Supplemental Data:

Supporting information

Materials and Methods

Bacterial strains and culture conditions. *Escherichia coli* strains used for cloning were maintained in Luria–Bertani (LB) agar at 37°C. Liquid cultures were grown in LB broth (Fisher scientific). *Brucella abortus* 2308 and its derivative strains were grown in Brucella broth (BD Difco) or on Schaedler agar (BD Difco) supplemented with 5% defibrinated bovine blood (SBA) at 37°C with 5% CO_2 (1). Antibiotics were added as required at the following concentrations: 50 μg ml^{-1} of kanamycin (Kan), 100 μg ml^{-1} of ampicillin (Amp). All studies in live *B. abortus* were performed at biosafety level 3 (BSL3) as per CDC select agent regulations at the University of Chicago H.T. Ricketts Laboratory or at East Carolina University School of Medicine.

Construction of *B. abortus* mutants and genetic complementation. To create *phyR*, *rpoE1,* and *nepR-rpoE1* deletion strains, 500 to 700 bp flanking regions of these genes were PCR-amplified from *B. abortus* 2308 using primers listed in Table S3 and ligated into the suicide plasmid pNPTS138 (2) that contains *sacB* for counterselection on 10% sucrose. The putative ClpX-recognition tag (VAA) at the C-terminus of PhyR was mutated to ADD by lacing PCR, followed by cloning into pNPTS138. Plasmids for gene deletion/allele replacement were transformed into *B. abortus* 2308 by electroporation. Genetic complementation of deletion strains involved ligation of the entire ORF (or operon) including plus ~250 bp upstream of the predicted start codon into either pNPTS138 and pNPT228. These complementation plasmids were transformed into *B. abortus* by electroporation.

Primary peritoneal macrophage infections. Resident peritoneal macrophages were isolated from either C57BL/6 mice or BALB/c mice and seeded into 96-well plates in Dulbecco's modified Eagle's medium with 5% fetal bovine serum. After an overnight incubation, macrophages were infected with opsonized brucellae at a multiplicity of infection of 50:1. Extracellular bacteria were killed after 2 hours with gentamicin (50 μg/ml). For the 2-hour time point, the macrophages were lysed with 0.1% deoxycholate in PBS; serial dilutions were plated on Schaedler blood agar (SBA). For the 24- and 48-hour time points, cells were washed with phosphate-buffered saline (PBS) following gentamicin treatment. Fresh cell culture medium containing gentamicin (20 μg/ml) was subsequently added to the monolayer. Macrophages were then lysed, and serial dilutions plated on SBA. Experiments for each Brucella strain, in each strain of macrophage were conducted in triplicate.

Recombinant protein purification. Genes encoding PhyR, NepR, and σ^{E1} were PCR-amplified and cloned into overexpression vectors, pETDuet-1 (Novagen) and/or pET151 Directional TOPO (Invitrogen). Expression of N-terminally His-tagged proteins was induced in *E. coli* BL21 (DE3) by addition of 250 μM of isopropyl β-D-1-thiogalactopyranoside (IPTG). After cell lysis and centrifugation, soluble recombinant proteins were affinity-purified by using Ni^{2+} Chelating Resin (GE Amersham Pharmacia) using a 250 to 500 mM imidazole gradient. Purity of the proteins was assessed to be ≥95% by SDS/PAGE with Coomassie blue staining.

LacZ promoter fusion and β-Galactosidase assays. A 240 bp fragment immediately upstream of *phyR* was cloned into pMR15-*lacZ* (Table 1S). Strains were grown at 37°C in GMM supplemented with 50µg/ml Kan and 5 mM H_2O_2 for 1 h. β-galactosidase assays were performed using the substrate o-nitrophenyl-β-D-galactopyranoside (ONPG) as described (3). Enzyme activity was measured in Miller units: (A_{420} of reaction x 10,000) / Log(CFU/ml) x *t* x *v*, where *t* = reaction time in minutes and *v* = volume of cells used in ml. The CFU at 1 hour after oxidative stress was determined (Figure S4A).

Affymetrix microarray protocol and array data analysis. Three independent replicate cultures each of wild-type strain *B. abortus* 2308 and 2308Δ*rpoE1* were grown to 10^{10} CFU/ml in Brucella broth. Cells were then subjected to 10 minutes of 5 mM hydrogen peroxide stress. Cells were disrupted by bead beating and RNA was extracted using Trizol and RNA integrity assessed using an Agilent Bioanalyzer (Agilent, Santa Clara, CA, USA). For each of the six RNA samples, 10 µg was processed to produce single-stranded cDNA; RNA was removed by addition of 1 N NaOH. cDNA was column-purified, fragmented using DNaseI (GE Life Sciences, Piscataway, NJ, USA), and end-labelled using GeneChip labelling reagent (Affymetrix, P/N 900542). Labelled cDNA was hybridized to GeneChip URMC_Dunman_Brucella_abortus_v1.0 according to GeneChip Expression analysis technical manual (Affymetrix, Santa Clara, CA, USA). Hybridization proceeded for 16 h at 50°C. Arrays were washed using protocol PRO-GE-W52-V3 and stained on a GeneChipFluidics Station (Affymetrix) according to the Genechip Expression analysis technical manual. The arrays were scanned using the Affymetrix Gene Chip Scanner 3000 7G and CEL intensity files were generated by GCOS (Gene Chip Operating Software) version 1.4. Microarray data analyses were performed using Partek Genomics Suite software, version 6.6 (Partek Incorporated). The probe signal intensities were normalized using Robust Multichip Average (RMA) and the dataset was corrected to remove unexpected batch effects before statistical analysis. The differentially expressed genes among *B. abortus* 2308 wild type and Δ*rpoE1* strains were identified using Analysis of Variance (ANOVA) followed by False Discovery Rate (FDR) calculation to correct false positive for p-vales calculated from ANOVA ($p<0.005$ and $FDR<0.05$). Datasets are available at the European Bioinformatics Institute (accession no. E-MEXP-3722).

