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Supplemental information

Sleeping ribosomes: Bacterial signaling triggers

RaiA mediated persistence to aminoglycosides

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SUPPLEMENTAL DATA

Supplementary figures:

Figure S1. Indole improves growth of *V. cholerae* in the presence of tobramycin. A. WT. B. Δ *raiA*. Growth is measured on a TECAN plate reader. IND: indole 350 μ M. TOB: tobramycin sub-MIC (0.6 μ g/ml). Experiments were performed in triplicates and geometric means are represented. Error bars represent the geometric standard deviation. Related to **Figure 1**.

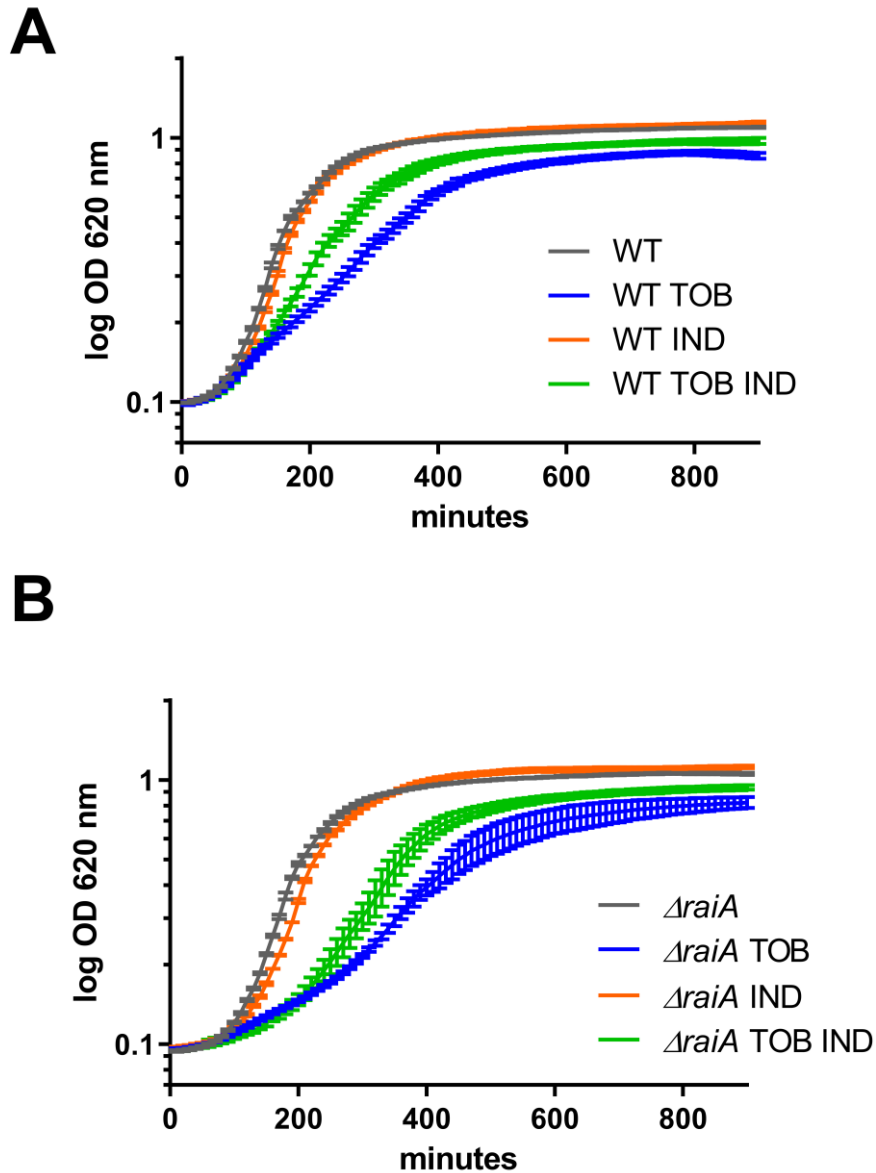


Figure S2. Persistence and the effect of indole. **A.** Kinetics of survival of WT *V. cholerae* to tobramycin 10 µg/ml in MH media. Time zero corresponds to the total number of CFU before addition of antibiotics, to an early exponential phase culture (OD 620 nm 0.25-0.3), as described in the methods section. The proportion of surviving cells is calculated after plating and counting growing colonies, and is represented for each time point. Curves represent geometric means of at least 3 replicates for each time point and error bars represent geometric standard deviation. **B.** Persistence of *V. cholerae* WT and $\Delta tnaA$ mutants in exponential phase (in LB instead of MH, to allow indole production, see methods) after 20 hours treatment with specified antibiotics. Tobramycin (TOB): 10 µg/ml, carbenicillin (CRB): 100 µg/ml. Related to **Figure 2**.

