi	16.33	0.37	25 / 100		-	<0.0001	<0.0001
	Neuronal *daf-2* KO; intestine ctrl RNAi	29.11	1.03	47 / 100			-	<0.0001
	Neuronal *daf-2* KO; intestine *daf-16* RNAi	19.39	0.61	53 / 100				-
# 3	**intestine ctrl RNAi**	18.74	0.29	65 /100	-	0.0001	<0.0001	0.0303
	intestine *daf-16* RNAi	16.96	0.27	64 /100		-	<0.0001	<0.0001
	Neuronal *daf-2* KO; intestine ctrl RNAi	28.32	0.99	56 /100			-	<0.0001
	Neuronal *daf-2* KO; intestine *daf-16* RNAi	21.09	0.78	72 /100				-

Trial	Strain	Mean lifespan (Days)	Std. error	Worms (n)	Bonferroni P-value			
					vs neuron ctrl RNAi	vs neuron *daf-16* RNAi	vs intestine *daf-2* KO; neuronctrl RNAi	vs intestine *daf-2* KO; neuron *daf-16* RNAi
# 1	**Neuron ctrl RNAi**	18.5	0.46	53 / 80	-	<0.0001	<0.0001	0.0002
	Neuron *daf-16* RNAi	14.78	0.27	56 / 80		-	<0.0001	0.0628
	Intestin *daf-2* KO; neuron ctrl RNAi	30.05	1.02	49 / 79			-	<0.0001
	Intestine *daf-2* KO; neuron *daf-16* RNAi	15.88	0.42	54 / 77				-
# 2	**Neuron ctrl RNAi**	17.76	0.31	38 / 81	-	0	<0.0001	8.00E-04
	Neuron *daf-16* RNAi	14.74	0.37	56 / 80		-	<0.0001	0.1849
	Intestin *daf-2* KO; neuron ctrl RNAi	30.11	1.03	53 / 81			-	<0.0001
	Intestine *daf-2* KO; neuron *daf-16* RNAi	15.47	0.61	58 / 80				-

Table S11. Statistics of the Independent Reproductive Span Experiments 2. Related to Figure S3

Trial	Strain	Mean reproductive span (Days)	Std. error	Worms (n)	Bonferroni P-value					
					vs intestine ctrl RNAi	vs intestine *daf-2* RNAi	vs intestine *daf-16* RNAi	vs neuronal *daf-2* KO; intestine ctrl RNAi	vs neuronal *daf-2* KO; intestine *daf-2* RNAi	vs neuronal *daf-2* KO; intestine *daf-16* RNAi
# 1	**intestine ctrl RNAi**	3.47	0.12	27/28	-	0.0015	1	<0.0001	<0.0001	1
	intestine *daf-2* RNAi	4.2	0.13	26/28		-	0.0006	0.7126	0.0151	0.1135
	intestine *daf-16* RNAi	3.38	0.13	28/30			-	<0.0001	<0.0001	1
	Neuronal *daf-2* KO; intestine ctrl RNAi	4.5	0.16	34/37				-	0.3223	0.0031
	Neuronal *daf-2* KO; intestine *daf-2* RNAi	4.95	0.19	36/40					-	<0.0001
	Neuronal *daf-2* KO; intestine *daf-16* RNAi	3.6	0.18	35/41						-
# 2	**intestine ctrl RNAi**	3.5	0.13	27/30	-		1	<0.0001		0.5807
	intestine *daf-16* RNAi	3.43	0.14	28/30			-	<0.0001		0.3975
	Neuronal *daf-2* KO; intestine ctrl RNAi	4.48	0.17	35/51				-		0.0484
	Neuronal *daf-2* KO; intestine *daf-16* RNAi	3.85	0.19	35/50						-

Table S12. (A) Statistics of the Brood Size of the Individuals. Related to Figure S3

VP303 / Ctrl RNAi	N-*daf-2* KO;VP303 / Ctrl RNAi	VP303 / *daf-16* RNAi	N-*daf-2* KO;VP303 / *daf-16* RNAi
231	291	181	201
266	260	192	243
181	273	282	241
176	257	173	256
222	257	278	334
209	233	197	178
197	200	244	293
238	180	184	225
210	235	263	221
203	253	203	227
246	267	241	271
123	133	208	179
207	261	224	205
234	318	269	251
309	239	240	240
251	200	228	196
200	266	231	159
138	255	214	190
258	241	213	271
209	193	181	206
157	229	235	258
204	221	117	202
223	266	244	234
218	224	201	155
	177	221	215
	230		127
	241		195
			206
			191
			255
			169
			233

