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**Supplemental Table 1. Labeled metabolites identified in wild type mouse red blood cells.**

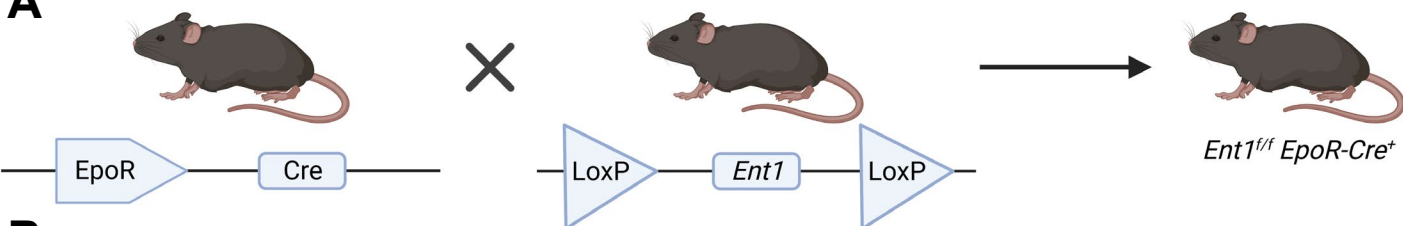
**Supplemental Table 2. Complete blood count of healthy controls and CKD patients**

**Supplemental Dataset 1. Metabolites identified in mouse red blood cells, plasma, kidney and urine (Excel File)**

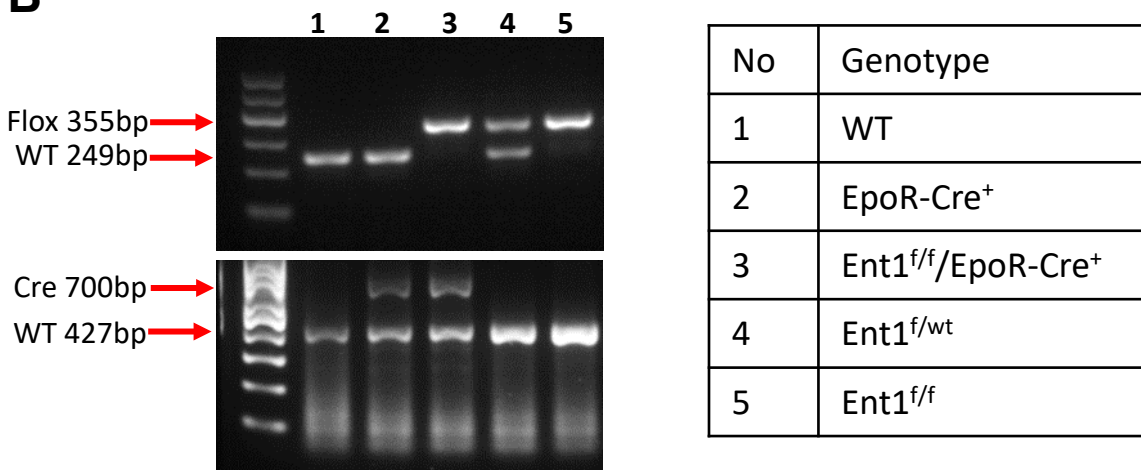
**Supplemental Dataset 2. Metabolites identified in mouse red blood cells, plasma and kidney (Excel File)**

# Figure S1

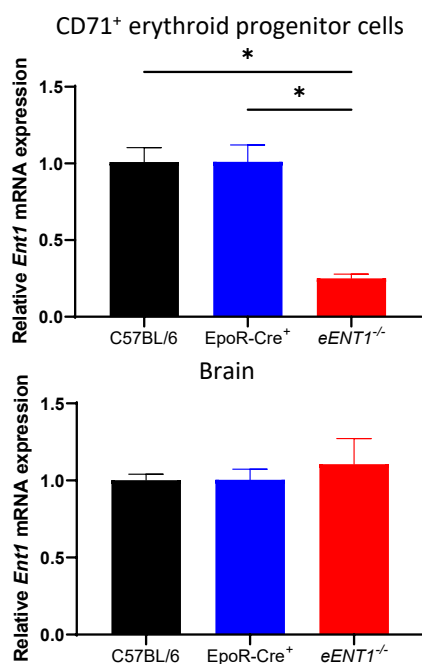
**A**



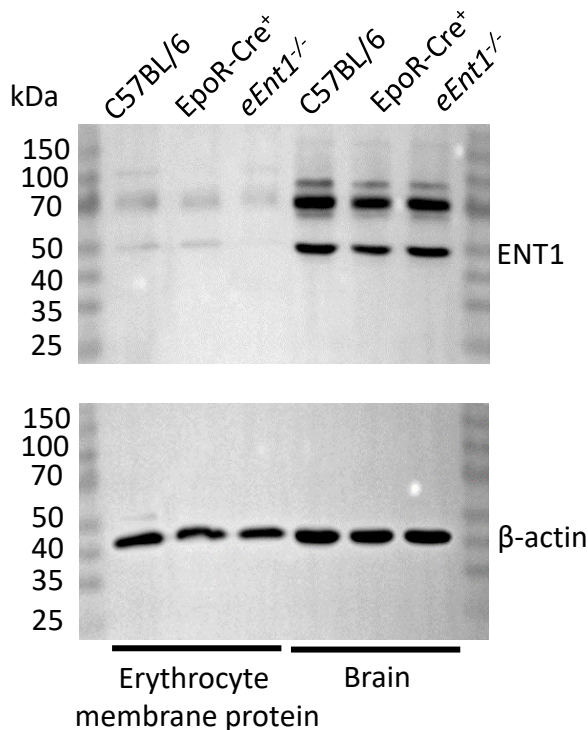
**B**



**C**



**D**



## Figure S1. Generation of mice with deletion of *Ent1* on erythroid cells.

**(A)** Schematic representation of erythrocyte specific *Ent1* deletion generation. **(B)** Representative result of mouse genotyping. **(C)** Relative *Ent1* mRNA levels in CD71 positive erythroid progenitor cells and brain of C57BL/6, *EpoR-Cre<sup>+</sup>*, *Ent1<sup>f/f</sup>/EpoR-Cre<sup>+</sup>* mice. Data are expressed as mean  $\pm$  SEM.  $n=3$  for each group.  $*P < 0.05$ . **(D)** Protein level of ENT1 in erythrocyte membranes and brain tissue.

