Supplementary material

Materials and Methods

qRT-PCR

Total RNA of flies, worms, mice tissue homogenate and cell lines was extracted using Trizol reagent (Ambion, USA). cDNA was synthesized with PrimeScriptRTase (TaKaRa, Japan). qRT-PCR was performed with SYBR Green Mix (TaKaRa, Japan) in Roche LightCycler480 (Roche, Mannheim, Germany) PCR instrument. The $2^{-\Delta\Delta CT}$ method was used to determine relative mRNA expression. The primer sequences used are listed below.

Primer	Sequences (5' to 3')
Humans	
universal Eubacteria 16s F (internal reference)	CGGCAACGAGCGCAACCC
universal Eubacteria 16s R (internal reference)	CCATTGTAGCACGTGTGTAGCC
B. adolescentis F	CTCCGCCGCTGATCCGGAAGTCG
B. adolescentis R	AACCAACTCGGCGATGTGGACGACA
Caenorhabditis elegans	
act-3 F (internal reference)	TCGGTATGGGACAGAAGGAC
act-3 R (internal reference)	CATCCCAGTTGGTGACGATA
sod-3 F	GCTGCAATCTACTGCTCGCACTG
sod-3 R	GGCTGATTACAGGTTCCAAATCTGC
ctl-2 F	TCCCAGATGGGTACCGTCAT
ctl-2 R	AAGTTGACCGGCCTTCTCC
daf-16 F	ATCATCTTTCCGTCCCCG
daf-16 R	TTGGAATTGCTGGAACCG

atg-18 F	AAGTTGGGGAGCTGATGACG
atg-18 R	CCGTCTGATGTAGCAGCCAT
aak-2 F	AGAGGTCACTGAGCGCTATC
aak-2 R	GTCCAGGTCCCTTATTCGGC
hsp-12.6 F	TGGAGTTGTCAATGTCCTCG
hsp-12.6 R	GACTTCAATCTCTTTTGGGAGG
lgg-1 F	ACGCATCCAACTTCGTCCA
lgg-1 R	GACCTCTCCTCCATACACACTT
phm-2 F	CCATCTCGTCCAGAGTTGATAC
phm-2 R	GAGCTCCGAAGTGCTAATGT
eat-2 F	AACGATGAAGAAGCTGGGGG
eat-2 R	TAAGTGCTGTGGTGCCGAAT
rsks-1 F	CAAATTTCTGCTCCACCGCC
rsks-1 R	GATTTGGCCATTGTCGGAGC
Drosophila melanogaster	
rp49 F (internal reference)	CACCGGATTCAAGAACTTCC
rp49 R (internal reference)	GACAATCTCCTTGCGCTTCT
sod-3 F	AATTGCAGCTCGGATGTGGA
sod-3 R	GCACGTACAAATTGCAAGCAC
cat F	CAACCCCTTCGATGTCACCA
cat R	TCTGCTCCACCTCAGCAAAG
Mouse/Mouse embryonic fibroblasts	
actin F (internal reference)	TCCGCTCTCTGTCAAAGTGTG
actin R (internal reference)	GGAGAGCATAGCCCTCGTAGAT
catalase-F	AGCGACCAGATGAAGCAGTG
catalase-R	TCCGCTCTCTGTCAAAGTGTG

Western blot

Total proteins were extracted from mice skeletal muscle and brain tissue homogenate or MEFs using RIPA lysis buffer and quantified using BCA Protein Assay Kit (Beyotime, China). Protein was separated by SDS-PAGE and electrotransferred onto a PVDF membrane. The membranes were blocked with 5% fat-free milk for 2 h at room temperature and then incubated with primary antibody catalase (catalog No. 21260-1-AP, Proteintech) at 4℃ overnight. Membranes were washed and incubated with anti-rabbit IgG antibody at room temperature for 2 h and the signal was detected using an ECL kit (Fdbio science, China). β-actin or GAPDH was used as a reference gene.

