Supplement

Figure S1

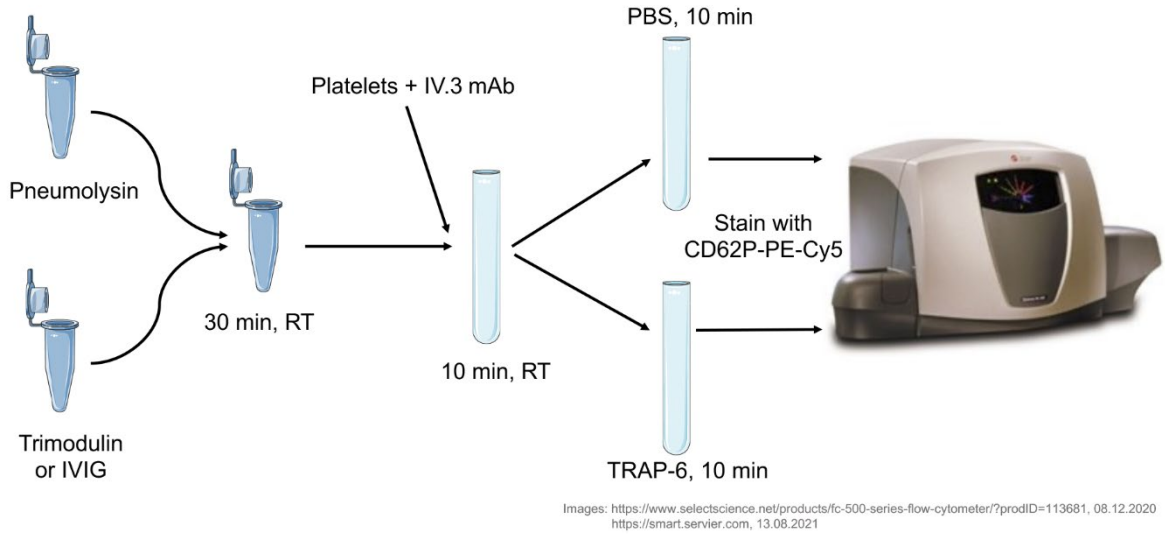

Figure S1: Experimental setup of CD62P assay.

Figure S2

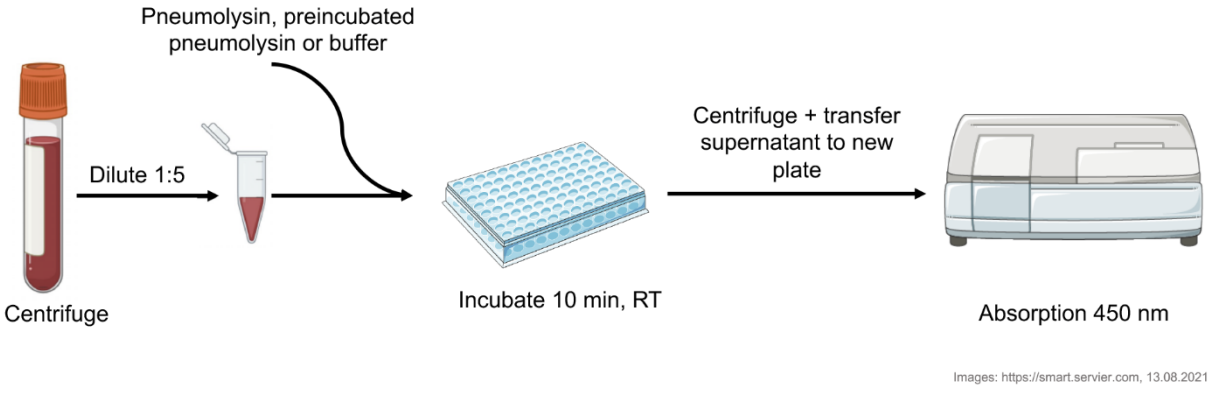

Figure S2: Experimental setup of the hemolysis assay.

