SUPPLEMENTARY MATERIAL

Platelet agonists

The following agonists were used: Crosslinked collagen-related peptide (CRP), with the sequence Gly-Cys-Hyp-(Gly-Pro-Hyp)$_{10}$-Gly-Cys-Hyp-Gly-NH2, was provided by Dr. Richard W. Farndale, from the University of Cambridge (UK). Collagen Reagent HORM® Suspension (KRH) was purchased from Takeda Austria GmbH (Austria). Thrombin and ADP were purchased from Sigma (Sigma-Aldrich, St. Louis, MO) and arachidonic acid from Cayman Chemical (Michigan, USA).

Rhodocytin was provided by Johannes A. Eble, from Center for Molecular Medicine, Excellence Cluster Cardio-Pulmonary System, Frankfurt University Hospital, Frankfurt am Main, Germany.

Supplementary Methods

2D-DIGE

Six gels (technical replicates) were run in the experiment with a total of 150 μg of mixed labelled protein per gel. These protein mixtures contained 50 μg of protein from each sample (10 obese pooled and 10 lean pooled matched-controls) randomly labeled with 400 pmol minimal CyDye DIGE fluors (Cy3 and Cy5), and 50 μg of a pool of both conditions (25 μg from obese patients and 25 μg from lean controls) labeled with 400 pmol Cy2 (internal standard). Labelling was performed for 30 min on ice in the dark. The reaction was stopped with 1 μl of 10 mM lysine acting for 10 min on ice in the dark. After labelling step, the three samples were pooled and an equal volume of 2× sample buffer was added (65 mM CHAPS, 2 M thiourea, 5 M urea, 0.15 M NDSB-

256, 130 mM DTT, 4 mM tributylphosphine, 1 mM sodium vanadate, 0.1 mM sodium fluoride, and 1 mM benzamidine).

After mixing, the tube was left for 10 min on ice in the dark. For reswelling, samples were diluted up to a total of 500 µl of 2D Sample buffer (5 M urea, 2 M thiourea, 2 mM tributylphosphine, 65 mM DTT, 65 mM CHAPS, 0.15 M NDSB-256, 1 mM sodium vanadate, 0.1 mM sodium fluoride, and 1 mM benzamidine, final concentration), and ampholytes (Servalyt 4–7) were added to a final concentration of 1.6% (v/v). IPG strips were rehydrated with the samples for 16 h in the dark. Isoelectric focusing (IEF) was run on 24 cm, pH 4–7 IPG strips (GE Healthcare) powered by a Multiphor II (GE Healthcare) for 64.9 kVh at 17 °C.

Following the first dimension, IPG strips were immediately equilibrated for 15 min in reduction buffer (6 M urea, 50 mM tris pH 8.8, 30% glycerol, 2% w/v SDS, 65 mM DTT and traces of bromophenol blue) and then for 15 min in alkylation buffer (6 M urea, 50 mM tris pH 8.8, 30% glycerol, 2% w/v SDS, 135 mM iodoacetamide and traces of bromophenol blue) with gentle agitation; all steps in the dark. IPG strips were washed out with ultrapure water and placed on top of the second dimension gels, embedded with 0.5% melted agarose.

Proteins were separated in the second dimension by SDS-polyacrylamide gel electrophoresis (PAGE) on 11% polyacrylamide gels at run conditions of 10°C, 20 mA per gel for 1 h, followed by 40 mA per gel for 4 h by using an Ettan Dalt 6 system (GE Healthcare). Following electrophoresis gels were scanned directly in a Typhoon FLA 7000 scanner (GE Healthcare). After imaging, gels were fixed in 10% methanol/ 7% acetic acid for 1 hour, and stained overnight with Sypro Ruby fluorescent dye for spot picking.

Differential Image Analysis

Scanned images were processed with Progenesis SameSpots software (v 4.5) from Nonlinear Dynamics Ltd. (Newcastle, UK) in order to find real differences between both conditions of study. Both manual and automatic alignment was used to align the images. SameSpots detects the spots simultaneously across all images generating a master gel list containing all the features. All gels were compared with each other and fold values as well as P-values of all spots were calculated by SamesSpots software using one way ANOVA analysis. Differential expression of a protein present in the gels was considered significant when the fold change was at least 1.2 and the P-value was below 0.05.

Mass Spectrometric Analysis

Protein identifications were by LC-MS/MS. Digested peptide mixtures dissolved in 0.1% formic acid were separated in an EASY-nLC (Proxeon, Bruker Daltonik GmbH) with a reverse phase nanocolumn (Easy column SC200) from Proxeon (see below for more details).

LC parameters for separation of tryptic peptides

LC settings

LC system	Easy-nLC PROXEON
Trap column	Easy-column SC001 L 2 cm, ID 100 um, 5 um, 120 A, C18-A1 from Proxeon
Analytical column	Easy column SC200 C18 3µm 120A 360 µm OD/75µm ID, L=10cm) from Proxeon
Flow rate	300 nl/min

Eluents	A:0.1% FA in water B:0.1%FA in ACN
Gradient	5% (t=0 min), 35% (t=32 min), 50% (t=37 min), 100% (t=38min)

Ionized peptides were analyzed in a CID-ETD ion trap mass spectrometer (Bruker Daltonics), equipped with a Nanosprayer ionization source that was used for data-dependent MS/MS experiments. Spectra were acquired in Enhanced Resolution mode. Further acquisition parameters are listed in below.

MS and MS/MS settings used for acquisition with the Amazon ETD

Acquisition parameters

Source	Nanosprayer®
	MS settings:
Scan mode	Enhanced resolution mode (8,100 m/z s^{-1})
Scan range	50-3000 Da
Spectra averages	5 (Rolling averaging: 1)
	MS/MS settings:
Scan mode	UltraScan
Scan range	100-3,000 m/z
No. of precursor ions	3 (active exclusion after 1 spectrum, release after 0.2 min; reconsider precursor, if current intensity/previous intensity >1%
Isolation width	4 m/z
Spectral averages	2
Fragmentation amplitude (CID)	30-300%
Fragmentation time (ETD)	100 ms

Automated analysis of mass data was achieved by Data Analysis 4.0 and BioTools 3.2 from Bruker Daltonik GmbH. Database search was performed with the Mascot v2.3.0 search tool (Matrix Science, London, UK) screening SwissProt (SwissProt_2016_09.fasta). Searches were restricted to human taxonomy allowing carbamidomethyl cysteine as a fixed modification and oxidized methionine as potential variable modification. Both the precursor mass tolerance and the MS/MS tolerance were set at 0.3 and 0.4 Da, respectively, allowing 1 missed tryptic cleavage site. All spectra and database results were manually inspected in detail using the above software, especially in the case of identifications based on one peptide hit. For the latter, positive identification by MS was only accepted when more than 50% y-ions (CID fragmentation) or z-ions (ETD fragmentation) were obtained for a peptide comprising at least eight amino acids long and no missed tryptic cleavage site. Positive hits corresponded to Mascot scores > 40 plus the fulfillment of the above criteria.

