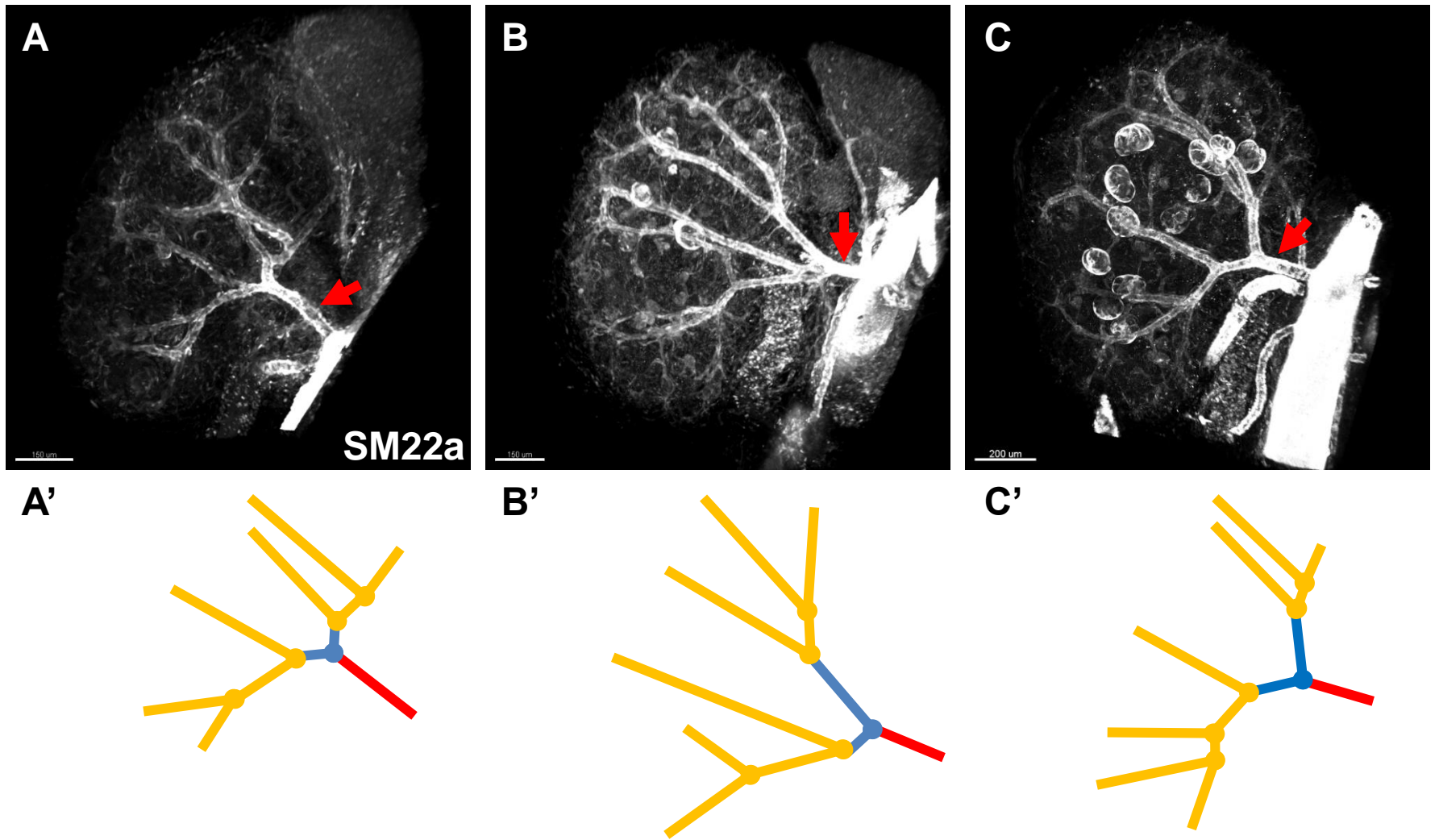
