Figure S1

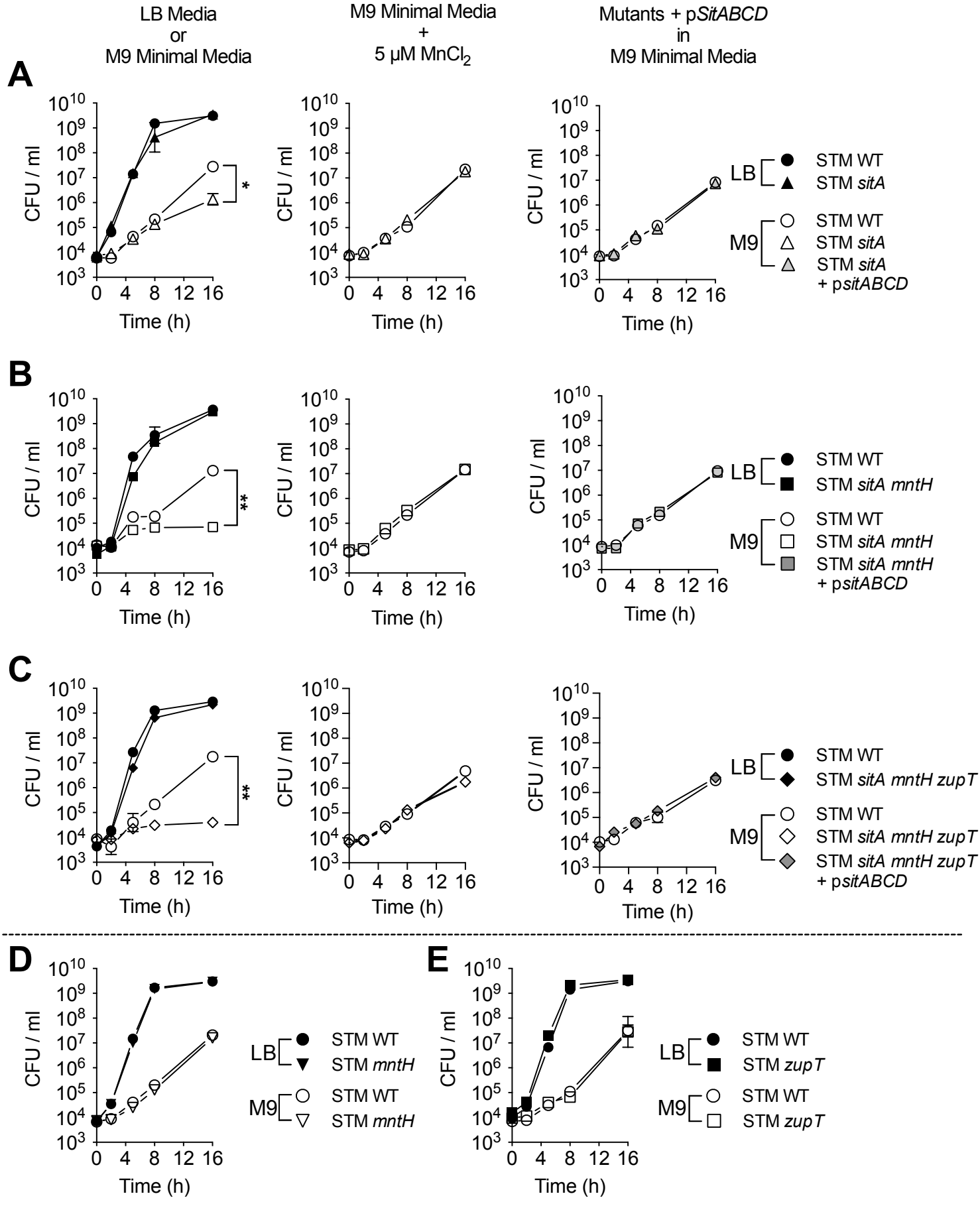

LB Media
or
M9 Minimal Media
M9 Minimal Media
+
5 μM MnCl2
Mutants + pSitABCD
in
M9 Minimal Media
A
B
C
D
E
CFU / ml
Time (h)
0 4 8 12 16
*
**
**
LB
M9
STM WT
STM sitA
STM sitA + psitABCD
STM sitA mntH
STM sitA mntH + psitABCD
STM sitA mntH zupT
STM sitA mntH zupT + psitABCD
STM mntH
STM zupT

Figure S1. (Related to Figure 1)

Growth of *S*. Typhimurium wild-type and mutants under metal rich and metal limiting conditions. *S*. Typhimurium wild-type (WT) and manganese transporter deficient strains (*sitA*, *mntH, zupT*, *sitA mntH*, and *sitA mntH zupT*) were grown in either LB or M9 minimal media (**A-C left panels, D and E**), or M9 minimal media supplemented with 5 μM $MnCl_2$ (**A-C middle panels**). The WT *S*. Typhimurium strain and mutant strains complemented with a plasmid expressing the *sit* operon (p*sitABCD*) were grown in M9 minimal media (**A-C, right panels**). Growth was determined by calculating the colony forming units (CFU) per ml of culture from plating dilutions of the cultures on LB at the indicated time points. Data represent the mean of 3 replicates ± SEM. Some error bars are not visible due to a small degree of variation between assays. A significant difference in growth between WT and the manganese transporter deficient mutants is indicated by * (*P* value ≤ 0.05) and ** (*P* value ≤ 0.01).

Figure S2

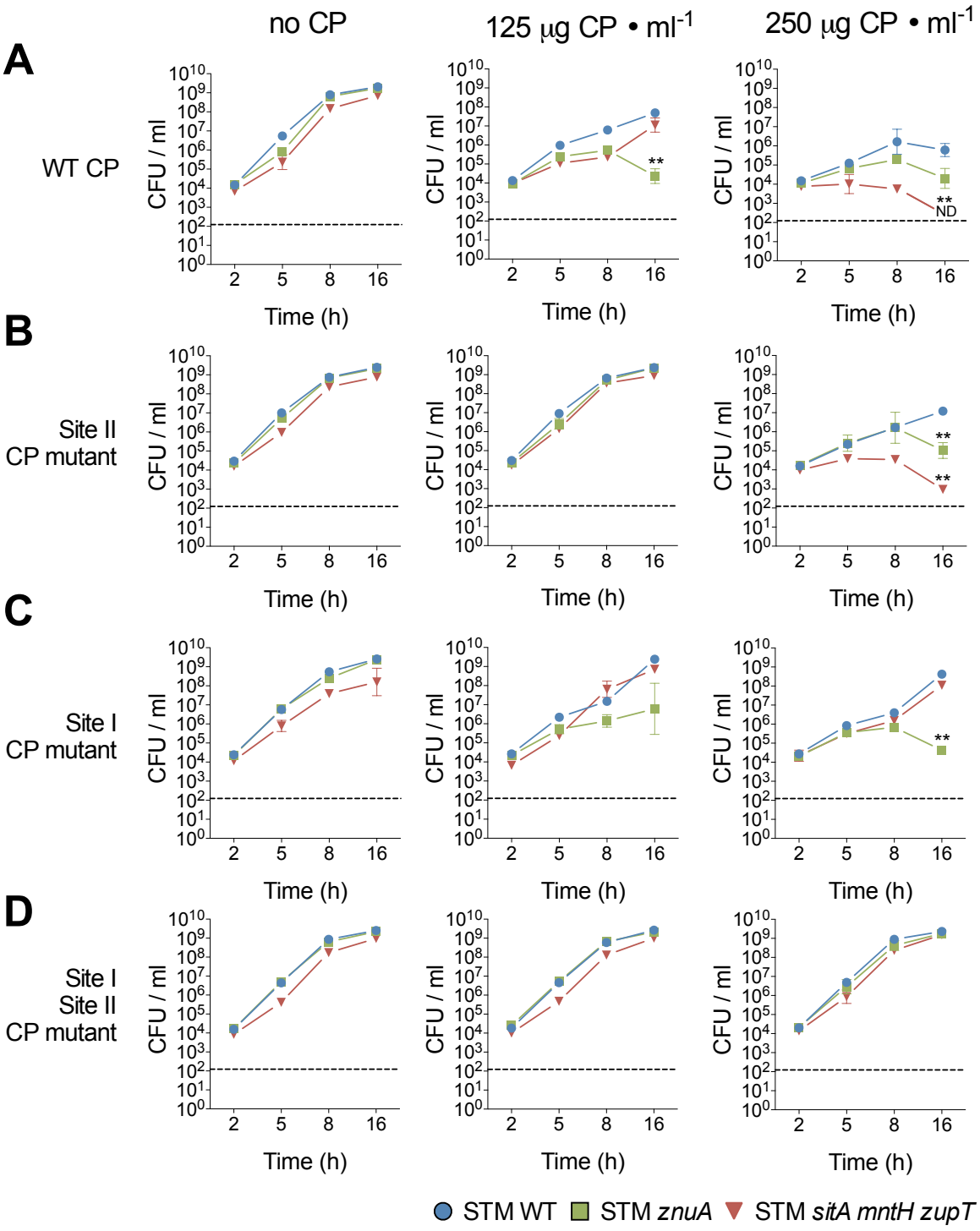

no CP
125 μg CP • ml-1
250 μg CP • ml-1
A
WT CP
B
Site II
CP mutant
C
Site I
CP mutant
D
Site I
Site II
CP mutant
CFU / ml
Time (h)
**
ND
STM WT
STM znuA
STM sitA mntH zupT

Figure S2 (Related to Figure 1)

***S*. Typhimurium utilizes manganese transporters to evade calprotectin-mediated growth inhibition *in vitro*.** *S*. Typhimurium WT, the *znuA* mutant and *sitA mntH zupT* mutant were grown in LB supplemented with either (**A**) wild-type calprotectin (CP), (**B**) a Site II CP mutant, (**C**) a Site I CP mutant, or (**D**) a Site I/Site II CP double mutant at the indicated CP concentrations. Growth was determined by calculating the CFUs per ml of culture from plating dilutions of the supplemented cultures on LB at 2, 5, 8, and 16 hours of incubation. A significant difference in growth between the WT and mutant strains is indicated by * (*P* value ≤ 0.05) or ** (*P* value ≤ 0.01). ND (not detected) indicates that viable bacteria were below the limit of detection (dashed line).

