Supplementary material

The updated German Diabetes Risk Score

The updated German Diabetes Risk Score (GDRS) is a risk score that was trained in 21845 participants of the prospective EPIC-Potsdam study with a mean follow-up time of 7 years (1). It was validated in 3625 participants of the German National Health Interview and Examination Survey 1998 (baseline) with follow up data in the German Health Interview and Examination Survey for Adults (follow-up) (2).

The score is based on clinical phenotypes and aims to identify those at risk of developing type 2 diabetes in the German population. It is built by assigning weights to each variable, which are derived from the regression model used to develop the score (1; 2).

Physical activity definition in both cohorts

In KORA, physical activity was assessed using a questionnaire with about weekly exercise frequency and duration in summer and winter (3). Potential answers were (1) > 2 hours, (2) 1–2 hours, (3) <1 hour and (4) none. Participants with a total score < 5, obtained by summing the numbers (1)–(4) relating to winter and summer, we considered to be 'physically active'. Those who walked at least 30 minutes a day were also considered 'physically active'. In HUNT, we applied the same definition to maintain consistency.

Validation of the SOMAscan protein measurements

We checked the performance of the SOMAscan platform in KORA by examining the correlation between SOMAscan measured biomarkers and their overlapping counterparts measured using other techniques.

These proteins were leptin measured in plasma by ELISA from Mercodia (Stockholm, Sweden), C-reactive protein (CRP), cystatin-C measured in plasma by a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer from Dade Behring (Erlangen, Germany) and 7 proteins measured by selected reaction monitoring mass spectrometry (SRM-MS).

We used Pearson correlation to test the concordance between proteins measured with SOMAscan and other techniques in KORA (Supplementary Figure S10, S11). CRP, leptin and cystatin-C showed good correlation, with their r ranging between 0.94 for CRP and 0.75 for cystatin-C. Proteins measured by SRM-MS showed good correlation for CRP, Mannose-binding protein C (MBL2), Thrombospondin-1 (THBS1), SHBG and adiponectin (ADIPOQ) with r ranging from 0.69 to 0.85 while RBP4 and CD5L showed lower r of 0.31 and 0.55 respectively.

Furthermore, we checked the validity of the replicated proteins by examining the data from Emilsson V. et al, where the authors validated the SOMAscan measurements using mass spectrometry (4). We found information on the validation for 22 of the unique 26 replicated proteins (Supplementary Table S10).

STRING protein-protein interaction network analysis

We queried STRING (5), the protein-protein interaction server, to visually assess the relationship between these candidate proteins and other proteins connected to type 2 diabetes. We used our replicated proteins and the type 2 diabetes associated proteins curated from UniProt as seed proteins and specified that no additional proteins (interactors) should be added by STRING to the network.

Among the type 2 diabetes associated proteins in UniProt (6) (Supplementary table S5; Protein origin: UniProtKB), 19 out of 23 proteins form a complex network with 17 of our 26 replicated proteins in STRING (Supplementary Figure S12).

Table S1: Baseline characteristics of the incident cohorts with those with type 2 diabetes at baseline excluded.

Variable	KORA (n=881)	HUNT (n=794)	p value*
Age † (years)	58.63 (43 - 75)	68.83 (31.6 - 99.4)	<0.001
Sex female ‡	464 (52.7 %)	199 (25.1 %)	<0.001
BMI § (kg/m2)	27.41 (4.41)	28.04 (3.69)	0.002
Waist circumference § (cm)	93.31 (13.55)	98.94 (10.49)	<0.001
Physical inactivity ‡	560 (63.6 %)	407 (52.2 %)	<0.001
Smoking ‡ Never smoker Former smoker Current smoker	382 (43.4 %) 367 (41.7 %) 132 (15.0 %)	192 (24.2 %) 430 (54.2 %) 172 (21.7 %)	<0.001
Family history of diabetes ‡	255 (34.8 %)	201 (26.8 %)	0.001
Hypertension ‡	316 (35.9 %)	304 (38.3 %)	0.331

* Continuous variables were tested for a difference between the two populations using t-tests and categorical variables with Chi-square tests with continuity correction.

† Mean (range); ‡ number (percentage); § mean ± standard deviation.

Table S2: KORA FDR significant results of prevalent type 2 diabetes in KORA, HUNT as well as their combined effect using a meta-analysis random effects model. Model was adjusted for age and sex, body mass index (BMI), smoking and hypertension.

Protein Full Name	UniProt ID	KORA (n = 993)		HUNT (n = 940)		Combined	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
72 kDa type IV collagenase	P08253	0.7 (0.56 - 0.87)	1.83E-03	0.89 (0.73 - 1.08)	2.36E-01	0.79 (0.63 - 1.01)	5.63E-02
Adiponectin	Q15848	0.57 (0.44 - 0.75)	3.96E-05				
Afamin	P43652	1.52 (1.22 - 1.92)	2.97E-04	1.11 (0.91 - 1.36)	3.20E-01	1.29 (0.95 - 1.77)	1.05E-01
Alpha-1-antichymotrypsin	P01011	0.71 (0.56 - 0.89)	3.07E-03	0.93 (0.77 - 1.11)	4.14E-01	0.82 (0.63 - 1.06)	1.35E-01
Alpha-1-antichymotrypsin complex	P01011	0.7 (0.56 - 0.88)	2.25E-03	1.09 (0.9 - 1.31)	3.49E-01	0.88 (0.57 - 1.36)	5.66E-01
Alpha-L-iduronidase	P35475	1.48 (1.2 - 1.84)	3.04E-04	1.44 (1.19 - 1.74)	1.59E-04	1.46 (1.26 - 1.68)	1.78E-07
Aminoacylase-1	Q03154	2.1 (1.64 - 2.71)	5.62E-09	1.49 (1.22 - 1.84)	1.26E-04	1.76 (1.26 - 2.46)	9.23E-04
Apolipoprotein B	P04114	0.48 (0.37 - 0.61)	4.19E-09	0.7 (0.57 - 0.84)	2.87E-04	0.58 (0.4 - 0.84)	3.63E-03
Arylsulfatase A	P15289	1.31 (1.09 - 1.57)	3.62E-03	1.13 (0.96 - 1.33)	1.37E-01	1.21 (1.05 - 1.39)	7.88E-03
Calpastatin	P20810	0.62 (0.46 - 0.82)	1.00E-03	0.86 (0.7 - 1.05)	1.44E-01	0.74 (0.54 - 1.03)	7.13E-02
Cathepsin D	P07339	1.39 (1.14 - 1.7)	1.13E-03	1.22 (1.01 - 1.48)	4.02E-02	1.3 (1.13 - 1.49)	1.90E-04
Cathepsin Z	Q9UBR2	1.41 (1.13 - 1.77)	2.27E-03	1.33 (1.1 - 1.62)	3.20E-03	1.37 (1.18 - 1.58)	2.39E-05
C-C motif chemokine 23	P55773	0.7 (0.56 - 0.88)	2.92E-03	0.81 (0.66 - 0.99)	3.99E-02	0.76 (0.65 - 0.89)	4.44E-04
CD97 antigen	P48960	0.66 (0.51 - 0.84)	1.14E-03	0.84 (0.68 - 1.02)	8.69E-02	0.75 (0.59 - 0.96)	2.03E-02
Cerebral dopamine neurotrophic factor	Q49AH0	0.64 (0.48 - 0.82)	7.25E-04	0.7 (0.55 - 0.87)	1.83E-03	0.67 (0.56 - 0.79)	4.89E-06
Chordin-like protein 1	Q9BU40	0.57 (0.43 - 0.74)	2.51E-05	0.79 (0.63 - 0.98)	2.94E-02	0.67 (0.49 - 0.93)	1.47E-02
Ciliary neurotrophic factor receptor subunit alpha	P26992	0.71 (0.57 - 0.89)	2.30E-03	0.81 (0.67 - 0.98)	3.16E-02	0.77 (0.66 - 0.88)	2.84E-04
Ck-beta-8-1	P55773	0.66 (0.53 - 0.82)	2.18E-04	0.79 (0.65 - 0.96)	1.58E-02	0.73 (0.61 - 0.86)	2.47E-04
Coagulation factor IX	P00740	1.67 (1.3 - 2.18)	1.10E-04	1.14 (0.94 - 1.41)	2.02E-01	1.37 (0.94 - 1.98)	9.88E-02
Coagulation factor IXab	P00740	1.69 (1.31 - 2.22)	9.18E-05	1.12 (0.93 - 1.37)	2.63E-01	1.36 (0.91 - 2.04)	1.34E-01
Coiled-coil domain-containing protein 80	Q76M96	0.62 (0.48 - 0.79)	1.05E-04	0.85 (0.68 - 1.07)	1.78E-01	0.73 (0.53 - 1)	5.15E-02
Collagenase 3	P45452	0.53 (0.36 - 0.76)	1.06E-03	0.96 (0.77 - 1.16)	6.89E-01	0.73 (0.41 - 1.3)	2.86E-01
Complement C2	P06681	2.01 (1.37 - 3.04)	6.63E-04	1.47 (1.2 - 1.82)	3.03E-04	1.63 (1.22 - 2.19)	9.06E-04
Complement C3b, inactivated	P01024	1.42 (1.15 - 1.77)	1.34E-03				
Complement component C9	P02748	0.72 (0.58 - 0.88)	1.71E-03	1.06 (0.87 - 1.3)	5.73E-01	0.87 (0.59 - 1.28)	4.84E-01
Cystatin-M	Q15828	0.68 (0.54 - 0.85)	8.95E-04	0.84 (0.69 - 1.02)	8.11E-02	0.76 (0.62 - 0.94)	1.01E-02
Dickkopf-like protein 1	Q9UK85	1.35 (1.14 - 1.66)	1.26E-03	1.08 (0.89 - 1.3)	4.33E-01	1.21 (0.97 - 1.51)	9.82E-02
Dickkopf-related protein 3	Q9UBP4	0.69 (0.54 - 0.87)	1.95E-03	0.86 (0.7 - 1.06)	1.70E-01	0.78 (0.62 - 0.97)	2.64E-02
Ectodysplasin-A, secreted form	Q92838	0.64 (0.49 - 0.83)	1.18E-03	0.85 (0.68 - 1.04)	1.30E-01	0.75 (0.57 - 0.98)	3.56E-02
Endoplasmic reticulum resident protein 29	P30040	1.44 (1.15 - 1.82)	1.97E-03	1.21 (1 - 1.45)	4.81E-02	1.3 (1.1 - 1.54)	2.64E-03