Total RNA isolation for qRT-PCR. The cells of *B. abortus* 2308 and $PhyR_{VAA/ADD}$ tail mutant grown in brucella broth were harvested and resuspended in PBS. After adding 0.5 volume of SDS-lysis buffer (2% SDS, 16mM EDTA, 200 mM NaCl), the suspension was incubated at 95°C for 5 min. One volume of hot acid phenol/chloroform (pH 4.5) was added followed by incubation at 65°C for 10 min. The aqueous phase was separated from the phenol phase by centrifugation at 2500 g and extracted again with the acid phenol/chloroform (pH 4.5) and then with chloroform. Total RNA was precipitated by adding 1 volume of ice-cold isopropanol and the precipitated RNA was recovered by centrifugation at 15,000 g for 15 min at 4°C. The pellet was washed with 70% ethanol, air-dried, and then resuspended in DEPC-treated water. To remove contaminating DNA, RNA preparations were treated with TURBO DNase™ (Ambion) according to the manufacturer's instructions. Gene-specific oligonucleotide primers (*rpoH1*_fwd 5'-AGTTCAAGCTGCCGATGAGT-3'; *rpoH1*_rev 5'-CCAGGATGCATAGGTCGAAA; *dps*_fwd 5'-ATCTTGCCCTCATCACCAAG-3'; *dps*_rev 5'-

CACATGGTCGTCGAGTTCTG-3') were designed to amplify approximately 100-bp. qRT-PCR was performed using *rplk* expression with primer pairs *rplK*_fwd (5'-CCGGTGACCTACTTCCTCAA-3') and *rplK*_rev (5'-ATCGAACGGGCAGAACCT-3') as a reference.

Protein immunoblot analysis. Rabbit PhyR polyclonal antiserum was raised against His6-PhyR, which was purified by serial affinity and size exclusion chromatographies. Rabbits were immunized with purified PhyR on days 0, 21, 35, 49, and 63. Total *B. abortus* protein from liquid cell culture was concentrated by precipitation with 10% trichloroacetic acid, separated by 12 % SDS/PAGE, and then transferred to a PVDF membrane (Millipore). The membrane was probed with the primary polyclonal anti-PhyR antiserum (1:1,000) followed by HRP-conjugated goat anti-rabbit secondary antibodies (1:10,000) (Thermo Scientific). Blots were developed using SuperSignal West Femto Substrate (Thermo Scientific). The membrane was stripped using Restore™ Western Blot Stripping Buffer (Thermo Scientific) to probe with rabbit polyclonal ClpX antiserum (4) as described above for anti-PhyR. The signal intensity was measured using ChemiDoc™ MP system (Bio-Rad) or ImageJ 1.44o (NIH) for the films of blots.

