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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 our Editorial Policies 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	All custom Chemprop code for molecular property prediction (antibiotic activity against A. baumannii) is freely available to download from https://github.com/chemprop/chemprop. Additionally, a snapshot of the version used in this paper is preserved and freely available from https://github.com/GaryLiu152/chemprop_abaucin
Data analysis	Molecular feature were calculated using the open-source package RDKit (https://www.rdkit.org) (version 2020.03.1.0). t-SNE plots were generated using scikit-learn (https://scikit-learn.org/stable/) (version 0.22.2.post1). SNP identification was conducted using BreSeq (https://barricklab.org/twiki/bin/view/Lab/ToolsBacterialGenomeResequencing) (v0.35.0). kallisto (https://pachterlab.github.io/kallisto/about) (v0.46.1) was used to compute mRNA abundances for RNA sequencing. DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html) was used to quantify drug-induced mRNA expression changes. EcoCyc (https://ecocyc.org) (v24.1) was used for GO term enrichment analyses. sgRNA design was performed using code from (https://gitlab.pasteur.fr/dbikard/ecowg1). A. baumannii LolE structural predictions were computed using RoseTTAfold through the Robetta platform (https://robetta.bakerlab.org/). Structural alignments were conducting using Maestro (version 12.9.137) and and ChimeraX (version 1.3rc202112020528).

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 and 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

GenBank accession numbers for sequencing of abaucin resistant mutants are: BankIt2629921 – OP677864, OP677865, OP677866, OP677867. GEO accession numbers for RNA sequencing datasets are GSE214305 – GSM6603484, GSM6603485, GSM6603486, GSM6603487, GSM6603488, GSM6603489, GSM6603490.

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	Mouse experiment sample size was determined using field convention group sizes for antibiotic efficacy evaluation (n=5 or n=6) (for example, see Stokes et al., 2020). All in vitro experiments were conducted using biologically independent replicates, also based on field convention. Stokes, J. M., Yang, K., Swanson, K., Jin, W., Cubillos-Ruiz, A., Donghia, N. M., MacNair, C. R., French, S., Carfrae, L. A., Bloom-Ackermann, Z., Tran, V. M., Chiappino-Pepe, A., Badran, A. H., Andrews, I. W., Chory, E. J., Church, G. M., Brown, E. D., Jaakkola, T. S., Barzilay, R., & Collins, J. J. (2020). A deep learning approach to antibiotic discovery. Cell, 180(4). https://doi.org/10.1016/j.cell.2020.01.021
Data exclusions	No data was excluded from any analyses.
Replication	All in vitro experiments were conducted using biologically independent replicates. We define biological independence as experimental replicates being conducted using identical protocols on different days.
Randomization	Randomization was only applicable to mouse studies . Before infection, mice were relocated at random from housing cages to single-occupancy control or treatment cages. For in vitro antibiotic testing, randomization is not necessary since the same bacterial culture is aliquoted and each aliquot received the desired treatment with the appropriate controls running side-by-side.
Blinding	Investigators were not blinded for any in vitro or animal experiments. We were analyzing the antibacterial properties of a new molecule against A. baumannii. Many of the analyses performed pertained to antibacterial efficacy, which is quantifiable using common laboratory approaches. Therefore, blinding was considered unnecessary.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Animals and other organisms

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

Laboratory animals	Six- to eight-week-old female C57BL/6N mice were purchased from Charles River Laboratories. Animals were housed in a specific

Laboratory animals	pathogen free barrier unit under Level 2 conditions, temperature controlled (21℃) 30-50% humidity, 12h light and dark cycle environment (dark from 7pm to 7am) and were fed regular chow ad libtum.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Mouse model experiments were conducted according to the guidelines set by the Canadian Council on Animal Care, using protocols approved by the Animal Review Ethics Board and McMaster University under Animal Use Protocol #20-12-41.

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