nature portfolio

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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	No commercial, open source, or custom code was used to collect data. Data was downloaded directly from sources: UNK iCLIP-seq data was obtained from E-MTAB-2279; RNA-seq data from ENCODE (see Data Availability for accession numbers); eCLIP data from ENCODE (see Data Availability for accession numbers); RiboSeq data was obtained directly from Jernej Murn (Shah et al. 2024).
Data analysis	Open source software and packages as well as custom code were utilized for data analysis. All custom code available on request. RStudio (version 2023.03.0) with R platform (version 4.2.2) was used for primary data analysis. RStudio packages were used as needed: AnnotationHub (version 3.8.0), ape (version 5.7), BSgenome.Hsapiens.NCBI.GRCh38 (version 1.3.1), BSgenome.Hsapiens.UCSC.hg38 (version 1.4.5), BSgenome.Mmusculus.UCSC.mm10 (version 1.4.3), cowplot (version 1.1.1) data.table (version 1.14.8), DESeq2 (version 1.40.1), dplyr (version 1.1.0), flextable (version 0.9.6), ggmsa (version 1.3.4), ggprism (version 1.0.4), ggplot2 (version 3.4.1), ggpattern (version 1.0.1), ggpubr (version 0.6.0), ggrepel (version 0.9.3), Hmisc (version 4.8.0), liftOver (version 1.24.0), lsr (version 0.5.2), magick (version 2.8.3), msa (version 1.30.1), org.Hs.eg.db (version 3.17.0), reshape2 (version 1.4.4), rstatix (version 0.7.2), scales (version 1.2.1), stringr (version 1.4.4), and VennDiagram (version 1.7.3). BEDtools (version 2.31.0), fastx_toolkit (version 0.0.14), GraphPad Prism (version 10), PHAST (version 1.5), RSEM (version 1.3.1), SAMtools (version 1.16), seqtk (version 2.3.0), STAR (version 2.7.10b), and Vienna RNAfold (version 2.5.1) were also used for data analysis as needed.

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

Final enrichments and fastqs for the nsRBNS and 100vertRBNS datasets as well as fastqs for the RBNS detailed here have been deposited in Gene Expression Omnibus (GSE262560).

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	No human participants, data, or biological material were used in this study.
Reporting on race, ethnicity, or other socially relevant groupings	No human participants, data, or biological material were used in this study.
Population characteristics	No human participants, data, or biological material were used in this study.
Recruitment	No human participants, data, or biological material were used in this study.
Ethics oversight	No human participants, data, or biological material were used in this study.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For large-scale natural sequence-based sequencing data, a sample size of two was utilized due to experimental cost. Low variability was observed (Pearson's correlation values reported throughout manuscript). For random sequence-based sequencing data, a sample size of one was used and validated via previously reported structural data. For in vitro binding validation, a sample size of three was used when little variance was expected (fluorescence polarization) whereas a sample size of five was used in experiments with high variance (qPCR binding assay).
Data exclusions	No data were excluded from the given study.
Replication	Large-scale sequencing experiments were performed in binding duplicate (full binding assays), with independent protein preparations. Pearson's correlation values reported throughout manuscript for replicate sequencing samples. Fluorescence polarization validation was performed in binding triplicate (full binding assays) with independent protein preparations. qPCR binding assay validation was performed in quintuplicate (full binding assays) with three independent protein preparations: technical duplicates for qPCR were performed due to qPCR-based variance.
Randomization	Randomization was not used in this study. For data selection, top-ranked data was included rather than randomized.
Blinding	Blinding not relevant to given study as study consists primarily of large-scale sequencing data.

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	No plants or plant-based materials were used in this study.
Novel plant genotypes	No plants or plant-based materials were used in this study.
Authentication	No plants or plant-based materials were used in this study.