SUPPLEMENTAL MATERIAL

Detailed Methods

Animals

Srf $^{fl/fl}$, *Mkl1* $^{-/-}$, *Mkl2* $^{fl/fl}$, *NPHS2-Cre* mice were characterized previously.[1-4] Homozygous floxed *Srf* (*Srf* $^{fl/fl}$) and *NPHS2-Cre* $^{+/-}$ mice were used to generate *Srf* $^{fl/fl}$ and *Srf* $^{fl/+}$/*NPHS2-Cre* controls and mice of *Srf* knockout genotype *Srf* $^{fl/fl}$/*NPHS2-Cre*. *Mkl1* $^{-/-}$, *Mkl2* $^{fl/fl}$, *NPHS2-Cre* mice were utilized to generate *Mkl1* single knockout (*Mkl1* $^{-/-}$, *Mkl1* $^{-/-}$/*Mkl2* $^{fl/+}$, *Mkl1* $^{-/-}$/*Mkl2* $^{fl/fl}$, or *Mkl1* $^{-/-}$/*NPHS2-Cre*), *Mkl2* single knockout (*Mkl2* $^{fl/fl}$/*NPHS2-Cre*), *Mkl1/Mkl2* double knockout (*Mkl1* $^{-/-}$/*Mkl2* $^{fl/fl}$/*NPHS2-Cre*) and 3-allele-knockout (*Mkl1* $^{+/-}$/*Mkl2* $^{fl/fl}$/*NPHS2-Cre* and *Mkl1* $^{-/-}$/*Mkl2* $^{fl/+}$/*NPHS2-Cre*). Genotyping PCR primers are listed in Supplemental Table 1. Animals were treated in accordance with NIH animal care guidelines and all animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Rochester School of Medicine and Dentistry. Sections of de-identified human kidney biopsies were obtained with approval from University of Rochester School of Medicine and Dentistry Research Subjects Review Board (RSRB00062813).

Urine, serum and body weight analysis

Urine from control and *Srf* KO mice at three, four, and six weeks was collected between 6:00AM and 8:00AM on each collecting day. Urine dipstick assay was

performed using Iris™ Diagnostics vChem™ Urine Chemistry Strips (Fisher Scientific, Hampton, NH). The protein level and number of white blood cells and red blood cells were analyzed to evaluate proteinuria, leukocyturia and hematuria. In order to semi-quantify the severity of these parameters, different indices were assigned to each corresponding range of readings in accordance with the manufacturer. To quantify protein levels in urine, urine samples were centrifuged at 500 × g for 5 minutes to remove cells and debris, and then a small volume (2 or 0.5 μL) of urine, or specified amount of bovine serum albumin standard, were denatured with sampling buffer and resolved by SDS-PAGE gel. The gel was then stained with SimplyBlue™ SafeStain (Invitrogen, Waltham, MA) according the manufacturer's instructions. The stained gel was documented with Gel Doc XR+ System (Bio-Rad, Hercules, CA). Blood from six-week control and *Srf* KO mice was harvested via heart puncture and immediately treated with anticoagulant. Samples were submitted to cobas® 6000 analyzer (Roche, provided by URMC Labs), to measure serum levels of blood urea nitrogen (BUN), creatinine, and albumin. Body weights of control and *Srf* KO mice were measured at three, four, and six weeks. All body weight data were collected from age-matched male mice.

Histopathology and immunofluorescence microscopy

Kidney tissues from mice of different genotypes were fixed and processed for paraffin sectioning. Tissues were sectioned at five μm thickness and slides were deparaffinized and rehydrated in PBS. Hematoxylin and Eosin (H&E) staining and Masson's trichrome staining (American MasterTech Lodi, CA) were conducted

according to standard procedures. For immunostaining, slides were immersed in Target Retrieval Solutions (Dako, Santa Clara, CA) and heated in a pressure cooker for 5-20 min to restore masked epitopes, and then blocked for 30 min with Protein block (Dako). Single or Mixed (double staining) primary antibodies were incubated at 4˚C overnight, washed in PBS three times for five minutes each, and incubated with appropriate fluorescently-labeled secondary antibodies for 30 min at room temperature. Slides were then washed in PBS and mounted with Prolong Gold Antifade reagent. Images were taken on a confocal microscope (Olympus FV 1000, Japan) with fixed parameters and stacked with 8-12 × 0.5 μm images. The primary antibodies and other detailed information for immunostaining are summarized in Supplemental Table 2. Quantitation of alterations in H&E staining and WT1+/SRF+ double positive cells in glomeruli were conducted by two independent observers blind to genotype.

