Supplementary material for the manuscript:

Hop derived fraction rich in beta acids and prenylflavonoids regulates the inflammatory response in dendritic cells differently from quercetin: unveiling metabolic changes by mass spectrometry-based metabolomics

S1: Hop bitter acid fractionation and characterization

Hop pellet is produced by pressing hop powders, which are subsequently packaged under vacuum and stored at -2 to +4°C and this material was used in this work. Hop pellets (Target T90) were kindly donated by a local brewery company (AEFFE, Castel San Giorgio, Salerno, Italy). The whole phytocomplex was extracted as follows. In detail, 1 g of sample was extracted with 12.5 mL of CH_3OH and kept under stirring for 15 minutes in the dark at room temperature. The operation was repeated three times. The supernatants were pooled, dried under reduced pressure and lyophilized. Sample was solubilized in methanol, filtered on a 0.45 μm nylon membrane prior injection in the LC system. Phytocomplex fractionation was performed on a semi-prep HPLC system LC20AP system (Shimadzu, Milan, Italy). A Luna® C18 column 250 mm × 10 mm, 5 μm was employed at a flow rate of 6 mL/min, mobile phase was: A) 0.1% CH_3COOH in H_2O, B) 0.1% CH_3COOH in ACN. Analysis was performed in gradient as follows: 0 min, 5%B, 10 min, 5-40%B, 15 min, 40-75%B, 17 min, 75-100%B, 22 min 100%B. Detection was performed by photo diode array (PDA) and chromatograms were extracted at 280 and 330 nm. Three fractions were collected based on the retention time. In order to assess the correct fractionation process, the obtained crude fractions were further screened by UHPLC-PDA-MS/MS on a UHPLC Nexera coupled to an IT-TOF mass spectrometer (Shimadzu, Milan, Italy). A Kinetex® EVO C18 150 × 2.1 mm, 2.6 μm (Phenomenex®) column was employed at flow rate of 0.5 mL/min. The separation was carried out with the following parameters: mobile phase was: A) 0.1 % CH_3COOH in H_2O *v/v*, B) ACN plus 0.1 % CH_3COOH, gradient: 0-15 min, 5-30%B, 15-20 min, 30-70 %B, 20-22 min, 70-95 %B, 22-25 min, 98-98%B, 25-30 min, 5%B. Column oven was set at 45°C, 2 μL of sample was injected. PDA detection parameters were: sampling rate 12 Hz, time constant 0.160 s and chromatograms were extracted at 280 and 330 nm. ESI- MS detection was performed as follows: detector, 1.60kV; CDL (curve desolvation line), 250 °C; heater block, 250 °C, nebulizer (N_2), 1.5 L/min, drying gas, 100 kPa. MS1 150-1500 m/z, ion accumulation time, 30 ms; IT, repeat =3. MS/MS data dependent acquisition precursor range: 150-900 m/z; peak width 3 Da, accumulation time 40 ms; CID energy, 50%; repeat =3. The confirmation of metabolite identity was assessed High resolution MS and MS/MS spectra, retention time of available standards, and comparison with online database (Mass bank of north America (MoNA) and PubChem) and previous MS/MS data obtained on the same MS device.

S2: UHPLC-PDA-MS/MS quantitative analysis of main compounds in Fraction B and C

International Calibration Extract 4 (ICE-4) for α- and β-acids (Labor Veritas, Zurich, Switzerland) was selected as external standard for the quantification of α and β acids isolated from hop fractions (HOP B, C). ICE-4 was reported to contain 10.88% cohumulone; 31.60% n+adhumulone; 13.02% colupulone; 13.52% n+adlupulone. Stock solution (10 mg/mL) was prepared weighing 150 mg in a 15-mL volumetric flask. 8 mL of CH_3OH were added to the flask, which was sonicated for 10 min

and filled to volume with CH_3OH. The ICE-4 stock solution was filtered through a 0.45 µm nylon filter and was diluted with CH_3OH to concentration of working solutions.
Xanthohumol was selected as external standard for its quantification in HOP C. Stock solution (1 mg/mL) of was prepared in CH_3OH.
Calibration curves in the range of 5–250 µg/mL for total ICE-4 and 10 – 250 µg/mL for Xanthohumol with five concentration levels were constructed from regression analysis ($R^2 \geq 0.999$)
Limits of detection (LODs) and quantification (LOQs) were calculated by the ratio between the standard deviation (SD) and analytical curve slope multiplied by 3 and 10, respectively.
Note that ach standard concentration level of ICE-4 contained different amounts of the individual analytes, as following reported:

Concentration Level	**5 µg/mL**	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**250 µg/mL**
Compound (%)	**Compound concentration in calibration curve level (µg/mL)**				
Cohumulone (10.88%)	0.794	3.971	7.943	15.885	39.714
n-Humulone (24%)	1.736	8.681	17.361	34.722	86.806
Adhumulone (7.6%)	0.550	2.749	5.498	10.995	27.488
Colupulone (13.02%)	0.942	4.709	9.418	18.837	47.092
n-Lupulone (10.30%)	0.745	3.725	7.451	14.902	37.251
Adlupulone (3.22%)	0.233	1.164	2.329	4.659	11.646

Table S1: Quantitative profile of α and β acids and xanthohumol isolated from hop fractions. Values are expressed as µg/mL and µg/mg of extract mean of three replicates ± standard deviation.

Compound	**Hop Fraction B (HOP B)**		**Hop Fraction C (HOP C)**		**LOD**	**LOQ**
	µg/mL ± SD	**µg/mg ± SD**	**µg/mL ± SD**	**µg/mg ± SD**	**µg/mL**	**µg/mL**
Oxo-Humulinone	3.529 ± 0.03	1.176 ± 0.01	-	-	0.022	0.073
Humulinone	20.14 ± 0.12	6.71 ± 0.03	-	-	0.022	0.073
Cohumulinone	5.183 ± 0.02	1.728 ± 0.01	-	-	0.016	0.054
Cohulupone	106.87 ± 0.3	35.63 ± 0.08	-	-	0.008	0.026
Deoxycohumulone	38.36 ± 0.26	12.79 ± 0.07	-	-	0.016	0.054
Xanthohumol	-	-	65.49 ± 0.04	21.83 ± 0.01	0.209	0.696
Lupulon E	-	-	1.862 ± 0.07	0.621 ± 0.02	0.034	0.112
Colupulone	-	-	0.933 ± 0.06	0.311 ± 0.01	0.008	0.026
Lupulone	-	-	0.693 ± 0.03	0.231 ± 0.01	0.034	0.112
Adlupulone	-	-	2.751 ± 0.03	0.917 ± 0.01	0.060	0.207

Figure S1. Fractionation of hop pellets phytocomplex in three different fractions by semi-preparative liquid chromatography (**a**) and UHPLC-UV-MS analysis of the different fractions (**b**).

