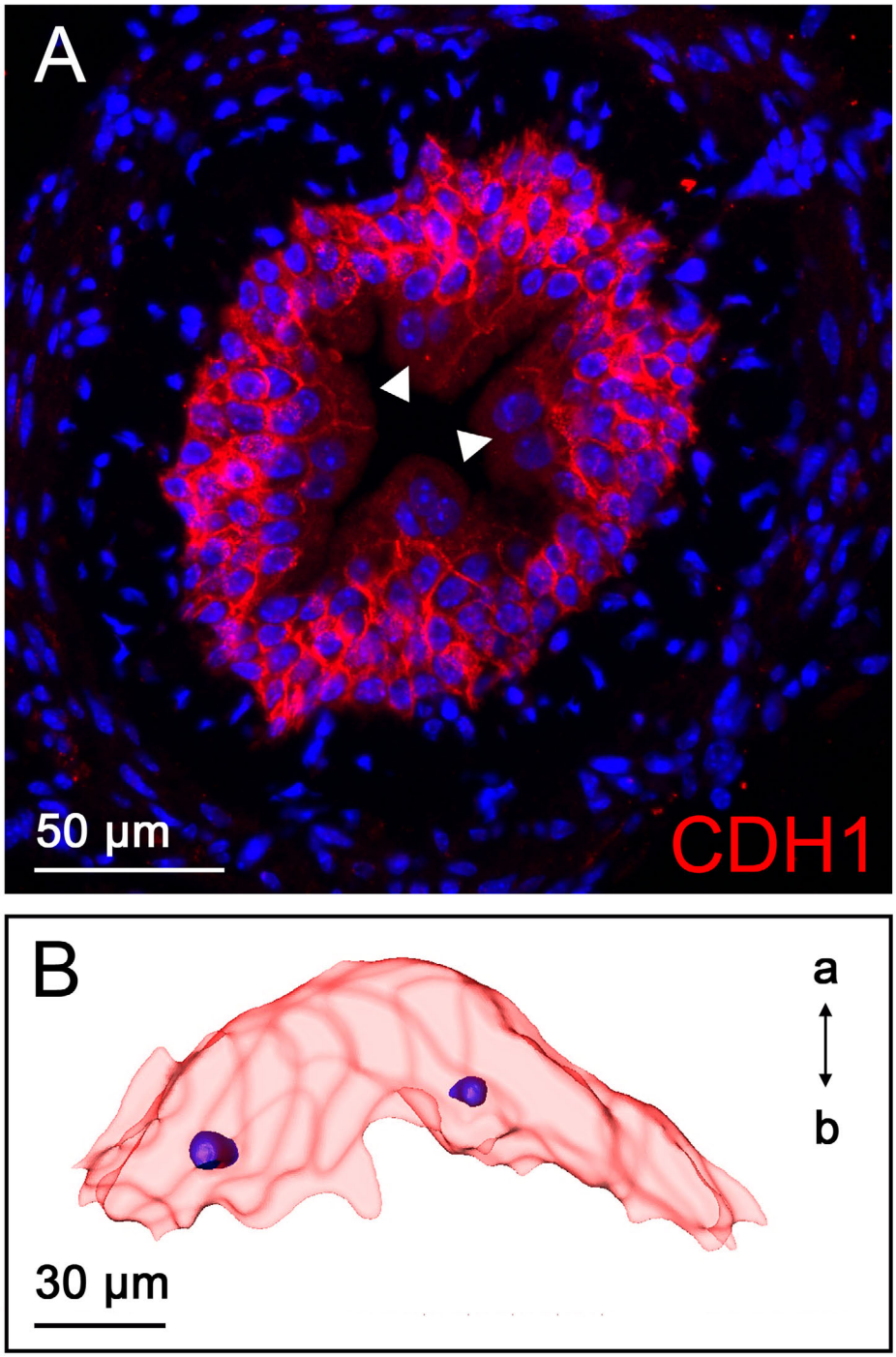