Figure S3

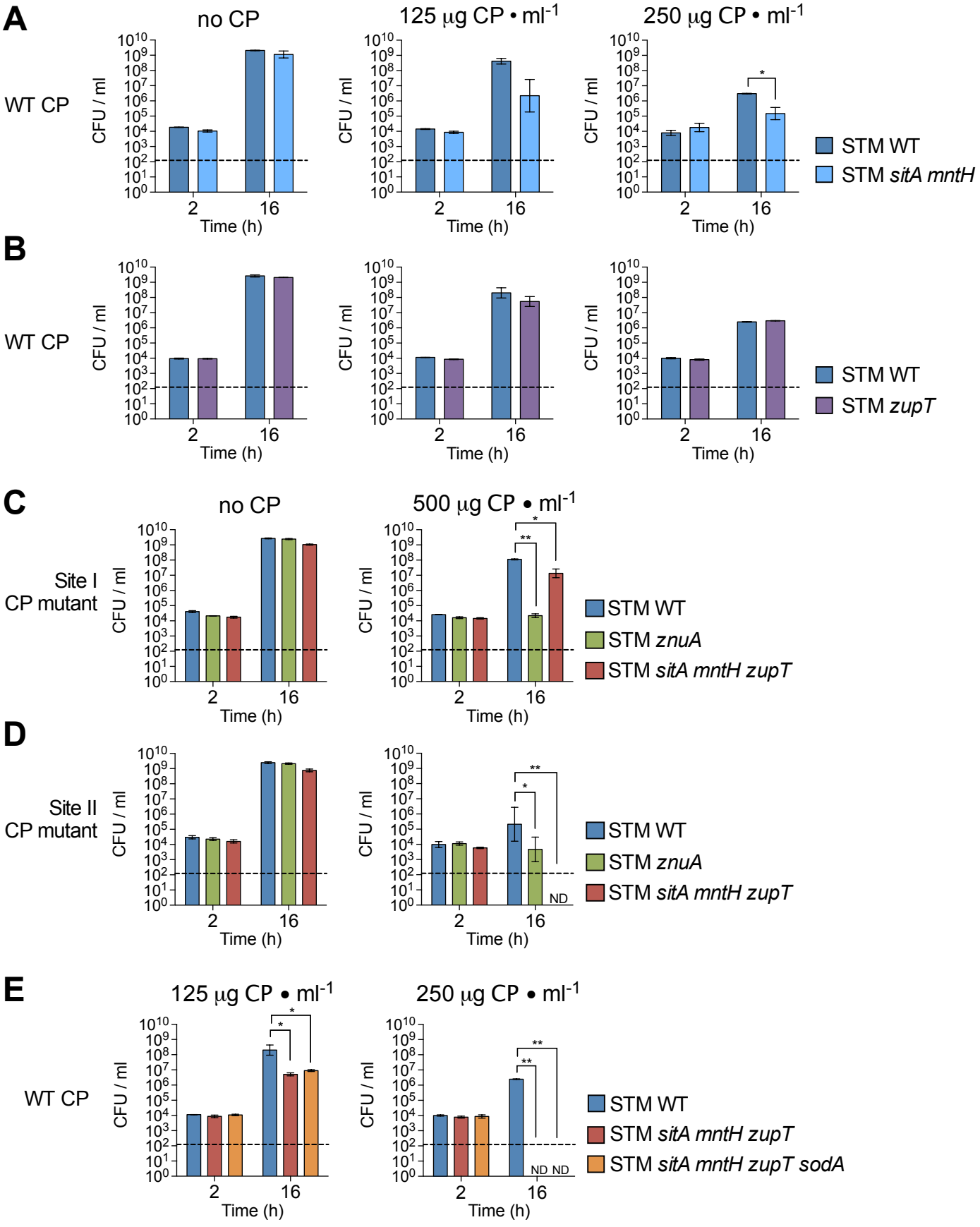

A
no CP
125 μg CP • ml-1
250 μg CP • ml-1
WT CP
CFU / ml
Time (h)
STM WT
STM sitA mntH
B
WT CP
STM WT
STM zupT
C
no CP
500 μg CP • ml-1
Site I
CP mutant
STM WT
STM znuA
STM sitA mntH zupT
D
Site II
CP mutant
ND
E
125 μg CP • ml-1
250 μg CP • ml-1
WT CP
STM WT
STM sitA mntH zupT
STM sitA mntH zupT sodA
ND ND

Figure S3 (Related to Figures 1 and 5)

Growth of manganese transporter mutants in LB media supplemented with calprotectin and LB media supplemented with high concentrations of calprotectin mutants. *S*. Typhimurium WT, (**A**) *sitA mntH*, and (**B**) the *zupT* mutants were grown in LB or LB supplemented with wild-type calprotectin (CP) at the indicated concentrations. *S*. Typhimurium WT, *znuA*, and the *sitA mntH zupT* mutants where grown in LB or LB supplemented with either (**C**) 500 µg of the site I CP mutant per ml or (**D**) 500 µg of the site II CP mutant per ml. (**E**) *S*. Typhimurium WT, *sitA mntH zupT*, and the *sitA mntH zupT sodA* mutants were grown in LB (data not shown) or LB supplemented with either 125 µg of wild-type CP per ml (left panel) or 250 µg of wild-type CP per ml (right panel). Growth was determined as previously described for the indicated incubation times. A significant difference in growth between the WT and mutant strains is indicated by * (*P* value ≤ 0.05) or ** (*P* value ≤ 0.01). ND (not detected) indicates that viable bacteria were below the limit of detection (dashed line).

