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**Supplemental information**

**Therapeutic silencing of SMOC2  
prevents kidney function loss in mouse  
model of chronic kidney disease**

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## SUPPLEMENTARY TABLES

ANTIBODY	DILUTION	IDENTIFIER	CAT. NO
SMOC2	1:250	Santa Cruz Biotechnology Inc.	sc-67396
$\alpha$ -SMA	1:500	Sigma-Aldrich	A2547
COLLAGEN 1	1:200	Abcam	ab600408
FIBRONECTIN	1:200	Abcam	ab23750
F4/80	1:200	Abcam	ab6640
PDGFR $\beta$	1:200	Abcam	ab32570

Supplemental table 1 (Table S1), related to STAR Methods, section of *In vivo* immunofluorescence.

List of primary antibodies for immunostaining.

ANTIBODY	DILUTION	IDENTIFIER	CAT. NO
P-P42/44 (T202/Y204)	1:1000	Cell Signaling	4377S
P-P38 (T180/Y182)	1:1000	Cell Signaling	9211S
P-JNK	1:1000	Cell Signaling	9251S
P-AKT (S473)	1:1000	Cell Signaling	9271S
P-MTOR	1:1000	Cell Signaling	2971S
P-SMAD 2 (S465/467)/3 (S423/425)	1:1000	Cell Signaling	13820S
SMOC2	1:1000	Santa Cruz Biotechnology Inc.	sc-67396
BCN1 (E-8)	1:1000	Santa Cruz Biotechnology Inc.	sc-48341
COLLAGEN 1	1:1000	Abcam	ab600408
FIBRONECTIN	1:1000	Abcam	ab23750
GAPDH	1:1000	Abcam	ab181602
$\alpha$ -SMA	1:5000	Sigma-Aldrich	A2547

Supplemental table 2 (Table S2), related to STAR Methods, section of Immunoblotting. List of primary antibodies for Western Blot.

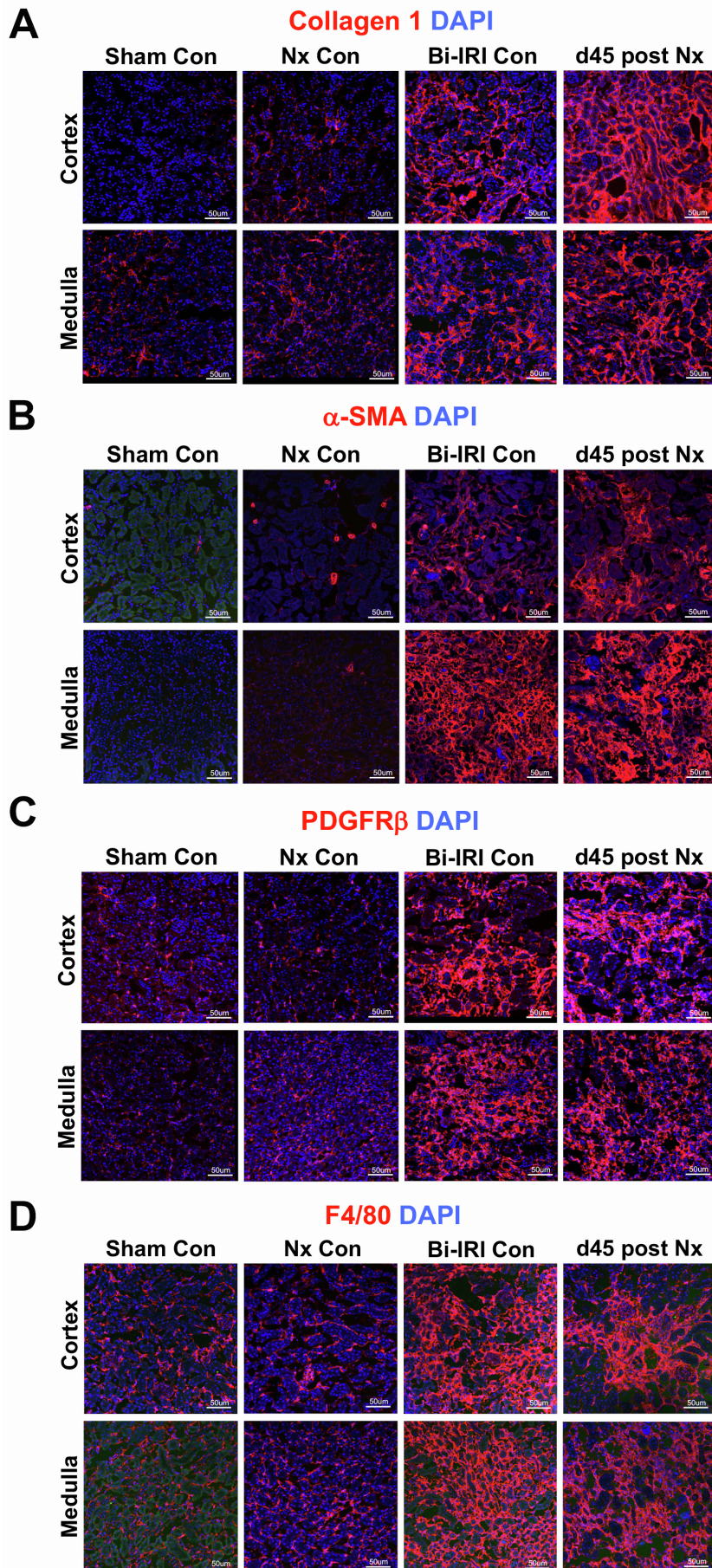
<b>INHIBITOR</b>	<b>INHIBITOR OF</b>	<b>IDENTIFIER</b>	<b>CAT. NO</b>
<b>ATN-161 (AC-PHSCN-NH<sub>2</sub>)</b>	Integrin $\alpha$ 5	Selleckchem	S8454
<b>CHLOROQUINE DIPHOSPHATE SALT</b>	Lysosome	Sigma	C 6628-50G
<b>GW788388</b>	ALK5/TGF $\beta$ RII	Selleckchem	S2750
<b>LY2109761</b>	TGF $\beta$ RII	Selleckchem	S2704
<b>LY294002</b>	Akt	GIBCO	PHZ1144
<b>3-MA</b>	Autophagy	Millipore Sigma	189490-100MG
<b>MHY1485</b>	mTOR Activator	Millipore Sigma	5005540001
<b>SB203580</b>	P38	Selleckchem	S1076
<b>SB273005</b>	Integrin	Selleckchem	S7540
<b>SB431542</b>	ALK5	Selleckchem	S1067
<b>SP600125</b>	JNK	Selleckchem	S1460
<b>U0126</b>	P42/44	EMD Millipore	19-147

