Conditional toxicity and synergy drive diversity among antibacterial effectors

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Bacteria in polymicrobial habitats contend with a persistent barrage of competitors, often under rapidly changing environmental conditions. The direct antagonism of competitor cells is thus an important bacterial survival strategy. Towards this end, many bacterial species employ an arsenal of antimicrobial effectors with multiple activities; however, the benefits conferred by the simultaneous deployment of diverse toxins are unknown. Here we show that the multiple effectors delivered to competitor bacteria by the type VI secretion system (T6SS) of Pseudomonas aeruginosa display conditional efficacy and act synergistically. One of these effectors, Tse4, is most active in high-salinity environments and synergizes with effectors that degrade the cell wall or inactivate intracellular electron carriers. We find Tse4 synergizes with these disparate mechanisms by forming pores that disrupt the ΔΨ component of the proton motive force. Our results provide evidence that the concomitant delivery of a cocktail of effectors serves as a bet-hedging strategy to promote bacterial competitiveness in the face of unpredictable and variable environmental conditions.

The T6SS is a functionally plastic pathway that is used by many bacteria to translocate toxic effector proteins into adjacent cells. Effectors that target bacteria generally degrade conserved, essential cellular structures, and, as such, single effectors are sufficient to kill or terminate growth. Despite the capacity of single effectors to kill target cells, the γ-proteobacterium Pseudomonas aeruginosa delivers a diverse cocktail of effectors that degrade, among other structures, phospholipids, peptidoglycan and nicotinamide adenine dinucleotides. The T6SSs of other experimentally characterized bacteria also deliver effectors that target multiple essential molecules. To estimate the generality of this phenomenon, we searched the genomes of 2,566 sequenced proteobacterial species for T6SS effectors with known activities. Only 42% (n = 474) of the species within this group contain the T6SS also contain an effector of known activity, suggesting that many as yet undescribed effectors exist. Nevertheless, we found that 40% (n = 196) of these species possess a second effector with unique biochemical activity, and 25% (n = 52) possess three or more (Supplementary Table 1). Such bacteria were identified in four of the five major classes of Proteobacteria. These data suggest that bacteria benefit from the coordinated delivery of effectors with diverse biochemical activities.

We considered the potential benefits that could select for and maintain a diversity of T6SS effectors. Utilizing multiple antibiotic mechanisms simultaneously is a well-documented strategy for minimizing the evolution of resistance. This strategy might also overcome potential intrinsic resistance mechanisms present in the wide phylogenetic range of bacteria targeted by the T6SS. Preventing resistance may contribute to effector expansion, but because of the essentiality and structural conservation of T6SS effector targets, we reasoned it is unlikely to be the major selective pressure driving the extent of effector diversity observed.

Two additional benefits conferred by a diverse effector arsenal could be that the toxins act with synergy on recipient cells or that they display conditional efficacy. The former is defined by instances in which the activity of two or more effectors on recipient cells is greater than the sum of their individual activities, whereas the latter is defined by effectors that contribute to recipient cell intoxication in a manner dependent on the environmental conditions. Synergy and conditional efficacy among T6 effectors have not been explored, and we reasoned that both offered a potential explanation for the effector diversity observed. It is worth noting that these scenarios are not mutually exclusive.

To interrogate T6 effectors in a high-throughput fashion, we developed a sequencing-based, pooled strategy for measuring their activity during interbacterial competition (Fig. 2a–c). Henceforth, we refer to this method as PAEE (Parallel Analysis of Effector Efficacy). Briefly, we introduced unique barcode sequences to a library of P. aeruginosa strains rendered susceptible to intoxication by one or two effectors of the Hcp Secretion Island I-encoded T6SS (H1-T6SS) through the deletion of effector–immunity gene pairs. Thus, only effectors with an experimentally defined cognate immunity determinant were included (Tse1–6). Prior studies established the antibacterial activity of these effectors, but the precise biochemical mechanisms of Tse2, Tse4 and Tse5 remain unknown (Fig. 2a). A pool containing the barcoded mutants and a barcoded toxin-resistant reference strain was then cultivated under a variety of conditions with an excess of the unmarked parental strain acting as a toxin donor. Susceptibility to intoxication was assessed by comparing the frequency of the barcode associated with a given mutant to the reference strain at the beginning and end of the experiment (Fig. 2b, c). To uncouple the potential contribution of regulation from the inherent biochemical capacity of effectors to act synergistically or conditionally, we utilized a background of P. aeruginosa (ΔretS) in which Tse1–6 are produced constitutively. Data presented herein derive from four independent repetitions of PAEE.

