Supporting Information

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SI Text

Cell Culture and Reagents. Hepa1–6 cells (C57L mouse liver hepatoma; ATCC) (37 °C, 95% humidity, 5% CO_2) in DMEM (Invitrogen), 10% FCS, 20 U/mL penicillin, and 20 U/mL streptomycin (Invitrogen). Human and mouse recombinant TNF (hTNF; R&D System), heme (hemin; Frontier Scientific), CHX, ActD, NAC (Sigma), 5-(and-6)-chloromethyl-2′7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA; Invitrogen), or Hoechst 33342 (Invitrogen) were used as indicated. All experiments in vitro were performed under pO_2 21%, CO_2 5%, and 95% humidity. Experiments illustrated under Figs. 5 and 6 were also performed under pO_2 2%, CO_2 5%, and 95% humidity with similar results.

Immunofluorescence. Hepa1–6 cells grown in coverslip-coated 6-well plates (150×10^3 cells/well) were fixed (4% paraformaldehyde; for 30 min at RT), washed twice (PBS), and stained with Hoechst 33342 (10 μg/mL; PBS; for 20 min.). Representative fluorescent images (400×) were captured with a fluorescence microscope (Leica; DMRA2) equipped with UV light.

Flow Cytometry. Hepa1–6 cells (100 g; for 3 min at 4 °C) were incubated with CM-H_2DCFDA (10 μM; for 15 min at 37 °C; 95% humidity; 5% CO_2), resuspended in PBS, and fluorescence was measured by flow cytometry on a FACSCalibur (Becton Dickinson).

In Vivo Treatments. Anti-TNF mAb purified in-house from the XT3.11 hybridoma (DNAX Research Institute), which secretes a neutralizing rat anti-murine TNF (IgG1), previously described (1). Purified rat IgG (Sigma) was used as isotype control. Anti-TNF mAb and rat IgG (0.2 mg/mouse/daily/i.p.) were administered from day 5 to 9 postinfection. TNF depletion was assessed by ELISA. NAC was administered (i.p.) starting at day 4 postinfection and every 12 h thereafter until day 15.

Serum Biochemistry. Mice were euthanized in CO_2, blood was collected by cardiac puncture in heparinized tubes, centrifuged ($1,500 \times g$ for 10 min), and plasma was frozen (−20 °C) until used. AST and ALT were determined as described (2). All measurements were done by spectrophotometric analysis (modular DP; Roche-Hitachi, Echevarne Laboratories).

Protein Extraction and Immunoblotting. Proteins were extracted, electrophoresed, and electrotransferred as described (3). Rabbit HO-1 (OSA-111, 1:5,000 dilution; Stressgen), HO-2 (OSA-200, 1:5,000 dilution; Stressgen), caspase-3 (9665, 1:1,000 dilution; Cell Signaling), and α-tubulin (T9026, 1:15,000 dilution; Sigma) antibodies were used, as described (4). Peroxidase conjugated secondary antibodies (1 h at RT) and developed with SuperSignal chemiluminescent detection kit (Pierce).

Statistical Analysis. Data analysis was performed using unpaired Student's *t*-test, when the data follows Gaussian distributions, an assumption tested using the Kolmogorov-Smirnov method. When the data does not follow Gaussian distributions, the Mann-Whitney *U* test was used instead. Log-rank test was used for all experiments in which survival was assessed as an end point.

1. Beretich GR, Jr, Carter PB, Havell EA (1998) Roles for tumor necrosis factor and gamma interferon in resistance to enteric listeriosis. *Infect Immun* 66:2368–2373.
2. Horder M, Elser RC, Gerhardt W, Mathieu M, Sampson EJ (1991) International Federation of Clinical Chemistry, Scientific Division Committee on Enzymes: Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes Part 7 IFCC method for creatine kinase (ATP: creatine N-phosphotransferase, EC 2732). *Eur J Clin Chem Clin Biochem* 29:435–456.
3. Pamplona A, et al. (2007) Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med* 13:703–710.
4. Brouard S, et al. (2000) Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. *J Exp Med* 192:1015–1026.

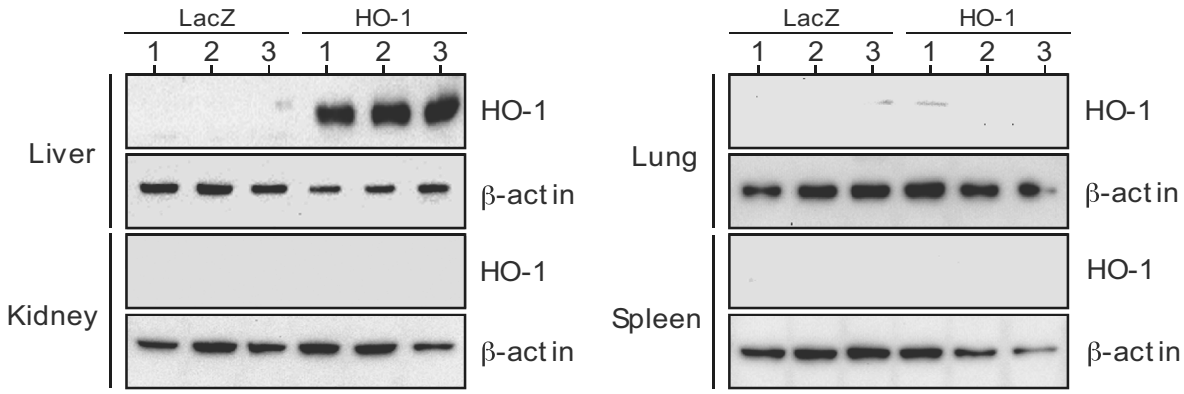

Fig. S1. HO-1 transduction in the liver. HO-1-HA-tagged and β-actin protein expression in liver, kidney, lung, and spleen of DBA/2 mice detected by western blot, 4 days after Rec.Ad. transduction. Numbers (1–3) indicate individual mice.

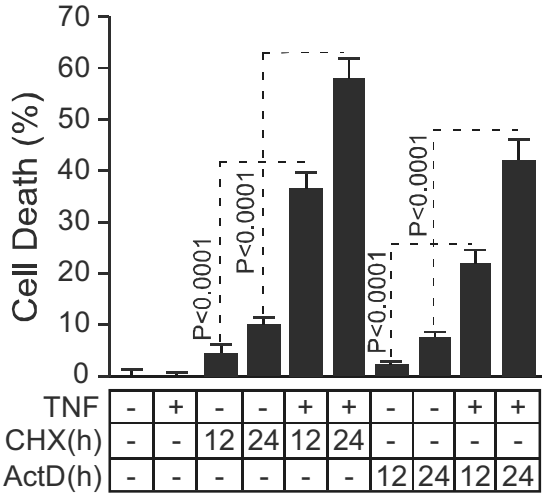

Fig. S2. Inhibition of mRNA (ActD) and/or protein synthesis (CHX) sensitizes hepatocytes to TNF-mediated apoptosis in vitro. Hepa1–6 cells were exposed to 50 ng/mL TNF, 5 μg/mL ActD, or 5 μg/mL CHX for the times indicated. Cytotoxicity was assessed by crystal violet staining. Results are expressed as mean $\pm$ SD, one experiment in sixtuplicate. Notice that when exposure to TNF was prolonged (i.e., 12–24 h), both ActD and CHX sensitized hepatocytes to apoptosis.