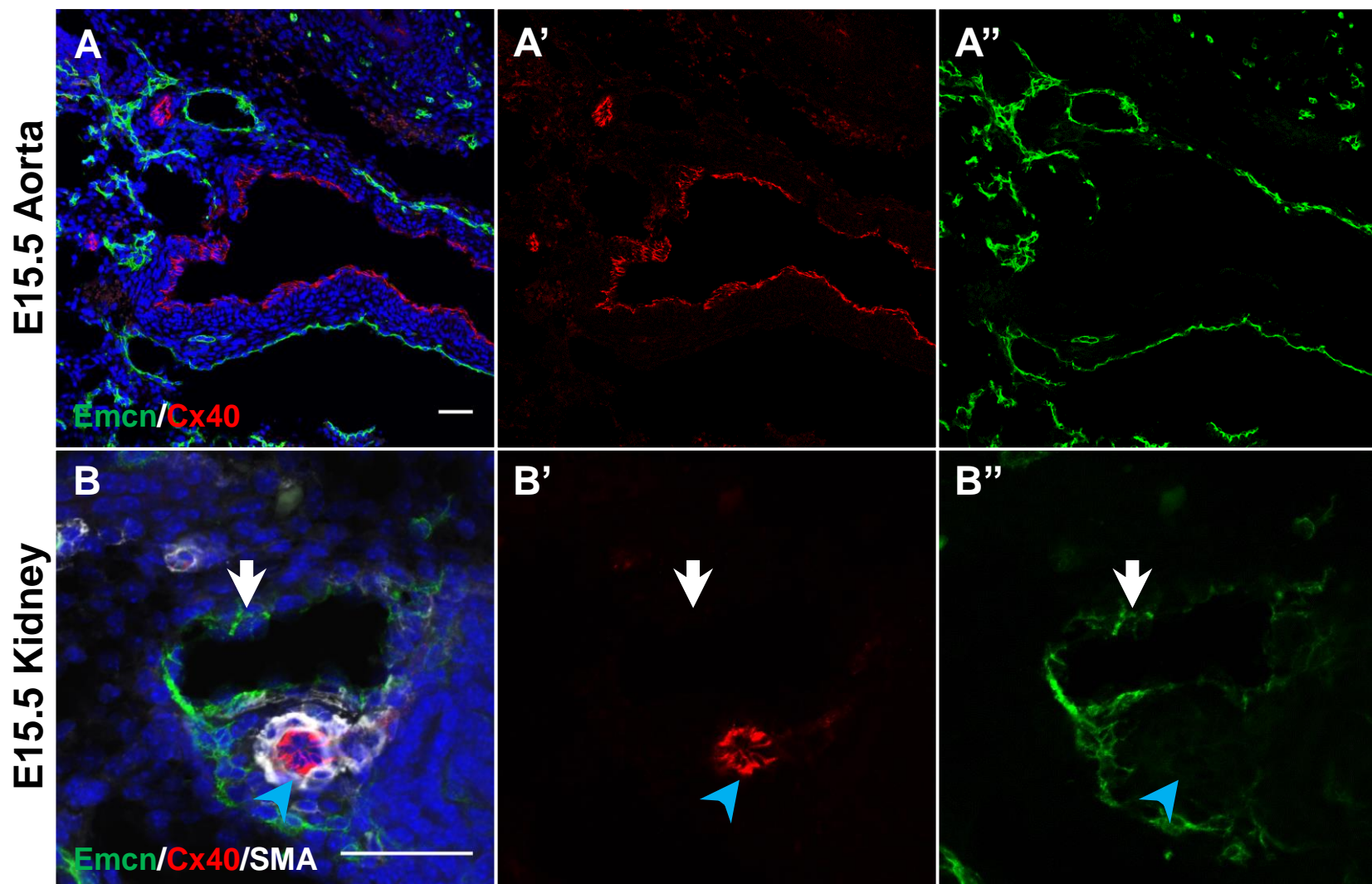