Prolonged cell–cell contact is critical for T6SS-mediated effector translocation. Conditions that do not permit extended contact, such as cultivation on semi-solid surfaces or in liquid, thus provide a convenient means of specifically evaluating the contribution of the T6SS to fitness. Consistent with this, we found that our barcoded effector-susceptible strains displayed fitness defects only when cultivated under T6S-conducive conditions (Supplementary Fig. 1).

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Moreover, we noted that the relative magnitude of individual effector activities under T6S-conducive conditions approximated those observed in earlier studies. With this validation of our method, we sought to examine whether T6SS effectors display conditional efficacy. We measured effector activity under growth conditions varying in parameters that are likely to be in flux across the environmental habitats where *P. aeruginosa* resides, including salinity, temperature, oxygen availability and pH. PAEE identified several conditions in which the relative efficacy of effectors varied compared with a reference condition, including high salinity, anaerobiosis, high temperature and alkalinity (Fig. 2d–g). For instance, between pH 6 and pH 8, the relative activity of Tse5 increases sevenfold, whereas that of Tse3 decreases by 50%. Importantly, these results reproduced in environments where *P. aeruginosa* naturally resides, including salinity, temperature, anaerobiosis, high temperature and alkalinity (Fig. 2d–g). For instance, between pH 6 and pH 8, the relative activity of Tse5 increases sevenfold, whereas that of Tse3 decreases by 50%. Importantly, these results reproduced in environments where *P. aeruginosa* naturally resides.

Next we used PAEE to ask whether effectors can act synergistically. We found striking differences in the capacity of, and frequency by which effector pairs exhibit this behavior (Fig. 3a–d, and Supplementary Fig. 3a–k). For example, the cumulative activity of Tse3 and Tse4 exceeds the sum of the two individual effectors in all conditions tested (Fig. 3a). On the contrary, we did not observe an instance of synergy between Tse2 and Tse4 (Fig. 3b). We found that effector pair synergy can also be conditional. For example, synergy between Tse1 and Tse4 was most pronounced in high salinity and at concentrations sub-inhibitory to the wild type (Fig. 4b). In contrast, significant growth inhibition was not observed on media containing sodium chloride (150 mM) or lithium chloride (20 mM) at concentrations sub-inhibitory to the wild type (Fig. 4b). 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that the proteins localize to the inner membrane of *P. aeruginosa* (Supplementary Fig. 4b)\(^1^7\). Tse4 is also characterized by a high content of glycine residues, many of which are in configurations reminiscent of glycine zipper motifs (Supplementary Fig. 4c). These sequences can promote multimerization of transmembrane segments and frequently occur in proteins that induce pores, including bacterial toxins\(^1^8\). To test the importance of these residues for Tse4 function, we generated a panel of mutants in which glycine residues predicted to form a transmembrane glycine zipper were substituted for valine. All of these mutants exhibited decreased Tse4-mediated intercellular intoxication (Supplementary Fig. 4d). Substitutions at G176 and G184 also impacted Tse4 secretion and stability, thereby complicating interpretation of the intoxication defects of these alleles (Supplementary Fig. 4e). However, proteins containing G180V or G186V substitutions were produced and secreted at wild-type levels, indicating specific disruption of toxin activity. Alleles encoding substitutions in adjacent non-glycine residues (A187V and A188V) had no impact on Tse4 function despite diminished secretion. These observations, taken together with the ion sensitivity we found is induced by Tse4, led us to hypothesize that the effector forms pores in the inner membrane of intoxicated cells.

