SUPPLEMENTAL MATERIALS:

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Supplementary figures:

Fig S1A

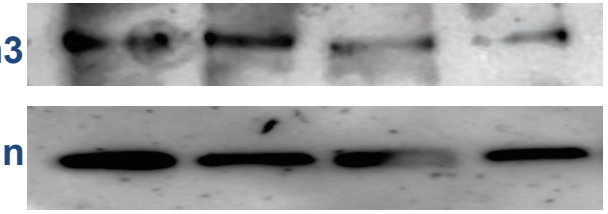
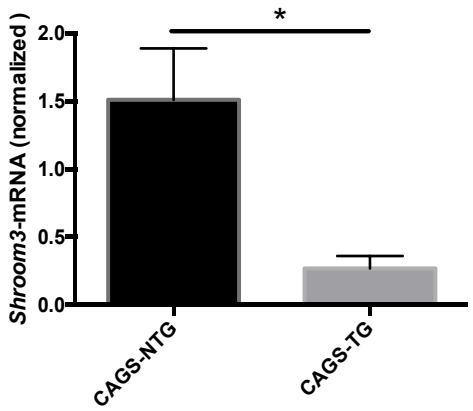
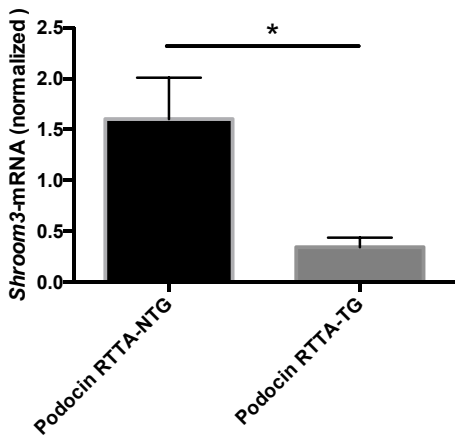


Fig S1B





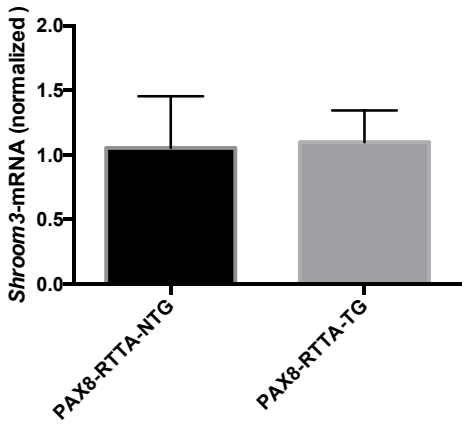


Fig S1C

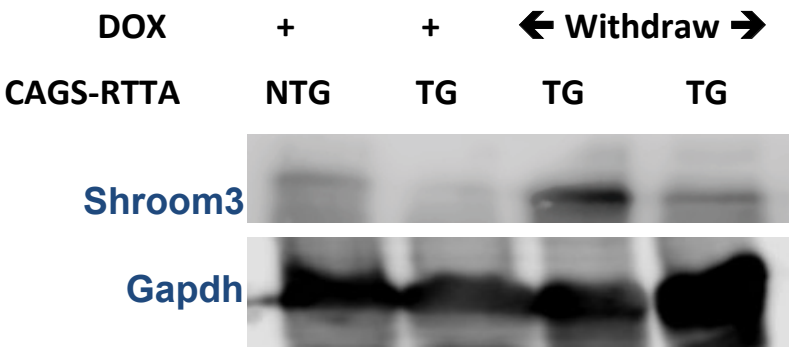


Fig S1D

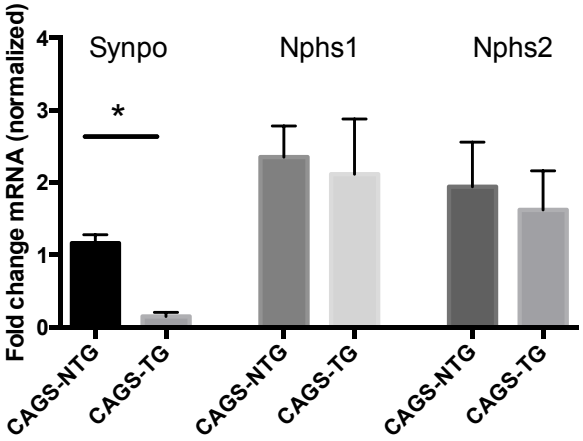


Fig S1 A-D: Glomerular Protein/RNA from DOX-fed Transgenic CAGS-RTTA/Podocin-RTTA & PAX8-RTTA mice (TG) and Non transgenic littermates (NTG) were extracted (6-weeks DOX; n=4 in each comparison). (1A) Figure displays Western blots of Shroom3 and β-Actin from Podocin-RTTA mice vs NTG. Bar graphs compares fold change (1B) *Shroom3*-mRNA & (1D) *Synpo*-, *Nphs1-, Nphs2*-mRNA by QPCR in NTG-glomeruli vs TG-glomeruli (normalized to *Gapdh*).(1C) WBs of total kidney lysates of CAGS-TG & NTG mice showing expression of Shroom3 after DOX withdrawal 3 wks)[error bars = mean±SEM]

Fig S1E

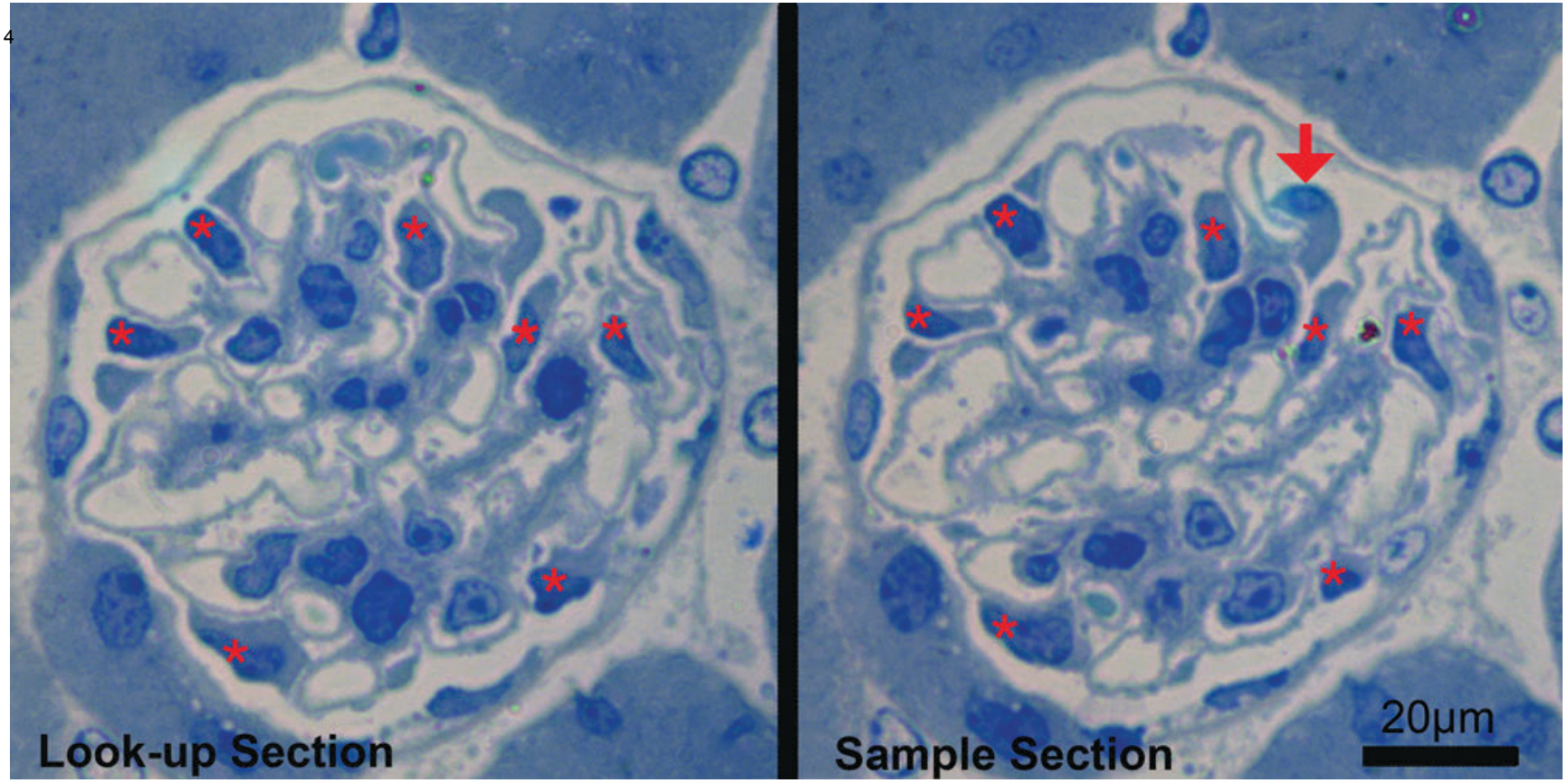


Figure S1E: A disector pair made up of a look-up section and a sample section used for counting podocyte number. Podocyte nuclei profiles are marked with a red asterisk in both the look-up section and the sample section. A red arrow points to the podocyte nuclei profile present in the sample section and not present in the look-up section and is thus counted a Q^-. Sections are 1-µm thick stained with toluidine blue.