Figure S3

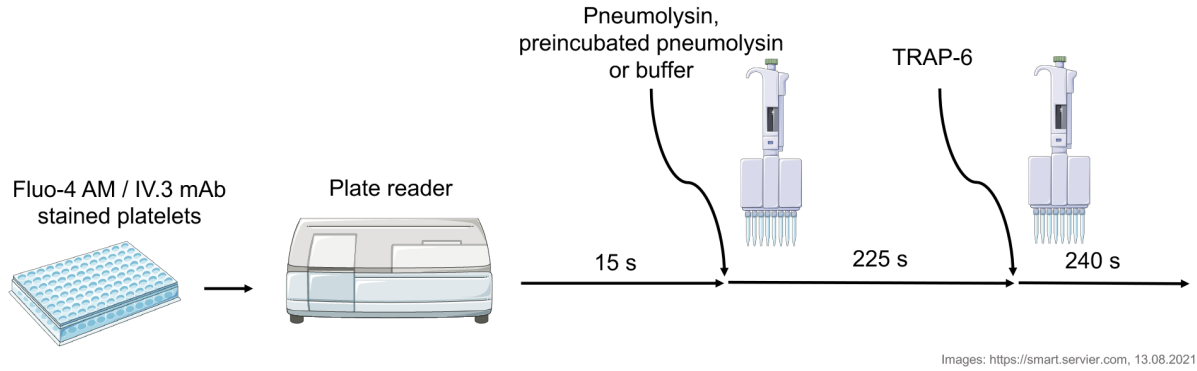

Figure S3: Experimental setup of the calcium release assay.

Figure S4

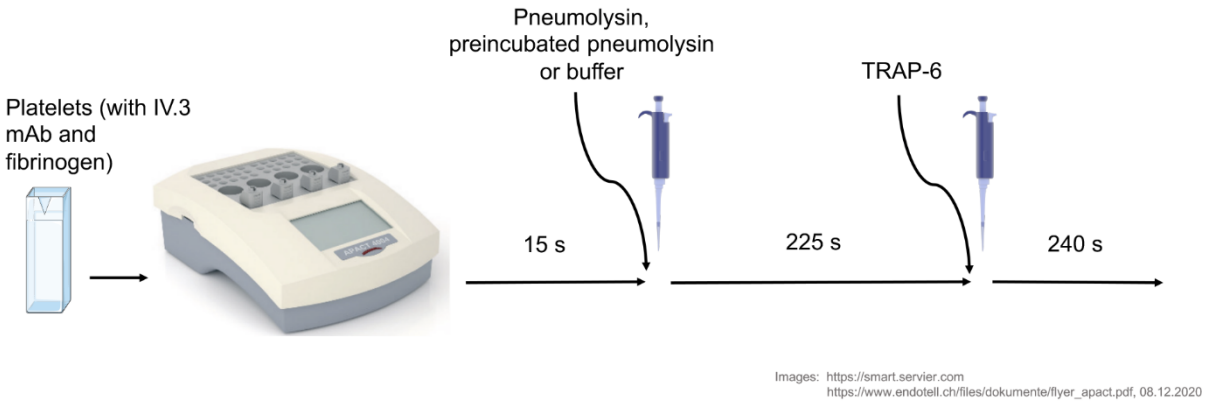

Figure S4: Experimental setup of aggregometry.

