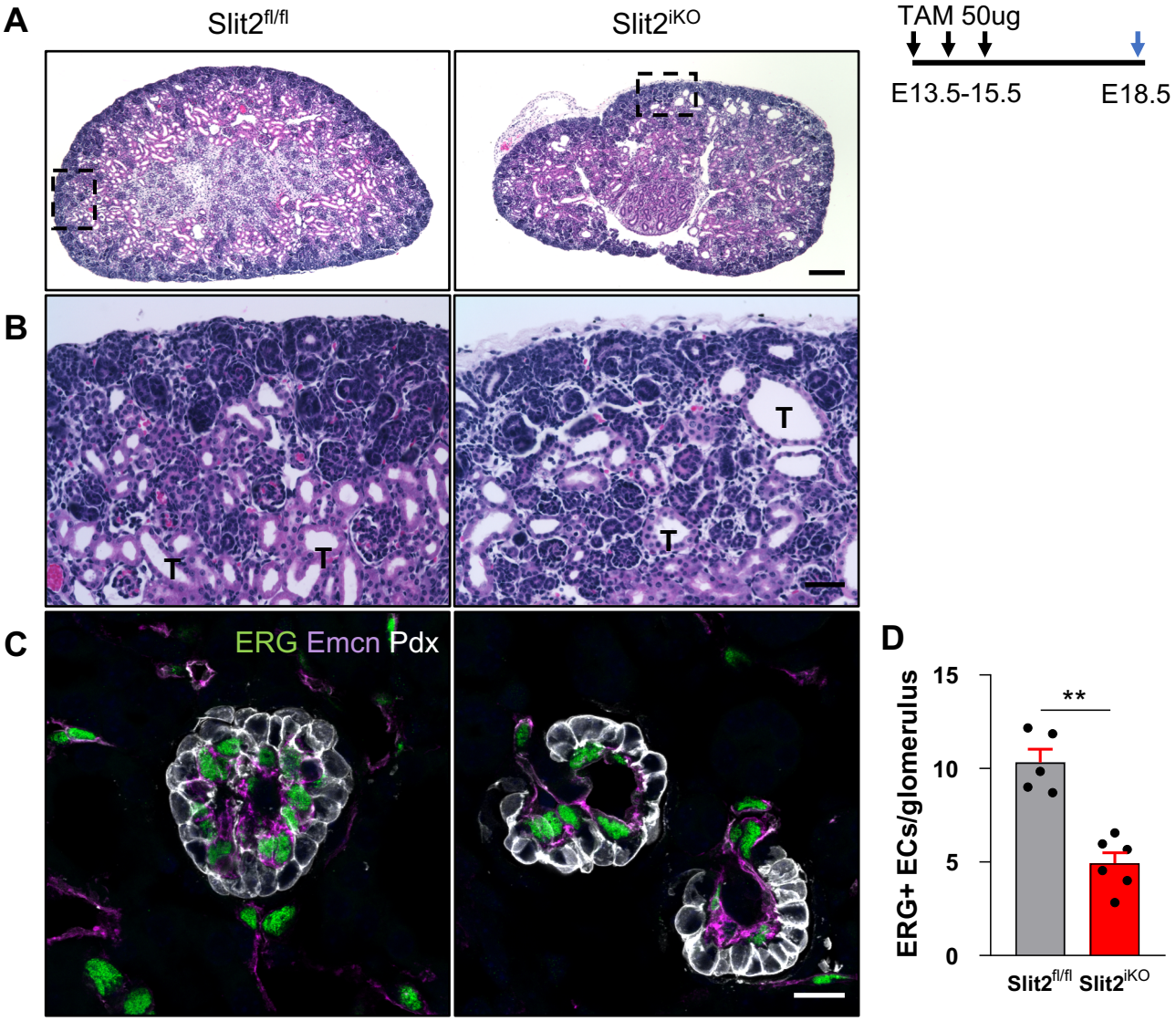