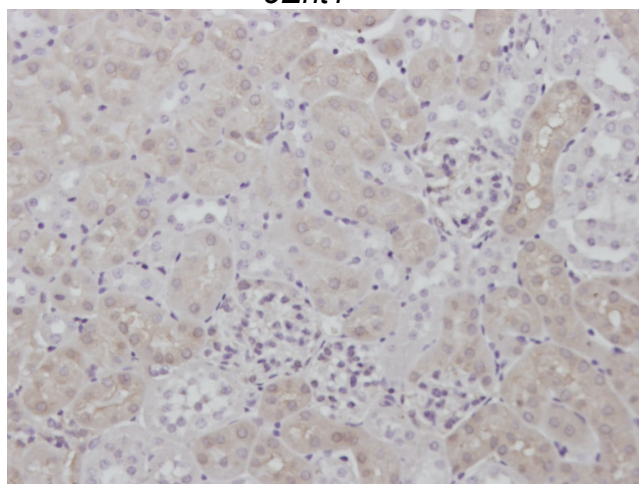
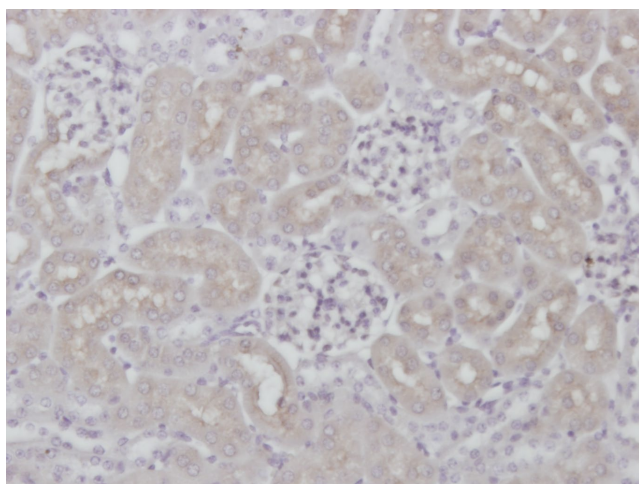
# Figure S2

**A**

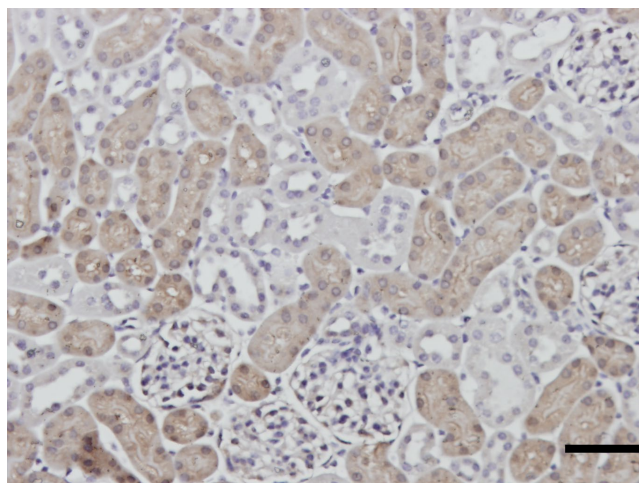
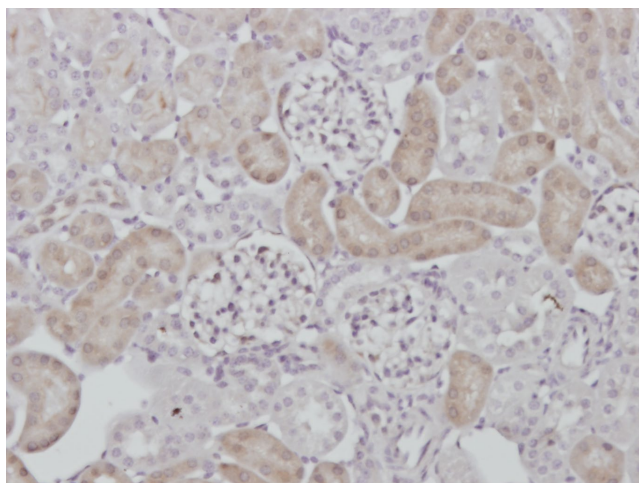
*EpoR-Cre*<sup>+</sup>

