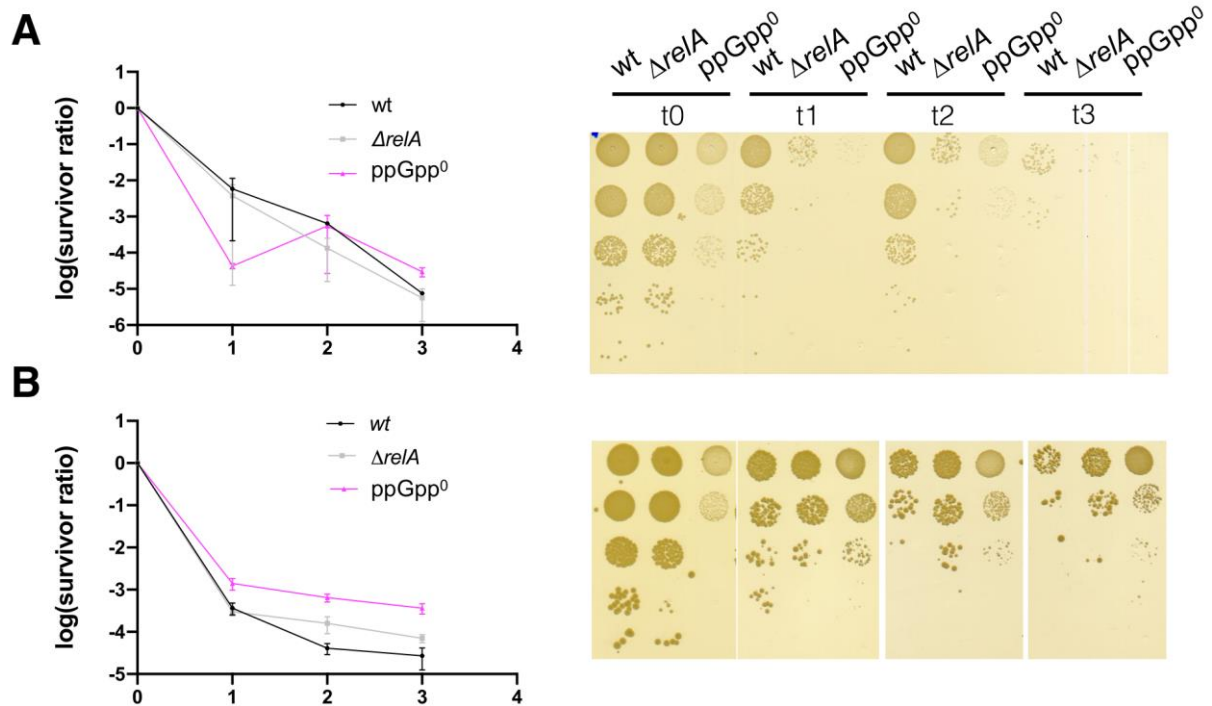
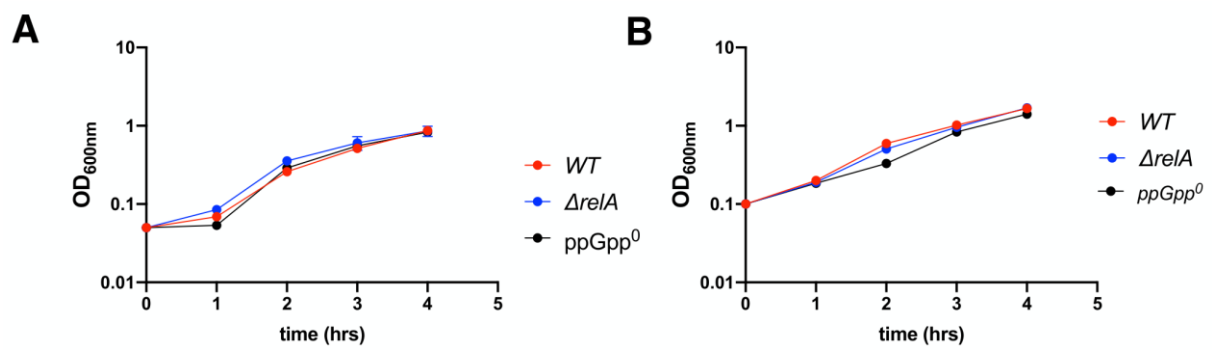