Figure S4

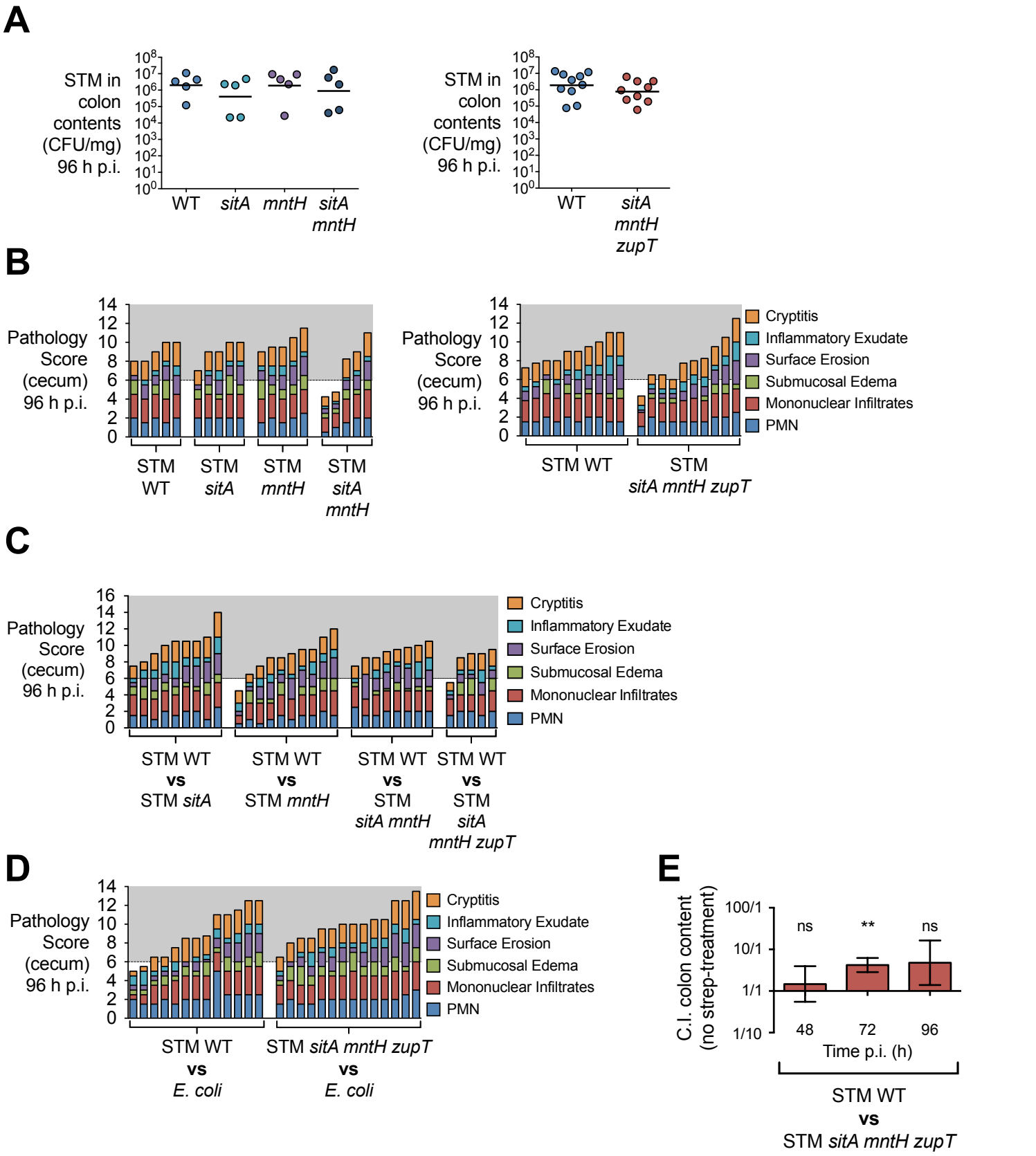
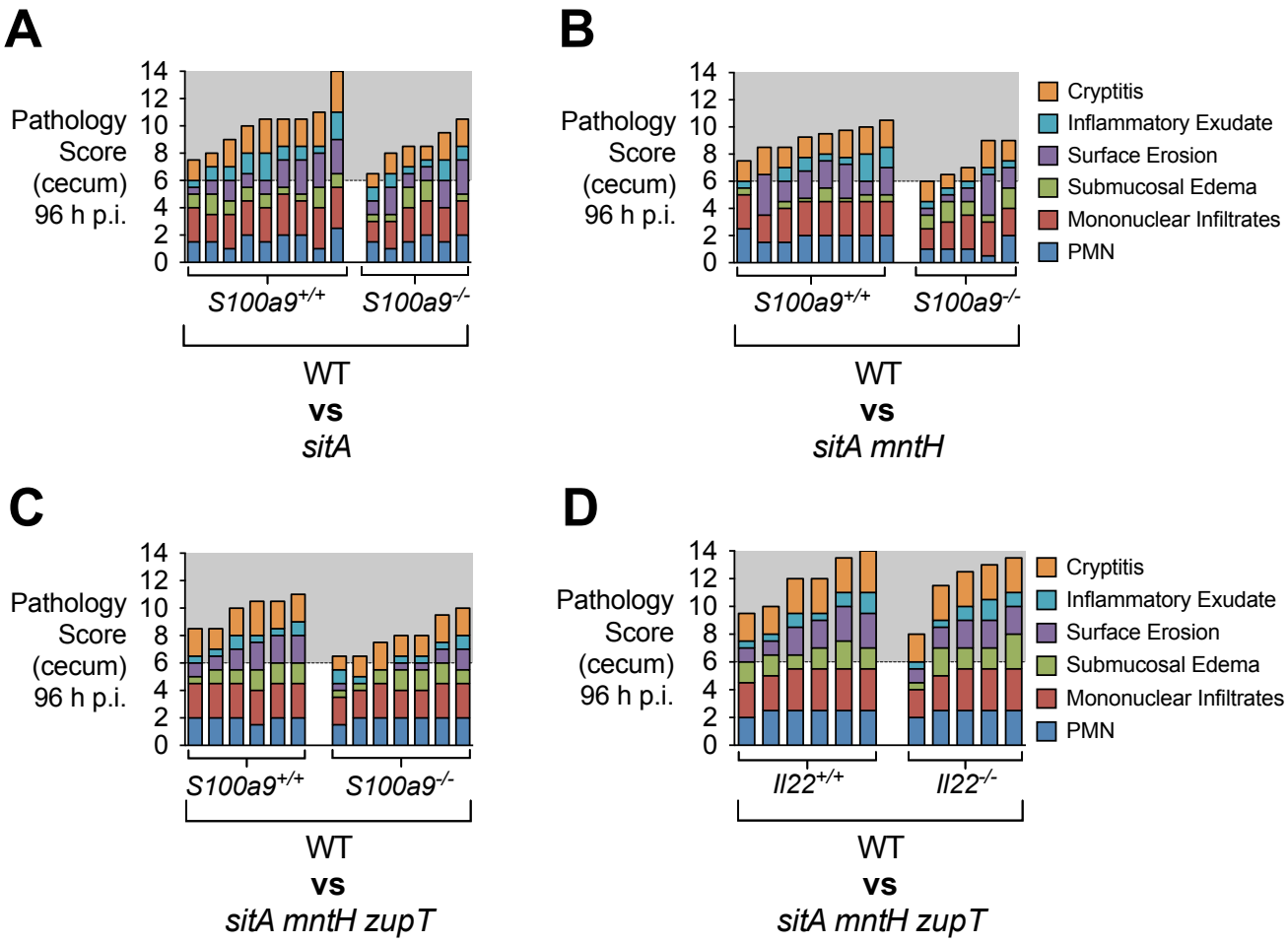

A
STM in colon contents (CFU/mg) 96 h p.i.
WT
sitA
mntH
sitA mntH
sitA mntH zupT
B
Pathology Score (cecum) 96 h p.i.
STM WT
STM sitA
STM mntH
STM sitA mntH
STM sitA mntH zupT
Cryptitis
Inflammatory Exudate
Surface Erosion
Submucosal Edema
Mononuclear Infiltrates
PMN
C
STM WT vs STM sitA
STM WT vs STM mntH
STM WT vs STM sitA mntH
STM WT vs STM sitA mntH zupT
D
STM WT vs E. coli
STM sitA mntH zupT vs E. coli
E
C.I. colon content (no strep-treatment)
100/1
10/1
1/1
1/10
ns
**
ns
48
72
96
Time p.i. (h)
STM WT vs STM sitA mntH zupT

Figure S4 (Related to Figure 2)

Single infections, histopathology of the cecum and colonization of the gut in the absence of intestinal inflammation. (**A**) C57BL/6 mice were infected with 10^9 bacteria of either the wild type (WT) or a manganese transporter deficient strain of *S*. Typhimurium. Circles represent the *S.* Typhimurium burden in colon contents of individual mice 96 hours post-infection (p.i.) and bars are the geometric mean of the group. Left panel is representative data from 2-3 experimental replicates (n = 5 mice per group per experiment). Right panel is data combined from 2 experiments (n = 10 mice infected with WT *S*. Typhimurium and n = 9 mice infected with the *sitA mntH zupT* mutant). (**B-D**) Blinded histopathology scores of the cecum of infected animals represented in Figures S4A, 2A, and 2D, respectively; each column represents an individual mouse, the gray quadrant includes scores indicative of moderate to severe inflammation. (**E**) Mice were co-infected with an equal mixture of WT *S*. Typhimurium and the *sitA mntH zupT* mutant (n = 5) in the absence of streptomycin pre-treatment. Colon contents were collected 48-96 hours p.i. and the competitive index (C.I.) was calculated by dividing the output ratio (CFU of the WT / CFU of the mutant) by the input ratio (CFU of the WT / CFU of the mutant). Bars represent the mean C.I. ± SEM. A significant difference in the competitive advantage of the WT strain over the *sitA mntH zupT* mutant strain is indicated by ** (*P* value ≤ 0.01).

Figure S5


A
B
C
D
Pathology Score (cecum) 96 h p.i.
Cryptitis
Inflammatory Exudate
Surface Erosion
Submucosal Edema
Mononuclear Infiltrates
PMN
S100a9+/+
S100a9-/-
Il22+/+
Il22-/-
WT
vs
sitA
sitA mntH
sitA mntH zupT

