Punicic acid immune-enhancing *in vitro* and *in vivo* effects and action mechanisms as revealed *via* microbiome and lipids profiling

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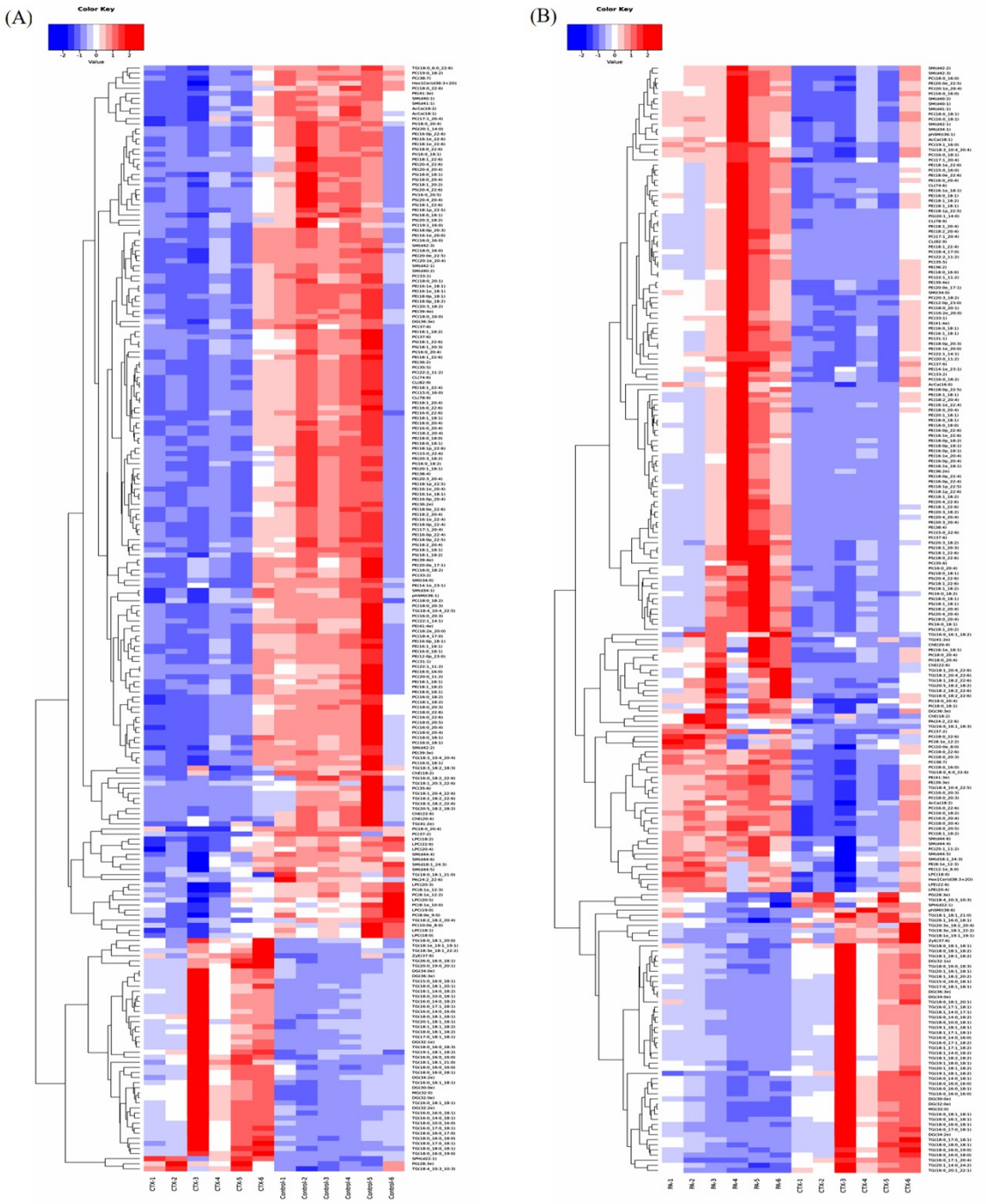

Figure S1. Effect of PA on serum lipid metabolism in immunocompromised mice (n = 6). (A) Statistical analysis of differentially abundant metabolites: Control group vs. CTX group, (B) CTX group vs. PA group. The blue and red colors indicate negative and positive correlation, respectively.

