# SUPPLEMENTAL TABLES AND FIGURES

Table S1: Physiological blood parameters from *Atp6v1b1*$^{+/+}$ and *Atp6v1b1*$^{-/-}$ mice fed a normal diet (0.3% $Na^+$) or a low $Na^+$ diet (0% $Na^+$)

| | **0.3 % $Na^+$** | | **0 % $Na^+$** | |
|---|---|---|---|---|
| | *Atp6v1b1*$^{+/+}$ | *Atp6v1b1*$^{-/-}$ | *Atp6v1b1*$^{+/+}$ | *Atp6v1b1*$^{-/-}$ |
| pH | 7.23 ± 0.01 (10) | 7.26 ± 0.01 (9) | 7.23 ± 0.01 (10) | 7.27 ± 0.01 (9)* |
| $PCO_2$, mmHg | 47.2 ± 1.8 (10) | 50.0 ± 1.2 (9) | 52.3 ± 1.8 (9) | 52.2 ± 1.3 (9) |
| c[$HCO_3^-$], mmol/L | 19.2 ± 0.9 (10) | 21.5 ± 0.6 (9)* | 21.2 ± 0.4 (10) | 23.3 ± 0.8 (9)* |
| $PO_2$ mmHg | 85.2 ± 1.8 (10) | 93.9 ± 3.2 (9)* | 83.3 ± 3.2 (10) | 85.0 ± 3.6 (9) |
| [$Na^+$], mmol/L | 147.8 ± 1.2 (9) | 147.3 ± 0.7 (8) | 147.1 ± 0.3 (10) | 144.3 ± 0.8 (9)** |
| [$K^+$], mmol/L | 4.04 ± 0.12 (10) | 3.57 ± 0.15 (9)* | 3.81 ± 0.18 (9) | 3.38 ± 0.07 (9)* |
| [$Cl^-$], mmol/L | 120.4 ± 0.9 (10) | 121.0 ± 0.7 (9) | 119.2 ± 0.4 (10) | 115.1 ± 1.0 (9)** |
| [Proteins], g/L | 45.7 ± 0.8 (10) | 50.2 ± 1.2 (9)** | 44.4 ± 1.3 (10) | 53.1 ± 2.5 (8)** |

Values are the means ± S.E (n), n= number of mice studied. Statistical significance between groups was assessed by unpaired Student’s t-test. * $p<0.05$ vs wild type mice fed same diet; ** $p<0.01$ vs wild type mice fed same diet.

