

## **Supplemental material:**

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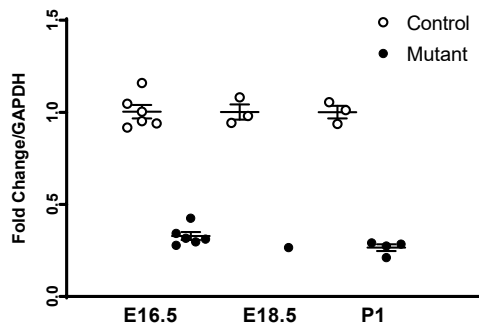
Supplemental Figure 11: **Histology of the Six2Cre mouse kidneys**

**Supplemental Table 1**

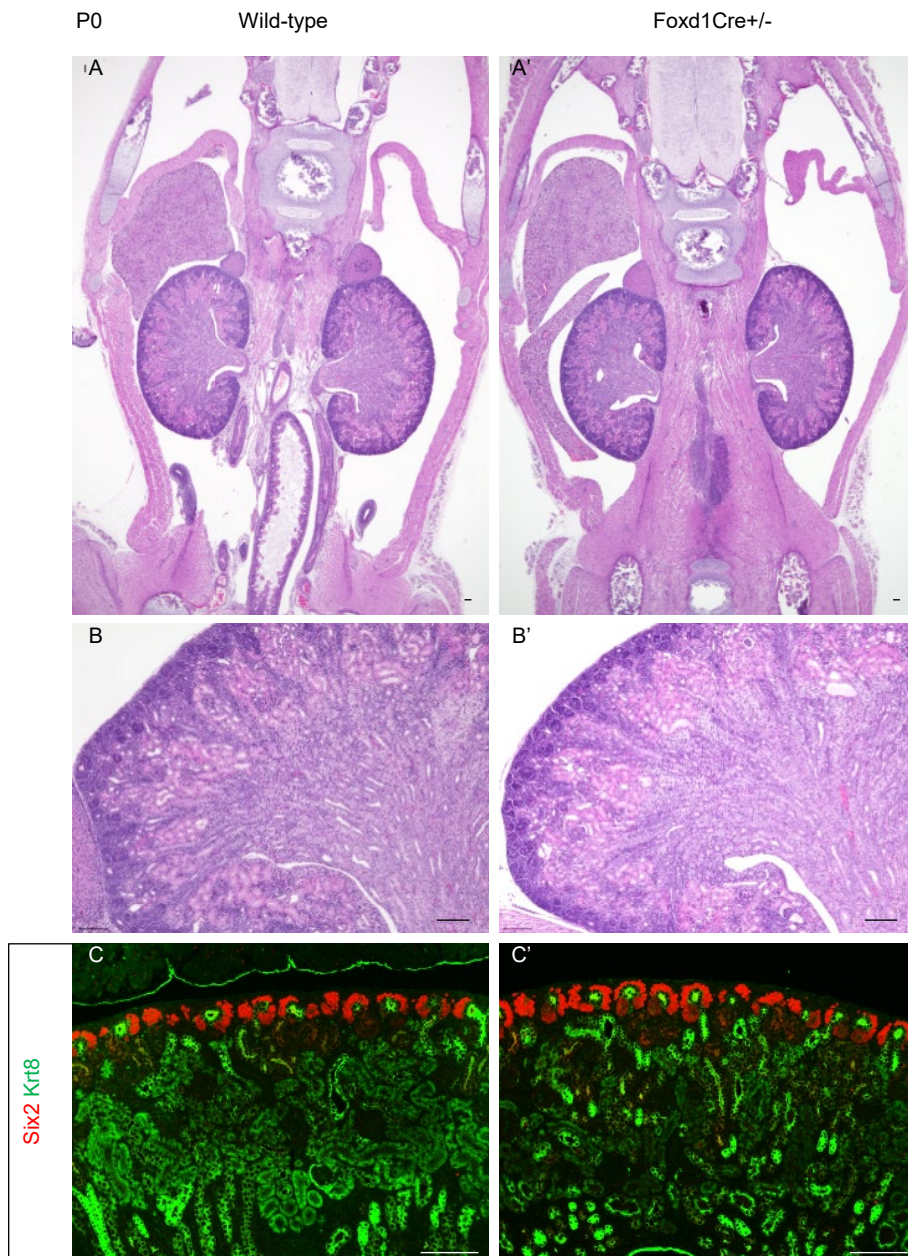
<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
Tcf21	GTC CAG CTA CAT CGC TCA CT	CAT AAA GGG CCA CGT CAG GTT
Cited1	GCT TCG TCC GTA CCT CAG C	AGC TGG GCC TGT TGG TCT
Wnt4	GTC AGG ATG CTC GGA CAA CAT	CAC GTC TTT ACC TCG CAG GA
Pla2g7	ACA ACT CCT GCA AGC TGG AAT	AAG TAA GTT GCC GAT GCA GA
Pax8	ATG CCT CAC AAC TCG ATC AGA	ATG CGT TGA CGT ACA ACT TCT
Tafa5	AAC CTG TGA GAT TGT GAC CCT	TCG AGT GGT GCC TGC TAT C
C1qdc2	ACA TGA CGT GGT TGA ACT TTG	CAG TAA GGC CTC TGG GGT AA
Axin2	GAG AGT GAG CGG CAG AGC	CGG CTG ACT CGT TCT CCT
Apcdd1	TGG CAC GGA GTT TGT GTTC	TTC CCA CTG AAG ACA TTG AGG
Nkd1	GAC ACC AAA CCC GCT GAG	TGG CTG TCA CCC TGG AAC
GAPDH	AAC TTT GGC ATT GTG GAA GG	GGA TGC AGG GAT GAT GTT CT

