Supplementary Materials

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Supplementary Methods

Antibodies

The used antibodies were the following : anti-Six2 (11562-1-AP, Proteintech), anti-beta Glactosidase (ab9361, Abcam), anti-Jagged1 (2620S, Cell signaling), anti-Calbindin-D-28K (C9848, Sigma Aldrich), anti-Cd31 (553370, BD Pharmingen), anti-Podocin (P0372, Sigma-Aldrich), anti-Desmin (ab15200, Abcam or IR606, Dako), anti-*Aquaporin*2 (sc-9882, Santa Cruz), anti-Tamm Horsfall Glycoprotein (BT-590, Biomedical Technologies

Inc), anti-Wt1(M3561, Dako), anti-Pax2 (PRB-276P, Covance), anti-Bmp4 antibody (MAB1049, Millipore), anti-Ecad antibody (R&D AF748)

Lectins

Biotinylated Dolichos Biflorus Agglutinin (B-1035, Vector Laboratories), FITC-labeled Lotus Tetragonolobus Lectin(FL-1321, Vector Laboratories),

RNA *in situ* hybridization

In situ hybridization probes were the following: Wnt11 (405021, ACD), Gdnf (421951, ACD), Ret (431791, ACD), and BMP4 (401301, ACD), Tcf21 (508661, ACD), Aldh1a2 (447391, ACD).

Supplementary Table 1

RNA scope probe

Mm-Gdnf-3UTR	ACD	421951
Mm-Ret	ACD	431791
Mm-Wnt11	ACD	405021
Mm-Tcf21	ACD	508661
Mm-Aldh1a2	ACD	447391
Mm-Bmp4	ACD	401301

Antibodies

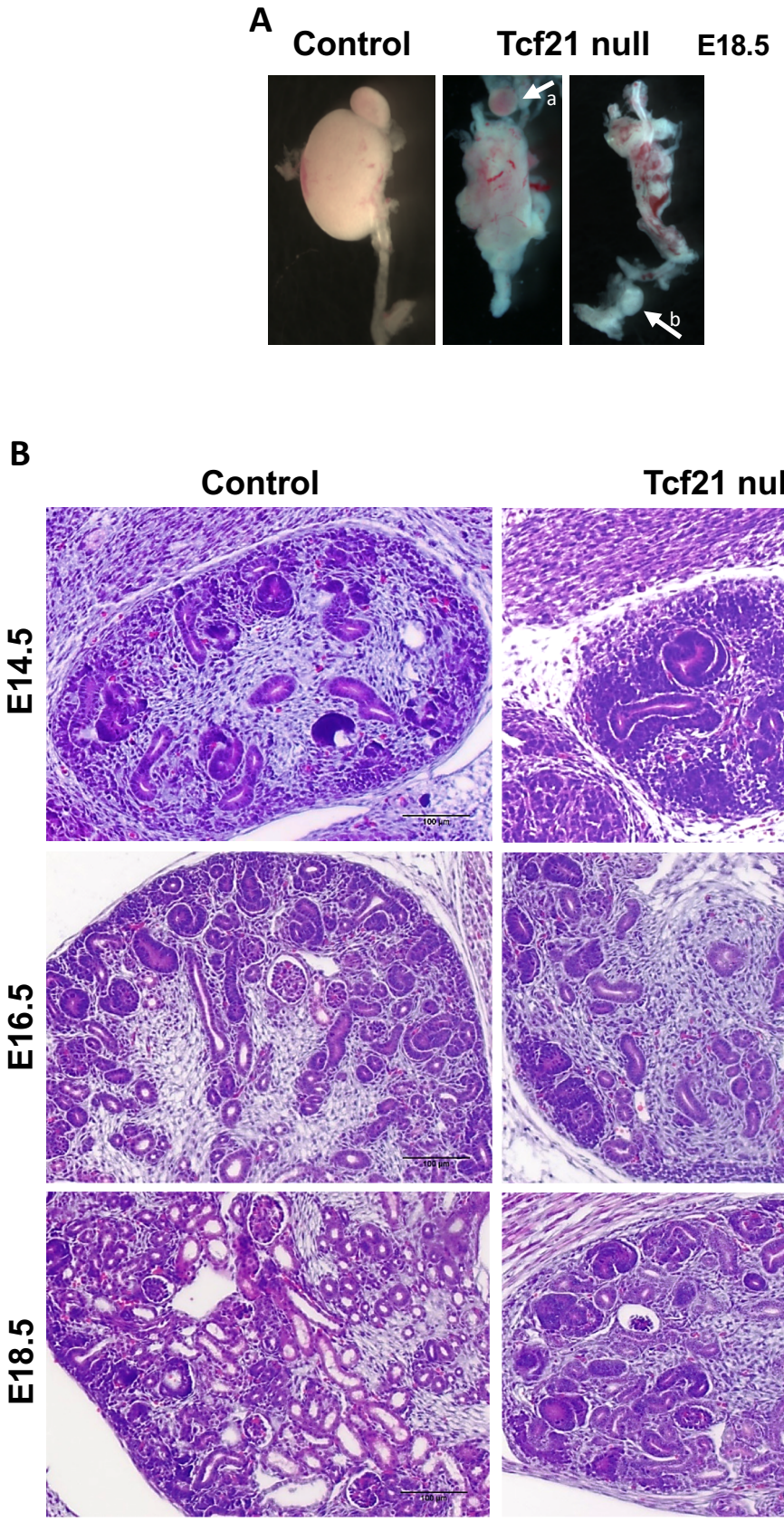
Calbindin-D-28K	Sigma Aldrich	C9848
Jagged1	Cell signaling	2620S
Gdnf	R&D	AF-212-NA
E-cadherin	R&D	AF748
Six2	Proteintech	115621-1-AP
Pax2	PRB-276P	Covance
Wt1	Dako	M3561
beta Galactosidase	Abcam	ab9361
Lotus Tetragonolobus Lectin	Vector Laboratories	FL-1321
Tamm Horsfall Glycoprotein	Biomedical Technologies	BT-590
Dolichos Biflorus Agglutinin	Vector Laboratories	B-1035
Aquaporin2	Santa Cruz	sc-9882
Ki67	Thermo Fisher	RM-9106
Cd31	BD Phamingen	553370
Pdgfr-b	Abcam	ab32570
Podocin	Sigma Aldrich	P0372
Desmin	Abcam	ab15200
Bmp4	Millipore	MAB1049
p-smad1/5	Cell signaling	41D10
Pbx1	Cell signaling	4342S

