Supplementary Information

Figures S1-S10
Tables S1-S4
Supplemental Materials and Methods
Supplemental References

Figure S1

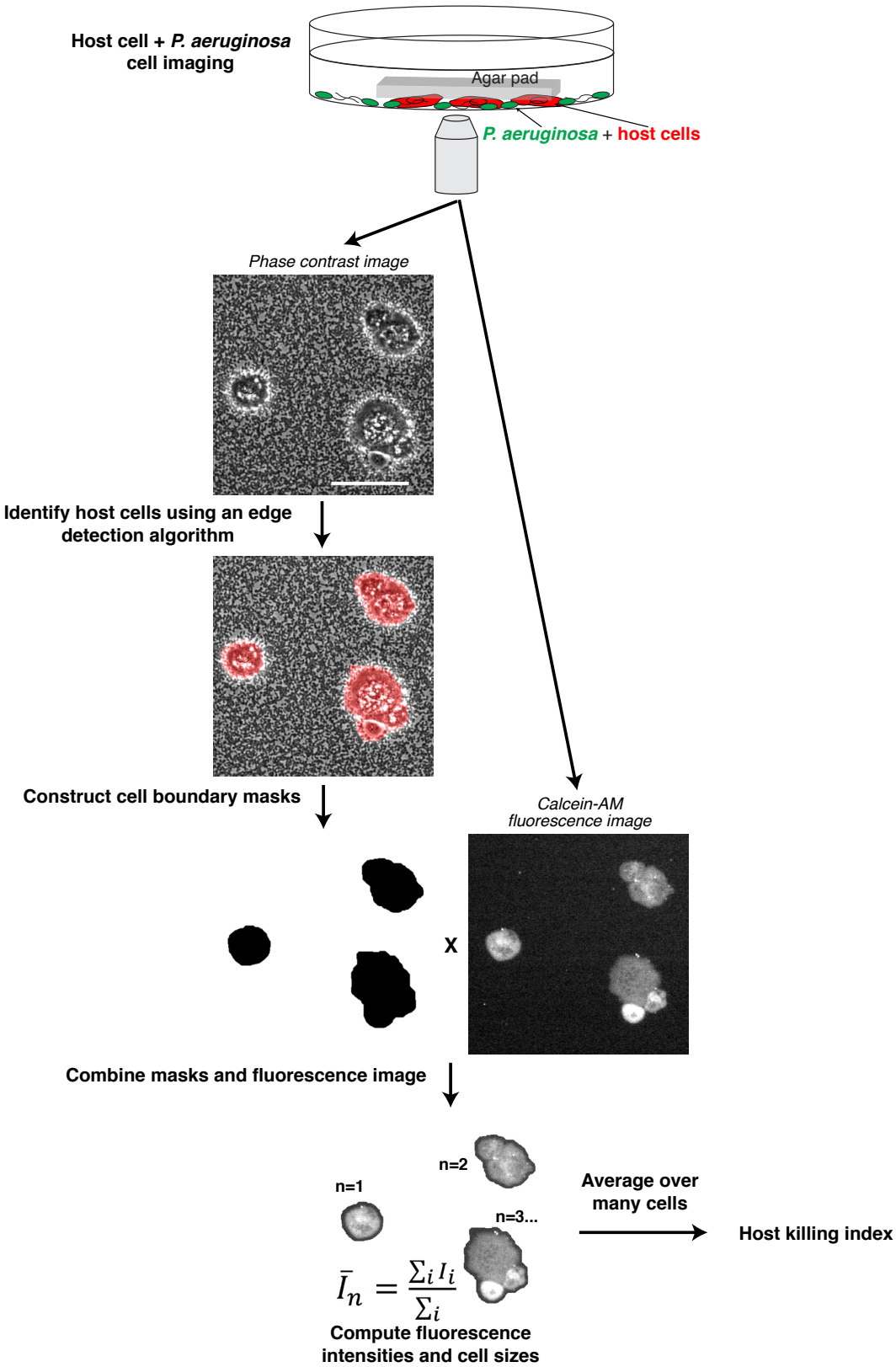


Figure S1. Schematic depicting software-based automated analysis of the rapid host killing assay. Phase contrast and calcein-AM fluorescence images are acquired of amoebae mixed with *P. aeruginosa* cells that are immobilized to a single imaging plane by an agar pad. Applying an edge detection algorithm to phase contrast images identifies the boundaries of host cells, which are used to construct cell boundary masks. The masks are combined with calcein-AM images in order to isolate the fluorescence of individual host cells. The average calcein-AM intensity ($\bar{I}_n$) for each host cell is computed by integrating the individual calcein-AM fluorescence pixel intensities (I_i) over the entire cell and dividing by the cell size. The host cell killing index is computed by averaging $\bar{I}_n$ over many cells.

Figure S2

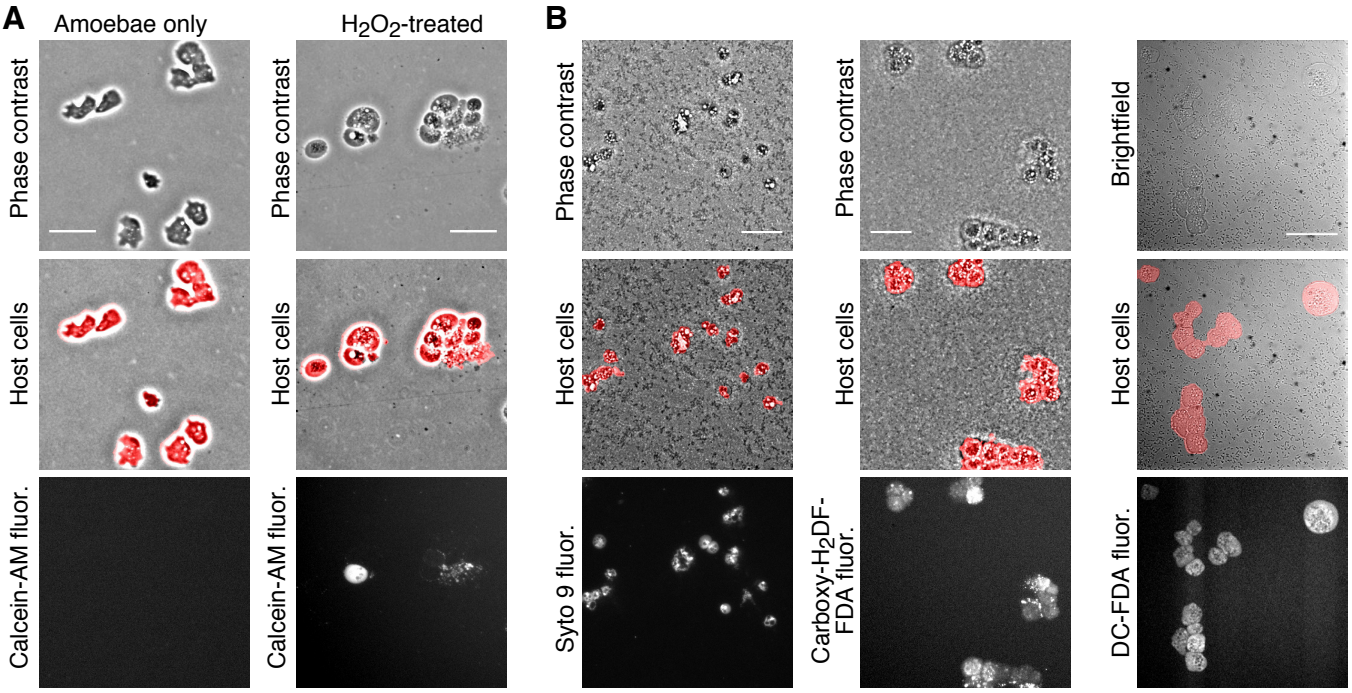


Figure S2. Using fluorescent markers to assess amoeba cell death. (A) Phase contrast and calcein-AM fluorescence images of untreated amoebae and amoebae treated with 1% H_2O_2. Host cells (red) are identified in phase contrast images using an edge detection algorithm. (B) Phase contrast or bright-field images and fluorescence images of amoebae that have been mixed with surface-attached *P. aeruginosa* cells and with Syto 9, which stains nucleic acids and does not significantly permeate healthy amoebae membranes, or carboxy-H_2DF-FDA or DC-FDA, which indicate the production of reactive oxygen species. Host cells (red) are identified in phase contrast or bright-field images using an edge detection algorithm. Scale bars represent 50 µm.

Figure S3

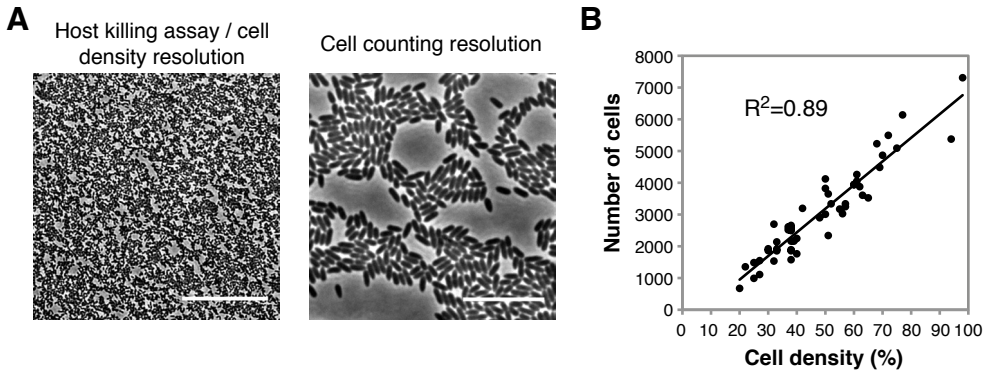


Figure S3. Correlation between surface cell density and cell number. (A) Phase contrast images at the resolution used for both host killing assays and measuring *P. aeruginosa* cell densities and at the resolution for counting individual *P. aeruginosa* cells. The scale bars for the cell density and cell counting images represent 50 µm and 10 µm, respectively. (B) A graph indicating the relationship between *P. aeruginosa* cell density and the number of cells in the same imaging field. The least squares fit to a line and coefficient of determination (R^2) are shown. Cell density and cell counting measurements are described in the Materials and Methods section.