Supplemental table 3 (Table S3), related to STAR Methods, section of *In vitro* inhibitional experiments. List of inhibitors.

<b>GENE</b>	<b>SEQUENCES</b>
<i>Collagen 1</i> Forward Reverse	5'- GAG CGG AGA GTA CTG GAT CG-3' 5'- GTT CGG GCT GAT GTA CCA GT-3'
<i>Fibronectin</i> Forward Reverse	5'- CCA CCC CCA TAA GGC ATA GG-3' 5'- GTA GGG GTC AAA GCA CGA GTC ATC-3'
<i><math>\alpha</math>-SMA</i> Forward Reverse	5'- ATC ATG CGT CTG GAC TTG G-3' 5'- AAT AGC CA GCT Cag TCA GG-3'
<i>PDGFR<math>\beta</math></i> Forward Reverse	5'- CAC CTT CTC CAG TGT GCT GA-3' 5'- GGA GTC CAT AGG GAG GAA GC-3'
<i>IL-1<math>\beta</math></i> Forward Reverse	5'- GCA CTA CAG GCT CCG AGA TGA AC-3' 5'- TTG TCG TTG CTT GGT TCT CCT TGT-3'
<i>IL-6</i> Forward Reverse	5'- GAG GAT ACC ACT CCC AAC AGA CC-3' 5'- AAG TGC ATA ATC GTT GTT CAT ACA-3'
<i>TNF<math>\alpha</math></i> Forward Reverse	5'- TAG CCA GGA GGG AGA ACA GA-3' 5'- TTT TCT GGA GGG AGA TGT GG-3'
<i>TGF<math>\beta</math>1</i> Forward Reverse	5'- GAA GGA CCT GGG TTG GAA GTG G-3' 5'- CGT AGT AGA CGA TGG GCA GTG G-3'
<i>GAPDH</i> Forward Reverse	5'- ATC TTG GGC TAC ACT GAG GA-3' 5'- CAG GAA ATG AGC TTG ACA AAG-3'

