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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	Custom written scripts in Micro-manager 1.4 for automated fluids delivery and image acquisitions.
Data analysis	Custom written scripts in MATLAB R2019a, Python (v3.8.3), Mathematica (v12.0), R (v3.6.3), PyMOL (v2.0) and ImageJ (v1.51s). In addition, following tools and algorithms were used, Bowtie2 (v2.3.1), Juicer tools (v1.5), Bedtools (v2.26.0), Seurat (v3.0), Cytoscape (v3.5.1), and 3D radial center algorithm (https://pages.uoregon.edu/raghu/particle_tracking.html). See the Methods section for the detail usages. The custom written scripts used in this study are available at https://github.com/CaiGroup/dna-seqfish-plus.

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

Additional processed data from this study is available at Zenode website (DOI: 10.5281/zenodo.3735329). All raw data obtained during this study are available from the corresponding author upon reasonable request. Publicly available datasets used in the study (GSE96107, 4DNESOJRTZZR, GSE17051, GSE102076, GSE48895, ENCSR000CFN, ENCSR000CGP, ENCSR000CGQ) are detailed in the Methods.

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 were chosen to stabilize the measurement distributions. Analyses showing high reproducibility and the agreement of measurements with literatures (Shen et al. 2012; Hormoz et al. 2016; Bonev et al. 2017; Quinodoz et al. 2018) indicate that the sample sizes are sufficient. See Figure legends for each experiment.
Data exclusions	No raw data was excluded from the analysis. For downstream analysis, cells at the corner of the fields of view were excluded due to the laser illumination bias, and several antibodies (H3, H3K4me1, H3K4me2 and H3K4me3) were excluded due to the quality of antibody staining with oligo-conjugation.
Replication	Two independent biological replicates with hundreds of cells each were imaged. High Pearson's correlation coefficient of 0.92 for chromosome contact frequencies indicates the reproducibility of the methods between the two replicates.
Randomization	Individual cells across 5 to 10 different field of views were chosen randomly for image acquisition for each sample.
Blinding	No blinding was necessary for this study, and biological samples were defined before the measurements. Image processing and analyses were performed in an automated and identical way across different samples.

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	mH2A1 (Abcam ab232602), E-Cadherin (R&D AF748), Fibrillarin (C13C3) (Cell Signaling 2639BF), Geminin (Abcam ab238988), GFP (Invitrogen G10362), H3 (Active Motif 39763), H3K27ac (Active Motif 39133), H3K27me2 (Cell Signaling 9728BF), H3K27me3 (Cell Signaling 9733BF), H3K4me1 (Cell Signaling 5326S), H3K4me2 (Cell Signaling 9725BF), H3K4me3 (Active Motif 39915), H3K9ac (Active Motif 91103), H3K9me2 (Abcam ab1220), H3K9me3 (Diagenode MAb-146-050), H3pSer10 (Millipore 05-806), H4K16ac (EMD Millipore 07-329), H4K20me1 (Abcam ab9051), H4K20me2 (Abcam ab9052), H4K20me3 (Active Motif 39671), Lamin B1 (Abcam ab220797), RNAPII Ser5-P (Abcam ab5408), SF3a66 (Abcam ab77800)
Validation	Localization patterns from oligo DNA conjugated antibodies were validated by comparing to those from unconjugated antibodies individually. All primary antibodies were purchased and validated by manufactures as follows. mH2A1 (Abcam ab232602) Application: Western Blot (WB), Immunohistochemistry (IHC-P), Immunocytochemistry (ICC)/Immunofluorescence (IF) Species reactivity: Mouse, Rat, Human E-Cadherin (R&D AF748) Application: WB, Simple Western, Flow Cytometry (Flow), IHC, CyTOF-ready, ICC Species reactivity: Human, Mouse

Fibrillarin (Cell Signaling 2639BF)
Application: WB, IF
Species reactivity: Human, Mouse, Rat, Monkey

Geminin (Abcam ab238988)
Application: IHC-P, WB, ICC/IF
Species reactivity: Human

GFP (Invitrogen G10362)
Application: ELISA, Flow, IHC, IP, WB
Species reactivity: Tag
Published species: C. elegans, Cat, Fruit fly, Human, Mouse, Non-human primate, Rat, Tag, Yeast, Zebrafish
The antibody is reported in the literature for IF application (list of publications on the manufacturer's website).

H3 (Active Motif 39763)
Application: Chromatin Immunoprecipitation (ChIP), ChIP-seq, IF, WB, ICC
Species reactivity: Human, Wide Range Predicted
The antibody is reported in the literature for Mouse reactivity (list of publications on the manufacturer's website).

