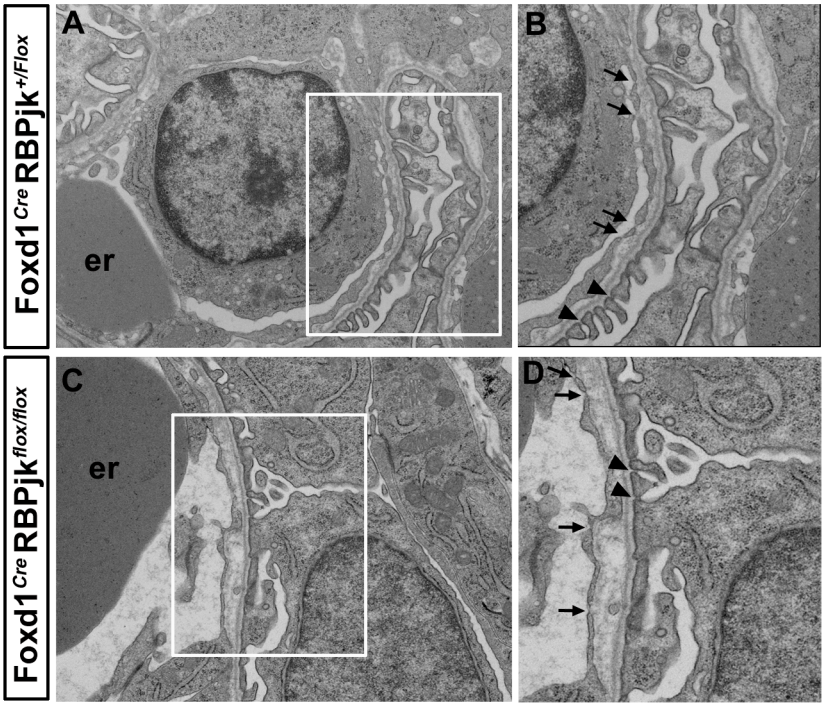

Figure S1. Fenestrated endothelial cells and podocyte slit diaphragms are present in *Foxd1^{tgCre} RBPjk$^{F/F}$ Rosa$^{+/eYFP}$* mice. To investigate whether the formation of fenestrated endothelial cells and podocyte slit diaphragms required mesangial cells we performed scanning electron microscopy on glomeruli from P0 control and *FoxD1^{tgCre} RBPjk$^{F/F}$ Rosa$^{+/eYFP}$* mice. **(A,B)** Single terminus of a capillary loop in control kidney showing an erythrocyte (er) passing through the filtration space. Endotheial fenestrations are noted with arrows and podocyte slit diaphragms are marked with arrowheads. **(C,D)** Mutant glomeruli contain a dilated vascular space but fenestrations (arrows) are still evident on endothelial cell membranes. Podocyte foot processes attach to the basement membrane (light gray strip) and slit diaphragms are present in the absence of mesangial cells (arrowheads).

