Supporting Information

Laminaria japonica polysaccharide prevents high-fat diet-induced insulin resistance in mice via regulating gut microbiota

Qiang-Ming Li[1,2,3,#], Xue-Qiang Zha[1,2,3,#,*], Wei-Nan Zhang[1,#], Jian Liu[1,2,3], Li-Hua Pan[1,2,3], Jian-Ping Luo[1,2,3,*]

[1]School of Food and Biological Engineering, Hefei University of Technology, Hefei 230009, People's Republic of China

[2]Engineering Research Center of Bio-process, Ministry of Education, Hefei University of Technology, Hefei, 230009, People's Republic of China

[3]Key Laboratory of Metabolism and Regulation for Major Diseases of Anhui Higher Education Institutes, Hefei University of Technology, Hefei, 230009, People's Republic of China

*Correspondence authors: X.-Q. Zha (zhaxueqiang@hfut.edu.cn) and J.-P. Luo (jianpingluo@hfut.edu.cn).

[#] These authors contributed equivalently.

Methods

Gut microbiota analysis

Gut microbiota composition in stool samples was determined by 16S rDNA amplicon sequencing and QIIME-based microbiota analysis. Total genome DNA from stool samples was extracted using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. All PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers (Primer: 16S V4: 515F-806R, 515F: 5'-GTGCCAGCMGC-CGCGGTAA-3', 806R: 5'-GGACTACHVGGGT-WTCTAAT-3'), and 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 ℃ for 1 min, followed by 30 cycles of denaturation at 98 ℃ for 10 s, annealing at 50 ℃ for 30 s, and elongation at 72 ℃ for 30 s, finally 72 ℃ for 5 min. PCR products were mixed with the same volume of 1X loading buffer (contained SYB green) and operated electrophoresis on 2 % agarose gel for detection. Samples with bright main strip between 400-450 bp were chosen for further experiments. PCR products was mixed in equidensity ratios and purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA) following manufacturer's recommendations and assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina HiSeq platform. Paired-end reads from the original DNA fragments were

merged by using FLASH[1], and then high-quality sequencing reads were obtained by the QIIME quality control process[2]. To remove chimeric sequences, the obtained reads were blasted with the reference database (Gold Database) using the UCHIME algorithm[3]. The sequences with ≥ 97% similarity were assigned to the same OTUs. Alpha diversity analysis including rarefaction analysis and Shannon index were calculated using QIIME[2]. Some statistical analysis methods, including T-test and MetaStat, were used to analyze the differences of microbial diversity.

References

[1] Magoc T., Salzberg S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, **2011**, *27*, 2957-2963.

[2] Caporaso J. G.*, et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, **2010**, *7*, 335-336.

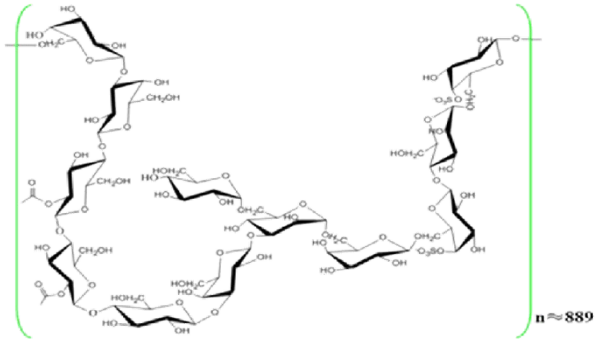
[3] Edgar R. C., Haas B. J., Clemente J. C., Quince C., Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **2011**, *27*, 2194-2200.

Supplementary Table S1. Diet composition used in this work.

