Supplementary Materials Provided

1. Supplementary Figures

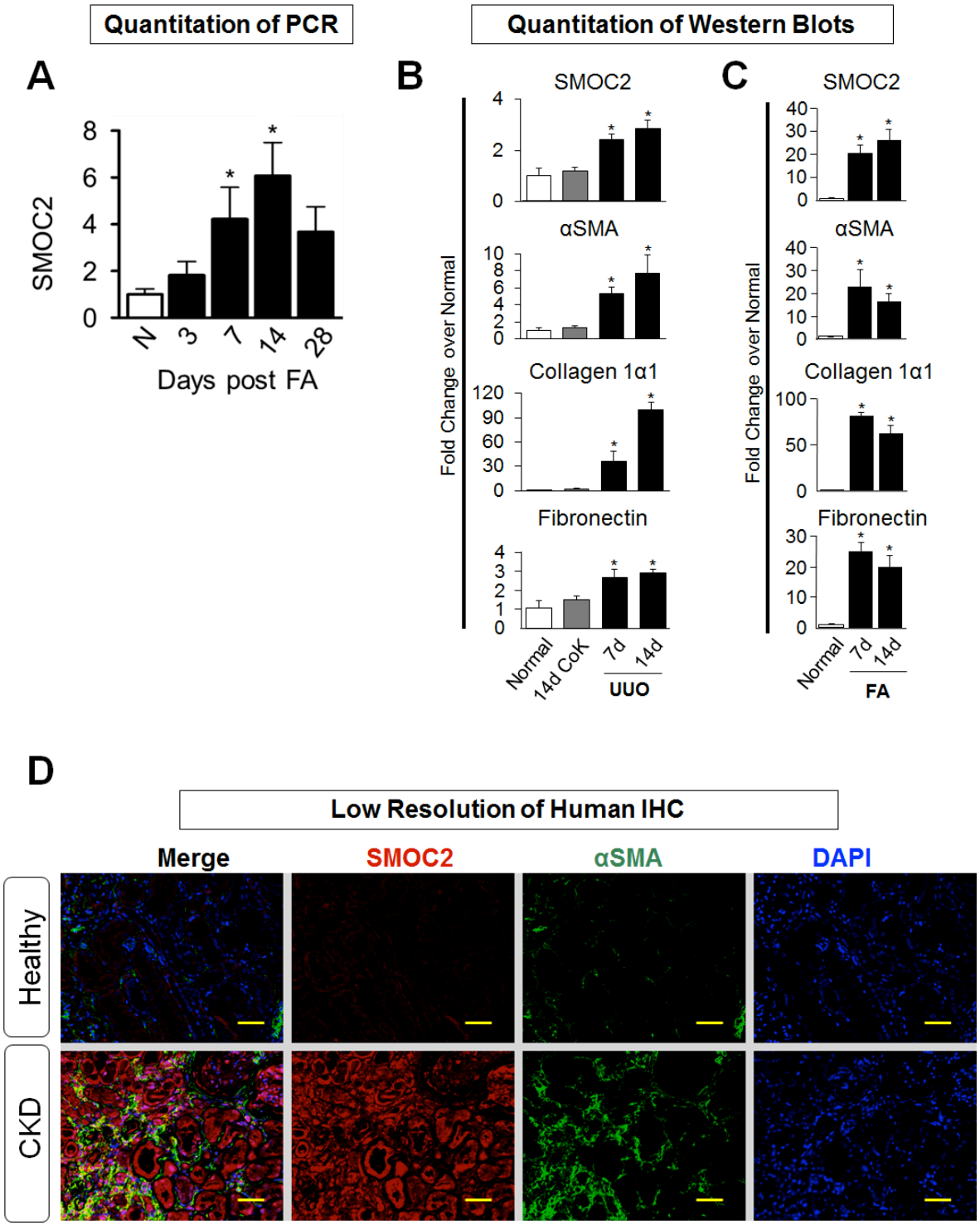
- **Supplementary Figure 1** Quantitation of SMOC2 protein expression along with fibrotic markers.
- **Supplementary Figure 2** TGFβ1 induces the expression of SMOC2 in fibroblasts and epithelial cells.
- **Supplementary Figure 3:** Quantitation of SMOC2 protein expression along with fibrotic markers in wild type and SMOC2 transgenic mice.
- **Supplementary Figure 4** Histological analysis of SMOC2 overexpressing tansgenic (SMOC2 Tg) mice.
- **Supplementary Figure 5** Quantitation of SMOC2 mRNA and protein levels along with fibrotic markers in mice following Unilateral Ureteral Obstruction.
- **Supplementary Figure 6** Quantitation of SMOC2 mRNA and protein levels along with fibrotic markers in mice following Folic acid administration.
- **Supplementary Figure 7** RNA sequencing analysis to identify differentially expressed pathways between SMOC2 Tg versus wild type mice following kidney fibrosis.
- **Supplementary Figure 8** In vitro profile of recombinant SMOC2 on NIH3T3 cells.
- **Supplementary Figure 9** SMOC2 transfected fibroblasts acquire an active phenotype.
- **Supplementary Figure 10** Histological analysis of SMOC2 knockout mice.
- **Supplementary Figure 11** Quantitation of Western blots for SMOC2 and fibrotic markers in SMOC2 knockout (KO) and wild type mice treated with folic acid.
- **Supplementary Figure 12** Quantitation of Western blots for SMOC2 and fibrotic markers in SMOC2 knockout (KO) and Wild type mice that underwent UUO surgery.
- **Supplementary Figure 13** Performance of SMOC2 siRNAs in NIH3T3 cells.
- **Supplementary Figure 14** Quantitation of Western blots for SMOC2 siRNA treatment of fibroblasts.

- **Supplementary Figure 15** Enrichment of siRNA in the mice kidneys following iv injection via the tail vein.
- **Supplementary Figure 16** Quantitation of Western blots for mice treated with SMOC2 siRNA followed by folic acid administration.

2. Supplementary Tables

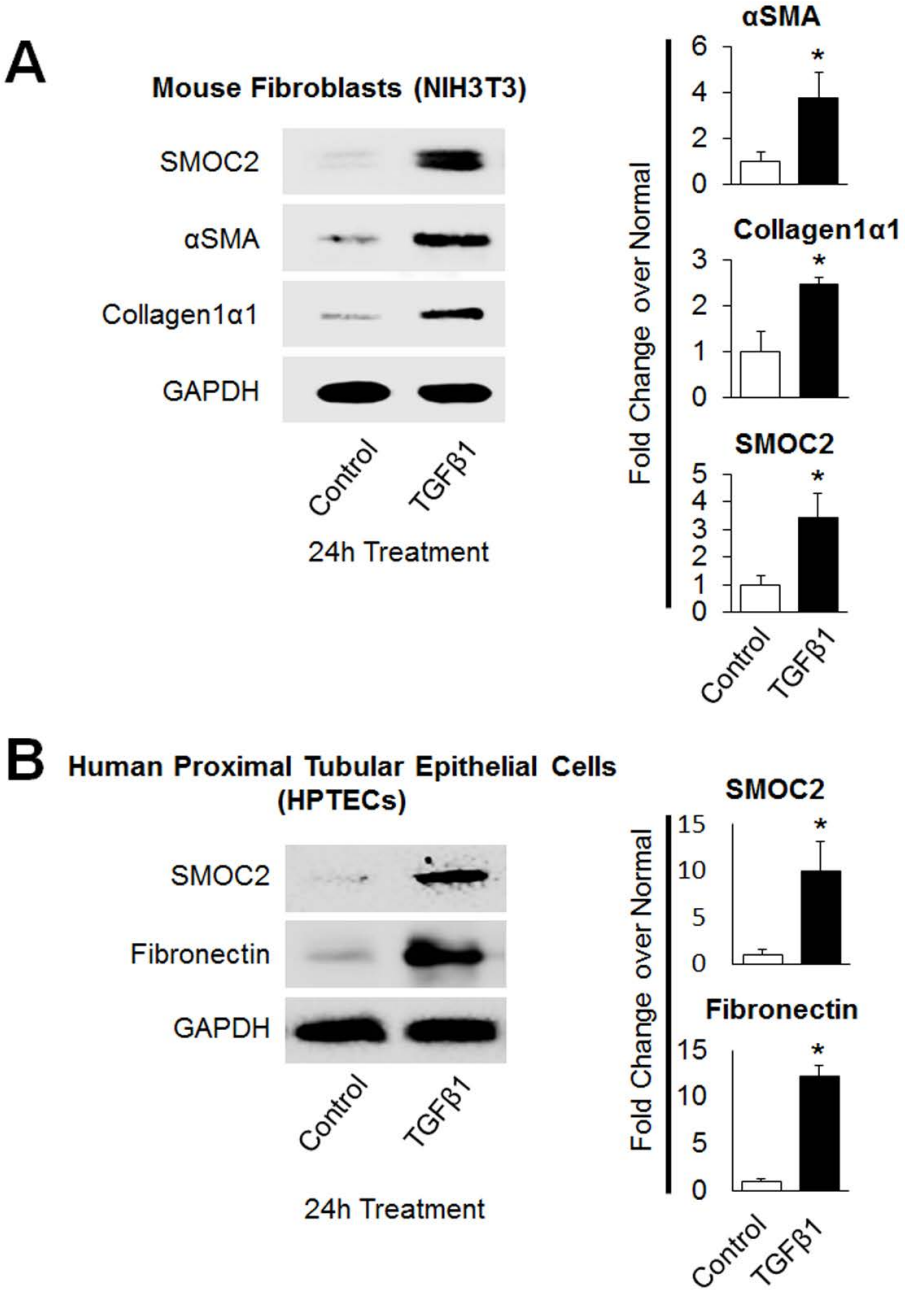
- **Supplementary Table 1** List of primers used for Genotyping
- **Supplementary Table 2** List of primers used for qRT-PCR