Fig S1F 5

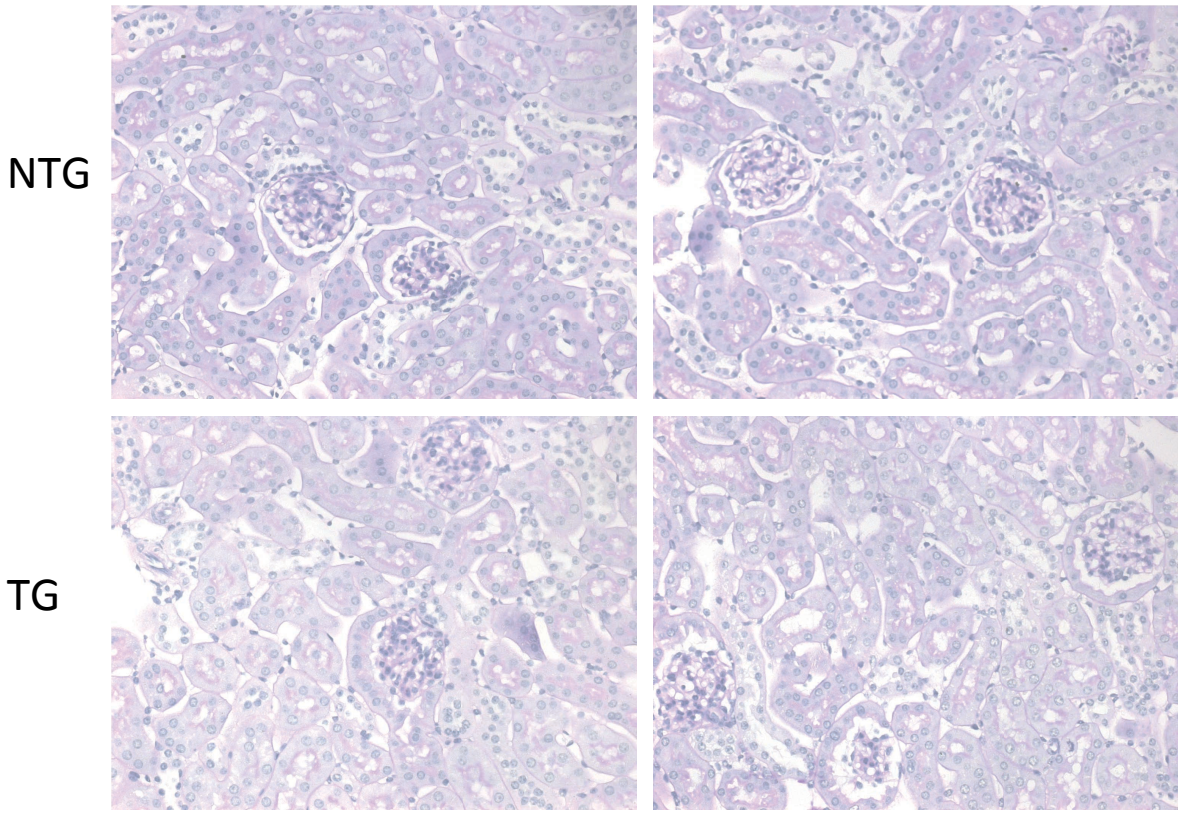


Fig S1F: CAGS-TG and NTG mice (8 weeks old) were fed DOX for 6 weeks. Representative 20X images (n=2 each group) of Per iodic acid Schiff stain are shown. No glomerulosclerosis, tubular atrophy or interstitial matrix expansion was identified by light microscopy at 6 weeks

Fig S1G [6]

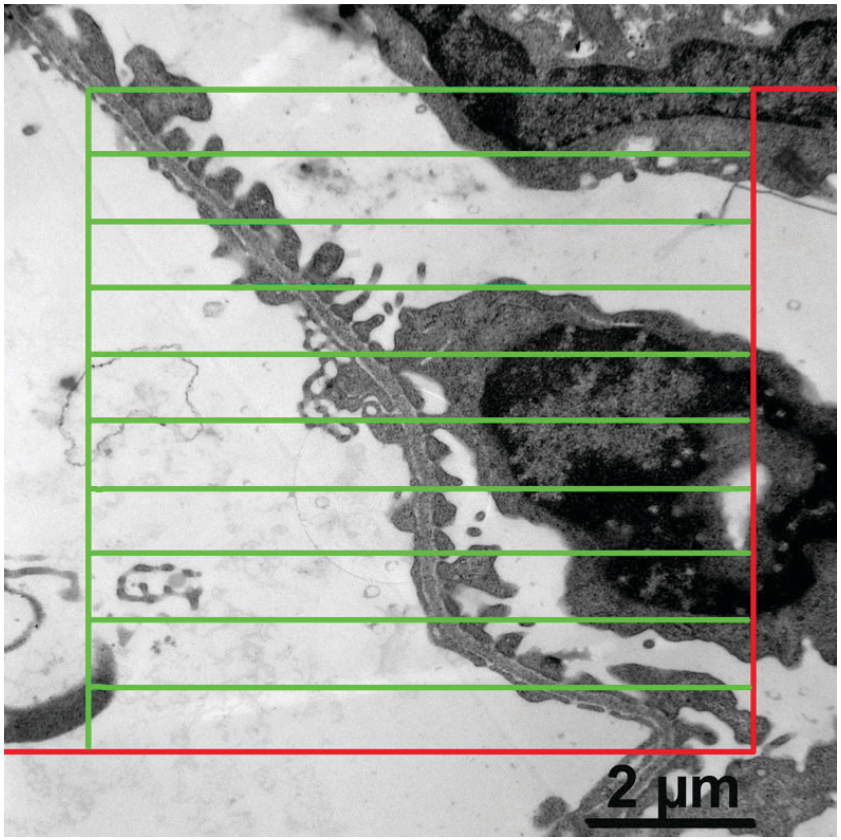


Fig S1G: Electron microscope image with superimposed unbiased 2-dimensional counting grid with 10 green parallel counting lines use to quantify length density of the slid diaphragm over peripheral glomerular basement membrane. Section stained with uranyl acetate and lead citrate.

Fig S2 [7]

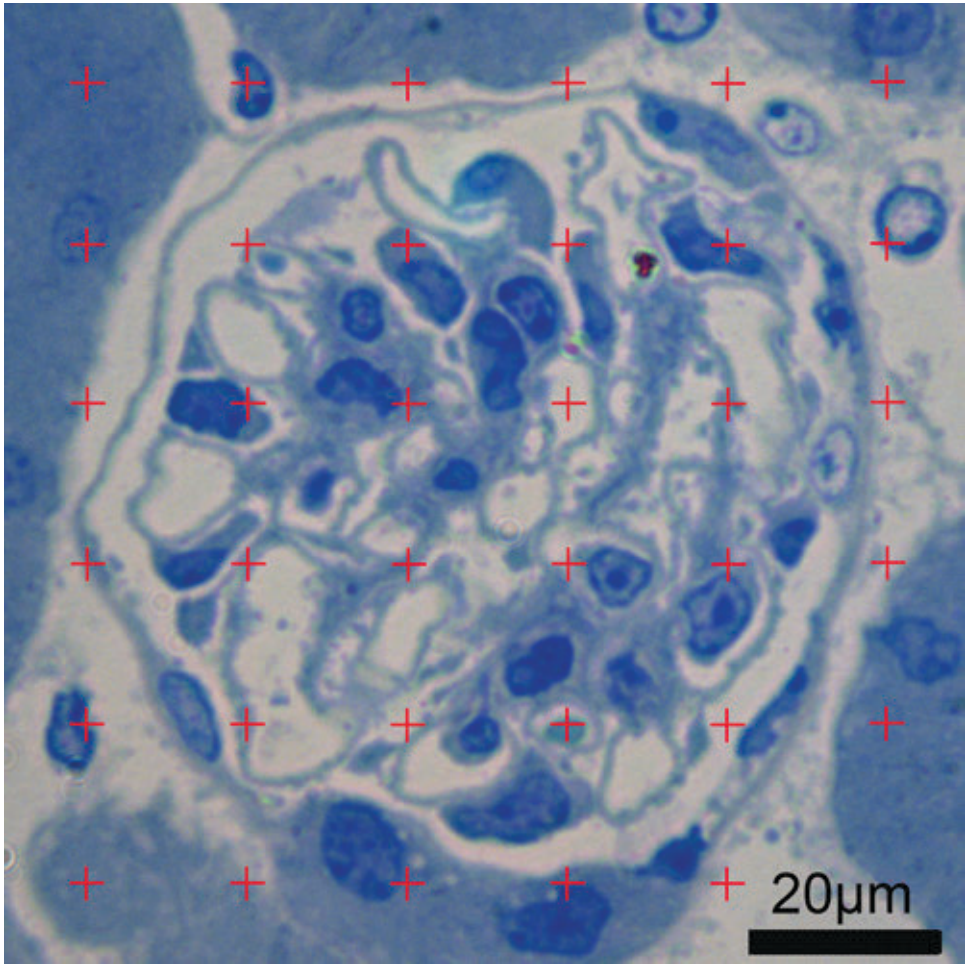
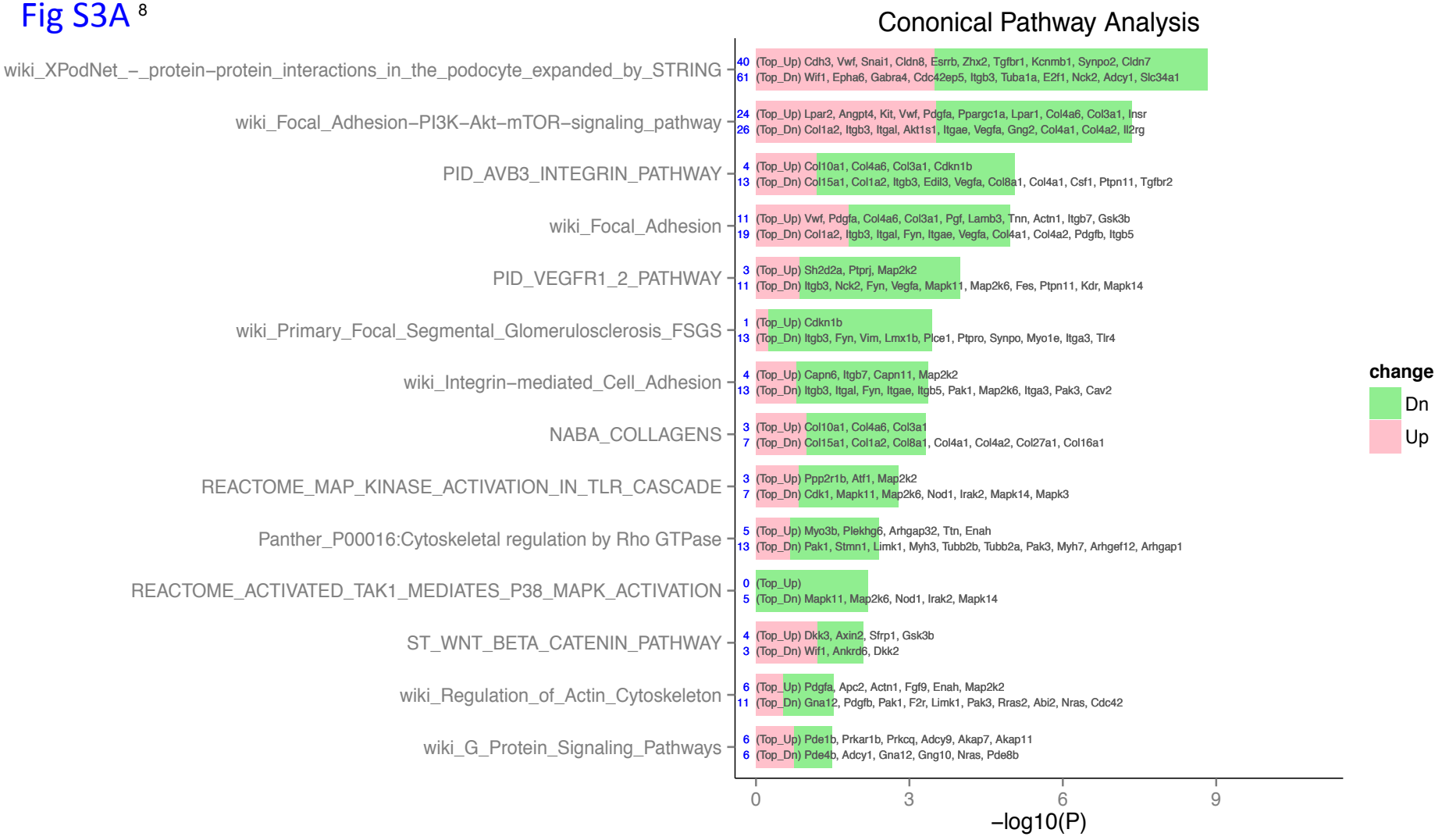


