## Supplementary Table 1: Assay characteristics

<table>
<thead>
<tr>
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<th><strong>Platelet micro aggregation test</strong></th>
<th><strong>Platelet reactivity assay</strong></th>
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<tbody>
<tr>
<td><strong>Patient material</strong></td>
<td>- 10 ml whole blood</td>
<td>- 150 µl whole blood</td>
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<td><strong>Pathways tested</strong></td>
<td>- GPIIbIIIa (PMA)</td>
<td>- P2Y₁ and P2Y₁₂ (ADP)</td>
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<td></td>
<td>- GPIbIX (ristocetin)</td>
<td>- GPVI (convulxin)</td>
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<td>- PAR1 (TRAP)</td>
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<td><strong>Material processing</strong></td>
<td>- Platelet isolation (1 hour)</td>
<td>- Incubation of whole blood (20 minutes)</td>
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<td>- Staining, incubation and fixation (1.5 hours)</td>
<td>- Fixation (10 minutes)</td>
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<td>- Lysis of erythrocytes (30 minutes)</td>
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<td><strong>Analysis</strong></td>
<td>- FACS analysis in a 96 well plate</td>
<td>- FACS analysis in a 96 well plate</td>
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<tr>
<td><strong>Read-out</strong></td>
<td>- Micro aggregation with control platelets</td>
<td>- Degranulation (P-selectin)</td>
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<td></td>
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<td>- Fibrinogen binding (GPIIbIIIa opening)</td>
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Supplementary Figure 1. Optimization of the PKH:CFSE ratio for the platelet microaggregation test. Different ratios ranging from 0.01:1 to 1:1 were tested to determine the lowest number of test platelets (PKH+) for detection of micro-aggregates after stimulation with PMA. Ratios of 0.06:1 to 0.5:1 resulted in reliable and comparable test outcomes with low background aggregation without stimulus, at time point 0. Using a ratio of 0.01:1 resulted in very few PKH+ events and thereby affected the determination of aggregation, and 1:1 lead to high numbers of PKH-stained events causing spillover in FACS analysis. A ratio of approximately 0.10:1 was considered optimal since this required a minimum number of test platelets while giving similar results.

Supplementary Figure 2: Healthy Controls reach similar aggregation levels in an autologous and allogenic setting. Platelets obtained from 2 HCs were isolated and tested in the platelet micro aggregation test to assess whether aggregation levels where influenced by the allogenic nature of the assay compared to the autologous healthy control. Two HCs were performed in an autologous setting as well as, crossed with each other to test the allogenic setting. No differences in aggregation scores were observed.

Supplementary Figure 3. Validation platelet reactivity assay with decreasing platelet number. Platelets from 3 control subjects were diluted to 250*10^9/L, 50*10^9/L, 25*10^9/L and 10*10^9/L, by diluting platelet rich plasma with platelet poor plasma. 5 µL of these samples were added to 50 µL of buffer containing RPE-labeled anti-P-selectin antibodies, Alexa Fluor 488-labeled fibrinogen, and serial concentrations of ADP, convulxin (CVX) or TRAP. Maximal mean fluorescence intensity (MFI) in arbitrary units (AU) and concentration of the agonists needed to obtain half maximal MFI are displayed for both platelet P-selectin expression (A, B, E, F, I, J) and opening of GPIIbIIIa receptor (C, D, G, H, K, L). Each line represents the results obtained for individual donor. These related samples were compared by Wilcoxon signed ranks tests, showing no significant differences when platelet number was reduced from 250*10^9/L to 10*10^9/L.

Supplementary Figure 4. Correlation platelet reactivity with platelet count within study population. No correlation is observed between platelet number and ADP induced maximal platelet reactivity (Spearmans rho 0.165, p= 0.367) within study population (n=33).
**Supplementary Figure 5. Severe phenotype patients have reduced aggregation levels compared to mild phenotype patients upon PMA stimulation.** Aggregation indexes were calculated by dividing the healthy control aggregation level by its patient aggregation level. An aggregation index of 1 indicates that the patient has an aggregation capacity equal to its healthy control (depicted by the dotted line), whereas a number <1 indicates diminished aggregation capacity. Upon PMA stimulation, severe phenotype patients exhibited a lower aggregation index compared to mild phenotype patients after five (0.57 [0.39-0.65] versus 0.94 [0.64-1.12]; p=.04) and ten minutes (0.77 [0.53-0.91] versus 0.97 [0.86-1.06]). No significant differences were observed upon stimulation with ristocetin, between severe and mild phenotype patients after five (0.78 [0.58-0.86] versus 1.07 [0.93-1.25]) and ten minutes (0.79 [0.63-0.89] versus 1.17 [0.74-1.22]).
Supplemental Figure 1. Optimizing PKH:CFSE ratio for the micro platelet aggregation test.
Supplementary Figure 2: Healthy Controls reach similar aggregation levels in an autologous and allogenic setting.
Supplementary Figure 3. Validation platelet reactivity assay with decreasing platelet number.

**P-selectin**

A. ADP max

B. ADP EC50

C. ADP max

D. ADP EC50

E. CVX MFI max P-selectin

F. CVX MFI EC50 P-selectin

G. CVX MFI max

H. CVX MFI EC50

I. TRAP max

J. TRAP EC50

K. TRAP max

L. TRAP EC50

**Open GPIIb/IIa**

A. ADP max

B. ADP EC50

C. ADP max

D. ADP EC50

E. CVX MFI max P-selectin

F. CVX MFI EC50 P-selectin

G. CVX MFI max

H. CVX MFI EC50

I. TRAP max

J. TRAP EC50

K. TRAP max

L. TRAP EC50
Supplementary Figure 4. Correlation platelet reactivity with platelet count within study population

![Graph showing correlation between platelet count and max P-selectin to ADP in AU.](image-url)
Supplementary Figure 5. Severe phenotype patients have reduced aggregation levels compared to mild phenotype patients upon PMA stimulation.