1. Supplementary Figures

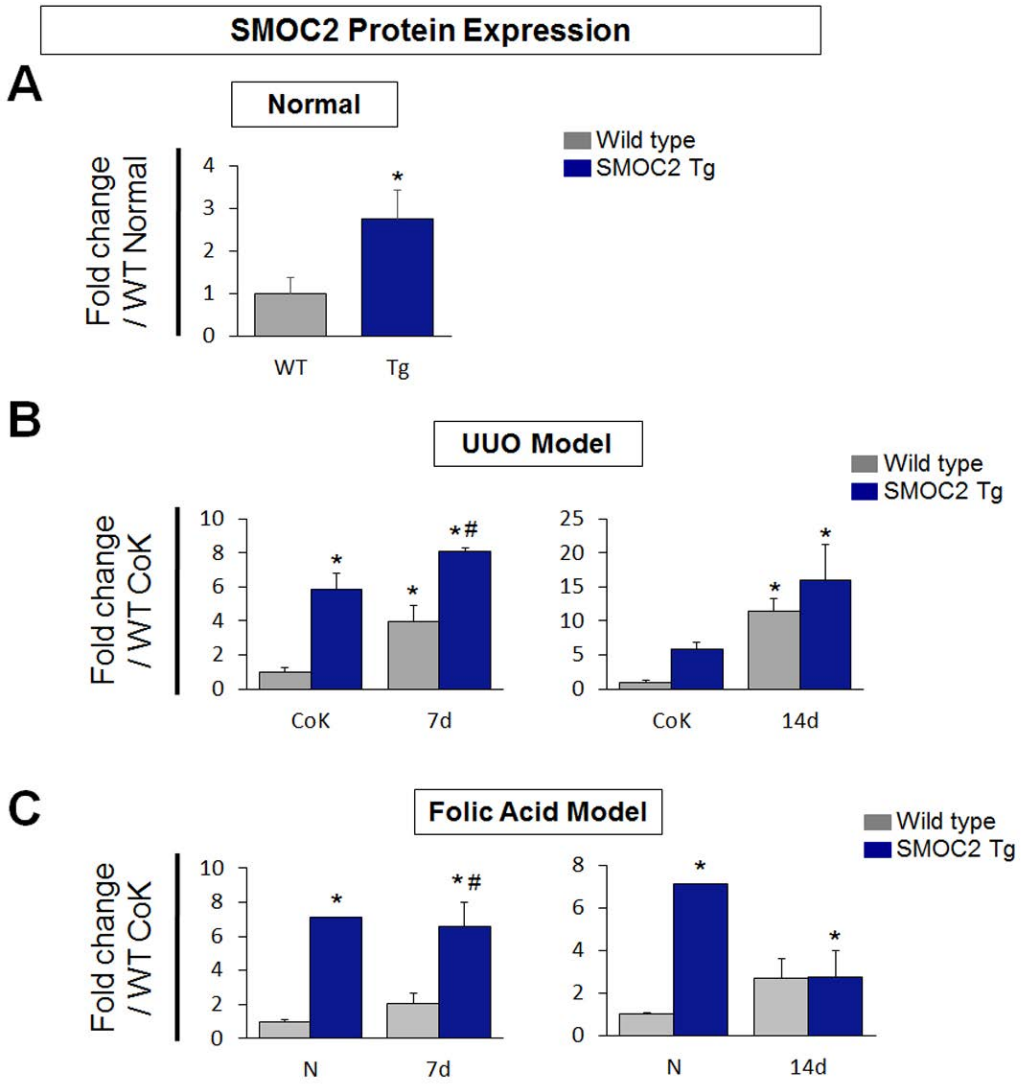


Supplementary Figure 1. Quantitation of SMOC2 protein expression along with fibrotic markers. FA treated mice SMOC2 levels by (**A**) qPCR. Mice were (**B**) subjected to Unilateral Ureteral Obstruction (UUO) or (**C**) treated with Folic Acid (FA), intraperitoneally, then sacrificed at 7 days and 14

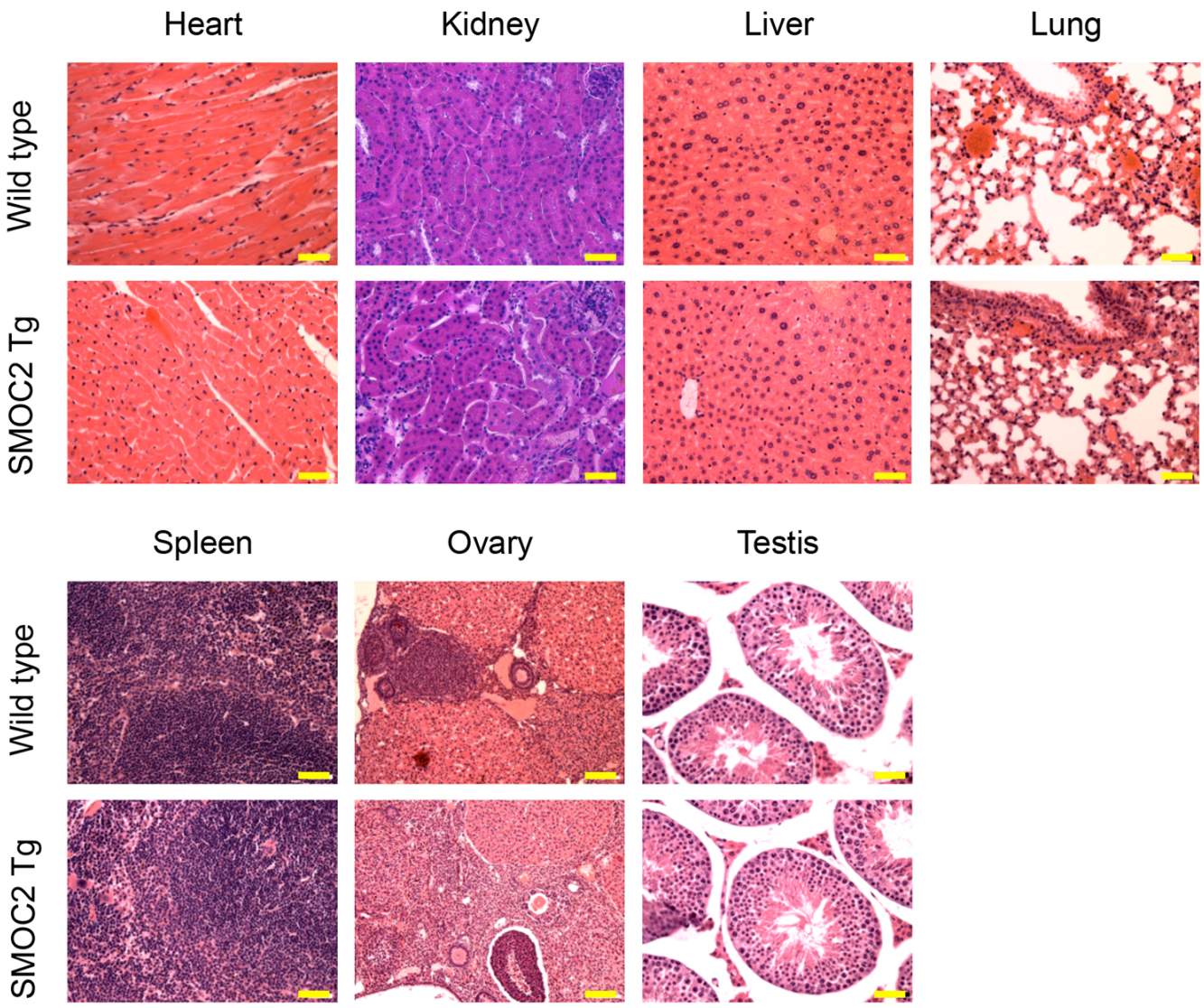
days. Western blotting was performed on kidney tissue lysates to measure established fibrotic markers such as α-smooth muscle actin (αSMA), collagen 1α1 and fibronectin. For the UUO model, Contralateral Kidney (CoK) tissue lysates were also included. Densitometry data are representative of Western blot images from Figure 1B (UUO) and Figure 1C (FA) which were normalized to sham/vehicle and represent mean ± SEM (n = 5 mice/group/time point). *P < 0.05 determined by t-test. (**D**) Low power resolution of CKD patient. Light microscopy images are 20X magnification; scale bar = 50uM.



