

Supplemental Material for:

***Pseudomonas aeruginosa* high-level resistance to polymyxin and other antimicrobial peptides requires *cprA*, a gene that is disrupted in the PAO1 strain**

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Table S1. Oligonucleotide primers used in this study

Primer name	Primer no	Sequence (5'-3')
For deletion constructs		
$\Delta cprA$ 5'outflank (F) attB1	SM 1692	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAATACCTCACCGAACAGGC
$\Delta cprA$ 3'outflank (R) attB2	SM 1694	GGGGACCACTTTGTACAAGAAAGCTGGGTGCTTCCATCCACGGTTATAC
$\Delta cprA$ 5' delete (R)	SM 1695	CCTGCCGTCACCTGAAGTC <u><i>AAGCTT</i></u> ATGCATGTTTCATGTCTTCC
$\Delta cprA$ 3' delete (F)	SM 1693	GGAAGACATGAACATGCAT <u><i>AAGCTT</i></u> GACTTCAAGTGACGGCAGG
$\Delta cprA$ confirmation (R)	SM 1684	AGTAGCTGGTGGCGAACAGT
$\Delta cprA$ confirmation (F)	SM 1685	TCATCGGTATCTGGCTGAACT
For sequencing internal <i>cprA</i> region		
<i>cprA</i> (R)	SM 1842	CGCGTCCAAGGCGGAAATCGA
<i>cprA</i> (F)	SM 1843	CGGCGGAGATATGATAGAGGCT
<i>cprA</i> (R)	SM 1844	AGGTGGAGCGGGTGATCAATT
For <i>in trans cprA</i> complementation		
<i>cprA</i> (F)	SM 1789	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTGAGGGGGGAAGACATGAAC
<i>cprA</i> (R)	SM 1790	GGGGACCACTTTGTACAAGAAAGCTGGGTTCGCGCCTGCCGTCACCTTGAA
For qPCR		
<i>cprA</i> (F)	SM 1870	ACTGAAGCGTTTCTCCAC
<i>cprA</i> (R)	SM 1871	CTTGGACGCGGTGTAGTC
<i>arnC</i> (F)	SM 1892	TGATCCTCAACCGCAACTAC
<i>arnC</i> (R)	SM 1893	GTTCTGCAGGTCGGCAT
PA4268 ORF (F)	SM 1872	AACTCGGCACTGCGTAAG
PA4268 ORF (R)	SM 1873	TGTGCTCTTGCAGGTTGT
For <i>cprA</i> allelic replacement		
$\Delta cprA$ 5'outflank (F) attB1	SM 1692	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAATACCTCACCGAACAGGC
$\Delta cprA$ 3'outflank (R) attB2	SM 1694	GGGGACCACTTTGTACAAGAAAGCTGGGTGCTTCCATCCACGGTTATAC
QuikChange F	SM 1968	CCGGCAGCATCTTCTGGGTCTTCCGCATGGGCTTC
QuikChange R	SM 1969	GAAGCCCATGCGGAAGACCCAGAAGATGCTGCCGG
<i>cprA</i> (R)	SM 1698	GGTCAACTGGACATTGTTTC
<i>cprA</i> (F)	SM 1701	GCGCAGTTGCTCGACTGCT
<i>cprA</i> (F)	SM 1686	CACCCTGATTCTCGCCCAT
<i>cprA</i> (F)	SM 1699	TCATCTCGGTGATGTTGCT
<i>cprA</i> (R)	SM 1842	CGCGTCCAAGGCGGAAATCGA
<i>cprA</i> (F)	SM 1843	CGGCGGAGATATGATAGAGGCT
<i>cprA</i> (R)	SM 1844	AGGTGGAGCGGGTGATCAATT

Underlined and italic HindIII cutting site. In bold attB1 and attB2 sequence.

Table S2. Amino acid changes in CprA and corresponding *cprA* allele designations for 9 newly sequenced and 22 publicly available *P. aeruginosa* genomes and one *P. entomophila* genome.

Strain	Database	Source of strain or data	Allele designation	Amino acid change relative to WT
<i>P. aeruginosa</i>				
1020 (CF isolate)	--	(1)	<i>cprA</i> ⁺	--
1603 (CF isolate)	--	(1)	<i>cprA</i> ⁺	--
1016 (CF isolate)	--	(1)	<i>cprA</i> ⁺	--
1611 (CF isolate)	--	(1)	<i>cprA</i> ⁺	--
2192	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
39016	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
PACS2	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
RP73	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
DK2	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
M18	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
LESB58	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
B136-33	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
NCGM2.S1	Pseudomonas.com	(2)	<i>cprA</i> ⁺	--
SCV20265	NCBI	(3)	<i>cprA</i> ⁺	--
LES431	NCBI	(3)	<i>cprA</i> ⁺	--
PA1	NCBI	(3)	<i>cprA</i> ⁺	--
PA1R	NCBI	(3)	<i>cprA</i> ⁺	--
1027 (PAO1 Lory)	--	(4)	<i>cprA1</i>	frameshift [V224S...V244X]
1555 (PAO1 Franklin)	--	(5)	<i>cprA1</i>	frameshift [V224S...V244X]
2114 (PAO1 Manoil)	--	(6)	<i>cprA1</i>	frameshift [V224S...V244X]
2178 (PAO1 Vasil)	--	(7)	<i>cprA1</i>	frameshift [V224S...V244X]
PAO1	Pseudomonas.com	(2, 4)	<i>cprA1</i>	frameshift [V224S...V244X]
PAO1-VE2	NCBI	(3)	<i>cprA1</i>	frameshift [V224S...V244X]
PAO1-VE13	NCBI	(3)	<i>cprA1</i>	frameshift [V224S...V244X]
PAO581	NCBI	(3)	<i>cprA1</i>	frameshift [V224S...V244X]
1026 (PAK)	--	(8)	<i>cprA2</i>	E192Q, G196D
MTB-1	NCBI	(3)	<i>cprA2</i>	E192Q, G196D
UCBPP_PA14	Pseudomonas.com	(2)	unassigned	A33S, A73T
C3719	Pseudomonas.com	(2)	unassigned	S347N
PA7	Pseudomonas.com	(2)	unassigned	I13T, Q35R, A39D, D71A, A73I, C75Y, L76M, R79G, A80T, L84I, P99L, D135E, E160Q, D239E, C262R, R275H, E298G, E304D, S355A, A356P
<i>P. entomophila</i>				
L48	Pseudomonas.com	(2)	unassigned	M3S, A5V, D6T, E8A, Q9P, I10H, T11A, A12L, I13H, D14Q, T15L, R16P, A33T, A39D, H41Q, G42A, S46C, G63D, M67Q, D71S, E72Q, C75N, R79G, A80E, L84I, S92T, A95P, E105D, D128E, F131Y, D135E, L137M, Q158E, A171E, E193Q, G196S, T212S, C262Q, S266D, R275G, A276S, G281A, R289L, A294Q, E304D, E310S, K313S, S317T, S355A, A356V, Q369R, A373G, P377A, A378T

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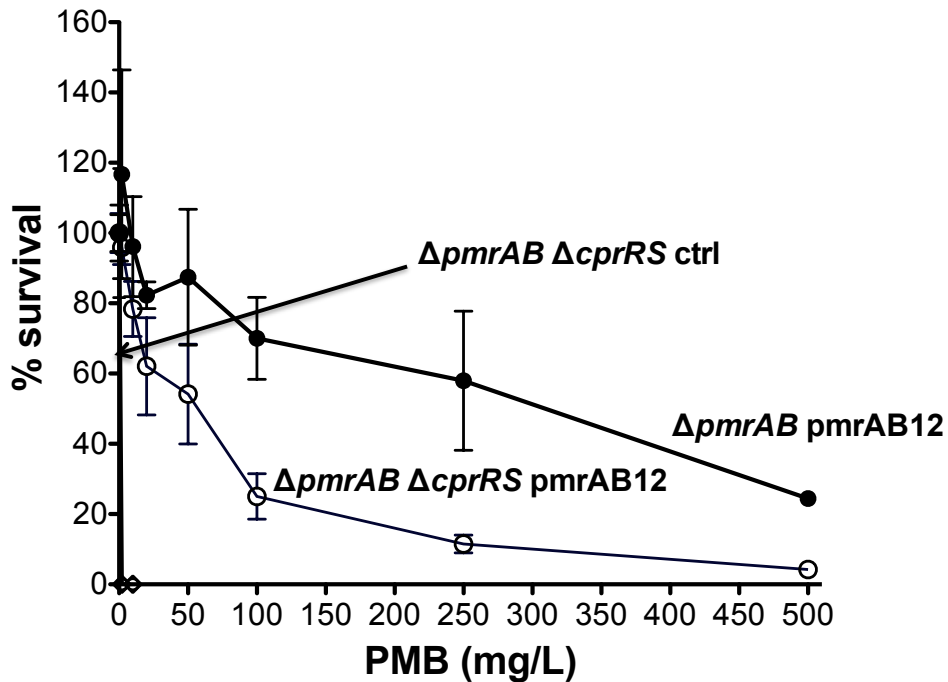


Figure S1. Effect of *cprRS* deletion on Pm resistance in a PAK $\Delta pmrAB$ strain over-expressing *pmrAB12*. Here and in Fig. S3, Pm resistance experiments were performed twice; if discrepancies were seen, the experiment was performed a third time. Each panel shows a representative experiment, with results expressed as means of three technical replicates. Error bars are \pm SD. The non-italicized allele name indicates the presence of an episomal version, i.e., an inducible expression strain; “ctrl” signifies empty vector control. PMB plate assay of strain 4232 ($\Delta pmrAB \Delta cprRS pmrAB12$) induced with 0.1% L-Ara for 24 h, with strain 2735 ($\Delta pmrAB pmrAB12$) as a positive control and strain 4234 ($\Delta pmrAB \Delta cprRS$ ctrl) as a negative control.

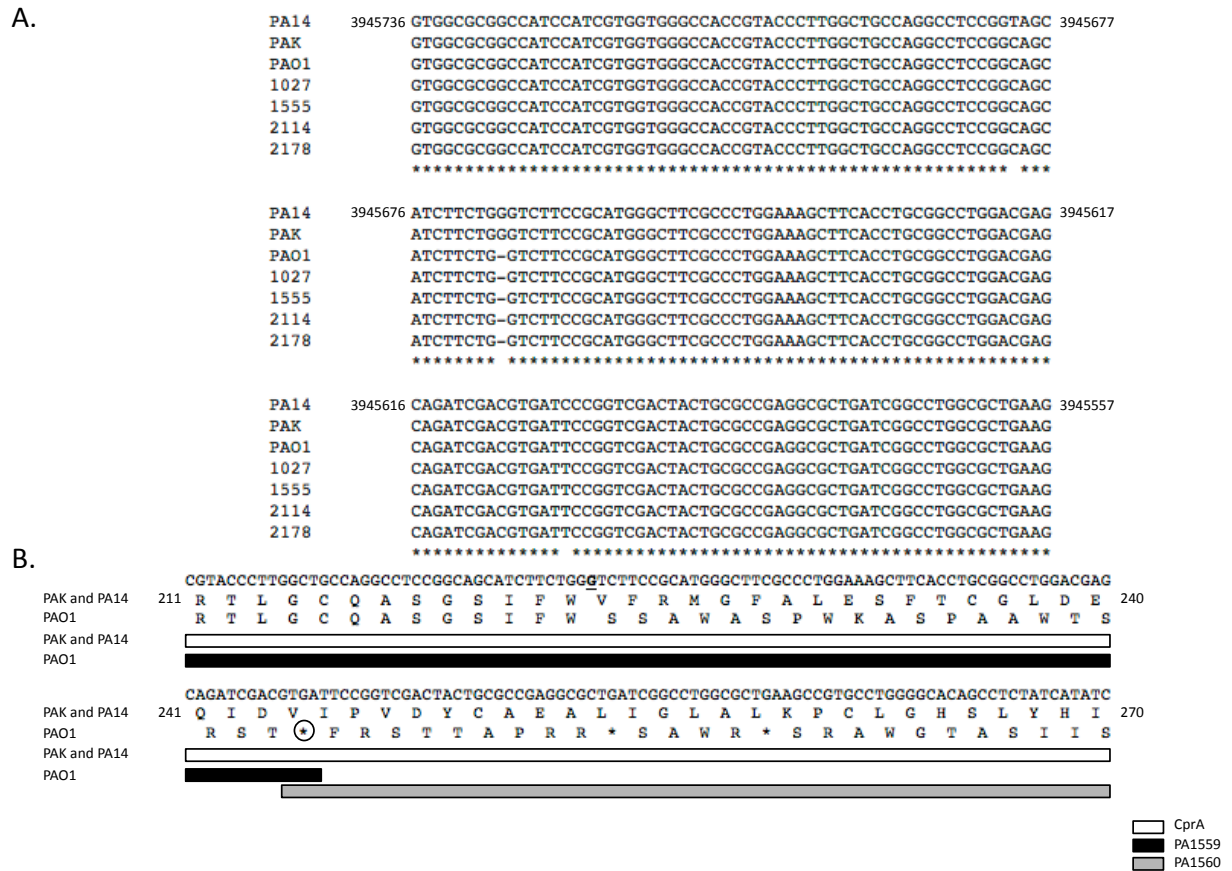


Figure S2. (A) Sequence alignment of the *cprA1* allele from the published genome of PAO1 strain (“PAO1”) and additional re-sequenced *cprA1* alleles of PAO1 substrains obtained from multiple laboratories (“1027”, S. Lory; “1555”, M. Franklin; “2114”, C. Manoil; “2178”, M. Vasil), with the *cprA* allele from the PA14_44311 strain and the *cprA2* allele from the PAK strain (“PAK”) as the reference sequence. A guanine nucleotide at position 3945668 of the PA14 genomic sequence is missing in all of the PAO1 strain backgrounds. (B) Diagram illustrating the consequence of the frameshift mutation in the *cprA1* allele of the PAO1 strain, resulting in tandem ORFs that have been annotated in the PAO1 genome as PA1559 and PA1560.

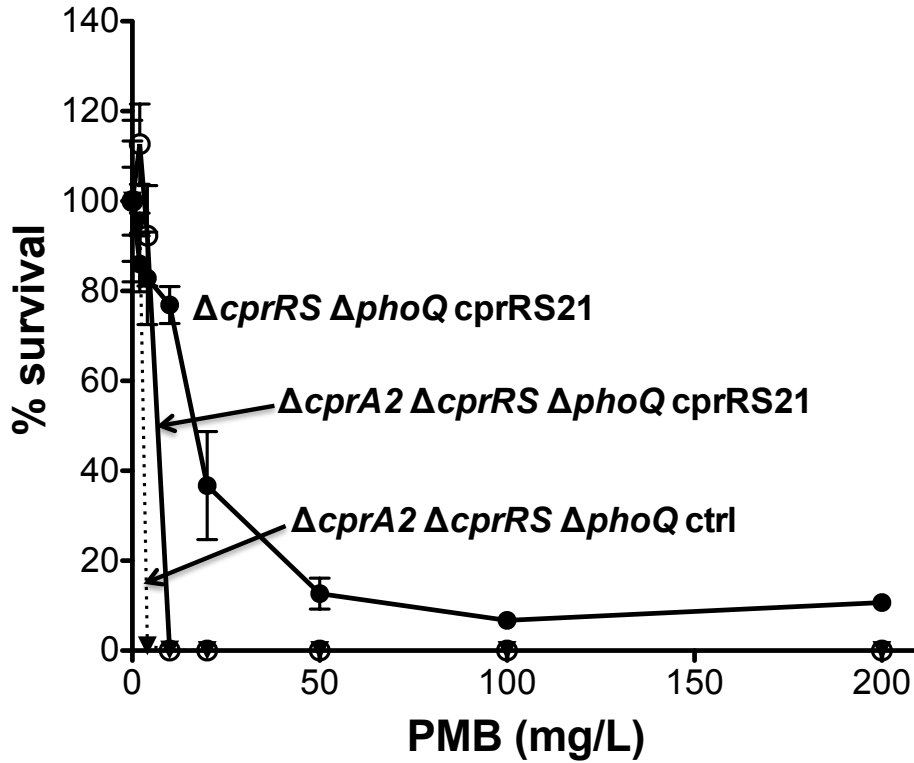


Figure S3. Effect of *cprA2* deletion on Pm resistance of the PAK $\Delta cprRS \Delta phoQ cprRS21$ strain. PMB plate assay data of strain 4608 ($\Delta cprA2 \Delta cprRS \Delta phoQ cprRS21$), induced with 0.1% L-Ara for 24 h, with strain 4200 ($\Delta cprRS \Delta phoQ cprRS21$) as a positive control and strain 4612 ($\Delta cprA2 \Delta cprRS \Delta phoQ ctrl$) as a negative control; the last of these had Pm susceptibility similar to the PAK parental strain (not shown).

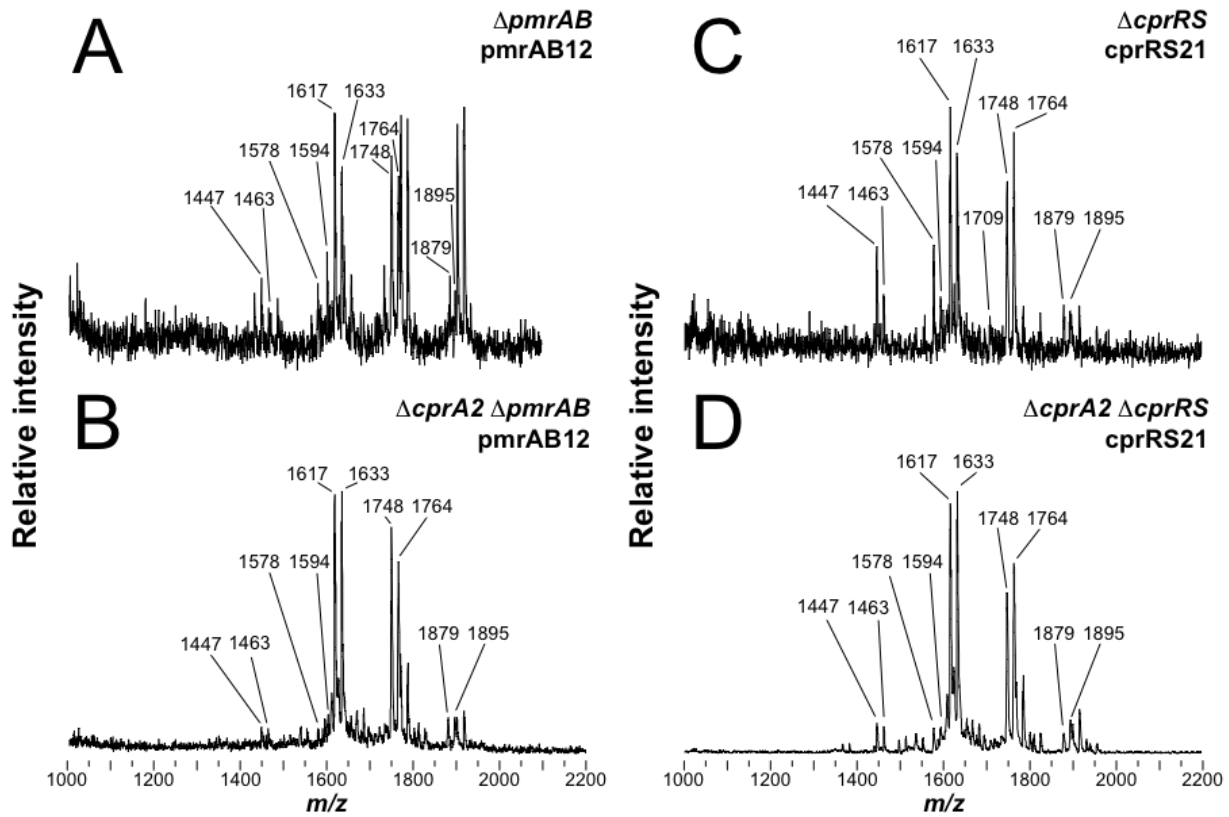


Figure S4. MALDI-TOF spectra of lipid A isolated from PAK strain background. (A) Strain 2735 ($\Delta pmrAB$ *pmrAB12*); (B) strain 4519 ($\Delta cprA2$ $\Delta pmrAB$ *pmrAB12*); (C) strain 4240 ($\Delta cprRS$ *cprRS21*); and (D) strain 4425 ($\Delta cprA2$ $\Delta cprRS$ *cprRS21*). The non-italicized allele name indicates the presence of an episomal version, i.e., an inducible expression strain.