

Online Supplement for:
**Longevity-Associated Core Gut Microbiota
Mining and Effect of Mediated Probiotic
Combinations on Aging Mice: Case Study of a
Long-Lived Population in Guangxi, China**

Rui-Ding Li, Wen-Xuan Zheng, Qin-Ren Zhang, Yao Song, Yan-Ting Liao,
Feng-Cui Shi, Xiao-Hui Wei, Fan Zhou, Xiao-Hua Zheng, Kai-Yan Tan and

Quan-Yang Li

Supplement Contents:

Table S1. Details of subjects in each of the categorical groups analyzed in this study.

Table S2. Primers and conditions.

Table S3. PCR reaction system for strain identification.

Table S4. PCR reaction conditions for strain identification.

Table S5. Top 10 in the mediumpurple3 network ranked by MNC method.

Table S6. Basic characteristics of the strains.

Figure S1. Diagram showing experimental design and animal groupings for mice.

Figure S2. Structural characteristics of the microbiota.

Figure S3. The selection process of a soft threshold.

Figure S4. Size of each module.

Figure S5. Functional prediction results.

Tables

Table S1. Details of subjects in each of the categorical groups analyzed in this study.

Sample ID	Essential information				Personal habits				Geographical position				
	Group	Age	Gender	BMI (kg/m ²)	Probiotic treatment (within the first 6 months)	Antibiotics and related medications (within the first 6 months)	Diseases	Staple Food Type	Smoking	Alcohol intake	Province	Urban/Rural	Area
YS1	YG	60	Female	22.27	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS10	YG	57	Male	25.10	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
YS11	YG	56	Male	23.38	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS12	YG	56	Male	20.43	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS13	YG	55	Male	21.50	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS14	YG	56	Male	23.06	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS15	YG	55	Male	21.94	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS16	YG	55	Female	22.63	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS17	YG	55	Female	24.48	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Areawith
YS18	YG	55	Male	21.81	Never	Never	None	Rice	Never	Never	GuangXi	Rural	high
YS19	YG	62	Male	22.06	Never	Never	None	Rice	Never	Never	GuangXi	Rural	longevity
YS2	YG	60	Male	21.12	Never	Never	None	Rice	Never	Never	GuangXi	Rural	in
YS20	YG	69	Male	23.17	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Guangxi
YS21	YG	66	Male	21.03	Never	Never	None	Rice	Never	Never	GuangXi	Rural	(LA)
YS22	YG	66	Male	20.28	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS23	YG	65	Male	24.47	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS24	YG	64	Female	23.50	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS25	YG	63	Male	20.88	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
YS26	YG	62	Female	21.71	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS27	YG	61	Female	23.74	Never	Never	Hypertension	Rice	Never	Never	GuangXi	Rural	
YS28	YG	61	Male	20.97	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	

YS29	YG	60	Male	23.83	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
YS3	YG	60	Male	24.83	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
YS30	YG	60	Male	23.84	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
YS31	YG	60	Male	24.47	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS32	YG	58	Female	22.48	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS33	YG	56	Female	22.24	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS34	YG	56	Female	25.38	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS35	YG	56	Female	22.30	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS36	YG	55	Male	25.17	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS4	YG	59	Male	24.45	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS5	YG	58	Female	23.71	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS6	YG	57	Male	24.79	Never	Never	Hypertension	Rice	Never	Never	GuangXi	Rural	
YS7	YG	55	Male	20.51	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Areawith high longevity in Guangxi (LA)
YS8	YG	57	Female	23.91	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
YS9	YG	57	Female	22.82	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE1	LG	116	Female	17.03	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
CE10	LG	102	Female	20.35	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE11	LG	102	Female	19.61	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE12	LG	101	Female	20.62	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE13	LG	101	Female	17.03	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE14	LG	101	Female	18.32	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE15	LG	101	Female	19.32	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE16	LG	118	Female	20.03	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE17	LG	111	Female	19.69	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE18	LG	108	Female	20.47	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE37	LG	103	Male	17.57	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
CE19	LG	108	Female	19.51	Never	Never	None	Rice	Never	Never	GuangXi	Rural	

