SUPPLEMENTARY METHODS

Animal treatment protocols.

To examine the expression of KLF4 in mice with proteinuria, 8 week-old male Balb/c mice and C57BL/6J mice were obtained from Sankyo Laboratory and injected with adriamycin (ADM). Preliminary dose-response studies confirmed that the optimal dose of ADM administration was 11 mg/kg iv for Balb/c mice, and 18 mg/kg iv for C57BL/6J mice and mixed strains as reported previously (1-4). Puromycin aminonucleotide (Sigma) was administered at a dose of 500 mg/kg sc (5, 6). For the experiments using podocyte-specific inducible KLF4 transgenic mice (Pod/Tet-O/KLF4), 8-week-old male mice and control littermates were injected with ADM (18 mg/kg iv) to induce nephropathy. The mice were administered doxycycline (2 mg/ml) 4 weeks after the ADM injection for 28 days to induce KLF4 expression in glomerular podocytes. In vivo siRNA experiments were performed using the method reported by Okamoto et al (7). In brief, KLF4 SiRNA (In vivo grade Mission siRNA^{TM}, Mm_KLF4_8987, Sigma-Aldrich) or control siRNA were dissolved in 0.8 ml PBS at a concentration of 500 ug/ml, and delivered by rapid tail injection to deliver 400 ug/mouse. Injections were performed twice with a 7-day interval between injections. The siRNA sequences are shown in **Supplementary Table 2**.

Cloning and methylation of the human nephrin gene promoter.

DNA fragments (A: bp-1884 to + 156, B: bp-767 to -377 and C: bp-520 to +280 relative to the transcription start site (TSS)) of the human nephrin gene were obtained by PCR, using human genomic DNA as the template. The generated DNA fragments were subcloned into pGL3-basic plasmid (Promega), and transfected into podocytes using lipofectamine. Luciferase assays were performed using a commercial kit (Promega), with Renilla luciferase as the transfection control. For the KRE deletion studies, the KRE in the nephrin promoter (shown boxed in **Supplementary Fig. 8**) was deleted from the promoter region by PCR-based mutagenesis using the In-Fusion HD Cloning system (Clontech), as recommended by the manufacturers. For the analysis of the effects of promoter methylation on nephrin promoter activity, DNA fragments were subcloned into a CpG-sequence free promoter plasmid (pCpGfree-promoter, InVivogen) which lacks intrinsic CpG sequences in the vector sequence, and therefore allows comparison of the effects of methylation on promoter activity. The plasmid DNA was methylated with the bacterial methyltransferase SssI, transfected into podocytes, and then SEAP activity was assayed using a commercial kit (Clontech).

Microarray-based genome-wide DNA methylation profiling and expression analysis.

Microarray-based DNA methylation profiling was performed on DNA samples from podocytes transfected with pCMV-SPORT6-KLF4 or empty vector using the HumanMethylation450 DNA Analysis BeadChip (Illumina) for the analysis of DNA methylation sites in the promoter and non-promoter regions, as defined by the manufacturer (8, 9). This array allows analysis of over 450,000 genome-wide methylation sites per sample at single-nucleotide resolution. Genomic DNA was subjected to bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research, Orange County, CA). Processed DNA samples were hybridized to the BeadChip, and the methylation level for each locus was determined by calculating the ratio of the fluorescent signals from the methylated versus unmethylated sites.

References

1. Wang Y, Wang YP, Tay YC, and Harris DC. Progressive adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. *Kidney international.* 2000;58(4):1797-804.
2. Dai C, Stolz DB, Kiss LP, Monga SP, Holzman LB, and Liu Y. Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. *J Am Soc Nephrol.* 2009;20(9):1997-2008.
3. Dai C, Saleem MA, Holzman LB, Mathieson P, and Liu Y. Hepatocyte growth factor signaling ameliorates podocyte injury and proteinuria. *Kidney international.* 2010;77(11):962-73.
4. Hayashi K, Sasamura H, Ishiguro K, Sakamaki Y, Azegami T, and Itoh H. Regression of glomerulosclerosis in response to transient treatment with angiotensin II blockers is attenuated by blockade of matrix metalloproteinase-2. *Kidney international.* 2010;78(1):69-78.
5. Wang L, Fields TA, Pazmino K, Dai Q, Burchette JL, Howell DN, Coffman TM, and Spurney RF. Activation of Galpha q-coupled signaling pathways in glomerular podocytes promotes renal injury. *J Am Soc Nephrol.* 2005;16(12):3611-22.
6. Wang L, Ellis MJ, Fields TA, Howell DN, and Spurney RF. Beneficial effects of the Rho kinase inhibitor Y27632 in murine puromycin aminonucleoside nephrosis. *Kidney & blood pressure research.* 2008;31(2):111-21.
7. Okamoto K, Tokunaga K, Doi K, Fujita T, Suzuki H, Katoh T, Watanabe T, Nishida N, Mabuchi A, Takahashi A, et al. Common variation in GPC5 is associated with acquired nephrotic syndrome. *Nature genetics.*

2011;43(5):459-63.

8. Wilop S, Fernandez AF, Jost E, Herman JG, Brummendorf TH, Esteller M, and Galm O. Array-based DNA methylation profiling in acute myeloid leukaemia. *Br J Haematol.* 2011;155(1):65-72.

9. Calvanese V, Fernandez AF, Urdinguio RG, Suarez-Alvarez B, Mangas C, Perez-Garcia V, Bueno C, Montes R, Ramos-Mejia V, Martinez-Camblor P, et al. A promoter DNA demethylation landscape of human hematopoietic differentiation. *Nucleic acids research.* 2012;40(1):116-31.

SUPPLEMENTARY TABLES

Supplementary Table 1. Clinical profiles of patients with proteinuric glomerular diseases.

	Normal (n=9)	**MCD** (n=10)	**FSGS** (n=11)	**DN** (n=9)
Age at biopsy (yr; mean and range)	53 (35-71)	48 (31-74)	50 (33-65)	52 (40-70)
Sex (male/ female)	7/2	6/4	8/3	8/1
Serum creatinine (mg/dl; mean±SEM)	1.06±0.11	1.01±0.09	1.17±0.10	1.24±0.20
Serum albumin (g/dl; mean±SEM)	4.30±0.11	3.06±0.42	3.58±0.26	3.54±0.17
Proteinuria (g/d; mean±SEM)	nd	2.75±0.87	2.58±0.50	3.48±1.00

MCD: minimal change disease, FSGS: focal segmental glomerulosclerosis, DN: diabetic nephropathy, nd: not detectable

Supplementary Table 2. Sequence of oligonucleotide primers.