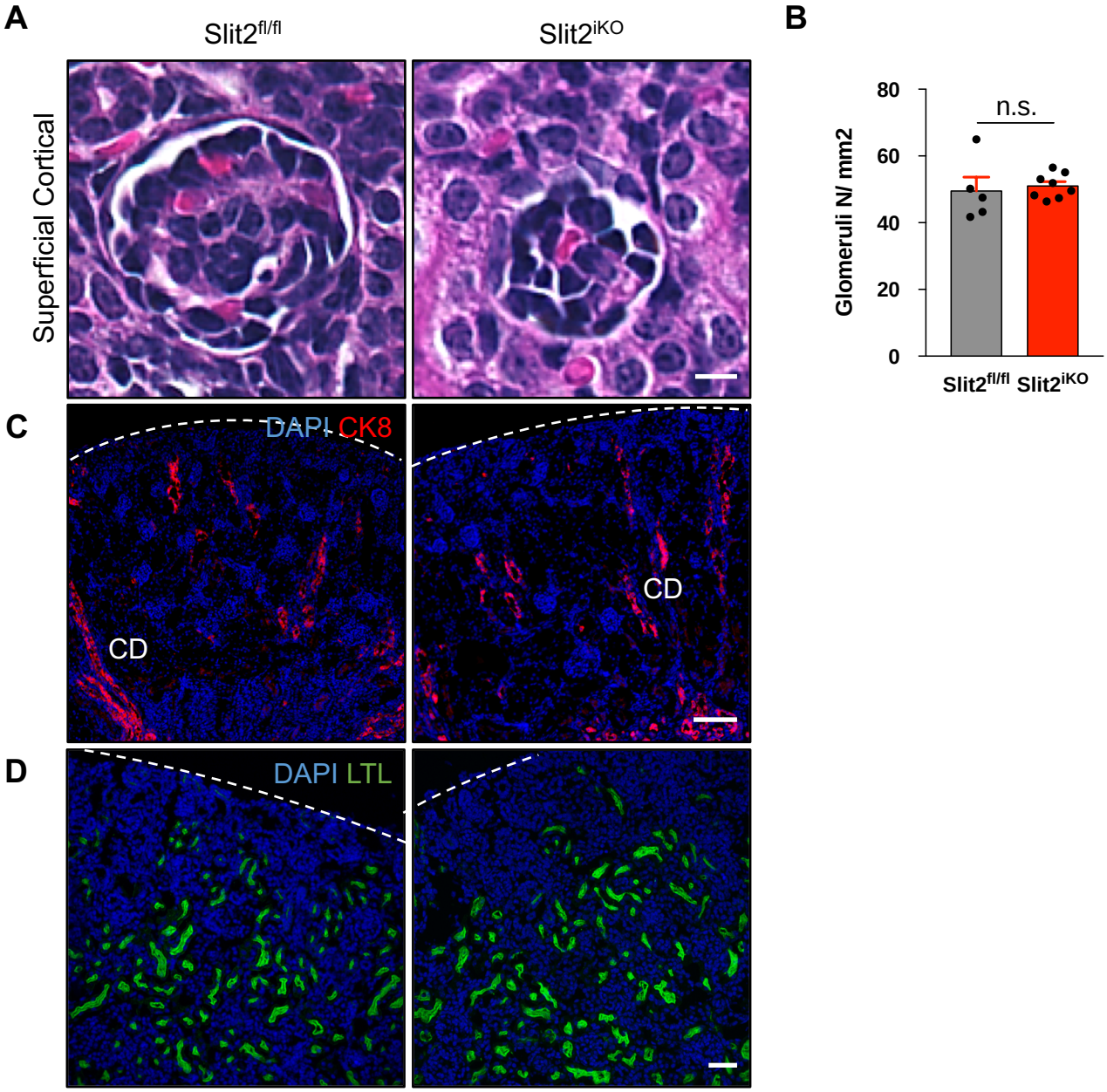
Supplemental material



