Supplemental Material

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Manuscript Title: Pulchinenoside B4 ameliorates oral ulcers in rats by modulating gut microbiota and metabolites

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H&E staining protocol

OU tissue sections were deparaffinized with xylene and washed with various levels of ethanol in the following steps: Sections were stained with hematoxylin solution for 3 to 5min, then differentiated with

differentiation solution, stained with back blue solution (0.4% ammonia), and finally rinsed with tap water. The rinsed sections were treated in absolute ethanol for 5 min(5 min each time, repeated 3 times) and xylene for 5 min(5 min each time, repeated 2 times) to make them transparent, and then the sections were dried and sealed with neutral glue and photographed under an electron microscope for histopathological changes.

Figures:

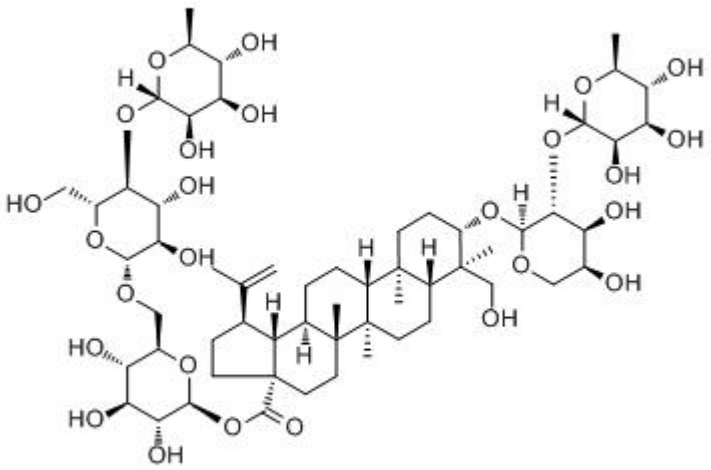


Fig. S1. *Pulsatilla* saponin B4 structure. Structure reference vital genesis network (https://www.chemsrc.com/cas/129741-57-7_895739.html)

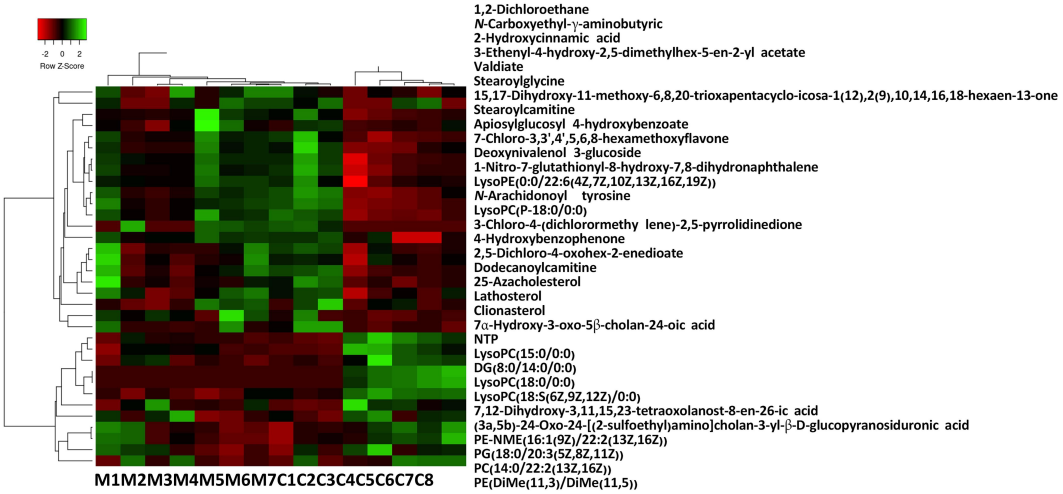


Fig. S2-1. Serum metabonomics study of rat oral ulcer model with clustering fever related to rats in normal and OU model groups.