Supplemental Figures

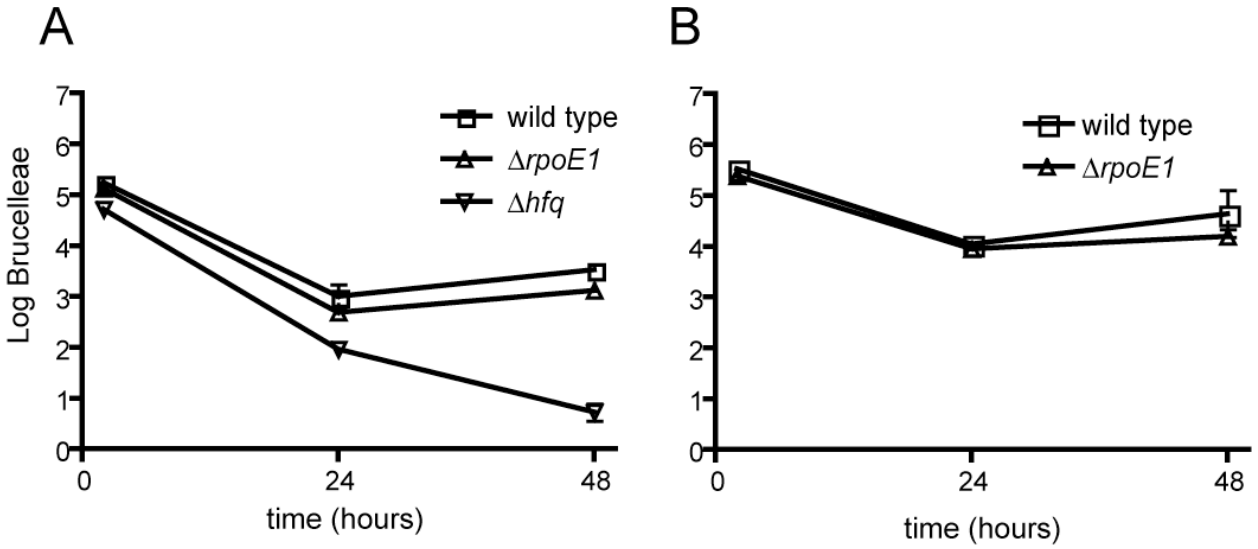

Figure S1. Primary murine peritoneal macrophage infection by *B. abortus* 2308 and an in-frame deletion mutant of *rpoE1* (Δ*rpoE1*). Peritoneal macrophages from (A) C57BL/6 mice and (B) BALB/c mice were infected with *B. abortus* 2308 and Δ*rpoE1*. A Δ*hfq* strain was included as an attenuated positive control (panel A). At marked times postinfection, macrophages were lysed, and the number of intracellular brucellae enumerated by serial dilution and plating on solid medium. Data represent the mean of 3 independent replicates; errors bars represent standard deviation.

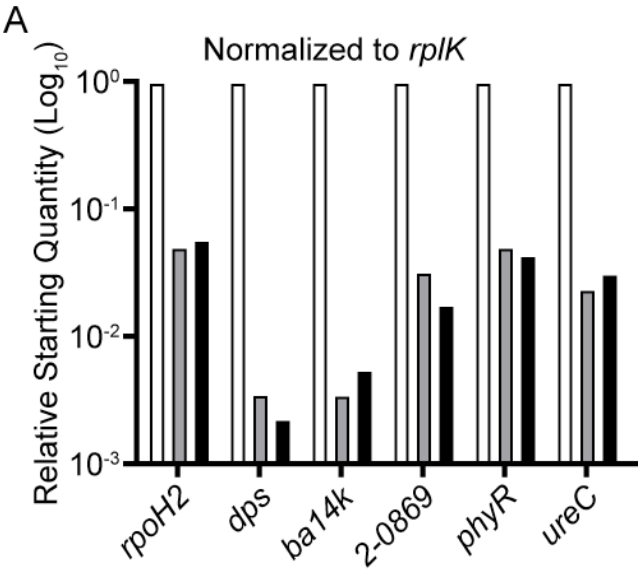

Figure S2. qRT-PCR experiment to confirm the Affymetrix GeneChip analysis of the GSR regulon. mRNA levels of *rpoH1, ba14k*, *BAB2_0869*, *phyR*, and *ureC* measured in wild-type (white bars), Δ*rpoE1* (grey bars), and Δ*nepR-rpoE1* (black bars) normalized to reference gene *rplK*

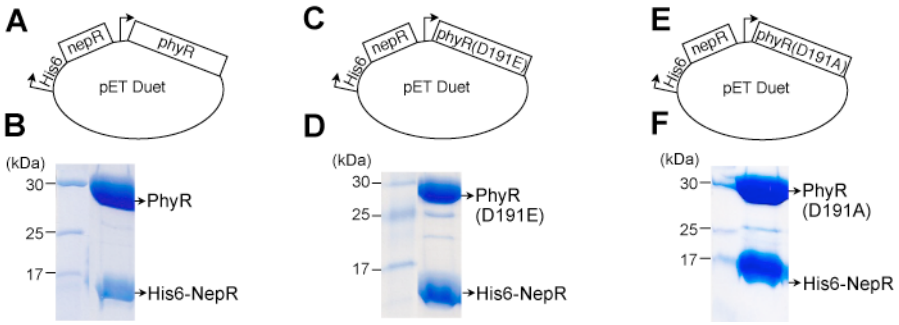

Figure S3. *B. abortus* His6-NepR co-purifies with wild-type *B. abortus* PhyR, PhyR(D191E), and PhyR(D191A). PhyR, PhyR(D191E), or PhyR(D191A) and NepR were individually cloned into and expressed from pETDuet (A, C, and E). The proteins were resolved on a 14% SDS-PAGE after purification on a Ni^{2+}-nitrilotriacetic acid (NTA) resin column (B, D, and F).