**Supplemental Figure S1.** Related to Figure 1. **(A)** The evolved (Evo) cells of the  $ppGpp^0$  strain, after 72 hrs growth in the presence of ampicillin (both 6.25 and 3.125  $\mu\text{g}/\text{ml}$ ), grew up earlier than the original (Ori)  $ppGpp^0$  strain. **(B, C, D)** Growth curves of the wt,  $\Delta relA$  and  $ppGpp^0$  cells in the presence of gentamycin (0.5, 0.25, 0.125  $\mu\text{g}/\text{ml}$ ). **(E, F, G)** Growth curves of the wt,  $\Delta relA$  and  $ppGpp^0$  cells in the presence of ofloxacin (0.125, 0.0625, 0.03125  $\mu\text{g}/\text{ml}$ ). Two biological replicates were performed, and the average and range were presented. **(H)** The evolved (Evo) cells of the  $ppGpp^0$  strain, after 72 hrs growth in the presence of gentamycin and ofloxacin (0.125 and 0.03125  $\mu\text{g}/\text{ml}$ , respectively), did not grow up earlier than the original (Ori)  $ppGpp^0$  strain.



**Supplemental Figure S2.** (A) The gentamycin (2.5 μg/ml) and (B) ofloxacin (0.625 μg/ml) killing curves of the exponentially growing wt,  $\Delta relA$  and ppGpp<sup>0</sup> strains in MOPSR-Lp. Two (gentamycin) and six (ofloxacin) biological replicates were performed, and the averages and SDs are shown. Representative plates of the surviving cells after killing are shown to the right side of each panel.



**Supplemental Figure S3.** Growth curves of the wt,  $\Delta relA$  and ppGpp<sup>0</sup> cells in the MOPSR-Lp medium from initial OD<sub>600nm</sub> of 0.05 (A) and 0.1 (B). Two biological replicates were performed, and the averages and SDs are shown.

**Supplemental TABLE S1. Bacterial strains used in this study.**

Strain	Relevant features	Reference
YZ142	MG1655, wild type <i>E. coli</i>	Laboratory stock
YZ38	MG1655 $\Delta$ <i>relA</i>	[21]
YZ62	MG1655 $\Delta$ <i>relA</i> <i>spoT207::cat</i> ; CamR	[21]
YZ313	MG1655 $\Delta$ <i>relA</i> , pWRG99	This study
YZ580	MG1655 $\Delta$ <i>relA</i> , <i>spoT</i> (T78-E319)::lscel-Cam; pWRG99	This study
YZ584	MG1655 $\Delta$ <i>relA</i> <i>spoT203</i> (R140C)	This study
YZ585	MG1655 $\Delta$ <i>relA</i> <i>spoT202</i> (T78I)	This study
YZ840	MG1655 $\Delta$ <i>relA</i> $\Delta$ <i>spoT</i> ( <i>colony-1</i> )	This study
YZ841	MG1655 $\Delta$ <i>relA</i> $\Delta$ <i>spoT</i> ( <i>colony-2</i> )	This study
YZ1094	MG1655 $\Delta$ <i>relA</i> $\Delta$ <i>spoT</i> <i>rpoBD675A</i> ( <i>colony-1</i> )	This study
YZ1097	MG1655 $\Delta$ <i>relA</i> $\Delta$ <i>spoT</i> <i>rpoBD675A</i> ( <i>colony-2</i> )	This study

**Supplemental TABLE S2. Primers used in this study.**

Number	Name	Sequence
pYZ69	DspoT-conf-F	gtcgtcgtaatcacaagc
pYZ71	spoT-seq6-R	gcactgcataagcgaagtcg
pYZ351	80mer-Is(Spot)ifdel	ACAAAGCGGGTCGCCctgtatctgtttgaaagcctgaatAAAGTCACCCGAAACCG AAATTAATGTTTTATGAACCCAA
pYZ395	60mer-spoTnt-ISceI-F	AACGCTGATGGCGGCGCTGCTGCATGACGTGATTGAAGATCTAGACTATATT ACCCTGTTATCCC
pYZ396	60mer-spoTnt-ISceI-R	TCTCCGCCATCTGGTCCATATCTTCGGTACGGATCTGGACATTTAAATGGCGC GCCTTAC
pYZ397	spoT-T78I-F2	GAAGATAtTCCCGCCACCTACCAG
pYZ398	spoT-T78I-R1	GGCGGGAaTATCTTCAATCACGTCATGCAG
pYZ399	spoT-R140C-R1	TGGGTACaGTCGGCAAGTTTGATGAGG
pYZ400	spoT-R140C-F2	TGCCGACTGTACCCACAACATGCGCA