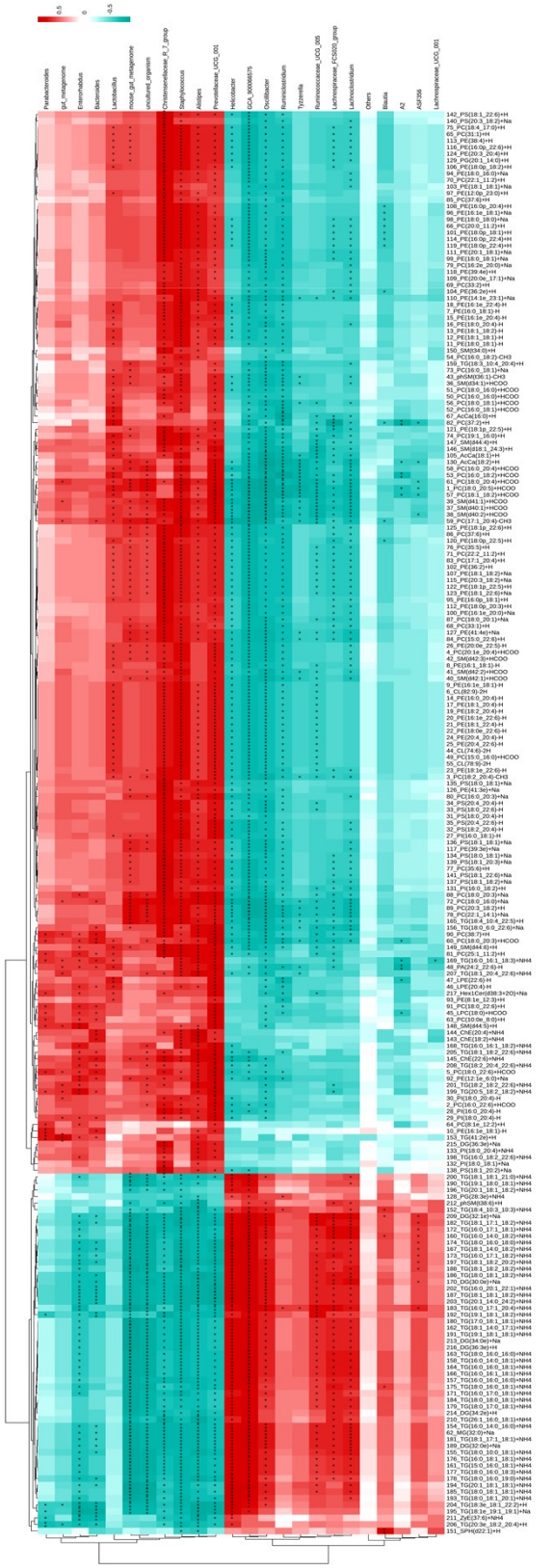

Figure S2. Spearman correlation between gut microbiota and parameters related to serum lipid metabolism. Blue and red colors indicate negative and positive correlations, respectively.

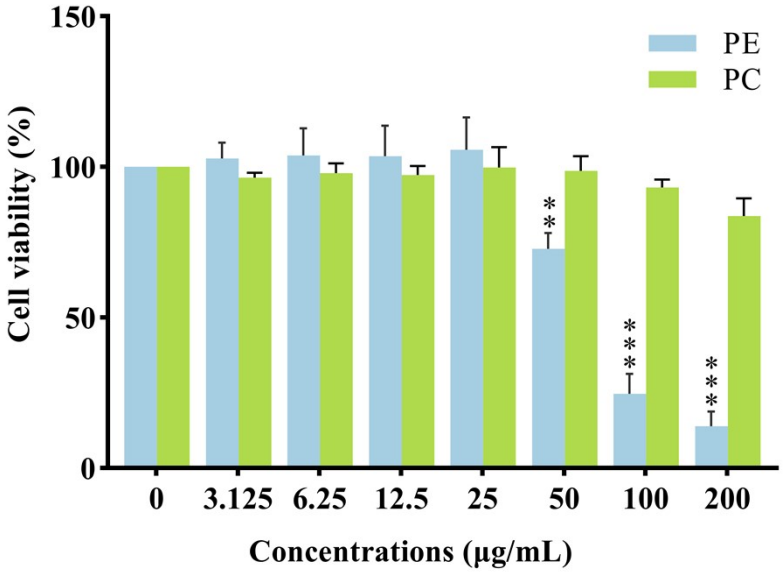

Figure S3. Effect of different concentrations of PC and PE on cell viability.

