Supplemental Materials: Tables and Figures.

Table S1. RNA-seq statistics	•
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Strain bioreplicate	Total number of	Total number of reads	Reads not mapped	Reads mapped >1x
& treatment	reads sequenced	mapped uniquely	to chromosome	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
∆ <i>rtcR</i> 1	46,571,576	39,022,270	4,440,113	3,109,193
∆rtcR 2	16,427,543	14,633,741	1,384,032	409,770
∆rtcR 3*	3,775,980	1,527,319	2,175,792	72,869
∆rtcR 1 +MMC	39,833,788	35,711,397	2,830,132	1,292,259
∆rtcR 2 +MMC	21,193,827	18,855,747	1,688,471	649,609
∆rtcR 3 +MMC	16,059,581	14,226,416	1,353,148	480,017
∆rsr 1	22,386,184	19,119,591	2,709,208	557,385
∆rsr 2	15,510,441	12,646,470	2,466,666	397,305
∆rsr 3	16,137,416	8,062,563	7,812,809	262,044
∆rsr 1 +MMC	16,845,332	14,966,926	1,380,774	497,632
∆rsr 2 +MMC	34,518,155	30,850,209	2,522,913	1,145,033
∆rsr 3 +MMC	14,983,343	13,383,635	1,146,785	452,923
∆ <i>rtcB</i> 1	19,899,438	18,018,089	1,411,306	470,043
∆rtcB 2	21,493,828	19,585,216	1,448,051	460,561
∆rtcB 3	19,875,919	17,728,551	1,661,371	485,997
∆rtcB 1 +MMC	23,946,132	21,203,786	1,797,872	944,474
∆rtcB 2 +MMC	16,631,197	14,857,557	1,293,566	480,074
∆rtcB 3 +MMC	18,257,370	15,983,836	1,728,604	544,930

\*Biological replicate  $\Delta rtcR$  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  $\Delta rtcR$  (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 <i>r</i>
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
ΔrtcR	
1 vs 2	0.97
Δ <i>rtcR</i> +MMC	•
1 vs 2	0.88
2 vs 3	0.98
1 vs 3	0.91
Δrsr	
1 vs 2	0.97
2 vs 3	0.97
1 vs 3	0.99
Δ <i>rsr</i> +MMC	
1 vs 2	0.84
2 vs 3	0.85
1 vs 3	0.98
ΔrtcB	
1 vs 2	0.94
2 vs 3	1.0
1 vs 3	0.96
Δ <i>rtcB</i> +MMC	
1 vs 2	1.0
2 vs 3	0.98
1 vs 3	0.98

S. Typhimurium ATCC 14028sWild-typeATCCDJS10114028s ΔrtcRThis studyDJS10214028s ΔrtcBThis studyCH0614028s ΔrtcBThis studyCH0614028s ΔrtcCThis studyCH0114028s ΔrtcCThis studyACB0114028s ΔrtcCThis studyACB0114028s ΔrtcAThis studyACB0114028s ΔrtcA:kanRef. (1)JE10649LT2 metE205 ara-9 ΔrecA::kanCourtesy of J. Escalante-SemerenaDS10314028s ΔrecA::kanThis studyJEK1714020s ΔrecA::kanThis studyJEK1714020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrecA::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref. (2)E. coliMG1655 (rph-1)rph-1MG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1gyrA96 deoR nupG purB20φ80dlacZM15 Δ(lacZYA-argF)U169, hsdR17(rk-mk*), λ <sup>-</sup> Ref. (4)PKD4Kan template with FLP recognition target sites for A-Red recombination, Kan <sup>R</sup> Ref. (4)pKD4Kan template with FLP recognition target sites for A-Red recombination, Kan <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)pSB306pBlueScript derivativ	Strain/Plasmid	Description or Construction	Source or reference
ATCCHid-typeATCCDJS10114028s Δ <i>tcR</i> This studyANE00414028s Δ <i>tcR</i> This studyDJS10214028s Δ <i>tcB</i> This studyCH0614028s Δ <i>tcB</i> This studyCH0114028s Δ <i>trC</i> This studyACB0114028s Δ <i>trC</i> This studyJE10649LT2 metE205 ara-9 ΔrecA::kanCourtesy of J. Escalante-SemerenaDS10314028s Δ <i>recA::kan</i> This studyJEK1714028s Δ <i>recA::kan</i> This studyJEK1714028s Δ <i>recA::kan</i> This studyJEK1714028s Δ <i>recA::kan</i> This studyJEK26JEK17 Δ <i>recA::kan</i> This studyJEK26JEK17 Δ <i>recA::kan</i> This studyJEK26JEK17 Δ <i>recA::kan</i> This