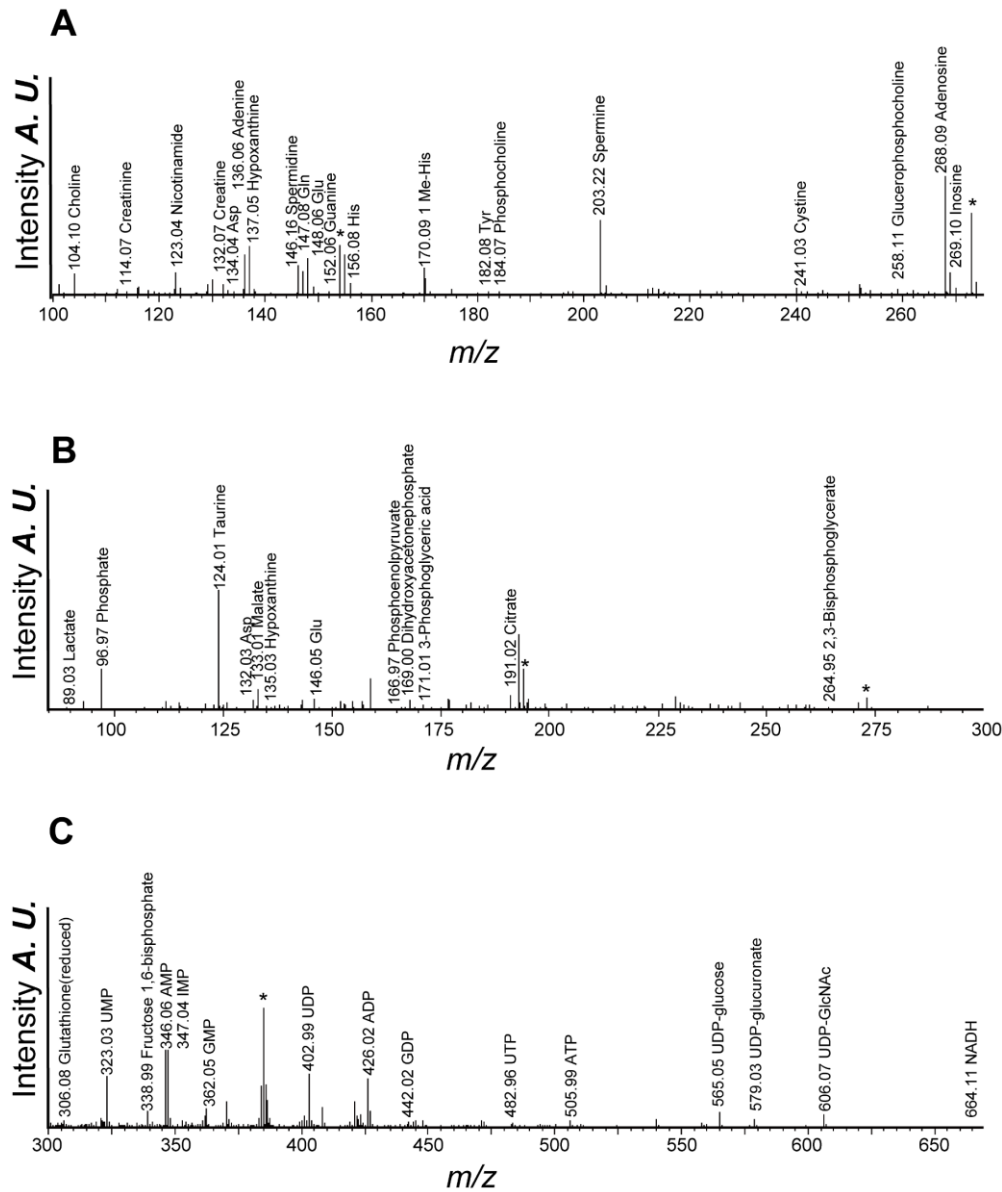


## Supplementary information.

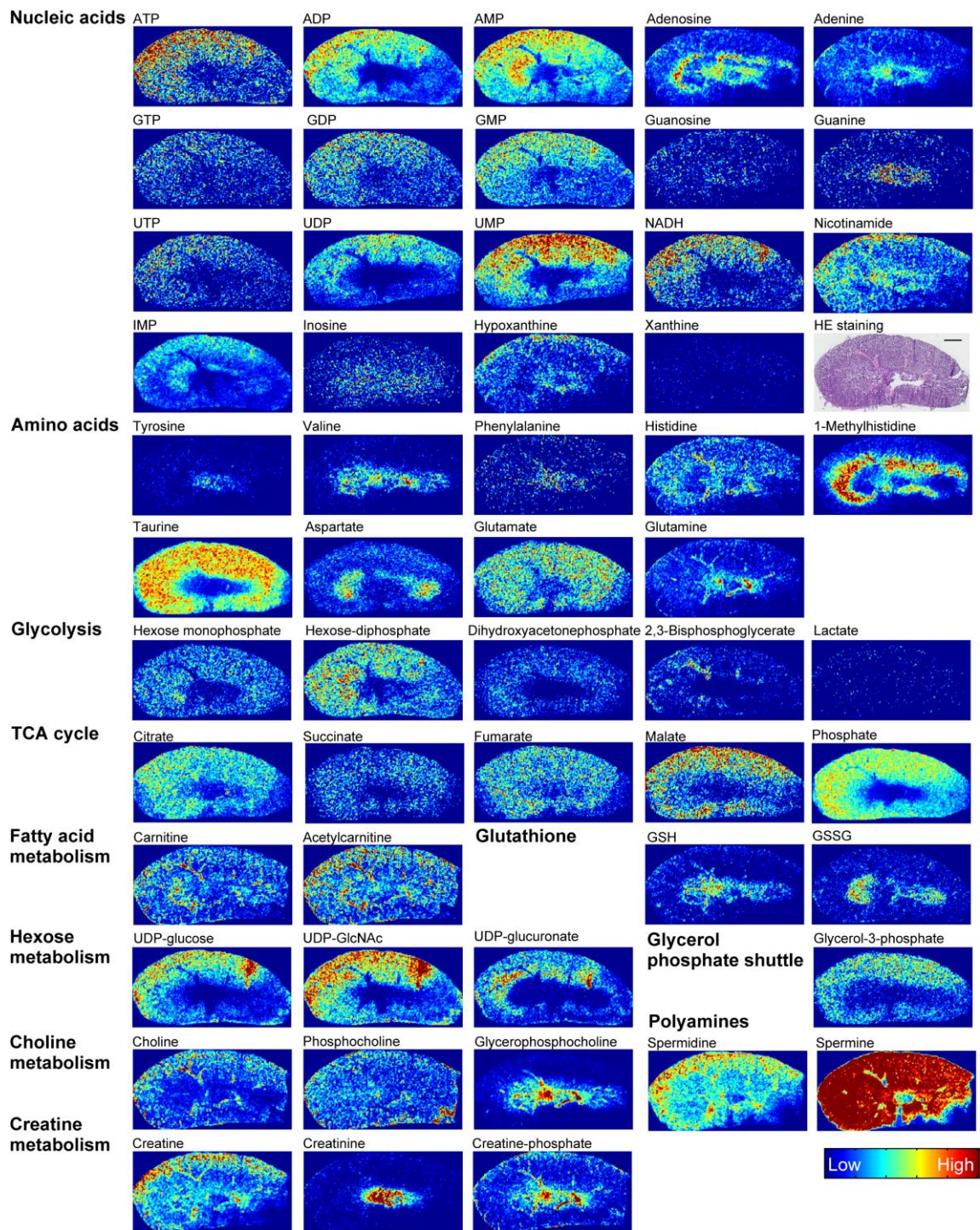
Supplementary Figures and Tables are provided