

## SUPPLEMENTAL MATERIAL

1. **Online Supplemental Methods** (methods taken directly from manufacturer's protocol from Olink; please see full legend for details)
2. **Supplementary Table I.** Proteins assayed on the Olink platform "Cardiometabolic" and "Cardiovascular Disease III" panel.
3. **Supplementary Table II.** Proteins assayed on the Luminex platform in FHS. Median value of coefficient of variation for FHS plasma samples is provided. (Number of FHS samples with measurable protein concentration varied by protein.)
4. **Supplementary Table III.** Characteristics of 181 proteins assayed in the Olink platform. In the "Missing Observations" column, the number in the parenthesis specifies the number of samples (participants) in whom the protein was measured below the detection limit of the assay. The "Included\_in\_analysis" tab specifies whether the given protein was included in the present analysis (e.g., whether the protein is expressed above detection limit in >50% of the overall samples assayed). The P values specified are Bonferroni adjusted for multiple comparisons for the 139 proteins that were included in the analysis. NA refers to those entries where all sample values were "missing."
5. **Supplementary Table IV.** Genes encoding 19 proteins differentially expressed after bariatric surgery, and ratio of expression in the top and next highest expressing tissue, expressed as fold change (log).
6. **Supplementary Table V.** STRING-expanded set of 26 proteins for pathway analysis. This list contains contains 16/19 proteins identified from the bariatric surgical cohort, "augmented" by 10 additional proteins identified by STRING. Details in text.
7. **Supplementary Table VI.** Characteristics of FHS participants stratified by new-onset diabetes or metabolic syndrome. Values are expressed as mean with standard deviation or frequency and percentage. Comparisons for continuous variables were performed age- and sex-adjusted via a linear regression. Comparisons for categorical variables were performed age- and sex-adjusted via a logistic regression. The \* and \*\* indicate  $P < 0.05$  for comparison between individuals with or without diabetes (\*) or metabolic syndrome (\*\*).
8. **Supplementary Figure I.** Selection scheme for bariatric surgery discovery cohort. Run 1 is comprised of 10 subjects assayed serially at Visits 1-3 with (N=4) and without (N=6) diabetes at baseline; subjects with diabetes resolved their diabetes at subsequent study visits. An additional 30 samples (baseline Visit 1) were assayed as part of other efforts in the laboratory not connected to the current project, and were therefore not included. Run 2 included samples from subjects with diabetes at baseline visit (N=13) and subjects without diabetes with greatest relative change in hemoglobin A1c over Visit 1 to Visit 2. \*In Run 2, one participant with diabetes and serial Visit 1-3 samples was excluded in error (with a subject with pre-diabetes in its place in error); an additional 2 subjects in the non-

diabetic cohort of Run 2 did not have samples run (in error) and 1 subject only had 2 study visits assayed. Additional details in text.

9. **Supplementary Figure II.** Principal component analysis plot showing raw protein expression exhibiting batch effects between Run 1 and Run 2 (left) and after correction of batch effects (right) for the Cardiometabolic panel (A) and the Cardiovascular III panel (B).
10. **Supplementary Figure III.** Boxplot of 19 plasma proteins differentially expressed (at a type 1 error Bonferroni adjusted  $P < 0.05$ ) between Visit 1 and both post-operative time points.

## Online Supplemental Methods

***The following methods are reproduced from Olink User Manual (and this statement serves as the direct reference attribution/credit to the primary source for this material:***

***<https://www.olink.com/products/document-download-center/>***. Of note, the wording may be slightly modified below (relative to the methods on the website).

### **Proximity Extension Assay Protocol**

**Principle:** The Proseek reagents are based on PEA, a Proximity Extension Assay technology, in which 96 oligonucleotide-labeled antibody pairs can bind to their respective protein targets in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event and is subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format with no need for washing steps. There are three core steps; incubation, extension and detection.

#### **Reagents and consumables:**

1. Proseek Multiplex Probe Kit
  - a. Incubation Solution contains components needed for the incubation reaction.
  - b. A-probes contains 96 antibody probes labeled with A oligos
  - c. B-probes contains 96 antibody probes labeled with B oligos
2. Proseek Multiplex Detection Kit
  - a. PEA Solution contains components needed for the extension reaction
  - b. PEA Enzyme for extension of A and B probes which are bound to their target
  - c. PCR Polymerase, for pre-amplification of the extension product created by the PEA enzyme
  - d. Detection Solution contains components needed for the detection reaction
  - e. Detection Enzyme for qPCR amplification
  - f. Primer Plate 96-well plate with ready-to-use primers for amplification of extension product
3. Proseek Multiplex Controls
  - a. Interplate Control for normalization between runs
  - b. Negative Control for determination of background levels
  - c. Incubation Stabilizer for stabilization of the incubation reaction

4. Dynamic Array 96.96 GE (Fluidigm, South San Francisco, CA, USA)

**Experimental Design:**

Ninety plasma samples (20 µl each) organized randomly in a 96 well PCR plate. Plate positions C12, D12 and E12 left empty for Negative Control, F12, G12 and H12 left empty for Interplate Control.

