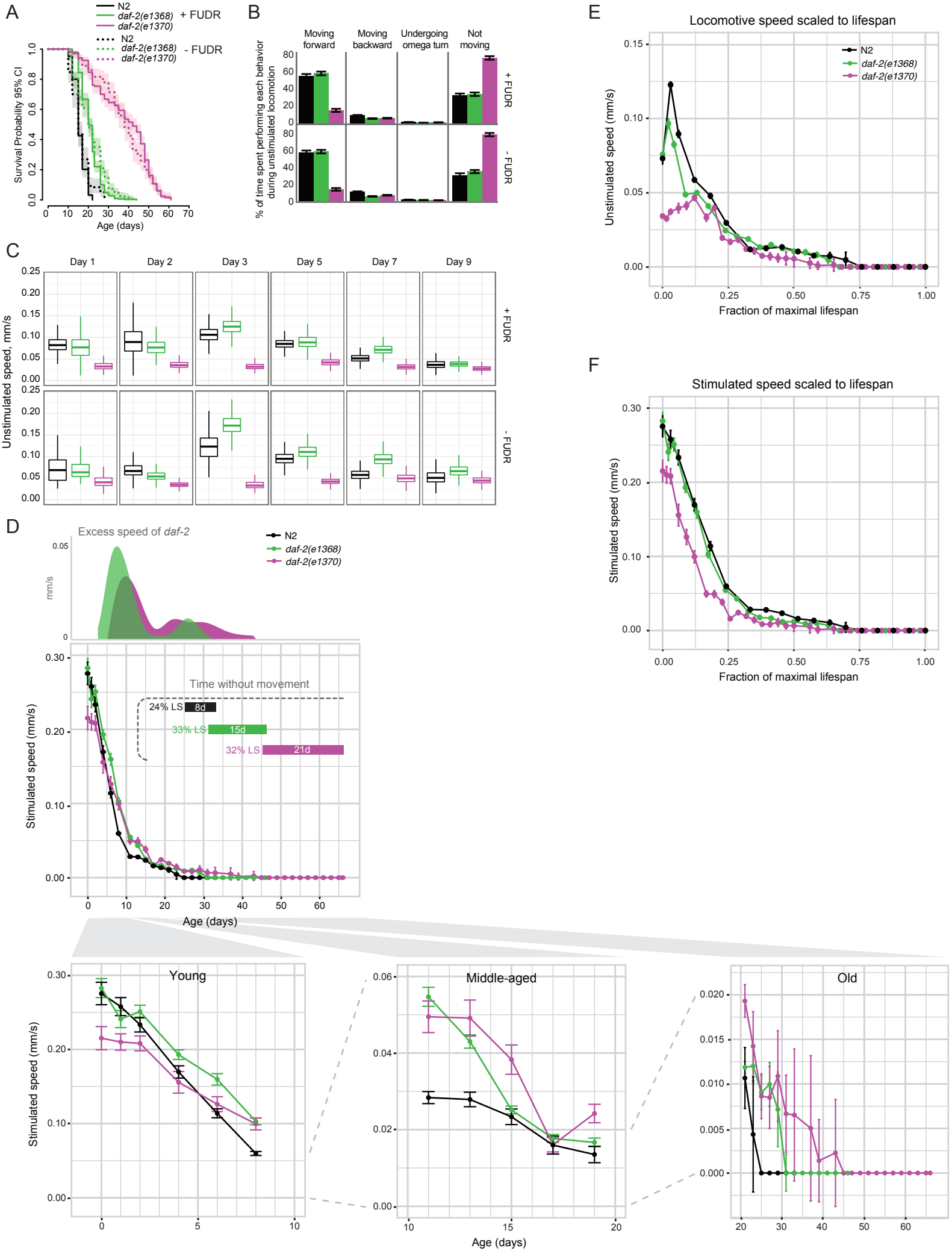
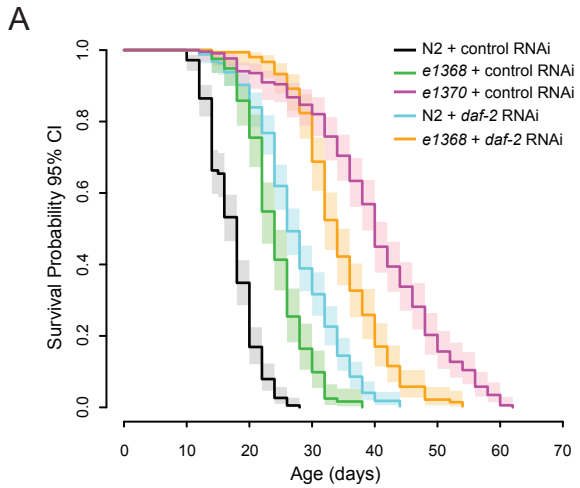
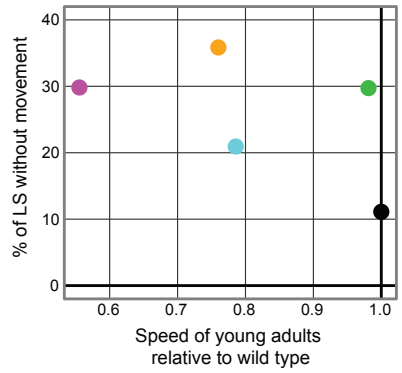
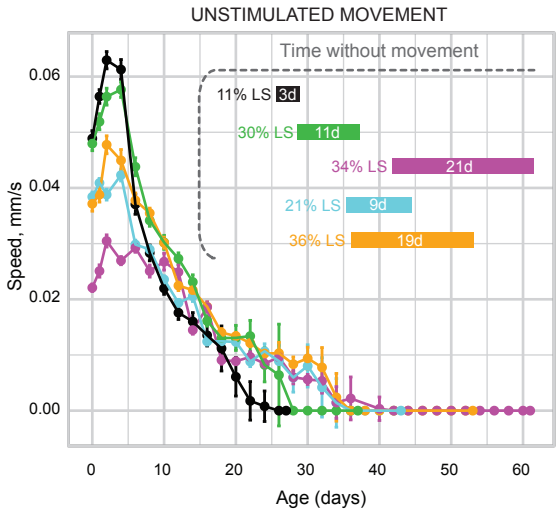
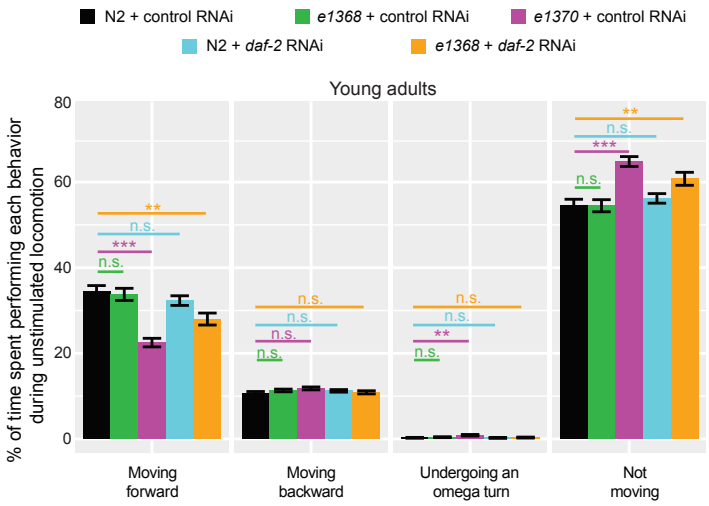