Fig S2: Glomerulus with superimposed counting grid used to quantify glomerular volume. Sections are 1-µm thick stained with toluidine blue.

Fig S3A [8]


Cononical Pathway Analysis
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61 (Top_Dn) Wif1, Epha6, Gabra4, Cdc42ep5, Itgb3, Tuba1a, E2f1, Nck2, Adcy1, Slc34a1
wiki_Focal_Adhesion–PI3K–Akt–mTOR–signaling_pathway
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Dn
Up

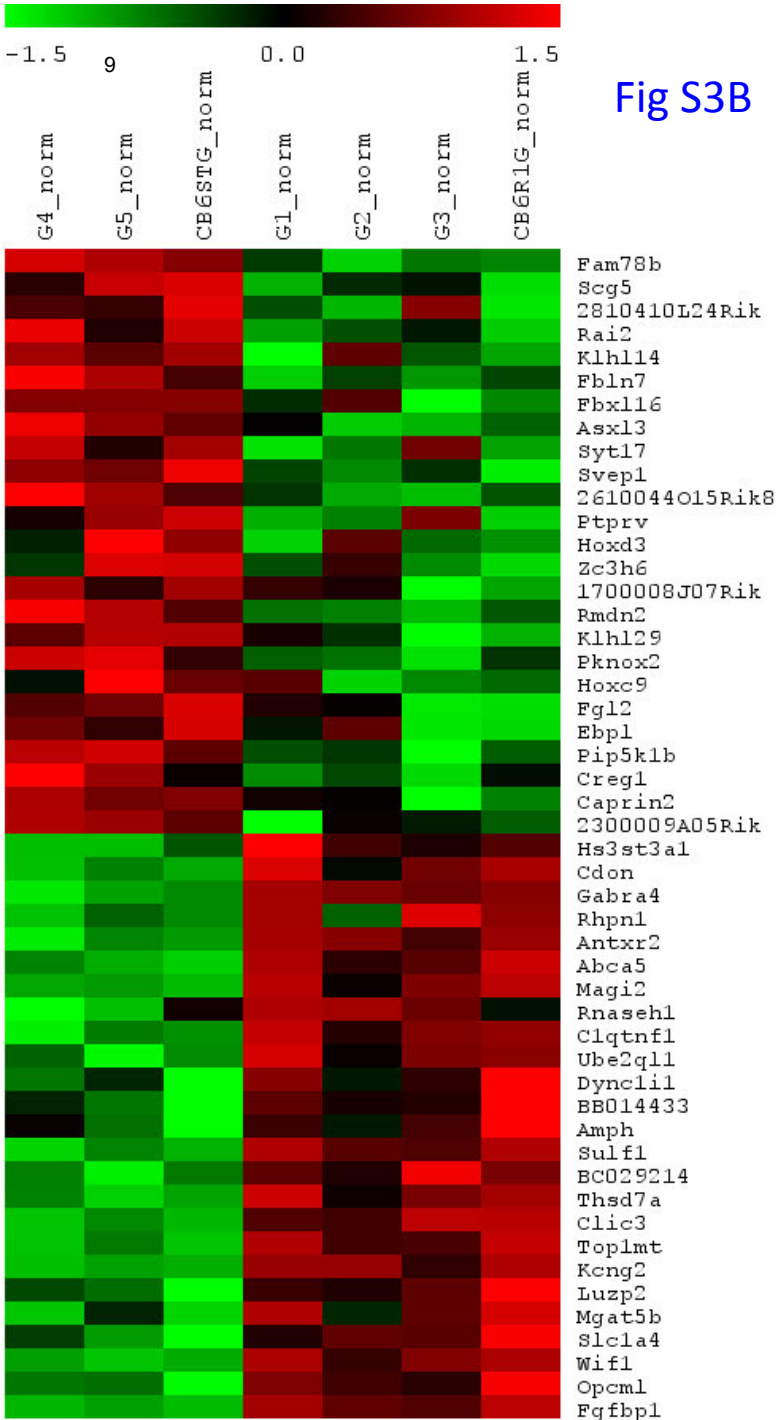
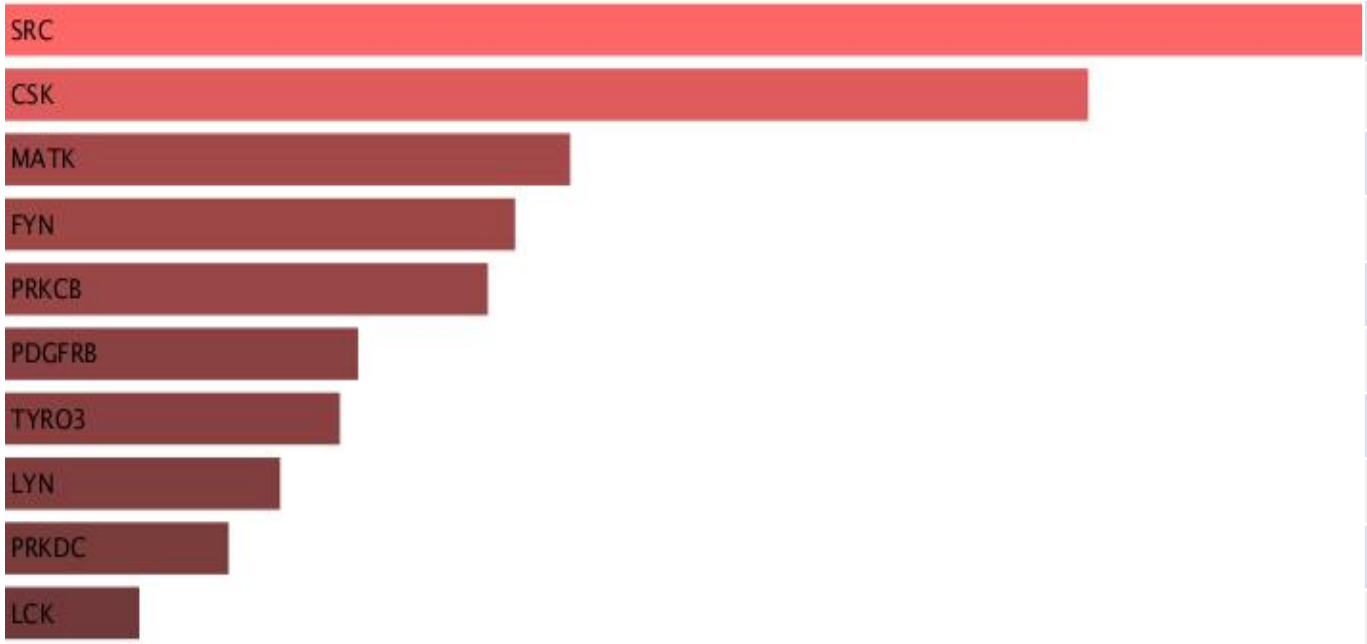


