Supplementary material

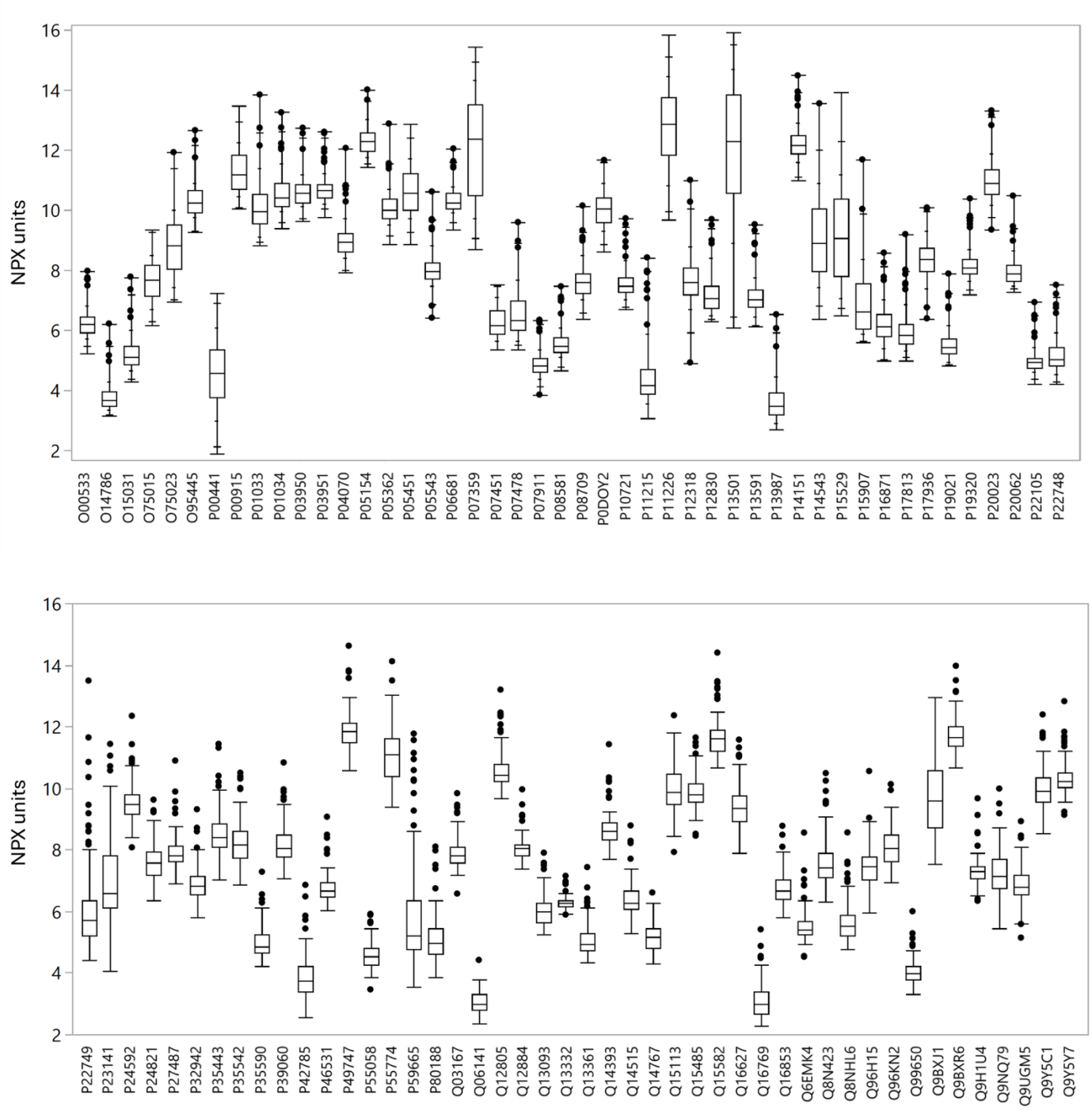


Figure S1: Box plot with the distribution of Normalized Protein eXpression (NPX) in the overall samples. Data are expressed as Olink's arbitrary unit in Log_2 scale. Please see **Table S3** for values in each group.