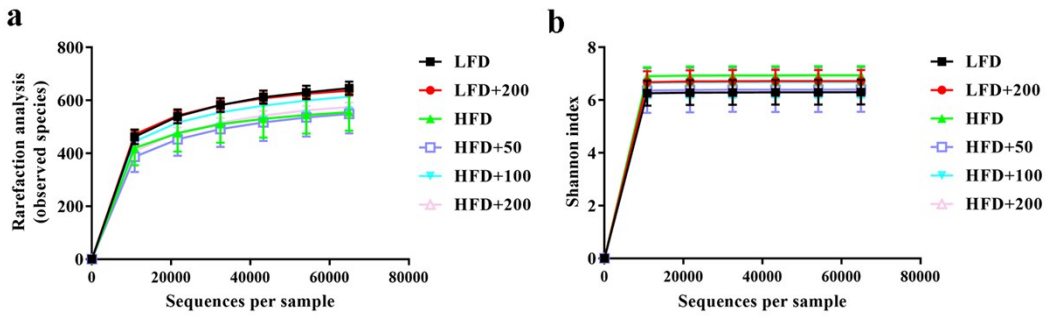
Ingredient	LFD diet	HFD diet
	g/kg dry weight	
Casein	189.6	222.9
Cystine	2.8	3.3
Soy oil	23.7	27.9
Cocoa butte	19.0	172.8
Corn Starch	355.4	236.3
Malto-dextrin	118.5	79.1
Sucrose	189.6	125.9
Cellulose	47.4	55.7
Mineral Mix S10001 (41)	9.5	11.2
Dicalcium phosphate	12.3	14.5
Calcium carbonate	5.2	6.1
Potassium citrate, monohydrate	15.6	18.4
Vitamin Mix V10001 (41)	9.5	11.2
Choline	1.9	2.2
Cholesterol	0.0	12.5
	% kcal	% kcal
Macronutrient content		
Protein	20	20
Carbohydrate	70	40
Lipid	10	40

Supplementary Table S2. Sequence of primers used in this work.

Primer	Forward sequence	Reverse sequence
TNF-α	TAGCCAGGAGGGAGAACAGA	TTTTCTGGAGGGAGATGTGG
IL-1β	TTGAAGAAGAGCCCATCCTC	CAGCTCATATGGGTCCGAC
IL-6	CCGGAGAGGAGACTTCAC	TCCACGATTTCCCAGAGA
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
ACC-1	GAGTGACTGCCGAAACATCTCTG	GCAAGGAGGACAGAGTTTATCGTG
FAS	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
SREBP-1c	GATGTGCGAACTGGACACAG	CATAGGGGGCGTCAAACAG
PPAR-γ	GCAGCTACTGCATGTGATCAAGA	GTCAGCGGGTGGGACTTTC
ZO-1	ACCCGAAACTGATGCTGTGGATAG	AAATGGCCGGGCAGAACTTGTGTA
Occludin	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTTGGGTCTGTAT
KLF4	TGCCAGACCAGATGCAGTCAC	GTAGTGCCTGGTCAGTTCATC
Muc2	GGCTCGGAACTCCAGAAAG	CCAGGGAATCGGTAGACATC
RegIIIγ	TTCCTGTCCTCCATGATCAAA	CATCCACCTCTGTTGGGTTC
GAPDH	GAAGGGTGGAGGCAAAAG	ACCAGTGGTTGCAGGGAT



Supplementary Figure S1 Chemical structure of LJP61A.



Supplementary Figure S2 Gut microbiota alpha diversity analysis of LJP61A treated mice. Mice were fed with LFD or HFD and administrated with 200 μL LJP61A (50, 100 or 200 mg/kg/day) or physiological saline for 8 weeks. Rarefaction analysis (a) and Shannon index (b) of fecal samples from all group mice were showed.