**Supplemental Table 1: Primer information**

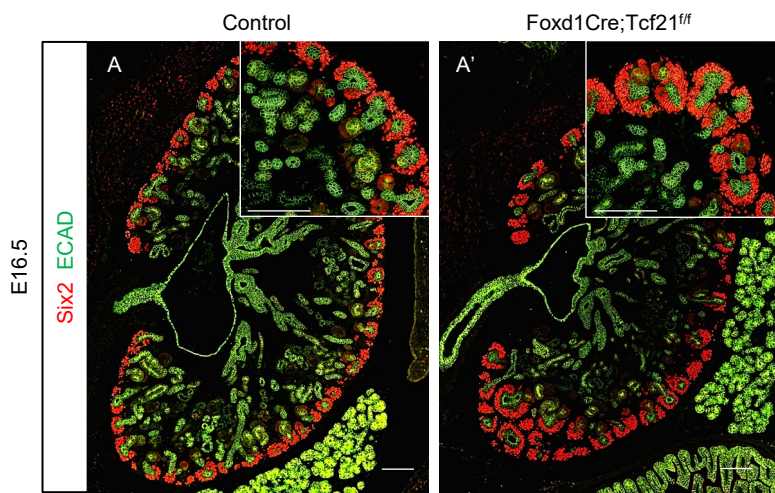
Tcf21 mRNA Expression in Foxd1CreTcf21<sup>ff</sup> Kidneys



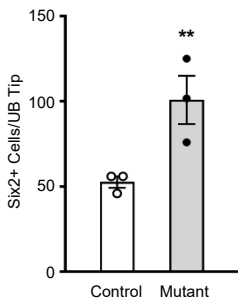
**Supplemental Figure 1: Tcf21 mRNA in Foxd1-eGFP-Cre;Tcf21<sup>ff</sup> kidney.** RT-qPCR of Tcf21 on whole kidney lysate indicates marked reduction in the Tcf21 mRNA in the Foxd1Cre; Tcf21<sup>ff</sup> kidneys compared to controls.



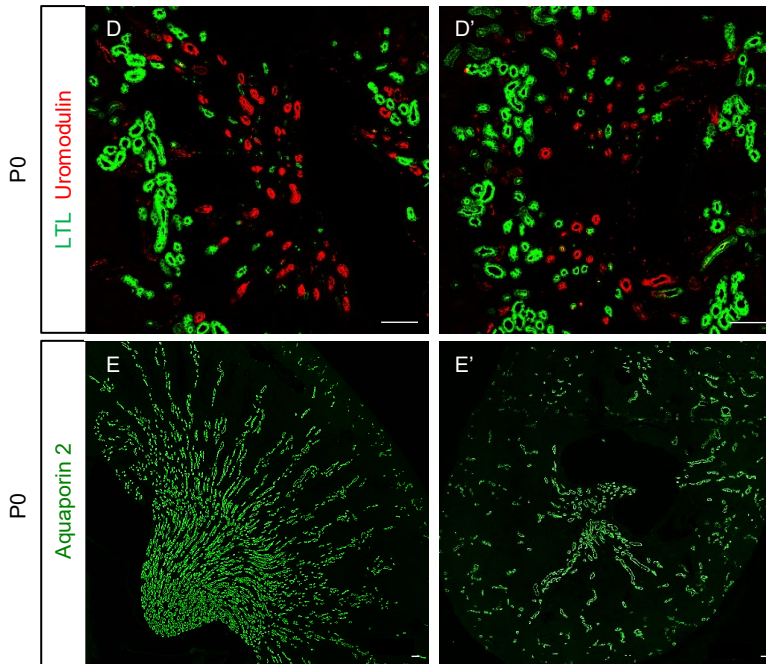
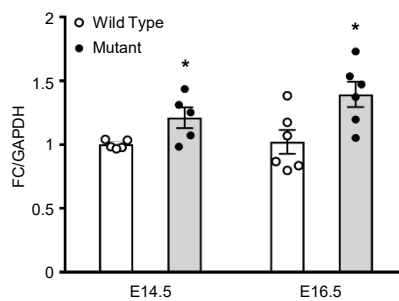
**Supplemental Figure 2: Histology of the Foxd1Cre** A, A', B, B': H&E gross anatomy, location, and architecture of Foxd1Cre mouse kidney shows preserved structures as in the wild-type. C, C': Immunofluorescence demonstrated preserved Six2+ nephron progenitor cells in the Foxd1Cre mouse kidney. Scale bar 100µM



