Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

LC-MS-based metabolomics reveals metabolic signatures related to glioma stem-like cell self-renewal and differentiation

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Fig. S1. Flowchart of the LC−MS-based metabolomics approach.

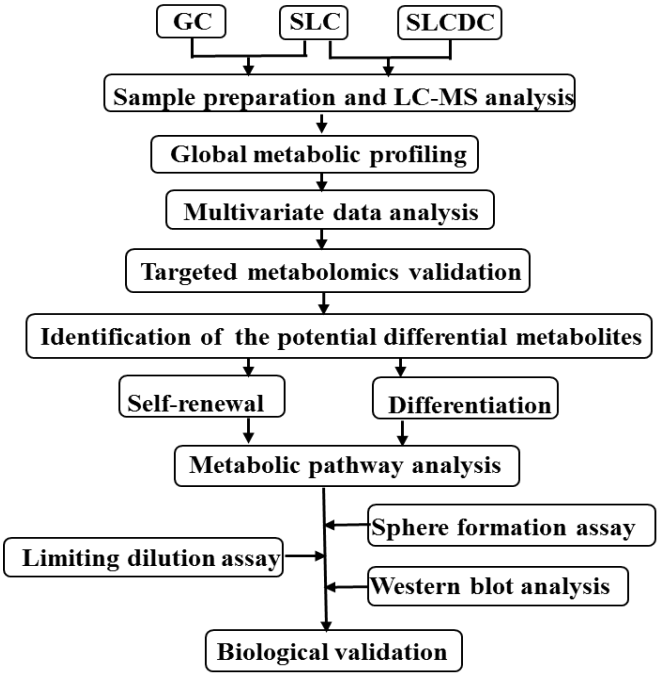
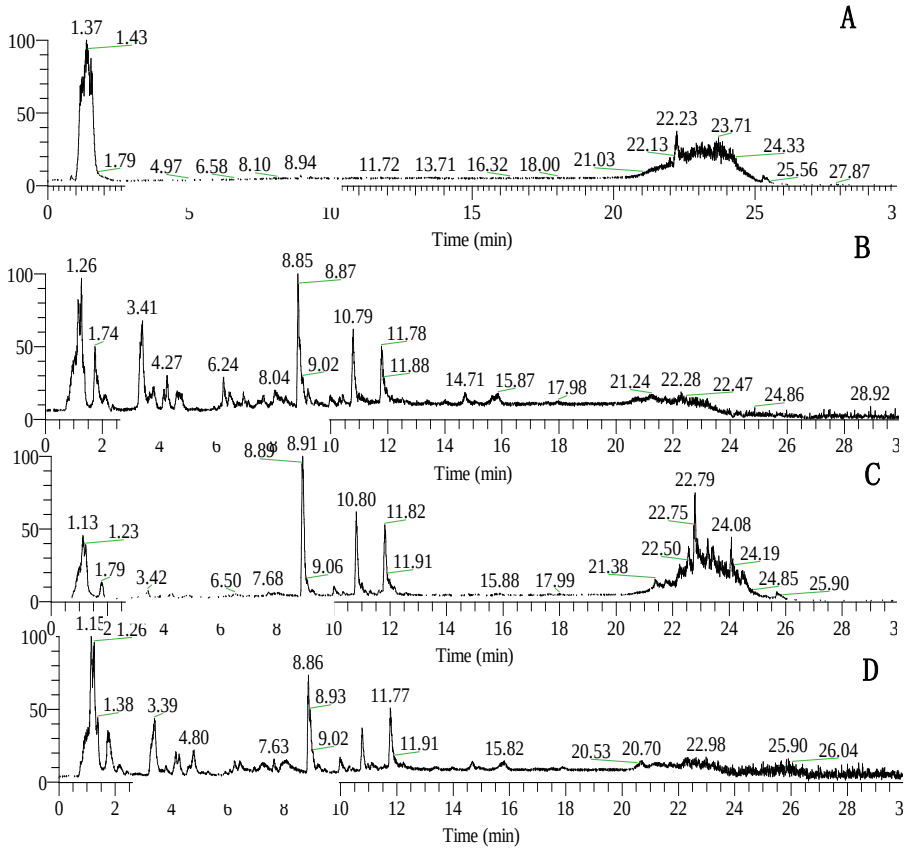


Fig. S2 The total ion chromatograms (TICs) of different cell samples. (A) Blank samples, (B) GC, (C) SLC, (D) SLCDC.

LC-(+) ESI/MS



LC-(-) ESI/MS

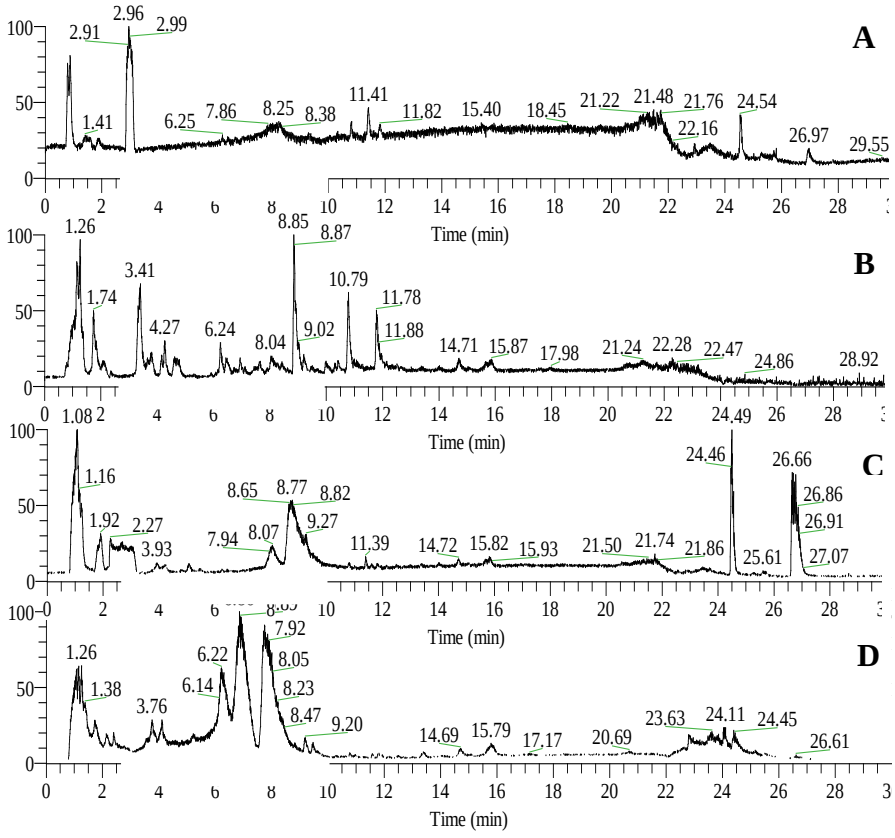


Fig. S3 The PCA score plots (95% confidence interval) of QC samples deriving from LC-(±) ESI-MS data.

