Supplementary Materials for

Circulating proteins protect against renal decline and progression to end stage renal disease in patients with diabetes

Materials and Methods

Joslin Kidney Study

Briefly, the Joslin Kidney Study (JKS) comprises two components, type 1 diabetes (T1D) and type 2 diabetes (T2D). Individuals in the T1D component were recruited consecutively from among 2,000 adults 18-64 years old with T1D who attended the Joslin Clinic between 1991 and 2009. According to the median values of ACR obtained during the 2-year period preceding enrollment (baseline examination), participants were classified into three sub-groups: those with macro-albuminuria (ACR $\geq$ 300 μg/mg), micro-albuminuria (30 $\leq$ ACR < 300 μg/mg), and normo-albuminuria (ACR < 30 μg/mg). We aimed to recruit into the JKS all of those with macro- and micro-albuminuria and a similar number of those with normo-albuminuria. In total, we enrolled 1884 participants; 526 with macro-albuminuria, 563 with micro-albuminuria and 795 with normo-albuminuria.

Individuals in the T2D cohort were recruited consecutively from among 1,500 adults 35-64 years old with T2D who attended the Joslin Clinic between 2003 and 2009. According to the median values of ACR obtained during the 2-year period preceding enrollment (baseline examination), participants were classified into three sub-groups as described above for T1D. We aimed to recruit into the JKS all those with macro- and micro-albuminuria and a similar number of participants with normo-albuminuria. In total, we enrolled 1,476 participants: 261 with macro-albuminuria, 482 with micro-albuminuria and 733 with normo-albuminuria.

All participants enrolled into the JKS had biannual examinations either during routine clinic visits or were invited for a special visit or were examined at their homes. These examinations were conducted until they developed ESRD, died, were lost to follow-up or until the end of follow-up in 2015. Biospecimens obtained at examinations were stored at -85°C. Serum creatinine was used to determine renal function at baseline and its changes during follow-up visits. Protocols to calibrate

serum creatinine measurements over time were described previously (*38*). Estimates of GFR were obtained using the Chronic Kidney Disease Epidemiology Collaboration formula (*39*).

To classify patterns of trajectories of renal function changes during follow-up, the first step was to determine whether they were linear or non-linear. Although most eGFR trajectories appeared linear on inspection, we sought to validate this impression statistically by fitting both linear and spline models to each patient's renal function trajectory. We applied an approach described by Jones and Molitoris (*40*) and used by Shah and Levey (*41*), to examine an individual's serial renal function changes during follow-up. Participants in our study had 5 or more eGFR determinations over 7-15 years of follow-up. The method represents each participant's renal function trajectory as a simple linear model and as a spline model with linear segments connected at an individually determined point. The linear and spline models were compared, and the linear model was rejected at a nominal significance of 0.05 and degrees of freedom determined by the number of spline segments (n-1). The majority had linear slopes. To determine the slope of eGFR decline, we extracted the linear component of each individual's trajectory to generate distribution of slopes of overall eGFR change during follow-up. Details of this approach are included in the methods section and were also described in our earlier publication (*38*).

All participants included in the JKS were queried every two years against rosters of the United States Renal Data System (USRDS) and the National Death Index (NDI) to ascertain patients who developed ESRD or died. The last inquiries were conducted in 2015. The USRDS maintains a roster of US patients receiving renal replacement therapy, which includes dates of dialysis and transplantation.

Plasma measurement of ANGPT1

Plasma ANGPT1 was measured using the Human Ang-1 MSD R-Plex assay (catalog number F21YQ-3, Meso Scale Diagnostics) according to the manufacturer's protocols. Briefly, an MSD GOLD Small Spot Streptavidin plate was coated with 100 µl of biotinylated Ang-1 capture antibody in coating diluent 100 and incubated for 1 hour at room temperature. The plate was washed with 150 µl/well of washing buffer (1X PBS-Tween 20), and duplicates of 25 µl of serially diluted standard from 100,000 pg to 24 pg/ml and 32 plasma samples from our study were all loaded onto the same plate. After 1 hour incubation with shaking at room temperature, the plate was washed and incubated with 50 µl of conjugated detection antibody (MSD GOLD SULFO-TAG^{TM}) for 1 hour at room temperature, then washed, and finally 150 µl/well of read buffer was added on the plate. The plate was loaded into an MSD instrument where a voltage was applied to the plate electrodes to measure to intensity of the emitted light and provided a quantitative measure of the analyte in the sample.