Figure S5

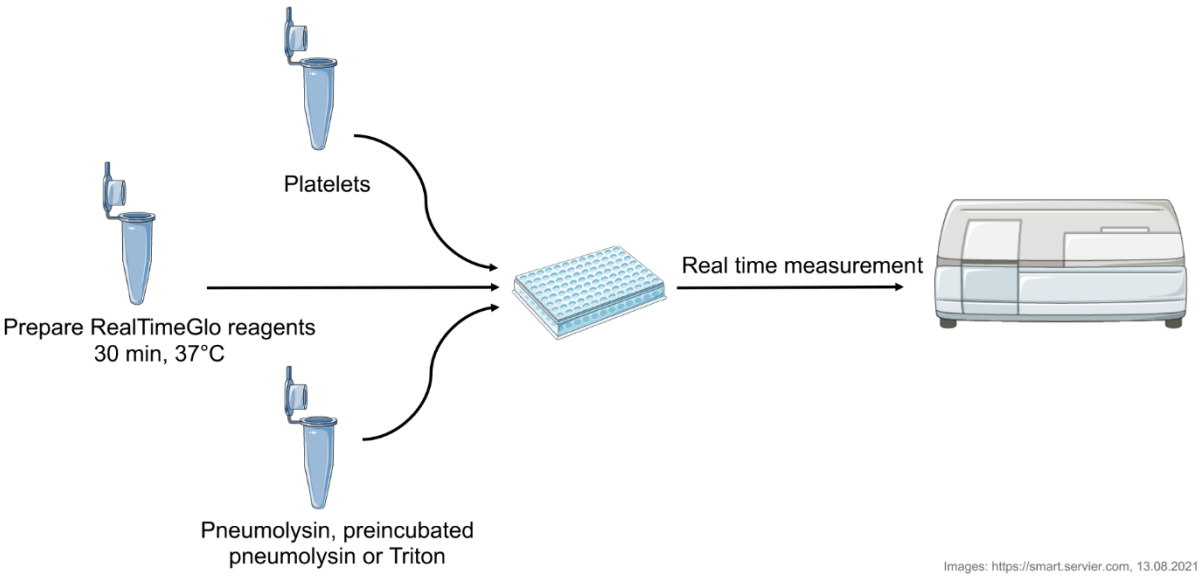

Figure S5: Experimental setup of the platelet viability assay.

Figure S6

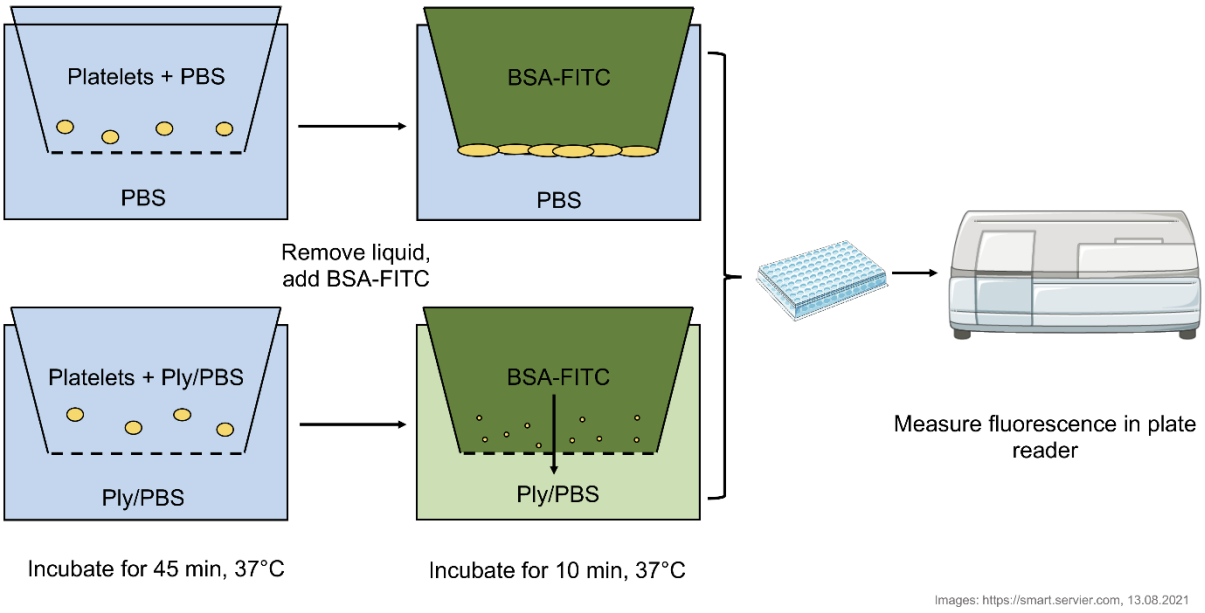

Figure S6: Experimental setup of the transwell assay.