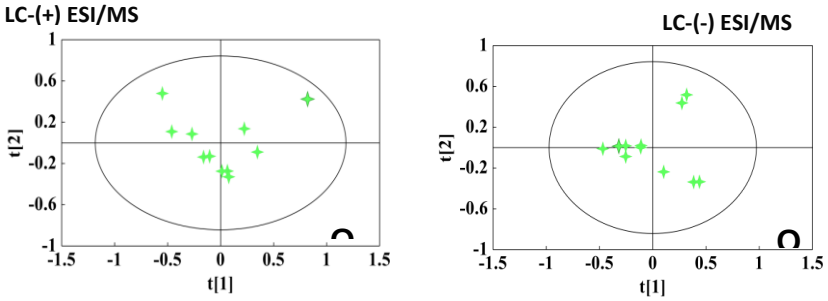


Fig. S4 Line plots of QC samples generated by PCA using component 1 and 2.

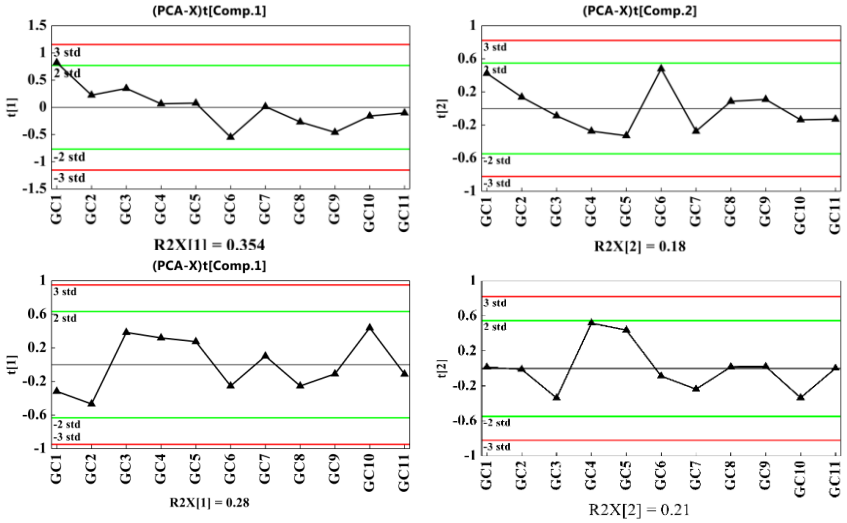


Fig. S5 The retention time deviation profiles deriving from LC-(±) ESI-MS.

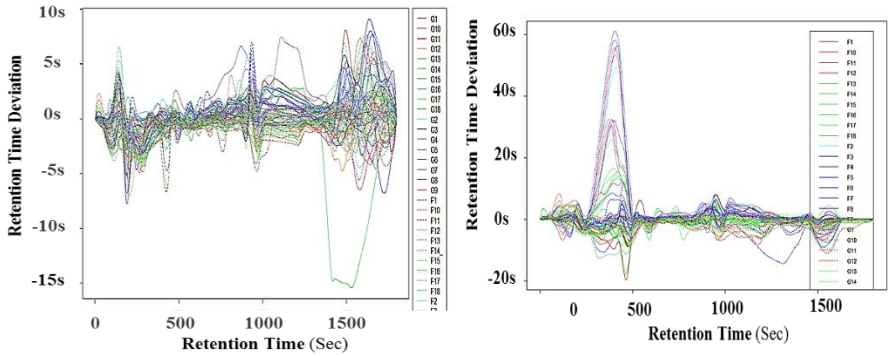


Fig. S6 Score plots of the OPLS-DA models (A, B) and plots of the permutation tests of the PLS-DA models (C,D) based on the LC-(-) ESI-MS data sets. (A, C) GCs vs SLCs, (B, D) SLCDCs vs SLCs, (green ●) GCs, (red ▲) SLCs, (purple■) SLCDCs.

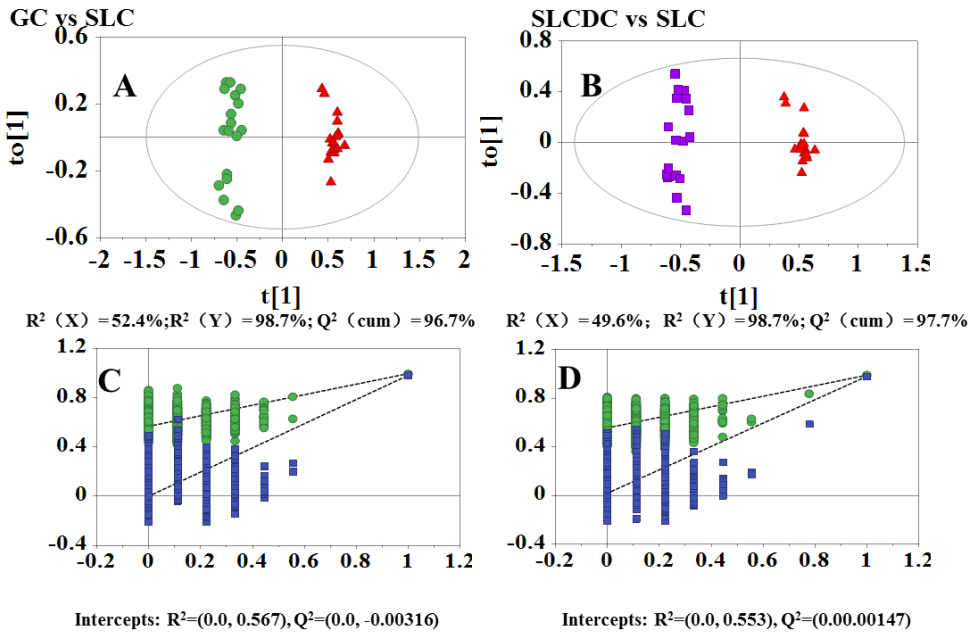


Fig. S7 The XICs of differential variables of the LC-MS/MS MRM analysis in positive ion mode (A) and negative ion mode (B).

