Supplementary figures

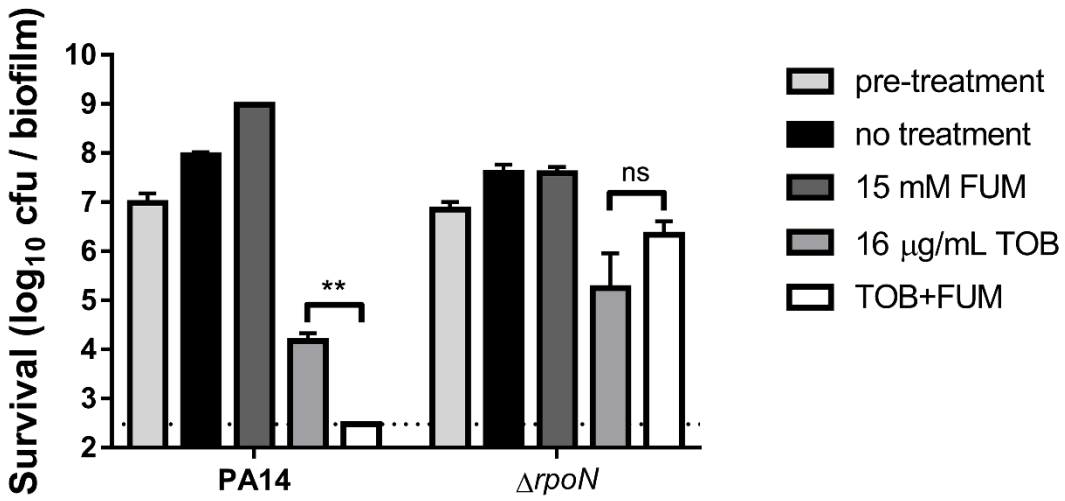

Figure S1. RpoN is required for colony biofilm susceptibility to TOB+FUM. Survival of 4 h-old PA14 wild-type and Δ*rpoN* colony biofilms (pre-treatment) that were treated for 20 h on M9 plates containing no supplements (no treatment), 15 mM FUM, 16 μg/mL TOB, or TOB+FUM. Data are presented as mean $\log_{10}$ cfu/biofilm ± SEM for three biological replicates. The dashed line indicates the limit of detection of the assay (all replicates for PA14 with TOB+FUM were below the limit of detection).

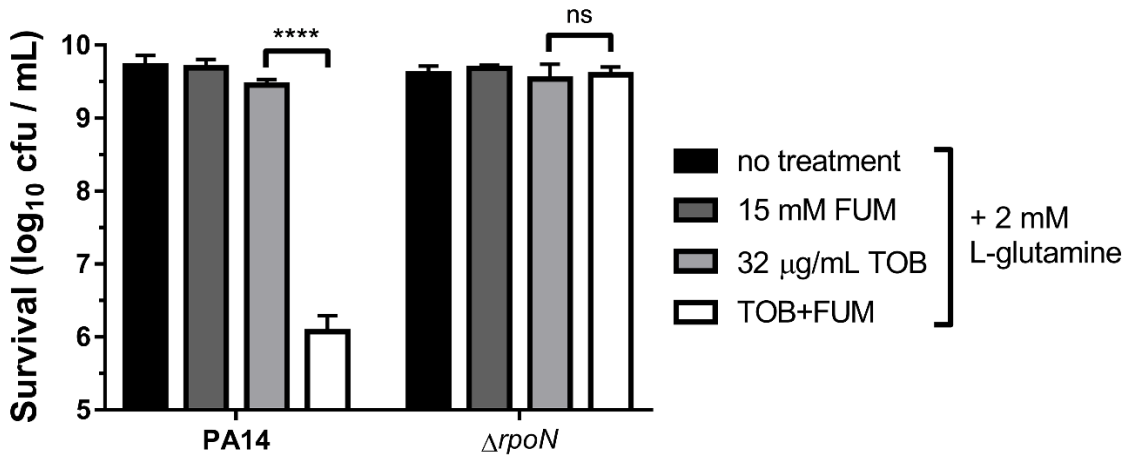

Figure S2. L-glutamine supplementation does not reverse non-susceptibility of Δ*rpoN* to TOB+FUM. Survival of PA14 wildtype and Δ*rpoN* stationary phase cells that were incubated for 4 h in the presence of 2 mM L-glutamine with no additional treatment, 15 mM FUM, 32 μg/mL TOB, or TOB+FUM. Data are presented as mean $\log_{10}$ cfu/mL ± SEM for three biological replicates.

