

Supplementary information

The amelioration of purified *Pleurotus abieticola* polysaccharide on atherosclerosis in ApoE^{-/-} mice

Lei Xing^{† a, b}, Fange Kong^{† a, b}, Chunxia Wang^{a, b}, Lanzhou Li^{a, b}, Shichao Peng^{a, b}, Di Wang^{* a}, Changtian Li^{* a}

a Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, 130118, China.

b College of Plant Protection, Jilin Agricultural University, Changchun, 130118, China.

Corresponding author:

Changtian Li, Ph.D., Professor;

Di Wang, Ph.D., Professor;

Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University

Changchun City, Jilin Province, P.R.China.

Email: lct@jlau.edu.cn

Email: wangdi@jlau.edu.cn

† These authors contributed equally to this work.

Lei Xing (L.X.), Email: xl@mails.jlau.edu.cn

Fange Kong (F.K.), Email: kongfange@mails.jlau.edu.cn

Chunxia Wang (C. W.), Email: wangchunxia@mails.jlau.edu.cn

Lanzhou Li (L.L.), Email: lilanzhou@jlau.edu.cn

Shichao Peng (S.P.), Email: pengshichao@mails.jlau.edu.cn

Methods for determination of UV–Vis and FT-IR

The ultraviolet–visible (UV–Vis) spectrum was used to analyze the presence of proteins and nucleic acids in PAPS2 ¹. Fourier transform infrared spectroscopy (FT-IR) was used to identify the functional groups of PAPS2 ².

References

1. S. Teng, Y. Zhang, X. Jin, Y. Zhu, L. Li, X. Huang, D. Wang and Z. Lin, Structure and hepatoprotective activity of Usp10/NF- κ B/Nrf2 pathway-related *Morchella esculenta* polysaccharide, *Carbohydrate Polymers*, 2023, **303**, 120453.
2. X. Zhang, Z. Cai, H. Mao, P. Hu and X. Li, Isolation and structure elucidation of polysaccharides from fruiting bodies of mushroom *Coriolus versicolor* and evaluation of their immunomodulatory effects, *International Journal of Biological Macromolecules*, 2021, **166**, 1387-1395.

Table list

Table S1 Detail information of antibodies used in western blot.

Antibody	Molecular weight	Catalog number	Dilution
Anti-Nrf2 ^b	100 kDa	a1244	1:1000
Anti-SOD2 ^b	16kDa	A1340	1:1000
Anti-HO-1 ^c	33kDa	AF5393	1:1000
Anti-TLR4 ^b	110k Da	A11226	1:1000
Anti- NF- κ B p65 (phosphor Ser536) ^c	65kDa	AF2006	1:1000
Anti-NF- κ B ^a	60 kDa	ab32536	1:1000
Anti-IL-6 ^d	23 kDa	bs-0782R	1:1000
Anti-TNF- α ^b	25 kDa	A0277	1:1000
Anti-NLRP3 ^b	110 kDa	A5652	1:1000
Anti-IL-1 β ^b	17 kDa	A16288	1:1000
Anti-IL-18 ^b	22 kDa	A1115	1:1000
Anti-GAPDH ^e	37 kDa	E-AB-48016	1:4000
goat anti-mouse ^e		E-AB-1001	1:4000
goat anti- rabbit ^e		E-AB-1003	1:4000

^a Abcam, Cambridge, MA, USA; ^b Wuhan ABclonal Technology Co., Ltd., Wuhan, Hubei, China; ^c Affinity Biosciences, Cincinnati, OH, USA; ^d Beijing bioss Biotechnology Co., Ltd, Beijing, China; ^e Elabscience Biotechnology Co., Ltd., Wuhan, China.

Table S2 Molecular characteristics of PAPS2.

Polydispersity	
Mw/Mn	1.750
Mz/Mn	14.293
Molar mass moments (10⁴g/mol)	
Mn	1.122
Mp	0.763
Mw	1.964
Mz	16.040

Note: Mn: number-average molecular weight; Mp: peak molecular weight; Mw: weight-average molecular weight; Mz: Z-average molecular weight.

Table S3 The information on the standard monosaccharide mixture and the results of the monosaccharide composition of PAPS2.

