biomedicines

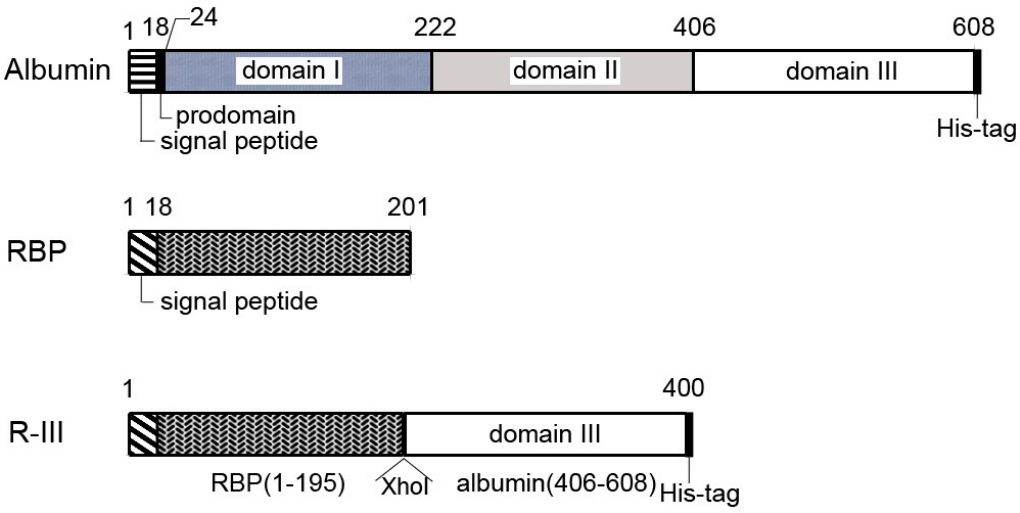
Supplemental Table 1. Primers used in this study.

Gene		5' to 3'
mβ-actin	F	GGACTCCTATGTGGGTGACG
	R	CTTCTCCATGTCGTCCCAGT
mCollagen I	F	CCAAAGGTGCTGATGGTTCT
	R	ACCAGCTTCACCCTTGTCAC
mMCP1	F	CTGGATCGGAACCAAATGAG
	R	CGGGTCAACTTCACATTCAA
mPAI-1	F	TCCTCATCCTGCCTAAGTTCTC
	R	GTGCCGCTCTCGTTTACCTC
mSTRA6	F	TGCTGGACTCTGGAGATG
	R	GTGATCACCTGCCCATC
mTGFβ1	F	AGCCCGAAGCGGACTACTAT
	R	CTGTGTGAGATGTCTTTGGTTTTC
rAlbumin	F	CGGTACCGGCACAATGAAGTGGGTAA
	R	GGTCTAGATTAGGCTAAGGCTTCTTTG
rAggf1	F	AAGGCCGGAAGATGTTGGAG
	R	CTCTCGTGCTTTGTCCCAGT
rα-SMA	F	TATCTGGGAAGGGCAGCAAA
	R	CCAGGGAAGAAGAGGAAGCA
rCEBPα	F	AAGATGCGCAACCTGGAGAC
	R	CCTTCTTCTGCAGCCGCTC
rCollagen I	F	GGAGAGTACTGGATCGAC
	R	CTGACCTGTCTCCATGTT
rCrbp1	F	CACTACCCACCCATTTCGCT
	R	GGGTGGAGGGGTAAGAAAGC
rGAPDH	F	GGTGGTCTCCTCTGACTTCAACA
	R	GTTGCTGTAGCCAAATTCGTTGT
rPPARγ	F	CACAATGCCATCAGGTTTGG
	R	GCTGGTCGATATCACTGGAGATG
rPDGF-β	F	TGGAGTCGAGTCGGAAAGCT
	R	GAAGTTGGCATTGGTGCGAT
rNG2	F	GTTTACCCTCACCACTCGGA
	R	TAAAGTTGCCACGCTTGTCC

