Reduced platelet glycoprotein Ibα shedding accelerates thrombopoiesis and COX-1 recovery: implications for aspirin dosing regimen

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Supplementary extended study participants, methods and results Appendix

Title: Reduced platelet glycoprotein Ibα shedding accelerates thrombopoiesis and COX-1 recovery: implications for aspirin dosing regimen

Short Title: Platelet lifespan imbalance and aspirin response

Online Supplementary Methods

Study participants

For all patients, inclusion criteria included: age between 45 and 80 years, body mass index (BMI) between 20 and 45 kg/m^2. A diagnosis of overt type 2 diabetes mellitus (T2DM), according with the ADA criteria[1], was also required for the T2DM group.

Exclusion criteria were poorly controlled hypertension or hypercholesterolemia, smoking, pregnancy or lactation, impaired liver or renal function, history of malignant neoplasms (diagnosed and treated within the last 5 years), history of malabsorption, regular (daily) consumption of alcohol, or treatment with non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants or other antiplatelet drugs. Type 1 diabetes was suspected and excluded with islet autoantibodies evaluation (anti-glutamic acid decarboxylase, islet cell cytoplasmic, and IA-2 antibodies), when one of the following applied: family history of type 1 diabetes, age lower than 40, lean phenotype, precocious requirement for insulin therapy. No patient was diagnosed as having MODY (Maturity Onset Diabetes of the Young).

Liver Ultrasound

The grading of liver steatosis has been obtained using a high-resolution ultrasound System (GE Heathcare), equipped with a 3.5-MHz convex- array probe. Subjects were examined in the supine position. Ultrasound features included liver brightness, contrast between the liver and the kidney, US appearance of the intrahepatic vessels, liver parenchyma and diaphragm. Steatosis was graded as follows: absent (score 0) when the echotexture of the liver is normal; mild (score 1), when there is a slight and diffuse increase of liver echogenicity with normal visualization of the diaphragm and of the portal vein wall; moderate (score 2), in case of a moderate increase of liver echogenicity with slightly impaired appearance of the portal vein wall and the diaphragm; severe (score 3), in case of marked increase of liver echogenicity with poor or no visualization of portal vein wall, diaphragm, and posterior part of the right liver lobe [2]. The examinations were assessed by the same operator, to reduce the variability.

Biochemical measurements and glycocalicin index (GCI)

Serum thromboxane B_2 ($sTXB_2$) produced during whole blood clotting *ex vivo*[3] was measured with

an enzyme-immunoassay (EIA) (Cayman Chemical, Ann Arbor, MI).[4] Urinary 11-dehydro-TXB_2, a major TXA_2 metabolite reflecting TXA_2 biosynthesis rate, was measured with validated liquid chromatography–tandem mass spectrometry (LC- MS-MS).[4]

Thrombopoietin (TPO) (R&D Systems, Minneapolis, MN) and glycocalicin (GC) (Cusabio Technology, Houston, TX), ELISA kits were used on ethylenediamine tetraacetic acid (EDTA) plasma.

The glycocalicin index (GCI), a parameter of platelet destruction,[5,6] was calculated as: *[GC concentration (μg/mL) x 250x10^9 platelet/L/individual platelet count x 10^9/L].*[7,5] Normal GCI is 0.7 (0.6-0.9).

Proteomics data processing and bioinformatics parameters

Proteomics analysis was performed on pooled platelet samples by selecting 10 patients (with or without T2DM) per group according to their homogeneous clinical and demographical parameters. Samples were analyzed in triplicate (third *vs.* first tertile) by LC-MS/MS and processed using MaxQuant 1.6.6.0 as previously reported.[8,9]

For protein identification and quantification carbamidomethylation of cysteines I was defined as fixed modification, while oxidation of methionines (M), deamidation of asparagines (N) and glutamines (Q) were set as variable modifications. Mass tolerances were set by default to 0.07 Da in the first search and 0.006 Da in the main search, while TOF MS/MS match tolerance was set to 0.05 Da. A retention time tolerance of 0.7 min was used to align any time shift in acquisition between samples. Match-between-runs (MBR) algorithm was used to transfer the peptide identifications from one LC-MS/MS run to all others using its default settings (match window of 0.7 min and alignment time of 20 min). False discovery rate (FDR) at the protein level was set at 2%, on the contrary at peptide level was set at 1%. Intensity-based absolute quantification (iBAQ) was used to quantify protein abundance in each sample in the bioinformatics analysis performed with Perseus,[10] version 1.6.10.50. (Max-Planck Institute for Biochemistry, Martinsried, Germany) uploading the protein groups generated by MaxQuant. Data were log_2 transformed in order to facilitate the calculation of the protein expression. Site only, reverse and contaminant peptides were removed from the dataset. Then, the missing and invalid values were removed.

The minimum number of valid values accepted was set at 2 in at least one treatment condition. In this way we have evaluated not only the different protein expression, but also the presence and absence of proteins between the different clinical conditions. The expression of common proteins between two clinical groups (third *vs.* first tertile) in both patients without T2DM (Online Supplementary Figure S1A) and with T2DM (Online Supplementary Figure S1B) was evaluated comparing the iBAQ (log_2 transformed) of the proteins in the density plot with Pearson correlation (R^2). A Volcano plot function was used to identify the differentially regulated proteins by performing a T-test with a false discovery rate (FDR) of 0.22 and a S_0 of 0.05 (Online Supplementary Figure S2).

The quality of our proteomic data is evaluated by using Platelet W–b - Systems Biology Workbench.

Gene Ontology and Comparison Analysis were performed using Ingenuity Pathway Analysis (IPA, Qiagen, Hilden, Germany) by loading the protein ratio for each comparison (third *vs.* first tertile) both for patients with T2DM and without T2DM. We considered molecules and/or relationships in all species and a confidence setting as "high predicted" or "experimental observed" (excluding medium predicted). The predicted activation or inhibition of each transcriptional regulator or downstream was inferred by the IPA-generated z-scores[11] (Online Supplementary Figure S2).

Immunoprecipitation

For immunoprecipitation, platelet lysates (200 μg of total protein) were precleared by incubation with protein A-Sepharose (Sigma Aldrich). Precleared lysates were incubated with 2 μg of anti-14-3-3ξ antibody (Santa Cruz Biotechnology) for 2 h at 4°C on a rotatory shaker, followed by adding 100 μL of 50 mg/ml protein A-Sepharose and incubation for 1 hour at 4°C on a rotatory shaker. Beads were washed three times in lysis buffer and samples were eluted with Laemmli buffer at 95°C for 5 minutes. Protein lysates were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane.

Megakaryocyte maturation

$CD45^+$ cells were separated from peripheral blood samples obtained from healthy subjects and patients by immunomagnetic bead selection (Miltenyi Biotech, Bologna, Italy) and cultured in Stem Span

medium (Stem Cell Technologies, Canada) supplemented with 1% L-glutamine, 1% penicillin-streptomycin, 10 ng/mL recombinant human thrombopoietin and 10 ng/mL recombinant human interleukin-11 (PeproTech, London, UK) for 14 days as previously described.[12] To evaluate Mk maturation, at the end of the culture, aliquots of 50 x 10^3 cells were collected, washed in PBS, and double-stained with the FITC-conjugated monoclonal antibody HIP8 against CD41 (eBioscience, ThermoFisher Scientific) and the PE-conjugated monoclonal antibody HIP1 against glycoprotein (GP)Ibα (CD42b, Abcam, Cambridge, UK). Cells were analyzed using a Navios flow cytometer (Beckman Coulter). A minimum of 10,000 events was acquired. Off-line data analysis was performed using the Beckman Coulter Navios software package. The maturation of Mks was measured as the percentage and mean fluorescence intensity of CD41 and GPIbα positive cells.[13]

Proplatelet formation (PPF) assay

PPF was investigated in adhesion to fibrinogen according to a previously described protocol [12]. At the end of the culture, large Mks were separated by a bovine serum albumin gradient (3-4%). 1 x 10^5 Mks were allowed to adhere at 37°C and 5% CO_2 for 16 hours onto glass coverslips coated with 100 µg/mL fibrinogen (Merck-Millipore, Milan, Italy). Samples were then fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, blocked with 5% BSA and stained with the anti-β1-tubulin antibody (Abcam). An Alexa Fluor 488-conjugated goat anti-rabbit antibody (Life Technologies, Monza, Italy) was used as secondary antibody. Hoechst was used for counterstaining nuclei. Proplatelet forming Mk were identified by fluorescence microscopy as the β1-tubulin positive cells displaying at least one proplatelet with respect to the total number of β1-tubulin positive cells. At least 10 fields per sample were analyzed using an Olympus IX53 microscope (Olympus Deutschland GmbH, Hamburg, Germany).

Western Blot Antibodies

The used primary antibodies were: anti-78 kDa glucose-regulated protein (GRP78, 1:1000; clone 76-E6, sc-13539, Santa Cruz Biotechnology), anti-β-actin (1:5000; Sigma Aldrich), anti-cyclooxygenase (COX)-1 (1:1000; clone CX111, Cayman Chemical), anti-Rab27B (1:1000; 44813 Cell Signaling), anti-GPIbα (1:10000; EPR6995, ab27901, Abcam), anti-a disintegrin and metalloprotease 17 **(**ADAM17,

1:1000; Ab2051, Abcam), and anti-neuraminidase (Neu)1 (1:1000; Invitrogen), anti-caspase 3 (Caspase 3, 1:1000; sc-7272, Santa Cruz). The used horseradish peroxidase-conjugated secondary antibodies were: goat anti-mouse (1:3000, Calbiochem) or goat anti-rabbit (1:5000, Calbiochem).