Electron microscopy

For scanning electron microscopy, the kidneys were bisected in half and fixed in 0.1M sodium cacodylate buffered 2.5% glutaraldehyde/4.0% paraformaldehyde fixative overnight at 4 ˚C, rinsed in two changes of buffer (15 min each), and trimmed to the cortical region containing the majority of glomeruli. Tissues were post-fixed 75 min in buffered 1.0% osmium tetroxide, rinsed in buffer then dehydrated at 60 min intervals in a graded series (50 to 100%, × 3 times) of ethanol, and the specimens were critical point dried. The kidney was fractured sagittally to reveal the glomeruli within the cortex on carbon sticky tape and aluminum stubs. The mounted tissue was sputter-coated with gold in a Denton vacuum evaporator for 90 seconds. A Zeiss Auriga Supra Field

Emission scanning microscope was used to photograph the glomeruli. For transmission electron microscopy, the kidneys were fixed and processed as above except the trimmed cortical tissue was cut into smaller pieces for a dehydration series ending in 100% ethanol (x 3 times), transitioned into propylene oxide (PO) at 60 minute intervals (1:1, 1:2, 100% PO, twice), PO/Epon/Araldite at 1:1, 1:2, 100% resin overnight, embedded in fresh resin into molds, and polymerized 48 hours at 65^{o}C. One micron semi-thin sections were stained with Toluidine blue to identify regions rich in glomeruli for thin sectioning (70 nm) using an ultramicrotome and diamond knife. The thin sections were placed onto nickel formvar/carbon slot grids, stained with aqueous uranyl acetate and lead citrate, and photographed using a Hitachi 7650 microscope with an attached Gatan Erlangshen 11-megapixel digital camera.

Quantitative real-time PCR

Total RNA was extracted from the aorta and kidney cortex of normal adult mice using the RNAeasy kit (Qiagen, Hiden, Germany), treated with DNAase I (Invitrogen), and reverse transcribed into cDNA with iScript™ Reverse Transcription Supermix (Bio-Rad). SYBR green supermix (Bio-Rad) was used for Quantitative PCR to detect cycle numbers. Relative mRNA levels to *Gapdh* or *Rhoa* were normalized to the control sample using $2^{-\Delta\Delta Ct}$ method. Real-time primer sequences are provided in Supplemental Table 3.

Cell culture, Srf knockdown and phalloidin staining

A conditionally-immortalized murine podocyte cell line (MPC) was kindly provided by the lab of Dr. Mundel. Undifferentiated cells were passaged and maintained at 33 ℃ in RPMI 1640 media containing 10% FBS and interferon-γ (Sigma-Aldrich, St. Louis, MO; cat. no. I4777) as described.[5] Podocyte differentiation occurs under interferon-γ withdrawal and a temperature shift to 37 ℃ for 12-14 days. 800,000 differentiated podocytes were plated in 6-well plates for 12 hours before transfection. For each well, cells were transfected in 2.75 mL complete media containing 125 pmol siRNA, siNC or siRNA (Thermo Fisher Scientific, Waltham, MA; cat. no. 4390771, ID s74391), and 7.5 μL TransIT-X2 (Mirus Bio, Madison, WI; cat. no. MIR6003) according to the manufacturer's instructions. After incubation for 48 hours, transfected podocytes were harvested for total RNA or plated for phalloidin staining.

Alexa Fuor 488 phalloidin (Invitrogen, cat. no. A12379) was used to stain the podocyte actin-cytoskeleton according to the manufacturer instruction. Briefly, podocytes were plated onto collagen-I (Gibco, Gaithersburg, MD; cat. no. A1048301) coated coverslip after differentiation and transfection. Cells were fixed in 3.7% formaldehyde for 10 min, permeabilized in 0.1% Triton X-100 for 3 min, stained with 1:40 diluted fluorescent phalloidin for 20 min, and mounted with ProLong Gold with DAPI, successively.

Western blot

Podocytes were lysed with Cell lysis buffer (Cell Signaling Technology, Danvers, MA; cat. no. 9803) supplemented with Proteinase Inhibitor cocktail tablet (Roche

Applied Sciences, Indianapolis, IN). Cell extracts were centrifuged at 1,200 × g for 15 min in the 4 ˚C centrifuge to remove insoluble fraction. 25 µg of total protein was loaded onto 15% SDS-PAGE gel for separation and transferred to PVDF membrane at 350 mA for 2.5 hours. The membrane was blotted with 5% milk in 1xTBST and incubated with primary antibody diluted in 1xTBST containing 5% BSA at 4 ˚C overnight and secondary antibody at room temperature for 1h. Membranes were then washed with 1xTBST 3 times for 10 min. Secondary antibodies labeled with HRP were detected with West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, cat. no. 32209). The following antibodies were used: Rabbit Anti-SRF 1:500 (Santa Cruz Biotechnology, Dallas, TX; cat. no. sc-335), Mouse Anti-GAPDH 1:10000 (Cell Signaling Technology, cat. no. 2118s).

Statistics

Data are shown as means ± standard deviation (SD). Student's *t* test was used in comparing continuous data between two groups. We used one-way analysis of variance for comparison among multiple groups with Tukey's multiple comparisons test for post-hoc testing. Mann-Whitney *U* test was utilized to compare dipstick indices of mutant groups to those of control groups at each time point considering dipstick data were discrete. A Chi-squared test was employed to compare observed offspring numbers of each genotype with expected numbers according to Mendelian genetics. A *p* value less than 0.05 was considered as statistical significance. Statistical analyses were performed with GraphPad Prism and Microsoft Excel.