Supplement Fig.1

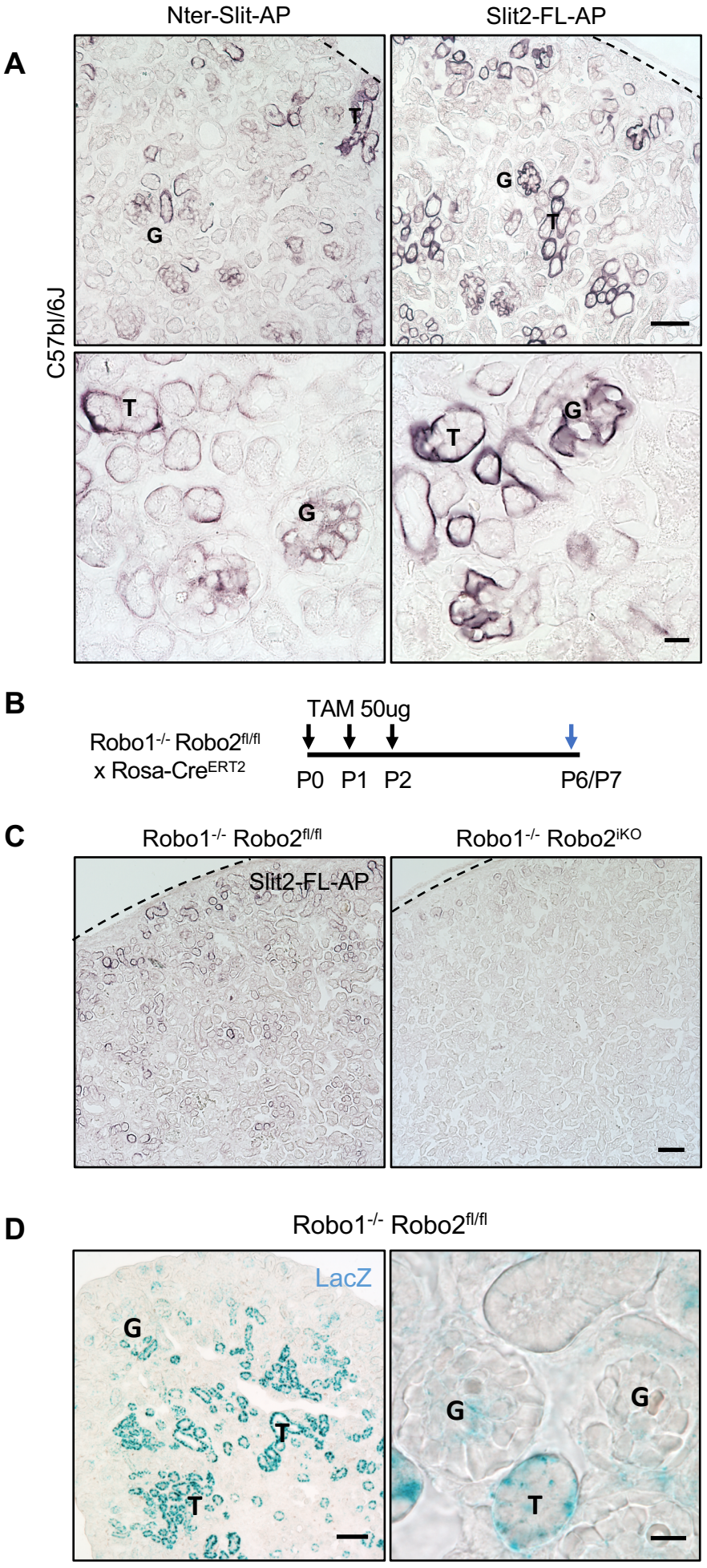
A. H&E staining of embryonic Slit2^{iKO} and control mice. **B.** Higher magnification of boxed areas in A. Note that Slit2^{iKO} have dilated tubules and disrupted overall morphology. T, tubules. Scale bar: 200μm (A), 50μm (B). **C.** E18.5 kidney section immuno-labeled with antibodies recognizing endothelial cells (ERG, Emcn) and podocytes (Pdx). Scale bar: 10μm. **D**. quantification of endothelial cells per glomerulus. Each dot represents average number of 15 glomeruli. n=5 Slit2$^{fl/fl}$ mice, 6 Slit2^{iKO} mice.



Supplement Fig.2

A. High magnification view of H&E staining of glomerulus of control and Slit2^{iKO} mice at P7 shown in Fig.1F. Scale bar: 10μm. **B.** Quantification of total number of glomeruli normalized to area of control and mutant mice at P7. Each dot represents individual mice. n=5 Slit2$^{fl/fl}$ mice, 8 Slit2^{iKO} mice. **C.** Representative image of CK8^{+} collecting ducts of P7 kidney quantified in Fig.1K. Scale bar: 50μm. **D.** Representative image showing LTL^{+} proximal tubules of P7 kidney quantified in Fig.1K. Scale bar: 50μm.

