Supplementary Information

Single-cell metabolite profiling enables information-rich classification of lymphocyte types and subtypes

Siyuan Pan,[a] Changyi Liu,[b] Huan Yao,[c] Xingyu Pan,[a] Jinhang Li,[d] Jinlei Yang,[a] Murong Du,[a] Peng Liu,[*b] Sichun Zhang[*a] and Xinrong Zhang[a]

[a.] Department of Chemistry, Tsinghua University, Beijing 100084, P.R. China. E-mail: sczhang@mail.tsinghua.edu.cn

[b.] State Key Laboratory of Complex, Severe, and Rare Diseases, Peking Union Medical College Hospital, Beijing 100730, P.R. China. Email: liupeng@pumch.cn

[c.] Division of Chemical Metrology and Analytical Science, National Institute of Metrology, Beijing 100029, P.R.China.

[d.] Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R.China.

Table of Contents

1. Experimental Section

1.1 Ethics Approval

This research complies with all relevant ethical regulations. The study of human samples was performed according to the Declaration of Helsinki and Good Clinical Practice and approved by the Ethnical Committee of Peking Union Medical College Hospital (grant no. I-23PJ1876). Informed written consent was obtained from all participants.

1.2 Chemical Reagents

Methanol (HPLC/UHPLC-UV grade) was purchased from fisher chemical. Ammonium formate (for LC-MS, ≥99.0%) was purchased from Sigma-Aldrich. PBMCs Ficoll was purchased from Dayou. RPMI 1640 medium and Dulbecco's phosphate buffered saline (DPBS) were purchased from Gibco Life Technologies. Ultrapure water (resistance ≥18 MΩ/cm) was prepared from the Milli-Q water purification system.

CD3 monoclonal antibody (OKT3), CD19 monoclonal antibody (HIB19), CD56 (NCAM) monoclonal antibody (CMSSB), CD14 monoclonal antibody (61D3), CD16 monoclonal antibody (eBioCB16 (CB16)) and LIVE/DEAD kit were all purchased from eBioscience. CD4 monoclonal antibody (A161A1) and CD8 monoclonal antibody (SK1) were purchased from Biolegend.

1.3 FCM Sorting of Lymphocytes

Whole blood was obtained from the volunteers and mixed with an equal volume of DPBS. PBMCs Ficoll was added to the blood sample and centrifuged. After obtaining the layered sample, the PBMCs layer was collected and washed with DPBS by centrifugation twice. In PBMCs study, CD3 antibody, CD19 antibody, CD56 antibody, CD14 antibody and CD16 antibody were used for staining. In lymphocyte study, CD3 antibody, CD19 antibody, CD56 antibody, CD4 antibody and CD8 antibody were used for staining. LIVE/DEAD kit was used to detect the viability of cells. Samples were incubated in the dark for 30 minutes and washed, then resuspended in RPMI 1640 medium and transferred to flow cytometry tubes for FCM. A Moflo EQ flow cytometer from Beckman was used. The samples were first compensated using single stained samples and then sorted. Live cells were gated by forward scatter (FSC), side scatter (SSC) and LIVE/DEAD kit. T cells and B cells were gated by $CD3^+$ and $CD19^+$, respectively. NK cells were gated from $CD3^-$ $CD19^-$ cells by $CD56^+$. Monocytes were gated as $CD14^+CD16^+$ cells from live cells. T cell subtypes were dsicriminated by $CD4^+CD8^-$ and $CD8^+CD4^-$ from T cells. Sorted cells were resuspended in ammonium formate solution (140 mM) and small molecules in the medium were removed by centrifugation twice. The concentration of the cell suspension before MS detection was controlled at 5×10^4 cells/mL.

1.4 Device Configuration and Optimization

The device consists of three parts: cell sampling system, online extraction system and MS analysis system. The schematic diagram and photos of the device are shown in Figure S1. The cell sampling system includes a sampling pump and a cell sampling bottle. The cell suspension was introduced into the next part of the device through a capillary by creating a pressure difference between the inside and outside of the cell sampling bottle with the sampling pump. The online extraction system includes a coaxial capillary device, a high-voltage power supply, an injection pump and auxiliary gas. Cells were transmitted in single-cell form through the inner capillary. Methanol sheath liquid was injected through the in-between capillary at 20 μL/min by the injection pump, and performed online extraction of the cell contents. N_2 was chosen as the auxiliary gas and was transmitted through the outer capillary at the pressure of 0.4 MPa, quickly evaporating the solution. The distance between inner capillary and in-between capillary was optimized to 2 mm to adjust to the size of lymphocytes. An QE-Orbitrap MS from Thermo Fisher Scientific was used in the MS analysis system to analyze the cell metabolites. Dead volume is avoided in the whole process.

Since lymphocytes are smaller in size and more complex in composition compared with epithelial cells, the ability of the device to analyze complex samples needs to be evaluated. Unsorted PBMCs were used for preliminary experiment. The concentration of the cell suspension was 5×10^4 cells/mL. Totally 679 cells were detected in 20 min, corresponding to 34 cells/min. The extracted ion chromatogram (EIC) of PC(34:1) ($[M+H]^+$ 760.58) representing cell events is shown in Figure S2a, proving that the optimized device has enough sensitivity for clinical samples. The heatmap of the retained 448 ions in cells after data processing indicates the complex composition of PBMCs (Figure S2b). To simulate the complex environment in human body, we established a cancer cell lines model by mixing HeLa cells and Hek293 cells at a ratio of 4:1. Then the sample was analyzed by MS, and 213 single-cell data with 170 HeLa cells and 43 Hek293 cells were obtained. As shown in Figure S2c-S2d, the metabolic information of different groups was visualized in the two-dimensional plane by UMAP algorithm, showing that 173 of them were HeLa cells and 40 of them were Hek293 cells in the mixed group, with the recovery rate of 102% and 93%. The results show that the optimized device is applicable for complex samples research. To further evaluate the stability of the device, sorted T cells and NK cells were used for clustering analysis, which showed great reliability of the discriminating results (Figure S3a-S3b).