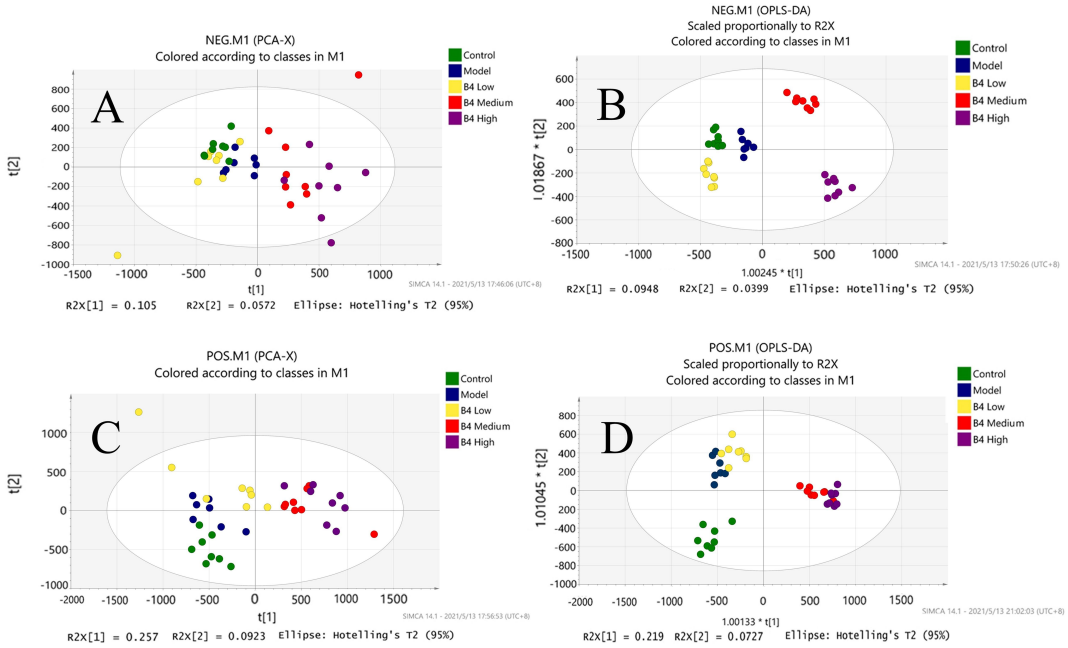


Fig. S2-2. Serum metabonomics study of rat oral ulcer model. A: PCA profile of each group of serum samples in negative ion mode. B: OPLS-DA profile of each group of serum samples in negative ion mode. C: PCA of each group of serum samples in positive ion mode contour map. D: OPLS-DA contour map of each group of serum samples in positive ion mode.

