Materials and Methods:

Collection for Circulating Blood Count. Patient (GT) and normal control (WT) whole blood (2-4 ml) was collected with informed consent by venipuncture into a vacutube containing 3.2% buffered sodium citrate. All samples were de-identified and assigned a unique GT number. Complete blood counts (CBC) and platelet counts were obtained from citrated whole blood using a Hemavet 950 (Drew Scientific, Miami Lakes, FL) or Clinical Lab Counter Sysmex XP300 (Sysmex America, Lincolnshire, IL) **(Table s1)**.

Blood Collection for Platelet Aggregation. To determine the responsiveness of patient platelets to different agonists, aggregations were performed as previously described[1]. Briefly, platelet-rich plasma (PRP) was isolated from citrated whole blood and adjusted to a platelet count of 250000/μL. Platelet aggregation in response to agonists Type 1 collagen (5μg/mL), thrombin (1U/ml) and ADP (20μM) was performed on a PAP-8E Platelet Aggregometer and analyzed on the systems software (Bio/Data Corporation, Horsham, PA, USA) **(Figure s1).** Platelet aggregation was measured at increasing Ristocetin concentrations at UCH Clinical Lab (Aurora, CO) **(Table s2).**

Antibodies and Molecules Used for Immuno-Cytometric Analysis (Method s1). Integrin αIIb and β3 goat-anti-human polyclonal Ab, fibrinogen (5C5) mouse-anti-human monoclonal antibody (mAb), donkey-anti-goat IgG-HRP polyclonal Ab and HRP-labelled m-IgGκ binding protein were purchased from Santa Cruz (Dallas, TX). AP3, mouse monoclonal IgG Ab (specific for β3) was kindly provided by Dr. Peter Newman (Versiti BloodCenter of Wisconsin, Milwaukee). 319.4Fab-647, 322.3IgG, 370.2IgG mouse mAbs (specific for ligand induced binding site "LIBS" activated form of β3) were kindly provided by Dr. Richard Aster (Versiti BloodCenter of Wisconsin, Milwaukee).[2,3] Murine anti-human mAbs recognizing GPIbα (6D1), αIIb (10E5) and αIIbβ3/αVβ3 complexes (7E3) were kindly provided by Dr. Barry S. Coller (Rockefeller University, NY, NY)[4]. PE-conjugated Mouse Anti-Human CD61 (VIPL2, β3) and CD41 (HIP8, αIIb) as well as PE-Conjugated Anti-Human CD62 (P-Selectin) and FITC-conjugated ligand-mimetic IgM mAb specific to activated form of αIIbβ3 (PAC-1) were purchased from BD Biosciences (San Jose, CA). Peroxidase-conjugated anti-rabbit IgG and FITC-conjugated goat anti–mouse IgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Goat IgG HRP-conjugated Ab was obtained from R&D systems (Minneapolis, MN). Alexa-Fluor-488-conjugated to Fibrinogen from human plasma was obtained from Invitrogen (Eugene, OR). Sheep anti-Human Fibrinogen polyclonal Ab (SAFG-AP 210R4) was purchased from Enzyme Research Laboratories (South Bend, IN). Alexa-488 donkey anti-sheep IgG (#A11015) was purchased from Invitrogen (Eugene, OR). The GRGDW (RGD) peptide (Versiti Blood Research Institute Peptide Core, MW 589.6 at 5 mg/vial) was used at 2.5mM final concentration in PBS at pH 7.4 to inhibit PAC-1 binding to αIIbβ3 in active conformation.

