**Supplementary Information**

# Hypoxanthine is a metabolic biomarker for inducing GSDME-dependent pyroptosis of endothelial cells during ischemic stroke

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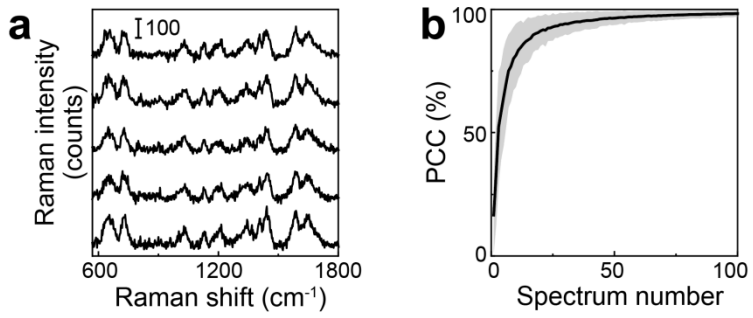


**Figure S1. Estimation of spectrum number per measurement for robust SERS profiling.** (a) Signal fluctuation across successively acquired spectra in one measurement. (b) Pearson's correlation coefficient (PCC) versus the spectrum number per measurement. The black line for the mean value in all samples and the gray shade for the standard deviation (n = 120).

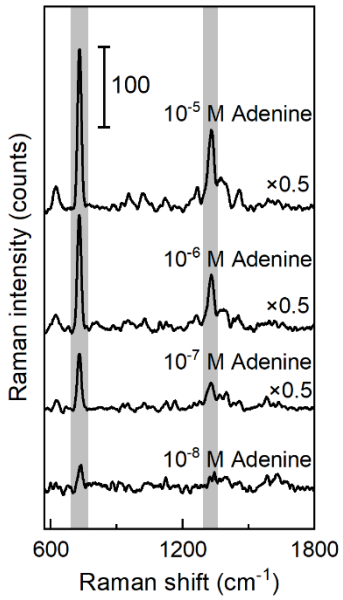


**Figure S2. SERS spectra of adenine at different concentrations.** The intensity of the characteristic peak of adenine as indicated by the grey shade is positively correlated with the concentration, indicating the capability of quantification even down to $10^{-8}$ M. While the peak position remains constant, validating the biomarker identification based on the characteristic peaks.

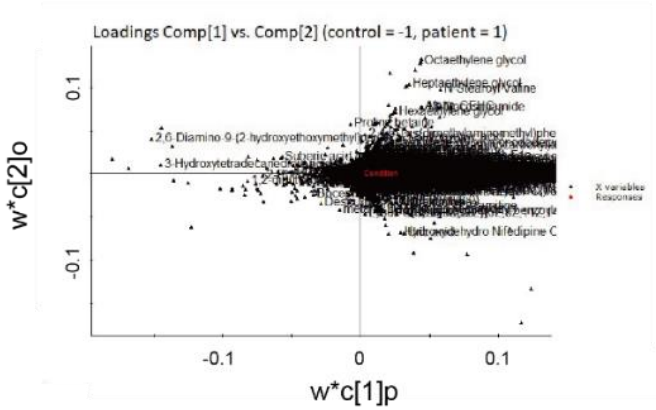


**Figure S3. Permutation test of OPLS-DA in Figure 2k.** The permutation plot displays the correlation coefficient between the original y variable and the permuted y variable on x axis versus the cumulative on the y axis, indicating the effective prediction ability.

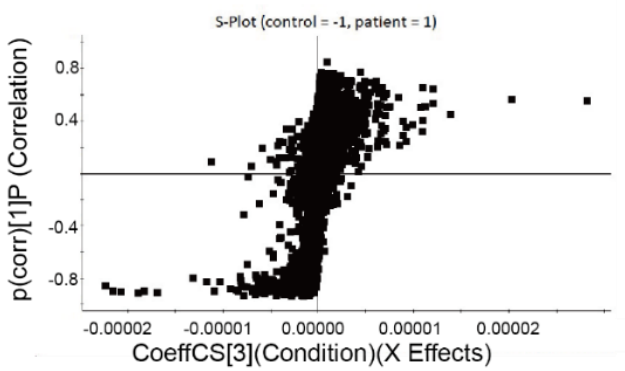


**Figure S4. Metabolite detection of human serum by the conventional HPLC-MS-MS in positive ion mode.** S-plots of the ischemic stroke group.

