Complete methods

Subjects

Renal biopsy samples derived from different human glomerular disease such as IgA nephropathy, lupus class II mesangial proliferative nephritis, early diabetic nephropathy (ne.) without nodular lesions, advanced diabetic nephropathy (ne.) with nodular lesions, membranous nephropathy (MN), and minimal change disease (MCD) were used in this study. The profiles of control patients and the patients with the diseases are shown below.

	Number (n)	Females (n)	Age (mean ± SD) (years)
Control	9	5	46.2 ± 18.8
IgA nephropathy	12	9	32.8 ± 16.8
lupus class II	6	5	44.5 ± 16.0
early diabetic ne.	6	1	64.2 ± 7.7
advanced diabetic ne.	6	0	59.5 ± 10.7
MN	9	3	60.2 ± 12.0
MCD	9	3	57.6 ± 19.1

Immunohistochemistry

Immunohistochemical analysis was performed on paraffin embedded section using indirect or direct immunohistochemistry procedure with the following primary antibodies: rabbit antibody against phospho-rpS6 (Cell Signaling Technology, Beverly, MA, USA), collagen I (EMD Millipore, Billerica, MA, USA), Ki67 (Leica biosystems, Nussloch, Eisfeld, Germany), mouse albumin (Bethyl Laboratories, Montgomery,TX, USA), goat antibody against collagen IV (SouthernBiotech, Birmingham, AL, USA). Following the first antibody, sections were incubated with Histofine SAB-PO (Nichirei Biosciences, Tokyo, Japan) and then with DAB (Wako Pure Chemical Industries, Osaka, Japan), or with TSA™ Biotin System (PerkinElmer Life Sciences, Boston, MA, USA) and then with Texas Red Streptavidin (Vector Laboratories, Burlingame, CA, USA) for phospho-rpS6. Alexa Fluor 488-conjugated donkey anti-rabbit or goat antibody (Invitrogen, Grand Island, NY, USA) were used to detect collagen I and Ki67 or collagen IV, respectively. FITC-conjugated α-SMA antibody was purchased from Sigma-Aldrich. Avidin/biotin blocking kit was obtained from Vector Laboratories.

DAPI was purchased from Wako Pure Chemical Industries. In order to visualize the expression of β-galactosidase, X-gal (Nacalai Tesque, Kyoto, Japan) was applied on paraformaldehyde-fixed frozen section. Subsequently, the following first antibodies were applied: rat anti-CD34 antibody, rabbit anti-WT1 antibody (Abcam, Cambridge, UK), or rat anti-PDGF receptor β antibody (APB5). Those frozen sections were incubated with Histofine SAB-PO (Nichirei Biosciences) and then DAB (Wako Pure Chemical Industries). Alexa Fluor 594-conjugated donkey anti-rabbit antibody and Alexa Fluor 488-conjugated goat antibody (Invitrogen) were used to visualize phospho-rpS6 and collagen IV in paraformaldehyde-fixed frozen section.

Western blotting

Mouse glomeruli at three months of age were collected by magnetic beads-based isolation. Briefly, the transcardiac perfusion was performed with phosphate buffered saline containing precleaned beads (Dynabeads, Invitrogen). The perfused renal cortex was briefly digested with collagenase A (Roche, Basel, Switzerland) and deoxyribonuclease I (Invitrogen), and the glomeruli stuffed with

beads were isolated by DYNAL (Invitrogen). Glomeruli or cultured mesangial cells were lysed using Mammalian Cell Extraction Kit (BioVision, Inc., Milpitas, CA, USA). Lysates of glomeruli were applied to SDS-PAGE and immunoblotted with the following primary antibodies: mouse antibody against β-actin (Sigma-Aldrich), rabbit antibody against phospho-rpS6, rpS6, and TSC1 (Cell Signaling Technology, Beverly, MA, USA). ECL select (GE healthcare Life Sciences, Chicago, IL, USA) was used to detect the blot signals using LAS-3000 (FUJIFILM, Tokyo, Japan).

Urine albumin and creatinine

Urine albumin and creatinine were determined using Albuwell M kit and Creatinine Companion kit (Exocell Inc., Philadelphia, PA, USA). Urine albumin was also evaluated by albumin immunohistochemistry.

