SUPPLEMENTAL MATERIAL

Branching Parameters During Kidney Development			
Embryonic Day	**Maximum Number Of Generations**	**Total Tip Number (±SD)**	**Total Glomeruli (±SD)**
E11.5	1	2	0
E12.5	5 ± 1	11 ± 1	0
E13.5	8 ± 1	116 ± 13	11 ± 2
E14.5	10 ± 1	298 ± 36	59 ± 12
E15.5	15 ± 1	587 ± 61	101 ± 22
E16.5	16 ± 1	873 ± 140	176 ± 34
E17.5	19 ± 2	1232 ± 256	228 ± 50
E18.5	22 ± 2	1772 ± 196	361 ± 82
E19.5	27 ± 3	3271 ± 425	956 ± 84

Table S1. Branching parameters during kidney development. The average number of maximal branching generations, total tip number and total glomerular number are shown for each embryonic day. N=5 for E11.5, N=5 E12.5, N=5 E13.5, N=6 E14.5, N=5 E15.5, N=3 E16.5, N=3 E17.5, N=3 E18.5 and N=3 for E19.5. Standard deviation (SD) is shown for total tip number and total glomeruli.

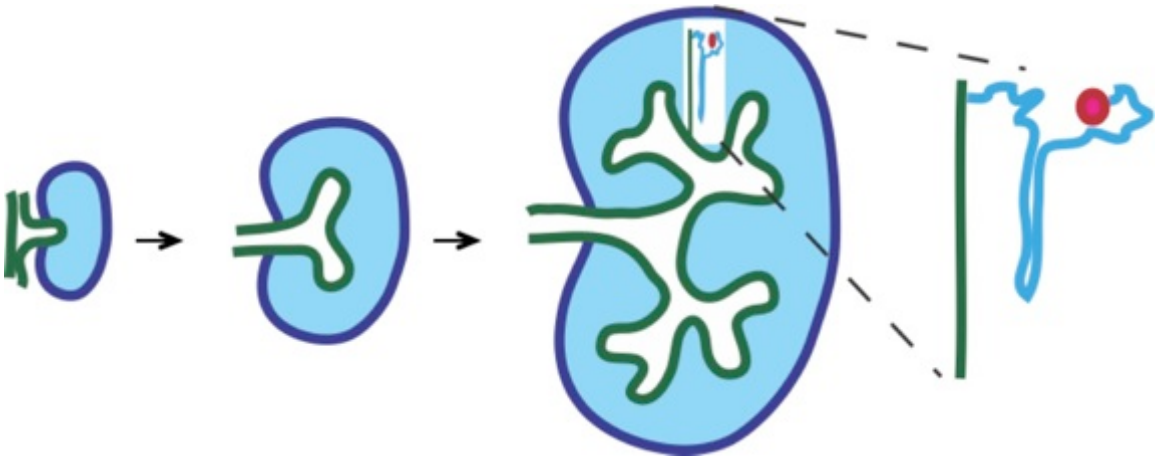

Figure S1. **Schematic representation of kidney development and organization**. Mouse intrauterine kidney development occurs between embryonic days 11.5 and 19.5. At E11.5, the ureteric bud (UB in green) is an outpouching that arises from the Wolffian duct and invades an adjacent collection of loose epithelial progenitor cells called the metanephric mesenchyme (MM in blue). Reciprocating signals induce the UB to branch while the MM simultaneously condenses at UB tips, epithelializes and organizes into nephron segments. Repeating UB branching events form the ureteric tree. One mature nephron is shown on the right with the ureteric tree in green and MM-derived structures in blue (proximal nephron) and red (glomerulus). Glomeruli are capillary networks that initiate the filtration process required to maintain homeostasis. In the adult kidney, on average one million nephrons are packed into a complex arrangement that also includes a parallel vascular supply. The final nephron count in the adult kidney is highly variable and reflects the efficiency of this branching and maturation process.