Figure S4

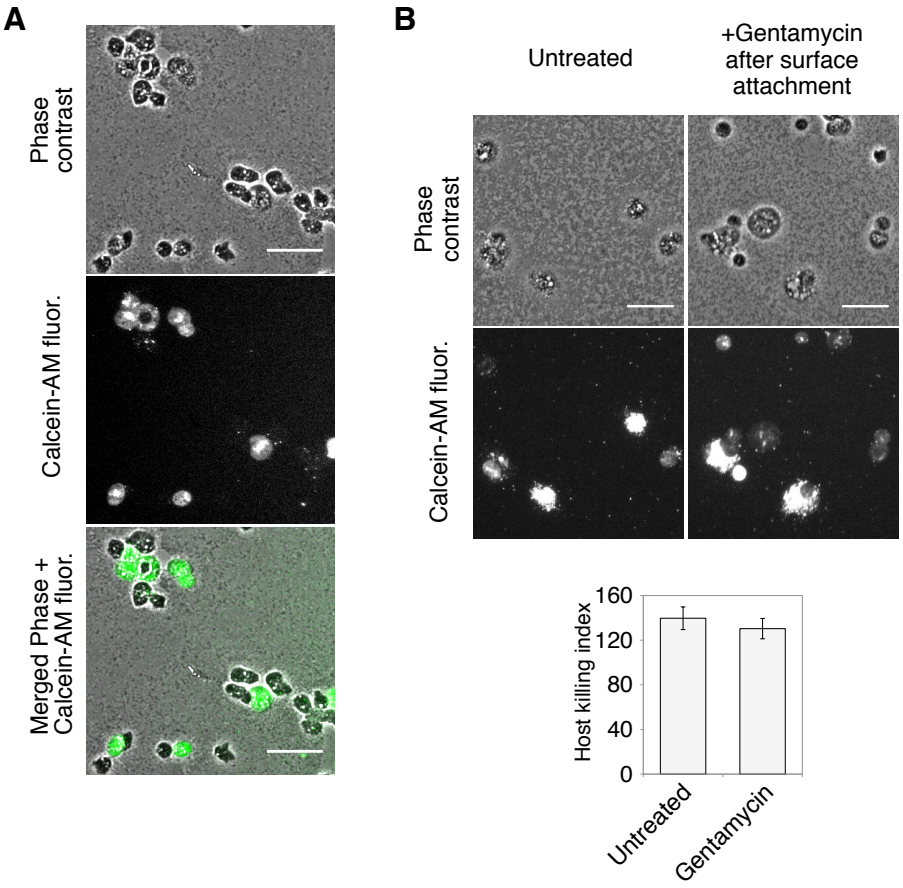


Figure S4. Effects of attachment time and protein synthesis inhibitors on surface-induced virulence. Phase contrast, calcein-AM fluorescence, and merged images of *P. aeruginosa* that were surface-attached for 45 minutes. Only a fraction of the amoebae are calcein-AM fluorescent (green in merged image), indicating that virulence induction is heterogeneous during this attachment period. (B) Phase contrast and calcein-AM fluorescence images and host killing indexes for *P. aeruginosa* cells that were surface-attached for more than 1 hour, treated with gentamycin, and mixed with amoebae, indicating that virulence is not inhibited by protein synthesis after it has been induced. Scale bars represent 50 µm. Bars represent the average of three independent experiments and error bars indicate standard deviation.

Figure S5

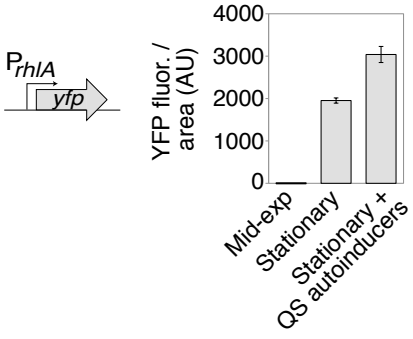


Figure S5. Hyper-activation of quorum sensing in planktonic cells. Fluorescence intensities of *P. aeruginosa* cells that contain a transcriptional fusion of the *rhlA* promoter to the gene encoding yellow fluorescent protein (YFP). The expression of YFP was measured in planktonic *P. aeruginosa* cells from mid-exponential or stationary phase cultures supplemented with either DMSO or 5 μM 3OC12-HSL and 10 μM C4-HSL. Bars represent the average of three independent experiments and error bars indicate standard deviation.

Figure S6

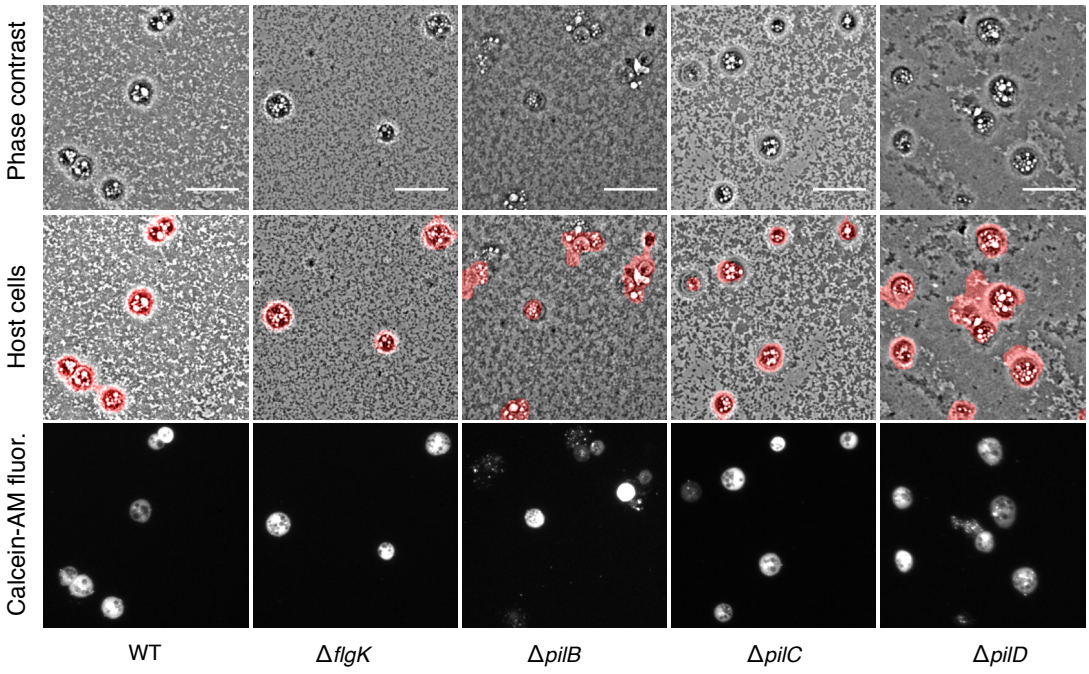
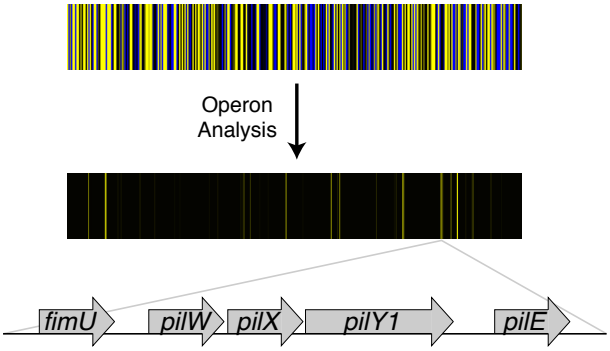


Figure S6. Virulence of surface-attached flagella and pilus mutants toward amoebae. Phase contrast, host-cell mask overlays (red), and calcein-AM fluorescence images of surface-attached wildtype, Δ*flgK*, Δ*pilB*, Δ*pilC*, or Δ*pilD P. aeruginosa* cells that have been mixed with amoebae. The scale bar represents 50 µm.

Figure S7

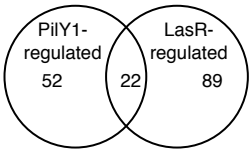
A



B

First gene locus	Last gene locus	Category	Average cluster score
PA14_62730	PA14_62890	Transcription, translation, and unknown	41.1
PA14_06690	PA14_06750	Energy metabolism	35.9
PA14_60300 (*pilX*)	**PA14_60320 (*pilE*)**	**Motility and attachment**	**33.2**
PA14_08670	PA14_08720	Transcription or translation	28.1
PA14_61750	PA14_61790	Transcription or translation	26.5
PA14_54340	PA14_54430	Secreted factors	24.4
PA14_28370	PA14_28800	Unknown	21.6
PA14_44050	PA14_44380	Energy metabolism	20.9
PA14_65230	PA14_65310	Transcription or translation	10.6