Bacteria concentrate potassium ions in their cytoplasm and thus their release to the milieu is a convenient measure of membrane pore formation. Therefore, we monitored potassium release from Tse4-intoxicated cells using inductively coupled plasma optical emission spectroscopy. One hour after Tse4 induction, the level of extracellular potassium detected from a strain lacking Tse4 approached that of mechanically lysed cells (Fig. 4c). Immunity
to Tse4 inhibited the accumulation of extracellular potassium and allowed cells to concentrate potassium intracellularly during this period. We next asked whether the action of Tse4 allows the translocation of larger, organic molecules. Neither propidium iodide (668 Da) nor α-nitrophenyl-β-galactopyranoside (301 Da) accessed the cytoplasm of Tse4-intoxicated cells (Fig. 4d). However, control cells treated with pyocin S5, a bacterial toxin previously shown to form large, non-selective pores, were permeable to both molecules. In summary, these findings suggest that Tse4 induces membrane pores that accommodate ions, but exclude larger organic molecules.

Tse4 induction leads to bacteriostasis and the permeability of the inner membrane to ions, suggesting that the toxin disrupts the proton motive force (PMF). To measure the ΔΨ component of the PMF, we used the membrane potential-sensitive fluorescent dye DiOC$_2$(3)19. Tse4 intoxication resulted in a dramatic decrease in ΔΨ, nearly to the level of the ionophore-treated control (Fig. 4e).

In contrast, by employing the pH-sensitive green fluorescent protein pHluorin20, we found that Tse4-intoxicated cells have no defect in the maintenance of the ΔpH component of the PMF (Fig. 4f). It is noteworthy that this result is consistent with our PAEE findings, which indicated that the efficacy of Tse4 is enhanced by sodium ions, but unaffected by changes in pH. In total, our data indicate that Tse4 promotes highly selective membrane permeability. Although ions, but unaffected by changes in pH. In total, our data indicate that Tse4 promotes highly selective membrane permeability. Although

Fig. 3 | Environmental conditions and effector activities influence synergy between T6SS effectors. a–d, Filled circles indicate observed (in PAEE screen)/expected (sum of individual effector activities measured by PAEE) activity for the indicated effector pairs. The ratios reflecting synergistic (green) or inhibitory (red) interactions are indicated. Single and paired effector activities calculated as in Fig. 2. The inhibitory interactions for each effector pair are calculated as the portion of the additive effect that is contributed by the more active effector (red bars). The values between red bars and the dashed line are additive. High temperature (H), 42 °C, low (L), 37 °C; high NaCl, 300 mM, low, 0 mM; anaerobic (An.). (n = 4 biologically independent experiments).

e, Average observed/expected activity for the indicated effectors paired with each other effector under a given condition. Measurements are plotted only for the conditions with the three highest average observed/expected values for a given effector. f, The effector expression levels (as measured by qRT–PCR, n = 2 biologically independent experiments) plotted against the effector activity level (as determined by PAEE, n = 4 biologically independent experiments) under the same set of conditions. The colours indicate effector identity (see Fig. 2). Pearson’s correlation coefficient is indicated. a–e, Data presented as mean values ± s.d.

Methods

Bacterial strains, plasmids and growth conditions. A detailed list of all strains and plasmids created in this study can be found in Supplementary Tables 2 and 3. All P. aeruginosa strains were derived from the sequenced strain PAO121 and were grown on Luria–Bertani (LB) medium at 37 °C supplemented as appropriate with 30 μg/ml gentamicin, 25 μg/ml kanamycin, 75 μg/ml tetracycline, 5% (w/v) sucrose, 0.2 mM IPTG (isopropyl-β-D-1-thiogalactopyranoside), 0.02% (w/v) arabinose and 40 μg ml$^{-1}$ X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside). Escherichia coli was grown in LB medium supplemented with 15 μg ml$^{-1}$ gentamicin, and 0.2 mM IPTG. E. coli strains DH5α, BL21 (DE3) and SM10 (Novagen) were used for plasmid maintenance, gene expression and conjugative transfer, respectively. Plasmids PSV39-CV and pETDual-1 were used for inducible expression in P. aeruginosa and E. coli respectively. Site-specific chromosomal insertions in P. aeruginosa were generated using pUC8-Tn7/pBAD-araE (gltS) or pMinCct1 (atB) as previously described13,25.

