

**Exploratory metabolomic analysis based on reverse phase liquid chromatography-mass spectrometry to study an *in vitro* model of hypoxia-induced metabolic alterations in HK-2 cells**

Samuel Bernardo-Bermejo<sup>1</sup>, Elena Sánchez-López<sup>2#</sup>, Lei Tan<sup>1,3</sup>, Selma Benito-Martínez<sup>4,5</sup>, Zhengjin Jiang<sup>3</sup>, María Castro-Puyana<sup>1,6</sup>, Francisco Javier Lucio Cazaña<sup>4</sup>,  
María Luisa Marina<sup>1,6\*</sup>

<sup>1</sup>Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares (Madrid), Spain.

<sup>2</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands.

<sup>3</sup>Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou, 510632, China.

<sup>4</sup>Universidad de Alcalá. Departamento de Biología de Sistemas, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain.

<sup>5</sup>Universidad de Alcalá. Ramón y Cajal” Health Research Institute (IRYCIS), Madrid, Spain.

<sup>6</sup>Universidad de Alcalá. Instituto de Investigación Química Andrés M. del Río. Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares (Madrid), Spain.

# **Current affiliation:** Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, the Netherlands.

**\*Correspondence:** Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871, Alcalá de Henares, Madrid, España.

**E-mail:** mluisa.marina@uah.es

**Fax:** +34-91 885 4971

**Tel:** +34-91 885 4935

## Figures

**Figure S1.** Permutation tests of PLS-DA models at short times.

**Figure S2.** Total ion chromatograms of four samples of the two metabolomic sequences at long times: A) Control sample at 48 h of intracellular fluid, B) Hypoxia sample at 48 h of intracellular fluid, C) Control sample at 48 h of extracellular fluid, D) Hypoxia sample at 48 h of extracellular fluid. Experimental conditions detailed on *section 3.5*.

**Figure S3.** Permutation tests of PLS-DA models at long times.

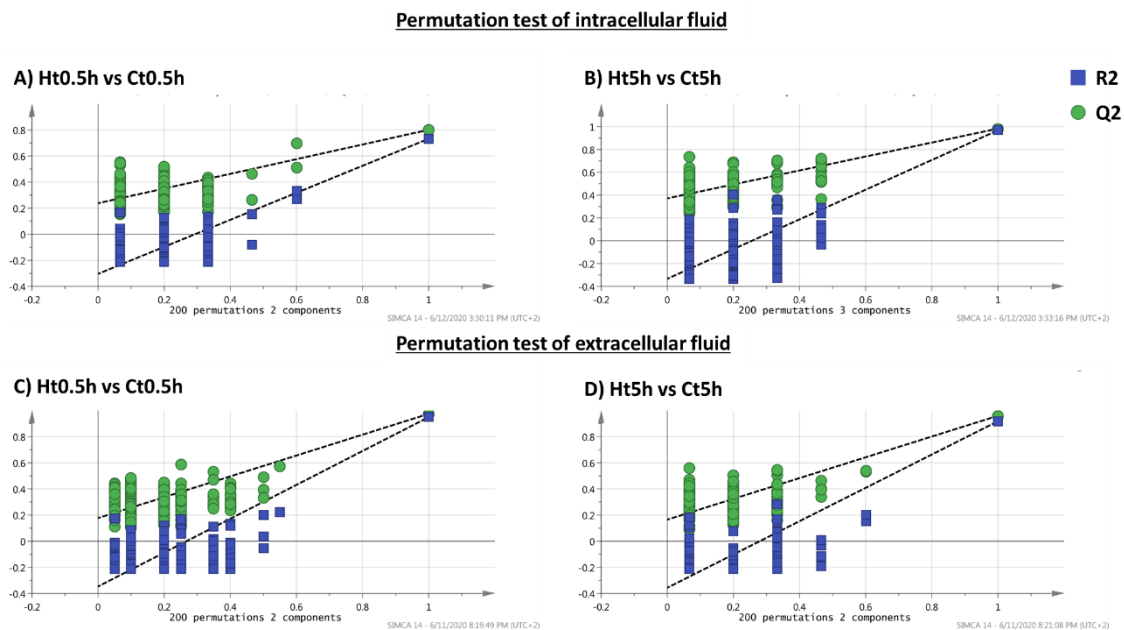
**Figure S4.** Box-plots of the common metabolites identified in the intracellular fluid at short and long times. The p-value of Mann Whitney U test is included.

**Figure S5.** Box-plots of the common metabolites identified in the extracellular fluid at short and long times. The p-value of Mann Whitney U test is included.

**Figure S6.** Box-plots of the metabolites identified in the intracellular fluid at short or long times. The p-value of Mann Whitney U test is included.

**Figure S7.** Box-plots of the common metabolites identified in the extracellular fluid at short and long times. The p-value of Mann Whitney U test is included.

