

**Supplementary Figure 1.** Screenshots of Integrative Genome Viewer<sup>1</sup> with tracks showing the binding profiles (tag density) as measured by ChIP-seq of NtrC (black) and RNAp (gray) in N non-starved (denoted as N+) and N starved (denoted as N-) *E. coli* aligned against the upstream regions of all the transcription units shown in Table 1. Tracks with the input DNA control tag density (denoted as input) and with the genomic loci bound by NtrC identified by

the SISSRS peak-calling algorithm  $^2$  at t=N- are also shown for comparison. Red arrow (panel "Peaks 12 & 13") denotes peak upstream from *argT* gene miss-called by SISSRS.



**Supplementary Figure 2.** Screenshots of Integrative Genome Viewer<sup>1</sup> with tracks showing the binding profiles (tag density) as measured by ChIP-seq of NtrC (black) and RNAp (gray) in N non-starved (denoted as N+) and N starved (denoted as N-) *E. coli* aligned against the upstream regions of transcription units *flgMN*, *dicC*, *fliC*, *ssrS*, *soxR* and *yjcZ-proP*. Here these are supplemented with representative autoradiographs of non-denaturing gels showing the binding of *in situ* phosphorylated NtrC to <sup>32</sup>P-labelled DNA probes with sequences corresponding to their respective upstream regions. Tracks with the input DNA control tag density (denoted as input) and with the genomic loci bound by NtrC identified by the SISSRS peak-calling algorithm <sup>2</sup> at t=N- are also shown for comparison.



**Supplementary Figure 3.** Screenshots of Integrative Genome Viewer<sup>1</sup> with tracks showing the binding profiles (tag density) as measured by ChIP-seq of RNAp with Rifampicin treatment (red) and RNAp in the absence of Rifampicin treatment (black) in N non-starved (denoted as N+) and N starved (denoted as N-) *E. coli* aligned against the upstream regions of all the transcription units shown in Table 1. Tracks with the input DNA control tag density (denoted as input) is shown for comparison.



**Supplementary Figure 4.** Transcription start site mapping and NtrC-activated  $\sigma^{54}$ -dependent transcription of *relA* from novel promoter P4 (A) *Left*. Representative image of agarose gels showing the 5'-RACE PCR products amplified from cDNA synthesised from total RNA

b

isolated from N starved wild-type NCM3722 and NCM3722:  $\Delta glnG E$ . coli cells. The presence or absence of the different transcripts originating from promoters P1-4 in the N starved wild-type NCM3722 and NCM3722: $\Delta g ln G E$ . coli is shown in the table below the agarose gel images. *Right*. DNA sequencing chromatograms (from FinchTV<sup>3</sup>) showing the transcription start sites (boxed) originating from promoters P1-4 in the regulatory region of relA determined by 5'-RACE analysis. (B) The DNA sequence of the relA regulatory region (from +240 to -920 with respect to the translation start site of RelA). The consensus -10/-35 of P1 and P2 promoters and -12/-24 elements of the P3 and P4 are shown in bold typeface and underlined; the CRP binding site is indicated and underlined. The transcription start sites of P1-P4 and the translation start site of RelA are indicated. The sequences of primers A and B used for making the cDNA for the 5'-RACE analysis are underlined and marked with an empty leftward pointing arrow. The DNA region (from -607 to -811 with respect to the translation start site of RelA) enriched in the immunoprecipitated sample by RNAp binding is highlighted in yellow. (C) Representative autoradiographs of 20% (w/v) denaturing urea gel showing the *in situ* phosphorylated NtrC dependent synthesis of CpUpGpG transcript (underlined nucleotides are  $\alpha^{32}$ P labelled) from the P4 promoter by  $\sigma^{54}$ -RNAp. The reaction components present in each lane are given at the top of the autoradiographs; lanes 6 and 7 are control lanes and show that the synthesis of CpUpGpG can be initiated from P4 by a non-DNA binding and phosphorylation-independent form of the  $\sigma^{54}$ -RNAp transcription activator PspF<sub>1-275</sub>.

Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	1 1 1 1 1	— ПСЛАСИСАРССССА — ЛСЛАСИСАРССССА Алсол Сапсарсссса — ЛССЛСАРСАРССССА — ПСЛСАРССРСССА — ССЛСАРССРСССА — ССЛСАРССРСССА — ССЛСАРССАРСССА — ССЛСАРССАРСССА — СЛСА — СЛСА — СЛСАРССАРСССАР	IGTTCCCTGGGGCTATC IGTTCCTGGGGCTATC IGCGTCCTGGGGTTATC IGGGCGGTGGGGTATC IGAGCAGACGACGATTC IGAGCAGCAGACGATTC IGAGCAGCAGACGATCC	CCCTCCCCCCCCT GCCCCCCCCCCCT GCCCCCCCCCC	TTAAGTCTGAACT/ TTAAGTCTGAACT/ TTAAGTCTGAACT/ TTAAGTCTCGAGT/ TTAAGTCTCCAGGATCA TTAAGCCTGATGT	ACTTACCGAAAAA ACTTACCGAAAAA GCCCGCCGGATAA ACCAGTCGAAAAA ATCATGCGCGCGAC AGGATAA <mark>GAAAA</mark>	ACAGCAACTTCAG ACAGCAACTTCAG -AACGCTGCTAA CCAACAACTGCAA - AATTGCAG GCA - AGCTGTAT TCAGCAACTACAA	ATGGGGTTT ATGGGGTT ATGGGTTT ATGGGTTT ATGGGTTT TTGGTCATGGGATTC TGGTCATGGGATTC	C C C C CAATGTGGGAGAATT C	SCA - AAGCGGG SCA - AAGCGGG SCA - AAGCGGG SCA - AGGCCGC SCC - AGGCCCA SCC - AGGCCAA SCC - GGACACA SCC - GGACACA	CTCCA CTCCA TTCCA CTCCA CTCCA CGGCA CTCTACTTTAA CTCTACTTTAA
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	111 111 110 111 34 125 95	- GTGACATTG-TCGAC- GTGACATTG-TCGAC- GCGATATCG-TCGAT- - GCGATATCG-TCGAT- - AAGGATCG-TCGAT TGAGCGATCG-TCGAT - AAGCGATCG-TCATC	TCAAACAATGCCCCAT TCAAACAATGCCCCAT TCGAACAGTGCCCCAT TCGTGCAGTGCCCCAT TGGTGCAGTGCCCCGT GCAAAAATGGCGCAAT TGACCCATGCCCCGT	TTTAGCGCCCCAACT TTTAGCGCCCCAACT TTTGGCGCCCCAGCT TTTGGCGCCCCAACT TTTGGTGCCCCAACT TGTAGTGCCCCAACT GCTGGAG-CCGAGT	TTGAA-GCATTGC TTGAA-GCATTGC TTGAG-GCATTGC TTGAG-GCGCCT TTGAG-GCATTGC CGC-GCATTGC TTAATGCGCTAT	TGCCCAAAGT TGCCCAAAGT TGCCCCGCAT TGCCCGATGT TGCCCGCCGT GACCCGCCAAAG TGCCTGATT7	CAGGCATGTCTG CAGGCATGTCTG CAGGCGTGTCTG CAGGCGTGCTG GCCCGAGTGCTTA CTGCAATGTGTG GAACGCTTTGTTG	SGCA GCTT GCA GCTT SCA AGTT AGCGCGTT AGCGCGCT AGCGCGCT AGCGCACCTCGGCTCCC AGTGA GTG 	ACAAGCTATG ACAAGCTATG GACGGCACC GACGGCACG GAGCGCATTA TTTGGGCAGGGGGGG GAGTCAACCTG 	CGCCATCT CGCCATCTT CGRCATCTT CGRCATCTC CGCCATCTC CGCCACATC CACGCCACATC AGCGGTTG	GGTCATGTTGA GGTCATGTTGA GGCCATGTTGA GGCCACGTTGA GGCCACGTCGA GGCAAGTCGT GGCCATGTCGA -10
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	229 228 229 152 263 213	ACTGGTACAGGCAACCA ACTGGTACAGGCAACCA GCTGGTACAGGCGGAA GCTGGTGCAGGCGGGAA GCTGGTGCAGGCGGGCA GTTTGGTGCGGAGAGTGTT ATTGGTGAAAGGGGATA	GC GCCACGC TGAT GC GGCACGC TGAT GC GGTACGC TGAT GC GCTACGC TGAT AT GGCCCGC TGAT GCCGGCCGAGC - AGGC AC ACTCGC GTGTT	GATTITGCGCCATAG GATTITGCGCCATAG GATTICGCGCCATAG GGTGCGCCCATAG GGCAATGCAGCGCATAG G <mark>GCAATGCAGCTGAGGCGAT</mark> TI	CGCACCGCTAAG CGCACCGCTAAG CGCGCGCATTAAG GGCGCGTTAAG CGCAGCCTGCC CGAAGACTGCC CGAAGAA-AAA TAGCCCCCCTGAT	TTCGGCAGATCG TTCGGCAGATCG TGCGGCGGATAAA CGCGGGGATCG GGCGACAGACA GATGGATCG TG <mark>AACAAGATCA</mark>	GAAAAACTGGAAC GAAAAACTGGAAC GAAAAACTGGAAC GAAAAACTGGAAC GAAAAACTGGAAC GAAAAACTGGAAC GAAAAACTGGAAC GAAGGGTTACCGA CAGGGGTTACCGA CAGGGGTTACCGA	GCTTTTC GCATTCTC GCTTTTC GCATTCTC GCTTTTC GCATTCTC GCTTTTC GCATTCTC GCTTTCGCATCTC GCTTTCGCAGCAAC	AAGGCCTGATCTGT AAGGCCTGATCTGT AGGGATCTGTCTGT GCCAACGGCCTTT ACGGTCTTCCCTGT AAGGGCCAACCATTT AAAGCCTAACCATTT	AT CTCC CCCCG AT CTCCCCCCG TC CTCCCCCCG AT CTCCCCCCCG AT CTCCCCCCCCCC AT CTCCCCCCCCCCCCCCCCCC	ATAGTGA GA-T ATAGTGA GA-T TTAGCGA GA-T CAGGCGA GA-T AAAGCGA GA-T GCAGCG GAGG A-AGC <mark>AG G</mark> CGA
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	364 363 364 287 400 348	ACT CGAAACC TCTCTG ACT CGAAACC TCTCTG ACT CGAAACC TCTCTG ACT CGAAACC TTTCA ACT CGAACC CTTTCA ACT CGACCAT TTCATG GCGAAAACT CTGT-GC GTTGCAACAT GTGCAAG	TGAGATGCCCTGGTAT TGAGATGCCCTGGTAT CGAGACCCCTGGTAT CCAGACCCCCTGGTAT CGAAGCCCCTGGTAT CGAAGCGCCATGGTAT GGGAAGCGCCATAGTAT TGAAGCGCCATACTGT	GACTCAAACGGGTTG GACTCAAACGGGTTG GATTCGCACGGGTTG GAGTCAACGGGCTA AGCTCAGACGGGCTA GAGGTCGACGGGCTA GAGGTCGACGGCCTA GAGGTCGACGGCTA	C-GCTTAACTTT C-GCTTAACTTT C-GTTTAACTT C-GCTTACACTT C-GCTTACACTT C-GCTTAGACTT C-GCTTAGACTT C-GCTTAGACTT	TA-GCCCGCGCG TA-GCCCCCCG TA-GTCCCCGA TA-GTCCCCGA TA-GTCCCCGGA CA-GTCCCCGGA CA-GTCCCCGGA CTTGCCCA-GCGA	TTTTATTCAGGTC: TTTTATTCAGGTC: GTTTATTCAGGTC: TTTTATTCAGGTC: TTTTATCCAGGTC: GTTTATTCAGGTC: GTTTATTCAGGTC:	AATGC GGGTGTGAAC AATGCGGGTGTGAAC AATGAGGGGTAAAC AACGAAGGGGTAAAC AACGACGGGGTAAAA AATGACGGCGTAAAA AATGACACGGCGTAAAA AATGCCGCCGCGTGAAT	CAAAAAATGGTAGCG CAAAAAATGGTAGCG CAGCAAATGGTGCG CAGCAATGGTGCG CAGCAGATGGT-GCG CAGCAAGATGGT-GCG CAGCAAATGGCGGCG CAGCAAATGGCGGCG CAGCA <mark>GATGGT</mark> CGTG(	CGT-GCGTTGGA CGT-GCGTTGGA CGC-GCGCTGGA CGC-GCGCTGGG TACCGCACTGGA CAG-GCCCTTGA CAG-GCCTTGA	ATGGCTGGATG ATGGCTGGATG GTGGCTTGATG GTGGCTGGATG GTGGCTGGATC ATGGCTGGACA TTGGCTTGACG
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	501 500 501 424 535 485	TGCAACCTGAAGATCGCC TGCAACCTGAAGATCGCC TGCGCGCAGGATCGGC TGCGCGACGACGACGGC TGCGCCGACGACGGC TGCGCCGAGGGACGGG TTGCGCCCAAAGATCGGC TTAGCCCAAAGATCGC	TACTGGATCTGTTCTG TACTGGATCTGTTCTG TCCTGGATCTGTTCTG TTCTGATCTGTTCTG TGCTGGATCTGTTCTG TATTGGACCTGTTCTG GCTGGATCTGTTCTG	CGGUATGGGC-AACT CGGTATGGGC-AACT CGGTATGGGC-AACT CGGCATGGGC-AACT