Figure S7

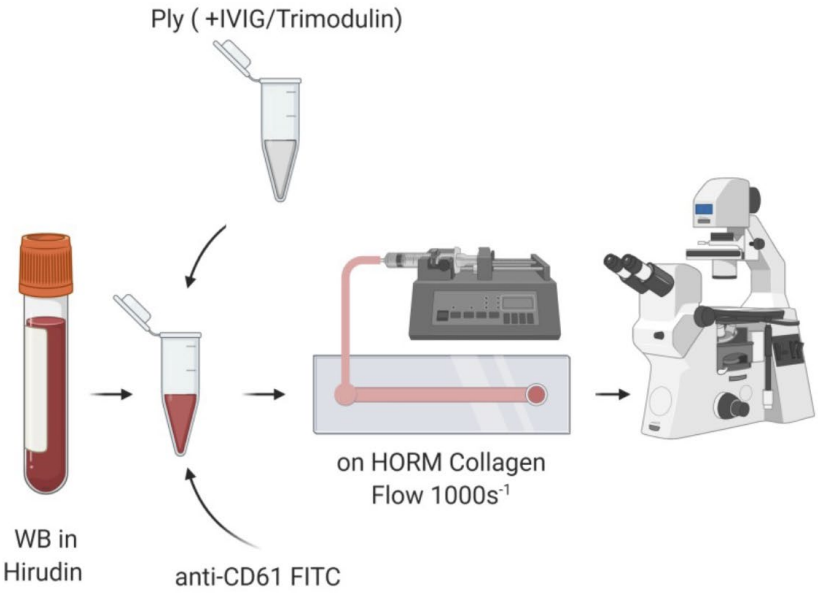

Figure S7: Experimental setup of the flow chamber assay.