C



D

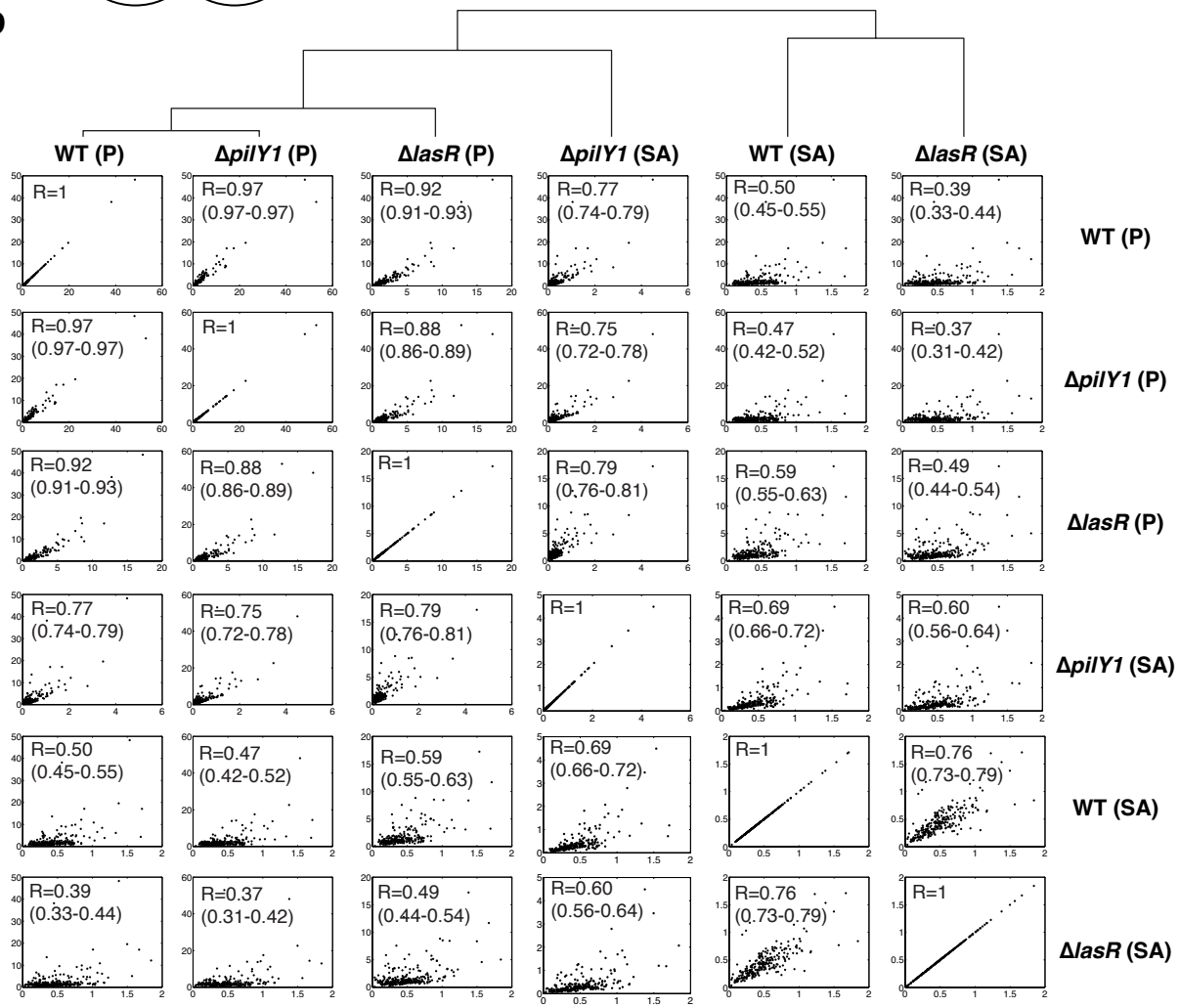


Figure S7. PilY1 mediates a transcriptional surface-attachment response. (A) Transcriptional profiling and operon analysis for the identification of operons activated by surface attachment. Microarray indicates expression of genes that are decreased (blue) or increased (yellow) by surface attachment. Operon analysis was performed to identify gene expression changes in operons, as described in the Materials and Methods. (B) The scores of the nine highest gene cluster expression changes are listed. (C) Diagram indicating the number of genes in surface-attached cells regulated at least 3-fold by PilY1, LasR, or by both. The genes regulated by PilY1 or LasR are shown in Tables S2 and S3, respectively. (D) Hierarchical clustering, correlation plots, and Pearson correlation coefficients for transcriptional profiles from microarrays for surface-attached (SA) or planktonic (P) sub-populations of wildtype, Δ*lasR*, and Δ*pilY1* cells for genes that are activated by at least 4-fold by surface attachment in overnight cultures. Axes of the correlation plots indicate fold upregulation compared against a common mixed reference. The 68% confidence interval ranges for each correlation coefficient are given in parentheses.

Figure S8

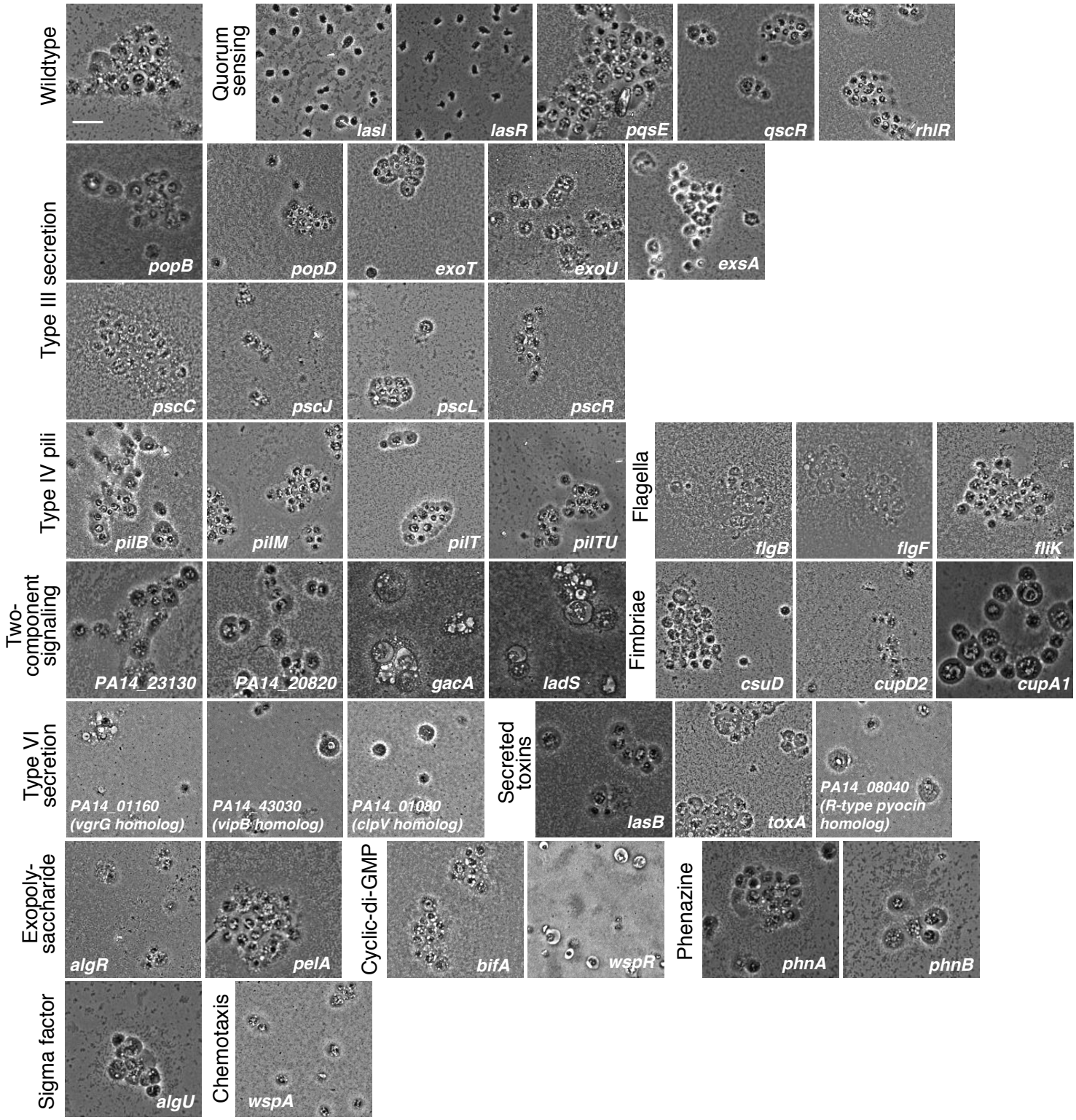


Figure S8. Targeted screen for loss of surface-activated virulence. Phase contrast images of amoebae that have been mixed with surface-associated wild-type or mutant *P. aeruginosa* strains. Cells round up when mixed with all mutants except for the LasR and LasI mutants. The scale bar for wildtype represents 50 μm and the magnification for images of all the mutants matches that of wildtype.

