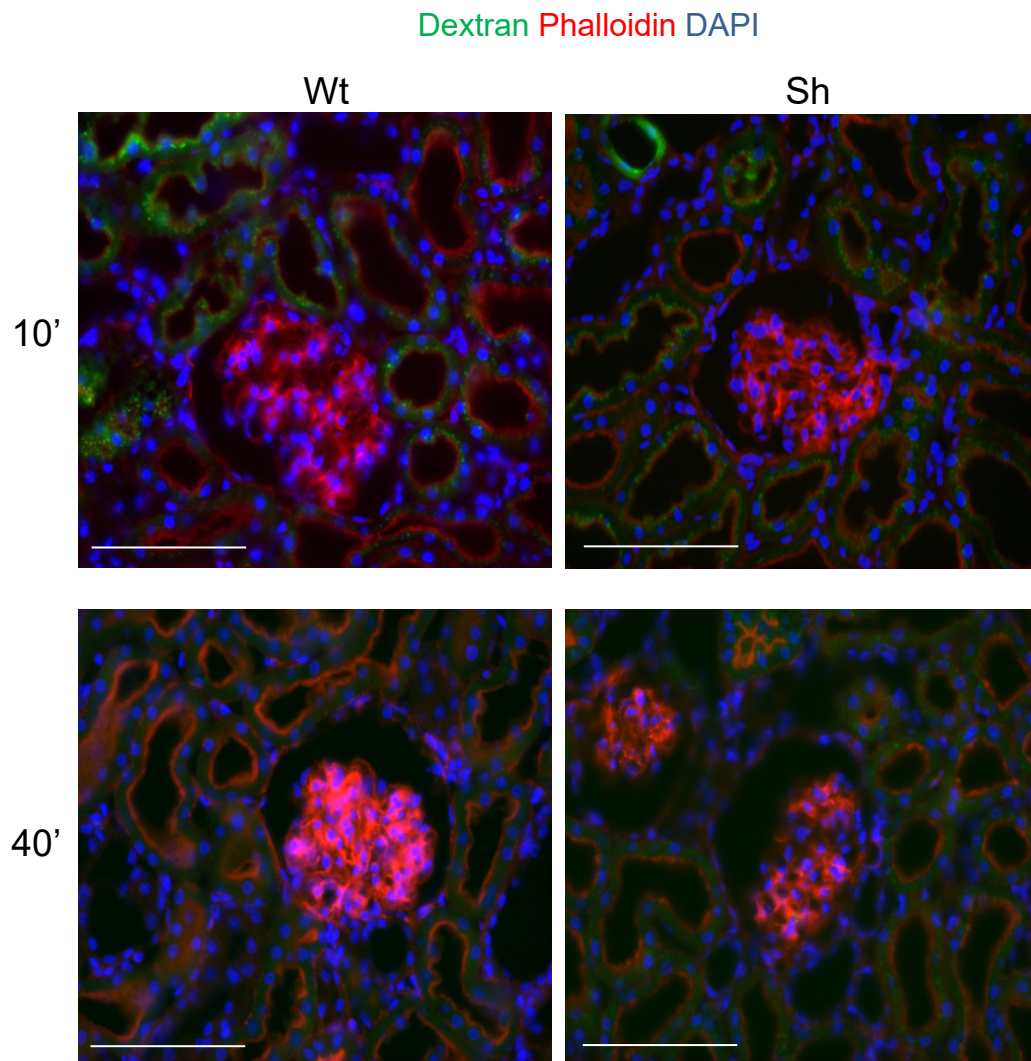
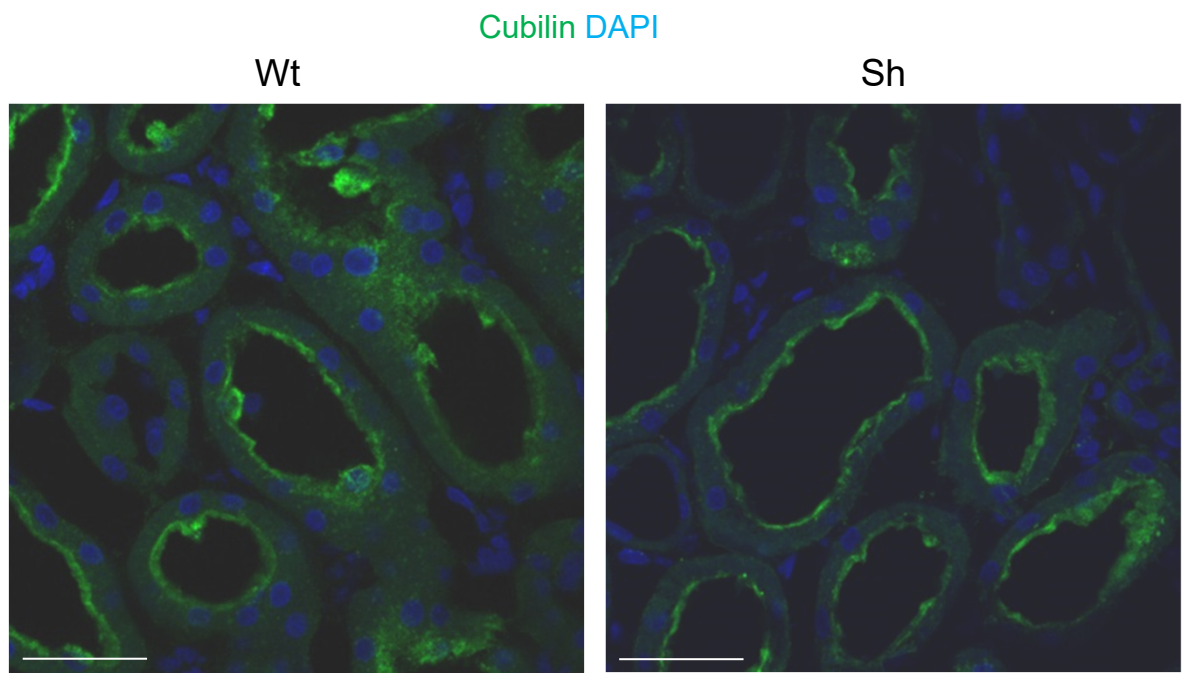