B Six2+ Cells Count

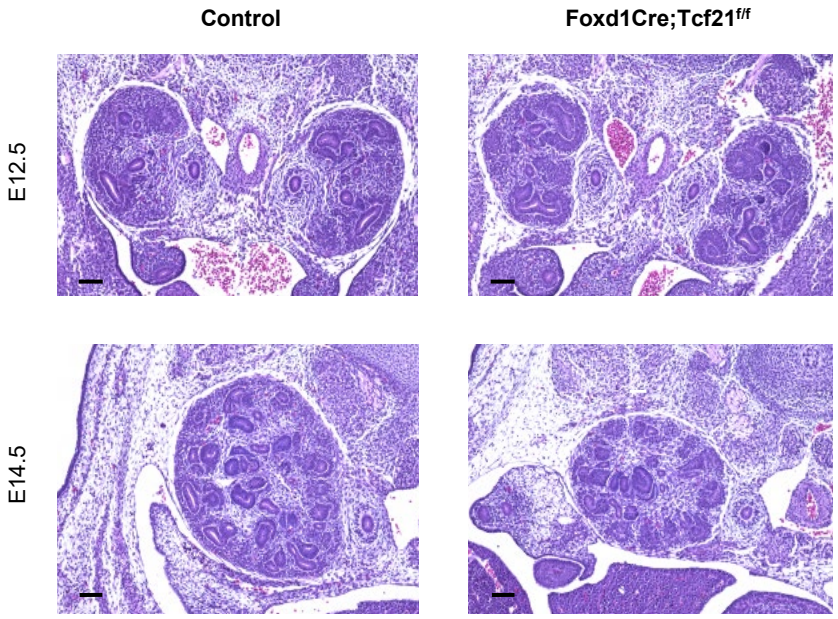


C Cited1 mRNA

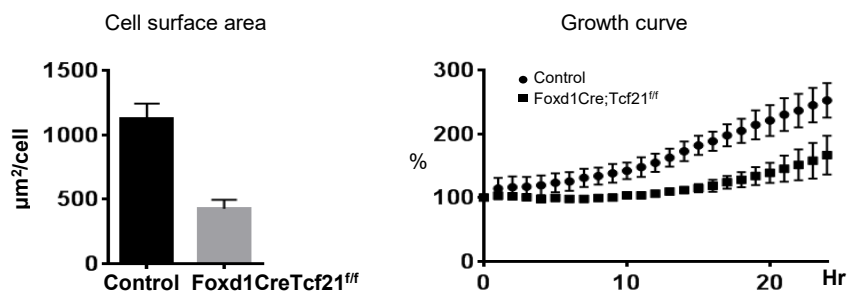
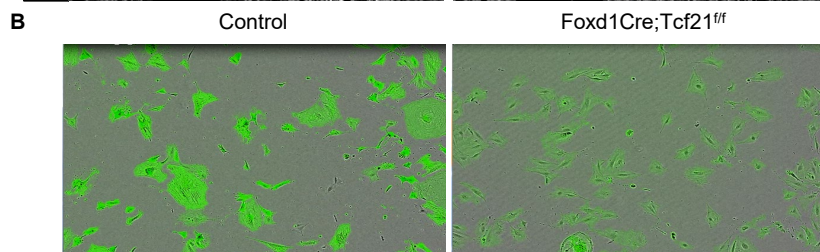
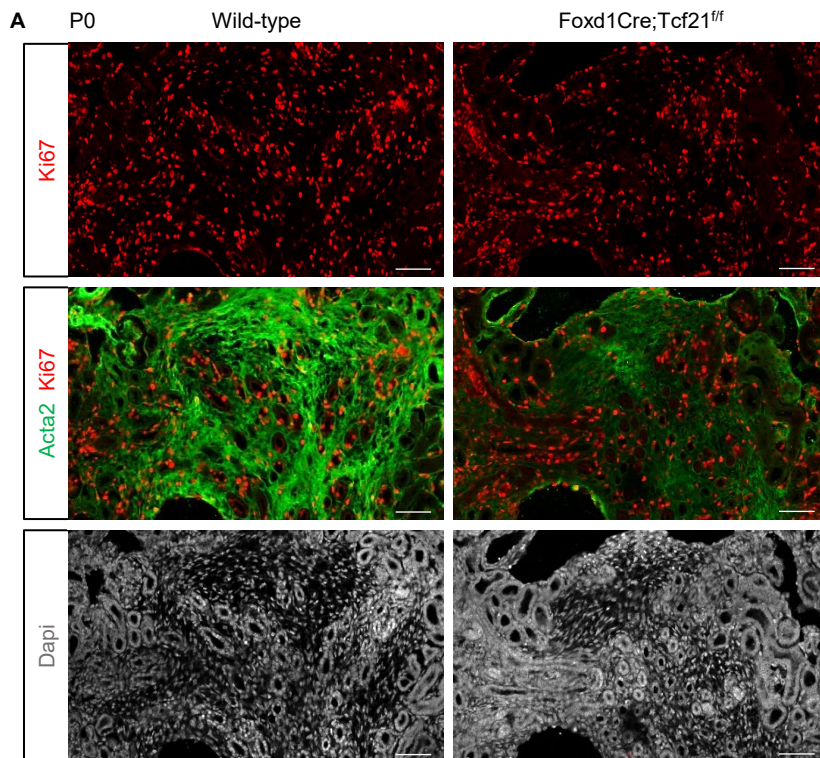