Electrophoresis/Immunoblot Quantitation Analysis for Platelet αIIbβ3 and Fibrinogen (Methods s2). Washed platelets were solubilized in 20mM Tris, 100mM sodium chloride (NaCl), 1% Triton X-100, 10 mM *N*-ethylmaleimide (NEM), 2mM of protease inhibitor phenylmethylsulfonyl fluoride, and 100 g/ml leupeptin at 4^0C as described previously[5]. After centrifugation at 15,000*g* for 30 min, protein concentration was determined by the micro-bicinchoninic acid protein assay using bovine serum albumin (BSA) as a protein standard[6]. Solubilized proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% resolving gels and a Tris-glycine running buffer, according to the method of Laemmli [7]. Fractionation was carried out using the Criterion Precast Gel Electrophoresis System (Bio-Rad, Hercules, CA, U.S.A.), followed by electrophoretic transfer to a nitrocellulose membrane using the Criterion Blotter (Bio-Rad) [8]. Non-specific binding sites were blocked by overnight incubation at 4°C with 5% nonfat dry milk in Tris-buffered saline (TBS; 25 mM Tris, pH 7.5, 150 mM NaCl). The blots were then incubated for 1 h at room temperature with primary antibody diluted 1:5000 in TBS containing 0.5% nonfat dry milk. After extensive washing with

several changes of TBS supplemented with 0.1% Tween-20, the blots were incubated for 1 h at room temperature with horseradish-conjugated goat anti-rabbit IgG diluted 1:10,000 in TBS containing 0.5% nonfat dry milk. Blots were then washed with several changes of TBS supplemented with 0.1% Tween-20 and processed for detection by enhanced chemiluminescence according to the manufacturer's instructions. The luminescence produced was detected by exposure of Fuji Super RX film (Fisher Scientific, Pittsburgh, PA) and after digitizing the image using a Hewlett Packard 6300C scanner (Boise, ID), the integrated OD of immunoreactive protein bands was determined using a Kodak DC120 digital camera and Digital Science 1D V 3.0 software (New Haven, CT). Some of the immunoblot analysis for platelet fibrinogen was directly analyzed on an Amersham Imager (Piscataway, NJ). For a standard curve, protein preparations representing 25, 50, 100, 250, and 500 ng of platelet protein lysate concentration was used to detect αIIb, β3, or GPIbα as indicated on each blot. Individual protein levels were determined by linear regression based on this standard curve (GraphPad Instat version 3.00, GraphPad Software, San Diego, CA). In all blots, bands corresponding to the protein of interest were identified by reference to the molecular weight standards.

Immunofluorescent Confocal Microscopy (Method s3). Human platelets were fixed with 3.7% (vol/vol) buffered formalin, permeabilized in 0.5% Triton X-100 (in 20 mmol/L Hepes, 300 mmol/L sucrose, 50 mmol/L NaCl, and 3mmol/L MgCl2, pH7.0), and blocked with 2.5% normal goat serum in HBSS as previously described.[9] Platelets were incubated with a sheep polyclonal 1°Ab to human fibrinogen overnight at 4°C.[10] The Alexa Fluor® 488-conjugated F(ab')$_2$ fragment of donkey anti-sheep IgG (H+L) was used as a 2°Ab (1:500 dilution) to detect fibrinogen for 30 min at 25°C. Platelets were mounted with Vectashield (Vector Labs, Burlingame, CA). Immunofluorescence was detected with a Zeiss LSM 510 Multiphoton Confocal Microscope (Carl Zeiss, Inc. Oberkochen, Germany).[11] Platelets treated with 2°Ab only were used as negative controls. Platelets were imaged by Z sections taken for each field and the entire Z series (12-25 images) combined into a stacked projection. The projections were merged using the Confocal Assistant software program (Bio-Rad). Computer-assigned colors were based on the intensities of bitmap overlaps, with Alexa488-fluorochrome represented by green pixels.

