The Tripartite Interaction of Phosphate, Autophagy and αKlotho in Health Maintenance

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One Sentence Summary: *Becn1* role in phosphate, αKlotho and lifespan

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Supplementary Information:

Supplementary Figure 1. Becn1F121A upregulates autophagic flux in vivo. *WT* (*Becn1*$^{WT/WT}$;GFP-LC3) and *BK/BK* (*Becn1*$^{FA/FA}$;GFP-LC3) mice at 12 weeks old were scarified 6 hours after chloroquine or vehicle (normal saline) treatment. There were 4 mice in each condition. (**A**) Representative microscopic images of GFP-LC3 immunofluorescence in the kidneys. Scale bar = 25 μm. Right panel is the summary of quantitation of GFP-LC3 puncta in renal tubules. Data are presented as mean ± S.D. for 4 mice per group. (**B**) Immunoblots of total kidney lysates for LC3 and p62 in the kidneys. Left panel is representative blot from 4 independent experiments. Right panel is summary and presented as mean ± S.D. *P<0.05, **P<0.01 between two groups by one-way ANOVA followed by Student-Newman-Keuls *post hoc* test for **A** and **B**. (**C**) Left panel is representative co-immunoprecipitation (Co-IP) of beclin 1 and BCL2 in total kidney lysates of mice at baseline. Right panel is representative blots of input protein from 3 independent Co-IP experiments.

Supplementary Figure 2. Characterize newly generated polyclonal rabbit anti-NaPi-2a and 2c antibodies. (**A**) Immunoblots for NaPi-2a and NaPi-2c in brush border membrane vesicles (BBMVs) from mouse kidney cortex and HEK-293 cells transiently transfected with empty eGFP vector, human eGFP-NaPi-2a- or 2c plasmids respectively. Two days later, cells were harvested. One representative blot is shown from 3 independent experiments. (**B**) Immunoblots for NaPi-2a and NaPi-2c in total kidney lysates of NaPi-2a/2c double null or 2c null mice and *WT* littermates at 8 weeks old. The representative blot from 4 mice in each genotyping group, respectively.

Supplementary Figure 3. Becn1F121A mice have lower apical membrane NaPi-2 transporters in the kidney than *WT* mice. *WT* mice (*Becn1*$^{WT/WT}$) and *BK/BK* (*Becn1*$^{F121A/F121A}$) mice at 12 weeks old were sacrificed and kidney sections were stained NaPi-2a (**A**) and 2c (**B**). The representative microscopic images were from 3 independent experiments. Scale bar = 25 μm. G: glomerulus.

Supplementary Figure 4. Becn1F121A upregulates renal αKlotho in growing *kl/kl* mice. Representative immunoblots of total kidney lysates for αKlotho protein in the kidney of 4 genotypes (*kl/kl*, *WT*, *BK/BK;kl/kl*, and *BK/BK* mice) at the age of 8 weeks. Hematoxylin was stain for contrast stain. Each genotype has 4 mice.

Supplementary Figure 5. Becn1FA upregulates renal αKlotho expression in *kl/kl* mice at 12 weeks old in a manner of Becn1-dose dependence. Representative immunoblots of total kidney lysates for αKlotho protein in the kidney of 4 genotypes (*WT, BK/BK, BK/+;kl/kl*, and *BK/BK;kl/kl* mice) at the age of 12 weeks. Each genotype has 4 - 6 mice.