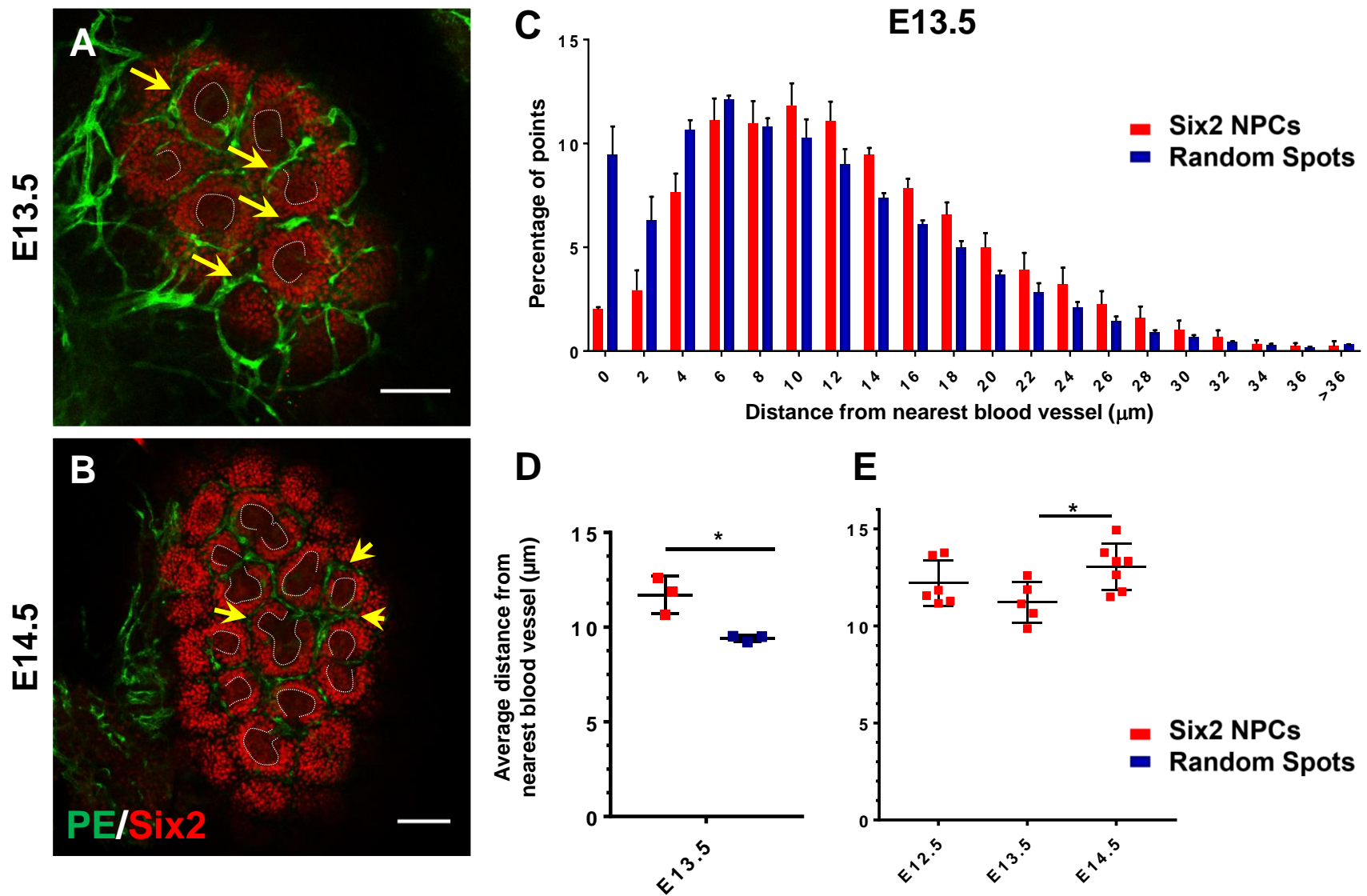
Supplemental Figure 1. ephrinB2-LacZ and EphB4-lacZ stains reveal developing AV structures. a-b) ephrinB2-LacZ X-gal stain marks both arterial and ureteric bud tree at E12.5 (**a**) and E14.5 (**b**). Arrow marks vascular cord that connects aorta and common iliac artery (**c-d**) EphB4-LacZ X-gal stain demonstrates developing venous tree absent at E12.5 but present at E14.5. E12.5 kidneys marked with dotted line. Scale bar = 100 μ m.