Figure S9

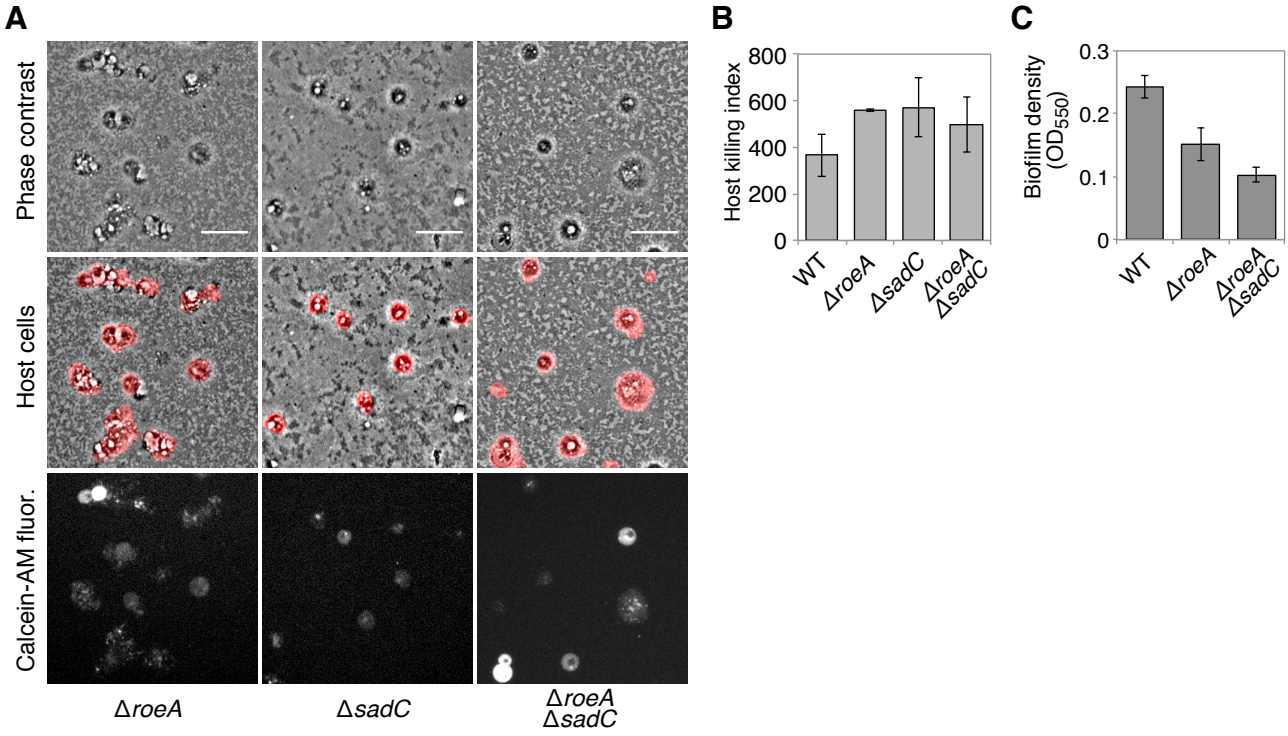


Figure S9. Surface virulence and biofilm formation in c-di-GMP mutants. (A) Phase contrast, host cell overlay (red), and calcein-AM images and (B) host killing indexes of surface-attached Δ*roeA*, Δ*sadC*, or Δ*roeA* Δ*sadC* double deletion *P. aeruginosa* mutants. (C) The biofilm density of Δ*roeA* and Δ*roeA* Δ*sadC* double deletion mutants, as measured by optical density at 550 nm of crystal violet stained surface-attached cells. Bars represent the average of three independent experiments and error bars indicate standard deviation.

Figure S10

Figure S10. The occurrence of PilY1 among bacteria, eukaryotes and archaea. The sequence similarity to *P. aeruginosa* PilY1 is indicated by a continuous scale indicating no homology (white), intermediate sequence conservation (red) and strong sequence conservation (black). The table was produced using the STRING database (24) version 9.1.

Table S1

Gene locus	Name	Description	Ratio (WT)	Ratio (Δ*pilY1*)
PA14_59630		hypothetical protein	3.7	2.1
PA14_28360		hypothetical protein	2.7	1.7
PA14_28030		hypothetical protein	2.6	1.3
PA14_25140		hypothetical protein	2.6	1.6
PA14_41610		hypothetical protein	2.5	1.2
PA14_60090		hypothetical protein	2.3	1.6
PA14_08690		tRNA-Trp	2.3	1.9
PA14_43680	*fabA*	3-hydroxydecanoyl-ACP dehydratase	2.3	1.4
PA14_03166		hypothetical protein	2.1	1.7
PA14_62880		hypothetical protein	2.1	1.2
PA14_70450	*rpoZ*	DNA-directed RNA polymerase subunit omega	2.1	1.3
PA14_61760		tRNA-Gln	2.1	1.6
PA14_08695	*secE*	preprotein translocase subunit SecE	2.0	1.6
PA14_31060		hypothetical protein	2.0	1.3
PA14_40560		hypothetical protein	2.0	1.2
PA14_57250		hypothetical protein	2.0	1.3
PA14_21760	*capB*	cold acclimation protein B	2.0	1.0
PA14_14710		Rrf2 family protein	1.9	1.4
PA14_17050	*map*	methionine aminopeptidase	1.9	1.3
PA14_57980		phosphoryl carrier protein	1.9	1.2
PA14_19130	*rhlI*	autoinducer synthesis protein RhlI	1.9	1.3
PA14_05580		hypothetical protein	1.9	1.3
PA14_29590		transcriptional regulator	1.9	1.3
PA14_59230		colicin immunity protein	1.9	1.5
PA14_16020		hypothetical protein	1.9	1.4
PA14_44690		GntR family transcriptional regulator	1.9	1.2
PA14_17190	*fabZ*	(3R)-hydroxymyristoyl-ACP dehydratase	1.8	1.4
PA14_59620		hypothetical protein	1.8	1.4
PA14_53410		transcriptional regulator	1.8	1.2
PA14_44490	*anr*	transcriptional regulator Anr	1.8	1.2
PA14_53610		hypothetical protein	1.8	1.9
PA14_12330		hypothetical protein	1.8	1.1
PA14_59610		hypothetical protein	1.8	1.4
PA14_56910		hypothetical protein	1.8	1.2
PA14_28790		hypothetical protein	1.8	1.7
PA14_57460		cell division protein MraZ	1.8	1.2
PA14_23980	*xcpP*	secretion protein XcpP	1.8	1.5
PA14_10380		hypothetical protein	1.8	1.2
PA14_60030		hypothetical protein	1.8	1.2
PA14_60100	*dtd*	deoxycytidine triphosphate deaminase	1.8	1.5
PA14_23380	*orfH*	UDP-N-acetyl-D-mannosaminuronate dehydrogenase	1.7	1.4
PA14_39480		hypothetical protein	1.7	1.3
PA14_69780		hypothetical protein	1.7	1.6
PA14_12560		hypothetical protein	1.7	1.2
PA14_51560		acetyltransferase	1.7	1.4
PA14_23370	*orfK*	UDP-N-acetylglucosamine 2-epimerase	1.7	1.4
PA14_30370		hypothetical protein	1.7	1.3
PA14_27590		hypothetical protein	1.7	1.1
PA14_07560	*rpsU*	30S ribosomal protein S21	1.7	1.3
PA14_62780		hypothetical protein	1.7	1.3
PA14_23450	*orfM*	NAD dependent epimerase/dehydratase	1.7	1.5
PA14_14540		hypothetical protein	1.7	1.7
PA14_17600		hypothetical protein	1.7	1.0
PA14_28800		hypothetical protein	1.7	1.3
PA14_35780		hypothetical protein	1.7	1.6
PA14_25100		hypothetical protein	1.7	1.4
PA14_49200	*oprH*	PhoP/Q and low Mg2+ inducible outer membrane prote	1.7	1.5
PA14_40710		hypothetical protein	1.7	1.3
PA14_53430	*slyD*	peptidyl-prolyl cis-trans isomerase SlyD	1.7	1.1
PA14_05960		cold-shock protein	1.7	1.1
PA14_25670	*acpP*	acyl carrier protein	1.6	1.3
PA14_69190	*rho*	transcription termination factor Rho	1.6	0.9
PA14_60110		hypothetical protein	1.6	1.4
PA14_57380	*mraY*	phospho-N-acetylmuramoyl-pentapeptide- transferase	1.6	1.2
PA14_18750	*gloA1*	lactoylglutathione lyase	1.6	1.0
PA14_54410	*mucB*	negative regulator for alginate biosynthesis MucB	1.6	1.0
PA14_67670	*ntrB*	two-component sensor NtrB	1.6	1.4
PA14_64090	*aroQ1*	3-dehydroquinate dehydratase	1.6	1.2
PA14_69630	*rnk*	nucleoside diphosphate kinase regulator	1.6	1.1
PA14_22800	*yciB*	intracellular septation protein A	1.6	1.3
PA14_16990		hypothetical protein	1.6	1.2
PA14_31420		hypothetical protein	1.6	1.3
PA14_54840		tRNA-Gly	1.6	1.6
PA14_23360	*wzz*	O-antigen chain length regulator	1.6	1.7
PA14_53840		hypothetical protein	1.6	1.0
PA14_54350	*lepB*	signal peptidase I	1.6	1.2
PA14_65260		hypothetical protein	1.6	1.1
PA14_28740		tRNA-Pro	1.6	1.6
PA14_19120	*rhlR*	transcriptional regulator RhlR	1.6	1.3
PA14_61200		hypothetical protein	1.6	1.2
PA14_07330		hypothetical protein	1.6	1.4
PA14_23440	*orfL*	group 1 glycosyl transferase	1.6	1.5
PA14_69200	*trxA*	Thioredoxin	1.6	1.3
PA14_65450	*motA*	flagellar motor protein MotA	1.6	1.1
PA14_60460	*rplU*	50S ribosomal protein L21	1.6	1.4
PA14_49920		hypothetical protein	1.6	1.0
PA14_14110	*pcs*	phosphatidylserine synthase	1.6	1.3
PA14_45630	*fliA*	flagellar biosynthesis sigma factor	1.6	1.3
PA14_22180		hypothetical protein	1.6	1.7
PA14_60320	*pilE*	type 4 fimbrial biogenesis protein PilE	1.6	1.3
PA14_56920	*inaA*	InaA protein	1.6	1.2
PA14_08370	*vfr*	cAMP-regulatory protein	1.6	1.3
PA14_52210	*cysM*	cysteine synthase B	1.6	1.0
PA14_51770		hypothetical protein	1.6	1.4
PA14_17270	*accA*	acetyl-CoA carboxylase carboxyltransferase subunit alpha	1.5	0.9
PA14_05660		transcriptional regulator	1.5	1.1
PA14_72470		cytochrome	1.5	1.2
PA14_60120	*dcd2*	deoxycytidine deaminase	1.5	1.3
PA14_09080	*rpsM*	30S ribosomal protein S13	1.5	1.2
PA14_50130	*fliG*	flagellar motor switch protein G	1.5	1.5
PA14_22190		hypothetical protein	1.5	1.7
PA14_57810	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	1.5	1.2
PA14_65310	*hfq*	RNA-binding protein Hfq	1.5	1.3
PA14_50440	*flgF*	flagellar basal body rod protein FlgF	1.5	1.1
PA14_45950	*rsaL*	regulatory protein RsaL	1.5	1.2
PA14_51690		hypothetical protein	1.5	1.2
PA14_53530		hypothetical protein	1.5	1.2
PA14_69970	*cycB*	cytochrome c5	1.5	1.2
PA14_45680	*flhA*	flagellar biosynthesis protein FlhA	1.5	1.1
PA14_63030	*omlA*	outer membrane lipoprotein OmlA precursor	1.5	1.2
PA14_40980		enoyl-CoA hydratase	1.5	1.2
PA14_15360		hypothetical protein	1.5	1.2
PA14_41240	*clpP*	ATP-dependent Clp protease proteolytic subunit	1.5	1.2
PA14_25770	*pilZ*	type 4 fimbrial biogenesis protein PilZ	1.5	1.2
PA14_51730	*tolA*	TolA protein	1.5	1.3
PA14_60380	*ribF*	bifunctional riboflavin kinase/FMN adenylyltransferase	1.5	1.3
PA14_23850	*trpF*	N-(5'-phosphoribosyl)anthranilate isomerase	1.5	1.1
PA14_08710	*nusG*	transcription antitermination protein NusG	1.5	1.3
PA14_41250	*tig*	trigger factor	1.5	1.3
PA14_66270	*glnE*	bifunctional glutamine-synthetase adenylyltransferase/deadenyltransferase	1.5	1.2
PA14_50470	*flgC*	flagellar basal body rod protein FlgC	1.5	1.1
PA14_26550		lipoprotein	1.5	1.2
PA14_28650	*thrS*	threonyl-tRNA synthetase	1.5	1.3
PA14_16970		arsenate reductase	1.5	1.1
PA14_21820		peptidyl-prolyl cis-trans isomerase, FkbP-type	1.5	1.0
PA14_56050		hypothetical protein	1.5	1.2
PA14_30690		hypothetical protein	1.5	1.3
PA14_55180		glycosyl transferase family protein	1.5	1.1
PA14_45610	*cheZ*	chemotaxis protein CheZ	1.5	1.2
PA14_62830	*tpiA*	triosephosphate isomerase	1.5	1.3
PA14_57260	*lpxC*	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	1.5	1.1
PA14_53600		hypothetical protein	1.5	1.7
PA14_50480	*flgB*	flagellar basal body rod protein FlgB	1.5	1.0
PA14_28830		hypothetical protein	1.5	1.4
PA14_35770		hypothetical protein	1.5	1.6
PA14_30650	*gacA*	response regulator GacA	1.5	1.1
PA14_17180	*lpxD*	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	1.5	1.3
PA14_44500	*apt*	adenine phosphoribosyltransferase	1.5	1.1
PA14_08990	*rpsH*	30S ribosomal protein S8	1.5	1.3
PA14_41930		hypothetical protein	1.5	1.2
PA14_16050	*dsbC*	thiol:disulfide interchange protein DsbC	1.5	1.0
PA14_50460	*flgD*	flagellar basal body rod modification protein	1.5	1.1
PA14_55090		hypothetical protein	1.5	1.4
PA14_66110		glycosyl transferase family protein	1.5	1.4
PA14_46550		ribonuclease	1.5	1.4
PA14_33510		hypothetical protein	1.5	1.1
PA14_66660	*pilM*	type 4 fimbrial biogenesis protein PilM	1.5	1.2
PA14_60290	*pilW*	type 4 fimbrial biogenesis protein PilW	1.5	1.3
PA14_01780		nucleoside 2-deoxyribosyltransferase	1.5	1.4
PA14_08540		hypothetical protein	1.5	1.3
PA14_25580	*rluC*	ribosomal large subunit pseudouridine synthase C	1.5	0.9
PA14_21910		ABC transporter ATP-binding protein	1.5	1.1
PA14_27950		hypothetical protein	1.5	1.2
PA14_20700		glycosyltransferase	1.5	1.3
PA14_20720	*flgN*	hypothetical protein	1.5	1.2
PA14_50160	*fliE*	flagellar hook-basal body protein FliE	1.5	1.1
PA14_27210	*efp*	elongation factor P	1.5	1.3
PA14_54430	*algU*	RNA polymerase sigma factor AlgU	1.5	1.1
PA14_48870		C32 tRNA thiolase	1.5	1.1
PA14_62740	*rbfA*	ribosome-binding factor A	1.5	1.2

