Electronic Supporting Information

Introducing Charge Tag via Click Reaction in Living Cells for Single Cell Mass Spectrometry

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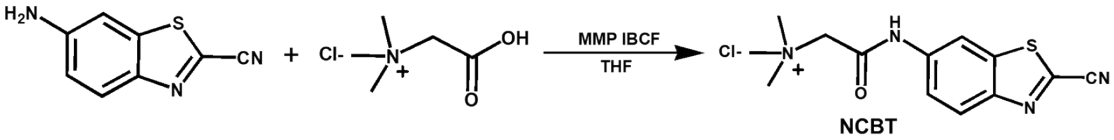
Syntheses of NCBT

Scheme S1. The synthetic route for NCBT



Synthesis of NCBT: Isobutyl chloroformate (IBCF, 20 µL, 0.15 mmol) was added to the mixture of betaine hydrochloride (23 mg, 0.15 mmol) and 4-methylmorpholine (MMP, 33 µL, 0.3 mmol) in tetrahydrofuran (THF, 2 mL). The mixture was stirred at 0 °C for 30 min. The solution of 2-cyano-6-aminobenzothiazole (CBT, 8.8 mg, 0.05 mmol) was added to the reaction mixture and further stirred at 0 °C for 1 h. Then the mixture was stirred at room temperature overnight. The pure product NCBT was obtained after high performance liquid chromatography purification. High performance liquid chromatography analyses were performed on an Agilent 1200 HPLC system equipped with a G1322A pump and in-line diode array UV detector using an Agilent Zorbax 300SB-C18 RP column with acetonitrile and water (0.1% of TFA) as the eluent.

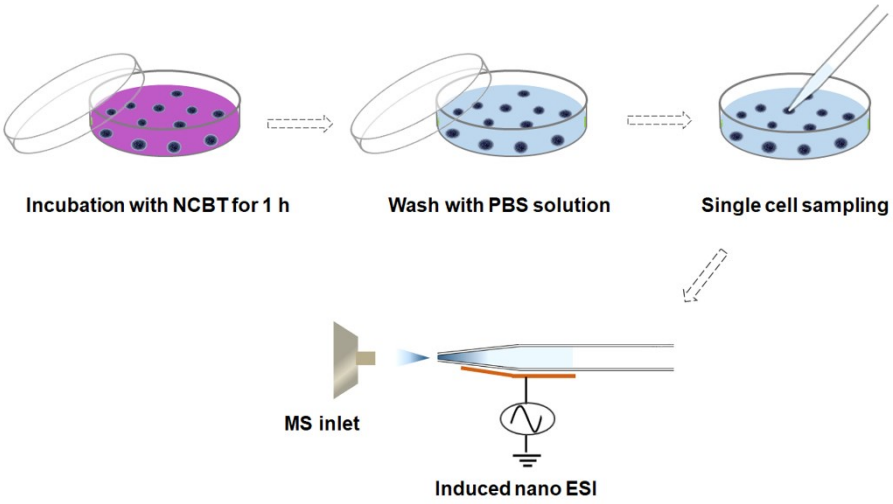


Fig. S1 Schematic of single cell experimental workflow for treatment of cells. i) incubation: The cells was incubated with 200 μM NCBT in serum-free medium (avoid Cys contained in serum that could react with NCBT) at 37 °C under 5% CO_2 for 1 h; ii) wash: The cells was washed by PBS solution three times and then subjected to single cell sampling; iii) sampling: The cytoplasm was withdrawn using borosilicate glass pipettes controlled with micromanipulator; iv) analysis: The micropipette after sampling was directed with InESI-MS.