Bioinformatic search for type VI effectors encoded by Proteobacteria. We generated custom hidden Markov model (HMM) profiles for experimentally validated effectors and a T6S structural gene (tssC) from homologue alignments using hmmscan from the HMMER 3.1b2 toolset. The effectors groups chosen for this search were Tae1, Tae2, Tae3, Tae4, Tga1, Tge2, Tga3, Tle1, Tle2, Tle3, Tle4, Tle5, VasX and Tse4, as well as proteins containing Tox46 (NAK[P] glycohydrolase) and Tox34 (FIM1 DNase) motifs13,26. For all proteobacterial species on the List of Prokaryotic names with Standing in Nomenclature (LPNS, http://www.bacterio.net/), a strain was selected at random and the corresponding genome downloaded from the NCBI complete genome database. Draft genomes were selected and translated if no complete genomes were available. These genomes were searched for proteins matching our custom HMM profiles. The results were binned by profile hits and filtered using $E$-value cutoffs. Appropriate cut-off values were selected by manually identifying the highest value for a given sequence that was found in the appropriate genomic context. For effectors, this included proximity of immunity scale, the concomitant delivery of multiple effectors with a range of conditional optimality may act as a bet-hedging strategy. As a corollary, the set of effectors a bacterium possesses should provide a window into the environmental conditions in which it encounters competitors. The benefits conferred by the delivery of toxins with conditional and synergistic activities underscores the utility of combination antibiotic therapy beyond the subversion of resistance13.
gene candidates, vgrG homologues, and genes encoding accessory proteins with T6S-related domains of unknown function. Hits for tscC were validated by identifying adjacent genes encoding other T6S structural components.

Parallel analysis of effector efficacy screen. Generation of barcoded mutant strains. The pEXG2 suicide vector was used for creating in-frame chromosomal deletions in P. aeruginosa as previously described. Single and pairwise deletions of the six β-haemolytic mutant alleles in P. aeruginosa was used to generate 21 different strains (see Fig. 1 and Supplementary Table 2). The integration vector pEXG1 was then used to insert a unique 18 base pair barcode at the neutral attB site in each mutant as well as the wild-type parent strain. The pEXG2 suicide vector was used for creating in-frame chromosomal deletions in P. aeruginosa H1-T6SS effector–immunity gene pairs in P. aeruginosa populations susceptible (Δtse4 Δtsi4) or resistant (Δtse4) to Tse4 intoxication. Extracellular K+ levels measured in the supernatants of Tse4-expressing cultures (susceptible, light grey; resistant, dark grey) (n = 4 biologically independent samples). Lysed cells provide the maximal concentration that can be released.

Intracellular uptake of ONPG or PI by cells rendered genetically susceptible to the non-selective pore-forming toxin pyocin S5 (dark grey bars) or by cells expressing Tse4 (light grey bars) (n = 3 biologically independent samples). ONPG uptake monitored through intracellular β-galactosidase-mediated release of o-nitrophenol (A405 nm); PI uptake monitored through intracellular fluorescence (617 nm). Membrane polarization of Tse4-expressing cultures (n = 3 biologically independent samples). CCCP-treated cultures (light grey) included as depolarized controls. Membrane potential is indicated by the ratio of fluorescence intensities (610/535 nm) emitted by cells treated with DiOC2(3). Intracellular pH change in Tse4-expressing cells incubated in buffers of varying pH (n = 3 biologically independent samples). Sodium-benzoate-treated populations included as a control for proton gradient dissipation. Intracellular pH indicated by ratio of excitation peaks (392/470 nm) exhibited by pHlucin2 expressed by each population. Data presented as mean values ± s.d. (error bars in f not visible due to overlap with symbols; *P < 0.05 (two-tailed t-test).

Bacterial growth competitions. The 22 barcoded strains were grown for 16 hours on LB NO salt (LBNS) medium agar plates, then resuspended in LBNS broth before pooling together at equal concentrations (normalized to Δtse4 Δtsi4) and mixed with an excess of a donor (P. aeruginosa PAO1 ΔretS, donor to recipient = 10:1). This mixture (5 μl) was spotted on a 0.2 μm nitrocellulose membrane placed on 3% (w/v) agar plates, except as noted in Supplementary Table 4. Plate composition, incubation times and other varied growth conditions are described in Supplementary Table 4. After the incubation period noted in Supplementary Table 4, competitions were harvested into LBNS broth and washed twice with the same medium. Cells were then incubated with 10 mg ml−1 benzozene (Sigma-Aldrich) for 30 min at 37 °C to remove extracellular DNA, and washed a final time with LBNS medium containing 5 mM EDTA to inactivate the benzozene.

