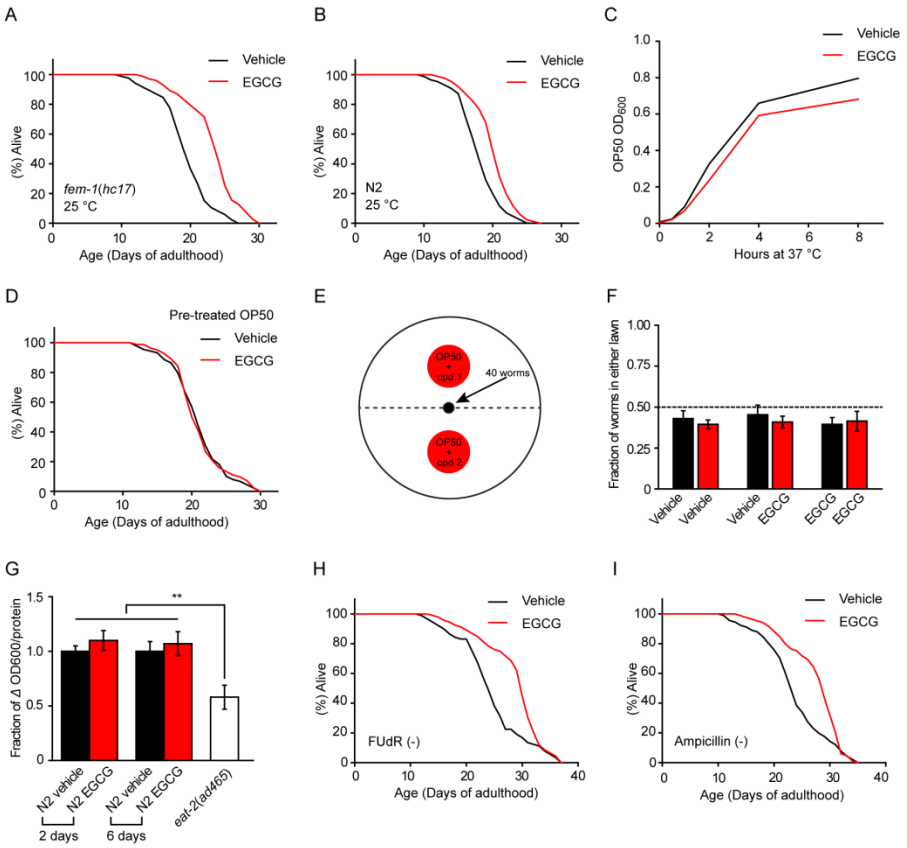
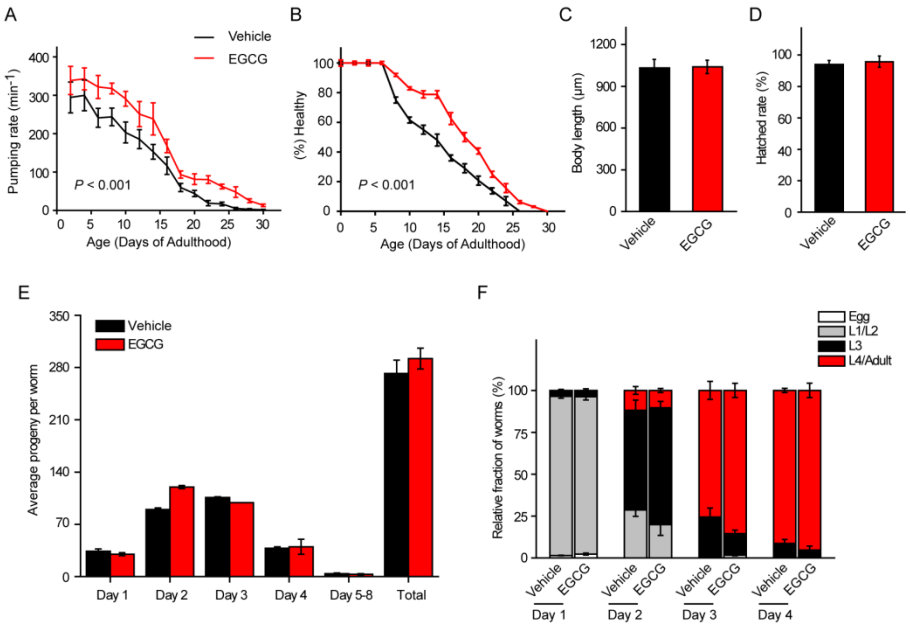
Supplementary information

