Supplementary Information

Supplementary Movie Legends

Movie S1. Swarming assays in which *P. aeruginosa* is spotted (A) without phage or (B) with phage. (C) Pairwise swarming assay in which *P. aeruginosa* is spotted (left) without phage or (right) with phage. Time-lapse images are acquired over the course of 23 hours at 37 °C

Movie S2. Six-way swarming assays in which *P. aeruginosa* is spotted at the center. The same strain is spotted (A) without phage or (B) with phage at the satellite positions. Time-lapse images are acquired over the course of 17 hours at 37 °C.

Movie S3. Swarming assays of *P. aeruginosa* in the center and (A) rhamnolipids, (B) PQS, and (C) HHQ at the satellite positions for concentrations indicated in millimolar units. Time-lapse images are acquired over the course of 17 hours at 37 °C.

Movie S4. Swarming assays of uninfected wild-type *P. aeruginosa* in the center. The Δ*rhlAB* strain is spotted (A) without phage or (B) with phage at the satellite positions. The Δ*rhlAB* Δ*pqsA* strain is spotted (C) without phage or (D) with phage at the satellite positions. Time-lapse images are acquired over the course of 17 hours at 37 °C.

Movie S5. Swarming assays of wild-type *P. aeruginosa* in the center and the (A) wild-type, (B) Δ*rhlAB*, or (C) Δ*rhlAB* Δ*pqsA* strain mixed with gentamycin and spotted at satellite positions. Time-lapse images are acquired over the course of 18 hours at 37 °C.

Figure S1

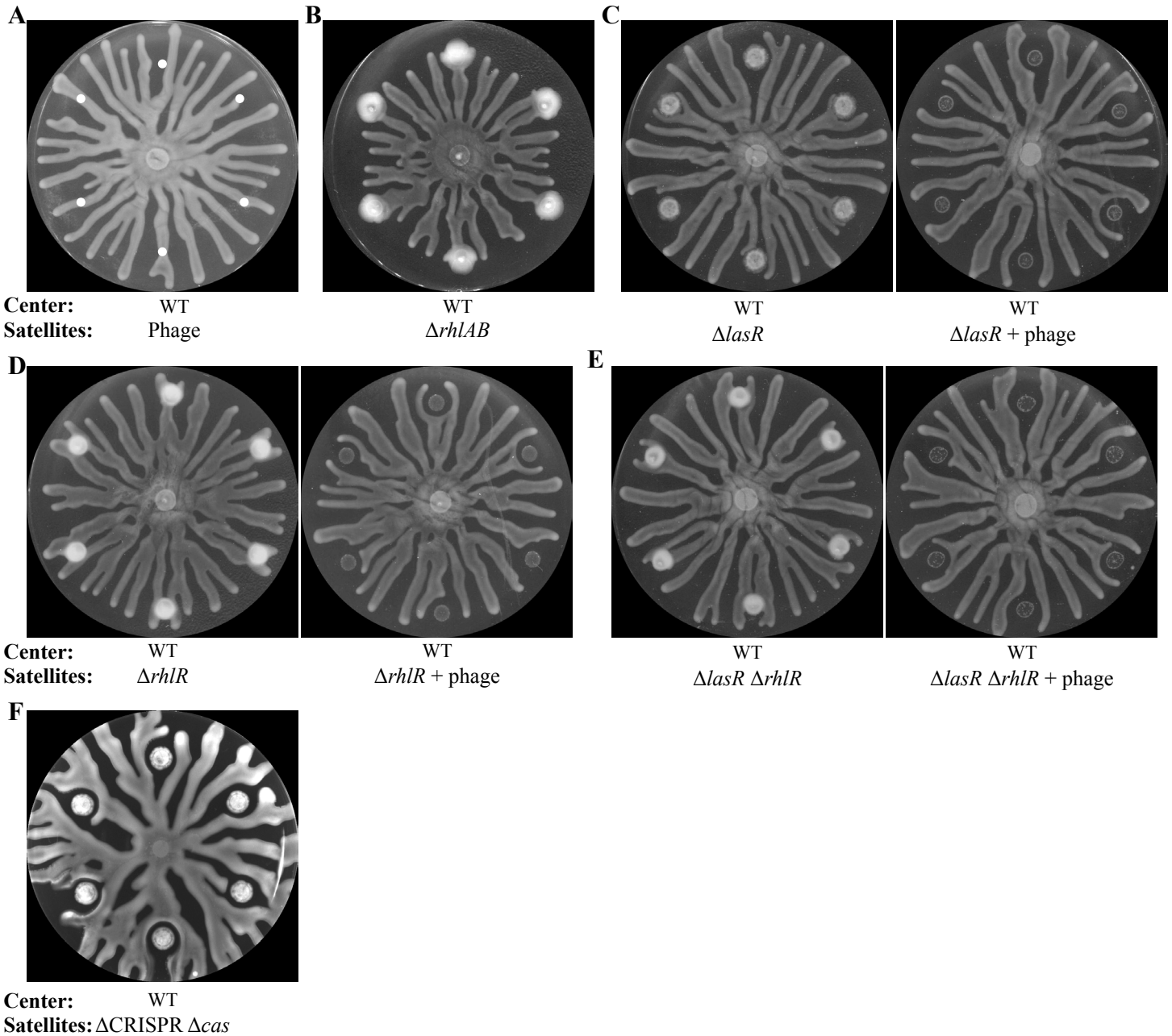
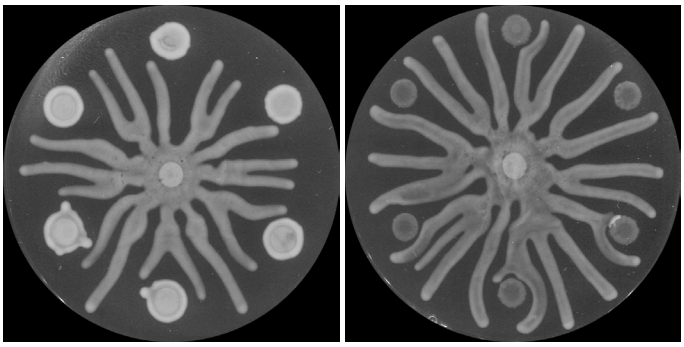


