Figure S1

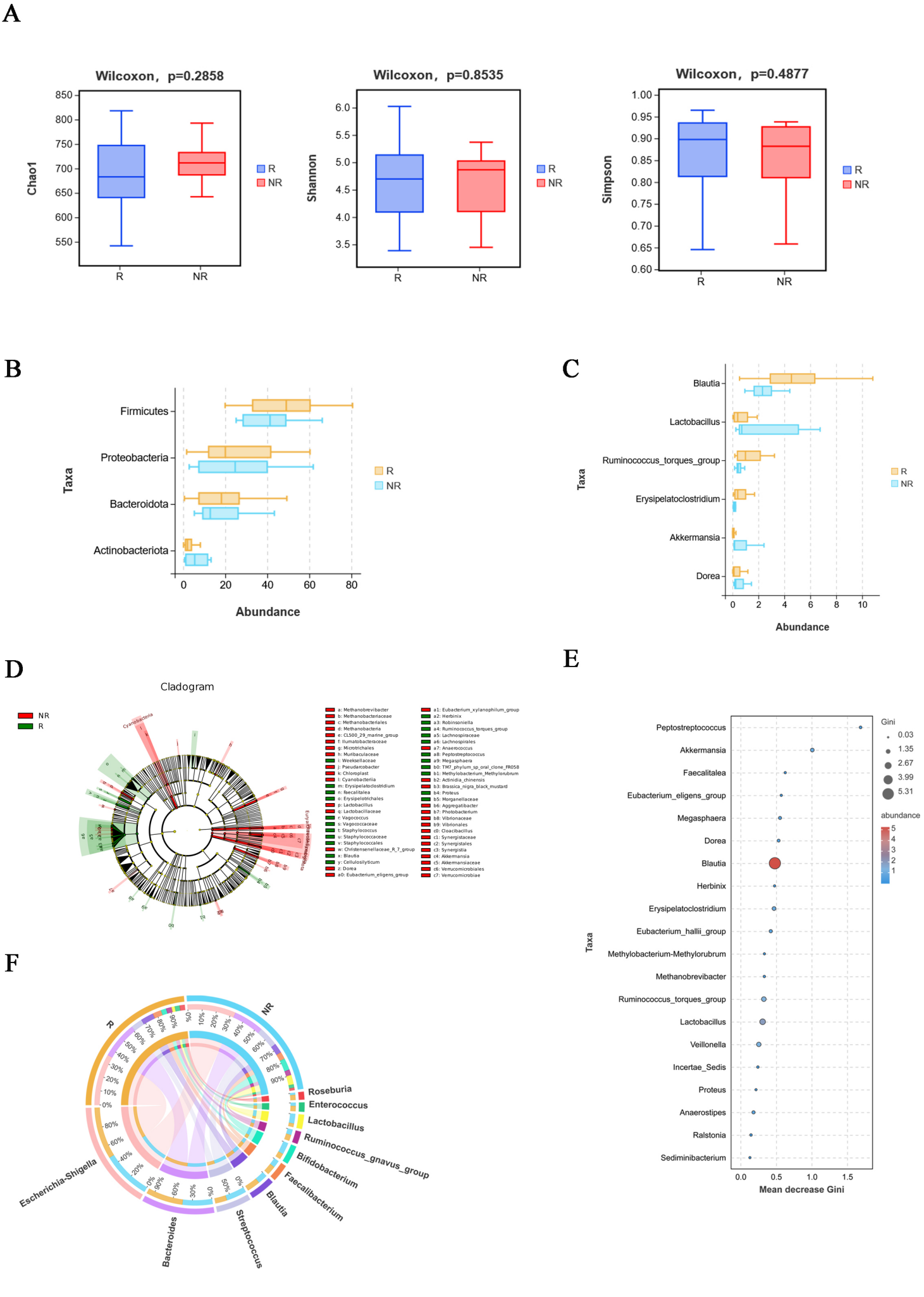

A
Wilcoxon, p=0.2858
Chao1
R
NR
Wilcoxon, p=0.8535
Shannon
Wilcoxon, p=0.4877
Simpson
B
Firmicutes
Proteobacteria
Bacteroidota
Actinobacteriota
Taxa
Abundance
C
Blautia
Lactobacillus
Ruminococcus_torques_group
Erysipelatoclostridium
Akkermansia
Dorea
D
Cladogram
E
Peptostreptococcus
Faecalitalea
Eubacterium_eligens_group
Megasphaera
Eubacterium_hallii_group
Methylobacterium-Methylorubrum
Methanobrevibacter
Veillonella
Incertae_Sedis
Proteus
Anaerostipes
Ralstonia
Sediminibacterium
Gini
abundance
Mean decrease Gini
F
Roseburia
Enterococcus
Ruminococcus_gnavus_group
Bifidobacterium
Faecalibacterium
Streptococcus
Escherichia-Shigella
Bacteroides

