

## SUPPORTING INFORMATION

### **Metabolomics analysis elucidates unique influences on purine / pyrimidine metabolism by xanthine oxidoreductase inhibitors in a rat model of renal ischemia-reperfusion injury**

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## SUPPLEMENTARY MATERIALS AND METHODS

### *Drugs*

Febuxostat (2-[3-cyano-4-isobutoxyphenyl]-4-methyl-1,3-thiazole-5-carboxylic acid; CAS: 144060-53-7) and allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine; CAS: 315-30-0) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and topiroxostat (4-(5-pyridin-4-yl-1H-[1,2,4] triazol-3-yl)pyridine-2-carbonitrile) was kindly provided by Fuji Yakuhin (Saitama, Japan). Each XOR inhibitor (5 mg) was pulverized in a mortar and suspended in a small amount of 0.5% methylcellulose. Subsequently, 0.5% methylcellulose was added up to 10 ml.

### *Standards*

For quantitative high-performance liquid chromatography (HPLC) and CE-TOFMS analyses, ATP disodium salt hydrate (CAS: 34369-07-8, F.W. 551.14, 27-1006-01; GE healthcare, Waukesha, WI, USA), ADP sodium salt (CAS: 20398-34-9, F.W. 427.20, A2754; Sigma-Aldrich), AMP disodium salt (CAS: 18422-05-4, F.W. 365.24, A2252; Sigma-Aldrich), hypoxanthine (CAS: 68-94-0, H9377; Sigma-Aldrich), xanthine (CAS: 69-89-6, 241-00013; Wako Pure Chemical Industries, Osaka, Japan), uric acid (CAS: 69-93-2, U2625; Sigma-Aldrich), oxypurinol (CAS: 2465-69-0, O6881; Sigma-Aldrich), and allopurinol (CAS: 315-30-0, A8003; Sigma-Aldrich) were used as standards.

### *Animals*

Male Sprague–Dawley rats weighing 180–210 g (CLEA Japan Inc., Tokyo, Japan) were housed in standard cages with wood chip bedding at constant ambient temperature (21–22°C) and humidity (40–50%) with a 12-h light cycle. All animals had free access to tap water and the assigned diet. Prior to the start of the study, all mice were acclimatized for 7 days.

### *Measurement of purine nucleotide concentration by HPLC*

Kidney extract (100 µl) was centrifugally concentrated and resuspended in 100 µL of buffer A (50 mM potassium phosphate buffer, pH 6.0). Samples were filtered through a 0.22-µm Millipore filter (Merck KGaA, Darmstadt, Germany), and 10 µl was injected into a HPLC system (ÄKTApurifier UPC 10; GE Healthcare UK/Amersham, Little Chalfont, Buckinghamshire, UK). A reverse-phase column (Supelcosil LC-18-T, 250 × 4.6 mm, 5 µm;

Sigma-Aldrich, Bellefonte, PA, USA) protected with a guard column (Supelguard LC-18-T, 20 × 4.0 mm; Sigma-Aldrich) was used. For peak analysis and quantification, UNICORN version 5.0 (GE Healthcare) was used. Chromatograms were obtained at wavelengths of 250, 260, and 295 nm. Buffer A and buffer B (50 mM potassium phosphate buffer and 10% methanol) were prepared in deionized water and filtered through a 0.22-μm filter before use. The flow rate was 1.3 ml/min, and the gradient profile was as follows: 9 min in 100% buffer A, 15 min to 25% buffer B, 17.5 min to 90% buffer B and maintained until 23.5 min, and 35 min to 0% buffer B. The run was maintained at 100% buffer B for an additional 3 min before it was completed and the gradient was returned to 100% buffer A. Concentrations were calculated by comparing sample peak areas with standard peak areas. Standard curves were drawn using 3.125 μM to 200 μM standard solutions prepared in buffer A. All concentrations are expressed as nmol/g wet weight.

#### *Measurement of metabolites*

Samples (600 μl each) were added to 240 μL of Milli-Q water containing internal standards (Solution ID: H3304-1002; Human Metabolome Technologies, Tsuruoka, Japan) on ice to inactivate enzymes. The mixture (800 μl) was filtered through a Millipore 5-kDa cutoff filter at 9,100 ×g and 4°C for 60 min to remove proteins. The filtrate was centrifugally concentrated and resuspended in 50 μL of Milli-Q water for CE-MS. Metabolome measurements were performed at Human Metabolome Technologies.

CE-TOFMS was performed on an Agilent CE Capillary Electrophoresis System equipped with an Agilent 6210 TOF mass spectrometer, Agilent 1100 isocratic HPLC pump, Agilent G1603A CE-MS adapter kit, and Agilent G1607A CE-ESI-MS sprayer kit (Agilent Technologies, Waldbronn, Germany). The systems were controlled by Agilent i ChemStation software version B.03.01 for CE (Agilent Technologies, Waldbronn, Germany). The metabolites were analyzed on a fused silica capillary (50 μm *i.d.* × 80 cm total length), with commercial electrophoresis buffer (Solution ID: H3301-1001 for cation analysis and H3302-1021 for anion analysis; Human Metabolome Technologies) as the electrolyte. The sample was injected at a pressure of 50 mbar for 10 s (~10 nL) in cation analysis and 25 s (~25 nL) in anion analysis. The scan range was *m/z* 50–1,000. Other conditions were as described previously (1-3).

#### *Data processing and analysis*

Peaks were extracted using MasterHands software (Keio University, Tsuruoka, Japan) to obtain peak information, including  $m/z$ , migration time for CE-TOFMS measurement (MT), and peak area (4, 5). Signal peaks corresponding to isotopomers, adduct ions, and other product ions of known metabolites were excluded, and the remaining peaks were annotated to putative metabolites from the HMT metabolite database based on their migration time (MT) and  $m/z$  values determined by TOFMS. The tolerance range for peak annotation was set at  $\pm 0.5$  min for MT and  $\pm 10$  ppm for  $m/z$ . In addition, the MT was normalized to those of internal standards, and peaks were aligned according to the  $m/z$  values and normalized MT values. Finally, peak areas were normalized to those of the internal standards, methionine sulfone and D-camphor-10-sulfonic acid, for cations and anions, respectively. The resultant relative area values were further normalized to sample amount. Annotation tables were produced by CE-TOFMS measurement of standard compounds and were aligned with the datasets according to similar  $m/z$  values and normalized MT values. The relative peak areas were calculated using the following equation:

$$\text{Relative peak area} = \frac{\text{Metabolite peak area}}{\text{Internal standard peak area} \times \text{Sample amount}}$$

The peak detection limit was determined based on the signal-noise ratio: S/N = 3.

