Supplemental Table S2. Bacterial strains and plasmids.

Strain or plasmid	Genotype or relevant characteristics	Source, Reference or Construction
Strain		
LB402	*Salmonella enterica* serovar Typhimurium wild type 14028s	ATCC
IB3	*hmp::FRTkanFRT*	(Bang et al., 2006)
IB682	*nsrR::FRTkanFRT*	(Bang et al., 2006)
CS015	*phoP102*::Tn*10d-Cam*	(Miller et al., 1989)
χ^{3642}	*invA::TnphoA(kan)*	(Galan and Curtiss, 1989)
JK339	14028s Δspi-1::*FRTkanFRT*	(Karlinsey et al., 2010)
LB40	*phoP102*::Tn*10d-Cam*	øP22-CS015 X LB402
JK911	14028s *hmp::FRTkanFRT*	øP22-IB3 X LB402
JK912	14028s *nsrR::FRTkanFRT*	øP22-IB682 X LB402
JK937	14028s *invA::TnphoA(kan)*	øP22-χ^{3642} X LB402
LB271	14028s *STM1808*::*FRTcatFRT*	This study
LB273	14028s *yeaR*::*FRTkanFRT*	This study
LB275	14028s *ytfE::cat*	This study
LB276	14028s *hcp*::*FRTkanFRT*	This study
JK944	14028s *ygbA::* *FRTkanFRT*	This study
HR264	14028s *Δhcp-hcr::FRTcatFRT*	This study
JK928	14028s *hmp:: FRTkanFRT STM1808*::*FRT*	This study
JK929	14028s *hmp:: FRTkanFRT yeaR*::*FRT*	This study
JK930	14028s *hmp:: FRTkanFRT hcp*::*FRT*	This study
JK938	14028s *hmp:: FRTkanFRT ytfE::cat*	This study
JK975	14028s *hmp:: FRTkanFRT ygbA::FRT*	This study
HR267	14028s *hmp:: FRTkanFRT Δhcp-hcr::FRTcatFRT*	This study
JK932	14028s *hmp:: FRTkanFRT nsrR::FRT*	This study
JK1052	14028s *hmp:: FRTkanFRT nsrR::FRT STM1808*::*FRTcatFRT*	This study
JK1053	14028s *hmp:: FRTkanFRT nsrR::FRT yeaR::FRT*	This study
JK1054	14028s *hmp:: FRTkanFRT nsrR::FRT ytfE::cat*	This study
JK1055	14028s *hmp:: FRTkanFRT nsrR::FRT*	This study

	hcp::FRT	
JK1056	14028s *hmp:: FRTkanFRT nsrR::FRT ygbA::FRT*	This study
HR256	14028s *hmp:: FRTkanFRT nsrR::FRT hcr::FRTcatFRT*	This study
HR269	14028s *hmp:: FRTkanFRT nsrR::FRT Δhcp-hcr::FRTcatFRT*	This study
JK1095	14028s wild type / pRB3	This study
JK1096	14028s *hmp:: FRTkanFRT* / pRB3	This study