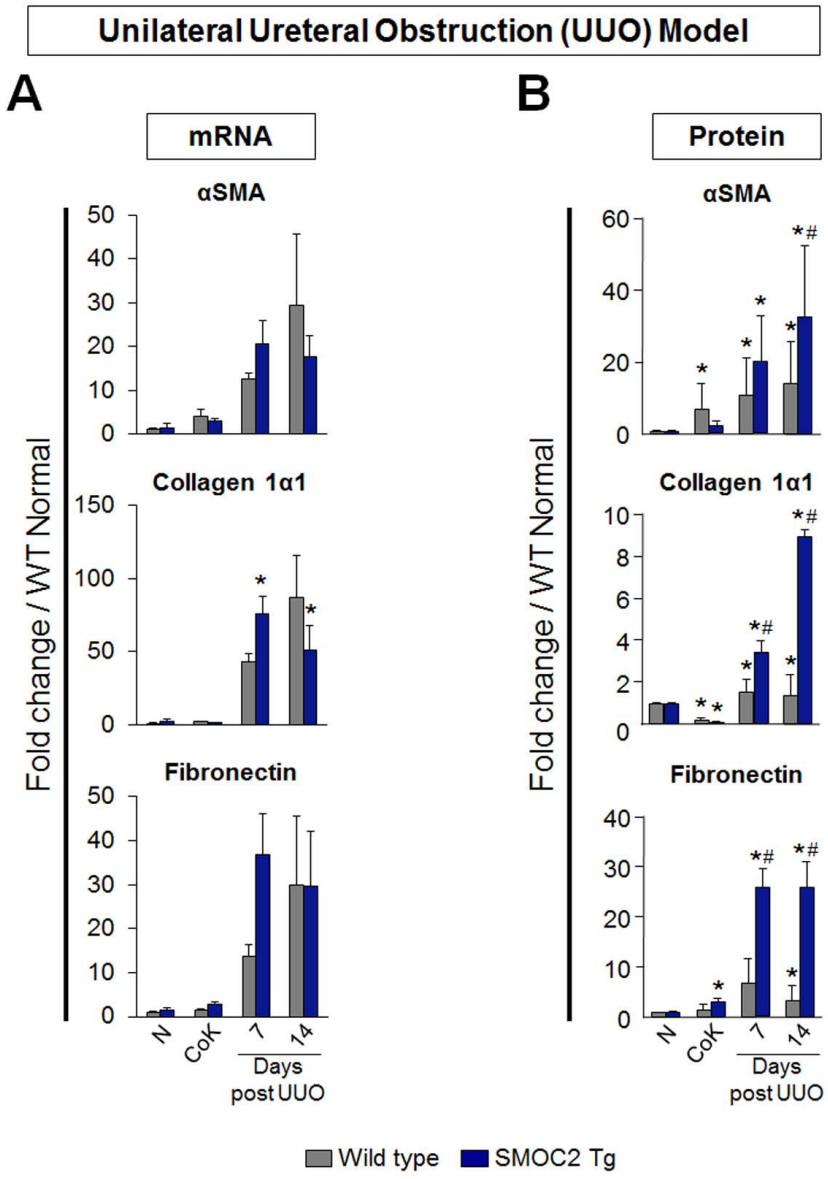
Supplementary Figure 2. TGFβ1 induces the expression of SMOC2 in fibroblasts and epithelial cells. NIH3T3 (**A,** n=4) and HPTEC cells (**B,** n=3) were incubated with 10ng/mL TGFβ1 for 24h. Protein expression of listed targets was determined by Western blot. Densitometry data are relative to control levels, normalized by GAPDH and represent Mean ± SEM. *P < 0.05 determined by t-test.



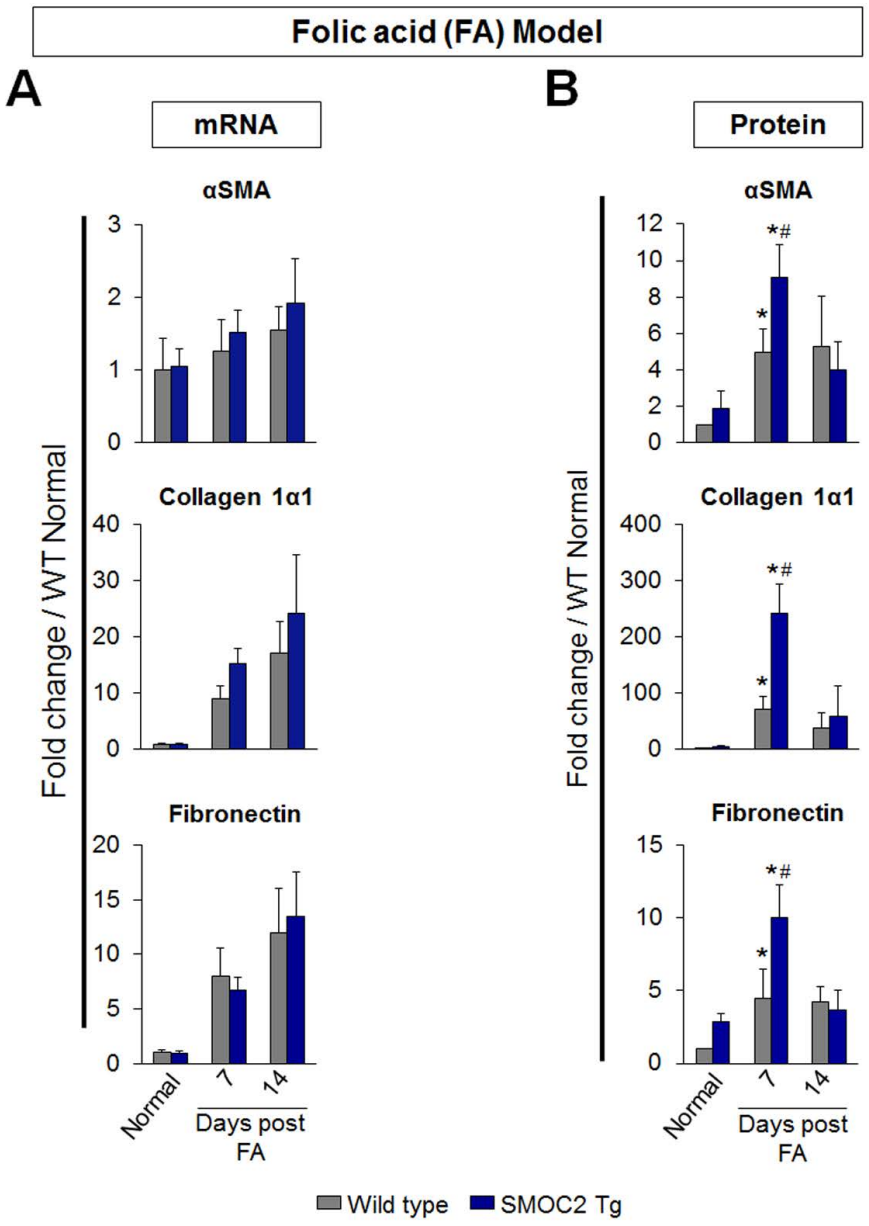
Supplementary Figure 3. Quantitation of SMOC2 protein expression along with fibrotic markers in wild type and SMOC2 transgenic mice. (**A**) Densitometry for SMOC2 expression in SMOC2 Tg and wild type (WT) mice (n = 4). (**B**) SMOC2 Tg and wild type (WT) mice were subjected to Unilateral Ureteral Obstruction (UUO), and protein expression from kidney tissue samples collected at 7 and 14 days following UUO were assessed by Western blot for SMOC2. (**C**) SMOC2 Tg and WT mice treated with Folic Acid (FA) and protein expression of αSMA, collagen 1α1, fibronectin and SMOC2 was assessed by Western blot from kidney tissue samples collected at 7 and 14 days post FA. Densitometry are representative of Western blot images from Figure 2B (UUO) and Figure 2D (FA) which were normalized to sham/vehicle and represent mean ± SEM (n = 3-4 mice/group/time point). *P < 0.05 determined by t-test.



