Supplemental Figure 1. Platelet gating on flow cytometry.

Washed platelets were treated with tirofiban (1 μM) and then stimulated with 10 μg/ml PGN pre-incubated with plasma at 37^{o}C for 30 min. After fixation, the cells were first analyzed based on forward and side scatter to distinguish platelets from PGN particles. Platelets were further analyzed based on CD41 expression and analyzed for PS exposure by annexin V or in other studies for PAC1 expression. The histogram below shows that PGN-stimulated platelets express PS to the same level as do ionomycin-stimulated platelets.

Supplemental Figure 2. Tirofiban (Aggrastat) blocks aggregation induced by PGN and thrombin but not PGN. ML161 blocks thrombin but not PGN-induced platelet aggregation.

Platelet-rich plasma was pretreated with buffer, tifobifan (1μM), RGD (100μM) or ML161 (100 μM) for 30 mins at room temperature. The treated platelet-rich plasma was then stimulated with either 1U/ml thrombin (Panel A) or 50 μg/ml PGN (Panel B), and aggregation was monitored at 37^{o}C in presence of 3 mM $CaCl_2$ at a constant stirring rate of 1200 rpm.

Supplemental Figure 3: ML161 blocks thrombin but not PGN-induced platelet PS exposure.

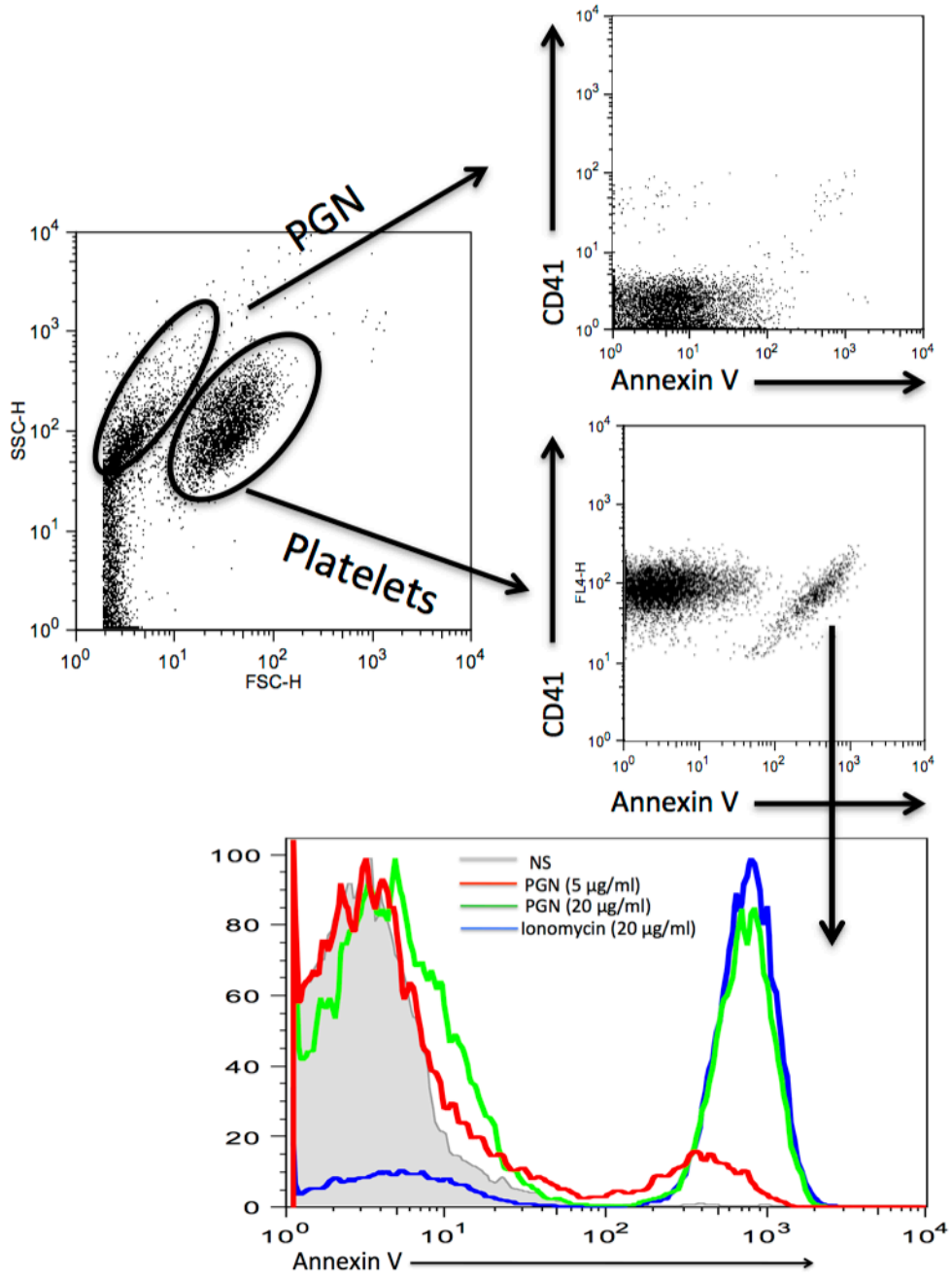
Isolated platelets were pretreated with buffer (Panels A, C, E) or ML161 (100 μM; Panels B, D, F) for 30 min at room temperature. Thrombin (1 U/ml; Panels C, D) or 10 ug/ml PGN pre-opsonized in plasma (Panels E, F) were added to platelets for 30 mins at 37^{o}C. After fixing and staining, annexin V, CD41 positive platelets were examined by flow cytometry.

Supplemental Figure 4: PS exposure is not a function of platelet concentration.

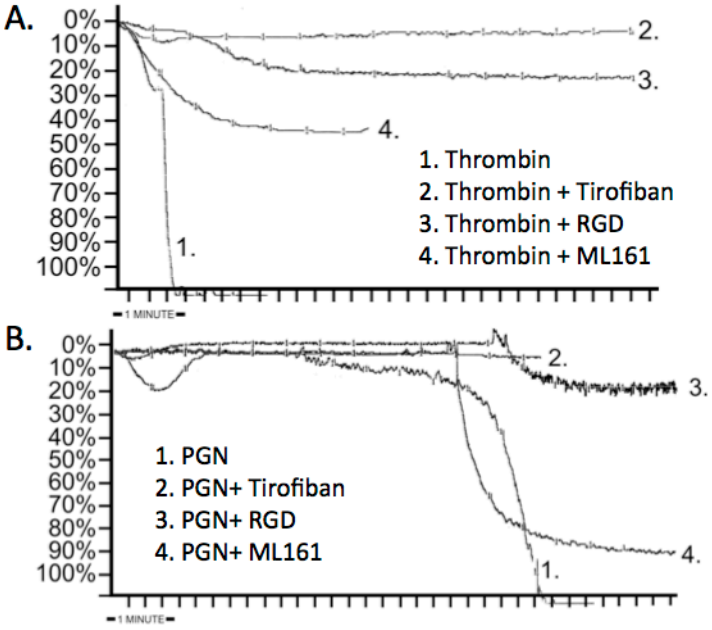
Platelet-rich plasma was pretreated with Tirofiban (1μM) for 30 mins at room temperature. The platelets were diluted with Tyrode's buffer to $400x10^6$ /ml, $40x10^6$/ml and $4x10^6$/ml. The platelets were stimulated with buffer, PGN (10 μg/ml) in plasma or ionomycin (10 μM) for 15 mins at 37°C. After fixation and staining, Annexin V+ platelets were measured by flow cytometry. The results are representative of three experiments.

Supplemental Figure 5: PGN-triggered expression of the PAC1 antigen on platelets is sensitive to the Syk inhibitor, piceatannol.

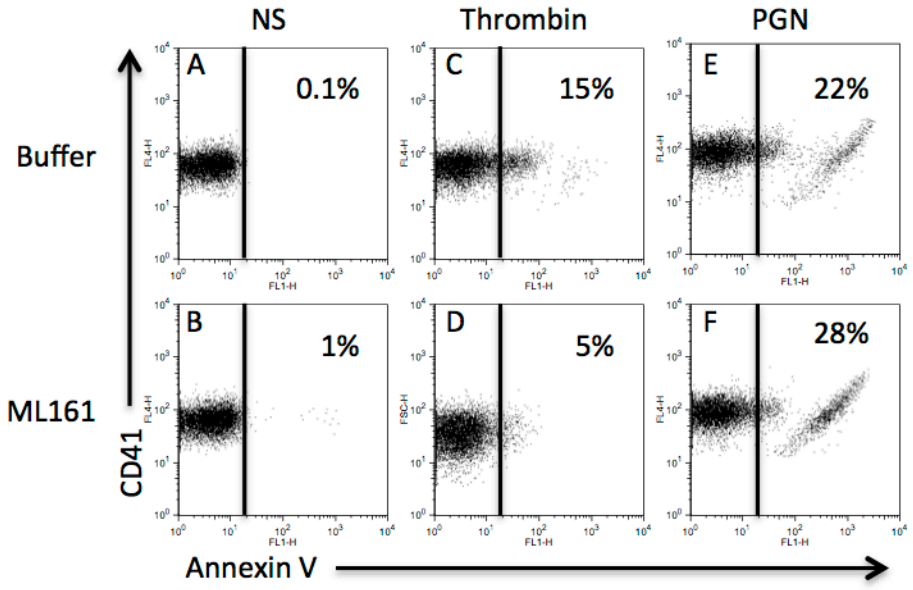
A. Platelets were pretreated with indicated amount of piceatannol for 30 mins at room temperature before stimulation with PGN (10 μg/mL) in the presence of 10% v/v human plasma for 15 mins at 37°C. After staining and fixation, staining with the PAC-1 antibody was measured by flow cytometry. The results are representative of three independent trials. B. Platelet-rich plasma was pretreated with tirofiban (1μM) and stimulated at 37°C for 10 mins with Na_3VO_4 or PGN (10 μg/ml) pre-opsonized with plasma. Platelets were lysed with RIPA lysis buffer, separated on SDS-PAGE and transferred to nitrocellulose filter. The filter was probed with antibodies to a tyrosine-phosphorylated peptide within human Syk.



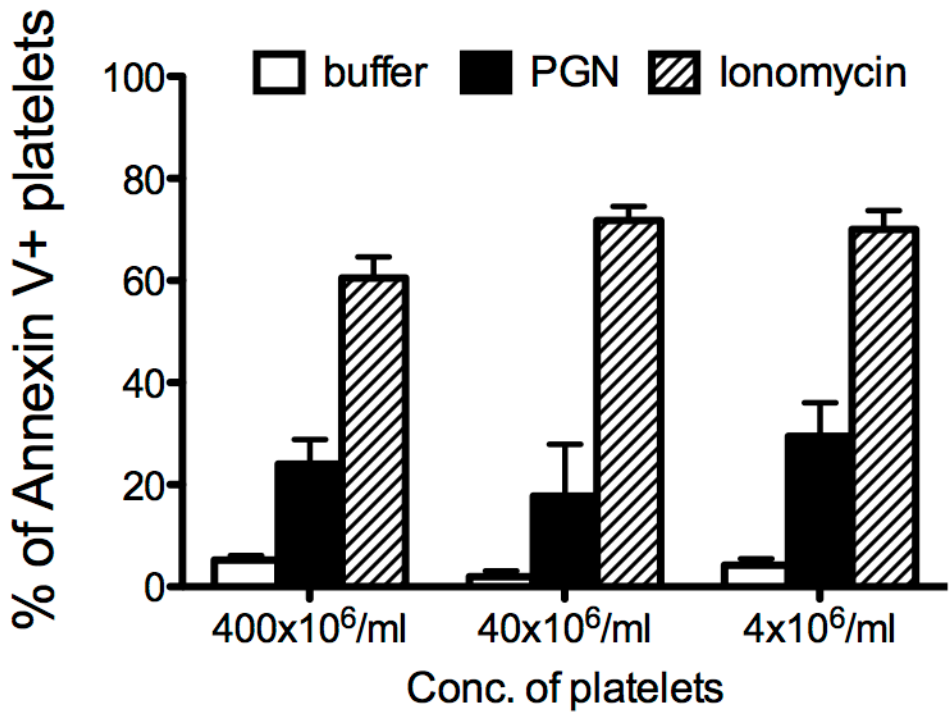
Supplemental Figure 1



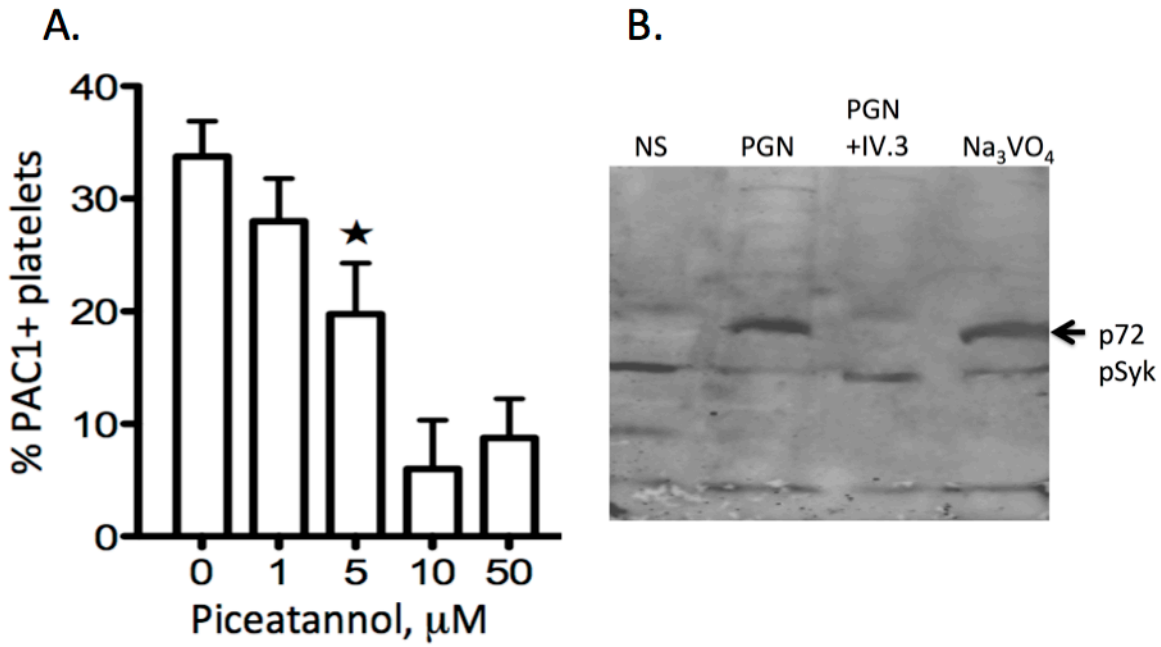
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5