Figure S1

Seth *et al*.

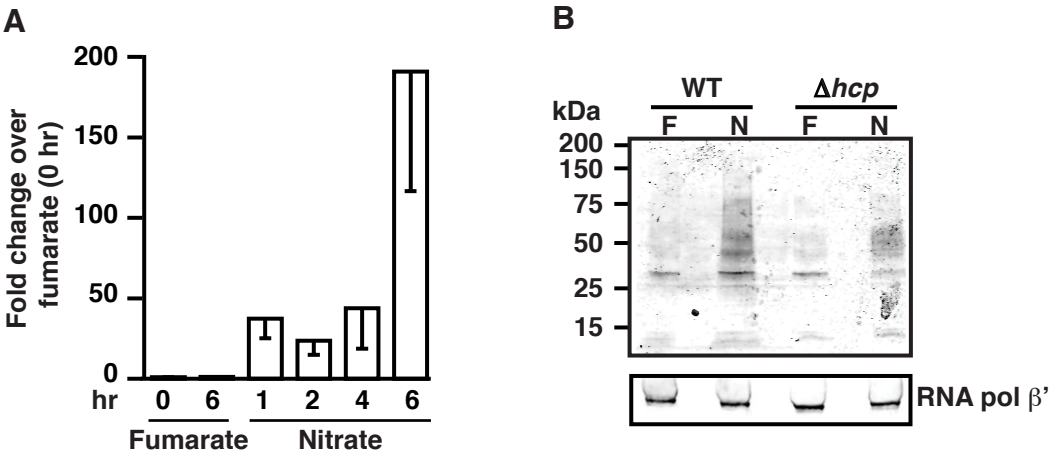

Figure S2

Seth *et al.*

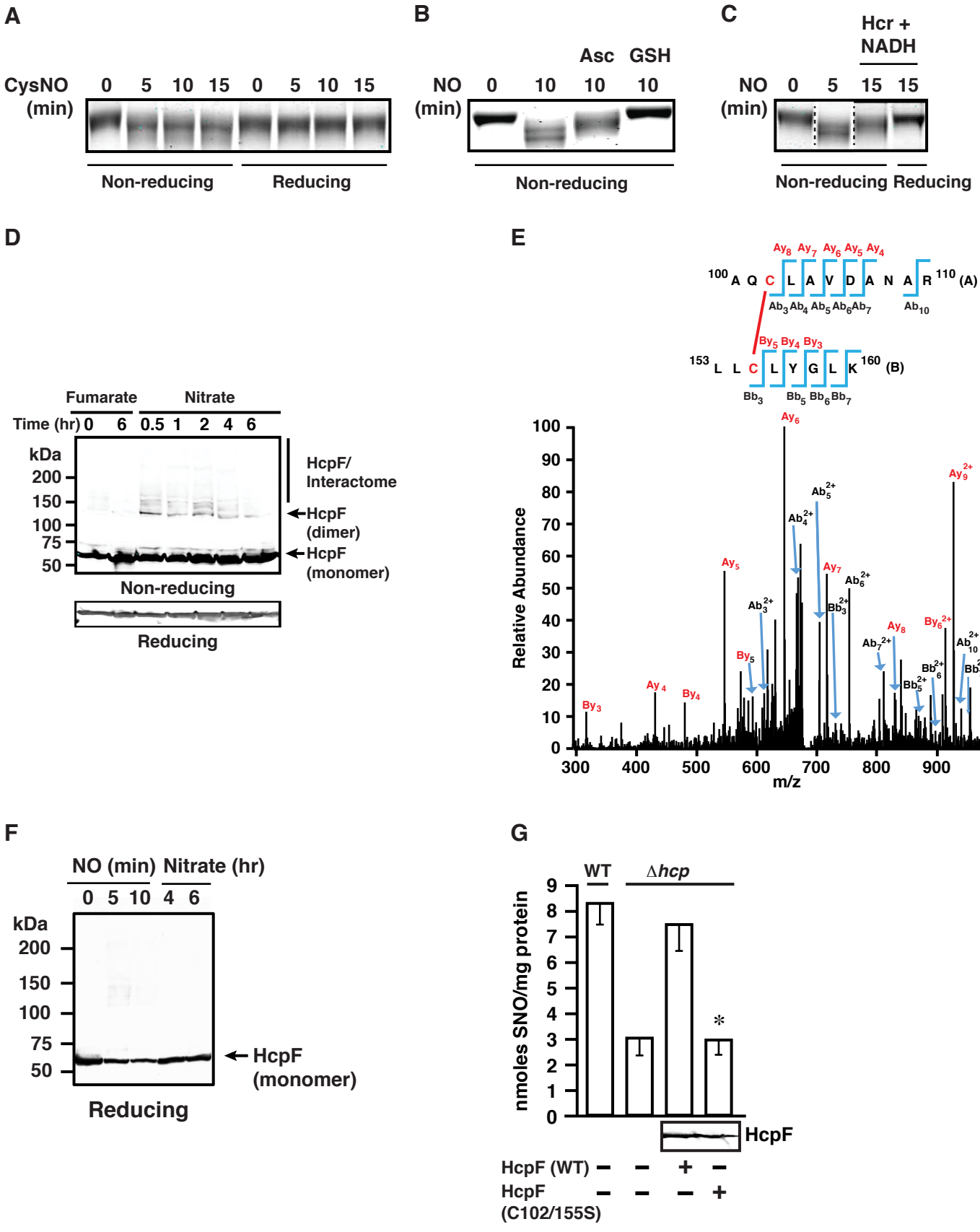

Figure S3
Seth *et al*.