Supplemental Table 1. List of genotyping primers.

Targets	PCR Primer sequences
Floxed *Srf*	Fwd: 5'-tgcttactggaaagctcatgg-3' Rev: 5'-tgctggtttggcatcaac t-3'
Mkl1 knockout	WT Fwd: 5'-ggcttcagtaccttcctaagctctgcag-3' Mutant Fwd: 5'-catggtggatcctgagactggcgaattc-3' Universal Rev: 5'-gttgctcagtcatgtgacacctgtacag-3'
Floxed *Mkl2*	Fwd: 5'-catggcgacttccttctcctcttctcaaggctg-3' Rev: 5'-ggcttagacaagatggttggtctggcactgc-3'
mTmG	Fwd: 5'- aacctcttcgagggacctaata-3' Rev: 5'- tctttgatgacggccatgt-3'
NPHS2-Cre	Fwd: 5'-gggacaaagtgtctcttcctaaa-3' Rev: 5'-atctcctggagggttcctaat-3'

Supplemental Table 2. Detailed parameters for immunostaining.

Staining	Primary antibodies	Secondary antibodies	Retrieval
α-actinin-4	Rabbit Anti-α-actinin-4 1:50 (Invitrogen, cat. no. 42-1400)	Anti-Rabbit Alexa fluor 594 1:200 (Life Technologies, cat. no. A11012)	High pH [a] 5 min
Synaptopodin Podocin	Mouse Anti-Synaptopodin 1:50 (American Research Products, cat. no. 03-61094) Rabbit Anti-Podocin 1:500 (Sigma-Aldrich, cat. no. P 0372)	Anti-Mouse Alexa fluor 488 1:200 (Life Technologies, cat. no. A11001) Anti-Rabbit Alexa fluor 594 1:200 (Life Technologies, cat. no. A11012)	Low pH [b] 7 min
Nephrin	Goat Anti-Nephrin 1:50 (R&D Systems, cat. no. AF3159)	Anti-Goat Alexa fluor 488 1:200 (Life Technologies, cat. no. A11055)	Low pH 7min
Ki-67	Mouse Anti-Ki-67 1:100 (BioLegend cat. no. 652402)	Anti-Mouse AlexFluo 488 1:200 (Life Technologies, cat. no. A11001)	Low pH 10min
CD45	Rat Anti-CD45 1:50 (BD pharmingen, cat. no. 550539)	Anti-Rat Alexa fluor 546 1:200 (Life Technologies, cat. no. A11081)	Low pH 5 min
SMA SRF	Mouse smooth muscle α-actin 1:200 (Sigma-Aldrich, cat. no. C6198) Rabbit Ant-SRF 1:500 (Santa Cruz, cat. no. sc-335)	Cy3 Labeled; no secondary Anti-Rabbit Alexa fluor 488 1:200 (Life Technologies, cat. no. A11008)	High pH 5 min
VWF WT1	Goat Anti-vWF 1:50 (Fitzgerald, cat. no. 20R-VG001) Mouse Anti-WT1 1:50 (Dako, cat. no. M3561)	Anti-Goat Alex fluor 594 1:200 (Life Technologies, cat. no. A11058) Anti-Mouse Alex fluor 488 1:200 (Life Technologies, cat. no. A11001)	High pH 5 min
Lectin	Biotinylated *Lotus tetragonolobus* lectin 1:200 (Vector Laboratories, cat. no. B-1325)	Texas Red avidin DCS 1:200 (Vector Laboratories, cat. no. A-2016)	High pH 4 min
SRF WT1	Rabbit Ant-SRF 1:500 (Santa Cruz, cat. no. sc-335) Mouse Anti-WT1 1:50 (Dako, cat. no. M3561)	Anti-Rabbit AlexFluo 594 1:200 (Life Technologies, cat. no. A11012) Anti-Mouse AlexFluo 488 1:200 (Life Technologies, cat. no. A11001)	High pH 5 min

[a] Target Retrieval Solution High pH (Dako, cat. no. K8004)
[b] Target Retrieval Solution Low pH (Dako, cat. no. GV805)

Supplemental Table 3. **List of qPCR primers**.

