**SUPPLEMENTARY INFORMATION**

Bacterial persistence induced by salicylate via reactive oxygen species
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## SUPPLEMENTARY METHODS

### Bacterial strains and growth conditions

Δ*marRAB* Δ*rob* Δ*soxSR* strain
We generated a PCR fragment of the kanamycin resistant gene flanked by FRT regions from pKD13 with extensions homologous to the regions adjacent to the operon or gene [1].

For deletion of the *marRAB* operon, we used the forward primer:
**CCAGCGATCTGTTCAATGAAATTATTCCATTGGGTCGCTTAATCCATATG**GTGTAGGCTGGAGCTGCTTC
and the reverse primer:
**AAGCCCCGAGATGTCGGGGCCAGAACAAACTACATAGCGTGTTGATTAT**AATTCCGGGGATCCGTCGACC.

For deletion of the *rob* gene, we used the forward primer:
**CGACGGATCGGAATCAGCAGTTCACAGCGTAGATTAATTGGGCGATCTC**CGTGTAGGCTGGAGCTGCTTC
and the reverse primer:
**ATGGATCAGGCCGGCATTATTCGCGACCTTTTAATCTGGCTGGAAGGTC**AATTCCGGGGATCCGTCGACC.

For deletion of the *soxSR* genes, we used the forward primer:
**AATTACAGGCGGTGGCGATAATCGCTGGGAGTGCGATCAAACTGCCGAC**GGTGTAGGCTGGAGCTGCTTC
and the reverse primer:
**TGTTCATCTTCCAGCAAGCGTGCGCCGGTACCTTCTTCTCCTAAGCGGTC**ATTCCGGGGATCCGTCGACC.

Bold letters indicate the homologous recombination extensions. We then followed the protocol from [1] using the phage lambda recombinase harbored on the low copy plasmid pSIM6. After recombination, the resistance marker gene was removed using the pCP20 helper plasmid encoding the FLP recombinase.

MarA$^+$ strain
The transversion mutations were derived from [2], where they are listed as "TV -14 to -18" and "TV +11 to 15." To construct this strain we synthesized a DNA fragment containing a modified version of the *marRAB* promoter:
GCATCGCATTGAACAAAACTTGAACCGATTTAGCAAAACGTGGCATCGGTCA
ATTCATTCATTTGACTTATACTTGCCTG**TTACC**TATTATCCCCTGCAACTAATT
AC**GGTAA**AGGGCAACTAATGTGAAAAGTACCAGCGATCTGTTCAATGAAATT
ATT.
Underlined letters indicate the MarR binding sites and bold letters indicate the transversion mutations. We introduced this modified promoter via homologous recombination following [1], then cured the resistance marker. To accomplish this, we ligated the modified *marRAB* promoter given above to the kanamycin resistance gene

flanked by FRT regions from pKD13. The ligated fragment containing the kanamycin resistance gene followed by the modified *marRAB* promoter was then amplified using the forward primer:
**GGGGTAAACAAGGATAAAGTGTCACTCTTTAGCTAGCCTTGCATCGCAT**
**T**GTGTAGGCTGGAGCTGCTTC
and the reverse primer:
AATAATTTCATTGAACAGATCGCTGGT.
Bold letters indicate the homologous recombination extensions. We followed the protocol for recombination and curing of the kanamycin marker as described above.

## SUPPLEMENTARY FIGURES

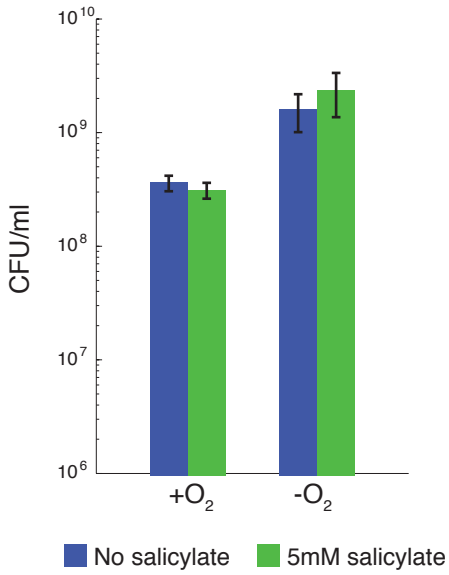


**Figure S1: Salicylate exposure does not affect initial cell counts before addition of antibiotics.**
Colony forming units per milliliter (CFU/ml) for cultures with and without 5 mM salicylate under aerobic conditions and anaerobic conditions before addition of antibiotics (t = 3 hours in aerobic conditions and t = 4 hours in anaerobic conditions). Error bars show standard errors from n = 12 biological replicates under aerobic conditions and from n = 6 biological replicates under anaerobic conditions.

