Supplementary information

(p)ppGpp-mediated stress response induced by defects in outer membrane biogenesis and ATP production promotes survival in *Escherichia coli*

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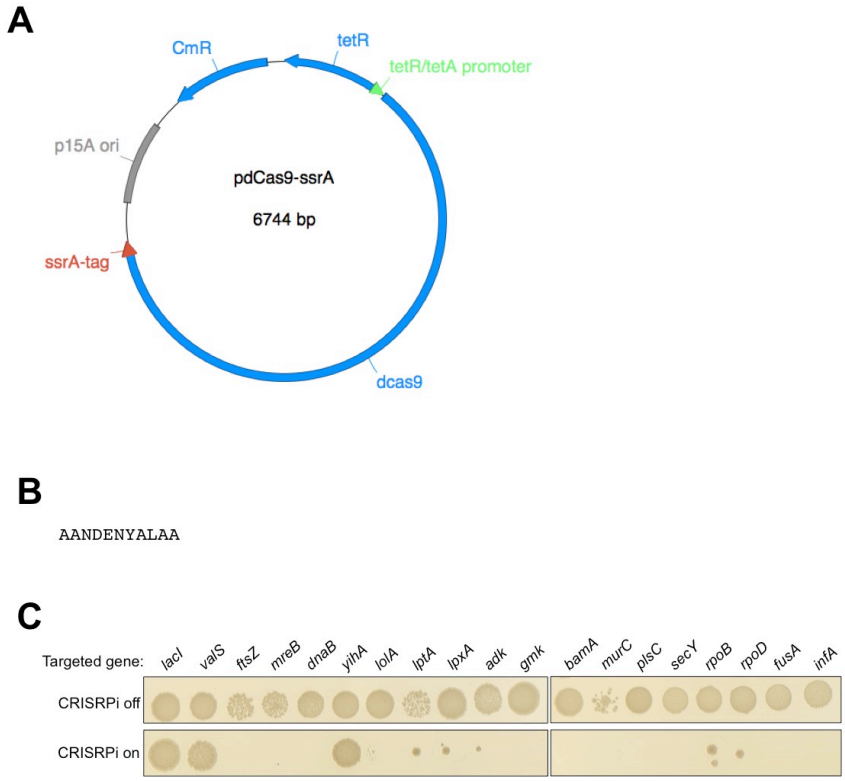
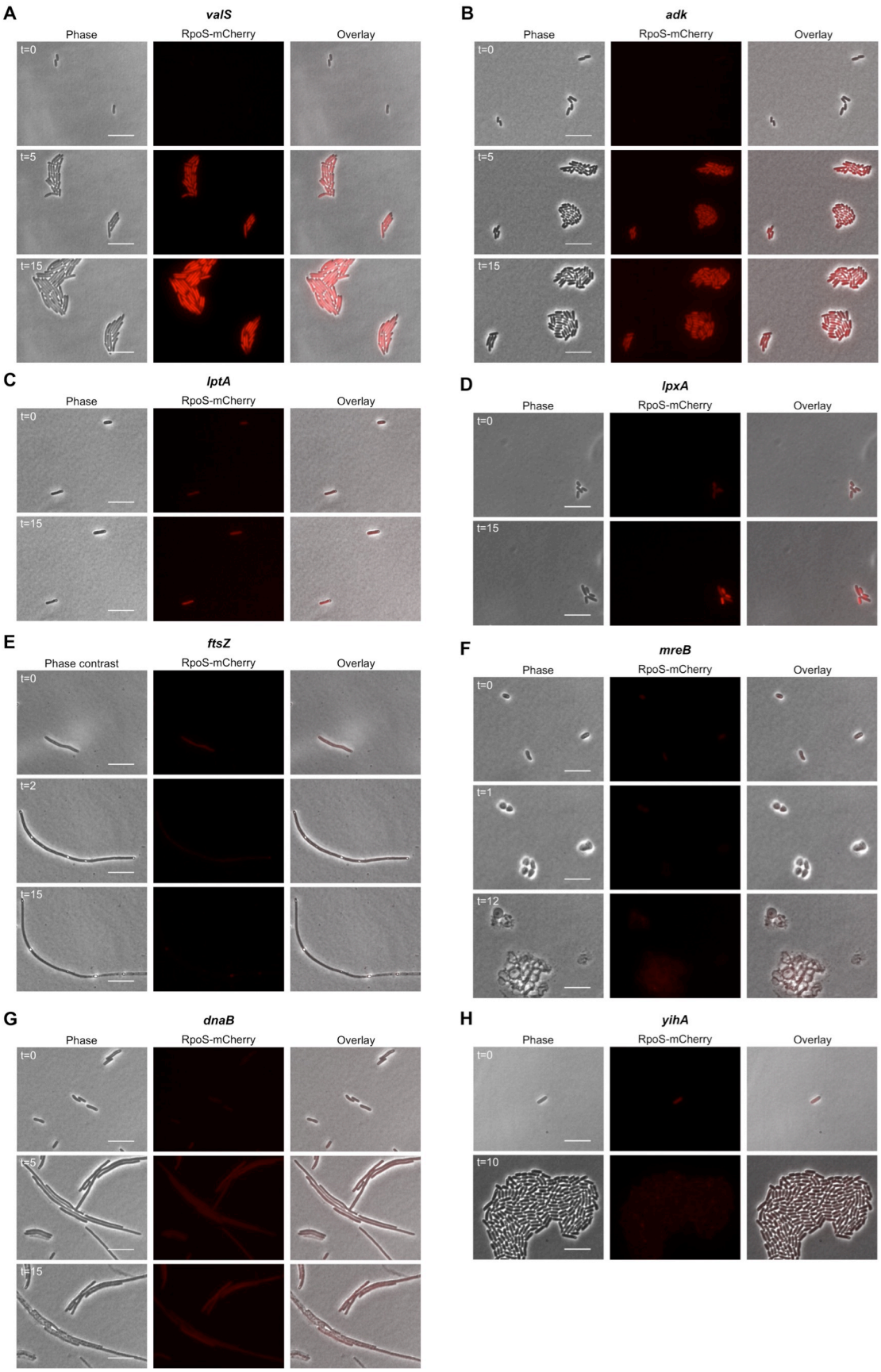