Figure S1



**Figure S2.**

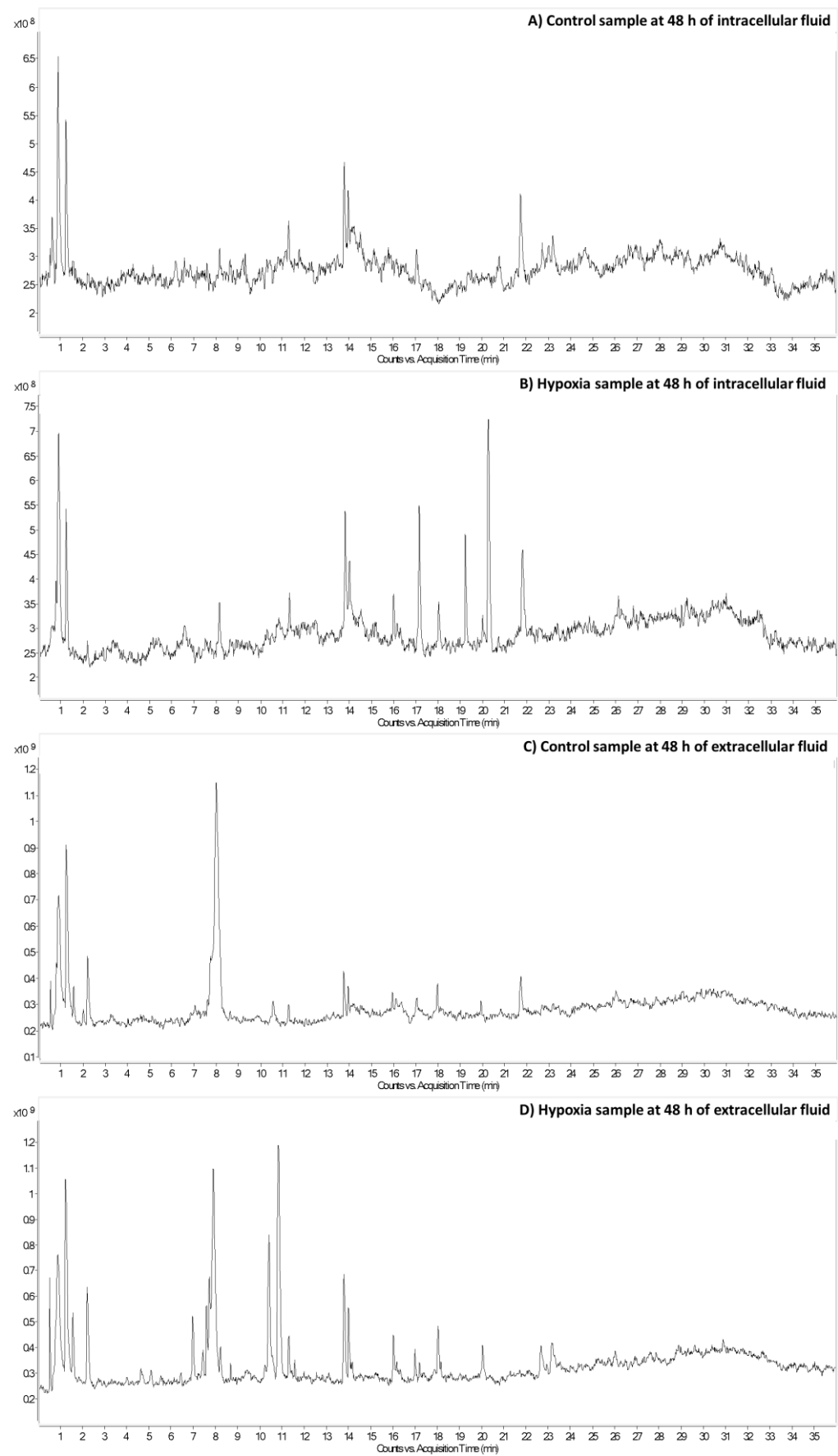


Figure S3

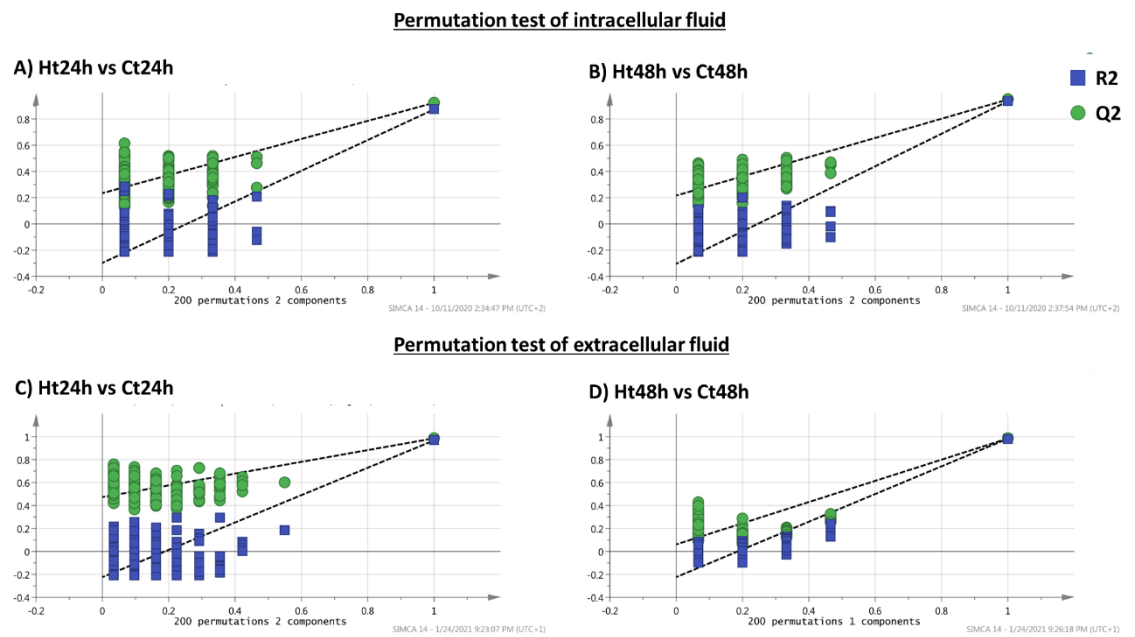


