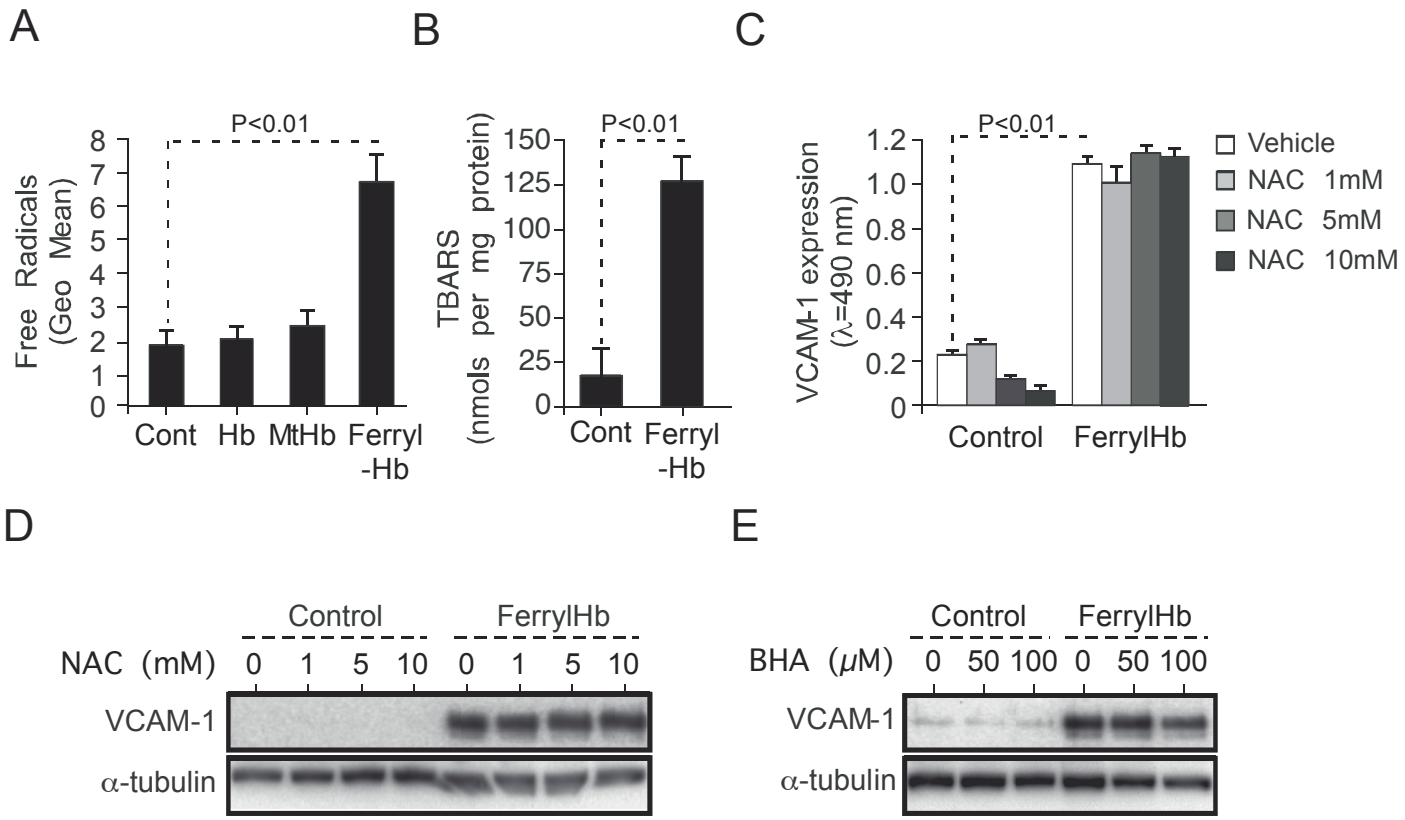
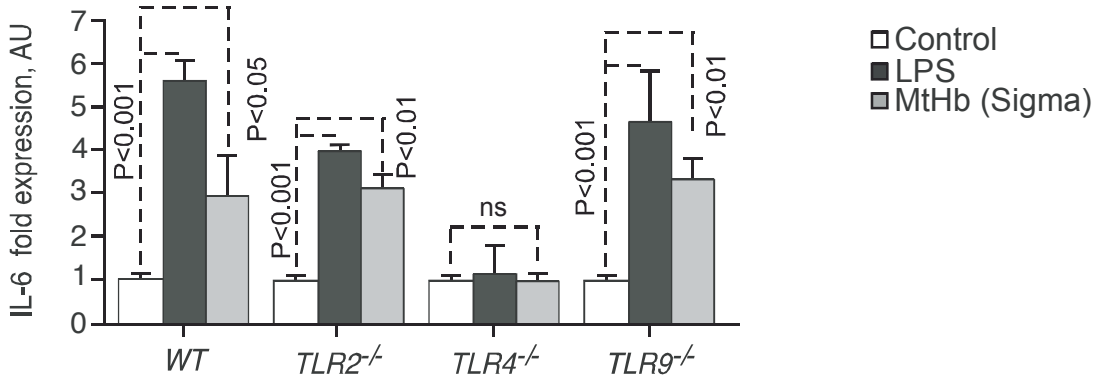
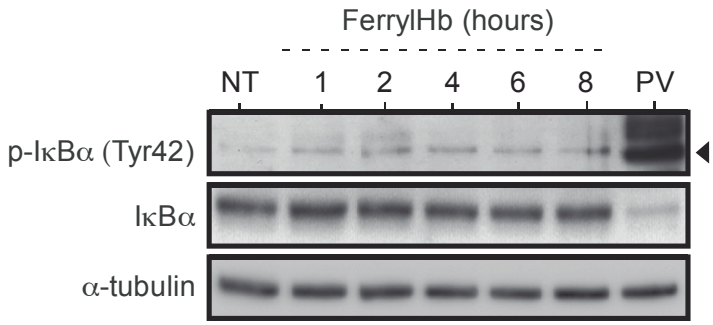