CE2	LG	109	Female	19.58	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
CE20	LG	107	Female	18.14	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
CE21	LG	107	Female	17.17	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
CE22	LG	106	Female	17.87	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE23	LG	104	Female	21.20	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural	
CE24	LG	104	Female	20.41	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE25	LG	103	Female	20.31	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE26	LG	102	Female	18.80	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE27	LG	102	Female	17.20	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE28	LG	102	Female	19.57	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Areawith high longevity in Guangxi (LA)
CE29	LG	102	Female	20.96	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE3	LG	108	Female	18.69	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural	
CE30	LG	102	Female	20.75	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE31	LG	101	Female	18.06	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE32	LG	101	Female	17.60	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE33	LG	108	Female	19.42	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural	
CE34	LG	108	Female	17.87	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE35	LG	105	Female	19.47	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE36	LG	104	Male	19.95	Never	Never	None	Rice	Never	Ever	GuangXi	Rural	
CE38	LG	103	Female	18.74	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE39	LG	101	Female	17.60	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE4	LG	105	Female	19.56	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
CE5	LG	105	Female	21.03	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural	
CE6	LG	105	Female	21.05	Never	Never	None	Rice	Never	Never	GuangXi	Rural	

CE7	LG	104	Female	19.30	Never	Never	None	Rice	Never	Never	GuangXi	Rural
CE8	LG	102	Female	21.02	Never	Never	None	Rice	Never	Never	GuangXi	Rural
CE9	LG	102	Female	20.78	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE1	YG	86	Male	23.41	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE10	YG	73	Male	23.84	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE11	YG	73	Female	24.69	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE12	YG	73	Male	21.59	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural
GE13	YG	72	Male	23.03	Never	Never	None	Rice	Ever	Never	GuangXi	Rural
GE14	YG	71	Female	21.65	Never	Never	None	Rice	Never	Ever	GuangXi	Rural
GE15	YG	71	Male	24.50	Never	Never	None	Rice	Never	Ever	GuangXi	Rural
GE16	YG	71	Male	24.45	Never	Never	None	Rice& Wheat	Never	Never	GuangXi	Rural
GE17	YG	80	Female	24.81	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE18	YG	76	Female	23.92	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE19	YG	72	Female	24.87	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE2	YG	86	Male	23.60	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE20	YG	86	Female	22.57	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE21	YG	85	Female	20.95	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural
GE3	YG	85	Female	20.73	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural
GE4	YG	84	Male	21.02	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE5	YG	80	Male	22.99	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE6	YG	79	Female	20.01	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE7	YG	76	Female	18.78	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE8	YG	75	Female	22.03	Never	Never	None	Rice	Never	Never	GuangXi	Rural
GE9	YG	73	Male	19.61	Never	Never	None	Rice	Never	Never	GuangXi	Rural
OS1	LG	98	Female	21.38	Never	Never	None	Rice	Never	Never	GuangXi	Rural
OS10	LG	92	Female	17.23	Never	Never	None	Rice	Never	Never	GuangXi	Rural

Areawith
high
longevity
in
Guangxi
(LA)

OS11	LG	91	Male	19.14	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS12	LG	91	Male	16.24	Never	Never	None	Rice&Wheat	Never	Never	GuangXi	Rural	
OS13	LG	100	Female	18.33	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Rural	
OS14	LG	100	Male	20.60	Never	Never	None	Rice&Wheat	Never	Never	GuangXi	Rural	
OS15	LG	100	Male	20.69	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS16	LG	98	Female	16.33	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS17	LG	96	Female	18.09	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS18	LG	95	Female	17.43	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS19	LG	94	Male	20.07	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS2	LG	98	Female	21.44	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Areawith high longevity in Guangxi (LA)
OS20	LG	93	Male	18.29	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS21	LG	93	Female	21.08	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS22	LG	93	Female	19.02	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS23	LG	93	Female	17.40	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS24	LG	92	Female	19.62	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS25	LG	92	Female	21.02	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS26	LG	92	Female	19.16	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS27	LG	92	Female	15.91	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS28	LG	91	Male	17.80	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS29	LG	91	Female	18.33	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS3	LG	96	Female	17.80	Never	Never	None	Rice&Wheat	Never	Never	GuangXi	Rural	
OS30	LG	91	Female	16.90	Never	Never	None	Rice&Wheat	Never	Never	GuangXi	Rural	
OS31	LG	91	Female	17.03	Never	Never	None	Rice&Wheat	Never	Never	GuangXi	Rural	