The used lectins were: biotinylated *Erythrina cristagalli* (ECL, 1:3000), and biotinylated *Ricinus communis agglutinin I* (RCA-I, 1:3000) as previously described.[14] Immunoreactive bands were detected by horseradish peroxidase-labeled streptavidin (1:3000).

Finally, the blots were developed using Western Lightning Chemiluminenscence Reagent (PerkinElmer) and immunoblot images were acquired with Alliance 4.7 (UVITEC, Cambridge, UK). The intensity of the relevant bands was evaluated using the ImageJ NIH Image Analysis Program.

Quantitative Real-time PCR (qRT-PCR)

Platelet RNA was extracted by using Total RNA Purification Kit (Norgen), and the retrotranscription of 500 ng was performed by Omniscript RT Kit (Qiagen). qRT-PCRs were performed using miSCRIPT Syber Green PCR Kit (Qiagen). Primers (5' to 3') used were:

-COX-1 forward TCATCAGGGAGTCTCGGGAG, reverse ATTCCTCCAACTCTGCTGCC;

-β2-microglobulin forward GCTCGCGCTACTCTCTCTTT, reverse TGTCGGATGGATGAAACCCA;

-thrombopoietin (TPO) forward, ACTGCTTCGTGACTCCCATG, reverse AGGAGGGATGAGAGGCAAGT;

-cyclophilin (cyclo) A forward, AGTCCATCTATGGGGAGAAATTTG reverse GCCTCCACAATATTCATGCCTTC.

Platelet Annexin V

Platelet staining for flow cytometry analyses were carried out by adding 5 μL of peripheral blood samples to the reagent mix detailed in Supplementary Table S3. 1 x 10^6 events/sample were acquired by flow cytometry (FACSVerse, BD Biosciences), and data were analyzed using FACSuite v 1.0.6.5230 and FlowJo X v 10.0.7 software (BD Biosciences). Platelets were identified on an FSC-H/SSC-H dot plot and then they were analyzed for their positivity to CD41a. CD41a+ platelets were gated and analyzed for their

Annexin V positivity[15].

Total and platelet derived Annexin V Extracellular vesicles

Extracellular-vesicles, total and platelet derived, for flow cytometry analyses were carried out by adding 5 µL of peripheral blood samples to the reagent mix[15] prepared by adding to 195 µL of binding buffer 1X (BD Biosciences) the dyes and antibodies (1 µl Lypophilic Cationic Dye (LCD) APC 626267 BD Biosciences; 5 µL CD41a BV510 563250 BD Biosciences; 1 µL AnnexinV V450 560506 BD Biosciences) and incubated for 45 minutes (RT, in the dark). Finally, samples were adequately diluted with binding buffer 1X (1:143), and no swarm effects occurred[15]when they were acquired (1 x 10^6 events/samples) by flow cytometry (FACSVerse, BD Biosciences). All requirements imposed for polychromatic flow cytometry EV analysis were considered[16]. The trigger threshold was placed on the channel in which the dye used to stain EVs emits (lipophilic cationic dye, allophycocyanin—APC—channel, threshold value = 200/262.144). For all used parameters the height (H) signals and bi-exponential or logarithmic modes were selected. Instrument performances, data reproducibility, and fluorescence calibrations were sustained by the Cytometer Setup & Tracking Module (BD Biosciences). The evaluation of non-specific fluorescence was obtained by acquiring fluorescence minus one control combined with the respective isotype control. Compensation was automatically calculated. Data were analyzed using FACSuite v 1.0.6.5230 (BD Biosciences) and Flow Jo X v 10.0.7 (BD Biosciences) software. EV concentrations were obtained by the volumetric count function[17]. EVs were identified and subtyped as already reported.[15] Briefly, within the gate of intact EVs (LCD+/Phalloidin-), platelets derived EVs were identified as CD41a+ events. Each of the above-mentioned EV subsets were also analyzed for the surface expression of phosphatidylserine (AnV+).

In vitro HepG2 cells treatment

Human HepG2 hepatocarcinoma cells were grown as previously reported[18] in DMEM high glucose, pyruvate (GIBCO, Thermo Fisher Scientific), 10% heat-inactivated fetal bovine serum (FBS; GIBCO, Thermo Fisher Scientific), 10,000 U ml-1 penicillin G and 10 mg ml-1 streptomycin sulphate. For *in vitro* assay, the HepG2 cells were transferred to 12-well plates (10^6 per well), allowed to adhere for 24 hours,

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and treated with a commercially available recombinant human GPIba peptide (rGC, 4067-GP R&D Systems) at the indicated concentration for 1 hour and for 24 hours at 37°C. DMSO was used as control. After the incubation period, the HepG2 cells were lysed in order to harvest the mRNA (after 1 hours) and culture supernatants (after 24 hours) were collected for TPO assay as described above. The rGC protein (His 17-Leu 505) was expressed in mouse myeloma cells (90-120 kDa apparent molecular weight under SDS-PAGE).

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Online Supplementary Results

Clinical characteristics

All subjects with T2DM were at high (n=64) or very high (n=36) CV risk, according to the 2019 ESC guidelines.[19] Among those without T2DM, 53 patients had very-high CV risk, 28 high risk, 18 moderate and 1 low risk. Patients without T2DM in primary prevention (n=78) had an average 10-year risk of fatal CVD of 12.3% according to European SCORE.[20]

Demographic, anthropometric, and clinical parameters of two groups of patients with *vs.* without diabetes were fairly balanced, except for higher BMI (p=0.026), HOMA-IR (p=0.038), higher prevalence of non-alcoholic-fatty-liver disease (NAFLD) (p<0.001) and retinopathy (p=0.007), lower total cholesterol (p=0.003) levels (with higher prevalence of statin treatment, p=0.015), lower total bilirubin (p=0.006), red blood cell count (p<0.001), hemoglobin (p<0.001), and hematocrit (p<0.001) in T2DM patients (Online Supplementary Table S1).

As expected, patients were significantly different for diabetes-specific treatment such as metformin (p<0.001), glinides (p=0.001), PPAR-γ agonists (p<0.001), insulin (p=0.029), for glycated hemoglobin % (p<0.001), and fasting plasma glucose (p<0.001). Notably, median HbA1c in T2DM patients was 51.0 mmol/mol (6.8%), reflecting good glycemic control in T2DM patients in the group of patients with diabetes (Online Supplementary Table S1).

T2DM patients of the third tertile had higher platelet counts (p=0.080 across the three groups, p=0.047 third *vs.* first tertile), a higher prevalence of NAFLD (p=0.04) and a nonsignificant trend for higher HOMA-IR (p=0.071) third *vs.* first tertile (Table 1).

Among patients without T2DM, while platelet count (p=ns) did not change across $sTXB_2$ recovery slope tertiles, MPV (p=0.029) was lower in patients of third *vs*. first tertile. Moreover, patients in the upper tertile had a lower prevalence of dyslipidemia (p=0.017) and lower rate of statin treatment (p=0.014) *vs.* first tertile (Table 1 and Online Supplementary Table S2).

$sTXB_2$ recovery slope was inversely related to age and directly related to BMI, waist circumference, estimated glomerular filtration rate (eGFR) in T2DM patients (Online Supplementary

TableS7). Among patients without T2DM, the $sTXB_2$ recovery slope was directly related to weight, waist circumference, WHR and diastolic arterial pressure (Online Supplementary TableS7).

Inhibited apoptosis in patients of the third tertile

We found no differences in annexin-V-positive, both total and platelet-derived, extracellular vesicles, in patients of first (n=35) *vs.* third (n=21) tertile (Online Supplementary Figure S5A), to corroborate that the faster recovery of COX-1 and differential PS exposure is not linked to different pro-coagulant properties of third tertile platelets.

In the intrinsic apoptotic pathway of PLTs, the activation of the effector caspases (Caspase-3/7) determines the subsequent exposure of PS on the membrane.[21–23] We observed lower levels of caspase 3 in patients of third *vs.* first tertile (p=0.042, Online Supplementary Figure S5B) and *vs.* healthy subjects (p=0.015, Online Supplementary Figure S5B), supporting inhibition of apoptosis in our patients with accelerated platelet COX-1 recovery.

Based on data showing "inhibition of apoptosis" and enhanced GPIbα receptor levels in poor aspirin responder, we investigated whether the different recovery of COX-1 activity reflected differences in 14-3-3ζ platelet immunoprecipitates of complexes 14-3-3ζ-GPIbα and 14-3-3ζ-COX-1 in patients of first (n=3) *vs.* third tertile (n=3) (Online Supplementary Figure S5C). We observed significantly lower levels of 14-3-3ξ:GPIbα complexes (p=0.016, Online Supplementary Figure S5C) and higher levels of 14-3-3ξ:COX-1 complexes (p=0.019, Online Supplementary Figure S5C) in patients of third *vs.* fist tertile, thus confirming inactivated apoptosis in patients of third tertile and providing a mechanistic link between COX-1 acetylation or inhibition and GPIbα clustering.

