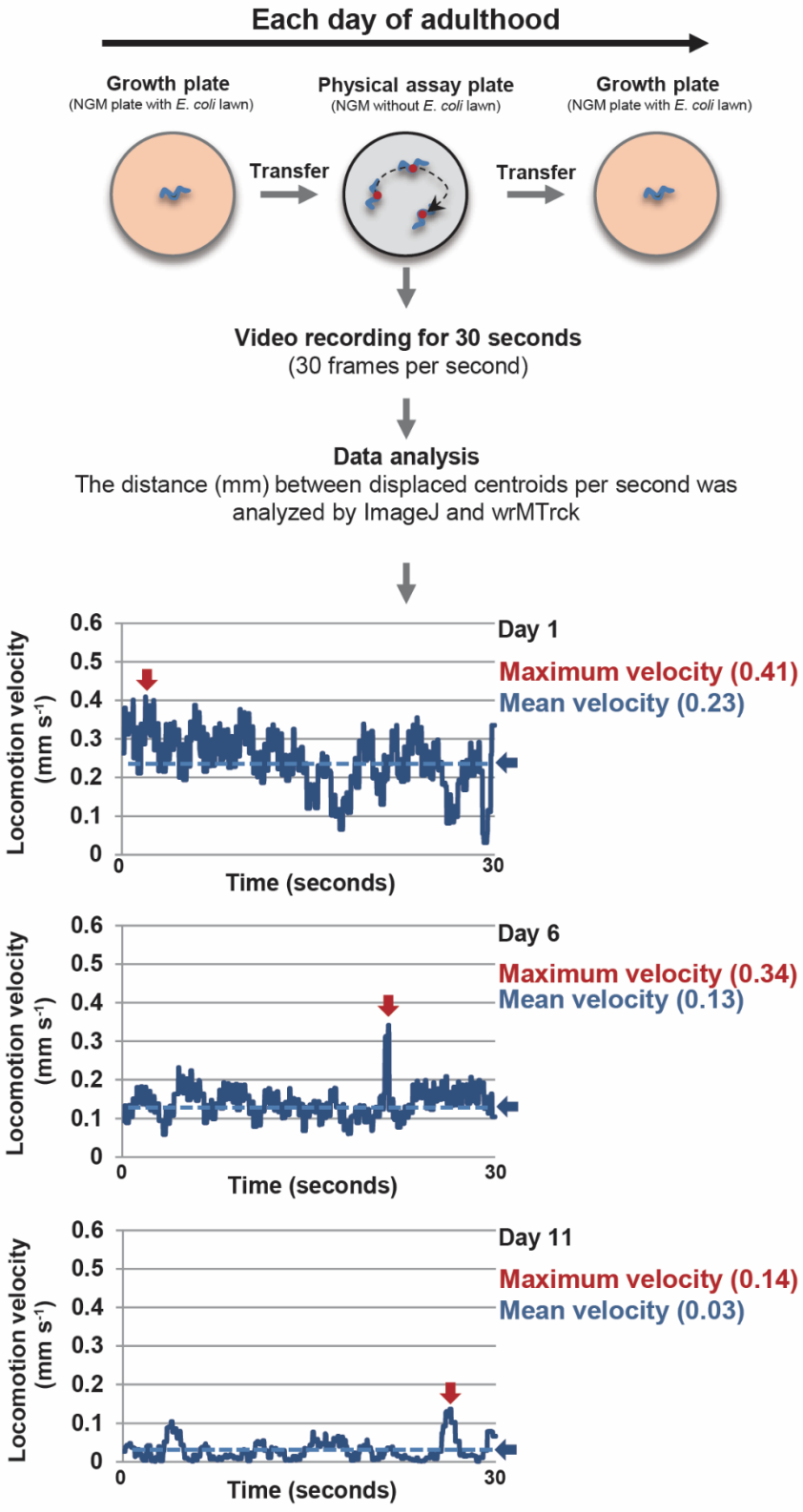
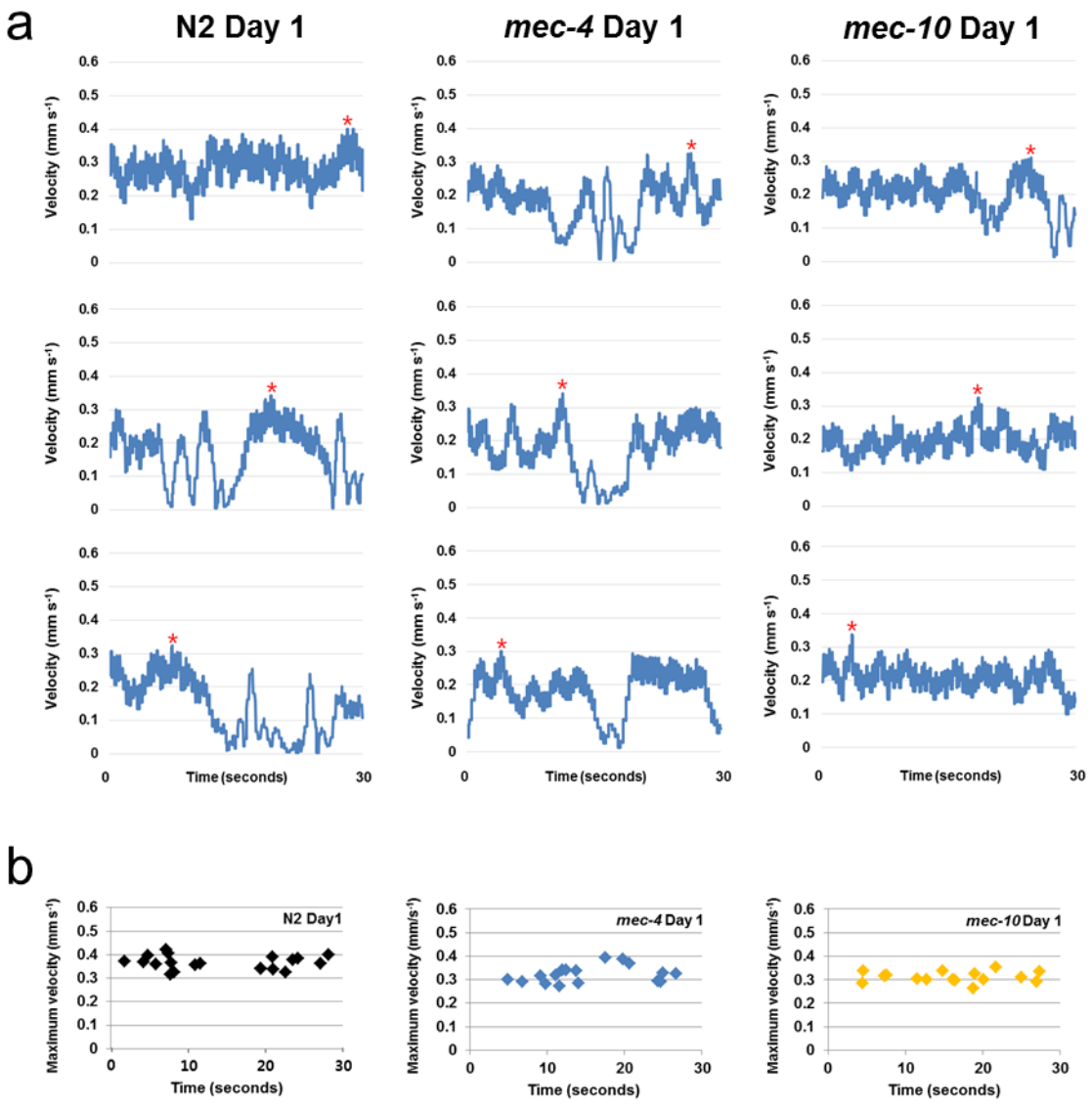
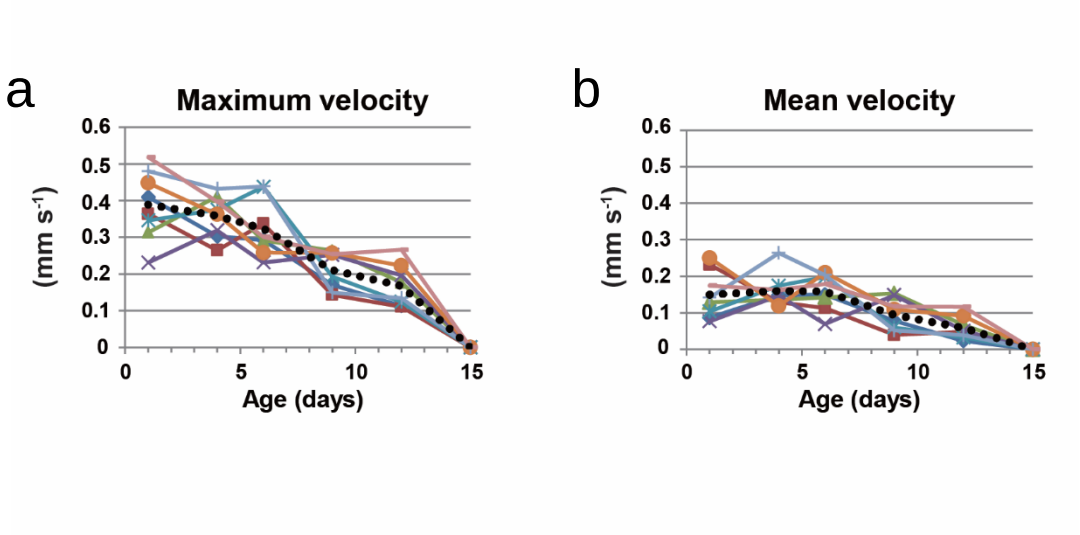