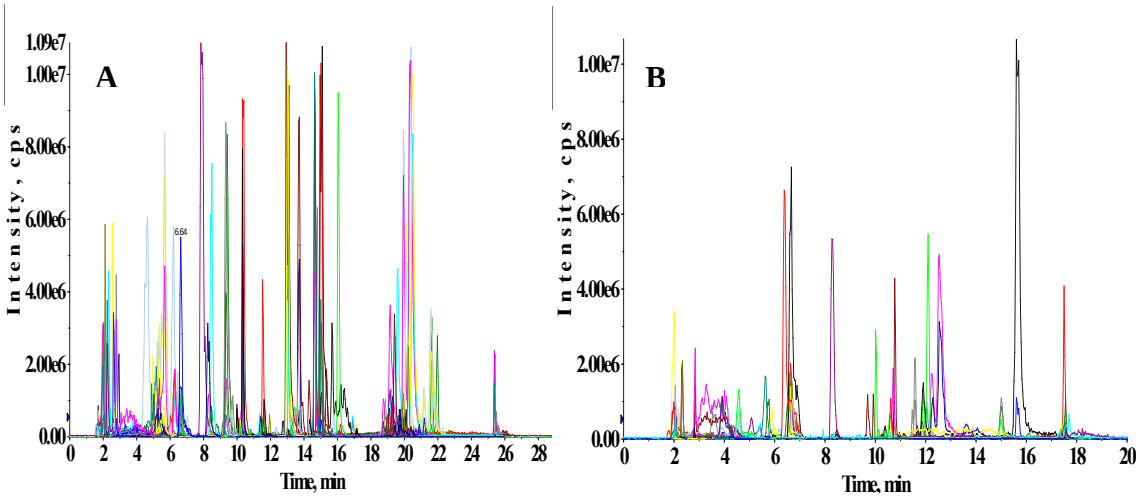


Fig. S8 The differences in the overlapping metabolites among the three cell types. (GCs vs SLCs and SLCDCs vs SLCs)

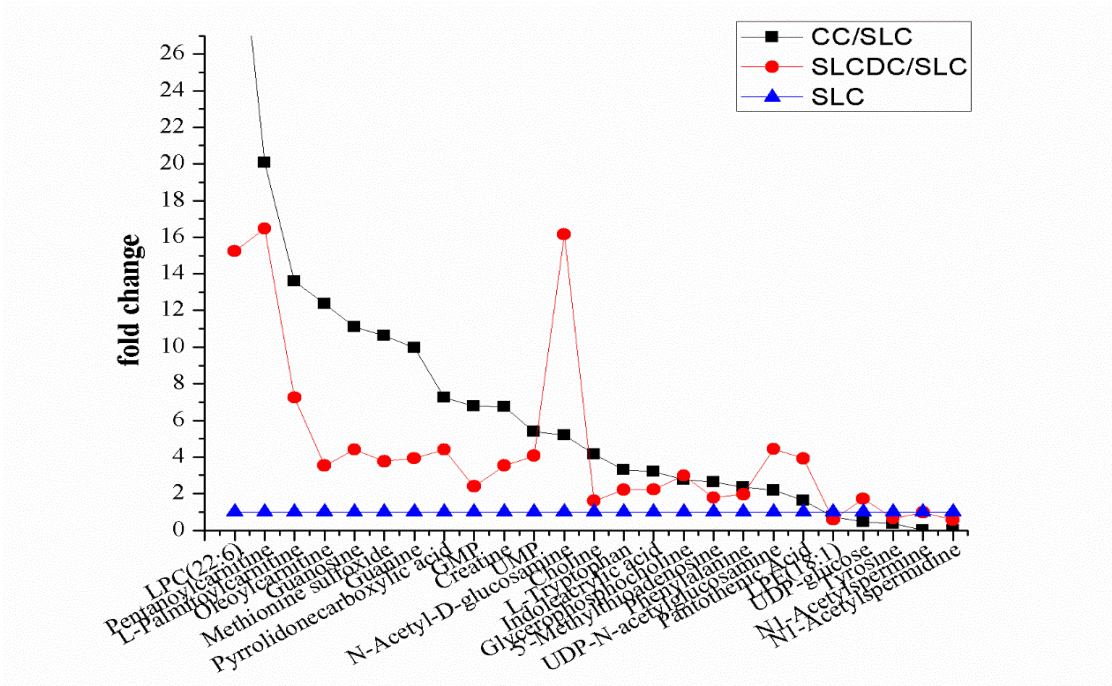


Fig. S9 The levels of intracellular ROS and of GSH and GSSG, which are related to glutathione metabolism.