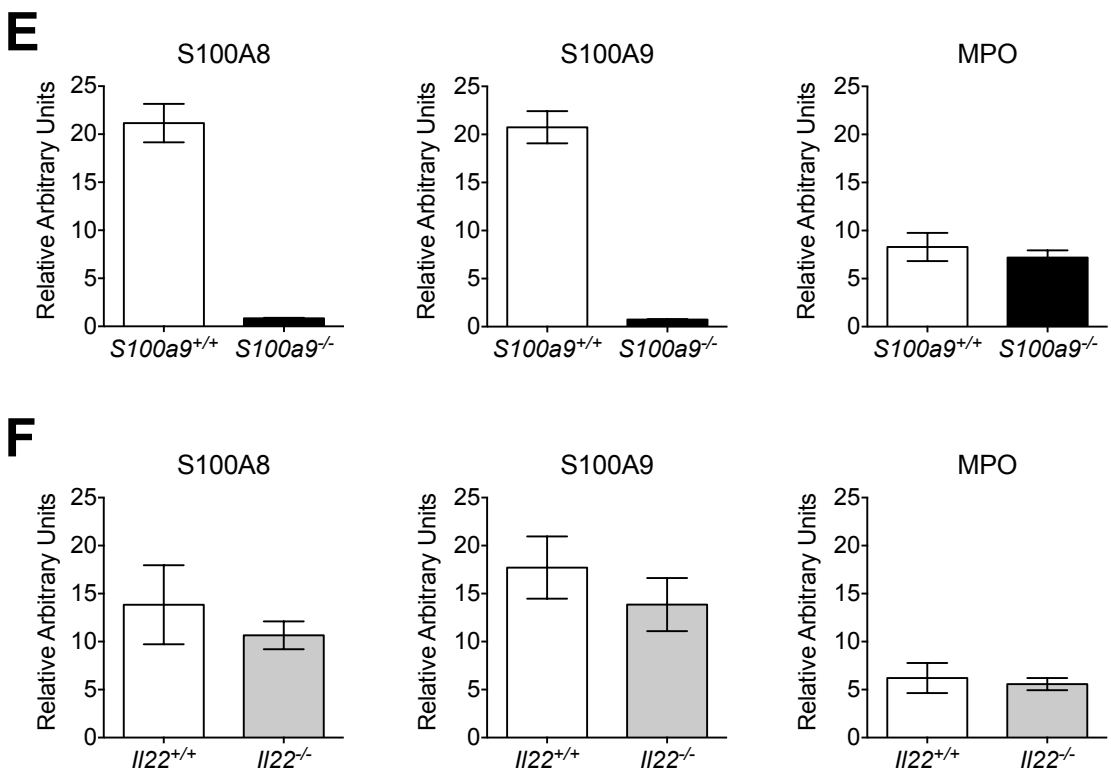
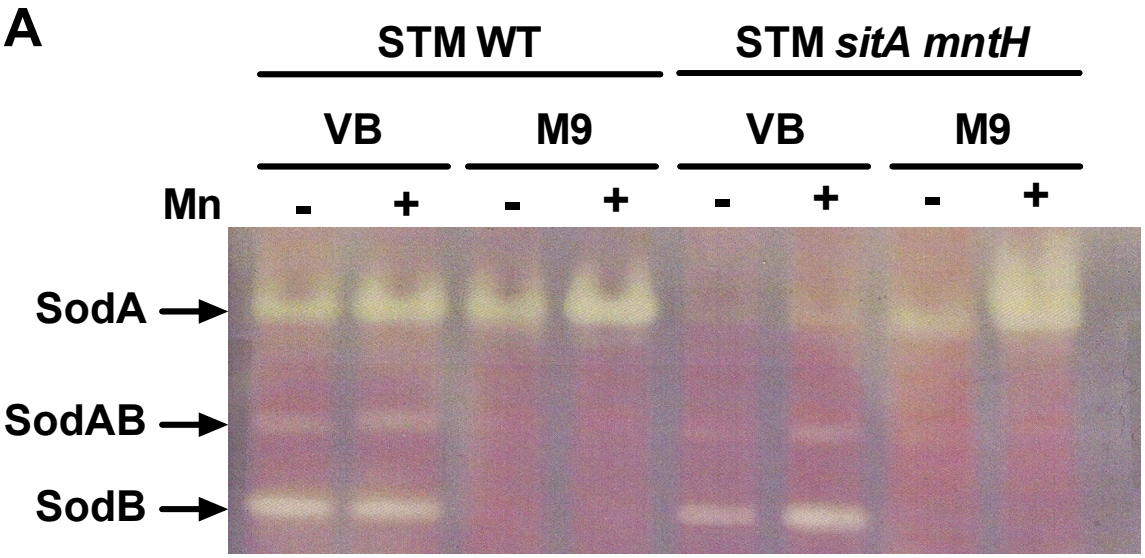
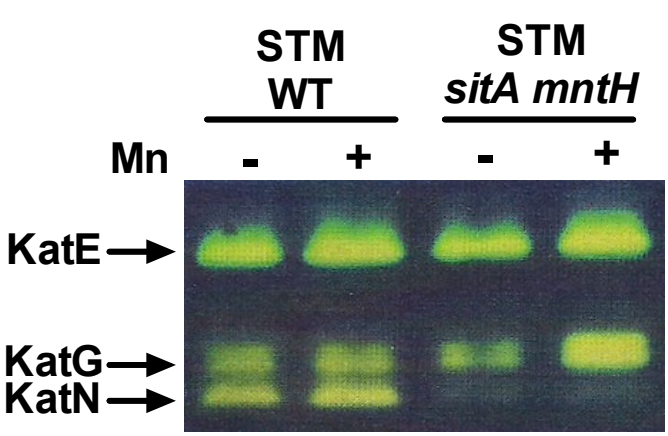

E
F
S100A8
S100A9
MPO
Relative Arbitrary Units
S100a9+/+
S100a9-/-
Il22+/+
Il22-/-

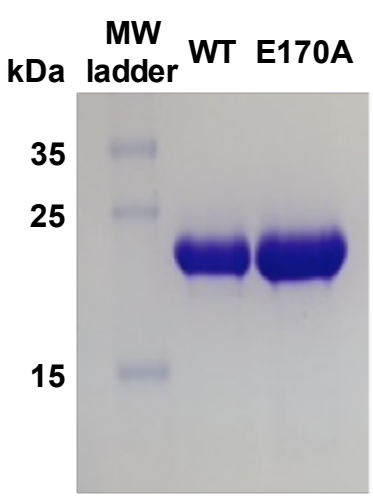
Figure S5 (Related to Figure 3)

Histopathology of the cecum and quantification of immunoblot. (**A-D**) Blinded histopathology scores of the cecum of *S100a9*$^{+/+}$, *S100a9*$^{-/-}$, *Il22*$^{+/+}$, and *Il22*$^{-/-}$ infected animals, represented in Figures 3A, 3B, 3C, and 3D; each column represents an individual mouse, and the gray quadrant includes scores indicative of moderate to severe inflammation. (**E** and **F**) Intensities of bands on immunoblots in Figure 3G and 3H were quantified using the Fuji ImageGauge/MultiGauge software (Fujifilm). Relative arbitrary units were calculated for S100A8, S100A9 and MPO by dividing the protein:tubulin band intensity ratio of infected animals by the protein:tubulin band intensity ratio of mock infected animals. Bars represent the mean relative arbitrary units ± SEM.

Figure S6


A
STM WT
STM sitA mntH
VB
M9
VB
M9
Mn
- + - + - + - +
SodA
SodAB
SodB


B
STM WT
STM sitA mntH
Mn
- + - +
KatE
KatG
KatN


C
kDa
MW ladder
WT
E170A
35
25
15

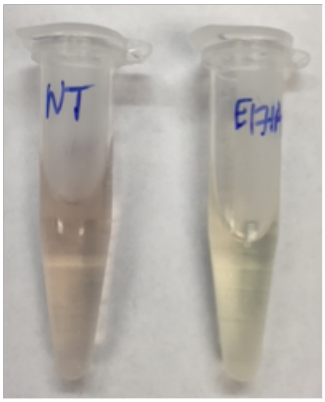

D
WT
E170A

Figure S6 (Related to Figure 6)

Metalation-dependent activity of SodA and KatN. (**A**) Superoxide dismutase activity from soluble protein extracts of STM WT or the *sitA mntH* mutant grown in Vogel-Bonner (VB) media or M9 media, with or without 3 μM $MnCl_2$ supplementation, was visualized as achromatic zones on a polyacrylamide gel that did not stain upon photochemical reduction of nitroblue tetrazolium. (**B**) Catalase activity from soluble protein extracts of STM WT or the *sitA mntH* mutant grown in LB media, with or without 3 μM $MnCl_2$ supplementation, was visualized as achromatic zones that prevented H_2O_2-dependent reduction of ferricyanideon on a polyacrylamide gel. (**C**) SDS-PAGE of pure C-terminal His-tagged SodA WT and E170A mutant (24.2 kDa) post Ni^{2+} chromatography. (**D**) Concentrated SodA WT and E170A (10 mg/ml) indicative of binding Mn(III) and Fe(III), respectively.