For MALDI analysis, dried peptides were dissolved in 4 μL of 0.5% formic acid. Equal volumes (0.5 μL) of peptide and matrix solution (3 mg alpha-cyano-4-hydroxycinnamic acid (α-CHCA) dissolved in 1 mL of 50% acetonitrile in 0.1% trifluoroacetic acid) were deposited using the thin layer method, onto a 384 Opti-TOF 123x81 mm MALDI plate (Applied Biosystems) and allowed to dry at room temperature. Mass spectrometric data were obtained in an automated analysis loop using a 4800 MALDI-TOF/ TOF analyzer (Applied Biosystems). Spectra were acquired in the reflector positive-ion mode with a Nd:YAG, 355nm wavelength laser, at 200 Hz laser frequency, and 1000 to 2000 individual spectra were averaged. The experiments were acquired uniform with fixed laser intensity. All MSMS spectra were performed by

selecting the precursors with a relative resolution of 300 (FWHM) and metastable suppression.

Automated analysis of mass data was achieved by using the 4000 Series Explorer Software V3.5 (Applied Biosystems). Internal calibration of MALDI-TOF mass spectra was performed using two trypsin autolysis ions with m/z = 842.510 and m/z = 2211.105. For MALDI-MS/MS, calibrations were performed with fragment ion spectra obtained for Angiotensin II (peptide mix, calibration standard, Bruker). MS and MS/MS spectra data were combined through the Protein Pilot Explorer Software v4.5. Database search was performed with the Mascot v2.1 search tool (Matrix Science, London, UK) screening SwissProt (release version 2016-05; February, 551193 entries). Searches were restricted to Human taxonomy allowing carbamidomethyl cysteine as a fixed modification and oxidized methionine as potential variable modification. Both the precursor mass tolerance and the MS/MS tolerance were set at 100 ppm and 0.3 Da, respectively, allowing 1 missed tryptic cleavage site. MALDI-MS(/MS) spectra and database search results were manually inspected in detail using the previous software. For combined MS and MS/MS data, identifications were accepted when Confidence Interval (C.I.%) of Protein Pilot software was 95% or higher. Since Protein Scores and Ion Scores from different searches cannot be directly compared, Protein Pilot software calculates this C.I.% in order to combine results from MS and MS/MS database searches. This coefficient value means that the probability that the observed match is a random event is lower than 5%. For PMF spectra, identifications were also accepted when (C.I.%) of Protein Pilot software was 99% or higher.

Immunoprecipitation

Basal and activated platelets (8×10^8platelets/mL, 500 µL per immunoprecipitation; activations with CRP-XL 1µg/mL, 90sec) were lysed with 500 µL NP40-based lysis buffer, (0.3M Sodium Chloride, 20mM Tris, 2mM EGTA, 2mM EDTA, 2% (v/v) NP-40, pH 7.5). Activations were under non-aggregating conditions in the presence of integrilin (9 µM). For phosphotyrosine (p-Tyr) immunoprecipitations, 5 µg of agarose-conjugated 4G10 monoclonal anti-phosphotyrosine antibody (EMD Millipore Corporation, Billerica, MA, USA) were added to the lysates per immunoprecipitation and samples rotated overnight at 4 °C. Before the addition of the antibodies, samples were precleared with 25 µL of Protein A-Sepharose (50% w/v in TBS-T (20mM Tris-HCl (pH 7.6), 137mM NaCl, and 0.1% v/v Tween 20)) at 4 °C for 60 minutes with end-over-end mixing.

After immunoprecipitations, proteins were eluted from the beads in 2X Laemmli sample buffer (4% w/v SDS, 10% v/v 2-mercaptoethanol, 20% v/v glycerol, 50 mM Tris, pH 6.8) and resolved on 4–12% NuPAGE Bis-Tris gradient gels (Invitrogen, Carlsbad, CA, USA) for western blotting.

Supplementary Table 1. Clinical characteristics of obese and lean-matched control- proteomics study.

Variable	Obese patients (N =10)	Lean-matched controls (N =10)	P value
Age (years)	33.50 ± SD 11.90	33.50 ± SD 11.30	1
Females (%)	90%	90%	1
BMI***	46.30 ± SD 6.07	21.83 ± SD 1.96	<0.001
Diabetes (%)	10%	0%	-
HbA1c (%)	5.39 ± SD 0.45 (n=7)	-	-
Laboratory Measurements			
Hemoglobin (g/dl)	13.46 ± SD 0.71	13.50 ± SD 0.85	0.939
Leukocytes/µL*	8.70 ± SD 2.62	7.75 ± SD 6.58	0.028
Platelets/µL	255400 ± SD 60110	242400 ± SD 60120	0.762
Mean Platelet Volume (fL)*	10.07 ± SD 1.60	8.70 ± SD 1.49	0.037
Glucose (mg/dl)	83.10 ± SD 13.33	83.60 ± SD 8.04	0.570
Creatinin (mg/dl)	1 ± SD 0.0	0.72 ± SD 0.09	0.058
Cholesterol (mg/dl)	175.10 ± SD 24.48	194.50 ± SD 37.88	0.211
Chronic Treatments			
Antiplatelets (%)	0%	0%	
ACE Inhibitors (%)	0%	0%	
Benzodiazepines (%)	0%	0%	
Celecoxib (%)	0%	0%	
Statins (%)	0%	0%	
Antidepressants (%)	0%	0%	
Muscle relaxant (%)	0%	0%	

a. Data are presented as the median ± SD or percentage of patients. * $p<0.05$.

b. Patients with glucose levels below 80 mg/dL were not requested HbA1c measuring.

Supplementary Table 2. Clinical characteristics of obese and lean-matched controls validation and functional studies.

