# Supporting Information

## Imperi et al. 10.1073/pnas.1222706110

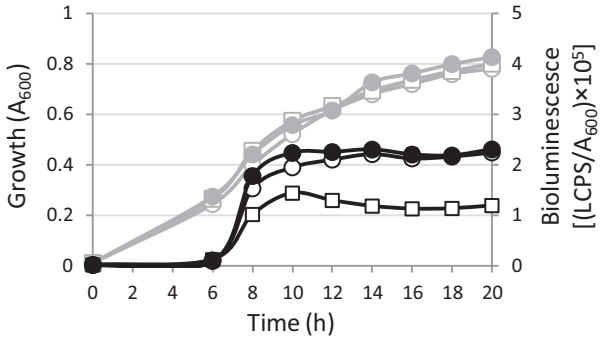


**Fig. S1.** Response of the PvdS-dependent *pvdE* promoter (P*pvdE*) *::lux* reporter to pyoverdine signaling. Bacterial growth (gray lines, left *y* axis) and bioluminescence emission (black lines, right *y* axis) of PAO1 P*pvdE::lux* (filled circles), PAO1*pvdA* P*pvdE::lux* (open squares), and PAO1*pvdA* P*pvdE::lux* plus 10 μM exogenously added pyoverdine (open circles) cultured in TSBD medium at 37 °C in microtiter plates. Values represent the mean of five independent assays (SD < 9% of the mean values).