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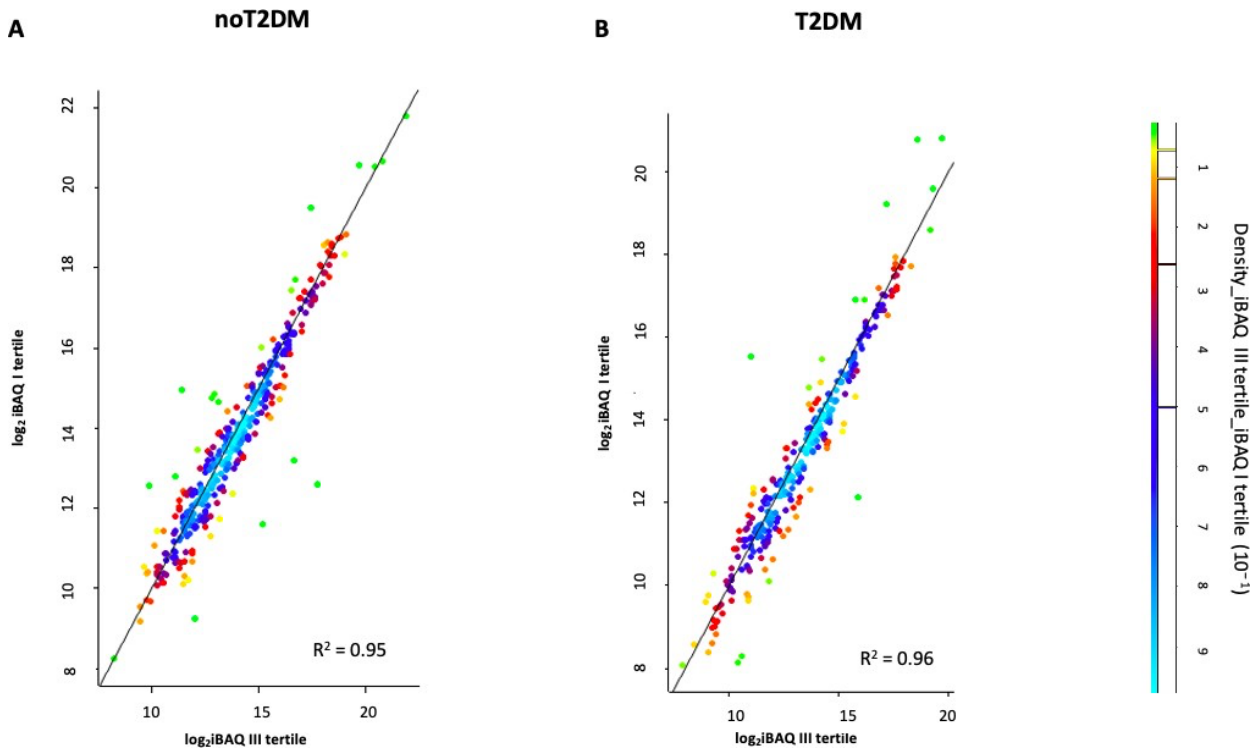
Online Supplementary Figure S1



Online Supplementary Figure S1: **Density Plot of protein expression in patients without T2DM (A) and with T2DM (B)**. Protein expression is reported as density value of iBAQ: data points with the highest density are light blue, on the contrary data points with lowest density are bright green. The color gradient is report in the legend made on comparison between third *vs.* first tertile both in patients without and with T2DM.

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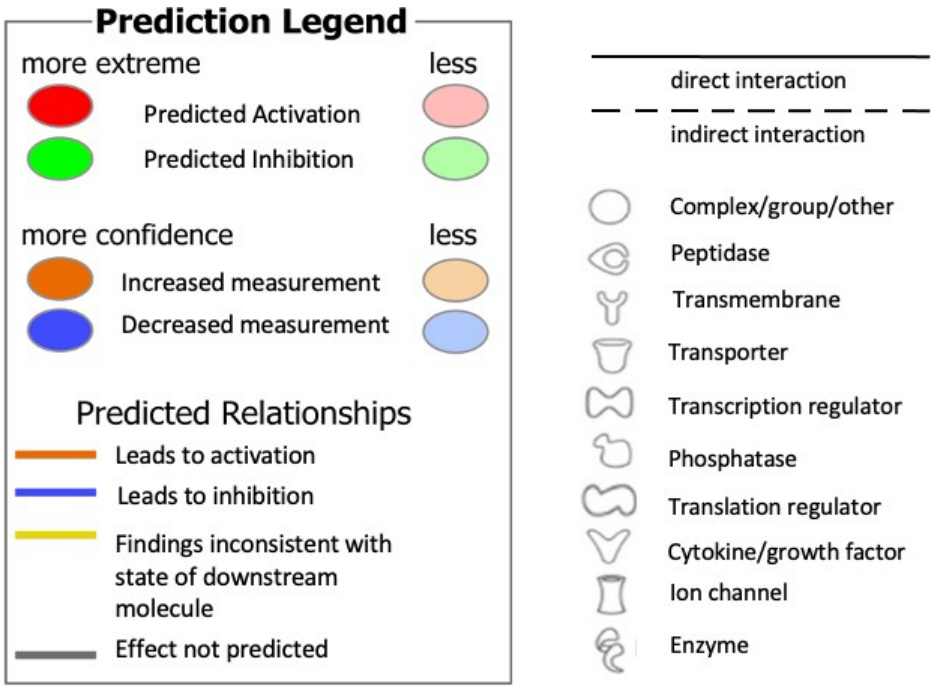
Online Supplementary Figure S2



Online Supplementary Figure S2: IPA networks legend. The figure shows the color and shape key to read the IPA networks reported in the work. Red and green color: increased or decreased measurements of quantified proteins. Blue and orange shapes: predicted inhibition (z-scores ≤ -2.0) and activation (z-scores ≥ 2.0). Direct activation: orange solid lines; direct inhibition: blue solid lines. Indirect relationships: dotted lines. Yellow and grey lines: "inconsistent" or "not predicted" relationship effects.

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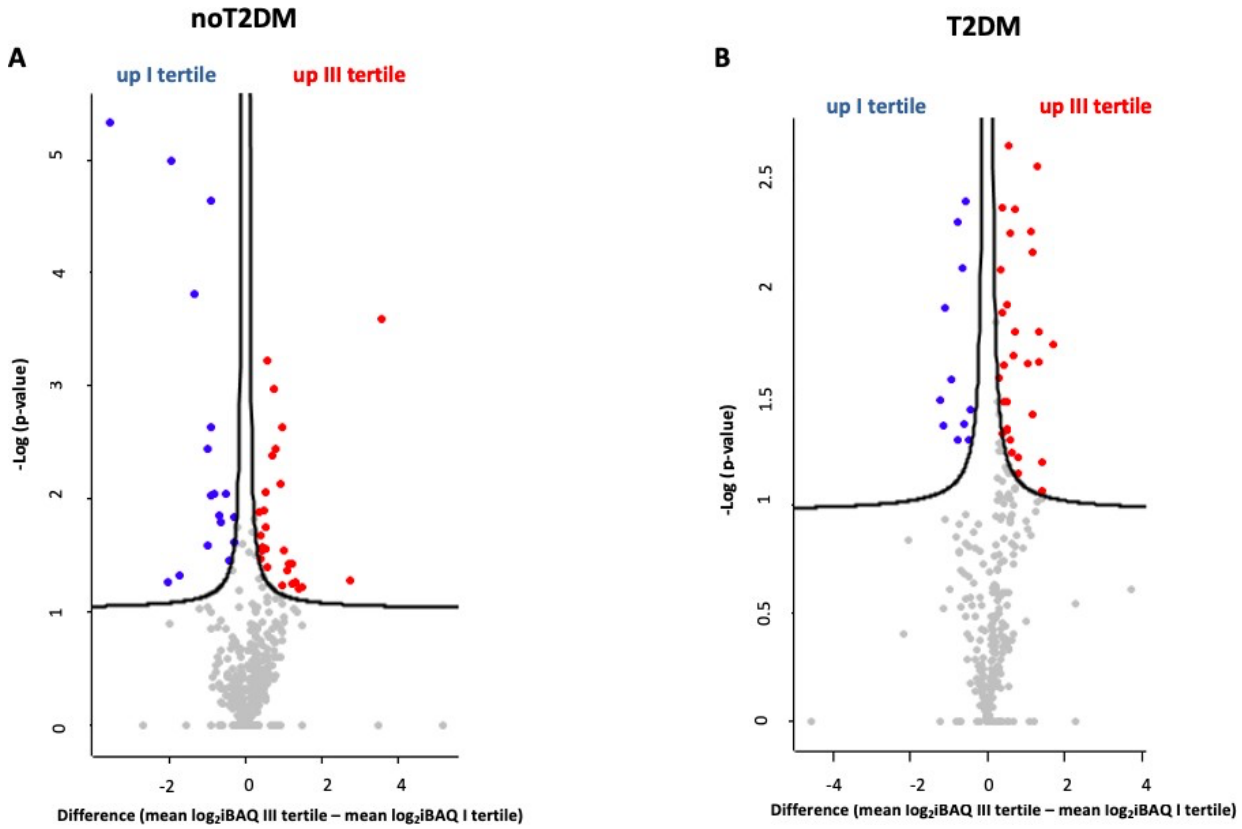
Online Supplementary Figure S3



Online Supplementary Figure S3: Volcano Plots of differentially expressed proteins in the different clinical groups, in patients without and with T2DM (A and B, respectively). Proteins were graphed by fold change (Difference) and -Log (p value) using a false discovery rate (FDR) of 0.22 and an S_0 of 0.05 for both graphs. Grey dots represent proteins that are not differentially expressed; while red and blue dots represent proteins that are significantly up-regulated in the pooled platelets of third tertile and up- regulated in the pooled platelets of first tertile, respectively, in both the clinical conditions.