Figure S4

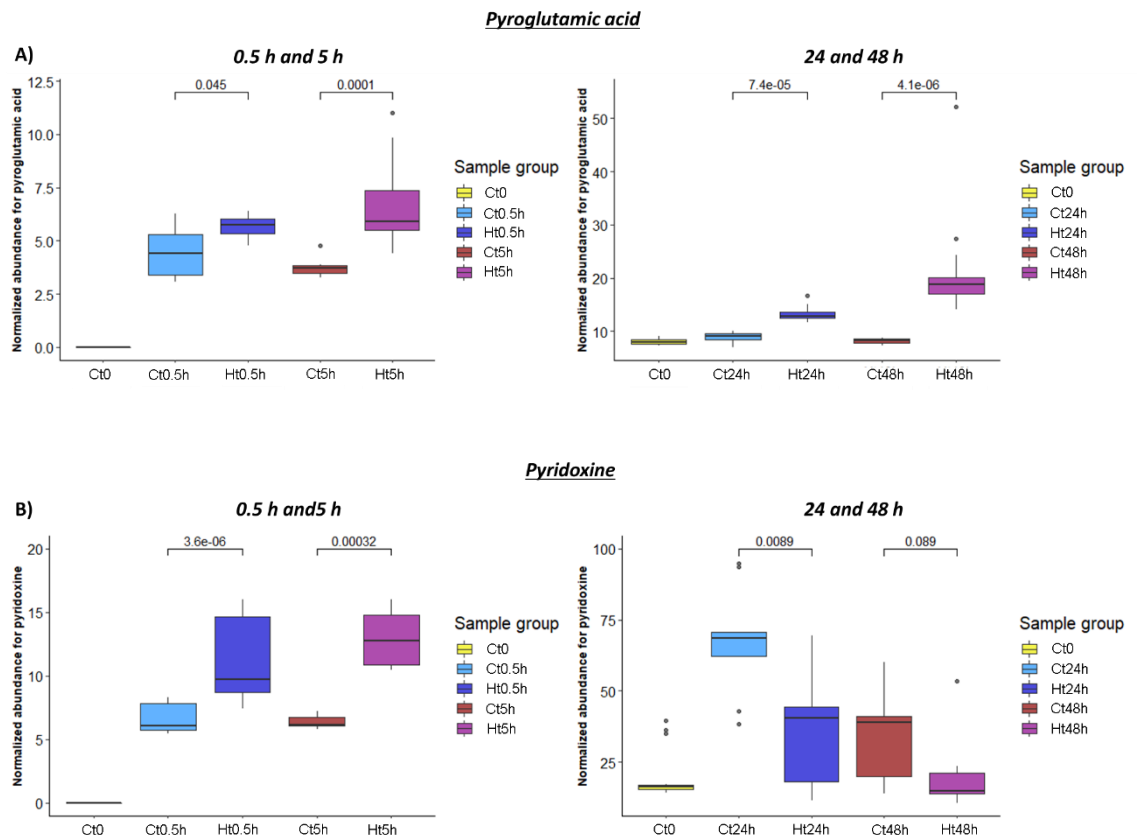


Figure S5

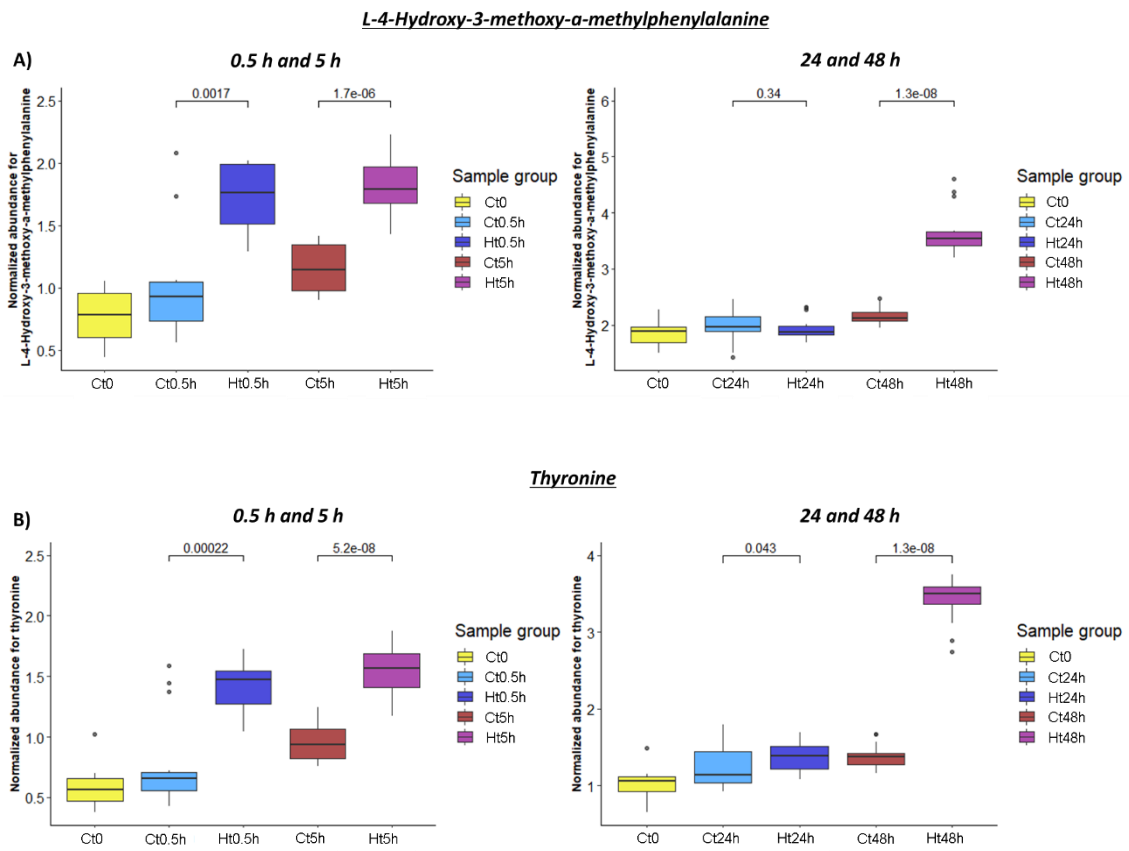