SUPPLEMENTARY FIGURE 1

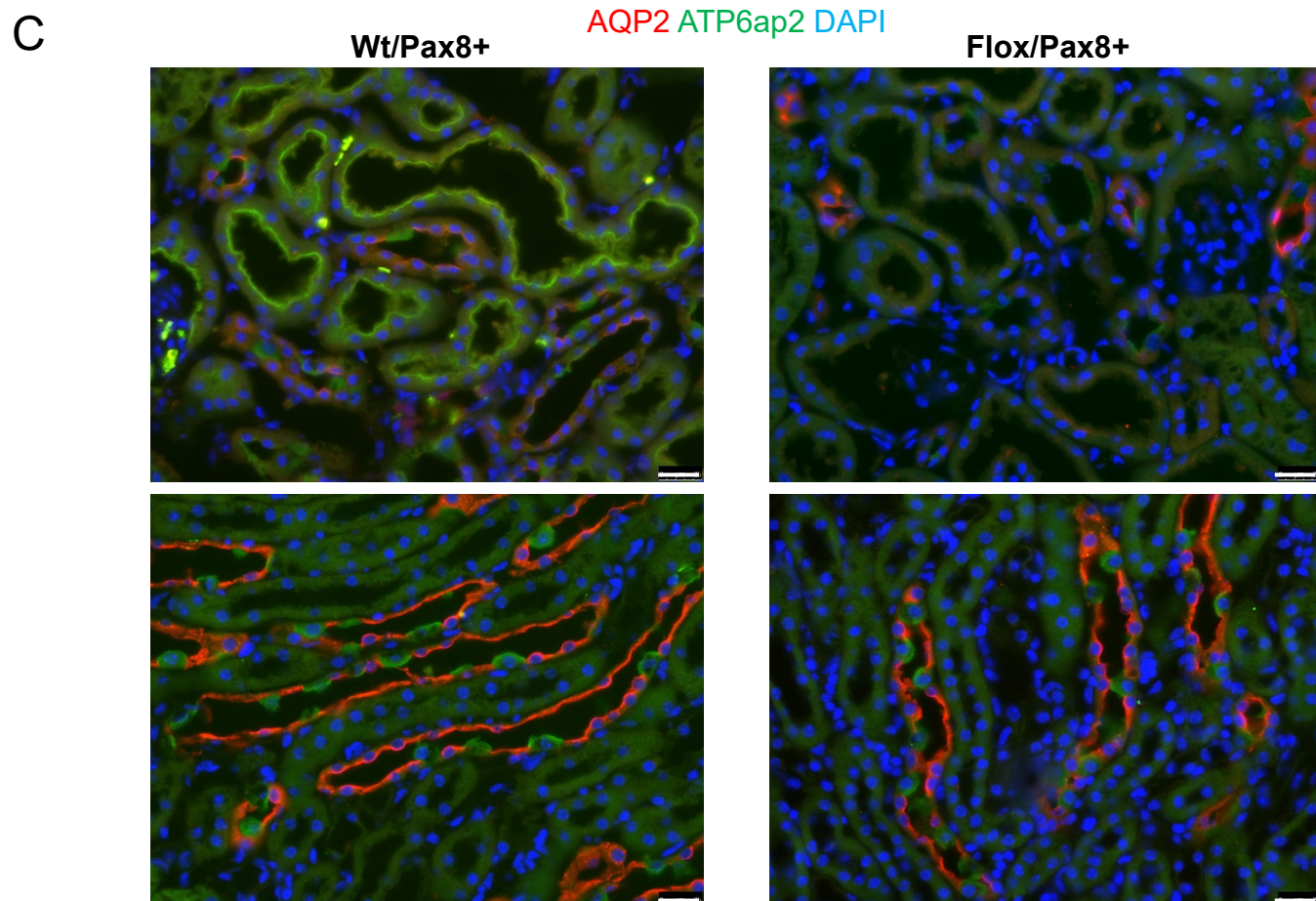
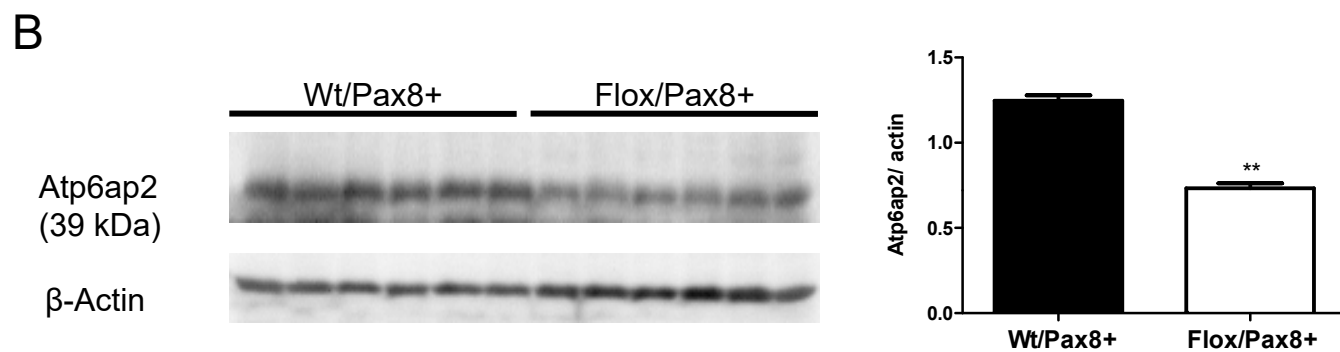
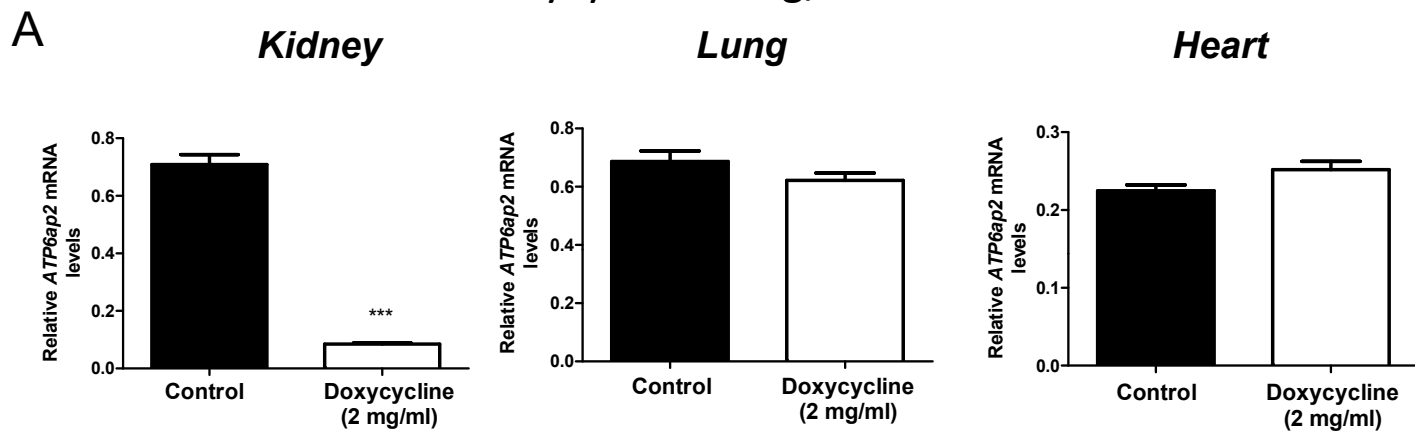


SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3

Doxycycline 2 mg/ml

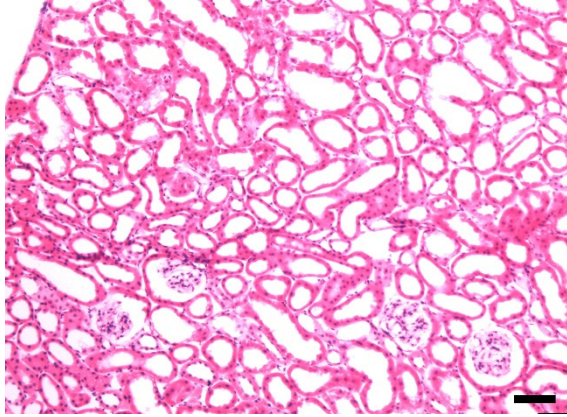


SUPPLEMENTARY FIGURE 3

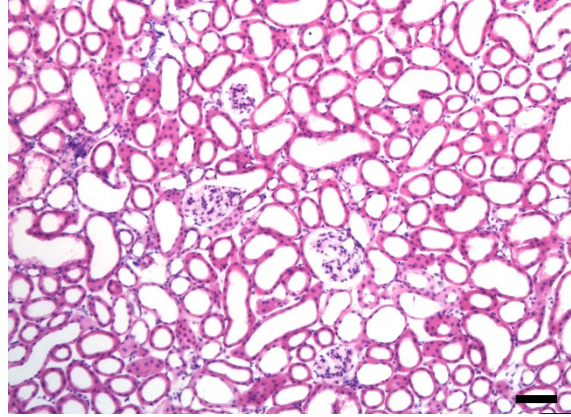
Doxycycline 2 mg/ml

D

Wt/Pax8+



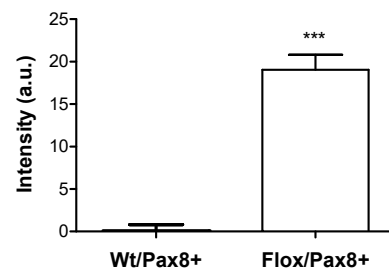
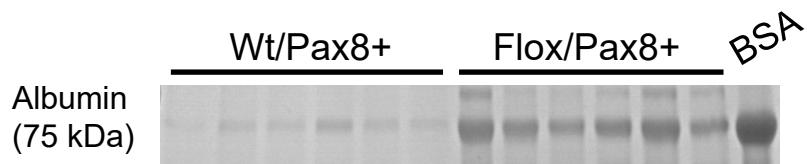
Flox/Pax8+



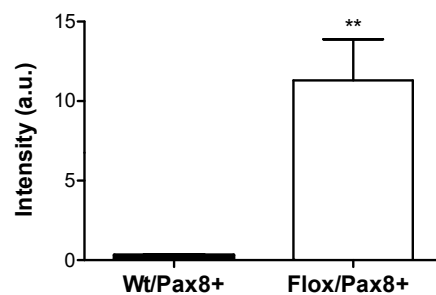
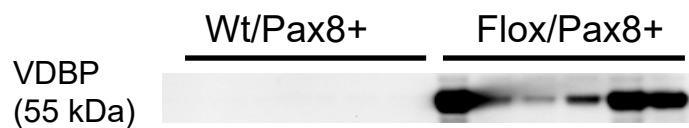
SUPPLEMENTARY FIGURE 4

Doxycycline 2 mg/ml

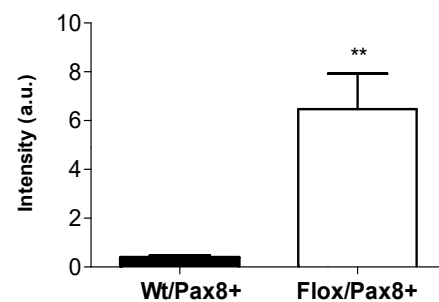
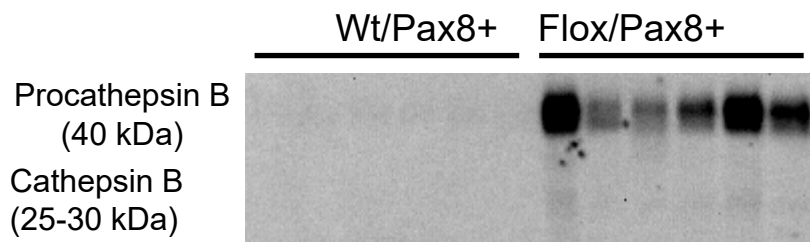
A



B



C

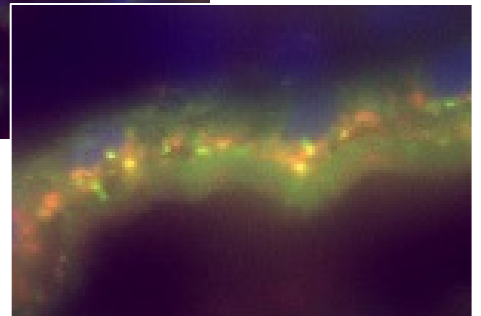
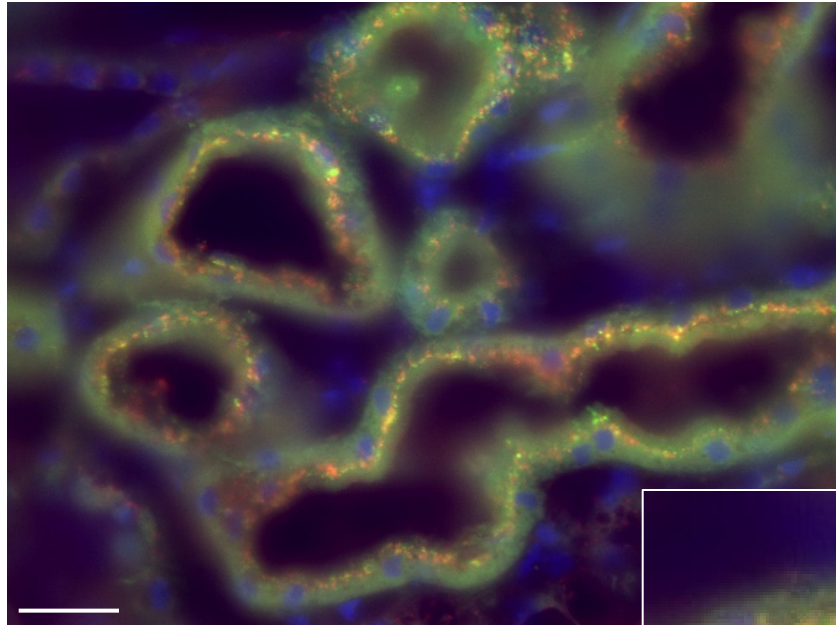


SUPPLEMENTARY FIGURE 5

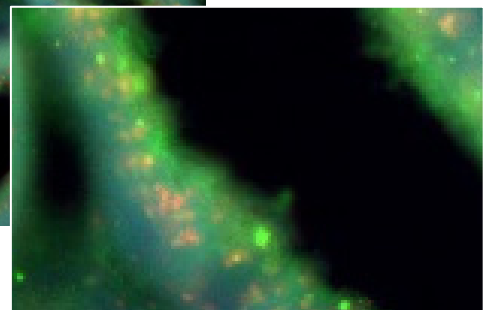
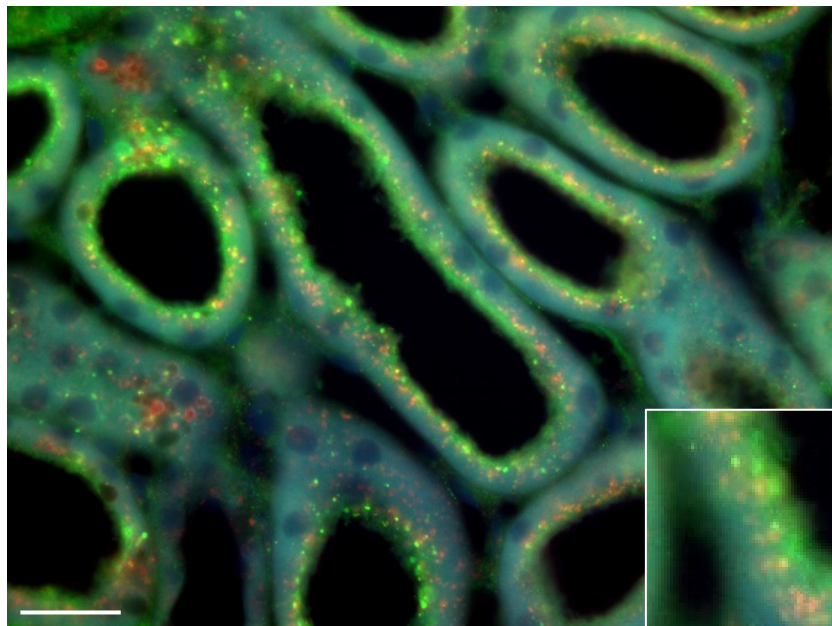
Doxycycline 2 mg/ml

Lamp-1 Transferrin DAPI

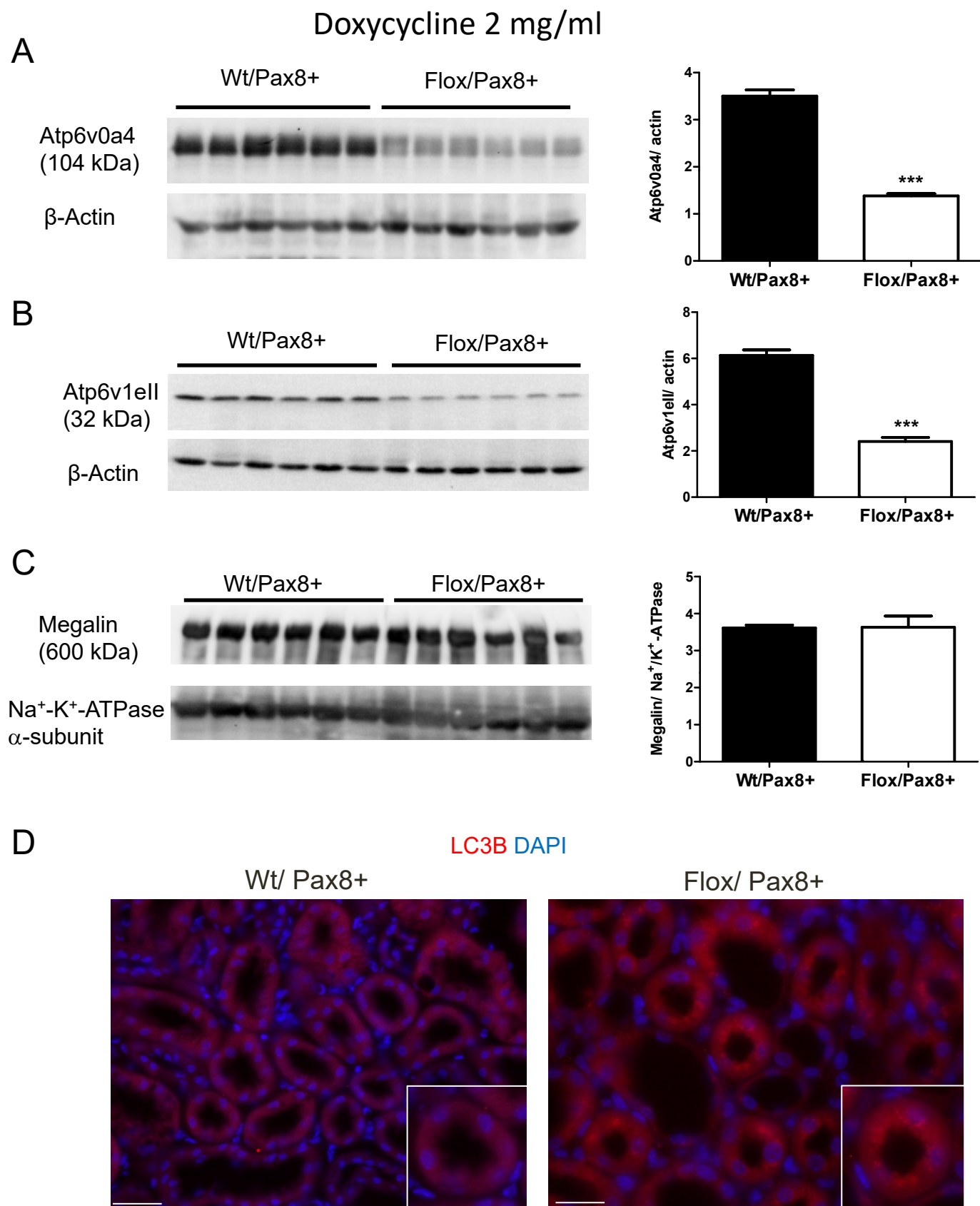
Wt/Pax8+



Flox/Pax8+



SUPPLEMENTARY FIGURE 6



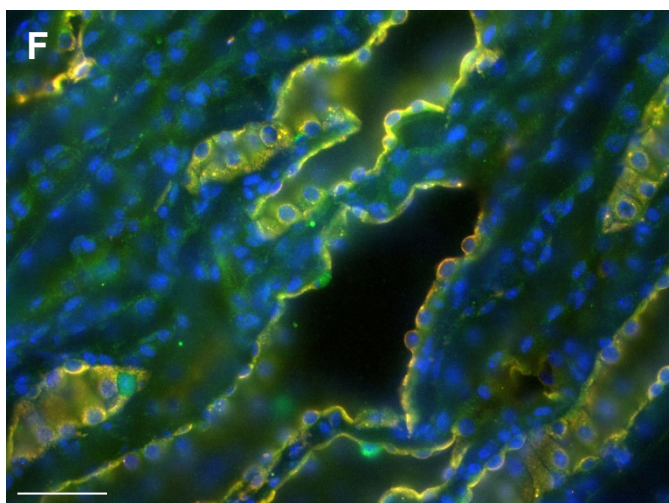
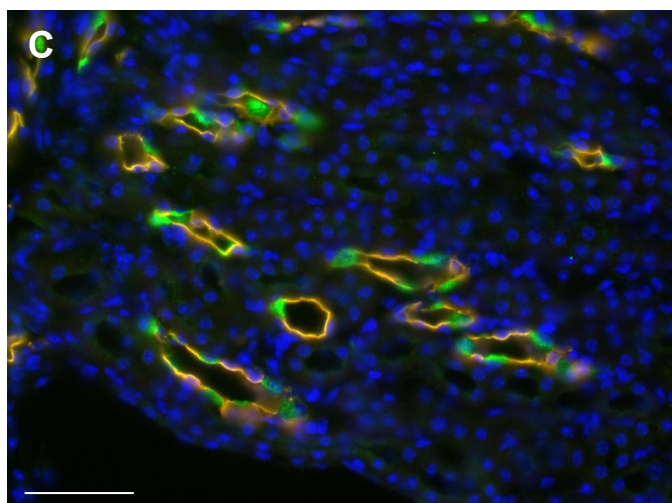