1.5 Data Processing

We used SCMeTA (https://www.sc-meta.com) to process single-cell data. First, the raw file was imported into the software and extracted into matrix form. Then, the extracted data were filtered through preprocessing process. In positive ion mode, m/z 760.58 is usually the peak with the highest intensity of all metabolites, and is commenly used to identify cell events, named refer m/z. Data with refer m/z intensity higher than a certain ratio of the maximum value were retained and considered as cell events. Peaks with high frequency of occurrence and signal-to-noise ratio greater than three in cell events were retained as ions. Further, two processing methods were used to normalize and standardize data, using refer m/z of each cell event for normalization or taking the logarithm base two of the absolute intensity of metabolites for standardization. Heatmap was used to visually display the differences in metabolites between different groups and within groups in the processed data. Each column represents a single cell, each row represents an ion, and the color shows the content of the ion in the cell. The data processing software includes clustering analysis methods, such as Principal Component Analysis (PCA), t-distributed Stochastic Neighbor Embedding (t-SNE) and UMAP. UMAP was mainly used in this work. UMAP is a non-linear dimensionality reduction technique that preserves both the local and global structure of the data by approximating the manifold on which the data lies and projecting it to a lower-dimensional space. It is faster and more scalable than PCA and t-SNE.

1.6 Metabolites Annotation and Analysis

The ions retained after data processing were compared with HMDB using accurate mass measurement (https://hmdb.ca/). Forms such as $[M+H]^+$ and $[M+Na]^+$ were used to determine the existence form of ions in the positive mode. Some lipids with isomers were annotated by general names. Ions that did not match in HMDB were considered as exogenous interference ions and were deleted in the further analysis. The metabolic pathway difference analysis was performed in MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/).

2. Supplementary Figures

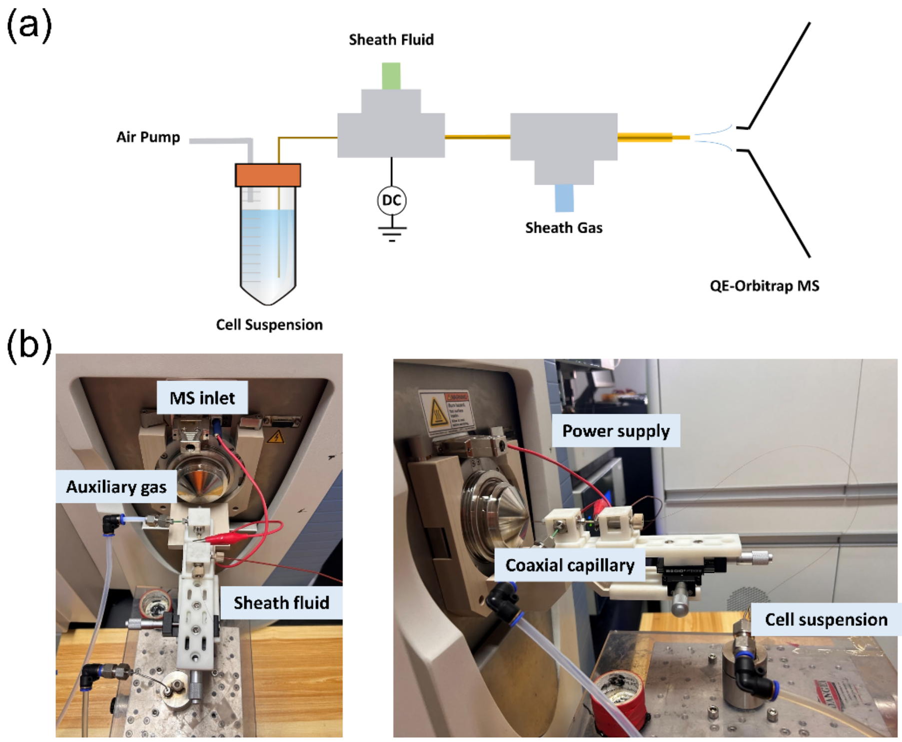

Figure S1. Schematic diagram and photos of the device. (a) Schematic diagram of the device. (b) Photos of the device showing its basic construction, including top view (left) and side view (right).