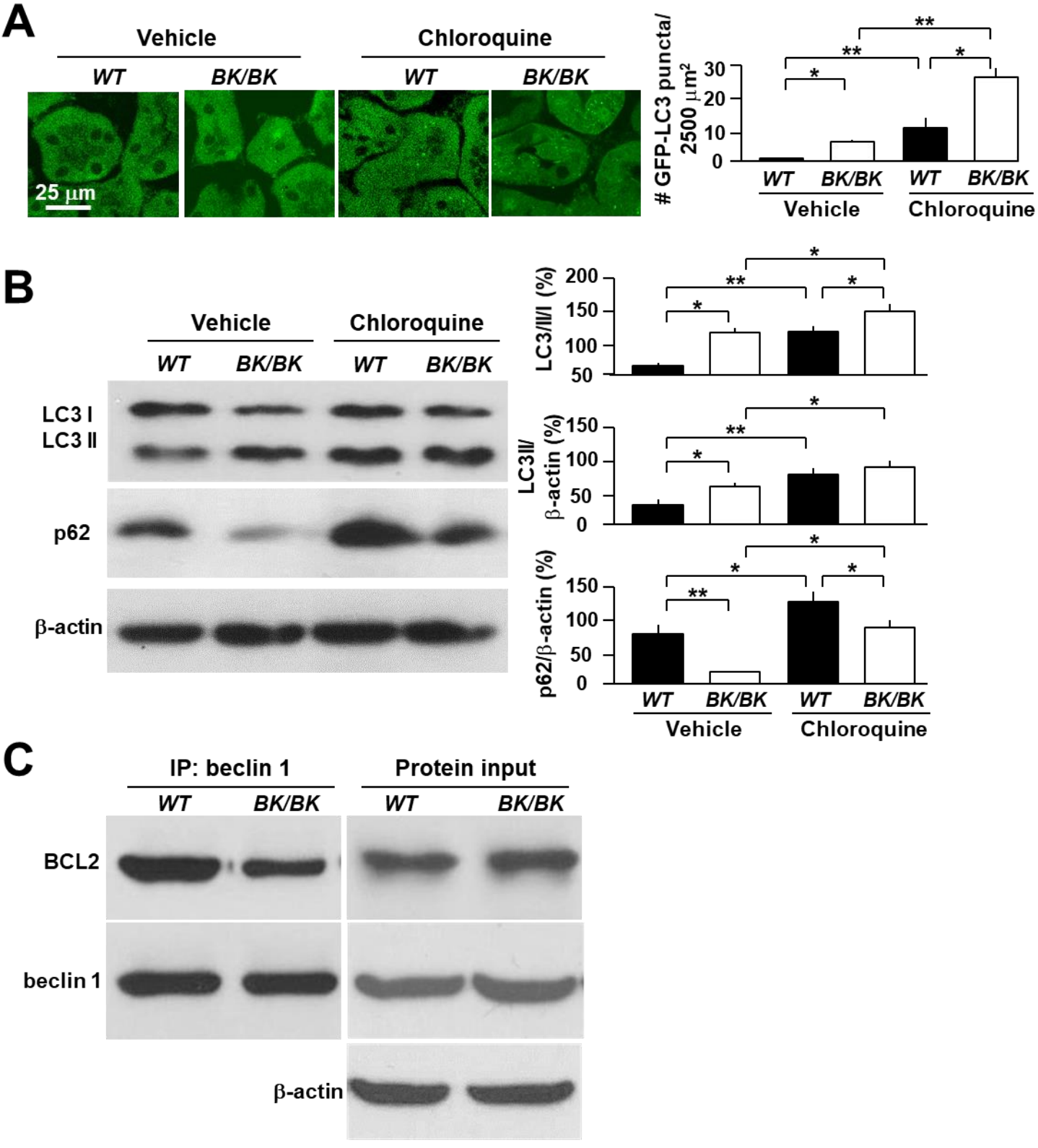
Supplementary Figure 6. Becn1F121A restores reproductive organs in *kl/kl* mice. (**A**) Comparison of female and male mouse body size among 4 genotypes (*kl/kl, WT, BK/BK;kl/kl,* and *BK/BK* mice) at 10 weeks old. Scale bar = 2 cm. (**B**) Comparison of female and male reproductive organs among 4 genotypes (*kl/kl, WT, BK/BK;kl/kl,* and *BK/BK* mice) at 10 weeks old. Scale bar = 0.3 cm. (**C**) Representative microscopic images of H&E stained gonad (Testis and ovary) sections of 4 genotypes (*kl/kl, WT, BK/BK;kl/kl,* and *BK/BK* mice). Scale bar = 100 μm. There are mature spermatids (black arrow heads) in well-developed seminiferous tubules of male *WT, BK/BK;kl/kl,* and *BK/BK* mice, but not in *kl/kl* mice. There are mature follicles (black arrows) in female *WT, BK/BK;kl/kl,* and *BK/BK* mice, but not in *kl/kl* mice. Each genotype has 4 male and 4 female mice for **A - C**.

Supplementary Figure 7. Trichrome stain in the kidney sections. Representative scanned images of Trichrome stain in the kidney sections encompassing cortex and outer medulla (OM) zones from 4 genotypes (*kl/kl, WT, BK/BK;kl/kl,* and *BK/BK* mice) at 10 weeks old. There are numerous trichrome stained areas (black arrows) in *kl/kl* mice, and a few in *BK/BK;kl/kl* mice, but not in *WT* and *BK/BK* mice. High-magnified figures are presented in Figure 3E. Each type has 4 male and 4 female mice. Scale bar = 500 μm.