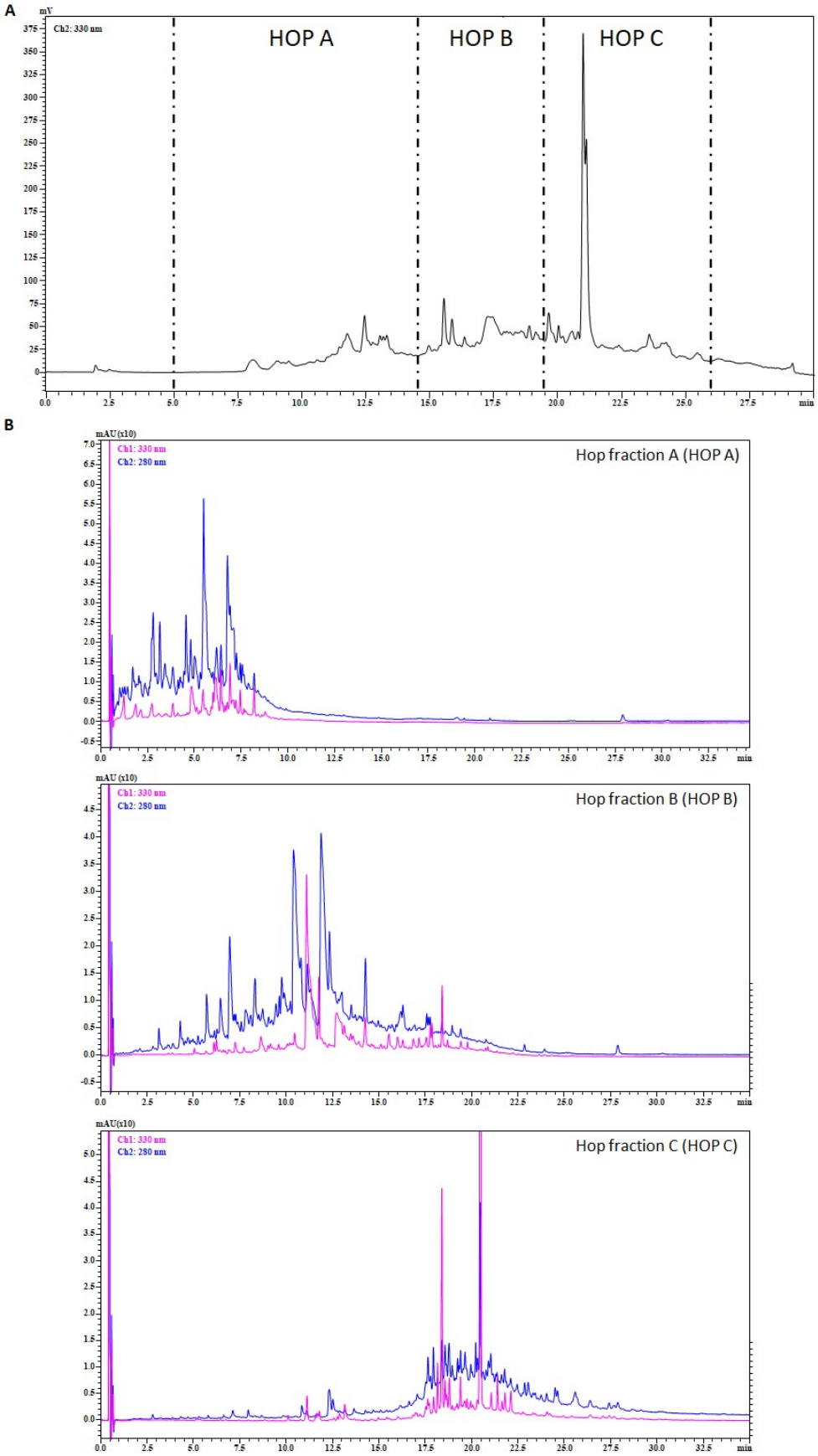


Table S2: UHPLC-MS/MS data of different Hop fractions

Peak	r_t (min)	Compound	$[M-H]^-$	[MS/MS]	Error (ppm)	PDA λ_{max} (nm)	Molecular Formula
Hop Fraction A (HOP A)							
Hydroxycinnamic acid							
1	1.83	3'- caffeolquinic acid (Chlorogenic acid) *	353.0850	191.0625, 179.0385	0.85	320	$C_{16}H_{18}O_9$
2	2.65	3'- coumaroylquinic acid	337.0922	163.0407, 119.0394	-2.67	304	$C_{16}H_{18}O_8$
3	3.52	3' - feruloylquinic acid	367.1040	193.0516, 134.0216	1.36	322	$C_{17}H_{20}O_9$
Flavonols							
4	6.50	Quercetin 3-O-(2-rhamnosyl) -hexoside	755.2036	301.0333, 255.0535 271.0211	-0.53	256, 299	$C_{33}H_{40}O_{20}$
5	6.99	Quercetin 3-O-galactoside*	463.0855	301.0346, 271.0255 255.0290	-3.89	255	$C_{21}H_{20}O_{12}$
6	7.48	Rutin*	609.1445	301.0362, 255.0306 271.0269	-4.43	353	$C_{27}H_{30}O_{16}$
7	7.78	Quercetin 3-O-glucoside*	463.0855	301.0346, 271.0255 255.0290	-4.53	255	$C_{21}H_{20}O_{12}$
8	8.33	Quercetin 3-O-(acetyl) -hexoside	505.0972	301.0381, 271.0204 255.0288	-3.17	311	$C_{23}H_{22}O_{13}$
9	863	Kaempferol 3-O-hexoside	635.1601	285.0384, 593.1373 255.0300 227.0416	0.63	262, 345	$C_{29}H_{32}O_{16}$
10	8.88	Kaempferol 3-O-di-hexoside	593.1413	285.0393, 255.0279 227.0656	-2.42	265, 344	$C_{27}H_{30}O_{15}$
11	8.94	Kaempferol 3-O-–(malonyl-hexoside)-O-rhamnoside	447.0949	285.0433, 255.0302 227.0741	4.03	264, 345	$C_{21}H_{20}O_{11}$
12	2.30	Epicatechin dimer	577.1247	407.0662, 289.0448	-4.97	279	$C_{30}H_{26}O_{12}$
13	3.10	Epicatechin dimer isomer II	577.1288	407.0729, 289.0619	1.73	279	$C_{30}H_{26}O_{12}$
Hop Fraction B (HOP B)							
α- and β- acids derivatives							
14	13.50	Oxy-humulinone	393.1912	349.2050, 263.1292 395.1898	-4.42	255, 323	$C_{21}H_{30}O_7$
15	14.00	Humulinone isomer	377.1928	263.1259, 221.0969	-5.04	285, 323	$C_{21}H_{30}O_6$
16	15.15	Oxidized humulinone derivative	409.1879	263.1285, 295.1130	0.98	285, 323	$C_{21}H_{30}O_8$
17	16.60	Oxidized humulinone derivative isomer	409.1879	263.1285, 295.1130	0.98	285, 323	$C_{21}H_{30}O_8$
18	17.69	Cohumulone	347.1862	278.1176, 235.0624	-0.58	285, 323	$C_{20}H_{28}O_5$
19	18.65	Cohumulinone	363.1814	278.1176, 235.0624	-1.38	275, 310	$C_{20}H_{28}O_6$
20	19.15	Humulinone	377.1968	292.1315, 249.0765	-5.04	285, 323	$C_{21}H_{30}O_6$
21	20.84	Cohumulone isomer	347.1862	235.0624,278.1176	-0.58	285, 323	$C_{20}H_{28}O_5$
22	21.40	Iso-α-ad/n-humulone	361.2015	265.1428, 363.1836	2.21	285, 323	$C_{21}H_{30}O_5$
23	21.70	Deoxycohumulone	331.1920	262.1292, 194.0621	1.51	290, 335	$C_{20}H_{28}O_4$
24	24.30	Prehumulone	375.1818	306.1319	2.40	285, 325	$C_{22}H_{32}O_5$
25	18.10	Cohulupone	317.1765	248.0959, 135.3119	2.21	280, 335	$C_{19}H_{26}O_4$
Hop Fraction C (HOP C)							
β- acids and derivatives							
26	21.70	Lupulone E	415.2489	259.1037, 303.1277	-0.24	235, 360	$C_{25}H_{36}O_5$
27	22.56	Postlupulone	385.1228	273.0903, 248.9524	-3.63	280, 335	$C_{24}H_{34}O_4$
28	24.10	Colupulone	399.2541	287.1253, 219.0712	-0.25	280, 335	$C_{25}H_{36}O_4$
29	26.10	Deoxyhumulone/deoxyadlupulone	345.2064	346.2135, 301.2297 221.0866	-2.03	290, 335	$C_{21}H_{30}O_4$
30	26.80	Lupulone	413.2698	301.1446, 344.1991, 233.0808	2.83	235, 360	$C_{26}H_{38}O_4$
31	26.95	Adlupolone	413.2698	301.1446, 344.1991, 233.0808	2.83	235, 360	$C_{26}H_{38}O_4$
Prenylflavonoids							
32	18.25	Ox-Xanthohumol	369.1336	249.0772	-5.40	255, 307	$C_{21}H_{22}O_6$

33	18.32	Isoxanthohumol	353.1405	233.0921, 189.0277 175.0147	-0.85	370	$C_{21}H_{22}O_5$
34	20.10	Xanthohumol*	353.1405	233.0921, 189.0277 175.0147	-0.85	370	$C_{21}H_{22}O_5$
35	25.10	Prenyl-naringenin	339.1229	219.0672, 245.0805	-1.75	294, 337	$C_{20}H_{20}O_5$

* Identified according to standard retention times.

Figure S2. DCs IL-6 secretion modulation by HOP A, B and C fractions used at different concentrations. * p-value <0.05, *** p-value <0.001, ns non-significant (A). 7-AAD staining showing the percentage of dead cells in control conditions and after the administration of LPS and/or HOP A, B or C fractions (B).