Fig S3B

Fig S3 A-B: Glomerular and non-Glomerular RNA from DOX-fed Transgenic CAGS-RTTA mice (TG) and Non transgenic littermates (NTG) were extracted using DYNA-bead perfusion and magnetic separation (6-weeks DOX; n=4 in each comparison). RNA-seq was performed on Poly-A selected Ribosomal RNA depleted, total RNA in Illumina NEXTSEQ sequencer (single end, 75 BP reads). Among significantly downregulated genelist (n= 1102 genes; LIMMA test $P<0.05$), genes uniquely downregulated in the glomerular fraction were identified and ranked by P-value (n=704 genes). (2A) Figure shows significant relevant Meta-pathway analysis terms represented by Up- or down-regulated genes (among top 50 pathways ranked by P-value(see Table S3). We utilized published data[21] to identify podocyte specific genes from the glomerular transcriptome. (2B) Figure shows the normalized heat map of top 50 differentially expressed genes. (G4, G5 & CB6ST =TG group; G1, G2, G3, CB6R1G = NTG group)

Fig S4 [10]

Kinase Enrichment Analysis



Src Kinase Family	SH3 domain	P Value
Y	Y	0.001
N	Y	0.001
N	Y	0.001
Y	Y	0.02
N	N	0.02
N	N	0.02
N	Y	0.001
Y	Y	0.03
N	N	0.04
Y	Y	0.04

Fig S4: Lysates of 293 cells overexpressing SHROOM3 or Control Vector were immuno-precipitated with Anti- V5 (overexpressed and control lysates), Anti-SHROOM3 (control lysates), and separated by PAGE. Lanes were separately analyzed by LCMS. Resulting peptide lists were filtered (see methods). Proteins with > 5 Spectral counts in both Over-expression and Endogenous lanes were ranked by fold change of spectral counts to controls (n=287). This protein list was input into ENRICHR database. Kinase enrichment results are displayed in order of combined score.[26]

Fig S5A

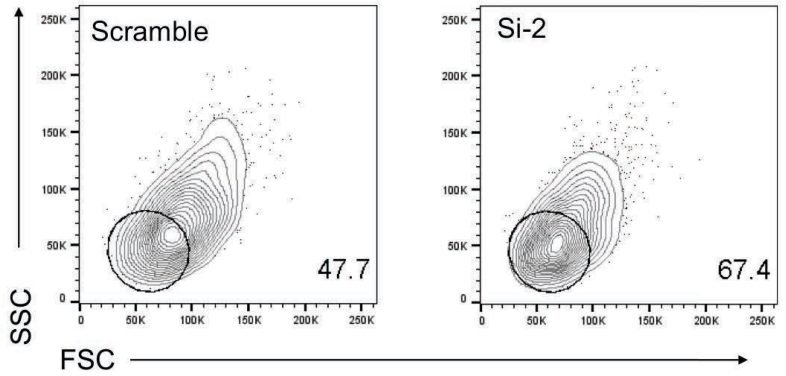


Fig S5B

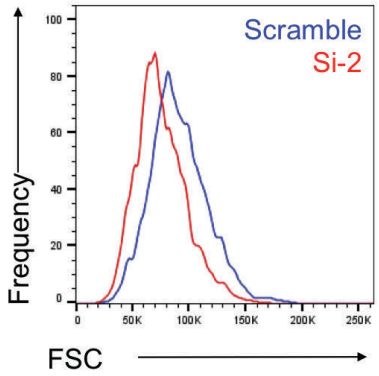


Fig S5C

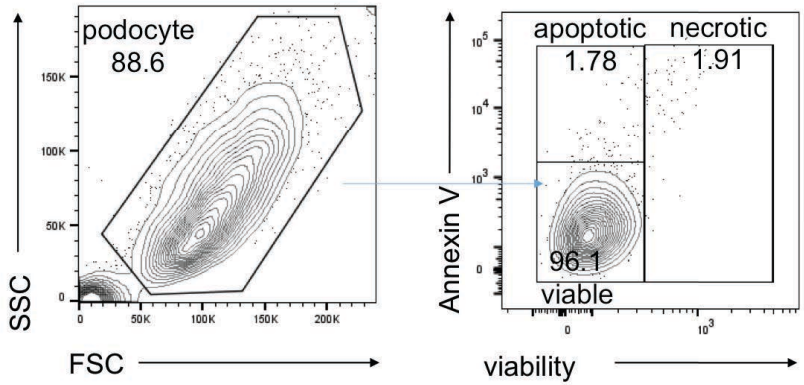


Fig S5D

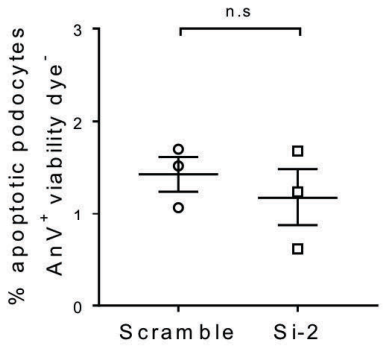


Fig S5F

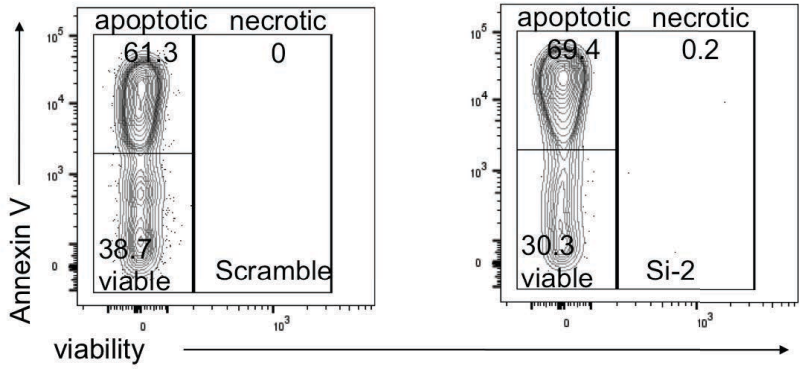


Fig S5E

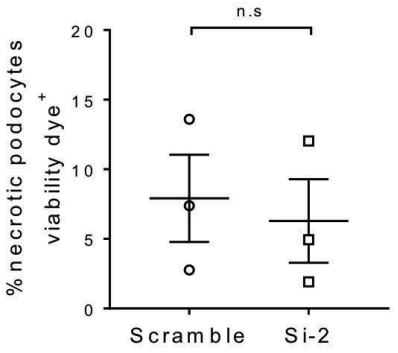


Fig S5 A-F: Differentiated human Scramble and Si-2 podocyte cell lines were grown in collagen-coated 6cm dishes at 37 ^{o}C. (A) FSC/SSC profile and (B) FSC MFI of Scramble and Si-2 podocytes by flow cytometry is shown. Si-2 podocytes cluster more compactly into an arbitrary gate as indicated. (C) Gating strategy used to define rates of podocyte apoptosis (D) and necrosis (E) using Anexin V and a viability dye, respectively. Annexin V threshold was determined on PBS controls. (F) Podocyte apoptosis induction with 25% w/v H_2O_2 incubation for 20min at 4^{o}C was used as positive control for Apoptosis and Annexin V stain. (n=3 independent experiments)

Fig S5G

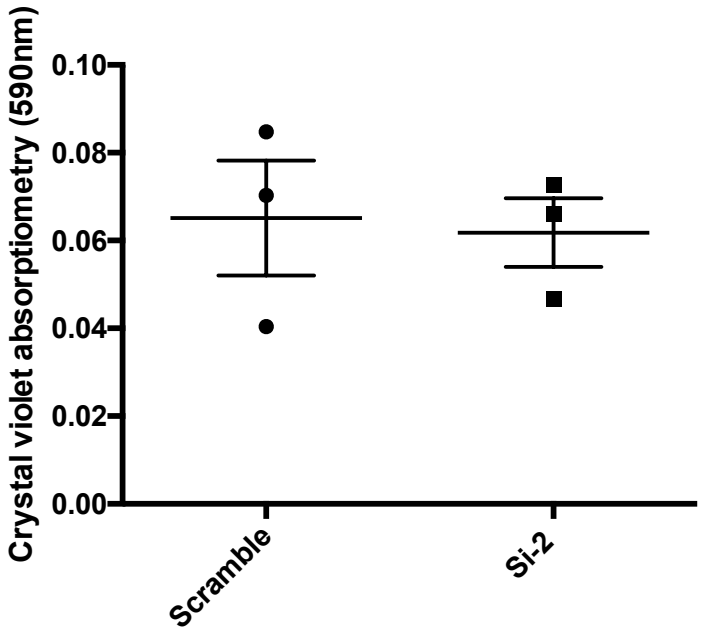


Fig S5G: Human Scramble and Si-2 podocyte cell lines were grown in collagen-coated 6-well plates (n=3 independent experiments). After complete differentiation at 37-degrees, cells were collagenized and replated in 96 well plates for 24 hours. After washing unattached cells, Crystal violet staining followed by detergent lysis (10%SDS) was performed. Colorimetry was done at 590 nm. Dot blots represent mean colorimetry readings from each experiment. [error-bars=mean±SEM]