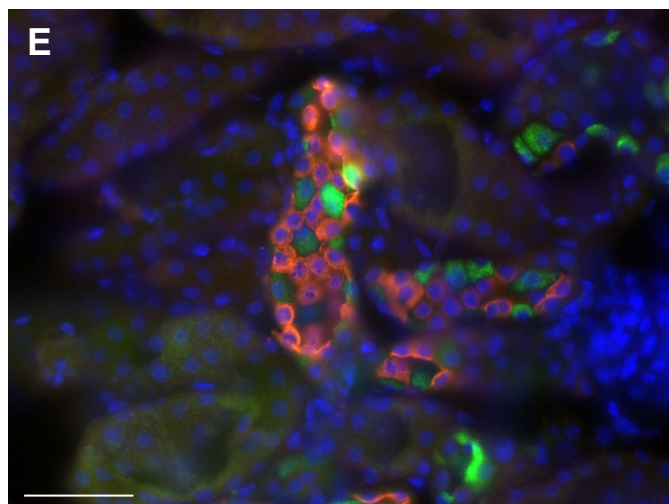
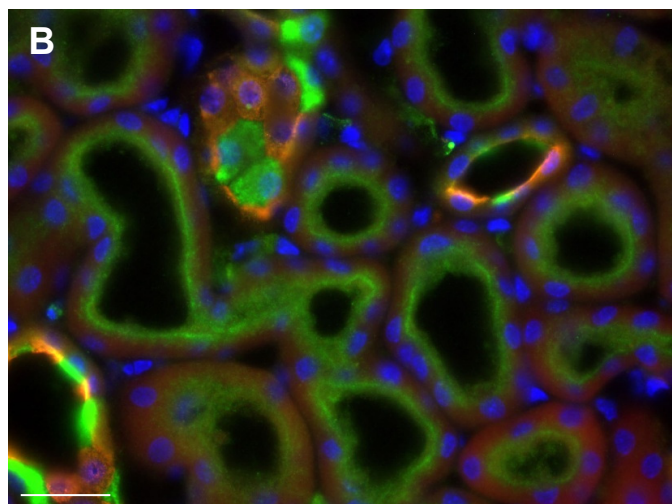
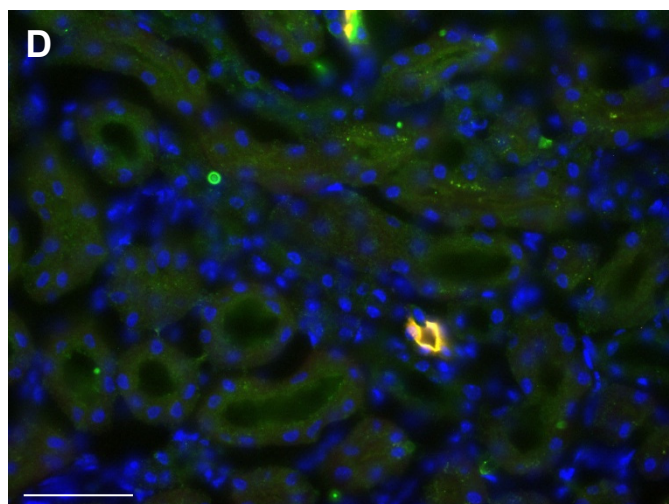
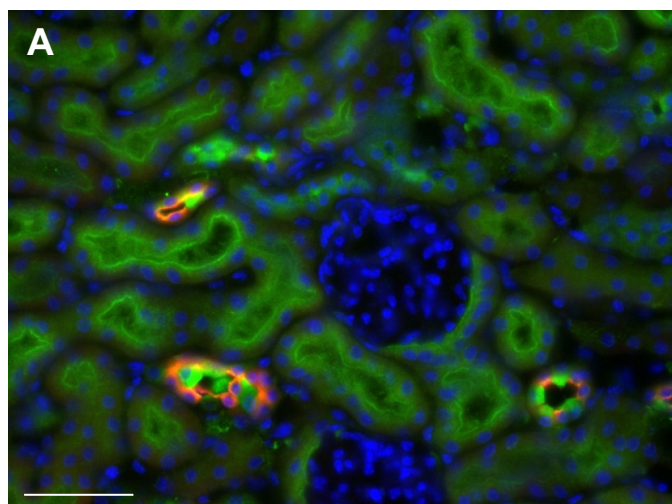
SUPPLEMENTARY FIGURE 7