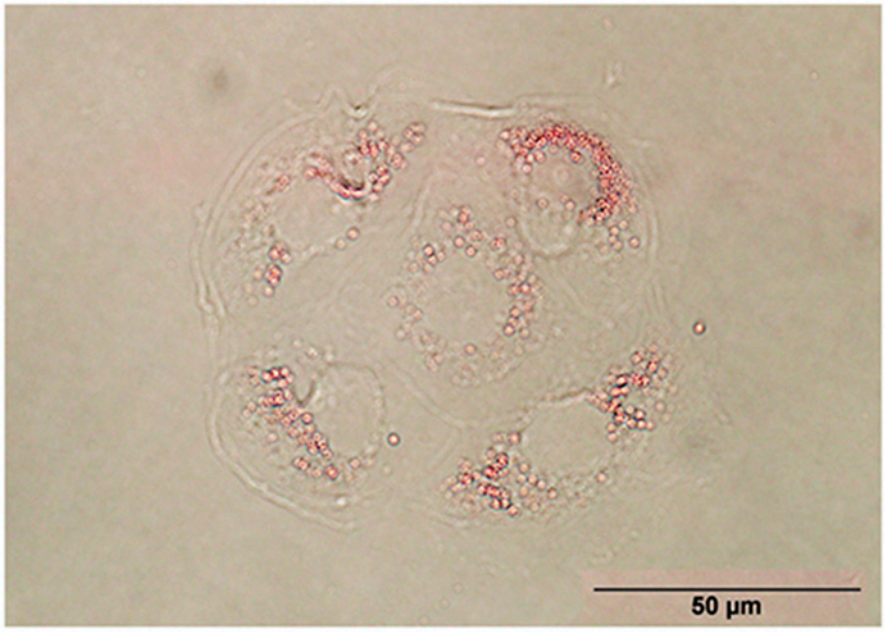
Supplemental Table 2. Primary antibodies used in this study.

Method	Primary antibody	Company and Country
Immunohistochemistry	α-SMA	Dako, Santa Clara, CA, USA
	CD31	Cell Signaling Technology, Beverly, MA, USA
	Collagen I	Abcam, Cambridge, MA, USA
	Collagen IV	Abcam, Cambridge, MA, USA
	Cygb/STAP	Generous gift from Dr. Norifumi Kawada
	Desmin	Dako, Carpinteria, CA, USA
	Fibronectin	Abcam, Cambridge, MA, USA
	F4/80	Serotec, Kidlington, Oxford, UK
	His-tag	Bioss, Woburn, MA, USA
	PAI-1	American Diagnostica, Stamford, CT, USA
	PDGFR-β	Enzo, Farmingdale, NY, USA
	STRA6	Bioss, Woburn, MA, USA
	TGF-β1	Santa Cruz Biotechnology, Santa Cruz, CA, USA
Western blotting	Albumin	Affinity Bioreagents, Rockford, Illinois, USA
	α-SMA	Sigma-Aldrich, St. Louis, MO, USA
	α-tubulin	Cell Signaling Technology, Beverly, MA, USA
	β-actin	Sigma-Aldrich, St. Louis, MO, USA
	E-cadherin	Abcam, Cambridge, MA, USA
	FSP-1	Abcam, Cambridge, MA, USA
	LRAT	IBL, Gunma, Japan

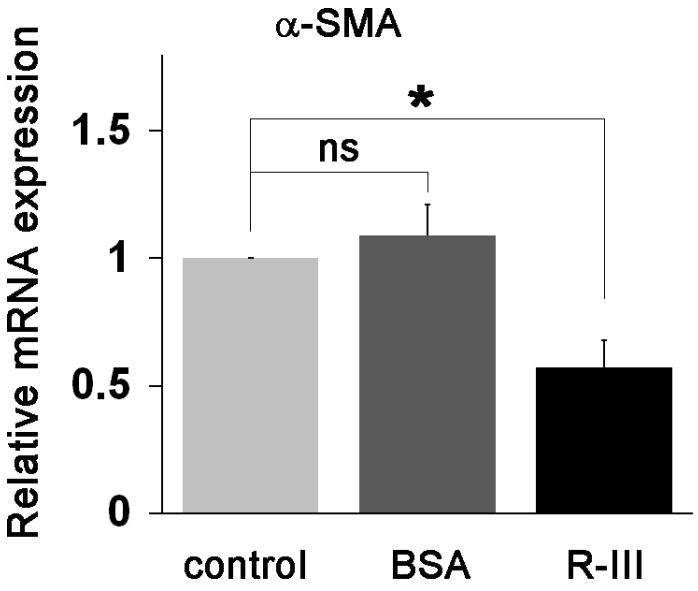
Supplemental Figure 1. Schematic diagram of R-III, a retinol-binding protein (RBP) - albumin domain III fusion protein, in comparison with full-length albumin and RBP. The numbers indicate amino acids.