Table S12. (B) Statistics of the Brood Size of the Individuals. Related to Figure 3

ANOVA summary	
F	1.775
P value	0.1566
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.0487

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VP303 / Ctrl RNAi vs. N-*daf-2* KO;VP303 / Ctrl RNAi	-24.12	-53.49 to 5.253	No	ns	0.1462
VP303 / Ctrl RNAi vs. VP303 / *daf-16* RNAi	-5.643	-35.56 to 24.28	No	ns	0.9606
VP303 / Ctrl RNAi vs. N-*daf-2* KO;VP303 / *daf-16* RNAi	-6.677	-34.95 to 21.60	No	ns	0.9266
N-*daf-2* KO;VP303 / Ctrl RNAi vs. VP303 / *daf-16* RNAi	18.48	-10.58 to 47.54	No	ns	0.35
N-*daf-2* KO;VP303 / Ctrl RNAi vs. N-*daf-2* KO;VP303 / *daf-16* RNAi	17.44	-9.917 to 44.80	No	ns	0.3476
VP303 / *daf-16* RNAi vs. N-*daf-2* KO;VP303 / *daf-16* RNAi	-1.034	-28.98 to 26.91	No	ns	0.9997

Table S13. (A) Statistics of the Independent DAF-16::GFP Nuclear Accumulation Experiments 2. Related to Figure 5 and S4

Ctrl RNAi

WT				**N-*daf-2* KO**				**I-*daf-2* KO**			
Head		**Intestine**		**Head**		**Intestine**		**Head**		**Intestine**	
Nuclear	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**
1	16	1	19	15	1	19	1	7	7	15	0
0	34	0	58	17	5	22	4	8	8	20	0
0	23	0	33	23	5	31	3	6	10	21	0
0	29	0	45	37	9	66	7	13	14	28	4

***daf-18* RNAi**

WT				**N-*daf-2* KO**				**I-*daf-2* KO**			
Head		**Intestine**		**Head**		**Intestine**		**Head**		**Intestine**	
Nuclear	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**	**Nuclear**	**Cytosolic**
0	26	0	49	19	1	13	7	4	6	7	3
0	32	0	48	5	6	6	10	4	7	11	2
0	31	0	52	21	10	32	18	7	12	19	5
0	26	0	52	21	18	42	17	12	20	39	12

Table S13. (B) Statistics of the Independent DAF-16::GFP Nuclear Accumulation Experiments 2. Related to Figure 5 and S4

Head

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	270.8	2	135.4	F (2, 18) = 1.425	P=0.2663
KO	21844	2	10922	F (2, 18) = 115.0	P<0.0001
RNAi	526.2	1	526.2	F (1, 18) = 5.540	P=0.0302
Residual	1710	18	95		

Sidak's multiple comparisons tes	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Ctrl RNAi - *daf-18* RNAi					
WT	1.471	-16.66 to 19.60	No	ns	0.9954
N-*daf-2* KO	17.89	-0.2437 to 36.02	No	ns	0.0538
I-*daf-2* KO	8.736	-9.398 to 26.87	No	ns	0.5275

Body

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	576.4	2	288.2	F (2, 18) = 4.836	P=0.0209
KO	34024	2	17012	F (2, 18) = 285.5	P<0.0001
RNAi	1323	1	1323	F (1, 18) = 22.19	P=0.0002
Residual	1073	18	59.6		

Sidak's multiple comparisons tes	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Ctrl RNAi - *daf-18* RNAi					
WT	1.25	-13.11 to 15.61	No	ns	0.9943
N-*daf-2* KO	23.98	9.616 to 38.34	Yes	**	0.0011
I-*daf-2* KO	19.31	4.949 to 33.67	Yes	**	0.007