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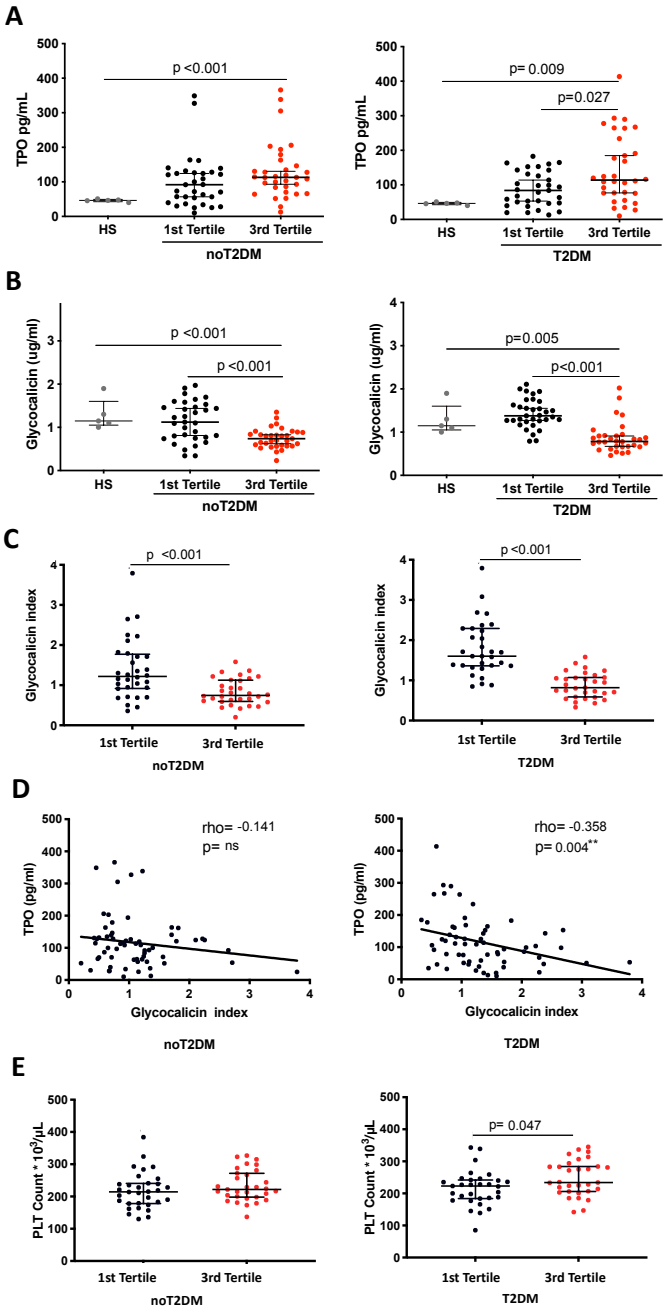
Online Supplementary Figure S4



Online Supplementary Figure S4: Higher circulating levels of thrombopoietin (TPO) and platelet (PLT) count and lower glycocalicin (GC) and glycocalicin index (GCI) at 24h after witnessed aspirin intake in patients with faster COX-1 recovery. Comparison of TPO (A), GC (B), GCI (C) and PLT count (E) between first and third $sTXB_2$ slope tertile in patients without (n=66) and with T2DM (n=66). Comparison of TPO (A) and GC (B) in third *vs*. first $sTXB_2$ slope tertile and *vs.* healthy subjects (HS, n=5) in patients without (n=66) and with T2DM (n=66). Significance was calculated by Mann-Whitney *U* test. Correlation between GCI and TPO in patients without and with T2DM (D). Spearman correlation coefficient and p-value are reported.

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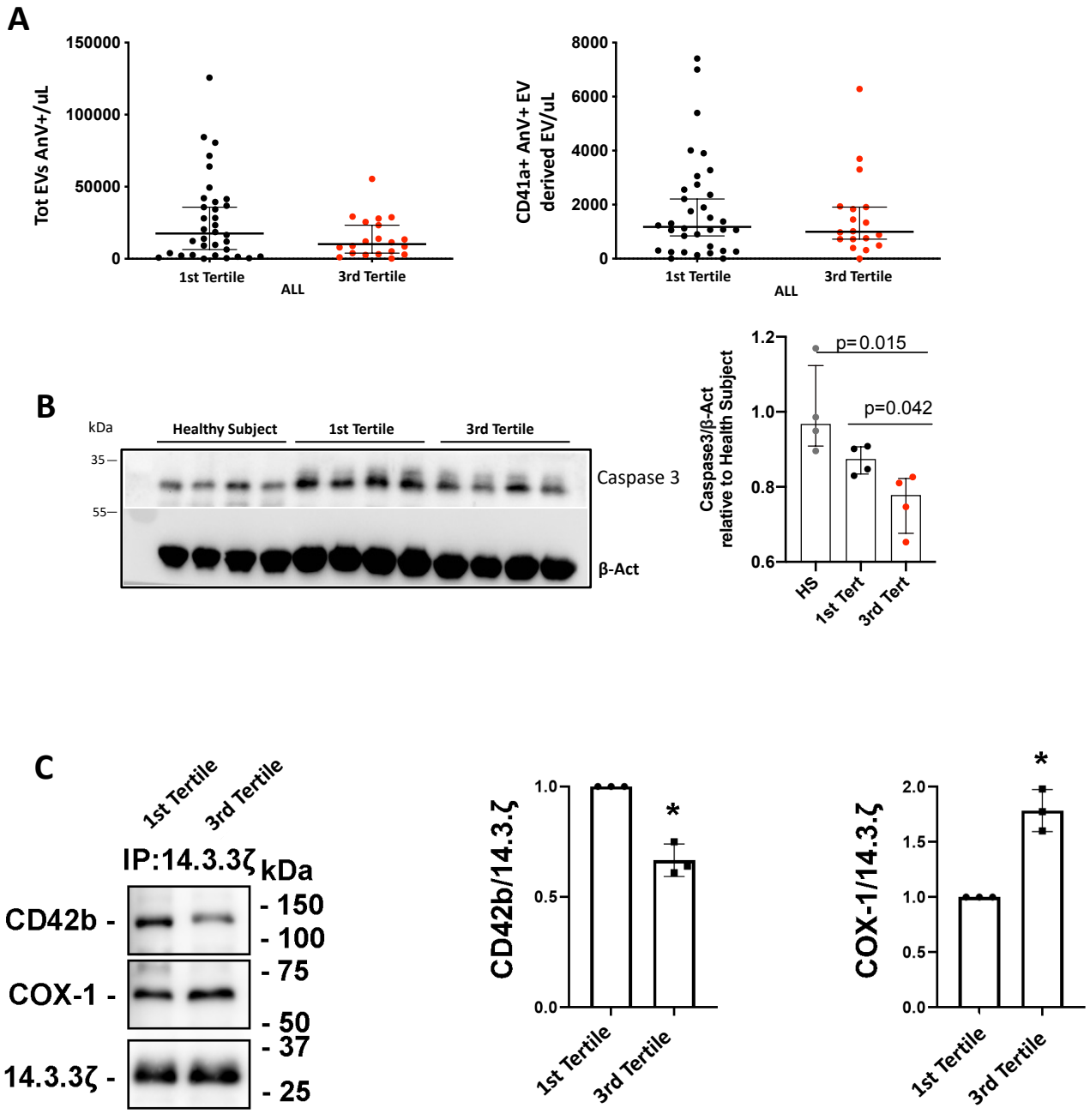
Online Supplementary Figure S5



Online Supplementary Figure S5: Inhibited apoptosis in patients of the third tertile. The number of AnnexinV-positive extracellular vesicles (Total EVs AnV+ and, CD41a+ PLT AnV+ derived) was measured in patients of first (n=35) and third tertile (n=21) (A); Expression levels of caspase 3 in healthy subjects (n=4), patients of first (n=4) and third tertile (n=4). β-Actin was used as loading control (B); Measurement in 14-3-3ζ platelet immunoprecipitates of complexes 14-3-3ζ-COX-1 and 14-3-3ζ-GPIba in patients of first (n=3) and third tertile (n=3) (C). Significance was calculated by Mann-Whitney *U* test or by Student's t-test.

