Supplemental Materials: Tables and Figures.

Table S1. RNA-seq statistics.

Strain bioreplicate & treatment	Total number of reads sequenced	Total number of reads mapped uniquely	Reads not mapped to chromosome	Reads mapped >1x to chromosome
WT 1	15,846,152	13,074,337	2,364,088	407,727
WT 2	15,735,848	13,004,425	2,333,234	398,189
WT 3	35,684,241	32,020,819	2,823,043	840,379
WT 1 +MMC	15,727,305	13,067,209	2,089,081	571,015
WT 2 +MMC	15,727,305	13,067,209	2,089,081	571,015
WT 3 +MMC	33,294,158	29,923,271	2,417,759	953,128
Δ*rtcR* 1	46,571,576	39,022,270	4,440,113	3,109,193
Δ*rtcR* 2	16,427,543	14,633,741	1,384,032	409,770
Δ*rtcR* 3*	3,775,980	1,527,319	2,175,792	72,869
Δ*rtcR* 1 +MMC	39,833,788	35,711,397	2,830,132	1,292,259
Δ*rtcR* 2 +MMC	21,193,827	18,855,747	1,688,471	649,609
Δ*rtcR* 3 +MMC	16,059,581	14,226,416	1,353,148	480,017
Δ*rsr* 1	22,386,184	19,119,591	2,709,208	557,385
Δ*rsr* 2	15,510,441	12,646,470	2,466,666	397,305
Δ*rsr* 3	16,137,416	8,062,563	7,812,809	262,044
Δ*rsr* 1 +MMC	16,845,332	14,966,926	1,380,774	497,632
Δ*rsr* 2 +MMC	34,518,155	30,850,209	2,522,913	1,145,033
Δ*rsr* 3 +MMC	14,983,343	13,383,635	1,146,785	452,923
Δ*rtcB* 1	19,899,438	18,018,089	1,411,306	470,043
Δ*rtcB* 2	21,493,828	19,585,216	1,448,051	460,561
Δ*rtcB* 3	19,875,919	17,728,551	1,661,371	485,997
Δ*rtcB* 1 +MMC	23,946,132	21,203,786	1,797,872	944,474
Δ*rtcB* 2 +MMC	16,631,197	14,857,557	1,293,566	480,074
Δ*rtcB* 3 +MMC	18,257,370	15,983,836	1,728,604	544,930

*Biological replicate Δ*rtcR* 3 gave insufficient reads for good coverage of the transcriptome, and so the data was not used in subsequent analyses of the RNA-seq data for Δ*rtcR* (see Materials and Methods).

Table S2. Correlation of FPKM data from RNA-seq with *S.* Typhimurium strain and condition biological replicates.

Strain & treatment Bioreplicates compared	Pearson's *r*
WT	
1 vs 2	0.98
2 vs 3	0.96
1 vs 3	0.97
WT+MMC	
1 vs 2	0.90
2 vs 3	0.95
1 vs 3	0.88
Δ*rtcR*	
1 vs 2	0.97
Δ*rtcR* +MMC	
1 vs 2	0.88
2 vs 3	0.98
1 vs 3	0.91
Δ*rsr*	
1 vs 2	0.97
2 vs 3	0.97
1 vs 3	0.99
Δ*rsr* +MMC	
1 vs 2	0.84
2 vs 3	0.85
1 vs 3	0.98
Δ*rtcB*	
1 vs 2	0.94
2 vs 3	1.0
1 vs 3	0.96
Δ*rtcB* +MMC	
1 vs 2	1.0
2 vs 3	0.98
1 vs 3	0.98

Table S3. Bacterial strains and plasmids used in this study.

