Supporting Information

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SI Materials and Methods

Targeting Vector and Generation of *Magi-2*$^{tm1Key/+}$ Mice. DNA clones corresponding to the *Magi-2* exon 4 locus were isolated from an isogenic 129 bacterial artificial chromosome genomic library. The targeting vector contains the PGKneo cassette inserted into *Magi-2* exon 4 flanked by a 3.7-kb EcoRI-KpnI fragment (5′ arm) and a 5.3-kb HpaI-XhoI fragment (3′ arm) in the backbone of a pBluescript vector with a diphtheria toxin cassette flanking the 3′ arm. The targeted allele inserts a PGKneo cassette in exon 4 with a 1.77-kb intragenic deletion of a 3′ fragment of exon 4 and intronic sequence 3′ to exon 4. Linearized targeting vector was electroporated into 129 LW1 ES cells and selected for G418 resistance. Genomic DNA was prepared for Southern blot analysis and screened with a PCR amplified probe designed outside the targeting region with the following primers: forward, AGACTTGGAGGGTATTCAAGTTTCAAGTAA; and reverse, TTTGCCTTGAATCTTGTTAGAGTTCTAGA. PstI and EcoRV digests of genomic DNA probed on Southern blot confirmed the correct intragenic deletion with PGKneo insertion (7.0-kb and 10.2-kb bands, respectively). ES cell clones heterozygous for the targeted *Magi-2* deletion were injected into C57BL/6 donor blastocysts and implanted into mice. Resulting chimeric male animals were mated to C57BL/6 or 129 female mice and genotyped for germ-line transmission of the targeted allele by Southern blot and PCR (as detailed later). The *Magi-2*^{tm1Key} allele has been submitted to the Mouse Genome Database (www.informatics.jax.org; MGI 5508897).

Mouse Genotyping. Genomic DNA isolated from tail or toe biopsies was used to genotype mice by using a 3-primer PCR strategy (common forward primer, distinct reverse primers) whereby the WT and KO allele products are distinguished by size. The WT allele product is 870 bp, and the KO product is 450 bp. Oligos were ordered from Operon Technologies: forward, GGATATCTGTTACAGACAACTACTACGGTACC; WT reverse, GGGGAGTGTCCTTG CCTAGCTTGCACGAG; KO (Neo) reverse, GGTCTTCTGAGGGGATCCGCTGTAAGTCTG.

Antibodies. Antibodies for MAGI-2 were generated by collecting serum from rabbits immunized with two different GST-tagged epitopes of the human MAGI-2 protein: (*i*) amino acids 1024–1139, the glutamine- and proline-rich region between PDZ domains 4 and 5 (called "Q" here; available purified from Millipore; AB9878), and (*ii*) amino acids 1143–1197, the entirety of PDZ domain 5. The Q antibody is specific to MAGI-2 and does not recognize MAGI-1 or -3, whereas the PDZ5 antibody recognizes all three MAGI proteins. The human and murine sequences of the Q region are identical outside of an 8-aa insertion in the human sequence at amino acid 1031, and the sequences of human and murine PDZ5 are identical.

Western Blot Analysis. Mouse tissue was homogenized by using RIPA lysis buffer containing 50 mM Tris, pH 8, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, and protease and phosphatase inhibitor mixtures (EMD Biosciences). Lysates were sonicated and centrifuged at 16,000 × *g* to remove insoluble material. The lysates were then run on 3–8% Tris·acetate or 10% Bis-Tris NuPAGE gels (Invitrogen), and transferred to PVDF membrane (Immobilon-P; Millipore). After probing with primary antibodies, antigen–antibody complexes were detected with HRP-labeled anti-rabbit, -mouse, -goat, or -guinea pig antibodies, and visualized by enhanced chemiluminescence (GE Healthcare).

Quantitative RT-PCR. Mouse tissue was lysed and homogenized in TRIzol Reagent (Invitrogen), and total RNA isolated by phenol-chloroform extraction. RNA was cleaned up and DNase treated with the RNeasy Mini Kit (Qiagen). cDNA was synthesized using the TaqMan Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed in the Mastercycler Realplex thermal cycler (Eppendorf) using Power SYBR Green PCR master mix (Applied Biosystems), and analysis was performed using the $2^{-\Delta Ct}$ method, normalizing to β-actin. Mouse nephrin, CIN85, and CD2AP primers were ordered from SA Biosciences. The following primer pairs were ordered from Operon (written 5′ to 3′): mouse β-actin (TGTTACCAACTGGGACGACA and GGGGTGTTGAAGGTCTCAAA) and Magi-2 (ACCACAAGGCCACATAAGGA and CCCTCAGCACTTGGAGTAGC).