Sequencing. Total DNA was extracted from washed, benzozene-treated cells using the DNeasy kit (Qiagen). The barcode region was amplified using primers containing an adaptor sequence and targeting conserved regions flanking the integrated barcodes. Amplification was monitored via SYBR Green incorporation and terminated prior to saturation. A second round of amplification was used to introduce Nextera sequencing adapters (Illumina) and unique indices for each library, and sequencing was performed using an Illumina MiSeq instrument (50 cycles program).

Analysis. Each read was assessed for an exact match at the barcode position using string matching, with no mismatches for any base allowed and excluding reverse complemented matches (reads that did not meet these criteria were filtered). To normalize values between different conditions, we converted the reads for each barcoded strain into a fraction of the total reads for each individual condition. Effector activity (E) was calculated by a metric analogous to a competitive index using the following equation:

\[
E = \log \left( \frac{\text{Final (ΔretS)\, \text{mutant}}}{\text{Initial (ΔretS)\, \text{mutant}}} \right)
\]

Synergy was calculated as the observed activity of an effector pair compared with the expected additive value (observed/expected, o/e). The expected additive value is the sum of the individual effector activities from single effector susceptible barcoded strains minus 1. Synergy was defined as any o/e value > 1, meaning the activity of the effector pair was greater than the sum of its individual effector activities. The bar for inhibition was calculated as the percentage contribution of the most active effector within the pair to the expected additive value. Partially additive activity was defined by o/e values greater than the inhibitory cutoff of 1.

Effector expression analysis. Expression of T6SS effector and structural genes was measured in wild-type P. aeruginosa PAO1. Cultures were grown for 16 h in LB broth, then cells (5 μl) were spotted on 0.2 μm nitrocellulose filters placed upon agar plates. Agar plate composition and incubation conditions are the same as described for the bacterial growth competitions.
same as employed in PAEE (Supplementary Table 4). Spots were harvested from nitrocellulose and resuspended into Bacterial RNA Protect buffer (Qiagen). Cells were lysed by lysozyme treatment (1 mg ml⁻¹ for 10 min) followed by sonication. RNA was purified using the RNeasy kit (Qiagen), the residual DNA was removed by Turbo DNase (Invitrogen) and the remaining RNA was purified and concentrated using the RNA Minelute kit (Qiagen). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher). Gene expression was measured using SYBR Green-based quantitative PCR with primers targeting each gene of interest, and normalized to the housekeeping gene psoD. Primer sequences are available upon request. Two independent experiments with three technical replicates included were performed for each measurement.

**Growth competition assays.** For *P. aeruginosa* competitions, the recipient strain contained lacZ (tetacycline resistant) inserted at the neutral phage attachment site (attB) to enable its differentiation or selection from the unlabelled donor strain when plated on LB containing X-gal (40 µg ml⁻¹) or tetracycline (75 µg ml⁻¹). Overnight cultures of donor and recipient strains were washed, A₅₆₀ standardized, and mixed at the ratios indicated. Competition mixtures (5 µl) were then spotted in triplicate on a 0.2 µm nitrocellulose membrane overlaid on a 3% (w/v) agar plate of the indicated medium and incubated face up at 37°C for 8h unless denoted otherwise. Competitions were harvested from the nitrocellulose membrane and resuspended into LB medium. Initial and final populations of donor and recipient cells were enumerated following serial dilution on appropriate selective or differentiation media. The competitive index (CI) is defined as:

\[ CI = \frac{Final \, donor \, \%}{Initial \, donor \, \%} \]

