Supplemental Information

Title:

O-linked sialic acid residues on platelet membrane glycoprotein (GP) IIb mask the human HPA-9b alloepitope

Short Title:

Enhanced detection of anti-HPA-9b alloantibody

Authors:

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HPA-9a, 3b, A845/847 gBlock fragment:

5’-

ggcgtcgaattcAGCACTTCAAGTGAACATGGaggAGCCAGAATCCAAACAGCAAGATTGTGCTGCTGGACGT
GCCGGTCCGGGCAGAGGCCCAAGTGGAGCTGCGAGGGTGAGAGGCCAGGGGTGGAGAAGGGAGAT
GGCATTCAGGGCTCTAAACTCCAGGGGGCGCTGGGGAAACCTCACAGGCCAATCAGGGCATCACACT
CTCTCTGGGGGTCTTGGGCACCTGCAGGAACTCCTTTCCAGCCTCCCTGGTGGTGGCAGCAGAAGAA
GGTGAGAGGGAGCAGAACAGCTTGGACAGCTGGGGACCCAAAGTGGAGCACACCTATGAGGTATTGG
GGAGCCTCGCGTCCCTGGCTGGGGTGAGCGGGTCCTCAGAACTCCGGGTGAGGCGCTAAGCTCCCC
ACACCCTGCCACCACCACCCCTTCAGCTCCACAACAATGGCCCTGGGACTGTGAATGGTCTTCACCTC
AGCATCCACCTTCCGGGACAGTCCCAGCCCTCCGACCTGCTCTACATCCTGGATATACAGCCCCAGGG
GGGCCTTCAGTGCTTCCCACAGCCTCCTGTCAACCCTCTCAAGGTAAGAGCTGGGTGGAAGAAAGAC
CTGGGAAGGCGGCCCCAGACCAACCAACGTTGCACCTCTGTGGGCTGGGGTTCGGGGGAGACCTGG
GCCTGACCACTCCTTTGCCCCCCCAGGTGGACTGGGGGCTGCCCAGCCCCGCTCCCGCTCCCATTCA
CCCGGCCCATCACAAGCGGGATCGCAGACAGATCTTCCTGCCAGAGCCCGAGCAGCCCTCGAGGCTT
CAGGATCCAGTTCTCGTAGTGAGCAGGCTCTCTGGTCTCTGGCCCGGCCTCCCCGGGACCCACGGGG
CAGAGGGGATGGGAGGAGGGAGAGGGGTCCGGGTGTGCTGTGGGCCTCTGTGGGCCACGCTTGGTC
CCTGGGAGCACTTCAATTGCAGTTGGAGTAGCATGCTGGCTTGTGTCTGGGGTGAGCTGAAAGACAC
TTGCACTTTTTAAAAGCTTCCCAGTACGTTAAGGAGCATAAAACAATGCCAAAGCAAGGTTATCATAGA
TCTGAGCATTGTGCGCTGGGGGATGACCCTCCCTGCATCTCTGGGACTATGTGAGCAAGCCCGTGGA
AAGACAGCATCCGAAGCTTGGATCCAAGGCCCTTCCTGATGGGAAGGCCACCGCTTCCTGAACCCCC
GGCCCCTTCTGCGTTGGGTCCTGGGGGTAAGGGGGTGGGGGATGATGGGGTGATGGGCCGGGACGG
GCTGGGGACTGACGATGCTTCCCCTCAGAGCTGCGACTCGGCGCCCTGTACTGTGGTGCAGTGTGAC
CTGCAGGAGATGGCGCGCGGGCAGCGGGCCATGGTCACGGTGCTGGCCTTCCTGTGGCTGCCCAGC
CTCTACCAGGTGGGGTGGGCCGTGGTGGGGCGGGGCCGGGCCTTCTGGGCGGGGACCACTTTGCTC
TGGGAGGGGCGGGGTTTGGTGTGGGAGGGCAGGAAGAGAGGGAAGGCAAGGTTTACTTTGGGGGAT
TGCAGTGGGATTAGGTCAGAGGCctCCATGTTCACTTGAAGTGCTgaattcgcagcg -3’

