SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Salmonella Infection *In Vitro*

If desired macrophages were stimulated with 50 µM FAC (ferric ammonium citrate), 50 µM CORM-2 (CO-releasing molecule), 100 µM NAC (N-acetyl-cystein), 10 µM bilirubin (all obtained from Sigma). Concentrations were previously tested according to published data for CORM-2 (Sawle *et al.*, 2005, Murray *et al.*, 2012), Bilirubin (Wang *et al.*, 2004) and FAC (Sohn *et al.*, 2011) and the above stated concentrations were used for experiments.

LDH Cytotoxicity Assay

RAW264.7 cells were seed in a 96-well plate at a density of 20.000 cells/ well. Cells were maintained in complete DMEM with 1% heat-inactivated FCS without antibiotics for 6 hours. Afterward *Salmonella* infection was performed as described in Material and Methods. After 12 and 20 hrs cell supernatants were subjected to Pierce LDH cytotoxicity assay kit (Thermo Scientific) according to the manufacturer’s protocol. Absorbance was measured at 490 nm and 680 nm and activity determined by subtracting the background (680 nm) from 490 nm and calculating % of cytotoxicity by normalizing the values to maximum LDH activity controls (lysed cells).

Nramp1 Cell line

RAW-264.7 murine macrophages originating from Nramp1-susceptible BALB/c mice were stably transfected with an expression vector containing Nramp1 in the sense direction (Nramp1 functional) or with a non-functional antisense construct (Nramp1 non-functional), as described previously (Atkinson *et al.*, 1999). *Salmonella in vitro* infections were exactly performed as described in Material and Methods.

Primers and Probes for qRT-PCR

The following primers and TaqMan probes were used for probe based real-time PCR (see Tabel S1).

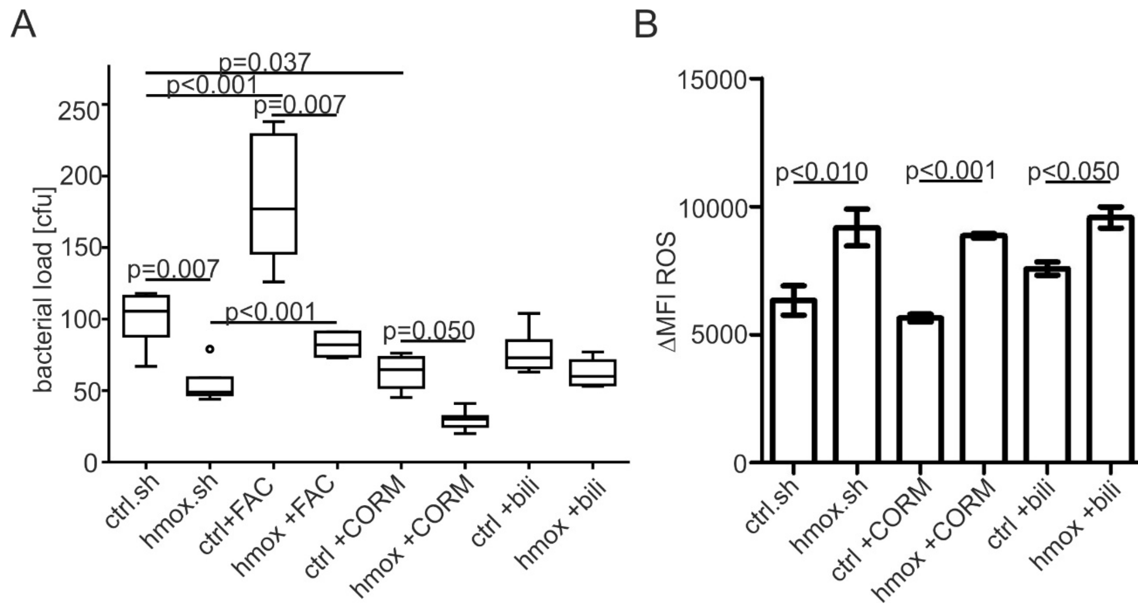

Figure S1. Impact of heme catabolism products on bacterial burden and ROS production.