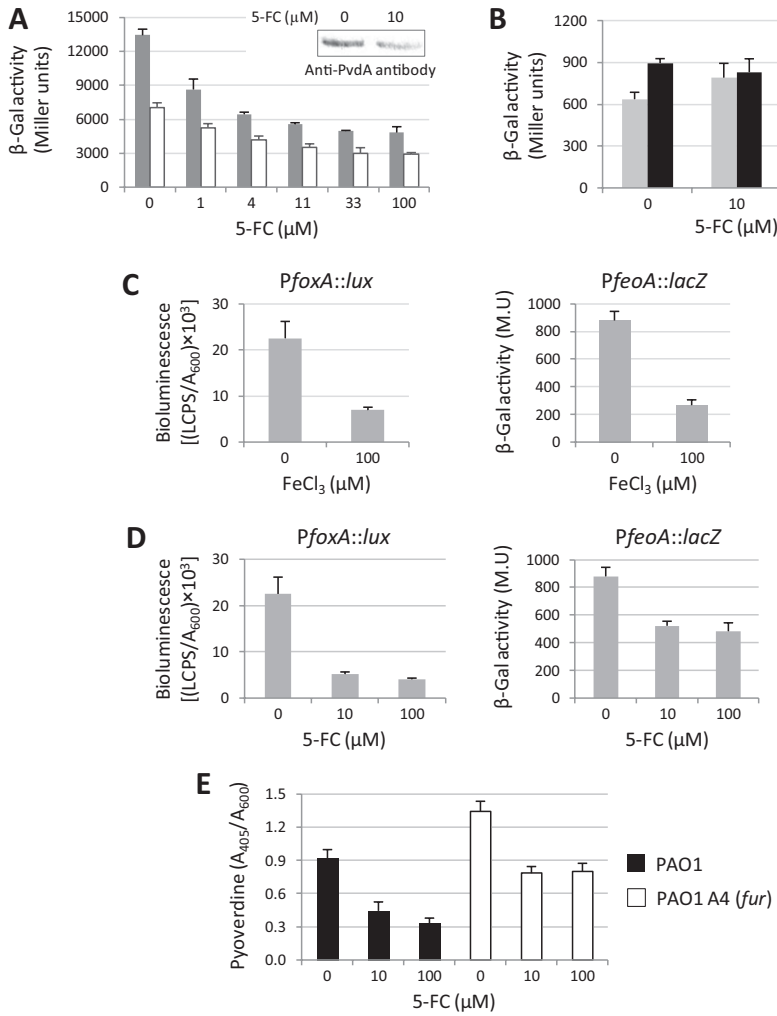


**Fig. S2.** 5-Fluorocytosine (5-FC) specifically inhibits transcription of iron-dependent genes. (*A*) Effect of 5-FC (0–100 μM) on β-gal expression by PAO1 P*pvdA*::*lacZ* (gray bars) and PAO1 P*pvdD*::*lacZ* (white bars) at 14 h of growth in TSBD. *Inset* shows a representative anti-PvdA Western blot on whole-cell lysates from 5-FC–treated or -untreated *Pseudomonas aeruginosa* PAO1 cells grown for 14 h in TSBD. (*B*) Effect of 5-FC (0–100 μM) on β-gal expression by PAO1 P*proC*::*lacZ* at 8 and 14 h of growth (gray and black bars, respectively) in TSBD. Values represent the mean (± SD) of at least three independent assays. (*C*) Bioluminescence emission by PAO1 P*foxA*::*lux* (*Left*) and β-gal expression by PAO1 P*feoA*::*lacZ* (*Right*) at 14 h of growth in TSBD supplemented or not supplemented with 100 μM $FeCl_3$. (*D*) Effect of 5-FC (0–100 μM) on bioluminescence emission by PAO1 P*foxA*::*lux* (*Left*) and β-gal expression by PAO1 P*feoA*::*lacZ* (*Right*) at 14 h of growth in TSBD. (*E*) Effect of 5-FC (0–100 μM) on pyoverdine production by PAO1 and the *fur* mutant PAO1 A4 expressing a Fur variant, which is strongly impaired in iron-dependent gene repression (1), at 14 h of growth in TSBD. Values represent the mean (± SD) of at least three independent assays.

1. Barton HA, Johnson Z, Cox CD, Vasil AI, Vasil ML (1996) Ferric uptake regulator mutants of Pseudomonas aeruginosa with distinct alterations in the iron-dependent repression of exotoxin A and siderophores in aerobic and microaerobic environments. *Mol Microbiol* 21(5):1001–1017.

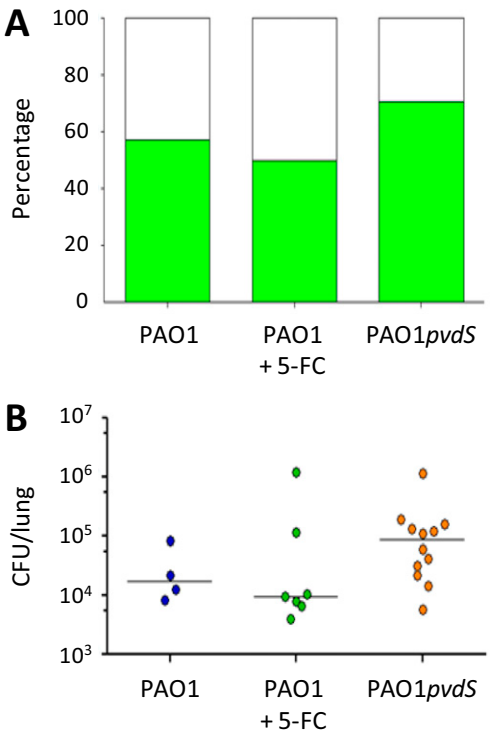


**Fig. S3.** 5-FC does not affect *P. aeruginosa* persistence in mouse lungs. (*A*) Percentage of mice showing $\geq 10^3$ viable *P. aeruginosa* cells per lung among mice surviving the pulmonary infection (corresponding to 100%) with *P. aeruginosa* PAO1 or the isogenic PAO1*pvdS* mutant treated or not treated with 5-FC (Fig. 4*A*). (*B*) Bacterial load [colony-forming unit (CFU) per lung] in the lungs of mice showing $\geq 10^3$ viable *P. aeruginosa* cells per lung. No statistically significant differences between groups ($0.16 \leq P \leq 1$) were observed (Fisher and unpaired two-tailed *t* tests for *A* and *B*, respectively).