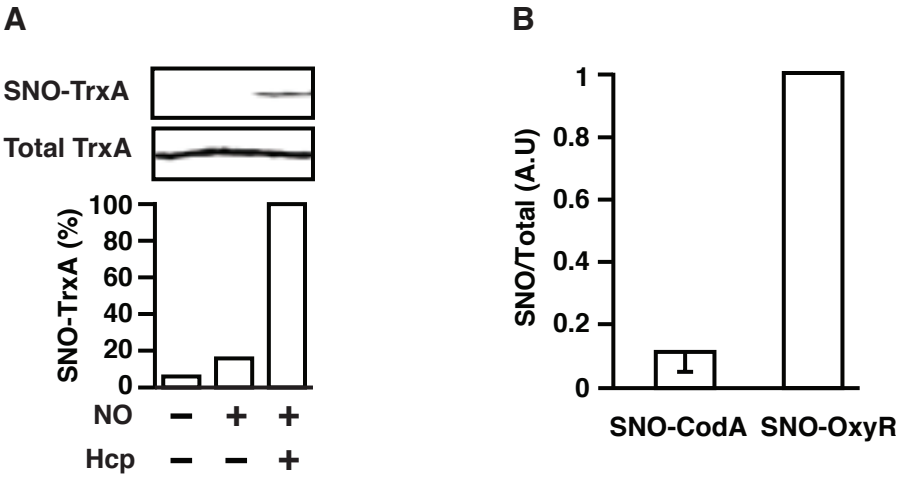

Figure S4

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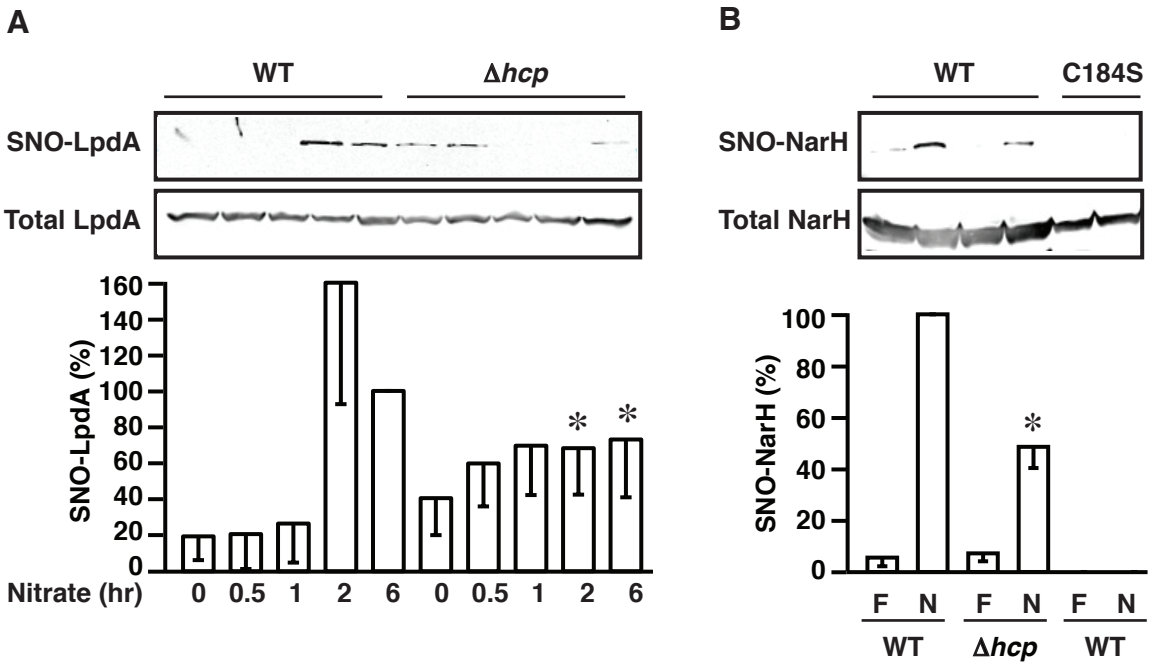
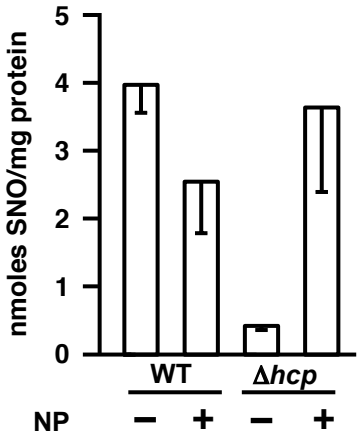


