High-resolution gene expression analysis of the developing mouse kidney defines novel cellular compartments within the nephron progenitor population.

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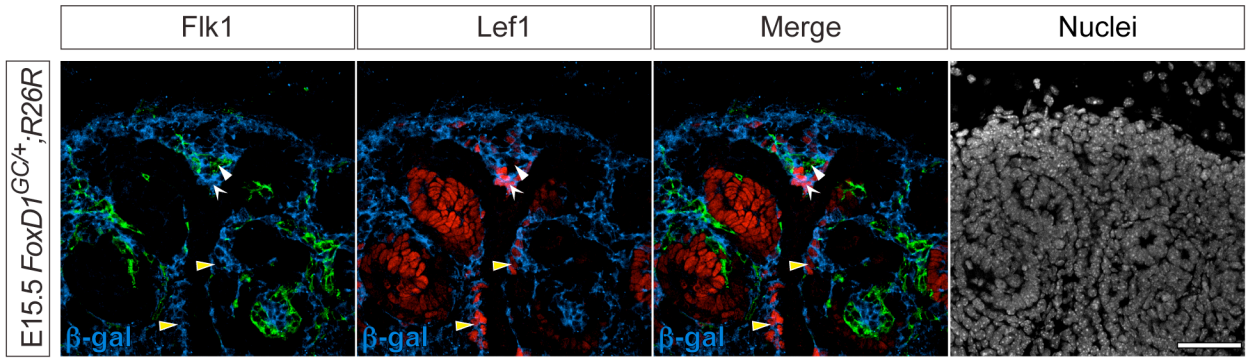
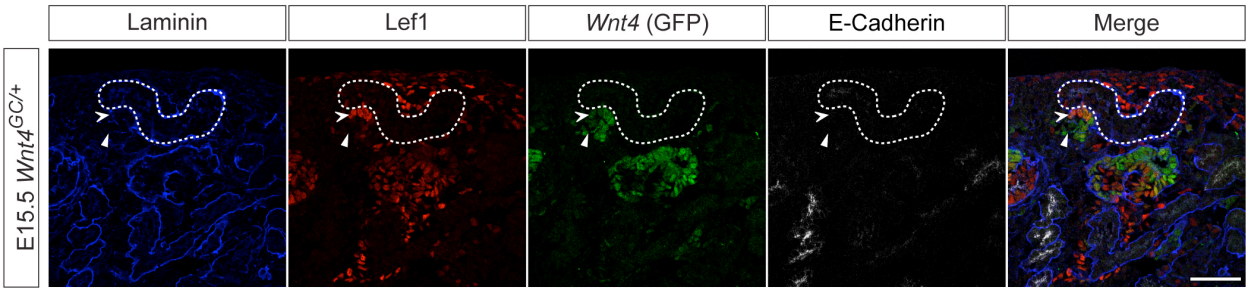
Supplemental Data

Figures



Supplemental Figure 1 – Lef1 is present in non-vascular *Foxd1* descendant interstitial cells. Confocal immunofluorescence of E15.5 *Foxd1*$^{GC/+}$;*R26R* metanephric kidneys immunostained for β-gal (blue), Flk1 (green) and Lef1 (red). Nuclei stained for Hoechst 33258 (white). White arrowheads, yellow arrowheads and concave arrowheads indicate Lef1^{-}, β-gal^{-}, Flk1^{+} vasculature, Lef1^{+}, β-gal^{+}, Flk1^{-} interstitial mesenchyme and Lef1^{+}, β-gal^{+}, Flk1^{-} cells surrounding the ureteric epithelium, respectively. Scale bars = 50 μm.



Supplemental Figure 2 – Wnt4 and Lef1 are differentially distributed in pre-tubular aggregates. Confocal immunofluorescence of E15.5 Wnt4$^{GC/+}$ metanephric kidneys immunostained for E-cadherin (white), Lef1 (red), GFP (green) and Laminin (blue). Dashed line indicates the ureteric epithelium. Arrowheads and concave arrowheads indicate Lef1^{-}, GFP^{+} and Lef1^{+}, GFP^{low} cells, respectively. Scale bars = 50 μm.

Materials and Methods

For *in situ* hybridization, embryos were collected in PBS, fixed in 4% paraformaldehyde in PBS (PFA) at 4 °C overnight, washed thoroughly in PBS and then cryopreserved overnight at 4 °C in 30% sucrose. Tissue was frozen in OCT (Tissue-Tek) on a dry ice/ethanol bath. 20 μm cryosections were collected on Superfrost coated slides (VWR), dried and used immediately or frozen at − 80 °C. Sections were fixed in 4% PFA for 10min, washed 3x in PBS and incubated in 10μg/ml ProteinaseK (Sigma) in PBS for 7 min. Sections were then immediately fixed in 4% PFA, washed in 3x PBS and acetylated (0.1M% Triethanolamine-HCl, 0.25% Acetic Anyhdride in H_2O) for 10min. Sections were then washed 3x in PBS, dehydrated in an ethanol series and dried for 30min. Appropriate DIG-labeled riboprobes were diluted to 500ng/ml in hybridization solution (50% formamide, 5xSSC pH 4.5, 50μg/ml yeast tRNA, 1%SDS, 50μg/ml Heparin) and applied to the sections. Parafilm was placed over the sections and these were incubated at 65˚C overnight in a humidified chamber.

