

1 **Supplemental Material**

2 **The Impact of a Transposon Insertion in *phzF2* on the Specialized Metabolite**
3 **Production and Interkingdom Interactions of *Pseudomonas aeruginosa***

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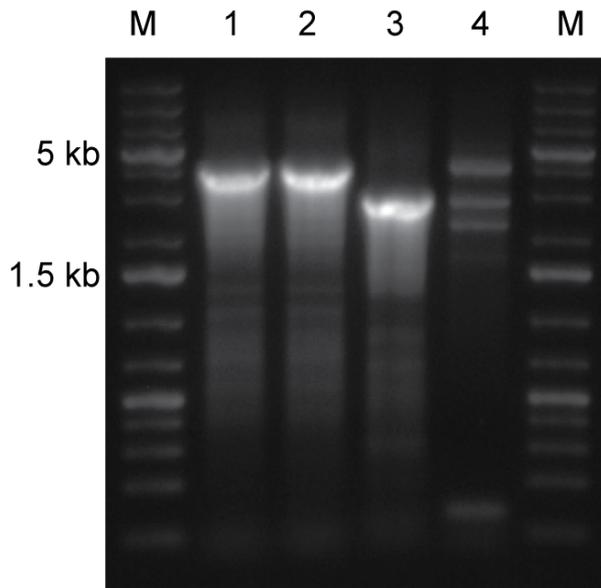
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19 **Running Title:** Impact of *phzF2* on Specialized Metabolite Production

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26 **Figure S1.** *P. aeruginosa* has two *phzF* genes (*phzF1* and *phzF2*) with 100% identity.

27 To determine whether the transposon insertion was in *phzF1* or *phzF2*, primers were

28 designed to specifically amplify each *phzF* gene. M: marker, 1: PAO1 with amplification

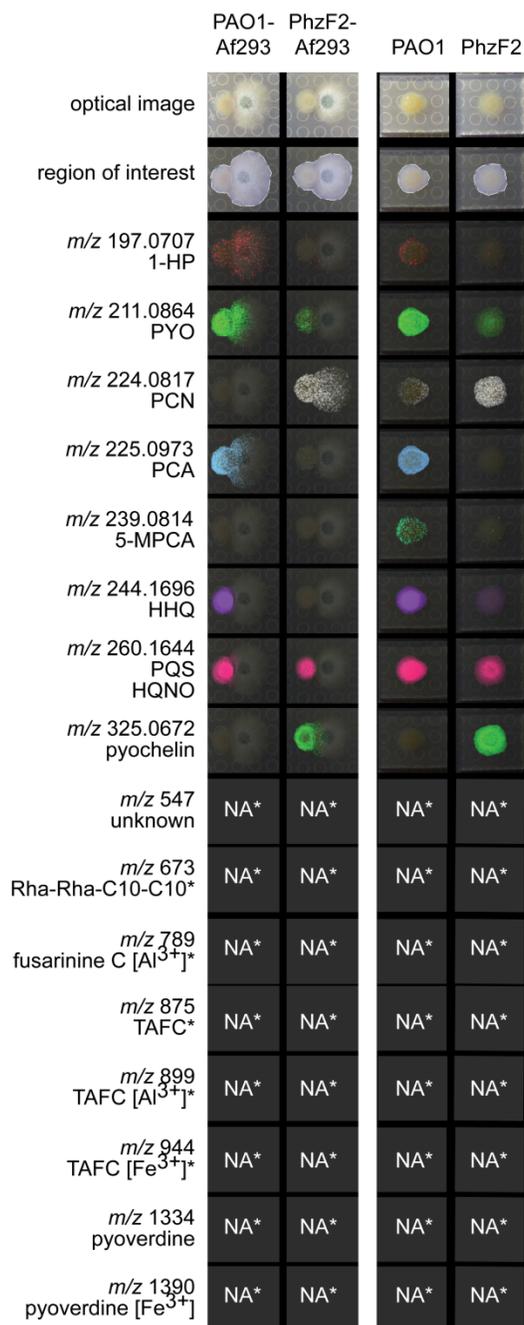
29 of the *phzF1* gene (expected product 3.7 kb), 2: the *phzF2* mutant with amplification of

30 the *phzF1* gene (expected product 3.7 kb), 3: PAO1 with amplification of the *phzF2*

31 gene (expected product 2.7 kb), 4: the *phzF2* mutant with amplification of the *phzF2*

32 gene (expected product 2.7 kb).

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35 **Figure S2.** Duplicate MALDI FT-ICR imaging data of the interactions between *P.*
36 *aeruginosa* PAO1 or the *phzF2* mutant and *A. fumigatus* Af293. The detected mass
37 range was 175 to 375. Spatial resolution is 200 μm . Region of interest corresponds to
38 the area measured. High resolution MALDI IMS is less sensitive than traditional IMS.
39 5-MPCA was not detected in the *P. aeruginosa* – *A. fumigatus* interaction because it

40 was produced at levels below the limit of detection for this mass spectrometer. NA*
41 denotes not analyzed.

