Supplementary Material

Supplementary Fig. S1 The direct thrombin inhibitors Refludan and dabigatran inhibit thrombin-induced platelet aggregation. Washed human platelets ($3 \times 10^8$/ml) were pre-incubated with Refludan (200 µg/mL) or Dabigatran (500 nM) for 4 minutes at room temperature and after 12 seconds α-thrombin was added. (A) Representative aggregation curves when 0.1 U/mL thrombin (thr) was added and (B) corresponding quantitative analysis from three independent experiments with three different donors ($n = 3$) at $t = 600$ seconds. (C) Representative aggregation curves of the effect of different dabigatran concentrations when 2 U/mL α-thrombin was used according to Wienen et al. Data are shown as mean ± standard deviation (SD); ***$p < 0.0001$. 

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Supplementary Fig. S2 The direct thrombin inhibitors lepirudin/Refludan, dabigatran and D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) do not affect ristocetin/von Willebrand factor (vWF)-mediated agglutination of human washed platelets (WP). (A, B) Human washed platelets (according to Gambaryan et al and Navdaev et al) (3 × 10⁸/mL) were pre-incubated with Aggrastat (1.25 µg/mL) for 1 minute at room temperature and then treated with vWF (15 µg/mL) plus ristocetin (1 mg/ml; vWF/R). Pre-incubation of washed platelets with echicetin monomer (25 µg/mL, EM) for 4 minutes at room temperature served as a control for competitive blocking of vWF binding to glycoprotein (GP)Ibα through EM. Representative curves of ristocetin/vWF-induced light transmission/platelet agglutination in the presence of (C) lepirudin/Refludan, (D) dabigatran and (E) PPACK. (F) Quantitative analysis of (C–E) including three independent experiments from three different donors at t = 600 seconds (n = 3). Data are shown as mean ± standard deviation (SD); n.s., not significant.
Supplementary Fig. S3 The direct thrombin inhibitors lepirudin/Refudan, dabigatran and D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) do not affect ristocetin-induced binding of von Willebrand factor (vWF) to human platelets. Diluted platelet-rich plasma (PRP) was pre-incubated with Refudan, dabigatran, PPACK and vehicle control for 4 minutes at room temperature and treated with ristocetin (1 mg/mL) for 6 minutes at room temperature. Binding of autologous vWF to human platelets was detected by flow cytometry after labelling formaldehyde-fixed platelets with anti-vWF-fluorescein isothiocyanate (FITC) antibody. (A) Representative flow cytometry histograms demonstrate fluorescence intensity of anti-vWF-FITC antibody-labelled platelets without ristocetin treatment (basal) and with ristocetin treatment in the presence of Refudan, dabigatran, PPACK and vehicle control (dimethyl sulfoxide [DMSO]), respectively. (B) Quantitative analysis of (A) including three independent experiments from three different donors (n = 3). Data are shown as mean ± standard deviation (SD); n.s., not significant; **p < 0.01.
Supplementary Fig. S4  Echicetin beads induce aggregation of mouse platelets through glycoprotein (GP)Iba. Washed platelets (WP, $3 \times 10^8$ platelets/mL) of interleukin (IL)4R/GPibα-transgenic- and C57BL6/J (BL6)-wild-type mice were treated with (A, B) 0.25 U/mL von Willebrand factor (vWF) and 0.25 mg/mL botrocetin or (C, D) echicetin beads (0.15 mg/mL; EB) in the aggregometer. (B) Quantitative analysis of (A) including three independent experiments at $t = 300$ seconds ($n = 3$). (D) Quantitative analysis of (C) including three independent experiments at $t = 600$ seconds ($n = 3$). Data are shown as mean ± standard deviation (SD); **** $p < 0.0001$, *** $p < 0.01$. 

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