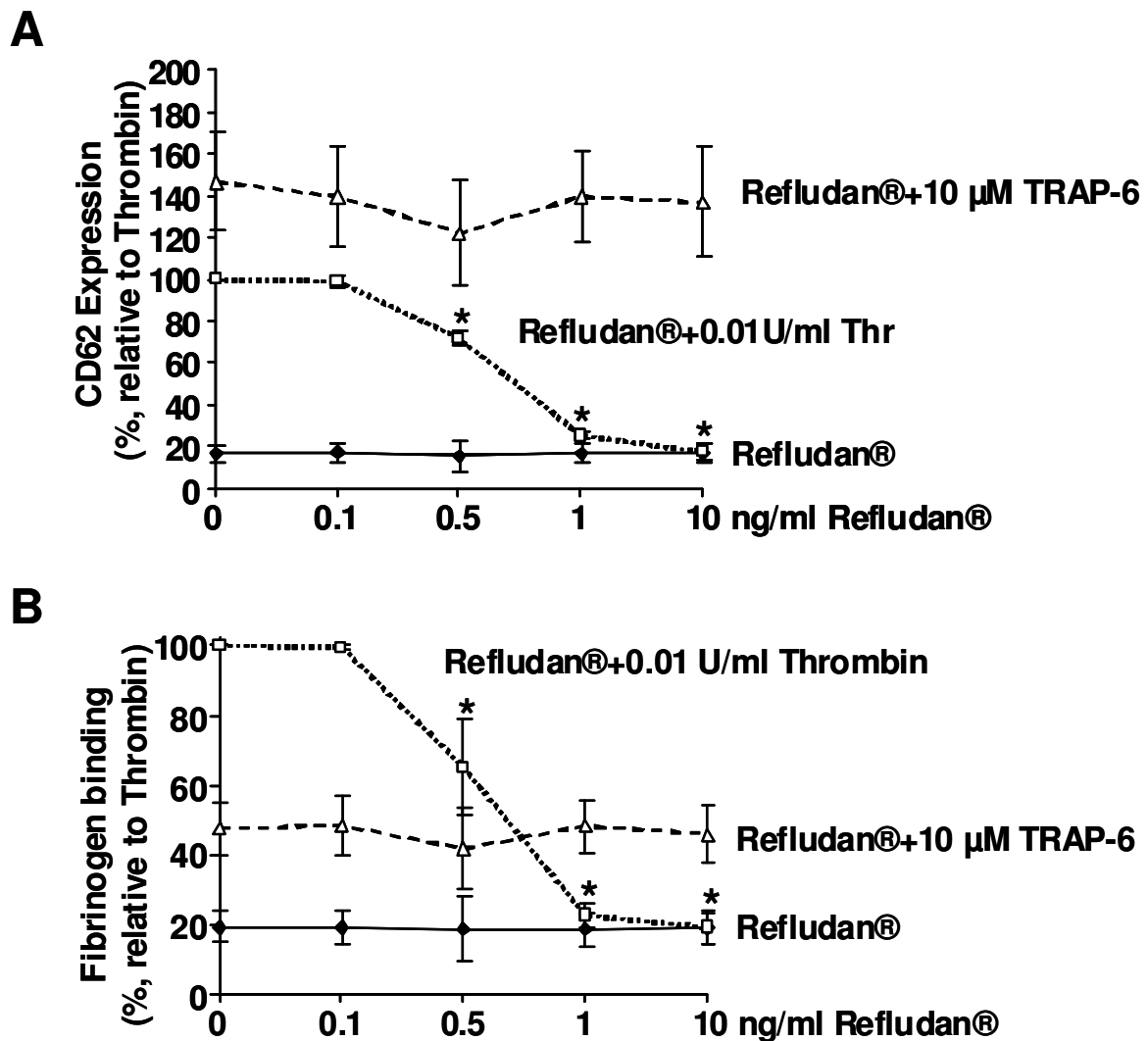


**Supplementary Figures to Kobsar, Koessler et al. “The thrombin inhibitors, hirudin und Refludan®, activate the soluble guanylyl cyclase and the cGMP pathway in washed human platelets” (Thromb Haemost 2012; 107.3)**