Each day of adulthood
Growth plate
(NGM plate with E. coli lawn)
Physical assay plate
(NGM without E. coli lawn)
Growth plate
(NGM plate with E. coli lawn)
Transfer
Transfer
Video recording for 30 seconds
(30 frames per second)
Data analysis
The distance (mm) between displaced centroids per second was analyzed by ImageJ and wrMTrck
Locomotion velocity (mm s-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
30
Time (seconds)
Day 1
Maximum velocity (0.41)
Mean velocity (0.23)
Day 6
Maximum velocity (0.34)
Mean velocity (0.13)
Day 11
Maximum velocity (0.14)
Mean velocity (0.03)

Supplementary Figure 1: The assay scheme for measuring the age-associated decline of short physical performances in *C. elegans*.

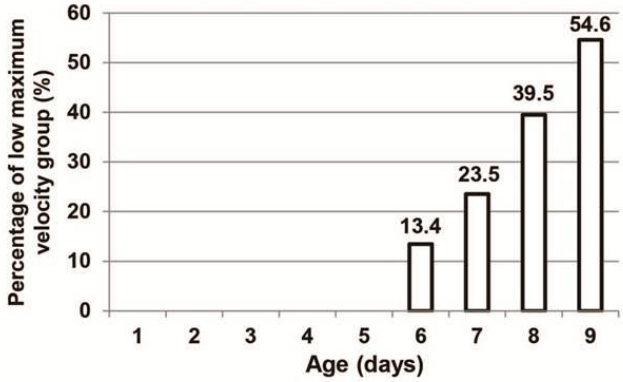
Worms were grown on a growth plate covered with a bacterial lawn. When measuring the physical performance, the worms were transferred to a 'physical assay plate' with no bacterial lawn and their movement was recorded for 30 seconds. The red dots indicate the centroids used to follow the movement of the worms. ImageJ and wrMTrck were used to calculate the locomotion velocity, which was expressed as mm s^{-1} [distance (mm) between centroid displacements per second]. The velocity profiles of a single worm at the three different ages (Day 1, 6, and 11 of adulthood) are shown at the bottom. The MV and mean velocity at the three different ages are noted.



