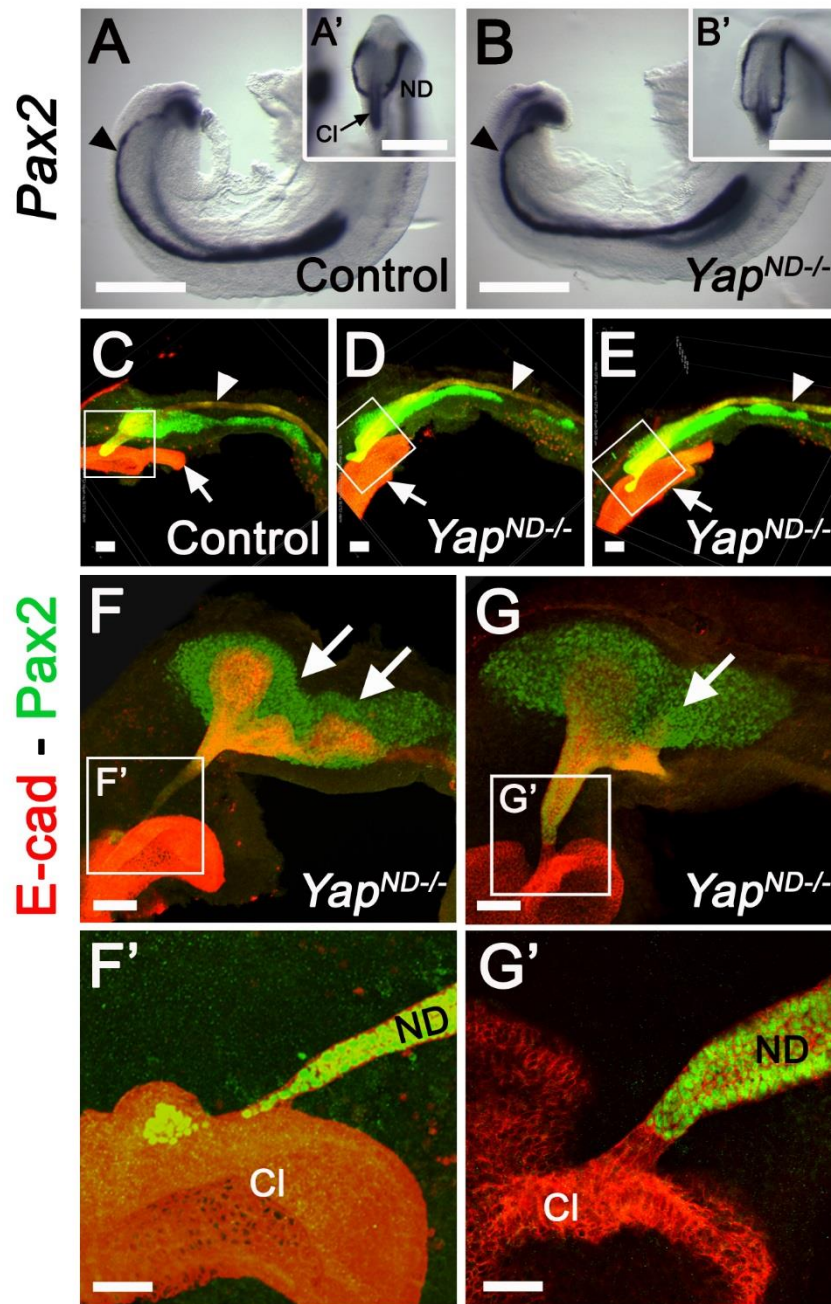


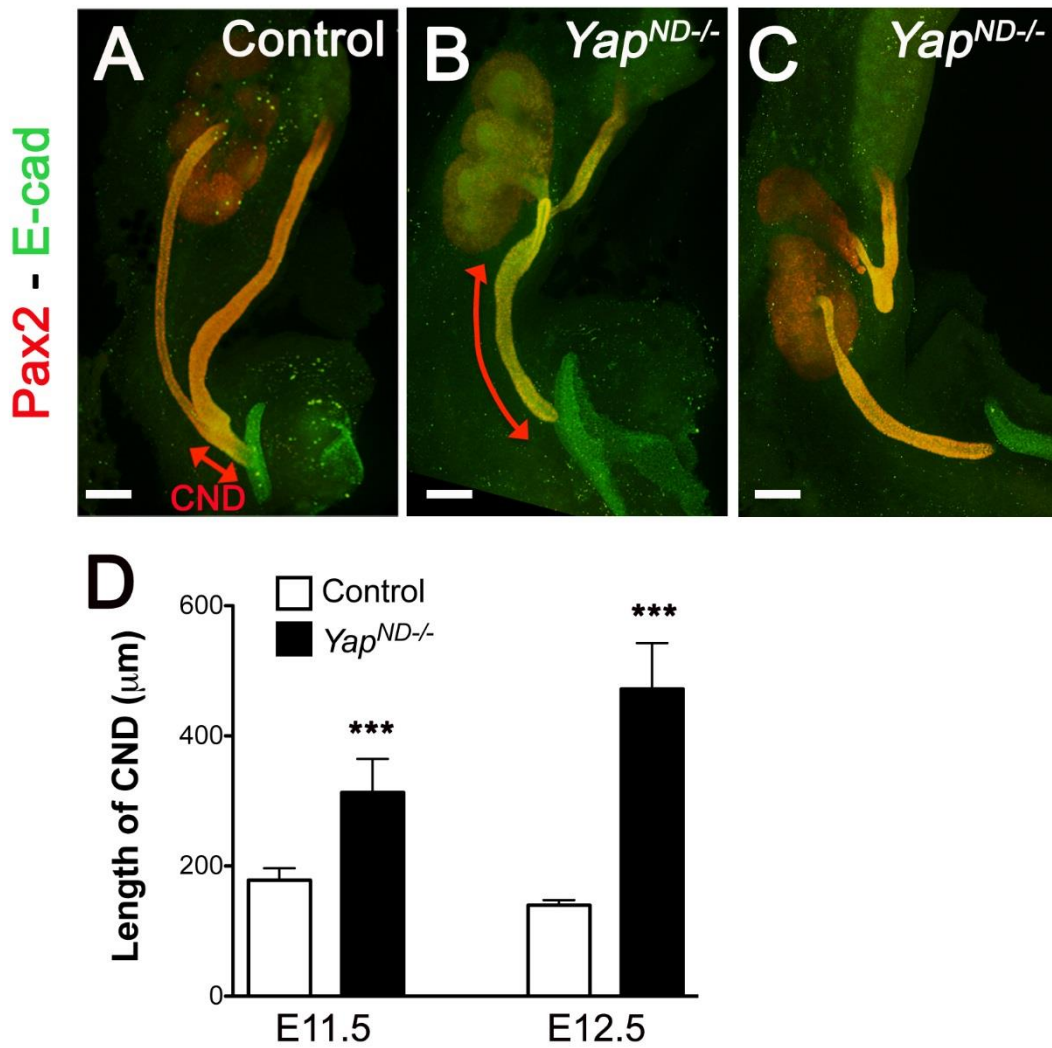
**Figure S1: *Yap*<sup>ND-/-</sup> escapers, Cre efficiency and smooth muscle differentiation.**

PAS staining of wild-type (A) and *Yap* mutant (B,C) kidneys at post-natal day 50. (D-F') Efficiency of the *Hoxb7:Cre* deletion on *Yap* conditional allele at E11.5. Yap antibody staining in controls (D) and *Yap* mutants (E) confirmed Yap deletion within the ND, UB and UB tips, whereas Yap staining in the cloaca (Cl) compartment persists. (F) Rarely, we observed mosaic efficiency of Cre deletion in the CND and the ureter which could explain why a few *Yap* cKO embryos can survive after birth. (G,H) PAS staining of E14.5 kidneys from wild-type and *Yap*<sup>ND-/-</sup> animals. (I,J) Alpha-smooth muscle actin immunostaining analysis on ureter cross-section of E16.5 animals showed normal differentiation of the smooth muscle along the ureter in both genotypes. Scale bars represent 1 mm (A-C) and 100  $\mu$ m (D-J).



