

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	NIS-Elements L/Lite software (Nikon ECLIPSE Ts2R microscope), Illumina NovaSeq X plus sequencing system
Data analysis	Trim-Garole (0.6.10), BBMap (v.39.01), 10X Genomics cellranger-atac (v2.0.0), runHiC (v.0.8.3-r1) (https://xiaotaowang.github.io/HiC_pipeline/), pairtools (v. 0.3.0)(https://pairtools.readthedocs.io/en/latest/), ArchR (https://www.archrproject.com/), AtlasXbrower (https://github.com/atlasxomics/AtlasXbrower), scHiCluster (v.1.3.5) (https://github.com/zhoujt1994/scHiCluster), SnapATAC2 (v. 2.6.4) (https://scverse.org/SnapATAC2/), Scanpy (v. 1.10.2), Seurat (v. 5.1.0) (https://github.com/satijalab/seurat), dcHiC (https://github.com/ay-lab/dcHiC), NeoLoopFinder (https://github.com/XiaoTaoWang/NeoLoopFinder)

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](#)

Raw sequencing data reported in this paper are deposited in the Gene Expression Omnibus (GEO) with accession code: GSE307620. In-situ Hi-C data from mouse cortex were downloaded from 4DN Data Portal with accession number: 4DNESUQT299T. The BICCN whole mouse brain snATAC-seq datasets were downloaded from Gene Expression Omnibus under accession number GSE246791.

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

Sex and gender were not relevant in this study. Patients were male and female. For patient samples:
 NU03747: Female.
 NU03766: Female.
 NU04041: Male.
 NU04741: Female
 NU04707: Female

Reporting on race, ethnicity, or other socially relevant groupings

Race, ethnicity, and other socially relevant groupings were not considered in and did not impact our study design.

Population characteristics

Population characteristics were not considered in and did not impact our study design.

Recruitment

Human brain cerebellum frozen slides and cerebral cortex frozen slides are commercially-available samples obtained from Zyagen. The glioma specimen samples were obtained from Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, and informed consent was obtained from the participating patient.

Ethics oversight

The glioma specimen collection was approved by the Institutional Review Board (IRB) at Northwestern University.

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

No sample size calculations were performed. Sample size were chosen primarily based on sample availability and sequencing costs. The current manuscript mainly described a new method for profiling spatially resolved 3D chromatin architecture and accessibility. The sample sizes are sufficient because each sample serves as a proof-of concept for the Spatial-Hi-C technology.

Data exclusions

No data were excluded from the study.

Replication

There are no replicates in this study, mainly due to the fact there are enough data collected from different types of tissues, which can support the feasibility of the current technique.

Randomization

There are no defined sample groups in this study because the focus of this paper is the development of a new method for profiling spatially resolved chromatin architecture and accessibility. Randomization is not relevant to this study.

Blinding

There are no defined sample groups in this study, so blinding is not relevant to this study.

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

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	The mouse brain samples: C57BL/6 mouse Anterior Hypothalamus Coronal frozen slides_Region.6 and C57BL/6 mouse Hippocamp/thalamus/hypothal Coronal frozen slides_Region.8 are commercially-available samples obtained from Zyagen.
Wild animals	The study did not involve wild animals.
Reporting on sex	N.A.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	The samples were obtained from a commercial source.

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

Plants

Seed stocks	N.A.
Novel plant genotypes	N.A.
Authentication	N.A.