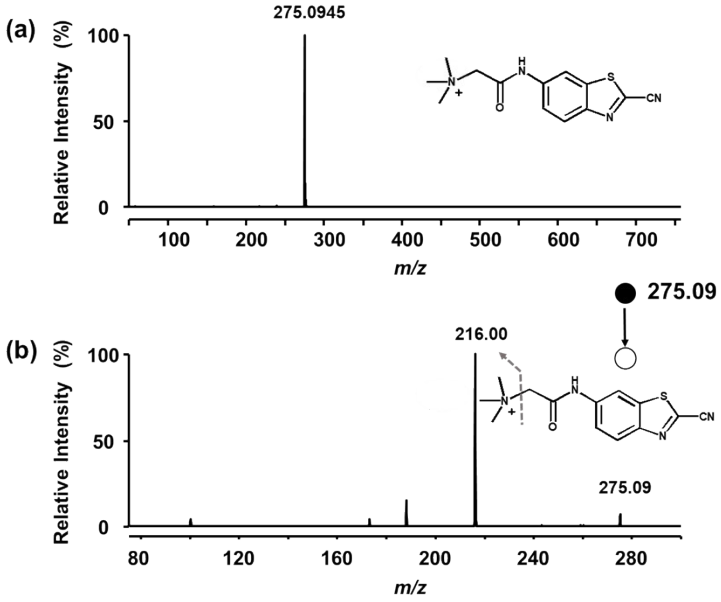


Fig. S2 Characterizations of NCBT. (a) MS spectra and (b) MS/MS spectra of synthetic NCBT.

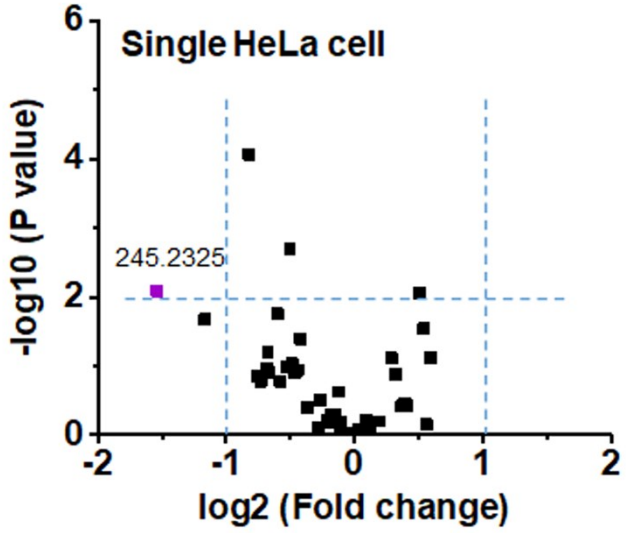


Fig. S3 Metabolites changes in single living HeLa cells with (n = 30) and without (n = 46) 200 μM NCBT for 1 h. The purple square denotes metabolite have significant change.

For single cell analysis, we visualized the data as a volcano plot and significant difference was termed as both P value < 0.01 and fold change > | 2 |.[1-2] As shown in Fig. S3, only one metabolites (m/z 245.2325) according to list in Table S2 (except m/z 379.0881) showed a significant downregulation after NCBT derivatization. Thus, NCBT has little effect on intracellular metabolites. In order to minimize the possible influence on metabolome, we recommend reducing the concentration of derivatization reagents (such as 50 μM) when conducting metabolite studies, and the reaction efficiency could be compensated by extending the incubation time (such as 2-3 h).

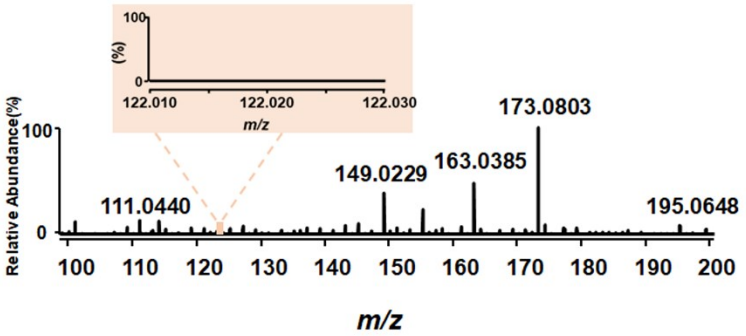


Fig. S4 MS spectra of 100 μM Cys in artificial intracellular solution. Insert: magnified Cys MS spectra signal. The theoretical m/z of $[Cys + H]^+$ is 122.0270, which showed no significant MS response under current condition.