Figure S2

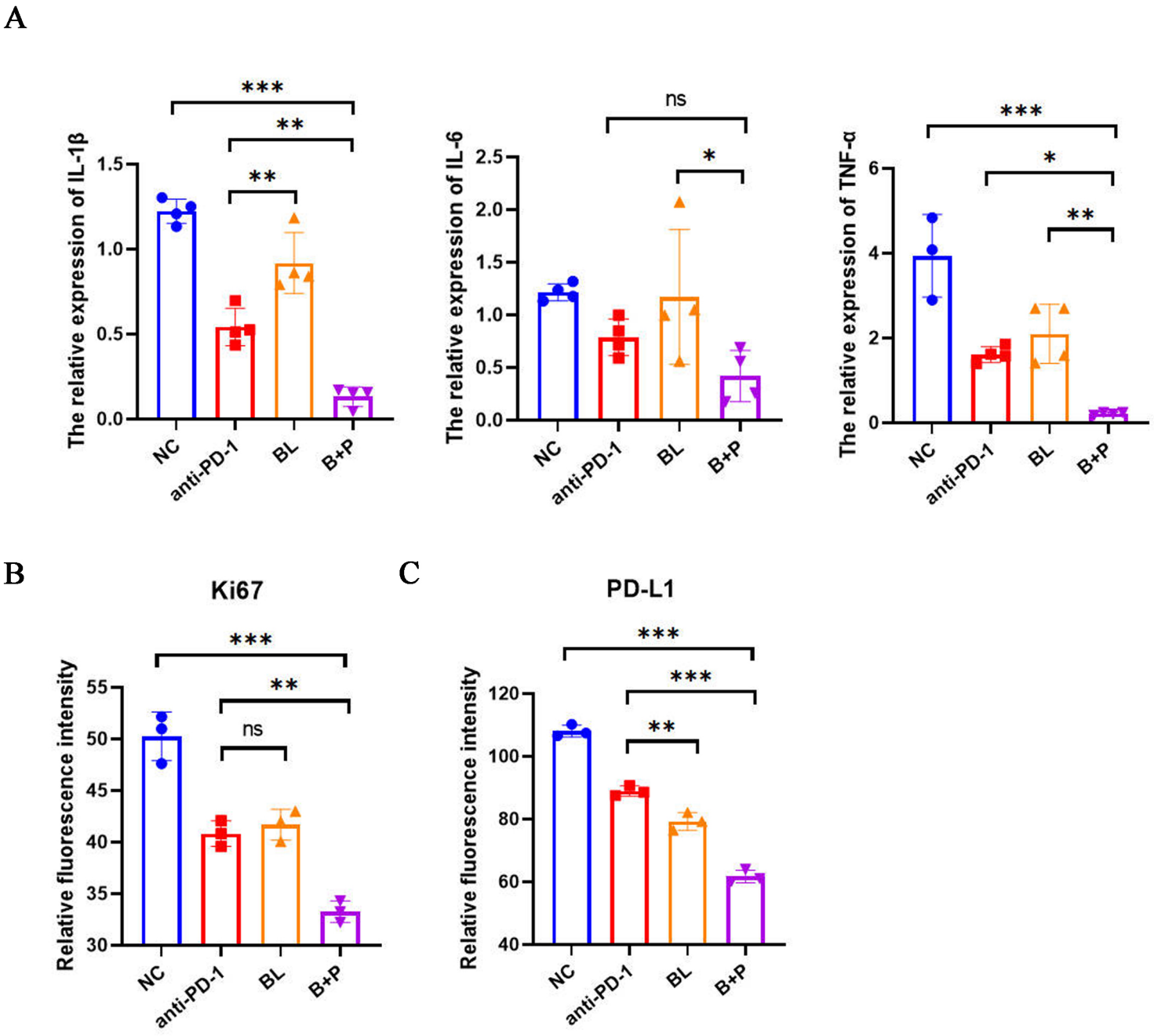
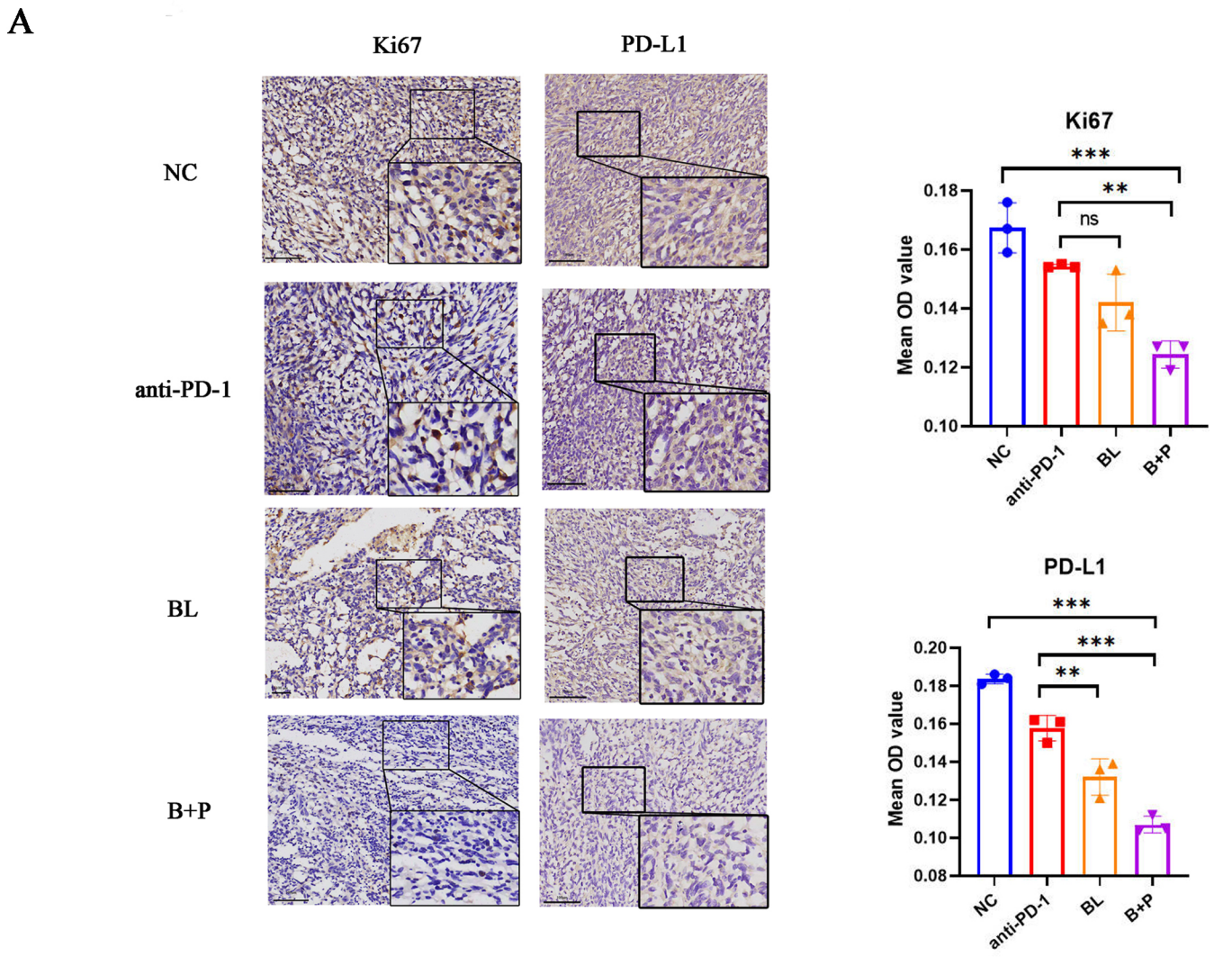
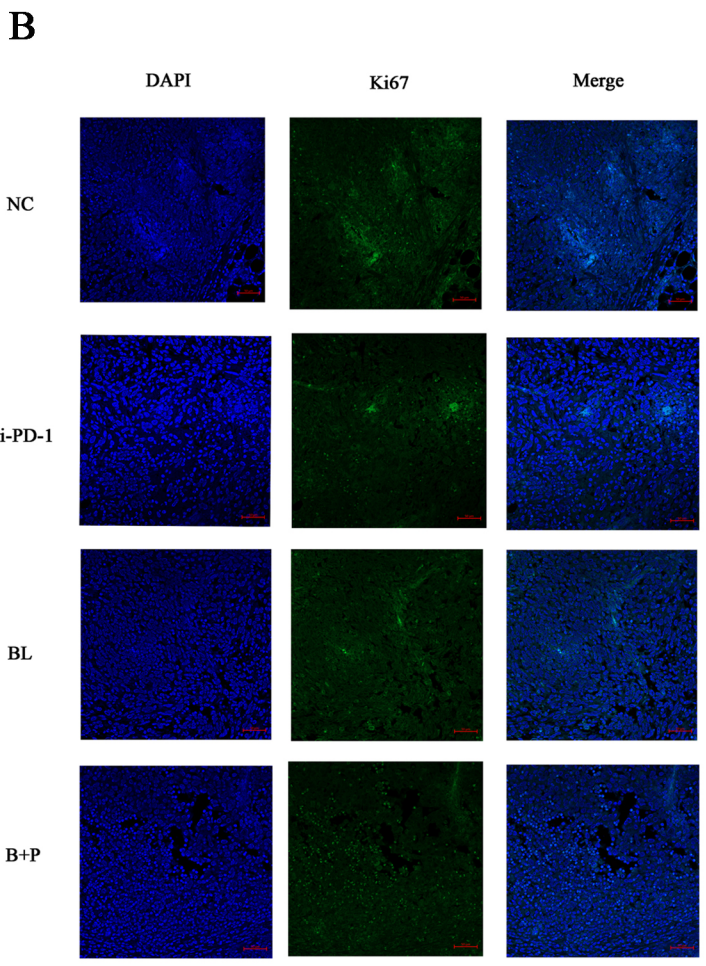