Technical validation of SOMAmer specificity by LC-MS/MS

To systematically assess SOMAscan platform specificity, we developed protocols using SOMAmer for affinity pull-down of intact proteins followed by digestion to peptides and analysis by untargeted mass spectrometry. The FGF20 SOMAmer reagent was thawed, vortexed and spun down for 2 minutes (min), heated to 100°C for 5 min in PCR machine, and then slowly cooled in a 25°C water bath. The FGF20 SOMAmer was diluted to 50mM AB Buffer (40 mM HEPES, 100 mM NaCl, 5 mM KCl, 5 mM MgCl2, 0.05% Tween-20 at pH 7.5), and then cooled in a water bath to 25°C for 20 min. Streptavidin agarose beads were diluted from 50mM to 7.5%, and then spun at 1000x*g* for 2 min. The 7.5% streptavidin agarose beads were washed with AB buffer, vortexed and centrifuged for 2 min at 1000x*g*. The liquid was vacuumed out and the washing was repeated once more for a

total of two times. SOMAmers were added to the beads and incubated for 20 min with shaking at 25°C. The tubes were spun for 2 min at 1000x*g* and the liquid was removed by vacuum. The beads were washed twice with 0-W buffer, and then washed twice with AB buffer. AB buffer, plasma and serum samples, and recombinant proteins were added to the appropriate tubes, along with 30 µl of SOMAmers bound beads. These tubes were shaken for 1.5 hours at room temperature. After incubation was completed, the tubes were spun down for 1 minute and the liquid was removed. The samples were washed once with 1-B blocker, shaken for 5 min at 800 rpm, and the liquid was removed. The samples were washed 6 times with AB buffer, and then frozen at -80°C. Four times the sample volume of acetone at -20°C was added to each tube. The tubes were quickly vortexed and incubated -20°C for 1 hour. The tubes were centrifuged for 10 min at 13,000x*g*, and the supernatant was vacuumed out.

An equal volume of 0.5 M ammonium carbonate pH 10.5 was added to each set of washed beads. Another equal volume of reduction/alkylation cocktail consisting of 2% (v/v) iodoethanol and 0.5% (v/v) triethylphosphine in 97.5% acetonitrile was then added to each sample. The solutions were capped and incubated for 1 hour at 37°C, after which they were speed-vacuumed to dryness. The resulting pellets were then redissolved in a trypsin solution (Pierce Trypsin Protease MS-Grade, in 100 mM Tris-HCl, pH 8.0). The digestion was carried out at 37°C overnight, after which the solutions were desalted using µC18 ZipTips (Millipore). The digested samples were analyzed with a Thermo Q-Exactive mass spectrometer using a Thermo EASY-nLC HPLC system. The separation was carried out with a 75 µm x 15 cm Thermo EASY-Spray C18 column. MS data were collected in data dependent acquisition mode with a full high resolution MS scan followed by MS/MS scans of the top 10 most intense precursor ions (within a mass range of 350-2000 m/z).

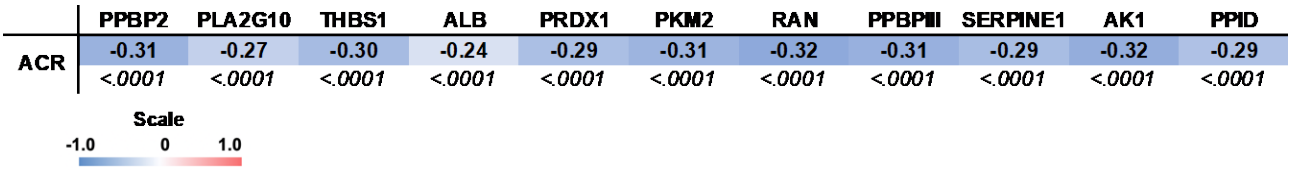
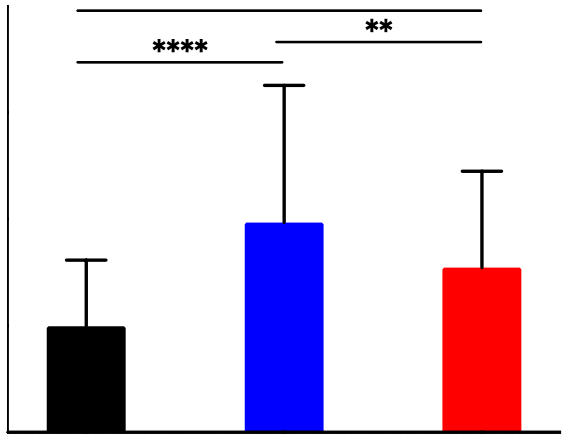


Fig. S1. Association of 11 candidate protective proteins with ACR concentrations.

