Supplementary Table 1. Strains and plasmids

Plasmid or Strain	Genotype	Source
pKD46	*bla araC*-P_{araB}-γβ *exo* oriR101 repA101ts	Datsenko and Wanner 2000
pKD3	*bla FRTcatFRT* PS1 PS2 oriRγ	Datsenko and Wanner 2000
pKD4	*bla FRTaphFRT* PS1 PS2 oriRγ	Datsenko and Wanner 2000
pKD13	*bla FRTaphFRT* PS1 PS4 oriRγ	Datsenko and Wanner 2000
pCP20	*bla cat* cI857 lPr *flp* PSC101 oriTS	Datsenko and Wanner 2000
pRB3-273C	*bla par* RK2 oriV *trfA*	Berggren et al. 1995
pJK671 (pZupT)	*bla par* RK2 oriV *tfrA* P_{zupT}-*zupT*	Karlinsey et al. 2010
pEF97 (pMntH)	*bla par* RK2 oriV *trfA* P_{mntH}-*mntH*	This study
pEF98 (pSitABCD)	*bla par* RK2 oriV *trfA* P_{sit}-*sitABCD*	This study
JK237 (WT)	14028s	ATCC
JK377	14028s *fliC5569::tetRA*(+1 UTR) Δ*fljBA::frt-kan-frt*	Frawley et al. 2018
JK601	*mntH11::kan*	Karlinsey et al. 2010
JK603	*sitA100::*Mu*d*Cm	Karlinsey et al. 2010
JK605	*mntH11::kan sitA100::*Mu*d*Cm	This study
JK715	Δ*zupT::frt-kan-frt*	Karlinsey et al. 2010
JK716	Δ*zupT::frt-cm-frt*	This study
JK719	*mntH11::kan* Δ*zupT::frt-cm-frt*	This study
JK720	*sitA100::*Mu*d*Cm Δ*zupT::frt-kan-frt*	This study
JK830	*sitA100::*Mu*d*Cm Δ*zupT::FRT*	This study
JK831	*mntH11::kan sitA100::*Mu*d*Cm Δ*zupT::FRT*	This study
JK895 (FLS187)	14028s/pRB3-273C	Richardson et al. 2011
EF394	Δ*fur::cm*	Frawley et al. 2013
EF610	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR)	This study

EF622	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) *mntH11::kan*	This study
EF623	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) *sitA100::*Mu*d*Cm	This study
EF624	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) *mntH11::kan sitA100::*Mu*d*Cm	This study
EF633	Δ*mntR::frt-kan13-frt*	This study
EF648	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) Δ*zupT::frt-kan-frt*	This study
EF649	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) Δ*zupT::FRT*	This study
EF650	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) Δ*zupT::FRT mntH11::kan*	This study
EF651	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) Δ*zupT::FRT sitA100::*Mu*d*Cm	This study
EF653	Δ*fljBA::FRT fliC5569::tetRA* (+1UTR) Δ*zupT::FRT mntH11::kan sitA100::*Mu*d*Cm	This study
EF664	*mntH11::kan sitA100::*Mu*d*Cm Δ*zupT::FRT* /pRB3-273C	This study
EF674	*mntH11::kan sitA100::*Mu*d*Cm Δ*zupT::FRT* /pEF97	This study
EF675	*mntH11::kan sitA100::*Mu*d*Cm Δ*zupT::FRT* /pEF98	This study
EF676	*mntH11::kan sitA100::*Mu*d*Cm Δ*zupT::FRT* /pJK671	This study
EF733	Δ*mntR::FRT*	This study
EF752	Δ*fur::cm* (anaerobic)	This study
EF753	Δ*mntR::FRT* Δ*fur::cm* (anaerobic)	This study