CGGCATGGGC-AACT TGGCATGGGC-AACT TGGCTTAGGC-AACT	TTACACTGCCAT TTACACTGCCAT TTACGCTACCCC TTACCCTGCCGC TTACCCTGCCGC TTGCCCTGCCGC TTGCCCTGCCGC TTGCCCCGCCCCC	TGGCGACACANGG TGGCGACACANGG TGGCGACGCGG TGGCGACGCACGG TGGCGACACGGG TGGCCAACGCGG TGGCCAAACGGG TGGCCAAACAAGG	TGCCAGTG-TGGT TGCCAGTG-TGGT GGCAAGCG-TGGT GGCAAGCG-TGGT CGCCATG-TGGT GGC	CGTTTAGAAGTGT CGTGTAGAAGGTGT AGGGSTTGAGGGGG GGCGTTGAGGGGG GGCGTGGAAGGGGT GGGTTTAGGGGTG GGCTTTGAGGGGTGT	TCCGGCGCTGGTGGA TCCGGCGCTGGTGGA TCGGCCCTGGTGGA ACCCCCGCGCGGGGA GCCGCGCGCGGGGGG GCCGGCGCTGGTGGA TGCCACGCTGGTGGA TGCCACGCTGGTGGA	AAAAGCCAGCA AAAAGCCCGTGA AAAAGGCCGTGA AAAAGGCCGGGA AAAGGGCGGGA AAAGGGCGGCAATA AGAGGGCACTCA	GAATGCGCGTC GAATGCGCGTC AAACGC-CATC AAATGCCCGGC AAATGCCCGGC TAATGCACATA TAACGCGAAAT
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	639 637 639 561 658 623	T - TAATGGCTTACAGAAT $T - TAATGGCTTACAGAAT CGGAATGGTTTACAGAAT A - AAATGGATTACACAAA CGCAATGGTTTATCAAA A - GAATAACTGAATAAA TGAACCAAATGA-ATAAA -35$	GTGACGTTTTATCACG GTGACGTTTTATCACG GTGACATTCTTGCACG SGGACATTCTTGCACG GTGACATTCTTGCATG GCCACGTTCTTGCATG GCCACGTTTTATCAGG	алалтстисалсало адалтстисалсало адалостселсало селасстисасалсало алалтстссаласало селатитссаласало селатитссаласало 10 о <sup>2</sup> 91	SATGTCACAAAGC; SATGTCACAAAGC; SATGTCACGAAGC; SATGTCACCGAAGC; SATGTCACCCGC; SACGTCACCCGCC; SACGTT <u>CACC</u> GCC; SATAT <mark>CACC</mark> AGCCC	AGCCGTGGGCGAA AGCCGTGGGCGAA AGCCGTGGGCGAA AGCCGTGGGCGAA AGCCTGGGCGAA AGCCTGGGCGAA AGCCTGGGCGCGC CCTCATGGGCGC	AAACGGCTTCGAT AAACGGCTTCGAT AAACGGCTTTGAG AAGCGGTTTGAG GCACGGCTTTGAT GCACGGCTTTGAT GCACGATATTTGCT	AAAGTGTTGCTGGAC AAAGTGTTGCTGGAC AAAGTGTTACTGGAC AAAGTGCTGCTGGAT AAAGTGCTGCTGGAC AAAGTGATGCTGCTGGAC AAAGTACTGCTGCAC	CCCCCCCACCACCACCA CCCCCCCCACCACCACCA CCCCCC	SCCGCAGGRGT SCCGCAGGRGT SCTACAGGAGTG SCCGCTGGCGTG SCCGCTGGCGTG SCGGTCGGCGTG SCCAGAAGGGATT	ATGCAGCANAT ATGCAGCANAT ATGCGACATAT ATGCAGCATAT ATGCCGCATAT ATGTCACATAG GTGGATCAAC
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	778 778 777 778 701 797 762	TATAAAA CTGGAA CTA TATAAAACTGGAA CTA TATAAAACTGGAA CTA TATAAAATTAAAA CTA TATAAAATTAAAACTGC AATAAAA CTGGCA CGC CTCTGCGTTAGGT CCA CTCTGCGTTAGGT CCA	TC GTATAGTTTATGTA TC GTATAGTTTATGTA TC GCATGTTATGTA TC GCATGTTATGTA TC GCGCAGTGTATGTA GC GGCGGGTGTATGTA AC GGGGAGGTTACGTT GC GGGTGTGTGTATGTA	TCCT STAACC TGC TCCT STAACC TGC TCCT STAACC GGC TCCT STAACC GGC TCCT GAACC GGC TCCT GAACC GCC TCTT STAACC TAC	ACGCTGGCTCGG ACGCTGGCTCGG ACGCTGGCGCGG