Cell culture and senescence

Telomerase RNA component deletion intercrossed mice ($Terc^{+/-}$) were used to derive MEFs as previously described (*5*). Cells were cultured in 37 °C, 5% CO_2 incubator. For replicative senescence, passage culture of MEFs with or without *B. adolescentis* (bacteria:cell,100:1) are maintained until passage 12 (p12) according to senescence-related morphologic alterations. As for induction senescence (*6, 7*), 40nM doxorubicin (SIGMA-ALDRICH, Germany) was added to culture medium for 3 days and then replaced with fresh complete culture medium for 3 days. The entire cycle lasted 12 days prior to co-cultured with PBS or *B. adolescentis* for 6 days. Senescence-associated β-galactosidase (SA-β-gal) staining assay was performed following the manufacturer's instructions.

Hematoxylin-Eosin staining and immunohistochemistry

Brain tissues were fixed in 4% paraformaldehyde at room temperature and HE staining was conducted to assess morphological changes. CA3 region of hippocampus was magnified and the number of surviving neurons were counted.

The paraffin embedded brain tissues were deparaffinized and rehydrated, followed by antigen retrieval. After blocking endogenous peroxidase activity, tissues were sealed with 3% BSA and incubated with primary and secondary antibody. Finally, the slices was stained with DAB, dehydrated and sealed with neutral gum. The images were scanned with Pannoramic Scanner (Pannoramic DESK, 3D HISTECH，Hungary) and observed with Caseviewer C.V 2.3.

***B. adolescentis* and *E. coli* OP50 culture and preparation**

Bifidobacterium adolescentis bio-67127（ATCC 15703）was obtained from Biobw Biotechnology Co., Ltd (Beijing, China) and cultured in modified reinforced clostridial medium (ATCC Medium 2107) with anaerobic gas mixture (80% N_2, 10% CO_2 and 10% H_2) at 37°C. *E. coli* OP50 was cultured in Luria-Bertani (LB) liquid medium at 37°C with shaking overnight.

For mice, *B. adolescentis* was heat-inactivated in 95°C water bath for 15 min and adjusted to a final concentration of 10^9 CFU/mL. Each mouse was gavaged with 200 μL sterile PBS or pretreated *B. adolescentis* every other day. For cell assay, MEFs were co-cultured with heat-inactivated *B. adolescentis* slurry in the ratio of

1:100 during subculture process. For *D. melanogaster*, *B. adolescentis* deposit was resuspended with 2.5% sucrose solution to the concentration of 10^{10} CFU/mL and then heat-inactivated. For intervention group, *B. adolescentis* slurry was mixed with standard cornmeal food to a final concentration of 10^9 CFU/mL. For control group, the same volume 2.5% sucrose solution was mixed with standard cornmeal food. For *C. elegans*, liquid *B. adolescentis* and OP50 cultures were washed, centrifuged and the deposits were adjusted to a final concentration of 0.04 mg/mL (wet weight) in M9 buffer and heat-inactivated. Worms were fed on OP50 alone or OP50 and *B. adolescentis* mixture.

D. melanogaster stocks and husbandry

Two wild type fly stocks: W^{1118} and Canton-S were reared on standard cornmeal medium (Nutri-Fly Bloomington formulation: 3.72g agar, 35.28g cornmeal, 35.28g inactivated dried yeast, 16 mL of a 10% solution of methyl-paraben in 85% ethanol, 36 mL fruit juice, 2.9 mL 99% propionic acid for 600 mL) at 25°C with a 12/12h light/dark cycle. *B. adolescentis* was supplemented at every food change.

D. melanogaster climbing assay

Assessment of climbing ability was performed similarly to Guo et al. (*1*) with minor modifications. Briefly, three vials of 30-day-old flies (approximately 15~20 flies per vial) were transferred to a tube vertically joined by two empty plastic vials,

respectively. After gently tapped down to the bottom, the number of flies that climbed above the 8-cm mark within 10 seconds were measured to calculate the climbing index (versus the total number of flies in the tube). Each experiment was performed with 3 replicates.

