Table S1. Strains, plasmids, and primers used in this study.

Strain name	Strain description	Ref. no.	Source / Reference
P. aeruginosa			
PAO1 WT	Wild-type	DH1856	(1)
PAO1 Δ*anr*	In-frame deletion of *anr*	DH2399	This study
PAO1-R1	PAO1 *lasR*:Tc	DH2400	(2)
PA14 WT	Wild-type	DH122	(3)
PA14 Δ*anr*	In-frame deletion of *anr*	DH1977	L. Dietrich
PA14 Δ*lasR*	In-frame deletion of *lasR*	DH164	(4)
PA14 Δ*lasR* Δ*anr*	In-frame deletions of *lasR*, *anr*	DH2401	This study
PA14 Δ*lasR rhlR:*Tc	In-frame deletion of *lasR*; gene replacement of *rhlR*	DH237	(5)
PA14 Δ*lasR rhlR:*Tc Δ*anr*	In-frame deletion of *anr* in DH237	DH2402	This study
PA14 Δ*lasI*	In-frame deletion of *lasI*	DH132	(4)
J215	CF clinical isolate	DH2403	This study
J215 Δ*anr*	In-frame deletion of *anr*	DH2404	This study
J215 Δ*anr*+*anr*	Complementation of *anr* at native locus	DH2405	This study
J215 Δ*cgrC*	In-frame deletion of *cgrC*	DH2406	This study
J215 Δ*cupA2*	In-frame deletion of *cupA2*	DH2407	This study
J215 ΔPA2133	In-frame deletion of PA2133	DH2408	This study
J215 Δ*dnr*	In-frame deletion of *dnr*	DH2409	This study
J215 Δ*anr* Δ*antA*	In-frame deletion of *antA* in DH2404	DH2410	This study
J215 ΔPhrS	In-frame deletion of PhrS	DH2411	This study
J215 *pqsA*:Tn*Mar*	Mariner transposon inserted into *pqsA*	DH2412	This study
J215 *pqsB*:Tn*Mar*	Mariner transposon inserted into *pqsB*	DH2413	This study
J215 *pqsH*:Tn*Mar*	Mariner transposon inserted into *pqsH*	DH2414	This study
NC-AMT0101-1	CF clinical isolate with frame-shift mutation in *lasR*	DH2415	(6)
NC-AMT0101-1 Δ*anr*	In-frame deletion of *anr* in DH2415	DH2416	This study
NC-AMT0101-2	CF clinical isolate. Parent of NC-AMT0101-1	DH2417	(6)
NC-AMT0101-2 Δ*anr*	In-frame deletion of *anr* in DH2417	DH2418	This study

AMT0047-3	CF clinical isolate with substitution in *lasR* resulting in STOP	DH1132	(6)
AMT0047-2	CF clinical isolate. Parent of AMT0047-3	DH1133	(6)
E. coli			
	SM10 λ*pir*	DH2419	
S. aureus			
	8325-4	DH2420	
Plasmids			
pMQ30	Shuttle vector for yeast cloning and Gram-negative allelic replacement; Gm^R		(7)
*anr*_del_pMQ30	For deleting *anr*; Gm^R		This study
*anr*_KON_pMQ30	For inserting *anr* at the native locus; Gm^R		This study
pEXG2-Δ*cupA2*	For deleting *cupA2*; Gm^R		S. Dove
pEXG2-Δ*cgrC*	For deleting *cgrC*; Gm^R		(1)
*dnr*_del_pMQ30	For deleting *dnr*; Gm^R		(8)
pEXG2-ΔPA2133	For deleting PA2133; Gm^R		S. Dove
pMQ70	Shuttle vector for yeast cloning and for arabinose-inducible gene expression; Amp^R		(7)
cgrABC-pMQ70	For expressing *cgrABC*; Amp^R		This study
pMQ123	Shuttle vector for yeast cloning and for IPTG-inducible gene expression; Amp^R		(7)
anr-pMQ123	For over-expressing *anr*; Amp^R		(8)
anr D149A-pMQ123	For over-expressing oxygen insensitive *anr allele* D149A; Amp^R		This study
pBT20	Vector carrying *mariner* transposon; Ap^R Gm^R (marker on transposon)		(9)
pMQ72	Shuttle vector for yeast cloning and for arabinose-inducible gene expression; Gm^R		(7)
gcbC E429R-pMQ72	For over-expressing hyperactive allele of DGC *gcbC*; Gm^R		G.A O'Toole
gcbC R366E-pMQ72	For over-expressing hyperactive allele of DGC *gcbC*; Gm^R		G.A O'Toole