Composition of standard monosaccharide mixture							
No.	Monosaccharide	Abbreviation	Retention time (min)	No.	Monosaccharide	Abbreviation	Retention time (min)
1	Fucose	Fuc	3.7668	8	Fructose	Fru	16.4002
2	Rhamnose	Rha	8.0252	9	Ribose	Rib	17.5418
3	Arabinose	Ara	8.3502	10	Galacturonic Acid	Gal-UA	33.0585
4	Galactose	Gal	9.9335	11	Glucuronic Acid	Glc-UA	33.5252
5	Glucose	Glc	11.7585	12	Mannuronic Acid	Man-UA	35.1502
6	Xylose	Xyl	13.8168	13	Guluronic Acid	Gul-UA	37.0168
7	Mannose	Man	14.8252				

Monosaccharide composition of PAPS2							
No.	Monosaccharide	Abbreviation	Content (%)	No.	Monosaccharide	Abbreviation	Content (%)
1	Fucose	Fuc	2.5	4	Mannose	Man	26.20
2	Galactose	Gal	37.98	5	Glucuronic Acid	Glc-UA	1.8
3	Glucose	Glc	31.45				

Table S4 Dominant nodes of intestinal flora in mice

Group	Taxa	Abundance	LDA_score	P value
HFD	Bacteria.Firmicutes.Clostridia.Clostridiales.Lachnospiraceae._ <i>Ruminococcus</i> _	4.23	3.83	0.018
HFD	Bacteria.Firmicutes.Bacilli.Turicibacterales.Turicibacteraceae. <i>Turicibacter</i>	4.60	4.27	0.012
HFD	Bacteria.Firmicutes.Bacilli.Turicibacterales	4.60	4.29	0.012
HFD	Bacteria.Firmicutes.Clostridia.Clostridiales.Lachnospiraceae._<i>Ruminococcus</i>_._<i>Ruminococcus</i>_ <i>gnavus</i>	4.23	3.82	0.018
HFD	Bacteria.Firmicutes.Clostridia	5.75	5.21	0.024
HFD	Bacteria.Firmicutes.Clostridia.Clostridiales.Lachnospiraceae	5.47	5.01	0.023
HFD	Bacteria.Firmicutes.Bacilli.Turicibacterales.Turicibacteraceae	4.60	4.29	0.012
HFD	Bacteria.Firmicutes.Clostridia.Clostridiales	5.75	5.23	0.024
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Bifidobacteriales.Bifidobacteriaceae. <i>Bifidobacterium</i> . <i>Bifidobacterium_pseudolongum</i>	3.58	3.35	0.010
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae. <i>Salinicoccus</i>	2.35	3.84	0.024
Ctrl	Bacteria.Firmicutes.Bacilli.Lactobacillales.Aerococcaceae. <i>Atopostipes</i>	3.26	3.22	0.005
Ctrl	Bacteria.Proteobacteria.Gammaproteobacteria	2.97	3.50	0.031
Ctrl	Bacteria.Actinobacteria.Actinobacteria	4.27	3.96	0.012
Ctrl	Bacteria.Firmicutes.Bacilli.Lactobacillales.Aerococcaceae	4.17	3.86	0.010
Ctrl	Bacteria.Bacteroidetes	5.45	4.94	0.024
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Planococcaceae	3.84	3.59	0.022
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae	3.69	3.44	0.026
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales	5.50	5.24	0.025
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Actinomycetales.Corynebacteriaceae	4.16	3.85	0.017
Ctrl	Bacteria.Bacteroidetes.Bacteroidia	5.45	4.94	0.024
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae. <i>Jeotgalicoccus</i> . <i>Jeotgalicoccus_psychrophilus</i>	3.48	3.25	0.044
Ctrl	Bacteria.Proteobacteria.Gammaproteobacteria.Pseudomonadales.Moraxellaceae	2.30	3.73	0.048

