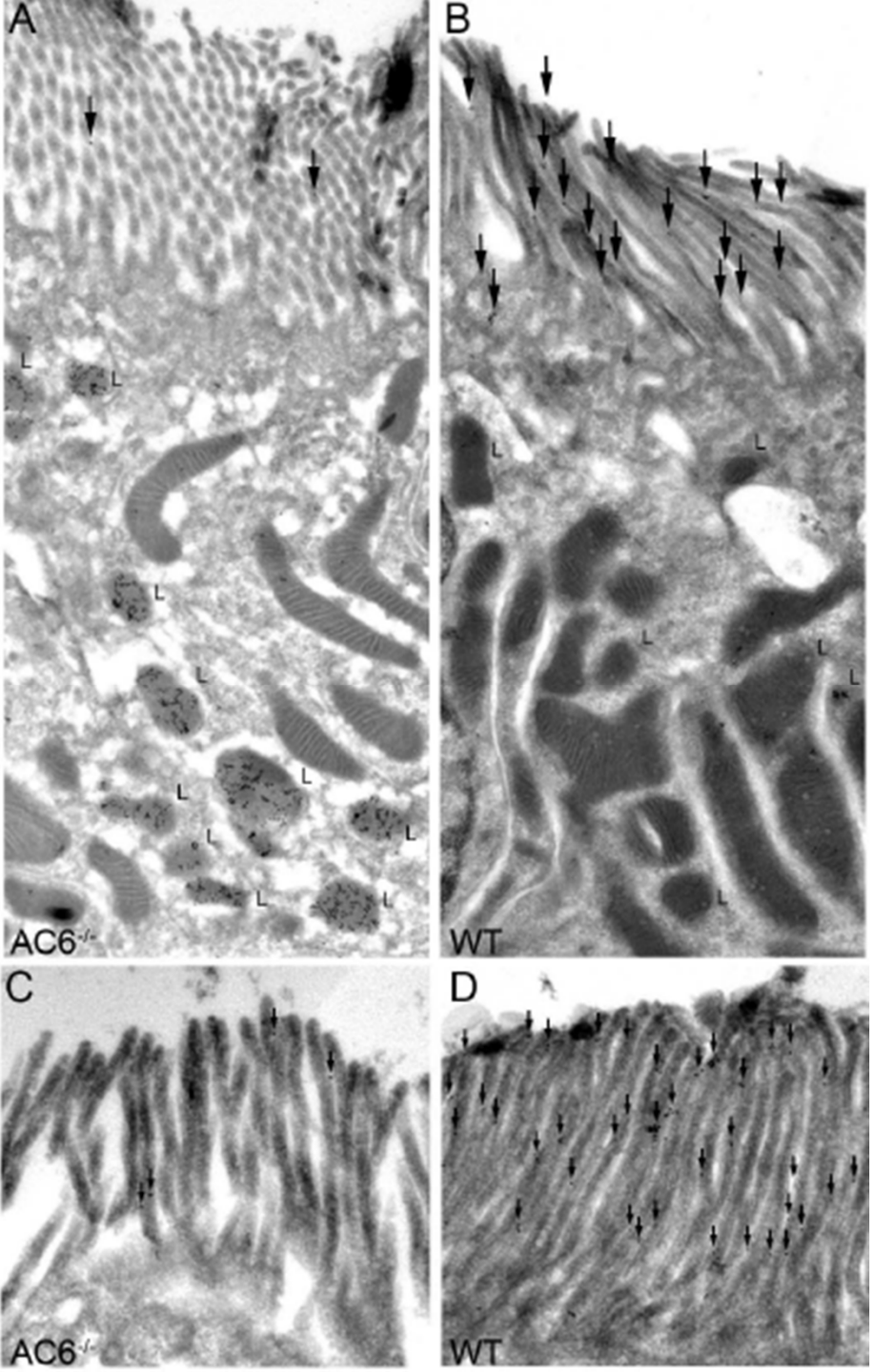
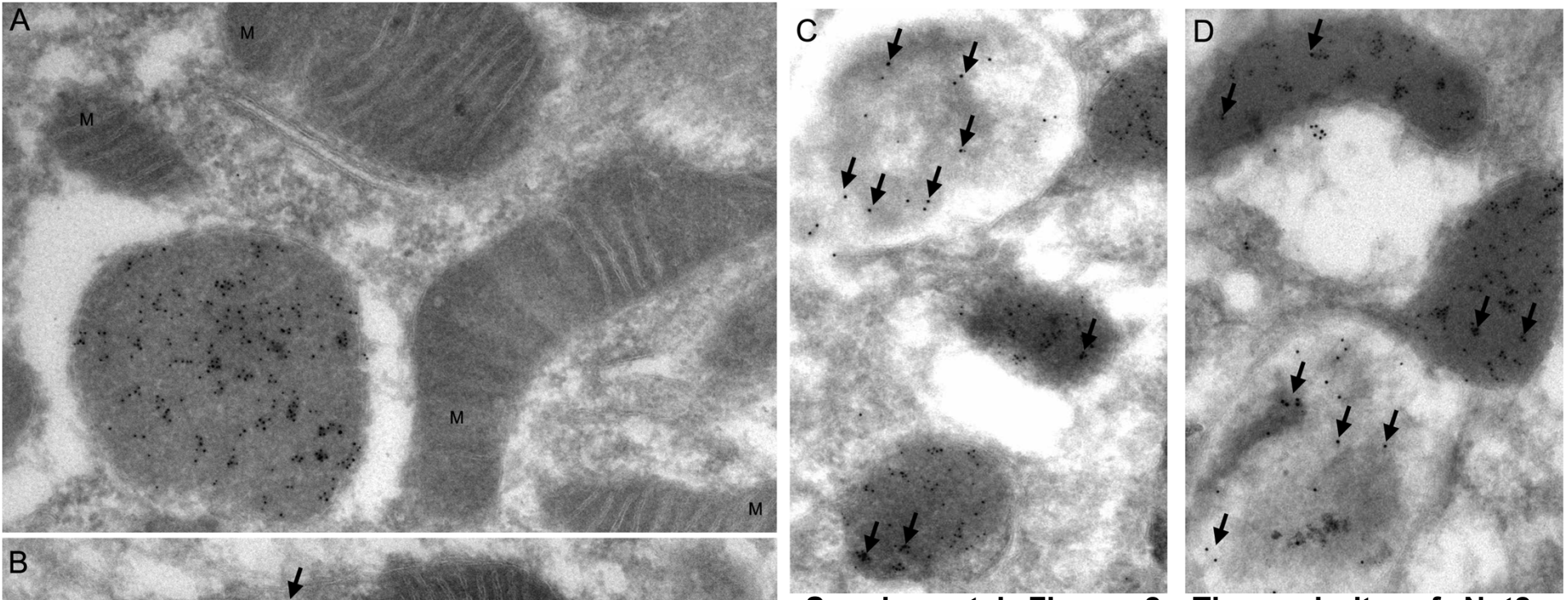
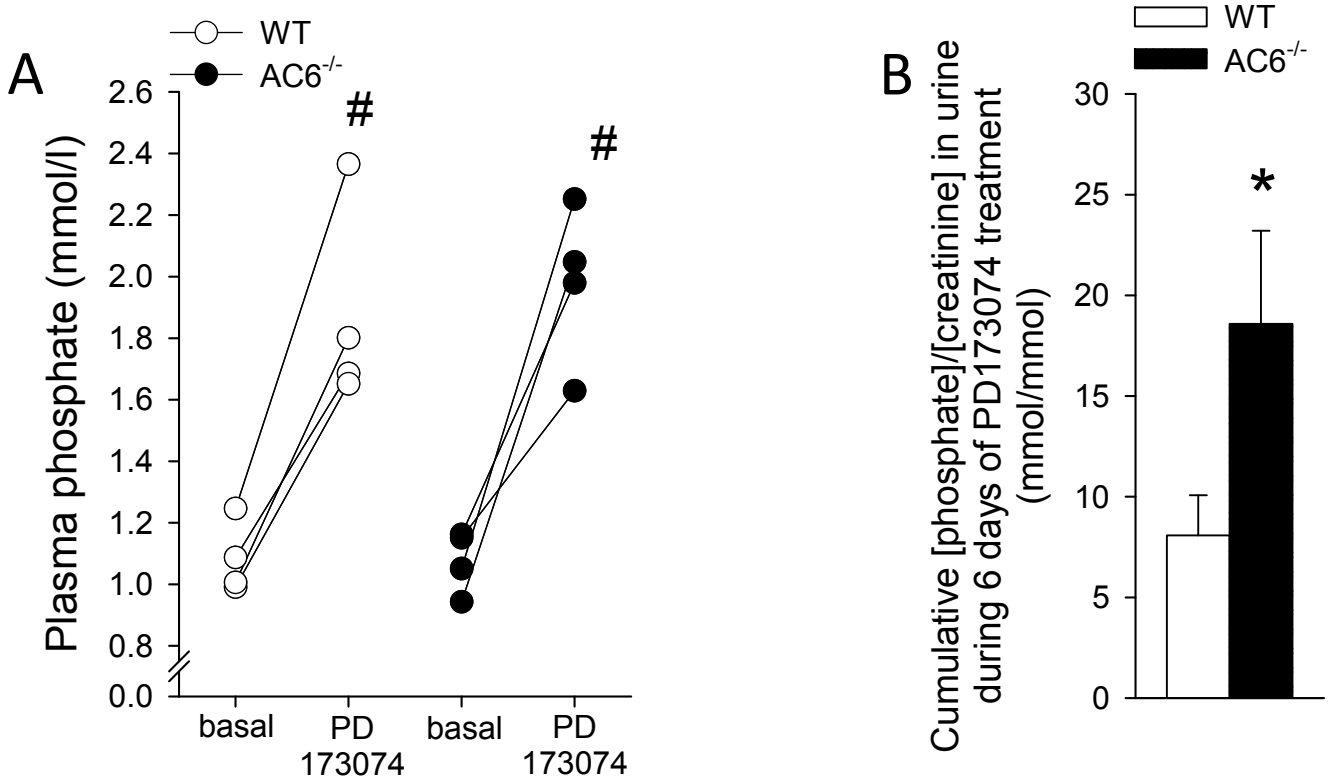