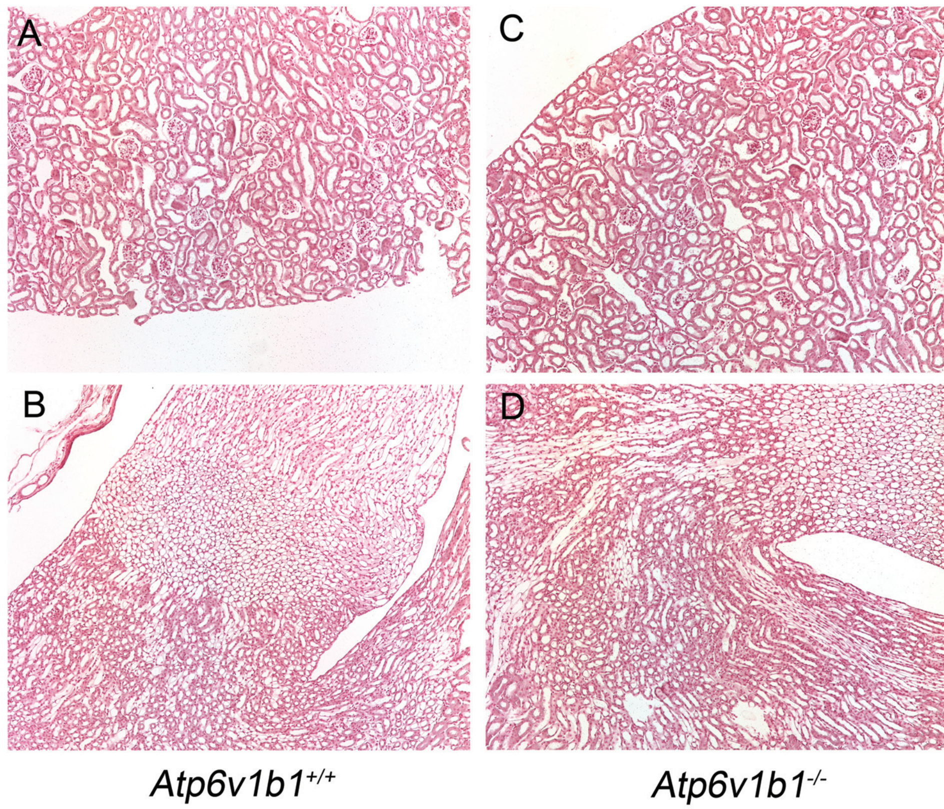



**Figure S1**

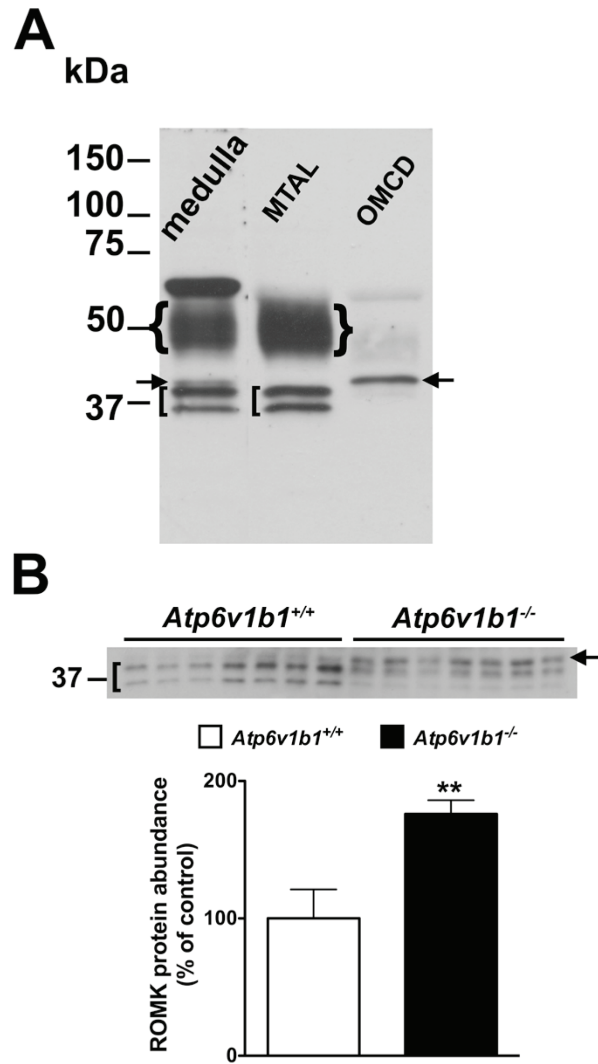
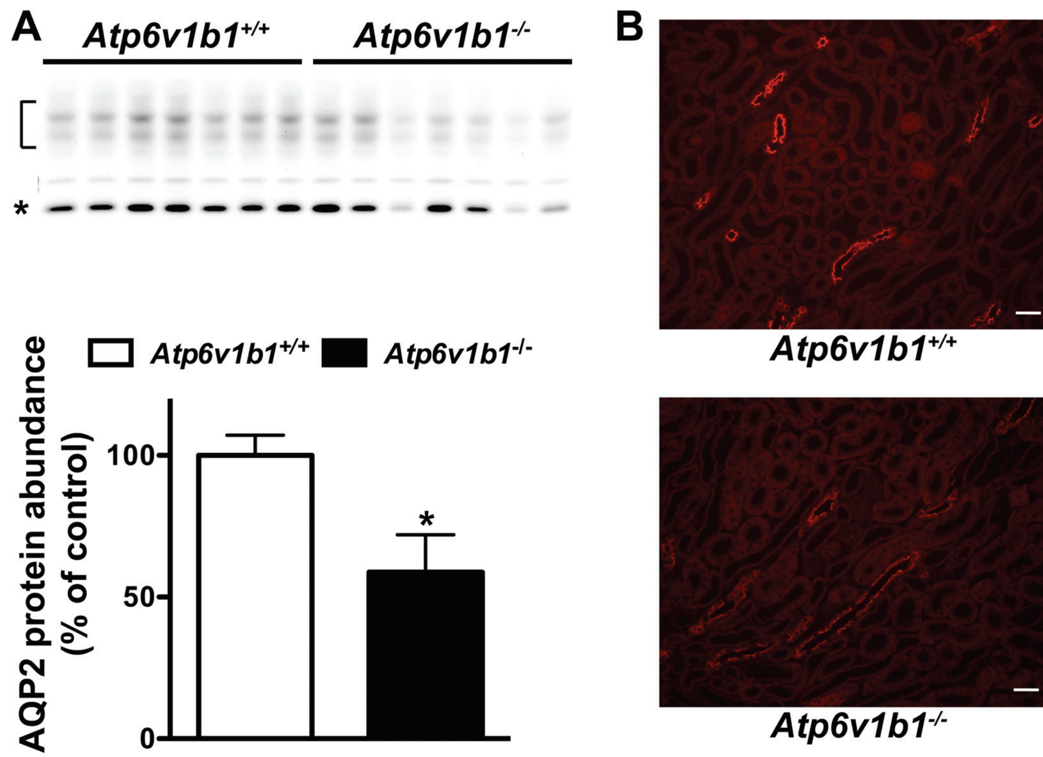
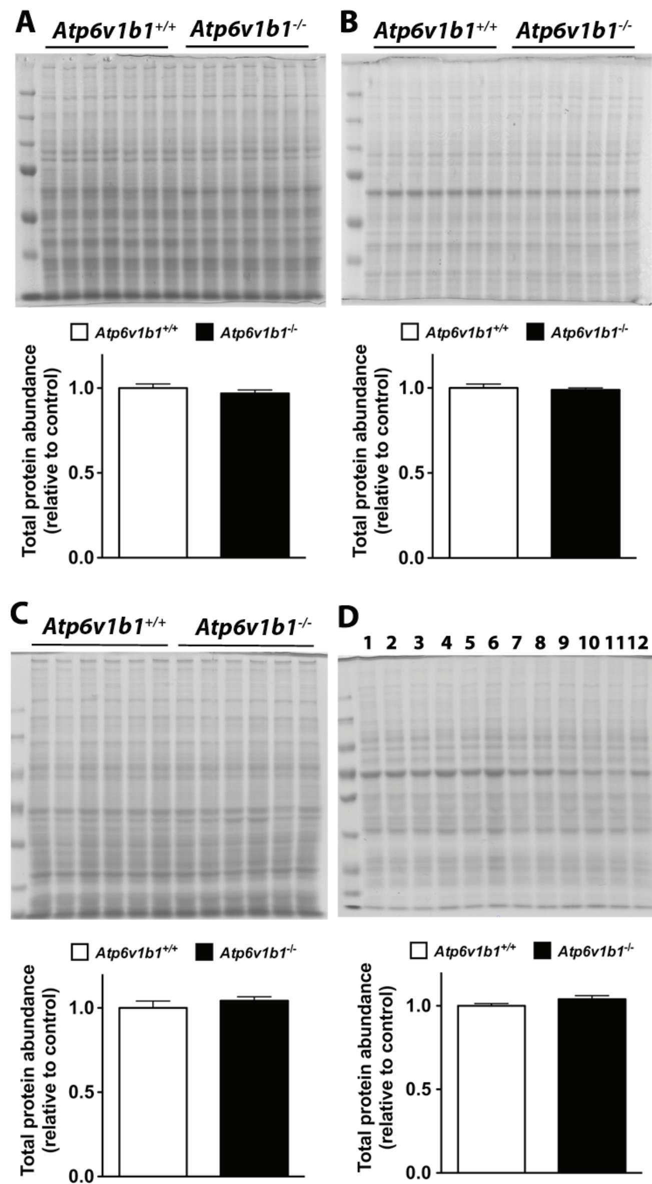



Figure S2




# Figure S3




# Figure S4

## LEGENDS FOR SUPPL. FIGURES

**Figure S1: Von Kossa stainings of sections of kidneys from *Atp6v1b1*$^{-/-}$ and *Atp6v1b1*$^{+/+}$**
A, renal cortex from *Atp6v1b1*$^{+/+}$ mouse, B renal medulla from *Atp6v1b1*$^{+/+}$ mouse, C renal cortex from *Atp6v1b1*$^{-/-}$ mouse, and D renal medulla from *Atp6v1b1*$^{-/-}$ mouse.