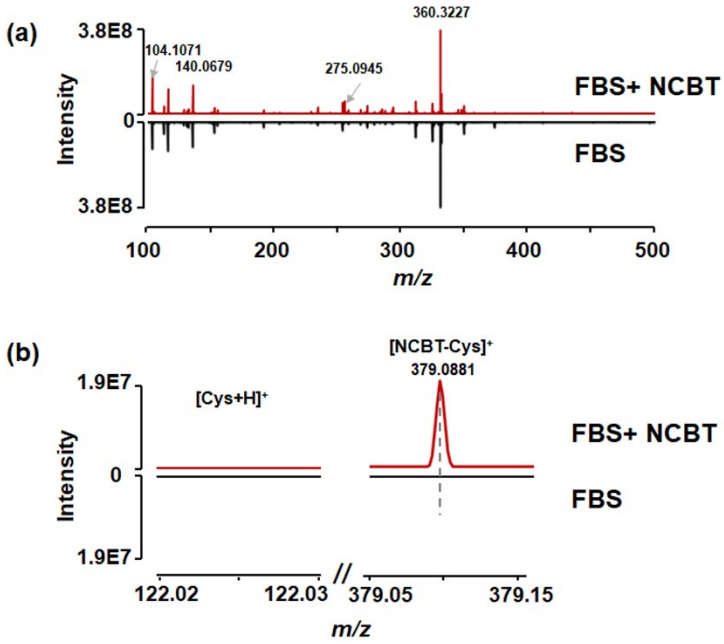


Fig. S5 Detection of total Cys in FBS samples. (a) MS spectra obtained from FBS samples with (FBS + NCBT) and without (FBS) NCBT derivatization. (b) The expand view of MS spectra of protonated Cys (m/z 122.0278) and NCBT-Cys (m/z 379.0881).

Total Cys (tCys) in plasma/serum is a risk factor for atherosclerosis in hyperlipidemic patients,[3] and tCys is also associated with vascular disease in the coronary, cerebral, and peripheral arteries.[4] tCys in FBS samples was tested and corresponding mass spectra was shown in Fig. S5a, with detailed spectra zoomed in Fig. S5b. It was found that, only for NCBT incubated FBS, NCBT-Cys (m/z 379.0881) could be detected with ion intensity of ~1.9E7. While raw Cys in FBS samples with or without NCBT incubation remained undetectable. Thus, the present method could significantly enhance the sensitivity for tCys in serum. In addition, a variety of other metabolite molecules in FBS samples could be detected in presence of excess NCBT, as listed in Table S1.

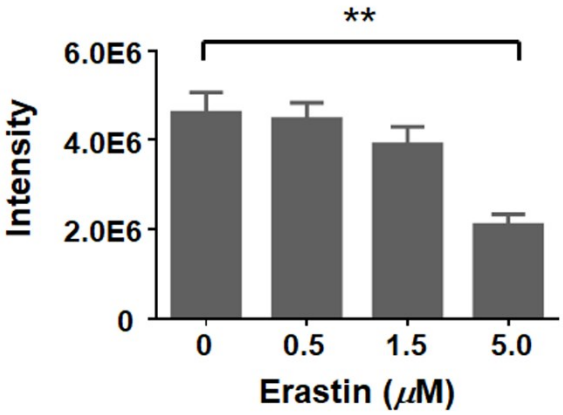


Fig. S6 The cell lysate experiments of erastin stimulation. The MS signal intensity of NCBT-Cys (m/z 379.0881) in HeLa cell after erastin stimulation at different concentrations. ($P < 0.05$ correlated by false discovery rate) **denotes $P < 0.01$; All error bars denote s.d.; n = 3. ~1E5 of HeLa cells incubated with different concentration erastin (0, 0.5, 1.5 and 5.0 μM) for 2 h, then the cells was incubated with 200 μM NCBT for 1 h. The cells were washed by PBS solution and lysed in 100 μL pure H_2O solution.

Compared with untreated cells, the level of NCBT-Cys showed no significant down-regulation for cell lysates with 0.5 and 1.5 μM erastin treatment ($P > 0.05$), and that level changed dramatically ($P < 0.01$) with erastin treatment up to 5.0 μM. Less generation of Cys with erastin was similar with single cell and cell lysate experiments.

