SUPPLEMENTARY APPENDIX

Platelet proteome and function in X–linked thrombocytopenia with thalassemia and *in silico* comparisons with gray platelet syndrome

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Platelet proteome and function in X−linked thrombocytopenia with thalassemia and *in silico* comparisons with gray platelet syndrome

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Supplementary Material – Supplementary Methods

Hematological and other blood tests

Standard hematology and other variables were analyzed at the routine laboratories at the respective Departments of Clinical Chemistry at Örebro University Hospital, Falun Hospital, or Karolinska University Hospital (Stockholm), all in Sweden. For thrombopoietin (TPO) measurements, serum from three male XLTT patients, one female carrier (mother of patient A:II)[1], and matched controls was collected and stored at -80ºC until analysis. TPO was measured using an ELISA method (Human Thrombopoietin Quantikine ELISA Kit, R&D Systems, Minneapolis, MN), following the manufacturer's instructions. The correlation between platelet counts (at sampling) and TPO levels was determined.

Transmission electron microscopy (TEM)

Blood, obtained by venipuncture without stasis, was collected in CTAD (citrate, theophylline, adenosine and dipyridamole) Vacutainer® tubes (Becton, Dickinson, Franklin Lakes, NJ). After centrifugation, platelet-rich plasma (PRP) was aliquoted and centrifuged to collect platelet pellets. Plasma was removed and platelet pellets were fixed with 2.5% glutaraldehyde and further processed for TEM as earlier described.[2] TEM was performed at the electron microscopy unit, Karolinska University Hospital Huddinge (Stockholm, Sweden).

Platelet proteomics

Platelet isolation and lysis. Isolation of platelet protein was done by collection of venous patient blood in EDTA coated vacutainer tubes. The tubes were kept in a rocker for approximately 20 min from the collection time before transferred into fresh tubes and centrifuged at 220 × g for 20 min in a swing out rotor at 22°C. The top third of the plasma (PRP) was transferred to propylene tubes in 1 mL aliquots and centrifuged in a swing-out rotor at 22°C at 480 × g for 20 min. The plasma was removed without disturbing the pellet and the pellet washed twice using 0.5 mL of Krebs–Ringer glucose buffer (KRG) without calcium. Any remaining KRG was removed with a filter paper. The remaining pellet was finally dissolved in 100µL of lysis solution (8 M Urea, 2% (W/V)

3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and protease inhibitor cocktail P1048 (Sigma-Aldrich Co USA) was added in a ratio 1:100 before storage in -80°C. Thawed samples were centrifuged for 10 min at 2,000 × g in 4°C and the supernatants were moved into new tubes. Total protein concentration of the lysed platelet pellets was determined with Pierce™ BCA Protein Assay (Thermo Scientific).

Samples for quantitative mass spectrometry (QMS). Samples were run in 10-plex and from practical reasons separated into different sets. A reference sample, made from a pool of platelet lysates with equal total protein amounts from patients with myeloproliferative neoplasms (N=13; 4 polycythemia vera, 4 essential thrombocythemia and 5 primary myelofibrosis) and healthy controls (N=5) from two earlier analyzed sets, were used in all assays to be able to compare samples between different runs.

Tryptic Digestion and TMT (tandem mass tags) labeling of proteins. Protein aliquots of each sample, and an equal amount of protein from the reference sample, were denatured and reduced by addition of 10% SDS and 0.5 M TCEP to a final concentration of 2% SDS and 10 mM TCEP and incubated at 37ºC for 2 h. The samples were trypsin digested using a modified filter-aided sample preparation (FASP) method.[3] In short, reduced samples diluted with 400 µl wash buffer (8 M urea in 100 mM TEAB) were applied on Nanosep 30k Omega filters (Pall Life Sciences) and wash buffer was used to repeatedly wash away the SDS. Alkylation was performed with methyl methane thiosulfonate (MMTS) 10 mM diluted in wash buffer, followed by repeated washing with wash buffer. Trypsin (Pierce Trypsin Protease, MS Grade, Thermo Fisher Scientific) in a ratio of 1:25 relative to protein amount was added with 100 mM TEAB to a pH of about 8 and the samples were incubated in 37°C for 3 h. Another portion of trypsin was added before incubation overnight at 37°C. The peptides were collected by centrifugation and subjected to isobaric mass tagging reagent TMT® according to the manufacturer's instructions (Thermo Scientific). In a set, each sample and a reference were labelled with a unique tag from a TMT 10plex isobaric mass tag labeling kit, and 5 sets were prepared in total to cover all samples. After TMT labeling, the samples in a set were pooled and acidified with formic acid to pH about 2.

Fractionation of TMT labelled peptides. The peptides were further purified and fractionated by Strong Cation Exchange Chromatography (ÄKTA-system, Amersham-Pharmacia) on a PolySULFOETHYL A™ column (100 × 2.1mm, 5µm 300Å, PolyLC inc.). Prior to fractionation the samples were concentrated to ~90µl in a vacuum centrifuge and diluted with 140 µl 10% formic acid in water,

120 µl acetonitrile and water to a final volume of 650 µl. Solvent A was 25 mM ammonium formate, pH 2.8 and solvent B was 500 mM ammonium formate, pH 2.8. The following gradient was run at 0.25 mL/min: 20% B over 20 min, 40% B over 10 min and finally 100% B over 10 min. A total of 28 fractions were collected and 20 fractions containing the peptides from each set were desalted using PepClean C18 spin columns (Thermo Fisher Scientific) according to the manufacturer's guidelines.

LC-MS/MS Analysis. The dried desalted 10-plexed TMT-labeled sample fractions were reconstituted with 15 µl of 0.1% formic acid (Sigma Aldrich) in 3% acetonitrile and analyzed on QExactive or Orbitrap Fusion Tribrid mass spectrometers interfaced to an Easy-nLC II (Thermo Fisher Scientific). Peptides (2 µL injection volume) were separated using an in-house constructed analytical column (300 × 0.075 mm I.D.) packed with 1.9 µm Reprosil-Pur C18-AQ particles (Dr. Maisch, Germany). Solvent A was 0.2% formic acid in water and solvent B was 0.2% formic acid in acetonitrile. The following gradient was run at 200 nL/min: 5−30% B over 75 min, 30−80% B over 5 min, with a final hold at 80% B for 10 min. Ions were injected into the mass spectrometer under a spray voltage of 1.8 kV in positive ion mode.