**Supplemental Figure 3:**  
**Non-cell-autonomous effects of stromal Tcf21 on development of the epithelial segments of the nephron.** A, B, Expansion of the Six2+ NPC in the cap-mesenchyme of the mutant compared to wild-type at E16.5 (IF, quantification in B). C, Cited1 (marker of uninduced NPC) mRNA is increased supporting NPC expansion. D, Severe reduction of loop of Henle (Uromodulin/THP) in the medullary and papillary domains of the stroma in Foxd1CreTcf21<sup>ff</sup> kidneys. E, Disruption of collecting duct anatomy (Aquaporin 2) in the medullary and papillary stroma. Scale Bar 100  $\mu$ M, \*  $p < 0.05$ , \*\*  $p < 0.01$

Supplemental Figure 4



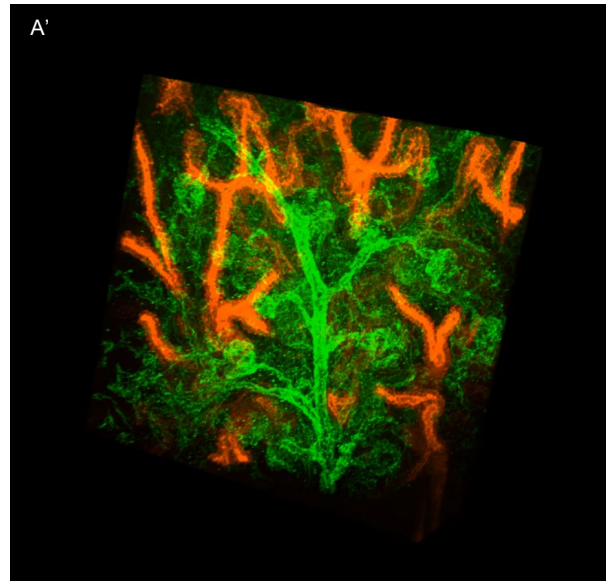
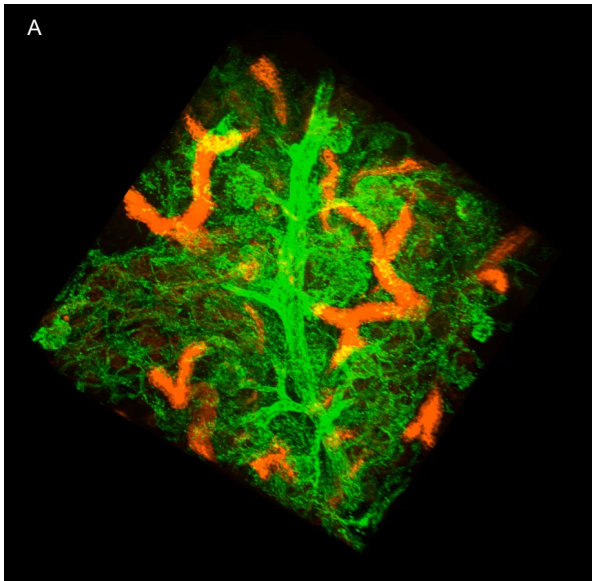
**Supplemental Figure 4: Phenotype of Stromal Tcf21 KO kidneys at E12.5 and E14.5.** The Foxd1-eGFP<sup>Cre</sup>;Tcf21<sup>ff</sup> kidney is smaller than control. Differences in the stromal compartment were not obviously detected at this stage. H&E. Scale bar: 100µm