Targets	qPCR primer sequences
Acta2	Fwd: 5'-gaagagcatccgacactgct-3'
	Rev: 5'-agagtccagcacaataccagtt-3'
Aif1l	Fwd: 5'-aacaggttccaaggagggaa-3'
	Rev: 5'-ttccggcaggttctcttcatc-3'
Arpc1a	Fwd: 5'-gatcgtacccagatcgccct-3'
	Rev: 5'-tctgccccacaagtgacaat-3'
Fgrf1	Fwd: 5'-ggcagcgataccacctactt-3'
	Rev: 5'-gctacaggcctacggtttgg-3'
Gapdh	Fwd: 5'-catggccttccgtgttcctac -3'
	Rev: 5'-cttcaccaccttcttgatgtcatc-3'
Golim4	Fwd: 5'-caagacgtcaagcaacagcat-3'
	Rev: 5'-gctcgactgggtcaggattt-3'
Gusb	Fwd: 5'-catcagaagccgattatccagag-3'
	Rev: 5'-tgtttccgattactctcagcg-3'
Mkl1	Fwd: 5'-tcccgtgctccctacaa-3'
	Rev: 5'-cggtgtttgtcgtttggattc-3'
Mkl2	Fwd: 5'-agccaacctggatgacatgaa-3'
	Rev: 5'-cgcaggcgttctatcagctc-3'
Myocd	Fwd: 5'-aaggtccattccaactgctc-3'
	Rev: 5'-ccatctctactgctgtcatcc-3'
RhoA	Fwd: 5'-agcttgtggtaagacatgcttg-3'
	Rev: 5'-gtgtcccataaagccaactctac-3'
Sept10	Fwd: 5'-tatgggcccagagaacaagc-3'
	Rev: 5'-ttggcctgaagctctctttcc-3'
Srf	Fwd: 5'-ccaccacagaccagagaatgag-3'
	Rev: 5'-tcttgagcacagtcccgttg-3'
Synpo	Fwd: 5'-gtacggtcaaggtgggattt-3'
	Rev: 5'-cagctgggctgcaatct-3'
Tsc22d1	Fwd: 5'-tctcttcccgttgaaggtgc-3'
	Rev: 5'-tcaccagatccatagcttgctc-3'
Wt1	Fwd: 5'-ttaaagggaatggctgctggg-3'
	Rev: 5'-tactgggcaccacagaggat-3'

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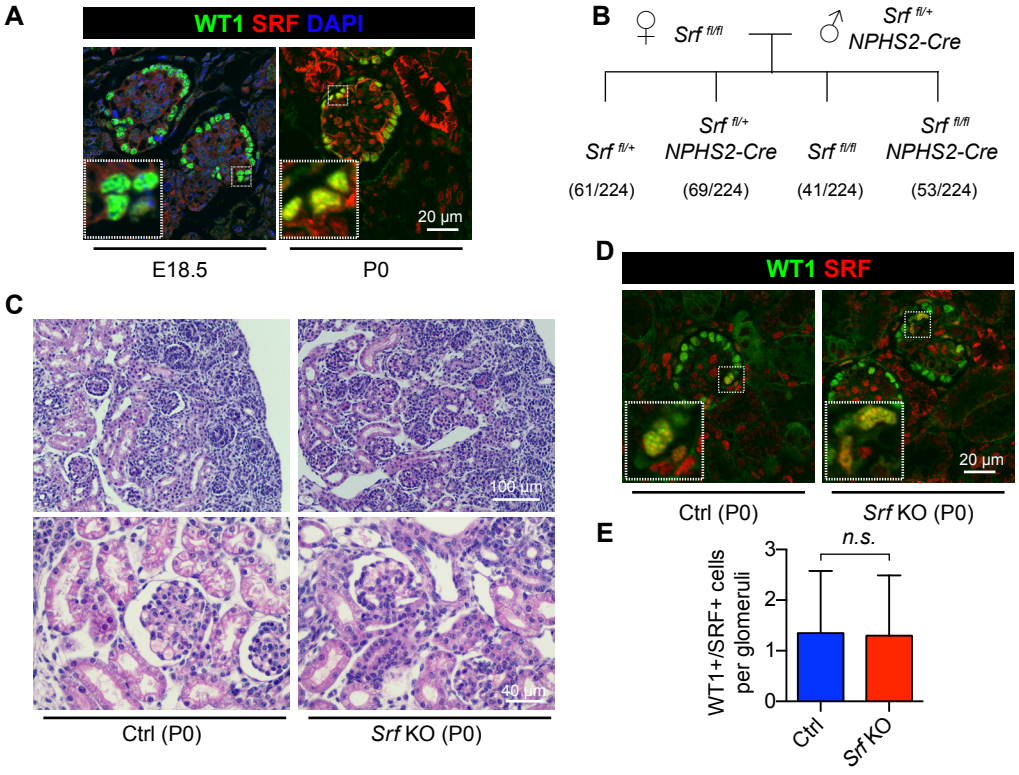
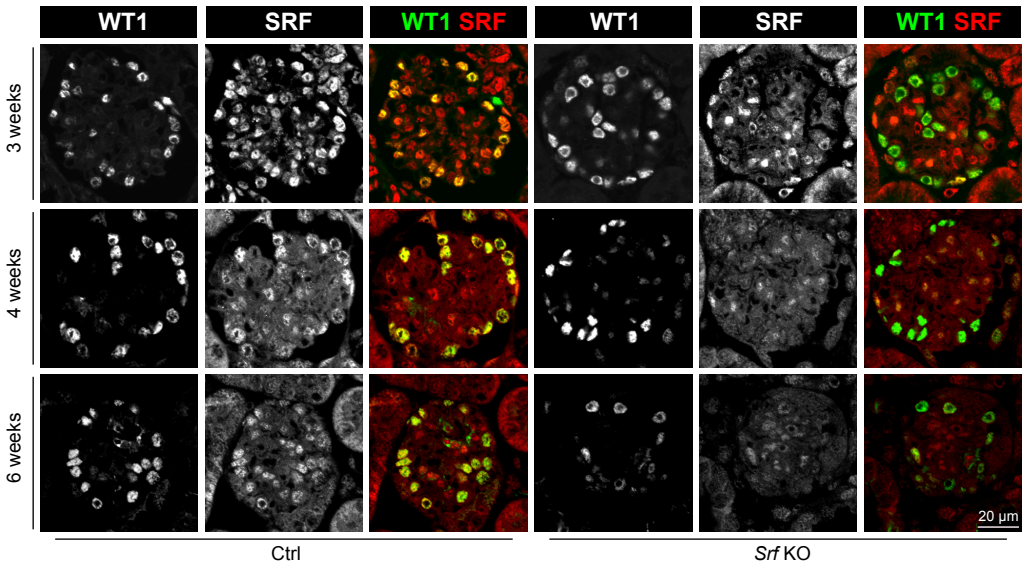
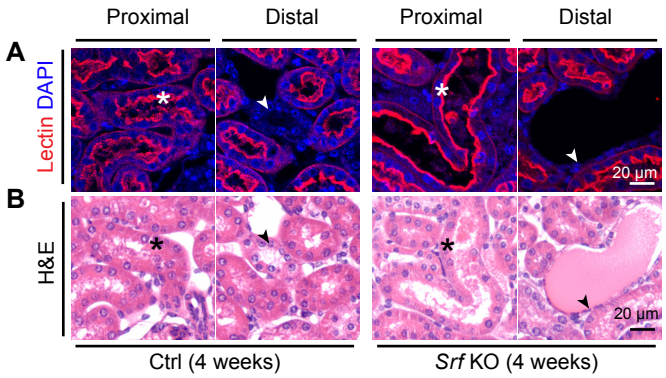
3. Li, S, Chang, S, Qi, X, Richardson, JA, Olson, EN: Requirement of a myocardin-related transcription factor for development of mammary myoepithelial cells. *Mol Cell Biol,* 26**:** 5797-5808, 2006
4. Mokalled, MH, Johnson, A, Kim, Y, Oh, J, Olson, EN: Myocardin-related transcription factors regulate the Cdk5/Pctaire1 kinase cascade to control neurite outgrowth, neuronal migration and brain development. *Development,* 137**:** 2365-2374, 2010
5. Mundel, P, Reiser, J, Zuniga Mejia Borja, A, Pavenstadt, H, Davidson, GR, Kriz, W, Zeller, R: Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. *Exp Cell Res,* 236**:** 248-258, 1997