MS scans on the QExactive were performed at 70,000 resolution with a mass range of m/z 400−1,600. MS/MS analysis was performed in a data-dependent mode, with the top ten most abundant doubly or multiply charged precursor ions in each MS scan selected for fragmentation (MS2) by stepped high energy collision dissociation (stepped HCD) of NCE-value of 30, 40 and 50. For MS2 scans the following parameters were employed: 1 microscan performed at 35,000 resolution, a fixed first mass of m/z 100, an isolation window of 2 Da, intensity threshold of 1.1e4 and a dynamic exclusion of 15 seconds, enabling most of the co-eluting precursors to be selected for MS2.

MS scans at the Fusion were performed at 120,000 resolution, m/z range 350−1,500. MS/MS analysis was performed in a data-dependent multinotch mode, with a top speed cycle of 3 s for the most intense doubly or multiply charged precursor ions. Ions in each MS scan over threshold 5,000 were selected for fragmentation (MS2) by collision induced dissociation (CID) for identification at 30% and detection in the ion trap, followed by multinotch (simultaneous) isolation of the top 10 MS2 fragment ions. Those with m/z 400−900 were selected for fragmentation (MS3) by high energy collision dissociation (HCD) at 55% and detection in the Orbitrap at 60,000 resolution, m/z range 100−500. Precursors were isolated in the quadrupole with a 1.6 m/z window, and dynamic exclusion within 10 ppm during 30 s was used for m/z-values already selected for fragmentation.

Database Search for protein TMT Quantification. MS raw data files for each TMT set were merged for relative quantification and identification using Proteome Discoverer version 1.4 (Thermo Fisher Scientific). A database search for each set was performed with the Mascot search engine (Matrix Science) using the Homo Sapiens Database version November 2014 (Swiss Institute of Bioinformatics, Switzerland) with MS peptide tolerance of 5 ppm and MS/MS tolerance for identification of 50 millimass units (mmu). Tryptic peptides were accepted with one missed cleavage and variable modifications of methionine oxidation, cysteine alkylation and fixed modifications of N-terminal TMT-label and lysine TMT-label were selected. The detected peptide threshold in the software was set to 1% False Discovery Rate by searching against a reversed database, and identified proteins were grouped by sharing the same sequences to minimize redundancy. For TMT quantification, the ratios of the TMT reporter ion intensities in HCD MS/MS spectra (m/z 126−131) from raw data sets were used. Ratios were derived by Proteome Discoverer using the following criteria: fragment ion tolerance as 3 mmu for the most confident centroid peak and missing values were replaced with minimum intensity. Only peptides unique for a given protein were considered for relative quantification, excluding those common to other isoforms or proteins of the same family. The quantification was normalized using the protein median.

Bioinformatics

Protein data were imported to the Ingenuity Pathway Analysis (IPA®) software (QIAGEN's Ingenuity Pathway Analysis, Redwood City, CA, USA), and analyzed[4] (13/05/2019).

By upstream analysis in IPA it is possible to identify potential upstream regulators that might be responsible for alterations recognized from the dataset. The analyses have two different statistical measures: the overlap *P*-value and the Z-score. The overlap *P*-value (calculated by a right-sided Fisher's exact test) measures whether there is significant overlap between the dysregulated dataset molecules and molecules that are known to be regulated in the diseases and functions or by the predicted upstream regulators. The Z-score is calculated to indicate the activation state, where an absolute Z-score ≥2.0 is considered significant. A positive Z-score predicts activation and a negative Z-score predicts inhibition based on the numbers of up- and downregulated molecules in the dataset. A protein−protein interaction network analysis was performed using STRING[5] (28/07/2020).

Immunohistochemistry

Paraffin embedded bone marrow biopsies from the four adult XLTT patients and 7 healthy controls were examined according to routine procedures, including evaluations of cellularity, megakaryocyte morphology, and fibrosis grade. The protein expressions of PTGS1/COX-1 and SLC35D3 were estimated in 4 µm tissue sections using immunohistochemistry (IHC). Following deparaffinization and rehydration, antigen retrieval was performed in Diva Decloaker buffer (pH 6.0) and Decloaking Chamber™ (Biocare Medical, Gothenburg, Sweden) for 10 min at 110°C. The polyclonal rabbit anti-PTGS1/COX-1 antibody (1 h at RT, 1:50, Atlas Antibodies Cat#HPA002834, RRID:AB_1079711, Sigma-Aldrich, Stockholm, Sweden) and the polyclonal rabbit anti-SLC35D3 antibody (30 min at RT, 1:1,000, Atlas Antibodies Cat#HPA030431, RRID:AB_10601716, Sigma-Aldrich, Stockholm, Sweden) were visualized by 3,3'-Diaminobenzidine (DAB, Biocare). The staining procedure was performed using the automated slide stainer instrument IntelliPATH FLX® (Biocare) with the MACH1™ Universal HRP-Polymer Detection system (Biocare) according to manufacturer's instructions. The sections were counterstained with Mayer's Hematoxylin, dehydrated and mounted using Pertex® mounting medium (Histolab, Gothenburg, Sweden). The IHC stained sections were digitalized using the Pannoramic 250 FLASH II Scanner (3DHistech Ltd, Budapest, Hungary), and the protein expression (i.e. staining) was assessed in a viewer software (CaseViewer, 3DHistech).

Flow cytometry

Venous blood was collected with minimal stasis into Vacutainer® tubes (Becton Dickinson (BD), Franklin Lakes, NJ, USA) anticoagulated with citrate. For measurements with annexin V where extracellular calcium is crucial, blood tubes with heparin or hirudin (Roche Diagnostics GmbH, Mannheim, Germany) were used. The blood was allowed to rest for 1 h before the start of experimental procedures.

