Supplementary Tables

Supplementary Table 1. Top Ten Upregulated Genes by Microarray Analysis of Mouse Kidney FAF Normalized to Mouse Kidney NF

GENES	FOLD	GENE DESCRIPTION
Wfdc2	37.21	Human Epididymis Protein 4 (HE4)
Bgn	32.92	Biglycan
Des	26.68	Desmin
Serpinf1	20.83	Serpin peptidase inhibitor, clade F, member 1
Dcn	19.75	Decorin
Actg2	16.53	Actin, gamma2, smooth muscle, enteric
Emp1	16.01	Epithelial membrane protein 1
Serpina10	14.44	Serpin peptidase inhibitor, clad A, member 10
Igfbp6	14.37	Insulin-like growth factor binding protein 6
Ptgis	14.32	Prostaglandin I2 (prostacyclin) synthase

Supplementary Table 2. Patient Information

Sera from: (F: female M: male)	Interstitial Fibrosis (%)	Age (years)	Other Renal Pathology	Other Medical History	BUN (mg/dL)	Albumin to Creatinine Ratio
Patient 1 (M)	20	75	Secondary FSGS	Monoclonal gammopathy, bladder cancer	22	296
Patient 2 (F)	20	74	Fibrillary GN	Breast cancer, obesity	30	46.3
Patient 3 (F)	20	56	Secondary FSGS, thin basement membranes		26	288.2
Patient 4 (M)	20	29	IgA nephropathy and interstitial nephritis		33	
Patient 5 (F)	30	59	Secondary FSGS, thrombotic angiopathy	Glucose intolerance, obesity	27	1799.4
Patient 6 (F)	60	56	Acute interstitial nephritis		19	3.5
Patient 7 (M)	60	58	Diabetic nodular sclerosis (early), phosphate nephropathy		26	61.3
Patient 8 (M)	40	59	Secondary FSGS		32	188.2
Patient 9 (M)	60	69	Chronic interstitial nephritis, immune complex GN	Bacteremia, colitis	38	428.4
Patient 10 (F)	20	55	IgA nephropathy, thin basement membrane, interstitial nephritis	Pernicious anemia, thyroiditis	36	NA
Patient 11 (F)	60	54	Secondary FSGS, vascular sclerosis	Pericarditis	35	NA
Control 1 (F)	NA	34	NA	Endometriosis	17	35.8
Control 2 (F)	NA	33	NA	Endometriosis	14	
Control 3 (F)	NA	58	NA	Cerebrovascular disease, lumpectomy, hysterectomy	17	NA
Control 4 (M)	NA	55	NA	Mitral regurgitation	18	NA
Control 5 (F)	NA	55	NA	Liver hemangiomas	15	NA

Supplementary Material and Methods

Generation of αSMA-RFP transgenic mice

αSMA-RFP mice were generated using an extended *αSMA* promoter containing approximately 2.4kb of the *αSMA* promoter plus exon 1, intron 1, and part of exon 2 (altogether 5.2kb) amplified with the following primers: 5' CAATGCATGCTGTACAAACATCAGG 3' (Forward); 5' AGCTGGAGCAGCGTCTCAGGGTTCTGC 3' (Reverse). The extended *αSMA* promoter was cloned into the pDsRed-Express-1 vector (Clontech) using EcoRI and SalI restriction sites, and the whole αSMA-RFP construct was released from the vector using EcoRI and DrdI before purification and injection into fertilized eggs. All transgenic mice were created in the Brigham and Women's Hospital Transgenic Core Facility on a FVB background and backcrossed at least 10 generations to a BALB/c background for experiments. Mouse studies followed the Institutional Animal Care and Use Guidelines at the BIDMC. Age-matched mice were used for all studies.

5/6 Nephrectomy

CD-1 mice were subjected to 5/6 nephrectomy as previously described[1]. Two weeks following completion of surgery, the mice were treated with anti-IgG (n=3) and anti-HE4 (n=3). 14mg/Kg BW of rabbit anti-HE4 antibody or rabbit IgG control antibody was administered i.p. every other day for two weeks. The mice were then euthanized and kidney histology assessed.