Table S1. Representative metabolites species detected in FBS with and without NCBT derivatization

No.	Measured m/z	Theoretical m/z	Relative error (ppm)	Formula	Ion type	Name
1	104.0708	104.0706	+1.92	$C_4H_9NO_2$	$[M+H]^+$	GABA
	126.0525	126.0525	0		$[M+Na]^+$	
2	104.1071	104.1070	+0.96	$C_5H_{14}NO$	$[M]^+$	Choline
3	112.0371	112.0369	+1.79	$C_3H_7NO_2$	$[M+Na]^+$	Alanine
4	112.0506	112.0505	+0.89	$C_4H_5N_3O$	$[M+H]^+$	Cytosine
5	114.0661	114.0662	-0.88	$C_4H_7N_3O$	$[M+H]^+$	Creatinine
	136.0477	136.0482	-3.67		$[M+Na]^+$	
	152.0218	152.0221	-1.97		$[M+K]^+$	
6	115.0366	115.0366	0	$C_3H_8O_3$	$[M+Na]^+$	Glycerol
7	116.0705	116.0706	-0.86	$C_5H_9NO_2$	$[M+H]^+$	Proline
	138.0523	138.0526	-2.17		$[M+Na]^+$	
8	118.0861	118.0863	-1.69	$C_5H_{11}NO_2$	$[M+H]^+$	Valine
	140.0678	140.0682	-2.86		$[M+Na]^+$	
	156.0418	156.0421	-1.92		$[M+K]^+$	
9	129.0656	129.0659	-2.32	$C_5H_8N_2O_2$	$[M+H]^+$	Pyroglutamine and isomers
	151.0475	151.0478	-1.99		$[M+Na]^+$	
10	130.0497	130.0499	-1.54	$C_5H_7NO_3$	$[M+H]^+$	Pyroglutamic acid
	152.0315	152.0318	-1.97		$[M+Na]^+$	
11	130.0859	130.0863	-3.07	$C_6H_{11}NO_2$	$[M+H]^+$	Pipecolic acid
	152.0679	152.0682	-1.97		$[M+Na]^+$	
12	132.0766	132.0768	-1.51	$C_4H_9N_3O_2$	$[M+H]^+$	Creatine
	154.0582	154.0587	-3.24		$[M+Na]^+$	
	170.0323	170.0326	-1.76		$[M+K]^+$	
13	132.1014	132.1019	-3.78	$C_6H_{13}NO_2$	$[M+H]^+$	Leucine and isomers
	154.0836	154.0839	-1.95		$[M+Na]^+$	
14	133.0970	133.0972	-1.50	$C_5H_{12}N_2O_2$	$[M+H]^+$	Ornithine
15	137.0450	137.0458	-5.84	$C_5H_4N_4O$	$[M+H]^+$	Hypoxanthine
16	141.0654	141.0659	-3.54	$C_6H_8N_2O_2$	$[M+H]^+$	1,3-Dimethyluracil Imidazolepropionic acid
17	142.0473	142.0475	-1.41	$C_4H_9NO_3$	$[M+Na]^+$	Threonine
18	146.1173	146.1175	-1.37	$C_7H_{16}NO_2$	$[M]^+$	Acetylcholine
19	147.0760	147.0764	-2.72	$C_5H_{10}N_2O_3$	$[M+H]^+$	Glutamine
	169.0581	169.0584	-1.77		$[M+Na]^+$	
20	147.1123	147.1128	-3.40	$C_6H_{14}N_2O_2$	$[M+H]^+$	Lysine
	169.0945	169.0948	-1.77		$[M+Na]^+$	
21	148.0036	148.0039	-2.03	$C_2H_7NO_3S$	$[M+Na]^+$	Taurine
22	150.0582	150.0583	-0.67	$C_5H_{11}NO_2S$	$[M+H]^+$	Methionine
	172.0400	172.0403	-1.74		$[M+Na]^+$	
23	155.0424	155.0427	-1.93	$C_4H_8N_2O_3$	$[M+Na]^+$	Asparagine
24	156.0763	156.0768	-3.20	$C_6H_9N_3O_2$	$[M+H]^+$	Histidine
	178.0576	178.0587	-6.18		$[M+Na]^+$	
25	160.1328	160.1332	-2.50	$C_8H_{17}NO_2$	$[M+H]^+$	2-Aminooctanoic acid
26	161.1282	161.1285	-1.85	$C_7H_{16}N_2O_2$	$[M+H]^+$	N(6)-Methyllysine
27	162.1120	162.1125	-3.08	$C_7H_{15}NO_3$	$[M+H]^+$	Carnitine
	184.0941	184.0944	-1.63		$[M+Na]^+$	
28	166.0859	166.0863	-2.41	$C_9H_{11}NO_2$	$[M+H]^+$	Phenylalanine
	188.0677	188.0682	-2.66		$[M+Na]^+$	
29	168.0628	168.0631	-1.79	$C_6H_{11}NO_3$	$[M+Na]^+$	Butyrylglycine and isomers
30	169.0581	169.0584	-1.77	$C_5H_{10}N_2O_3$	$[M+Na]^+$	Glutamine
31	170.0421	170.0424	-1.76	$C_5H_9NO_4$	$[M+Na]^+$	Glutamic acid
32	170.0921	170.0924	-1.76	$C_7H_{11}N_3O_2$	$[M+H]^+$	Methylhistidine
	192.0740	192.0744	-1.56		$[M+Na]^+$	