**Figure S2: *Yap* signaling is essential for the ND insertion into the cloaca.**

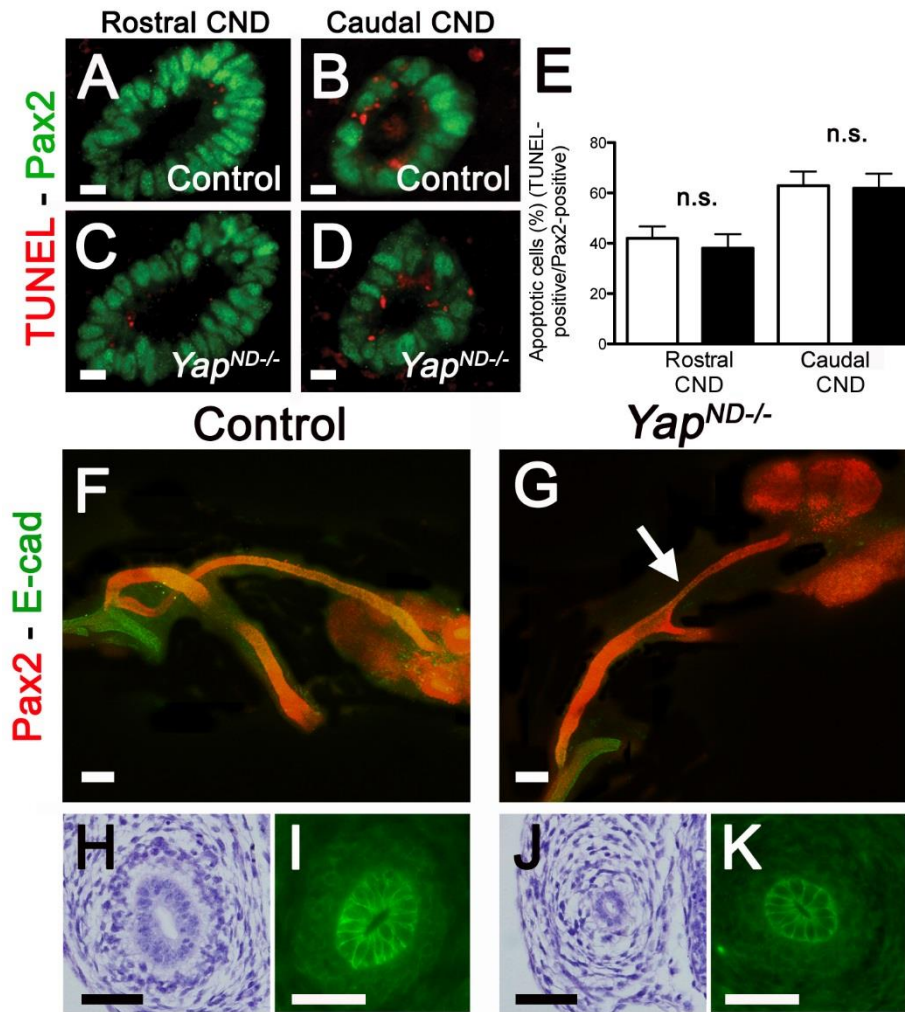
(A,B) WM ISH using *Pax2* riboprobe to visualize both ND and cloaca at E9.5 showed normal migration of the ND towards the cloaca in both genotypes. (C-E) Low magnification views of the ND - cloaca connection at E10.5 (shown in Fig. 2B-D). Arrowheads point to the ND and arrows to the cloaca. (F-G') *Pax2*/E-cadherin WM IF staining at E11.5 revealed the extent of the ND - cloaca connection in *Yap* mutants compared to controls. Scale bars represent 500  $\mu\text{m}$  (A,B), 100  $\mu\text{m}$  (C-G) and 50  $\mu\text{m}$