Figure S1. Fusion of ssrA degradation-tag to *dcas9*. (**A**) Schematic plasmid map of pdCas9-ssrA. (**B**) The amino acid sequence of the ssrA degradation-tag fused to dCas9. (**C**) Viability of *E. coli* MG1655 containing pdCas9-ssrA and sgRNA against the indicated genes. Overnight cultures were diluted 1000x and 3 µl spotted on *dcas9-ssrA* non-inducing (CRISPRi off) and inducing (CRISPRi on) solid LB media.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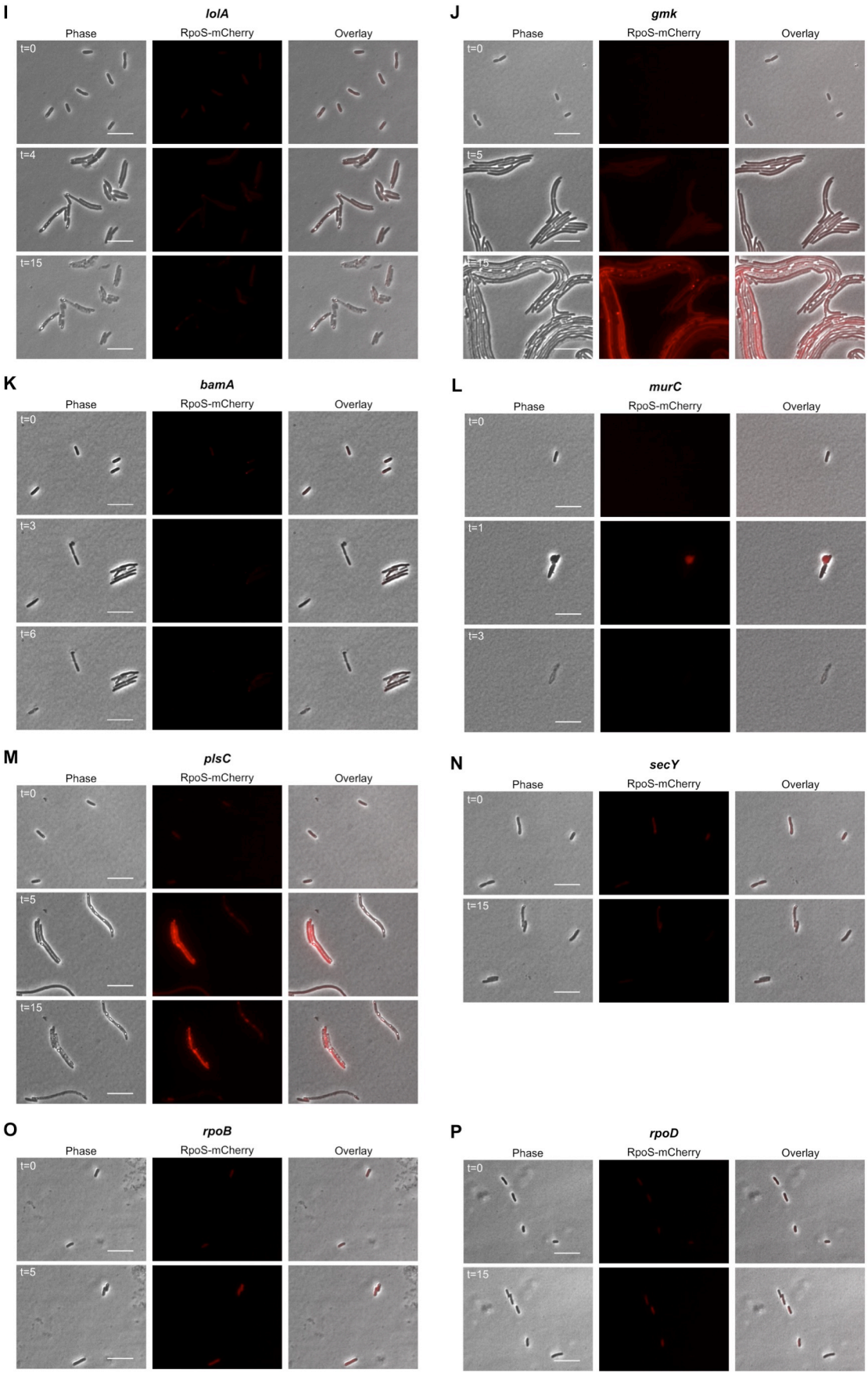
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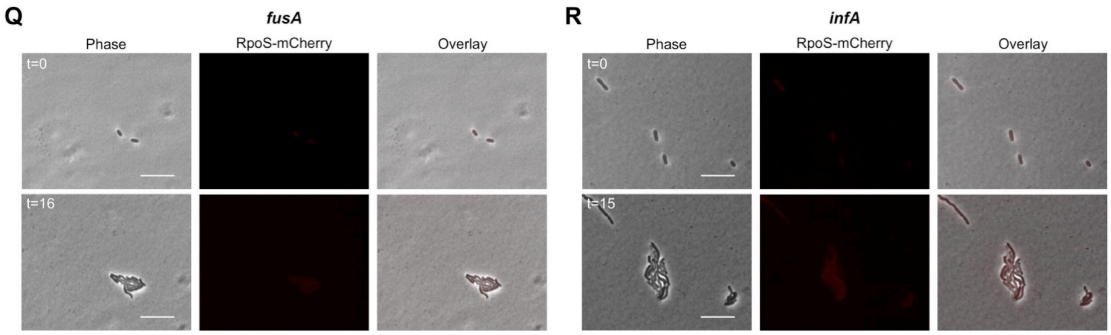