(A-B) Ctrl.sh and hmox.sh cells infected with *Salmonella* Typhimurium wt, were treated with 100 µM FAC, 50 µM CORM-2 and 5 µM bilirubin. (A) pathogen burden was determined after 20 hrs from the cell lysates (B) ROS levels (mean fluorescence intensity – MFI) were measured via FACS analysis in ctrl.sh and hmox.sh cells infected with *Salmonella* at 3 hrs post infection using CellROX™. (A-B) Data are shown as mean ± SEM from at least three independent experiments performed in triplicate. P-Values are reported as determined by ANOVA using Bonferroni correction.

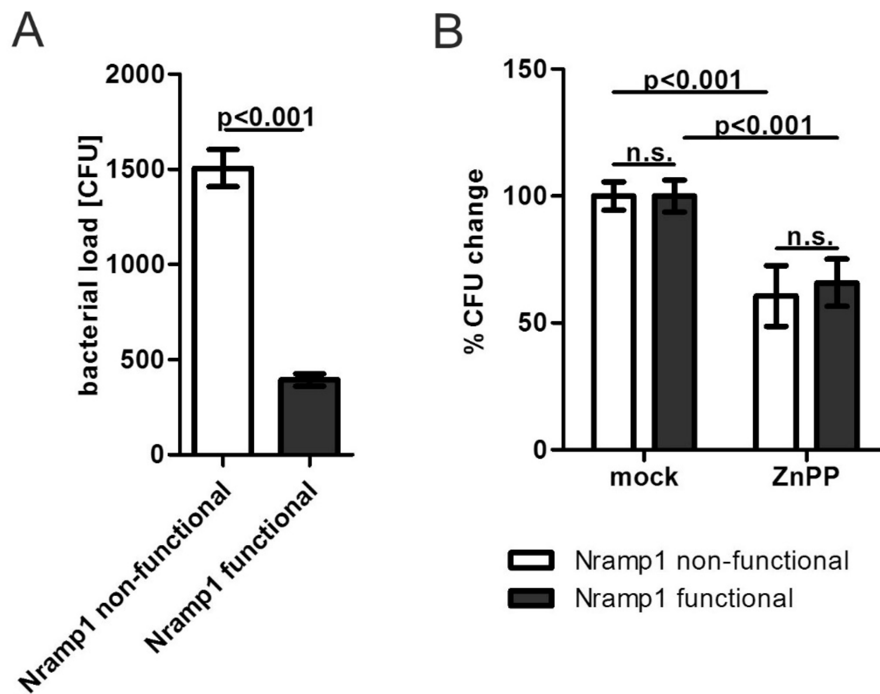

Figure S2. Nramp1 functionality does not affect HO-1 mediated regulation of intracellular Salmonella replication

(A-B) RAW 264.7 cells stably transfected with expression vector containing functional or non-functional Nramp1 were infected with *Salmonella* Typhimurium wt at a MOI of 10 as described in Material and Methods. (A) Influence of Nramp1 cells on *Salmonella* load (CFU) was measured. (B) Bacterial burden (CFU) was determined 20 hrs post-infection in the presence of 10 µM HO-1 inhibitor ZnPP or solvent. For better comparability of the effects of Nramp1 on HO-1 mediated control of infection Nramp1 non-functional and Nramp1 functional bacterial loads, were normalized to 100%, in the absence of ZnPP (mock). (A-B) Data are shown as mean ± SEM from at least three independent experiments. P-Values as determined by (A) t-test or (B) ANOVA using Bonferroni's correction.