Spearman's rank correlation matrix among 11 candidate protective proteins with ACR adjusted for type of diabetes. Correlation coefficients (r_s) are presented as shades of red (positive) and blue (negative) which correspond to the magnitude of the effect size.

(A)



(B)

(C)

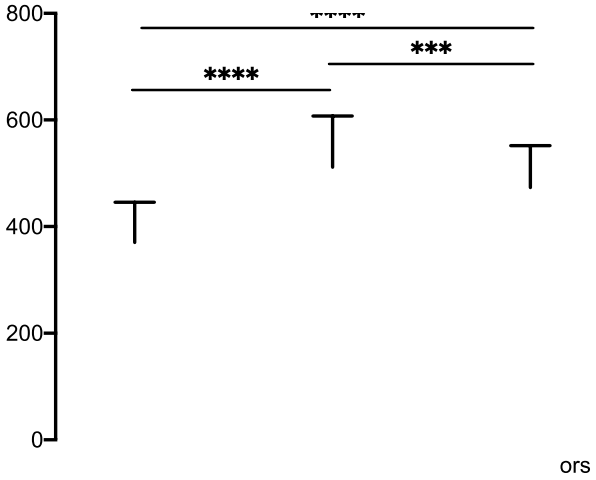


Fig. S2. Plasma concentrations of exemplar protective proteins in non-diabetics and diabetic individuals.

Plasma concentrations of (A) ANGPT1, (B) TNFSF12, (C) FGF20 in the combined Joslin cohorts, for non-progressors and progressors, compared to non-diabetics. Bars depict the mean ± standard deviations. One-way ANOVA with Dunn's multiple comparisons test. ***P*<0.01; ****P*<0.001; *****P*<0.0001; ns, not significant.

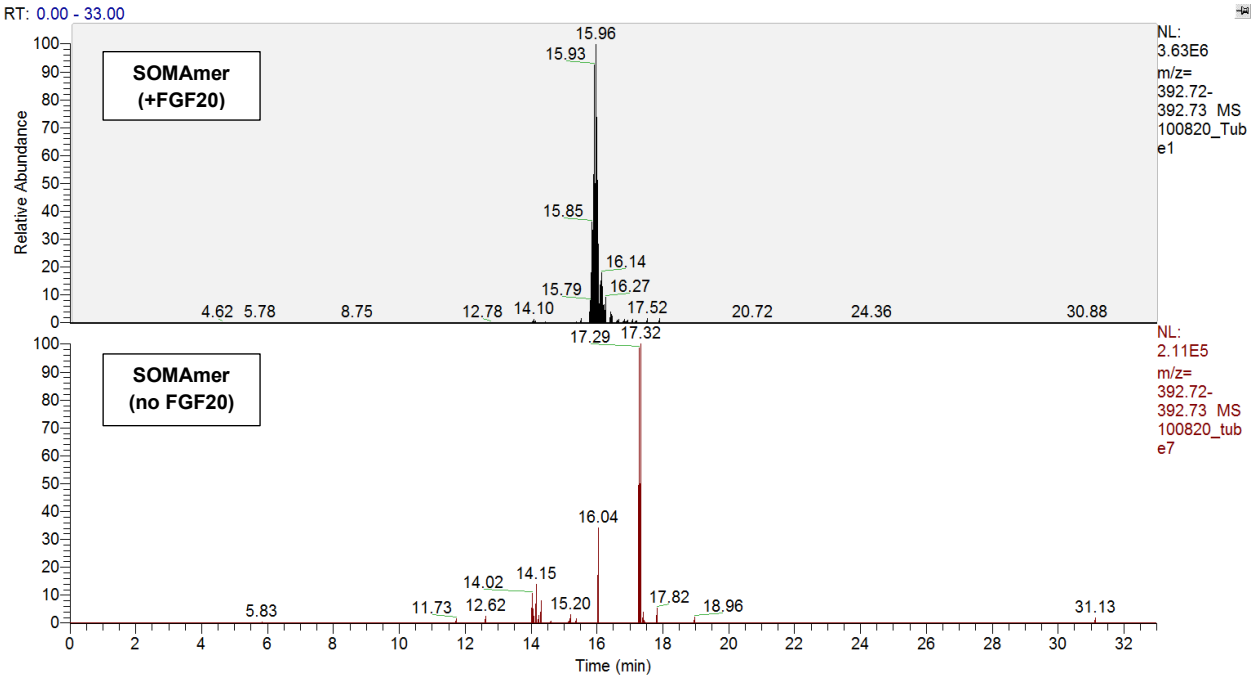


Fig. S3. Mass spectrometry verification of SOMAmer-protein binding.

