# Figure S1

Supplementary Figure 1: *RARRES1* **mRNA expression in CKD cohort in NEPTUNE dataset.** The association of glomerular *RARRES1* expression level with eGFR (A), serum creatinine (B), and BUN (C) in NEPTUNE CKD dataset.

Figure S2

A   RARRES1 Over-Expression in Diabetic Nephropathy vs. Healthy Living Donor

Woroniecka Diabetes Glom (22)
Human | microarray | Glomeruli

Reporter:
221872_at

n=	22
p Value:	8.18e-4
Fold Change:	9.419
t Statistic:	4.928
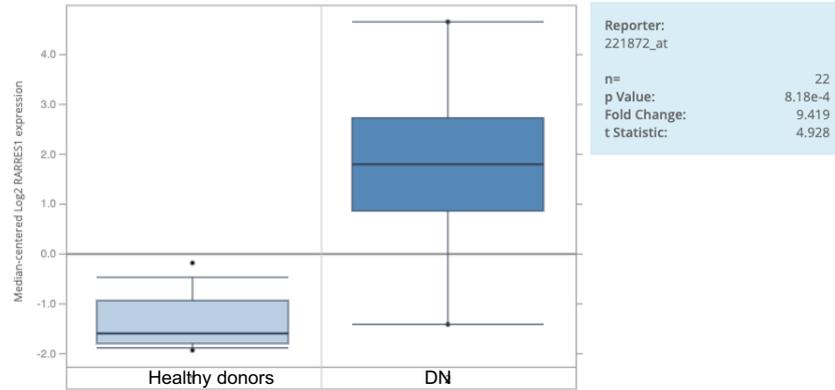
B

RARRES1 Expression Correlated with GFR (All Measured Samples)

Ju CKD Glom (199)
Human | microarray | Glomeruli

Reporter:
5918

n=	192
p Value:	2.31e-17
r Value:	-0.562
r² Value:	0.316
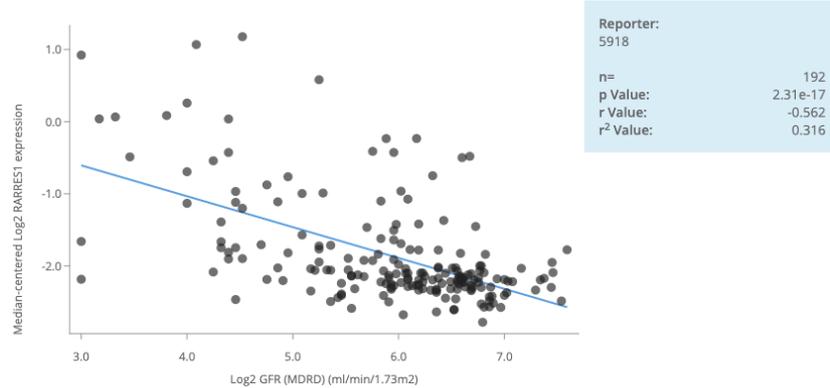
C

### RARRES1 Expression Correlated with GFR (All Measured Samples)

Woroniecka Diabetes Glom (22)
Human | microarray | Glomeruli

Reporter:
221872_at

|	
n=	22
p Value:	1.81e-5
r Value:	-0.781
r² Value:	0.610
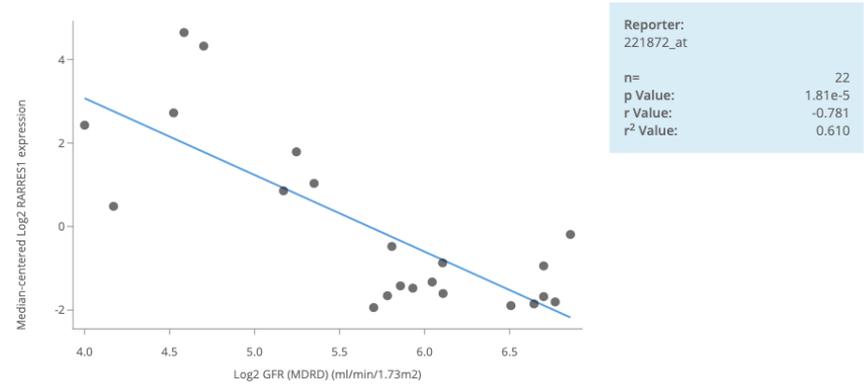
Supplementary Figure 2: *RARRES1* mRNA expression in CKD in Nephroseq dataset (nephroseq.org). (A) *RARRES1* expression in the glomeruli of DN patients and living donors. (B) Glomerular *RARRES1* expression correlates with eGFR in CKD cohort. (C) Glomerular *RARRES1* expression correlates with eGFR in DN cohort.