To activate the platelets, agonists used were adenosine diphosphate (ADP) from Sigma (St. Louis, MO, USA), peptides activating platelet thrombin receptor PAR1 (PAR-1-AP, also known as TRAP, amino acid sequence SFLLRN-OH[6] or PAR4 (PAR4-AP, sequence AYPGKF-NH2,[7] both from JPT Peptide Technologies GmbH (Berlin, Germany) and cross-linked collagen-related peptide (CRP-XL, sequence Gly-Cys-Hyp-(Gly-Pro-Hyp)10-Gly-Cys-Hyp-Gly-NH2), purchased from Prof. Richard W. Farndale (Cambridge, UK).[8] In some samples, apyrase (Grade VII) from Sigma was added to degrade ADP released from the dense granule of activated platelets. This was done to investigate whether defects in release or content of dense granule were present, as previously described.[9, 10]

For flow cytometry, 3 µL of blood was added to 33 µL of HEPES buffer (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 5.6 mM glucose, 1 g/L bovine serum albumin, 20 mM HEPES, pH 7.4, chemicals from Sigma) containing platelet agonists and antibodies for identification of platelets (0.03 µg/mL PE-mouse-anti-human-CD41 (BD, clone H1P8) or 2.5 µg/mL PE-mouse-anti-human-CD42b (Dako, Glostrup, Denmark, clone AN51) and detection of platelet activation (0.02 µg/mL APC- mouse-anti-humanCD62P (P-selectin, BD, clone AK-4), and FITC-chicken-anti-human-fibrinogen (saturating concentration, Diapensia HB, Linköping, Sweden). In complementing experiments, mouse-anti-human-LAMP-1 (CD107a, BD, clone H4A3, conjugated to PE-Cy5 or PE-Cy7, final concentration 0.5 µg/mL) and Annexin V (BD, conjugated to V450 or APC, final concentration 2 or 0.11 µg/mL, respectively) was used to investigate capacity of exocytosis of lysosomes and potential to expose the procoagulant phospholipid phosphatidylserine (PS) upon platelet activation. When PS expression was investigated, HEPES buffer supplemented with 1.5 mM of Ca2+ was used, as binding of the PS marker annexin V is dependent on Ca2+.

To determine background fluorescence levels for gating, negative controls were always run in parallel with the same blood but using isotype antibodies with the same concentrations and fluorophores as the P-selectin and LAMP-1-antibodies. For annexin V, the background control was created by omitting Ca2+ in the buffer as this is crucial for annexin V binding. For the anti-fibrinogen antibody, the background control was created by addition of 10 mM EDTA to the buffer, as this will disrupt the structure of the platelet fibrinogen receptor GPIIb/IIIa and thus prevents binding of fibrinogen.[11] On all occasions, a sample with buffer instead of platelet agonist was included to ensure that the sampling procedure and handling did not cause significant pre-analytical platelet activation.

The samples were incubated for 10 min at room temperature before dilution in buffer (final dilution at least 1:20) and analysis using a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA). The acquisition threshold was set on PE (FL2), to exclude non-platelet particles from analysis.

Gating and analysis. Platelets were identified by their characteristic forward scatter and PE-fluorescence. All data was further analyzed using KaluzaTM software (Beckman Coulter). To determine the percentage of platelets positive for the different platelet activation markers, gates were set with 1−2% positive platelets in the background fluorescence control samples, as previously recommended.[12] Examples of dot plots for Patient V and a normal donor analyzed at the same occasion are shown in *Supplementary Figure S1*.

Statistical analyses

The difference in S-TPO level between XLTT family members and controls was evaluated by Student's t-test. Relative protein levels (fold change compared to the pooled reference sample where the level for no difference was set to 1) from QMS of platelets from 5 XLTT patients and 5 healthy controls were compared using 2-tailed t-tests and corrected for multiple comparisons by estimation of q-values.[13] Protein abundance alterations with $q < 0.05$ and FC $\geq \pm 1.2$ were regarded as statistically significant and biologically relevant, given successful quantification in at least three patients and controls. However, for upstream analyses and comparison analyses (to another dataset), all proteins with FC $\geq \pm 1.2$ and successful quantification in at least two patients and controls were included, regardless of q- or *P*-values. Statistical evaluation of IHC staining results was performed by Mann-Whitney U-test comparing the megakaryocyte H-scores[14] for XLTT patients and controls. The software IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) was used for computations.

Supplementary References

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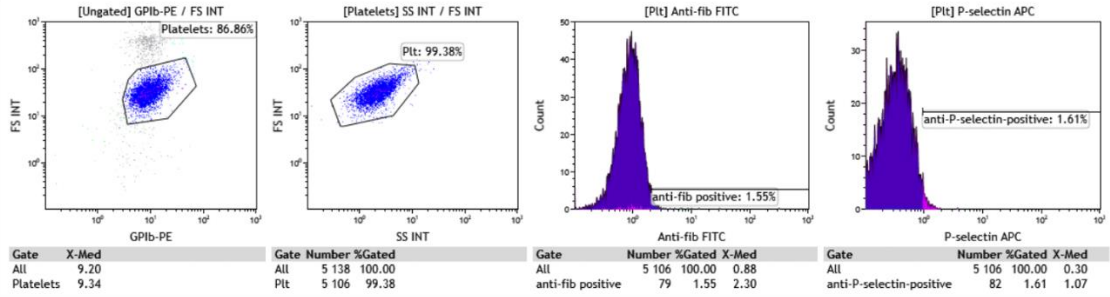
Supplementary Figures

Resting platelets: Normal donor.
Negative (isotype) control for gating



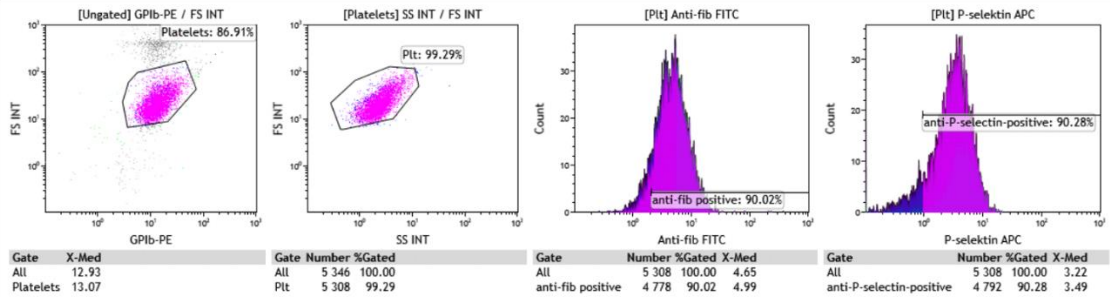
Gate	X-Med
All	9.20
Platelets	9.34

Gate	Number	%Gated
All	5 138	100.00
Plt	5 106	99.38

Gate	Number	%Gated	X-Med
All	5 106	100.00	0.88
anti-fib positive	79	1.55	2.30

Gate	Number	%Gated	X-Med
All	5 106	100.00	0.30
anti-P-selectin-positive	82	1.61	1.07

Platelets activated with PAR1-AP (10 µM): Normal donor.