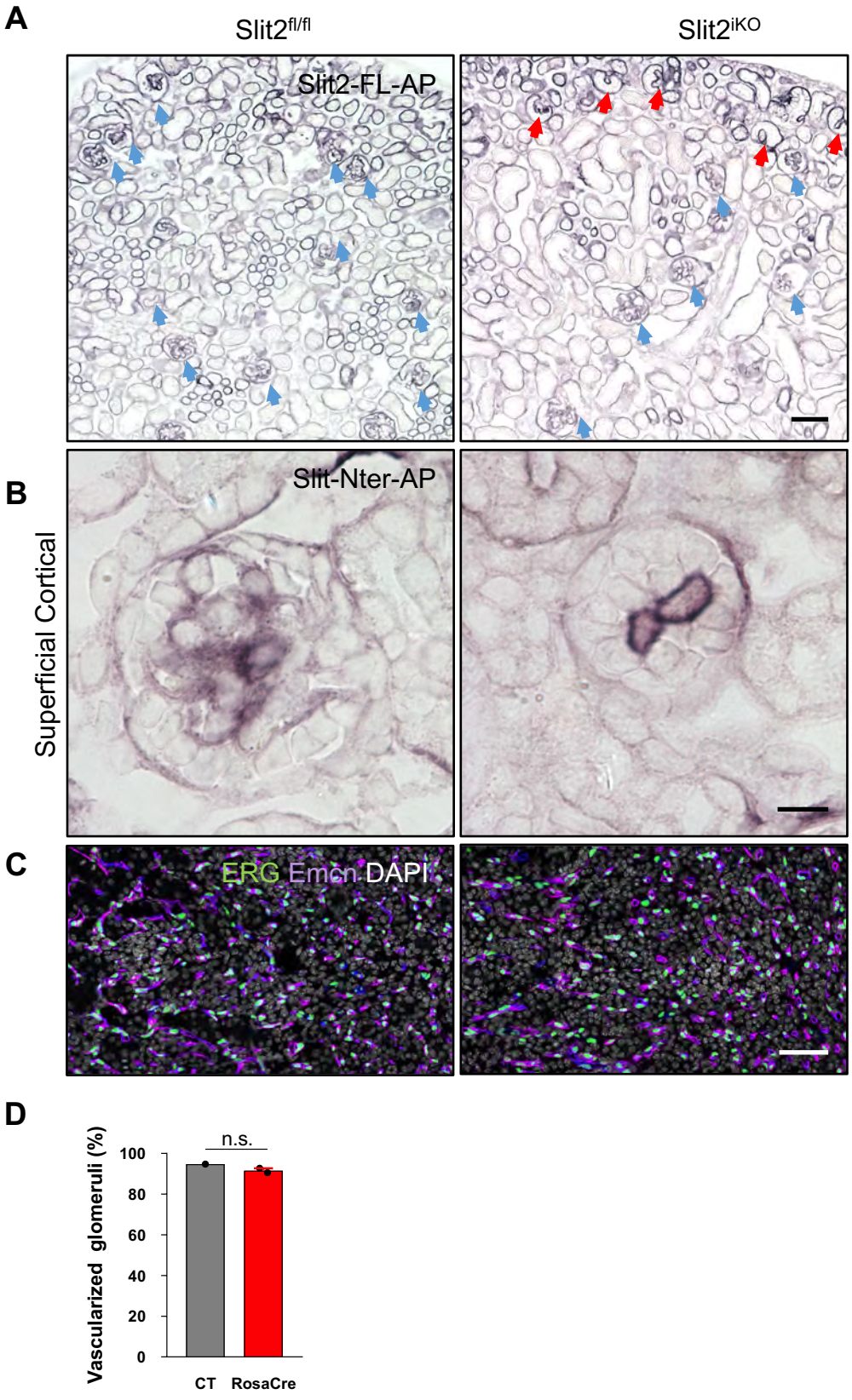
Li et al, Supplement Fig.3



Supplement Fig.3

A. Alkaline phosphatase staining with the Robo binding Slit-Nter-AP and the full length Slit2-FL-AP of P7 wild type mice. Note the same pattern of Slit-Nter-AP and Slit2 FL-AP. Scale bar: 50μm (upper panel); 10μm (lower panel). **B.** Timeline for Rosa-Cre^{ERT2} mediated global deletion of the *Robo2* fl/fl allele on a *Robo1* null background by IP injection of 50ug TAM to neonates at P0-P2. Samples were collected at P6-7**. C.** Slit2-FL-AP staining of control and Robo1,2^{iKO}. Note the abolished Slit2-FL-AP binding in Robo1,2^{iKO}, and reduced binding to *Robo1* null kidneys. Scale bar: 50μm. **D.** LacZ staining of *Robo1* null mice with LacZ reporter, showing expression of Robo1 within the glomerulus and renal tubules. T, tubules; G, glomerulus. Scale bar: 50μm (left panel), 10μm (right panel)