A
The relative expression of IL-1β
***
**
**
1.5
1.0
0.5
0.0
NC
anti-PD-1
BL
B+P
The relative expression of IL-6
ns
*
2.5
2.0
1.5
1.0
0.5
0.0
NC
anti-PD-1
BL
B+P
The relative expression of TNF-α
***
*
**
6
4
2
0
NC
anti-PD-1
BL
B+P
B
Ki67
Relative fluorescence intensity
***
**
ns
55
50
45
40
35
30
NC
anti-PD-1
BL
B+P
C
PD-L1
Relative fluorescence intensity
***
***
**
120
100
80
60
40
NC
anti-PD-1
BL
B+P

Figure S3


A
Ki67
PD-L1
NC
anti-PD-1
BL
B+P
Ki67
Mean OD value
0.18
0.16
0.14
0.12
0.10
***
**
ns
NC
anti-PD-1
BL
B+P
PD-L1
Mean OD value
0.20
0.18
0.16
0.14
0.12
0.10
0.08
***
***
**
NC
anti-PD-1
BL
B+P


B
DAPI
Ki67
Merge
NC
anti-PD-1
BL
B+P

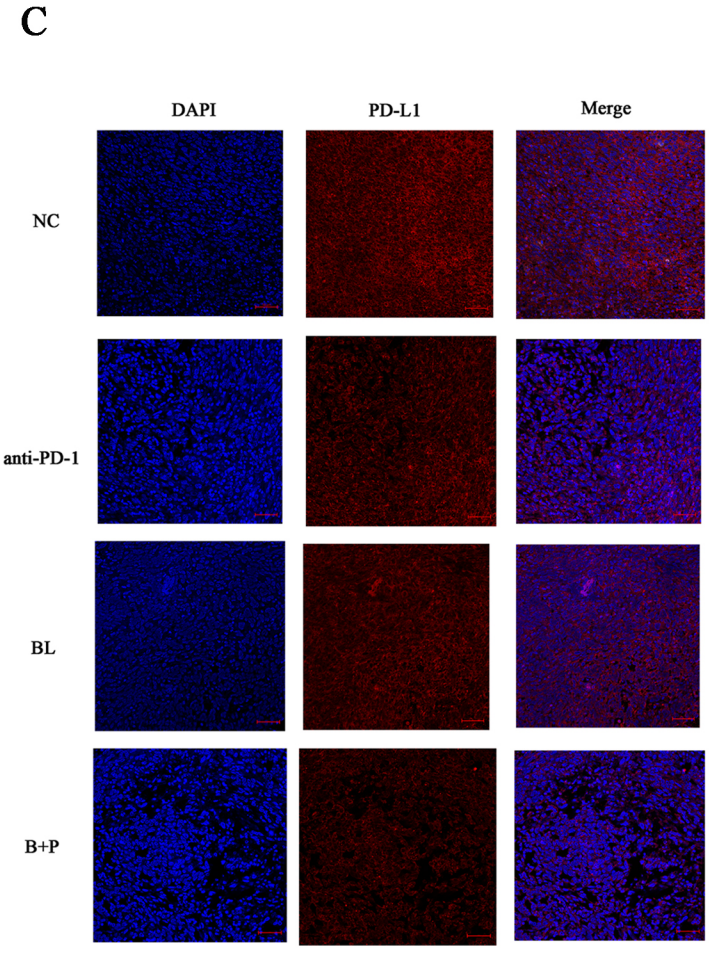

C
DAPI
PD-L1
Merge
NC
anti-PD-1
BL
B+P

Figure S4

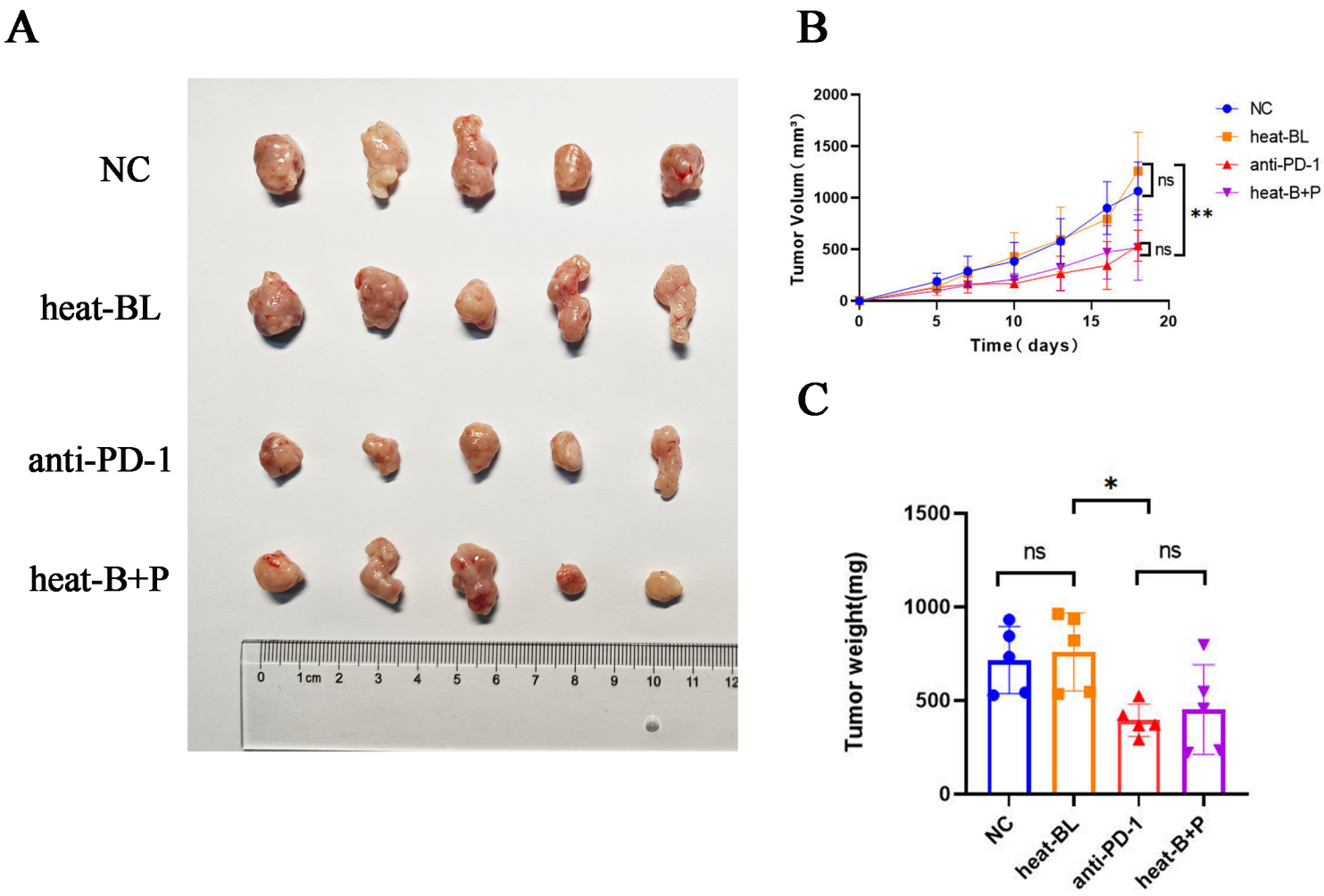

A
NC
heat-BL
anti-PD-1
heat-B+P
0 1cm 2 3 4 5 6 7 8 9 10 11 12
B
Tumor Volum (mm³)
2000
1500
1000
500
0
0 5 10 15 20
Time (days)
NC
heat-BL
anti-PD-1
heat-B+P
ns
ns
**
C
Tumor weight(mg)
1500
1000
500
0
ns
ns
*
NC
heat-BL
anti-PD-1
heat-B+P