### *Western blotting*

We followed the method used in our previous reports, with minor modifications (6, 7). Frozen tissue samples were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.8) containing 0.4 mM EDTA and complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). Tissue lysates were centrifuged (10 min, 4°C, 20,670  $\times g$ ), and the supernatants were used for western blotting and XOR activity assay. The total protein concentration was determined using a DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, USA). After boiling at 95°C for 5 min, 10  $\mu g$  of each protein sample was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Mini-PROTEAN TGX 4–15%, Bio-Rad Laboratories), and bands were electrophoretically transferred to a 0.2- $\mu m$ -thick polyvinylidene fluoride membrane (Trans-Blot Turbo; Bio-Rad Laboratories). The membrane was blocked in 5% skim milk TBS-T (50 mM Tris-buffered saline with 0.05% Tween-20) for 1 h. After washing with TBS-T, polyclonal rabbit antibody (6) was added for 1 h (1:1,000). The membrane was washed and incubated with horseradish peroxidase-conjugated polyclonal goat anti-rabbit

immunoglobulin antibody (1:10,000; Dako Denmark A/S, Glostrup, Denmark) for 1 h. Signals were visualized with an ECL kit (GE Healthcare, Fairfield, CT, USA), and analyzed with the ChemiDoc XRS Plus System with Image Lab SVP Software (Bio-Rad Laboratories).

#### *Quantitative reverse-transcription (qRT-)PCR*

RNA was extracted from frozen tissue samples using an RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The RNA concentration was determined on a NanoDrop 2000 (Thermo Scientific, Delaware, ME, USA). Complementary DNA was generated with oligo-dT primers using the ReverTra qPCR RT Kit (Toyobo Co., Osaka, Japan) according to the manufacturer's protocol. qPCR amplification was carried out using the TaqMan Fast Advanced Master Mix (Life Technologies, Carlsbad, CA, USA) and TaqMan gene expression assay probes in reaction mixtures of 20  $\mu$ L. Probes for 18S rRNA (Rn01428913\_gH), xanthine dehydrogenase (Rn00567654\_m1), adenine phosphoribosyl transferase (Rn01432775\_m1), HGPRT 1 (Rn01527840\_m1), and kidney injury molecule 1 (Rn00597703\_m1) were purchased from Life Technologies. The TaqMan probes have a FAM fluorochrome reporter tag at the 5'-end and an MGB quencher at the 3'-end. Amplification was performed in 96-well optical plates on an ABI 7500 Fast Real-Time PCR System (Life Technologies), with one cycle at 95°C for 20 s, followed by 50 cycles at 95°C for 3 s and 60°C for 30 s. Each sample was analyzed in triplicate. Products were analyzed using the manufacturer's software (SDS 1.1). Gene expression was normalized to levels of 18S rRNA as an internal control and expressed as fold increases using the  $\Delta\Delta C_t$  method.

1. Soga T, Heiger DN. Amino acid analysis by capillary electrophoresis electrospray ionization mass spectrometry. *Analytical chemistry*. 2000;72(6):1236-41.
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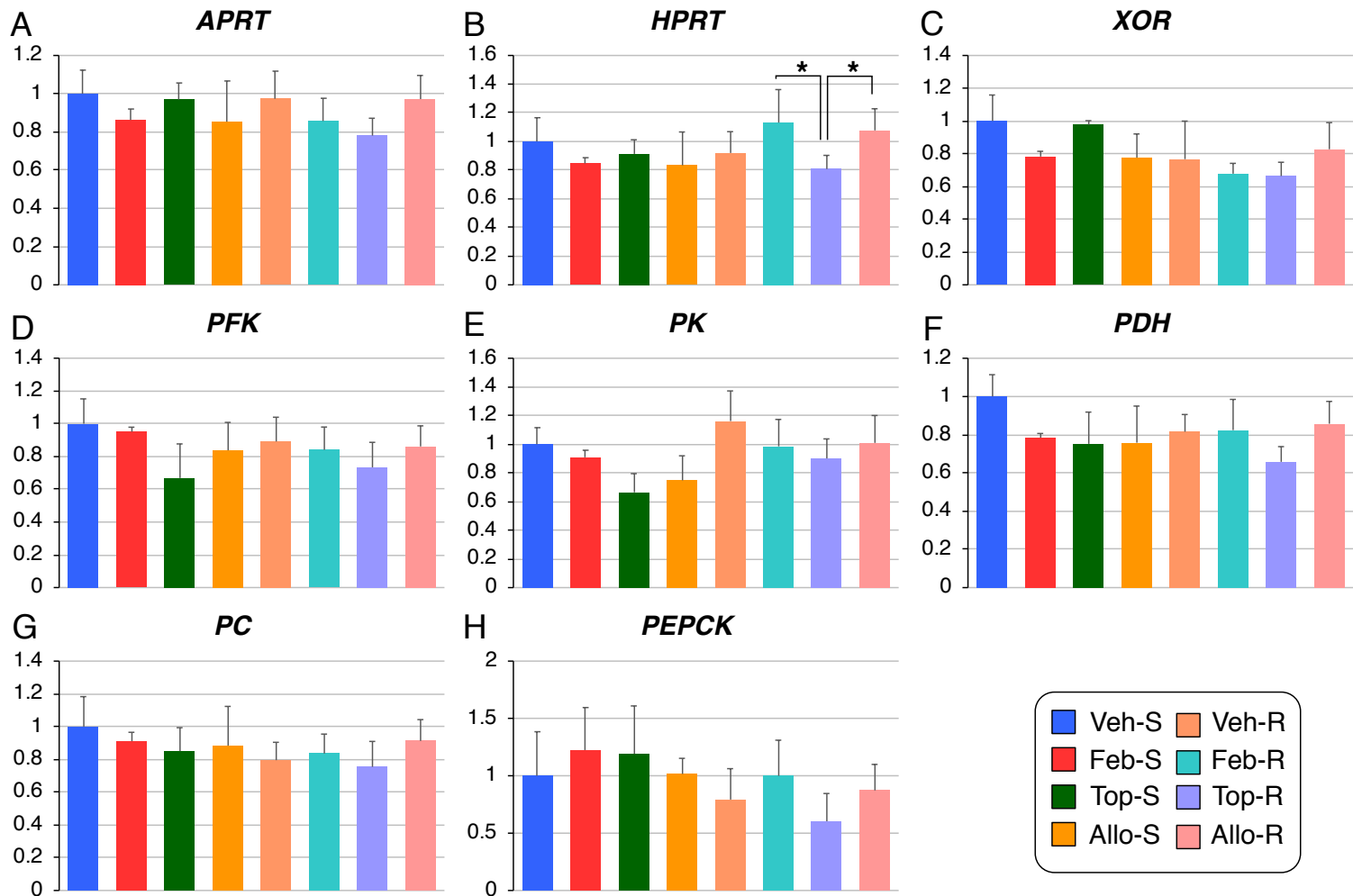
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**Figure S1** Gene expression of key enzymes for purine metabolism.

