Podocyte p53 limits the severity of experimental Alport syndrome

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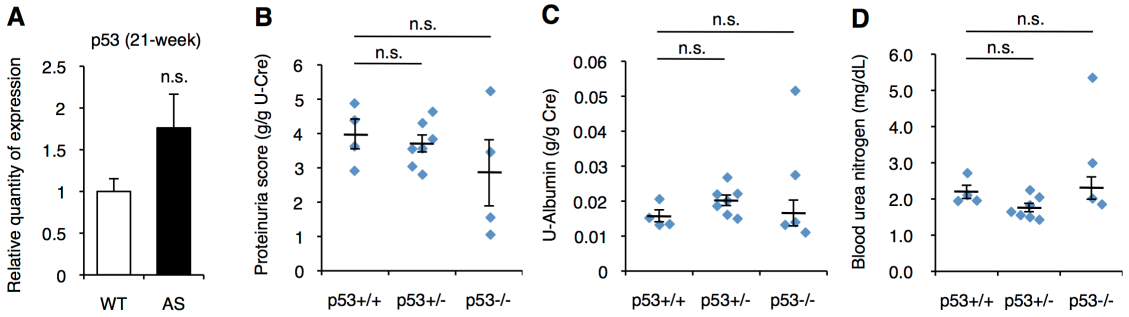
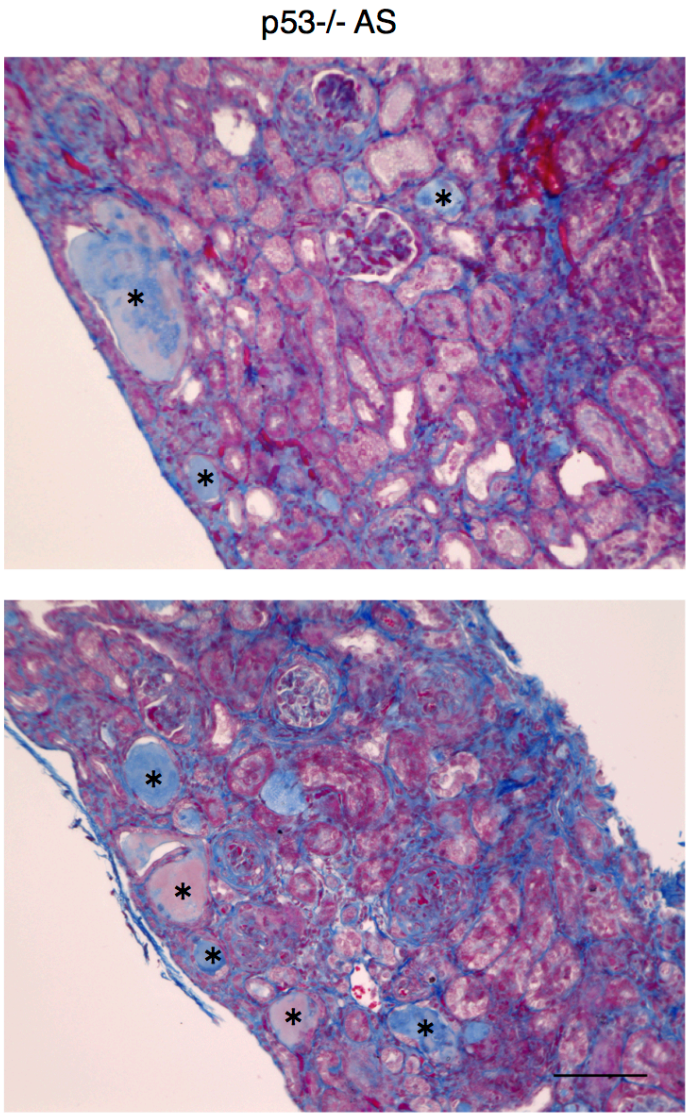
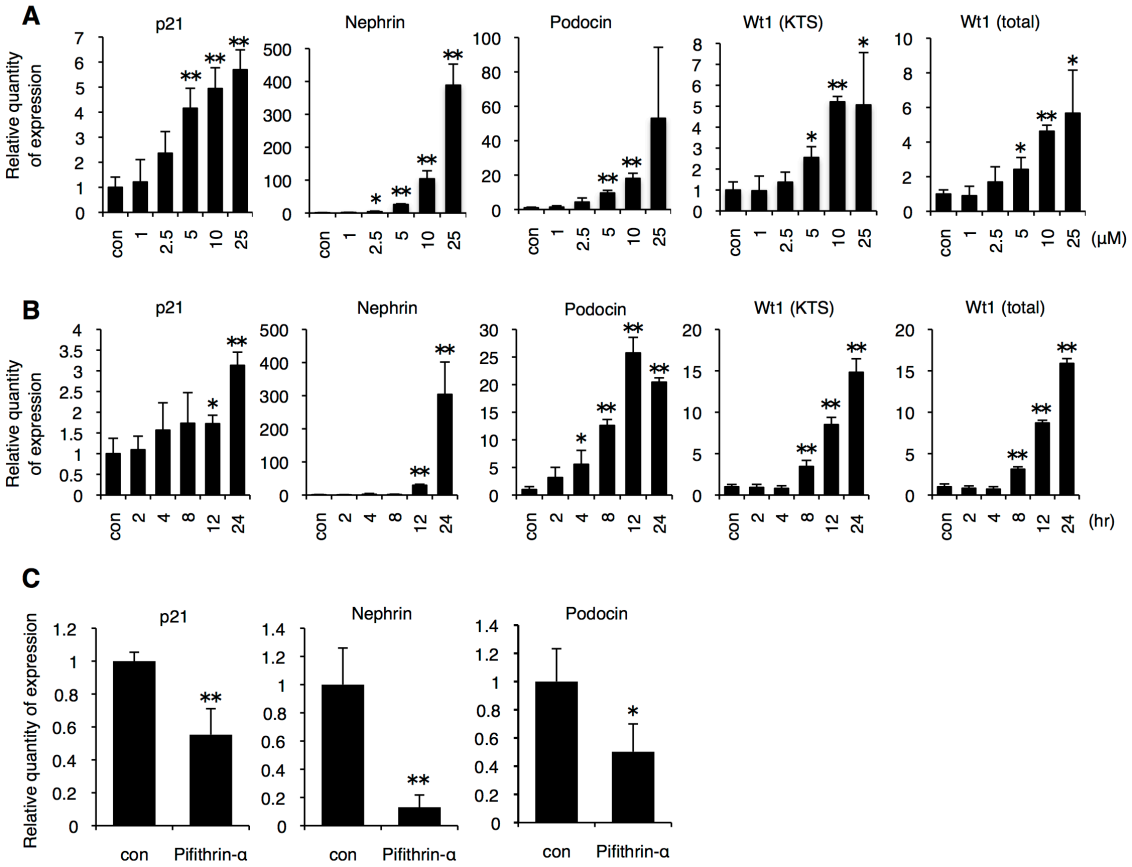