Variable	Obese patients (N =34)	Lean-matched controls (N =34)	P value
Age (years)	38.97 ± SD 12.29	38.05 ± SD 11.44	0.854
Females (%)	82.35%	82.35%	1
BMI***	46.58 ± SD 6.62	22.14 ± SD 1.75	<0.0001
Diabetes (%)	2.94%	0%	-
HbA1c (%)	5.3 ± SD 0.44 (n=29)	-	-
Laboratory Measurements			
Hemoglobin (g/dl)	14.11 ± SD 1.02	13.75 ± SD 1.24 (n=32)	0.167
Leukocytes/µL*	8.65 ± SD 2.22	6.8 ± SD 3.99 (n=32)	0.0133
Platelets/µL	259350 ± SD 60980	242750 ± SD 51570 (n=32)	0.205
Mean Platelet Volume (fL)**	8.47 ± SD 1.16	9.63 ± SD 1.44 (n=31)	0.0022
Glucose (mg/dl)	88.76 ± SD 14.23	83.97 ± SD 8.45 (n=31)	0.111
Creatinin (mg/dl)	0.68 ± SD 0.14	0.74 ± SD 0.12 (n=31)	0.083
Cholesterol (mg/dl)	186.8 ± SD 33.27	197.37 ± SD 34.54 (n=32)	0.197
Chronic Treatments			
Antiplatelets (%)	0%	0%	
ACE Inhibitors (%)	6.25%	0%	
Benzodiazepines (%)	21.8%	0%	
Celecoxib (%)	3.1%	0%	
Statins (%)	3.1%	3.1%	
Antidepressants (%)	6.25%	3.1%	
Muscle relaxants (%)	3.1%	0%	

a. Data are presented as the median ± SD or percentage of patients. * $p<0.05$; ** $p<0.001$; *** $p<0.0001$.

b. Patients with glucose levels below 80 mg/dL were not requested HbA1c measuring.

Supplementary Table 3: Platelet proteins differentially regulated in obese patients versus lean healthy controls.

Uniprot code	Name	Biological process	Spot	Fold Change
ACTB_HUMAN	Actin, cytoplasmic 1	Platelet aggregation	4405 4325 2021 2550 4461	+1.3 +1.3 +1.2 +1.2 +1.2
ACTG_HUMAN	Actin, cytoplasmic 2	Platelet aggregation	2550 4461	+1.2 +1.2
ACTN1_HUMAN	Alpha-actinin-1	Platelet degranulation and platelet formation	4465 4405 4403	+1.2 +1.3 +1.3
ALBU_HUMAN	Serum albumin	Regulation of the colloidal osmotic pressure of blood	4424 4356	-1.2 +1.3
ANXA5_HUMAN	Annexin A5	Hemostasis	4356	+1.3
ARK72_HUMAN	Aflatoxin B1 aldehyde reductase member 2	Carbohydrate metabolic process	2898	-1.2
DCTN2_HUMAN	Dynactin subunit 2	Cell proliferation	4354	-1.2
DPYL2_HUMAN	Dihydropyrimidinase-related protein 2	Endocytosis	1858	-1.2
FIBB_HUMAN	Fibrinogen beta chain	Platelet aggregation	2547 2077	+1.2 +1.2
FIBG_HUMAN	Fibrinogen gamma chain	Platelet aggregation	4465 4325 2232 4304 4344	+1.2 +1.3 +1.3 +1.2 +1.2
GELS_HUMAN	Gelsolin	Actin filament capping	4508 4505	-1.2 -1.2
GPDM_HUMAN	Glycerol-3-phosphate dehydrogenase, mitochondrial	Triglyceride catabolic process	4400	-1.4
HEM2_HUMAN	Delta-aminolevulinic acid dehydratase	Response to oxidative stress	2932	-1.2
HSPB1_HUMAN	Heat shock protein beta-1	Negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	4361 3287	-1.2 -1.2
ITA2B_HUMAN	Integrin αIIb	Platelet aggregation	993 1006	+1.2 +1.2
LYSC_HUMAN	Lysozyme C	Inflammatory response	4320	-1.3
ODO2_HUMAN	Dihydrolipoyllysine-residue succinyltransferase	Catalytic activity	2077	+1.2

	component of 2-oxoglutarate dehydrogenase complex, mitochondrial			
PDLI1_HUMAN	PDZ and LIM domain protein 1	Response to oxidative stress	2782	-1.2
PDIA6_HUMAN	Protein disulfide-isomerase A6	Apoptotic cell clearance	4354	-1.2
PGM2_HUMAN	Phosphoglucomutase-2	Glucose metabolic process	1711	-1.2
PI42A_HUMAN	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	Phospholipid metabolic process	4359	-1.2
PP1R7_HUMAN	Protein phosphatase 1 regulatory subunit 7	Positive regulation of preotein dephosphorylation	4465	+1.2
SEPT2_HUMAN	Septin-2	Regulation of L-glutamate transport	2547	+1.2
SEP11_HUMAN	Septin-11	Cell cycle	4359	-1.2
STIP1_HUMAN	Stress-induced-phosphoprotein 1	Protein complex	1858	-1.2
TBB1_HUMAN	Tubulin beta-1 chain	Spindle assembly	4354	-1.2
TPM1_HUMAN	Tropomyosin alpha-1 chain	Actin-binding	4424 4348	-1.2 -1.2
TSP1_HUMAN	Thrombospondin-1	Platelet desgranulation, platelet activation and reactive oxygen species metabolic process	993 4405 4321 4311 4403	+1.2 +1.3 +1.2 +1.2 +1.3
TXNL1_HUMAN	Thioredoxin-like protein 1	Cell redox homeostasis	2867	+1.2
TYPH_HUMAN	Thymidine phosphorylase	Angiogenesis	2021	+1.2
VINC_HUMAN	Vinculin	Platelet aggregation and platelet degranulation	933 1006	+1.2 +1.2
VP37B_HUMAN	Vacuolar protein sorting-associated protein 37B	Involved in cell growth and differentiation	2932	-1.2
ZYX_HUMAN	Zyxin	Regulation of inflammatory response	4491 4339	-1.2 -1.3

[a.] A positive fold change indicates that the protein feature is up-regulated in obese patients, whereas a negative fold change indicates that the spot is down-regulate.