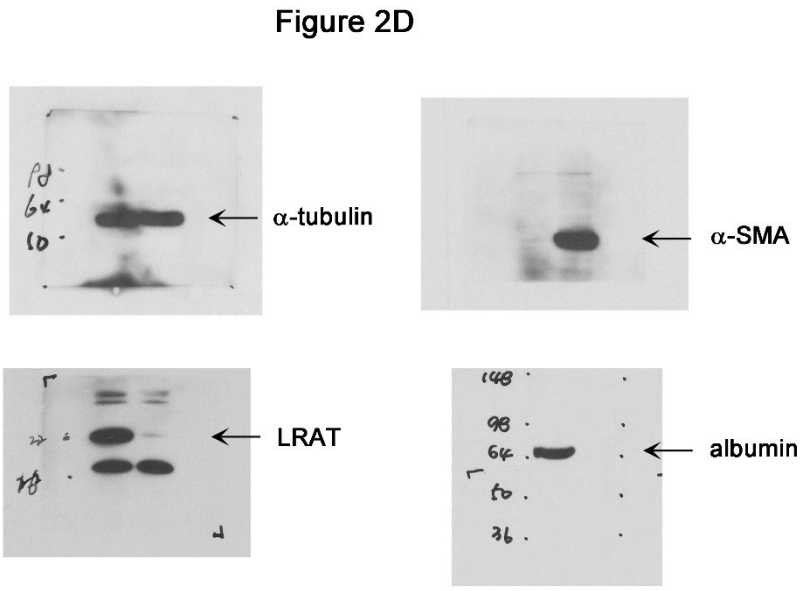
Supplemental Figure 2. Differential interference contrast images of oil red O staining for renal stellate cells on day 3 after seeding.



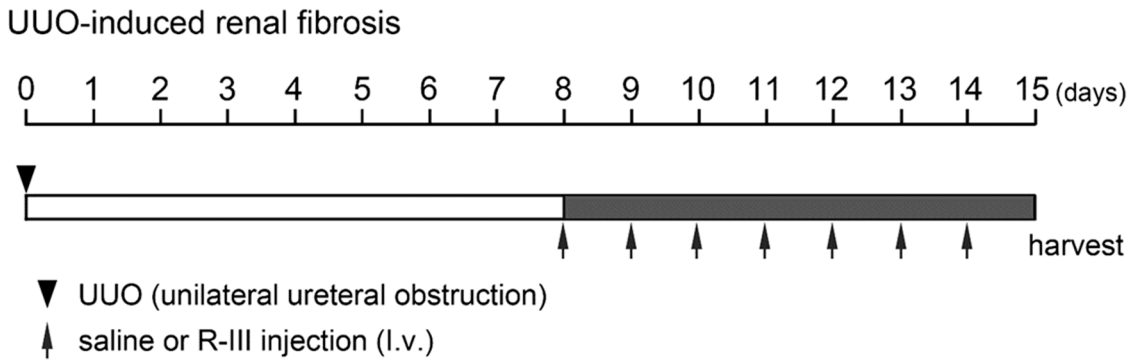
Supplemental Figure 3. Renal stellate cells after passage 2 were treated with bovine serum albumin (BSA; 0.5 μM) or R-III (0.5 μM) for 20 h, and α-smooth muscle actin (α-SMA) expression was analyzed by real-time PCR. The data represent the means ± SD for three independent experiments. *P*-value was estimated using paired *t*-test (compared with the untreated cells). $^{**}P < 0.01$. ns = not significant.