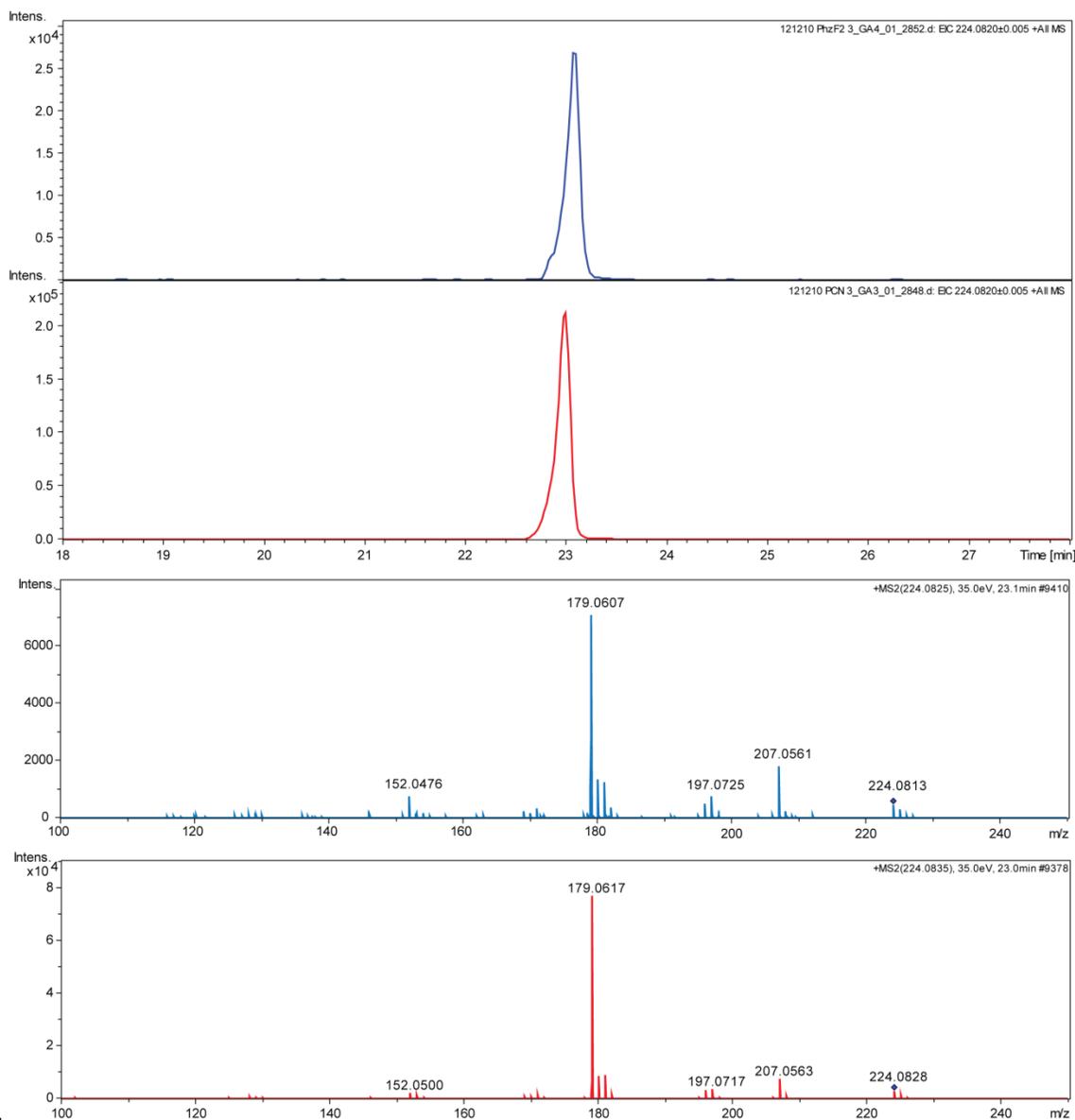
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43 **Table S1. Metabolite Production by PAO1 and the *phzF2* Mutant in an Interaction**
44 **with Af293**

Metabolite	MPAO1-Af923 Peak Area ¹	PhzF2-Af293 Peak Area ¹	Fold Change
1-HP	1348479	90378	-14.9
PYO	971184	83432	-11.6
PCN	106497	695734	+6.5
PCA	1739523	112756	-15.4
5-MPCA*			
HHQ	4711651	1059701	-4.4
HQNO	5494695	2330215	-2.4
Pyochelin	2581	2392412	+926.9
Rha-Rha-C10-C10 Na ⁺ salt	283659	1596761	+5.6
Pyoverdine*			

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*not measured. ¹Average of three independent cultures



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48 **Figure S3. Confirmation of PCN production by the *phzF2* mutant.** Top: Extracted ion
 49 chromatograms for PCN for both the *phzF2* mutant (blue) and commercial standard (red).
 50 Bottom: MS/MS of PCN for both the *phzF2* mutant (blue) and commercial standard (red). The
 51 retention times, measured exact mass and MS/MS patterns are the same between PCN
 52 produced by the *phzF2* mutant and a commercial PCN standard.