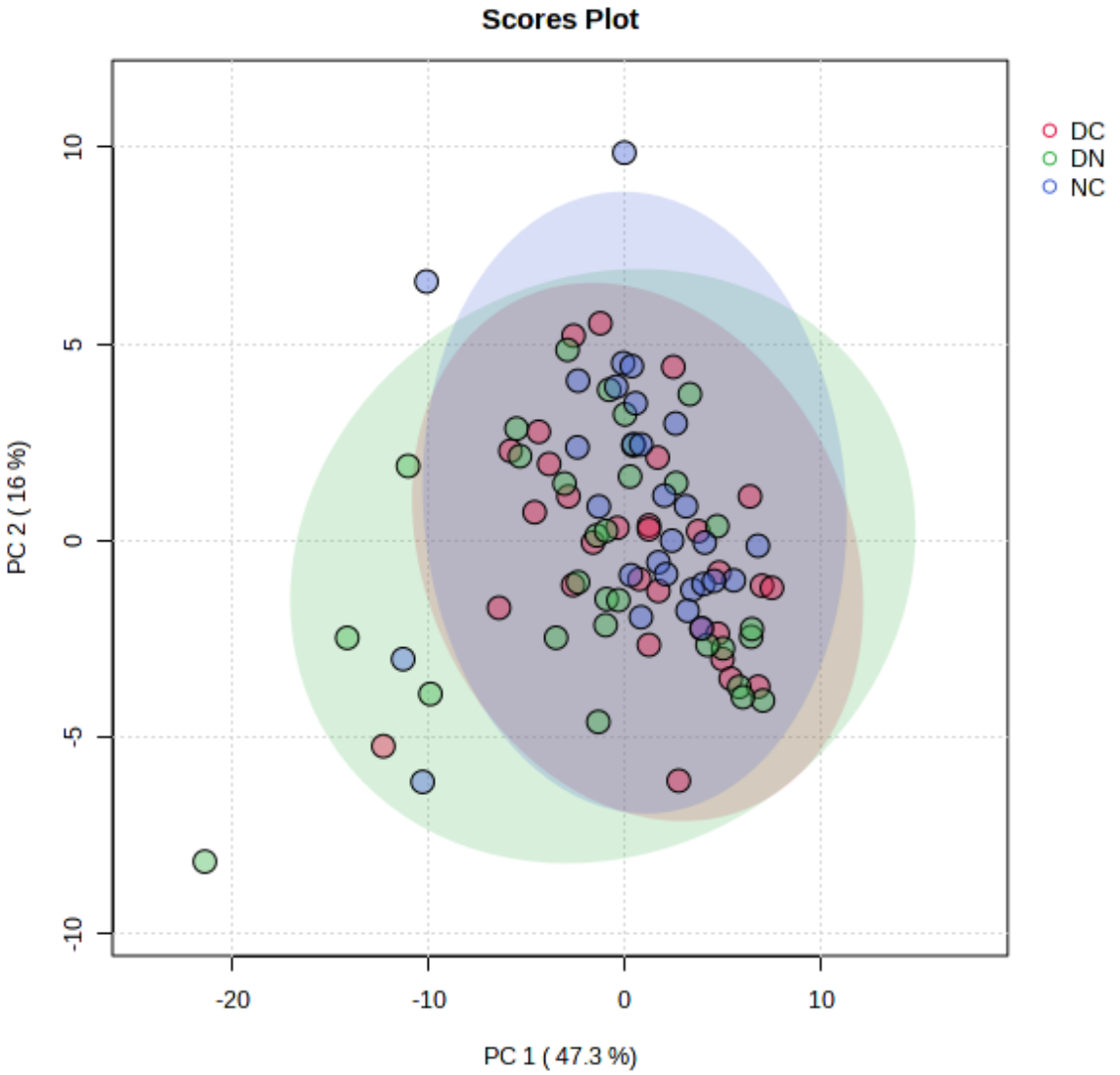


Figure S2: PCA analysis using the 92 measured proteins, indicating no specific distinction between the three patients' groups.

