SUPPLEMENTARY DATA

Supplementary Table 1.
Characteristics of each group of mice

	Control			STZ		
	WT	CreTg	CreTg X Tg#5	WT	CreTg	CreTg X Tg#5
Number	6	6	6	8	8	10
BW(g)	42.9±9.0	44.8±6.0	48.8±8.4	30.2±3.5*	28.9±4.2*	31.3±2.4*
HbA1c (NGSP)(%)	2.0±0.3	2.7±0.3	2.5±0.4	8.0±0.5*	8.4±0.5*	8.6±0.7*
HbA1c (IFFC)(mmol/mol)	21.9±3.3	29.5±3.3	27.3±4.4	64±5.5*	68±5.5*	70±7.7*
rKW/BW (mg/g)	5.4±1.8	5.0±0.2	4.8±0.2	8.7±1.1*	9.8±1.1*	8.7±1.0*
CCr(ml/m/10gBW)	0.14±0.03	0.11±0.03	0.12±0.02	0.32±0.08*	0.29±0.04*	0.28±0.07*

*p<0.05 versus control

SUPPLEMENTARY DATA

Supplementary Table 2.
Primers for real-time RT-PCR

Gene	Sequence of primers (5'-3')	
18s rRNA	TCAACTTTCGATGGTGGTCG	TTCCTTGGATGTGGTAGCCGT
Col1a1	TGGACATTAGGCGCAGGAA	GAACTGGTACATCAGCCCGAA
Col4a1	TTCAGATTCCGCAGTGCCCTA	TTCTCATGCACACTTGGCAGC
Col4a2	TGGCTGAGGAGGAAATCAAGC	AATGGCGTTGCACGGAAGT
Col4a3	ACCAGGATGCAACGGATCTAA	TCCTATCACGCTATCGCCCAT
BMP2	TCGACCATGGTGGCCGGGACCCG	TGTTCCCGGAAGATCTGGAGT
BMP4	CGAGCCAACACTGTGAGGAGTT	GCTGCTGAGGTTGAAGAGGAAA
BMP7	TGTGGCAGAAAACAGCAGCA	TCAGGTGCAATGATCCAGTCC
BMPRII	AGCGAATTGTGCCAACCTCAC	TAACTGGAAATCGGCTGGTGC
ALK3	TGAGGACATGCGTGAGGTTGT	GCTCGAAGACATTCATCGCTG
Smad1	ATGGTTTCACAGATCCGTCCA	TCCCAATATGTCGCCTGGTGT

SUPPLEMENTARY DATA

Supplementary Table 3.

Antigen	Company (Cat. No)	Dilution	Fixation	Section	Ag retrieval	Amplification
Smad1	Abnova (1D3)	1:15	FA	Paraffin	10mM citrate pH6.0	SA-HRP
Type IV Collagen	Progen	1:250	Carnoy	Paraffin	Protease VIII (SIGMA)	ABC (Vector)
αSMA	Neo Markers (MS-113B)	1:400	FA	Paraffin	10mM citrate pH6.0	SA-HRP, TSA
Type I Collagen	Carbiochem	1:25	FA	Paraffin	10mM citrate pH6.0	ABC (Vector)
pSmad1/5/8	Chemicon	1:400	FA	Paraffin	10mM citrate pH6.0	SA-HRP, TSA
BMP4	Abgent	1:50	FA	Paraffin	10mM citrate pH6.0	ABC (Vector)
BMPR1a (ALK3)	Abgent	1:20	Zamboni	Frozen	N/A	N/A
Desmin	DAKO	1:100	Zamboni	Frozen	N/A	N/A
Nephrin	Gift from Dr Tsukaguchi	1:200	Acetone	Frozen	N/A	N/A
vVF	SIGMA	1:100	Acetone	Frozen	N/A	N/A
PDGFRβ	eBioscience	1:100	Zamboni	Frozen	N/A	N/A

SA-HRP: Streptavidin-horseradish Peroxidase (Perkin Elmer), TSA: Tyramide Signal Amplification (Parkin Elmer)

SUPPLEMENTARY DATA

Supplementary Table 4.
Characteristics of non-diabetic (Control) and streptozotocin induced diabetic (STZ) wild type (WT) and Smad1 hetero KO (Smad1 KO) mice

