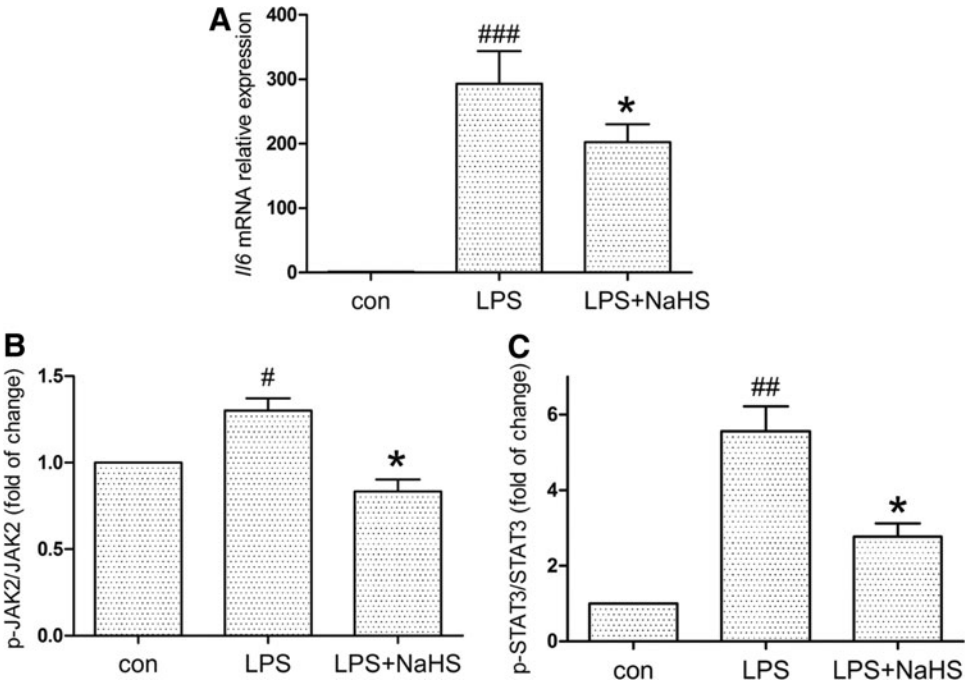
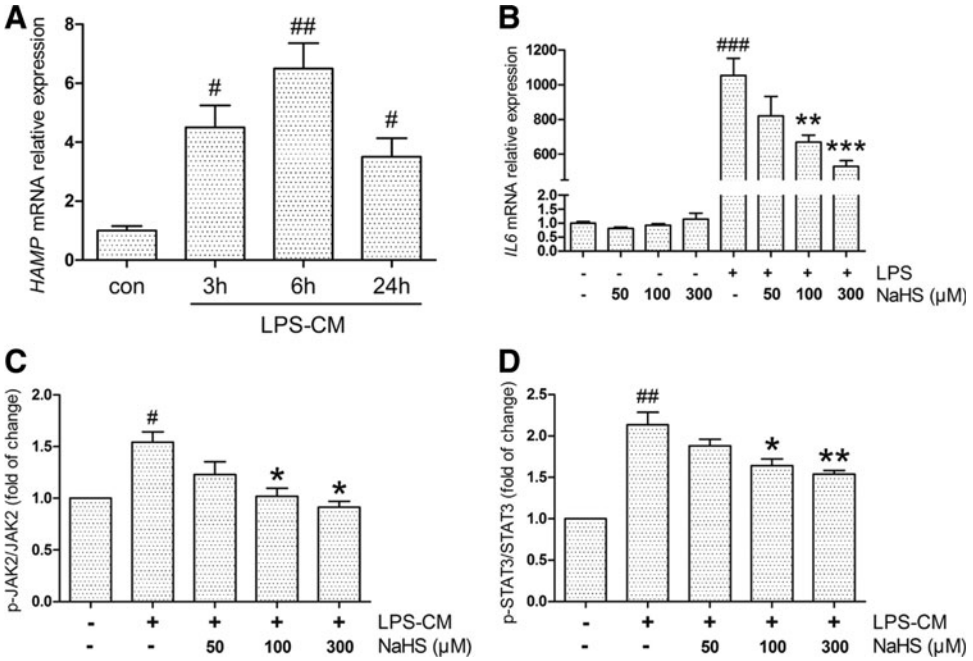
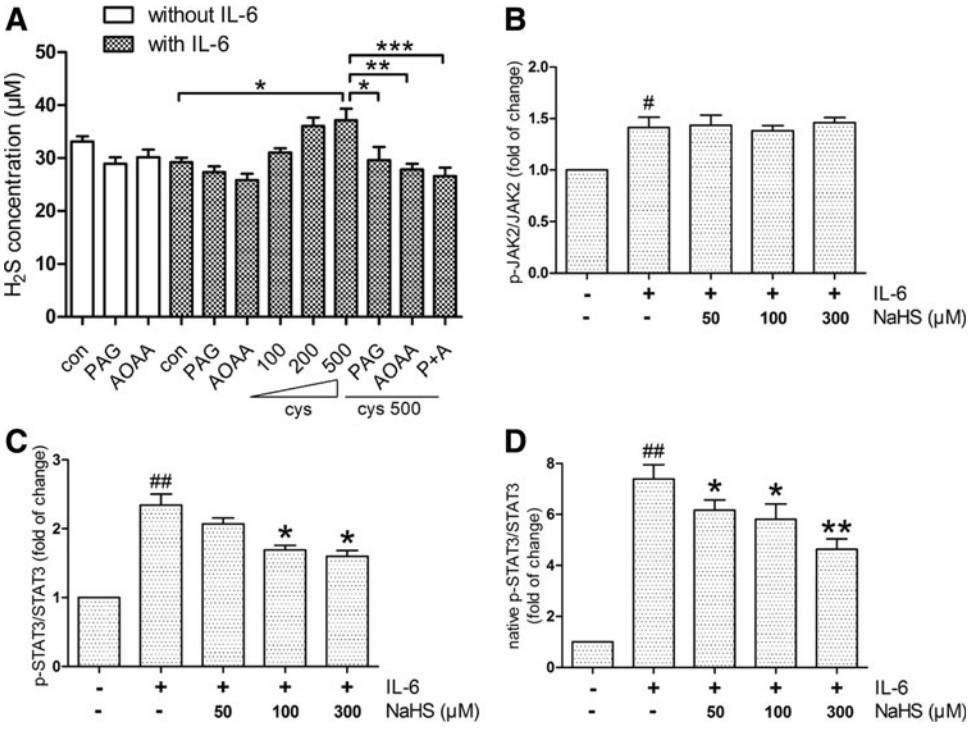
Supplementary Data



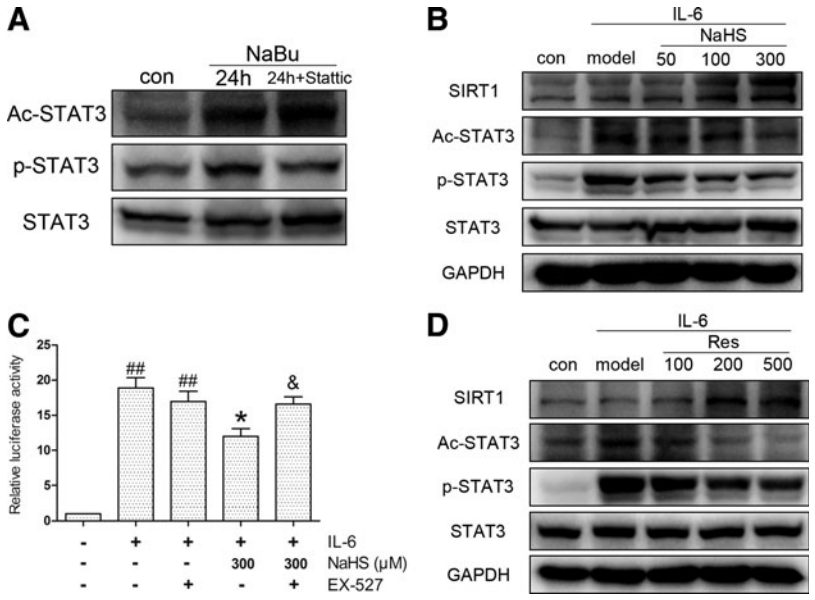
SUPPLEMENTARY FIG. S1. NaHS suppressed the hepatic *Il-6* mRNA level and JAK2/STAT3 phosphorylation in LPS-challenged C57BL/6 mice. (A) The hepatic *Il-6* mRNA level was dramatically induced by LPS (0.5 mg/kg), but suppressed by NaHS (6 mg/kg) treatment ($n = 6$). **(B, C)** Densitometry analysis of immunoblots with hepatic JAK2/STAT3 phosphorylation ($n = 6$). Data are represented as the mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with the control group; $^{*}p < 0.05$, compared with the LPS group. JAK2, Janus kinase 2; LPS, lipopolysaccharide; STAT3, signal transducer and activator of transcription 3.



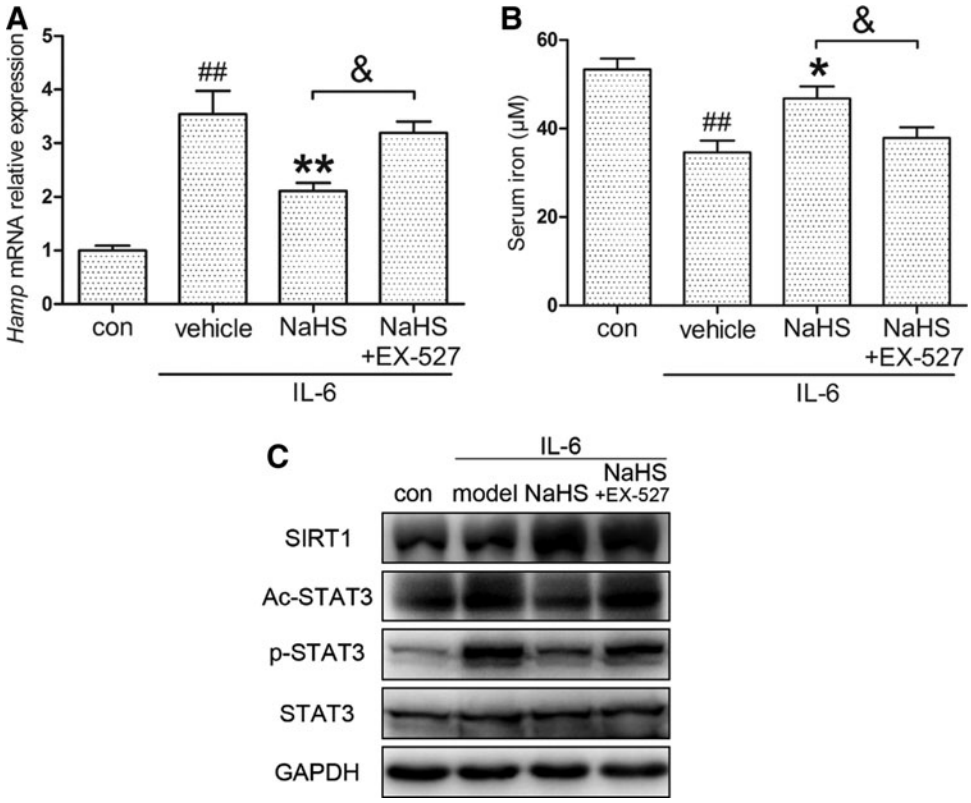
SUPPLEMENTARY FIG. S2. NaHS decreased IL-6 expression and JAK2/STAT3 activation in the pretreatment model. **(A)** Time course valuation of *HAMP* induction by LPS-CM. *HAMP* mRNA level in Huh7 cells was analyzed after incubation with LPS-CM for different hours ($n=3$). **(B)** *IL6* mRNA level in THP-1 cells treated with NaHS in the absence or presence of LPS. NaHS suppressed *IL6* mRNA expression induced by LPS (1 μg/ml), while elicited no significant effect without LPS stimulation ($n=3$). **(C, D)** Densitometry analysis of JAK2/STAT3 activation in Huh7 cells treated by LPS-CM and NaHS ($n=4$). Data are represented as the mean ± SEM of three individual experiments. $^{\#}p<0.05$, $^{\#\#}p<0.01$, and $^{\#\#\#}p<0.001$ compared with the control group; $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared with the LPS or LPS-CM group. CM, conditioned medium; IL-6, interleukin-6.



