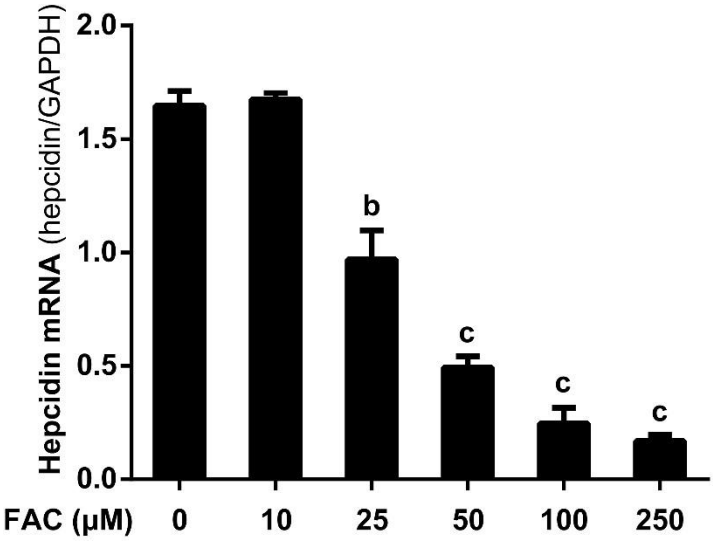
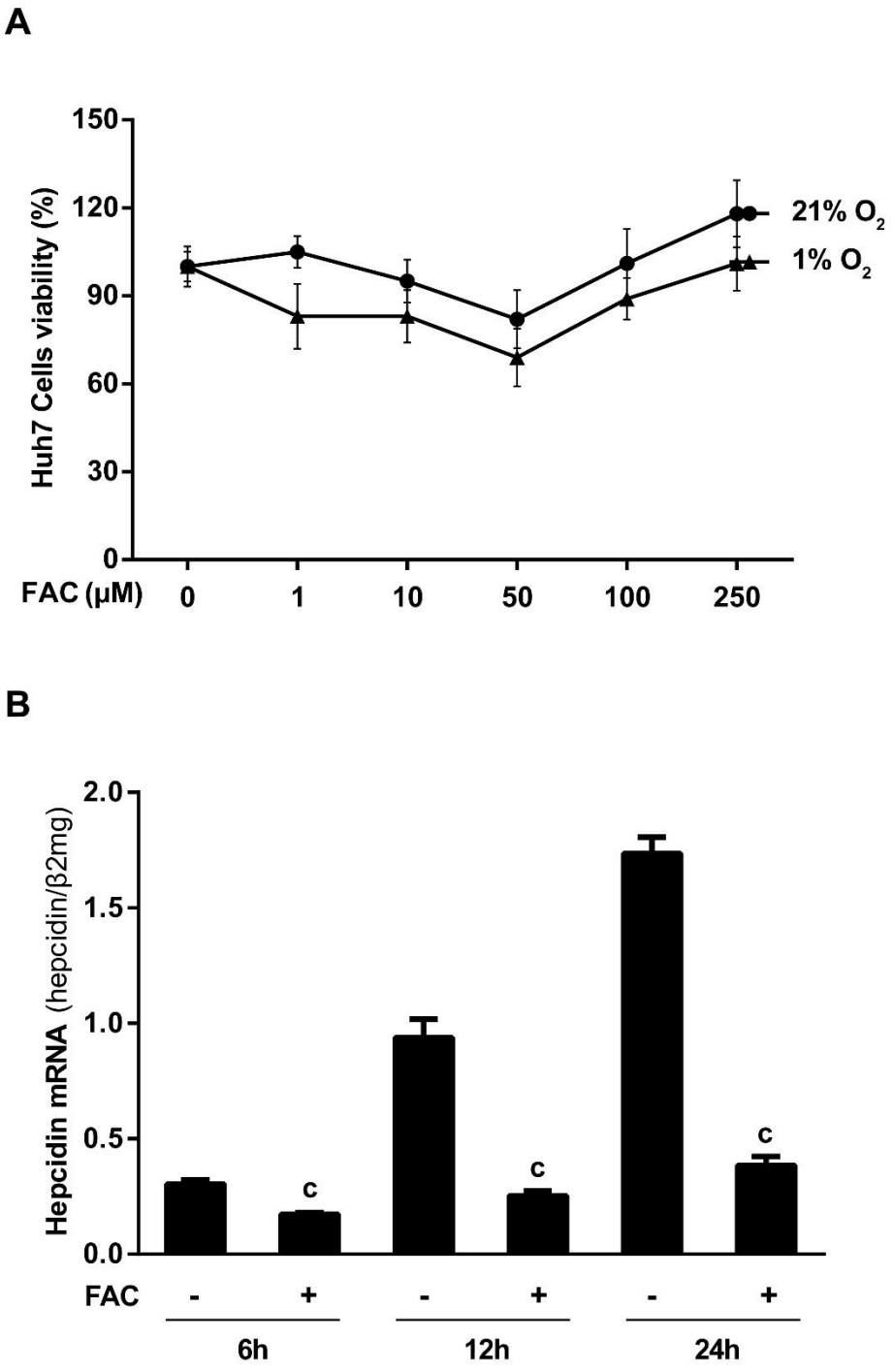
Supplementary Figures

