Supplementary Material

Tocotrienol-rich fraction of palm oil improves behavioral impairments and regulates metabolic pathways in AβPP/PS1 mice

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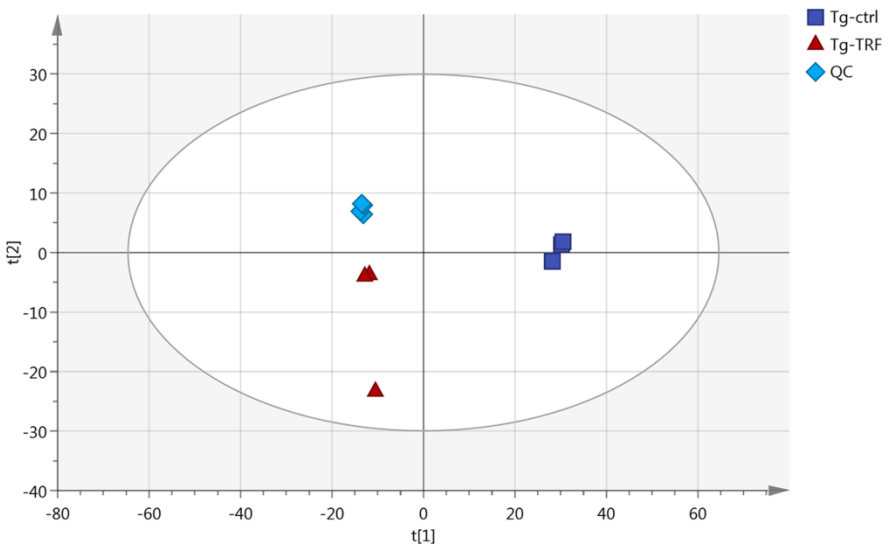
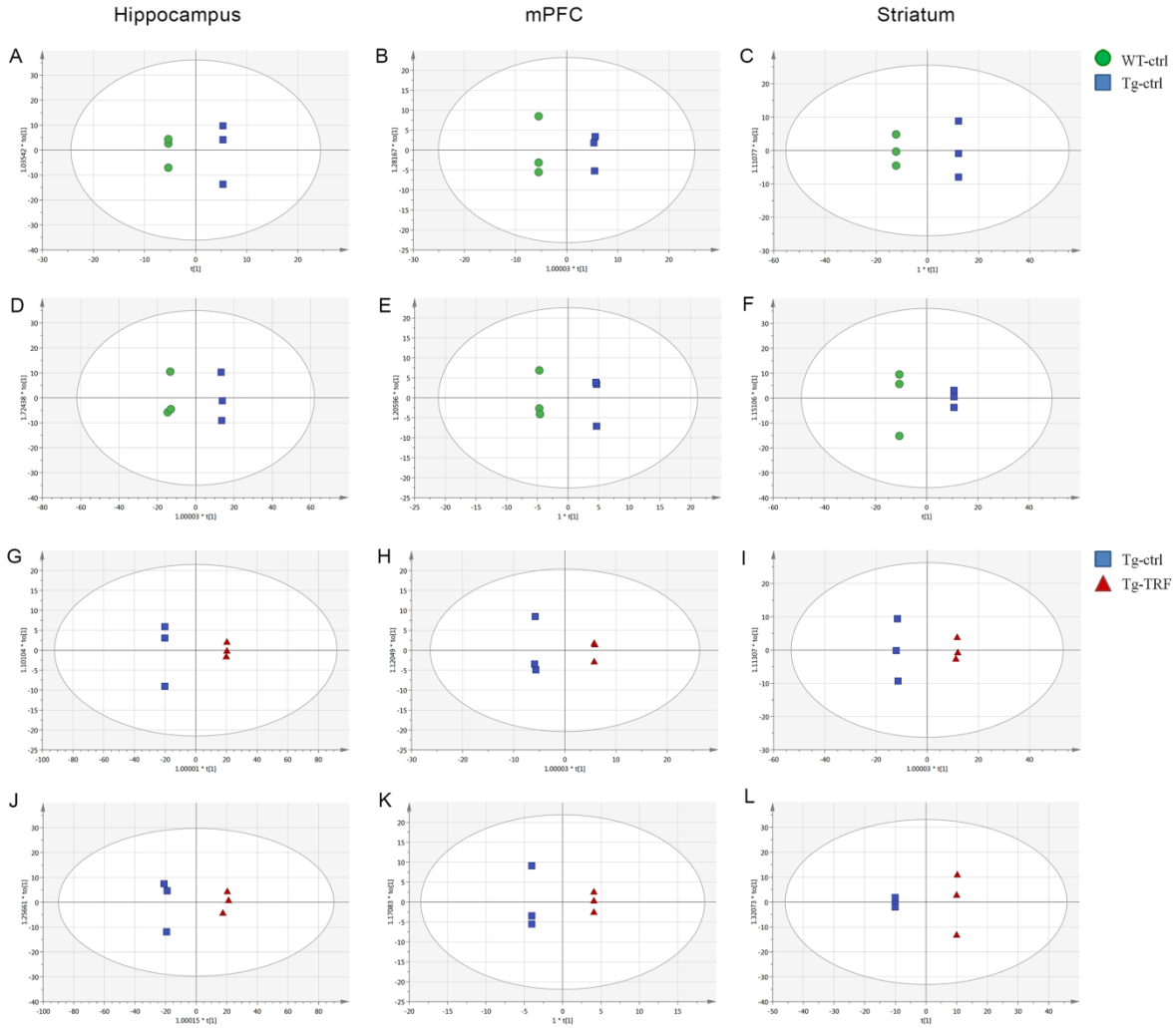
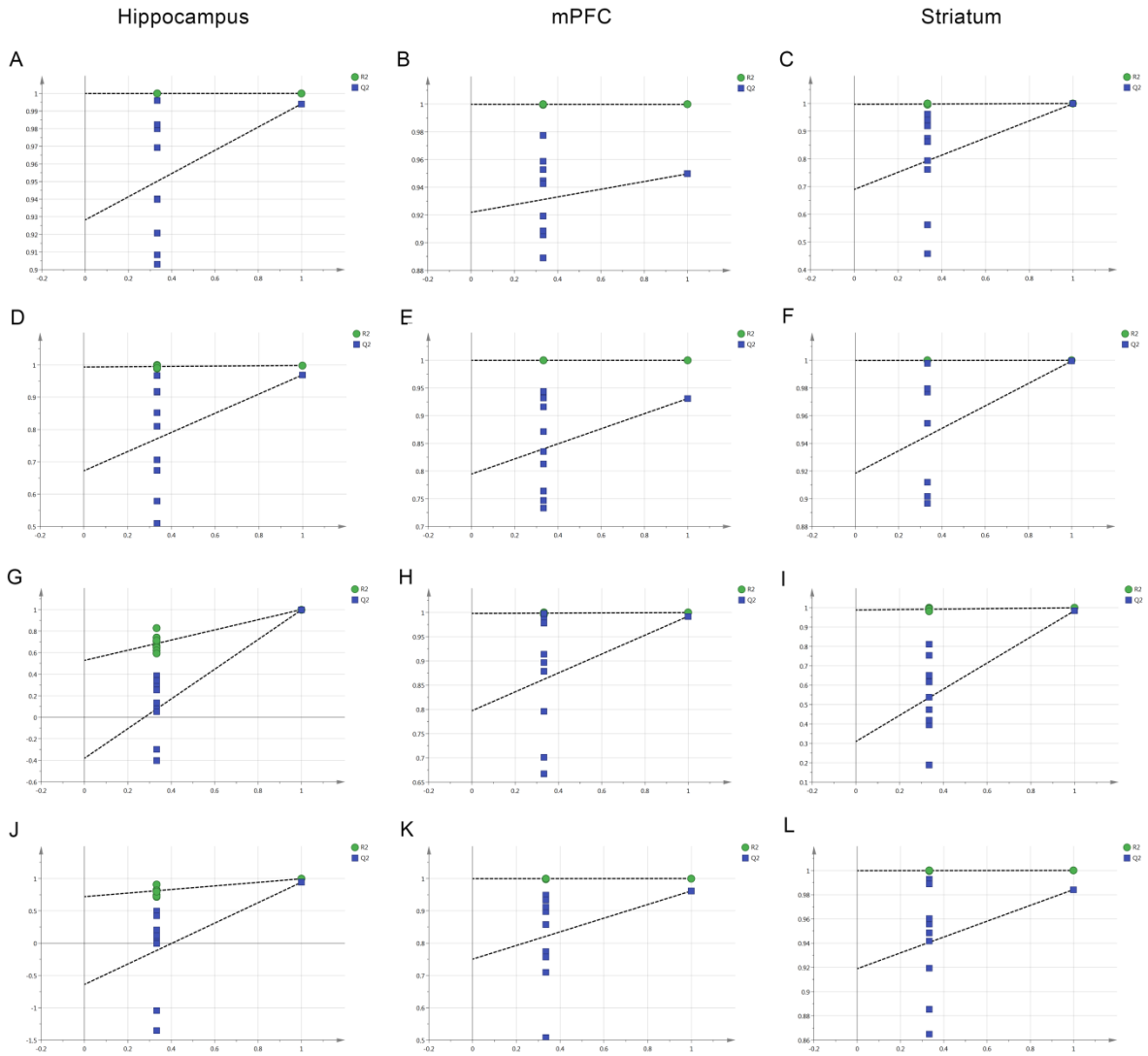