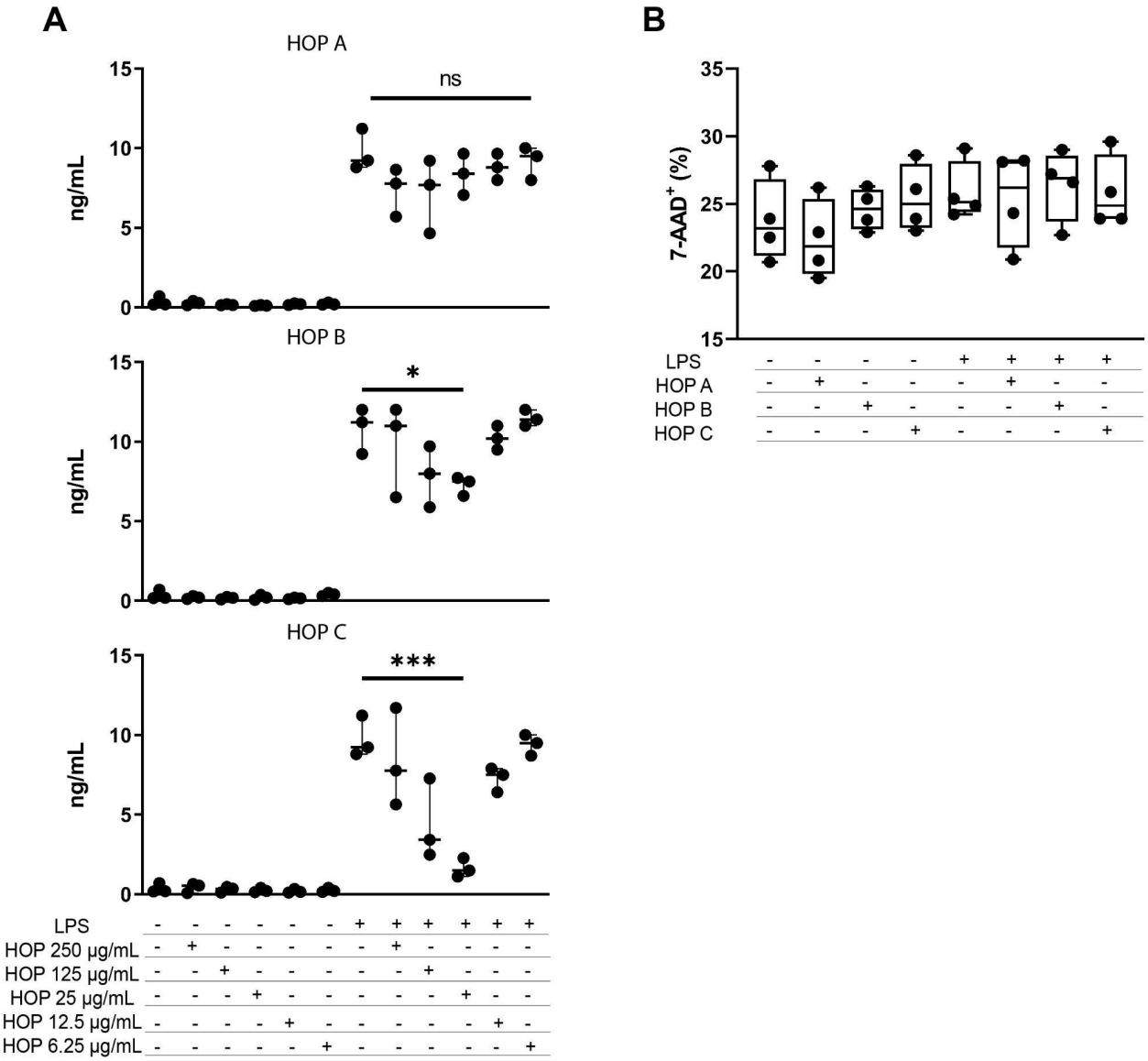


Table S3: List of all metabolites annotated with MSI level 2 or 3 by DI-FT-ICR and/or HILIC UHPLC-HRMS/MS and listed in alphabetical order of biochemical class.

m/z meas.	Compound name (HMDB or LIPIDMAPS)	BIOCHEMICAL CLASS/SUB CLASS	Mol. Formula	Error [ppm]	QC CV%
162.11525	Nicotine imine	Alkaloids and derivatives	$C_{10}H_{13}N_2$	0.66	4.14
104.07071	4-Aminobutanoate	Amino acids, peptides, and analogues	$C_4H_9NO_2$	0.69	6.80
116.07082	5-Aminopentanoic acid	Amino acids, peptides, and analogues	$C_5H_{11}NO_2$	0.88	1.99
130.0497	5-oxo-L-Proline	Amino acids, peptides, and analogues	$C_5H_7NO_3$	0.30	4.16
90.05508	Alanine (Ala)	Amino acids, peptides, and analogues	$C_3H_7NO_2$	4.9	0.48
159.07658	Alanylalanine (Ala-Ala)	Amino acids, peptides, and analogues	$C_6H_{12}N_2O_3$	0.93	2.48
175.11917	Arginine (Arg)	Amino acids, peptides, and analogues	$C_6H_{14}N_4O_2$	0.77	0.63
133.06032	Asparagine (Asn)	Amino acids, peptides, and analogues	$C_4H_8N_2O_3$	0.49	1.32
168.00691	Aspartic acid (Asp)	Amino acids, peptides, and analogues	$C_4H_7NO_4$	0.02	3.36
176.10236	Citrulline	Amino acids, peptides, and analogues	$C_6H_{13}N_3O_3$	0.61	5.94
130.0622	Creatine	Amino acids, peptides, and analogues	$C_4H_9N_3O_2$	0.01	3.65
274.14082	Epsilon-(gamma-Glutamyl)-lysine (gamma-Glu-epsilon-lys)	Amino acids, peptides, and analogues	$C_{11}H_{21}N_3O_5$	-0.09	0.95
168.03022	Furoylglycine	Amino acids, peptides, and analogues	$C_7H_7NO_4$	-0.04	1.29
181.03853	Glutamine (Gln)	Amino acids, peptides, and analogues	$C_5H_{10}N_2O_3$	-0.11	0.62
182.02256	Glutamic acid (Glu)	Amino acids, peptides, and analogues	$C_5H_9NO_4$	0.06	2.15
306.07651	Glutathione (GSH)	Amino acids, peptides, and analogues	$C_{10}H_{17}N_3O_6S$	0.12	2.53
190.03886	Histidine (His)	Amino acids, peptides, and analogues	$C_6H_9N_3O_2$	-0.01	4.92
112.08692	Histamine	Amino acids, peptides, and analogues	$C_5H_9N_3$	0.04	0.93
130.05112	Hydroxyproline (Hyp)	Amino acids, peptides, and analogues	$C_5H_9NO_3$	0.08	2.31
158.08227	Isovalerylglycine	Amino acids, peptides, and analogues	$C_7H_{13}NO_3$	0.05	1.32