*eEnt1*<sup>-/-</sup>

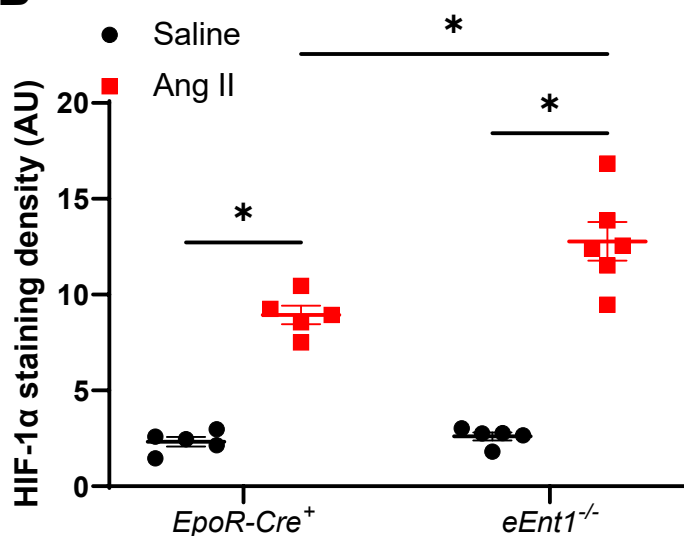
Saline



Ang II



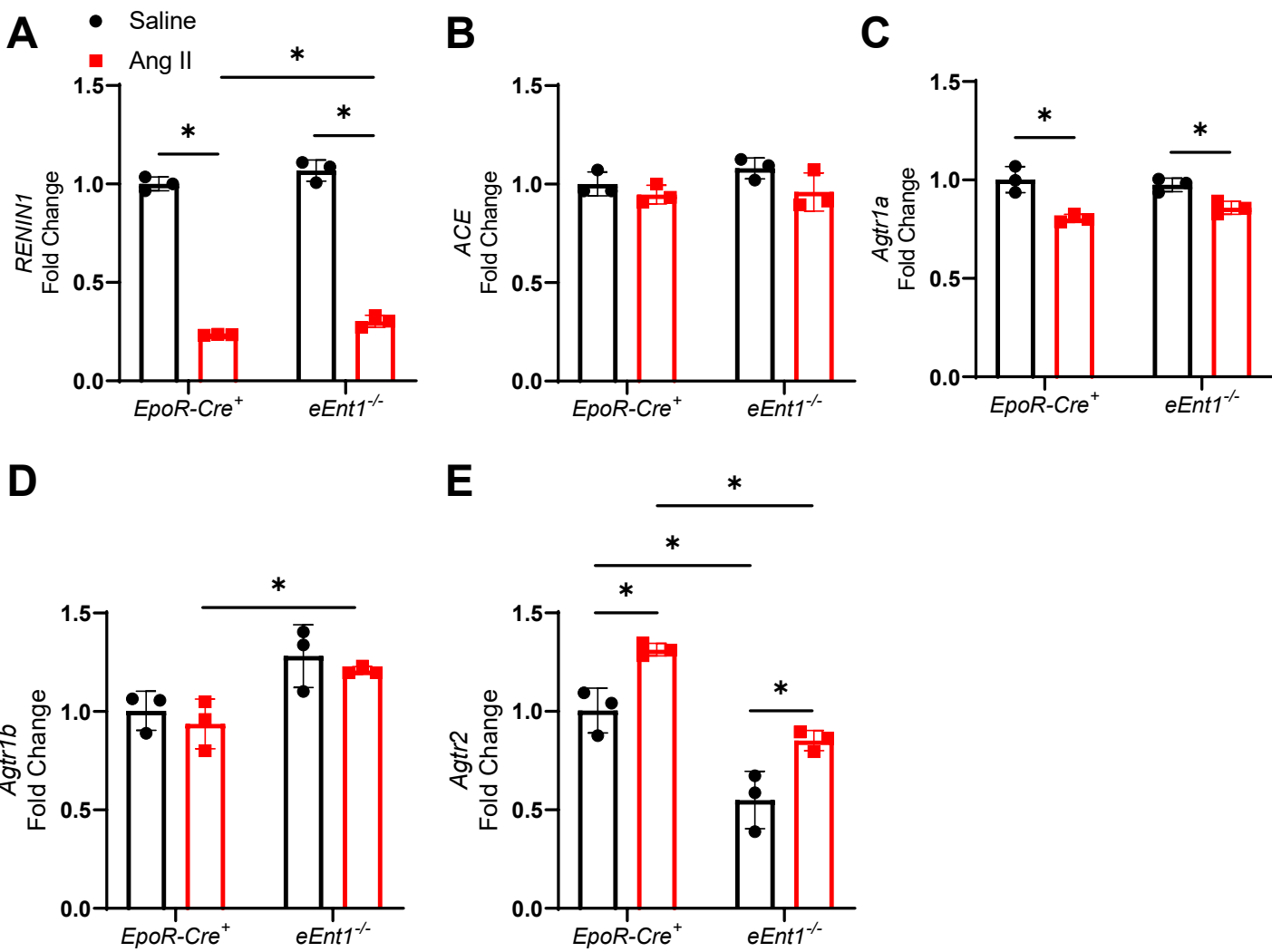
**B**



## Figure S2. HIF-1α immunohistochemistry staining.

**(A)** Representative immunohistochemistry staining of HIF-1α. **(B)** Quantitative analysis of HIF-1α expression in the kidneys of *EpoR-Cre*<sup>+</sup> and *Ent1*<sup>ff</sup>/*EpoR-Cre*<sup>+</sup> mice with or without Ang II infusion. Scale bar = 50 μm. Data are expressed as mean ± SEM. \**P* < 0.05.

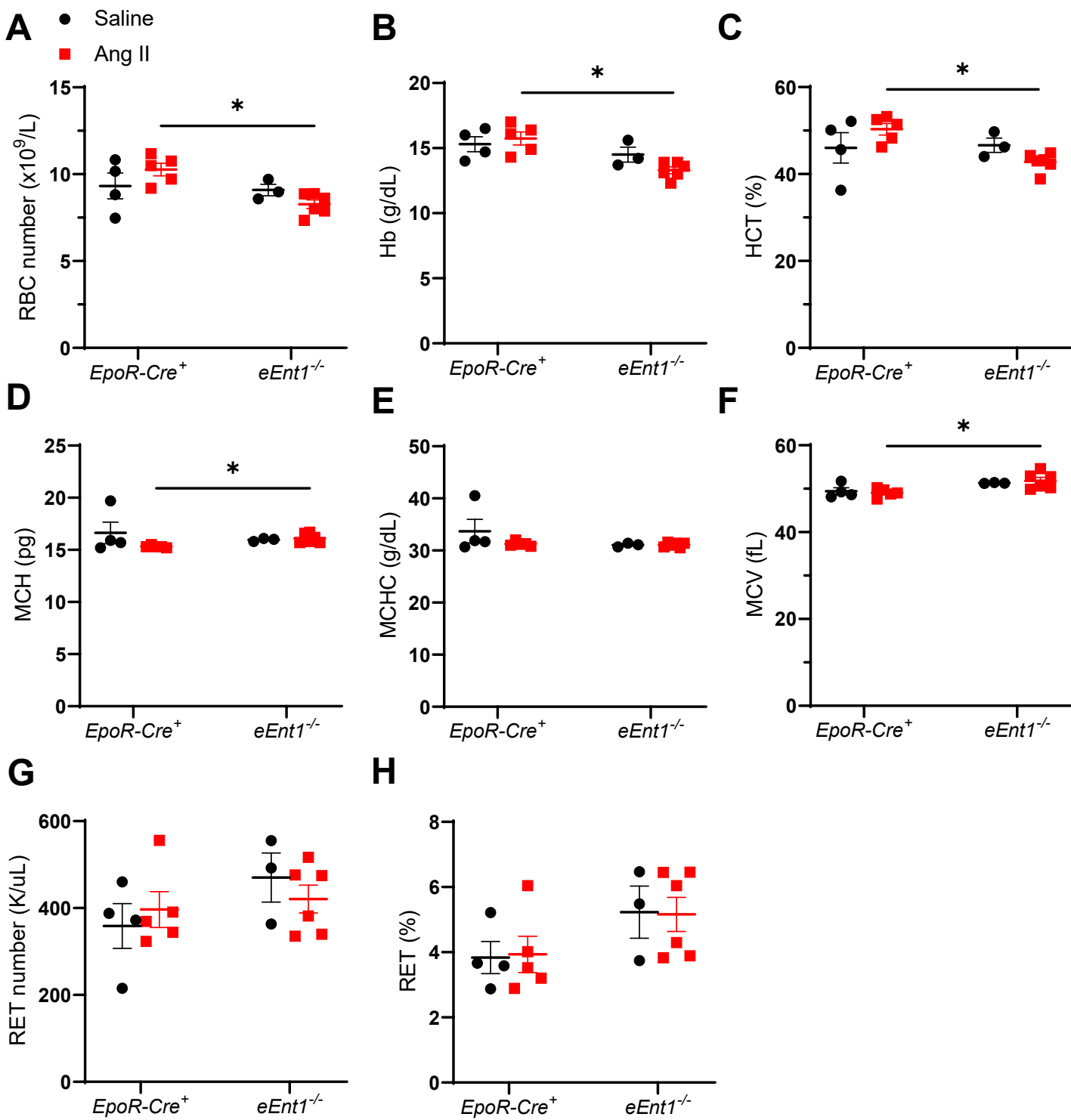
## Figure S3



### Figure S3. Renin-Angiotensin System (RAS) related gene expression in Kidney.

(A-E) *Renin1*, Angiotensin-converting enzyme (*ACE*), Angiotensin II receptor type 1a (*Agtr1a*), Angiotensin II receptor type 1b (*Agtr1b*), Angiotensin II receptor type 2 (*Agtr2*) mRNA levels in the mouse kidney. Data are expressed as mean  $\pm$  SEM.  $n = 3$  for each group.  $P$  values determined by one-way ANOVA with a post-hoc *Holm-Sidak* test for multiple comparisons. \* $P < 0.05$ .