Supplemental Figure 2. Renal arterial tree forms in a predictable but not stereotyped pattern. a-c) Whole mount imaging of 3 separate E14.5 kidneys were stained with the smooth muscle marker Sm22a to visualize the arterial tree. Red arrows mark the renal artery extending from the aorta. Balloon-like structures are parietal epithelial cells of Bowman's capsule. **a', b', c')** Simplified 2D representation of the arterial branching pattern for each kidney. Each bar corresponds to a branch of the arterial tree and each circle is a branch point. Red branch represents the renal artery, blue branches represent the first major branching event, and the orange branches represent the lobar arteries. Lengths and angles of each branching event is roughly proportional to the in vivo stain, demonstrating the heterogeneity in the branching pattern.

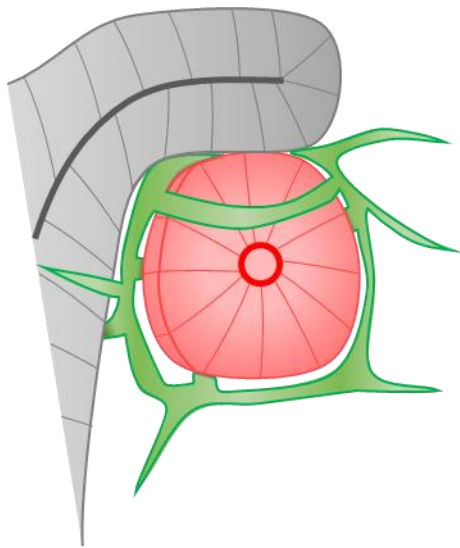


Supplemental Figure 3. Arteries and veins express distinct genes in the midgestation kidney. a-a'') E15.5 aorta stained for Emcn and Cx40 demonstrating that the gene expressions are mutually exclusive in the aorta. **b-b'')** E15.5 kidney artery and vein doublet. Arteries and veins are marked with a blue arrowhead and a white arrow, respectively. Mature arteries express Cx40, but not Emcn, and are surrounded by SMA⁺ cells. Mature veins express only Emcn and are not surrounded by any SMA⁺ cells. Scale bar = 50 μ m.

