SUPPLEMENTARY DATA

Details of COBRA analysis

Tissue of inner medulla, 400-800 pieces of proximal or non-proximal tubules, and 80–100 glomeruli were directly subjected to bisulfite conversion using the DNA Methylation Direct Kit (Zymo Research, Irvine, CA, USA). PCR was performed using Qiagen HotStarTaq DNA polymerase (Qiagen K.K., Tokyo, Japan) under the following conditions: 95 °C for 15 min; 48 cycles at 94 °C for 45 s, 57 °C for 45 s, and 72 °C for 1 min; the final extension step at 72 °C for 10 min. The EpiTect PCR Control DNA Set (Qiagen K.K., Tokyo, Japan) was used for the generation of defined proportions of methylated DNA. Samples were measured in duplicates and methylation values were averaged. Measurements taken from distinct samples were regarded as N = 1. Methylation levels of cytosine located at the *SMTNL2* locus (chromosome 17, position 4510743 of the GRCh37/hg19) were analyzed using the primers: 5'-GGTTTTTGGGAATTTTTGGTTGT -3' (forward) and 5'-CAATCCAAACTCAAAAACTTTACTC -3' (reverse), which amplify the bisulfite-converted DNA of chr17: 4510532 - 4510801 (GRCh37/hg19). The following primers were used for the analysis of cytosine located at the *G6PC* locus (chromosome 17, position 41053025 of the GRCh37/hg19): PCR primers 5'-TAGGGTTGGGTTGATTTGAATATTG -3' (forward) and 5'-ATCTCCAATCACAACTACCCAAAAA -3' (reverse), which amplify the bisulfite-converted DNA of chr17: 41052751 – 41053100 (GRCh37/hg19). The PCR products were digested with HpyCH4IV, a methylation-sensitive restriction enzyme, and were analyzed using MultiNA, a microchip electrophoresis system (Shimadzu, Kyoto, Japan). In 101 recruited patients, methylation values of 8 urine samples were undetectable with no anticipated PCR product. In 2 urine samples, the absolute methylation differences exceeded 15% points between the duplicate measurements. These results of methylation measurements were likely to be caused by insufficient amounts of DNA, and were excluded from further analysis.

Supplementary Table 1. Characteristics of the control and diabetic patients in the kidney study

Variable	Infinium	Laser micro-dissection studies	
	normal (N=5)	normal (N=5)	diabetes (N=4)
Age (years)	59.4 ± 5.7	59.8 ± 1.1	60.5 ± 4.2
Female sex, N (%)	2 (40)	2 (40)	0 (0)
HbA_{1c} (%)	n.d.*	n.d.*	8.6 ± 1.7
Systolic blood pressure	127.6 ± 6.7	133.6 ± 4.6	126.5 ± 9.3
Antihypertensive drug use, N (%)	2 (40)	1 (20)	3 (75)
eGFR (ml/min/1.73 m^2)	59.8 ± 6.0	60.4 ± 10.1	54.0 ± 6.5
Protein/Cr (g/g)	n.d.	n.d.	1.5 ± 0.4

Abbreviations: HbA_{1c}, glycated hemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; Protein/Cr, urinary protein-to-creatinine ratio; n.d., not done.

Continuous characteristics are represented as mean ± S.E.M. Categorical characteristics are represented as N (%). Kidney tissue obtained from one patient was used for both Infinium and laser micro-dissection studies.

* Absence of diabetes was confirmed by blood sugar levels of less than 126 mg/dl in all subjects of the normal group.