JK1097	14028s *nsrR:: FRTkanFRT* / pRB3	This study
JK1098	14028s *hmp:: FRTkanFRT nsrR::FRT* / pRB3	This study
JK1099	14028s *hmp:: FRTkanFRT nsrR::FRT hcp::FRT* / pRB3	This study
JK1111	14028s *hmp:: FRTkanFRT nsrR::FRT hcr::FRTcatFRT* / pRB3	This study
JK1102	14028s *hmp:: FRTkanFRT nsrR::FRT Δhcp-hcr::FRTcatFRT* / pRB3	This study
JK1100	14028s *hmp:: FRTkanFRT nsrR::FRT hcp::FRT* / pJK693	This study
JK1112	14028s *hmp:: FRTkanFRT nsrR::FRT hcr::FRTcatFRT* / pJK693	This study
JK1103	14028s *hmp:: FRTkanFRT nsrR::FRT Δhcp-hcr::FRTcatFRT* / pJK693	This study
JK1109	14028s *hmp:: FRTkanFRT nsrR::FRT hcp::FRT* / pJK694	This study
JK1113	14028s *hmp:: FRTkanFRT nsrR::FRT hcr::FRTcatFRT* / pJK694	This study
JK1110	14028s *hmp:: FRTkanFRT nsrR::FRT Δhcp-hcr::FRTcatFRT* / pJK694	This study
VT84	14028s STM1808-H31A	This study
VT85	14028s STM1808-H32A	This study
VT86	14028s STM1808-H82A	This study
VT87	14028s STM1808-H95A	This study
VT88	14028s STM1808-H102A	This study
BL21(DE3)	*E. coli B* F– *dcm ompT hsdS*(r_B^- m_B^-) gal λ(DE3)	Novagen
JK953	BL21(DE3) / pGEX-2T	This study
JK954	BL21(DE3) / pJK678	This study
JK962	BL21(DE3) / pJK681	This study
Plasmid		
pKD3	*bla FRTcatFRT* PS1 PS2 ori6K	(Datsenko and Wanner, 2000)
pKD4	*bla FRTkanFRT* PS1 PS2 ori6K	(Datsenko and Wanner, 2000)
pKD46	*bla araC-P*araB γ β *exo* oriR101 repA101ts	
pCP20	*bla cat* cI857 lPr *flp* PSC101 oriTS	(Datsenko and

		Wanner, 2000)
pRB3	*par* RK2 *bla* stable low copy cloning vector	(Berggren et al., 1995)
pJK693	pRB3-P$_{hcp}$-*hcp-hcr*	This study
pJK694	pRB3-P$_{hcp}$-*hcp*	This study
pGEX-2T	P$_{tac}$-GST *bla* colE1	Pharmacia Biotech
pJK678	pGEX-2T-STM1808	This study
pJK681	pGEX-2T-STM1808-H82A	This study

Supplemental Table S3. Primers used in this study.

Primer	Primer sequence (5'-3')	Use for
1808-d1	TGTCTTATGTCCCACTTACGCATCCCGGCAAACTGGAAAGAAGCCACTGGAGCACCTCAA	STM1808 mutant