Sections were then washed in pre-warmed 5xSSC, pH 4.5 for 5min at 65˚C for 5min and then for 30min in 50% formamide, 1xSSC, pH4.5 at 65˚C. Slides were incubated in TNE (10mM Tris, pH 7.5, 500mM NaCl, 1mM EDTA) at 37˚C for 10min, then in 5μg/ml RNaseA in TNE for 15min at 37˚C and then twice in TNE (5min each) at 37˚C. Sections were then washed in 2xSSC, pH 4.5 for 20min at 65˚C and then twice in 0.2xSSC, pH4.5 for 20min each wash at 65˚C. Sections were equilibrated into MBST (100mM Maleic acid pH 7.5, 150mM NaCl, 0.1% Tween-20) and then blocked in blocking solution (2% Boehringer Mannheim blocking reagent, 20% heat inactivated sheep serum in MBST) for 1.5hrs. Sections were then incubated in antibody solution (Roche sheep-anti-DIG 1:4000, 2% Boehringer Mannheim blocking reagent, 1% heat

inactivated sheep serum in MBST) overnight at 4˚C. Sections were then washed in 3x MBST and equilibrated into NTMT (100mM NaCl, 100mM Tris pH 9.5, 50mM $MgCl_2$, 2mM Levimasole, 0.1% Tween-20) and then stained with BMPurple (Boehringer Mannheim) until specific signal was detected above background (up to 7 days) depending on the probe. Sections were then washed in PBS pH 4.5, fixed in PFA + 0.25% gluteraldehyde for 1hr., dehydrated into 70% ethanol, dried, mounted in Glycergel (Dako) and imaged.

For X-gal staining, embryos were dissected in PBS, fixed for 1 h on ice in cold 4% PFA, washed thoroughly in PBS and cryopreserved overnight at 4 °C in 30% sucrose. Tissue was frozen in OCT (Tissue-Tek) on a dry ice/ethanol bath. 20 µm cryosections were collected on Superfrost coated slides (VWR), dried and used immediately or frozen at − 20 °C. Slide mounted sections were incubated in PBS for 5 min in PBS, fixed for 10min in 4% PFA and washed thoroughly in PBS. Staining was carried out in staining solution (5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, 0.01% NaDeoxycholate, 0.02% NP-40, 1 mg/ml X-gal) at 37 °C for 6–8 h. Staining was stopped by thorough washing in PBS followed by fixation in 4% paraformaldehyde + 0.02% gluteraldehyde at 4 °C overnight. Samples were then counter-stained with Nuclear Fast Red (Sigma), dehydrated into ethanol, washed twice with xylenes and coverslipped in Permount (Daigger).

For immunofluorescence, embryos were dissected in PBS, fixed for 1 h on ice in cold 4% PFA, washed thoroughly in PBS and cryopreserved overnight at 4 °C in 30% sucrose. Tissue was frozen in OCT (Tissue-Tek) on a dry ice/ethanol bath. 20 µm cryosections were collected on

Superfrost coated slides (VWR), dried and used immediately or frozen at − 20 °C. Slide mounted sections were incubated in PBS for 5 min, blocked for 30 min in 3%BSA, 1% serum in PBS + 0.25% TritonX100 and incubated with combinations of appropriate primary antibodies in block solution overnight at 4 °C. Sections were washed 3 times in PBS + 0.25% TritonX100 (PBTx) and then incubated at room temperature with appropriate conjugated secondary antibodies in block solution for 1.5 h. Sections were washed 3 times in PBTx, rinsed once in PBS, stained with 1 µg/ml Hoechst 33342 (Invitrogen) for 5 min and rinsed once in PBS. Sections were coverslipped in either Vectashield (Vector Labs) or ProLong Gold (Invitrogen) and imaged as described.

For *in situ* hybridization with immunohistochemistry, the protocol for *in situ* hybridization was carried out first, except sections were incubated in cold 80% Acetone/H_2O at -20˚C for 10min instead of ProteinaseK. Though detection of the riboprobe took longer in this case (up to 10 days), we did not see any difference between the Acetone or ProteinaseK methodologies. After detection of the *in situ* probe and final wash in PBS pH 4.5, sections were washed in PBT, treated with 1% H_2O_2 in PBS for 10min, rinsed in PBTx, blocked for 30 min in 3%BSA, 1% serum in PBTx and incubated with anti-Pax2 (1:250) in block solution overnight at 4 °C. Sections were washed 3 times in PBTx and then incubated at room temperature with HRP-conjugated anti-rabbit secondary antibodies (Invitrogen, 1:500) in block solution for 1.5 h. Sections were then washed 3x in PBTx and rinsed in PBS. HRP was detected with 3,3'-Diaminobenzidine. Slides were then dehydrated into ethanol, washed twice with xylenes and coverslipped in Permount (Daigger).