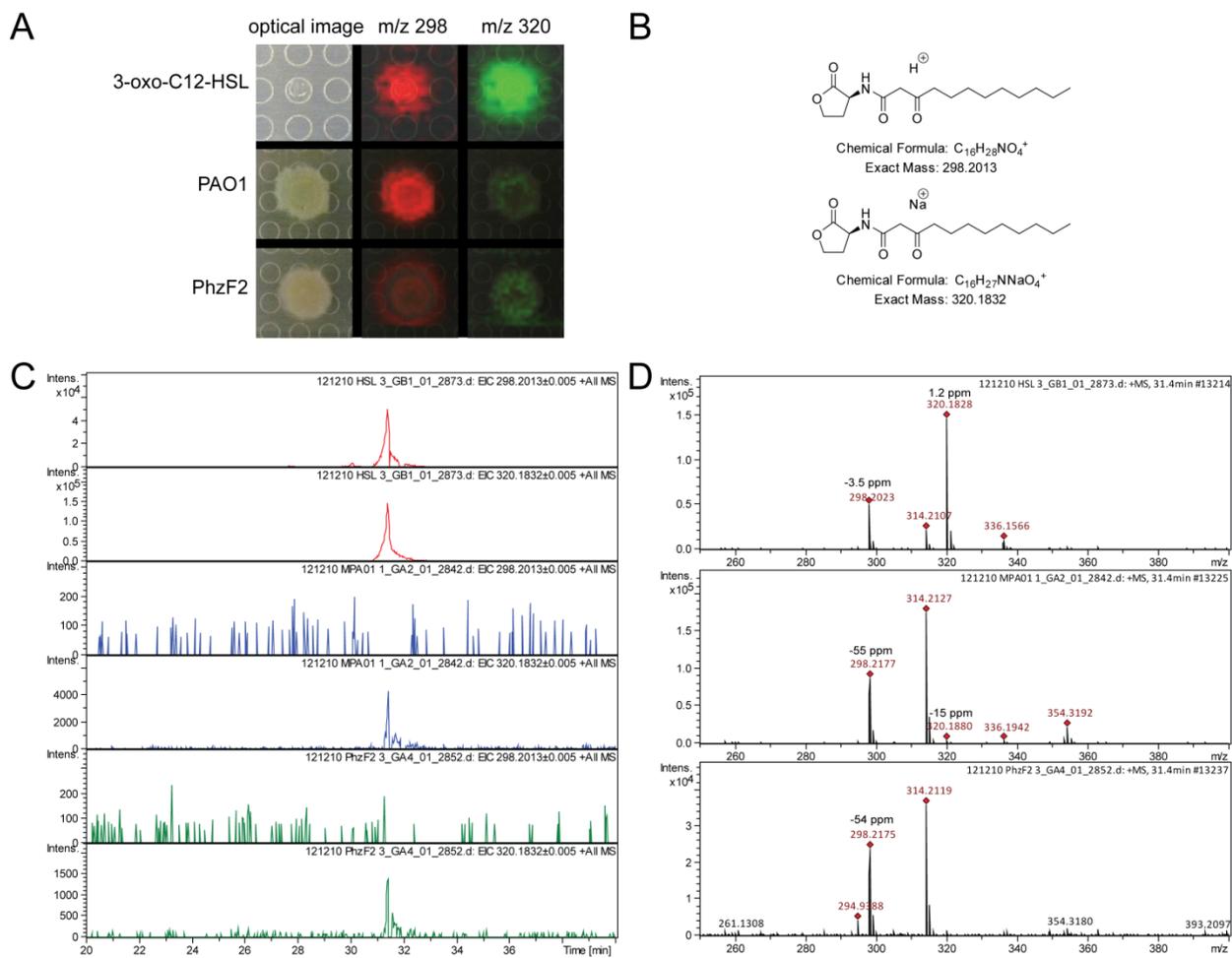
53 **Table S2. Metabolite Production by PAO1 and the *phzF2* mutant**

Metabolite	Calculated Mass	Measured Mass	ppm error	MPAO1 Peak Area ¹	PhzF2 Peak Area ¹
1-HP	197.0709	197.0713	2.03	140095	37361
PYO	211.0866	211.0875	4.26	143637	24695
PCN	224.0818	224.0820	0.89	133941	274048
PCA	225.0659	225.0662	1.33	11898123	454998
5-MPCA*					
HHQ	244.1696	244.1708	4.91	2615233	503902
HQNO	260.1645	260.1651	2.31	6615501	1912010
Pyochelin	325.0675	325.0681	1.85	5464	263419
Rha-Rha-C10-C10 Na ⁺ salt	673.3770	673.3766	-0.59	1843045	3359381
Pyoverdine*					

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*not measured. ¹Average of three independent cultures



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57 **Figure S4. (A)** 3-oxo-C12-HSL is detected by IMS as a spotted standard on ISP2 agar.

58 Both PAO1 and PhzF2 produce metabolites corresponding to m/z 298. **(B)** The

59 structures and exact masses for the $[M+H]^+$ and $[M+Na]^+$ forms of 3-oxo-C12-HSL are

60 shown. **(C)** Extracted ion chromatograms for m/z 298.2013 and 320.1832 for 3-oxo-

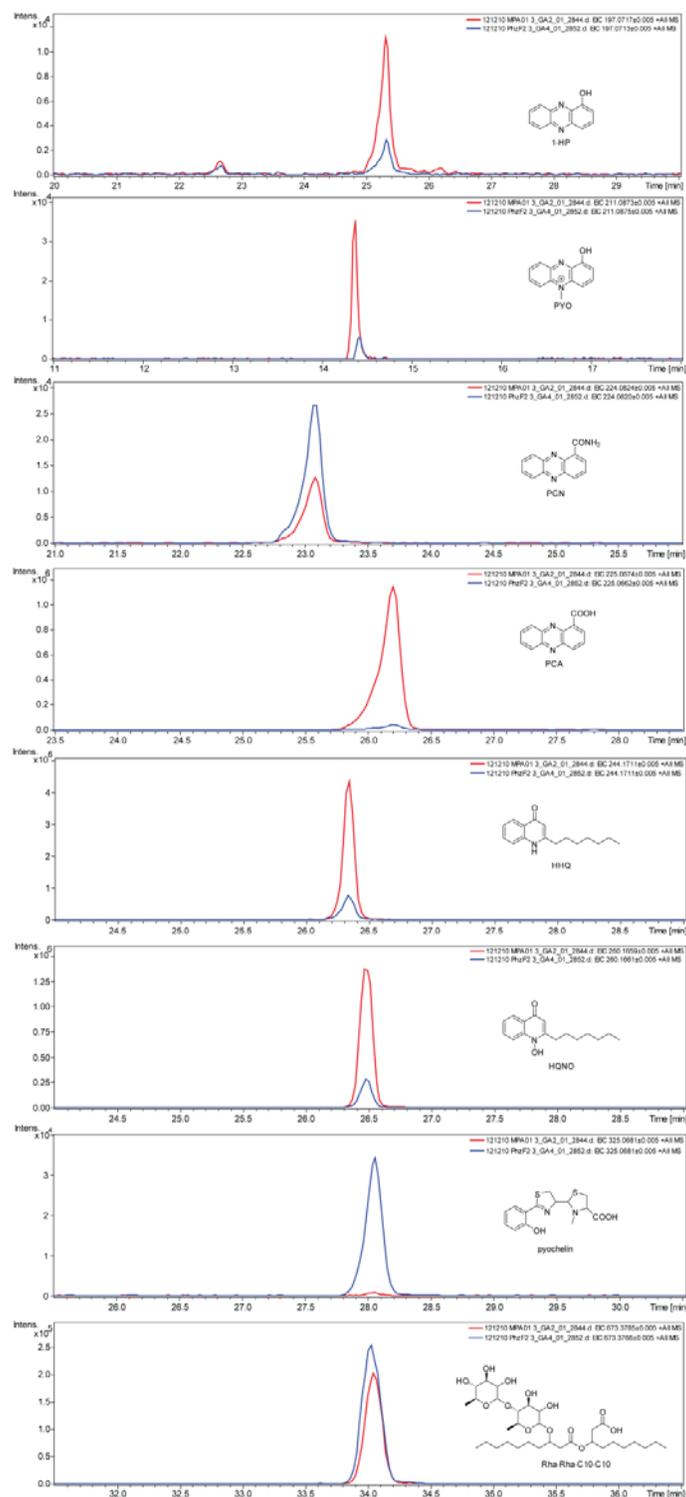
61 C12-HSL alone, PAO1 and PhzF2. **(D)** Mass spectra for 3-oxo-C12-HSL alone, PAO1