PCR Amplification of *ITGB2* and *ITGB3* (Methods s4). PCR amplification of *ITGB2* and *ITGB3* DNAs was accomplished using paired sets of primers designed to amplify all 30 exons and flanking splice acceptor and donor site sequences for each exon of the αIIb gene, and all 15 exons and immediate flanking sequences of the β3 gene, based on published genomic DNA sequences.[12,13] The sequences of the primers employed for PCR amplification of the exons, adjacent intronic regions, and 5' and 3' untranslated regions of the β3 and αIIb gene are shown in **Table s3 A-B**. Reactions were performed using 50 ng of genomic DNA and 1 unit of Platinum *Taq* DNA polymerase in a final volume of 25 µl containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate, and 0.2 µM of each primer. The sequences of the primers employed for PCR amplification of the β3 and αIIb cDNA are shown in **Table s3 C-D**. Briefly, all polymerase chain reactions had an initial denaturation step at 94°C for 2 min, followed by: a. 30 cycles of denaturation at 94°C for 15s for *ITGB2* exons 1, 2-4, 5-7, 8-10, 11-12, 19-20, 23-26, 27, 28 and 30, and *ITGB3* exons 1, 3, 4, 5, 8, 9, 10, 11, 13 and 14, or 30 cycles of denaturation at 94°C for 30s for *ITGB2* exons 13-14, 15-17, 18, 21-22 and 29, and *ITGB3* exons 2, 6-7, and 12. b. annealing at 55°C for 30 s for *ITGB2* exons 1, 2-4, 5-7, 8-10, 11-12, 19-20, 23-26, 27, 28 and 30, and *ITGB3* exons 1, 3, 4, 5, 8, 9, 10, 11, 13 and 14, or annealing at 65°C for 30 s for *ITGB2* exons 13-14, 15-17, 18, 21-22 and 29, and *ITGB3* exons 2, 6-7, and 12. c. elongation at 68°C for 90s for *ITGB2* exons 1, 8-10, 11-12, 19-20, 27, 28 and 30, and *ITGB3* exons 1, 8, 9, 10, 11, 13 and 14, or elongation at 68°C for 60s for *ITGB2* exons 2-4, 5-7 and 23-26, and *ITGB3* exons 3, 4 and 5, or elongation at 72^{0}C for 60s for *ITGB2* exons 13-14, 15-17, 18, 21-22 and 29, and *ITGB3* exons 2, 6-7, and 12. The final elongation step was performed at 68°C for 3 min for *ITGB2* exons 1, 8-10, 11-12, 19-20, 27, 28 and 30, and *ITGB3* exons 1, 8, 9, 10, 11, 13 and 14, or at 68°C for 2 min for *ITGB2* exons 2-4, 5-7 and 23-26, and *ITGB3* exons 3, 4 and 5, or at 72^{0}C for 2 min for *ITGB2* exons 13-14, 15-17, 18, 21-22 and 29,

and *ITGB3* exons 2, 6-7, and 12. For PCR reactions requiring betaine (*ITGB2* exons 2-4 and 5-7) 2.5 µl of 5M betaine was add to the reaction mixture.

Nucleotide Sequence Analysis (Method s4). Analysis of gDNA and cDNA was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions. Briefly, a 5-µl aliquot of the DNA amplification reaction was transferred to a 96-well plate and treated with exonuclease I and shrimp alkaline phosphatase (ExoSAP-IT) to degrade any residual single-stranded DNA and hydrolyze remaining deoxynucleotide triphosphates. Sequencing reactions were performed on an Eppendorf thermal cycler after the addition of Big Dye reagent to each well and the appropriate sequencing primer (either the forward or reverse PCR primer at the concentration recommended in the Big Dye kit protocol) in a final volume of 20 µl. All sequencing reactions included 30 cycles of denaturation at 96°C for 20 s, annealing at 50°C for 20 s, and elongation at 60°C for 4 min. Unincorporated primer and nucleotides were removed from the reactions using CleanSEQ magnetic beads according to the manufacturer's instructions. Cycle sequencing reactions were performed with the same primer pairs used for amplification. For DNA sequencing analysis of plasmids, heat inactivation of 250 ng of the plasmid for 5 min at 96°C was followed by sequencing reactions as described above on an Eppendorf thermal cycler. The plasmid sequencing reactions included 50 cycles of denaturation at 96°C for 20 s, annealing at 50°C for 20 s, and elongation at 60°C for 4 min. Sequences were compared with human genomic and cDNA sequences of the *ITGA2B* gene with accession numbers NT_010783 and NM_000419 respectively, and the human genomic and cDNA sequences of the *ITGB3* gene with accession numbers NT_010783 and NM_000212 respectively. The nomenclature for the mutations was organized according to the Human Genome Variation Society recommendations. To confirm that the mutations were located on separate alleles, *ITGB3* genomic DNA primers were designed to amplify the region from intron 3 to intron 5 (g.30431-32779). The PCR product (2373 kb) was then cloned into the TOPO vector using the TA cloning kit per manufacturer's instructions. Twenty clones were analyzed by DNA sequence identity comparison.