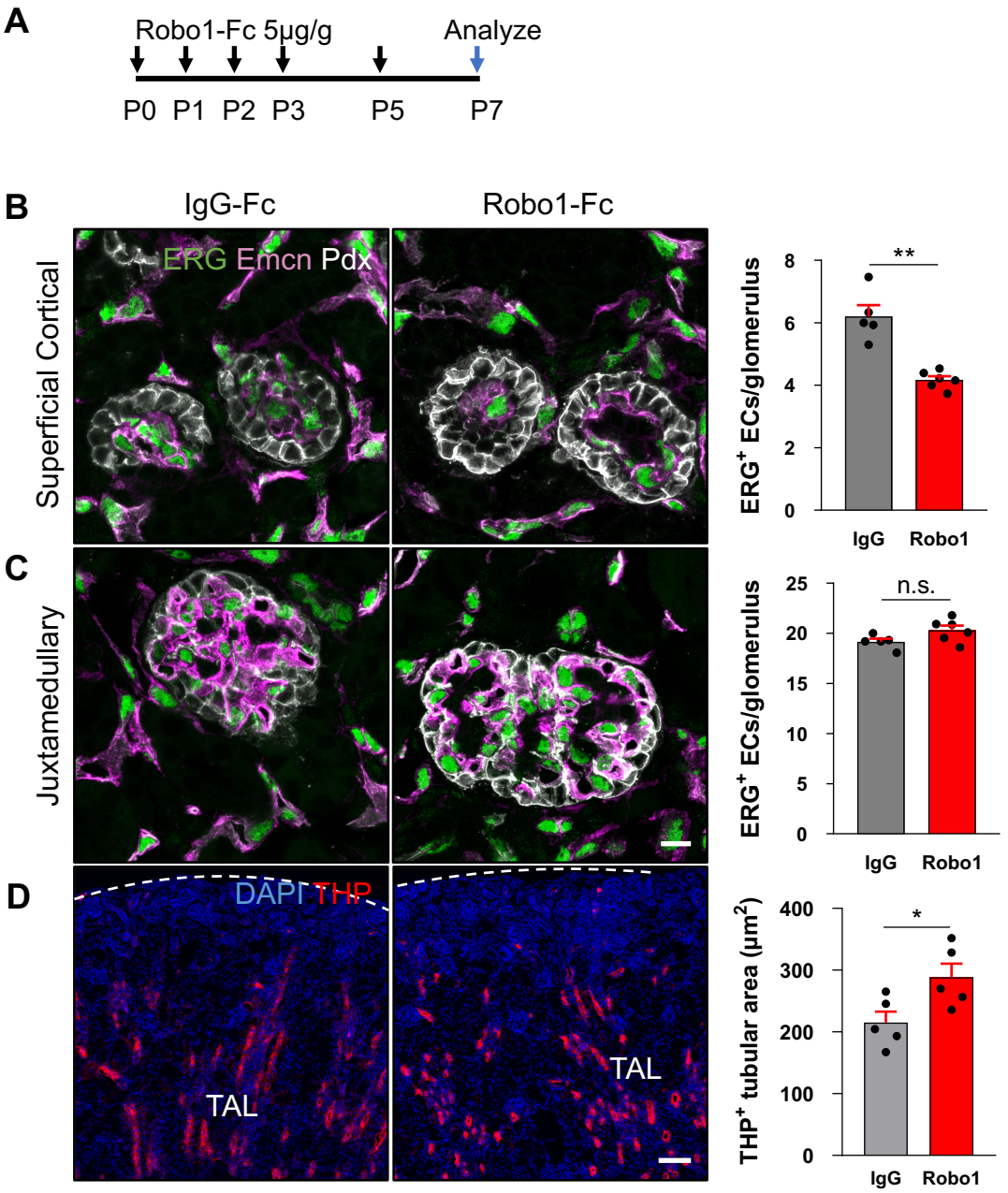
Li et al, Supplement Fig.4



Supplement Fig.4

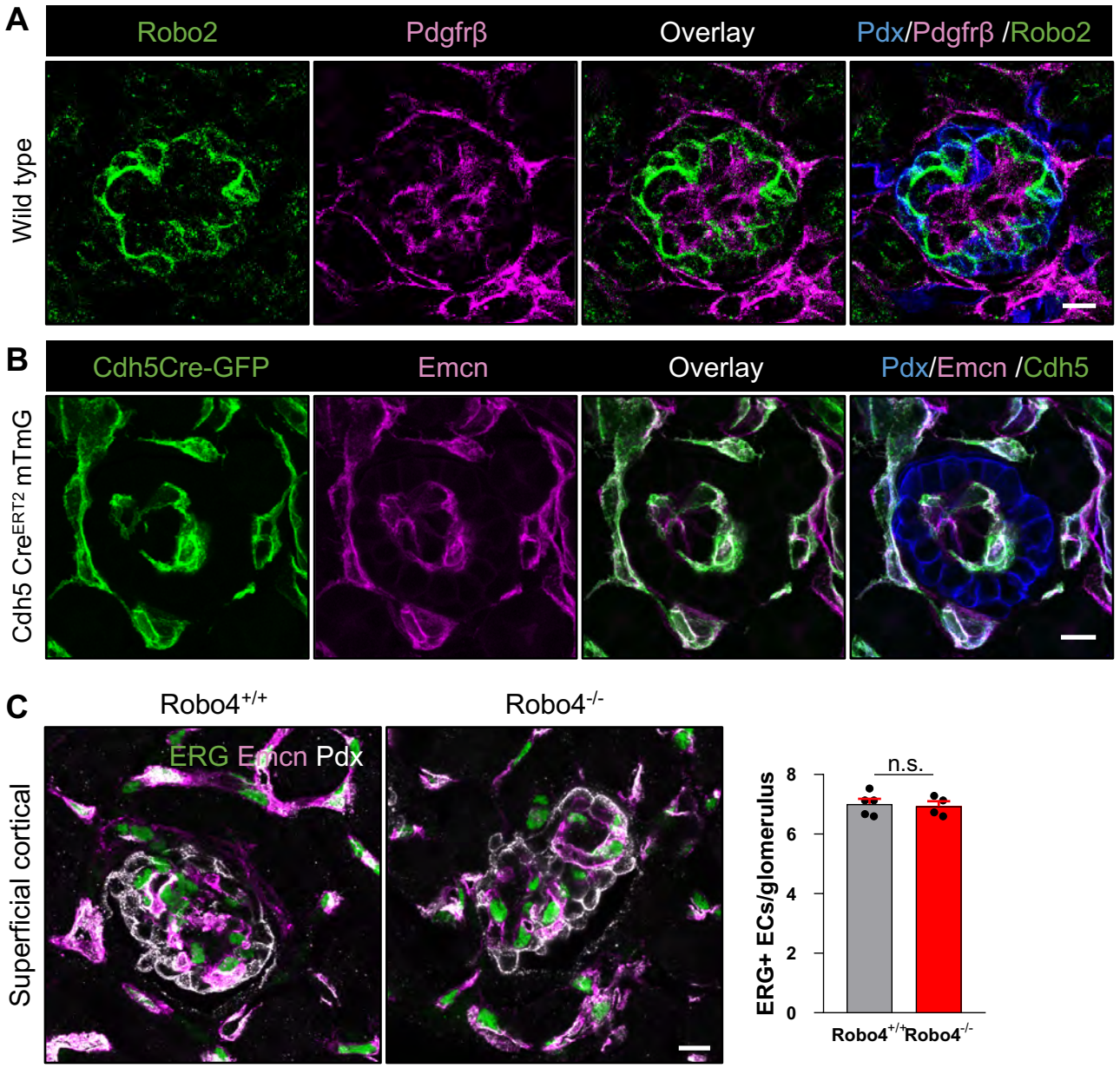
A. Slit2-FL-AP staining of P7 control and Slit2^{iKO} kidneys. Slit2-FL-AP binds to glomerular capillary loops, and reveals normal glomeruli of controls (blue arrows, left panel), and poorly vascularized glomeruli (red arrows, right panel) in the superficial cortical region of Slit2^{iKO} kidneys. Glomeruli in the juxtamedullary region were similar in controls and of Slit2^{iKO} (blue arrows). Scale bar: 50μm. **B.** High-magnification images of Slit-Nter-AP stained superficial cortical glomerulus of control and Slit2^{iKO} kidneys. Note the reduction of glomerular capillary in the mutant. Scale bar: 10μm. **C.** Representative images of peritubular capillary of P7 kidneys stained with endothelial marker endomucin (Emcn) and ERG. Scale bar: 20μm. **D.** Quantification of vascularized glomeruli of RosaCre-positive flox-negative mice and littermate control. Each dot represents individual mice.