as Supplementary information.

### [Supplementary Figures]



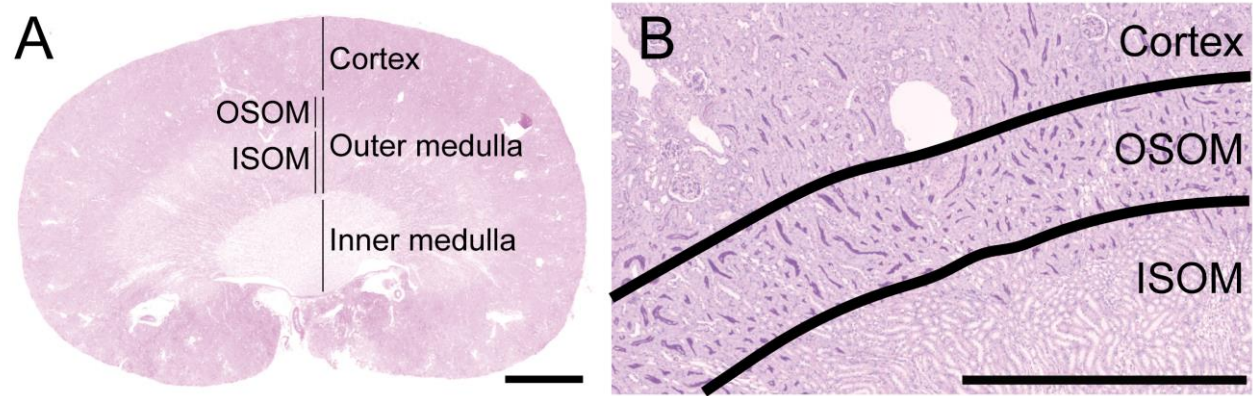
Supplementary Figure 1. Mass spectrum of murine kidney.