Genotype Primer

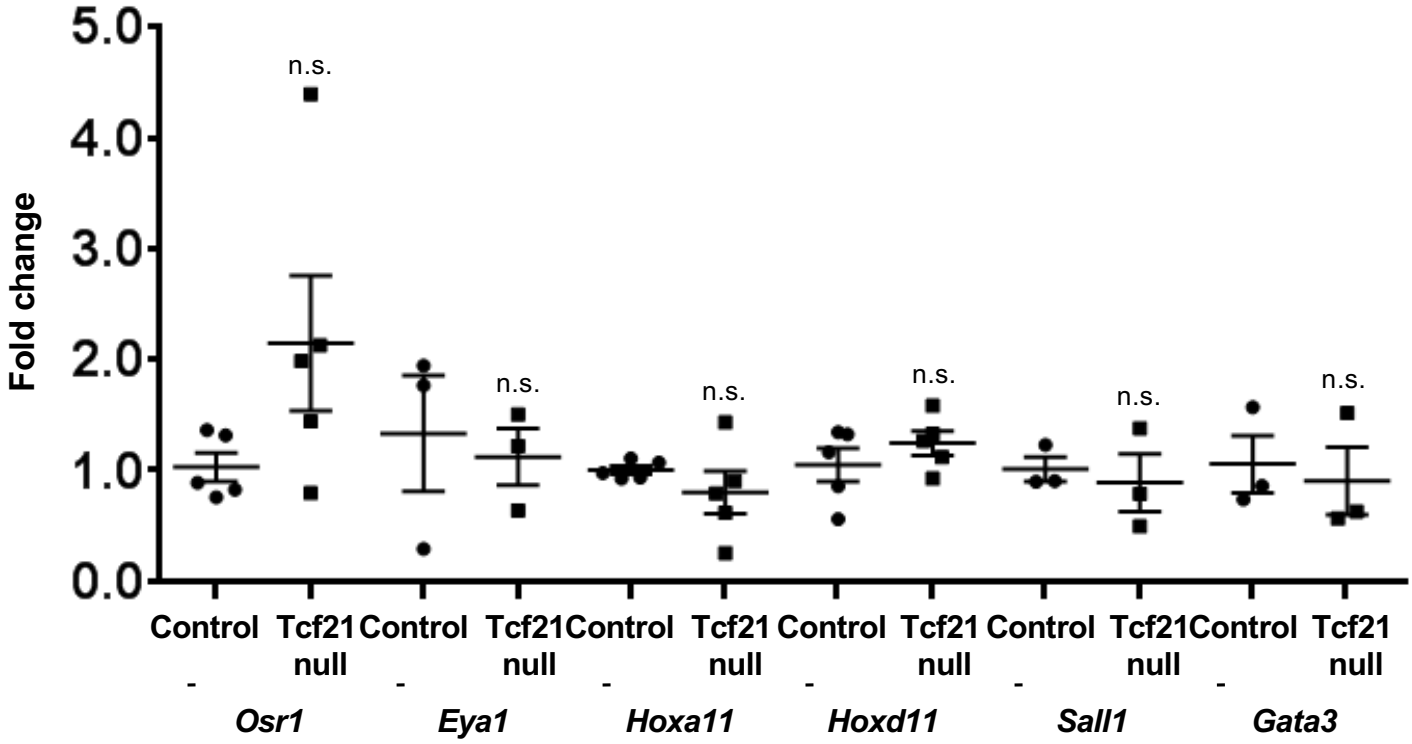
Tcf21-floxed Forward	5-GTGTGCATTTCTGTGGTTGTCTCTG-3
Tcf21-floxed Reverse	5-CTGTTGTTTGTGCAGGTGGAGA-3
Tcf21-LacZ Forward	5-CGGGACTGCCAGATCCCACCTC-3
Tcf21-LacZ Reverse1	5-CCTGCTTGCCCTCCTGGCTGAC-3
Tcf21-LacZ Reverse2	5-AGTAACAACCCGTCGGATTCTCC-3
Cre Forward	5-GTGCAAGTTGAATAACCGGAAATGG-3
Cre Reverse	5-AGAGTCATCCTTAGCGCCGTAAATCAAT-3

