

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	NA
Data analysis	Guppy (version 6.0.6+8a98bbc and model template_rna_r9.4.1_70bps_hac.jsn) Taiyaki (version 5.3.0) Bonito (version 0.8.1) Dorado (ersion 0.8.1) Minimap2 (version 2.24-r1122) Remora (version 3.2.0)Samtools (version 1.16) The Taiyaki iterative basecalling framework can be found at: https://github.com/wangziyuan66/iterative-labeling-toolkit-taiyaki . The Bonito iterative basecalling framework can be found at: https://github.com/wangziyuan66/iterative-labeling-toolkit-bonito .

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](#)

The BioRNA nanopore sequencing data was deposited at NCBI under the BioProject PRJNA1155679 (reviewer link: <https://dataview.ncbi.nlm.nih.gov/object/PRJNA1155679?reviewer=tmp2haiuk3d28bd7q25p15ula0>). The RNA oligo nanopore sequencing data was downloaded from NCBI under the BioProject PRJNA1050579 (runs SRR27324841, SRR27324838 and SRR27324839). Corresponding reference sequences were downloaded from the Table S2 of 8. The yeast native rRNA nanopore sequencing data was downloaded from ENA under accession number PRJEB48183 (samples ERR7162388, ERR7162390 and ERR7162391). Corresponding reference sequences were downloaded from https://github.com/adbailey4/yeast_rna_modification_detection/tree/main/notebooks/data. The yeast native tRNA nanopore sequencing data was downloaded from ENA under accession number PRJEB55684 (samples ERR10224971 and ERR10224975). Corresponding reference sequences and modification annotations were downloaded from <https://github.com/novoalab/Nano-tRNAseq/tree/main/ref>. The mRNA vaccine nanopore sequencing data was downloaded from NCBI under the BioProject PRJNA856796 (runs SRR22888949 and SRR22888950). Corresponding reference sequence was downloaded from <https://github.com/scchess/Mana/tree/main/data>. The basecalling sequencing summary files were uploaded to Figshare: <https://figshare.com/s/f39c893a85928aefd10c>, and the associated metadata is provided in Table S4.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	NA
Data exclusions	NA
Replication	NA
Randomization	NA
Blinding	NA

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	NA
Novel plant genotypes	NA
Authentication	NA