Supplemental Material to Bertling et al. “Candida albicans and its metabolite gliotoxin inhibit platelet function via interaction with thiols” (Thromb Haemost 2010; 104.2)

Legends:

**Suppl. Figure 1. Inhibition of fibrinogen binding to platelets by gliotoxin.** FACS-analysis of the concentration-dependent effect of gliotoxin on fibrinogen-FITC binding to platelets. In the presence of the FITC-coupled fibrinogen, platelets in PRP were preincubated with gliotoxin for 30 minutes at room temperature. Afterwards, platelets were stimulated by (A) ADP, (B) collagen, (C) thrombin or (D) TRAP for 5 minutes. The total fluorescence of platelet bound fibrinogen-FITC fluorescence was expressed as the percentage of the fluorescence in the absence of the mycotoxin, defined as 100 %. Data are mean ± SD from 3 different independent experiments (* P<0.05, ** P<0.01, *** P<0.005). Histograms were created using WinMDI 2.9.

**Suppl. Figure 2. Inhibition of PAC-1 binding to platelets by gliotoxin.** FACS-analysis of the concentration-dependent effect of gliotoxin on PAC-1-FITC binding to platelets. Platelets in PRP were preincubated with gliotoxin for 30 minutes at room temperature. Afterwards, platelets were stimulated by (A) ADP, (B) collagen, (C) thrombin or (D) TRAP for 5 minutes. After 30 minutes of incubation with PAC-1-FITC fluorescence was measured by FACS. The total fluorescence of platelet bound PAC-1-FITC was expressed as the percentage of the fluorescence in the absence of the mycotoxin, defined as 100 %. Data are mean ± SD from 3 different independent experiments (* P<0.05, ** P<0.01, *** P<0.005). Histograms were created using WinMDI 2.9.

**Suppl. Figure 3. Inhibition of agonist induced platelet aggregation by gliotoxin.** PRP was preincubated with gliotoxin for 30 minutes at room temperature and aggregation induced by
(A) ADP, (B) collagen, (C) thrombin and (D) TRAP was assessed at 37 °C using light transmission aggregometry.

**Suppl. Figure 4. Inhibition of coagulation factor VIII binding to platelets by gliotoxin.**
FACS-analysis of the concentration-dependent effect of gliotoxin on coagulation factor VIII-FITC binding to platelets. Gel-filtered platelets were preincubated with gliotoxin for 30 minutes at room temperature. Afterwards, platelets were stimulated by thrombin plus collagen for 20 minutes. After addition of factor VIII-FITC and incubation time of 15 minutes, fluorescence was measured by FACS. The total fluorescence of platelet bound factor VIII-FITC was expressed as the percentage of the fluorescence in the absence of the mycotoxin, defined as 100%. Data are mean ± SD from 3 different independent experiments (* P<0.05, ** P<0.01, *** P<0.005). Histograms were created using WinMDI 2.9.

**Suppl. Figure 5. Agonist induced platelet CD62P expression is not affected by gliotoxin.**
FACS-analysis of the concentration-dependent effect of gliotoxin on thrombin induced CD62P expression on the platelet surface. Platelets in PRP were preincubated with gliotoxin for 30 minutes at room temperature. Afterwards, platelets were stimulated by thrombin for 5 minutes. After incubation with monoclonal FITC-conjugated anti-CD62P antibody, fluorescence was measured by FACS. The total fluorescence of platelet bound anti-CD62P-FITC was expressed as the percentage of the fluorescence in the absence of the mycotoxin, defined as 100%. Data are mean ± SD from 3 different independent experiments (n.s. not significant). Histograms were created using WinMDI 2.9.
suppl. figure 1
suppl. figure 2
suppl. figure 3
**Suppl. Figure 4**

**0.5 U/ml thrombin + 1 µg/ml collagen**

The diagram illustrates the residual binding of factor VIII-FITC against gliotoxin concentration. The x-axis represents the gliotoxin concentration in µM, while the y-axis shows the residual binding in %.

- **0 U/ml thrombin; 0 µg/ml collagen**
- **0.5 U/ml thrombin; 1 µg/ml collagen**

Significance levels are indicated as:
- **p < 0.01 (**)**
- **p < 0.001 (***)**

The data points and error bars are shown to reflect the variability in the measurements.
suppl. figure 5