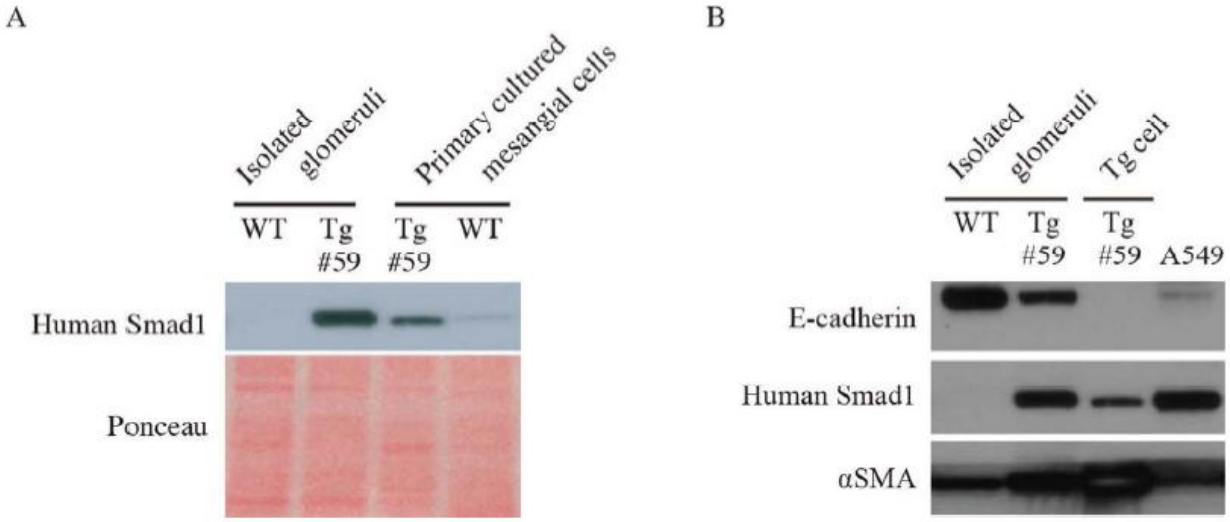
	Control		STZ	
	WT	Smad1 KO	WT	Smad1 KO
Number	5	4	6	5
BW (g)	44.8±8.3	43.1±3.8	55.5±10.9	65.4±9.5*
HbA1c(NGSP) (%)	4.4±0.4	4.2±0.2	12.1±1.0*	11.7±1.4*
HbA1c(IFCC) (mmol/mol)	25.0±4.4	22±2.2	109±11*	104±15*
SBP (mmHg)	120±1.6	123±3.7	120±4.6	120±7.7
rKW/BW (mg/g)	4.5±0.7	4.4±0.5	5.7±1.4	4.8±0.7

*p<0.05 versus control. BW, body weight; SBP, systolic blood pressure; rKW, right kidney weight

SUPPLEMENTARY DATA

Supplementary Figure 1.

A: Establishment of primary cultured mesangial cells from Smad1-Tg mice
Left: Western blotting of Smad1 protein in isolated glomeruli and primary cultured mesangial cells from wild type and Smad1-Tg mice. Mesangial cells were established from glomeruli isolated from a 4-week-old wild type or Smad1-Tg mice (C57BL/6J) as described. The cultured cells fulfilled the criteria generally accepted for glomerular mesangial cells. Note that Smad1 protein from transgene was also expressed in mesangial cells from Smad1-Tg mice. Human Smad1 specific antibody was used.
B: E-cadherin expression of primary cultued cells from Smad1-Tg#59
Cells and tissues were homogenized in RIPA buffer and subjected to immunoblotting. Fifteen μg of lysate was loaded in each lane. Cell lysate from A549, human alveolar adenocarcinoma cell line was used as positive control of Smad1 and E-cadherin. Anti E-cadherin (1:1000) antibody was from BD Bioscience (San Jose, CA). Anti-Smad1 antibody (#9512, 1:500) was from Cell signaling (Danvers, MA). Anti-αSMA (1:1000) antibody was from SIGMA (St. Louis, MO).



SUPPLEMENTARY DATA

Supplementary Figure 2.

Generation of Inducible Smad1 overexpressing mice

A: Generation of inducible Smad1 overexpressing mice. Ubiquitously expressed MCM protein leads to the excision of the "floxed" *GFP* and *polyA*, resulting in the expression of *Smad1* after the treatment of tamoxifen.

GFP (green) was seen in glomeruli of inducible Tg mice (B, E, and H). The same section was immunostained (Red) for von willbrand factor (C), podocin (F), and desmin (I), which are the markers for endothelial cells, podocytes, and mesangial cells respectively. Overlay of GFP and each staining was seen (D, G, and J). Note that GFP positive area were not in endothelial cells, but mainly in podocytes. Moreover, part of mesangium was also positive for GFP (J, K). Bar 50 μm.

L: Western blot analyses of Smad1 protein extracted from various tissues before and after tamoxifen treatment. Eighty μg of protein obtained from each sample was loaded. Smad1 expression was markedly induced after tamoxifen in many tissues including psoas muscle, heart, liver, and stomach. Note that transgene was slightly induced in kidney and ovary.

M: Inducible Smad1-Tg#5 mice also showed mesangial matrix expansion after induction of diabetes.

Morphometric analysis of mesangial matrix expansion in wild type mice, *MerCreMer*-Tg mice, and double transgenic mice with or without diabetes is shown.

All the groups of mice were treated with tamoxifen. Mesangial matrix fraction was measured at 36 weeks after STZ treatment in each group of mice. Glomerular surface area and PASM-positive area were determined as described in Research design and methods. The mesangial sclerotic fraction was determined as percentage of mesangial matrix area per total glomerular surface area. The number of each group of mice was listed in supplemental table 1. Comparisons were made by the Tukey's HSD test. # $p < 0.05$, versus non-diabetic mice in the same genotype. * $p<0.05$, versus diabetic WT mice in the same diabetic condition.

N: Inducible Smad1-Tg#5 mice did not show more albuminuria after induction of diabetes.

Urinary albumin excretion 36 weeks after diabetes in each group of mice was shown. The number of each group of mice was listed in supplemental table 1. Comparisons were made by the Tukey's HSD test. # $p < 0.05$, versus non-diabetic mice in the same genotype.

SUPPLEMENTARY DATA