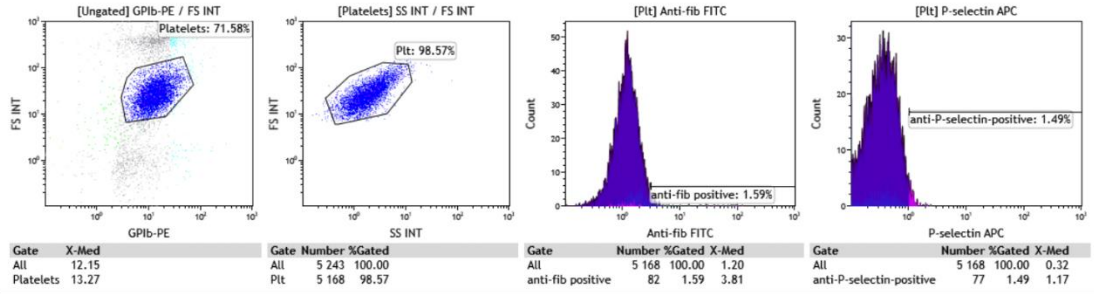
Gate	X-Med
All	12.93
Platelets	13.07

Gate	Number	%Gated
All	5 346	100.00
Plt	5 308	99.29

Gate	Number	%Gated	X-Med
All	5 308	100.00	4.65
anti-fib positive	4 778	90.02	4.99

Gate	Number	%Gated	X-Med
All	5 308	100.00	3.22
anti-P-selectin-positive	4 792	90.28	3.49

Resting platelets: Patient V.
Negative (isotype) control for gating



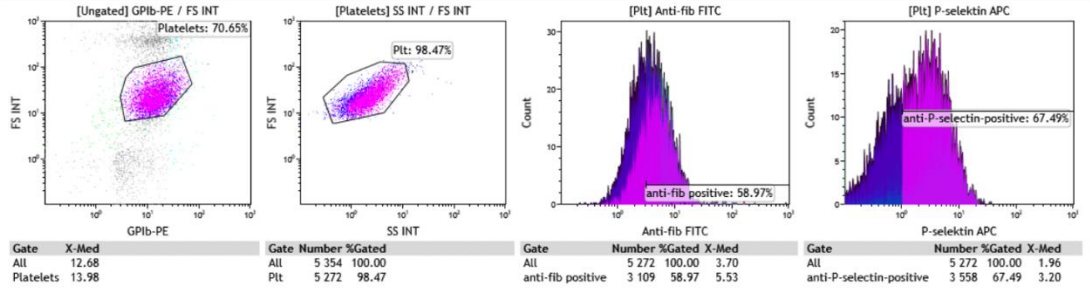
Gate	X-Med
All	12.15
Platelets	13.27

Gate	Number	%Gated
All	5 243	100.00
Plt	5 168	98.57

Gate	Number	%Gated	X-Med
All	5 168	100.00	1.20
anti-fib positive	82	1.59	3.81

Gate	Number	%Gated	X-Med
All	5 168	100.00	0.32
anti-P-selectin-positive	77	1.49	1.17

Platelets activated with PAR1-AP (10 µM): Patient V.



