Supplementary Material

Supplementary Table S1. List of Renal Tubular Biomarkers Assayed

Biomarker	Sample Type	Analysis Platform	Inter-assay CVs
EGF	Urine	ELISA – R&D Systems	10.0% at 12.6 pg/mL and 4.8% at 75.7 pg/mL
MCP1	Urine	ELISA – R&D Systems	6.6% at 121 pg/mL and 5.7% at 760 pg/mL
sTNFR1	Serum/plasma	ELISA – R&D Systems	9.1% at 19.1 pg/mL and 4.1% at 300 pg/mL
KIM-1	Serum/plasma	ELISA – R&D Systems	9.2% at 42.7 pg/mL and 4.4% at 295 pg/mL

Biomarker	Sample Type	Analysis Platform	Inter-assay CVs		
			Serum/plasma	Urinary 10x dilution	Urinary 50x dilution
Hippurate	Urine, serum/plasma	Mass spectrometry	2.6-7.0% at 2.4-17.4 µM	-	5.5-9.7% at 397.2-7013.9 µM
Phenylacetyl glutamine	Urine, serum/plasma	Mass spectrometry	3.4-4.0% at 0.4-5.7 µM	8.2-9.3% at 754.1-2092.6 µM	4.0-6.2% at 730.7-2924.4 µM
Cinnamoylglycine	Urine, serum/plasma	Mass spectrometry	3.2-3.4% at 0.06-0.55 µM	2.7-4.2% at 3.2-68.8 µM	3.9-4.5% at 3.2-69.3 µM
m-Hydroxy hippurate	Urine, serum/plasma	Mass spectrometry	3.4% at 0.1-2.1 µM	2.7-4.3% at 78.9-805.8 µM	4.1-4.3% at 79.3-810.3 µM
2-Furoylglycine	Urine, serum/plasma	Mass spectrometry	3.0-3.6% at 0.1-2.6 µM	3.5-3.8% at 21.9-330.0 µM	4.2-4.4% at 22.0-333.3 µM
Tiglylglycine	Urine, serum/plasma	Mass spectrometry	3.8-4.9% at 0.05-0.52 µM	3.4-4.8% at 7.9-83.7 µM	4.9-5.2% at 7.9- 84.7 µM
Indoxyl sulfate	Urine, serum/plasma	Mass spectrometry	5.1-5.5% at 2.6-22.3 µM	2.9-5.7% at 76.2-1089.2 µM	4.0-4.6% at 77.4-1152.5 µM
1,3,7 trimethyluric acid	Urine, serum/plasma	Mass spectrometry	4.4-4.5% at 0.03-0.26 µM	4.8-4.9% at 2.7-12.1 µM	6.1-6.9% at 2.7-12.3 µM
1,7 dimethyluric acid	Urine, serum/plasma	Mass spectrometry	6.0-8.8% at 0.12-2.21 µM	6.2-6.5% at 19.0-297.9 µM	6.7-8.3% at 19.7-303.1 µM
Adipic acid	Urine, serum/plasma	Mass spectrometry	3.9-9.9% at 0.5-4.2 µM	2.9-4.1% at 61.2-362.1 µM	4.4-4.7% at 61.1-362.9 µM
P-cresol sulfate	Urine, serum/plasma	Mass spectrometry	3.6-4.9% at 2.6-44.9 µM	3.3-4.7% at 81.2-5359.1 µM	4.8-6.5% at 75.8-5619.7 µM

Supplementary Table S2. Correlations between composite tubular Secretion Score and Components of the Tubular Secretion Score