Endostatin	P39060	0.7 (0.57 - 0.86)	6.07E-04	1.04 (0.85 - 1.27)	7.21E-01	0.85 (0.58 - 1.26)	4.17E-01
Endothelial cell-specific molecule 1	Q9NQ30	0.62 (0.47 - 0.8)	4.30E-04	0.8 (0.62 - 1)	6.68E-02	0.71 (0.55 - 0.91)	6.96E-03
Endothelin-converting enzyme 1	P42892	0.71 (0.57 - 0.88)	1.66E-03	0.84 (0.7 - 1)	5.24E-02	0.78 (0.66 - 0.92)	3.17E-03
Ephrin type-B receptor 2	P29323	0.68 (0.55 - 0.84)	4.68E-04	0.97 (0.79 - 1.18)	7.41E-01	0.81 (0.57 - 1.15)	2.40E-01
Ephrin type-B receptor 6	O15197	0.7 (0.55 - 0.88)	2.26E-03	0.96 (0.78 - 1.16)	6.86E-01	0.82 (0.6 - 1.13)	2.25E-01
Fibroblast growth factor 19	O95750	0.6 (0.47 - 0.75)	1.19E-05	0.87 (0.72 - 1.04)	1.35E-01	0.73 (0.5 - 1.05)	8.71E-02
Fibronectin Fragment 4	P02751	0.7 (0.56 - 0.87)	1.27E-03	0.82 (0.68 - 0.99)	3.65E-02	0.76 (0.65 - 0.89)	7.03E-04
Ficolin-3	O75636	1.46 (1.15 - 1.87)	2.46E-03	1.15 (0.94 - 1.42)	1.70E-01	1.28 (1.02 - 1.62)	3.18E-02
Galectin-3-binding protein	Q08380	1.6 (1.27 - 2.01)	5.04E-05	1.43 (1.2 - 1.72)	9.47E-05	1.5 (1.3 - 1.72)	2.40E-08
Galectin-4	P56470	1.33 (1.1 - 1.59)	2.29E-03	1.18 (1.01 - 1.39)	3.36E-02	1.24 (1.1 - 1.39)	3.39E-04
GDNF family receptor alpha-2	O00451	0.63 (0.5 - 0.78)	5.02E-05	0.83 (0.69 - 1.01)	5.86E-02	0.73 (0.55 - 0.96)	2.45E-02
Gelsolin	P06396	0.55 (0.43 - 0.69)	4.31E-07	0.66 (0.54 - 0.81)	4.31E-05	0.61 (0.51 - 0.73)	1.06E-07
Hepatocyte growth factor receptor	P08581	0.62 (0.49 - 0.78)	4.89E-05	0.78 (0.65 - 0.92)	4.93E-03	0.7 (0.57 - 0.88)	1.58E-03
Iduronate 2-sulfatase	P22304	0.69 (0.54 - 0.87)	1.77E-03	0.85 (0.71 - 1.02)	7.03E-02	0.77 (0.63 - 0.95)	1.35E-02
Insulin-like growth factor-binding protein 2	P18065	0.42 (0.32 - 0.55)	1.53E-09	0.76 (0.59 - 0.97)	2.57E-02	0.57 (0.32 - 1)	5.19E-02
Interleukin-11 receptor subunit alpha	Q14626	0.58 (0.44 - 0.75)	3.23E-05	0.82 (0.67 - 1)	5.49E-02	0.7 (0.49 - 0.98)	3.98E-02
Interleukin-22 receptor subunit alpha-2	Q969J5	0.72 (0.57 - 0.9)	3.42E-03	0.93 (0.78 - 1.11)	4.07E-01	0.82 (0.64 - 1.06)	1.26E-01
Kallikrein-7	P49862	0.59 (0.46 - 0.75)	1.47E-05	0.67 (0.54 - 0.82)	1.95E-04	0.63 (0.54 - 0.74)	1.49E-08
Kynureninase	Q16719	1.35 (1.1 - 1.65)	3.44E-03	1.11 (0.92 - 1.33)	2.66E-01	1.22 (1 - 1.48)	4.53E-02
Legumain	Q99538	1.41 (1.18 - 1.74)	3.80E-04	1.17 (0.97 - 1.4)	8.96E-02	1.28 (1.07 - 1.54)	7.56E-03
Leucine carboxyl methyltransferase 1	Q9UIC8	0.48 (0.32 - 0.72)	4.68E-04	0.87 (0.69 - 1.07)	1.90E-01	0.66 (0.37 - 1.18)	1.59E-01
Lysosomal protective protein	P10619	1.54 (1.24 - 1.92)	8.51E-05	1.32 (1.09 - 1.6)	5.48E-03	1.42 (1.21 - 1.65)	1.05E-05
Matrilin-2	O00339	0.62 (0.49 - 0.77)	2.77E-05	0.7 (0.57 - 0.86)	7.17E-04	0.66 (0.57 - 0.77)	1.05E-07
Melanoma-derived growth regulatory protein	Q16674	0.69 (0.55 - 0.87)	2.09E-03	0.79 (0.65 - 0.96)	1.75E-02	0.75 (0.65 - 0.87)	1.47E-04
Muellerian-inhibiting factor	P03971	0.72 (0.57 - 0.9)	3.85E-03	1.02 (0.84 - 1.21)	8.70E-01	0.86 (0.61 - 1.21)	3.81E-01
Myoglobin	P02144	0.68 (0.53 - 0.86)	1.51E-03	0.81 (0.66 - 0.99)	4.18E-02	0.75 (0.63 - 0.89)	1.16E-03
NADPH--cytochrome P450 reductase	P16435	1.43 (1.14 - 1.78)	1.52E-03	1.08 (0.9 - 1.31)	4.00E-01	1.24 (0.94 - 1.62)	1.23E-01
Netrin receptor UNC5D	Q6UXZ4	0.55 (0.43 - 0.71)	3.04E-06	0.82 (0.69 - 0.97)	2.28E-02	0.68 (0.46 - 1)	4.98E-02
Neurexin-1-beta	P58400	0.59 (0.42 - 0.81)	1.97E-03	0.79 (0.62 - 0.98)	4.66E-02	0.7 (0.53 - 0.92)	1.21E-02
Neurogenic locus notch homolog protein 1	P46531	0.7 (0.56 - 0.88)	1.99E-03	0.83 (0.69 - 1)	5.41E-02	0.77 (0.66 - 0.91)	1.93E-03
Osteomodulin	Q99983	0.61 (0.47 - 0.77)	3.89E-05	0.64 (0.52 - 0.78)	1.22E-05	0.62 (0.54 - 0.73)	2.03E-09
Pappalysin-1	Q13219	0.71 (0.57 - 0.88)	1.88E-03	1.06 (0.87 - 1.28)	5.87E-01	0.87 (0.59 - 1.28)	4.77E-01

