## SUPPLEMENTARY FIGURE LEGENDS

**Suppl. Figure 1 Usage of IL-1RA and Reparixin in Huh7 cells efficiently blocked IL-1β and MCHM-induced IL-1 and IL-8 signaling pathways. A**) IL-1RA treatment inhibits the induction of IL-1β-mediated downstream signaling protein phospho-c-Jun as well as IL-6 mRNA in Huh7 cells. B) Treatment of Huh7 cells with Reparixin completely abolished MCHM-mediated AKT induction (AKT is also known as protein kinase. IL-6 mRNA level was quantified by RT-PCR and presented as mean IL-6 mRNA normalized to β2-microglobulin ± SD.

**Suppl. Figure 2 Differentiation of monocytes into macrophages leads to transcriptional activation of ferritin and suppression of ferroportin (FPN).** PMA-mediated differentiation of THP-1 monocytes into macrophages caused a significant increase of ferritin (heavy and light chain) and suppression of FPN mRNA expression during 24 h. Transferrin receptor 1 mRNA expression remains unchanged. mRNAs were quantified by qRT-PCR and the results are represented as mean of mRNA levels normalized to β2-microglobulin ± SD.

**Suppl. Figure 3 NOX1 expression is not further induced during monocyte to macrophage differentiation.** THP-1 cells were differentiated into macrophages for 24 h using PMA and NOX1 protein expression was determined in THP-1 monocytes vs. differentiated macrophages. Densitometric analysis of NOX1 protein expression normalized β-actin ± SD from three independent experiments is shown.

**Suppl. Figure 4 Catalase significantly inhibited differentiation-induced intracellular ROS accumulation and hepcidin induction.** A) Increased intracellular ROS accumulation in THP-1 cells is inhibited by catalase during 24 h of PMA-induced differentiation. THP-1 monocytes were pre-loaded with dihydrofluorescein diacetate (DHFDA) and differentiated with PMA and exposed to catalase for 24 h. Intracellular ROS were detected by measuring the fluorescence at 540 nm and the mean fluorescence intensity is shown in relative fluorescence units (RFU) after subtraction of the control in the absence of PMA (RFU ± SD). B) Differentiation induced hepcidin mRNA expression is significantly inhibited by co-treatment with catalase. Hepcidin mRNA was quantified by quantitative real-time PCR and the results are represented as mean of hepcidin mRNA normalized to β2-microglobulin ± SD. Significant differences are marked by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

**Suppl. Figure 5 Basal hepcidin mRNA levels are 8 times higher in hepatocytes compared to macrophages.** Hepcidin mRNA levels in $1x10^5$ Huh7 cells, naïve THP-1 cells and PMA-differentiated THP-1 cells are shown. Hepcidin mRNA was quantified by quantitative real-time PCR and the results are represented as mean of hepcidin mRNA normalized to β2-microglobulin ± SD.

**Suppl. Figure 6 Hypoxia is not inducing hepcidin in macrophages.** A) Hepcidin and NOX2 mRNA expression level in PMA-differentiated THP-1 macrophages under different $O_2$ levels. THP-1 macrophages were conditioned for 24 h to 1%, 5% or 21% $O_2$ after differentiation. No significant differences were observed between normoxia and hypoxia (1% and 5% $O_2$). B) Hypoxia does not induce hepcidin in human primary monocyte-derived macrophages (MDM). NOX2 and hepcidin mRNA levels were assessed after exposure of MDM to 5% $O_2$for 24 h. mRNAs were quantified by qRT-

PCR and the results are represented as mean of mRNA levels normalized to β2-microglobulin ± SD.

**Suppl. Figure 7 Hepcidin mRNA is induced in hepatocyte monocultures, but suppressed in unphysiological high hepatocyte/macrophage co-cultures under hypoxia.** Huh7 cells were cultivated alone or with macrophage-conditioned medium for 24 h under 1% $O_2$. At unphysiologic hepatocyte/macrophage cell ratio of 1:1.6 was used. Hepcidin mRNA was quantified by quantitative real-time PCR and the results are represented as mean of hepcidin mRNA normalized to β2-microglobulin ± SD. Significant differences in relation to the respective normoxic control are marked by asterisks (**, $P < 0.01$)

**Suppl. Figure 8 NOX2 mRNA is upregulated in hepatocyte/macrophage co-cultures by hypoxia.** Huh7 cells were cultivated alone or with macrophages at 10:1 and 4:1 cell ratio under 21%, 5% and 1% $O_2$. NOX2 mRNA was quantified by qRT-PCR and the results are represented as mean of NOX2 mRNA normalized to β2-microglobulin ± SD. Significant differences are marked by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

**Suppl. Figure 9 Significant correlation between hepcidin mRNA and pSTAT3/STAT3 expression level.** Spearman correlation analysis of hepcidin mRNA expression and densitometric pSTAT3/STAT3 expression levels.