Supplemental Figures

Figure S1



6 Figure S2



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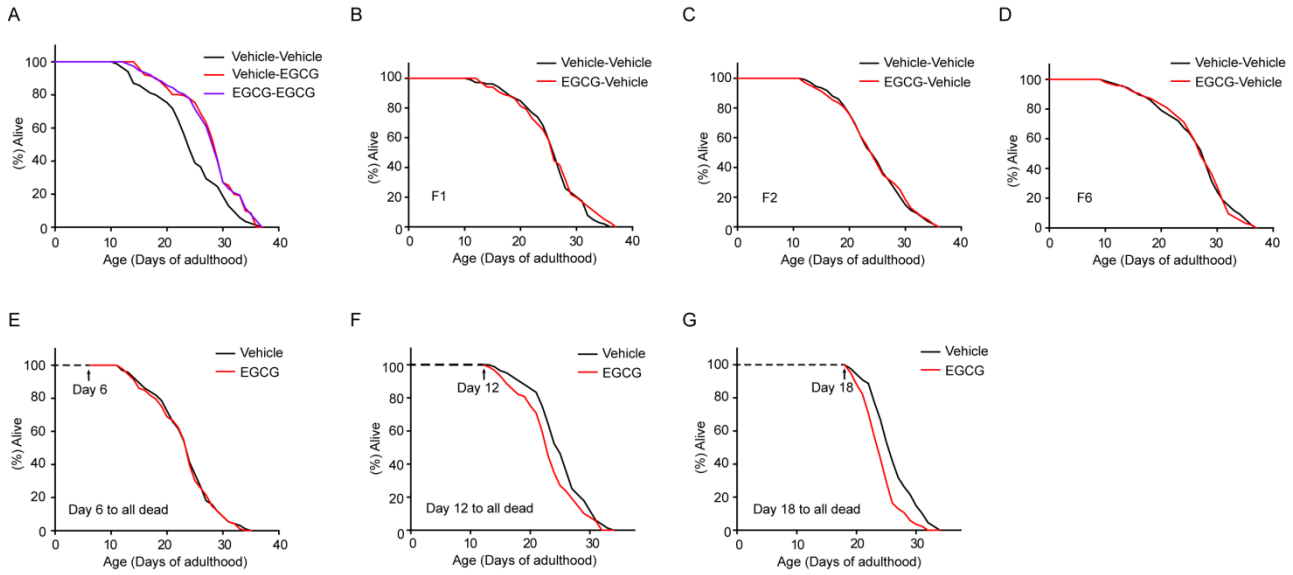
9 Figure S3



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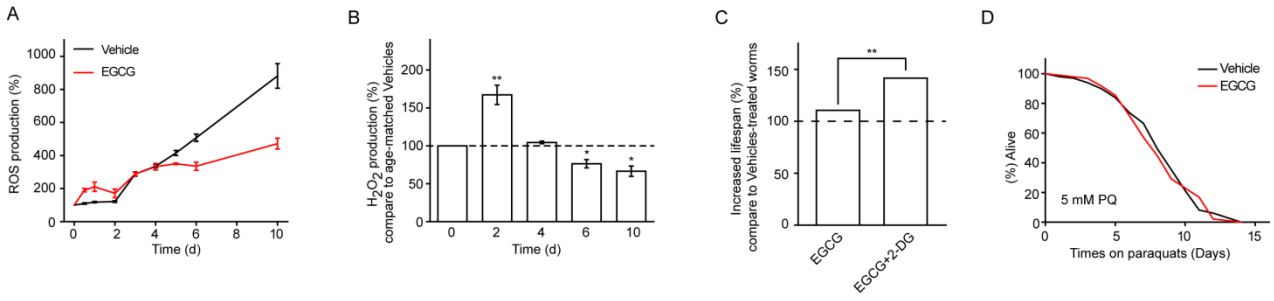
12 Figure S4