**Table S1. *P. aeruginosa* cystic fibrosis isolates used in this study**

| Strain* | Source |
|---|---|
| BT2 | 1 |
| BT73 | 1 |
| KK1 | 1 |
| KK28 | 1 |
| KK71 | 1 |
| KK72 | 1 |
| TR1 | 1 |
| TR66 | 1 |
| FM-01 | 2 |
| FM-02 | 2 |
| FM-04 | 2 |
| FM-11 | 2 |
| FM-12 | 2 |
| FM-13 | 2 |
| FM-14 | 2 |
| FM-15 | 2 |
| FM-17 | 2 |
| FM-19 | 2 |
| FM-20 | 2 |
| FM-21 | This work |

*Numbers of FM isolates refer to the sputum samples and thus, patients from whom strains have been isolated (described in ref. 2). FM-21 was isolated from the sputum sample of a CF patient chronically infected by *P. aeruginosa* and hospitalized at the Policlinico Umberto I Hospital (Rome, Italy).

1. Bragonzi A, et al. (2009) *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am J Respir Crit Care Med* 180(2): 138–145.
2. Massai F, et al. (2011) A multitask biosensor for micro-volumetric detection of N-3-oxo-dodecanoyl-homoserine lactone quorum sensing signal. *Biosens Bioelectron* 26(8):3444–3449.

**Table S2. Bacterial strains and plasmids used in this study**

| | Genotype and/or relevant characteristics | Source |
|---|---|---|
| Strain | | |
| *P. aeruginosa* | | |
| PAO1 | WT (prototroph), type I pyoverdine producer | ATCC |
| 7NSK2 | Type II pyoverdine producer (type IIa pyoverdine receptor) | 1 |
| ATCC27853 | Type II pyoverdine producer (type IIb pyoverdine receptor) | 1 |
| LESB58 | Type III pyoverdine producer | 2 |
| PAO1*pvdS* | PAO1 Δ*pvdS,* $Gm^R$ | This work |
| PAO1*codA* | PAO1 Δ*codA*, in-frame deletion mutant in the *codA* (PA0437) gene | This work |
| PAO1*codB* | PAO1 Δ*codB*, in-frame deletion mutant in the *codB* (PA0438) gene | This work |
| PAO1*pvdA* | PAO1 Δ*pvdA* | 3 |
| K1660 | K767 Δ*fpvA* | 4 |
| PAO1*fpvR* | PAO1 *fpvR*::$Km^R$ | 5 |
| PAO1 A4 | PAO1 carrying a *fur* mutation resulting in the H86→Y substitution in the Fur protein | 6 |
| *Escherichia coli* | | |
| DH5αF′ | *recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1* Δ(*lacZYA-argF*)U169 [Φ*80*d*lacZ*ΔM15] $Nal^R$ | 7 |
| S17-1 λ*pir* | *TpR SmR recA, thi, pro, hsdR*-M+RP4: 2-Tc:Mu: $K_m$ Tn*7* λ*pir* | 8 |
| Plasmid | | |
| pEX18Tc | pMB1 replicon, *oriT sacB lacZ*α, *Mob*$^+$ $Tc^r$, allelic exchange vector | 9 |
| pPS858 | $Ap^r$; source of $Gm^r$-GFP cassette | 9 |
| pUCP18 | *E. coli–Pseudomonas* shuttle vector; Ap/$Cb^R$ | 10 |
| pDM4 | Suicide vector; *sacBR*, *oriR6K*; $Cm^R$ | 11 |
| Mini-CTX-*lux* | Promoter-probe vector containing the *luxCDABE* operon; $Tc^R$ | 12 |
| Mini-CTX-P*pvdE*::*lux* | Plasmid to insert a P*pvdE*::*lux* fusion into the chromosome of *P. aeruginosa*; $Tc^R$ | This work |
| pEXΔ*pvdS* | pEX18Tc derivative carrying the $Gm^r$-GFP cassette between flanking regions of the *pvdS* coding sequence | This work |
| pDM4Δ*codA* | pDM4 derivative carrying the flanking regions of the *codA* coding sequence | This work |
| pDM4Δ*codB* | pDM4 derivative carrying the flanking regions of the *codB* coding sequence | This work |
| pUCP*codA* | pUCP18 derivative carrying the entire *codA* coding sequence under the control of the $P_{T5}$ *lacO* region | This work |
| pUCP*codB* | pUCP18 derivative carrying the entire *codB* coding sequence under the control of the $P_{T5}$ *lacO* region | This work |
| pUCP*pvdS* | pUCP18 derivative carrying the entire *pvdS* coding sequence under the control of the $P_{T5}$ *lacO* region; $Ap^R$ | 13 |
| pMP220::P*pvdA* | pMP220 derivative carrying a P*pvdA*::*lacZ* transcriptional fusion; $Tc^R$ | 13 |
| pMP190::P*pvdD* | pMP190 derivative carrying a P*pvdD*::*lacZ* transcriptional fusion; $Cm^R$ | 14 |
| pMP190::P*pvdE* | pMP190 derivative carrying a P*pvdE*::*lacZ* transcriptional fusion; $Cm^R$ | 14 |
| pPZ-*pvdS* | pPZTC derivative carrying a P*pvdS*::*lacZ* transcriptional fusion; $Cb^R$ | 15 |
| pPZ-*toxA* | pPZ20 derivative carrying a P*toxA*::*lacZ* translational fusion; $Cb^R$ | 5 |
| pPZ-*prpL* | pPZTC derivative carrying a P*prpL*::*lacZ* transcriptional fusion; $Cb^R$ | 5 |
| pMP220::P*pchR* | pMP220 derivative carrying a P*pchR*::*lacZ* transcriptional fusion; $Tc^R$ | This work |
| pMP220::P*feoA* | pMP220 derivative carrying a P*feoA*::*lacZ* transcriptional fusion; $Tc^R$ | This work |
| pME6425 | Translational *pchE′-′lacZ* fusion, $Tc^R$ | 16 |
| pME3641 | Plasmid carrying a translational P*proC*::*lacZ* fusion, $Cb^R$ | 17 |
| Mini-CTX-*lux*::P*foxA* | Plasmid to insert a P*foxA*::*lux* fusion into the chromosome of *P. aeruginosa*; TcR | 18 |