Tubular Secretion Score Component*	1, 7-Dimethyluric Acid	Adipic Acid	Cinnamoyl-glycine	Hippuric Acid	Indoxyl Sulfate	p-Cresol Sulfate	Phenylacetyl-glutamine	Tiglylglycine	Composite Tubular Secretion Score
1, 7-Dimethyluric Acid	1.000	0.30	0.36	0.38	0.41	0.42	0.43	0.44	0.66
Adipic Acid	0.30	1.000	0.27	0.20	0.30	0.30	0.24	0.35	0.54
Cinnamoylglycine	0.36	0.27	1.000	0.47	0.35	0.35	0.42	0.54	0.68
Hippuric Acid	0.38	0.20	0.47	1.000	0.43	0.35	0.48	0.35	0.64
Indoxyl Sulfate	0.41	0.30	0.35	0.43	1.000	0.73	0.72	0.55	0.75
p-Cresol Sulfate	0.42	0.30	0.35	0.35	0.73	1.000	0.74	0.51	0.73
Phenylacetylglutamine	0.43	0.24	0.42	0.48	0.72	0.74	1.000	0.57	0.75
Tiglylglycine	0.44	0.33	0.54	0.35	0.55	0.51	0.57	1.000	0.74
Tubular Secretion Score	0.66	0.54	0.68	0.64	0.75	0.73	0.75	0.74	1.000

*All components of the tubular secretion score are included as standardized clearances.

Supplementary Table S3. Descriptions and Associated Outcome Windows for Tubular Biomarker Assessments

Measurement	Formula	Associated Outcome Window
Single Biomarker	$Log(B_i)$	t_i to t_{i+1}
Mean of Consecutive Biomarkers	$Log((B_{i+1}+B_i)/2)$	t_{i+1} to t_{i+2}
Slope	$Log(B_{i+1}/B_i)/(t_{i+1}-t_i)$	t_{i+1} to t_{i+2}
DCCT Baseline or Year 1	$Log(B_o)$ or $Log(B_1)$	After t_o or t_1

B_i- Biomarker level at study visit *i*.

t_i- Years of follow-up at study visit *i*.

Supplemental Table S4. Associations of mean consecutive tubular biomarkers with risk of incident macroalbuminuria or sustained low eGFR.

	Unadjusted model		Minimally-adjusted model		Intermediately-adjusted model		Fully-adjusted model	
A. Macroalbuminuria	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
KIM-1 (pg/mL)	**1.25 (1.19, 1.31)**	**<0.0001***	**1.18 (1.12, 1.25)**	**<0.0001***	**1.18 (1.11, 1.25)**	**<0.0001***	1.06 (0.99, 1.14)	0.11
sTNFR1 (pg/mL)	**1.33 (1.16, 1.53)**	**<0.0001***	**1.28 (1.12, 1.48)**	**0.0005***	**1.27 (1.11, 1.45)**	**0.0007***	1.08 (0.95, 1.24)	0.24
EGF to MCP-1 ratio MCP1 excretion rate (ng/day)	**1.15 (1.10, 1.20)**	**<0.0001***	**1.09 (1.04, 1.14)**	**0.0002***	**1.06 (1.01, 1.12)**	**0.0257**	0.98 (0.94, 1.03)	0.41
EGF to MCP-1 ratio EGF excretion rate (ng/day)	1.05 (0.97, 1.14)	0.24	1.05 (0.95, 1.15)	0.33	0.96 (0.87, 1.06)	0.46	0.94 (0.86, 1.03)	0.16
EGF to MCP-1 ratio (ng/ng)	**0.90 (0.86, 0.94)**	**<0.0001***	**0.94 (0.90, 0.98)**	**0.0054***	**0.94 (0.90, 0.99)**	**0.0207***	0.99 (0.95, 1.03)	0.50
Tubular secretion score	1.28 (0.89, 1.83)	0.18	1.40 (0.90, 2.20)	0.14	0.93 (0.59, 1.48)	0.76	1.16 (0.83, 1.63)	0.37
B. Sustained low eGFR								
KIM-1 (pg/mL)	**1.30 (1.24, 1.37)**	**<0.0001***	**1.25 (1.18, 1.32)**	**<0.0001***	**1.24 (1.16, 1.32)**	**<0.0001***	**1.13 (1.05, 1.22)**	**0.0013***
sTNFR1 (pg/mL)	**1.92 (1.63, 2.26)**	**<0.0001***	**1.66 (1.38, 1.99)**	**<0.0001***	**1.58 (1.33, 1.86)**	**<0.0001***	1.07 (0.87, 1.33)	0.51
EGF to MCP-1 ratio MCP1 excretion rate (ng/day)	**1.18 (1.13, 1.24)**	**<0.0001***	**1.14 (1.08, 1.20)**	**<0.0001***	**1.13 (1.06, 1.20)**	**0.0002***	1.05 (0.99, 1.11)	0.10
EGF to MCP-1 ratio EGF excretion rate (ng/day)	**0.92 (0.84, 1.00)**	**0.0482**	0.95 (0.87, 1.05)	0.33	**0.86 (0.77, 0.97)**	**0.0110***	0.88 (0.78, 1.01)	0.06
EGF to MCP-1 ratio (ng/ng)	**0.83 (0.78, 0.88)**	**<0.0001***	**0.88 (0.83, 0.93)**	**<0.0001***	**0.87 (0.82, 0.93)**	**<0.0001***	**0.93 (0.87, 0.99)**	**0.0261**
Tubular secretion score	0.80 (0.63, 1.02)	0.07	0.86 (0.61, 1.22)	0.40	0.69 (0.45, 1.06)	0.09	0.81 (0.49, 1.33)	0.40