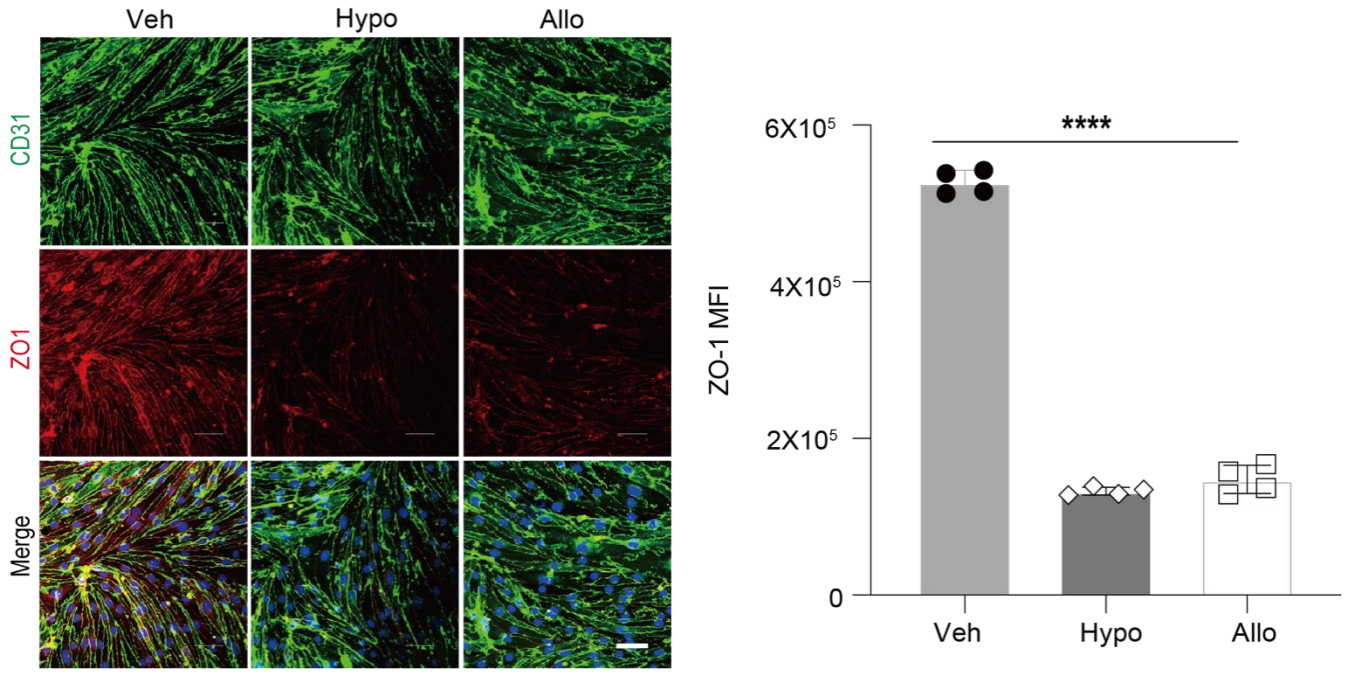


**Figure S5. Hypoxanthine treatment reduced the expression of tight junction protein ZO-1 in endothelial cells.** Immunostaining and quantification of tight junction protein ZO-1 expression in endothelial cells treated with vehicle (Veh), hypoxanthine (Hypo) or allopurinol (Allo). Scale bar, 100 μm. One-way ANOVA with Dunnett's multiple comparisons test. N = 4. Data are mean ± SD. ****: $p < 0.0001$, vs vehicle.

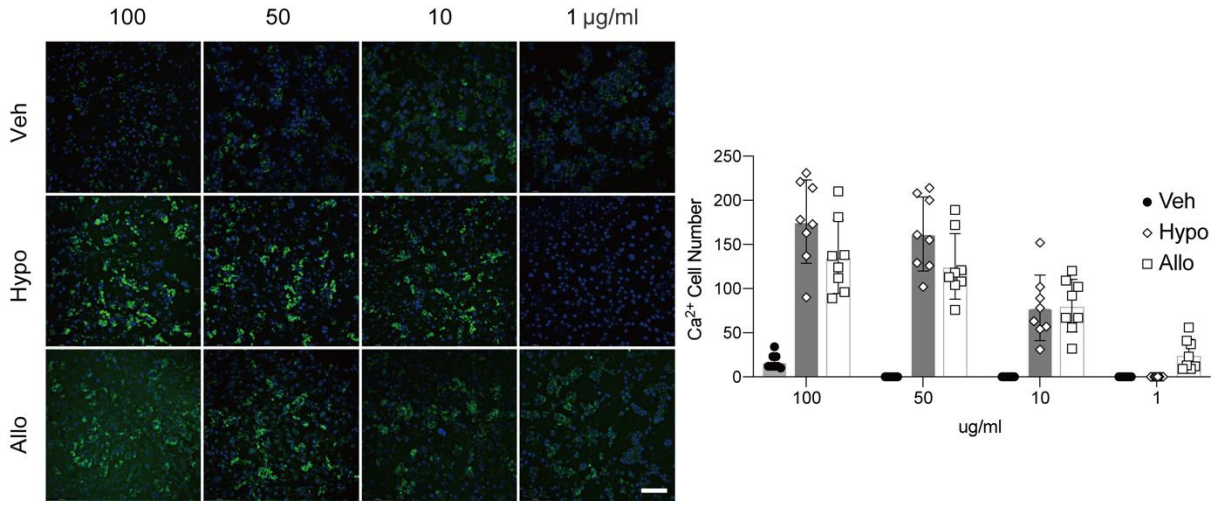


**Figure S6. Hypoxanthine and allopurinol induced intracellular $Ca^{2+}$ accumulation in endothelial cells in a dose-dependent manner.** Representative images of $Ca^{2+}$ signal in bEnd.3 cells treated with different concentrations of vehicle, hypoxanthine, or allopurinol at 24 h after OGD. Scale bar, 100 µm. Two-way ANOVA with Bonferroni multiple comparisons test. N = 8. Data are mean ± SD.

**Table S1. Primer sequence.**

| | |
|---|---|
| β-actin-F | 5’-GGCTGTATTCCCCTCCATCG-3’ |
| β-actin-R | 5’-CCAGTTGGTAACAATGCCATGT-3’ |
| IL-1β-F | 5’-AGAGCCCATCCTCTGTGACT-3’ |
| IL-1β-R | 5’-GCTCATATGGGTCCGACAGC-3’ |
| IL-18-F | 5’-GACTCTTGCGTCAACTTCAAGG-3’ |
| IL-18-R | 5’-CAGGCTGTCTTTTGTCAACG-3’ |
| caspase 3-F | 5’-CTCTGGTTTTCGGTGGGTGT-3’ |
| caspase 3-R | 5’-CTTCCATGTATGATCTTTGGTTCC-3’ |
| GSDMD-F | 5’-TCTGCCCTCAACACTTCTGG-3’ |
| GSDMD-R | 5’-TGCAGCCACAAATAACTCAGC-3’ |
| GSDME-F | 5’-TGCAACTTCTAAGTCTGGTGACC-3’ |
| GSDME-R | 5’-CTCCACAACCACTGGACTGAG-3’ |