243.17151	Isoleucylisoleucine	Amino acids, peptides, and analogues	$C_{12}H_{24}N_2O_3$	0.09	2.89
245.18481	Leucylleucine (LeuLeu)	Amino acids, peptides, and analogues	$C_{12}H_{24}N_2O_3$	2.19	0.55
147.11285	Lysine (Lys)	Amino acids, peptides, and analogues	$C_6H_{14}N_2O$	0.06	1.81
150.05788	Methionine (Met)	Amino acids, peptides, and analogues	$C_5H_{11}NO_2S$	0.12	0.96
200.01534	Methionine sulfoxide	Amino acids, peptides, and analogues	$C_5H_{11}NO_3S$	-0.11	3.87
209.06981	N-Acetylornithine	Amino acids, peptides, and analogues	$C_7H_{14}N_2O3$	-0.10	2.98
203.15018	N,N-Dimethylarginine	Amino acids, peptides, and analogues	$C_8H_{18}N_4O_2$	-0.49	1.32
223.08548	N6-Acetyl-L-lysine	Amino acids, peptides, and analogues	$C_8H_{16}N_2O_3$	0.07	4.50
132.1019	Norleucine	Amino acids, peptides, and analogues	$C_6H_{13}NO_2$	0.03	1.07
164.0717	Phenylalanine (Phe)	Amino acids, peptides, and analogues	$C_9H_{11}NO_2$	0.01	0.68
192.06587	Phenylacetylglycine	Amino acids, peptides, and analogues	$C_{10}H_{11}NO_3$	0.75	1.32
164.01199	Pyroglutamic acid	Amino acids, peptides, and analogues	$C_5H_7NO_3$	-0.14	2.19
116.07062	Proline (Pro)	Amino acids, peptides, and analogues	$C_5H_9NO_2$	0.02	1.51
104.03423	Serine (Ser)	Amino acids, peptides, and analogues	$C_3H_7NO_3$	0.15	0.29
152.00231	Sulfinoalanine	Amino acids, peptides, and analogues	$C_3H_7NO_4S$	-0.06	1.51
118.04976	Threonine (Thr)	Amino acids, peptides, and analogues	$C_4H_9NO_3$	0.08	2.82
216.04327	Tyrosine (Tyr)	Amino acids, peptides, and analogues	$C_9H_{11}NO_3$	-0.23	2.43
118.08654	Valine	Amino acids, peptides, and analogues	$C_5H_{11}NO_2$	0.30	1.84
139.0502	Urocanic acid	Azoles	$C_6H_6N_2O_2$	0.14	0.70
181.0710	Iditol	Carbohydrates and carbohydrate conjugates	$C_6H_{14}O_6$	0.76	1.47
259.02283	Mannose 6-phosphate	Carbohydrates and carbohydrate conjugates	$C_6H_{13}O_9P$	0.40	2.02
259.02243	Fructose 6-phosphate	Carbohydrates and carbohydrate conjugates	$C_6H_{13}O_9P$	-0.09	3.16
338.98876	Fructose 1,6-bisphosphate	Carbohydrates and carbohydrate conjugates	$C_6H_{14}O_{12}P_2$	0.15	11.20