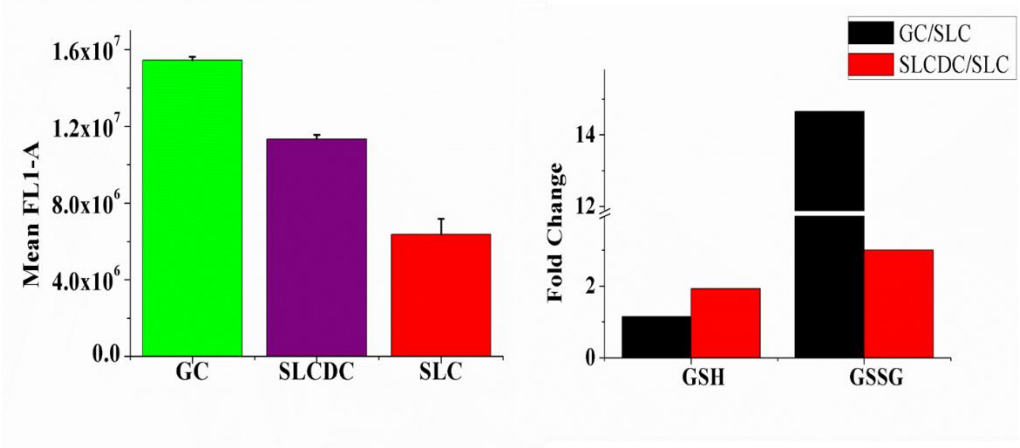
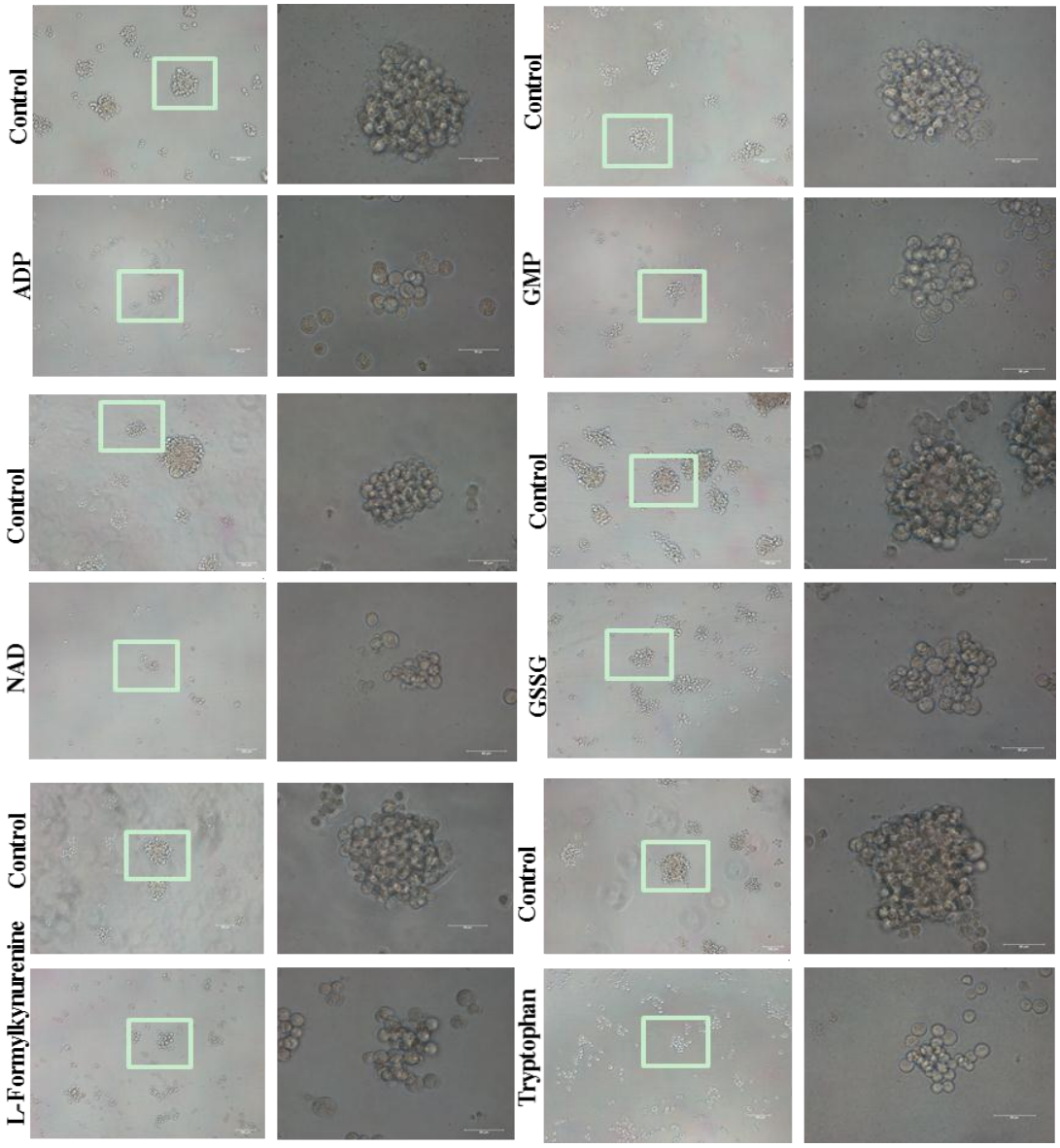


Fig. S10 Neurosphere formation assay in SLCs. Phase contrast photomicrographs of SLC treated with metabolites (Bar = 100 µm, left; Bar = 50 µm, right)



Sphere formation assay of SLC treated with metabolites, neurospheres with diameters larger than 50 μm were counted. The values shown are the means ± SD of at least 3 independent experiments.