C. elegans strains and synchronization

N2 Bristol strain (*Caenorhabditis* Genetics Center, CGC) was used as wild type *C. elegans*. Mutants *sod-3* (*tm760*) and *ctl-2* (*ok1137*) were provided by the CGC. Worms were maintained at 20°C on nematode growth medium (NGM) plates seeded with OP50 strain of *Escherichia coli* before intervention. From adult day 2 (D2)，worms were maintained on peptone-free modified NGM (mNGM) plates (*2*) seeded with different heat-inactivated bacteria. To isolate eggs，gravid adult worms were bleached in NaClO solution. Then the eggs were cultured at NGM plates to obtain a synchronized population of L4 worms.

C. elegans locomotion assay

1200 adult worms synchronized as described in online supplementary methods were evenly picked to 20 control plates and 20 intervention plates. Then they were cultured at 20 °C and transferred to new plates every other day. Locomotion ability of worms can be measured by two indicators: movement speed and movement trajectory. On day 2, 8 and 16, 50 nematodes were randomly selected to measure the number of sine

waves(≥1 mm) that they crawled out in 30s for each group. The movement trajectory assay of worms at different time-points was conducted using a scoring method described in a previous report (*3, 4*). Experiments were conducted at least three times independently.

C. elegans heat stress resistance assay

On day 4, 8 and 12, worms in each group as aforementioned were transferred from 20°C to 37°C and assayed for their response every two hours. Dead worms were counted, and the mean survival time was then calculated. Experiments were conducted three times independently.

CAT activity measurement

Skeletal muscle and brain tissues were harvested and homogenized with iced PBS (25% w/v). The supernatant was used to measured CAT activity with commercial kit according to the manufacturer's instructions (Jiancheng, Nanjing).

UHPLC-QE-MS

Metabolites extraction

25 mg of sample was weighted to an EP tube, and 500 μL extract solution (acetonitrile: methanol: water = 2: 2: 1, with isotopically-labelled internal standard mixture) was added. After 30 s vortex, the samples were homogenized at 35 Hz for 4

min and sonicated for 5 min in ice-water bath. The homogenization and sonication cycle was repeated for 3 times. Then the samples were incubated for 1 h at -40°C and centrifuged at 12000 rpm for 15 min at 4°C. The resulting supernatant was transferred to a fresh glass vial for analysis. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatants from all of the samples.

LC-MS/MS analysis

LC-MS/MS analyses were performed using an UHPLC system (Vanquish, Thermo Fisher Scientific) with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) coupled to Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 ammonia hydroxide in water (pH = 9.75) (A) and acetonitrile (B). The analysis was carried with elution gradient as follows: 0~0.5 min, 95%B; 0.5~7.0 min, 95%~65% B; 7.0~8.0 min, 65%~40% B; 8.0~9.0 min, 40% B; 9.0~9.1 min, 40%~95% B; 9.1~12.0 min, 95% B. The column temperature was 30°C. The auto-sampler temperature was 4°C, and the injection volume was 2 μL.

The QE HFX mass spectrometer was used for its ability to acquire MS/MS spectra on information-dependent acquisition (IDA) mode in the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software continuously evaluates the full scan MS spectrum. The ESI source conditions were set as following: sheath gas flow rate as 50 Arb, Aux gas flow rate as 10 Arb, capillary temperature 320°C, full MS resolution as 60000, MS/MS resolution as 7500, collision energy as

10/30/60 in NCE mode, spray Voltage as 3.5 kV (positive) or -3.2 kV (negative), respectively.

Data preprocessing and annotation

The raw data were converted to the mzXML format using ProteoWizard and processed with an in-house program, which was developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. Then an in-house MS2 database (BiotreeDB) was applied in metabolite annotation. The cutoff for annotation was set at 0.3.

References

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Supplementary Table 1 Baseline characteristics of study participates

Characteristics	**20-40 yrs (n=46)**	**40-60 yrs (n=77)**	**60+ yrs (n=43)**	**P-value**
Age(years)[a]	29.52 ± 4.58	49.35 ± 5.57	65.91 ± 5.20	<0.001
BMI[a]	21.84 ± 3.10	23.01 ± 2.36	22.80 ± 2.81	0.063
Gender				0.095
Male[b]	19 (41.3%)	46 (59.7%)	26 (60.5%)	
Female[b]	27 (58.7%)	31 (40.3%)	17 (39.5%)	

BMI, body mass index.

a Data are presented as mean ± standard deviation (SD).

b Data are presented as n (%).