Supplementary Figure 8. High Pi diet downregulates renal αKlotho mRNA expression. *WT* mice at 10 weeks old were fed with normal (N Pi) or high Pi (H Pi) diet for 2 weeks and αKlotho mRNA expression in the kidney was quantified with RT-qPCR. Data are presented as mean ± S.D. from 6 mice per group. **P<0.01 between two groups by unpaired *t*-test.

Supplementary Figure 9. High Pi induces cell injury *in vitro*. OK cells were treated normal Pi (0.96 mM) media or high Pi (2.0 and 3.0 mM) media for 24 hours after cells were confluent. The media were harvested for measurement of lactate dehydrogenase (LDH), cell injury marker, with LDH cytotoxicity Assay Kit. The data is presented as mean ± S.D. from 4 independent experiments. *P<0.05, **P<0.01 between two groups by one-way ANOVA followed by Student-Newman-Keuls *post hoc* test.

Supplementary Figures



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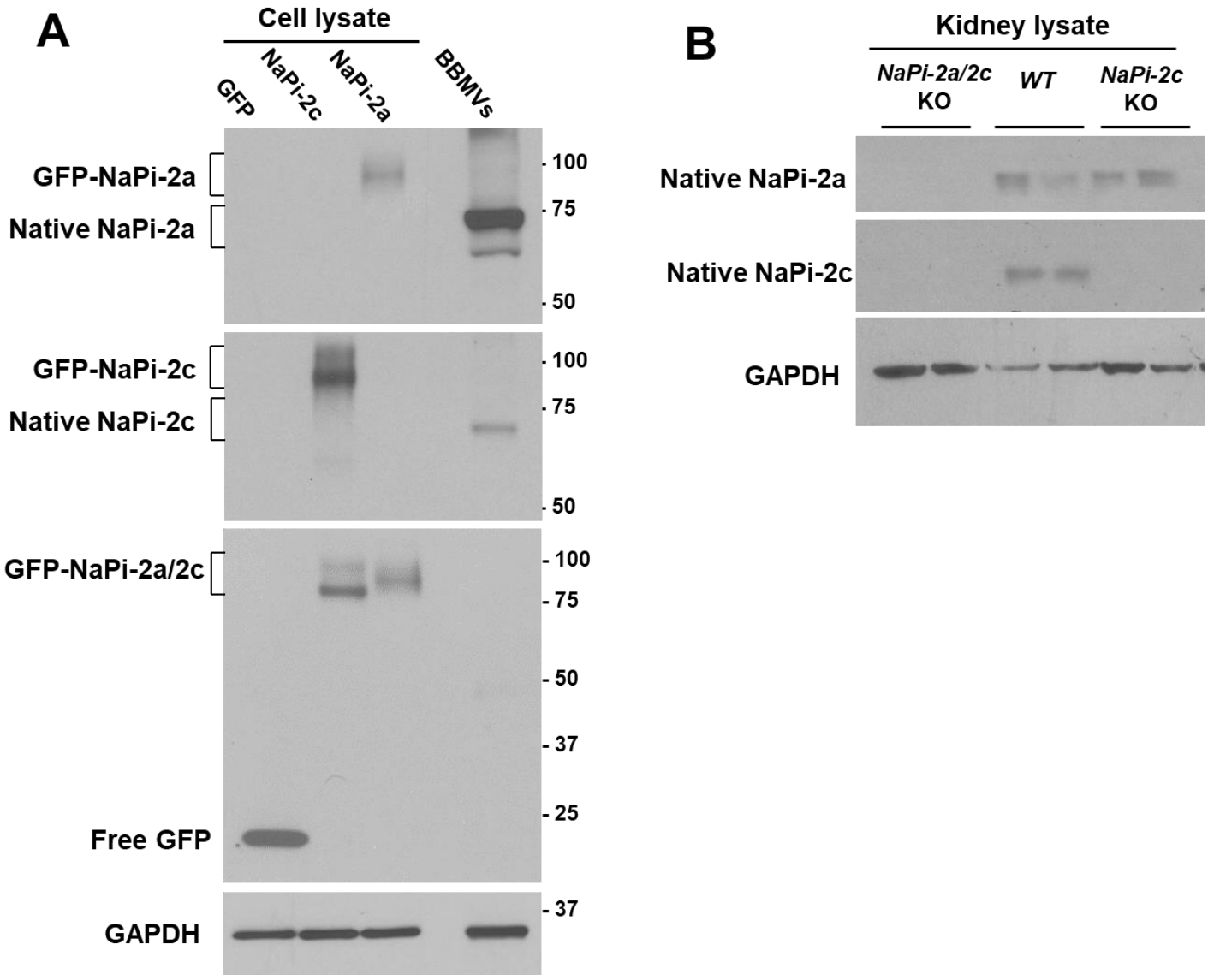