Table S14. Strain List. Related to STAR Method

Strain	Genotype	Comment
N2		Wild type
NL2098	*rrff-1(pk1417) I*	Germline specific RNAi
NR222	*rde-1(ne219) V* (outcrossed 3x)*; kzIs9[lin26p::nls::gfp + lin-26p::rde-1 + rol-6(su1006)]*	Hypodormis specific RNAi
NR350	*rde-1(ne219) V* (outcrossed 1x)*; kzIs20[hlh-1p::rde-1 + sur-5p::nls::gfp]*	Muscle specific RNAi
TU3401	*sid-1(pk3321) V* (outcrossed 0x)*; uIs69[myo-2p::mCherry + unc-119p::sid-1]*	Neuron specific RNAi
VP303	*rde-1(ne219) V* (outcrossed 3x)*; kbIs7[nhx-2p::rde-1; rol-6(su1006)]*	Intestine specific RNAi
KN259	*huIs33[sod-3::gfp + rol-6(su1006)]*	
TJ356	*zIs356[daf-16::gfp + rol-6(su1006)]*	
FJ224	*unc-31(e298) IV; dpy-11(e224) V* (outcrossed 3x)	
DCL569	*mkcSi13 [sun-1p::rde-1::sun-1 3′ UTR + unc-119(+)] II; rde-1(ne219) V*	Germline specific RNAi
NIS1700	*kyEx1700[myo-2p::mCherry + U6p::sgRNA(empty) + eft-3p::Cas9 + dpy-30p::wGxxFP(empty)]*	N2 mock #1
NIS1701	*kyEx1701[myo-2p::mCherry + U6p::gfp sgRNA + eft-3p::Cas9 + dpy-30p::wGxxFP(empty)]*	N2 mock #2
NIS1702	*kyEx1702[myo-2p::mCherry + U6p::daf-2 sgRNA + dpy-5p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	hypodormis specific KO
NIS1703	*kyEx1703[myo-2p::mCherry + U6p::daf-2 sgRNA + unc-54p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	muscle specific KO
NIS1704	*kyEx1704[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	neuron specific KO
NIS1705	*kyEx1705[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	intestine specific KO
NIS1706	*kyEx1706[myo-2p::mCherry + U6p::daf-2 sgRNA + unc-54p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	
NIS1707	*kyEx1707[myo-2p::mCherry + U6p::daf-2 sgRNA + ehn-3p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	
NIS1708	*unc-31(e298) V (outcrossed 3x); kyEx1704[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	
NIS1709	*unc-31(e298) V (outcrossed 3x); kyEx1705[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9 + dpy-30p::wGxxFP(daf-2)]*	
NIS1710	*huIs33[sod-3::gfp + rol-6(su1006)]; kyEx1704[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]*	
NIS1711	*huIs33[sod-3::gfp + rol-6(su1006)]; kyEx1705[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]*	
NIS1712	*huIs33[sod-3::gfp + rol-6(su1006)]; kyEx1702[myo-2p::mCherry + U6p::daf-2 sgRNA + dpy-5p::Cas9]*	
NIS1713	*zIs356[daf-16::gfp + rol-6(su1006)]; kyEx1704[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]*	
NIS1714	*zIs356[daf-16::gfp + rol-6(su1006)]; kyEx1705[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]*	
NIS1715	*huIs33[sod-3::gfp + rol-6(su1006)]; kyEx1708[myo-2p::mCherry + eft-3p::Cas9]*	sod-3::gfp mock
NIS1716	*rde-1(ne219) V* (outcrossed 3x)*; kbIs7[nhx-2p::rde-1; rol-6(su1006)]; kyEx1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]*	neuron specific KO and intestine specific RNAi
NIS1717	*sid-1(pk3321) V* (outcrossed 0x)*; uIs69[myo-2p::mCherry + unc-119p::sid-1]; kyEx1706[myo-3p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]*	intesitine specific KO and neuron specific RNAi
NIS1718	*huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]* (outcrossed 0x) *#1*	
NIS1719	*huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]* (outcrossed 0x) *#2*	
NIS1720	*huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1706[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]* (outcrossed 0x) *#1*	
NIS1721	*huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1706[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]* (outcrossed 0x) *#2*	
NIS1722	*huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1706[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]* (outcrossed 0x) *#3*	
NIS1723	*zIs356[daf-16::gfp + rol-6+C22:C40(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9] (outcrossed 0x)*	
NIS1724	*sid-1(qt9) V* (outcrossed x1); *huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]* (outcrossed 2x)	
NIS1725	*unc-31(e298) IV* (outcrossed 5x)	
NIS1726	*unc-31(e298) IV* (outcrossed 6x); *huIs33[sod-3::gfp + rol-6(su1006)]*	
NIS1727	*unc-31(e298) IV* (outcrossed 6x); *huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]* (outcrossed 2x)	
NIS1728	*unc-64(e246) III* (outcrossed 1x); *huIs33[sod-3::gfp + rol-6(su1006)]*	
NIS1729	*unc-64(e246) III* (outcrossed 1x); *huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1705[myo-2p::mCherry + U6p::daf-2 sgRNA + rgef-1p::Cas9]* (outcrossed 2x)	
NIS1730	*unc-64(e246) III* (outcrossed 1x); *huIs33[sod-3::gfp + rol-6(su1006)]; kyIs1706[myo-2p::mCherry + U6p::daf-2 sgRNA + gly-19p::Cas9]* (outcrossed 2x)	

