Supplemental Material For Mao et al, A Fat4-Dchs1 signal between stromal and cap mesenchyme cells influences nephrogenesis and ureteric bud branching

Supplementary Methods

Primer sequences:

GapdhF AGGTCGGTGTGAACGGATTTG

GapdhR TGTAGACCATGTAGTTGAGGTCA

six2F: CACCTCCACAAGAATGAAAGCG

six2R: CTCCGCCTCGATGTAGTGC

wnt7bF: TTTGGCGTCCTCTACGTGAAG

wnt7bR: CCCCGATCACAATGATGGCA

retF: GCATGTCAGACCCGAACTGG

retR: CGCTGAGGGTGAAACCATCC

sox9F: GAGCCGGATCTGAAGAaGGA

sox9R: GCTTGACGTGTGGCTTGTTC

E-CadF: CAGGTCTCCTCATGGCTTTGC

E-cadR: CTTCCGAAAAGAAGGCTGTCC

Ecm1F: GGGACCGTATCCAGAGCAG

Ecm1R: GCTGGTCTGAAGCCTTGAAG

DcnF: TCTTGGGCTGGACCATTTGAA

DcnR: CATCGGTAGGGGCACATAGA

Primers sequences were designed using http://pga.mgh.harvard.edu/primerbank/.

Supplementary Figures

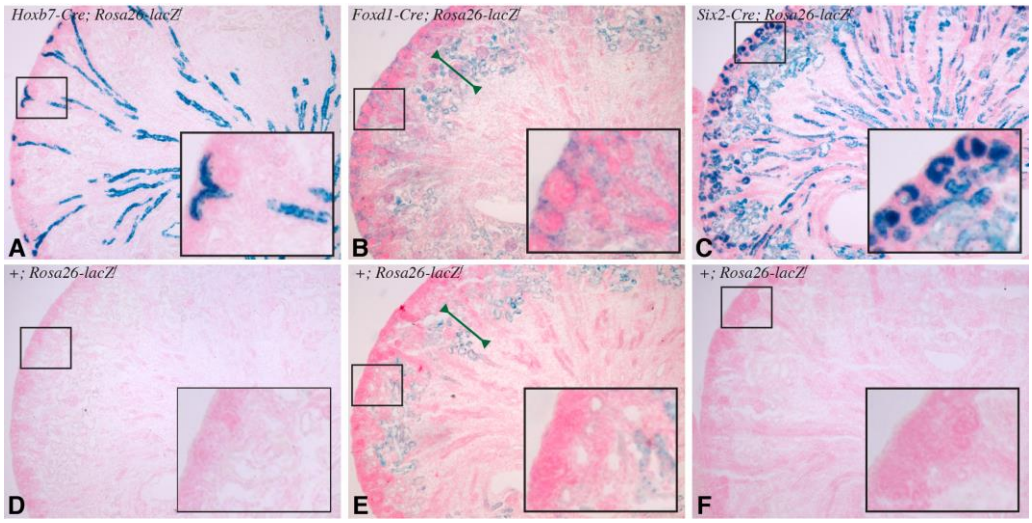

Figure S1. Confirmation of tissue-specific Cre activity

Sections of Newborn (P0) mouse kidneys subject to histochemical staining for ß-galactosidase activity expressed from a conditional Rosa26-lacZ allele. A) Hoxb7-Cre B) Foxd1-Cre C) Six2-Cre. D-F) show sibling controls that lack Cre-expressing alleles. Insets show higher magnification of the boxed region encompassing uteric distal tip and nephrogenic precursors. Sections were counterstained with nuclear Fast Red.

