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Reporting Summary

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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 was performed on the Illumina Hi-Seq4000.
Images were collected using Leica LAS X v1.9.0.
E13 NPCs were sorted using FACS Aria III flow cytometer (Becton, Dickinson, Biosciences, USA).
Raw sequencing data was generated using illumina Hi-seq4000 platform.

Data analysis

Pseudoalignment of RNA-seq data was performed using Kallisto v0.43.1. Differential RNA-seq expression was computed using Sleuth v0.30.0 in the R (v.3.6.3) computing environment (www.r-project.org). R (v.3.6.3) was also used to perform student's t-tests, permutation analysis, pearson correlations, and for the creation of plots. deepTools (v 3.2.0) was used for analysis of sequencing trace files.
Genomic compartment enrichment was performed with Garfield (V2).
Images were processed using ImageJ software (Version: 2.0.0-rc-65/1.52q, build: 961c5f1b7f). For DNA-FISH quantifications, 3D reconstructions of nuclei were conducted via the Imaris x64 (v 9.2.1) software (Bitplane AG, Switzerland).
Mouse, human and macaque data were aligned to mm10, hg38 and rheMac8 reference genomes, respectively.

Other softwares used:
Adapter trimming: bbduk (Dec 13,2018 update)
Alignment: bowtie2 (v2.2.6)
Duplicate Removal: PicardTools MarkDuplicated (v2.15.0-snapshot)
LAD calling: EED (v1.1.19)
LAD/SPAD calling: EPIC2 (v0.0.41)
NGS trace file visualization: deeptools (v.3.1.3)
UCSC genome browser

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

Mouse and macaque datasets generated in this study are available on GEO repository with the following accession number:

Human data generated in this study is available on dbGAP repository with the following accession number:

The following previously published datasets were used in the study:
1. DamID data of MEFs, NPCs, ASCs, ESCs and cLADs (GSE17051).https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17051
2. 3T3 MEFs RNA-seq (PRJNA269376). https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA269376.
3. DamID data of human cell lines, HCT116, RPE, H1hESCs, U2OS, K562, HAP1. (https://data.4dnucleome.org/publications/5a5117c9-face-4648-bbb7-d54e468af2ba/#overview).
4. Mouse enhancer data (http://chromosome.sdsc.edu/mouse/download.html).
5. GW12 human brain ChIP-seq data (GSE63634).https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63634.
6. K562 SON TSA-seq (GSE81553). https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81553.
7. E13.5 mouse forebrain ATAC-seq (Encode - ENCFF059AWY). https://www.encodeproject.org/experiments/ENCSR903GMO/.
8. E13.5 mouse forebrain Whole Genome bisulfite sequencing (WGBS) (Encode - ENCFF254BTJ). https://www.encodeproject.org/experiments/ENCSR141ZVB/.
9. Human neuronal stem cells originated from H1, Whole Genome bisulfite sequencing (WGBS) (Encode- ENCFF458SQS). https://www.encodeproject.org/experiments/ENCSR540ONK/.
10. GW19 fetal cortex ATAC-seq (GSE149268). https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE149268

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

Mouse and human data was generated for two biological replicates. These biological replicates showed high concordance. For mouse studies, 4-5 embryos were pooled for each replicate. For human studies, replicates were from brain tissue obtained from two identified subjects. Macaque data was generated for one replicate due to limited tissue availability. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications:
https://www.nature.com/articles/nature06947.
https://www.sciencedirect.com/science/article/pii/S1097276510003217
https://rupress.org/jcb/article/217/11/4025/120670/Mapping-3D-genome-organization-relative-to-nuclear