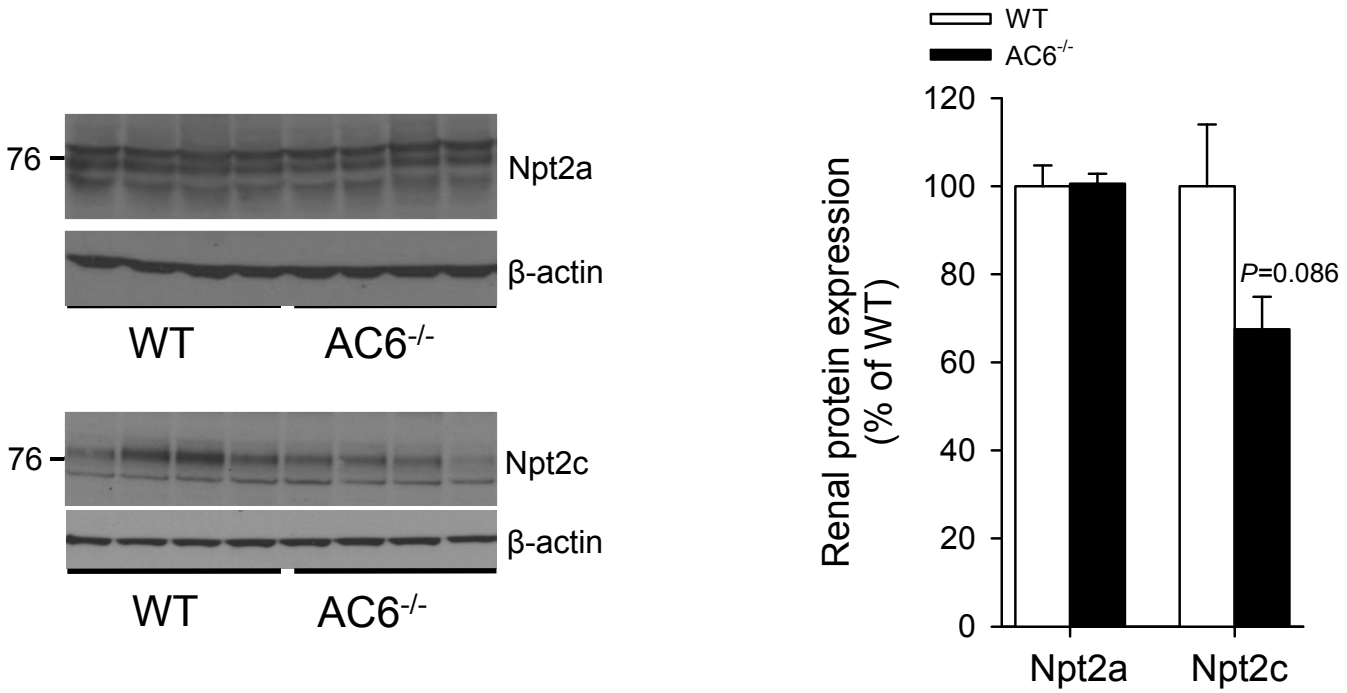
Supplemental Figure 1. Immunogold electron microscope localization of Npt2a in proximal tubule of wild-type (WT) and AC6 knockout ($AC6^{-/-}$) mice. A) Low magnification cross section of early proximal tubule cell demonstrating that gold particles representing Npt2a are abundantly observed in structures morphologically resembling lysosomes (L), with little labeling associated with the microvilli (arrows) in $AC6^{-/-}$ mice. B) In contrast, in WT mice gold particles representing Npt2a are abundantly observed in the microvilli (arrows), with little labeling of lysosomes. C and D) Higher magnification demonstrating little Npt2a labeling is associated with the microvilli (arrows) of $AC6^{-/-}$ mice compared to WT mice. Gold particles are 10 nm in diameter.