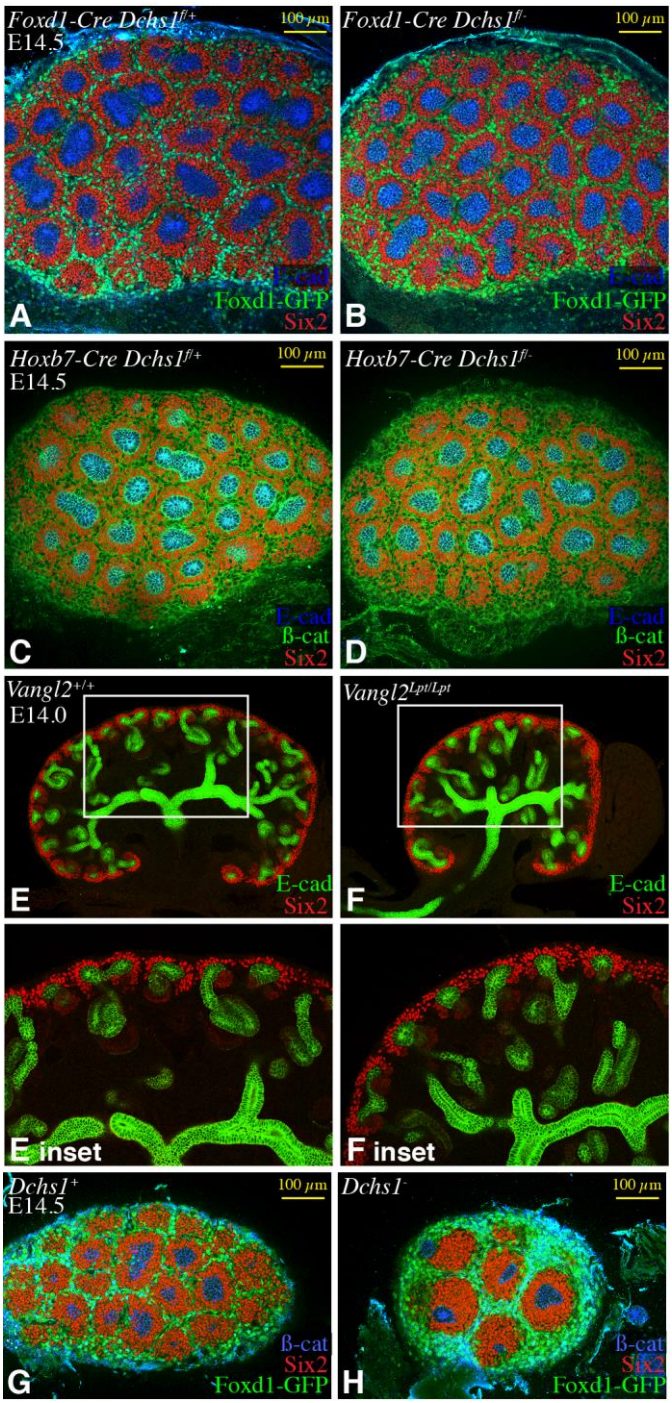

Figure S2. Lack of requirement for Dchs1 in stroma or UB

Embryonic kidneys stained for Six2 (red) to reveal CM, together with E-cad (blue, expressed in UB), and either ß-cat (green, expressed in all cells) or GFP (green). Scale bar (yellow) is 100 μm. A,B) Surface sections of E14.5 kidney from sibling control (A, Foxd1-Cre Dchs1$^{f/+}$), or mouse with conditional deletion of Dchs1 in stroma (B, Foxd1-

Cre Dchs1$^{f/-}$). C,D) Surface sections of E14.5 kidney from sibling control (C, Hoxb7-Cre Dchs1$^{f/+}$), or mouse with conditional deletion of Dchs1 in UB (D, Hoxb7-Cre Dchs1$^{f/-}$). E,F) E14.0 *Vangl2*^{Lpt} (F) and wild-type sibling (E), stained for E-cad (green) and Six2 (red). Lower panels (-inset) show higher magnifications of the boxed regions. (G,H) Surface section through sibling control (G) and *Dchs1*$^{-/-}$ (H) E14.5 kidneys imaged for ß-cat (blue), Six2 (red), and Foxd1-GFP (green).