Hazard Ratios are per 20% higher biomarker mean values. All models employ a robust sandwich standard error. Asterisks indicate significant associations accounting for multiple testing using the Holm-Bonferroni Method. Models shown are: unadjusted; minimally adjusted for age and mean updated HbA1c; intermediately adjusted for age, mean updated HbA1c, DCCT treatment group, sex, diabetes duration, systolic blood pressure, pulse, mean updated triglycerides, and history of hypertension, calcium channel blocker use, or beta blocker use; fully adjusted for all variables in intermediately adjusted models plus concurrent mean eGFR and AER.

Supplementary Table S5. Associations of tubular biomarker slopes with risk of incident macroalbuminuria or sustained low eGFR.

	Unadjusted model		Minimally-adjusted model		Intermediately-adjusted model		Fully-adjusted model	
A. Macroalbuminuria	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
KIM-1 (pg/mL)	**2.76 (2.14, 3.55)**	**<0.0001***	**2.47 (1.95, 3.13)**	**<0.0001***	**2.30 (1.81, 2.93)**	**<0.0001***	**1.81 (1.40, 2.34)**	**<0.0001***
TNFR1 (pg/mL)	**3.10 (1.94, 4.96)**	**<0.0001***	**2.35 (1.43, 3.88)**	**0.0008***	**2.43 (1.46, 4.05)**	**0.0007***	**1.95 (1.18, 3.21)**	**0.0089***
Urinary MCP1 excretion rate (ng/day)	**1.15 (1.03, 1.28)**	**0.0092***	**1.17 (1.05, 1.30)**	**0.0043***	**1.13 (1.00, 1.27)**	**0.0410**	0.98 (0.89, 1.09)	0.75
Urinary EGF excretion rate (ng/day)	1.01 (0.78, 1.31)	0.94	1.10 (0.84, 1.45)	0.48	1.16 (0.87, 1.54)	0.31	0.87 (0.68, 1.11)	0.25
EGF to MCP-1 ratio (ng/ng)	**0.85 (0.77, 0.94)**	**0.0017***	**0.86 (0.77, 0.97)**	**0.0123***	0.90 (0.80, 1.01)	0.07	0.98 (0.89, 1.09)	0.75
Tubular secretion score	0.51 (0.21, 1.20)	0.12	0.84 (0.30, 2.34)	0.73	0.72 (0.25, 2.10)	0.55	0.75 (0.26, 2.18)	0.60
B. Sustained eGFR<60								
KIM-1 (pg/mL)	**3.14 (2.34, 4.22)**	**<0.0001***	**3.02 (2.30, 3.96)**	**<0.0001***	**2.63 (1.97, 3.53)**	**<0.0001***	**2.26 (1.65, 3.09)**	**<0.0001***
TNFR1 (pg/mL)	**6.36 (3.48, 11.64)**	**<0.0001***	**4.51 (2.38, 8.55)**	**<0.0001***	**4.59 (2.38, 8.85)**	**<0.0001***	**2.94 (1.39, 6.23)**	**0.0049***
Urinary MCP1 excretion rate (ng/day)	1.02 (0.91, 1.15)	0.71	1.04 (0.92, 1.17)	0.50	1.03 (0.90, 1.17)	0.70	0.94 (0.81, 1.09)	0.43
Urinary EGF excretion rate (ng/day)	0.90 (0.69, 1.16)	0.40	1.09 (0.83, 1.42)	0.54	1.09 (0.83, 1.44)	0.53	1.13 (0.84, 1.51)	0.42
EGF to MCP-1 ratio (ng/ng)	0.95 (0.82, 1.09)	0.43	0.98 (0.84, 1.13)	0.78	1.00 (0.86, 1.16)	0.97	1.10 (0.94, 1.29)	0.23
Tubular secretion score	0.80 (0.26, 2.46)	0.70	1.85 (0.61, 5.60)	0.27	1.29 (0.41, 4.01)	0.66	2.13 (0.77, 5.92)	0.15

