Supplementary figure S1. Release of α-granule by stimulation with PAR1-AP and PAR4-AP.

Heparinized whole blood was diluted (1:12) in HEPES buffer containing agonists, anti-GPIIb and anti-P-selectin. Samples were incubated for 10 minutes at room temperature, diluted 1:20 in HEPES buffer and then analysed on a Coulter Epics XL.MCL flow cytometer (Beckman Coulter). The agonist concentrations shown are chosen to induce approximately 20% (low), 50% (medium) or 100% (high) release of α-granules (percentage of P-selectin-positive platelets). Data is expressed as means ± SEM, (n=5).

Supplementary figure S2. Stimulation with PAR1-AP and PAR4-AP for 55 min induces lysosomal exocytosis in a fraction of human platelets.

Platelets in diluted whole blood were stimulated for 55 minutes in room temperature with low, medium and high concentration of PAR1-AP (2.5, 5 or 30 µM) and PAR4-AP (25, 50 or 150 µM). After incubation with anti-LAMP-1, the fraction of platelets exposing LAMP-1 on the surface was analysed by flow cytometry. Data is expressed as means ± SEM, (n=5)**.** Samples were compared to control using repeated measures ANOVA followed by Dunnett's multiple comparison tests, significant differences are indicated by *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Surface expression of LAMP-1 upon PAR1-AP and/or PAR4-AP stimulation was compared, for low, medium and high concentrations respectively, using repeated measures ANOVA followed by Bonferroni's multiple comparison tests, significant differences are indicated by # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.

Supplementary figure S3. The effect of cangrelor on thrombin-induced lysosomal exocytosis from human platelets is maintained at longer stimulation.

Platelets were incubated with ASA (100 µM, 15 min) and cangrelor (5 µM, 5 min) alone or in combination and then stimulated with thrombin (0.3 or 1 U/ml). **(A)** Isolated platelets ($3 \cdot 10^8$ platelets/ml) were stimulated with thrombin for 5 min and NAG activity was analysed in the supernatant. **(B-C)** Platelets in diluted whole blood were stimulated with thrombin for 10 min after which the fraction of (B) LAMP-1 or (C) P-selectin positive platelets was determined using flow cytometry. Data are expressed as means ± SEM (n=5). For statistical analysis repeated measures ANOVA, followed by Dunnett's multiple comparison tests against the respective control was used. Significant differences are indicated by *$p < 0.05$, *** $p < 0.001$.

Supplementary figure S4. SNAP prevents thrombin-induced lysosomal and α-granule exocytosis from human platelets in platelet-rich plasma but not in diluted whole blood.

Platelets were incubated with the NO-donor SNAP (10 or 30 µM, 2 min) and stimulated with thrombin (0.3 or 1 U/ml, 1 min). **(A-B)** The fraction of platelets positive for LAMP-1 in diluted whole blood (A) or platelet-rich plasma (PRP) (B) determined using flow cytometry. **(C-D)** P-selectin expression on platelets in diluted whole blood (C) or PRP (D) determined using flow cytometry. Data are expressed as means ± SEM (n=5). For statistical analysis repeated measures ANOVA was followed by Dunnett's multiple comparison tests against the respective control. Significant differences are indicated by *$p < 0.05$, *** $p < 0.001$.

Supplementary figure S5. SNAP- and PGI_2-prevented thrombin-induced lysosomal exocytosis from human platelets is maintained at longer stimulation.

Platelets were incubated with the NO-donor SNAP (10 µM, 2 min) and PGI_2 (1 µM, 2 min) alone or in combination and stimulated with thrombin (0.3 or 1 U/ml). **(A)** Isolated platelets ($3 \cdot 10^8$ platelets/ml) were stimulated with thrombin for 5 min and NAG activity was analysed in the supernatant. **(B)** Platelets in diluted whole blood were stimulated with thrombin (10 min) and the fraction of LAMP-1 positive platelets was determined using flow cytometry. **(C)** The same experiment as in B performed in PRP. **(D)** Platelets in diluted whole blood were stimulated with thrombin (10 min) and the fraction of P-selectin positive platelets was determined using flow cytometry. **(E)** The same experiment as in D performed in PRP. Data are expressed as means ± SEM (n=5). For statistical analysis repeated measures ANOVA was followed by Dunnett's multiple comparison tests against the respective control. Significant differences are indicated by *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.