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2. Kukavica-Ibrulj I, et al. (2008) In vivo growth of *Pseudomonas aeruginosa* strains PAO1 and PA14 and the hypervirulent strain LESB58 in a rat model of chronic lung infection. *J Bacteriol* 190(8):2804–2813.
3. Imperi F, et al. (2008) Membrane-association determinants of the omega-amino acid monooxygenase PvdA, a pyoverdine biosynthetic enzyme from *Pseudomonas aeruginosa*. *Microbiology* 154(Pt 9):2804–2813.
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6. Barton HA, Johnson Z, Cox CD, Vasil AI, Vasil ML (1996) Ferric uptake regulator mutants of *Pseudomonas aeruginosa* with distinct alterations in the iron-dependent repression of exotoxin A and siderophores in aerobic and microaerobic environments. *Mol Microbiol* 21(5):1001–1017.
7. Liss L (1987) New M13 host: DH5 F′ competent cells. *Focus* 9:13.
8. Simon R, Priefer U, Puhler A (1983) A broad host range mobilization system for in vivo genetic engineering: Transposon mutagenesis in Gram-negative bacteria. *Biotechnology (NY)* 1:784–791.
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13. Ambrosi C, Tiburzi F, Imperi F, Putignani L, Visca P (2005) Involvement of AlgQ in transcriptional regulation of pyoverdine genes in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* 187(15):5097–5107.
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15. Ochsner UA, Wilderman PJ, Vasil AI, Vasil ML (2002) GeneChip expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: Identification of novel pyoverdine biosynthesis genes. *Mol Microbiol* 45(5):1277–1287.
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17. Savioz A, Zimmermann A, Haas D (1993) *Pseudomonas aeruginosa* promoters which contain a conserved GG-N10-GC motif but appear to be RpoN-independent. *Mol Gen Genet* 238(1–2):74–80.
18. Mettrick KA, Lamont IL (2009) Different roles for anti-sigma factors in siderophore signalling pathways of *Pseudomonas aeruginosa*. *Mol Microbiol* 74(5):1257–1271.