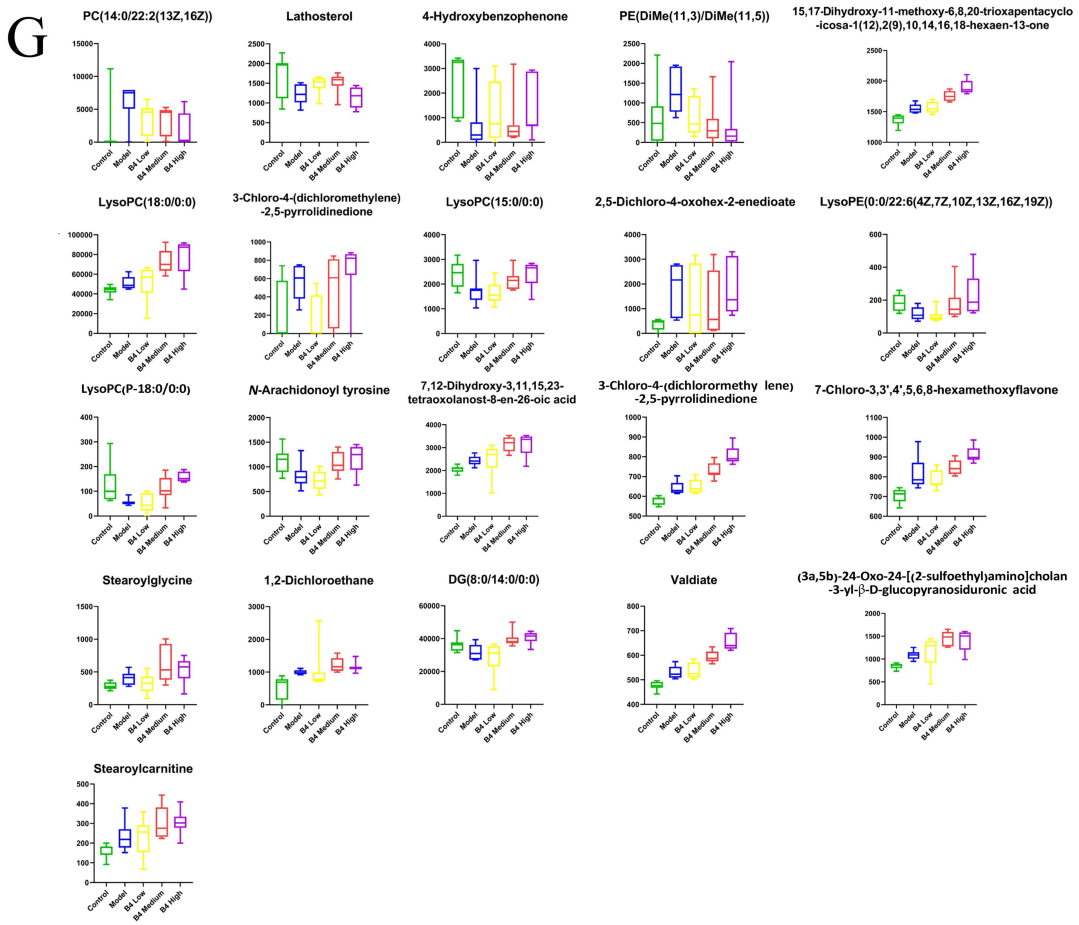


Fig. S2-3. Serum metabonomics study of rat oral ulcer model. G: Box graph to study the regulation of each group of biomarkers after administration.