308.0987	N-Acetylneuraminic acid	Carbohydrates and carbohydrate conjugates	$C_{11}H_{19}NO_9$	0.34	4.72
275.01732	Phosphogluconic acid	Carbohydrates and carbohydrate conjugates	$C_6H_{13}O_{10}P$	-0.08	1.49
229.01189	Ribulose 5-phosphate	Carbohydrates and carbohydrate conjugates	$C_5H_{11}O_8P$	-0.05	3.50
289.03302	Sedoheptulose 7-phosphate	Carbohydrates and carbohydrate conjugates	$C_7H_{15}O_{10}P$	0.11	6.33
173.00914	Aconitic acid	Carboxylic acids and derivatives	$C_6H_6O_6$	-0.11	1.76
191.01971	Citric acid	Carboxylic acids and derivatives	$C_6H_8O_7$	-0.05	6.52
131.03371	Glutaric acid	Carboxylic acids and derivatives	$C_5H_8O_4$	1.28	5.53
117.01822	Succinic acid	Dicarboxylic acids and derivative	$C_4H_6O_4$	1.11	8.76
145.04979	2-Methylglutaric acid	Fatty acids and conjugates	$C_6H_{10}O_4$	0.84	4.63
253.08336	FA 9:0 + 1O, sulfate	Fatty Acyls [FA]	$C_6H_{15}N_4O_5P$	6.65	3.40
227.20107	FA 14:0	Fatty Acyls [FA]	$C_{14}H_{28}O_2$	0.59	5.60
241.21729	FA 15:0	Fatty Acyls [FA]	$C_{15}H_{30}O_2$	-0.10	6.63
291.20962	FA 16:0	Fatty Acyls [FA]	$C_{16}H_{32}O_2$	-0.04	2.11
289.19403	FA 16:1	Fatty Acyls [FA]	$C_{16}H_{30}O_2$	0.104	2.39
269.24976	FA 17:0	Fatty Acyls [FA]	$C_{17}H_{34}O_2$	1.16	4.33
315.17323	FA 17:3;O	Fatty Acyls [FA]	$C_{17}H_{28}O_3$	-0.20	4.36
283.2648	FA 18:0	Fatty Acyls [FA]	$C_{18}H_{36}O_2$	0.55	7.70
281.24855	FA 18:1	Fatty Acyls [FA]	$C_{18}H_{34}O_2$	-0.05	7.70
279.23294	FA 18:2	Fatty Acyls [FA]	$C_{18}H_{32}O_2$	-0.03	5.85
547.61929	FA 18:4;O	Fatty Acyls [FA]	$C_{39}H_8O$	0.98	0.13
309.28098	FA 20:1	Fatty Acyls [FA]	$C_{20}H_{38}O_2$	1.07	4.75
341.2253	FA 20:3	Fatty Acyls [FA]	$C_{20}H_{34}O_2$	-0.03	2.33
339.20965	FA 20:4	Fatty Acyls [FA]	$C_{20}H_{32}O_2$	0.06	2.09
355.20458	FA 20:4;O	Fatty Acyls [FA]	$C_{20}H_{32}O_3$	0.14	3.54
337.19401	FA 20:5	Fatty Acyls [FA]	$C_{20}H_{30}O_2$	0.13	2.46
365.22533	FA 22:5	Fatty Acyls [FA]	$C_{22}H_{34}O_2$	0.34	3.29
363.20963	FA 22:6	Fatty Acyls [FA]	$C_{22}H_{32}O_2$	0.03	5.14
132.10203	FA 24:9;O	Fatty Acyls [FA]	$C_6H_{13}NO_2$	0.63	5.04
397.40375	FA 26:0	Fatty Acyls [FA]	$C_{26}H_{52}O_2$	-0.62	6.06
410.31847	FA 28:7	Fatty Acyls [FA]	$C_{28}H_{42}O_2$	0.22	6.41
152.99601	FA 4:1;O2	Fatty Acyls [FA]	$C_4H_6O_4$	0.02	0.64
115.04008	FA 5:1;O	Fatty Acyls [FA]	$C_5H_8O_3$	0.03	0.62
129.01934	FA 5:2;O2	Fatty Acyls [FA]	$C_5H_6O_4$	0.06	2.15
173.08091	FA 8:2;O2	Fatty Acyls [FA]	$C_8H_{12}O_4$	0.15	2.53
379.20459	17-HDoHE	Fatty Acyls [FA]	$C_{22}H_{32}O_3$	0.19	8.55
353.18892	5-HEPE	Fatty Acyls [FA]	$C_{20}H_{30}O_3$	0.04	4.92
204.12315	Acetylcarnitine	Fatty Acyls [FA]	$C_9H_{18}NO_4$	0.67	6.60
147.0299	Citramalic acid	Fatty Acyls [FA]	$C_5H_8O_5$	-0.01	3.84
129.01794	Mesaconic acid	Fatty Acyls [FA]	$C_5H_6O_4$	1.89	4.48
786.16479	Flavin-adenin-dinucleotide	Flavin nucleotides	$C_{27}H_{33}N_9O_{15}P_2$	0.36	1.54
783.59881	MGDG 36:1	Glycerolipids [GL]	$C_{45}H_{82}O_{10}$	0.99	3.29
835.68001	TG 51:7	Glycerolipids [GL]	$C_{54}H_{90}O_6$	-0.90	1.75
854.61206	IPC 38:0;O3	Glycerophospholipids [GP]	$C_{44}H_{88}NO_{12}P$	0.40	1.62
480.3097	LPC 15:0	Glycerophospholipids [GP]	$C_{23}H_{48}NO_7P$	0.33	6.44
496.33844	LPC 16:0	Glycerophospholipids [GP]	$C_{24}H_{50}NO_7P$	1.77	1.55
522.3526	LPC 18:1	Glycerophospholipids [GP]	$C_{26}H_{52}NO_7P$	3.66	3.68