SUPPLEMENTARY FIG. S3. H_2S attenuated STAT3 activation *via* a JAK2-independent manner in the post-treatment model. (A) H_2S concentration in culture medium of Huh7 cells ($n=3$). H_2S level was increased by L-cysteine (100–500 μM), but inhibited by PAG (2 mM) and AOAA (10 μM). **(B, C)** Densitometry analysis of JAK2/STAT3 phosphorylation of Huh7 cells in the post-treatment model ($n=4$). **(D)** Densitometry analysis of native p-STAT3 dimers in Huh7 cells ($n=4$). Data are represented as the mean ± SEM of three individual experiments. $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared with the control group; $*p<0.05$, $**p<0.01$, and $***p<0.001$ compared with the IL-6 group unless indicated. P and A stand for PAG and AOAA, respectively. AOAA, aminooxyacetic acid; PAG, propargylglycine.



SUPPLEMENTARY FIG. S4. Evaluation of STAT3 acetylation and SIRT1 in the induction of hepcidin. **(A)** NaBu (1 m*M*), an HDAC inhibitor, increased the STAT3 acetylation level and added to STAT3 phosphorylation as well ($n = 3$). The induction in p-STAT3 was abolished by stattic (10 μM). **(B)** NaHS promoted SIRT1 expression and suppressed IL-6-induced STAT3 acetylation and phosphorylation in mouse primary hepatocytes ($n = 3$). **(C)** EX-527 (10 μM) blocked the inhibition of NaHS on STAT3 transcriptional function assessed by SIE reporter ($n = 5$). **(D)** Resveratrol pretreatment, similar to NaHS, promoted SIRT1 and inhibited STAT3 acetylation and phosphorylation irritated by IL-6 ($n = 3$). GAPDH served as the loading control. Representative immunoblots are presented. Data are represented as the mean ± SEM of three individual experiments. $^{\#\#}p < 0.01$ compared with the control group; $^{*}p < 0.05$ compared with the IL-6 group; $^{\&}p < 0.05$ compared with the IL-6 + NaHS group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SIRT1, sirtuin 1.



SUPPLEMENTARY FIG. S5. NaHS inhibited hepatic hepcidin *via* SIRT1 in mice injected with IL-6. C57BL/6 mice were pretreated with NaHS for 3 days (6 mg/kg/day). EX-527 (10 mg/kg) was applied 1 h before the last NaHS injection. Mice were sacrificed 6 h after IL-6 (25 μg/g) challenge. **(A)** NaHS attenuated the hepatic *Hamp* level, which was abolished by EX-527 ($n = 6$). **(B)** EX-527 diminished the improvement of serum iron by NaHS ($n = 6$). **(C)** Inhibition of SIRT1 by EX-527 reversed STAT3 acetylation and phosphorylation ($n = 6$). GAPDH served as the loading control. Representative immunoblots are presented. Data are represented as the mean ± SEM of three individual experiments. $^{\#\#}p < 0.01$ compared with the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the IL-6 group; $^{\&}p < 0.05$ compared with the IL-6 + NaHS group.