Protein	ID	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Peptide YY	P10082	1.34 (1.1 - 1.62)	3.36E-03	1.53 (1.27 - 1.86)	9.26E-06	1.43 (1.25 - 1.64)	1.77E-07
Periostin	Q15063	0.54 (0.43 - 0.68)	1.52E-07	0.75 (0.62 - 0.92)	5.50E-03	0.64 (0.47 - 0.88)	6.59E-03
Peroxiredoxin-1	Q06830	1.41 (1.15 - 1.72)	9.65E-04	1.01 (0.83 - 1.21)	9.51E-01	1.19 (0.85 - 1.65)	3.09E-01
Plasma protease C1 inhibitor	P05155	0.67 (0.53 - 0.84)	5.39E-04	0.76 (0.61 - 0.93)	9.66E-03	0.72 (0.61 - 0.83)	2.10E-05
Plasminogen activator inhibitor 1	P05121	1.46 (1.16 - 1.83)	1.19E-03	1.17 (0.97 - 1.42)	1.03E-01	1.29 (1.05 - 1.6)	1.71E-02
Protein S100-A9	P06702	1.53 (1.24 - 1.88)	6.16E-05				
Proto-oncogene tyrosine-protein kinase receptor Ret	P07949	1.39 (1.13 - 1.7)	1.75E-03	1.26 (1.02 - 1.56)	3.04E-02	1.33 (1.14 - 1.54)	1.73E-04
Pulmonary surfactant-associated protein D	P35247	1.43 (1.16 - 1.77)	6.82E-04	0.94 (0.77 - 1.12)	4.96E-01	1.16 (0.76 - 1.75)	4.96E-01
Renin	P00797	1.61 (1.32 - 1.99)	5.48E-06	1.45 (1.21 - 1.74)	5.16E-05	1.52 (1.33 - 1.74)	1.54E-09
Retinol-binding protein 4	P02753	1.3 (1.08 - 1.55)	3.68E-03				
RGM domain family member B	Q6NW40	0.64 (0.49 - 0.81)	3.52E-04	0.73 (0.59 - 0.9)	3.25E-03	0.69 (0.59 - 0.81)	5.33E-06
Sex hormone-binding globulin	P04278	0.62 (0.47 - 0.8)	2.41E-04	0.63 (0.51 - 0.77)	1.12E-05	0.62 (0.53 - 0.73)	1.04E-08
SLIT and NTRK-like protein 5	O94991	0.6 (0.47 - 0.76)	3.81E-05	0.78 (0.66 - 0.93)	4.38E-03	0.7 (0.54 - 0.9)	5.11E-03
SPARC-like protein 1	Q14515	0.69 (0.55 - 0.86)	1.22E-03	0.99 (0.82 - 1.21)	9.37E-01	0.83 (0.58 - 1.19)	3.10E-01
Transforming growth factor beta receptor type 3	Q03167	0.58 (0.46 - 0.73)	4.45E-06	0.74 (0.61 - 0.89)	1.40E-03	0.66 (0.52 - 0.85)	9.40E-04
Trypsin-1	P07477	0.63 (0.5 - 0.78)	4.06E-05	0.7 (0.58 - 0.84)	2.19E-04	0.67 (0.58 - 0.77)	4.35E-08
Tumor necrosis factor receptor superfamily member 11B	O00300	0.7 (0.58 - 0.86)	5.86E-04				
Tumor necrosis factor receptor superfamily member 13C	Q96RJ3	1.33 (1.12 - 1.57)	7.26E-04	0.88 (0.69 - 1.07)	2.43E-01	1.09 (0.72 - 1.63)	6.90E-01
Tumor necrosis factor-inducible gene 6 protein	P98066	0.58 (0.45 - 0.74)	2.27E-05	0.7 (0.57 - 0.85)	3.96E-04	0.65 (0.54 - 0.77)	1.28E-06
Tyrosine-protein kinase JAK2	O60674	0.72 (0.58 - 0.89)	2.54E-03	0.9 (0.76 - 1.07)	1.71E-01	0.81 (0.66 - 1.01)	5.64E-02
Tyrosine-protein kinase transmembrane receptor ROR1	Q01973	0.65 (0.49 - 0.84)	1.26E-03	0.95 (0.77 - 1.15)	6.12E-01	0.79 (0.54 - 1.15)	2.23E-01
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2	Q8TEU8	0.69 (0.54 - 0.87)	2.10E-03	0.85 (0.7 - 1.04)	1.13E-01	0.77 (0.63 - 0.95)	1.31E-02
Wnt inhibitory factor 1	Q9Y5W5	0.5 (0.37 - 0.66)	2.97E-06	0.65 (0.54 - 0.79)	2.21E-05	0.58 (0.45 - 0.76)	6.44E-05

Table S3: KORA FDR significant results of Incident type 2 diabetes in KORA, HUNT as well as their combined effect using a meta-analysis random effects model. Model was adjusted for age and sex, body mass index (BMI), smoking and current hypertension status.

Protein Full Name	UniProt ID	KORA (N = 881)		HUNT (N = 794)		Combined	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	Pvalue
Adiponectin	Q15848	0.41 (0.29 - 0.57)	1.84E-07				
Afamin	P43652	2.18 (1.64 - 2.94)	1.31E-07	1.09 (0.86 - 1.4)	4.90E-01	1.53 (0.78 - 3.03)	2.18E-01
Aminoacylase-1	Q03154	1.78 (1.34 - 2.37)	7.15E-05	1.6 (1.26 - 2.04)	1.27E-04	1.67 (1.39 - 2.01)	3.97E-08
Growth hormone receptor	P10912	1.74 (1.31 - 2.38)	2.43E-04	1.42 (1.07 - 1.88)	1.37E-02	1.56 (1.28 - 1.91)	1.64E-05
Insulin-like growth factor-binding protein 2	P18065	0.47 (0.34 - 0.65)	6.07E-06	0.57 (0.42 - 0.77)	2.91E-04	0.52 (0.42 - 0.65)	1.01E-08
Netrin receptor UNC5D	Q6UXZ4	0.57 (0.41 - 0.78)	4.37E-04	0.89 (0.71 - 1.12)	2.88E-01	0.72 (0.46 - 1.11)	1.39E-01
Pappalysin-1	Q13219	0.59 (0.45 - 0.77)	1.02E-04	1.02 (0.8 - 1.3)	8.45E-01	0.78 (0.45 - 1.34)	3.67E-01
Scavenger receptor cysteine-rich type 1 protein M130	Q86VB7	1.56 (1.23 - 2.01)	3.68E-04	0.98 (0.79 - 1.22)	8.53E-01	1.23 (0.78 - 1.95)	3.70E-01
Tartrate-resistant acid phosphatase type 5	P13686	1.74 (1.31 - 2.34)	1.97E-04	1.13 (0.9 - 1.44)	3.14E-01	1.39 (0.91 - 2.12)	1.27E-01
Transforming growth factor beta receptor type 3	Q03167	0.58 (0.44 - 0.77)	1.38E-04	0.83 (0.68 - 1.02)	5.55E-02	0.7 (0.5 - 0.99)	4.35E-02

Table S4: Overlap between our replicated proteins with type 2 diabetes associated genes from Xue et al, and Human Diabetes Proteome Project (HDPP) lists: 1000 diabetes related proteins list, islet of Langerhans proteome, rodent beta cell proteome and blood glycated proteins database.

Full Name	UniProt ID	Entrez Gene	Outcome in our study	T2D genes	HDPP lists				Any
					T2D 1000 proteins	Islet proteome	Rodent beta cell proteome	Glycated proteins	
Aminoacylase-1	Q03154	ACY1	Both			Y			Y
Growth hormone receptor	P10912	GHR	Incident		Y				Y
Insulin-like growth factor-binding protein 2	P18065	IGFBP2	Incident		Y	Y			Y
Alpha-L-iduronidase	P35475	IDUA	Prevalent			Y			Y
Apolipoprotein B	P04114	APOB	Prevalent		Y	Y			Y
Cathepsin Z	Q9UBR2	CTSZ	Prevalent			Y			Y
Cerebral dopamine neurotrophic factor	Q49AH0	CDNF	Prevalent						
Complement C2	P06681	C2	Prevalent	Y					Y
Galectin-3-binding protein	Q08380	LGALS3BP	Prevalent			Y			Y
Gelsolin	P06396	GSN	Prevalent			Y			Y
Hepatocyte growth factor receptor	P08581	MET	Prevalent			Y			Y
Kallikrein-7	P49862	KLK7	Prevalent						
Lysosomal protective protein	P10619	CTSA	Prevalent			Y			Y
Matrilin-2	O00339	MATN2	Prevalent						
Osteomodulin	Q99983	OMD	Prevalent						
Peptide YY	P10082	PYY	Prevalent		Y				Y
Periostin	Q15063	POSTN	Prevalent			Y			Y
Plasma protease C1 inhibitor	P05155	SERPING1	Prevalent			Y			Y
Renin	P00797	REN	Prevalent		Y				Y
RGM domain family member B	Q6NW40	RGMB	Prevalent						
Sex hormone-binding globulin	P04278	SHBG	Prevalent		Y				Y
SLIT and NTRK-like protein 5	O94991	SLITRK5	Prevalent						

Transforming growth factor beta receptor type 3	Q03167	TGFBR3	Prevalent						
Trypsin-1	P07477	PRSS1	Prevalent		Y	Y			Y
Tumor necrosis factor-inducible gene 6 protein	P98066	TNFAIP6	Prevalent			Y			Y
Wnt inhibitory factor 1	Q9Y5W5	WIF1	Prevalent						

Table S5: Results of the data analytics of replicated proteins:

(Excel sheet)

Table S6: Comparison between original model and drug-adjusted model with replicated prevalent type 2 diabetes proteins showing those that lost significance or showed different direction of effect estimate in any of the drug-adjusted models:

Drug (number of participants taking the drug)	C2		PYY		CATZ		N
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	
Original Result	2.01 (1.37 - 3.04)	6.63E-04	1.34 (1.1 - 1.62)	3.36E-03	1.41 (1.13 - 1.77)	2.27E-03	993
Antidiabetic Medications (52)	1.33 (0.9 - 2.18)	2.21E-01	0.9 (0.67 - 1.19)	4.71E-01	1.15 (0.86 - 1.54)	3.30E-01	993
Insulin (11)	1.93 (1.3 - 2.97)	1.81E-03	1.33 (1.08 - 1.63)	5.26E-03	1.39 (1.1 - 1.75)	5.32E-03	993
Oral antidiabetics (49)	1.34 (0.91 - 2.17)	2.02E-01	0.89 (0.66 - 1.17)	3.97E-01	1.19 (0.89 - 1.58)	2.31E-01	993
Oral antidiabetics without Metformin (27)	1.61 (1.08 - 2.52)	2.78E-02	1.14 (0.9 - 1.42)	2.63E-01	1.25 (0.97 - 1.6)	8.26E-02	993
Metformin (40)	1.37 (0.94 - 2.17)	1.44E-01	0.93 (0.71 - 1.21)	6.07E-01	1.27 (0.98 - 1.66)	7.51E-02	991

Table S7: Mendelian randomization results of the direction with type 2 diabetes as the exposure and the individual proteins as the outcome.