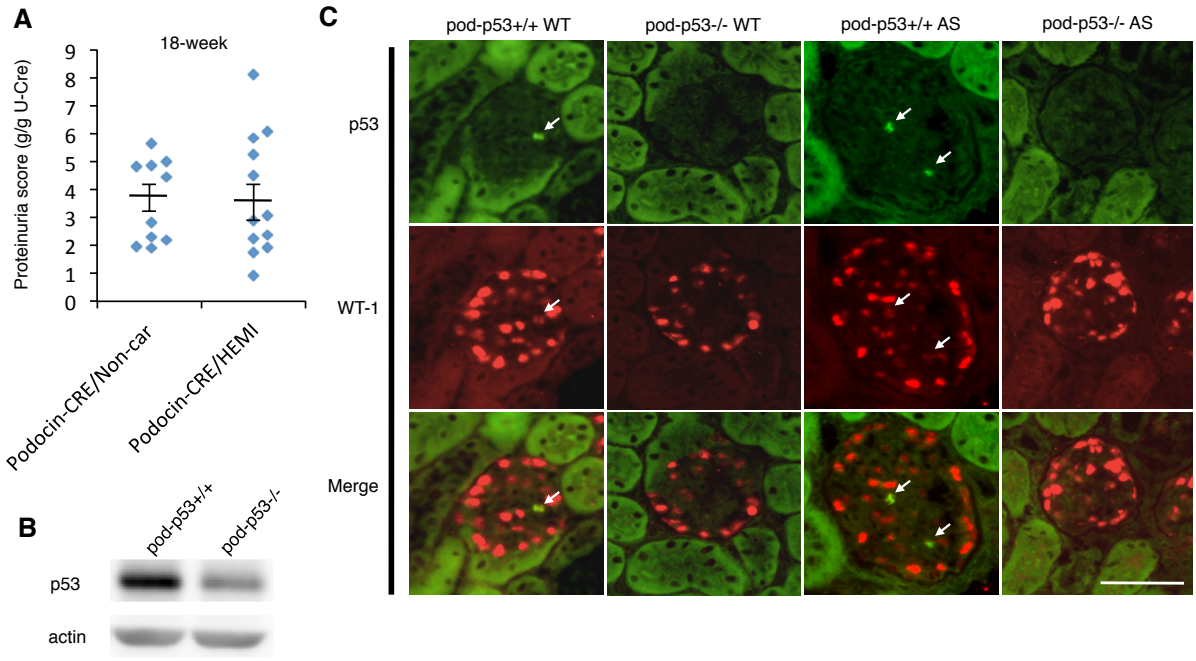
Supplementary Figure 1. Whole-body p53 deletion has no effect on renal function. (**A**) p53 mRNA expression in the glomeruli of 21-week-old WT and AS mice was determined by real time RT-PCR. mean ± s.e.m., n=3. Urine and plasma samples were obtained from 15-week-old p53+/+, +/- and -/- mice. (**B**) Proteinuria, (**C**) Urine-albumin and (**D**) BUN score were measured. (mean ± s.e.m., n=3-7). There were no differences in renal function between each p53 genotype. n.s., not significant.