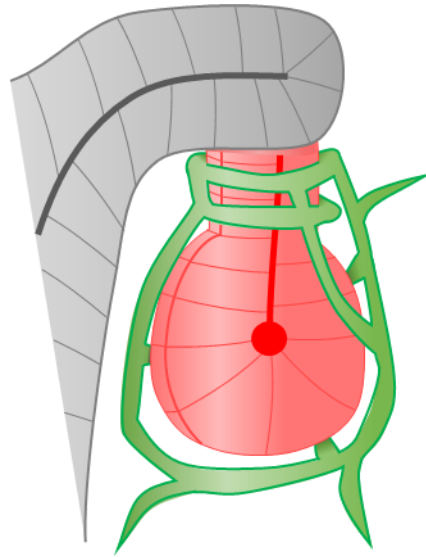


Supplemental Figure 4. Endothelial cells avoid Six2⁺ cells and circumscribe progenitor caps. **a-b** Whole mount imaging of outer cortex in E13.5 (**a**) and E14.5 (**b**) kidneys. White dashed lines demarcate ureteric bud tips. Yellow arrows mark ECs circling the Six2⁺ caps. **c** Histogram of the distance between Six2⁺ NPC or randomly generated spots and the nearest blood vessel. Histogram represents the average distributions across 3 different E13.5 kidneys. **d** Average distance of all Six2⁺ NPC and randomly generated spots to the nearest blood vessel at E13.5. n=3. **e** Average distance of all Six2⁺ NPCs to the nearest blood vessel from E12.5-E14.5. n=5-7. Error bars show standard deviation. Scale bar = 100 μm , * P <0.05.

