table S1: strains and plasmids

Strain or plasmid	Description	Reference
Strains		
E. coli		
TOP10	Competent *E. coli* for plasmid transformation	Invitrogen
BL21	BL21(DE3)pLysS strain for protein expression	Promega
BL21-121	BL21 with *relP* gene under P_{IPTG} control, amp	This work
BL21-122	BL21 with *relQ* gene under P_{IPTG} control, amp	This work
S. aureus		
RN4220	Restriction deficient *S. aureus* strain, r^-	(1)
RN4220-55	RN4220 with *rsh* gene under P*spac* control in the chromosome, *ermC*	(2)
HG001	*rsbU* restored RN1 (8325), previously named RN1HG	(3, 4)
HG001-55	HG001 with *rsh* gene under P*spac* control in the chromosome, *ermC*	(2)
HG001-86	HG001 rsh_{Syn} (Δ942-950nt)	(2)
HG001-229	HG001 *relP* (Δ450-536nt)	This work
HG001-230	HG001 *relQ* (Δ343-429nt)	This work
HG001-229-230	HG001 *relP/ relQ* double mutant	This work
HG001-229-230- 263	HG001 *rsh/relP/relQ* triple mutant referred as (p)ppGpp^0	This work
USA300JE2 NE554	vraR ::Tn bursa aurealis mutant	(5)
HG001 NE554	vraR ::Tn bursa aurealis mutant	This work
Plasmids		
pET15b	Protein expression vector,P_{IPTG}, amp	Novagen
pMUTIN4	Integrative vector including the IPTG-inducible promoter Pspac, (Ap^r, Em^r)	(6)
pKOR1	AHT inducible suicide mutagenesis vector	(7)
pCG86	pKOR1 with mutated *rsh* synthase domain (Δ942-950nt)	(2)
pCG263	pKOR1 with mutated *rsh* (Δ249-951nt)	This work
pCG229	pKOR1 with mutated *relP* synthase (Δ450-536nt)	This work
pCG230	pKOR1 with mutated *relQ* synthase (Δ343-429nt)	This work
pCG121	*relP* gene in protein expression vector pET15b	This work
pCG122	*relQ* gene in protein expression vector pET15b	This work
pCG248	anhydrotetracycline (ATc) inducible vector	(8, 9)
pCG258	ATc inducible vector with *relP*	(8, 9)
pCG259	ATc inducible vector with *relQ*	(8, 9)
pCG328	ATc inducible vector with rsh_{Hyd}	(8, 9)

References:

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5. **Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW.** 2013. A genetic resource for rapid and comprehensive phenotype screening of nonessential Staphylococcus aureus genes. MBio **4:**e00537-00512.
6. **Vagner V, Dervyn E, Ehrlich SD.** 1998. A vector for systematic gene inactivation in *Bacillus subtilis*. Microbiology **144 (Pt 11):**3097-3104.
7. **Bae T, Schneewind O.** 2006. Allelic replacement in *Staphylococcus aureus* with inducible counter-selection. Plasmid **55:**58-63.
8. **Schroder W, Goerke C, Wolz C.** 2013. Opposing effects of aminocoumarins and fluoroquinolones on the SOS response and adaptability in Staphylococcus aureus. The Journal of antimicrobial chemotherapy **68:**529-538.
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table S2: oligonucleotides