studyJEK26JEK17 Δ <i>recA::kan</i> This studyMS1868LT2 <i>leuA</i> 414 <i>hsdL</i> Fels2Ref. (2)E. coliMG1655 ( <i>rph-1</i> ) <i>rph-1</i> Courtesy of S. KushnerJW3385BW25113 Δ <i>rtcR</i> Ref. (3)ACK206MG1655 <i>ph-1</i> Δ <i>rtcR</i> This studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20φ80d <i>lacZ</i> ΔM15 Δ( <i>lacZYA-argF</i> )U169, hsdR17( <i>r<sub>K</sub>m<sub>k</sub></i> <sup>+</sup> ), λ <sup>-</sup> Ref. (4)Plasmidspromoter, Amp <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal induced FLP recombinatee, sepression vector, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	S. Typhimurium	•	
DJS10114028's ΔrtcRThis studyANE00414028's ΔrtcRThis studyDJS10214028's ΔrtcBThis studyCH0614028's ΔrtcBThis studyCH0114028's ΔrtcCThis studyACED114028's ΔrtcCThis studyACED114028's ΔrtcACourtesy of J. Escalante- SemerenaDS10314028's ΔrecA::kanCourtesy of J. Escalante- SemerenaDS10314028's ΔrecA::kanThis studyJEK1714028's ΔrecA::kanThis studyJEK1714020's ΔrecA::kanThis studyJEK21DJS101 Δrsc::xylE-kanThis studySGD_recA_Kan14020's ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (2)E. coliMG1655 (rph-1)rph-1JW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRCourtesy of C. MorangyrA96 deoR nupG purB20 φ80d/acZΔM15 Δ(lacZYA-argF)U169, hsdR17(rk/mk*), λ-Ref. (4)PKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>areB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)pSB306pSB306pSB306Refizien theriza	ATCC 14028s	Wild-type	ATCC
ANE00414028s ΔrsrThis studyDJS10214028s ΔrtcBThis studyCH0614028s ΔrtcBThis studyCH0114028s ΔntrCThis studyACB0114028s ΔrpoNRef. (1)JE10649LT2 metE205 ara-9 ΔrecA::kanCourtesy of J. Escalante-SemerenaDS10314028s ΔrecA::kanThis studyJEK1714028s ΔrecA::kanThis studyJEK1714028s ΔrecA::kanThis studyJEK21DJS101 Δrsr::xylE-kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrecA::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliMG1655 rph-1 ΔrtcRCourtesy of S. KushnerMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1gyrA96 deoR nupG purB20φ80dlaczΔM15 Δ(lacZYA-argF)U169, hsdR17(rk-mk <sup>+</sup> ), λ <sup>-</sup> Ref. (4)PKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (5)pSB306pBlueScrift derivative with xv/E geneRef. (6)	DJS101	14028s Δ <i>rtcR</i>	This study
DJS10214028s Δ <i>rtcB</i> This studyCH0614028s Δ <i>rtcC</i> This studyCH0114028s Δ <i>rtC</i> This studyACB0114028s Δ <i>rpoN</i> Ref. (1)JE10649LT2 metE205 ara-9 Δ <i>recA::kan</i> Courtesy of J. Escalante-SemerenaDS10314028s Δ <i>recA::kan</i> This studyJEK1714028s Δ <i>recA::kan</i> This studyJEK1714028s Δ <i>recA::kan</i> This studyJEK21DJS101 Δ <i>rsr::xylE-kan</i> This studySGD_recA_Kan14020s Δ <i>recA::kan</i> BEI Resources, ATCCJEK26JEK17 Δ <i>rtcR::kan</i> This studyJEK41JEK17 Δ <i>rtcR::kan</i> This studyMS1868LT2 <i>leuA</i> 414 <i>hsdL</i> Fels2Ref. (2)E. coliMG1655 ( <i>rph-1</i> ) <i>rph-1</i> MG1655 ( <i>rph-1</i> ) <i>rph-1</i> Courtesy of S. KushnerPW3385BW25113 Δ <i>rtcR</i> Ref. (3)ACK206MG1655 <i>rph-1</i> Δ <i>rtcR</i> This studyDH5αF - endA1 gln/44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80d/acZΔM15 Δ( <i>lacZYA-argF</i> )U169, hsdR17( <i>r<sub>K</sub> rm</i> , <i>k</i> ), <i>λ</i> -PlasmidspKD4 <i>kan</i> template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>areB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	ANE004	14028s Δ <i>rsr</i>	This study