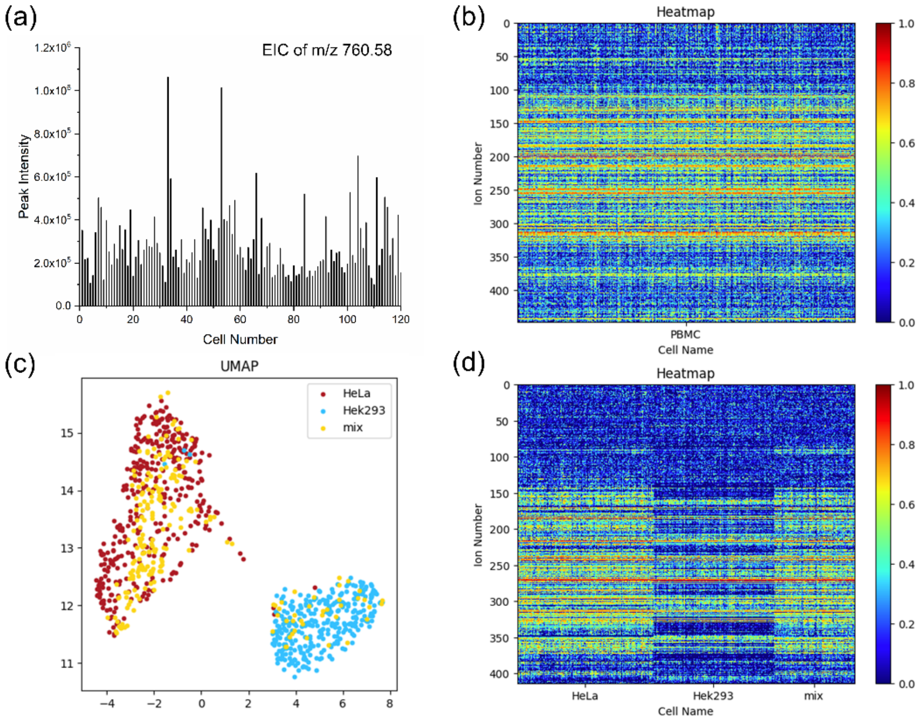

Figure S2. Acquisition of metabolite profiles from single cells. (a) EIC of PC (34:1) of PBMCs in the positive ion mode. (b) Heatmap of the ions detected in 679 PBMCs. (c) UMAP analysis of HeLa cells, Hek293 cells and mixed cells. (d) Heatmap of the ions detected in mixed cancer cells of Hek293 cells and HeLa cells.

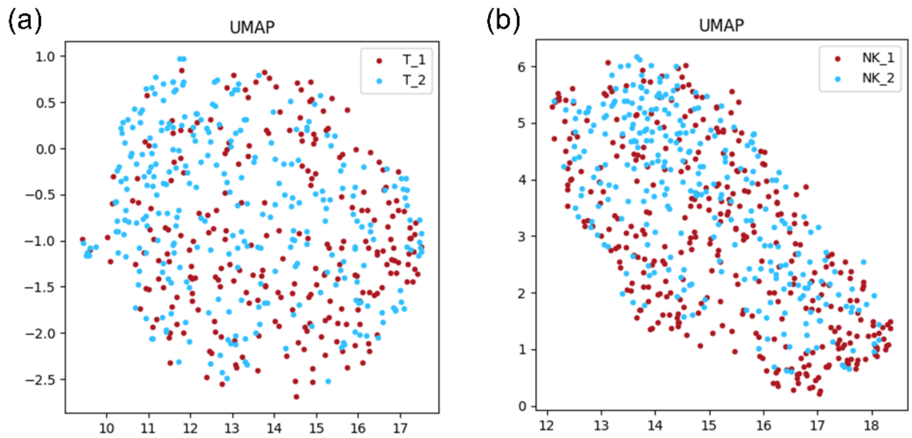

Figure S3. Proof of stability. (a) UMAP analysis of T cells from different batches. (b) UMAP analysis of NK cells from different batches.

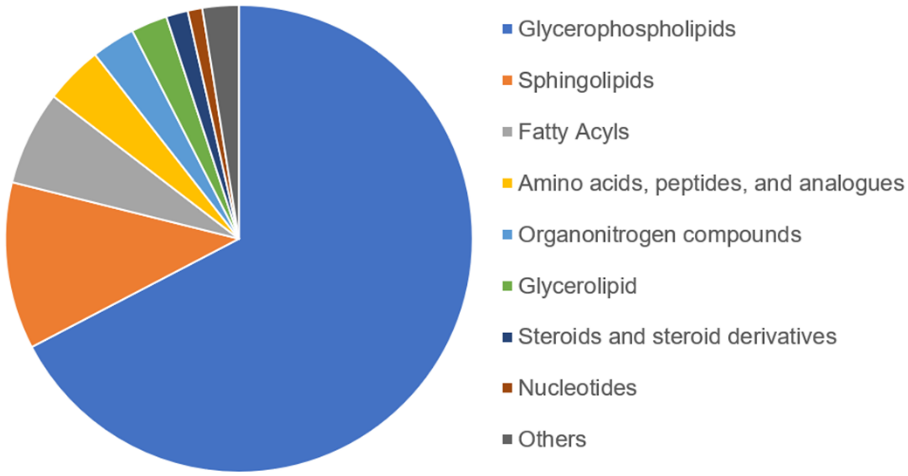

Figure S4. Classification of detected metabolites in lymphocytes in the positive mode.

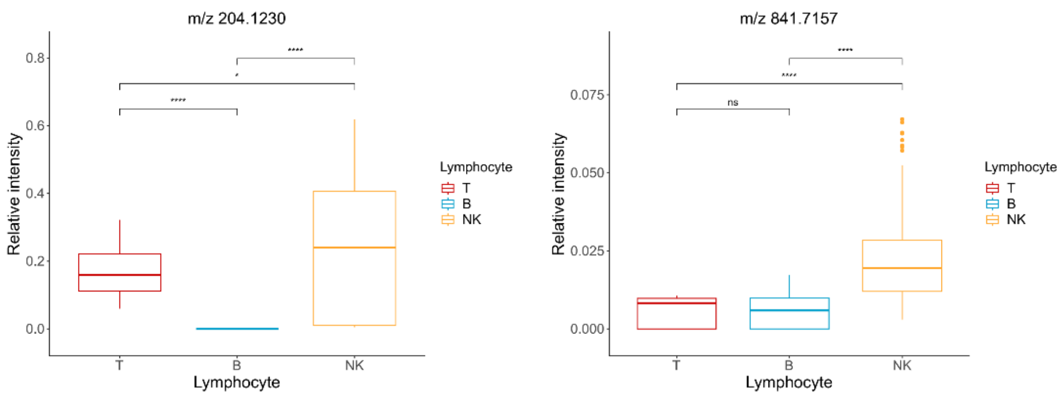

Figure S5. Distribution of acetylcarnitine (left) and SM(d44:2) (right) in T cells, B cells and NK cells.