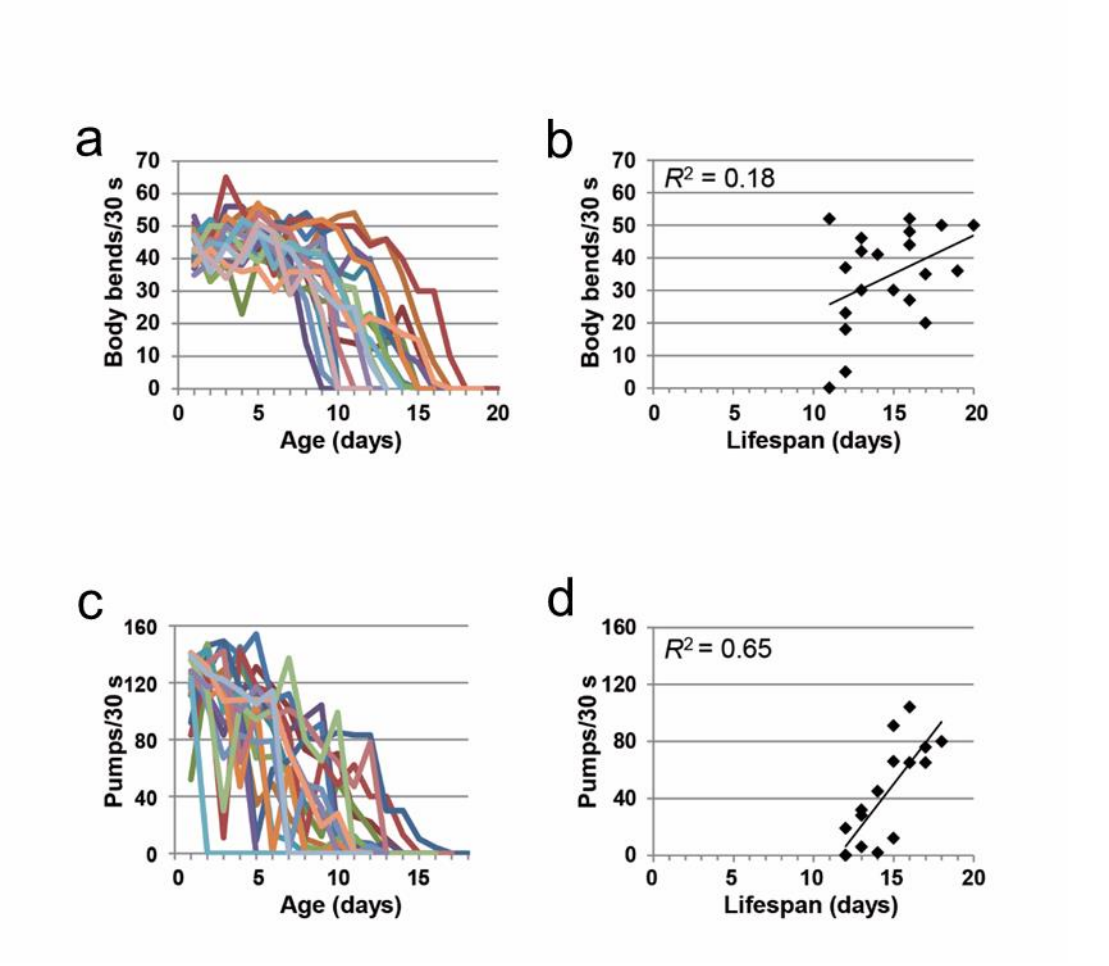
Supplementary Figure 2: Harsh-touch mutants do not behave differently from wild-type in the maximum velocity (MV) assay. (**a**) Mechanosensory mutants (*mec-4* and *muc-10*) show a velocity pattern similar to that of wild type in the MV assay. Red asterisk represents maximum velocity. (**b**) Maximum velocity appeared randomly on the 30 seconds interval of the MV assay in both wild type and mechanosensory mutants.



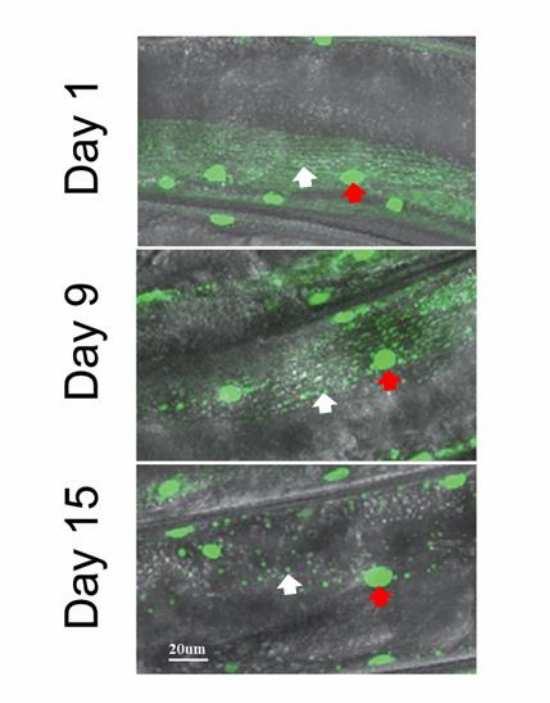
Supplementary Figure 3: Age-dependent changes of maximum (a) and mean (b) velocity of individual worms. Black dots represent the average value at each age (*n* = 8).



Supplementary Figure 4: Age-associated increase of percentage of low MV group (*n* = 119).