**Table S2. Ischemic stroke patients information**

| **Characteristic** | **Stroke (n=51)** | **Control (n=69)** | **P value** |
|---|---|---|---|
| Age, year median ± SD | 74 ±11.309 | 50 ± 8.217 | 0.3192 |
| Sex, male, n (%) | Male, 26 (51) | Male, 40 (58) | 0.8973 |
| **TOAST, n (%)** | LAA, 30(55.6)<br>CE, 15 (27.8)<br>SAO, 8 (14.8) | Ns | Ns |
| **Therapy** | tPA, 29 (57)<br>Thrombectomy, 22 (42) | Ns | Ns |
| **Medical history** | | | |
| Previous stroke, n (%) | 6 (11) | Ns | Ns |
| Hypertension, n (%) | 38 (75) | 30（43） | 0.3506 |
| Diabetes Mellitus, n (%) | 16 (31) | 25（36） | 0.8156 |
| Arial fibrillation, n (%) | 12 (23) | 22（22） | 0.5001 |
| **On admission** | | | |
| NIHSS score, median (SD) | 9 (5.69) | Ns | Ns |

SD (Standard Deviation); TOAST (Trial of Org 10172 in Acute Stroke Treatment); LAA (large-artery atherosclerosis); CE (cardio embolism); SAO (small artery occlusion); SUE (stroke of undemonstrated etiology); tPA (Tissue plasminogen activator); NIHSS (National Institute of Health Stroke scale).

**Table S3. Supplemental materials**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Antibodies** | | |
| Anti- Caspase 3 antibody | Cell Signaling Technology | Cat#9662 |
| Anti- Cleaved Caspase-3 (Asp175) antibody | Cell Signaling Technology | Cat#9661 |
| Anti-cleaved GSDME N-terminal | Abcam | Cat# ab222407 |
| Anti- DFNA5/GSDME Ab antibody | Affinity Biosciences | Cat#AF4016 |
| Anti- IL-1 B/IL1B Ab (B122)antibody | Santa Cruz | Cat#sc-12742 |
| Anti-DFNA5/GSDME Ab antibody | Abcam | Cat#a ab21591 |
| Anti- GSDMDC1 AB (64-Y) antibody | Santa Cruz | Cat# sc-81868 |
| Anti- Caspase-8 (1.1.40) antibody | Santa Cruz | Cat# sc-81656 |
| Anti- Caspase-1 antibody | Santa Cruz | Cat# sc-56036 |
| Anti- Caspase 1 rabbit pAb | Zenbio | Cat#342947 |
| Anti- Cleaved-Caspase 1 rabbit pAb | Zenbio | Cat#341030 |
| Anti- Cleaved-Caspase 3 rabbit pAb | Zenbio | Cat#R23727 |
| Anti- Xanthine Oxidase antibody | Santa Cruz | Cat# sc-398548 |
| Anti-Xanthine Oxidase [EPR4605] | Abcam | Cat#ab109235 |
| Anti-ZO-1antibody | Invitrogen | Cat#61-7300 |
| Anti- CD31 antibody | R&D | Cat#AF3628 |
| Anti-CD31 antibody | R&D | Cat#AF806 |
| horseradish peroxidase-conjugated goat anti-rabbit IgG | HUABIO | Cat#HA1001 |
| horseradish peroxidase-conjugated goat anti-mouse IgG | HUABIO | Cat#HA1006 |
| horseradish peroxidase-conjugated rabbit anti-goat IgG | HUABIO | Cat#HA1005 |
| Second antibodies donkey anti-mouse alexa fluor 594 | Invitrogen | Cat#A21203 |
| Second antibodies donkey anti-goat alexa fluor 647 | Invitrogen | Cat#A21447 |
| **Chemicals, peptides, and recombinant proteins** | | |
| DMEM/F12 | Gibco | Cat#11330-032 |
| DMEM Ca2+ free medium | Gibco | Cat#21068028 |
| Hypoxanthine | Sigma-Aldrich | Cat#H9337 |
| Citrate trisodium (98%) | Aladdin | Cat#S189183 |
| Silver nitrate (AR, 99.8%) | Aladdin | Cat# S116264 |