An extracted ion chromatogram of FGF20 tryptic peptide GGPGAAQLAHLHGILR (amino acids 50-65). The FGF20 SOMAmer plasma pull-downs in the presence (top) or absence (bottom) of recombinant FGF20.

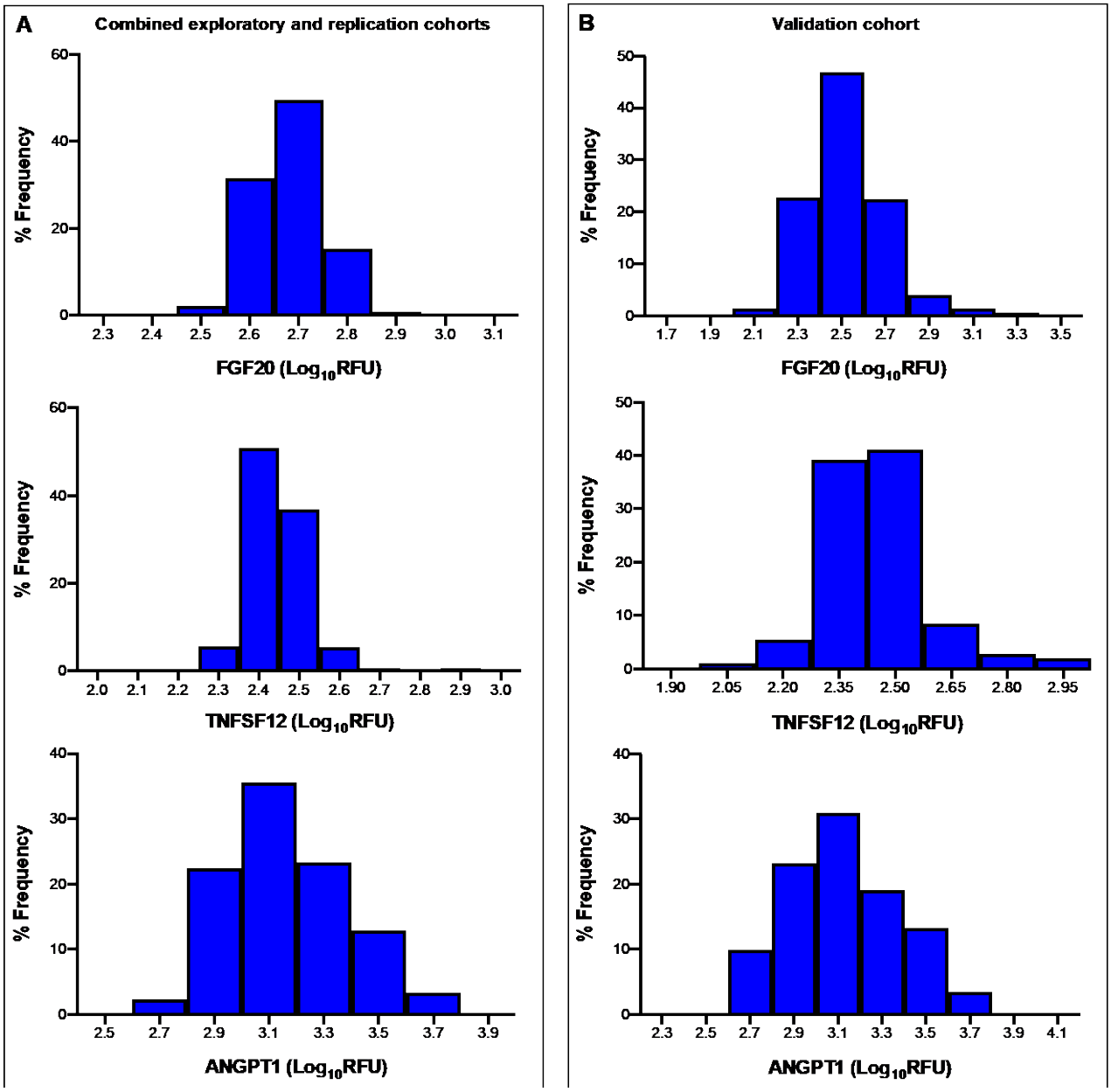


Fig. S4. Histograms showing the distribution of the top 3 protective protein candidates after $\log_{10}$ transformation in (A) the combined T1D discovery and T2D replication cohorts and in (B) the T1D validation cohort.

