Supplemental Methods

Existing genetically altered mice

Mice carrying the Col4a3 null mutation (*Col4a3*^tm1Jhm^), the Nphs2-rtTA transgene (Tg(NPHS2-rtTA2*M2)1Jbk, obtained from Dr. Jeffrey Kopp, NIH/NIDDK), the T-HISGFP transgene (Tg(tetO-HIST1H2BJ/GFP)47Efu/J, purchased from The Jackson Laboratory, JAX stock #005104), the T-hLAMA5 transgene, and the Rosa26-rtTA insertion (Gt(Rosa)26Sor^tm1(rtTA,EGFP)Nagy, JAX stock #005670), have been described. The latter, designed for tissue specific rtTA expression, was bred to a Sox2-Cre transgenic mouse (JAX stock #008454) to delete the neo cassette interrupting rtTA expression in the germ line, so the resulting mice expressed rtTA ubiquitously.

Analysis of urine

To estimate the level of urinary albumin, 1 µL of urine was analyzed by SDS-PAGE and Coomassie Brilliant Blue staining with 1 and 5 µg BSA standards.

Antibodies and immunofluorescence

Rabbit anti-mouse COL4A4 NC1 (serum 356), rat IgG2a anti-human COL4A2 NC1 (clone H22, a gift from Y. Sado, Shigei Medical Research Institute, Okayama, Japan), mouse IgG1 anti-human COL4A3 NC1 (clone 8D1, a gift from D-B Borza, Vanderbilt University), and mouse IgG1 anti-bovine collagen α3α4α5(IV) hexamer (clone 26-20, also a gift from D-B Borza) have been previously described; all have been documented to recognize the orthologous mouse proteins and were found by us to stain normal but not Alport mouse kidney basement membranes. Rabbit anti-WT1 was
purchased from Santa Cruz Biotechnology. Immunostaining of 7 µm frozen sections was performed as described,\textsuperscript{10} except that serum 356 and clone H22 required acid urea-glycine denaturation of frozen sections, as described.\textsuperscript{6}

**Light and electron microscopy**

For light microscopy, kidneys were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with PAS or H&E by standard methods. Transmission electron microscopy was performed by standard methods with resources in the Washington University O’Brien Center Renal Disease Models Core. For scanning electron microscopy, acellular kidney samples were prepared as described.\textsuperscript{11} Kidney cortices were cut into 2 mm cubes and incubated with vigorous shaking in three different solutions consecutively: 4 mM EDTA, 3% triton X-100, and 0.05% type I deoxyribonuclease (Sigma, St. Louis, MO) in 1 M sodium chloride with 0.1% sodium azide at 4°C for 24 hrs. Samples were then incubated with 4% sodium deoxycholate for 4 hrs at room temperature and placed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate. Samples were triple rinsed with distilled water between each step. Fixed kidney samples were processed for scanning electron microscopy as previously described.\textsuperscript{12} Images were captured using FEI Nova NanoSEM 2300 in the Washington University Nano Research Facility.
References