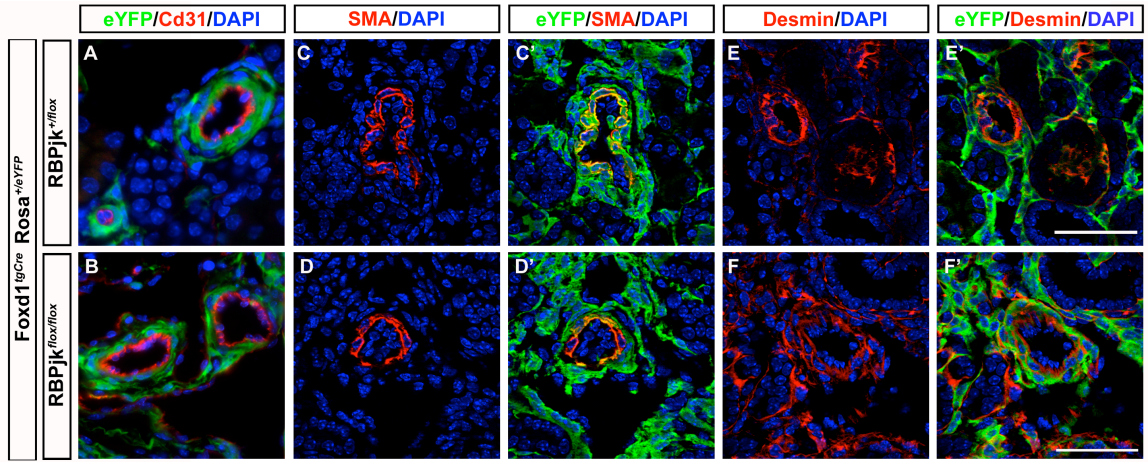

Figure S2. VSMCs differentiate normally in *Foxd1^{tgCre} RBPjk$^{F/F}$ Rosa$^{+/eYFP}$* kidneys. **(A,B)** In both control (A) and mutant (B) kidneys, CD31+ (red) endothelial cells are surrounded by SM derived cells that have expressed Foxd1^{tgCre} (green). To ask if these are normally differentiated VSMC we looked at expression of smooth muscle alpha actin (SMA) and desmin, hallmarks of VSMCs. **(C-F')** SM derived vessels in both control (C,C'; E,E') and mutant (D,D', F,F') kidneys express SMA (C,D, red) and desmin (E,F, red).