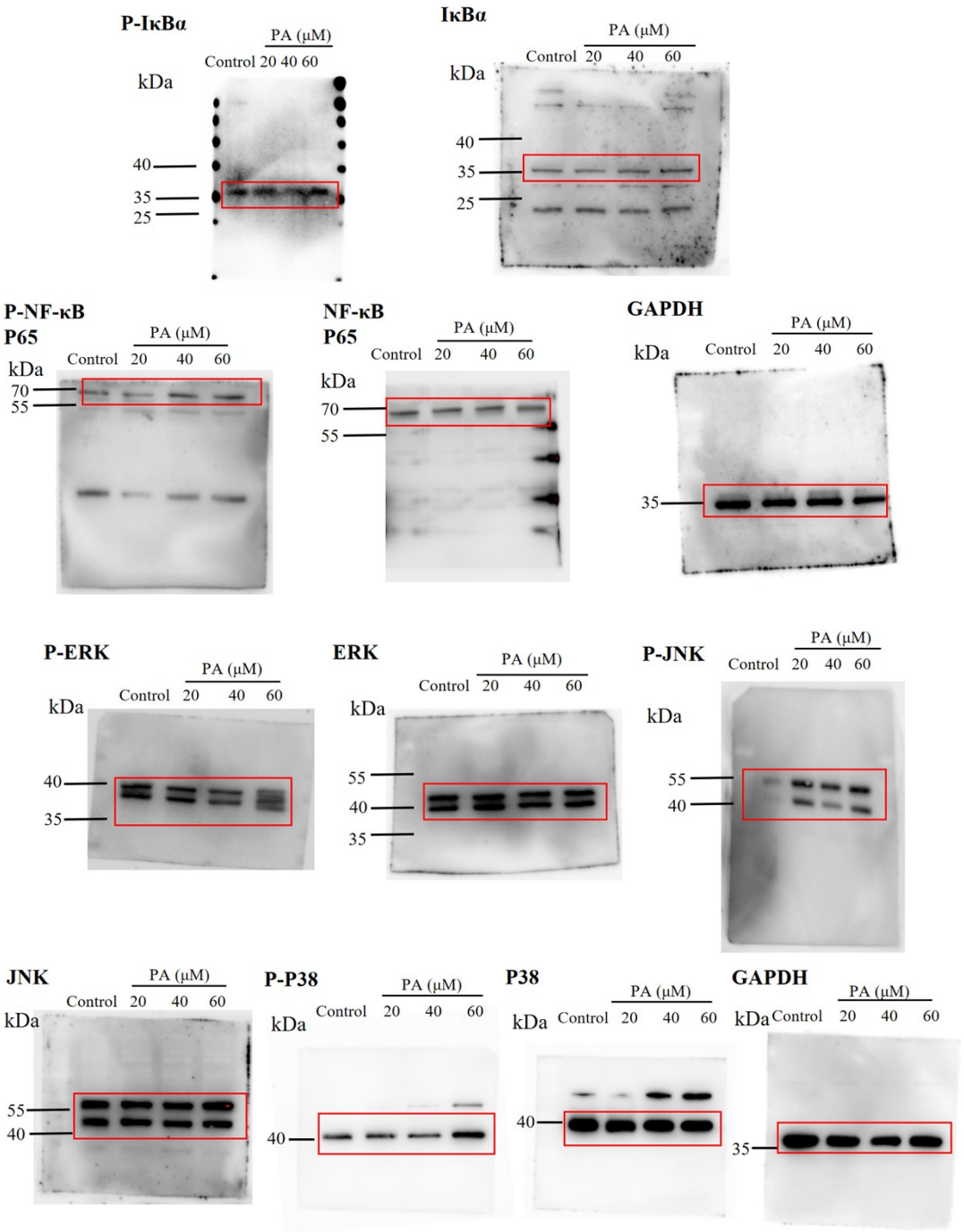

Figure S4. The image of the uncropped and unprocessed Western blot labelled with closest molecular mass markers in Figure 3.