62 and PhzF2. The m/z values observed in PAO1 and PhzF2 samples do not correlate to

63 3-oxo-C12-HSL. They correlate to the quinolone C11:db UHQ ($[M+H] = 298.2165$).

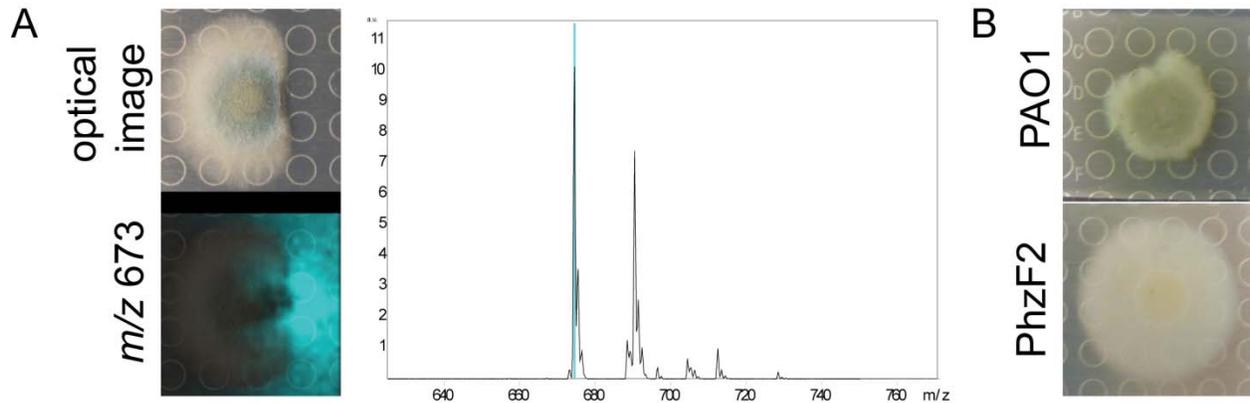
64 Expected ppm error is within 5 ppm for the settings used for mass spectrometry

65 analysis.



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 67 **Figure S5.** Extracted ion chromatograms from LC-MS/MS analysis of PAO1 and the
 68 *PhzF2* mutant. The area under the curve was calculated and fold changes of metabolite
 69 production were determined as displayed in Figure 2 of the main text.