OS32	LG	91	Female	20.13	Never	Never	None	Rice	Never	Never	GuangXi	Rural	Areawith high longevity in Guangxi (LA)
OS33	LG	90	Female	21.37	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS34	LG	100	Male	18.76	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS35	LG	99	Male	17.16	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS36	LG	98	Female	16.67	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS37	LG	95	Female	18.87	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS38	LG	95	Male	18.91	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS39	LG	90	Female	21.08	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS4	LG	96	Female	16.41	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS5	LG	94	Female	21.36	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS6	LG	93	Female	17.22	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS7	LG	92	Female	16.95	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS8	LG	92	Female	20.77	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
OS9	LG	92	Female	17.74	Never	Never	None	Rice	Never	Never	GuangXi	Rural	
NLA1	NLA	61	Female	22.11	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA2	NLA	61	Male	24.36	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA3	NLA	63	Male	20.94	Never	Never	None	Rice	Ever	Never	GuangXi	Urban	
NLA4	NLA	64	Male	20.67	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA5	NLA	65	Male	23.40	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA6	NLA	66	Female	20.66	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA7	NLA	70	Male	23.15	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA8	NLA	70	Female	21.47	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA9	NLA	71	Male	20.51	Never	Never	None	Rice	Ever	Never	GuangXi	Urban	
NLA10	NLA	72	Female	23.88	Never	Never	None	Rice	Never	Ever	GuangXi	Urban	
NLA11	NLA	80	Male	24.06	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Urban	
NLA12	NLA	82	Female	19.86	Never	Never	Hypertersion	Rice	Never	Never	GuangXi	Urban	
NLA13	NLA	53	Male	24.61	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA14	NLA	54	Female	20.00	Never	Never	None	Rice	Never	Never	GuangXi	Urban	

NLA15	NLA	54	Female	22.06	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA16	NLA	55	Male	25.22	Never	Never	None	Rice	Never	Ever	GuangXi	Urban	
NLA17	NLA	56	Male	24.42	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA18	NLA	56	Female	22.34	Never	Never	None	Rice	Ever	Never	GuangXi	Urban	NLA
NLA19	NLA	56	Male	19.90	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA20	NLA	56	Male	25.20	Never	Never	None	Rice	Never	Ever	GuangXi	Urban	
NLA21	NLA	59	Female	22.11	Never	Never	None	Rice	Never	Never	GuangXi	Urban	
NLA22	NLA	59	Female	20.42	Never	Never	None	Rice	Never	Never	GuangXi	Urban	

Table S2. Primers and conditions.

ID	Primer Sequence (5'-3')	Annealing Temperature (°C)
Total Bacteria	F: ACTCCTACGGCAGGCAGCAGT R: ATTACCGCGGCTGCTGGC	65
<i>Alistipes</i>	F: CTATGCCGTGCCGTTTCGATACC R: AGTAATCCTTGTCGTCCTCCTCCTG	60
<i>Bacteroides</i>	F: GTCACACTCAGTCACCGACAACAC R: GAGCACGGATACTCTGAACAGCATC	60
<i>Blautia</i>	F: GTCACACTCAGTCACCGACAACAC R: GAGCACGGATACTCTGAACAGCATC	60
<i>Lachnospiraceae bacterium</i> NK4A136	F: AGATCAGAATGCTGCGGTGAATACG R: CCCAGTTATCAATCCTGCCTTCGG	62
<i>Lactobacillus</i>	F: AGATCAGAATGCTGCGGTGAATACG R: CCCAGTTATCAATCCTGCCTTCGG	62
27F	AGAGTTTGATCCTGGCCTCA	55
1492R	GGTTACCTTGTTTGTTACGACTT	55

Table S3. PCR reaction system for strain identification.