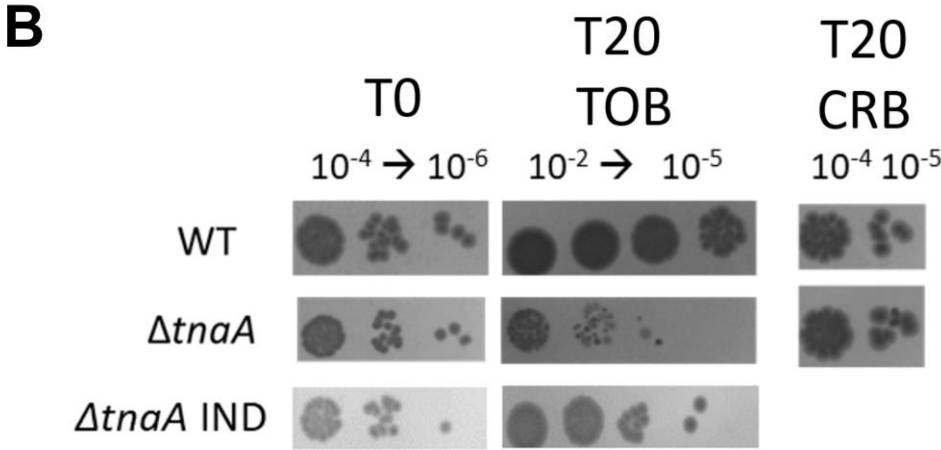
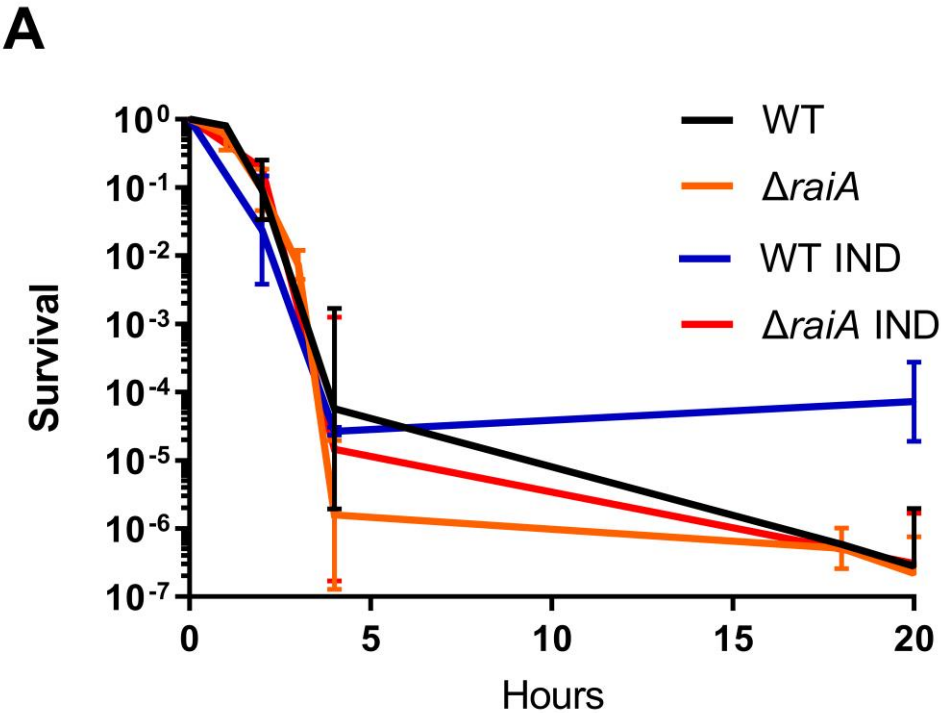


Figure S3. Indole does not apparently influence aminoglycoside entry and resistance in *V. cholerae* WT and Δ *raiA*. **A.** Intracellular level of neomycin coupled to the fluorophore Cy5 measured by fluorescence associated flow cytometry. Error bars represent standard deviation. **B.** Minimal inhibitory concentrations of tobramycin (TOB) and gentamicin (GEN) measured using *etests* in *V. cholerae*, in the absence (MH) and presence of indole (IND), and indicated in μ g/ml by a numeral on each image. Related to **Figure 2**.

