

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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

1. Thermo XCalibur: Mass spectrometry [Commercial]
2. Agilent Chemstation: HPLC data [Commercial]
3. Agilent MassHunter: Mass spectrometry [Commercial]
4. Softmax Pro 5.3: Enzyme and whole-cell fluorescence and absorbance data [Commercial]
5. Cary WinUV: UV-Vis spectra [Commercial]
6. Cary e2 software: Fluorescence spectra of purified F420 [Commercial]
7. Crystallography data was collected on the MX1 beamline at the Australian Synchrotron

Data analysis

1. XDS: Macromolecular diffraction indexing and processing
2. Aimless: Macromolecular data scaling
3. Phaser: Molecular replacement
4. REFMAC (version 5.8.0230): Refinement of crystallographic structures
5. COOT: Macromolecular model building
6. AceDRG: Creation of ligand restraint dictionaries
7. Desmond: Molecular dynamics software for energy minimization [Commercial]
8. OPLS3e: Forcefield used for parameterizing docked ligands [Commercial]
9. GraphPad Prism (version 7.04): Calculation of enzyme kinetics [Commercial]
10. Maestro (version 11.0.015 2016-4 release): Visualization of molecular structures [Commercial]
11. SHELXD: Molecular substructure determination
12. T-Coffee: Multiple sequence alignment
13. Molprobit: Structure validation

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

Structural factors and coordinated have been deposited in the Protein Data Bank under accession codes 6BWH and 6BWG for the PEP-bound and apo structures of Rv2983, respectively. All other data are available from the corresponding authors upon 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](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 sample size calculation was performed.
Data exclusions	No data were excluded from the analysis.
Replication	Enzyme kinetic assays and whole-cell assays were carried out in triplicate. Coupled enzyme assays were repeated on two or more occasions.
Randomization	No randomization was performed.
Blinding	No blinding was performed.

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

Antibodies

Antibodies used	Anti-FLAG conjugated to HRP (ThermoFischer Scientific cat# MA1-91887-HRP)
Validation	Validation was performed by ThermoFischer Scientific