(A) A positive ion mode ( $m/z$  100 to  $m/z$  270) mass spectrum of murine kidney. (B) A negative ion mode ( $m/z$  85 to  $m/z$  300) mass spectrum of murine kidney. (C) A negative ion mode ( $m/z$  300 to  $m/z$  670) mass spectrum of murine kidney. Peaks labeled with asterisks are matrix-origin artifacts.



Supplementary Figure 2. Mass spectrometric images of murine kidney

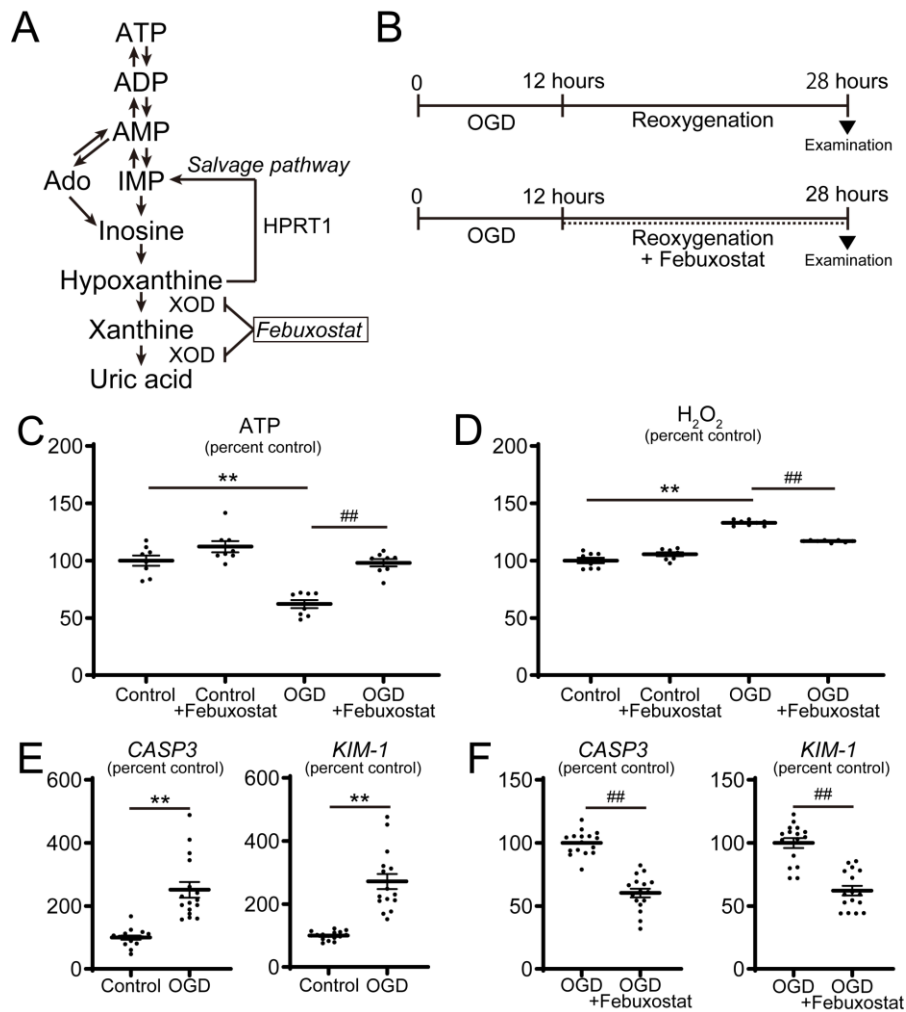
Images were obtained from three sequential sections, two of which were for negative ion mode and another was for positive ion mode. IMP, inosine monophosphate. GSH, reduced glutathione. GSSG, oxidized glutathione. Scale bar: 1mm



	whole	cortex	OSOM	ISOM	IM
Volume (mm <sup>3</sup> )	181 ± 8.4	124 ± 5.9	32.8 ± 3.1	23.4 ± 1.8	3.8 ± 0.2
Relative volume (%)	100	68.5 ± 2.38	18.0 ± 1.55	12.9 ± 0.65	2.1 ± 0.19

Supplementary Figure 3. A PAS-stained tissue sections of murine kidney

A PAS-stained tissue section of murine kidney is shown at (A) a low-power field, and (B) a high-power field. The region of OSOM is outlined by the black lines. The four segments of the kidney (cortex, OSOM, ISOM and IM) were identified by the histological characteristics. (C) Relative volume of each area in murine kidney. n=4 animals for each group. Values are shown as mean ± SEM. OSOM, outer stripes of outer medulla, ISOM, inner stripes of outer medulla. IM, inner medulla. Bar: 1 mm.