476.27908	LPE 18:2	Glycerophospholipids [GP]	$C_{23}H_{44}NO_7P$	2.99	4.35
436.28337	LPE 16:0	Glycerophospholipids [GP]	$C_{21}H_{44}NO_6P$	-0.06	1.38
555.27295	LPG 22:6	Glycerophospholipids [GP]	$C_{28}H_{45}O_9P$	-0.58	8.75
619.28886	LPI 20:4	Glycerophospholipids [GP]	$C_{29}H_{49}O_{12}P$	0.34	2.09
524.29946	LPS 18:0	Glycerophospholipids [GP]	$C_{24}H_{48}NO_9P$	0.15	3.54
524.29837	LPS 18:1	Glycerophospholipids [GP]	$C_{24}H_{46}NO_9P$	0.43	2.46
425.23017	PA 16:0	Glycerophospholipids [GP]	$C_{19}H_{37}O_8P$	0.67	5.14
709.48193	PA 37:4	Glycerophospholipids [GP]	$C_{40}H_{71}O_8P$	0.87	5.04
761.60541	PA 40:0	Glycerophospholipids [GP]	$C_{43}H_{85}O_8P$	-0.13	6.06
783.58926	PA 42:3	Glycerophospholipids [GP]	$C_{45}H_{83}O_8P$	-0.12	6.41
817.66783	PA 44:0	Glycerophospholipids [GP]	$C_{47}H_{93}O_8P$	-0.64	0.64
809.60633	PA 44:4	Glycerophospholipids [GP]	$C_{47}H_{85}O_8P$	0.98	0.62
685.51872	PA O-36:2	Glycerophospholipids [GP]	$C_{39}H_{75}O_7P$	0.89	2.15
678.54412	PC 29:0	Glycerophospholipids [GP]	$C_{37}H_{76}NO_7P$	0.72	2.53
804.64689	PC 37:0	Glycerophospholipids [GP]	$C_{45}H_{90}NO_8P$	-0.62	8.55
840.58778	PC 37:1	Glycerophospholipids [GP]	$C_{45}H_{88}NO_8P$	-0.16	9.30
826.63135	PC 39:3	Glycerophospholipids [GP]	$C_{47}H_{88}NO_8P$	-0.40	4.92
824.61583	PC 39:4	Glycerophospholipids [GP]	$C_{47}H_{86}NO_8P$	-0.45	8.74
822.60015	PC 39:5	Glycerophospholipids [GP]	$C_{47}H_{84}NO_8P$	-0.58	6.91
846.69391	PC 40:0	Glycerophospholipids [GP]	$C_{48}H_{96}NO_8P$	-0.59	2.46
844.67848	PC 40:1	Glycerophospholipids [GP]	$C_{48}H_{94}NO_8P$	-0.57	2.11
842.66339	PC 40:2	Glycerophospholipids [GP]	$C_{48}H_{92}NO_8P$	-0.01	1.75
840.64776	PC 40:3	Glycerophospholipids [GP]	$C_{48}H_{90}NO_8P$	0.21	5.91
796.62038	PC O-38:4	Glycerophospholipids [GP]	$C_{46}H_{86}NO_7P$	-0.94	7.79
791.59198	PC O-42:3	Glycerophospholipids [GP]	$C_{45}H_85O_7P$	-0.31	2.31
500.27849	PE 20:4	Glycerophospholipids [GP]	$C_{25}H_{44}NO_7P$	0.48	1.86
844.58521	PE 44:8	Glycerophospholipids [GP]	$C_{49}H_{82}NO_8P$	-0.21	6.52
722.51292	PE O-36:5	Glycerophospholipids [GP]	$C_{41}H_{74}NO_7P$	-0.28	1.41
780.58965	PE O-40:4	Glycerophospholipids [GP]	$C_{45}H_{82}NO_7P$	-0.22	3.82
804.59091	PE O-42:7	Glycerophospholipids [GP]	$C_{47}H_{82}NO_7P$	0.84	1.76
747.51838	PG 34:1	Glycerophospholipids [GP]	$C_{40}H_{77}O_{10}P$	-0.06	3.66
773.53427	PG 36:2	Glycerophospholipids [GP]	$C_{42}H_{79}O_{10}P$	0.89	5.17
819.51802	PG 40:7	Glycerophospholipids [GP]	$C_{46}H_{77}O_{10}P$	-0.47	4.23
719.52324	PG O-33:1	Glycerophospholipids [GP]	$C_{39}H_{77}O_9P$	0.06	4.61
829.62952	PG O-39:0	Glycerophospholipids [GP]	$C_{45}H_{91}O_9P$	0.30	2.15
184.07303	Phosphocholine	Glycerophospholipids [GP]	$C_5H_{15}NO_4P$	0.26	6.32
883.53529	PI 38:5	Glycerophospholipids [GP]	$C_{47}H_{81}O_{13}P$	0.94	6.09
877.61726	PI O-38:2	Glycerophospholipids [GP]	$C_{47}H_{89}O_{12}P$	0.95	2.19
766.46595	PS 35:5	Glycerophospholipids [GP]	$C_{41}H_{70}NO_{10}P$	-0.08	3.73
830.59041	PS 39:2	Glycerophospholipids [GP]	$C_{45}H_{84}NO_{10}P$	-0.28	9.55
848.63757	PS 40:0	Glycerophospholipids [GP]	$C_{46}H_{90}NO_{10}P$	-0.36	4.66
828.61012	PS O-40:3	Glycerophospholipids [GP]	$C_{46}H_{86}NO_9P$	-0.85	5.69
826.59464	PS O-40:4	Glycerophospholipids [GP]	$C_{46}H_{84}NO_9P$	-0.80	4.29
854.62672	PS O-42:4	Glycerophospholipids [GP]	$C_{48}H_{88}NO_9P$	0.16	6.38
834.65893	PS O-40:0	Glycerophospholipids [GP]	$C_{46}H_{92}NO_9P$	0.90	2.43
830.62581	PS O-40:2	Glycerophospholipids [GP]	$C_{46}H_{88}NO_9P$	-0.99	2.95