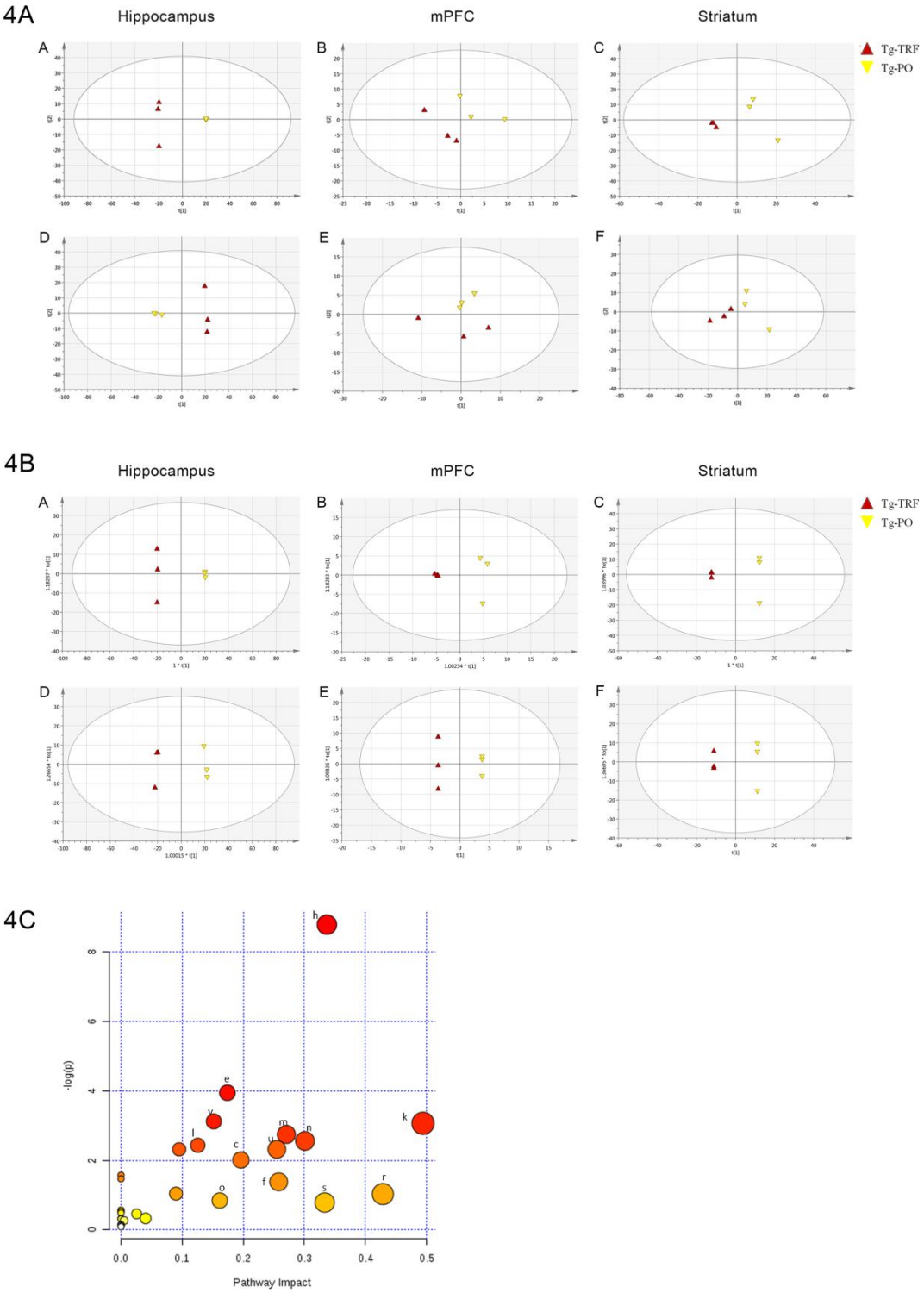
Supplementary Fig. 1. A good clustering of quality control samples indicates stability during the analyses.