Supplementary Figure 4. Februxostat promotes the re-synthesis of adenylates and suppresses the expression of injury marker genes.

(A) Scheme of the nucleoside salvage pathway. (B) Scheme of the present study. Human kidney-2 (HK-2) cells were exposed to hypoxia (5% CO<sub>2</sub>, 1% O<sub>2</sub>) for 12 hours followed by 16 hours of reoxygenation (5% CO<sub>2</sub>, 21% O<sub>2</sub>). During hypoxia, the culture medium was replaced with DMEM containing no glucose (oxygen and glucose deprivation, OGD). (C) The contents of ATP and total adenylates were measured from cell lysates prepared after 24-hour recovery. Data are shown as relative value against control. n = 8 for each group. (D) The contents of H<sub>2</sub>O<sub>2</sub> were measured after 16 hours of reoxygenation. Data are shown as relative value against control. n = 8 for each group. (E) Gene expression of *CASP3* and *KIM-1* in HK-2 cells. #: *P* < 0.05, ##: *P* < 0.01 control vs. OGD. n = 16 for each group. (F) Gene expression of *CASP3* and *KIM-1* in HK-2 cells. \*\*: *P* < 0.01 control vs. OGD. ##: *P* < 0.01 OGD vs. OGD+Februxostat. NS; not significant (*P* > 0.05). n = 16 for each group. Differences in mean values were statistically analyzed by Student's two-tailed *t* test. HPRT1, hypoxanthine phosphoribosyl transferase 1. Ado, adenosine. XOD, xanthine oxidase.

## [Supplementary Tables]

Supplementary Table 1. Metabolites detected in MALDI-IMS experiments

Metabolite	<i>m/z</i> observed	ion	identification
Citrate	191.019	[M-H] <sup>-</sup>	Tandem MS
Succinate	117.019	[M-H] <sup>-</sup>	Tandem MS
Fumarate	115.003	[M-H] <sup>-</sup>	Tandem MS
Malate	133.015	[M-H] <sup>-</sup>	Tandem MS
Lactate	89.024	[M-H] <sup>-</sup>	Tandem MS
Glycerol-3-phosphate	171.006	[M-H] <sup>-</sup>	Tandem MS
Hexose-diphosphate	338.988	[M-H] <sup>-</sup>	Tandem MS
Hexose-phosphate	259.022	[M-H] <sup>-</sup>	Tandem MS
Dihydroxyacetonephosphate	168.998	[M-H] <sup>-</sup>	Tandem MS
Phosphate	96.969	[M-H] <sup>-</sup>	Tandem MS
Aspartate	132.031	[M-H] <sup>-</sup>	Tandem MS
Glutamate	146.047	[M-H] <sup>-</sup>	Tandem MS
Glutamine	147.077	[M+H] <sup>+</sup>	Tandem MS
Taurine	124.011	[M-H] <sup>-</sup>	Tandem MS
Valine	118.086	[M+H] <sup>+</sup>	Tandem MS
Tyrosine	182.078	[M+H] <sup>+</sup>	Tandem MS
Phenylalanine	166.087	[M+H] <sup>+</sup>	Tandem MS
Histidine	156.076	[M+H] <sup>+</sup>	Tandem MS
1-Methylhistidine	170.090	[M+H] <sup>+</sup>	Tandem MS
ATP	505.988	[M-H] <sup>-</sup>	Tandem MS
ADP	426.031	[M-H] <sup>-</sup>	Tandem MS
AMP	346.094	[M-H] <sup>-</sup>	Tandem MS
Adenosine	268.099	[M+H] <sup>+</sup>	Tandem MS
Adenine	136.063	[M+H] <sup>+</sup>	Tandem MS
GTP	521.983	[M-H] <sup>-</sup>	Tandem MS
GDP	442.018	[M-H] <sup>-</sup>	Tandem MS
GMP	362.097	[M-H] <sup>-</sup>	Tandem MS
Guanosine	284.082	[M+H] <sup>+</sup>	Tandem MS
Guanine	152.057	[M+H] <sup>+</sup>	Tandem MS
IMP	347.090	[M-H] <sup>-</sup>	Tandem MS
Inosine	267.0737	[M-H] <sup>-</sup>	Tandem MS
Hypoxanthine	137.046	[M+H] <sup>+</sup>	Tandem MS
Xanthine	151.026	[M-H] <sup>-</sup>	Tandem MS