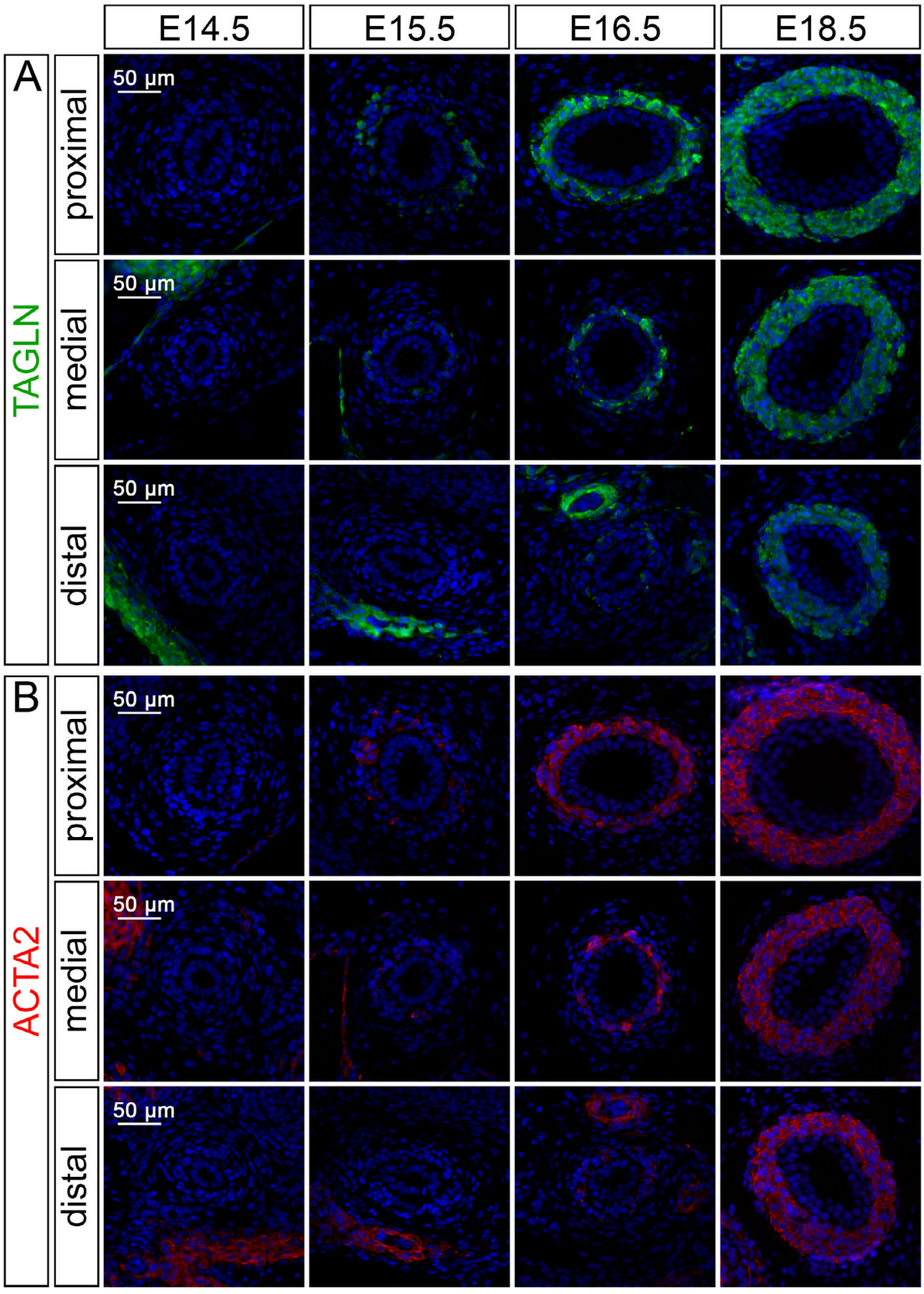
Supplemental Data