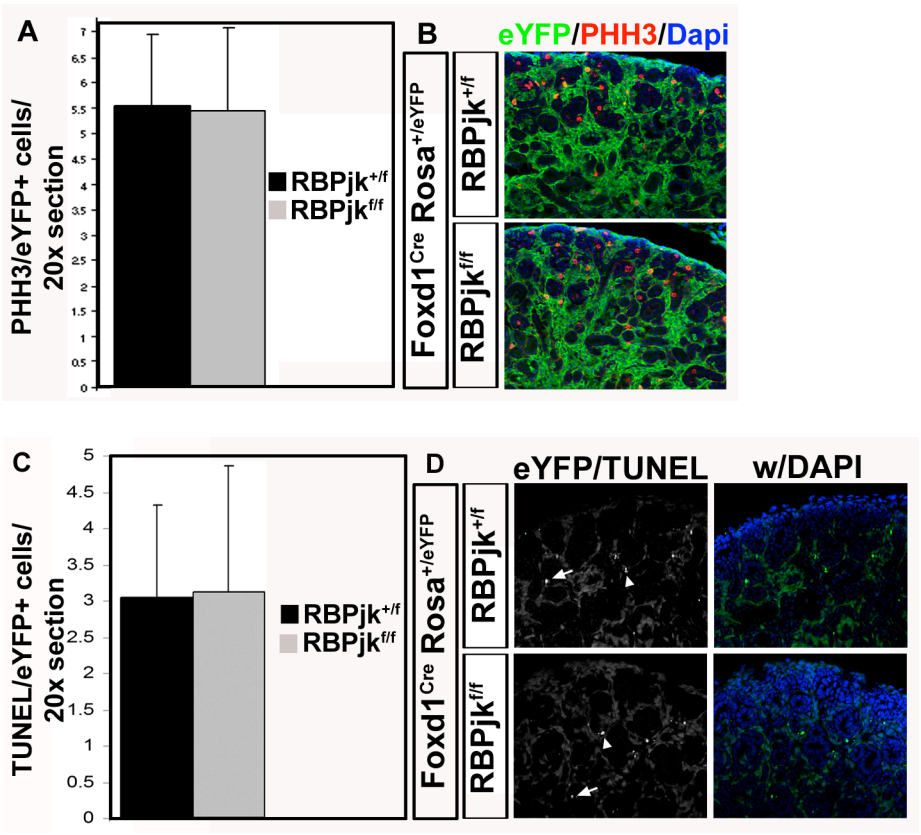

Figure S3. Analysis of proliferation and apoptosis in control and Foxd1^{tgCre} RBPjk$^{f/f}$ Rosa $^{+/eYFP}$ kidneys. 20X images were collected from each e18.5 kidneys isolated from FoxD1^{tgCre} RBPjk$^{+/f}$ Rosa $^{+/eYFP}$ (n=4 animals) and Foxd1^{tgCre} RBPjk$^{f/f}$ Rosa $^{+/eYFP}$ (n=5 animals). The tissues were stained as noted in the methods and analyzed for proliferation and apoptosis in 20 sections for control and 26 sections for mutant. **A.** PHH3/eYFP double positive cells in the stromal mesenchyme and nephrogenic were counted in each section. In both control and mutant kidneys an average of 5.5 cells were observed to be undergoing active proliferation. **B.** Representative images of PHH3 staining in control and mutant kidneys. **C.** TUNEL/eYFP double positive cells in the stromal mesenchyme and nephrogenic were counted in 4 sections from each of 4 control animals and 5-6 sections in each mutant animal. In both control and mutant kidneys an average of ~3

TUNEL positive cells/section were observed. **D.** Representative images of TUNEL staining. A green TUNEL label (bright dots) was imaged on top of live eYFP. TUNEL+ cells derived from the stromal mesenchyme (eYFP+, arrowheads) could easily be distinguished from those in epithelial compartments (arrows).

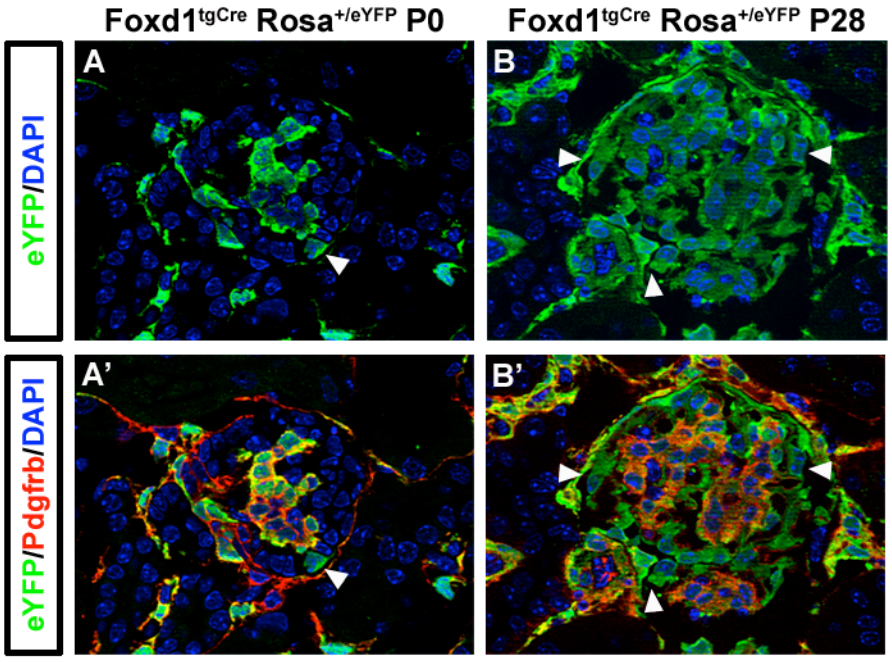

Figure S4. *Foxd1*^{tgCre} does not delete in podocytes until after glomerular development is complete. Data from GUDMAP indicates that *Foxd1* mRNA is expressed in podocytes during development (Brunskill et al. 2011), which could be a confounding factor in our analysis based on the known role of Notch in podocytes (Cheng et al., 2007; Niranjan et al., 2008). To address this we looked at the pattern of eYFP expression in *Foxd1*^{tgCre} *Rosa*$^{+/eYFP}$ mice at P0 and P28. (**A,A'**) At P0 recombination (eYFP, green) has occurred almost exclusively in SM derivatives including mesangium and interstitium (Pdgfrb, red), with only a rare podocyte labeled (arrowhead). (**B,B'**) By P28 several cells within the glomerulus outside of the mesangium and consistent with podocyte morphology are labeled by *Foxd1*^{tgCre}, arrowheads shoe examples.