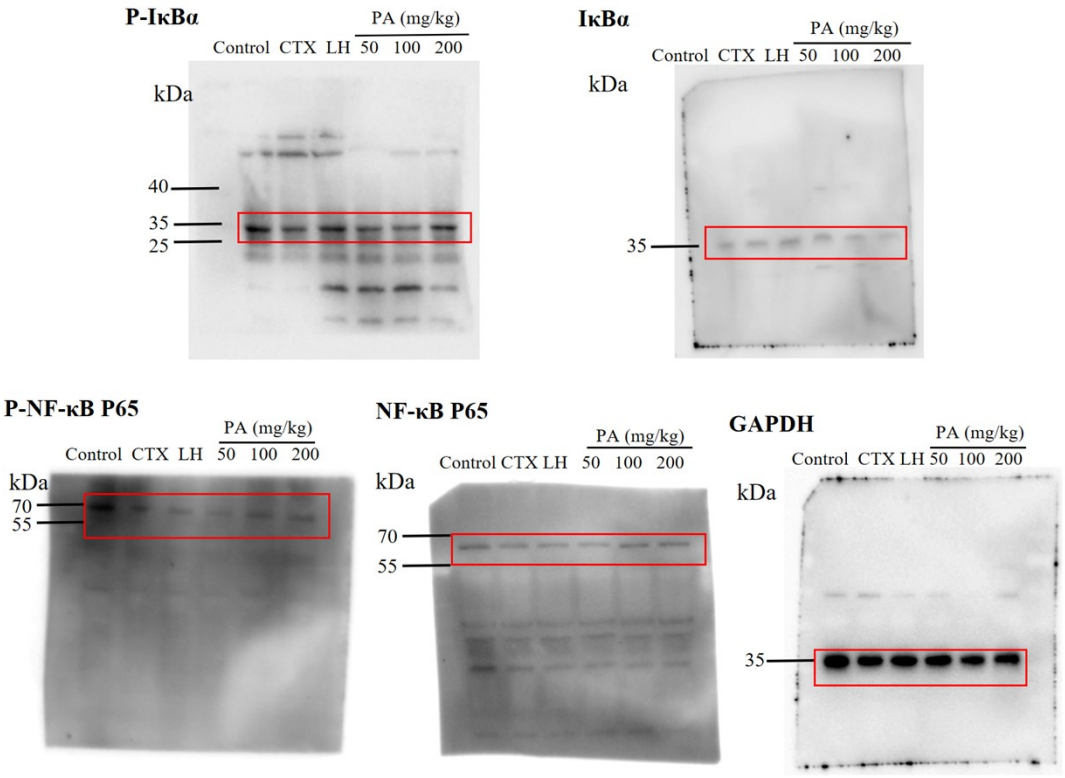

Figure S5. The image of the uncropped and unprocessed Western blot labelled with closest molecular mass markers in Figure 5.

Table S1

Statistical analysis of differential metabolites identified by lipidomics (PA vs CTX).