Figure S5
Seth *et al.*

A



B

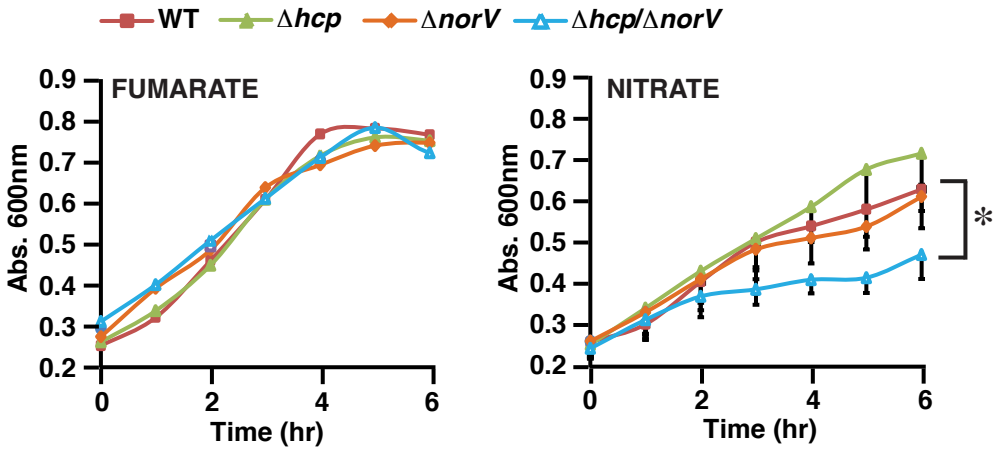


Table S1. Nitrosylation, Growth and Nitrite Measurements in *E. coli* Strains Mutant for OxyR-dependent, Nitrate-induced Genes During ARN, Related to Figure 1