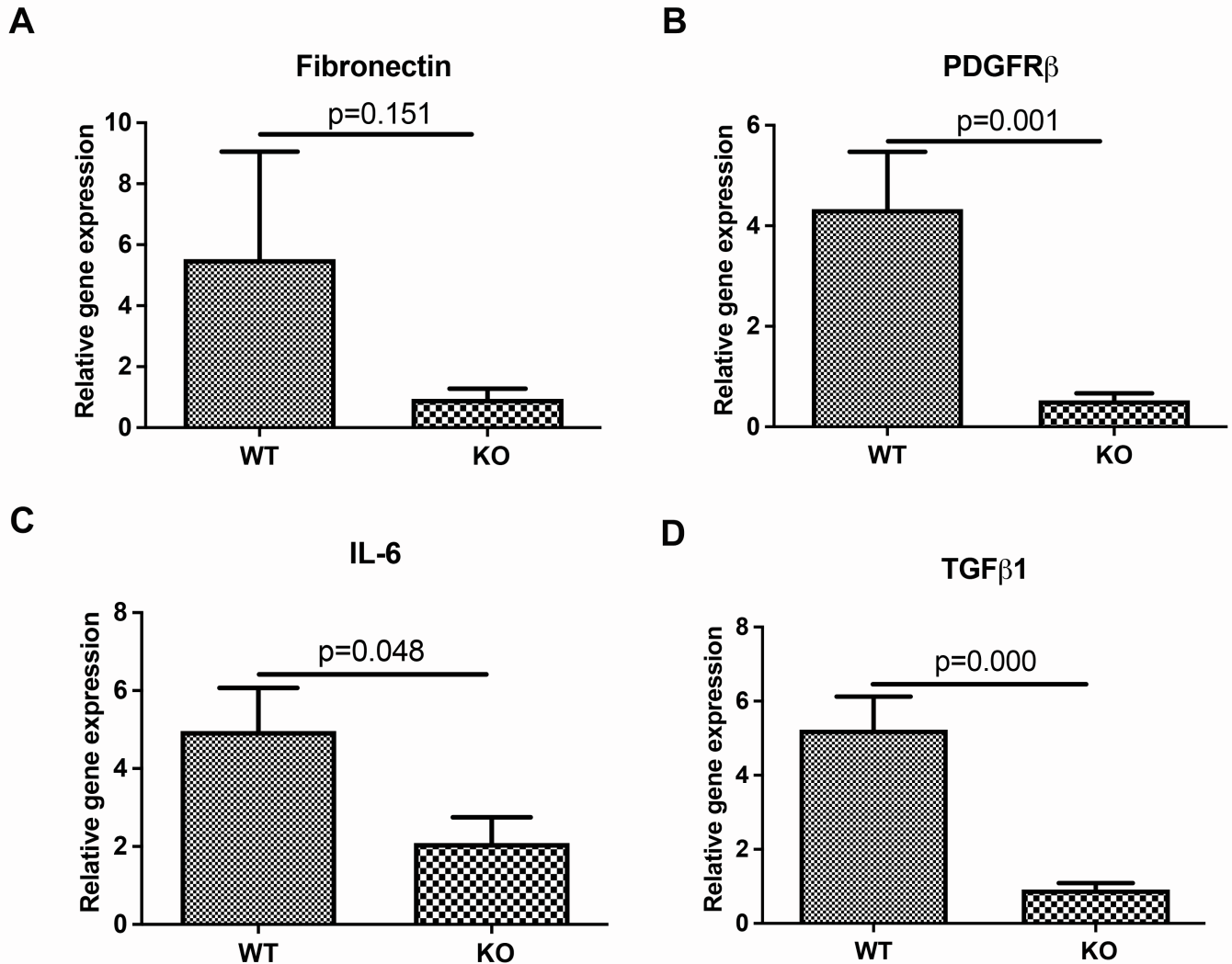
Supplemental table 4 (Table S4), related to STAR Methods, section of Quantitative PCR. List of primer sequences.