Fig S6A

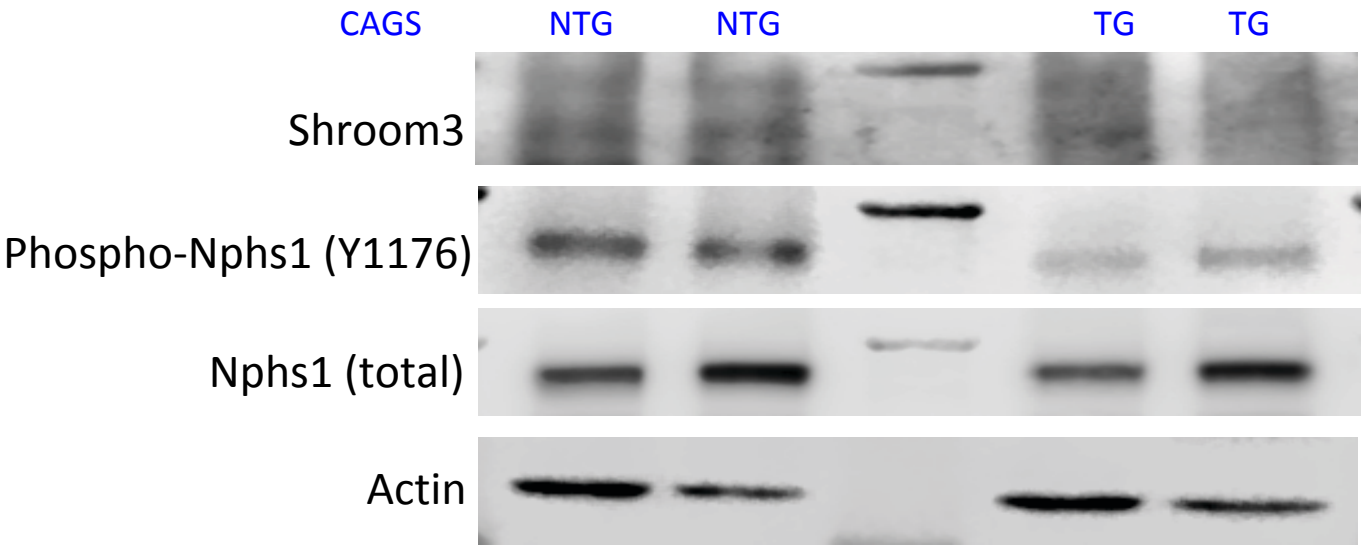


Fig S6A: Glomerular protein Lysates of additional CAGS-NTG & TG mice (n=2 each) were run on 8% PAGE gels (Phosphatase/Protease inhibitors added to lysis buffer). Results of immunoblotting with SHROOM3, Phospho Nphs1 (Y1193/1176), Total Nphs1, & Actin are shown. Two Isoform bands of Shroom3 are visualized. The phospho NPHS1:Nphs1 data shown here were used for densitometry shown in Fig 6C.

Fig S6B

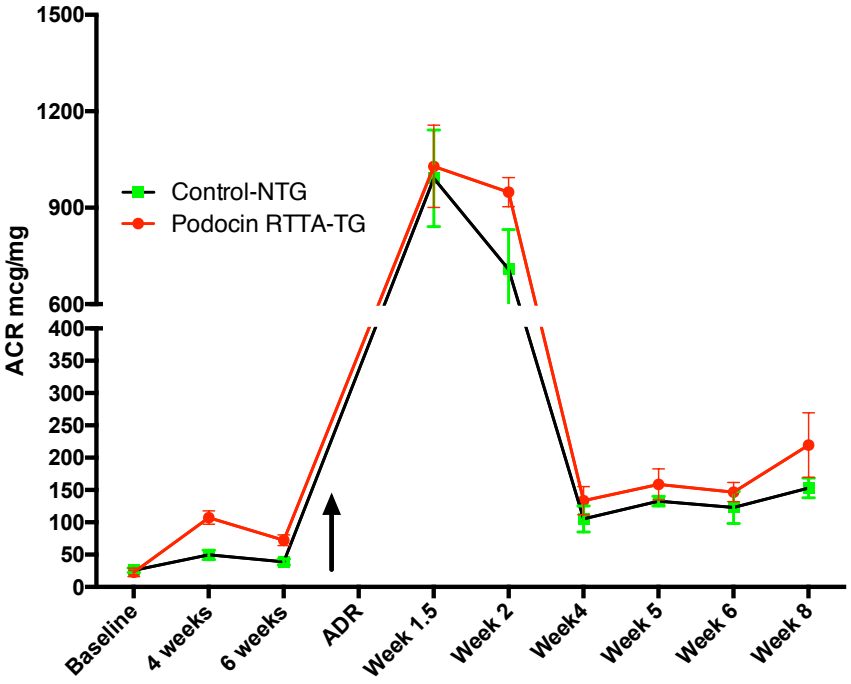


Fig S6C

NTG

TG

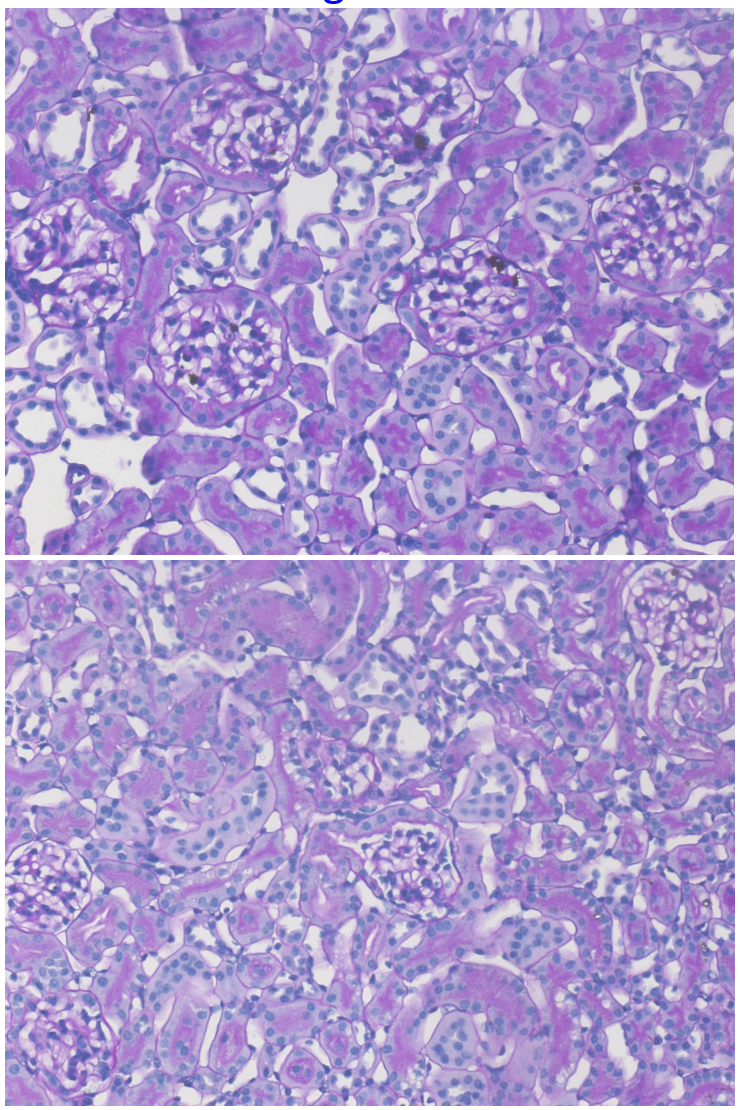


Fig S6B-C: DOX-fed Podocin-RTTA mice and littermates (n=5 each group) were injected with Adriamycin (high dose protocol: 18 mg/kg; Arrow in figure). Albuminuria was measured weekly (mcg/mg creatinine). (5B) Figure shows trend of Albuminuria (Dot/Whiskers=Mean/SEM), while (5C) shows representative 20X-images of PAS stained sections.