Hazard ratios (HR) are per 20% increase in biomarker fold-change over consecutive visits (ratio of biomarker at time X+1 over time X). All models employ a robust sandwich standard error. Asterisks indicate significant associations accounting for multiple testing using the Holm-Bonferroni Method. Models shown are: unadjusted; minimally adjusted for age and mean updated HbA1c; intermediately adjusted for age, mean updated HbA1c, DCCT treatment group, sex, diabetes duration, systolic blood pressure, pulse, mean updated triglycerides, and history of hypertension, calcium channel blocker use, or beta blocker use; fully adjusted for all variables in intermediately adjusted models plus concurrent eGFR slope and AER slope.

Supplementary Table S6. Associations of tubular biomarker slopes with risk of incident macroalbuminuria or sustained low eGFR, in fully-adjusted models adjusted for mean consecutive eGFR and AER instead of eGFR and AER slope.

	Fully Adjusted with Mean Consecutive AER and eGFR	
A. Macroalbuminuria	**HR (95% CI)**	**P-value**
KIM-1 (pg/mL)	**1.91 (1.50, 2.44)**	**<0.0001***
sTNFR1 (pg/mL)	1.53 (0.90, 2.59)	0.12
Urinary MCP1 excretion rate (ng/day)	1.03 (0.93, 1.14)	0.57
Urinary EGF excretion rate (ng/day)	1.15 (0.91, 1.45)	0.26
EGF to MCP-1 ratio (ng/ng)	1.00 (0.89, 1.11)	0.96
Tubular secretion score	1.04 (0.38, 2.86)	0.94
B. Sustained low eGFR		
KIM-1 (pg/mL)	**2.30 (1.69, 3.13)**	**<0.0001***
sTNFR1 (pg/mL)	**3.37 (1.67, 6.77)**	**0.0007***
Urinary MCP1 excretion rate (ng/day)	1.02 (0.88, 1.18)	0.82
Urinary EGF excretion rate (ng/day)	1.10 (0.82, 1.47)	0.53
EGF to MCP-1 ratio (ng/ng)	1.01 (0.87, 1.17)	0.92
Tubular secretion score	1.05 (0.35, 3.17)	0.93

Hazard ratios (HR) are per 20% increase in biomarker fold-change over consecutive visits (ratio of biomarker at time X+1 over time X). All models employ a robust sandwich standard error. Asterisks indicate significant associations accounting for multiple testing using the Holm-Bonferroni Method. Models shown are fully adjusted for age, mean updated HbA1c, DCCT treatment group, sex, diabetes duration, systolic blood pressure, pulse, mean updated triglycerides, and history of hypertension, calcium channel blocker use, or beta blocker use, plus concurrent mean consecutive eGFR and mean AER.

