

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

Gel images and autoradiographs of membranes in binding assays were captured using FLA-7000 image analyzer (Fujifilm). MS data were obtained by Xcalibur for LTQ Orbitrap XL (Thermo Fisher Scientific) and Q Exactive hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Cryo-EM grids were prepared using Vitrobot Mark IV (Thermo Fisher Scientific). Automated cryo-EM data acquisition was performed by EPU 2.9 software (Thermo Fisher Scientific) on a Krios G4 transmission electron microscope (FEI) equipped with a K3 direct electron detector (Gatan).

Data analysis

Canvas X (version 20) and ChemDraw (20.1) were used to create figures. UCSF Chimera (version 1.15) and UCSF ChimeraX (version 1.2) were used to analyze and prepare figures of cryo-EM maps and atomic models. Microsoft Excel for Microsoft 365 MSO was used for statistical analysis. GraphPad Prism ver 7.04 was used to draw bar graphs of binding assay results. Multi Gauge Version 3.0 was used to quantify the radioactivity in binding assays. Xcalibur Qual Browser 4.4 was used to analyze LC/MS data. Phenix (1.19.2) and Coot (version 0.9.4) were used for model building. RELION 3.1.3 and cryOLO (1.9.1) were used for cryo-EM image processing. x3dna-dssr (version 1.9.9) was used to calculate torsion angles in the atomic models.

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](#)

Publicly available datasets from Protein Data Bank (7K00, 4V8N, and 4V5R) were used for atomic model building and comparison.

Cryo-EM maps and atomic coordinates of the reported structures were deposited in Electron Microscopy Data Bank (EMDB) and Protein Data Bank, respectively, with the following accession codes; EMD-35001 and 8HSP (EctRNAIle2 with t6A37); EMD-35022 and 8HU1 (EctRNAIle2 with ct6A37); EMD-35020 and 8HTZ (HmtRNAIle2).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

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	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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