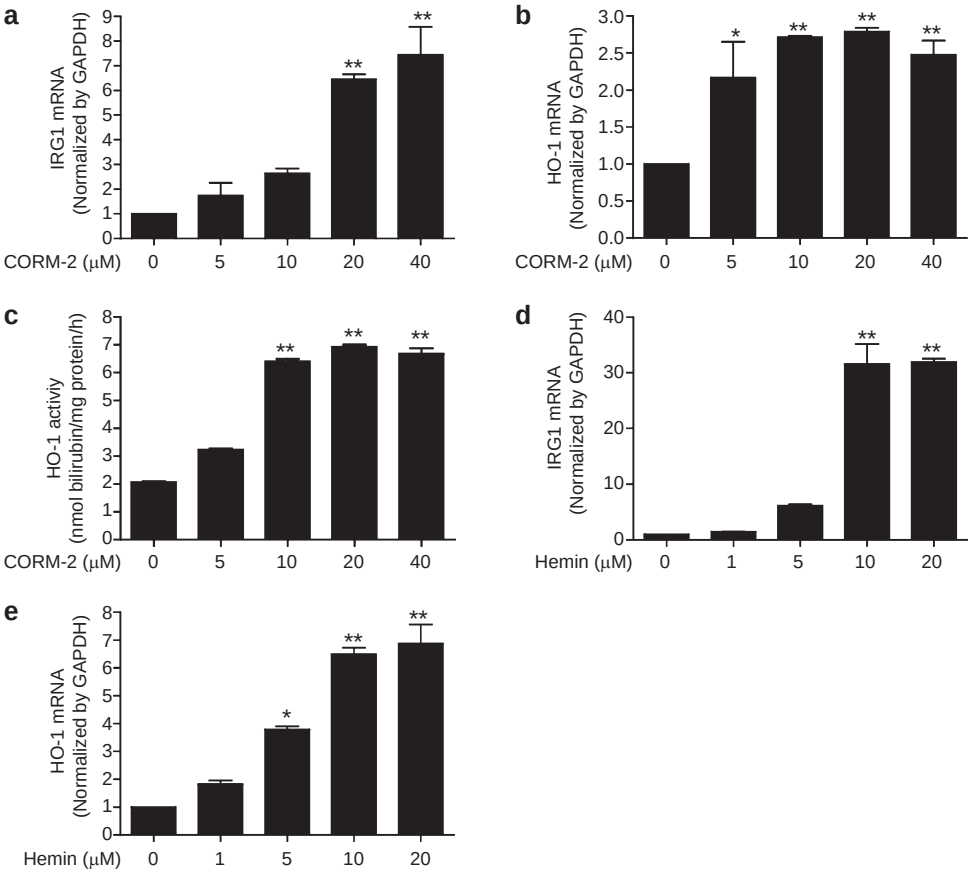
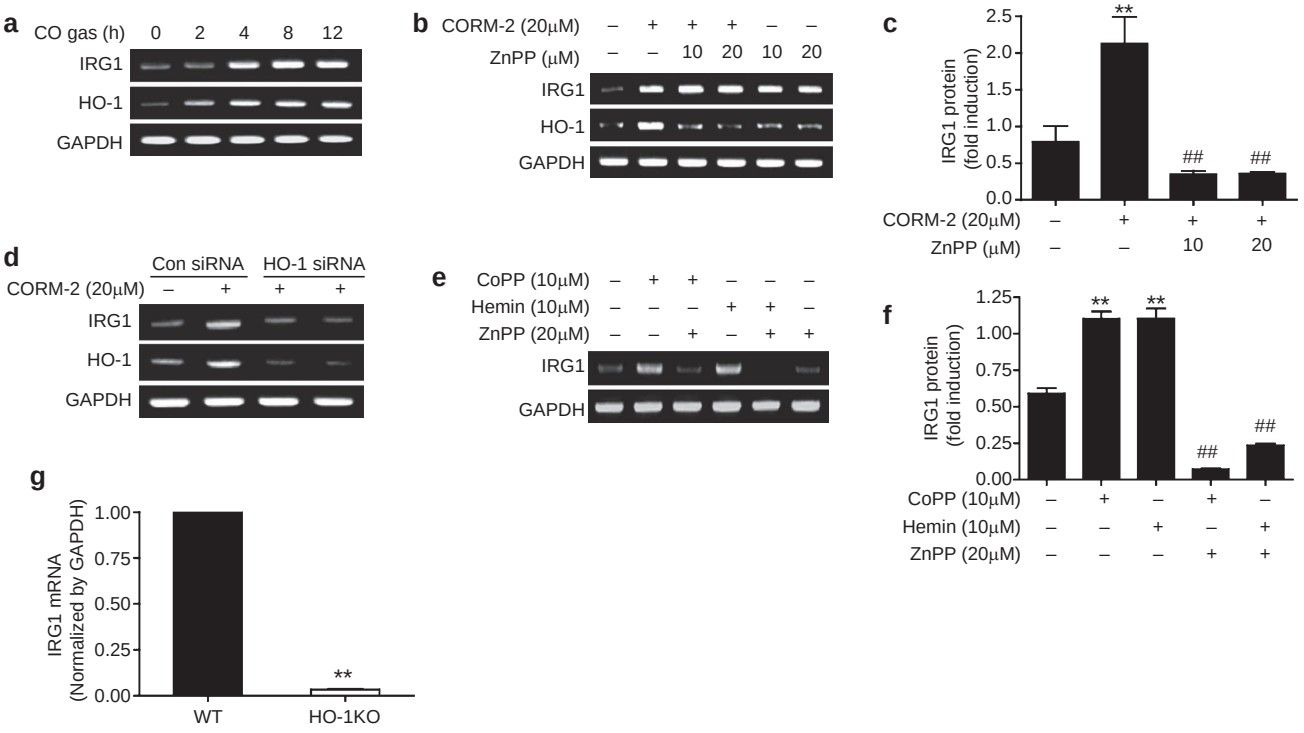
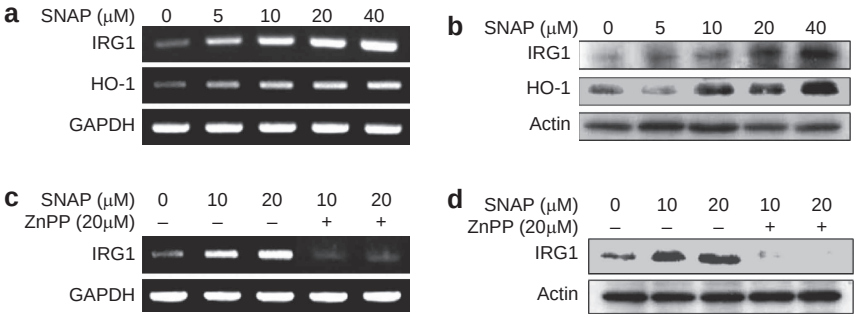


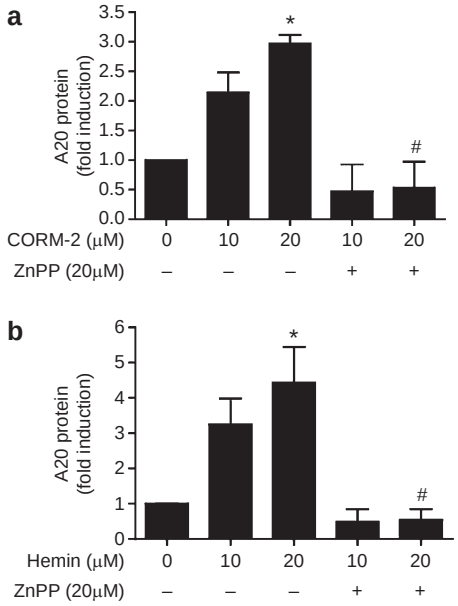
Supplemental Figure 1 CO and HO-1 inducers increase IRG1 expression in RAW264.7 macrophages. (**a** and **b**) RAW264.7 cells were treated with CORM-2 in a dose-dependent manner (0, 5, 10, 20 and 40 μM) for 8 h or 16 h. (**a**) IRG1 and (**b**) HO-1 mRNA was measured at 8 h by real-time RT-PCR, and (**c**) HO-1 activity was measured at 16 h. (**d** and **e**) Cells were treated with hemin (0, 1, 5, 10 and 20 μM) for 8 h and IRG1 (**c**) and HO-1(**d**) mRNA levels were measured by real-time RT-PCR analysis. Data represents mean±s.e.m. (n=3), *P<0.05 and **P<0.001 as compared with control.