Supplementary Table 2 Bacteria species with significant different abundance in two age groups

Species name	Group	Mean	LDA_value	P-value	-log10(P-value)
s__Bifidobacterium_adolescentis	20-40 yrs	3.0446355	2.671129472	0.00085369	3.068699785
s__Ruminococcus_lactaris_CC59_002D	20-40 yrs	3.172895537	2.61724204	0.001072556	2.969580223
s___Clostridium__scindens	20-40 yrs	2.216776533	2.010199039	0.001345179	2.871219962
s__Anaerostipes_hadrus_g__Anaerostipes	20-40 yrs	3.358276655	2.869865052	0.00548915	2.260494873
s__Sutterellaceae_bacterium_YL45	20-40 yrs	2.243045036	1.904929128	0.009347217	2.029317673
s__Odoribacter_splanchnicus_DSM_20712	20-40 yrs	3.486786784	2.676308673	0.010302087	1.98707478
s__Bacillus_litoralis_g__Bacillus	40+ yrs	1.528816393	1.541356879	0.010584609	1.975325199
s__Streptococcus_salivarius_subsp__salivarius	20-40 yrs	3.716383502	3.179962646	0.011039753	1.957040642
s__Sporosarcina_globispora_g__Sporosarcina	40+ yrs	1.114084366	1.631389744	0.01867647	1.728705201
s__Faecalibacterium_prausnitzii_g__Alistipes	20-40 yrs	2.648454781	2.060953239	0.019540852	1.709056505
s__Desulfovibrio_legallii	20-40 yrs	1.878034249	1.630443484	0.024698262	1.607333599
s__Lactococcus_raffinolactis	20-40 yrs	0.628050502	2.393339787	0.030727524	1.512472428
s__Clostridiales_bacterium_10_3b	20-40 yrs	0.605430199	2.786545889	0.030727524	1.512472428
s__gut_metagenome_g__norank_f__Ruminococcaceae	20-40 yrs	3.454330982	2.522238798	0.033792072	1.471185173
s__Butyricimonas_synergistica	20-40 yrs	1.683921766	1.600661619	0.038071926	1.419395157
s__Ruminococcaceae_bacterium_GD6	20-40 yrs	1.668610887	1.533225097	0.041676262	1.380111243
s__Faecalitalea_cylindroides	20-40 yrs	0.762015992	2.130551051	0.041910532	1.377676828

s__Ruminococcus_bicirculans	20-40 yrs	3.456784215	2.743933257	0.04193292	1.377444899
s__Ruminococcus_sp__16442	20-40 yrs	2.046634834	1.567285539	0.04232817	1.373370503
s__Paenibacillus_castaneae	40+ yrs	0.629221281	1.940092166	0.043578596	1.360726768
s__Peptoniphilus_sp__BV3C26	20-40 yrs	0.637049309	2.332493876	0.046376721	1.333699961
s__intestinal_bacterium_CG19_1	20-40 yrs	0.296939101	2.327615817	0.047584161	1.322537581
s__Prevotella_colorans	20-40 yrs	0.668697915	2.005607282	0.049103783	1.308885045

Supplementary Table 3 Increase of lifespan in wile-type fly strains

Fly Strain	Median Lifespan (days)	% Increase in Median Lifespan	Mean lifespan (days)	% Increase in Mean Lifespan	Mean Maximum Lifespan (days)	% Increase in Mean Maximum Lifespan
w^{1118} ♂	46.00 vs 38.00	21.05%	45.00 vs 35.78	25.77%	64.20 vs 58.20	10.31%
w^{1118} ♀	60.00 vs 49.00	22.45%	56.53 vs 45.67	23.78%	68.00 vs 62.40	8.97%
Canton S ♂	46.00 vs 37.00	24.32%	44.88 vs 37.99	18.14%	60.70 vs 59.30	3.36%
Canton S ♀	42.00 vs 34.00	23.53%	40.02 vs 33.97	17.81%	57.00 vs 55.20	3.26%