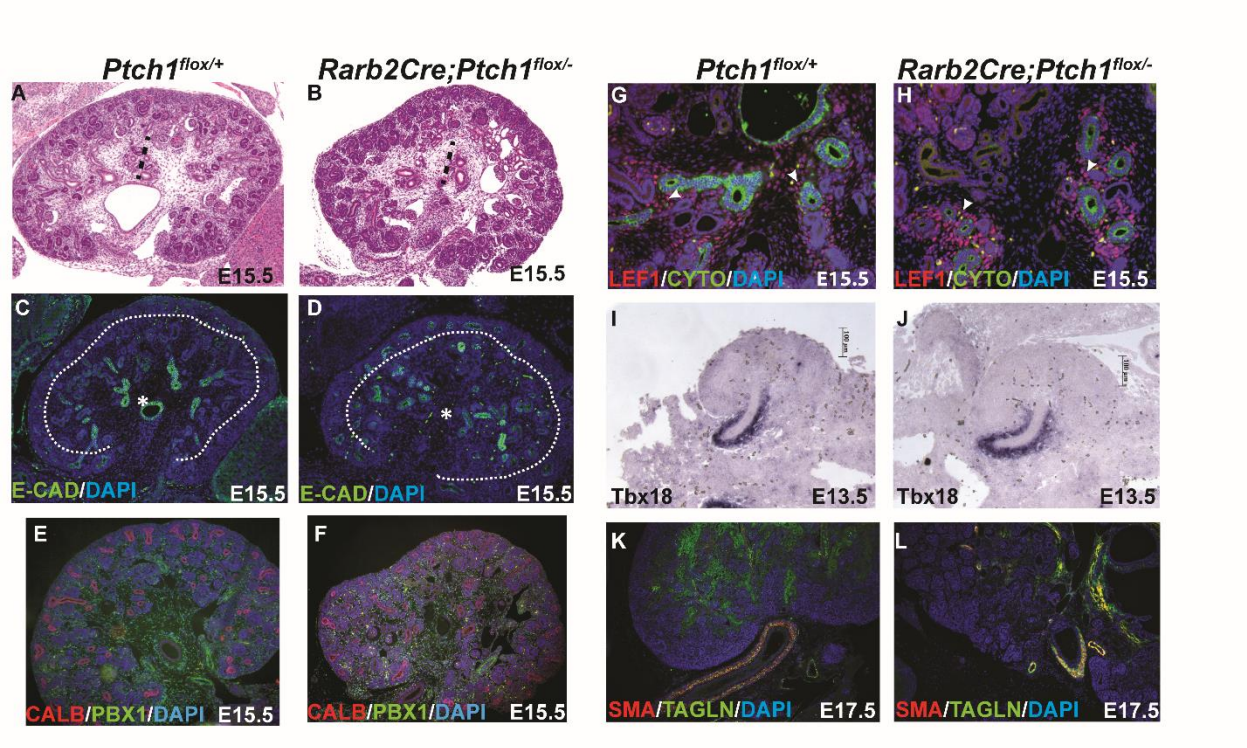
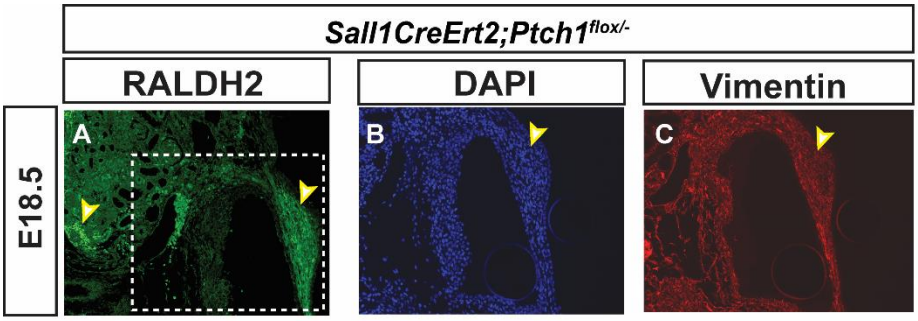
Supplemental Figures



Supplemental Fig. 1. Development of the renal medulla and smooth muscle lineage is not disrupted. A,B. H&E staining shows of E15.5 kidney sections from from *Rarb2Cre;Ptc1*$^{-/-MM}$ mutants and littermate controls. The renal medullary compartment (dashed-line) was evident in both mutants and controls. Pelvis was not formed completely in the mutants and there is no clear ureter to pelvis junction in the mutants at E15.5. **C,D.** E-cad expression (green) in the renal medulla in both WT and controls indicate distal tubule formation. (asterisk shows the pressumptive pelvis and dashed line separate nephrogenic zone from the medulla) **E,F.** Calbindin (CALB, red) and PBX1 (green) expression in the renal medulla indicate formation of nascent collecting duct and normal presence of interstitial cells in the mutants at E15.5. **G,H.** LEF1 expression (red) in the interstitial cells surrounding the collecting ducts marked by Cytokeratin (CYTO, green) is comparable between the WT and mutant at E15.5. **I,J**. *In situ* hybdrization analysis shows that *Tbx18* mRNA expression in the ureteric mesenchyme is unchanged in mutants compared to controls at E13.5. **K,L**. SMA (red) and Transgelin (TAGLN, green), two key markers of differentiated smooth muscle cells are expressed together in ureter in both WT and mutants at E17.5.