realtime PCR Primer

Sox9 Forward	5-GTACCCGCATCTGCACAAC-3
Sox9 Reverse	5-CTCCTCCACGAAGGGTCTCT-3
Wnt7b Forward	5-TTTGGCGTCCTCTACGTGAAG-3
Wnt7b Reverse	5-CCCCGATCACAATGATGGCA-3
E-cadherin Forward	5-CAGTTCCGAGGTCTACACCTT-3
E-cadherin Reverse	5-TGAATCGGGAGTCTTCCGAAAA-3
Aqp2 Forward	5-TTGCCATGTCTCCTTCCTTC-3
Aqp2 Reverse	5-GGTCAGGAAGAGCTCCACAG-3
ENaC Forward	5-ATGCCAGTGAAGAAGTACCTCC-3
ENaC Reverse	5-GATGGCCTCCACCTCACTGT-3
Na-H-ATPase Forward	5-GGCTGGTGAAGAAATCCAA-3
Na-H-ATPase Reverse	5-CACACTGGTAGGCAAGGAAC-3
Gdnf Forward	5-ACGCTTGGTGGTTGATTCTGGA-3
Gdnf Reverse	5-AACTTGCTTCCTGTTTCTGAGGGC-3
Ret Forward	5-CATCAGCGGAAATGACCTTCTG-3
Ret Reverse	5-CTTGAAAGAGTCCACAGGAACC-3
Wnt11 Forward	5-GCTGCGTCTGGAAGAAGCTA-3
Wnt11 Reverse	5-TGGATAGGGAGAGTGCGGAA-3
FoxD1 Forward	5-CTACTCGTACATCGCGCTCA-3
FoxD1 Reverse	5-CTCCCGGTAGTAAGGGAAGC-3
Tbx18 Forward	5-CAGGCTTACCAACCAGAGCA-3
Tbx18 Reverse	5-ACTGTGCAATCGGAAGGTGT-3
Ecm1 Forward	5-CTCTTGCTTCTGCTGCCTCT-3
Ecm1 Reverse	5-GGGTGCTGCATAACCTTCAT-3
Fgf7 Forward	5-TATTCATGAACACCCGGGGC-3
Fgf7 Reverse	5-CAGTTCACACTCGTAGCCGT-3
Fgf10 Forward	5-GGAGCTATCCAGAAGCCACC-3
Fgf10 Reverse	5-GAGGTGATTGTAGCTCCGCA-3
Aldh1a2 Forward	5-GGTATTATGCAGGCTGGGCT-3
Aldh1a2 Reverse	5-ACGGTGTTACCACAGCACAA-3
Rara Forward	5-TTCAGCCTCTGCACGTGACTC-3
Rara Reverse	5-CAAAGAGGATGCCACTCCCAG-3
Pbx1 Forward	5-AGGACATCGGGGACATTTTAC-3
Pbx1 Reverse	5-CATTAAACAAGGCAGGCTTCA-3
Meis1 Forward	5-GCATGCAGCCAGGTCCAT-3
Meis1 Reverse	5-TAAAGCGTCATTGACCGAGGA-3
Meis2 Forward	5-CTATGGCCACCACGACTTC-3
Meis2 Reverse	5-TGTCAGTAGGTGTTGGCAGG-3
Etv4 Forward	5-CAAAGGAAATGCACCAATCAGC-3
Etv4 Reverse	5-CCGGGGGATGACACAACTAA-3
Etv5 Forward	5-GCCTTCCCTGCAGGCTTTTA-3
Etv5 Reverse	5-AAGCAGCCCTTCGAGTTCAA-3