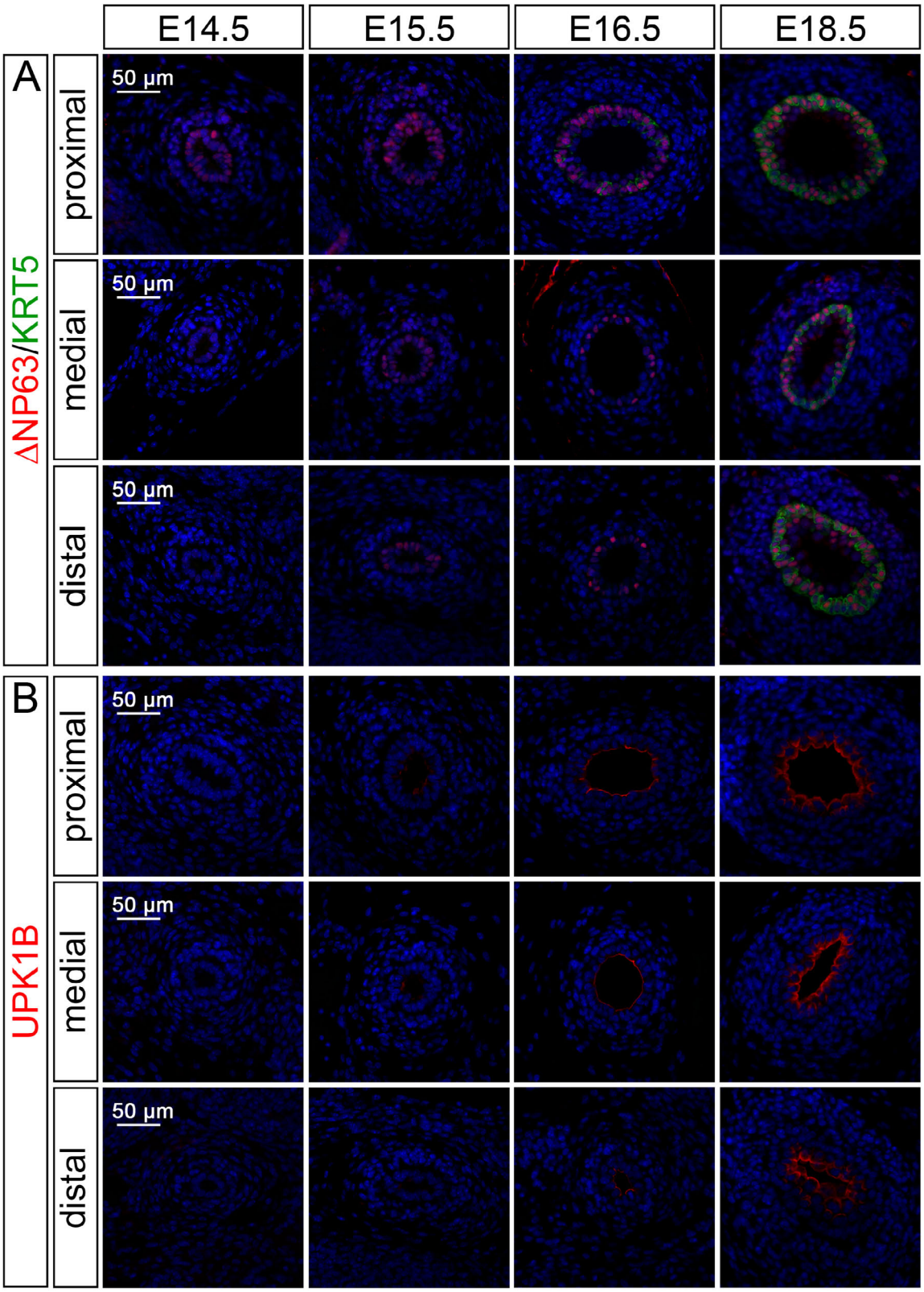
Supplemental Figures



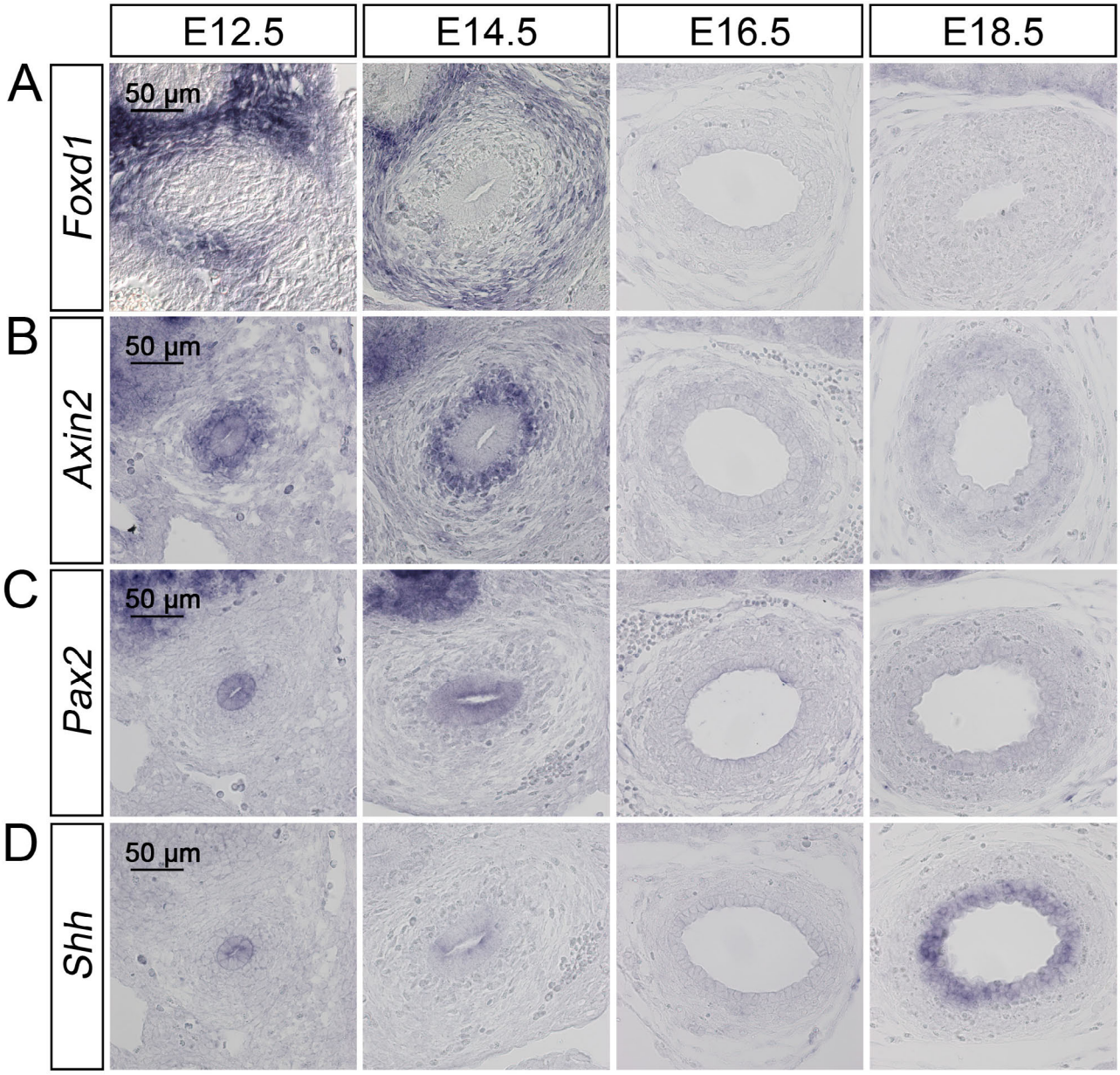
Supplemental Figure 1. S-cells in the ureter are binucleate. (A) Immunofluorescence analysis of CDH1 expression with nuclear DAPI counterstain (blue) on transverse sections of a proximal ureter at P220. White arrowheads point towards binucleated S-cells. (B) Three-dimensional reconstruction of a single S-cell harboring two nuclei based on serial sections of an adult ureter stained as in (A). a, apical; b, basal.