1808-d2	TCGCCCTGATGGTCTTCCTCTACCCAAAAATGAATGTTGAACGGGGAGAGCCTGAGCAAA	
YeaR-d1	GAAGCAAATGCGGCAAATTCCACAAAATCATATTCATACAGTGTAGGCTGGAGCTGCTTC	*yeaR* mutant
YeaR-d2	CCGTGACGGTATAAGTAGCTTTTCCCATTATTATTCTCTCCATATGAATATCCTCCTTAG	
YgbA-d1	GGGCGCTTATCGTCGATAAGGTGCCGCACGGTCAGGACAGGTGTAGGCTGGAGCTGCTTC	*ygbA* mutant
YgbA-d2	ATGATCGCGCTGTATGAAAGCCAGTGCCCACAGGCGTCAACATATGAATATCCTCCTTAG	
YtfE-d1	TATTCCCCGGCCAGCGCGCGTGGGAACAGCACGTTATTCTAAGCCACTGGAGCACCTCAA	*ytfE* mutant
YtfE-d2	CTATGGCTTATCGCGATCAACCTTTAGGCGAACTGGCGCTACGGGGAGAGCCTGAGCAAA	
JKP404	ACAAACCATCCGTACACCAGCCGGAAACGGCTGCTCCTACGTGTAGGCTGGAGCTGCTTC	Δ*hcp* and Δ*hcp-hcr* mutant
HP72	GCCAACCTCACAGTGTCCCTGGCGAATGCAGGTGCATCACGTGTAGGCTGGAGCTGCTTC	Δ*hcr* mutant
HP73	AGATCGCCCTGCGGATGGCAGGAGCAGGCCAGTACGTAACCATATGAATATCCTCCTTAG	
1808tetRA-f	TTGCGAATGAAACCGCGACAGAACCCGAAGTGAAAGTGGTTTAAGACCCACTTTCACATT	STM1808::tetRA
1808tetRA-r	GTATTGCGGCGGACTGGTGGCGAATTGCCCGGCATTAATCCTAAGCACTTGTCTCCTG	
JKP327	AACCAAGCTTTCAGGCTTTTTTCTGTTGATAC	STM1808 His to Ala mutants
JKP335	CACCACATCAGGGAGATGTC	
1808H31A-f	CTTTTTCACCAAAGAGAATGTTCCGGCGGCACTGCTCTCTGCACACAACACAGCGGCAGGC	STM1808-H31A
1808H32A-r	TTTCACCAAAGAGAATGTTCCGGCGGCACTGCTCTCTCATGCAAACACAGCGGCAGGCGTT	STM1808-H32A
1808H82A-r	AATGAATGTTGAACCGCGCATCATCGCTCAGCTCTACCCGTGCCCAGTATTGCGGCGGACT	STM1808-H82A
1808H95A-r	GATACATTTCCTCGCCCTGATGGTCTTCCTCTACCCAAAATGCAATGTTGAACCGCGCATC	STM1808-H95A
1808H102A-r	GATGTCAGGCTTTTTTCTGTTGATACATTTCCTCGCCCTGTGCGTCTTCCTCTACCCAAAA	STM1808-H102A
JKP395	AACCAAGCTTACGCGCTCAGCAATTGCT	pJK694

JKP397	AACCAAGCTTATGCGAGAACAAGATCGC	pJK693
JKP408	AACCGGATCCGCTTTATCCTCAGCCTGCTG	pJK693 and pJK694
JKP341	AACCGGATCCATGTCCCACTTACGCATCCC	pJK678 and pJK681
JKP342	AACCGAATTCTCAGGCTTTTTTCTGTTGATAC	
qRT-hcpf	GGTGAAACCACCAAATACGG	qRT-PCR *hcp*
qRT-hcpr	CCGACCAGATGCTTGAATTT	
qRT-yeaRf	ATCCTCGTCTCTCGGTGATG	qRT-PCR *yeaR*
qRT-yeaRr	CACTTTTCCGGAGGAAACAC	
