

1 **Supplementary Materials**

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3 **Hfq-binding small RNA PqsS regulates *Pseudomonas aeruginosa* pqs quorum**
4 **sensing system and virulence**

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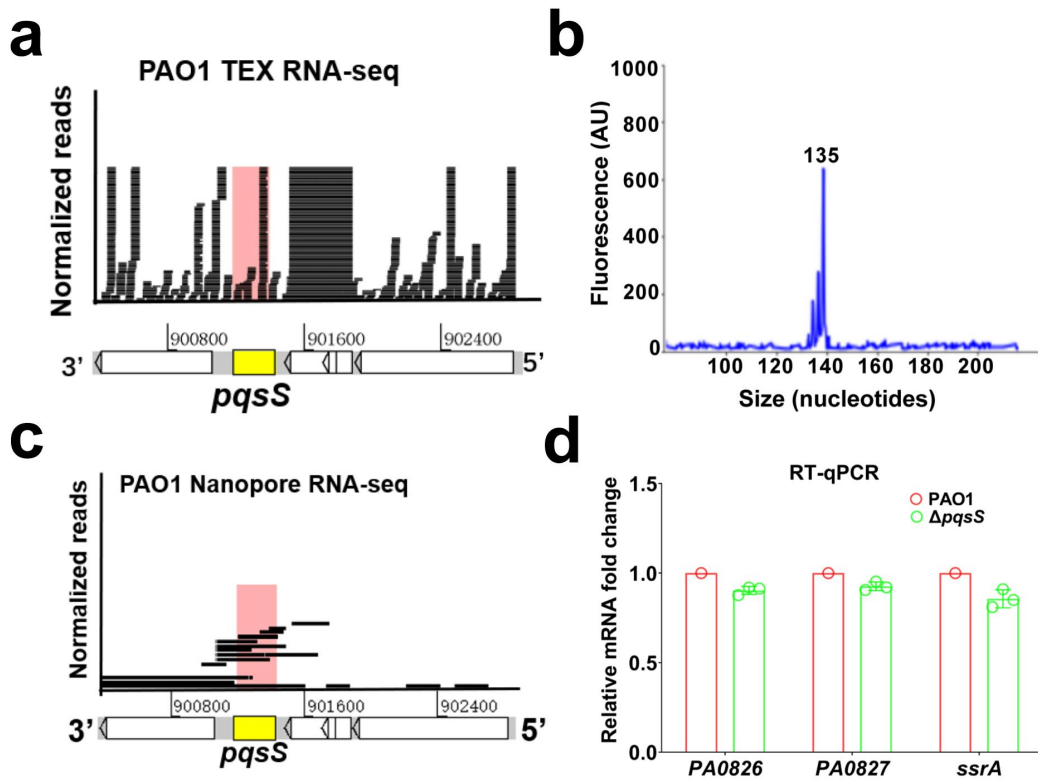
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18 **This PDF file includes:**

19 Supplementary Fig. 1 to 7

20 Supplementary Tables 1 to 6



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41 **Supplementary Fig. 1.** *P. aeruginosa* PAO1 sRNA PqsS identifications. (a)

42 Transcription of the *PA0826-PA0827* region as analyzed in the previous PAO1 TEX

43 RNA-seq data³⁷. (b) Determination of the transcription start site of *pqsS* via

44 fluorescent primer extension and comparing the retention time of the 6-

45 carboxyfluorescein (FAM)-labeled primer extension product with that of DNA size

46 standards. A FAM-labeled primer complementary to the sequence 99 to 135 bp

47 downstream *pqsS* transcription start site (TSS) (underlined in Figure 2G) was used. (c)

48 Transcription of all, full-length size RNAs within the *PA0826-PA0827* region as

49 analyzed in PAO1 WT Nanopore RNA-seq data. (d) Expression of *PA0826*, *PA0827*

50 and *ssrA* in $\Delta pqsS$ was analyzed using real-time quantitative polymerase chain

51 reaction (RT-qPCR). Data are presented as means $\pm$ SD (n=3).

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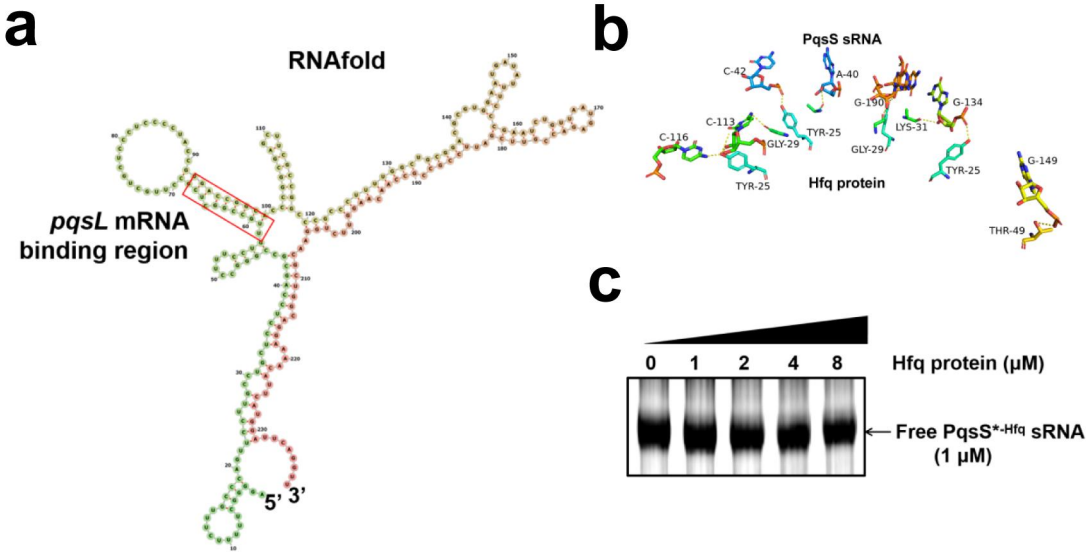
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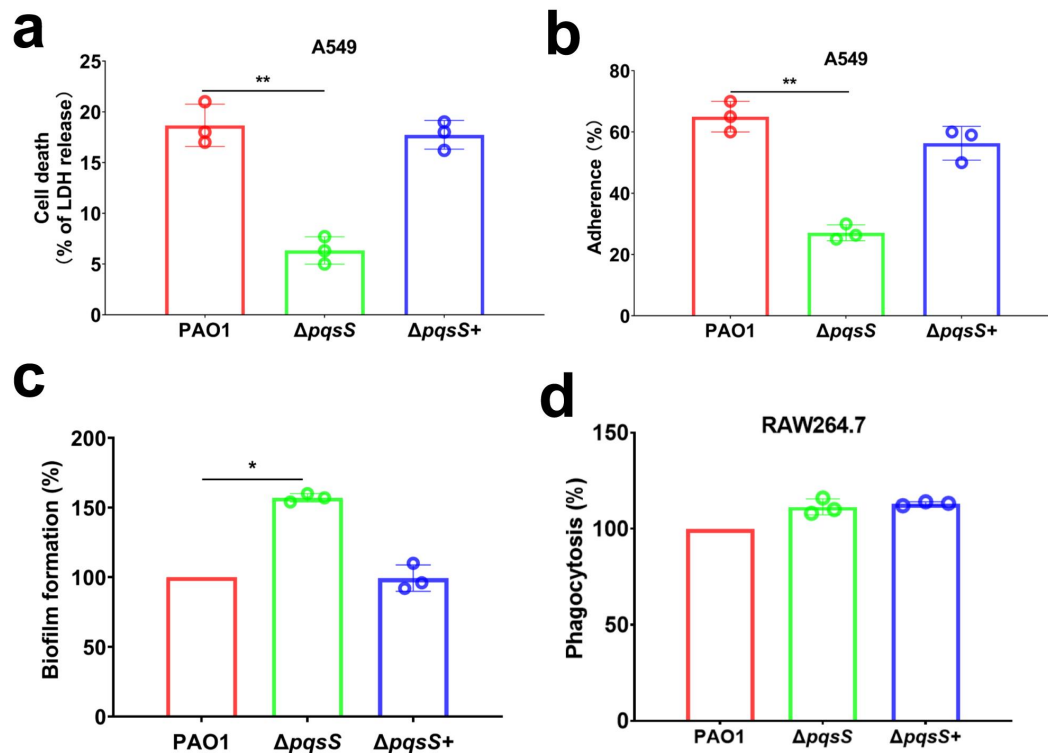
67 **Supplementary Fig. 2.** PqsS sRNA-Hfq protein interactions. (a) The predicted
68 secondary structure of PqsS sRNA was determined using RNAfold and depicted as a
69 color gradient from green to red, representing 5' to 3' ends of PqsS sRNA,
70 respectively. The *pqsL* mRNA binding region is highlighted in the red box. (b)
71 Interactive model between seven bases of PqsS sRNA and seven amino acids of Hfq
72 protein. (c) EMSA with PqsS*-*pqsL* sRNA in the presence of Hfq protein.