A
B
C
P<0.01
Free Radicals (Geo Mean)
Cont Hb MtHb Ferryl -Hb
TBARS (nmols per mg protein)
Cont Ferryl -Hb
VCAM-1 expression (λ=490 nm)
Control FerrylHb
Vehicle
NAC 1mM
NAC 5mM
NAC 10mM
D
E
Control
FerrylHb
NAC (mM) 0 1 5 10 0 1 5 10
BHA (μM) 0 50 100 0 50 100
VCAM-1
α-tubulin

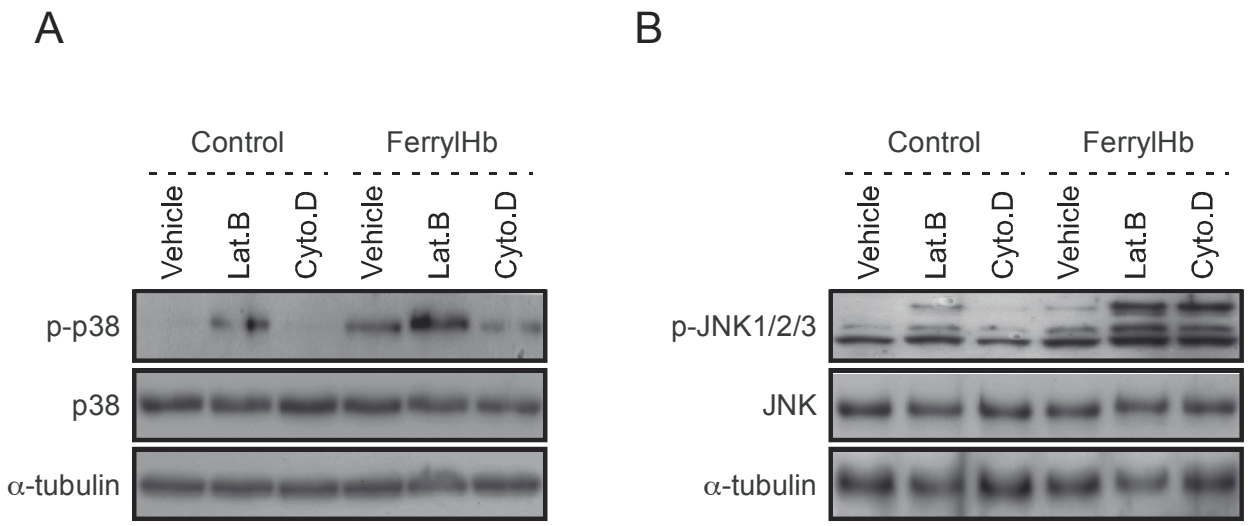
G.Silva et al. Supplementary Figure 2



G.Silva et al. Supplementary Figure 3



G.Silva et al. Supplementary Figure 4



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1: The pro-inflammatory effect of FerrylHb acts via a mechanism distinct from its pro-oxidant effect. A) Confluent HUVEC were either not treated or exposed (4h) to Hb, MtHb, or FerrylHb (20 μM). B) Confluent HUVEC were either not treated or exposed to FerrylHb (20 μM; 8h). C) Confluent HUVEC were exposed to N-acetyl-L-cysteine (NAC; 4h) (Sigma-Aldrich) or vehicle and not further treated (Control) or exposed (8h) to FerrylHb (20 μM). Expression of VCAM-1 was detected by cellular ELISA. Results in (A-C) are the mean values ± standard deviation (n=3), from one out of three independent experiments. P values were calculated with ANOVA and the Tukey-Kramer multiple comparison test. D) HUVEC were treated as in (C) and proteins were detected in whole cell lysates by Western blotting. E) HUVEC were exposed to butylated hydroxyanisole (BHA; 4h; Sigma-Aldrich) or vehicle (0) and not further treated (Control) or exposed (8h) to FerrylHb (20 μM) and proteins were detected in whole cell lysates by Western blotting. Immunoblots in (D) and (E) are representative of at least two independent experiments.

Supplementary Fig. 2: The pro-inflammatory effect of Sigma MtHb is abrogated in *TLR4* deficient cells. Primary kidney fibroblasts isolated from *C57BL/6*, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$ and *Tlr9*$^{-/-}$ mice were either untreated (control), exposed (24h) to MtHb (2.6 μM, Sigma-Aldrich) or to LPS (100 ng/ml). IL-6 concentrations were measured in the cell culture supernatants by ELISA. Results are shown as mean ± standard deviations (n=3), from one representative experiment out of three. P values were calculated using ANOVA and the Tukey-Kramer multiple comparison test.

Supplementary Fig. 3. FerrylHb induces low level of Tyr 42 IκBα phosphorylation in EC. Confluent HUVEC were either not-treated (NT), exposed to FerrylHb (20 μM) or to pervanadate (PV; 1mM; 30 min), used as a positive control to induce IκBα phosphorylation at Tyr-42. Total IκBα P-Tyr-42 IκBα and α-tubulin proteins were detected by western blotting.

Supplementary Fig. 4: Effect of actin polymerization on MAPK activation by FerrylHb. A) and B) Confluent HUVEC were exposed to latrunculin B (lat.B; 0.5 μM; 30 min), cytochalasin D (cyto.D; 0.5 μM; 30 min) or vehicle and either not further treated (control) or exposed (8h) to FerrylHb (20 μM). Proteins were detected in whole cell lysates by Western blotting. Immunoblots are representative of two independent experiments.