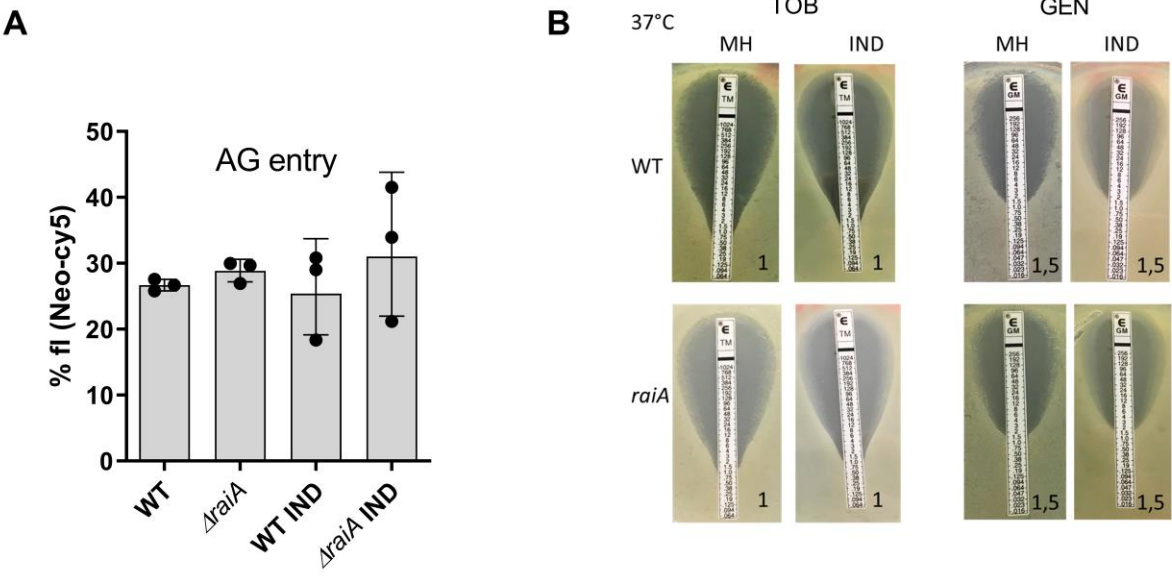


Figure S4. Fluorescence detection from the *raiA* promoter-*gfp* fusion by flow cytometry. Representative acquisitions are shown. Each plot represents one experiment. Each experiment was performed at least 3 times and data and statistical significance are shown in the histograms in the main manuscript. **A:** histogram curves: GFP fluorescence is represented in the x-axis (FITC channel), the y-axis represents the number of events corresponding to the number of cells, normalized to height (same number of total cells for both conditions). **B:** dot plots: Bacterial cells' size and shape (rugosity). FSC: forward scatter. SSC: size scatter. The SSC (size scatter) vs FSC (forward scatter) graphs show the distribution of cells by size and shape and no major difference in cell size/shape is observed between the WT and mutants with or without indole. Related to **Figure 4**.

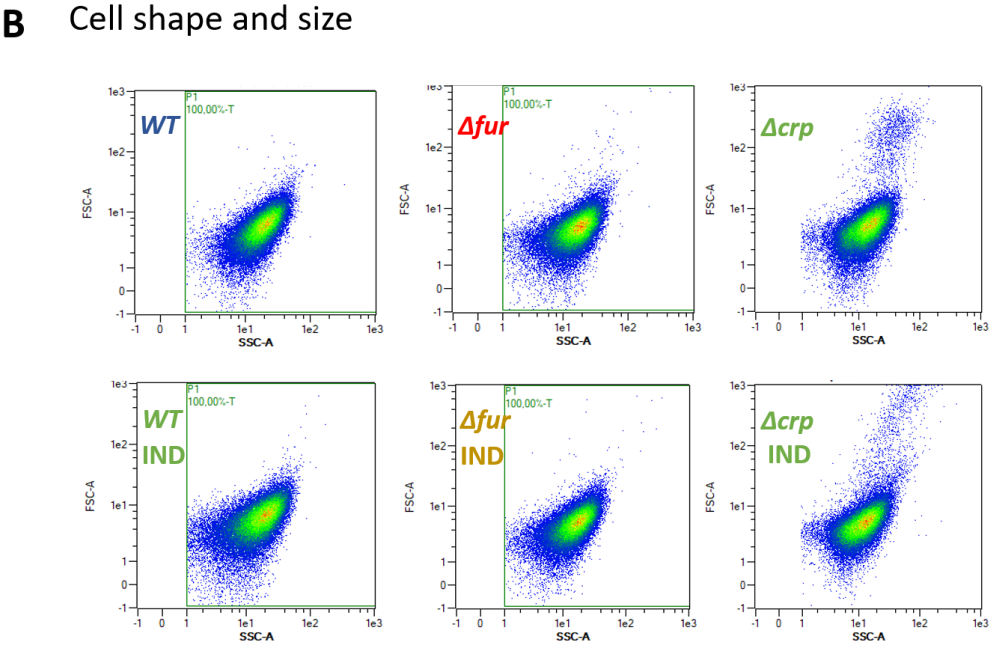
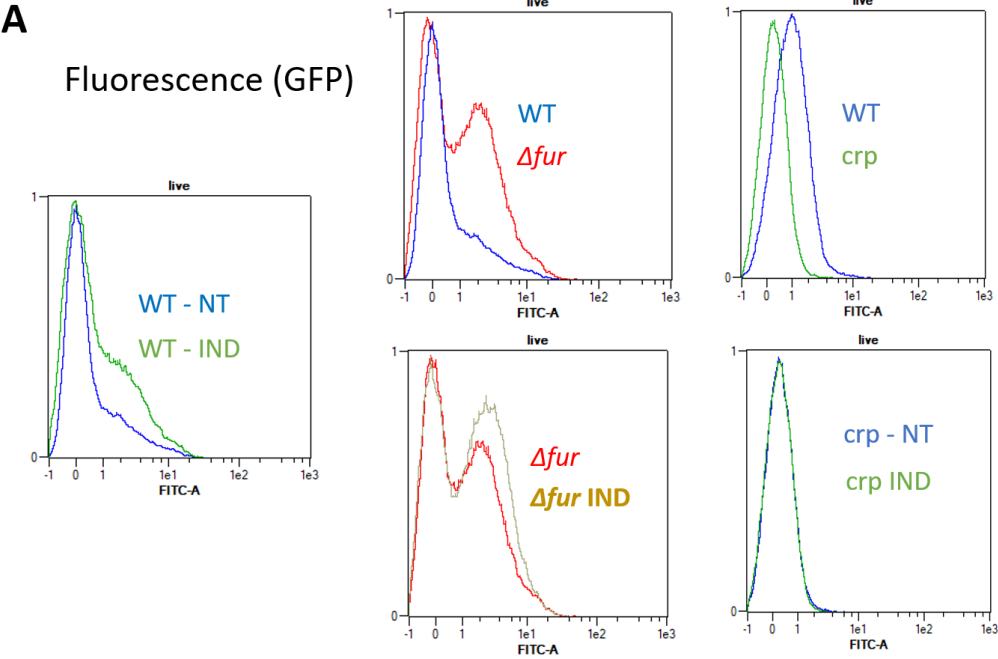