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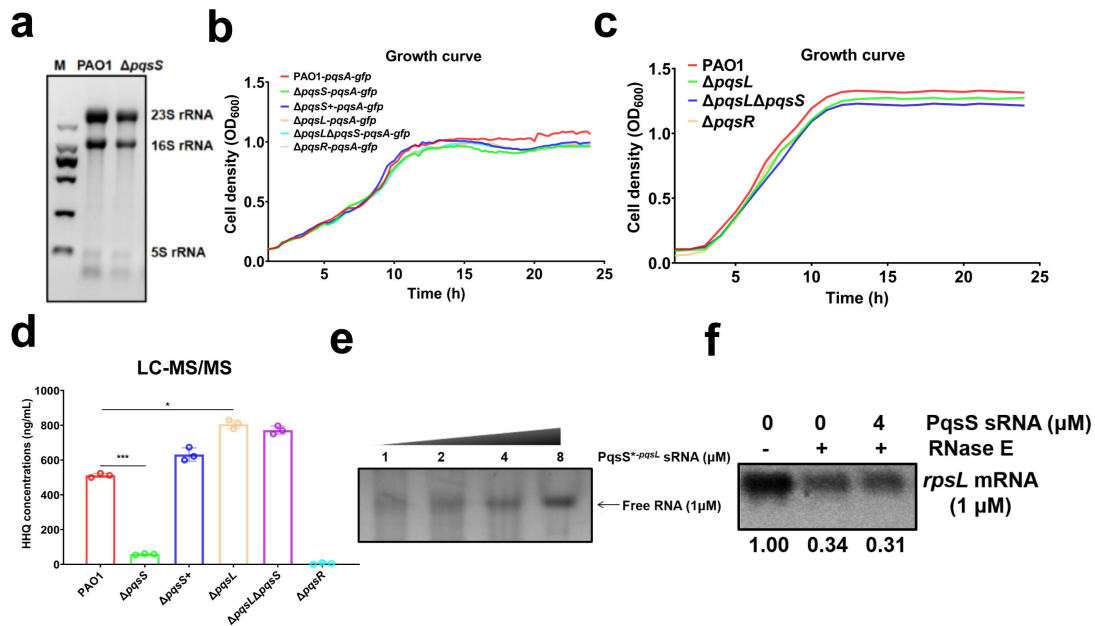
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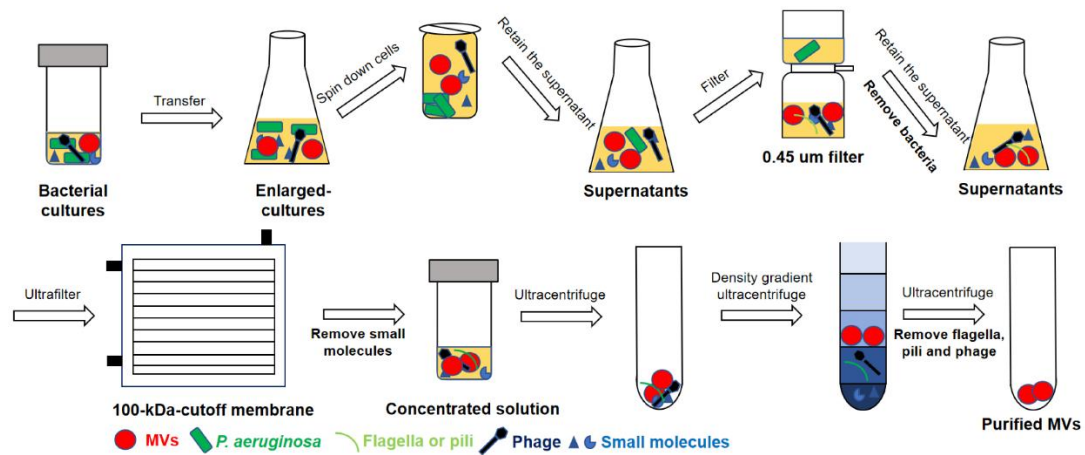
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Supplementary Fig. 3. PqsS promotes A549 human lung epithelial cell death. (a) LDH assay for cell death in A549 cells infected with PAO1 WT, $\Delta pqsS$, and $\Delta pqsS^+$ strains. Data represent the means $\pm$ SD ($n = 3$, $**P \leq 0.01$, Mann-Whitney U test). (b) Percent adherence of PAO1 WT, $\Delta pqsS$, and $\Delta pqsS^+$ strains compared to inocula by plate counts following washing and lysis with 1% Triton. Data represent the means $\pm$ SD ($n = 3$, $**P \leq 0.01$, Mann-Whitney U test). (c) Biofilm formation assay of PAO1 WT, $\Delta pqsS$, and $\Delta pqsS^+$ strains grown in LB was displayed with crystal violet staining (down) and quantified with optical density measurement (up) ($n = 3$, $*P \leq 0.05$, Mann-Whitney U test). (d) Phagocytosis of bacteria in RAW264.7 cells after 30 min internalization. Results are shown as a percentage of phagocytic bacteria compared to the number of bacteria before phagocytosis. Data were presented as means $\pm$ SD ($n = 3$).

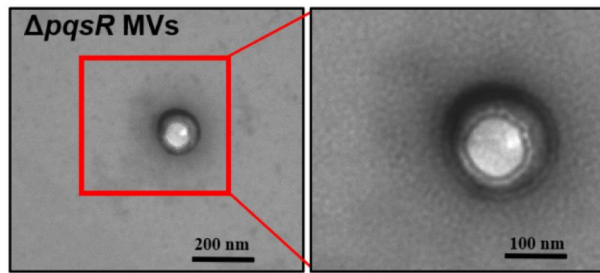


Supplementary Fig. 4. PqsS promotes HHQ productions. (a) Gel picture of total RNAs of PAO1 WT and $\Delta pqsS$ strains before RNA-seq. (b) Growth curves of PAO1-*pqsA-gfp*, $\Delta pqsS$ -*pqsA-gfp*, $\Delta pqsS^+$ -*pqsA-gfp*, $\Delta pqsL$ -*pqsA-gfp*, $\Delta pqsL\Delta pqsS$ -*pqsA-gfp*, and $\Delta pqsR$ -*pqsA-gfp* strains. Data represent the means $\pm$ standard deviations (SD) (n = 6). (c) Growth curves of PAO1 WT, $\Delta pqsL$, $\Delta pqsL\Delta pqsS$, and $\Delta pqsR$ strains. Data represent the means $\pm$ standard deviations (SD) (n = 6). (d) Qualification of HHQ concentrations measured in the supernatants of PAO1 WT, $\Delta pqsS$, $\Delta pqsS^+$, $\Delta pqsL$, $\Delta pqsL\Delta pqsS$, and $\Delta pqsR$ cultures. Data were presented as means $\pm$ SD (n = 3, * $P \leq 0.05$ and *** $P \leq 0.001$, Mann-Whitney *U* test). (e) REMSA with a fixed amount of the *pqsL* mRNA but an increasing amount of PqsS*-*pqsL* sRNA. (f) *In vitro* cleavage of *rpsL* mRNA by RNase E in the absence or presence of PqsS sRNA (n=3). Densitometric analyses of the bands were performed with ImageJ.