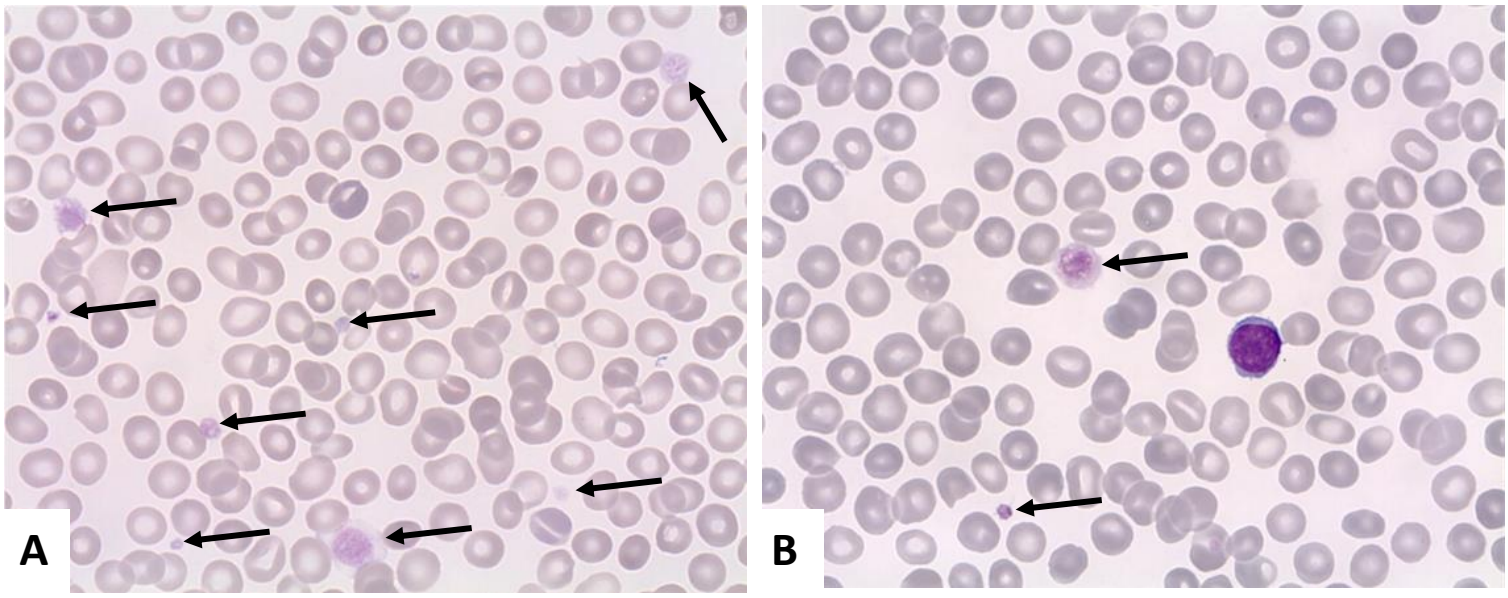
Gate	X-Med
All	12.68
Platelets	13.98

Gate	Number	%Gated
All	5 354	100.00
Plt	5 272	98.47

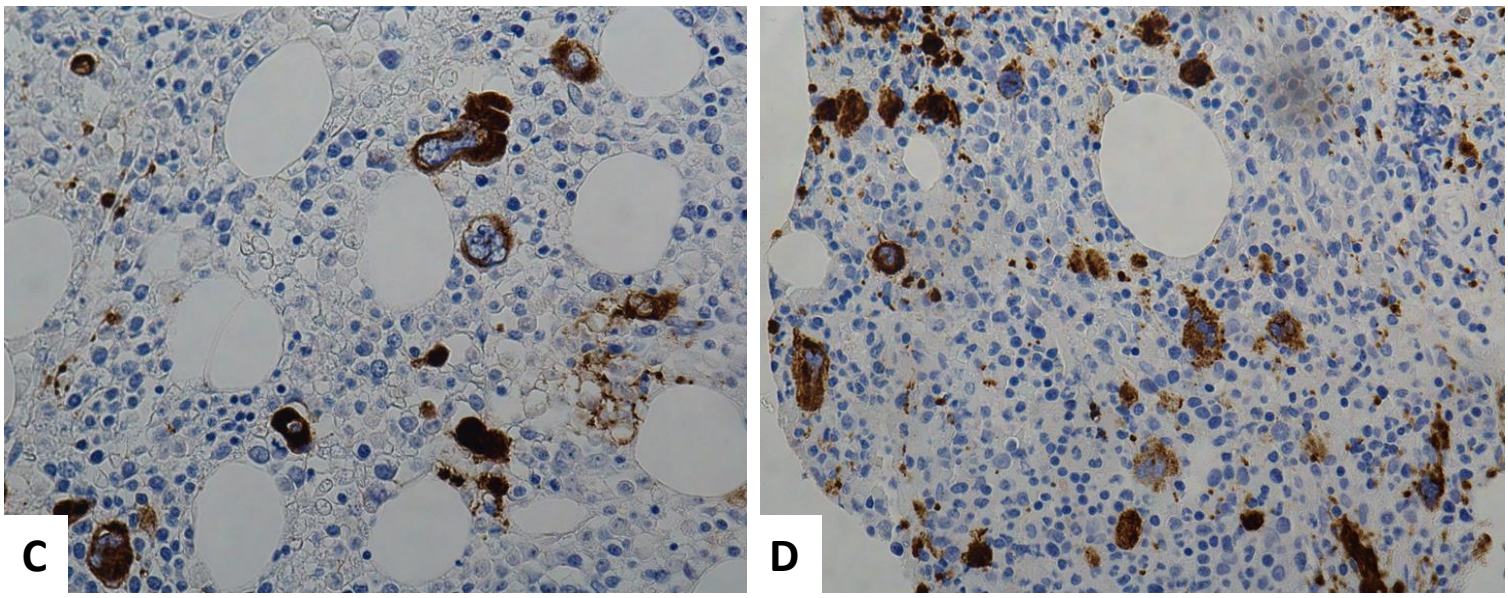
Gate	Number	%Gated	X-Med
All	5 272	100.00	3.70
anti-fib positive	3 109	58.97	5.53

Gate	Number	%Gated	X-Med
All	5 272	100.00	1.96
anti-P-selectin-positive	3 558	67.49	3.20

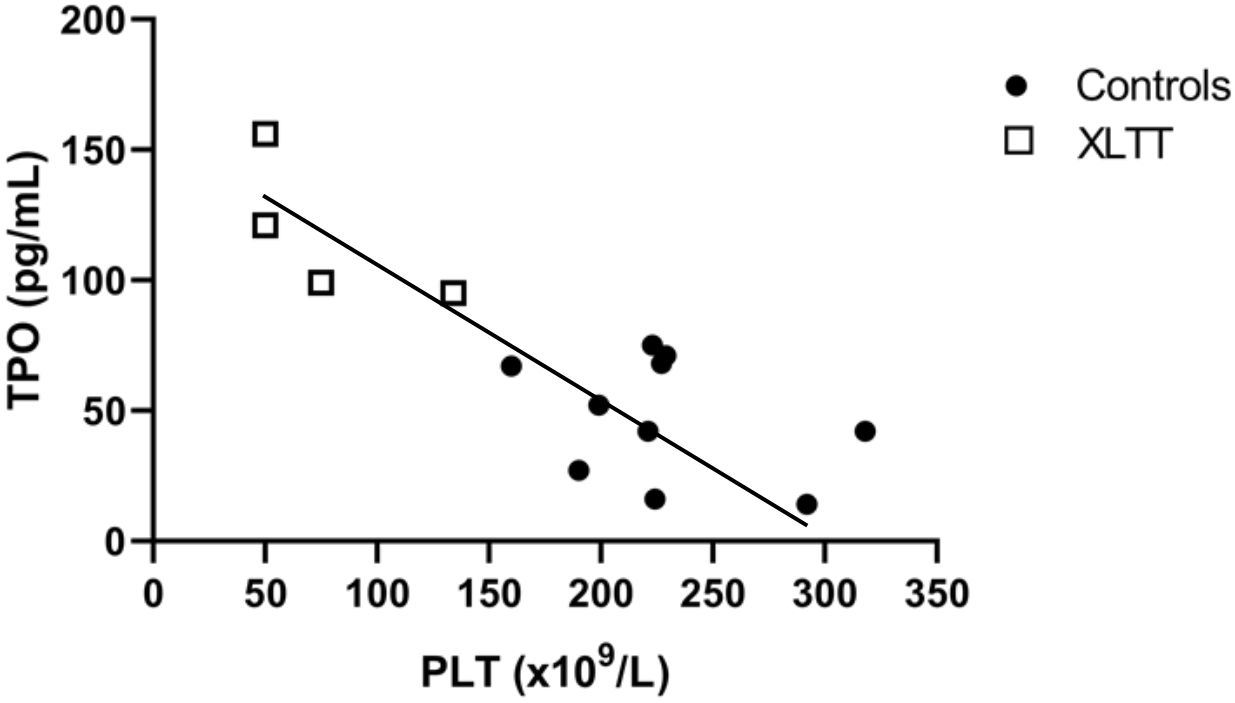
Supplementary Figure S1. Gating strategy for platelet function analysis by flow cytometry, and example plots from patient V and normal donor analyzed at the same occasion.