## SUPPLEMENTARY FIGURES AND LEGENDS



**Supplemental Figure 1 (Fig S1), related to Figure 1. Mouse chronic kidney disease (CKD) model.**

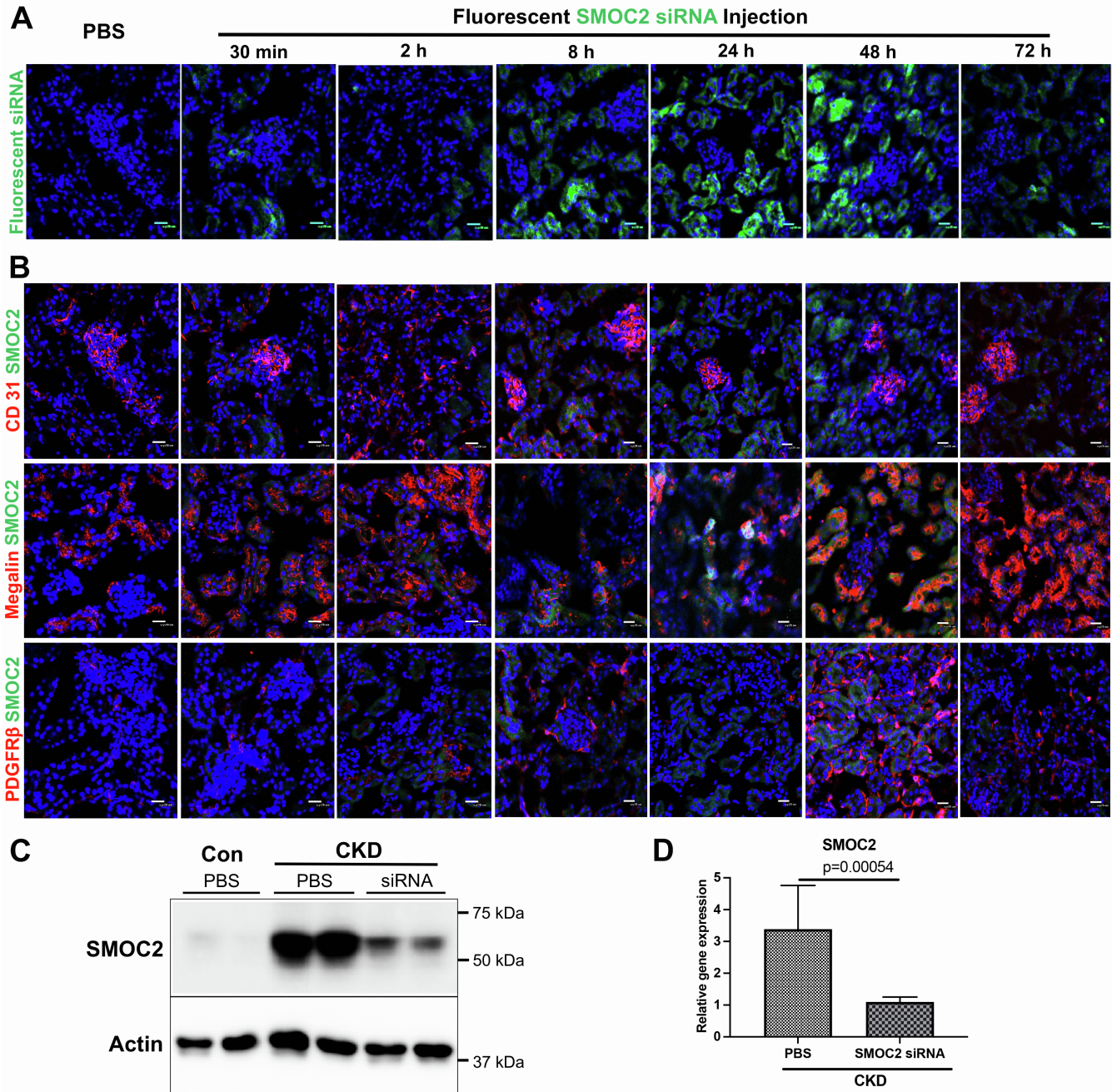
Immunofluorescent staining of different markers (20x magnification and scale bar, 50um) in cortex and medulla of kidney in mouse CKD model compare with sham as indicated (A) collagen 1; (B)  $\alpha$ -SMA; (C) PDGFR $\beta$ ; (D) F4/80. n=6/group unless otherwise stated.



**Supplemental Figure 2 (Fig S2), related to Figure 3. Genes expression in whole CKD kidney tissue of SMOC2 KO compared with WT.**

(A-D) Quantitative PCR for transcripts of matrix proteins, fibrotic factors and inflammatory factors.

n=6/group unless otherwise stated. Data are represented as mean +/- SEM.



