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	Sequencing data were collected by Nanopore direct RNA sequencing at the Genomic Facility of the European Institute of Oncology (IEO).
Data analysis	FAST5s obtained from dRNA-seq were basecalled using Guppy 6.2.1 FASTQs filtered with NanoFilt 2.8.0 The selected reads were aligned using minimap 2.0.1 Samtools (v1.6) was used for reads filtering Intronic and exonic regions were defined based on the TxDb.Hsapiens.UCSC.hg38.knownGene object from Bioconductor 3.17 and the Bioconductor package GenomicFeatures (v1.48.1) Reads annotation was performed with the Bioconductor package GenomicAlignments (v1.32.1) Gene expression quantification was performed with the Bioconductor package DESeq2 (v1.38.3) Genomic sequences were extracted from BED files with bedtools (v2.30.0) Sequences entropy was estimated with the R package DescTools (v 0.99.49) Structural identifiability was tested with the SIAN software (v 1.1) RNA dynamics profiling with INSPEcT was performed with the software version 1.28 GSEA analyses were performed with the Bioconductor package clusterProfiler (v4.6.2) Nascent reads were in silico profiled with nano-ID Nanopolish 0.13.3 had been used to profile transcripts polyA tail length R 4.2 has been used to perform all the analyses Spearman correlation significance was estimated using the default options of the R 4.2 cor.test function from the stats package (algorithm AS 89 for n < 1290 or via the asymptotic t approximation otherwise)

Nanodynamo source code is available at https://github.com/mfurla/Nanodynamo.git

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 sequencing data generated in this study have been deposited in the SRA database under the accession code PRJNA1023045 [https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1023045].

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	The study aims at studying the dynamics of RNA metabolism. The data were obtained using SUM159 cells, an in vitro model of triple negative breast cancer derived from a primary human anaplastic breast carcinoma. We have no reasons to expect that the dynamics of RNA metabolism would depend on cells gender.
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	The optimum sample size (i.e. number of replicates and labeling time-points) was determined based on simulated data analyses.
Data exclusions	No data were excluded.
Replication	The reproducibility of expression data and kinetic rates was assessed through correlative analyses across two biological replicates; all attempts were successful.
Randomization	Samples allocation and randomization is not relevant for this study
Blinding	Our project did not require group allocation thus blinding was not possible.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used: Vinculin (Cell Signaling #13901), LaminB1 (Cell signaling #12586), Histone H3 (Santa Cruz, sc-517576)

Validation: Vinculin anti-Rabbit: see manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=13901&images=1&size=A4
LaminB1 anti-Rabbit: see manufacturer's datasheet: https://www.cellsignal.com/datasheet.jsp?productId=12586&images=1&size=A4
Histone H3 anti-Mouse: see manufacturer's datasheet: https://www.scbt.com/it/p/ac-histone-h3-antibody-ah3-120

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): SUM159PT (RRID:CVCL_5423 - Female): Asterand (BioIVT) HUMANSUM-0003006: https://bioivt.com/sum-breast-cancer-cell-lines
K562 (RRID:CVCL_0004 - Female): acquired from Kristian Helin lab

Authentication: SUM159PT and K562 cell lines were not authenticated.

Mycoplasma contamination: SUM159PT and K562 Cell lines were tested for mycoplasm contamination at the time of purchasing and regularly thereafter, as a service from the cell culture facility of our Institution.

Commonly misidentified lines (See ICLAC register): No misidentified cell lines were used in this study

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A