ACGCTGGCGCGG ACGCTGGCGCGCA GCGCTGGCACGG ACATTAGCACGA	GATAGCGAAGCG GATAGCGAAGCGG GATAGTGAAGCGG GATAGCGAGGAAGCG GACAGCGAAAGCC GGCAGCAAAGCG GACAGTCAAAGC	TATTANAAGCAGG TATTAAAAGCAGG TGGTCAATGCGGG TGGTGCGCGCGGG TGGTGCAGGCGGG TGCTGCAGGCGG TGCTGCAGGCGG TGCTGGAGCCAAGG	ATATA CATTGEGE ATATACCATTGEGEG ATATGAGGTAGEGE ATACGAGATTCAGEG ATATCAGATTCAGEG TTATCGETTGCAEG ATTTCGETTAGAGAA	actggcgatgctgga Actggcgatgctgga Tttagcgatgctgga Getgcgatgctgga Cetggcgatgctgga Cetggcgatgttgga Actggcgatgttgatga	PATGTTCCCACA PATGTTCCCACA PATGTTCCCGCA PATGTTCCCGCA PATGTTCCCGCA PATGTTCCCGCA PATGTTCCCGCA	CACGGGACATC CACGGGACATC CACAGGACATC CACTGGACACC TACGGCCATC TACGGGCCATC CACCAGTCATC
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	918 918 917 918 841 937 902	-24 TGGAATCGATGCTACTT TGGAATCGATGCTACTT TGGAATCGATGCTCCT TGGAATCGATGCGTCCT TGGAATCGATGCGTCCT TGGAGTCGATGCGCCTCT TGGAGTCGATGCCTTTCT	-12 TCTCGCCGGTTA TCTCGCCGCGTTA TCGAGCCATGT TTGAGCACAAGT TTGAGCGCAAGCCTTACJ TTGAGCGCAGCCTTACJ TTG GARAMAGG	ATAAGGTCATTCACA	AAT AAT AAT AAT AAT AAT AAT AAT AAT AT A	AGTTGC GTTAC GTTACC ATTACC ACGTCC AGCCACCCTGCC AGCCACCCTGCC AGCGATT	ATGAATGCTGACG	CGACCTGCAGGATGA	САТДТАДАААДТТДА	SATTTGCC SGCTTACC SGCTTACC SGCTTGCT SGCTTGCT SGCTTGCC ACTGCCGATAC	- GATTTCGCC- - GATTTCGCC- - GACTTCGCT- - GAATCCGCC- - GACTTCGCC- TGGGTCGCCC GAGCGTCAGTC
Escherichia Shigella Salmonella Citrobacter Klebsiella Serratia Vibrio	974 974 975 975 975 984 966	- AGGTCT - GGTCCC- - AGGTCT - GGTCCC- - AGGCCT - GGTCCC- - AGGCG - GGTCCC- - AGGCG - GGTCCC- - GGTCCC- GAAGGCAA - AGT GCAA - AGT GAAGGCTAAAAATACAAT	Ke 	A SIGT ATCGTTGCGGTAAGA ATCGTTGCGCGTAAGA ATCGTTGCGGTAGGA ATCGTTGCGGTAGGA ATCGTTGCGGTAAGA ATCGTTGCGGTAGGA	AGTGCACATATC AGTGCACATATC AGTGCACATAT AGTGCACATTTA AGTGCACATTTA AGTGCACATCTG AGTGCACATCTG AGG <mark>C</mark> GCACACTTA	AATAAGGCTGGTG AATAAGGCTGGTG AATAAAGCTGGTG AATAAAGCTGGTG AATAAAGCTGGTG AACACGGCTGGTG AA <mark>CACG</mark> ACG <mark>ACCAAC</mark>	AATTTGATCCGGAA AATTTGATCCGGAA AATTTGATCCGAA AATTTGATCCGAA AATTTGACCCGAA AATTTGCCGAA AATTTGCTCGAGCTAGA	AAAATGGATCGCAAG AAAATGGATCGCAAG GAAGTGGATCGCAAG AAATGGATCGCAAG GAATGGATGGATGCCAG CGAGTGGATGCCAG AA <mark>C</mark> ATGGATACCAG	TCTGGGTATTACCA- TCTGGGTATTACCA- CCTGGGATTTCCA- TCTGGGATTTCCA- TCTGGGATTCCA- CTTGGGGCTACCA- CTTGGGGCTACCTA- CCTGACCCA	GCCAGAA GCCAGAA GCCAGCA GCCAGCA GCCAGCA GCCAGCA ACCCGCA SAGGG <mark>G</mark> AAAACG	GTCGTGTGAGN GTCGTGTGAGC GTCGTGTGAGC GTCGTGTGAGC GTCGTGTGAGC GTCATGTGAGC GCCGC-TAAA-