Fig S6D

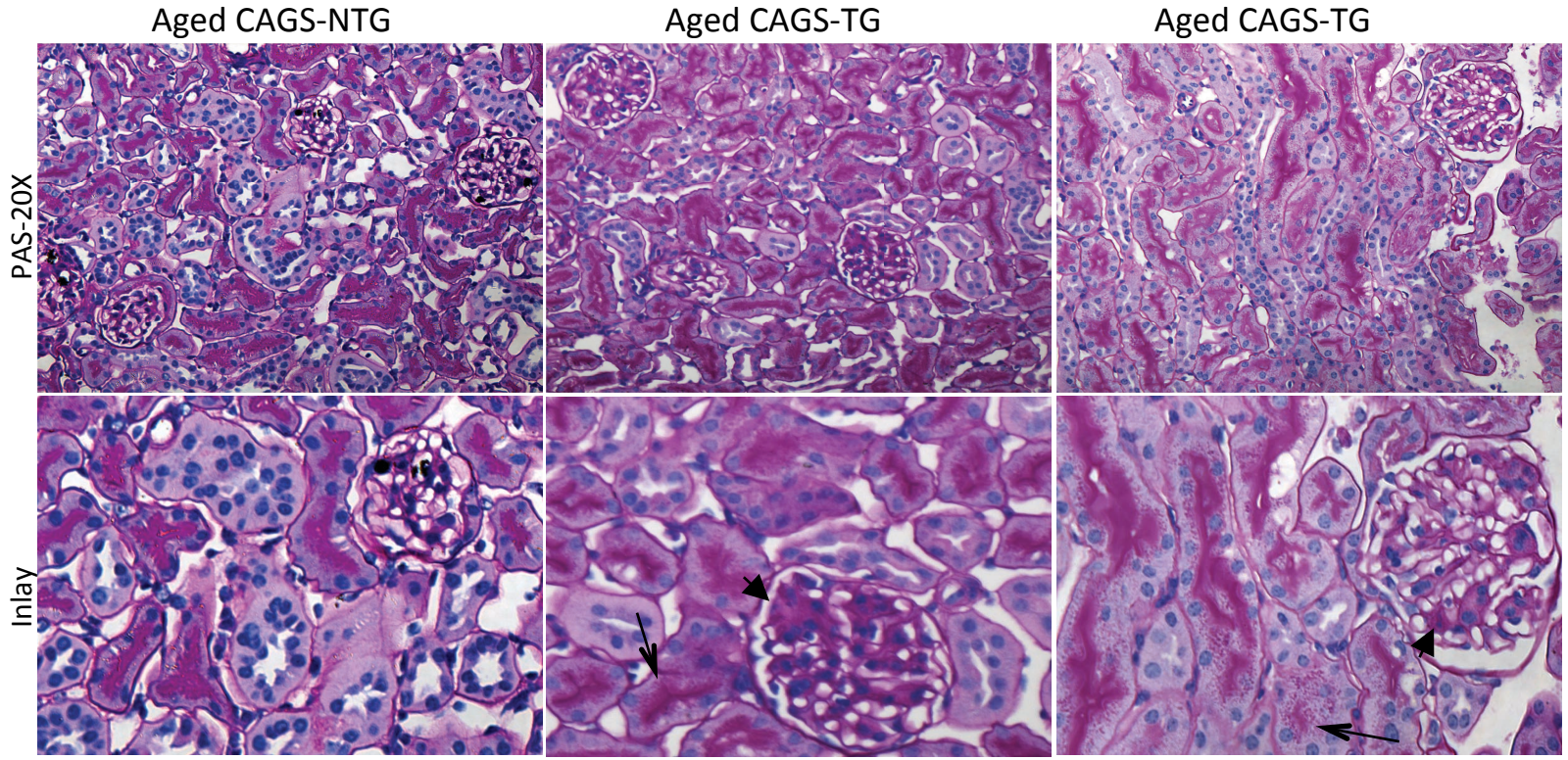


Fig S6E

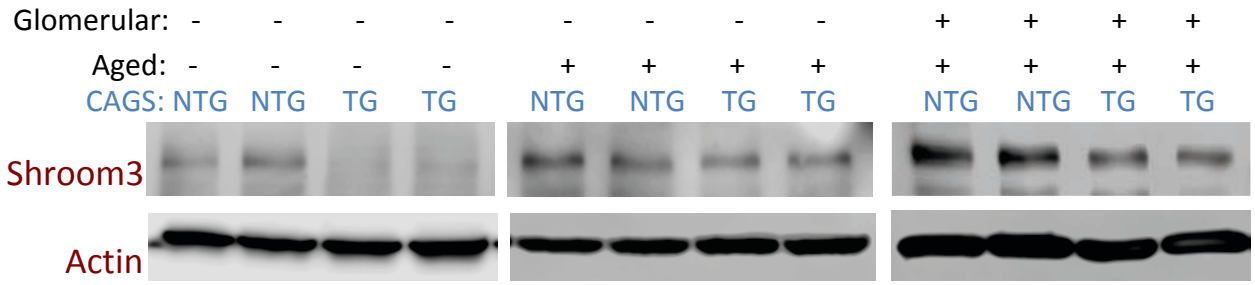


Fig S6F

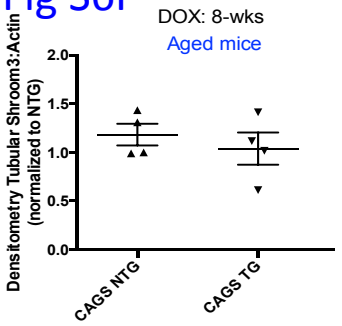


Fig S6D-F: CAGS-TG & -NTG mice were aged >1year (n=4 each); and mice were sacrificed after DOX feeding for 8 weeks to examine the age-dependent effect of Shroom3. (6D): Six panels show PAS-stained sections (20X), of representative CAGS-NTG &TG mice. Arrows in inlay show mesangeal sclerosis (arrowhead) & tubular protein reabsorption droplets (arrows). (6E): WBS show Shroom3 and Actin from non glomerular lysates of representative young (left) or aged NTG/ TG mice. (6F): Dot-plots show relative density of Shroom3 normalized to Actin in non-glomerular lysates. [Bars = SEM].

Fig S6G

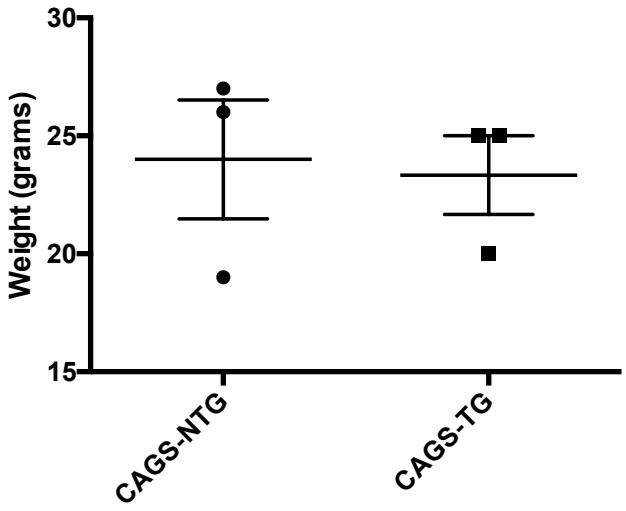


Fig S6G: CAGS-TG & -NTG female mice were aged >1year (n=3 each); and mice were sacrificed after DOX feeding for 12 weeks to study effect of age on Shroom3 knockdown. Figure shows weights of study animals at sacrifice.

Table S1: Primer sequences used in QPCR

		Reverse primer (5'➔ 3')
GAPDH	TGTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG
SHRM3	CCCTCTCGGGGCGTCTAGCC	GCCCAGCACTACTCGCTCCC
Gapdh	GCCATCAACGACCCCTTCAT	ATGATGACCCGTTTGGCTCC
Shrm3	CACACTGGCGGTCCCTGTGC	CTGAGCCGTTCCAGCAGGGC
Synpo	CTTTGGGGAAGAGGCCGATTG	GTTTTCGGTGAAGCTTGTGC
Nphs1	GTGCCCTGAAGGACCCTACT	CCTGTGGATCCCTTTGACAT
Nphs2	CTTGGCACATCGATCCCTCA	CGCACTTTGGCCTGTCTTTG

Table S2: Top 50 downregulated Gene Ontology biologic functions (glomerular unique differentially expressed genelist)

GO:0001525~angiogenesis	22	1.60E-10
GO:0007242~intracellular signaling cascade	60	9.68E-09
GO:0009100~glycoprotein metabolic process	15	2.88E-05
GO:0016477~cell migration	20	3.15E-05
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	17	3.94E-05
GO:0007265~Ras protein signal transduction	10	3.92E-05
GO:0009101~glycoprotein biosynthetic process	13	4.32E-05
GO:0006486~protein amino acid glycosylation	11	8.76E-05
GO:0043413~biopolymer glycosylation	11	8.76E-05
GO:0070085~glycosylation	11	8.76E-05
GO:0007264~small GTPase mediated signal transduction	21	9.74E-05
GO:0007155~cell adhesion	35	0.0001
GO:0007167~enzyme linked receptor protein signaling pathway	20	0.0001
GO:0022610~biological adhesion	35	0.0001
GO:0034655~nucleobase, nucleoside, nucleotide and nucleic acid catabolic process	7	0.0001
GO:0010647~positive regulation of cell communication	16	0.0002
GO:0006897~endocytosis	16	0.0002
GO:0009967~positive regulation of signal transduction	15	0.0002
GO:0010324~membrane invagination	16	0.0002
GO:0033674~positive regulation of kinase activity	13	0.0003
GO:0051347~positive regulation of transferase activity	13	0.0004
GO:0001763~morphogenesis of a branching structure	12	0.0004
GO:0009166~nucleotide catabolic process	6	0.0004
GO:0048754~branching morphogenesis of a tube	10	0.0004
GO:0043062~extracellular structure organization	13	0.0004
GO:0031663~lipopolysaccharide-mediated signaling pathway	4	0.0006
GO:0044270~nitrogen compound catabolic process	7	0.0005
GO:0045860~positive regulation of protein kinase activity	12	0.0006
GO:0006468~protein amino acid phosphorylation	36	0.0006
GO:0007266~Rho protein signal transduction	6	0.0006
GO:0001570~vasculogenesis	7	0.0007
GO:0007229~integrin-mediated signaling pathway	9	0.0008

GO:0043085~positive regulation of catalytic activity	19	0.0008
GO:0016044~membrane organization	19	0.0010
GO:0048870~cell motility	20	0.0010
GO:0051674~localization of cell	20	0.0010
GO:0048661~positive regulation of smooth muscle cell proliferation	4	0.0011
GO:0006793~phosphorus metabolic process	45	0.0013
GO:0006796~phosphate metabolic process	45	0.0013
GO:0050798~activated T cell proliferation	3	0.0017
GO:0006928~cell motion	23	0.0020
GO:0044093~positive regulation of molecular function	20	0.0022
GO:0006817~phosphate transport	3	0.0022
GO:0032755~positive regulation of interleukin-6 production	4	0.0024
GO:0046578~regulation of Ras protein signal transduction	13	0.0024
GO:0000165~MAPKKK cascade	10	0.0026
GO:0007243~protein kinase cascade	16	0.0026
GO:0030198~extracellular matrix organization	9	0.0027
GO:0045785~positive regulation of cell adhesion	6	0.0025
GO:0051270~regulation of cell motion	12	0.0034

Table S3: Top 20 proteins ranked in order of spectral count ratio (Overexpression lane: control lane)