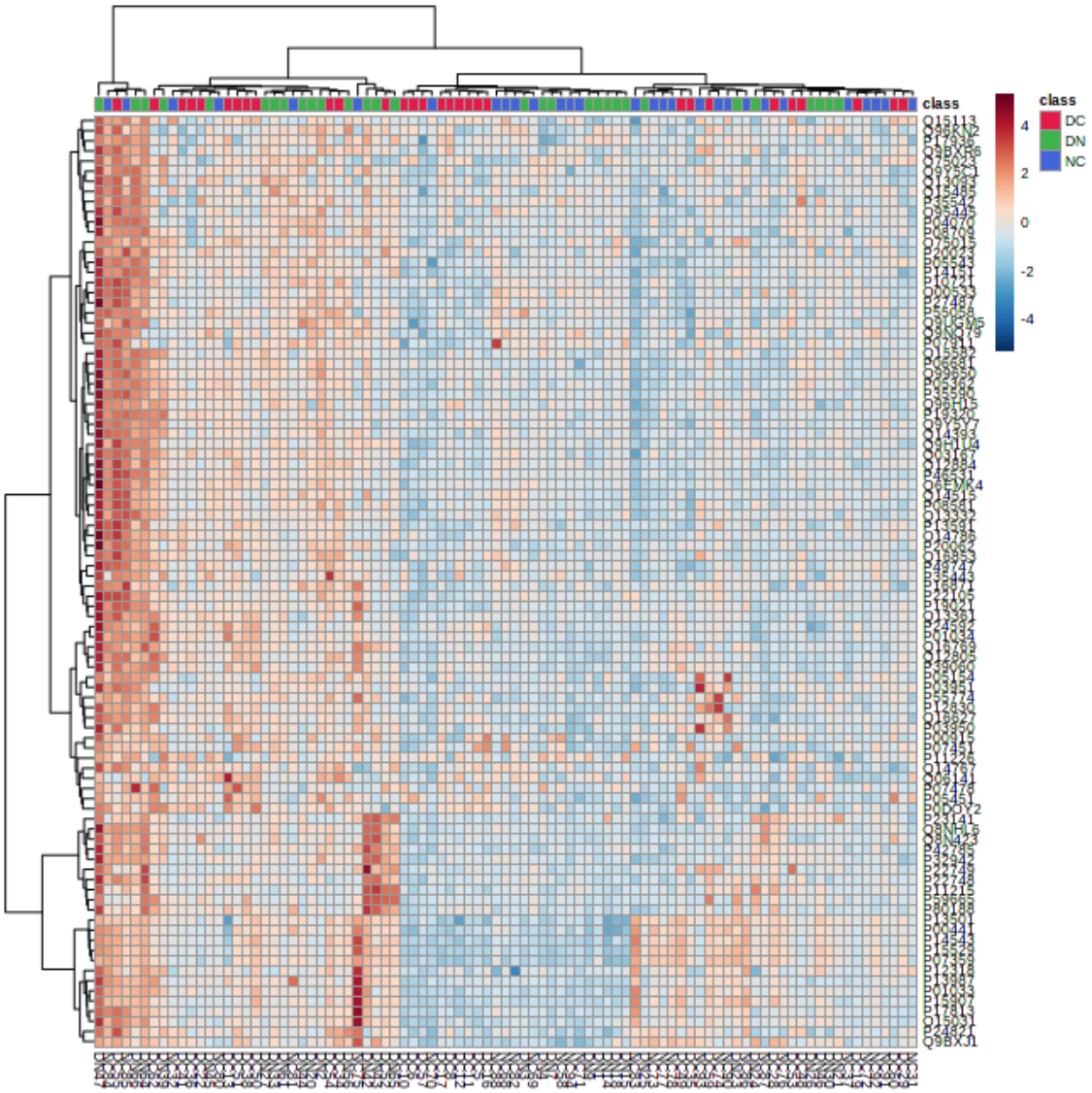
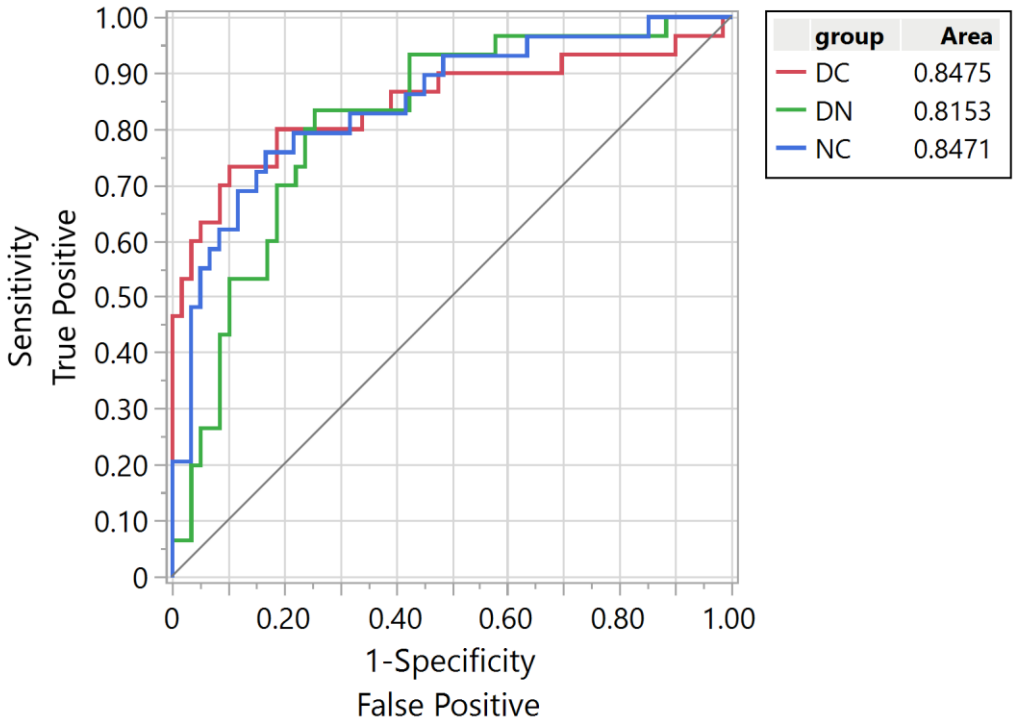


Figure S3: Heatmap from cluster analysis using the 92 measured proteins, indicating no specific distinction between the three patients' groups.

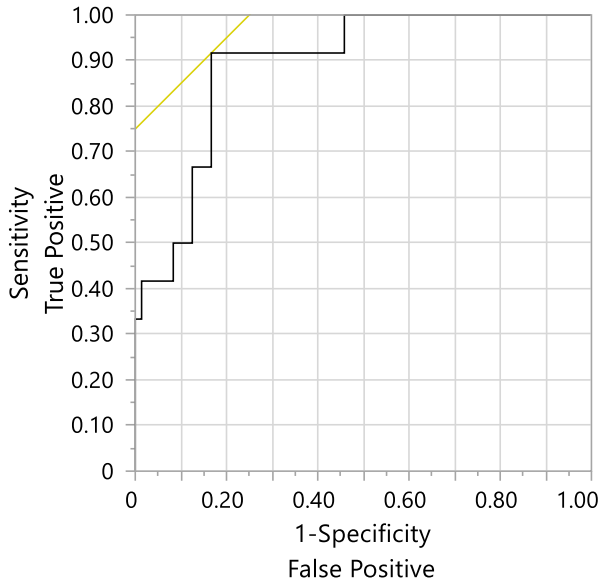


Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	26.438320	12	52.87664	<.0001*
Full	71.326895			
Reduced	97.765215			

Source	Logworth	*P* Value
P05451	1.857	0.01389
P23141	1.340	0.04568
P0DOY2	1.224	0.05964
P32942	1.044	0.09040
P06681	0.529	0.29612
P42785	0.056	0.87955

Figure S4: ROC analysis obtained with nominal logistic plot using the six significant proteins for distinguishing among the three groups. The table below the graph indicates the effect summary with logworth and *p* value for each protein contribution.