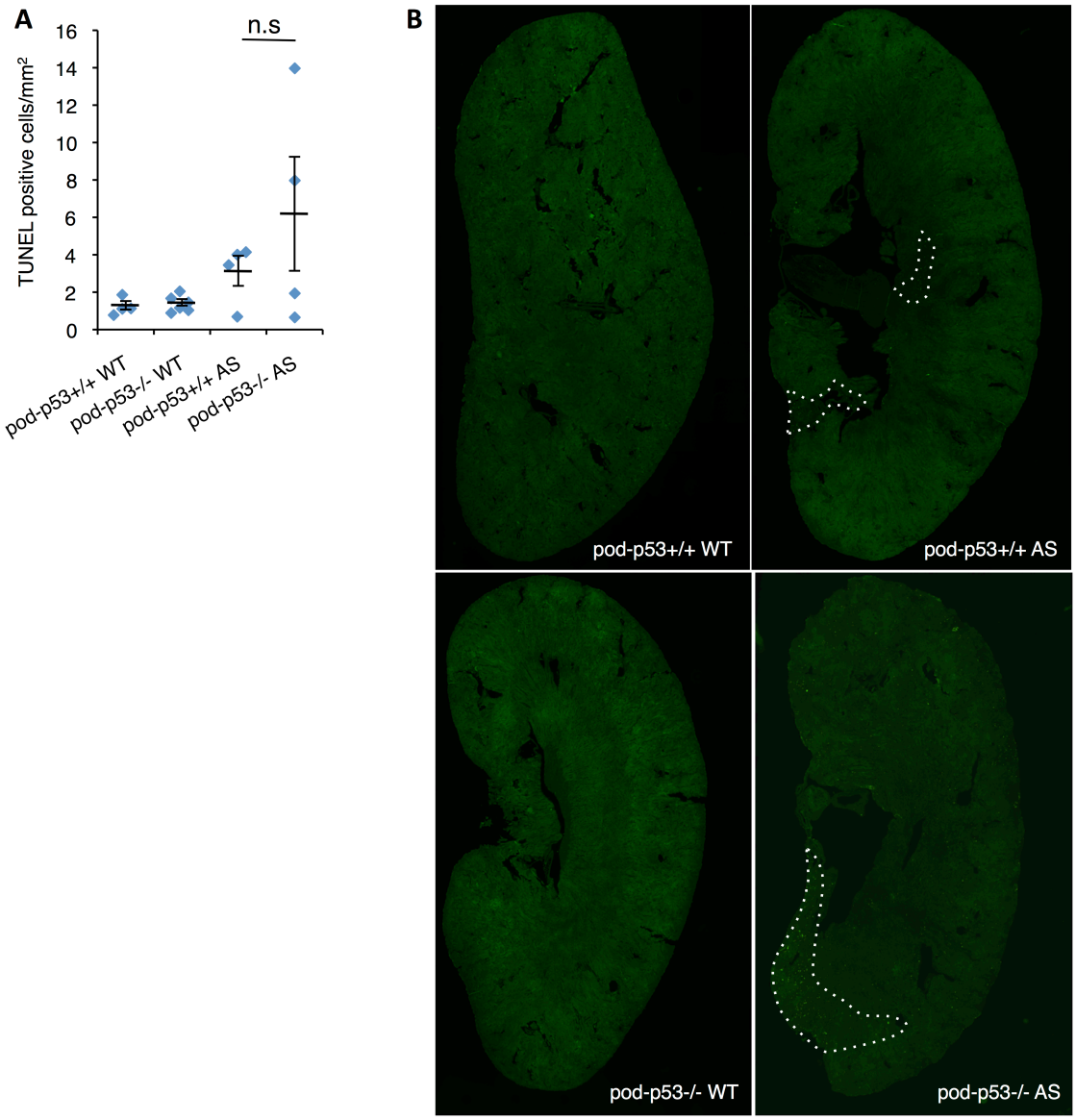
Supplementary Figure 2. Whole-body p53 knockout dramatically enhanced renal injury in AS mouse. Masson's trichrome staining was performed in 15-week-old p53-/- AS mouse. Severe renal fibrosis was observed. Tubular dilation and protein cast formation (marked as *) was dramatically enhanced. Scale bar, 100 μm.