Supplementary Table 4. Additional data on MS protein identification

Spot	MS method	N†/ % ‡	Mascot Score	Peptides identified by MS		Identified protein	Accession number	MW (exp) / MW(theo)	pI (exp) / pI(theo)	Fold change
				M + H	Sequence					
993	LC-MS	9/10,7	519	561 666 458 594 518.301 611.7923 539.564 751.8823 803.3217	ALSNVEGFER FGSAIAPLGDLDR AEAQVELR DGYNDIAVAAPYGGPSGR VYLFLQPR NVGSQTLQTFK HDLLVGAPLYMESR GQVLVFLGQSEGLR TPVGSCFLAQPESGR	Integrin alpha-IIb	ITA2B_HUMAN	141.3/113.3	4.78/5.1	+1.2
		5/5.9	270.1	530.2687 808.8687 623.8222 625.9483 604.2803	KDHSGQVFSVVSNGK GGVNDNFQGVLQNVR TIVTTLQDSIR FTGSQPFGQGVEHATANK SITLFVQEDR	Thrombospondin-1	TSP1_HUMAN	141.3/129.3	4.78/4.6	
		4/4.3	215.6	553,3259 729,3563 587,3057 635,3227	SLGEISALTSK AQQVSQGLDVLTAK ALASQLQDSLK AVAGNISDPGLQK	Vinculin	VINC_HUMAN	141.3/123.7	4.78/5.4	
1006	LC-MS	3/2.9	120.9	437 635 560	GNDIIAAAK AVAGNISDPGLQK STVEGIQASVK	Vinculin	VINC_HUMAN	140.20/123.7	4.81/5.4	+1.2
		2/1.7	81.0	458 561,2335	AEAQVELR ALSNVEGFER	Integrin alpha-IIb	ITA2B_HUMAN	140.20/113.3	4.81/5.1	
1711	LC-MS	5/7.5	215.1	600 528 668 728 438,2078	IVLANDPDADR DLTTGYDDSQPDKK DTYMLSSTVSSK DLTTGYDDSQPDK AAMGPGISR	Phosphoglucomutase-2	PGM2_HUMAN	67.34/32.72	6.08/6.15	-1.2
1858	LC-MS	4/6.3	161.0	601 532 503 644	ETKPEPMEEDLPENK EGLQNMEAR AAALEFLNR KETKPEPMEEDLPENK	Stress-Induces-phosphoprotein	STIP1_HUMAN	62.85/62.6	6.08/6.4	-1.2
2021	LC-MS	2/7.7	95.1	652	VAPEEHPVLLTEAPLNPK	Actin, Cytoplasmic 1	ACTB_HUMAN	60.17/41.7	5.2/5.2	+1.2

				600	DSYVGDEAQSK					
		1/2.9	52.8	477	VAAALTAMDKPLGR	Tymidine Phosphorylase	TYPH_HUMAN	63.54/49.9	5.2/5.2	
2077	LC-MS	4/12.4	199.2	885 513 651 655	DNENVVNEYSSELEK AHYGGFTVQNEANK EEAPSLRPAPPPISGGGYR QGFGNVATNTDGK	Fibrinogen Beta	FIBB_HUMAN	59.06/55.9	5.5/9.3	+1.2
		1/2.9	62.9	885	AKPAEAPAAAAPK	Dihydrolipoyllysine-Residue Succinyltransferaes	ODO2_HUMAN	59.06/48.7	5.5/9.9	
2232	LC-MS	6/17.2	254.2	345 576 846 498 757,3377 431,8508	VGPEADKYR TSTADYAMFK AIQLTYNPDESSKPNMIDAATLK YEASILTHDSSIR YLQEIYNSNNQK QSGLYFIKPLK	Fibrinogen Gamma	FIBG_HUMAN	50.54/51.5	5.03/5.3	+1.3
2547	LC-MS	49.8	219.1	513 655 359 618	AHYGGFTVQNEANK QGFGNVATNTDGK ISQLTR TMTIHNGMFFSTYDR	Fibrinogen Beta	FIBB_HUMAN	30.32/55.9	6.06/9.3	+1.2
		3/12.2	146.6	816,6186 401,89 451,5414	MQAQMQMQMQGGDGDGGALG HHV YLHDESGLNR ILDEIEEHNIK	Septin-2	SEPT2_HUMAN	30.32/41.5	6.06/6.1	
2550	MALDI	9/24	136	795,54 976,54 1132,63 1198,81 1791,04 1954,23 2231,23	IIAPPER AGFAGDDAPR GYSFTTTAER AVFPSIVGRPR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTMYPGIADR	Actin, Cytoplasmic 1	ACTB_HUMAN	30.16/42.05	5.26/5.29	+1.2 OR
		9/24	136	795,54 976,54 1132,63 1198,81 1791,04 1954,23	IIAPPER AGFAGDDAPR GYSFTTTAER AVFPSIVGRPR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK	Actin, Cytoplasmic 2	ACTG_HUMAN	30.16/41.79	5.26/5.31	

				2231,23	DLYANTVLSGGTTMYPGIADR					
2787	LC-MS	3/10.9	201.0	800 480 588	SAMPFTASPASSTTAR VAASIGNAQK DFEQPLAISR	PDZ and LIM domain protein 1	PDLI1_HUMAN	13.71/36.07	6.18/6.56	-1.2
2867	LC-MS	6/28	278.7	637 573 565 681 584,7476 733,6486	QHLENDPGSNEDTDIPK FQGPDNGQGPK SMDFEEAER GYMDLMPFINK IAPAFSSMSNK VGVKPVGSDPDFQPELSGAGSR	Thioredoxin-like	TXNL1_HUMAN	13.60/32.2	4.92/4.7	+1.2
2898		5/11.4	265.4	301 355 411 642 538,2157	SLKPDSVR SQLETSLKR RMDAPASAAAVR VASVLGTMEMGR MDAPASAAAVR	Aflatoxin B1 aldehyde reductase member 2	ARK72_HUMAN	14.21/39.58	6.37/6.70	-1.2
2932	LC-MS	2/7.7	122.5	451 663	MEETQNVQLNK IEEDTENMAEK	Vacuolar Protein	VP37B_HUMAN	15.39/31.3	6.21/7.6	-1.2
		2/6.4	86.8	525 598	LAEVALAYAK AAVLEAMTAFR	Delta-aminolevulinc	HEM2_HUMAN	15.39/36.3	6.21/6.4	
3287	MALDI	12/42	383	831,58 987,68 1075,66 1104,6 1163,72 1643,95 1784,06 1906,15	VPFSLLR RVPFSLLR QLSSGVSEIR QDEHGYISR LFDQAFGLPR AQLGGPEAAKSDETAAK VSLDVNHFAPDELTVK LATQSNEITIPVTFESR	Heat shock protein beta-1	HSPB1_HUMAN	24.74/22.86	5.89/5.98	-1.2
4304	MALDI	12/26	98	851,5 1034,61 1037,63 1045,66 1117,62 1150,61 1194,59 1293,86 1513,85	NWIQYK VGPEADKYR KMLEEIMK TSEVKQLIK VELEDWNGR TSTADYAMFK DNCCILDER QSGLYFIKPLK YLQEIYNSNNQK	Fibrinogen Gamma	FIBG_HUMAN	49.13/52.101	4.87/ 5.37	+1.2