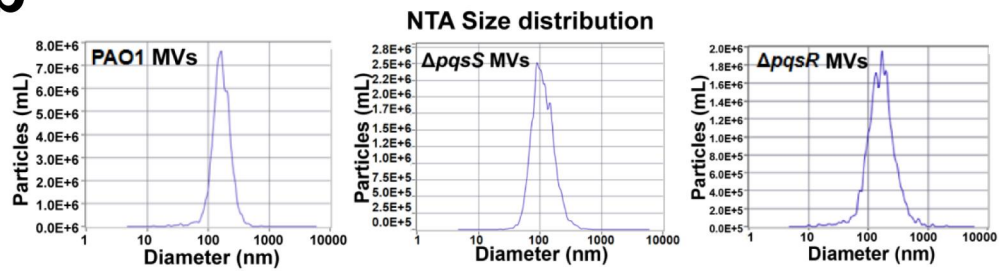


Supplementary Fig. 5. Optimized *P. aeruginosa* PAO1 MVs purification procedure. After filtering and ultrafiltering, bacteria and small molecules can be removed. After density gradient ultracentrifuging, flagella, pili, and phage can be removed. Then the purified MVs samples can be obtained.

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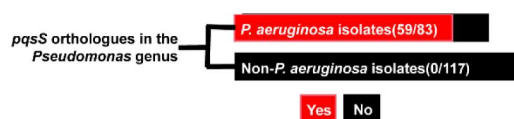


Supplementary Fig. 6. PqsS promotes MVs productions. (a) TEM identification of MVs in PAO1 $\Delta pqsR$ strain (n = 3). Scale bar = 200/100 nm. (b) NTA identification of MVs sizes in PAO1 WT, $\Delta pqsS$, and $\Delta pqsR$ strains (n = 3).

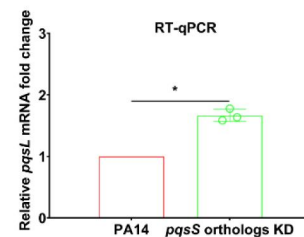
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Supplementary Fig. 7. PqsS is widespread and specifically exists in *P. aeruginosa* strains. (a) A maximum-likelihood tree was constructed using PhyML, based on 83 *P. aeruginosa* strains. PqsS is present in strains marked in red but absent in strains marked in black. (b) *pqsS* orthologues are only found in *P. aeruginosa*. Red and black bars indicate the presence and absence of orthologues, respectively. (c) The relative expression difference of *pqsL* in PA14 $\Delta pqsS$ orthologs was analyzed using RT-qPCR. Data are plotted as the relative transcript abundance of the indicated genes compared to the transcript for *rpsL* using the comparative Ct method ($2^{-\Delta\Delta CT}$). Data are presented as means $\pm$ SD (n=3, * $P \leq 0.05$, Mann-Whitney *U* test).

Supplementary Tables

Supplementary Table 1. Bacterial strains, plasmids, and primers.

Strain	Genotype or description	Source or reference
PAO1 WT	Wild-type <i>Pseudomonas aeruginosa</i> PAO1 strain	ATCC ^a
PA14	Wild-type <i>Pseudomonas aeruginosa</i> PA14 strain	ATCC ^a
PAK	Wild-type <i>Pseudomonas aeruginosa</i> PAK strain	ATCC ^a
PAO1 $\Delta pqsS$	$\Delta pqsS$ deletion mutant in PAO1 WT	This study
PAO1 $\Delta pqsR$	$\Delta pqsR$ deletion mutant in PAO1 WT	This study
PAO1 Δhfq	Δhfq deletion mutant in PAO1 WT	This study
PAO1 $\Delta pqsL$	$\Delta pqsL$ deletion mutant in PAO1 WT	This study
PAO1 $\Delta pqsS\Delta pqsL$	$\Delta pqsS$ and $\Delta pqsL$ deletion mutant in PAO1 WT	This study
PAO1 pUCP20- <i>gfp</i>	PAO1 strain containing plasmid pUCP20- <i>gfp</i> ; Cb ^R	This study
PAO1 pUCP20- <i>PpqsA-gfp</i>	PAO1 strain containing plasmid pUCP20- <i>PpqsA-gfp</i> ; Cb ^R	This study
PAO1 $\Delta pqsR$ pUCP20- <i>PpqsA-gfp</i>	PAO1- $\Delta pqsR$ strain containing plasmid pUCP20- <i>PpqsA-gfp</i> ; Cb ^R	This study
PAO1 $\Delta pqsS$ pMiniCTX1- <i>PqsS</i> +pUCP20- <i>PpqsA-gfp</i>	PAO1- $\Delta pqsS$ strain containing plasmid pMiniCTX1- <i>pqsS</i> and pUCP20- <i>PpqsA-gfp</i> ; Tc ^R +Cb ^R	This study
PAO1 $\Delta pqsS$ +pMiniCTX1- <i>PqsS</i> ($\Delta pqsS$ +))	PAO1- $\Delta pqsS$ strain containing plasmid pMiniCTX1- <i>pqsS</i> ; Tc ^R	This study
PAO1 $\Delta PqsS$ +pJV300- <i>PqsS</i>	PAO1- $\Delta pqsS$ strain containing plasmid pJV300- <i>pqsS</i> without promoter; Cb ^R	This study
PA14 <i>pqsS</i> KD	PA14 strain knocking down <i>pqsS</i> gene by Crispr; Apr ^R	This study
PAO1 $\Delta pqsR$ pUCP20 <i>PpqsA-gfp</i>	PAO1- $\Delta pqsR$ strain containing plasmid pUCP20- <i>PpqsA-gfp</i> ; Cb ^R	This study
PAO1 Δhfq +pMiniCTX1- <i>hfq</i>	PAO1- Δhfq strain containing plasmid pMiniCTX1- <i>hfq</i> ; Tc ^R	This study
PAO1 pUCP20- <i>pqsA-flag</i>	PAO1 strain containing plasmid pUCP20- <i>pqsA-flag</i> ; Cb ^R	This study
PAO1 $\Delta pqsS$	PAO1- $\Delta pqsS$ strain containing plasmid pMiniCTX1- <i>pqsS</i> and	This

pMiniCTX1- <i>pqsS</i> +pUCP20 <i>pqsA</i> - <i>flag</i>	pUCP20 <i>pqsA</i> - <i>flag</i> ; Tc ^R +Cb ^R	study
PAO1 $\Delta pqsS$ pUCP20- <i>pqsL</i> - <i>flag</i>	PAO1- $\Delta pqsS$ strain containing plasmid pUCP20- <i>pqsL</i> - <i>flag</i> ; Cb ^R	This study
PAO1 $\Delta pqsS$ pMiniCTX1- <i>pqsS</i> +pUCP20- <i>pqsL</i> - <i>flag</i>	PAO1- $\Delta pqsS$ strain containing plasmid pMiniCTX1- <i>pqsS</i> and pUCP20- <i>pqsL</i> - <i>flag</i> ; Cb ^R +Tc ^R	This study
PAO1 $\Delta pqsL$ pUCP20- <i>PpqsA</i> - <i>gfp</i>	PAO1- $\Delta pqsL$ strain containing plasmid pUCP20- <i>PpqsA</i> - <i>gfp</i> ; Cb ^R	This study

Plasmid	Description	Source or reference
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pK18mobsacB	Cloning vector; Gm ^R	Lab collection
pUCP20	Expression vector; Cb ^R	Lab collection
pMiniCTX1	Complement vector; Tc ^R	Lab collection
pJV300	Promoter-less vector; Cb ^R	Lab collection