Li et al, Supplement Fig.5



Supplement Fig. 5

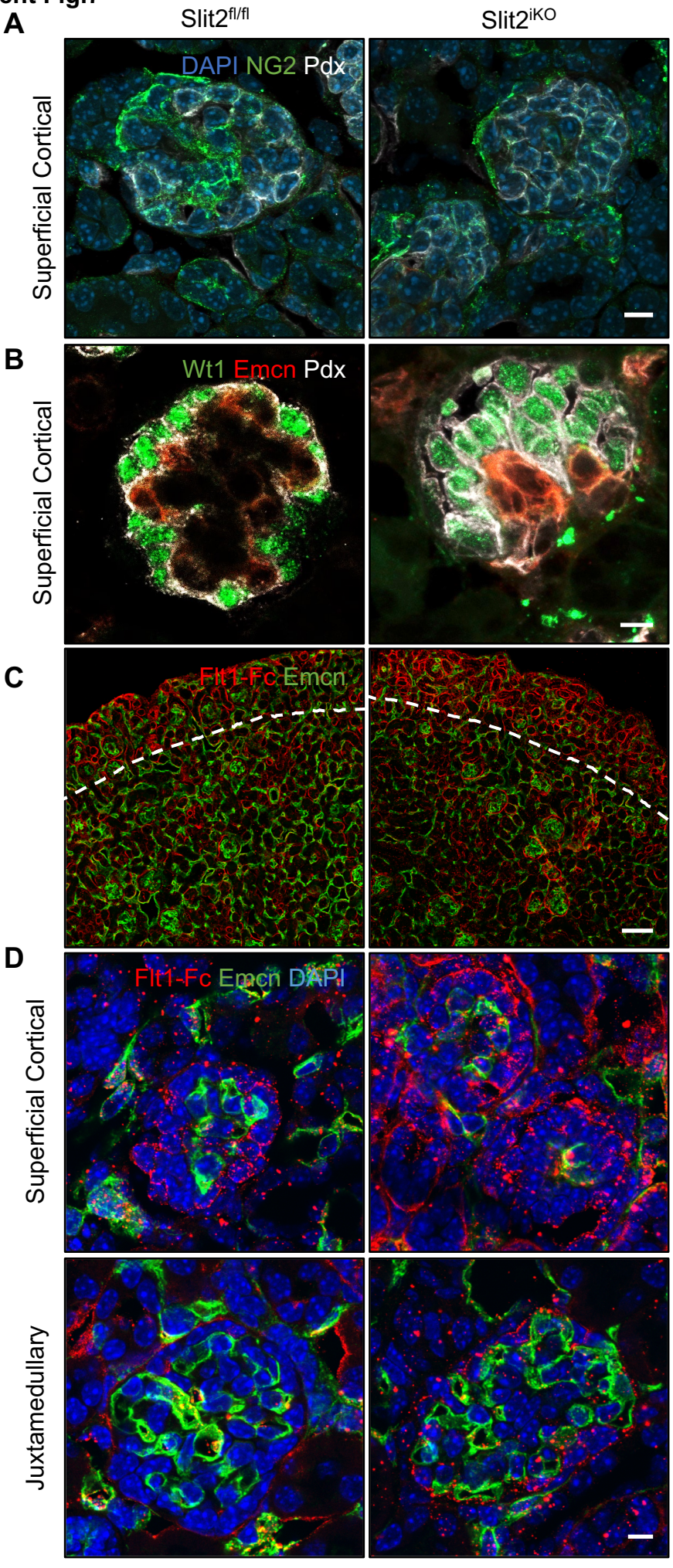
A. Neonatal wildtype C57/Bl6 mice were treated with Robo1-Fc protein or control IgG-Fc (5ug/g, IP) for P0-3, P5 and sacrificed at P7. **B,C.** High magnification views of glomeruli labeled with the indicated antibodies in the superficial cortical (B) and juxtamedullary region (C). Right panel shows quantification of ERG^+ endothelial cell number in individual glomeruli at the superficial cortex versus the juxtamedullary region. Scale bar: 10μm. Each dot represents the average number of endothelial cells from 15 glomeruli of individual mice. n=5 IgG-Fc treated, 6 Robo1-Fc treated mice. **D.** Immuno-staining of TALs, and quantification of THP+ tubular area. Each dot represents individual mice. Scale bar: 50μm.