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15 Figure S5

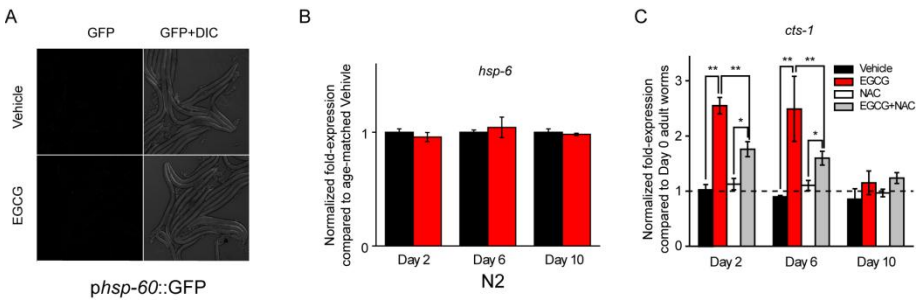

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Figure S6

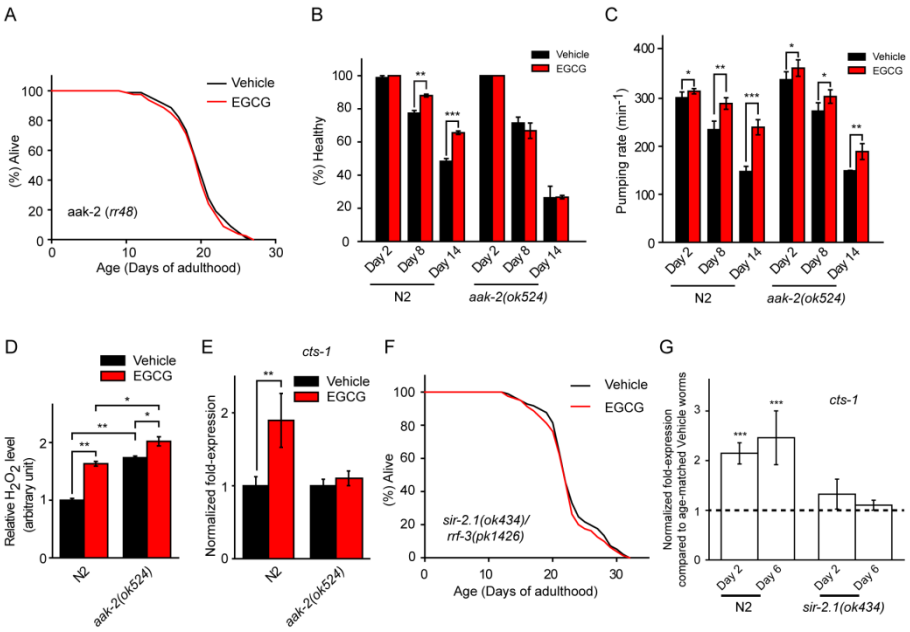
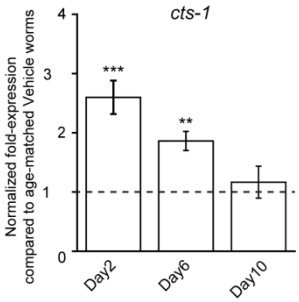

A
(%) Alive
Vehicle
EGCG
aak-2 (rr48)
Age (Days of adulthood)
B
(%) Healthy
Vehicle
EGCG
Day 2
Day 8
Day 14
N2
aak-2(ok524)
C
Pumping rate (min-1)
Vehicle
EGCG
N2
aak-2(ok524)
D
Relative H2O2 level (arbitrary unit)
Vehicle
EGCG
N2
aak-2(ok524)
E
cts-1
Normalized fold-expression
Vehicle
EGCG
N2
aak-2(ok524)
F
(%) Alive
Vehicle
EGCG
sir-2.1(ok434)/ rrf-3(pk1426)
Age (Days of adulthood)
G
cts-1
Normalized fold-expression compared to age-matched Vehicle worms
Day 2
Day 6
N2
sir-2.1(ok434)

Figure S7


cts-1
Normalized fold-expression
compared to age-matched Vehicle worms
4
3
2
1
0
***
**
Day2
Day6
Day10

Supplemental Figure Legends

Figure S1 Supplementation with EGCG extends lifespan directly. (A-B) EGCG extended lifespan of *fem-1(hc17)* (A) and N2 (B) worms at 25 °C. (C) Addition of EGCG slightly slowed the growth of bacterial strains OP50. (D) EGCG-pretreatment of OP50 did not affect lifespan. (E-F) EGCG was not repulsive for the worms. Schematic representation of food preference assay (E); worms did not have any preference between EGCG and vehicle as measured during the bacterial avoidance assay (F). (G) Food uptake after treatment with EGCG (for 2 or 6 days) in N2 worms and in untreated *eat-2(ad465)* mutants (Day 0 adults). (H-I), FUdR and ampicillin did not affect the life extension induced by EGCG.

Bar graphs are expressed as mean ± SEM, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure S2 EGCG improves fitness without side effects in *C.elegans*. (A) Pharyngeal pumping rate on EGCG was significantly altered. (B) Healthspan comparison on EGCG and vehicle plate as assessed 30 days. (C-F) EGCG had no significant impact on body length (C), hatched rate (D), brood size (E) and development (F).