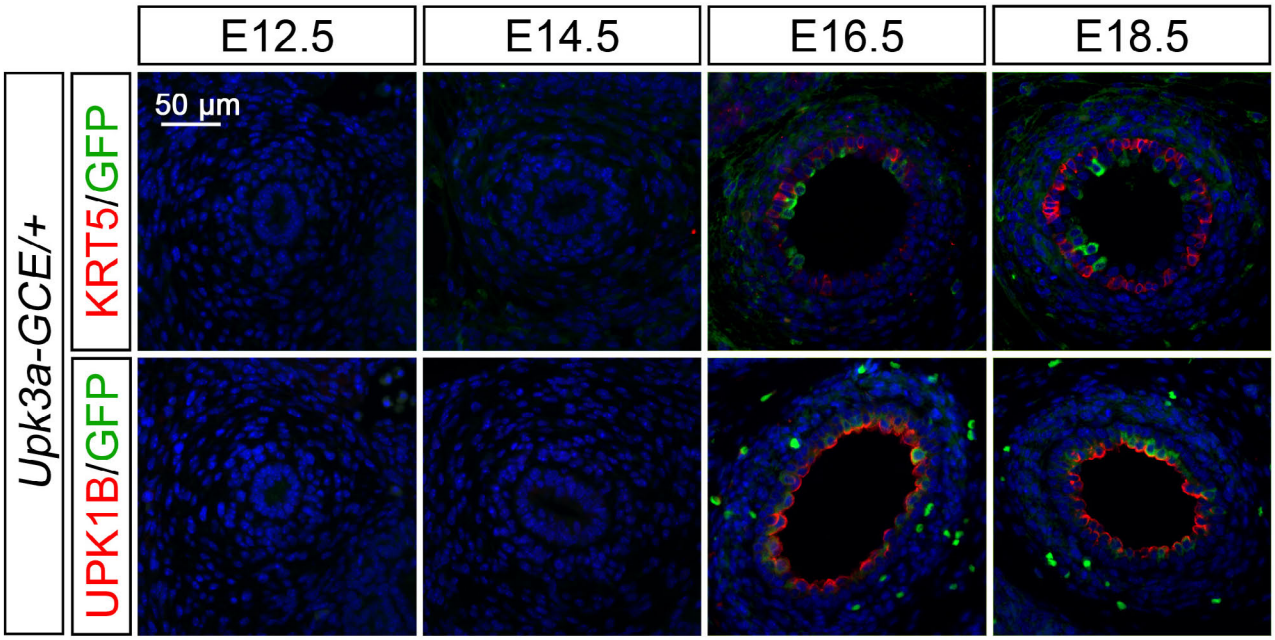
Supplemental Figure 2. Cellular differentiation in the ureteric mesenchyme proceeds in a proximal to distal fashion. Immunofluorescence analysis of the SMC markers TAGLN (A) and ACTA2 (B) with nuclear DAPI (blue) on transverse sections of E14.5, E15.5, E16.5 and E18.5 ureters sectioned each at a proximal (upper row), medial (middle row) and distal (lower row) level. Expression of differentiation markers at the proximal ureter level precedes their expression at more distal levels by approximately one day.