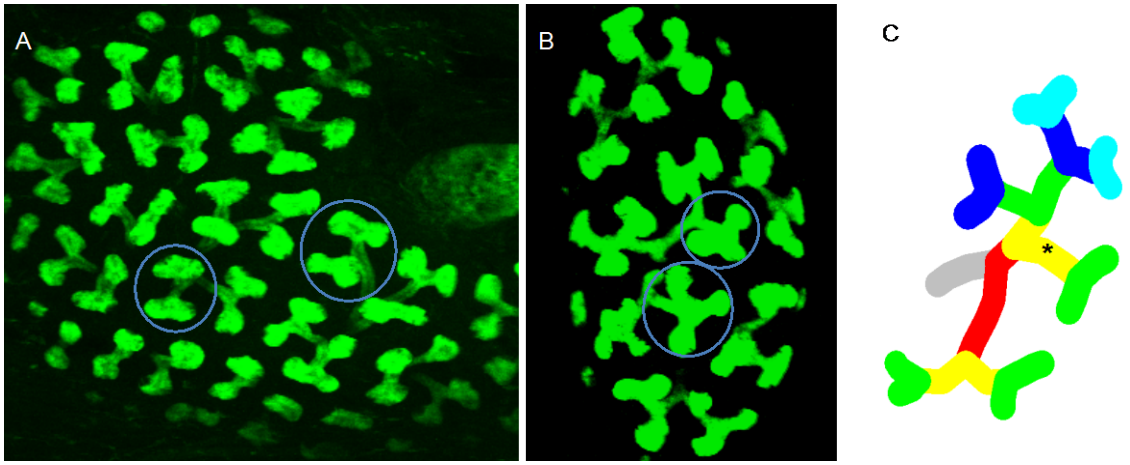

Figure S2. **Bifurcating, trifurcating and lateral branching mechanisms**. (A) Projection of confocal image stacks (generated using LSM Image Browser) showing the surface of an age E15.5 kidney. Circled areas indicate terminal branches that bifurcate. The average local branch angle is 56.3^{o} +/- 27.7^{o}. (B) Projection of E13.5 kidney demonstrates trifurcations at the surface. (C) Tracing of the E12.5 ureteric tree demonstrates a lateral branch (*) that arises from the upper main stem (order number 1 is shown in red). This branching event was preserved in every kidney studied and established the first element of asymmetry between upper and lower poles.

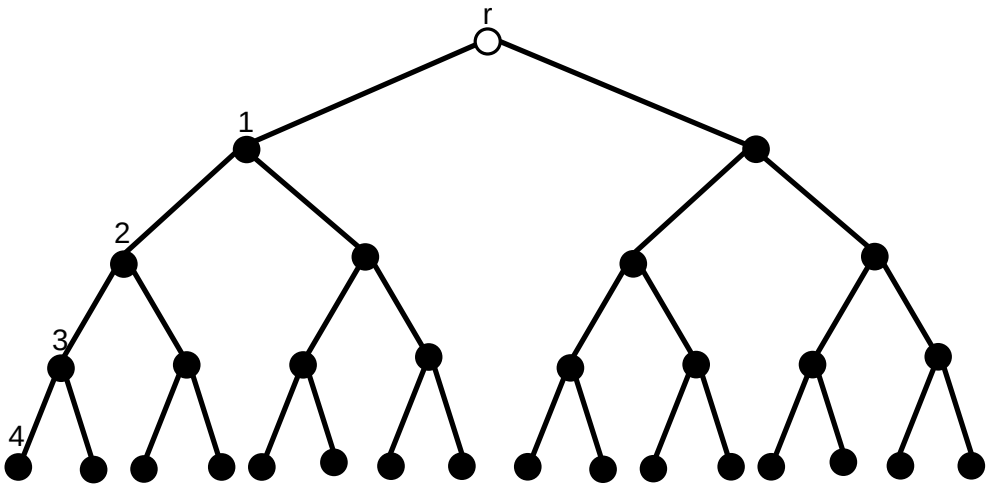

Figure S3. **Definition of a complete binary tree**. The tree shown has height h = 4. All circles represent vertices (or nodes) and the open circle represents the root that corresponds to the first division of the ureteric bud. Every level is filled in the figure shown, thus forming a complete binary tree with height h and 2^{h+1} -1 vertices.