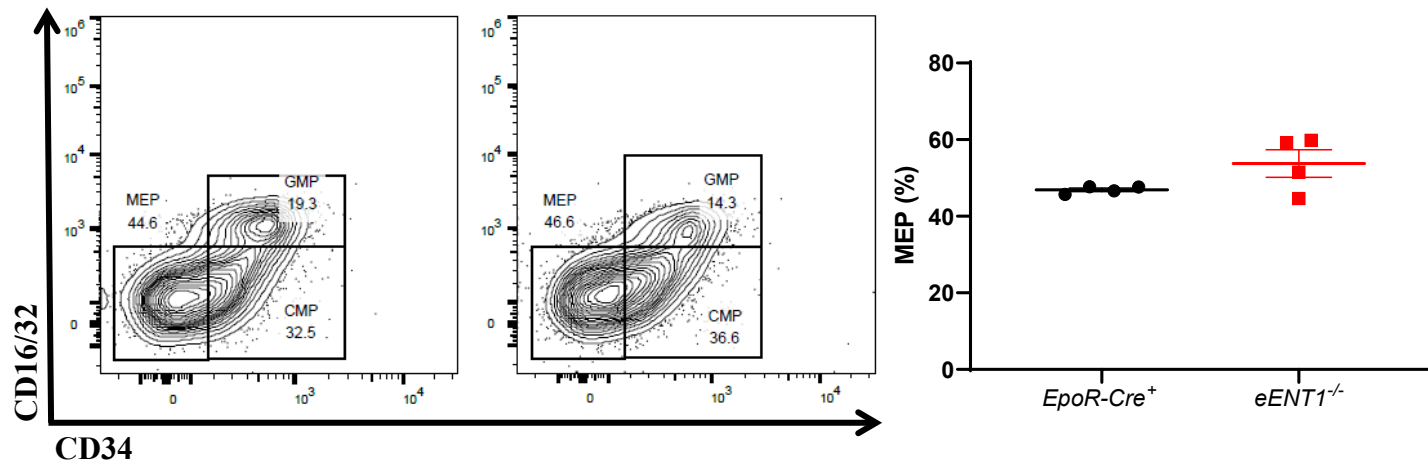
## Figure S4



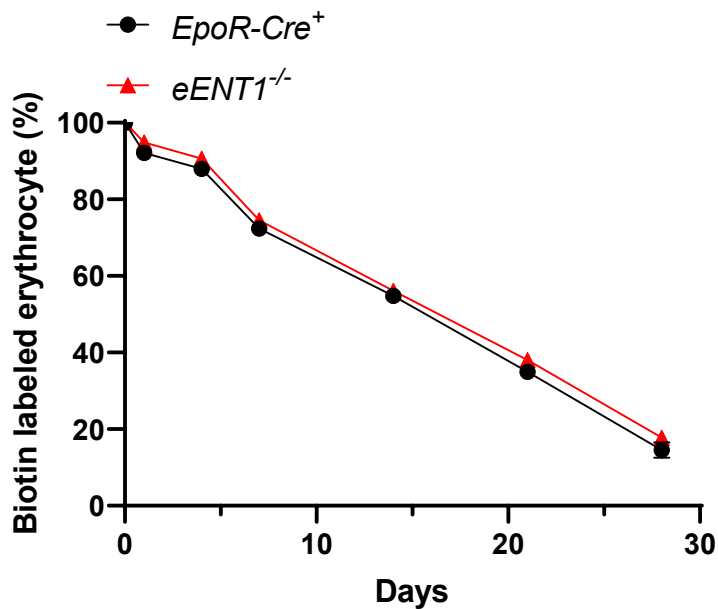
**Figure S4. Mouse complete blood count.** (A) Red Blood Cell (RBC) Number. (B) Hemoglobin (Hb) levels. (C) Hematocrit (HCT). (D) Mean Corpuscular Hemoglobin (MCH). (E) Mean Corpuscular Hemoglobin Concentration (MCHC). (F) Mean Corpuscular Volume (MCV). (G) Reticulocyte (RET) Number. (H) RET percentage. Data are expressed as mean  $\pm$  SEM, n = 3-6 mice each group. *P* values determined by one-way ANOVA with a post-hoc *Holm-Sidak* test for multiple comparisons. \**P* < 0.05.

## Figure S5

**A**



**B**

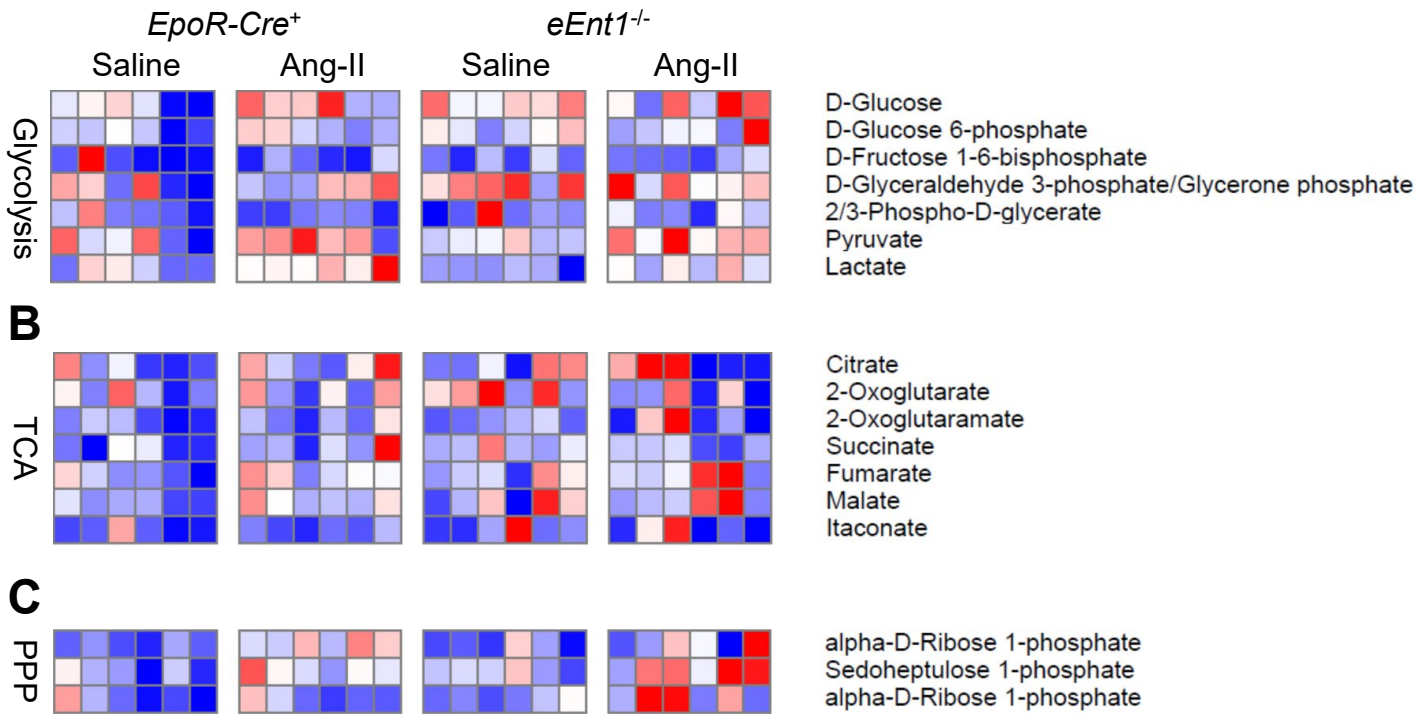


**Figure S5. Flow cytometry analysis of hematopoietic progenitor cells of bone marrow and the life span of erythrocytes.**

**(A)** Representative flow cytometry analysis of hematopoietic progenitor cells of bone marrow of 8-12 weeks *EpoR-Cre*<sup>+</sup> and *eEnt1*<sup>-/-</sup> mice. Data are expressed as mean  $\pm$  SEM.  $n = 4$  for each group. **(B)** Percentage of biotin-labeled erythrocytes of 8-12 weeks *EpoR-Cre*<sup>+</sup> and *eEnt1*<sup>-/-</sup> mice after biotin labelling. Data are expressed as mean  $\pm$  SEM.  $n = 4$  for each group.