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Online Supplementary Table S1. Characteristics of patients with and without type 2 diabetes mellitus (T2DM)

Variable	noT2DM	T2DM	P value*
N.	100	100	1.000
Age – years	69.0 (63.0-74.0)	68.0 (64.0-72.8)	0.989
Male gender, n.	57	64	0.386
Smoke, n.	10	13	0.658
Weight – Kg	77.0 (67.0-85.8)	80.0 (72.4-91.8)	0.084
BMI - Kg/m^2	27.9 (25.2-31.1)	29.9 (25.8-33.0)	0.026
Waist circumference – cm	100.0 (92.3-110.0)	104.0 (96.0- 111.0)	0.069
WHR	1.0 (0.9-1.0)	1.0 (0.9-1.0)	0.290
Obesity, n.	40	49	0.255
Systolic arterial pressure – mmHg	140.0 (128.3-153.8)	146.0 (134.3-152.0)	0.120
Diastolic arterial pressure – mmHg	77.0 (70.0-83.0)	75.0 (70.0-80.0)	0.626
Hypertension, n.	80	90	0.073
Glycated hemoglobin - mmol/mol - %	38.8 (35.5-42.1) 5.7 (5.4-6.0)	50.8 (45.4-58.5) 6.8 (6.3-7.5)	0.000
Diabetes duration – years	-	5.0 (2.0-10.0)	-
Total cholesterol – mmol/L	4.9 (4.1-5.5)	4.4 (3.8-5.0)	0.003
HDL cholesterol – mmol/L	1.3 (1.1-1.5)	1.3 (1.0-1.5)	0.160
Triglycerides – mmol/L	1.3 (0.9-1.7)	1.4 (1.0-1.9)	0.198
Dyslipidemia, n	54	60	0.475
AST - U/L	24.0 (21.0-28.0)	24.0 (20.0-31.0)	0.851
ALT - U/L	28.5 (24.0-33.0)	31.0 (25.0-40.8)	0.076
Total Bilirubin – umol/L	12 (10-15)	10 (9-14)	0.006
NAFLD, n.	52	76	0.000
hs-C-reactive protein – nmol/L	19.0 (9.5-38.1)	19.0 (9.5-47.6)	0.619
Red Blood Cell count, $x10^{12}/L$	4.9 (4.5-5.1)	4.6 (4.4- 4.9)	0.000
Hemoglobin – g/dL	14.3 (13.5-15.3)	13.7 (12.8-14.4)	0.000
Hematocrit – %	42.6 (40.0-45.1)	38.3 (40.1-42.4)	0.000
Red cell Distribution Width –	13.2 (12.8-13.8)	12.8 (13.3- 13.9)	0.491

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%			
Platelet count – ´10^9/L	223.5 (189.3-266.0)	227.0 (191.0-264.3)	0.963
Platelet Distribution Width – fL	13.7 (12.3-15.0)	13.8 (12.4-15.4)	0.538
Mean Platelet Volume – fL	11.2 (10.5-11.7)	11.3 (10.6-11.9)	0.500
White Blood Cell count – ´10^9/L	6.6 (5.6-7.8)	7.0 (5.8-8.3)	0.130
Fasting plasma glucose – mmol/L	5.29 (4.89-5.67)	6.75 (5.79-7.61)	0.000
Fasting plasma Insulin – pmol/L	67.4 (54.2-95.8)	72.9 (51.4-102.7)	0.784
HOMA-IR	2.4 (1.7-3.2)	3.2 (1.9-5.0)	0.038
Serum Creatinine – umol/L	70.4 (61.6-88.0)	70.4 (61.6-79.2)	0.221
eGFR – ml/min	88.0 (72.5-99.6)	88.8 (78.0-98.6)	0.222
Uric Acid – umol/L	327.1 (267.7-410.4)	333.1 (273.6-386.6)	0.990
Aspirin treatment duration - years	4.0 (2.0-8.0)	5.0 (2.0-10.0)	0.190
Cardiovascular disease, n.			
Stable CAD	6	11	0.311
Carotid stenosis (>50%), n.	30	42	0.105
MI or revascularization	11	16	0.308
Stroke, TIA or revascularization	11	9	0.814
Peripheral artery disease	1	6	0.118
Diabetic microvascular disease, n.			
Retinopathy	0	8	0.007
Nephropathy	0	2	0.497
Neuropathy	0	1	1.000
Therapy, n.			
Metformin	1	63	0.000
Glinide	0	11	0.001
PPAR-γ agonists	0	12	0.000
Sulfonylurea	0	4	0.121
Insulin	0	6	0.029

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sGLT2 inhibitors	0	3	0.246
GLP-1 RA	0	2	0.497
DPP-IV	0	5	0.059
Acarbose	0	2	0.497
ACE-I	27	31	0.640
ARBs	34	41	0.381
Diuretics	28	31	0.757
β-blockers	34	32	0.881
CCA	22	26	0.602
Other antihypertensives	3	9	0.134
Statins	36	54	0.015
Fibrates	1	2	1.000
Ezetimibe	8	8	1.000
Omega 3	4	3	1.000
Proton Pump Inhibitors	45	39	0.474
ASA	100	100	1.000

Abbreviations: BMI= body mass index, WHR= Waist to Hip ratio, HDL= high-density lipoproteins, AST= Aspartate Aminotransferase, ALT= Alanine amino Transferase, NAFLD= non alcoholic fatty liver disease, HOMA-IR= Homeostatic Model Assessment of Insulin Resistance, eGFR= estimated glomerular filtration rate, CAD= coronary artery disease, CCA= Common carotid artery, MI= myocardial infarction, TIA= transient ischemic attack, PPAR-Y= peroxisome proliferator-activated receptor gamma, SGLT2= Sodium-glucose co-transporter-2, GLP1 RA= glucagon-like peptide 1 receptor agonist, DPP-IV= dipeptidyl peptidase IV, ACE-I= angiotensin-converting-enzyme -inhibitors, ARBs= angiotensin receptor blockers, CCA= calcium channel antagonists, ASA= acetylsalicylic acid.

Data are median (25th – 75th percentile). †Determined by Kruskal-Wallis or Mann-Whitney or Chi Square test, as appropriate.

Online Supplementary Table S2. Characteristics of patients with and without type 2 diabetes in relation to tertiles of $sTXB_2$ recovery slope

Variable	T2DM				noT2DM			
	1st tertile	2nd tertile	3rd tertile	P value	1st tertile	2nd tertile	3rd tertile	P value
Cardiovascular disease, n. (%)								
Carotid stenosis (>50%)	12 (36.4)	15 (44.1)	15 (45.5)	0.721	10 (30.3)	0 (0.0)	11 (33.3)	0.828
MI or revascularization	6 (18.2)	3 (8.8)	7 (21.2)	0.329	8 (24.2)	9 (26.5)	2 (6.1)	0.011
Stroke, TIA or revascularization	4 (12.1)	1 (2.9)	4 (12.1)	0.315	1 (3.0)	3 (8.8)	7 (21.2)	0.054
Peripheral artery disease	3 (9.1)	2 (5.9)	1 (3.0)	0.584	0 (0.0)	0 (0.0)	1 (3.0)	0.359
Diabetic microvascular disease, n. (%)								
Retinopathy	3 (9.1)	1 (2.9)	4 (12.1)	0.368	-	-	-	-
Nephropathy	0 (0.0)	0 (0.0)	2 (6.1)	0.126	-	-	-	-
Neuropathy	0 (0.0)	0 (0.0)	1 (3.0)	0.359	-	-	-	-
Therapy, n. (%)								
Metformin	21 (63.6)	21 (61.8)	21 (63.6)	0.983	-	-	-	-
Glinide	4 (12.1)	3 (8.8)	4 (12.1)	0.883	-	-	-	-
PPAR-γ agonists	4 (12.1)	4 (11.8)	4 (12.1)	0.999	-	-	-	-
Sulfonylurea	2 (6.1)	0 (0.0)	2 (6.1)	0.342	-	-	-	-
Insulin	1 (3.0)	2 (5.9)	3 (9.1)	0.584	-	-	-	-
sGLT2 inhibitors	0 (0.0)	1 (2.9)	2 (6.1)	0.353	-	-	-	-
GLP-1 RA	0 (0.0)	1 (2.9)	1 (3.0)	0.605	-	-	-	-
DPP-IV	2 (6.1)	1 (2.9)	2 (6.1)	0.795	-	-	-	-
Acarbose	0 (0.0)	2 (5.9)	0 (0.0)	0.138	-	-	-	-
ACE-I	8 (24.2)	9 (26.5)	14 (42.4)	0.218	6 (18.2)	12 (35.3)	9 (27.3)	0.288
ARBs	12 (36.4)	16 (47.1)	13 (39.4)	0.656	11 (33.3)	9 (26.5)	14 (42.4)	0.385
Diuretics	8 (24.2)	11 (32.4)	12 (36.4)	0.555	10 (30.3)	12 (35.3)	6 (18.2)	0.278
β-blockers	12 (36.4)	11 (32.4)	9 (27.3)	0.730	12 (36.4)	12 (35.3)	10 (30.3)	0.857
CCA	9 (27.3)	8 (23.5)	9 (27.3)	0.922	7 (21.2)	8 (23.5)	7 (21.2)	0.965
Other antihypertensives	2 (6.1)	4 (11.8)	3 (9.1)	0.717	0 (0.0)	2 (5.9)	1 (3.0)	0.369
Statins	16 (48.5)	20 (58.8)	18 (54.4)	0.695	17 (51.5)	6 (17.6)	13 (39.4)	0.014
Fibrates	0 (0.0)	2 (5.9)	0 (0.0)	0.138	0 (0.0)	0 (0.0)	1 (3.0)	0.359
Ezetimibe	4 (12.1)	2 (5.9)	2 (6.1)	0.566	6 (18.2)	1 (2.9)	1 (3.0)	0.031
Omega 3	1 (3.0)	2 (5.9)	0 (0.0)	0.373	3 (9.1)	1 (2.9)	0 (0.0)	0.157
Proton Pump Inhibitors	12 (36.4)	16 (47.1)	11 (33.3)	0.480	16 (48.5)	15 (44.1)	14 (42.4)	0.878
ASA	33 (100.0)	34 (100.0)	33(100.0)	-	33 (100.0)	34 (100.0)	33 (100.0)	-
Aspirin treatment duration – year	5.0 (2.0-10.0)	4.0 (2.0-10.0)	5.0 (2.0-9.5)	0.579	4.0 (1.5-10.0)	3.0 (1.0-7.3)	5.0 (2.0-9.0)	0.464

Abbreviations: CAD= coronary artery disease, CCA= Common carotid artery, MI= myocardial infarction, TIA= transient ischemic attack, PPAR-Y= peroxisome proliferator-activated receptor gamma, SGLT2= Sodium-glucose co-transporter-2, GLP1 RA= glucagon-like peptide 1 receptor agonist, DPP-IV= dipeptidyl peptidase IV, ACE-I= angiotensin-converting-enzyme - inhibitors, ARBs= angiotensin receptor blockers, CCA= calcium channel antagonists, ASA= acetylsalicylic acid. Data are median (25th – 75th percentile).