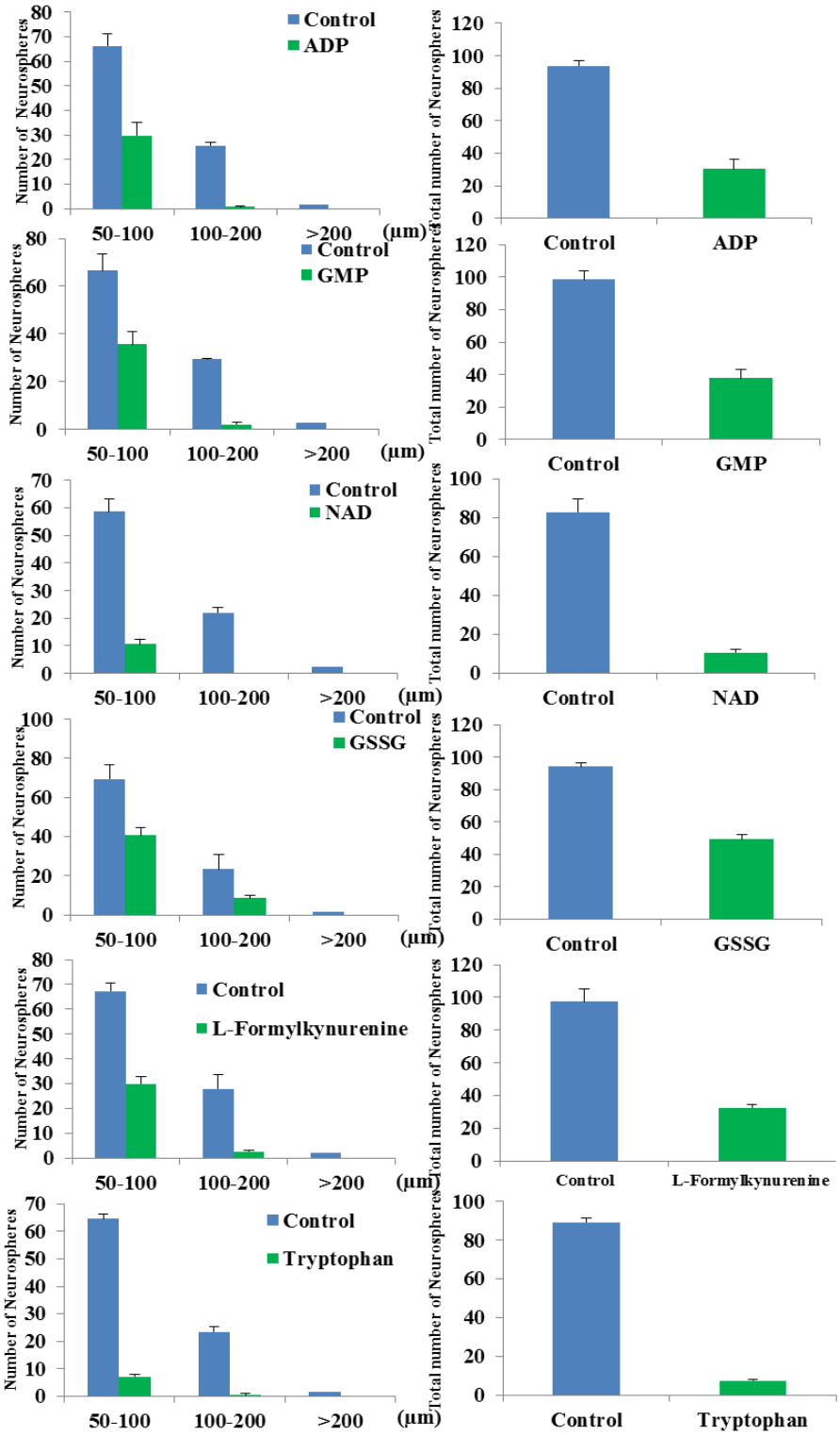


Fig. S11 Limiting dilution neurosphere assay in SLCs.

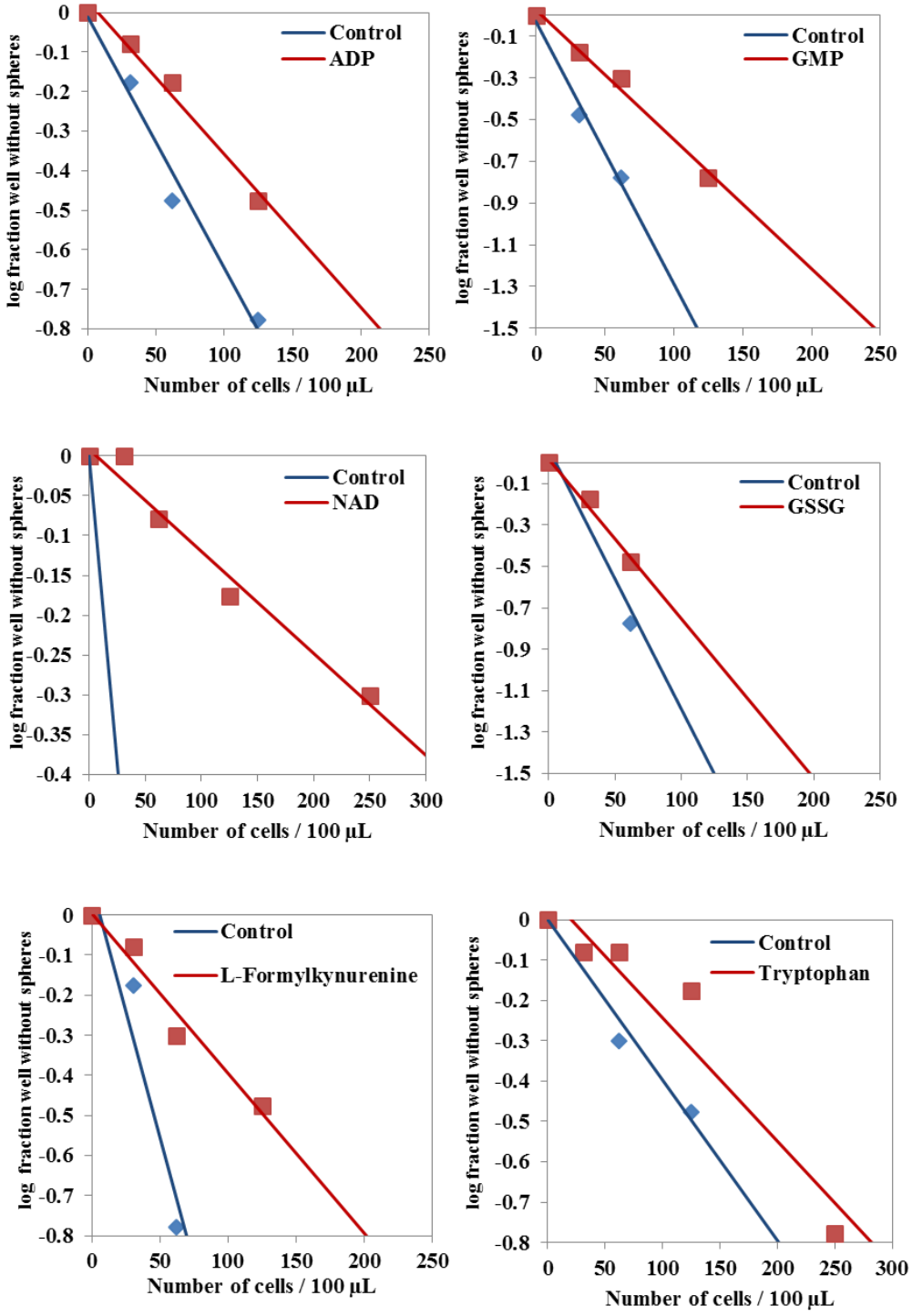


Table S1. The differential metabolites between GC and SLC group obtained by LC-(±) MS/MS analysis.