Supplemental Fig. 2. Ptc2-LacZ is expressed at the presumptive UPJ in E12.5 mutant kidneys. Whole-mount β-galactosidase staining of E12.5 kidney tissue dissected from control and mutant kidneys demonstrates ectopic expression of *Ptc2-lacZ* at the presumptive UPJ (arrowhead).



Supplemental Fig 3. Vimentin and Raldh2 co-localize in the UPJ obstructing tissue in Ptc1$^{-/-MM}$ kidneys. A. Immunostaining showing Raldh2 expression in the cortical stroma and ectopic expression in the tissue obstructing the UPJ (UPJO, outlined by dashed square) in E18.5 mutant kidneys. **B,C.** DAPI shows the nuclei of the cells present in the UPJO (arrowhead). Vimentin expression is found in the same UPJO cells (arrowhead) that ectopically express *Raldh2*.

Supplemental Methods

Mice

Ptch1$^{loxP/loxP}$*;Ptch2*$^{LacZ/LacZ}$ mice were generated by crossing *Ptch1*$^{loxP/loxP}$ with *Ptch2*$^{LacZ/LacZ}$ mice, followed by interbreeding the double-heterozygote progenies [*Ptch2*$^{LacZ/LacZ}$ provided by CC. Hui]. *Rarb2Cre;Ptch1*$^{+/-}$ mice were mated to *Ptch1*$^{loxP/loxP}$*;Ptch2*$^{LacZ/LacZ}$ to generate *Ptch1*$^{-/-MM}$*;Ptch2*$^{LacZ/+}$ embryos.

Gli3$^{\Delta 699/\Delta 699}$ mice were mated to Ptch1$^{loxP/loxP}$ mice to generate *Ptch1*$^{loxP/+}$*;Gli3*$^{\Delta 699/+}$ progeny and then bred with *Rarb2Cre;Ptch1*$^{+/-}$ mice to ultimately generate *Ptch1*$^{-/-MM}$*;Gli3*$^{\Delta 699/+}$ embryos (termed *Gli3* compound mutants).

Ptch1$^{loxP/loxP}$*;Rosa26*$^{tdT/tdT}$ mice were generated by crossing *Ptch1*$^{loxP/loxP}$ with *Rosa26*$^{tdT/+}$, followed by selecting for and crossing the double-heterozygote male and female progenies. *Sall1CreErt2;Ptc1*$^{+/-}$ mice were mated to *Ptch1*$^{loxP/loxP}$*;Rosa26*$^{tdT/tdT}$ to generate *Sall1CreErt2;Ptc1*$^{loxP/-}$*;Rosa26*$^{tdT/+}$ embryos.

Immunostaining

Paraffin-embedded sections were first deparaffinised in xylene and rehydrated through ethanol gradient. Subsequently, antigen retrieval was carried out using citrate buffer (pH=6) in a pressure cooker. Frozen sections were incubated with PBS-triton solution for one hour prior to protein blocking. Non-specific antigens were blocked at room temperature using DAKO serum-free protein block followed by primary antibody overnight incubation at 4^{o}C. Antibodies used include anti–SMA (1:300, Sigma A2547), anti–UPK-III (1:200, Biotechnik 651108), anti–RALDH2 (1:200, Abcam ab96060), anti–E-CAD (1:200, Cell Signaling 24E10) anti–PBX1 (1:200, Cell

Signaling 4342), anti–pan-cytokeratin (1:200, Sigma C5992), anti-vimentin (1:200, Abcam ab92547), anti- calbindin (1:200, Sigma C7354), anti-LEF1 (1:100, Cell Signaling C12A5), anti-transgelin (1:200, Abcam ab14106). DAPI, Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 647 goat anti-mouse, goat anti-rat, or goat anti-rabbit (Invitrogen, 1:1,000 dilution) were used as secondary antibodies.

Immunohistochemical staining of human specimens were performed according to published protocols (Orlando et al. 2013). Briefly, after tissue rehydration, antigen retrieval and protein blocking steps, slides were incubated with primary antibodies, including rabbit polyclonal anti-human PTCH2 antibody (1:180, LS-B301) or goat polyclonal anti human FOXD1 antibody (1:50, sc-47585). Endogenous peroxidase activity was blocked by incubating in hydrogen peroxide for 15 minutes. For horseradish peroxidase (HRP) detection, sections were incubated with appropriate biotinylated- secondary antibody (Vector) for 1 hour at room temperature. Next, in a separate reaction, avidin and biotinylated peroxidase (Vector ABC Kit) were mixed according to manufacturer instructions such that some of the binding sites on avidin were left unoccupied. This complex was then incubated with the tissue sections. The unoccupied biotin-binding sites on the complex bind to the biotinylated secondary antibody, amplifying the signal. The brown product of the enzyme was developed using 3,3'- Diaminobenzidine (DAB) substrate (Vector). Cell nuclei were counterstained with hematoxylin.

B-galactosidase staining

The following solutions were used for β-galactosidase staining procedure: LacZ rinse buffer (0.1 M phosphate buffer, PH 7.3, 0.02% NP-40, 0.01% sodium deoxycholate, 2 mM $MgCl_2$), and staining solution (0.1 M $NaPO_4$ buffer, PH 7.3, 2 mM $MgCl_2$, 0.02% NP-40, 0.01% sodium deoxycholate, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 0.1% X-gal).