Ctrl	Bacteria.Firmicutes.Bacilli.Lactobacillales.Aerococcaceae. <i>Atopostipes</i> . <i>Atopostipes_suicloacalis</i>	3.26	3.73	0.005
Ctrl	Bacteria.Firmicutes.Bacilli.Lactobacillales.Aerococcaceae. <i>Facklamia</i>	3.34	3.13	0.005
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae	5.43	5.13	0.025
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Actinomycetales. <i>Corynebacteriaceae</i> . <i>Corynebacterium</i>	4.16	3.86	0.017
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae. <i>Staphylococcus</i> . <i>Staphylococcus_sciuri</i>	4.45	4.17	0.020
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales	5.45	4.92	0.024
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Bifidobacteriales.Bifidobacteriaceae. <i>Bifidobacterium</i> . <i>Bifidobacterium_bifidum</i>	2.12	3.60	0.005
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Actinomycetales. <i>Corynebacteriaceae</i> . <i>Corynebacterium</i> . <i>Corynebacterium_stationis</i>	4.15	3.84	0.012
Ctrl	Bacteria.Firmicutes.Erysipelotrichi.Erysipelotrichales.Erysipelotrichaceae. <i>Clostridium</i>	2.94	3.87	0.009
Ctrl	Bacteria.Firmicutes.Erysipelotrichi.Erysipelotrichales.Erysipelotrichaceae. <i>Clostridium</i> . <i>Clostridium_cleatum</i>	2.86	3.99	0.009
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Planococcaceae. <i>Sporosarcina</i>	2.82	3.42	0.015
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Planococcaceae. <i>Staphylococcus</i> . <i>Staphylococcus_saprophyticus</i>	3.77	3.51	0.022
Ctrl	Bacteria.Firmicutes.Bacilli	5.55	5.17	0.037
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae. <i>Jeotgalicoccus</i>	3.48	3.18	0.044
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Staphylococcaceae. <i>Staphylococcus</i>	5.42	5.13	0.023
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Bifidobacteriales	3.61	3.42	0.017
Ctrl	Bacteria.Proteobacteria.Gammaproteobacteria.Pseudomonadales	2.30	3.77	0.048
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae.Bacteroides	3.69	3.43	0.026
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Actinomycetales	4.16	3.84	0.023
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae. <i>Bacteroides</i> . <i>Bacteroides_acidifaciens</i>	3.50	3.22	0.043
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae. <i>Parabacteroides</i>	3.35	3.14	0.048
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Bifidobacteriales.Bifidobacteriaceae	3.61	3.41	0.017
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales._Odoribacteraceae_	4.06	3.70	0.015

Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales._Odoribacteraceae_. <i>Odoribacter</i>	4.06	3.72	0.015
Ctrl	Bacteria.Actinobacteria.Actinobacteria.Bifidobacteriales.Bifidobacteriaceae. <i>Bifidobacterium</i>	3.61	3.37	0.017
Ctrl	Bacteria.Firmicutes.Bacilli.Gemellales	2.93	3.14	0.009
Ctrl	Bacteria.Firmicutes.Bacilli.Bacillales.Planococcaceae. <i>Staphylococcus</i>	3.77	3.49	0.022
Ctrl	Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae	3.35	3.14	0.048
HFD+PAPS2	Bacteria.Firmicutes.Erysipelotrichi.Erysipelotrichales.Erysipelotrichaceae. <i>Allobaculum</i>	4.64	4.34	0.025
HFD+PAPS2	Bacteria.Deferribacteres	3.15	3.04	0.024
HFD+PAPS2	Bacteria.Firmicutes.Erysipelotrichi	4.65	4.28	0.025
HFD+PAPS2	Bacteria.Firmicutes.Clostridia.Clostridiales.Lachnospiraceae. <i>Coprococcus</i>	4.29	3.90	0.021
HFD+PAPS2	Bacteria.Firmicutes.Clostridia.Clostridiales.Lachnospiraceae.Roseburia	3.19	3.52	0.009
HFD+PAPS2	Bacteria.Deferribacteres.Deferribacteres.Deferribacterales.Deferribacteraceae	3.15	3.07	0.024
HFD+PAPS2	Bacteria.Firmicutes.Erysipelotrichi.Erysipelotrichales.Erysipelotrichaceae	4.65	4.31	0.025
HFD+PAPS2	Bacteria.Firmicutes.Bacilli.Lactobacillales.Lactobacillaceae. <i>Lactobacillus.Lactobacillus_vaginalis</i>	4.21	3.93	0.022
HFD+PAPS2	Bacteria.Deferribacteres.Deferribacteres.Deferribacterales.Deferribacteraceae. <i>Mucispirillum.Mucispirillum_schaedleri</i>	3.15	3.03	0.024
HFD+PAPS2	Bacteria.Firmicutes.Erysipelotrichi.Erysipelotrichales	4.65	4.29	0.025
HFD+PAPS2	Bacteria.Deferribacteres.Deferribacteres	3.15	3.04	0.024
HFD+PAPS2	Bacteria.Deferribacteres.Deferribacteres.Deferribacterales.Deferribacteraceae. <i>Mucispirillum</i>	3.15	3.04	0.024
HFD+PAPS2	Bacteria.Deferribacteres.Deferribacteres.Deferribacterales	3.15	3.06	0.024