Figure S3 EGCG extends *C.elegans* lifespan independent on the larval stage and progressively left shift elicited this kind of shift at late-onset administration. (A) EGCG treatment (200 μM) beginning at the egg stage and that beginning in adulthood produced identical lifespan increase. (B-D) Treatment with EGCG (200 μM) during the egg and larval stages had no effect on adult lifespan of F1 (B), F2 (C) or F6 (D). (E-G) Lifespan of wild-type N2 nematodes exposed to 400 μM EGCG at Day 6 (E), 12 (F) and 18 (G) adult stages.

Figure S4 EGCG induces a transient ROS at early adulthood. (A) EGCG-treated worms exhibited lower ROS level later in life. (B) EGCG-induced H_2O_2 production increased after 2 days treated worms and decreases after 6 days treated worms. (C) Treatment with 2-DG increases the lifespan of EGCG-treated worms. (D) EGCG did not increase oxidative stress resistance induced by 5 mM PQ after 2 days EGCG treatment.

Bar graphs are expressed as mean ± SEM, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure S5 EGCG induces mitochondrial biogenesis at early stages. (A) EGCG did not induce the mitochondrial unfolded protein response (UPR^{mt}) (*hsp-60* reporter) at Day 2 worms. (B) EGCG did not affect *hsp-6* mRNA levels at Day 2, 6 and/or 10 in the wild type worms. (C) EGCG increased *cts-1*, a mitochondrial biogenesis marker, expression and partially affected by NAC.

Bar graphs are expressed as mean ± SEM, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure S6 AAK-2/AMPK is necessary for EGCG's beneficial effects on lifespan and age-dependent decline. (A) EGCG did not extend lifespan in the *aak-2(rr48)* mutants. (B) EGCG delayed the age-dependent decline in healthspan comparison, as evidenced by the locomotor activity in an *aak-2*-dependent manner. (C) EGCG altered pharyngeal pumping rate in wild type and *aak-2(ok524)* worms. (D) Total hydrogen peroxide level in wild type worms and *aak-2(ok524)* mutants upon EGCG treatment were measured with an Amplex Red assay. (E) EGCG up-regulated *cts-1* expression depended on AAK-2. (F) EGCG did not extend lifespan in

68 the *sir-2.1(ok434), rrf-3(pk1426)* mutants. (G) EGCG up-regulated *cts-1* expression dependent

69 on SIR-2.1.

70

71 Figure S7 EGCG up-regulated *cts-1* mRNA level declined with age.

72 Bar graphs are expressed as mean ± SEM, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table S1 List of primers used for the quantitative real-time reverse transcription-polymerase chain reaction

Gene name	Primer sequence
sod-1	Forward: CCGACACGCTCGTCACGCTT
	Reverse: ACTGGGGAGCAGCGAGAGCA
sod-2	Forward: CCGACACGCTCGTCACGCTT
	Reverse: TCCTTTGGAGACCGCCTCGTGA
sod-3	Forward: CTAAGGATGGTGAACCTTCA
	Reverse: CGCGCTTAATAGTGTCCATCAG
sod-4	Forward: ATGTGGAACTATCGGAATTGTG
	Reverse: GGTTGAGATTGTGTAACTGGA
sod-5	Forward: ATGGAGACTCAACCGATGG
	Reverse: GACCACGGAATCTCTTCCT
ctl-1	Forward: CCAATGCTCATGCAAGATGT
	Reverse: TTGCGTCACGAATGAAGAAG
cl-2	Forward: ACGTCCTTGGAGCATCTTGT
	Reverse: GCAAGATGGTGCTGAACAGA
ctl-3	Forward: CTTCCCCACATGGTCAATCT
	Reverse: TGTCCTGCATTAGCATTGGA
prdx-2	Forward: TTGTGCTCGCCGCTTCCACC
	Reverse: GGGTCGATGATGAAGAGTCCACGG
prdx-3	Forward: GTTCCGTTCTCTTGGAGCTG
	Reverse: CTTGTTGAAATCAGCGAGCA
prdx-6	Forward: GGAGAACAATGGCTGATGC
	Reverse: ATCTGAACATGGCGTTTGC
trx-1	Forward: CGTCAACATCCGGAGAAGAT
	Reverse: AATTGCGTCTCCATTTTGG
trx-2	Forward: CCATCTCCGTCAAACCAACT
	Reverse: TGGCGAGAAGAACACTTCCT
trx-3	Forward: GCAGGAGAGCACTTGAAAGG
	Reverse: TCCAACTGGAATCGCATGTA
daf-16	Forward: TTTCCGTCCCCGAACTCAA
	Reverse: ATTCGCCAACCCATGATGG
hsp-6	Forward: AACCACCGTCAACAACGCCG
	Reverse: AGCGATGATCTTATCTCCAGCGTCC
cts-1	Forward: CTCGACAACTTCCCAGATAACC
	Reverse: GGTACAGGTTGCGATAGATGATAGC