				1683,1 2536,46	IHLISTQSAIPYALR AIQLTYNPDESSKPNMIDAATLK					
4311	LC-MS	1/0.9	45.5	624	TIVTTLQDSIR	Thrombospondin-1	TSP1_HUMAN	129.61/129.3	4.95/4.6	+1.2
4320	MALDI	6/26	72	788,44 1012,49 1179,64 1400,72	AWVAWR WESGYNTR VFERCELAR STDYGIFQINSR	Lysozyme C	LYSC_HUMAN	77.72/16.98	4.35/9.38	-1.3
4321	LC-MS	4/4	200.7	530 516 373 809	KDHSGQVFSVVSNGK GPDPSSPAFR DLASIAR GGVNDNFQGVLQNVR	Thrombospondin-1	TSP1_HUMAN	127.83/129.3	4.99/4.6	+1.2
4325	LC-MS	7/25.3	374.6	896 506 589 323 566,7203 652,0153 744,3243	SYELPDGQVITIGNER QEYDESGPSIVHR EITALAPSTMK LDLAGR GYSFTTTAER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTMYPGIADR	Actin, Cytoplasmic 1	ACTB_HUMAN	20.71/41.7	5.49/5.2	+1.3
		2/5.3	82.8	497,8928 431,9183	YEASILTHDSSIR QSGLYFIKPLK	Fibrinogen Gamma	FIBG_HUMAN	20.71/51.5	5.49/5.3	
4339	MALDI	12/20	214	749,46 775,49 881,5 1037,67 1039,71 1515,84 1710,03 1989,16 2597,49	FTPVASK FSPVTPK CHQPLAR SPGAPGPLTLK FGPVVAPKPK FSPGAPGGSGSQPNQK QHPVPPPAQNQNQVR VNPFRPGDSEPPPAPGAQR LGHPEALSAGTGSPQPPSFTYAQQ R	Zyxin	ZYX_HUMAN	89.89/62.43	6.21/8.59	-1.3
4344	MALDI	6/15	62	1045,71 1293,92 1491,96 1513,94 2536,6	TSEVKQLIK QSGLYFIKPLK YEASILTHDSSIR YLQEIYNSNNQK AIQLTYNPDESSKPNMIDAATLK	Fibrinogen gamma	FIBG_HUMAN	22.18/52.101	5.45/5.37	+1.2

4348	MALDI	17/34	166	722,41 744,51 777,41 810,46 875,53 894,55 926,52 1073,66 1109,59 1186,77 1243,76 1314,87 1475,88 1728,04	AEFAER LATALQK AADESER KMQMLK SLEAQAEK YEEVARK HIAEDADR LDKENALDR AADESERGMK LVIIESDLER IQLVEEELDR KLVIIESDLER MEIQEIQLKEAK IQLVEEELDRAQER	Tropomyosin alpha-1 chain	TPM1_HUMAN	13.10/32.74	4.71/4.69	-1.2
4354	LC-MS	7/20.4	351.8	708 473 848 668 779,9624 544,6017 580,3036	EVDQQLLSVQTR GASALQLER ALSVAELTQQMFDAR ISVYYNEAYGR AVLEEDEEVTEEAEMEPEDK AVLVDLEPGTMDSIR LAVNMVPFPR	Tubulin beta-1	TBB1_HUMAN	57.70/50.3	5.01/4.9	+1.2
		4/10.5	163.6	384,8861 405,163 516,2628 883,4082	QQLVASHLEK GLDFSDR LTELETAVR ENLATVEGNFASIDER	Dynactin Subunit2	DCTN2_HUMAN	57.70/44.2	5.01/5	
		3/8.6	145.7	397,8601 808,3937 576,1825	NRPEDYQGGR GSTAPVGGGAFPTIVER GESPVDYDGGR	Protein Disulfide-isomerase	PDIA6_HUMAN	557.70/48.1	5.01/4.8	
4356	MALDI	22/54	275	744,44 775,46 826,54 893,54 903,56 954,61 1001,68 1014,59	TPEELR ADAETLR SVSHLRK QEISAAFK ADAETLRK FITIFGTR VLTEIIASR LYDAYELK	Annexin A5	ANXA5_HUMAN	18.05/35.97	4.85/4.93	+1.3

				1106,67 1143,78 1155,67 1172,76 1234,77 1290,73 1340,71 1705,02 1750 2888,45	SEIDLFNIR LIVALMKPSR GAGTDDHTLIR MLVVLLQANR SEIDLFNIRK NFATSLYSMIK GTVTDFPGFDER GLGTDEESILTLLTSR SIPAYLAETLYYAMK QVYEEEYGSSLEDDVVGDTSGYYQR					
		6/9	121	927,57 1149,69 1640,06 1911,03	YLYEIAR LVNEVTEFAK KVPQVSTPTLVEVSR RPCFSALEVDETYVPK	Serum Albumin	ALBU_HUMAN	18.05/69.3	4.85/5.9	
4359	LC-MS	6/15.4	340.2	653 696 690 644 519,2193 612,7728	AAAQLLQSQAQQSGAQQTK KAAAQLLQSQAQQSGAQQTK ELEEEVNNFQK STLMDTLFNTK FESDPATHNEPGVR SYELQESNVR	Septin-11	SEP11_HUMAN	54.15/49.39	6.62/6.36	-1.2
		2/5.2	88.7	590,2191 578,24	DNDFINEGQK SAPLPNDSQAR	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	PI42A_HUMAN	54.15/46.22	6.62/6.50	
4361	MALDI	12/42	383	831,58 987,68 1075,66 1104,6 1163,72 1643,95 1784,06 1906,15	VPFSLLR RVPFSLLR QLSSGVSEIR QDEHGYISR LFDQAFGLPR AQLGGPEAAKSDETAAK VSLDVNHFAPDELTVK VSLDVNHFAPDELTVK LATQSNEITIPVTFESR	Heat shock protein beta-1	HSPB1_HUMAN	24.29/22.86	5.89/5.98	-1.2
4400	LC-MS	9/10.9	484.7	392 581 633	SSYVLSK MNLAIALTAAR SMAEDTINAAVK	Glycerol-3-phosphate dehydrogenase, mitochondrial	GPDM_HUMAN	64.70/80.82	6.37/7.58	-1.4