Microarray analysis and gene expression analyses

Total RNA was isolated from mouse fibroblasts using RNeasy Plus Mini Kit (Qiagen) and submitted to the Molecular Genetics Core Facility at Children's Hospital (Boston, MA). Microarray analysis was performed using Mouse Ref8 Gene Expression BeadChip (Illumina), and gene expression was determined by MetaCore (GeneGo) and Genome Studio (Illumina) software. For gene expression analysis by Real time PCR, the following primers were used:

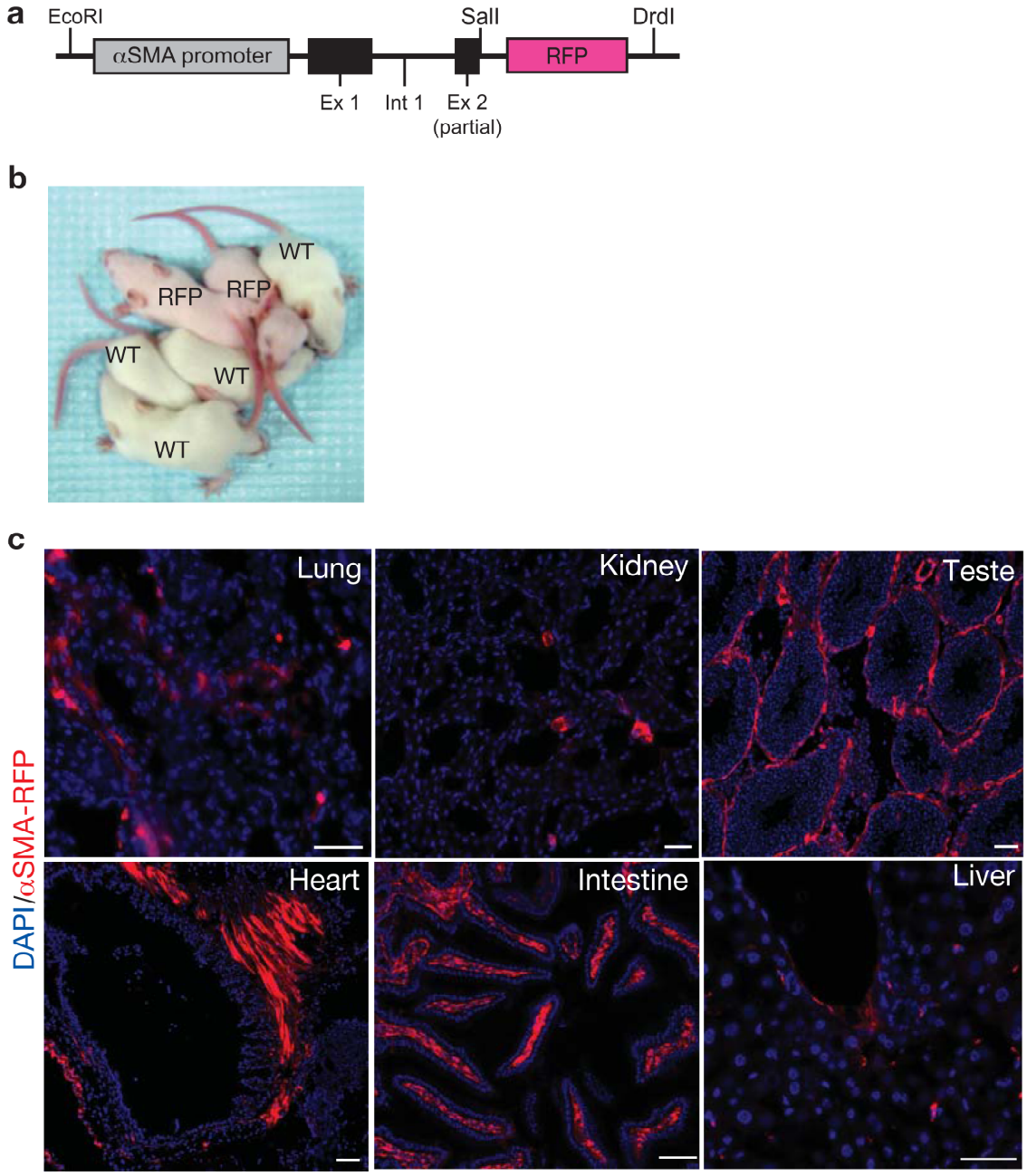
Mouse Col1a1	5'-GCTCCTCTTAGGGGCCACT-3' 5'-CCACGTCTCACCATTGGGG-3'
Mouse HE4	5'-AACCAATTACGGACTGTGTGTT-3' 5'-TCGCTCGGTCCATTAGGCT-3'
Mouse Prss23	5'-GGCAGACTTCGACGCCAAA-3' 5'-TGTTCCCTTGTGACACTGAGG-3'
Mouse Prss35	5'-TCTCTGATGGGTCGGAAACAG-3' 5'-ACTCACAACCTGGGGTATTCT-3'
Mouse Acta2	5'-CCAGTTGTACGTCCAGAGGC-3' 5'-GGTGATGATGTCCCAGGGC-3'
Mouse β-actin	5'-GGCTGTATTCCCCTCCATCG-3' 5'-CCAGTTGGTAACAATGCCATGT-3'
Human HE4	5'-CGACAACCTCAAGTGCTGC-3' 5'-AGAAATCTCCCAGAGCCTCC-3'
Human PRSS23	5'-CAGTGTCATAAGGGAACTCCAC-3' 5'-CCTGAGTCTCGGTGTTGGG-3'
Human PRSS35	5'-CATCGAATGCCAGAAAGAACTCC-3' 5'-GGTTAAGGTTCGGGTGCCA-3'
Human β2-microglobulin	5'-CCAGCAGAGAATGGAAAGTC-3' 5'-GATGCTGCTTACATGTCTCG-3'