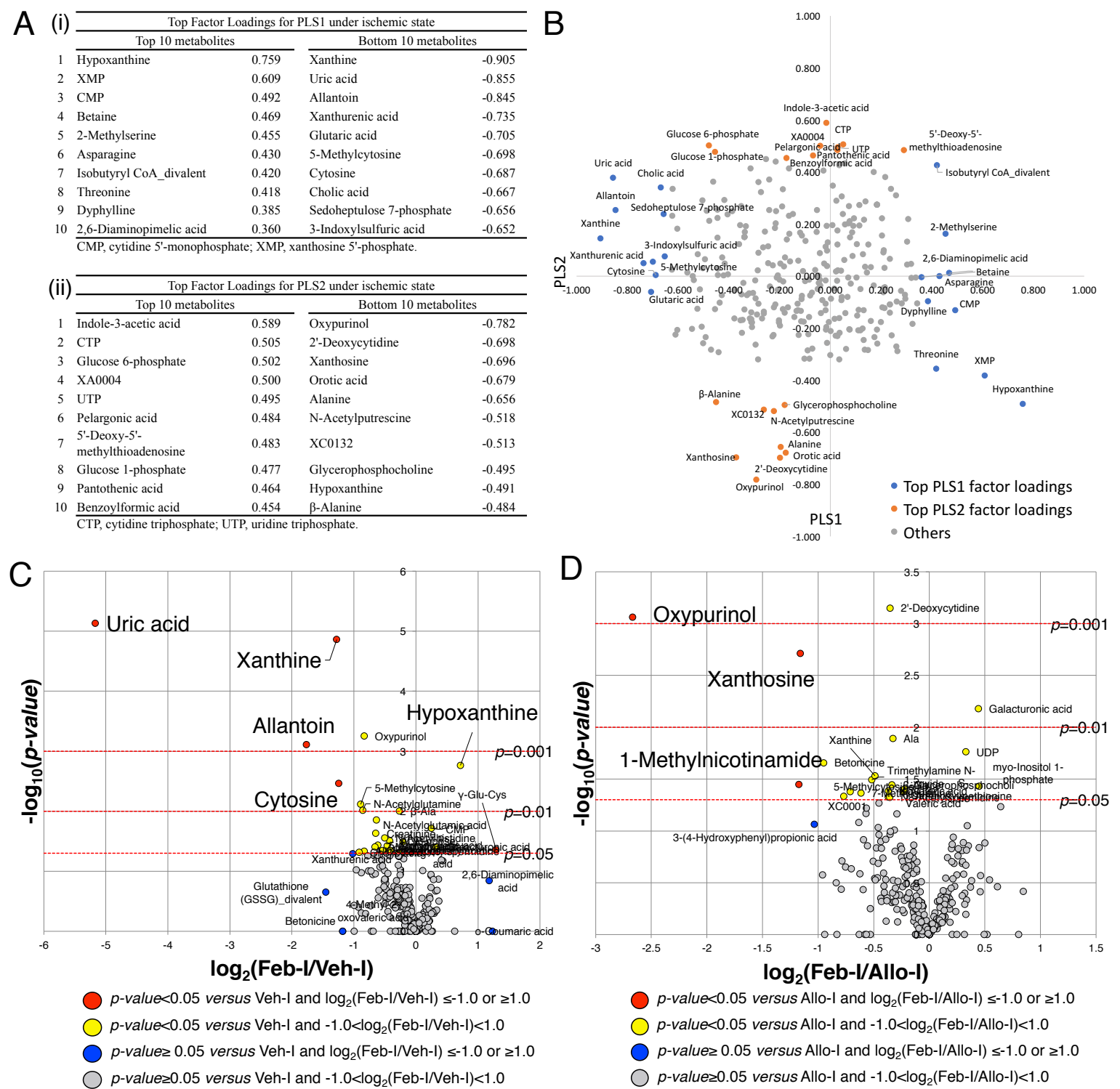


We evaluated the mRNA expression of key enzymes in the stationary and reperused phases: **(A)** mRNA expression levels of adenine phosphoribosyltransferase (*APRT*), **(B)** hypoxanthine phosphoribosyltransferase (*HPRT*), **(C)** xanthine oxidoreductase (*XOR*), **(D)** phosphofructokinase (*PFK*), **(E)** pyruvate kinase (*PK*), **(F)** pyruvate dehydrogenase kinase (*PDH*), **(G)** pyruvate carboxylase (*PC*), and **(H)** phosphoenolpyruvate carboxykinase (*PEPCK*) were determined by qPCR in triplicate, normalized to 18S rRNA levels, and expressed relative to expression levels in the Veh-S group, set as 1. **(B)** Gene expression of *HPRT* was decreased in the Top-R group as compared to the Feb-R and Allo-R groups. No other significant differences within groups in the stationary and reperused phases were observed. Data represent the mean  $\pm$  SEM (n = 5–6). \* $P < 0.05$  \*\* $P < 0.01$ ; non-repeated measures ANOVA.



