

Supplementary material

Screening of *Lactobacillus salivarius* strains from the feces of Chinese populations and the evaluation of their effects against intestinal inflammation in mice

Qixiao Zhai^{1,2,6}, Xudan Shen^{1,2}, Shi Cen^{1,2}, Chengcheng Zhang^{1,2}, Fengwei Tian^{1,2,4}, Jianxin Zhao^{1,2}, Hao Zhang^{1,2,3,4,5}, Yuzheng Xue^{7*}, Wei Chen^{1,2,3,8*}

¹State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China

²School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China.

³National Engineering Research Centre for Functional Food, Wuxi, Jiangsu 214122, China

⁴Wuxi Translational Medicine Research Center and Jiangsu Translational Medicine Research Institute Wuxi Branch.

⁵(Yangzhou) Institute of Food Biotechnology, Jiangnan University, Yangzhou 225004, China.

⁶International Joint Research Laboratory for Probiotics at Jiangnan University, Wuxi, Jiangsu 214122, China

⁷Department of Gastroenterology, Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu 214122, China

⁸Beijing Innovation Centre of Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, China

*Corresponding author:

Yuzheng Xue, E-mail: xueyz001@163.com, Phone: (86)0510-81195162

Wei Chen, E-mail: chenwei66@jiangnan.edu.cn, Phone: (86)510-85912155

Figures

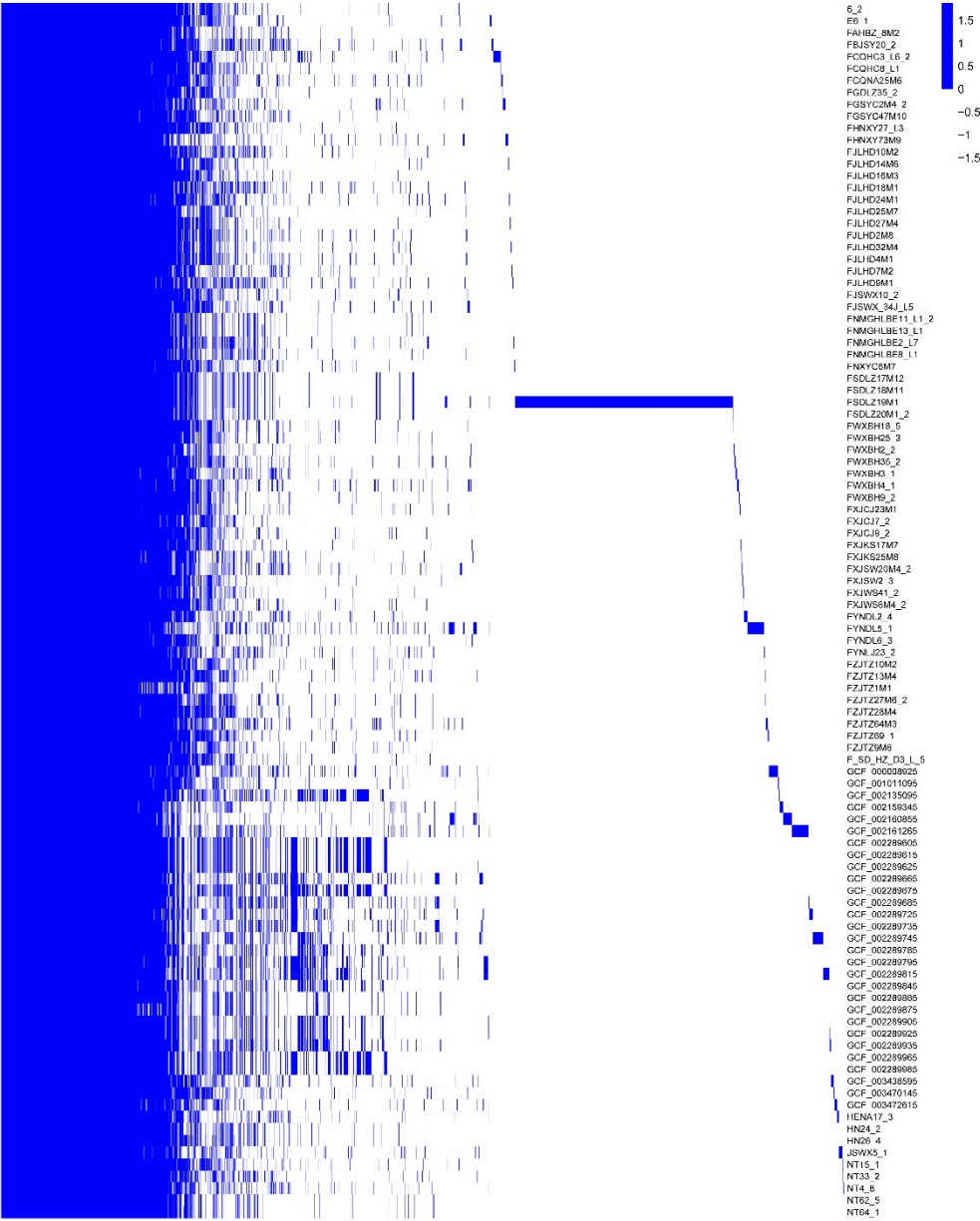


Fig. S1. Gene presence and absence among 102 *L. salivarius* strains.

