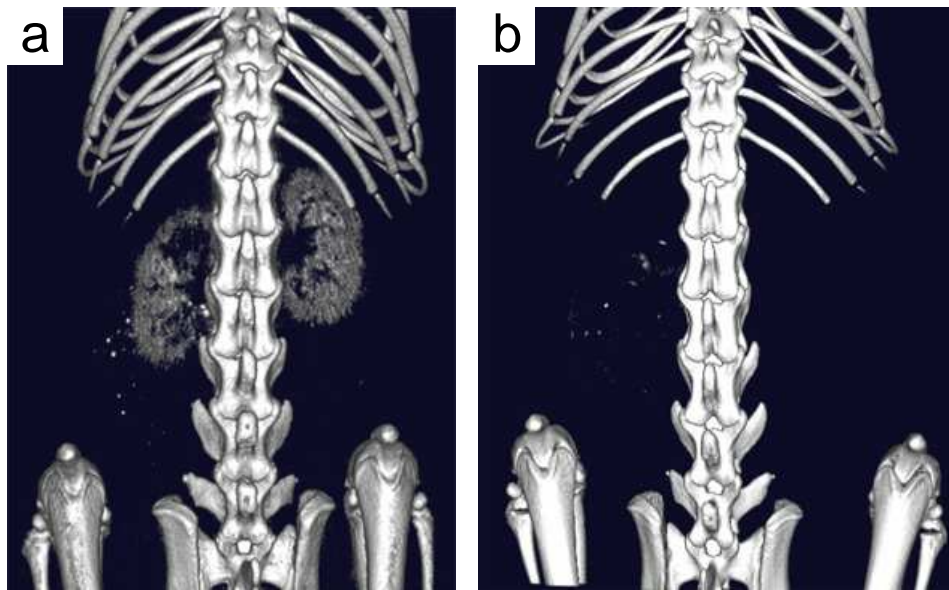
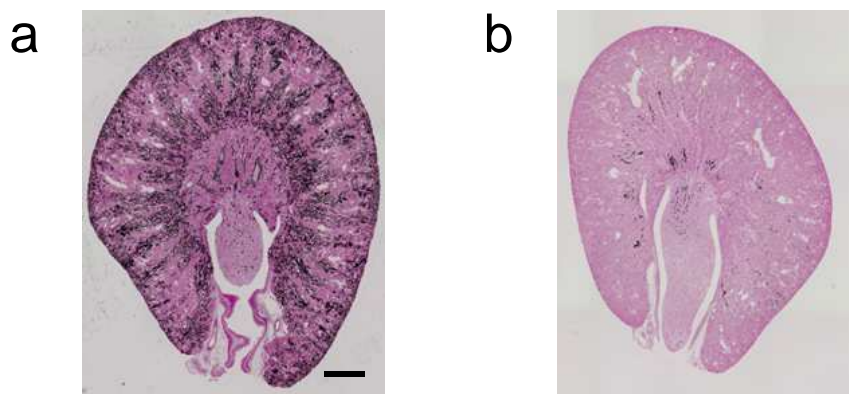