**Table S3. Primers used in this study**

| Primer name | Sequence* | Restriction site | Application |
|---|---|---|---|
| *pvdS* mut_UP_FW | 5′-CGGAATTCCGTCTCCTGCTGCGC-3′ | EcoRI | Generation of the PAO1*pvdS* deletion mutant |
| *pvdS* mut_UP_RV | 5′-ACGCGTCGACCGACATGGAAATCACCTTG-3′ | SalI | Generation of the PAO1*pvdS* deletion mutant |
| *pvdS* mut_DOWN_FW | 5′-ACGCGTCGACTGACGGCGGCGAGCATTC-3′ | SalI | Generation of the PAO1*pvdS* deletion mutant |
| *pvdS* mut_DOWN_RV | 5′-CCCAAGCTTCCGTCCCCAGCCTC-3′ | HindIII | Generation of the PAO1*pvdS* deletion mutant |
| *codA* mut_UP_FW | 5′-GCTCTAGACCGCCGTGGTGGTGTGC-3′ | XbaI | Generation of the PAO1*codA* in-frame deletion mutant |
| *codA* mut_UP_RV | 5′-CGGAATTCGCGGCCGCGCAGGC-3′ | EcoRI | Generation of the PAO1*codA* in-frame deletion mutant |
| *codA* mut_DOWN_FW | 5′-CGGAATTCGACGCGGTGCGCCG-3′ | EcoRI | Generation of the PAO1*codA* in-frame deletion mutant |
| *codA* mut_DOWN_RV | 5′-GGCCTGCTCGAGGTCTGG-3′ | XhoI | Generation of the PAO1*codA* in-frame deletion mutant |
| *codB* mut_UP_FW | 5′-GCTCTAGATGGACAGCCATGGCTAC-3′ | XbaI | Generation of the PAO1*codB* in-frame deletion mutant |
| *codB* mut_UP_RV | 5′-CGGAATTCCGCCAGCGGGACCGG-3′ | EcoRI | Generation of the PAO1*codB* in-frame deletion mutant |
| *codB* mut_DOWN_FW | 5′-CGGAATTCGCTTGCGGCACCGCC-3′ | EcoRI | Generation of the PAO1*codB* in-frame deletion mutant |
| *codB* mut_DOWN_RV | 5′-GCGCACCTCGAGCATGGC-3′ | XhoI | Generation of the PAO1*codB* in-frame deletion mutant |
| *codA*_FW | 5′-CGGGATCCCCCACGGAGACTCGCG-3′ | BamHI | Construction of *codA*-complementing plasmid |
| *codA*_RV | 5′-CCCAAGCTTGTCAATCAGCATGGAGGAC-3′ | HindIII | Construction of *codA*-complementing plasmid |
| *codB*_FW | 5′-CGGGATCCAGAAGAGGTCCTCCATG-3′ | BamHI | Construction of *codB*-complementing plasmid |
| *codB*_RV | 5′-CCCAAGCTTCGCTAGAAGGCTCCGG-3′ | HindIII | Construction of *codB*-complementing plasmid |
| P*pchR*_FW | 5′-GGAAGATCTACCGTGTCGCCATGTG-3′ | BglII | Construction of P*pchR*::*lacZ* transcriptional fusion |
| P*pchR*_RV | 5′-GCGGAATTCGATGTGCGCGACGCC-3′ | EcoRI | Construction of P*pchR*::*lacZ* transcriptional fusion |
| P*feoA*_FW | 5′-CGGAATTCGACAACCGCCCGGCC-3′ | EcoRI | Construction of P*feoA*::*lacZ* transcriptional fusion |
| P*feoA*_RV | 5′-GCTCTAGATGCGGTAGGAACGGGAC-3′ | XbaI | Construction of P*feoA*::*lacZ* transcriptional fusion |

All PCRs were performed using the genomic DNA of the PAO1 strain as template.
*The restriction site used for cloning is underlined in the primer sequence.