†Determined by Kruskal-Wallis or x^2 test, as appropriate.

Online Supplementary Table S3. List of flow cytometry specificities and reagents.

Detection	Fluorochrome	Vendor	Ab Clone	Catalog	Amount per Test
Lipophilic Cationic Dye (LCD)	-	BD Biosciences	-	626267 custom	0.5
Phalloidin-FITC	FITC	BD Biosciences	-	626267 custom	0.5
CD41a	PE	BD Biosciences	HIP8	555467	2,5 µl
AnnexV	PerCP-Cy5.5	BD Biosciences		561431	0.25 µl

Keys: Peridinin-Chlorophyll-protein-Cyanine 5.5 (PerCP-Cy5.5). Becton Dickison (BD) Bioscience (San Jose, CA, USA)

Online Supplementary Table S4. Significantly differential proteins at univariate statistical analysis (Volcano Plot) in pooled patients without diabetes (noT2DM). A positive value of "Difference" indicates an up-regulation in third tertile respect to first tertile, while a negative value indicates a down-regulation in the same comparison.

Protein IDs	Protein names	Gene names	-LOG(P-value)	Difference	Peptides	Score	log2 iBAQ 1st tertile noT2DMC 02	log2 iBAQ 1st tertile noT2DMC 03	log2 iBAQ 1st tertile noT2DMC 04	log2 iBAQ 3rd tertile noT2DMC 02	log2 iBAQ 3rd tertile noT2DMC 03	log2 iBAQ 3rd tertile noT2DMC 04
P05976	Myosin light chain 1/3, skeletal muscle isoform	MYL1	5.332177	-3.54049	1	2.6151	14.95606	15.01581	14.9036	0	11.4417	11.3943
P69905	Hemoglobin subunit alpha	HBA1	1.258716	-2.03383	10	192.99	19.16416	19.6849	19.60449	16.31771	17.19017	18.84417
P10124	Serglycin	SRGN	5.001477	-1.95195	2	24.377	14.80972	14.86767	14.88216	12.88108	0	12.92139
Q9Y6C2	EMILIN-1	EMILIN1	1.325754	-1.71139	13	25.248	12.65988	13.05371	12.66831	11.8011	9.91247	11.53416
P04275	von Willebrand factor	VWF	3.813111	-1.31896	43	89.557	13.47193	13.36782	13.50643	11.96391	12.24487	12.18053
P62328	Thymosin beta-4	TMSB4X	2.448961	-0.98798	5	21.013	17.6404	17.49748	17.97146	16.63254	16.87183	16.64104
P11216	Glycogen phosphorylase, brain form	PYGB	1.596722	-0.9733	10	17.113	12.34452	12.59038	12.37278	11.74239	10.9257	11.71969
P29350	Tyrosine-protein phosphatase non-receptor type 6	PTPN6	2.641331	-0.90101	3	25.222	11.43327	11.48553	11.40243	10.69427	10.28575	10.63816
P02042	Hemoglobin subunit delta	HBD	4.641929	-0.8987	12	31.915	15.97123	16.09809	16.00904	15.14534	15.13495	15.10198
Q13576	Ras GTPase-activating-like protein IQGAP2	IQGAP2	2.029018	-0.89803	4	8.8637	10.49725	10.50661	10.62388	9.438646	9.477212	10.01778
P68871	Hemoglobin subunit beta	HBB	2.040805	-0.8288	14	323.31	20.89386	20.37571	20.37103	19.69129	19.696	19.76691
P12259	Coagulation factor V	F5	1.848569	-0.67294	17	39.206	12.35113	12.71031	12.17068	11.67424	11.75951	11.77956

Q93084	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	ATP2A3	1.796691	-0.62606	21	145.32	14.84711	15.00325	15.33494	14.31465	14.49648	14.49598
P35579	Myosin-9	MYH9	2.044834	-0.52842	130	323.31	18.54762	18.53727	18.62724	18.15837	17.82706	18.14146
P02679	Fibrinogen gamma chain	FGG	1.457894	-0.42144	20	323.31	18.73218	18.62607	18.52187	18.0146	18.17524	18.42595
P05106	Integrin beta-3	ITGB3	1.846059	-0.30173	29	323.31	17.77462	17.72521	17.6447	17.37653	17.32869	17.53412
Q7Z406	Myosin-14	MYH14	1.613068	-0.29379	11	3.7569	12.92928	0	12.99435	12.76754	12.63411	12.60242
P02656	Apolipoprotein C-III	APOC3	1.882155	0.352379	1	14.545	12.80306	12.70016	12.64908	12.95456	13.06056	13.19431
Q71U36	Tubulin alpha-1A chain	TUBA1A	1.674371	0.378992	17	3.33	12.98865	12.87233	12.77748	13.37041	13.30834	13.09668
P06753	Tropomyosin alpha-3 chain	TPM3	1.475808	0.384619	12	59.094	17.01944	17.19806	17.42431	17.65536	17.58461	17.5557
P06733	Alpha-enolase	ENO1	1.57891	0.430053	16	228.3	16.76811	16.55817	16.38956	17.0268	16.88691	17.09228
P52907	F-actin-capping protein subunit alpha-1	CAPZA1	1.546087	0.458621	6	33.976	14.11772	14.45725	14.1055	14.54352	14.78948	14.72334
P04406	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.894752	0.490143	13	317.94	17.77514	17.63616	17.91992	18.33704	18.10835	18.35625
P61225	Ras-related protein Rap-2b	RAP2B	1.556436	0.523111	2	3.8337	0	11.65374	11.37488	12.03738	12.143	11.93188
P01876	Ig alpha-1 chain C region	IGHA1	1.751109	0.525279	4	12.817	13.47814	13.50569	13.88408	14.21371	14.12444	14.10558
P00491	Purine nucleoside phosphorylase	PNP	2.055927	0.539813	6	28.786	13.73904	13.78187	13.80413	14.50265	14.3246	14.11724
O15511	Actin-related protein 2/3 complex subunit 5	ARPC5	1.394136	0.578421	4	14.082	14.54702	15.14669	14.88741	15.53992	15.5073	15.26916
O00299	Chloride intracellular channel protein 1	CLIC1	3.224727	0.58839	10	79.555	16.41005	16.51297	16.32745	16.95651	17.04655	17.01258
P14625	Endoplasmin	HSP90B1	2.384741	0.719527	22	120.9	13.91083	14.27801	14.2746	14.8825	14.872	14.86752
P13716	Delta-aminolevulinic acid dehydratase	ALAD	2.98044	0.75905	2	6.0967	0	11.66787	11.67225	0	12.45358	12.40464

P60174	Triosephosphate isomerase	TPI1	2.451393	0.806739	11	37.299	14.78872	14.59881	15.00233	15.71893	15.57344	15.5177
P23284	Peptidyl-prolyl cis-trans isomerase B	PPIB	2.130531	0.895937	8	21.934	15.38903	14.9917	15.55408	16.12155	16.16938	16.33169
Q99832	T-complex protein 1 subunit eta	CCT7	1.242115	0.948009	5	7.8265	10.90516	0	10.95768	0	11.64372	12.11514
Q15084	Protein disulfide-isomerase A6	PDIA6	2.629273	0.951978	7	41.456	14.77427	14.43169	14.6775	15.39238	15.66828	15.67874
P11021	78 kDa glucose-regulated protein	HSPA5	1.553379	0.99763	22	126.49	14.71505	15.56066	14.91961	16.35741	15.86375	15.96705
P61158	Actin-related protein 3	ACTR3	1.363059	1.08742	11	32.722	14.38283	13.67937	14.89174	15.64931	15.24481	15.32207
Q9H0U4	Ras-related protein Rab-1B	RAB1B	1.426842	1.140889	7	36.248	15.7072	14.72051	14.6625	16.46415	15.94622	16.10251
P13489	Ribonuclease inhibitor	RNH1	1.253269	1.229427	4	11.132	10.11048	9.931639	10.93921	11.48472	0	11.62835
P40227	T-complex protein 1 subunit zeta	CCT6A	1.421799	1.232392	5	8.5281	10.25136	11.46817	10.27053	11.88023	11.95143	11.85557
P55209	Nucleosome assembly protein 1-like 1	NAP1L1	1.260657	1.288467	2	13.287	13.74599	13.83368	15.22596	15.58942	15.53898	15.54264
P11234	Ras-related protein Ral-B	RALB	1.206523	1.410002	4	6.1192	12.44964	12.97061	11.71643	0	13.62605	13.95174
P29401	Transketolase	TKT	1.216089	1.498017	3	4.0548	0	10.21359	10.18339	12.08348	0	11.30953
O60610	Protein diaphanous homolog 1	DIAPH1	1.277175	2.734471	13	22.023	0	8.606442	9.923476	10.96441	12.90834	12.12554
P04179	Superoxide dismutase [Mn], mitochondrial	SOD2	3.592551	3.566264	4	13.7	11.7945	11.41891	0	15.04286	15.32024	15.15581

Online Supplementary Table S5. Significantly differential proteins at univariate statistical analysis (Volcano Plot) in pooled patients with diabetes (T2DM). A positive value of "Difference" indicates an up-regulation in third tertile respect to first tertile, while a negative value indicates a down-regulation in the same comparison.