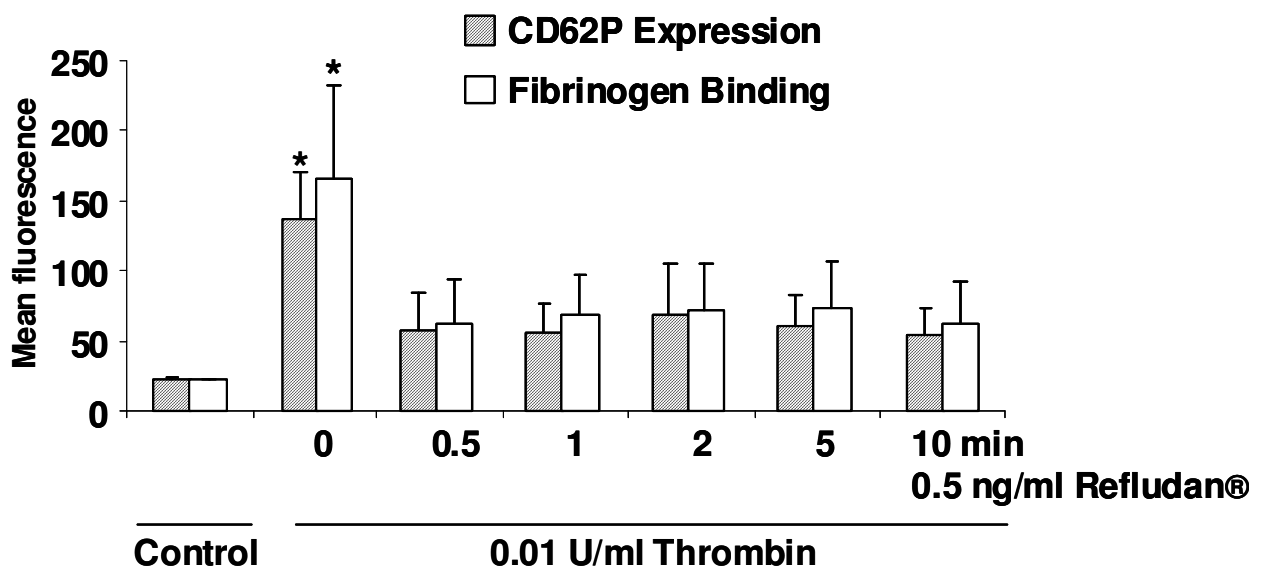
Suppl. Figure 1



Refludan® reduces thrombin-, but not TRAP-6 induced CD62P-activation and fibrinogen-binding dose-dependently. Washed human platelets ( $3 \times 10^8$ /ml) were mixed with 3 μg/ml mouse anti-CD62-FITC antibodies (A) and 10 μg/ml fibrinogen-Alexa Fluor 647 (B) and then incubated for 10 min with different concentrations of

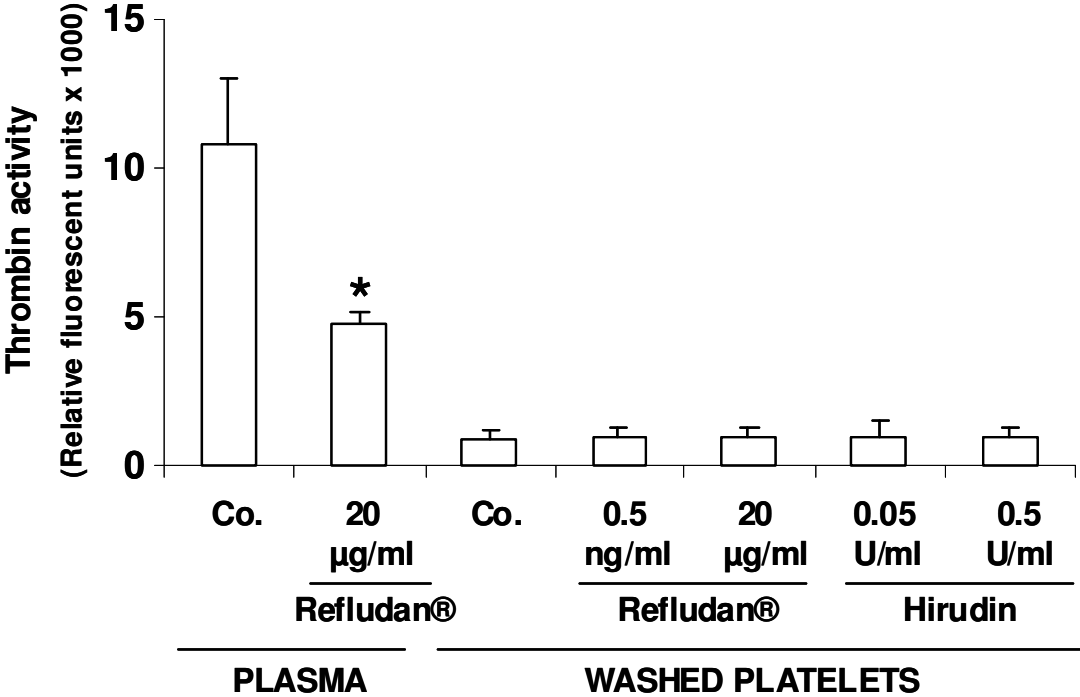
Refludan® as indicated, followed by 2 min stimulation with 0.01 U/ml Thrombin or 10 µM TRAP-6. Analysis was done by flow cytometry. Results are presented in % relative to thrombin induced stimulation (taken as 100%) in the absence of Refludan® (mean±SEM; n=5; \*: p < 0.05 compared to no Refludan®).