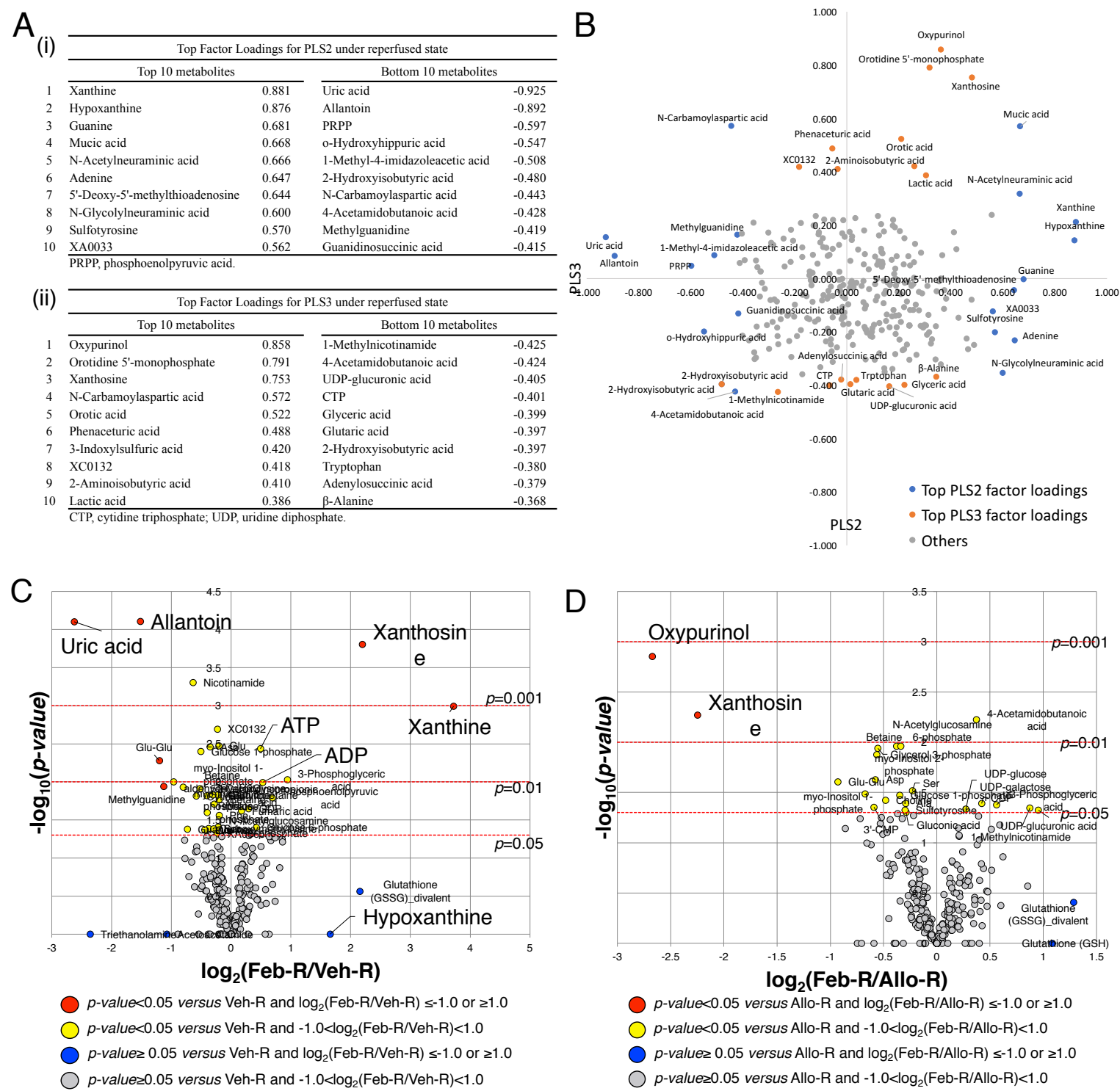


**Figure S4** Top/Bottom Factor Loadings for PLS1/2 and Volcano plot analysis under ischemic state.



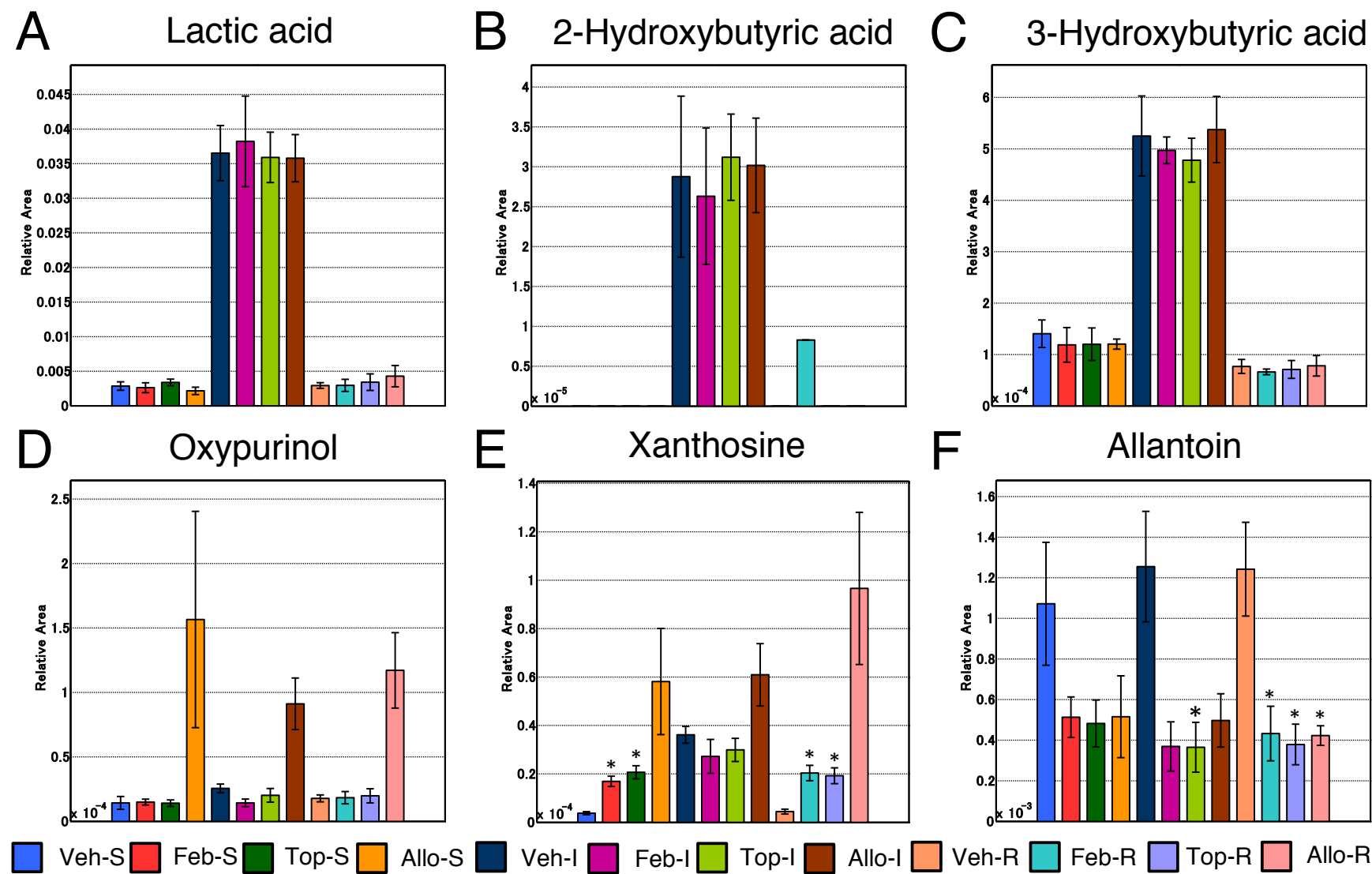
(A) Top/Bottom 10 factor loadings for PLS1 and PLS2 under ischemic state by partial least squares with rank order of groups (PLS-ROG) analysis. (B) The loadings bi-plot of the top ranked loadings with the PLS-ORG scores. (C) In the volcano plot, more than two-fold decreases in cytosine, xanthine, allantoin, and uric acid levels in the Feb-I group as compared to the Veh-I group were observed. (D) The Feb-I showed more than two-fold decreases in xanthosine, oxypurinol, and 1-methylnicotinamide levels as compared to the Allo-I group.