Supplementary Figure S2. Examples of platelets and megakaryocytes from XLTT patients.
(A-B) Light microscopy of peripheral blood smears from patients II and V with thrombocytopenia. The platelets (marked by arrows) are poorly stained (characteristic of "gray platelets"). Some of them are macrothrombocytes of similar size as the red blood cells.
May-Grunewald-Giemsa staining (×100).

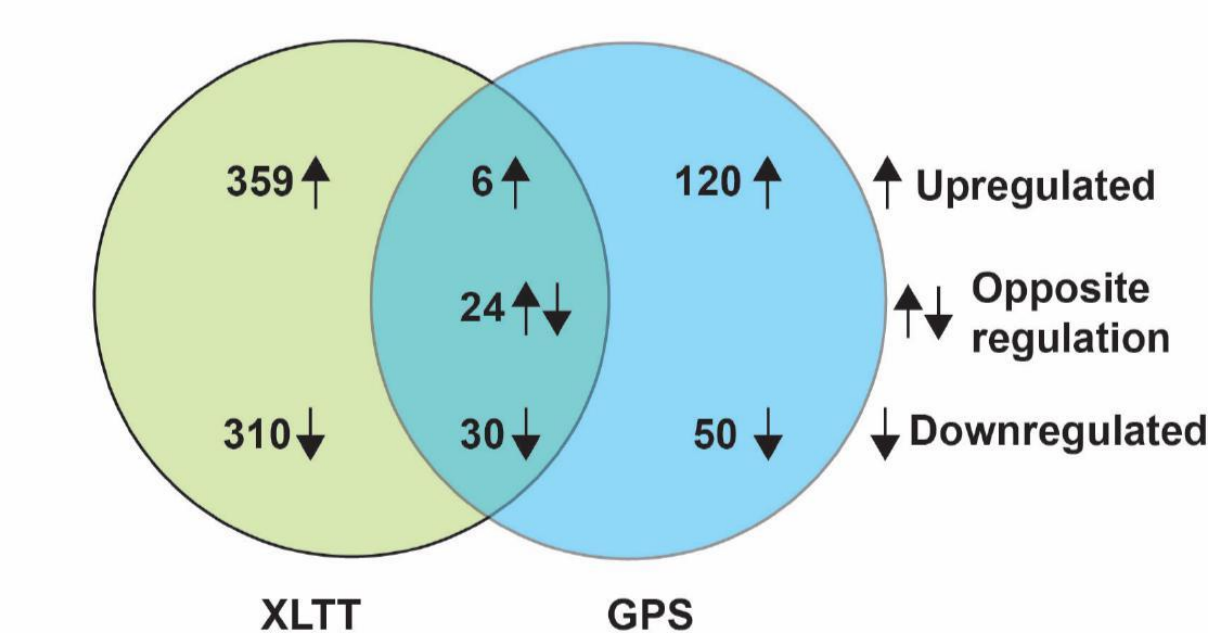


Supplementary Figure S2 ***(continued)***
(C-D) CD61 immunohistochemistry staining of bone marrow biopsies from patients II and III (×40). Megakaryocytes are abundant and more than 90% of them stain intensively for CD61.



Supplementary Figure S3. Correlation between S-TPO and platelet counts.

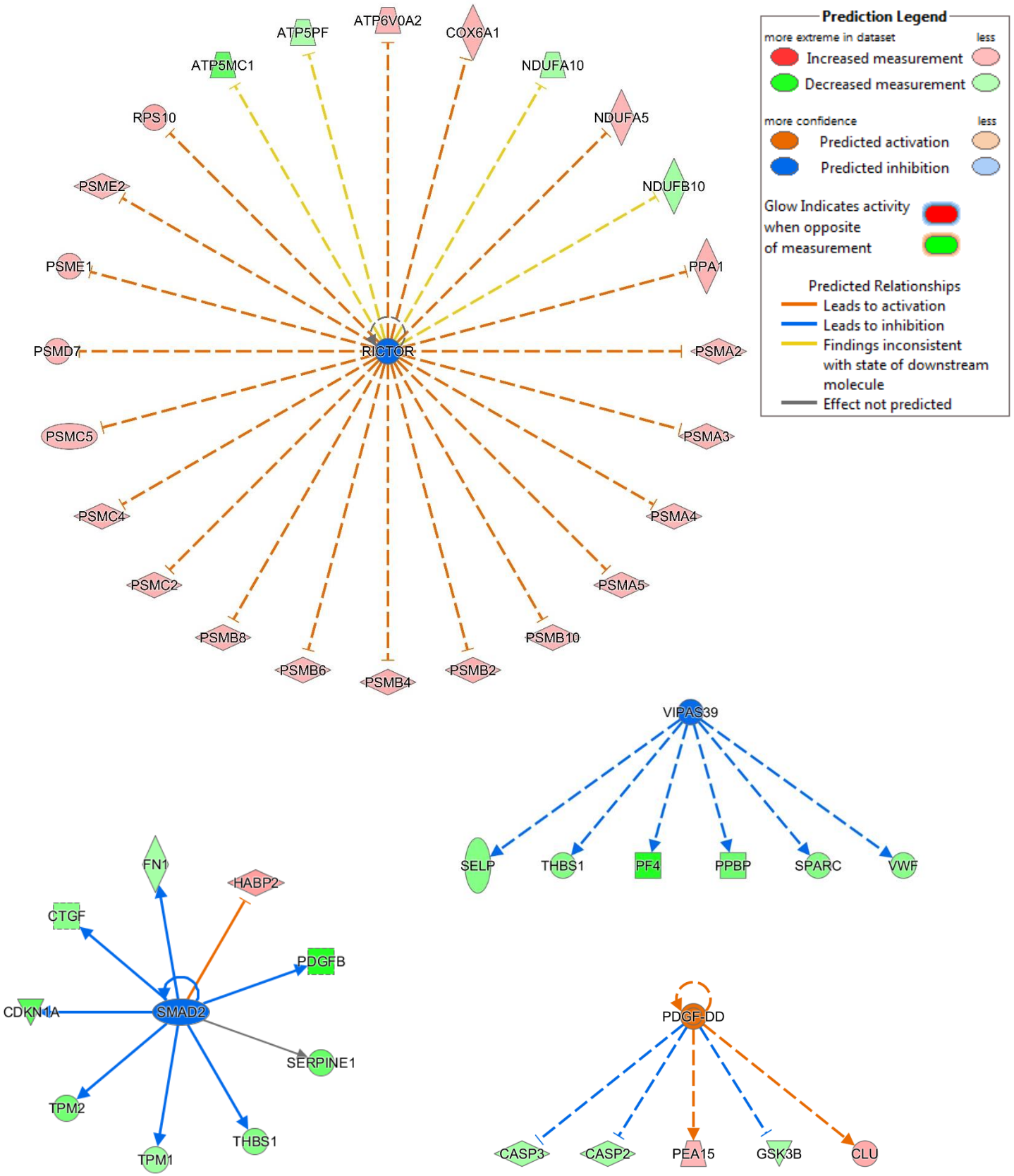
S-Thrombopoietin (TPO) values (pg/mL) and platelet counts (PLT) (× 10^9/L) in three adult XLTT males and one female carrier as well as 10 healthy blood donors of comparable ages.



