

**A purified fraction of polysaccharides from the fruits of *Lycium barbarum* L.
improves glucose homeostasis and intestinal barrier function in high-fat diet
induced mice**

Wangting Zhou ^a, Peiyun Liu ^a, Weiqi Xu ^a, Linwu Ran ^b, Yamei Yan ^c, Lu Lu ^c,
Xiaoxiong Zeng ^{a,*}, Youlong Cao ^{c,*}, Jia Mi ^{c,*}

^a College of Food Science and Technology, Nanjing Agricultural University, Nanjing
210095, Jiangsu, China

^b Laboratory animal center of Ningxia Medical University, Yinchuan, 750004, Ningxia,
China

^c Institute of wolfberry Engineering and Technology, Ningxia Academy of Agriculture
and Forestry, Yinchuan, 750004, Ningxia, China

*To whom correspondence should be addressed. Tel & Fax: +86 25 84396791, E-mail: zengxx@njau.edu.cn (X. Zeng); +86 951 6886783, E-mail address: youlongckh@126.com (Y. Cao); +86 951 6886783, E-mail address: lorna0102@126.com (J. Mi)

Table S1. Formula of standard maintenance diet and high-fat diet

Ingredient (gram)	High-fat diets (D12492)	Standard maintenance diet
Casein	200	200
L-Cystein	3	3
Corn starch	0	506.2
Maltodextrin 10	125	125
Sucrose	68.8	68.8
Cellulose, BW200	50	50
Soybean Oil	25	25
Lard	245	20
Mineral Mix S10026	10	10
DiCalcium Phosphate	13	13
Calcium Carbonate	5.5	5.5
Potassium Citrate. 1H ₂ O	16.5	16.5
Vitamin Mix, V10001	10	10
Choline Bitartrate	2	2
FD&C Blue Dye No.1	0.05	0.01
FD&C Yellow Dye No.5	0	0.4

Table S2. Histopathological scoring standard

Score	Crypt damage	Surface epithelial injury
0	None	Absent
1	Loss of one-third of the crypts	Present
2	Loss of two-thirds of the crypts	
3	The lamina propria is covered with a single layer of epithelium and mild inflammatory infiltrate is present	
4	Erosions and large amount of infiltration are present	

Table S3. Nucleotide sequences of primers used for RT-qPCR analysis

Target genes	Primer Sequence
zonula occludens 1	FW: TTTTGGACAGGGGGAGTGG RV: TGCTGCAGAGGTCAAAGTTCAAG
Occludin	FW: ATGTCCGGCCGATGCTCTC RV: TTTGGCTGCTCTTGGGTCTGTAT
Claudin-1	FW: AGCTGCCTGTTCCATGTACT RV: CTCCCATTTGTCTGCTGCTC
MUC2	FW: TGTGGCCTGTGTGGGAAC TTT RV: CATAGAGGGCCTGTCCTCAGG
GAPDH	FW: AGGTCGGTGTGAACGGATTTG RV: TGTAGACCATGTAGTTGAGGTCA

Table S4. Alpha diversity of the gut microbiota in mice of different treatment groups.

	NC	HFD	LBP5-4
Observed Species	269.63 ± 17.66 b	213.63 ± 34.33 a	234.63 ± 25.45 a
Chao 1	272.90 ± 17.82 b	217.30 ± 34.74 a	239.01 ± 26.52 a
ACE	272.42 ± 17.85 bc	216.49 ± 35.76 a	238.32 ± 25.94 ab
Shannon	4.12 ± 0.18 a	3.87 ± 0.23 a	3.97 ± 0.28 a
Simpson	0.040 ± 0.10 a	0.047 ± 0.011 a	0.046 ± 0.013 a

Values are expressed as mean ± standard deviation. The means with a row not sharing the same letters are significantly different ($p < 0.05$).

Table S5. Effects of FMT on alpha diversity of gut microbiota of HFD-fed recipient mice.