A
N2
daf-2(e1368)
daf-2(e1370)
+ FUDR
- FUDR
Survival Probability 95% CI
Age (days)
B
Moving forward
Moving backward
Undergoing omega turn
Not moving
% of time spent performing each behavior during unstimulated locomotion
+ FUDR
- FUDR
C
Day 1
Day 2
Day 3
Day 5
Day 7
Day 9
Unstimulated speed, mm/s
D
Excess speed of daf-2
mm/s
Time without movement
24% LS 8d
33% LS 15d
32% LS 21d
Stimulated speed (mm/s)
Age (days)
Young
Middle-aged
Old
E
Locomotive speed scaled to lifespan
Unstimulated speed (mm/s)
Fraction of maximal lifespan
F
Stimulated speed scaled to lifespan
Stimulated speed (mm/s)
Fraction of maximal lifespan

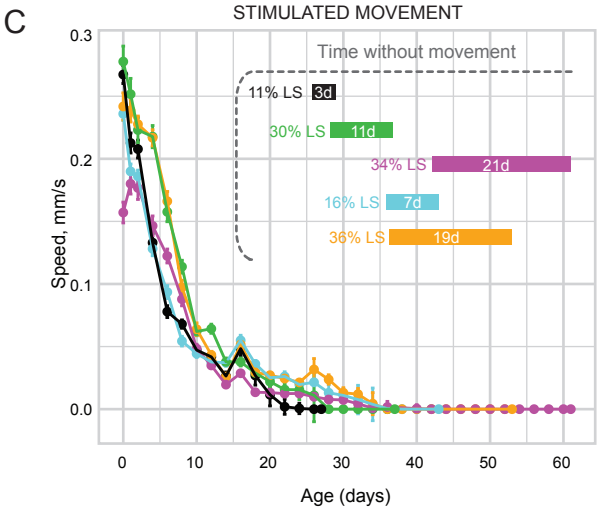
Figure S1. Additional analyses of locomotary behavior changes with age. Related to Figure 1 and Figure 2. **a)** Survival analysis of wild type and *daf-2* mutants carried out at 20°C in the presence or absence of 50 μM FUDR. To avoid selective handling, animals in both treatment groups were transferred to new plates daily during their reproductive periods and every 2-3 days thereafter. **b)** Time spent by each young adult performing each of the four stereotypical behaviors during unstimulated locomotion. **c)** Box and whisker plots showing distributions of unstimulated movement speeds on days 1, 2, 3, 5, 7, and 9 of adulthood. $n > 130$. In the absence of FUDR, both *daf-2* mutants exhibited a longer period without any detectable movement in proportion to lifespan than did wild type (data not shown). **d)** Tap stimulus induces an increase in locomotive speed, but young *daf-2(e1370)* mutants still move more slowly than young wild-type or *daf-2(e1368)* mutants. Both *daf-2* mutants respond to a tap stimulus better than wild type during mid-life, but spend an extended duration of time and percentage of life without detectable movement following stimulation. Excess speed of *daf-2* mutants was calculated by subtracting wild-type speed from mutant speed (above). The period of life when there is no more detectable movement in the population (no worms have moved at a speed of at least 0.02mm/s at least once in a period of 2 minutes) is indicated with boxes. Data are shown as means ± s.e. $n > 200$. Stimulated speed was calculated over a 1 s time interval following the tap. **e)** Unstimulated movement speed normalized to lifespan. **f)** Stimulated movement speed normalized to lifespan.