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
4.8	114.0916	$[M+H-H_2O]^+$	$C_6H_{13}NO_2$	L-Leucine[a]	96.1, 105, 79.1, 69.1, 55.1	< 0.001	0.59	6.57
1.26	116.0709	$[M+H]^+$	$C_5H_9NO_2$	L-Proline[a]	70.1, 68.05	< 0.001	1.28	3.35
1.25	118.0865	$[M+H]^+$	$C_5H_{11}NO_2$	Valine[a]	72.1, 55.1	< 0.001	0.8	2.96
3.4	166.0861	$[M+H]^+$	$C_9H_{11}NO_2$	Phenylalanine[a]	120.1, 103.1, 107.0, 131, 149.1	< 0.001	2.34	15.7
1.26	182.0812	$[M+H]^+$	$C_9H_{11}NO_3$	L-Tyrosine[a]	136.1, 391.1, 119.0, 123.0, 147.0, 165.1	< 0.001	0.38	3.33
4.28	205.0971	$[M+H]^+$	$C_{11}H_{12}N_2O_2$	L-Tryptophan[a]	188.1, 146.1, 159.1, 144.1, 118.1, 115.1	< 0.001	3.29	7.36
1.27	132.0769	$[M+H]^+$	$C_4H_9N_3O_2$	Creatine[a]	90.1, 114.1, 115.1, 86.1, 87.1	< 0.001	6.74	7.76
4.16	220.1179	$[M+H]^+$	$C_9H_{17}NO_5$	Pantothenic Acid[a]	90.1, 485.1, 124.1, 142.1, 160.1, 184.1, 202.1	< 0.001	1.63	3.4
2.38	130.0500	$[M+H]^+$	$C_5H_7NO_3$	Pyrrolidonecarboxylic acid[a]	84.0, 56.1, 102.1	< 0.001	7.25	4.93
1.75	148.0426	$[M+H-H_2O]^+$	$C_5H_{11}NO_3S$	Methionine sulfoxide[a]	84.0, 102.1, 130.0, 56.1, 74.0	< 0.001	10.64	5.62
4.28	188.0706	$[M+H]^+$	$C_{11}H_9NO_2$	Indoleacrylic acid[a]	146.1, 115.1, 170.1	< 0.001	3.2	8.44
3.4	131.0492	$[M+H-H_2O]^+$	$C_9H_8O_2$	Trans-Cinnamic acid[a]	103.1, 77.0, 131.0	< 0.001	2.44	2.04
1.93	165.0546	$[M+H]^+$	$C_9H_8O_3$	2-Hydroxycinnamic acid[a]	123.0, 91.1, 95.0, 103.1, 147.0	< 0.001	0.64	2.2
2.45	611.1441	$[M-H]^-$	$C_{20}H_{32}N_6O_{12}S_2$	Oxidized glutathione[a]	306.1, 338.0, 272.1, 254.1, 160.0, 482.1, 593.1	< 0.001	14.65	2.99
0.86	188.1756	$[M+H]^+$	$C_9H_{21}N_3O$	N1-Acetylspermidine	72.1, 171.1, 117.1, 100.1, 58.1	< 0.001	0.02	13.89
0.85	245.2333	$[M+H]^+$	$C_{12}H_{28}N_4O$	N1-Acetylspermine[a]	100.1, 112.1, 129.1, 171.1, 84.1, 70.1	< 0.001	0.03	3.19
7.22	204.0865	$[M+H-H_2O]^+$	$C_8H_{15}NO_6$	N-Acetyl-D-glucosamine[a]	96.0, 109.0, 126.1, 138.1, 144.1, 168.1, 186.1	< 0.001	5.18	5.65

Table S1. Continued.

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
4.1	246.1698	$[M+H]^+$	$C_{12}H_{23}NO_4$	Pentanoylcarnitine	187.1, 144.1, 60.1, 85.0	< 0.001	20.07	3.94
11.02	398.3261	$[M+H]^+$	$C_{23}H_{43}NO_4$	trans-2-Hexadecenoyl-carnitine	85.0, 339.3, 237.2, 144.1, 95.1, 60.1	< 0.001	9.51	2.5
11.95	400.3417	$[M+H]^+$	$C_{23}H_{45}NO_4$	L-Palmitoylcarnitine	85.0, 341.3, 239.2, 144.1, 95.1, 60.1	< 0.001	13.59	4.18
12.37	426.3573	$[M+H]^+$	$C_{25}H_{47}NO_4$	Oleoylcarnitine	85.0, 367.3, 265.3, 60.1, 144.1, 95.1	< 0.001	12.35	5.3