# Figure S6

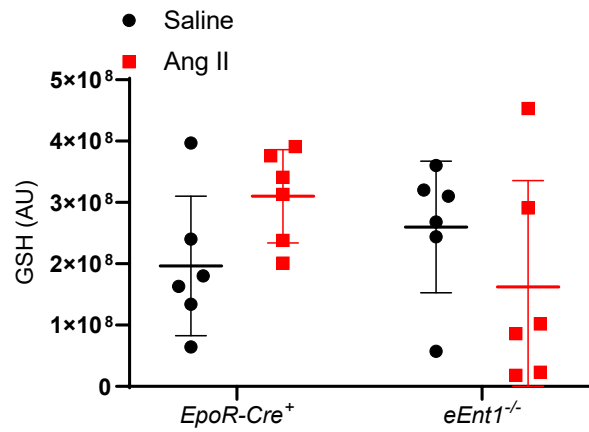
**A**



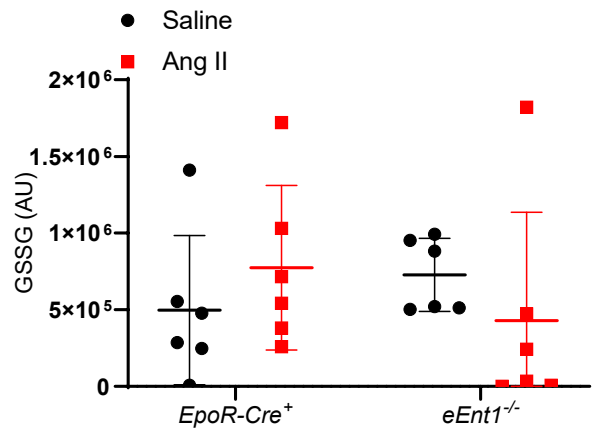
**Figure S6. Mouse kidney glycolysis, TCA cycle and PPP pathway heatmap.** Heatmap showing relative abundance of metabolites in **(A)** glycolysis **(B)** TCA cycle **(C)** PPP pathway in the kidney of *EpoR-Cre<sup>+</sup>* or *eENT1<sup>-/-</sup>* mice with saline or Ang II infusion. n = 6 mice each group.