Migration and proliferation assays

Mouse FAFs were assessed for migration in Boyden chambers as previously described[2] and proliferation using MTT assay under standard condition using DMSO as solubilizing agent.

Supplementary Figures

Supplementary Figure 1

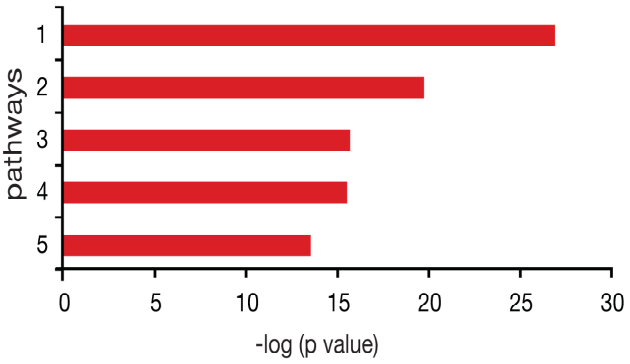


Supplementary Figure 1. Characterization of αSMA-RFP transgenic mice.

a. Construct map of the αSMA-RFP transgene injected into zygotes for the production

of transgenic mice. Ex: exon, Int: intron. **b.** αSMA-RFP transgenic mice (RFP) express red fluorescent protein (dsRed) in αSMA^+ cells, resulting in a demonstrably pink hue when compared to wild-type (WT) mice because αSMA^+ cells within the hair follicle dermis cast a red tint. **c**. $\alpha SMA\text{-}RFP^+$ cells from lung, kidney, teste, heart, intestine and liver in αSMA-RFP mice. DAPI (Blue): nuclei. Scale bar: 50μm.