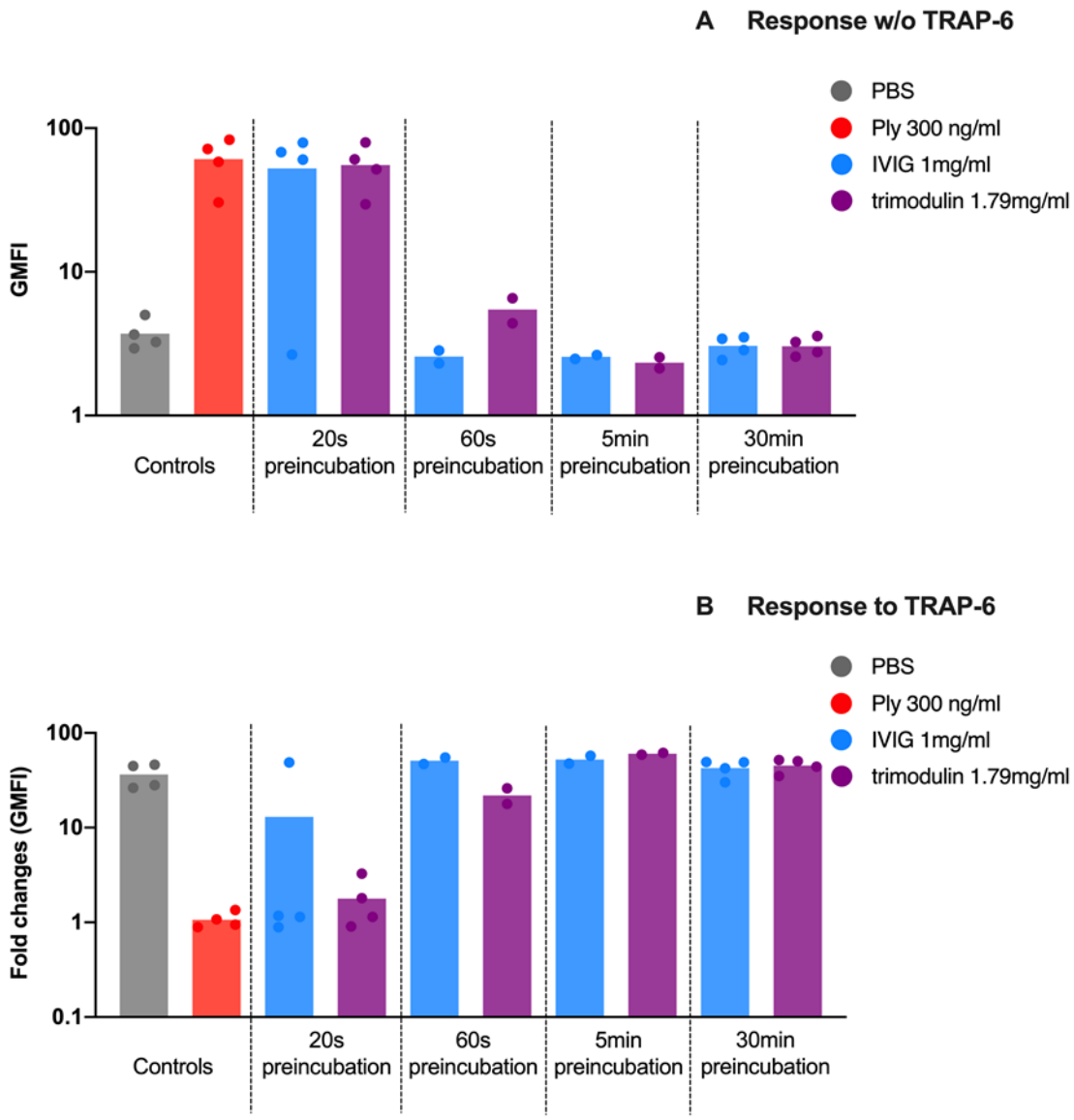

Figure S8: CD62P staining in platelets upon pneumolysin treatment.

(A) CD62P staining upon treatment with pneumolysin or pneumolysin preincubated with IVIG or trimodulin. CD62P staining is presented as geometric mean fluorescence intensity (GMFI) of CD62P-positive gated events. As a control, samples were incubated only with PBS.

(B) CD62P staining upon TRAP-6 (20 µM) addition after preincubation of platelets with pneumolysin or pneumolysin preincubated with IVIG or trimodulin. Response to TRAP-6 is presented as a fold change of the GMFI of CD62P-positive gated events. As a control, samples were incubated with PBS only.