Supplemental Figure Legends

**Supplemental Figure 1. Establishment of a mouse model which has β-galactosidase expression in mesangial cells. (*Foxd1-GCE$^{(+)}$ R26R-LacZ*

floxed/WT **mice).**

(A) Illustration of the mating strategy to generate *Foxd1-GCE* $^{(+)}$ *R26R-LacZ* $^{floxed/WT}$ mice. Tamoxifen-induced Cre expression from Foxd1 promoter removes the loxP-STOP-loxP sequence, leading to permanent heritable β-galactosidase expression in mesangial cells. (B) In order to visualize the expression of β-galactosidase, X-gal was applied on the paraformaldehyde fixed frozen sections prepared from Foxd1ER(-) or (+) ROSA mice. Subsequently, WT1 (a podocyte marker), CD34 (an endothelial cell marker), and PDGF receptor β (a mesangial cell marker) were immunostained. The expression of β-galactosidase was merged with that of PDGF receptor β, suggesting mesangium-specific expression of Cre recombinase in glomeruli in Foxd1ER(+) ROSA mice. (C) Representative pictures of β-galactosidase expression in Foxd1ER(-) or (+) ROSA mice. Focal β-galactosidase expression was observed in mesangial cells, vascular smooth muscle cells and pericytes. At 100X magnification. (D) The percentage of glomeruli in which β-galactosidase was induced. Cre recombinase was induced focally in kidney. Each dot represents the percentage of

β-galactosidase positive glomeruli among 50 glomeruli in each mouse. A horizontal bar shows a mean value. Mann-Whitney's U tests were used for statistical analysis. n = 6 per each group. *P <0.01.

Supplemental Figure 2. Representative pictures of western blot analysis of cultured mesangial cell protein established from Foxd1ER(-) or (+) TSC1 mice.

A significant difference of TSC1 expression was detectable in cultured mesangial cells established from Foxd1ER(+) TSC1 mice compared to those established from Foxd1ER(-) TSC1 mice. However, the increase of phosphorylated ribosomal protein S6 was not significant in mesangial cells derived from Foxd1ER(+) TSC1 mice (n = 4). rpS6; ribosomal protein S6.

Supplemental Figure 3. Representative pictures of albumin immunohistochemistry in Foxd1ER(+) TSC1 mice.

Immunohistochemical analysis was performed on paraffin embedded section using direct immunohistochemistry procedure with horseradish peroxidase-conjugated anti-mouse albumin antibody. Foxd1-Cre(+) TSC1 mice

at three months of age were used as positive controls. Foxd1 stroma-derived cells is observed in whole glomeruli in adult mice (Kobayashi et al. *J Clin Invest* 126: 1926-1938, 2016). Therefore, the kidney pathological changes in Foxd1-Cre(+) TSC1 mice have the similar phenotype with those in Podocin-Cre(+) TSC1 mice, which recapitulate many diabetic nephropathy features, including significant albuminuria (Inoki et al. *J Clin Invest* 121: 2181-2196, 2011). Albumin was not detected in the kidney tissue derived from Foxd1ER(+) TSC1 mice at one year of age. At 40X maginification.

Supplemental Figure 4. Representative PCR bands to distinguish *Foxd1-GCE, R26R-LacZ,* and *TSC1 floxed* mice.

The primers, WT forward : CTC CTC CGT GTC CTC GTC, Mutant forward : GGG AGG ATT GGG AAG ACA AT, Common : TCT GGT CCA AGA ATC CGA AG can detect both mutant and wild type bands for *FoxD1-GCE* mice. The primer pair, Rosa 5′ : AAA GTC GCT CTG AGT TGT TAT, Rosa 3′ : GCG AAG AGT TTG TCC TCA ACC provides a mutant band for *R26R-LacZ* mice. The primer pair, TSC1 forward : GTC ACG ACC GTA GGA GAA GC, TSC1 reverse :

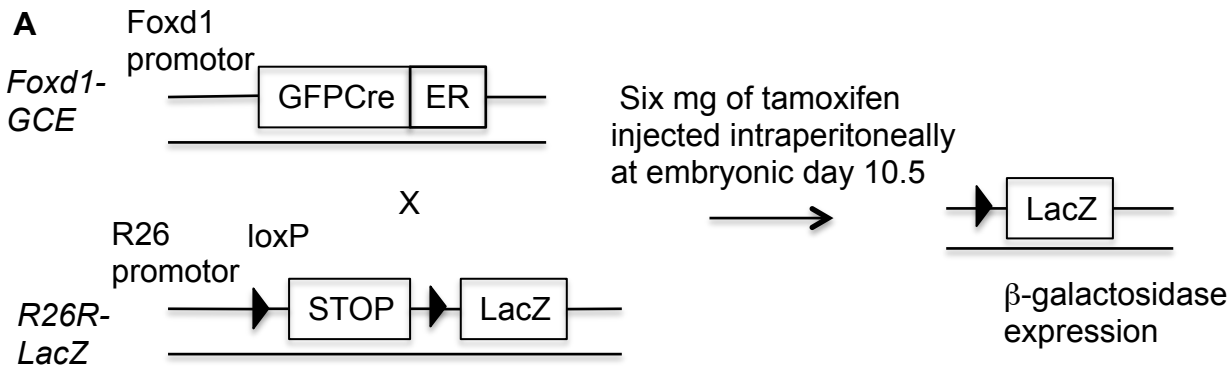
GAA TCA ACC CCA CAG AGC AT can detect both mutant and wild type bands for *TSC1 floxed* mice.