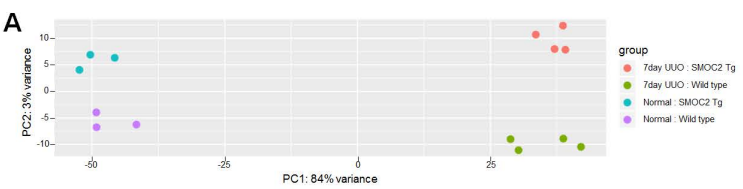
Supplementary Figure 4. Histological analysis of SMOC2 overexpressing tansgenic (SMOC2 Tg) mice. Light microscopy 20X H&E sections of tissues from 8-week old Wild type and SMOC2 Tg mice. Representative images of n=3; Scale bars, 50 μm.



Supplementary Figure 5. Quantitation of SMOC2 mRNA and protein levels along with fibrotic markers in mice following Unilateral Ureteral Obstruction. SMOC2 Tg and Wild type (WT) mice were subjected to Unilateral Ureteral Obstruction (UUO) then sacrificed at 7 and 14 days. (**A**) Quantitative rtPCR and (**B**) Western blot analysis were performed on kidney tissue lysates to measure the expression of αSMA, collagen 1α1, and fibronectin (Densitometry data from Figure 2B Western blots). Contralateral Kidney (CoK) tissue lysates were also included. The expression was normalized to house keeping gene GAPDH and values are represented as fold change over WT normal. Mean ± SEM (n=5 mice/group/time point). *P < 0.05 (WT Normal) and #P < 0.05 (WT at respective time point) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.



Supplementary figure 6. Quantitation of SMOC2 mRNA and protein levels along with fibrotic markers in mice following Folic acid administration. SMOC2 Tg and Wild type (WT) mice were subjected to Folic acid (FA), intraperitoneally, treatment then sacrificed at 7 and 14 days. (**A**) Quantitative rtPCR and (**B**) Western blot analysis were performed on kidney tissue lysates to measure the expression of αSMA, collagen 1α1, and fibronectin (Densitometry data from Figure 2D Western blots). Quantitative data are relative to WT normal levels, normalized by GAPDH. Mean ± SEM (n=5 mice/group/time point). *P < 0.05 (WT Normal) and #P < 0.05 (WT at respective time point) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.



B

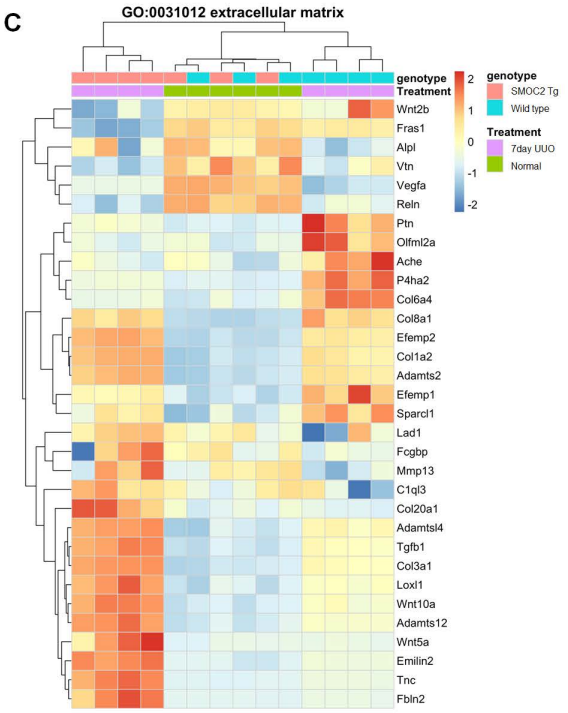
Cellular Components

	p.geomean	stat.mean	p.val	q.val	set.size	exp1
GO: 0031012 extracellular matrix	0	5.709648	0	0.0000043	314	0
GO: 0005578 proteinacious extracellular matrix	0	5.433926	0	0.0000101	280	0
GO: 0009897 external side of plasma membrane	0.0000122	4.280318	0.0000122	0.0019048	212	0.0000122
GO: 0009986 cell surface	0.0000358	3.990163	0.0000358	0.0042034	473	0.0000358
GO: 0005887 integral to plasma membrane	0.000075	3.807584	0.000075	0.0070312	465	0.000075
GO: 0044420 extracellular matrix part	0.0003842	3.397763	0.0003842	0.0292318	165	0.0003842
GO: 0005581 collagen	0.0004396	3.408533	0.0004396	0.0292318	77	0.0004396
GO: 0031225 anchored to membrane	0.0004986	3.342041	0.0004986	0.0292318	103	0.0004986
GO: 0034702 ion channel complex	0.0006113	3.279654	0.0006113	0.0318578	118	0.0006113
GO: 0044456 synapse part	0.0007779	3.178557	0.0007779	0.0364838	324	0.0007779
GO: 0045211 postsynaptic membrane	0.0015247	2.991452	0.0015247	0.0620092	142	0.0015247
GO: 0097060 synaptic membrane	0.0015866	2.973377	0.0015866	0.0620092	172	0.0015866
GO: 0045202 synapse	0.002217	2.852691	0.002217	0.0799831	468	0.002217
GO: 0034703 cation channel complex	0.0045306	2.648541	0.0045306	0.1517756	77	0.0045306