nd-1	Forward: AGCGTCATTTATTGGGAAGAAGAC
	Reverse: AAGCTTGTGCTAATCCCATAAATGT
MTCE.26	Forward: GGTTGTGGGACTAGGTGAACA
	Reverse: CAGGGTGCCCCATTGTTCTT
act-3	Forward: TGCGACATTGATATCCGTAAGG
	Reverse: GGTGGTTCTCCGGAAAGAA
18s	Forward: GCGAAAGCATTTGCCAAGAA
	Reverse: ATCGCGAGATGGCATCGTT
act-1	Forward: GCTGGACGTGATCTTACTGATTACC
	Reverse: GTAGCAGAGCTTCTCCTTGATGTC
ama-1	Forward: CTGACCCAAAGAACACGGTGA
	Reverse: TCCAATTCGATCCGAAGAAGC

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Table S2 EGCG robustly extends lifespan dependent on concentrations, related to Figure 1A, 1B, S1A, S1B, S1D, S1H and S1I.

Regimens	EGCG Concentra-tions (μM)	Mean lifespan(d) ±SEM (*P* Value)	Max lifespan(d) ±SEM (*P* Value)[#]	N (trials/n)
Arrested OP50 (N2/20 °C)	0	25.3 ± 0.6 (n.s.)	35.0 ± 0.7 (n.s.)	111 (1)
	10	25.6 ± 0.5 (n.s.)	35.1 ± 0.4 (n.s.)	108 (1)
	25	25.4 ± 0.6 (n.s.)	35.0 ± 0.3 (n.s.)	110 (1)
	50	26.6 ± 0.5 (n.s.)	36.0 ± 0.7 (n.s.)	106 (1)
	100	28.7 ± 0.5 (<0.001)	35.8 ± 0.4 (n.s.)	111 (1)
	200	29.5 ± 0.5 (<0.001)	36.1 ± 0.6 (n.s.)	108 (1)
	300	27.1 ± 0.5 (<0.01)	36.4 ± 0.6 (n.s.)	107 (1)
	400	25.3 ± 0.7 (n.s.)	34.8 ± 0.9 (n.s.)	107 (1)
	500	25.2 ± 0.4 (n.s.)	34.6 ± 0.1 (n.s.)	108 (1)
	600	24.7 ± 0.8 (n.s.)	34.1 ± 0.2 (n.s.)	112 (1)
	800	23.3 ± 0.5 (<0.001)	31.2 ± 0.6 (<0.001)	106 (1)
	1000	21.1 ± 0.5 (<0.001)	30.4 ± 1.0 (<0.001)	106 (1)
Live OP50 (N2/20 °C)	0	19.5 ± 0.4	26.8 ± 0.2	274 (3)
	200	23.9 ± 0.4 (<0.001)	28.0 ± 0.1 (n.s.)	264 (3)
Arrested OP50 (BA17/25 °C)	0	19.4±0.7	25.1 ± 0.5	165 (2)
	200	23.4±0.5 (<0.001)	26.9 ± 0.9 (n.s.)	182 (2)
Arrested OP50 (N2/25 °C)	0	18.1 ± 0.3	23.9 ± 1.0	198 (2)
	200	20.2 ± 0.3 (<0.001)	25.1 ± 1.5 (n.s.)	187 (2)
EGCG pretreated OP50 (Live) (N2/20 °C)	0	21.0 ± 0.4	27.6 ± 0.6	174 (2)
	200	21.2 ± 0.5(n.s.)	27.3 ± 0.4(n.s.)	182 (2)
Arrested OP50 No Ampicillin (N2/20 °C)	0	23.6 ± 1.6	34.0 ± 0.6	163 (2)
	200	27.9 ± 0.6 (<0.001)	34.6 ± 0.5 (n.s.)	182 (2)
Arrested OP50 No FUdR (N2/20 °C)	0	24.6 ± 0.6	34.5 ± 0.5	169 (2)
	200	28.7±0.6 (<0.001)	34.1 ± 0.7 (n.s.)	142 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S3 - EGCG extends lifespan independent on the larval stage, related to Figure S3A, S3B, S3C and S3D (Arrested OP50/20 °C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	Max lifespan(d) ±SEM (p Value)[#]	N (trials)
The whole adulthood	0	24.0 ± 1.1	35.0 ± 0.9	186 (2)
	200	28.6 ± 1.3 (<0.001)	36.3 ± 1.7(n.s)	175 (2)
From eggs to all dead	0	23.5 ± 0.8	33.8±0.3	160 (2)
	200	27.9±0.3 (<0.001)	34.3±0.7(n.s)	164 (2)
F1 From eggs to L4	0	25.3±0.6	35.1 ± 0.7	201 (2)
	200	25.9±0.5 (n.s.)	35.3 ± 0.6 (n.s.)	178 (2)
F2 From eggs to L4	0	24.3±0.5	35.6 ± 0.6	184 (2)
	200	24.7±0.5 (n.s.)	34.9 ± 0.4(n.s.)	194 (2)
F6 From eggs to L4 for 5 generations	0	25.8±0.6	35.9 ± 0.6	163 (2)
	200	25.3±0.4 (n.s.)	35.5 ± 0.4 (n.s.)	182 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S4 – EGCG induced lifespan extension is timing requirement, related to Figure 1C, 1D, 1E, 1F, 1G, 1H, 1I, S3E, S3F and S3G (Arrested OP50/20 °C)