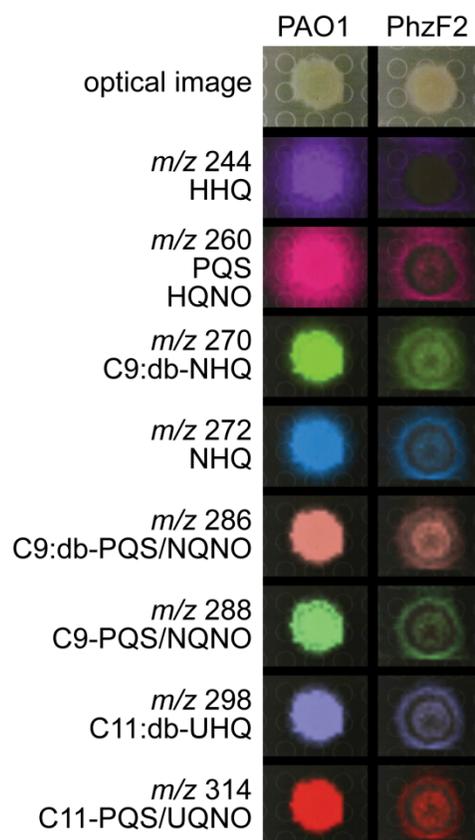
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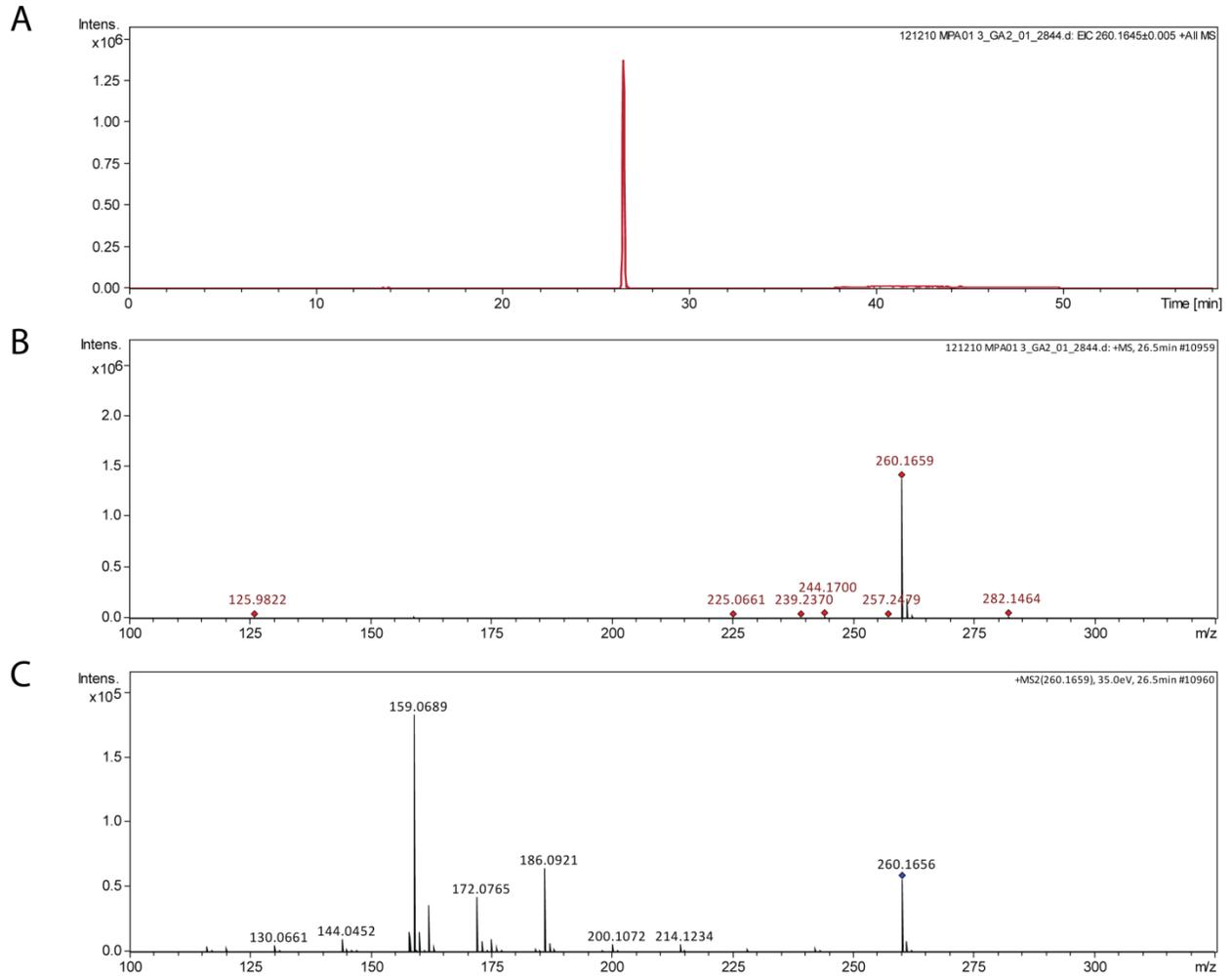
74 **Figure S6. (A)** Inhibition of *A. fumigatus* Af293 by chemical complementation with
75 rhamnolipids. The distribution of Rha-Rha-C10-C10 (Na^+ salt) is represented in the IMS
76 image. **(B)** The observed increase in surface area of mutants affecting the phenazine
77 biosynthetic gene clusters is recapitulated. Optical images of PAO1 and the *phzF2*
78 mutant are shown after 4 days of growth on ISP2 media.

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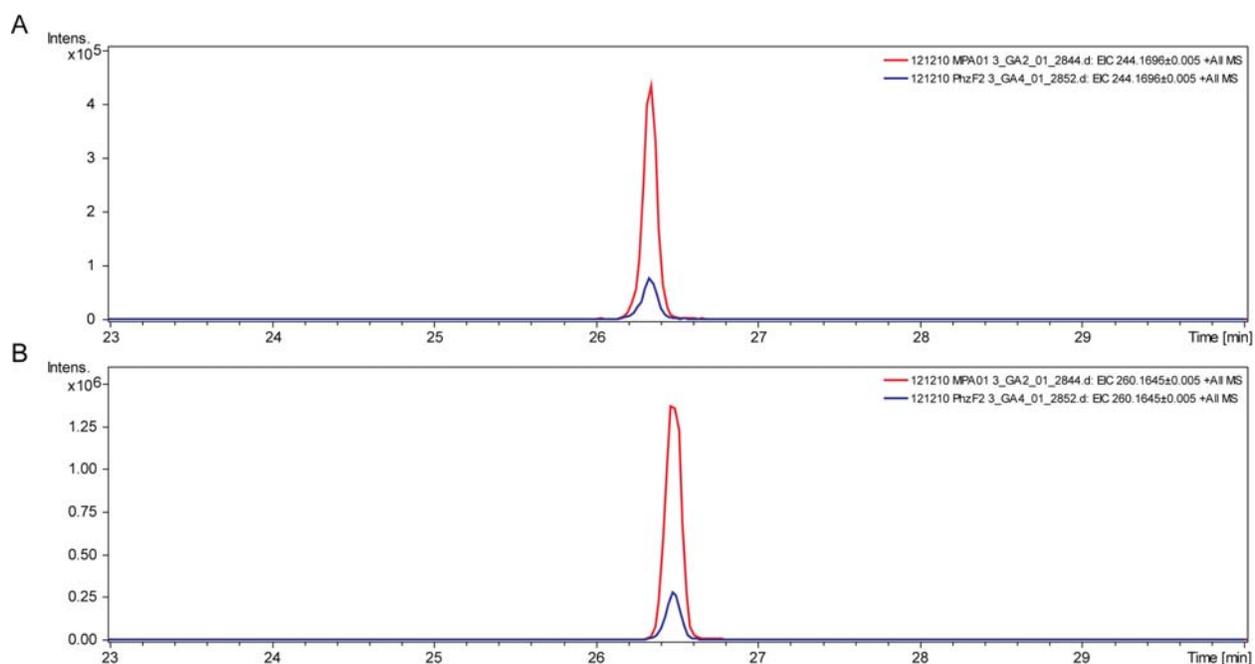
81 **Figure S7.** Approximately 12 quinolones were detected by IMS. All quinolones had
 82 similar distribution patterns.