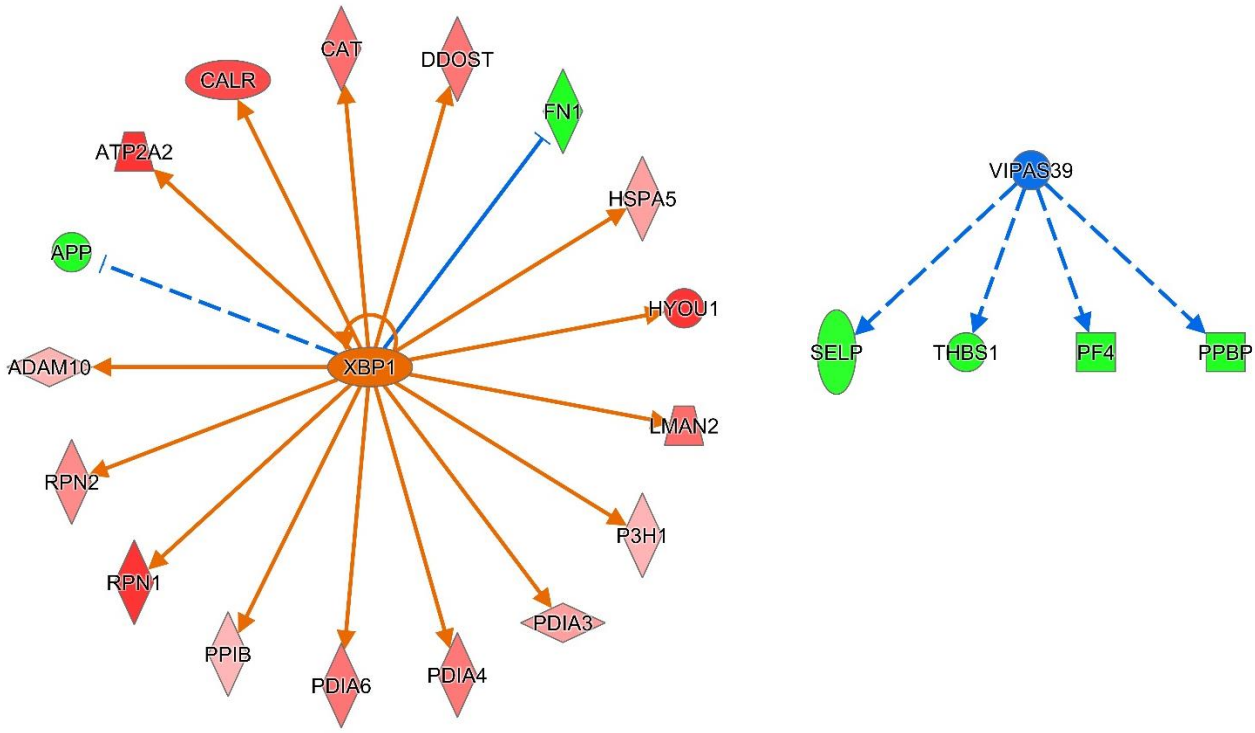
Supplementary Figure S4. Venn diagram of overlap between XLTT and GPS.
Proteins altered by FC ≥±1.2, without regard to statistical significance, comparing XLTT and GPS (729 and 230 proteins respectively). The names and regulations of the 60 proteins in the overlapping section of the diagram are displayed in *Supplementary Table S1*.

Supplementary Figure S5

A



B



Supplementary Figure S5. Predicted upstream regulators for XLTT and GPS platelet proteomes. (A) In XLTT platelets, the strongest predicted inhibition among upstream regulators was seen for RICTOR. Downstream dysregulated proteins in XLTT platelets (reaching FC ≥±1.2) predicting inhibited status of RICTOR, SMAD2 and VIPAS39 as well as activated status of PDGF-DD are displayed.

(B) In GPS platelets, the strongest predicted activation among upstream regulators was seen for XBP1, with downstream proteins reaching FC ≥±1.2 shown here. Also the four downregulated granule proteins predicting the inbibited status of VIPAS39 are shown, all of them overlapping with the 6 corresponding proteins in XLTT (Supplementary Figure S3A).

Supplementary Table S1. Sixty commonly dysregulated proteins in GPS and XLTT with FC ≥±1.2 regardless of statistical significance.