Figure S5. RaiA is involved in persistence in *V. cholerae* and *E. coli*. **A.** Increase of surviving colonies (persisters) after 20 hours of treatment with tobramycin (TOB) 10 µg/ml (ratio over WT surviving colonies) in *V. cholerae*. Error bars represent geometric standard deviation **B.** Persistence of *E. coli* WT and *raiA* mutant, at late exponential phase (OD 620nm 0.5), after 20 hours treatment with 10 µg/ml tobramycin (TOB) in MH media. Serial dilutions are spotted on plates for estimation of survival. Spot assays were performed at least 3 times. Related to **Figure 2**.

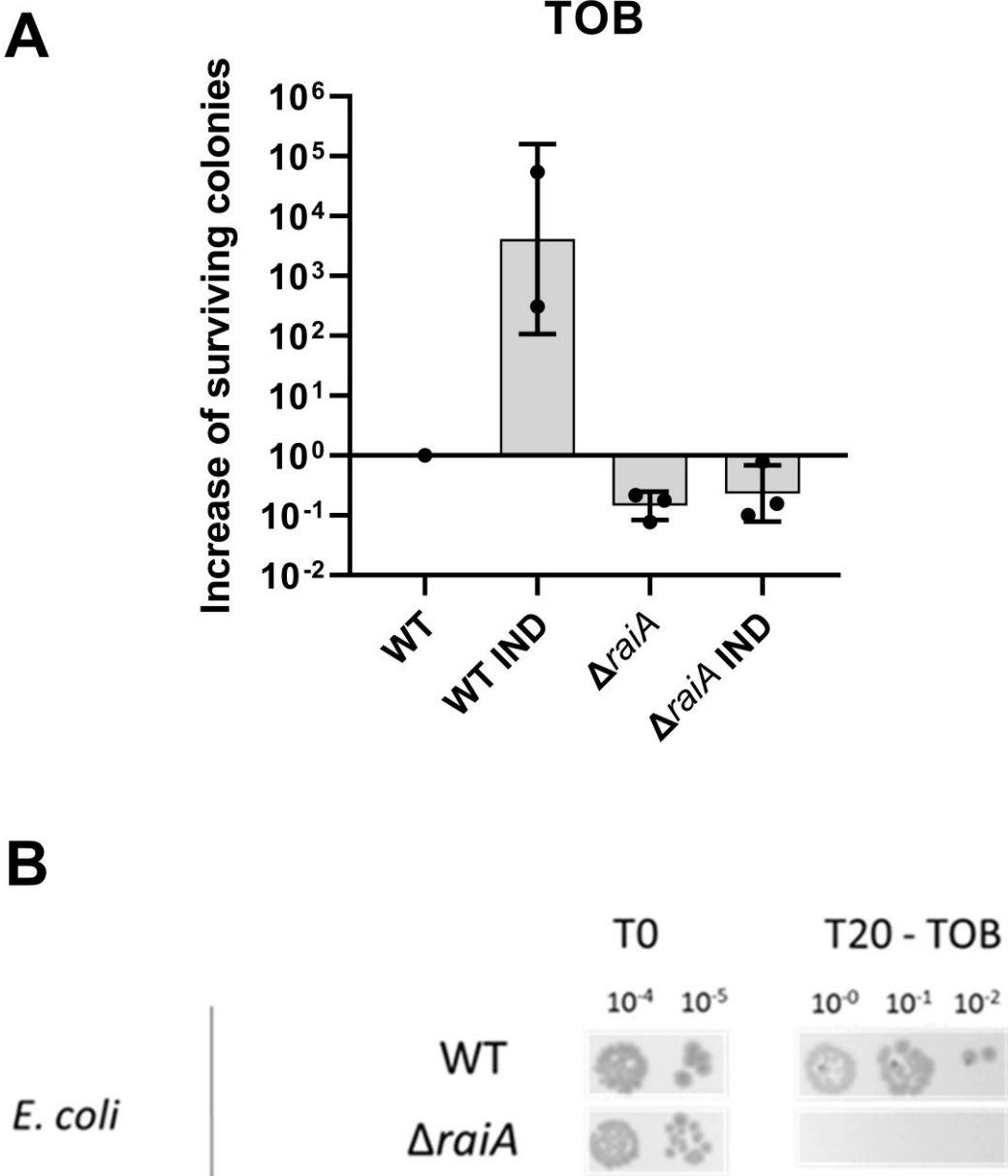


Figure S6. Influence of RaiA on the length of lag phase upon growth restart after the stationary phase. **A.** Experimental set-up. Repression was achieved with 1% glucose (GLC), induction was achieved with 0.2% arabinose (ARA). **B.** Lag time (minutes) defined as time to reach OD 620 nm 0.15 in WT *V. cholerae* carrying empty vector (p0) or pBAD-RaiA vector (see ABCD in Figure 6). **C.** Lag time (in minutes) defined as time to reach OD 620 nm 0.15 in *V. cholerae* Δ raiA, Δ rmf and Δ hpf and WT *V. cholerae* carrying empty vector (p0) or hibernation factors (pRaiA, pRmf, pHpf) (Figure 7AB) **D.** Lag time (in minutes) defined as time to reach OD 620 nm 0.15 in WT *P. aeruginosa* (Figure 9A) carrying empty vector (p0) or pBAD-RaiA vector. Experiments were performed in triplicates and statistical analysis was performed (**: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$; ns: not significant). Related to **Figure 6, 7AB** and **9A**.