Figure S1. Swarming of wild-type *P. aeruginosa* at the center and (A) phage only at satellite positions, imaged after 16 hours of growth or (B) the Δ*rhlAB* strain at the satellite positions, imaged after 14 hours of growth. The experiment in (B) is identical to that in Fig. 3B except that image was acquired at an earlier time point. Swarming assays of wild-type *P. aeruginosa* at the center and (C) Δ*lasR*, (D) Δ*rhlR*, and (E) Δ*lasR* Δ*rhlR* spotted at the satellite positions (left images) without phage or (right images) with phage. (F) Swarming assays of wild-type *P. aeruginosa* at the center and ΔCRISPR Δ*cas* at the satellite positions.

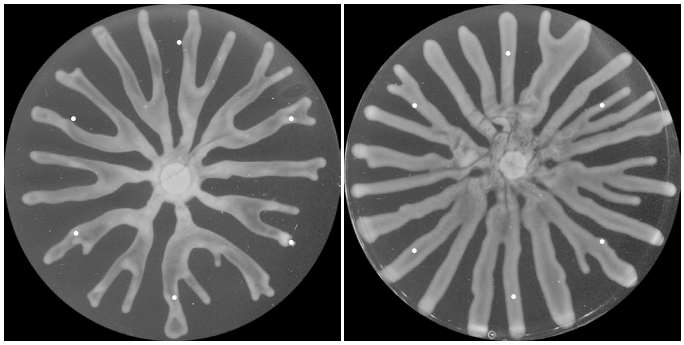
Figure S2

A



Center: WT — WT
Satellites: Δ*pqsA* — Δ*pqsA* + phage

B



Center: WT — WT
Satellites: Δ*rhlAB* filtered sup. — Δ*rhlAB* sonicated & filtered sup.

Figure S2. (A) Swarming assay of wild-type *P. aeruginosa* at the center and the Δ*pqsA* strain (left) without phage or (right) with phage. (B) Swarming assay of wild-type *P. aeruginosa* at the center and (left) filtered overnight culture or (right) sonicated and filtered overnight culture at the satellite positions. White dots at the satellite positions indicate the precise centers of positions where the cultures were spotted.