Protein IDs	Protein names	Gene names	-LOG(P-value)	Difference	Peptides	Score	log2 iBAQ 3rd tertile T2DM 01	log2 iBAQ 3rd tertile T2DM 02	log2 iBAQ 3rd tertile T2DM 03	log2 iBAQ 1st tertile T2DM 01	log2 iBAQ 1st tertile T2DM 02	log2 iBAQ 1st tertile T2DM 03
P02042	Hemoglobin subunit delta	HBD	1.485667	-1.23763	12	56.441	14.08697	13.70271	14.92105	15.6418	15.58552	15.19629
O75915	PRA1 family protein 3	ARL6IP5	1.362992	-1.13315	2	19.28	14.20533	13.84353	12.92151	14.88832	14.65654	14.82496
P47755	F-actin-capping protein subunit alpha-2	CAPZA2	1.907236	-1.123	2	6.7226	11.48165	11.20316	10.67384	12.28326	12.042	12.40237
P01009	Alpha-1-antitrypsin	SERPINA1	1.574752	-0.94506	2	31.074	11.17636	11.09764	10.7151	11.54868	12.36506	11.91053
P40197	Platelet glycoprotein V	GP5	1.30206	-0.77677	6	35.965	12.77569	12.31967	12.54134	13.801	13.18587	12.98014
P32119	Peroxiredoxin-2	PRDX2	2.307579	-0.76107	5	31.728	13.78259	13.64959	13.40248	14.33427	14.52099	14.26261
P01877	Ig alpha-2 chain C region	IGHA2	2.090789	-0.65005	1	6.7504	10.4292	10.28019	10.62571	11.19722	10.92072	11.16729
Q71U36	Tubulin alpha-1A chain	TUBA1A	1.374359	-0.60008	16	9.8418	11.23829	10.81306	10.70977	11.7608	11.45389	11.34668
Q00325	Phosphate carrier protein, mitochondrial	SLC25A3	2.397486	-0.56923	1	7.3322	10.81137	10.69357	10.94017	11.31882	11.32193	11.51205
Q93084	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	ATP2A3	1.297035	-0.46689	14	143.33	14.22362	14.07079	13.82665	14.72542	14.49611	14.30021
P61026	Ras-related protein Rab-10	RAB10	1.439938	-0.44629	4	19.822	12.94171	12.84223	13.16101	13.50581	13.56546	13.21256
A0A087WV48	Peptidyl-prolyl cis-trans isomerase FKBP1A	FKBP1A	1.582264	0.314351	1	7.7167	13.10617	13.36318	13.38073	12.96842	12.93257	13.00605
P21926	CD9 antigen	CD9	2.086679	0.347439	2	11.463	14.79563	14.76523	14.94105	14.43111	14.57831	14.45018

P11021	78 kDa glucose-regulated protein	HSPA5	2.371004	0.385367	14	95.684	14.86104	14.86868	14.79609	14.41283	14.57837	14.3785
P11142	Heat shock cognate 71 kDa protein	HSPA8	1.890217	0.39384	17	128.76	15.40171	15.65749	15.52616	15.02484	15.19568	15.18332
P09486	SPARC	SPARC	1.325489	0.39593	4	30.946	15.64329	15.3549	15.20201	15.09959	14.99643	14.91639
P06703	Protein S100-A6	S100A6	1.478966	0.402231	1	6.0352	13.07494	0	13.29519	12.68753	12.788	12.87298
P14770	Platelet glycoprotein IX	GP9	1.647101	0.413659	4	29.441	15.80735	15.66069	15.77316	15.17094	15.53162	15.29767
P19105	Myosin regulatory light chain 12A	MYL12A	1.475887	0.425207	8	84.722	17.65425	17.72507	17.58152	17.34906	16.97455	17.36162
P61224	Ras-related protein Rap-1b	RAP1B	1.345179	0.486839	10	88.933	17.68414	17.37101	17.82637	17.34577	17.03596	17.03928
P30101	Protein disulfide-isomerase A3	PDIA3	1.477568	0.506003	6	40.336	13.37545	13.65508	13.15477	13.00692	12.78195	12.87842
P06753	Tropomyosin alpha-3 chain	TPM3	1.351964	0.510431	11	63.08	15.52506	15.46531	15.33696	15.08986	15.10906	14.59712
Q14766	Latent-transforming growth factor beta-binding protein 1	LTBP1	1.924697	0.51086	12	96.839	12.99327	12.7426	12.85722	12.48902	12.39218	12.17932
P01834	Ig kappa chain C region	IGKC	2.657308	0.559691	1	7.796	13.25441	13.36932	13.51015	12.75776	12.84015	12.85689
Q9UBW5	Bridging integrator 2	BIN2	1.300584	0.563637	8	51.553	13.58848	14.01498	13.77982	13.44061	13.33818	12.91358
P10124	Serglycin	SRGN	2.25759	0.569873	2	20.092	15.6434	15.67777	15.55597	14.98686	14.93111	15.24956
P62491	Ras-related protein Rab-11A	RAB11A	1.239257	0.610461	4	29.32	14.61896	14.54243	14.53491	14.35218	13.95647	13.55627
Q9NZN3	EH domain-containing protein 3	EHD3	1.691269	0.683522	10	73.056	14.34935	13.89945	13.85565	13.52906	13.21193	13.31288
P10809	60 kDa heat shock protein, mitochondrial	HSPD1	1.801825	0.692291	4	27.759	12.35508	11.79576	12.12069	11.51062	11.36008	11.32396
P09493	Tropomyosin alpha-1 chain	TPM1	2.366772	0.721876	7	6.4116	11.78324	11.6475	11.86213	11.14275	11.15526	10.82925
P06744	Glucose-6-phosphate isomerase	GPI	1.218757	0.781909	2	12.827	10.86751	11.75121	11.7309	10.8157	10.6359	10.55229
P78417	Glutathione S-transferase omega-1	GSTO1	1.145498	0.792027	5	30.831	14.34748	14.42947	14.20358	13.89851	13.80564	12.9003

P09211	Glutathione S-transferase P	GSTP1	1.654674	1.026776	3	21.064	14.82063	14.62445	14.0612	13.72973	13.53782	13.1584
P10720	Platelet factor 4 variant	PF4V1	2.258485	1.125004	3	38.881	14.25274	14.58959	14.52326	13.02649	13.64611	13.31798
P0DP25			1.418752	1.156385	3	18.644	15.72355	15.71473	15.74405	15.32952	14.21021	14.17344
P04632	Calpain small subunit 1	CAPNS1	2.16573	1.166851	3	21.31	13.11399	12.97302	13.41389	11.9115	12.35824	11.7306
O15144	Actin-related protein 2/3 complex subunit 2	ARPC2	2.560653	1.274261	6	35.028	12.30318	12.80613	12.29809	11.07808	11.38306	11.12348
P07237	Protein disulfide-isomerase	P4HB	1.661223	1.309026	5	31.242	12.37648	12.74201	12.8865	11.75104	11.61305	10.71382
Q13561	Dynactin subunit 2	DCTN2	1.801636	1.312723	2	12.557	12.29769	11.72626	11.85981	10.84101	10.45605	0
P09972	Fructose-bisphosphate aldolase C	ALDOC	1.194812	1.396106	3	8.3192	13.33762	0	12.77604	11.36271	12.33829	11.28118
P30740	Leukocyte elastase inhibitor	SERPINB1	1.061225	1.397216	7	41.743	13.28944	13.97127	13.84931	12.00748	11.48109	13.4298
Q01813	ATP-dependent 6-phosphofructokinase, platelet type	PFKP	1.737466	1.69225	3	17.686	11.95815	11.64273	0	10.596	10.03974	9.688828

Online Supplementary Table S6. Characteristics of patients with and without type 2 diabetes analyzed for MK and proplatelet studies in relation to tertiles of $sTXB_2$ recovery slope.