Supplementary Figure 2

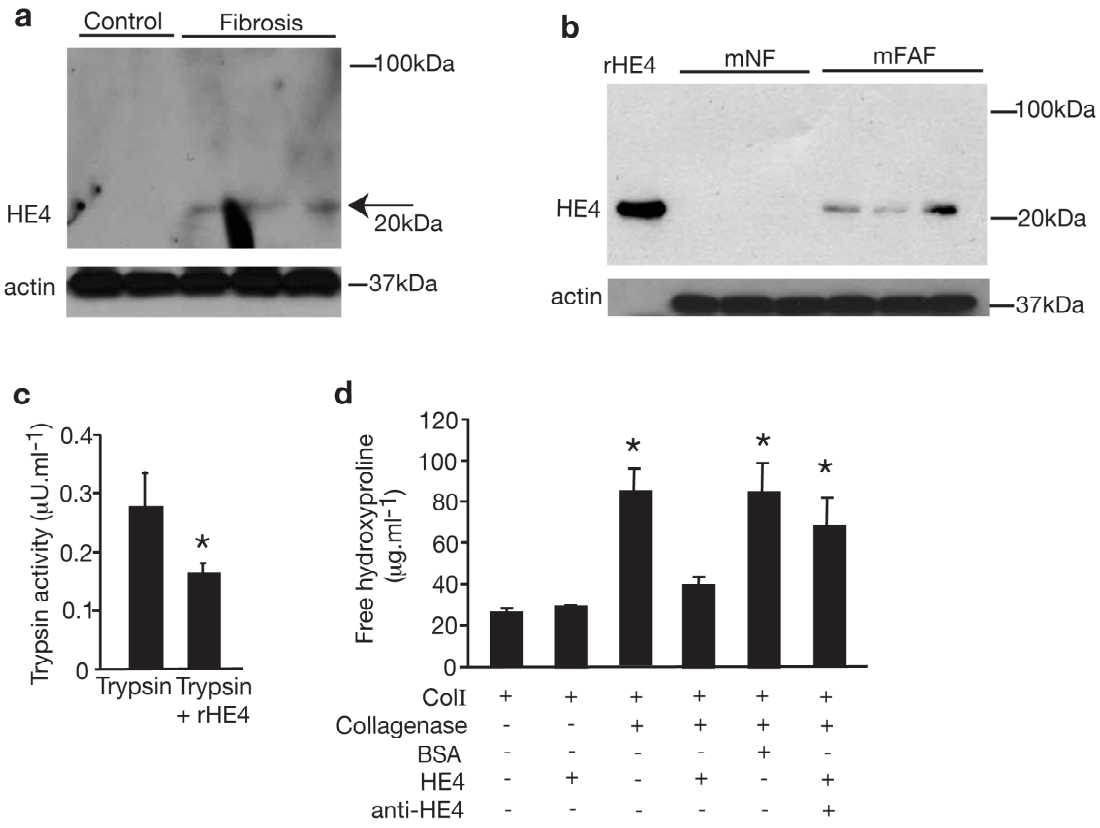


	pValue
1. TGF mediated cytoskeleton remodeling	**1.222e-27**
MAPKKK cascade	3.149e-28
Transforming growth factor beta receptor signaling pathway	1.658e-22
Positive regulation of mesenchymal cell proliferation	4.994e-14
2. Cytoskeleton remodeling	**1.862e-20**
Cell migration	3.044e-17
Cell-matrix adhesion	4.325e-14
Integrin-mediated signaling pathway	6.334e-13
3. Developmental regulation of epithelial-to-mesenchymal transition (EMT)	**1.968e-16**
Wound healing	2.202e-23
Positive regulation of cell division	2.584e-22
Positive regulation of epithelial to mesenchymal transition	3.443e-22
4. Cell adhesion	**2.913e-16**
Caveola assembly	5.986e-11
Negative regulation of fibrinolysis	1.074e-10
Chemotaxis	1.696e-11
5. Transport of Clathrin-coated vesicle cycle	**2.890e-14**
Clathrin coat assembly	2.429e-21
Vesicle-mediated transport	2.185e-92
Cellular membrane organization	1.682e-22

Supplementary Figure 2. Pathway analysis from microarray gene expression studies in mouse fibrosis-associated fibroblasts compared to normal fibroblasts.

Pathways analysis from differential gene expression and p-values are depicted.