Strain	Nitrosylation (nmoles/mg protein)	A600	Nitrite (mM)	Strain	Nitrosylation (nmoles/mg protein)	A600	Nitrite (mM)
hcp	0.58	0.98	6.10	***kgtP***	4.26	0.82	6.08
ymgA	1.63	1.23	6.37	***gst***	4.31	0.95	4.99
ymgC	2.11	1.15	6.38	***gcvP***	4.35	0.98	6.49
ybaY	2.20	1.09	6.75	***ynhG***	4.39	1.13	5.05
yeaC	2.26	1.08	6.94	***acnB***	4.43	0.79	4.23
lpd	2.53	0.83	7.59	***fdnH***	4.52	1.07	5.43
yjcB	2.56	0.99	6.30	***yobA***	4.54	0.96	6.00
speD	2.59	0.74	3.76	***fdoG***	4.58	1.12	6.50
hslV	2.69	0.95	5.40	***nuoI***	4.71	0.94	5.15
tpx	3.03	1.29	6.54	***ilvM***	4.73	1.07	6.16
yifK	3.15	1.04	5.48	***dadX***	4.78	0.81	8.27
yciW	3.19	1.08	6.67	***nrdD***	4.89	0.80	2.68
thiH	3.22	0.40	0.62	***napB***	4.97	1.00	6.00
metH	3.26	0.90	5.89	***sdhB***	5.06	1.14	6.34
glpF	3.28	0.87	5.41	***mdh***	5.08	1.03	5.93
cbl	3.33	1.19	6.89	***maeB***	5.12	0.93	5.51
psiE	3.35	0.86	5.75	**WT**	5.19	0.88	6.11
tauA	3.40	0.91	7.22	***glpD***	5.25	0.93	5.46
ycgZ	3.41	1.19	7.20	***ylaC***	5.42	1.05	6.10
yfiM	3.47	0.68	6.22	***ydcN***	5.53	1.07	6.19
ybiJ	3.64	0.88	3.06	***acnA***	5.63	1.19	6.59
ilvG	3.70	1.05	5.64	***fdnI***	5.89	1.04	4.51
yqhA	3.73	0.85	5.55	***yhjE***	5.94	0.89	5.90
hslU	3.77	0.81	5.14	***ynaJ***	6.10	0.96	5.08
ssuE	3.77	0.91	11.80	***deaD***	6.15	1.14	6.28
proP	3.82	0.99	5.41	***osmB***	6.23	0.97	5.68
fdoI	3.87	0.93	5.91	***yeeE***	6.27	0.90	5.75
pdhR	3.91	1.08	5.45	***clpA***	6.30	0.76	6.12
cyoD	3.96	1.07	6.91	***cyoE***	6.49	0.96	5.30
gcd	3.99	1.32	7.64	***intF***	6.58	1.13	6.45
aceA	4.00	1.09	6.28	***glpE***	6.82	1.14	6.74
yzgL	4.02	1.15	6.54	***tauB***	6.86	0.80	5.52
ybiC	4.03	0.86	7.59	***aceB***	6.98	0.99	5.33
yceI	4.05	0.88	5.47	***bhsA***	7.18	1.00	6.49
fdnG	4.07	1.11	5.36	***ylbA***	7.49	1.07	6.12
msrB	4.21	0.87	5.92	***fumA***	7.55	1.08	6.28
yebZ	4.24	0.89	5.44	***pgm***	8.81	0.91	4.99

Table S1. *E. coli* cultures were started in minimal medium with fumarate and sub-cultured into medium with nitrate (10 mM) for 6 hrs. Nitrosylation was measured by photolysis/chemiluminescence. Growth and nitrite levels were measured at A600 and by Griess assay respectively (n=3 to 21).

Table S2. Hcp-independent S-nitrosylation of Proteins During ARN, Related to Figure 3

Protein	Uniprot	Fold change WT	Fold change Δ*hcp*	WT/ Δ*hcp*	Interact with Hcp
KatG Catalase-peroxidase	P13029	2.2	1.9	1.1	Y
CysJ Sulfite reductase [NADPH] flavoprotein alpha-component	P38038	3.2	2.9	1.1	
PurA Adenylosuccinate synthetase	P0A7D4	2.0	1.8	1.1	Y
CarA Carbamoyl-phosphate synthase small chain	P0A6F1	3.2	3.0	1.1	
GltD Glutamate synthase [NADPH] small chain	P09832	2.1	2.0	1.1	Y
InfB Translation initiation factor IF-2	P0A705	1.9	2.0	1.0	
FabB 3-oxoacyl-[acyl-carrier-protein] synthase 1	P0A953	2.3	2.4	1.0	
HchA Molecular chaperone Hsp31 and glyoxalase 3	P31658	2.2	2.5	0.9	
FabZ 3-hydroxyacyl-[acyl-carrier-protein] dehydratase	P0A6Q6	4.6	5.9	0.8	
Hcp Hydroxylamine reductase	P75825	16.4			

Table S2. Proteins that are S-nitrosylated during ARN in WT were identified as proteins that were pulled down by SNO-RAC ≥1.5 fold on nitrate versus fumarate. Proteins listed are S-nitrosylated comparably in WT and Δ*hcp*. Proteins present in the Hcp interactome (Table S3) are identified with Y.

