

Reporting Summary

Nature Portfolio 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 Portfolio 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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | 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

EPU v3.3.1.5184REL from ThermoFisher; SerialEM v4.0.6
Synergy H1 Plate Reader (BioTek) was used for growth inhibition and intracellular ATP measurement assays.
Seahorse XFe96 Analyzer was used to measure the oxygen consumption rate.

Data analysis

1. Drift correction: MotionCor2 version 1.6.3
2. CTF estimation: Ctffind version 4.1.13
3. Particle picking: CryoSPARC Version4.6.0
4. 2D and 3D Reconstruction, 3D refinement, post-processing: CryoSPARC Version4.6.0
5. Local resolution estimation: CryoSPARC Version4.6.0
6. Model visualization: ChimeraX v1.8
7. 3D volume visualization: Chimera version 1.16, ChimeraX v1.8
8. Manual model building: Coot 0.9.8.1
9. Structure refinement: Phenix version 1.20.1-4487
10. Figure generation: ChimeraX v1.6.1
11. FSC plot generation: CryoSPARC Version4.6.0
12. Stereochemistry analysis: MolProbity web server (v4.5.2)
13. Graph plot generation: GraphPad Prism (version 7.0.4)

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The coordinates of the PDBs and EMDB maps are provided.

Atomic structures are deposited with PDB IDs:

Inhibitor-free structure	9WCX	https://www.rcsb.org/structure/9WCX
ND-011458-bound structure	9WCY	https://www.rcsb.org/structure/9WCY

Corresponding maps are deposited with EMD IDs:

EMD-65878	https://www.ebi.ac.uk/emdb/EMD-65878
EMD-65879	https://www.ebi.ac.uk/emdb/EMD-65879

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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

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](https://www.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

No explicit sample size calculations were implemented for the cryo-EM studies in this work. The number of micrographs and particles are sufficient for obtaining reliable classifications and reconstructions as indicated by the data processing.

The drug dose-response assays presented in the manuscript are derived from a minimum of three independent experiments. Checkerboard assays were performed in duplicate, and MIC assays were conducted in at least biological duplicate. The kill kinetics experiment was performed in triplicates.

Data exclusions	Particles with lesser quality were excluded by iterative 2D and 3D classifications as is generally endorsed by cryo-EM studies. No data were excluded from the microbiological experiments.
Replication	No replications were applied in this work. In cryo-EM studies, statistical analyses and validation procedures are generally sufficient to evaluate the quality and reproducibility of cryo-EM results. In the case of the microbiological assays, the frequency of each experiment is detailed in the figure legends and/or the corresponding Methods sections.
Randomization	As is generally demanded by cryo-EM studies, randomization was performed at 3D reconstruction stage for the calculation of FSC curves illustrated in supplementary figures. Randomization was not considered for the in vitro experiments conducted in this study.
Blinding	Blinding is not generally feasible for structural studies as a specific protein subject is being studied in a defined environment/condition. Blinding was not considered necessary for the microbiological assays.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	The Rabbit Polyclonal DDDDK tag antibody from abcam (ab2493) was used as a primary antibody for the detection of FLAG® tag sequence at a dilution of 1:2000. Link: https://www.abcam.com/en-us/products/primary-antibodies/hrp-ddddk-tag-binds-to-flag-tag-sequence-antibody-ab2493
Validation	The antibodies were detected using SuperSignal West Pico Plus chemiluminescent substrate (Thermo Scientific™, Catalog number: 34580) according to the manufacturer's protocol producing a signal in the presence of the FLAG® tag, confirming its validity. The supplier of the primary antibody also provided validation data on their website. Link: https://www.abcam.com/en-us/products/primary-antibodies/hrp-ddddk-tag-binds-to-flag-tag-sequence-antibody-ab2493

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A