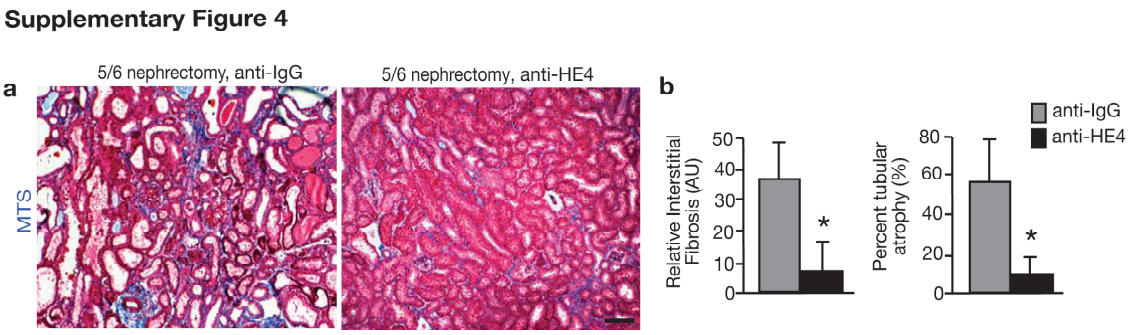
Supplementary Figure 3



Supplementary Figure 3. HE4 inhibits collagenase activity.

a. Western blot for HE4 in mouse control and fibrotic kidneys. Actin was used as an internal control. Arrow points to a single band for HE4 detection in fibrotic kidney lysates. **b**. Western blot for HE4 in mouse normal (mNF) and fibrosis associated fibroblasts (mFAF). Actin was used as an internal control. **c**. Trypsin activity assayed with and without recombinant HE4 protein (rHE4). **d**. Hydroxyproline release assay: free hydroxyproline ($\mu g.ml^{-1}$) from type I collagen digestion: type I collagen digested by bacterial collagenase with and without

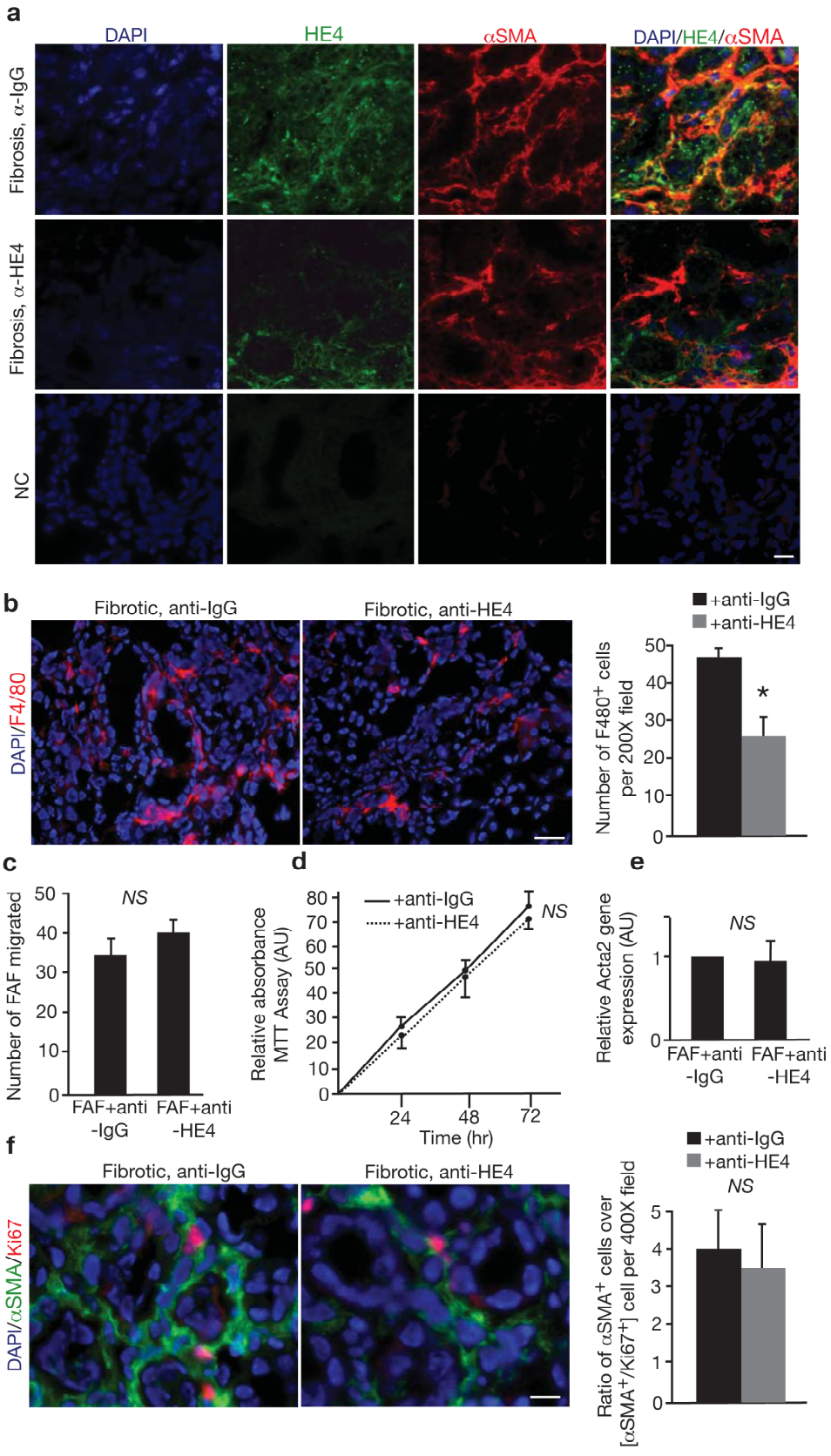
HE4, with and without anti-HE4 antibody. Data shown as mean +/- sem. *p<0.05.