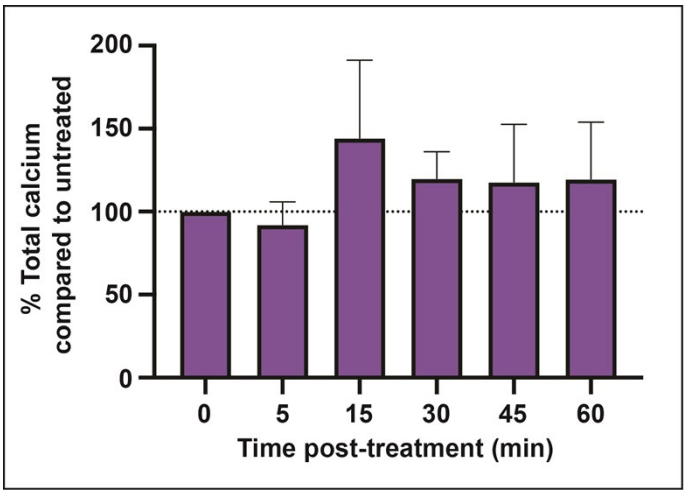
Supplementary Table 2. Primer sequences

Primer	Sequence 5'-3'	Purpose
EF292	TGCCCAGACAACTCTGGTTTACAATGAGCGCTCTGTCAGC GTGTAGGCTGGAGCTGCTTC	Creation of EF633
EF293	CAGCCAGCCAACCCTTAGCGGGCAAATACGTAAGCTGGAA GTGTAGGCTGGAGCTGCTTC	Creation of EF633
EF300	ATGTTTGCTGGGGGCAGTGATGTGTTTAATGGATACCCC GGTGTAGGCTGGAGCTGCTTC	Creation of EF635
EF301	TTAACCGTGAAAATGCGTCCAGAGGATCTGGACGCCAA TTCATATGAATATCCTCCTTAG	Creation of EF635
EF302	ACCTCCTGATCTTTCTATCCCATCG	EF633 validation
EFP306	ATGTTAATGTTGCGCCGTCAATTGG	EF635 validation
EFP307	ACCGGATCCTCGTGCACATTCTATGCAACAGCC	Creation of pEF97
EFP308	ACCAAGCTTTTATGACAACCCCATCACCGTTCC	Creation of pEF97
EFP309	CAAGGTACCCACTGCATATTGTCGCCATTTCGC	Creation of pEF98
EFP310	ACCAAGCTTTTAACCTTGCGCCTCTGGTACGATTTC	Creation of pEF98
EFP321	CGCGATAACGACATGAAAGAGC	Sequencing of pEF98
EFP322	CATTGCATCGCCCATTAACG	Sequencing of pEF98
JKP227	ACTCATTAGGCACCCCAGGC	Sequencing of pEF97, pEF98
JKP244	CTCTTCGCTATTACGCCAGC	Sequencing of pEF97, pEF98
JKP272	ACCACTTATTCTGACCTTACTGGCGGGCGCCGCCACCT TTGTGTAGGCTGGAGCTGCTTC	Creation of JK716 Karlinsey et al. 2010
JKP273	CTATCGTCTGCAAAATGACGAGACTGAGCCCCATGATG GACATATGAATATCCTCCTTAG	Creation of JK716 Karlinsey et al. 2010
mntH qPCR F	CTGACAATCGCGTAGAGAATAGCAGC	*mntH* qPCR
mntH qPCR R	CCTGAATATTGGTCGCGAAGTTAC	*mntH* qPCR
sitA qPCR F	GACGAATCTACATCGTCTGAAAAC	*sitA* qPCR
sitA qPCR R	CGCCATGTCGGCAATAACGGTAAAC	*sitA* qPCR
zupT qPCR F	GATCATGCTGCTTATCTCGCTG	*zupT* qPCR
zupT qPCR R	CCAGATCCTGCGGATGAGCGTG	*zupT* qPCR