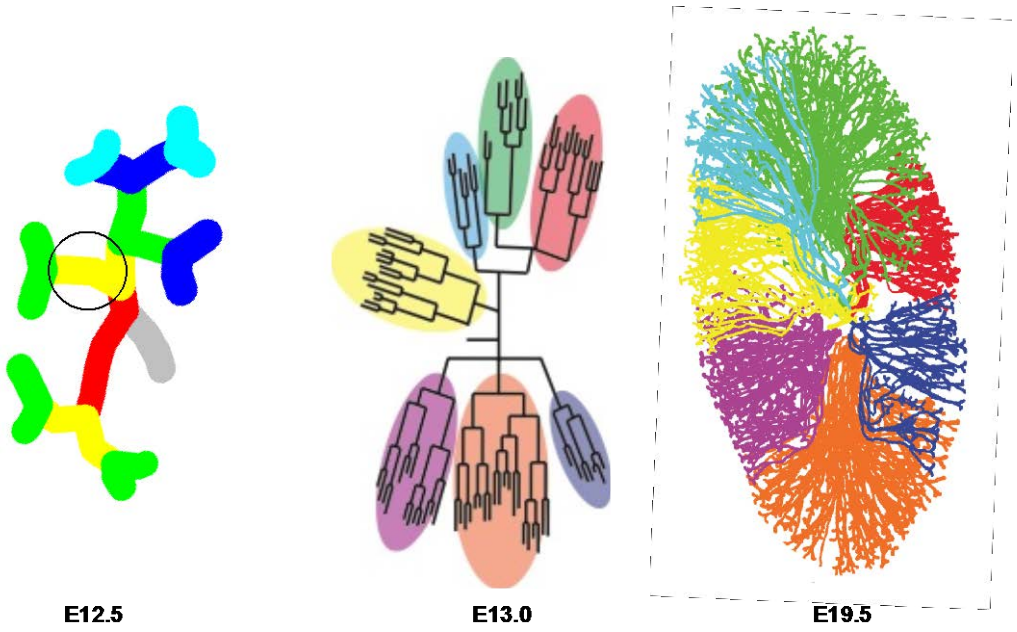

Figure S4. Persistence of the first lateral branch throughout gestation. The first lateral branch noted at E12.5 is circled (yellow). Descendants of this branch are shown at E13.0 within the yellow ellipse and at E19.5 by the subtree traced in yellow. The color-coded lobular arrangement seen at E13.0 remains conserved throughout E19.5. At E19.5, each color represents the corresponding individual tree with independent origin that is established by remodeling and pruning of the first several branch orders.

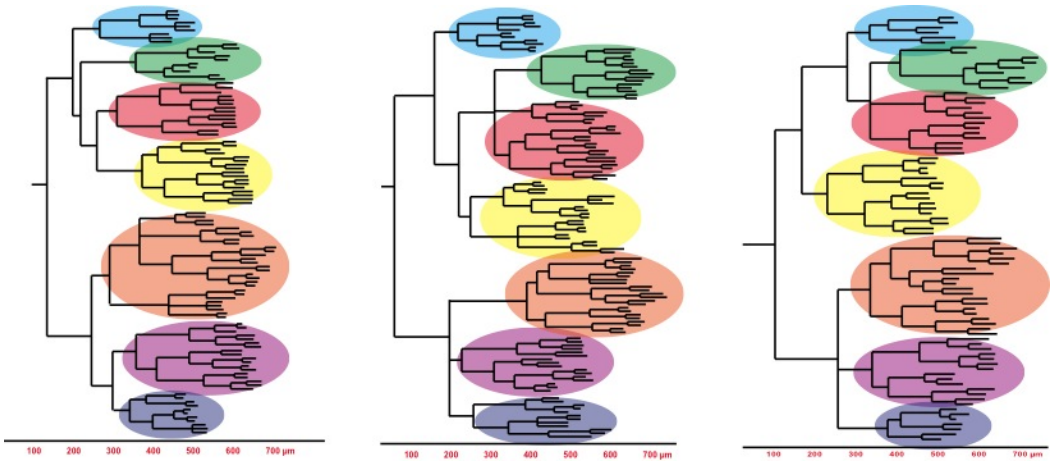

Figure S5. Branching dendrograms of littermates at E13.5. Dendrograms show that basic kidney scaffolding and upper vs. lower pole asymmetry are conserved in kidneys extracted from three littermates. Patterns within ellipses demonstrate variability in node number, segment lengths and topology. Although the kidneys appear identical in shape and gross size, the branching patterns and lineage are variable.