A
N2 + control RNAi
e1368 + control RNAi
e1370 + control RNAi
N2 + daf-2 RNAi
e1368 + daf-2 RNAi
Survival Probability 95% CI
Age (days)


D
% of LS without movement
Speed of young adults
relative to wild type


B
UNSTIMULATED MOVEMENT
Time without movement
11% LS 3d
30% LS 11d
34% LS 21d
21% LS 9d
36% LS 19d
Speed, mm/s
Age (days)


E
N2 + control RNAi
e1368 + control RNAi
e1370 + control RNAi
N2 + daf-2 RNAi
e1368 + daf-2 RNAi
Young adults
% of time spent performing each behavior during unstimulated locomotion
Moving forward
Moving backward
Undergoing an omega turn
Not moving


C
STIMULATED MOVEMENT
Time without movement
11% LS 3d
30% LS 11d
34% LS 21d
16% LS 7d
36% LS 19d
Speed, mm/s
Age (days)

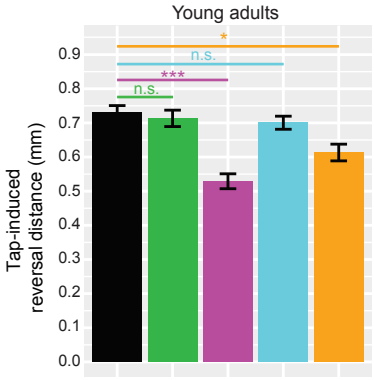
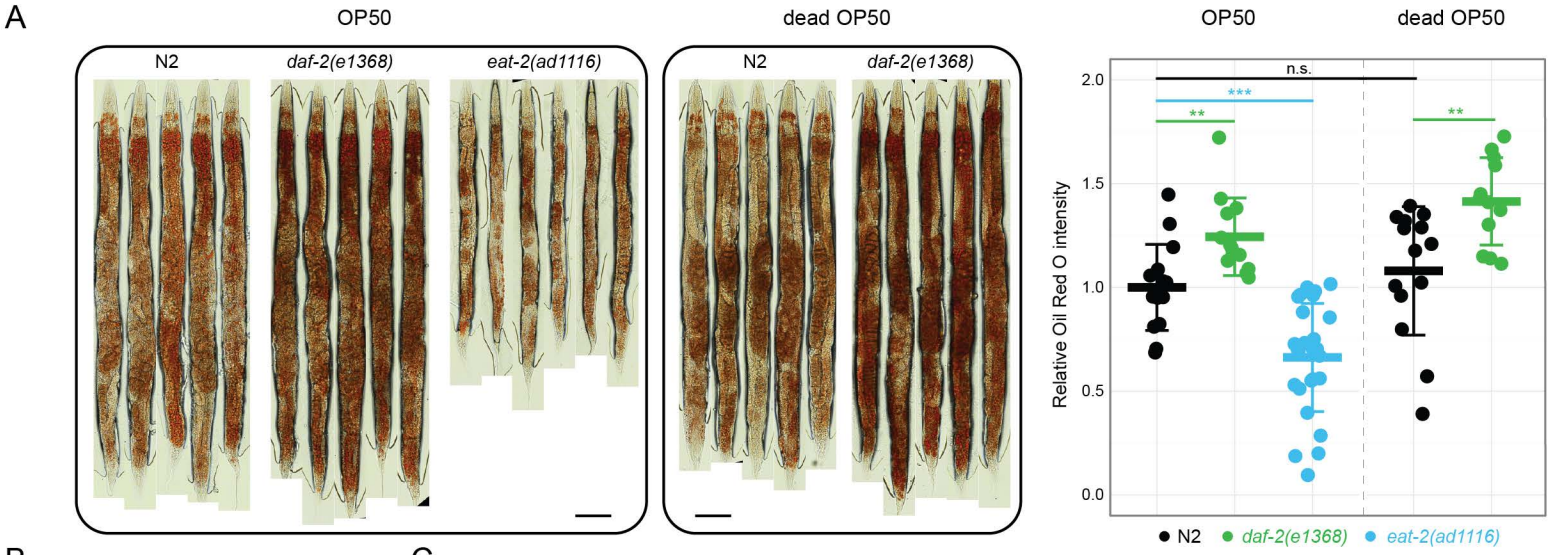
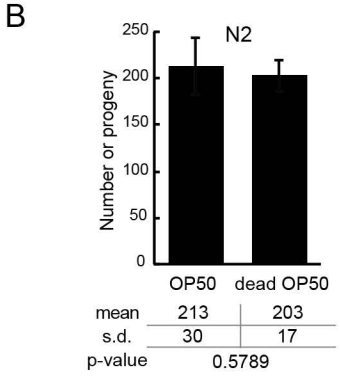
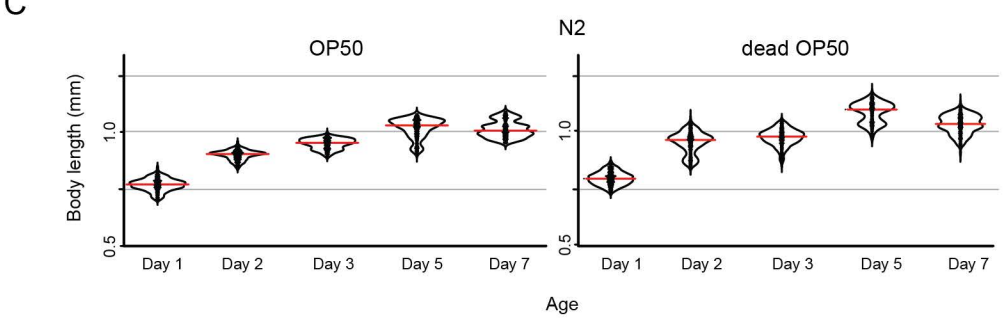
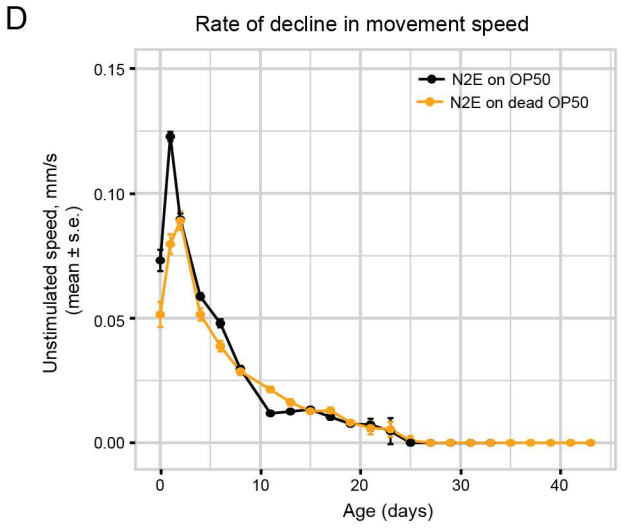