Regimens (Adulthood)	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	Max lifespan(d) ±SEM (p Value)[#]	N (trials)
The first 6 days	0	23.9 ± 0.5	32.5 ± 0.3	189 (2)
	200	26.2 ± 0.4 (<0.001)	33.3 ± 0.7 (n.s)	178 (2)
From the 6th to 12th days	0	18.3 ± 0.7	25.8 ± 0.5	201 (3)
	200	20.9 ± 0.9 (n.s)	25.9 ± 0.6 (n.s.)	178 (3)
From the 6th days to all dead	0	17.3 ± 0.5	26.4 ± 0.6	164 (2)
	200	19.8 ± 0.5 (<0.01)	27.3 ± 0.4 (n.s.)	174 (2)
	400	17.1 ± 0.6 (n.s.)	26.9 ± 0.2 (n.s.)	168 (2)
From the 12th days to all dead	0	17.3 ± 0.5	20.1 ± 0.6	189 (2)
	200	19.8 ± 0.5 (<0.05)	20.9 ± 0.4(n.s.)	196 (2)
	400	11.2 ± 0.4 (<0.01)	18.6 ± 0.3 (<0.05)	157 (2)
From the 18th days to all dead	0	8.2 ± 0.6	13.8 ± 0.6	209 (3)
	200	7.6 ± 0.4 (n.s.)	12.9 ± 0.4 (n.s.)	212 (3)
	400	6.2 ± 0.3 (<0.001)	12.1 ± 0.1 (<0.01)	231 (3)
The whole adulthood	0	23.1 ± 0.6	34.1 ± 0.6	161 (2)
	200	27.0 ± 0.5 (<0.001)	35.0 ± 1.0 (n.s.)	166 (2)
The first 15 days of adulthood	200	27.1 ± 0.6 (<0.001)	35.4 ± 0.6 (n.s.)	177 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S5 – The antioxidant abolishes the lifespan-extending effects of EGCG, related to Figure 2B (Arrested OP50/20 °C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	Max lifespan(d) ±SEM (p Value)[#]	N (trials)
	0	24.8±0.6	33.9±0.4	82 (1)
	200	28.5±0.5 (<0.001)	34.0±0.3 (n.s)	86 (1)
NAC (5 mM)	0	24.6±0.6	34.3 ± 0.7	186 (2)
	200	24.9±0.5 (n.s)	33.8 ± 0.6 (n.s.)	178 (2)
BHA (25 μM)	0	24.7±0.7	34.6 ± 0.7	174 (2)
	200	24.7±0.8 (n.s.)	34.8 ± 0.4 (n.s.)	164 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S6 – Paraquats and 2-deoxy-D-glucose promotes the lifespan-extending effects with EGCG synergistically, related to Figure 2C and S4C (Arrested OP50/20 °C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	Max lifespan(d) ±SEM (p Value)[#]	N (trials)
	0	24.8 ± 0.5	34.6±0.4	105 (1)
	200	27.4 ± 0.5 (<0.001)	36.0±0.7 (n.s)	95 (1)
Paraquats (200 μM)	0	30.9 ± 0.5 (<0.001)	39.3 ± 0.9 (<0.001)	105 (1)
	200	34.7 ± 0.6 (<0.001)	42.6 ± 1.5 (<0.001)	90 (1)
2-deoxy-D-glucose(5 mM)	0	31.3 ± 0.2 (<0.001)	38.5 ± 0.5 (<0.001)	115 (1)
	200	35.1 ± 1.1 (<0.001)	43.1 ± 1.0 (<0.001)	98 (1)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S7 – The antioxidant abolished the oxidative stress resistance effects of EGCG, related to Figure S4D and 2D (Arrested OP50/20 ^{o}C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	N (trials)
EGCG for 2 d	0	8.3 ± 0.9	248 (3)
	200	8.2 ± 1.1 (n.s.)	264 (3)
EGCG for 6 d	0	6.8 ± 0.6	306 (4)
	200	8.3 ± 0.9 (< 0.001)	291 (4)
EGCG & NAC (5 mM) for 6 d	0	6.3 ± 0.7	154 (2)
	200	6.8 ± 0.8 (n.s.)	154 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S8 - EGCG does not change or shortens lifespan in the ETC mutants, related to Figure 3G, 3H, 3I, 3J and 3K (Arrested OP50/20 °C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	Max lifespan(d) ±SEM (p Value)[#]	N (trials)
CW152	0	18.0 ± 0.5	24.4 ± 0.3	81 (1)
	200	15.6 ± 0.4 (<0.05)	22.1 ± 0.4 (<0.01)	76 (1)
MQ1333	0	33.7 ± 0.7	44.8 ± 0.9	88 (1)
	200	33.5 ± 0.5 (n.s.)	46.1 ± 1.1 (n.s.)	79 (1)
TK22	0	18.4 ± 0.4	26.2 ± 0.5	88 (1)
	200	15.1 ± 0.5 (<0.001)	23.1 ± 0.1(<0.001)	98 (1)
MQ887	0	35.0 ± 0.8	47.3 ± 0.5	86 (1)
	200	34.4 ± 0.8 (n.s.)	47.0 ± 0.4 (n.s.)	93 (1)
MQ130	0	29.9 ± 0.6	39.9 ± 0.5	84 (1)
	200	27.3 ± 0.7 (<0.001)	36.7 ± 0.7 (<0.01)	74 (1)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S9 – EGCG does not increase healthspan of *aak-2*- or *sir-2.1*-deficient mutants, related to Figure 4A, 4D 4G 4I, S6A and S6F(Arrested OP50/20 °C)