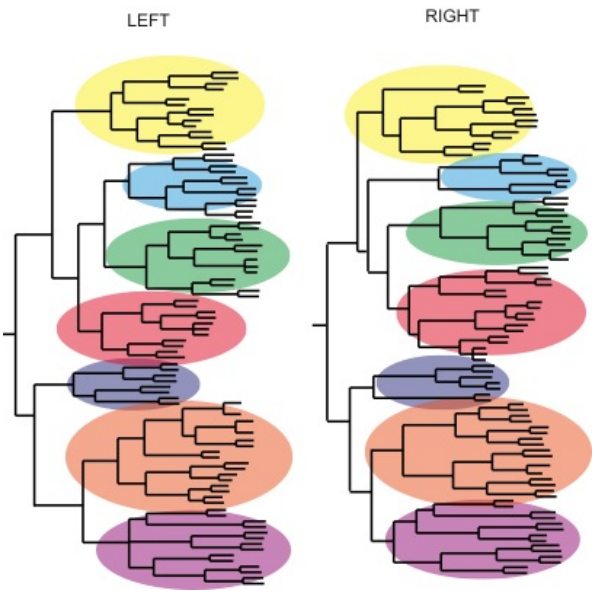

Figure S6. **Branching dendrograms of left and right kidneys from a single embryo at E13.0**. Left and right kidneys dissected from a single embryo begin to show variability in branch patterns and lineage. The left and right have 88 and 85 total tips respectively.