Figure S7

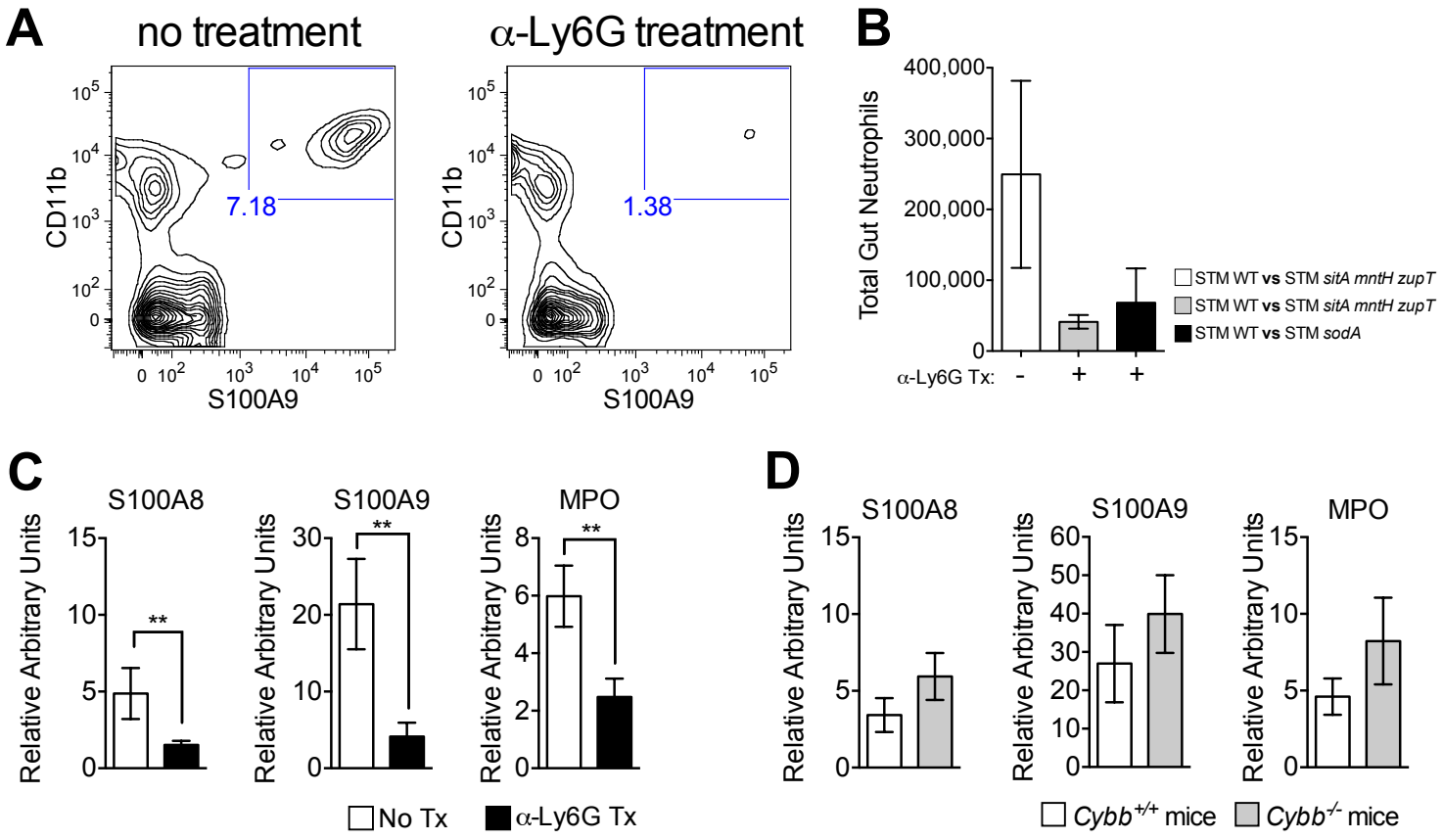

A
no treatment
α-Ly6G treatment
CD11b
S100A9
7.18
1.38
B
Total Gut Neutrophils
400,000
300,000
200,000
100,000
0
α-Ly6G Tx: - + +
STM WT vs STM sitA mntH zupT
STM WT vs STM sitA mntH zupT
STM WT vs STM sodA
C
S100A8
S100A9
MPO
Relative Arbitrary Units
**
No Tx
α-Ly6G Tx
D
S100A8
S100A9
MPO
Relative Arbitrary Units
Cybb+/+ mice
Cybb-/- mice

Figure S7 (Related to Figure 7)

Quantification of total gut neutrophils and immunoblots. (**A**) Representative contour plots of intestinal neutrophils in untreated and α-Ly6G treated animals identified through successive gating for singlets, live cells, CD11c- CD49b- F4/80-, Gr1+, and CD11b+ S100A9+. (**B**) Total gut neutrophils from untreated (white bar, n = 2), α-Ly6G treated animals co-infected with equal numbers of *S*. Typhimurium WT and the *sitA mntH zupT* mutant (grey bar, n = 4), and α-Ly6G treated animals co-infected with equal numbers of *S*. Typhimurium WT and the *sodA* mutant (black bar, n = 4). Bars represent the mean intestinal neutrophil count ± SEM. (**C**-**D**) Intensities of bands on immunoblots in Figure 7C and 7F were quantified using the Fuji ImageGauge/MultiGauge software (Fujifilm). Relative arbitrary units were calculated for S100A8, S100A9 and MPO by dividing the protein:tubulin band intensity ratio of infected animals by the protein:tubulin band intensity ratio of mock infected animals. Bars represent the mean relative arbitrary units ± SEM.

Table S1 (Related to Figure 1) Strains and plasmids used in this study.

Designation	Genotype	Source or Reference
Escherichia coli strains		
CC118 λ_{pir}	F- *araD139* Δ(*ara*, *leu*)7697 Δ*lacX74 phoA*Δ20 *galE galK thi rpsE rpoB argE*^{am} *recA1* λ_{pir}	(Herrero et al., 1990)
DH5α MCR	F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) F80d*lacZ*M15 (*lacZYA-argF*)*U169 deoR recA1 endA1 phoA supE44*l- *thi-1 gyrA*96 *relA*1	Gibco BRL
S17-1 λ_{pir}	F- *recA thi pro* rK- mK+ RP4:2-Tc::*Mu*Km Tn7 λ_{pir}	(Herrero et al., 1990)
JB2	Wild type (commensal isolated from mouse gut)	(Behnsen et al., 2014)
BL21-Gold(DE3)	F- *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ *Tet*^{r} *gal* λ(DE3) *endA* Hte	Agilent Technologies
Salmonella enterica serovar *Typhimurium* strains		
IR715	ATCC 14028, Nal^R derivative	(Stojiljkovic et al., 1995)
VDO25	IR715 Δ*sitA* (+1 to +913)::KSAC	This study
VDO29	IR715 Δ*mntH* (-120 to +1117)::KSAC	This study
VDO31	IR715 Δ*mntH* (-120 to +1117)::*scar*	This study
VDO32	IR715 Δ*mntH* (-120 to +1117)::*scar* Δ*sitA* (+1 to +913)::KSAC	This study
VDO43	IR715 Δ*mntH* (-120 to +1117)::*scar* Δ*sitA* (+1 to +913)::*tetRA* Δ*zupT* (+29 to +733)::KSAC	This study
VDO38	IR715 Δ*sodA* (-98 to +583)::*kan*	This study
VDO39	IR715 Δ*katN* (+48 to +829)::*kan*	This study
DL2	IR715 Δ*sodA* (-98 to +583)::*scar* Δ*katN* (+48 to +829)::*kan*	This study
JZL3	IR715 Δ*znuA*::Cm	(Liu et al., 2012)
AP3	IR715 Δ*zupT* (+29 to +733)::KSAC	(Cerasi et al., 2014)

Plasmids		
pCR2.1	TOPO Cloning Vector (Carb^R, Kan^R)	Invitrogen
pBS34	pBluescript II KS (+), (Carb^R, Kan^R, KSAC cassette)	
pSPN23	pBluescript II KS (+), (Carb^R, Tc^R, *tetRA* cassette)	(Raffatellu et al., 2009)
pBKS	pBluescript II KS (-), Carb^R, *lacZα*	
pRDH10	*oriR6K*, Cm^R, TC^R, *sacRB*	(Kingsley et al., 1999)
pHP45Ω	Strep^R, Carb^R	(Prentki and Krisch, 1984)
pACYΩ	Strep^R, Cm^R	Takeshi Haneda and Andreas Bäumler
pWKS30	pSC101 *ori*, Carb^R, *lacZα*	(Wang and Kushner, 1991)
pVO1	pCR2.1::FR1::FR2 *sitA*	This study
pVO2	pRDH10::FR1::FR2 *sitA*	This study
pVO3	pVO2::*tetRA*	This study
pVO4	pCR2.1::FR1 *sitA* probe	This study
pVO5	pCR2.1::*sitABCD*	This study
pVO6	pWKS30::*sitABCD*	This study
pVO7	pCR2.1::FR1::FR2 *mntH*	This study
pVO8	pRDH10::FR1::FR2 *mntH*	This study
pVO10	pCR2.1::FR1 *mntH* probe	This study
pVO11	pVO2::KSAC	This study
pVO12	pVO8::KSAC	This study
pVO13	pCR-Blunt II-TOPO::*sodA*	This study
pVO14	pWKS30::*sodA*	This study
pVO15	pCR-Blunt II-TOPO::*sodA* E170A	This study
pVO16	pWKS30::*sodA* E170A	This study

pVO17	pET28a::*sodA*	This study
pVO18	pET28a::*sodA* E170A	This study
pAP4	pRDH10::FR1::FR2 *zupT*	(Cerasi et al., 2014)
pAP7	pAP4::KSAC	(Cerasi et al., 2014)
pKD4	pANTSγ, (Kan^R)	(Datsenko and Wanner, 2000)

Table S2 (Related to Figure 1) Primers used in this study.