qRT-STM1808f	AACCCGAAGTGAAAGTGGTG	qRT-PCR STM1808
qRT-STM1808r	GATACATTTCCTCGCCCTGA	
qRT-hmpf	CTTGACGCACAAACCATCGCT	qRT-PCR *hmp*
qRT-hmpr	CGGGTTATGCGTAAACATACG	
qRT-ygbAf	CGTATTTGGCGAGGAAAAAC	qRT-PCR *ygbA*
qRT-ygbAr	CCAACGCATAATCTGCTTCA	
qRT-ytfEf	ATTCTGCAGGCAACCAAAGT	qRT-PCR *ytfE*
qRT-ytfEr	TTGATCATGGGGAACAGGAT	
qRT-invAf	GCCATGGTATGGATTTGTCC	qRT-PCR *invA*
qRT-invAr	GTCACGATAAAACCGGCACT	
qRT-siiCf	GGCAGCACTTTATCCTACCCTG	qRT-PCR *siiC*
qRT-siiCr	TCACTCCAAAATCCGTTATTCG	
qRT-tehAf	GAGCGCTCGGTTACAATGAT	qRT-PCR *tehA*
qRT-tehAr	TACGCAGTCGCTGCAAAATA	
qRT-tehBf	CGTATTAAAGCGGCAGAAGG	qRT-PCR *tehB*
qRT-tehBr	AATCGTATTCGCCGTCAAAG	
qRT-dsdXf	ACTTACCCACACCGAAAACG	qRT-PCR *dsdX*
qRT-dsdXr	GCCCGCTACTTTTGAGAATG	
qRT-dsdAf	CAAAAACGGCTGGAAAAAGA	qRT-PCR *dsdA*
qRT-dsdAr	GCGCTTTAATGGAACCAGAG	

|		
qRT-STM1276f	GTACTGGCAATCCTTTTTCTGC	qRT-PCR STM1276
qRT-STM1276r	TGCTGCGATTGCAATAGTTC	
entCf	GGTTTAGTTCGCTGCCGTTA	qRT-PCR *entC*
entCr	GATCAACTGCGGAGAGGAAG	
norVf	ACGCAAATCGTGGAACTGTA	qRT-PCR *norV*
norVr	GGTCCACTTCGTTGATACCC	
soxSf	AACATTGATGTGGTGGCAAA	qRT-PCR *soxS*
soxSr	CTGCGATACATAGCCCAGGT	
rpoDf	GTGAAATGGGCACTGTTGAACTG	qRT-PCR *rpoD*
rpoDr	TTCCAGCAGATAGGTAATGGCTTC	

Figure S1
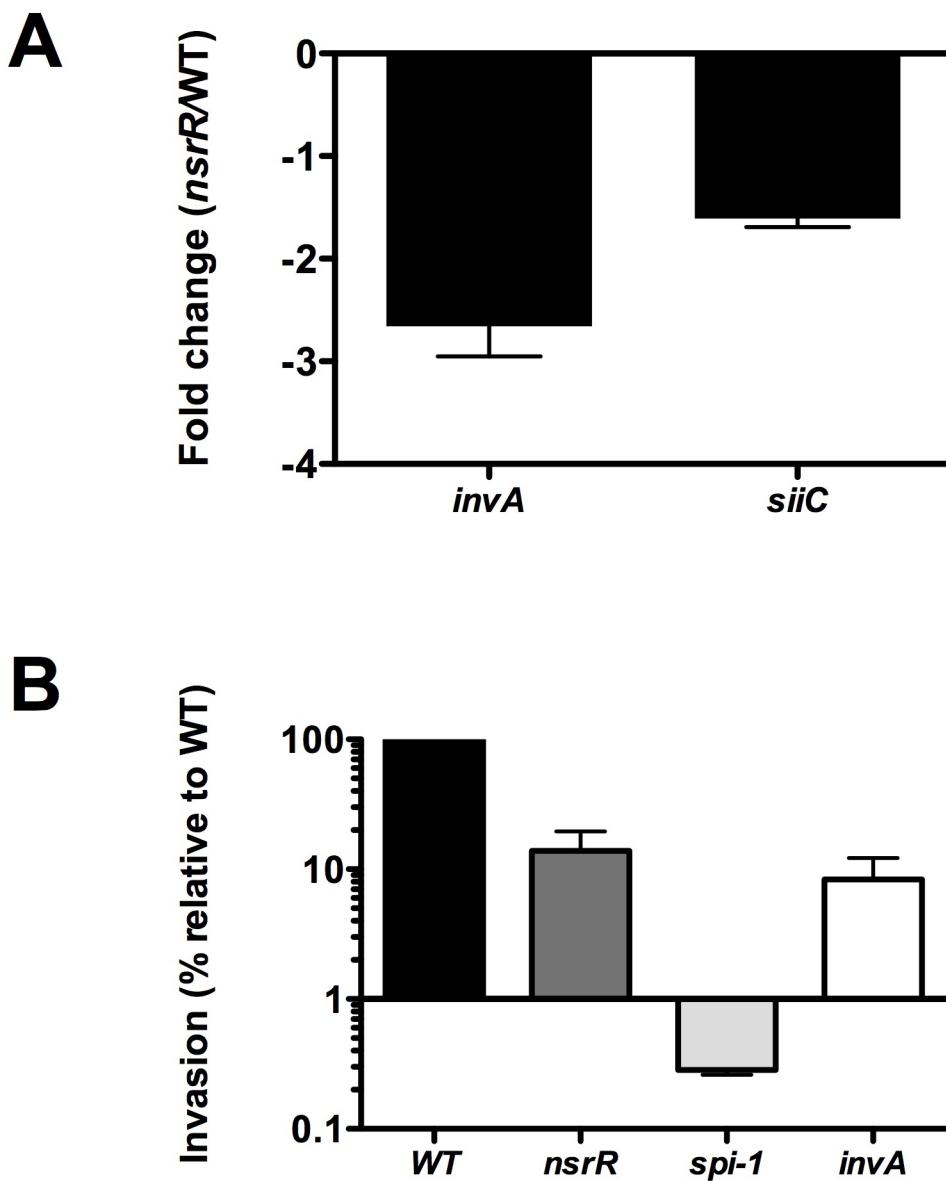
Supplemental Figure S1. NsrR regulates *Salmonella* Pathogenicity Island-1 and *Salmonella* Pathogenicity Island-4 gene expression in *S.* Typhimurium 14028s and is important for epithelial cell invasion. A. Fold change *nsrR*/WT mRNA of representative genes from SPI1 (*invA*) and SPI4 (*siiC*) as measured by quantitative RT-PCR (Experimental Procedures). **B.** Invasion into HeLa epithelial cells by wild-type (black bar), *nsrR* (dark grey bar), *Δspi-1* (light grey bar) or *invA* (white bar) *S.* Typhimurium (Experimental Procedures). Invasion of mutant *S.* Typhiumurium strains are reported relative to wild-type invasion into HeLa cells.