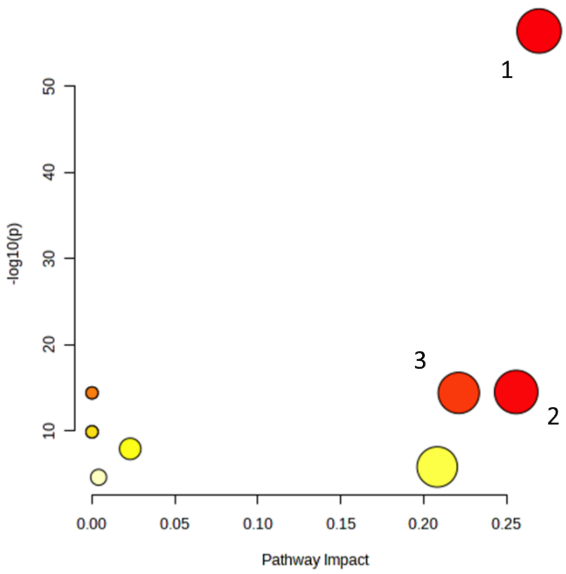

Figure S6. The metabolic pathway difference between T cells and B cells, including (1) sphingophospholipid metabolism pathway, (2) glutathione metabolism pathway and (3) histidine metabolism pathway.

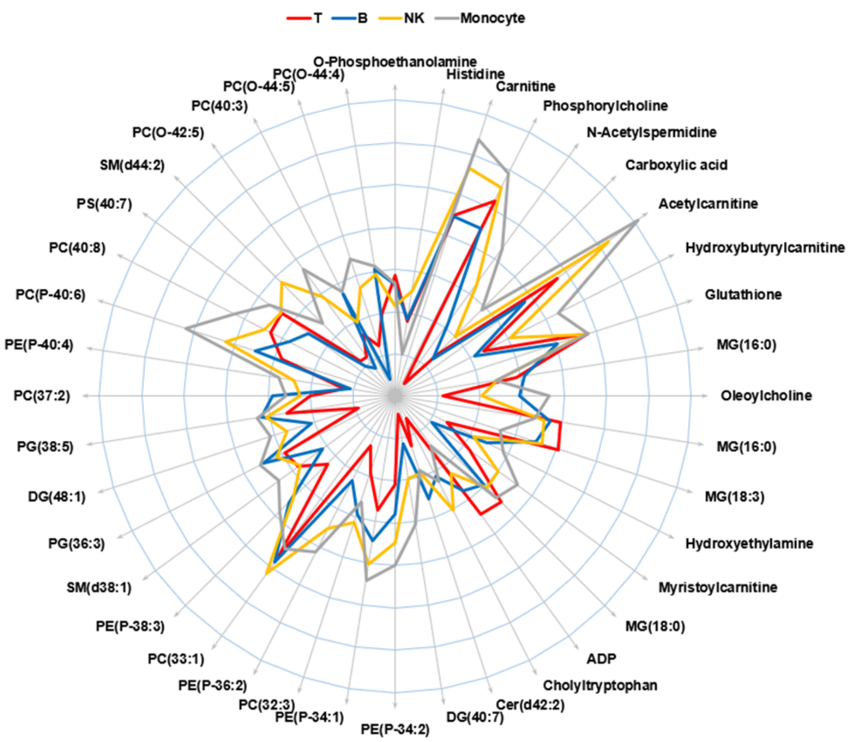

Figure S7. Logarithmic radar chart of 40 differential metabolites in T cells, B cells, NK cells and monocytes.

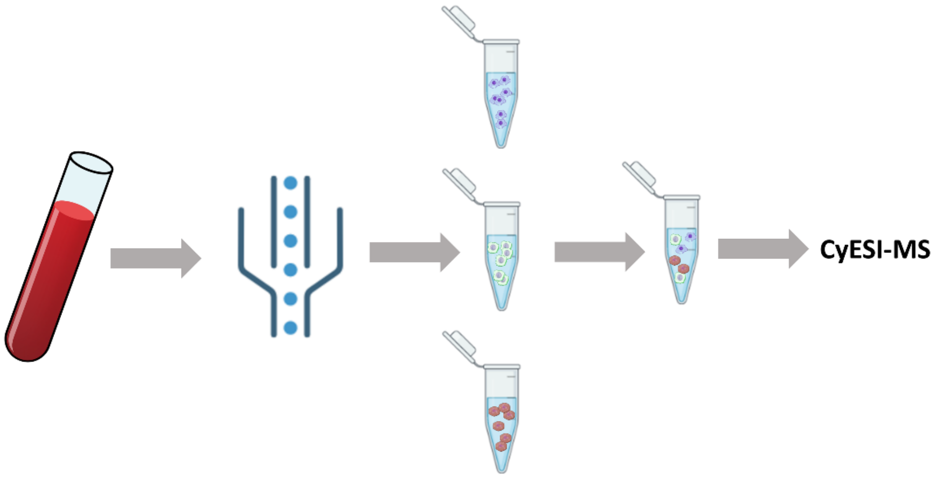

Figure S8. Schematic diagram of the procedure of preparing mixed lymphocytes.