Reaction System	Volume (μ L)
2 \times Master Mix	12.50
Primer 1	0.75
Primer 2	0.75
DNA template	1.00
ddH ₂ O	10.00

Table S4. PCR conditions system for strain identification.

Temperature (°C)	Reaction	Reaction time	Number of cycles
94	pre-denaturation	3 min	1
94	denaturation	30 s	30
55	Annealing	30 s	30
72	Extension	1 min	30
72	Re-extension	5 min	1

Table S5. Size of each module.Top 10 in the mediumpurple3 network ranked by MNC method.

Rank	OTU_ID	Taxonomy
1	OTU_46016	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Blautia; uncultured bacterium</i>
2	OTU_45904	<i>Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; uncultured bacterium</i>
2	OTU_46034	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; [Eubacterium] coprostanoligenes group; uncultured bacterium</i>
4	OTU_46057	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Christensenellaceae; Christensenellaceae R-7 group; uncultured bacterium</i>
4	OTU_46004	<i>Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides; uncultured bacterium</i>
4	OTU_45797	<i>Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; Alistipes; Alistipes finegoldii DSM 17242</i>
4	OTU_45857	<i>Bacteria; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium; Bifidobacterium longum NCC2705</i>
8	OTU_45834	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminiclostridium 5; uncultured bacterium</i>
8	OTU_46033	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnospiraceae NK4A136 group; uncultured bacterium</i>
10	OTU_45907	<i>Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcaceae UCG-004; uncultured bacterium</i>

Table S6. Basic characteristics of the strains.

	Survival rate after simulated gastrointestinal fluid transfer(%)	Self-agglutination rate (5h, %)	Percentage of adhesion (%)
<i>Lactobacillus fermentum</i> LTP1806	67.65 ± 0.06	76.89 ± 2.52	76.13 ± 1.10
<i>Lactobacillus fermentum</i> LTP1805	6.53 ± 0.02	64.68 ± 0.72	59.54 ± 1.43
<i>Lactobacillus fermentum</i> LTP1804	9.09 ± 0.00	57.96 ± 9.50	15.82 ± 0.37
<i>Lactobacillus fermentum</i> LTP1334	88.92 ± 1.26	81.34 ± 0.13	76.68 ± 0.02
<i>Lactobacillus fermentum</i> LTP1332	90.23 ± 2.86	78.88 ± 0.01	81.77 ± 0.02
<i>Lactobacillus fermentum</i> LTP1333	91.29 ± 0.85	71.50 ± 0.72	67.06 ± 0.80
<i>Lactobacillus fermentum</i> LTP1848	30.51 ± 0.45	63.04 ± 6.43	55.43 ± 1.14
<i>Lactobacillus fermentum</i> LTP1832	72.58 ± 0.15	72.63 ± 0.32	42.60 ± 6.55
<i>Bacteroides fragilis</i> LTBF34	33.75 ± 1.04	35.04 ± 0.07	22.29 ± 0.11
<i>Bacteroides fragilis</i> LTBF32	35.62 ± 4.58	36.71 ± 0.48	23.18 ± 0.53
<i>Bacteroides fragilis</i> LTBF31	55.88 ± 1.27	43.98 ± 0.92	45.75 ± 0.03
<i>Bacteroides fragilis</i> LTBF13	64.46 ± 0.55	58.67 ± 0.21	56.08 ± 0.58
<i>Bacteroides fragilis</i> LTBF12	79.07 ± 2.67	51.60 ± 0.52	64.97 ± 0.30
<i>Bacteroides fragilis</i> LTBF11	68.00 ± 3.46	56.51 ± 2.08	44.07 ± 0.26

All values are presented as mean (SD).

Figures

Figure S1. Diagram showing experimental design and animal groupings for mice.