Supplementary Table S7. Associations of tubular biomarkers ascertained at DCCT baseline or year 1 with risk of incident macroalbuminuria or sustained low eGFR.

	Unadjusted model		Minimally-adjusted model		Intermediately-adjusted model		Fully-adjusted model	
A. Macroalbuminuria	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
KIM-1 (pg/mL)	1.04 (0.98, 1.10)	0.1531	1.02 (0.95, 1.09)	0.6744	1.01 (0.94, 1.09)	0.7592	1.00 (0.93, 1.08)	0.9572
sTNFR1 (pg/mL)	1.08 (0.95, 1.23)	0.2211	1.12 (0.98, 1.29)	0.0960	1.14 (0.97, 1.33)	0.1036	1.14 (0.98, 1.33)	0.0895
Urinary MCP1 excretion rate (ng/day)	**1.04 (1.00, 1.07)**	**0.0251**	1.02 (0.99, 1.06)	0.1677	1.02 (0.99, 1.06)	0.2307	1.01 (0.97, 1.05)	0.6126
Urinary EGF excretion rate (ng/day)	1.05 (0.98, 1.12)	0.1919	1.03 (0.96, 1.11)	0.4437	1.04 (0.95, 1.13)	0.3756	1.02 (0.94, 1.11)	0.6066
EGF to MCP-1 ratio (ng/ng)	0.96 (0.93, 1.00)	0.0561	0.98 (0.94, 1.02)	0.2368	0.98 (0.94, 1.02)	0.3668	0.99 (0.95, 1.04)	0.7751
Tubular secretion score	**1.64 (1.10, 2.46)**	**0.0164**	**1.51 (1.01, 2.27)**	**0.0444**	1.47 (0.97, 2.22)	0.0714	**1.57 (1.02, 2.43)**	**0.0413**
B. Sustained eGFR<60								
KIM-1 (pg/mL)	1.06 (1.00, 1.12)	0.0550	0.99 (0.92, 1.07)	0.8422	0.96 (0.88, 1.04)	0.3260	0.94 (0.86, 1.03)	0.1886
sTNFR1 (pg/mL)	**1.17 (1.03, 1.33)**	**0.0192**	**1.27 (1.10, 1.46)**	**0.0012**	**1.33 (1.14, 1.54)**	**0.0002***	**1.32 (1.14, 1.53)**	**0.0002***
Urinary MCP1 excretion rate (ng/day)	1.02 (0.99, 1.05)	0.2663	1.02 (0.98, 1.06)	0.2846	1.02 (0.98, 1.06)	0.2895	1.02 (0.98, 1.06)	0.2591
Urinary EGF excretion rate (ng/day)	0.95 (0.88, 1.04)	0.2595	0.97 (0.89, 1.06)	0.4536	0.96 (0.87, 1.05)	0.3784	0.96 (0.88, 1.06)	0.4383
EGF to MCP-1 ratio (ng/ng)	0.96 (0.92, 1.00)	0.0793	0.97 (0.93, 1.01)	0.1376	0.97 (0.93, 1.01)	0.1246	0.97 (0.93, 1.01)	0.1216
Tubular secretion score	0.98 (0.68, 1.40)	0.9002	0.91 (0.61, 1.38)	0.6679	0.87 (0.55, 1.37)	0.5563	0.86 (0.55, 1.35)	0.5139

Hazard ratios (HR) are per 20% increase in biomarker values. All models employ a robust sandwich standard error. Asterisks indicate significant associations accounting for multiple testing using the Holm-Bonferroni Method. Models shown are: unadjusted; minimally adjusted for age and mean updated HbA1c; intermediately adjusted for age, mean updated HbA1c, DCCT treatment group, sex, diabetes duration, systolic blood pressure, pulse, mean updated triglycerides, and history of hypertension, calcium channel blocker use, or beta blocker use; fully adjusted for all variables in intermediately adjusted models plus concurrent AER and eGFR.