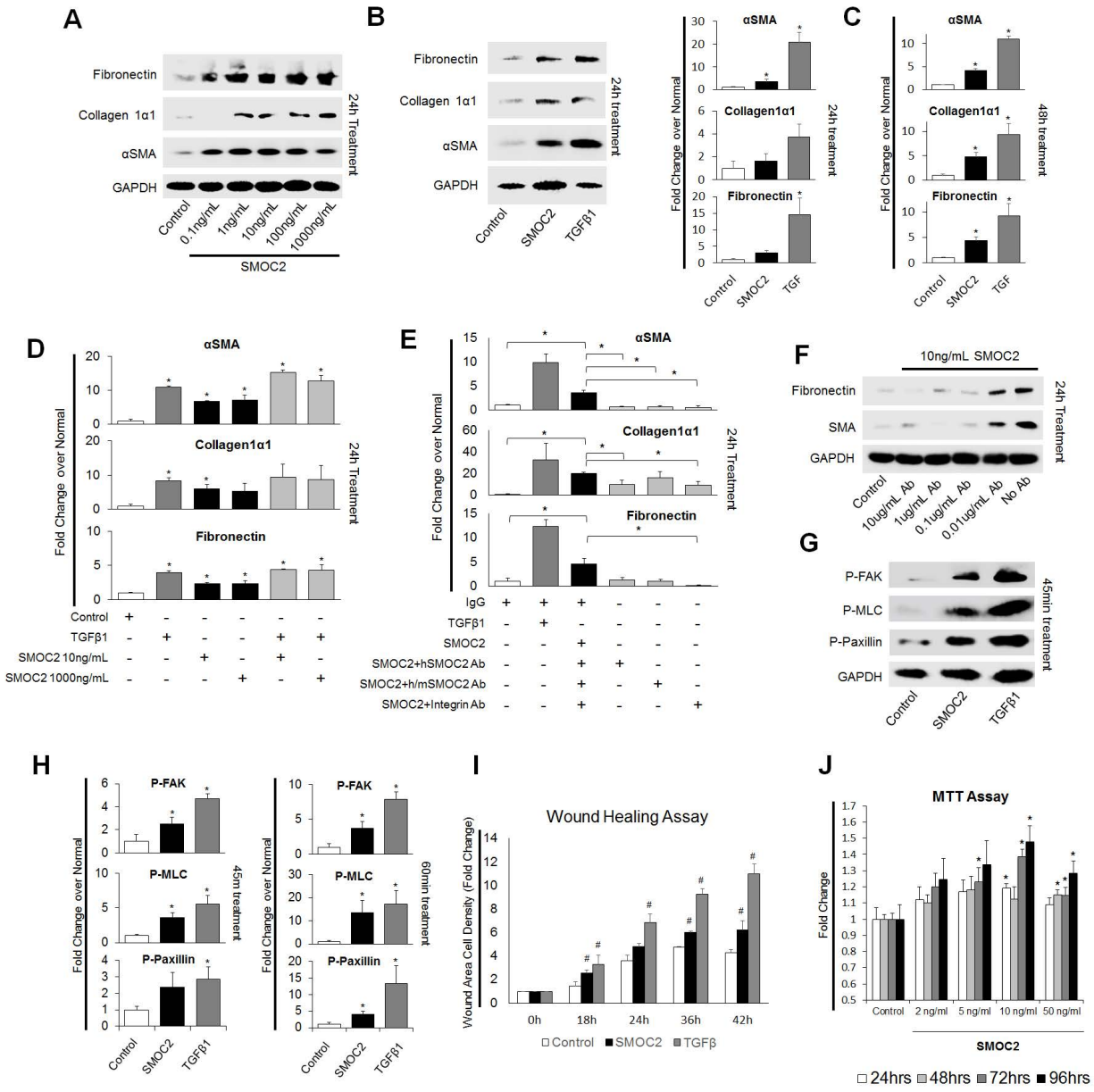
D

Biological Processes

	p.geomean	stat.mean	p.val	q.val	set.size	exp1
GO: 0007186 G-protein coupled receptor signaling pathway	0.0000012	4.750792	0.0000012	0.0047358	445	0.0000012
GO: 0006935 chemotaxis	0.0000465	3.934712	0.0000465	0.0668553	316	0.0000465
GO: 0042330 taxis	0.0000507	3.91363	0.0000507	0.0668553	317	0.0000507
GO: 0043269 regulation of ion transport	0.0001407	3.651542	0.0001407	0.1392421	349	0.0001407
GO: 0007610 behavior	0.0002487	3.495883	0.0002487	0.1969084	431	0.0002487

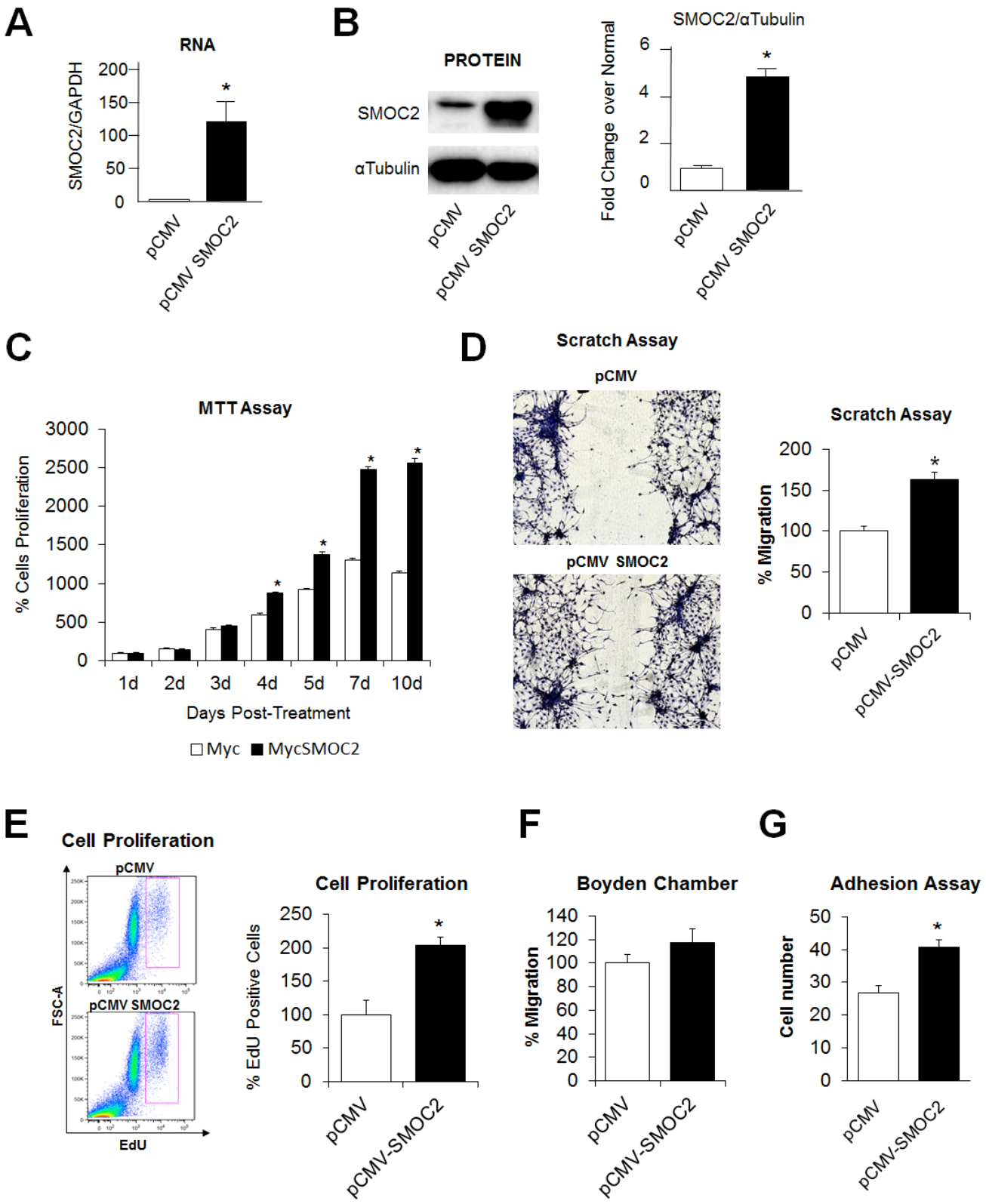


Supplementary figure 7. RNA sequencing analysis to identify differentially expressed pathways between SMOC2 Tg versus Wild type mice following kidney fibrosis. (**A**) The principal component analysis (PCA) analysis shows clear separation of sample genotypes and treatments. Axes height is proportional to the amount of variance explained by each principal component, with the two highest sources of variance explained by UUO treatment and genotype, respectively. A cut-off-free gene set enrichment analysis (GSEA) for gene ontology (GO) and KEGG terms was performed on the fold change values derived from DESeq2 using GAGE, and enriched GO categories are represented as categorized by (**B**) cellular components. (**C**) Heatmaps of the expression patterns of genes within selected enriched gene ontology categories. Only the top decile of genes are shown, as determined by the DESeq2 derived p-value for differential expression. All expression values were centered and scaled to their respective genotype's untreated samples mean expression values. Both columns and rows were clustered by similarity. (**D**) Gene Ontology categories for Biological Processes with an FDR-adjusted (q.val) pvalue (p.val) of at most 0.2 are shown.



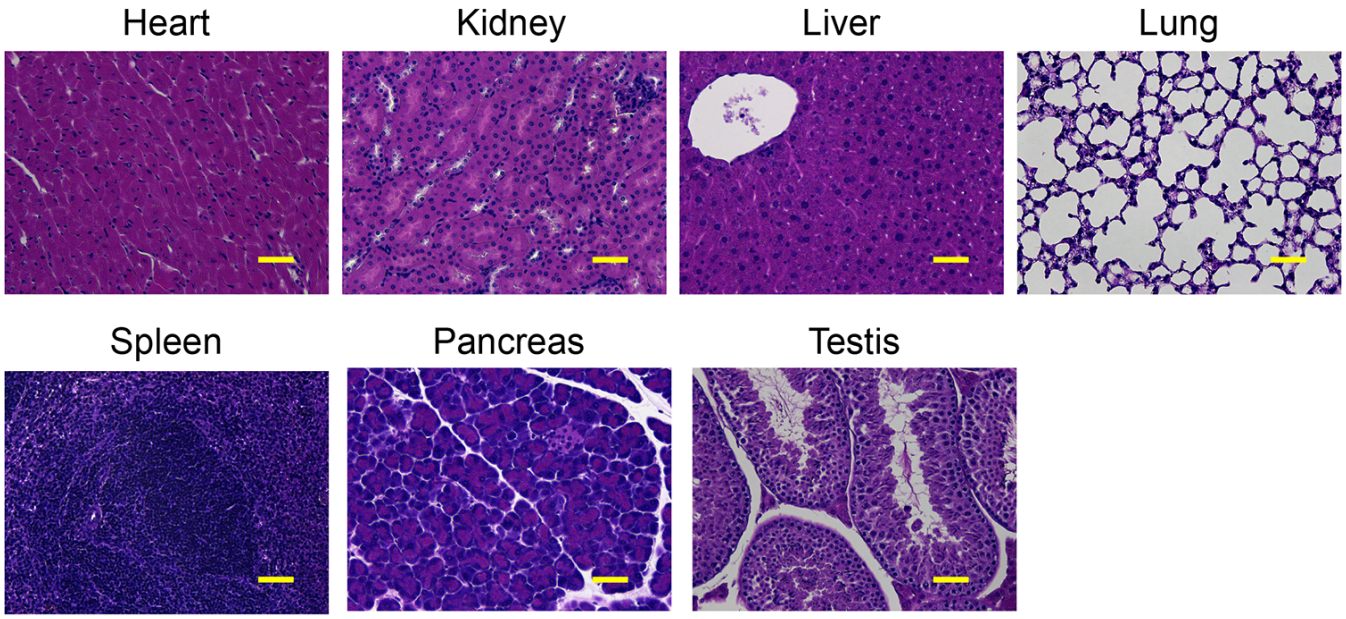
Supplementary Figure 8. In vitro profile of recombinant SMOC2 on NIH3T3 cells. (**A**) Serum deprived NIH3T3 cells treated with varying concentrations of SMOC2 for 24h and measured for αSMA, collagen 1α1 and fibronectin expression by Western blot. (**B**) Western blot images with respective densitometry (n = 4) showing fibrotic markers from quiescent primary human kidney fibroblasts treated with 10ng/mL SMOC2 or 5ng/mL TGFβ1 for 24h. (**C**) Densitometry data for Figure 3B showing 48h SMOC2 treatment on primary human kidney fibroblasts (n = 3). (**D**) Compared to profibrotic TGFβ (10ng/mL), densitometry data for Figure 3C Western blots show the expression levels of myofibroblast markers αSMA, collagen 1α1 and fibronectin from serum deprived NIH3T3 fibroblasts treated for 24h