| | | |
|---|---|---|
| Cysteamine (95%) | Aladdin | Cat# C106461 |
| Phenylalanine (HPLC, > 98.0%) | Aladdin | Cat# P103489 |
| Hypoxanthine (99%) | Aladdin | Cat# H108384 |
| Uric acid (99%) | Aladdin | Cat# U105582 |
| Riboflavin (99.55%) | MedChemExpress | Cat#HY-B0456 |
| Adenine (HPLC, ≥ 99.5%) | Macklin | Cat# A800685 |
| Nicotinic acid (≥ 99%) | Yuanye | S13023 |
| Mass spectrometry metabolite library | Sigma-Aldrich | MSMLS |
| 8-Aminoguanine | Sigma-Aldrich | Cat#SML1856 |
| Allopurinol | Sigma-Aldrich | Cat#A8003 |
| JC-1(Synonyms:CBIC2) | MedChemExpress | Cat#HY-15534 |
| Fluo-3AM | MedChemExpress | Cat#HY-D0716 |
| penicillin/streptomycin | Meilunbio | Cat#MA0110-1 |
| Accutase | STEMCELL | Cat#07920 |
| poly-l-ornithine | Sigma-Aldrich | Cat#L4544 |
| mTeSR™ medium | STEMCELL Technologies | Cat#85850 |
| Matrigel | Corning | Cat#356231 |
| ReleSR | STEMCELL | Cat#100-0484 |
| Y-27632 | STEMCELL Technologies | Cat#72308 |
| Mesodermal induction medium[APEL2] | STEMCELL | Cat#05270 |
| CHIR99021 | STEMCELL | Cat#72054 |
| VEGF | YEASEN | Cat#91502ES10 |
| BMP4 | YEASEN | Cat#92053ES10 |
| bFGF | YEASEN | Cat#91330ES10 |
| EGM medium | Lonza | Cat#CC-3162 |
| Tamoxifen | MedChemExpress | Cat# HY-13757A |
| Tetrazolium chloride | Sigma-Aldrich | Cat#T-8877 |
| TRI reagent | MRC | Cat#TR118 |
| RIPA lysis buffer | EMD Millipore | Cat#20-188 |
| phosphatase inhibitor cocktail | ROCHE | Cat#11697498001 |
| Proteinase inhibitor cocktail | ROCHE | |
| **Critical commercial assays** | | |
| Xanthine/Hypoxanthine Assay | Abcam | Cat#ab155900 |
| Xanthine Oxidase Activity | Abcam | Cat#ab102522 |
| Uric Acid Assay | Abcam | Cat# ab65344 |
| Hifair II 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) | YEASEN | Cat#11123ES60 |
| Hieff qPCR SYBR Green master mix (High rox) | YEASEN | Cat#11203ES08 |
| TRIzol reagent box | Thermo Fisher Scientific | Cat#15596018 |

| | | |
|---|---|---|
| **Experimental models: Organisms** | | |
| C57BL/6J mice | Beijing Charles River | N/A |
| **Software and algorithms** | | |
| Lax | | |
| ImageJ software | ImageJ | N/A |
| Prism v.7 software | GraphPad | N/A |
| Xcalibur 3.0 | Thermo fisher Scientific | N/A |
| Progenesis QI v 2.3 | Waters | N/A |
| Ezinfor V3.0.3 | Umetrics | N/A |
| Python (version 3.9.16) | Python Software Foundation | N/A |
| LabSpec 6 (version 6.4.4.10) | Horiba Scientific | N/A |
| Matlab (version R2022b) | MathWorks | N/A |

