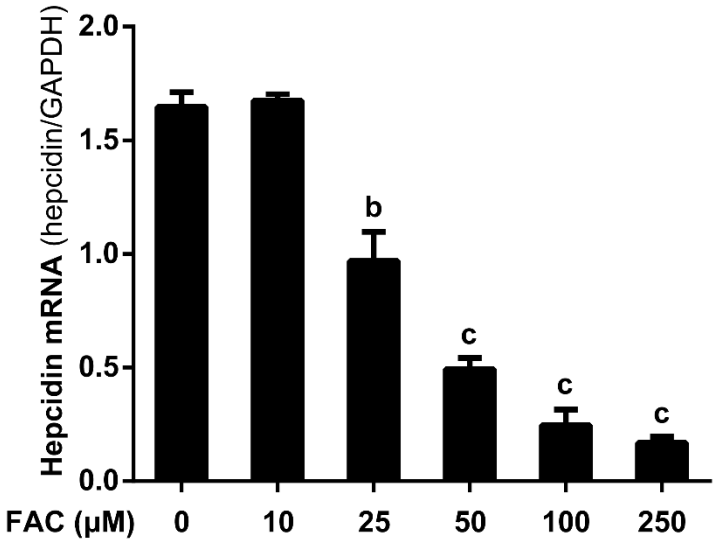
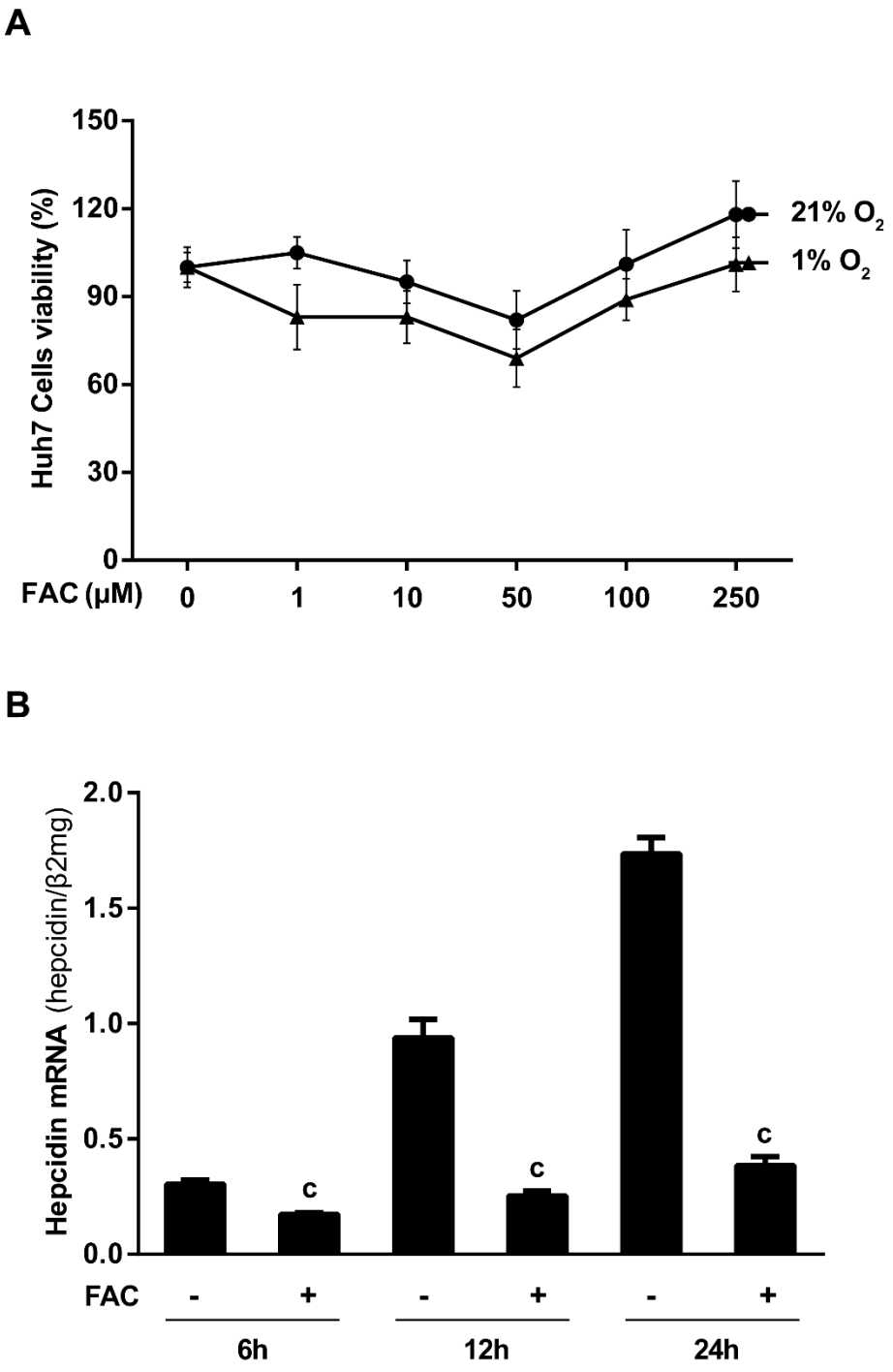
Supplementary Figures