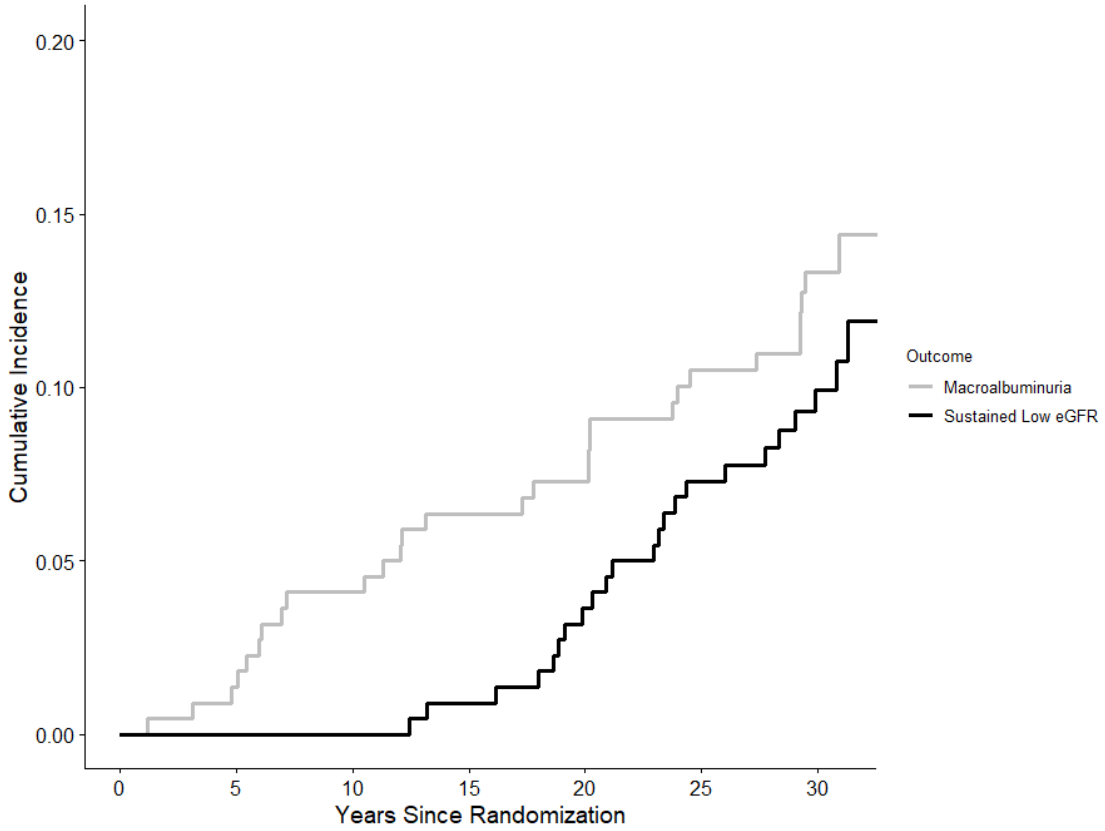
Supplementary Table S8. Associations of mean-updated HbA1c with subsequent outcomes in models with and without adjustment for tubular biomarkers