	HFD-FMT	LBP _s -4-FMT
Observed Species	245.50 ± 11.99	272.75 ± 20.37
Chao 1	249.55 ± 12.00	275.83 ± 19.60
ACE	248.99 ± 12.21	275.87 ± 20.10
Shannon	4.02 ± 0.20	4.06 ± 0.29
Simpson	0.045 ± 0.016	0.054 ± 0.021

Table S6. The contents of SCFAs in feces of mice in the HFD-FMT group and LBPs-4-FMT group

Groups	HFD-FMT	LBPs-4-FMT
Acetic acid	7.65 ± 1.24	10.50 ± 0.63*
Propionic acid	0.61 ± 0.28	0.91 ± 0.20
i-Butyric acid	0.23 ± 0.12	0.34 ± 0.08
n-Butyric acid	0.55 ± 0.23	0.97 ± 0.18 *
i-Valeric acid	0.63 ± 0.18	0.68 ± 0.16
n-Valeric acid	0.70 ± 0.17	0.84 ± 0.17
Total SCFAs	10.36 ± 1.25	14.25 ± 0.64 *

The statistical significance of two groups was measured using Student's t test. * indicates a significant difference between the two groups ($p < 0.05$)

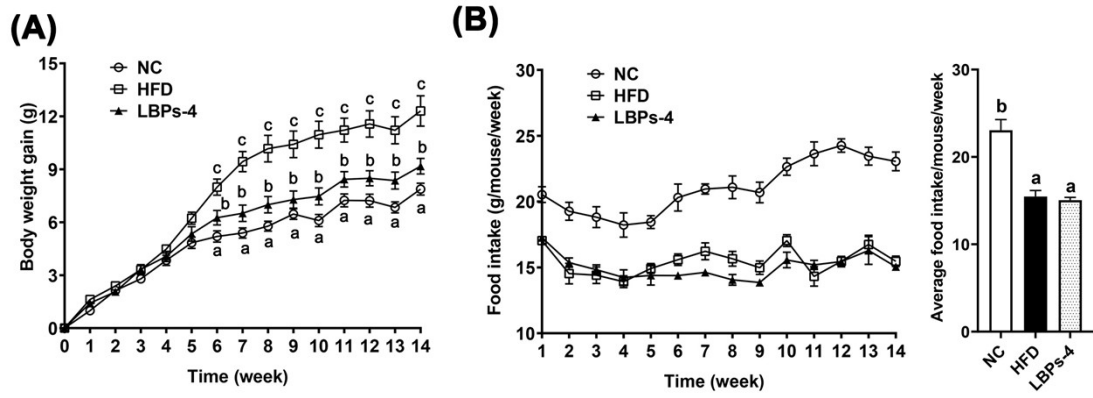


Fig. S1. Changes in body weight gain (A) and weekly food consumption (B) of mice in the NC group, HFD group and LBPs-4 group throughout the experimental period. Different letters indicate significant differences ($p < 0.05$) between the groups.

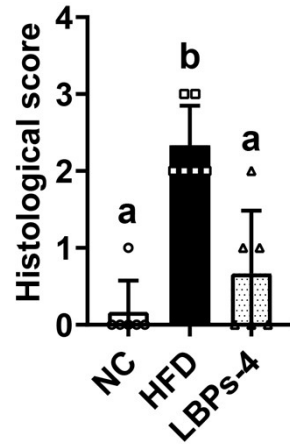


Fig. S2. Histopathological scores of colonic sections for NC group, HFD group and LBPs-4 group, respectively.

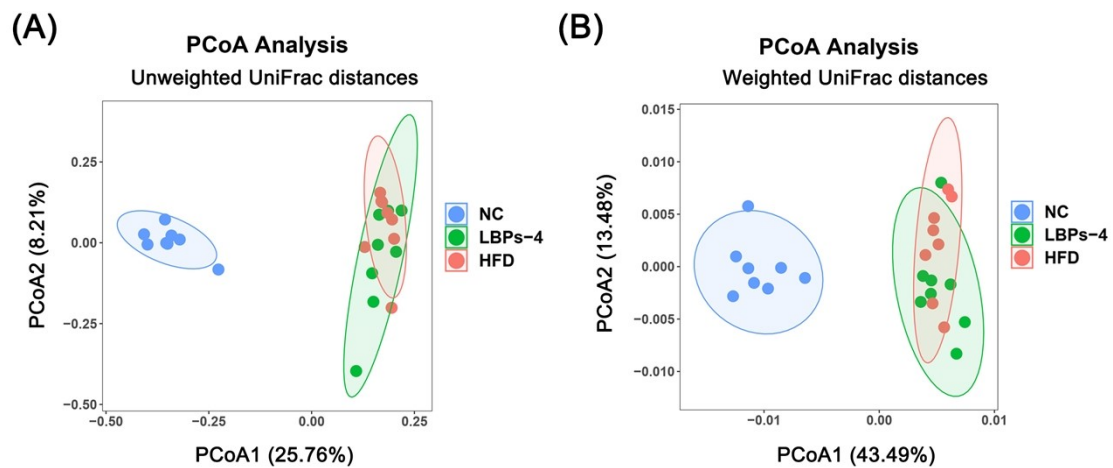


Fig. S3. PCA plot based on unweighted UniFrac distances (A) and weighted UniFrac distances (B).