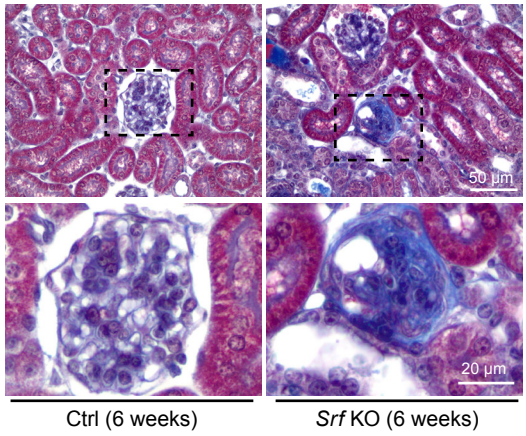
Supplemental Figure 1. **Embryonic and neonatal expression of SRF in podocytes.** (A) Immunofluorescence microscopy shows undetectable SRF protein in podocytes (WT1+) at embryonic day 18.5 (E18.5) and weak staining at postnatal day 0 (P0). (B) Expected Mendelian ratio of genotypes from *Srf* $^{fl/fl}$ female x *Srf* $^{fl/+}$ /*NPHS2-Cre* male mice cross (p = 0.46675, Chi-squared test). (C) H&E staining of control and *Srf* KO mice at P0. Representative immuno-fluorescence images (D) and quantitation (E) demonstrating indistinguishable WT1 and SRF co-localization in newborn mice (n ≥ 10 fields). Scale bars, 100 μm (C top panel), 40 μm (C bottom panel), 20 μm (A, D). *n.s.*, not statistically significant.