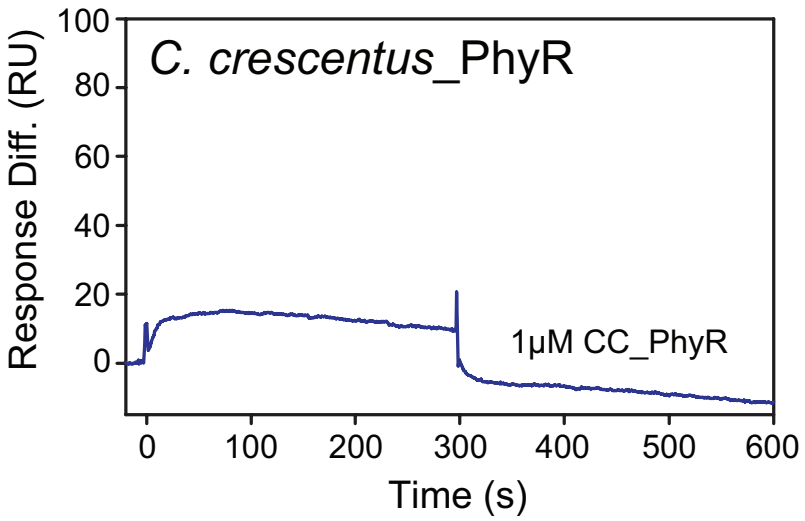

Figure S4. SPR experiment testing *C. crescentus* PhyR binding to *B. abortus* NepR. 1 μM of *C. crescentus* PhyR was flowed across a NTA biosensor chip on which His6-NepR of *B. abortus* was immobilized (R_{ligand} of His6-NepR binding to NTA chip = 505; theoretical $R_{max} \approx 1300$). Association measured from 0-300 seconds; dissociation phase was measured from 300 to 600 seconds. These data provide evidence that there is no specific binding between *C. crescentus* PhyR and *B. abortus* NepR at 1 μM *C. crescentus* PhyR.

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B. abortus       1 -----MTLSTRIAPHLPYLRRFSRALTGSQSSGDAYVAAALEALIADVGIFPEASSDRIG
S. meliloti      1 -----MPLSTRIAPHLPYLRRYARAVTGSQAAGDAYVAAVLEALIDDLELFPAASNDRIG
C. crescentus    1 -----MSLLARLAPHLPYIRRYARALTGDQATGDHYVRVALEALAAGELVLDANLSPRVA
M. extorquens    1 ------------MQHLPYLRRYARALTGSQVAGDAYVAATLETLVNEPETLGRSTNVKAD
B. japonicum     1 MNGVGMSRSQLVAEHLPLLRRYARALTGSQASGDAYVAAMLEAMLGDPAVLDESHGPRAG

B. abortus      56 LYRLFCNLYKNASIRMPETSSE-FAWETHAARNLAHIAPLPRQAFLLVAVEGFNDHEAAE
S. meliloti     56 LYKLFCSLFEKLKIDRTQLASP-FAWEQRAASNLSLIAPPQRQAFLLVALEGFSTGEAAE
C. crescentus   56 LYRVFHAIWLSSGAQLEVGHDQGLHAGDDAAQRLMRIAPRSRQAFLLTALEGFTPTEAAQ
M. extorquens   49 LFRVFTRIWNSLSVN---GHSDQVQHDLPAEVRLGQITPLPRQAFLLSCLEGFSEEDAGV
B. japonicum    61 LFRLFTQIWNSVSVN---DDSEVTTLPMPPERRLSNITPLPRQAFLLLSLEGFSEEEVAF

B. abortus     115 ILEVDQAEFGRLLSTASGEISRQVATRLMIIEDEPLIAMDIEQMVESLGHEVVGIARTKD
S. meliloti    115 IMGLDQGAFGKLLTSASEEISRQIATDVMIIEDEPLIALDIEDMVTSLGHRVTGIARTRR
C. crescentus  116 ILDCDFGEVERLIGDAQAEIDAELATEVLIIEDEPVIAADIEALVRELGHDVTDIAATRG
M. extorquens  106 ILDVDVSKVRDLVDEAGRELAADMATEILIIEDEPLIAMDLEALVEGLGHNVIGVARTRT
B. japonicum   118 ILSTDVAETRRLADAAGREMAAEIATDVLIIEDETFIAMDLESLVKNLGHNVVGVARTHA

B. abortus     175 EALALYE-KEKPRMVLADIQLADGSSGIDAVNEILHDNTIPVIFITAFPERLLTGERPEP
S. meliloti    175 EALNLYH-QTSPKMVLADIQLADGSSGIDAVNDILTQAAVPVIFITAFPERLLTGKKPEP
C. crescentus  176 EALEAVT-RRTPGLVLADIQLADGSSGIDAVKDILGRMDVPVIFITAFPERLLTGERPEP
M. extorquens  166 EAVKIASESKRPGLILADIQLADGSSGLDAVNDLLKTFEVPVIFITAYPERFLTGERPEP
B. japonicum   178 DAVALAK-NRRPGLILADIQLADGSSGLDAVNELLRTFEVPVVFITAYPERFLTGERPEP

B. abortus     234 TFLVTKPFNPDMVKALISQALFFEEASQVAA------
S. meliloti    234 AFLVTKPFNPETVKALISQALFFDEHAQAGAS-----
C. crescentus  235 TFLITKPFQPETVKAAIGQALFFHPRRTAKAA-----
M. extorquens  226 AFLIAKPFQPANVSAVISQALFFQQSARRREAHNA--
B. japonicum   237 AFLISKPFQPAMVSAVASQALFFQRNSRNRTPKAPAA
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Figure S5. Alignment of 5 different PhyR proteins. The highlighted red sequence of *B. abortus* PhyR indicates the putative *ssrA*-like ClpXP degron tag.