Supplementary Figure 1



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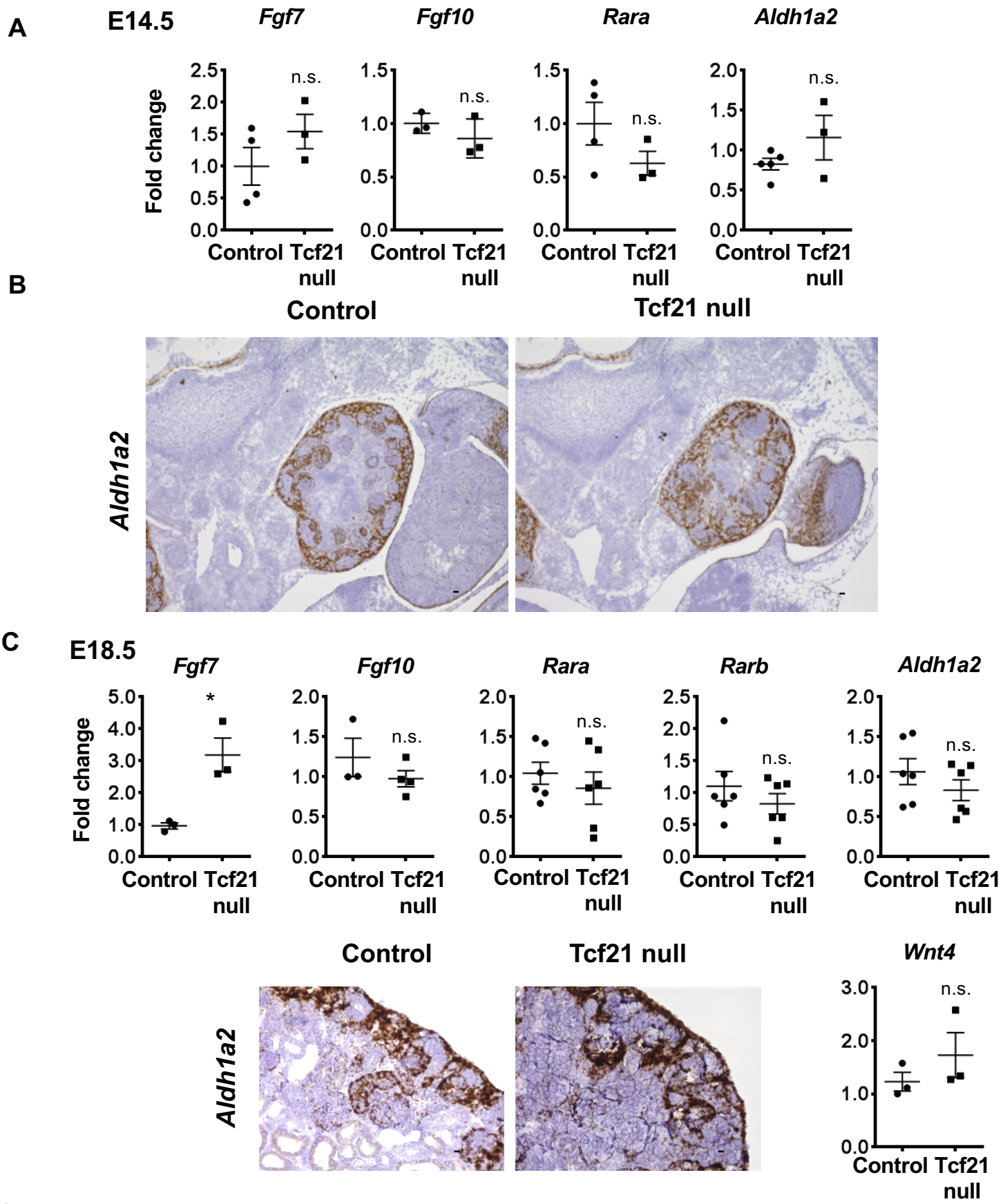

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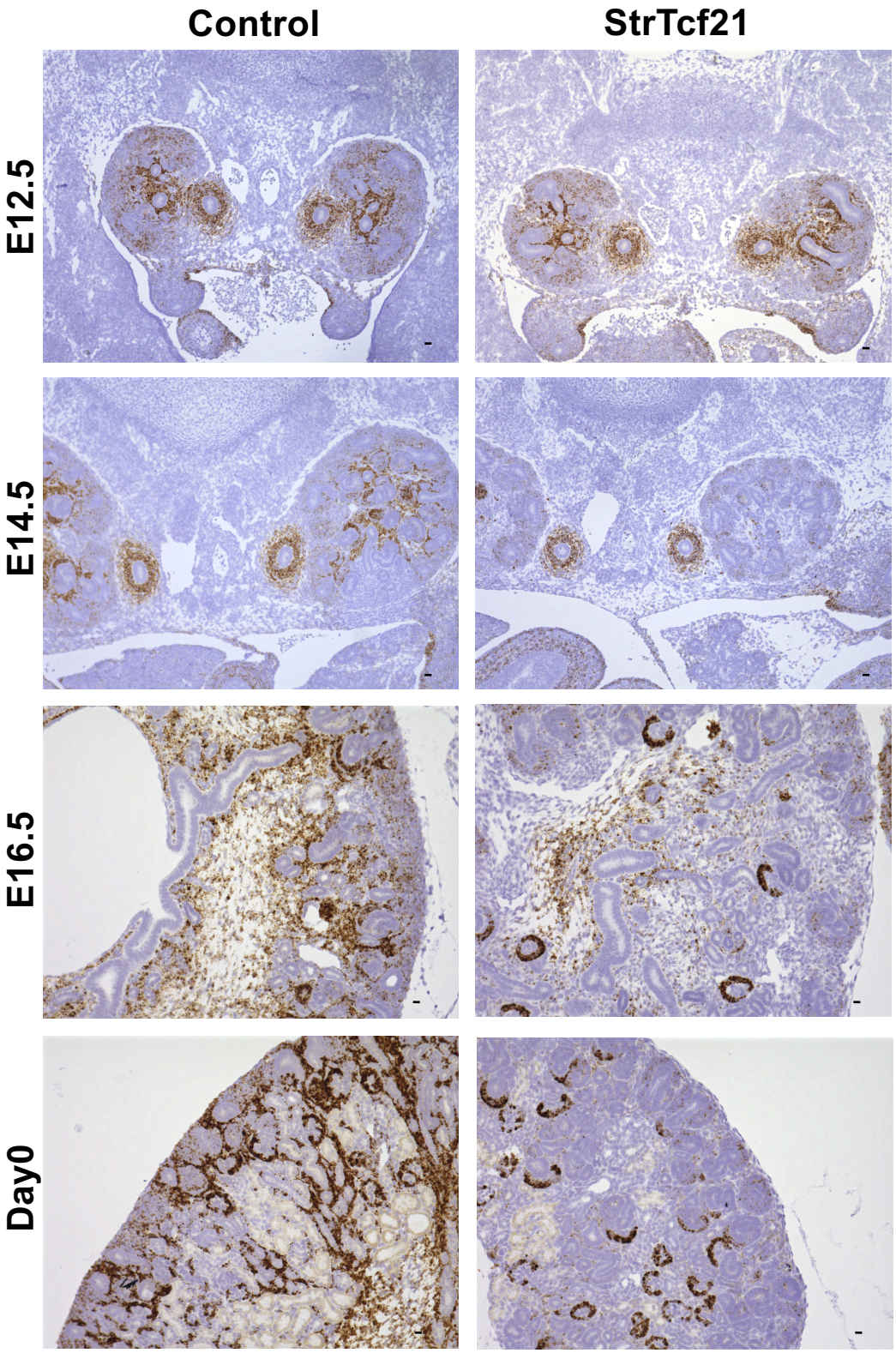