				488 417,2137 474,2226 574,3066 332,6644 529,6788	QEQLETAR IVELMGR TAEENLDR GFITIVDVQR AFEVAK DDFSSGTSSR					
4403	LC-MS	7/7.3	129.30	826 626 667 516 422,7228 531,7262 530,2393	DCVGDVTENQICNK FTGSQPFGQGVEHATANK LCNNPTPQFGGK GPDPSSPAFR ELANELR FQDLVDAVR KDHSGQVFSVVSNGK	Thrombospondin-1	TSP1_HUMAN	124.87/129.3	5.08/4.6	+1.3
		2/2.7	103	493,1916 677,7483	LSNRPAFMPSEGR GISQEQMNEFR	Alpha-actinin-1	ACTN1_HUMAN	124.87/103	5.08/5.1	
4405	LC-MS	7/7.8	414.7	624 809 667 516 625,9361 604,2892 530,2655	TIVTTLQDSIR GGVNDNFQGVLQNVR LCNNPTPQFGGK GPDPSSPAFR FTGSQPFGQGVEHATANK SITLFVQEDR KDHSGQVFSVVSNGK	Thrombospondin-1	TSP1_HUMAN	126.35/129.3	5.04/4.6	+1.3
		2/7.5	74.5	652,0124 488,7376	VAPEEHPVLLTEAPLNPK AGFAGDDAPR	Actin, Cytoplasmic 1	ACTB_HUMAN	126.35/41.7	5.04/5.2	
		1/1.6	55.2	769,3351	FAIQDISVEETSAK	Alpha-actinin-1	ACTN1_HUMAN	126.35/103	5.04/5.1	
4424	LC-MS	8/20.8	403.6	574 373 825 404 358,5302 622,3107 657,8473 593,8076	MEIQEIQLK LATALQK DEEKMEIQEIQLK EDRYEEEIK LDKENALDR IQLVEEELDR KLVIIESDLER LVIIESDLER	Trompomyosin Alpha-1	TPM1_HUMAN	22.02/32.7	4.71/4.5	-1.2
		1/ 2.5	73.4	547,3077	KVPQVSTPTLVEVSR	Serum Albumin	ALBU_HUMAN	22.02/69.3	4.71/5.9	
4461	MALDI	15/32	416	795,53	IIAPPER	Actin, Cytoplasmic 1	ACTB_HUMAN	20.99/42.05	5.29/5.29	+1.2

				923,63 1014,57 1132,61 1177,69 1516,82 1791,03 1911,01 1954,21 2231,23	IIAPPERK DLTDYLMK GYSFTTTAER EITALAPSTMK QEYDESGPSIVHR SYELPDGQVITIGNER MDDDIAALVVDNGSGMCK VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTMYPGIADR					OR
				795,53 923,63 1014,57 1132,61 1177,69 1516,82 1791,03 1851,98 1954,21 2231,23	IIAPPER IIAPPERK DLTDYLMK GYSFTTTAER EITALAPSTMK QEYDESGPSIVHR SYELPDGQVITIGNER EEEIAALVIDNGSGMCK VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTMYPGIADR	Actin, Cytoplasmic 2	ACTG_HUMAN	20.99/42.05	5.29/5.31	
4465	LC-MS	6/13.5	300.7	441 757 346 432 351,8449 497,9094	LDGSVDFK YLQEIYNSNNQK VGPEADKYR QSGLYFIKPLK KMLEEIMK YEASILTHDSSIR	Fibrinogen Gamma	FIBG_HUMAN	49.29/51.5	4.81/5.3	+1.2
		2/6.7	124.1	555,2467 575,8878	SLETVYLER GAGQQQSQEMMEVDR	Protein Phosphatase 1	PP1R7_HUMAN	49.29/41.5	4.81/4.7	
		2/5.1	100.8	452,1944 442,5648	GISQEQMNEFR RDQALTEEHAR	Alpha-actinin-1	ACTN1_HUMAN	49.29/103	4.81/5.1	
4491	LC-MS	4/10.8	213.0	571 539 664 506	QHPVPPPAQNQNQVR GPPASSPAPAPK VNPFRPGDSEPPPAPGAQR FSPGAPGGSGSQPNQK	Zyxin	ZYX_HUMAN	91.07/61.2	6.13/6.2	-1.2
4505	MALDI	23/20	262	747,44 839,46 839,46	ANSAGATR MDAHPPR LFACSNK	Gelsolin	GELS_HUMAN	102.33/86.04	5.5/5.9	-1.3

				847,47 850,54 859,51 887,57 913,66 967,56 998,62 1074,62 1078,61 1165,69 1254,8 1275,83 1319,81 1349,76 1830,11 2479,41	LFQVKGR KGGVASGFK GIRDNER TGAQELLR RTPITVVK KMDAHPPR EPGLQIWR EGGQTAPASTR YIETDPANR LFACSNKIGR AGKEPGLQIWR HVVPNEVVVQR AGALNSNDAFVLK YIETDPANRDR QTQVSVLPEGGETPLFK VSNGAGTMSVSLVADENPFAQG ALK					
4508	LC-MS	8/11.1	85.6	379 444 556 426 610,6398 660,3282 378,2259 539,7373	TPITVVK TGAQELLR DSQEEEKTEALTSAK HVVPNEVVVQR QTQVSVLPEGGETPLFK AGALNSNDAFVLK AVEVLPK.A R.YIETDPANR	Gelsolin	GELS_HUMAN	102.93/85.6	5.5/5.9	-1.2

a. Identifications were by MALDI-MS/MS in 11 spots and by LC-MS/MS in 24 spots. † Number of matched peptides. ‡ % coverage of full length protein by tryptic peptides. All differential proteins have a p value lower than 0.05.

Supplementary Table 5. Aggregation data corresponding to Figure 3, representing agonists, condition, doses, cohort and p value (Mann-Whitney test).