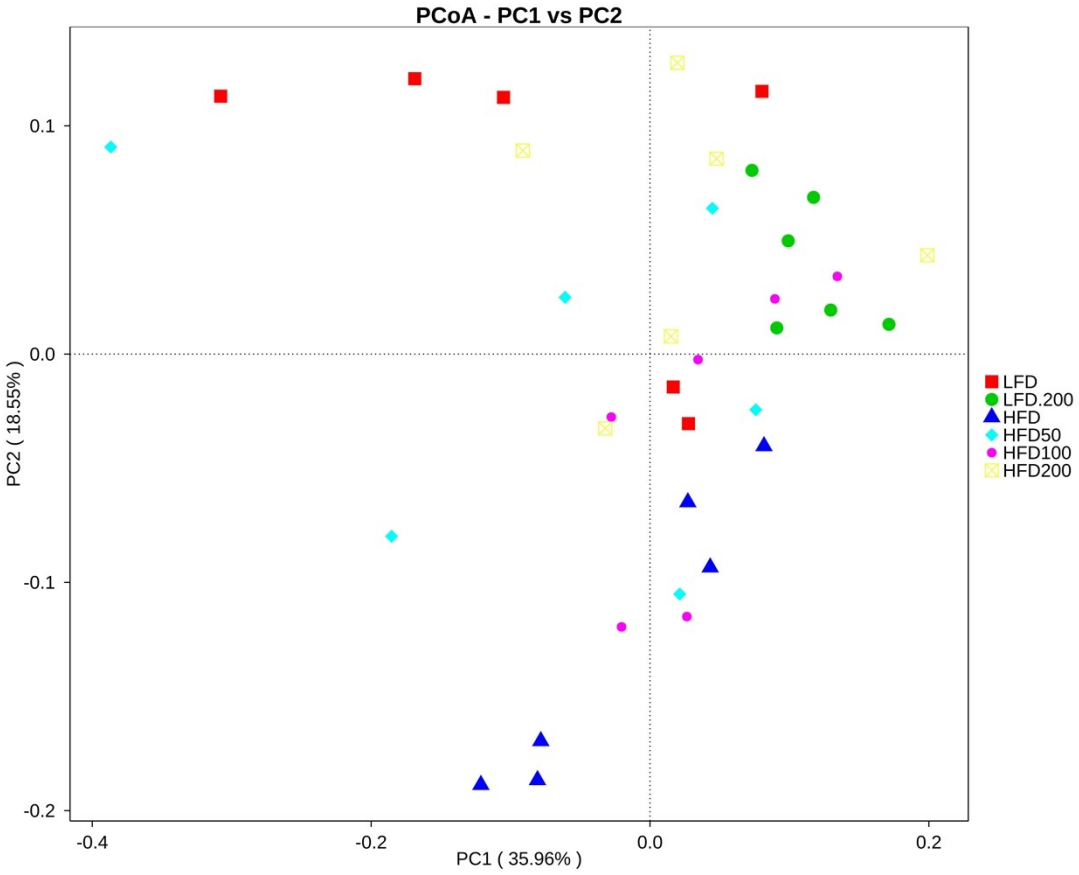


Figure S3 Gut microbiota UniFrac-based PCoA of LJP61A treated mice.

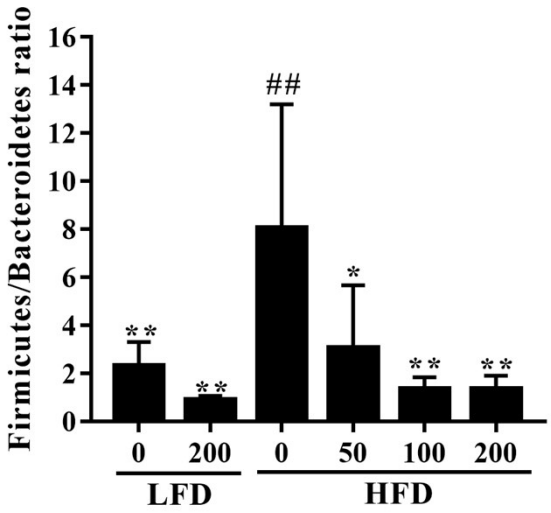
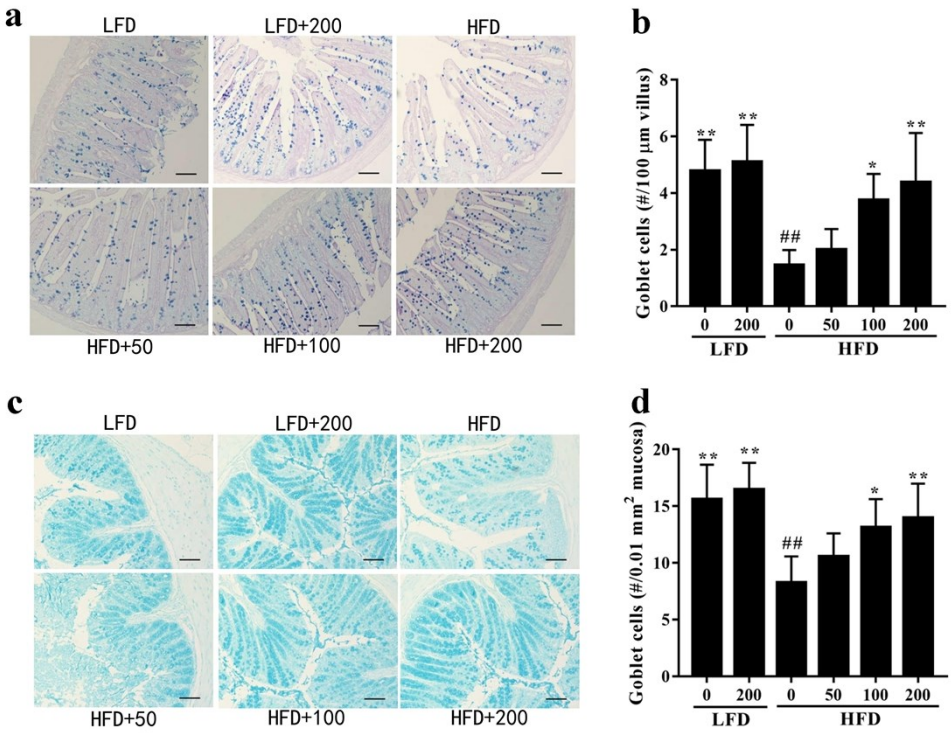
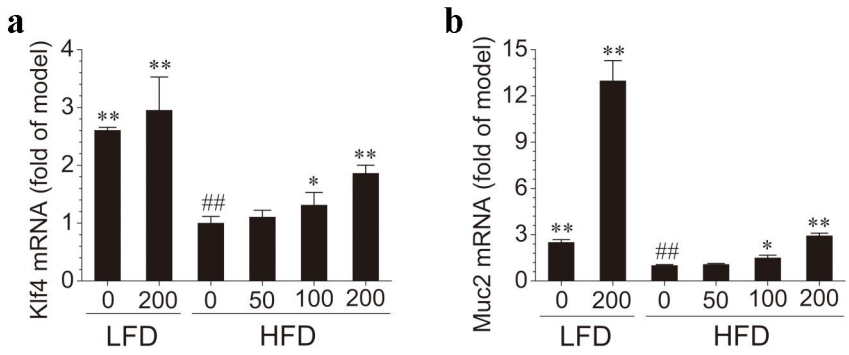