Protein	IVW Beta (SE)	IVW p-value	n SNPs	Pleiotropy test *	Protein summary statistics origin
Aminoacylase-1	-0.02 (0.07)	7.51E-01	96	2.01E-01	Suhre K
Growth hormone receptor	0.02 (0.04)	6.86E-01	120	5.60E-01	Sun BB
Insulin-like growth factor-binding protein 2	-0.05 (0.05)	2.47E-01	120	6.97E-01	Sun BB
Apolipoprotein B	-0.07 (0.04)	1.12E-01	120	3.24E-01	Sun BB
Cerebral dopamine neurotrophic factor	0.02 (0.04)	5.54E-01	120	8.09E-01	Sun BB
Plasma protease C1 inhibitor	-0.05 (0.04)	2.35E-01	120	7.84E-01	Sun BB
Complement C2	-0.02 (0.08)	8.35E-01	96	1.65E-01	Suhre K
Lysosomal protective protein	-0.003 (0.04)	9.39E-01	120	6.42E-01	Sun BB
Cathepsin Z	0.13 (0.04)	2.00E-03	120	1.80E-01	Sun BB
Gelsolin	0.03 (0.08)	6.61E-01	96	3.21E-01	Suhre K
Alpha-L-iduronidase	0.02 (0.04)	6.82E-01	120	6.87E-01	Sun BB
Kallikrein-7	-0.04 (0.04)	2.29E-01	120	9.56E-01	Sun BB
Galectin-3-binding protein	0.13 (0.07)	8.38E-02	96	9.82E-01	Suhre K
Matrilin-2	0.03 (0.04)	5.14E-01	120	1.24E-01	Sun BB
Hepatocyte growth factor receptor	-0.09 (0.05)	1.11E-01	120	4.38E-01	Sun BB
Osteomodulin	-0.02 (0.04)	5.70E-01	120	2.20E-01	Sun BB
Periostin	0.01 (0.04)	7.98E-01	120	7.68E-01	Sun BB
Peptide YY	0.05 (0.04)	1.70E-01	120	3.37E-01	Sun BB
Renin	0.08 (0.04)	3.15E-02	120	1.05E-01	Sun BB
RGM domain family member B	-0.01 (0.04)	8.52E-01	120	5.97E-01	Sun BB
Sex hormone-binding globulin	-0.01 (0.08)	8.55E-01	96	1.89E-02	Suhre K
SLIT and NTRK-like protein 5	0.01 (0.04)	7.49E-01	120	1.43E-02	Sun BB
Transforming growth factor beta receptor type 3	-0.01 (0.04)	8.30E-01	120	5.10E-01	Sun BB
Trypsin-1	-0.01 (0.04)	8.08E-01	120	6.16E-01	Sun BB
Tumor necrosis factor-inducible gene 6 protein	0.05 (0.04)	2.63E-01	120	5.41E-01	Sun BB
Wnt inhibitory factor 1	0.04 (0.04)	2.98E-01	120	1.72E-01	Sun BB

* Pleiotropy p value represents the p value of the intercept of Egger's regression.

Table S8: Mendelian randomization results of the direction with the proteins as the exposure and type 2 diabetes as the outcome. All causal effects were tested using Wald statistics.

Protein	Beta	SE	p value	SNP (IV)	IV Origin
Alpha-L-iduronidase	0.03	0.05	4.93E-01	rs76650972	Sun BB
Apolipoprotein B	0.05	0.03	1.16E-01	rs679899	Emilsson V
Cerebral dopamine neurotrophic factor	0.00	0.05	9.75E-01	rs11814337	Sun BB
Growth hormone receptor	-0.03	0.05	5.35E-01	rs34742008	Sun BB
Hepatocyte growth factor receptor	-0.01	0.05	8.07E-01	rs437	Sun BB
Kallikrein-7	0.01	0.05	7.74E-01	rs2691258	Sun BB
Matrilin-2	-0.06	0.05	2.77E-01	rs17831160	Sun BB
Peptide YY	0.03	0.05	5.84E-01	rs8074783	Emilsson V
Periostin	-0.04	0.03	1.91E-01	rs962462	Emilsson V
Plasma protease C1 inhibitor	0.00	0.01	7.62E-01	rs11229075	Sun BB
RGM domain family member B	0.02	0.04	5.65E-01	rs1563317	Sun BB
Sex hormone-binding globulin	-0.09	0.04	2.95E-02	rs858519	Emilsson V
Tumor necrosis factor-inducible gene 6 protein	-0.03	0.01	5.41E-02	rs289828	Sun BB

Table S9: Information on replicated proteins' validation adapted from Emilsson V. et al.:

| Target Full Name	UniProt	Gene Symbol	DDA	MRM	Cis effect	Cis-Trans effects	Protein module
Matrilin-2	O00339	MATN2	MATN2				PM26
SLIT and NTRK-like protein 5	O94991	SLITRK5			SLITRK5		PM26
Renin	P00797	REN	REN	REN			No module
Apolipoprotein B	P04114	APOB	APOB	APOB	APOB	APOB	PM11
Sex hormone-binding globulin	P04278	SHBG	SHBG		SHBG	SHBG	No module
Plasma protease C1 inhibitor	P05155	SERPING1	SERPING1		SERPING1	SERPING1	PM27
Gelsolin	P06396	GSN	GSN		GSN		PM27
Complement C2	P06681	C2	C2		C2	C2	No module
Trypsin-1	P07477	PRSS1					No module
Hepatocyte growth factor receptor	P08581	MET		MET	MET	MET	PM27
Peptide YY	P10082	PYY			PYY		PM26
Lysosomal protective protein	P10619	CTSA	CTSA			CTSA	No module
Growth hormone receptor	P10912	GHR			GHR		No module
Insulin-like growth factor-binding protein 2	P18065	IGFBP2	IGFBP2				PM10
Alpha-L-iduronidase	P35475	IDUA		IDUA	IDUA		No module
Kallikrein-7	P49862	KLK7	KLK7		KLK7		PM27
Tumor necrosis factor-inducible gene 6 protein	P98066	TNFAIP6			TNFAIP6		PM27
Aminoacylase-1	Q03154	ACY1	ACY1	ACY1			PM23
Transforming growth factor beta receptor type 3	Q03167	TGFBR3					PM26
Galectin-3-binding protein	Q08380	LGALS3BP			LGALS3BP		PM6
Periostin	Q15063	POSTN			POSTN		PM13
Cerebral dopamine neurotrophic factor	Q49AH0	CDNF			CDNF		PM2
RGM domain family member B	Q6NW40	RGMB					PM26
Osteomodulin	Q99983	OMD					PM27
Cathepsin Z	Q9UBR2	CTSZ			CTSZ		PM26
Wnt inhibitory factor 1	Q9Y5W5	WIF1				WIF1	PM13
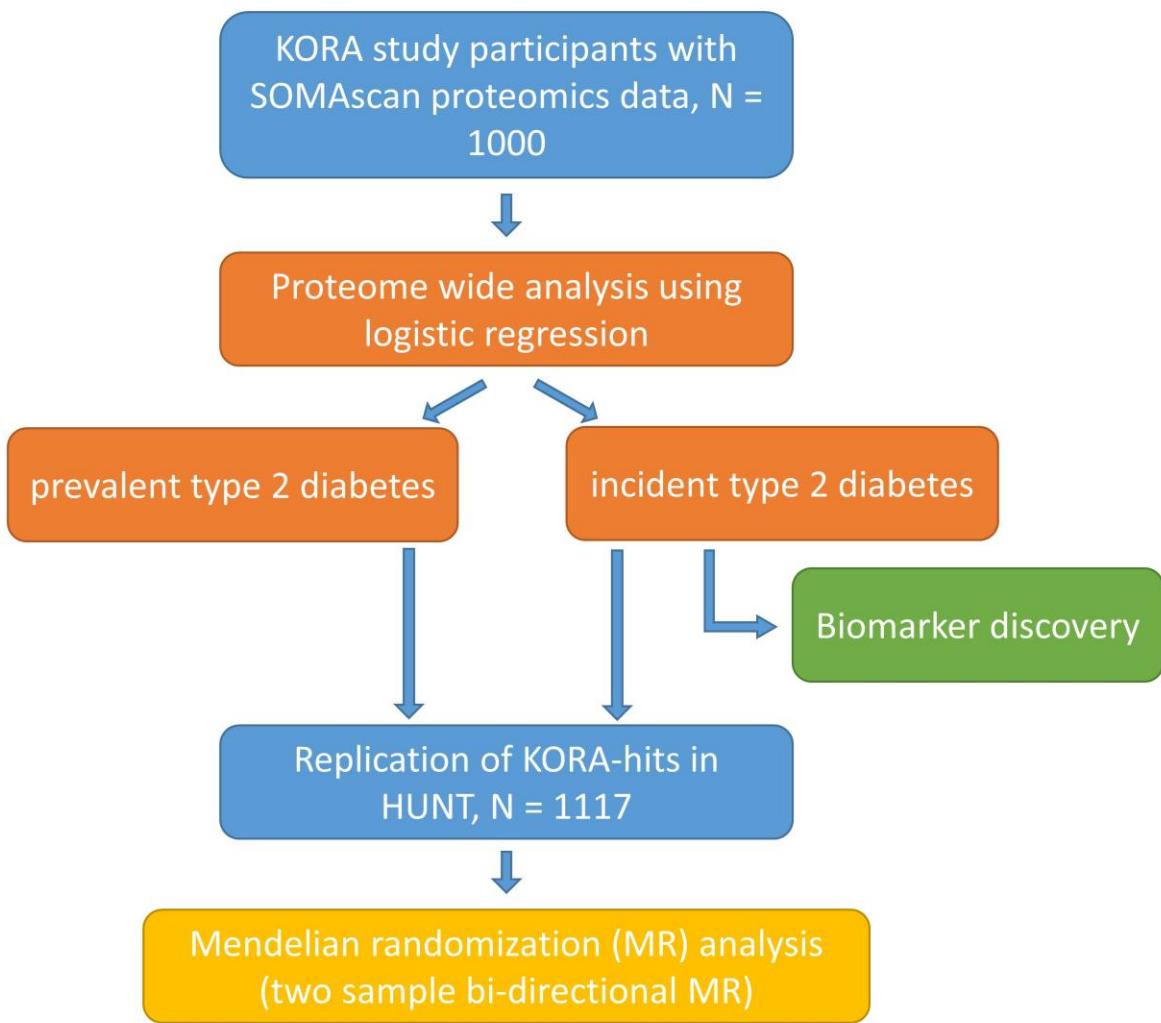