Figure S2
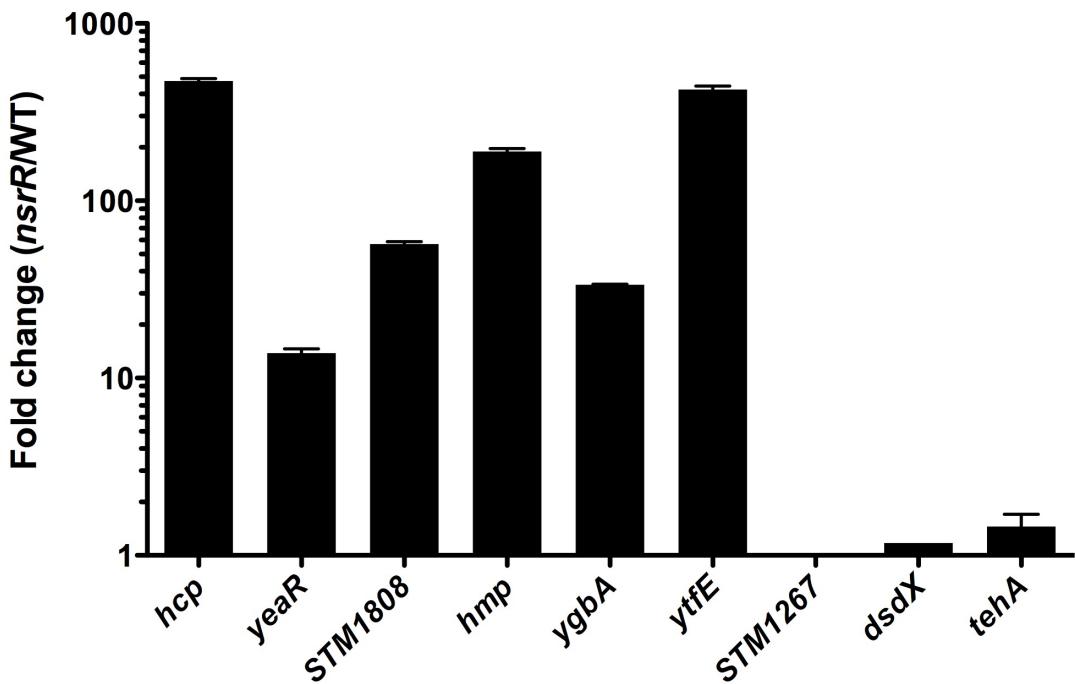
Supplemental Figure S2. Validation of the *S*. Typhimurium NsrR regulon by real-time quantitative RT-PCR. Fold change of *nsrR*/WT mRNA corresponding to the *hcp, yeaR*, STM1808, *hmp, ygbA*, *ytfE,* STM1274, *dsdX* and *tehA* operons was measured by real-time quantitative reverse transcription PCR (Experimental Procedures). Each bar represents fold change of *nsrR*/WT mRNA from triplicate samples.