Supplementary Figure 3. p53 positively regulates podocyte-specific genes in MPC-5 and primary GECs. (**A**) Differentiated MPC-5 cells were treated with the indicated concentration of nutlin-3α for 24 hr or (**B**) treated with 10 μM nutlin-3α for 2 to 24 hr. Real time RT-PCR was performed to analyze the expression of genes. *WT-1* (KTS) means transcriptional variant, which contains KTS region that is important for the regulation of renal function genes. Primers used for detecting *WT-1* (total) recognize all transcriptional variants of *WT-1*. (**C**) Primary GECs were treated with 50 μM pifithrin-α for 24 hr to suppress p53 function. Expression of *p21*, *Nephrin* and *Podocin* was analyzed by real time RT-PCR. *Gapdh* was used as internal control. (mean ± s.e.m., n=3). *P < 0.05, **P < 0.01.

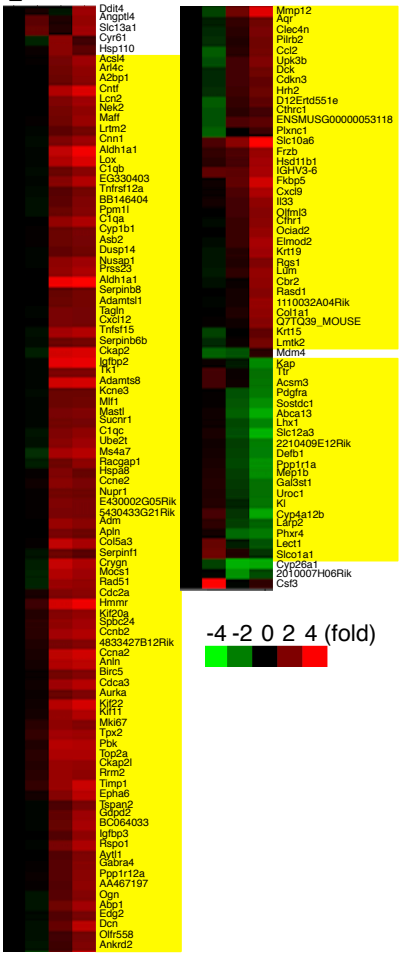


Supplementary Figure 4. Influence of podocyte-specific gene editing on the renal phenotype. (**A**) Proteinuria score was measured and compared between *Podocin*-Cre recombinase non-carrier mice (*Podocin*-Cre/non-car) and *Podocin*-Cre recombinase carrier mice (*Podocin*-Cre/HEMI). 18-week-old littermate mice were separated in each group. Urine samples collected for 24 hr were used for assay. (mean ± s.e.m., n=10, 12). (**B**) p53 expression in the glomeruli of pod-p53+/+ or pod-p53-/- mouse was analyzed by Western blotting. Glomeruli were isolated from 24-week-old mice by magnetic beads method. (**C**) Frozen sections of renal cortex harvested from 15-week-old mice were stained for immunofluorescence with antibodies against p53 (green) and WT-1 (red) or with type-IV collagen (green) and WT-1 (red). White arrows indicate p53, WT-1 and merged cells. Scale bars, 50 μm.