Data are presented as mean (n = 4) and were analyzed using a one-way ANOVA test followed by post-hoc Dunn's multiple comparison test. The size of dominant nodes was proportional to the relative abundance of the operational taxonomic units (measured as log 10 of median reads). The LDA score and p-value were calculated.

Table S5 Differential lipids in serum lipid metabolism in mice.

	name	Class	Normalized intensity ($\times 10^6$)			HFD vs. Ctrl		HFD+PAPS2 vs. HFD	
			Ctrl	HFD	HFD+PAPS2	Fold change	P value	Fold change	P value
1	AcCa(12:1)	AcCa	0.15	0.09	0.13	1.76	0.013	1.46	0.010
2	BisMePA(20:5_18:2)	BisMePA	0.47	0.05	0.10	9.91	0.004	2.14	0.048
3	Cer(d38:0)	Cer	0.44	0.25	0.33	1.74	0.035	1.29	0.045
4	ChE(22:4)	ChE	1.20	0.44	0.65	2.73	0.000	1.48	0.012
5	CmE(18:2)	CmE	1.88	0.83	1.51	2.26	0.005	1.82	0.000
6	CmE(20:4)	CmE	0.95	0.07	0.12	13.89	0.001	1.75	0.010
7	CmE(22:6)	CmE	0.82	0.06	0.08	13.35	0.000	1.37	0.036
8	MePC(30:4e)	MePC	0.04	0.02	0.03	2.26	0.037	1.68	0.046
9	MePC(38:5)	MePC	0.05	0.02	0.05	2.89	0.000	2.74	0.010
10	PC(20:3)	PC	0.19	0.07	0.25	2.74	0.013	3.67	0.034
11	PC(33:5)	PC	0.03	0.01	0.02	2.85	0.033	2.10	0.048
12	PC(35:6)	PC	0.73	0.07	0.16	9.91	0.004	2.14	0.048
13	PC(38:4)	PC	224.59	145.77	180.73	1.54	0.001	1.24	0.001
14	PC(41:6)	PC	0.43	0.28	0.45	1.54	0.025	1.62	0.038
15	PE(16:1_18:2)	PE	0.11	0.02	0.04	5.47	0.010	2.15	0.026
16	SPH(d20:0)	SPH	2.25	1.42	2.41	1.59	0.015	1.70	0.005
17	SiE(20:4)	SiE	2.07	0.76	1.13	2.72	0.000	1.49	0.013
18	LPC(14:0)	LPC	0.07	0.05	0.08	1.41	0.002	1.73	0.028
19	LPC(18:3)	LPC	0.48	0.30	0.47	1.62	0.005	1.59	0.032
20	LdMePE(16:1)	LdMePE	0.04	0.03	0.04	1.47	0.017	1.53	0.048
21	PC(18:0_20:4)	PC	9.44	6.62	8.85	1.43	0.005	1.34	0.039
22	dMePE(18:1_18:2)	dMePE	0.04	0.03	0.04	1.72	0.011	1.57	0.022
23	BisMePA(30:1_18:1)	BisMePA	0.05	0.14	0.06	0.32	0.018	0.40	0.033