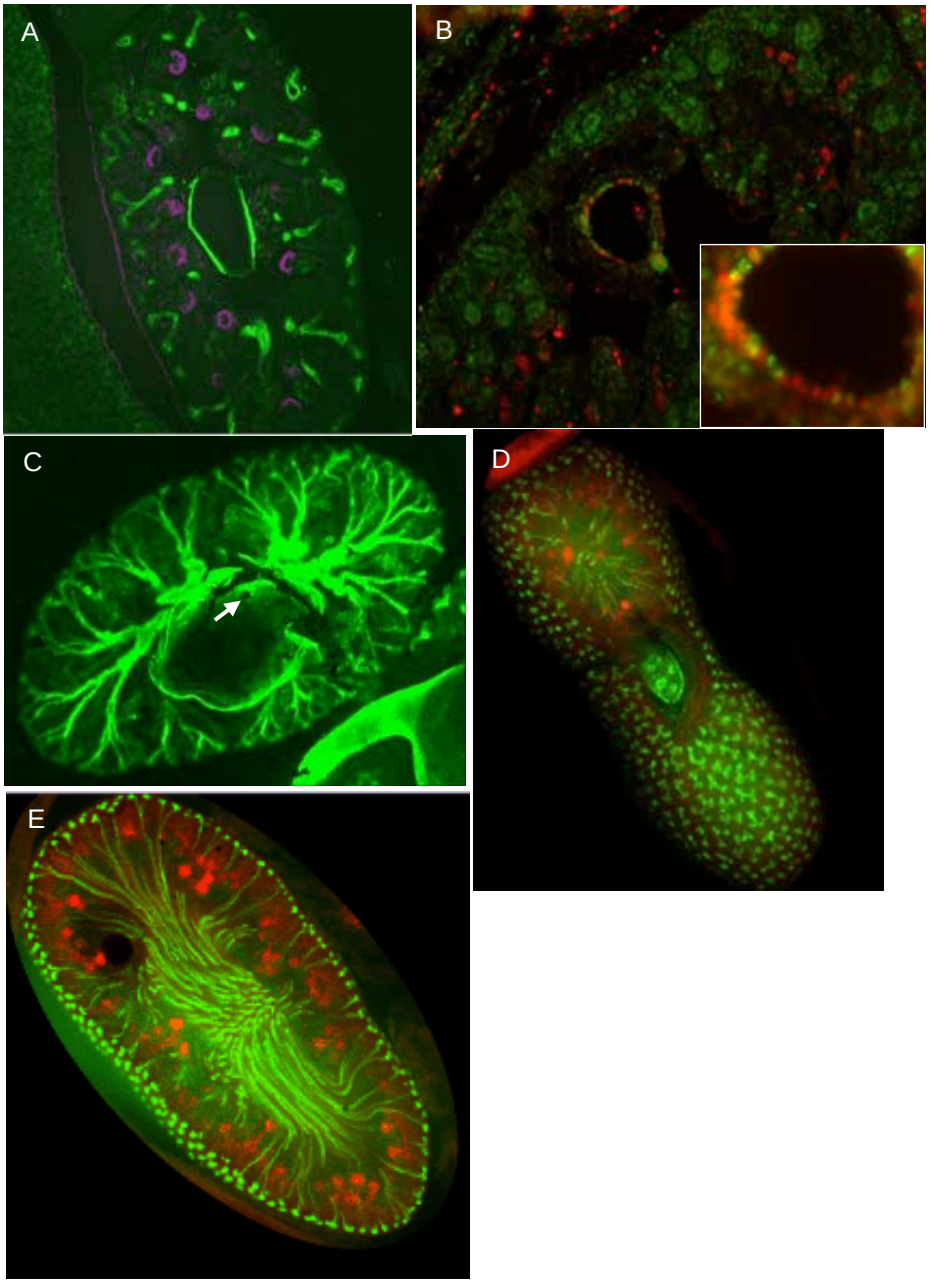

Figure S7. **Remodeling during the late branching phase.** (A) A central cavity begins to form and expand at E15.5 (glomeruli shown in purple). (B) Apoptosis (TUNEL) and proliferation (Ki-67) markers show concomitant cell growth and apoptosis in the origin (first branching bifurcation) of the ureteric tree at E15.5 (inset; magnification 400x). (C) Kidney bisected at E16.5 showing further cavity expansion. Arrow indicates the origin of a resorbing branch. (D) Lateral view of the kidney at E19.5 shows some of the separate collecting ducts exiting the papilla. These are created by erosion of the first 2 to 3 branching orders. (E) Kidney at E18.5 shows segment lengthening in the longitudinal axis.