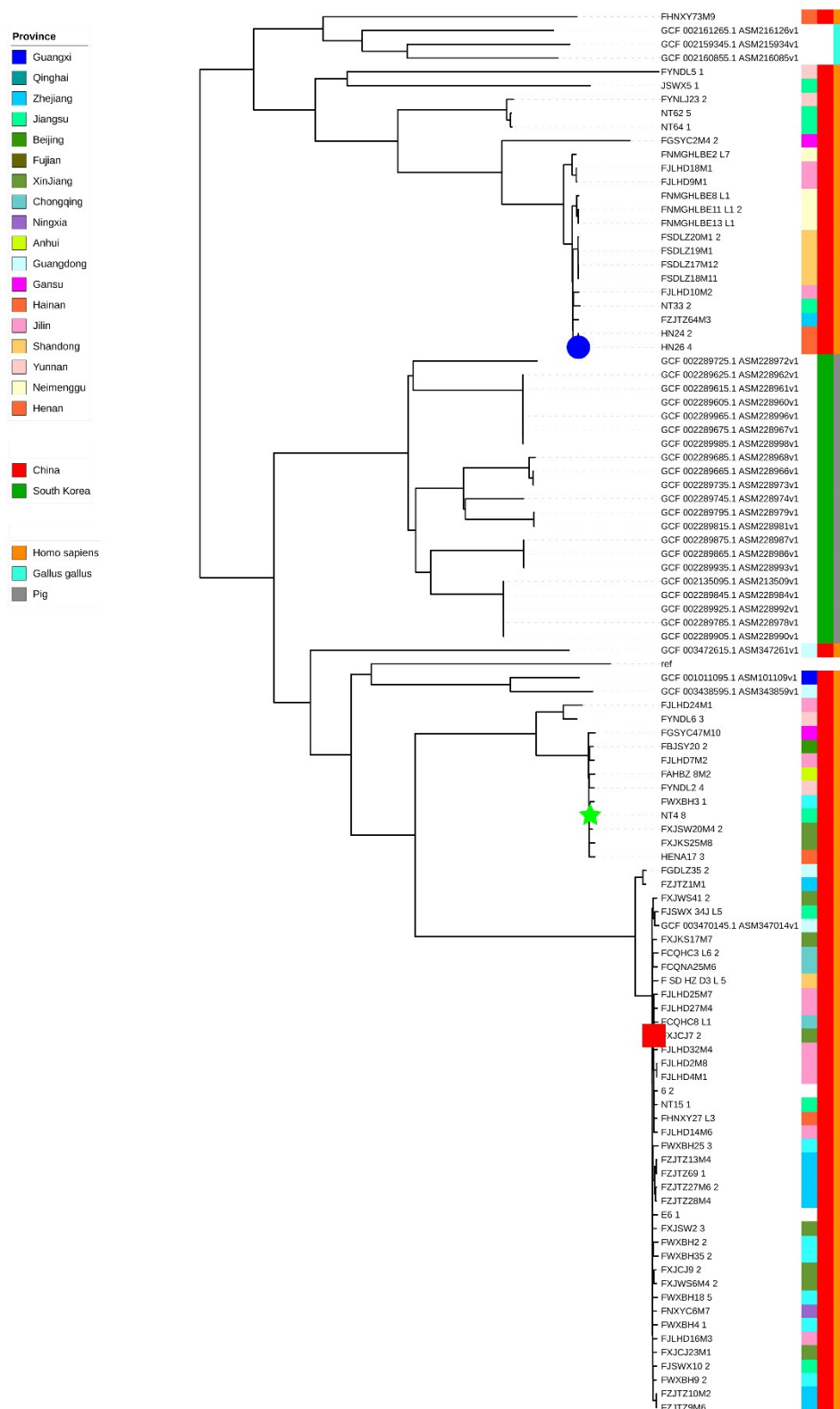


Fig. S2. The phylogenetic tree of *L. salivarius* constructed by neighbor-joining method based on the bi-SNPs in the core-genome of 102 strains. The three strains selected for the subsequent animal experiment were marked on the corresponding clades.

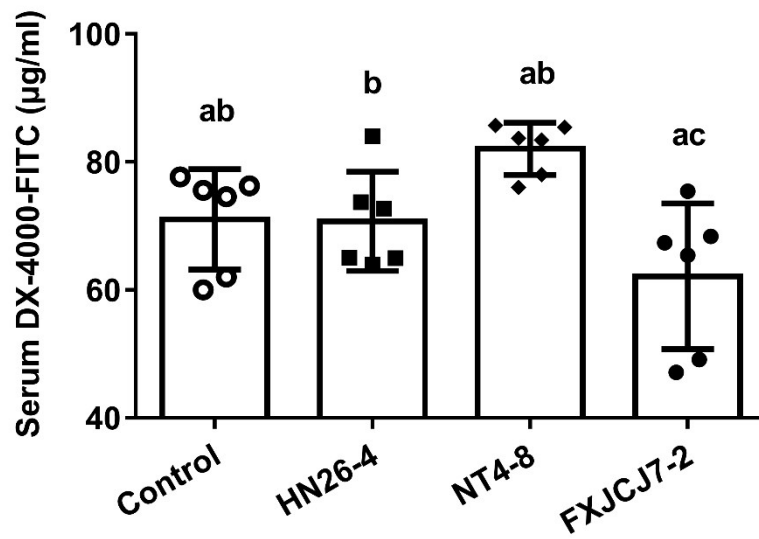


Fig. S3. Effects of *L. salivarius* administration on intestinal permeability of mice, as expressed by the alterations in the DX-4000-FITC levels in the serum. Significant differences ($P < 0.05$) between groups are indicated with different letters (a–c) above the bars. Values are mean $\pm$ SD values of six mice per group.

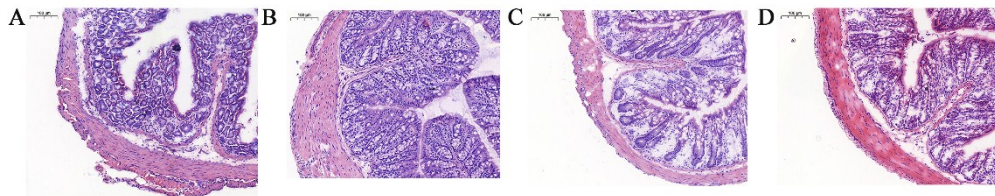


Fig. S4. Representative photomicrographs of colonic tissue of mice (H&E staining; magnifications, $\times 10$). (A) Control group. (B) HN26-4 group. (C) NT4-8 group. (D) FXJCJ7-2 group.