Histology, Immunohistochemistry, and Transmission EM. After dissection, kidneys for histological analysis were fixed overnight in 4% paraformaldehyde in PBS solution and paraffin-embedded. Sections (4 μm) were stained with periodic acid–Schiff or, after antigen retrieval (pressure cooker or microwave in citrate buffer, pH 6), with antibodies against PAX2 (Invitrogen), Synaptopodin (provided by P.M.), KI67 (Abcam), or CD68 (Abcam), followed by HRP-conjugated secondary antibodies (Dako), and counterstained with hematoxylin. Images were taken with an Olympus BX53 microscope with DP72 camera and processed using Adobe PhotoShop software. Transmission EM of renal tissue was performed as previously described (1). The sections were quantitatively analyzed by a blinded renal pathologist (A.W.), and the results were subjected to statistical analysis (ANOVA and *t* test) using GraphPad Prism software.

Isolation of Glomeruli from Mouse Kidneys. Mice were killed and kidneys were harvested under sterile conditions. The kidneys were kept on ice, in cold PBS solution, decapsulated, and finely minced using sterile razors. The homogenate was then gently pressed through a 100-μm nylon cell strainer, and the membrane washed several times with cold PBS solution. The flow-through was then poured over a 40-μm nylon cell strainer and washed several times with cold PBS solution. The fraction that remained on top of the cell strainer was collected, and an aliquot was examined under the microscope to ensure that it contained primarily glomeruli. The glomeruli were then spun at 500 × *g* for 5 min, and the concentrated glomeruli were then lysed for subsequent protein or RNA extraction.

1. Barisoni L, Kriz W, Mundel P, D'Agati V (1999) The dysregulated podocyte phenotype: A novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 10(1):51–61.

Fig. S1. *Magi-2* transcript expression in the brain. Real-time quantitative-PCR was used to analyze *Magi-2* transcript expression in the brains of WT *Magi-2*$^{+/+}$, *Magi-2*$^{\Delta/+}$, and *Magi-2*$^{\Delta/\Delta}$ mice (n = 6 of each genotype). Transcript levels were normalized to a single mouse in the WT group.

Fig. S2. Homogenous and early-onset proteinuria in 129 background *Magi-2*$^{\Delta/\Delta}$ mice. SDS/PAGE analysis of urine from WT ($^{+/+}$) and *Magi-2*$^{\Delta/\Delta}$ ($^{\Delta/\Delta}$) littermates from the 129 background at 10 d, 2 wk, or 3–4 wk of age. BSA (5 μg) was loaded as a control.

Fig. S3. Elevated serum blood urea nitrogen (BUN) and creatinine in proteinuric *Magi-2*$^{\Delta/\Delta}$ mice. (*A*) Serum BUN and (*B*) creatinine levels were measured in *Magi-2*$^{+/+}$, *Magi-2*$^{\Delta/+}$, and *Magi-2*$^{\Delta/\Delta}$ mice in the B6/129 background (n = 14 for each genotype). Only four proteinuric *Magi-2*$^{\Delta/\Delta}$ mice displayed elevated levels of BUN (normal range, 8–33 mg/dL) and creatinine (normal range, 0.2–0.9 mg/dL).

Fig. S4. Impaired survival and low-grade proteinuria in B6/129 background *Magi-2*$^{\Delta/\Delta}$ mice (*A*). Kaplan–Meier survival curve showing significant survival impairment of *Magi-2*$^{\Delta/\Delta}$ mice (n = 41) in the B6/129 background, with no significant difference observed in WT ($^{+/+}$; n = 53) and heterozygous ($^{\Delta/+}$; n = 117) littermates. The median survival for the KO mice was 169 d, but was not reached for WT or heterozygous mice (*$P < 0.001$, log-rank test). (*B*) SDS/PAGE analysis of urine from WT ($^{+/+}$) and *Magi-2*$^{\Delta/\Delta}$ ($^{\Delta/\Delta}$) littermates from the B6/129 background at 3 mo of age. BSA (5 μg) was loaded as a control.