**Sample Dilutions:**

Plasma samples diluted 1:100 for Cardiovascular III panel (Catalog No: 94600) with Sample Diluent and 1:2025 for Cardiometabolic Panel (Catalog No: 94360).

**Incubation:**

1. Plasma samples thawed, vortexed and spun down at 150 xg for 1 min at room temperature and diluted (see above Sample Dilution section).
2. Incubation Stabilizer thawed, vortexed and spun briefly.
3. Interplate Control and Negative Control tubes thawed, vortexed and spun briefly.
4. Following Incubation mix prepared in a microcentrifuge tube.

Incubation Solution	280.0 µl
Incubation Stabilizer	40.0 µl
A-probes	40.0 µl
B-probes	40.0 µl
<b>Total</b>	<b>400.0 µl</b>

5. This incubation mix vortexed briefly and spun down the content and 47 µL of this mixture transferred into each well of an 8-well strip.
6. Three µL of the Incubation mix from the 8-well strip to the bottom of each well of a 96-well plate pipetted by a multichannel pipet.
7. One µL of each diluted plasma samples added to the bottom of the well of the Incubation Plate according to our plate layout.
8. One µL of Negative Control added to the bottom of each Negative Control well in positions (C12, D12 and E12 according to the plate layout).

9. One 1  $\mu\text{L}$  of Interplate Control added to the bottom of each Negative Control well in positions (F12, G12 and H12 according to the plate layout).
10. Incubation Plate sealed with an adhesive plastic film and plate spun at 150 xg for 1 min at room temperature.
11. Incubation Plate incubated overnight at +4°C.

**Extension:**

12. PEA Solution thawed, vortexed and spun briefly.
13. Extension mix in a centrifuge tube prepared according to following pipetting schedule in a freezing block.

High Purity Water	9,385.0 $\mu\text{l}$
PEA Solution	1,100.0 $\mu\text{l}$
PEA Enzyme	55.0 $\mu\text{l}$
PCR Polymerase	22.0 $\mu\text{l}$

**Total            10,562.0  $\mu\text{l}$**

14. Extension mix vortexed.
15. Incubation Plate brought to room temperature, spun down at 150 x g for 1 min at room temperature.
16. Extension mix poured into a multi-channel pipette reservoir.
17. Plastic adhesive film carefully removed from the Incubation Plate.
18. Ninety-six  $\mu\text{L}$  of Extension mix pipetted into to each well of the Incubation Plate by using reverse pipetting within 5 min.
19. New adhesive film sealed to the Incubation Plate.
20. Plate vortexed gently and spun down at 150 xg for 1 min at room temperature.
21. After the 5 min, Incubation Plate placed in the thermal cyclor and following PEA program performed.

Extension	50°C	20 min
Hot start	95°C	5 min
PCR Cycle	95°C	30 s
	54°C	1 min
	60°C	1 min total of 17 cycles
Hold	10°C	∞

**Detection:**

22. Control fluid injected to a Dynamic Array 96.96 GE and placed on an IFC Controller HX and primed.

23. Primer Plate thawed, vortexed and spun at 150 xg for 1 min at room temperature.

24. Detection Solution thawed, vortexed and spun briefly and Detection Mix in a centrifuge tube is prepared according to following pipetting schedule in a freezing block.

Detection Solution	550.0 µl
High Purity Water	230.0 µl
Detection Enzyme	7.8 µl
PCR Polymerase	3.1 µl

**Total            790.9 µl**

25. Detection mix spun briefly and transferred 95 µL into each well of an 8-well strip.

26. 7.2 µl transferred into each well of a new 96-well plate by reverse pipetting.

27. Incubation Plate removed from the thermal cycler, vortexed and spun briefly.

28. Plastic film carefully removed and 2.8 µL transferred from each well of the Incubation Plate to the Sample Plate prepared above.

29. Sample Plate sealed with a new plastic adhesive film, vortexed and spun at 150 xg for 1 min at room temperature.

30. Primer Plate seal gently removed to avoid contamination between wells.

31. 5 µl from each well of the Primer Plate transferred into Assay Inlets of the primed Dynamic Array 96.96 GE by using reverse pipetting.
32. 5 µl from each well of the Sample Plate transferred into the Sample Inlets of the Dynamic Array 96.96 GE by using reverse pipetting.
33. Dynamic Array 96.96 GE placed on IFC Controller HX and load program started.
34. When load completed Dynamic Array 96.96 GE removed from IF Controller HX and placed on BioMark HD System.
35. Olink Protein Expression 96×96 Program started on BioMark Data Collection Software.
36. When program completed Fluidigm Real Time PCR Analysis Software started and in sample inlets sample names entered. For Assay names “.plt” file which recognized by Fluidigm Real Time PCR Analysis Software downloaded from [www.olink.com/products/proseek-multiplex/downloads/data-analysis-files](http://www.olink.com/products/proseek-multiplex/downloads/data-analysis-files)
37. Quality Treshold set to 0.5 and Linear mode and Auto (global) selected under Analysis Settings. Then Analysis performed. When analysis completed file saved and exported as a Heat Map Results.