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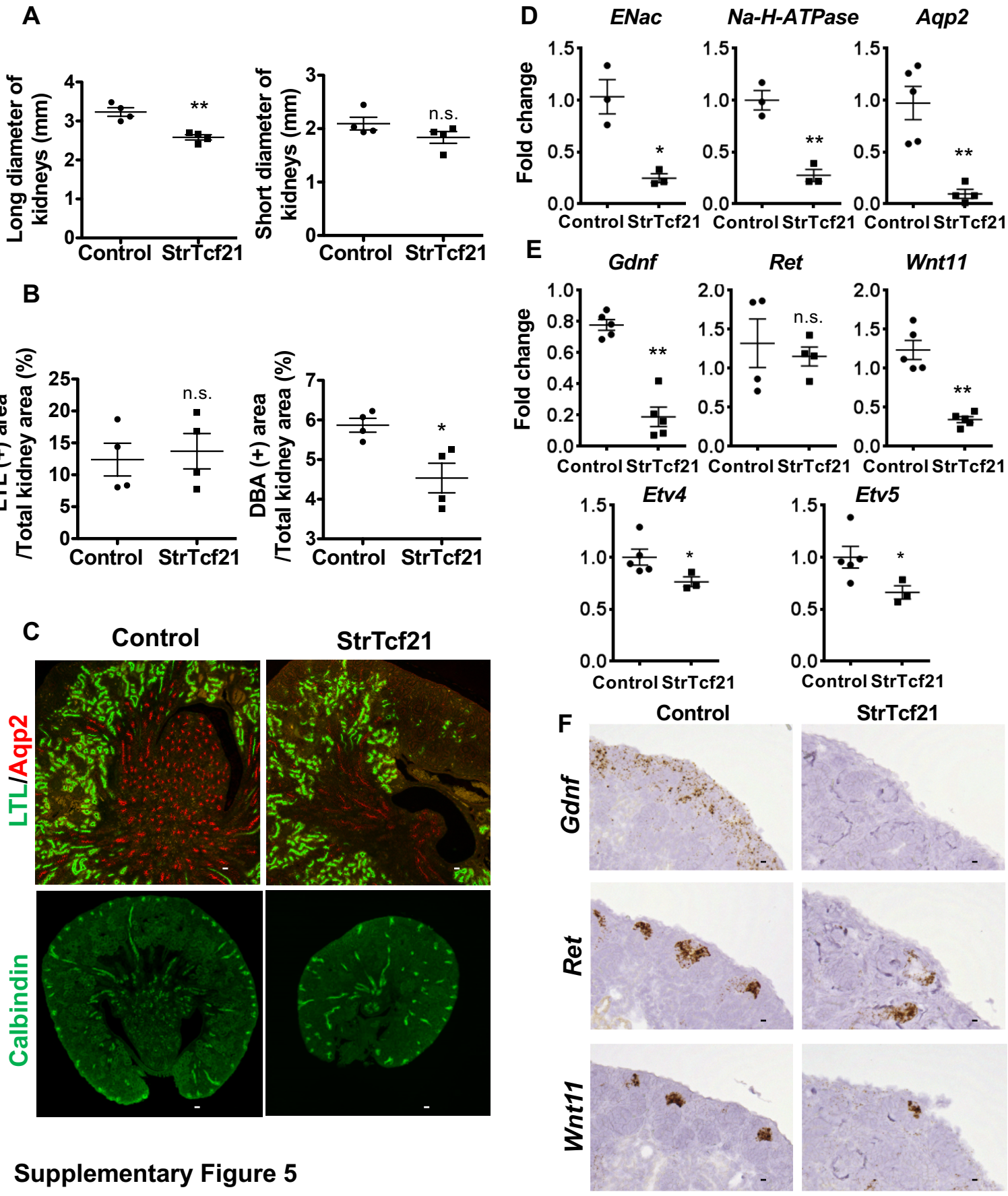