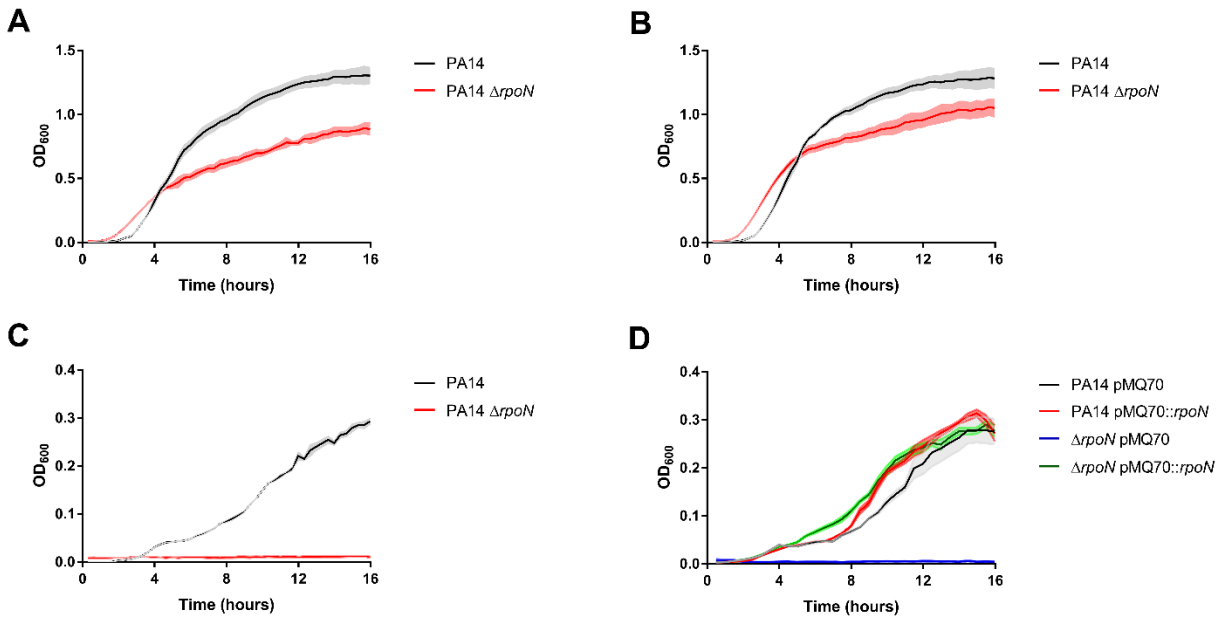

Figure S3. RpoN is required for growth with FUM as the sole carbon source. Growth curves of indicated strains in (A) LB, (B) LB with 2 mM L-glutamine, (C) M9 with 30 mM FUM and 2 mM L-glutamine, and (D) M9 with 30 mM FUM, 0.2% L-arabinose, and 2 mM L-glutamine. Lines represent the mean OD_{600} of two biological replicates, each with six technical repeats. Lighter shading represents the SEM.