LipidIon	Fold Change	P-value	VIP
PC(18:0_20:5)+HCOO	2.273860516	0.000295779	1.178257721
PC(16:0_22:6)+HCOO	2.030546955	0.001532839	2.452433711
PC(18:2_20:4)-CH_3	3.541732438	0.022009754	1.025632595
PC(20:1e_20:4)+HCOO	2.201328746	0.004322606	2.318124215
PC(18:0_22:6)+HCOO	2.399119492	0.000294322	1.767417525
CL(82:9)-2H	3.830984346	0.019214482	1.183213374
PE(16:0_18:1)-H	2.759647842	0.004808806	2.051641544
PE(16:1_18:1)-H	2.783712317	0.009985485	1.262516086
PE(16:1e_18:1)-H	3.240859604	0.009247672	1.577796753
PE(16:1e_18:1)-H	2.300387648	0.043434633	1.284361159
PE(18:0_18:1)-H	2.963172871	0.007765657	1.359405785
PE(18:1_18:1)-H	2.977726915	0.012326686	1.380462792
PE(18:1_18:2)-H	3.021896082	0.01166319	1.641236867
PE(16:0_20:4)-H	3.3909048	0.00676216	1.987405398
PE(16:1e_20:4)-H	4.088196702	0.032307545	1.786585157
PE(18:0_20:4)-H	3.246613152	0.012709569	2.07344808
PE(18:1_20:4)-H	3.706254826	0.011039614	2.760465684
PE(16:1e_22:4)-H	3.96598196	0.013003191	1.368074444
PE(18:2_20:4)-H	4.235044004	0.009290523	1.971624634
PE(16:1e_22:6)-H	4.249363314	0.015329298	1.820379212
PE(18:1_22:4)-H	3.864635497	0.018576418	1.189275601
PE(18:0e_22:6)-H	4.051723455	0.006173782	1.259350889
PE(18:1e_22:6)-H	4.472843648	0.006882576	1.518781554
PE(20:4_20:4)-H	5.458318244	0.01073223	2.082209236
PE(20:4_22:6)-H	5.153314478	0.017008059	1.211834365
PE(20:0e_22:5)-H	2.171939087	0.004364251	2.267284066
PI(16:0_18:1)-H	3.722482605	0.003507724	1.172267495
PI(16:0_20:4)-H	2.487263577	0.00734249	1.28308471
PI(18:0_20:4)-H	2.246687861	0.004497943	3.136553246
PI(18:0_20:4)-H	2.267877273	0.003221685	3.244990253
PS(18:0_20:4)-H	3.198005938	0.003916564	1.456550874
PS(18:2_20:4)-H	3.128941151	0.004144049	1.084689913
PS(18:0_22:6)-H	4.623542138	0.007646275	1.108524191
PS(20:4_20:4)-H	3.364528133	0.006660297	1.649691349
PS(20:4_22:6)-H	4.05333803	0.007269321	1.250100999
SM(d34:1)+HCOO	2.075286282	0.008724115	1.780371092
SM(d40:1)+HCOO	2.390127195	0.001182715	2.131382248
SM(d40:2)+HCOO	2.46469021	0.001366378	1.392980588
SM(d41:1)+HCOO	2.45714391	0.001026116	1.258417673
SM(d42:1)+HCOO	2.409271525	0.003854307	2.589613585

SM(d42:2)+HCOO	2.160654055	0.003635418	2.492665793
SM(d42:3)+HCOO	2.231004466	0.002521059	2.052970096
phSM(t36:1)-CH_3	2.062630071	0.009914872	1.761273489
CL(74:6)-2H	3.394947139	0.008004916	1.313653666
LPC(18:0)+HCOO	1.648029156	0.010460816	2.547553231
LPE(20:4)-H	1.636458393	0.005852764	1.231416847
LPE(22:6)-H	1.693551549	0.008978645	1.02376265
PA(24:2_22:6)-H	2.961018435	0.010406482	1.201893142
PC(15:0_16:0)+HCOO	3.556755463	0.00933882	1.683233238
PC(16:0_16:0)+HCOO	2.055738972	0.004268278	4.420212991
PC(18:0_16:0)+HCOO	2.106022622	0.004238178	2.705071705
PC(16:0_18:1)+HCOO	1.842611254	0.003396063	3.061668052
PC(16:0_18:2)+HCOO	1.731281676	0.001402239	4.614126913
PC(16:0_18:2)-CH_3	2.238478396	0.019144422	1.110901371
CL(78:9)-2H	3.706162818	0.011178586	2.761311127
PC(18:0_18:1)+HCOO	2.05838385	0.003807972	1.93010537
PC(18:1_18:2)+HCOO	1.983537445	0.000396939	1.417100559
PC(16:0_20:4)+HCOO	1.964154894	0.000736509	2.828735681
PC(17:1_20:4)-CH_3	2.178126315	0.001031182	2.060497794
PC(18:0_20:3)+HCOO	2.751518375	1.83086E-05	1.428837234
PC(18:0_20:4)+HCOO	2.19413252	0.00025781	2.679294718
MG(32:0)+Na	0.456073052	0.003802099	2.343069489
PC(10:0e_8:0)+H	1.574705324	0.035934273	5.850437041
PC(8:1e_12:2)+H	1.973219912	0.048400281	1.381018966
PC(31:1)+H	2.874678506	0.007485279	3.280302265
PC(20:0_11:2)+H	2.876863758	0.004370486	2.020167399
AcCa(16:0)+H	1.666727386	0.02469168	1.63625103
PC(33:1)+H	2.635168864	0.006705956	1.581124477
PC(33:2)+H	2.546284749	0.01867107	1.751998837
PC(22:1_11:2)+H	3.444923752	0.015450613	2.707791969
PC(22:2_11:2)+H	3.536127606	0.013674537	3.581935965
PC(18:0_16:0)+Na	2.896136343	3.79537E-05	6.575353874
PC(16:0_18:1)+Na	1.695181106	0.003365839	10.34457648
PC(19:1_16:0)+H	3.088566405	0.000512495	1.416425625
PC(18:4_17:0)+H	4.1509684	0.007797253	1.282963025
PC(35:5)+H	3.927745828	0.015177714	5.134738417
PC(35:6)+H	6.062112646	0.036504616	3.533464296
PC(22:1_14:1)+Na	2.952925435	0.001106409	2.608048622
PC(16:2e_20:0)+Na	3.062867988	0.01093413	2.04306307
PC(16:0_20:3)+Na	2.194240211	0.001328961	9.517184958
PC(25:1_11:2)+H	1.305724662	0.00266126	1.008641682
PC(37:2)+H	3.757668541	0.013285839	2.464543006
PC(17:1_20:4)+H	3.843126629	0.015759334	1.948738425
PC(15:0_22:6)+H	4.514854403	0.010432908	1.000925855