name	Class	Normalized intensity ($\times 10^6$)			HFD v.s. Ctrl		HFD+PAPS2 v.s HFD		
		Ctrl	HFD	HFD+PAPS2	Fold change	P value	Fold change	P value	
24	TG(18:0_16:0_16:0)	TG	6.37	30.90	18.45	0.21	0.000	0.60	0.034
25	TG(16:1_16:1_18:3)	TG	0.27	0.40	0.30	0.68	0.031	0.76	0.040
26	TG(16:0_17:0_18:1)	TG	2.59	3.83	2.52	0.68	0.028	0.66	0.039
27	TG(18:1e_16:0_18:1)	TG	0.03	3.20	1.44	0.01	0.001	0.45	0.020
28	TG(16:0_18:1_19:0)	TG	1.43	2.81	2.04	0.51	0.001	0.73	0.006
29	TG(18:4_18:1_18:2)	TG	0.55	1.39	0.98	0.39	0.000	0.70	0.004
30	TG(20:0_16:0_20:4)	TG	0.44	0.94	0.56	0.46	0.006	0.59	0.023
31	TG(18:1_18:3_20:5)	TG	0.27	0.46	0.33	0.58	0.001	0.72	0.006
32	TG(16:0_20:1_21:0)	TG	0.48	1.10	0.82	0.44	0.002	0.75	0.020
33	TG(18:1_18:1_23:0)	TG	0.78	1.64	1.11	0.48	0.003	0.68	0.031
34	TG(60:5e)	TG	0.43	3.54	1.68	0.12	0.000	0.47	0.000
35	TG(25:1_18:1_18:1)	TG	0.24	0.38	0.24	0.63	0.033	0.64	0.021
36	TG(26:1_18:1_18:1)	TG	0.38	0.92	0.54	0.42	0.005	0.59	0.017
37	TG(28:1_18:1_18:2)	TG	0.08	0.15	0.08	0.54	0.028	0.50	0.004
38	TG(29:1_18:1_18:1)	TG	0.07	0.29	0.22	0.24	0.000	0.74	0.035
39	TG(30:1_18:1_18:2)	TG	0.07	0.26	0.16	0.25	0.000	0.60	0.008
40	TG(30:1_18:2_18:2)	TG	0.03	0.06	0.01	0.53	0.033	0.21	0.004
41	TG(26:1_18:2_24:2)	TG	0.11	0.29	0.20	0.40	0.000	0.68	0.036
42	TG(70:5)	TG	0.06	0.19	0.10	0.31	0.001	0.55	0.044

Data were presented as mean (n=4). Statistically significant differences using a T-TEST ($p < 0.05$) in metabolite levels and fold change ≥ 1.5 or ≤ 0.667 were regarded as the standard for differential lipids.

Figure list

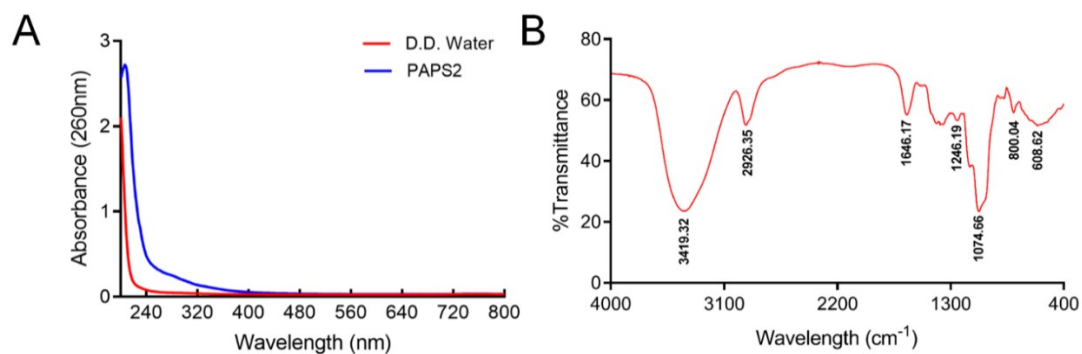


Fig. S 1 The UV-Vis and FT-IR spectrums of PAPS2. (A) The UV-Vis spectrum of PAPS2 at the wavelength range of 200–800 nm. The blue line represents PAPS2; the red line represents the negative control (D.D. water). (B) The FT-IR spectrum of PAPS2 at the wavelength range of 4000–400 cm⁻¹.