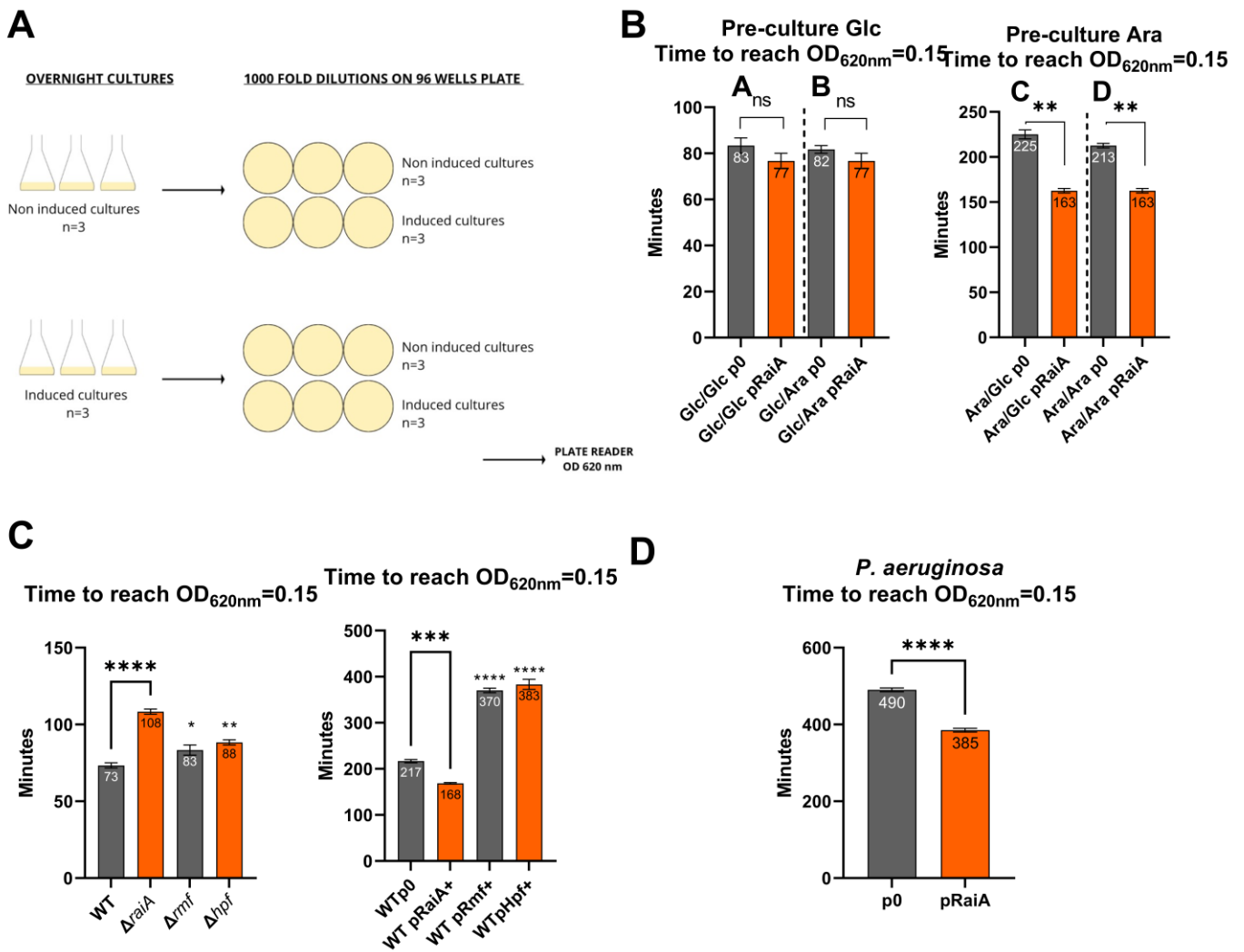


Figure S7. Influence of RaiA, Rmf and Hpf on persistence. Persistence of *V. cholerae* WT and mutants in early exponential phase, after 20 hours treatment with 10 µg/ml tobramycin (TOB) in