Primer	Sequence (5'→3')	Application
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PK18- <i>pqsR</i> -F	CGGCGCAGACGGTGGCATGCAAGCTTGGCA	<i>pqsR</i> deletion
PK18- <i>pqsR</i> -R	CGAGCAGGCAGAACTTCATGTCATAGCTGT	<i>pqsR</i> deletion
<i>pqsR</i> -P1	ACAGCTATGACATGAAGTTCTGCCTGCTCG	<i>pqsR</i> deletion
<i>pqsR</i> -P2	TAAAAGGAATAAGGGAGCGTTCTCCAGCAG	<i>pqsR</i> deletion
<i>pqsR</i> -P3	CTGCTGGAGAACGCTCCCTTATTCCCTTTTA	<i>pqsR</i> deletion
<i>pqsR</i> -P4	TGCCAAGCTTGCATGCCACCGTCTGCGCCG	<i>pqsR</i> deletion
PK18- <i>pqsL</i> -F	TCAACGGCGCGTTCTGGAACAT	<i>pqsL</i> deletion
PK18- <i>pqsL</i> -R	GCGACTACCTGCTGAACAAGGT	<i>pqsL</i> deletion
<i>pqsL</i> -P1	GAGCTCGGTACCCGGGGATCTGTTCCGCGAGGAAATCTTC	<i>pqsL</i> deletion
<i>pqsL</i> -P2	CGCTCCGGCCGCGCTGGCGGGTGGATGAGTCTCCGTGTC GT	<i>pqsL</i> deletion
<i>pqsL</i> -P3	ACGACACGGAGACTCATCCACCCGCCAGCGCGGCCGGAG CG	<i>pqsL</i> deletion
<i>pqsL</i> -P4	ACGACGGCCAGTGCCAAGCTGCAGGGCTTCTTCTACGTG	<i>pqsL</i> deletion
<i>pqsL</i> -RT-F	GCCGGCTGCTCGATATCGATGTGG	RT-PCR
<i>pqsL</i> -RT-R	GCTCTCGTCGCCGACGAAGCGTTG	RT-PCR
<i>pqsA</i> -RT-F	CCTGCGCAACACGCTCGGTTTCTG	RT-PCR

<i>pqsA</i> -RT-R	CGGAAGGCGACCAGGTTCTCCAGA	RT-PCR
<i>rpsL</i> -RT-F	GTGGCGTATGCACCCGCGTATACAC	RT-PCR
<i>rpsL</i> -RT-R	CCTTTACACGACCGCCACGGATCAG	RT-PCR
<i>pqsB</i> -RT-F	CCATCTGATCGAGAACCGCGACAT	RT-PCR
<i>pqsB</i> -RT-R	ACTCGCTGTCCACTTCCAATCCCT	RT-PCR
<i>pqsC</i> -RT-R	GAACGGCGAGAACGAGTTCAGCCT	RT-PCR
<i>pqsC</i> -RT-F	AATCCAATGGCAGCGCCCGACTCA	RT-PCR
<i>pqsD</i> -RT-F	GCCAGGTCAGCAATCATGACCTGG	RT-PCR
<i>pqsD</i> -RT-R	GGTGTTACCAGCAACAGGTCGAT	RT-PCR
<i>pqsE</i> -RT-F	ATCAGCCGGGATGCCGAATTGGTT	RT-PCR
<i>pqsE</i> -RT-R	AACAGTTGCCGGTTCAAGCGCTCG	RT-PCR
<i>pqsS</i> -MINI-F	AGGTCGACGGTATCGTGCTTGACGAAAAGT	<i>pqsS</i> cloning
<i>pqsS</i> -MINI-R	AAAGCTGGAGCTCCACCGGGCCTTTCCTGT	<i>pqsS</i> cloning
MINI- <i>pqsS</i> -F	ACAGGAAAGGCCCGGTGGAGCTCCAGCTTT	<i>pqsS</i> cloning
MINI- <i>pqsS</i> -R	ACTTTTCGTCAAGCACGATACCGTCGACCT	<i>pqsS</i> cloning
<i>pqsS</i> -PJV-F	GAATTGTGAGCGGATAACAAGCTCCAAGCACCCCTGCCAC	<i>pqsS</i> cloning
<i>pqsS</i> -PJV-R	TCTTTCGACTGAGCCTTTCGTGCTTGACGAAAAGTATGCG	<i>pqsS</i> cloning
PJV- <i>pqsS</i> -F	CGCATACTTTTCGTCAAGCACGAAAGGCTCAGTCGAAAG	<i>pqsS</i> cloning
PJV- <i>pqsS</i> -R	AGTGGCAGGGTGCTTGGAGCTTGTTATCCGCTCACAATTC	<i>pqsS</i> cloning
<i>pqsS</i> -TRANS-F	TAATACGACTCACTATAGGGTGCTTGACGAAAAGTATGCG TCGGTTTCTCGGAGGCTCGTTTTCTTGGGCGGGGAGTTTC GAACCTG	<i>In vitro</i> transcription
<i>pqsS</i> -TRANS-R	AAACAAACAGATAAAAAGAACAGATGAAAGAACAGATAA GAAAACAGATGGAACCGGCTCGCCTTGCTGCTCCCTCCC CTACCGTGACCGG	<i>In vitro</i> transcription
PqsS- <i>pqsL</i> *- TRANS-R	AAACAAACAGATAAAAAGAACAGATGAAAGAACAGATAA GAAAACAGATGGAACCGGCTCGCCTACGACGACCCTCCC CTACCGTGACCGG	<i>In vitro</i> transcription
<i>pqsL</i> -pucp20-flag--R	TCACTTGTCGTCATCGTCCTTGTAGTCGCCGAGCGGCGCC GGCGACC	Western
<i>pqsL</i> -pucp20-flag-F	GACTACAAGGACGATGACGACAAGTGAGGCACTGGCCGT CGTTTTAC	Western
5S rRNA probe	GGGGAGACCCCACTACCATCGGCGATGCGTCGTTTCAC TTCTGAGTTCGGG	Northern probe
PqsS sRNA probe	ATGAGACCGTTCGATTCCGCCGCCAACAAGGTTCTGGAA CGCTGGCAGGA	Northern probe

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Supplementary Table 2. The sRNAs were screened by analysing previous Hi-GRIL-seq data.

Start	End	Length	Strand	Potential sRNA Name
901047	901519	472	-	IG_minus_901047_901519 (PqsS)
785571	785968	397	+	IG_plus_785571_785968
785571	785968	397	+	IG_plus_785571_785968
5308994	5309325	331	+	IG_plus_5308994_5309325
5308994	5309325	331	+	IG_plus_5308994_5309325
5131156	5131427	271	+	IG_plus_5131156_5131427
5131156	5131427	271	+	IG_plus_5131156_5131427
5131156	5131427	271	+	IG_plus_5131156_5131427
5131156	5131427	271	+	IG_plus_5131156_5131427
5131156	5131427	271	+	IG_plus_5131156_5131427
5131156	5131427	271	+	IG_plus_5131156_5131427
124600	124809	209	+	IG_plus_124600_124809

200 **Supplementary Table 3.** The *pqs* QS-related and other differentially expressed genes
 201 in the *P. aeruginosa* $\Delta pqsS$ RNA sequencing data.