F
Young adults
Tap-induced reversal distance (mm)

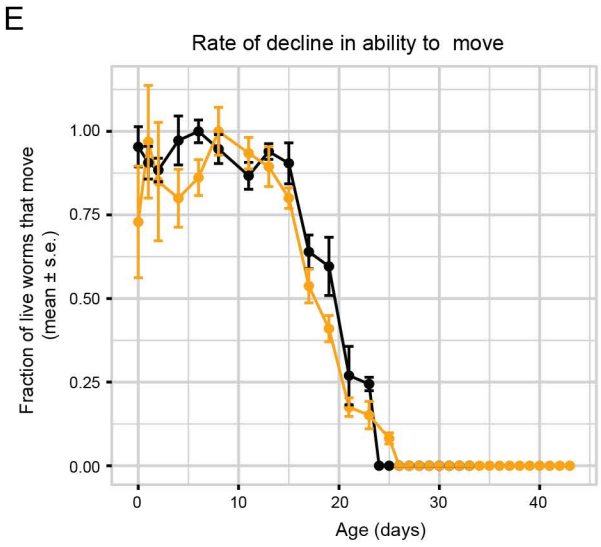
Figure S2. Severity of early-life movement phenotypes of *daf-2* mutants does not correlate with the duration of end-of-life decrepitude. Related to Figure 1 and Figure 2.
a) Survival analysis of N2 (wild type), *daf-2(e1368)*, *daf-2(e1370)*, *daf-2*(RNAi) and *daf-2(e1368); daf-2*(RNAi). Animals were grown on control or *daf-2* RNAi-expressing bacteria starting at L1 at 20°C. Speed of **(b)** unstimulated and **(c)** stimulated movement. Horizontal bars indicate the period of life without any detectable movement within the population. **d)** Correlation between movement speed during early adulthood (normalized to wild type) and the percent of lifespan spent without movement. **e)** Time spent by each young adult animal performing each of four stereotypical behaviors during unstimulated locomotion. **f)** Magnitude of the stereotypical response to a mechanical tap stimulus, measured as a distance traveled backward immediately following a tap and before resuming forward locomotion. Data are shown as mean ± s.e.; $n > 160$. * - $p < 0.05$ ** - $p < 0.005$; *** - $p < 0.0005$; n.s. – not significant.


A
OP50
dead OP50
N2
daf-2(e1368)
eat-2(ad1116)
N2
daf-2(e1368)
OP50
dead OP50
n.s.
***
**
**
Relative Oil Red O intensity
2.0
1.5
1.0
0.5
0.0
N2
daf-2(e1368)
eat-2(ad1116)


B
N2
Number or progeny
250
200
150
100
50
0
OP50
dead OP50
mean 213 203
s.d. 30 17
p-value 0.5789


C
N2
OP50
dead OP50
Body length (mm)
1.0
0.5
Day 1
Day 2
Day 3
Day 5
Day 7
Age


D
Rate of decline in movement speed
N2E on OP50
N2E on dead OP50
Unstimulated speed, mm/s
(mean ± s.e.)
0.15
0.10
0.05
0.00
0
10
20
30
40
Age (days)


E
Rate of decline in ability to move
Fraction of live worms that move
(mean ± s.e.)
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
Age (days)

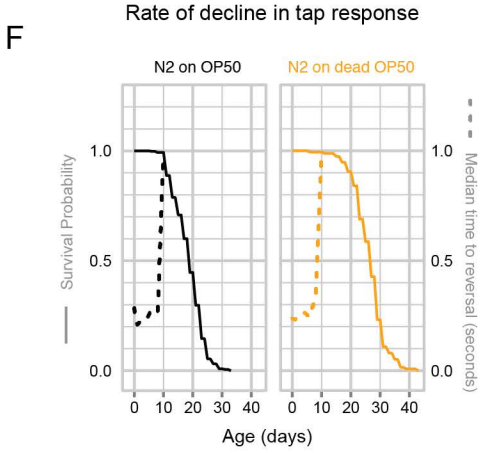

F
Rate of decline in tap response
N2 on OP50
N2 on dead OP50
Survival Probability
Median time to reversal (seconds)
1.0
0.5
0.0
0
10
20
30
40
Age (days)

Figure S3. Dead OP50 *E.coli* does not induce dietary restriction or affect the rate of behavioral aging in *C. elegans*. Related to Figure 3 and Figure 5.

a) Fat content in day-7 adults fed live or dead (gentamicin-killed) OP50 from hatching as determined by Oil Red O staining. Representative images and quantification of whole-body Oil Red O staining intensity normalized to body size in at least 12 worms are shown. Scale bar = 100 μm. Each dot represents a worm, horizontal bars are means and error bars are s.d. ** - $p < 0.005$; *** - $p < 0.0005$; n.s. – not significant. **b)** Brood sizes of wild-type worms grown on live or dead OP50. $n = 10$. **c)** Body lengths as measured by the MWT. Data are shown as beanplots, each consisting of a mirrored density trace, a one-dimensional scatter plot of individual length measurements, and a population median shown in red. $n > 200$. **d)** Unstimulated locomotive speed declines at the same rate if wild-type worms are grown on live or dead OP50, but on dead OP50 animals stay alive longer without any detectable movement. **e)** The ability of live worms to move declines at the same rate on live and dead OP50. **f)** Reaction time in response to a tap stimulus is lost on the same day on live and dead OP50. After day 10, the time to a reversal following a tap is no shorter than the time between reversals during unstimulated locomotion under both growth conditions.