MH media. Serial dilutions are spotted on plates for estimation of survival. Spot assays were performed at least 3 times. Related to **Figure 7**.

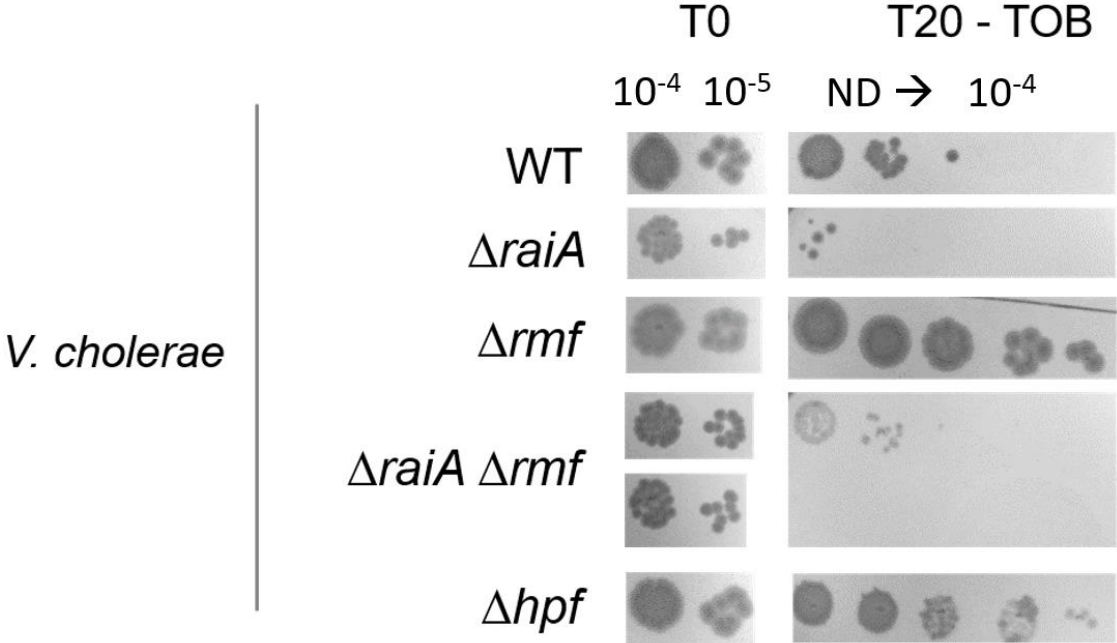
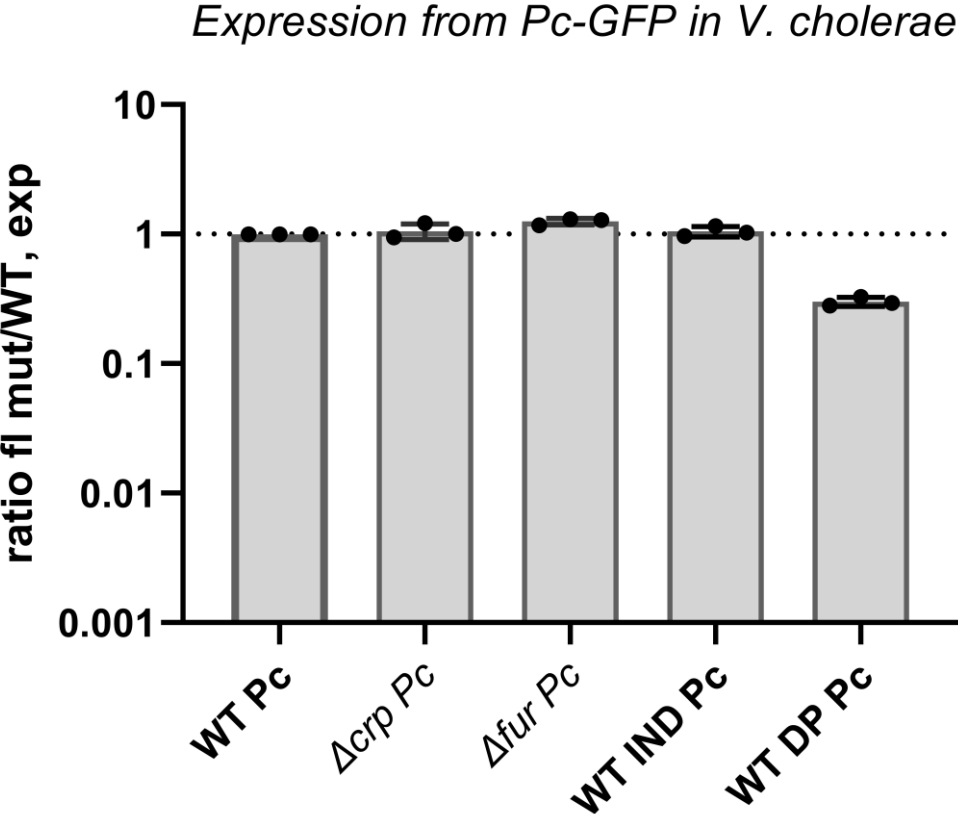