Cloning primers

Name	F/R	Target sequence
Human nephrin promoter A	F	CGACGCGTGGTGACAGAGCAAGACTCCC (with a MluI restriction site)
	R	CCCTCGAGCACAGGTCCCCCTACTGTG (with a XhoI restriction site)
Human nephrin promoter B	F	TTACGCGTGTATCAGGGCAAGGAAGAGCTGG (with a MluI restriction site)
	R	TACTCGAGTCTCACTACTCACAGCCTTTCAAGA (with a XhoI restriction site)
Human nephrin promoter C	F	TACTCGAGTTGCACTGTGAGAATGAGCT (with XhoI restriction site)
	R	CGAAGCTTGTCTAGGGAAGGTAAGTG (with HindIII restriction site)
Human nephrin promoter A (SEAP)	F	ATAGTACTGGTGACAGAGCAAGACTCCC (with a ScaI restriction site)
	R	ATGGATCCCACAGGTCCCCCTACTGTG (with a BamHI restriction enzyme cutting site)

MSP/BGS primers

Name	F/R	Target sequence
Human nephrin promoter MSP (Methyl primer) (Unmethyl primer)	F	AGTTTGAGTAATAGAGTAAGAT
	R	CACATACCAAACGCGTTCCTCG
	R	CACATACCAAACACATTCCTCA
Human nephrin promoter MSP control primer	F	CATCATTTAGGCACTGATGTCTACT
	R	CTTGTCTCACTACTCACAGCCTTTC
Human nephrin promoter BGS1	F	AGAGATTTGTTAAGGTTATGTATTAGG
	R	TCAATCTCTATCTTCTCTCCTACCACC
Human nephrin promoter BGS2	F	GTATTAGGGTAAGGAAGAGTTGG
	R	TCTCACTACTCACAACCTTTCAAAA
Human vimentin promoter BGS1	F	AAAGAGGTTTGTTTAAAGAAGTTAGA
	R	AACTCAATAATCAATAATTTTTACCCTA
Human vimentin promoter	F	AGAATTAATTTTATAGTGGTTTTGTAGT

BGS2	R	ACAACAATACACAATACAAAATTCAC

ChIP primers

Name	**F/R**	**Target sequence**
Human nephrin promoter B	F	GTATCAGGGCAAGGAAGAGCTGG
	R	TCTCACTCATCACAGCCTTTCAAGA
Human nephrin promoter (negative control)	F	TCTTGAAAGGCTGTGAGTAGTGAGA
	R	CACAGGTCCCCCTACTGTG
Human vimentin promoter	F	AGAATCAATTTTACAGTGGTTCTGCAGT
	R	ACAGCAATGCACAGTACAGGGTTCAC

Real-time PCR primers

Name	**No.**
KLF4	ABI Hs00358836_m1, Mm00516104_m1
NPHS1 (Nephrin)	ABI Hs00190446_m1, Mm01176615_g1
NPHS2 (Podocin)	ABI Hs00922492_m1, Mm01292252_m1
CDH3 (P-cadherin)	ABI Hs00999918_m1
WT1	ABI Hs01103751_m1
SYNPO (Synaptopodin)	ABI Mm03413333_m1
ACTA2 (α SMA)	ABI Hs00909449_m1
VIM (Vimentin)	ABI Hs00185584_m1, Mm01333430_m1
KLF2	ABI Hs00360439_g1
KLF3	ABI Hs00974383_g1, Mm00492956_m1
KLF6	ABI Hs00810569_m1, Mm00516184_m1
KLF15	ABI Hs00362736_m1, Mm00517792_m1
GAPDH	ABI Hs00266705_g1, Mm99999915_g1

siRNA oligonucleotides

Name	**Sequence**
KLF4-HSS190182s	UUCAGCACGAACUUGCCCAUCAGCC
KLF4-HSS190182as	GGCUGAUGGGCAAGUUCGUGCUGAA
Mm_Klf4_8987s	rGrGrCUrGrAUrGrGrGrCrArArGUUUrGUTT
Mm_Klf4_8987_as	rArCrArArArCUUrGrCrCrCrAUrCrArGrCrCTT

EMSA oligonucleotides

Name	**Sequence**
KRE (s)	CACCGAGGAACGCGCCTGGCATGTG
KRE deletion oligo (s)	CACCGAGGAACGCATGTG

Supplementary Table 3. Antibodies for immunofluorescent labeling and Western blot analysis.

Name	**Source**	**Type**
Anti-KLF4 antibody	Sigma-Aldrich	Goat, polyclonal
Anti-KLF4 antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-Nephrin antibody	PROGEN	Guinea-pig, polyclonal
Anti-Podocin antibody (IF)	IBL	Rabbit, polyclonal
Anti-Podocin antibody (Western)	Abcam	Rabbit, polyclonal
Anti-P-cadherin antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-WT1 antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-Synaptopodin antibody	Synaptic systems	Rabbit, polyclonal
Anti-Vimentin antibody	Sigma-Aldrich	Goat, polyclonal
Anti-Alpha-SMA antibody	Sigma-Aldrich	Mouse, monoclonal
Anti-vWF antibody	Dako	Rabbit, polyclonal
Anti-Desmin antibody	Dako	Mouse, monoclonal
Anti-Acetyl-H3K9 antibody	Abcam	Rabbit, polyclonal
Anti-Dnmt1 antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-Dnmt3a antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-Dnmt3b antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-CtBP antibody	Santa Cruz Biotechnology	Rabbit, polyclonal
Anti-Alpha-tubulin antibody	Sigma-Aldrich	Mouse, monoclonal

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. KLF4 expression is decreased in proteinuric glomerular disease models.

Details of the experimental protocol are provided in **Fig. 1(D-G)**. (Upper panel) western blots and (lower panel) real-time PCR analysis of KLF4 and nephrin expression in the kidney cortex in ADM nephropathy model of Balb/c mice (A) or C57BL/6J mice (B), PAN nephropathy model (C) and diabetic nephropathy model (db/db) (D) (n=5-6 per time points).

Supplementary Figure 2. Gene transfer of KLF4 attenuates proteinuria and restores nephrin expression in adriamycin nephropathy in Balb/c mice.