PC(37:6)+H	3.089967932	0.026356125	1.25245477
PC(37:6)+H	4.904903509	0.022896926	1.582009075
PC(18:0_20:1)+Na	3.569016409	0.008574281	1.284642585
PC(18:0_20:3)+Na	2.786406146	0.00012303	6.522208076
PC(20:3_18:2)+H	3.752905716	0.00332452	1.274492534
PC(38:7)+H	3.213072527	0.000124368	1.282285323
PC(18:0_22:6)+H	2.138375492	0.004126626	1.413526947
PE(12:1e_6:0)+Na	1.497375538	0.00495727	1.174238865
PE(8:1e_12:3)+H	1.419689455	0.013211267	1.065650659
PE(18:0_16:0)+Na	3.44524608	0.015423289	3.829447216
PE(16:0p_18:1)+H	3.415345162	0.013468981	2.172710633
PE(16:1e_18:1)+Na	4.166728338	0.039533433	2.746975417
PE(12:0p_23:0)+H	2.873289303	0.007790519	1.400293233
PE(18:0_18:0)+Na	3.384308765	0.017122089	1.199152637
PE(18:0_18:1)+Na	3.488338731	0.016650659	3.410235255
PE(16:1e_20:0)+Na	3.337338633	0.006096761	1.237218639
PE(18:0p_18:1)+H	3.045611085	0.016688048	1.146137332
PE(36:2)+H	3.90743231	0.014963944	5.135498429
PE(18:1_18:1)+Na	3.824350827	0.038617608	1.351766382
PE(36:2e)+H	3.916522476	0.036741589	2.192681395
AcCa(18:1)+H	1.791994549	0.003932188	1.378800789
PE(18:0p_18:2)+H	3.376492782	0.0213628	1.15611652
PE(18:1_18:2)+Na	4.875701768	0.027166133	3.290582025
PE(16:0p_20:4)+H	4.104739193	0.047135972	2.790749817
PE(20:0e_17:1)+Na	2.814768682	0.010507291	2.363817218
PE(14:1e_23:1)+Na	2.458672124	0.007284802	1.481828193
PE(20:1_18:1)+Na	3.773705036	0.012897077	1.104591441
PE(18:0p_20:3)+H	3.331468673	0.006171167	1.236132196
PE(38:4)+H	4.114328639	0.014522322	4.07714134
PE(16:0p_22:4)+H	4.249825567	0.021713929	2.031872703
PE(20:3_18:2)+Na	5.570800988	0.01713719	3.521768381
PE(16:0p_22:6)+H	4.619210376	0.012857105	2.972377555
PE(39:3e)+Na	2.752275498	0.001077441	1.559348624
PE(39:4e)+H	2.811369262	0.011146634	2.384214162
PE(18:0p_22:4)+H	4.072292931	0.022723212	1.21059563
PE(18:0p_22:5)+H	3.881718094	0.013722279	1.658514343
PE(18:1p_22:5)+H	4.733532267	0.014182693	1.113474201
PE(18:1p_22:5)+H	4.775458008	0.010646305	2.291377644
PE(18:1_22:6)+Na	6.732231909	0.017114982	2.145185275
PE(20:3_20:4)+H	3.906117172	0.013444855	4.357887358
PE(18:1p_22:6)+H	5.555130694	0.028314842	1.591573931
PE(41:3e)+Na	2.914655025	0.000538246	1.052421048
PE(41:4e)+Na	3.094444855	0.004341036	1.228812146
PG(28:3e)+NH_4	0.741921851	0.027259215	1.36812327