Table S3. Nitrate-dependent Hcp Interactome, Related to Figure 3

Protein	Uniprot	Protein	Uniprot
NarG Respiratory nitrate reductase 1 alpha chain	P09152	**MetE** 5-methyltetrahydropteroyltriglutamate	P25665
IlvB Acetolactate synthase isozyme 1 large subunit	P08142	**GltD** Glutamate synthase [NADPH] small chain	P09832
MiaB tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase	P0AEI1	**MetA** Homoserine O-succinyltransferase	P07623
OmpA Outer membrane protein A	P0A910	**Gcp** tRNA N6-adenosine threonylcarbamoyltransferase	P05852
AccD Acetyl-coenzyme A carboxylase carboxyl transferase	P0A9Q5	**YcfP** UPF0227 protein	P0A8E1
LpdA Dihydrolipoyl dehydrogenase	P0A9P0	**DeaD** ATP-dependent RNA helicase	P0A9P6
AsnB Asparagine synthetase B	P22106	**AccA** Acetyl-coenzyme A carboxylase carboxyl transferase	P0ABD5
EttA Energy-dependent translational throttle protein	P0A9W3	**DnaB** Replicative DNA helicase	P0ACB0
RplN 50S ribosomal protein L14	P0ADY3	**PurA** Adenylosuccinate synthetase	P0A7D4
PyrG CTP synthase	P0A7E5	**IspG** 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	P62620
IlvI Acetolactate synthase isozyme 3 large subunit	P00893	**Hcr** NADH oxidoreductase	P75824
NirB Nitrite reductase (NADH) large subunit	P08201	**AroG** Phospho-2-dehydro-3-deoxyheptonate aldolase	P0AB91
GapA Glyceraldehyde-3-phosphate dehydrogenase A	P0A9B2	**GyrB** DNA gyrase subunit B	P0AES6
SurA Chaperone	P0ABZ6	**DnaK** Chaperone protein	P0A6Y8
SecD Protein translocase subunit	P0AG90	**AceF** Dihydrolipoyllysine-residue acetyltransferase (PyrDH complex)	P06959
MetL Bifunctional aspartokinase/homoserine	P00562	**RpoA** DNA-directed RNA polymerase subunit alpha	P0A7Z4
AceE Pyruvate dehydrogenase E1 component	P0AFG8	**CysC** Adenylyl-sulfate kinase	P0A6J1
IlvA L-threonine dehydratase biosynthetic	P04968	**AroF** Phospho-2-dehydro-3-deoxyheptonate aldolase	P00888
MurC UDP-N-acetylmuramate--L-alanine ligase	P17952	**AccC** Biotin carboxylase	P24182
OppF Oligopeptide transport ATP-binding protein	P77737	**MreB** Rod shape-determining protein	P0A9X4
RpsG 30S ribosomal protein S7	P02359	**MetF** 5,10-methylenetetrahydrofolate reductase	P0AEZ1
RplE 50S ribosomal protein L5	P62399	**Pur7**Phosphoribosylaminoimidazole -succinocarboxamide synthase	P0A7D7
GpmI 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P37689	**ArgB** Acetylglutamate kinase	P0A6C8
CysH Phosphoadenosine phosphosulfate reductase	P17854	**ClpX** ATP-dependent Clp protease ATP-binding subunit	P0A6H1
Upp Uracil phosphoribosyltransferase	P0A8F0	**LeuC** 3-isopropylmalate dehydratase large subunit	P0A6A6
FtsZ Cell division protein	P0A9A6	**EF-Tu1** Elongation factor Tu 1	P0CE47
OmpR Transcriptional regulatory protein	P0AA16	**TtcA** tRNA 2-thiocytidine biosynthesis protein	P76055
MinD Septum site-determining protein	P0AEZ3	**ObgE** GTPase CgtA	P42641

NusG Transcription termination/antitermination protein	P0AFG0	**LysC** Lysine-sensitive aspartokinase 3	P08660
Rho Transcription termination factor	P0AG30	**IlvC** Ketol-acid reductoisomerase	P05793
RplF 50S ribosomal protein L6	P0AG55	**Tpx** Thiol peroxidase	P0A862
RecA Protein RecA	P0A7G6	**NarL** Nitrate/nitrite response regulator protein	P0AF28
RplJ 50S ribosomal protein L10	P0A7J3	**NarH** Respiratory nitrate reductase 1 beta chain	P11349
RpsE 30S ribosomal protein S5	P0A7W1	**IlvH** Acetolactate synthase isozyme 3 small subunit	P00894
RpsB 30S ribosomal protein S2	P0A7V0	**OmpF** Outer membrane protein F	P02931
RpsC 30S ribosomal protein S3	P0A7V3	**DnaJ** Chaperone protein	P08622
RpsK 30S ribosomal protein S11	P0A7R9	**LeuA** 1 2-isopropylmalate synthase	P09151
ArgG Argininosuccinate synthase	P0A6E4	**ArgA** Amino-acid acetyltransferase	P0A6C5
Tsf Elongation factor Ts	P0A6P1	**HslU** ATP-dependent protease ATPase subunit	P0A6H5
HisD Histidinol dehydrogenase	P06988	**PyrI** Aspartate carbamoyltransferase regulatory chain	P0A7F3
RplM 50S ribosomal protein L13	P0AA10	**RplK** 50S ribosomal protein L11	P0A7J7
PyrB Aspartate carbamoyltransferase catalytic chain	P0A786	**RplS** 50S ribosomal protein L19	P0A7K6
GlyQ Glycine--tRNA ligase alpha subunit	P00960	**RplI** 50S ribosomal protein L9	P0A7R1
GatY D-tagatose-1,6-bisphosphate aldolase subunit	P0C8J6	**RpsJ** 30S ribosomal protein S10	P0A7R5
TrpG Bifunctional protein	P00904	**RpsD** 30S ribosomal protein S4	P0A7V8
GuaA GMP synthase [glutamine-hydrolyzing]	P04079	**Tig** Trigger factor	P0A850
TyrR Transcriptional regulatory protein	P07604	**PflA** Pyruvate formate-lyase 1-activating enzyme	P0A9N4
RplT 50S ribosomal protein L20	P0A7L3	**ArcA** Aerobic respiration control protein	P0A9Q1
HflK Modulator of FtsH protease	P0ABC7	**FdnH** Formate dehydrogenase, nitrate-inducible, iron-sulfur subunit	P0AAJ3
SucB Dihydrolipoyllysine-residue succinyltransferase component of 2-	P0AFG6	**FabG** 3-oxoacyl-[acyl-carrier-protein] reductase	P0AEK2
IbpB Small heat shock protein	P0C058	**NtrC** Nitrogen regulation protein NR(I)	P0AFB8
FdnG Formate dehydrogenase, nitrate-inducible, major subunit	P24183	**SecY** Protein translocase subunit	P0AGA2
RplB 50S ribosomal protein L2	P60422	**CysA** Sulfate/thiosulfate import ATP-binding protein	P16676
Aas Bifunctional protein	P31119	**ThiE** Thiamine-phosphate synthase	P30137
AcnB Aconitate hydratase 2	P36683	**RavA** ATPase	P31473
KatG Catalase-peroxidase	P13029	**RplD** 50S ribosomal protein L4	P60723
		YdgH Protein YdgH	P76177

Table S3. The Hcp interactome was identified following ARN for 1 hr (n=3) or 6 hr (n=3) using the criteria described in Materials and Methods. Proteins listed were identified at least twice at either of the two time points. Proteins that are also S-nitrosylated in an Hcp-dependent manner are indicated in red.

Table S4. NO-dependent Hcp Interactome, Related to Figure 3

Protein	Uniprot	Nitrate interactome
OppD Oligopeptide transport ATP-binding protein	P76027	
YdiJ Uncharacterized protein	P77748	
IspG 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	P62620	Y
MetG Methionyl-tRNA synthetase	P00959	
GltD Glutamate synthase [NADPH] small chain	P09832	Y
GltB Glutamate synthase [NADPH] large chain	P09831	
FdhF Formate dehydrogenase H	P07658	
CysI Sulfite reductase [NADPH] hemoprotein beta-component	P17846	
AceE Pyruvate dehydrogenase E1 component	P0AFG8	Y
Aas Bifunctional protein	P31119	Y
NuoC NADH-quinone oxidoreductase subunit C/D	P33599	
KatG Catalase-peroxidase	P13029	Y
MetE 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	P25665	Y
CysN Sulfate adenylyltransferase subunit 1	P23845	
AccA Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	P0ABD5	Y
MiaB tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase	P0AEI1	Y
OppF Oligopeptide transport ATP-binding protein	P77737	Y
TrpG Bifunctional protein	P00904	Y
MetH Methionine synthase	P13009	
AccD Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	P0A9Q5	Y
MbhL Hydrogenase-1 large chain	P0ACD8	
UspA Universal stress protein A	P0AED0	
ProB Glutamate 5-kinase	P0A7B5	
LeuC 3-isopropylmalate dehydratase large subunit	P0A6A6	Y
GatY D-tagatose-1,6-bisphosphate aldolase subunit	P0C8J6	Y
NuoG NADH-quinone oxidoreductase subunit G	P33602	
AceF Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P06959	Y
IlvC Ketol-acid reductoisomerase	P05793	Y
LpdA Dihydrolipoyl dehydrogenase	P0A9P0	Y
SucA 2-oxoglutarate dehydrogenase E1 component	P0AFG3	

Table S4. The Hcp interactome was identified following treatment with DEANO (100 μM, 10 min.) using the criteria described in Materials and Methods. Proteins listed were identified in at least 2 independent experiments. Proteins present in the nitrate-dependent Hcp interactome (Table S3) are identified with Y.