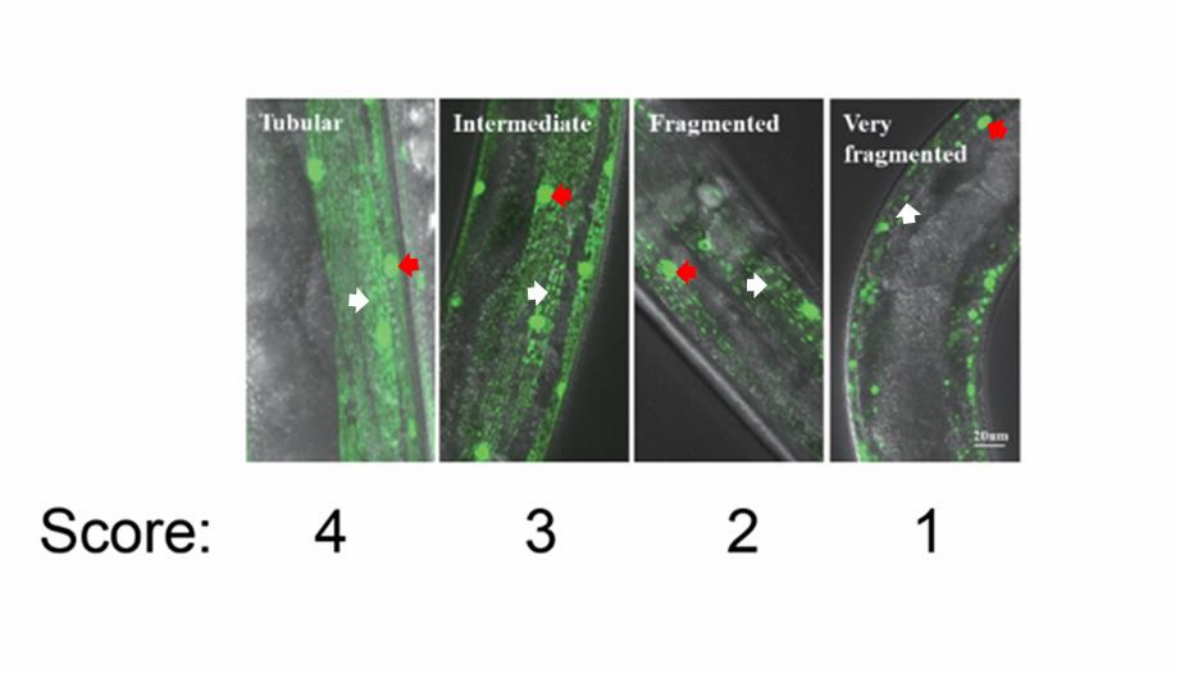


Supplementary Figure 5: Age-dependent changes in the thrashing and pharyngeal pumping. (**a**) Age-associated change of thrashing. Thrashing of each worm is noted as body bends/30 s. (**b**) Correlation between thrashing and lifespan of individual worms. Thrashing of individual worms was measured at Day 9 of adulthood and their lifespans were measured thereafter. R^2, coefficient of determination. n = 20. (**c**) Age-associated change of pharyngeal pumping rate. (**d**) Correlation between pharyngeal pumping and lifespan of individual worms. The pumping rates of individual worms were measured at Day 9 of adulthood and their lifespans were measured thereafter. R^2, coefficient of determination. n = 19. Note that, although the pharyngeal pumping rates showed a coefficient of determination value of 0.65, they showed highly irregular patterns along aging and thus do not serve as a good age-associated indicator.

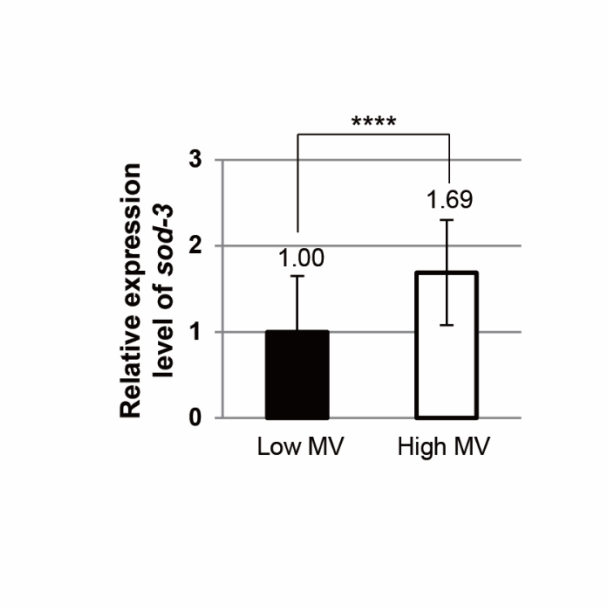


Supplementary Figure 6: Age-dependent changes of mitochondrial morphology in wild-type worms.

Top: mitochondria in the muscle cell of a worm at Day 1 of adulthood show tubular and intermediate morphology. Middle: fragmented mitochondria appear in a worm at Day 9 of adulthood. Bottom: mitochondria in a worm at Day 15 of adulthood are very fragmented with a few globular organelles. Colored green is the green fluorescence protein produced in mitochondria (white arrow) and nucleus (red arrow) in body wall muscles of PD4251 worms.

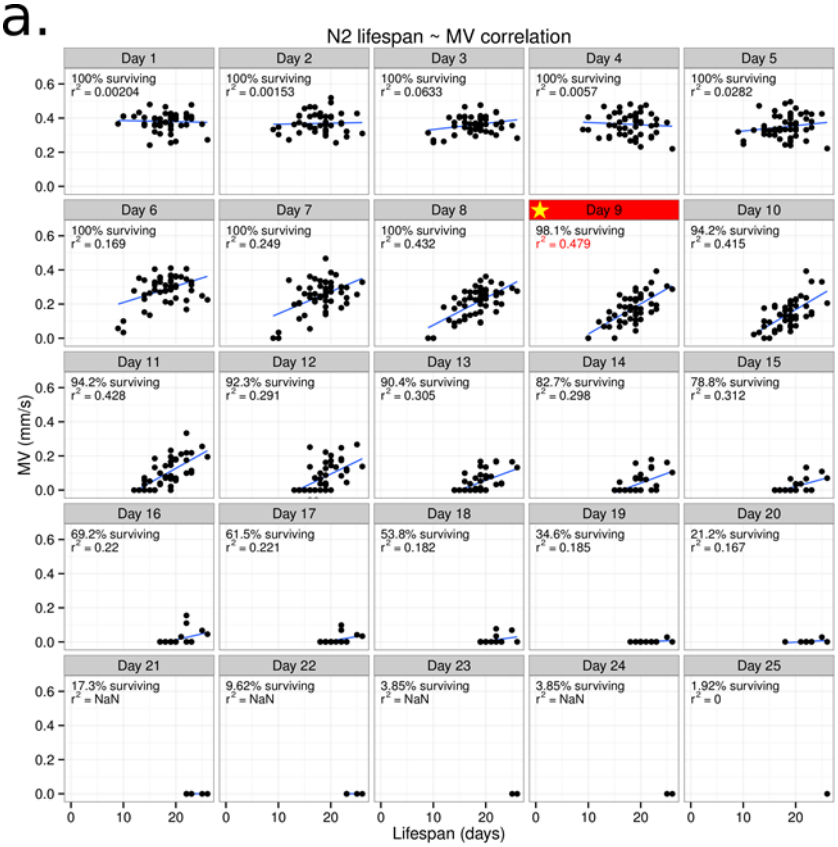


Supplementary Figure 7: Scoring of mitochondrial morphologies for mitochondrial integrity. To assay mitochondrial integrity, the scores of 1, 2, 3, and 4 were given to mitochondria with very fragmented, fragmented, intermediate, and tubular morphologies, respectively. Colored green is the green fluorescence protein produced in mitochondria (white arrow) and nucleus (red arrow) in body wall muscles of PD4251 worms.