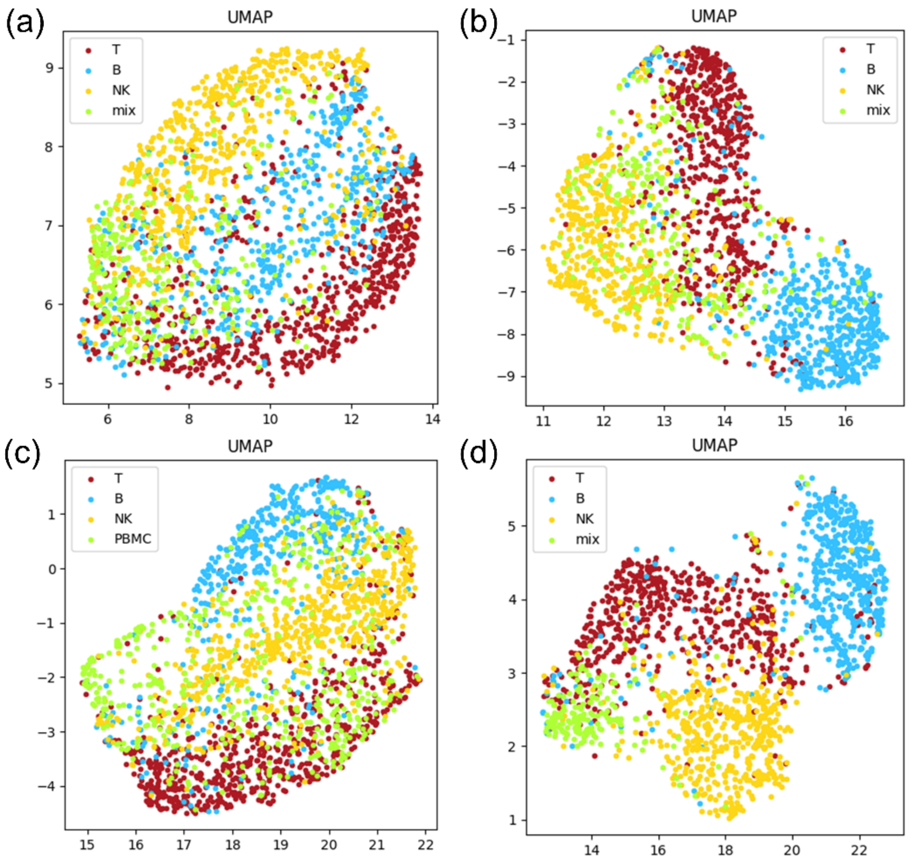

Figure S9. Proof of accuracy and repeatability. (a, b) UMAP analysis of mixed cells on the same day. (c) UMAP analysis of PBMC on the same day. (d) UMAP analysis of mixed cells on different days.

3. Supplementary Tables

Table S1. 177 metabolites assigned from lymphocytes at single-cell level in positive ion mode (scan range m/z 150-1000).

No.	Metabolites assignment	Chemical formula	Observed mass, m/z	Theoretical mass, m/z	Formula	Error, ppm
1	Histidine	C6H9N3O2	156.0772	156.0768	M+H	2.56
2	L-Carnitine	C7H15NO3	162.1128	162.1125	M+H	1.85
			184.0947	184.0944	M+Na	1.63
3	O-Phosphoethanolamine	C2H8NO4P	164.0085	164.0083	M+Na	1.22
4	DL-Glutamate	C5H9NO4	170.0424	170.0424	M+Na	0.00
5	Phosphorylcholine	C5H15NO4P	184.0737	184.0739	M+H	1.09
6	N-Acetylspermidine	C9H21N3O	188.1761	188.1757	M+H	2.13
7	1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid	C10H11NO2	200.0686	200.0682	M+Na	2.00
8	Acetylcarnitine	C9H17NO4	204.1234	204.1230	M+H	1.96
9	Phosphorylcholine	C5H15NO4P	206.0558	206.0558	M+Na	0.00
10	Orgothionenine/Ergothioneine	C9H15N3O2S	230.0960	230.0958	M+H	0.87
			252.0781	252.0777	M+Na	1.59
11	Hydroxybutyrylcarnitine	C11H21NO5	248.1497	248.1492	M+H	2.01
12	3-Methyl-5-pentyl-2-furanpentanoic acid	C15H24O3	253.1802	253.1798	M+H	1.58
13	Palmitic amide	C16H33NO	256.2633	256.2635	M+H	0.78
14	Glycerophosphorylcholine	C8H20NO6P	280.0916	280.0920	M+Na	1.43
15	Tyrosyl-Cysteine	C12H16N2O4S	284.1064	284.1058	M+NH4-H2O	2.11
16	hydroxyisovaleroyl carnitine	C12H23NO5	284.1474	284.1468	M+Na	2.11
17	Glutathione	C10H17N3O6S	308.0911	308.0911	M+H	0.00
			330.0730	330.0730	M+Na	0.00
18	methylnonadecanoic acid	C20H40O2	330.3369	330.3367	M+NH4	0.61
19	MG(16:0/0:0/0:0)	C19H38O4	331.2845	331.2843	M+H	0.60
20	Oleoylcholine	C23H46NO2	332.3315	332.3323	M+H-2H2O	2.41
21	hydroxyphytanic acid	C20H40O3	346.3314	346.3316	M+NH4	0.58
22	AMP	C10H14N5O7P	348.0706	348.0704	M+H	0.57
23	MG(16:0/0:0/0:0)	C19H38O4	348.3106	348.3108	M+NH4	0.57
24	MG(18:3/0:0/0:0)	C21H36O4	353.2671	353.2686	M+H	4.25
25	MG(18:0/0:0/0:0)	C21H42O4	359.3161	359.3156	M+H	1.39
			381.2982	381.2975	M+Na	1.84