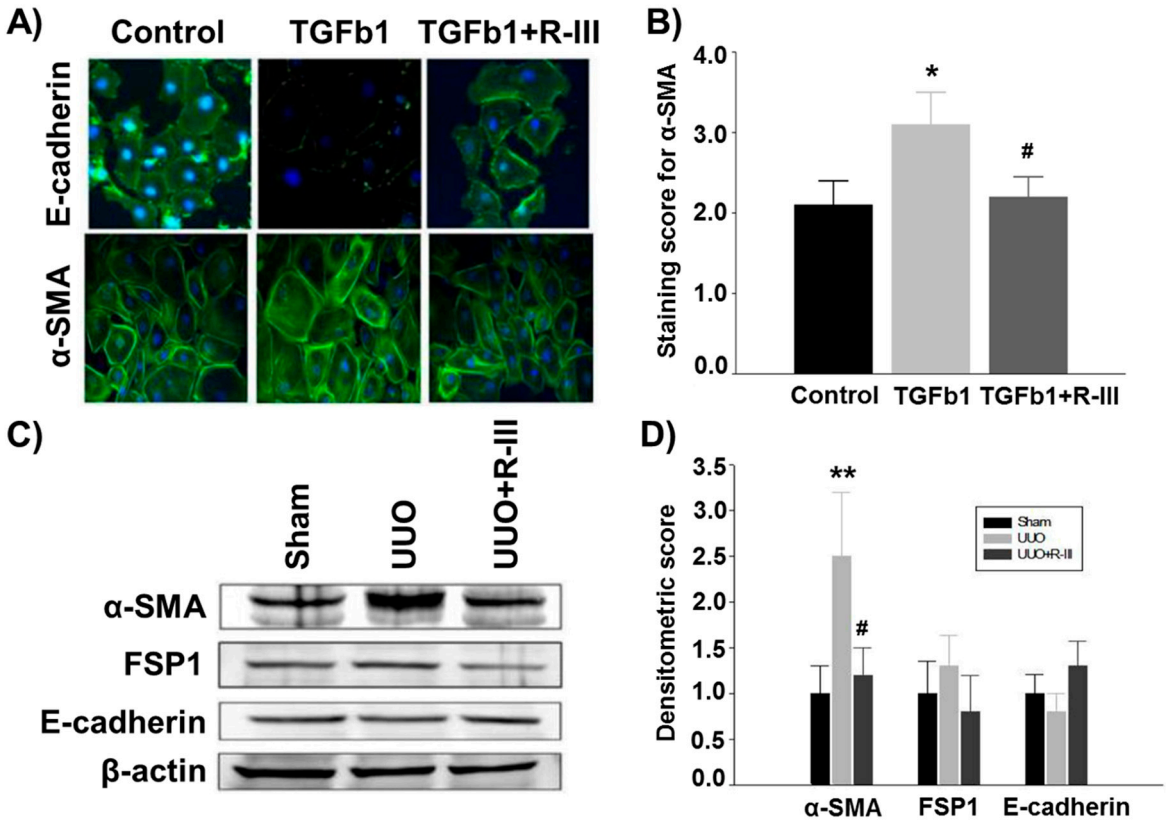
Supplemental Figure 4. Uncropped full-length blot images.



Supplemental Figure 5. Schedules for developing the murine kidney injury model using unilateral ureteral obstruction (UUO). Male C57BL/6 mice were subjected to UUO, and were intravenously administered saline or R-III (30 μg) dissolved in saline daily at 8–14 days after UUO (*hatched box, black arrows*).



Supplemental Figure 6. Effects of R-III on epithelial-mesenchymal transition (EMT) *in vitro* and *in vivo*. (A) NRK-52E cells were treated with TGF-β1 (50 ng/ml) for 5 days in the presence or absence of R-III (0.5 μM) and subjected to immunofluorescence using antibodies against E-cadherin or α-SMA. (B) Quantitative assessment of the intensity of α-SMA staining. The data represent the means ± SD for five independent experiments. *P*-value was determined using paired *t*-test. **P* < 0.05 vs. control, #*p* < 0.05 vs. TGF-β1-treated cells. (C) Tissue lysates were prepared from the kidneys of sham-, UUO-, and UUO+R-III-treated mice and analyzed by western blotting for the expression of EMT markers. (D) Densitometric analysis of the bands on the western blot. Data are expressed as the means ± SD (*P*-value; Kruskal–Wallis test, followed by DSCF multiple comparison test). ***P* < 0.01 vs. Sham, #*p* < 0.05 vs. UUO treatment.



Supplemental Methods

Epithelial-mesenchymal transition (EMT)

NRK-52E cells were seeded on 0.1% gelatin-coated coverslips in a 24-well plate and grown in Dulbecco`s Modified Eagle Media (DMEM) containing 5% fetal bovine serum (FBS). Subconfluent cells were starved for 24 h by incubating with DMEM containing 1% FBS, and then cultured for five days in the presence of recombinant mouse TGF-β1 (50 ng/ml, Cell Signaling Technology, Danvers, MA, USA) to induce EMT. To examine the effects of R-III, cells were treated with R-III (0.5 μM) by adding it to the culture medium during the last 24 h of TGF-β1 treatment.