Supplementary Figure 5. p53 deletion promoted apoptosis in partial regions of renal tissue in AS mouse. Apoptotic cells in the renal tissue of 15-week-old pod-p53+/+ or -/- WT and AS mice were analyzed by TUNEL assay. (**A**) TUNEL-positive cells / mm^2 of renal tissue were quantified. (mean ± s.e.m., n=4-6). There were no statistical differences among groups. (**B**) Images of TUNEL-stained renal tissue revealed that apoptotic cells in pod-p53+/+ and pod-p53-/- AS groups were localized in atrophic regions (shown by broken line).

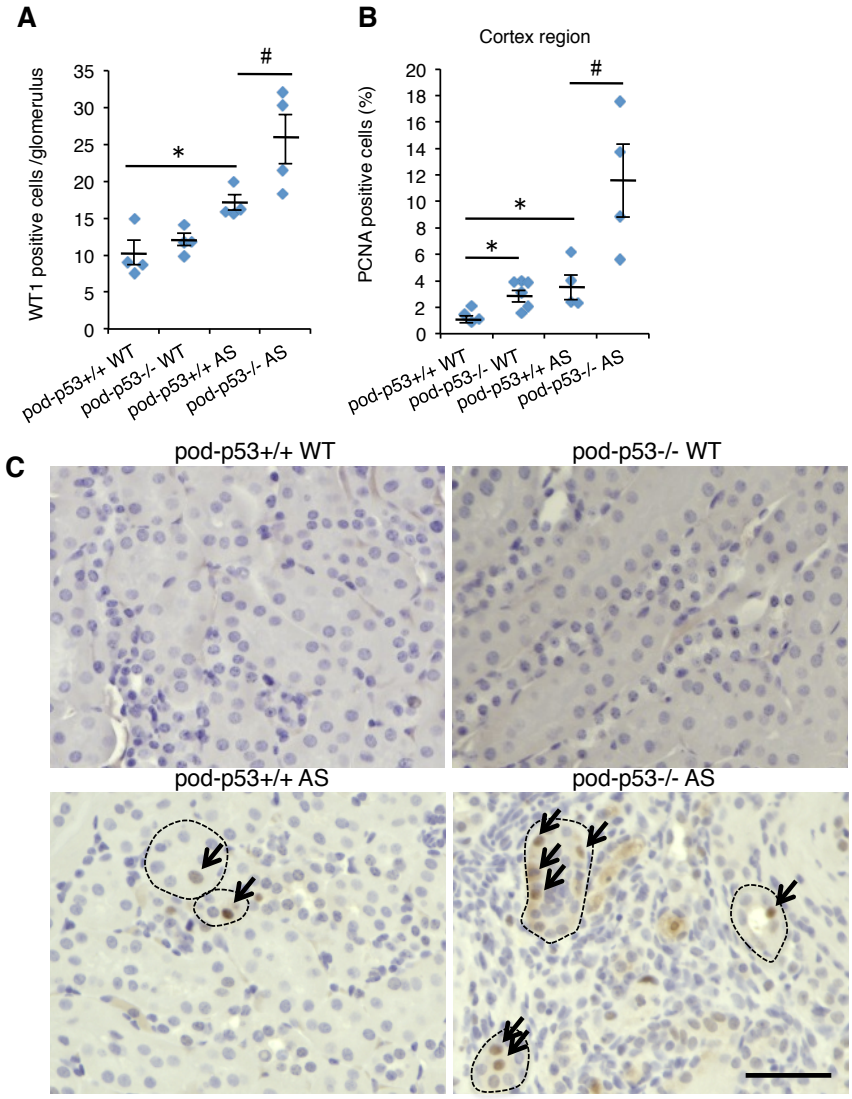
A



B

	Whole kidney (vs. pod-p53+/+ WT, n=4)		
	WT	AS	
pod-p53	-/-	+/+	-/-
n=	7	3	4
Cyp1b1	0.85(±0.07)	1.35(±0.17)	1.9(±0.23)*
Dusp14	1.17(±0.06)	1.22(±0.08)*	1.63(±0.05)**,##
Serpinb8	0.93(±0.05)	1.1(±0.14)	1.71(±0.24)*
Tnfsf15	0.75(±0.07)*	2.21(±0.34)**	2.62(±0.37)**
Elmod2	1.07(±0.06)	1.31(±0.08)	1.53(±0.14)*
Rasd1	0.92(±0.07)	1.91(±0.32)*	2.91(±1.06)
Col1a1	0.96(±0.13)	5.61(±0.98)**	11.83(±2.34)**
Lmtk2	1.02(±0.07)	0.96(±0.02)	0.82(±0.05)
Crygn	N.A.	N.A.	N.A.
Rad51	1.25(±0.12)	1.49(±0.12)	1.85(±0.14)**
Cdc2a	0.76(±0.05)*	3.7(±0.30)**	10.17(±2.83)*
Kif20a	1(±0.14)	3.3(±0.08)**	5.6(±0.64)**,#
Igfbp3	1.28(±0.09)	0.49(±0.03)*	0.34(±0.05)**
Rspo1	1.19(±0.09)	5.86(±0.90)**	7.49(±2.35)*
Abp1	0.97(±0.09)	11(±2.19)**	33.64(±6.67)**,#
Kcne3	1.11(±0.06)	1.72(±0.52)	1.88(±0.34)
Ube2t	1.11(±0.11)	3.07(±0.81)*	5.4(±1.14)**
Racgap1	1.04(±0.08)	2.8(±0.40)**	4.92(±0.77)**
Ccne2	1.16(±0.15)	2.2(±0.14)**	3.32(±0.45)**
Kif22	1.18(±0.18)	3.63(±0.29)**	7.31(±1.37)**
Kif11	1.2(±0.15)	3.61(±0.53)**	7.54(±1.64)**
Pbk	1.31(±0.25)	4.41(±0.84)**	6.91(±0.46)**,#
Ckap2l	1.08(±0.14)	3.97(±0.66)**	7.35(±1.29)**
Upk3b	1.39(±0.36)	2.5(±0.53)*	3.33(±2.15)
Cdkn3	1.26(±0.23)	4.73(±0.48)**	9(±1.58)**
Frzb	1.1(±0.08)	1.85(±0.02)**	1.93(±0.28)*
Fkbp5	1.19(±0.13)	1.41(±0.15)	1.35(±0.09)
Cntf	0.96(±0.09)	1.65(±0.18)*	2.57(±0.60)*