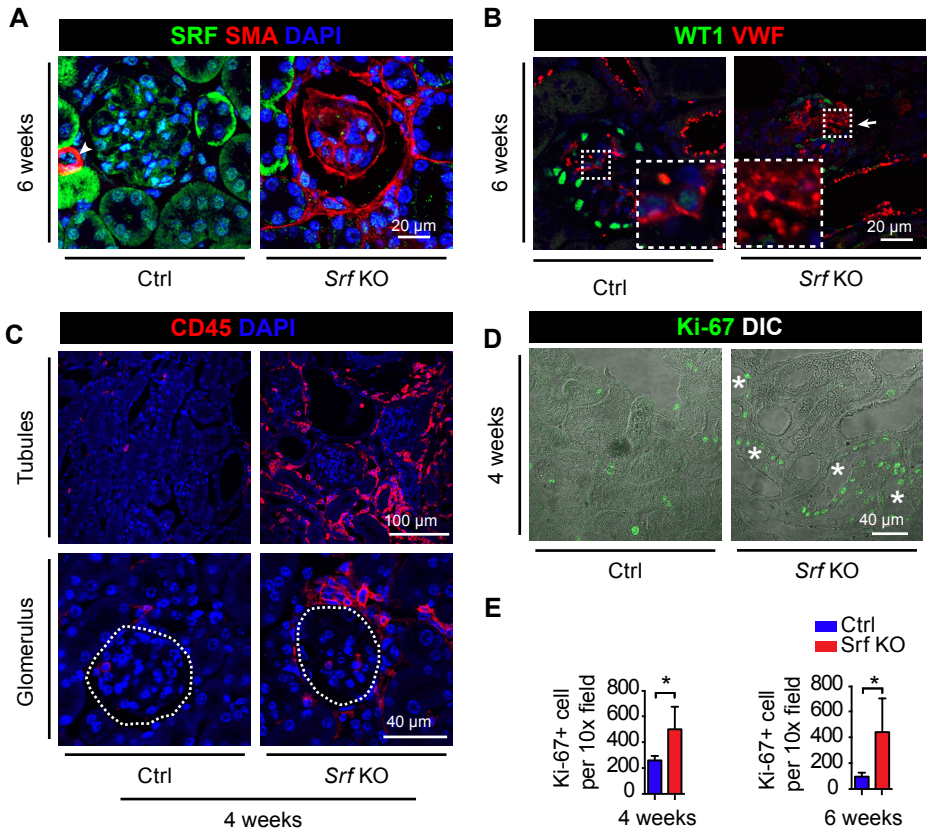
Supplemental Figure 2. Postnatal expression of SRF and WT1 in podocytes. Single channel and merged images of Figure 1C. Scale bar, 20 µm.



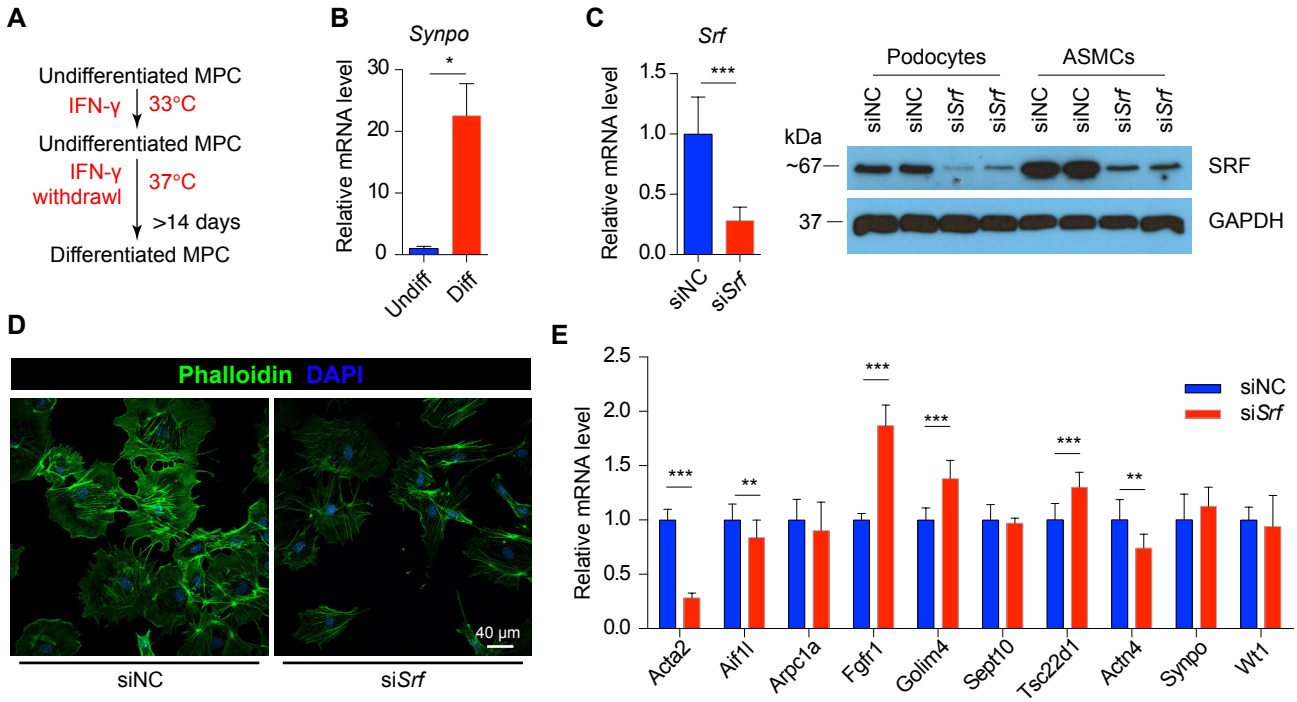
Supplemental Figure 3. Proximal and distal tubular dilation in *Srf* KO mice. Adjacent sections from *Srf* KO and control mice stained with *Lotus tetragonolobus* lectin (A) or H&E (B) at four weeks of age. Asterisks and arrowheads indicate proximal tubules (lectin positive) and distal tubules (lectin negative), respectively. Scale bars, 20 μm.