# Figure S7

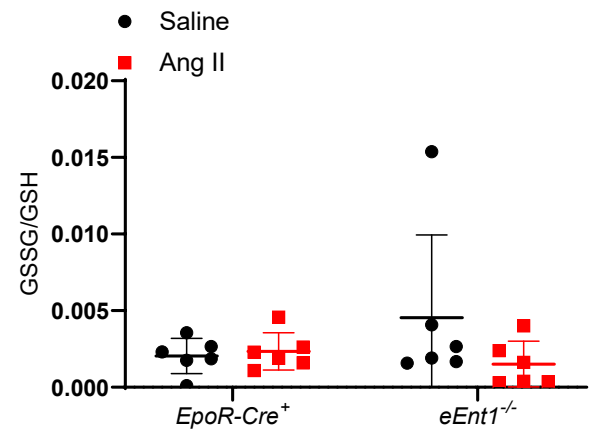
**A**



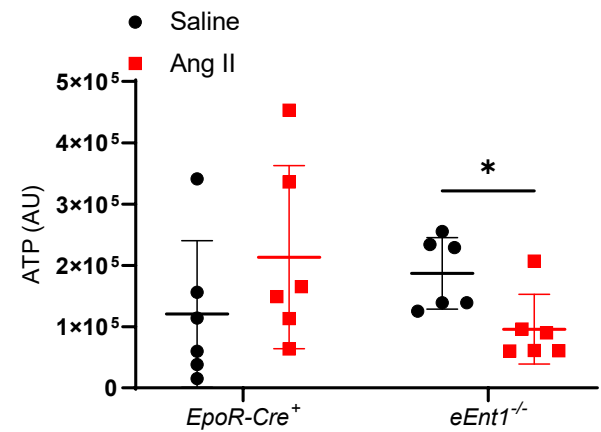
**B**



**C**

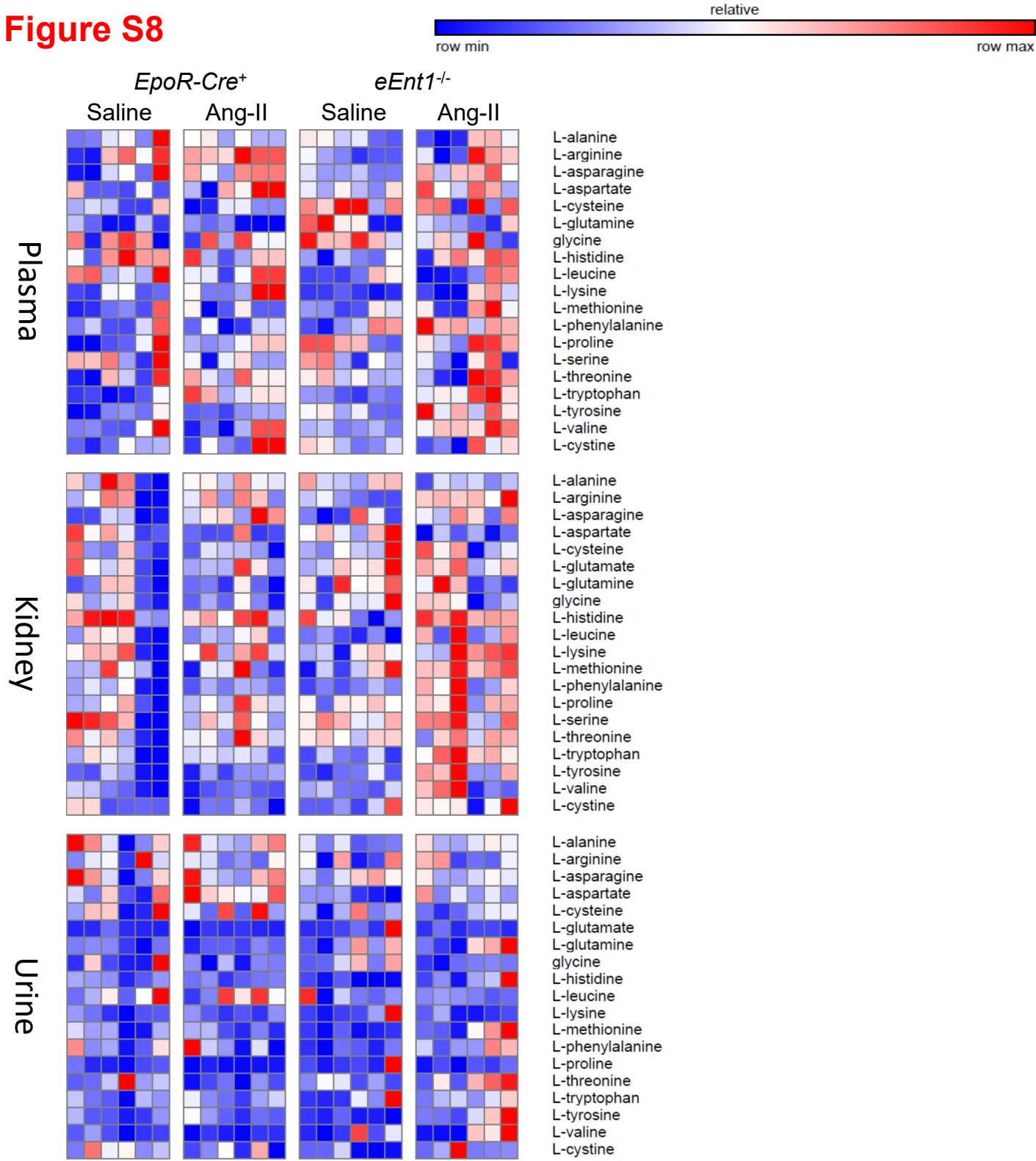


**D**



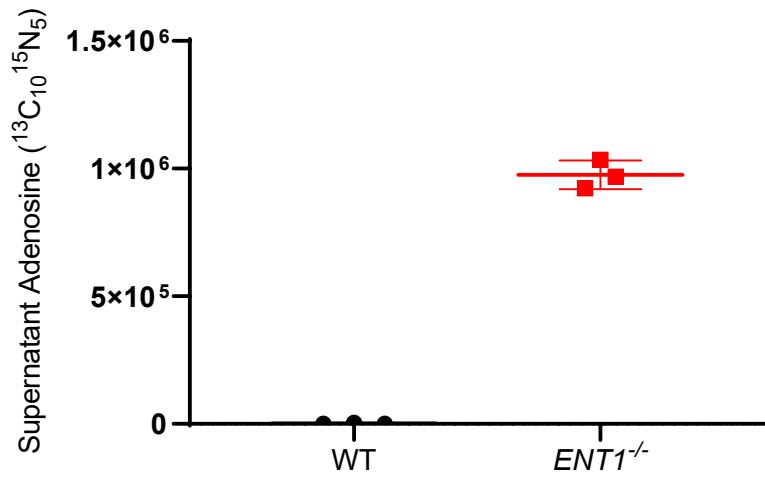
**Figure S7. GSH, GSSG, GSSG/GSH and ATP levels in mouse kidney.** (A) GSH (B) GSSG (C) the ratio of GSSG to GSH (D) ATP in the kidney of *EpoR-Cre<sup>+</sup>* or *eENT1<sup>-/-</sup>* mice with saline or Ang II infusion.  $n = 6$  mice each group. Data are expressed as mean  $\pm$  SEM. \* $P < 0.05$ .



**Figure S8**

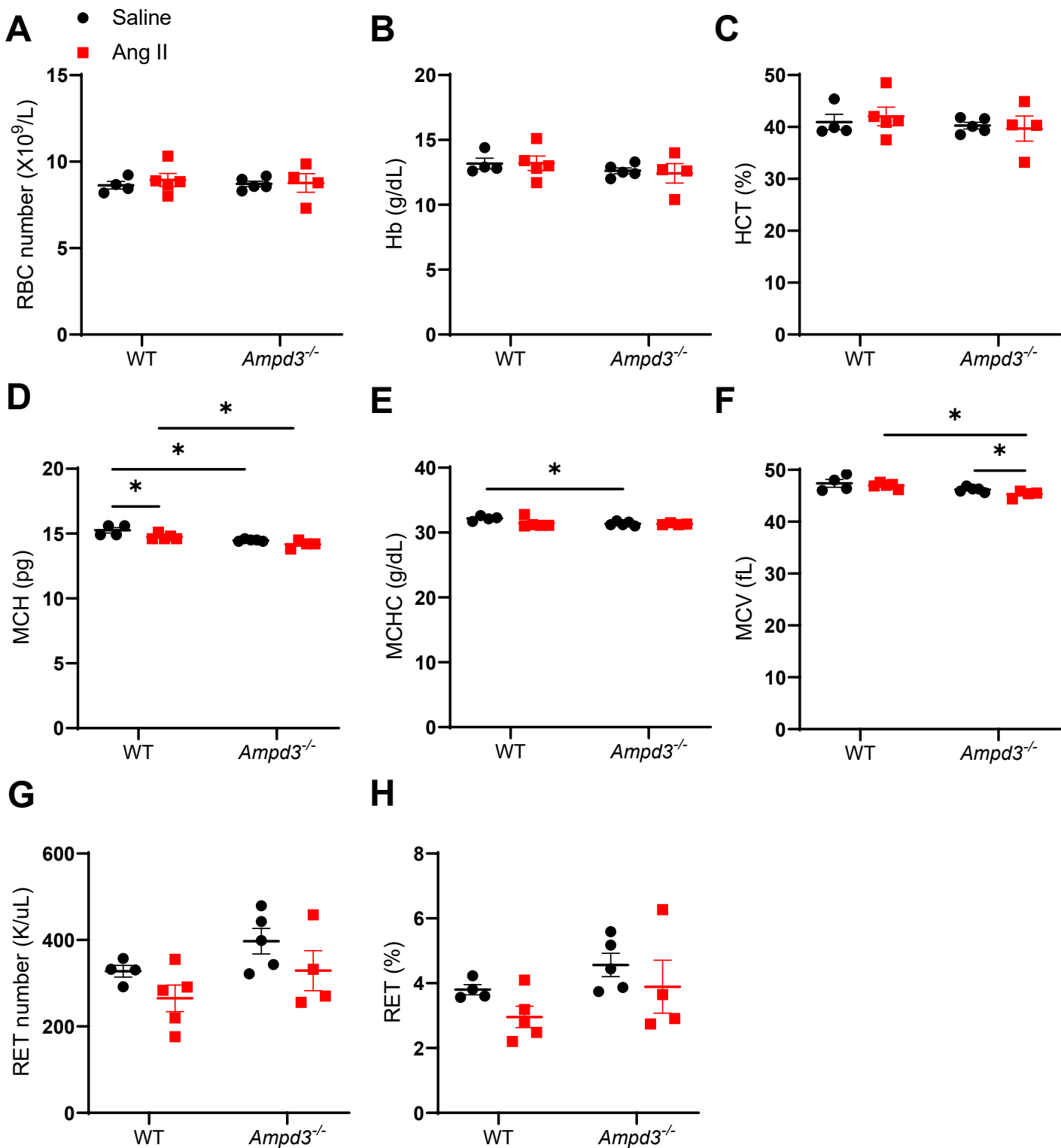
**Figure S8. Mouse plasma, kidney and urine amino acids heatmap.** Heatmap showing relative abundance of amino acids in the plasma, kidney and urine of *EpoR-Cre<sup>+</sup>* or *eENT1<sup>-/-</sup>* mice with saline or Ang II infusion. n = 6 mice each group.