Nominal Logistic Fit for eGFR<60 ml/min - Effect Summary. AUC= 0.89 (whole model test: *p*=0.0003)

Source	Logworth	*P* Value
P0DOY2 (IGLC2)	3.286	0.00052
P42785 (PRCP)	1.337	0.04598
P05451 (REG1A)	0.366	0.43077
P23141 (CES1)	0.175	0.66758
P32942 (ICAM3)	0.096	0.80158
P06681 (C2)	0.001	0.99704

Figure S5: ROC analysis obtained with nominal logistic plot using the six significant proteins on prediction of eGFR impairment (eGFR<60 ml/min), considering all the subjects together. The table below the graph indicates the effect summary with logworth and *p* value for each protein contribution.

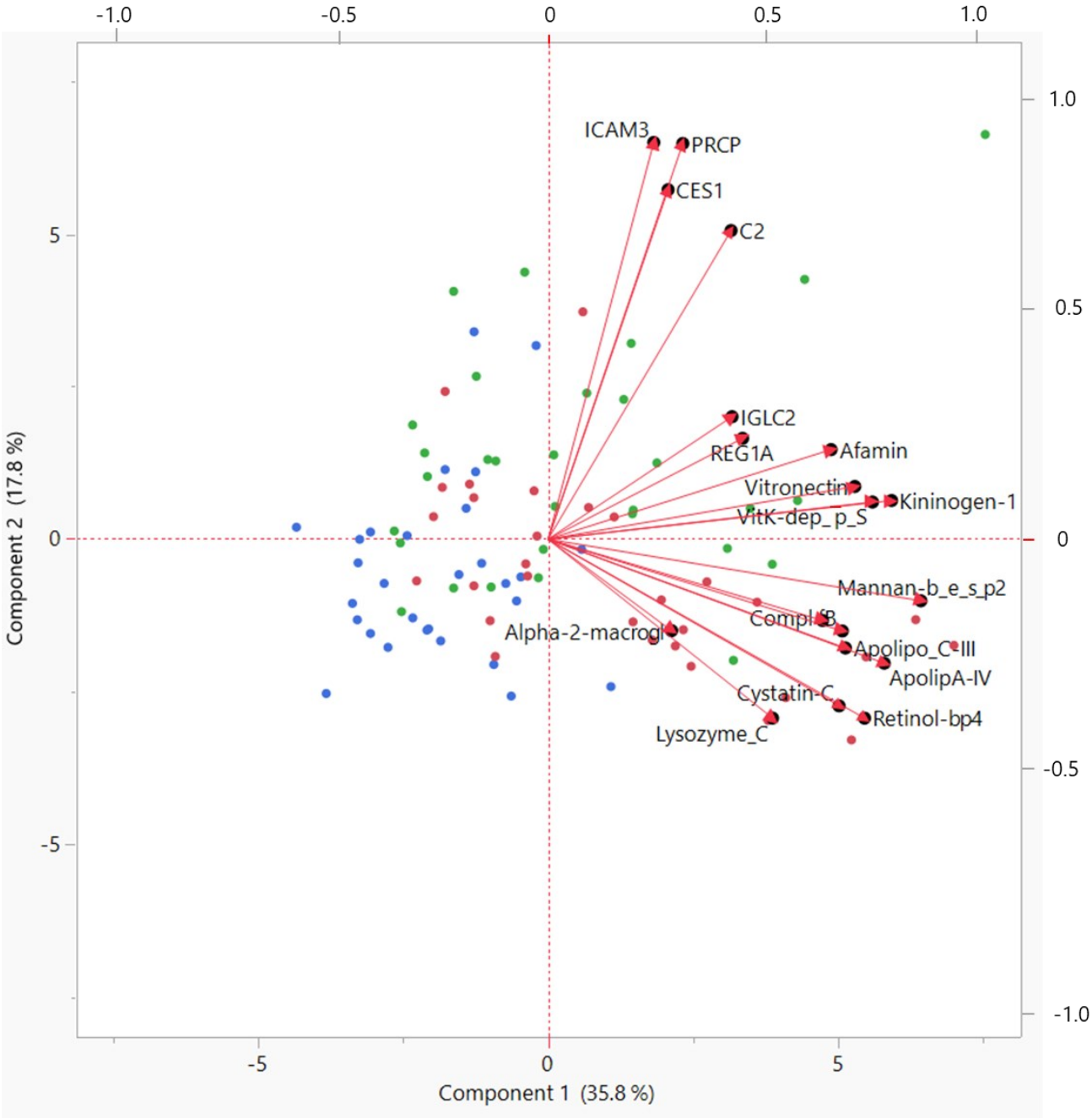


Figure S6. Biplot from PCA analysis on the 6 identified significant proteins together the 11 identified proteins found in a previous investigation on the same subjects using Multiplexed MRM-based proteomics [9]. The left and bottom axes show principal component scores; the top and right axes indicate the loadings. Red dots: DC group; green: DN group; blue: NC group.