	noT2DM 1st tertile	noT2DM 3rd tertile		T2DM 1st tertile	T2DM 3rd tertile	
N.	4	6	**P value**	4	5	**P value**
Age – years	58.0 (44.3-73.3)	66.5 (53.8-68.0)	0.521	67.0 (60.8-73.3)	68.0 (60.5-70.0)	0.902
Male gender, n. (%)	3 (75)	4 (66)	1.000	2 (50)	4 (20)	0.524
Weight – Kg	72.0 (51.0-98.3)	79.5 (68.8-107.0)	0.522	82.5 (74.3-93.8)	100.0 (84.5-101.5)	0.110
BMI - Kg/m^2	25.4 (20.3-33.4)	27.0 (25.6-33.9)	0.522	30.2 (26.8-37.0)	32.2 (30.9-34.8)	0.462
Waist circumference – cm	96.5 (79.3-116.8)	103.5 (98.0-117.3)	0.240	103.5 (90.8-121.5)	107.0 (106.5-116.0)	0.621
WHR	0.97 (0.86-1.07)	0.99 (0.96-1.01)	0.667	0.92 (0.88-0.98)	0.97 (0.95-1.02)	0.110
Obesity, n. (%)	1 (25)	2 (33)	1.000	2 (50)	5 (100)	0.167
Systolic arterial pressure – mmHg	128.5 (113.8-168.0)	155.0 (140.5-167.3)	0.201	143.0 (135.5-152.8)	148.0 (137.0-170.0)	0.461
Diastolic arterial pressure – mmHg	78.0 (63.0-90.0)	88.5 (84.3-94.0)	0.281	75.5 (70.8-79.5)	79.0 (74.5-83.5)	0.327
Hypertension, n. (%)	2 (50)	6 (100)	.133	3 (75)	4 (20)	1.000
Glycated hemoglobin - mmol/mol	39 (36-44)	39 (33-45)	0.747	53 (41-72)	56 (50-64)	0.461
Glycated hemoglobin - %	5.7 (5.4-6.2)	5.7 (5.2-6.3)	0.747	7.0 (5.9-8.7)	7.3 (6.7-8.0)	0.461
Dyslipidemia, n. (%)	2 (50)	3 (50)	1.000	4 (100)	4 (20)	1.000
AST - U/L	30.0 (21.0-42.8)	27.0 (21.3-31.8)	0.669	20.5 (16.8-25.8)	25.0 (20.0-26.0)	0.537
ALT - U/L	43.0 (30.5-54.0)	31.0 (27.0-38.3)	0.240	28.0 (19.8-37.8)	32.0 (26.5-41.5)	0.268
Total Bilirubin – umol/L	0.65 (0.50-0.88)	0.95 (0.55-1.20)	0.333	0.50 (0.43-0.88)	0.60 (0.45-0.60)	0.702
NAFLD, n. (%)	4 (100)	3 (50)	.444	3 (75)	5 (100)	.126
hs-C-reactive protein – nmol/L	0.27 (0.07-0.60)	0.23 (0.06-0.40)	0.670	0.08 (0.05-0.30)	0.23 (0.09-0.29)	0.389
Red Blood Cell count, $x10^{12}/L$	4.8 (4.5-5.0)	5.4 (4.7-5.5)	0.219	4.6 (4.5-4.7)	4.7 (4.3-4.9)	0.624
Hemoglobin – g/dL	14 (14-15)	15 (14-16)	0.806	13 (13-14)	14 (13-15)	0.142
Hematocrit – %	43 (42-45)	45 (42-48)	0.221	39 (38-41)	41 (40-42)	0.142
Red cell Distribution Width – %	13.7 (12.5-14.3)	14.3 (12.9-14.8)	0.387	14.1 (13.7-14.4)	13.2 (13.2-13.6)	0.026
Platelet count – $10^9/L$	201 (138-348)	224 (213-229)	1.000	230 (169-241)	228 (194-259)	1.000
Platelet Distribution Width – fL	13.6 (10.8-16.8)	14.7 (13.5-15.0)	0.461	12.5 (11.6-15.2)	15.3 (12.9-16.1)	0.221
Mean Platelet Volume – fL	11.6 (9.7-12.7)	11.7 (11.1-11.8)	0.806	10.7 (10.1-11.9)	11.7 (10.9-16.6)	0.176
White Blood Cell count – $10^9/L$	6.44 (5.08-8.81)	7.02 (5.27-9.58)	0.624	6.36 (4.68-9.26)	6.22 (5.52-6.76)	0.806
Fasting plasma glucose – mmol/L	86.5 (77.8-100.5)	90.5 (86.8-104.3)	0.454	130.0 (96.0-150.5)	130.0 (110.5-147.5)	0.806
Fasting plasma Insulin – pmol/L	10.2 (7.27-10.37)	10.2 (7.67-20.57)	0.67	6.4 (4.5-12.57)	14.2 (11.8-23.85)	0.086
HOMA-IR	2.21 (1.4-3.33)	2.45 (1.69-4.97)	0.67	2.32 (1.43-2.99)	4.8 (3.81-7.1)	0.014
Serum Creatinine – umol/L	61.6 (48.4-88.0)	74.8 (66.0-96.8)	0.190	83.6 (52.8-83.6)	79.2 (55.4-83.6)	0.537
eGFR – ml/min	109.5 (73.5-126.1)	90.8 (74.4-102.0)	0.394	91.0 (79.8-97.8)	87.0 (82.4-104.0)	0.806

Uric Acid – umol/L	285.5 (279.6-297.4)	333.1 (237.9-535.3)	0.453	321.1 (243.9-362.8)	327.1 (303.3-440.1)	0.624
Cardiovascular disease, n. (%)	2 (50)	2 (33)	1.000	2 (50)	4 (20)	0.524
Stable CAD	0 (0)	0 (0)		1 (25)	1 (20)	1.000
Carotid stenosis (>50%)	0 (0)	2 (33)	0.467	0 (0)	2 (40)	0.444
MI or revascularization	1 (25)	0 (0)	0.400	1 (25)	1 (20)	1.000
Stroke, TIA or revascularization	1 (25)	1 (16)	1.000	1 (25)	1 (20)	1.000
Peripheral artery disease	0 (0)	0 (0)		1 (25)	0 (0)	0.444
Therapy, n. (%)						
Metformin	0 (0)	0 (0)	-	4 (100)	3 (60)	0.444
Glinide	0 (0)	0 (0)	-	1 (25)	1 (20)	1.000
PPAR-γ agonists	0 (0)	0 (0)	-	1 (25)	1 (20)	1.000
Sulfonylurea	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Insulin	0 (0)	0 (0)	-	0 (0)	0 (0)	-
sGLT2 inhibitors	0 (0)	0 (0)	-	0 (0)	1 (20)	1.000
GLP-1 RA	0 (0)	0 (0)	-	0 (0)	0 (0)	-
DPP-IV	0 (0)	0 (0)	-	1 (25)	0 (0)	0.444
Acarbose	0 (0)	0 (0)	-	0 (0)	0 (0)	-
ACE-I	1 (25)	1 (16)	1.000	0 (0)	1 (20)	-
ARBs	1 (25)	2 (33)	1.000	1 (25)	2 (40)	1.000
Diuretics	0 (0)	1 (16)	1.000	0 (0)	2 (40)	0.444
β-blockers	1 (25)	5	0.190	2 (50)	2 (40)	1.000
CCA	1 (25)	1 (16)	1.000	2 (50)	1 (20)	0.524
Other antihypertensives						-
Statins	2 (50)	3 (50)	1.000	3 (75)	1 (20)	0.206
Fibrates	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Ezetimibe	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Omega 3	0 (0)	0 (0)	-	1 (25)	0 (0)	0.444
Proton Pump Inhibitors	3 (75)	3 (50)	0.571	1 (25)	1 (20)	1.000
ASA	4 (100)	6 (100)	1.000	4 (100)	5 (100)	-
$sTXB_2$ T0	3.20 (1.17-5.56)	12.74 (8.31-18.06)	0.055	1.42 (1.05-2.50)	4.88 (3.76-12.77)	0.014
$sTXB_2$ T10	1.57 (0.35-2.47)	3.53 (1.56-5.16)	0.088	0.88 (0.47-1.52)	1.99 (1.93-5.48)	0.014
$sTXB_2$ T24	2.71 (0.95-3.49)	7.72 (5.37-11.46)	0.011	1.51 (1.26-2.58)	5.83 (5.12-13.91)	0.014
Slope_$sTXB_2$ $ngmL^{-1}h^{-1}$	0.07 (0.03-0.09)	0.270.25-0.51)	0.011	0.05 (0.04-0.08)	0.28 (0.22-0.60)	0.014

Reference range of "normal values": AST, 5-34 U/L; ALT, 0-55 U/L; hs-C-reactive protein, 0-47.62 nmol/L; Platelet count, 150-450 x109/L; Total cholesterol, 0.0-5.17 mmol/L; HDL cholesterol, 1.03-1.55 mmol/L; Triglycerides, 0-1.69 mmol/L; Fasting plasma glucose, 4.61-6.11 mmol/L; Serum Creatinine, 50.2-97.7 umol/L; Fasting plasma glucose, 3.9-5.5 mmol/L; eGFR, >60 mL/min.

Abbreviations: BMI= body mass index, WHR= Waist to Hip ratio, HDL= high-density lipoproteins, AST= Aspartate Aminotransferase, ALT= Alanine amino Transferase, NAFLD= non alcoholic fatty liver disease, HOMA-IR= Homeostatic Model Assessment of Insulin Resistance, eGFR= estimated glomerular filtration rate, CAD= coronary artery disease, CCA= Common carotid artery, MI= myocardial infarction, TIA= transient ischemic attack, PPAR-Y= peroxisome proliferator-activated receptor gamma, SGLT2= Sodium-glucose co-transporter-2, GLP1 RA= glucagon-like peptide 1 receptor agonist, DPP-IV= dipeptidyl peptidase IV, ACE-I= angiotensin-converting-enzyme -inhibitors, ARBs= angiotensin receptor blockers, CCA= calcium channel antagonists, ASA= acetylsalicylic acid.

Data are median (25th – 75th percentile). †Determined by Kruskal-Wallis or Mann-Whitney or Chi Square test, as appropriate.

Online Supplementary Table S7. Correlations between $sTXB_2$ recovery slope and clinical variables in patients with and without type 2 diabetes mellitus.

T2DM				
	Age	**BMI**	**WC**	**eGFR**
$sTXB_2$ slope	rho=-0.215 p=0.030	rho=0.217 p=0.027	rho=0.229 p=0.020	rho=0.256 p=0.009
noT2DM				
	Weight	**WHR**	**WC**	**DAP**
$sTXB_2$ slope	rho=0.217 p=0.029	rho=0.267 p=0.007	rho=0.211 p=0.034	rho=0.227 p=0.022

Abbreviations: BMI=body mass index; WC=waist circumference; eGFR=estimated glomerular filtration rate; WHR=waist-to-hip ratio; DAP=diastolic arterial pressure.

Spearman correlation coefficient and p-value are reported.