nature portfolio

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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 *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	The CDS sequences of Gapdh, Rif1, Wnt7b, Sox4, Klf4, Cdc73, Cdkn1a, Mdm2, Cdk6 and Cdk4 were collected from the database of National Center for Biotechnology Information (NCBI). And their forward/reward primer were collected from Sangon Biotech. The sequence information in this study was included in Supplementary Table 2.
Data analysis	Statistical analysis were performed using GraphPad Prism 8.2.1; Flow cytometry results were analyzed by FlowJo V10; Curves were fitted with Origin 2022; Fluorescence images were analyzed through Image J 1.52a or Spectrum Living Image 4.0; The semi-quantitative analysis of western blot images were performed by TanonImage 1.00; NMR spectrawere analyzed using MestReNova 6.1.0.

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 datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

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

Sample size	Sample size was chosen to ensure reproducibility of the experiments in accordance with the replacement, reduction and refinement principles of animal ethics regulation.
Data exclusions	No data was excluded in this study.
Replication	All experimental findings were reliably reproduced. At least three independent samples were performed for each experiment. All experiments were performed as biological replications as appropriate for the experiment design. Details of experimental replicates are given in the figure legends.
Randomization	All experimental samples or models including in vitro cells and in vivo mice were randomly allocated to each group.
Blinding	No blinding was used throughout experiments. The investigators should keep careful track of protocols because that most of the experiments needed multiple treatments (including formulation, cells or mouse tumor treatment, sample collection, and so on). Hence, it would be difficult to blind the investigators to group allocation during data collection and analysis.

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

Peroxidase-Conjugated Goat Anti-Rabbit IgG (H+L) (1:5000, CAT:33101ES60) and Peroxidase-Conjugated Goat Anti-Mouse IgG (H+L) (1:5000, CAT:33201ES60) were purchased from YEASEN (Shanghai, China). Anti-BRD4 antibody (1:1000, ab128874) , Anti-caspase-3 antibody (1:1000, ab184787), Anti-GAPDH antibody (1:1000, ab8245), Anti-β-actin antibody (1:1000, ab8226), Anti-β-tubulin antibody (1:1000, ab78078), Anti-CDK4 antibody (1:1000, ab199728), Anti-CDK6 antibody (1:1000, ab241554), Anti-P21 antibody (1:1000, ab109199), Anti-Oct4 antibody (1:100, ab200834), Anti-Sox2-Alexa Fluor-647 antibody (1:100, ab279687), Anti-Nanog antibody (1:100, ab109250), Anti-CD133-Alexa Fluor-647 antibody (1:100, ab252127) were all purchased from Abcam (Shanghai). Anti-CD44-APC antibody (1:50, 70-AH04405-100) was purchased from MultiSciences Biotech Co., Ltd. Anti-CD24-PerCP Cy5.5 antibody (1:100, 11-0247-42) was purchased from eBioscience Inc.

