Supplemental Figure 1

A

PSI Hybrid

EGF1

B

Mouse ITGB3

(exon 3)
(exon 10)

M

5’-CCAGGGCTGGCTGGGGTCCATGTGTGAGTGCTCTGAGGAGGATTACCGAC-3’

3’-GGTCCCGACCGACCCCAGGTACACACTCACGAGACTCCTCCTAATGGCTG-5’

PAM Guide RNA target

C

Cytoplasmic injection

Cas9 protein
gRNAs

M470Q.ssODN

APLD Embryo

D

APLD mouse allele

W L G S M C E C S E

APLDQ mouse allele

W L G S M Q C E C S E

G C T G C T G C G G T T C ATG T G T G A G G A G A T T A C C G A C - 3’

3’-GGTCCCGACCGACCCCAGGTACACACTCACGAGACTCCTCCTAATGGCTG-5’

M470Q HDR template

5’-82bp TCC CAA TGC GAG TGC TCT GAG G

S M Q C E

W L G S Q C E C S E

ATG>CAA M470Q TGT>TGC SM
Supplemental Figure 1– CRISPR-mediated generation of the APLDQ humanized transgenic mouse. (A) Three-dimensional structure of the GPIIIa PSI domain, showing the location of the residues M470 in EGF1 domains that was mutated to Q in the APLD murine GPIIIa protein. (B) Schematic illustration of the ITGB3 locus, showing the location of the gRNA binding site (red bar), the protospacer adjacent motif (PAM) sequence (magenta bar) and the Cas9 cleavage site (red arrow heads). A 167 bp Homology Directed Repair (HDR) template was designed to introduce the M to Q amino acid substitutions (mutated nucleotides labeled in red) flanked by 82 and 77 nucleotides homology arms. The HDR template also introduces silent mutation (nucleotides in green) to prevent re-cleavage by Cas9. (C) Cytoplasmic of APLD C57BL/6N fertilized eggs were microinjected with Cas-9 protein, gRNAs along with the HDR template to generate the humanized APLDQ mouse. (D) The ITGB3 locus surrounding the genomic editing site was PCR-amplified from genomic DNA of Pup and subjected to DNA sequence analysis, confirming precise heterozygous integration of the HDR sequence into one alleles of murine ITGB3.
Supplemental Figure 2 - Antigen-capture ELISA analysis of anti-HPA-1a maternal alloantisera binding to human and murine forms of GPIIb-IIIa. Sixteen different human FNAIT alloantisera or PTP alloantisera were incubated with human or murine platelets of the indicated phenotype. Platelet/antibody complexes were then detergent lysed and added to microtiter wells that had been coated with either anti-mouse CD41 to capture immune complexes from mouse platelets, or mAb AP2 to capture immune complexes from human platelets. Note that human FNAIT alloantisera 2, 3, 4, 7, 11, 12, 13 and PTP alloantisera 2 and 3 react similarly with human GPIIb-IIIa and APLD murine GPIIb-IIIa, while human FNAIT alloantisera 1, 5, 9, 10 react poorly with murine APLD GPIIb-IIIa, suggesting that the preponderance of the HPA-1a-specific alloantibodies present in these polyclonal sera have more complex epitope requirements. None of the FNAIT alloantisera react with wild-type murine GPIIb-IIIa, as expected.
Supplemental Figure 3

Supplemental Figure 3 - Inhibition of PAC-1 binding to human αIIbβ3 by Type II, but not Type I, anti-HPA-1a alloantibodies. HEK293FT cells were transfected with wild-type human αIIbβ3 plus EGFP. Cells were pre-incubated with either the Type I mAbs SZ21, the Type II mAbs B2G1 and 26.4, or purified IgG fractions from a previously-characterized Type I PTP antisera (PTP-1), or previously characterized Type II FNAIT antisera (FNAIT-5 and FNAIT-9). Following pre-incubation, the fibrinogen ligand-mimetic mAb PAC-1 was added in a buffer containing 0.2 mM Ca+2 and 2 mM Mn+2. EGFP-positive cells were analyzed by flow cytometry for the binding of PAC-1. PAC-1 binding was normalized to total β3 surface expression and presented as a percentage of buffer control. Data are mean ± SD (n≥2). Note that both monoclonal and polyclonal Type II antibodies inhibit PAC-1 binding to various extents, while Type I antibodies are largely without effect.