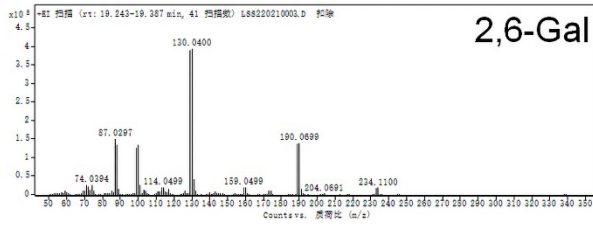
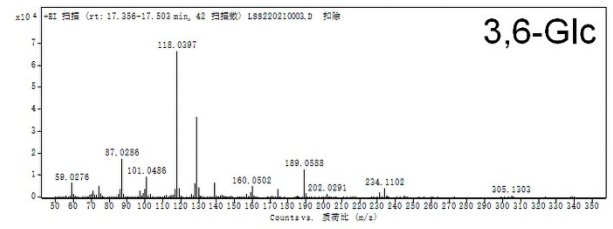
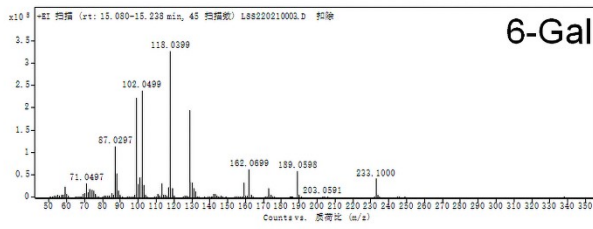
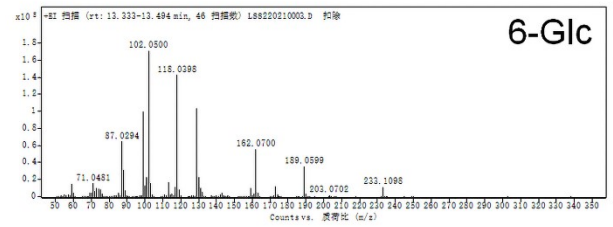
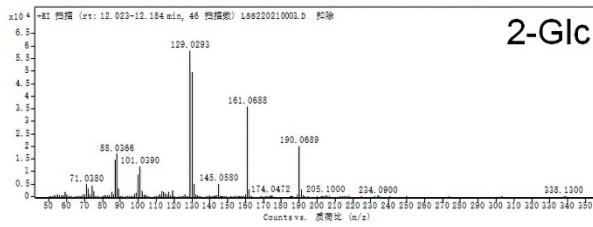
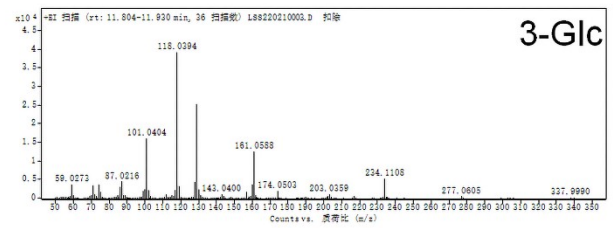
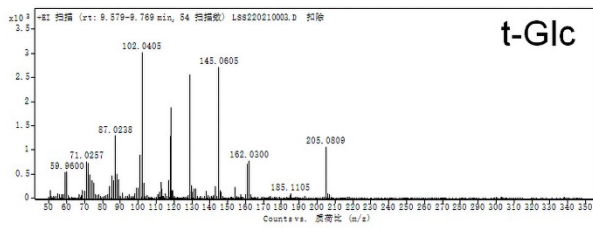
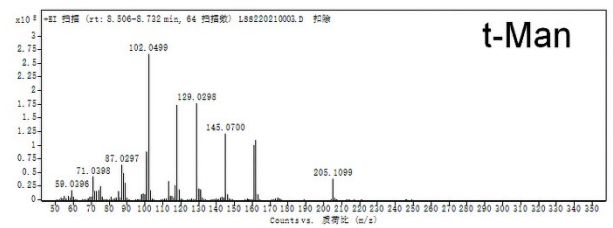
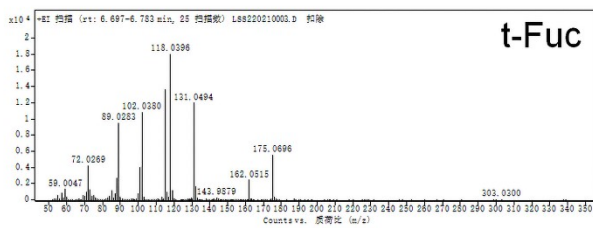
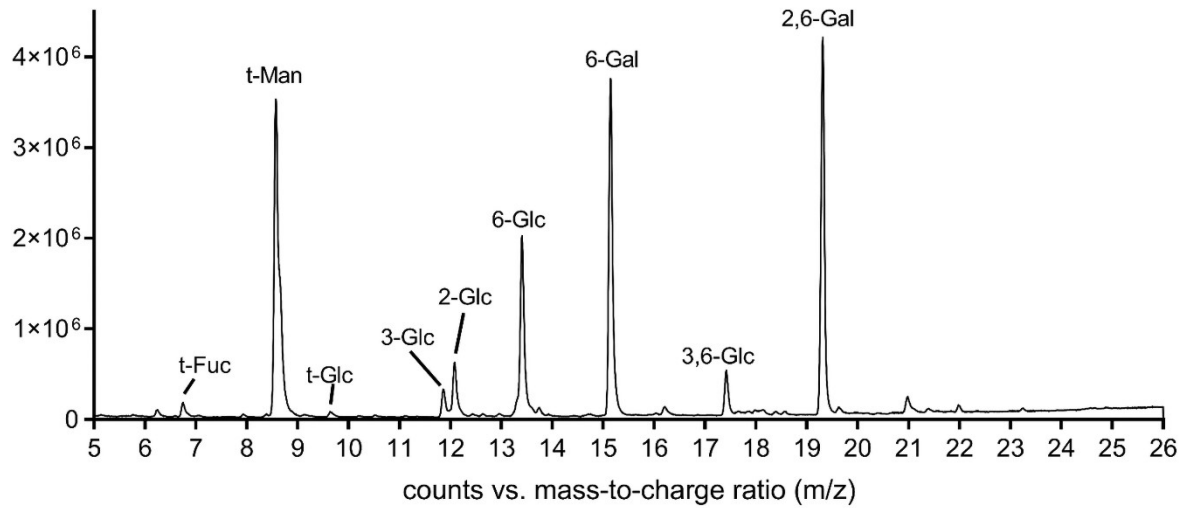