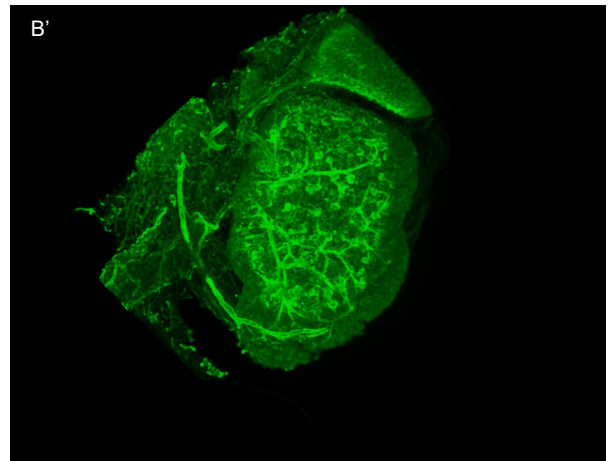
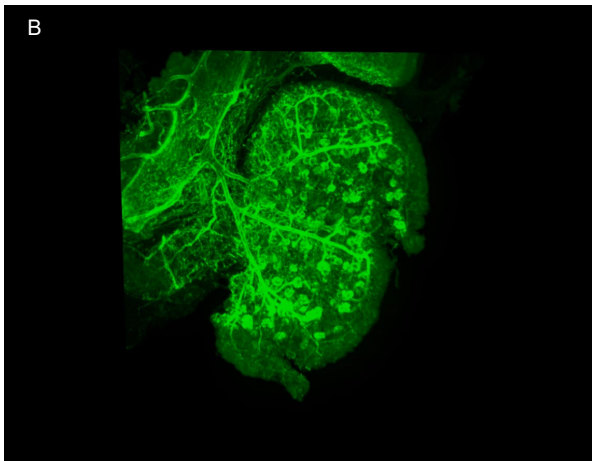
**Suppl Figure 5: Ki67 and cell growth of stromal Tcf21 deficient kidneys** A, Ki67 staining of Foxd1Cre;Tcf21<sup>ff</sup> kidney at P0 shows reduced signal in the medullary stroma compared to wild-type (top panel), adjacent to the reduction in Acta2 (middle panel). DAPI (bottom panel) staining is shown for structural background and overall cell density. B, Analysis of fibroblasts isolated from Foxd1Cre;Tcf21<sup>ff</sup> mTmG and control kidneys at P0 (after tissue dissociation). The mutant fibroblasts cells are smaller and have delayed growth (analysis by incucyte). Scale bar 50µm

E15.5

Control

Foxd1Cre; Tcf21<sup>ff</sup>

CD31/KRT8 x40 objective

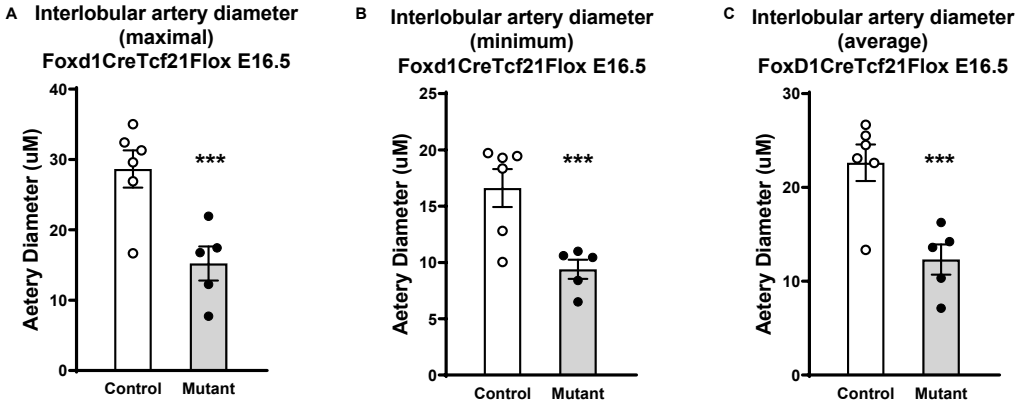


CD31 x10 objective

**Supplemental Figure 6: Abnormal arterial tree of stromal Tcf21 deficient kidneys.**

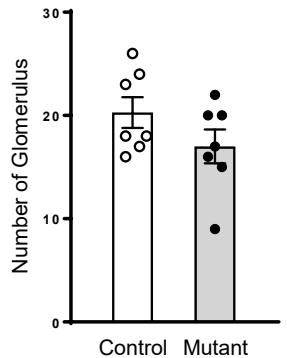
Immunostaining and tissue clearing of E15.5 Foxd1Cre-Tcf21<sup>ff</sup> and controls with the pan-endothelial marker CD31. A, A' Stromal Tcf21 deficient kidneys show distorted pattern of the arterial tree with disorganized, shorter, and thinner arterioles. B, B' The mutant kidney vessel distribution is abnormally centripetal and shows abnormal vessel branching with aberrant ramification leading into the renal capsule at E16.5