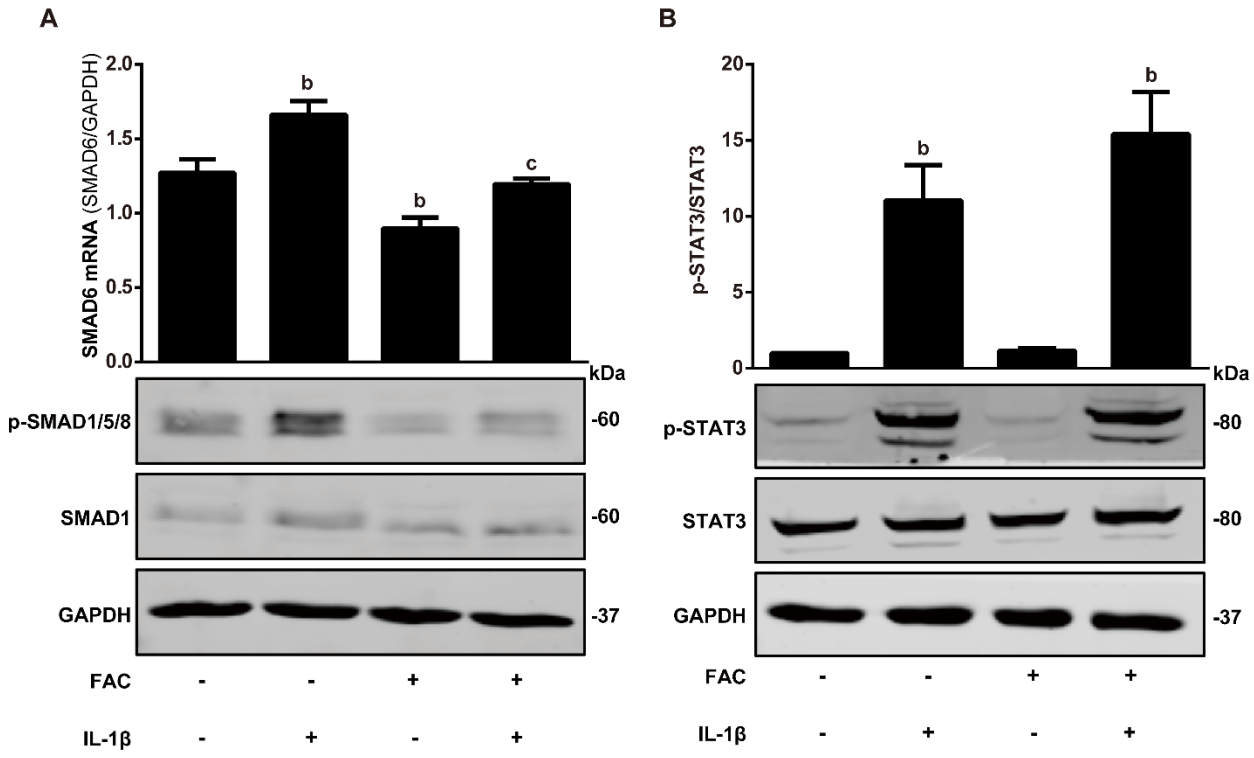


Supplementary Figure S1. The inhibiting effect of iron was observed over a wide concentration range. Huh7 cells were treated with FAC (10, 25, 50, 100 and 250 μM) for 24 h. Total RNA was extracted from Huh7. FAC inhibits hepcidin mRNA expression in Huh7 cells in a concentration-dependent manner. Hepcidin mRNA levels were determined by qRT-PCR, normalized to GAPDH. Data are presented as mean ± SD. $^{b}P < 0.01$ *vs* control; $^{c}P < 0.001$ *vs* control. FAC, Ferric ammonium citrate.