Primers	**Primer sequence (5’-3’)**[a]
*lasI*_Seq_1	GCTCCAGAAAGTTTCCTGGCTTTCCC
*lasI*_Seq_2	GAGTCCGGAGCGGGTCGGAC
*lasR*_Seq_1	CAAACGCTGCGGTCTATTGTTAAGTG
*lasR*_Seq_2	CAGTCGTTTCGAGAATGGCGAGAAC
*rhlI*_Seq_1	CTGGCTGCCGCCTACGCC
*rhlI*_Seq_2	GCGGCCAAATCCCGGAATGCAG
*rhlIR*_Seq_1	CAACCGCACAGTATCGCTTGCG
*rhlR*_Seq_2	CTCTCAGTCGGAGGACATACCAGC
*anr*_KO_pMQ30_1	tgcgttctgatttaatctgtatcaggctgaTGTTCATGAACTGGGTCATGAA GGGTTGGC
*anr*_KO_pMQ30_2	GTGCCTTTAACCTAGCAAGGACCCCTCAAGCGCCTGCGAA CCGCCAAC
*anr*_KO_pMQ30_3	GTTGGCGGTTCGCAGGCGCTTGAGGGGTCCTTGCTAGGTT AAAGGCAC
*anr*_KO_pMQ30_4	tgagcggataacaatttcacacaggaaacagctatgTCTGCCACTTCGAACT GGCCTTCG
pMQ70_*cgrABC*_1	tctgtatcaggctgaaaatcttctctcatccgccCTACTCCCAGGAGCGGGA ATACACG
pMQ70_*cgrABC*_2	cgttttttgggctagcccaaggaagcacaaccATGGCAGGCAAGCATTAC CAGGATGC
*anr*_pMQ123_1	ccgccaaaacagccaagcttgcatgcctgcagactagtTCAGCCTTCCAGCT GGCCGCC
*anr*_pMQ123_2	ctagcgaattcgagctcggtacccggggaaggagatatacatATGGCCGAAAC CATCAAG
*antA*_KO_pMQ30_1	agaccgcttctgcgttctgatttaatctgtatcaggctgaCAGCAGGTAGTGCG GCGGGC
*antA*_KO_pMQ30_2	GCGGTACTGCAGTCCGGCGTGCGTTCTCACCCTTGTGCGT TGTC
*antA*_KO_pMQ30_3	GACAACGCACAAGGGTGAGAACGCACGCCGGACTGCAGTA CCGC
*antA*_KO_pMQ30_4	cacaggaaacagctatgggcggtcgagcagttcctgggtcTGCGGATTCGGC CGGTTGGC

PhrS_KO_pMQ30_1	agaccgcttctgcgttctgatttaatctgtatcaggctgaCCTGCATCGACGCCG GATCG
PhrS_KO_pMQ30_2	GCGCCTCGCTAATCTGAGCCCGCGCCTGGAAAAACACCGG CG
PhrS_KO_pMQ30_3	CGCCGGTGTTTTTCCAGGCGCGGGCTCAGATTAGCGAGGC GC
PhrS_KO_pMQ30_4	ttgtgagcggataacaatttcacacaggaaacagctatgCGGCCTTGCGGCG TAGTAACC

[a] In primer sequences, upper case letters indicate *Pseudomonas*-specific genomic sequence, and lower case letters indicate sequence identity to the cloning vector.

Table S2. Mutations in quorum sensing-related genes in J215.
From cycle sequencing.

Gene	Sequence Difference from PAO1
lasR	
	Nonsynonymous mutation 588G →T (**E196D**) [a]
lasI	
	Synonymous mutation 432 C→T
rhlR	
	Synonymous mutation 147 C → T
	Synonymous mutation 426 C → T
	Synonymous mutation 453 C → A
	Synonymous mutation 591 T → C
	Synonymous mutation 717 T → C
rhlI	
	Synonymous mutation 105 C → T
	Synonymous mutation 138 A → G
	Nonsynonymous mutation 184 A → G (**S62G**)
	Synonymous mutation 196 C → T
	Synonymous mutation 207 C → T
	Synonymous mutation 217 C → T
	Nonsynonymous mutation 249 C → A (**D82E**)
	Synonymous mutation 282 G → C
	Synonymous mutation 312 C → T
	Synonymous mutation 342 G → A

[a] Indicates a previously identified loss-of-function mutation (10).

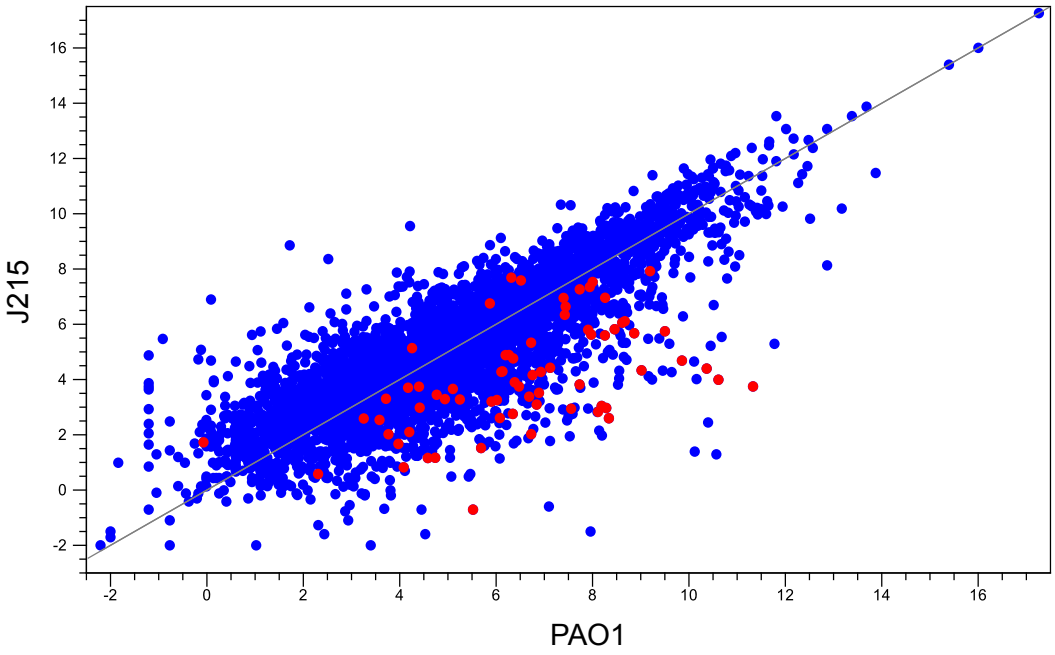

FIG S1. Expression of LasR-controlled genes is reduced in J215 compared with PAO1. Scatterplot of the number of reads for each *P. aeruginosa* transcript in J215 and PAO1. Reads were quantile normalized and log2 transformed. Genes directly regulated by LasR as determined through LasR-ChIP experiments (11) are marked in red.

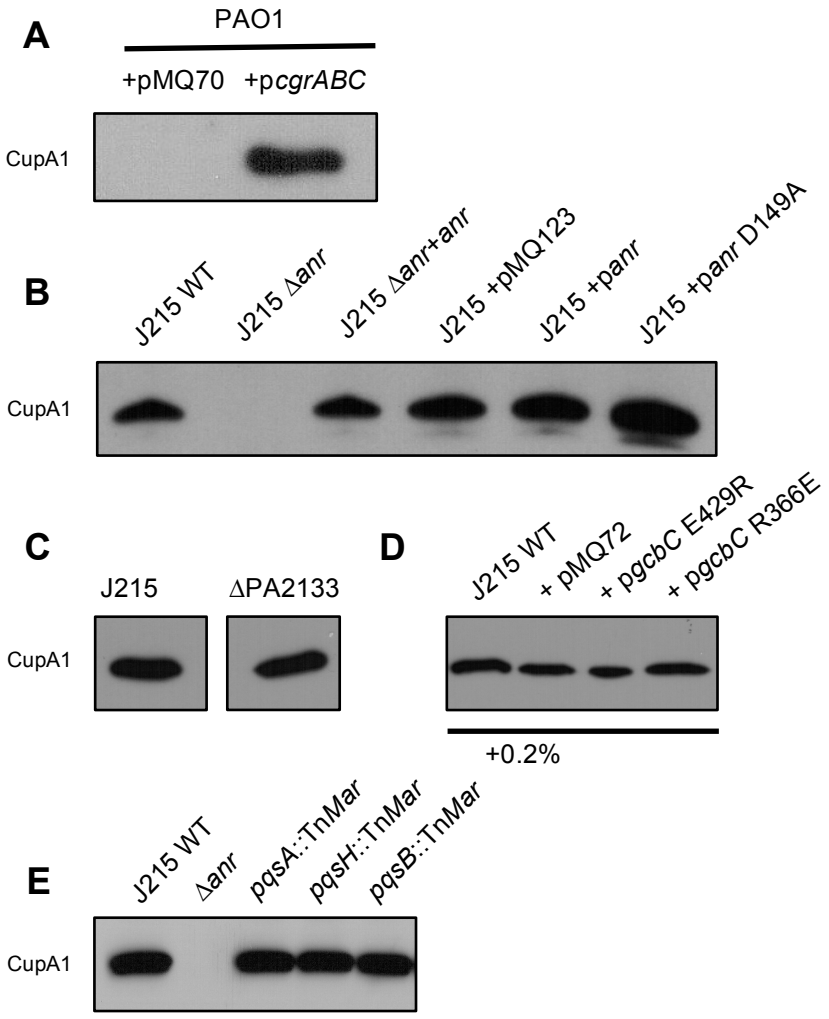

FIG S2. Measurement of CupA1 production in PAO1 and J215. Western blots of whole cell lysates from colony biofilms (A,E, : 3 days, B-D : 24 hours) grown in 1% O_2 (A, C-E) or 21% O_2 (B) on T-broth agar. **(A)** Production of fimbriae by PAO1 transformed with either a plasmid to overexpress *cgrABC* or with vector control pMQ70. **(B)** J215 transformed with constructs to express either wild-type *anr* or *anr* D149A, or with pMQ123 vector control. **(C)** Wild-type J215 and a phosphodiesterase PA2133 mutant. **(D)** J215 transformed with plasmids to express constitutively active alleles of *gcbC* from *Pseudomonas fluorescens* or with vector control pMQ72. Lane 3 showed less protein loading by coomassie stain. **(E)** J215 wild-type and Δ*anr* compared with mutants in the HHQ/PQS biosynthetic pathway.

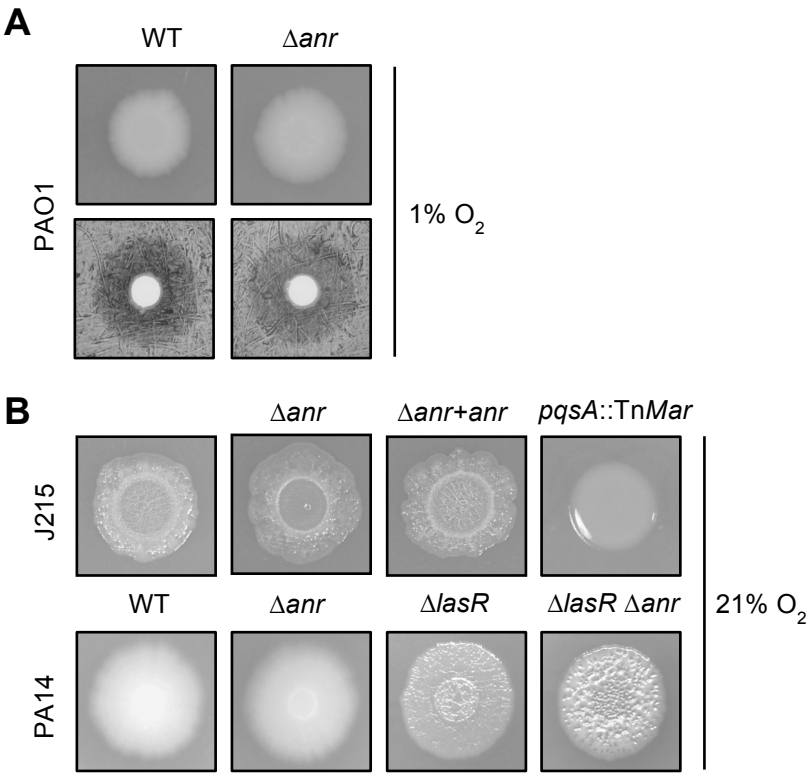

FIG S3. Effects of Anr on HAQ production. (A) Colony sheen and *S. aureus* inhibition by PAO1 and Δ*anr* mutant. **(B)** HHQ sheen formed by Δ*anr* mutants in either J215 or PA14 Δ*lasR* background in 21% O_2.

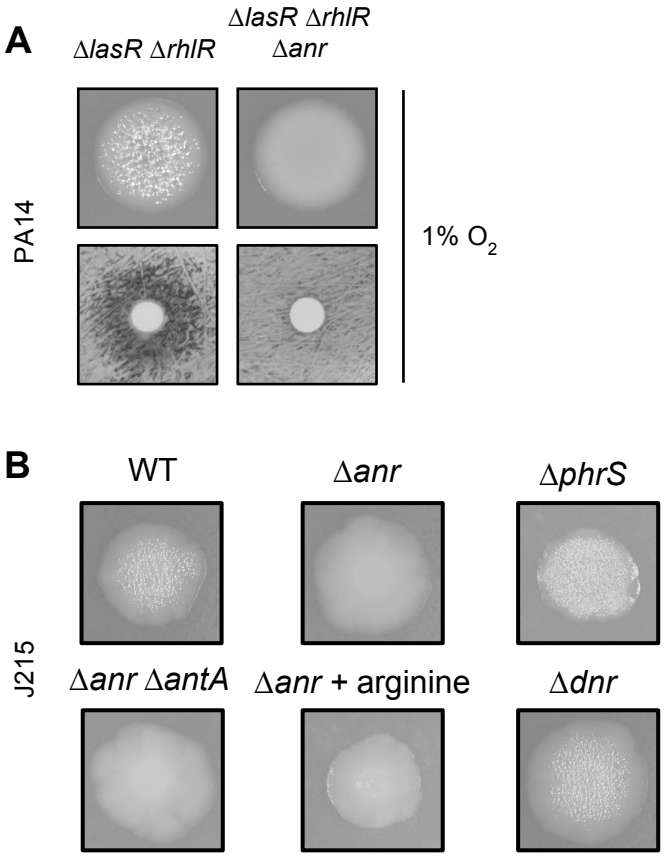

FIG S4. The Δ*anr* HAQ defect is not due to the activity of RhlR, Dnr, PhrS, or AntA, and is not rescued by excess arginine. (A) Colony morphology and *S. aureus* inhibition by PA14 Δ*lasR* Δ*rhlR* and Δ*lasR* Δ*rhlR* Δ*anr*. **(B)** J215 mutant colony morphologies. Arginine was supplied in the media at a concentration of 0.4% in the indicated panel.

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