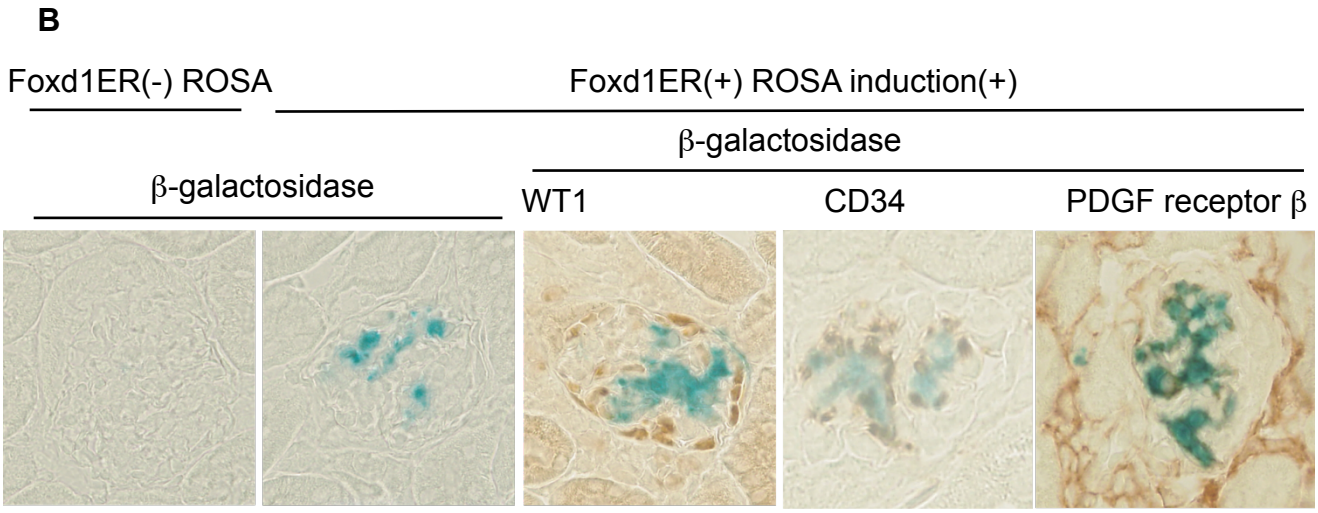
Supplemental Figure 5. A representative picture of collected glomeruli choked with magnetic beads.

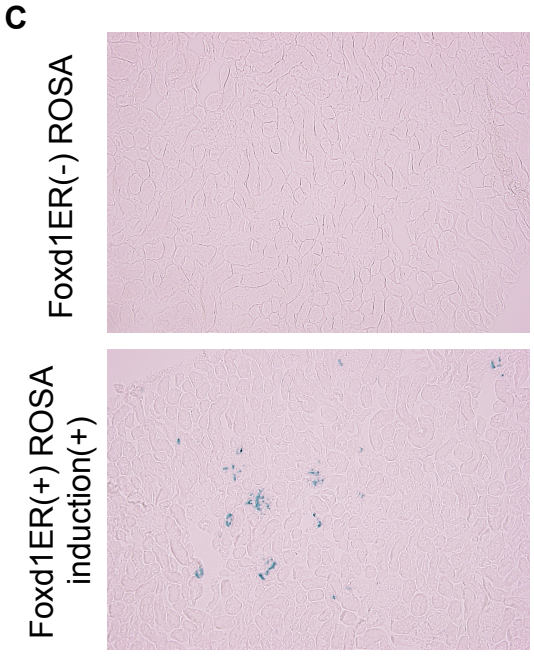
Mouse glomeruli at three months of age were collected by magnetic beads-based Isolation. Briefly, the transcardiac perfusion was performed with phosphate buffered saline containing precleaned magnetic beads. The perfused renal cortex was briefly digested with collagenase A and deoxyribonuclease I, and the glomeruli stuffed with beads were isolated by a magnet.