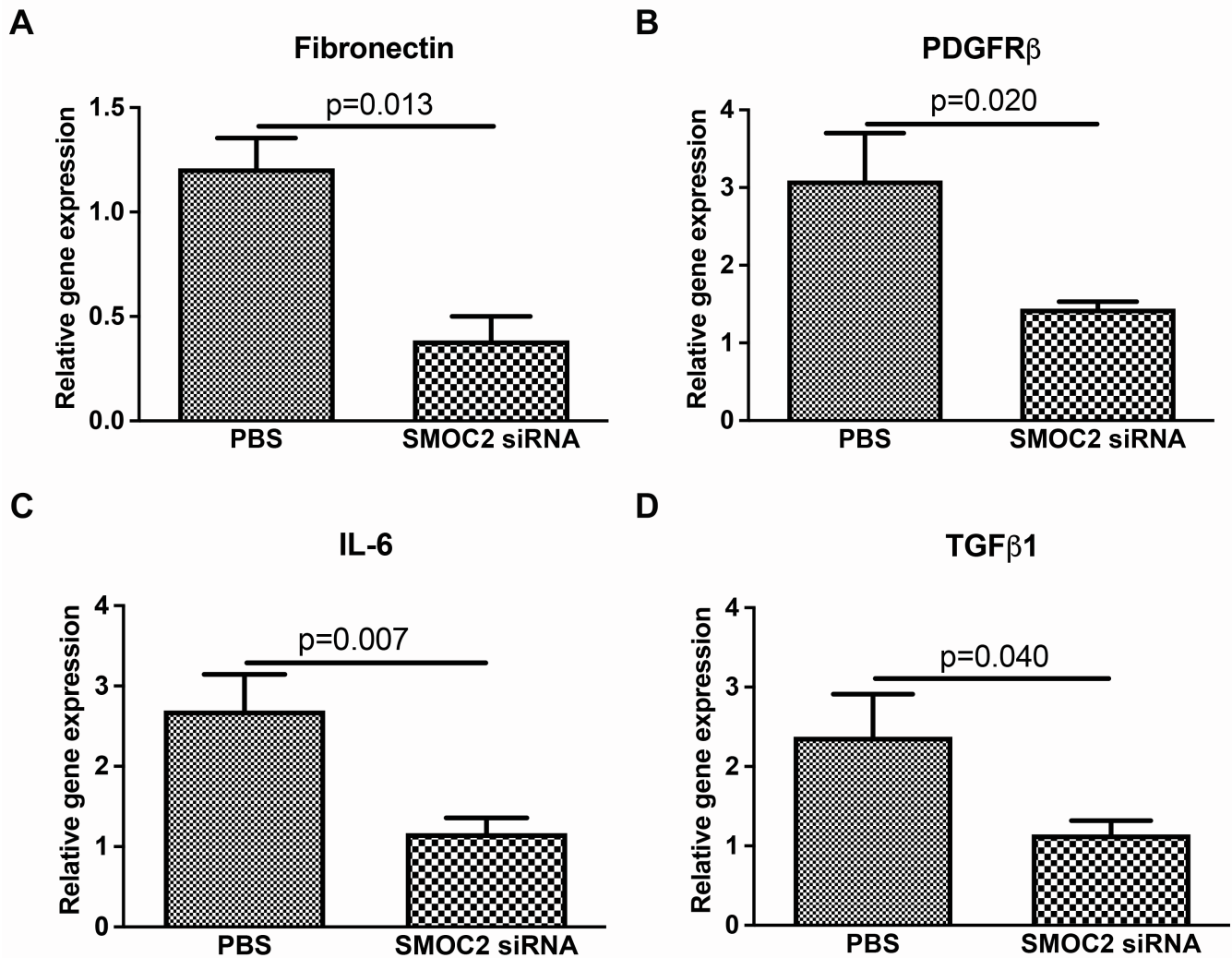
Supplemental Figure 3 (Fig S3), related to Figure 4. SMOC2 siRNA delivery and distribution in mouse kidney.

(A,B) C57Bl6 mice were administrated with PBS or fluorescent SMOC2 siRNA (30 mg/200ul) via retro-orbital vein injection. Kidneys were harvested at 30 min, 2 h, 8 h, 24 h, 48 h and 72 h. (A) Fluorescent SMOC2 siRNA deposition in the kidney and (B) SMOC2 siRNA distribution in proximal tubules (Megalin+) and identified in endothelial cells (CD31+) and glomerular cells (PDGFRβ+)

SMOC2, a therapeutic target for CKD

(C,D) Mice subjected to experimental CKD then received either SMOC2 siRNA (30 mg/200ul) or PBS at day 21 post nephrectomy surgery twice/week until day 43. SMOC2 depletion was confirmed at protein (C) and mRNA (D) level.

Immunofluorescence staining with 40x magnification and scale bar, 20um. n=6/group unless otherwise stated. Data are represented as mean +/- SEM.