Suppl. Figure 2



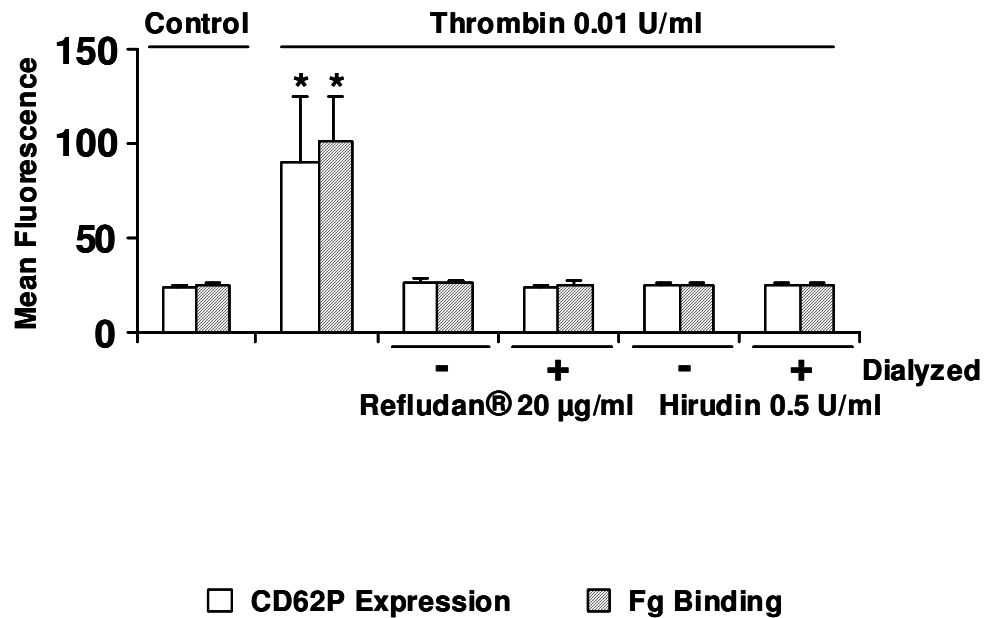
**Time curve of Refludan®-induced inhibition of Thrombin-stimulated CD62P-activation and fibrinogen-binding in human washed platelets.** Washed human platelets ( $3 \times 10^8$ /ml) were mixed with 3 µg/ml mouse anti-CD62-FITC antibodies (A) and 10 µg/ml fibrinogen-Alexa Fluor 647 (B) and then incubated for the time as indicated with 0.5 ng/ml Refludan®, followed by 2 min stimulation with 0.01 U/ml Thrombin. Analysis was done by flow cytometry, the results are presented as MFI values (mean ± SEM; n=5; \*: p < 0.05 compared to no Refludan®).

Suppl. Figure 3



**Washed human platelets do not contain any intrinsic thrombin activity.** Blood plasma and washed human platelets ( $3 \times 10^8$ /ml) were incubated for 10 min with buffer or indicated concentrations of thrombin inhibitors before the measurement of thrombin activity. Results are presented as relative fluorescent units (mean±SEM; n=10; \*: p <0.05 compared to control).

Suppl. Figure 4



**Dialysis does not influence the potential of Refludan® and hirudin to inhibit CD62P-activation and fibrinogen-binding in Thrombin-stimulated human platelets.** Washed human platelets ( $3 \times 10^8$ /ml) were mixed with 3 µg/ml mouse anti-CD62-FITC antibodies and 10 µg/ml fibrinogen-Alexa Fluor 647 and then incubated for 10 min with different concentrations of undialyzed or dialyzed Refludan® and hirudin as indicated, followed by 2 min stimulation with 0.01 U/ml Thrombin. Analysis was done by flow cytometry. Results are presented as MFI values (mean±SEM: n=3; \*: p < 0.05 compared to control).