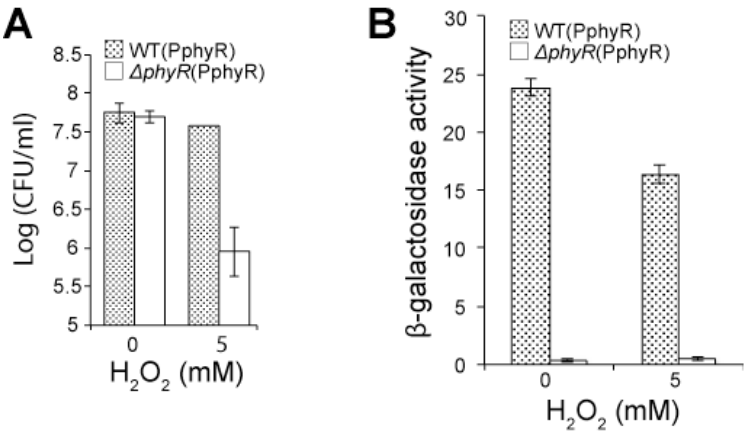

Figure S6. Effects of oxidative stress on expression of the P_{phyR}–*lacZ* transcriptional fusion (P_{phyR}-pMR15). *B. abortus* 2308 and Δ*phyR* mutant strains carrying P_{phyR}–*lacZ* were grown in GMM in the presence or absence of 5 mM H_2O_2, as indicated. Samples were harvested at 1 hour after oxidative stress exposure and assayed for β-galactosidase activity. The data presented represent three experimental trials. Error bars represent standard deviation.

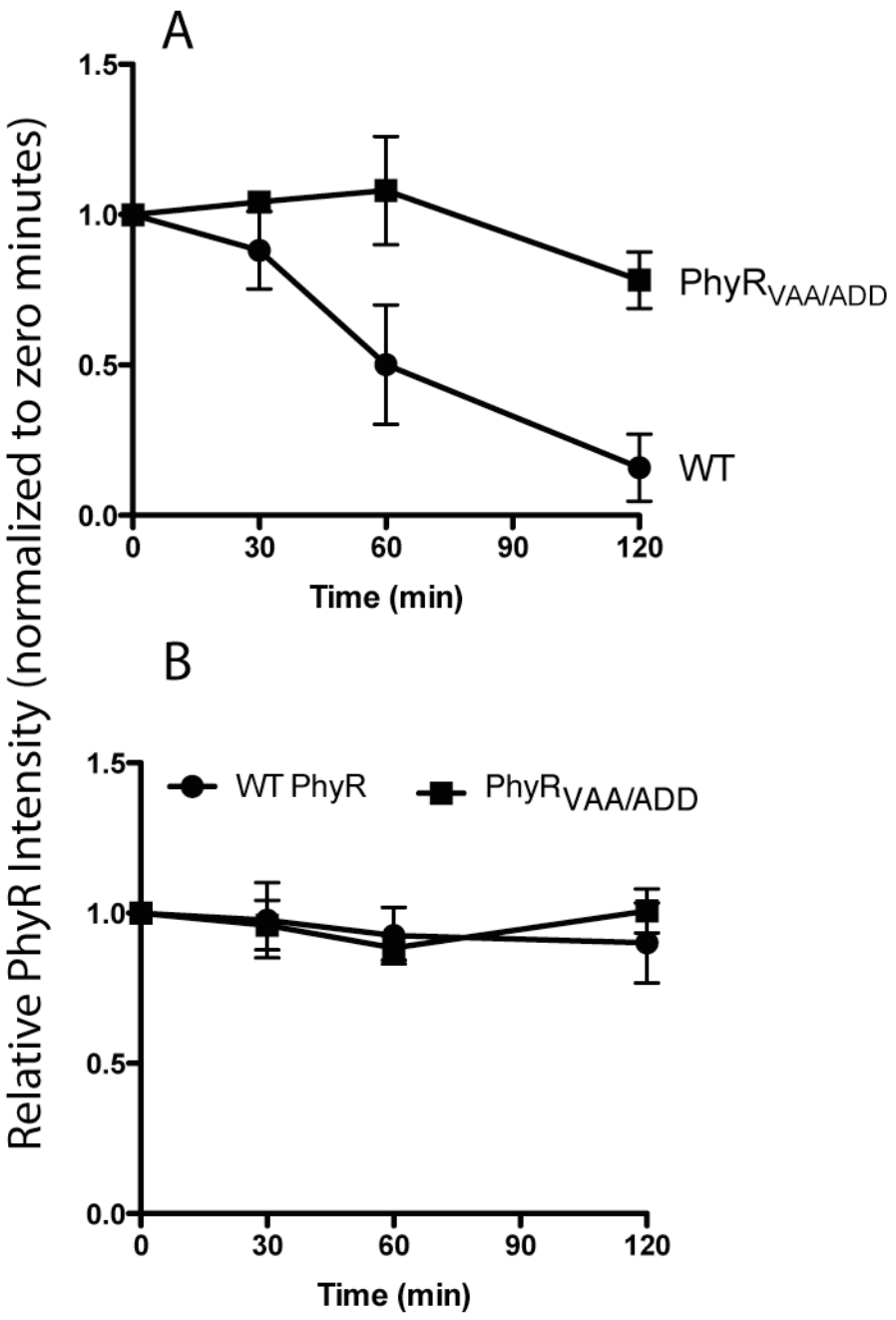

Figure S7. Quantification of PhyR protein degradation. (A) Turnover of wild-type PhyR and the C-terminal mutant PhyR$_{VAA/ADD}$ protein during logarithmic growth, in the absence of stress. The Western blot signal of wild-type PhyR and mutant was quantified as described in Supplemental Materials and Methods above. (B) Turnover of wild-type PhyR and the C-terminal mutant PhyR$_{VAA/ADD}$ after treatment of the cell with an oxidative stressor (hydrogen peroxide). All data are normalized to the signals from non-specific control bands on the same biological samples (*n*=3) (see Figure 5 in the main manuscript for blot images). Error bars refer to standard error of the mean.