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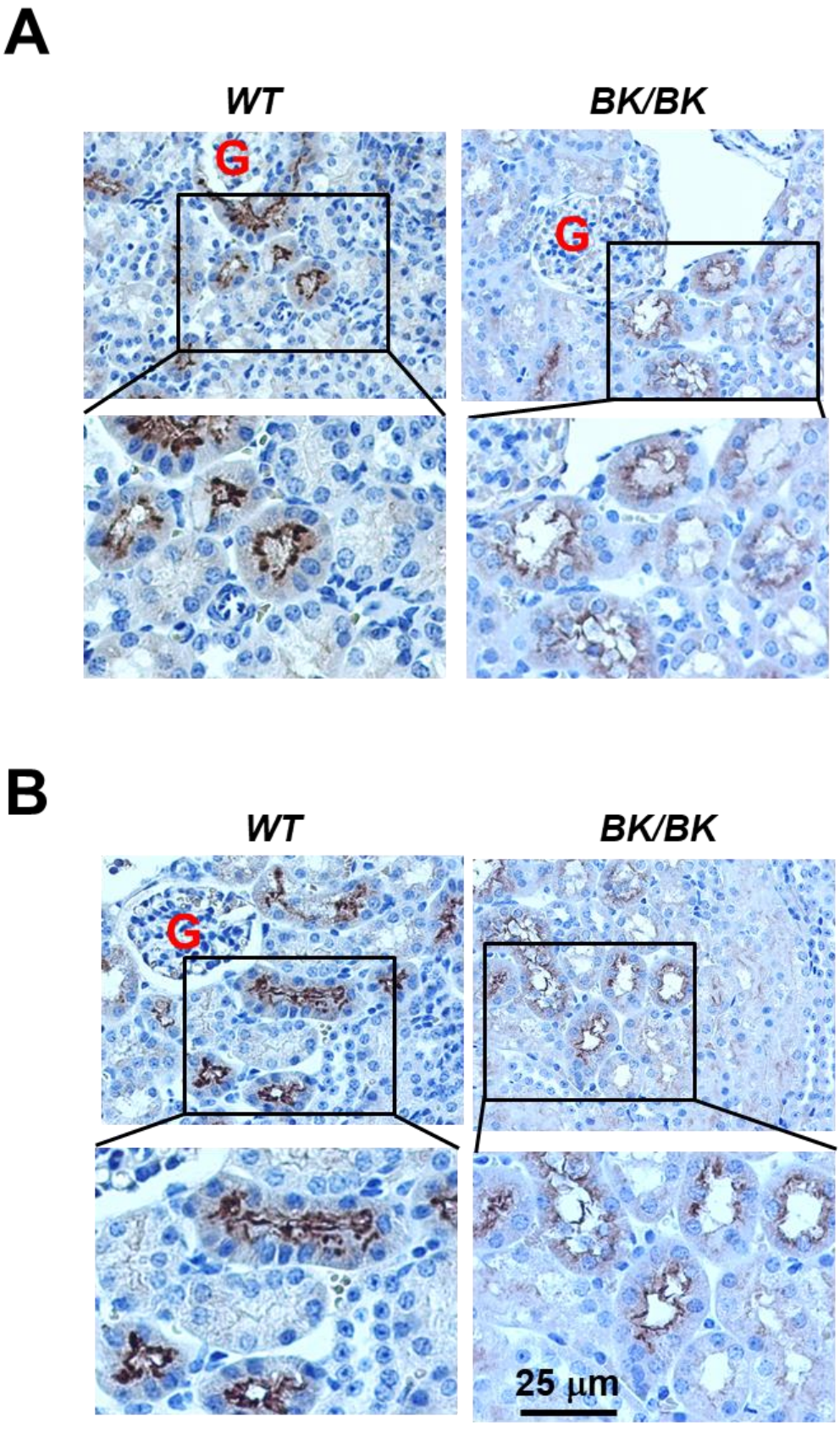