# Supplemental Figure 1. Shimomura *et al.*

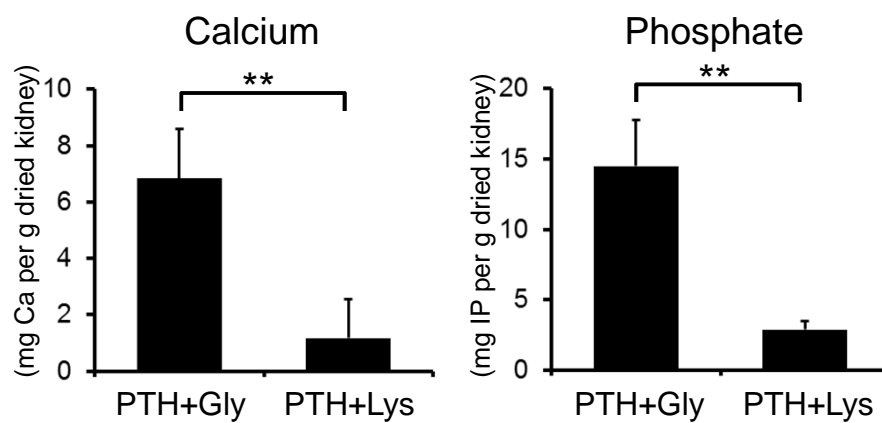
## A



## B

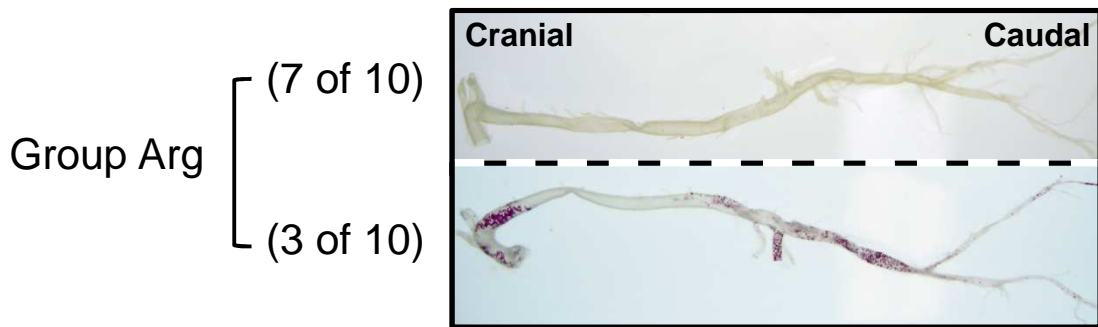


## C

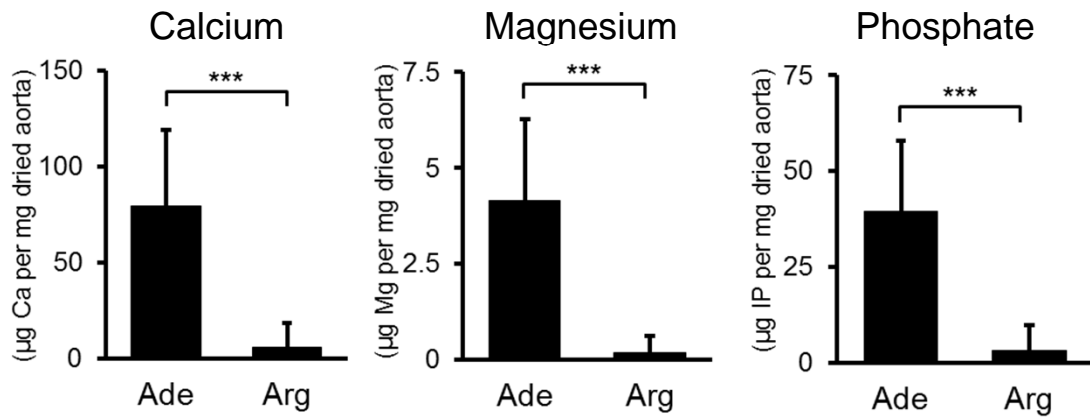


## Supplemental Figure 2. Shimomura *et al.*

### A

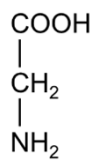


### B

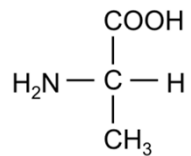


## Supplemental Figure 3. Shimomura *et al.*

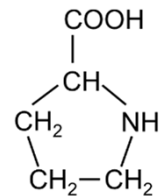
**Gly**



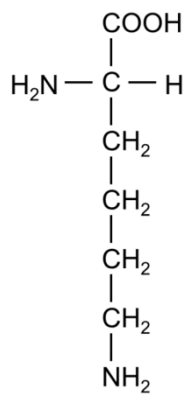
**Ala**



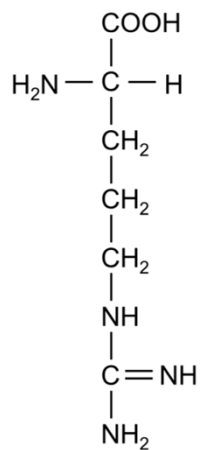
**Pro**



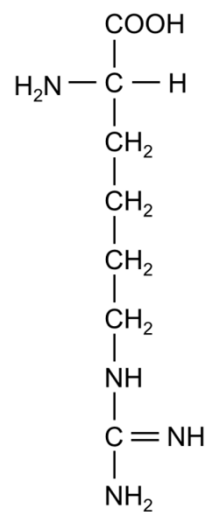
**Lys**



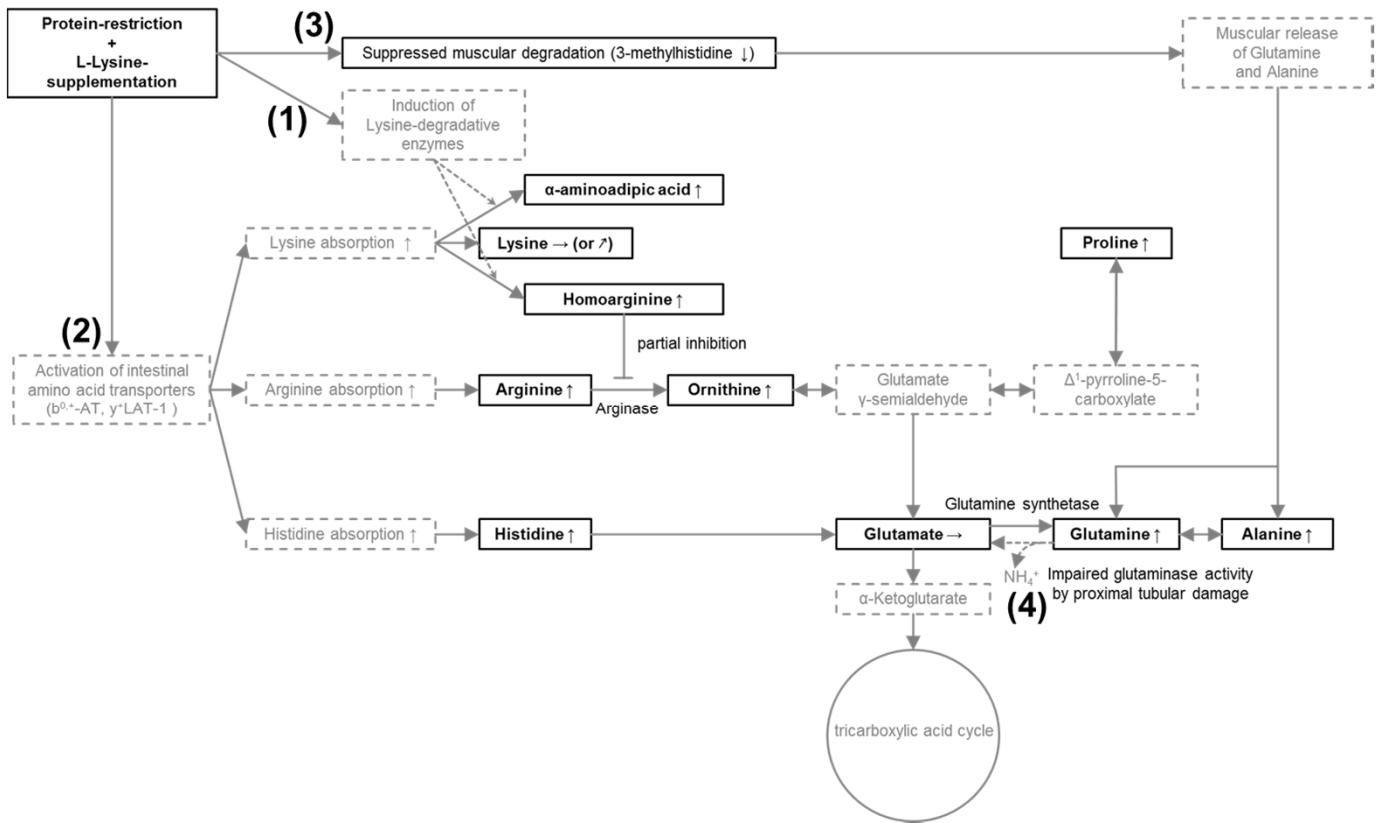
**Arg**



**Homo-Arg**



# Supplemental Figure 4. Shimomura *et al.*



## FIGURE LEGENDS

### Supplemental Figure 1

#### **L-Lys ameliorated nephrocalcinosis in rats.**

Six-week-old male Wistar rats were divided randomly into two groups: PTH+Gly and PTH+Lys. (A) Micro X-ray computed tomography and (B) von Kossa staining of the kidney revealed that (a) group PTH+Gly, but not (b) group PTH+Lys, developed nephrocalcinosis. (Scale bar: 1.0 mm) (C) Quantification of calcium and phosphate in the kidney confirmed that L-Lys suppressed nephrocalcinosis. All results are presented as means  $\pm$  SD (N=4 in each group: \*\*  $P < 0.01$ , non-paired  $t$  test).

### Supplemental Figure 2

#### **Dietary supplementation of L-Arg prevented arteries from calcification in adenine-induced uremic rats.**

Adenine rats supplemented with L-Arg (LP diet + 0.75% adenine + 2.5% L-arginine•HCl (group Arg)) were prepared in a similar way as the rats in Figure 1. (A) Alizarin red staining of the aorta revealed that the majority (7 of 10 animals) of L-Arg-supplemented adenine rats had no calcification. Only 3 of 10 animals developed partial vascular calcification. (B) The levels of calcium, magnesium, and phosphate of the aorta were quantified. The results were compared with the corresponding values of the rats in group Ade, as shown in Figure 2C. All results are presented as means  $\pm$  SD (N=10: \*\*\*  $P < 0.001$ , non-paired  $t$  test).