**Figure S3: Increased CND length in *Yap* mutants.**

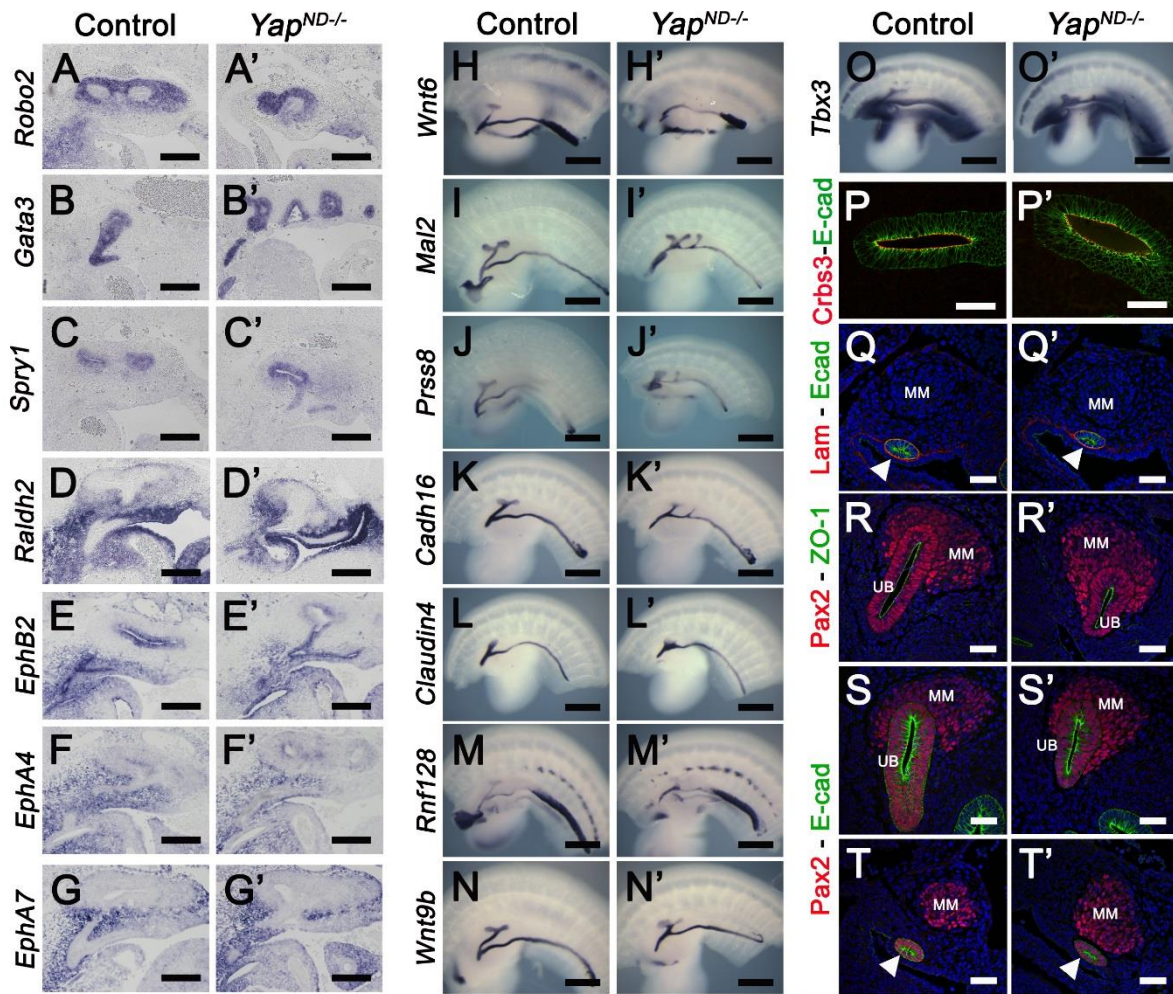
(A-C) Pax2/E-cadherin WM IF staining at E12.5 revealed the extent of kidney abnormalities in *Yap* mutants compared to controls. (D) Quantification of the length of the CND at E11.5 and E12.5 in controls (white) and *Yap* mutants (black). Error bars indicate the Standard Deviation (SD). \*\*\*:  $p < 0.0001$ . Scale bars represent 100 μm. (F',G').