Table S1. List of proteins analyzed using proximity extension assay (PEA).

Protein	Acronym	Uniprot ID
Angiogenin	ANG	P03950
Angiopoietin-related protein 3	ANGPTL3	Q9Y5C1
Alipoprotein M	APOM	O95445
Beta-Ala-His dipeptidase	CNDP1	Q96KN2
Beta-galactoside alpha-2,6-sialyltransferase 1	ST6GAL1	P15907
Cadherin-1	CDH1	P12830
Carbonic anhydrase 1	CA1	P00915
Carbonic anhydrase 3	CA3	P07451
Carbonic anhydrase 4	CA4	P22748
Cartilage acidic protein 1	CRTAC1	Q9NQ79
Cartilage oligomeric matrix protein	COMP	P49747
C-C motif chemokine 5	CCL5	P13501
C-C motif chemokine 14	CCL14	Q16627
C-C motif chemokine 18	CCL18	P55774
CD59 glycoprotein	CD59	P13987
Coagulation factor VII	F7	P08709
Coagulation factor XI	F11	P03951
Collagen alpha-1 (XVIII) chain	COL18A1	P39060
Complement C1q tumor necrosis factor-related protein 1	C1QTNF1	Q9BXJ1
Complement C2	C2	P06681
Complement factor H-related protein 5	CFHR5	Q9BXR6
Complement receptor type 2	CR2	P20023
Cystatin-C	CST3	P01034
Dipeptidyl peptidase 4	DPP4	P27487
EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	Q12805
Endoglin	ENG	P17813
Fetuin-B	FETUB	Q9UGM5
Ficolin-2	FCN2	Q15485
Glutaminyl-peptide cyclotransferase	QPCT	Q16769
Granulysin	GNLY	P22749
Growth arrest-specific protein 6	GAS6	Q14393
Hepatocyte growth factor receptor	MET	P08581
Ig lambda-2 chain C regions	IGLC2	P0CG05
Insulin-like growth factor-binding protein 3	IGFBP3	P17936

Protein	Acronym	Uniprot ID
Insulin-like growth factor-binding protein 6	IGFBP6	P24592
Integrin alpha-M	ITGAM	P11215
Intercellular adhesion molecule 1	ICAM1	P05362
Intercellular adhesion molecule 3	ICAM3	P32942
Interleukin-7 receptor subunit alpha	IL7R	P16871
Latent-transforming growth factor beta-binding protein 2	LTBP2	Q14767
Leukocyte immunoglobulin-like receptor subfamily B member 1	LILRB1	Q8NHL6
Leukocyte immunoglobulin-like receptor subfamily B member 2	LILRB2	Q8N423
Leukocyte immunoglobulin-like receptor subfamily B member 5	LILRB5	O75023
Lithostatine-1-alpha	REG1A	P05451
Liver carboxylesterase 1	CES1	P23141
Low affinity immunoglobulin gamma Fc region receptor II-a	FCGR2A	P12318
Low affinity immunoglobulin gamma Fc region receptor III-B	FCGR3B	O75015
L-selectin	SELL	P14151
Lymphatic vessel endothelial hyaluronic acid receptor 1	LYVE1	Q9Y5Y7
Lysosomal Pro-X carboxypeptidase	PRCP	P42785
Mannose-binding protein C	MBL2	P11226
Mast/stem cell growth factor receptor Kit	KIT	P10721
Membrane cofactor protein	CD46	P15529
Membrane primary amine oxidase	AOC3	Q16853
Metalloproteinase inhibitor 1	TIMP1	P01033
Microfibrillar-associated protein 5	MFAP5	Q13361
Multiple epidermal growth factor-like domains protein 9	MEGF9	Q9H1U4
Neural cell adhesion molecule 1	NCAM1	P13591
Neural cell adhesion molecule L1-like protein	CHL1	O00533
Neurogenic locus notch homolog protein 1	NOTCH1	P46531
Neuropilin-1	NRP1	O14786
Neutrophil defensin 1	DEFA1	P59665
Neutrophil gelatinase-associated lipocalin	LCN2	P80188
Nidogen-1	NID1	P14543
Oncostatin-M-specific receptor subunit beta	OSMR	Q99650
Peptidyl-glycine alpha-amidating monooxygenase	PAM	P19021
Phospholipid transfer protein	PLTP	P55058
Plasma serine protease inhibitor	SERPINA5	P05154
Platelet glycoprotein Ib alpha chain	GP1BA	P07359