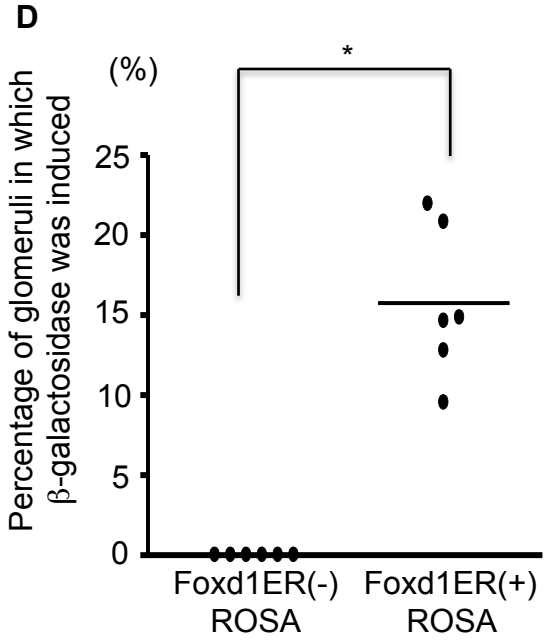
Supplemental Figure 1.

Establishment of a mouse model which has β-galactosidase expression in mesangial cells. (*Foxd1-GCE* $^{(+)}$ *R26R-LacZ* $^{floxed/WT}$ mice).



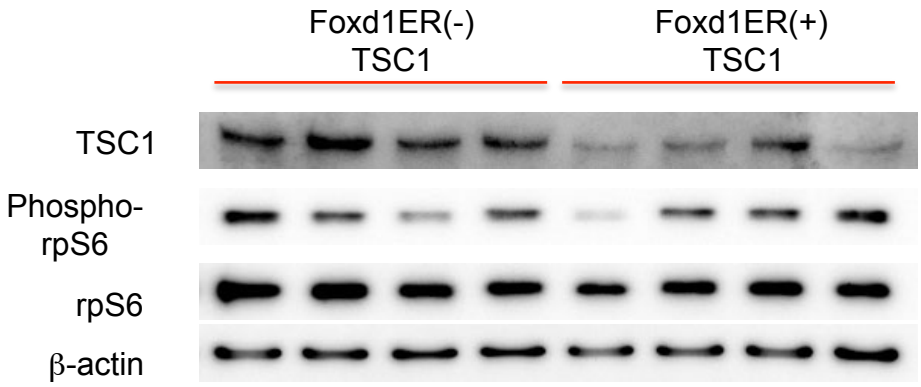






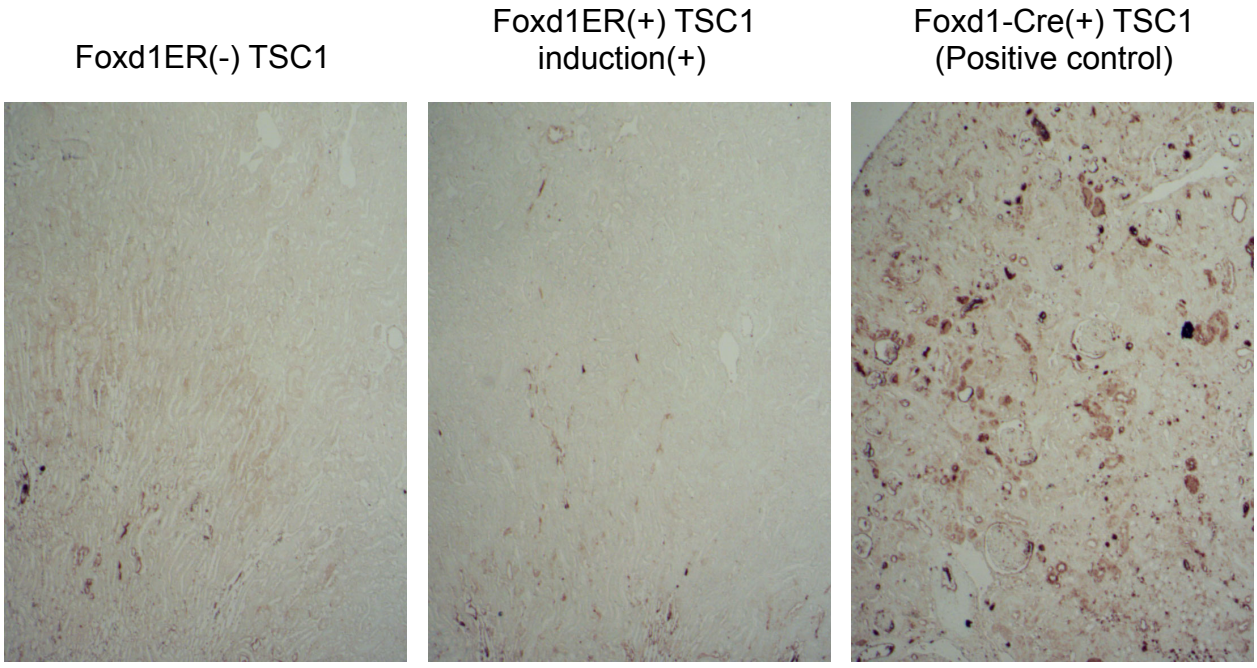
Supplemental Figure 2.