Figure S3
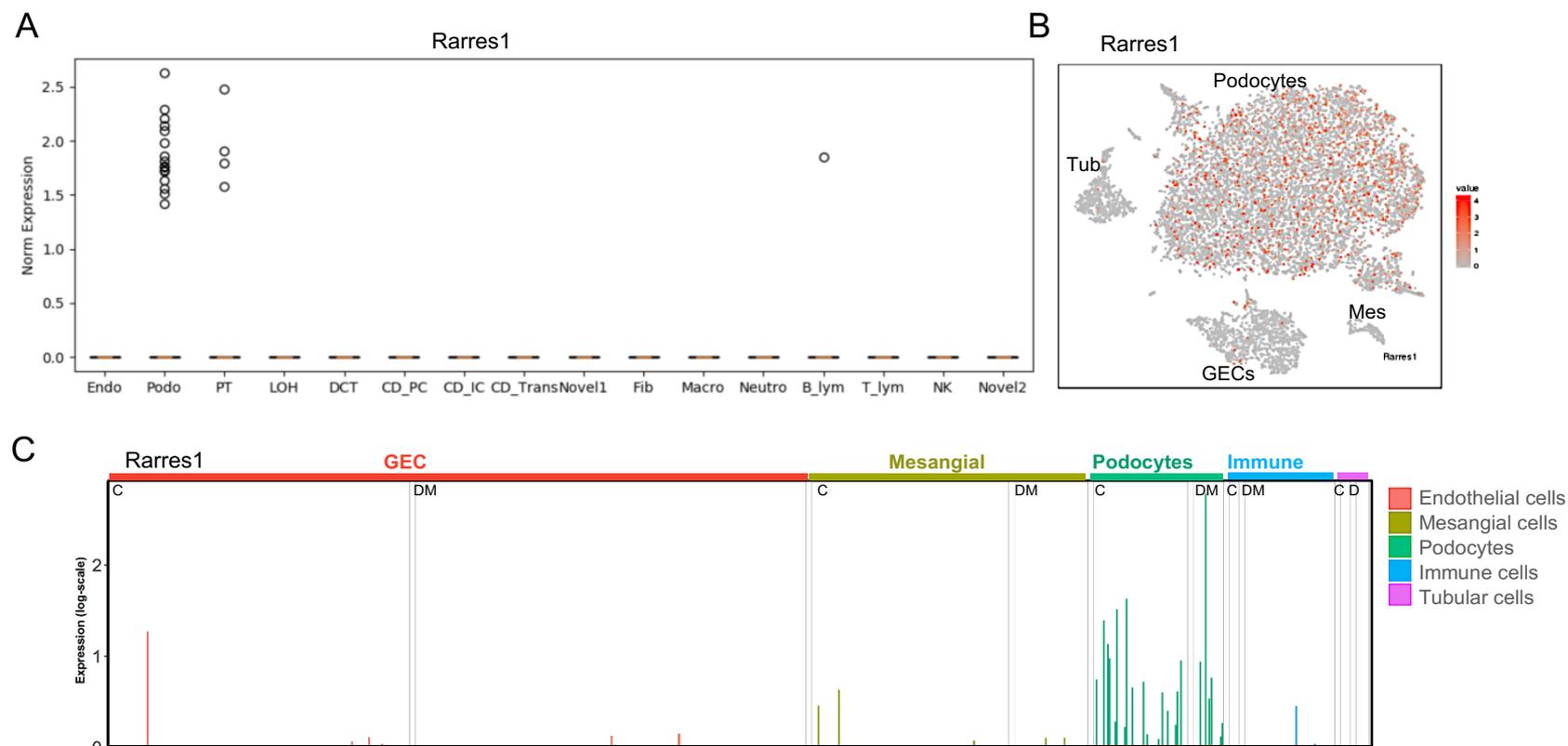
A

B

C

Supplementa
et al. 2018). (
endothelial cells; Tub, tubular cells
control; DM, diabetic.