with 10ng/mL SMOC2 (n = 3). (**E**) Densitometry data representing Figure 3D (n = 3) antibody blocking. (**F**) Antibody blocking titration of SMOC2 treated NIH3T3 cells. (**G**) Western blot images with respective densitometry (**H**) showing phosphoactivating profibrotic signals Phospho(P)-Focal Adhesion Kinase (FAK) Y925, P-Myosin Light Chain (MLC) Ser19 and P-Paxillin Tyr118 from quiescent NIH3T3 fibroblasts treated with 10ng/mL SMOC2 or 5ng/mL TGFβ1 for 45min (**H** left, densitometry; n = 5) and 60 minutes (**H** right, densitometry data from Figure 3F Western blots; n=5). (**J**) Quantification of the NIH3T3 cell density into the wound area of a migration assay over a time course. (**K**) Metabolic activity of NIH3T3 cells treated with various concentrations of SMOC2 over a time course were measured by MTT assay (n=5). Densitometry data are relative to control levels, normalized by GAPDH and represent Mean ± SEM. **P* < 0.05 determined by t-test. #*P* < 0.05 (Control at respective time point).

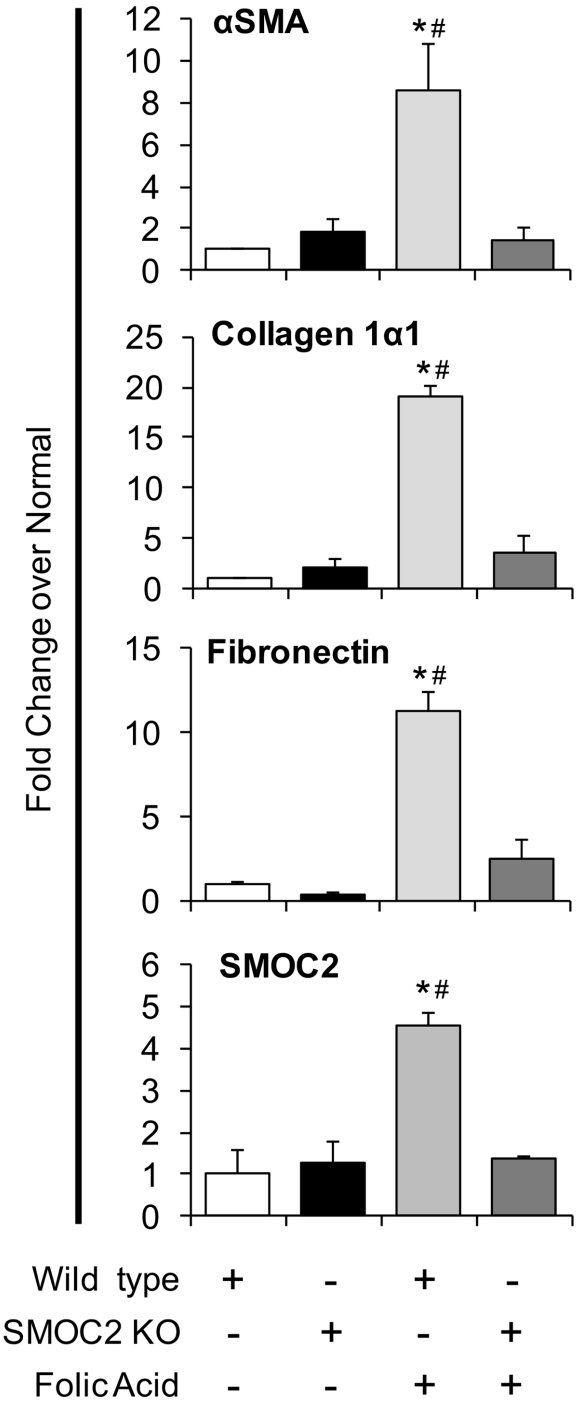


Supplementary Figure 9. SMOC2 transfected fibroblasts acquire an active phenotype. Quantification of RNA expression (**A**) and protein expression (**B**) of SMOC2 by rtPCR and Western blot

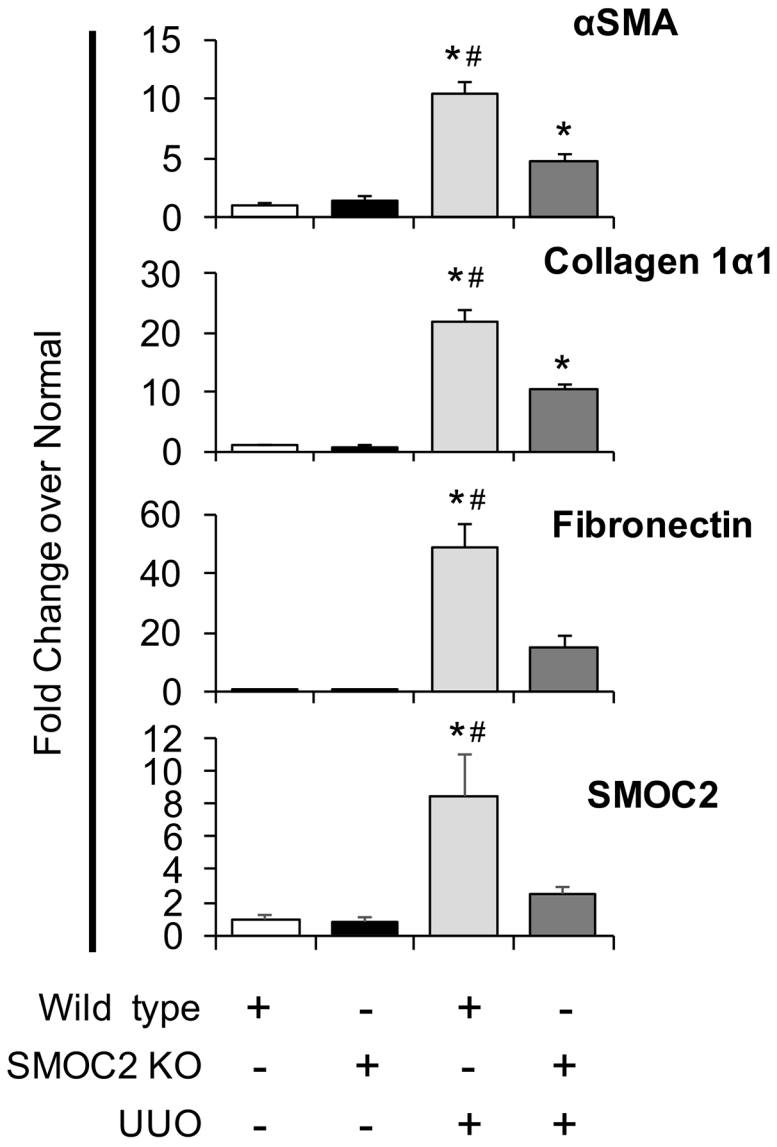
in pCMV and pCMV-SMOC2 transfected NIH3T3 cells. Quantitative rtPCR and densitometry data are relative to pCMV control levels, normalized by GAPDH and represent Mean ± SEM (RNA n=3, 2 technical replicates; Protein n=3). (**C**) Metabolic activity of pCMV control and pCMV-SMOC2 transfected NIH3T3 cells were measured by MTT assay over listed days (n=12/time point, % relative to day 1). (**D**) The wound healing influence of SMOC2 transfection on fibroblasts was analyzed by a scratch assay. Equally dispersed cells were inflicted with a scratch to evaluate the restorative capacity between the 24h post-SMOC2 transfected NIH3T3 cells and its pCMV control. The difference in healing was calculated as a percentage of pCMV-SMOC2 over pCMV transfected cells. Representative images (10X; scale bar = 50μM) have been stained with methylene blue at 24h for increased contrast. (**E**) NIH3T3 cells were transfected with pCMV or pCMV-SMOC2 for 24h. Cell proliferation and cell cycle progression were measured by EdU labeling and subsequent cell cycle analysis by fluorescence-activated cell sorting (FACS). (**F**) The migration potential of SMOC2 transfected NIH3T3 cells was evaluated using the Boyden Chamber assay to determine the percentage of migrating cells. (**G**) NIH3T3 cells were transfected with pCMV and pCMV-SMOC2 for 24h, after which cells were harvested by trypsin and reseeded. After 1h, unattached cells were washed and cell numbers were quantified for adherence (n=3). $*P < 0.05$ determined by t-test.