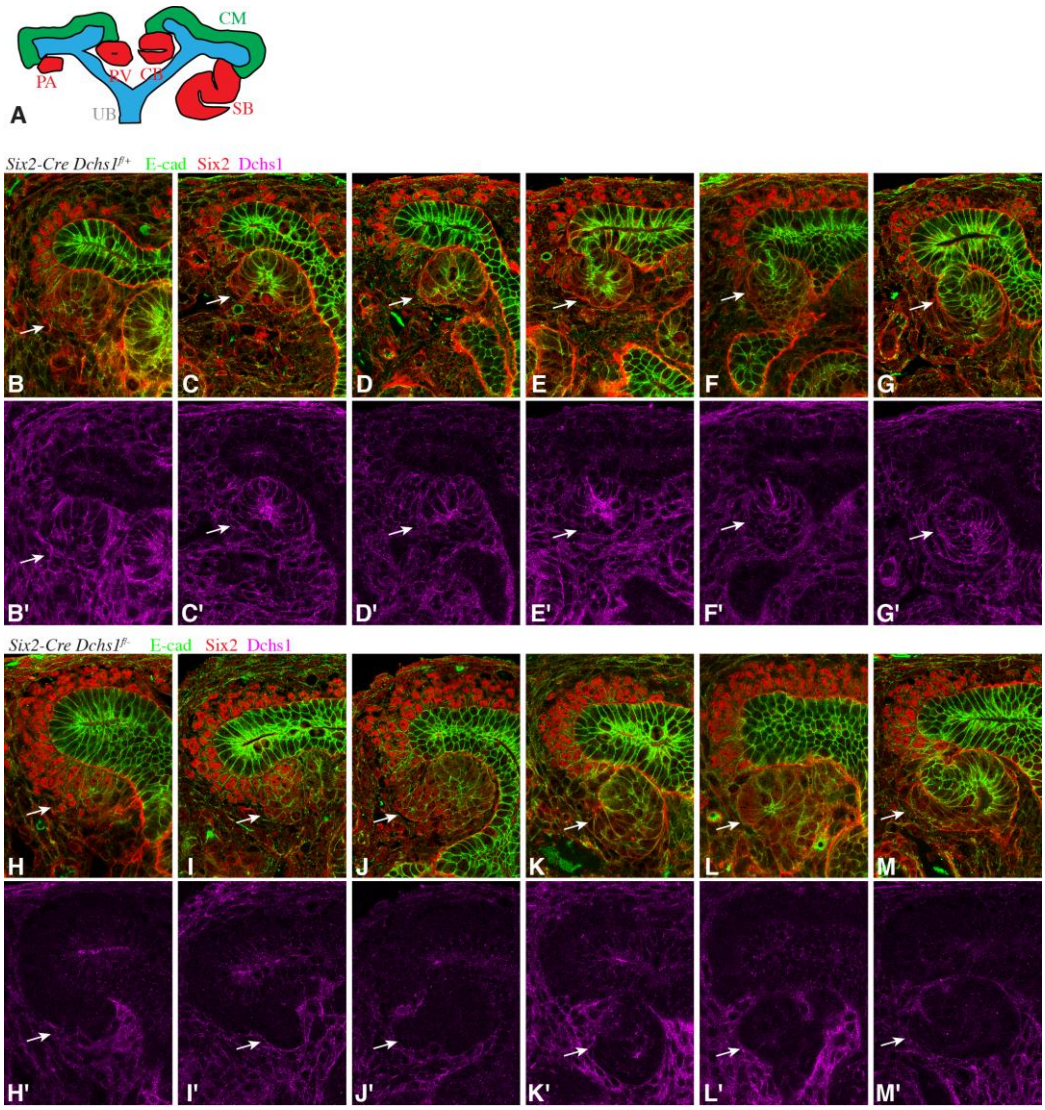

Figure S3. Abnormal initiation of nephrogenesis after conditional deletion of Dchs1 in CM

A) Schematic illustrating progressive stages of early nephrogenesis, from PA (pre-cellular aggregate) to RV (renal vesicle) to CB (comma shaped body) to SB (S shaped body). B-M) Examples from sibling controls (B-G, *Six2-Cre Dchs1$^{f/+}$*) or kidneys with conditional deletion of Dchs1 in CM (H-M, *Six2-Cre Dchs1$^{f/-}$*), stained for E-cad (green), Six2 (red), and Dchs1 (magenta, in panels marked by prime symbols). Several examples

are shown because the defects are heterogeneous. White arrows indicate the forming PA/RV/CB. Defects visible here include persistence of strong nuclear Six2 expression on the proximal side of the UB (H,I), irregular size and shape of the RV/CB (J-M, cf C-G), and irregular location and orientation of connecting tubules (L,M, cf E-G), see also Fig. 4. Dchs1 staining in A-G reveals distinct apical accumulation of Dchs1 in RV. Lack of Dchs1 staining in H-M identifies CM derivatives even after Six2 expression declines.