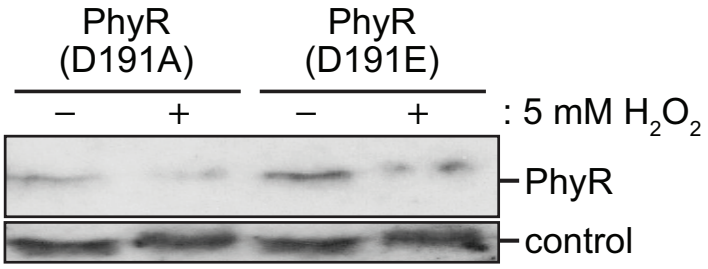

Figure S8. Mutation of the conserved aspartyl phosphorylation site (D191) on the PhyR receiver domain to either alanine (D191A) or glutamic acid (D191E) decreased PhyR protein level in the cell regardless of stress. Protein samples were prepared at 1 hr after oxidative stress exposure. Immunoblot analysis was performed using a rabbit PhyR polyclonal antiserum.

Table S1. Genes directly and indirectly regulated by *Brucella abortus* σ^{E1} upon oxidative stress treatment (using a p <0.005 cutoff as determined by Partek analysis). Replicate Affymetrix tiled microarrays were used compare gene expression in wild type relative to a Δ*rpoE1* null strain; both strains were grown to the same density and subjected to equivalent oxidative (H_2O_2) stress (see Materials and Methods). Negative fold change indicates genes that have decreased transcription in a Δ*rpoE1* null mutant relative to wild-type; positive fold change indicates genes that have increased transcription in Δ*rpoE1* relative to wild-type. Fold change value is the mean from three replicate array experiments.

Locus ID	Annotation	*p*-value	Fold change
BAB1_1775	RNA polymerase factor sigma-32 RpoH1	1.00E-06	-56.3
BAB1_2150	DNA starvation/stationary phase protection protein Dps	5.27E-06	-44.0
BAB2_0505	immunoreactive 14 kDa protein BA14k	6.84E-09	-32.6
BAB2_0696	hypothetical protein	1.48E-06	-25.8
BAB2_0869	secretion protein HlyD	1.77E-06	-25.2
BAB1_1672	RNA polymerase sigma factor RpoE1	2.60E-04	-20.1
BAB2_0755	hypothetical protein BAB2_0755	1.01E-09	-19.3
BAB1_1389	camphor resistance protein CrcB	1.44E-05	-18.1
BAB1_1390	camphor resistance CrcB protein	1.02E-05	-17.9
BAB2_0866	glutamate decarboxylase alpha	4.60E-05	-16.4
BAB1_1070	TrpR binding protein WrbA	4.24E-06	-16.4
BAB1_1670	Conserved Hypothetical - Likely integral membrane protein	3.76E-06	-14.1
BAB2_0868	hypothetical protein	5.51E-07	-14.0
BAB1_1213	hypothetical protein	1.21E-06	-13.6
BAB2_0836	putative phosphoketolase	5.77E-07	-13.6
BAB2_0835	acetate kinase	3.53E-05	-10.5
BAB1_1391	hypothetical protein	4.28E-05	-8.6
BAB2_0620	hypothetical protein	7.03E-07	-8.5
BAB1_1375	hypothetical protein	9.94E-05	-7.8
BAB1_1378	urease subunit alpha	6.51E-06	-7.7
BAB1_1376	urease subunit gamma	6.32E-05	-7.6
BAB2_0648	hypothetical protein	9.01E-07	-7.4
BAB1_1377	urease subunit beta	1.68E-04	-7.3
BAB1_1674	hypothetical protein	5.14E-05	-7.2
BAB2_0865	pyridoxal-dependent decarboxylase	5.70E-05	-6.7
BAB2_0504	hypothetical protein	2.55E-07	-6.0
BAB1_1405	hypothetical protein	1.27E-04	-5.6
BAB1_0224	ABC transporter ATPase	2.52E-05	-5.6
BAB2_0272	hypothetical protein	3.34E-06	-5.6
BAB1_1380	urease accessory protein UreF	7.95E-04	-5.0
BAB2_1108	putative integral membrane protein	8.20E-07	-5.0
BAB1_0225	binding-protein dependent transport system inner membrane protein	1.21E-04	-4.9
BAB1_0226	substrate-binding region of ABC-type glycine betaine transport system	6.53E-05	-4.8
BAB1_1381	urease accessory protein UreG	1.76E-04	-4.7
BAB1_1495	antifreeze protein, type I	4.42E-07	-4.5
BAB1_0099	response regulator receiver	4.76E-07	-4.5