Strain/Plasmid	Description or Construction	Source or reference
***S.* Typhimurium**		
ATCC 14028s	Wild-type	ATCC
DJS101	14028s Δ*rtcR*	This study
ANE004	14028s Δ*rsr*	This study
DJS102	14028s Δ*rtcB*	This study
CH06	14028s Δ(*dinJ-yafQ*)	This study
CH01	14028s Δ*ntrC*	This study
ACB01	14028s Δ*rpoN*	Ref. (1)
JE10649	LT2 *metE*205 *ara*-9 Δ*recA*::*kan*	Courtesy of J. Escalante-Semerena
DS103	14028s Δ*recA*::*kan*	This study
JEK17	14028s Δ*rsr*::*xylE*	This study
JEK21	DJS101 Δ*rsr*::*xylE-kan*	This study
SGD_recA_Kan	14020s Δ*recA*::*kan*	BEI Resources, ATCC
JEK26	JEK17 Δ*recA*::*kan*	This study
JEK41	JEK17 Δ*rtcR*::*kan*	This study
MS1868	LT2 *leuA*414 *hsdL* Fels2	Ref.(2)
E. coli		
MG1655 (*rph*-1)	*rph-1*	Courtesy of S. Kushner
JW3385	BW25113 Δ*rtcR*	Ref. (3)
ACK206	MG1655 *rph*-1 Δ*rtcR*	This study
DH5α	F^- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^- m_K^+$), λ^-	Courtesy of C. Moran
Plasmids		
pKD4	*kan* template with FLP recognition target sites for λ-Red recombination, Kan^R	Ref. (4)
pKD46	Expresses λ-Red genes *γ, β*, and *exo* from arabinose-inducible P_{araB} promoter, Amp^R	Ref. (4)
pCP20	Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp^R	Ref. (5)
pSB306	pBlueScript derivative with *xylE* gene from *Pseudomonas putida*, Amp^R	Ref. (6)
pCR2.1	TA cloning vector, Amp^R Kan^R	Invitrogen; Carlsbad, CA
pCR4	TA cloning vector, Amp^R Kan^R	Invitrogen; Carlsbad, CA

pKH66	pSC101 derivative with P_{taq}-Hin, Str^R Spc^R	Ref. (7)
pSRK-Tc	Derivative of pBBR1, expression vector containing a reengineered *lacI*^{q}-*lac* promoter-operator complex for tight repression in absence of inducer, Tet^R	Ref. (8)
pBAD30	Derivative of pACYC-184, expression vector with arabinose-inducible P_{BAD} promoter, Amp^R	Ref. (9)
pDS171	pCR2.1 with 197 bp 14028s *rpoD* fragment	This study
pDS185	pCR4 with 180 bp 14028s *rtcA* fragment	This study
pDS181	pCR4 with truncated 14028s *rtcR* (to produce $RtcR_{con}$)	This study
pDS183	pHK66 digested with BamHI-HindIII, with BamHI-HindIII *rtcR*$_{con}$ fragment from pDS181	This study
pCH11	pCR2.1 with full-length 14028s *rtcR*	This study
pCH12	pSRK-Tc with NdeI-XhoI *rtcR* fragment from pCH11	This study
pJK3	pSRK-Tc with NdeI-XhoI *rtcR* fragment, amplified from MG1655 genomic DNA	This study
pJK20	pBAD30 with EcoRI-HindIII *yafQ* fragment, amplified from 14028s genomic DNA	This study

Table S4. Oligonucleotides used in this study.

Number	Name	Sequence
Deletion strain generation:		
1	rtcR-RED F	GCCTTTGGTTTTGTCGGTACGGTACTCGACTATGCATATGTAG GCTGGAGCTGCTTCG
2	rtcR-RED R	ATTCTGTAAAACGTCCCACGTCAGCCCAAAACGCGCCATATGA ATATCCTCCTTAG
3	rsr-RED F	CATGGAGAATAACGGAAATGGGAAAAACAATGTGTAGGCTGGA GCTGCTTC
4	rsr-RED R	GGCCAGCATTCGCGCCGCGCTGAATACTCTCATATGAATATCC TCCTTA
5	rtcB-RED F	CAGAACGCACCGGTAAAAATGTGGACCAAAGGCGTATGTGTAG GCTGGAGCTGCTTCG
6	rtcB-RED R	TCCTTTAACGCACACCACCTGCCGCAGGGCGTACATCATATGA ATATCCTCCTTAG
7	dinJ-yafQ red F	ATTTGAACTGTATAGAGACACAGTACAGGAGACTAATCGTGTGT AGGCTGGAGCTGCTTC
8	dinJ-yafQ red R	CCTGAAAGCACCGGTTAACATATGGGCAAAAACGGCGCTTCAT ATGAATATCCTCCTTA

