

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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 All data analyzed within this manuscript are publicly available. No additional software was used for the data collection process.

Data analysis We performed all the data analysis using our developed eSpatial software (Version: 1.0.0) in this paper, which is available at <https://github.com/xmuhuanglab/eSpatial>, Zenodo (<https://doi.org/10.5281/zenodo.15383127>) and CodeOcean (<https://codeocean.com/capsule/2493559/tree/v1>). eSpatial has multiple dependencies, including R version 4.2.2, ArchR v1.0.2, Signac v1.11.0 and Seurat v4.4.0.

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 spatial-ATAC-RNA seq data of the P22 mouse brain, e13 mouse embryo, and e11 mouse embryo used in this study are available in the Gene Expression Omnibus (GEO) under accession codes GSE205055 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205055>] and GSE171943 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171943>]

www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171943. The MISAR-seq data of the e13.5 mouse fetal brain used in this study are available in the National Genomics Data Center under accession number OEP003285 (www.biosino.org/node/project/detail/OEP003285). The H3K27ac MERFISH data of the e13.5 mouse fetal brain used in this study are available in Zenodo under the DOI <https://doi.org/10.5281/zenodo.7075964>. The Slide-tags snRNA+snATAC data of human melanoma used in this study are available in the Broad Institute Single Cell Portal under the accession number SCP2176 [https://singlecell.broadinstitute.org/single_cell/study/SCP2176/slide-tags-multiomic-snrna-seq-snatac-seq-on-human-melanoma]. The spatial-ATAC data of human breast cancer used in this study were kindly shared by the authors of the original study. The experimentally validated VISTA enhancers in human and mouse noncoding fragments with gene enhancer activity assessed in transgenic mice are accessible through the VISTA Enhancer Browser at <https://enhancer.lbl.gov/>. The Allen Brain Reference Atlases ISH data used in this study are accessible through the Allen Brain Atlas at <https://atlas.brain-map.org/>.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

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	E0-KO, E2-KO, E3-KO, and E0+3-DKO mice are from our lab. These mice are created from strain ICR. Embryos were dissected at e11.
Wild animals	The study dose not involve wild animals.
Reporting on sex	Sex was not considered in study design.
Field-collected samples	Mice were housed in a standard ventilated cage with a 12h light/12h dark cycle. 3-4 mice were housed in a cage. The ambient temperature is 25°C with relative humidity of 50%. All mice ad libitum access to water and chow.
Ethics oversight	The care and use of mice were followed by the United States National Institutes of Health Guidelines and the Chinese Ministry of Public Health Guidelines. All the mouse experiments were performed according to the ethical guidelines established by the Animal Care and Use Committee of the Institute of Genetics and Development Biology, Chinese Academy of Sciences

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

Plants

Seed stocks	This study doses not involve the use of plant seed stocks or other plant materials.
Novel plant genotypes	This study dose not involve the generation or use of novel plant genotypes.
Authentication	This study dose not involve the authentication of plant seed stocks or novel genotypes.