HPA-9b, 3b, A845/847 gBlock fragment:

5’-

ggcgtcgaattcAGCACTTCAAGTGAACATGGaggAGCCAGAATCCAAACAGCAAGATTGTGCTGCTGGACGT
GCCGGTCCGGGCAGAGGCCCAAGTGGAGCTGCGAGGGTGAGAGGCCAGGGGTGGAGAAGGGAGAT
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CTCTCTGGGGGTCTTGGGCACCTGCAGGAACTCCTTTCCAGCCTCCCTGGTGGTGGCAGCAGAAGAA
GGTGAGAGGGAGCAGAACAGCTTGGACAGCTGGGGACCCAAAGTGGAGCACACCTATGAGGTATTGG
GGAGCCTCGCGTCCCTGGCTGGGGTGAGCGGGTCCTCAGAACTCCGGGTGAGGCGCTAAGCTCCCC
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GGGCCTTCAGTGCTTCCCACAGCCTCCTGTCAACCCTCTCAAGGTAAGAGCTGGGTGGAAGAAAGAC
CTGGGAAGGCGGCCCCAGACCAACCAACGTTGCACCTCTGTGGGCTGGGGTTCGGGGGAGACCTGG
GCCTGACCACTCCTTTGCCCCCCCAG<u>ATG</u>GACTGGGGGCTGCCC<u>AGC</u>CCC<u>GCT</u>CCC<u>GCT</u>CCCATTCA
CCCGGCCCATCACAAGCGGGATCGCAGACAGATCTTCCTGCCAGAGCCCGAGCAGCCCTCGAGGCTT
CAGGATCCAGTTCTCGTAGTGAGCAGGCTCTCTGGTCTCTGGCCCGGCCTCCCCGGGACCCACGGGG
CAGAGGGGATGGGAGGAGGGAGAGGGGTCCGGGTGTGCTGTGGGCCTCTGTGGGCCACGCTTGGTC
CCTGGGAGCACTTCAATTGCAGTTGGAGTAGCATGCTGGCTTGTGTCTGGGGTGAGCTGAAAGACAC
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AAGACAGCATCCGAAGCTTGGATCCAAGGCCCTTCCTGATGGGAAGGCCACCGCTTCCTGAACCCCC
GGCCCCTTCTGCGTTGGGTCCTGGGGGTAAGGGGGTGGGGGATGATGGGGTGATGGGCCGGGACGG
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CTGCAGGAGATGGCGCGCGGGCAGCGGGCCATGGTCACGGTGCTGGCCTTCCTGTGGCTGCCCAGC
CTCTACCAGGTGGGGTGGGCCGTGGTGGGGCGGGGCCGGGCCTTCTGGGCGGGGACCACTTTGCTC
TGGGAGGGGCGGGGTTTGGTGTGGGAGGGCAGGAAGAGAGGGAAGGCAAGGTTTACTTTGGGGGAT
TGCAGTGGGATTAGGTCAGAGGCctCCATGTTCACTTGAAGTGCTgaattcgcagcg -3’

