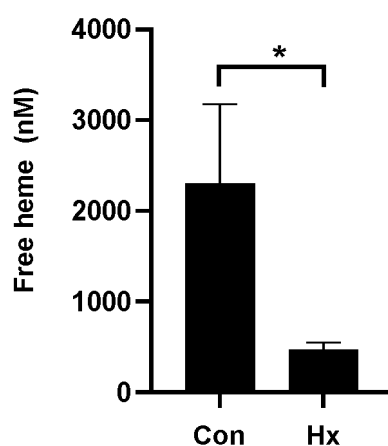


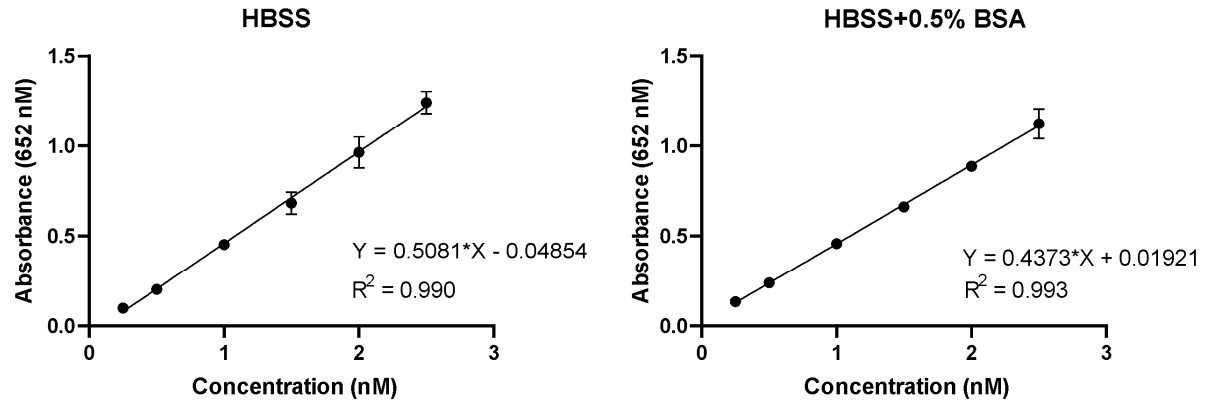
## Determination of free heme in stored red blood cells with an apo-horseradish peroxidase-based assay

Vijith Vijayan, Robert Greite, Sebastian Schott, Julian Doricic, Kuku Madyaningrana, Pooja Pradhan, Jörg Martens, Rainer Blasczyk, Sabina Janciauskiene and Stephan Immenschuh

### Supplementary material



**Supplementary Figure 1:** Specificity of apo-HRP based heme assay for free heme. Supernatants from 5 different RBC units collected at day 42 was incubated with or without Hx (3  $\mu$ M) for 30 min and used for the apo-HRP based heme assay.



**Supplementary Figure 2:** Standardization of the apoHRP heme assay.

Hemin standard concentrations (0.25 to 2.5 nM) prepared in HBSS or HBSS+0.5% BSA were plotted against the respective absorbance values corresponding to the HRP activity obtained after reconstitution of apoHRP to holoHRP at 652 nm. A representative plot with the absorbance values  $\pm$  SD from two independent assays is shown.