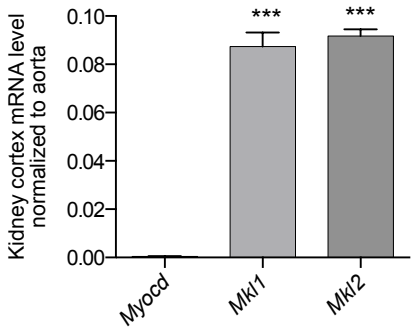
Supplemental Figure 4. Glomerulosclerosis in podocyte-specific *Srf* KO mice. Trichrome staining of control (left) and *Srf* KO (right) mice shows little evidence of interstitial fibrosis at 6 weeks of age. Only occasional instances of glomerulosclerosis were evident as indicated by the dark blue staining within the glomerulus of a mutant mouse (bottom right). Scale bars, 50 µm (top), 20 µm (bottom).



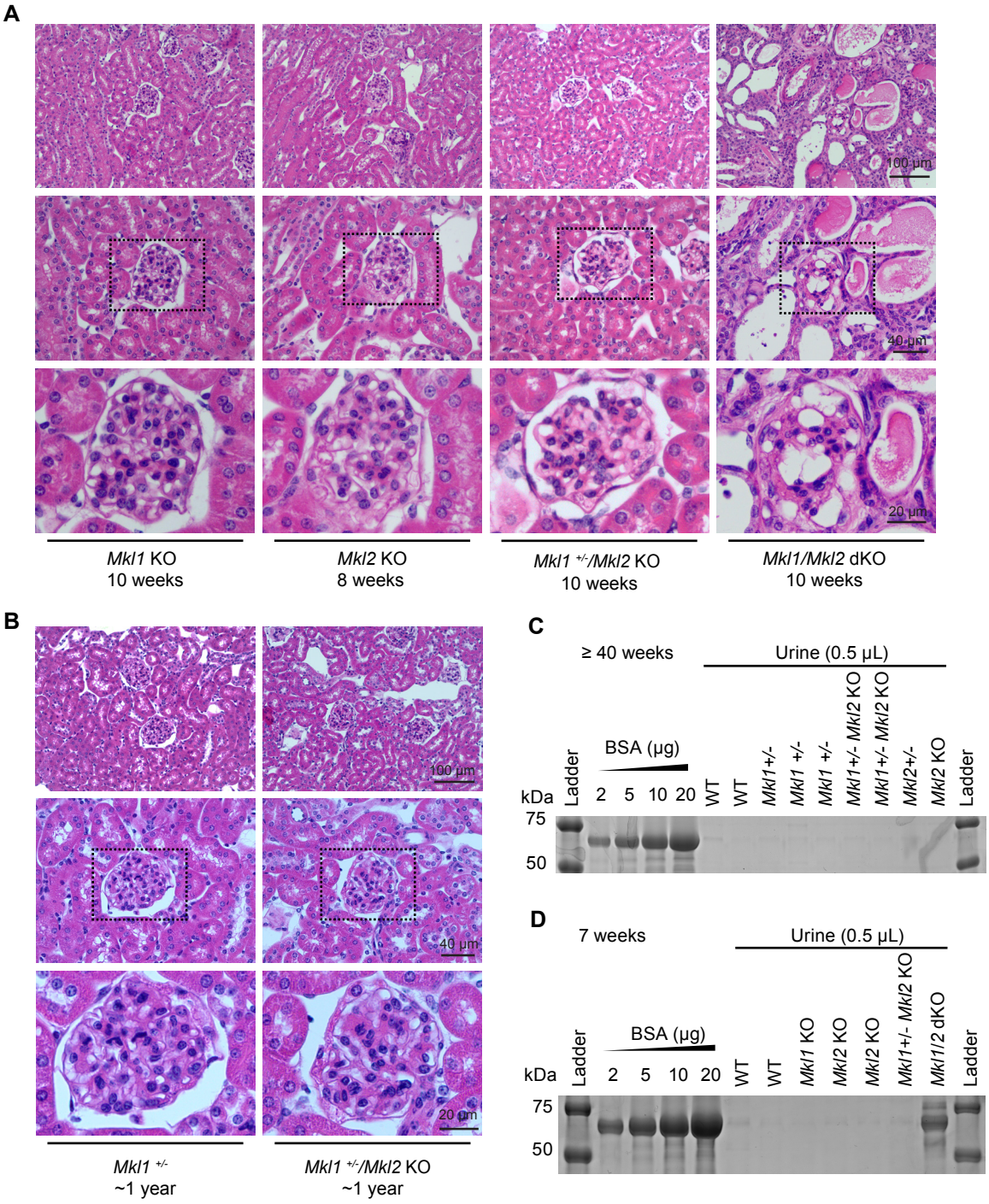
Supplemental Figure 5. Reactive changes in glomeruli and tubulointerstitium. (A-B) Immunofluorescence microscopy showing increases in the mesangial marker, smooth muscle actin (SMA, A), and endothelial cell marker, von Willebrand factor (VWF, B), in glomeruli of six-week-old *Srf* KO mice. The arrowhead indicates a small blood vessel; the arrow indicates elevated VWF staining. (C-D) Increased staining of CD45 (C) and Ki-67 (D) in tubulointerstitium and tubular epithelium, respectively, of *Srf* KO mice at six weeks of age. Bottom panel of (C) shows the absence of CD45 signal in glomeruli (dotted circle); differential interference contrast (DIC) phase microscopy merged with Ki-67 immunostaining (D) localizes Ki67 staining to tubular epithelial cells (asterisks). Ki-67 positive cells were quantified in 10x fields of control and *Srf* KO (n = 3) at four and six weeks of age (E). Scale bars, 20 μm (A, B), 40 μm (C bottom, D), 100 μm (C top panel). * $p < 0.05$.