(A) Experimental protocol. 2 weeks after injection of Balb/c mice with ADM (11 mg/kg iv), either pCMV-Vector (Vector) or pCMV-KLF4 (KLF4) were administered by tail vein injection, as described in Methods. Mice per group were sacrificed before ADM injection, and at days 0, 1, 7, 14, and 28 after plasmid injection in ADM nephropathy (n=5-6 per time point). (B) (Left panels) Representative photomicrographs of immunofluorescence staining of KLF4 and nephrin in mice injected with pCMV-KLF4 or control vector. (Right panels) Quantification of the immunolabeled area. (C) Time course of changes in albuminuria in mice injected with pCMV-KLF4 or control vector. (D) (Left panel) Representative photomicrographs and (right panel) quantification of

podocyte foot process effacement in mice injected with pCMV-KLF4 or control vector. Scale bar, 5 um. (E) (Upper panel) Representative photomicrographs of light microscopy of glomeruli (PAS staining) and (lower panel) quantification of PAS-positive area in mice injected with pCMV-KLF4 or control vector. (F) (Upper panel) Representative photomicrographs of light microscopy of glomeruli (WT1-staining) and (lower panel) quantification of WT-1 positive cells per glomerulus at 14 days after plasmid injection. *: $p<0.05$, **: $p<0.01$ vs controls.

Supplementary Figure 3. Gene transfer of KLF4 attenuates proteinuria and restores nephrin expression in adriamycin nephropathy in C57BL/6J mice.

(A) Experimental protocol. 4 weeks after injection of C57BL/6J mice with ADM (18 mg/kg iv), either pCMV-Vector (Vector) or pCMV-KLF4 (KLF4) were administered by tail vein injection, as described in Methods. Mice were sacrificed at days 0, 1, 7, 28, 84, and 168 after plasmid injection (n=5-6 per time point). (B) Representative photomicrographs and (C) quantification of immunofluorescence staining of KLF4, nephrin, podocin, synaptopodin, and vimentin in pCMV-KLF4-injected mice. (D) Representative low power photomicrograph of kidney KLF4 immunofluorescence staining 1 day after injection of pCMV-KLF4 (Scale bar, 100 um). (E) (Upper panel) Western blot and (Lower panel) real-time RT-PCR analysis of KLF4 and nephrin

expression in the kidney cortex at the indicated days after injection of pCMV-KLF4. *: $p<0.05$, **: $p<0.01$ vs day 0. (F) Time course of changes in albuminuria in mice injected with pCMV-KLF4 or control vector. *: $p<0.05$, **: $p<0.01$ vs controls.

Supplementary Figure 4. Gene transfer of KLF4 attenuates proteinuria and restores nephrin expression in diabetic nephropathy in db/db mice.

(A) Experimental protocol. db/db mice were administered either pCMV-Vector (Vector) or pCMV-KLF4 (KLF4) by tail vein injection, as described in Methods. Mice were sacrificed at days 0, 1, 7, 14, and 28 after plasmid injection (n=5-6 per time point). (B) (Left panels) Representative photomicrographs of immunofluorescence staining of KLF4 and nephrin in mice injected with pCMV-Vector (Vector) or pCMV-KLF4 (KLF4). (Right panels) Quantification of the immunolabeled area. (C) Time course of changes in albuminuria in mice injected with pCMV-Vector (Vector) or pCMV-KLF4 (KLF4). (D) (Left panel) Representative photomicrographs of light microscopy of glomeruli (PAS staining) and (right panel) quantification of PAS-positive area in mice injected with pCMV-KLF4 or control vector. (E) (Upper panel) Representative photomicrographs of light microscopy of glomeruli (WT1-staining) and (lower panel) quantification of WT-1 positive cells per glomerulus at 28 days after plasmid injection. *: $p<0.05$, **: $p<0.01$ vs controls.

Supplementary Figure 5. Restoration of podocyte KLF4 expression in diseased glomeruli alters phenotype marker expression.

Details of the experiment protocol are provided in **Fig. 2**. (Upper panel) Western blot and (lower panel) real-time RT-PCR analysis of podocin, synaptopodin and vimentin expression in the kidney cortex of KLF4 transgenic mice or controls at the indicated times after starting Dox treatment (n=5-6 per time points).

Supplementary Figure 6. Transient overexpression of KLF4 results in sustained changes in podocyte morphology and nephrin expression.

Serial changes in (upper panel) cell morphology and (lower panel) Western blots of KLF4, nephrin, αSMA, and vimentin in podocytes with tetracycline-inducible KLF expression (Tet-O/KLF4) and control cells (Tet-O/Vector) after 7 days treatment with doxycycline (1 ug/ml), followed by 7 days wash-out.

Supplementary Figure 7. Transient overexpression of KLF4 results in changes in promoter methylation of epithelial and mesenchymal genes.