**Figure S5** Top/Bottom Factor Loadings for PLS2/3 and Volcano plot analysis under reperfed state.



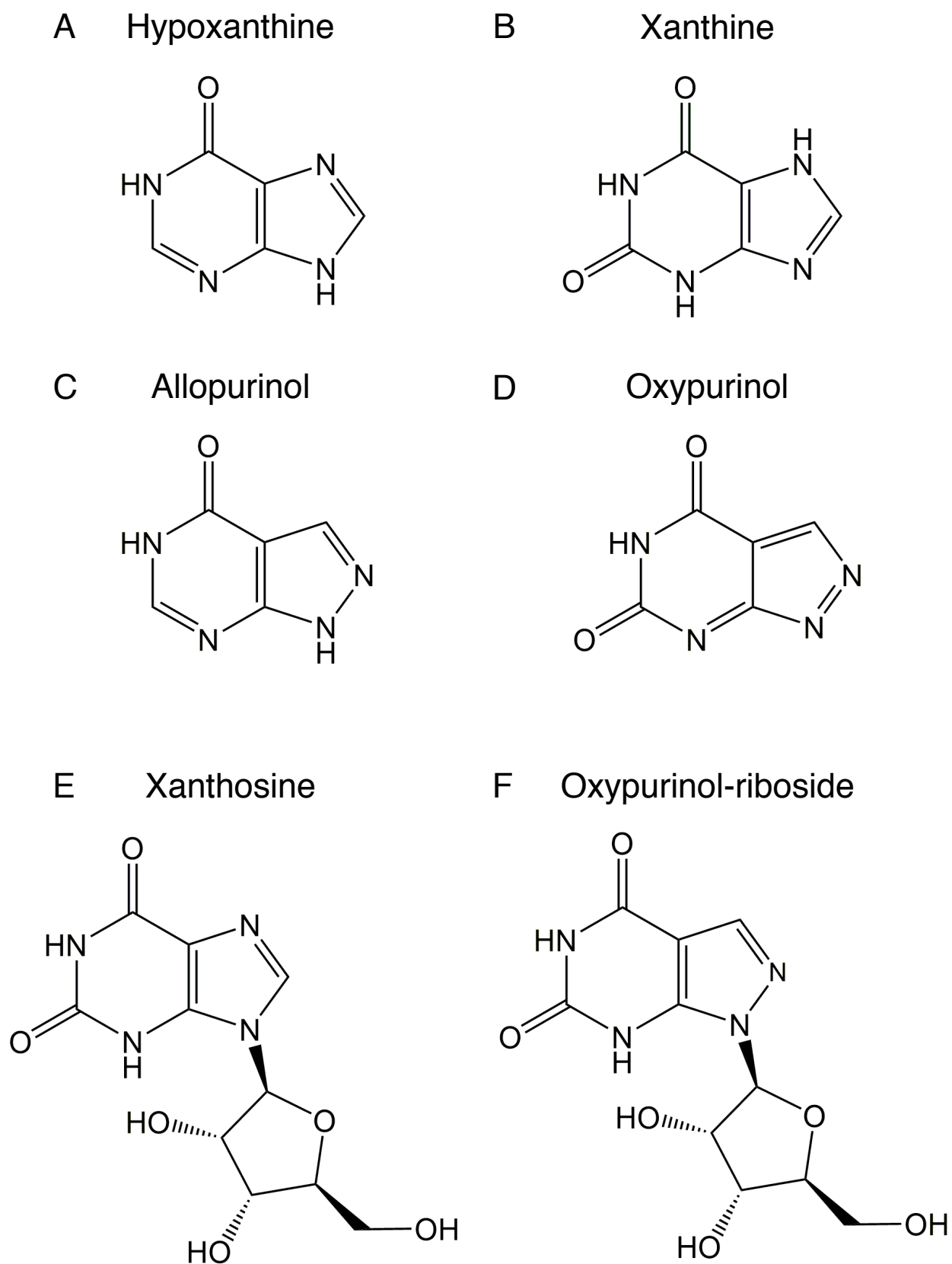
**(A)** Top/Bottom 10 factor loadings for PLS2 and PLS3 under reperfed state by partial least squares with rank order of groups (PLS-ROG) analysis. **(B)** The loadings bi-plot of the top ranked loadings with the PLS-ORG scores. **(C)** In the volcano plot, more than two-fold increases/decreases in xanthosine, xanthine, allantoin, and uric acid levels in the Feb-R group compared to the Veh-R group were observed. **(D)** The Feb-R group showed more than two-fold decreases in xanthosine and oxypurinol as compared to Allo-R.