PG(20:1_14:0)+H	4.967094638	0.010342138	2.102914036
AcCa(18:2)+H	1.573605384	0.00624907	1.005711726
PI(16:0_18:2)+H	3.597671992	0.00329203	1.023521761
PI(18:0_18:1)+Na	1.364214909	0.042861365	1.005025147
PI(18:0_20:4)+NH_4	2.304041615	0.015208284	2.587955793
PS(18:0_18:1)+Na	2.886345396	0.005902755	2.491620413
PS(18:0_18:1)+Na	2.762062298	0.00635114	1.161781782
PS(18:1_18:1)+Na	3.447678862	0.004817563	1.315355152
PS(18:1_18:2)+Na	2.675531032	0.01135185	1.432634786
PS(18:1_20:2)+Na	2.440897276	0.008669289	1.798682472
PS(18:1_20:3)+Na	3.683792877	0.01195356	1.41204874
PS(20:3_18:2)+Na	3.074192217	0.009457895	2.143772823
PS(18:1_22:6)+Na	2.863601025	0.015352309	1.598013296
PS(18:1_22:6)+H	3.514073646	0.013155774	1.440227036
ChE(18:2)+NH_4	4.738392942	0.013992332	1.790279507
ChE(20:4)+NH_4	6.114578864	0.006158792	1.76909169
ChE(22:6)+NH_4	6.463489147	0.003079309	1.75382041
SM(d18:1_24:3)+H	1.450755627	0.007214888	1.011529513
SM(d44:4)+H	1.847429353	0.001022672	1.660501698
SM(d44:5)+H	1.339653003	0.020458969	1.126290804
SM(d44:6)+H	1.602835171	0.001986802	1.207966477
SM(t34:0)+H	5.556848389	0.008973127	2.33864615
SPH(d22:1)+H	0.859566491	0.046400728	1.297120231
TG(18:4_10:3_10:3)+NH_4	0.683461262	0.006968601	1.747226543
TG(41:2e)+H	3.530987233	0.025259091	1.143407277
TG(16:0_14:0_16:0)+NH_4	0.266623337	0.04937003	1.027993505
TG(18:0_10:0_18:1)+NH_4	0.23698908	0.032040687	2.576083601
TG(18:0_6:0_22:6)+Na	1.970185158	0.000256439	2.059957206
TG(16:0_16:0_16:0)+NH_4	0.438714224	0.005051083	1.338068207
TG(16:0_14:0_18:1)+NH_4	0.439296153	0.007976335	3.207017749
TG(18:3_10:4_20:4)+H	1.658611959	0.00305657	1.67674838
TG(16:0_14:0_18:2)+NH_4	0.27103057	0.038684155	3.787430626
TG(15:0_16:0_18:1)+NH_4	0.375442783	0.033307388	1.275873743
TG(18:1_14:0_17:1)+NH_4	0.338188188	0.04758452	1.254263982
TG(18:0_16:0_16:0)+NH_4	0.592009079	0.008196223	2.015937438
TG(16:0_16:0_18:1)+NH_4	0.564340166	0.008687765	5.497331142
TG(18:4_10:4_22:5)+H	2.308310942	0.00061887	1.758174014
TG(16:0_16:1_18:1)+NH_4	0.433913059	0.008941659	7.722706724
TG(18:1_14:0_18:2)+NH_4	0.344916184	0.028733835	5.711433439
TG(16:0_16:1_18:2)+NH_4	7.712309413	0.011964489	3.793246083
TG(16:0_16:1_18:3)+NH_4	18.163591	0.005405065	2.812407876
DG(30:0e)+Na	0.328742103	0.002985397	1.250168764
TG(16:0_17:0_18:1)+NH_4	0.378685742	0.008809729	3.738587914
TG(16:0_17:1_18:1)+NH_4	0.316791308	0.036477299	3.124895827