Figure S4
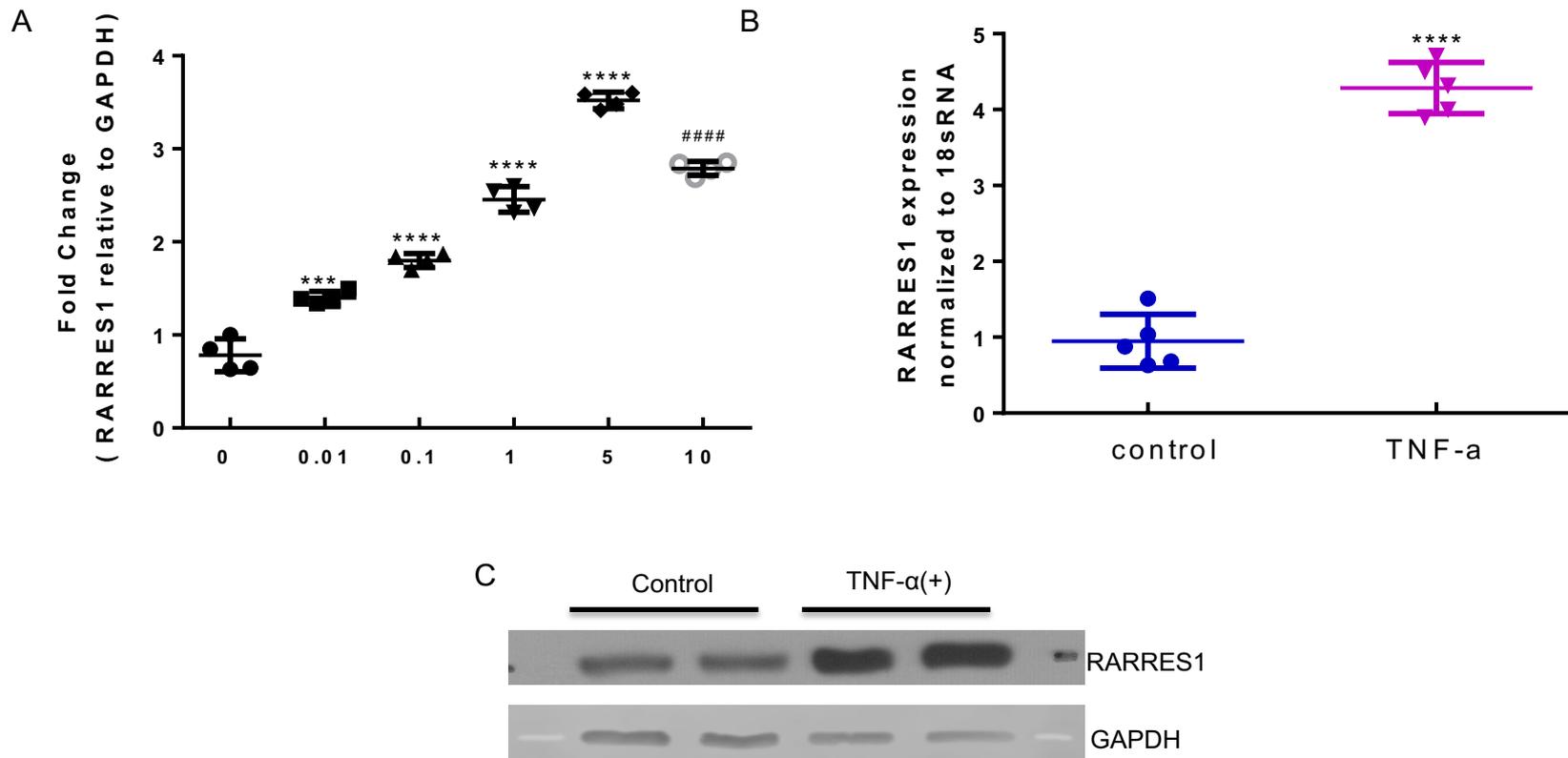
Supplementary Figure 4: RARRES1 is regulated by RA and TNF-α. (A) Real time PCR of RARRES1 with increasing doses of RA (μM) in cultured human podocytes. (B-C) TNF-α