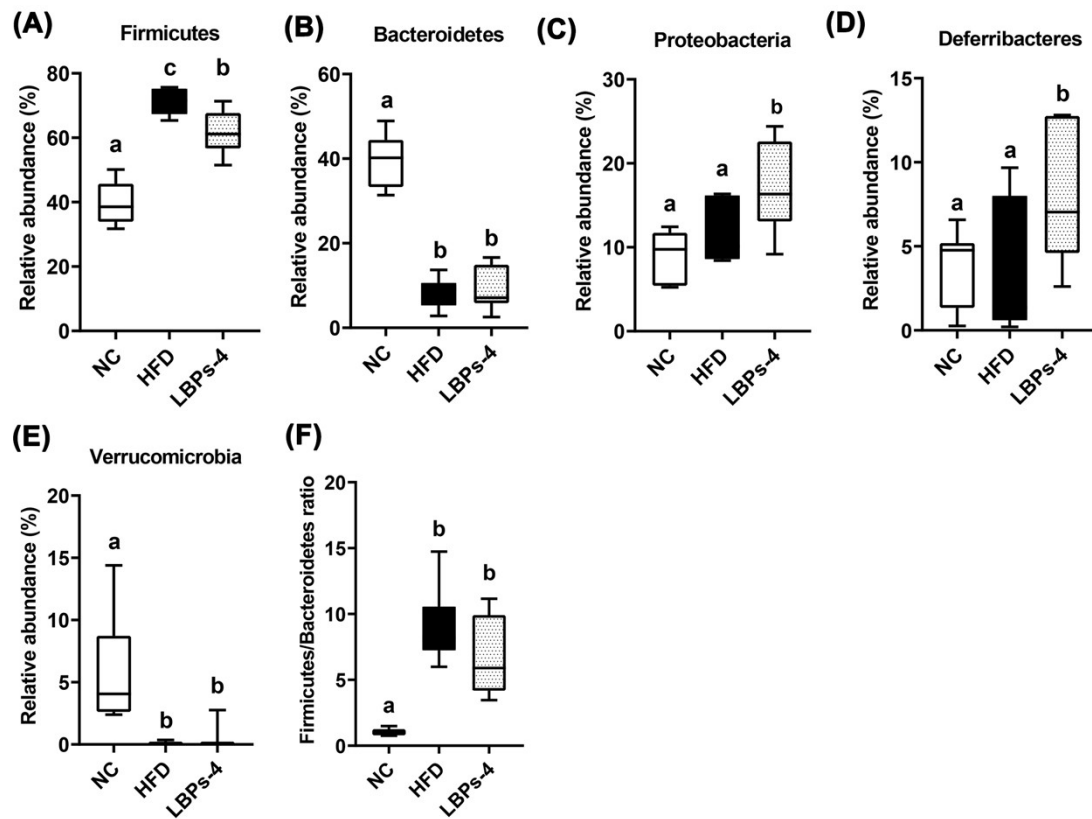


Fig. S4. Comparative analysis of the relative abundances of gut microbiota at phylum level. The relative abundances of Firmicutes (A), Bacteroidetes (B), Proteobacteria (C), Defferribacteres (D) and Verrucomicrobia (E) at the different treatment groups. (F) The ratio of Firmicutes/Bacteroidetes. Different letters represents significant differences ($p < 0.05$) between the different treatment groups.