CH0614028s Δ(dinJ-yafQ)This studyCH0114028s ΔntrCThis studyACB0114028s ΔntrCThis studyJE10649LT2 metE205 ara-9 ΔrecA::kanCourtesy of J. Escalante-SemerenaDS10314028s ΔrecA::kanThis studyJEK1714028s ΔrecA::kanThis studyJEK21DJS101 Δrsr::xylE-kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref. (2)E. coliMG1655 rph-1This StudyMS1868LT2 leuA414 hsdL Fels2Ref. (3)This studyCourtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF= endA1 glnV44 thi-1 recA1 relA1gyrA96 deoR nupG purB20φ80dlaczΔM15 Δ(lacZYA-argF)U169, hsdR17(r <sub>K</sub> m <sub>K</sub> *), λ^-Ref. (4)PlasmidspKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (5)pSB306pBlueScript derivative with xv/E neneRef. (6)	DJS102	14028s ∆ <i>rtcB</i>	This study
CH0114028s $\Delta ntrC$ This studyACB0114028s $\Delta rpoN$ Ref. (1)JE10649LT2 metE205 ara-9 $\Delta recA::kan$ Courtesy of J. Escalante-SemerenaDS10314028s $\Delta recA::kan$ This studyJEK1714028s $\Delta recA::kan$ This studyJEK1714020s $\Delta recA::kan$ This studyJEK21DJS101 $\Delta rsr::xylE-kan$ This studySGD_recA_Kan14020s $\Delta recA::kan$ BEI Resources, ATCCJEK26JEK17 $\Delta recA::kan$ This studyJEK41JEK17 $\Delta recA::kan$ This studyJEK46JEK17 $\Delta recA::kan$ This studyJEK26JEK17 $\Delta recA::kan$ This studyJEK26JEK17 $\Delta recA::kan$ This studyJEK41JEK17 $\Delta recA::kan$ This studyJEK26JEK17 $\Delta recA::kan$ This studyMS1868LT2 leuA414 hsdL Fels2Ref. (2)E. coliMG1655 $rph-1$ $drcR$ MG1655 $rph-1$ $drcR$ Ref. (3)This studyCourtesy of S. KushnerJW3385BW25113 $\Delta rtcR$ Ref. (3)DH5 $\alpha$ F <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\phi 80dlacZ\DeltaM15 \Delta(lacZYA-argF)U169,$ hsdR17( $r_K m_K^+$ ), $\lambda^-$ Ref. (4)PlasmidsFfrom arabinose-inducible $P_{araB}$ promoter, Amp <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)pSB306pBlueScript derivative with $xu/E$ geneRef. (6)	CH06	14028s Δ( <i>dinJ-yaf</i> Q)	This study
ACB0114028s $\Delta rpoN$ Ref. (1)JE10649LT2 metE205 ara-9 $\Delta recA::kan$ Courtesy of J. Escalante-SemerenaDS10314028s $\Delta recA::kan$ This studyJEK1714028s $\Delta recA::kan$ This studyJEK21DJS101 $\Delta rsr::xylE-kan$ This studyJEK26JEK17 $\Delta recA::kan$ BEI Resources, ATCCJEK26JEK17 $\Delta rtcR::kan$ This studyJEK41JEK17 $\Delta rtcR::kan$ This studyMS1868LT2 leuA414 hsdL Fels2Ref. (2)E. coliCourtesy of S. KushnerMG1655 (rph-1)rph-1JW3385BW25113 $\Delta rtcR$ Ref. (3)ACK206MG1655 rph-1 $\Delta rtcR$ This studyDH5aF <sup>-</sup> endA1 glinV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\varphi 80dlacZ\DeltaM15 \Delta(lacZYA-argF)U169$ , hsdR17( $r_K m_K^+$ ), $\lambda^-$ PlasmidspKD4kan template with FLP recognition target sites for $\lambda$ -Red recombination, Kan <sup>R</sup> pKD46Expresses $\lambda$ -Red genes $\gamma$ , $\beta$ , and exo from arabinose-inducible $P_{araB}$ promoter, Amp <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)pSB306pBlueScript derivative with xv/E geneRef. (6)	CH01	14028s ΔntrC	This study
JE10649LT2 metE205 ara-9 ΔrecA::kanCourtesy of J. Escalante-SemerenaDS10314028s ΔrecA::kanThis studyJEK1714028s ΔrecA::xanThis studyJEK21DJS101 Δrsr::xylE-kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliMG1655 (rph-1)rph-1MG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rk <sup>-</sup> mk <sup>+</sup> ), λ <sup>-</sup> Courtesy of C. MoranPlasmidspKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>araB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	ACB01	14028s Δ <i>rpoN</i>	Ref. (1)
