

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	Olympus FV1000 confocal microscope; CytoFLEX flow cytometer; StepOnePlus qPCR system
Data analysis	GraphPad Prism (v9.5); ImageJ; FastQC (v0.12.1); Fastp (v0.23.4); BWA (v0.7.17); SAMtools (v1.18); MACS2 (v2.2.7.1); STAR (v2.7.11); edgeR; DeepTools (v3.5.4); S3V2-IDEAS; ChIPseeker; FlowJo

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](#)
- RNA-seq, ChIP-seq, and ATAC-seq data have been deposited in the GEO database under accession numbers GSE259215, GSE257544, and GSE257545, respectively.

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

No sample size calculations were performed. Sample sizes ($n \geq 3$ independent experiments) were chosen based on standard practices in the field and practical considerations of experimental feasibility. Exact sample sizes are indicated in figure legends as discrete numbers.

Data exclusions

No data were excluded from the analyses.

Replication

All experiments were replicated at least three times ($n \geq 3$) in independent settings. All attempts at replication were successful.

Randomization

For in vitro biological experiments, samples were randomly distributed across wells/plates. For animal experiments, mice were randomly assigned to experimental groups.

Blinding

Investigators were not blinded during data collection and analysis, as quantitative measures were performed and experimental conditions were predetermined

Behavioural & social sciences study design

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

Study description

Research sample

Sampling strategy

Data collection

Timing

Data exclusions

Non-participation

Randomization

Ecological, evolutionary & environmental sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	
Blinding	

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	
Location	
Access & import/export	
Disturbance	

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	Primary antibodies for WB:< • Top2a (Beyotime, AF0303, 1:1000; Abcam, ab52934, 1:5000) • Trim28/KAP1 (Abcam, ab22553, ab70369, 1:5000; CST, 4123S, 1:1000) • Nanog (Bethyl Laboratories, A300-397A) • Oct4 (CST, D6C8T, 1:1000; Santa Cruz, sc-5279, 1:1000) • Sox2 (Abcam, ab97959, 1:5000) • H3K4me3 (Abcam, ab8580, 1:5000) • H3K9me3 (CST, 13969S, 1:1000; Abcam, ab8898, 1:5000)
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- H3K27me3 (CST, 9733S, 1:1000)
- H3K9ac (CST, 9649S, 1:1000)
- HP1α (Abcam, ab77256, 1:5000)
- HP1γ (Santa Cruz, sc-398562, 1:1000)
- H3 (Millipore, 07-690, 1:50000)
- β-actin (internal control)
- Chd3 (CST, 4241, 1:1000), Chd4 (CST, 11912, 1:1000), Hdac1 (CST, 5356, 1:1000), Hdac2 (CST, 5113, 1:1000), Mta1 (CST, 5647, 1:1000), Mbd3 (CST, 14540, 1:1000), Gatad2a (Abcam, ab87663, 5000), Gatad2b (Abcam, ab76925, 1:5000), Rbbp7 (CST, 6882T, 1:1000)
- Ezh1 (CST, 42088, 1:1000), Ezh2 (CST, 5246, 1:1000), Suz12 (CST, 3737, 1:1000), Eed (CST, 51673, 1:1000), Jarid2 (CST, 13594, 1:1000), Kdm5b (CST, 3273T, 1:1000)
- His-tag (Genscript, A00186-100, 1:1000)
- α-tubulin (CST, 2144S, 1:1000)
- P53 (Santa Cruz, sc-6243, 1:1000)
- Ki67 (Sigma, SAB5700770, 1:1000)
- ALDH1 (Abclonal, A1802, 1:1000)

Primary antibodies for IF:<

Top2a(Beyotime, AF0303, 1:100); KAP1 (Abcam, ab22553, 1:500); TIF1b (CST, 4123S, 1:200); Oct4 (CST, D6C8T, 1:200); Nanog (Bethyl Laboratories, A300-397A, 1:100); H3K9me3 (CST, 13969S, 1:200)

Secondary antibodies for WB:<

- Goat anti-rabbit IgG-HRP (Invitrogen, 65-6120, 1:3000)
- Goat anti-mouse IgG-HRP (Invitrogen, 62-6520, 1:3000)
- Donkey anti-goat IgG-HRP (Santa Cruz, sc-2020, 1:3000)
- Peroxidase AffiniPure Goat Anti-Mouse IgG (Jackson Immuno Research, 115-035-174, 1:3000)

Secondary antibodies for IF:<

- AlexaFluor 594-Donkey anti-Rabbit IgG (Invitrogen, A21207, 1:1000)
- AlexaFluor 488-Goat anti-mouse IgG (Invitrogen, A28175, 1:1000)

Flow cytometry antibodies:<

- FITC-conjugated anti-CD44 (eBioscience, 11-0441-82)
- APC-conjugated anti-CD24 (eBioscience, 17-0247-42)

Validation

All commercial antibodies were validated by the manufacturers and used according to their specifications. The experimenters further validated antibodies by Western blot, immunofluorescence, and ChIP-qPCR as shown in the manuscript figures. Custom antibodies were not used in this study.

Eukaryotic cell lines

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

Cell line source(s)

- R1/E mouse embryonic stem cells (mESCs)
- Primary mouse embryonic fibroblasts (MEFs) derived from 13.5-day embryos of C57BL/6 background crossed with Tg(Pou5f1-EGFP)2Mnn (OG2) transgenic mice
 - Oct4-EGFP (OG2) MEFs
 - LentiX-293T (Takara, 632180)
 - Plat-E (Cell Biolabs, RV-10)
 - MDA-MB-231 human triple-negative breast cancer cells (ATCC, HTB-26)

Authentication

MDA-MB-231 cells were obtained from ATCC. MEFs were derived in-house from genetically defined mouse strains. ESC lines were maintained as previously established. No additional authentication procedures were performed.