Designation	Purpose	Sequence (5' to 3')
1	5' flanking region of *sitA* construct	ATATGGATCCGGCGTAATATCGACAGCA
2	5' flanking region of *sitA* construct	ACTCATTCTAGAAGTATCCCTCGCAACAATGTG
3	3' flanking region of *sitA* construct	GATACTTCTAGAATGAGTCAATCTGCGATTACC
4	3' flanking region of *sitA* construct	TATAGGATCCGACGCCAGGCACAATAGAGTG
5	5' flanking region of *mntH* construct	TAATGGATCCTTTACCGTGGCGTGATGAA
6	5' flanking region of *mntH* construct	CATCGTTCTAGATTGTCGTGCTGGTCGTC
7	3' flanking region of *mntH* construct	CGACAATCTAGAACGATGACGACCTGTATCAG
8	3' flanking region of *mntH* construct	TAATGGATCCGAGATGCTGTAGCCGAACC
9	5' flanking region of *zupT* construct	GCCATAGGATCCCGAGATGTTTTCCACC
10	5' flanking region of *zupT* construct	CTTCAGTCTAGAGTAAGGTCAGAATAAGTG
11	3' flanking region of *zupT* construct	TTTCTTTCTAGAGGCTCAGTCTCGTCAT
12	3' flanking region of *zupT* construct	ATTCTTGGATCCTGCCGTCTTCTTTGCG
13	confirming *sitA* deletion by Southern blot analysis	CGCTTCCCCTTCACTTCTTG
14	confirming *mntH* deletion by Southern blot analysis	GGAGAGAAAGTATGTTCAGG

Designation	Purpose	Sequence (5' to 3')
15	confirming *zupT* deletion by Southern blot analysis	CCCACGCTTCTTTATCG
16	confirming *zupT* deletion by Southern blot analysis	ACTGACATCCATTACTC
17	pWSK30::*sitABCD* expression vector	ACTTGGATCCCGTGCTCTCTCCGAACATTA
18	pWSK30::*sitABCD* expression vector	TAACGAATTCATAAGGTTGCCTGCCAGA
19	confirming *sodA* deletion by PCR	AACAGGCGGTTTCGGTTGGA
20	confirming *sodA* deletion by PCR	TGCATCTGCTGCTCCTTACG
oli228	*sodA* H1+P1, one step inactivation	GCGGATGCCGTAACGTTTATAACCCTGGAAAA AGTACGGCTGTAGGCTGGAGCTGCTTCG
oli229	*sodA* H2+P2, one step inactivation	AATTATTTTTTAGCGGCGAAACGCGCTGCTGCT TCGTCCCCATATGAATATCCTCCTTAG
oli230	*sodA* K1 primer pair	CGGTTGGAGGCGTAAAAAAC
K1	antibiotic resistance cassette, internal primer	CAGTCATAGCCGAATAGCCT
K2	antibiotic resistance cassette, internal primer	CGGTGCCCTGAATGAACTGC
M13 Fw	sequencing	TGTAAAACGACGGCCAGT
M13 Rv	sequencing	CAGGAAACAGCTATGACC
21	pET28a::*sodA* expression vector	CGCCATGGGCAGTTATACACTGCCATCCCTGC CGTACGCTTATGATG
22	pET28a::*sodA* expression vector	CGCTCGAGTTTTTTAGCGGCGAAACGCGCTGC TGCTTCG
23	*sodA* site-directed mutagenesis	CCTGGACGTGTGGG**CG**CACGCTTACTACCTG
24	*sodA* site-directed mutagenesis	CAGGTAGTAAGCGT**GC**GCCCACACGTCCAGG
25	pWSK30::*sodA* expression vector	ACTTGGATCCTAAGGCCAACGTAGCCAGAC
26	pWSK30::*sodA* expression vector	TAACGATATCCAGACTCGCTTCTACAGACG

Table S3 (Related to Figure 3) Quantitative Real-time PCR Primers

Species	**Target**	**Primer Pairs**
Mus musculus	*Il22*	5'- GGCCAGCCTTGCAGATAACA -3' 5'- GCTGATGTGACAGGAGCTGA -3'
Mus musculus	*Il17a*	5'-GCTCCAGAAGGCCCTCAGA-3' 5'-AGCTTTCCCTCCGCATTGA-3'
Mus musculus	*Cxcl-1*	5'-TGCACCCAAACCGAAGTCAT-3' 5'-TTGTCAGAAGCCAGCGTTCAC-3'
Mus musculus	*ActB*	5'-GGCTGTATTCCCCTCCATCG-3' 5'-CCAGTTGGTAACAATGCCATGT-3'

Supplemental Experimental Procedures

Bacterial Strains and Culture Conditions

A complete list of strains, plasmids, and primers used in all experiments is provided in **Tables S1 and S2**. Unless otherwise noted, all cultures of *S.* Typhimurium and *Escherichia coli* were incubated aerobically at 37°C in Luria-Bertani (LB) broth (per liter: 10 g tryptone, 5 g yeast extract, 10 g NaCl) or on LB agar plates (1.5% Difco agar). *S*. Typhimurium wild-type and mutants were tested for their ability to grow under nutrient rich (LB) and nutrient limiting conditions (M9 minimal media per liter; 7.5g Na_2HPO_4, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl, 0.1mM $CaCl_2$, 0.5mM $MgSO_4$, 0.2% glucose) as previously described (Liu et al., 2012). Antibiotics and other chemicals were added at the following concentrations (mg/l) as needed: carbenicillin (Carb), 100; chloramphenicol (Cm), 30; kanamycin (Km), 100; nalidixic acid (Nal), 50; 5-bromo-4-choloro-3-indoyl-b-D-galactopyranoside (Xgal), 40.

Bacterial Growth Assays in LB and M9 Minimal Media

Inocula were prepared from overnight cultures of the strains grown aerobically in LB at 37 ºC with agitation. Absorbance (λ=600nm) of the overnight cultures was determined by spectrophotometry and used to calculate the volume required to obtain 10^9 cells. To prepare LB inocula, 10^9 cells were harvested by centrifugation, resuspended in 1 ml of LB, and then serially diluted. To prepare M9 minimal media inocula, 10^9 cells were washed twice in M9, resuspended in 1 ml of M9, and serially diluted. LB and M9 minimal media were inoculated with a starting culture of 10^4 cells/ml. Growth was monitored by determining the number of colony forming units (CFUs) per

ml of culture at 0, 2, 5, 8, and 16 hours of incubation at 37 ºC with agitation. When indicated, M9 was also supplemented with 5 μM $MnCl_2$. Each experiment was repeated a minimum of three times.

Construction of mutants in *S.* Typhimurium

To construct *S*. Typhimurium mutant strains carrying a deletion of *sitA, mntH* and *zupT,* DNA regions flanking the gene of interest (5' flanking region, FR1; 3' flanking region, FR2) of approximately 1000 bp in length were amplified by PCR with primers listed in **Table S2**. A *Bam*HI restriction site was added to the 5' end of the external primers and an *Xba*I restriction site was added to the 5' end of the internal primers. To facilitate amplifying FR1 and FR2 in tandem by nested PCR six complementary nucleotides were added on the internal primers upstream and downstream the *Xba*I site. For nested PCR, FR1 and FR2 were first PCR amplified in separate reactions. The PCR products were then diluted 10-fold and used in a second round of PCR containing the external primers to amplify FR1 and FR2 in tandem (FR1-FR2). A PCR product of the predicted size was gel purified and ligated to the pCR2.1 vector (TOPO TA Cloning Kit, Invitrogen). The recombinant vector reaction was used to transform *E. coli* TOP10 cells and transformants were plated on LB+Carb+Xgal agar. Plasmid DNA was purified from white colonies (QIAPrep Spin Miniprep Kit, QIAGEN) and digested with *Eco*RI to screen for the correct linearized plasmid and insert fragment sizes. Positive clones were confirmed by sequencing the insert with M13 forward and reverse universal primers. The correct plasmids were digested with *Bam*HI and the FR1-FR2 fragment were gel purified and ligated to *Bam*HI digested suicide vector pRDH10. *E. coli* CC118 λ_{pir} cells were transformed with the ligation and transformants were plated on LB+Cm agar to

select for the Cm resistance encoded by pRDH10. The pBS34 plasmid containing a genetic cassette for Km resistance (KSAC) was digested with *Xba*I and the resistance cassette was purified then ligated into the suicide vectors digested with *Xba*I. *E. coli* S17-1 λ_{pir} cells were transformed with the resulting plasmids and transformants were plated on LB+Km agar for selection. The resulting suicide vectors were then introduced into *S*. Typhimurium IR715 cells by conjugation. *S.* Typhimurium transconjugants were positively selected using LB+Nal+Km, and colonies with double cross-over events were screened for by sensitivity to Cm. Mutants were then further confirmed by Southern blot analysis.

To generate an *S*. Typhimurium mutant with deletions in both *sitA* and *mntH,* sucrose selection (Reyrat et al., 1998) was first used to produce a mutant with a clean deletion of *mntH* (Δ*mntH*), yielding VDO31 after Southern blot confirmation. Next, to generate a deletion of *sitA,* plasmid pVO11 was introduced into VDO31 by conjugation. The strain was termed VDO32 after both the Δ*mntH* and Δ*sitA*(+1 to +913)::KSAC mutations were confirmed by Southern blot analysis.

