

1 **On-line Supplement for:**

2

3 **PhoQ mutations promote lipid A modification and polymyxin resistance of**

4 ***Pseudomonas aeruginosa* found in colistin-treated cystic fibrosis patients**

5

6 Amanda K. Miller, Mark K. Brannon, Laurel Stevens, Helle Krogh Johansen, Sara E. Selgrade,

7 Samuel I. Miller, Niels Høiby, and Samuel M. Moskowitz*

8

9 ***Corresponding author:** Division of Pediatric Pulmonary Medicine

10 Massachusetts General Hospital

11 175 Cambridge Street

12 Boston, Massachusetts 02114

13 Telephone: 617-643-7232; Fax: 617-643-7234

14 Email: smoskowitz@partners.org

15

16

16

17 **Table S1.** Oligonucleotide primers for sequencing.

Primer name	Primer no.	Sequence (5' to 3')
pDONR201 attL1 (F)	SM877	TCGCGTTAACGCTAGCATGGATCTC
pDONR201 attL2 (R)	SM878	GTAACATCAGAGATTTTGAGACAC
in <i>phoPQ</i> – 1 (F)	SM1010	CTGGAAGCGCGCCTGAACG
in <i>phoPQ</i> – 2 (F)	SM1011	GGCGGTGCTGTTCATGCTGG
in <i>phoPQ</i> – 3 (F)	SM1012	GCAGCTCTACCTGTGGCTCG
in <i>phoPQ</i> – 4 (F)	SM1013	TGCGACGCGCTGGACAAGG
in <i>phoPQ</i> – 1 (R)	SM1014	GATGGTCGACTGGACGAACC
in <i>phoPQ</i> – 2 (R)	SM1015	TCCAGGGCGATGCCGAAGG
in <i>phoPQ</i> – 3 (R)	SM1016	CCCCAGGTCAGACCCAGCC
in <i>phoPQ</i> – 4 (R)	SM1017	TGGACGGCGAGAAGTCCCG

18

Table S2. Oligonucleotide primers for deletion constructs.

Primer name	Primer no.	Sequence (5' to 3')
<i>ΔphoPQ</i> - 5' flanking	SM936	GGAACAGGCAGATCACGAGAA
<i>ΔphoPQ</i> - 5' delete (F) + attB1	SM937	GGGGACAAGTTTGTACAAAAAAGC AGGCTGACTGTAGAAAGCCTAGACCC
<i>ΔphoPQ</i> - 5' delete (R)	SM938	GTCTCAGACTGTAGCGAAACGAAGC TTTACCAGCAGTTTCATGAG
<i>ΔphoPQ</i> - 3' delete (F)	SM939	CTCATGAAACTGCTGGTAAAGCTTC GTTTCGCTACAGTCTGAGAC
<i>ΔphoPQ</i> - 3' delete (R) + attB2	SM940	GGGGACCACTTTGTACAAGAAAGC TGGGTAGCACGGCGATGAAACAGGTG
<i>ΔphoPQ</i> - 3' flanking	SM941	GCGGGAATGAGGGTCAAGGC
<i>ΔphoPQ</i> - 5' delete (F)	SS97	GGCTAGTCTAGACAGATCACGAGAAACAGG
<i>ΔphoPQ</i> - 5' delete (R)	SS99	GAACGGCCGCCAAGTCTCAGGATCCCATGAG GTTCTCCGGATG
<i>ΔphoPQ</i> - 3' delete (F)	SS98	CATCCGGAGGAACCTCATGGGATCCTGAGAC TTGGCGGCCGTTT
<i>ΔphoPQ</i> - 3' delete (R)	SS93	CTCCAAGCTTCATGATCACCGAGACCAG
<i>ΔphoQ</i> - 5' flanking	SM942	ATGTCGGCTACGCTGCCGGG
<i>ΔphoQ</i> - 5' delete (F) + attB1	SM943	GGGGACAAGTTTGTACAAAAAAGC AGGCTAGAATGCCTCGATCGAAGGCG
<i>ΔphoQ</i> - 5' delete (R)	SM944	GTCTCAGACTGTAGCGAAACGAA GCTTCAGGGAACGGATCACCGG
<i>ΔphoQ</i> - 3' delete (F)	SM945	CCGGTGATCCGTTCCCTGAAGCTTC GTTTCGCTACAGTCTGAGAC
<i>ΔphoQ</i> - 3' delete (R) + attB2	SM946	GGGGACCACTTTGTACAAGAAAGC TGGGTCCGGGAATGAGGGTCAAGGCC
<i>ΔphoQ</i> - 3' flanking	SM947	CGGCTGCGCTCCATGATCAC

Table S3. Oligonucleotide primers for expression constructs.

Primer name*	Primer no.	Sequence (5' to 3')
<i>phoPQ</i> (F) + attB1	SM855	GGGGACAAGTTTGTACAAAAAAGC AGGCTCTCATCCGGAGGAACCTCATGAAA
<i>phoPQ</i> (R) + attB2	SM856	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCTCAGACTGTAGCGAAACGTAT
<i>phoP</i> (R) + attB2	SM1022	GGGGACCACTTTGTACAAGAAAGC TGGGTTCGGATCACCGGCAGCGCTCGG
<i>phoPQ11</i> (R) + attB2	SM1023	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCTCAGTCCAGCGCCACGGCCAGGCC
<i>phoPQ12</i> (R) + attB2	SM1024	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCTCAGTCCAGGCTCAGTTCGCCGTC
<i>phoP</i> ⁺ <i>phoQ24</i> SOE 5' (R)	SM1036	CCGCGCACCGTATCGATCG
<i>phoP</i> ⁺ <i>phoQ24</i> SOE 3' (F)	SM1037	CGATCGATACGGTGCGCGG

*SOE = splicing by overlap extension

Table S4. Oligonucleotide primers for promoter fusion construct.

Primer name	Primer no.	Sequence (5' to 3')
<i>P_{pmrH}</i> (F)	SS100	GAGAGAGAATTCTGGTCGCCGGCAGCCGCTGAAG
<i>P_{pmrH}</i> (R)	SS101	AGAGAGGGATCCTCACCGGTGGTGATCCAGCCGGAAC
CTX - 5' flanking	SS102	CGAGTGGTTTAAGGCAACGGTCTTGA
CTX - 3' flanking	SS103	AGTTCGGCCTGGTGGAACAACCTCG