Gene name	Function	Fold change	P value ^a
The <i>pqs</i> QS-related genes:			
<i>pqsL</i>	Monooxygenase	5.20930	2.85E-05
<i>pqsA</i>	anthranilate--CoA ligase and AMP-binding enzyme	0.16521	4.96E-05
<i>pqsB</i>	Hypothetical protein	0.14374	2.31E-10
<i>pqsC</i>	Hypothetical protein	0.17314	5.19E-06
<i>pqsD</i>	3-oxoacyl-ACP synthase	0.15197	6.38E-10
<i>pqsE</i>	Thioesterase	0.15214	3.23E-06
162 upregulated differentially expressed genes:			
<i>PA3298</i>	2-2C4-dienoyl-CoA reductase family	259.46712	2.59E-06
<i>PA1568</i>	Porin	193.34762	4.96E-05
<i>PA5404</i>	Hypothetical protein	31.62348	3.28E-07
<i>PA1955</i>	Transporter	25.59257	5.19E-06
<i>PA1918</i>	ABC transporter permease	20.09092	2.43E-11
<i>hisQ</i>	Hypothetical protein	18.28614	1.66E-08
<i>PA5405</i>	ABC transporter permease	17.24208	8.17E-05
<i>pvdN</i>	Flavohemo protein	16.98193	4.31E-08
<i>PA2058</i>	Hypothetical protein	16.33314	8.40E-10
<i>PA0240</i>	Porin	16.29055	1.20E-12
<i>PA3379</i>	Hypothetical protein	15.77791	1.67E-08
<i>PA2340</i>	Porin	15.48133	2.75E-06
<i>pvcB</i>	ABC transporter	15.35166	2.14E-07
<i>PA0443</i>	Hypothetical protein	14.61569	2.68E-11
<i>fadH2</i>	4-hydroxybenzoate transporter	14.17679	5.02E-13
<i>PA4149</i>	Tonb-dependent receptor	14.06633	7.72E-11
<i>PA2213</i>	Major facilitator superfamily transporter	14.05869	8.83E-09
<i>PA0692</i>	Major facilitator superfamily transporter	14.00294	3.14E-10
<i>PA4178</i>	Hypothetical protein	13.80823	1.33E-07
<i>PA4860</i>	Hypothetical protein	13.67893	1.27E-10
<i>PA3953</i>	Phosphate transporter	13.26758	2.26E-08
<i>PA4137</i>	Aminotransferase	12.40604	7.35E-10
<i>xphA</i>	Porin	11.96166	3.30E-07
<i>PA3897</i>	Lipolytic protein	11.95069	4.79E-09
<i>PA2292</i>	Secretion protein	11.60204	7.20E-06
<i>PA2792</i>	Hypothetical protein	11.43901	9.11E-06
<i>PA1956</i>	Resistance-nodulation-cell division (RND) efflux transporter	11.37976	7.63E-05
<i>PA3773</i>	Hypothetical protein	11.32294	3.80E-10
<i>PA2367</i>	Alcohol dehydrogenase	11.13849	0.000102
<i>pfeA</i>	Major facilitator superfamily transporter	11.08291	2.64E-08
<i>PA1873</i>	Histidine ABC transporter permease	10.89424	3.93E-07
<i>PA2836</i>	Carbon-phosphorus lyase complex subunit	10.89347	1.01E-08
<i>PA2421</i>	Aldehyde dehydrogenase	10.79535	4.69E-07
<i>PA1236</i>	Dehydrogenase	10.67718	3.15E-09

<i>PA4859</i>	Short-chain dehydrogenase	10.67324	6.63E-11
<i>toxR</i>	Hypothetical protein	10.42564	1.43E-07
<i>PA3772</i>	Hypothetical protein	10.30225	8.21E-08
<i>cupB4</i>	Ferric enterobactin receptor	10.20939	2.86E-08
<i>PA2209</i>	Chaperone cupb4	10.20202	9.65E-07
<i>pscT</i>	Major facilitator superfamily transporter	10.11509	3.15E-06
<i>PA0528</i>	Type II secretion system protein	10.08935	1.10E-07
<i>PA4364</i>	Extracellular DNA degradation protein	9.90535	1.39E-06
<i>pcaK</i>	Multidrug resistance efflux pump	9.83746	1.51E-09
<i>PA4540</i>	Major facilitator superfamily transporter	9.80456	1.02E-08
<i>PA2472</i>	Pyoverdine biosynthesis protein	9.76589	4.09E-09
<i>phnE</i>	Transporter	9.76456	3.25E-07
<i>PA0189</i>	2-2C5-diketo-D-gluconate reductase B	9.71486	3.65E-10
<i>PA2068</i>	Major facilitator superfamily transporter	9.63888	4.17E-08
<i>PA4883</i>	Hydrolase	9.21436	1.29E-07
<i>PA2339</i>	Psl and pyoverdine operon regulator	9.18613	9.01E-07
<i>PA1144</i>	Major facilitator superfamily transporter	8.85214	1.41E-08
<i>PA2006</i>	Major facilitator superfamily transporter	8.24561	4.92E-08
<i>PA2916</i>	LysE type translocator	8.19541	5.76E-07
<i>PA2880</i>	Chlorhexidine efflux transporter	8.10445	1.08E-06
<i>PA1019a</i>	thioesterase	8.04521	2.30E-05
<i>oprP</i>	Phosphate-specific outer membrane porin OprP	7.95210	2.17E-06
<i>xyly</i>	Ring hydroxylating beta subunit	7.85214	4.09E-09
<i>PA2672</i>	Type II secretion system protein	7.84578	3.09E-09
<i>PA0682</i>	HxcX atypical pseudopilin	7.83654	1.16E-07
<i>PA0824</i>	Hypothetical protein	7.82145	2.60E-06
<i>PA1352</i>	Major Facilitator Superfamily	7.81452	4.43E-09
<i>braZ</i>	Branched-chain amino acid transport protein	7.80541	1.17E-07
<i>PA4181</i>	Hypothetical protein	7.74102	1.35E-08
<i>PA4088</i>	Aminotransferase	7.65412	7.64E-09
<i>PA4097</i>	Alcohol dehydrogenase	7.52145	1.35E-08
<i>PA4652</i>	Outer membrane usher protein	7.32541	1.37E-09
<i>PA5341</i>	LysE type translocator	7.21456	2.61E-08
<i>pvcC</i>	4-hydroxyphenylacetate 3-hydroxylase	7.15842	1.69E-07
<i>PA2933</i>	Major facilitator superfamily transporter	7.05412	3.21E-08
<i>PA4087</i>	Molybdenum cofactor biosynthesis protein F	6.97412	2.18E-07
<i>PA0687</i>	Type II secretion system protein	6.95214	3.54E-08
<i>PA2474</i>	Hypothetical protein	6.85120	7.37E-07
<i>PA1237</i>	Multidrug resistance efflux pump	6.82415	4.32E-07
<i>PA2803</i>	Haloacid dehalogenase-like hydrolase	6.75412	2.15E-06
<i>PA4319</i>	Stage II sporulation protein M	6.75101	6.53E-06
<i>PA1891</i>	Hypothetical protein	6.65511	1.44E-07
<i>PA0678</i>	Type II transport protein GspH	6.64210	2.11E-08
<i>PA0800</i>	Hypothetical protein	6.62014	8.02E-07
<i>PA4099</i>	Carbohydrate-selective porin	6.60247	9.17E-06
<i>endA</i>	DNA-specific endonuclease I	6.56521	6.17E-08
<i>PA0450</i>	Phosphate transporter	6.52145	3.21E-08
<i>PA4167</i>	Diketo-D-gluconate reductase B	6.50214	3.82E-07
<i>PA0192</i>	TonB-dependent receptor	6.45871	9.37E-09
<i>PA0170</i>	Hypothetical protein	6.35412	9.17E-06