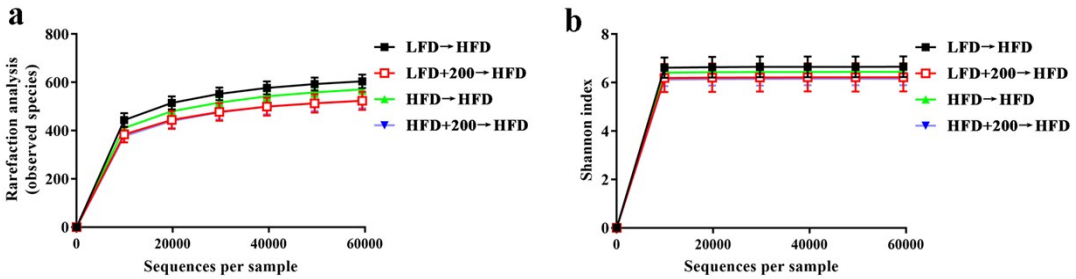
Figure S4 LJP61A modulates the Firmicutes/Bacteroidetes ratio of HFD-fed mice. Mice were treated as described in Figure S1. Gut microbiota composition in stool samples of mice in all groups was determined by 16S rDNA amplicon sequencing and QIIME-based microbiota analysis. Experiments data were showed as the mean ± SD. *$p < 0.05$ and **$p < 0.01$ versus HFD group. ##$p < 0.01$ versus LFD group.



Supplementary Figure S5 LJP61A modulates the number of goblet cells in HFD-fed mice. Mice were fed and administrated as described in Figure S2. The effects of LJP61A on the number of goblet cells in ileum (**a** and **b**) and colon (**c** and **d**) of HFD-fed mice were showed. The number of goblet cells in ileum (**a**) and colon (**b**) were determined by PAS-Alcian blue and Alcian blue staining, respectively. Scale bar in **a** and **b** is 100 μm. Experiments data were showed as the mean ± SD (n ≥ 3). *$p < 0.05$ and **$p < 0.01$ as compared with HFD group. ##$p < 0.01$ as compared with LFD group.



Supplementary Figure S6 LJP61A modulates the mRNA levels of Klf4 (**a**) and Muc2 (**b**) in the colon of HFD-fed mice. Experiments data were showed as the mean ± SD (n ≥ 3). **p* < 0.05 and ***p* < 0.01 as compared with HFD group. ##*p* < 0.01 as compared with LFD group.



Supplementary Figure S7 Gut microbiota alpha diversity analysis of faecal transplant mice. HFD-fed mice were administrated with different faecal transplant materials for 2 months. Rarefaction analysis (a) and Shannon index (b) of fecal samples from all group mice were showed.