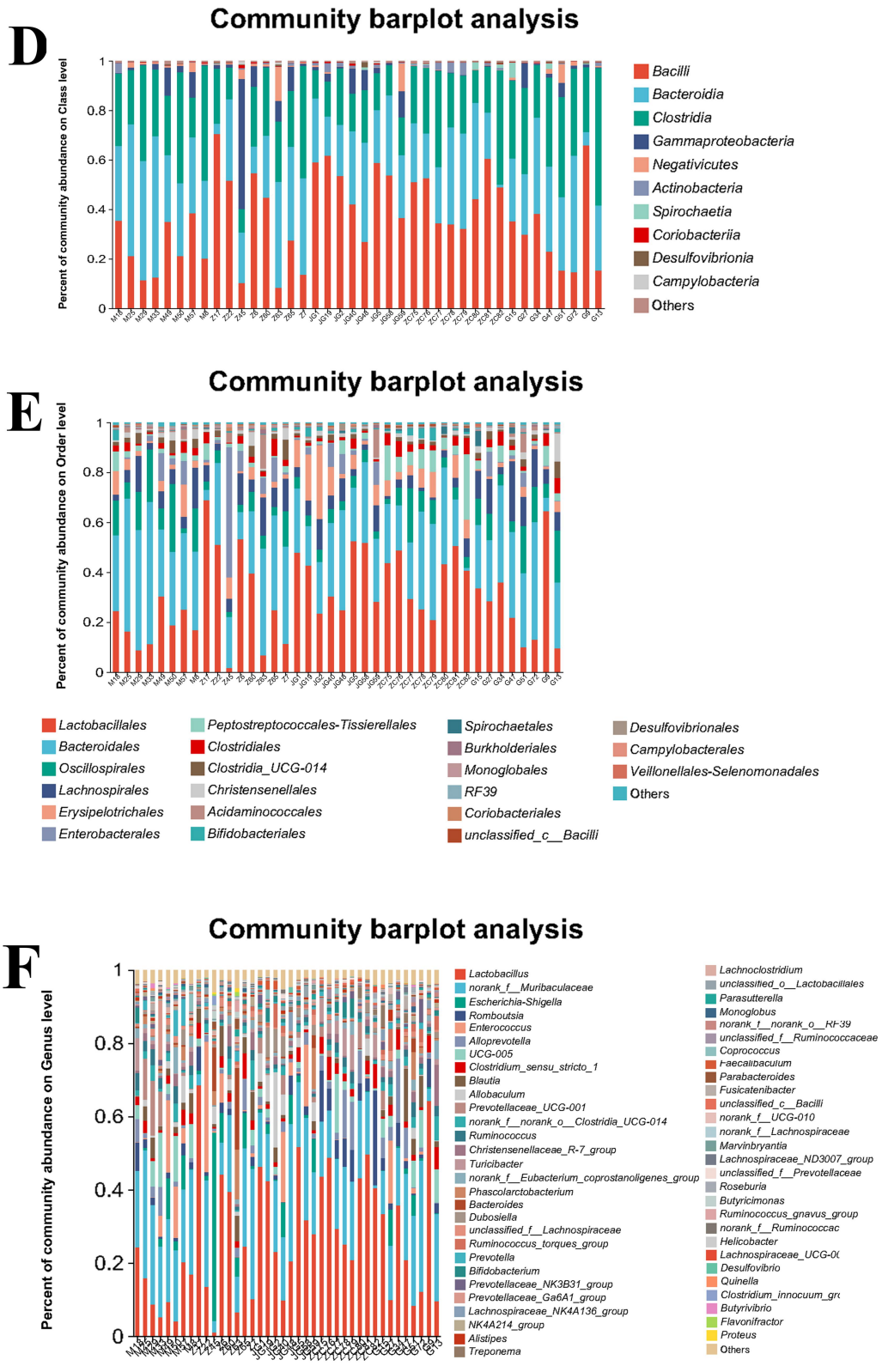
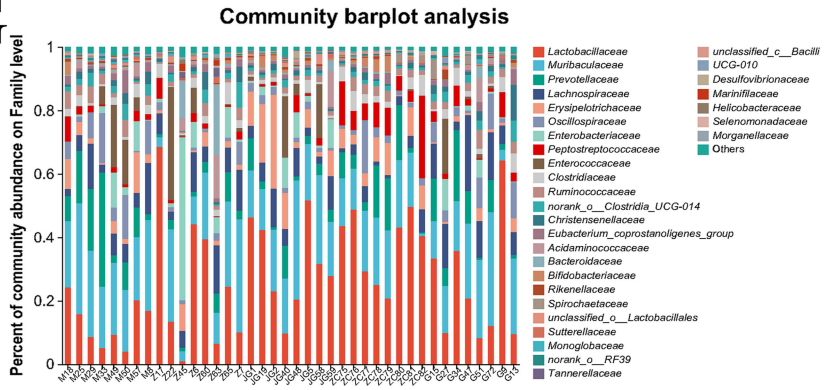
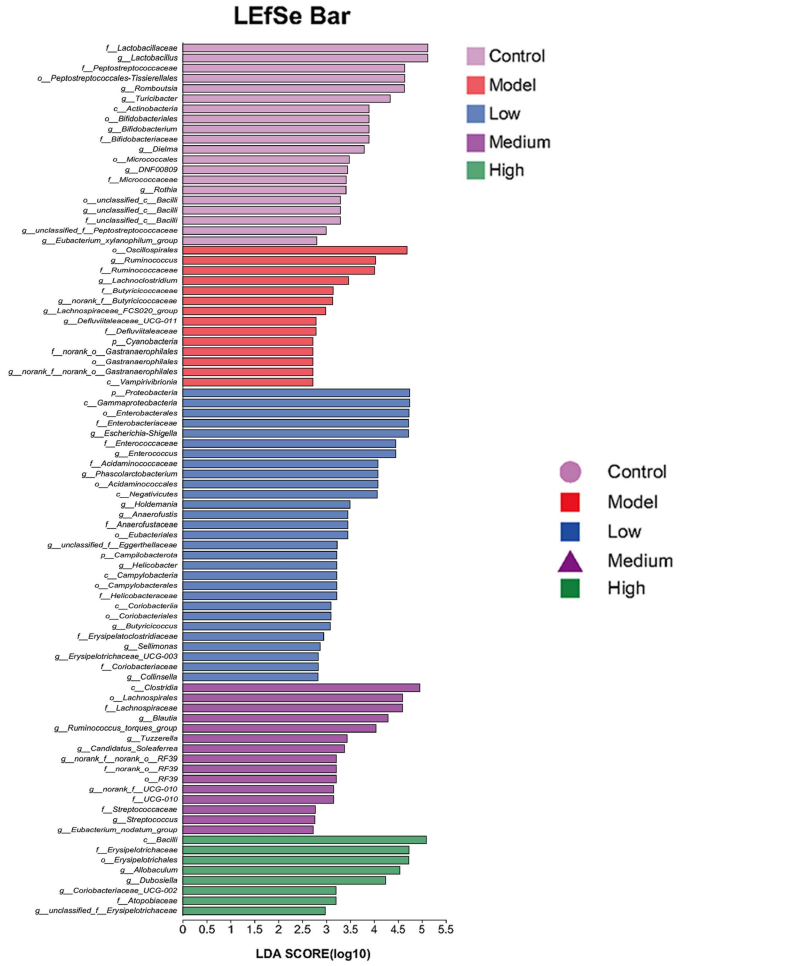


Fig. S3-1. Evaluation of the overall sample of rat intestinal microbiota.D-F: Bacteria at the level of phylum, family, class, order, and genus analysis of the overall species abundance of the population.