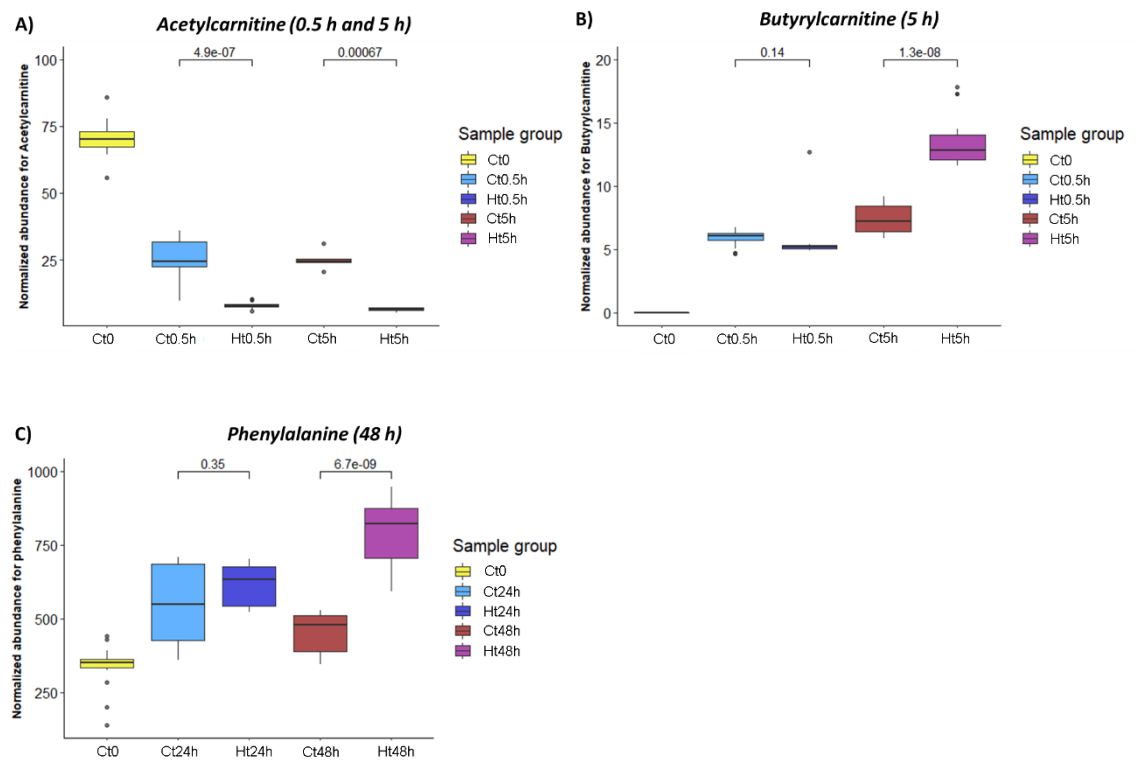
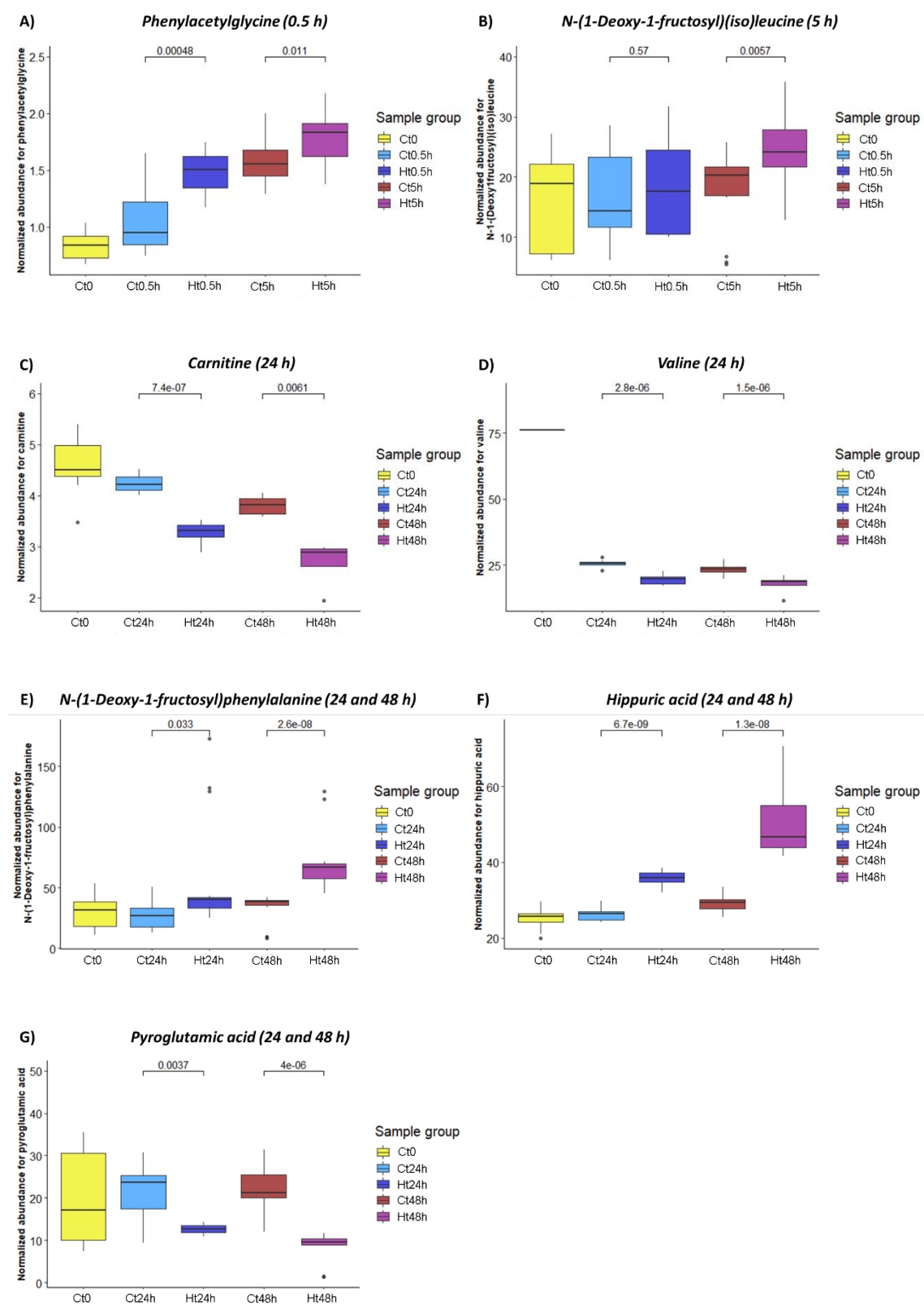