Supplementary Table 1 (continued). Metabolites detected in MALDI-IMS experiments

UTP	482.961	[M-H] <sup>-</sup>	Tandem MS
UDP	402.996	[M-H] <sup>-</sup>	Tandem MS
UMP	323.049	[M-H] <sup>-</sup>	Tandem MS
UDP-glucose	565.047	[M-H] <sup>-</sup>	Tandem MS
UDP-glucuronate	579.026	[M-H] <sup>-</sup>	Tandem MS
UDP-GlcNAc	606.074	[M-H] <sup>-</sup>	Tandem MS
NADH	664.112	[M-H] <sup>-</sup>	Tandem MS
Nicotinamide	123.056	[M+H] <sup>+</sup>	Tandem MS
Carnitine	162.113	[M+H] <sup>+</sup>	Tandem MS
Acetylcarnitine	204.124	[M+H] <sup>+</sup>	Tandem MS
Glutathione (oxidized)	611.144	[M-H] <sup>-</sup>	Tandem MS
Glutathione (reduced)	306.076	[M-H] <sup>-</sup>	Tandem MS
Choline	104.107	M <sup>+</sup>	Tandem MS
Phosphocholine	184.070	[M+H] <sup>+</sup>	Tandem MS
Glycerophosphocholine	258.104	[M+H] <sup>+</sup>	Tandem MS
2,3-Bisphosphoglyceric acid	264.952	[M-H] <sup>-</sup>	Tandem MS
Creatine	132.070	[M+H] <sup>+</sup>	Tandem MS
Creatinine	114.067	[M+H] <sup>+</sup>	Tandem MS
Creatine-phosphate	212.044	[M+H] <sup>+</sup>	Tandem MS
Spermidine	146.164	[M+H] <sup>+</sup>	Tandem MS
Spermine	203.218	[M+H] <sup>+</sup>	Tandem MS

[M+H]<sup>+</sup> : protonated positive ion, M<sup>+</sup> : permanent cation, [M-H]<sup>-</sup> : deprotonated negative ion. MS: mass spectrometry.

Supplementary Table 2. The regional content of adenine nucleotides, the breakdown products, glutathione and NADH in the kidney on normal condition quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )					The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total	EC Value	Adenosine	IMP	Inosine	Hypoxanthine
Cortex	3027 $\pm$ 45	638 $\pm$ 14	254 $\pm$ 5	3919 $\pm$ 62	0.85 $\pm$ 0.00	25.1 $\pm$ 2.0	71.1 $\pm$ 1.5	13.7 $\pm$ 0.1	5.5 $\pm$ 0.1
OSOM	2571 $\pm$ 91	540 $\pm$ 28	231 $\pm$ 12	3342 $\pm$ 128	0.85 $\pm$ 0.00	85.8 $\pm$ 9.4	50.2 $\pm$ 3.5	13.3 $\pm$ 0.2	4.7 $\pm$ 0.2
ISOM	2195 $\pm$ 255	541 $\pm$ 86	287 $\pm$ 28	3023 $\pm$ 368	0.81 $\pm$ 0.01	33.6 $\pm$ 4.3	92.3 $\pm$ 9.5	12.7 $\pm$ 0.3	4.8 $\pm$ 0.4
IM	1139 $\pm$ 148	101 $\pm$ 24	115 $\pm$ 21	1300 $\pm$ 209	0.86 $\pm$ 0.03	18.1 $\pm$ 10.0	33.2 $\pm$ 3.6	13.5 $\pm$ 1.2	7.7 $\pm$ 1.2

	GSH ( $\mu\text{M}$ )	GSSG ( $\mu\text{M}$ )	NADH ( $\mu\text{M}$ )
Cortex	71.0 $\pm$ 1.0	2009 $\pm$ 105	56.8 $\pm$ 1.3
OSOM	68.6 $\pm$ 2.2	1745 $\pm$ 135	49.6 $\pm$ 1.6
ISOM	70.3 $\pm$ 6.7	3231 $\pm$ 643	51.9 $\pm$ 5.3
IM	56.7 $\pm$ 6.8	1753 $\pm$ 372	35.4 $\pm$ 18.5

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. ATP, adenosine triphosphate. ADP, adenosine diphosphate. AMP, adenosine monophosphate. Total, total adenylates (ATP+ADP+AMP). EC value, energy charge value  $((\text{ATP} + 1/2 \text{ADP})/(\text{ATP}+\text{ADP}+\text{AMP}))$ . IMP, inosine monophosphate. GSH, reduced glutathione. GSSG, oxidized glutathione. n=9. The results are also shown in Figure 2 and 4 as bar graphs.

