

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	The authors did not generate new computer code
Data analysis	1) Bulk RNAseq data: Raw RNAseq data were pre-processed using the RNAsik pipeline (v1.5.5) Raw counts were used for downstream differential expression analysis using the Degust web tool. Differential expression analysis used Limma-voom workflow. By thresholding the gene ontology and pathway analyses used the Metascape webtool. 2) Bulk ATACseq: Raw sequencing reads were processed through the nf-core/atacseq pipeline (v1.2.1). The GRCm38/mm10 genome was used for the sequence alignment performed using BWA; and the peaks called using MACS2 were then quantified using featureCounts to create a raw peak count matrix and used for differential chromatin accessibility analysis using the Degust webtool. The peaks that were differentially accessible were annotated using the Genomic Regions Enrichment of Annotations Tool (GREAT). 3) ChIPseq and CUT&RUN: Raw sequencing results of the ChIPseq and CUT&RUN were processed through the nf-core/chipseq (v1.2.2) and nf-core/cutandrun (v3.2.2) 56 pipeline, respectively, to generate peaks sets and assess quality. BWA was used for sequence alignment of the ChIPseq pipeline and Bowtie2 for the CUT&RUN pipeline, both using the GRCm38/mm10 mouse genome from ENSEMBL as a reference. Bigwig files were generated and peaks were called against the IgG samples using SEACR. The consensus peak sets of each replicate, and the consensus of H3K27ac, ATACseq, and DLX2 CUT&RUN were performed using Bedtools intersect. The genomic region annotation of these peak sets was then performed in R (v4.1.2) using clusterProfiler (v4.2.2), while gene association and GSEA was performed using GREAT. HOMER (v4.1.1) was then used to perform motif enrichment analysis, using findMotifsGenome.pl. 4) 10x Multiome data: Raw sequencing fastq files of Gene Expression and ATAC libraries were processed using Cell Ranger Arc (v2.0.2) with the mm10 reference genome. 5) snRNAseq data: Downstream gene expression data pre-processing utilised Scanpy (v1.10.1). Individual data sets were integrated using scanpy.external.pp.harmony_integrate. The UMAP was calculated using default parameters, and Leiden unsupervised clustering was performed.

6) snATACseq data: Downstream ATAC pre-processing used pycisTopic (v1.03). Using the annotated snRNAseq data generated previously, pseudobulk ATAC fragment profiles for each cell type were generated and peaks were called using MACS for each pseudobulk profile. cisTopic object was created for individual data sets, including filtering of GRCm38/mm10 genome blacklist regions, obtained from the UCSC database. cisTopic objects were merged according to genotype and data sets were individually integrated using Harmony.

7) Differentiation trajectory analysis of snRNAseq data used scFates (v1.0.7). Multiscale diffusion space was computed using Palantir (v1.3.3) then ForceAtlas2 embedding. The differentiation tree used scFates.tl.tree with the simplept option. The pseudotime and dendrogram of the differentiation tree were then calculated using scFates.tl.pseudotime and scFates.tl.dendrogram, respectively.

8) eRegulon analysis used the SCENIC+ (v1.01a1) pipeline (eRegulons) and pre-processed snRNAseq data. The pre-processing ATAC data as the cisTopic object was used as snATACseq data input, along with the topic set that was generated from the pycisTopic processing. pycisTarget (v1.0.2) was used to generate custom databases of scores of a set of given motifs in the regulatory regions identified in the data set created. The eRegulon specificity score was determined using scenicplus.RSS.regulon_specificity_scores function.

9) snRNAseq Pseudobulk expression and TF activity analysis: The pseudobulk profile of the snRNAseq data was obtained using Decoupler (v1.6.0). Differential expression of the filtered genes were computed using pydeseq2 (v0.4.4). The networks were visualized using decoupler.plot_network.

10) Pre-processing analysis of spatial transcriptomics data: Stitched images taken during tissue processing were processed through ImageStudio to transform into files compatible with the SAW pipeline (v6.12). Files were aligned to the reference genome GRCm39. Downstream analysis was performed in Stereopy (v1.4.0). The data was normalised and log transformed, followed by PCA using 50 principal components and ARPACK SVD solver. Neighbours were calculated followed by gaussian smoothing calculated with n_neighbours=10. The data was then exported into Scanpy and Squidpy compatible anndata objects.

11) Spatial transcriptomics analysis: Individually, the pre-processed data were loaded into Squidpy (v1.2.3), where neighbours were calculated using n_neighbors=10 and n_pcs=20, and UMAP and Leiden clustering were subsequently performed. The data was next loaded into Stereopy creating a multi-slice object; the data was integrated using stereopy.tl.batches_integrate. Marker genes were identified using stereopy.tl.find_marker_genes. Corresponding dot plot figures were generated using stereopy.pl.cluster_gene_scatter. The cell communities were computed using stereopy.tl.ms_community_detection and the agglomerative algorithm. The cell communication analysis was then performed using stereopy.tl.cell_cell_communication with the Liana database.