Supplementary Table 2. Candidate CpG sites of proximal tubule-specific DNA methylation

Gene symbol	Target ID	CHR	MAPINFO	Mean methylation (%)		*q* value
				Cortex	Inner medulla	
MSRA	cg20710386	8	10228431	66.4	96.6	3.74×10^{-3}
SMTNL2	cg20818528	17	4510743	66.8	95.1	3.60×10^{-3}
GNA12	cg14668632	7	2872130	65.6	90.8	4.80×10^{-3}
HAO2	cg06613817	1	119910670	60.0	90.7	5.17×10^{-3}
REXO1	cg18677278	19	1827752	61.0	90.7	4.50×10^{-3}
PLCB2	cg08779207	15	40586496	62.1	90.6	5.02×10^{-3}
TRAPPC9	cg11739041	8	140980978	58.3	90.2	6.46×10^{-3}

Target ID indicates the probe ID of Infinium MethylationEPIC Kit. Methylation levels are calculated from β values.

CHR, chromosome; MAPINFO, position on the chromosome

Significant *q* values <0.01

Supplementary Table 3. Characteristics of the diabetic patients in the urinalysis study

Variable	N = 91
Age (years)	67.9 ± 1.1
Female sex, N (%)	26 (28.6)
HbA_{1c} (%)	7.5 ± 0.1
Systolic blood pressure	135.5 ± 1.4
eGFR (ml/min/1.73 m^2)	67.5 ± 2.6
eGFR group, N (%)	
<30	5 (5.5)
30–60	29 (31.9)
≧60	57 (62.6)
eGFR change (ml/min/1.73 m^2/year)	−0.59 ± 0.17
RAAS inhibitor use, N (%)	70 (77.0)
Alb/Cr (mg/g)	21.6 (7.5–92.3)
Alb/Cr group, N (%)	
<30	50 (54.9)
30–299	27 (29.7)
≧300	14 (15.4)
G6PC (%)	72.5 ± 1.5
SMTNL2 (%)	85.4 ± 1.0
L-FABP/Cr (ng/mg)	3.8 (2.4–8.1)
NAG/Cr (mU/mg)	9.9 (6.2–12.8)

Abbreviations: HbA_{1c}, glycated hemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; RAAS, renin angiotensin aldosterone system; Alb/Cr, urinary albumin-to-creatinine ratio; G6PC, DNA methylation levels of *G6PC* in urine sediment; SMTNL2, DNA methylation levels of *SMTNL2* in urine sediment; L-FABP/Cr, urinary liver-type fatty acid-binding protein-to-creatinine ratio; NAG/Cr, urinary N-acetyl-β-D-glucosaminidase-to-creatinine ratio.

Continuous characteristics are represented as mean ± S.E.M. or median (25th–75th percentile). Categorical characteristics are represented as N (%).

Supplementary Table 4. Pearson's correlation coefficients for DNA methylation levels of *SMTNL2* and *G6PC* in urine sediment and clinical variables

Urinary marker	Age	SBP	HbA_{1c}	eGFR	Alb/Cr	NAG/Cr	L-FABP/Cr	G6PC
SMTNL2	−0.160	−0.039	0.011	0.063	−0.228[a]	−0.029	−0.148	0.337[b]
G6PC	−0.158	0.108	0.003	0.050	0.115	0.146	0.186	
L-FABP/Cr	0.206[a]	0.204	0.122	−0.228[a]	0.659[b]	0.430[b]		
NAG/Cr	0.072	0.158	0.075	0.129	0.500[b]			

Abbreviations: SBP, systolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; Alb/Cr, log-transformed urinary albumin-to-creatinine ratio; NAG/Cr, log-transformed urinary N-acetyl-β-D-glucosaminidase-to-creatinine ratio; L-FABP/Cr, log-transformed urinary liver-type fatty acid-binding protein-to-creatinine ratio; SMTNL2, DNA methylation levels of *SMTNL2* in urine sediment; G6PC, DNA methylation levels of *G6PC* in urine sediment

[a]p <0.05.
[b]p <0.01.