Figure S1: Analysis flowchart.
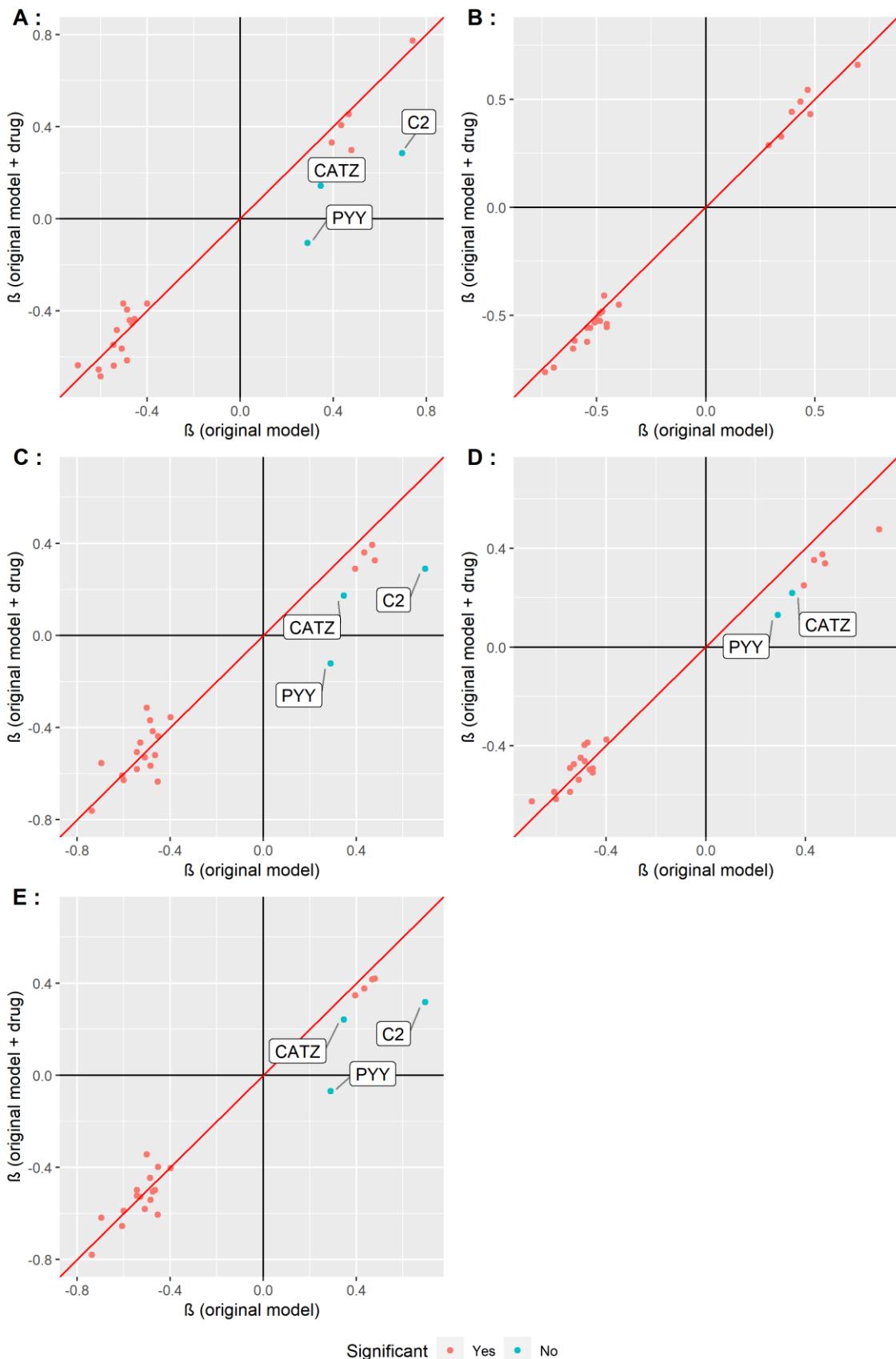
Figure S2: Results of drug-adjusted model of replicated prevalent type 2 diabetes associations. (A: Antidiabetic Medications, B: Insulin, C: Oral antidiabetics, D: Oral antidiabetics without Metformin, E: Metformin).
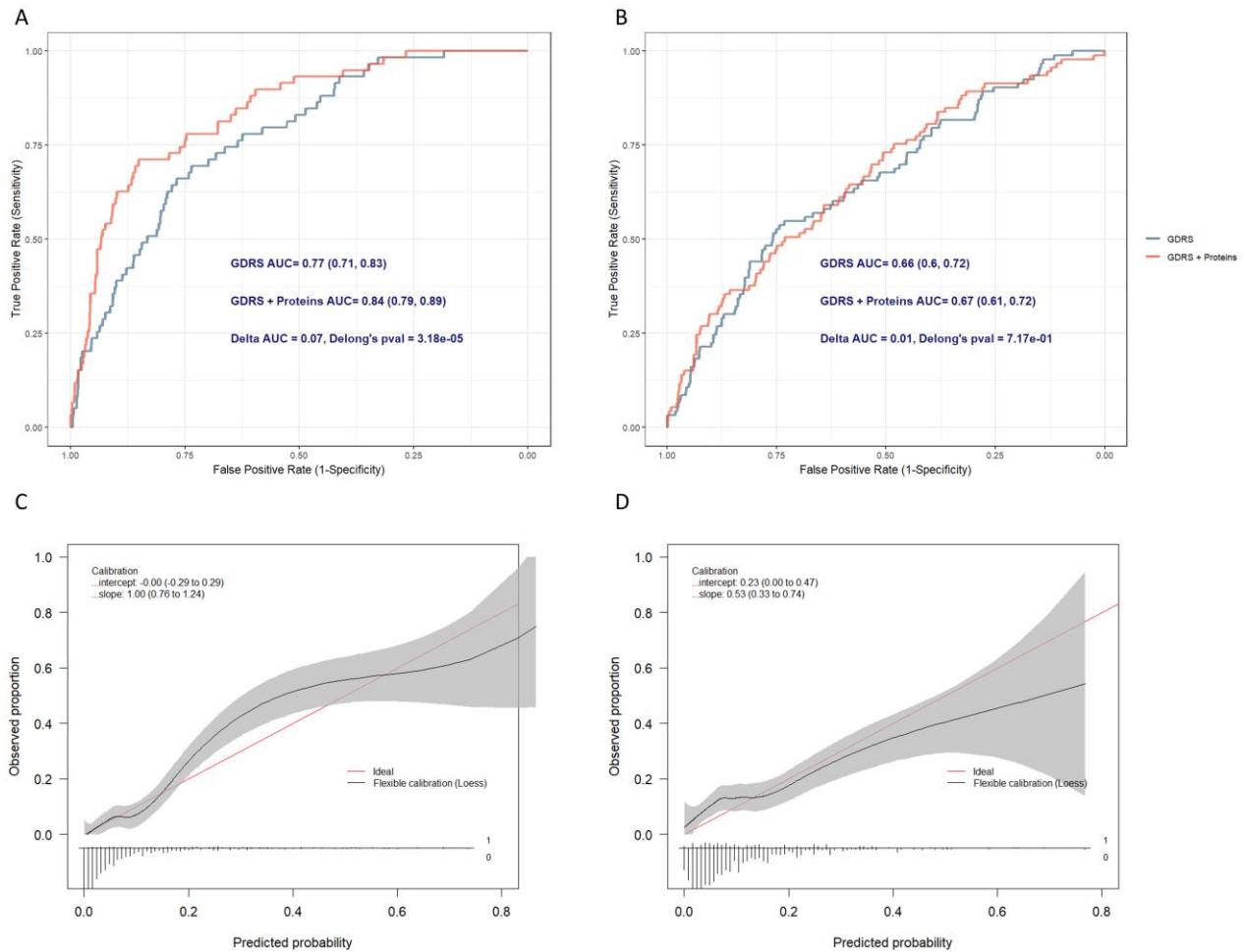
Figure S3: Performance of LASSO selected biomarkers on top of GDRS compared to GDRS in KORA and HUNT: assessing discrimination using receiver operating characteristic curve and c-statistic (A: KORA, B: HUNT), and assessing calibration (C: KORA, D: HUNT).
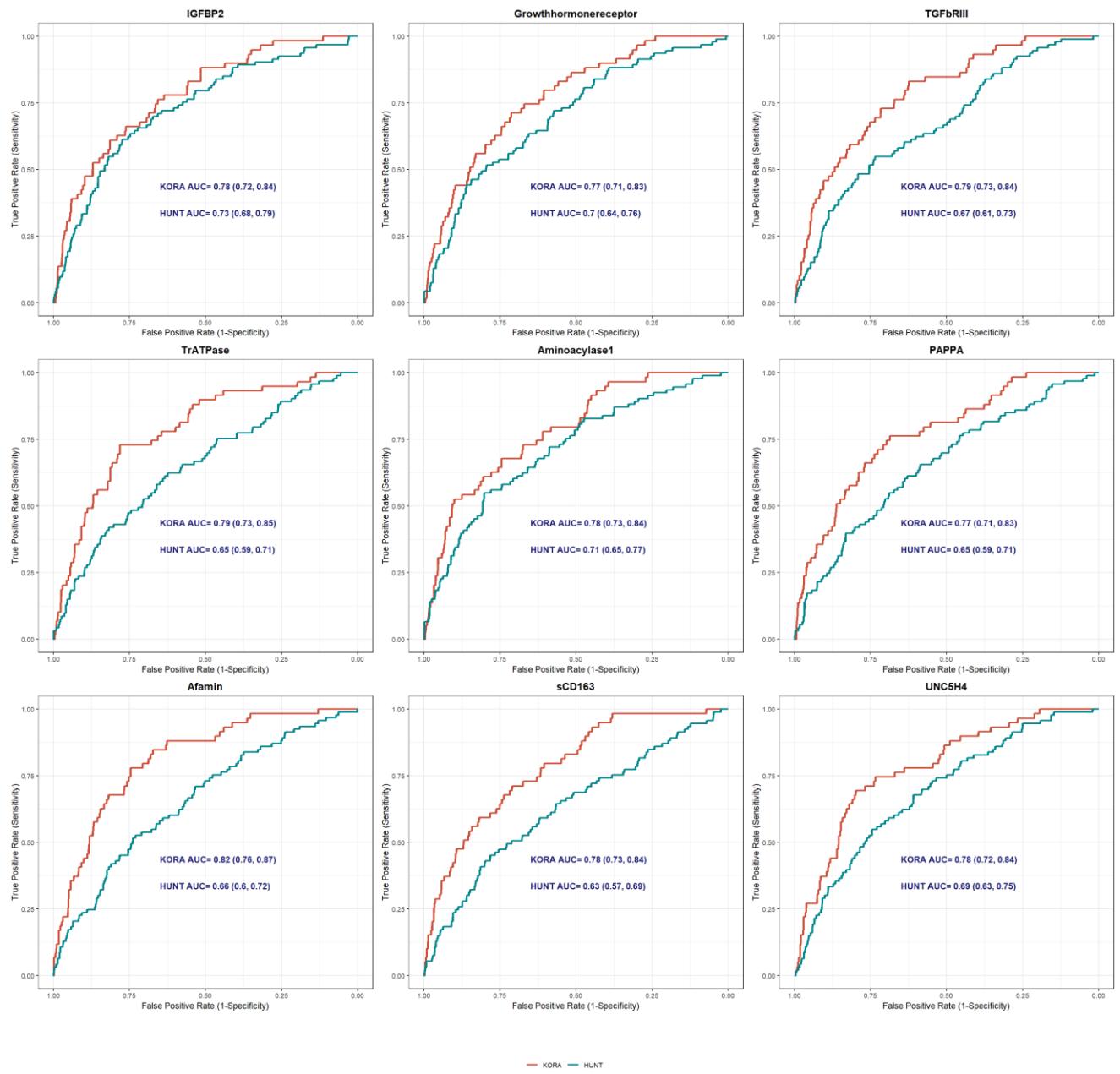
Figure S4: Performance of individual proteins on top of GDRS in both KORA and HUNT.
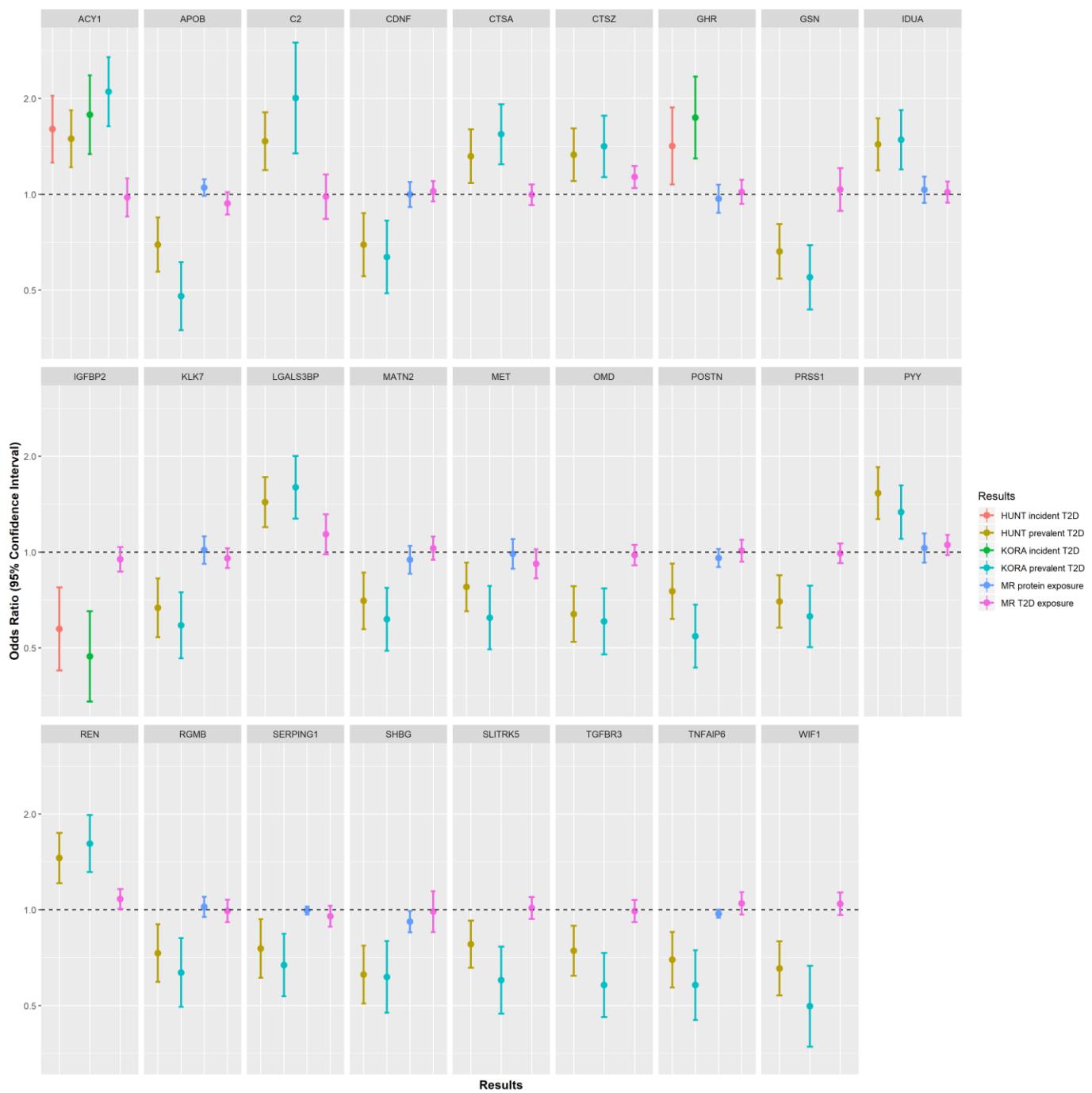
Figure S5: Forest plot of proteome wide analysis in KORA and HUNT as well as Mendelian randomization results for replicated proteins. Odds ratio are per SD change of protein level.
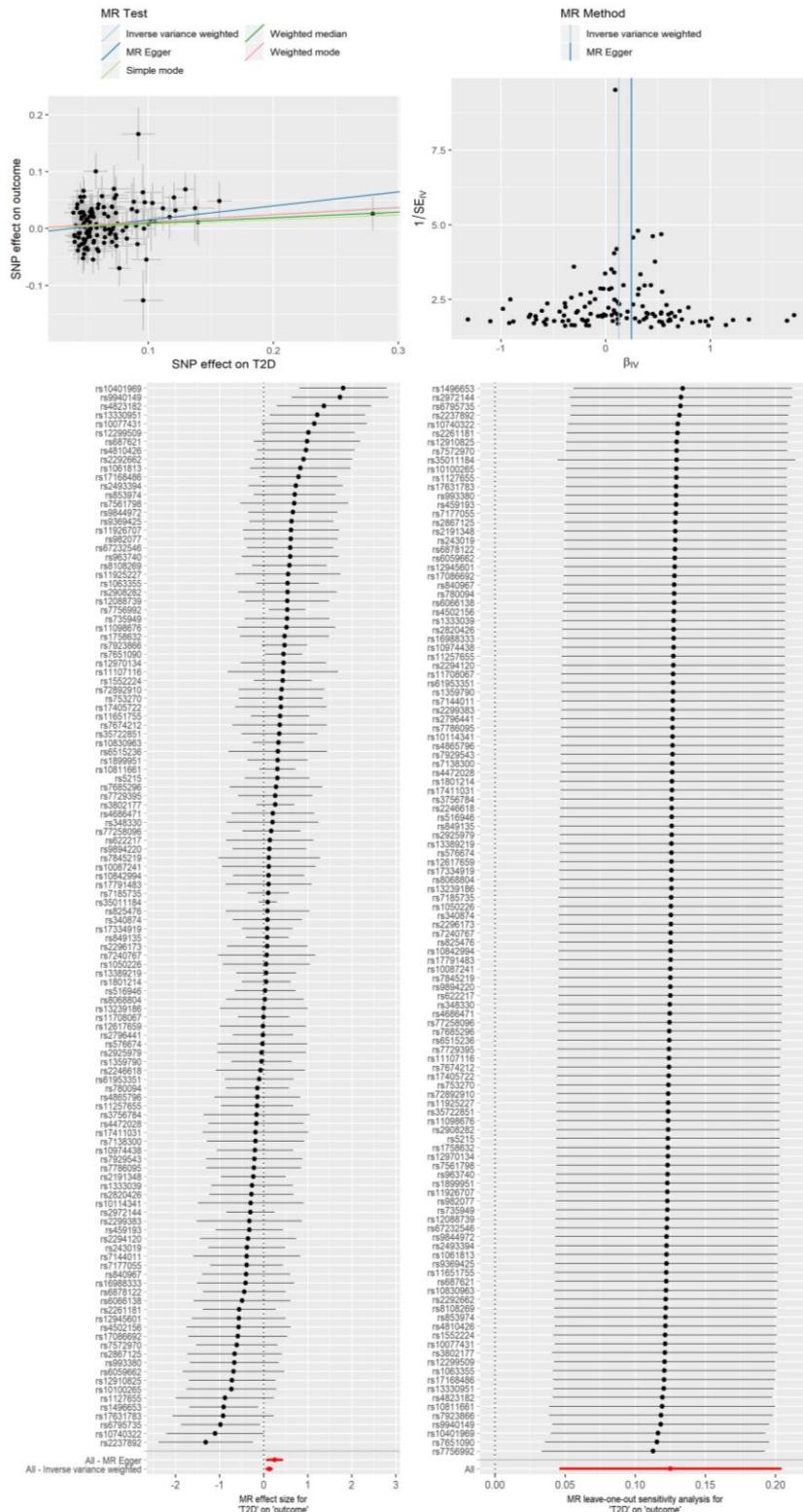
Figure S6: Sensitivity analyses of the Mendelian randomization analysis with cathepsin Z as the outcome. From top left to right: a: Scatter plot of the IVs' effects on exposure and outcome with causal analysis fitted lines plotted to check if there are any outliers driving the results; b: Funnel plot of the IVs' effects on the exposure to check for pleiotropy; c: Forest plot of the casual effect of each SNP to check for any potential outliers driving the results; d: Leave-one-out analysis to check if the results are dependent on any specific IV.