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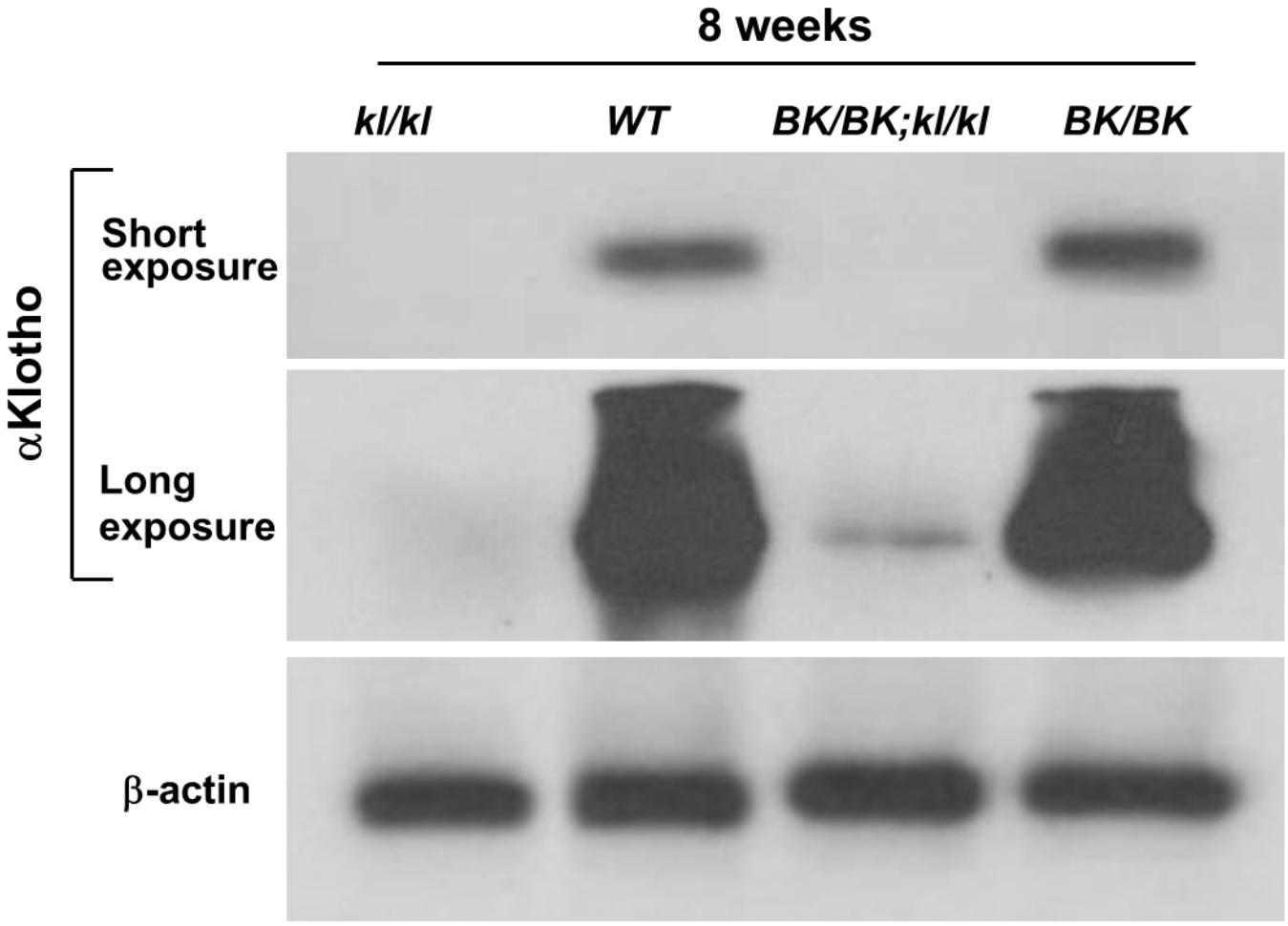