Supplementary Fig. 2. Score plots of the OPLS-DA model for the hippocampus, mPFC, and striatum showing a clear separation between groups. A-C) OPLS-DA score plot model of the WT-ctrl and Tg-ctrl mice from ESI (+) analysis. D-F) OPLS-DA score plot model of the WT-ctrl and Tg-ctrl mice from ESI (-) analysis. G-I) OPLS-DA score plot model of the Tg-ctrl and Tg-TRF mice from ESI (+) analysis. J-L) OPLS-DA score plot model of the Tg-ctrl and Tg-TRF mice from ESI (-) analysis.

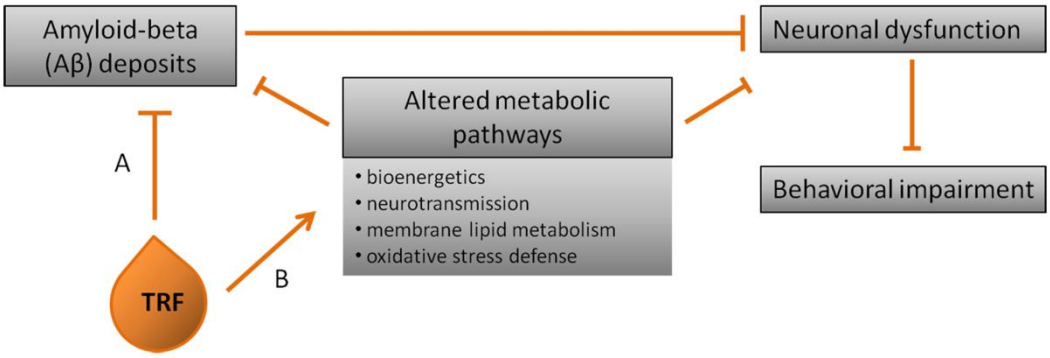


Supplementary Fig. 3. Permutation analysis of OPLS-DA model derived from WT-ctrl versus Tg-ctrl and Tg-TRF versus Tg-ctrl. Statistical validation of the OPLS-DA model by permutation analysis using 500 different model permutations. $R^2Y_{(cum)}$ and $Q^2_{(cum)}$ of the original model are indicated on the far right. A-C) Permutation analysis of OPLS-DA model derived from WT-ctrl versus Tg-ctrl from ESI (+) analysis. D-F) Permutation analysis of OPLS-DA model derived from WT-ctrl versus Tg-ctrl from ESI (-) analysis. G-I) Permutation analysis of OPLS-DA model derived from Tg-TRF versus Tg-ctrl from ESI (+) analysis. J-L) Permutation analysis of OPLS-DA model derived from Tg-TRF versus Tg-ctrl ESI (-) analysis.