9	NtrC::KanF	CGAGTTTTCGGTTTACCTGCCGATTCGGAAATAGAGGTGTTGT GTAGGCTGGAGCTGCTTC
10	NtrC::KanR	CGCGGGTAATGTTTACTCCATTCCCAGCTCTTTCAACTTCCATA TGAATATCCTCCTTA
11	rsr42-xylE-F	CAATAACCATGGCATGGAGAATAACGGAAATGGGAAAAACAAA TGAACAAAGGTGTAATGCGACC
12	KanR-xylE-R	AAGCAGCTCCAGCCTACACATCAGGTCAGCACGGTCATG
13	xylE-KanR-F	TGCTGACCTGATGTGTAGGCTGGAGCTGCTTC
14	rsr42-KanR-R	AGATGTAATCCCGGCCAGCATTCGCGCCGCGCTGAATACTCTC ATATGAATATCCTCCTTA
Deletion strain verification:		
15 (11)	ckrtcR-F	CCATTTCCGTTATTCTCCATGCC
16 (12)	ckrtcR-R	GTGATGCAGGTGTTGACGG
17 (13)	ckSTM3521-F	GCAGATCGATACACACCAGGC
18 (14)	ckSTM3521-R	TTTAATGACTACCGTGCAGGC
19 (15)	ckrtcB-O	ATCTGCCCGCCGCCCTCTCC
20 (16)	ckrtcB-R	TCTACATTTGTTGCGGGTTCGAGACCC
21 (17)	ck dinJ-yafQ F	GTTTGTATGTGTGTCTCAGTTGAGC
22 (18)	ck dinJ-yafQ R	CTGAAAGCACCGGTTAACATATGG
23 (19)	ck recA F	CGGTTCAATACCAAGTTGCATGAC
24 (20)	ck recA R	GAATGGCGGCTTCGTTTTGC
25 (21)	ntrC-Fwd1	CGAGTTTTCGGTTTACCTGCCGATTCGGAAATAGAGGTGTTGT GTAGGCTGGAGCTGCTTC
26 (22)	ntrC-Rev	CGCGGGTAATGTTTACTCCATTCCCAGCTCTTTCAACTTCCATA TGAATATCCTCCTTA
27	RTCR-192	AGGTGCTGACGGACCACCAT
28	RTCR-1886	CGTCCGTAACCGGAGATTTC
qRT-PCR:		
29	rpoD-RT F	AACGAATAAGTGTGGATACCG
30	rpoD-RT R	TCTTCCATTACCTGAATACCC

31	rtcA-RT F	CTGGTTAGCTACCGCTTCCG
32	rtcA-RT F	CGAACGTGAAGTCGCAACGC
33	rtcA F1	GCTGGGCTCGCAACAGTTGC
34	rtcA R1	GGACGGTTTGCAGCACCAGC
35	rtcB Q2 F	GTGATGAGCCGAACGAAAGC
36	rtcB Q2 R	CCGCATCAATATCTTTATACGCC
37	kdgR RT F	CGGATGTACTCACGTATTGGGCG
38	kdgR RT R	CAGTGCTGGTAATCGTGCGTCC
39	glnK RT F	CGTTCAAGCTGGAGGACGTGC
40	glnK RT R	GCGATACAGTTCGGCGTGTCC
41	gltU,V,W F	GTCCCCTTCGTCTAGAGGCCC
42	gltU,V,W R1	TGGCGTCCCCTAGGGGATTC
43	EcRpoD-RT F	GCTGAAACTTCTTGTCACCC
44	EcRpoD-RT R	CATCAGATCATCGGCATCCG
45	EcRtcA-RT F	CGTGAAATCGCTACACTGGC
46	EcRtcA-RT R	CCTCTTTCACCAACTGTGCC
47	E.coli_mdh-F	TGACCAAACGCATCCAGAAC
48	E.coli_mdh-R	GCACGAACCAGAGACAGACC
49	StMdh-RT-F	GCCGAGCTGACTAAACGTATTC
50	StMdh-RT-R	GAACCAGAGAAAGACCGAAACG
Cloning:		
51	rtcRcon-F2	ATTATTGGATCCTAAAGAGGTATATATTAATGCTCAACTTCCTG AAGT
52	rtcR-R	AAGCTTAATTCTGTAAAACGTCCC
53	NdeI-RtcR F	ACGCATATGCGAAAAACGGTGGCCTTTGG
54	XhoI-RtcR R	AAGCTCGAGTTAATTCTGTAAAACGTCCCACGTCAGCC
55	NdeI-EcRtcR-F	ATCACTCATATGCGTAAAACAGTGGCTTTTGG
56	XhoI-EcRtcR-R	ATCACTCTCGAGTCAACTGGAGCTGTGCTGATC
57	yafQ-EcoRI-F	ATCACTGAATTCATGGGGCAAAGGGAAATTG

