

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	guppy_basecaller 3.2.2+9fe0a78 ((C) Oxford Nanopore Technologies, Limited)
Data analysis	minimap2 2.18-r1015 samtools 1.11 (Copyright (C) 2020 Genome Research Ltd.) scikit-learn 1.2.0 tensorflow 2.11.1 Gene Ontology Resource (http://geneontology.org) NanoSPA (https://github.com/sihaohuanguc/NanoSPA)

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 nanopore seq data of this study have been submitted to NCBI GEO (accession number: GSE230936). The published nanopore WT samples (replicate 1 and 2) used to validate the m6A model are GSM5467024 and GSM5467025 under GSE180656. The published WT GLORI (GSM6432590, GSM6432591) and eTAM-seq data (GSE211303, GSE211303_hela.polya.wt.ftom.ftop.rep1.deep.hits.txt.gz) are from GEO database. The mESC nanopore sample (GSM5841801) and miCLIP2 sample (GSE163500) are from GEO database. H uman genome (GRCh38.p13) and annotation (gencode.v41.annotation.gff3) and mouse genome (GRCm38.primary_assembly.genome.fa) are from GENCODE database (<https://www.genencodegenes.org>). Gene ontology information is from Gene Ontology Resource (<http://geneontology.org>).

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

Methods

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

Antibodies

Antibodies used

Primary Antibodies:

Mouse anti-actin (Supplier: EMP Milipore/Sigma-Aldrich; clone C4 MAB1501; catalog number: MAB1501; lot number: GR3375496-17; Clone: C4)

Rabbit anti-METTL3 -- Supplier: Abcam; catalog number: [EPR18810] ab195352; lot number: GR3375496-17)

Rabbit anti-TRUB1 -- Supplier: ProteinTech; catalog number: 12520-1-AP; Lot Number: 00010789)

Secondary Antibodies:

Sheep anti-mouse IgG conjugated to HRP (Supplier: Cytiva, Catalog Number: NA931V, lot number: 17288406)

Donkey anti-rabbit IgG conjugated to HRP (Supplier: Cytiva ; Catalog Number: NA934V; lot number: 17365256)

Validation

Primary Antibodies:

Anti-Actin (MAB1501 clone C4) --

Manufacturer validation: Tested in HeLa cells, confirmed for use in ELISA, ICC, IF, IHC, IH(P), WB

Citations: Commonly cited antibody (www.millipore.com/catalogue/item/mab1501)

Anti-METTL3 ([EPR18810] ab195352) --

Manufacturer validation: WB, IHC-P, ICC/IF, IP, Flow Cyt (intra).

Citations: At least 165 literature citations of ab195352 have been found.

Anti-TRUB1 (1250-1-AP) --

Manufacturer validation - Positive WB detected in HEK-293T, HepG2; Positive IP in mouse liver tissue; positive IHC human kidney tissue, mouse testis tissue.

Citations: Limited published examples of available anti-TRUB1 antibodies. Blotting control HEK293T cells matched to reported size and banding pattern provided by manufacturer.

Secondary Antibodies:

Sheep anti-mouse IgG-HRP (NA931V) --

Manufacturer Validation: "Highly species-specific... designed for applications such as protein blotting, ELISA, and immunocytochemistry."

Citations: At least 128 literature citations have been found including our group's own (Watkins, C.P., Zhang, W., Wylder, A.C. et al. A multiplex platform for small RNA sequencing elucidates multifaceted tRNA stress response and translational regulation. Nat Commun 13, 2491 (2022). <https://doi.org/10.1038/s41467-022-30261-3>)

Donkey anti-rabbit IgG-HRP (NA934V) --

Manufacturer Validation: "Highly species-specific... designed for applications such as protein blotting, ELISA, and immunocytochemistry."

Citations: At least 155 literature citations have been found including our group's own (Watkins, C.P., Zhang, W., Wylder, A.C. et al. A multiplex platform for small RNA sequencing elucidates multifaceted tRNA stress response and translational regulation. Nat Commun 13, 2491 (2022). <https://doi.org/10.1038/s41467-022-30261-3>)

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

HEK293T /17 (CRL11268); Homo sapiens, kidney. ATCC. HeLa (ATCC).

Authentication

Morphology and doubling time

Mycoplasma contamination

These cells have not been verified against Mycoplasma contamination besides no observed change in morphology. Pan lab checks for mycoplasma contamination as a routine lab procedure, so far no contamination has been found.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines used