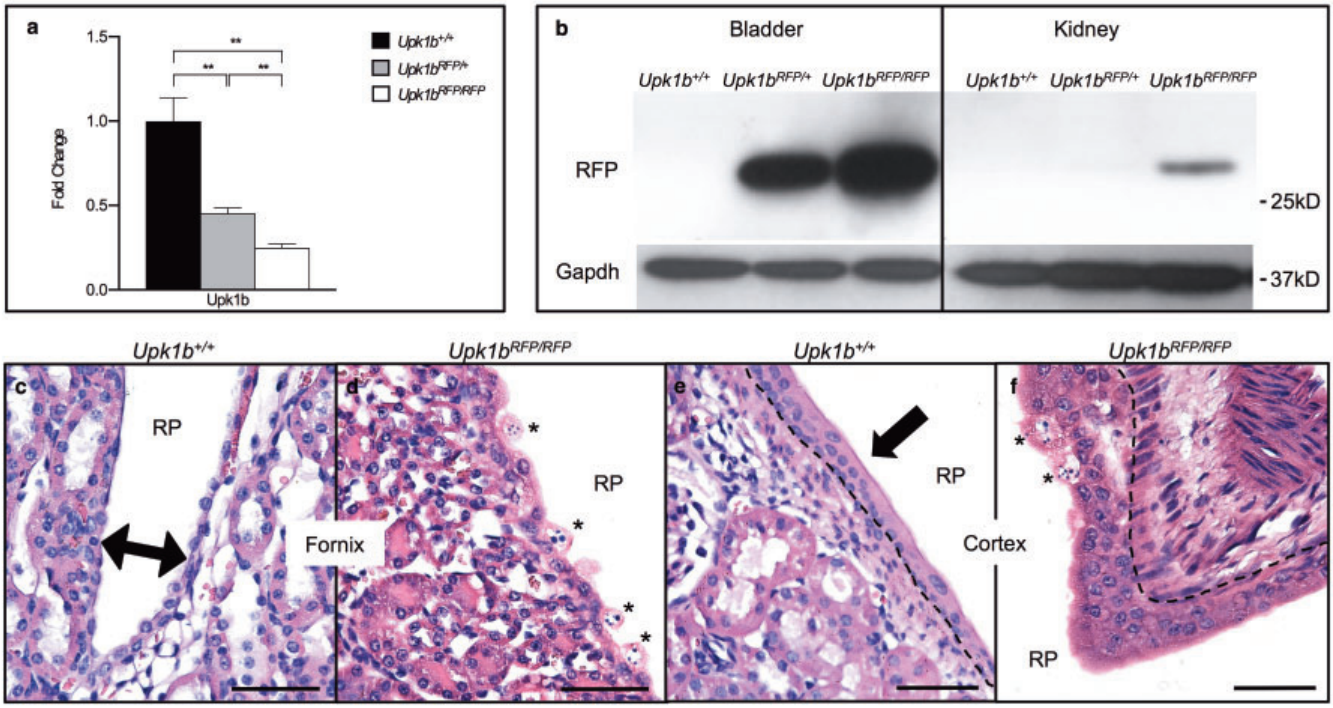
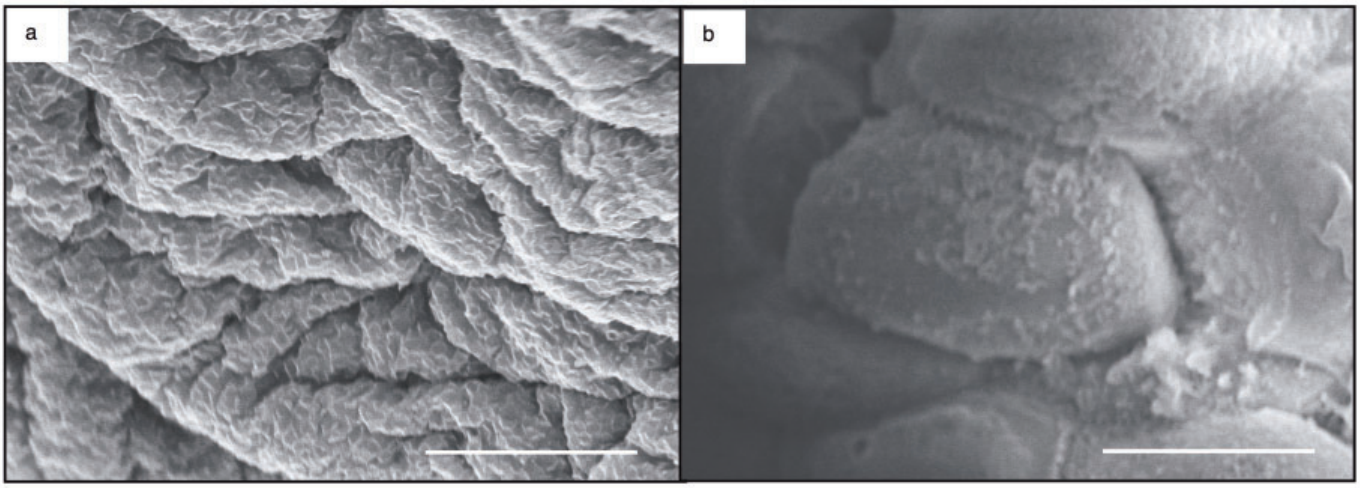
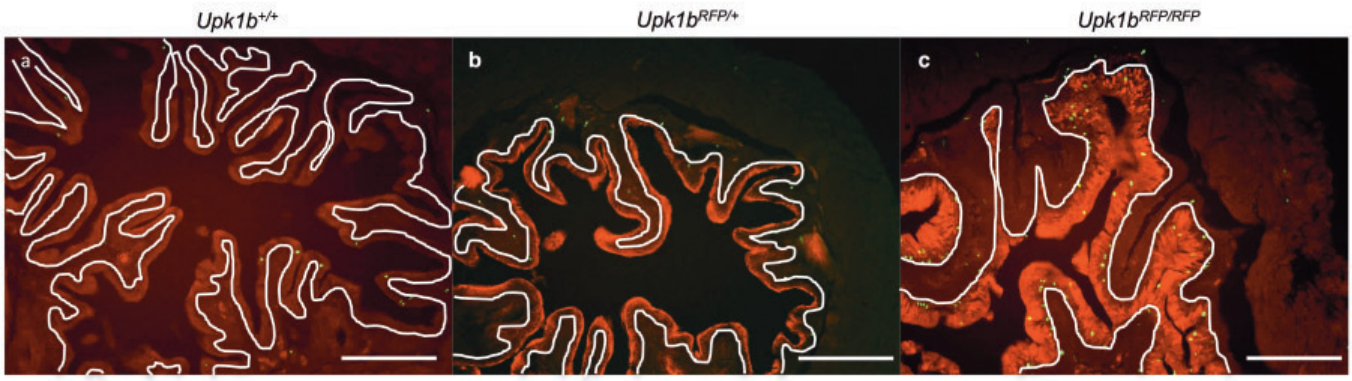
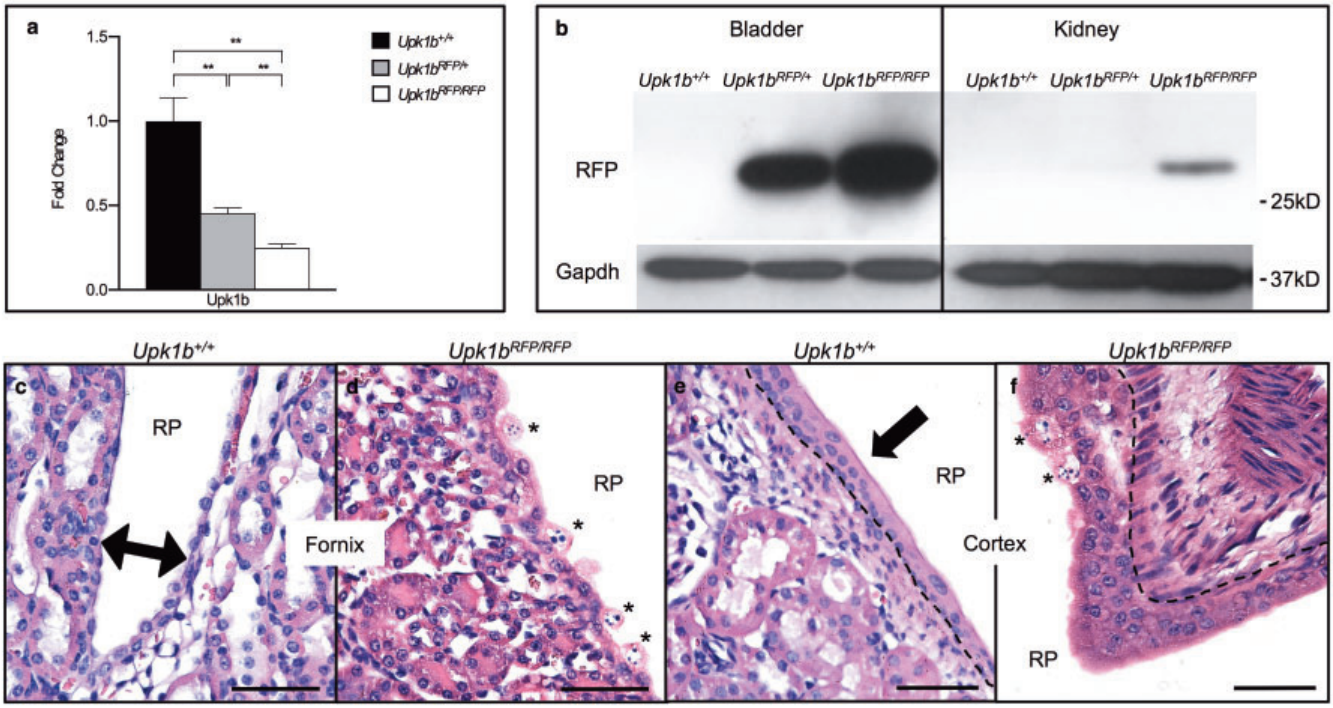
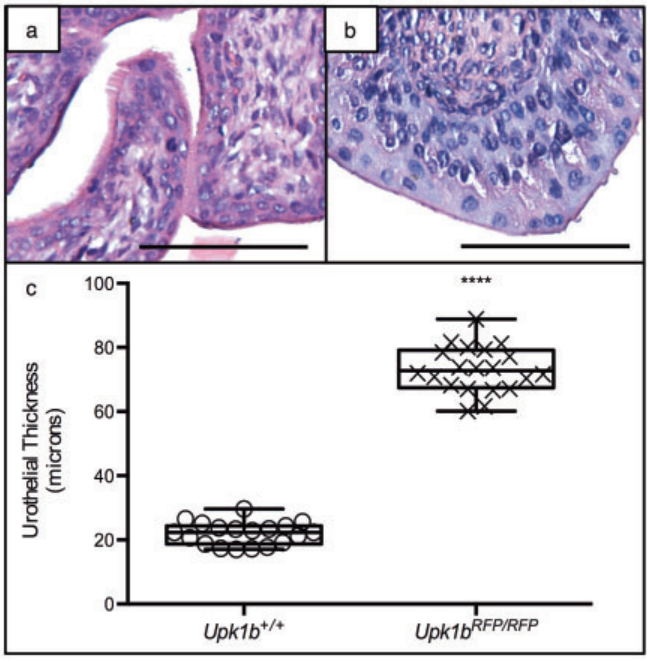