TG(16:0_17:1_18:2)+NH_4	0.288419783	0.047275435	2.226209018
TG(18:0_16:0_18:0)+NH_4	0.585099289	0.006039249	4.604461905
TG(18:0_16:0_18:1)+NH_4	0.535495895	0.011128728	10.12535461
TG(16:0_18:1_18:1)+NH_4	0.502325862	0.004302	13.56217292
TG(18:0_16:0_18:3)+NH_4	0.479949896	0.015635783	11.06452036
TG(18:0_16:0_19:0)+NH_4	0.386614773	0.005587115	1.005271424
TG(18:0_17:0_18:1)+NH_4	0.381533963	0.020469253	1.905904902
TG(17:0_18:1_18:1)+NH_4	0.304540034	0.018866793	3.29766581
TG(18:1_17:1_18:1)+NH_4	0.270495204	0.036452293	3.194179739
TG(18:1_17:1_18:2)+NH_4	0.260973977	0.04122066	2.295682884
TG(16:0_17:1_20:4)+NH_4	0.275273741	5.65242E-05	1.109707685
TG(18:0_18:0_18:1)+NH_4	0.382620889	0.010722223	6.010211252
TG(18:0_18:1_18:1)+NH_4	0.455054097	0.022938648	8.043080869
TG(18:0_18:1_18:2)+NH_4	0.428556207	0.015424068	7.531032351
TG(18:1_18:1_18:2)+NH_4	0.430330797	0.01297518	9.813680611
TG(18:1_18:2_18:2)+NH_4	0.428112418	0.046322364	8.052691217
DG(32:0e)+Na	0.45526656	0.003964946	2.361555604
TG(19:1_18:0_18:1)+NH_4	0.419586562	0.043076073	1.001768709
TG(19:1_18:1_18:1)+NH_4	0.353593743	0.035146536	1.209280367
TG(19:1_18:1_18:2)+NH_4	0.299692877	0.001046576	1.180296933
TG(18:0_18:1_20:1)+NH_4	0.336194256	0.031230312	3.908217791
TG(20:1_18:1_18:1)+NH_4	0.389496313	0.013097157	2.768174511
TG(18:1e_19:1_19:1)+Na	0.079985593	0.026414342	2.631382169
TG(20:1_18:1_18:2)+NH_4	0.470609219	0.046402587	3.41385532
TG(18:1_18:2_20:2)+NH_4	0.472821855	0.023290366	2.919813956
TG(16:0_18:2_22:6)+NH_4	3.656892735	0.014002584	5.22896585
TG(20:5_18:2_18:2)+NH_4	3.358293238	0.007382507	1.648657143
TG(18:1_18:1_21:0)+NH_4	0.602145526	0.026171821	1.30778726
TG(18:2_18:2_22:6)+NH_4	3.128596439	0.016338288	1.713187242
TG(16:0_20:1_22:1)+NH_4	0.699780251	0.000689474	1.219200085
TG(20:1_14:0_24:2)+NH_4	0.629390985	0.002472959	1.142995477
TG(18:3e_18:1_22:2)+H	0.09592332	0.030674587	2.524887486
TG(18:1_18:2_22:6)+NH_4	3.614791294	0.006682302	2.357687599
TG(20:3e_18:2_20:4)+H	0.244248373	0.041597208	1.587024027
TG(18:1_20:4_22:6)+NH_4	5.315130744	0.008805571	1.065621753
TG(18:2_20:4_22:6)+NH_4	4.166550708	0.01019471	1.146503062
DG(32:1e)+Na	0.439267641	0.013754635	1.372936389
TG(26:1_16:0_18:1)+NH_4	0.610763056	0.002549271	1.04808408
ZyE(37:6)+NH4	0.245552219	0.002521174	1.721523377
phSM(t38:6)+H	0.329118105	0.01918305	1.398620522
DG(34:0e)+Na	0.415405143	0.017655013	1.470679842
DG(34:2e)+H	0.478010578	0.026153417	1.394883132
DG(36:3e)+Na	1.350466	0.029608531	1.373240329
DG(36:3e)+H	0.417082555	0.017174029	1.475316293

Hex1Cer(d38:3+2O)+Na	1.551163924	0.002888394	1.405673011

Note: Fold Change means ratio of metabolite content in the PA and CTX groups, VIP means variable importance in the projection.