**Table S4. Comparison of serum metabolites between ischemic stroke patient and control**

| Accepted Compou | Accepted Description | Anova (p) | q Value | Max Fold Change |
|---|---|---|---|---|
| HMDB0001933 | Furosemide | 0.00048635 | 0.000471154 | 588.8124158 |
| HMDB0255703 | Non-sulfonylurea | 0.00053061 | 0.000510471 | 175.4223065 |
| HMDB0252034 | Ethyl 6-chlorochroman-2-carboxylate | 4.28E-05 | 5.14E-05 | 127.9387857 |
| HMDB0029722 | 1,2,5-Trimethyl-1H-pyrrole | 7.94E-05 | 9.02E-05 | 90.98485273 |
| HMDB0240252 | Salicyluric beta-D-glucuronide | 3.91E-07 | 7.17E-07 | 75.0981604 |
| HMDB0242638 | Doramectin | 5.82E-05 | 6.83E-05 | 63.20475577 |
| HMDB0038738 | Ethyl 3-hydroxyoctanoate O-[glucosyl-(1->6)-glucoside] | 0.00010382 | 0.000115036 | 57.71727194 |
| HMDB0038574 | Lansiumamide A | 9.30E-06 | 1.28E-05 | 57.31824833 |
| HMDB00157 | Hypoxanthine | 2.16E-12 | 8.14E-12 | 51.68461924 |
| HMDB0255805 | Chloro-{methyl-[1-(2-methyl-5,6-dihydro-[1,4]oxathiin-3-yl)-methanoy | 0.00079019 | 0.000731158 | 50.02769421 |
| HMDB0247526 | N(G)-Nitroarginine-4-nitroanilide | 1.28E-11 | 4.41E-11 | 45.11379552 |
| HMDB0062570 | Erythro-5-hydroxy-L-lysinium(1+) | 1.62E-13 | 6.84E-13 | 32.40426589 |
| HMDB0033757 | Marcanine A | 1.47E-05 | 1.94E-05 | 31.3487609 |
| HMDB0038315 | 28-Galloylglucosylpomolate 3-arabinoside | 0.00022175 | 0.000232197 | 30.27811136 |
| HMDB0242124 | Dibutyl sulfosuccinate | 2.92E-07 | 5.47E-07 | 26.71026234 |
| HMDB0257086 | N-(1-Carboxy-3-carboxanilidopropyl)alanylproline | 1.61E-05 | 2.11E-05 | 16.07329022 |
| HMDB0014905 | Benzquinamide | 1.30E-09 | 3.43E-09 | 15.74153395 |
| HMDB0037153 | S-2,5-Dimethyl-3-furanyl 3-methylbutanethioate | 5.68E-12 | 2.04E-11 | 15.44445283 |
| HMDB0246169 | 2-Mercapto-3-phenylthieno[3,2-d]pyrimidin-4(3H)-one | 2.93E-05 | 3.62E-05 | 14.33613768 |
| HMDB0011654 | 2-(3-Carboxy-3-(methylammonio)propyl)-L-histidine | 1.19E-06 | 1.98E-06 | 13.0437765 |
| HMDB0256350 | Perphenazine decanoate | 3.81E-11 | 1.23E-10 | 12.68713325 |
| HMDB0244626 | Desmethyl rizatriptan | 1.49E-06 | 2.41E-06 | 11.65040594 |
| HMDB0245935 | 3-Methylsulfonyl-4-piperidin-1-ylbenzoyl guanidine | 1.11E-16 | 6.05E-16 | 10.78803898 |
| HMDB0255366 | 2-[(3-Iodophenyl)methyl]-1-[[N'-[(3-iodophenyl)methyl]carbamimidoyl] | 0.0004774 | 0.00046346 | 10.70725048 |
| HMDB0033483 | 6,7-Dihydro-4-(hydroxymethyl)-2-(p-hydroxyphenethyl)-7-methyl-5H- | 0.00088677 | 0.000810212 | 10.58700997 |
| HMDB0250537 | Crimidine | 4.67E-13 | 1.89E-12 | 10.34897315 |
| HMDB0010320 | Cortolone-3-glucuronide | 3.10E-11 | 1.01E-10 | 10.01399877 |
| HMDB0249821 | ceritinib | 8.22E-07 | 1.42E-06 | 10.00085324 |
| HMDB0244021 | 1,1-Dichloroheptane | 5.32E-14 | 2.37E-13 | 9.855229195 |
| HMDB0256196 | Chembl4238926 | 5.65E-13 | 2.26E-12 | 9.697804361 |
| HMDB0000739 | Isodesmosine | 9.57E-05 | 0.000106872 | 9.527567954 |
| HMDB0255616 | Niobium(V) oxide | 1.76E-09 | 4.56E-09 | 8.757590741 |
| HMDB0094704 | N-Propionylmethionine | 1.11E-16 | 6.05E-16 | 8.420112093 |
| HMDB0014644 | Cimetidine | 1.11E-16 | 6.05E-16 | 7.917252929 |
| HMDB0005076 | 13,14-Dihydro PGF-1a | 7.32E-14 | 3.21E-13 | 7.872678857 |
| HMDB0256990 | pyroglutamyl-histidyl-glycine | 3.71E-12 | 1.37E-11 | 7.793671825 |
| HMDB0059977 | 4-Hydroxy-5-(dihydroxyphenyl)-valeric acid-O-methyl-O-sulphate | 8.22E-12 | 2.90E-11 | 7.469368416 |
| HMDB0060834 | N-Acetylserotonin sulfate | 1.22E-11 | 4.21E-11 | 7.281641988 |
| HMDB0247747 | Abecarnil | 1.11E-12 | 4.31E-12 | 7.266604938 |
| HMDB0029367 | Sanguinarine | 9.11E-09 | 2.17E-08 | 7.195765477 |
| HMDB0247589 | 9-Deaza-9-(3-thienylmethyl)guanine | 3.10E-11 | 1.01E-10 | 7.141102735 |
| HMDB0249909 | Galactocerebroside | 2.52E-09 | 6.39E-09 | 7.100953573 |
| HMDB0033335 | Antibiotic X 14889C | 4.10E-11 | 1.31E-10 | 7.00717749 |
| HMDB0244433 | Vorozole | 4.74E-10 | 1.33E-09 | 6.966408545 |
| HMDB0258388 | Sorbinil | 1.28E-10 | 3.87E-10 | 6.650528508 |
| HMDB0252020 | Ethoprophos | 7.95E-14 | 3.47E-13 | 6.544754665 |
| HMDB0029367 | Sanguinarine | 6.51E-09 | 1.57E-08 | 6.493889878 |
| HMDB0247498 | Butyryl trihexyl citrate | 2.78E-10 | 8.00E-10 | 6.441786698 |