a
1.5
1.0
0.5
0.0
Fold Change
**
**
**
Upk1b
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
b
Bladder
Kidney
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
RFP
Gapdh
-25kD
-37kD
Upk1b+/+
Upk1bRFP/RFP
Upk1b+/+
Upk1bRFP/RFP
c
RP
Fornix
d
RP
e
RP
Cortex
f
RP


a
b


Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
a
b
c


a
1.5
1.0
0.5
0.0
Fold Change
Upk1b
**
**
**
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
b
Bladder
Kidney
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
Upk1b+/+
Upk1bRFP/+
Upk1bRFP/RFP
RFP
Gapdh
-25kD
-37kD
Upk1b+/+
Upk1bRFP/RFP
Upk1b+/+
Upk1bRFP/RFP
c
RP
Fornix
d
RP
e
RP
Cortex
f
RP


a
b
c
Urothelial Thickness (microns)
100
80
60
40
20
0
****
Upk1b+/+
Upk1bRFP/RFP

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *Upk1b*$^{RFP/RFP}$ Bladders Exhibit Altered Urothelial Morphology.

(A-C) RFP expression in *Upk1b*$^{+/+}$, *Upk1b*$^{RFP/+}$, and *Upk1b*$^{RFP/RFP}$ bladders was analyzed by fluorescence imaging (D) *Upk1b* mRNA expression in *Upk1b*$^{+/+}$, *Upk1b*$^{RFP/+}$ and *Upk1b*$^{RFP/RFP}$ bladders (*n*=3) was analyzed by qPCR. A One-Way ANOVA and Tukey's multiple comparison post-hoc test were used to evaluate statistical significance. ****p < 0.0001. Error bars represent standard error. (E) RFP and Gapdh expression in *Upk1b*$^{+/+}$, *Upk1b*$^{RFP/+}$ and *Upk1b*$^{RFP/RFP}$ bladders was analyzed by immunoblotting. (F-H) Urothelial morphology in *Upk1b*$^{+/+}$ *Upk1b*$^{RFP/+}$, and *Upk1b*$^{RFP/RFP}$ bladders was evaluated by H&E staining. L: Bladder Lumen, Dotted White Line: Apical Urothelial Surface, Solid White and Solid Black Lines: Basement Membrane, V: Vacuole, Black Arrow: Umbrella Cell, White Arrow: Abnormal Superficial Cell. Scale bars indicate 62.5μm (A-C) and 100μm (F-H)

Supplemental Figure 2. Scanning Electron Microscopy Confirms Absence of the Urotheilal Plaque

(A) Scanning electron micrographs demonstrate ultrastructural analysis of the apical surface of the urothelial superficial cells in *Upk1b*$^{+/+}$ (A) and *Upk1b*$^{RFP/RFP}$ (B) bladder. Scale bars indicate 15μm (A, B).

Supplemental Figure 3. Absent *Upk1b* and Urothelial Plaque Increase Urothelial Renewal.

(A-C) Urothelial proliferation in $Upk1b^{+/+}$, $Upk1b^{RFP/+}$ and $Upk1b^{RFP/RFP}$ bladders was analyzed by EdU incorporation and detection (green cells). Solid white line indicates basement membrane. Scale bar indicates 250μm.

Supplemental Figure 4. $Upk1b^{RFP/RFP}$ Kidneys Exhibit Altered Urothelial Morphology.

(A) *Upk1b* mRNA expression in $Upk1b^{+/+}$, $Upk1b^{RFP/+}$ and $Upk1b^{RFP/RFP}$ kidneys (n=3) was analyzed by qPCR. A One-Way ANOVA and Tukey's multiple comparison post-hoc test were used to evaluate statistical significance. **$p < 0.01$. Error bars represent standard error. (B) RFP and Gapdh expression in $Upk1b^{+/+}$, $Upk1b^{RFP/+}$ and $Upk1b^{RFP/RFP}$ bladders and kidneys was analyzed by immunoblotting. (C-F) Renal urothelial morphology in $Upk1b^{+/+}$ and $Upk1b^{RFP/RFP}$ fornix and cortex was evaluated by H&E staining. RP: Renal Pelvis, Double Sided Arrow: Single Cell Layer Medullary Urothelium/Renal Fornix, Asterisk: Apoptotic Bodies, Black Arrow: Multiple Urothelial Cell Layer Cortical Urothelium, Dotted Black Line: Urothelial Basement Membrane. Scale bars indicate 50μm (C-F).

Supplemental Figure 5. Altered Urothelial Morphology is Evident Prior to the Onset of Hydronephrosis.

(A, B) *Upk1b*$^{RFP/RFP}$, but not *Upk1b*$^{+/+}$, bladder urothelium is thickened prior to the onset of hydronephrosis. (C) A box and whiskers plot displays the average urothelial thickness (microns) per section in *Upk1b*$^{+/+}$ and *Upk1b*$^{RFP/RFP}$ bladders (*n*=4). Whiskers represent the minimum and maximum. An unpaired, one-tailed t test was used to calculate the p-value. ****p < 0.0001. Scale bars indicate 100μm (A, B).