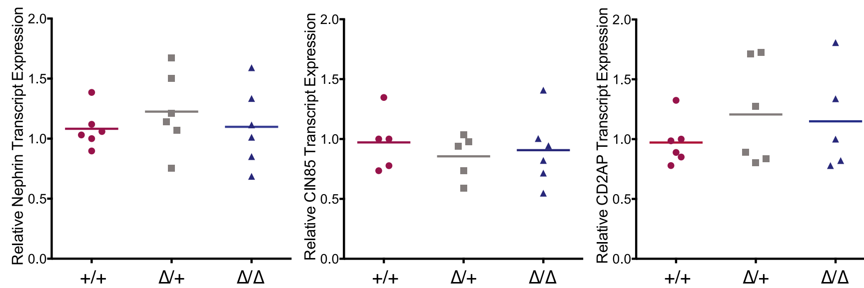

Fig. S5. Transcript expression of *Nephrin*, *CIN85*, and *CD2AP* in glomeruli. Real-time quantitative PCR was used to assess transcript levels of *Magi-2* in glomeruli isolated from littermates of each genotype: WT (*Magi-2*$^{+/+}$), heterozygous (*Magi-2*$^{\Delta/+}$), and KO (*Magi-2*$^{\Delta/\Delta}$); ($n = 5$–6 of each genotype). Transcript levels were normalized to a single mouse in the WT group.