Protein	Acronym	Uniprot ID
Platelet-activating factor acetylhidrolase	PLA2G7	Q13093
Plexin-B2	PLXNB2	O15031
Procollagen C-endopeptidase enhancer 1	PCOLCE	Q15113
Prolyl endopeptidase FAP	FAP	Q12884
Receptor-type tyrosine-protein phosphatase S	PTPRS	Q13332
Regenerating islet-derived protein 3-alpha	REG3A	Q06141
Serum amyloid A-4 protein	SAA4	P35542
SPARC-like protein 1	SPARCL1	Q14515
Superoxide dismutase [Cu-Zn]	SOD1	P00441
T-cell immunoglobulin and mucin domain-containing protein 4	TIMD4	Q96H15
Tenascin	TNC	P24821
Tenascin-X	TNXB	P22105
Thrombospondin-4	THBS4	P35443
Thyroxine-binding globulin	SERPINA7	P05543
Transcobalamin-2	TCN2	P20062
Transforming growth factor beta receptor type 3	TGFBR3	Q03167
Transforming growth factor-beta-induced protein ig-h3	TGFBI	Q15582
Trypsin-2	PRSS2	P07478
Tyrosine-protein kinase receptor Tie-1	TIEI1	P35590
Uromodulin	UMOD	P07911
Vascular cell adhesion protein 1	VCAM1	P19320
Vasorin	VASN	Q6EMK4
Vitamin K-dependent protein C	PROC	P04070

Table S2. Clinical and metabolic parameters of subjects in study.

	DC (n=30)	**DN (n=30)**	**NC (n=29)**
Gender (M/F)	27/3	22/8	21/8
Age (y)	65.8±8.7**	60.3±5.8†	65.5±6.1
Diabetes duration (y)	10.9±7.6***	1.9±0.9	---
BMI (kg/m^2)	29.5±3.9	29.4±3.3†	26.7±5.2‡
FPG (mg/dl)	143.2±43.0	144.9±36.7†††	94.1±12.7‡‡‡
HbA1c (%)	7.1±0.8	6.8±0.8†††	5.8±0.2‡‡‡
Total cholesterol (mg/dl)	152±36***	191±36††	155±41
LDL cholesterol (mg/dl)	82±32**	112±40††	84±31
HDL cholesterol (mg/dl)	42±10	48±9	53±16‡‡‡
Triglycerides (mg/dl)	167±80	139±73††	87±32‡‡‡

Data are expressed as mean ±SD.
For each continuous variable, symbols indicate significant differences between means by the Tukey's HSD test. ***p<0.001, **p<0.01, DC *vs* DN; $^{\dagger\dagger\dagger}p$<0.001, $^{\dagger\dagger}p$<0.01, $^{\dagger}p$<0.05, DN *vs* NC; $^{\ddagger\ddagger\ddagger}p$<0.001, $^{\ddagger\ddagger}p$<0.01, $^{\ddagger}p$<0.05, DC *vs* NC.

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein.

Full information regarding patients' cohort has been previously published in Piarulli et al. (2022). Methods used for metabolic parameters determination are described in the mentioned paper. [Piarulli F, Banfi C, Brioschi M, Altomare A, Ragazzi E, Cosma C, Sartore G, Lapolla A. The Burden of Impaired Serum Albumin Antioxidant Properties and Glyco-Oxidation in Coronary Heart Disease Patients with and without Type 2 Diabetes Mellitus. *Antioxidants (Basel),* 2022;11(8):1501. doi: 10.3390/antiox11081501].

Table S3: Values of Normalized Protein eXpression (NPX) in the three groups of patients. Data are expressed as Olink's arbitrary unit in Log_2 scale.

Protein	NPX units (mean+ SD)		
	DC group	DN group	NC group
O00533	6.09±0.44	6.32±0.49	6.27±0.5
O14786	3.8±0.47	3.82±0.57	3.75±0.43
O15031	5.14±0.5	5.31±0.57	5.19±0.67
O75015	7.46±0.73	7.87±0.7	7.63±0.63
O75023	8.82±0.97	9.06±1.12	8.57±0.99
O95445	10.23±0.53	10.56±0.67	10.22±0.54
P00441	4.29±1.02	4.45±1.21	4.88±1.08
P00915	11.3±0.78	11.38±0.79	11.11±0.63
P01033	10.06±0.77	10.12±0.91	10.15±1.03
P01034	10.81±0.64	10.56±0.76	10.36±0.56
P03950	10.71±0.42	10.56±0.59	10.56±0.6
P03951	10.64±0.36	10.77±0.5	10.72±0.59
P04070	8.92±0.49	9.24±0.8	8.92±0.57
P05154	12.27±0.34	12.42±0.47	12.24±0.61
P05362	10.07±0.46	10.22±0.72	9.91±0.53
P05451	11.08±0.76	10.66±0.74	10.17±0.61
P05543	7.93±0.49	8.27±0.69	7.87±0.61
P06681	10.39±0.32	10.49±0.48	10.1±0.42
P07359	11.8±1.79	12.16±1.78	12.38±1.66
P07451	6.35±0.59	6.25±0.56	6.25±0.51
P07478	6.89±0.86	6.53±0.82	6.24±0.54
P07911	4.67±0.44	4.98±0.33	4.93±0.45
P08581	5.46±0.48	5.66±0.48	5.54±0.41
P08709	7.6±0.48	7.91±0.73	7.41±0.55
P0DOY2	10.37±0.63	10.03±0.57	9.76±0.58
P10721	7.41±0.55	7.75±0.58	7.61±0.55
P11215	4.31±0.91	4.86±1.56	4.25±0.49
P11226	12.6±1.28	13.03±1.41	12.78±1.23
P12318	7.64±0.63	7.79±0.84	7.64±1.13
P12830	7.32±0.66	7.21±0.67	7.08±0.78
P13501	11.59±2.5	12.06±2.52	12.52±1.63
P13591	7.06±0.62	7.11±0.63	7.18±0.61
P13987	3.56±0.57	3.58±0.74	3.65±0.82
P14151	12.01±0.46	12.47±0.62	12.2±0.6
P14543	8.83±1.3	8.98±1.28	9.15±1.47
P15529	8.78±1.44	9.11±1.59	9.39±1.65
P15907	6.77±0.91	7±1.05	7.07±1.32
P16871	6.06±0.65	6.28±0.63	6.24±0.72
P17813	5.83±0.6	6.02±0.62	6.11±0.85
P17936	8.15±0.67	8.6±0.76	8.28±0.62
P19021	5.48±0.5	5.55±0.64	5.57±0.55
P19320	8.2±0.5	8.28±0.7	8.1±0.52
P20023	10.87±0.62	11.23±0.73	10.84±0.73