<i>PA2784</i>	Hypothetical protein	6.25411	2.82E-10
<i>PA0790</i>	Sulfite exporter TauE/SafE	6.25745	3.82E-07
<i>PA1334</i>	NADH:flavin oxidoreductase	6.14545	4.73E-06
<i>PA1577</i>	Hypothetical protein	6.10247	4.73E-06
<i>alg44</i>	Alginate biosynthesis protein Alg44	6.05241	1.97E-08
<i>PA1143</i>	Haloacid dehalogenase-like hydrolase	5.96521	5.95E-07
<i>opdE</i>	Transcriptional regulator OpdE	5.75521	1.29E-05
<i>PA3592</i>	CoA-transferase family III	5.62145	4.73E-06
<i>PA5031</i>	Short-chain dehydrogenase	5.54122	1.90E-08
<i>xylX</i>	Toluene dioxygenase subunit alpha	5.52147	1.27E-07
<i>PA0711</i>	Acetyltransferase (GNAT) domain	5.45214	6.65E-07
<i>cdhA</i>	3-hydroxybutyryl-CoA dehydrogenase	5.32457	7.49E-07
<i>PA2935</i>	Hypothetical protein	5.28478	2.44E-05
<i>PA4148</i>	Short-chain dehydrogenase	5.26541	2.21E-08
<i>PA5384</i>	Lipolytic protein	5.25641	2.07E-06
<i>PA4095</i>	Hypothetical protein	5.24571	9.66E-08
<i>fhp</i>	Flavo-hemoprotein	5.24358	6.52E-06
<i>PA2181</i>	Glutamate--cysteine ligase	5.24102	2.97E-05
<i>PA0677</i>	HxcW pseudopilin	5.23854	2.38E-06
<i>PA4193</i>	ABC transporter permease	5.23447	4.52E-08
<i>PA4100</i>	Dehydrogenase	5.22412	2.19E-06
<i>PA1972</i>	Hypothetical protein	5.22354	1.21E-08
<i>PA1845</i>	Hypothetical protein	5.22021	2.18E-05
<i>PA3219</i>	Hypothetical protein	5.21985	7.05E-07
<i>PA0219</i>	Aldehyde dehydrogenase	5.21854	1.13E-08
<i>PA4187</i>	Major facilitator superfamily transporter	5.21457	3.11E-08
<i>pqsL</i>	Monoxygenase	5.20930	2.85E-05
<i>PA5325</i>	Hypothetical protein	5.19656	7.10E-08
<i>PA5440a</i>	Hypothetical protein	5.18452	5.91E-08
<i>PA1154</i>	Hypothetical protein	5.17852	2.50E-06
<i>PA0695</i>	Hypothetical protein	5.16584	2.60E-05
<i>PA1146</i>	Iron-containing alcohol dehydrogenase	5.15452	9.14E-06
<i>PA4177</i>	Glutathione-dependent formaldehyde enzyme	5.14784	3.34E-05
<i>cdhB</i>	Carnitine dehydrogenase	5.13451	8.17E-05
<i>potB</i>	Polyamine ABC transporter permease PotB	5.12547	4.27E-06
<i>PA0166</i>	Transporter	5.11478	9.90E-07
<i>PA3136</i>	Secretion protein	5.10245	1.51E-05
<i>cupA2</i>	Chaperone CupA2	5.09851	3.34E-05
<i>PA2050</i>	RNA polymerase sigma factor	5.08954	2.26E-06
<i>PA0864</i>	Transcriptional regulator	5.07841	2.34E-05
<i>PA3522</i>	Resistance-nodulation-cell division	5.06587	8.30E-06
<i>ureA</i>	Urease subunit gamma	5.05478	5.06E-07
<i>PA1258</i>	ABC transporter permease	5.04784	3.54E-05
<i>PA4096</i>	Major facilitator superfamily transporter	5.03547	3.90E-08
<i>ppyR</i>	Psl and pyoverdine operon regulator PpyR	5.02457	8.30E-06
<i>PA4145a</i>	Hypothetical protein	5.01477	6.26E-05
<i>pvcD</i>	Paerucumarin biosynthesis protein PvcD	4.98511	6.26E-05
<i>PA5388</i>	Hypothetical protein	4.68954	9.29E-07
<i>potC</i>	Polyamine ABC transporter permease PotC	4.56874	1.17E-05

<i>PA5530</i>	MFS dicarboxylate transporter	4.52547	1.34E-05
<i>PA4098</i>	Short-chain dehydrogenase	4.14784	6.26E-05
<i>PA3037</i>	Metallo-beta-lactamase superfamily	4.09544	9.05E-07
<i>PA0685</i>	Type II secretion system protein	4.05747	3.86E-06
<i>eddB</i>	Extracellular DNA degradation protein EddB	4.01477	1.29E-05
<i>gpuP</i>	3-guanidinopropionate transporter	3.98541	1.41E-05
<i>PA0324</i>	ABC transporter permease	3.89541	3.34E-05
<i>PA0220</i>	Amino acid APC family transporter	3.85214	2.34E-05
<i>PA2004</i>	Hypothetical protein	3.78410	3.25E-05
<i>czcC</i>	Outer membrane protein CzcC	3.65412	3.11E-06
<i>PA3619</i>	Hypothetical protein	3.56541	8.17E-05
<i>mexE</i>	Resistance-nodulation-cell division	3.45711	3.39E-05
<i>PA5156</i>	Methyltransferase domain	3.35478	3.64E-05
<i>PA3926</i>	Major facilitator superfamily transporter	3.24478	1.79E-06
<i>mtlY</i>	Xylulose kinase	3.15277	3.40E-05
<i>PA1108</i>	Major facilitator superfamily transporter	3.01457	2.34E-05
<i>PA4008</i>	Hydrolase	2.99741	1.40E-05
<i>PA2835</i>	Major facilitator superfamily transporter	2.97842	1.35E-05
<i>cupA3</i>	Usher CupA3	2.89651	5.79E-07
<i>czcB</i>	Resistance-nodulation-cell division	2.85412	2.40E-05
<i>PA2276</i>	Transcriptional regulator	2.71453	2.40E-05
<i>PA3359</i>	Hypothetical protein	2.65477	2.46E-06
<i>PA2057</i>	Hypothetical protein	2.56412	9.14E-06
<i>PA4622</i>	Major facilitator superfamily transporter	2.41547	3.34E-05
<i>hasD</i>	Transporter HasD	2.35478	8.17E-05
<i>PA4194</i>	ABC transporter permease	2.25647	1.40E-05
<i>PA0205</i>	ABC transporter permease	2.14578	4.19E-05
<i>PA3774</i>	Acetylpolyamine aminohydrolase	2.02145	1.47E-05

130 downregulated differentially expressed genes:

<i>mmsB</i>	3-hydroxyisobutyrate dehydrogenase	0.05806	3.14E-18
<i>mmsA</i>	Methylmalonate-semialdehyde dehydrogenase	0.06860	7.96E-17
<i>PA1784</i>	Hypothetical protein	0.07722	3.40E-15
<i>PA1190</i>	Hypothetical protein	0.09246	4.19E-14
<i>PA0776</i>	Hypothetical protein	0.07961	1.34E-13
<i>mdcD</i>	Malonate decarboxylase subunit beta	0.09863	2.53E-13
<i>PA0736a</i>	Hypothetical protein	0.10409	3.21E-13
<i>PA0588</i>	Hypothetical protein	0.10698	5.30E-13
<i>PA5183</i>	Hypothetical protein	0.10244	5.64E-13
<i>PA5182</i>	Hypothetical protein	0.11459	2.21E-12
<i>aprA</i>	Alkaline metalloproteinase	0.11234	3.03E-12
<i>PA3417</i>	Pyruvate dehydrogenase E1 component subunit alpha	0.11523	3.21E-12
<i>pstS</i>	Phosphate ABC transporter substrate-binding protein	0.11923	5.00E-12
<i>PA0737</i>	Hypothetical protein	0.11972	5.38E-12
<i>popB</i>	Translocator protein	0.12084	8.03E-12
<i>PA1041</i>	Hypothetical protein	0.12121	8.32E-12
<i>PA1888</i>	Hypothetical protein	0.12134	9.02E-12
<i>PA3334</i>	Acyl carrier protein	0.12436	1.16E-11
<i>PA2562</i>	Hypothetical protein	0.12516	1.28E-11