58	yafQ-HindIII-R	ATCACTAAGCTTTTAAAATAAATCGGCATGCGTTC
Sequencing:		
59	pSRK Insert F	CCGGCTCGTATGTTGTGTGG
60	pSRKTc Rev	CAAGGCGATTAAGTTGGGTAACG
61	M13F(-21)	TGTAAAACGACGGCCAGT
62	M13R	CAGGAAACAGCTATGAC
63	pBAD Forward	ATGCCATAGCATTTTTATCC
64	pBAD Reverse	GATTTAATCTGTATCAGG
Northerns and *dinJ-yafQ* transcript analyses:		
65	T-A transc R	ATCCGCTATATGAACCTTGTAACG
66	T-A transc F1	TCGGCGTAAAAGGCACCAGC
67	T-A transc F2	AGCGACTAAGTCATCTTTACGAGC
68	T-A transc F3	GCTTGTTCGTGCCCGTATCG
69	valV	TGTCAAGGTGGTGCTCTAACCAAC
70	ARGX-89	TCCGGAGGGCAGCGCTCTAT
71	GLTmature	ACCCCTGTTACCGCCGTGAA
72	rho1 RT R	TCAACTCATGTCTGTTCGCTGTC
73	LPP-1	TCAGCTGGTCAACTTTAGCG
74	rsr Q1 R	GCACAAAGTTACGCAGCATCC
75	rtcB Q1 R	GGTGGCACGAATTTGATCG
76	rtcA probe	TCCACCACCGCAATATTGGTA
77	DINJ6350	CACGCGCAACTTTAGTCAGT
78	YRLB-3594	TAGCCGGGATGATACGCATC
79	Pseudo-tRNA R	CTGACGGGTCTCGAACCC
Additional qRT-PCR Primers:		
80	rsr-RT1-F	TTGGTAAGCCGTGCGATAAG
81	rsr-RT1-R	AAACGACACATCGGGTAAGG

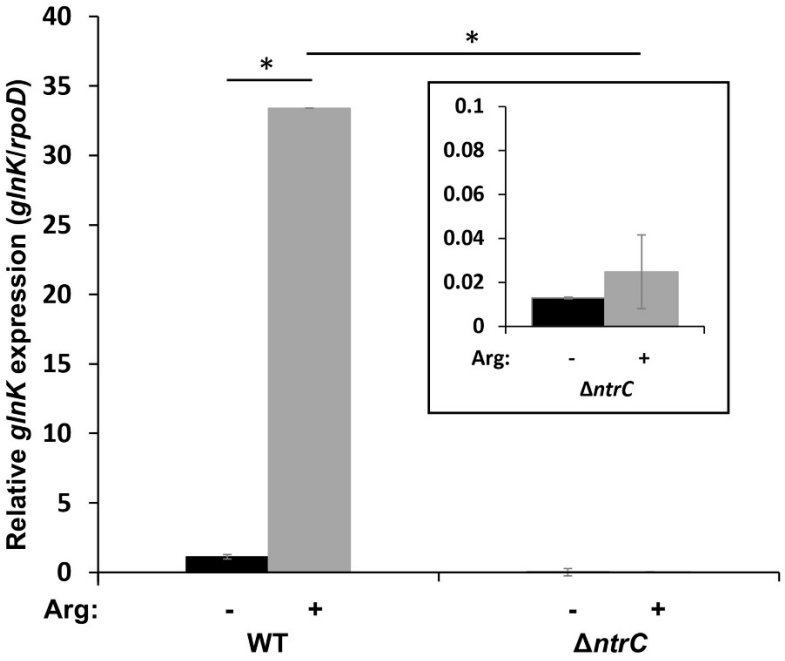

FIG S1. Nitrogen-limiting conditions activate NtrC to stimulate expression of *glnK*. NtrC activation by the nitrogen-limiting conditions (90-min growth in minimal medium containing arginine as the sole nitrogen source) was assayed using qRT-PCR to measure *glnK* transcript levels (normalized to *rpoD* levels) in WT and the Δ*ntrC* mutant. Significant differences in relative *glnK* expression between treated and untreated samples and between the treated WT and Δ*ntrC* strains are indicated (**P* <0.0001). Data shown are for 3 biological replicates for each strain; error bars represent ±1 standard deviation.