Purpose and Description	Template	Name	Sequence
Mutagenesis			
relP mutant	H G001	attB1relP2-for	GGGGACAAGTTTGTACAAAAAA GCAGGCTTGGTCACTATCACA CGAA
		relPmut-rev	ACTTGCCCACATATCCATACCT ATCCTAGGACCATTTTCTTTAG GGTGCTGAAT
		relPmut-for	ATAGGTATGGATATGTGGGCA
		attB2relP2-rev	GGGGACCACTTTGTACAAGAA AGCTGGGTTGGGCGAGTATTT AGCAA
relQ mutant	HG001	attB1relQ-for	GGGGACAAGTTTGTACAAAAAA GCAGGCTTAAAGGTTGATGAT GTTCG
		relQmut-rev	CGTTGCCCAGAAATTCATTGCT AACCTAGGACCACTTTCTTTAG TGTTACGAAT
		relQmut-for	TTAGCAATGAATTTCTGGGCA
		attB2relQ-rev	GGGGACCACTTTGTACAAGAA AGCTGGGTCGCGGTAAATGAA TTTCTAA
relP, relQ, rsh mutant ((p)ppGpp^0)	HG001	attB1rel-for	GGGGACAAGTTTGTACAAAAAA GCAGGCTATTAGGCGGTATCG TAGT
		rshmut-rev	GCCTACTACTGTAGTATGCAAC AAAAAACCTGCGACAAT
		rshmutfor	TTGCATACTACAGTAGTAGGC
		attB2relrev1	GGGGACCACTTTGTACAAGAA AGCTGGGTGCTGCGAATAAAT CATCTT
Construction of hybridization probes			
vraR	HG001	vraRDIG-for	GGAATTTCAAGTTATCTATCAA
	HG001	vraRDIG-rev	TGAGTAACCTTTCGCAATCA
relP	HG001	relPDIG-for	GTCGCACATTCTTTCAGT
	HG001	relPDIG-rev	CGTTATTAGGTTTCGTAGAGTT
relQ	HG001	relQDIG-for	AAGCGGTTGATGAGTTGA
	HG001	relQDIG-rev	AAATACGCTGCTTCTGCC

rsh	HG001 HG001	rshDIG-for rshDIG-rev	CGGCTCTTCGTTATATTGATAA GGCAACTCAATAACATCAC
Protein purification			
RelP	HG001	XhoIpETrelP-for	CCCCCTCGAGATGTATGTAGATCGAAAACCAT
	HG001	BamHIpETrelP-rev	GGGGGGATCCCTACTCTGTTATTTCAGAATGAA
RelQ	HG001	XhoIpETrelQ-for	GGGGCTCGAGATGAATCAATGGGATCAGTT
	HG001	BamHIpETrelQ-rev	GGGGGGATCCTTAATCATTTTCATGTTTTTT
ATc inducible plasmids			
pRelP	HG001	SalIrelPfor	CCGGGTCGACAAAATGTTAGGTACAATAAAGA
	HG001	SalIrelPrev	CCGGGTCGACGAACACACCTACTAAACAT
pRelQ	HG001	SalIreQfor	AATTGTCGACTGCAACCGTTGAAGAAACAT
	HG001	SalIrelQrev	TTAAGTCGACCGCATGATTTATAACACCTCG
$pRSH_{Hyd}$	HG001	EcoRIrel1151	AAAAGAATTCGTACCTAAATCATTGTTTAAGGCG
	HG001	HDreplaceASrev	ATACGGTGTATCTTCAATTACGGAGGCCAAAAAACCTGC
	HG001	HDreplaceASfor	GTAATTGAAGATACACCGTAT
	HG001	EcoRIrelrev	CCCCGAATTCCCGATACCGACTAATAAACAATA

Underlined: artificial restriction sites

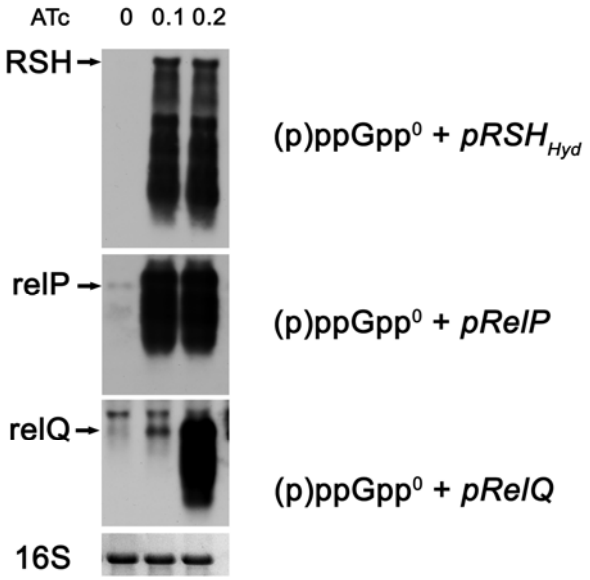

Figure S1: Overexpression of the three (p)ppGpp Synthases.

(p)ppGpp^0 mutants complemented with *pRSH*$_{Hyd}$, *pRelP*, or *pRelQ* synthases were grown in CYPG to the exponential growth phase followed by further incubation with and without ATc (µg/ml) for one hour. RNA was hybridized with digoxigenin-labeled PCR fragments. The 16S rRNA detected in the ethidium bromide-stained gels is indicated as loading control in the lower lane.