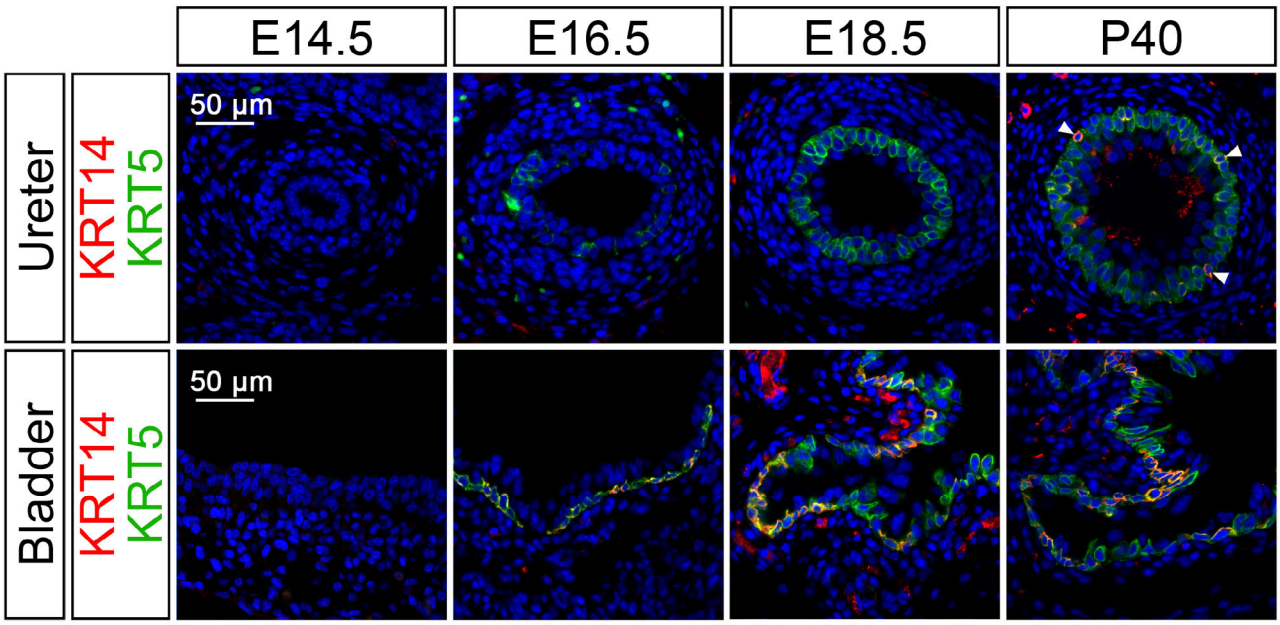
Supplemental Figure 3. Cellular differentiation in the ureteric epithelium proceeds in a proximal to distal fashion. Immunofluorescence analysis of the urothelial markers ΔNP63/KRT5 (A) and UPK1B (B) with nuclear DAPI (blue) on transverse sections of E14.5, E15.5, E16.5 and E18.5 ureters sectioned each at a proximal (upper row), medial (middle row) and distal (lower row) level. Expression of differentiation markers at the proximal ureter level occurs approximately one day earlier than at the distal level.



Supplemental Figure 4. Gene expression during ureter development confirms specificity of *cre* driver lines. (A-D) *In situ* hybridization analysis of gene expression on proximal sections of the mouse ureter at the indicated stages. (A) *Foxd1* expression is restricted to the outer mesenchymal domain at E12.5 and E14.5 while (B) *Axin2* mRNA is found only in the inner mesenchymal domain at these two stages. (C) *Pax2* expression is confined to the ureteric epithelium at E12.5 and E14.5. (D) *Shh* mRNA is expressed in the ureteric epithelium from E12.5 to E18.5 with reduced expression at E16.5.



Supplemental Figure 5. The *Upk3a-GCE* driver line mediates *loxP*-recombination in I- and S-cells but not in B-cells. Co-immunofluorescence analysis of expression of cytoplasmic GFP in the GFP-creRT2 fusion protein expressed from the *Tg(Upk3a-GFP/cre/ERT2)26Amc (Upk3a-GCE*) transgene with nuclear DAPI (blue) and the B-cell marker KRT5 (upper row) and the I- and S-cell marker UPK1B (lower row) on proximal sections of a ureter at the indicated embryonic stages. GFP and KRT5 expression is mutually exclusive while GFP overlaps with UPK1B showing that the GFP-creERT2 fusion protein, thus, cre activity is confined to I- and S-cells.