Figure S6



**Figure S7**



**Table S1.** Unknown molecular features (identification level of 4 according to [19]). in the intracellular and extracellular fluid analysis at 0.5 and 5 h.

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend*)	
				Ht0.5h vs Ct0.5h	Ht5h vs Ct5h
Intracellular fluid					
Significant metabolites at 0.5 h					
1	2.9	113.0842	55.0537, 69.0685	1.30 (↑)	0.04 (↑)
2	1.1	161.0520	-	1.17 (↓)	0.94 (↓)
3	10.6	366.1278	160.0421, 217.0634	1.03 (↑)	0.13
Significant metabolites at 5 h					
4	0.7	103.1011	-	0.19 (↓)	1.09 (↓)
5	14.0	273.2696	-	0.73	1.59 (↓)
6	14.2	317.2957	88.0756, 57.0696, 70.0643	0.21	1.37 (↓)
7	16.2	301.3001	256.2625, 88.0756, 219.1733	0.05	1.24 (↓)
Significant metabolites at 0.5 h and 5 h					
8	1.7	219.1123	90.0547. 72.0439. 98.0230	1.21 (↓)	1.59 (↓)
9	7.6	760.1953	298.0556. 473.1246	2.51 (↑)	1.56 (↑)
10	8.0	308.1218	174.0576. 128.0519	1.05 (↑)	1.77 (↑)
11	8.1	352.1120	174.0578. 160.0421. 128.0525	1.83 (↑)	1.63 (↑)
12	9.4	455.1216	-	1.44 (↓)	1.35 (↓)
13	17.5	303.2956	91.0544. 58.0658	2.52 (↓)	2.19 (↓)
14	18.3	329.3319	87.0748. 70.0647. 106.0854	1.08 (↓)	1.63 (↓)
15	19.5	331.3270	91.0546. 58.0660	2.44 (↓)	2.33 (↓)
16	20.6	325.3745	186.2201	2.57 (↓)	2.32 (↓)
Extracellular fluid					
Significant metabolites at 0.5 h					
17	1.0	562.2164	-	1.46 (↑)	0.70 (↑)
18	7.1	415.0885	176.07139, 241.0301	1.80 (↑)	0.98 (↑)
19	7.1	240.0257	-	1.74 (↑)	0.93 (↑)
20	15.3	386.1751	105.0696, 121.0646	1.45 (↑)	0.43
Significant metabolites at 5 h					
21	0.9	85.0896	56.0478, 57.0555	0.26	1.01 (↓)
22	1.2	248.1280	29.1219, 174.1030, 189.1112	0.33	1.08 (↓)
23	1.3	349.1103	143.0296, 230.0785, 201.0580	0.35	1.30 (↑)
24	1.4	138.1147	83.0592	0.44	1.98 (↓)
25	6.3	480.2100	84.0805, 147.1162, 130.0862	0.00	2.52 (↑)
26	6.3	187.1216	56.0544, 83.0849, 97.1010	0.09	1.25 (↓)