**Supplementary Figure 5.** The alignment of DNA sequences of *relA* regulatory regions (corresponding to positions –1,000 to +100 of RelA transcription start site of *E. coli*) from representatives of the Enterobacteriaceae family (*Shigella, Salmonella, Citrobacter, Klebsiella, Serratia* and *Vibrio*). The regions corresponding to the NtrC binding region and the promoters P1-4 are boxed in cyan (NtrC), red (P1 and P2) orange (P3 and P4) and the translation start site of RelA is indicated.

#### Transcription





### Translation



(0-2163) RNAp N+	(0-1936) RNAp N+	RNAp N+	RNAp N+
(0-2163)	(0-1938)	(0-2384)	(0-1902)
RNAp N-	RNAp N-	RNAp N-	RNAp N-
dnaA rpmH mpA yidD	cmk rpsA	rpii rpmi infC thrs yniD	ygjD <b>rpsU</b> dnaG
(0-670)	(0-1976)	(0-750)	(0-1536)
RNAp Input	RNAp Input	RNAp Input	RNAp Input
(0-670)	(0-1976)	(0-750)	(0-1536)
RNAp N+	RNAp N+	RNAp N+	RNAp N+
(0-670)	(0-1976)	(0-750)	(0-1536)
RNAp N-	RNAp N-	RNAp N-	RNAp N-
rpsK rpsM rpmJ secY ⇔ ⇔ ⇔ ⇔	rrsH ileV	insA <b>rpsT</b> yaaY ribF	rpID rpIC rpsJ gspB
(0-2242)	(0-3641)	(0-629)	(0-2747)
RNAp Input	RNAp Input	RNAp Input	RNAp Input
(0-2242)	(0-3641) RNAp N+	(0-629)	(0-2747)
RNAp N+		RNAp N+	RNAp N+
(0-2242)	(0-8641) RNAp N-	(0-629)	(0-2747)
RNAp N-		RNAp N-	RNAp N-
secE rpIA	rrsG clpB	prmA dusB fis	rrsD yrdA
(0-3406)	(0-2943)	(0-2708)	(0-972)
RNAp Input	RNAp Input	RNAp Input	RNAp Input
(0-3406)	(0-2943)	(0-2708)	(0-972)
RNAp N+	RNAp N+	RNAp N+	RNAp N+
(0-3406)	(0-2943)	(0-2708)	(0-972)
RNAp N-	RNAp N-	RNAp N-	RNAp N-
yieP rrsC	hemG rrsA	murl rrsB	purH rrsE

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(0-3098) RNAp <mark>N-</mark>	

## Metabolism



**Cell Division** 



### **DNA** replication



**Supplementary Figure 6**. Screenshots of Integrative Genome Viewer<sup>1</sup> with tracks showing the binding profiles (tag density) as measured by ChIP-seq of RNAp binding in N nonstarved (denoted as N+) and N starved (denoted as N-) *E. coli* aligned against the upstream regions of all known ppGpp responsive promoters grouped into key cellular processes. A track with the input DNA control tag density (denoted as input) is shown for comparison. The screenshots in the green and red boxes denote promoters at which RNAp binding is positively

and negatively, respectively, affected by ppGpp during N starvation. The screenshots in gray boxes denote promoters at which RNAp binding remains unchanged at t=N+ and N-.



**Supplementary Figure 7.** Screenshots of Integrative Genome Viewer<sup>1</sup> with tracks showing the binding profiles (tag density) as measured by ChIP-seq of RNAp binding in N non-starved (denoted as N+) and N starved (denoted as N-) *E. coli* aligned against the upstream regions of toxin-antitoxin pair genes. A track with the input DNA control tag density (denoted as input) is shown for comparison. The screenshots in the green boxes denote promoters at which RNAp binding is positively affected by ppGpp during N starvation. The screenshots in gray boxes denote promoters at which RNAp binding at which RNAp binding is positively affected by ppGpp during N starvation. The screenshots in gray boxes denote promoters at which RNAp binding remains unchanged at t=N+ and N-.



**Supplementary Figure 8.** Entire gel image of the RelA Western blot showing expression of RelA proteins in cells sampled at t=N- (as in Fig. 2C). Lane 1 contains the molecular weight marker and lane 6 contains purified *E. coli* RelA-6xHis protein.

Gene	Functional group	Increased ppGpp effect (predicted) <sup>a</sup>	RNAp ChIP binding N- vs N+	Fold change gene expression N- vs N+
rpoS	Transcription	+	+	<b>↑</b> 4.59 ±0.75
crp	Transcription	+	+	<b>↑</b> 2.41 ±0.36
leuO	Transcription	+	+	♥ 2.88 ±1.03
rpoE	Transcription	+	+	<b>↑</b> 2.06 ±0.30
pspB	Transcription	+	+	<b>↑</b> 5.88 ±1.05
rpoH	Transcription	+	+	<b>↑</b> 7.23 ±1.04
yddM	Transcription	+	+	<b>↑</b> 2.78 ±0.88
rsd	Transcription	+	nc	<b>↑</b> 2.77 ±0.60
flhD	Transcription	-	nc	♥ 3.76 ±0.77
lrp	Transcription	+	nc	0.67 ±0.14
stpA	Transcription	-	nc	<b>↑</b> 2.28 ±0.52
iraD	Transcription	+	nc	1.06 ±0.36
zntR	Transcription	+	nc	<b>↑</b> 2.81 ±0.76
rpoZ	Transcription	-	• • • •	0.76 ±0.21
phoB	Transcription	+	-	<b>↑</b> 2.23 ±0.09
iraP	Transcription	+	•	<b>↑</b> 3.23 ±1.29
pcnB	Transcription	-		0.64 ±0.06
bolA	Stress adaptation	+	+	<b>↑</b> 5.69 1.80
relB	Stress adaptation	+	+	<b>↑</b> 3.65 ±1.50
relA	Stress adaptation	+	+	<b>↑</b> 6.78 ±0.75
mazE	Stress adaptation	-	nc	<b>↑</b> 3.68 ±0.50
grxB	Stress adaptation	+	+	1.86 ±0.18
lexA	Stress adaptation	+	nc	<b>↑</b> 6.01 ±0.58
dksA	Stress adaptation	-	nc	1.31 ±0.40
rmf	Translation	+	+	<b>↑</b> 4.09 ±1.84
rplJ	Translation	-	+	<b>↓</b> 4.33 ±1.30
fis	Translation	-	nc	♥ 18.83 ±3.52
rpsP	Translation	-	nc	♥ 8.69 ±1.51
rplN	Translation	-	nc	0.64 ±0.17
rpmH	Translation	-	-	♥ 8.01 ±1.78
rpsA	Translation	-		♥ 4.98 ±2.06
thrS	Translation	-		<b>↑</b> 3.73 ±0.71
rpsU	Translation	-		<b>↓</b> 21.70 ±3.36
rpsM	Translation	-		0.56 ±0.13
rrsH	Translation	-		No array data
rpsT	Translation			
rpsJ	Translation			<b>↓</b> 12.62 ±6.99
rplK	Translation	-		♥ 4.84 ±1.81
rrsG	Translation			No array data
dusB	Translation			<b>♦</b> 26.35 ±4.17
rrsD	Translation			No array data
rrsC	Translation			No array data
rrsA	Translation	-		No array data