Fold up-regulation: 1.7 ± 0.02 | 1.3 ± 0.02

Correlation coefficient: 0.46; (0.39-0.52)

Table S1. Genes that are induced by surface attachment in wildtype and Δ*pilY1* cells determined by microarray analysis. The ratio of gene expression f surface attached cells to that of planktonic cells from the same culture for 1 hour of surface attachment beginning at late-exponential phase is shown for all predicted genes where the ratio is at least 1.5. Gene expression ratios are the average of three independent experiments. The average up-regulation and corresponding standard error for each cell type are given. The Pearson correlation coefficient between wildtype and Δ*pilY1* cells and the range of correlation coefficients for a 68% confidence interval are shown in parentheses.

Table S2

Gene locus	Name	Description	Ratio
PA14_60290	*pilW*	type 4 fimbrial biogenesis protein PilW	+212.7
PA14_60300	*pilX*	type 4 fimbrial biogenesis protein PilX	+109.1
PA14_60280	*fimU*	type 4 fimbrial biogenesis protein FimU	+104.9
PA14_60320	*pilE*	type 4 fimbrial biogenesis protein PilE	+37.7
PA14_18020		PhzF family phenazine biosynthesis protein	+20.9
PA14_54490		hypothetical protein	+17.7
PA14_43310		hypothetical protein	+16.9
PA14_69480	*algZ*	alginate biosynthesis protein AlgZ/FimS	+10.5
PA14_18040		hypothetical protein	+7.0
PA14_62190		hypothetical protein	+6.0
PA14_68300	*arcD*	arginine/ornithine antiporter	+5.5
PA14_69470	*algR*	alginate biosynthesis regulatory protein AlgR	+5.2
PA14_51480		hypothetical protein	+5.2
PA14_48870		C32 tRNA thiolase	+5.1
PA14_05110		hypothetical protein	+5.0
PA14_46990		two-component response regulator	+4.9
PA14_22620	*cyaB*	hypothetical protein	+4.9
PA14_11250		hypothetical protein	+4.8
PA14_48860		hypothetical protein	+4.6
PA14_22250		hypothetical protein	+4.4
PA14_11670		hypothetical protein	+4.3
PA14_54800		pseudouridylate synthase	+4.3
PA14_70570	*recG*	ATP-dependent DNA helicase RecG	+4.3
PA14_11660	*aqpZ*	aquaporin Z	+4.1
PA14_11260		epimerase	+3.7
PA14_59790	*pvrR*	two component response regulator	+3.6
PA14_62180		hypothetical protein	+3.6
PA14_17120	*cdsA*	phosphatidate cytidylyltransferase	+3.2
PA14_71880		hypothetical protein	+3.2
PA14_46160		hypothetical protein	+3.2
PA14_67300		ABC transporter substrate-binding protein	+3.1
PA14_72090		hypothetical protein	+3.1
PA14_11270	*oprG*	outer membrane protein OprG precursor	+3.1
PA14_64060		chemotaxis transducer	+3.0
PA14_22880		Fe-S protein	+3.0
PA14_72260		hypothetical protein	+3.0
PA14_00640	*phzH*	potential phenazine-modifying enzyme	-3.0
PA14_06160		hydroxamate-type ferrisiderophore receptor	-3.1
PA14_45940	*lasI*	autoinducer synthesis protein LasI	-3.2
PA14_33590		hypothetical protein	-3.2
PA14_11010		hypothetical protein	-3.2
PA14_33260	*pvdS*	extracytoplasmic-function sigma-70 factor	-3.2
PA14_55160	*toxR*	transcriptional regulator ToxR	-3.3
PA14_09270	*pchE*	dihydroaeruginoic acid synthetase	-3.3
PA14_37745		carbamoyl transferase	-3.6
PA14_09210	*pchA*	salicylate biosynthesis isochorismate synthase	-3.6
PA14_13130		hypothetical protein	-3.7
PA14_24650	*rmf*	ribosome modulation factor	-3.8
PA14_09900	*prpL*	Pvds-regulated endoprotease, lysyl class	-3.8
PA14_31170		hypothetical protein	-3.9
PA14_39960	*phzB2*	phenazine biosynthesis protein	-3.9
PA14_10240		branched-chain alpha-keto acid dehydrogenase subunit E2	-3.9
PA14_56830	*icmP*	metalloproteinase outer membrane	-3.9
PA14_58730	*pilA*	type IV pilin structural subunit	-4.1
PA14_39250		double-glycine peptidase	-4.5
PA14_62790		tRNA-Met	-4.5
PA14_11600		ABC transporter	-4.7
PA14_39970	*phzA2*	phenazine biosynthesis protein	-4.7
PA14_52320		tRNA-Met	-4.9
PA14_13140		hypothetical protein	-5.3
PA14_33050		hypothetical protein	-5.5
PA14_39240		hypothetical protein	-5.6
PA14_10260		dehydrogenase E1 component	-5.7
PA14_10370		hypothetical protein	-5.9
PA14_10350		secretion protein	-6.5
PA14_06180		RNA polymerase sigma factor	-6.7
PA14_39230		hypothetical protein	-6.9
PA14_10330		outer membrane protein	-7.4
PA14_20500		tRNA-Arg	-7.4
PA14_60310	*pilY1*	type 4 fimbrial biogenesis protein PilY1	-7.9
PA14_10230	*adh*	2,3-butanediol dehydrogenase	-8.1
PA14_10360		hypothetical protein	-12.0
PA14_49310		hypothetical protein	-23.0
PA14_10380		hypothetical protein	-30.3

Table S2. Genes that are regulated in surface-attached Δ*pilY1* cells. The ratio of gene expression in Δ*pilY1* cells to that of wildtype cells is shown for all predicted genes that are regulated at least 3-fold in surface-attached cells from cultures that were grown overnight. The inverse of the ratio is shown for down-regulated genes and is indicated by a minus sign. Gene expression ratios are the average of three independent experiments.