**Figure S6** Relative peak areas of metabolites in kidney lysates as analyzed by CE-TOFMS.



(A,B,C) Lactic acid, 2-hydroxybutyric acid, 3-hydroxybutyric acid levels were greatly increased during ischemia with no significant differences between groups. (D) Oxypurinol tended to be increased only in allopurinol-administrated groups. (E) Levels of xanthosine tended to be increased by XOR inhibitor administration, especially allopurinol, at all time points: This may be due to the co-existence of oxypurinol-riboside in the renal tissue, which is an isomer of xanthosine and the metabolite of allopurinol. (F) Levels of allantoin showed tendency of decreases by XOR inhibitor administration at all time points. *q*-value was quantified by Benjamini-Hochberg procedure and was used for the statistical cut-off of significance: \**q* < 0.05 versus Veh group.

**Figure S7** Structural similarities between allopurinol-associated metabolites and purine metabolites.



Structural similarities between hypoxanthine, xanthine, allopurinol and oxypurinol (**A,B,C,D**), and xanthosine and allopurinol riboside (**E,F**) are shown.

Table S1 Concentrations of purine nucleotide as measured by HPLC system

|                  | Stationary |                          |                          |                          | Ischemic   |                         |                       |                         | Reperfused |                          |                        |                          |
|------------------|------------|--------------------------|--------------------------|--------------------------|------------|-------------------------|-----------------------|-------------------------|------------|--------------------------|------------------------|--------------------------|
|                  | Veh-S      | Feb-S                    | Top-S                    | Allo-S                   | Veh-I      | Feb-I                   | Top-I                 | Allo-I                  | Veh-R      | Feb-R                    | Top-R                  | Allo-R                   |
| Energy Charge(%) | 73.9 ±5.5  | 74.0 ±2.1                | 76.4 ±2.2                | 75.1 ±4.6                | 38.5 ±5.5  | 38.0 ±7.4               | 34.4 ±3.8             | 33.0 ±3.5               | 68.7 ±5.2  | 69.2 ±1.4                | 70.3 ±6.0              | 68.7 ±2.2                |
| TAN              | 1293 ±158  | 1503 ±161 <sup>**</sup>  | 1485 ±43 <sup>*</sup>    | 1187 ±53 <sup>##</sup>   | 349 ±74    | 341 ±111                | 349 ±62               | 357 ±84                 | 455 ±68    | 650 ±128 <sup>*</sup>    | 599 ±138               | 551 ±41                  |
| ATP              | 744 ±61    | 889 ±115 <sup>**</sup>   | 908 ±30 <sup>**</sup>    | 711 ±39 <sup>##</sup>    | 70.4 ±10.1 | 64.7 ±6.9               | 63.8 ±8.7             | 56.8 ±4.6               | 238 ±38    | 341 ±68 <sup>*</sup>     | 316 ±68                | 284 ±21                  |
| ADP              | 410 ±102   | 448 ±31                  | 451 ±33 <sup>#</sup>     | 357 ±39                  | 121 ±28    | 114 ±19                 | 109 ±36               | 116 ±31                 | 148 ±32    | 218 ±47                  | 199 ±56                | 188 ±18                  |
| AMP              | 140 ±59    | 166 ±36                  | 126 ±26                  | 119 ±50                  | 157 ±49    | 162 ±94                 | 176 ±37               | 184 ±54                 | 69.4 ±18.0 | 91.5 ±16.4               | 82.8 ±42.6             | 79.2 ±12.2               |
| Hypoxanthine     | N.D        | N.D                      | 12.5 ±15.3 <sup>**</sup> | N.D <sup>#</sup>         | 620 ±23    | 1025 ±101 <sup>**</sup> | 1060 ±52 <sup>*</sup> | 999 ±55 <sup>**</sup>   | N.D        | 8.7 ±10.7                | 9.8 ±12.0              | 5.7 ±11.4                |
| Xanthine         | N.D        | 73.8 ±50.0 <sup>**</sup> | 68.0 ±19.5 <sup>**</sup> | 122.8 ±45.5 <sup>*</sup> | 466 ±9     | 195 ±52 <sup>**</sup>   | 201 ±28 <sup>*</sup>  | 240 ±32 <sup>**</sup>   | N.D        | 92.7 ±18.5 <sup>**</sup> | 111 ±27 <sup>**</sup>  | 89.5 ±21.6 <sup>**</sup> |
| Uric acid        | 83.5 ±31.2 | 5.58 ±4.2 <sup>**</sup>  | 0.73 ±0.9 <sup>**</sup>  | 3.85 ±1.3 <sup>*</sup>   | 363 ±25    | 11.0 ±3.3 <sup>**</sup> | 3.8 ±1.3 <sup>*</sup> | 10.5 ±1.5 <sup>**</sup> | 87.9 ±12.7 | 13.5 ±4.9 <sup>**</sup>  | 3.7 ±0.8 <sup>**</sup> | 13.7 ±2.2 <sup>**</sup>  |

Energy Charge and total adenine nucleotide were calculated by the following formula; energy charge =ATP+0.5ADP/ATP+ADP+AMP, TAN=ATP+ADP+AMP. Concentrations are expressed as nmol/g wet weight; the data are represented as the mean ± SEM (n=5~6); N.D: not detected. \*,  $p<0.05$  and \*\*,  $p<0.01$  versus Vehicle group; #,  $p<0.05$  and ##,  $p<0.01$  versus Allopurinol group; all  $p$ -values are evaluated by non-repeated measures ANOVA.