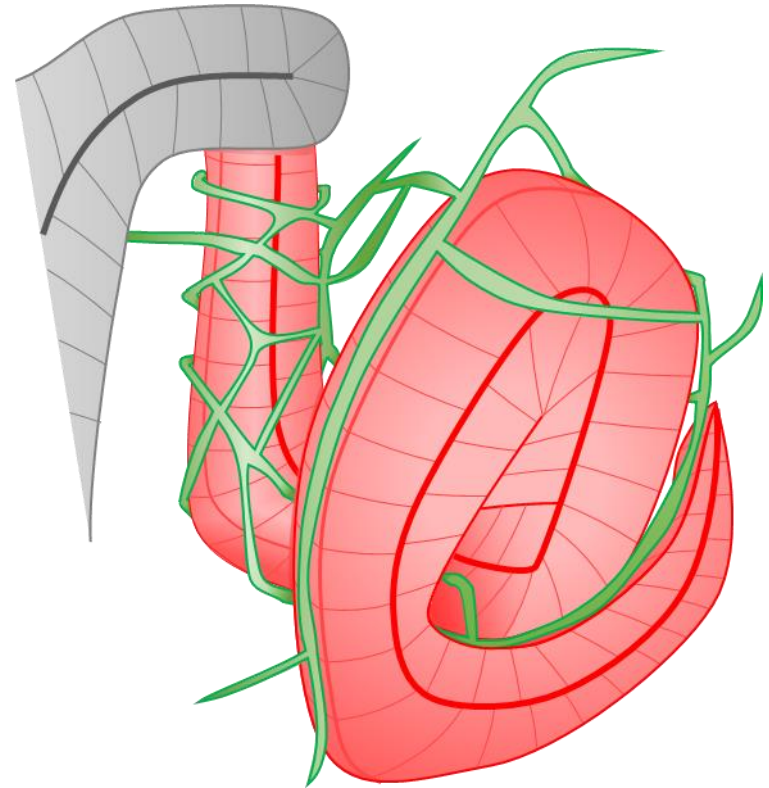
A Renal Vesicle



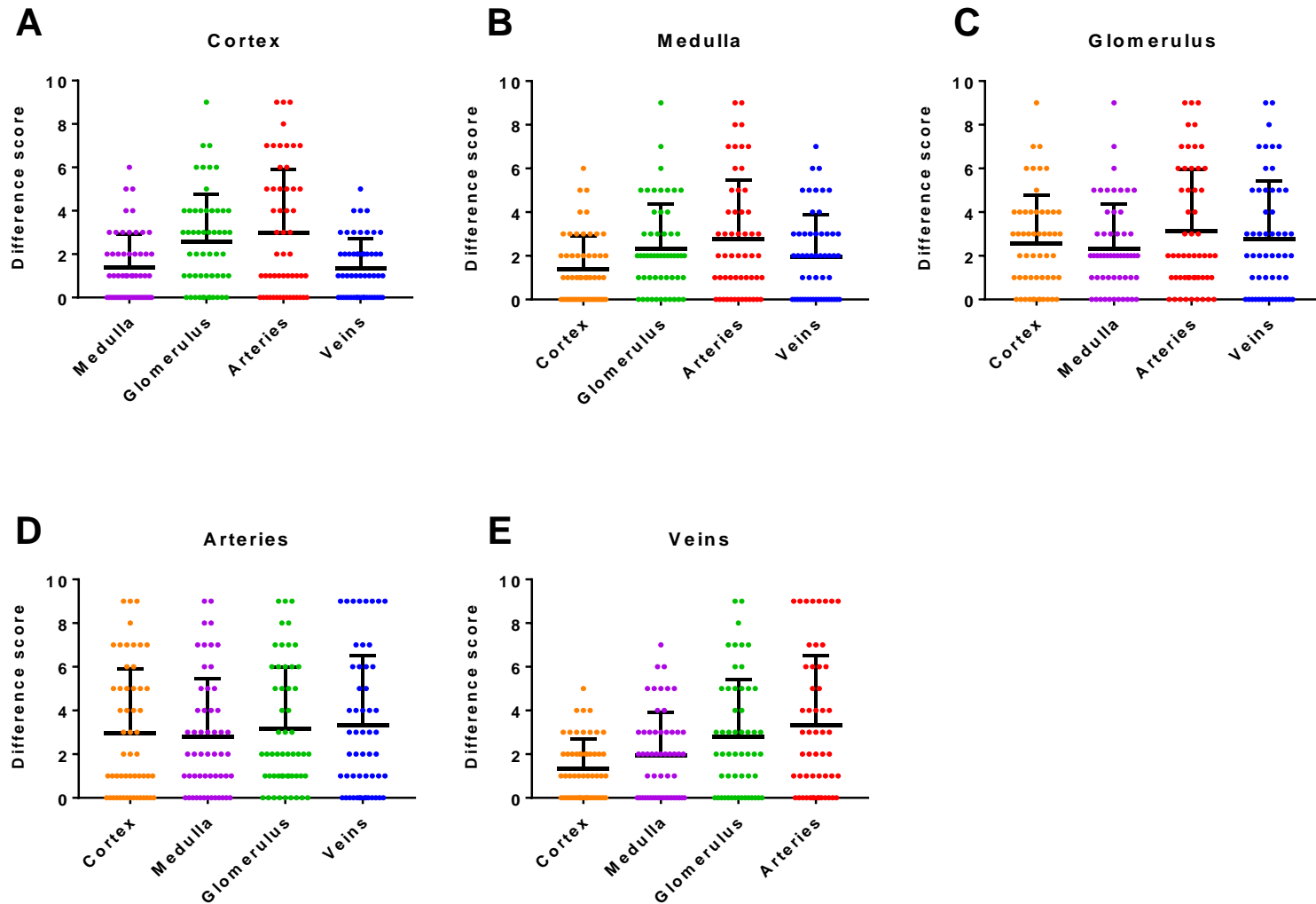
B Late Renal Vesicle



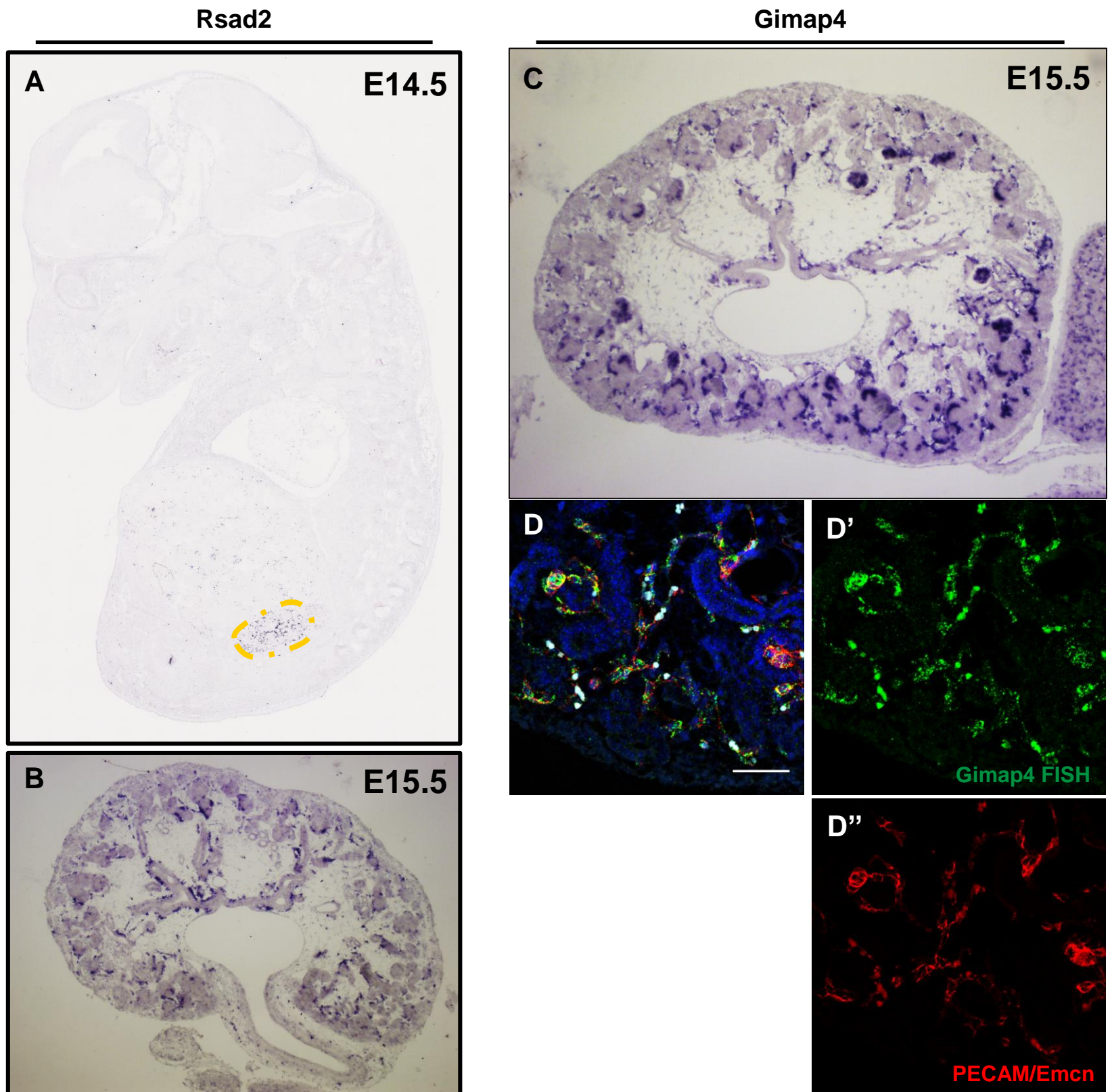
C S-Shaped Body



Supplemental Figure 5. Models for endothelial organization around the developing nephron. Developing nephrons undergo multiple morphologic steps to form a mature nephron. (a) At the RV stage, ECs (green) form a plexus around the developing nephron structure (red) including in between the RV and UB (grey). (b) By the late RV stage when the RV elongates and attaches to the UB, the endothelial plexus begins to show signs of enrichment along the area of the vesicle destined to become the distal tubule. (c) Once the RV has reached the S-shaped body stage, the endothelial plexus has completely enveloped the distal part of the S-shaped body but is relatively sparse surrounding the proximal tubule portion (note that the proximal tubule portion has been slightly displaced to better visualize the distal tubule portion). The ECs that form along the cleft of the S-shaped body connect through the nephron to the rest of the endothelial plexus. At all stages, the plexus that surrounds the developing nephron is continuous with the rest of the cortical endothelium.