Figure S3

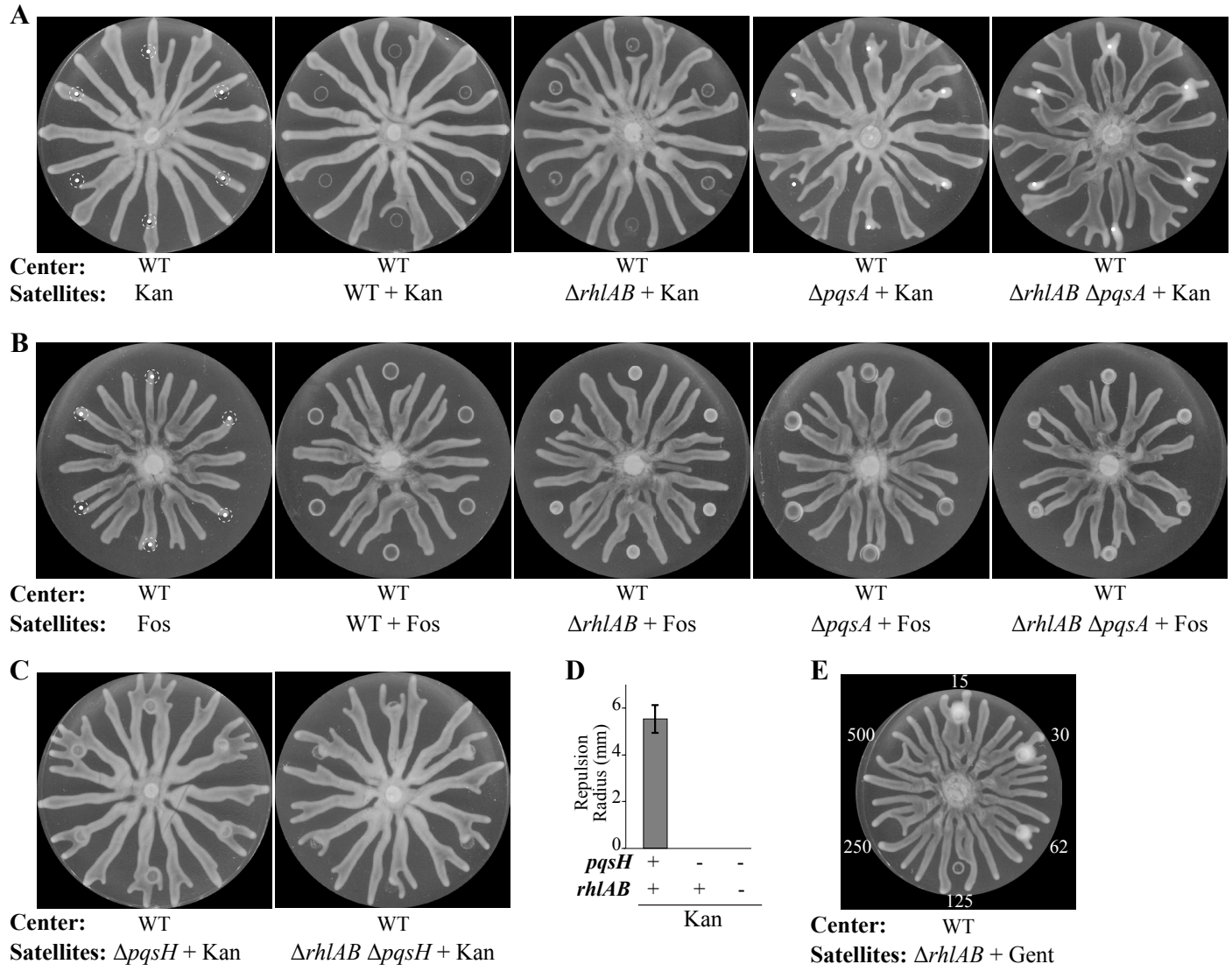


Figure S3. Swarming assay of wild-type *P. aeruginosa* at the center and 6 μL of (A) 25 mg / mL kanamycin or (B) 40 mg / mL fosfomycin spotted alone or with wild-type, Δ*rhlAB*, Δ*pqsA*, or Δ*rhlAB* Δ*pqsA P. aeruginosa* at the satellite positions on antibiotic-free plates. (C) Swarming assay with 6 μL of 25 mg / mL kanamycin spotted with the Δ*pqsH* or Δ*rhlAB* Δ*pqsH* strains at the satellite positions and (D) the corresponding repulsion radii at the satellite positions. (E) Swarming assay and (F) corresponding repulsion radii in which 6 μL of the initial culture of Δ*rhlAB* is spotted with gentamycin (concentrations in μg / mL) at the satellite positions. White dots on the satellite positions indicate the precise centers of positions where the cultures or antibiotics were spotted. The dashed lines indicate the boundaries of the initial spots. Bars are the average of at least 6 positions and error bars indicate standard deviation.