Figure S8. Expression of *gfp* from constitutive promoter (Pc) in conditions where expression from the *raiA* promoter is up or down-regulated. Fluorescence quantification of GFP expression from constitutive promoter by flow cytometry in MH media in exponential phase, in WT and indicated *V. cholerae* deletion mutants. IND: indole (350 μ M), DP: 2,2'-Dipyridyl (500 μ M). The Y axis represents fluorescence ratio of the mutant over wild type (WT) strain. Error bars represent standard deviation. Related to **Figure 4**.



Supplementary table:

Table S2. Strains and plasmids. Related to STAR Methods.

Strain	Strain number	Construction
<i>Vibrio cholerae</i>		
N16961 wt strain		
N16961 hapR+ wt strain	F606	Gift from Melanie Blokesch
Δ <i>raiA</i> (VC0706)	J251	PCR amplification of 500 bp up and down regions of VC0706 using primers ZIP381/384 and ZIP382/383. PCR amplification of <i>aadA7</i> conferring spectinomycin resistance on pAM34 using ZB47/48. PCR assembly of the VCA0706:: <i>aad7</i> fragment using ZIP383/384 and allelic exchange by natural transformation, as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>rmf</i> (VC1484)	L557	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L045, replacing the gene with <i>frt::aph::frt</i> as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>hpf</i> (VC2530)	M566	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 M472, replacing the gene with <i>frt::aph::frt</i> as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>raiA</i> Δ <i>rmf</i>	L790	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 L045 in J251, replacing the gene with <i>frt::aph::frt</i> as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>crp</i>	9950	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 8348, replacing the gene with <i>aad7</i> (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>fur</i>	N541	allelic exchange by integration and excision of conjugative suicide plasmid pMP7 N450, replacing the gene with <i>frt::aph::frt</i> as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
Δ <i>rpoS</i>	A321	Baharoglu <i>et al.</i> , 2013
Δ <i>tnaA</i> (VC0161)	J253	PCR amplification of 500 bp up and down regions of VC0161 using primers ZIP93/94 and ZIP95/96. PCR amplification of <i>aadA7</i> conferring spectinomycin resistance on pAM34 using ZIP97/98. PCR assembly of the VCA0161:: <i>spec</i> fragment using ZIP93/96 and allelic exchange by natural transformation, as described previously (Val <i>et al.</i> , 2012, Negro <i>et al.</i> , 2019)
<i>Escherichia coli</i>		
MG1655 wt strain		laboratory collection
Δ <i>raiA</i>	J237	P1 transduction from KEIO strain JW2578-1
<i>Pseudomonas aeruginosa</i>		
PAO1		laboratory collection
Plasmids		
pBAD43		spectinomycin resistant. Carries Para promoter repressed by glucose 1% and induced by arabinose 0.2%. (Guzman, 1995)

pBAD43-RaiA+	L907	<i>raiA</i> (VC0706) amplified using primers <i>raiAeco/raiAxba</i> and cloned under Pbad using 5' EcoRI and 3' XbaI restriction sites
pBAD43-Hpf+	M834	<i>hpf</i> (VC2530) amplified using primers <i>hpfeco/hpfxba</i> and cloned under Pbad promoter using 5' EcoRI and 3' XbaI restriction sites
pBAD43-Rmf+	M831	<i>rmf</i> (VC1484) amplified using primers <i>rmfeco/rmfxba</i> and cloned under Pbad using 5' EcoRI and 3' XbaI restriction sites
pSC101-PraiA-gfp	L707	<i>raiA</i> promoter region (500 bp) was amplified using primers ZIP383/446 and <i>gfp</i> was amplified using primers zip443/444. 2 fragments were assembled by PCR assembly using primers ZIP383/ZIP443, so that <i>gfp</i> is fused to the <i>raiA</i> promoter at the ATG start codon of <i>raiA</i> . The assembled fragment was cloned into pTOPO and extracted using EcoRI and cloned into pSC101 low copy plasmid (carbenicillin resistant).
pSC101-Pc-gfp	N110	<i>gfp</i> was amplified using primers carrying the promoter zip513/200. The fragment was cloned into pTOPO and extracted using EcoRI and cloned into pSC101 low copy plasmid (carbenicillin resistant).
pMP7- Δ hpf::kan	M472	gibson assembly using primers MV450/451 for the amplification of pMP7 vector, primers VC2530hpf5bis/7 and VC2530hpf6bis/8bis for up and down regions of the gene, and primers MV268/269 on pKD4 plasmid for the resistance gene (frrt::kan::frrt).
pMP7- Δ rmf::kan	L045	gibson assembly using primers MV450/451 for the amplification of pMP7 vector, primers VC1484rmf5/7 and VC1484rmf6/8 for up and down regions of the gene, and primers MV268/269 on pKD4 plasmid for the resistance gene (frrt::kan::frrt).
pMP7- Δ crp::aad7	8348	Baharoglu <i>et al.</i> , 2012
pMP7- Δ fur::kan	N450	gibson assembly using primers MV450/451 for the amplification of pMP7 vector, primers VC2106fur5/7 and VC2106fur9/8 for up and down regions of the gene, and primers MV268/269 on pKD4 plasmid for the resistance gene (frrt::kan::frrt).
Primers		
raiAecoRI		ggaattcaccATGAAAATCAACATCACTGGTAA
raiAxba		gctctagaTTATTCCAATTCTTCGCTCAG
hpfeco		ggaattcaccATGCAAATCAACATTCAAGGCC
hpfxba		gctctagaTTAATGACTACTTAGCTTTTCTTT
rmfeco		ggaattcaccATGAAGAGACAAAAGCGTGAT
rmfxba		gctctagaTTATTTGCAGAGACCAGAAAGTTT
ZB47		CCCGTTCCATACAGAAGCTGGGCGAACAACGATGCTCGC
ZB48		GACATTATTTGCCGACTACCTTGGTGATCTCGCCTTTCAG
ZIP381		GCGAGCATCGTTTGTTCGCCAGCTTCTGTATGGAACGGGTAAATAGAATGATGGGAGATAGCGC
ZIP382		CGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGTCAATGCTTTTTCC TCTGTGTCATCCCTTATGG
ZIP383		AACCTATCCAGATACCGAAGCGGC
ZIP384		GTTTTGCAGATAGGATTGCTGAAGC
ZIP93		GTCGAATGCCAAACGAAAGCGGAAAATACC
ZIP94		GCGAGCATCGTTTGTTCGCCAGCTTCTGTATGGAACGGGTACGGTATTGAAA AAGTGCAGCCTGC
ZIP95		CGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAATGTCGCTGGGTATTCC TAAAAATAAAATATAAAGTCATGC

ZIP96	CACAACAACCTCTAGTATCTGGTTTACCCTCG
ZIP97	GGCAGCCTATGCAGGCTGCACTTTTTCAATACCGTCCCGTCCATACAGAAGCT GGGCGAACAAACGATGCTCGC
ZIP98	ATGACTTTATATTTTAATTTTTAGGAATACCCAGCGACATTATTTGCCGACTACC TTGGTGATCTCGCCTTTCACG
ZIP443	TTATTTGTATAGTTCATCCATGCCATGTGTAATCCCAGC
ZIP444	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCC
ZIP446	GGACAACCTCCAGTGAAAAGTTCTTCTCCTTTACGCATAATGCTTTTTCTCTGTG TCATCCCTTATGG
VC2530hpf5bis	CTATTATTTAAACTCTTTCgccgatggtgctcaacgatg
VC2530hpf7	CTACACAATCGCTCAAGACGTGagacttctctctagtttagg
VC2530hpf6bis	TACGTAGAATGTATCAGACTcttcacactgcaatagcacg
VC2530hpf8bis	CTAATTTCCCATGTCAGCCGTGCGCCAGATCGCCGAAGCTA
VC1484rmf5	CTATTATTTAAACTCTTTCggcttggtgtagatgatc
VC1484rmf7	CTACACAATCGCTCAAGACGTGagttctgtcctcatacggta
VC1484rmf6	TACGTAGAATGTATCAGACTgttgatgatggatcgattacc
VC1484rmf8	CTAATTTCCCATGTCAGCCGTttccatacaacaagcttga
VC2106fur5	CTATTATTTAAACTCTTCCAAGCGGATGCGAACTTCGC
VC2106fur7	CTACACAATCGCTCAAGACGTGATACTTTCCTGTTGATGTTCTGC
VC2106fur8	CTAATTTCCCATGTCAGCCGTGCTCACAAGCCGAAGAAATAA
VC2106fur9	TACGTAGAATGTATCAGACTccacaaatcgatcagtttatgg
ZIP200	TATCAAGCTTATTTGTATAGTTCATCCATGCC
ZIP513	GAG CTG TTG ACA ATT AAT CAT CCG GCT CGT ATA ATG TGT GGA ATT GTG AGC GGA TAA CAA TTT CAC ACA GGA AAC ACA TAT GCG TAA AGG AGA AGA AC