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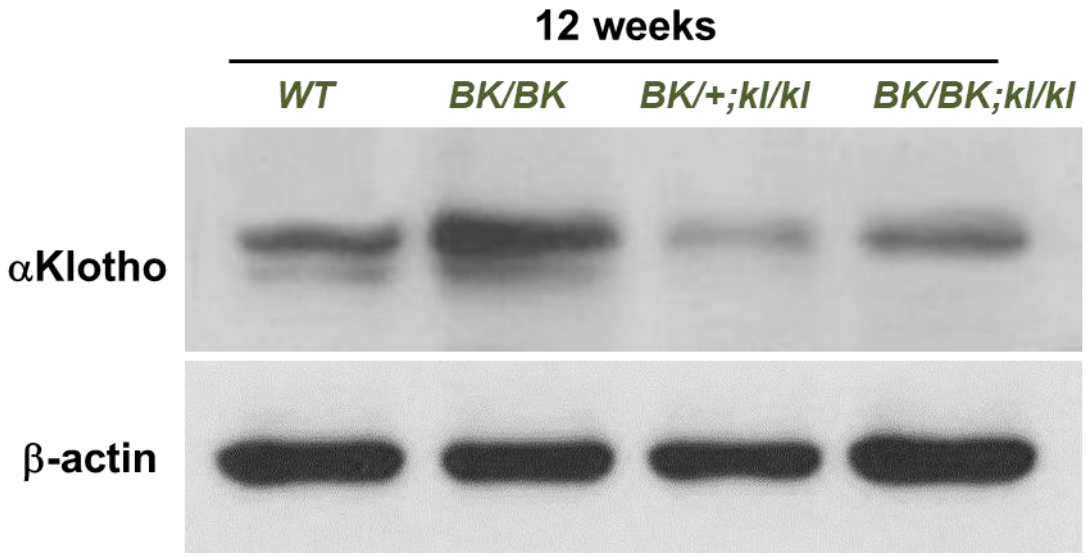

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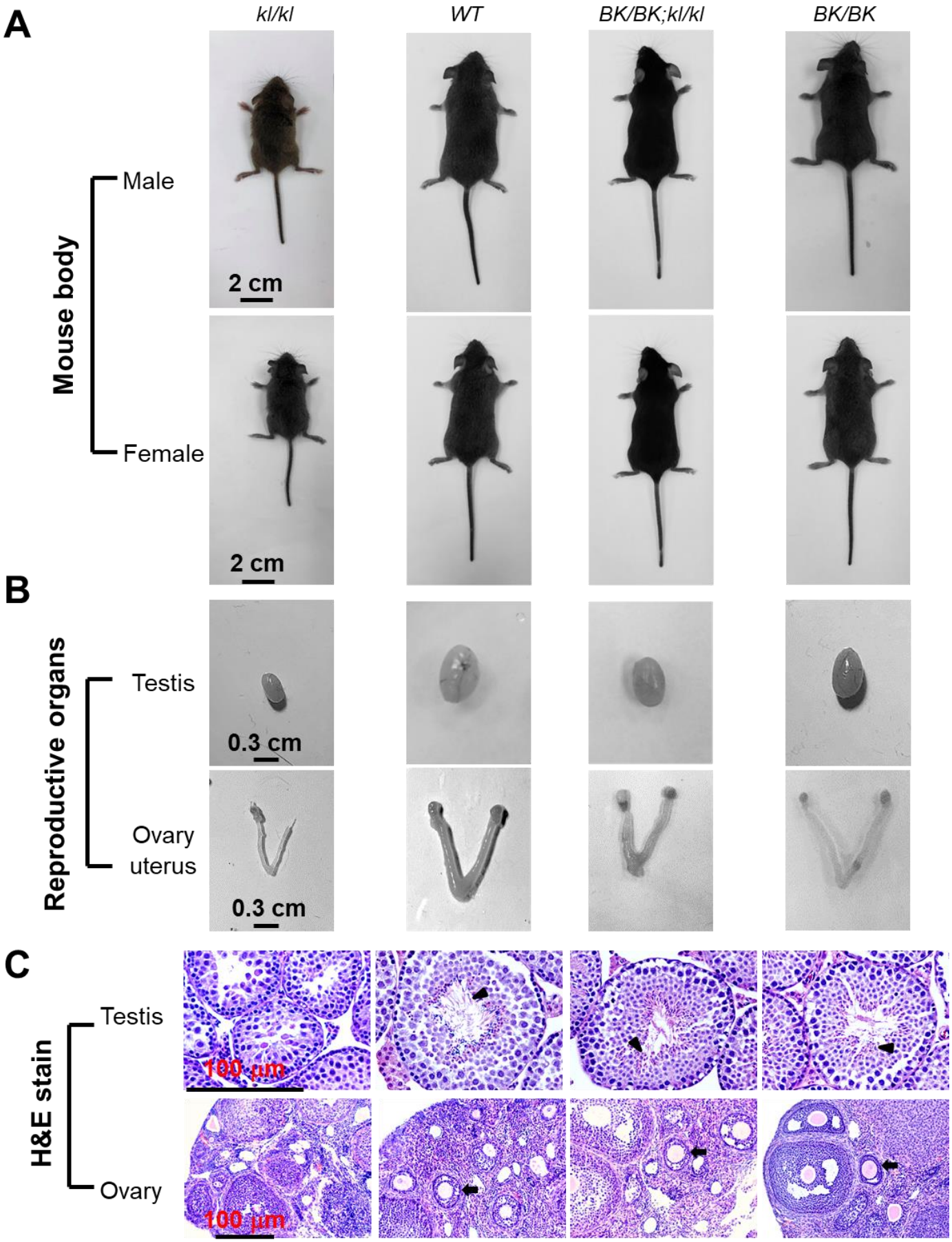