Supplementary Table 3. The regional content of adenine nucleotides and the breakdown products in the kidney on 1 minute ischemic condition quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )				EC Value	The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total		Adenosine	IMP	Inosine	Hypoxanthine
Cortex	1631 $\pm$ 11	984 $\pm$ 10	984 $\pm$ 10	3517 $\pm$ 23	0.59 $\pm$ 0.00	54.8 $\pm$ 3.9	315.3 $\pm$ 2.7	54.8 $\pm$ 0.5	48.5 $\pm$ 1.1
OSOM	1441 $\pm$ 61	852 $\pm$ 15	851 $\pm$ 15	3019 $\pm$ 98	0.60 $\pm$ 0.01	84.4 $\pm$ 13.3	205.8 $\pm$ 10.2	53.9 $\pm$ 1.3	37.6 $\pm$ 1.6
ISOM	1646 $\pm$ 109	1353 $\pm$ 45	1353 $\pm$ 45	4035 $\pm$ 222	0.53 $\pm$ 0.03	92.0 $\pm$ 9.4	297.5 $\pm$ 12.1	53.4 $\pm$ 1.7	53.0 $\pm$ 4.0
IM	849 $\pm$ 211	554 $\pm$ 171	554 $\pm$ 171	1657 $\pm$ 463	0.59 $\pm$ 0.04	112.3 $\pm$ 23.0	134.4 $\pm$ 32.7	51.3 $\pm$ 2.7	88.9 $\pm$ 14.2

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. Total, total adenylylates (ATP+ADP+AMP). EC value, energy charge value ((ATP + 1/2 ADP)/(ATP+ADP+AMP)). IMP, inosine monophosphate. n=5. The results are also shown in Figure 2 as bar graphs.

Supplementary Table 4. The regional content of adenine nucleotides, the breakdown products, glutathione and NADH in the kidney on 10 minutes ischemic condition quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )					The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total	EC Value	Adenosine	IMP	Inosine	Hypoxanthine
Cortex	466 $\pm$ 21	408 $\pm$ 26	1233 $\pm$ 84	2107 $\pm$ 130	0.32 $\pm$ 0.010	73.5 $\pm$ 3.7	627 $\pm$ 35	250.1 $\pm$ 11.9	261.4 $\pm$ 13.8
OSOM	461 $\pm$ 26	403 $\pm$ 26	1452 $\pm$ 88	2315 $\pm$ 126	0.29 $\pm$ 0.01	121.2 $\pm$ 3.7	503 $\pm$ 32	227.9 $\pm$ 13.9	178.0 $\pm$ 32.5
ISOM	673 $\pm$ 137	728 $\pm$ 122	2738 $\pm$ 325	4139 $\pm$ 580	0.24 $\pm$ 0.01	109.4 $\pm$ 13.7	1187 $\pm$ 148	283.7 $\pm$ 36.2	267.5 $\pm$ 42.0
IM	318 $\pm$ 44	198 $\pm$ 46	853 $\pm$ 221	1369 $\pm$ 280	0.33 $\pm$ 0.06	134.5 $\pm$ 35.0	507 $\pm$ 111	303.3 $\pm$ 62.3	409.5 $\pm$ 96.4

	GSH ( $\mu\text{M}$ )	GSSG ( $\mu\text{M}$ )	NADH ( $\mu\text{M}$ )
Cortex	131.5 $\pm$ 7.5	2129 $\pm$ 180	267.0 $\pm$ 16.7
OSOM	108.0 $\pm$ 2.9	1506 $\pm$ 172	275.9 $\pm$ 12.9
ISOM	124.4 $\pm$ 29.8	3218 $\pm$ 890	463.9 $\pm$ 85.4
IM	135.8 $\pm$ 52.6	2947 $\pm$ 1163	114.6 $\pm$ 31.4

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. Total, total adenylates (ATP+ADP+AMP). EC value, energy charge value  $((\text{ATP} + 1/2 \text{ADP})/(\text{ATP}+\text{ADP}+\text{AMP}))$ . IMP, inosine monophosphate. GSH, reduced glutathione. GSSG, oxidized glutathione. n=4. The results are also shown in Figure 2 and 4 as bar graphs.