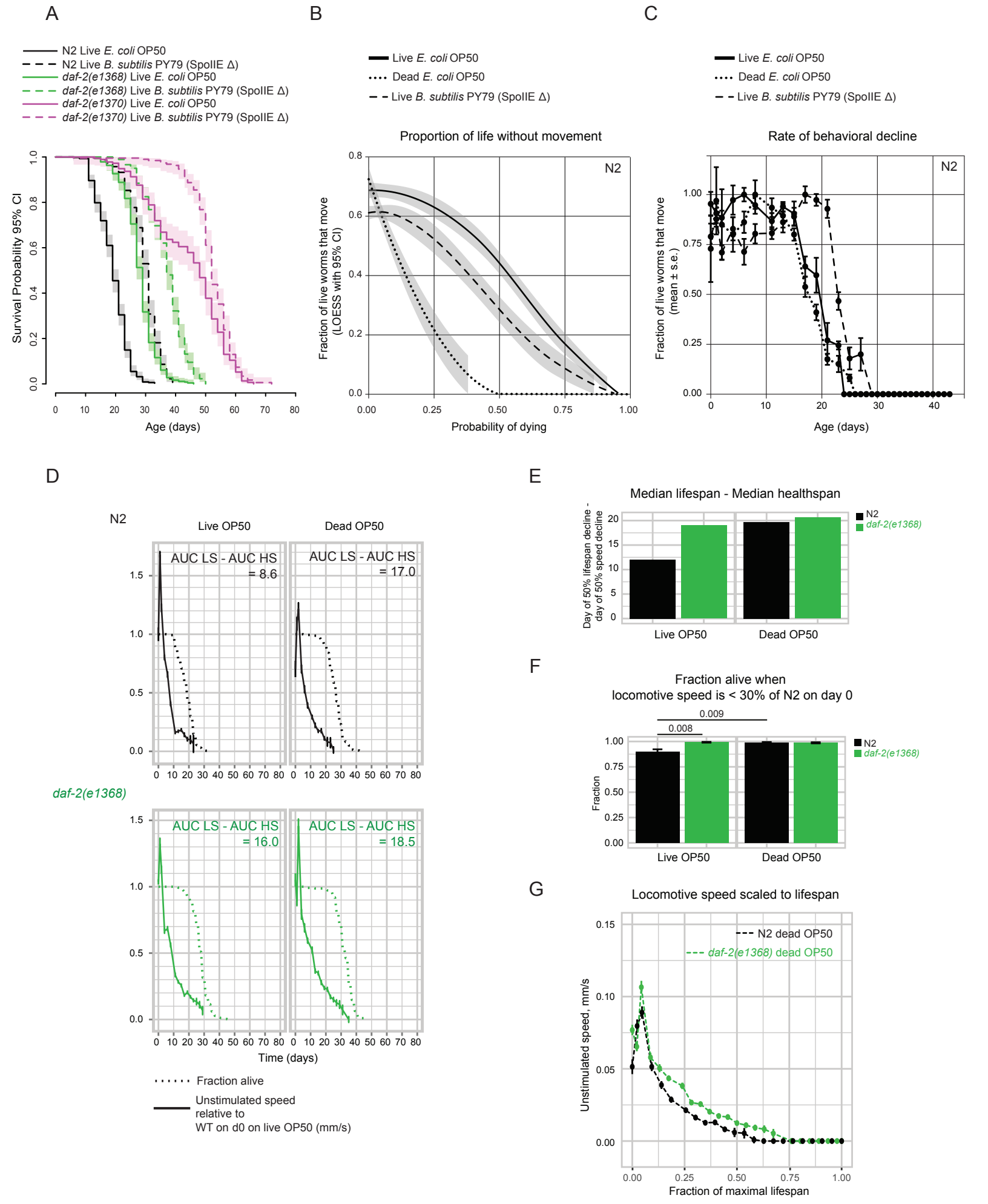

A
N2 Live E. coli OP50
N2 Live B. subtilis PY79 (SpoIIE Δ)
daf-2(e1368) Live E. coli OP50
daf-2(e1368) Live B. subtilis PY79 (SpoIIE Δ)
daf-2(e1370) Live E. coli OP50
daf-2(e1370) Live B. subtilis PY79 (SpoIIE Δ)
Survival Probability 95% CI
Age (days)
B
Live E. coli OP50
Dead E. coli OP50
Live B. subtilis PY79 (SpoIIE Δ)
Proportion of life without movement
N2
Fraction of live worms that move (LOESS with 95% CI)
Probability of dying
C
Live E. coli OP50
Dead E. coli OP50
Live B. subtilis PY79 (SpoIIE Δ)
Rate of behavioral decline
N2
Fraction of live worms that move (mean ± s.e.)
Age (days)
D
N2
Live OP50
Dead OP50
AUC LS - AUC HS = 8.6
AUC LS - AUC HS = 17.0
daf-2(e1368)
AUC LS - AUC HS = 16.0
AUC LS - AUC HS = 18.5
Time (days)
Fraction alive
Unstimulated speed relative to WT on d0 on live OP50 (mm/s)
E
Median lifespan - Median healthspan
Day of 50% lifespan decline - day of 50% speed decline
N2
daf-2(e1368)
Live OP50
Dead OP50
F
Fraction alive when locomotive speed is < 30% of N2 on day 0
0.009
0.008
Fraction
N2
daf-2(e1368)
Live OP50
Dead OP50
G
Locomotive speed scaled to lifespan
N2 dead OP50
daf-2(e1368) dead OP50
Unstimulated speed, mm/s
Fraction of maximal lifespan