DS10314028s ΔrecA::kanThis studyJEK1714028s Δrsc7::xylEThis studyJEK21DJS101 Δrsr::xylE-kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rk <sup>-</sup> mk <sup>+</sup> ), λ <sup>-</sup> Ref. (4)PlasmidspKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>araB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	JE10649	LT2 metE205 ara-9 ∆recA::kan	Courtesy of J. Escalante- Semerena
JEK1714028s Δrsr::xy/EThis studyJEK21DJS101 Δrsr::xy/E-kanThis studySGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliCourtesy of S. KushnerMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Gourtesy of C. MorangyrA96 deoR nupG purB20 $\varphi 80dlacZ\DeltaM15 \Delta(lacZYA-argF)U169$ , hsdR17( $r_K m_K^+$ ), $\lambda^-$ Ref. (4)PlasmidspKD4kan template with FLP recognition from arabinose-inducible ParaB promoter, Amp <sup>R</sup> Ref. (4)pCP20Temperature-sensitive origin, thermal 	DS103	14028s ΔrecA::kan	This study
JEK21DJS101 $\Delta rsr::xylE-kan$ This studySGD_recA_Kan14020s $\Delta recA::kan$ BEI Resources, ATCCJEK26JEK17 $\Delta recA::kan$ This studyJEK41JEK17 $\Delta rtcR::kan$ This studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 $\Delta rtcR$ Ref. (3)ACK206MG1655 rph-1 $\Delta rtcR$ This studyDH5aF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\phi$ 80dlacZ $\Delta$ M15 $\Delta$ (lacZYA-argF)U169, hsdR17( $r_K \cdot m_K^+$ ), $\lambda^-$ PlasmidspKD4kan template with FLP recognition target sites for $\lambda$ -Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses $\lambda$ -Red genes $\gamma$ , $\beta$ , and exo from arabinose-inducible $P_{araB}$ promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)pSB306pBlueScript derivative with $xv/F$ geneRef. (6)	JEK17	14028s Δ <i>rsr</i> :: <i>xylE</i>	This study
SGD_recA_Kan14020s ΔrecA::kanBEI Resources, ATCCJEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2) <b>E. coli</b> mG1655 (rph-1)rph-1Courtesy of S. KushnerMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\phi$ 80d/acZΔM15 Δ(lacZYA-argF)U169, hsdR17(rk <sup>-</sup> mk <sup>+</sup> ), λ <sup>-</sup> Ref. (4)PlasmidspKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>araB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	JEK21	DJS101 ∆ <i>rsr∷xylE-kan</i>	This study
JEK26JEK17 ΔrecA::kanThis studyJEK41JEK17 ΔrtcR::kanThis studyMS1868LT2 leuA414 hsdL Fels2Ref.(2) <b>E. coli</b> MG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 ΔrtcRRef. (3)ACK206MG1655 rph-1 ΔrtcRThis studyDH5αF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\varphi$ 80d/acZΔM15 Δ(lacZYA-argF)U169, hsdR17(rκ <sup>-</sup> mκ <sup>+</sup> ), λ <sup>-</sup> Ref. (4)PlasmidspKD4kan template with FLP recognition target sites for λ-Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses λ-Red genes γ, β, and exo from arabinose-inducible P <sub>araB</sub> promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	SGD_recA_Kan	14020s ΔrecA::kan	BEI Resources, ATCC