G


Community barplot analysis
Percent of community abundance on Family level
1
0.8
0.6
0.4
0.2
0
Lactobacillaceae
Muribaculaceae
Prevotellaceae
Lachnospiraceae
Erysipelotrichaceae
Oscillospiraceae
Enterobacteriaceae
Peptostreptococcaceae
Enterococcaceae
Clostridiaceae
Ruminococcaceae
norank_o__Clostridia_UCG-014
Christensenellaceae
Eubacterium_coprostanoligenes_group
Acidaminococcaceae
Bacteroidaceae
Bifidobacteriaceae
Rikenellaceae
Spirochaetaceae
unclassified_o__Lactobacillales
Sutterellaceae
Monoglobaceae
norank_o__RF39
Tannerellaceae
unclassified_c__Bacilli
UCG-010
Desulfovibrionaceae
Marinifilaceae
Helicobacteraceae
Selenomonadaceae
Morganellaceae
Others

H


LEfSe Bar
f__Lactobacillaceae
g__Lactobacillus
f__Peptostreptococcaceae
o__Peptostreptococcales-Tissierellales
g__Romboutsia
g__Turicibacter
c__Actinobacteria
o__Bifidobacteriales
g__Bifidobacterium
f__Bifidobacteriaceae
g__Dielma
o__Micrococcales
g__DNF00809
f__Micrococcaceae
g__Rothia
o__unclassified_c__Bacilli
g__unclassified_c__Bacilli
f__unclassified_c__Bacilli
g__unclassified_f__Peptostreptococcaceae
g__Eubacterium_xylanophilum_group
o__Oscillospirales
g__Ruminococcus
f__Ruminococcaceae
g__Lachnoclostridium
f__Butyricicoccaceae
g__norank_f__Butyricicoccaceae
g__Lachnospiraceae_FCS020_group
g__Defluviitaleaceae_UCG-011
f__Defluviitaleaceae
p__Cyanobacteria
f__norank_o__Gastranaerophilales
o__Gastranaerophilales
g__norank_f__norank_o__Gastranaerophilales
c__Vampirivibrionia
p__Proteobacteria
c__Gammaproteobacteria
o__Enterobacterales
f__Enterobacteriaceae
g__Escherichia-Shigella
f__Enterococcaceae
g__Enterococcus
f__Acidaminococcaceae
g__Phascolarctobacterium
o__Acidaminococcales
c__Negativicutes
g__Holdemania
g__Anaerofustis
f__Anaerofustaceae
o__Eubacteriales
g__unclassified_f__Eggerthellaceae
p__Campilobacterota
g__Helicobacter
c__Campylobacteria
o__Campylobacterales
f__Helicobacteraceae
c__Coriobacteriia
o__Coriobacteriales
g__Butyricicoccus
f__Erysipelatoclostridiaceae
g__Sellimonas
g__Erysipelotrichaceae_UCG-003
f__Coriobacteriaceae
g__Collinsella
c__Clostridia
o__Lachnospirales
f__Lachnospiraceae
g__Blautia
g__Ruminococcus_torques_group
g__Tuzzerella
g__Candidatus_Soleaferrea
g__norank_f__norank_o__RF39
f__norank_o__RF39
o__RF39
g__norank_f__UCG-010
f__UCG-010
f__Streptococcaceae
g__Streptococcus
g__Eubacterium_nodatum_group
c__Bacilli
f__Erysipelotrichaceae
o__Erysipelotrichales
g__Allobaculum
g__Dubosiella
g__Coriobacteriaceae_UCG-002
f__Atopobiaceae
g__unclassified_f__Erysipelotrichaceae
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
LDA SCORE(log10)
Control
Model
Low
Medium
High
Control
Model
Low
Medium
High