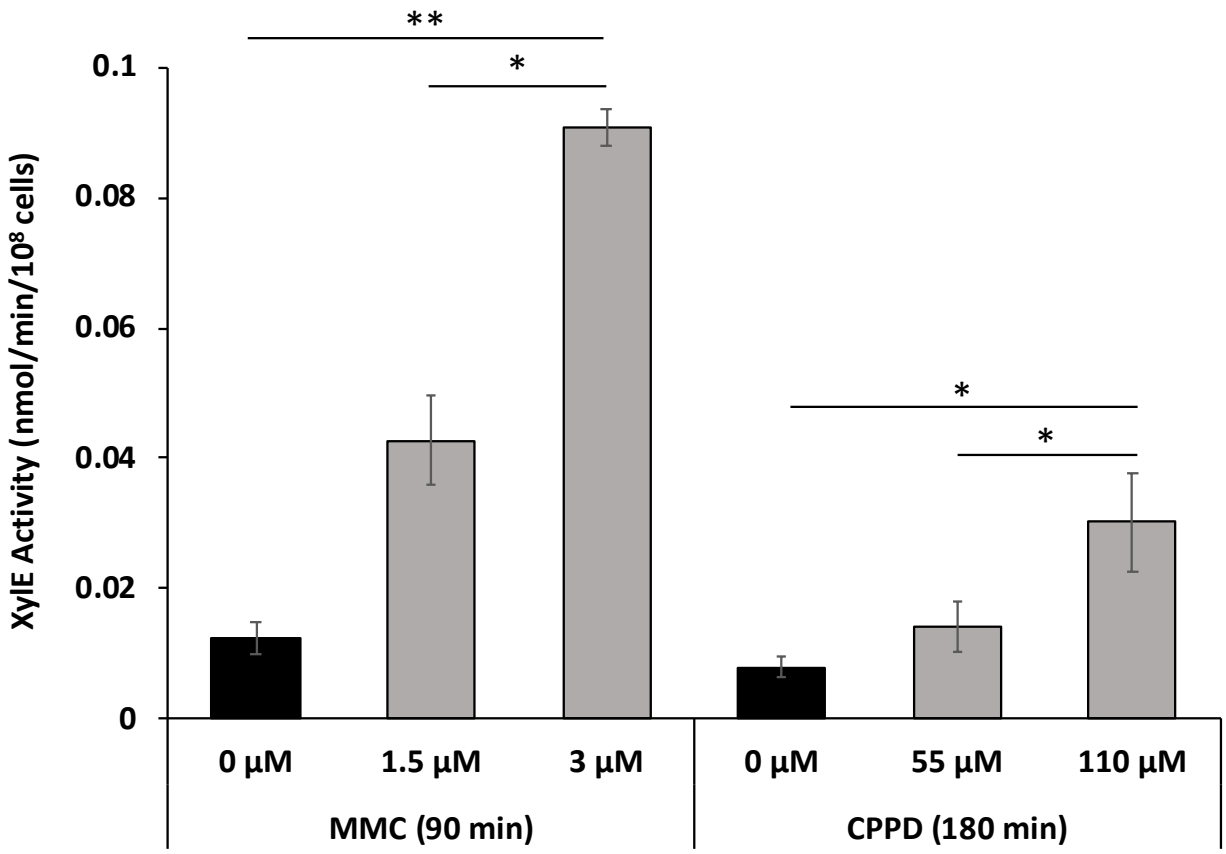

FIG S2. Induction of the RNA repair operon is proportionate to the level of nucleotide damage incurred. Biological replicates of the reporter strain JEK17 (WT Δ*rsr*::*xylE*) were grown to mid-log phase and split into three cultures: one remained untreated, one received an intermediate dose of MMC or CPPD, and one received a high dose of MMC or CPPD (concentrations specified on the x-axis). Cells were treated for 90 min (MMC) or 180 min (CPPD) and promoter activity at P_{rsr} was inferred by assaying cells for XylE activity. Data are given as nmol of catechol oxidized per minute (normalized to 10^8 cells). Each bar represents the average of three biological replicates; error bars represent ±1 standard deviation. Significant differences are indicated (* P <0.05, ** P <0.001).