Table S5. Primers Used for Cloning and QPCR, Related to STAR Methods

Primer name	Primer sequence
Hcp-pUC19-F	ATTAGGATCCTTTAAGAAGGAGATATACATATGTTTTGTGTGCAATG
Hcp-pUC19-R	CGATCAAGCTTTTACTTGTCATCGTCGTCCTTGTAGTCCGCGCTCAACAGTTGC
OxyR-pUC19-F	ATTAGGATCCCTTTAAGAAGGAGATATACATATGAATATTCG
OxyR-pUC19-R	GCATAAGCTTTTACTTGTCATCGTCGTCCTTGTAGTCAACCGCCTGTTTTAAAAC
LpdA-pUC19-F	ATTAGGATCCTTTAAGAAGGAGATATACATATGAGTACTGAAATCAAAACTC
LpdA-pUC19-R	CGATCAAGCTTTTACTTGTCATCGTCGTCCTTGTAGTCCTTCTTCTTCGCTTTCG
Hcp-pET21b-F	ATATACATATGTTTTGTGTGCAATGTGAACAAAC
Hcp-pET21b-R	TAATAAGCTTCGCGCTCAACAGTTGCTTCATG
Hcr-pET21b-F	ATATACATATGACGATGCCAACGAATC
Hcr-pET21b-R	ATTGCAAGCTTTGCGAGAACCAAATCC
OxyR-pET21b-F	ACTATCATATGAATATTCGTGATCTTG
OxyR-pET21b-R	TGAATCTCGAGAACCGCCTGTTTTAAAACTTTATCG
LpdA-pET21b-F	ATCATACATATGAGTACTGAAATCAAAACTCAGG
LpdA-pET21b-R	GACTGAAAGCTTCTTCTTCTTCGCTTTCGGGTTCGG
GapA-pET21b-F	ATCATACATATGACTATCAAAGTAGGTATCAACG
GapA-pET21b-R	GACTGAAAGCTTTTTGGAGATGTGAGCGATCAGG
CodA-pET21b-F	ATCATAGCTAGCGTGTCGAATA ACGCTTTACA AACAATTATT AACG
CodA-pET21bR	GACTGAAAGCTTACGTTTGTAATCGATGGCTTCTGG
NapA-pET21b-F	ATCATAGCTAGCATGAAACTCA GTCGTCGTAG CTTTATGAAA GC
NapA-pET21b-R	GACTGAAAGCTTCACCTTCTCCAGTTTGACCGCGC
hcp-F (QPCR)	TGACCTTCAGGATTTACTCATCGCGG
hcp-R (QPCR)	GATGATGCCGTATTCACGCGCTTT

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Induction of *hcp* and Comparison of SNO-proteins Between WT and Δ*hcp* During ARN, Related to Figure 1 and Figure 3

(A) Cells were grown anaerobically on nitrate or fumarate prior to RNA extraction and QPCR. Fold-expression of *hcp* on nitrate was calculated relative to expression on fumarate using the comparative C_t method. Expression in each sample was normalized to that of 16s rRNA (n=3, ±SEM).

(B) *E. coli* were grown anaerobically in minimal media containing either fumarate or nitrate (10 mM) for 6 hr and lysates were subjected to SNO-RAC followed by SDS-PAGE and Coomassie staining. Representative gel, n=4.

Figure S2. SNO-dependent Intramolecular Disulfide Formation in Hcp *In Vitro* and *In Vivo*, Related to Figure 3

(A) Hcp (1.8 μM) was treated anaerobically with CysNO (200 μM), followed by SDS-PAGE and Coomassie staining. As for exposure to NO (Figure 3B), exposure to the S-nitrosylating agent CysNO resulted in a decrease in m_r in non-reduced samples, indicative of disulfide formation, which was reversed by reduction. Representative gel, n=2.

(B) The decrease in m_r of Hcp induced by anaerobic treatment with NO (DEANO, 200 μM, 10 min) was diminished when ascorbate (Asc) was included in the reaction mixture as a selective reductant of SNO, and was eliminated when glutathione (GSH) was included as a reductant of SNO and disulfide. Representative gel, n=2.