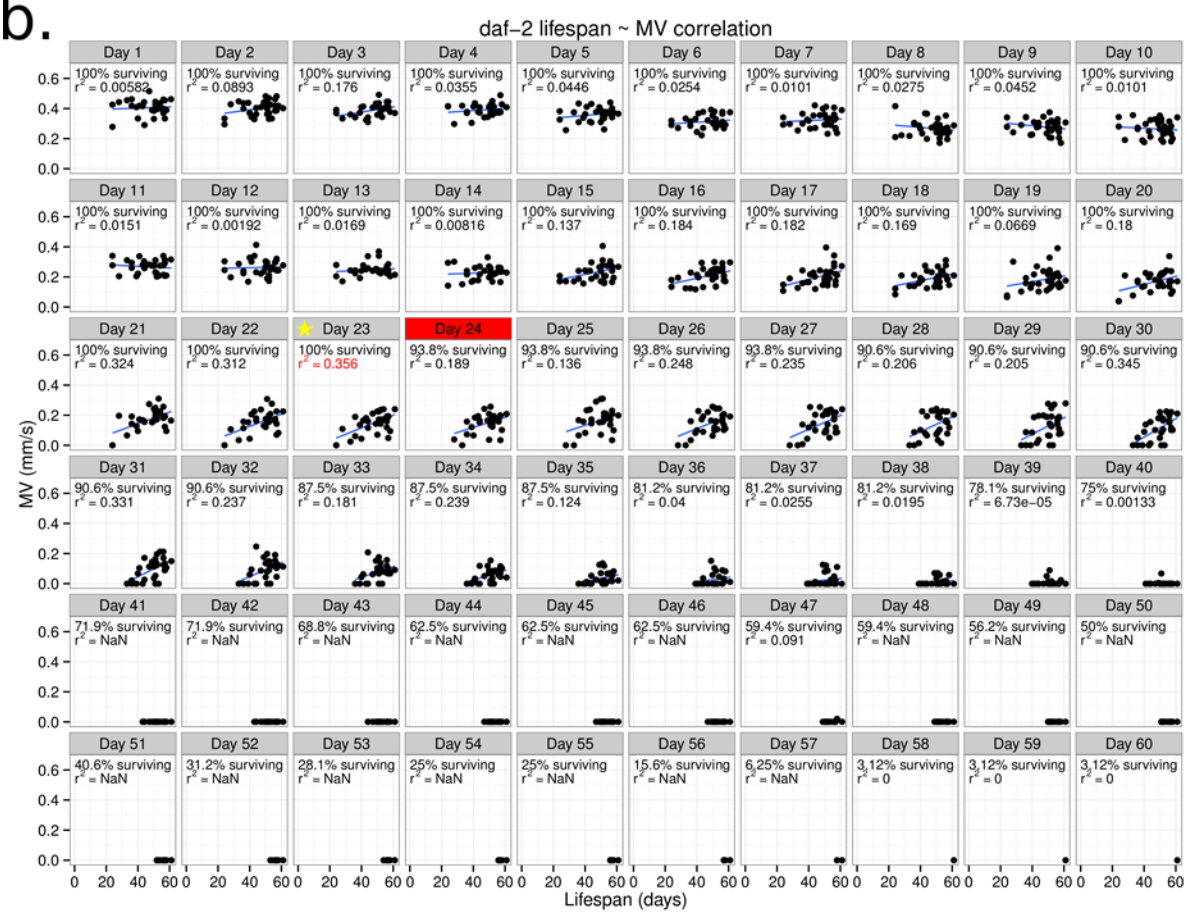


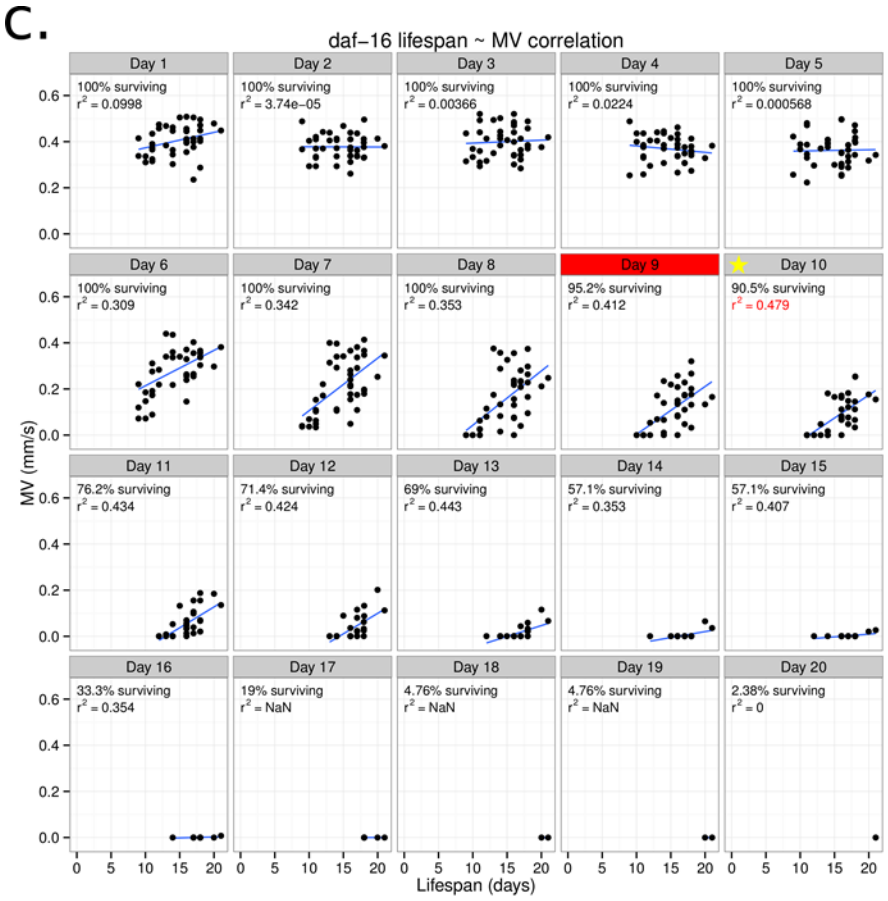
Supplementary Figure 8: Correlation of *sod-3* gene expression with MV in the low and high MV groups at Day 9 of adulthood. Significance was determined using a two-tailed, unpaired *t*-test. ****$P<0.0001$. Error bars represent s.d.

a.