33	175.1090	175.1077	+7.42	$C_7H_{14}N_2O_3$	$[M+H]^+$	N-Acetylornithine
	197.0893	197.0897	-2.03		$[M+Na]^+$	
34	175.1184	175.1190	-3.43	$C_6H_{14}N_4O_2$	$[M+H]^+$	Arginine
	197.1006	197.1009	-1.52		$[M+Na]^+$	
35	182.0784	182.0788	-2.19	$C_7H_{13}NO_3$	$[M+Na]^+$	Methyl 5-(hydroxymethyl)pyrrolidine-3-carboxylate
36	188.1754	188.1757	-1.59	$C_9H_{21}N_3O$	$[M+H]^+$	N1-Acetylspermidine and isomers
37	203.0522	203.0526	-1.97	$C_6H_{12}O_6$	$[M+Na]^+$	Hexose
	219.0261	219.0265	-1.83		$[M+K]^+$	
38	203.1499	203.1503	-1.97	$C_8H_{18}N_4O_2$	$[M+H]^+$	Dimethylarginine
39	204.0628	204.0631	-1.47	$C_9H_{11}NO_3$	$[M+Na]^+$	Tyrosine
40	204.1225	204.1230	-2.45	$C_9H_{17}NO_4$	$[M+H]^+$	Acetylcarnitine
	226.1045	226.1050	-2.21		$[M+Na]^+$	
41	205.0968	205.0972	-1.95	$C_{11}H_{12}N_2O_2$	$[M+H]^+$	Tryptophan
	227.0786	227.0791	-2.20		$[M+Na]^+$	
42	218.1379	218.1387	-3.67	$C_{10}H_{19}NO_4$	$[M+H]^+$	Propionylcarnitine
43	227.0786	227.0791	-2.20	$C_{11}H_{12}N_2O_2$	$[M+Na]^+$	Tryptophan
44	232.1536	232.1543	-3.02	$C_{11}H_{21}NO_4$	$[M+H]^+$	Butyrylcarnitine and isomers
45	234.1332	234.1336	-1.71	$C_{10}H_{19}NO_5$	$[M+H]^+$	Hydroxypropionylcarnitine
46	242.0993	242.0999	-2.48	$C_9H_{17}NO_5$	$[M+Na]^+$	Pantothenic acid
47	245.0778	245.0768	+4.08	$C_9H_{12}N_2O_6$	$[M+H]^+$	Uridine
48	256.2628	256.2635	-2.73	$C_{16}H_{33}NO$	$[M+H]^+$	Palmitic amide
	278.2446	278.2455	-3.23		$[M+Na]^+$	
	294.2189	294.2194	-1.70		$[M+K]^+$	
49	265.0789	265.0795	-2.26	$C_{10}H_{14}N_2O_5$	$[M+Na]^+$	Thymidine
50	280.2628	280.2635	-2.50	$C_{18}H_{33}NO$	$[M+H]^+$	Linoleamide
	302.2447	302.2454	-2.32		$[M+Na]^+$	
51	282.2785	282.2791	-2.13	$C_{18}H_{35}NO$	$[M+H]^+$	Oleamide
	304.2602	304.2611	-2.96		$[M+Na]^+$	
52	284.2941	284.2948	-2.46	$C_{18}H_{37}NO$	$[M+H]^+$	Octadecanamide
	306.2760	306.2767	-2.29		$[M+Na]^+$	
53	300.2890	300.2897	-2.33	$C_{18}H_{37}NO_2$	$[M+H]^+$	Sphingosine and isomers
	322.2717	322.2716	-2.79		$[M+Na]^+$	
54	302.3046	302.3054	-1.33	$C_{18}H_{39}NO_2$	$[M+H]^+$	Sphinganine
55	318.2996	318.3003	-2.20	$C_{18}H_{39}NO_3$	$[M+H]^+$	Phytosphingosine
	340.2815	340.2822	-2.06		$[M+Na]^+$	
56	324.2885	324.2897	-3.70	$C_{20}H_{37}NO_2$	$[M+H]^+$	Linoleoyl ethanolamide
	346.2711	346.2717	-1.73		$[M+Na]^+$	
57	348.2865	348.2873	-2.30	$C_{20}H_{39}NO_2$	$[M+Na]^+$	N-Oleoylethanolamine
58	379.0881	379.0893	-3.17	$C_{16}H_{19}N_4O_3S_2$	$[M]^+$	NCBT-Cys
59	386.3253	386.3265	-3.11	$C_{22}H_{43}NO_4$	$[M+H]^+$	Pentadecanoylcarnitine
	408.3075	408.3084	-2.20		$[M+Na]^+$	
	424.2815	424.2824	-2.12		$[M+K]^+$	