Supplemental Figure 6. Differences in gene expression score between each region. Data summarized in **Fig. 5r-s** organized by each region compared to the other 4 regions to better compare region expression patterns. All scores for all 3 independent analyses are shown. Line in each graph represents arithmetic mean and standard deviation. Each region is color-matched: cortex (**a**, orange), medulla (**b**, purple), glomerulus (**c**, green), arteries (**d**, red), and veins (**e**, blue).



Supplemental Figure 8. Validation of Genepaint.org data. a) E14.5 in situ of *Rsad2* from genepaint.org demonstrating enriched expression in the kidney with some expression in the liver. Kidney is outlined in orange dotted line. b) in situ hybridization of *Rsad2* on E15.5 kidney. c-d) *In situ* hybridization of *Gimap4* on E15.5 kidneys to further validate WGCNA analysis on E16.5 kidneys. d-d'') Fluorescent *in situ* hybridization of *Gimap4* demonstrate co-expression with PECAM/*Emcn*. White cells are autofluorescent blood cells. Scale bar = 50 μ m.

Gene	Company	Catalog no.	Dilution	Antigen Retrieval
CD34	Abcam	ab8158	1:100	
Claudin-5	Invitrogen	34-1600	1:100	+
Connexin 40	Santa Cruz	sc-20466 (C-20)	1:100	+
Cytokeratin	Sigma Aldrich	C2562	1:100	+
Endoglin	Rolf Brekken	N/A	1:100	
Endomucin	Santa Cruz	sc-65495 (V.7C7)	1:200	
GFP	Aves	GFP-1020	1:200	
Icam1	Proteintech	10020-1-AP	1:100	
Icam2	BD Pharmingen	553927	1:100	
Meis1/2/3	Active Motif	39795	1:100	+
N-Cam	Sigma Aldrich	C9672	1:100	+
Nrp1	R&D Systems	AF566	1:100	+
Nrp2	Cell Signaling	3366s	1:100	+
PECAM	BE Pharmingen	55370 (MEC13.3)	1:200	
Plvap	BD Biosciences	550563	1:100	+
Podocalyxin1	R&D Systems	AF1556	1:100	
Six2	Proteintech	11562-1-AP	1:200	
SM22a	Abcam	ab14106	1:100	
SMA	Sigma Aldrich	A5528	1:100	+
Tie2	R&D Systems	AF313-SP	1:100	+
VE-Cad	Santa Cruz	sc-6458 (C-19)	1:100	+
Vegfr1	R&D Systems	AF471	1:100	+
Vegfr3	BD Biosciences	552857	1:100	+
vWF	Dako	A0082	1:100	

Supplementary Table 1. List of antibodies

Movie Titles and Captions

Movie 1. 360° rotational view of vasculature around the E10.5 metanephros. Stained for Six2 (red) and PECAM/Emcn (green)

Movie 2. 360° rotational view of vasculature around the E11.5 metanephros. Stained for Six2 (red) and PECAM/Emcn (green)

Movie 3. 360° rotational view of vasculature around the E12.5 metanephros. Stained for Six2 (red) and PECAM/Emcn (green)

Movie 4. 360° rotational view of vasculature around the E13.5 metanephros. Stained for Six2 (red) and PECAM/Emcn (green)

Movie 5. Stack of z-plane images through E14.5 renal vesicle. Stained for N-Cam (red) and PECAM/Emcn (green). White arrow indicates EC in between UB and renal vesicle. Scale bar = 50 μm

Movie 6. Stack of z-plane images through E14.5 late renal vesicle. Stained for N-Cam (red) and PECAM/Emcn (green). White arrows point to ECs forming collar around the late renal vesicle. Orange arrow indicates areas devoid of direct epithelial contact as part of the vascular basket. Scale bar = 50 μm

Movie 7. Stack of z-plane images through E14.5 S-shaped body. Stained for N-Cam (red) and PECAM/Emcn (green). Scale bar = 50 μm