(C) Hcp was treated anaerobically with DEANO for 5 min, and Hcr (1.8 μM) and NADH (100 μM) were added to an aliquot for an additional 10 min. The NO-induced decrease in m_r is substantially diminished by Hcr+NADH, consistent with Hcr-catalyzed disulfide reduction

with NADH as co-factor. Representative gel, n=3. Dashed lines indicate in-gel cuts for clarity.

(D) Hcp dimerization and interactome formation *in vivo* occur rapidly during ARN. WT/HcpF were grown in the presence of either nitrate or fumarate (10 mM). Lysates were analyzed by SDS-PAGE. Hcp was visualized by Western blotting with anti-FLAG antibody. Representative gel, n=2.

(E) MS/MS (LC-MS/MS) spectra of the disulfide bond formed between Cys102-Cys155 in Hcp during ARN. A representative annotated MS/MS fragmentation spectrum is shown illustrating the Cys102- and Cys155-containing peptides (labeled as A and B respectively) with disulfide bond between Cys102-155. Peptide sequences are shown at the top of the spectrum, with the annotation of the identified matched amino-terminus containing ions (*b* ions) in black and the carboxyl terminus ions (*y* ions) in red. For clarity, only major identified peaks are labeled.

(F) Dimerization and interactome formation by Hcp is abrogated under reducing conditions. As in Figure 3C, WT *E. coli* overexpressing FLAG-tagged Hcp (WT/HcpF) were grown anaerobically either in nitrate (10 mM, 6 hr), or in fumarate followed by treatment with DEANO (100 μM). Lysates were analyzed by reducing SDS-PAGE and Hcp was visualized by Western blotting with anti-FLAG antibody. Representative gel, n=3.

(G) WT, Δ*hcp*, Δ*hcp*/HcpF, Δ*hcp* /HcpF C102/155S were grown anaerobically in LB medium in the presence of nitrate (10 mM, 2.5 hrs) and SNO levels were measured by Hg^{2+}-coupled photolysis/chemiluminescence (n=5, ±SEM). * differs from Δ*hcp*/HcpF, $p < 0.05$.

Figure S3. Trans-nitrosylases Propagate Hcp-dependent S-nitrosylation, Related to Figure 4

(A) TrxA (4.8 μM) was treated with DEANO (50 μM) in the presence or absence of Hcp (1.2 μM) as indicated for 15 min at room temperature under anaerobic conditions. The reactions were subjected to SNO-RAC and TrxA was visualized by Western blotting using anti-thioredoxin antibody (n=2).

(B) CodA (1.6 μM) or OxyR (1.6 μM) was treated with DEANO (50 μM) and Hcp (200 nM) anaerobically *in vitro*. S-nitrosylation of CodA and OxyR was assessed by SNO-RAC followed by western blotting and densitometry. The yield of SNO-protein (SNO-protein/total) was calculated and data are presented re SNO-OxyR (n=3, ±SEM).

Figure S4. S-nitrosylation of LpdA and NarH During ARN is Decreased in the Absence of Hcp, Related to Figure 5

(A) WT/LpdA-FLAG and Δ*hcp*/LpdA-FLAG cells were grown anaerobically on 10 mM nitrate. Lysates were analyzed by SNO-RAC (n= 3, ± SEM). * differs from 2 hrs WT/LpdA-FLAG by ANOVA with Dunnett's test, $p < 0.05$.

(B) WT/NarH-FLAG, Δ*hcp*/NarH-FLAG, and WT/NarH C184S-FLAG cells were grown anaerobically on nitrate (N) or fumarate (F) (10 mM, 6 hr). Lysates were analyzed by SNO-RAC (n=3, ±SEM). * differs from nitrate grown WT/NarH-FLAG by ANOVA with Dunnett's test, $p < 0.05$.

Figure S5. Hcp Plays a Role in Protection from Nitrosative Stress, Related to Figure 5

(A) *E. coli* cultures were started in minimal medium including nitrate (10 mM) at A600 of 0.1. Where indicated sodium nitroprusside (NP; 2 mM) was added and cells were grown anaerobically for 6 hr. SNO levels were measured by Hg^{2+}-coupled photolysis/chemiluminescence (n=3, ±SEM).

(B) *E. coli* cultures were started in minimal medium including 10 mM fumarate or 10 mM nitrate at A600 of 0.2. At various time points, cultures were fixed in 0.5% formaldehyde and A600 was measured (n=3, ±SEM). * $p<0.05$ WT vs *Δhcp*/*ΔnorV*.