	Number of Biomarker Measurements	Association of HbA1c with kidney outcomes without adjustment for individual biomarker		Association of HbA1c with kidney outcomes with adjustment for individual biomarker		Percent Reduction in Hazard Ratio with Biomarker*
A. Macroalbuminuria		**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	
KIM-1 (pg/mL)	1603	2.39 (2.09, 2.73)	<0.0001	1.99 (1.74, 2.28)	<0.0001	28.7%
sTNFR1 (pg/mL)	1603	2.39 (2.09, 2.73)	<0.0001	2.36 (2.07, 2.70)	<0.0001	1.9%
Urinary MCP1 excretion rate	1587	2.44 (2.10, 2.84)	<0.0001	2.29 (1.96, 2.67)	<0.0001	10.7%
Urinary EGF excretion rate (ng/day)	1587	2.44 (2.10, 2.84)	<0.0001	2.49 (2.15, 2.89)	<0.0001	-3.5%
EGF to MCP-1 ratio (ng/ng)	1587	2.44 (2.10, 2.84)	<0.0001	2.24 (1.91, 2.62)	<0.0001	14.2%
Tubular secretion score	1425	2.56 (2.19, 2.98)	<0.0001	2.58 (2.22, 3.01)	<0.0001	-1.7%
eGFR (ml/min/1.73m^2)	1767	2.31 (2.03, 2.63)	<0.0001	2.31 (2.03, 2.63)	<0.0001	0.2%
AER (mg/day)	1767	2.31 (2.03, 2.63)	<0.0001	1.71 (1.48, 1.98)	<0.0001	45.5%
All tubular biomarkers**	1423	2.55 (2.18, 2.98)	<0.0001	2.04 (1.73, 2.42)	<0.0001	32.7%
B. Sustained eGFR<60						
KIM-1 (pg/mL)	1746	2.35 (1.91, 2.89)	<0.0001	1.57 (1.22, 2.01)	0.0004	58.0%
sTNFR1 (pg/mL)	1746	2.35 (1.91, 2.89)	<0.0001	2.04 (1.63, 2.56)	<0.0001	22.9%
Urinary MCP1 excretion rate	1736	2.40 (1.91, 3.01)	<0.0001	2.23 (1.76, 2.82)	<0.0001	12.3%
Urinary EGF excretion rate	1736	2.40 (1.91, 3.01)	<0.0001	2.35 (1.88, 2.93)	<0.0001	3.4%
EGF to MCP-1 ratio (ng/ng)	1736	2.40 (1.91, 3.01)	<0.0001	2.00 (1.55, 2.59)	<0.0001	28.5%
Tubular secretion score	1558	2.33 (1.86, 2.93)	<0.0001	2.32 (1.86, 2.91)	<0.0001	0.6%
eGFR (ml/min/1.73m^2)	1926	2.41 (1.96, 2.97)	<0.0001	2.12 (1.65, 2.74)	<0.0001	20.4%
AER (mg/day)	1926	2.41 (1.96, 2.97)	<0.0001	1.56 (1.16, 2.09)	0.0030	60.4%
All tubular biomarkers**	1533	2.65 (2.15, 3.28)	<0.0001	1.53 (1.20, 1.95)	0.0007	68.0%

Hazard ratios (HR) are presented per 1-percentage increase (such as from 7% to 8%) in mean updated HbA1c. All models are further adjusted for current age.
*Percent reduction in hazard ratio is calculated as [(HR without biomarker)-(HR with biomarker)]/(HR without biomarker-1)*100.
**Includes all biomarkers listed in the table except for eGFR and AER.

Supplementary Figure S1. Incidence of macroalbuminuria and sustained low eGFR in the study cohort. (A) Cumulative incidence curve of kidney outcomes in the DCCT/EDIC random subcohort. (B) Number of kidney events and event rate per 1000 person-years in the random subcohort, each outcome subgroup, and the source DCCT/EDIC cohort.

A.



B.

	Number of Events (Event Rate per 1000 person-years)			
Outcome	**Source DCCT/EDIC cohort (n=1266)**	**Random Subcohort (n=220)**	**Macroalbuminuria Subgroup (n=355)**	**Sustained Low eGFR Subgroup (n=320)**
Macroalbuminuria	165 (4.45)	30 (4.77)	165 (4.55)	153 (4.18)
Sustained eGFR<60	123 (3.18)	23 (3.47)	113 (2.95)	123 (3.22)