Supplemental Information

(Six Figures and Seven Movies)

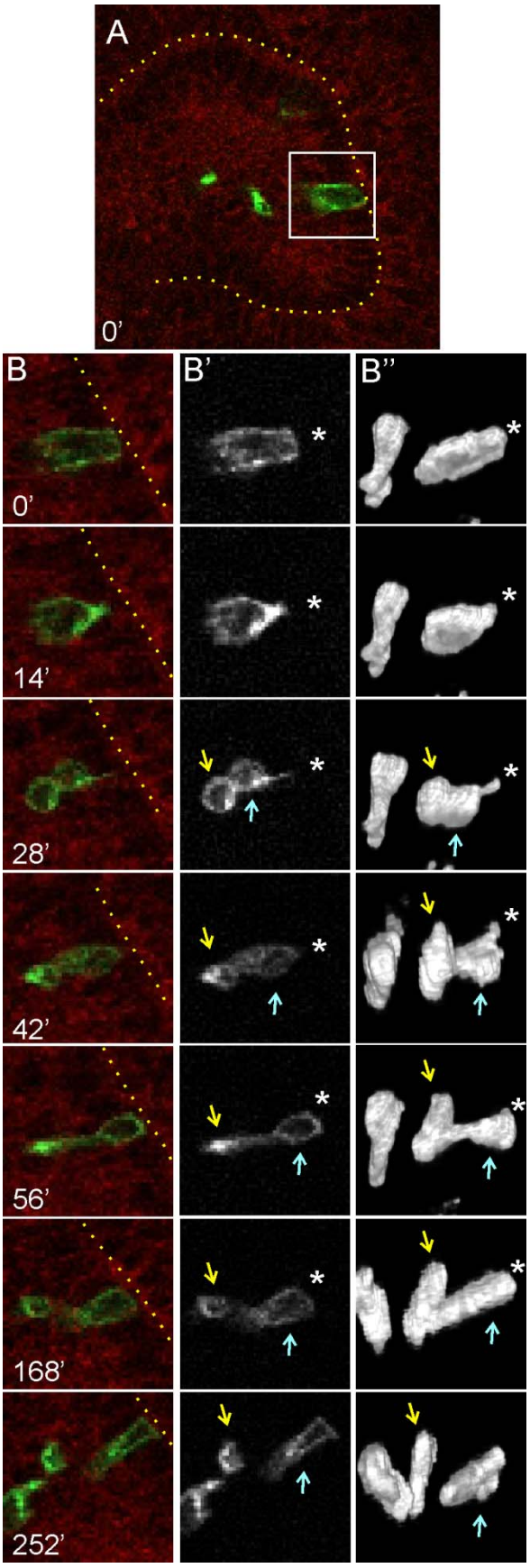

Figure S1, related to Figure 3. A second example of UB tip cell mitosis in a kidney culture, using dual-colored, membrane-targeted fluorescent markers and 4D confocal microscopic analysis. **A,** an optical section through a branching ureteric bud ampulla (at a z-level bisecting the lumen) in a *mTmG*/+, *Ret-CreERT2*/+ kidney. The kidney was explanted at E12.5, treated with 4-OH tamoxifen, and cultured overnight before confocal images stacks (0.75 μm spacing) were collected at 14 min intervals. All cells express mTomato, except for rare recombinant clones in the UB tips that switch to express mGFP. The yellow dotted lines in **A** and **B** indicate the basal surface of the UB tip epithelium, and the white box in **A** highlights an mGFP-positive UB cell about to undergo mitosis. **B, B'** and **B''** show time-lapse sequences of UB cell delamination, division and reinsertion. **B**, optical sections showing red/green merge; **B'**, optical sections showing mGFP channel only; **B''**, 3D rendering of mGFP channel (the 3D rendering shows an additional cell, at left, not visible in some of the optical sections of B and B'). At 0', the pre-mitotic cell has elongated into the lumen, but retains extensive contact with the basal surface (asterisk); at 14', the cell is has rounded and moved away from the basal surface; at 28', the cell has started to divide, and one daughter cell (blue arrow) is tethered to the basal surface

by a thin process (asterisk), while the other daughter lacks a basal process; at 42’ – 56’, the tethered cell (blue arrow) reinserts at the original position in the surface epithelium (asterisk); at 168’ this cell (blue arrow) has reinserted at its original position (asterisk), while the second daughter cell (yellow arrow) is reinserting at a different position, while the cells remain connected at their apical ends; at 252’, cytokinesis is complete and the two daughter cells are located at separate sites.