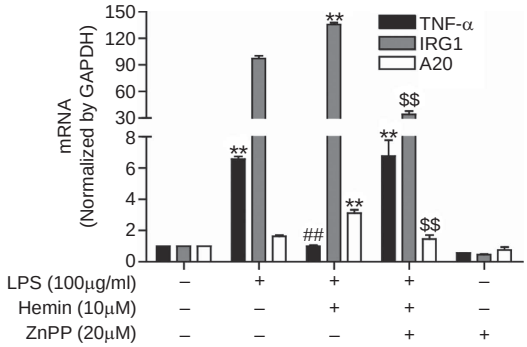
Supplemental Figure 2 Increased level of IRG1 expression is dependent on HO-1. (**a**) RAW 264.7 cells were incubated with CO gas (250 ppm) in time-dependent manner (0, 2, 4, 8 and 12 h) and harvested cells were analyzed for IRG1 and HO-1 mRNA using RT-PCR. (**b**) Cells were pre-treated with ZnPP (0, 10 and 20 μM) for 0.5 h and then treated with or without CORM-2 (20 μM) for 8 h, and HO-1 and IRG1 mRNA levels were checked by RT-PCR. (**c**) Cells were pre-treated with ZnPP (0, 10 and 20 μM) for 0.5 h and then treated with CORM-2 (20 μM) for 16 h and, after western blotting, bands from Figure 2d were analyzed using ImageJ software. (**d**) Cells were transfected with HO-1 siRNA or control siRNA (Con siRNA). After treatment with 20 μM CORM-2 for 8 h, cells were harvested and mRNA levels of HO-1 and IRG1 were performed by RT-PCR. (**e**) Cells were pre-treated with or without ZnPP (20 μM) for 0.5 h and treated with 10 μM hemin and 10 μM CoPP for 8 h. RT-PCR analysis was performed to determine mRNA levels of IRG1. (**f**) Cells were pre-treated with or without ZnPP (20 μM) for 0.5 h and then treated with 10 μM hemin and 10 μM CoPP for 16 h. Densitometric quantitation of immunoblotting presented in Fig. 2F were analyzed by using ImageJ software. (**g**) Liver tissues of Balb/c wild-type and HO-1 KO mice (female, 7–8 weeks of age, 20–25 g) were used to measure mRNA level of IRG1 by real-time RT-PCR. Data represents mean±s.e.m. (n=2), *P<0.05 and **P<0.001 as compared with control/wild type (WT); and $^{\#}P$<0.05 and $^{\#\#}P$<0.001 as compared with the cells exposed to only CORM-2 or hemin or CoPP.



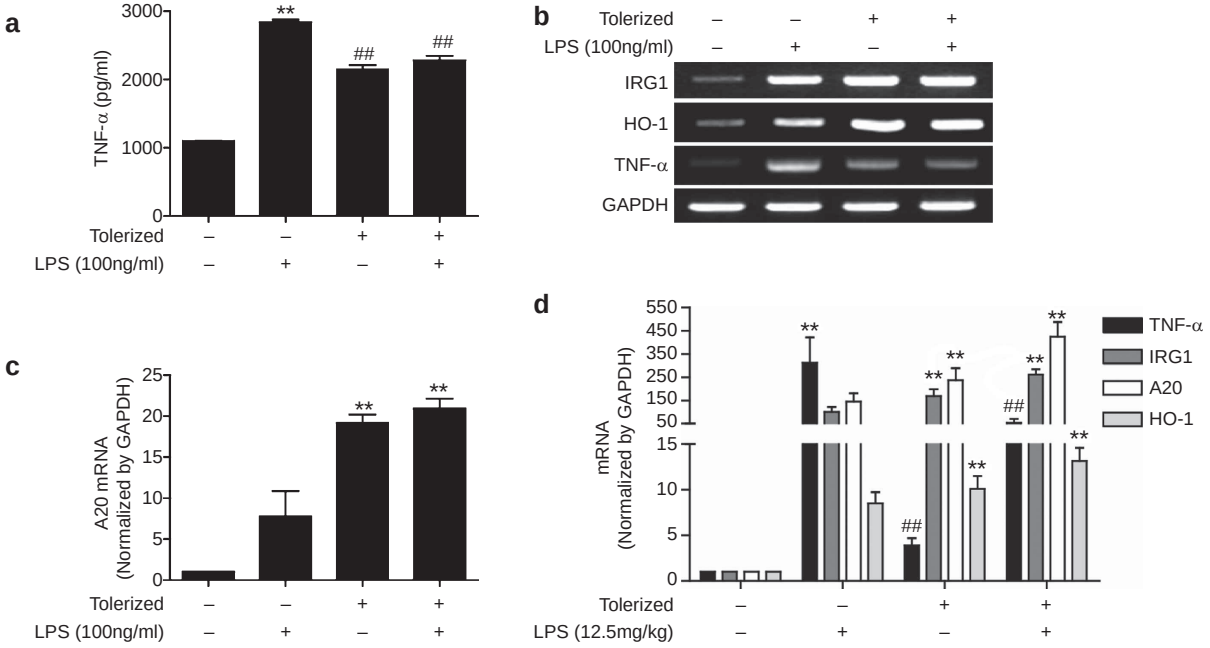
Supplemental Figure 3 NO increases IRG1 expression in a HO-1 dependent manner in RAW 264.7 macrophages. (**a** and **b**) RAW 264.7 cells were treated with NO donor, SNAP at the indicated concentrations (0, 5, 10, 20 and 40 μM) for 8 or 16 h. (**a**) IRG1 and HO-1 mRNA were analyzed at 8 h by RT-PCR; (**b**) IRG1 and HO-1 proteins were analyzed at 16 h by western blotting. (**c** and **d**) Cells were pre-treated with or without ZnPP (20 μM) for 0.5 h and then treated with 10 and 20 μM SNAP for 8 or 16 h. (**c**) After 8 h, IRG1 mRNA was analyzed by RT-PCR; (**d**) IRG1 protein level was analyzed after 16 h by western blotting.