Figure S4

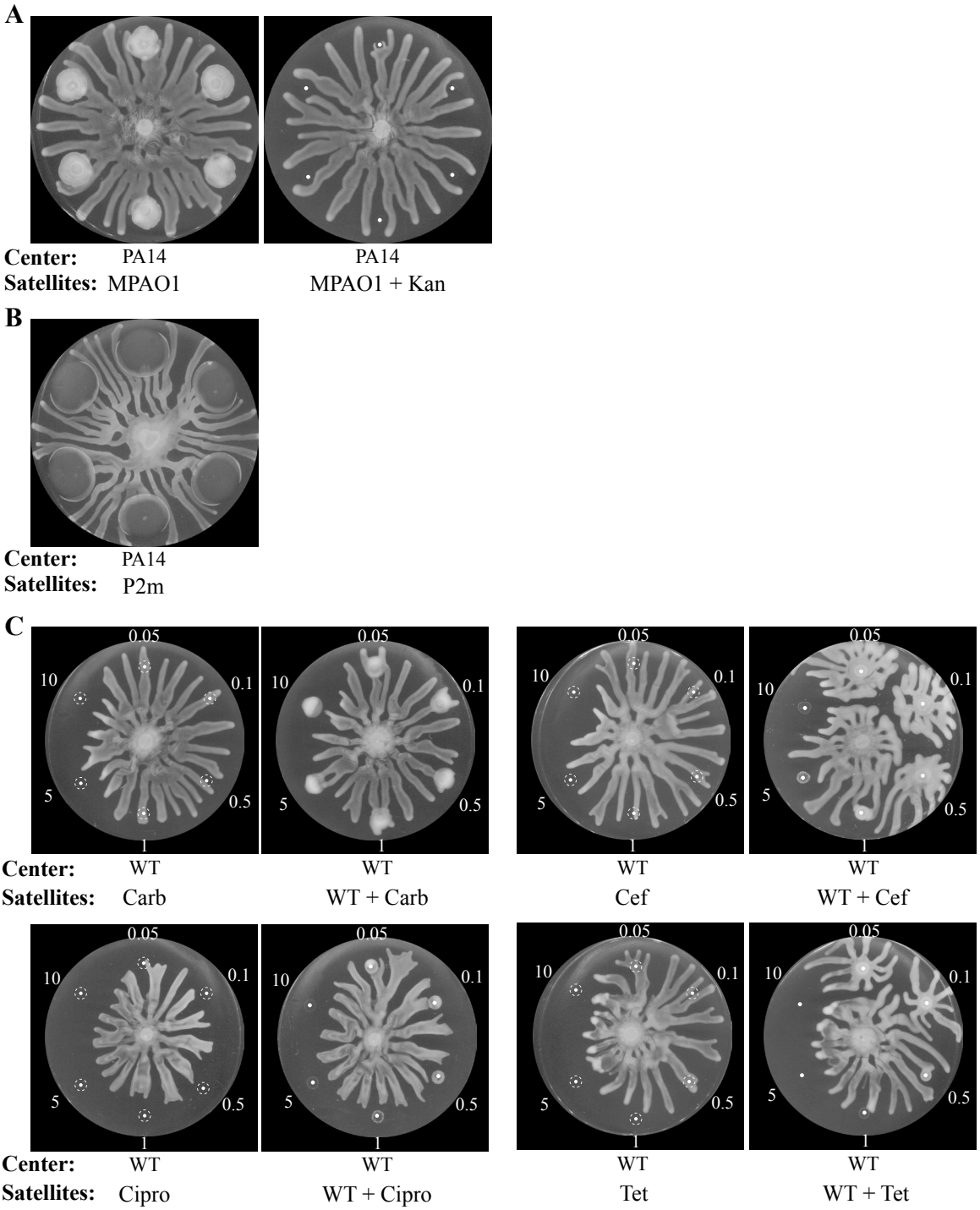


Figure S4. Swarming assay of wild-type *P. aeruginosa* PA14 at the center and 6 μL at the satellite positions on antibiotic-free plates of (A) *P. aeruginosa* MPAO1 alone or with 25 mg / mL of kanamycin or (B) *P. aeruginosa* P2m. (C) Swarming assay with carbenicillin, cefsulodin, ciprofloxacin, or tetracycline (concentrations in mg / mL) spotted alone or with wild-type *P. aeruginosa* PA14 at the satellite positions on antibiotic-free plates. White dots at the satellite positions indicate the precise centers of positions where the cultures or antibiotics were spotted. The dashed lines indicate the boundaries of the initial spots.