1.28	258.1099	$[M+H]^+$	$C_8H_{20}NO_6P$	Glycerophosphocholine	104.1, 60.1, 86.1, 125.0, 184.1	< 0.001	2.78	3.34
1.16	104.1074	$[M+H]^+$	$C_5H_{13}NO$	Choline[a]	60.1, 58.1, 59.1	< 0.001	4.14	10.31
11.91	468.3080	$[M+H]^+$	$C_{22}H_{46}NO_7P$	LysoPC(14:0)	184.1, 450.3, 125.0, 104.1	0.0158	2.9	4.04
13.03	482.3238	$[M+H]^+$	$C_{23}H_{48}NO_7P$	LPC(15:0)[a]	184.1, 464.3, 125.0, 104.1, 86.1	< 0.001	21.13	2.95
21.28	550.3863	$[M+H]^+$	$C_{28}H_{56}NO_7P$	LPC(20:1)	184.1, 532.4, 104.1, 86.1,125.0	< 0.001	2.16	2.53
13.35	568.3391	$[M+H]^+$	$C_{30}H_{50}NO_7P$	LPC(22:6)	184.1, 550.4, 104.1, 125.0	< 0.001	35.53	2.54
15.67	480.3081	$[M+H]^+$	$C_{23}H_{46}NO_7P$	LPE(18:1)[a]	69.1, 95.1, 135.1, 265.3, 308.3, 339.3, 419.3	0.0144	1.63	4.7
3.33	152.0567	$[M+H]^+$	$C_5H_5N_5O$	Guanine[a]	135.0, 110.0, 107.0, 82.0	< 0.001	9.5	3.87
2.38	259.0219	$[M-H]^-$	$C_6H_{13}O_9P$	D-Glucose 6-phosphate[a]	78.96, 96.97, 138.98, 199.0, 222.8, 241.0	< 0.001	0.12	2.41
4.16	298.0966	$[M+H]^+$	$C_{11}H_{15}N_5O_3S$	5'-Methylthioadenosine[a]	136.1, 145.0, 97.0, 75.0, 61.0	< 0.001	2.63	6.58
22.09	326.3049	$[M+H]^+$	$C_{20}H_{39}NO_2$	N-Oleoylethanolamine	62.1, 309.3, 69.1, 95.1, 135.1, 163.1, 265.3	< 0.001	3.79	2.11
0.95	146.1651	$[M+H]^+$	$C_7H_{19}N_3$	Spermidine[a]	72.1, 58.1, 75.1, 84.1, 112.1, 129.1	0.0019	3.97	6.71
7.12	606.0735	$[M-H]^-$	$C_{17}H_{27}N_3O_{17}P_2$	UDP-N-acetylglucosamine	384.98,402.99,362.0,282.0,158.9,176.9,78.96	< 0.001	2.15	16.36
8.03	565.0471	$[M-H]^-$	$C_{15}H_{24}N_2O_{17}P_2$	UDP-glucose[a]	323.0,384.98,241.0,158.9,402.99,96.97,78.96	< 0.001	0.47	2.62

Table S1. Continued.

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
4.61	426.0218	$[M-H]^-$	$C_{10}H_{15}N_5O_{10}P_2$	ADP[a]	78.96,134.0,158.9,272.96,328.0,408.0,346.1,96.97	0.0394	3.83	5.5
4.14	347.0392	$[M-H]^-$	$C_{10}H_{13}N_4O_8P$	IMP[a]	78.96,96.97,135.0,150.98,211.0	< 0.001	0.29	3.59
3.67	362.0503	$[M-H]^-$	$C_{10}H_{14}N_5O_8P$	GMP[a]	78.96,96.97,150.0,211.0	< 0.001	6.73	5.29
4.25	363.0344	$[M-H]^-$	$C_{10}H_{13}N_4O_9P$	XMP	211.0,78.96,96.7,108.0,151.0	< 0.001	33.98	3.39
3.68	283.0680	$[M-H]^-$	$C_{10}H_{12}N_4O_6$	Xanthosine[a]	151.0,108.0	< 0.001	37.38	3.2
3.89	323.0280	$[M-H]^-$	$C_9H_{13}N_2O_9P$	UMP[a]	78.96,96.97,111.0,150.98,192.99,211.0,280.0	< 0.001	5.26	4.21
3.32	284.0986	$[M+H]^+$	$C_{10}H_{13}N_5O_5$	Guanosine[a]	152.1,135.0,110.0	< 0.001	11.09	3.52

Table S2. The differential metabolites between SLCDC and SLC group obtained by LC-(±) MS/MS analysis.

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
2.38	130.0500	$[M+H]^+$	$C_5H_7NO_3$	Pyrrolidonecarboxylic acid[a]	84.0,56.1,102.1	0.0014	4.41	3.96
1.27	132.0769	$[M+H]^+$	$C_4H_9N_3O_2$	Creatine[a]	90.1,114.1,86.1,73.0,60.1	0.0017	3.53	5.57
3.4	166.0861	$[M+H]^+$	$C_9H_{11}NO_2$	Phenylalanine[a]	120.1,103.1,107.0,131.0,149.1	0.0029	1.97	15.99
4.28	205.0971	$[M+H]^+$	$C_{11}H_{12}N_2O_2$	L-Tryptophan[a]	188.1,146.1，159.1,144.1,118.1,115.1	< 0.001	2.21	6.39
1.26	182.0811	$[M+H]^+$	$C_9H_{11}NO_3$	L-Tyrosine[a]	136.1,91.1,119.05,123.0,147.0,165.1	0.0408	0.66	2.59
4.7	261.1444	$[M+H]^+$	$C_{11}H_{20}N_2O_5$	Gamma-Glu-Leu	86.1,132.1,198.1,244.1	< 0.001	7.85	2.89
3.3	209.0919	$[M+H]^+$	$C_{10}H_{12}N_2O_3$	Kynurenine[a]	146.1,94.1,136.1,192.1,174.1,150.1,120.0,99.0,	< 0.001	58.84	3.39

Table S2. Continued.