Figure S5

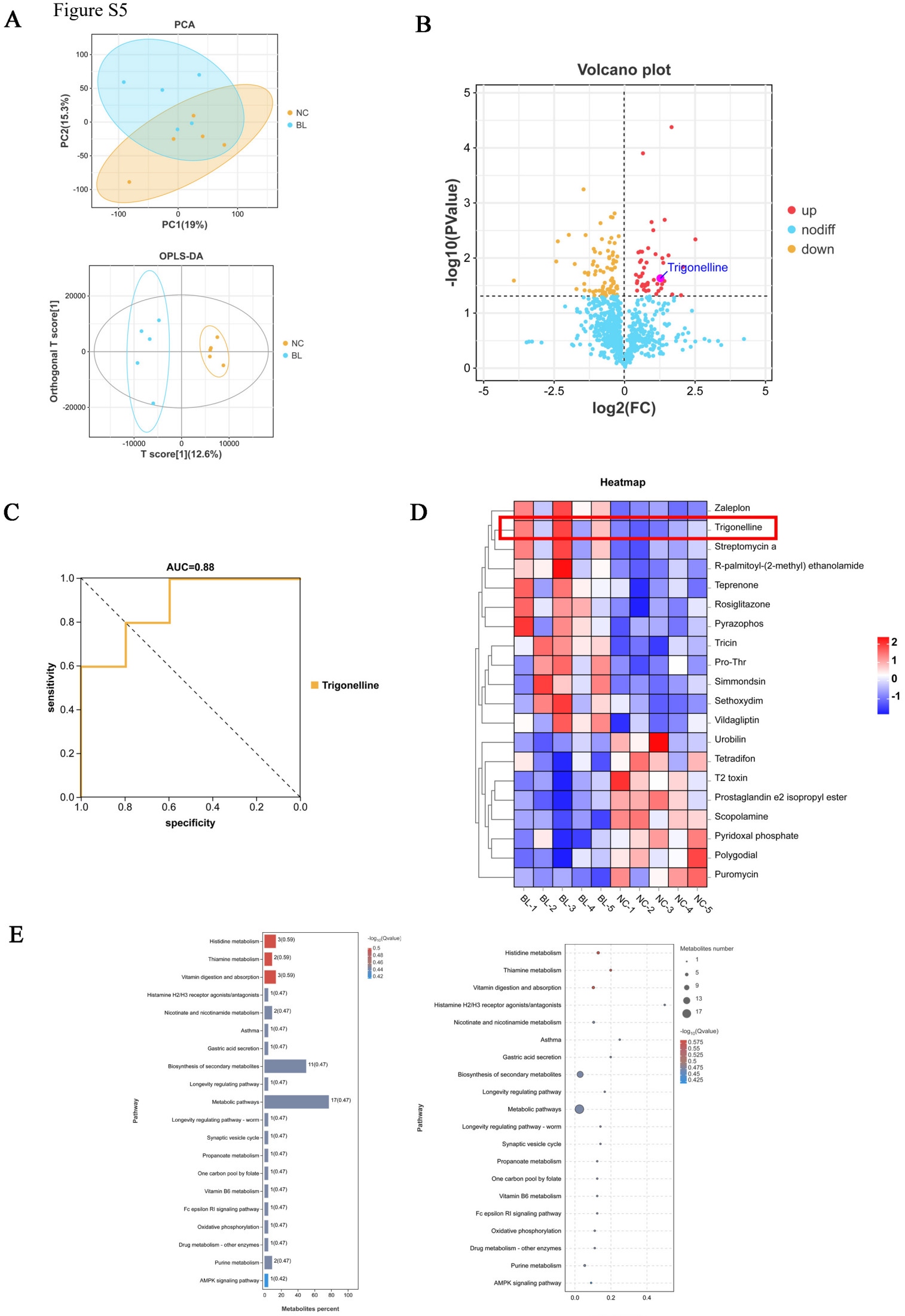
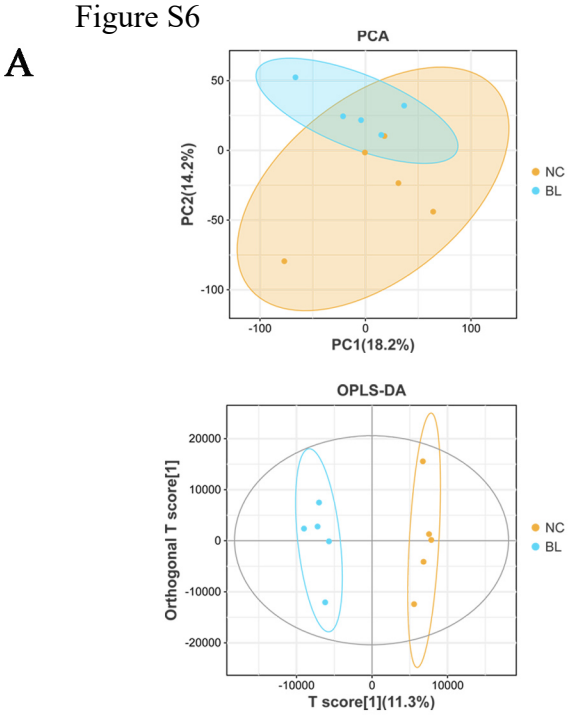
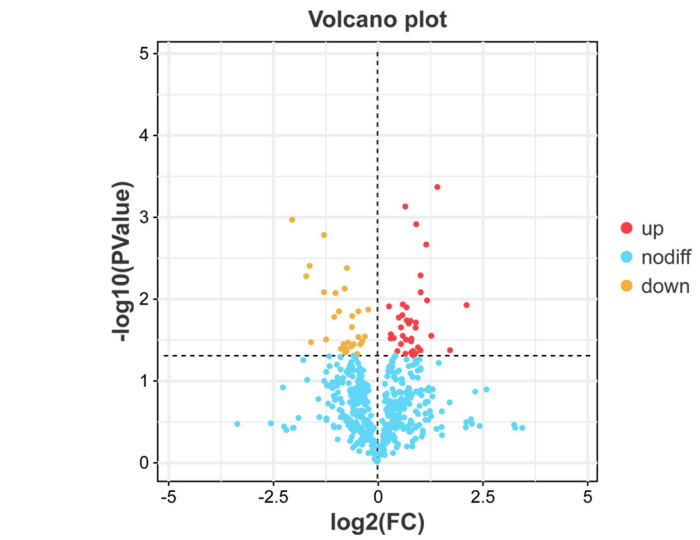
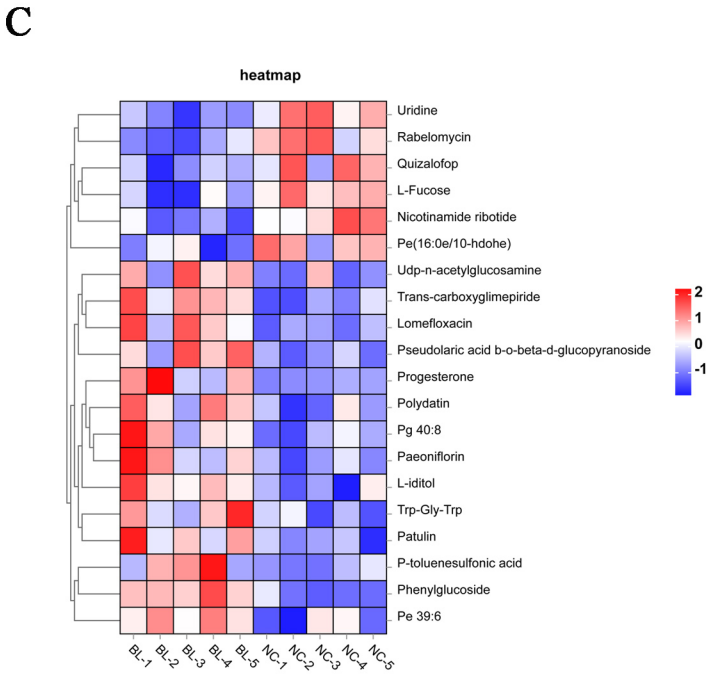
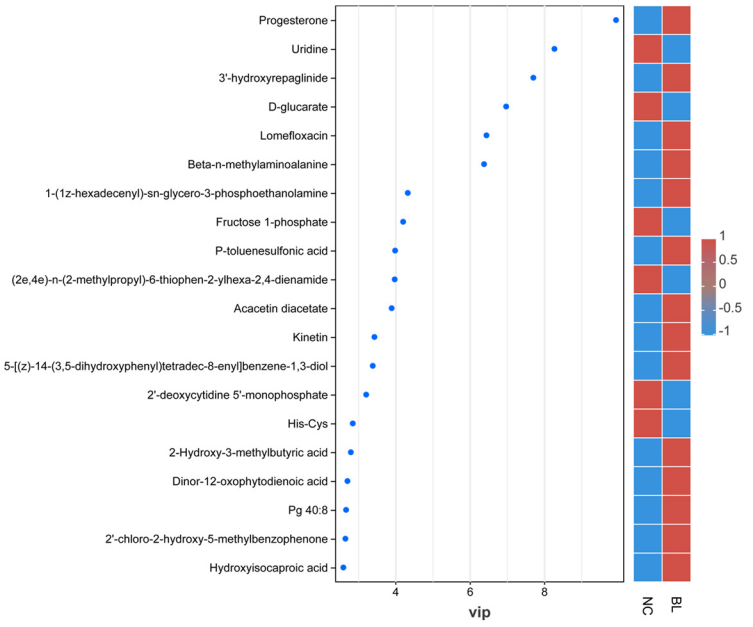

A
PCA
PC2(15.3%)
PC1(19%)
NC
BL
OPLS-DA
Orthogonal T score[1]
T score[1](12.6%)
NC
BL
B
Volcano plot
-log10(PValue)
log2(FC)
Trigonelline
up
nodiff
down
C
AUC=0.88
sensitivity
specificity
Trigonelline
D
Heatmap
Zaleplon
Trigonelline
Streptomycin a
R-palmitoyl-(2-methyl) ethanolamide
Teprenone
Rosiglitazone
Pyrazophos
Tricin
Pro-Thr
Simmondsin
Sethoxydim
Vildagliptin
Urobilin
Tetradifon
T2 toxin
Prostaglandin e2 isopropyl ester
Scopolamine
Pyridoxal phosphate
Polygodial
Puromycin
BL-1
BL-2
BL-3
BL-4
BL-5
NC-1
NC-2
NC-3
NC-4
NC-5
E
Histidine metabolism
Thiamine metabolism
Vitamin digestion and absorption
Histamine H2/H3 receptor agonists/antagonists
Nicotinate and nicotinamide metabolism
Asthma
Gastric acid secretion
Biosynthesis of secondary metabolites
Longevity regulating pathway
Metabolic pathways
Longevity regulating pathway - worm
Synaptic vesicle cycle
Propanoate metabolism
One carbon pool by folate
Vitamin B6 metabolism
Fc epsilon RI signaling pathway
Oxidative phosphorylation
Drug metabolism - other enzymes
Purine metabolism
AMPK signaling pathway
Pathway
Metabolites percent
Metabolites Ratio
Metabolites number
-log10(Qvalue)