## Figure S9



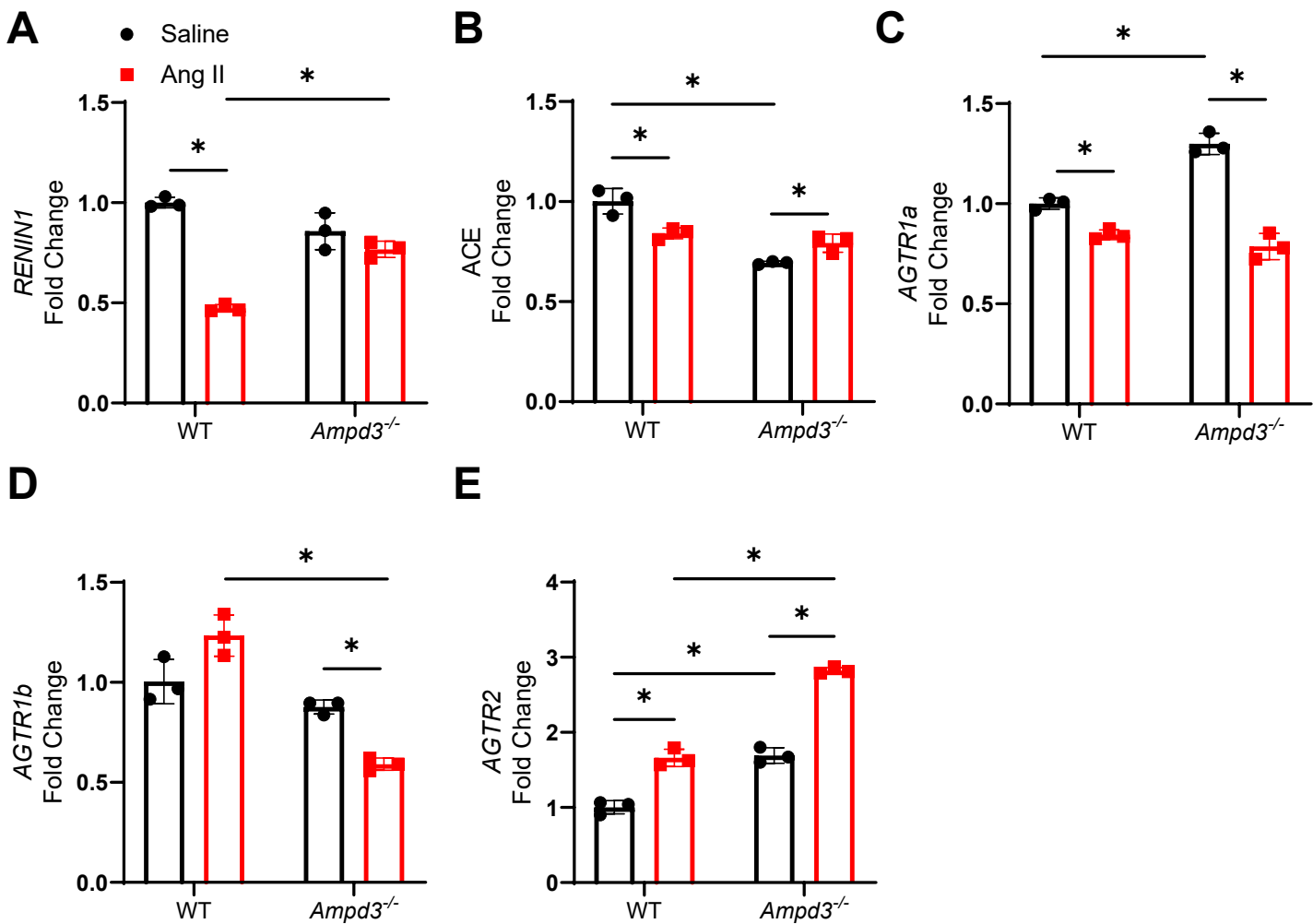
**Figure S9. Labeled adenosine in supernatant.** Intensity of labeled adenosine ( $^{13}\text{C}_{10} \text{ } ^{15}\text{N}_5$ ) in supernatant in WT and  $ENT1^{-/-}$  mice red blood cell culture medium. n = 3 mice each group.

# Figure S10



**Figure S10. Mouse complete blood count.** (A) Red Blood Cell (RBC) Number. (B) Hemoglobin (Hb) levels. (C) Hematocrit (HCT). (D) Mean Corpuscular Hemoglobin (MCH). (E) Mean Corpuscular Hemoglobin Concentration (MCHC). (F) Mean Corpuscular Volume (MCV). (G) Reticulocyte (RET) Number. (H) RET percentage. Data are expressed as mean  $\pm$  SEM, n = 5 mice each group. P values determined by one-way ANOVA with a post-hoc *Holm-Sidak* test for multiple comparisons. \*P < 0.05.

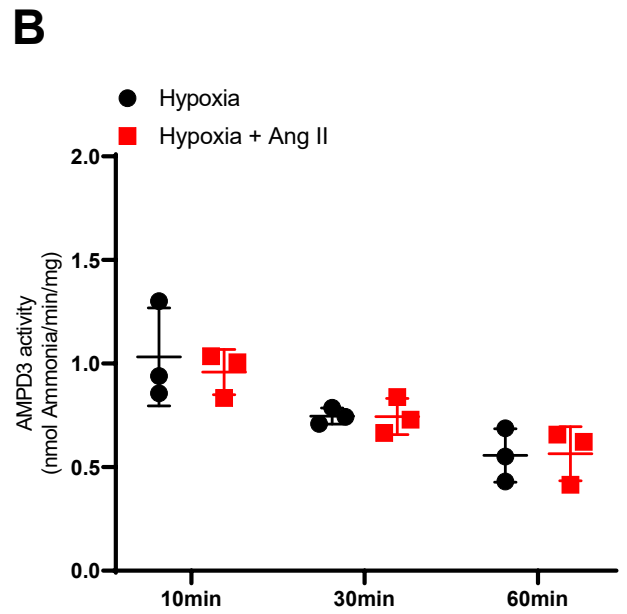
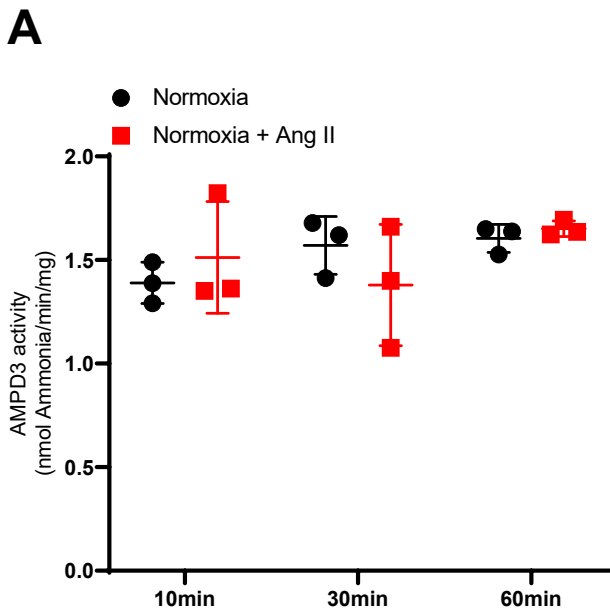
# Figure S11