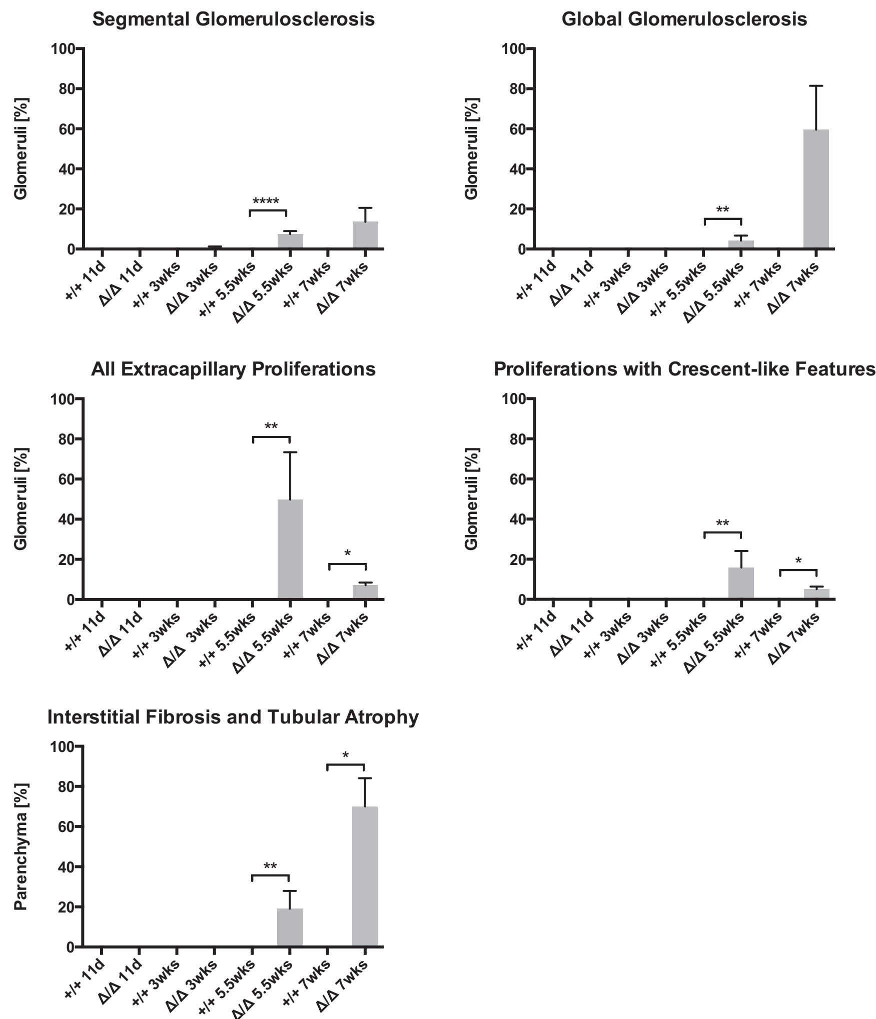

Fig. S6. Quantitative analysis of histologic abnormalities. The kidneys of WT ($^{+/+}$) mice showed no morphologic abnormalities in glomeruli, tubulointerstitium, or vasculature at any age (11 d, $n = 3$; 3 wk, $n = 3$; 5.5 wk, $n = 5$; 7 wk, $n = 2$). *Magi-2*$^{\Delta/\Delta}$ kidneys displayed no light microscopic changes by 11 d of age ($n = 4$). By 3 wk of age, only mild increase in extracellular matrix accumulation in the mesangium was seen, whereas the tubulointerstitium appeared normal (not quantified). By 5.5 wk, the majority of glomeruli showed substantial extracapillary epithelial cell proliferations (*Magi-2*$^{\Delta/\Delta}$: 49.78 ± 10.54%, $n = 5$, vs. *Magi-2*$^{+/+}$: none, $n = 5$; $^{**}P = 0.0015$), sometimes resembling cellular or fibrocellular crescents (*Magi-2*$^{\Delta/\Delta}$: 15.86 ± 3.69%, $n = 5$, vs. *Magi-2*$^{+/+}$: none, $n = 5$; $^{**}P = 0.0026$), notably in the absence of glomerular inflammation or hypercellularity. Few glomeruli were globally (*Magi-2*$^{\Delta/\Delta}$: 4.2 ± 1.12%; $n = 5$, vs. *Magi-2*$^{+/+}$: none, $n = 5$; $^{**}P = 0.0056$) or segmentally hyalinized (*Magi-2*$^{\Delta/\Delta}$: 7.44 ± 0.69%; $n = 5$, vs. *Magi-2*$^{+/+}$: none, $n = 5$; $^{****}P < 0.0001$), and the tubulointerstitium showed mild tubular atrophy and interstitial fibrosis (*Magi-2*$^{\Delta/\Delta}$: 19 ± 4%; $n = 5$, vs. *Magi-2*$^{+/+}$: none, $n = 5$; $^{**}P = 0.0014$). By 7 wk, the kidneys of *Magi-2*$^{\Delta/\Delta}$ mice showed widespread chronic changes of the tubulointerstitium (*Magi-2*$^{\Delta/\Delta}$: 70 ± 10%; $n = 2$, vs. *Magi-2*$^{+/+}$: none, $n = 2$; $^{*}P = 0.0198$), and the majority of glomeruli were globally hyalinized or sclerosed (*Magi-2*$^{\Delta/\Delta}$: 59.5 ± 15.5%; $n = 2$, vs. *Magi-2*$^{+/+}$: none, $n = 2$; $P = 0.0617$) with focal residual fibrous or fibrocellular extracapillary lesions (*Magi-2*$^{\Delta/\Delta}$: 7.00 ± 1.00; $n = 2$, vs. *Magi-2*$^{+/+}$: none, $n = 2$; $^{*}P = 0.0198$).

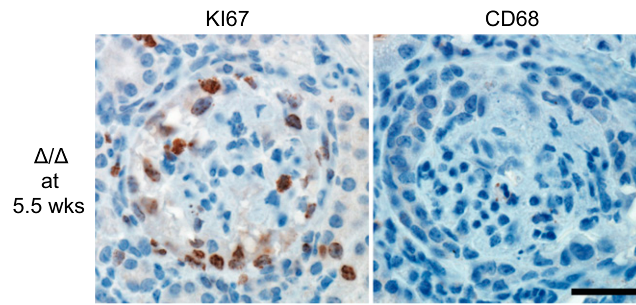

Fig. S7. KI67 and CD68 expression in glomeruli of *Magi-2*$^{\Delta/\Delta}$ mice at 5.5 wk of age. The proliferative epithelial cells seen in glomeruli of *Magi-2*$^{\Delta/\Delta}$ mice show positive staining for KI67, indicating cell cycle activation. A marker for macrophages, CD68, is negative in the cellular crescents. (Scale bars: 20 μm.)