N2 lifespan ~ MV correlation
Day 1
100% surviving
r² = 0.00204
Day 2
100% surviving
r² = 0.00153
Day 3
100% surviving
r² = 0.0633
Day 4
100% surviving
r² = 0.0057
Day 5
100% surviving
r² = 0.0282
Day 6
100% surviving
r² = 0.169
Day 7
100% surviving
r² = 0.249
Day 8
100% surviving
r² = 0.432
Day 9
98.1% surviving
r² = 0.479
Day 10
94.2% surviving
r² = 0.415
Day 11
94.2% surviving
r² = 0.428
Day 12
92.3% surviving
r² = 0.291
Day 13
90.4% surviving
r² = 0.305
Day 14
82.7% surviving
r² = 0.298
Day 15
78.8% surviving
r² = 0.312
Day 16
69.2% surviving
r² = 0.22
Day 17
61.5% surviving
r² = 0.221
Day 18
53.8% surviving
r² = 0.182
Day 19
34.6% surviving
r² = 0.185
Day 20
21.2% surviving
r² = 0.167
Day 21
17.3% surviving
r² = NaN
Day 22
9.62% surviving
r² = NaN
Day 23
3.85% surviving
r² = NaN
Day 24
3.85% surviving
r² = NaN
Day 25
1.92% surviving
r² = 0
MV (mm/s)
Lifespan (days)

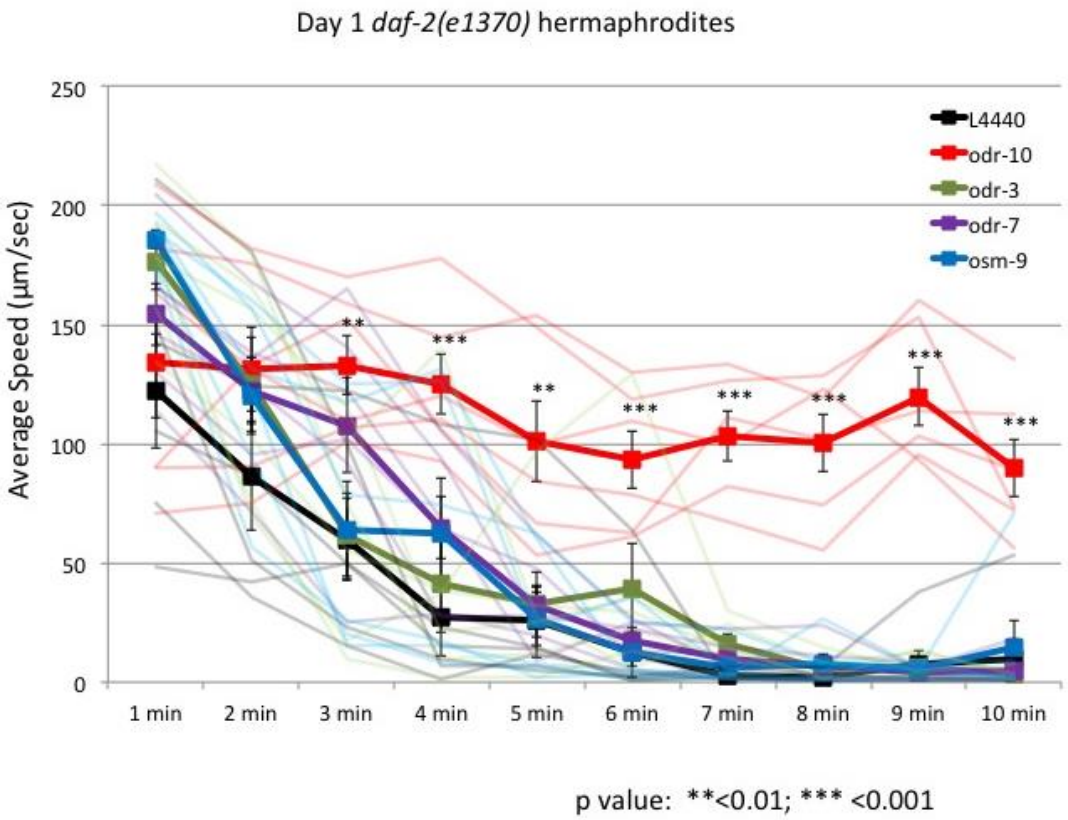
b.


daf−2 lifespan ~ MV correlation
Day 1
100% surviving
r² = 0.00582
Day 2
100% surviving
r² = 0.0893
Day 3
100% surviving
r² = 0.176
Day 4
100% surviving
r² = 0.0355
Day 5
100% surviving
r² = 0.0446
Day 6
100% surviving
r² = 0.0254
Day 7
100% surviving
r² = 0.0101
Day 8
100% surviving
r² = 0.0275
Day 9
100% surviving
r² = 0.0452
Day 10
100% surviving
r² = 0.0101
Day 11
100% surviving
r² = 0.0151
Day 12
100% surviving
r² = 0.00192
Day 13
100% surviving
r² = 0.0169
Day 14
100% surviving
r² = 0.00816
Day 15
100% surviving
r² = 0.137
Day 16
100% surviving
r² = 0.184
Day 17
100% surviving
r² = 0.182
Day 18
100% surviving
r² = 0.169
Day 19
100% surviving
r² = 0.0669
Day 20
100% surviving
r² = 0.18
Day 21
100% surviving
r² = 0.324
Day 22
100% surviving
r² = 0.312
Day 23
100% surviving
r² = 0.356
Day 24
93.8% surviving
r² = 0.189
Day 25
93.8% surviving
r² = 0.136
Day 26
93.8% surviving
r² = 0.248
Day 27
93.8% surviving
r² = 0.235
Day 28
90.6% surviving
r² = 0.206
Day 29
90.6% surviving
r² = 0.205
Day 30
90.6% surviving
r² = 0.345
Day 31
90.6% surviving
r² = 0.331
Day 32
90.6% surviving
r² = 0.237
Day 33
87.5% surviving
r² = 0.181
Day 34
87.5% surviving
r² = 0.239
Day 35
87.5% surviving
r² = 0.124
Day 36
81.2% surviving
r² = 0.04
Day 37
81.2% surviving
r² = 0.0255
Day 38
81.2% surviving
r² = 0.0195
Day 39
78.1% surviving
r² = 6.73e−05
Day 40
75% surviving
r² = 0.00133
Day 41
71.9% surviving
r² = NaN
Day 42
71.9% surviving
r² = NaN
Day 43
68.8% surviving
r² = NaN
Day 44
62.5% surviving
r² = NaN
Day 45
62.5% surviving
r² = NaN
Day 46
62.5% surviving
r² = NaN
Day 47
59.4% surviving
r² = 0.091
Day 48
59.4% surviving
r² = NaN
Day 49
56.2% surviving
r² = NaN
Day 50
50% surviving
r² = NaN
Day 51
40.6% surviving
r² = NaN
Day 52
31.2% surviving
r² = NaN
Day 53
28.1% surviving
r² = NaN
Day 54
25% surviving
r² = NaN
Day 55
25% surviving
r² = NaN
Day 56
15.6% surviving
r² = NaN
Day 57
6.25% surviving
r² = NaN
Day 58
3.12% surviving
r² = 0
Day 59
3.12% surviving
r² = 0
Day 60
3.12% surviving
r² = 0
MV (mm/s)
Lifespan (days)