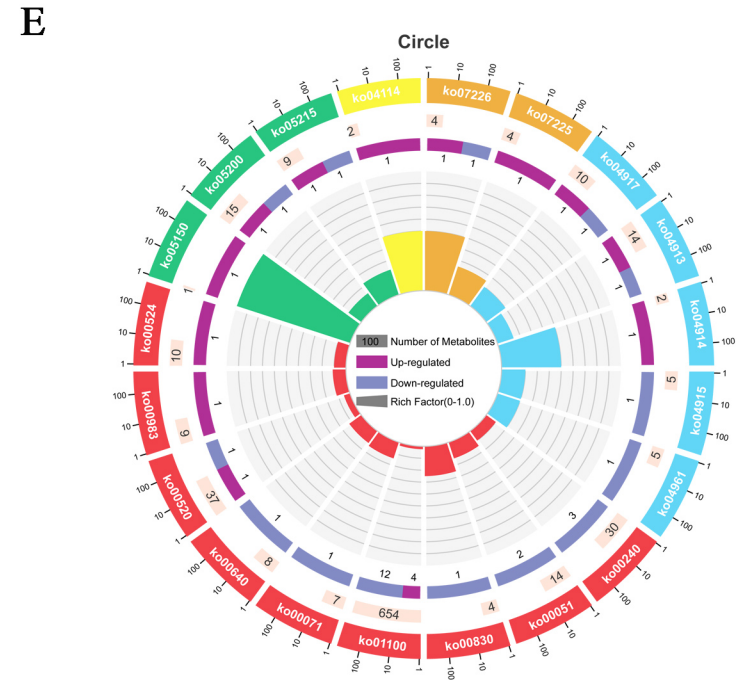
Figure S6







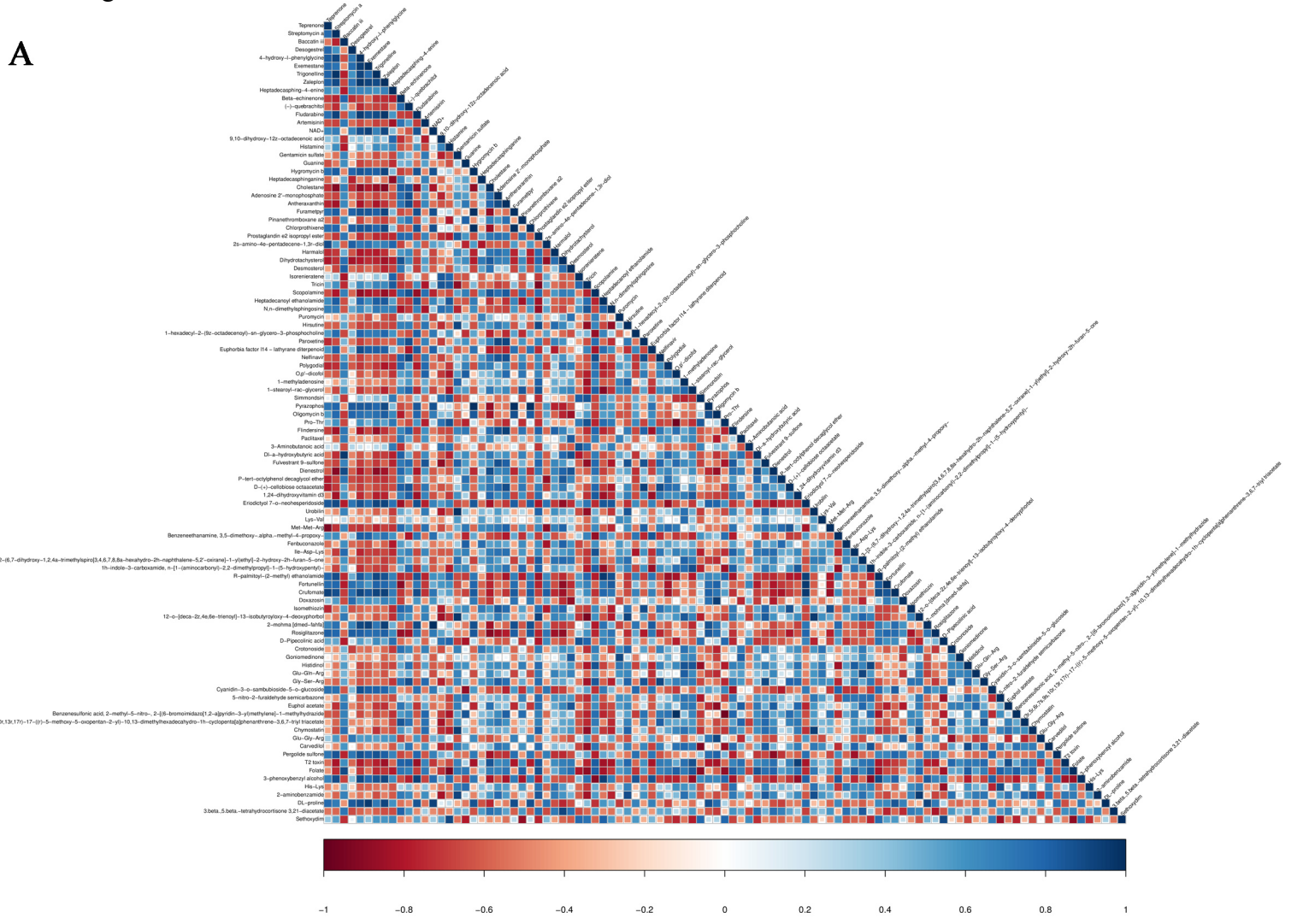




pathway top20

ID	Descrption
ko07226	Progesterone, androgen and estrogen receptor agonists/antagonists
ko05215	Prostate cancer
ko04917	Prolactin signaling pathway
ko00240	Pyrimidine metabolism
ko05150	Staphylococcus aureus infection
ko00051	Fructose and mannose metabolism
ko04913	Ovarian steroidogenesis
ko05200	Pathways in cancer
ko04114	Oocyte meiosis
ko04914	Progesterone-mediated oocyte maturation
ko00830	Retinol metabolism
ko07225	Glucocorticoid and mineralocorticoid receptor agonists/antagonists
ko01100	Metabolic pathways
ko04915	Estrogen signaling pathway
ko04961	Endocrine and other factor-regulated calcium reabsorption
ko00071	Fatty acid degradation
ko00640	Propanoate metabolism
ko00520	Amino sugar and nucleotide sugar metabolism
ko00983	Drug metabolism - other enzymes
ko00524	Neomycin, kanamycin and gentamicin biosynthesis