| HMDB0247875 | (Z)-7-[(1S,4R,6R)-4-[(E)-Oct-6-enyl]-2,3-diazabicyclo[2.2.1]hept-2-en- | 8.00E-11 | 2.48E-10 | 6.398357155 |
| HMDB0252347 | Fluocinolone | 1.48E-05 | 1.96E-05 | 6.385914174 |
| HMDB0241595 | (5Z,8Z,10E,14Z,17Z)-12-Hydroxyicosa-5,8,10,14,17-pentaenoylcarni | 2.43E-05 | 3.06E-05 | 6.336730384 |
| HMDB0259276 | Tris(2-chloroethyl) phosphate | 6.95E-06 | 9.89E-06 | 6.303539505 |
| HMDB0029782 | 6-Epi-7-isocucurbic acid glucoside | 2.33E-14 | 1.07E-13 | 6.269225917 |
| HMDB0246374 | 4-Azidobenzenesulfonamide | 3.33E-16 | 1.76E-15 | 6.195299824 |
| HMDB0303432 | Calcium citrate | 0.00016688 | 0.00017848 | 6.174775172 |
| HMDB0259625 | 2,4-Pyrimidinediamine, 5-(5-(1-piperazinylmethyl)-1,3,4-oxadiazol-2- | 1.94E-14 | 8.98E-14 | 6.170533447 |
| HMDB0246374 | 4-Azidobenzenesulfonamide | 2.55E-11 | 8.48E-11 | 6.16886088 |
| HMDB0248879 | Batelapine | 2.59E-05 | 3.24E-05 | 6.160594357 |
| HMDB0000394 | 3-Hydroxytetradecanedioic acid | 9.99E-16 | 5.14E-15 | 6.125766202 |
| HMDB0259952 | 1-Cycloheptyl-1-(9H-fluoren-2-ylmethyl)-3-(2,4,6-trimethylphenyl)urea | 9.97E-11 | 3.05E-10 | 5.990684565 |
| HMDB0248956 | Benoxaprofen | 1.08E-08 | 2.54E-08 | 5.98503962 |
| HMDB0259712 | urapidil | 1.11E-16 | 6.05E-16 | 5.920339535 |
| HMDB0255529 | Neosalacinol | 2.23E-13 | 9.28E-13 | 5.919354842 |
| HMDB0040824 | Tsangane L 3-glucoside | 1.21E-11 | 4.19E-11 | 5.817481926 |
| HMDB0246720 | 5-(4-Fluorophenyl)-3-(3-nitrophenyl)-1,2,4-oxadiazole | 8.58E-13 | 3.37E-12 | 5.754720603 |
| HMDB0031885 | 6-Hydroxypentadecanedioic acid | 1.08E-12 | 4.21E-12 | 5.727348964 |
| HMDB0060538 | Nordiazepam | 1.07E-10 | 3.28E-10 | 5.657318625 |
| HMDB0254042 | Leupeptin | 1.06E-11 | 3.70E-11 | 5.488533065 |
| HMDB0258508 | Estreptoquinasa | 3.92E-09 | 9.72E-09 | 5.284556616 |
| HMDB0259434 | 4H-Thieno(2,3-b)thiopyran-2-sulfonamide, 5,6-dihydro-6-(3-methoxy | 0.00046725 | 0.00045471 | 5.218390683 |
| HMDB0015150 | Sulfamethoxazole | 1.67E-08 | 3.83E-08 | 5.141039278 |
| HMDB0256398 | Phenazocine | 1.68E-11 | 5.69E-11 | 5.139415331 |
| HMDB0039947 | Pisumionoside | 4.87E-09 | 1.20E-08 | 5.023945225 |
| HMDB0059931 | 2,3-Diacetoxypropyl stearate | 1.14E-07 | 2.29E-07 | 5.011095801 |
| HMDB0012087 | SM(d18:0/18:0) | 1.97E-06 | 3.13E-06 | 4.920443622 |
| HMDB0014769 | Clofarabine | 9.65E-09 | 2.29E-08 | 4.86302871 |
| HMDB0248054 | N-(3-Chlorophenyl)-6,7-dimethoxyquinazolin-4-amine | 2.56E-05 | 3.21E-05 | 4.796348887 |
| HMDB0248566 | 1,3(2H,4H)-Isoquinolinedione, 2-(2-(4-(2-methoxyphenyl)-1-piperazi | 5.80E-11 | 1.82E-10 | 4.791507273 |
| HMDB0033757 | Marcanine A | 2.89E-15 | 1.43E-14 | 4.753547005 |
| HMDB0041946 | N-Nitrosoproline | 1.93E-10 | 5.69E-10 | 4.65400618 |
| HMDB0094708 | Tetraethylene glycol | 9.29E-09 | 2.20E-08 | 4.623660124 |
| HMDB0059609 | albendazole S-oxide | 9.37E-08 | 1.91E-07 | 4.559820672 |
| HMDB0029887 | Sorbitan palmitate | 2.42E-10 | 7.03E-10 | 4.528262602 |
| HMDB0032660 | (2Z,4'Z)-2-(5-Methylthio-4-penten-2-ynylidene)-1,6-dioxaspiro[4.4]no | 1.11E-08 | 2.60E-08 | 4.474122729 |
| HMDB0242753 | Vertilmicin | 9.86E-11 | 3.02E-10 | 4.461910364 |
| HMDB0259640 | Benzonitrile, 3-amino-4-((2-((dimethylamino)methyl)phenyl)thio)- | 2.22E-16 | 1.19E-15 | 4.44949376 |
| HMDB0247381 | H-Arg(NO2)-Obzl | 3.33E-16 | 1.76E-15 | 4.438178874 |
| HMDB0303294 | Sodium decylbenzenesulfonate | 0.00029675 | 0.000303084 | 4.410913229 |
| HMDB0304801 | Ile-Val-Val | 1.62E-07 | 3.17E-07 | 4.395228641 |
| HMDB0304214 | 5-hydroxy-2-oxo-4-ureido-2,5-dihydro-1H imidazole-5-carboxylate | 1.89E-15 | 9.52E-15 | 4.37227215 |
| HMDB0254628 | Methyldopate | 3.45E-05 | 4.20E-05 | 4.292747842 |
| HMDB0032740 | N-Hexadecanoylpyrrolidine | 0.0003834 | 0.000380525 | 4.26403845 |
| HMDB0029367 | Sanguinarine | 1.65E-06 | 2.64E-06 | 4.223441177 |
| HMDB0242731 | Arteether | 6.35E-10 | 1.75E-09 | 4.211254162 |
| HMDB0032959 | 1-Octen-3-yl glucoside | 1.45E-06 | 2.37E-06 | 4.209389482 |
| HMDB0010320 | Cortolone-3-glucuronide | 2.41E-06 | 3.75E-06 | 4.157546813 |
| HMDB0246686 | 2-(4-Amino-2-chlorophenyl)-2-phenylacetonitrile | 8.15E-08 | 1.69E-07 | 4.109561525 |
| HMDB0257893 | D-Phe-L-Pro-L-Arg-(Chloromethyl) ketone | 3.47E-06 | 5.25E-06 | 4.016594789 |