Table S2 Concentrations of purine nucleotide as measured by CE-TOF MS.

|                  | Stationary |                |               |               | Ischemic   |              |              |              | Reperused  |             |                  |            |
|------------------|------------|----------------|---------------|---------------|------------|--------------|--------------|--------------|------------|-------------|------------------|------------|
|                  | Veh-S      | Feb-S          | Top-S         | Allo-S        | Veh-I      | Feb-I        | Top-I        | Allo-I       | Veh-R      | Feb-R       | Top-R            | Allo-R     |
| Energy Charge(%) | 75.2 ±5.4  | 78.8 ±1.9      | 78.2 ±2.6     | 78.6 ±5.7     | 40.0 ±7.1  | 38.5 ±6.7    | 35.5 ±5.5    | 33.8 ±4.7    | 76.6 ±4.6  | 75.3 ±2.5   | 76.2 ±6.8        | 74.7 ±4.0  |
| TAN              | 1476 ±222  | 1612 ±105      | 1494 ±134     | 1433 ±291     | 387 ±124   | 385 ±160     | 403 ±90      | 407 ±97      | 487 ±86    | 699 ±109 ** | 607 ±162 *       | 613 ±88    |
| TAN'             | 792 ±332   | 783 ±140       | 891 ±96       | 945 ±181      | 2147 ±118  | 2033 ±309    | 2124 ±103    | 2132 ±91     | 635 ±96    | 501 ±157    | 833 ±229 \$      | 608 ±13    |
| TAN+TAN'         | 2268 ±235  | 2395 ±96       | 2384 ±88      | 2378 ±132     | 2535 ±120  | 2418 ±231    | 2527 ±100    | 2538 ±55     | 1122 ±67   | 1200 ±85    | 1440 ±167 **\$## | 1221 ±66   |
| ATP              | 908 ±55    | 1067 ±22 ***   | 972 ±64       | 948 ±75       | 89 ±18.2   | 85 ±17.8     | 78 ±9.1      | 73 ±4.0      | 310 ±49    | 435 ±54 **  | 385 ±67          | 377 ±38 *  |
| ADP              | 402 ±89    | 407 ±54        | 392 ±38       | 358 ±124      | 132 ±33    | 126 ±43      | 131 ±21      | 128 ±21      | 127 ±20    | 182 ±30 *   | 154 ±47          | 163 ±27 *  |
| AMP              | 166 ±78    | 138 ±29        | 129 ±32       | 127 ±93       | 166 ±73    | 174 ±99      | 195 ±60      | 205 ±72      | 50.4 ±17.5 | 81.2 ±24.8  | 67.6 ±48.4       | 73.4 ±24   |
| dATP             | 1.41 ±0.5  | 1.27 ±0.1      | 1.27 ±0.2     | 1.37 ±0.3     | N.D        | N.D          | N.D          | N.D          | 0.80 N.A   | N.D         | 1.09 ±0.3        | N.D        |
| PRPP             | 2.30 ±1.8  | N.D            | N.D           | N.D           | N.D        | N.D          | N.D          | N.D          | 5.19 ±3.6  | N.D         | N.D              | N.D        |
| Adenosine        | 215 ±118   | 96.4 ±32.2     | 222 ±40       | 190 ±71       | 56.3 ±24.0 | 65.6 ±40.9   | 84.3 ±53.8   | 88.8 ±49.9   | 156 ±38    | 133 ±44     | 185 ±51          | 133 ±20    |
| Adenine          | 3.73 ±0.9  | 3.72 ±1.17     | 4.17 ±0.3     | 3.07 ±0.5     | 3.83 ±1.4  | 4.36 ±1.2    | 4.73 ±0.5    | 4.00 ±0.6    | 1.09 ±0.2  | 1.39 ±0.3   | 1.86 ±0.4 **     | 1.55 ±0.5  |
| Inosine          | 434 ±280   | 508 ±161       | 503 ±140      | 541 ±184      | 530 ±113   | 611 ±223     | 589 ±117     | 538 ±158     | 369 ±105   | 215 ±122    | 484 ±223         | 329 ±22    |
| IMP              | 41.4 ±24.4 | 43.6 ±13.7     | 38.7 ±9.4     | 32.3 ±16.6    | 76.3 ±27.5 | 73.1 ±34.6   | 90.0 ±19.9   | 90.1 ±27.9   | 17.0 ±9.2  | 24.4 ±5.5   | 19.7 ±16.5       | 18.5 ±4.0  |
| Hypoxanthine     | 12.7 ±4.7  | 51.1 ±10.4 *** | 52.8 ±9.9 *** | 51.3 ±9.3 *** | 652 ±37    | 1073 ±138 ** | 1151 ±59 *** | 1160 ±34 *** | 6.8 N.A    | 21.3 ±6.1   | 26.8 ±6.3        | 22.7 ±7.3  |
| Xanthine         | N.D        | 73.8 ±50.0     | 68.0 ±19.5    | 123 ±45.5     | 466 ±8.9   | 195 ±53      | 201 ±28      | 240 ±32      | N.D        | 92.7 ±18.5  | 111 ±27.0        | 89.5 ±21.6 |
| Uric Acid        | 84 ±31     | 5.6 ±4.2       | 0.7 ±0.9      | 3.9 ±1.3      | 363 ±24.7  | 11.0 ±3.3    | 3.8 ±1.3     | 10.5 ±1.5    | 87.9 ±12.7 | 13.5 ±4.9   | 3.7 ±0.8         | 13.7 ±2.2  |