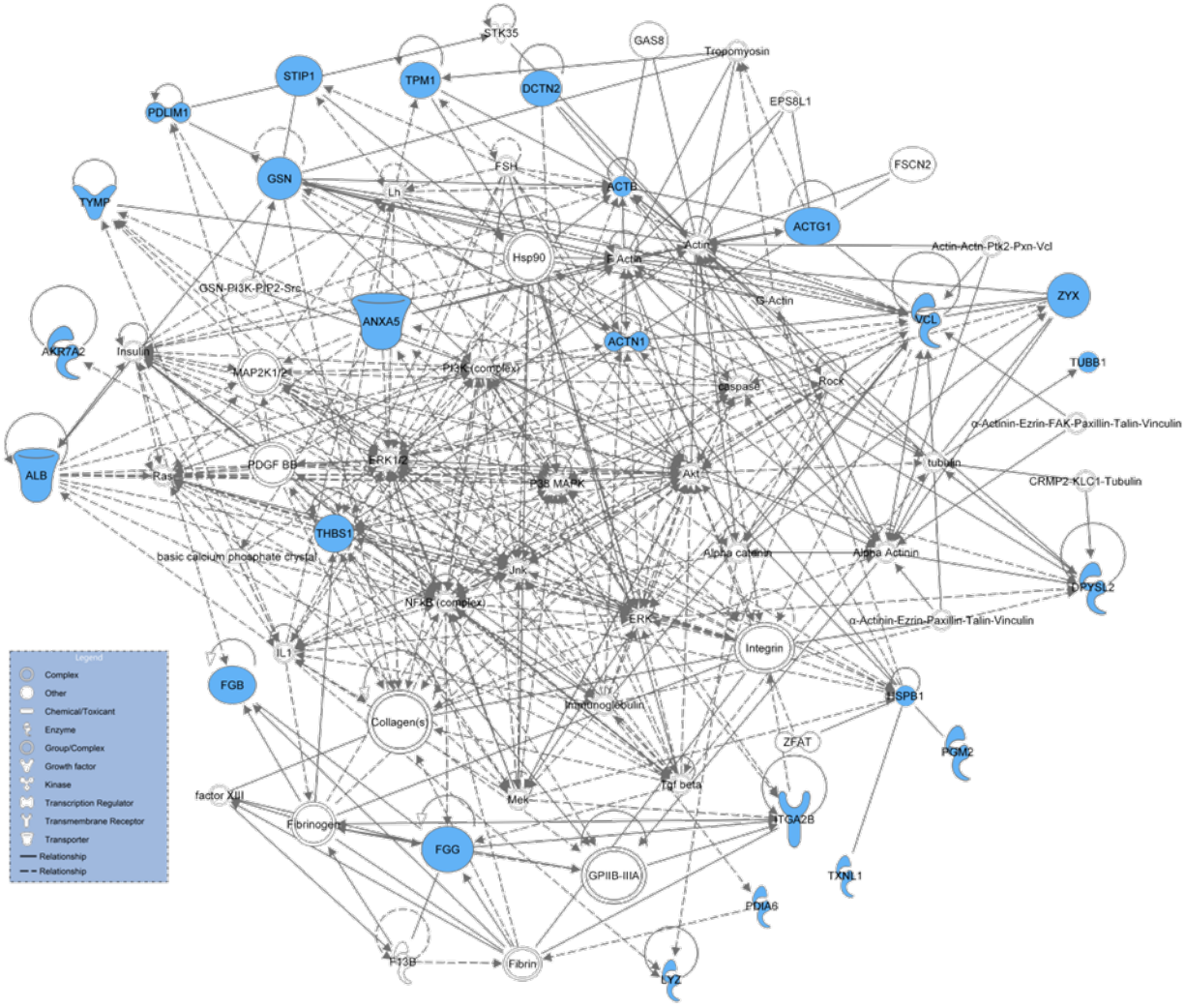
	Condition	Doses (µg/mL)	n	p-value
CRP	PRP	0.1 0.15 0.2	18 20 18	0.049 0.0048 0.0083
CRP	Washed platelets	0.4 0.5 1	20 20 20	0.356 0.092 0.0015
COLL	PRP	0.5 0.75 1	20 20 17	0.059 0.0002 0.022
COLL	Washed platelets	1 2 3	20 19 19	0.56 0.81 0.60

a. CRP: collagen-related peptide; COLL: collagen.

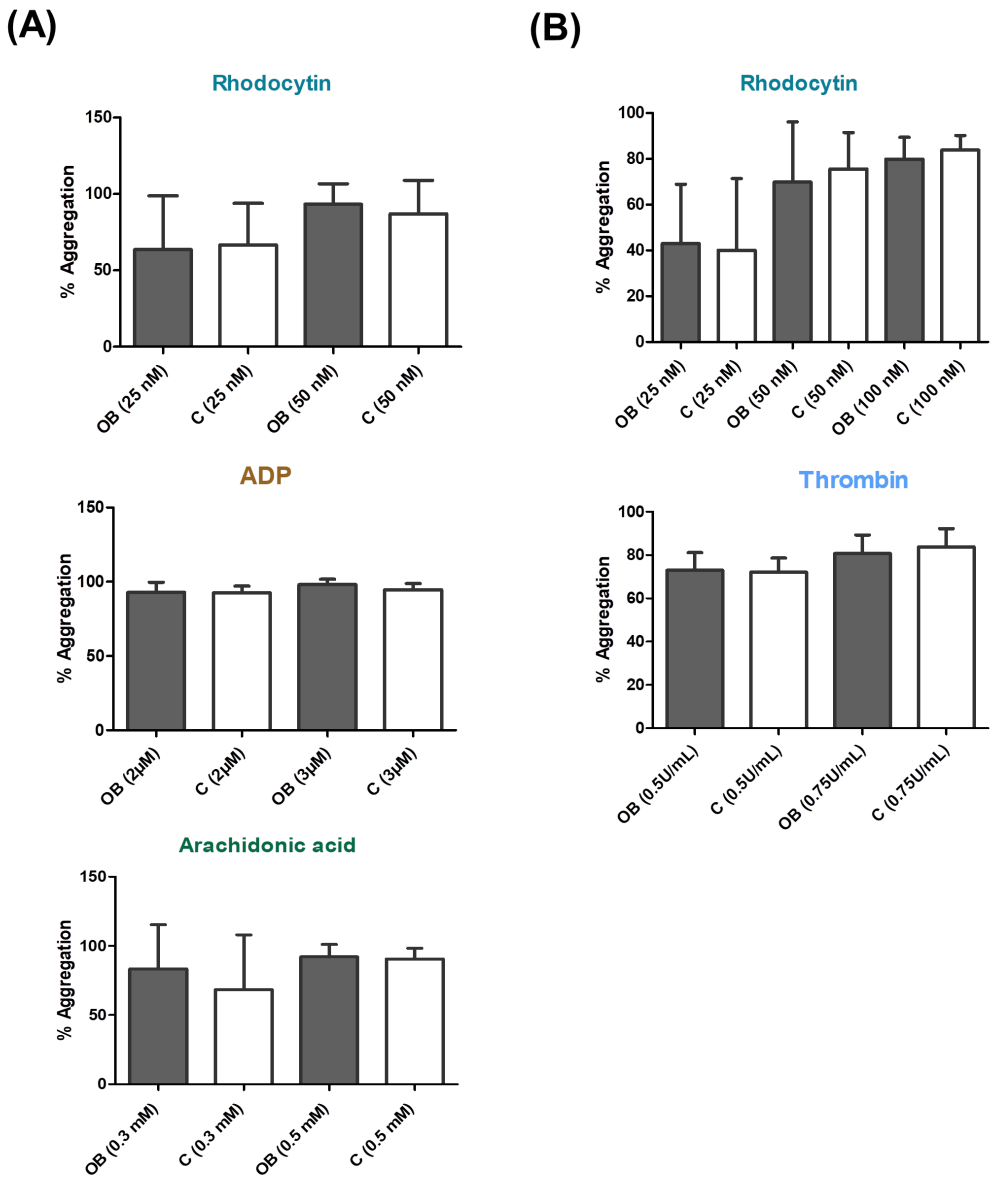
Supplementary Table 6. Aggregation data corresponding to Supplementary Figure 2, representing agonists, condition, doses, cohort and p value (Mann-Whitney test).

