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Corresponding author(s): Siyuan Wang
Last updated by author(s): Jan 21, 2021

Reporting Summary

Nature Research 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 Research 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	Data are collected with open source python codes from https://github.com/ZhuangLab/storm-control. No other software is used.
Data analysis	The ProbeDealer package can be downloaded from https://campuspress.yale.edu/wanglab/probedealer. The MinaAnalyst package can be downloaded from https://campuspress.yale.edu/wanglab/mina-analyst/. Data analysis codes were deposited at https://github.com/SiyuanWangLab/MINA. MATLAB version R2019b was used for data analysis.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The ProbeDealer package can be downloaded from https://campuspress.yale.edu/wanglab/probedealer. The MinaAnalyst package can be downloaded from https://campuspress.yale.edu/wanglab/mina-analyst/. An example raw dataset of two imaging fields are downloadable from https://campuspress.yale.edu/wanglab/mina-analyst/. The full datasets used to generate Fig. 7 and Fig. 8 are not uploaded online due to the prohibitively large size. The full datasets are available from the corresponding author upon request.

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 data presented in the manuscript represents one replicate from the published MINA work. The sample size for Fig.7b is: 9,335 copies of mChr19. The sample sizes for Fig.8b are: 789 copies and 3,386 copies of chromatin traces in hepatocytes and non-hepatocytes respectively. Sample sizes were counted from images. The sample sizes are large enough as the conclusions derived from each replicate in the published MINA work are repeatable.
Data exclusions	The data presented in the manuscript represents one replicate from the published MINA work. In MERFISH analysis of single-cell RNA expression, we excluded "cells" smaller than 2,500 pixels as these were usually non-cell particles. We excluded 'cells' larger than 20,000 pixels in area as these were usually empty regions in tissue sections. We excluded cells with less than 10 detected RNA molecules to ensure high quality in the cell type identification analyses. These exact exclusion criteria were not pre-established but the concepts of these exclusion criteria are similar to those generally used in previous single cell RNA sequencing analyses.
Replication	The data presented in the manuscript represents one replicate from the published MINA work.
Randomization	Mouse E14.5 fetal livers were randomly selected for performing MINA experiments. Imaging fields of view were randomly selected across the tissue sections.
Blinding	No blinding was performed as no comparisons were made between different groups.

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
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

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

Antibodies

Antibodies used	anti-fibrillarin primary antibody (Abcam Cat# ab5821); Alexa Fluor 647-conjugated anti-rabbit secondary antibody (Molecular Probes Cat# A-31573)
Validation	The anti-fibrillarin antibody has been tested for specificity by the manufacturer through western blot and blocking by immunising peptide.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The IMR90 cell line used was purchased from ATCC (Cat# CCL-186).
Authentication	The cell line used was not authenticated.
Mycoplasma contamination	The cell line was not tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was used in the study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Pregnant female C57BL/6 mice aged 8-15 weeks from the Jackson Laboratory were used for all MINA experiments. All mice were maintained under 12 h light/12 h darkness cycles with constant conditions of temperature (22 °C) and humidity (40%-60%).
Wild animals	No wild animals were involved in the study.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	All animal experiments described here were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.