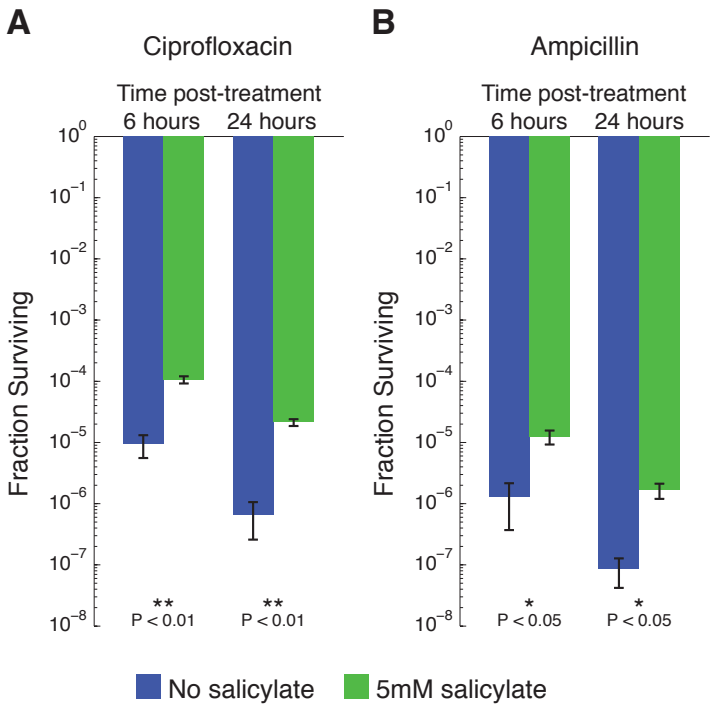


**Figure S2: Salicylate increases survival rate after 6 and 24 hours of ciprofloxacin or ampicillin treatment.**

Fraction of E. coli MG1655 surviving after 6 and 24 hours of (A) 5 μg/ml ciprofloxacin or (B) 100 μg/ml ampicillin treatment with and without 5 mM salicylate. Error bars show standard error of n = 3 biological replicates. We used the Student's t-test to test for statistical significance.

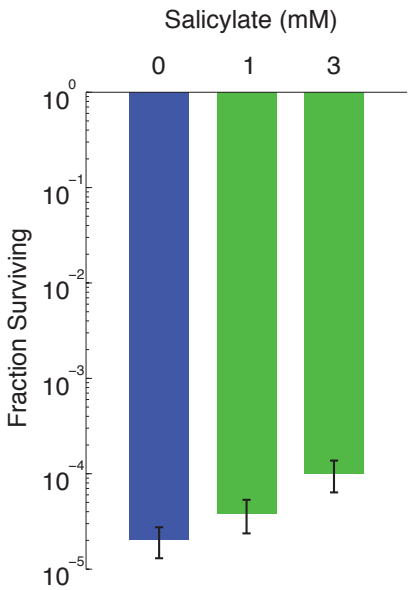


**Figure S3: 1 and 3 mM salicylate increase persister levels.**
Survival after 6 hours of 5 μg/ml ciprofloxacin treatment with and without 1 or 3mM salicylate. Error bars show standard errors from n = 3 biological replicates.

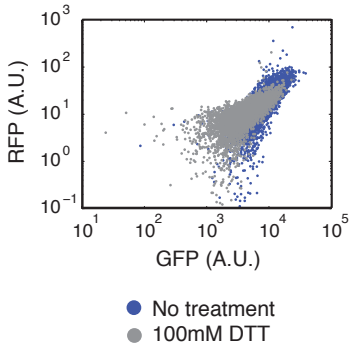


**Figure S4: Effect of DTT on membrane potential.**
Red vs. green fluorescence scatter plots showing individual cells treated with the membrane potential indicator DiOC2(3) with and without 100 mM DTT.

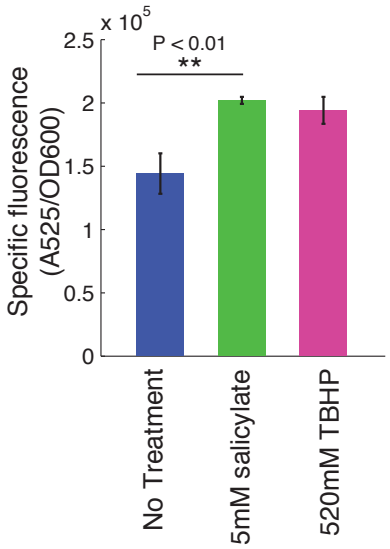


**Figure S5: Salicylate induces ROS in Δ*marRAB* Δ*rob* Δ*soxSR* strain.**
Green fluorescence divided by $OD_{600nm}$ for Δ*marRAB* Δ*rob* Δ*soxSR* cultures treated with the general ROS indicator carboxy-H2DCFDA. TBHP is a positive control for ROS. Error bars show standard deviation from n = 3 biological replicates. We used the Student's t-test to test for statistical significance.

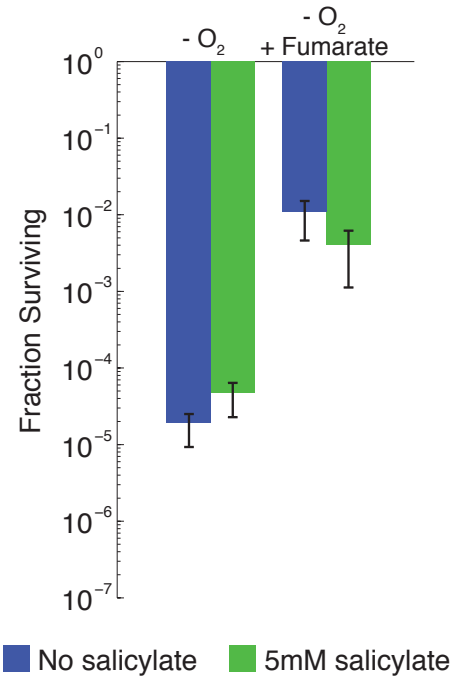


**Figure S6: Salicylate-induced persistence in ampicillin requires oxygen.**
Survival with and without 5 mM salicylate after 6 hours of 100 μg/ml ampicillin treatment under anaerobic conditions with and without 40 mM fumarate. Error bars show standard errors from n = 3 biological replicates.

## SUPPLEMENTARY REFERENCES


1 Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 6640-6645, doi:10.1073/pnas.120163297 (2000).

2 Martin, R. G. & Rosner, J. L. Transcriptional and translational regulation of the *marRAB* multiple antibiotic resistance operon in *Escherichia coli*. *Molecular microbiology* **53**, 183-191, doi:10.1111/j.1365-2958.2004.04080.x (2004).