Identified Proteins (1828)	Accession Number	Molecular Weight	Spectral counts Overexpressed Shroom3:Control	Spectral counts Endogenous Shroom3:Control
Protein Shroom3 OS=Homo sapiens GN=SHROOM3 PE=1 SV=2	SHRM3_HUMAN	217 kDa	74.5	9.5
Rho-associated protein kinase 2 OS=Homo sapiens GN=ROCK2 PE=1 SV=4	ROCK2_HUMAN	161 kDa	30	4.0
Keratin, type I cytoskeletal 15 OS=Homo sapiens GN=KRT15 PE=1 SV=3	K1C15_HUMAN	49 kDa	15	16.5
14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1	1433Z_HUMAN	28 kDa	8	4.0
Tyrosine-protein kinase Fyn OS=Homo sapiens GN=FYN PE=1 SV=3	FYN_HUMAN	61 kDa	7.5	5.0
E3 ubiquitin-protein ligase MYCBP2 OS=Homo sapiens GN=MYCBP2 PE=1 SV=3	MYCB2_HUMAN	510 kDa	6.6	2.2
14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1	1433T_HUMAN	28 kDa	6.33	3.0
ADP/ATP translocase 1 OS=Homo sapiens GN=SLC25A4 PE=1 SV=4	ADT1_HUMAN	33 kDa	6	4.5
Heterogeneous nuclear ribonucleoprotein D-like OS=Homo sapiens GN=HNRNPDL PE=1 SV=3	HNRDL_HUMAN	46 kDa	6	4.5
Elongation factor 1-alpha 2 OS=Homo sapiens GN=EEF1A2 PE=1 SV=1	EF1A2_HUMAN	50 kDa	6	5.0
Creatine kinase B-type OS=Homo sapiens GN=CKB PE=1 SV=1	KCRB_HUMAN	43 kDa	5.5	2.0
Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2	PPIA_HUMAN	18 kDa	5.5	5.0
Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5	DYHC1_HUMAN	532 kDa	4.666666667	1.3
Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3	LPPRC_HUMAN	158 kDa	4.4	2.6
14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2	1433G_HUMAN	28 kDa	4	4.0
14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1	1433E_HUMAN	29 kDa	3.8	2.0
Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	UBA1_HUMAN	118 kDa	3.8	2.4
40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4	RSSA_HUMAN	33 kDa	3.75	2.5
Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	PDIA1_HUMAN	57 kDa	3.666666667	2.3
Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform OS=Homo sapiens GN=PPP2R1A PE=1 SV=4	2AAA_HUMAN	65 kDa	3.5	2.5

Concise Methods:

GOCAR Study: Ultrasound-guided, renal allograft biopsies were obtained at 0, 3, 12, and 24 months post transplant, at 3 of 5 clinical sites. Histology and CADI score were reported by a central pathology core lab at Massachusetts General Hospital to identify glomerular lesions in GoCAR Sinai patients with nephrotic proteinuria. eGFR was calculated using Modified diet in renal disease equation (MDRD). Albumin:Creatinine ratio and Protein:Creatinine ratios were collected by Chart review from clinical patient charts from Westmead hospital, Sydney, & the Mount Sinai hospital in New York.

SHROOM3 SNP analysis: Targeted SNP genotyping for rs17319721 was done using Taqman SNP analysis assay (# 4351379 Applied Biosystems, Foster City, CA). DNA was extracted from pre-implantation biopsies or blood for donor SNP and from peripheral blood for recipient SNP assay (~20ng/sample).

Cell Culture: Human podocyte cell line (Generous gift of Dr Moin Saleem), & HEK-293 T cells were expanded using RPMI-1640 (1% ITS) and DMEM (GIBCO) media, respectively. Podocytes were differentiated in Collagen coated culture plates/flasks. Cell adhesion assay was performed by coating 1000-, 2000-, 3000-podocytes per well in beta-Laminin-coated 96 well plates as described [12].

Reverse transcription: For *in vitro* studies we used Superscript-III (Invitrogen- Life technologies, Grand Island, NY) with starting total RNA ~ 1000 ng.

Quantitative-PCR: *SHROOM3* expression was assayed invitro/invivo by real-time polymerase chain reaction (Q-PCR) (Applied Biosystems 7500). Amplification curves were analyzed using automated 7500 software platform, via the delta-delta CT method. Human Glyceraldehyde phosphate dehydrogenase (*GAPDH*) was used as endogenous control. Similarly, primers were designed for mouse Gapdh, Nphs1, Podocin, and Synaptopodin (Table S3).

Western Blotting: Cells were lysed with a buffer containing 1% Triton, a protease inhibitor mixture and tyrosine and serine/threonine phosphorylation and phosphatase inhibitors. Lysates were subjected to immunoblot analysis using polyclonal Rabbit anti-SHROOM3 (#SAB3500818, Sigma), anti-V5 tag antibody (A01724, Genscript Inc), Phospho-SRC family Y416 Rabbit monoclonal antibody (#2101S, Cell Signaling), non Phosphorylated Mouse monoclonal Ab (#2102S, Cell Signaling), Fyn Rabbit polyclonal antibody (#4023S, Cell Signaling), Mouse monoclonal Fyn (#610163, BD Biosciences), Phospho Nephrin (Y1176/1193; #Ab80299, Abcam), Total Nephrin (Gift from Dr Lawrence Holzman to CJH), Actin (Mouse Monoclonal; Sigma # A5441). Anti-V5 magnetic beads (MBL international) were used for immunoprecipitation. Densitometry was performed on images of western blots using ImageJ software.

Overexpression studies: A human *SHROOM3* construct (Open Biosystems, Lafayette, CO) was cloned into mammalian expression vector PC-DEST40 (Invitrogen, Carlsbad, CA) with C-terminal V5 and Histidine tags using the Clonase-II recombinase (Invitrogen) system. Site specific mutagenesis was performed replacing -**C**CC/-**C**CA (Proline) sequences with -**G**CC/-**G**CA (Alanine) by proprietary recombinase (Genescript technologies inc, NJ, USA). Construct Sequencing of 1KB region around mutagenesis site confirmed substitution mutations in M1, M2, and M1+2 constructs. Transfection was performed in 293 cells using Polyjet reagent (SignaGen labs, Rockville, MD) as described previously[4].

Mass Spectrometry: PCDEST SHROOM3 and PCDEST 40 (control vector) were overexpressed in 293 T cells. Protein lysates after 48hrs transfection were immunoprecipitated with Anti-V5-tag, anti-SHROOM3 or control IgG , and run on PAGE gels. Three resultant lanes were sent for Mass spectrometry (Protein Mixture Identification by LC/MS/MS(LTQ Orbitrap Velos, ThermoFisher Scientific, USA). Gel code Blue stain was done on gels to reversibly visualize protein bands. Immunoprecipitated lysates (V5, SHROOM3- & IgG controls) were run on PAGE gels and individual lanes analyzed separately as high & low abundance bands, after trypsin proteolysis. Data was first filtered to remove proteins with high control spectral counts (>5) with low Test:Control ratio (<1.5). Proteins with test:control ratios 1.2 or greater, and identified in both overexpression and endogenous lanes were selected. These 287 unique IDs were ranked by Spectral Count ratio values of Overexpression:Control, and used for further analyses.

shRNA suppression studies: Human *SHROOM3* short hairpin clones (Open Biosystems, Lafayette, CO) were tested for optimal suppression in 293-T cells. The selected GFP-tagged hairpins were used to generate a mammalian VSV pseudotyped lentiviral expression construct. Lentiviral medium was used to

infect human podocytes at 33 degrees. Cells were passaged in puromycin (200 ng/ml)-RPMI 1640 for experiments after 7-days differentiation at 37 degrees.

Immunofluorescence: PC-*SHROOM3* and PC-DEST40 transfected cells were plated in 12-cm wells on collagenised cover slips (20% rat-tail collagen, BD biosciences, San Jose CA) for 36-48 hours, formalin-fixed (36% HCHO, 0.1% TritonX100 in PBS). F-Actin staining for cytoskeletal changes in SHROOM3-transfected cells was done using Cy5-conjugated Phalloidin (Alexa-Fluor 647 Phalloidin, Invitrogen, Life technologies, Grand Island NY) at 1:40 dilution in 1% BSA-PBS. **Quantitative Image Analysis:** Cells were segmented from intensity-normalized Phalloidin stain images by thresholding combined with an algorithm to find connected-pixel objects. For each cell, the surface area was calculated, and an edge mask obtained by substracting the cell mask from a contour-eroded inside mask. The mean fluorescence intensity (MFI) of the pixels belonging to the edge mask was calculated. All scripts were programmed in Matlab (http://www.mathworks.com)

Murine *Shroom3* knockdown model: Tetracycline-responsive, shRNAmir-mediated *Shroom3* knockdown mouse strain based on tested shRNA guide sequences was developed with Mirimus Inc, NY. In the double-transgenic CAGS-rtTA/Podocin-RTTA/ PAX8-RTTA;Shroom3 RNAi mice, shRNAmir-mediated knockdown was driven by the respective universal or tissue-specific promoters and, inducible by Doxycycline feeding (DOX)[4]. CAGS-RTTA animals were backcrossed into BALBC background. In the PAX8-RTTA animals, the shRNA expression was pantubular. Male mice (7-8 week old) were DOX-fed for 6 weeks (600mg/g DOX chow; Envigo inc), and subjected to weekly urine collection (n=4 mice /group in all data). Non transgenic DOX-fed littermates were used as controls. At 6- or 8-weeks DOX kidney tissues were collected for histology, immunofluorescence (snap frozen for IF) as well as RNA isolation for QPCR/RNAseq, protein extraction for WB, and Immunoperoxidase (Formalin fixed IHC). Glomeruli & non-glomerular fractions were extracted using DYNA-bead perfusion. For IF, snap frozen kidney-sections were formalin-fixed and treated with anti-WT1 and SYNPO (1:100 each; Gift from Kirk Campbell) overnight, and fluorescence microscopy. >30 glomeruli per mouse were assayed in 40X images for WT-1/DAPI/SYNPO costaining. **Adriamycin injection study:**Eight-week old male Podo-RTTA mice(C57B6/129SVJ/FVB background) and littermates were fed DOX (6 weeks), and were injected with retrobulbar injection (ADR 15 g/kg). DOX was continued. Urine was collected 2-weekly till sacrifice at 8 weeks post injection. **Aged mice:** CAGS-TG mice were aged to 12 months on regular chow. DOX was initiated at 12months for 12 weeks (n=3 each). Kidney tissue was collected for microscopy and IHC (P57). $P57^{kip2}$ is a Cyclin kinase inhibitor which is specific for podocyte nuclei, colocalizing with WT1[44]. p57 positivity was counted in each glomeruli by two blinded observers and results expressed as mean/SD. To assess the glomerular size, glomeruli were outlined using imageJ software (40X images), thresholded, and area of SYNPO-staining (snap-frozen sections) were quantified and expressed as percent of total area/glomerulus using Metamorph (Molecular Devices, LLC, Sunnyvale, CA. All glomeruli in the biopsy tissues of the mice were included (ranging from 24-58, average of 44 glomeruli per mouse). Scale was set at 50um equivalent to 50,000nm. The results are expressed as mean in micrometer-square and standard deviation.