Supplement Table s1. Complete Blood Count Parameters Show Low End of Normal Platelet Count and a Mean Platelet Volume (MPV) within the Normal Range.

ID / DATE	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW %	PLT	MPV
GT 09/2013	6.9	5.41	16.1	47.7	88.2	29.8	33.8	12.7	**146**	**10.3**
GT 02/2018	6.02	4.76	13.3	38.4	80.5	27.8	34.6	13.9	**151**	**9.4**
GT 09/2022	5.8	5.22	14.9	47.8	91.6	28.5	31.2	13.7	**155**	**11.3**

[i]**PLT:** Platelet: Normal Range (150-450 10e3/µl)

[ii]**MPV:** Mean Platelet Volume: Normal Range (9 -11.8 fL)

Supplement Table s2. GT Platelet Aggregation Detected at Increasing Ristocetin Concentration *(n=1 shown at 4 different concentrations)*

ID / DATE	1	1.2	1.5	1.8	Ristocetin Concentration (mg/ml)
WT 03/2010	25	77	75	83	% Peak Aggregation
GT 03/2010	2	3	17	57	% Peak Aggregation

Supplement Table s3. Primers used for β3 and αIIb gDNA and cDNA amplification

A. *ITGB3* gDNA amplification primers

Name	Exon	Sequence
GPIIIa_exon1_forward	1	5'-CAA TAG TTT CCC ACC GCT CCC TC-3'
GPIIIa_exon1_reverse	1	5'-CAC GCT CTC ACC CAG GAA GCT ACA G-3'
GPIIIa_exon2_forward	2	5'-CGT CTG TGG TAT GGT TAG GGT GTG-3'
GPIIIa_exon2_reverse	2	5'-GTT AGT ATT CCC AGC AAC ATC CCC ATG ACA GC-3'
GPIIIa_exon3_forward	3	5'-CTC CAA TGT ACG GGG TAA ACT CTT AGC-3'
GPIIIa_exon3_reverse	3	5'-TTG AGC ACC TAC TAT GTC TGA AGG G-3'
GPIIIa_exon4_forward	4	5'-GGA GGG TCA AGA GAT TAG AAG AGT AAT AG-3'
GPIIIa_exon4_reverse	4	5'-CTT AGG CTC CAT TGA CAC AAC ACT CCA GAT CC-3'
GPIIIa_exon5_forward	5	5'-GGA GTC TGA ACT GTC TGG GTA ACT GTG G-3'
GPIIIa_exon5_reverse	5	5'-GGA ACA TCT GAG ACC ACT GAG GTT C-3'
GPIIIa_exon6_forward	6	5'-GAA GAG AAT GAA ACT CCT GAA CTG G-3'
GPIIIa_exon6_reverse	6	5'-GTG ACA GTG AAG GCA GTG ATA ACA CC-3'
GPIIIa_exons7-8_forward	7 & 8	5'-TTG TAG AGA TGG GGT CTT GCT ATG TTG C-3'
GPIIIa_exons7-8_reverse	7 & 8	5'-GAG CAC ACG GAG AAG GCA GTA AGA C-3'
GPIIIa_exon9_forward	9	5'-TAG ACG CAA GTG GAG ATG GAA AGA GG-3'
GPIIIa_exon9_reverse	9	5'-CTT ACG CAA CAG AAG CCT CAG-3'
GPIIIa_exon10_forward	10	5'-TTC ATA TAG GGA AGG CTG AGG AAC TCC-3'
GPIIIa_exon10_reverse	10	5'-CGT GAA AAG GGG ATT GGT CC-3'
GPIIIa_exon11_forward	11	5'-ATC TTT TGC TCT ACA GTG GCT CCG-3'
GPIIIa_exon11_reverse	11	5'-GGA GGT TTG TAG TAG TTT CAC AGA GTG TCC-3'
GPIIIa_exon12_forward	12	5'-AAT GAA GGA CCT GGG AGG CTT CTG GCT G-3'
GPIIIa_exon12_reverse	12	5'-ACT TGC TCC CTT TGC TTT GCA GGC TC-3'
GPIIIa_exon13_forward	13	5'-TAC ATC AGT GAG TGT CAG TGA GTG GC-3'
GPIIIa_exon13_reverse	13	5'-GTA GAG ACA GGG TTT CAC CAT GTT GG-3'
GPIIIa_exon14_forward	14	5'-GAC TTC TGA TTT GAG CCA CTC AGT G-3'
GPIIIa_exon14_reverse	14	5'-ATA GTG AGA CCA TCT CTC CCA GTT TCC-3'
GPIIIa_exon15_forward	15	5'-GAA ACG GTG GCA GGA TGG CAT TCT ACC-3'
GPIIIa_exon15_reverse	15	5'-CAC AGA CCC ACA CTT CCA CAT ACT GAC AT-3'

