nature portfolio

Corresponding author(s): Thomas J. Santangelo

Last updated by author(s): 2024-06-2018

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	Software was not used to collect data.
Data analysis	MiniMap2 (v2.26-r1175), Agilent MassHunter WorkStation Quantitative Analysis for QQQ version 11.1, Trimmomatic v0.39, BSseeker2 v2.1.8, bowtie2 v2.2.14, Samtools v1.17, CGmaptools v0.1.2, R v4.2.2 software, tidyverse v2.0.0, dplyr v1.1.0, readr v2.1.4, forcats v1.0.0, stringr v1.5.0, ggplot2 v3.4.1, tibble v3.1.8, lubridate v1.9.2, tidyr v1.3.0, and purrr v1.0.1, pwr v1.3.0, Python2, Python3, Custom R and python scripts and associated files are provided on Github at https://github.com/kscott94/The_m5C-epitranscriptome_of_Thermococcus_kodakarensis, or on Zenodo at https://zenodo.org/doi/10.5281/zenodo.11556635.

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

The Bisulfite-sequencing fastq files generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA937301 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA937301). Whole genome sequencing fastq files generated in this study have been deposited in the NCBI SRA under BioProject PRJNA1125032 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1125032). RNA-seq Data presented in Fig. 1d and Supplementary Fig. 1d were queried from NCBI Sequence Read Archive under accession codes SRR9966705, SRR9966714, SRR9966715, SRR9966721. The CryoEM structure of the T. kodakarensis ribosome was queried from the Protein Data Bank under accession code 6TH6. The original agarose gel electrophoresis image represented in Fig. 2a is provided in Source Data File 1.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	sample sizes of n=2 for bisulfite sequencing and and n=3 for methyltransferase andmass spec. assays.
Data exclusions	N/A
Replication	Bisulfite-seq was done in duplicate, methyltransferase assays were done in triplicate.
Randomization	Randomly assigned
Blinding	N/A

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	N/A
Novel plant genotypes	N/A
Authentication	N/A