#### **Data Analysis:**

Exported csv files in Heat Map Result form from BioMark Real Time PCR Analysis Software imported into Olink NPX Manager Software v1.0.0.2 and NPX values obtained by normalizing raw Cq values to inter-plate controls.

#### **References**

1. Lundberg, M., et.al. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low abundant proteins in human blood. *Nucleic Acid Res* 6 June (2011). doi: 10.1093/nar/gkr424.
2. Assarsson E., et.al. Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. *PLOS One* 6 April (2014)

Supplementary Table I.

Cardiometabolic Panel			
Protein Name (Short Name)	Uniprot Nr.	Protein Name (Short Name)	Uniprot Nr.
Angiogenin (ANG)	P03950	Low affinity immunoglobulin gamma Fc region receptor II-a (FCGR2A)	P12318
Angiopoietin-related protein 3 (ANGPTL3)	Q9Y5C1	Low affinity immunoglobulin gamma Fc region receptor III-B (FCGR3B)	O75015
Apolipoprotein M (APOM)	O95445	Lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1)	Q9Y5Y7
Beta-Ala-His dipeptidase (CNDP1)	Q96KN2	Lysosomal Pro-X carboxypeptidase (PRCP)	P42785
Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)	P15907	Mannose-binding protein C (MBL2)	P11226
C-C motif chemokine 14 (CCL14)	Q16627	Mast/stem cell growth factor receptor Kit (KIT)	P10721
C-C motif chemokine 18 (CCL18)	P55774	Membrane cofactor protein (CD46)	P15529
C-C motif chemokine 5 (CCL5)	P13501	Membrane primary amine oxidase (AOC3)	Q16853
Cadherin-1 (CDH1)	P12830	Metalloproteinase inhibitor 1 (TIMP1)	P01033
Carbonic anhydrase 1 (CA1)	P00915	Microfibrillar-associated protein 5 (MFAP5)	Q13361
Carbonic anhydrase 3 (CA3)	P07451	Multiple epidermal growth factor-like domains protein 9 (MEGF9)	Q9H1U4
Carbonic anhydrase 4 (CA4)	P22748	Neural cell adhesion molecule 1 (NCAM1)	P13591
Cartilage acidic protein 1 (CRTAC1)	Q9NQ79	Neural cell adhesion molecule L1-like protein (CHL1)	O00533
Cartilage oligomeric matrix protein (COMP)	P49747	Neurogenic locus notch homolog protein 1 (NOTCH1)	P46531
CD59 glycoprotein (CD59)	P13987	Neuropilin-1 (NRP1)	O14786
Coagulation factor VII (F7)	P08709	Neutrophil defensin 1 (DEFA1)	P59665
Coagulation factor XI (F11)	P03951	Neutrophil gelatinase-associated lipocalin (LCN2)	P80188
Collagen alpha-1(XVIII) chain (COL18A1)	P39060	Nidogen-1 (NID1)	P14543
Complement C1q tumor necrosis factor-related protein 1 (C1QTNF1)	Q9BXJ1	Oncostatin-M-specific receptor subunit beta (OSMR)	Q99650