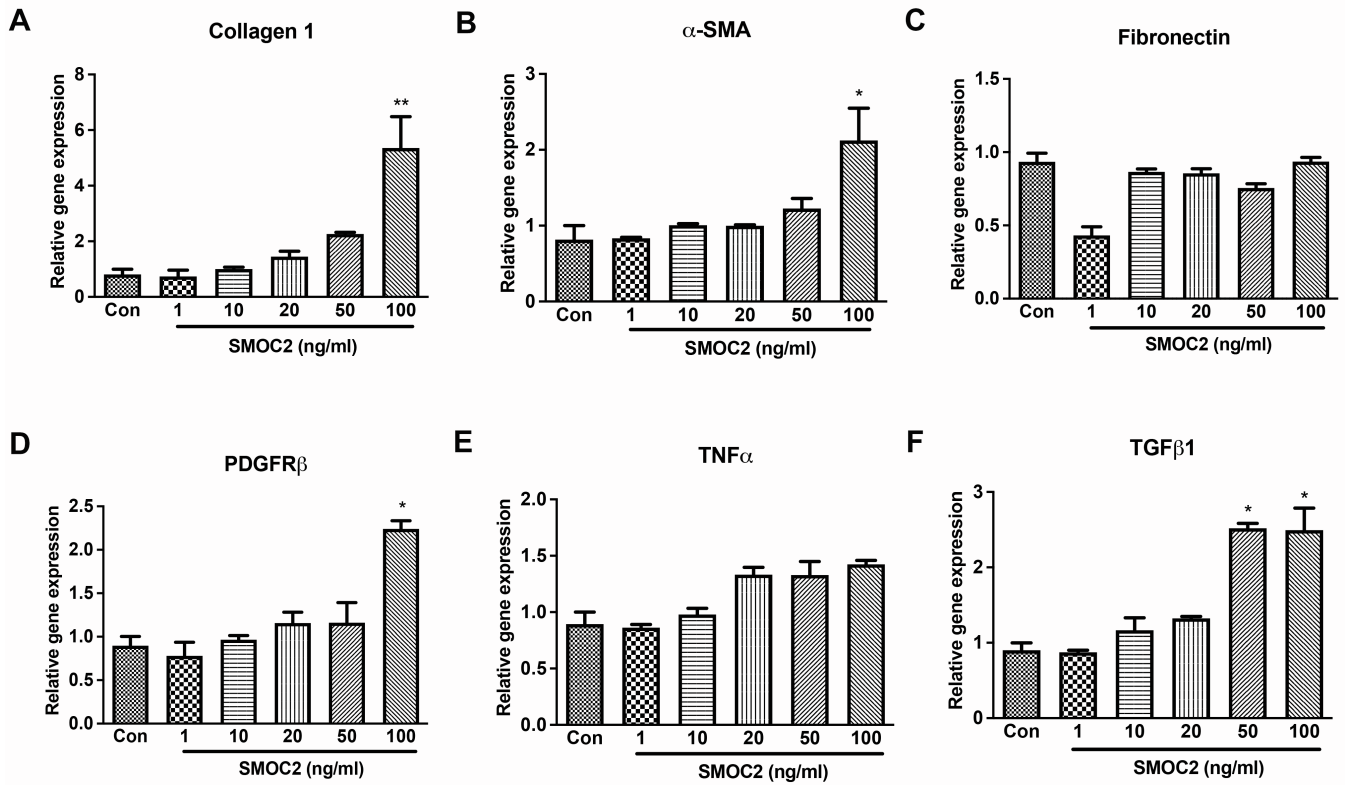


Supplemental Figure 4 (Fig S4), related to Figure 4. Genes expression in whole CKD kidney tissue of SMOC2 siRNA treatment compared with vehicle treatment.

(A-D) Quantitative PCR for transcripts of matrix proteins, fibrotic factor and inflammatory factors.

n=6/group unless otherwise stated. Data are represented as mean +/- SEM.

