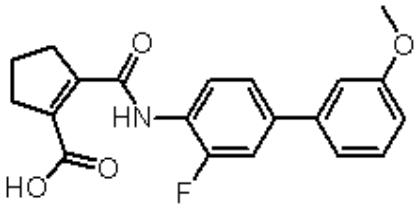
SDC – SUPPLEMENTAL DIGITAL CONTENT

Supplemental Figure S1. Chemical structure of 4SC-101.



Supplemental Table S1. Body weights, blood urea nitrogen (BUN), serum creatinine and creatinine clearance in rats 12 weeks after 5/6 nephrectomy. *P<0.05 vs. placebo treated group.

Treatment group	Body weight (g)	BUN (mM)	Serum creatinine (uM)	Creatinine clearance (ml/min)
Placebo	523 ± 48	9.98 ± 1.7	67.8 ± 4.8	0.99 ± 0.45
4SC-101 20 mg/kg	528 ± 67	10.47 ± 0.5	65 ± 3.22	0.79 ± 0.44

Supplemental methods

Functional Measurements

Serum creatinine and blood urea nitrogen levels were analyzed by the time of harvesting by an automatic laboratory analyzer (Synchron CX5, Beckman Coulter, Krefeld, Germany) in rats who underwent 5/6 nephrectomy.

Body weights of animals who underwent 5/6 nephrectomy were measured and 24-hr urine samples were collected using metabolic cages with urine-cooling systems every 4 week. Urinary protein concentrations were measured (Beckmann Coulter CX5, Krefeld, Germany) and proteinuria was calculated. Creatinine clearance at the time of harvesting was calculated according to the formula: $CrCl=U_{crea}$ (mg/dL)×diuresis (mL)/S_{crea} (mg/dl)×1440 (min)/weight (kg).

Immunohistochemistry

Immunohistochemical studies were performed on frozen sections fixed in acetone (4°C). Sections were incubated with mouse primary monoclonal antibodies against the macrophage marker CD68 (clone ED1) and CD5 positive T cells (clone OX19; Biozol, Eching, Germany) followed by washing the sections and incubation with a secondary rabbit anti mouse antibody (Dako A/S, Glostrup, Denmark).

After washing, sections were incubated with alkaline phosphatase antialkaline phosphatase (APAAP) complex (Dako A/S, Glostrup, Denmark). Binding of antibodies was visualized by development with Fast Red chromogene solution (Dako A/S, Glostrup, Denmark). Infiltration of CD68, CD5 and CD3 immunoreactive cells were assessed on a scale of 1 to 3.
For Interleukin-17 (IL-17) immunohistochemistry, rabbit polyclonal antibody from Santa Cruz Biotechnology Inc. was used (sc-7927), and signal was visualized by usage of biotinylated anti rabbit Vector BA-1000, Streptavidin HRP-POD Vector, and Nov Red POD-Substrate Vector SK4800 (Vector Laboratories, Burlingame USA).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)
TUNEL analysis was performed on frozen sections fixed in paraformaldehyde 4% as described previously ([i]).

RNA Extraction and Quantitative Real-Time Reverse-Transcriptase Polymerase Chain Reaction
RNeasy Mini Kit (Qiagen) was used for total mRNA extraction from snap-frozen organs. The RT (reverse transcription) protocol was according to the user manual of Bio-Rad cDNA synthesis kit (iScript cDNA Synthesis Kit, BIO-RAD; RNA amount: 250 ng). Quantitative Real-Time PCR (RT-PCR) was performed with IQ SYBR Green Supermix (Bio-Rad) using gene-specific primers. Primers against the selected genes are given in **Supplemental Table S2 (below).** Glycerinaldehyd-3-phosphat-Dehydrogenase (GAPDH) was used as a housekeeping gene. For IL-2 PCR analysis, primer pairs were ordered from R&D Systems Inc (R&D Systems, Inc. Minneapolis, USA). The primer concentrations were adjusted to 200 nM. The number of PCR cycles for the listed genes was 40.

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Supplemental Table S2. Primer sequences for real-time PCR analysis

	primer pairs
TGF-beta	Forward: 5'-`-GACCCTTCCTGCTCCTCAT -3' Reverse: 5'-GTGTCCAGGCTCCAAATGTA -3'
Collagen I	Forward: 5'-GCAAGGAACAGTCGATTCA-3' Reverse: 5'-GGTCTTGGTGGTTTTGTATTCGA -3'
Collagen III	Forward: 5'-TCCTGAAGATGTCCTTTGATGTA-3' Reverse: 5'-TTCAGAGACTTCTTTACATTGCC-3'
IL-17	Forward: 5'- GAAGAGGGAGCCTGAGAAGT-3' Forward: 5'- GCATGGCGGACAATAGAGGAA-3'
GAPDH	Forward: 5'-ATG CTT GTG ATG GGT GTG AA-3' Forward: 5'-GGA TGC AGG GAT GAT GTT CT-3'

[i]. Rusai K, Wagner B, Roos M *et al.* The serum and glucocorticoid-regulated kinase 1 in hypoxic renal injury. *Cell Physiol Biochem*. 2009; 24: 577-84.