Supplement Table s3. Primers used for β3 and αIIb gDNA and cDNA amplification

B. *ITGA2B* gDNA amplification primers

Name	Exon	Sequence
GPIIb_ exon1_forward	1	5'-CCT GGC AAT TCT AGC CAC CAT GAG TC-3'
GPIIb_ exon1_reverse	1	5'-GTC TTC CCT TTG TTC TTG CTG TAG-3'
GPIIb_exons2-4_forward	2-4	5'-TGA TTG CCC TCG CTG AGA GTC GGT TTC-3'
GPIIb_exons2-4_reverse	2-4	5'-AGG AGA CAA GGA GGA GGG GTC AG-3'
GPIIb_exons5-7_forward	5-7	5'-GGA ACA CCC TGA GCC GCA TTT ACG-3'
GPIIb_exons5-7_reverse	5-7	5'-GCA AAT TAG TCT TTT CCA GGG GAG G-3'
GPIIb_exons8-10_forward	8-10	5'-GCT CCC TTC CAC TGC GGA CTC GTA GC-3'

GPIIb_exons8-10_reverse	8-10	5'-GTG ACA GCC ACT GAA TGC CCA AAA TAC G-3'
GPIIb_exons11-12_forward	11 & 12	5'-GGG TAC AAG AAT GAT GCT CTC GCC-3'
GPIIb_exons11-12_reverse	11 & 12	5'-CTC AGC ACC CCA TGT GTC TAA GCC AC-3'
GPIIb_exons13-14_forward	13 & 14	5'-AAC AAT CCT ACT TGG CAG GTC CCT CTC CC-3'
GPIIb_exons13-14_reverse	13 & 14	5'-GTC TTG GTC TGA GGT AGG ACA CAG C-3'
GPIIb_exons15-17_forward	15-17	5'-GAT CCC ATG CCC TAA TCG CCA ATT C-3'
GPIIb_exons15-17_reverse	15-17	5'-ATC TTG CTA CCA TAC ACA TCC CAC CTT C-3'
GPIIb_exon18_forward	18	5'-CTG AAC CTG GAT CTG GGC GGA AAG CAC AG-3'
GPIIb_exon18_reverse	18	5'-CAA TCT GCC TGC CTC GGT CTC CCA AAG TGC-3'
GPIIb_exons19-20_forward	19 & 20	5'-CTC TGA TGT AAA TTG ACA AAC CCT GAC-3'
GPIIb_exons19-20_reverse	19 & 20	5'-TGA CAG CAA AGC AGA AGA GAA GAG G-3'
GPIIb_exons21-22_forward	21 & 22	5'-CAC ACT AGC ATG TGA CAG TCC CTT GAG-3'
GPIIb_exons21-22_reverse	21 & 22	5'-GAT CCT TCT GCC TTG GCC TCG CAA C-3'
GPIIb_exons23-26_forward	23-26	5'-GCC TGG TTC CCT TTC TTC CTC AGA G-3'
GPIIb_exons23-26_reverse	23-26	5'-TGC TCC TCC ATG TTC ACT TGA AG-3'
GPIIb_exons27_forward	27	5'-CAT CTC TGG GAC TAT GTG AGC AAG C-3'
GPIIb_exons27_reverse	27	5'-TGA AAG CCG TTT ACA CCC ACA AGA GG-3'
GPIIb_exon28_forward	28	5'-CAC TTT GCT CTG GGA GGG GCG GGG TTT-3'
GPIIb_exon28_reverse	28	5'-AGC ACA CCC ACC AGC ACC CAC CAG ATT G-3'
GPIIb_exon29_forward	29	5'-TGG TAC GGG TGG GTG GCA TGG CTG GAG-3'
GPIIb_exon29_reverse	29	5'-GAC CTC AGG TGA TCT AAA CGC TTT GG-3'
GPIIb_exon30_forward	30	5'-CTC TGT ACT CCC TGA CAC TCC CCA AAG ACA G-3'
GPIIb_exon30_reverse	30	5'-AGG CAT CCA TTT GTG AGT ACA GTG GGC-3'