Figure S5

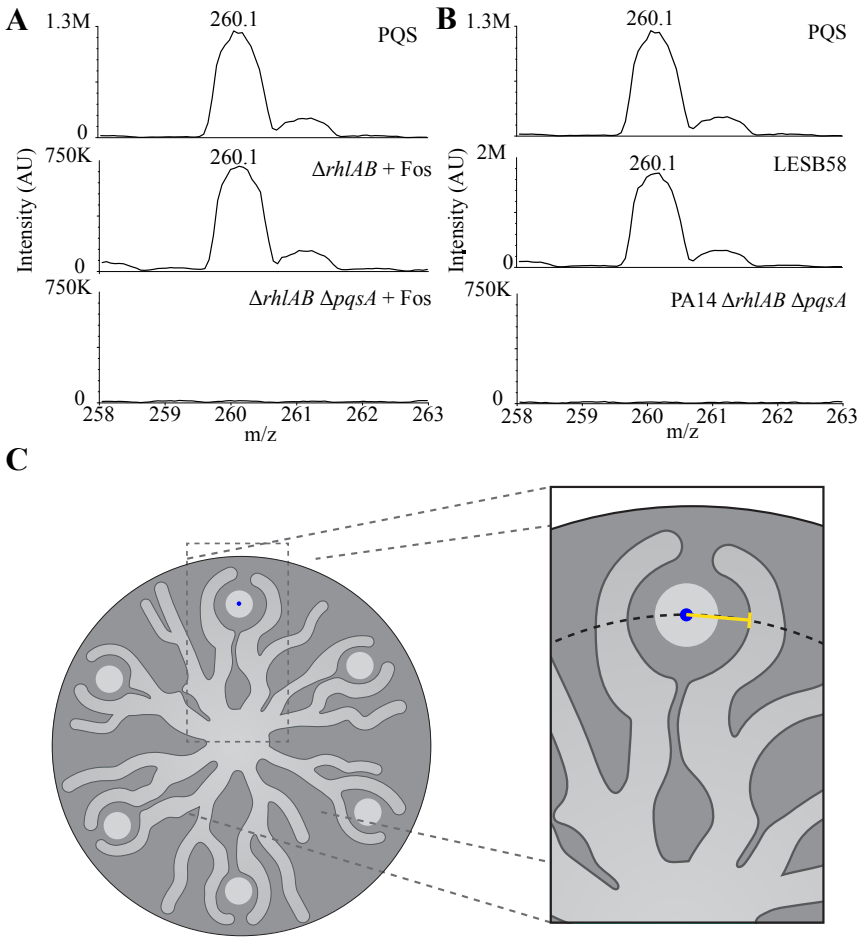


Figure S5. (A) LC-MS analysis of agar extracts for PA14 Δ*rhlAB* or PA14 Δ*rhlAB* Δ*pqsA* in which cultures are mixed with 40 mg / mL of fosfomycin. 6 µL of the mixture is spotted onto antibiotic-free swarming medium, incubated for 16-18 hours, and the area surrounding the colony is extracted. Pure 10 µM PQS, which is not spotted onto agar, is provided as a reference. (B) LC-MS analysis of agar extracts surrounding the LESB58 or PA14 Δ*rhlAB* Δ*pqsA* strains. (C) Schematic indicating the arc (dashed line) along which the radius of repulsion is measured (yellow). The blue dot indicates the center of the satellite colony.