**Table S4. Mass spectrometry identified distinct serum metabolites between ischemic stroke patient and control** (ANOVA, $p < 0.05$, fold change >4, OPSLSDA mode VIP >1, coefficient of variation (CV) <30%)

# Table S5. Comparison of metabolites between tMCAO and control

| Accepted Compound | Accepted Description | Anova (p) | q Value | Max Fold Chang |
|---|---|---|---|---|
| HMDB0246184 | LysoPC | 2.97E-11 | 1.72E-10 | 37.84162681 |
| HMDB00157 | Hypoxanthine | 8.50E-07 | 7.24E-07 | 20.13678571 |
| HMDB0032305 | N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide | 4.44E-16 | 5.03E-15 | 15.16865249 |
| HMDB0032807 | Niazirin | 0 | 0 | 14.81950769 |
| HMDB0304915 | 2-(4-Methoxyphenyl)ethyl hydrogen sulfate | 0 | 0 | 13.93617529 |
| HMDB0060017 | Pyrogallol-2-O-glucuronide | 3.36E-12 | 2.19E-11 | 10.0253476 |
| HMDB0240444 | 3-Methylgallic acid 5-sulfate | 0 | 0 | 8.736265284 |
| HMDB0252413 | Fluroxene | 0 | 0 | 7.547056851 |
| HMDB06317 | trans-Hexadec-2-enoyl carnitine | 0 | 0 | 7.335896696 |
| HMDB05066 | Tetradecanoylcarnitine | 0 | 0 | 7.294610977 |
| HMDB02302 | 3-Indolepropionic acid | 3.65E-10 | 1.82E-09 | 6.932168296 |
| HMDB05066 | Tetradecanoylcarnitine | 0 | 0 | 6.750587765 |
| HMDB02005 | Methionine sulfoxide | 0 | 0 | 6.373197406 |
| HMDB02014 | cis-5-Tetradecenoylcarnitine | 0 | 0 | 6.054708103 |
| HMDB0244869 | 1H-1,2,4-Triazole-3-carboxamide | 0 | 0 | 6.019038576 |
| HMDB00696 | L-Methionine | 0 | 0 | 5.792575532 |
| HMDB01830 | Progesterone | 0 | 0 | 5.789676533 |
| HMDB02183 | Docosahexaenoic acid | 0 | 0 | 5.734095832 |
| HMDB00222 | L-Palmitoylcarnitine | 0 | 0 | 5.520786596 |
| HMDB0032369 | L-Menthyl acetoacetate | 0 | 0 | 5.176578666 |
| HMDB03312 | Daidzein | 1.81E-05 | 4.74E-05 | 4.831319683 |
| HMDB00222 | L-Palmitoylcarnitine | 0 | 0 | 4.817864744 |
| HMDB00651 | Decanoylcarnitine | 9.77E-14 | 7.90E-13 | 3.993656393 |
| HMDB00549 | Gamma-Butyrolactone | 0 | 0 | 3.931542412 |
| HMDB00824 | Propionylcarnitine | 5.55E-16 | 6.17E-15 | 3.858051084 |
| HMDB00532 | Acetylglycine | 0 | 0 | 3.826942408 |
| HMDB03312 | Daidzein | 1.81E-09 | 8.20E-09 | 3.661825007 |
| HMDB0242752 | 6-Methylthioinosine | 0.013426 | 0.017192 | 3.555867934 |
| HMDB04620 | N-a-Acetyl-L-arginine | 0 | 0 | 3.476938929 |
| HMDB0033679 | Pelargonidin 3-sophoroside | 0.003255 | 0.005078 | 3.414373717 |
| HMDB11628 | Glycyrrhetinic acid | 0.030999 | 0.034582 | 3.321408117 |
| HMDB0247731 | Pentaleno(1,6a-c)pyran-5-carboxylic acid, 1-(chloromethyl)-1,2,4,4a,6a,7-hexahydro-1-hydroxy-7,8-dimeth | 0 | 0 | 3.250240432 |
| HMDB01358 | Retinal | 0 | 0 | 2.920665612 |
| HMDB0248182 | Aloxistatin | 0 | 0 | 2.855392917 |
| HMDB0011155 | PA(P-16:0/18:2(9Z,12Z)) | 3.71E-09 | 1.62E-08 | 2.763217454 |
| HMDB02088 | N-Oleoylethanolamine | 0 | 0 | 2.760627419 |
| HMDB00162 | L-Proline | 0 | 0 | 2.721266639 |
| HMDB05045 | 15(S)-Hydroxyeicosatrienoic acid | 0 | 0 | 2.699661526 |
| HMDB00630 | Cytosine | 0 | 0 | 2.577363033 |
| HMDB06406 | Ecgonine methyl ester | 1.11E-16 | 1.36E-15 | 2.573102315 |
| HMDB00848 | Stearoylcarnitine | 0 | 0 | 2.463812696 |
| HMDB00526 | 5a-Tetrahydrocortisol | 1.19E-14 | 1.09E-13 | 2.444152079 |
| HMDB0254486 | Mesulergine | 0 | 0 | 2.380445216 |
| HMDB00014 | Deoxycytidine | 9.82E-12 | 6.02E-11 | 2.331847291 |
| HMDB00721 | Glycylproline | 0 | 0 | 2.330325402 |
| HMDB00210 | Pantothenic acid | 0 | 0 | 2.282453164 |
| HMDB03464 | 4-Guanidinobutanoic acid | 1.07E-09 | 4.99E-09 | 2.207425943 |
| HMDB00273 | Thymidine | 5.75E-11 | 3.19E-10 | 2.165495926 |
| HMDB02183 | Docosahexaenoic acid | 3.19E-14 | 2.71E-13 | 2.140799218 |
| HMDB0245977 | 3-Propyl-1H-pyrazole | 2.23E-05 | 5.78E-05 | 2.110894024 |
| HMDB10391 | LysoPC(20:1(11Z)) | 3.33E-16 | 3.87E-15 | 2.100295069 |