To generate an *S*. Typhimurium mutant with deletions in *sitA*, *mntH* and *zupT,* the *tetRA* cassette from pSPN23 was inserted into the Δ*sitA* suicide vector between the flanking regions of the *sitA* gene to give rise to the plasmid pVO3. This plasmid was then conjugated into strain VDO31, yielding strain VDO40 after the Δ*mntH* and *sitA*(+1 to +913)::*tetRA* mutations were confirmed by Southern blot analysis. To generate a deletion of *zupT,* plasmid pAP7 was introduced into VDO40 by conjugation, producing strain VDO43 after the Δ*mntH,* Δ*sitA*(+1 to +913)::*tetRA*, and Δ*zupT*(+29 to +733)::KSAC mutations were confirmed by Southern blot analysis.

To construct an *S*. Typhimurium strain carrying a deletion of *sodA* or *katN* we followed the one-step inactivation protocol (Datsenko and Wanner, 2000) using the *sodA* targeting primers oli228 and oli229, the *katN* targeting primers oPF12 and oPF13 (Pacello et al., 2012), and the pKD4 template plasmid. Deletions were confirmed by PCR with primers annealing upstream of the mutated *sodA* (oli230) and *katN* (oPF37) alleles and an internal primer annealing within the inserted Km resistance cassette (K1). The Δ*sodA*::Km and Δ*katN*::Km regions were then transduced into a clean *S*. Typhimurium IR715 background by generalized transduction with phage P22 HT 105/1 *int-201.* To construct an *S*. Typhimurium mutant carrying a deletion in both *sodA* and *katN* we first removed the Km resistance gene in the *sodA* mutant by transforming this strain with the temperature-sensitive Ap^R plasmid pCP20 that encodes the FLP recombinase. Carbenicillin resistant transformants were selected at 30 °C, colony-purified once, non-selectively, at 37 °C, and tested for sensitivity to Km and Ap. Generation of the Δ*sodA*::scar mutant, termed DL1, was confirmed by PCR using oligos flanking *sodA*. The *sodA katN* double mutant was then generated by generalized transduction of the mutated *katN* allele onto the Δ*sodA*::scar background with phage P22 HT 105/1 *int-201*. The *sodA katN* double mutant, termed DL2, was confirmed by PCR using oligos that flank the *sodA* and *katN* alleles (oligos 19-24, **Table S2**).

An *S*. Typhimurium quadruple mutant carrying deletions in *sitA*, *mntH*, *zupT* and *sodA* was generated by first constructing a *sodA*::*cat* Cm^R mutant with the one-step inactivation protocol (Datsenko and Wanner, 2000) using the *sodA* targeting primers oli228 and oli229 and the pKD3 plasmid. The *sodA*::*cat* mutation was confirmed by PCR with primers annealing upstream of the mutated *sodA* (oligo 19) and an internal primer

annealing within the inserted antibiotic resistance cassette (C1). The *sitA mntH zupT sodA* mutant was then generated by generalized transduction of the mutated *sodA* allele onto the VDO43 background with phage P22 HT 105/1 *int-201*. The mutant was termed VDO45 after the mutation in the *sodA* allele was confirmed as described.

Genetic Complementation of the Manganese Transporter Deficient Strains

To complement the manganese transporter deficient strains, the *sitABCD* operon, including the promoter region, was amplified by PCR using primers 17 and 18 (**Table S2**) and ligated to the pCR2.1 vector (TOPO TA Cloning Kit, Invitrogen) to generate pVO5. The region containing the *sitABCD* operon was excised from pVO5 using BamHI and EcoRI double-digestion and ligated into BamHI and EcoRI digested pWSK30 (a low-copy vector) to generate pVO6. Next, pVO6 was electroporated into *S*. Typhimurium strains for complementation *in trans*.

Bacterial Growth Assays in LB Supplemented with Calprotectin

S. Typhimurium wild-type and mutants were tested for their ability to grow in LB supplemented with either wild-type or mutant calprotectin (CP) with or without 50 μM $MnCl_2$. To prepare the inocula, each strain was grown in M9 minimal media at 37 ºC for 20 hours with agitation. Absorbance (λ=600nm) of the overnight cultures was determined by spectrophotometry and used to calculate the volume required to obtain 10^9 cells. Resuspended cells were serially diluted 10,000 fold in M9 minimal media and 10 μl were then used to inoculate the wells of a 96-well Nunclon Surface plate (Nunc). Each well contained 100 μl of a 10:28:62 ratio of inoculum to LB media to CP buffer (20

mM Tris pH 7.5, 100 mM β-mercaptoethanol, 3 mM $CaCl_2$). Recombinant wild-type and mutant CP were produced as described in (Damo et al., 2013; Kehl-Fie et al., 2011; Pacello et al., 2012). Wild-type and mutant CP was added to the media to final concentrations of 0, 62.5, 125, 250, and 500 µg of the protein per ml prior to inoculation. The 96-well plate was incubated at 37 ºC with 5% CO_2. Growth of the wild-type and mutant *S*. Typhimurium strains was monitored by determining the CFUs per ml of culture at 2, 5, 8, and 16 hours of incubation. For growth under anaerobic conditions (3% hydrogen, 5% carbon dioxide, and 92% nitrogen), inocula were prepared inside a Bactron II Anaerobic Chamber (Shell Lab). Strains were initially grown under anaerobic conditions in M9 minimal media at 37 ºC for 24 hours without agitation. Wild-type CP was added to LB media to final concentrations of 0, 125, and 250 µg of the protein per ml prior to inoculation. The 96-well plate was placed in a moist chamber and incubated in anaerobic conditions at 37 ºC. Growth of the wild-type and mutant *S*. Typhimurium strains was monitored by determining the CFUs per ml of culture at 2 and 16 hours of incubation. Each experiment was repeated a minimum of three times.

Measurement of Manganese in Fecal Samples by ICP-MS

C57BL/6 mice were treated with streptomycin and mock infected or infected with the wild-type strain of *S*. Typhimurium as described. At 96 hours post-infection, fecal pellets were collected with plastic forceps and placed in glass containers that were previously cleaned with nitric acid to remove metal contamination. The fecal pellets were then autoclaved to kill all bacteria. Sample analysis was performed by Applied Speciation (Bothell, WA) as described in (Corbin et al., 2008). Briefly, the fecal samples from 4 infected and 4 mock-infected mice were digested by boiling in nitric acid and

hydrochloric acid. The samples were then resuspended in water and analyzed by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). Aliquots of each sample are introduced into a radio frequency (RF) plasma where the energy transfer process causes desolvation, atomization, and ionization. The ions were extracted from the plasma through a differentially-pumped vacuum interface and traveled through a pressurized chamber (DRC) containing a specific reactive gas which preferentially reacts with interfering ions of the same target mass to charge ratios (m/z). A solid-state detector detected ions transmitted through the mass analyzer, on the basis of their mass-to-charge ratio (m/z), and the resulting current was processed by a data handling system. The results were reported as manganese per kg of dry weight.

Animal Infections

One day prior to infection, mice were given streptomycin (1 mg per gram body weight) by oral gavage. Twenty-four hours after streptomycin treatment, mice were infected with either $1x10^9$ CFU of a single *S*. Typhimurium strain or co-infected with $1x10^9$ CFU of a mixture of two *S*. Typhimurium strains at a 1:1 ratio. Colon contents were collected 48-96 hours post-infection (p.i.), serially diluted, and plated on selective media to determine the CFUs of each strain of *S*. Typhimurium used in the infection. The cecum was also harvested 96 hours p.i. for mRNA, protein, and histopathology. In mice co-infected with two strains of *S*. Typhimurium the competitive index was calculated by dividing the output ratio (CFU of wild type divided by CFU of the mutant) by the input ratio (CFU the wild type divided by CFU of the mutant). Mice co-infected with a mixture of *S.* Typhimurium and *E. coli* were given $1x10^9$ CFU of a 1:1 ratio of *S.* Typhimurium (either wild-type or the *sitA mntH zupT* mutant) and *E. coli* (JB2) twenty-

four hours post-streptomycin treatment. Colon contents were collected 48-96 hours p.i., serially diluted, and plated on selective media to determine the CFUs of the *Salmonella* strain and *E. coli*. The competitive index was calculated by dividing the output ratio (CFU of *Salmonella* strain by CFU of *E. coli*) by the input ratio (CFU of the *Salmonella* strain by the CFU of *E. coli*).