Complement C2 (C2)	P06681	Peptidyl-glycine alpha-amidating monooxygenase (PAM)	P19021
Complement factor H-related protein 5 (CFHR5)	Q9BXR6	Phospholipid transfer protein (PLTP)	P55058
Complement receptor type 2 (CR2)	P20023	Plasma serine protease inhibitor (SERPINA5)	P05154
Cystatin-C (CST3)	P01034	Platelet glycoprotein Ib alpha chain (GP1BA)	P07359
Dipeptidyl peptidase 4 (DPP4)	P27487	Platelet-activating factor acetylhydrolase (PLA2G7)	Q13093
EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1)	Q12805	Plexin-B2 (PLXNB2)	O15031
Endoglin (ENG)	P17813	Procollagen C-endopeptidase enhancer 1 (PCOLCE)	Q15113
Fetuin-B (FETUB)	Q9UGM5	Prolyl endopeptidase FAP (FAP)	Q12884
Ficolin-2 (FCN2)	Q15485	Receptor-type tyrosine-protein phosphatase S (PTPRS)	Q13332
Glutamyl-peptide cyclotransferase (QPCT)	Q16769	Regenerating islet-derived protein 3-alpha (REG3A)	Q06141
Granulysin (GNLY)	P22749	Serum amyloid A-4 protein (SAA4)	P35542
Growth arrest-specific protein 6 (GAS6)	Q14393	SPARC-like protein 1 (SPARCL1)	Q14515
Hepatocyte growth factor receptor (MET)	P08581	Superoxide dismutase [Cu-Zn] (SOD1)	P00441
Ig lambda-2 chain C regions (IGLC2)	P0CG05	T-cell immunoglobulin and mucin domain-containing protein 4 (TIMD4)	Q96H15
Insulin-like growth factor-binding protein 3 (IGFBP3)	P17936	Tenascin (TNC)	P24821
Insulin-like growth factor-binding protein 6 (IGFBP6)	P24592	Tenascin-X (TNXB)	P22105
Integrin alpha-M (ITGAM)	P11215	Thrombospondin-4 (THBS4)	P35443
Intercellular adhesion molecule 1 (ICAM1)	P05362	Thyroxine-binding globulin (SERPINA7)	P05543
Intercellular adhesion molecule 3 (ICAM3)	P32942	Transcobalamin-2 (TCN2)	P20062
Interleukin-7 receptor subunit alpha (IL7R)	P16871	Transforming growth factor beta receptor type 3 (TGFB3)	Q03167
L-selectin (SELL)	P14151	Transforming growth factor-beta-induced protein ig-h3 (TGFB1)	Q15582
Latent-transforming growth factor beta-binding protein 2 (LTBP2)	Q14767	Trypsin-2 (PRSS2)	P07478
Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1)	Q8NHL6	Tyrosine-protein kinase receptor Tie-1 (TIE1)	P35590
Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2)	Q8N423	Uromodulin (UMOD)	P07911
Leukocyte immunoglobulin-like receptor subfamily B member 5 (LILRB5)	O75023	Vascular cell adhesion protein 1 (VCAM1)	P19320
Lithostathine-1-alpha (REG1A)	P05451	Vasorin (VASN)	Q6EMK4
Liver carboxylesterase 1 (CES1)	P23141	Vitamin K-dependent protein C (PROC)	P04070