## Figure S11. Renin-Angiotensin System (RAS) related gene expression in Kidney.

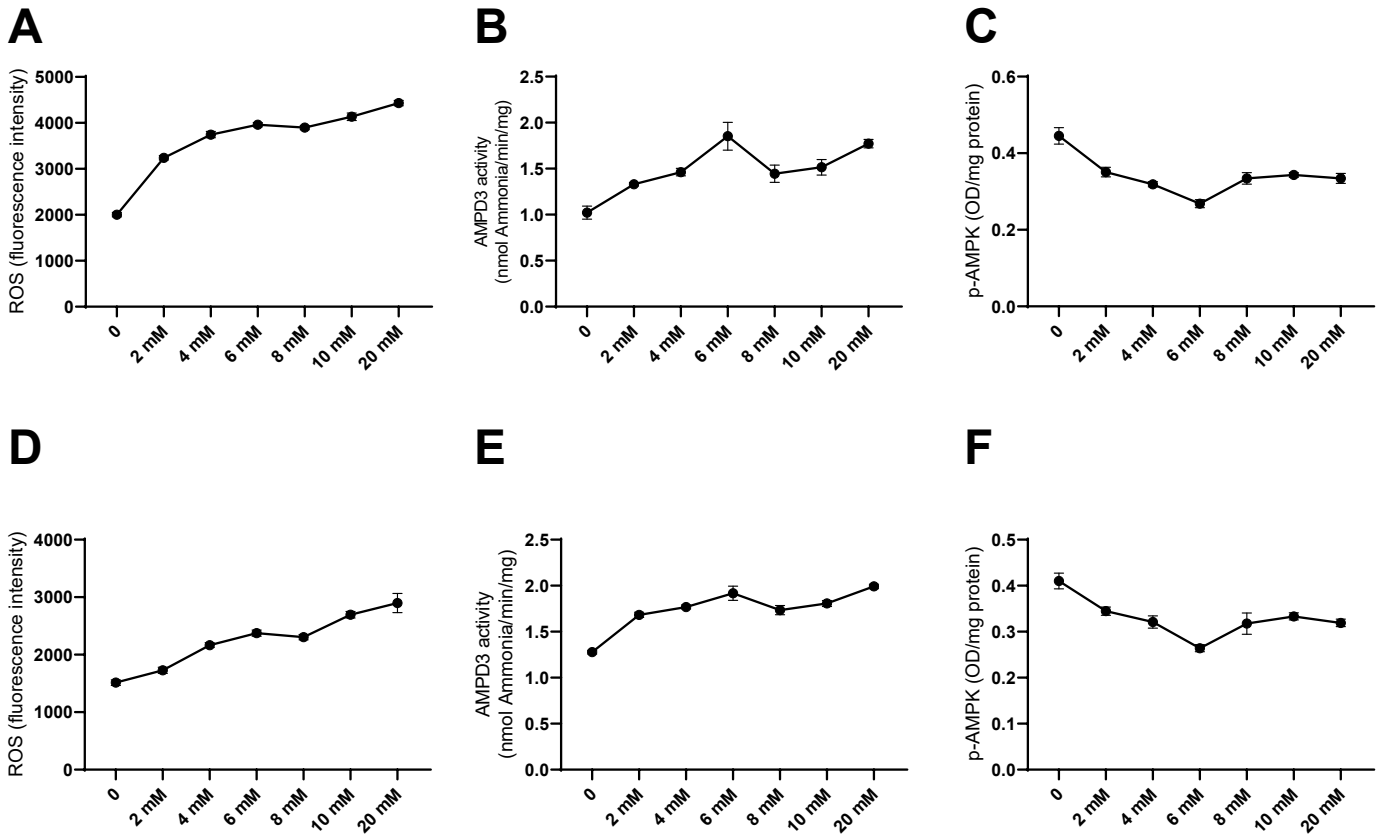
(A)-(E) *Renin1*, Angiotensin-converting enzyme (*ACE*), Angiotensin II receptor type 1a (*Agtr1a*), Angiotensin II receptor type 1b (*Agtr1b*), Angiotensin II receptor type 2 (*Agtr2*) mRNA levels in the mouse kidney. Data are expressed as mean  $\pm$  SEM.  $n = 3$  for each group.  $P$  values determined by one-way ANOVA with a post-hoc *Holm-Sidak* test for multiple comparisons. \* $P < 0.05$ .

# Figure S12



**Figure S12. Mouse erythrocyte AMPD3 activity with or without Ang II under normoxia and hypoxia. (A)** Erythrocyte AMPD3 activity was measured with or without Ang II treatment under normoxia at 10, 30, 60 minutes. **(B)** Erythrocyte AMPD3 activity was measured with or without Ang II treatment under hypoxia at 10, 30, 60 minutes. n = 3 mice each group.

# Figure S13



**Figure S13. Mouse and human erythrocyte ROS level, AMPD3 activity and AMPKα activity with different concentration of H<sub>2</sub>O<sub>2</sub> treatment. (A) Mouse erythrocyte ROS level was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. (B) Mouse erythrocyte AMPD3 activity was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. (C) Mouse erythrocyte AMPKα activity was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. (D) Mouse erythrocyte ROS level was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. (E) Mouse erythrocyte AMPD3 activity was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. (F) Mouse erythrocyte AMPKα activity was measured with different concentration of H<sub>2</sub>O<sub>2</sub> treatment at 10 minutes. n = 3 mice or human each group.**

**Table S1**

Labeled Compound	AMP	Adenine	IMP	ADP	ATP	Inosine
WT N 10min. R1	856446.2	27389.04	253304	1478987	1502641	14685.42
WT N 10min. R2	768739.2	46224.64	214455.8	1465670	1281770	24325.12
WT N 10min. R3	1268998	94082.62	340077.7	1319270	1006145	0
WT H 10min. R1	2392486	144981.7	547267.7	1796135	968660	0
WT H 10min. R2	3032987	149119.7	792336.1	1690684	905248.2	0
WT H 10min. R3	2633496	211939.1	490186.4	1338014	852200	0
WT N 1 hr R1	401720.5	36901.58	170650.2	989343.3	777339.3	158696.3
WT N 1 hr R2	489408.8	36592.4	121661.6	1065253	875691	150128.2
WT N 1 hr R3	479059.8	123076.3	179737.3	1114323	1009959	151427
WT H 1 hr R1	3548102	290653.6	683273.4	1280432	668606.2	0
WT H 1 hr R2	1683508	88235.59	405154.4	745291.8	563798.2	0
WT H 1 hr R3	3592224	233798.6	1003835	1348081	558957.9	0
WT N 3hr R1	382211.7	0	118971.7	652281.3	651283.3	133048.1
WT N 3hr R2	300379.2	8482.306	178773.6	600852.8	516259.1	85968.74
WT N 3hr R3	316595.5	0	122503.3	625472.6	678343.4	121861.5
WT H 3hr R1	1506548	88141.46	455583.2	756039.8	660038.1	0
WT H 3hr R2	1502416	31828.39	306142.2	705408.8	602694.1	0
WT H 3hr R3	1815642	118708.4	392742.3	836237.8	798567.3	0
WT N 6hr R1	164483	9073.736	61942.14	479862.2	586259.5	0
WT N 6hr R2	205080.9	42625.99	88256.48	502793	609464.8	7558.439
WT N 6hr R3	204389.9	8463.1	103211.5	592944.8	623387.9	22394.12
WT H 6hr R1	1362960	126338.8	288023.1	730116.7	687524.6	0
WT H 6hr R2	1547580	93597.38	447135	782447.5	876728	6778.015
WT H 6hr R3	1471977	149639.5	349832.5	834915.8	691197.9	0

**Labeled metabolites identified in wild type mouse red blood cells.** The table shows intensity of labeled metabolites in wild type mouse red blood cells treated with  $^{13}\text{C}_{10}$ ,  $^{15}\text{N}_5$ -adenosine at different time points (10min, 1 hour, 3 hours and 6 hours). n = 3 each group.

## Table S2

Variable	Control (n=18)	CKD (n=18)
WBC (10 <sup>9</sup> /L)	6.35±1.55	5.71±0.24
PLT (10 <sup>9</sup> /L)	236.29±43.3	190.17±60.04*
NEUT (10 <sup>9</sup> /L)	3.58±1.24	3.55±1.17
LYMPH (10 <sup>9</sup> /L)	2.07±0.38	1.26±0.58*
MCV (fl)	89.52±2.37	91.15±0.17
MCH (g/L)	30.28±0.77	29.89±0.30
MCHC (g/L)	338.26±4.31	328.02±10.01*

Data were expressed as mean±SD. *P* values were assessed by two-tailed unpaired t-test with Welch correlation. WBC, white blood cell; PLT, platelet; NEUT, neutrophil; LYMPH, lymphocyte; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

## Complete blood count of healthy controls and CKD patients