26	(5Z,7E)-9,10-Seco-5,7,10(19)-cholestatriene	C27H44	369.3522	369.3516	M+H	1.62
27	Myristoylcarnitine	C21H41NO4	372.3110	372.3108	M+H	0.54
28	Hydroxydocosanoic acid	C22H44O3	374.3632	374.3629	M+NH4	0.80
29	Palmitoylcarnitine	C23H45NO4	400.3425	400.3421	M+H	1.00
30	Pentacosanoylglycine	C27H53NO3	404.3893	404.3898	M+H-2H2O	1.24
31	ADP	C10H15N5O10P2	428.0363	428.0367	M+H	0.93
32	LysoPC(16:0/0:0)	C24H50NO7P	496.3403	496.3398	M+H	1.01
33	LysoPC(18:3/0:0)	C26H48NO7P	518.3226	518.3241	M+H	2.89
34	LysoPC(18:1/0:0)	C26H52NO7P	522.3568	522.3554	M+H	2.68
35	LysoPC(18:0/0:0)	C26H54NO7P	524.3721	524.3711	M+H	1.91
36	Cer(d18:1/16:0)	C34H67NO3	538.5193	538.5194	M+H	0.19
			560.5009	560.5013	M+Na	0.71
37	LysoPC(20:4/0:0)	C28H50NO7P	544.3395	544.3398	M+H	0.55
38	LysoPC(20:3/0:0)	C28H52NO7P	546.3546	546.3554	M+H	1.46
39	Cholyltryptophan	C35H50N2O6	577.3647	577.3642	M+H-H2O	0.87
40	DG(38:4)	C41H72O5	627.5350	627.5353	M+H-H2O	0.48
41	Cer(d42:2)	C42H81NO3	648.6293	648.6289	M+H	0.62
42	DG(40:7)	C43H70O5	667.5289	667.5296	M+H	1.05
43	SM(d32:1)	C37H75N2O6P	675.5441	675.5436	M+H	0.74
44	SM(d33:1)	C38H77N2O6P	689.5603	689.5592	M+H	1.60
45	PE(P-34:2)	C39H74NO7P	700.5281	700.5276	M+H	0.71
46	SM(d34:2)	C39H77N2O6P	701.5605	701.5592	M+H	1.85
47	PE(P-34:1)	C39H76NO7P	702.5430	702.5432	M+H	0.28
48	SM(d34:1)	C39H79N2O6P	703.5763	703.5749	M+H	1.99
49	PC(30:0)	C38H76NO8P	706.5393	706.5381	M+H	1.70
50	PE(34:2)	C39H74NO8P	716.5225	716.5225	M+H	0.00
51	SM(d35:1)	C40H81N2O6P	717.5915	717.5905	M+H	1.39
52	PC(31:1)	C39H76NO8P	718.5388	718.5381	M+H	0.97
53	PC(O-32:0)	C40H80NO7P	718.5757	718.5745	M+H	1.67
54	PC(31:0)	C39H78NO8P	720.5555	720.5538	M+H	2.36
55	DG(41:3-2OH)	C44H80O7	721.5950	721.5977	M+H	3.74
56	PE(P-36:4)	C41H74NO7P	724.5281	724.5276	M+H	0.69
57	PC(32:3)	C40H74NO8P	728.5220	728.5225	M+H	0.69
58	PE(P-36:2)	C41H78NO7P	728.5593	728.5589	M+H	0.55
59	SM(d36:2)	C41H81N2O6P	729.5912	729.5905	M+H	0.96
60	PC(32:2)	C40H76NO8P	730.5391	730.5381	M+H	1.37

61	PC(P-33:1)	C41H80NO7P	730.5741	730.5745	M+H	0.55
62	SM(d36:1)	C41H83N2O6P	731.6081	731.6062	M+H	2.60
			753.5892	753.5881	M+Na	1.46
63	PC(32:1)	C40H78NO8P	732.5549	732.5538	M+H	1.50
64	PC(32:0)	C40H80NO8P	734.5703	734.5694	M+H	1.23
65	PC(P-34:4)	C42H76NO7P	738.5441	738.5432	M+H	1.22
66	PC(33:4)	C41H74NO8P	740.5242	740.5225	M+H	2.30
67	PC(P-34:3)	C42H78NO7P	740.5580	740.5589	M+H	1.22
68	PC(33:3)	C41H76NO8P	742.5382	742.5381	M+H	0.13
69	PC(P-34:2)	C42H80NO7P	742.5742	742.5745	M+H	0.40
70	PC(33:2)	C41H78NO8P	744.5544	744.5538	M+H	0.81
71	PC(P-34:1)	C42H82NO7P	744.5901	744.5902	M+H	0.13
72	PC(33:1)	C41H80NO8P	746.5699	746.5694	M+H	0.67
73	PC(P-34:0)	C42H84NO7P	746.6072	746.6058	M+H	1.88
74	PG(34:2)	C40H75O10P	747.5157	747.5171	M+H	1.87
75	PE(P-38:6)	C43H74NO7P	748.5287	748.5276	M+H	1.47
			770.5107	770.5095	M+Na	1.56
76	PC(33:0)	C41H82NO8P	748.5834	748.5851	M+H	2.27
77	PC(O-34:0)	C42H86NO7P	748.6215	748.6215	M+H	0.00
78	PG(34:1)	C40H77O10P	749.5323	749.5327	M+H	0.53
79	PE(P-38:5)	C43H76NO7P	750.5440	750.5432	M+H	1.07
			772.5262	772.5252	M+Na	1.29
80	PG(34:0)	C40H79O10P	751.5472	751.5484	M+H	1.60
81	PE(P-38:4)	C43H78NO7P	752.5602	752.5589	M+H	1.73
82	PC(34:4)	C42H76NO8P	754.5372	754.5381	M+H	1.19
83	PE(P-38:3)	C43H80NO7P	754.5694	754.5745	M+H	6.76
84	PC(34:3)	C42H78NO8P	756.5528	756.5538	M+H	1.32
85	PC(34:2)	C42H80NO8P	758.5710	758.5694	M+H	2.11
86	SM(d38:1)	C43H87N2O6P	759.6378	759.6375	M+H	0.39
87	PC(34:1)	C42H82NO8P	760.5851	760.5851	M+H	0.00
88	PE(38:6)	C43H74NO8P	764.5230	764.5225	M+H	0.65
89	PC(35:5)	C43H76NO8P	766.5386	766.5381	M+H	0.65
90	PC(P-36:4)	C44H80NO7P	766.5748	766.5745	M+H	0.39
			788.5530	788.5565	M+Na	4.44
91	PC(35:4)	C43H78NO8P	768.5548	768.5538	M+H	1.30
92	PC(P-36:3)	C44H82NO7P	768.5912	768.5902	M+H	1.30
93	PC(35:3)	C43H80NO8P	770.5688	770.5694	M+H	0.78
94	PC(P-36:2)	C44H84NO7P	770.6049	770.6058	M+H	1.17
95	PC(35:2)	C43H82NO8P	772.5856	772.5851	M+H	0.65
96	PC(P-36:1)	C44H86NO7P	772.6203	772.6215	M+H	1.55