Fig. S2 Methylation GC-MS chart of PAPS2.

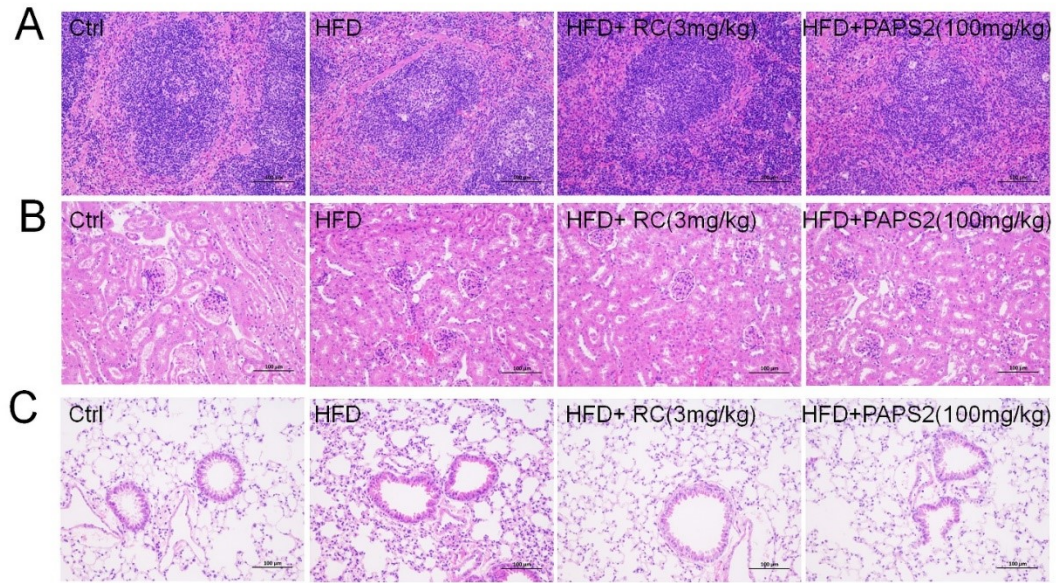


Fig. S3 PAPS2 shows no significant effect on the (A) spleen and (B) kidney, and relived the inflammatory infiltration in (C) lungs in HFD-fed ApoE^{-/-} mice (n=3).