Supplementary Fig. 4. Metabolomics analysis of Tg-TRF versus Tg-PO. 4A) Principal component analyses (PCA) score plots. 4B) Score plots of the OPLS-DA model for the hippocampus, mPFC, and striatum. A-C) Results obtained by ESI (+) and (D-F) by ESI (-) modes. 4C) Pathway analysis overview showing altered metabolic pathways in the hippocampus region

from Tg-TRF versus Tg-PO. (c) Alanine, aspartate and glutamate metabolism; (e) Glycerophospholipid metabolism; (f) Glyoxylate and dicarboxylate metabolism; (k) Glutathione metabolism; (l) Citrate cycle (TCA cycle); (m) Pyrimidine metabolism; (n) Pentose phosphate pathway; (o) Cysteine and methionine metabolism; (r) Taurine and hypotaurine metabolism; (s) Valine, leucine and isoluecine biosynthesis; (u) Fructose and mannose metabolism; (v) Amino sugar and nucleotide sugar metabolism. Each node represents an altered metabolic pathway in the hippocampus of both groups. The color and size of each node is based on *p*-value and pathway impact-value, respectively.



Supplementary Fig. 5. Proposed mechanisms of TRF action in improving neuronal dysfunction and behavioral impairment (see Results and Discussion sections for details of biochemical changes). Aβ deposits and/or altered metabolic pathways cause neuronal dysfunction leading to behavioral impairment. (A) TRF modulates Aβ deposition [19] and so improves neuronal dysfunction and reduces impairment of behavior; (B) TRF modulates metabolic pathways dependent on and/or independent of Aβ interaction, and so improves neuronal dysfunction and reduces impairment of behavior.

Putative ID Hippocampus	ESI	Formula	m/z	rt	Δ Mass [Da]	Δ Mass [ppm]	MS/MS Fragment (Intensity)	Identifier
L-Aspartic acid	+	C4 H7 N O4	134.0444	1.020	4.32E-04	3.247	70.02901 (72561) 74.02393 (1071012) 88.03948 (846268) 116.03433 (129193) 134.04495 (59817)	462 (mzCloud)
N-Acetylaspartic acid	-	C6 H9 N O5	174.03961	0.964	1.07E-03	6.097	58.02878 (1836288) 59.01279 (749707) 70.02876 (184283) 71.01277 (395320) 88.03926 (4929591) 89.04241 (82518) 96.99199 (190332) 112.03925 (216181) 114.0185 (585333) 115.00252 (510750) 130.04985 (666340) 132.0291 (59357) 156.02907 (161178) 174.03986 (436207)	1376 (mzCloud)
L-Tyrosine	-	C9 H11 N O3	180.06551	1.440	1.01E-03	5.593	72.00792 (17331) 93.03358 (11032) 119.04909 (38250) 163.03903 (43128) 180.06589 (31250)	2255 (mzCloud)
L-Glutamic acid	-	C5 H9 N O4	146.0448	0.510	1.16E-03	7.909	102.05487 (4142793) 103.05834 (84777) 128.03416 (1410582) 146.04471 (327154)	470 (mzCloud)
DL-Glutamine	+	C5 H10 N2 O3	147.0759	0.497	5.08E-04	3.480	84.04458 (10953530) 101.07116 (400909) 112.57800 (256098) 130.04964 (7827806) 147.07579 (272782)	2967 (mzCloud)
L-Phenylalanine	+	C9 H11 N O2	166.08585	2.255	4.10E-04	2.484	103.05431 (180995) 107.04904 (31552) 120.08064 (2327546) 131.04875 (32395) 166.08615 (28407)	8 (mzCloud)
L-Histidine	-	C6 H9 N3 O2	154.06114	0.466	1.06E-03	6.822	78.95803 (7829) 93.04461 (17724) 96.95924 (2607) 110.98306 (7891) 137.03464 (9854) 154.06146 (15558)	473 (mzCloud)
Pyroglutamate	+	C5 H7 N O3	130.0495	0.498	3.46E-04	2.681	56.04991 (1147593) 84.04457 (60139000) 85.02853 (3600213) 130.04961 (16462560)	1831 (mzCloud)
Creatinine	+	C4 H7 N3 O	114.06602	0.516	1.32E-04	1.164	72.04445 (43885) 86.07138 (517373) 114.06604 (5075335) 115.06945 (52813)	375 (mzCloud)
AMP	+	C10 H14 N5 O7 P	348.0689	1.068	1.37E-03	3.951	97.02847 (293650) 119.03502 (40680) 136.06131 (5068331) 250.09258 (6041) 348.06921 (300388)	252 (mzCloud)
IMP	-	C10 H13 N4 O8 P	347.03943	1.318	5.57E-04	1.600	78.95795 (832014) 96.96847 (264402) 135.03011 (151816) 138.97932 (10941) 150.97920 (18203) 192.98981 (4927) 211.00082 (32360) 347.03937 (188306)	452 (mzCloud)