Supplement Table s3. Primers used for β3 and αIIb gDNA and cDNA amplification

C. β3 cDNA amplification primers

Name	**Exon**	**Sequence**
GPIIIa_3-23_forward	1 to 6	5'-CCG CGG GAG GCG GAC GAG ATG-3'
GPIIIa_970-950_reverse	1 to 6	5'-GAG GGA TAA TCC ATG GTA GTG-3'
GPIIIa_328-349_forward	3 to 11	5'-GCT CCC AGG TCA CTC AAG TCA G-3'
GPIIIa_1795-1771_reverse	3 to 11	5'-GTG TCA GTA CGC GTG GTA CAG TTG C-3'

GPIIIa_907-930_forward	7 to 11	5'-ACG GGC AGT GTC ATG TTG GTA GTG-3'
GPIIIa_1793-1772_reverse	7 to 11	5'-GTC AGT ACG CGT GGT ACA GTT G-3'
GPIIIa_1204-1230_forward	10 to 15	5'-CCT TCA ATG CCA CCT GCC TCA ACA ATG-3'
GPIIIa_2463-2437-reverse	10 to 15	5'-GCT CTG GCG CGT TCT TCC TCA AAT TTA G-3'

D. αIIb cDNA amplification primers

Name	**Exon**	**Sequence**
GPIIb_35-57_forward	1 to 6	5'-GGC CAG AGC TTT GTG TCC ACT GC-3'
GPIIb_810-786_reverse	1 to 6	5'-CTG GGT TGC TGG AGT CAA AGG AGA G-3'
GPIIb_634-656_forward	6 to 16	5'-GCT TCA GCT CCG TGG TCA CTC AG-3'
GPIIb_1651-1625_reverse	6 to 16	5'-TGC AGC TCG GCA TTT AGG GAT AGC TTC-3'
GPIIb_1226-12491_forward	13 to 20	5'-CCG GGA TGG CTA CAA TGA CAT TGC-3'
GPIIb_2078-2056_reverse	13 to 20	5'-CAC GGC CAG CTC TGC TTC ATA GG-3'
GPIIb_1893-1919_forward	18 to 27	5'-ACC CAT GTG CAG GAG CAG ACA CGA ATC-3'
GPIIb_2853-2832_reverse	18 to 27	5'-ACA GGA AGG CCA GCA CCG TGA C-3'
GPIIb_2421-2460_forward	25 to 30	5'-GAG AGG GAG CAG AAC AGC TTG GAC AG-3'
GPIIb_3232-3207_reverse	25 to 30	5'-GCT TGG AGG CAA CTT GTT GGA GAA GG-3'

Supplement Table s4. Constructs for mammalian cell expression

Construct	**Mutation**
Human αIIb/β3	None
Human αIIb/β3 F153S	F153S
Human αIIb/β3 F153W	F153W
Human αIIb/β3 F153A	F153A
Human αIIb/β3 F153Y	F153Y