Supplemental Figure 1

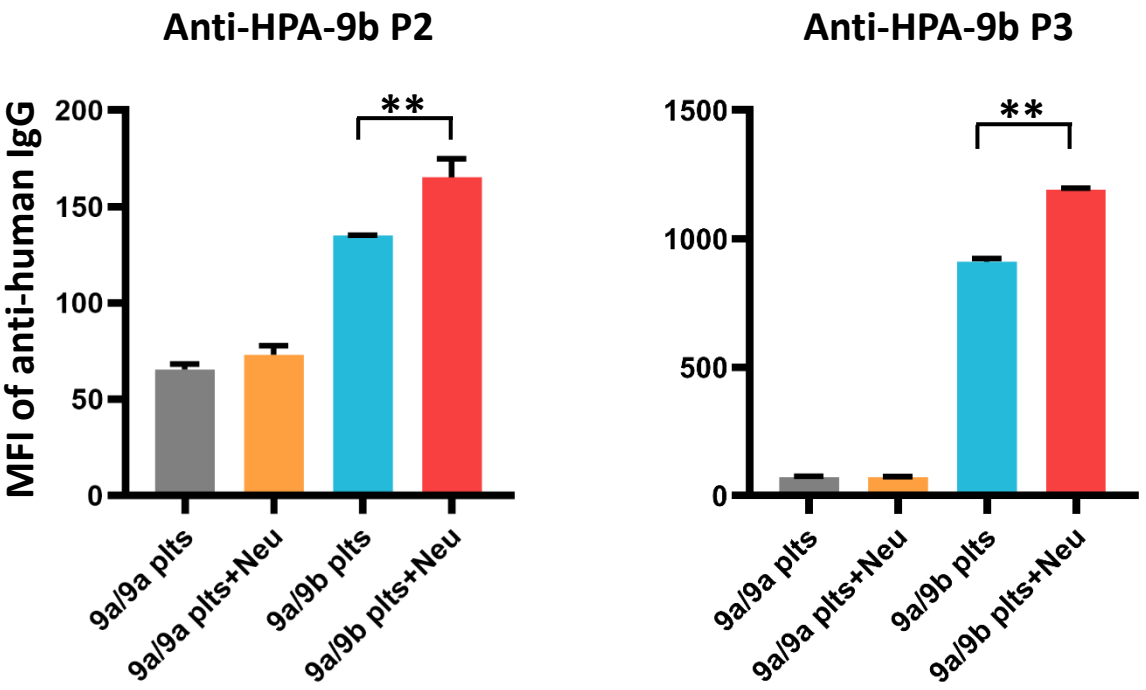

Figure S1. Neuraminidase treatment of human platelets enhanced HPA-9b alloantibody detection in clinical diagnostic platelet antibody bead array (PABA). HPA-9a/9a or HPA-9a/9b group O donor platelets were treated with or without 30 mU/ml neuraminidase before incubation with two different anti-HPA-9b patient sera. PABA was done as previously described.[26] Data show the median fluorescence intensity of anti-human IgG detected from beads conjugated with AP2 (anti-GPIIb/IIIa) antibody. Values represent the means ± SD. ** P<0.01 by 1-way ANOVA with Dunnett's test.