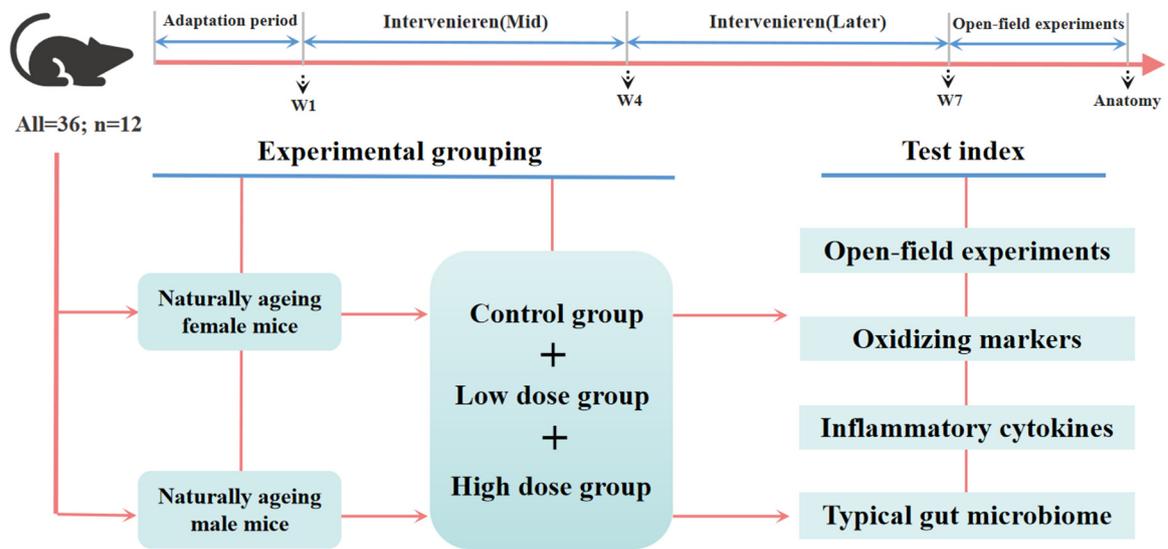


Figure S1. After one week of acclimatization feeding, mice were randomly divided into three groups: control (C) mice were gavaged with sterile saline (same volume as used in the probiotic combination) daily; low-dose (L) group was gavaged with 1×10^7 CFU/mL *Lactobacillus fermentum* LTP1332 + 1×10^7 CFU/mL *Bacteroides fragilis* LTBF12 daily; high-dose (H) group was gavaged with 1×10^9 CFU/mL *Lactobacillus fermentum* LTP1332 + 1×10^9 CFU/mL *Bacteroides fragilis* LTBF12 daily, 12 males and females in each group for 6 weeks. The overall intervention process is shown in the diagram below.

Figure S2. Structural characteristics of the microbiota.

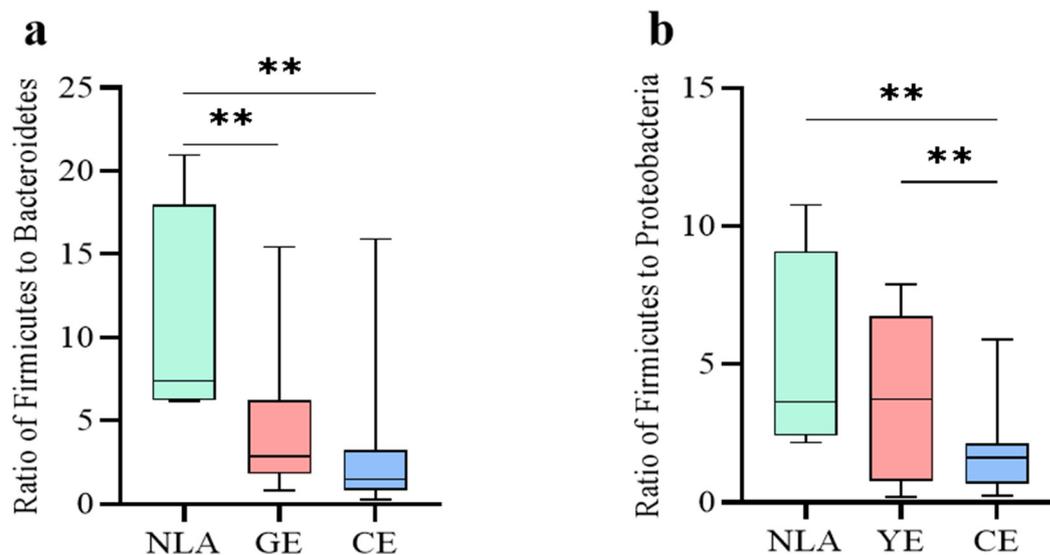


Figure S2. Ratio of typical bacterial phylum in the centenarian (CE) to the general elderly (GE) in the longevity area and the 70-86 year old group in the non-longevity area (NLA). **(a)** Indicates the ratio of *Firmicutes* to *Bacteroides* (F/B); **(b)** Indicates the ratio of *Firmicutes* to *Proteobacteria* (F/P). ** indicates $p < 0.01$ (Wilcoxon rank sum test).