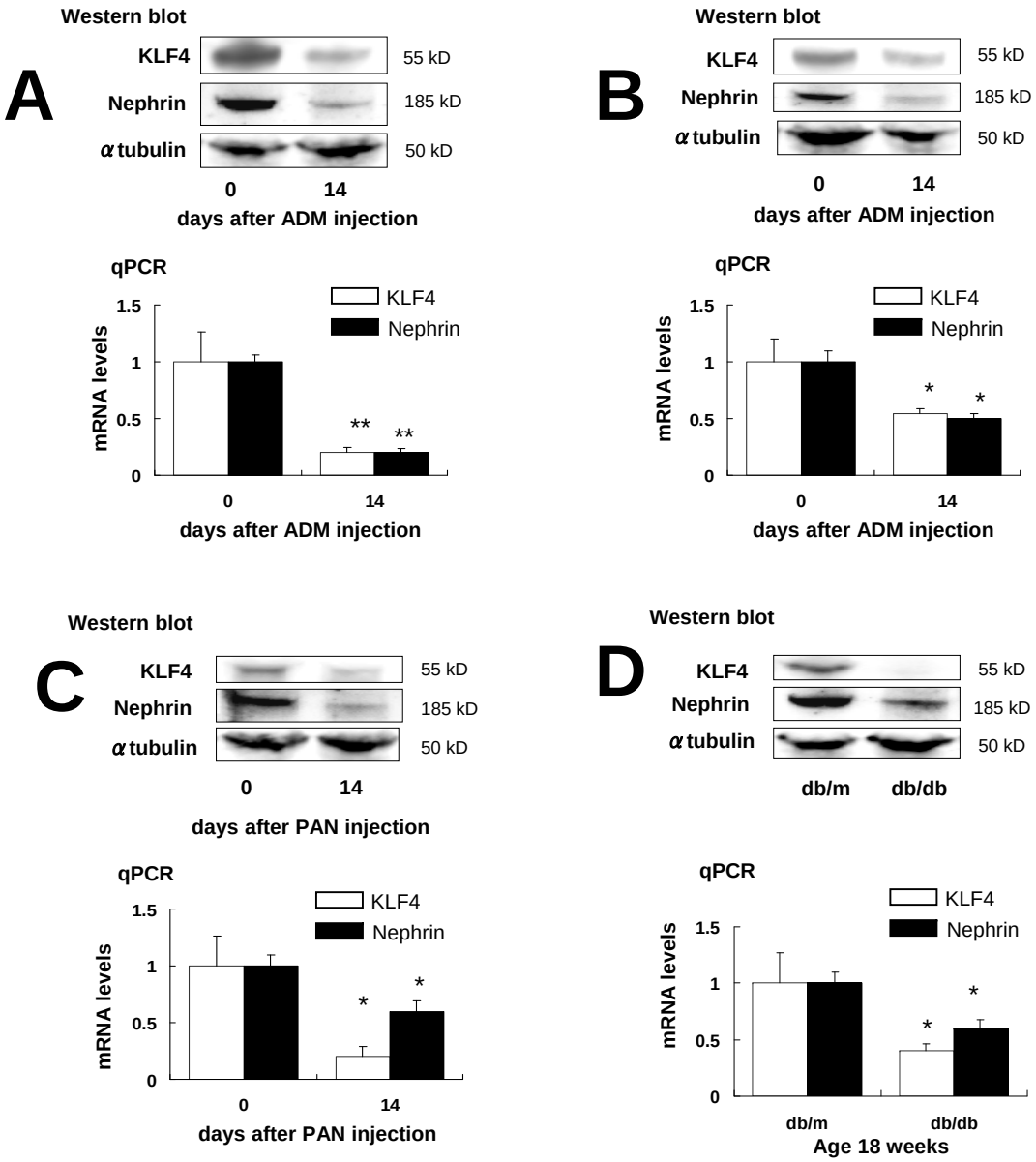
DNA samples from KLF4-expressing podocytes and their controls were subjected to microarray-based DNA methylation profiling. Changes in methylation of (A) promoter regions and (B) non-promoter regions of (upper panel) all genes included in the array or (lower panel) epithelial and mesenchymal marker genes are shown.

Supplementary Figure 8. Nucleotide sequences of the human nephrin promoter and human vimentin promoter region adjacent to the transcription start site (TSS) examined by BGS and ChIP assays.

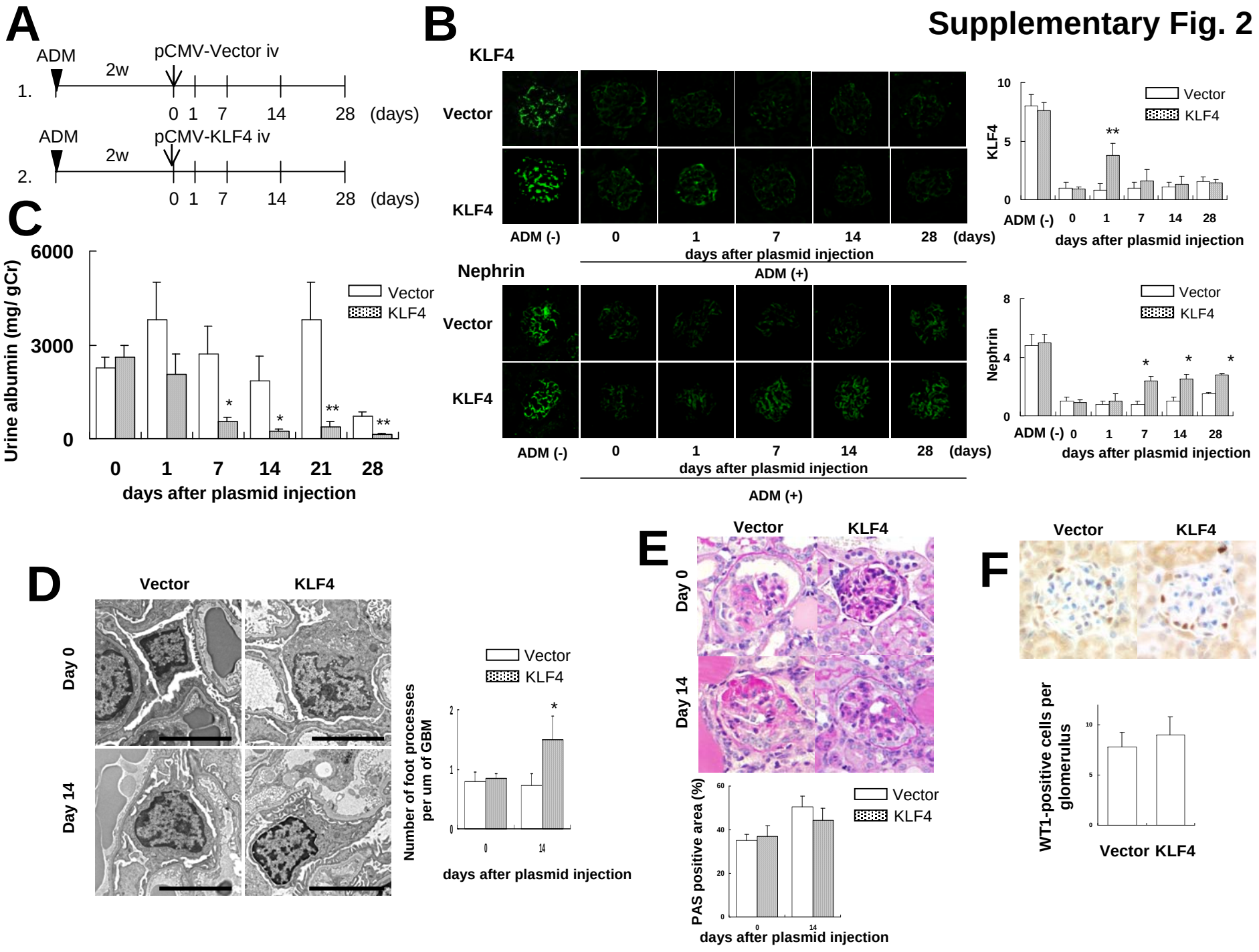
In both cases, KLF4-response elements (KRE) were identified (boxed), and several CpG binucleotides were found in the analyzed region.