stimulate RARRES1 mRNA expression (B) and protein expression (C) in human podocytes. (***p<0.01 and ****p<0.001 VS control, ####p<0.05 vs 5μM atRA)
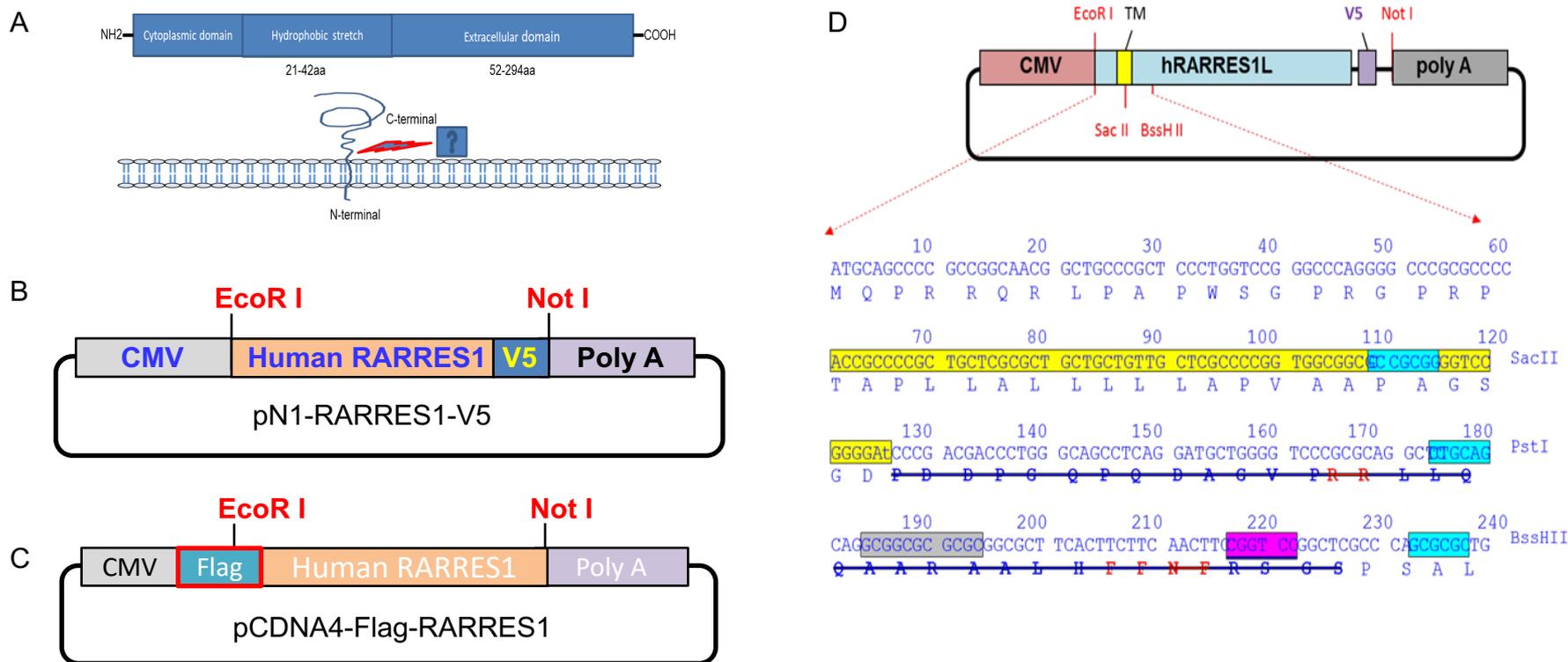
Figure S5