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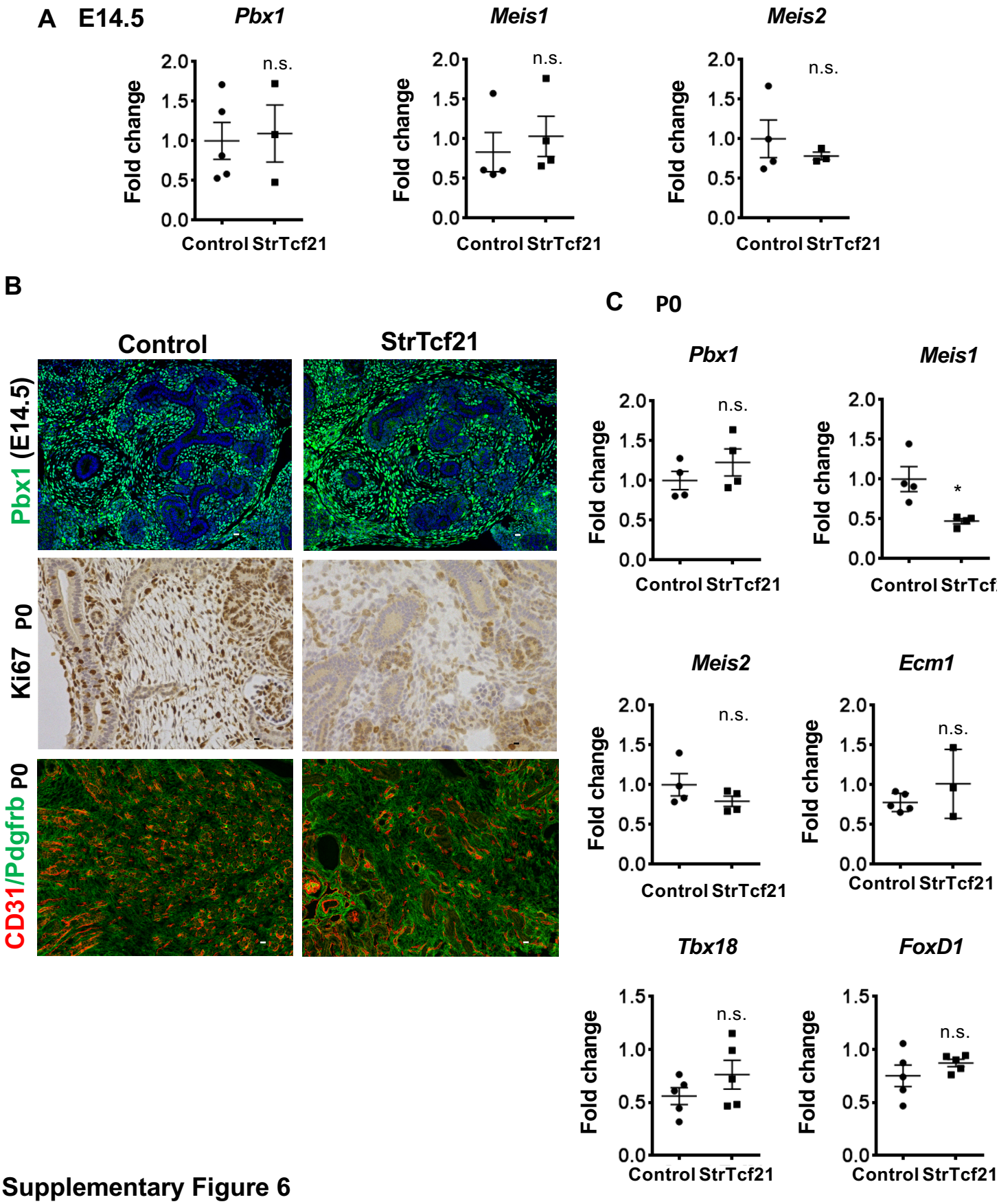