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84 **Figure S8. (A)** Extracted ion chromatogram for m/z 260.1645 from PAO1 grown on

85 ISP2 agar media. **(B)** MS analysis of peak at 26.5 minutes. **(C)** MS2 of m/z 260.1659.



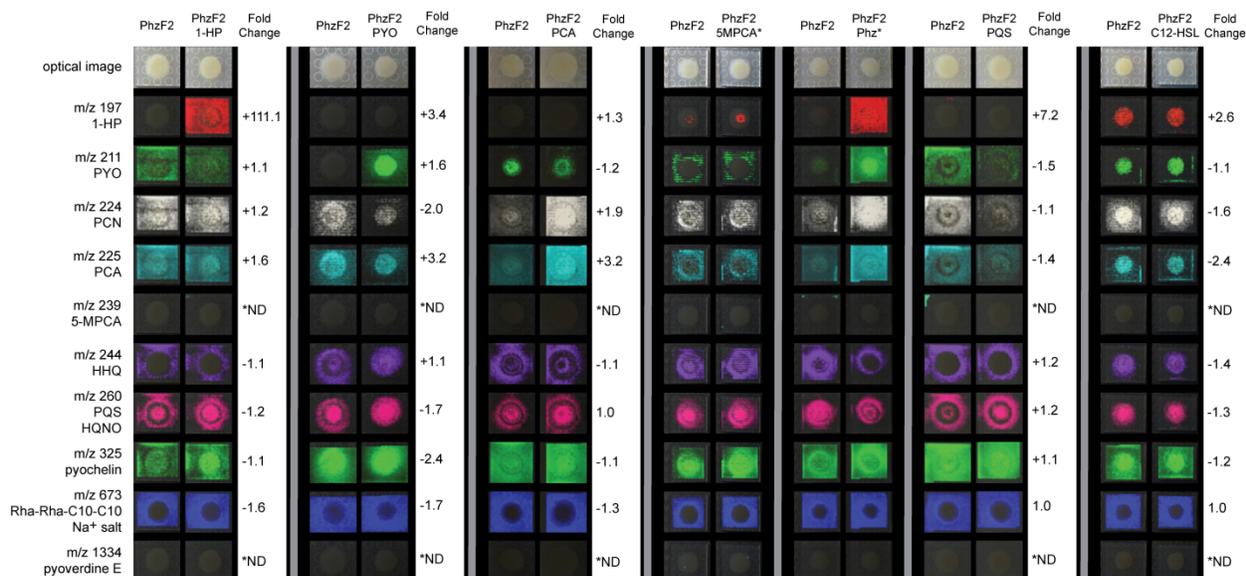
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 87 **Figure S9.** Extracted ion chromatograms of HHQ (**A**, m/z 244.1696) and HQNO (**B**, m/z
 88 260.1645) produced by PAO1 (red) and the *phzF2* mutant (blue). Production of both
 89 HHQ and HQNO is reduced in the *phzF2* mutant.

90 **Table S3. Metabolite Production of PCN Complemented PAO1.**

Metabolite	MPAO1	MPAO1 PCN
1-HP	140095	281062
PYO	143637	157422
PCN	133941	2011030
PCA	11898123	8896510
5-MPCA*		
HHQ	2615233	2311794
HQNO	6615501	9072170
Pyochelin	5464	1193
Rha-Rha-C10-C10 Na ⁺ salt	1843045	1941404
Pyoverdine*		

*not measured. All values are averages of peak areas from three independent cultures.

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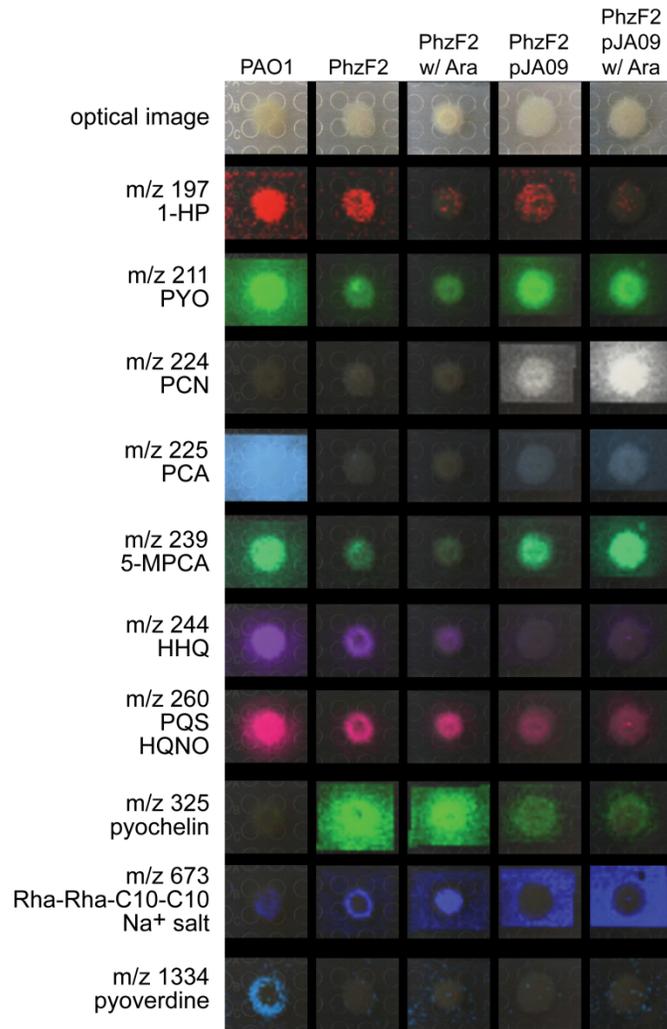