**Table S1** (continued)

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend*)	
				Ht0.5h vs Ct0.5h	Ht5h vs Ct5h
27	7.1	256.0974	186.1047	0.55 (↓)	1.27 (↓)
28	7.5	159.0359	87.0257, 70.0641, 114.0353	0.00	1.31 (↑)
29	7.7	606.1010	-	0.00	1.45 (↑)
30	8.2	453.1583	160.0413, 277.1199	0.00	1.2 (↑)
31	8.9	453.1067	91.0548, 156.0852	0.00	1.5 (↑)
32	9.2	354.0587	261.0211, 273.0901	0.91 (↑)	1.02 (↑)
33	11.0	159.0358	114.0371, 87.0257	0.73	2.61 (↑)
34	11.0	1161.1360	-	0.84 (↑)	1.18 (↑)
35	14.3	289.2633	242.2479, 88.0750, 56.0493	0.29 (↑)	1.02 (↑)
36	14.6	449.3731	344.3134	0.43 (↑)	1.15 (↑)
<b>Significant metabolites at 0.5 and 5 h</b>					
37	1.0	296.0949	122.0707	2.29 (↓)	1.82 (↓)
38	1.1	159.0689	130.0648, 143.0700, 115.0526	1.13 (↑)	2.30 (↑)
39	1.3	264.0709	120.0783, 175.0158	1.12 (↑)	2.15 (↑)
40	2.1	301.1897	-	1.58 (↑)	1.56 (↑)
41	2.9	113.0844	55.0538, 69.0697, 79.0539	1.99 (↑)	1.24 (↑)
42	4.9	179.0956	91.0527, 62.0591	1.14 (↑)	2.09 (↑)
43	5.7	239.1171	194.1175, 126.0534	1.79 (↑)	1.45 (↑)
44	5.9	554.2221	247.1024	1.80 (↑)	1.78 (↑)
45	6.5	508.2161	201.0982	2.67 (↑)	2.63 (↑)
46	6.6	480.2109	322.1757, 147.1114, 84.0803	2.72 (↑)	2.77 (↑)
47	6.6	321.1699	84.0786, 130.0855, 147.1179	2.65 (↑)	2.69 (↑)
48	6.6	159.0358	114.0372, 87.0273	2.62 (↑)	2.62 (↑)
49	6.6	489.1730	156.0766, 331.1418	2.53 (↑)	2.18 (↑)
50	7.1	574.1303	416.09380, 241.0301	1.88 (↑)	1.07 (↑)
51	7.1	159.0358	114.0349, 87.0267	1.85 (↑)	1.03 (↑)
52	7.3	480.2097	84.0807, 130.0852	3.13 (↑)	2.83 (↑)
53	7.5	574.1280	241.0289, 160.0402	2.83 (↑)	1.45 (↑)
54	7.5	480.1702	147.0756	2.38 (↑)	2.53 (↑)
55	7.9	439.1425	160.0412, 114.0364	2.55 (↑)	1.65 (↑)
56	8.1	409.1321	160.0418, 114.0371	2.46 (↑)	2.68 (↑)
57	8.3	242.0818	198.0626, 172.0862	1.15 (↑)	1.12 (↑)
58	18	356.2719	-	1.31 (↑)	1.22 (↑)
59	20.3	357.3628	88.0738, 54.0757, 71.0780	1.46 (↑)	1.24 (↑)

\*\*↑: The metabolite (on average) is more abundant in hypoxia condition; ↓: The metabolite (on average) is less abundant in hypoxia condition.

**Table S2.** Unknown molecular features (identification level of 4 according to [19]) in the intracellular and extracellular fluid analysis at 24 and 48 h.

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend)	
				Ht24h vs Ct24h	Ht48h vs Ct48h
Intracellular fluid					
Significant metabolites at 24 h					
1	8.1	217.2042	70.0646, 88.0754, 57.0699	1.17 (↑)	0.99 (↑)
Significant metabolites at 48 h					
2	0.7	161.1040	90.0545	0.25 (↑)	1.01 (↑)
3	0.9	131.0941	86.0963, 69.0698	0.32 (↑)	1.02 (↑)
4	8.1	352.1092	174.0578, 160.0424, 128.0524	0.56 (↓)	1.55 (↑)
5	11.3	245.2356	88.0757, 57.0701, 70.0654	0.90 (↑)	1.00 (↑)
Significant metabolites at 24 and 48 h					
6	8.6	261.2304	80.0757, 70.0656, 57.0701	1.11 (↑)	1.06 (↑)
7	13.8	273.2668	88.07533, 57.0700, 70.0650, 106.0858	1.30 (↑)	1.10 (↑)
8	14.0	317.2929	256.2630, 88.0756	1.21 (↑)	1.04 (↑)
9	16.0	301.2984	-	1.22 (↑)	1.16 (↑)
10	17.2	303.2930	91.0540, 58.0653, 212.2367	3.11 (↑)	1.90 (↑)
11	18.0	329.3297	88.0755, 57.0702	1.34 (↑)	1.36 (↑)
12	19.2	331.3244	91.0538, 58.0653, 240.2672	2.25 (↑)	1.93 (↑)
Extracellular fluid					
Significant metabolites at 24 h					
1	0.8	132.0223	87.0989, 70.0702	1.04 (↑)	0.73 (↑)
2	1.2	281.1115	150.0731	1.74 (↑)	0.00
3	1.6	446.2217	-	1.12 (↑)	0.41 (↑)
4	1.7	443.1282	-	1.02 (↑)	0.00
5	3.7	191.0577	-	1.12 (↑)	0.78 (↑)
6	4.2	245.1617	85.0279	1.07 (↑)	0.62 (↓)
7	6.1	246.1686	85.0289	1.05 (↑)	0.49 (↑)
8	6.2	466.1885	308.1602, 70.0645	2.35 (↑)	0.34 (↑)
9	6.2	307.1536	-	1.95 (↑)	0.75 (↑)
10	6.4	230.1733	-	1.17 (↑)	0.50 (↑)
11	6.4	321.1683	84.0811, 130.0891, 141.1048	1.49 (↑)	0.89 (↑)
12	6.4	480.2040	322.1754, 84.0808, 147.1121	1.57 (↑)	0.97 (↑)
13	6.8	485.1283	454.1079	3.28 (↓)	0.00 (↓)