Supplementary Figure 9. (A) Chromatin immunoprecipitation (ChIP) assay for DNMT1 in the promoter regions of nephrin, vimentin, and the control promoter. The control was an adjacent region of the nephrin promoter (-401 ~ +156 relative to the TSS), which does not contain the KRE. The amplified fragment for nephrin corresponds to region B in **Fig. 8A**. The bar graph shows the quantification of DNMT1 /input intensity (n=4). (B) ChIP assay for DNMT1, 3a, and 3b in the promoter regions of nephrin, vimentin, and control promoter. (Upper panel of DNMT1 is the same figure as **Supplementary Fig. 9A**.) (C) ChIP assay for the acetylated H3K9 in the promoter regions of nephrin, vimentin, and control promoter. The bar graph shows the quantification of acetyl-H3K9 /input intensity (n=4). KLF4: KLF4-overexpressing podocytes; Empty: empty vector-transfected control podocytes. *: $p<0.05$, **: $p<0.01$ vs controls. ND: not detectable.

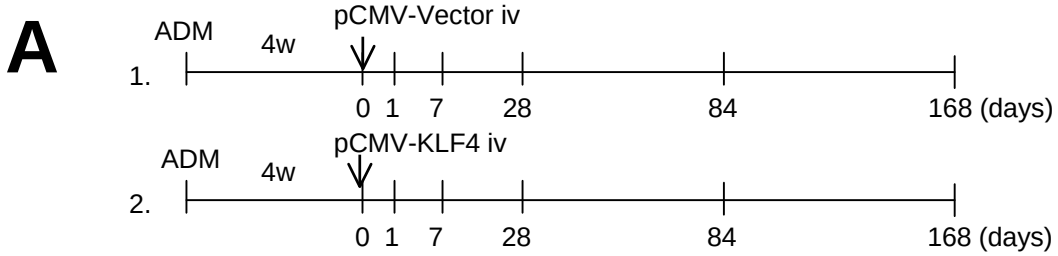
Supplementary Fig. 1

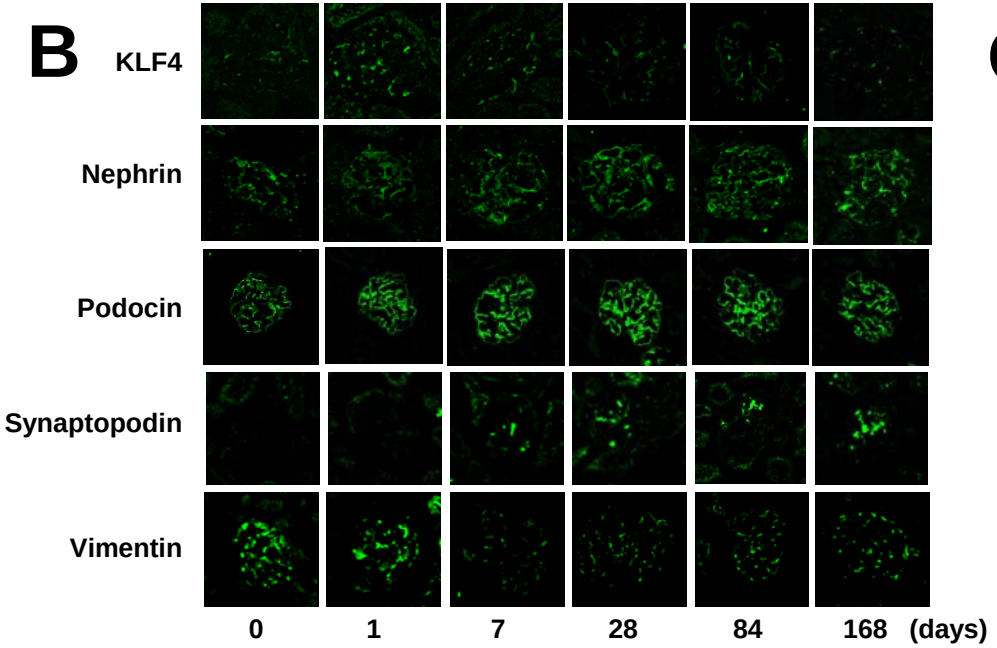


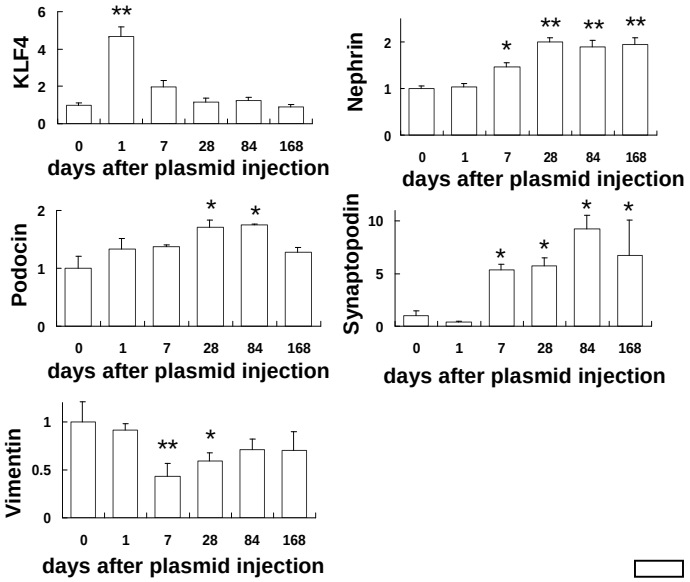
Supplementary Fig. 2

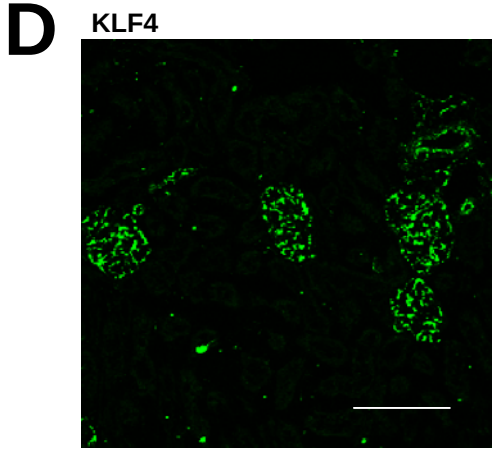


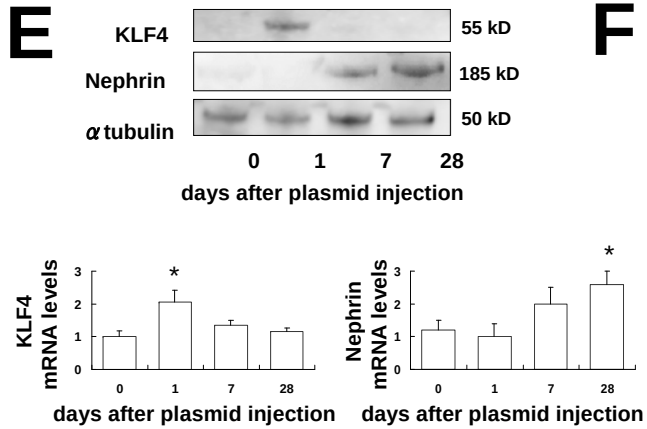
Supplementary Fig. 3

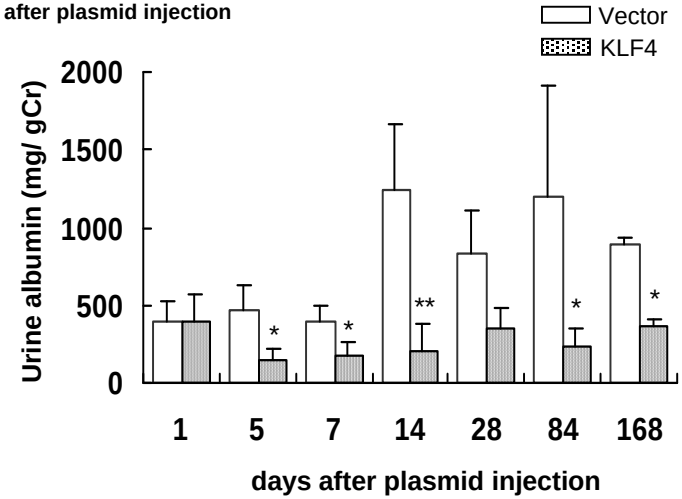




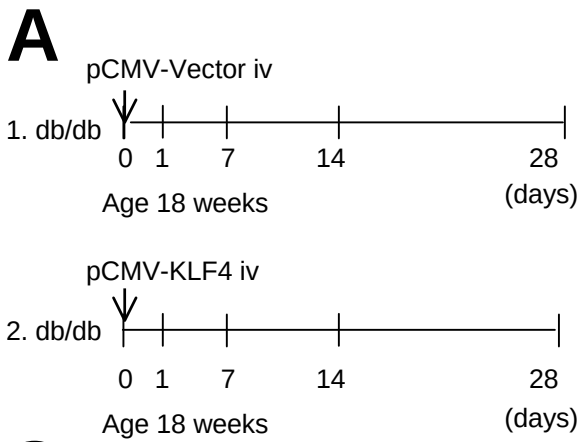


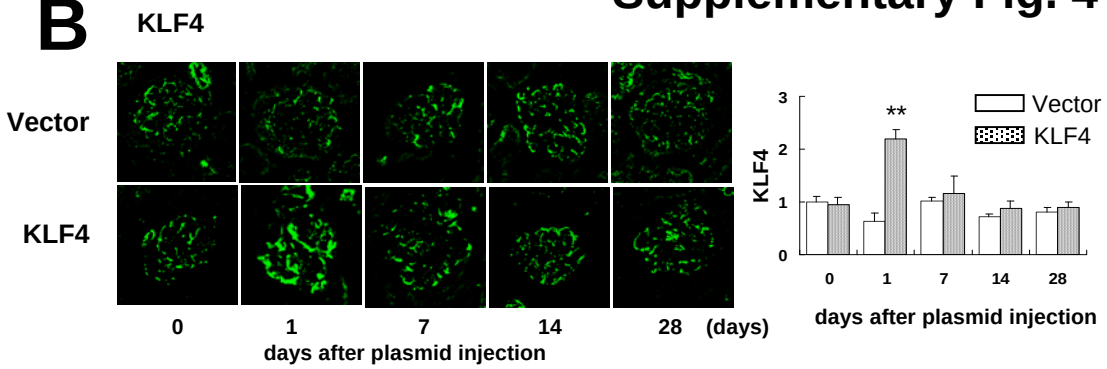


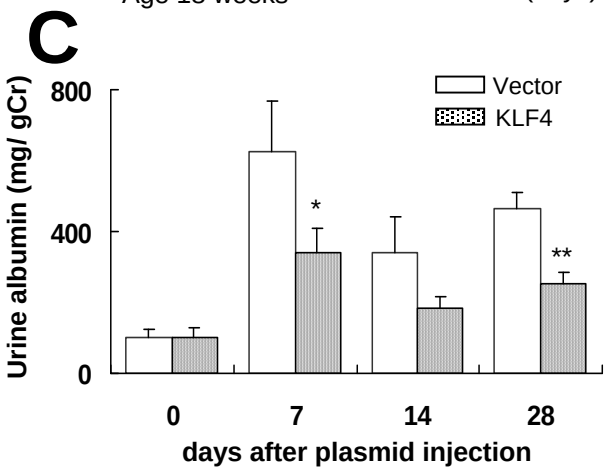


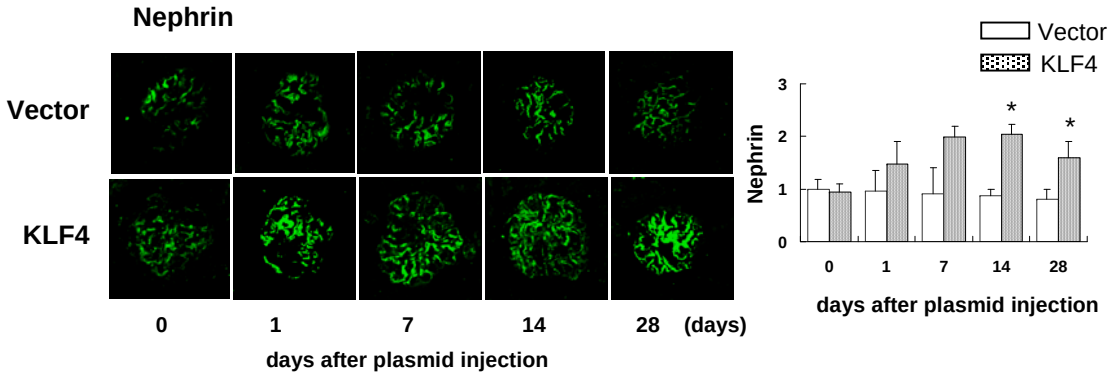


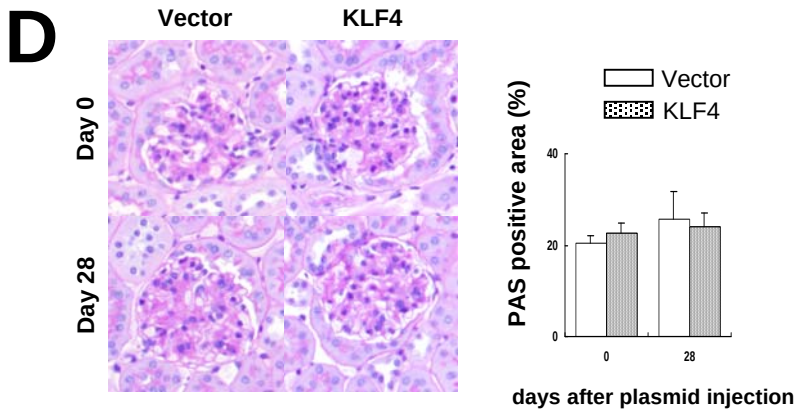