Novus Biologicals

ATP6ap2 AQP2 DAPI

Wt/ Pax8+

Flox/ Pax8+



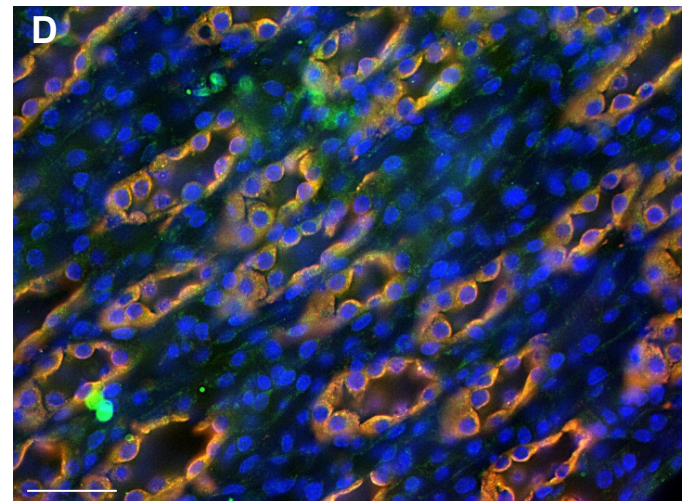
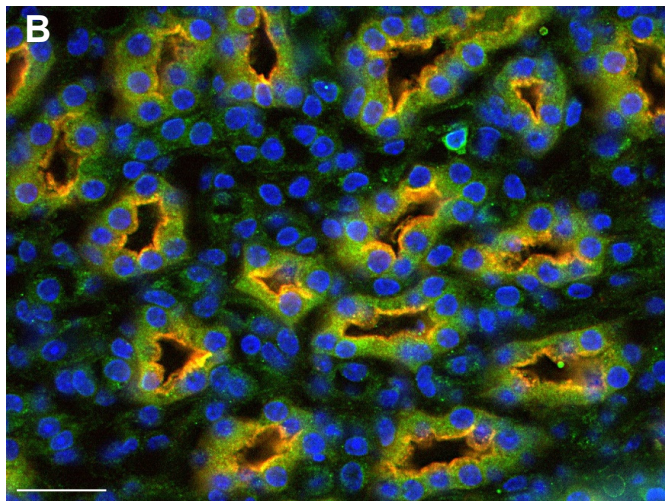
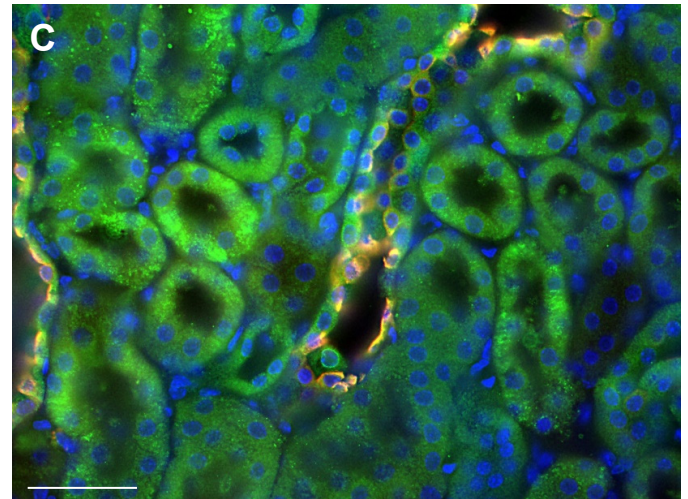
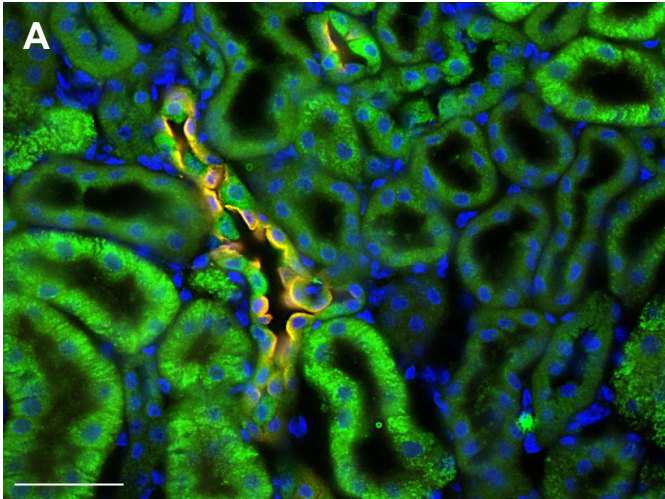
SUPPLEMENTARY FIGURE 8

R&D Systems

ATP6ap2 AQP2 DAPI

Wt/ Pax8+

Flox/ Pax8+



Supplementary figure 1. Dextran-FITC staining intensity and localization is similar in WT and shRNA rats at 10 min and 40 min time points.