JEK41JEK17 $\Delta rtcR::kan$ This studyMS1868LT2 leuA414 hsdL Fels2Ref.(2)E. coliMG1655 (rph-1)rph-1Courtesy of S. KushnerJW3385BW25113 $\Delta rtcR$ Ref. (3)ACK206MG1655 rph-1 $\Delta rtcR$ This studyDH5aF <sup>-</sup> endA1 glnV44 thi-1 recA1 relA1Courtesy of C. MorangyrA96 deoR nupG purB20 $\phi 80dlacZ\DeltaM15 \Delta(lacZYA-argF)U169,$ hsdR17( $r_{K} m_{K}^{+}$ ), $\lambda^{-}$ Courtesy of C. MoranPlasmidspKD4kan template with FLP recognition target sites for $\lambda$ -Red recombination, Kan <sup>R</sup> Ref. (4)pKD46Expresses $\lambda$ -Red genes $\gamma$ , $\beta$ , and $exo$ from arabinose-inducible $P_{araB}$ promoter, Amp <sup>R</sup> Ref. (5)pCP20Temperature-sensitive origin, thermal induced FLP recombinase expression vector, Amp <sup>R</sup> Ref. (6)	JEK26	JEK17 ΔrecA::kan	This study
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vector, Amp <sup>R</sup> pSB306 pBlueScript derivative with <i>xvIF</i> gene Ref. (6)	μομέ	induced ELD recombined a syntaxion	Rel. (3)
pSB306 pBlueScript derivative with $xy/F$ gene Ref. (6)		voctor Amp <sup>R</sup>	
	nSB306	PRIveScript derivative with xu/E gape	Pof( <b>6</b> )
from Pseudomonas nutida Amp <sup>R</sup>	h20200	from Pseudomonas putida Amp <sup>R</sup>	
nCR2.1 TA cloning vector Amp <sup>R</sup> Kan <sup>R</sup> Invitrogen: Carlshad, CA	nCR21	TA cloning vector $\Delta m n^R Kan^R$	Invitrogen: Carlshad, CA
nCR4 TA cloning vector Amp <sup>R</sup> Kan <sup>R</sup> Invitrogen, Carlsbad, CA	nCR4	TA cloning vector Amp <sup>R</sup> Kan <sup>R</sup>	Invitrogen: Carlsbad, CA

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

pSC101 derivative with P <sub>taq</sub> -Hin, Str <sup>R</sup> Spc <sup>R</sup>	Ref. (7)
Derivative of pBBR1, expression vector containing a reengineered <i>lacl</i> <sup>q</sup> - <i>lac</i> promoter-operator complex for tight repression in absence of inducer, Tet <sup>R</sup>	Ref. (8)
Derivative of pACYC-184, expression vector with arabinose-inducible P <sub>BAD</sub> promoter, Amp <sup>R</sup>	Ref. (9)
pCR2.1 with 197 bp 14028s <i>rpoD</i> fragment	This study
pCR4 with 180 bp 14028s rtcA fragment	This study
pCR4 with truncated 14028s $rtcR$ (to produce RtcR <sub>con</sub> )	This study
pHK66 digested with BamHI-HindIII, with BamHI-HindIII <i>rtcR<sub>con</sub></i> fragment from pDS181	This study
pCR2.1 with full-length 14028s rtcR	This study
pSRK-Tc with Ndel-Xhol <i>rtcR</i> fragment from pCH11	This study
pSRK-Tc with NdeI-XhoI <i>rtcR</i> fragment, amplified from MG1655 genomic DNA	This study
pBAD30 with EcoRI-HindIII <i>yafQ</i> fragment, amplified from 14028s genomic DNA	This study
	pSC101 derivative with P <sub>taq</sub> -Hin, Str <sup>R</sup> Spc <sup>R</sup> Derivative of pBBR1, expression vector containing a reengineered <i>lacl</i> <sup>q</sup> - <i>lac</i> promoter-operator complex for tight repression in absence of inducer, Tet <sup>R</sup> Derivative of pACYC-184, expression vector with arabinose-inducible P <sub>BAD</sub> promoter, Amp <sup>R</sup> pCR2.1 with 197 bp 14028s <i>rpoD</i> fragment pCR4 with 180 bp 14028s <i>rtcA</i> fragment pCR4 with truncated 14028s <i>rtcR</i> (to produce RtcR <sub>con</sub> ) pHK66 digested with BamHI-HindIII, with BamHI-HindIII <i>rtcR<sub>con</sub></i> fragment from pDS181 pCR2.1 with full-length 14028s <i>rtcR</i> pSRK-Tc with NdeI-XhoI <i>rtcR</i> fragment, amplified from MG1655 genomic DNA pBAD30 with EcoRI-HindIII <i>yafQ</i> fragment, amplified from 14028s genomic DNA

Table 34. Uliuuliuuleuliues useu III lilis sluuv	Table S4.	Oligonucleotides	used in	this study.	