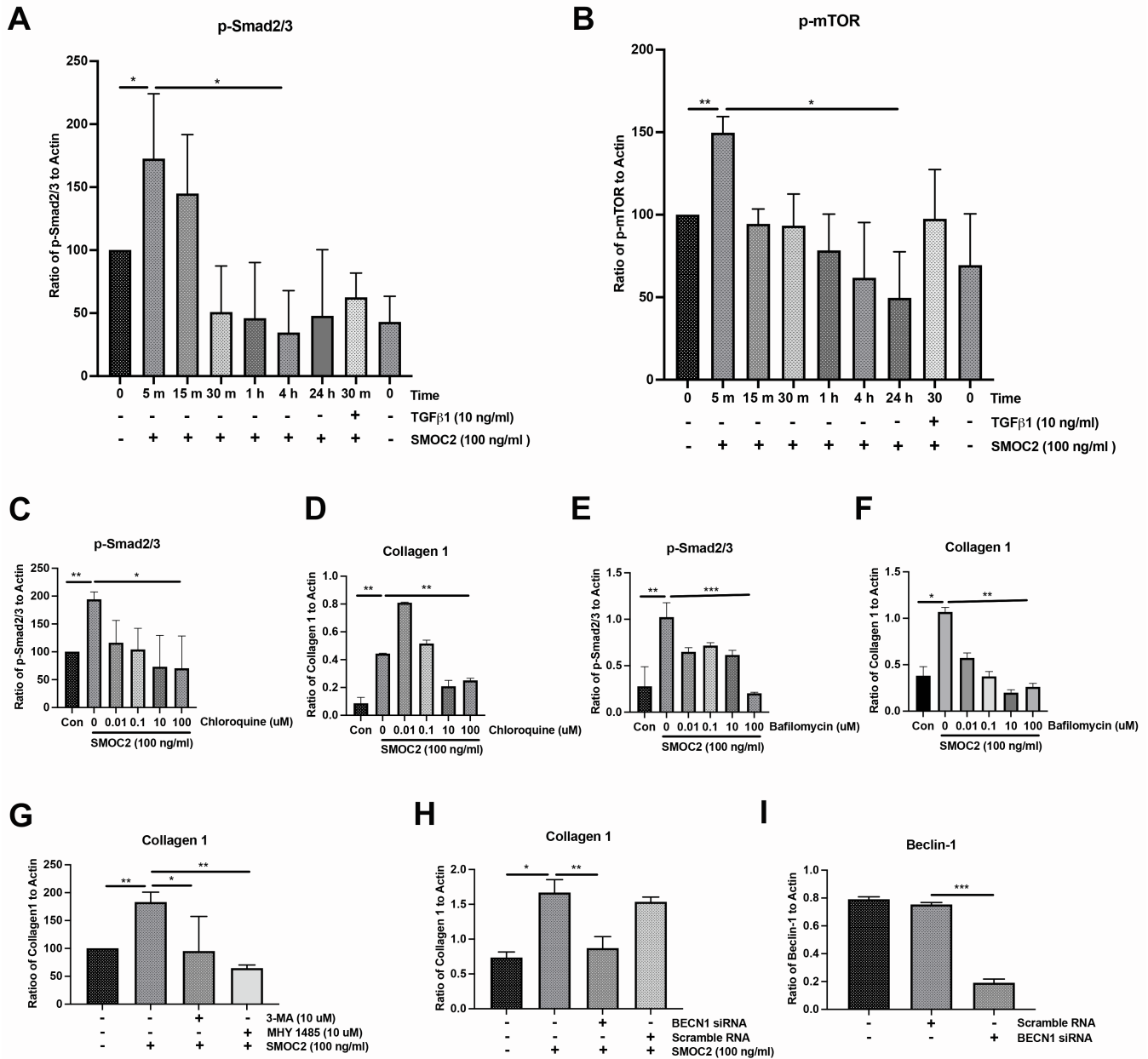




**Supplemental Figure 5 (Fig S5), related to Figure 5. SMOC2 induced genes expression.**

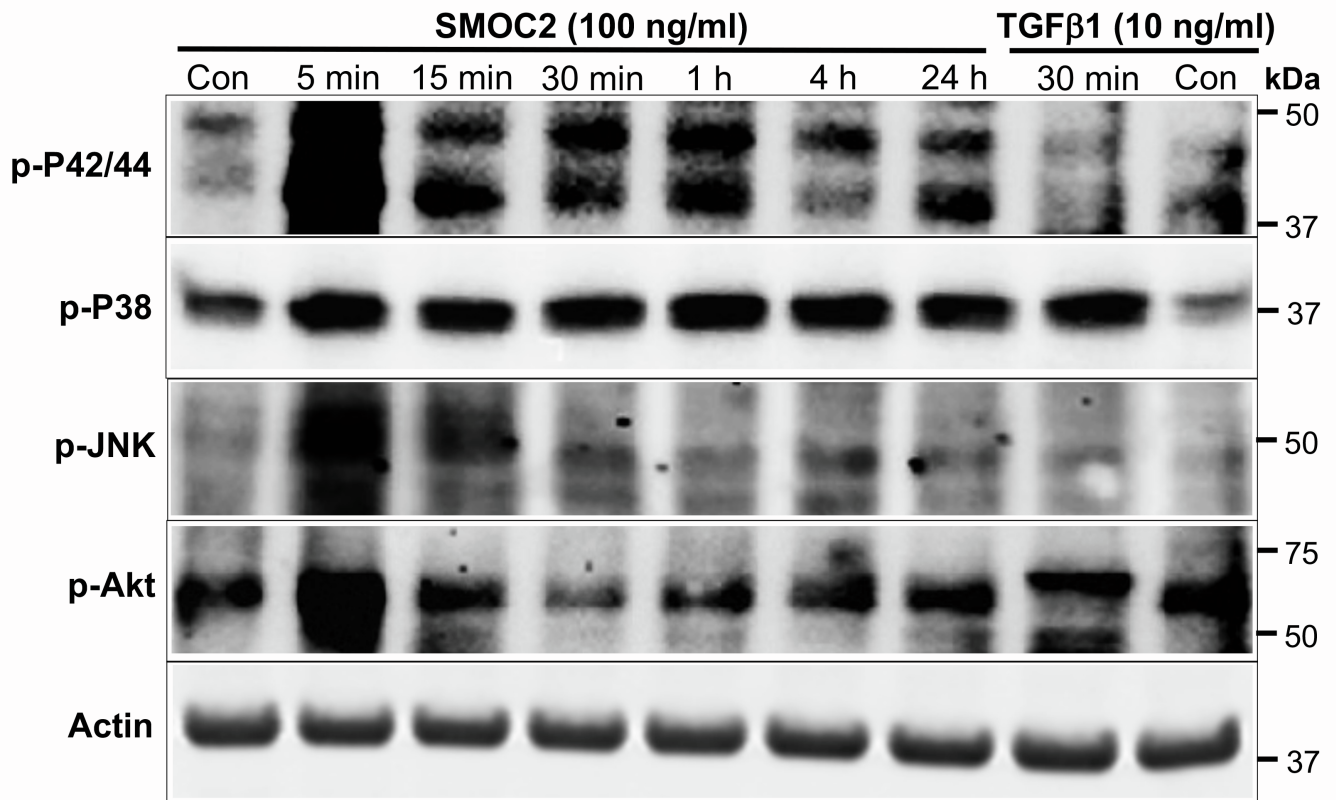
NIH/3T3 cells were incubated with SMOC2 recombinant protein for 24 hours with 1, 10, 20, 50, 100 ng/ml. Quantitative PCR for transcripts of matrix proteins, fibrotic factors and inflammatory factors (A-F). Quantification are representative of three independent experiments. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001. Data are represented as mean +/- SEM.