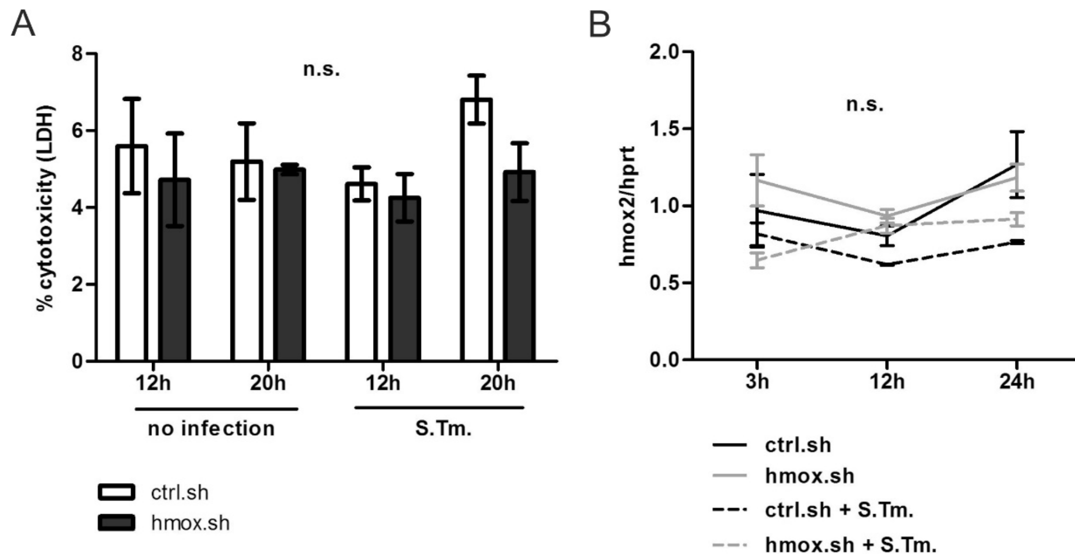

Figure S3. Effect of *hmox1* knockdown on the cytotoxicity during *Salmonella* infection and *hmox2* expression.

(A) Ctrl.sh and hmox.sh cells were infected with *Salmonella* Typhimurium wt or left untreated for 12 and 20 hrs. Thereafter cell supernatants were subjected to LDH cytotoxicity test and % cytotoxicity was calculated by normalizing to maximum LDH activity of lysed control cells. (B) Ctrl.sh (black line) and hmox.sh (grey line) macrophages infected with *Salmonella* (black or grey dashed line) were subjected to qRT-PCR at indicated time points. Expression of *hmox2* gene was normalized to *hprt* expression and fold-change relative to untreated controls is shown. (A-B) Error bars represent means ± SEM, values were determined by ANOVA using Bonferroni's correction.

Table S1. Primers used for qRT-PCR. Annealing temperature was 60°C for all primer pairs.

Primer/ Probe Name	Sequence (5‘-3‘)
mu hmox1 fw	GTGATGGAGCGTCCACAGC
mu hmox1 probe	CGACAGCATGCCCCAGGATTTGTC
mu hmox1 rv	TGGTGGCCTCCTTCAAGG
mu hmox2 fw	TGGCCCATGCTTATACTCGTT
mu hmox2 rv	GCTGGGAAGTTTTAGTGCCCT
mu hprt fw	GACCGGTCCCGTCATGC
mu hprt probe	ACCCGCAGTCCCAGCGTCGTC
mu hprt rv	TCATAACCTGGTTCATCATCGC
mu IL-10 fw	CCAGAGCCACATGCTCCTAGA
mu IL-10 probe	TGCGGACTGCCTTCAGCCAGG
mu IL-10 rv	TGGTCCTTTGTTTGAAAGAAAGTCT
mu IL-6 fw	TGTTCTCTGGGAAATCGTGGA
mu IL-6 probe	ATGAGAAAAGAGTTGTGCAATGGCAAT
mu IL-6 rv	AAGTGCATCATCGTTGTTCATACA
mu inos fw	CAGCTGGGCTGTACAAACCTT
mu inos probe	CGGGCAGCCTGTGAGACCTTTGA
mu inos rv	CATTGGAAGTGAAGCGTTTCG
mu lcn2 fw	GCCTCAAGGACGACAACATCA
mu lcn2 probe	TTCTCTGTCCCCACCGACCAATGC
mu lcn2 rv	CACCACCCATTCAGTTGTCAAT
mu p47phox fw	GAGGCGGAGGATCCGG
mu p47phox probe	CAACTACGCAGGTGAACCGTATGTAAC
mu p47phox rv	TCTTCAACAGCAGCGTACGC
mu tfr1 fw	CGCTTTGGGTGCTGGTG
mu tfr1 probe	CCCACACTGGACTTCGCCGCA
mu tfr1 rv	GGGCAAGTTTCAACAGAAGACC
mu tnfa probe	CTCAGATCATCTTCTCAAAATTCGAGT
mu tnfa rv	TTGCTACGACGTGGGCTACA
mu tnfa fw	TTCTATGGCCCAGACCCTCA

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