Supplementary Figure 5: Schematics of RARRES1 wildtype and mutant expression plasmids (A) The structure of the RARRES1 isoform 1 precursor, which is a type I transmembrane protein. (B) The schema of C-terminal V5- tagged RARRES1 overexpression plasmid. (C) The schema of N-terminal FLAG tagged RARRES1 overexpression plasmid. (D) Region surrounding aa 43-76 residues for constructing deletions or point mutation of RARRES1 as shown in Figure 3.

Figure S6
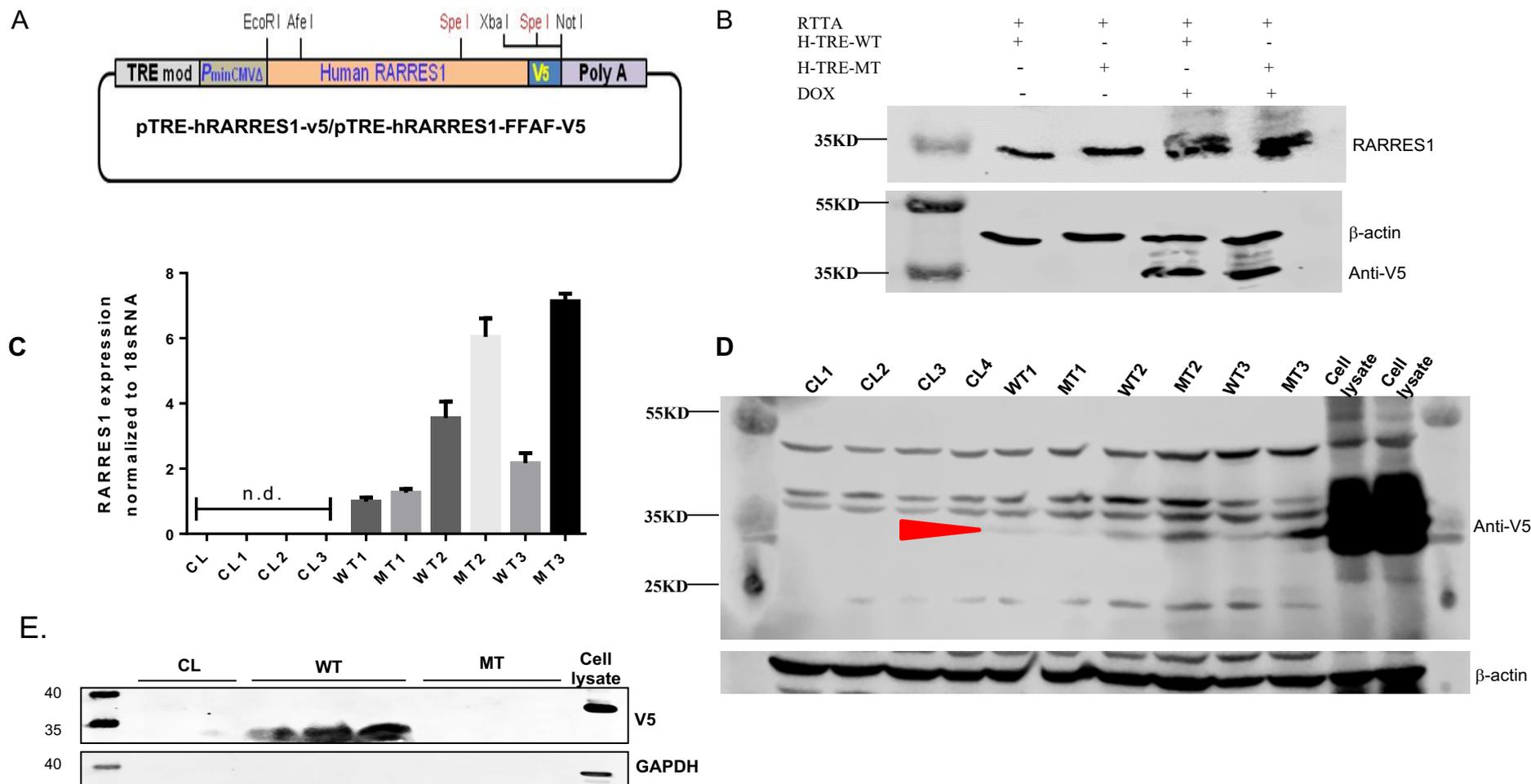
Supplementary Figure 6: Transgenic overexpression of RARRES1^WT and RARRES1^MT in mice. (A) tetracycline-inducible RARRES1 wild and mutant) overexpression plasmid. (B) pN1-RARRES1(FFNF)-v5 and pN1-RARRES1(FFAF)-v5 was transfected into Tet-U2OS cells. Induction with doxycycline resulted in the expression of RARRES1(FFNF)-V5 protein and RARRES1 (FFAF)-V5 protein. (C-D) The induction of Human RARRES1 gene expression in Nphs1-rtTA;TRE-RARRES1^WT and Nphs1-rtTA;TRE-RARRES1^MT mice was confirmed by real-time PCR (C) and western blot (D) analyses. E. RARRES1 was detected in the urine of RARRES1^WT mice but not in RARRES1^MT mice.

Figure S7

A LPE Rarres1_1170: two independent founders
   Guide sequence: UUCAGCAACAUAAAAUAACCAC
   miRE sequence: GAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCG ATGGTTATTTTATGTTGCTGAA TAGTGAAGCCACAGATGTA TTCAGCAACATAAAA TAACCACTGCCTACTGCCTCGGACTTCAAGGGGCTAGAATTCGAGCA