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 raw and processed spatial transcriptomics data generated in this study has been deposited in the GEO database under accession code GSE295815. The raw and processed snMultiome data used in this study are available in the GEO database under accession code GSE292680. The raw and processed bulk ATACseq data used in this study are available in the GEO database under accession code GSE292442. The raw and processed bulk RNAseq data used in this study are available in the GEO database under accession code GSE292443. The raw and processed CUT&RUN and ChIPseq data used in this study are available in the GEO database under accession code GSE292536.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	Spatial transcriptomics: Mouse embryonic ganglionic eminences: E13.5 n=2, E12.5/E14.5 n=1. For each embryonic age, comparisons were made between wild-type and Dlx1/Dlx2 double knockout mouse tissues. With StereoSeq, a relatively new whole genome based single cell resolution platform, single sections per embryo is the current standard, comparing experimental (Dlx1/Dlx2 double knockout) versus control (wild-type). DLX2 CUT&RUN (n=3), H3K27ac ChIPseq (n=3), RNAseq (n=3) and ATACseq (n=3). Sample sizes were by convention for these genomics-based assays.
Data exclusions	None
Replication	All replications of experiments were successful.
Randomization	Samples were allocated to include litter mate controls/sample
Blinding	Blinding was not performed as it was not relevant to the study as our analysis was not biased by embryonic age or Dlx1/Dlx2 mutational status.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National

Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

Sampling strategy *Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*

Data collection *Describe the data collection procedure, including who recorded the data and how.*

Timing and spatial scale *Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*

Data exclusions *If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*

Reproducibility *Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*

Randomization *Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*

Blinding *Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☒ No

Field work, collection and transport

Field conditions *Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*

Location *State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*

Access & import/export *Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*

Disturbance *Describe any disturbance caused by the study and how it was minimized.*

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

Antibodies

Antibodies used *Rabbit anti-DLX2 (proprietary, Prof David Eisenstat, 1:250); Rabbit anti-OLIG2 (Merck AB9610, 1:100); Goat anti-SOX10 (R&D Systems AF2864, 1:100); Alexa Fluor 488 (ThermoFisher Scientific AF11055, 1:1000); Alexa Fluor 568, A10042, 1:1000).*

Validation *Each antibody lot was validated for use in IHC/IF. Also please refer to Figure 4 of the manuscript, and Eisenstat D et al [PMID 10516593], and to the manufacturer's websites.*

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293 (ATCC catalogue #CRL-1573).
Authentication	We used STR analysis to authenticate this cell line.
Mycoplasma contamination	This cell line tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Palaeontology and Archaeology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>
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Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Timed pregnant CD1 mice and the Dlx1/Dlx2 double knockout mouse maintained on a CD1 mouse background strain
Wild animals	N/A
Reporting on sex	Only embryonic time-points from mid-gestation (E12.5-E14.5) were utilised. Sex of each embryo was not determined upon collection as this can only be determined postnatally or by sequencing. Hence, embryo sex was determined by sequencing data where possible: Spatial transcriptomics: E12.5 Wild-type (WT) 1 male; Dlx1/Dlx2 double knockout (DKO) 1 male. E13.5 WT 1 female, 2 male; DKO 1 female, 2 male. E14.5 WT 1 male; DKO 1 female. snMultiome: E12.5 WT 1 female; DKO 1 female. E13.5 WT 1 female 1 male; DKO 1 female, 1 male. E14.5 WT 1 male; DKO 1 male. RNAseq (E13.5): WT 2 female, 1 male; DKO 1 female, 2 male. ATACseq (E13.5): WT 1 female, 2 male; DKO 2 female, 1 male. CUT&RUN: 3 female. ChIPseq: 3 female.
Field-collected samples	N/A
Ethics oversight	Animal Ethics Committee (AEC), Murdoch Children's Research Institute, Parkville VIC 3052 Australia; AEC Project number A981

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

Leung RF et al.2025 Table S5 DLX2 CUT&RUN H3K27ac ChIPseq ATAC consensus peaks

Files in database submission

GSE292536 (CUT&RUN & ChIPseq)

Genome browser session

(e.g. [UCSC](#))

[https://urldefense.com/v3/__https://genome.ucsc.edu/cgi-bin/hgTracks?hubUrl=https:**Ausegalaxy.org.au*api*dasetsets*a6e389a98c2d16780726048a194b913a*display*to_ext=txt&genome=mm10&position=lastDbPos__;Ly8vLy8vPw!!dXLKk1Z!UUyDjX5PXyL4qKgmljVpnYWEPHOeJdi9eGOaZlMr53nAvAVMWohKsEJuLu8o8ZTxZPwnvdZw6uqSuYni_g4GqhABOfw\\$](https://urldefense.com/v3/__https://genome.ucsc.edu/cgi-bin/hgTracks?hubUrl=https:**Ausegalaxy.org.au*api*dasetsets*a6e389a98c2d16780726048a194b913a*display*to_ext=txt&genome=mm10&position=lastDbPos__;Ly8vLy8vPw!!dXLKk1Z!UUyDjX5PXyL4qKgmljVpnYWEPHOeJdi9eGOaZlMr53nAvAVMWohKsEJuLu8o8ZTxZPwnvdZw6uqSuYni_g4GqhABOfw$)

Methodology

Replicates

3

Sequencing depth

150 bp single end and targeted recovery of 30 million reads per sample.

Antibodies

Anti-H3K27ac (Abcam ab4729); Anti-IgG control (ThermoFisher Scientific 026102)

Peak calling parameters

The raw sequencing results of ChIPseq were processed through the nf-core/chipseq (v1.2.2) pipeline to generate peaks sets and assess the quality of the raw sequencing results. BWA was used for the sequence alignment of the ChIPseq pipeline using the GRCm38/mm10 mouse genome from ENSEMBL as a reference. Bigwig files were generated for visualisation and peaks were called against the IgG samples using SEACR. The consensus peak sets of each replicate were performed using Bedtools. The genomic region annotation of these peak sets was then performed in R (v4.1.2) using clusterProfiler (v4.2.2), while the gene association and subsequent GSEA was performed using GREAT. The set association was performed with the basal plus extension setting of 5kb proximal, 1kb downstream and up to 1000kb distal. HOMER (v4.1.1) was then used to perform motif enrichment analysis, using findMotifsGenome.pl with GRCm38/mm10 genome and 'size' parameter set as 100.

Data quality

See above

Software

See above