**Table S2.** (continued)

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend)	
				Ht24h vs Ct24h	Ht48h vs Ct48h
14	7.3	452.3350	86.0966, 309.8652	1.18 (↑)	0.82 (↑)
15	7.8	439.1406	160.0429	2.54 (↑)	0.20
16	8.1	646.2235	315.0941	2.48 (↓)	0.00
17	8.3	1471.5847	-	1.07 (↑)	0.20
<b>Intracellular fluid</b>					
18	10.4	159.0349	114.0370, 87.0271	2.11 (↑)	0.84 (↑)
19	16.0	257.2717	62.0621, 174.9822	1.21 (↑)	0.33 (↑)
20	16.1	473.2521	60.0554	1.01 (↑)	0.66 (↑)
21	16.2	317.2923	270.2830, 88.0750	1.11 (↑)	0.60 (↑)
22	17.2	303.2928	91.0557, 212.2369	2.35 (↑)	0.00
23	18.0	329.3294	88.0757, 57.0700	1.37 (↑)	0.18 (↑)
24	18.2	345.3236	298.3080, 74.0568, 56.0478	1.17 (↑)	0.46 (↑)
25	18.2	528.5210	256.2639	1.57 (↑)	0.12 (↑)
26	18.7	399.3712	338.3439, 57.0681, 102.0951	1.58 (↑)	0.38 (↑)
27	19.0	399.3698	338.3389, 88.0738, 100.9248	1.32 (↑)	0.98 (↑)
28	19.9	556.5537	256.2631, 284.2921	1.20 (↑)	0.47 (↓)
29	26.0	330.2740	57.0705, 71.0840, 95.0817	1.47 (↑)	0.04 (↑)
30	26.7	530.2727	119.0852	1.08 (↑)	0.07
31	31.0	679.3579	-	1.71 (↑)	0.42 (↑)
<b>Significant metabolites at 48 h</b>					
32	0.5	262.1387	86.0956, 132.0998	0.73 (↑)	1.05 (↑)
33	0.5	595.2805	-	0.47	1.11 (↑)
34	0.8	244.1412	-	0.41	1.26 (↑)
35	0.9	175.0615	70.0627, 60.0529	0.67 (↑)	1.21 (↑)
36	0.9	153.0756	-	0.82 (↑)	1.33 (↑)
37	1.3	583.1634	210.0453	0.61	1.47 (↑)
38	1.3	593.1157	210.0518	0.05 (↓)	1.16 (↑)
39	1.3	330.1541	120.0802, 166.0910	0.84 (↑)	1.06 (↑)
40	1.3	367.1228	203.0509, 188.0679	0.28	1.44 (↓)
41	1.4	250.0642	137.0442	0.62 (↑)	1.08 (↑)
42	1.6	219.1096	-	0.94 (↑)	1.22 (↑)
43	1.7	246.1204	86.0947	0.87	1.42 (↑)
44	2.2	408.1789	188.0698	0.62	1.23 (↑)
45	2.2	242.0453	-	0.47	1.41 (↑)
46	2.2	226.0714	-	0.62	1.31 (↑)

**Table S2.** (continued)

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend)	
				Ht24h vs Ct24h	Ht48h vs Ct48h
47	2.3	287.1155	-	0.85 (↑)	1.33 (↑)
48	3.5	246.1372	146.0573, 188.0718	0.82	1.05 (↑)
49	3.5	257.0724	164.0642, 126.0526	0.38	1.30 (↑)
50	3.6	877.3352	107.04627, 182.0818, 165.0543	0.02	1.51 (↑)
51	3.7	329.1584	106.04820, 197.1235, 86.0935	0.24 (↑)	1.27 (↑)
52	4.3	135.0680	91.0538	0.60	1.27 (↑)
53	4.4	265.1310	85.0273	0.54 (↑)	1.55 (↑)
54	4.6	412.1338	136.0605, 281.0978, 162.0403	0.91 (↑)	1.33 (↑)
55	4.7	266.0904	120.0442	0.09	1.12 (↑)
56	5.5	239.1158	194.1179, 126.0536	0.32	1.14 (↑)
57	6.1	187.1204	83.0849, 97.1003, 55.0541	0.70	1.13 (↑)
58	6.1	334.0982	289.10003, 128.0521, 159.0895, 91.0534	0.87	3.13 (↑)
59	7.0	159.0352	114.0370, 87.0268	0.89	1.85 (↑)
60	7.0	578.1194	243.0216, 418.0825	0.00	1.76 (↑)
61	7.1	480.2039	84.0804, 147.1116, 130.0856	0.87	1.86 (↑)
62	7.4	159.0351	114.0385, 87.0245	0.00	2.44 (↑)
63	7.6	606.0944	448.0646, 273.0032	0.00	1.37 (↑)
64	7.7	330.1010	253.1240	0.46	1.18 (↑)
65	7.8	346.0747	-	0.28	1.33 (↑)
66	7.8	638.2195	331.1081	0.01	1.74 (↑)
67	7.8	616.2380	309.1263	0.28	1.56 (↑)
68	8.2	334.0983	217.0644, 202.0300	0.53	1.38 (↑)
69	8.2	393.1468	-	0.58	1.95 (↑)
70	8.8	453.1027	91.0534, 139.0849, 176.0679	0.82	1.72 (↑)
71	9.8	231.2189	70.0633, 106.0843, 85.0276	0.70	1.07 (↑)
72	11.3	245.2353	88.0751, 70.0650, 57.0701	0.86 (↑)	1.26 (↑)
73	11.3	201.2088	57.0681	0.90	1.44 (↑)
74	11.4	465.1926	160.0411, 132.1014, 86.0950	0.68	2.00 (↑)
75	11.8	333.2875	272.2540, 228.2313, 70.0644	0.29	1.03 (↑)
76	12.1	499.1768	166.0836, 160.0415	0.84	1.08 (↑)
77	12.6	259.2507	57.0677, 70.0651, 95.0761	0.99	1.66 (↑)
78	13.1	465.3079	-	0.73	1.14 (↑)
79	14.3	449.3712	344.3135	0.90 (↑)	1.25 (↑)
80	14.5	493.3977	388.3389, 344.3175	0.66 (↑)	1.14 (↑)
81	27.9	539.5277	256.2628, 311.2939, 284.2938	0.64	1.10 (↑)