**CVD III Panel (CCL2 removed from the panel by Olink)**

<b>Protein Name (Short Name)</b>	<b>Uniprot Nr.</b>	<b>Protein Name (Short Name)</b>	<b>Uniprot Nr.</b>
Aminopeptidase N (AP-N)	P15144	Matrix metalloproteinase-2 (MMP-2)	P08253
Azurocidin (AZU1)	P20160	Matrix metalloproteinase-3 (MMP-3)	P08254
Bleomycin hydrolase (BLM hydrolase)	Q13867	Matrix metalloproteinase-9 (MMP-9)	P14780
C-C motif chemokine 15 (CCL15)	Q16663	Metalloproteinase inhibitor 4 (TIMP4)	Q99727
C-C motif chemokine 16 (CCL16)	O15467	Monocyte chemotactic protein 1 (MCP-1)	P13500
C-C motif chemokine 24 (CCL24)	O00175	Myeloblastin (PRTN3)	P24158
C-X-C motif chemokine 16 (CXCL16)	Q9H2A7	Myeloperoxidase (MPO)	P05164
Cadherin-5 (CDH5)	P33151	Myoglobin (MB)	P02144
Carboxypeptidase A1 (CPA1)	P15085	N-terminal prohormone brain natriuretic peptide (NT-proBNP)	NA
Carboxypeptidase B (CPB1)	P15086	Neurogenic locus notch homolog protein 3 (Notch 3)	Q9UM47
Caspase-3 (CASP-3)	P42574	Osteopontin (OPN)	P10451
Cathepsin D (CTSD)	P07339	Osteoprotegerin (OPG)	O00300
Cathepsin Z (CTSZ)	Q9UBR2	P-selectin (SELP)	P16109
CD166 antigen (ALCAM)	Q13740	Paraoxonase (PON3)	Q15166
Chitinase-3-like protein 1 (CHI3L1)	P36222	Peptidoglycan recognition protein 1 (PGLYRP1)	O75594
Chitotriosidase-1 (CHIT1)	Q13231	Perlecan (PLC)	P98160
Collagen alpha-1(I) chain (COL1A1)	P02452	Plasminogen activator inhibitor 1 (PAI)	P05121
Complement component C1q receptor (CD93)	Q9NPY3	Platelet endothelial cell adhesion molecule (PECAM-1)	P16284
Contactin-1 (CNTN1)	Q12860	Platelet-derived growth factor subunit A (PDGF subunit A)	P04085
Cystatin-B (CSTB)	P04080	Proprotein convertase subtilisin/kexin type 9 (PCSK9)	Q8NBP7
E-selectin (SELE)	P16581	Protein delta homolog 1 (DLK-1)	P80370
Elafin (PI3)	P19957	Pulmonary surfactant-associated protein D (PSP-D)	P35247
Ephrin type-B receptor 4 (EPHB4)	P54760	Resistin (RETN)	Q9HD89
Epidermal growth factor receptor (EGFR )	P00533	Retinoic acid receptor responder protein 2 (RARRES2)	Q99969
Epithelial cell adhesion molecule (Ep-CAM)	P16422	Scavenger receptor cysteine-rich type 1 protein M130 (CD163)	Q86VB7
Fatty acid-binding protein, adipocyte (FABP4)	P15090	Secretoglobin family 3A member 2 (SCGB3A2)	Q96PL1
Galectin-3 (Gal-3)	P17931	Spondin-1 (SPON1)	Q9HCB6
Galectin-4 (Gal-4)	P56470	ST2 protein (ST2)	Q01638
Granulins (GRN)	P28799	Tartrate-resistant acid phosphatase type 5 (TR-AP)	P13686
Growth/differentiation factor 15 (GDF-15)	Q99988	Tissue factor pathway inhibitor (TFPI)	P10646
Insulin-like growth factor-binding protein 1 (IGFBP-1)	P08833	Tissue-type plasminogen activator (t-PA)	P00750
Insulin-like growth factor-binding protein 2 (IGFBP-2)	P18065	Transferrin receptor protein 1 (TR)	P02786
Insulin-like growth factor-binding protein 7 (IGFBP-7)	Q16270	Trefoil factor 3 (TFF3)	Q07654
Integrin beta-2 (ITGB2)	P05107	Trem-like transcript 2 protein (TLT-2)	Q5T2D2
Intercellular adhesion molecule 2 (ICAM-2)	P13598	Tumor necrosis factor ligand superfamily member 13B (TNFSF13B)	Q9Y275
Interleukin-1 receptor type 1 (IL-1RT1)	P14778	Tumor necrosis factor receptor 1 (TNF-R1)	P19438
Interleukin-1 receptor type 2 (IL-1RT2)	P27930	Tumor necrosis factor receptor 2 (TNF-R2)	P20333
Interleukin-17 receptor A (IL-17RA)	Q96F46	Tumor necrosis factor receptor superfamily member 10C (TNFRSF10C)	O14798
Interleukin-18-binding protein (IL-18BP)	O95998	Tumor necrosis factor receptor superfamily member 14 (TNFRSF14)	Q92956
Interleukin-2 receptor subunit alpha (IL2-RA)	P01589	Tumor necrosis factor receptor superfamily member 6 (FAS )	P25445
Interleukin-6 receptor subunit alpha (IL-6RA)	P08887	Tyrosine-protein kinase receptor UFO (AXL)	P30530
Junctional adhesion molecule A (JAM-A)	Q9Y624	Tyrosine-protein phosphatase non-receptor type substrate 1 (SHPS-1)	P78324
Kallikrein-6 (KLK6 )	Q92876	Urokinase plasminogen activator surface receptor (U-PAR)	Q03405
Low-density lipoprotein receptor (LDL receptor)	P01130	Urokinase-type plasminogen activator (uPA)	P00749
Lymphotoxin-beta receptor (LTBR)	P36941	von Willebrand factor (vWF)	P04275
Matrix extracellular phosphoglycoprotein (MEPE)	Q9NQ76	C-C motif chemokine 22 (CCL2)	O00626