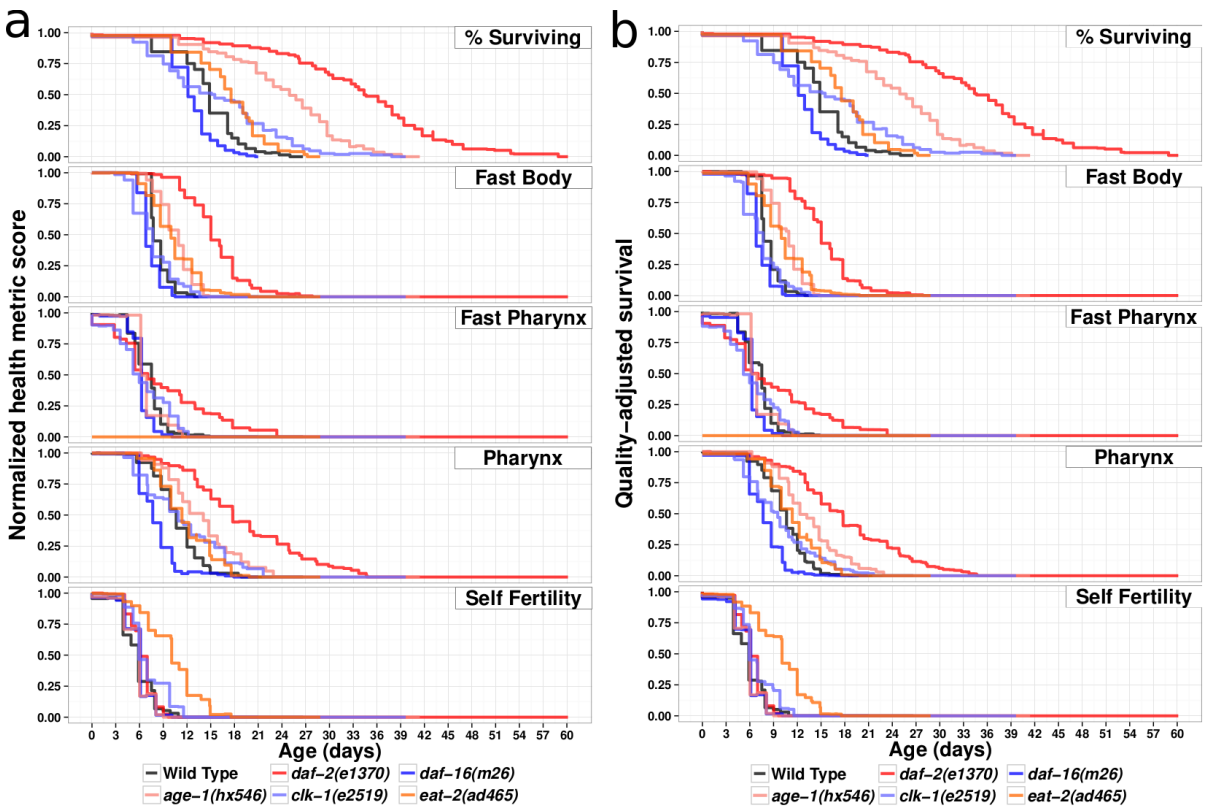


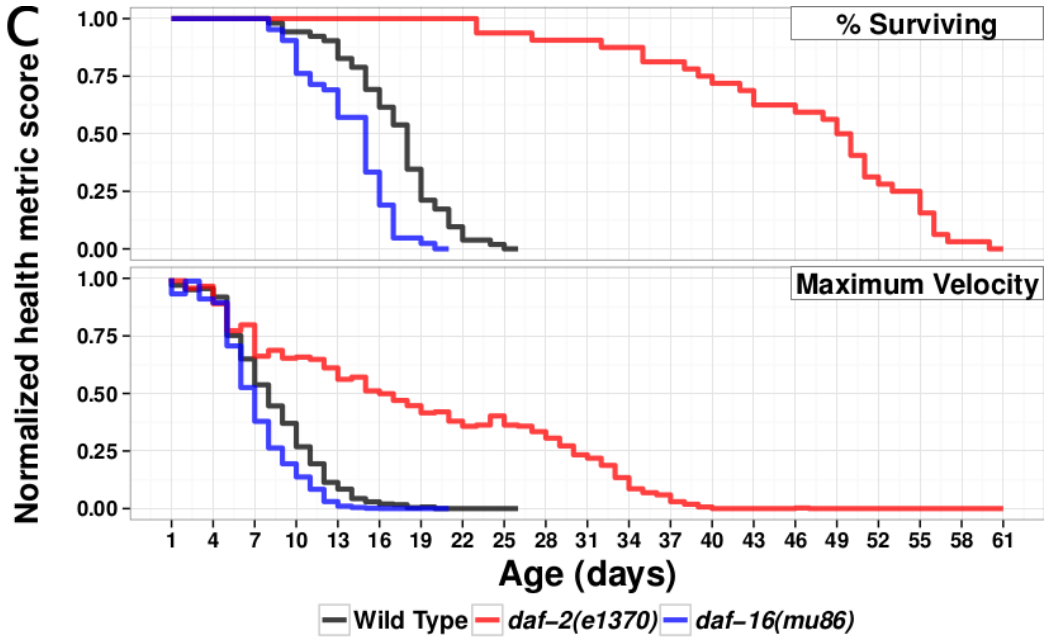
Supplementary Figure 9: Correlation between MV and lifespan for a cohort of worms tracked longitudinally through their lifespan.