Regimens /Strains	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (*p* Value)	Max lifespan(d) ±SEM (*p* Value)[#]	N (trials)
Lifespan /RB754	0	19.0 ± 1.8	25.4 ± 1.4	242 (3)
	200	19.1 ± 2.5 (n.s.)	26.1 ± 1.3 (n.s)	226 (3)
Lifespan /MR507	0	20.0 ± 0.3	25.8 ± 0.7	79 (1)
	200	19.6 ± 0.4 (n.s.)	25.1 ± 0.6 (n.s.)	79 (1)
Lifespan /VC199	0	22.0 ± 0.5	29.1 ± 0.5	175 (2)
	200	22.5 ± 0.5 (n.s.)	29.3 ± 0.2 (n.s.)	173 (2)
Lifespan /IU7	0	22.9 ± 0.4	28.8 ± 0.3	97 (1)
	200	22.3 ± 0.4 (n.s.)	28.5 ± 0.1 (n.s.)	80 (1)
Lifespan /MIR13	0	17.6 ± 0.4	22.4 ± 0.4	74 (1)
	200	17.2 ± 0.3 (n.s.)	22.1 ± 0.6 (n.s.)	70 (1)
Exposure to 5 mM paraquats after 6 d EGGC treatment/RB754	0	3.8±0.4	6.1 ± 0.7	201 (2)
	200	3.7±0.2 (n.s.)	5.4 ± 0.4 (n.s.)	209 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S10 – EGCG increases lifespan dependent on *daf-16*, independent of *daf-2* or *age-1* related to Figure 5A, 5B, 5C and 5G (Arrested OP50/20 °C)

Regimens /Strains	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (*p* Value)	Max lifespan(d) ±SEM (*p* Value)[#]	N (trials)
CF1038	0	18.2 ± 0.4	24.9 ± 0.1	93 (1)
	200	18.4 ± 0.4 (n.s.)	25.3 ± 0.4 (n.s)	87 (1)
CB1370	0	47.3 ± 1.0	64.9 ± 0.6	84 (1)
	200	51.6 ± 1.0 (<0.001)	65.3 ± 0.6 (n.s.)	101 (1)
TJ1052	0	32.5 ± 0.8	42.9 ± 0.6	93 (1)
	200	35.4 ± 0.8 (<0.001)	43.3 ± 0.6 (n.s.)	86 (1)
CF1558	0	19.1 ± 0.3	23.2 ± 0.1	81 (1)
	200	19.6 ± 0.3 (n.s.)	23.3 ± 0.4 (n.s.)	76 (1)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Table S11 – The oxidative stress resistance is weaker at older worms after EGCG treatment for 6 days, related to Figure 6C (Arrested OP50/20 ^{o}C)