Supplementary figure 4. Anti-HE4 neutralizing antibody improves renal histology following 5/6 nephrectomy

a. Representative Masson Trichrome (MTS) of fibrotic kidneys from mice with 5/6 nephrectomy treated with anti-HE4 (n=3) or anti-IgG (n=3) antibody. Scale bar: 50μm. **b**. Morphometric analyses for relative interstitial fibrosis and tubular atrophy based on MTS staining shows reduced fibrosis and tubular atrophy in anti-HE4-treated mice. AU: Arbitrary Unit. Data shown as mean +/- sem. *p<0.05.

Supplementary Figure 5



Supplementary Figure 5. HE4 does not directly alter migration and proliferation of fibrosis-derived fibroblasts.

a. Immunolabeling for HE4 and αSMA in fibrotic kidneys from mice treated with anti-HE4 or anti-IgG control antibodies. DAPI (blue): nuclei. NC: Negative control, 2^{ry} antibody only. Scale bar: 50μm. **b**. Immunolabeling for F4/80 (labeling macrophage) in fibrotic kidneys from mice treated with anti-HE4 or anti-IgG control antibodies. DAPI (Blue): nuclei. Scale bar: 50μm. Histogram represents the relative number of F4/80^{+} cells per field of view at 200X magnification. **c**. FAF migration assay incubated with anti-IgG or anti-HE4. **d**. Relative absorbance for MTT assay (proliferation) of FAF incubated with anti-IgG or anti-HE4. **e.** Relative *Acta2* gene expression in mouse NFs and FAFs (normalized to NF set arbitrarily to 1). AU, arbitrary units. **f**. Immunolabeling for KI67 and αSMA in fibrotic kidneys from mice with UUO and treated with anti-HE4 or anti-IgG control antibodies. DAPI (Blue): nuclei. Scale bar: 10μm. Histogram represents the relative number of αSMA^{+}/KI67^{+} (double positive) cells per field of view at 400X magnification. Data shown as mean +/- sem. *p<0.05, *NS*: not significant.

Supplementary references:

1. Leelahavanichkul, A.e.a. Rapid CKD progression in a new mouse kidney remnant model: strain dependent resistance is overcome by angiotensin II. *Kidney Interntational* **78**, 1136-1153 (2010).
2. Zeisberg, M., Maeshima, Y., Mosterman, B. & Kalluri, R. Renal fibrosis. Extracellular matrix microenvironment regulates migratory behavior of activated tubular epithelial cells. *The American journal of pathology* **160**, 2001-2008 (2002).