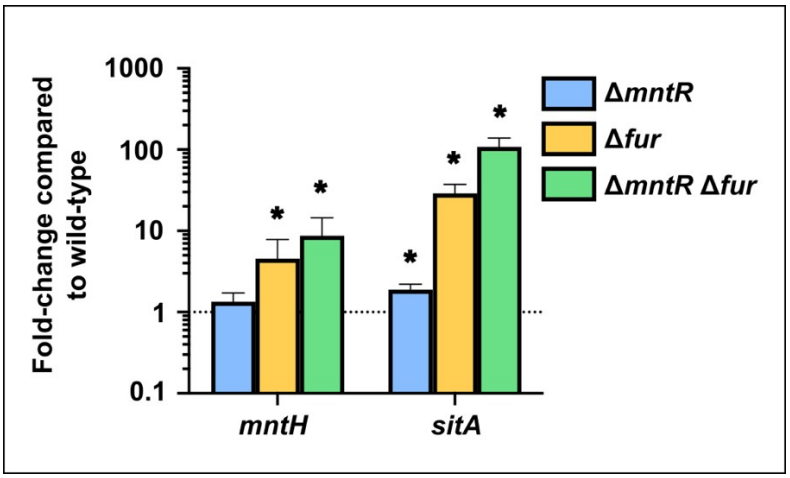
Supplementary Table 3. Mean ICP-MS values with standard deviation

	WT aMoles/ cell	*mntH* aMoles/ cell	*sitA* aMoles/ cell	*mntH sitA* aMoles/ cell	*mntH* Δ*zupT* aMoles/ cell	*sitA* Δ*zupT* aMoles/ cell	*mntH sitA* Δ*zupT* aMoles/ cell
0 min	0.08 +/- 0.01	0.09 +/- 0.04	0.09 +/- 0.01	0.09 +/- 0.01	0.06 +/- 0.01	0.06 +/- 0.01	0.07 +/- 0.03
5 min	0.10 +/- 0.02	0.11 +/- 0.01	0.11 +/- 0.02	0.10 +/- 0.02	0.06 +/- 0.01	0.06 +/- 0.01	0.08 +/- 0.02
15 min	0.15 +/- 0.02	0.15 +/- 0.02	0.19 +/- 0.01	0.15 +/- 0.01	0.10 +/- 0.05	0.08 +/- 0.03	0.09 +/- 0.01
30 min	0.22 +/- 0.03	0.22 +/- 0.03	0.28 +/- 0.06	0.19 +/- 0.02	0.17 +/- 0.04	0.15 +/- 0.08	0.08 +/- 0.01
45 min	0.13 +/- 0.03	0.16 +/- 0.03	0.18 +/- 0.02	0.12 +/-0.03	0.17 +/- 0.07	0.16 +/- 0.04	0.09 +/- 0.02
60 min	0.11 +/- 0.03	0.11 +/- 0.01	0.12 +/- 0.01	0.10 +/- 0.02	0.09 +/- 0.01	0.09 +/- 0.03	0.08 +/- 0.01