**Potassium release assays.** *P. aeruginosa* strains were grown overnight in a low K⁺ media (10 mM bis-tris propane pH 7.0, 5 mM succinate, 2 mM MgCl₂, 5 mM NH₄Cl, 1 mM NaH₂PO₄, 10 µM Fe(NH₄)₂SO₄, 0.1% (w/v) tryptone, 0.005% (w/v) yeast extract). Overnight cultures were back diluted 1:100 in low K⁺ media, grown to mid-log phase, pelleted by centrifugation at 4,000 x g for 10 min, and pellets resuspended in 2 ml of Wash Buffer (50 mM Tris-HCl pH 7.5, 500 mM NaCl, 2% glycerol) with 1 mg ml⁻¹ lysozyme, 0.5 mg ml⁻¹ DNase, and 1% Triton X-100. The resuspension was manually lysed through sonication, centrifuged at 4°C for 30 min at 16,000g, and lysates applied to 250 µl of Ni-NTA beads (Qiagen). Beads were incubated with rotation for 1 h at 4°C, washed 3 times in 10 ml of Wash Buffer with 0.1% Triton X-100, and protein was eluted with 300 µl of Wash Buffer containing 500 mM imidazole pH 7.4 and 0.1% Triton X-100. For secretion assays, samples were prepared as previously described. All protein samples were analyzed by SDS-PAGE and western blotting as previously described using rabbit α-VSVG (1:5000, Sigma) and mouse α-RNAP (1:1000, Upstate) antibodies and then detected with rabbit and α-mouse horseradish peroxidase-conjugated antibodies (1:5000, Sigma). Western blots were developed using chemiluminescent substrate (SuperSignal West Pico Substrate, Thermo Scientific) and imaged with a FluorChemQ (ProteinSimple). The original western blot images are provided in Supplementary Fig. 5.

**Statistics.** All statistical tests were performed in GraphPad Prism 7.0 with α = 0.05; P < 0.05 is indicated by an asterisk in the figures.

**Life Sciences Reporting Summary.** Further information on experimental design is available in the Life Sciences Reporting Summary.

**Data availability.** The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files.

**Code availability.** Computer code generated for this study is available upon request.

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Author contributions
K.D.L., S.B.P., H.D.K. and J.D.M. designed the study. K.D.L., S.B.P., H.D.K., M.C.R., R.K. and J.D.M. performed experiments, and K.D.L., S.B.P., H.D.K., M.C.R., R.K. and J.D.M. performed experiments, and K.D.L., S.B.P., H.D.K., M.C.R. and J.D.M. analysed data. K.D.L., S.B.P., and J.D.M. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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### Experimental design

1. **Sample size**
   - **Describe how sample size was determined.**
   - No size calculation was performed prior to experimental design. We used 3-4 biological replicates for experiments as that is standard practice for most biochemical or microbiological assays.

2. **Data exclusions**
   - **Describe any data exclusions.**
   - No data were excluded from analysis.

3. **Replication**
   - **Describe whether the experimental findings were reliably reproduced.**
   - All experimental findings reported in the paper were reliably reproduced during replicate experiments.

4. **Randomization**
   - **Describe how samples/organisms/participants were allocated into experimental groups.**
   - Randomization was not used within this study. Randomization was not needed because we were using specific genotype backgrounds for our experiments, and not applying a treatment to a larger subset of species/strains.

5. **Blinding**
   - **Describe whether the investigators were blinded to group allocation during data collection and/or analysis.**
   - Investigators were not blinded during data collection or analysis since there was not group allocation.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - **n/a** Confirmed
   - **☐** The exact sample size \( (n) \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - **☐** A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - **☐** A statement indicating how many times each experiment was replicated
   - **☐** The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - **☐** A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - **☐** The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted
   - **☐** A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - **☐** Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study.

Prism 7, Microsoft Excel for Mac 2017, HMMER3.1b2 tool set (online), FlowJo Version 4.10.1

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There are no restrictions on material availability.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

rabbit anti-VSV-G (supplier: Sigma Aldrich, catalog number: V4888-200UG, 1.28 mg/mL stock) validated by Western Blot in Pseudomonas aeruginosa; anti-HIS-HRP (Supplier: BioLegend, catalog number: 663903, lot number: B219269) validated by Western Blot in Escherichia coli; HRP-conjugated anti-rabbit secondary (Supplier: Sigma Aldrich, catalog number: A6154-1mL, lot number:SLBP3451V) validated by Western Blog in Pseudomonas aeruginosa, anti-mouse (Sigma) validated by Western blot in Pseudomonas aeruginosa.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.