Symbol	Entrez Gene Name	GPS FC	XLTT FC	XLTT q	XLTT *P*
ABHD11	abhydrolase domain containing 11	-1.60	1.21	0.4720	0.316726
ACAA1	acetyl-CoA acyltransferase 1	1.25	-1.34	0.1052	**0.012307**
ALDOC	aldolase. fructose-bisphosphate C	5.64	-1.59	0.2052	0.053102
ANO6	anoctamin 6	-2.88	-1.31	**0.0043**	**0.000023**
APP	amyloid beta precursor protein	-35.67	-1.58	**0.0194**	**0.000397**
ATP2A2	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2	4.71	-1.23	**0.0333**	**0.001496**
ATP2A3	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 3	3.90	-1.30	**0.0103**	**0.000161**
ATP2C1	ATPase secretory pathway Ca2+ transporting 1	2.09	-1.33	0.0569	**0.004040**
ATP8A1	ATPase phospholipid transporting 8A1	-1.43	-1.36	0.1164	**0.016076**
CCL5	C-C motif chemokine ligand 5	-2.82	-1.61	0.0680	**0.005659**
CLU	clusterin	-6.19	1.43	0.2503	0.077768
CNN2	calponin 2	1.31	-1.34	0.1357	**0.022272**
CSRP1	cysteine and glycine rich protein 1	2.06	-1.25	0.3651	0.171540
F13A1	coagulation factor XIII A chain	-1.60	-1.39	**0.0220**	**0.000649**
FERMT3	fermitin family member 3	1.81	-1.22	0.1637	**0.032975**
FHL1	four and a half LIM domains 1	1.47	-1.77	**0.0285**	**0.001050**
FN1	fibronectin 1	-11.86	-1.29	0.4693	0.312757
HLA-A	major histocompatibility complex. class I. A	1.63	-6.20	0.4056	0.221382
HSD17B4	hydroxysteroid 17-beta dehydrogenase 4	-1.59	-1.32	**0.0220**	**0.000640**
IGHG2	immunoglobulin heavy constant gamma 2 (G2m marker)	-1.46	1.40	0.5523	0.483146
IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)	-1.98	1.26	0.6287	0.651127
IGHG4	immunoglobulin heavy constant gamma 4 (G4m marker)	-1.38	-1.82	0.3671	0.175857
JAM3	junctional adhesion molecule 3	-2.35	-1.22	0.3207	0.129354
KRT1	keratin 1	-1.21	-3.23	0.5324	0.429448
KRT2	keratin 2	-1.44	-3.61	0.5092	0.375371
KRT5	keratin 5	-2.27	-2.26	0.6067	0.605168
KRT9	keratin 9	-1.63	-4.65	0.5143	0.386226
LASP1	LIM and SH3 protein 1	1.83	-1.27	0.3923	0.201879
LDHB	lactate dehydrogenase B	2.77	1.30	0.1099	**0.013868**
LTBP1	latent transforming growth factor beta binding protein 1	-99.06	-2.11	**0.0028**	**0.000009**

MLEC	malectin	2.74	-1.29	**0.0413**	**0.002095**
MMRN1	multimerin 1	-11.71	-1.69	**0.0220**	**0.000664**
MYL9	myosin light chain 9	-1.49	-1.20	0.2966	0.110348
MYO1C	myosin IC	-1.67	-1.40	0.3270	0.137700
NIPSNAP3A	nipsnap homolog 3A	-1.82	1.23	0.3736	0.182781
PF4	platelet factor 4	-4.09	-3.56	0.2649	0.086287
PF4V1	platelet factor 4 variant 1	-5.08	-2.30	0.4278	0.248248
PFKP	phosphofructokinase. platelet	-2.31	-1.21	0.1859	**0.042660**
PKM	pyruvate kinase M1/2	1.82	-1.45	0.0569	**0.004022**
PLEK	pleckstrin	1.33	-1.34	0.0510	**0.003110**
PPBP	pro-platelet basic protein	-3.60	-2.08	**0.0103**	**0.000147**
PRDX2	peroxiredoxin 2	1.35	2.56	0.1535	**0.028622**
PSTPIP2	proline-serine-threonine phosphatase interacting protein 2	3.01	1.25	0.0569	**0.004044**
PTPRJ	protein tyrosine phosphatase. receptor type J	-2.08	-1.22	0.0680	**0.005610**
RAB5B	RAB5B. member RAS oncogene family	1.52	1.21	0.4140	0.230434
RAP1B	RAP1B. member of RAS oncogene family	-1.49	-1.26	0.4608	0.295873
RHOG	ras homolog family member G	2.00	-1.28	0.3208	0.130360
RSU1	Ras suppressor protein 1	1.25	-1.30	0.4596	0.293241
SACM1L	SAC1 like phosphatidylinositide phosphatase	1.89	1.29	**0.0330**	**0.001376**
SELP	selectin P	-3.01	-1.74	**0.0040**	**0.000017**
SFXN1	sideroflexin 1	-1.39	-1.50	0.1972	**0.048408**
SLC2A3	solute carrier family 2 member 3	-1.49	-1.35	0.1138	**0.015015**
STOM	stomatin	-3.15	-1.29	0.0935	**0.010324**
SYTL4	synaptotagmin like 4	-2.34	-1.22	0.1150	**0.015495**
THBS1	thrombospondin 1	-18.42	-1.78	**0.0088**	**0.000093**
TREML1	triggering receptor expressed on myeloid cells like 1	-1.34	-1.35	0.2200	0.060135
TUBA4A	tubulin alpha 4a	4.07	-1.22	0.0680	**0.005556**
WDR1	WD repeat domain 1	1.62	-1.30	**0.0330**	**0.001395**
YWHAG	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma	1.93	1.20	0.1241	**0.018504**
YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta	2.44	-1.37	**0.0061**	**0.000038**

30	DOWN in GPS. DOWN in XLTT.
19	UP in GPS. DOWN in XLTT.
5	DOWN in GPS. UP in XLTT.
6	UP in GPS. UP in XLTT.

Supplementary Table S2. Results for analysis of lysosomal exocytosis (LAMP-1 expression) and procoagulant platelet (Annexin V-positive) formation for patient II and V, as compared to results for normal donors. Note that the patients were not analyzed at the same occasion or with the same protocol, as this was still under development, which means that fluorophores and agonist concentrations are not the same in these experiments. This means that data should only be compared to the results for the normal donors analyzed with exactly the same settings.

Analysis	Agonist	Patient II	Patient V	Corresponding results in normal donors (mean ± SD)
LAMP-1 expression	PAR4-AP 50 µM	14.3		13.8 ± 10.7 (n=4)
LAMP-1 expression	PAR4-AP 100 µM		14.1	47.3 ± 14.0 (n=20)
Procoagulant (Annexin V-positive) platelets	CRP-XL 0.9 µg/ml + PAR1-AP 30 µM	33.4		18.5 ± 3.6 (n=4)
Procoagulant (Annexin V-positive) platelets	CRP-XL 2 µg/mL + PAR1-AP 10 µM		17.4	27.6 ± 10.1 (n=20)