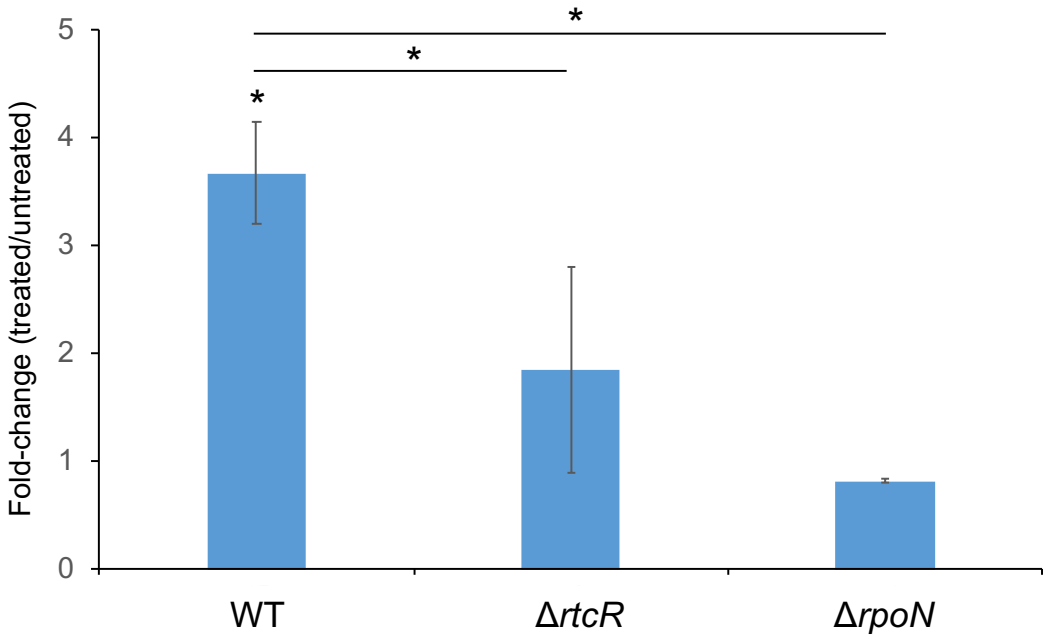

FIG S3. Analysis of *rsr* expression during low- level peroxide stress. 14028s WT, Δ*rtcR*, or Δ*rpoN* strains were grown to mid-log phase in LB and split into two cultures. One culture was treated with 1 mM H_2O_2 for 20 min, while the other remained untreated. Expression of *rsr* was assessed by qRT-PCR; transcript levels were normalized to expression of the reference gene *kdgR*. The data denote the fold-increase in expression of H_2O_2-treated cells vs. untreated cells. Statistically significant differences between treated and untreated cells and between different strains are indicated (* $P < 0.05$); error bars represent ±1 standard deviation.