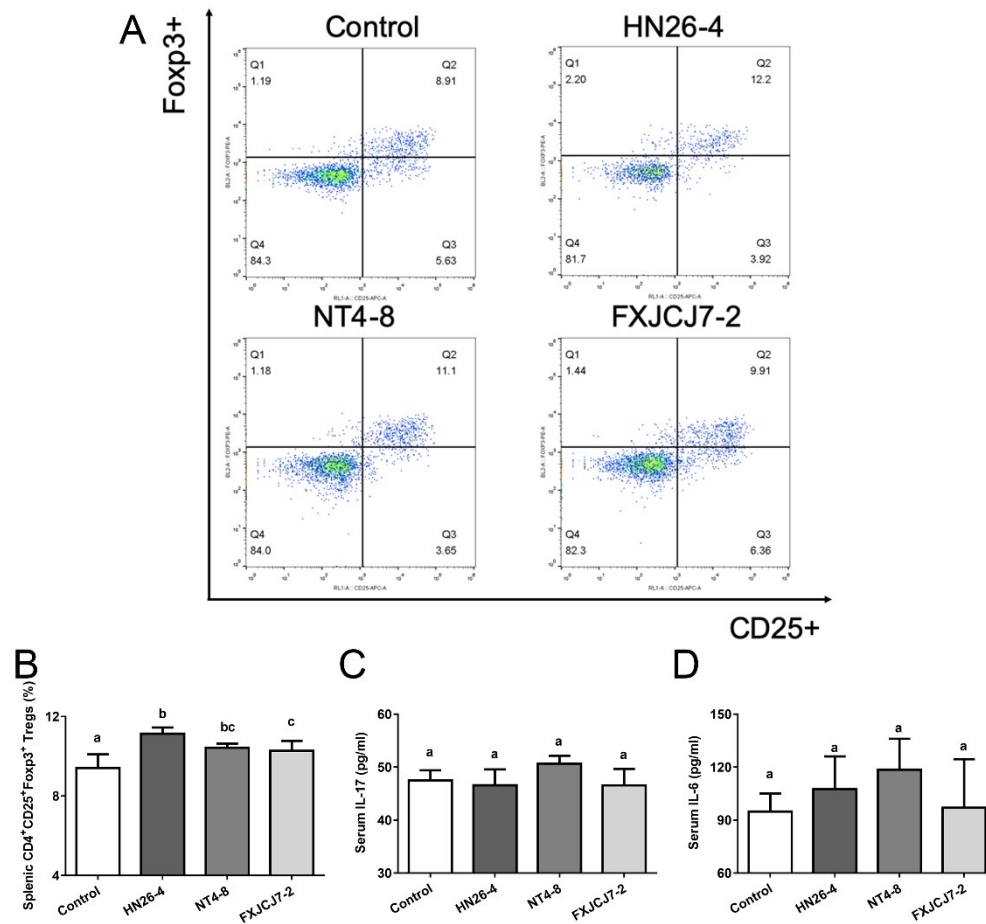


Fig. S5. Effects of *L. salivarius* administration on Treg/Th-17 balance of mice. (A) Representative histogram of flow cytometric analysis for splenic Tregs. (B) Percentages of splenic Tregs. (C) Levels of serum IL-17. (D) Levels of serum IL-6. Significant differences ($P < 0.05$) between groups are indicated with different letters above the bars. Values are mean $\pm$ SD values of six mice per group.