Supplementary Table 5. The regional content of adenine nucleotides and the breakdown products in the sham operated kidney quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )					The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total	EC Value	Adenosine	IMP	Inosine	Hypoxanthine
Cortex	3097 $\pm$ 52	766 $\pm$ 11	405 $\pm$ 7	4267 $\pm$ 69	0.82 $\pm$ 0.00	37.5 $\pm$ 1.8	141.9 $\pm$ 2.6	25.9 $\pm$ 0.4	12.5 $\pm$ 0.3
OSOM	2257 $\pm$ 137	559 $\pm$ 31	329 $\pm$ 9	3146 $\pm$ 177	0.81 $\pm$ 0.00	72.4 $\pm$ 8.0	94.4 $\pm$ 2.0	25.0 $\pm$ 0.7	10.7 $\pm$ 0.7
ISOM	2027 $\pm$ 199	665 $\pm$ 42	438 $\pm$ 30	3130 $\pm$ 269	0.75 $\pm$ 0.01	49.3 $\pm$ 4.5	134.8 $\pm$ 11.2	24.1 $\pm$ 1.5	11.3 $\pm$ 0.6
IM	1001 $\pm$ 127	114 $\pm$ 25	146 $\pm$ 35	1261 $\pm$ 163	0.84 $\pm$ 0.02	134.5 $\pm$ 35.0	45.5 $\pm$ 10.3	21.8 $\pm$ 1.6	18.3 $\pm$ 3.9

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. Total, total adenylates (ATP+ADP+AMP). EC value, energy charge value ((ATP + 1/2 ADP)/(ATP+ADP+AMP)). IMP, inosine monophosphate. n=5 in each group. The results are also shown in Figure 3 as bar graphs.

Supplementary Table 6. The regional content of adenine nucleotides, the breakdown products, glutathione and NADH in the kidney after 10 minutes ischemia and 24 hours reperfusion quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )					The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total	EC Value	Adenosine	IMP	Inosine	Hypoxanthine
Cortex	2332 $\pm$ 40	664 $\pm$ 14	377 $\pm$ 10	3302 $\pm$ 99	0.79 $\pm$ 0.00	38.3 $\pm$ 2.2	113.4 $\pm$ 1.3	18.9 $\pm$ 0.3	11.7 $\pm$ 0.4
OSOM	1681 $\pm$ 82	480 $\pm$ 29	320 $\pm$ 15	2542 $\pm$ 136	0.77 $\pm$ 0.00	80.2 $\pm$ 5.5	70.1 $\pm$ 4.8	19.2 $\pm$ 0.5	10.1 $\pm$ 0.3
ISOM	1826 $\pm$ 91	646 $\pm$ 40	478 $\pm$ 29	3064 $\pm$ 169	0.73 $\pm$ 0.00	54.1 $\pm$ 5.7	143.3 $\pm$ 6.7	21.0 $\pm$ 0.8	12.6 $\pm$ 1.5
IM	831 $\pm$ 150	68 $\pm$ 7	147 $\pm$ 27	1074 $\pm$ 189	0.83 $\pm$ 0.00	52.6 $\pm$ 7.8	32.1 $\pm$ 5.5	23.4 $\pm$ 2.7	19.3 $\pm$ 4.3

	GSH ( $\mu\text{M}$ )	GSSG ( $\mu\text{M}$ )	NADH ( $\mu\text{M}$ )
Cortex	38.5 $\pm$ 0.3	1293 $\pm$ 35	265.2 $\pm$ 5.1
OSOM	38.2 $\pm$ 1.3	1215 $\pm$ 53	246.6 $\pm$ 8.9
ISOM	44.3 $\pm$ 2.0	2122 $\pm$ 206	203.7 $\pm$ 17.3
IM	39.6 $\pm$ 2.3	1454 $\pm$ 179	87.8 $\pm$ 12.2

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. Total, total adenylates (ATP+ADP+AMP). EC value, energy charge value  $((\text{ATP} + 1/2 \text{ADP})/(\text{ATP}+\text{ADP}+\text{AMP}))$ . IMP, inosine monophosphate. GSH, reduced glutathione. GSSG, oxidized glutathione. n=5 for adenylates, n=14 for glutathione and NADH in each group. The results are also shown in Figure 3 and 4 as bar graphs.