Supplemental Figure 2

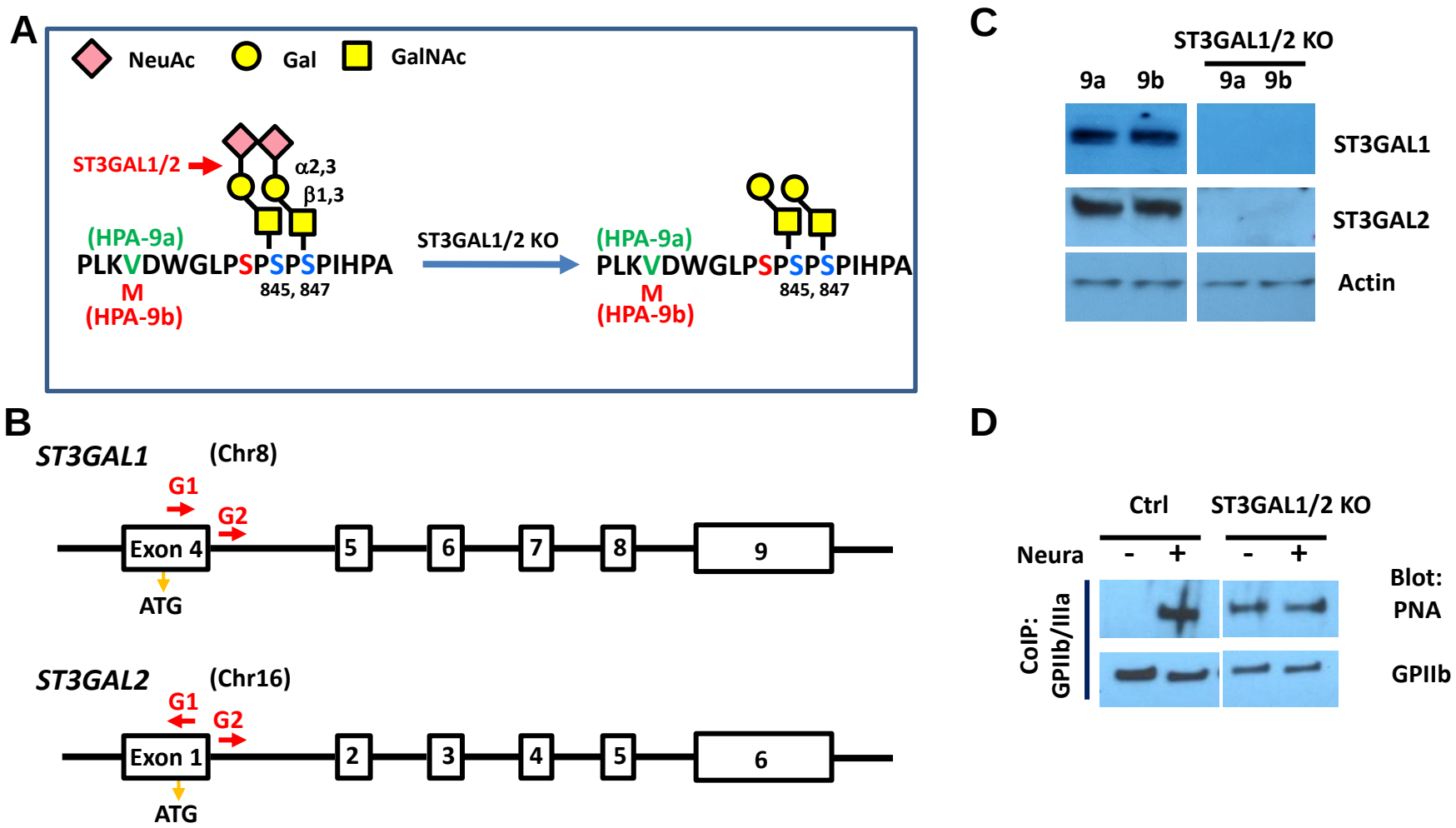

Figure S2. Generation of ST3GAL1/2-deficient, HPA-9 allele-specific, iPSCs. (A) Schematic of local alloantigenic peptide and O-glycan modification of GPIIb from ST3GAL1/2-deficient iPSC-derived MKs. **(B)** Schematic illustration of the ST3GAL1 and ST3GAL2 locus, showing the location of the gRNA binding sites (red arrows) to guide Cas9 to its cleavage site. The ATG start codon for gene translation is marked by a yellow arrow. **(C)** Western blot demonstrating the loss of expression of ST3GAL1 and ST3GAL2 in the corresponding KO iPSC lines. **(D)** Co-immunoprecipitation of GPIIb-IIIa from iPSC-derived MKs further confirming that ST3GAL1/2 KO MKs express GPIIb with a completely exposed, unsialylated Core-1 structure, as indicated by comparable levels of PNA binding in the absence or presence of neuraminidase treatment.