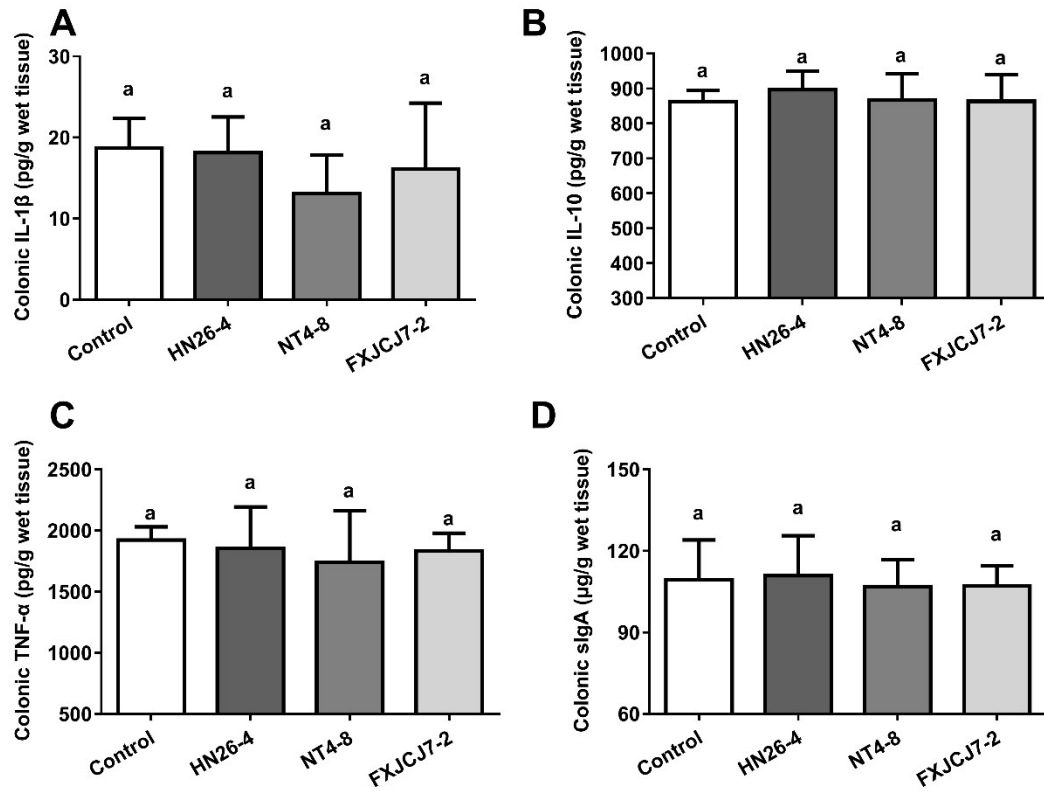


Fig. S6. Effects of *L. salivarius* administration on the levels of immunomodulatory indicators in the colon of mice. (A) Levels of IL-1 β . (B) Levels of IL-10. (C) Levels of TNF- α . (D) Levels of sIgA. Significant differences ($P < 0.05$) between groups are indicated with different letters above the bars. Values are mean $\pm$ SD values of six mice per group.