Putative ID Hippocampus	ESI	Formula	m/z	rt	Δ Mass [Da]	Δ Mass [ppm]	MS/MS Fragment (Intensity)	Identifier
UMP	-	C9 H13 N2 O9 P	323.02802	0.952	3.03E-04	0.935	78.95794 (3908848) 96.96848 (1921299) 111.01881 (413424) 138.97920 (156423) 150.97896 (140122) 192.99040 (57262) 211.00058 (281547) 280.02084 (125773) 323.02814 (986284)	1405 (mzCloud)
Adenosine	+	C10 H13 N5 O4	268.10309	1.952	9.37E-04	3.507	92.02445 (7280) 109.05083 (19368) 135.63580 (60255) 136.06136 (37938320) 268.10352 (2302890)	297 (mzCloud)
Adenine	+	C5 H5 N5	136.0614	0.781	4.01E-04	2.969	94.04015 (19085) 109.01020 (3980) 119.03522 (65555) 136.06139 (700796) 137.06464 (10352)	296 (mzCloud)
Inosine	+	C10 H12 N4 O5	269.08713	2.025	8.90E-04	3.322	94.04008 (11449) 110.03481 (27618) 119.03503 (32021) 136.06128 (10655) 137.04535 (2868747)	1234 (mzCloud)
Guanine	+	C5 H5 N5 O	152.05634	1.244	3.91E-04	2.585	109.05067 (12500) 110.03490 (73542) 128.04521 (33436) 134.04561 (4597) 135.02986 (152069) 136.01338 (3026) 152.05626 (825161) 153.04027 (206137)	436 (mzCloud)
Uridine	-	C9 H12 N2 O6	243.06168	1.641	5.32E-04	2.178	66.03380 (52017) 82.02872 (95611) 94.02869 (13652) 110.02361 (359505) 111.01904 (17044) 122.02360 (35150) 124.03923 (15376) 140.03418 (37072) 152.03438 (63228) 153.02966 (25435) 200.05579 (108735) 243.06180 (67361)	1408 (mzCloud)
DL-Malic acid	-	C4 H6 O5	133.01306	0.711	1.12E-03	8.365	71.01279 (129810) 72.99209 (27651) 87.00748 (2163) 89.02341 (7529) 115.00258 (134890) 133.01329 (17939)	1796 (mzCloud)
Choline	+	C5 H13 N O	104.10697	0.520	3.68E-05	0.357	58.06544 (412233) 60.08112 (5030140) 104.10701 (42907310) 105.11051 (339415)	886 (mzCloud)
Acetylcholine	+	C7 H15 N O2	146.11714	0.611	4.19E-04	2.885	60.08110 (100435) 87.04420 (467344) 146.11710 (303644)	885 (mzCloud)
S-Adenosylmethionine	+	C15 H22 N6 O5 S	399.14316	0.485	1.06E-03	2.671	74.02385 (99571) 74.06051 (108351) 84.04464 (79892) 97.02847 (2587430) 102.05499 (1131514) 136.06136 (3961221) 145.03169 (99633) 163.04179 (88410) 232.08234 (23260) 250.09261 (3997892) 264.08871 (210626) 298.09598 (708748) 399.14557 (37313)	896 (mzCloud)