Figure S4. Other approaches to reducing food pathogenicity and quantifying healthspan. Related to Figure 5.

a) Feeding wild-type and *daf-2* mutant animals live *Bacillus subtilis* increases their lifespans. Worms were grown on a sporulation-deficient mutant (SpoIIE deletion) of the wild-type PY79 *B. subtilis* strain starting at L1. **b)** *B. subtilis* diet increases the proportion of life spent without movement in wild-type animals, as demonstrated by the decreased fraction of live worms exhibiting detectable movement at a given survival probability. If every animal exhibited movement right until death regardless of whether it died early or late (i.e. there were no late-life decrepitude), this plot would be a straight horizontal line. Local polynomial regression was fit to the data collected from 8 independent replicate plates with 30-50 worms per plate on day 0 and is shown with a 95% confidence interval. The increase in decrepitude achieved by *B. subtilis* was smaller than that achieved by dead OP50, possibly because live *B. subtilis*, while less pathogenic than *E. coli*, is still able to establish some degree of colonization in old animals. Furthermore, unlike dead OP50, *B. subtilis* delayed behavioral aging **(c),** possibly via production on nitric oxide (Gusarov et al., 2013), thereby extending the duration of healthy life. **d)** The total amount of life and health in a population can be quantified by calculating area under the survival curve and area under a health metric curve (in this case, change in locomotive speed), respectively. The difference between the two areas could be taken to represent the amount of life without health. *daf-2* mutants spend a greater amount of life than wild-type animals without health, and feeding wild-type animals dead OP50 increases the amount of life without health to levels comparable to those observed in *daf-2* mutants. **e)** The amount of life without health can also be represented by the difference between median lifespan and median healthspan. This approach shows that in wild-type animals, lifespan declines by 50% 11 days after locomotive speed declines by 50%, whereas in *daf-2* mutants the difference is almost 20 days. When fed dead OP50, the two strains look very similar. **f)** In wild type, 90% of animals are alive when locomotive speed has declined by 70%, whereas 100% of *daf-2* mutants are alive when the same state of locomotive decline is reached. When fed dead bacteria, 100% of wild-type and *daf-2* are alive. **g)** When both strains are fed dead bacteria, *daf-2(e1368)* mutant animals are more vigorous than wild-type animals at every percentile of lifespan. $n > 200$.