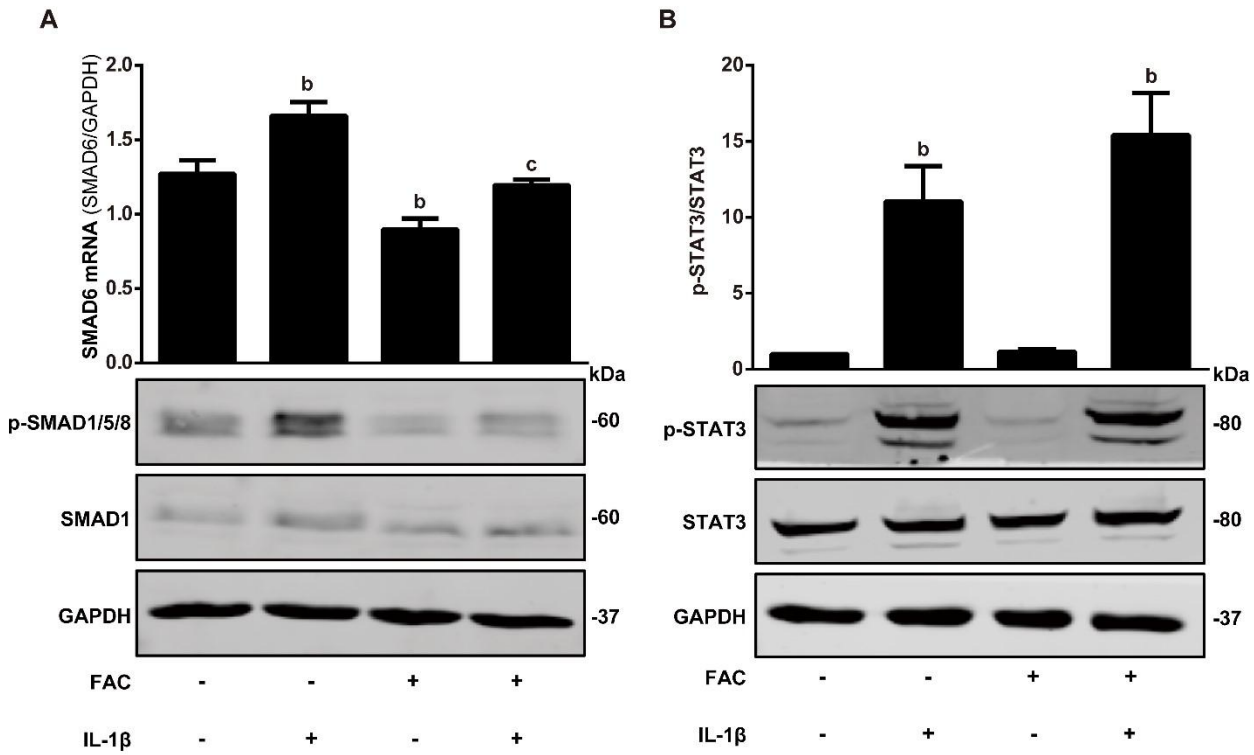


Supplementary Figure 1 The inhibiting effect of iron was observed over a wide concentration range. Huh7 cells were treated with ferric ammonium citrate (FAC) (10, 25, 50, 100 and 250 μM) for 24 h. Total RNA was extracted from Huh7. FAC inhibits hepcidin mRNA expression in Huh7 cells in a concentration-dependent manner. Hepcidin mRNA levels were determined by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are presented as mean ± SD. $^{b}P < 0.01$ *vs* control; $^{c}P < 0.001$ *vs* control.