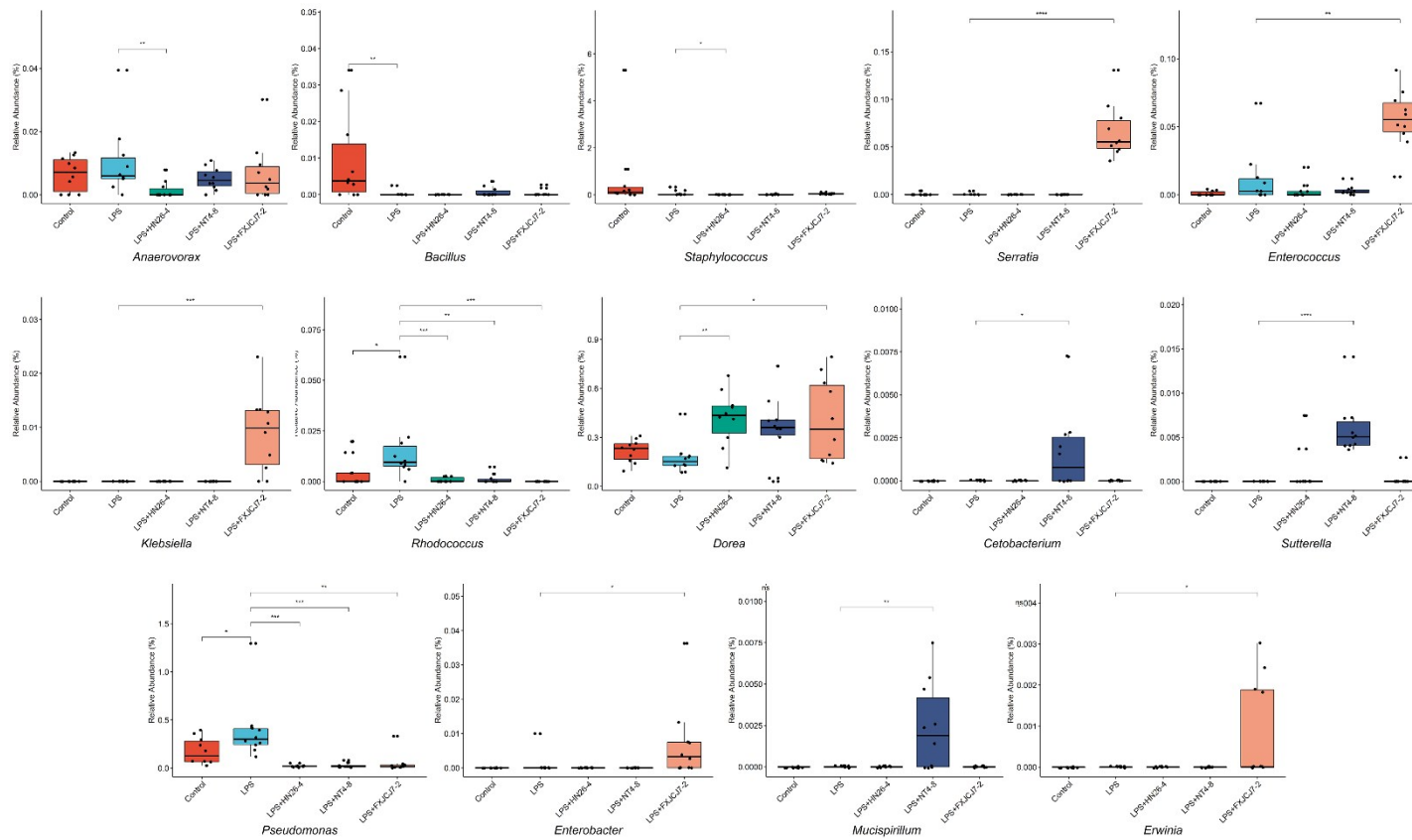


Fig. S7. Other microbial genera whose relative abundance values were significantly altered by *L. salivarius* administration. Values are mean $\pm$ SD values of ten mice per group. *, **, and *** indicate significant differences ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively) between groups. n.s. indicates no significant differences ($P > 0.05$) between groups.

Tables

Table S1 Basic information of 29 *Lactobacillus salivarius* strains recorded in NCBI database

Strain	Assembly No.	Size (Mb)	GC%	Genes	Level	Location	Host	Isolation source
Ren	GCA_001011095.1	1.97836	33.0757	1990	Complete Genome	Guangxi, China	Homo sapiens	Feces
KLW001	GCA_002135095.1	2.32671	33	2360	Contig	South Korea	Pig	Porcine feces
An63	GCA_002159345.1	1.8076	32.7	1774	Contig	N/A	Gallus gallus	Caecum
An128	GCA_002160855.1	1.88402	32.7	1857	Contig	N/A	Gallus gallus	Caecum
An84	GCA_002161265.1	2.09004	32.7	2078	Contig	N/A	Gallus gallus	Caecum
KLA003	GCA_002289605.1	2.26392	32.8	2352	Contig	South Korea	Pig	Porcine feces
KLA002	GCA_002289615.1	2.25711	32.8	2321	Contig	South Korea	Pig	Porcine feces
KLA004	GCA_002289625.1	2.26601	32.8	2328	Contig	South Korea	Pig	Porcine feces
KLF002	GCA_002289665.1	2.17667	32.8	2233	Contig	South Korea	Pig	Porcine feces
KLA006	GCA_002289675.1	2.3669	32.9	2432	Contig	South Korea	Pig	Porcine feces
KLF003	GCA_002289685.1	2.20692	32.7	2287	Contig	South Korea	Pig	Porcine feces
KLF005	GCA_002289725.1	2.1547	32.7	2184	Contig	South Korea	Pig	Porcine feces
KLF004	GCA_002289735.1	2.1157	32.7	2205	Contig	South Korea	Pig	Porcine feces
KLF007	GCA_002289745.1	2.22216	32.9	2254	Contig	South Korea	Pig	Porcine feces
KLW002	GCA_002289785.1	2.0913	32.8	2095	Contig	South Korea	Pig	Porcine feces
KLW004	GCA_002289795.1	2.33817	32.8	2361	Contig	South Korea	Pig	Porcine feces
KLW003	GCA_002289815.1	2.36801	32.9	2406	Contig	South Korea	Pig	Porcine feces
KLW005	GCA_002289845.1	2.07512	32.8	2096	Contig	South Korea	Pig	Porcine feces

