## Title: Structural and functional studies on *Pseudomonas aeruginosa* DspI:

## implications for its role in DSF biosynthesis

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## **Supplementary Figure Legends**



Fig. S1: Statistical results of swarming motility assays presented as the length of the colony tendril growth. Data shown are represent of three independent experiments. *P* values for comparison of two groups were determined by 2-tailed Student's *t* test (\*P<0.05, \*\*P<0.01 vs wild-type PA14).



**Fig. S2:** The swarming-defective phenotype of PA14 mutations could be recovered after the exogenous CDA supplied. PA14 as well as strains carrying mutations in genes were incubated at 37  $^{\circ}$ C for 16 h in modified M8 plates supplemented with CDA at 500 nM.



Fig. S3: Statistical results of SEM and TEM assays presented as the size of the bacteria



**Fig. S4:** The PA14 mutation strains exhibited no growth difference relative to wild-type PA14. Wild-type PA14 as well as mutation strains were serially ten-fold dilution to 10<sup>-6</sup>. Dispense 10 microliters of the last dilution onto the PIA solid medium plates and then take it picture.



**Fig. S5:** Mice were infected with mutation strains in lung exhibited almost no difference relative to wild-type PA14. Mice were infected with  $5\sim10 \times 10^6$  CFU/lung of PA14 or mutation strains embedded in agar beads, three days later, the lung was harvested, homogenized, and counted respectively.



**Fig. S6:** Possible reaction mechanism for DspI based on its three-dimensional X-ray structure and AutoDock suite.

Application		oligos and sequences (5' to 3')
Protein expression	dspI	pET22b- DspI-FOR: CTTTAAGAAGGAGATATACATATGATGAACACTGCCGTCGAACCC
		pET22b- DspI-REV: TCAGTGGTGGTGGTGGTGGTGGTGGTGGCAGGCAGTTGCGCCACTTGGG
	pET22b (+)	pET22b-line-FOR: ATGGTGAGCAAGGGCGAGGAGCTGTTCACC
		pET22b-line- REV: CATATGTATATCTCCTTCTTAAAGTTAAAC
Site-directed mutagenesis	PEX18Gm	PEX18Gm- DspI-FOR: AACGACGGCCAGTGCCAAGCTTGGCGACGAATACGTGCTGGACGG
		PEX18Gm- DspI- REV: AATTCGAGCTCGGTACCCGGGGATCGAGCAGTGCGTCCAGGCGCG
		PEX18Gm-line-FOR: ATGGTGAGCAAGGGCGAGGAGCTGTTCACC
		PEX18Gm-line-REV: CATATGTATATCTCCTTCTTAAAGTTAAAC
	E126A	E126A- FOR: ACGCCATGGGCGGCGGCCTGGCCTGCGCCCTCGCCTGC
		E126A- REV: AGCCGTTGATCGCCGCGATCGACACCCCGCGGAAATCGC
	E146A	E146A- FOR: ATGGCCCTGCCGGCCGCCGCGGTGGGGCCTGCTGCCCTG
		E146A- REV: CTGCGCCTGGCGCTCGGCGATGCGGATGTCGCAGGCGAGG
	C127S/C131S	C127S/C131S- FOR: TGGAGTCCGCCCTCGCCTCCGACATCCGCATCGCCGAGCGCCAGG
		C127S/C131S- REV: TCGGCGATGCCGGATGTCGGAGGCGAGGCGGACTCCAGGCCGCCG
	C154G	C154G-FOR: TGCTGCCCGGGGGCCGGGGGCCCAGGCGC
		C154G-REV: TCCCGCCGGGCCCCGGGCAGCAGGCCCACCG
	⊿DspI	ΔPA0745- FOR: GCCTTCCCATCGAACCCGGCGGGCTGCGCC
		ΔPA0745- REV: GAAGTCCCTCACTGGATCATGGGCCGGGGGGGT

Table. S1. Related to experimental procedures oligo primers used in this study