H3K27ac (Active Motif 39133)
Application: ChIP, ChIP-seq, IF, WB, Dot Blot (DB), IHC, ICC
Species reactivity: Budding Yeast, Human, Wide Range Predicted
IF profiles obtained in this study were validated by ChIP-seq data (encodeproject.org, accession ENCSR000CGQ) in Extended Data Fig. 6a, b.

H3K27me2 (Cell Signaling 9728BF)
Application: WB, IP, IF, Flow, ChIP
Species reactivity: Human, Mouse, Rat, Monkey

H3K27me3 (Cell Signaling 9733BF)
Application: WB, IHC, IF, Flow, ChIP, CUT&RUN
Species reactivity: Human, Mouse, Rat, Monkey

H3K4me1 (Cell Signaling 5326S)
Application: WB, IF, Flow, ChIP
Species reactivity: Human, Mouse, Rat, Monkey

H3K4me2 (Cell Signaling 9725BF)
Application: WB, IP, IHC, IF, Flow, ChIP
Species reactivity: Human, Mouse, Rat, Monkey

H3K4me3 (Active Motif 39915)
Application: ChIP, ChIP-seq, WB, IF, DB, ICC
Species reactivity: Budding Yeast, Human, Wide Range Predicted
The antibody is reported in the literature for Mouse reactivity (list of publications on the manufacturer's website).

H3K9ac (Active Motif 91103)
Application: ChIP, ChIP-seq, WB, IF, ELISA, ICC
Species reactivity: Human, Wide Range Predicted
IF profiles obtained in this study were validated by ChIP-seq data (encodeproject.org, accession ENCSR000CGP) in Extended Data Fig. 6a, b.

H3K9me2 (Abcam ab1220)
Application: WB, ELISA, IHC-P, ChIP
Species reactivity: Rat, Chicken, Cow, Human, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Corn, Common marmoset, Rice
Predicted species reactivity: Mouse, Sheep, Saccharomyces cerevisiae, Other species
The antibody is reported in the literature for IF application and Mouse reactivity (list of publications on the manufacturer's website).

H3K9me3 (Diagenode MAb-146-050)
Application: ChIP, DB, WB
Species reactivity: Human, Fungi
The antibody is reported in the literature for IF application and Mouse reactivity (list of publications on the manufacturer's website).

H3pSer10 (Millipore 05-806)
Application: Flow, ICC, IF, Platelet Immuno-Assay (PIA), WB, multiplexing (Mplex)
Species reactivity: Human
The antibody is reported in the literature for IF application and Mouse reactivity (list of publications on the manufacturer's website).

H4K16ac (EMD Millipore 07-329)
Application: WB, Mplex, PIA, DB, ChIP-seq, ChIP
Species reactivity: Human, Mouse, Rat
The antibody is reported in the literature for IF application (list of publications on the manufacturer's website).

H4K20me1 (Abcam ab9051)
Application: IHC-P, WB, ICC/IF, ChIP
Species reactivity: Mouse, Cow, Human

H4K20me2 (Abcam ab9052)
Application: IP, WB, ICC/IF, IHC-P
Species reactivity: Mouse, Cow, Schizosaccharomyces pombe, Toxoplasma gondii

H4K20me3 (Active Motif 39671)
Application: ChIP, WB, DB, ICC
Species reactivity: Human, Mouse, Wide Range Predicted

Lamin B1 (Abcam ab220797)
Application: ICC, IP, WB, IHC-P
Species reactivity: Mouse, Rat, Human
BSA and Azide containing batches (Abcam ab133741) are validated for IF by the manufacturer.

RNAPII Ser5-P (Abcam ab5408)
Application: Flow, ICC/IF, DB, ChIP, WB, ELISA
Species reactivity: Mouse, Rat, Human

SF3a66 (Abcam ab77800)
Application: IHC-P, WB, ICC/IF, Flow
Species reactivity: Rat, Human, Monkey
IF profiles obtained in this study were validated by SPRITE data (data.4dnucleome.org, accession 4DNESOJRTZZR) in Extended Data Fig. 6a, b.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	E14 mESCs (E14Tg2a.4) from Mutant Mouse Regional Resource Centers was used in this study.
Authentication	The cell lines were authenticated by DNA seqFISH+ (Extended Data Fig. 3a-g), multiplexed immunofluorescence (Extended Data Fig. 6a-f), and RNA seqFISH (Extended Data Fig. 9a-c), all of which gave results consistent with the embryonic stem cell identity.
Mycoplasma contamination	The cell line was not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	The cell line used was not listed in the ICLAC database.