## SMOC2, a therapeutic target for CKD



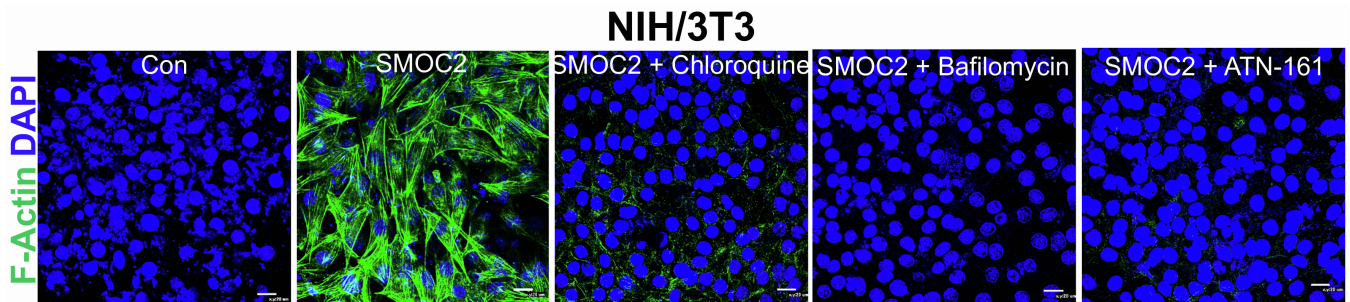
Supplemental Figure 6 (Fig S6), related to Figure 5. Western Blots analysis of lysosome-autophagosome signaling pathways involved in SMOC2-induced kidney fibrosis.

(A-I) Ratio of target protein expression to Actin. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001. Data are represented as mean +/- SEM.



**Supplemental Figure 7 (Fig S7), related to Fig 6. SMOC2 induced phosphorylation of MAPKs and Akt.**

NIH/3T3 cells were treated with either SMOC2 (100 ng/ml) at 5 min, 15 min, 30 min, 1 h, 4 h and 24 h, or TGFβ1 (10 ng/ml). Cell lysates were performed by Western Blotting analysis and showed phosphorylation of MAPKs (p-P42/44, p-P38, p-JNK) and Akt (p-Akt), and control of Actin expression showed same as in Fig 5A. Blots are representative of three independent experiments.



**Supplemental Figure 8 (Fig S8), related to Figure 6. SMOC2 triggered cell differentiation.**

NIH/3T3 cells were co-incubated with SMOC2 (100 ng/ml, 24 h) and lysosome inhibitors of Chloroquine (10 uM, 24 h), Bafilomycin (10 uM, 24 h) and Integrin  $\alpha$ 5 inhibitor of ATN-161 (10 uM, 24 h), cell differentiation was represented by F-Actin (Green) staining. Immunofluorescence staining with 40x magnification and scale bar, 20um. Images are representative of three independent experiments.