Supplemental Figure 6. KRT14 expression marks a small subpopulation of KRT5^{+} B-cells in the adult ureter. Co-immunofluorescence analysis of expression of KRT14 and the B-cell marker KRT5 with nuclear DAPI counterstain (blue) on transverse sections of the proximal ureter (upper row) and on mid-sagital sections of the bladder (lower row) at the indicated stages. In the ureter, KRT14 expression is found in few KRT5^{+} B-cells in the P40 adult only (white arrowheads) whereas KRT14 marks a large subpopulation of KRT5^{+} B-cells in the bladder from E16.5 onwards.

Supplemental Tables

Stage	Undiff. (%)	TA-cells (%)	SM-cells (%)	LP-cells (%)
E12.5	100	0	0	0
E13.5	57.1 ± 6.1	42.9 ± 6.1	0	0
E14.5	47.8 ± 3.3	52.2 ± 3.3	0	0
E15.5	34.6 ± 8.1	54.3 ± 2.2	11.1 ± 8.3	0
E16.5	15.5 ± 7.2	49.2 ± 4.5	26.2 ± 6.2	9.1 ± 1.1
E18.5	0	53.7 ± 3.6	37.0 ± 2.3	9.3 ± 1.4
P40	0	15.0 ± 1.1	62.6 ± 2.9	22.4 ± 2.8

Supplemental Table 1A. Distribution of mesenchymal cell types during ureter development. Values are displayed in % as mean ± sd of undifferentiated cells (Undiff.), adventitial cells (TA-cells), smooth muscle cells (SM-cells) and *Lamina propria* cells (LP-cells) with respect to the total cell number. Quantification is based on 6 sections from three individuals at each stage.

Stage	Undiff. (%)	B-cells (%)	I-cells (%)	S-cells (%)
E12.5	100	0	0	0
E13.5	97.4 ± 4.3	0	2.6 ± 4.3	0
E14.5	17.5 ± 8.9	0	82.5 ± 8.9	0
E15.5	0	0	95.5 ± 1.6	4.5 ± 1.6
E16.5	0	37.9 ± 9.8	39.5 ± 8.8	22.5 ± 2.4
E18.5	0	53.8 ± 5.1	26.9 ± 4.8	19.4 ± 3.8
P40	0	71.3 ± 3.2	21.8 ± 3.6	6.9 ± 1.5

Supplemental Table 1B. Distribution of epithelial cell types during ureter development. Values are displayed in % as mean ± sd of undifferentiated cells (Undiff.), basal cells (B-cells), intermediate cells (I-cells) and superficial cells (S-cells) with respect to the total cell number. Quantification is based on 6 sections from three individuals at each stage.

Stage	IM	OM	UE
E12.5	0.236 ± 0.030	0.217 ± 0.017	0.228 ± 0.028
E14.5	0.198 ± 0.009	0.202 ± 0.014	0.230 ± 0.013

Supplemental Table 2A. Proliferation rates in the developing ureter at E12.5 and E14.5. Shown are the BrdU indices for the inner mesenchymal domain (IM), the outer mesenchymal domain (OM) and the ureteric epithelium (UE). The BrdU index is displayed as mean ± sd, 12 sections from three individuals were used for quantification.

Stage	LP	SMC	TA
E16.5	0.233 ± 0.022	0.191 ± 0.008	0.122 ± 0.007
E18.5	0.267 ± 0.025	0.134 ± 0.009	0.081 ± 0.004
P40	0.003 ± 0.002	0	0.008 ± 0.003

Supplemental Table 2B. Proliferation rates in the ureteric mesenchyme at E16.5, E18.5 and at P40. Shown are the BrdU indices for the inner *Lamina propria* (LP), the smooth muscle cells (SMC) and the *Tunica adventitia* (TA). The BrdU index is displayed as mean ± sd, 8 sections from three individuals were used for quantification.