Figure S3
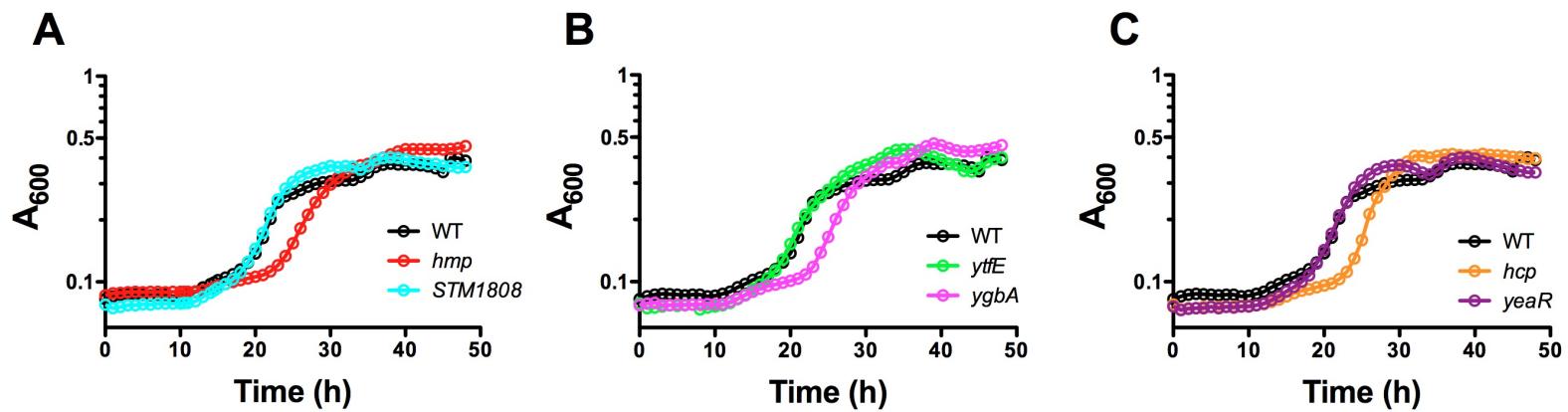
Supplemental Figure S3. The NsrR-regulated *hmp*, *ygbA* and *hcp* genes are important for *S*. Typhimurium growth in M9 minimal medium + 0.2% glucose under nitrosative stress. Overnight cultures of *S*. Typhimurium 14028s were inoculated 4 x 10^7 cfu/ml in M9 minimal media + 0.2% glucose with 0.5mM Sper/NO and growth was monitored for 48h at 37°C. Strains with mutations in individual genes of the NsrR regulon were compared to wild-type (WT,black). **A.** *hmp* (red) and STM1808 (blue). **B.** *ytfE* (green) and *ygbA* (light purple). **C.** *hcp* (orange) and *yeaR* (dark purple).

Figure S4
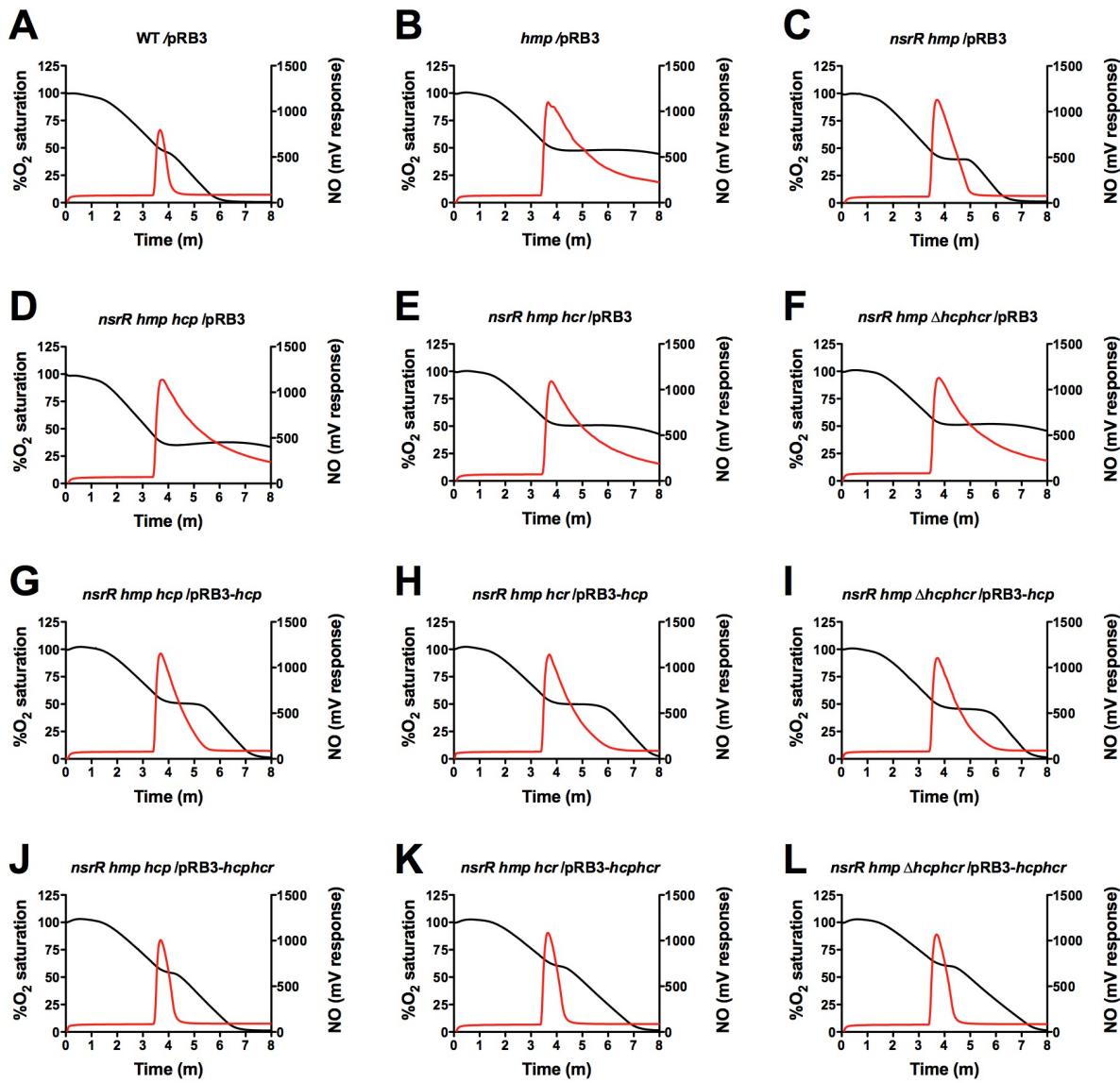