Figure S2. Time-lapse fluorescence microscopy images during repression of the surveyed genes. CRISPRi-mediated shut down of expression of the indicated genes in a MG1655-derivate strain harboring the intracellular (p)ppGpp-reporter *rpoS-mCherry*. Phase contrast (left panels), the (p)ppGpp-reporter RpoS-mCherry fluorescence (middle panels) and the merged overlay images (right panels) capturing cells throughout the duration of the experiment. Scale bars = 10 µm. The repressed genes are indicated in bold text: (**A**) *valS* (**B**) *adk* (**C**) *lptA* (**D**) *lpxA* (**E**) *ftsZ* (**F**) *mreB* (**G**) *dnaB* (**H**) *yihA* (**I**) *lolA* (**J**) *gmk* (**K**) *bamA* (**L**) *murC* (**M**) *plsC* (**N**) *secY* (**O**) *rpoB* (**P**) *rpoD* (**Q**) *fusA*, and (**R**) *infA*.

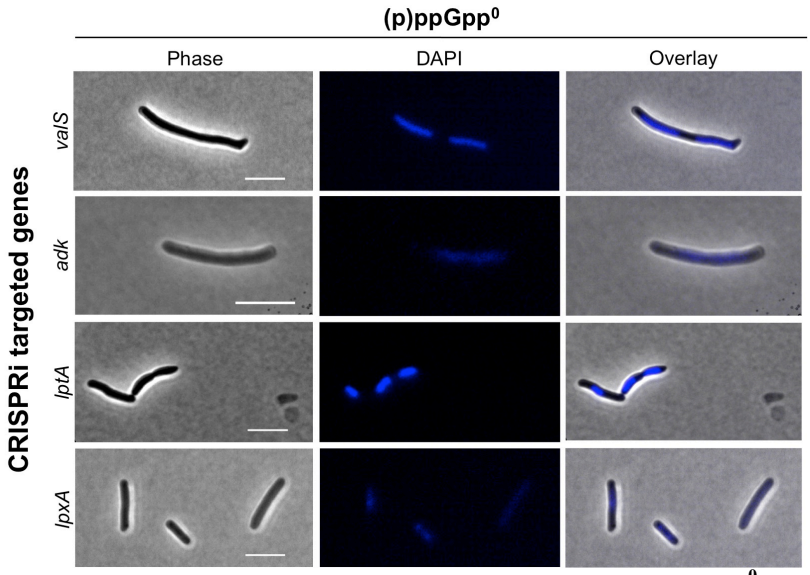

Figure S3. The nucleoid localizes differently in (p)ppGpp^0 cells during repression of *valS*, *adk*, *lptA* and *lpxA*. Fluorescence microscopy imaging of DAPI-stained Δ*relA/spoT* cells 3h post CRISPRi-mediated transcriptional repression of the indicated genes. Phase contrast (left panels), DAPI fluorescence (middle panels) and their merged overlay (right panels) are shown. Scale bars = 10 µm.

Table S1. The selected cellular processes targeted in this study. The involved genes, corresponding functions, locus tags and the gRNA targeting regions are listed.