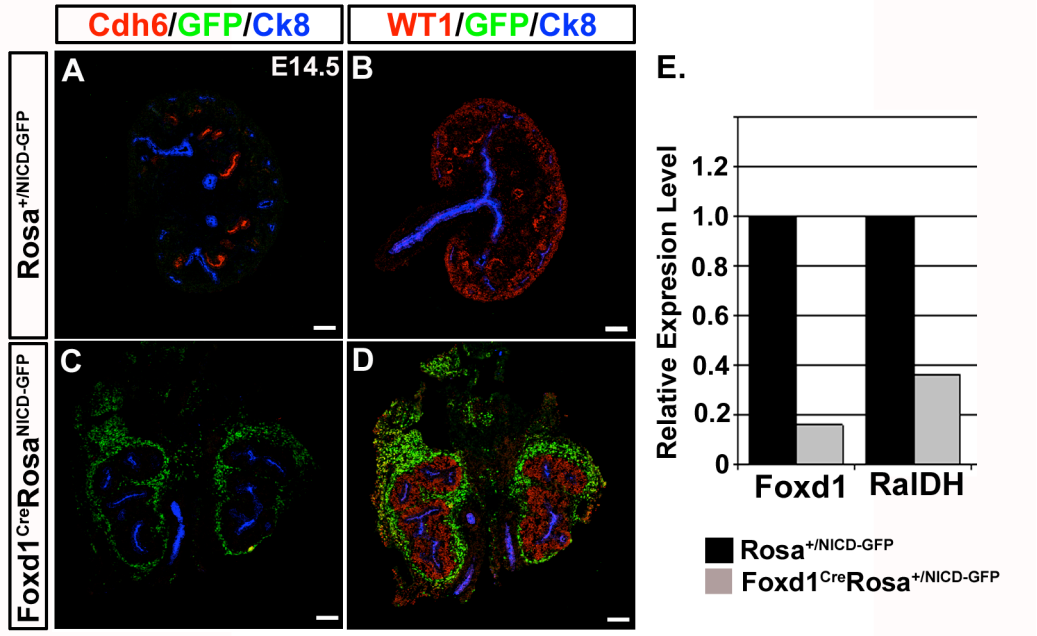

Figure S5. Overexpression of constitutively active Notch1 in the stromal mesenchyme suppresses nephron differentiation from the cap mesenchyme. A, B. In control kidneys nephron differentiation is evident at e14.5 with Cadherin 6+ proximal tubules (A, Red) and WT1+ podocytes (B, red interior crescents). Normal UB branching is demonstrated with multiple Cytokeratin 8+ tips at the periphery of the kidney (A/B, blue) surrounded thin layers of WT1+ cap mesenchyme (B, red periphery). **C,D**. In Foxd1^{Cre} Rosa $^{NICD-GFP}$ kidneys Cadherin 6 and WT1+ differentiated structures are absent, UB branching is significantly reduced and WT1+ cap mesenchyme cells (D, red) 'pile up' around UB branch points. **E.** This phenotype is reminiscent of that observed in the Foxd1 loss of function mutant. To determine if expression of active Notch1 suppressed Foxd1 mRNA we preformed qRTPCR on total RNA from e14.5 control and Foxd1^{Cre} Rosa $^{NICD-GFP}$. We observed a >80% reduction in FoxD1 mRNA in mutant animals. Other gene expression changes observed in Foxd1 null mice were also seen in Foxd1^{Cre}

Rosa $^{NICD\text{-}GFP}$ kidneys, including a 60% reduction in RalDH, another gene known to play a role in the stromal mesenchyme.