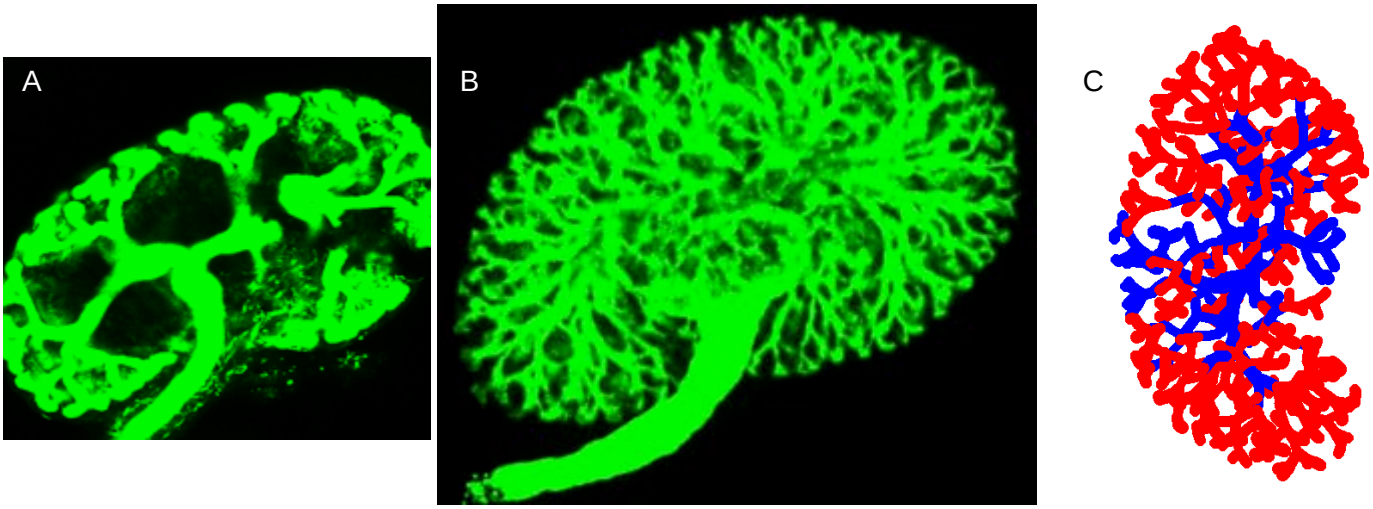

Figure S8. Protein deficient and wild type kidneys at E15.5. (A) A confocal section of protein deficient kidney at E15.5 shows truncated branching orders as compared to wild type (shown in panel B). Figures to scale. (C) Tracing colored by stereotyped (blue) and variable (red) branching patterns shows a deficit in segments that specify kidney circumference (compare to wild type pattern arrangement at E13.5 in Fig. 3C).