Figure S3. The selection process of a soft threshold.

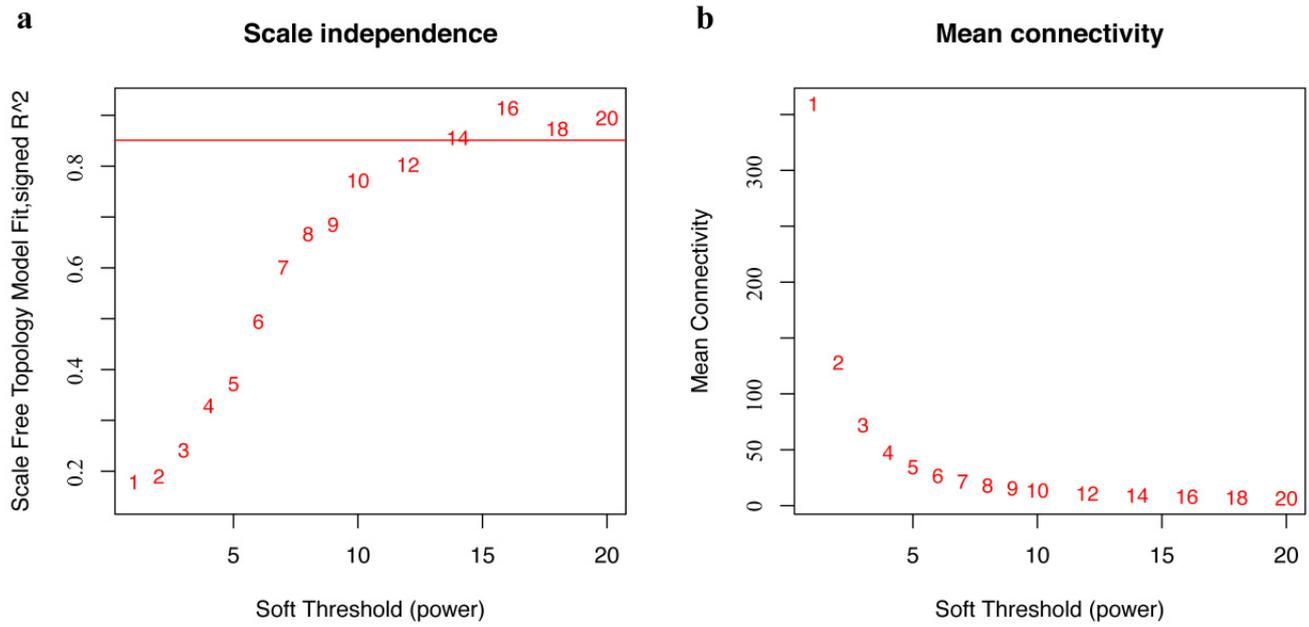


Figure S3. (a) The abscissa represents the value of the power index, and the ordinate represents the scale-free topological fitting index R^2 . When R^2 is close to 0.85, the established network is closer to the scale-free network. (b) The abscissa represents the value of the power index, and the ordinate represents the corresponding average number of left and right nodes. The average connectivity gradually approaches zero, indicating that the average connectivity of the network is good.

Figure S4. Size of each module.

