

Potassium depletion stimulates Na-Cl cotransporter *via* phosphorylation and  
inactivation of the ubiquitin ligase Kelch-like 3

Supplemental Information

Kenichi Ishizawa<sup>a, f</sup>, Ning Xu<sup>a, b, f</sup>, Johannes Loffing<sup>c</sup>, Richard P. Lifton<sup>d</sup>, Toshiro Fujita<sup>e</sup>,  
Shunya Uchida<sup>a</sup>, Shigeru Shibata<sup>a, e</sup>

<sup>a</sup>Division of Nephrology, Department of Internal Medicine, Teikyo University School of  
Medicine, Tokyo, Japan. <sup>b</sup>Department of Nephrology, Tianjin First Central Hospital, Tianjin,  
China. <sup>c</sup>Institute of Anatomy, University of Zurich, Zurich, Switzerland. <sup>d</sup>Department of  
Genetics, Yale University School of Medicine, Connecticut, U.S.A. <sup>e</sup>Division of Clinical  
Epigenetics, Research center for Advanced Science and Technology, The University of Tokyo,  
Japan. <sup>f</sup>These authors equally contributed to the work.

All correspondence to: Shigeru Shibata, M.D., Ph.D.

Division of Nephrology, Department of Internal medicine, School of Medicine, Teikyo  
University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan.

Tel: 81-3-3964-1211; Fax: 81-3-3964-8942; e-mail: shigeru.shibata@med.teikyo-u.ac.jp

## **Supplemental Figure Legends**

### **Supplemental Figure 1**

#### **KLHL3<sup>S433-P</sup> antibody recognizes phosphorylated but not non-phosphorylated KLHL3 peptide.**

Monoclonal  $\alpha$ -KLHL3<sup>S433-P</sup> antibody was incubated with phosphorylated and non-phosphorylated KLHL3 peptides on a nitrocellulose membrane, followed by the incubation with peroxidate-conjugated anti-mouse antibody. The signal was visualized by ECL reagents.

### **Supplemental Figure 2**

#### **Specificity of WNK4 antibody.**

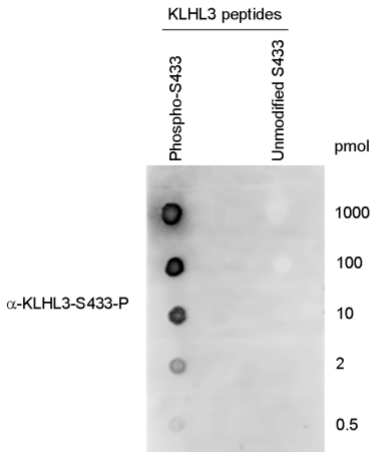
HEK cell lysates expressing WNK4-HA or no WNK4 were analyzed by Western blotting using  $\alpha$ -WNK4 antibody.

### **Supplemental Figure 3**

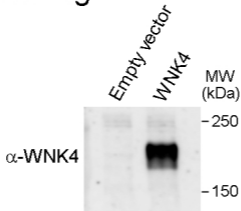
#### **KLHL3<sup>S433-P</sup> staining in the kidney of mice on a normal-K<sup>+</sup> or a low-K<sup>+</sup> diet.**

Kidney sections stained for  $\alpha$ -KLHL3<sup>S433-P</sup> (green, indicated by arrows) and  $\alpha$ -aquaporin 2 (AQP2, a marker for principal cells of the collecting duct, blue) in the indicated mice. The increase in KLHL3<sup>S433-P</sup> by low K<sup>+</sup> was not evident in the AQP2-positive principal cells (arrowheads). Scale bars represent 50  $\mu$ m. Glo, glomeruli.

# Supplemental Fig.1



# Supplemental Fig.2



Supplemental Fig.3