Validation

All antibodies were commercially available and were validated by the supplier. All antibodies were used in the study according to the profile of manufacturers. All validation statements are available on the antibody websites, respectively.
1. Peroxidase-Conjugated Goat Anti-Rabbit IgG (H+L): https://www.yeasen.com/products/detail/319
2. Peroxidase-Conjugated Goat Anti-Mouse IgG (H+L): https://www.yeasen.com/products/detail/407
3. Anti-BRD4 antibody (ab128874): https://www.abcam.com/brd4-antibody-epr51502-ab128874.html
4. Anti- caspase-3 antibody (ab 184787): https://www.abcam.com/caspase-3-antibody-epr18297-ab184787.html
5. Anti-GAPDH antibody (ab8245): https://www.abcam.com/gapdh-antibody-6c5-loading-control-ab8245.html
6. Anti-β-actin antibody (ab8226): https://www.abcam.com/beta-actin-antibody-mabcam-8226-loading-control-ab8226.html
7. Anti-β-tubulin antibody (ab78078): https://www.abcam.com/beta-iii-tubulin-antibody-2g10-neuronal-marker-ab78078.html
8. Anti-CDK4 antibody (ab199728): https://www.abcam.cn/products/primary-antibodies/cdk4-antibody-epr17525-ab199728.html
9. Anti-CDK6 antibody (ab241554): https://www.abcam.cn/products/primary-antibodies/cdk6-antibody-98d-ab241554.html
10. Anti-p21 antibody (ab109199): https://www.abcam.cn/products/primary-antibodies/p21-antibody-epr3993-ab109199.html
11. Anti-Oct4 antibody (ab200834): https://www.abcam.cn/products/primary-antibodies/oct4-antibody-epr17980-ab200834.html
12. Anti-Sox2-Alexa Fluor-647 antibody (ab279687): https://www.abcam.cn/products/primary-antibodies/alexa-fluor-647-sox2-antibody-sp76-ab279687.html
13. Anti-Nanog antibody (ab109250): https://www.abcam.cn/products/primary-antibodies/nanog-antibody-epr20272-ab109250.html
14. Anti-CD133-Alexa Fluor-647 antibody (ab252127): https://www.abcam.cn/products/primary-antibodies/alexa-fluor-647-cd133-antibody-epr20980-104-ab252127.html
15. Anti-CD44-APC antibody (70-AH04405-100): https://www.liankebio.com/product/anti-human-mouse-cd44-apc-clone-im7-16029.html
16. Anti-CD24-PerCP Cy5.5 antibody (11-0247-42): https://www.thermofisher.cn/cn/zh/antibody/product/CD24-Antibody-clone-M1-69-Monoclonal/A14790

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

MDA-MB-231 human breast cancer cell line (CRM-HTB-26) and HN30 human oral squamous cancer cell line (TCP-1012) were obtained from the ATCC (Shanghai, China).

Authentication

These cell lines were authenticated using STR analysis and the last authentication testing time of MDA-MB-231 is 2/4/2024, the HN30 is 20/12/2023.

Mycoplasma contamination

All cell lines were tested negative for mycoplasma contamination. Contamination was detected by the Hoechst DNA stain method, qPCR-based assay.

Commonly misidentified lines
(See ICLAC register)

These all cell lines that we used were not listed in commonly misidentified lines in ICLAC register.

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	Balb/c nude mice, 5-week-old, 18 ~ 20 g. Animals were housed under SPF conditions in groups of 4–5 mice per cage, and maintained at a temperature of ~25 °C in a humidity-controlled environment with a 12 h light/dark cycle, with free access to standard food and water.
Wild animals	No wild animals were used in this study.
Reporting on sex	Female
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal procedures were carried out under the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Institute of Material Medica, 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	*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.*
Novel plant genotypes	*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.*
Authentication	*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, mosiacism, off-target gene editing) were examined.*

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	In the flow cytometric analysis in vitro, the pretreated cells were detached by trypsin-EDTA, washed 3 times with PBS, resuspended and stained with the indicated antibodies for another 1 hours, washed 3 times with PBS, then suspended cells were passed through 70-μm nylon cell strainers and further analyzed by flow cytometry. In the flow cytometric analysis in vivo, the tumor tissues were cut into small pieces and then added with mixed enzyme solution (collagenase IV, Hyaluronidase and DNase I), digested through tissue processor (Gentle MACS Dissociator, Militenyi Biotec GmbH, Germany) following the provided operating instructions. The single cells were obtained by filtering the aforementioned suspension through a 70 μm filter membrane, and further stained with fluorescence-labeled antibody according to the manufacturer's protocol, further examined through flow cytometry.
Instrument	BD FACS Fortessa, BD, USA
Software	FlowJo V10
Cell population abundance	No cell sorting was performed.
Gating strategy	The preliminary FSC/SSC gates were determined by the blank cell samples and the single stained cell samples.

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