Supplementary Table 5. Association between annual change in eGFR and variables in patients with eGFR more than 30 ml/min/1.73 m^2 (N = 86)

Variable	Univariate		Multivariate		Multivariate without SMTNL2	
	coefficient (S.E.)	*P*-value	coefficient (S.E.)	*P*-value	coefficient (S.E.)	*P*-value
Age	−0.01 (0.02)	0.47				
SBP	0.00 (0.01)	0.98				
HbA_{1c}	0.18 (0.17)	0.31				
eGFR	0.03 (0.01)	0.0001	0.03 (0.01)	0.0002	0.03 (0.01)	0.0003
Alb/Cr	−0.10 (0.23)	0.67				
SMTNL2	0.04 (0.02)	0.01	0.04 (0.02)	0.02		
G6PC	0.03 (0.01)	0.02			0.02 (0.01)	0.05
L-FABP/Cr	−0.15 (0.47)	0.75				
NAG/Cr	0.82 (0.67)	0.22				

Abbreviations: HbA_{1c}, glycated hemoglobin A_{1c}; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; Alb/Cr, urinary log-transformed albumin-to-creatinine ratio; SMTNL2, DNA methylation levels of *SMTNL2* in urine sediment; G6PC, DNA methylation levels of *G6PC* in urine sediment; L-FABP/Cr, urinary log-transformed liver-type fatty acid-binding protein-to-creatinine ratio; NAG/Cr, urinary log-transformed N-acetyl-β-D-glucosaminidase-to-creatinine ratio.

Values mentioned here are regression coefficients (standard error). Analysis without *SMTNL2* was also performed since methylation levels of *SMNTL2* and *G6PC* correlated with each other ($r = 0.32$, $p < 0.01$).

Supplementary Table 6. Characteristics of the diabetic patients for validation study

Variable	N = 22
Age (years)	67.7 ± 2.5
Female sex, N (%)	7 (31.8)
HbA_{1c} (%)	7.4 ± 0.2
Systolic blood pressure	134.5 ± 3.2
eGFR (ml/min/1.73 m^2)	64.1 ± 4.2
eGFR group, N (%)	
<30	1 (4.5)
30–60	10 (45.5)
≧60	11 (50.0)
eGFR change (ml/min/1.73 m^2/year)	−1.52 ± 0.51
RAAS inhibitor use, N (%)	12 (54.6)
Alb/Cr (mg/g)	28.1 (8.0–63.7)
Alb/Cr group, N (%)	
<30	11 (50.0)
30–299	8 (36.4)
≧300	3 (13.6)
SMTNL2 (%)	81.1 ± 1.8

Abbreviations: HbA_{1c}, glycated hemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; RAAS, renin angiotensin aldosterone system; Alb/Cr, urinary albumin-to-creatinine ratio; SMTNL2, DNA methylation levels of *SMTNL2* in urine sediment.

Continuous characteristics are represented as mean ± S.E.M. or median (25th–75th percentile). Categorical characteristics are represented as N (%).

Supplementary Table 7. Pearson's correlation coefficients for DNA methylation levels of *SMTNL2* in urine sediment and clinical variables in validation cohort

Urinary marker	Age	SBP	HbA_{1c}	eGFR	Alb/Cr
SMTNL2	0.303	−0.038	−0.423*	0.123	−0.622†

Abbreviations: SBP, systolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; Alb/Cr, log-transformed urinary albumin-to-creatinine ratio; SMTNL2, DNA methylation levels of *SMTNL2* in urine sediment

*p <0.05.
†p <0.01.

Supplementary Figure 1. Correlations of urinary DNA methylation of *SMTNL2*, eGFR, urinary albumin, and annual change in eGFR in the validation cohort.

Scatter plots for DNA methylation levels of *SMTNL2* and eGFR (**A**), and urinary albumin-to-creatinine ratio (**B**). Scatter plots for annual eGFR change and eGFR (**C**), urinary albumin-to-creatinine ratio (**D**), and DNA methylation levels of *SMTNL2* (**E**). Pearson's correlation coefficients (*r*) and *p* values are shown.