Supplement Table s5. Mutagenesis primers

Mutation	**Forward Primer**	**Reverse Primer**
F153S	535-575for_556TC CCAGTAACCTGCGGATTGGCTCCGGGG CATTTGTGGACAAG	575-535rev_556TC CTTGTCCACAAATGCCCCGGAGCCAATCCGCAG GTTACTGG
F153W	535-575for_556TG_557CG CCAGTAACCTGCGGATTGGCTGGGGGG CATTTGTGGACAAG	575-535rev_556TG_557CG CTTGTCCACAAATGCCCCCCAGCCAATCCGCAG GTTACTGG
F153A	535-575for_555TG_556TC CCAGTAACCTGCGGATTGGCGCCGGGG CATTTGTGGACAAG	575-535rev_555TG_556TC CTTGTCCACAAATGCCCCGGCGCCAATCCGCAG GTTACTGG
F153Y	535-575for_556TA CCAGTAACCTGCGGATTGGCTACGGGG CATTTGTGGACAAG	575-535rev_556TA CTTGTCCACAAATGCCCCGTAGCCAATCCGCAG GTTACTGG

Figure s1.

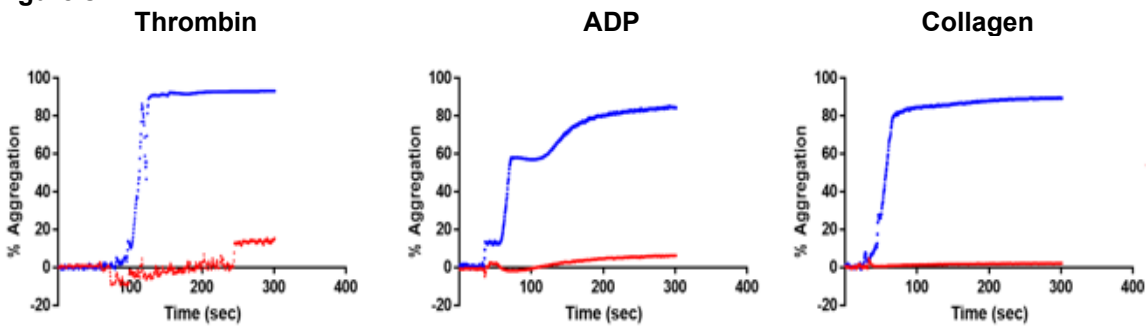

Figure s1. Agonist Stimulated Platelet Aggregation from PRP Isolated from GT Patient and WT Control Whole Blood. Platelet-rich plasma (PRP) was isolated from citrated whole blood and adjusted to a platelet count of 250,000/μL. Light transmission platelet aggregation measured on a PAP-8E Platelet Aggregometer in response to Thrombin (1 U/ml), Adenosine Diphosphate (ADP, 20 μM) and Collagen (5 μg/ml) using PRP from the GT Patient (red) showed defective aggregation in response to each physiological agonist of activation compared to a normal WT Control (Blue). The graphs show percent (%) platelet aggregation with time (seconds) in each experiment run in duplicate, which is representative of n=2 separate experiments.

Figure s2.