97	PG(36:3)	C42H77O10P	773.5306	773.5327	M+H	2.71
98	PE(P-40:7)	C45H76NO7P	774.5433	774.5432	M+H	0.13
			796.5271	796.5252	M+Na	2.39
99	PC(35:1)	C43H84NO8P	774.6006	774.6007	M+H	0.13
100	PC(P-36:0)	C44H88NO7P	774.6372	774.6371	M+H	0.13
			796.6210	796.6191	M+Na	2.39
101	PG(36:2)	C42H79O10P	775.5466	775.5484	M+H	2.32
102	PE(P-40:6)	C45H78NO7P	776.5590	776.5589	M+H	0.13
			798.5417	798.5408	M+Na	1.13
103	PG(36:1)	C42H81O10P	777.5630	777.5640	M+H	1.29
104	PE(P-40:5)	C45H80NO7P	778.5754	778.5745	M+H	1.16
			800.5568	800.5565	M+Na	0.37
105	PG(36:0)	C42H83O10P	779.5795	779.5797	M+H	0.26
106	PC(36:5)	C44H78NO8P	780.5529	780.5538	M+H	1.15
107	PE(P-38:1)	C43H84NO7P	780.5892	780.5878	M+Na	1.79
108	PC(36:4)	C44H80NO8P	782.5698	782.5694	M+H	0.51
109	PC(36:3)	C44H82NO8P	784.5850	784.5851	M+H	0.13
			806.5678	806.5670	M+Na	0.99
110	SM(d40:2)	C45H89N2O6P	785.6523	785.6531	M+H	1.02
111	PC(36:2)	C44H84NO8P	786.6019	786.6007	M+H	1.53
112	SM(d40:1)	C45H91N2O6P	787.6693	787.6688	M+H	0.63
			809.6503	809.6507	M+Na	0.49
113	PS(36:2)	C42H78NO10P	788.5466	788.5436	M+H	3.80
114	PC(36:1)	C44H86NO8P	788.6169	788.6164	M+H	0.63
115	PS(36:1)	C42H80NO10P	790.5623	790.5593	M+H	3.79
116	DG(48:1)	C51H98O5	791.7484	791.7487	M+H	0.38
117	PC(37:6)	C45H78NO8P	792.5562	792.5538	M+H	3.03
118	PC(P-38:5)	C46H82NO7P	792.5902	792.5902	M+H	0.00
119	PG(37:0)	C43H85O10P	793.5943	793.5953	M+H	1.26
120	PC(37:5)	C45H80NO8P	794.5701	794.5694	M+H	0.88
121	PC(P-38:4)	C46H84NO7P	794.6061	794.6058	M+H	0.38
122	PC(37:4)	C45H82NO8P	796.5843	796.5851	M+H	1.00
123	PG(38:5)	C44H77O10P	797.5316	797.5327	M+H	1.38
124	PC(35:0)	C43H86NO8P	798.5967	798.5983	M+Na	2.00
125	PC(P-38:2)	C46H88NO7P	798.6369	798.6371	M+H	0.25
126	PG(38:4)	C44H79O10P	799.5448	799.5484	M+H	4.50
127	PC(37:2)	C45H86NO8P	800.6153	800.6164	M+H	1.37
128	PC(P-38:1)	C46H90NO7P	800.6530	800.6528	M+H	0.25
129	SM(d41:1)	C46H93N2O6P	801.6835	801.6844	M+H	1.12
130	PE(P-40:4)	C45H82NO7P	802.5723	802.5721	M+Na	0.25