Intestinal Cell Isolation, Antibody Staining, and Flow Cytometry Analysis

Large intestine (colon and cecum) was collected and kept in IMDM medium (10% FBS, 1% antbiotic/antimycotic) at 4 ºC until further processing. Next, the intestine was cut open longitudinally, washed in 15 mM HEPES/ 1% antibiotic/antimycotic in 1x HBSS, (all Invitrogen), until the supernatant appeared clear. Then the intestine was shaken in 10 ml of 1x HBSS/ 15 mM HEPES/ 5 mM EDTA/ 10% FBS solution at 37 ºC in a water bath for 15 min. Supernatant was removed and kept on ice. Remaining tissue was cut into small pieces and submerged in a 10 ml mixture of collagenase (Sigma, Type VII, 1 mg/ml), Liberase (20 ug/ml), and DNAse (0.25 mg/ml) in warmed IMDM medium and run once on a GentleMACS dissociator (Miltenyi Biotech) using program spleen_04.01, digested for 15 min in a shaking water bath, followed by another run on the GentleMACS dissociator (program spleen_04.01). Afterwards, both fractions were strained through a 70 µm cell strainer (BD Biosciences) and pooled, followed by cell counting using a hemocytometer. $1x10^6$ extracted large intestinal cells were used for flow cytometry staining. Intestinal cells were stained with a fixable viability dye, antibodies against CD11b and Gr-1 to identify neutrophils, and also stained for CD11c,

CD49b, and F4/80 (all eBioscience) for exclusion of dendritic cells, NK cells, and macrophages. S100A9 antibody was conjugated in-house to the Pacific Blue fluorophore (R&D Systems, LifeTechnologies). All flow cytometry data was obtained using a BD LSR-II and analyzed using FlowJo (Treestar).

Western Blotting

Total protein was extracted from mouse cecal tissue using Tri-Reagent (Molecular Research Center). Protein concentration was determined by colorimetric spectrophotometry using the Micro BCA Protein Assay Kit (Thermo Fischer) and 15 µg of total protein was separated by 15% SDS-PAGE. Protein was then electroblotted to PVDF membranes, blocked with 2% non-fat dried milk in 1X PBS and incubated overnight at 4 °C with polyclonal goat anti-mouse S100A8, polyclonal goat anti-mouse S100A9 (R&D Systems), polyclonal goat anti-human and mouse myeloperoxidase (R&D Systems), or polyclonal rabbit anti-mouse α/β-tubulin (Cell Signaling Technology) in 5% BSA in 1X PBS. The following day blots were washed and then incubated for 1 hour with horseradish peroxidase conjugated anti-rabbit or anti-goat secondary antibodies (Jackson Immuno Research). The blots were washed then developed using the Immobilon Western Luminol Reagent and Peroxide Solution (Millipore) as per manufacture's instructions. Digital images of the blots were captured using a Fujifilm LAS 400. Band intensity on immunoblots in Figure 3G, 3H, 7C and 7H were quantified using the Fuji ImageGauge/MultiGauge software (Fujifilm). Relative arbitrary units were calculated for S100A8, S100A9 and MPO by dividing the protein:tubulin band intensity

ratio of infected animals by the protein:tubulin band intensity ratio of mock infected animals. Bars represent the mean relative arbitrary units ± SEM.

Quantitative Real-Time PCR

Total RNA was extracted from mouse cecal tissue using Tri-Reagent (Molecular Research Center). Reverse transcription of 1 μg of total RNA was performed using the Transcription First Strand cDNA synthesis kit (Roche). Quantitative real-time PCR (qRT-PCR) for the expression of *ActB*, *Il17a*, *Il22*, and *Cxcl1* (**Table S3**) were performed using the LightCycler 480 SYBR Green Master on the LightCycler 480 II (Roche). Conditions for qRT-PCR were 95 °C for 5 minutes followed by 45 cycles of 95 °C for 10 seconds, 60 °C for 10 seconds, and 72 °C for 15 seconds. Gene expression was graphed as fold-change in expression relative to uninfected controls as calculated by the *ΔΔ* Ct method after normalizing to β-actin expression.

Histopathology

Tissue samples were fixed in formalin, processed according to standard procedures for paraffin embedding, sectioned at 5 μm, and stained with hematoxylin and eosin. The pathology score of cecal samples was determined by blinded examinations of cecal sections from a board-certified pathologist using previously published methods (Barthel et al., 2003; Liu et al., 2012; Raffatellu et al., 2009). Each section was evaluated for the presence of neutrophils, mononuclear infiltrate,

submucosal edema, surface erosion, inflammatory exudates, and cryptitis. Inflammatory changes were scored from 0 to 4 according to the following scale: 0 = none; 1 = low; 2 = moderate; 3 = high; 4 = extreme. The inflammation score was calculated by adding up the scores obtained for each parameter and interpreted as follows: 0-2 = within normal limit; 3-5 = mild; 6-8 = moderate; 8+ = severe.

SodA Cloning, Expression, Purification and Complementation

The *sodA* gene was PCR-amplified from *S*. Typhimurium IR715 genomic DNA using primers 21 and 22 (**Table S2**) and subsequently cloned into pET28a (Novagen) using *NcoI* and *XhoI* (Fermentas Scientific) to generate pVO17. BL21-Gold (DE3) cells were transformed with the resulting pET28a::*sodA* plasmid and grown at 37 ºC in LB medium supplemented with 30 µg/mL kanamycin and 200 µM manganese chloride. C-terminal His_6-tagged SodA expression was induced when cells reached OD600 of 0.8 by the addition of 1 mM IPTG and cells were harvested after 3 hours by centrifugation at 5,100 rpm for 20 minutes, followed by resuspension in 50 mM Tris, pH 7.4, 350 mM NaCl and 10 mM imidazole. Cells were then lysed by sonication after addition of hen egg lysozyme (5 mg, Sigma) with phenylmethylsulfonyl fluoride (40 µM, Sigma) and the cell lysate centrifuged at 14,000 rpm for 20 minutes. The supernatant was filtered using a 0.45 µm membrane, loaded onto a Ni^{2+}-charged HisTrap column (GE Healthcare), and eluted with a linear imidazole gradient. Fractions containing SodA (between 100 – 250 mM imidazole) were visualized by SDS-PAGE, pooled and dialyzed against 50 mM potassium phosphate pH 7.0 and eventually concentrated using an Amicon centrifugal

filter (10 kD cut-off, Millipore) to 10 mg/mL (0.4 mM) for UV/vis spectrometry and inductively coupled plasma mass spectrometry analyses (conducted by UCLA Environmental Health Sciences ICP-MS facility). The plasmid encoding the *sodA* E170A mutant (pVO18, **Table S1**) was generated with the QuikChange II kit (Agilent) using primers 23 and 24 (**Table S2**) and plasmid pVO17. Expression and purification for SodA E170A proceed as with the wild-type protein.

To complement the *sodA* deficient strain, the *sodA* gene, including putative regulatory elements (Compan and Touati, 1993), was amplified by PCR using primers 25 and 26 (**Table S2**) and ligated to the pCR-Blunt II-TOPO vector (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) to generate pVO13. The region containing the *sodA* gene was excised from pVO13 using BamHI and EcoRV double-digestion and ligated into BamHI and EcoRV digested pWSK30 (a low-copy vector) to generate pVO14. The *sodA* E170A mutation was generated from pVO13 with the QuikChange II (Agilent) and primers 23 and 24 (**Table S2**) to generate pVO15. The *sodA* mutant gene was subcloned in pWSK30 as with the wild-type to generate pVO16. Next, pVO14 and pVO16 were electroporated into *S*. Typhimurium strains for complementation *in trans*.

Superoxide Dismutase and Catalase Activity Assays

To determine superoxide dismutase activity under metal restrictive conditions, wild-type *S.* Typhimurium and the *sitA mntH* mutant were cultured overnight in M9 minimal medium with or without 3 μM $MnCl_2$ supplementation. Vogel Bonner (VB) media was used to enhance iron uptake and promote SodB expression. Cells were harvested

by centrifugation, resuspended in 1 ml of Lysis Buffer (100 mM NaCl, 1 mM EDTA, and 50 mM Tris, pH 8.0) and lysed by sonication. To prevent inactivation of heat-labile enzymes, cells were lysed with four rounds of 15-s pulses with a Branson B-12 sonicator and a microtip at a power setting of 6 and kept on ice between rounds. Soluble proteins were separated from debris by centrifugation at 4 °C for 20 minutes at 17,000 x g. Protein concentration was determined by the method of Lowry et al. (1951). Superoxide dismutase activity was detected by loading 50 µg of protein per lane on a nondenaturing polyacrylamide gel and visualized according to the method of Beauchamp and Fridovich (1971). SODs were identified as achromatic zones in the gel that did not stain upon photochemical reduction of nitroblue tetrazolium to formazan blue.

To determine catalse activity of wild-type *S.* Typhimurium and the *sitA mntH* mutant these strains were cultured overnight in LB and their soluble proteins were extracted as described immediately above. Catalase activity was detected by loading 50 µg of protein per lane on a nondenaturing polyacrylamide gel and visualized according to the method of Woodbury et al. (1971). Gels were washed in distilled water and then incubated with 0.003% hydrogen peroxide for 10 minutes. After a rapid rinse with distilled water gels were placed in a solution containing 1% ferric chloride and 1% potassium ferricyanide (III) for 5 minutes. Achromatic zones against the dark background are due to the activity of catalases, which prevent the H_2O_2-dependent reduction of ferricyanide to ferrocyanide .

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