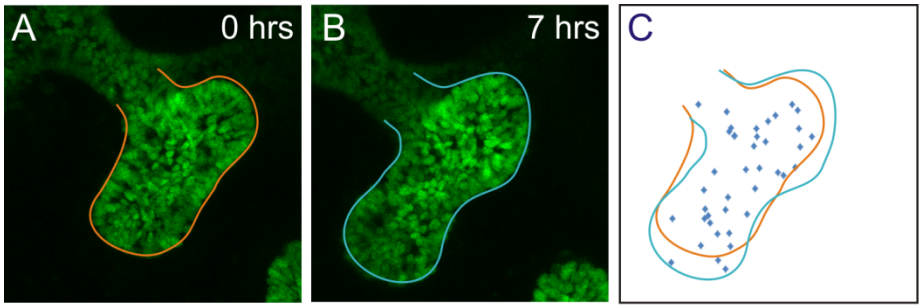

Figure S2, related to Figure 4. Mitotic events are widely dispersed throughout ureteric bud tips. **A** and **B** show maximal projections of the z-stacks at time 0 and at 7 hrs of culture, from the *TcfLEF-H2BGFP* transgenic kidney culture analyzed in **Fig. 3**. C shows the outline of the branching UB tip at time 0 (orange) and 7 hrs (blue). The x,y coordinates of each mitotic event in this time interval (in all z-levels) were recorded, and are plotted in **C** (blue diamonds), to show the distribution of mitotic events within the branching UB ampulla.

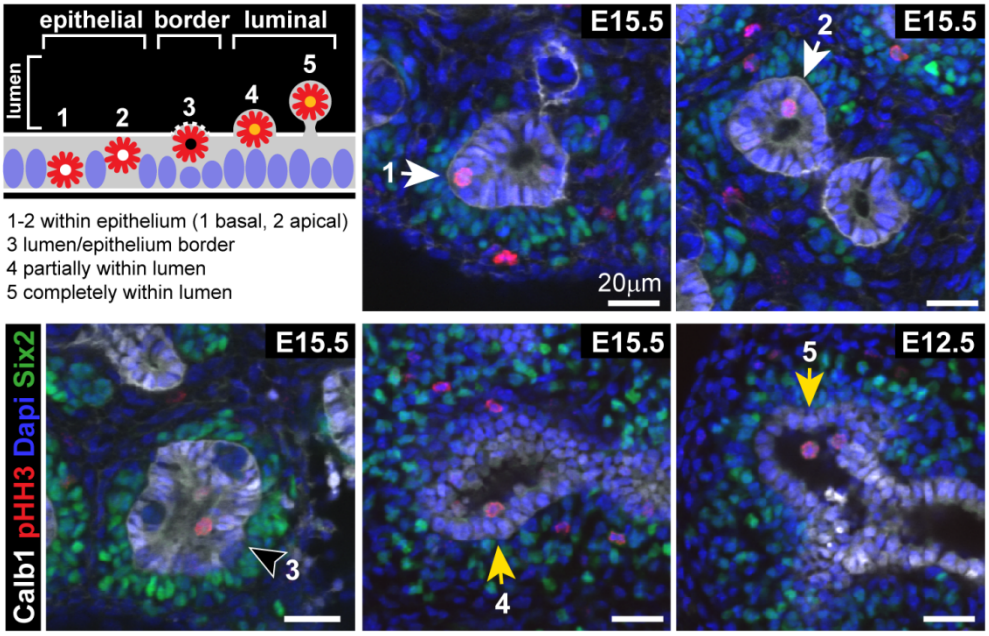

Figure S3, related to Figure 5. Methods to analyze the location of mitotic cells in the ureteric tip and trunk *in vivo*. Whole mouse kidneys from E11.75 to E15.5 were fluorescently labeled with markers for ureteric epithelium (anti-Calbindin1, white), mitotic cells (anti-pHH3, red), cap mesenchyme cells (anti-GFP to detect Six2GFP, green) and all nuclei (DAPI, blue), and imaged by confocal microscopy. The location of pHH3+ cells was scored "epithelial" if located in either a basal (1) or apical (2) position in the Calbindin1+ epithelium, scored "border" (3) if located at the lumen/epithelium border or ambiguous lumen edge, or scored "luminal" if part (4) or all of the pHH3+ signal (5) was present within the lumen. Representative optical sections of each type are shown for the ureteric tip (stages are indicated). Results for tips and trunks are shown in **Fig. 5.** Cells scored at the border/ambiguous were most often seen in tips at E15.5 when the lumens were smaller and often showed a less defined Calbindin1+ apical surface signal (see example 3, E15.5). Completely luminal cells (5) were most often seen at E12.5 when lumens were largest. These completely luminal cells retained contact with the epithelium surface (Calbindin1 expression) even though in some optical sections these cells appeared fully within the lumen (see example 5, E12.5, and **Supplementary Fig. S6C**).