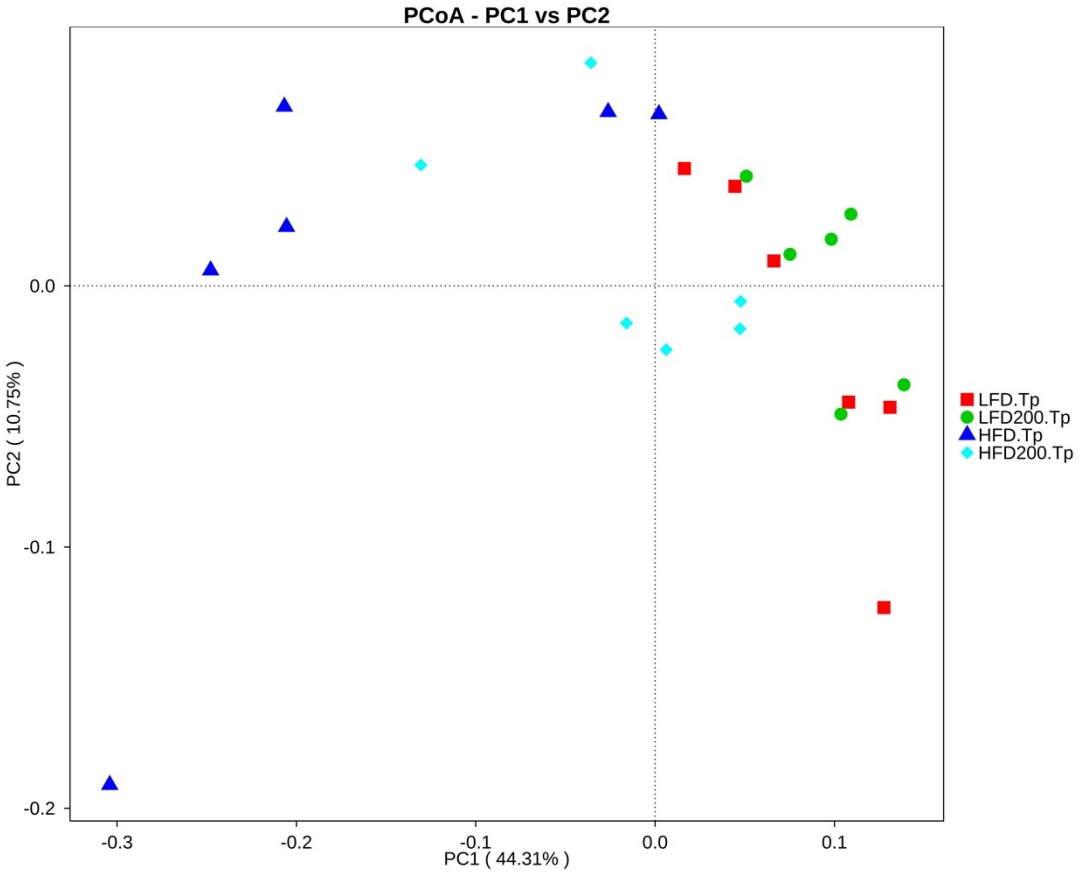


Figure S8 Gut microbiota UniFrac-based PCoA of LJP61A faecal transplant mice.

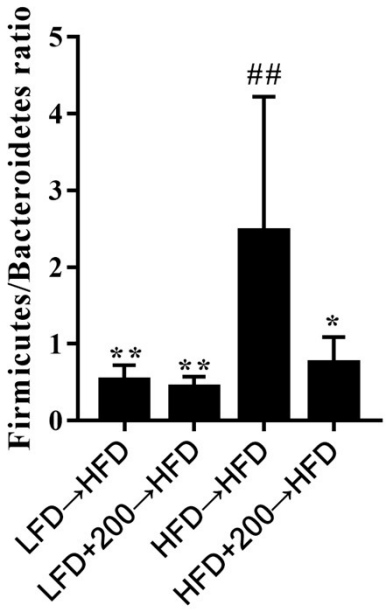
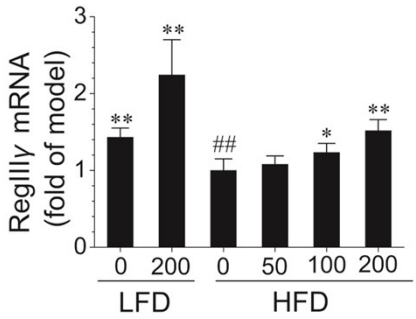


Figure S9 LJP61A faecal transplant modulates the Firmicutes/Bacteroidetes ratio of HFD-fed mice. Mice were treated as described in Figure S6. Gut microbiota composition in stool samples of mice in all groups was determined by 16S rDNA amplicon sequencing and QIIME-based microbiota analysis. Experiments data were showed as the mean ± SD. *$p < 0.05$ and **$p < 0.01$ versus HFD group. ##$p < 0.01$ versus LFD group.



Supplementary Figure S10 LJP61A increases the mRNA expression of RegIIIγ in the colon of HFD-fed mice. Mice were fed and administrated as described in Figure S2. Experiments data were showed as the mean ± SD (n ≥ 3). *p < 0.05 and **p < 0.01 as compared with HFD group. ##p < 0.01 as compared with LFD group.