# Supplementary Table 1. Gene expression analysis of ppGpp-dependent promoters

rrsB	Translation	-	-	No array data
rrsE	Translation	-	-	No array data
rpsL	Translation	-	-	<b>↓</b> 5.77 ±1.64
hisG	Metabolism	+	+	<b>↑</b> 2.63 ±0.45
livJ	Metabolism	+	+	<b>↑</b> 3.92 ±1.71
thrA	Metabolism	+	+	♥ 4.33 ±2.4
glgC	Metabolism	+	nc	1.75 ±0.47
argI	Metabolism	+	nc	<b>↑</b> 2.52 ±0.60
ppx	Metabolism	-	-	<b>↓</b> 4.88 ±0.74
pyrF	Metabolism	-	-	<b>♦</b> 6.41 ±1.72
purU	Metabolism	-	-	<b>↓</b> 2.71 ±0.10
apt	Metabolism	-	-	<b>↓</b> 56.18 ±5.37
speC	Metabolism	-	-	0.74 ±0.05
pyrL	Metabolism	-	-	0.64 ±0.10
ftsQ	Cell Division	+	nc	1.10 ±0.18
ftsZ	Cell Division	+	nc	<b>↑</b> 4.16 ±1.44
holA	DNA replication		nc	<b>↓</b> 3.04 ±0.56

Red indicates decrease, green indicates increase & grey indicates no change

<sup>a</sup> Predictions of changes in gene expression from an increase in cellular ppGpp levels are

derived from Ecocyc<sup>4</sup>

Supplementary Table 2. TaqMan *E. coli* gene expression primer and probe sequences used in this study

Gene	Forward primer (5'-3')	Reverse Primer (5'-3')	FAM Reporter probe (5'-3')
glnK	GGGCATGCCGAGCTGTA	ATCAGCAATCGCCACATCAATTTT	CAGCGTCAATTTCCTG
relA	CGGGACATGAAGACCGGATT	ATCTCTTCCTGCCACGCAAT	CCTGGCTGCGTAAACT
16S RNA	CCCCCTGGACGAAGACTGA	GTGGACTACCAGGGTATCTAATCCT	TCCCCACGCTTTCG