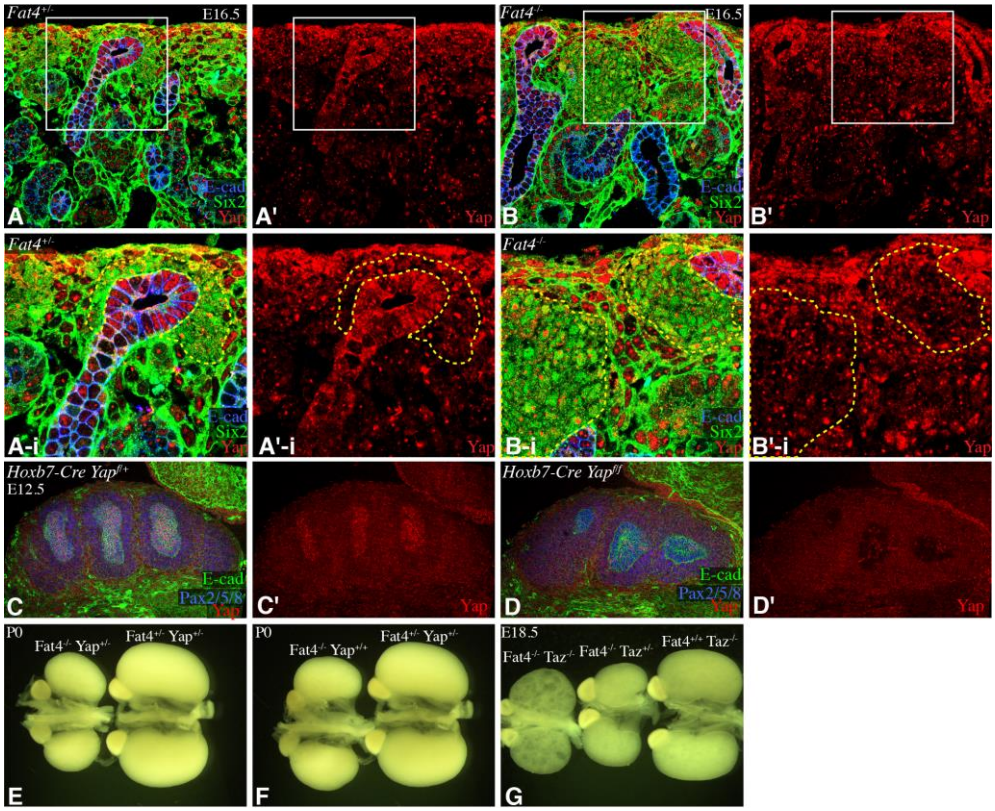

Figure S4. Lack of interaction of *Fat4* mutation with Yap or Taz

A,B) Confocal images of E16.5 kidneys stained for Yap using tyrimide signal amplification (red), Six2 (green), E-cad (blue). The Six2 antisera used here has high non-specific background, but Six2-specific staining is nuclear. The boxed regions are shown at high magnification below (panels labeled by "i"). Prime symbols identify panels with only a subset of stains shown. Dashed yellow line outlines CM, which shows similar levels of heterogeneous Yap nuclear stain in wild-type and mutants. C,D) Whole E12.5 kidneys stained for Yap (red) Pax2/5/8 (blue) and E-cad (green) for confirmation of Yap antisera specificity, from sibling control (C, Hoxb7-Cre $Yap^{f/+}$), or mouse with conditional deletion of Yap in UB (D, Hoxb7-Cre $Yap^{f/f}$). E-G) Whole kidneys from mice with combinations of *Fat4*, *Yap*, and *Taz* alleles. E) Comparison of P0 kidneys from $Fat4^{-/-}$ $Yap^{+/-}$ and $Fat4^{+/-}$ $Yap^{+/-}$ siblings. F) Comparison of P0 kidneys from $Fat4^{-/-}$ $Yap^{+/+}$ and $Fat4^{+/-}$ $Yap^{+/-}$ siblings. G) Comparison of E18.5 kidneys from $Fat4^{-/-}$ $Taz^{-/-}$, $Fat4^{-/-}$ $Taz^{+/-}$, $Fat4^{+/+}$ $Taz^{-/-}$ siblings.