	Condition	Doses	n	p-value
RHO	PRP	25 nM 50 nM	20 20	0.61 0.08
RHO	Washed platelets	25 nM 50 nM 100 nM	20 20 18	0.71 0.91 0.35
ADP	PRP	2 µM 3 µM	11 8	0.7 0.1
AA	PRP	0.3 mM 0.5 mM	9 5	0.15 0.66
THR	Washed platelets	0.5 u/mL 0.75 u/mL	14 20	0.8 0.3

a. RHO: rhodocytin; AA: arachidonic acid; THR: thrombin.

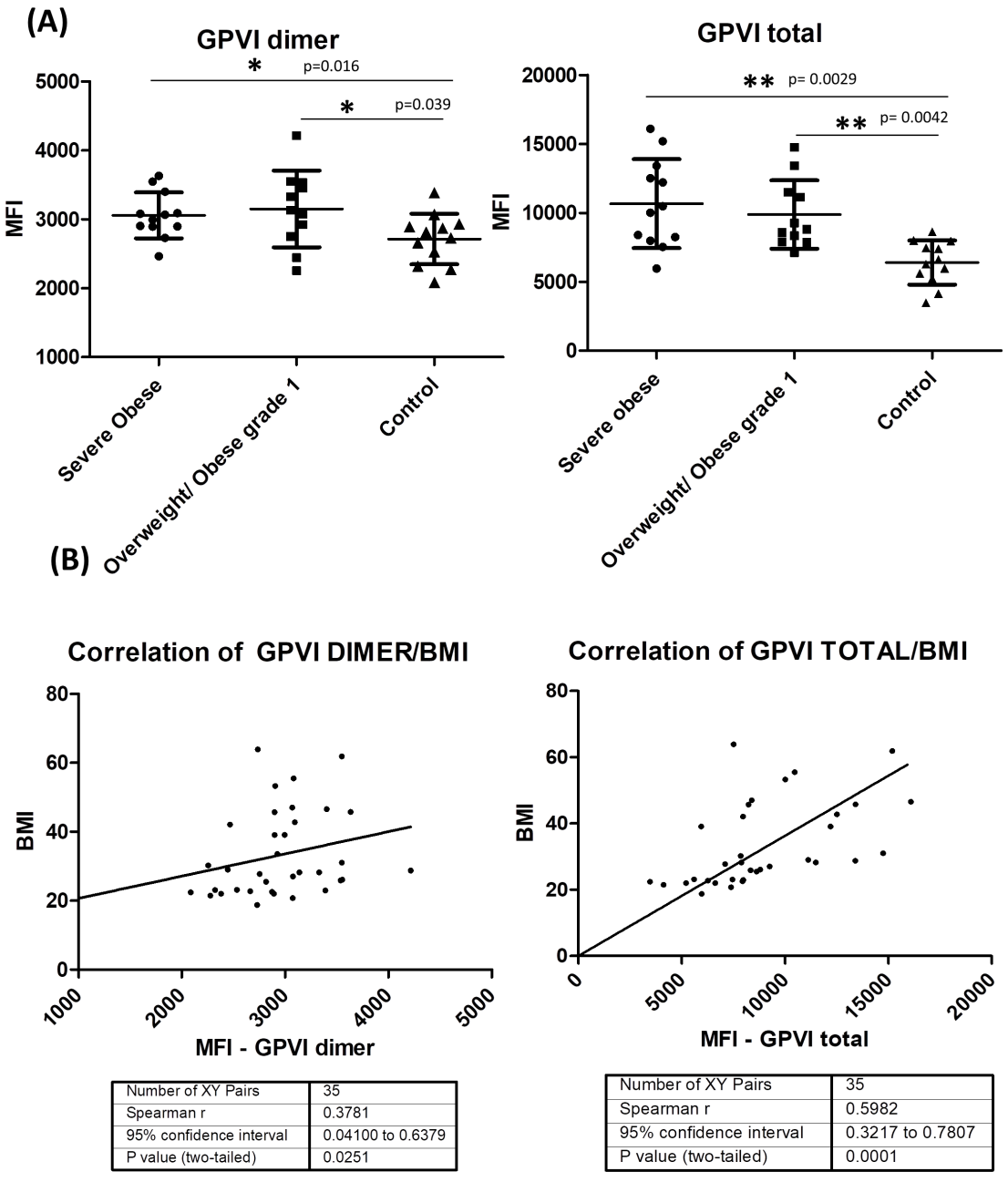


Supplementary Fig. 1: Ingenuity Pathway Analysis (IPA) of the proteomic results identifies a protein network related to Cell-To-Cell Signaling and Interaction, Hematological System Development and Function and Inflammatory Response altered in obesity. Proteins identified by differential analysis are shown as shaded nodes with their gene names. Solid lines represent direct interactions, dotted represent indirect interactions.



Supplementary Fig. 2: Aggregation studies showing no differences between platelets from obese and lean individuals in response to various agonists. PRP and washed platelets were stimulated with rhodocytin (CLEC-2 agonist); or with non-SFKs-mediated signaling pathways agonists such as ADP, arachidonic acid and thrombin (A) PRP was stimulated with rhodocytin (25nM and 50nM), ADP (2 and 3 μM) and arachidonic acid (0.3 and 0.5 mM) to trigger aggregation. (B) Washed platelets were stimulated with rhodocytin (25, 50 and 100nM) and thrombin (0.5 and 0.75 u/mL).

Results are presented as mean ±SD; P-values and cohort data are shown in Supplementary Table 6. OB: obese; C: control.



Number of XY Pairs	35
Spearman r	0.3781
95% confidence interval	0.04100 to 0.6379
P value (two-tailed)	0.0251

Number of XY Pairs	35
Spearman r	0.5982
95% confidence interval	0.3217 to 0.7807
P value (two-tailed)	0.0001

Supplementary Fig. 3: Higher expression levels of GPVI (total and dimer) in the surface of platelets from obese and overweight individuals compared to lean controls, with a positive correlation with BMI. The study was performed with a

subgroup of 12 severe obese patients, their 12 lean matched-controls, and an additional group of 11 overweight and obese grade 1 individuals (BMI between 26 and 35). (A) GPVI dimer and total levels of GPVI are increased in the obese groups. Fluorescence values are shown as mean ± SD. Measures were done in triplicate. Significant differences are indicated as follow: *$p<0.05$, **$p<0.01$. (B) Positive correlations between BMI and total GPVI and GPVI dimer were found using Spearman's test.