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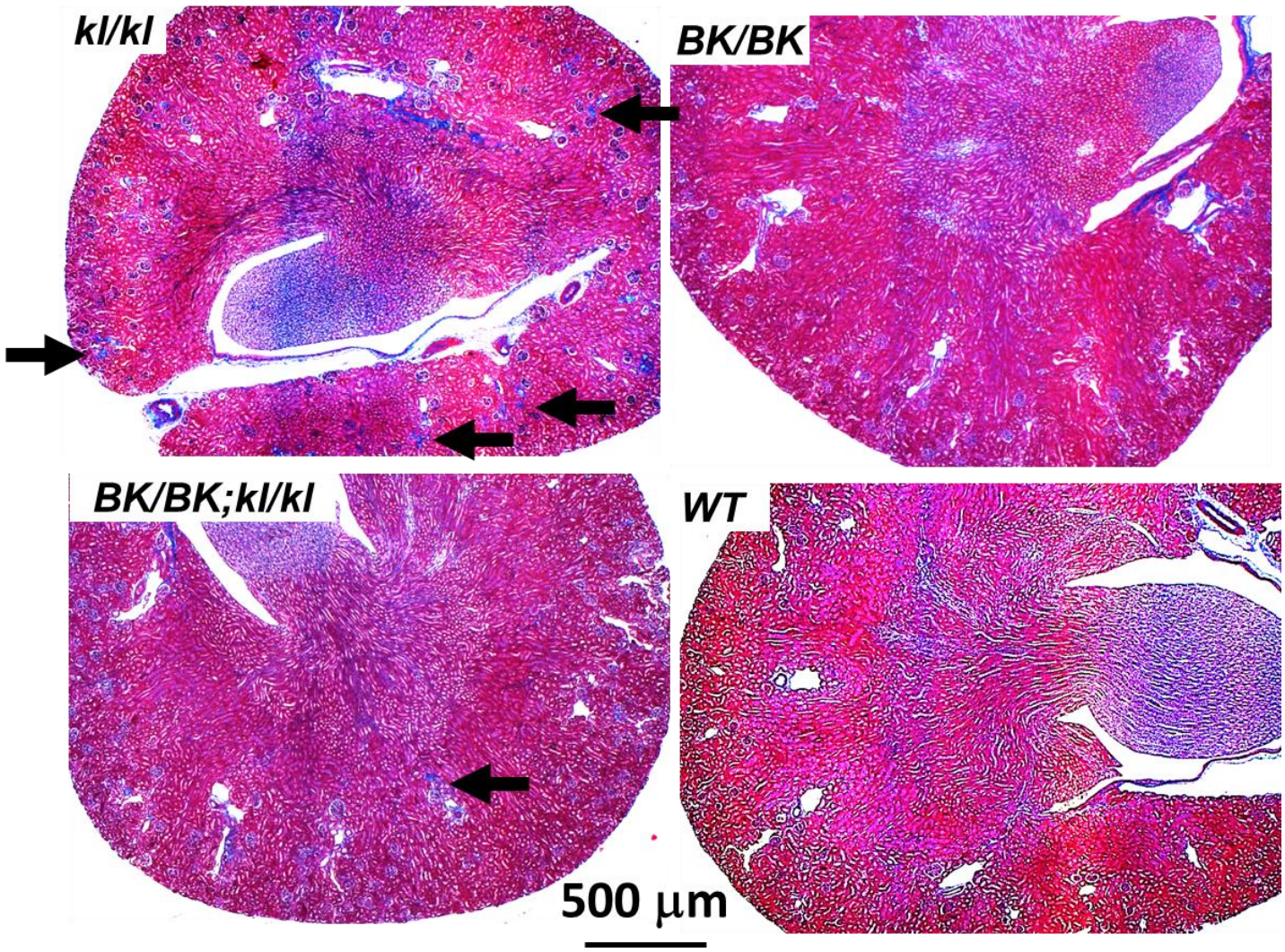