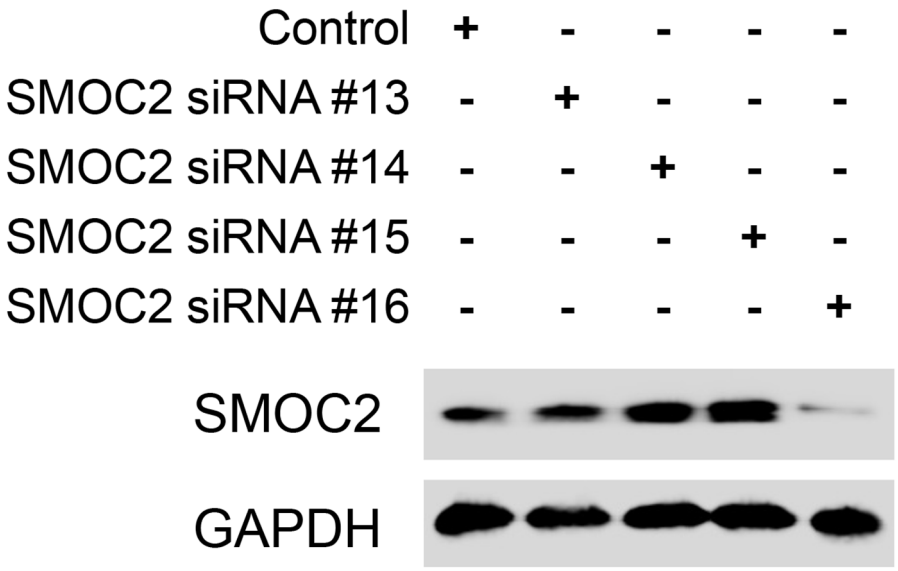
Supplementary Figure 10. Histological analysis of SMOC2 knockout mice. Light microscopy 20X H&E sections of tissues from 8 weeks old SMOC2 KO mice, which were confirmed by a pathologist to be normal. Representative images of n=3. Scale bars, 50 μm.



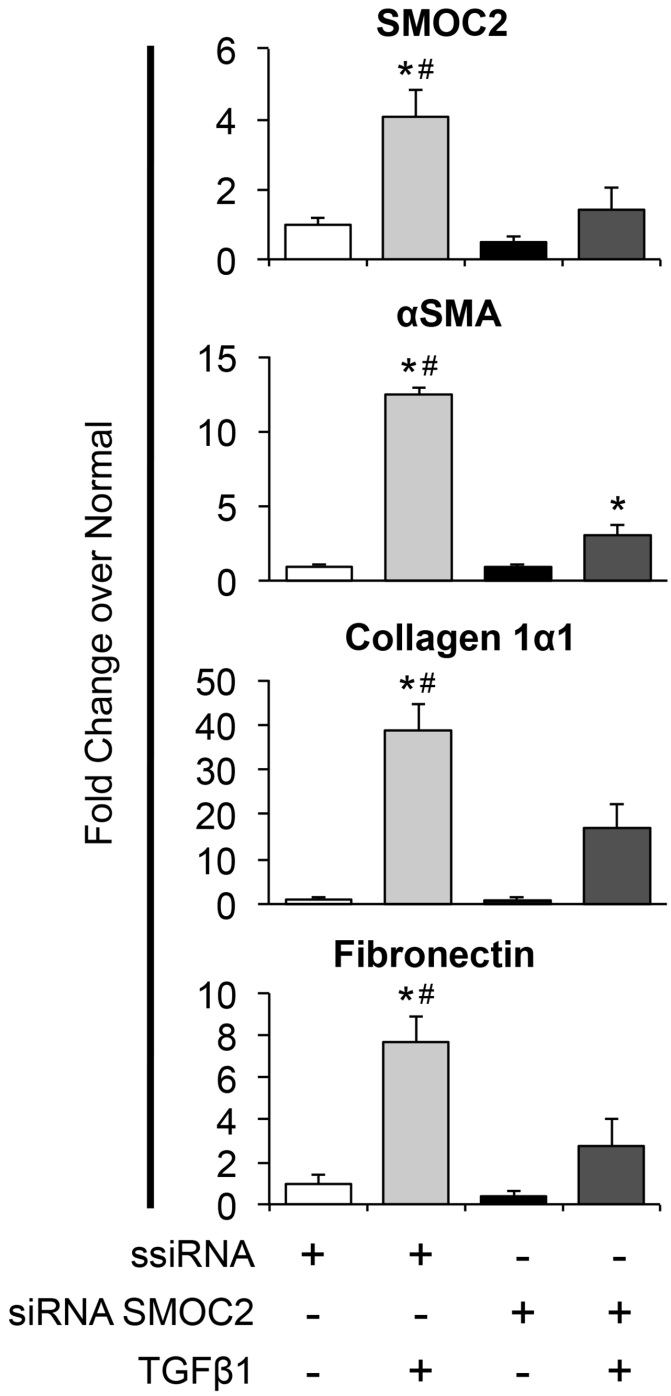
Supplementary Figure 11. Quantitation of Western blots for SMOC2 and fibrotic markers in SMOC2 knockout (KO) and Wild type mice treated with folic acid. Densitometry data representing Figure 5B which is relative to normal Wild type (WT) mice, normalized to GAPDH and represent Mean ± SEM (n = 4). *P < 0.05 (normal WT) and #P < 0.05 (WT at respective treatment) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.