Energy charge, total adenine nucleotide and TAN' were calculated by the following formula; energy charge =ATP+0.5ADP/ATP+ADP+AMP, TAN=ATP+ADP+AMP and TAN' = dATP+phosphoribosyl diphosphate(PRPP)+adenosine+adenine+inosine+inosine monophosphate(IMP)+hypoxanthine+xanthine+uric acid. Concentrations are expressed as nmol/g wet weight; the data are represented as the mean ± SEM (n=5-6); N.D: not detected. \*,  $P<0.05$ , \*\*,  $P<0.01$  and \*\*\*,  $P<0.001$  versus Vehicle group; \$,  $P<0.05$  versus Febuxostat group; #,  $P<0.05$  and ##,  $P<0.01$  versus Allopurinol group; all  $P$ -values are evaluated by non-repeated measures ANOVA.

Table S3. Top/Bottom Factor Loadings for PC1 (Figure 5B)

|    | Top 20 metabolites     |       | Bottom 20 metabolites           |        |
|----|------------------------|-------|---------------------------------|--------|
| 1  | Ornithine              | 0.104 | L-Glutamine                     | -0.102 |
| 2  | L-Isoleucine           | 0.103 | CTP                             | -0.100 |
| 3  | Uracil                 | 0.103 | 5'-Deoxy-5'-methylthioadenosine | -0.099 |
| 4  | O-Succinylhomoserine   | 0.102 | Mucic acid                      | -0.097 |
| 5  | Lactic acid            | 0.102 | GTP                             | -0.096 |
| 6  | L-Leucine              | 0.102 | 4-Methyl-2-oxovaleric acid      | -0.094 |
| 7  | L-Tryptophan           | 0.102 | S-Adenosylmethionine            | -0.093 |
| 8  | L-Phenylalanine        | 0.102 | CDP                             | -0.092 |
| 9  | Succinic acid          | 0.101 | ATP                             | -0.089 |
| 10 | S-Adenosylhomocysteine | 0.101 | Lauric acid                     | -0.088 |
| 11 | N-Acetylgalactosamine  | 0.101 | Citric acid                     | -0.088 |
| 12 | Isobutyric acid        | 0.101 | Glyceric acid                   | -0.088 |
| 13 | Hypoxanthine           | 0.101 | Argininosuccinic acid           | -0.088 |
| 14 | L-Valine               | 0.101 | Fumaric acid                    | -0.088 |
| 15 | L-Asparagine           | 0.100 | Phosphoenolpyruvic acid         | -0.087 |
| 16 | 2-Hydroxybutyric acid  | 0.100 | Malic acid                      | -0.086 |
| 17 | 3-Hydroxybutyric acid  | 0.100 | Glucuronic acid                 | -0.086 |
| 18 | 5-Hydroxylysine        | 0.099 | Glucose 6-phosphate             | -0.086 |
| 19 | Thiamine               | 0.098 | UTP                             | -0.085 |
| 20 | L-Methionine           | 0.098 | Cysteinesulfinic acid           | -0.085 |

ATP, adenosine triphosphate; CDP, cytidine 5'-diphosphate; CTP, cytidine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate.

Table S4. Top/Bottom Factor Loadings for PC2 (Figure 5B)

| Top 20 metabolites |                                 |       | Bottom 20 metabolites              |        |
|--------------------|---------------------------------|-------|------------------------------------|--------|
| 1                  | NAD <sup>+</sup>                | 0.120 | Kynurenine                         | -0.088 |
| 2                  | XA0033                          | 0.117 | Citrulline                         | -0.080 |
| 3                  | UDP-glucose                     | 0.115 | 2-Oxoglutaric acid                 | -0.073 |
| 4                  | UDP-N-acetylgalactosamine       | 0.113 | N-Acetylalanine                    | -0.072 |
| 5                  | Ethanolamine phosphate          | 0.112 | 1-Methyladenosine                  | -0.071 |
| 6                  | N-Acetylneuraminic acid         | 0.112 | Pyridoxal                          | -0.067 |
| 7                  | Cystathionine                   | 0.111 | N-Carbamoylaspartic acid           | -0.065 |
| 8                  | NADP <sup>+</sup>               | 0.110 | Dyphylline                         | -0.064 |
| 9                  | N-Glycolylneuraminic acid       | 0.106 | N1-Methyl-4-pyridone-5-carboxamide | -0.063 |
| 10                 | O-Acetylhomoserine              | 0.106 | Phosphocreatine                    | -0.061 |
| 11                 | L-Aspartic acid                 | 0.105 | XC0126                             | -0.056 |
| 12                 | Daminozide                      | 0.104 | 4-Aminohippuric acid               | -0.056 |
| 13                 | 3-Hydroxy-3-methylglutaric acid | 0.102 | 1-Methylnicotinamide               | -0.053 |
| 14                 | Cysteic acid                    | 0.101 | Lauric acid                        | -0.052 |
| 15                 | Isoglutamic acid                | 0.098 | 1-Pyrroline-5-carboxylic acid      | -0.050 |
| 16                 | Creatine                        | 0.098 | N6-Methyllysine                    | -0.049 |
| 17                 | 7-Methylguanine                 | 0.098 | Ergothioneine                      | -0.046 |
| 18                 | Gulonic acid                    | 0.097 | myo-Inositol 2-phosphate           | -0.045 |
| 19                 | Sulfotyrosine                   | 0.096 | Nicotinamide                       | -0.042 |
| 20                 | 4-Acetamidobutanoic acid        | 0.096 | 2-Oxoisovaleric acid               | -0.041 |

UDP, uridine diphosphate. XA0033 and XC0126 are undetermined peaks, but candidate metabolic molecules are predicted as follows. XA0033, cytidine; XC0126, N-acetylneuraminic acid.