Supplementary Tables

Table S1. Strains and plasmids

Strain or plasmid name	Lab strain name	Genotype	Reference / source
Strains			
WT	AFS27E.1	PA14 *att*Tn7::[$P_{A1/04/03}$-mCherry] *aacC1*::FRT	This study
ΔCRISPR Δ*cas*	NMHK326	PA14 ΔCRISPR Δ*cas*	(1)
AFS27E	AFS27E	PA14 *att*Tn7::[$P_{A1/04/03}$-mCherry] *aacC1*$^{+}$	(2)
Δ*lasR*	AFS20.1	PA14 Δ(*lasR*)::FRT	(3)
rhlR	SM32	PA14 Δ*rhlR*	(4)
Δ*lasR rhlR*	AFS49H	PA14 Δ(*lasR*)::FRT *rhlR*::MAR2xT7	(3)
Δ*rhlAB*	BR04.1	PA14 Δ(*rhlAB*)::FRT	This study
Δ*pqsA*	AFS79.1	AFS27E.1 Δ(*pqsA*)::FRT	This study
Δ*rhlAB* Δ*pqsA*	AFS82.1	PA14 Δ(*rhlAB*)::FRT Δ(*pqsA*)::FRT	This study
pqsH	AFS77	AFS27E.1 *pqsH*::Mar2xT7	This study
Δ*rhlAB pqsH*	BR07	PA14 Δ(*rhlAB*)::FRT *pqsH*::Mar2xT7	This study
LESB58		Hyper-virulent *P. aeruginosa* clinical isolate	(5)
P2m		Mucoid *P. aeruginosa* cystic fibrosis isolate PAmFLR02	(6)
MPAO1		*P. aeruginosa* strain MPAO1	(7), from Gitai lab
Plasmids			
pFLP2		Plasmid expressing Flp to recombine FRT sites	(8)
pAS03		Plasmid containing FRT-*aacC1*-FRT template	(9)
pUCP18-RedS		λ Red recombineering vector	(10)
Phage			
DMS3vir	DMS3vir		(11)

Table S2. Primers

Primer name	Sequence
Lambda red primers	
rhlA-lred-u1	CCCTCGAGTTCTCCAATACC
rhlA-lred-l1	CATCTCACACCTCCCAAAAATTTTC
rhlA-lred-u2	GCTGTTTGCCTGTTCGAAAATTTTTGGGAGGTGTGAGATGattccggggatccgtcgacc
rhlB-lred-l2	TCTGTTATGCCAGCACCGTTCAGGACGCAGCCTTCAGCCAtgtaggctggagctgcttcg
rhlB-lred-u3	TGGCTGAAGGCTGCGTCCTGA
rhlB-lred-l3	TCGAAGCTGGAGATGTTCTG
pqsA-lred-u1	CGACCGGCGATTCCATTTTC
pqsA-lred-l1	CATGACAGAACGTTCCCTCT
pqsA-lred-u2	ATCCGGATGCATATCGCTGAAGAGGGAACGTTCTGTCATGattccggggatccgtcgacc
pqsA-lred-l2	TTCACCCCCACAGCCTGAATCAACATGCCCGTTCCTCCGGtgtaggctggagctgcttcg
pqsA-lred-u3	CCGGAGGAACGGGCATGTTG
pqsA-lred-l3	GTTGAGGTGTCCCTTGACGT
pqsH-u1	CCTGGATGATCGTCGTGGGC
pqsH-l1	CCAACTCCTCGAGGTCGTTGT
qPCR primers	
pqsA_qPCR_u2	AGGCGGTTCTGGTTCCTAC
pqsA_qPCR_l2	GCATGTGCGAGGGAATCTG
pqsB_qPCR_u1	CTCTACGTGGTCGATACCCTCG
pqsB_qPCR_l1	CAGACTCGCTGTCCACTTCCAA
pqsH_qPCR_u1	TTCCAGTCTGGGTGGGAGTTC
pqsH_qPCR_l1	TCGAGGATCTGCACGATGGAG
rhlA_qPCR_u1	GCGCTTCGAGGTCAATCAC
rhlA_qPCR_l1	AGCATCGCCTGGTTCAGTC
rhlB_qPCR_u1	CGGCGACGTATTTCCCTTCATC
rhlB_qPCR_l1	TAGGTCAGTTCGTCGCTCAG
5S_qPCR_u1	GAACCACCTGATCCCTTCCC
5S_qPCR_l1	TAGGAGCTTGACGATGACCT

Supplementary References

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11. Cady KC, Bondy-Denomy J, Heussler GE, Davidson AR, O'Toole GA. 2012. The CRISPR/Cas Adaptive Immune System of Pseudomonas aeruginosa Mediates Resistance to Naturally Occurring and Engineered Phages. Journal of Bacteriology 194:5728–5738.