Data exclusions	"No data were excluded from the analyses".
Replication	Mouse GO-CaRT experiments were repeated two times using ~100,000 FAC sorted cells/antibody. Human GO-CaRT experiments were performed on freshly dissociated cells (~75,000) from two different subjects for each antibody. Macaque GO-CaRT experiments were performed on freshly dissociated cells (using ~100,000 cells/antibody). GO-CaRT experiments on mouse 3T3-MEFs and HEK293T cells were repeated two times. Mouse and human RNA-seq data was generated from two biological replicates. Data from two biological replicates was highly reproducible.
Randomization	In experiments with mouse, embryos from 3-5 female mice were pooled and randomly allocated to experimental groups. In experiments with human, brain tissue was obtained from de-identified donors with similar developmental age (Gestation week 20).
Blinding	DNA-FISH imaging and quantification was assessed blinded by two scientists. One of them was blinded to experimental 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 LaminB1 antibody, Rabbit polyclonal (Abcam# ab16048) was used at 1:100 dilution for GO-CaRT, 1:500 dilution combined Immunocytochemistry and DNA-FISH experiments . Vendor: https://www.abcam.com/lamin-b1-antibody-nuclear-envelope-marker-ab16048.html

anti-SON antibody, Rabbit polyclonal (Atlas antibodies #HPA031755) was used at 1:100 dilution for GO-CaRT, 1:500 dilution for combined Immunocytochemistry and DNA-FISH experiments . Vendor: https://www.atlasantibodies.com/products/antibodies/primary-antibodies/triple-a-polyclonals/son-antibody-hpa031755/

anti-H3K9me2 antibody, Rabbit polyclonal (Active Motif #39239) was used at 1:100 dilution for CUT&RUN analysis. Vendor: https://www.activemotif.com/catalog/details/39239/histone-h3-dimethyl-lys9-antibody-pab

anti-H3K9me3, Rabbit polyclonal (Abcam# ab8898) was used at 1:100 for CUT&RUN analysis. Vendor: https://www.abcam.com/histone-h3-tri-methyl-k9-antibody-chip-grade-ab8898.html

anti-H3K27me3, Rabbit monoclonal (Cell signaling technologies #9733) was used at 1:100 for CUT&RUN analysis. Validated by vendor: https://www.cellsignal.com/products/primary-antibodies/tri-methyl-histone-h3-lys27-c36b11-rabbit-mab/9733

Normal Rabbit IgG antibody (Cell signaling technologies #2729) was used at 1:100 for GO-CaRT and CUT&RUN analysis. Vendor: https://www.cellsignal.com/products/primary-antibodies/normal-rabbit-igg/2729

anti- GFP antibody, Chicken polyclonal (abcam #ab13970) was used at 1:500 dilution for immunohistochemistry. Vendor: https://www.abcam.com/gfp-antibody-ab13970.html

ATTO647N, Goat, anti-Rabbit, secondary antibody (Active Motif #15048) was used at 1:250 dilution for combined Immunocytochemistry and DNA-FISH experiments. Vendor: https://www.activemotif.com/catalog/details/15048/atto-647n-sted-gsd-goat-anti-rabbit-igg.

Validation

All antibodies have previously been validated for immunodetection of their corresponding proteins (see publications below).

anti LaminB1 antibody, Rabbit polyclonal (Abcam# ab16048): Validation: Poleshko, A. et al. Genome-Nuclear Lamina Interactions Regulate Cardiac Stem Cell Lineage Restriction. Cell 171, 573-587 e514, doi:10.1016/j.cell.2017.09.018 (2017).

anti-SON antibody, Rabbit polyclonal (Atlas antibodies #HPA031755) was used at 1:100 dilution for GO-CaRT, 1:500 dilution for combined Immunocytochemistry and DNA-FISH experiments . Validation: Chen, Y. et al. Mapping 3D genome organization relative to nuclear compartments using TSA-Seq as a cytological ruler. J Cell Biol 217, 4025-4048, doi:10.1083/jcb.201807108 (2018).

anti-H3K9me2 antibody, Rabbit polyclonal (Active Motif #39239) was used at 1:100 dilution for CUT&RUN analysis. Validation:

Poleshko, A. et al. Genome-Nuclear Lamina Interactions Regulate Cardiac Stem Cell Lineage Restriction. Cell 171, 573-587 e514, doi:10.1016/j.cell.2017.09.018 (2017).

anti-H3K9me3, Rabbit polyclonal (Abcam# ab8898) was used at 1:100 for CUT&RUN analysis. Vendor: https://www.abcam.com/histone-h3-tri-methyl-k9-antibody-chip-grade-ab8898.html: Validation: Poleshko, A. et al. Genome-Nuclear Lamina Interactions Regulate Cardiac Stem Cell Lineage Restriction. Cell 171, 573-587 e514, doi:10.1016/j.cell.2017.09.018 (2017).

anti-H3K27me3, Rabbit monoclonal (Cell signaling technologies #9733) was used at 1:100 for CUT&RUN analysis. Validation: Skene, P. J. & Henikoff, S. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. Elife 6, doi:10.7554/eLife.21856 (2017).

