Supplementary Information

Immunohistochemistry

Kidney explant cultures were fixed on filters with 100% methanol for 10 minutes and rinsed with a solution of 0.1% Tween-20 in PBS (PBST) for 10 minutes. Anti-pan-cytokeratin (Sigma, 1:50), anti–laminin A (Sigma, 1:200), anti-E-cadherin (Sigma, 1:500), anti-Cadherin-6 (1:200), and anti-Wt1 (Santa Cruz, 1:200) primary antibodies were diluted in PBST with 2% serum and incubated overnight at 4 °C. The explants were washed with PBST three times for 10 minutes each with another wash with PBST overnight at 4 °C. Explants were then incubated with the secondary antibodies Cy3-conjugated AffiniPure anti-rabbit (Jackson ImmunoResearch, 1:400), Alexa 488 anti-rat (Molecular Probes, 1:200), and Alexa 488 anti-mouse (Molecular Probes, 1:400) diluted in PBST with 2% fetal calf serum for 3 hours at room temperature. The explants were then washed three times for 10 minutes in PBST and imaged with a Zeiss 510 NLO multiphoton microscope. The dimensions of the z-stack projections in Figure 3 are as follows: Figure 3A is 921.4 microns (X) by 921.4 microns (Y) by 44 microns (X), Figure 3B is 921.4 microns (X) by 921.4 microns (Y) by 40 microns (X), Figure 3C is 921.4 microns (X) by 921.4 microns (Y) by 22.8 microns (X), Figure 3D is 921.5 microns (X) by 921.4 microns (Y) by 27.1 microns (Z), Figure 3E is 921.4 microns (X) by 921.4 microns (Y) by 64 microns (X), Figure 3F is 921.4 microns (X) by 921.4 microns (Y) by 40 microns (X), Figure 3G is 921.4 microns (X) by 921.4 microns (Y) by 48 microns (X), and Figure 3H is 921.4 microns (X) by 921.4 microns (Y) by 46 microns (X).

Embryos were fixed with 4% paraformaldehyde, cryopreserved with 30% sucrose, and cryosectioned for immunohistochemical analysis. Anti-Pax2 (Zymed, 1:200), anti-Wt1 (Santa Cruz, 1:200), anti-Cadherin-6 (1:200), anti-Sall1 (a kind gift from Michael Rauchman, 1:2000), anti-E-cadherin (Sigma, 1:500) and anti-Six2 (1:200) antibodies were detected by Alexa 488 anti-rabbit (Molecular Probes, 1:200), Cy3-conjugated AffiniPure anti-rabbit (Jackson ImmunoResearch, 1:200), Alexa 488 anti-rat (Molecular Probes, 1:200), or Cy3-conjugated AffiniPure Fab Fragment Goat anti-rabbit IgG (Jackson ImmunoResearch, 1:200) antibodies or by Biotin-SP-conjugated AffiniPure anti-rabbit (Jackson ImmunoResearch, 1:200) with diaminobenzidine and the Vectastain ABC Kit (Vector Labs). Double labeling of two antibodies in Figure 1D was performed

using diaminobenzidine (anti-Six2) and nickel sulfate-diaminobenzidine (Cadherin-6) substrates.

TUNEL and Proliferation Studies

TUNEL staining was performed with ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Intergen), and nuclei were counterstained with 0.5% methyl green. For proliferation analysis, anti-phosphohistone H3 (Upstate, 1:200) and anti-Pax2 antibodies were added to serial sections of wild-type and *Six2*-null embryos. The number of PH3+ cells present within the MM and UB were quantified and compared.