852.61055	PS P-42:4	Glycerophospholipids [GP]	$C_{48}H_{86}NO_9P$	-0.52	6.70
804.57552	PT 18:0/18:1	Glycerophospholipids [GP]	$C_{43}H_{82}NO_{10}P$	0.74	7.92
133.01425	Malic acid	Hydroxy acids and derivatives	$C_4H_6O_5$	0.06	1.52
135.03117	Hypoxanthine	Imidazopyrimidines	$C_5H_4N_4O$	0.05	1.15
187.00282	Xanthine	Imidazopyrimidines	$C_5H_4N_4O_2$	-0.09	0.98
131.04622	Ureidopropionic acid	Organic carbonic acids and derivatives	$C_4H_8N_2O_3$	0.133	2.08
124.00739	Taurine	Organic sulfonic acids and derivatives	$C_2H_7NO_3S$	0.06	3.17
162.11235	Carnitine	Organonitrogen compounds	$C_7H_{15}NO_3$	0.12	1.09
185.08125	Phosphorylcholine	Organonitrogen compounds	$C_3H_7O_7P$	0.49	2.31
150.11188	Triethanolamine	Organonitrogen compounds	$C_6H_{15}NO_3$	0.69	1.87
184.98567	Phosphoglyceric acid	Organooxygen compounds	$C_3H_7O_7P$	-0.09	3.51
218.1034	Pantothenic acid	Organooxygen compounds/ Alcohols and polyols	$C_9H_{17}NO_5$	0.01	3.03
165.05449	Phenylpyruvic acid	Phenylpyruvic acid derivatives	$C_9H_8O_3$	-0.26	7.88
489.11526	Cytidine 5'-diphosphocholine	Pterins and derivatives	$C_{14}H_{26}N_4O_{11}P_2$	0.67	0.66
268.1037	Adenosine	Purine nucleosides	$C_{10}H_{13}N_5O_4$	0.18	4.17
303.05018	Inosine	Purine nucleosides	$C_{10}H_{12}N_4O_5$	-0.04	0.36
346.0558	Adenosine monophosphate (AMP)	Purine ribonucleotides	$C_{10}H_{14}N_5O_7P$	-0.03	2.10
347.03981	Inosinic acid	Purine ribonucleotides	$C_{10}H_{13}N_4O_8P$	-0.04	1.67
362.05077	Guanosine monophosphate (GMP)	Purine ribonucleotides	$C_{10}H_{14}N_5O_8P$	0.04	3.32
166.0146	Quinolinic acid	Pyridines and derivatives	$C_7H_5NO_4$	0.20	7.17
123.05499	Nicotinamide	Pyridine nucleotides	$C_6H_6N_2O$	0.30	1.92
112.0507	Cytosine	Pyrimidines and pyrimidine derivatives	$C_4H_5N_3O$	0.16	1.38
265.11081	Thiamine	Pyrimidines and pyrimidine derivative	$C_{12}H_{17}N_4OS$	0.43	1.57
111.01873	Uracil	Pyrimidines and pyrimidine derivatives	$C_4H_4N_2O_2$	1.27	0.27
242.07832	Cytidine	Pyrimidine nucleosides	$C_9H_{13}N_3O_5$	0.07	1.26
323.02855	Orotidine	Pyrimidine nucleosides	$C_{10}H_{12}N_2O8$	-0.72	2.56
565.04834	Uridine diphosphate glucose UDP-D-glucose	Pyrimidine nucleotides	$C_{15}H_{24}N_2O_{17}P_2$	0.61	2.87
606.07312	Uridine 5'-diphospho-N-acetylglucosamine (UDP-Glc-NAc)	Pyrimidine nucleotides	$C_{17}H_{27}N_3O_{17}P_2$	1.16	2.60
323.02963	Uridine 5'-monophosphate	Pyrimidine nucleotides	$C_9H_{13}N_2O_9P$	1.04	2.48
243.0619	Uridine	Pyrimidine nucleosides	$C_9H_{12}N_2O_6$	0.36	3.88
608.08728	Uridine diphosphate-N-acetylgalactosamine (UDP-GalNAc)	Pyrimidine nucleotides	$C_{17}H_{27}N_3O_{17}P_2$	1.65	2.29
188.03533	Kynurenic acid	Quinoline carboxylic acids	$C_{10}H_7NO_3$	-0.01	5.05
608.59766	Cer 39:1;O2	Sphingolipids [SP]	$C_{39}H_{77}NO_3$	0.07	2.42
700.56567	CerP 40:1;O2	Sphingolipids [SP]	$C_{40}H_{80}NO_6P$	0.65	2.90
670.52582	HexCer 32:1;O2	Sphingolipids [SP]	$C_{38}H_{73}NO_8$	-0.97	9.21
744.59858	HexCer 36:1;O3	Sphingolipids [SP]	$C_{42}H_{81}NO_9$	0.03	1.41
742.58258	HexCer 36:2;O3	Sphingolipids [SP]	$C_{42}H_{79}NO_9$	0.24	6.60
740.60316	HexCer 37:2;O2	Sphingolipids [SP]	$C_{43}H_{81}NO_8$	-0.43	9.76
756.59815	HexCer 37:2;O3	Sphingolipids [SP]	$C_{43}H_{81}NO_9$	-0.44	9.19
754.61911	HexCer 38:2;O2	Sphingolipids [SP]	$C_{44}H_{83}NO_8$	0.15	4.97
786.68187	HexCer 40:0;O2	Sphingolipids [SP]	$C_{46}H_{91}NO_8$	0.45	0.66
784.64567	SM 40:2;O2	Sphingolipids [SP]	$C_{45}H_{89}N_2O_6P$	0.14	7.33
783.63721	SM 40:3;O2	Sphingolipids [SP]	$C_{45}H_{87}N_2O_6P$	0.18	10.88
455.3363	ST 26:0;O6	Sterol Lipids [ST]	$C_{26}H_{46}O_6$	0.11	2.11