**Figure S2: Western blots for ROMK on kidney samples. A.** Molecular profiling of ROMK was assessed by Western blots in renal medullary homogenate (medulla), medullary thick ascending limbs (MTAL) or outer medullary collecting ducts (OMCD) from *Atp6v1b1*$^{+/+}$ mice. In MTAL, anti ROMK antibody detects a broad band centered at 50kDa (curly bracket) and a doublet at 37 kDa (square bracket). In OMCD, anti ROMK antibody detects a major band that migrated above 37 kDa (arrow). A broad 50 kDa band was barely detected. In medulla, anti ROMK antibody detects bands that were seen in MTAL (the broad band centered at 50 kDa –curly bracket- and the doublet centered at 37 kDa – square bracket-) plus the band above 37kDa (arrow) that was seen in OMCD. Anti ROMK antibody also detects an additional sharp 65 kDa band (asterisk). 5 µg proteins of medullary homogenate or approximatively 40 tubule segments were loaded per gel lane. **B.** Effects of *Atp6v1b1* disruption on ROMK protein expression in medullary collecting ducts. ROMK protein abundance was assessed by Western blots of medullary homogenates from *Atp6v1b1*$^{-/-}$ and *Atp6v1b1*$^{+/+}$. Each lane was loaded with a protein sample from a different mouse. 5 µg proteins were loaded per gel lane. Equal loading was confirmed by parallel Coomassie-stained gels. Anti ROMK antibody detects a triplet around 37 kDa in which the upper band corresponds to the molecular form of ROMK expressed exclusively in the collecting duct (arrow), whereas the two lower bands are polypeptides whose expression is restricted to the thick ascending limb (square bracket). Bar graph shows summary of densitometric analyses of the upper band (arrow). Statistical significance was assessed by unpaired Student's t-test. ** $P<0.01$ vs. *Atp6v1b1*$^{+/+}$

**Figure S3: AQP2 expression assessed in *Atp6v1b1*$^{-/-}$ and *Atp6v1b1*$^{+/+}$ mice by Western blot on renal cortex (A) or immunohistochemistry on kidney sections (B).** A. Each lane was loaded with a protein sample from a different mouse. Equal loading was confirmed by parallel Coomassie-stained gels. 15 µg proteins were loaded per gel lane. Bracket and asterisk show the glycosylated 37 kDa and the unglycosylated 25 kDa forms of AQP2, respectively.

Bar graph shows summary of densitometric analyses of the bracketed and asterisked bands. Statistical significance was assessed by unpaired Student's t-test. * $P<0.05$ vs. *Atp6v1b1*$^{+/+}$. B. Renal cortex from *Atp6v1b1*$^{+/+}$ (upper panel) and *Atp6v1b1*$^{-/-}$ (lower panel) mice. Scale bars: 100 μm.

**Figure S4: Representative Coomassie blue-stained polyacrylamide gels used to control protein loading.** Protein samples loaded on gels are the same as those used for subsequent Western blots. A. Whole cortex homogenates from *Atp6v1b1*$^{+/+}$ or *Atp6v1b1*$^{-/-}$ mice. B. Whole medulla homogenates from *Atp6v1b1*$^{+/+}$ or *Atp6v1b1*$^{-/-}$ mice. C. Whole cortex homogenates from indomethacin treated *Atp6v1b1*$^{+/+}$ or *Atp6v1b1*$^{-/-}$ mice. D. Whole medulla homogenates from indomethacin treated *Atp6v1b1*$^{+/+}$ (odd numbers) or *Atp6v1b1*$^{-/-}$ (even numbers) mice. 15 μg proteins per lane were loaded on gels A and C. 5 μg proteins per lane were loaded on gels B and D. Bands from these gels were analyzed using densitometry to provide quantitative assessment of loading. Density values were normalized by dividing values by the mean value for the entire set of samples. Thus the mean for the entire set of samples is defined as 1. Normalized density values were never below 0.85 or higher than 1.15. To facilate comparisons between groups, density values were normalized by dividing by the mean value for the control group. Thus the mean for the control group is defined as 1. Bar graphs show summary of these densitometric analyses. Normalized band densities for mutants were compared with controls using an unpaired *t*-test. Values are means ± S.E.. In all gels, no difference was noted between control and mutant mice. These loading gels established that subsequent immunoblots (loaded identically) were uniformly loaded.