Table S3

Gene locus	Name	Description	Ratio
PA14_22570	*csaA*	CsaA protein	+16.0
PA14_51640		hypothetical protein	+9.7
PA14_39230		hypothetical protein	+7.9
PA14_15030	*leuA*	2-isopropylmalate synthase	+7.0
PA14_60140		xerD-like integrase	+6.9
PA14_23750	*leuC*	isopropylmalate isomerase large subunit	+6.8
PA14_23760	*leuD*	isopropylmalate isomerase small subunit	+6.4
PA14_39240		hypothetical protein	+6.1
PA14_38310		hypothetical protein	+5.5
PA14_35500	*bkdB*	branched-chain alpha-keto acid dehydrogenase subunit E2	+5.5
PA14_11790		amino acid transporter	+5.4
PA14_19590		molybdopterin-binding protein	+5.4
PA14_35530	*bkdA1*	2-oxoisovalerate dehydrogenase subunit alpha	+5.1
PA14_11770	*eutB*	ethanolamine ammonia-lyase large subunit	+5.0
PA14_38850	*exaB*	cytochrome c550	+4.9
PA14_38460	*gnyB*	acyl-CoA carboxyltransferase subunit beta	+4.7
PA14_31510		short-chain dehydrogenase	+4.6
PA14_46910		ABC transporter substrate-binding protein	+4.5
PA14_27100	*lipA*	lactonizing lipase	+4.4
PA14_34900		oxidoreductase	+4.3
PA14_34880		GntR family transcriptional regulator	+4.3
PA14_53940	*prpB*	2-methylisocitrate lyase	+4.3
PA14_29070		hypothetical protein	+4.2
PA14_53950	*prpC*	methylcitrate synthase	+4.2
PA14_28130		hypothetical protein	+4.2
PA14_51650		integrase	+4.2
PA14_34920		ferredoxin	+4.1
PA14_38440	*gnyD*	citronelloyl-CoA dehydrogenase, GnyD	+4.0
PA14_19540		hypothetical protein	+3.9
PA14_39270		hypothetical protein	+3.8
PA14_46950		ABC transporter ATP-binding protein	+3.7
PA14_21000		hypothetical protein	+3.7
PA14_00620		hypothetical protein	+3.6
PA14_34940		hypothetical protein	+3.6
PA14_50530	*braD*	branched-chain amino acid transport protein BraD	+3.6
PA14_23790	*leuB*	3-isopropylmalate dehydrogenase	+3.5
PA14_50520	*braC*	branched-chain amino acid transport protein BraC	+3.5
PA14_19530		NAD(P)H-dependent FMN reductase	+3.4
PA14_23030	*oprB*	glucose/carbohydrate outer membrane porin OprB precursor	+3.4
PA14_38800	*pqqC*	pyrroloquinoline quinone biosynthesis protein PqqC	+3.4
PA14_58990		DNA helicase	+3.4
PA14_38930		transcriptional regulator	+3.4
PA14_60130		hypothetical protein	+3.4
PA14_46030		chemotaxis transducer	+3.3
PA14_39260		hypothetical protein	+3.3
PA14_31540		acyl-CoA dehydrogenase	+3.3
PA14_38470	*gnyH*	gamma-carboxygeranoyl-CoA hydratase	+3.2
PA14_39220		hypothetical protein	+3.2
PA14_46970	*ansB*	glutaminase-asparaginase	+3.2
PA14_39250		double-glycine peptidase	+3.1
PA14_35520	*bkdA2*	2-oxoisovalerate dehydrogenase subunit beta	+3.1
PA14_33000	*gcvP2*	glycine dehydrogenase	+3.0
PA14_10700		bacteriophytochrome	-3.0
PA14_40300		hypothetical protein	-3.1
PA14_37790	*pcoA*	copper resistance protein A	-3.2
PA14_31160		hypothetical protein	-3.2
PA14_37780		hypothetical protein	-3.2
PA14_63170		transcriptional regulator	-3.2
PA14_00640	*phzH*	potential phenazine-modifying enzyme	-3.3
PA14_60450	*rpmA*	50S ribosomal protein L27	-3.3
PA14_64840		short-chain dehydrogenase	-3.4
PA14_23970	*xcpQ*	general secretion pathway protein D	-3.4
PA14_32150	*antB*	anthranilate dioxygenase small subunit	-3.5
PA14_31170		hypothetical protein	-3.5
PA14_43680	*fabA*	3-hydroxydecanoyl-ACP dehydratase	-3.6
PA14_44520		drug efflux transporter	-3.6
PA14_67440		N-formimino-L-glutamate deiminase	-3.6
PA14_38270		hypothetical protein	-3.7
PA14_18630		serine protease	-3.8
PA14_53840		hypothetical protein	-4.0
PA14_50640		hypothetical protein	-4.0
PA14_30720		tRNA-Cys	-4.2
PA14_34800		amino acid transporter LysE	-4.2
PA14_59850		hypothetical protein	-4.2
PA14_62790		tRNA-Met	-4.3
PA14_23980	*xcpP*	secretion protein XcpP	-4.4
PA14_01490		hemolysin	-4.4
PA14_33290		hypothetical protein	-4.5
PA14_20500		tRNA-Arg	-4.5
PA14_30620		AraC family transcriptional regulator	-4.6
PA14_19120	*rhlR*	transcriptional regulator RhlR	-4.6
PA14_52320		tRNA-Met	-4.6
PA14_05500		hypothetical protein	-4.7
PA14_10260		dehydrogenase E1 component	-5.0
PA14_39960	*phzB2*	phenazine biosynthesis protein	-5.0
PA14_01780		nucleoside 2-deoxyribosyltransferase	-5.0
PA14_30630	*pqsH*	FAD-dependent monooxygenase	-5.8
PA14_19100	*rhlA*	rhamnosyltransferase chain A	-5.8
PA14_37760		MFS transporter	-6.2
PA14_10240		branched-chain alpha-keto acid dehydrogenase subunit E2	-6.4
PA14_72060		hypothetical protein	-6.7
PA14_11600		ABC transporter	-6.7
PA14_37770		hydrolase	-6.8
PA14_59840		hypothetical protein	-6.9
PA14_45950	*rsaL*	regulatory protein RsaL	-7.0
PA14_28360		hypothetical protein	-7.6
PA14_53260		hypothetical protein	-7.6
PA14_10370		hypothetical protein	-9.7
PA14_32160	*antA*	anthranilate dioxygenase large subunit	-10.3
PA14_10350		secretion protein	-11.3
PA14_39970	*phzA2*	phenazine biosynthesis protein	-12.0
PA14_18800		hypothetical protein	-13.7
PA14_45960	*lasR*	transcriptional regulator LasR	-14.1
PA14_37745		carbamoyl transferase	-15.2
PA14_10330		outer membrane protein	-17.1
PA14_09900	*prpL*	Pvds-regulated endoprotease, lysyl class	-20.4
PA14_16250	*lasB*	elastase LasB	-26.9
PA14_49310		hypothetical protein	-29.1
PA14_10360		hypothetical protein	-30.9
PA14_10230	*adh*	2,3-butanediol dehydrogenase	-35.7
PA14_10380		hypothetical protein	-96.6

Table S3. Genes that are regulated in surface-attached Δ*lasR* cells. The ratio of gene expression in Δ*lasR* cells to that of wildtype is shown for all predicted genes that are regulated at least 3-fold in surface-attached cells from cultures that were grown overnight. The inverse of the ratio is shown for down-regulated genes and is indicated by a minus sign. Gene expression ratios are the average of three independent experiments.

Table S4

Strain	Description	Reference
***D. discoideum* strain**		
AX3	NC-4 strain derivative capable of axenic growth	(1), from E.C. Cox, Princeton University
***E. coli* strain**		
B/r	*E. coli B* strain with high UV resistance	(2)
***P. aeruginosa* strains**		
PA14	Wildtype	(3)
bifA	PA14 Δ*bifA*	(4)
fimU	PA14 Δ*fimU*	(5)
flgK	PA14 *flgK*::Tn5	(6)
lasR	PA14 Δ*lasR*::*aacC1*	(7)
lasR pilY1	PA14 Δ*pilY1* Δ*lasR*::*aacC1*	This study
pelA	PA14 Δ*pelA*	(8)
pilC	PA14 *pilC*::Tn5	(6)
pilD	PA14 Δ*pilD*	(5)
pilE	PA14 Δ*pilE*	(5)
pilTU	PA14 Δ*pilTU*::*FRT*	(9)
pilW	PA14 Δ*pilW*	(5)
pilX	PA14 Δ*pilX*	(5)
pilY1	PA14 Δ*pilY1*	(10)
roeA	PA14 Δ*roeA*	(11)
sadC	PA14 Δ*sadC*	(11)
roeA sadC	PA14 Δ*roeA* Δ*sadC*	(11)
Single mutants of: *algU, algR, csuD, cupD2, exoT, exoU, exsA, flgF, fliK, gacA, ladS, lasB, lasI, PA14_01160, PA14_43030, PA14_01080, PA14_08040, PA14_23130, PA14_20820, phnA, phnB, pilB, pilM, pilT, popB, popD, pscC, pscJ, pscL, pscR, pqsE, qscR, toxA, wspA, wspR*	*MAR2xT7* insertion into the specified gene in PA14	(12)
UTD5.2 (P_{rhlA}-*yfp*)	PA14 *attB*::[P_{rhlA}-*yfp*] *glmS*::[P_{tetA}-*mCherry*]	This study
AFS56 (Empty vector)	PA14 Δ*pilY1 glmS*::[empty vector]	This study
AFS64 (PA14 GFP)	PA14 *glmS*::[$P_{A1/04/03}$-*gfp*]	This study
AFS72-1 (WT PilY1)	PA14 Δ*pilY1 glmS*::[P_{lac}-*pilY1*-His]	This study
AFS72-3 (PilY1 ΔSS)	PA14 Δ*pilY1 glmS*::[P_{lac}-*pilY1*-His ΔSS (Δ(1-29))]	This study
AFS72-4 (PilY1 ΔN-term)	PA14 Δ*pilY1 glmS*::[P_{lac}-*pilY1*-His ΔN-term (Δ(30-584))]	This study
AFS72-5 (PilY1 ΔPilC)	PA14 Δ*pilY1 glmS*::[P_{lac}-*pilY1*-His ΔPilC (Δ(589-999))]	This study
AFS72-6 (PilY1 ΔVWFa)	PA14 Δ*pilY1 glmS*::[P_{lac}-*pilY1*-His ΔVWFa (Δ(115-362))]	This study
***M. musculus* strain**		
J774A.1	Macrophage cell line derived from a tumor in a female BALB/c mouse	(13)
Plasmid	**Description**	**Reference**
mini-CTX2	Self-proficient integration vector containing *tet*, *int*, and *lacI*^{q}	(14)
pFLP2	Plasmid expressing Flp to recombine FRT sites	(15)
pMQ80	Shuttle vector for cloning in yeast and for arabinose-inducible gene expression	(16)
pPilY1-His	pMQ80-derived vector expressing PilY1-His	(10)
pSMC263 (pPilY1-His ΔSS)	pMQ80-derived vector expressing PilY1-His ΔSS	(10)
pSMC264 (pPilY1-His ΔN)	pMQ80-derived vector expressing PilY1-His ΔN	(10)
pSMC265 (pPilY1-His ΔPilC)	pMQ80-derived vector expressing PilY1-His ΔPilC	(10)
pSMC266 (pPilY1-His ΔVWFa)	pMQ80-derived vector expressing PilY1-His ΔVWFa	(10)
pSW(GFP-PilT)	mini-CTX plasmid containing a GFP-PilT translational fusion under control of the P_{BAD} promoter	(17)
pTNS2	Mini-Tn7 Integration helper plasmid containing *tnaA, B, C* and *D*	(18)
pUC18-mini-Tn7T-Gm	Mini-Tn7 delivery vector	(18)
pUC18-mini-Tn7-Lac	Mini-Tn7 delivery vector	(18)
pUCP18-RedS	λ Red recombineering vector	(19)