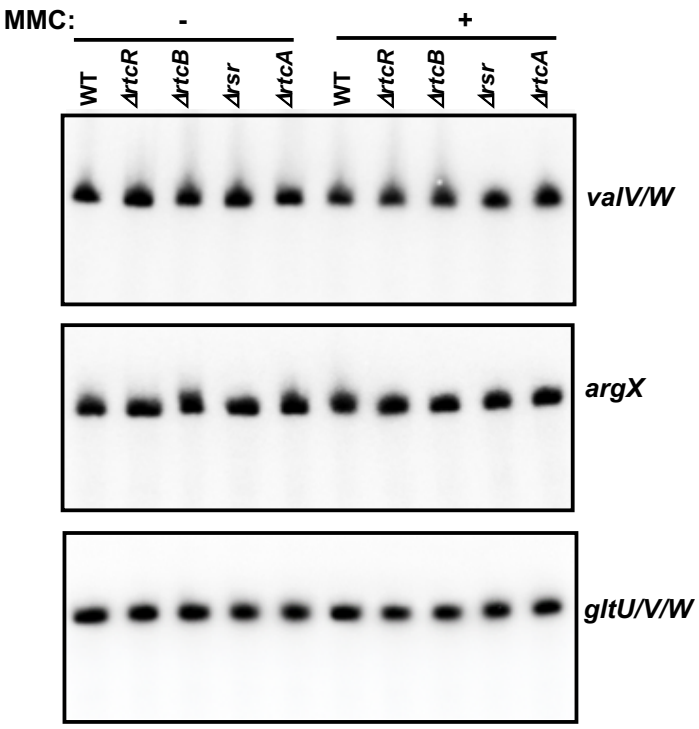

FIG S4. Northern blot analysis of transcripts for *valV, argX,* and *gltU/V/W*. The Northern blots were prepared with equal amounts of total steady state RNA from *S.* Typhimurium 14028s WT, Δ*rtcR,* Δ*rtcB,* Δ*rsr,* and Δ*rtcA* strains (untreated and treated with MMC) electrophoresed on a 8% polyacrylamide gel. Each membrane was probed for *valV/W, argX*, or *gltU/V/W*. These Northern blots are representative of results from 2 to 3 biological replicates.

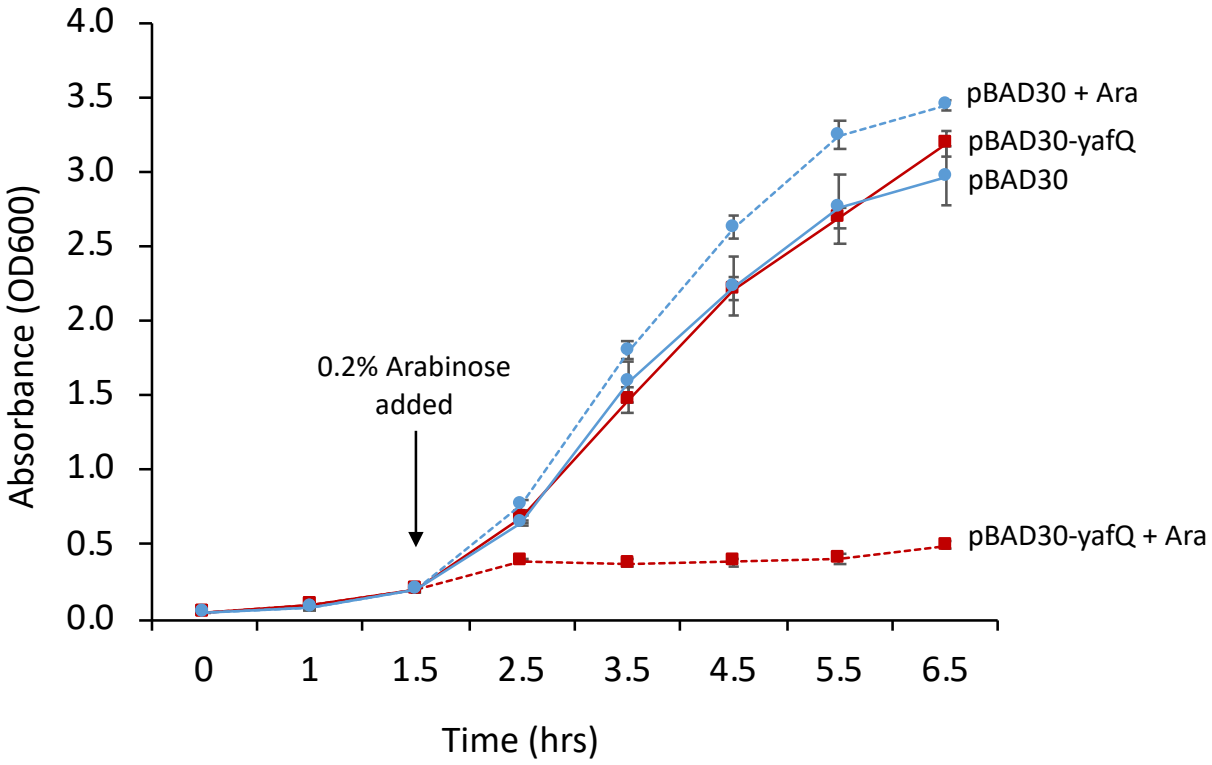

FIG S5. The RNA-cleaving toxin YafQ represses growth in *S*. Typhimurium. Growth curve assays were performed to verify the activity of YafQ *in vivo*. WT cultures containing pJK20 (pBAD30-yafQ) or pBAD30 empty vector were split and either induced with 0.2% arabinose (Ara) or remained untreated. OD_{600} readings were taken every hour for up to 5 hours after induction. The data shown are the average of 3 biological replicates for each strain; error bars represent ±1 standard deviation.