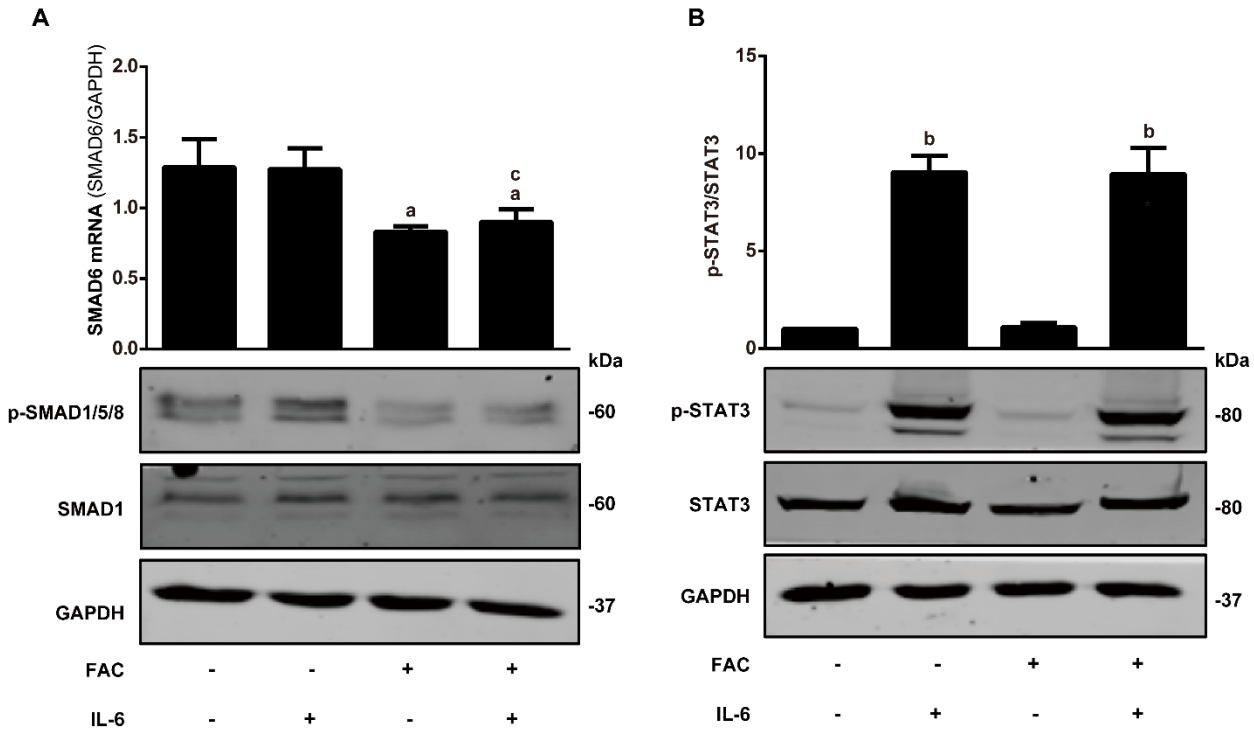


Supplementary Figure S2. Efficient suppression of hepatocellular hepcidin by higher FAC levels. A) Huh7 cells were treated with FAC (1, 10, 50, 100 and 250 μM) under normoxia (21% oxygen level, 21% O_2) or hypoxia (1% oxygen level, 1% O_2) for 24h. The viability of Huh7 cells was determined by MTT assay. Results were presented as the