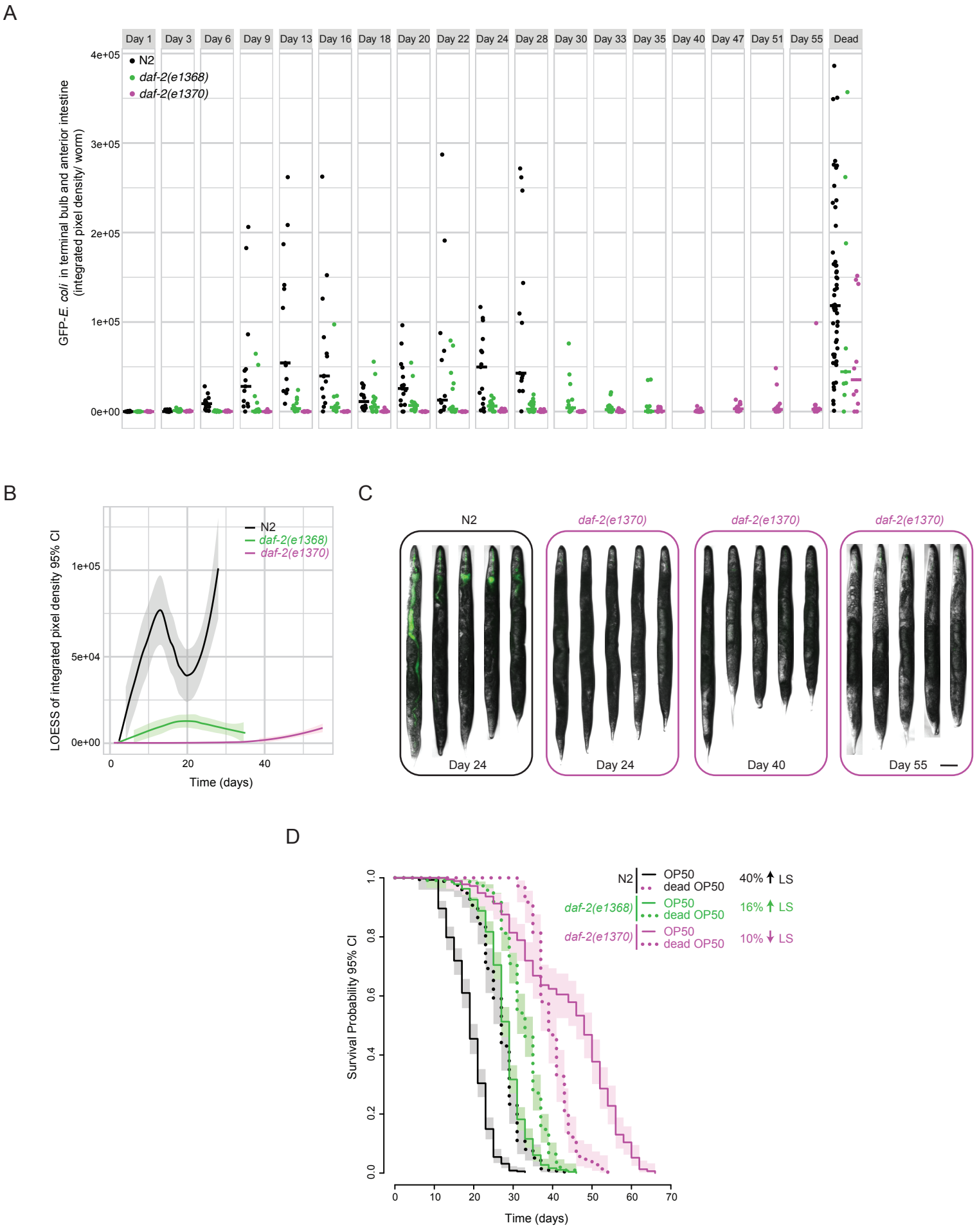

A
Day 1
Day 3
Day 6
Day 9
Day 13
Day 16
Day 18
Day 20
Day 22
Day 24
Day 28
Day 30
Day 33
Day 35
Day 40
Day 47
Day 51
Day 55
Dead
N2
daf-2(e1368)
daf-2(e1370)
GFP-E. coli in terminal bulb and anterior intestine (integrated pixel density/ worm)
4e+05
3e+05
2e+05
1e+05
0e+00
B
LOESS of integrated pixel density 95% CI
Time (days)
C
N2
daf-2(e1370)
Day 24
Day 24
Day 40
Day 55
D
Survival Probability 95% CI
Time (days)
N2 OP50
dead OP50
40% LS
daf-2(e1368) OP50
dead OP50
16% LS
daf-2(e1370) OP50
dead OP50
10% LS

Figure S5. *daf-2(e1370)* mutants are very resistant to bacterial colonization. Related to Figure 4 and Figure 5.

a) *daf-2(e1370)* mutants show a strongly delayed and reduced terminal bulb and proximal intestine colonization by OP50 *E. coli*. $n > 15$. **b)** Local regression of the data for live worms in (a). **c)** Representative images of wild-type and *daf-2(e1370)* on day 24 of adulthood and of *daf-2(e1370)* at later ages, when wild type are dead. Almost no colonization is observed in these mutants even late in life. Scale bar = 100 μm. **d)** Survival of *daf-2(e1368)* and *daf-2(e1370)* mutants on dead OP50 *E. coli*. **e)** A diet of dead OP50 markedly increases the proportion of life wild-type animals spend without detectable movement, but *daf-2(e1368)* and *daf-2(e1370)* are largely unaffected by this treatment.

Table S1 Adult lifespans of strains in this study
Related to Figures 2, 3, 5, S4 and S5

Strain	Bacteria	Events/ *N* initial	Mean lifespan +/- s.e.m. (Days)	Median lifespan	% mean lifespan change vs. N2 on same food	*P*-value (log-rank) vs. N2 on same food	% mean lifespan change vs. Live OP50	*P*-value (log-rank) vs. Live OP50	Fig.
N2E	OP50	361/430	19.04 ± 0.25	19					2a
daf-2(e1368)	OP50	365/476	28.04 ± 0.27	29	47.30	< 0.0001			2a
daf-2(e1370)	OP50	157/210	44.09 ± 0.99	48	131.57	< 0.0001			2a
N2E	Dead OP50	139/173	26.71 ± 0.44	27			40.28	< 0.0001	5a; S5d
daf-2(e1368)	Dead OP50	204/231	32.63 ± 0.38	33	22.18	< 0.0001	16.36	< 0.0001	5a; S5d
daf-2(e1370)	Dead OP50	106/138	39.9 ± 0.45	39	49.40	< 0.0001	-9.50	< 0.0001	S5d
N2E	*B. subtilis*	225/407	29.57± 0.32	31			55.30	< 0.0001	S4a
daf-2(e1368)	*B. subtilis*	226/374	36.86 ± 0.42	37	24.65	< 0.0001	31.46	< 0.0001	S4a
daf-2(e1370)	*B. subtilis*	184/208	52.26 ± 0.52	52	76.73	< 0.0001	18.53	< 0.0001	S4a

Strain	Bacteria	Events/ *N* initial	Mean lifespan +/- s.e.m. (Days)	Median lifespan	% mean lifespan change vs. N2	*P*-value (log-rank) vs. N2	Fig.
N2E + empty vector	HT115	211/271	17.31 ± 0.26	18			S2a
N2E + daf-2(RNAi)	HT115	222/256	27.51 ± 0.45	26	58.93	< 0.0001	S2a
daf-2(e1368) + empty vector	HT115	127/200	24.15 ± 0.42	24	39.51	< 0.0001	S2a
daf-2(e1368) + daf-2(RNAi)	HT115	146/168	34.51 ± 0.57	34	99.36	< 0.0001	S2a

Strain	OP50	Colonization	Events/ *N* initial	Mean lifespan +/- s.e.m. (Days)	Median lifespan	% mean lifespan change vs. Colonized on day 9	*P*-value (log-rank) vs. Colonized on day 9	Fig.
N2E	Live	Colonized on day 9	183/203	16.64 ± 0.49	15			3c
N2E	Live	Not colonized on day 9	132/137	23.46 ± 0.52	23	41.01	< 0.0001	3c

Strain	OP50	Colonization	Treatment	Events/ *N* initial	Mean lifespan +/- s.e.m. (Days)	Median lifespan	% mean lifespan change vs. Untreated	*P*-value (log-rank) vs. Untreated	Fig.
N2E	Live	Colonized on day 9	Untreated	83/101	13.23 ± 0.36	12			3d
N2E	Live	Colonized on day 9	Gentamicin	34/83	17.35 ± 0.39	n/a	31.09	< 0.0001	3d
N2E	Live	Not colonized (ever)	Untreated	0/129	n/a	n/a			3d
N2E	Live	Not colonized on day 9	Gentamicin	5/101	n/a	n/a	n/a		3d
N2E	GmR	n/a	Untreated	95/153	20.43 ± 0.42	20			3g
N2E	GmR	n/a	Gentamicin	42/170	19.5 ± 0.58	18	-4.77	0.195	3g