I

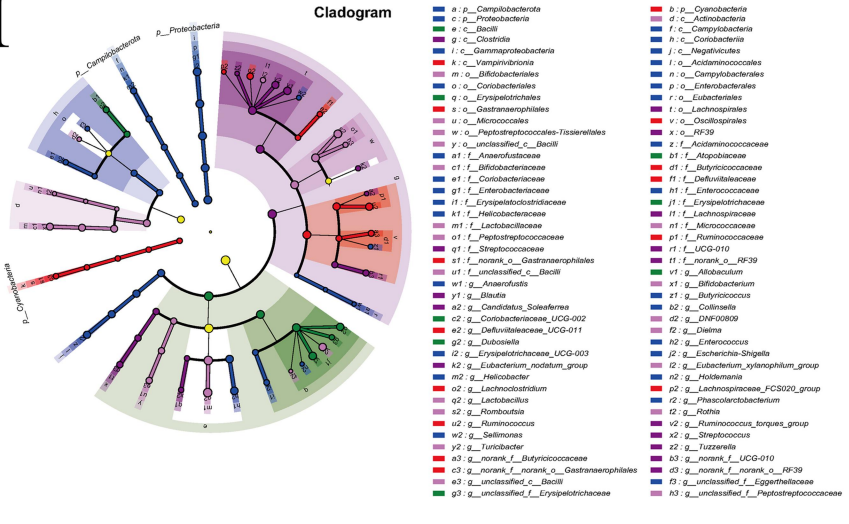

Cladogram
p__Campilobacterota
p__Proteobacteria
p__Cyanobacteria
a : p__Campilobacterota
c : p__Proteobacteria
e : c__Bacilli
g : c__Clostridia
i : c__Gammaproteobacteria
k : c__Vampirivibrionia
m : o__Bifidobacteriales
o : o__Coriobacteriales
q : o__Erysipelotrichales
s : o__Gastranaerophilales
u : o__Micrococcales
w : o__Peptostreptococcales-Tissierellales
y : o__unclassified_c__Bacilli
a1 : f__Anaerofustaceae
c1 : f__Bifidobacteriaceae
e1 : f__Coriobacteriaceae
g1 : f__Enterobacteriaceae
i1 : f__Erysipelatoclostridiaceae
k1 : f__Helicobacteraceae
m1 : f__Lactobacillaceae
o1 : f__Peptostreptococcaceae
q1 : f__Streptococcaceae
s1 : f__norank_o__Gastranaerophilales
u1 : f__unclassified_c__Bacilli
w1 : g__Anaerofustis
y1 : g__Blautia
a2 : g__Candidatus_Soleaferrea
c2 : g__Coriobacteriaceae_UCG-002
e2 : g__Defluviitaleaceae_UCG-011
g2 : g__Dubosiella
i2 : g__Erysipelotrichaceae_UCG-003
k2 : g__Eubacterium_nodatum_group
m2 : g__Helicobacter
o2 : g__Lachnoclostridium
q2 : g__Lactobacillus
s2 : g__Romboutsia
u2 : g__Ruminococcus
w2 : g__Sellimonas
y2 : g__Turicibacter
a3 : g__norank_f__Butyricicoccaceae
c3 : g__norank_f__norank_o__Gastranaerophilales
e3 : g__unclassified_c__Bacilli
g3 : g__unclassified_f__Erysipelotrichaceae
b : p__Cyanobacteria
d : c__Actinobacteria
f : c__Campylobacteria
h : c__Coriobacteriia
j : c__Negativicutes
l : o__Acidaminococcales
n : o__Campylobacterales
p : o__Enterobacterales
r : o__Eubacteriales
t : o__Lachnospirales
v : o__Oscillospirales
x : o__RF39
z : f__Acidaminococcaceae
b1 : f__Atopobiaceae
d1 : f__Butyricicoccaceae
f1 : f__Defluviitaleaceae
h1 : f__Enterococcaceae
j1 : f__Erysipelotrichaceae
l1 : f__Lachnospiraceae
n1 : f__Micrococcaceae
p1 : f__Ruminococcaceae
r1 : f__UCG-010
t1 : f__norank_o__RF39
v1 : g__Allobaculum
x1 : g__Bifidobacterium
z1 : g__Butyricicoccus
b2 : g__Collinsella
d2 : g__DNF00809
f2 : g__Dielma
h2 : g__Enterococcus
j2 : g__Escherichia-Shigella
l2 : g__Eubacterium_xylanophilum_group
n2 : g__Holdemania
p2 : g__Lachnospiraceae_FCS020_group
r2 : g__Phascolarctobacterium
t2 : g__Rothia
v2 : g__Ruminococcus_torques_group
x2 : g__Streptococcus
z2 : g__Tuzzerella
b3 : g__norank_f__UCG-010
d3 : g__norank_f__norank_o__RF39
f3 : g__unclassified_f__Eggerthellaceae
h3 : g__unclassified_f__Peptostreptococcaceae

Fig. S3-2. Evaluation of the overall sample of rat intestinal microbiota. G: Intestinal bacteria at the level of phylum, family, class, order, and genus analysis of the overall species abundance of the population; H: LEfSe analysis of the effect of species abundance on the difference effect; I: Cladogram diagram to explain the significant difference dominant species.