BAB1_0458	DedA family protein	8.68E-05	-4.4
BAB1_1206	7-cyano-7-deazaguanine reductase	2.30E-05	-4.1
BAB2_0568	hypothetical protein	1.32E-06	-4.1
BAB2_0697	hypothetical protein	5.45E-08	-3.9
BAB1_1675	DNA gyrase, subunit B	3.42E-05	-3.9
BAB2_0187	hypothetical protein	8.12E-06	-3.6
BAB1_1669	PAS domain-containing protein	4.00E-06	-3.6
BAB2_0758	flavin-containing monooxygenase FMO	5.33E-05	-3.4
BAB1_1382	urease accessory protein UreD	3.58E-04	-3.3
BAB1_1383	Urea transporter	1.00E-03	-3.2
BAB1_0223	binding-protein-dependent transporter inner membrane protein	1.21E-04	-3.1
BAB1_0055	Phosphoglucomutase Pgm	6.61E-05	-3.1
BAB2_0759	hypothetical protein	2.77E-05	-2.9
BAB2_0727	cytochrome bd ubiquinol oxidase, subunit II CydB	5.10E-06	-2.8
BAB2_0728	cytochrome bd ubiquinol oxidase, subunit I CydA	9.35E-06	-2.7
BAB1_2064	hypothetical protein	2.08E-05	-2.7
BAB1_1471	major facilitator superfamily sugar transporter	1.85E-05	-2.7
BAB1_0643	major facilitator transporter	3.24E-05	-2.5
BAB2_0863	glutaminase	5.06E-05	-2.5
BAB1_1217	glutamate racemase	1.80E-03	-2.5
BAB2_0726	cytochrome bd oxidase CydX	1.45E-07	-2.5
BAB1_0981	transglycosylase-associated protein	7.90E-05	-2.3
BAB2_0449	carbonic anhydrase	5.19E-03	-2.3
BAB1_0758	hypothetical protein	1.27E-04	-2.3
BAB1_1274	CBS domain-containing protein	1.33E-05	-2.2
BAB1_1421	hypothetical protein	1.40E-04	-2.1
BAB1_1335	hypothetical protein	1.05E-05	-2.1
BAB1_1335	hypothetical protein	1.29E-06	-2.1
BAB2_0864	aromatic amino acid ABC transporter permease	6.48E-06	-2.1
BAB1_1884	hypothetical protein	2.48E-04	-2.1
BAB1_1676	hypothetical protein	1.46E-06	-2.1
BAB1_1061	Beta-lactamase	1.67E-05	-2.1
BAB1_0103	hypothetical protein	4.71E-05	-2.0
BAB1_1336	hypothetical protein	8.14E-06	-2.0
BAB1_2139	hypothetical protein	1.07E-03	-2.0
BAB1_1208	hypothetical protein	1.35E-05	-2.0
BAB1_0400	hypothetical protein	1.83E-04	-2.0
BAB2_0729	transcriptional regulator LysR	3.68E-05	2.1
BAB2_0730	ABC transporter ATPase	1.30E-05	2.1
BAB2_0436	nickel transporter permease NikC	5.63E-05	2.1
BAB1_0802	sugar transferase	1.19E-04	2.2
BAB2_0259	RNA recognition motif-containing protein	2.92E-04	2.4
BAB1_0200	glycerol-3-phosphate dehydrogenase	2.71E-03	2.6
BAB2_0435	nickel transporter permease NikB	4.48E-06	2.8
BAB1_1461	SLT domain-containing protein	6.43E-05	3.6

Table S2. A MAST search was performed to search for conserved sequences in the region upstream of genes regulated by σ^{E1}. The first 200 nucleotides upstream of the annotated translational start of any gene regulated by greater than 1.5 fold was used in this analysis. The genes listed in this table contain a defined σ^{E1} (i.e. σ^{EcfG}) regulatory sequence (5) in their promoters; this provides evidence this gene set is directly regulated by σ^{E1}.

Gene Number	Description	-35 region	-N-	-10 region	Distance from Translational Start[1]	Fold Regulation by RpoE1[2]
BAB1_1775	RpoH1	GGAACC	14	GCGTT	167	-56.3
BAB1_2150	DNA starvation/stationary phase protection protein Dps	GGAACC	15	CGTT	169	-44.0
BAB2_0696	Conserved Hypothetical Protein	TGAACC	14	GCGTT	32	-25.8
BAB2_0505	Ba14K	GGAACA	15	CGTT	31	-32.6
BAB1_1070	TrpR-binding protein WrbA	GCAACC	15	CGTT	85	-16.4
BAB2_0835	Acetate Kinase AckA	GGAACC	15	TGTT	92	-10.5
BAB2_0620	Conserved Hypothetical Protein	GGATCA	15	CGTT	30	-8.5
BAB1_1391	Conserved Hypothetical Protein	GGAACC	15	CATT	99	-8.6
BAB1_1405	Conserved Hypothetical Protein	GCAACC	15	CGTT	56	-5.6
BAB1_1471	Major facilitator superfamily sugar transporter	GGAACC	15	TTTT	19	-2.7
BAB1_0103	Conserved Hypothetical Protein	GCAACG	15	CGTT	146	-2.0

[1]This value is based on the annotated translational start site for the *Brucella abortus 2308* genome.
[2]Fold regulation determined from microarray data, Δ*rpoE1*/WT.