Table S2. Global proteomic profiling data of the circulating plasma proteins in the exploratory cohort of 214 T1D individuals and in the replication cohort of 144 T2D individuals. Spearman's rank correlation coefficients (r_S) between baseline concentration of 73 proteins and eGFR slope.

			Joslin T1D Cohort		**Joslin T2D Cohort**	
Target Full Name	**UniProt ID**	***Gene Symbol***	r_S	***P*-value***	r_S	***P*-value***
Serum albumin	P02768	***ALB***	0.33	9.20E-07	0.18	3.01E-02
Tumor necrosis factor ligand superfamily member 12	O43508	***TNFSF12***	0.32	2.00E-06	0.23	5.40E-03
Secreted protein acidic and rich in cysteine	P09486	***SPARC***	0.29	1.50E-05	0.21	1.15E-02
Adenylate kinase isoenzyme 1	P00568	***AK1***	0.27	6.60E-05	0.18	3.04E-02
Connective tissue-activating peptide III	P02775	***CTAPIII***	0.27	6.70E-05	0.18	2.65E-02
Neutrophil-activating peptide 2	P02775	***NAP2***	0.26	9.60E-05	0.19	2.18E-02
C-C motif chemokine 5	P13501	***CCL5***	0.26	1.30E-04	0.23	5.30E-03
Thrombospondin-1	P07996	***THBS1***	0.24	3.20E-04	0.17	4.35E-02
Amyloid beta A4 protein	P05067	***APP***	0.24	3.60E-04	0.21	1.34E-02
Platelet factor 4	P02776	***PF4***	0.23	6.00E-04	0.21	1.17E-02
Fibroblast growth factor 20	Q9NP95	***FGF20***	0.23	6.20E-04	0.18	2.71E-02
Angiopoietin-1	Q15389	***ANGPT1***	0.23	6.80E-04	0.23	6.10E-03
DnaJ Heat Shock Protein Family Member C19	Q96DA6	***DNAJC19***	0.23	7.70E-04	0.17	4.09E-02
Group 10 secretory phospholipase A2	O15496	***PLA2G10***	0.23	8.90E-04	0.28	6.00E-04
Peptidyl-prolyl cis-trans isomerase D	Q08752	***PPID***	0.23	9.00E-04	0.18	3.41E-02
Plasminogen activator inhibitor 1	P05121	***SERPINE1***	0.22	9.90E-04	0.17	3.88E-02
GTP-binding nuclear protein Ran	P62826	***RAN***	0.22	1.40E-03	0.17	4.44E-02
Peroxiredoxin-1	Q06830	***PRDX1***	0.20	3.30E-03	0.17	4.72E-02
Pyruvate kinase PKM	P14618	***PKM2***	0.21	2.00E-03	0.11	2.09E-01
Alanine aminotransferase 1	P24298	*GPT*	0.31	5.10E-06	0.07	3.91E-01
Metalloproteinase inhibitor 3	P35625	*TIMP3*	0.30	7.10E-06	0.10	2.27E-01