Supplemental Figure S4. Resistance to NO· inhibition of respiration is restored in *hcp* and *hcr* mutants *in trans* with Hcp and Hcr containing plasmids. Concentrations of O_2 (black line) and NO· (red line) were measured using O_2 and NO· probes as described in Experimental Procedures. Respiration was stimulated in *S*. Typhimurium cells by the addition of 0.1% glucose at T = 1m. After 50% of the saturated oxygen was consumed, 5mM ProliNO, a rapidly releasing NO donor, were added and the O_2 and NO· concentrations monitored. **A.** Wild-type (WT) /pRB3. **B.** *hmp*/pRB3. **C.** *nsrR hmp*/pRB3. **D.** *nsrR hmp hcp*/pRB3. **E.** *nsrR hmp hcr*/pRB3. **F.** *nsrR hmp Δhcphcr*/pRB3. **G.** *nsrR hmp hcp*/pRB3-*hcp*. **H.** *nsrR hmp hcr*/pRB3-*hcp*. **I.** *nsrR hmp Δhcp-hcr*/pRB3-*hcp*. **J.** *nsrR hmp hcp*/pRB3-*hcp-hcr*. **K.** *nsrR hmp hcr*/pRB3-*hcp-hcr*. **L.** *nsrR hmp Δhcp-hcr*/pRB3-*hcp-hcr*.