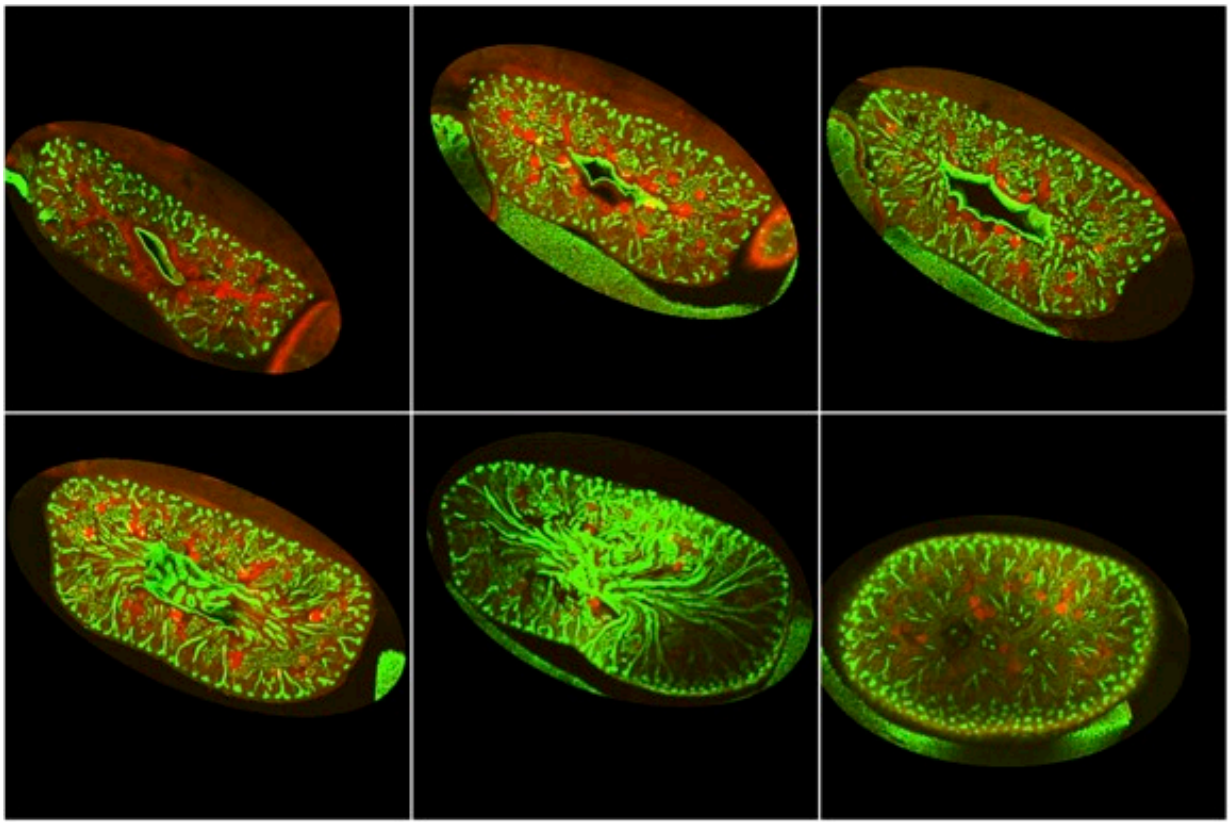

Figure S9. **Series of z-stacks**. Stacks are obtained from 150 micron sections of an E17.5 kidney stained with cytokeratin-8 (green) and podocalyxin (red).

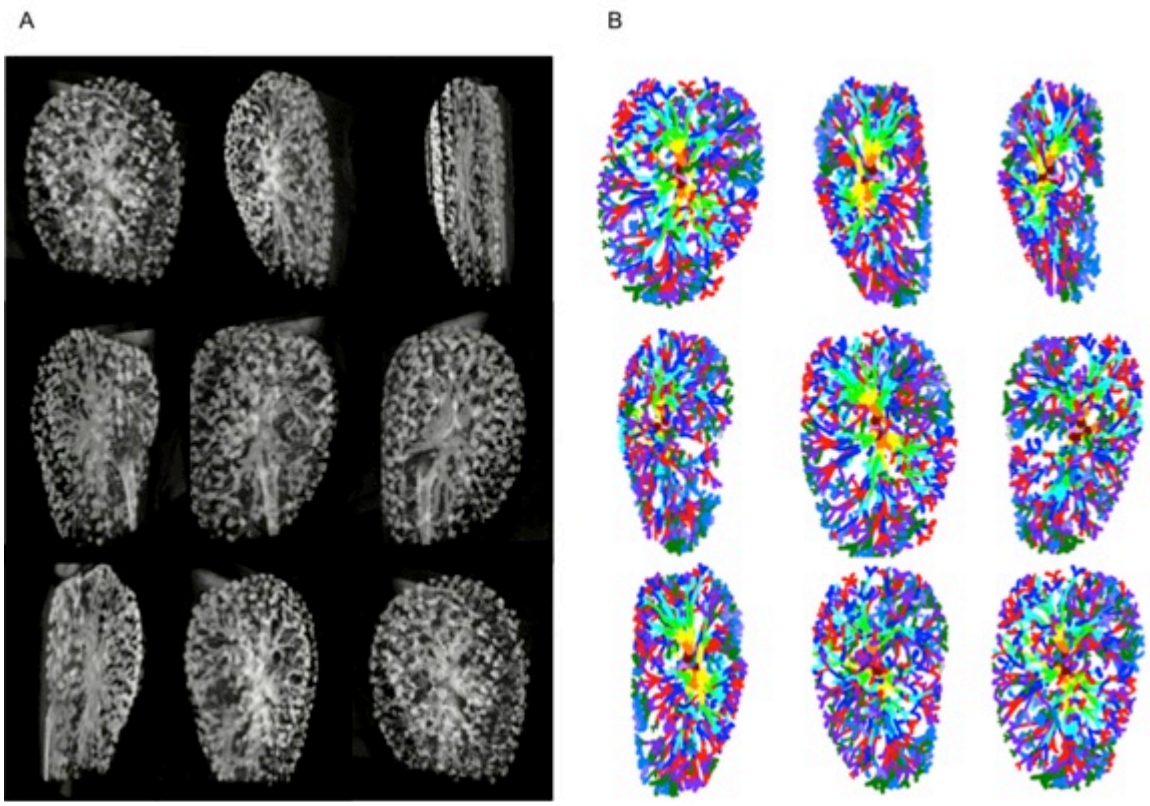

Figure S10. **3-dimensional reconstruction and tracing of wild type E15.5 kidney**. (A) 150 micron thick serial sections were imaged and layered using ImageJ[34] to reconstruct the whole embryonic kidney. Rotated views are represented. (B) Corresponding tracings of branching processes generated by the Neurolucida program.