**Table S2.** (continued)

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend)	
				Ht24h vs Ct24h	Ht48h vs Ct48h
82	30.1	595.5914	313.3260	0.45 (↓)	1.87 (↓)
<b>Significant metabolites at 24 and 48 h</b>					
83	0.7	166.0814	81.0450, 122.0709, 139.0994	1.55 (↓)	1.00 (↓)
84	0.7	382.1053	-	1.53 (↓)	1.65 (↓)
85	0.7	204.0556	-	1.13 (↑)	1.09 (↑)
86	0.8	282.9963	226.0389	1.37 (↑)	1.36 (↑)
87	0.9	386.0913	176.0641	1.18 (↑)	1.21 (↑)
88	0.9	284.1697	154.0840	1.04 (↑)	1.21 (↑)
89	1.0	248.1269	219.1219, 174.1019, 186.0999, 137.0764	2.09 (↓)	1.71 (↓)
90	1.6	482.1827	264.0794	1.17 (↑)	1.31 (↑)
91	1.7	231.1459	85.0273	2.04 (↑)	1.66 (↑)
92	1.9	301.1877	102.0903	1.53 (↑)	1.53 (↑)
93	2.2	201.0785	184.0762, 156.0800	1.06 (↑)	1.29 (↑)
94	2.2	187.0629	118.0642, 146.0591	2.07 (↑)	1.61 (↑)
95	2.3	177.1257	91.05667	1.30 (↑)	1.43 (↑)
96	2.4	260.1364	-	1.65 (↑)	1.51 (↑)
97	2.5	297.0890	136.0622	1.93 (↑)	1.01 (↑)
98	2.7	289.0945	244.1010, 126.0534, 110.0374	1.38 (↑)	1.02 (↑)
99	2.8	260.1353	86.0964, 132.1010	2.20 (↑)	1.24 (↑)
100	2.9	243.1464	-	1.21 (↑)	1.49 (↑)
101	3.7	245.1619	85.0280	1.46 (↑)	1.65 (↑)
102	3.8	294.1210	120.0805, 166.0870	2.35 (↑)	1.61 (↑)
103	4.0	104.0264	77.0378, 51.0223	1.14 (↑)	1.11 (↑)
104	4.7	441.1390	295.0925	1.05 (↑)	1.21 (↑)
105	5.1	75.0323	-	1.05 (↑)	1.69 (↑)
106	5.1	193.0748	-	1.27 (↑)	2.62 (↑)
107	5.7	1354.5665	-	1.14 (↑)	1.29 (↑)
108	6.2	480.2036	322.1745, 147.1118, 84.0802	1.93 (↑)	1.41 (↑)
109	6.3	508.2099	201.0954, 175.1178	1.66 (↑)	1.33 (↑)
110	6.4	159.0353	114.0369	1.54 (↑)	1.13 (↑)
111	6.4	489.1673	156.0765, 110.0704	1.46 (↑)	1.69 (↑)
112	7.0	508.2101	201.0950, 175.1193	1.51 (↑)	2.11 (↑)
113	7.0	415.0869	241.0339, 151.9829, 122.0254	2.80 (↑)	1.80 (↑)
114	7.0	574.1220	416.0927, 241.0300	3.62 (↑)	2.34 (↑)
115	7.4	574.1221	416.0932, 241.0298	2.76 (↑)	2.98 (↑)



**Table S2.** (continued)

#	RT (min)	Monoisotopic mass (Da)	Main fragments (MS/MS)	VIP (Trend)	
				Ht24h vs Ct24h	Ht48h vs Ct48h
116	7.4	415.0871	241.0300, 176.0705, 122.0309, 161.9846	2.08 (↑)	2.39 (↑)
117	7.7	670.1566	289.0257, 363.0442, 192.0071	1.16 (↓)	1.61 (↑)
118	7.9	481.1533	160.0424, 148.0622	1.26 (↑)	1.10 (↑)
119	8.0	409.1304	160.0423, 176.0694	2.25 (↑)	1.06 (↑)
120	8.1	453.1563	160.0438, 277.1150, 129.1042, 95.2478	2.12 (↑)	1.53 (↑)
121	8.2	439.1410	160.0435	1.16 (↑)	1.67 (↑)
122	8.3	1605.6796	-	1.27 (↑)	1.09 (↑)
123	8.4	423.1459	160.0422, 176.0682	1.85 (↑)	1.36 (↑)
124	8.8	449.1615	116.0703, 160.0420	1.35 (↑)	2.00 (↑)
125	10.4	424.1300	160.0420, 217.0634	4.03 (↑)	3.23 (↑)
126	10.9	393.1462	60.0553	2.72 (↑)	2.83 (↑)
127	11.4	357.3597	-	1.35 (↑)	1.55 (↑)
128	11.8	319.2872	91.0536, 228.2333, 58.0651	2.98 (↑)	2.64 (↑)
129	11.9	319.2874	91.05307, 58.0649, 228.2335	3.03 (↑)	2.40 (↑)
130	13.8	273.2667	88.0753, 57.0698, 70.0653, 106.0862	1.19 (↑)	1.47 (↑)
131	13.8	229.2404	57.0700	1.06 (↑)	1.38 (↑)
132	14.0	317.2931	-	1.01 (↑)	1.59 (↑)
133	14.1	361.3192	300.2883, 70.0649	1.31 (↑)	2.06 (↑)
134	14.2	405.3450	344.3126, 300.2946	1.08 (↑)	1.41 (↑)

\*\*↑: The metabolite (on average) is more abundant in hypoxia condition; ↓: The metabolite (on average) is less abundant in hypoxia condition.

**Table S3.** Cell number and protein content of the sample groups at short times.

Sample group	Ct0	Ct0.5	Ct5h	Ht0.5	Ht5h
Cell number	180000	162000	161000	14900	153000
Protein content (mg/500µL)	0.286	0.291	0.248	0.228	0.242

**Table S4.** Cell number and protein content of the sample groups at long times.

Sample group	Ct0	Ct24h	Ct48h	Ht24h	Ht48h
Cell number	221000	220000	224000	217000	180000
Protein content (mg/500µL)	1.076	1.062	1.130	1.085	0.984