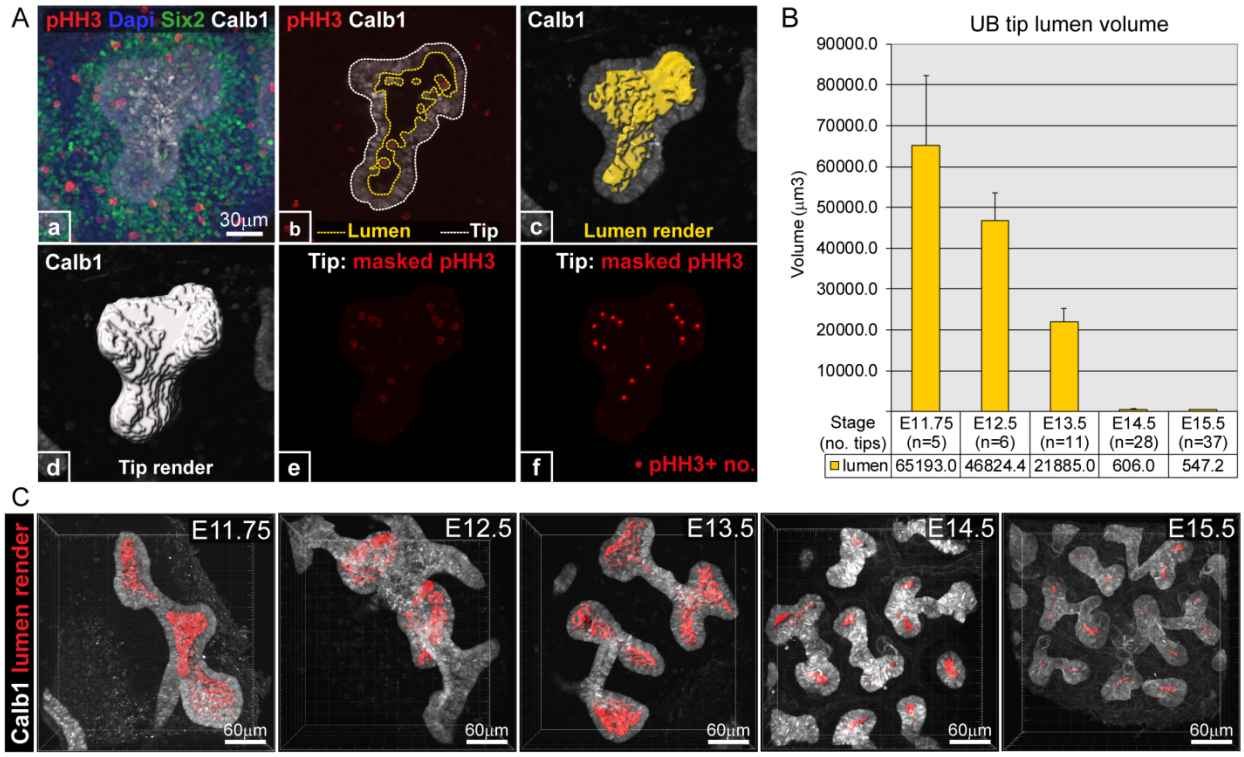

Figure S4, related to Figure 5. Lumen size of ureteric tips and methods to analyze the number of mitotic cells *in vivo*. The 3D wholemount kidney confocal data was analyzed using Imaris. **A,** a representative ureteric tip at E12.5 illustrates the method used to analyze the lumen volume and the number of pHH3+ cells per tip. (**a**) shows all fluorescent channels. Using the automated Imaris Isoline function, the edge of the Calb1 signal was used to draw both the outer surface of the ureteric epithelium (dotted white line, **b**) and the ureteric tip lumen (dotted yellow line, **b**) on each Z slice. The resulting 3D surface renders of the lumen (yellow, **c**) and tip render (white, **d**) were used to measure lumen volumes (results are shown in **B**). To separate ureteric trunks from tips, the Calb1 rendered 3D surface of the tree was manually cut perpendicular to where the tip joined the trunk (see example in **Fig. 5G''**). The ureteric tip 3D surface render was then used to mask the pHH3 fluorescent channel (**e**) followed by Imaris automated spot count function to count the number of pHH3+ cells (**f**) per tip (results are shown in **Fig. 5C**). **B,** the average ureteric tip lumen volume decreased dramatically from E11.75 to E15.5, and is illustrated with examples of each stage, **C** (rendered tip lumens are red). Error bars in **B** indicate standard error of the mean.