Supplement Fig.6

A. Robo2 and Pdgfrβ co-staining of C57/Bl6 superficial cortical glomeruli. Scale bar: 10μm. **B.** Linage tracing of Cdh5CremTmG expression showing GFP recombination in Emcn+ endothelial but not other cell types. Scale bar: 10μm. **C.** Representative images of glomeruli in Robo4$^{-/-}$ and littermate control stained with the indicated markers. Right panel shows quantification of ERG^{+} endothelial cells per glomerulus. Scale bar: 10μm. Each dot represents individual mice. n=5 Robo4$^{+/+}$, 4Robo4$^{-/-}$ mice.

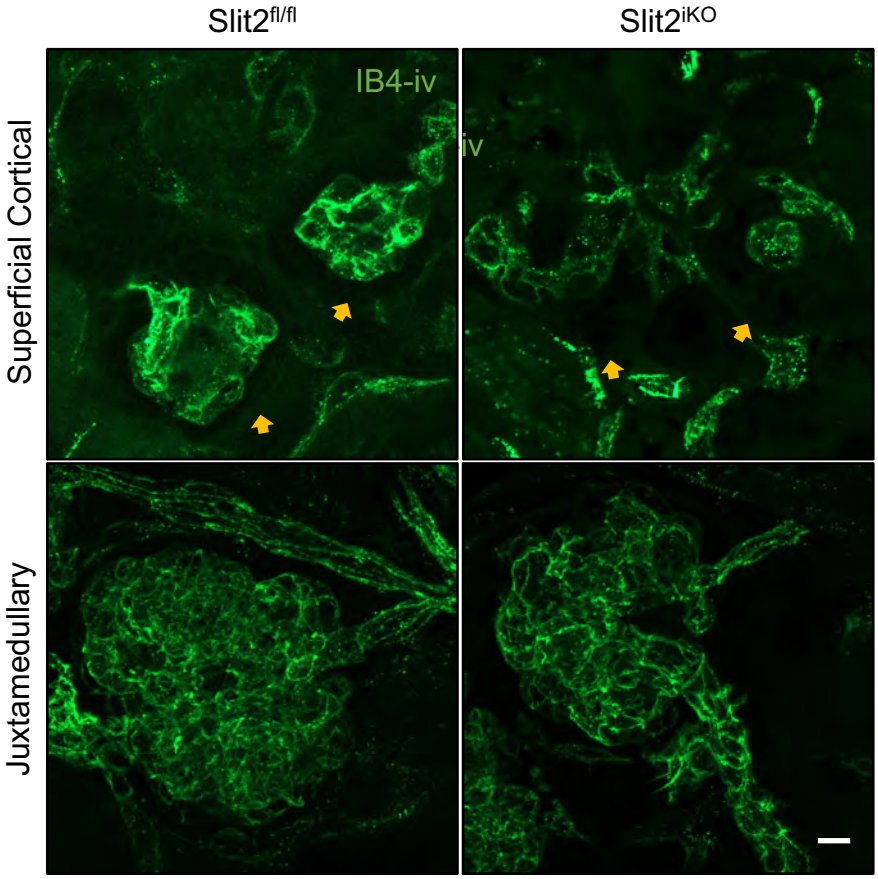
Li et al, Supplement Fig.7



Supplement Fig.7

A. Immunostaining of mesangial cells on P7 cryosectioned kidneys with anti-NG2 antibody quantified in Fig. 6B. Scale bar: 10μm. **B.** Immunostaining of P7 cryosectioned kidneys with podocyte (Wt1, Pdx) and endothelial markers (Emcn) quantified in Fig. 6C. Scale bar: 10μm. **C,D.** Representative images showing Flt1-Fc staining of Vegfa. Note the increase of Flt1-Fc at the superficial cortex of Slit2^{iKO} kidney. Scale bar: 50 μm (C); 10 μm, (D).

Li et al, Supplement Fig.8



Supplement Fig.8
Blood perfusion of glomeruli visualized by IV-injected IB4-488 dye. Arrow (upper panel) position corresponding to Fig.7B. Scale bar: 10μm.