Stage	BC	IC	SC
E16.5	0.203 ± 0.040	0.098 ± 0.008	0.175 ± 0.022
E18.5	0.035 ± 0.012	0.014 ± 0.009	0.087 ± 0.024
P40	0.001 ± 0.002	0	0

Supplemental Table 2C. Proliferation rates in the ureteric epithelium at E16.5, E18.5 and at P40. Shown are the BrdU indices for basal cells (BC), intermediate cells (IC) and superficial cells (SC). The BrdU index is displayed as mean ± sd, 8 sections from three individuals were used for quantification.

Stage	IM (%)	OM (%)	Numbers
E12.5 + 1d	76.7 ± 5.3	23.3 ± 5.3	269
E13.5 + 1d	94.3 ± 3.5	5.7 ± 3.5	129

Supplemental Table 3A. Lineage labelling in the ureteric mesenchyme of *Axin2$^{creERT/+}$;R26$^{mTmG/+}$* embryos that were Tamoxifen-induced at E12.5 or E13.5 and analysed for distribution of recombined GFP^{+} cells 24 h later. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the inner (IM) or outer (OM) mesenchymal compartment of the ureter. Additionally, the total number of GFP^{+} cells detected in a total of four individuals is displayed.

Stage	LP (%)	SMC (%)	TA (%)	Numbers
E12.5 + 6d	18.0 ± 2.8	70.7 ± 0.6	11.3 ± 2.6	521
E13.5 + 5d	17.1 ± 4.4	73.2 ± 4.0	9.7 ± 4.6	583

Supplemental Table 3B. Lineage tracing in the ureteric mesenchyme of *Axin2$^{creERT/+}$;R26$^{mTmG/+}$* embryos that were tamoxifen-induced at E12.5 or E13.5 and analysed for distribution of recombined GFP^{+} cells 6 or 5 days later. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the *Lamina propria* (LP), smooth muscle layer (SMC) or *Tunica adventitia* (TA) of the ureter. Additionally, the total number of GFP^{+} cells detected in a total of four individuals is displayed.

Stage	KRT5^{+} UPK1B^{-} (%)	KRT5^{-} UPK1B^{+} (%)	Numbers
E14.5 + 10d	0	0	0
E16.5 + 8d	100	0	135
E18.5 + 6d	100	0	409
Adult + 4w	100	0	552

Supplemental Table 4A. Lineage tracing in *Krt5$^{creERT2/+}$;R26$^{mTmG/+}$* ureter explants from E14.5, E16.5 and E18.5 mice cultured for 10 d, 8 d, and 6 d, respectively, and in adult mice after 4 weeks of labelling. First row: KRT5^{+}UPK1B^{-}. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the KRT5^{+}UPK1B^{-} basal cell layer. Second row: KRT5^{-}UPK1B^{+}. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the KRT5^{-}UPK1B^{+} intermediate and superficial cell layers. Third row: Numbers. Total number of GFP^{+} cells detected in a total of three individual ureters.

Stage	KRT5^{+} UPK1B^{-} (%)	KRT5^{-} UPK1B^{+} (%)	Numbers
E14.5 + 10d	31.7 ± 14.4	68.3 ± 14.4	113
E16.5 + 8d	57.1 ± 9.4	42.9 ± 9.4	271
E18.5 + 6d	55.0 ± 13.4	45.0 ± 13.4	384
Adult + 4w	41.7 ± 1.2	58.3 ± 1.2	652

Supplemental Table 4B. Lineage tracing in *Upk3a-GCE/+;R26$^{mTmG/+}$* ureter explants from E14.5, E16.5 and E18.5 mice cultured for 10 d, 8 d, and 6 d, respectively, and in adult mice after 4 weeks of labelling. First row: KRT5^{+}UPK1B^{-}. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the KRT5^{+}UPK1B^{-} basal cell layer. Second row: KRT5^{-}UPK1B^{+}. Values are displayed in % as mean ± sd of all GFP-labelled cells found in the KRT5^{-}UPK1B^{+} intermediate and superficial cell layers. Third row: Numbers. Total number of GFP^{+} cells detected in a total of three individual ureters.