Table S3. *E. coli* TOP10 strains bearing plasmids used in this study

Strain # (FC)	Plasmid name	Primer sequence (5'→3', underlines indicate restriction enzyme sites)
1099	pET151 Directional TOPO-*phyR*	CACCATGACGTTATCGACGCGTATAGCG TCAGGCAGCAACTTGCGATGCTTC
1229	pET151 Directional TOPO-*nepR*	CACCATGACTCAAAGAAACAACCT TC AACCGACGGACCTGTTGTTCTGCC
1256	pET151 Directional TOPO-*rpoE1*	CACCGTGGGCAGAACAACAGGTCCGT TCAGGCGAAGCTTCTGAGGGT CGCA
1091	pET151 Directional TOPO-*nepR-rpoE1*	CACCATGACTCAAAGAAACAACCTTC TCAGGCGAAGCTTCTGAGGGTCGCA
1224	pETDuet-*nepR-phyR*(D191E)	CCAGGATCCGATGACTCAAAGAAACAACCTTC CATGAGCTCTCAGGCGAAGCTTCTGAGGGT AGCCATATGACGTTATCGACGCGTATAGCG GTCGGCAAGCTGGATCTCGGCGAGAACCATGCG CGCATGGTTCTCGCCGAGATCCAGCTTGCCGAC GTGCTCGAGTCAGGCAGCAACTTGCGATGCTT
1225	pETDuet-*nepR-phyR*(D191A)	CCAGGATCCGATGACTCAAAGAAACAACCTTC CATGAGCTCTCAGGCGAAGCTTCTGAGGGT AGCCATATGACGTTATCGACGCGTATAGCG GTCGGCAAGCTGGATAGCGGCGAGAACCATGCG CGCATGGTTCTCGCCGCTATCCAGCTTGCCGAC GTGCTCGAGTCAGGCAGCAACTTGCGATGCTT
1393	pETDuet-*phyR-nepR*	TCGAGCTCGATGACGTTATCGACGCGTATAGC TTGTCGACTCAGGCAGCAACTTGCGATGCTTC GCCATATGACTCAAAGAAACAACCTTC AACTCGAGTCAACCGACGGACCTGTTGTTCTGCC
1415	pMR15-P*phyR*	GTAAGCTTCATACTAGGCTTTCGGCCCAAC ATGAATTCGATTCAGGGACTCCTCAATTACCG
1879	pNTPS138-Δ*phyR*	ATACTAGTTCCGTATCCTGCACCTCGCGG TGCTGCCTGCCAGCGGATTCAGGGACTCCTCAATTACC CGCTGGCAGGCAGCACAACACAG GTCTGCAGTCGACATTCTGGGCAATTCCACT
1887	pNTPS138-Δ*rpoE1-nepR*	ATATACTAGTAATGAGGGGTTCATCC AGGCGAATGAGTCATATTTGATCATA TGACTCATTCGCCTGACCAAACGAT ATATCTCGAGGTTCTTCCGCATCGAT
1640	pNTPS138-*phyR*(D191E)	ATACTAGTTCCGTATCCTGCACCTCGCGG GTCGGCAAGCTGGATCTCGGCGAGAACCATGCG CGCATGGTTCTCGCCGAGATCCAGCTTGCCGAC GTCTGCAGTCGACATTCTGGGCAATTCCACT

1627	pNTPS138-*phyR*(D191A)	ATACTAGTTCCGTATCCTGCACCTCGCGG GTCGGCAAGCTGGATAGCGGCGAGAACCATGCG CGCATGGTTCTCGCCGCTATCCAGCTTGCCGAC GTCTGCAGTCGACATTCTGGGCAATTCCACT
1880	pNTPS138-*phyR*$_{VAA/ADD}$	GCACTAGTGCATCAGCACTCGAAAGGTTCTCT GAGGAAGCATCGCAAGCTGATGACTGACGCTGGCAGG- CAGCACAACA TGTTGTGCTGCCTGCCAGCGTCAGGCAGCAACTTGCG- ATGCTTCCTC GTCTGCAGTCGACATTCTGGGCAATTCCACT

Table S4. *B. abortus* and *C. crescentus* strains used in this study

Strain #	Strain name
CBA 1	*B. abortus* 2308
CBA 2	2308 Δ*phyR*
CBA107	2308 Δ*rpoE1*
CBA108	2308 Δ*rpoE-nepR*
CBA 3	2308 *phyR*$_{VAA/ADD}$
CBA 15	2308 Δ*phyR*::pNPTS138-*phyR*$^{+}$
CBA109	2308 Δ*rpoE1-nepR*::pNPTS138-*rpoE1-nepR*$^{+}$
CBA 19	2308 *phyR*(D191E)
CBA 20	2308 *phyR*(D191A)
FC 19	*C. crescentus* CB15
FC 799	CB15 Δ*phyR*

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