			Joslin T1D Cohort		Joslin T2D Cohort	
Target Full Name	**UniProt ID**	***Gene Symbol***	r_s	***P*-value***	r_s	***P*-value***
Immunoglobulin G	P01857	*IGHG1 IGHG2*	0.30	9.10E-06	0.16	5.45E-02
6-phosphogluconate dehydrogenase, decarboxylating	P52209	*PGD*	0.28	4.20E-05	0.15	7.19E-02
Gro-beta/gamma	P19876 P19875	*CXCL3 CXCL2*	0.27	8.10E-05	0.15	7.65E-02
Dipeptidyl peptidase 2	Q9UHL4	*DPP7*	0.25	2.10E-04	0.09	2.67E-01
Hepatocyte growth factor	P14210	*HGF*	0.25	2.30E-04	0.03	7.38E-01
Tumor necrosis factor receptor superfamily member 13C	Q96RJ3	*TNFRSF13C*	0.25	2.70E-04	0.07	4.26E-01
Ubiquitin+1, truncated mutation for UbB	P62979	*RPS27A*	0.24	3.00E-04	0.02	8.05E-01
Malate dehydrogenase, cytoplasmic	P40925	*MDH1*	0.24	3.30E-04	0.02	8.47E-01
MAP kinase-activated protein kinase 2	P49137	*MAPKAPK2*	0.24	4.90E-04	0.11	2.07E-01
Drebrin-like protein	Q9UJU6	*DBNL*	0.24	5.20E-04	0.04	6.24E-01
Annexin A2	P07355	*ANXA2*	0.23	5.70E-04	-0.05	5.84E-01
Calpain I	P07384 P04632	*CAPN1 CAPNS*	0.23	5.90E-04	0.11	1.95E-01
Peroxiredoxin-6	P30041	*PRDX6*	0.23	6.20E-04	0.09	2.71E-01
Transketolase	P29401	*TKT*	0.23	6.40E-04	0.02	8.10E-01
Ribosome maturation protein SBDS	Q9Y3A5	*SBDS*	0.23	6.60E-04	0.14	1.04E-01
Dual specificity protein phosphatase 3	P51452	*DUSP3*	0.23	7.00E-04	0.08	3.68E-01
Hemoglobin	P69905, P68871	*HBA1 HBB*	0.23	8.50E-04	0.06	4.43E-01
Ubiquitin-conjugating enzyme E2 N	P61088	*UBE2N*	0.22	9.20E-04	0.03	7.13E-01
Alcohol dehydrogenase [NADP(+)]	P14550	*AKR1A1*	0.22	9.30E-04	-0.05	5.22E-01
Leukotriene A-4 hydrolase	P09960	*LTA4H*	0.22	9.40E-04	-0.32	<.0001
Growth/differentiation factor 9	O60383	*GDF9*	0.22	9.50E-04	0.02	8.28E-01
Histone acetyltransferase type B catalytic subunit	O14929	*HAT1*	0.22	9.80E-04	0.10	2.35E-01
Mitogen-activated protein kinase 13	O15264	*MAPK13*	0.22	1.10E-03	-0.05	5.20E-01
Rab GDP dissociation inhibitor beta	P50395	*GDI2*	0.22	1.10E-03	0.02	8.10E-01
Proto-oncogene tyrosine-protein kinase Src	P12931	*SRC*	0.22	1.20E-03	0.08	3.27E-01

			Joslin T1D Cohort		Joslin T2D Cohort	
Target Full Name	**UniProt ID**	***Gene Symbol***	r_s	***P*-value***	r_s	***P*-value***
Mesothelin	Q13421	*MSLN*	0.22	1.20E-03	0.00	1.00E+00
Catalase	P04040	*CAT*	0.22	1.30E-03	-0.01	9.52E-01
Triosephosphate isomerase	P60174	*TPI1*	0.22	1.30E-03	0.01	9.08E-01
Tumor necrosis factor receptor superfamily member 25	Q93038	*TNFRSF25*	0.22	1.30E-03	0.11	1.71E-01
Nucleoside diphosphate kinase B	P22392	*NME2*	0.22	1.40E-03	0.06	4.88E-01
Ferritin	P02794 P02792	*FTH1 FTL*	0.22	1.40E-03	0.11	2.06E-01
Proteasome activator complex subunit 1	Q06323	*PSME1*	0.22	1.50E-03	0.00	9.78E-01
Peptidyl-prolyl cis-trans isomerase A	P62937	*PPIA*	0.21	1.60E-03	0.09	2.63E-01
C-C motif chemokine 20	P78556	*CCL20*	0.21	1.70E-03	0.05	5.46E-01
Casein kinase II 2-alpha':2-beta heterotetram	P19784 P67870	*CSNK2A2 CSN*	0.21	1.70E-03	0.19	1.17E-01
N-acylethanolamine-hydrolyzing acid amidase	Q02083	*NAAA*	0.21	1.70E-03	0.07	3.92E-01
Inorganic pyrophosphatase	Q15181	*PPA1*	0.21	1.80E-03	0.04	6.29E-01
Bcl-2-related protein A1	Q16548	*BCL2A1*	0.21	1.90E-03	0.03	7.24E-01
Stress-induced-phosphoprotein 1	P31948	*STIP1*	0.21	2.10E-03	0.09	2.77E-01
Eukaryotic translation initiation factor 5A-1	P63241	*EIF5A*	0.21	2.20E-03	0.06	4.82E-01
Histone H2A.z	P0C0S5	*H2AFZ*	0.21	2.20E-03	-0.10	2.37E-01
cAMP-regulated phosphoprotein 19	P56211	*ARPP19*	0.21	2.50E-03	0.07	4.25E-01
cAMP-dependent protein kinase catalytic subunit alpha	P17612	*PRKACA*	0.21	2.50E-03	0.03	6.81E-01
Phosphatidylethanolamine-binding protein 1	P30086	*PEBP1*	0.21	2.60E-03	-0.02	7.77E-01
Cofilin-1	P23528	*CFL1*	0.21	2.60E-03	-0.06	4.78E-01
Alpha-soluble NSF attachment protein	P54920	*NAPA*	0.21	2.60E-03	0.14	8.46E-02
Calcium/calmodulin-dependent protein kinase kinase 1	Q8N5S9	*CAMKK1*	0.20	2.70E-03	0.11	1.81E-01
Ras-related C3 botulinum toxin substrate 1	P63000	*RAC1*	0.20	2.70E-03	0.15	8.25E-02
Ubiquitin	P62979	*RPS27A*	0.20	2.90E-03	-0.02	8.13E-01