Figure S9

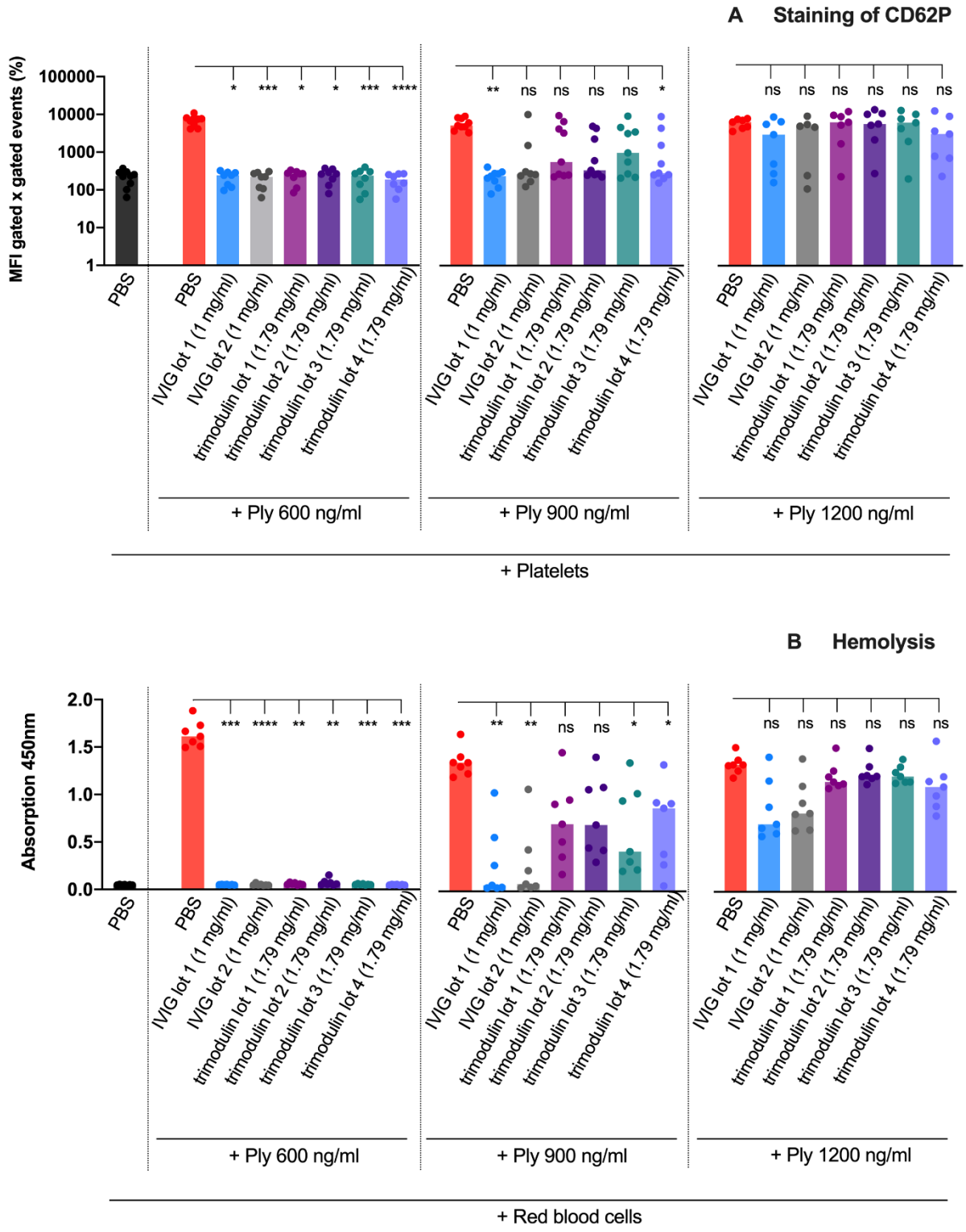
A Staining of CD62P
MFI gated x gated events (%)
100000
10000
1000
100
10
1
* *** * * *** ****
** ns ns ns ns *
ns ns ns ns ns ns
PBS
IVIG lot 1 (1 mg/ml)
IVIG lot 2 (1 mg/ml)
trimodulin lot 1 (1.79 mg/ml)
trimodulin lot 2 (1.79 mg/ml)
trimodulin lot 3 (1.79 mg/ml)
trimodulin lot 4 (1.79 mg/ml)
+ Ply 600 ng/ml
+ Ply 900 ng/ml
+ Ply 1200 ng/ml
+ Platelets
B Hemolysis
Absorption 450nm
2.0
1.5
1.0
0.5
0.0
*** **** ** ** *** ***
** ** ns ns * *
ns ns ns ns ns ns
+ Red blood cells

Figure S9: Batch-to-batch variability of immunoglobulin preparations used for neutralization of pneumolysin

(A) CD62P staining in platelets upon pneumolysin (Ply) treatment. CD62P staining is presented as the mean fluorescence intensity of CD62P-positive events multiplied with the percentage of positive gated events. As control, platelets were incubated only with PBS. Bars represent the median.

(B) Hemolysis upon pneumolysin treatment. Hemolysis was quantified by absorption measurement at 450 nm as a measure for hemoglobin content in the supernatant (B). As control, erythrocytes were incubated only with PBS. Bars represent the median.

Statistical analysis was performed using Friedman test followed by uncorrected Dunn's test for multiple comparisons. A p-value < 0.05 was considered to be significant (* > 0.033, ** > 0.002 , *** > 0.001).