B
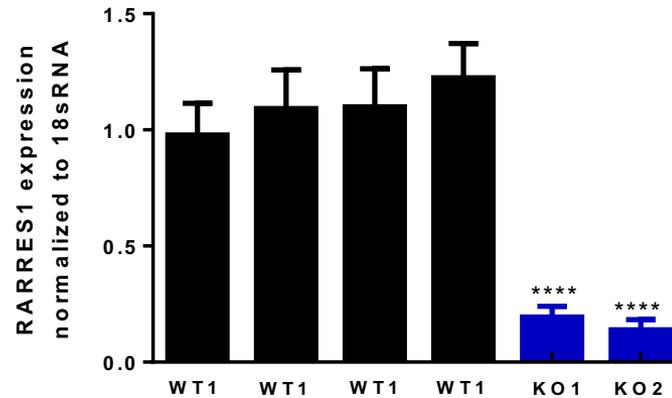
Supplementary Figure 7: RARRES1^KD in vivo. (A) *Rarres1* shRNA expression sequences for in vivo knockdown in mice are shown (procured from Mirimus Inc.). (B) *Rarres1* knockdown validation after Dox induction by real time PCR in two independent founders. ****P<0.0001 vs. WT controls.

Table S1

Gene	EntrezGene	correlation-slope	correlation-GFR	correlation-PCR
ALDH1A1	216	-0.382	0.032	-0.114
ALDH1A3	220	-0.190	-0.205	0.050
CACNA1C	775	0.139	-0.081	0.158
CCND2	894	-0.260	-0.183	0.358
CLCN3	1182	-0.046	0.024	0.189
CRABP	1381	0.096	0.099	-0.181
CYP1A1	1543	-0.024	0.045	-0.130
CYP1A2	1544	-0.039	-0.047	-0.046
CYP1B1	1545	0.014	-0.109	0.247
CYP26A1	1592	-0.124	-0.099	-0.103
DMD	1756	0.192	-0.148	0.091
FOXC1	2296	0.496	0.242	-0.044
GATA3	2625	0.340	0.026	-0.084
GDNF	2668	0.027	-0.031	-0.001
GSR	2936	-0.462	-0.048	0.038
GSTA4	2941	0.303	0.161	-0.249
GSTM2	2946	0.191	0.069	-0.126
Hox	3198	0.131	0.134	0.108
IFRD1	3475	-0.115	0.006	0.070
MCAM	4162	-0.284	-0.269	0.174
METTL1	4234	-0.269	0.037	0.057
PDE4	5141	0.358	0.162	0.090
PITX2	5308	0.025	-0.050	-0.022
PLAUR	5329	-0.223	-0.246	0.265
PPARg	5468	-0.100	-0.202	0.109
RARa	5914	-0.042	-0.073	0.010
RARb	5915	0.126	0.007	-0.036
RARg	5916	0.125	0.004	-0.035
RARRES1	5918	-0.588	-0.361	0.128
CRBP	5947	-0.214	-0.160	0.191
RBP4	5950	0.135	0.150	-0.115
RDH5	5959	0.021	0.002	-0.067
RHD1	6007	-0.032	-0.165	0.098
RORA	6095	0.136	0.336	-0.291
RXRa	6256	-0.056	0.162	-0.156
RXRb	6257	0.149	0.162	-0.069
RXRg	6258	-0.093	-0.017	-0.186
SIAT1	6480	0.189	0.076	0.073
VDR	7421	-0.271	-0.008	-0.193
YWHAZ	7534	-0.146	-0.038	0.028
FGF18	8817	-0.247	-0.167	-0.003
WISP1	8840	0.355	-0.006	-0.036
ALDH1A2	8854	0.276	0.127	0.161
PER2	8864	0.216	0.007	-0.167
LRAT	9227	-0.241	-0.080	-0.019
ISGF3G	10379	0.035	0.118	0.148
GABARAP	23710	0.092	0.172	0.053
KLF15	28999	-0.046	0.175	-0.258
CYP26B1	56603	0.154	0.111	-0.002
GNB4	59345	-0.068	-0.164	0.185
Stra6	64220	-0.054	0.088	-0.234
RDH10	157506	-0.214	0.083	-0.085

Table S1: Correlation of glomerular expression of RA-related genes with clinical outcomes. Living donors: n=6; MN: n=49; MCD: n=48; FSGS: n=50; Other (IgA etc) n=57. Correlation with GFR (glomerular filtration rate) slope, GFR and PCR (protein creatinine ratio).