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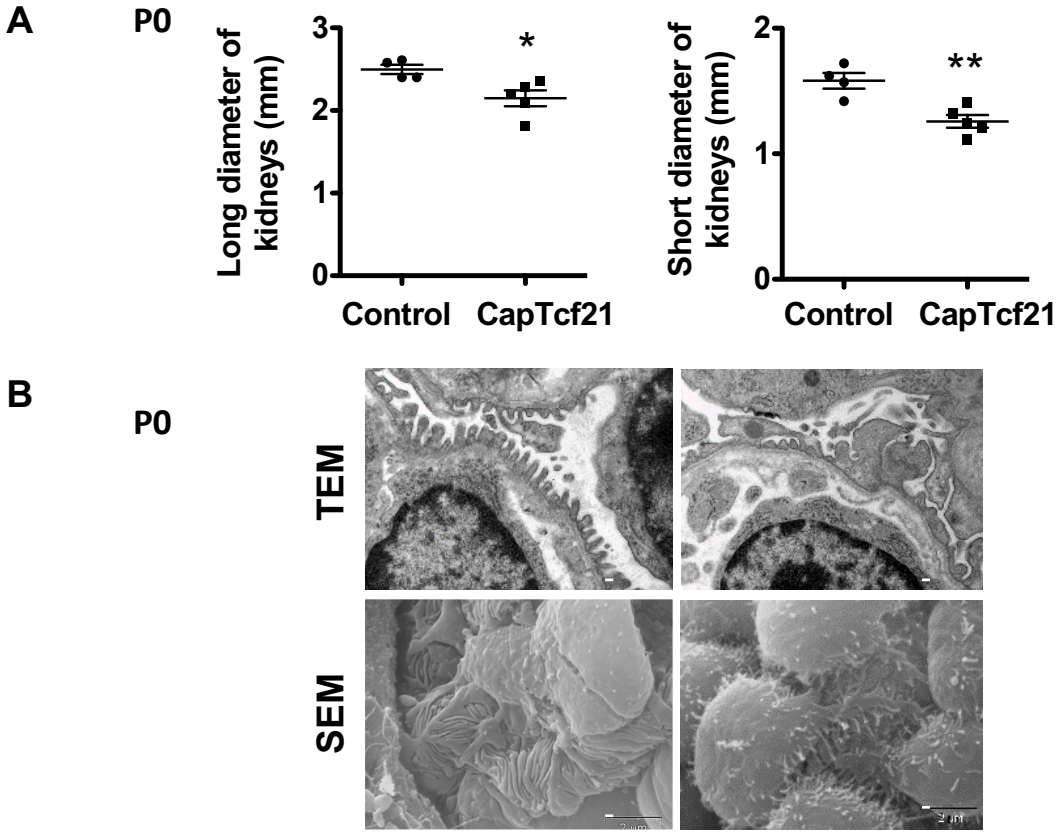

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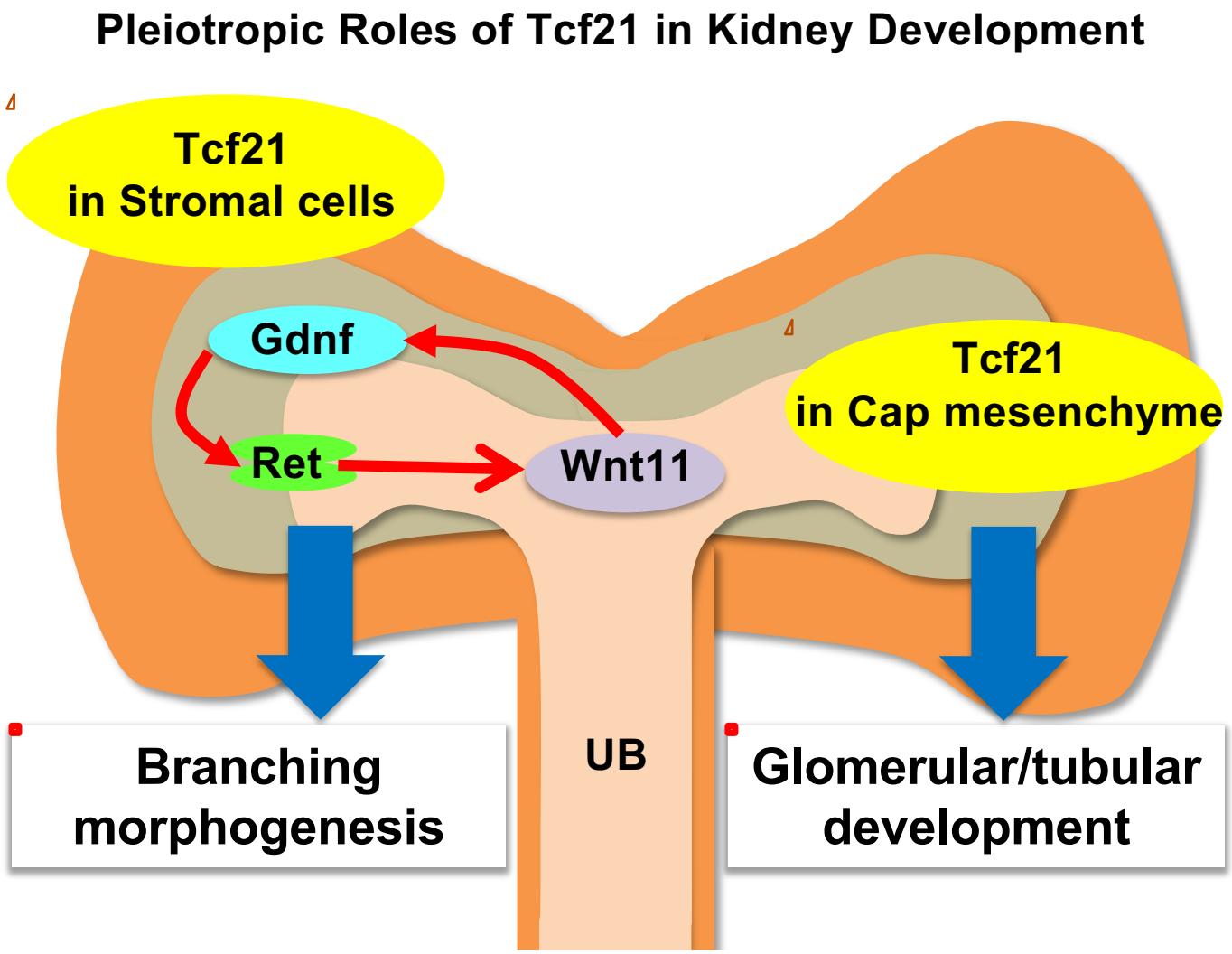