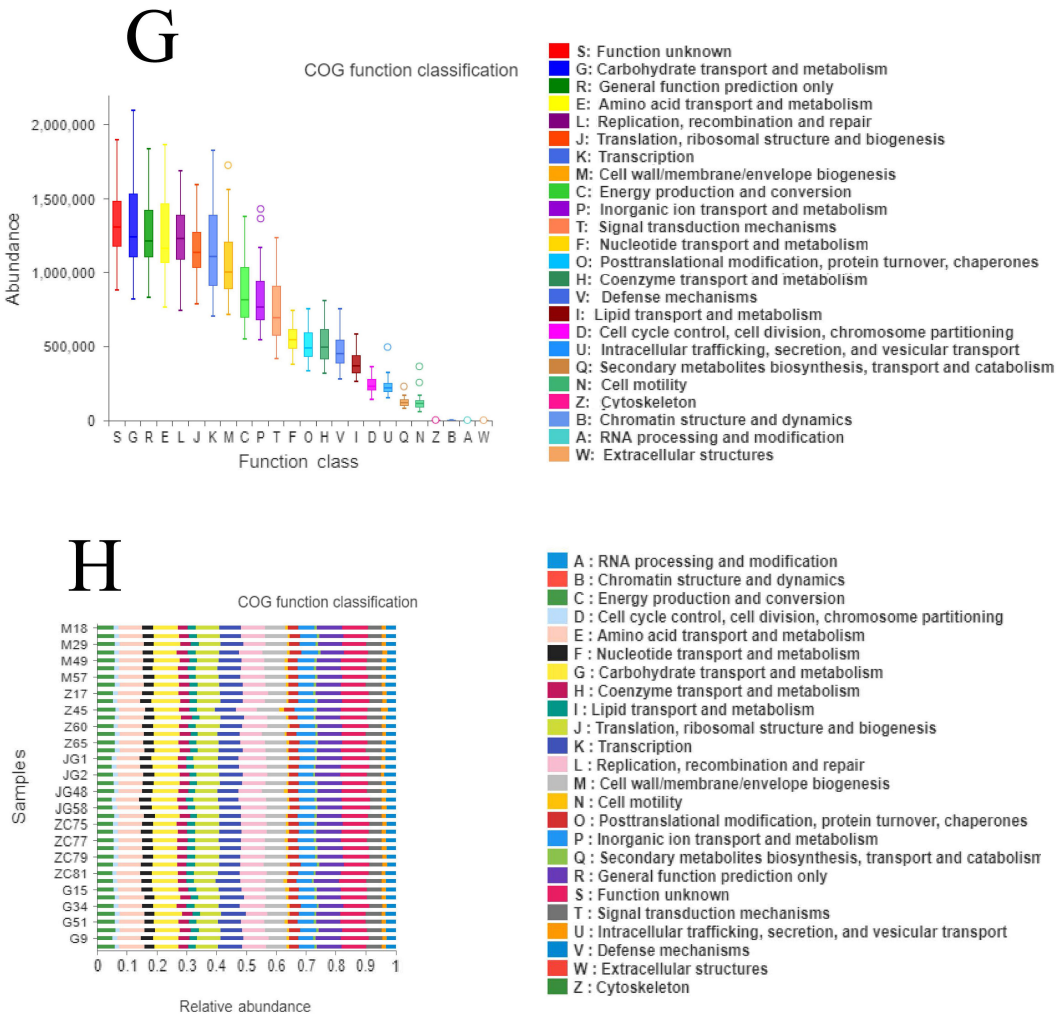


Fig. S4. Microbiota data analysis and function classification. G-H: COG function classification.