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95 **Figure S10.** Chemically complementing the *phzF2* mutant with 1-HP, PYO, PCA, 5-
96 MPCA, Phz (a mixture of the phenazines), PQS and 3-oxo-C12-HSL had no discernible
97 effect on specialized metabolite production. 5-MPCA was not detected in the 5-MPCA
98 complemented IMS image due to diffusion into the agar as it is water soluble. * denotes
99 that fold-changes were not measured.

100 **Table S4. Metabolite Production of Chemically Complemented *phzF2* mutant**

Metabolite	PhzF2	PhzF2 1-HP	PhzF2 PYO	PhzF2 PCA	PhzF2 5-MPCA*	PhzF2 Phz*	PhzF2 PQS	PhzF2 C12-HSL
1-HP	37361	4150093	126201	47152			268900	98133
PYO	24695	26076	38624	21274			16204	21721
PCN	274048	327511	139949	509673			257034	171236
PCA	454998	739591	1447421	1466002			335011	192218
5-MPCA*								
HHQ	503902	441973	546950	470971			590989	347883
HQNO	1912010	1630795	1155720	2001222			2292609	1471249
Pyochelin	263419	237735	108072	247010			232553	220535
Rha-Rha-C10-C10 Na ⁺ salt	3359381	2068990	1951854	2675212			3424335	3269245
Pyoverdine*								

101
102 *not measured. All values are averages of peak areas from three independent cultures.



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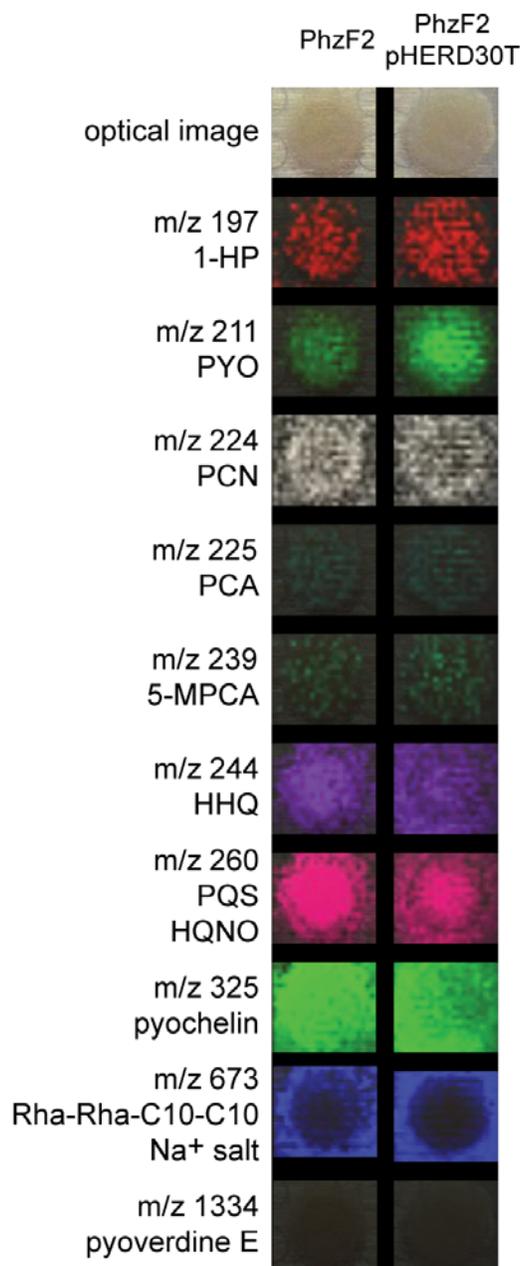
104 **Figure S11.** IMS analysis of genetic complementation of the *phzF2* mutant revealed
 105 some metabolite production was recovered although full complementation was not
 106 achieved with pJA09. Ara denotes addition of 0.2% L-arabinose to the culture medium
 107 to induce production of the *phzF* gene within the pJA09 construct. The *phzF2* mutant is
 108 still producing PCN. However, due to the comparative visual nature of IMS, the higher
 109 production of PCN by *phzF2* pJA09 and *phzF2* pJA09 w/ Ara gives the visual
 110 impression that the *phzF2* mutant is not producing PCN.

111 **Table S5. Metabolite Production of Genetically Complemented *phzF2* mutant**

Metabolite	MPAO1	PhzF2	PhzF2 w/ Ara	PhzF2 pJA09	PhzF2 pJA09 w/ Ara
1-HP	140095	37361	21430	37234	74876
PYO	143637	24695	12628	45788	46624
PCN	133941	274048	268586	1636317	3484371
PCA	11898123	454998	83136	327070	661642
5-MPCA*					
HHQ	2615233	503902	276109	312072	478828
HQNO	6615501	1912010	952103	1724175	1935011
Pyochelin	5464	263419	77875	42609	31800
Rha-Rha-C10-C10 Na ⁺ salt	1843045	3359381	4354715	6747226	8390807
Pyoverdine*					

* not measured. All values are averages of peak areas from three independent cultures.

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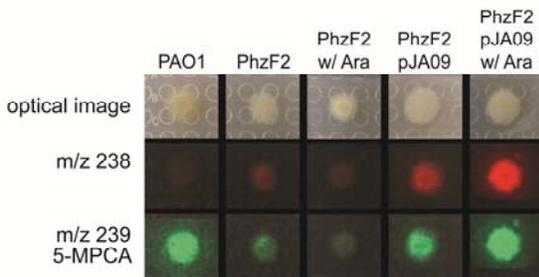
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116 **Figure S12.** IMS analysis of the *phzF2* mutant complemented with the empty

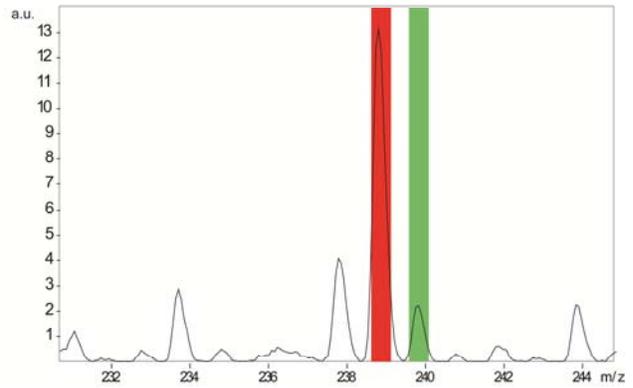
117 pHERD30T vector (pJA09 without *phzF*).

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A



B



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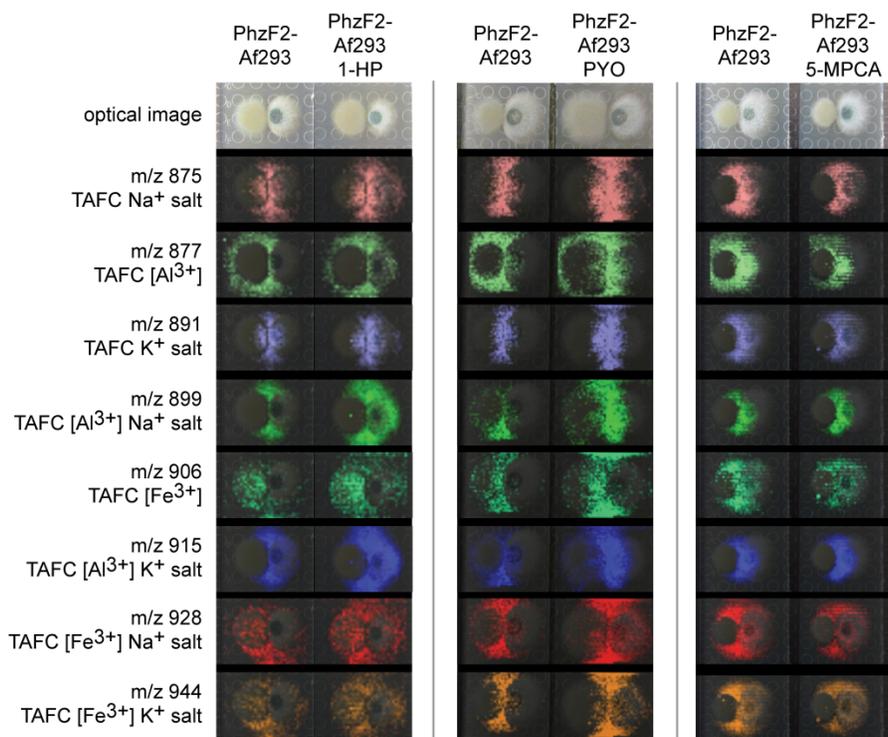
120 **Figure S12. (A)** IMS images of the genetic complement, m/z 238 and m/z 239.

121 Although the images show possible production of 5-MPCA by the genetically

122 complementing the *phzF2* mutant with pJA09, the mass spectrum shows a peak at m/z

123 238 **(B)** indicating that the m/z 239 observed is an isotope of m/z 238 and not 5-MPCA.

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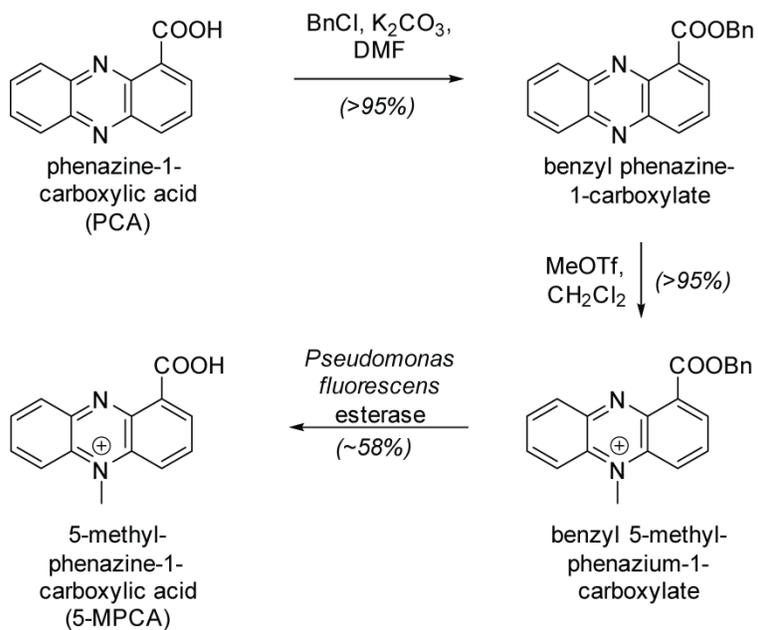
126 **Figure S13.** Chemical complementation of the interaction between *A. fumigatus* and the
 127 *phzF2* mutant by 1-HP, PYO and 5-MPCA causes small changes in triacetylfusarinine
 128 production by *A. fumigatus*.

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132 **Scheme S1. Synthesis of 5-MPCA**



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Proton NMR of Benzyl 1-phenazinecarboxylate