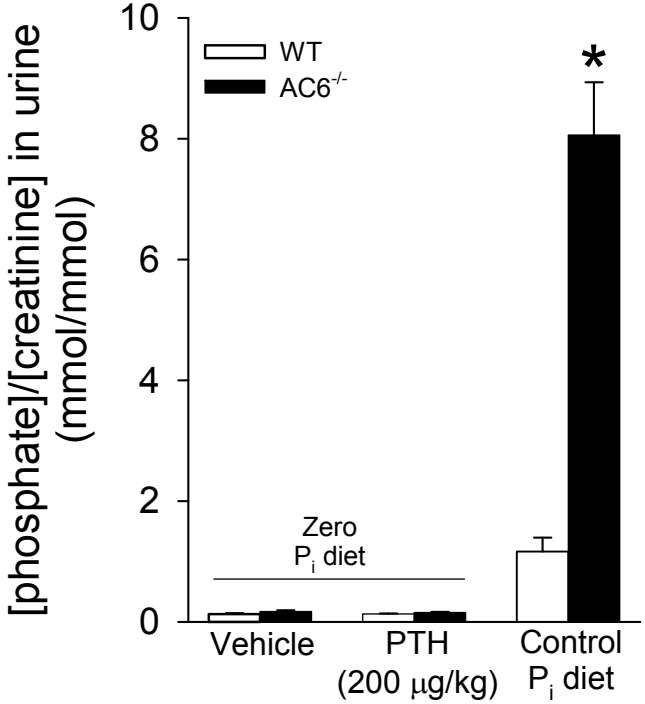
Supplemental Figure 2. The majority of Npt2a resides in secondary lysosomes within proximal tubule cells of AC6$^{-/-}$ mice. (A) Immunogold electron microscopy image demonstrating an abundance of gold particles representing Npt2a (10 nm gold particles) in electron dense structures morphologically resembling lysosomes, and distinct from other electron dense structures such as mitochondria (M). (B) Double labelling immunogold electron microscopy with Npt2a (5nm gold particles) and AtpB (beta subunit of ATP synthase, 10nm gold particles, arrows) confirms Npt2a labelling is not in mitochondria. In contrast, Npt2a (5nm gold particles) co-distributes to some extent with (C) LAMP2 or (D) Cathepsin B (10 nm gold particles, arrows) positive structures, suggesting that Npt2a is confined to a cohort of secondary lysosomes.