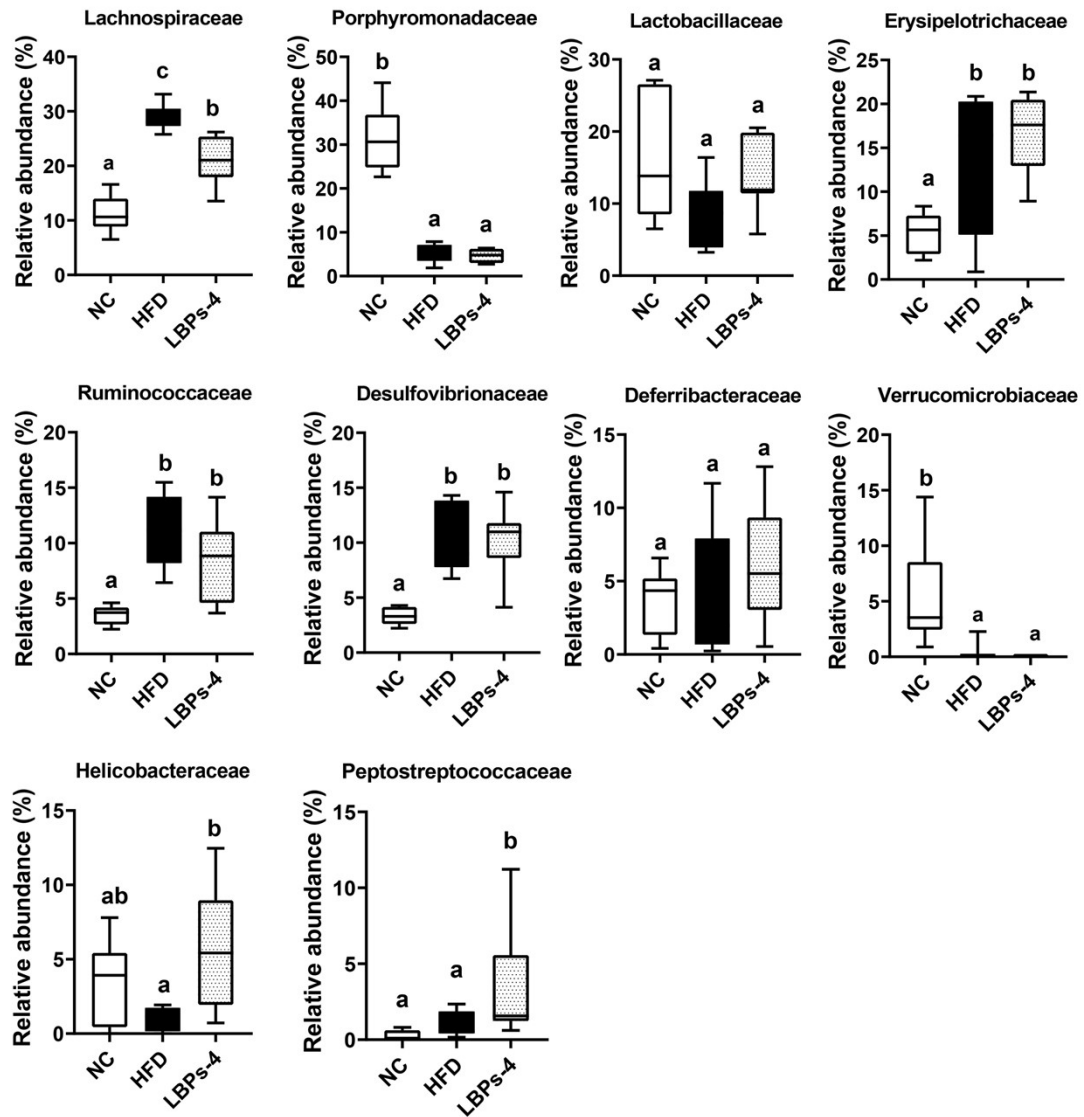


Fig. S5. The relative abundance of the gut microbiota at family level. Different letters indicate significant differences ($p < 0.05$) between the different treatment groups.