131	PC(38:7)	C46H78NO8P	804.5532	804.5538	M+H	0.75
132	PC(38:6)	C46H80NO8P	806.5671	806.5694	M+H	2.85
133	PC(38:5)	C46H82NO8P	808.5848	808.5851	M+H	0.37
134	PC(38:4)	C46H84NO8P	810.6008	810.6007	M+H	0.12
135	SM(d42:3)	C47H91N2O6P	811.6695	811.6688	M+H	0.86
			833.6502	833.6507	M+Na	0.60
136	PS(38:4)	C44H78NO10P	812.5453	812.5436	M+H	2.09
137	PC(38:3)	C46H86NO8P	812.6150	812.6164	M+H	1.72
138	SM(d42:2)	C47H93N2O6P	813.6846	813.6844	M+H	0.25
			835.6667	835.6663	M+Na	0.48
139	PS(38:3)	C44H80NO10P	814.5591	814.5593	M+H	0.25
140	PC(38:2)	C46H88NO8P	814.6306	814.6320	M+H	1.72
141	SM(d42:1)	C47H95N2O6P	815.6994	815.7001	M+H	0.86
			837.6820	837.6820	M+Na	0.00
142	PC(P-40:7)	C48H82NO7P	816.5894	816.5902	M+H	0.98
143	PC(38:1)	C46H90NO8P	816.6483	816.6477	M+H	0.73
144	PC(P-40:6)	C48H84NO7P	818.6047	818.6058	M+H	1.34
145	PC(P-40:5)	C48H86NO7P	820.6217	820.6215	M+H	0.24
146	PC(P-40:4)	C48H88NO7P	822.6370	822.6371	M+H	0.12
147	PC(P-40:3)	C48H90NO7P	824.6524	824.6528	M+H	0.49
148	PC(P-40:3)	C48H90NO7P	846.6333	846.6347	M+Na	1.65
149	PG(40:5)	C46H81O10P	825.5602	825.5640	M+H	4.60
150	PC(P-40:2)	C48H92NO7P	826.6683	826.6684	M+H	0.12
			848.6512	848.6504	M+Na	0.94
151	PC(40:9)	C48H78NO8P	828.5530	828.5538	M+H	0.97
152	PC(P-40:1)	C48H94NO7P	828.6827	828.6841	M+H	1.69
153	PC(40:8)	C48H80NO8P	830.5696	830.5694	M+H	0.24
154	PC(40:7)	C48H82NO8P	832.5840	832.5851	M+H	1.32
155	PS(40:7)	C46H76NO10P	834.5271	834.5280	M+H	1.08
156	PC(40:6)	C48H84NO8P	834.6005	834.6007	M+H	0.24
157	PS(40:6)	C46H78NO10P	836.5455	836.5436	M+H	2.27
158	PC(40:5)	C48H86NO8P	836.6168	836.6164	M+H	0.48
			858.6002	858.5983	M+Na	2.21
159	PI(34:1)	C43H81O13P	837.5486	837.5488	M+H	0.24
160	PS(40:5)	C46H80NO10P	838.5595	838.5593	M+H	0.24
161	PC(40:4)	C48H88NO8P	838.6302	838.6320	M+H	2.15
162	PI(34:0)	C43H83O13P	839.5637	839.5644	M+H	0.83
163	PS(40:4)	C46H82NO10P	840.5743	840.5749	M+H	0.71
164	PC(38:0)	C46H92NO8P	840.6442	840.6453	M+Na	1.31
165	SM(d44:2)	C49H97N2O6P	841.7151	841.7157	M+H	0.71

166	PG(42:8)	C48H79O10P	847.5479	847.5484	M+H	0.59
167	PC(O-42:5)	C50H92NO7P	850.6668	850.6684	M+H	1.88
168	PC(O-42:4)	C50H94NO7P	852.6851	852.6841	M+H	1.17
169	PC(42:10)	C50H80NO8P	854.5698	854.5694	M+H	0.47
170	PC(42:9)	C50H82NO8P	856.5849	856.5851	M+H	0.23
171	PC(42:7)	C50H86NO8P	860.6153	860.6164	M+H	1.28
172	PC(40:3)	C48H90NO8P	862.6285	862.6296	M+Na	1.28
173	PC(O-44:6)	C52H94NO7P	876.6850	876.6841	M+H	1.03
174	PC(O-44:5)	C52H96NO7P	878.7000	878.6997	M+H	0.34
175	PC(O-44:4)	C52H98NO7P	880.7172	880.7154	M+H	2.04
176	PI(38:4)	C47H83O13P	887.5659	887.5644	M+H	1.69
			909.5466	909.5463	M+Na	0.33
177	PC(44:9)	C52H86NO8P	906.6007	906.5983	M+Na	2.65

Table S2. Repeated results of percentage calculation of lymphocytes.

	Cell name	Actual Percentage (%)	Calculated Percentage (%)	Error (%)
Repeated group 1	T	35.00	33.23~36.92	-1.77~1.92
	B	25.00	24.31~26.77	-0.69~1.77
	NK	40.00	40.62~42.46	0.62~2.46
Repeated group 2	T	35.00	32.31~35.00	-2.69~0.00
	B	15.00	12.69~16.15	-2.31~1.15
	NK	50.00	46.54~50.77	-3.46~0.77
Repeated group 3	T	53.19	50.44~56.64	-2.75~3.45
	B	15.34	14.45~18.88	-0.89~3.54
	NK	31.48	30.97~37.46	-0.51~5.98
Repeated group 4	T	70.00	72.92~79.17	2.92~9.17
	B	0.00	0.52~2.08	0.52~2.08
	NK	30.00	26.04~32.29	-3.96~2.29

Table S3. Results of calculation of CD4/CD8 ratio.

	Actual value	Calculated value	Error
Mixed cells	2	1.80~2.16	-0.20~0.16
PBMCs	1.77	1.48~1.84	-0.29~0.07