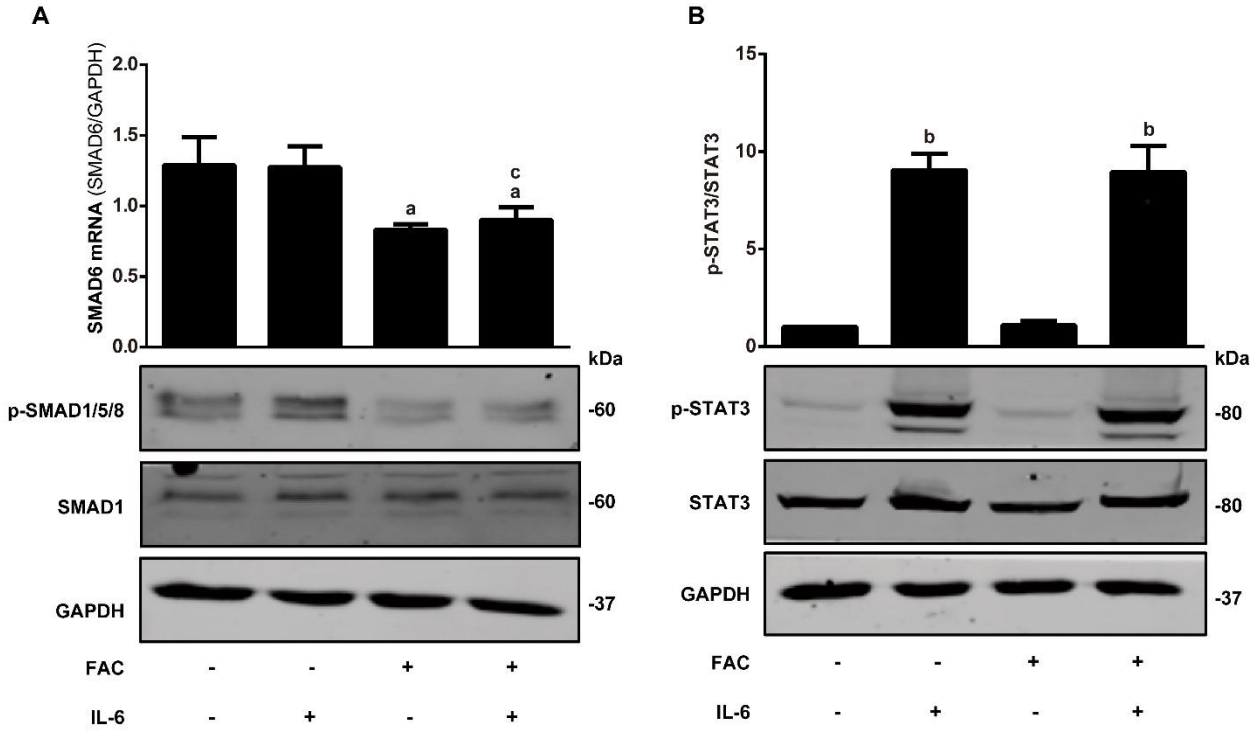


Supplementary Figure 2 Efficient suppression of hepatocellular hepcidin by higher ferric ammonium citrate levels. A: Huh7 cells were treated with ferric ammonium citrate (FAC) (1, 10, 50, 100 and 250 μM) under normoxia (21% oxygen level, 21% O_2) or hypoxia (1% oxygen level, 1% O_2) for 24 h. The viability of Huh7 cells was determined by MTT