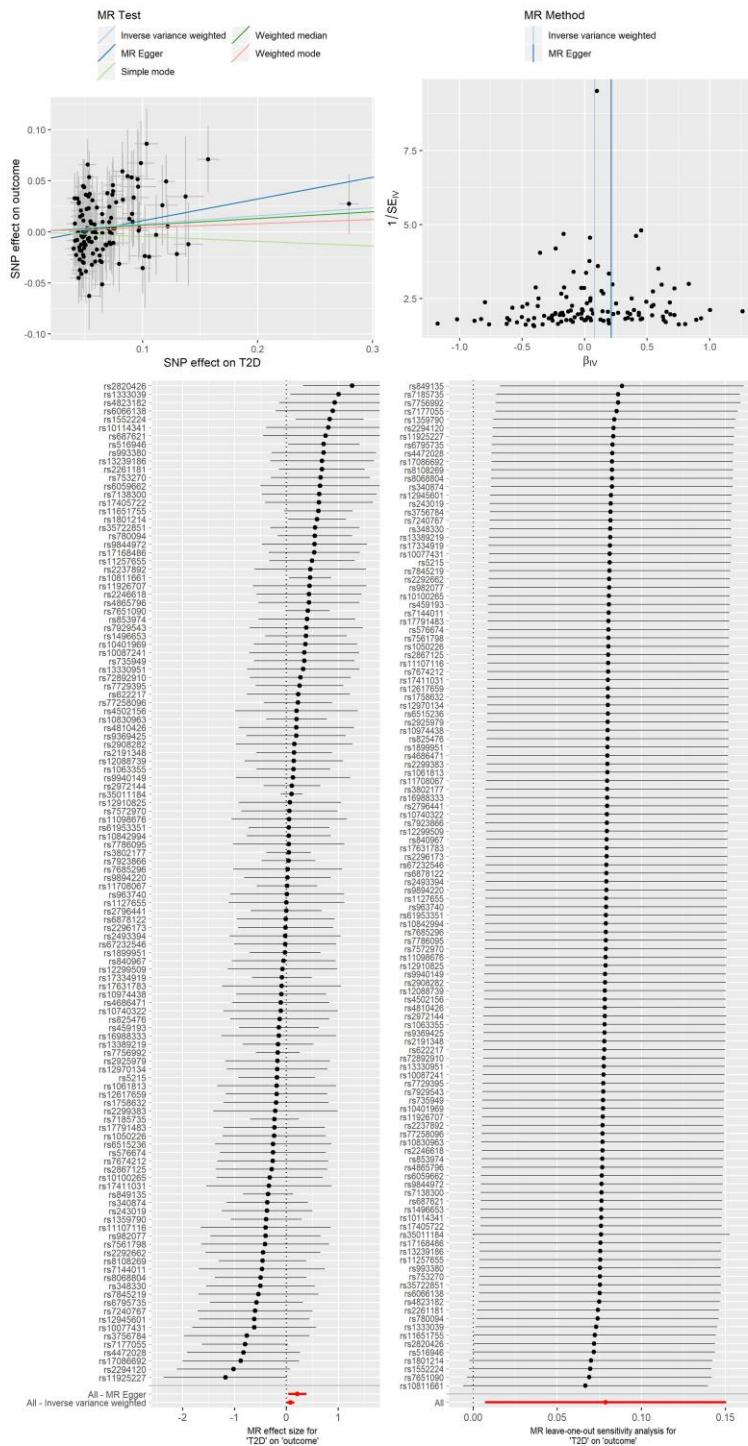
Figure S7: Sensitivity analyses of the Mendelian randomization analysis with renin as the outcome. From top left to right: a: Scatter plot of the IVs' effects on exposure and outcome with causal analysis fitted lines plotted to check if there are any outliers driving the results; b: Funnel plot of the IVs' effects on the exposure to check for pleiotropy; c: Forest plot of the casual effect of each SNP to check for any potential outliers driving the results; d: Leave-one-out analysis to check if the results are dependent on any specific IV.
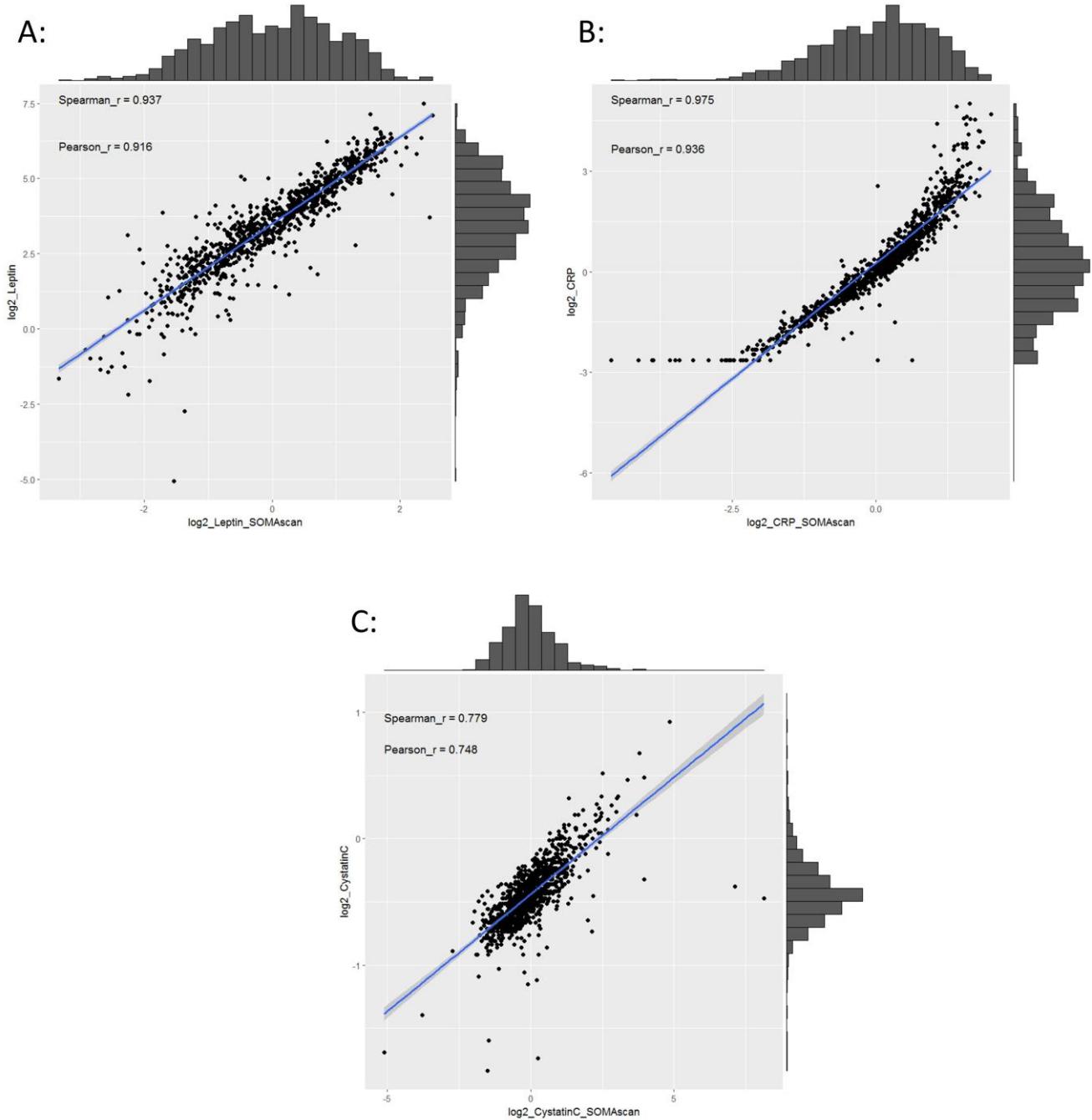
Figure S8: Correlation between SOMAscan measured and otherwise measured biomarkers in KORA (A: leptin by ELISA; B: CRP by nephelometry; C: cystatin-C by nephelometry.
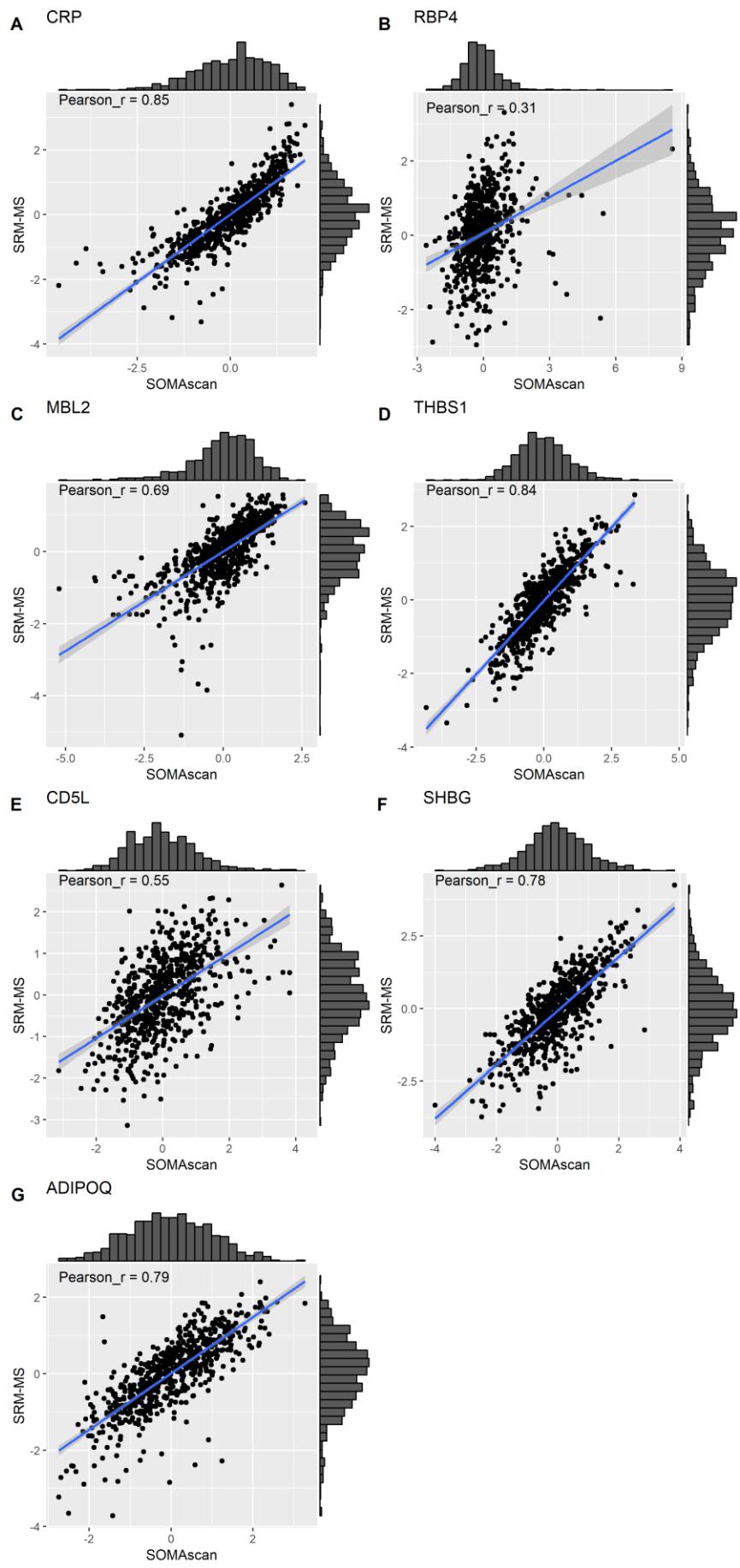
Figure S9: Correlation between SOMAscan measured and SRM-MS measured biomarkers in KORA
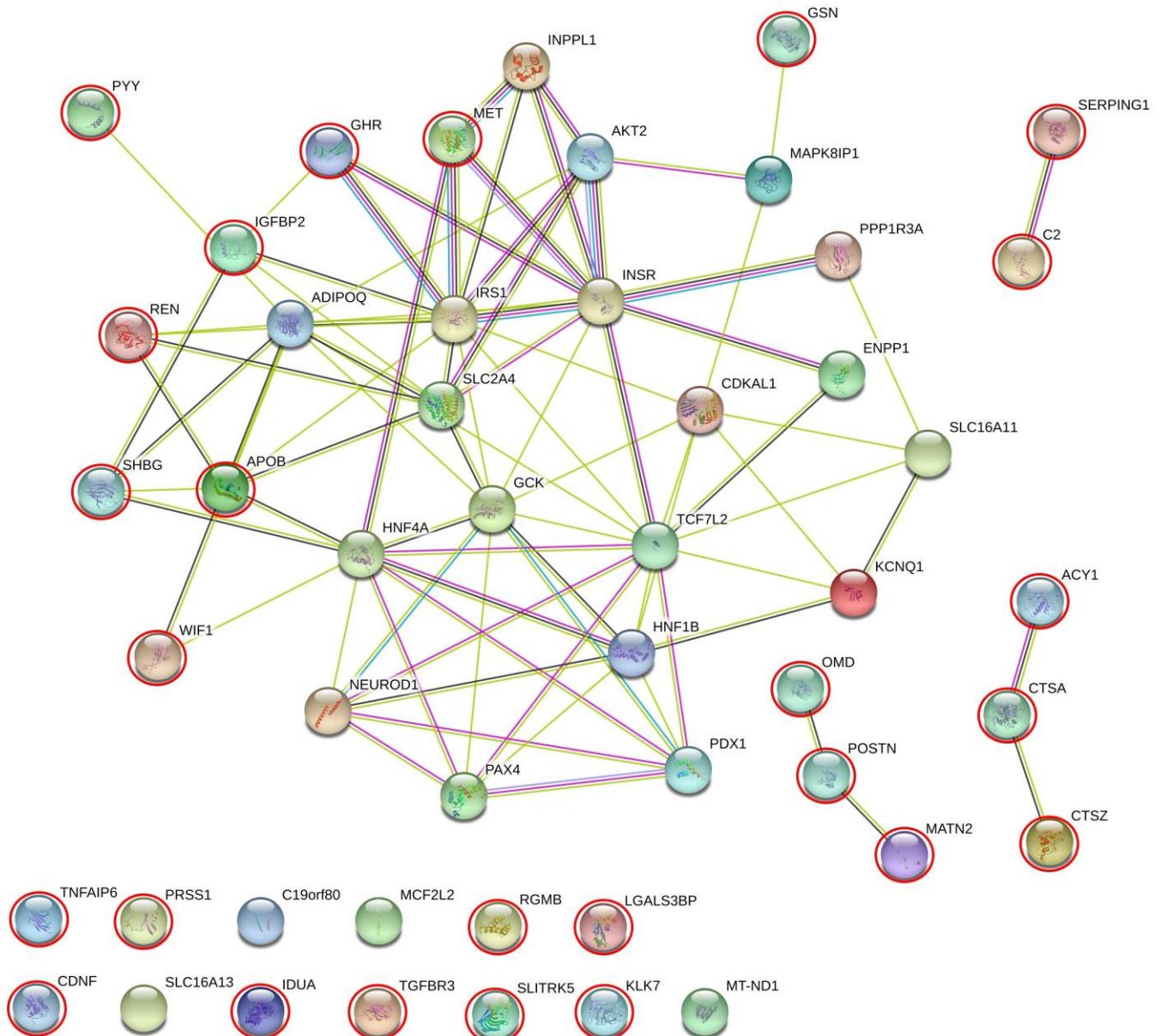
Figure S10: Network analysis using STRING featuring our replicated proteins, which are marked by red circles, and UniProt reported proteins associated with type 2 diabetes (https://version-11-0.string-db.org/cgi/network.pl?networkId=OzpbBXbgS2PW). Of the UniProt curated proteins, only two proteins were measured by SOMAscan. Adiponectin was significant in KORA but failed quality control in HUNT and could not be replicated and insulin receptor was not significant in our results.