Protein	NPX units (mean+ SD)		
	DC group	DN group	NC group
P20062	8±0.46	8.02±0.6	7.91±0.43
P22105	4.86±0.41	5.09±0.49	5.04±0.44
P22748	5.1±0.54	5.36±0.8	5.02±0.39
P22749	6.19±1.33	6.52±2.17	5.97±1.18
P23141	6.88±1.03	7.93±1.65	6.36±1.23
P24592	9.73±0.61	9.47±0.78	9.45±0.49
P24821	7.66±0.63	7.52±0.66	7.6±0.62
P27487	7.73±0.51	8.17±0.66	7.91±0.56
P32942	6.84±0.47	7.24±0.72	6.72±0.45
P35443	8.52±0.92	8.69±0.85	8.44±0.57
P35542	8.24±0.82	8.46±0.71	8.04±0.74
P35590	4.94±0.4	5.14±0.58	4.9±0.5
P39060	8.39±0.66	8.19±0.73	8.02±0.58
P42785	3.81±0.53	4.29±0.93	3.57±0.63
P46531	6.74±0.44	6.91±0.58	6.71±0.44
P49747	11.78±0.68	11.91±0.71	11.95±0.62
P55058	4.5±0.47	4.67±0.47	4.58±0.44
P55774	11.19±0.79	11.2±0.75	10.97±1.11
P59665	5.98±1.81	6.44±2.43	5.16±0.68
P80188	5.17±0.62	5.37±1.12	4.86±0.52
Q03167	7.88±0.5	7.96±0.57	7.86±0.55
Q06141	3.1±0.43	3.02±0.3	2.96±0.31
Q12805	10.74±0.65	10.55±0.7	10.55±0.63
Q12884	8.01±0.42	8.17±0.46	8.03±0.32
Q13093	5.96±0.52	6.27±0.57	5.92±0.43
Q13332	6.26±0.2	6.31±0.25	6.27±0.2
Q13361	5.11±0.54	5.12±0.63	5.03±0.47
Q14393	8.61±0.49	8.8±0.7	8.69±0.55
Q14515	6.3±0.53	6.56±0.63	6.35±0.55
Q14767	5.15±0.44	5.18±0.45	5.15±0.44
Q15113	10.1±0.68	9.72±0.87	10.1±0.76
Q15485	9.77±0.52	9.99±0.65	9.81±0.54
Q15582	11.63±0.57	11.97±0.73	11.49±0.55
Q16627	9.45±0.68	9.39±0.69	9.38±0.83
Q16769	3.16±0.48	3.25±0.66	2.95±0.57
Q16853	6.87±0.58	6.82±0.57	6.68±0.54
Q6EMK4	5.52±0.49	5.65±0.71	5.44±0.48
Q8N423	7.68±0.8	7.94±1.11	7.43±0.67
Q8NHL6	5.58±0.49	5.89±0.86	5.54±0.57
Q96H15	7.42±0.6	7.64±0.89	7.46±0.55
Q96KN2	8.01±0.6	8.36±0.69	7.88±0.49
Q99650	4.02±0.32	4.19±0.48	3.95±0.39
Q9BXJ1	9.68±1	9.8±0.97	9.54±1.28
Q9BXR6	11.73±0.6	11.83±0.68	11.71±0.53
Q9H1U4	7.25±0.5	7.52±0.59	7.22±0.41
Q9NQ79	6.91±0.72	7.38±0.82	7.52±0.77

Protein	NPX units (mean+ SD)		
	DC group	DN group	NC group
Q9UGM5	6.7±0.63	7.11±0.74	6.82±0.59
Q9Y5C1	9.83±0.6	10.24±0.76	9.89±0.63
Q9Y5Y7	10.33±0.5	10.41±0.66	10.23±0.52

Table S4: Linear correlation between the six identified significant proteins with the *p* values for statistical significance of the correlations. See also Figure 3 in the manuscript. *P* values highlighted in red color and marked with an asterisk indicate significant correlations.