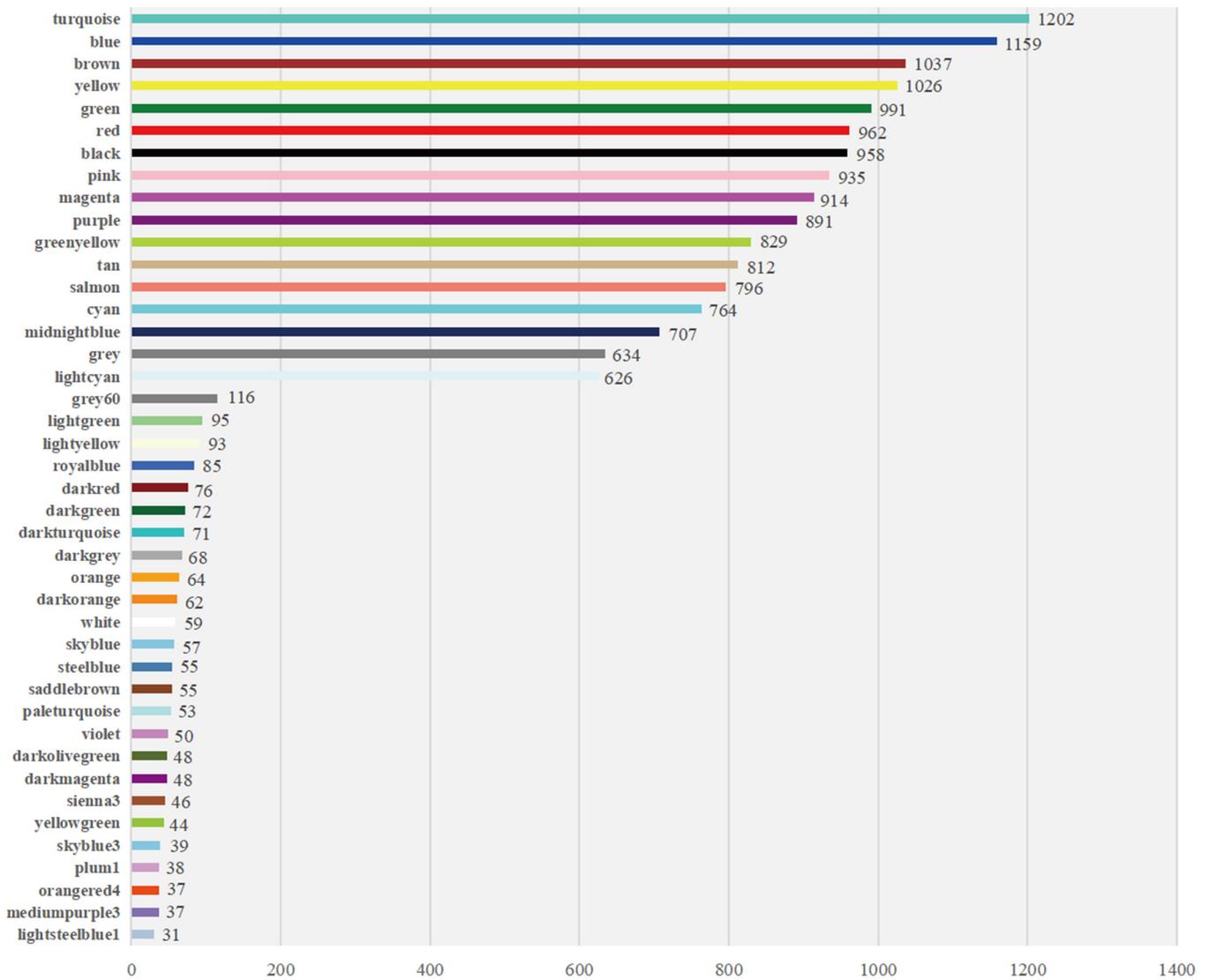


Figure S4. The bar graph with different colours reflects the number of OTU clusters in different modules. The number of clusters in the module varies from 31 to 1202. Of all the taxons, only 634 taxons (3.79%) are not included in any colour module. These taxons are classified into the grey module by default.

Figure S5. Functional prediction results.