The peak assignments were based on accurate mass measurements, comparison of isotopic patterns, information found in databases, such as Human metabolome database (http://www.hmdb.ca/), and some previous related literatures. The relative error of m/z values was set as < 10 ppm. Protonated ion $[M + H]^+$, sodium adduct ion $[M + Na]^+$, and potassium adduct ion $[M + K]^+$ peaks would be counted as one chemical.

Table S2. Representative metabolites species detected in single HeLa cell with and without NCBT derivatization

No.	Measured m/z	Theoretical m/z	Relative error (ppm)	Formula	Ion type	Name
1	104.0708	104.0706	+1.92	$C_4H_9NO_2$	$[M+H]^+$	GABA
2	104.1071	104.1070	+0.96	$C_5H_{14}NO$	$[M]^+$	Choline
3	114.0661	114.0662	-0.88	$C_4H_7N_3O$	$[M+H]^+$	Creatinine
	136.0477	136.0482	-3.67		$[M+Na]^+$	
4	115.0366	115.0366	0	$C_3H_8O_3$	$[M+Na]^+$	Glycerol
5	116.0705	116.0706	-0.86	$C_5H_9NO_2$	$[M+H]^+$	Proline
6	118.0861	118.0863	-1.69	$C_5H_{11}NO_2$	$[M+H]^+$	Valine
	140.0678	140.0682	-2.86		$[M+Na]^+$	
7	120.0654	120.0655	-0.83	$C_4H_9NO_3$	$[M+H]^+$	Threonine
8	129.0656	129.0659	-2.32	$C_5H_8N_2O_2$	$[M+H]^+$	Pyroglutamine and isomers
9	130.0497	130.0499	-1.54	$C_5H_7NO_3$	$[M+H]^+$	Pyroglutamic acid
10	130.0859	130.0863	-3.07	$C_6H_{11}NO_2$	$[M+H]^+$	Pipecolic acid
11	131.1177	131.1179	-1.53	$C_6H_{14}N_2O$	$[M+H]^+$	N-Acetylputrescine
12	132.0766	132.0768	-1.51	$C_4H_9N_3O_2$	$[M+H]^+$	Creatine
	154.0582	154.0587	-3.24		$[M+Na]^+$	
13	132.1014	132.1019	-3.78	$C_6H_{13}NO_2$	$[M+H]^+$	Leucine and isomers
14	146.1173	146.1175	-1.37	$C_7H_{16}NO_2$	$[M]^+$	Acetylcholine
15	146.1650	146.1652	-1.37	$C_7H_{19}N_3$	$[M+H]^+$	Spermidine
	168.1465	168.1471	-3.57		$[M+Na]^+$	
16	147.0760	147.0764	-2.72	$C_5H_{10}N_2O_3$	$[M+H]^+$	Glutamine
17	147.1123	147.1128	-3.40	$C_6H_{14}N_2O_2$	$[M+H]^+$	Lysine
18	156.0763	156.0768	-3.20	$C_6H_9N_3O_2$	$[M+H]^+$	Histidine
	178.0576	178.0587	-6.18		$[M+Na]^+$	