Supplemental Experimental Procedures

C. elegans strains and maintenance

Hermaphrodite worms were cultured under standard growth conditions at 20°C (Brenner, 1974). The wild-type strain was N2 Bristol (Brenner, 1974). CF4088: *daf-2(e1368)* III outcrossed 12 times from DR1572 (from CGC). CF4087: *daf-2(e1370)* III outcrossed 12 times from CB1370 (from CGC). Animals were cultured under standard growth conditions for at least 3 generations before use in any assay. Synchronized populations were obtained by hypochlorite treatment of gravid adults, overnight egg hatching in M9 buffer, and recovery of arrested L1 larvae on seeded plates.

Analysis of behavioral data

Analysis of movement parameters generated by Choreography was conducted in R (v 3.2.1) through the RStudio environment (v 0.99.467) and using *ggplot2* package for graph generation (Wickham, 2009) and *plyr* package for dataframe handling (Wickham, 2011). Because the process of being put under the tracker stimulates movement (presumably due to mechanical force associated with handling as well as changes in light, oxygen and temperature), to measure calmed, unstimulated speed of explorative locomotion, we only used data after the population speed no longer decreased (800 s from the start of tracking). Unstimulated speed was calculated as mean population speed over a 10 s time interval of calmed locomotion. MWT keeps track of individual animal identity during a single tracking session until the tracked worm collides with another worm. Percentage of time spent moving forward, backward, not moving and turning was calculated using data from individual animals that were detected over an entire 60 s window of calmed locomotion. For spontaneous reversal calculation, the number of reversals per worm was calculated over a 60 s time window of calmed locomotion and then percentage of worms reversing at least once was calculated. Fraction of worms reversing and reversal distance following a tap were calculated using data from individual animals that were detected as not moving backward before the first tap and moving backward immediately after the tap. Time to a reversal was the time it took an animal that was not moving backward prior to the tap to start going backward. Mean stimulated speed was calculated over a 1 s time window immediately following the first tap. Differences between behavioral metrics of different worm populations were compared using a two-tailed unpaired heteroscedastic Student's *t*-test.

Bacterial plate preparation and antibiotic treatment

OP50 and GFP-OP50 *E. coli* strains were obtained from CGC. GFP-OP50 contains a pFVP25.1 plasmid with an ampicillin-resistance cassette (Labrousse et al., 2000). Overnight cultures of OP50 or GFP-OP50 were 2x concentrated, spread on NGM plates and used the following day. To prepare plates with dead bacteria, overnight cultures were diluted to OD of 0.1, grown to OD of 1.5, incubated for 5 hours with 120 µg/ml gentamicin at 37°C, washed, concentrated 50x and spread on NGM plates containing 10 µg/ml gentamicin the day before adding worms. Killing was confirmed by streaking lawns on antibiotic-free LB plates. For treatment of colonized worms with gentamicin, day-9 adults grown on live GFP-OP50 since L1 were separated based on the presence of any detectable GFP in the grinder or proximal intestine and transferred to plates seeded with gentamicin-killed GFP-OP50 and containing 10 µg/ml gentamicin and 50 µM FUDR. In addition, 120 µl of 20 µg/ml gentamicin was added to the newly-transferred worms.

Fluorescence microscopy and image analysis

Non-confocal fluorescence images were acquired using Leica DFC3000 G camera attached to a Leica M165 FC stereo-microscope using ET GFP band-pass filter (excitation 470 nm, emission 525 nm) for GFP detection. GFP-OP50 fluorescence as a function of worm age was quantified using these images by measuring raw integrated pixel density within a region of a constant size containing the grinder and a region contacting the anterior 100 µm of the intestine (starting at the pharyngeal-intestinal valve). Fluorescence within an equivalent region but without any apparent bacteria was subtracted to correct for the age-associated increase in worm auto-fluorescence. Confocal and DIC images were captured using the Nikon Ti-E microscope with Yokogawa CSU-X1 spinning disk, Andor iXon Ultra camera and Piezo Z-drive. The laser 488 nm laser was used at 3.75 V. The objective used was Plan Apo 40x/0.95 with an additional 1.5x zoom. Images were analyzed in Fiji (version 2.0.0-rc-43/1.50e).

Oil Red O fat staining and quantification

Fat staining with Oil Red O was done as previously described (Wahlby et al., 2014). Images were acquired using Nikon Ti-E microscope and Nikon DS-Ri2 color camera. Whole-body intensity of the red stain was quantified in the following way. Worms were segmented and pixel intensity in each channel (red, green and blue) was calculated per

worm, normalized to worm size and background corrected. Red color is produced by reduction in the contribution of the other two colors (blue and green) to the total pixel intensity. Thus, to quantify red intensity, the change in the green channel intensity between unstained and stained worms was added to the change in the blue channel intensity between unstained and stained worms.

Brood size analysis
Single synchronized adults were transferred to fresh plates every 24 hours until cessation of reproduction and the number of progeny produced by each worm was counted.

Construction of gentamicin-resistant OP50
Gentamicin resistant OP50 (GmR OP50) was made by inserting gentamicin acetyltransferase-3-I (aacC1) gene driven by a synthetic promoter CP6 (GenBank DQ530421.1) into a pBR322 vector (NEB) using NEBuilder HiFi DNA Assembly Kit (NEB) and transfecting the construct into OP50 made chemically competent using Mix & Go *E. coli* Transformation Kit (Zymo Research).

Supplemental References

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