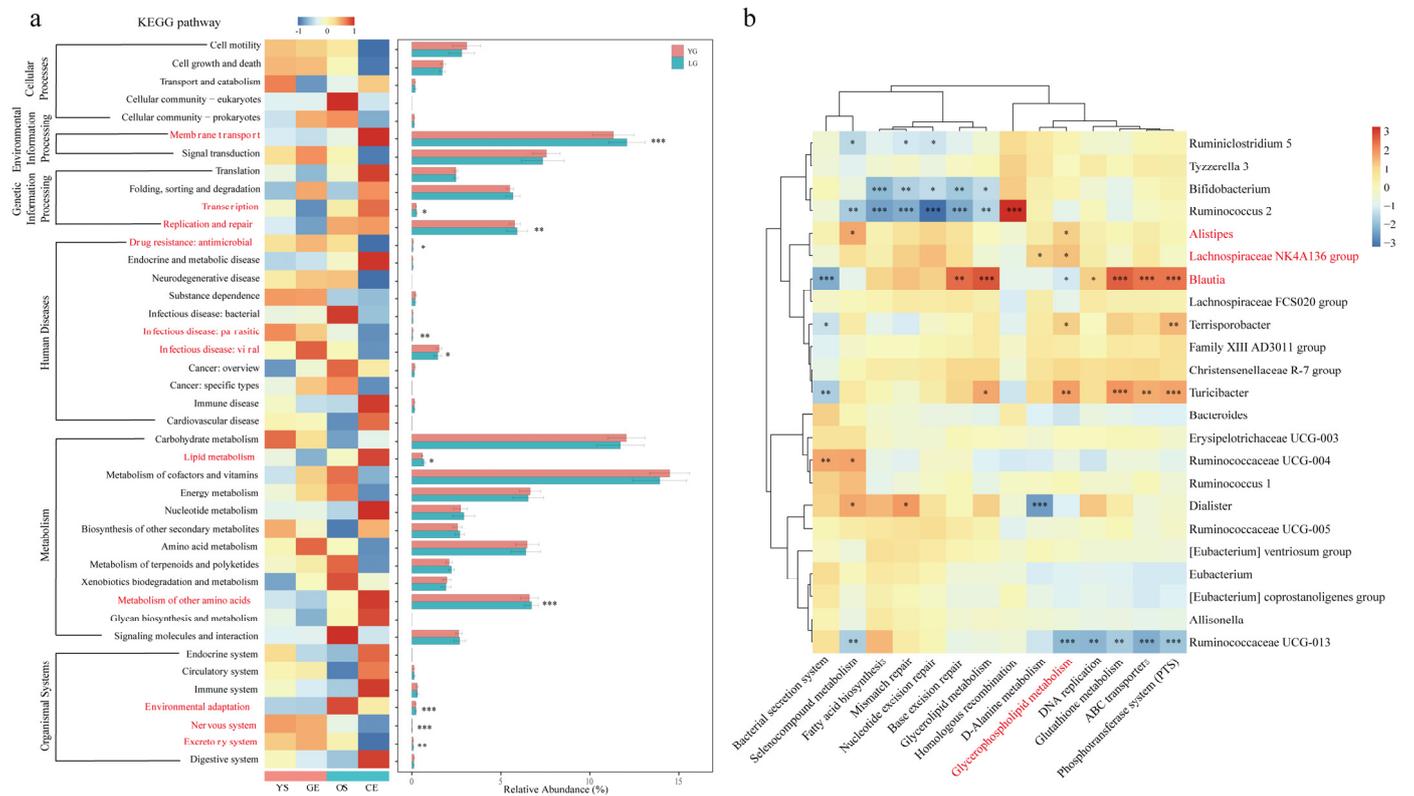


Figure S5. Functional predictions within key modules. (a) Functional predictions of the first versus second cascade and results of inter-group variance analysis. The left side of the image depicts the biological function prediction of the intestinal flora in the Guangxi longevity zone based on the KEGG database, where the first-tier metabolic pathway and the second-tier metabolic pathway of KEGG are included. The right side of the image depicts the relative abundance and differences in KEGG second-level metabolic pathways between the LG and YG groups. A heat map showing the correlation between the relative abundance of KEGG second-level metabolic pathways and each age grouping is shown in the middle of the image. **(b)** Heat map of the correlation between the abundance of the tertiary metabolic pathways with significant differences of >1% and the abundance of the genera within the module, calculated using Pearson. * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$ (Wilcoxon rank sum test).