Mycoplasma contamination

All cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

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

Ethics oversight

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	3- to 4-week-old female athymic BALB/c nude mice were used for xenograft tumor experiments.
Wild animals	No wild animals were used.
Reporting on sex	Female mice were used for xenograft studies. Sex was not considered a variable in this study.
Field-collected samples	No field-collected samples were used.
Ethics oversight	All animal procedures were approved by the Animal Care and Use Committee of Southern Medical University, Guangzhou, China (SYXK (Guangdong) 2016-0167).

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	
Study protocol	
Data collection	
Outcomes	

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

Novel plant genotypes

Authentication

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.<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE257545>

Files in database submission

GSM8107956 R1/E cells siControl, H3K9ac
 GSM8107957 R1/E cells siTop2a, H3K9ac
 GSM8107958 R1/E cells shControl, H3K9ac
 GSM8107959 R1/E cells shTrim28, H3K9ac
 GSM8107960 R1/E cells siControl, H3K4me3
 GSM8107961 R1/E cells siTop2a, H3K4me3
 GSM8107962 R1/E cells shControl, H3K4me3
 GSM8107963 R1/E cells shTrim28, H3K4me3
 GSM8107964 R1/E cells siControl, H3K9me3
 GSM8107965 R1/E cells siTop2a, H3K9me3
 GSM8107966 R1/E cells shControl, H3K9me3
 GSM8107967 R1/E cells shTrim28, H3K9me3
 GSM8107968 R1/E cells siControl, H3K27me3
 GSM8107969 R1/E cells siTop2a, H3K27me3
 GSM8107970 R1/E cells shControl, H3K27me3
 GSM8107971 R1/E cells shTrim28, H3K27me3

Genome browser session

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

no longer applicable

Methodology

Replicates

ChIP-seq experiments were conducted using biological replicates. Independent chromatin preparations from distinct cell cultures of R1/E cells were performed for each condition (siControl, siTop2a, shControl, shTrim28) and each histone mark (H3K9ac, H3K4me3, H3K9me3, H3K27me3). Each experimental group was analyzed with multiple biological replicates (n = 2–3 per group). Replicate agreement was verified after S3V2-IDEAS normalization, and the mean signal across replicates was computed to generate representative profiles for downstream heatmap and profile analyses. High reproducibility and concordance were observed among replicates for both activating and repressive histone modifications across all genetic perturbations.

Sequencing depth

Following the DNA Sequencing Data Preprocessing, we employed MACS2 (version 2.2.7.1) for peak calling. Specifically, for paired-end data, we ran MACS2 using the parameters “-f BAMPE -q 0.05.” For single-end data, MACS2 was executed with the parameters “-q 0.05 --nomodel --extsize 300”. Peak annotations were conducted using the ChIPseeker package

Antibodies

H3K4me3 (Abcam, ab8580,1:50), H3K27me3 (CST, 9733S,1:50), H3K9ac (CST, 9649S,1:50), H3K9me3 (Abcam, ab8898,1:50), Trim28 (Abcam, ab22553,1:50), Top2a (Beyotime, AF0303,1:50)

Peak calling parameters

MACS2 (v2.2.7.1): paired-end data used “-f BAMPE -q 0.05”; single-end data used “-q 0.05 --nomodel --extsize 300”

Data quality

FastQC (v0.12.1) and Fastp (v0.23.4) were used for quality control. Reads were aligned to GRCm38 mouse genome (release-101) using BWA (v0.7.17).

Software

MACS2, BWA, SAMtools, Bedtools, DeepTools (v3.5.4), S3V2-IDEAS, ChIPseeker, edgeR, STAR (v2.7.11)

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

Cells were harvested using 0.25% Trypsin-EDTA, washed twice with PBS, and resuspended in FACS buffer (PBS supplemented with 2% FBS) at a density of 1×10^6 cells/mL. After filtration through a 70 μ m cell strainer, single-cell suspensions were incubated with FITC-conjugated anti-CD44 (eBioscience, 11-0441-82) and APC-conjugated anti-CD24 (eBioscience, 17-0247-42) antibodies on ice for 30 min in the dark, with matched isotype controls included for background calibration. Cells were then washed three times with FACS buffer to remove unbound antibodies and resuspended in 300 μ L buffer for immediate analysis. Flow cytometric acquisition was performed on a CytoFLEX instrument (Beckman Coulter), collecting $\geq 10,000$ events per sample. Data were analyzed using FlowJo software, with sequential gating on FSC-A/SSC-A to exclude debris, FSC-A/FSC-H to eliminate doublets, and CD44-FITC versus CD24-APC plots to quantify the CD44⁺/CD24^{-low} cancer stem cell population. All experiments were conducted in triplicate.

Instrument

CytoFLEX system (Beckman, AS27202)

Software

FlowJo software

Cell population abundance

CD44⁺/CD24^{-low} population was gated and quantified.

Gating strategy

Standard gating strategy was applied. FACS staining followed standard flow cytometric protocols using FITC-anti-CD44 and APC-anti-CD24 antibodies.

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

Magnetic resonance imaging

Experimental design

Design type

Design specifications

Behavioral performance measures

Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See [Eklund et al. 2016](#))

Correction

Models & analysis

n/a

Involved in the study

☐

☐ Functional and/or effective connectivity

☐

☐ Graph analysis

☐

☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis