

Electronic Supplementary Material

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Key role of integrin $\alpha_{IIb}\beta_3$ signaling to Syk kinase in tissue factor-induced thrombin generation

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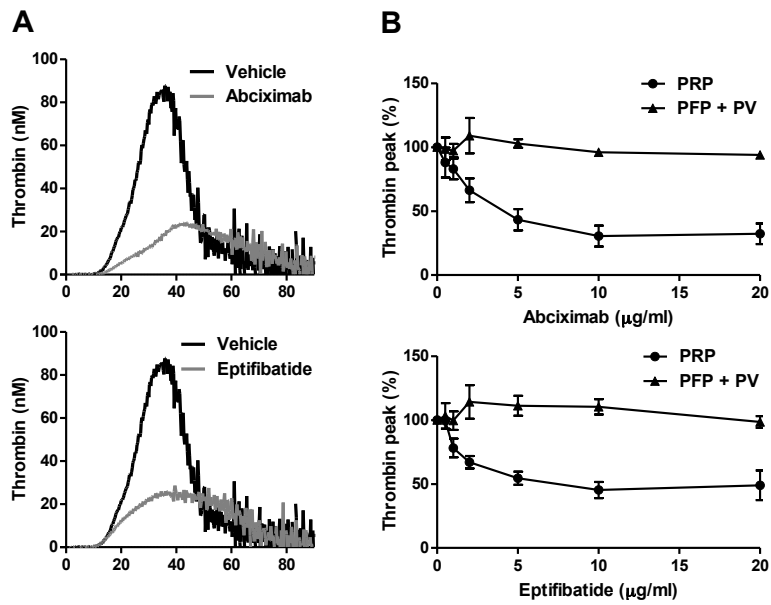


Fig. 1 Blocking of $\alpha_{IIb}\beta_3$ suppresses tissue factor-triggered thrombin generation in the presence of platelets. Human PRP (1.0×10^8 platelets/mL) or PFP supplemented with phospholipid vesicles (PV, 10 μ M) was preincubated with vehicle, abciximab (0.5-20 μ g/mL) or eptifibatide (0.5-20 μ g/mL) for 20 minutes. Thrombin generation was stimulated with tissue factor (1 pM) and CaCl_2 . **a** Representative thrombin generation curves with PRP, showing comparable effects of all integrin blockers. **b** Dose-dependent inhibition of thrombin generation only in the presence of platelets. Means \pm SEM ($n=3-9$)

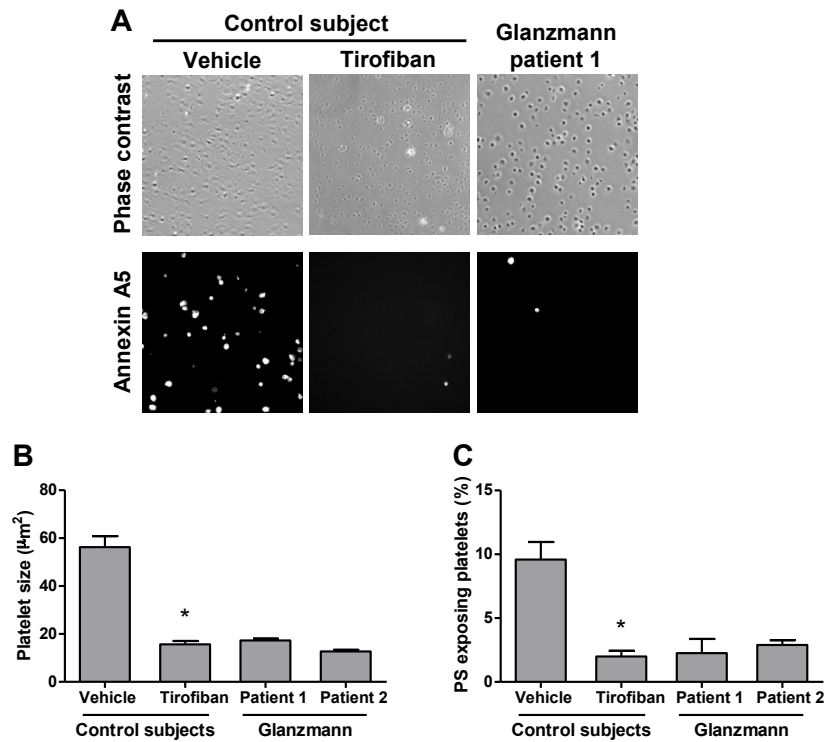


Fig. 2 Impaired spreading and thrombin activation of Glanzmann platelets. Platelets from healthy control subjects and Glanzmann patients were adhered to fibrinogen in the absence (control) or presence of tirofiban (1 $\mu\text{g}/\text{mL}$) for 30 minutes, and then stimulated with thrombin (10 nM) for another 10 minutes. **a** Representative phase contrast (120 \times 120 μm) and FITC-annexin A5 fluorescence (150 \times 150 μm) images after thrombin activation. **b** Averaged platelet size; **c** fractions of PS-exposing platelets after activation. Means \pm SEM ($n=3-6$); * $p<0.05$ vs. vehicle

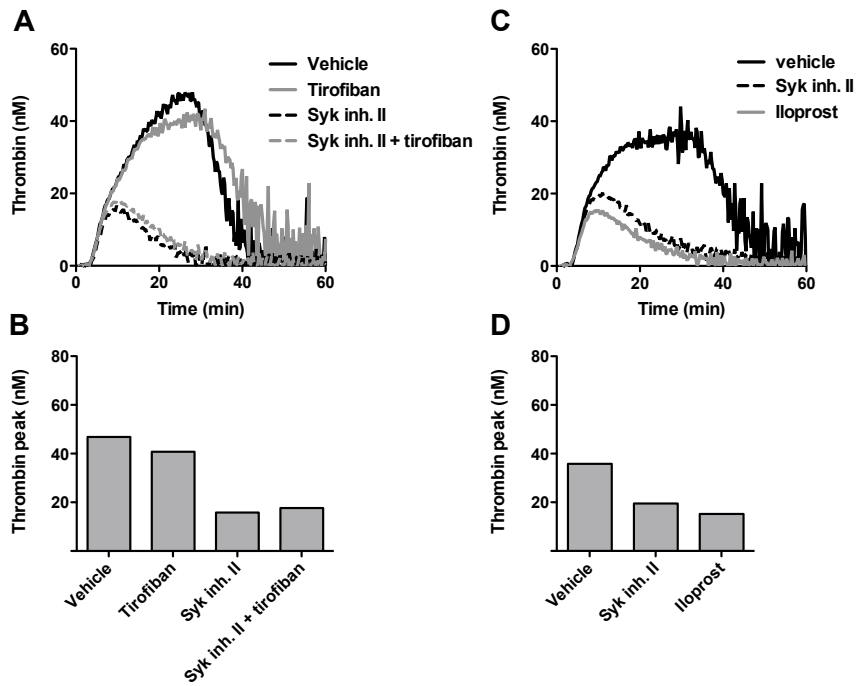


Fig. 3 Syk kinase is responsible for thrombin generation in tissue factor-stimulated PRP of a Glanzmann patient. PRP was incubated with vehicle, Syk inhibitor II (10 μ M), iloprost (1 μ M) and/or tirofiban (1 μ g/mL), and then stimulated with tissue factor (1 pM) and CaCl_2 . Cangrelor (AR-C, 10 μ M) was present to eliminate P2Y_{12} -dependent signaling events. **a, b** Effect of inhibitors on tissue factor-induced thrombin generation in PRP from blood samples collected on citrate, and **c, d** on citrate and tirofiban (1 μ g/mL). Representative thrombin generation curves and mean thrombin peak heights of triplicate measurements are shown.