References

1. Mühlenbruch K, Joost H-G, Boeing H, Schulze M. Risk prediction for type 2 diabetes in the German population with the updated German Diabetes Risk Score (GDRS). Ernährungs Umschau 2014;61:90-93

2. Paprott R, Mühlenbruch K, Mensink GBM, Thiele S, Schulze MB, Scheidt-Nave C, Heidemann C. Validation of the German Diabetes Risk Score among the general adult population: findings from the German Health Interview and Examination Surveys. BMJ Open Diabetes Research & Care 2016;4:e000280

3. Perna L, Mielck A, Lacruz ME, Emeny RT, von Eisenhart Rothe A, Meisinger C, Ladwig K-H. The association between resilience and diabetic neuropathy by socioeconomic position: Cross-sectional findings from the KORA-Age study. Journal of Health Psychology 2013;20:1222-1228

4. Emilsson V, Ilkov M, Lamb JR, Finkel N, Gudmundsson EF, Pitts R, Hoover H, Gudmundsdottir V, Horman SR, Aspelund T, Shu L, Trifonov V, Sigurdsson S, Manolescu A, Zhu J, Olafsson O, Jakobsdottir J, Lesley SA, To J, Zhang J, Harris TB, Launer LJ, Zhang B, Eiriksdottir G, Yang X, Orth AP, Jennings LL, Gudnason V. Co-regulatory networks of human serum proteins link genetics to disease. Science (New York, NY) 2018;361:769-773

5. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic acids research 2019;47:D607-d613

6. Consortium TU. UniProt: a worldwide hub of protein knowledge. Nucleic acids research 2019;47:D506-D515