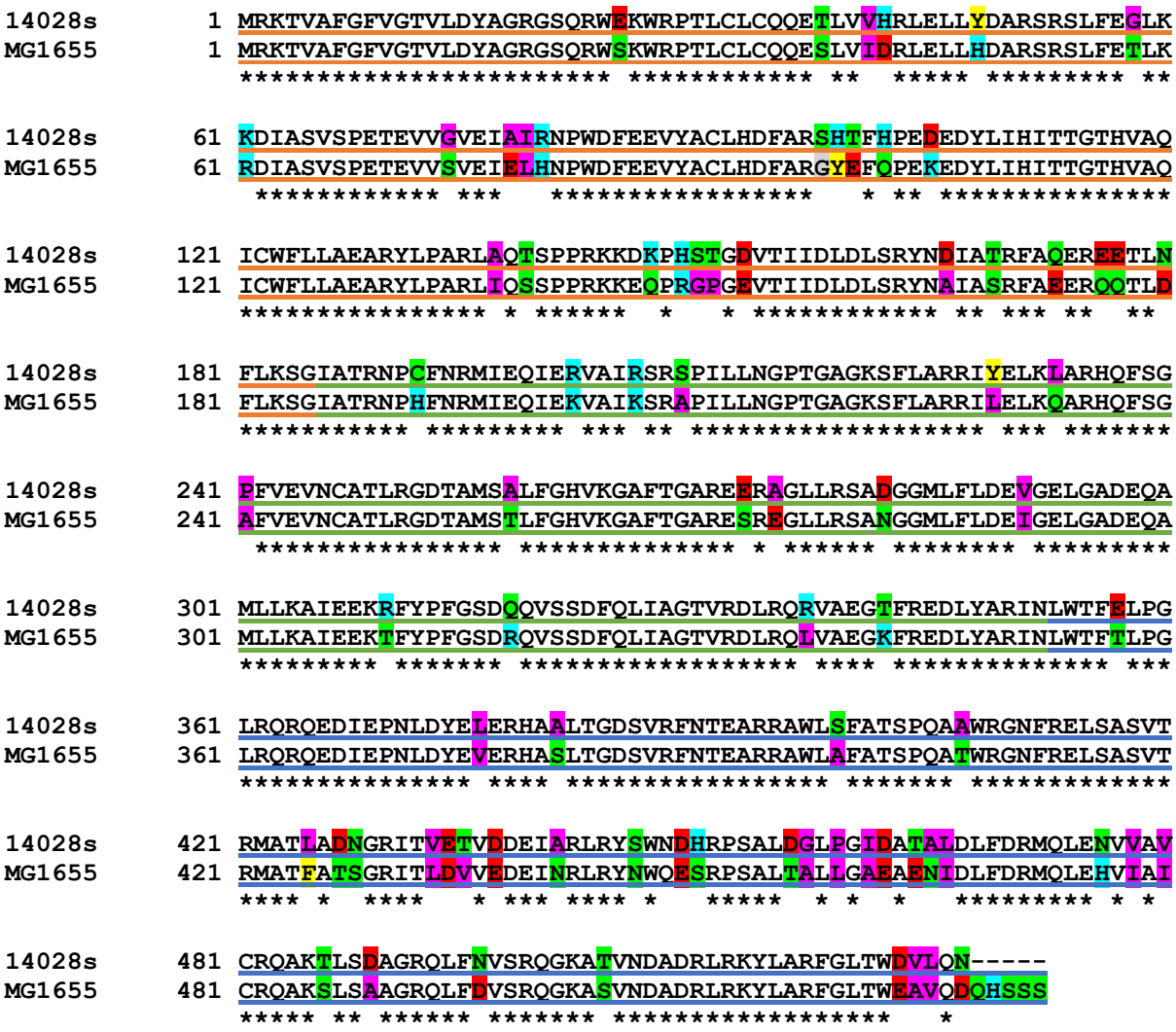

Figure S6. Amino acid sequence alignment of *S.* Typhimurium 14028s and *E. coli* MG1655 RtcR. The two proteins share 83.6% total amino acid identity, with 83.7% identity in the regulatory domain (underlined in orange), 94% identity in the AAA+ ATPase domain (underlined in green), and 77.1% identity in the DNA binding domain (underlined in blue). Amino acid substitutions are highlighted with colors denoting their side chain properties: negatively charged (red), positively charged (turquoise), polar (green), nonpolar (magenta), or aromatic (yellow).

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