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
4.16	220.1179	$[M+H]^+$	$C_9H_{17}NO_5$	Pantothenic Acid[a]	90.1,85.1,124.1,142.1,160.1,184.1,202.1	< 0.001	3.92	9.79
4.15	218.1023	$[M-H]^-$	$C_9H_{17}NO_5$	Pantothenic Acid[a]	88.0,146.1,71.0,99.0,116.1	0.0179	2.9	6.49
3.72	237.0869	$[M+H]^+$	$C_{11}H_{12}N_2O_4$	L-Formylkynurenine[a]	146.1,136.1,174.1,192.1,202.05,94.1	0.0097	24.55	2.34
4.28	188.0706	$[M+H]^+$	$C_{11}H_9NO_2$	Indoleacrylic acid[a]	146.1,115.1,170.1	< 0.001	2.23	7.47

0.86	188.1756	$[M+H]^+$	$C_9H_{21}N_3O$	N1-Acetylspermidine	72.1,171.1,117.1，100.1，58.1	< 0.001	0.57	10.24
1.16	245.2333	$[M+H]^+$	$C_{12}H_{28}N_4O$	N1-Acetylspermine[a]	100.1,112.1,129.1,171.1,84.1,70.1	0.0019	2.83	2.46
1.77	306.076	$[M-H]^-$	$C_{10}H_{17}N_3O_6S$	Glutathione[a]	143.0,128.0,160.0,210.1,99.1,254.1	< 0.001	1.92	5.43
7.22	204.0865	$[M+H-H_2O]^+$	$C_8H_{15}NO_6$	N-Acetyl-D-glucosamine[a]	138.1,144.1,126.1,168.1,186.1,109.0,96.0,84.0	< 0.001	16.15	13.34
4.16	298.0966	$[M+H]^+$	$C_{11}H_{15}N_5O_3S$	5'-Methylthioadenosine[a]	136.1,145.0,97.0,75.0,61.0	< 0.001	1.79	5.38
1.12	162.1124	$[M+H]^+$	$C_7H_{15}NO_3$	L-Carnitine[a]	103.0,85.0,60.1	< 0.001	6.41	3.98
1.27	204.1231	$[M+H]^+$	$C_9H_{17}NO_4$	Acetylcarnitine[a]	85.0,145.0，60.1,102.1	0.0018	6.97	2.18
1.26	218.1386	$[M+H]^+$	$C_{10}H_{19}NO_4$	Propionylcarnitine	85.0,159.1,144.1,60.1	0.0049	13.95	3.42
4.1	246.1698	$[M+H]^+$	$C_{12}H_{23}NO_4$	Pentanoylcarnitine	187.1,85.0,144.1,60.1	0.0026	16.47	4.15
10.71	372.3104	$[M+H]^+$	$C_{21}H_{41}NO_4$	Tetradecanoylcarnitine	85.0,60.1,95.1，144.1,313.2,211.2	0.0044	5.02	2.09
11.95	400.3417	$[M+H]^+$	$C_{23}H_{45}NO_4$	L-Palmitoylcarnitine	85.0,341.3,239.2,144.1,95.1,60.1	< 0.001	7.25	3.63
12.37	426.3573	$[M+H]^+$	$C_{25}H_{47}NO_4$	Oleoylcarnitine	85.0,367.3,265.3,60.1,144.1,95.1	0.0017	3.53	2.99
14.5	428.3730	$[M+H]^+$	$C_{25}H_{49}NO_4$	Stearoylcarnitine	85.0,369.3,267.3,144.1,95.1,60.1	< 0.001	15.53	2.44
1.28	258.1099	$[M+H]^+$	$C_8H_{20}NO_6P$	Glycerophosphocholine	104.1,60.1,86.1,125.0,184.1	< 0.001	2.99	3.77

Table S2. Continued

RT	m/z	Adduct ion	Elemental composition	Metabolite identification[a]	Product ions	P value	Fold Change	VIP
1.16	104.1074	$[M+H]^+$	$C_5H_{13}NO$	Choline[a]	60.1,58.1,59.1	0.0466	1.61	5.01
20.69	524.3707	$[M+H]^+$	$C_{26}H_{54}NO_7P$	LPC(18:0)	184.1, 506.36, 125.0, 104.1, 86.1	< 0.001	3.36	7.53
13.35	568.3391	$[M+H]^+$	$C_{30}H_{50}NO_7P$	LPC(22:6)	184.1, 550.4, 104.1, 125.0	< 0.001	15.24	2.08
14.9	480.3081	$[M+H]^+$	$C_{23}H_{46}NO_7P$	LPE(18:1)[a]	339.3, 419.3, 462.3, 308.3, 265.3, 69.1,	< 0.001	0.59	2.36
20.54	482.3237	$[M+H]^+$	$C_{23}H_{48}NO_7P$	LPE(18:0)	341.3, 310.3, 421.3, 464.3, 267.3, 69.1	0.0016	2.04	4.21
2.4	664.1154	$[M+H]^+$	$C_{21}H_{28}N_7O_{14}P_2$	NAD[a]	136.1, 232.1, 348.1, 428.0, 97.0	< 0.001	3.71	2.84
3.32	284.0986	$[M+H]^+$	$C_{10}H_{13}N_5O_5$	Guanosine[a]	152.1, 135.0, 110.0	< 0.001	4.4	2.67
3.9	325.0428	$[M+H]^+$	$C_9H_{13}N_2O_9P$	UMP[a]	97.0, 113.0, 227.1, 69.0	0.0063	6.31	2.06
3.67	362.0503	$[M-H]^-$	$C_{10}H_{14}N_5O_8P$	GMP[a]	78.96, 96.97, 150.0, 211.0	< 0.001	2.4	2.67
8.03	565.0471	$[M-H]^-$	$C_{15}H_{24}N_2O_{17}P_2$	UDP-glucose[a]	323.0, 384.98, 241.0, 158.9, 402.99,	< 0.001	1.73	2.67
7.12	606.0735	$[M-H]^-$	$C_{17}H_{27}N_3O_{17}P_2$	UDP-N-acetylglucosamine	384.98,402.99,362.0,282.0,158.9,176.9	< 0.001	4.43	29.66
3.33	152.0567	$[M+H]^+$	$C_5H_5N_5O$	Guanine[a]	135.0, 110.0, 107.0, 82.0	0.0389	4.1	3.09
1.84	137.0458	$[M+H]^+$	$C_5H_4N_4O$	Hypoxanthine[a]	119.0, 110.0, 94.0, 82.0, 67.0, 55.0	0.0331	0.68	2.6
1.75	148.0426	$[M+H-H_2O]^+$	$C_5H_{11}NO_3S$	Methionine sulfoxide[a]	84.0, 102.1, 130.05, 56.1, 74.0	< 0.001	3.76	2.9