Online Methods:

Animal studies

αSMA-RFP[17], αSMA-Cre, αSMA-tk, NG2-YFP, NG2-Cre, NG2-tk, *Pdgfrb*-tk and *Pdgfrb*-RFP transgenic mice were generated in our laboratory. NG2-tk, NG2-YFP, *Pdgfrb*-RFP and *Pdgfrb*-tk mice were recently described[24]. *Col4a3*$^{-/-}$ mice were previously described[18]. Details on the construct to generate αSMA-Cre, αSMA-tk, and NG2-Cre mice are listed in the Supplementary Methods section. *Pdgfrb*-Cre mice were kindly provided by R. Adams, Max Planck Institute for Molecular Biomedicine, Münster, Germany. γGT-Cre mice[9] were kindly provided by E. Neilson, Northwestern University, Chicago, IL. R26R-LoxP-Stop-LoxP-YFP (YPF$^{f/f}$) and *Cdh5*-Cre mice were purchased from Jackson Laboratories. *Tgfbr2*$^{f/f}$ mice were kindly provided by Dr. Harold Moses, Vanderbilt University, Nashville, TN. Unilateral ureteral obstruction (UUO) was performed as previously described[33] and the mice were euthanized two, five or ten days later. Both male and female mice were used. Some of the mice received daily intraperitoneal (i.p.) injections with 50 mg.kg^{-1} of ganciclovir (GCV, Invivogen). For *Col4a3*$^{-/-}$;αSMA-tk and *Col4a3*$^{-/-}$;NG2-tk mice, GCV was administered daily at 8 weeks of age and mice were euthanized at 12 weeks of age. All mice used were on BALB/c genetic background or mixed BALB/c genetic background (αSMA-Cre;*Tgfbr2*$^{f/f}$, *Col4a3*$^{-/-}$;αSMA-tk and *Col4a3*$^{-/-}$;NG2-tk) and genetic backgrounds were identical when comparing experimental groups. Bone marrow transplant studies were performed as previously described[18] and mice were maintained 4-5 weeks post bone marrow transplant and before UUO. All mice were housed under standard

conditions at the Beth Israel Deaconess Medical Center animal facility. All animal procedures were reviewed and approved by the Animal Care and Use Committee of the Beth Israel Deaconess Medical Center.

Cell Culture

MSCs were purified and expanded as previously described[39]. For TGFβ1 stimulation, 10 ng.ml^{-1} human recombinant TGFβ1 (R&D Systems) was added to serum free culture media for the indicated times.

Histological processing, light microscopy, and morphometric analyses

All procedures were performed as previously described[18,19].

Immunohistochemistry

Kidneys were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. The deparaffinised sections were incubated in 10 mM citrate buffer (pH 6.0) for one hour at boiling temperature prior blocking with 1% BSA in TBS for 30 min. The sections were immunolabeled using anti-Collagen I antibody (Southern Biotech, 1:100) or anti-αSMA (Sigma, 1:200) overnight at 4 ˚C. The immunobound antibodies were detected using biotin-conjugated secondary antibodies and ABC reagent (Vector Laboratories) and the sections were developed by DAB staining and counterstained with haematoxylin. The number of DAB^{+} (brown) cells was analyzed in 5-8 visual fields from each kidney section by NIH ImageJ analysis software and double-blind cell counting using light microscopy. Additionally, 5 random fields of view were photographed and the

number of αSMA$^+$ cells were counted for each imaged 200x field of view. For Collagen I staining, a grid intersection analysis was also performed on photographed images. Both manual and computerized quantification gave similar results.

Immunostaining

Frozen section of harvested kidneys embedded in O.C.T. medium were immunostained using antibodies against NG2 (Chemicon/Millipore, 1:200), PDGFRβ (eBiosciences, 1:50), αSMA (Sigma, 1:200), CD31 (BD Pharmingen, 1:100), CD34 (abcam, 1:100), phospho-Smad2 (Cell Signal, 1:100), CD11b (eBiosciences, 1:100), BrdU (Roche Applied Science, 1:100), F4/80 (Hycult, 1:50) and secondary fluorescent antibodies. Pictures were taken using the Axioskop 2 fluorescent microscope, AxioCam HRC camera and the Axiovision 4.3 software. Pictures were also taken using the Zeiss LSM 510 Meta confocal microscope. For quantitation of immunostained or fluorescent images, 5 random fields of view were photographed and the number of cells immunolabeled counted per 200x field of view.

FISH for Y-chromosome

To assess percent chimerism in bone marrow transplant studies, the bone marrow of sex-mismatched transplanted female mice was harvested at the experimental time point and cyto-spinned onto a microscope glass slide. Star*FISH mouse Y-chromosome probes (Cambio) were used to label the Y-

chromosome in bone marrow cells according to the manufacturer's directions. 100 nuclei were counted in three fields of view from each of the three bone marrow transplanted mice evaluated, and Y-chromosome positivity expressed as a percentage of the total number of nuclei counted.

YFP and RFP visualization

Mouse kidneys were fixed in 4% PFA overnight at 4 °C and equilibrated in 30% sucrose overnight at 4 °C. The kidneys were then embedded in O.C.T compound and frozen sections (5 μm) were mounted with Vectashield Mounting Medium with DAPI (Vectashield) and a glass coverslip and visualized under YFP or RFP fluorescent filter.

Quantitative Real time PCR analysis

MSCs and whole kidneys were homogenized in TRIzol® (Invitrogen), extracted according to the manufacturer's directions, and cDNA synthesis was performed using Applied Biosystems cDNA synthesis kit according to the manufacturer's directions. Real-time PCR was carried out for mouse *Acta2* (αSMA) and *Col1a1*. Primers were utilized with SYBR Green PCR Master Mix in a 7300 Sequence Detector System (Applied Biosystems) and measurements were standardized to expression of the β-actin housekeeping gene. Relative gene expression is reported by normalizing to untreated MSCs. Primer sequences are listed in the Supplementary Methods section.

39. Tondreau, T.*, et al.* Isolation of BM mesenchymal stem cells by plastic adhesion or negative selection: phenotype, proliferation kinetics and differentiation potential. *Cytotherapy* **6**, 372-379 (2004).