Supplementary Fig. 4

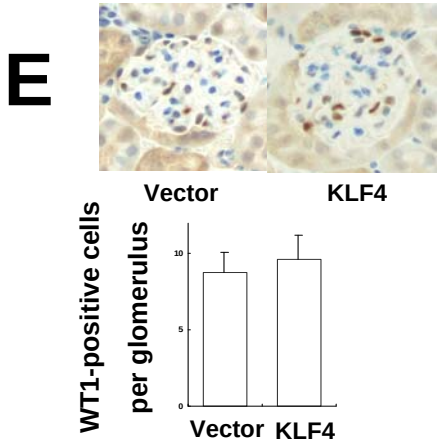




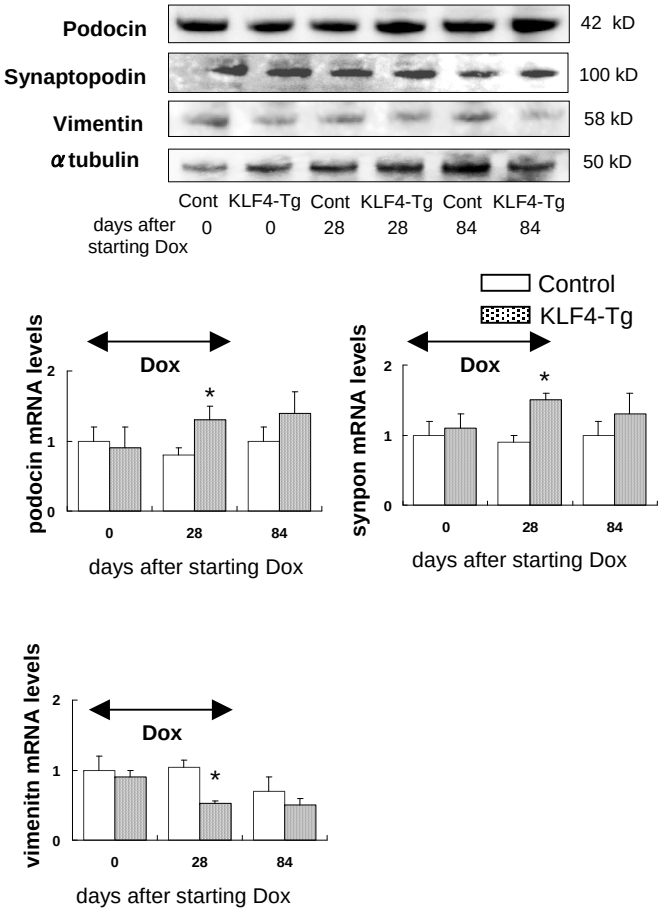




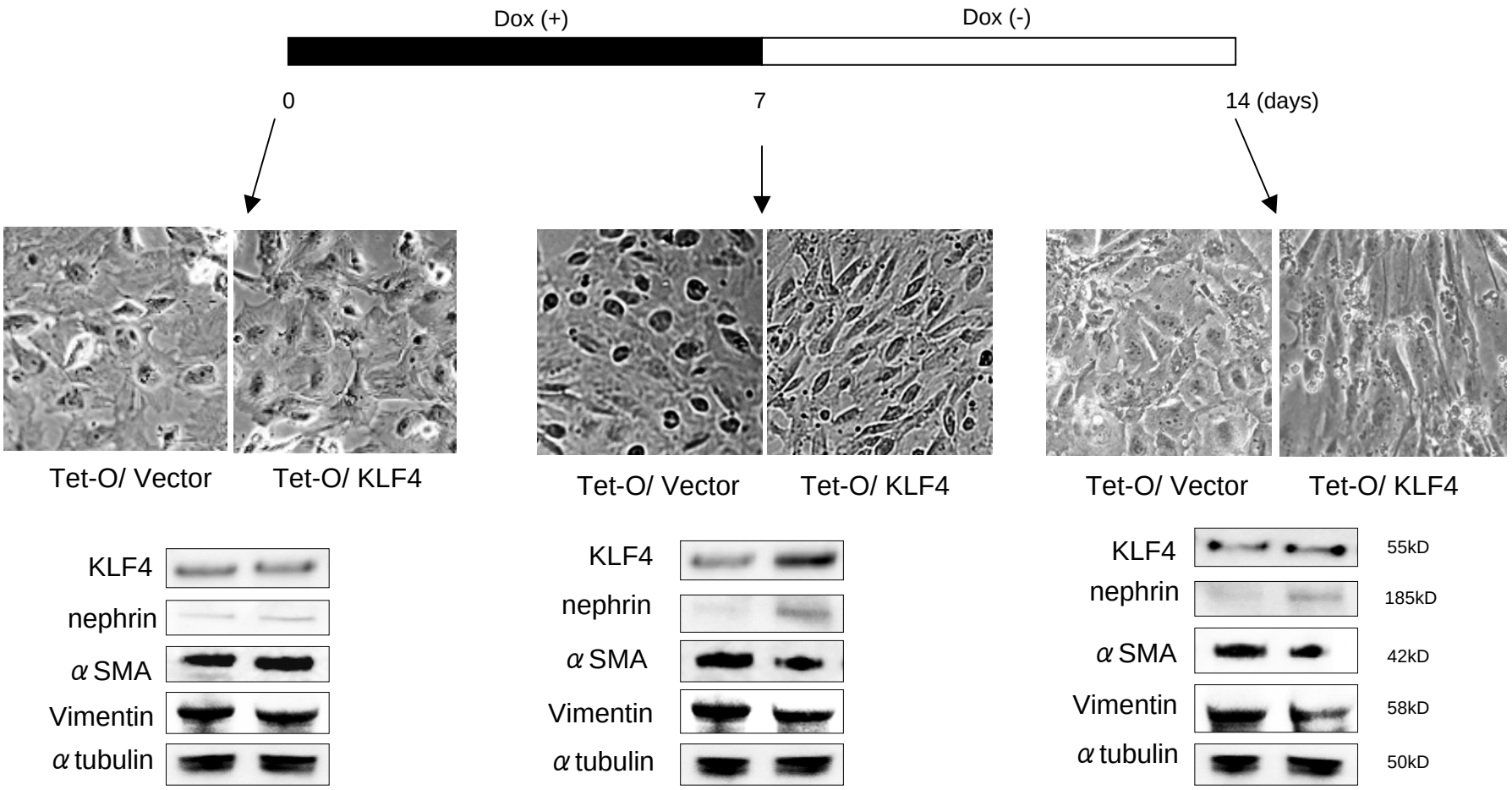




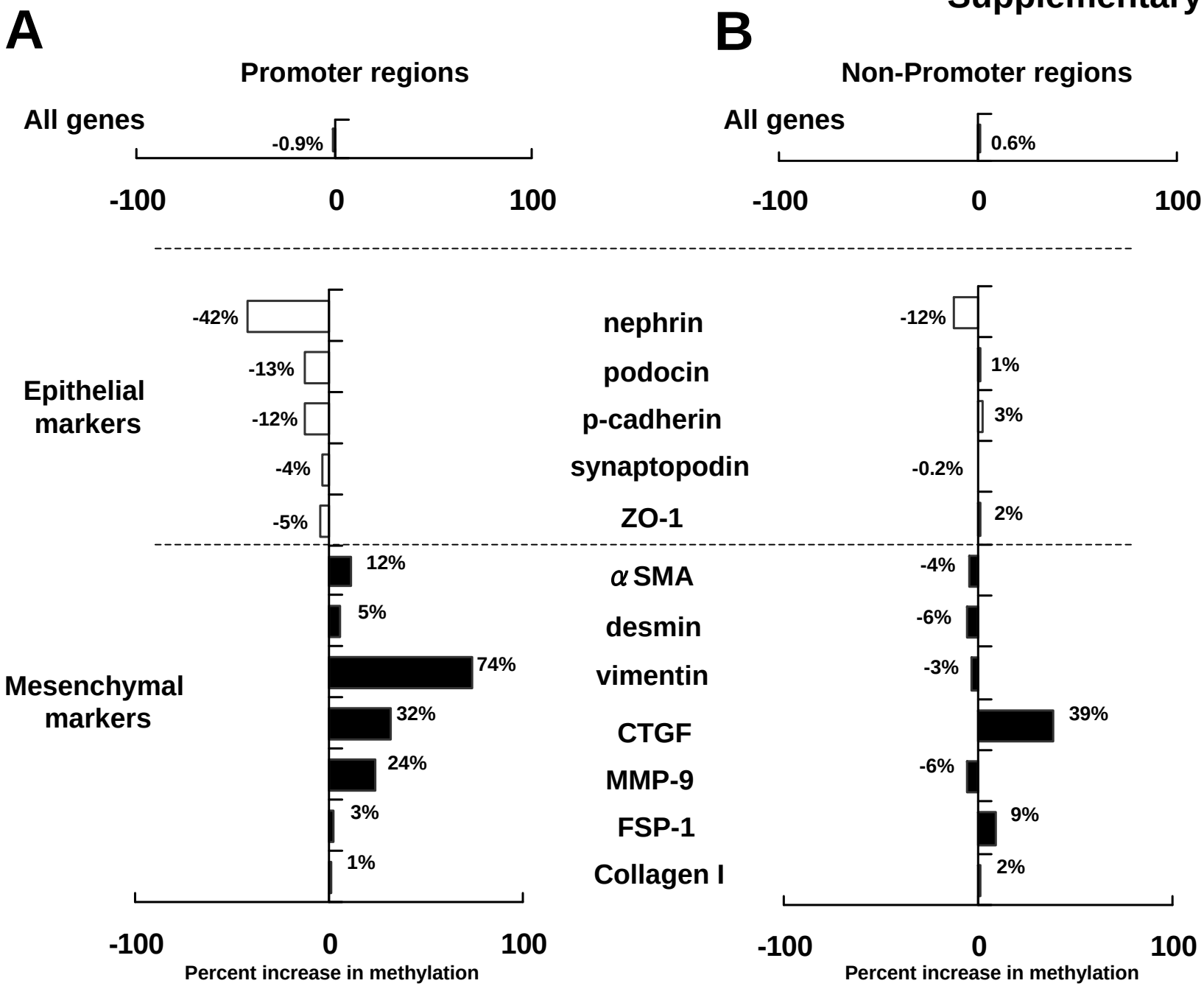
Supplementary Fig. 5



Supplementary Fig. 6



Supplementary Fig. 7



Supplementary Fig. 8

Nephrin promoter

-787 agagacttg ccaaggtcat gtatcagggc aaggaagagc tgggggccca gctggctgct cccctgctga

-717 gctgggagac caccttgatc tgacttctcc catcttccca gcctaagcca ggccctgggg tca**cg**gaggc

CpG1

KRE

-647 tggggaggca c**cg**aggaa**cg** **cg**cctggcat gtgctgacag gggattttat gctccagctg ggccagctgg

CpG2 CpG3 CpG4

-577 gaggagcctg ctgggcagag gccagagctg ggggctctgg aaggtacctg ggggaggttg cactgtgaga

-507 atgagctcaa gctgggtcag agagcagggc tgactctgcc agtgcctgca tcagcctcat **cg**ctctccta

CpG5

-437 ggctcctggc ctgctggact ctgggctgca ggtccttctt gaaaggctgt gagtagtgag a

Vimentin promoter

-736 agaatcaatt ttacagtggt tctgcagtta atcctttcag tacacc**a**taa atctaaatac tcaaaaaaac

-666 ctgtgccttt tcaattgcta ctaaatca**cg** agaagactga tttacatagt ctccttttat ctcccttgg**c**

CpG1 CpG2

-596 **g**ggtaagtac tcagctctgc t**cg**ttactaa tattgaaaca acagcccttg aattgagtga tttccctaga

CpG3

-526 aaggtt**aagg tgac**cgaatc tgaacactcc ctccatgtct tggaca**cg**aa gttttttgct g**cg**tagacag

KRE CpG4 CpG5 CpG6

-456 ttttatcccc ctcaccccaa ggtcaattgc a**cg**aattctt ttggaaaaca ggacctatgg catttcccag

CpG7

-386 acaaatcacc gtgaaccctg tactgtgcat tgctgt

KRE: KLF4 responsive element

BGS primers MSP primers

Supplementary Fig. 9