Supplementary Table 7. The regional content of adenine nucleotides, the breakdown products, glutathione and NADH in the kidney after 10 minutes ischemia and 24 hours reperfusion with febuxostat treatment quantified by metabolome analyses.

	Adenine nucleotides ( $\mu\text{M}$ )					The breakdown products ( $\mu\text{M}$ )			
	ATP	ADP	AMP	Total	EC Value	Adenosine	IMP	Inosine	Hypoxanthine
Cortex	3402 $\pm$ 58	737 $\pm$ 14	297 $\pm$ 6	4554 $\pm$ 72	0.85 $\pm$ 0.00	23.1 $\pm$ 0.6	117.9 $\pm$ 5.0	18.3 $\pm$ 0.1	40.9 $\pm$ 1.6
OSOM	2309 $\pm$ 202	524 $\pm$ 24	246 $\pm$ 12	3158 $\pm$ 240	0.83 $\pm$ 0.01	31.0 $\pm$ 1.6	79.1 $\pm$ 6.9	18.4 $\pm$ 0.4	27.1 $\pm$ 2.4
ISOM	3259 $\pm$ 322	703 $\pm$ 65	357 $\pm$ 17	4434 $\pm$ 385	0.83 $\pm$ 0.01	25.3 $\pm$ 2.0	115.1 $\pm$ 16.5	18.0 $\pm$ 0.6	38.4 $\pm$ 6.0
IM	1595 $\pm$ 175	219 $\pm$ 35	176 $\pm$ 17	2048 $\pm$ 155	0.85 $\pm$ 0.02	23.1 $\pm$ 4.4	58.9 $\pm$ 10.3	21.0 $\pm$ 1.1	62.9 $\pm$ 6.8

	GSH ( $\mu\text{M}$ )	GSSG ( $\mu\text{M}$ )	NADH ( $\mu\text{M}$ )
Cortex	198.7 $\pm$ 1.9	1344 $\pm$ 33	332.9 $\pm$ 5.5
OSOM	212.1 $\pm$ 7.3	1468 $\pm$ 61	310.9 $\pm$ 11.5
ISOM	259.9 $\pm$ 11.6	2721 $\pm$ 217	311.2 $\pm$ 18.2
IM	256.1 $\pm$ 13.3	1589 $\pm$ 155	158.7 $\pm$ 16.4

The values are shown as mean  $\pm$  SEM. OSOM, outer stripes of outer medulla. ISOM, inner stripes of outer medulla. IM, inner medulla. Total, total adenylates (ATP+ADP+AMP). EC value, energy charge value  $((\text{ATP} + 1/2 \text{ADP})/(\text{ATP}+\text{ADP}+\text{AMP}))$ . IMP, inosine monophosphate. GSH, reduced glutathione. GSSG, oxidized glutathione. n=5 for adenylates, n=12 for glutathione and NADH in each group. The results are also shown in Figure 3 and 4 as bar graphs.

Supplementary Table 8. The content of glutathione and NADH in the whole kidney quantified by metabolome analyses.

	GSH ( $\mu\text{M}$ )	GSSG ( $\mu\text{M}$ )	NADH ( $\mu\text{M}$ )
Intact	25.7 $\pm$ 9.6	2111.8 $\pm$ 193.7	54.5 $\pm$ 6.7
10 minutes ischemia	126.5 $\pm$ 31.2	2164.0 $\pm$ 197.4	290.4 $\pm$ 44.3
IR	39.2 $\pm$ 25.6	1387.7 $\pm$ 152.8	250.4 $\pm$ 61.4
IRF	76.0 $\pm$ 24.3	1545.9 $\pm$ 151.6	322.6 $\pm$ 80.2

The values are shown as mean  $\pm$  SEM. GSH, reduced glutathione. GSSG, oxidized glutathione. n=5 in each group.

Supplementary Table 9. The primer sequences used in the real-time PCR experiment.

Gene	Forward	Reverse
<i>CASP3</i>	AAGGCAGAGCCATGGACCAC	CTGGCAGCATCATCCACACATAC
<i>KIM-1</i>	AGCTGTGTCTGACAGTGGCGTA	GAACAGTCGTGACGGTTGGAA
<i>HPRT1</i>	GGCAGTATAATCCAAAGATGGTCAA	GTCAAGGGCATATCCTACAACAAAC
<i>RPS18</i>	TTTGCGAGTACTCAACACCAACATC	GAGCATATCTTCGGCCCACAC

*CASP3*, caspase 3, *KIM-1*, kidney injury molecule 1. *HPRT1*, hypoxanthine phosphoribosyl transferase 1, *RPS18*, *Homo sapiens* ribosomal protein S18.