Normal Rabbit IgG antibody (Cell signaling technologies #2729) was used at 1:100 for GO-CaRT and CUT&RUN analysis. Vendor: https://www.cellsignal.com/products/primary-antibodies/normal-rabbit-igg/2729. Validation: Hoffmann et al. TMEM41B Is a Pan-flavivirus Host Factor, Cell, Volume 184, 133-148.e20. https://doi.org/10.1016/j.cell.2020.12.005.

Anti- GFP antibody, Chicken polyclonal (abcam #ab13970) was used at 1:500 dilution for immunohistochemistry. Vendor: https://www.abcam.com/gfp-antibody-ab13970.html. Validation: Li C et al. Comprehensive transcriptome analysis of cochlear spiral ganglion neurons at multiple ages. Elife 9:N/A (2020).

ATTO647N, Goat, anti-Rabbit, secondary antibody (Active Motif #15048) was used at 1:250 dilution for combined Immunocytochemistry and DNA-FISH experiments. Vendor validated: https://www.activemotif.com/catalog/details/15048/atto-647n-sted-gsd-goat-anti-rabbit-igg

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	NIH-3T3 MEFs and HEK293 lines were obtained from ATCC.
Authentication	No cell lines were authenticated
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

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

Laboratory animals	Experiments were conducted on C57BL/6-Tg(Nes-TK*,-EGFP)145Sker/J reporter mice (Stock No: 029671, Jackson Laboratory, USA) at embryonic day 13 (E13). Gender of the mice were not determined. . All mice were maintained under standard housing conditions with a 12 hour light/12 hour dark cycle , 40-60% humidity and a temperature of 65-75 fahrenheit as approved by the Institutional Animal Care and Use Committee (IACUC).
Wild animals	Study did not involve wild animals
Field-collected samples	Study did not involve samples collected from the field
Ethics oversight	All mice were maintained under protocols approved by the Institutional Animal Care and Use Committee (IACUC).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Because of the sensitivity of the samples, no population characteristics are known or recorded.
Recruitment	No recruitment criteria other than consent were required. Primary human brain tissue (Gestational week 20) was obtained from de-identified donors with previous patient consent in strict observance of the legal and institutional ethical regulations.
Ethics oversight	Protocols were approved by the Human Gamete, Embryo, and Stem Cell Research Committee (institutional review board) at the University of California, San Francisco. All experiments were performed in accordance with protocol guidelines. Informed consent was obtained before sample collection and use for this study.

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

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Dorsal (Cortex) and ventral (Ganglionic eminence) regions of the embryonic forebrain were microdissected from C57BL/6-Tg(Nes-TK*,-EGFP)145Sker/J reporter mice. Tissue was cut into small pieces and dissociated into single cells using papain tissue dissociation kit (Worthington Biochemical Corporation) following manufacturer's instructions. Dissociated cells were passed through a cell strainer (70 micron, BD Biosciences) to obtain single cell suspension. Cells were resuspended in PBS containing 1% BSA and sorted for GFP positive cells. Sorted cells were directly processed for LaminB GO-CaRT.
Instrument	FACS Aria III cytometer (Becton, Dickinson Biosciences) with 85-micron size nozzle.
Software	No data analyses was required
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Cells were gated first using forward and side scatter pulse area parameters (FSC-A and SSC-A) excluding debris, followed by exclusion of aggregates using pulse width (FSC-W and SSC-W). Cells dissociated from wildtype dorsal and ventral forebrain regions (GFP negative) were used to set GFP+ gates.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.