Cellular process	Gene	Function	Locus tag	sgRNA N_{20} region	Ref
tRNA-charging (positive control)	*valS*	Valine-tRNA ligase	b4258	gcggctgttcgatatcttgt	1
Cell division	*ftsZ*	Septal ring protein	b0095	ctttcagcagtttgtcgttc	2
Cytoskeleton	*mreB*	Structural cytoskeletal protein	b3251	ttgtccttttacataaatga	3
DNA replication	*dnaB*	Replicative DNA helicase	b4052	tcagcctgctgtttgttgaa	4
Essential GTPases	*yihA*	Function not fully established	b3865	gataagctgggtgcgccctg	5
Lipoprotein insertion in outer membrane	*lolA*	Outer membrane lipoprotein carrier protein	b0891	ccgtagcttgctcaacgaac	6
LPS biosynthesis/transport	*lptA*	Periplasmic subunit of the Lpt LPS-transport system	b3200	attaattttgatggtgccct	7
LPS biosynthesis/transport	*lpxA*	UDP-N-acetylglucosamine acyltransferase, lipid A biosynthesis pathway	b0181	accctcaccaatttcgacat	8
Nucleotide metabolism	*adk*	Adenylate kinase	b0474	ctgcctgcggaatggtacgc	9
Nucleotide metabolism	*gmk*	Guanylate kinase	b3648	ggatttacccgcgccactgg	10
Outer membrane protein assembly	*bamA*	Essential BAM system component for assembly of proteins in the outer membrane	b0177	gcgcaccggcatactgagga	11
Peptidoglycan synthesis	*murC*	UDP-*N*-acetylmuramate—L-alanine ligase, lipid II biosynthesis pathway	b0091	tggcatcacgtacgttttcc	12
Phospholipid biosynthesis	*plsC*	1-acylglycerol-3-phosphate *O*-acyltransferase, phospholipid biosynthesis pathway	b3018	ccacatgtttcgggttacgc	13
Secretion system	*secY*	Sec translocon subunit SecY	b3300	gtacagcggcatcaatacca	14
Transcription	*rpoB*	RNA polymerase subunit β	b3987	taccgctgtagctctgaatc	15
Transcription	*rpoD*	Sigma 70, exponential growth sigma factor	b3067	gaagtttcagctgtgactgc	16
Translation	*fusA*	Translation elongation factor G	b3340	atgttacggtagcgtgcgat	17
Translation	*infA*	Translation initiation factor IF-1	b0884	attaggcaacgtttcaagaa	18
Lactose metabolism (negative control)	*lacI*	Transcriptional repressor of the *lac*-operon	b0345	gctggcctggttcaccacgc	19

Table S2. Strains and plasmids used in this study.

Strain/plasmid	Genotype	Source
Strains		
E. coli K-12 MG1655	Wild-type	Laboratory collection
::rpoS-mCherry	MG1655 *rpoS::mcherry-frt-aphA-frt*	[20]
Δ*relA*	Δ*relA::*FRT	This study
Δ*relA/spoT*	Δ*relA*::FRT/*spoT::zeoR*	This study
Plasmids		
pdCas9-ssrA	P_{tet}*::dcas9-ssrA*	This study
pgRNA-lacI	See Table S1 for N_{20} matching sequences	This study
pgRNA-valS	See Table S1 for N_{20} matching sequence	This study
pgRNA-adk	See Table S1 for N_{20} matching sequence	This study
pgRNA-lptA	See Table S1 for N_{20} matching sequence	This study
pgRNA-lpxA	See Table S1 for N_{20} matching sequence	This study
pgRNA-ftsZ	See Table S1 for N_{20} matching sequence	This study
pgRNA-mreB	See Table S1 for N_{20} matching sequence	This study
pgRNA-dnaB	See Table S1 for N_{20} matching sequence	This study
pgRNA-yihA	See Table S1 for N_{20} matching sequence	This study
pgRNA-lolA	See Table S1 for N_{20} matching sequence	This study
pgRNA-gmk	See Table S1 for N_{20} matching sequence	This study
pgRNA-bamA	See Table S1 for N_{20} matching sequence	This study
pgRNA-murC	See Table S1 for N_{20} matching sequence	This study
pgRNA-plsC	See Table S1 for N_{20} matching sequence	This study
pgRNA-secY	See Table S1 for N_{20} matching sequence	This study
pgRNA-rpoB	See Table S1 for N_{20} matching sequence	This study
pgRNA-rpoD	See Table S1 for N_{20} matching sequence	This study
pgRNA-fusA	See Table S1 for N_{20} matching sequence	This study
pgRNA-infA	See Table S1 for N_{20} matching sequence	This study

Supplementary References

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