Supplementary Figure 8

Supplementary Figure 1: Germline deletion of *Tcf21* leads to hypodysplastic kidneys. (A) Gross dissection of Tcf21 null kidneys shows smaller and mal-shaped organs. The adrenal gland (a) and urinary bladder (b) are marked for orientation. (B) Hematoxylin and eosin (HE) staining of sections of Tcf21 null and control kidneys at E14.5, E16.5, and E18.5. At E14.5 the null kidney is smaller and has fewer UB tips. The mesenchyme of the null kidney shows condensation however mesenchymal to epithelial transition is delayed. At E18.5 Tcf21 null kidneys have very few and abnormal glomeruli. Scale bars: 100μm

Supplementary Figure 2: Gdnf's Inducers and signals up-stream of Gdnf show normal or high expression in Tcf21 null kidneys at E18.5. In contrast to the low expression of *Gdnf-Ret-Wnt11* transcripts, the expression of factors that are critical for promoting and maintaining the expression of *Gdnf* in the metanephric mesenchyme are not reduced in Tcf21 null kidneys. RT-qPCR normalized to GAPDH.

Supplementary Figure 3: Expression of non-GDNF signaling pathways important for UB branching. mRNA expression by RT-qPCR and in-situ hybridization of members of the retinoic acid, fibroblast growth factor (Fgf), and canonical Wnt signaling in Tcf21 null and control kidneys. Note no change in transcript expression with the exception of elevated

level of Fgf7 at E18.5, but not at E14.5. *Aldh1a2, Aldehyde dehydrogenase family 1*, *subfamily a2* , *Rara,* retinoic acid receptor alpha, *Rarb*, retinoic acid receptor beta. Scale bar, B:100μm, C: 50μm.

Supplementary Figure 4: Tcf21 deletion from the stroma of StrTcf21 starting at E14.5. In-situ hybridization of *Tcf21* in StrTcf21 kidney where *Tcf21* is deleted under *FoxD1*-Cre driver. Note the diminished *Tcf21* mRNA expression in the stroma of StrTcf21 beginning at E14.5, while the remaining Tcf21 expression occurs mainly in glomeruli. Scale bar: 50μm

Supplementary Figure 5: Deletion of *Tcf21* in Stromal cells leads to severe decrease of collecting ducts in inner medulla, and abnormal Gdnf-axis at postnatal day 0 (A) Long diameters and short diameters of StrTcf21 kidneys at postnatal day 0. Long diameters were reduced compared to controls. (B) Quantification of areas that were stained by LTL for proximal tubules or DBA for ureteric buds. Area stained with LTL were not different, but calbindin-stained area was decreased. (C) High magnification picture of LTL/Aquaporin2 staining, and DBA staining showed reduced collecting ducts. Especially, inner medulla was almost absent. Scale bar: 100 μm, 500μm. (D) Markers for collecting ducts, Epithelial

sodium channel (*Enac), Na-H ATPase, Aquaporin 2* were largely decreased by RT-qPCR of whole kidneys. (E,) Decreased expression of *Gdnf*, *Wnt11, Etv4 and Etv5* in StrTcf21 kidneys shown by RT-qPCR. (F) In-situ hybridization for *Gdnf*, *Ret*, and *Wnt11*. Scale bar: 50μm.

Supplementary Figure 6: Stromal changes in StrTcf21 kidneys at E14.5 and P0. (A) Transcript levels of the stromal markers Pbx1, Meis1 and Meis2 at E14.5 showing no change compared to controls. (B) No change in protein expression of Pbx1 in StrTcf21(E14.5, upper panel). Reduced proliferation of inner medullary cells in StrTcf21 kidneys by Ki67 staining (P0, middle panel). Abnormal vascular patterning in the medullary stroma of StrTcf21 kidneys by immunostaining for CD31 and Pdgf receptor beta (P0, lower panel). (C) Transcript levels of stromal markers at day 0. Only Meis1 was decreased by RT-qPCR, but none of the others were significantly changed. Scale bar:100μm.

Supplementary Figure. 7: Absence of Tcf21 from the cap mesenchyme leads to reduction in proximal tubular mass but no change in collecting ducts. (A) At P0 CapTcf21 kidneys are 20% smaller in short diameter compared to control kidneys at P0. (B) Ultrastructure of

CapTcf21 kidneys showing effacement and disorganization of the podocyte foot processes. Scale bar: 2μm (C) RT-qPCR analysis of UB markers, Wnt7b and Sox9, and epithelial marker Ecad did not show decrease in CapTcf21 compared to controls. (D) Tcf21 transcript level is 65% decreased in CapTcf21 whole kidney lysate at E14.5, on the other hand, there was no decrease in the stromal marker *FoxD1* evaluated by RT-qPCR.

Supplementary Figure 8: proposed model for Tcf21's pleiotropic action in kidney development: Tcf21 in the stroma regulates factor/s to allow normal branching morphogenesis through Gdnf-Ret-Wnt11 axis. In the Cap mesenchyme, Tcf21 controls factor/s critical for normal glomerular and tubular development.