### Supplemental Figure 3

#### **Structures of Gly, Ala, Pro, Lys, Arg, and Homo-Arg are summarized.**

Precipitate-inhibitory amino acids (that is, Lys, Arg, and Homo-Arg) have two amino groups (-NH<sub>2</sub>).

### Supplemental Figure 4

#### **Putative metabolic pathways in group Lys are summarized.**

Several putative pathways regulated by dietary supplementation of L-Lys — (1) induction of lysine-degradative enzymes; (2) activation of intestinal amino acid transporters; and (3) suppression of muscular degradation — may explain the reason why the rats in group Lys had elevated plasma Ala, Pro, Arg, and Homo-Arg but not Lys. The solid boxes are experimentally confirmed changes, whereas the dotted boxes are

putative mediators. (4) Impaired glutaminase activity in the damaged proximal tubules may explain the reason, at least in part, why plasma glutamate levels were not elevated in group Lys.

### Supplemental Table 1.

Plasma amino acid levels of rats fed low protein (LP) diet containing 2.5% L-lysine•HCl without 0.75% adenine

Amino Acid (nmol/mL)	Values
Glycine (Gly)	250.0±45.6 <sup>NS</sup>
Alanine (Ala)	708.1±94.6 <sup>**</sup>
Serine (Ser)	421.6±53.5 <sup>**</sup>
Threonine (Thr)	38.3±6.8 <sup>*</sup>
Valine (Val)	112.2±17.9 <sup>***</sup>
Isoleucine (Ile)	52.9±12.4 <sup>**</sup>
Leucine (Leu)	95.6±20.9 <sup>*</sup>
Lysine (Lys)	1061.3±113.0 <sup>***</sup>
Arginine (Arg)	155.3±20.8 <sup>***</sup>
Histidine (His)	62.0±10.3 <sup>*</sup>
Tyrosine (Tyr)	41.8±13.8 <sup>*</sup>
Phenylalanine (Phe)	41.0±8.1 <sup>***</sup>
Tryptophan (Trp)	70.5±14.6 <sup>***</sup>
Methionine (Met)	32.6±5.6 <sup>***</sup>
Cystin (Cys2)	37.9±7.4 <sup>***</sup>
Proline (Pro)	243.5±43.5 <sup>**</sup>
Glutamine (Gln)	800.8±109.5 <sup>***</sup>
Glutamic acid (Glu)	99.8±19.6 <sup>*</sup>
Asparagine (Asn)	43.1±5.2 <sup>**</sup>
Asparatic acid (Asp)	9.3±1.4 <sup>**</sup>
α-Aminoadipic acid	7.3±2.0 <sup>***</sup>
Homoarginine (Homo-Arg)	11.8±3.7 <sup>***</sup>
α-aminobutyric acid	6.5±1.1 <sup>***</sup>
β-aminoisobutyric acid	Not detectable <sup>NS</sup>
γ-aminobutyric acid	Not detectable <sup>NS</sup>

Plasma amino acid levels of rats fed LP diet containing 2.5% L-lysine•HCl without 0.75% adenine are summarized. Because these rats had high plasma Lys, six of the ten measurements in plasma Lys exceeded the calibration range. All results are presented as means ± SD. Each value was compared with the corresponding value of group LP shown in Table 4. Statistical significance was evaluated by non-paired *t* test. (*N*=10: \* *P*<0.05; \*\* *P*<0.01; \*\*\* *P*<0.001)

## Supplemental Table 2.

Plasma ornithine and 3-methylhistidine levels of the adenine rats at 5 weeks

Amino Acid (nmol/mL)	Group LP	Group Ade	Group Gly	Group Lys
Ornithine (Orn)	43.4±7.5 <sup>NS</sup>	29.2±4.9 <sup>Ref</sup>	30.9±20.6 <sup>NS</sup>	45.8±20.1 <sup>*</sup>
3-methylhistidine (3MeHis)	2.0±0.5 <sup>***</sup>	27.1±4.9 <sup>Ref</sup>	30.8±7.1 <sup>NS</sup>	16.7±5.9 <sup>***</sup>

All results are presented as means ± SD. Statistical significance was evaluated by Dunnett's test. The values of Group Ade served as references. (N=10 in each group: \*  $P<0.05$ ; \*\*\*  $P<0.001$ )