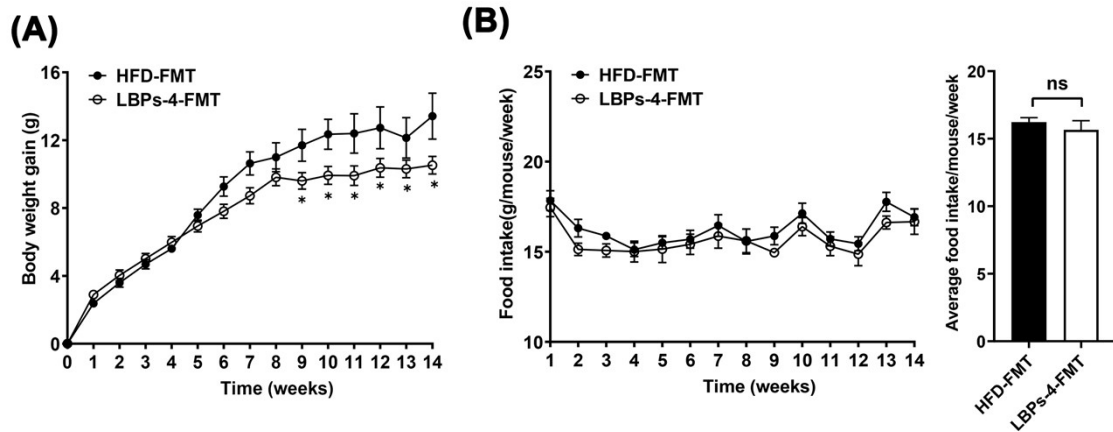


Fig. S6. Changes in body weight gain (A) and weekly food consumption (B) of mice in the HFD-FMT group and LBPs-4-FMT group over 14 weeks. Different letters indicate significant differences ($p < 0.05$) between the groups. * $p < 0.05$ versus the HFD-FMT group. Ns, no significant.