Adamtsl1	1.19(±0.08)	2.46(±0.28)**	4.34(±1.46)
Lhx1	0.91(±0.05)	0.93(±0.04)	0.77(±0.12)
Larp2	0.89(±0.03)	1.06(±0.07)	0.93(±0.07)
Pdgfra	1.3(±0.09)	1.91(±0.29)*	3.43(±1.12)

Supplementary Figure 6. (**A**) Microarray analysis of glomerular gene expression pattern. Microarray analysis of mRNA extracted from the glomeruli of indicated mouse group. Cluster analysis was performed using Cluster 3.0 software. The whole image is shown. Details of the gene cluster in yellow boxes are shown in main Figure 6B. (**B**) Total RNA was extracted from whole kidneys of 15-week-old pod-p53+/+ WT, pod-p53-/- WT, p53+/+ AS and p53-/- AS mice. The mRNA expression level of the indicated genes was analyzed by quantitative RT-PCR. *Gapdh* was used as internal control. The values are mean ± s.e.m. (n=4). *P < 0.05, **P < 0.01 vs. pod-p53+/+ WT. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 vs. pod-p53+/+ AS.



Supplementary Figure 7. Podocyte-p53 deletion promoted proliferation of podocytes and non-glomerular cells in kidneys of AS mice. (**A**) Immunofluorescent staining against WT-1 antigen was performed on renal sections of 15-week-old mice shown in Figure 4B (WT-1). WT-1-positive cells per glomeruli were counted. WT-1-positive cells were counted in 30 to 113 glomeruli per mouse. (mean ± s.e.m., n=4-6). (**B**) PCNA-positive cells in the cortex region were counted and quantified from the result of immunohistochemical staining. PCNA-positive cells were frequently observed in non-glomerular region such as tubular cells of pod-p53-/- AS mouse group. (mean ± s.e.m., n=3-6). *P < 0.05 vs. pod-p53+/+ WT. $^{\#}$P < 0.05 vs. pod-p53+/+ AS. (C) Renal cortex sections from 15-week-old mice of the indicated genotype were stained with PCNA and counterstained with hematoxylin. Black arrows indicate PCNA-positive cells. Tubular region is indicated by black broken lines. Scale bars, 50 μm