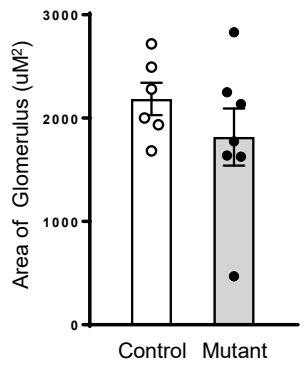


**Supplemental Figure 7: Arterial diameter of Stromal Tcf21 KO kidneys.** A, B, C Measurement of interlobular artery diameter stained with CD31 of Foxd1Cre<sup>+</sup>Tcf21<sup>ff</sup> and controls at E16.5.

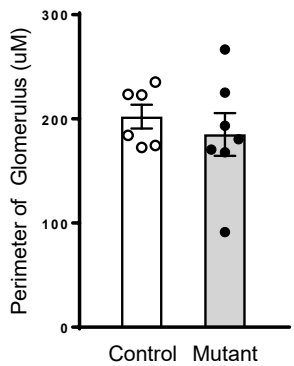
A Number of Glomeruli in Kidney Cross-section  
Foxd1CreTcf21Flox E16.5



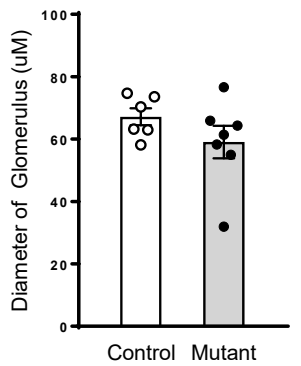
B Area of Glomerulus  
Foxd1CreTcf21Flox E16.5



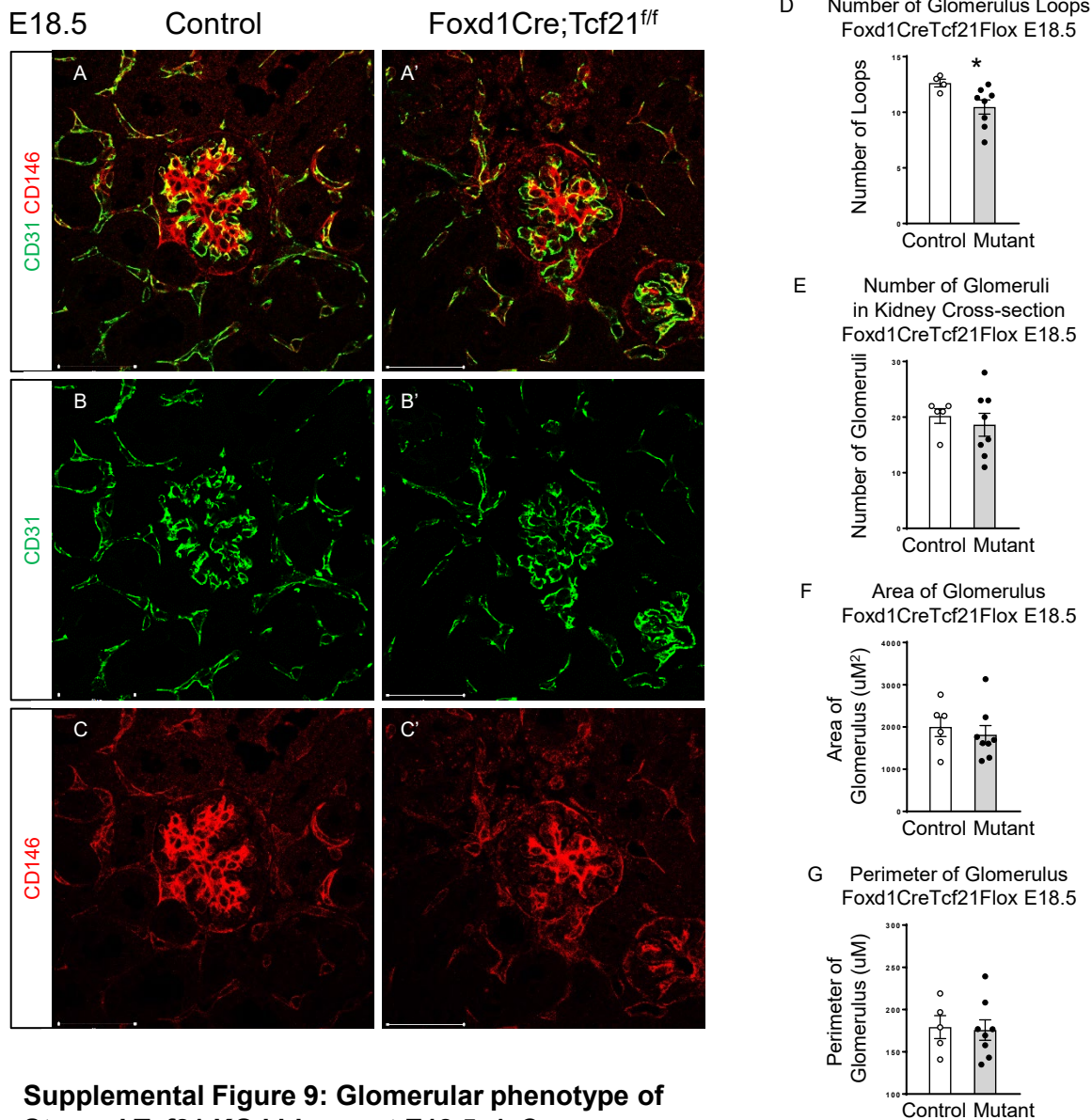
C Perimeter of Glomerulus  
Foxd1CreTcf21Flox E16.5