Table S4. Strains and plasmids used in this study. Details of strain and plasmid construction are described in the Supplemental Materials and Methods section.

SUPPLEMENTAL MATERIALS AND METHODS

Media

PS:DB medium consists of Development Buffer (DB) (5 mM KH_2PO_4, 5 mM Na_2HPO_4, 2 mM $MgCl_2$, 1 mM $CaCl_2$, pH 6.5) containing 10% (v/v) PS medium (10 g/L Special Peptone LP0072 (Oxoid, Hampshire, United Kingdom), 7 g/L Yeast Extract LP0021 (Oxoid, Hampshire, United Kingdom), 10 mM KH_2PO_4, 0.45 mM Na_2HPO_4, 1.5% (w/v) D-glucose, 30 nM Vitamin B12, 180 nM Folic acid, pH 6.5). GYP plates consist of 1 g/L D-glucose, 2 g/L Bacto Peptone, 0.25 g/L Yeast Extract, 31 mM KH_2PO_4, 19 mM Na_2HPO_4, 2.5% agar. PS:DPBS medium is a 1:9 mixture of PS and DPBS (Gibco, Grand Island, NY) supplemented with 1 mM $MgCl_2$ and 1 mM $CaCl_2$.

To test for dependence of surface-activated virulence on protein synthesis and cell division, gentamycin at 100 µg/mL, tetracycline at 200 µg/mL, or fosfomycin at 100 µg/mL were added 5.5 hours following dilution of cultures into petri dishes and allowed 6 hours of additional incubation with drugs with shaking at 37°C (Fig. 3C). To test the effect of gentamycin on the host cell killing process (Fig. S4 in Supplementary Information), gentamycin was added at 17 hours following dilution and given 3 hours of additional incubation with shaking at 37°C

For experiments in Supplementary Information Fig. S2B, calcein-AM was replaced in agar pads by 5 µM Syto 9 (Invitrogen), 0.1 mM carboxy-difluorodihydrofluorescein diacetate (carboxy-H_2DF-FDA) (Invitrogen) or by direct addition of 1 mM dichlorofluorescein diacetate (DC-FDA) (Sigma-Aldrich, St. Louis, MO) to the amoebae culture.

Strain construction

A strain that constitutively expresses GFP was constructed by amplifying the hybrid sequence corresponding to the -51 to 0 region of $P_{A1/04}$ (20) and the +1 to +28 region of $P_{A1/03}$ (20) (herein referred to as the $P_{A1/04/03}$ promoter) from a PA14-derived strain (gift of Kolter lab). The promoter sequence was joined with the sequence encoding mCherry using overlap extension, cloned into the KpnI and HindIII sites in the plasmid pUC18-mini-Tn7T-Gm (18), and integrated into the chromosome of PA14 using pTNS2 (18). The sequence encoding mCherry was replaced with that of GFP by flipping out the drug resistance marker of the chromosomal integration with pFLP2 and electroporating a PCR product containing the sequence that encodes GFP and a drug resistance marker using the λ Red recombination plasmid pUCP18-RedS (19), producing AFS64.

The Δ*lasR*::*aacC1* allele was moved into the Δ*pilY1* strain using λ Red recombination as described previously (19). To construct a strain that expresses a transcriptional fusion of the *rhlA* promoter to the gene encoding yellow fluorescent protein (YFP), the region encoding P_{rhlA}-*yfp* was cloned into the mini-CTX2 plasmid using overlap extension and integrated into the chromosome of a strain that constitutively expresses mCherry under the control of the *tetA* promoter, yielding UTD5.2.

The regions encoding wildtype PilY1-His, or the ΔSS, ΔN-term, ΔPilC, and ΔVWFa mutant versions of PilY1-His were amplified from pPilY1-His, pSMC263, pSMC264, pSMC265, pSMC266, respectively, using the upper primer 5'-CATAACTAGTTGGAGCCAGCGCATGATCCACCAGATTACCCG-3' (or 5'-CATAACTAGTTGGAGCCAGCGCATGATCGCCACGGCCCTGAATG-3' to amplify pSMC263) and lower primer 5'-CATACCCGGGAAGAGCTGTGGCGAGAAGAC-3', cloned into the SpeI and XmaI sites in the mini-Tn7 delivery vector pUC18-miniTn7-Lac, and integrated into the Δ*pilY1* strain by co-transforming with pTNS2, resulting in AFS72-1, AFS72-3, AFS72-4, AFS72-5, and AFS72-6, respectively. Sequencing revealed that the plasmid containing the ΔVWFa deletion

also contains a point mutation in the PilC domain at G677D. The plasmid pUC18-miniTn7-Lac was co-transformed with pTNS2 into the Δ*pilY1* strain, yielding the empty vector strain AFS56.

Phase contrast and fluorescence microscopy

Imaging was performed using a Nikon Ti-E microscope (Nikon, Melville, NY), a 10X Plan Fluor Ph1 Nikon objective (0.3 NA), a 20X Super Plan Fluor Ph1 Nikon objective (0.45 NA) or 100X Plan Apo VC Nikon objective (1.4 NA), a Prior Lumen 200 Pro, and an Andor Clara camera, an Andor iXon DU-897 EMCCD, a Hamamatsu Orca-R2 (Hamamatsu, Bridgewater, NJ) camera, or a QImaging Rolera-XR (QImaging, Surey, Canada BC) camera. DAPI, Calcein-AM, or propidium iodide/mCherry fluorescence were imaged using the 89014 filter set (Chroma, Bellows Falls, VT). The ET402/15x excitation and the ET455/50m emission filters were used to image DAPI fluorescence. The ET490/20x excitation and the ET535/50m emission filters were used to image Calcein-AM fluorescence. The ET572/35x excitation and the ET632/60m emission filters were used to image propidium iodide/mCherry fluorescence. CFP or YFP fluorescence was imaged using the 89002 filter set (Chroma) and ET430/24x excitation and the ET470/24m emission filters or the ET500/20x excitation and ET535/30m emission filters, respectively.

Amoeba cell viability assay

Dictyostelium discoideum AX3 cells were inoculated from frozen stocks into an overnight LB culture of *E. coli* B/r. The mixture was plated on GYP plates and incubated for 4-6 days at 22°C until *D. discoideum* spores formed. Individual spores were picked, inoculated into PS medium supplemented with Antibiotic-Antimycotic solution (GIBCO, Grand Island, NY) that was diluted to 1/4 of the working concentration, and grown axenically at 22°C to an optical density measured at 600 nm (OD_{600}) of 0.2 to 0.5, at which time *D. discoideum* cells were mixed with *P. aeruginosa* for host killing assays.

Agar pads were prepared by pouring molten 1% (w/v) Bacto Agar (BD Bioscience, San Jose, CA) in DB buffer and containing 1 μM Calcein-AM (Invitrogen, Grand Island, NY) on top of a glass surface and cutting the pad into individual 1.5 cm x 1.5 cm sections. *P. aeruginosa* cultures were picked from individual colonies on agar plates and grown overnight from at 37°C to saturation, diluted 1:100 or 1:1000 into PS:DB, and shaken in petri dishes on a benchtop rotator at 100 rpm at 37°C. Surface-attached cells were isolated by removing all the liquid medium from petri dishes and rinsing with DB buffer. Planktonic cells were isolated by transferring 10 μL of culture from petri dishes to a clean petri dish. Pre-conditioned media was isolated by centrifuging cultures at 13 k x g and filtering the supernatant with a 0.2 μm filter. 10 μL of amoebae were added to surface-attached or planktonic cells, confined to the same plane as *P. aeruginosa* by placing an agar pad on top, and imaged using fluorescence microscopy (details in Supplementary Information). Attachment to chemically distinct surfaces was performed by first pouring molten agar or polyacrylamide into petri dishes and soaking overnight in water, and inoculating cultures as described above. Attachment to glass or plant leaf surfaces was performed by adding a glass coverslip or live *Epipremnum aureum* plant leaf mounted on a glass coverslip (details in Supplementary Information), respectively, to cultures that were shaken in petri dishes.