	primer FW	primer RV
mCyp1b1	CACCAGCCTTAGTGCAGACAG	GAGGACCACGGTTTCCGTTG
mDusp14	TTGCTCAGATCACCTCCTCTC	AGTACAGTCTAATGGGGGCAT
mSerpinb8	AGGCTGGACTAGAAGAGCTGT	CGTGTACTTTCGGTCAAACTGA
mAdamtsl1	CTGTGAGGCGGTAGGCAAC	TCCCAAGGCATTTGTGGCATT
mTnfsf15	TCTGGTCAGAAGGGATCAGAAG	GTCCTGCGAGGATGGGAAATG
mKcne3	ATGGAGACTTCCAACGGGACT	GCCCGACGATCCTCAGTTTG
mUbe2t	GTGCTGGCAGGAAAAGGATCA	ATCGGACCTGTGGAGGTTCAA
mRacgap1	TGCCCGTAATCAAGTGGACG	CTTGGCCTCGGTTGAGGAAAG
mCcne2	GGGAGACATTTTACCTTGCCC	TCTTGGAGTTTAGGAGCGTAGAT
mCrygn	GCCAGTGCCTAGAGTTCGTG	AAGCAGCGGTAGTCTCCTCTC
mRad51	CGGGAGTTGGTGGGTTATCC	CCGGCACATCTTGGTTTATTTGT
mCdc2a	GCAATGGGGACCCCTTTTTC	GGTGTAGATGTTCCTAACCGC
mKif20a	CAGCGGGCTTACTCTCTGATG	TCCTCCAGTAGAGCCTGCTTG
mKif22	CTGCTCTCTTGAAGTGGCTAAC	CCATAGGCAAGTACACTGGCAT
mKif11	GGCTGGTATAATTCCACGCAC	CCGGGGATCATCAAACATCTG
mPbk	TGGGCCGTGAAAAAGATAAGTC	CTGGCTTCAGTAAAAGCACGATA
mCkap2l	CAGCCAAGGGAAAGCTAAAGG	TGCTAGGCAAAACATGATTGGA
mIgfbp3	CCAGGAAACATCAGTGAGTCC	GGATGGAACTTGGAATCGGTCA
mRspo1	GGGATCAAGGGCAAGAGACAG	CTGGCGGATGTCGTTCCTC
mAbp1	GCGTGTTGCCTATGAGGTCAG	AAAGCATCCAGGAAAGTAGCG
mUpk3b	AGACCTGATTGCCTACGTGC	GGTGTCCTTAGTTGAGACATGCT
mCdkn3	ATGAAGCCGCCCATTTCAATA	GGAAGAGCACATAAACCGAGAA
mFrzb	GCTGTGCAAGTCCCTTCCC	TGCAAATGGGTGCGTACATTG
mFkbp5	TGAGGGCACCAGTAACAATGG	CAACATCCCTTTGTAGTGGACAT
mElmod2	TGGACACTTTTTCCGATTTTGGA	ACTCGTGTTGCATTCTGTAGGA
mRasd1	GATGTGCCCAAGCGACTCT	TGAGGAAGCGCGACACAAT
mCol1a1	CTGGCGGTTCAGGTCCAAT	TTCCAGGCAATCCACGAGC
mLmtk2	GCAGGGGAAGTACCTGTTGTA	CTGGTGGGGTGAAGTCTATCT
mCntf	TAGAGCGGCTACAGAGGTCC	CAAACCAGCTCACTTGTTTCC
mPdgfra	GGAGACTCAAGTAACCTTGCAC	TCAGTTCTGACGTTGCTTTCAA
mLhx1	CCCATCCTGGACCGTTTCC	CGCTTGGAGAGATGCCCTG
mLarp2	ACACGGGGTCTCAGAGTGTTA	TCACATCATCTAAGTGGAGTGGT

Supplementary Table 1. Sequences of primers used for quantitative RT-PCR to confirm the mRNA expression of altered genes in the microarray analysis.