Supplemental Figure 3. Effect of FGF receptor blockade on plasma phosphate and urinary P_i excretion in wild-type (WT) and AC6 knockout (AC6$^{-/-}$) mice. WT and AC6$^{-/-}$ mice were treated with the FGF receptor inhibitor PD173074 (50 mg/kg in Cremophor EL/ethanol/water; 7.5:2.5:90 via oral gavage, 1% of body weight) every 12 hours for 6 days.[1,2] Blood was collected before and 6 days after treatment, spontaneous urine was collected daily. A) Treatment with PD173074 significantly increased plasma phosphate in both genotypes. B) Of note, cumulative urinary phosphate/creatinine remained significantly higher in AC6$^{-/-}$ versus WT mice. $^{\#}P<0.05$ versus basal same genotype, $^{*}P<0.05$ versus WT.



Supplemental Figure 4. Effect of FGF receptor blockade on Npt2a and Npt2c protein expression in wild-type (WT) and AC6 knockout (AC6$^{-/-}$) mice. WT and AC6$^{-/-}$ mice were treated with the FGF receptor inhibitor PD173074 (50 mg/kg in Cremophor EL/ethanol/water; 7.5:2.5:90 via oral gavage, 1% of body weight) every 12 hours for 6 days.[1,2] After 6 days of treatment, Npt2a and Npt2c abundances in membrane fractions of renal cortex were analyzed via Western blotting. Whereas treatment with PD173074 did not significantly affect Npt2a between genotypes, Npt2c tended to be lower in AC6$^{-/-}$ mice, giving a possible explanation for the higher urinary phosphate/creatinine (Supplemental Figure 3).



Supplemental Figure 5. Effect of acute PTH application on urinary P_i excretion in wild-type (WT) and AC6 knockout (AC6$^{-/-}$) mice. WT and AC6$^{-/-}$ mice were fed a zero P_i diet for 7 days. On day 7 urine was collected 2 hours after intravenous application of vehicle (0.85% NaCl, 2 µl/g bw) or PTH (200 µg/kg bw). As comparison urinary P_i/creatinine on a control diet is shown on the right side. We found that all mice developed PTH resistance because urinary phosphate/creatinine did not increase after PTH administration. Renal PTH resistance in rats[3-5] and mice[6] on low P_i intake was described before; however, it was never clarified what caused PTH-resistance under these conditions. *n*=6/genotype, **P*<0.05 versus WT.

Supplemental References

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