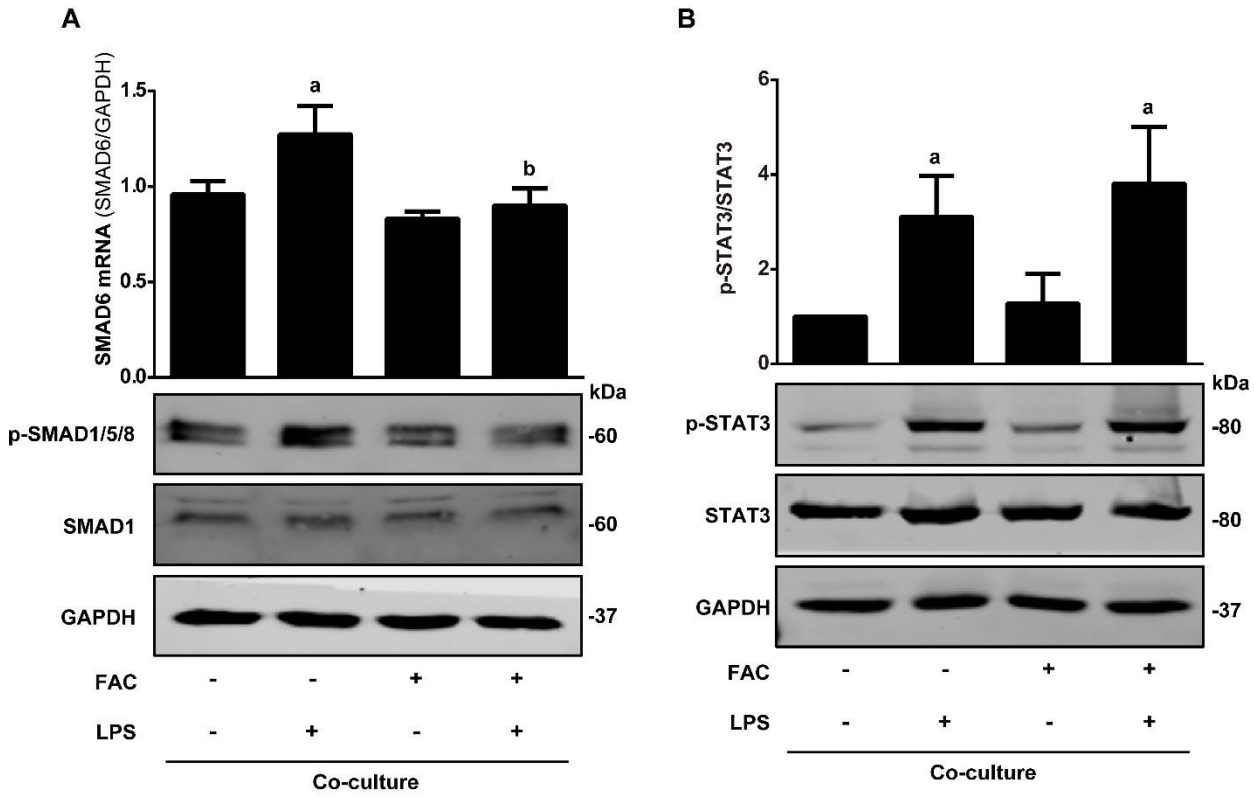
assay. Results were presented as the mean ± SD; B: Huh7 cells were treated with FAC (50 μM) for 6, 12, 24 h. Total RNA was extracted from Huh7. The response of hepcidin transcription blockage by iron has been significant starting from 6 h. Hepcidin mRNA levels were determined by qRT-PCR, normalized to β2-microglobulin (β2mg). Data are presented as mean ± SD. $^{c}P < 0.001$ *vs* control (Corresponding time point).



Supplementary Figure 3 Ferric ammonium citrate bocks the induction of small mothers against decapentaplegic signaling mediated by interleukin-1β. Huh7 cells were treated with or without interleukin (IL)-1β (10 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50 μM) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased the basal and IL-1β-induced small mothers against decapentaplegic (SMAD6) mRNA and phospho (p)-SMAD1/5/8 protein expression; B: IL-1β induced p-signal transducer and activator of transcription (STAT)3 protein expression, while FAC has no significant effect on p-STAT3 protein expression in the presence or absence of IL-1β. STAT3, p-STAT3, SMAD1, p- SMAD1/5/8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western blots are representatives of three independent experiments. Data are presented as mean ± SD. $^{b}P < 0.01$ *vs* control; $^{c}P < 0.01$ *vs* IL-1β group.



Supplementary Figure 4 Ferric ammonium citrate blocks small mothers against decapentaplegic signaling without affecting interleukin-6/signal transducer and activator of transcription 3 signaling. Huh7 cells were treated with or without IL-6 (10 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50 μM) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased small mothers against decapentaplegic (SMAD)6 mRNA and phospho (p)-SMAD1/5/8 protein expression in the presence or absence of IL-6; B: Interleukin (IL)-6 induced p-signal transducer and activator of transcription (STAT)3 protein expression, while FAC has no significant effect on p-STAT3 protein expression in the presence or absence of IL-6. STAT3, p-STAT3, SMAD1, p- SMAD1/5/8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western blots are representatives of three independent experiments. Data are presented as mean ± SD. $^{a}P < 0.05$ *vs* control; $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$ *vs* IL-6 group.



Supplementary Figure 5 Ferric ammonium citrate blocks the induction of small mothers against decapentaplegic signaling mediated by lipopolysaccharide in a macrophage-hepatocyte co-culture model. Huh7 cells were directly co-cultured with THP-1 macrophages according to pathophysiological macrophage / hepatocyte cell ratio (1:4) and then treated with or without LPS (500 ng/mL) for 24 h in the presence or absence of ferric ammonium citrate (FAC) (50 μM). Total RNA and protein were extracted from Huh7 cells and THP-1 macrophages. A: FAC decreased the basal and LPS-induced small mothers against decapentaplegic (SMAD)6 mRNA and phospho (p)-SMAD1/5/8 protein expression in co-culture; B: LPS induced p-signal transducer and activator of transcription (STAT)3 protein expression, while FAC has no significant effect on p-STAT3 protein expression in the presence or absence of LPS in co-culture. STAT3, p-STAT3, SMAD1, p- SMAD1/5/8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western blots are representatives

of three independent experiments. Data are presented as mean ± SD. [a]$P < 0.05$ *vs* control; [b]$P < 0.05$ *vs* LPS group.