Glomerular Morphometry:

Tissue Processing: Kidneys were perfused with PBS for 5 minutes. One-millimeter cubes were cut from the cortex and placed in glutaraldehyde. Samples were processed as described [13].

Podocyte Number: The fractionator/disector method was used to count podocytes [14, 15]. Podocyte nuclei were surrogates for podocytes assuming only one nucleus per podocyte. Using an ultramicrotome, serial 1-µm thick sections were cut from an embedded tissue block. Using Adobe Photoshop® the images from each pair of sections were view together. Podocyte profiles from glomerulus 1 seen in the second section of a pair (the sample section) but not present in the first section (the look-up section) were counted as a Q^-s [Figure S1D]. This counting was repeated for all the pairs of sections from a glomerulus. The number of podocytes in a glomerulus was calculated with the equation, Podocyte Number = $\sum Q^- \times 10$ (where $\sum Q^-$ is the sum of the podocyte profiles seen in the sample section but not in the look-up section, and 10 is the reciprocal of the fraction of the glomerulus sampled). An average of 7.4 glomeruli was measured per

kidney. An average of 6.2 pairs of sections were measured per glomerulus. An average of 109.2 Q^- were counted per kidney.

Glomerular & Glomerular component Volumes: The Cavalieri Principle was used to measure glomerular volume [16, 17]. Using the Layers function of Photoshop® a grid of points (100 mm apart) was superimposed over only the sample section from each section pair from a glomerulus [see previous paragraph]. The intersection of two perpendicular lines was defined as a grid point [Figure S4]. The number of grid points falling on a glomerulus for each look-up image was counted. Glomerular volume was calculated with the equation, Glomerular Volume = $\sum$Points x 10 x $(100{,}000/\text{mag})^2$ μm^3 (where $\sum$Points is the sum of grid points falling on all the glomerular profiles from a glomerulus, 10 is the distance in micrometers between the sections sampled, 100,000 is the distance in micrometers between the points of the grid and mag is the magnification of the images measured). An average of 7.4 glomeruli was measured per kidney. An average of 841.5 points was counted per kidney.

The glomerulus was divided into four components, podocytes, mesangium, capillary lumens + endothelial space and "other". The "other" component was defined as Bowman's space, glomerular basement membrane, and non-resolvable areas within the glomerulus, and were not used in glomerular component analysis. Using Photoshop's® Layers function a grid of points was superimposed over only the sample image from an image pair [Figure S4]. The number of grid points falling on each component was counted[18]. The volume density of each component X was calculated using the equation, V_v(Component X/Glomerulus) = $\sum$Points X/$\sum$Points Total $\mu m^3/\mu m^3$, (where $\sum$Points X is the sum of grid points falling on component X for all the sample images from a glomerulus). $\sum$Points Total is the sum of grid points falling on all four components for all the sample images from a glomerulus. The volume for each component is calculated by multiplying the glomerular volume by the appropriate volume density for each of the components. The unit for component volume is μm^3. For all the measured images from a kidney an average of 187, 235, 149, and 102 grid points were counted on podocyte, mesangium, capillary lumen, and other respectively.

F**oot Process Width:** Average foot process width was determined by first measuring the length density of the slit diaphragm as it lies on the peripheral glomerular basement membrane [L_s(SD/PGBM)] [19]. Images were taken in a meandering pattern with random start from throughout the glomerulus. Using the Layers function of Photoshop® an unbiased 2-dimensional counting frame was superimposed over the glomerular images[20]. The counting frame consisted of 10 parallel counting lines 18 mm apart with additional forbidden lines [Figure S1G]. The number of times a grid counting line intersected the interface between a podocyte foot process and the peripheral glomerular basement membrane was counted. Next the number of slid diaphragm profiles (spaces) between foot processes on peripheral glomerular basement membrane was counted. The L_s(SD/PGMB) was calculated with the equation, L_s(SD/PGBM = ($\sum$SD/$\sum$I PGBM)/(1,800,000/Mag) $\mu m/\mu m^2$ (where $\sum$SD is the sum over all the images from a kidney of the slit diaphragm profiles, $\sum$I PGBM is the sum of the intersections of grid lines with the interface of foot processes and peripheral glomerular basement membrane, 1,800,000 is the distance in nanometers between the grid lines, and mag is the magnification of the images). Average foot process equals the reciprocal of the L_s(SD/PGBM).

RNA-sequencing: Total RNA was isolated from glomerular and non-glomerular tissue fractions after homogenization [Qiagen RNEAsy kit]. Ribosomal RNA depletion was performed before Poly-A selection to improve quality. Single-ended sequencing with 75 bp read length was carried out on NEXTSEQ (Illumina Inc.). Single reads split by barcode into individual files using PERL script. The reads with good quality were aligned to reference sequence databases of mouse (mm10) genome, RefSeq exons, splicing junctions and contamination databases including ribosome and mitochondria sequences using BWA alignment algorithm and the alignment files in SAM format were generated. After filtering reads mapped to contamination databases, the reads that have one or no mismatch and are uniquely aligned to each exon and splicing-junction sites were extracted and then counted. The read count for each RefSeq transcript was calculated by combining the counts for exons and splicing junctions of corresponding transcript. The read count at exon, splice-junction, transcript and gene levels were summarized and normalized to the similar level across the samples and further corrected for batch effect in order to

compare transcription level in different groups. The differential analysis between TG and NTG samples by paired LIMMA was performed to identify significantly dysregulated genes at p value <0.05 which were then subjected to Gene Ontology function and Canonical pathway enrichment analysis by Fisher exact test. Genes uniquely and exclusively downregulated in glomerular fraction, but not in non glomerular fraction were identified and compared via Gene Ontology function and Canonical pathway enrichment analysis. Podocyte enriched genes were similarly compared based on prior data [21].

Statistical analysis: Descriptive statistics (means and standard deviations) were used to summarize the baseline characteristics of donors and recipients, and were compared using the chi-square test and Fisher's exact test. Univariate comparisons of continuous variables were done using unpaired T-test (Mann-Whitney test for corresponding non-parametric analysis). Spearman's correlation was used to determine relationship between *SHROOM3* expression, and eGFR (Nephroseq datasets).

Multi-variable linear regression was performed with donor- and recipient covariates with Albumin:Creatinine ratio and Protein:Creatinine ratios, as outcomes respectively. Predictors assessed were based on baseline demographic and clinical variables. All analyses were completed using SPSS Statistics, Version 23 (IBM corp, USA). For *in vitro and invivo* experiments, unpaired *t* test was used to analyze data between two groups and analysis of variance followed by Bonferroni correction was used when more than two groups were present. Invitro Experiments were repeated at least three times to obtain standard deviations, and representative experiments are shown. Statistical significance was considered with two-tailed $P<0.05$.

Study approval: Institutional IRB approval for the human subjects in the study protocol was obtained from all 5 participating institutions and informed written consent was taken from all participants (Living donors and Recipients). All animal studies were performed in accordance with guidelines and protocols approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai.

STROBE Statement—***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Study design: Prospective cohort study GoCAR (Introduction Para 2, Line 2 Abstract)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found In Abstract Lines 1, 4-5
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Introduction Paragraphs – 2 & 3
Objectives	3	State specific objectives, including any prespecified hypotheses Introduction Para 4, Line 4
Methods		
Study design	4	Present key elements of study design early in the paper -Methods – GoCAR study Line 1 (Reference 4 Menon M, 2015 }
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Reference 4 Menon M, 2015 } & Supplemental Methods under GoCAR Study
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (Reference 4 Menon M, 2015 } & Supplemental Methods under GoCAR Study
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Reference 4 Menon M, 2015 } & Supplemental Methods under GoCAR Study
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Reference 4 Menon M, 2015 } & Supplemental Methods under GoCAR Study results: Under Para 2 Shroom3 SNP rs17319721 in the donor associates with reduced albuminuria after first transplant year:
Bias	9	Describe any efforts to address potential sources of bias NA
Study size	10	Explain how the study size was arrived at Use of data from GoCAR study – Power/sample size calculations were done for original GoCAR study
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Under Methods – Statistical Analysis and Supplemental Methods – Statistical analysis
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Under Methods – Statistical Analysis and Supplemental Methods – Statistical analysis
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed – Only patients with covariate data and

		outcomes were included, Others were excluded.
		(*d*) If applicable, explain how loss to follow-up was addressed - NA
		(*e*) Describe any sensitivity analyses - NA
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Table 1 A & 1B – Shows demographics, covariates and results of albuminuria
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Table 1 A & 1B – Shows demographics, covariates and results of albuminuria
		(b) Indicate number of participants with missing data for each variable of interest Table 1 A & 1B – Shows demographics, covariates and results of albuminuria
		(c) Summarise follow-up time (eg, average and total amount) Supplemental Methods – GoCAR study and Statistical analysis
Outcome data	15*	Report numbers of outcome events or summary measures over time Table 1 A shows mean ACR and PCR – outcomes , by SNP type
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Table 1A – unadjusted means; 1B – adjusted means
		(*b*) Report category boundaries when continuous variables were categorized NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Reported as coefficients Table 1-B
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses NA
Discussion		
Key results	18	Summarise key results with reference to study objectives Paragraph 1 in discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Paragraph 5 in discussion - Limitations
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Paragraph 2 discusses future experiments that are needed, Paragraph 2-3 discuss other relevant mechanistic data
Generalisability	21	Discuss the generalisability (external validity) of the study results Paragraph 2 discusses future experiments that are needed, and challenges for external validity of findings. Also in Para 5 Line-1
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based In Acknowledgements and Title page

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