

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

- ☒ ☒ 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	X-ray diffraction data was collected at beamlines 24ID-C and 24ID-E at the Advanced Photon Source (Argonne National Laboratory) using NE-CAT Remote Access software v6.2.3.
Data analysis	Raw X-ray diffraction crystallographic data were integrated and scaled using XDS software (version Jan 10, 2022). Molecular replacement was performed using PHASER from the CCP4 program suite (version 7.0). All structures were refined using PHENIX Refine software (version 1.17). Structural models were built in Coot (version 0.8.2). Structural models and restraints for IBX and TYL were generated using PHENIX eLBOW software (version 1.17). All figures showing atomic models were generated using PyMol software (version 1.8).

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](#)

Coordinates and structure factors were deposited in the RCSB Protein Data Bank with accession codes:

- 9O3H for the wild-type *T. thermophilus* 70S ribosome in complex with mRNA, A-site Lys-tRNA^{Lys}, P-site fMRC-peptidyl-tRNA^{iMet}, and erythromycin;
- 9O3I for the wild-type *T. thermophilus* 70S ribosome in complex with mRNA, A-site Lys-tRNA^{Lys}, P-site fMRC-peptidyl-tRNA^{iMet}, and telithromycin;
- 9O3J for the wild-type *T. thermophilus* 70S ribosome in complex with mRNA, A-site Lys-tRNA^{Lys}, and P-site fMRC-peptidyl-tRNA^{iMet};
- 9O3K for the wild-type *T. thermophilus* 70S ribosome in complex with mRNA, A-site Lys-tRNA^{Lys}, P-site fMAC-peptidyl-tRNA^{iMet}, and erythromycin;
- 9O3L for the wild-type *T. thermophilus* 70S ribosome in complex with mRNA, A-site deacylated tRNA^{Phe}, P-site fMRC-peptidyl-tRNA^{iMet}, and erythromycin;

All previously published structures that were used in this work for structural comparisons were retrieved from the RCSB Protein Data Bank: PDB entries 6XHX, 6XHY, 7NSP, 7NSQ, and 8CVK.

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	Not Applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not Applicable
Population characteristics	Not Applicable
Recruitment	Not Applicable
Ethics oversight	Not Applicable

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	Not Applicable
Data exclusions	No data were excluded from the analyses.
Replication	The toe-printing assays shown in Supplementary Figure 2 represent one of the two independent experiments. All attempts at replication were successful.
Randomization	Randomization in general is not relevant to this study. For cross-validation during crystallographic model building and refinement, an R-free set was used. Other parts of model building and refinement did not require randomization because the outcomes of these experiments are not dependent on the judgment of the researcher and depend on the reference experiments that cannot be affected by unconscious bias.
Blinding	Blinding was not used in the in vitro toe-printing assays because the outcomes (differences in the positions of the primer extension termination sites) are evident upon inspection of the unprocessed gel images. Blinding was not used in X-ray crystallographic experiments, because the outcomes of these experiments are not dependent on the judgment of the researcher and depend on the reference experiments that cannot be affected by unconscious bias.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Plants

Seed stocks	Not Applicable
Novel plant genotypes	Not Applicable
Authentication	Not Applicable