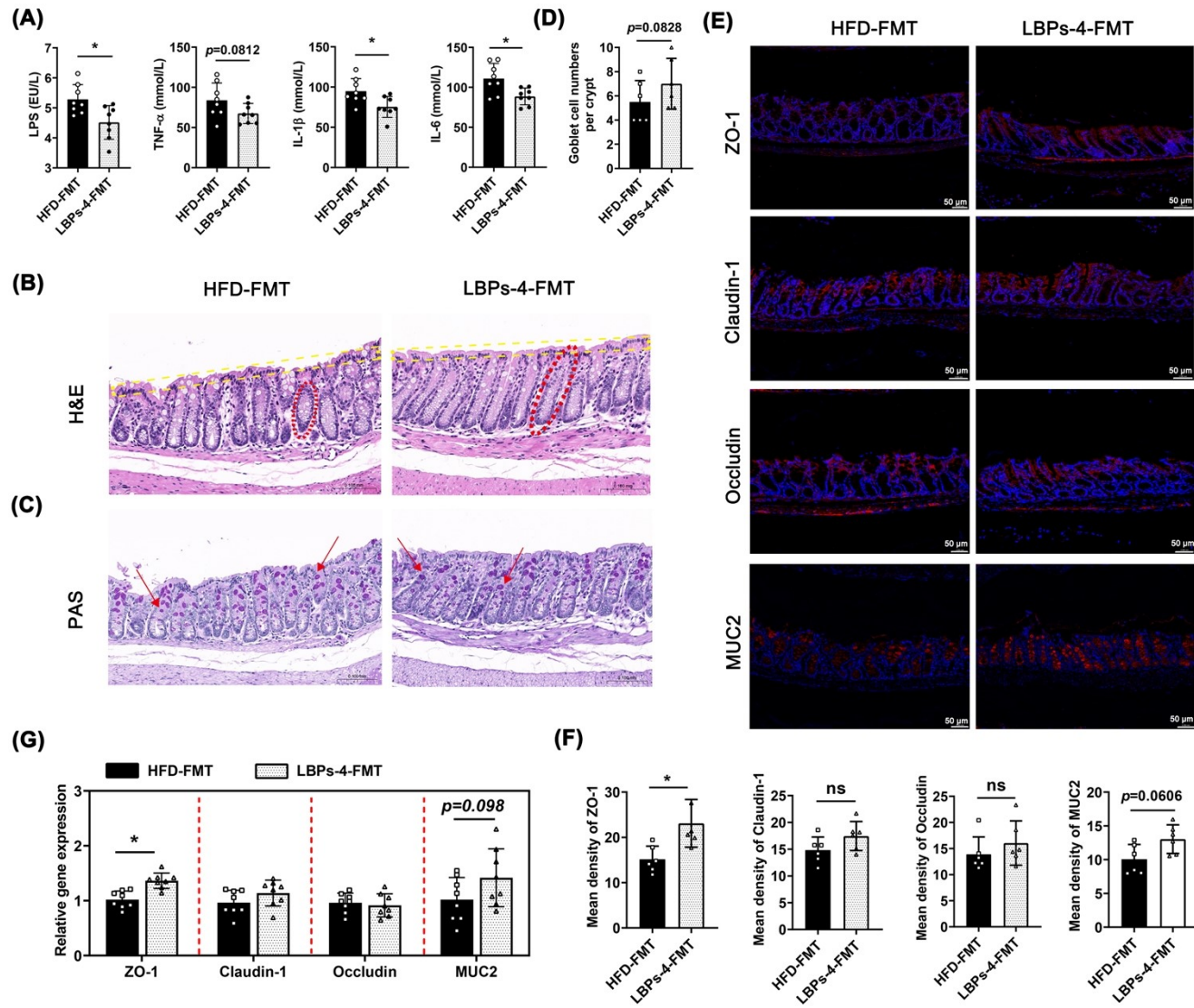


Fig. S7. Effects of the FMT treatment on systemic inflammation and intestinal barrier function in HFD-fed recipient mice. (A) content of lipopolysaccharide, TNF- α , IL-1 β and IL-6 in the plasma. (B-C) Histological sections of colonic tissue stained with hematoxylin and eosin and periodic acid-Schiff (scale=100 μ m), respectively. Red ellipse and yellow box in (B) indicate crypt and surface epithelial, respectively. (D) The number of goblet cells per crypt. (E) Representative images of immunofluorescence of zonula occludens 1, claudin-1, occludin and MUC2 for the two groups (scale=50 μ m). The positive proteins are stained red, and nuclei are counterstained blue. (F) Mean density of these positive proteins analyzed by Image J. (n = 6 per group). (G) mRNA level of zonula occludens 1, claudin-1, occludin and MUC2 in the colon tissues. * represent $p < 0.05$ compared with the HFD-FMT group.

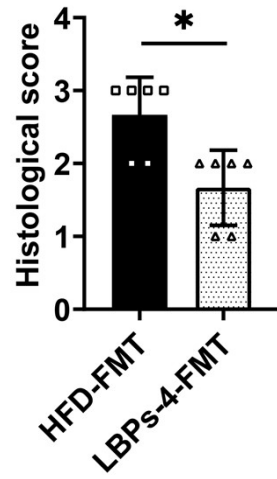


Fig. S8. Histopathological scores of colonic sections for HFD-FMT group and LBPs-4-FMT group, respectively.

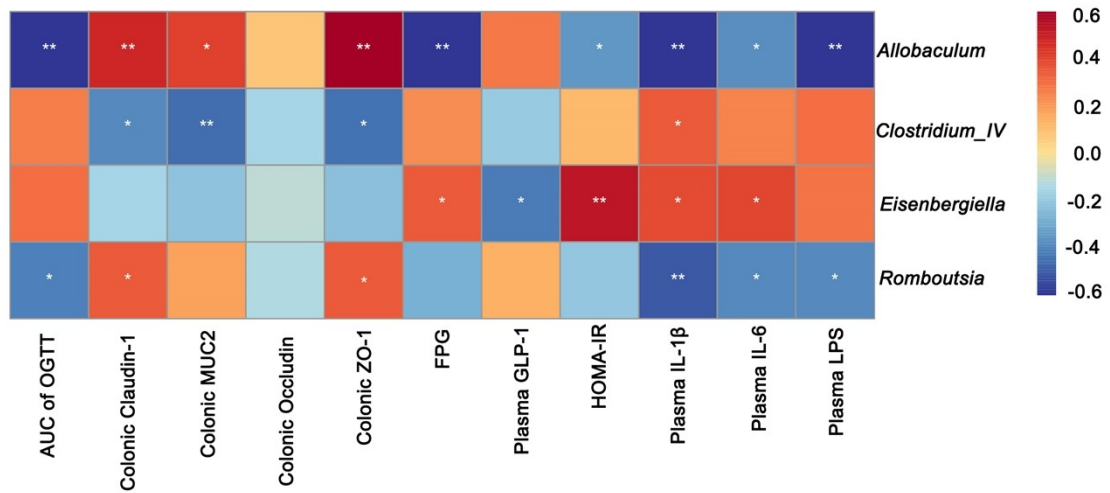


Fig. S9. Spearman correlation analysis between the four genera (*Allobaculum*, *Romboutsia*, *Clostridium_IV*, *Eisenbergiella*) and several parameters of glucose homeostasis or intestinal barrier function. * and ** indicate the associations significant ($p < 0.05$ and $p < 0.01$, respectively).