<i>mdcE</i>	Malonate decarboxylase subunit gamma	0.12637	1.34E-11
<i>pprB</i>	Two-component response regulator	0.12708	1.47E-11
<i>lecB</i>	Fucose-binding lectin PA-IIL	0.12793	4.29E-11
<i>PA3963a</i>	Hypothetical protein	0.13499	5.53E-11
<i>PA3416</i>	Pyruvate dehydrogenase E1 component subunit beta	0.13476	7.31E-11
<i>rmf</i>	Ribosome modulation factor	0.13800	9.87E-11
<i>PA2573</i>	Chemotaxis transducer	0.13716	1.12E-10
<i>popD</i>	Translocator outer membrane protein	0.13835	1.48E-10
<i>pstB</i>	Phosphate ABC transporter ATP-binding protein	0.13904	1.49E-10
<i>aer2</i>	Aerotaxis transducer Aer2	0.14159	1.76E-10
<i>pcrH</i>	Regulatory protein	0.14153	1.77E-10
<i>pqsB</i>	Hypothetical protein	0.14374	2.31E-10
<i>pcrV</i>	Type III secretion protein	0.15052	2.74E-10
<i>ldh</i>	Leucine dehydrogenase	0.15057	2.91E-10
<i>PA5061</i>	Hypothetical protein	0.15065	4.50E-10
<i>PA1728</i>	Hypothetical protein	0.15075	5.07E-10
<i>mdcA</i>	Malonate decarboxylase subunit alpha	0.15087	5.09E-10
<i>PA3332</i>	Hypothetical protein	0.15092	5.55E-10
<i>pqsD</i>	3-oxoacyl-ACP synthase	0.15197	6.38E-10
<i>pqsE</i>	Thioesterase	0.15214	3.23E-06
<i>PA1343</i>	Hypothetical protein	0.15254	9.73E-10
<i>PA0209</i>	2-(5"-triphosphoribosyl)-3'-dephosphocoenzyme-A synthase	0.15277	1.03E-09
<i>PA3415</i>	Branched-chain alpha-keto acid dehydrogenase subunit E2	0.15372	1.24E-09
<i>PA2260</i>	Hypothetical protein	0.15679	1.30E-09
<i>PA2504</i>	Hypothetical protein	0.15738	1.53E-09
<i>PA1545</i>	Hypothetical protein	0.15806	1.54E-09
<i>PA3723</i>	FMN oxidoreductase	0.16021	2.06E-09
<i>PA0743</i>	NAD-dependent L-serine dehydrogenase	0.16195	2.09E-09
<i>PA2618</i>	Arginyl-tRNA--protein transferase	0.16369	2.19E-09
<i>phnA</i>	Anthranilate synthase component I	0.16449	2.39E-09
<i>pqsA</i>	Anthranilate--CoA ligase and AMP-binding enzyme	0.16521	4.96E-05
<i>pqsC</i>	Hypothetical protein	0.17314	5.19E-06
<i>PA1753</i>	Hypothetical protein	0.17411	6.01E-08
<i>PA2024</i>	Ring-cleaving dioxygenase	0.18541	6.01E-08
<i>PA2261</i>	2-ketogluconate kinase	0.19634	6.42E-08
<i>PA1494</i>	Hypothetical protein	0.20147	7.23E-08
<i>PA2815</i>	Acyl-CoA dehydrogenase	0.21437	8.29E-08
<i>fliC</i>	B-type flagellin	0.22471	1.02E-07
<i>PA3205</i>	Hypothetical protein	0.23541	1.06E-07
<i>PA0587</i>	Hypothetical protein	0.24123	1.25E-07
<i>PA4607</i>	Hypothetical protein	0.25412	1.29E-07
<i>PA4782</i>	Hypothetical protein	0.26541	1.43E-07
<i>PA1323</i>	Hypothetical protein	0.27412	1.43E-07
<i>cioB</i>	Cyanide insensitive terminal oxidase	0.28541	1.85E-07
<i>exoS</i>	Exoenzyme S	0.29563	1.85E-07
<i>PA4702</i>	Hypothetical protein	0.30217	1.91E-07
<i>PA0747</i>	Aldehyde dehydrogenase	0.30314	2.05E-07

<i>PA1324</i>	Hypothetical protein	0.30589	2.11E-07
<i>PA0586</i>	Hypothetical protein	0.30895	2.15E-07
<i>fliD</i>	B-type flagellar hook-associated protein	0.30985	2.24E-07
<i>PA1887</i>	Hypothetical protein	0.30995	2.56E-07
<i>PA3691</i>	Hypothetical protein	0.31123	2.65E-07
<i>fabH2</i>	3-oxoacyl-ACP synthase III	0.31245	2.78E-07
<i>PA4357</i>	Hypothetical protein	0.31358	2.81E-07
<i>cioA</i>	Cyanide insensitive terminal oxidase	0.31852	2.95E-07
<i>PA2384</i>	Hypothetical protein	0.32147	5.07E-10
<i>PA2604</i>	Hypothetical protein	0.33024	5.09E-10
<i>PA1471</i>	Hypothetical protein	0.33457	5.55E-10
<i>PA3023</i>	Lipid kinase	0.33687	6.38E-10
<i>mucA</i>	Sigma factor AlgU negative regulator	0.34241	7.61E-10
<i>PA0738</i>	Hypothetical protein	0.34814	9.73E-10
<i>PA1118</i>	Hypothetical protein	0.34851	1.03E-09
<i>PA3919</i>	Hypothetical protein	0.34895	1.24E-09
<i>PA3330</i>	Short-chain dehydrogenase	0.35123	1.30E-09
<i>lptF</i>	Outer membrane porin F	0.35245	1.53E-09
<i>PA0327</i>	Hypothetical protein	0.35365	1.54E-09
<i>PA1852</i>	Hypothetical protein	0.35457	2.06E-09
<i>PA1559</i>	Male sterility protein	0.35587	2.09E-09
<i>pchE</i>	Dihydroaeruginosic acid synthetase	0.35684	2.19E-09
<i>phoU</i>	Phosphate uptake regulatory protein	0.35784	2.39E-09
<i>PA1093</i>	Hypothetical protein	0.35795	2.50E-09
<i>aprI</i>	Alkaline proteinase inhibitor AprI	0.35799	2.57E-09
<i>exoT</i>	Exoenzyme T	0.36021	2.73E-09
<i>PA4573</i>	Hypothetical protein	0.36035	3.20E-09
<i>mdcC</i>	Malonate decarboxylase acyl carrier	0.36085	3.37E-09
<i>PA0366</i>	Coniferyl aldehyde dehydrogenase	0.36123	3.59E-09
<i>pmrA</i>	Two-component regulator system	0.36158	3.67E-09
<i>pstA</i>	Phosphate ABC transporter permease	0.36201	3.91E-09
<i>PA3796</i>	Hypothetical protein	0.35784	3.98E-09
<i>PA3207</i>	Hypothetical protein	0.35862	4.31E-09
<i>PA3260</i>	Transcriptional regulator	0.36012	4.43E-09
<i>pchF</i>	Pyochelin synthetase	0.36514	4.56E-09
<i>PA2621</i>	ATP-dependent Clp protease adapter	0.36841	4.60E-09
<i>phhA</i>	Phenylalanine 4-monooxygenase	0.36952	4.68E-09
<i>PA2864</i>	Hypothetical protein	0.37012	4.89E-09
<i>PA0213</i>	Phosphoribosyl-CoA transferase	0.37125	4.89E-09
<i>PA4692</i>	Sulfite oxidase subunit YedY	0.37225	4.96E-09
<i>PA2433</i>	Hypothetical protein	0.37365	5.22E-09
<i>PA1344</i>	Short-chain dehydrogenase	0.37414	5.45E-09
<i>phhB</i>	Carbinolamine dehydratase	0.37568	5.61E-09
<i>phhC</i>	Aromatic amino acid aminotransferase	0.38012	5.71E-09
<i>bdIA</i>	Biofilm dispersion protein	0.38245	6.13E-09
<i>phnB</i>	Anthranilate synthase component II	0.38356	6.42E-09
<i>flp</i>	Type IVb pilin Flp	0.38478	7.44E-09
<i>PA0060</i>	Hypothetical protein	0.37414	8.04E-09
<i>PA5546</i>	Hypothetical protein	0.37685	8.09E-09
<i>exsC</i>	Exoenzyme S synthesis protein ExsC	0.37854	8.26E-09

<i>PA5285</i>	Hypothetical protein	0.38210	8.65E-09
<i>fliE</i>	Flagellar hook-basal body complex	0.38358	2.53E-08
<i>PA3441</i>	Molybdopterin-binding protein	0.39011	2.72E-08
<i>mucD</i>	Serine protease MucD	0.39895	2.75E-08
<i>algR</i>	Alginate biosynthesis protein AlgR	0.40257	3.11E-08
<i>PA3335</i>	Hypothetical protein	0.41254	3.35E-08
<i>PA1930</i>	Chemotaxis transducer	0.42157	3.76E-08
<i>PA2263</i>	2-hydroxyacid dehydrogenase	0.43578	3.97E-08
<i>PA0329</i>	Hypothetical protein	0.44512	4.41E-08
<i>PA0038</i>	Hypothetical protein	0.45124	4.81E-08
<i>PA0745</i>	Enoyl-CoA hydratase	0.46574	4.94E-08
<i>PA4495</i>	Hypothetical protein	0.47541	5.05E-08
<i>PA2288</i>	Hypothetical protein	0.48514	5.41E-08
<i>PA2262</i>	2-ketogluconate transporter	0.49512	5.72E-08

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Supplementary Table 4. Putative mRNA targets of PqsS sRNA determined by TargetRNA2.