			Joslin T1D Cohort		Joslin T2D Cohort	
Target Full Name	**UniProt ID**	***Gene Symbol***	r_s	***P*-value***	r_s	***P*-value***
Allograft inflammatory factor 1	P55008	*AIF1*	0.20	2.90E-03	-0.07	3.91E-01
Proliferation-associated protein 2G4	Q9UQ80	*PA2G4*	0.20	3.00E-03	0.07	3.73E-01
Insulin-degrading enzyme	P14735	*IDE*	0.20	3.00E-03	0.06	4.78E-01

*Threshold for the significance used in cohort with T1D: FDR adjusted *P*-value < 0.005 in the exploratory T1D cohort and a nominal *P-value* < 0.05 in the replication T2D cohort. Coefficients (r_s) are presented below and corresponding two-sided P values have been provided. Gene symbols indicated in **bold** were examined in the present study.

Table S3. Selection of potential covariates into the logistic regression model.

	Maximum Likelihood Estimates			
Potential covariates	**Estimate**	**Standard Error**	**Wald Chi-square**	***P*-value**
Age	-0.02	0.02	0.94	0.33
Gender	0.04	0.27	0.02	0.90
Ethnicity	0.96	0.74	1.68	0.20
Insulin Rx	-0.27	0.45	0.36	0.55
Renoprotection Rx	0.18	0.35	0.26	0.61
Duration of diabetes	-0.02	0.02	0.67	0.41
BMI	-0.01	0.02	0.33	0.56
Systolic BP	-0.001	0.01	0.01	0.93
Diastolic BP	0.01	0.02	0.16	0.69
HbA1c	0.24	0.09	6.85	**0.0089**
ACR	1.10	0.19	33.96	**<.0001**
eGFR	-0.05	0.01	15.11	**0.0001**

BMI, Body mass index; BP, Blood pressure; Rx, treatment; Renoprotection, Prescription of angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB); HbA1c, Hemoglobin A1c; ACR, Albumin-to-creatinine ratio; eGFR, Estimated glomerular filtration rate.

The criteria to retain a covariate in the final model were statistical significance at nominal $P < 0.05$ and by inspection of β estimates, such that a change of β of 20% or higher was considered non-negligible.

Table S4. Logistic regression models examining the association of 19 circulating plasma proteins and progressive renal decline in the combined Joslin cohorts with T1D and T2D.

	Model 1	**Model 2**
Gene symbol for proteins	**OR (95% CI)**	**OR (95% CI)**
ALB	0.71 (0.58, 0.87)	0.83 (0.66, 1.05)
TNFSF12	**0.61 (0.50, 0.75)**	**0.75 (0.59, 0.95)***
SPARC	**0.66 (0.54, 0.81)**	**0.75 (0.59, 0.95)***
AK1	0.72 (0.59, 0.87)	0.89 (0.70, 1.02)
PPBPIII	0.70 (0.57, 0.85)	0.83 (0.65, 1.04)
PPBP2	0.69 (0.56, 0.84)	0.81 (0.64, 1.02)
CCL5	**0.70 (0.58, 0.86)**	**0.75 (0.60, 0.95)***
THBS1	0.75 (0.62, 0.92)	0.88 (0.70, 1.11)
APP	**0.70 (0.58, 0.86)**	**0.78 (0.61, 0.98)***
PF4	**0.69 (0.56, 0.84)**	**0.78 (0.62, 0.99)***
FGF20	**0.66 (0.54, 0.81)**	**0.69 (0.54, 0.88)***
ANGPT1	**0.68 (0.56, 0.83)**	**0.72 (0.57, 0.91)***
DNAJC19	**0.68 (0.55, 0.83)**	**0.78 (0.62, 0.99)***
PLA2G10	0.63 (0.52, 0.78)	0.80 (0.63, 1.01)
PPID	0.70 (0.58, 0.86)	0.88 (0.70, 1.10)
SERPINE1	0.75 (0.62, 0.92)	0.92 (0.73, 1.17)
RAN	0.73 (0.60, 0.88)	0.90 (0.71, 1.15)
PRDX1	0.79 (0.65, 0.96)	1.03 (0.82, 1.30)
PKM2	0.74 (0.61, 0.90)	0.91 (0.72, 1.15)

The effect is shown as an odds ratio (95% CI) per one quartile increase in circulating concentration of the relevant protein. Model 1: Unadjusted; Model 2: Adjusted for baseline eGFR, HbA1c and ACR. All models were adjusted by type of diabetes. *Proteins in **bold** are significant (P<0.05) in both models.