Representative pictures of western blot analysis of cultured mesangial cell protein established from Foxd1ER(-) or (+) TSC1 mice.



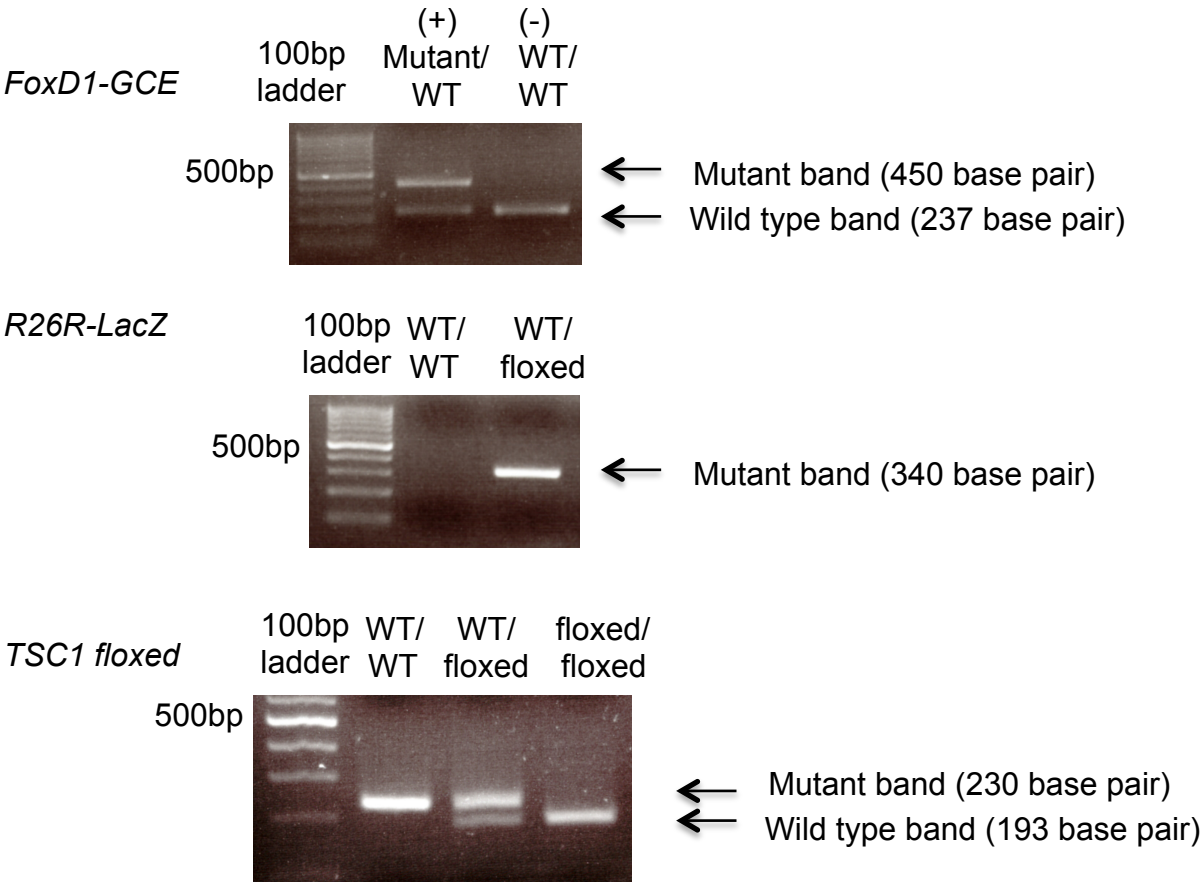
Supplemental Figure 3.

Representative pictures of albumin immunohistochemistry.



Supplemental Figure 4.

Representative PCR bands to distinguish *Foxd1-GCE, R26R-LacZ,* and *TSC1 floxed* mice.



Supplemental Figure 5.

A representative picture of collected glomeruli choked with magnetic beads.