Supplemental Figure 4 CORM-2 and hemin increase A20 in a HO-1 dependent manner in macrophages. (**a**) RAW 264.7 cells were pre-treated with ZnPP (20 μM) for 30 min and then further incubated with CORM-2 (20 μM) for 16 h. Densitometric quantitation of immunoblots for A20 expression presented in Figure 3e was performed. (**b**) Cells were pre-treated with ZnPP (20 μM) for 30 min and further incubated with hemin (10 μM) for 16 h. Densitometric quantitation of immunoblot s for A20 expression as presented in Figure 3g was performed. Bands were analyzed by using ImageJ software. Data represent mean ± s.e.m. (n=2), $^{*}P<0.05$ as compared with control; and $^{\#}P<0.05$ as compared with the cells exposed to CORM-2/hemin + ZnPP.



Supplemental Figure 5 Hemin induced IRG1 decreases inflammation *via* A20 expression in RAW 264.7 macrophages. RAW 264.7 cells were pre-treated with ZnPP (20 μM) for 0.5 h and then treated with hemin (10 μM) for 1 h and then incubated with 100 ng/ml LPS for 8 h. Cell lysate was analyzed by real-time RT-PCR analysis to determine the mRNA expression of TNF-α, IRG1 and A20. Data represent mean±s.e.m. (n=3), $^{*}P<0.05$ and $^{**}P<0.001$ as compared with control; $^{\#}P<0.05$ and $^{\#\#}P<0.001$ as compared with the cells exposed to only LPS and $^{\$\$}P<0.001$ as compared with the cells exposed to LPS+hemin.



Supplemental Figure 6 IRG1 expression is mediated in a HO-1 dependent manner in the endotoxin tolerance state. (**a** and **c**) RAW 264.7 cells were tolerized with LPS (100 ng/ml) for 12 h and then re-stimulated with a second round of LPS (100 ng/ml) for 8 or 12 h. (**a**) TNF-α production from cell supernatants was measured at 12 h by using ELISA. (**b**) RT-PCR analysis with IRG1, HO-1 and TNF-α mRNA were performed at 8 h. (**c**) Real-time RT-PCR was conducted to determine A20 mRNA at the 8 h time point. (**d**) Wild-type 7-week-old male C57BL/6 mice were tolerized with LPS (5 mg/ml, i.p.) for 24 h and then re-stimulated with a second round LPS (12.5 mg/ml, i.p.) for 16 h. Liver tissues were analyzed for TNF-α, IRG1, A20 and HO-1 mRNA by using real-time RT-PCR analysis. Data represent mean±s.e.m. (n=3), $^{*}P<0.05$ and $^{**}P<0.001$ as compared with control; and $^{\#}P<0.05$ and $^{\#\#}P<0.001$ as compared with the cells exposed to only LPS.