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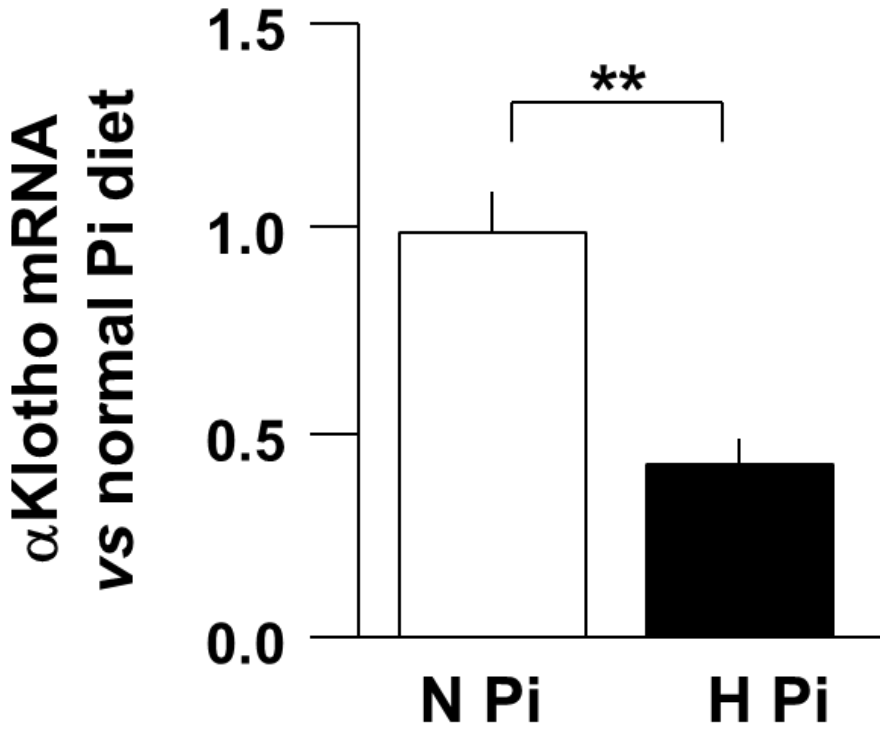

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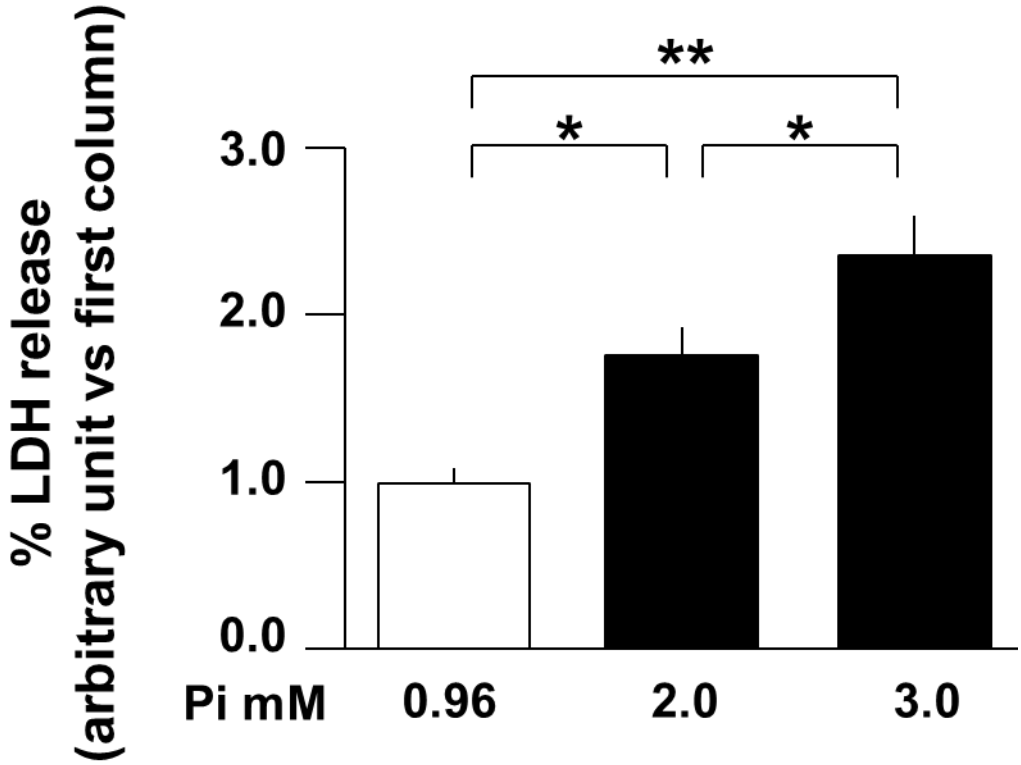

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