Supplemental Figure 3

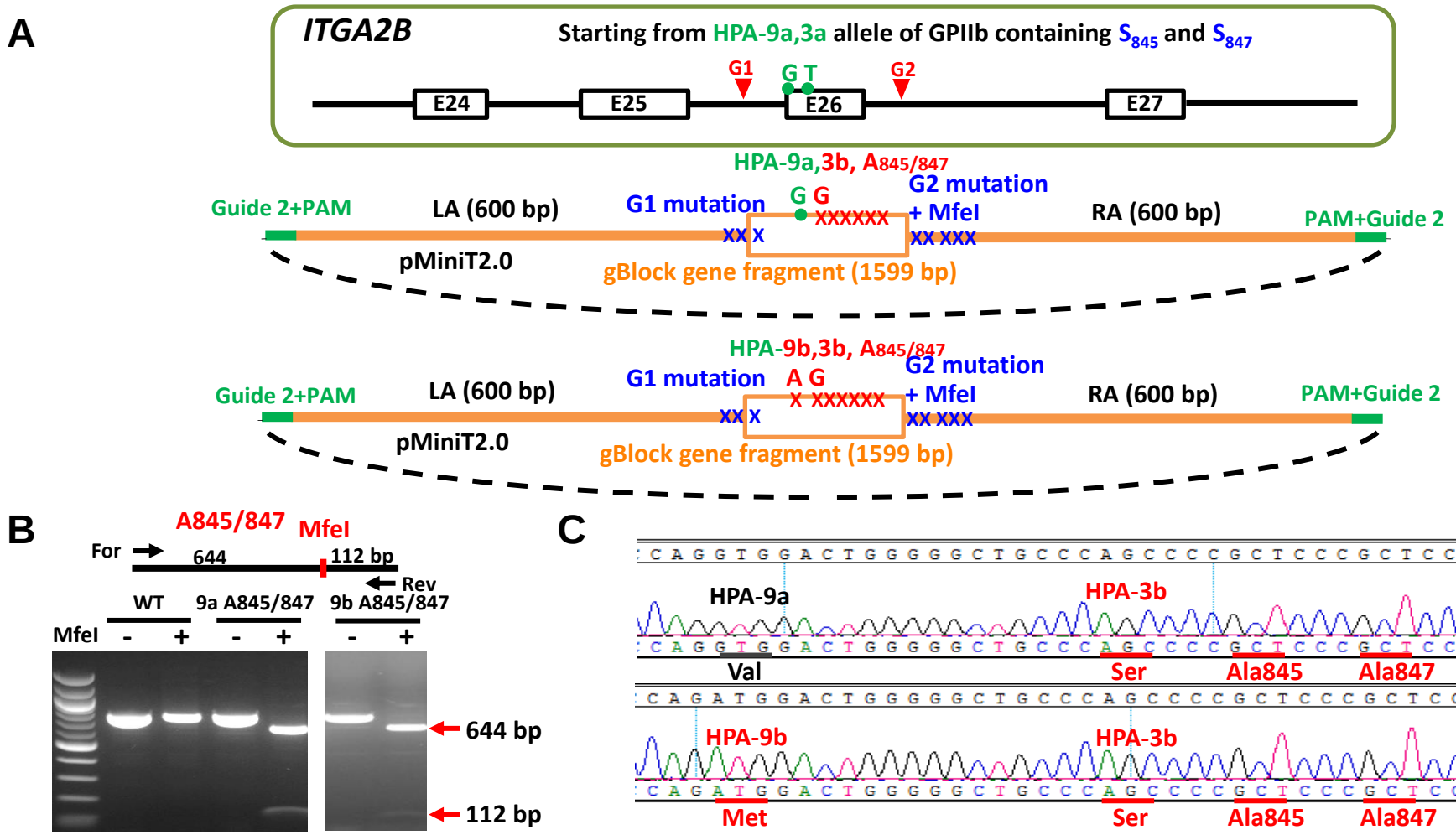

Figure S3. Generation of HPA-9 allele-specific A845/847 mutant iPSC clones. (A) Schematic illustration of donor plasmid and targeting strategy for generating HPA-9a or -9b iPSC lines with Ala845/847 mutations. Red triangles flanking exon 26 of the ITGA2B gene indicate the two gRNA binding sites that guide Cas9 to remove the entire exon encoding HPA-9a, -3a and Ser845/847. The HDR donor plasmid encodes either the HPA-9a, 3b epitopes, with alanines at residues 845 and 847, or the HPA-9b, 3b epitopes with alanines at residues 845 and 847. The recognition sequence and the PAM sequence of guide 2 (green line) are added to both ends of the homology arms for linearizing the donor templates in the transfected cells. Donor plasmids also contain silent mutations (blue X) to prevent re-cleavage by Cas9, and that generate an MfeI site for genotyping. **(B)** Genomic DNA isolated from puromycin-resistant iPSC clones was PCR-amplified and digested with MfeI, which differentiates HDR-directed repair from the WT allele. Red arrows indicate the expected fragment sizes of a typical clone with A845/847 mutations. **(C)** Sequencing data confirmed the targeted point mutations in CRISPR-edited iPSC lines.

