

## Supplemental tables

### Discovery and validation of metabolite markers in bloodstain for bloodstain age estimation

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**Table S1.** Demographic information of subjects

Experiment set	Discovery set (n=20)	Validation set (n=40)
Age (mean $\pm$ standard deviation)	Young: 24 $\pm$ 3 The elderly: 68 $\pm$ 9	Young: 24 $\pm$ 2 The elderly: 68 $\pm$ 10
Sex (male/female)	10/10	16/24
Disease	4	12
Smoking	1	3
> 8 hours fasting		yes

**Table S2.** MRM transition parameters

Analyte	Q1	Q3	DP	EP	CE	CXP
Adenosine 5'-monophosphate	347.992	136.1	111	10	27	20
	347.992	119.2	111	10	77	10
Adenosine 5'-monophosphate 15N5	352.952	141.1	116	10	25	8
	352.952	123.1	116	10	79	8
Choline	104.957	45	86	10	31	6
	104.957	59.3	86	10	37	22
Choline d4	109.07	61.1	116	10	25	8
	109.07	59.1	116	10	35	6
Ergothioneine	229.969	186.1	46	10	17	12
	230.066	127.1	206	10	25	12
Ergothioneine d9	239.124	127.1	106	10	27	10
	239.124	195.2	106	10	17	12
Hypoxanthine	136.909	119.1	106	10	29	8
	136.909	110.1	106	10	29	8
Hypoxanthine 13C2 15N	139.974	113.1	136	10	29	8
	139.974	122.1	136	10	29	10
L-Isoleucine	131.999	86.1	26	10	15	10
	131.991	69	51	10	23	10
L-Isoleucine d10	142.04	96.2	111	10	15	8
	142.04	78.1	111	10	25	10
L-Tryptophan	204.989	146.1	46	10	23	10
	204.989	118.1	46	10	35	6
L-Tryptophan d5	210.09	192.1	111	10	15	12
	210.09	150.1	111	10	25	10
Pyroglutamic acid	129.965	84	76	10	23	34
	129.965	56.1	76	10	31	8

**Table S3.** MFs list integrated from volcano plot analysis and sPLS-DA loading components

No.	Molecular feature		Day 0 vs Day 7	Day 0 vs Day 14	Day 0 vs Day 21	Day 0 vs Day 28	Day 7 vs Day 14	Day 7 vs Day 21	Day 7 vs Day 28
	Mass	Retention time							
1	382.1088	0.66							
2	165.0459	0.66							
3	455.2362	7.72							
4	513.3877	15.43							
5	557.4137	15.48							
6	264.0158	0.65							
7	761.5489	17.04							
8	619.8613	10.52							
9	511.9784	10.61							
10	185.178	13.14							
11	805.5756	17.04							
12	603.8296	11.91							
13	777.5451	15.66							
14	254.1397	0.63							
16	131.0134	4.07							
17	676.885	10.87							
18	596.8219	11.18							
19	586.3307	10.65							
20	583.8509	10.65							
21	433.1242	16.94							
22	199.0417	16.80							
23	603.8273	5.52							
24	459.1291	16.27							
25	746.4115	7.09							
26	321.1145	1.36							
27	468.9993	14.28							
28	428.0938	16.48							
29	460.1197	17.04							
31	518.1693	18.20							
32	520.7913	5.47							
33	514.0979	16.74							
34	573.0619	16.94							
35	244.0161	16.94							
36	545.8156	0.59							
37	285.2668	17.46							
38	737.8846	12.43							
39	544.8169	10.86							
40	113.0841	2.61							
41	103.0997	0.63							
42	306.076	0.81							
43	129.0426	0.96							
44	202.0453	0.66							
46	730.3702	13.20							
47	884.5306	19.03							
48	526.6535	10.63							
49	164.1049	2.08							
50	347.0631	0.80							
51	254.2121	9.80							
52	669.4298	15.66							
53	625.476	13.41							
54	266.1729	7.20							
55	370.2203	6.65							
56	217.1314	1.02							
57	529.8413	0.59							

Red shades indicate up fold-change, and blue shades indicate down fold-change.

**Table S4.** Statistical analysis to compare metabolite levels on each day

Day combination	5'-AMP	Choline	Pyroglutamic acid
Day 0 vs. Day 7	****	ns	ns
Day 0 vs. Day 14	*	****	****
Day 0 vs. Day 21	**	****	****
Day 0 vs. Day 28	ns	****	****
Day 7 vs. Day 14	**	***	***
Day 7 vs. Day 21	****	**	****
Day 7 vs. Day 28	***	***	****
Day 14 vs. Day 21	ns	ns	ns
Day 14 vs. Day 28	ns	ns	ns
Day 21 vs. Day 28	ns	ns	ns

\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and \*\*\*\*P < 0.0001.

**Table S5.** Metabolites selected as candidates for estimation of bloodstain age

No.	Matched name	Mass	Formula	CAS No.	HMDB ID	KEGG ID
1	Adenosine 5'-monophosphate	347.063	C <sub>10</sub> H <sub>14</sub> N <sub>5</sub> O <sub>7</sub> P	61-19-8	HMDB0000045	C00020
2	Choline	104.108	C <sub>5</sub> H <sub>14</sub> NO	62-49-7	HMDB0000097	C00114
3	Pyroglutamic acid	129.043	C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	98-79-3	HMDB0000267	C01879

**Table S6.** Comparison of concentration of seven metabolites for each day

Day combination	5'-AMP	Choline	Ergothioneine	Hypoxanthine	L-isoleucine	L-tryptophan	Pyroglutamic acid
Day 0 vs. Day 7	****	****	**	**	ns	ns	****
Day 0 vs. Day 14	**	****	ns	****	***	***	ns
Day 0 vs. Day 21	**	****	ns	****	**	**	*
Day 0 vs. Day 28	ns	****	ns	****	**	**	ns
Day 7 vs. Day 14	****	*	ns	**	****	****	****
Day 7 vs. Day 21	****	ns	ns	**	****	****	****
Day 7 vs. Day 28	****	*	*	***	****	****	****
Day 14 vs. Day 21	ns	ns	ns	ns	ns	ns	ns
Day 14 vs. Day 28	ns	ns	ns	ns	ns	ns	ns
Day 21 vs. Day 28	ns	ns	ns	ns	ns	ns	ns

\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and \*\*\*\*P < 0.0001.