Table S5. Ranking of proteins/clinical covariates for elimination from the multivariable logistic regression analysis using backward elimination procedure. Proteins with $\alpha > 0.1$ were eliminated from the final logistic regression model.

	Summary of backward elimination	
Proteins/Clinical covariates	**Wald Chi-Square**	***P*-value**
Eliminated proteins		
PF4	0.073	0.79
SPARC	0.18	0.67
CCL5	0.58	0.45
APP	0.57	0.45
DNAJC19	0.76	0.39
Selected proteins/covariates in the final model		
eGFR	5.21	*0.022*
HbA1c	8.43	*0.0037*
ACR	35.81	*<.0001*
TNFSF12	2.84	*0.092*
ANGPT1	5.73	*0.017*
FGF20	6.48	*0.011*

Table S6. Demographics and clinical characteristics of an independent validation cohort of T1D individuals with normal renal function.

	Validation Cohort
Characteristics	**Joslin T1D CKD12 Cohort (*N* = 294)**
At baseline	
Male (%)	55
Age (years)	38 (32, 45)
Duration of diabetes (years)	25 (17, 32)
HbA1c (%)	8.8 (7.9, 9.8)
eGFR (ml/min/1.73m^2)	100 (82, 114)
ACR (μg/mg creatinine)	491 (112, 1099)
During follow-up	
eGFR slope (ml/min/1.73m^2/year)	-2.6 (-7.1, -1.1)
*Non-progressors** (%)	53
*Progressors** (%)	47
New cases of ESRD within 10 years follow-up (%)	19

T1D, Type 1 diabetes; CKD, Chronic Kidney Disease; HbA1c, Hemoglobin A1c; eGFR, Estimated glomerular filtration rate; ACR, Albumin-to-creatinine ratio; ESRD, End-stage renal disease. Non-progressors were defined as eGFR loss < 3.0 ml/min/1.73m^2/year and progressors as eGFR loss ≥ 3.0 ml/min/1.73m^2/year. Data presented as median (25th, 75th percentile) or count (proportion) measures.

Table S7. Logistic regression models comparing the protective effect of ANGPT1, the risk effect of ANGPT2 and the effect of ANGPT1/ANGPT2 ratio on the risk of progressive renal decline in the combined Joslin cohorts.

	Model 1	**Model 2**
Protein	**OR (95% CI)**	**OR (95% CI)**
ANGPT1	0.68 (0.56, 0.83)	0.72 (0.57, 0.91)
ANGPT2	1.48 (1.21, 1.81)	1.19 (0.95, 1.51)
ANGPT1/ANGPT2 Ratio	0.68 (0.55, 0.82)	0.79 (0.63, 1.01)

ANGPT1, Angiopoietin-1; ANGPT2, Angiopoietin-2. Model 1: Unadjusted; Model 2: Adjusted for baseline eGFR, HbA1c and ACR. All models were adjusted by type of diabetes.

Table S8. Circulating plasma concentrations of top 3 protective proteins in non-diabetic parents of two categories of T1D probands.

Characteristics	Normoalbuminuria (*N* = 40)	Proteinuria or ESRD (*N* = 39)
At baseline		
Male, n (%)	50%	51%
Age, years	61 ± 6	62 ± 5
eGFR (ml/min/1.73m^2)	75 ± 13	71 ± 13
HbA1c (%)	5.4 ± 0.3	5.4 ± 0.4
ACR (µg/mg creatinine)	5.9 ± 3.4	9.4 ± 13.5
Baseline plasma concentrations (RFU)		
ANGPT1	771 (577, 1185)	746 (658, 1203)
TNFSF12	266 (242, 283)	273 (240, 303)
FGF20	392 (351, 449)	337 (298, 383)**

ESRD, end-stage renal disease; HbA1c, hemoglobin A1c; ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; RFU, relative fluorescent unit.

Data presented as mean ± standard deviation, median (25th, 75th percentile) or count (proportion) measures. Differences between the two groups were tested using the Wilcoxon-rank-sum test for continuous variables. ***P* < 0.01.