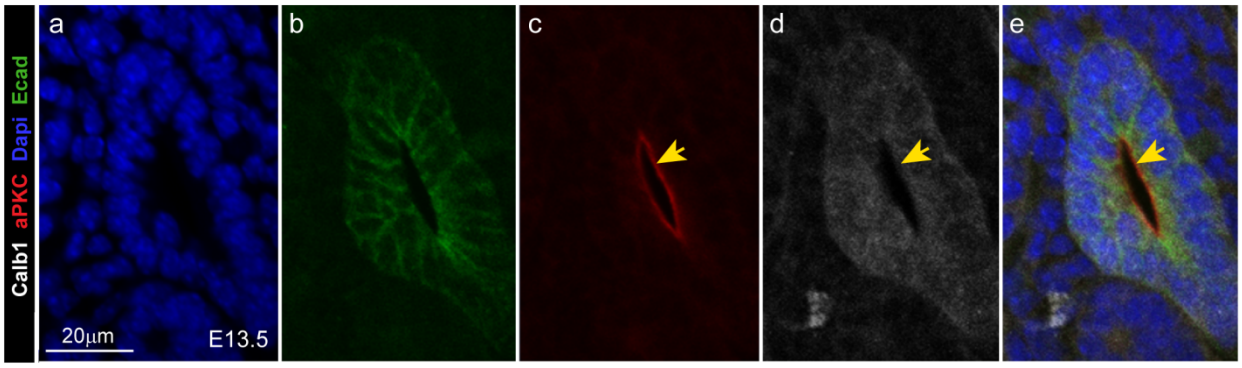

Figure S5, related to Figure 6. Calbindin1 expression in the ureteric tip extends to the apical surface. Optical section of E13.5 wholemount mouse kidney fluorescently labeled with **a**: DAPI (blue, nuclear marker), **b**: E-cadherin (green, epithelial marker), **c**: aPKC (red, apical surface marker), **d**: Calbindin1 (white) and **e**: merge. Yellow arrows (**c-e**) show that the Calb1 expression extends to the apical surface of the tip epithelium, defined by aPKC expression.