Table S2

Patient #	Gender	Age	Pathology	eGFR ml/min/ 1.73m^2	24h-UP mg/day	UACR mg/g	CRP mg/L	Albumin g/L	TP g/L	BUN mmol/L	Serum Creatinine mmol/L
Patient 1	M	17	MCD	131.5	887.4	6206.7	0.35	14.90	42.90	4.03	70.5
Patient 2	M	16	MCD	147.99	4414.1	3128.0	0.17	12.50	32.50	5.40	53.8
Patient 3	F	29	MCD	141.255	7584.3	2372.4	0.31	13.50	33.80	3.20	35.4
Patient 4	F	17	MCD	108.99	11969.4	3661.5	6.09	18.57	48.40	2.55	70.4
Patient 5	F	45	MCD	118.193	1574.2	1095.1	0.21	28.80	51.50	4.40	43.2
Patient 6	F	56	FSGS	118.067	3412.5	1762.2	0.37	34.50	66.80	3.20	34.3
Patient 7	F	47	FSGS	128.405	537.1	276.2	2.87	40.10	69.10	3.30	32.2
Patient 8	M	31	FSGS	112.343	880.4	438.7	1.03	43.00	76.40	6.40	80.2
Patient 9	M	39	FSGS	74.669	3388.8	1726.6	0.15	40.10	78.00	8.70	112.5
Patient 10	M	30	FSGS	86.529	1864.6	304.9	0.27	35.90	58.80	5.40	100.2
Patient 11	F	56	DKD	58.88	4629.7	3221.6	1.35	31.50	60.50	10.95	93.4
Patient 12	M	69	DKD	83.11	1944.7	1414.1	8.39	32.97	59.66	10.49	82.5
Patient 13	F	45	DKD	112.24	1025.6	551.2	1.31	39.40	63.70	5.28	50.0
Patient 14	M	70	DKD	67.94	1505.8	532.6	5.34	38.37	67.82	6.49	96.9
Patient 15	M	37	DKD	70.993	856.8	276.3	0.28	45.40	79.40	9.40	113.3

Table S2: Clinical parameters of biopsied patients. eGFR, estimated glomerular filtration rate; 24h-UP, 24-hour urine protein; UACR, urine albumin-to-creatinine ratio; CRP, c-reactive protein; TP, total protein; BUN, blood urea nitrogen.

Table S3

GO Term	P value
GO:0051270~regulation of cell motion	6.748175
GO:0030334~regulation of cell migration	6.065517
GO:0042981~regulation of apoptosis	**5.894912**
GO:0043067~regulation of programmed cell death	**5.840816**
GO:0010941~regulation of cell death	**5.814847**
GO:0040012~regulation of locomotion	5.678864
GO:0010033~response to organic substance	5.407627
GO:0008219~cell death	5.397598
GO:0019220~regulation of phosphate metabolic process	5.300013
GO:0051174~regulation of phosphorus metabolic process	5.300013
GO:0016265~death	5.26169
GO:0006915~apoptosis	5.236821
GO:0012501~programmed cell death	5.040145
GO:0042325~regulation of phosphorylation	4.962365
GO:0001944~vasculature development	4.60758
GO:0042127~regulation of cell proliferation	4.239477
GO:0043549~regulation of kinase activity	4.211206
GO:0001568~blood vessel development	4.197242
GO:0033273~response to vitamin	4.193605
GO:0051338~regulation of transferase activity	4.178514

Table S4

GO Term	P value
GO:0000279~M phase	**4.51E-11**
GO:0007049~cell cycle	**8.48E-11**
GO:0022403~cell cycle phase	**1.87E-10**
GO:0022402~cell cycle process	**2.92E-10**
GO:0048285~organelle fission	2.96E-10
GO:0000280~nuclear division	4.10E-10
GO:0007067~mitosis	4.10E-10
GO:0000087~M phase of mitotic cell cycle	6.78E-10
GO:0000278~mitotic cell cycle	1.21E-09
GO:0051301~cell division	1.95E-09
GO:0030198~extracellular matrix organization	3.85E-08
GO:0006260~DNA replication	6.52E-08
GO:0051726~regulation of cell cycle	2.08E-07
GO:0006259~DNA metabolic process	3.55E-07
GO:0000122~negative regulation of transcription from RNA poly II promoter	1.61E-06
GO:0006974~response to DNA damage stimulus	2.01E-06
GO:0043062~extracellular structure organization	2.66E-06
GO:0042127~regulation of cell proliferation	2.74E-06
GO:0031327~negative regulation of cellular biosynthetic	1.18E-05
GO:0033554~cellular response to stress	1.39E-05

Table S3: RNA-seq between control and RARRES1 overexpressing podocytes (GO Biological Process for limma p005fc1.5 up DEGs).
Table S4 DEGs stimulated by TNF-α but down-regulated by RARRES1 KD（GO Biological Process for limma p005fc1.5 down DEGs).