Variable	by Variable	Correlation *r*	Count	Lower 95%	Upper 95%	*P*
P06681 (C2)	P05451 (REG1A)	0.5403	89	0.3741	0.6728	<.0001*
P0DOY2 (IGLC2)	P05451 (REG1A)	0.5456	89	0.3806	0.6770	<.0001*
P0DOY2 (IGLC2)	P06681 (C2)	0.6099	89	0.4601	0.7259	<.0001*
P23141 (CES1)	P05451 (REG1A)	0.1540	89	-0.0560	0.3510	0.1495
P23141 (CES1)	P06681 (C2)	0.3786	89	0.1849	0.5439	0.0003*
P23141 (CES1)	P0DOY2 (IGLC2)	0.1111	89	-0.0994	0.3121	0.2999
P32942 (ICAM3)	P05451 (REG1A)	0.3571	89	0.1608	0.5262	0.0006*
P32942 (ICAM3)	P06681 (C2)	0.6750	89	0.5430	0.7744	<.0001*
P32942 (ICAM3)	P0DOY2 (IGLC2)	0.3739	89	0.1796	0.5401	0.0003*
P32942 (ICAM3)	P23141 (CES1)	0.7123	89	0.5918	0.8016	<.0001*
P42785 (PRCP)	P05451 (REG1A)	0.2746	89	0.0704	0.4568	0.0092*
P42785 (PRCP)	P06681 (C2)	0.6224	89	0.4759	0.7354	<.0001*
P42785 (PRCP)	P0DOY2 (IGLC2)	0.3296	89	0.1303	0.5033	0.0016*
P42785 (PRCP)	P23141 (CES1)	0.7906	89	0.6971	0.8577	<.0001*
P42785 (PRCP)	P32942 (ICAM3)	0.8267	89	0.7470	0.8829	<.0001*

Table S5. Parameters obtained with ROC analysis with each of the six identified proteins to distinguish between the three groups of patients.

NC vs DN						
Parameter	**Protein**					
	P0DOY2 (IGLC2)	**P05451 (REG1A)**	**P32942 (ICAM3)**	**P06681 (C2)**	**P42785 (PRCP)**	**P23141 (CES1)**
AUC	0.6391	0.7000	0.7494	0.7747	0.7655	0.7897
P†	0.0311	0.0055	0.0008	0.0007	0.0005	<0.0001
Cut-off	9.79	10.32	7.04	10.23	3.65	7.40
Sensitivity, %	66	69	86	83	72	86
Specificity, %	63	67	60	63	83	60
PPV, %	63	67	68	69	81	68
NPV, %	66	69	82	79	76	82
FDR, %	37	33	32	31	19	32

DC vs DN						
Parameter	**Protein**					
	P0DOY2 (IGLC2)	**P05451 (REG1A)**	**P32942 (ICAM3)**	**P06681 (C2)**	**P42785 (PRCP)**	**P23141 (CES1)**
AUC	0.7022	0.6644	0.6789	0.5378	0.6700	0.6967
P†	0.0289	0.0340	0.0109	0.3370	0.0127	0.0036
Cut-off	10.28	10.66	7.14	10.73	3.65	6.95
Sensitivity, %	80	60	50	27	83	70
Specificity, %	67	70	83	97	53	63
PPV, %	71	67	75	89	64	66
NPV, %	77	64	63	57	76	68
FDR, %	29	33	25	11	36	34

DC vs NC						
Parameter	**Protein**					
	P0DOY2 (IGLC2)	**P05451 (REG1A)**	**P32942 (ICAM3)**	**P06681 (C2)**	**P42785 (PRCP)**	**P23141 (CES1)**
AUC	0.7885	0.8310	0.6012	0.7908	0.6460	0.6437
P†	0.0002	<0.0001	0.2890	0.0022	0.1101	0.0764
Cut-off	10.24	10.43	6.94	10.17	3.41	6.03
Sensitivity, %	86	72	79	72	52	48
Specificity, %	70	87	43	83	80	83
PPV, %	74	84	58	81	71	74
NPV, %	84	76	68	76	63	63
FDR, %	26	16	43	19	29	26

† Model significance, chi-square test.

Table S6. Performance of ROC analysis on prediction of eGFR impairment (eGFR<60 ml/min) considering all the subjects together. Results are presented for the model including all six proteins, and separately for the three proteins resulted as main contributors in the overall logistic regression.

Parameter	**All 6 proteins**	**P0DOY2 (IGLC2)**	**P42785 (PRCP)**	**P05451 (REG1A)**
AUC	0.89	0.81	0.58	0.72
$P^{\dagger}$	0.0003	0.0006	0.1697	0.0074
Sensitivity, %	92	83	100	92
Specificity, %	83	82	24	50
PPV, %	48	43	18	23
NPV, %	98	97	100	97
FDR, %	52	57	82	77
Probability threshold	0.17	0.17	0.10	0.10
Cut-off for NPX value	--	10.39	4.36	10.51

† Model significance, chi-square test.

Table S7. Correlation of protein P42785 (PRCP) with BMI and glycemia (FPG) in the three groups of subjects. *P* value highlighted in red color and marked with an asterisk indicates significant correlation.

Group	Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
DC	P42785	FPG	0.0488	30	-0.3171	0.4020	0.7981
DC	P42785	BMI	-0.0751	30	-0.4239	0.2931	0.6934
DN	P42785	FPG	-0.0560	30	-0.4081	0.3105	0.7687
DN	P42785	BMI	-0.3957	30	-0.6617	-0.0414	0.0304*
NC	P42785	FPG	-0.1300	29	-0.4739	0.2484	0.5016
NC	P42785	BMI	0.2825	29	-0.0937	0.5881	0.1376