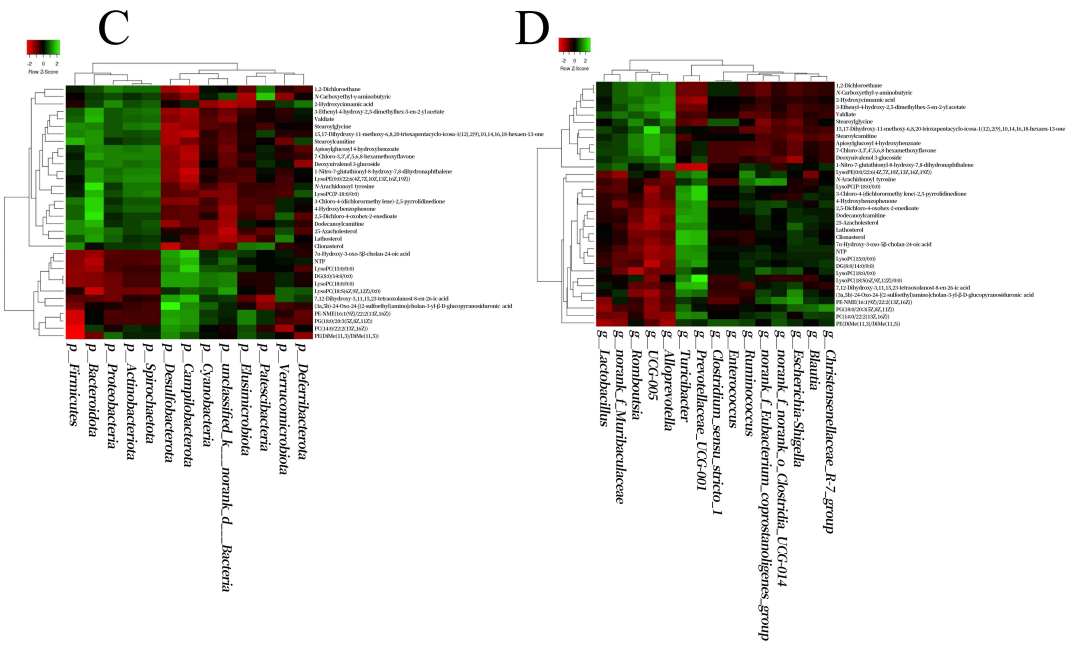


Fig. S5. Analysis of the correlation between biomarkers and intestinal microbiota. C: The difference between the phylum level and the serum biomarker analysis of the association of substances; D: Analysis of the association between different bacterial groups and serum biomarkers at the genus level.

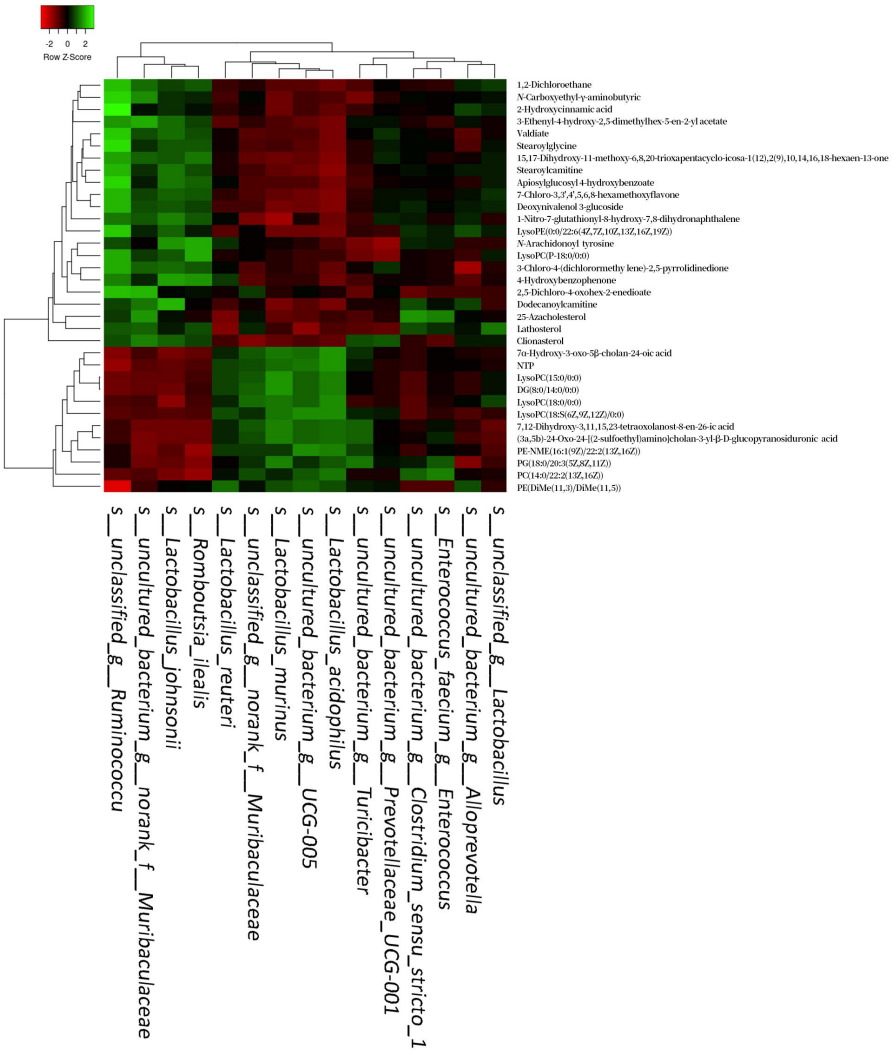


Fig. S6. Gut microbiota analysis. Species level callback