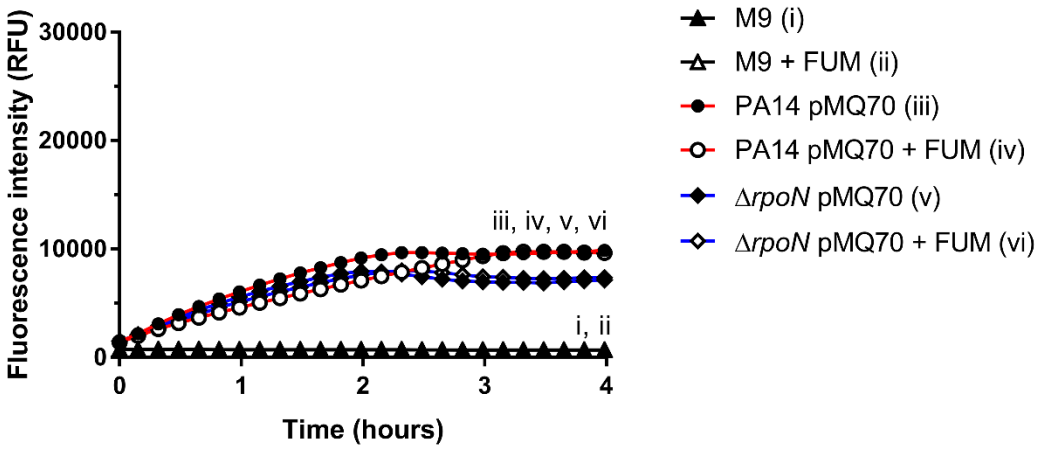

Figure S4. FUM-dependent increase in resazurin reduction is dependent on the ETC. Reduction of resazurin to resorufin was measured over time in NaN_3-treated stationary phase cells of the indicated strains in the presence or absence of 15 mM FUM. Data are shown as mean fluorescence intensity ± SEM for two independent experiments with three technical replicates per experiment. Since some data sets were obscured by others on the graph, Roman numerals were used to indicate the locations of the data sets.

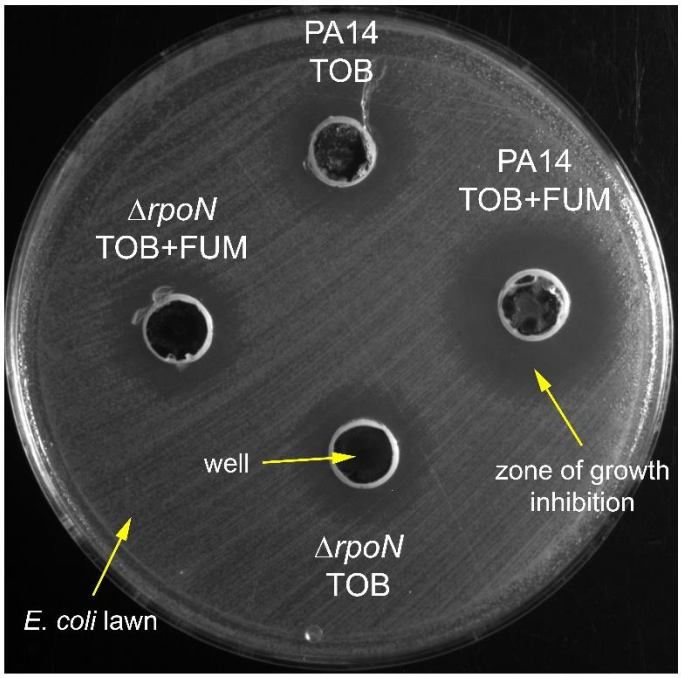

Figure S5. FUM increases TOB accumulation in wildtype but not Δ*rpoN* stationary phase cells. Image of a representative experiment showing zones of *E. coli* DH5α growth inhibition caused by lysates from PA14 and Δ*rpoN* cells treated with TOB or TOB+FUM.

Supplementary tables

Table S1. Antibiotic MICs (µg/mL) determined in LB with 2 mM L-glutamine for strains used in this study

Strain	TOB	TOB+FUM[a]	GEN	GEN+FUM[a]	AMK	AMK+FUM[a]
PA14	2	2	2	2	2	2
PA14-*lacZ*	2	2	ND[b]	ND[b]	ND[b]	ND[b]
Δ*rpoN*	2	2	2	2	2	2
PA14/pMQ70	4	4	4	4	4	4
PA14/pMQ70::*rpoN*	4	4	4	4	4	4
PA14/pMQ70::*dctA*	4	4	4	4	4	4
Δ*rpoN*/pMQ70	2	2	4	4	4	4
Δ*rpoN*/pMQ70::*rpoN*	4	4	4	4	4	4
Δ*rpoN*/pMQ70::*dctA*	4	4	4	4	4	4

[a]FUM added at a final concentration of 15 mM

[b]ND = not determined

Table S2. Bacterial strains and plasmids

Name	Genotype or description	Reference
Strains		
PA14	Wildtype *P. aeruginosa*; burn wound isolate	(1)
Δ*rpoN*	Unmarked deletion of *rpoN* in PA14	This study
PA14-*lacZ*	PA14 with chromosomal *lacZ* and TET^R markers	(2)
DH5α	*E. coli* strain; *supE44* Δ*lacU169*(φ80 *lacZ*ΔM15) *recA1 hsdR17 thi-1 relA1*	Invitrogen
S17-1	*E. coli* strain; *recA pro hsdR* RP42Tc::Mu-Km::Tn7	(3)
Plasmids		
pEX18Gm	Allelic exchange suicide vector; *sacB*, GEN^R	(4)
pEX18Gm::Δ*rpoN*	pEX18Gm derivative for unmarked deletion of *rpoN*; GEN^R	This study
pMQ70	Expression plasmid with arabinose-inducible P_{BAD} promoter; CAR^R	(5)
pMQ70::*rpoN*	pMQ70 carrying *rpoN* from wildtype PA14; CAR^R	This study
pMQ70::$rpoN^{L343S}$	pMQ70 carrying PA14 *rpoN* with engineered L343S mutation; CAR^R	This study
pMQ70::$rpoN^{L419P}$	pMQ70 carrying PA14 *rpoN* with engineered L419P mutation; CAR^R	This study
pMQ70::$rpoN^{A449V}$	pMQ70 carrying PA14 *rpoN* with engineered A449V mutation; CAR^R	This study
pMQ70::*dctA*	pMQ70 carrying *dctA* from wildtype PA14; CAR^R	This study

Table S3. Oligonucleotides

Name	Sequence (5'→3')[a]	Purpose
*rpoN*delUF	TCAG**aagctt**GCCGAAATTCATCCTCCTCGA	pEX18Gm::Δ*rpoN* construction
*rpoN*delUR	TCAG**ctgcag**GGCTGAGGGCTTAGTACCTTATTCG	pEX18Gm::Δ*rpoN* construction
*rpoN*delDF	TCAG**ctgcag**TGACTGACTGACGTTGATCCACGCCAAGGT	pEX18Gm::Δ*rpoN* construction
*rpoN*delDR	TCAG**ggatcc**TTGTTCGAGTACGCGTTTCTTGCT	pEX18Gm::Δ*rpoN* construction
*rpoN*DxF	GAATTCCACATCCACCAC	Δ*rpoN* confirmation
*rpoN*DxR	GGCGATGCCATTGCCGAA	Δ*rpoN* confirmation
pMQ*rpoN*F	CATCAT**gagctc**gaaggagatatacatATGAAACCATCGCTAGTC	pMQ70::*rpoN* construction
pMQ*rpoN*R	CATCAT**aagctt**TCACACCAGTCGCTTGCGCTC	pMQ70::*rpoN* construction
L343S_F	CAACCAGT**C**GCAGGAAGC	pMQ70::*rpoN*^{L343S} construction
L343S_R	GCTTCCTGC**G**ACTGGTTG	pMQ70::*rpoN*^{L343S} construction
L419P_F	GGCATTTTCGAGC**C**GAAATATTTC	pMQ70::*rpoN*^{L419P} construction
L419P_R	GAAATATTTC**G**GCTCGAAAATGCC	pMQ70::*rpoN*^{L419P} construction
A449V_F	CTGGTGG**T**CGCGGAAAATGC	pMQ70::*rpoN*^{A449V} construction
A449V_R	GCATTTTCCGCG**A**CCACCAG	pMQ70::*rpoN*^{A449V} construction
pMQ*dctA*F	CATCAT**gagctc**gaaggagatatacatATGACCAAACAACCCTTC	pMQ70::*dctA* construction
pMQ*dctA*R	CATCAT**aagctt**CGAACAGGTTTGAGCTTAGAC	pMQ70::*dctA* construction

pMQseqF	GGACCAAAGCCATGACAAAA	pMQ70 derivative sequencing
pMQseqR	TTAATCTGTATCAGGCTG	pMQ70 derivative sequencing

[a] Restriction sites are bolded and in lowercase. Engineered ribosomal binding sites are underlined and in lowercase. Engineered point mutations are bolded and underlined.

References for supplementary material

1. Rahme L, Stevens E, Wolfort S, Shao J, Tompkins R, Ausubel F. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. Science (80-) 268:1899–1902.
2. Wong A, Rodrigue N, Kassen R. 2012. Genomics of Adaptation during Experimental Evolution of the Opportunistic Pathogen Pseudomonas aeruginosa. PLoS Genet 8:e1002928.
3. Simon R, Priefer U, Pühler A. 1983. A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. Bio/Technology 1:784–791.
4. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. Gene 212:77–86.
5. Shanks RMQ, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. 2006. Saccharomyces cerevisiae-based molecular tool kit for manipulation of genes from gram-negative bacteria. Appl Environ Microbiol 72:5027–36.