**Supplementary Table II.** Proteins assayed on the Luminex platform in FHS. Median value of coefficient of variation for FHS plasma samples is provided. (Number of FHS samples with measurable protein concentration varied by protein.)

Plasma Protein		FHS samples for incident diabetes			FHS samples for incident metabolic syndrome		
Abbreviation	Protein name	N	Mean CV	Median CV	N	Mean CV	Median CV
CD163	Cluster of differentiation 163	4876	2.98	2.40	3970	2.95	2.40
GMP140	Granule membrane protein 140	4875	5.53	4.17	3969	5.45	4.13
IGFBP2	Insulin-like growth factor binding protein 2	4866	3.89	3.00	3963	3.80	2.90
IGFBP1	Insulin-like growth factor-binding protein 1	4649	2.66	2.00	3849	2.57	1.90
PAI1	Plasminogen activator inhibitor 1	4877	3.55	2.37	3971	3.59	2.41
LDLR	LDL receptor	4873	3.06	2.20	3968	3.09	2.25



CES1	32 (23)	32 (30)	32 (31)	1.75	1.24	1.56	0.43	0.02	NA	1.72 (1.47 - 1.91)	1.24 (1.23 - 1.24)	1.56 (1.56 - 1.56)	NO	NA	NA	NA	NA
CHL1	32 (22)	32 (14)	32 (20)	1.35	1.41	1.44	0.14	0.17	0.16	1.31 (1.26 - 1.35)	1.37 (1.27 - 1.52)	1.47 (1.31 - 1.53)	NO	NA	NA	NA	NA
DFAL	32 (22)	32 (24)	32 (22)	1.1	0.72	0.65	1.35	0.14	0.09	0.65 (0.61 - 0.81)	0.68 (0.64 - 0.76)	0.61 (0.59 - 0.72)	NO	NA	NA	NA	NA
ENG	32 (18)	32 (18)	32 (21)	0.16	0.12	0.13	0.21	0.16	0.2	0.12 (-0.04 - 0.33)	0.09 (-0.03 - 0.29)	0.09 (-0.01 - 0.24)	NO	NA	NA	NA	NA
EPH4	32 (20)	32 (18)	32 (15)	0.57	0.5	0.56	0.11	0.09	0.13	0.59 (0.48 - 0.63)	0.49 (0.45 - 0.52)	0.56 (0.48 - 0.62)	NO	NA	NA	NA	NA
FAP	32 (25)	32 (25)	32 (25)	0.93	0.96	1	0.2	0.18	0.19	0.89 (0.76 - 1.1)	0.98 (0.81 - 1.08)	0.92 (0.86 - 1.14)	NO	NA	NA	NA	NA
FCGR2A	32 (0)	32 (0)	32 (0)	-0.05	-0.24	-0.23	0.46	0.47	0.55	0.07 (-0.18 - 0.23)	-0.13 (-0.43 - 0.12)	-0.09 (-0.5 - 0.22)	NO	NA	NA	NA	NA
FTUB	32 (0)	32 (0)	32 (0)	-0.99	-1.12	-1.18	0.48	0.42	0.52	-1 (-1.22 - -0.72)	-1.21 (-1.42 - -0.88)	-1.28 (-1.54 - -0.99)	NO	NA	NA	NA	NA
GNLY	32 (26)	32 (28)	32 (27)	0.42	0.4	0.44	0.14	0.03	0.13	0.36 (0.35 - 0.44)	0.4 (0.38 - 0.41)	0.46 (0.32 - 0.48)	NO	NA	NA	NA	NA
ICAM3	32 (20)	32 (20)	32 (19)	0.98	1.01	0.97	0.19	0.2	0.22	0.95 (0.81 - 1.16)	0.99 (0.82 - 1.18)	0.91 (0.81 - 1.07)	NO	NA	NA	NA	NA
IL7R	32 (28)	32 (29)	32 (29)	0.52	0.83	0.69	0.05	0.25	0.2	0.53 (0.49 - 0.57)	0.97 (0.75 - 0.97)	0.62 (0.58 - 0.77)	NO	NA	NA	NA	NA
ITGAM	32 (27)	32 (26)	32 (27)	0.65	0.65	0.77	0.13	0.15	0.2	0.64 (0.58 - 0.7)	0.68 (0.52 - 0.78)	0.81 (0.59 - 0.84)	NO	NA	NA	NA	NA
IT	32 (20)	32 (18)	32 (21)	1.84	1.94	2.05	0.23	0.21	0.26	1.78 (1.68 - 1.98)	1.95 (1.76 - 2.11)	1.95 (1.89 - 2.24)	NO	NA	NA	NA	NA
IIRB1	32 (0)	32 (0)	32 (0)	-1.46	-1.68	-1.52	0.29	0.3	0.33	-1.47 (-1.63 - -1.26)	-1.64 (-1.85 - -1.47)	-1.54 (-1.75 - -1.29)	NO	NA	NA	NA	NA
LTRP2	32 (32)	32 (31)	32 (32)	NA	1.8	NA	NA	NA	NA	NA (NA - NA)	1.8 (1.8 - 1.8)	NA (NA - NA)	NO	NA	NA	NA	NA