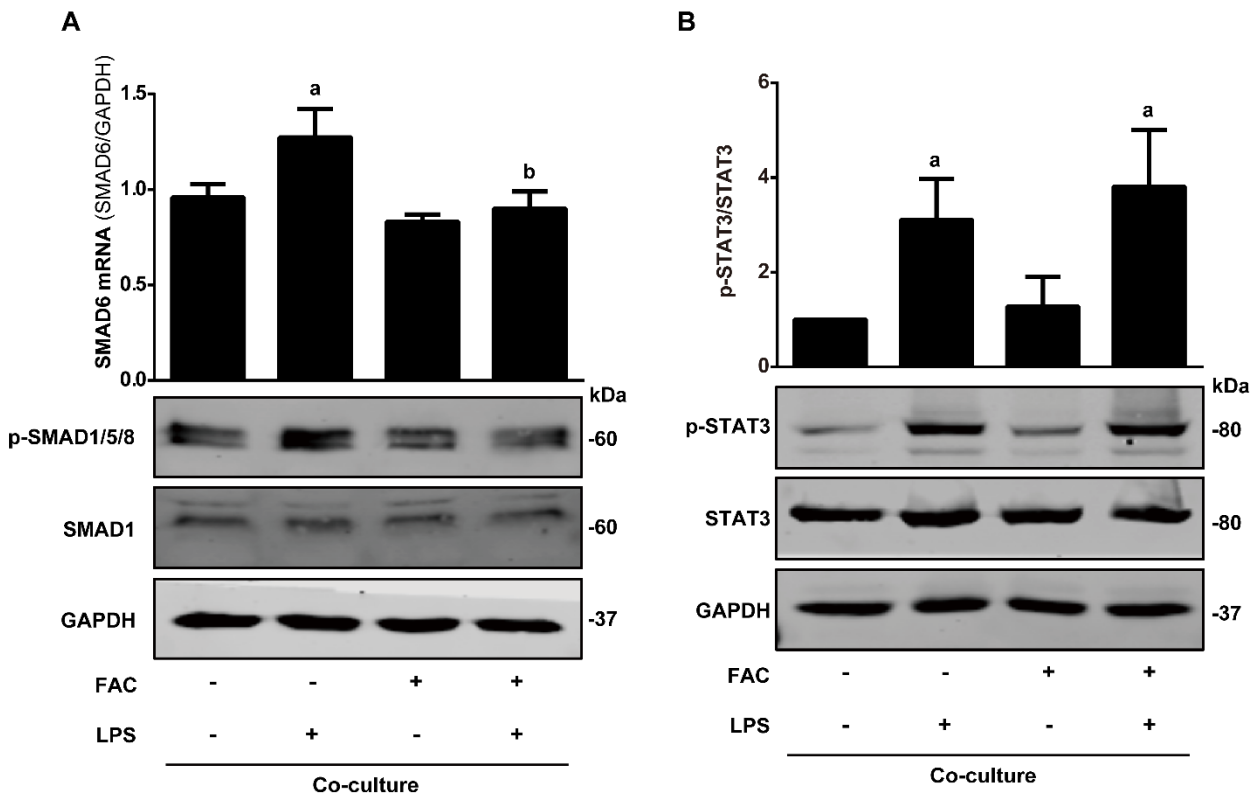
mean ± SD. B) Huh7 cells were treated with FAC (50 µM) for 6, 12, 24 h. Total RNA was extracted from Huh7. The response of hepcidin transcription blockage by iron has been significant starting from 6 h. Hepcidin mRNA levels were determined by qRT-PCR, normalized to β2mg. Data are presented as mean ±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



Supplementary Figure S3. FAC bocks the induction of SMAD signaling mediated by IL-1β. Huh7 cells were treated with or without IL-1β (10 ng/ml) in the presence or absence of FAC (50 μM) for 24h. Total RNA and protein were extracted from Huh7 cells. A) FAC decreased the basal and IL-1β-induced SMAD6 mRNA and p-SMAD1/5/8 protein expression. B) IL-1β induced p-STAT3 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 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. FAC: Ferric ammonium citrate; IL-1β: Interleukin 1β; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.



Supplementary Figure S4. FAC blocks SMAD signaling without affecting IL-6/STAT3 signaling. Huh7 cells were treated with or without IL-6 (10 ng/ml) in the presence or absence of FAC (50 µM) for 24h. Total RNA and protein were extracted from Huh7 cells. A) FAC decreased SMAD6 mRNA and p-SMAD1/5/8 protein expression in the presence or absence of IL-6. B) IL-6 induced p-STAT3 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 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. FAC: Ferric ammonium citrate; IL-6: Interleukin 6; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.



Supplementary Figure S5. FAC blocks the induction of SMAD signaling mediated by LPS 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 24h in the presence or absence of FAC (50 μM). Total RNA and protein were extracted from Huh7 cells and THP-1 macrophages. A) FAC decreased the basal and LPS-induced SMAD6 mRNA and p-SMAD1/5/8 protein expression in co-culture. B) LPS induced p-STAT3 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 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. FAC: Ferric ammonium citrate; LPS:

Lipopolysaccharide; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.