Figure S10

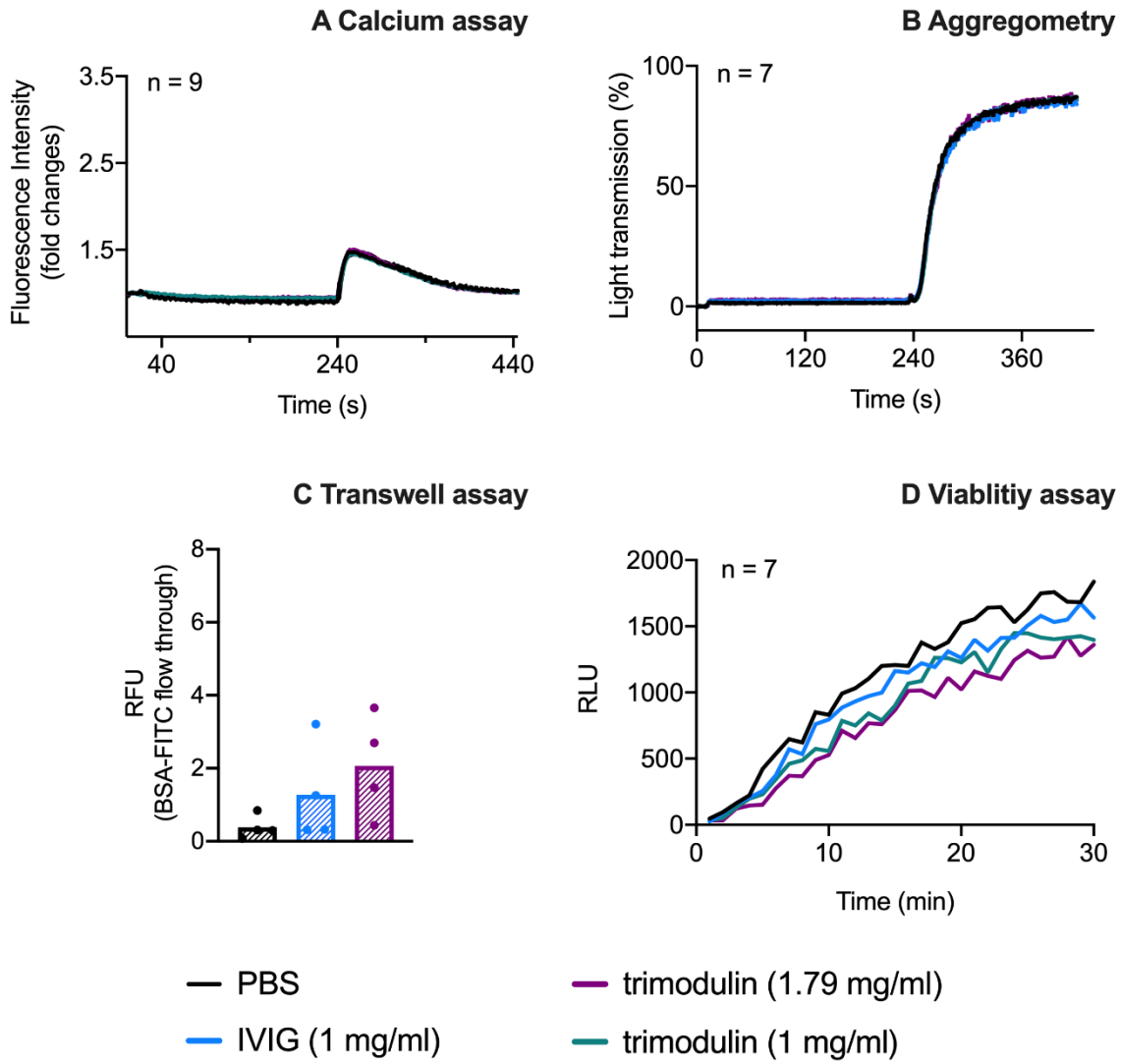

Figure S10: Controls of IVIG and trimodulin in the aggregometry, calcium -, transwell - and viability - assays.

(A) Incubation of the IVIG or trimodulin with platelets in the calcium assay. Curves represent the median of platelets of 9 donors.

(B) Incubation of the IVIG or trimodulin with platelets in aggregometry. Curves represent the median of platelets of 7 donors.

(C) Incubation of the IVIG or trimodulin with platelets in the transwell assay. Bars represent the mean of platelets of 4 donors.

(D) Incubation of the IVIG or trimodulin with platelets in the viablity assay.

Controls of the immunoglobulin preparations in the CD62P assay, thrombus formation assay and hemolysis assay are presented in Figure 1, 5 and S1.