Supplemental materials.

Table S1. Summary of mutations identified by targeted sequencing survey of *P. aeruginosa* mutants selected on LpxC inhibitors.

Strain used	Mutation or protein alterations identified	selection
K767	NfxB$_{T51P}$	4xMIC comp. 2
	fabG C403T (2 isolates)	2xMIC comp. 2
	NfxB$_{T51P}$	"
	mexR T insertion at 81bp, frameshift	"
PAO1V	C to A, 11bp upstream of lpxC start site	8xMIC comp. 2
	nfxB C del frameshift 357-359bp (5 isolates)	4xMIC comp. 2
	fabG C494T	2xMIC comp. 2
	mexR G deletion frameshift at 300-304	"
	mexR T170G	"
K767*	[a]LpxC$_{A214V}$	8xMIC comp. 3
	nfxB, 12 bp deletion from 147-158	4xMIC comp. 3
PAO1V	[b]LpxC$_{A214V}$	4xMIC comp. 3
	mexR G deletion, frameshift G run at 300-304	"
	nfxB C del frameshift 357-359bp	"
	nfxB C277T (early stop codon)	"
PAO1V	LpxC$_{A214V}$ (2 isolates)	8xMIC comp. 4
K2153	LpxC$_{A214V}$	8 and 4xMIC comp. 4
	mexS (5 of nine isolates, not shown)	4xMIC compound 4

*putative *mexR* and *fabG* mutants were also seen based on phenotypes (selected on 2X MIC); [a]the mutant with this alteration is K767-CDJ0037 (Table 1); [b]the mutant with this alteration is PAO1V-CDJ0042 (Table 1).

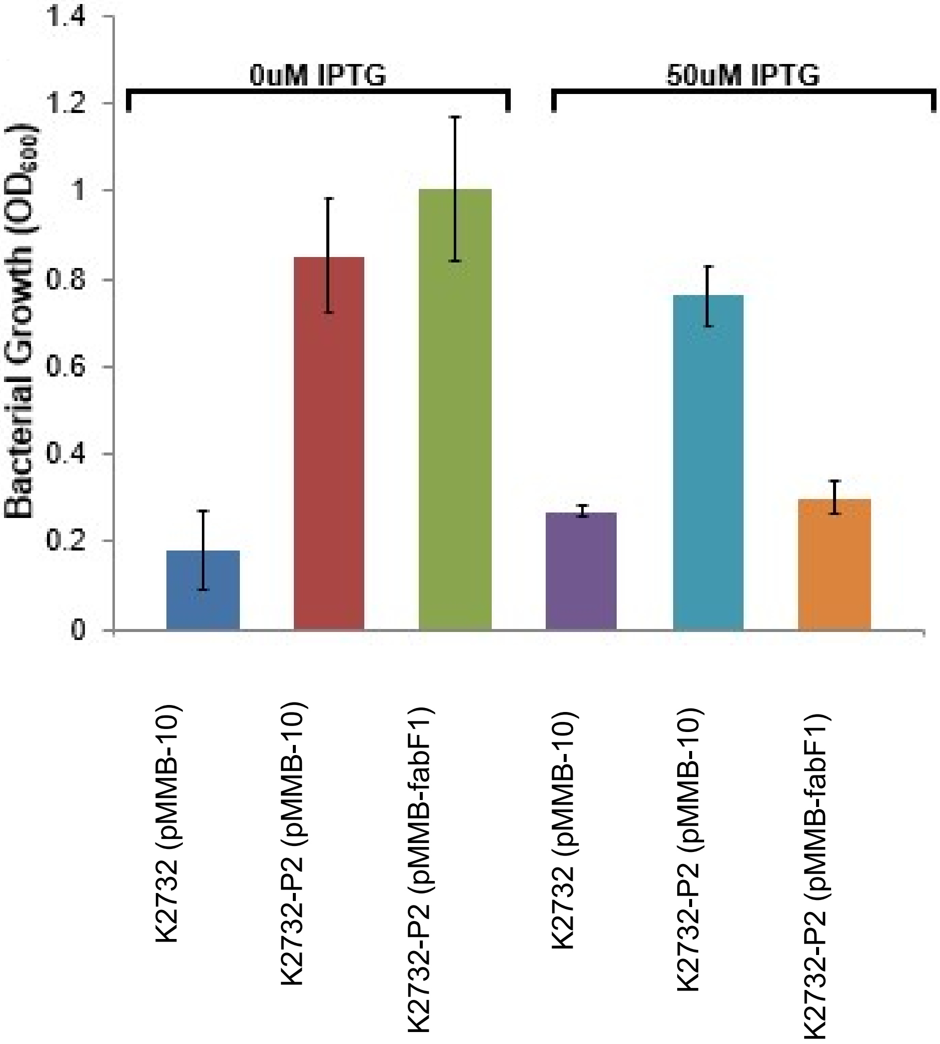

Figure S1. Growth of PAO1 strain K2732 (parent) or K2732-P2 (FabF1 $_{T306A}$) strains harboring either an empty tightly regulated IPTG inducible vector (pMMB-10) or vector with wild type *fabF1* (pMMB-FabF1) in 1 µg/mL CHIR-090 for 17 hours. *P. aeruginosa* mutant K2732-P2 grows better in CHIR-090 than the parent strain (compare dark blue and red bars or purple and light blue bars). Complementation of the *fabF1* mutation by induction of wild type FabF1 (orange bar) restored susceptibility (compared green and orange bars).

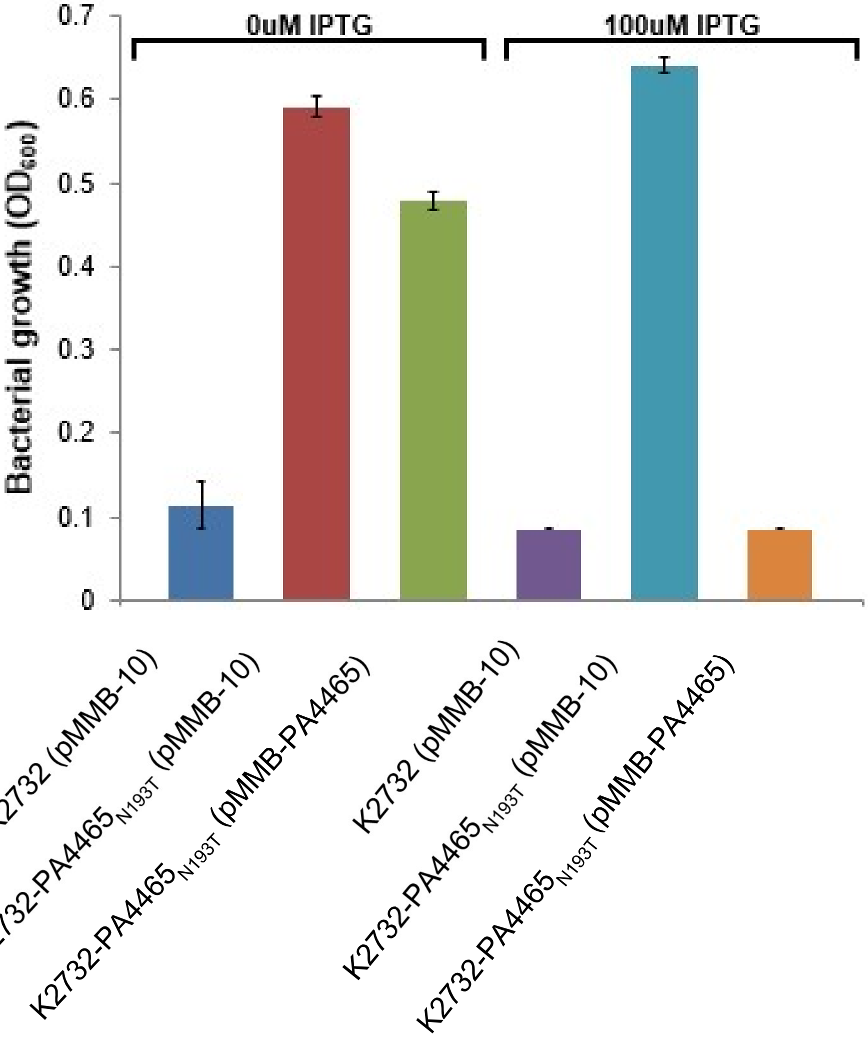

Figure S2. Growth of K2732 (parent) or K2732-PA4465$_{N193T}$ (engineered) strains harboring either an empty tightly regulated IPTG inducible vector (pMMB-10) or vector with wild type PA4465 (pMMB-PA4465) in 0.5 µg/mL CHIR-090 for 17 hours. *P. aeruginosa* mutant K2732-PA4465$_{N193T}$ grows better in CHIR-090 than the parent strain (compare dark blue and red bars or purple and light blue bars). Complementation of the PA4465 mutation by induction of wild type PA4465 (orange bar) restored susceptibility (compare green and orange bars).