Putative ID Hippocampus	ESI	Formula	m/z	rt	Δ Mass [Da]	Δ Mass [ppm]	MS/MS Fragment (Intensity)	Identifier
Spermidine	+	C7 H19 N3	146.16478	0.365	4.05E-04	2.792	72.08102 (234730) 84.08090 (26529) 112.11225 (102006) 129.13849 (23127)	9 (mzCloud)
γ-Aminobutyric acid	+	C4 H9 N O2	104.07059	0.475	1.72E-04	1.665	68.04982 (235966) 69.03378 (2796529) 86.06027 (2521131) 87.04420 (13690050) 88.04769 (67834) 104.07070 (725285)	796 (mzCloud)
Uric acid	-	C5 H4 N4 O3	167.02002	1.163	1.01E-03	6.009	80.96395 (2139) 96.01920 (40808) 124.01412 (147489) 167.02008 (189653)	753 (mzCloud)
Nicotinamide	+	C6 H6 N2 O	123.05505	1.162	2.27E-04	1.862	78.03405 (25147) 79.05419 (3350) 80.04965 (544907) 81.05305 (4864) 96.04448 (204205) 105.04488 (4283) 106.02873 (63079) 123.05506 (2645485) 124.05951 (41312)	517 (mzCloud)
Putative ID Striatum	ESI	Formula	m/z	rt	Δ Mass [Da]	Δ Mass [ppm]	MS/MS Fragment (Intensity)	Identifier
γ-Glutamylcysteine	+	C8 H14 N2 O5 S	251.06882	0.949	7.75E-04	3.098	76.02179 (71877) 84.04455 (539899) 86.99010 (22744) 102.05510 (13007) 105.00049 (77426) 122.02686 (578442) 130.04958 (303728) 142.03177 (122712) 188.03696 (111468) 233.05818 (12337) 234.04202 (106039) 251.06908 (190576)	427 (mzCloud)
γ-L-Glutamyl-L-glutamic acid	+	C10 H16 N2 O7	277.1022	0.682	7.58E-04	2.747	84.04459 (383115) 85.02856 (14166) 102.05505 (114513) 130.04959 (240801) 148.06000 (361355) 168.06526 (49144) 196.05957 (15074) 214.06999 (31927) 260.07578 (31516) 277.10263 (80568)	1223 (mzCloud)
ADP ribose	-	C15 H23 N5 O14 P2	558.0636	0.847	4.45E-04	0.796	78.95797 (589954) 96.96850 (157642) 150.97948 (12357) 158.92430 (80790) 211.00092 (20022) 272.95782 (8417) 346.05551 (184471) 408.01239 (5145) 558.06329 (44432)	1708 (mzCloud)
Acetyl-L-carnitine	+	C9 H17 N O4	204.12244	0.799	5.76E-04	2.835	60.08109 (176410) 85.02856 (2945815) 144.10155 (131517) 145.04921 (417959) 204.12250 (1271263)	879 (mzCloud)
6β-Prostaglandin I1	-	C20 H34 O5	353.2333	6.225	1.60E-04	0.452	193.12254 (9851) 317.20859 (2821) 353.23349 (63875)	4594 (mzCloud)

Supplementary Table 1. Putatively identified metabolites with MS/MS fragment information.