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Number	Name	Sequence
Deletion	strain generation	1:
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-xyIE-F	CAATAACCATGGCATGGAGAATAACGGAAATGGGAAAAACAAA TGAACAAAGGTGTAATGCGACC
12	KanR-xylE-R	AAGCAGCTCCAGCCTACACATCAGGTCAGCACGGTCATG
13	xylE-KanR-F	TGCTGACCTGATGTGTAGGCTGGAGCTGCTTC
14	rsr42-KanR-R	AGATGTAATCCCGGCCAGCATTCGCGCCGCGCTGAATACTCTC ATATGAATATCCTCCTTA
Deletion	strain verification	n:
15 (11)	ckrtcR-F	CCATTTCCGTTATTCTCCATGCC
16 (12)	ckrtcR-R	GTGATGCAGGTGTTGACGG
17 (13)	ckSTM3521-F	GCAGATCGATACACACCAGGC
18 (14)	ckSTM3521-R	TTTAATGACTACCGTGCAGGC
19 (15)	ckrtcB-O	ATCTGCCCGCCGCCTCTCC
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-PCF	र:	
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	Ndel-RtcR F	ACGCATATGCGAAAAACGGTGGCCTTTGG
54	Xhol-RtcR R	AAGCTCGAGTTAATTCTGTAAAACGTCCCACGTCAGCC
55	Ndel-EcRtcR-F	ATCACTCATATGCGTAAAACAGTGGCTTTTGG
56	Xhol-EcRtcR-R	ATCACTCTCGAGTCAACTGGAGCTGTGCTGATC
57	yafQ-EcoRI-F	ATCACTGAATTCATGGGGCAAAGGGAAATTG

58	yafQ-HindIII-R	ATCACTAAGCTTTTAAAATAAATCGGCATGCGTTC
Sequenc	ing:	
59	pSRK Insert F	CCGGCTCGTATGTTGTGTGG
60	pSRKTc Rev	CAAGGCGATTAAGTTGGGTAACG
61	M13F(-21)	TGTAAAACGACGGCCAGT
62	M13R	CAGGAAACAGCTATGAC
63	pBAD Forward	ATGCCATAGCATTTTTATCC
64	pBAD Reverse	GATTTAATCTGTATCAGG
Northerr	ns and <i>dinJ-yaf</i> Q f	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
Addition	al qRT-PCR Prime	ers:
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  $\Delta ntrC$  mutant. Significant differences in relative *glnK* expression between treated and untreated samples and between the treated WT and  $\Delta ntrC$  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  $\Delta rsr::xylE$ ) 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<sub>rsr</sub> was inferred by assaying cells for XylE activity. Data are given as nmol of catechol oxidized per minute (normalized to 10<sup>8</sup> 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,  $\Delta rtcR$ , or  $\Delta rpoN$  strains were grown to mid-log phase in LB and split into two cultures. One culture was treated with 1 mM H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>-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,  $\Delta rtcR$ ,  $\Delta rtcB$ ,  $\Delta rsr$ , and  $\Delta rtcA$  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.

