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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist .

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Xcalibur software (Thermo Fisher Scientific Inc.) was used for mass spectrometry analysis. Schrodinger 2018-2 was used for molecular modeling. SPAdes 3.11 was used to assemble the Photorhabdus genome. antiSMASH v4 was used to analyze the genome for BGCs. BLAST was used to search for homologous operons. Zen Software v14.03.201 was used to acquire fluorescent microscopy images. IsobarQuant and Mascot 2.4 were used to generate proteomic data. MassHunter B0.5 was used to quanitfy darobactin peaks in quantitative mass spectrometry experiments.
Data analysis	Microsoft Excel and GraphPad Prism 8.2 were used to plot graphs. Geneious 11.0.4 was used to find mutations in the resistant mutants. Fiji v1.52i software was used to process microscopy images. R v3.5.1 was used to analyze the transcriptomic and proteomic data, and Python v3.6.6 was used to plot transcriptomic data. AAT Bioquest was used to plot the BAM folding assay ITC data was analyzed and fit to the independent binding model using the NanoAnalyze software package.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The genome of P. khanii HGB1456 has been deposited to Genbank with identifier WHZZ00000000.
The transcriptomic dataset (Extended Data Figure 7) has been deposited to NCBI Sequence Read Archive with identifier PRJNA530781.
The proteomics (Extended Data Figure 8) data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with

the dataset identifier PXD013319.
All other data available from corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For animal studies, sample size was chosen based on prior experience with variability between mice for infection models. For survival analysis in septicemia, three mice were used as difference in survival with treatment is dramatic. For the thigh infection model, five mice per group was used due to known variability in infection burdens in untreated mice. No sample size calculation was performed.
Data exclusions	No data was excluded
Replication	For standard microbiological assays, MICs and time-kill, experiments were done in triplicate to ensure findings were reproducible. Resistant mutants were also generated from three independent cultures. In animal studies, multiple mice were included in each group.
Randomization	Mice were randomly assigned to groups for infection and treatment, and were then housed as a group in a cage for the duration of the experiment.
Blinding	Blinding was deemed not relevant to mouse work, as outcomes were quantitative and not subjective.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	All cell lines were obtained from ATCC or GenTarget in 2018
Authentication	None of the cell lines were authenticated
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Female CD-1 mice, 6 weeks old were used for all animal studies
Wild animals	The study did not involve wild animals

Field-collected samples	Photorhabdus and Xenorhabdus strains were received from Heidi Goodrich-Blair, grown at 28 C on LB agar, then stored at -80 C in glycerol stocks.
Ethics oversight	Animal studies were approved by the Northeastern University IACUC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.