Supplemental Figure 4

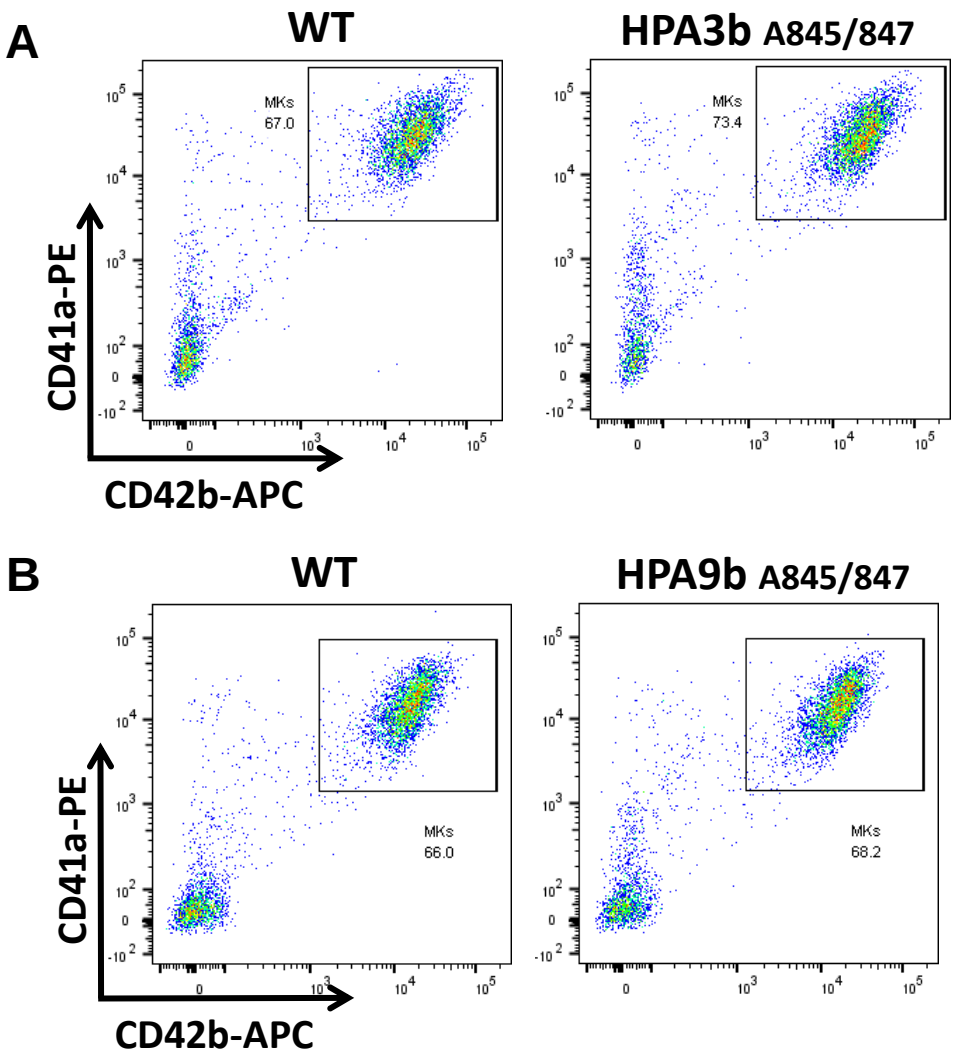

Figure S4. Loss of O-glycosylation on GPIIb does not affect MK differentiation or GPIIb surface expression. Flow cytometric analysis showed production of $CD41^{+}/CD42b^{+}$ MKs from different iPSC lines.

Supplemental Table 1

Table S1: gRNA sequences

	Sequence from 5' to 3'
Guide 1 targeting ST3GAL1	GAACTACTCCCACACCATGG
Guide 2 targeting ST3GAL1	GGGGTCTGGTAATGAGAGTG
Guide 1 targeting ST3GAL2	CGGAGAGGAACCACACCCGC
Guide 2 targeting ST3GAL2	GTGAGGAGTACAGCCATGGG
Guide 1 targeting ITGA2B	CGGCCCCAGACCAACCACCG
Guide 2 targeting ITGA2B	AGCACTTCAAGTGAACATGG