Rank	Gene name	Alias	Energy	Pvalue ^a
1	-	PA1699	-26.41	0.0001
2	<i>liuE</i>	PA2011	-25.99	0.0002
3	-	PA3169	-24.68	0.0002
4	<i>CupB5</i>	PA4082	-21.95	0.0002
5	-	PA3845	-21.31	0.0003
6	<i>pqsL</i>	PA4190	-20.81	0.0003
7	-	PA4517	-20.11	0.0003
8	<i>recB</i>	PA4284	-19.92	0.0004
9	<i>febC</i>	PA4158	-19.42	0.0004
10	-	PA0789	-19.27	0.0004
11	-	PA3949	-19.07	0.0005
12	<i>wspA</i>	PA3708	-19.01	0.0005
13	<i>motA</i>	PA4954	-18.05	0.0005
14	<i>alkB1</i>	PA2574	-18.05	0.0006
15	-	PA3185	-17.34	0.0007
16	-	PA4962	-16.99	0.0007
17	-	PA2724	-16.85	0.0007
18	-	PA5431	-16.56	0.0008
19	-	PA2115	-16.17	0.0008
20	-	PA4802	-15.55	0.0008
21	<i>ubiA</i>	PA5358	-14.94	0.0008
22	-	PA4093	-14.80	0.0009
23	-	PA4927	-14.79	0.0010
24	-	PA0386	-14.70	0.0015
25	-	PA3093	-13.77	0.0020
26	<i>algQ</i>	PA5255	-13.45	0.0032
27	-	PA3481	-12.75	0.0043
28	-	PA2403	-12.48	0.0056
29	-	PA2771	-12.32	0.0062
30	-	PA2117	-12.16	0.0070
31	-	PA2777	-12.00	0.0075

^a: P_{value}<0.01.

Strains
<i>P. aeruginosa</i> strain 12-4-4(59)
<i>P. aeruginosa</i> PAO1
<i>P. aeruginosa</i> strain W16407
<i>P. aeruginosa</i> strain H47921
<i>P. aeruginosa</i> strain 97
<i>P. aeruginosa</i> strain F5677
<i>P. aeruginosa</i> strain Y82
<i>P. aeruginosa</i> strain RIVM-EMC2982
<i>P. aeruginosa</i> strain Y89
<i>P. aeruginosa</i> strain WCHPA075019
<i>P. aeruginosa</i> strain PA1242
<i>P. aeruginosa</i> strain PA1088
<i>P. aeruginosa</i> strain 763266
<i>P. aeruginosa</i> strain X78812
<i>P. aeruginosa</i> LES431
<i>P. aeruginosa</i> DHS01
<i>P. aeruginosa</i> strain CCUG 70744
<i>P. aeruginosa</i> strain PA_150577
<i>P. aeruginosa</i> strain NHmuc
<i>P. aeruginosa</i> DK2
<i>P. aeruginosa</i> SCV20265
<i>P. aeruginosa</i> strain W45609
<i>P. aeruginosa</i> strain W60856
<i>P. aeruginosa</i> strain Pa1207
<i>P. aeruginosa</i> strain S86968
<i>P. aeruginosa</i> strain P9670
<i>P. aeruginosa</i> strain T38079
<i>P. aeruginosa</i> strain F22031
<i>P. aeruginosa</i> strain CF39S
<i>P. aeruginosa</i> strain PA121617
<i>P. aeruginosa</i> strain H27930
<i>P. aeruginosa</i> strain F23197
<i>P. aeruginosa</i> strain PB354
<i>P. aeruginosa</i> strain PB353
<i>P. aeruginosa</i> PA1
<i>P. aeruginosa</i> strain PA_154197
<i>P. aeruginosa</i> B136-33
<i>P. aeruginosa</i> strain MRSN12280
<i>P. aeruginosa</i> strain E6130952
<i>P. aeruginosa</i> strain PB367
<i>P. aeruginosa</i> strain PB350
<i>P. aeruginosa</i> strain BAMC 07-48
<i>P. aeruginosa</i> strain PB369
<i>P. aeruginosa</i> strain PB368
<i>P. aeruginosa</i> UCBPP-PA14
<i>P. aeruginosa</i> strain M37351
<i>P. aeruginosa</i> strain M1608
<i>P. aeruginosa</i> strain Pa58
<i>P. aeruginosa</i> strain PASGNM699
<i>P. aeruginosa</i> strain PASGNM345
<i>P. aeruginosa</i> strain PA34
<i>P. aeruginosa</i> strain PA_D22
<i>P. aeruginosa</i> strain PA_D5
<i>P. aeruginosa</i> strain PA_D1
<i>P. aeruginosa</i> strain PA_D9
<i>P. aeruginosa</i> strain PA_D2
<i>P. aeruginosa</i> strain PA_D21

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Supplementary Table 6. The *pqsS* was highly expressed in published clinical RNA-seq.

Samples	Reads	RPKM ^a
Human sputum ^b	16	3.98024
Human sputum ^c	11	/
SARS-CoV2 infected human sputum ^d	9	/
Pig lung ^e	9	4.50312
Mouse eyeball ^f	2	18.55972

^a RPKM, Reads per kilobase of transcript per million mapped reads.

^bCornforth, D. M., Dees, J. L., Ibberson, C. B., Huse, H. K., Mathiesen, I. H., Kirketerp-Møller, K., . . . Whiteley, M. (2018). *Pseudomonas aeruginosa* transcriptome during human infection. *Proc Natl Acad Sci U S A*, 115(22), E5125-e5134. doi:10.1073/pnas.1717525115.

^cRossi, E., Falcone, M., Molin, S., & Johansen, H. K. (2018). High-resolution in situ transcriptomics of *Pseudomonas aeruginosa* unveils genotype independent patho-phenotypes in cystic fibrosis lungs. *Nat Commun*, 9(1), 3459. doi:10.1038/s41467-018-05944-5.

^dQu, J., Cai, Z., Liu, Y., Duan, X., Han, S., Liu, J., . . . Yang, L. (2021). Persistent Bacterial Coinfection of a COVID-19 Patient Caused by a Genetically Adapted *Pseudomonas aeruginosa* Chronic Colonizer. *Front Cell Infect Microbiol*, 11, 641920. doi:10.3389/fcimb.2021.641920.

^eHarrington NE, Littler JL, Harrison F. Transcriptome Analysis of *Pseudomonas aeruginosa* Biofilm Infection in an Ex Vivo Pig Model of the Cystic Fibrosis Lung. *Appl Environ Microbiol*. 2022 Feb 8;88(3):e0178921. doi: 10.1128/AEM.01789-21.

^fYam JKH, Aung TT, Chua SL, Cheng Y, Kohli GS, Zhou J, Constancias F, Liu Y, Cai Z, Salido MMS, Drautz-Moses DI, Rice SA, Schuster SC, Boo ZZ, Wu B, Kjelleberg S, Tolker-Nielsen T, Lakshminarayanan R, Beuerman RW, Yang L, Givskov M. Elevated c-di-GMP Levels and Expression of the Type III Secretion System Promote Corneal Infection by *Pseudomonas aeruginosa*. *Infect Immun*. 2022 Aug 18;90(8):e0006122. doi: 10.1128/iai.00061-22.