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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.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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 |
| ☒ | ☐ | An indication of 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<br>*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 statistics 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<br>*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 |
| ☒ | ☐ | Clearly defined error bars<br>*State explicitly what error bars represent (e.g. SD, SE, CI)* |

*Our web collection on statistics for biologists may be useful.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Diffraction data collection was performed at the Canadian LIght Source using the Macromolecular Crystallography Data Collector software and at the Advanced Photon Source using CONSOLE and RAPD. |
| Data analysis | Crystallography data were processed and analyzed using DIALS v1.10, CCP4 Suite v7.0 (including iMosflm, Pointless, Scala, Phaser, Aimless, Coot, REFMAC5, TopDraw), Phenix v1.13 (including phenix.refine, Phaser, Sculptor, AutoBuild), PyMOL v1.8.2.<br>Mass spectrometry data were processed with Bruker Compass DataAnalysis v4.2 (including SmartFormula) or Agilent OpenLAB CDS software v2.3. |

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 upon request. 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

Source data for all figures are available from the corresponding authors upon reasonable request. The models and structure factors for the crystal structures are deposited in at the Protein Data Bank with accession numbers 6ECB, 6ECC, 6ECD, 6ECE and 6ECF.

# Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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. Because the variation in the assays used is small and we are interested in large effects, the sample sizes used, as indicated in the manuscript, were deemed appropriate. |
| Data exclusions | There are no data exclusions |
| Replication | Biochemical assays were replicated at least twice. The exact number of replicates is stated in the relevant legend. All attempts at replication were successful. |
| Randomization | No randomization. This is not relevant because the samples form defined groups. |
| Blinding | No blinding. Blinding is not relevant to the study. The groups were defined and studied by the investigator using standard protocols. |

# Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |

### Methods

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

## Unique biological materials

Policy information about availability of materials

| | |
|---|---|
| Obtaining unique materials | The following unique genetic materials are reported and are available from the corresponding authors upon reasonable request: The DAPRS/tRNA pair, TEV mutants, VlmTE and VlmTE amber mutant. |

## Antibodies

| | |
|---|---|
| Antibodies used | Strep-Tactin-HRP conjugate (α Strep): [IBA Lifesciences] (article number: 2-1502-001; lot number 1502-5051; Dilution: 1:5'000) - note this is not an antibody but a streptavidin derivative.<br>P4D1 antibody (α Ub, mouse monoclonal) [Enzo Life Sciences] (article number: BML-PW0935-0025; lot number 08101528; clone |

number: P4D1; Dilution: 1:5'000).
Anti-His-HRP (α His6 C-term., mouse monoclonal): [Miltenyl Biotech] (article number: 130-092-783; lot number 5140228100; clone number: GG11-8F3.5.2; Dilution: 1:10'000)

Validation

Antibodies were used to detect purified recombinant proteins without further in-house validation. Below are some descriptions of specificty from the suppliers' websites:
α Strep: https://www.iba-lifesciences.com/strep-tactin-system-technology.html
α Ub: Enzo website: "Recognizes mono- and poly-ubiquitin protein conjugates, free polyubiquitin chains and free ubiquitin."
α His6 C-term.: Miltenyi Biotec website: "The monoclonal Anti-His antibody specifically recognizes proteins that contain a polyhistidine tag at their N- and C-terminus. However, the Anti-His-HRP (C-term.) antibody shows higher sensitivity and specificity for C-terminal His-tagged proteins."