Table S15. Primer List. Related to STAR Method

Primer	Sequence
daf-2 **RNAi Fw**	5'-ATCCCTCCGATCAATGCGAC-3'
daf-2 **RNAi Rv**	5'-TGCTGTTGGAGAGCTGCTGC-3'
daf-16 **RNAi Fw**	5'-AGAATGAAGGAGCGGAAAG-3'
daf-16 **RNAi Rv**	5'-ACTGTTCAACTCGTGGTATG-3'
pDD162_non-sgRNA Fw	5'-AAGTGGCACCGAGTCGGTGCACACCTTAAAGGCGCA-3'
pDD162_non-sgRNA Rv	5'-GCACCGACTCGGTGCCTGCGCCTTTAAGGTGTACAG-3'
empty sgRNA Fw	5'-GGGAGACCGGCAGATACTTTATTGTGCCGCCAAGA-3'
empty sgRNA Rv	5'-TATCGATAAGCTTGAAGTGCACCATATGCGGTGTG-3'
daf-2 **sg RNA Fw**	5'-CGGACAACAGTGTGGAGGTTTTAGAGCTAGAAATAGC-3'
daf-2 sg RNA **Rv**	5'-CACACTGTTGTCCGAGCCAAGAACATCTCGCAATAGGA-3'
P*rgef-1* **Fw**	5'-ATGATTACGCCAAGCGATAAACCGTTTGGATGAGC-3'
P*rgef-1* **Rv**	5'-GTATTTTTTGTCCATCGTCGTCGTCGTCGATGCCG-3'
P*gly-19* **Fw**	5'-ATGATTACGCCAAGCCAAATATTCTCATTTCAAAA-3'
P*gly-19* **Rv**	5'-GTATTTTTTGTCCATCTGGAAATTTAAATTTAATT-3'
P*dpy-5* **Fw**	5'-CATGATTACGCCAAGCCATAGAAGTATGGGCCAAAA-3'
P*dpy-5* **Rv**	5'-GTATTTTTTGTCCATTTTCTGAAAACACAGAGACT-3'
P*ehn-3* **Fw**	5'-ATGATTACGCCAAGCTAATCTAGAAAAATACGACA-3'
P*ehn-3* **Rv**	5'-GTATTTTTTGTCCATTTTGTAATTTGGAAGCTGGG-3'
wGxxFP insert Fw	5'-CCGCTGCAGTCGACAGATCTTGCACTACTGGAAAACTACC-3'
wGxxFP insert Rv	5'-CAATTGGAGTATTTTGTTGA-3'
wGxxFP vector Fw	5'-AAAATACTCCAATTGGCGAT-3'
wGxxFP vector Rv	5'-TGTCGACTGCAGCGGCCGCGTTGATAATGGTCTGCTAGT-3'
wGxxFP (*daf-2*) Fw	5'-ATTATCAACGCGGCCATGACGCCAGGCTTCTTCAC-3'
wGxxFP (*daf-2*) Rv	5'-CAGTAGTGCAAGATCGAGATAGTCACGGAGATTTC-3'