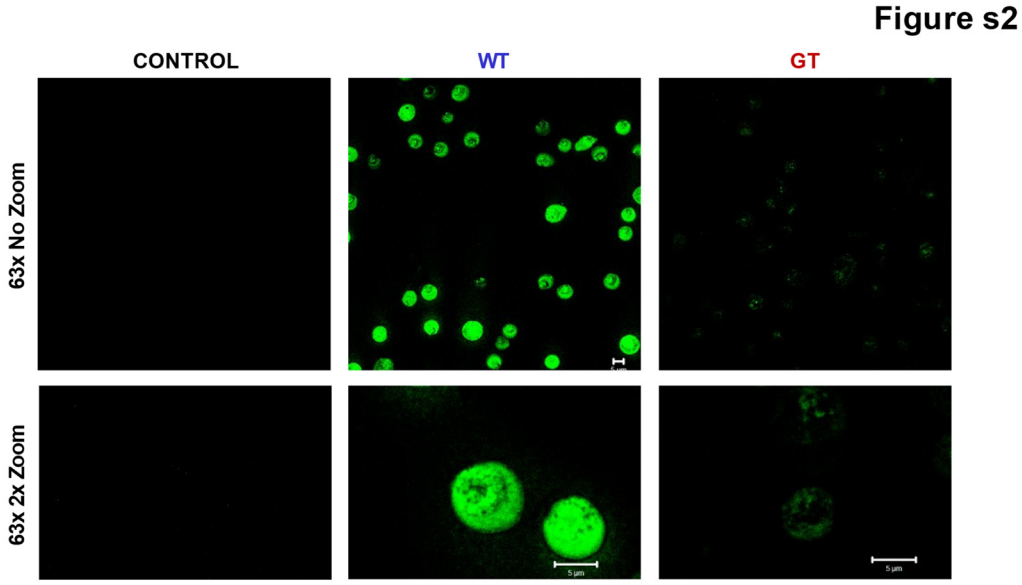

Figure s2. Confocal Microscopy Localized Fibrinogen within Fixed/Permeabilized Platelets. Peripheral blood platelets were isolated from human whole blood, washed, fixed, permeabilized and examined by indirect immunofluorescence analysis for fibrinogen distribution with a sheep anti-human fibrinogen polyclonal 1°Ab (SAFG-AP 210R4) and an Alexa-488 donkey anti-sheep IgG 2°Ab. Shown are representative fields from an experiment performed with WT (n=2) and GT (n=1) capturing ≥2 images in the field of interest detected with a Zeiss LSM 510 Multiphoton Confocal Microscope (Carl Zeiss, Inc. Oberkochen, Germany) using a 10x eye piece and 63x water objective (top row) and a 2x digital zoom (bottom row) of platelets isolated from a normal control **WT** and the **GT** patient. Fibrinogen appears as a dispersed granulated pattern in WT and GT throughout (top), and the digital zoom highlights two platelets in each field of WT and GT at reduced intensity consistent with immunoblot result in Figure 2CD.The negative **CONTROL** used 2° Ab only. The white scale bar is 5μm in length.

Supplemental References

1. Kastelowitz N, Tamura R, Onasoga A, et al. Peptides derived from MARCKS block coagulation complex assembly on phosphatidylserine. *Sci Rep*. 2017;7(1):4275.
2. Lin FY, Li J, Xie Y, et al. A general chemical principle for creating closure-stabilizing integrin inhibitors. *Cell*. 2022;185(19):3533-3550 e3527.
3. Zhang C, Liu J, Jiang X, et al. Modulation of integrin activation and signaling by alpha1/alpha1'-helix unbending at the junction. *J Cell Sci*. 2013;126(Pt 24):5735-5747.
4. Coller BS, Peerschke EI, Scudder LE, Sullivan CA. Studies with a murine monoclonal antibody that abolishes ristocetin-induced binding of von Willebrand factor to platelets: additional evidence in support of GPIb as a platelet receptor for von Willebrand factor. *Blood*. 1983;61(1):99-110.
5. Wang R, McFarland JG, Kekomaki R, Newman PJ. Amino acid 489 is encoded by a mutational "hot spot" on the beta 3 integrin chain: the CA/TU human platelet alloantigen system. *Blood*. 1993;82(11):3386-3391.
6. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985;150(1):76-85.
7. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-685.
8. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A*. 1979;76(9):4350-4354.
9. Du LM, Nurden P, Nurden AT, et al. Platelet-targeted gene therapy with human factor VIII establishes haemostasis in dogs with haemophilia A. *Nat Commun*. 2013;4:2773.
10. Rosenberg JB, Foster PA, Kaufman RJ, et al. Intracellular trafficking of factor VIII to von Willebrand factor storage granules. *J Clin Invest*. 1998;101(3):613-624.
11. Wilcox DA, White 2nd GC. Gene therapy for platelet disorders: studies with Glanzmann's thrombasthenia. *Journal of thrombosis and haemostasis : JTH*. 2003;1(11):2300-2311.
12. Heidenreich R, Eisman R, Surrey S, et al. Organization of the gene for platelet glycoprotein IIb. *Biochemistry*. 1990;29(5):1232-1244.
13. Zimrin AB, Gidwitz S, Lord S, et al. The genomic organization of platelet glycoprotein IIIa. *J Biol Chem*. 1990;265(15):8590-8595.