Regimens	EGCG Concentrations (μM)	Mean lifespan(d) ±SEM (p Value)	N (trials)
Day 0-Day 6	0	6.7 ± 0.2	161 (2)
	200	8.4 ± 0.3 (< 0.001)	143 (2)
Day 6-Day 12	0	4.6 ± 0.2	147 (2)
	200	5.3 ± 0.3 (< 0.05)	135 (2)

n.s.= not significant

N= total worm number

P value compared to the Vehicle (0) group

Supplemental Experimental Procedures

Bacterial growth assays

Liquid bacterial growth was performed as described previously [1]. Overnight *E.coli* was diluted (1:100) in LB (with and without EGCG) at pH = 7.0 over an 8-h period with shaking at 37 °C. Cell density was monitored at different time intervals by measuring OD600.

Measurement of pharyngeal pumping rates and locomotion

Pumping of nematodes was determined in a 1-min period [2]. Eight to ten worms in each condition were scored, beginning at Day 2 of the experiment and continued every other day until day 30.

A further index of age-associated decline in physiological capacity, based on measurement of the progressive functional decline of locomotion was used [3]. Briefly, worms (> 200) were observed for locomotor activity and subdivided into three groups: class A worms were healthy, showed spontaneous movements, and highly mobile; class B worms showed movement only after prodding; and class C worms movement was restricted to the head or tail upon prodding with a platinum wire. Only A type worms were counted as healthy.

Food preference assay

Protocol adapted as described previously [4]. A 100 mm NGM plate was seed with two spots of OP50. After letting the OP50 lawns dry over 2 days at room temperature, Vehicle (H_2O/0 µM EGCG) or EGCG (200 µM) was added to the top of the lawn and allowed to dry. Approximately 50-100 synchronized prefertile young adult worms were placed onto the center of the plate and their preference for either bacterial lawn was record after 3 h at room temperature.

Food uptake quantification

To analyze the total amount of incorporated food, worms were pretreated with EGCG as described previously with little modification [5]. Next, worms were transferred to plates spotted with a defined volume of heat killed OP50. Worms were allowed to consume OP50 for 6 h. afterwards, the remaining OP50 and worms were thoroughly removed and transferred in a reaction tube. Worms were spun down at low speed and an aliquot of the supernatant was removed for a subsequent optical density (OD600) determination. An empty reference plate was equally handled.

Body length measurement

Body length was measured as described previously [6]. L1 worms were cultured in the presence of EGCG for 72 h. At the end of the exposure period, eight to ten worms per condition were heated to approximately 50 °C and the length of the body was measured using the distance measurement of the software in an invert fluorescent microscope (Olympus X71).

Reproduction assay

The assay was conducted as described previously [2]. To obtain synchronised worms for reproduction assays, eight to ten gravid worms were grown on NGM plates with or without EGCG, allowing them to lay eggs for one hour. To determine the average brood size per worm, ten pretreated L4 worms were transferred to NGM without EGCG and moved to a fresh plate each day until reproduction ceased. The offspring of each worm was scored at the L2 or L3 stage.

To determine fertility of worms [7], eight to ten worms at the egg-laying period were placed on NGM plates per condition and were removed after an incubation period of 4 hr. The progeny were allowed to develop for 48 h, and infertile eggs and hatched worms were counted.

Larval development in the presence of EGCG

Larval development was assayed as described previously [8]. The eggs per plate were obtained by incubating ten egg-laying (gravid) worms. Adult worms were discarded from plates (after 2 h), and the eggs were used for experiments as subsequently described. The eggs were incubated with EGCG, and development from eggs to adult worms was monitored on NGM plates. The developmental stage of each worm was recorded daily for 4 days.

Transgenerational impacts assay

This assay was performed by the method as described previously with some modification [9]. Eggs were transferred to an NGM plate with EGCG, which were designated as the first generation (F1). When F1 reached young adult stage, several F1 worms of each group were picked and transferred to fresh EGCG treatment plates correspondingly, allowed to lay eggs (F2) for some hours and then picked off the plates. Subsequently, F2 worms were treated following this method until F5. We used synchronous F2 (only treat with EGCG for F1) and F6 worms for the lifespan assays. F2 and F6 worms were incubated on the NGM without EGCG from the prefertile young adult stage until the end of the assays.

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