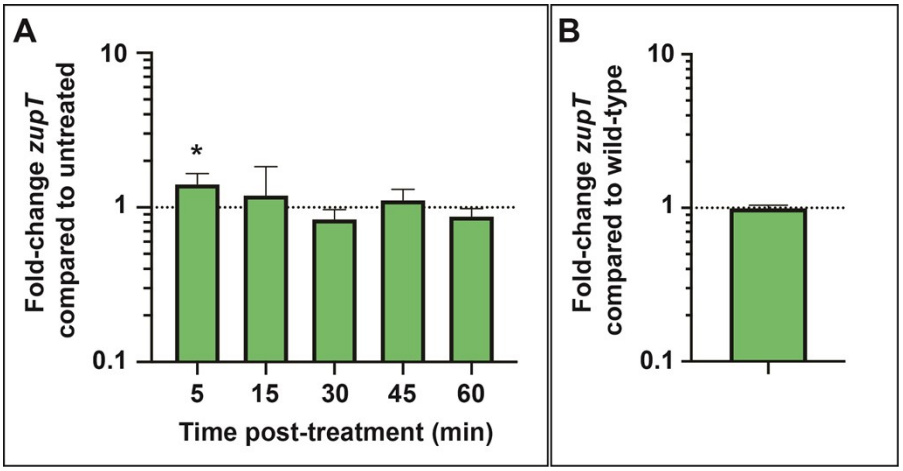
Supplementary Material



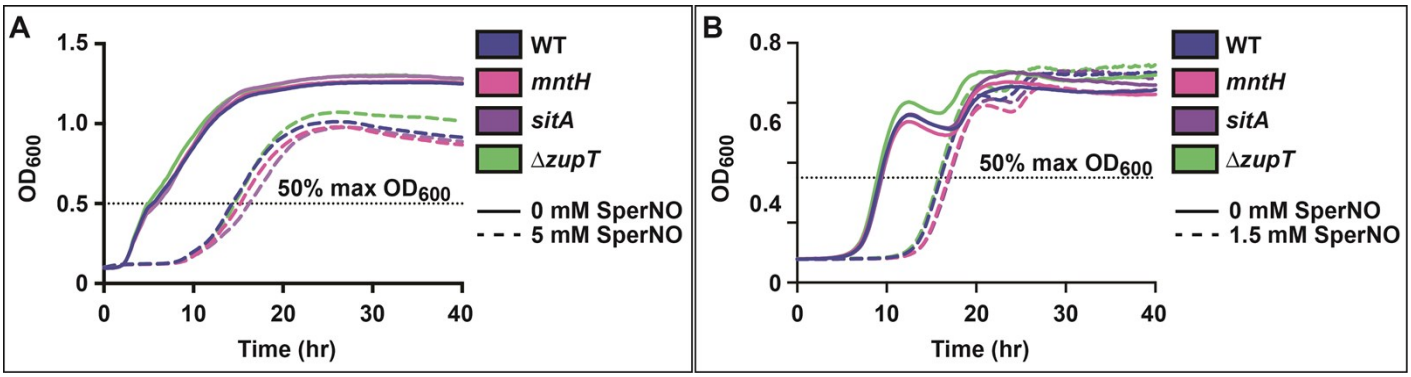
Supplementary Figure 1. Total calcium levels do not vary significantly in response to nitric oxide. Total cellular calcium was determined by inductively-coupled plasma mass spectrometry (ICP-MS) following addition of 2 mM diethylamine NONOate (DEANO) to $OD_{600} \approx 1$ *S.* Typhimurium cultures and compared to that of untreated cells. At no time post-treatment did calcium levels differ significantly from those in untreated cells.



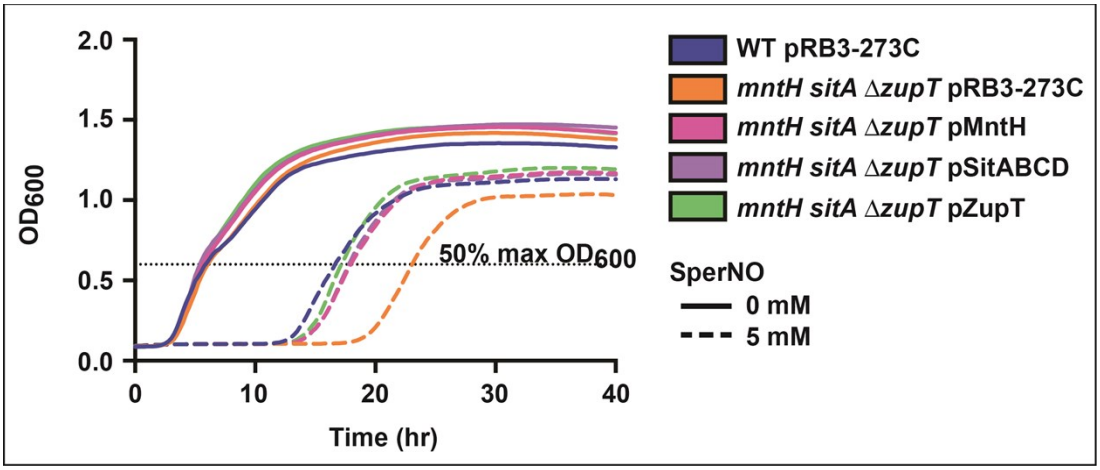
Supplementary Figure 2. Deletion of *mntR* and *fur* leads to derepression of *mntH* and *sitA*. Total RNA was isolated from untreated $OD_{600} \approx 1$ *S.* Typhimurium cultures and expression of *mntH* and *sitA* was determined by qPCR. Data are presented as positive mean fold-change compared to wild-type. Error bars represent standard deviation. Statistical significance (*) was determined by a one-sample t-test to a hypothetical mean of 1, indicating no difference in expression between mutant and wild-type. In the absence of repressor *mntR* (blue), expression of *sitA* is increased compared to wild-type ($p=0.001$). In the absence of *fur* (gold), expression of both *mntH* and *sitA* is increased ($p=0.047$, $p<0.001$). The greatest depression for *mntH* and *sitA* was observed in the absence of both *mntR* and *fur* (green, $p<0.001$, $p=0.01$).