| HMDB0032369 | L-Menthyl acetoacetate | 3.33E-16 | 3.87E-15 | 2.095794455 |
| HMDB00205 | Phenylpyruvic acid | 0 | 0 | 2.03813274 |
| HMDB11718 | 4-Hydroxybenzaldehyde | 0 | 0 | 2.014165724 |
| HMDB00089 | Cytidine | 4.64E-14 | 3.91E-13 | 1.989578973 |
| HMDB0257361 | RUCAPARIB | 1.33E-13 | 1.06E-12 | 1.989057877 |
| HMDB00036 | Taurocholic acid | 0.000244 | 0.000517 | 1.92639328 |
| HMDB0257242 | Rislenemdaz | 0 | 0 | 1.908893558 |
| HMDB03357 | N-Acetylornithine | 0 | 0 | 1.905533393 |
| HMDB04827 | Proline betaine | 1.33E-10 | 7.03E-10 | 1.905380572 |
| HMDB0028887 | Histidylhistidine | 1.39E-13 | 1.10E-12 | 1.868926681 |
| HMDB03337 | Oxidized glutathione | 0.013558 | 0.017313 | 1.849489643 |
| HMDB0242455 | (2-((3,5-Di-t-butyl-4-hydroxyphenyl)thio)-1-methylpropoxy)acetic acid | 2.80E-09 | 1.25E-08 | 1.783920521 |
| HMDB00630 | Cytosine | 7.33E-15 | 6.85E-14 | 1.783012352 |
| HMDB0303202 | Calcium oleate | 5.94E-12 | 3.76E-11 | 1.765939304 |
| HMDB10390 | LysoPC(20:0) | 1.45E-11 | 8.74E-11 | 1.731046553 |
| HMDB10392 | LysoPC(20:2(11Z,14Z)) | 1.52E-08 | 6.04E-08 | 1.730144617 |
| HMDB10399 | LysoPC(22:1(13Z)) | 2.88E-06 | 8.51E-06 | 1.710024451 |
| HMDB0258894 | Tetraglyme | 0.000585 | 0.001126 | 1.688142339 |
| HMDB0247995 | Androstane-3,17-diol 17-sulfate | 1.16E-09 | 5.39E-09 | 1.673623638 |
| HMDB0248697 | Atevirdine | 1.26E-09 | 5.83E-09 | 1.663703726 |
| HMDB0257361 | RUCAPARIB | 7.00E-09 | 2.93E-08 | 1.601790448 |
| HMDB10407 | LysoPC(P-16:0) | 9.76E-10 | 4.59E-09 | 1.601619737 |
| HMDB0240598 | LysoPE(P-18:0/0:0) | 8.71E-08 | 3.18E-07 | 1.600137868 |
| HMDB00630 | Cytosine | 2.70E-12 | 1.78E-11 | 1.595443544 |
| HMDB0256571 | Pipequaline | 7.16E-10 | 3.42E-09 | 1.594029939 |
| HMDB00517 | L-Arginine | 9.37E-07 | 2.96E-06 | 1.565056597 |
| HMDB0005076 | 13,14-Dihydro PGF-1a | 0 | 0 | 1.55599257 |
| HMDB03229 | Palmitoleic acid | 4.23E-13 | 3.15E-12 | 1.527138706 |
| HMDB00929 | L-Tryptophan | 8.08E-09 | 3.35E-08 | 1.523817777 |
| HMDB11738 | Gamma-Glutamylglutamine | 0.000214 | 0.000458 | 1.516337559 |
| HMDB0000734 | Indoleacrylic acid | 7.82E-09 | 3.26E-08 | 1.513546669 |
| HMDB10401 | LysoPC(22:4(7Z,10Z,13Z,16Z)) | 1.03E-05 | 2.82E-05 | 1.494166904 |
| HMDB0032740 | N-Hexadecanoylpyrrolidine | 0.001781 | 0.003012 | 1.480650114 |
| HMDB10397 | LysoPC(20:5(5Z,8Z,11Z,14Z,17Z)) | 0.000146 | 0.000325 | 1.446103937 |
| HMDB00688 | Isovalerylcarnitine | 1.29E-06 | 4.00E-06 | 1.433225589 |
| HMDB00064 | Creatine | 3.94E-13 | 2.94E-12 | 1.431932095 |
| HMDB01406 | Niacinamide | 0.000199 | 0.000428 | 1.429724651 |
| HMDB0255383 | 2-Hexadec-7-enylicosa-8,11-dienedioic acid | 8.45E-09 | 3.48E-08 | 1.374633444 |
| HMDB0034625 | Vinaginsenoside R2 | 1.36E-12 | 9.39E-12 | 1.34409516 |
| HMDB00086 | Glycerophosphocholine | 0.016565 | 0.020478 | 1.336686582 |
| HMDB0246853 | DhInl | 2.59E-14 | 2.26E-13 | 1.320675299 |
| HMDB0303802 | Campesterol glucoside | 3.52E-07 | 1.20E-06 | 1.320211401 |
| HMDB0243890 | 1-O-Hexadecyl-sn-glycero-3-phosphocholine | 4.86E-05 | 0.000119 | 1.290822925 |
| HMDB0033641 | (3b,21b)-12-Oleanene-3,21,28-triol 28-[arabinosyl-(1->3)-arabinosyl-(1->3)-arabinoside] | 3.87E-06 | 1.13E-05 | 1.287380051 |
| HMDB10384 | LysoPC(18:0) | 2.47E-07 | 8.51E-07 | 1.286140236 |
| HMDB0249823 | Cerivastatin lactone | 4.47E-09 | 1.94E-08 | 1.277002039 |
| HMDB10384 | LysoPC(18:0) | 2.48E-08 | 9.68E-08 | 1.274356141 |
| HMDB0012109 | 5,6-Dihydroxyprostaglandin F1a | 1.38E-12 | 9.55E-12 | 1.273620911 |
| HMDB00251 | Taurine | 1.03E-05 | 2.83E-05 | 1.26175498 |

**Table S5. Mass spectrometry identified distinct serum metabolites between tMCAO mice and control** (ANOVA, $p < 0.05$, fold change >1.25, OPSLSDA mode VIP >1, coefficient of variation (CV) <30%)