Images were analyzed using software written in Matlab (Mathworks, Natick, MA) that was modified from (21). An edge detection algorithm was applied to phase contrast images to construct masks of amoebae and isolate the fluorescence of individual cells (Fig. S1). The average calcein-AM intensity for each amoeba was computed by integrating the calcein-AM fluorescence intensities over the entire cell and dividing by the cell size. The host cell killing

index was computed as the average of the calcein-AM intensities of at least 100 amoebae in experiments that were performed in triplicate unless otherwise noted.

Macrophage cell death assay

Cells from the mouse macrophage line J774A.1 (TIB-67 (authenticated by Cytochrome Oxidase I testing and verified to be mycoplasma free), ATCC, Manassas, VA) were grown at 37°C with 5% CO_2 in DMEM (with 4.5 g/L D-Glucose, and L-Glutamine) (Gibco) with 10% fetal bovine serum and Penicillin-Streptomycin solution (Invitrogen, Grand Island, NY). Cells were passed by scraping cells from the surface when confluence reached 70-90%.

P. aeruginosa cultures were grown in PS:DPBS (recipe in Supplementary Information) overnight on a benchtop rotator at 100 rpm at 37°C in polystyrene multi-well culture plates in which the bottom was lined with 1% agar made with PS:DPBS medium. Planktonic cells were taken from liquid portion of the culture. Surface-attached cells were isolated by removing the liquid portion, washing with DPBS, and cutting a slice of the agar pad from the bottom of the well. Propidium iodide was added at 1 µg/mL (accounting for the volume of the agar pad) and the pad was inverted and placed on top of macrophages that had been cultured in separate culture plates. Time-lapse imaging was performed at room temperature and approximately 250 cells were analyzed for experiments that were repeated in duplicate.

Surface cell density, cell number, MOI

The density of *P. aeruginosa* cells on surfaces was measured from amoebae cell viability assay images that were acquired using a 10X or 20X objective. The IJ_Isodata algorithm (ImageJ 1.44o) was applied to phase contrast images to construct cell boundary masks. The cell density was computed by dividing the area above the threshold in the mask by the total area. To determine the relationship between the cell density and the number of cells, planktonic *P. aeruginosa* cells were prepared as described in the amoebae viability assay except that no amoebae cells were added. Phase contrast and GFP fluorescence images of *P. aeruginosa* cells (strain AFS64) of the same imaging field were acquired using both 20X and 100X objectives. Cell density was measured as described above from phase contrast images acquired with the 20X objective. The number of cells was measured from phase contrast and GFP fluorescence images acquired using the 100X objective. Fluorescence was quantified using our own software written in Matlab modified from (21). The number of cells was computed by dividing the total cell fluorescence by the fluorescence of a single cell.

The multiplicity of infection (MOI) for planktonic cells for experiments was increased by concentrating cells from liquid cultures by centrifugation at 8 k x g. Cells in all virulence assays were kept at room temperature and imaged using phase contrast and fluorescence techniques typically 1 to 2 hours after the agar pad was added.

Plant surface attachment virulence assay

Saturated *P. aeruginosa* PS:DB cultures were diluted 1:1000 into petri dishes and grown to an OD_{600} of 0.3. Live *Epipremnum aureum* plant leaves were cut in 1.5 x 1.5 cm squares, mounted onto slides using molten valap (vaseline/lanolin/paraffin mixture), and added to the bottom of petri dishes containing *P. aeruginosa* cultures. Cultures were shaken at 100 rpm at 37°C until they reached an OD_{600} of 0.9, at which time the plant sections were removed and rinsed with DB to remove unattached cells. Axenically growing amoebae in PS medium were supplemented with 0.04 µm orange fluorescent (540/560) FluoSpheres (Invitrogen/Life Technologies) and shaken at 22°C for 1 hour before use. The virulence of plant surface attached *P. aeruginosa* was assayed by adding amoebae to the surface of the plant leaf, and immobilizing cells using a 1% agar pad containing 5 mM Calcein-AM Violet (Invitrogen/Life

Technologies). The virulence of planktonic *P. aeruginosa* cells was assayed on leaves by mixing amoebae with planktonic cells, adding the mixture to leaves that were mounted on slides but not placed in cultures, and immobilizing cells on the leaf surface with the agar pad. Amoebae cell bodies were imaged using a YFP filter and Calcein-AM Violet fluorescence was imaged using a CFP filter (Fig. 2C). Amoeba host killing indexes were computed as described above for experiments repeated in biological triplicate. Amoebae and plant surface-attached *P. aeruginosa* were imaged together (Fig. 2B) by adding a mixture of amoebae that had been grown with orange FluoSpheres and *P. aeruginosa* that had been labeled with AlexaFluor 488 carboxylic acid, succinimidyl ester (Invitrogen/Life Technologies) to a mounted plant leaf. The sample was imaged using DAPI (for plant surface autofluorescence), mCherry (for amoebae containing beads), and GFP (for *P. aeruginosa*) filters. Images from different z-planes were assembled by identifying the regions in focus in each plane using the Canny detection algorithm and combining in-focus regions from different z-planes into a single image using our own software written in Matlab.

Hyper-stimulation of quorum sensing

P. aeruginosa cells were inoculated into LB, grown overnight to saturation, diluted 1:1000 into PS:DB supplemented with DMSO or 5 µM 3OC12-HSL (22) and 10 µM C4-HSL (23), and either grown to mid-exponential phase and harvested or grown in petri dishes for 16 hours, at which time planktonic and surface-attached cells were isolated for fluorescence measurements and assayed for virulence towards amoebae. Cells containing a P_{rhlA}-*yfp* transcriptional reporter (UTD5.2) were imaged using single-cell fluorescence techniques by immobilizing cells on agarose pads as described previously (21).

Biofilm density quantification

The density of biofilms in polystyrene petri dishes was measured by staining dishes with crystal violet, washing with water and transferring the stain to a cuvette using 95% ethanol. The absorbance at 550 nm was measured using a spectrophotometer.

RNA isolation and microarray analysis

P. aeruginosa cells were inoculated into LB, grown overnight to saturation and diluted 1:1000 into PS:DB medium. Cultures were grown overnight in petri dishes shaken at 100 rpm and harvested for RNA. Planktonic cells were isolated from the liquid medium, pelleted and snap frozen over liquid nitrogen. Surface-attached cells were isolated by washing petri dishes with DB to remove unattached cells, treating with DB supplemented with approximately 100 µg/mL of alginate lyase (Sigma-Aldrich, St. Louis, MO), and removing cells from the surface using a cell scraper (Fisher Scientific, Pittsburgh, PA). Cells were pelleted and snap frozen over liquid nitrogen. To create a mixed reference, a portion of all harvested cells were mixed together. Total RNA was prepared using the RNeasy Mini Kit (Qiagen, Valencia, CA), treated with RNase-Free DNaseI (Ambion/Life Technologies, Grand Island, NY), and re-purified using the RNeasy Mini Kit (Qiagen). To characterize the transcriptional response to 1 hour of surface attachment, PS:DB cultures were grown to an OD_{600} of 0.9, transferred to a petri dish containing a glass surface, and shaken at 100 rpm for 1 hour. Planktonic and surface-attached populations were harvested for RNA instead using a lysozyme solution with 1% SDS and hot phenol extraction. For all microarray experiments, complementary DNA (cDNA) libraries containing Cy3- or Cy5- labeled dUTP (Enzo Life Sciences) were synthesized from the purified RNA using SuperScript III Reverse Transcriptase (Invitrogen/Life Technologies, Grand Island, NY). RNA was degraded by adding sodium hydroxide and the reaction was subsequently neutralized by addition of hydrochloric acid. The library was purified using the PCR Purification Kit (Qiagen)

and measured for Cy3 and Cy5 incorporation using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE). Experimental libraries were normalized for cDNA concentration, combined with mixed reference libraries, and hybridized to customized Agilent microarrays (designs 28678 or 43307, Agilent Technologies, Santa Clara, CA) using the Agilent Gene Expression Hybridization Kit. Microarrays were designed using the Agilent eArray tool (https://earray.chem.agilent.com/earray/) and contain on average 2 probes per gene. Samples were hybridized for 17 hours at 65°C with continuous rotation at 10 rpm. Microarrays were scanned using an Agilent G2505B scanner and analyzed using Agilent Feature Extract software versions 9.5 and 11. Resulting microarray intensity data were submitted to the PUMA Database (https://puma.princeton.edu/cgi-bin/publication/viewPublication.pl?pub_no=561) for archiving. Operon analysis was performed by sorting microarray data by gene locus and applying the metric $c\prod_{j=-2}^{1} I_j$ at each gene locus, where $I = log_{10}\left({R_{SA}}/{R_{Planktonic}}\right)$, *c* is a fixed scaling factor, *j* denotes the relative position from each locus, and *R* is the log_{10} of the ratio of the expression of the experimental group (surface-attached or planktonic) to that of the mixed reference. The resulting scores were averaged and sorted, producing a list of genes ranked by gene cluster score (Fig. S7B). The linear correlation between expression profiles (Fig. S7D) was determined by computing the Pearson correlation coefficient for genes that are activated by surface attachment. A threshold of 4-fold activation was imposed in order to reduce the contribution of noise on the correlation coefficient while allowing for sufficient gene sampling (N > 250). A 68% confidence interval for each correlation coefficient was computed using Fisher's r-to-z transformation, for which the standard error was computed using $\sigma_z = {1}/{\sqrt{N-3}}$, where N is the number of genes used for the correlation. A hierarchical clustering tree was constructed in Matlab using the shortest distance linkage method and the Pearson correlation metric.

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