Table S5

Construct	Linker primer (LF)	Linker primer (LR)
RARRES1△aa43-76-V5	5'-GGGGTCCGGGGATCCCAG-3'	5'-CGCGCTGGGATCCCCGGACCCCGC-3'
RARRES1△aa43-55-V5	5'-GGGGTCCGGGGACCGCAGGCTCCTGCA-3'	5'-GGAGCCTGCGGTCCCCGGACCCCGC-3'
RARRES1△FFNF-V5	5'-GCAGGCGGCGAGGGCGGCGCTTCACAGATCTGGCTCG CCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGTGAAGCGCCGCCCTCGC CGCCTGCTGCA-3'
pN1-AAAA-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACGCGGCTGCTGCTAGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTAGCAGCAGCCGCGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'
pN1-AFNF-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACGCTTTCAACTTCAGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGAAGTTGAAAGCGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'
pN1-FANF-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACTTCGCCAACTTC AGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGAAGTTAAGCGAAGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'
pN1-FFAF-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACTTCTTCGCTTTCAGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGAAAGCGAAGAAGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'
pN1-FFNA-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACTTCTTCAACGCCAGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGGCGTTGAAGAAGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'
pN1-FFDF-v5	5'-GCAGGCGGCGAGGGCGGCGCTTCACTTCTTCGATTTCAGATCTGGCTCGCCTAG-3'	5'-CGCGCTAGGCGAGCCAGATCTGAAATCGAAGAAGTGAAGCGCCGCCCTCGCCGCCTGCTGCA-3'

Table S5: Primers for construction of human RARRES1 expression plasmids

Members of the Nephrotic Syndrome Study Network (NEPTUNE)
NEPTUNE Enrolling Centers

Case Western Reserve University, Cleveland, OH: J Sedor[*], K Dell[**], M Schachere[#]
Children's Hospital, Los Angeles, CA: K Lemley[*], L Whitted[#]
Children's Mercy Hospital, Kansas City, MO: T Srivastava[*], C Haney[#]
Cohen Children's Hospital, New Hyde Park, NY: C Sethna[*], K Grammatikopoulos[#]
Columbia University, New York, NY: G Appel[*], M Toledo[#]
Emory University, Atlanta, GA: L Greenbaum[*], C Wang[**], B Lee[#]
Harbor-University of California Los Angeles Medical Center: S Adler[*], C Nast[*‡], J La Page[#]
John H. Stroger Jr. Hospital of Cook County, Chicago, IL: A Athavale[*], M Itteera[#]
Johns Hopkins Medicine, Baltimore, MD: A Neu[*], S Boynton[#]
Mayo Clinic, Rochester, MN: F Fervenza[*], M Hogan[**], J Lieske[*], V Chernitskiy[#]
Montefiore Medical Center, Bronx, NY: F Kaskel[*], N Kumar[*], P Flynn[#]
NIDDK Intramural, Bethesda MD: J Kopp[*], E Castro-Rubio[#], E Brede[#]
New York University Medical Center, New York, NY: H Trachtman[*], O Zhdanova[**], F Modersitzki[#], S Vento[#]
Stanford University, Stanford, CA: R Lafayette[*], K Mehta[#]
Temple University, Philadelphia, PA: C Gadegbeku[*], D Johnstone[**], Z Pfeffer[#]
University Health Network Toronto: D Cattran[*], M Hladunewich[**], H Reich[**], P Ling[#], M Romano[#]
University of Miami, Miami, FL: A Fornoni[*], L Barisoni[*], C Bidot[#]
University of Michigan, Ann Arbor, MI: M Kretzler[*], D Gipson[*], A Williams[#], R Pitter[#]
University of North Carolina, Chapel Hill, NC: P Nachman[*], K Gibson[*], S Grubbs[#], Anne Froment[#]
University of Pennsylvania, Philadelphia, PA: L Holzman[*], K Meyers[**], K Kallem[#], FJ Cerecino[#]
University of Texas Southwestern, Dallas, TX: K Sambandam[*], E Brown[**], N Johnson[#]
University of Washington, Seattle, WA: A Jefferson[*], S Hingorani[**], K Tuttle[**§], K Klepach[#], S Dismuke[#], A Cooper[#§]
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The National Center for Advancing Translational Sciences (NCATS) Program Office: T Urv, PJ Brooks

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