MEGF9	32 (32)	32 (30)	32 (30)	NA	1.85	1.92	NA	0.05	0.04	NA (NA - NA)	1.85 (1.83 - 1.86)	1.92 (1.91 - 1.94)	NO	NA	NA	NA	NA
MET	32 (27)	32 (21)	32 (24)	1.41	1.35	1.42	0.14	0.18	0.17	1.41 (1.28 - 1.48)	1.29 (1.21 - 1.45)	1.4 (1.35 - 1.45)	NO	NA	NA	NA	NA
MFAP5	32 (24)	32 (20)	32 (22)	1.33	1.38	1.39	0.23	0.27	0.23	1.36 (1.24 - 1.39)	1.31 (1.18 - 1.55)	1.34 (1.18 - 1.58)	NO	NA	NA	NA	NA
NRP1	32 (0)	32 (13)	32 (8)	-0.57	-0.57	-0.54	0.14	0.14	0.12	-0.59 (-0.7 - -0.48)	-0.61 (-0.68 - -0.47)	-0.53 (-0.63 - -0.46)	NO	NA	NA	NA	NA
OSMR	32 (1)	32 (0)	32 (0)	-0.83	-0.89	-0.87	0.28	0.24	0.24	-0.79 (-1.08 - -0.65)	-0.89 (-1.04 - -0.73)	-0.85 (-1.06 - -0.78)	NO	NA	NA	NA	NA
PAM	32 (0)	32 (0)	32 (0)	-0.33	-0.42	-0.41	0.28	0.31	0.25	-0.37 (-0.51 - -0.15)	-0.39 (-0.67 - -0.28)	-0.45 (-0.58 - -0.28)	NO	NA	NA	NA	NA
PLA2G7	32 (24)	32 (23)	32 (23)	1.42	1.37	1.36	0.13	0.08	0.19	1.4 (1.35 - 1.48)	1.4 (1.28 - 1.45)	1.36 (1.19 - 1.47)	NO	NA	NA	NA	NA
PLTP	32 (29)	32 (31)	32 (29)	1.55	1.62	1.59	0.01	NA	0.06	1.55 (1.54 - 1.56)	1.62 (1.62 - 1.62)	1.59 (1.56 - 1.62)	NO	NA	NA	NA	NA
PLXNB2	32 (24)	32 (22)	32 (22)	0.09	0.07	0.08	0.12	0.13	0.14	0.08 (0.04 - 0.12)	0.03 (0 - 0.09)	0.1 (-0.04 - 0.15)	NO	NA	NA	NA	NA
PRCP	32 (20)	32 (29)	32 (23)	0.36	0.19	0.17	0.23	0.08	0.11	0.3 (0.24 - 0.42)	0.2 (0.15 - 0.23)	0.13 (0.09 - 0.22)	NO	NA	NA	NA	NA
PSP.D	32 (23)	32 (20)	32 (22)	1.24	1.18	1.12	0.29	0.26	0.31	1.12 (1.08 - 1.46)	1.18 (0.92 - 1.35)	1.09 (0.89 - 1.19)	NO	NA	NA	NA	NA
PTPRG	32 (18)	32 (21)	32 (14)	-0.13	-0.12	-0.1	0.15	0.14	0.15	-0.14 (-0.23 - -0.04)	-0.18 (-0.25 - -0.02)	-0.11 (-0.24 - 0)	NO	NA	NA	NA	NA
REG3A	32 (32)	32 (32)	32 (32)	NA	NA	NA	NA	NA	NA	NA (NA - NA)	NA (NA - NA)	NA (NA - NA)	NO	NA	NA	NA	NA
SOD1	32 (32)	32 (32)	32 (32)	NA	NA	NA	NA	NA	NA	NA (NA - NA)	NA (NA - NA)	NA (NA - NA)	NO	NA	NA	NA	NA
SPARC1	32 (25)	32 (22)	32 (24)	0.91	0.86	0.98	0.15	0.15	0.19	0.89 (0.87 - 1)	0.87 (0.73 - 0.99)	0.96 (0.89 - 1.07)	NO	NA	NA	NA	NA
SPOW1	32 (23)	32 (24)	32 (21)	0.64	0.76	0.76	0.43	0.52	0.45	0.4 (0.34 - 1.15)	0.51 (0.33 - 1.24)	0.55 (0.35 - 1.19)	NO	NA	NA	NA	NA
TGFBF3	32 (31)	32 (27)	32 (28)	2.03	1.99	2.02	NA	0.1	0.19	2.03 (2.03 - 2.03)	2.05 (1.89 - 2.06)	1.96 (1.89 - 2.09)	NO	NA	NA	NA	NA
TIE1	32 (0)	32 (0)	32 (0)	-0.03	-0.15	-0.13	0.23	0.25	0.25	0 (-0.19 - 0.06)	-0.09 (-0.34 - 0)	-0.16 (-0.35 - 0)	NO	NA	NA	NA	NA
TNC	32 (1)	32 (0)	32 (0)	-0.09	-0.03	-0.1	0.43	0.5	0.48	-0.15 (-0.33 - 0.06)	-0.08 (-0.36 - 0.25)	-0.11 (-0.38 - 0.19)	NO	NA	NA	NA	NA
TVXB	32 (24)	32 (21)	32 (23)	-0.09	-0.07	-0.05	0.11	0.1	0.2	-0.09 (-0.18 - -0.01)	-0.09 (-0.15 - -0.01)	-0.08 (-0.24 - 0.08)	NO	NA	NA	NA	NA
UMOD	32 (29)	32 (30)	32 (30)	0.27	0.23	0.37	0.08	0.02	0.13	0.23 (0.22 - 0.29)	0.23 (0.22 - 0.23)	0.37 (0.32 - 0.41)	NO	NA	NA	NA	NA
VASN	32 (16)	32 (19)	32 (21)	-0.23	-0.14	-0.18	0.13	0.11	0.17	-0.25 (-0.32 - -0.16)	-0.1 (-0.23 - -0.09)	-0.24 (-0.32 - -0.03)	NO	NA	NA	NA	NA

