

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 |
| <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: CryoEM data were collected using the EPU software version 2.8 (FEI, Netherlands)

Data analysis: Cryo-EM: RELION v3.0, Gctf v1.06, and Gautomatch v0.56 for processing micrographs, picking particles, classification and refining cryo-EM maps. Coot v0.8 and v0.92 for model building and Phenix (dev-2947-000) for model refinement and statistics. Figures were generated using Chimera v1.14, ChimeraX v1.0. and PyMOL 1.7.7.1.
Sequencing data were analyzed using the Biopython package, assembled in PEAR v0.9.10, trimmed and analyzed with Enrich2 v1.0.0.
MD simulations were prepared, performed and analyzed using GROMACS 2018.8 with the implemented LINCS and GENION versions. For preparation WHATIF 20071220-093, and for further analysis GROMACS 2019, Python 2.7.13, Matplotlib 1.4.3 were used.

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

The cryo-EM maps of the ErmDL-ERY-P-tRNA-SRC, ErmDL-ERY-A-P-tRNA-SRC and ErmDL-TEL-A-P-tRNA-SRC and the associated molecular models have been deposited in the Protein Data Bank and Electron Microscopy Data Bank with the accession codes EMDB-12573 (<https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/12573/>), EMDB-12574 (<https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/12574/>), EMDB-12575 (<https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/12575/>)

and PDB ID 7NSO (<https://www.rcsb.org/structure/7NSO>), 7NSP (<https://www.rcsb.org/structure/7NSP>), 7NSQ (<https://www.rcsb.org/structure/7NSQ>) respectively. Sequencing data from the inverse toeprinting analysis have been deposited in the National Center for Biotechnology Information Short Read Archive with the accession code PRJNA623725 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA623725>). Source data are provided with this paper

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	Inverse toeprinting assays were performed in duplicate as per convention
Data exclusions	Micrographs with low estimated resolution or poorly fitted CTFs were excluded from further processing, as were particles that clustered into poorly defined classes during 2D and 3D classification.
Replication	Toeprinting and Inverse toeprinting assays were performed in duplicate as per convention and all replications were successful. For each system, 20 independent MD simulation were performed.
Randomization	For 3D refinement in RELION, particles are randomly placed in one of two subsets. These subsets are maintained for CTF refinement. For inverse toeprinting data were allocated into experimental groups based on sequence identity i.e.complementarity.
Blinding	No blinding was performed as blinding is not possible or not applicable for the experiments.

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 checked="" type="checkbox"/>	<input 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"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

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