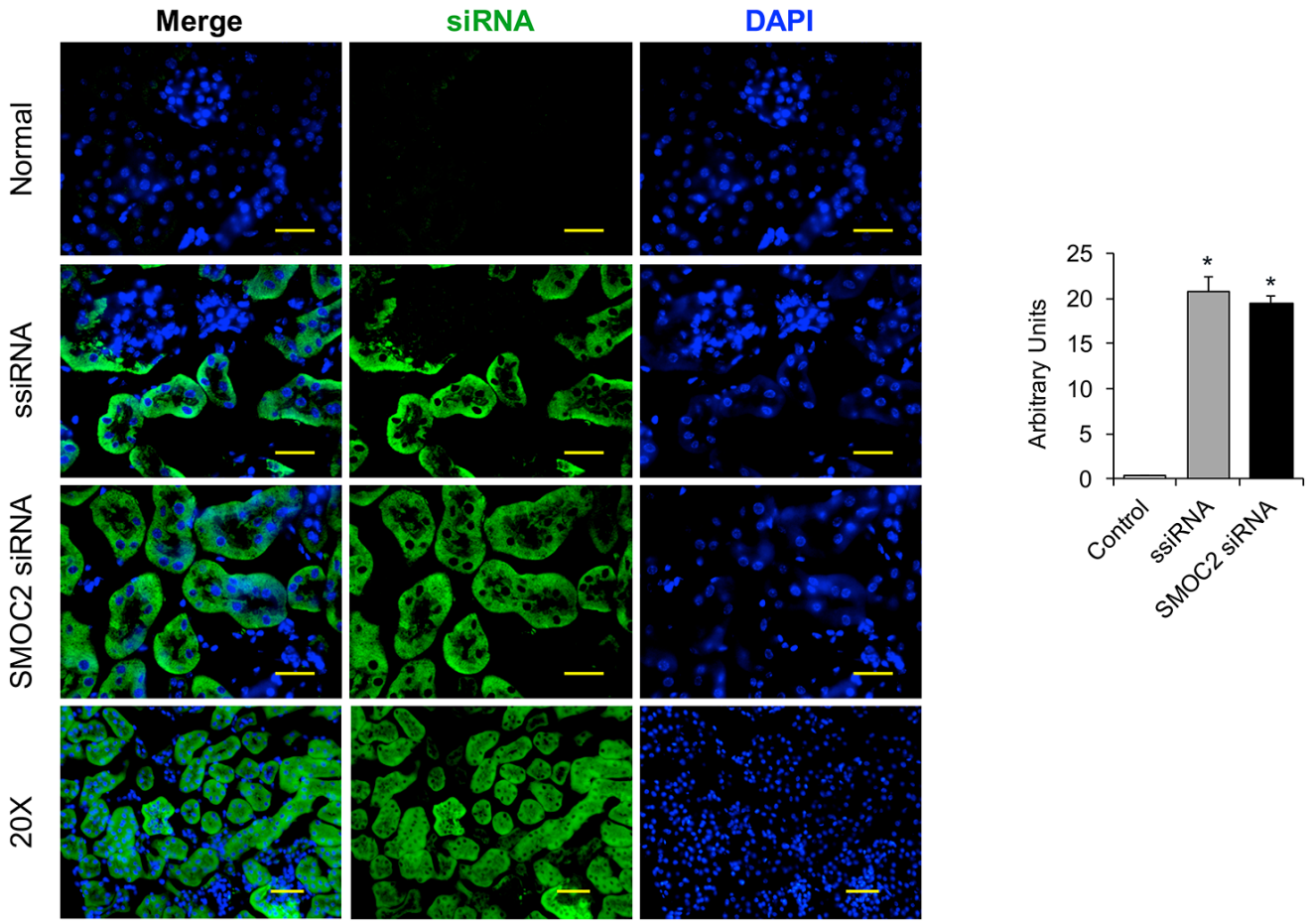
Supplementary Figure 12. Q**uantitation of Western blots for SMOC2 and fibrotic markers in SMOC2 knockout (KO) and Wild type mice that underwent UUO surgery.** Densitometry data representing Figure 6A which is relative to Wild type (WT) CoK mice, normalized to GAPDH and represent Mean ± SEM (n = 5). *P < 0.05 (WT CoK) and #P < 0.05 (WT at respective treatment) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.



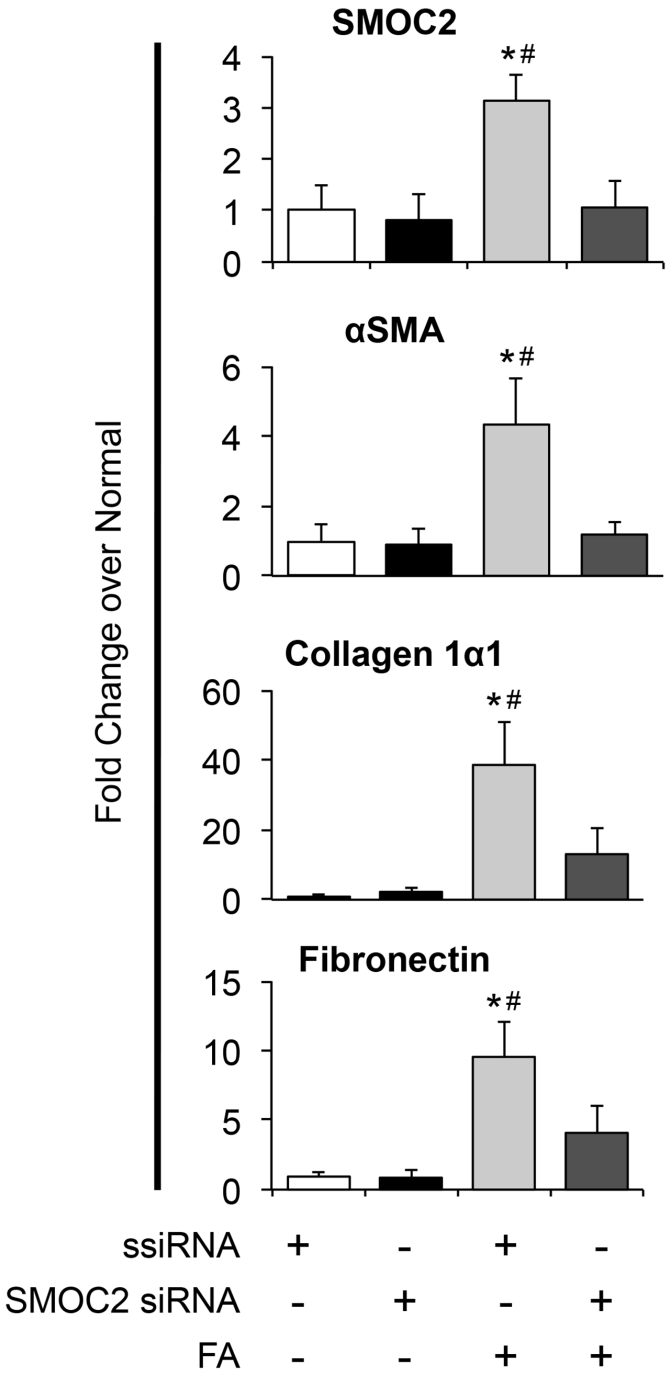
Supplementary Figure 13. Performance of SMOC2 siRNAs in NIH3T3 cells. NIH3T3 cells treated with various SMOC2 siRNA for 24h and measured for SMOC2 production by Western blot.



Supplementary Figure 14. Quantitation of Western blots for SMOC2 siRNA treatment of fibroblasts. Densitometry data representing Figure 7A which is relative to untreated ssiRNA transfected NIH3T3 cells, normalized by GAPDH and represent Mean ± SEM (n=3). *P < 0.05 (untreated ssiRNA cells) and #P < 0.05 (ssiRNA cells at respective treatment) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.



Supplementary Figure 15. Enrichment of siRNA in mice kidneys following iv injection via the tail vein. Mice were injected intravenously with 30µg/200uL of SMOC2 siRNA or ssiRNA 4h before and 2, 4 and 6 days and sacrificed on day 7. siRNA oligonucleotides were synthesized as Fluorescein conjugate; hence, visualized to evaluate siRNA delivery by 40X (above 3 rows, Scale = 30µm) and 20X (bottom row, Scale = 50µm) confocal microscopy. Images are representative of 10 visual fields/mouse (n=5 mice/group). Quantification is represented in a bar graph as arbitrary units (Mean ± SEM, n=5 mice/group, 10 visual fields/mice).



Supplementary Figure 16. Quantitation of Western blots for mice treated with SMOC2 siRNA followed by folic acid administration. Densitometry data representing Figure 7B which are relative to untreated ssiRNA injected mice, normalized to GAPDH and represent Mean ± SEM (n=5). *P < 0.05 (ssiRNA normal) and #P < 0.05 (ssiRNA at respective treatment) determined by one-way analysis of variance (ANOVA) with Tukey post-hoc analysis.

Supplementary Table 1: List of primers used for Genotyping

Gene	F/R	Sequence
Wild type	F	TCC TTC TCC AGC ACC AAG TC
	R	TGA TCC AAA AGT GCC TCC TC
KO	F	CGG TCG CTA CCA TTA CCA GT
	R	CAT GCT CTG AGA AAT AAT TAC CAA
Transgenic	F	TGA CAG CAG CAG CGG CAG TT
	R	TAG CGG CTG AAG CAC TGC A

Supplementary Table 2: List of primers used for qRT-PCR

Gene	F/R	Sequence
αSMA	F	GTC CCA GAC ATC AGG GAG TAA
	R	TCG GAT ACT TCA GCG TCA GGA
Fibronectin	F	ATG TGG ACC CCT CCT GAT AGT
	R	GCC CAG TGA TTT CAG CAA AGG
Smoc2	F	CCG TAC AAG AAC TGA TGG GC
	R	CTT TCA GCA TGA CCT CTG GG
Col1a1	F	TGA CTG GAA GAG CGG AGA GT
	R	GTT CGG GCT GAT GTA CCA GT
GAPDH	F	ATT GCC CTC AAC GAC CAC TTT G
	R	TCT CTC TTC CTC TTG TGC TCT TGC