19	160.1328	160.1332	-2.50	$C_8H_{17}NO_2$	$[M+H]^+$	2-Aminooctanoic acid
20	162.1120	162.1125	-3.08	$C_7H_{15}NO_3$	$[M+H]^+$	Carnitine
21	166.0859	166.0863	-2.41	$C_9H_{11}NO_2$	$[M+H]^+$	Phenylalanine
22	175.1184	175.1190	-3.43	$C_6H_{14}N_4O_2$	$[M+H]^+$	Arginine
23	184.0727	184.0739	-6.52	$C_5H_{15}NO_4P$	$[M+H]^+$	Phosphorylcholine
	206.0545	206.0558	-6.31		$[M+Na]^+$	
24	188.1754	188.1757	-1.59	$C_9H_{21}N_3O$	$[M+H]^+$	N1-Acetylspermidine and isomers
	210.1562	210.1577	-7.14		$[M+Na]^+$	
25	203.2223	203.2230	-3.44	$C_{10}H_{26}N_4$	$[M+H]^+$	Spermine
	225.2040	225.2050	-4.44		$[M+Na]^+$	
26	203.0518	203.0526	-3.94	$C_6H_{12}O_6$	$[M+Na]^+$	Hexose
	219.0260	219.0265	-2.28		$[M+K]^+$	
27	204.1225	204.1230	-2.45	$C_9H_{17}NO_4$	$[M+H]^+$	Acetylcarnitine
28	218.1379	218.1387	-3.67	$C_{10}H_{19}NO_4$	$[M+H]^+$	Propionylcarnitine
29	232.1536	232.1543	-3.02	$C_{11}H_{21}NO_4$	$[M+H]^+$	Butyrylcarnitine and isomers
30	234.1332	234.1336	-1.71	$C_{10}H_{19}NO_5$	$[M+H]^+$	Hydroxypropionylcarnitine
31	245.2325	245.2336	-4.49	$C_{12}H_{28}N_4O$	$[M+H]^+$	N1-Acetylspermine
32	246.1695	246.1670	-2.03	$C_{12}H_{23}NO_4$	$[M+H]^+$	Valerylcarnitine and isomers
33	258.1090	258.1101	-4.26	$C_8H_{20}NO_6P$	$[M+H]^+$	Glycerophosphocholine
34	302.3039	302.3054	-4.96	$C_{18}H_{39}NO_2$	$[M+H]^+$	Sphinganine
35	318.2989	318.3003	-4.40	$C_{18}H_{39}NO_3$	$[M+H]^+$	Phytosphingosine
36	379.0881	379.0893	-3.17	$C_{16}H_{19}N_4O_3S_2$	$[M]^+$	NCBT-Cys

The peak assignments were based on accurate mass measurements, comparison of isotopic patterns, information found in databases, such as Human metabolome database (http://www.hmdb.ca/), and some previous related literatures. The relative error of m/z values was set

as < 10 ppm. Protonated ion $[M + H]^+$, sodium adduct ion $[M + Na]^+$, and potassium adduct ion $[M + K]^+$ peaks would be counted as one chemical.

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