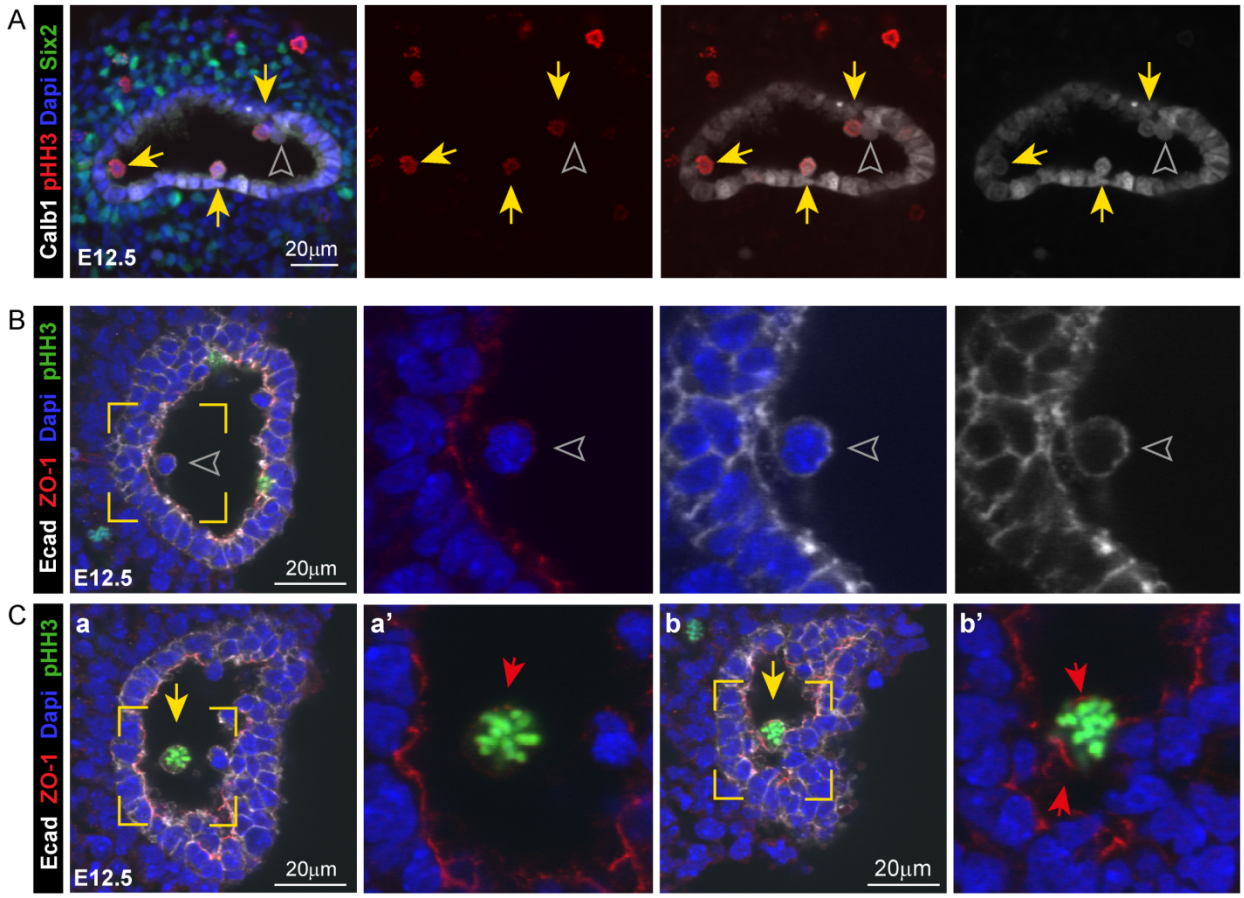

Figure S6, related to Figure 6. Calbindin1, ZO1 and E-cadherin expression in luminal mitotic cells in the ureteric tip epithelium *in vivo*. **A**, optical section of a ureteric tip from 3D confocal data of a wholemount mouse kidney at E12.5 (left panel is from Figure 5A), fluorescently labeled with markers for ureteric epithelium (anti-Calbindin1, white), mitotic cells (anti-pHH3, red), cap mesenchyme cells (anti-GFP to detect Six2GFP) and nuclei (DAPI, blue). Mitotic pHH3+ cells, seen here within the tip lumen (yellow arrows), expressed Calb1. Occasional Calb1+ tip cells that were pHH3-negative were seen within the tip lumen (grey open arrowheads). **B** and **C**, expression of epithelial surface marker E-cadherin and apical marker ZO-1 shown in optical sections of a E12.5 ureteric tip, fluorescently labeled with antibodies for E-cadherin (white), ZO-1 (red), pHH3 (green) and DAPI (blue). **B**, an example of a pHH3-negative tip cell located within the lumen (grey open arrowheads) expressing ZO-1 and E-cadherin. **C**, two different Z slices through an E12.5 tip (**a** and **b** and enlarged in **a'** and **b'**), demonstrate that the luminal pHH3+ tip cell (yellow arrows) seen completely within the lumen in **a** remains in contact with the tip surface (**b**). Red arrows indicate domains of ZO-1 expression on the pHH3+ luminal cell surface.

Supplemental Movies (MP4 format)

Movie S1, related to Figure 1. This movie shows the sequences of time-lapse images of ureteric bud cell divisions, from which the panels of **Figure 1A – 1D** were derived.

Movie S2, related to Figure 2. This movies shows the z-stack from which the images in **Figure 2B** were derived.

Movie S3, related to Figure 2. This movie shows the sequences of time-lapse images of ureteric bud cell divisions, from which the panels of **Figure 2C – 2F** were derived.

Movie S4, related to Figure 3. This movie shows a 3D rendering of the cell division from Figure 3B''. The asterisk at 0' (see Figure 3 for time course) marks the basal surface of the cell that is about to divide, as in Figure 3B''. The 3D image at 28' is rotated 360 degrees to show that the basal process is inherited by only one daughter cell.

Movie S5, related to Figure 4B, C. This movie is a 4D rendering of the kidney culture shown in **Figure 4B** and **4C**.

Movie S6, related to Figure 4B, C. This movie shows a time sequence of optical sections at the level of the epithelium (left) or at a level bisecting the lumen (right).

Movie S7, related to Figure 4D, E. This movie shows the sequences of time-lapse images of ureteric bud cell divisions, from which the panels of **Figure 4D and 4E** were derived. The dividing cells of interest are pseudo-colored green.