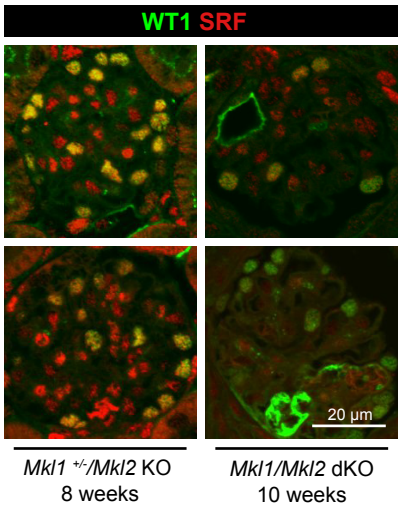
Supplemental Figure 6. The effects of *Srf* knockdown on actin-cytoskeleton and the expression of podocyte-enriched genes in a mouse podocyte cell line. (A) Experimental protocol for inducing a podocyte phenotype in conditional, immortalized MPC cell line. (B) RT-qPCR showing increased expression of *Synpo* in differentiated podocytes. (C) Verification of *Srf* knockdown in MPC podocyte cell line by RT-qPCR (left) and Western blot (right). Primary mouse aorta smooth muscle cells (ASMCs) serve as a control. *Srf* siRNA, si*Srf*; negative control siRNA, siNC. (D) Phalloidin staining of podocytes with *Srf* knockdown. (E) mRNA levels of podocyte-enriched genes in control and *Srf*-knockdown podocytes. Scale bar, 40 μm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplemental Figure 7. Relative mRNA expression of Myocardin family members in kidney cortex. *Myocd*, *Mkl1*, and *Mkl2* mRNA levels in normal adult kidney cortex determined by quantitative real-time PCR and normalized to aorta. *** $p < 0.001$.



Supplemental Figure 8. Genetic compensation of *Mkl1* and *Mkl2* in podocytes. (A) H&E staining of *Mkl1* and *Mkl2* single KO, *Mkl1*/*Mkl2* three-allele KO, and *Mkl1*/*Mkl2* dKO mice at 8-10 weeks of age. Note the normal histology in all genotypes save the *Mkl1*/*Mkl2* dKO that phenocopies *Srf* KO mice (compare with Figure 2F). (B) H&E staining of *Mkl1* $^{+/-}$ mice and *Mkl1*/*Mkl2* three-allele KO shows no observable abnormalities at ~1 year of age. (C) Urinary protein detection by Coomassie Blue staining in indicated genotypes of mice at more than 40 weeks of age. Compare negative data shown here with the overt proteinuria of *Srf* KO mice (see Figure 2C). (D) Urinary protein in *Mkl1*/*Mkl2* dKO mice. Scale bars, 100 μm (A top, B top), 50 μm (A middle, B middle), 20 μm (A bottom, B bottom).



Supplemental Figure 9. WT1 and SRF protein expression in podocytes of *Mkl1*/*Mkl2* dKO mice. Immunofluorescence microscopy shows co-localization of SRF and WT1 within podocytes of three-allele KO mice (left) with an apparent reduction of SRF in *Mkl1*/*Mkl2* dKO mice (right). Scale bar, 20 µm.