Probe name	Probe sequence 5'-3' (Primer sequences bold and underlined)
<i>glnA</i> probe (344bp)	<b>GTCCCTTTGTGATCGCTTTCA</b> CGGAGCATAAAAAGGGTTATCCAAAGGTCATTGCACCAA CATGGTGCTTAATGTTTCCATTGAAGCACTATATTGGTGCAACATTCACATCGTGGTGCA GCCCTTTTGCACGATGGTGCGCATGATAACGCCTTTTAGGGGCAATTTAAAAGTTGGCAC AGATTTCGCTTTATCTTTTTTACGGCGACACGGCCAAAATAATTGCAGATTTCGTTACCA CGACGACCATGACCAATCCAGGAGAGTTAAAGTATGTCCGCTGAACACGTACTGACGATG CTGAACGAGCACGAAGTGAAGT
<i>relA</i> probe (336 bp)	<b>CACAGCAACTTCAGATGGGG</b> TTTCGCAAAGCGGGCTCCAGTGACATTGTCGACGTCAAAC AATGCCCCATTTTAGCGCCCCAACTTGAAGCATTGCTGCCCAAAGTCAGGGCATGTCTGG GCAGCTTACAAGCTATGCGCCATCTTGGTCATGTTGAACTGGTACAGGCAACCAGCGGCA CGCTGATGATTTTGCGCCATACCGCACCGC
<i>dicC</i> probe (347 bp)	ACGAATACCTGCTGCTTGTGCAAGTTTTGTTTTGTACCGAAATACAAAAGAGCGTCAGT         TTTAAGCATTTAAAACACCTTTATTGTTAGTCATAACTAAC
<i>flgMN</i> probe (312 bp)	CTTGCTGCGCTTCGTTGATCGTTACGAATCGCCAGTTTTAACGCTTCGACACGTTCAAGATTGATATCACTGCTGCCGGGTTGCATCAGTTTTGCTTGCGCGCGTCGCTCGACACGTCCAGGTGCGGGGGGGG
<i>ynfB</i> probe (330 bp)	ATCACTCTCAGCAAACGAATCG GCCTGCTCGCTATTCTGCTGCCTTGCGCACTGGCATTG AGCACAACTGTTCATGCCGAAACTAACAAACTGGTGATTGAGTCTGGCGACAGTGCACAA AGCCGCCAGCACGCCGCTATGGAAAAAGAGCCAATGGAATGACACGCGCCAATCTGCGCCAG AAAGTGAATAAACGCACTGAAAAAGAGTGGGATAAAGCCGACGCCGCTTTTGATAACCGC GATAAATGTGAGCAAAGCGCCAACATCAATGCCTACTGGGAGCCCAATACTTTGCGCTGC CTGCACCGTCCAACTCCCCCCTTATTACC
<i>fliC</i> probe (359 bp)	TTAGTACCGGTAGTGGCCTG         TTAGTACCGGTAGTGGCCTG         TCGGACAGCGCGCCTTCGGTGGTCTGCGCAACGGAGATACCGTCGTTGGCGTTACGGGCC         GCCTGAGTCAGGCCTTTAATGTTAGAGGTGAAACGGTTAGCAATCGCCTGACCCGCTGCG         TCATCCTTCGCGCTGTTAATACGCAAGCCAGAAGACAGAC
ssrS probe (346 bp)	<b>TTGAACAAGGTCGCATCACC</b> GAAAAAACTAACCAAAACTTTGAATGACACTTTTCGGTTTACTGTGGTAGAGTAACCGTGAAGACAAAATTTCTCTGAGATGTTCGCAAGCGGGCCAGTCCCCTGAGCCGATATTTCATACCACAAGAATGTGGCGCTCCGCGGTTGGTGAGCATGCTCGGTCCGTCCGAGAAGCCTTAAAACTGCGACGACACATTCACCTTGAACCAAGGGTTCAAGGGTTACAGCCTGCGGCGGCATCTCGGAGATTCCCTTCTTATCTGGCACCAGCCATGACGCAACTACCAGAACTCCCACTGACATTATCCGCAAA
serA probe (331 bp)	TCAGCCAGAATGCCCAATTGCCGCGCGCTTCAAAAGAACCCGCCACGTTTGTTCCACACGCCACGGTGCGCTTTAGCATTGGCTTCCGGCACGCCGCGCAATAGCAGCAGCAGTTCGCCAATCACCAGCTCCGCAACAGAGCGCGTATTTGAGAACGGTGCGTTAAATACCGGGATCCCGCGCTTTGCCGCCGCATCCAGATCAACCTGGTTTGTTCCGATACAGAAACAGCCAATAGCGACCAGTTTTTCTGCGGCGTTGATCACGTCTTCAGTCAGATGGGTACGGG
<i>yjcC</i> probe (340 bp)	<b>CGGAACACATCATCGAGCTG</b> GCGCACAGCCTGGGGTTAAAAACGATCGCTGAAGGCGTCG AAACTGAGGAGCAGGTTAACTGGCTGCGCAAACGCGGCGTGCGCTATTGCCAGGGATGGT TCTTTGCGAAGGCGATGCCGCCGCAGGTGTTTATGCAATGGATGG

# Supplementary Table 3. EMSA probe and primer sequences

Primer	Primer sequence (5'-3')
GSP1_relA (set A)	CGTGTCAATGTCCATACT
GSP2_relA (set A)	CGAGAGGATCTCCACCATCTCA
GSP3_relA (set A)	CATCTCAACACCACGCCAC
GSP1_relA2 (set B)	CCAGCAACACTTTATCG
GSP2_ <i>relA2</i> (set B)	GGCTGCTTTGTGACATCTTCTTC
GSP3_relA2 (set B)	CTGTAAGCCATTAAGACGCGC

## Supplementary Table 4. Primer sequences for 5'RACE PCR used in this study

#### **Supplementary References**

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- 2 Narlikar, L. & Jothi, R. ChIP-Seq data analysis: identification of protein-DNA binding sites with SISSRs peak-finder. *Methods in molecular biology (Clifton, N.J.)* **802**, 305-322, doi:10.1007/978-1-61779-400-1\_20 (2012).
- 3 FinchTV (Geospiza Inc. <u>http://www.geospiza.com/Products/finchtv.shtml</u>).
- 4 Keseler, I. M. *et al.* EcoCyc: fusing model organism databases with systems biology. *Nucleic acids research* **41**, D605-612, doi:10.1093/nar/gks1027 (2013).