Immunohistochemistry for dextran-FITC, 10 kDa (green), DAPI (blue), and actin/phalloidin (red) in kidney slices from Wt and shRNA rats 10 and 40 min after injection. Scale size bar 100 μ m.

Supplementary figure 2. Cubilin localization in control and shRNA rat kidneys.

Immunohistochemistry for cubilin (green) and DAPI (blue) in kidney slices from Wt and shRNA rats. Scale size bar 50 μ m.

Supplementary figure 3. Generation of kidney epithelial cell specific ATP6ap2 ablation in mouse using the higher doxycycline protocol.

Mice received 2 mg/ml doxycycline as described in the methods section and ablation of *ATP6ap2* in kidney was examined. **(A)** RT-qPCR analysis of total mRNA of kidneys, lungs and hearts from Wt and Flox/Pax8+ mice. *Atp6ap2* mRNA abundance was normalized to *HPRT*. Statistical analysis was performed using Student's *t*-test (Wt/Pax8+: n = 6 and Flox/Pax8+: n = 6) *** $p < 0.001$. **(B)** Western blotting for ATP6ap2 in total membrane preparations from kidney of Wt and Flox/Pax8+ mice and summary of data (normalized to β -actin-loading) as bar graph. Statistical analysis was performed using Student's *t*-test (Wt/Pax8+: n = 6 and Flox/Pax8+: n = 6) ** $p < 0.01$. **(C)** Immunohistochemistry for ATP6ap2 (green), AQP2 (red), and DAPI (blue) in kidney sections from Wt/Pax8 and Flox/Pax8+ mice. Scale size bar 25 μ m. **(D)** H&E stained kidney sections from Wt/Pax8 and Flox/Pax8+ mice. Scale size bar 25 μ m.

Supplementary figure 4. Albuminuria and low molecular weight proteinuria in the absence of the ATP6ap2

Animals were treated with the higher dose of doxycycline (2 mg/ml). 24 hrs urine samples were normalized to creatinine. Bovine serum albumin (BSA, 7 mg/ml) was loaded as positive control. **(A)** Albumin was detected by coomassie blue staining whereas **(B)** vitamin D binding protein (VDBP) or **(C)** (Pro)cathepsin B were revealed by immunoblotting. Data were summarized as bar graphs (n = 6/genotype. Student's *t*-test ** $p < 0.01$, *** $p < 0.001$).

Supplementary figure 5. Delayed receptor-mediated endocytosis in ATP6ap2 deleted mice

WT/Pax8+ and Flox/Pax8+ mice were pretreated with 2 mg/ml doxycycline. Mice were injected with

human recombinant transferrin and kidneys collected 40 min after injection. Immunohistochemistry for human transferrin (green), DAPI (blue), and the lysosomal protein Lamp-1 (red) was performed. Inserts show higher magnifications. Scale size bar 25 μm .

Supplementary figure 6. Deletion of the ATP6ap2 alters expression of H⁺-ATPase subunits and autophagosome subunit

Mice were treated with the higher dose of doxycycline (2 mg/ml). **(A-C)** Total membrane preparations from kidneys of Wt/Pax8⁺ and Flox/Pax8⁺ mice were blotted for the H⁺ATPase $\alpha 4$ (ATP6V0a4) and E11 (ATP6V1E11) subunits as well as for megalin and the α subunit of the Na⁺/K⁺-ATPase as loading control. Densitometries were normalized to β -Actin or Na⁺/K⁺-ATPase (loading control). Student's *t*-test (*n* = 6 per group), *** *p* \leq 0.001 **(D)** Immunohistochemistry for the Microtubule-associated proteins 1A/1B light chain 3B (LC3-B) (red) and DAPI (blue) in kidneys from Wt and Flox/Pax8⁺ mice, insert shows higher magnification. Scale size bar 25 μm .

Supplementary figure 7. Detection of ATP6ap2 in mouse kidneys with various antibodies

Kidneys from wildtype (Wt/ Pax8⁺) **(A-C)** and Flox/Pax8⁺ **(D-F)** mice were stained with antibodies from Novus Biologicals against the (P)RR/ATP6ap2 (green), AQP2 (red), and with DAPI (blue) to detect nuclei. In wildtype kidney, strong staining with antibodies against the (P)RR/ATP6ap2 was detected in proximal tubules and in collecting ducts, in Flox/Pax8⁺ mouse kidney, the staining was weaker in the proximal tubule but preserved in collecting ducts. Of note, all cell types in the collecting duct were stained. Scale size bar: A,C,D,E: 100 μm , B,F: 50 μm .

Supplementary figure 8. Detection of (P)RR/ATP6ap2 in mouse kidneys with various antibodies

Kidneys from wildtype (Wt/ Pax8⁺) **(A-B)** and Flox/Pax8⁺ **(C-D)** mice were stained with antibodies from R&D Systems against the (P)RR/ATP6ap2 (green), AQP2 (red), and with DAPI (blue) to detect nuclei. In wildtype kidney, strong staining with antibodies against the (P)RR/ATP6ap2 was detected in proximal tubules and in collecting ducts while in the Flox/Pax8⁺ mouse kidneys, staining in the proximal tubule and collecting ducts remained unchanged. In contrast to staining with other antibodies, staining with R&D Systems antibodies was mostly intracellular. Scale size bar: A,C: 100 μm , B,D: 50 μm .