S4: Metabolomics data processing

Thermo RAW. data files were converted to ABF format using Reifycs Abf (Analysis Base File) converter, subesequently alignement of Profile Q-Exactive files was performed with MS tolerance set at 0.01 for MS and 0.025 for MS/MS with retention time tolerance of 0.1 min. Minimum peak height for detection was set to 10000 amplitude value. Lipid identification was performed with internal Lipidblast library while metabolites with internal libraries ESI POS and ESI NEG All_Public_MS/MS. All metabolites were annotation were based on mass accuracy, isotopic pattern and spectral matching, rev.dot product, score cut off was 70%. All reported spectral matches were manually revised for correct assignment, adducts with sodium, formate and acetate were allowed for adduct correction. The alignement was performed only on matched MS/MS metabolites blank corrected. Direct infusion nano-electrospray was performed by an automated multisample chip-based nESI sample ionization platform TriVersa NanoMate (Advion BioSciences Ltd, Ithaca NY, U.S.A), which was operated with the following parameters: gas pressure (nitrogen) was 0.3 psi, spray voltage 1.45kV, sample volume was 5 µL, sample plate temperature was set to 10°C, 5 µm nominal internal diameter nozzle chip was used. DI-FT-ICR data analysis was performed with Metaboscape 2021 (Bruker). The first step is the creation of a matrix (bucket table) using the T-ReX 2D algorithm. The T-Rex 2D algorithm for feature extraction from FT-ICR single spectra extracts m/z / intensity pairs (peaks) from acquired raw data and subjects them to deisotoping in order to create features consisting of isotope patterns. Subsequently, spectra alignment, filtering, normalization were performed. The spectra were processed in positive mode using H^+ as the primary ion, Na^+ and NH_4^+ as a potential adducts, while in negative mode H^- was set as the primary ion and Cl^- as a potential adduct. For metabolite annotation, assignment of the molecular formula was performed for the detected features using Smart Formula™ (SF), isotopic fine structure (ISF) and data recalibration. The bucket table was annotated with a list of metabolites and lipids obtained respectively from the HMDB (https://hmdb.ca/) and LIPIDMAPS database (www.lipidmaps.org). Annotation was performed with 0.2 ppm (narrow) or 1 ppm (wide) mass tolerance and a mSigma value below 200, molecular formula were manually inspected taking into account the most probable adduct form.

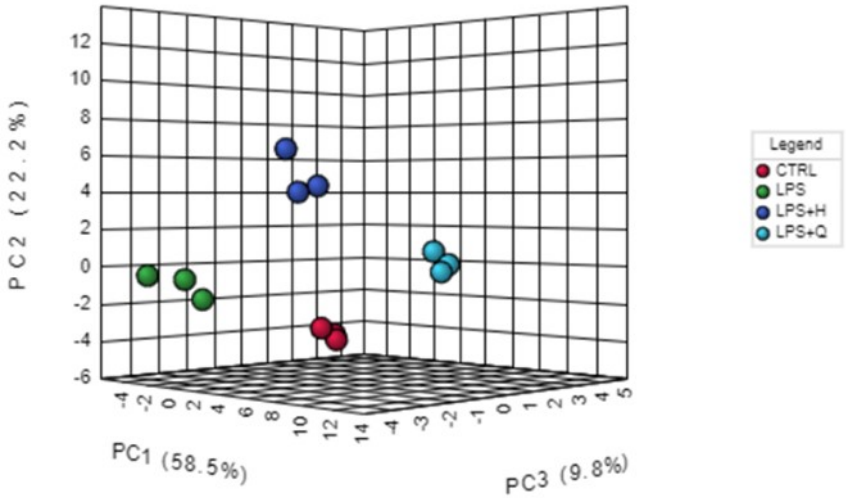


Figure S3: PCA 3D-score plot

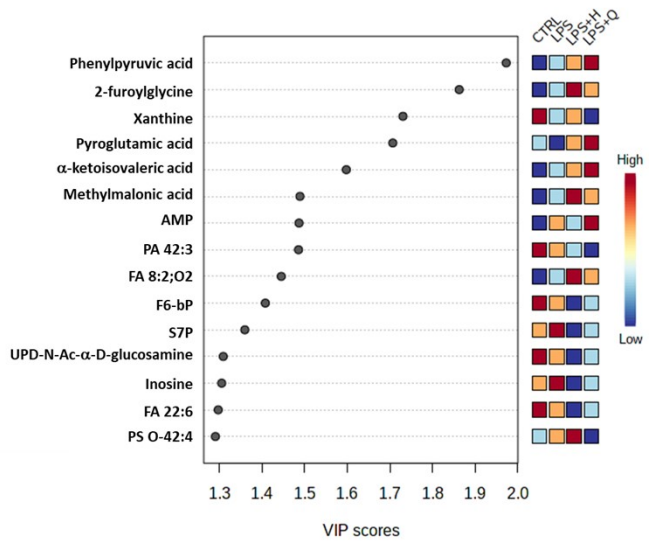
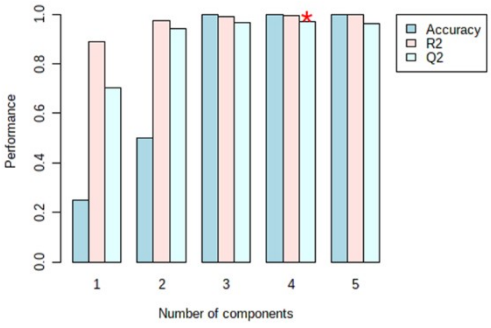
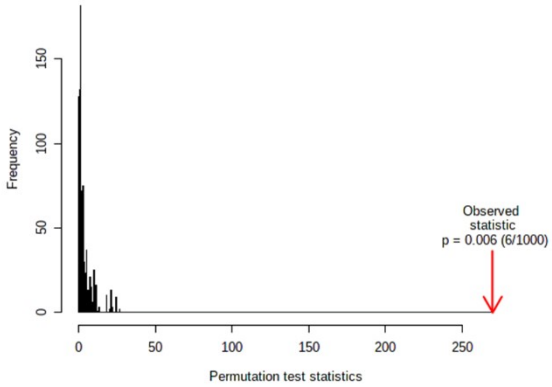


Figure S4: 15 high scoring VIP metabolites (VIP > 1.3) derived from PLS-DA analysis





PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.25	0.5	1.0	1.0	1.0
R2	0.88932	0.97527	0.99178	0.99509	0.99917
Q2	0.70159	0.94172	0.96723	0.97038	0.9612

Figure S5: Cross validation values and permutation test of PLS-DA model