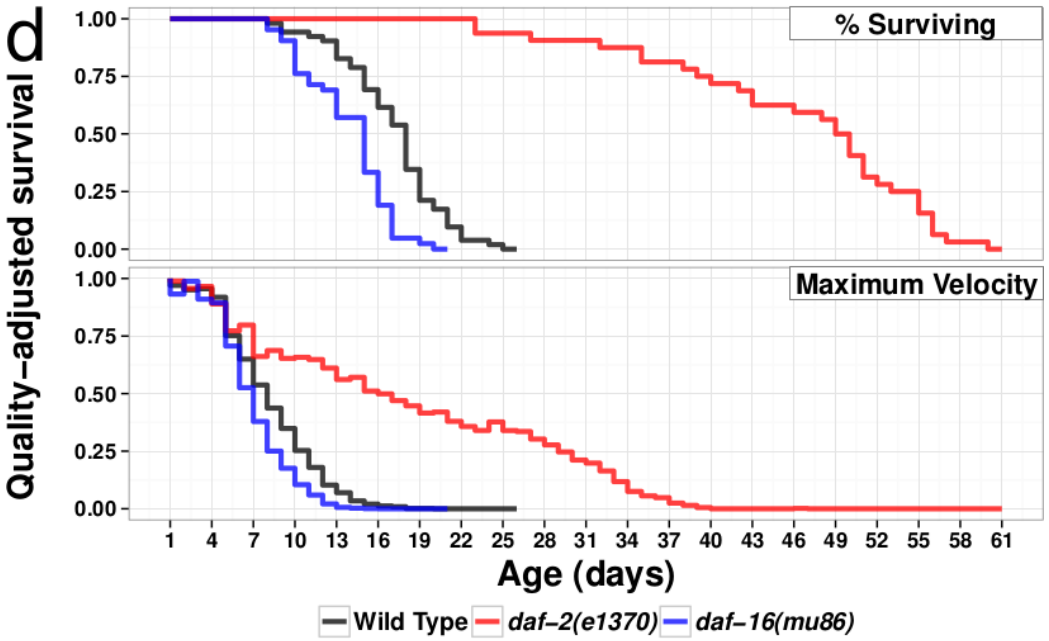
The day on which the first worm of the cohort dies is highlighted in red, and the day on which MV best correlates with lifespan is denoted with a star. In all cases tested, the best correlation between MV and lifespan occurs within a day of the first death of the cohort. (**a**) N2 worms tracked in Fig. 1a. The best correlation between MV and lifespan occurs on day 9, which is also when the first worm of the cohort dies. (**b**) *daf-2(e1370)* worms tracked in Fig. 2b. The best correlation between MV and lifespan occurs on day 23, the day before the first worm of the cohort dies. (**c**) *daf-16(mu86)* worms tracked in Fig. 2c. The best correlation occurs on day 10, the day after the first worm of the cohort dies.

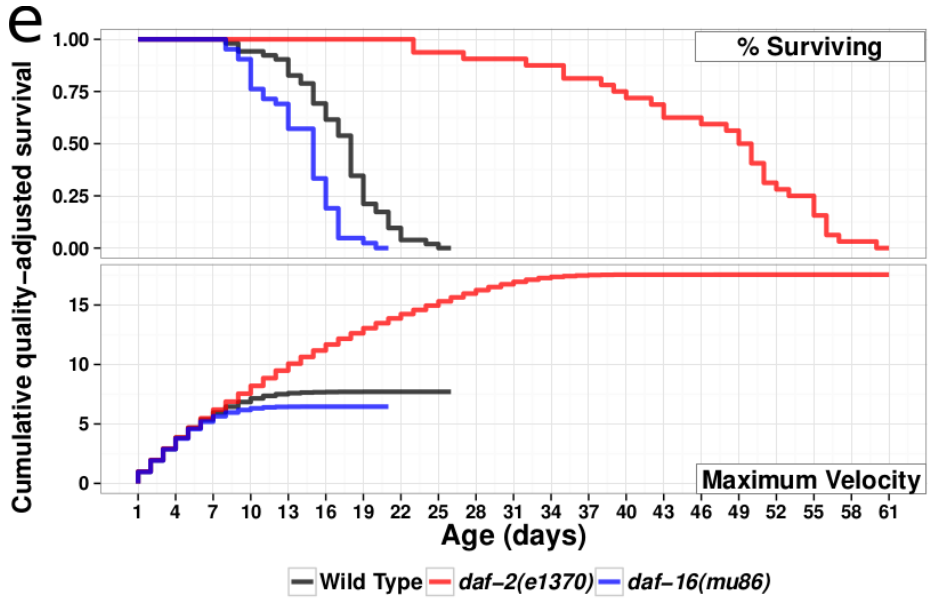


Supplementary Figure 10: On-bacteria motility assay. *daf-2(e1370)* worms after being raised on L4440 (control), *odr-3, odr-7*, or *osm-9* RNAi slow down soon after being placed onto bacteria, while *daf-2(e1370)* worms treated with *odr-10* RNAi (red) maintain a higher speed over the entire 10 min of the assay.


a
b
% Surviving
Fast Body
Fast Pharynx
Pharynx
Self Fertility
Normalized health metric score
Quality−adjusted survival
1.00
0.75
0.50
0.25
0.00
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
Age (days)
Wild Type
daf−2(e1370)
daf−16(m26)
age−1(hx546)
clk−1(e2519)
eat−2(ad465)


C
Normalized health metric score
% Surviving
Maximum Velocity
1.00
0.75
0.50
0.25
0.00
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61
Age (days)
Wild Type
daf−2(e1370)
daf−16(mu86)


D
Quality−adjusted survival
% Surviving
Maximum Velocity
1.00
0.75
0.50
0.25
0.00
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61
Age (days)
Wild Type
daf−2(e1370)
daf−16(mu86)



Supplementary Figure 11: Additional quality-adjusted health metrics

daf-2(e1370) mutants are healthier and have higher quality lives as measured in previous surveys of healthspan. (**a**) Quality-adjusted lifespan curves for the health categories, as scored by Huang et al.,[1] with categories separated by the health categories used in the study. (**b**) The Quality-adjusted lifespan curve is the survival rate multiplied by the health measurement, normalized to the maximum value attained by the wild type. From our own data on MV with age, we find *daf-2* mutants have higher quality life than wild type. (**c**)**,** Quality-adjusted lifespan curves for MV as measured in this study. (**d**) Cumulative area under curve of the quality-adjusted MV lifespan, indicating higher total quality for *daf-2* mutants, and lower total quality for *daf-16(mu86)* mutants as compared to wild type. (**e**) Cumulative area under curve of the quality-adjusted MV.

Supplementary Reference

1. Huang C, Xiong CJ, Kornfeld K. Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. *Proc Natl Acad Sci U S A* **101**, 8084-8089 (2004).