14028s	1	MRKTVAFGFVGTVLDYAGRGSQRW <mark>E</mark> KWRPTLCLCQQE <mark>TLVVH</mark> RLELL <mark>Y</mark> DARSRSLFE <mark>G</mark> LK
MG1655	1	MRKTVAFGFVGTVLDYAGRGSQRW <mark>S</mark> KWRPTLCLCQQE <mark>S</mark> LV <mark>ID</mark> RLELLHDARSRSLFE <mark>T</mark> LK
		***************************************
14028s	61	KDIASVSPETEVVGVEI <mark>AIR</mark> NPWDFEEVYACLHDFAR <mark>SHT</mark> FHPEDEDYLIHITTGTHVAQ
MG1655	61	RDIASVSPETEVV <mark>S</mark> VEI <mark>ELH</mark> NPWDFEEVYACLHDFARG <mark>YE</mark> F <mark>Q</mark> PE <mark>K</mark> EDYLIHITTGTHVAQ
		****** *** *** ************************
14028s	121	ICWFLLAEARYLPARLAQTSPPRKKDKPHSTGDVTIIDLDLSRYNDIATRFAQEREETLN
MG1655	121	ICWFLLAEARYLPARL <mark>IQS</mark> SPPRKKE <mark>QPRGP</mark> GEVTIIDLDLSRYNAIASRFAEERQQTLD
		************* * ****** * * ************
14028s	181	FLKSGIATRNP <mark>C</mark> FNRMIEQIE <mark>R</mark> VAI <mark>R</mark> SR <mark>S</mark> PILLNGPTGAGKSFLARRI <mark>Y</mark> ELK <mark>L</mark> ARHQFSG
MG1655	181	FLKSGIATRNPHFNRMIEQIEKVAIKSRAPILLNGPTGAGKSFLARRILELKQARHQFSG
		********* ********* *** ** ** *********
14028s	241	PFVEVNCATLRGDTAMS <mark>A</mark> LFGHVKGAFTGARE <mark>E</mark> R <mark>A</mark> GLLRSA <mark>D</mark> GGMLFLDE <mark>V</mark> GELGADE <u>Q</u> A
MG1655	241	<mark>A</mark> FVEVNCATLRGDTAMS <mark>T</mark> LFGHVKGAFTGARE <mark>S</mark> R <mark>E</mark> GLLRSA <mark>N</mark> GGMLFLDE <mark>I</mark> GELGADEQA
		************** ************************
14028s	301	MLLKAIEEK <mark>R</mark> FYPFGSD <mark>Q</mark> QVSSDFQLIAGTVRDLRQ <mark>R</mark> VAEG <mark>T</mark> FREDLYARINLWTF <mark>E</mark> LPG
MG1655	301	MLLKAIEEK <mark>T</mark> FYPFGSD <mark>R</mark> QVSSDFQLIAGTVRDLRQ <mark>L</mark> VAEG <mark>K</mark> FREDLYARINLWTF <mark>T</mark> LPG
		******* ****** ************************
14028s	361	LRQRQEDIEPNLDYE <mark>L</mark> ERHA <mark>A</mark> LTGDSVRFNTEARRAWL <mark>S</mark> FATSPQA <mark>A</mark> WRGNFRELSASVT
MG1655	361	LRQRQEDIEPNLDYE <mark>V</mark> ERHA <mark>S</mark> LTGDSVRFNTEARRAWL <mark>A</mark> FATSPQA <mark>T</mark> WRGNFRELSASVT
		************* **** ********************
14028s	421	RMATLADNGRITVETVDDEIARLRYSWNDHRPSALDGLPGIDATALDLFDRMQLENVVAV
MG1655	421	RMATFATSGRITLDVVEDEINRLRYNWQESRPSALTALLGAEAENIDLFDRMQLEHVIAI
		**** * **** * *** * * **** * * * * * ****
14028s	481	CRQAK <mark>T</mark> LS <mark>D</mark> AGRQLF <mark>N</mark> VSRQGKA <mark>T</mark> VNDADRLRKYLARFGLTW <mark>DVL</mark> QN
MG1655	481	<u>CRQAK<mark>S</mark>LSAAGRQLFDVSRQGKA<mark>S</mark>VNDADRLRKYLARFGLTW<mark>EAV</mark>QDQHSSS</u>
		**** ** ***** ****** **************

**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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