Figure S7



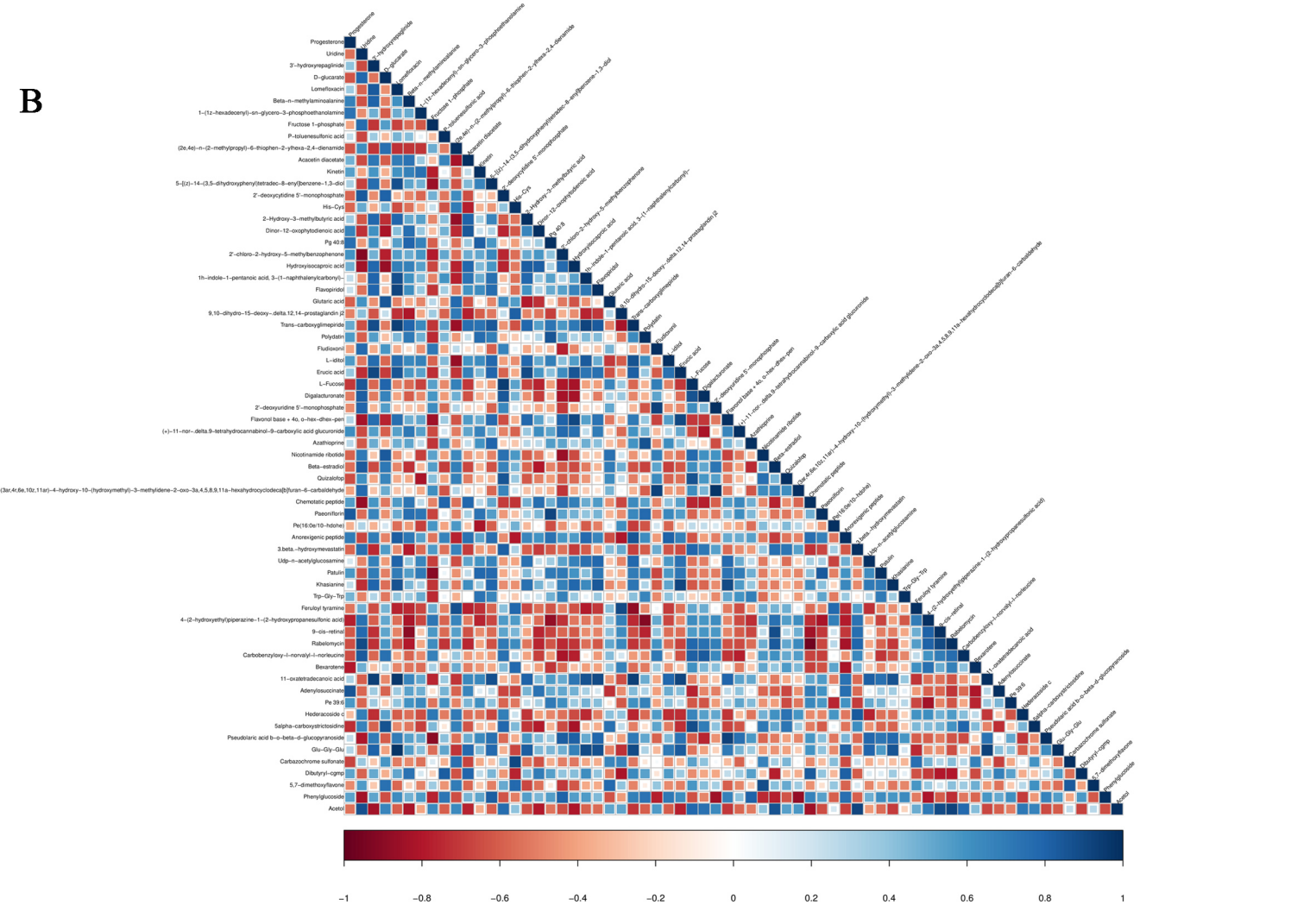
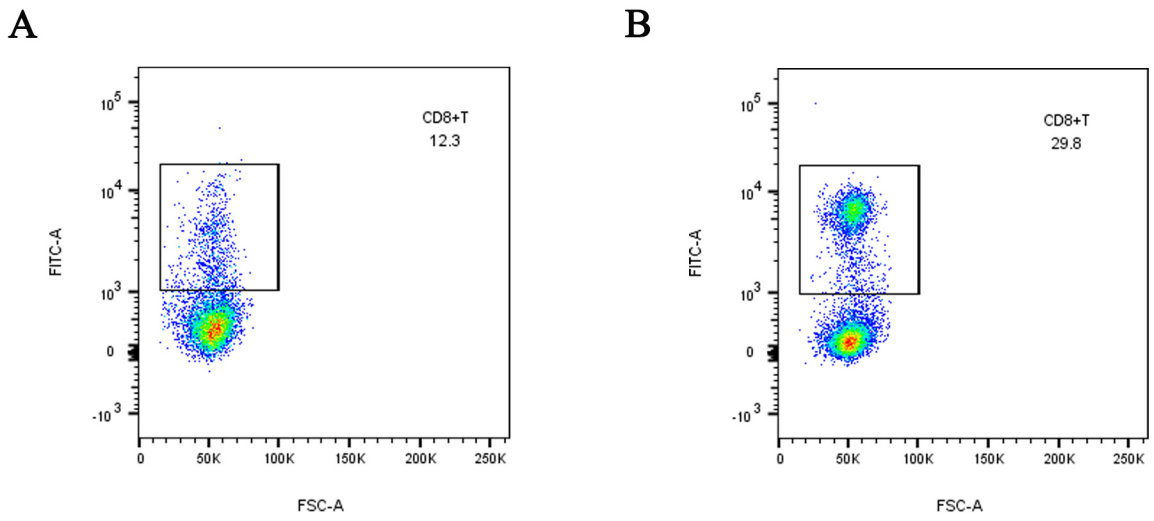