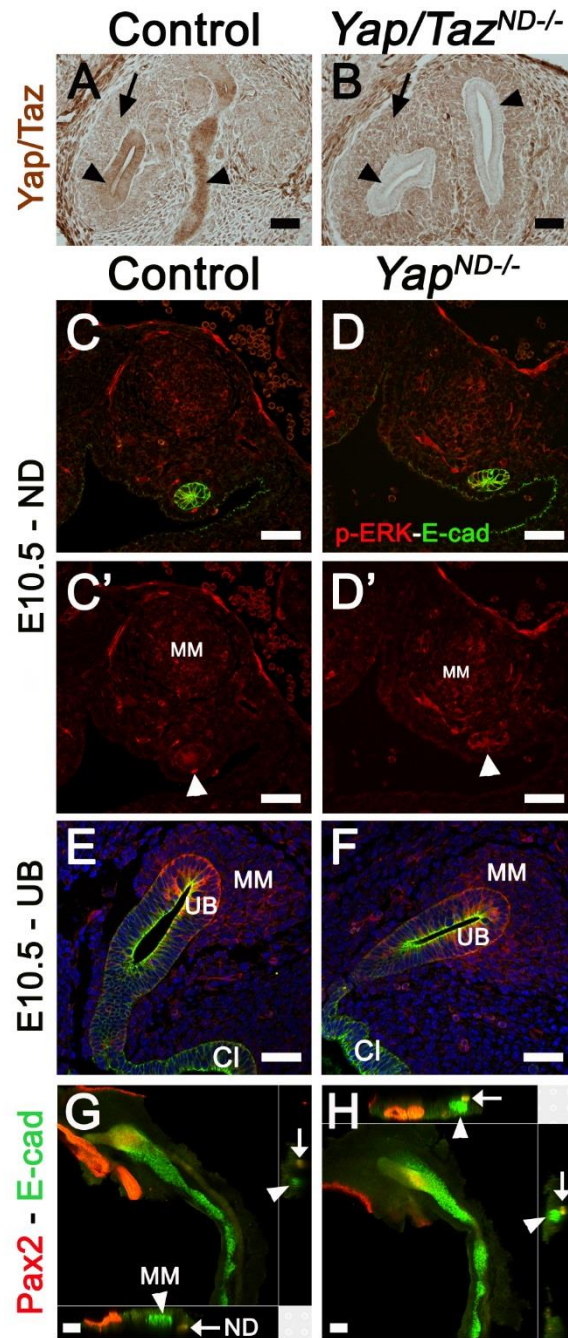
**Figure S4: Altered morphology of the ureter in *Yap* mutants.**

(A-D) TUNEL (red) and Pax2 (green) staining on E12.5 transverse sections of rostral CND (closest to ureter) and caudal CND (closest to bladder) of controls and *Yap* mutants. (E) Quantitative analysis of the cell death observed in control and *Yap* mutant CNDs at E12 revealed no significant (n.s.) changes. (F,G) Pax2/E-cadherin WM IF at E12.5 revealed thinning of the ureter diameter (arrow in G). This is confirmed by PAS staining (H,J) and E-cadherin (I,K) staining of ureter cross-sections at E14.5. Scale bars represent 10  $\mu$ m (A-D), 100  $\mu$ m (F,G) and 50  $\mu$ m (H-K).



**Figure S5: Cell polarity and specification is not affected by *Yap* deletion.**

(A-G') *In situ* hybridization on sections showed no change in *Robo2*, *Gata3*, *Sprouty1*, *Raldh2*, *EphB2*, *EphA4* and *EphA7* expression between controls and *Yap* mutants at E11.5. (H-O') WM ISH showed normal expression of *Wnt6*, *Mal2*, *Prss8*, *Cadherin16*, *Claudin4*, *Rnf128*, *Wnt9b* and *Tbx3* in the ND of controls and mutants at E11.5. (P-T') *Yap* mutant cells, like wild-type, acquired cell polarity as shown by *Crumbs3*, *Laminin*, *ZO-1* and *E-cadherin* staining at E10.5 (Q,T) and E11.5 (P,R,S). Scale bars represent 200 μm (A-G'), 500 μm (H-O') and 50 μm (P-T').



**Figure S6: Efficient Cre excision of *Yap/Taz* flox alleles, p-ERK analysis and abnormal mesenchymal disposition.**

(A,B) Immunostaining at E12.5 with a Yap/Taz antibody confirmed the absence of Yap/Taz expression in the ureter/UB compartment (arrowhead) of *Yap/Taz* double knockout while expression in the mesenchyme persists (arrow). (C-F) Phospho-ERK staining in controls and *Yap* mutants in the ND and UB at E10.5 and E11.5. E-cadherin counterstaining was used to label the epithelial structures. (G,H) Metanephric mesenchyme is malpositioned in *Yap* mutants. Insets represent 3D cross-sectional renderings of confocal images displayed on two axes clearly depicting the close apposition of the MM (arrowhead) and ND (arrow) in *Yap*<sup>ND-/-</sup> mutants compared to controls. ND: nephric duct, Cl: cloaca, UB: ureteric bud, MM: metanephric mesenchyme. Scale bars represent 50  $\mu$ m (A-F) and 100  $\mu$ m (G,H).