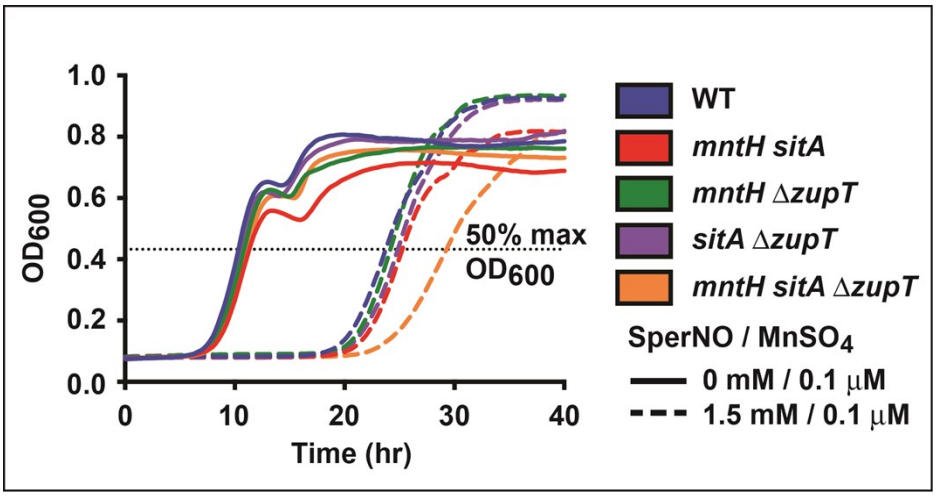
Supplementary Figure 3. Expression of *zupT* is not altered in an *mntH sitA* mutant. Expression of *zupT* was determined by qPCR following isolation of total RNA from untreated $OD_{600} \approx 1$ *S.* Typhimurium cultures and cultures treated with 2 mM DEANO. Data are shown as the positive mean fold-change with error bars representing standard deviation. (A) Expression of *zupT* increased 1.4-fold (p=0.04) at 5 min post-treatment with 2 mM DEANO in the *mntH sitA* mutant but was no different than untreated *mntH sitA* at any other time. (B) Expression of *zupT* was not significantly different in the *mntH sitA* mutant compared to expression in wild-type.



Supplementary Figure 4. *S.* Typhimurium mutants lacking a single manganese transporter are not sensitive to NO·. Mutants lacking the function of a single manganese transporter were tested for sensitivity to NO· during growth using the sustained release donor SperNO. (A) In LB, treatment with 5 mM SperNO increased the time to reach 50% maximum optical density compared to untreated cultures but none of the mutant strains were significantly different than wild-type. (B) In M9 minimal medium with no manganese, time to reach 50% maximum optical density in the presence of 1.5 mM SperNO was not significantly different for any mutant compared to wild-type.



Supplementary Figure 5. Plasmid-based expression of MntH, SitABCD or ZupT from the native promoter complements the growth defect of a *mntH sitA* Δ*zupT* strain in the presence of NO·. In the presence of NO· (dashed lines), an *mntH sitA* Δ*zupT* strain containing empty plasmid (orange) displayed a delay in growth ($p<0.001$). Expression of MntH (pink), SitABCD (purple) or ZupT (green) restored growth similar to that of wild-type (blue).



Supplementary Figure 6. Only the *mntH sitA* Δ*zupT* transport mutant is more sensitive to NO· when M9 medium is supplemented with manganese. Growth of wild-type and mutant *S.* Typhimurium was monitored in M9 medium supplemented with 0.1 μM $MnSO_4$, with and without the addition of 1.5 mM SperNO. In the absence of SperNO, all strains grew similarly. In the presence of SperNO, supplementation with $MnSO_4$ was sufficient to complement the growth defects of all mutants except for that of the *mntH sitA* Δ*zupT* mutant ($p<0.001$).