Figure S8



Supplement Table 1　The primary sequences list of qPCR

Primer name	Primer sequence
IL-1β	F：CAGGCAGGCAGTATCACTCA
	R：AGCTCATATGGGTCCGACAG
IL-6	F：GSGCCCACCAAGAACGATAG
	R：TCCACGATTTCCCAGAGAAC
TNF-α	F：AGGGTCTGGGCCATAGAACT
	R：CGGACCACAGTGGACTACCT
β-catenin	F: AACGGCTTTCGGTTGAGCTG
	R: TGGCGATATCCAAGGGCTTC
β-actin	F：CGTGCGTGACATCAAAGAGAA
	R：TGGATGCCACAGGATTCCAT

supplement Table 2 Potential biomarkers identified in BL group compared with NC group in POS ion model

No.	Metabolite	m/z	Log_2FC	VIP	Formula	mode	Variation	*p* value
1	Teprenone	313.2739	0.8669	23.5512	$C_{23}H_{38}O$	$[M+H]^+$	↑	0.04
2	Streptomycin	246.0973	1.1794	12.9396	$C_{21}H_{39}N_7O_{12}$	$[M+H]^+$	↑	0.03
3	Desogestrel	311.2582	0.6550	10.6413	$C_{22}H_{30}O$	$[M+H]^+$	↑	0.02
4	4-hydroxy-l-phenylglycine	168.06529	1.0347	10.5020	$C_8H_9NO_3$	$[M+H]^+$	↑	0.00
5	Exemestane	297.19074	5.5430	9.8018	$C_{20}H_{24}O_2$	$[M+H]^+$	↑	0.00
6	Trigonelline	138.05442	1.2884	8.4819	$C_7H_7NO_2$	$[M+H]^+$	↑	0.02
7	Zaleplon	264.10759	1.4394	8.2399	$C_{17}H_{15}N_5O$	$[M+H]^+$	↑	0.00
8	Heptadecasphing-4-enine	286.27413	0.6694	7.3823	$C_{17}H_{35}NO_2$	$[M+H]^+$	↑	0.00
9	Fludarabine	286.0892	1.3560	6.3937	$C_{10}H_{12}N_5O_4F$	$[M+H]^+$	↑	0.01
10	9,10-dihydroxy-12z-octadecenoic acid	315.25258	0.4615	5.1648	$C_{18}H_{34}O_4$	$[M+H]^+$	↑	0.04
11	Baccatin III	387.18044	-0.3943	10.9282	$C_{31}H_{38}O_{11}$	$[M+H]^+$	↓	0.01
12	β-echinenone	551.42347	-0.3356	7.2044	$C_{40}H_{54}O$	$[M+H]^+$	↓	0.00
13	(-)-quebrachitol	141.06544	-0.4217	7.1890	$C_7H_{14}O_6$	$[M+H]^+$	↓	0.02
14	Artemisinin	265.15365	-2.4072	6.0898	CHO_5	$[M+H]^+$	↓	0.01
15	Gentamicin sulfate	593.3318	-1.4140	4.9196	$C_{69}H_{127}N_{15}O_{26}S$	$[M+H]^+$	↓	0.03
16	Guanine	152.05671	-0.4674	4.7472	$C_5H_5N_5O$	$[M+H]^+$	↓	0.03
17	Heptadecasphinganine	288.28971	-0.1907	4.3304	$C_{17}H_{37}NO_2$	$[M+H]^+$	↓	0.00
18	Cholestane	411.36075	-0.2410	4.3290	$C_{27}H_{48}$	$[M+H]^+$	↓	0.01
19	Adenosine 2'-monophosphate	330.05832	-3.9105	4.2045	$C_{10}H_{16}N_5O_8P$	$[M+H]^+$	↓	0.02
20	Antheraxanthin	585.42567	-0.2438	4.0820	$C_{40}H_{56}O_3$	$[M+H]^+$	↓	0.04

Supplement Table 3 Potential biomarkers identified in BL group compared with NC group in NEG ion model

No.	Metabolite	m/z	Log_2FC	VIP	Formula	mode	Variation	*p* value
1	Progesterone	313.2380	0.7879	9.9350	C21H30O2	[M-H]-	↑	0.02
2	3-hydroxyrepaglinide	467.2770	0.5610	7.7105	C5H11NO	[M-H]-	↑	0.04
3	Lomefloxacin	350.1088	1.0301	6.4505	C17H19F2N3O3	[M-H]-	↑	0.01
4	β-N-methylamino-alanine	117.0554	0.3185	6.3862	C4H9NO2	[M-H]-	↑	0.03
5	1-(1z-hexadecenyl)-sn-glycero-3-phospho ethanolamine	436.2812	0.5076	4.3354	C41H80NO7P	[M-H]-	↑	0.02
6	P-toluenesulfonic acid	170.9959	0.8578	3.9946	C7H8O3S	[M-H]-	↑	0.05
7	Acacetin diacetate	367.0985	0.8099	3.9023	C20H18O7	[M-H]-	↑	0.03
8	Kinetin	214.0175	0.3141	3.4379	C10H9N5	[M-H]-	↑	0.03
9	5-[(z)-14-(3,5-dihydroxyphenyl)tetradec-8 -enyl]benzene-1,3-diol	411.3453	1.0276	3.3932	C27H38O4	[M-H]-	↑	0.01
10	2-Hydroxy-3-methylbutyric acid27	117.0554	0.6023	2.8043	C5H10O3	[M-H]-	↑	0.03
11	Uridine	279.0380	-1.2758	8.2783	C9H12N2O6	[M-H]-	↓	0.00
12	D-glucarate	209.0121	-0.4550	6.9797	C6H14O6	[M-H]-	↓	0.01
13	Fructose 1-phosphate	259.0127	-1.004	4.2101	C6H13O9P	[M-H]-	↓	0.01
14	(2e,4e)-N-(2-methylpropyl)-6-thiophen-2-ylhexa-2,4-dienamide	248.0910	-1.6205	3.9833	C16H29NO	[M-H]-	↓	0.00
15	2'-deoxycytidine-5'-monophosphate	194.9958	-0.2998	3.2168	C9H14N3O7P	[M-H]-	↓	0.03
16	His-Cys	257.0769	-0.9203	2.8559	C9H14N4O3S	[M-H]-	↓	0.01
17	Glutaric acid	131.0340	-0.8110	2.3343	C5H8O4	[M-H]-	↓	0.04
18	9,10-dihydro-15-deoxy-Δ12,14-prostaglan din j2	635.4490	-0.5705	2.2183	C20H28O3	[M-H]-	↓	0.04
19	Fludioxonil	247.0127	-0.4846	2.0247	CH6F2N2O2	[M-H]-	↓	0.05
20	L-Fucose	163.0599	-0.7849	1.9525	C6H12O5	[M-H]-	↓	0.01