**Supplementary Table IV.** Genes encoding 19 proteins differentially expressed after bariatric surgery, and ratio of expression in the top and next highest expressing tissue, expressed as fold change (log).

<b>Gene</b>	<b>Comparison between top expressed tissue and next expressed</b>	<b>log-FC</b>
C1QTNF1	adipose_tissue-heart	0.88
ITGB2	bone_marrow-lymph_node	1.11
CNDP1	cerebral_cortex-liver	0.19
SPP1	gall_bladder-kidney	0.50
COMP	gall_bladder-smooth_muscle_tissue	0.64
COL1A1	gall_bladder-smooth_muscle_tissue	0.63
FCN2	liver-adrenal_gland	3.97
LDLR	liver-adrenal_gland	0.99
SERPINE1	liver-gall_bladder	0.68
IGFBP1	liver-kidney	10.57
CTSD	liver-lung	0.16
RARRES2	liver-pancreas	0.32
SAA4	liver-saliva_secreting_gland	8.31
PON3	liver-saliva_secreting_gland	4.03
IGFBP2	pancreas-liver	0.67
PLAT	urinary_bladder-gall_bladder	0.91
SELE	urinary_bladder-smooth_muscle_tissue	0.50
SELP	urinary_bladder-smooth_muscle_tissue	0.24
CD163	vermiform_appendix-smooth_muscle_tissue	0.24

**Supplementary Table V.** STRING-expanded set of 26 proteins for pathway analysis. This list contains 16/19 proteins identified from the bariatric surgical cohort, “augmented” by 10 additional proteins identified by STRING. Details in text.

COL1A1  
LDLR  
IGFBP1  
ITGB2  
CTSD  
IGFBP2  
SELP  
SPP1  
SELE  
SERPINE1  
CD163  
PLAT  
RARRES2  
COMP  
FCN2  
PON3  
ALB  
IL6  
EGF  
VEGFA  
PLG  
INS  
VWF  
IGF1  
TGFB1  
ITGA2B

**Supplementary Table VI.** Characteristics of FHS participants stratified by new-onset diabetes or metabolic syndrome. Values are expressed as mean with standard deviation or frequency and percentage. Comparisons for continuous variables were performed age- and sex-adjusted via a linear regression. Comparisons for categorical variables were performed age- and sex-adjusted via a logistic regression. The \* and \*\* indicate P<0.05 for comparison between individuals with or without diabetes (\*) or metabolic syndrome (\*\*).

Characteristics	Diabetes cohort (N=4877)		Metabolic syndrome cohort (N=3971)	
	Participants without diabetes (N=4685)	New-onset diabetes (N=192)*	Participants without metabolic syndrome (N=3543)	new-onset metabolic syndrome(428)**
Age, year	48.1±12.8	56.3±11.6*	46.6±12.7	52.1±11.6**
Women (n, %)	2529 (54%)	81 (42%)*	1983 (56%)	222 (52%)
Framingham cohort (% in the Offspring cohort, versus Third Generation )	1971 (42%)	125 (65%)	1335 (38%)	243 (57%)
Systolic blood pressure, mmHg	119.7±15.9	132.7±17.6*	116.7±14.6	124.7±15.3**
Diastolic blood pressure, mmHg	74.8±9.5	78.8±9.9*	73.4±9.0	77.0±9.0**
Current cigarette smoker (n, %)	594 (13%)	22 (11%)	423(12%)	77 (18%)
Body mass index, kg/m <sup>2</sup>	27.0±5.0	32.4±6.3*	25.7±4.1	29.8±4.8**
Waist circumference, cm	82.9±12.2	96.3±12.9*	79.4±10.6	90.2±10.8**
Sedentary status (n, %)	411 (9%)	14 (7%)	289 (8%)	53 (12%)
High school degree or less (n, %)	1022 (22%)	67 (35%)*	663 (19%)	133 (31%)
Some college education (n, %)	1402 (30%)	62 (32%)	1040(29%)	139 (32%)
College graduate or more (n, %)	2261 (48%)	63 (33%)	1840(52%)	156 (36%)
Drinks per week, cups	5.4±7.7	4.9±7.3*	5.4±7.3	5.2±7.0
Insulin perSTD ,pmol/l	51.3±39.4	96.7±70.5*	42.2±29.8.	66.8±41.4**
Fasting glucose mg/dl	94.5±8.7	110.2±9.6*	92.7±8.0	97.1±8.1**

High density lipoprotein, mg/dl	54.8±16.2	46.8±15.2*	57.9±15.8	50.7±15.4**
Triglycerides, mg/dl	118.3±76.2	173.8±127.7*	98.1±53.9	140.6±73.0**
Insulin-like growth factor binding protein 2 (IGFBP2), pg/mL	11013991.5±6540219.4	8199166.7±5776225.2*	11793590.2±6665711.4	9288644.9±5762659.5**
Insulin-like growth factor binding protein 1, pg/mL	11445.2±14155.2	7535.9±8598.0*	12866.0±15141.6	8849.9±11498.5**
P-selectin (GMP-140), pg/ml	16147.8±4997.9	17194.2±5215.9	15760±4889.1	16523.9±4570.1**
Plasminogen activator inhibitor-1 (PAI-1), pg/ml	18220.8±10129.6	25164.4±12683.0*	16467.5.4±6665711.4	20776.4±10503.4**
Cluster of differentiation 163 (CD163), pg/ml	113173.9±71974.8	122132.3±56538.7	110007.8±15141.6	113899.3±66050.5
Low density lipoprotein receptor (LDL-R), pg/ml	235232.4±302173.4	234412.0±257652.8	235611.1±315201	208365.2±206933.5