KLW006	GCA_002289865.	2.03485	32.7	2003	Contig	South Korea	Pig	Porcine feces
KLW007	GCA_002289875.	1.96532	32.8	1955	Contig	South Korea	Pig	Porcine feces
KLW008	GCA_002289905.	2.13784	32.9	2130	Contig	South Korea	Pig	Porcine feces
KLW009	GCA_002289925.	2.07127	32.9	2088	Contig	South Korea	Pig	Porcine feces
KLW010	GCA_002289935.	2.38939	32.7	2392	Contig	South Korea	Pig	Porcine feces
KLA005	GCA_002289965.	2.25857	32.8	2310	Contig	South Korea	Pig	Porcine feces
KLA001	GCA_002289985.	2.26806	32.8	2338	Contig	South Korea	Pig	Porcine feces
OM08-20	GCA_003438595.	2.01106	32.8	2070	Scaffold	Shenzhen, China	Homo sapiens	Feces
AM25-8	GCA_003470145.	1.96949	32.8	1990	Scaffold	Shenzhen, China	Homo sapiens	Feces
AM09-40	GCA_003472615.	1.88884	32.8	1868	Scaffold	Shenzhen, China	Homo sapiens	Feces
UCC118 (reference)	GCA_000008925.	2.13398	33.0066	2182	Complete Genome	N/A	N/A	N/A

Table S2 Concentration of SCFAs in the colonic contents of mice

SCFAs (μmol/g)	Control	LPS	LPS+HN26-4	LPS+NT4-8	LPS+FXJCJ7-2
Acetic acid	60.36 ± 8.35 ^a	34.76 ± 6.97 ^b	47.22 ± 13.46 ^b	44.16 ± 8.14 ^b	67.25 ± 8.42 ^a
Propanoic acid	6.52 ± 0.45 ^a	5.49 ± 0.82 ^a	9.98 ± 0.77 ^b	9.80 ± 1.16 ^b	10.01 ± 0.62 ^b
Butanoic acid	1.13 ± 0.65 ^a	1.06 ± 0.40 ^a	1.92 ± 0.40 ^b	1.43 ± 0.29 ^c	2.57 ± 1.10 ^d
Isobutyric acid	0.77 ± 0.10 ^a	0.56 ± 0.30 ^b	1.08 ± 0.09 ^c	1.03 ± 0.16 ^c	1.28 ± 0.35 ^c
Pentanoic acid	0.92 ± 0.51 ^{ab}	0.71 ± 0.24 ^b	1.32 ± 0.42 ^a	1.12 ± 0.31 ^a	1.29 ± 0.38 ^a
Total SCFAs	69.69 ± 8.05 ^a	42.58 ± 7.05 ^b	61.52 ± 12.30 ^a	57.48 ± 7.82 ^{ab}	82.32 ± 8.52 ^c

Values are mean ± SD values with six mice in each group. The superscript letters a, b and c indicate statistically significant differences at *P* values of < 0.05 among groups.