D Diameter of Glomerulus  
Foxd1CreTcf21Flox E16.5

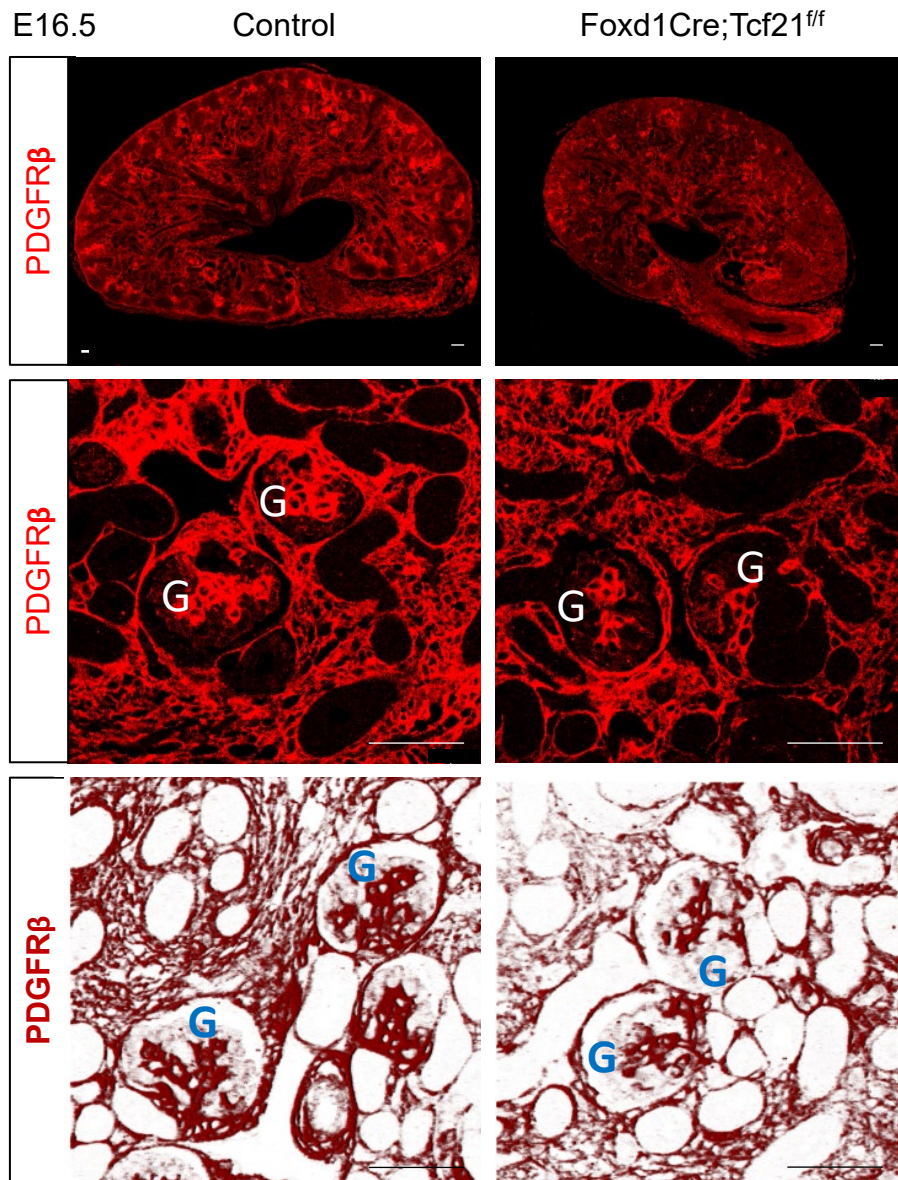


**Supplemental Figure 8: Glomerular phenotype of Stromal Tcf21 KO kidneys at E16.5.** Measurement of the number of glomeruli (A) and glomerular area (B, C, D) of stromal Tcf21 KO and control kidneys stained with CD31 and CD146 at E16.5 shows no difference in glomerular number and size.

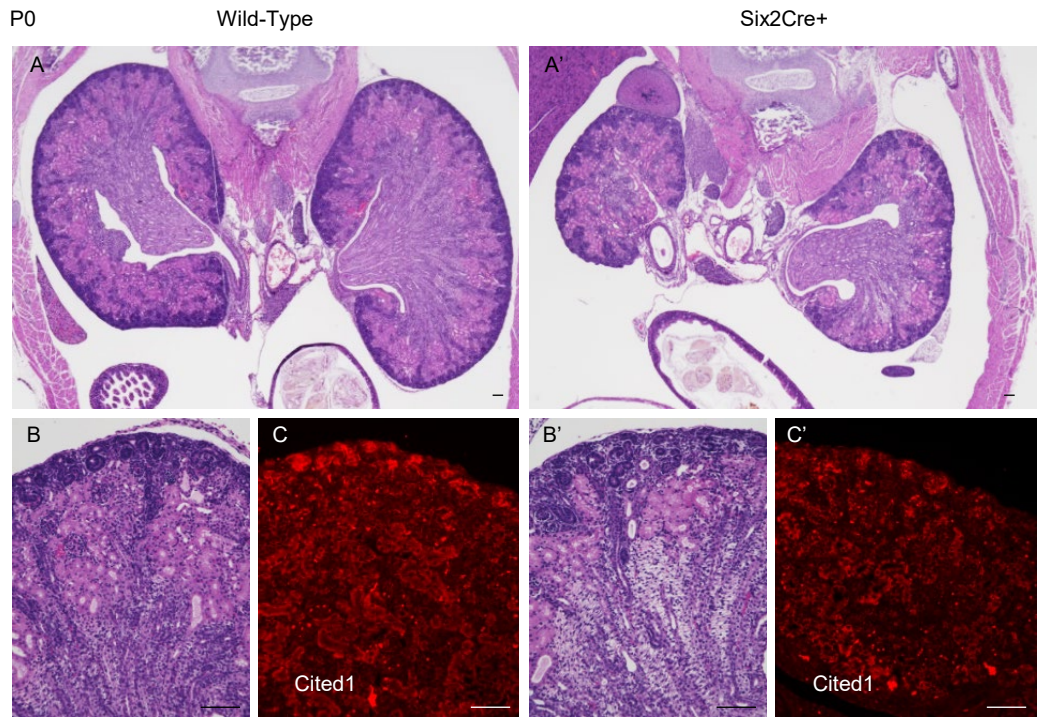


**Supplemental Figure 9: Glomerular phenotype of Stromal Tcf21 KO kidneys at E18.5. A-C:**

Immunostaining for CD31 and CD146 shows abnormal glomerular capillary loops in stromal Tcf21 mutant. Quantification in D. E-H: Measurement of the number of glomeruli, glomerular area, and size shows no difference in stromal Tcf21 mutant compared to control. Scale bar 10µM. \*p<0.05



**Supplemental Figure 10: PDGFR $\beta$  expression in Stromal Tcf21 KO kidneys.** Platelet Derived Growth Factor Receptor Beta (PDGFR $\beta$ ) which marks mesangial cells of the glomerulus, cortical fibroblasts, pericyte cells, and VSMC, all derived from Foxd1+ cells. PDGFR $\beta$  was globally reduced in the mutant kidney (top panel), specifically from the mesangial cells of the glomerulus (G) at E16.5. G- glomerulus. Scale bar 100um



**Supplemental Figure 11: Histology of the Six2Cre mouse kidney.** A, A': gross anatomy of the Six2Cre mouse kidney shows dramatically smaller kidney than the wild-type. B, B': the sub-cortex of the Six2Cre kidney is thinner. Some of the tubules and glomeruli are replaced by stroma, consistent with hypodysplasia. C, C': Expression of Cited1+ NPC (uninduced nephron progenitors) in the Six2Cre kidney is significantly diminished compared to the wild-type (red). H&E and IF. Scale bar 100uM