nature portfolio

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Last updated by author(s): Nov 28, 2024

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	We generated H3K27ac and ATF4 ChIP-Seq in LX-2 cell line using Illumina NovaSeq 6000 platform. The data was demultiplexed using bcl2fastq(v2.2.0).
Data analysis	NGS data software we used Cutadapt(v3.1), Bowtie2(v2.2.9), samtools(v1.9), HOMER, Bedtools(v2.1.0), deeptools(v3.5.0), IGV(v2.8.13).

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

Bulk RNA-seq and ChIP-seq data from this study have been deposited in NCBI with accession number: GSE235786 (https://www.ncbi.nlm.nih.gov/geo/query/

acc.cgi?acc=GSE235786; reviewer token: wzejimounxgjjab). The human scRNA-seq data used in this study are available in the GEO DataSets under accession code GSE136103. All remaining data can be found in the Article, Supplementary, or Source data files. All other data and materials are available from the corresponding author upon request. Source data are provided with this paper.

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	Liver tissues from both male and female patients were collected and analyzed. The number of male and female patients involved in this study was reported in the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	The patients' clinical features, including gender, age, and disease stage, were included and reported in the manuscript.
Recruitment	All patients selected for the study were informed and they gave informed consent.
Ethics oversight	The study was approved by the Ethical Committee of First Affiliated Hospital, Zhejiang University School of Medicine.

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	No statistical methods were used to determine the sample size. The sample size was chosen based on preliminary experiments and previously published results. The group size for each individual experiment is mentioned in the figure legend.
Data exclusions	There were no deliberate data exclusions.
Replication	Reproducibility of experimental findings (wherever applicable) was verified by either considering analyses of multiple patient samples or equal to no less than 3 biologically independent experiments.
Randomization	Experiment animals were randomized according to the weight of animals at the time when treatment started.
Blinding	N/A

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

1.Western blot:
anti ATF4, Cell Signaling Technology, 11815, 1:1000 dilution
anti ATF4, Santa Cruz Biotechnology, sc-390063, 1:1000 dilution
anti ZEB1,Cell Signaling Technolog, 70512,1:1000 dilution
anti E-Cadherin, Cell Signaling Technology, 3195, 1:1000 dilution
anti N-Cadherin, Cell Signaling Technology, 13116, 1:1000 dilution
anti Vimentin, Cell Signaling Technology, 5741, 1:1000 dilution
anti Snail , Cell Signaling Technology, 3879, 1:1000 dilution
anti Slug , Cell Signaling Technology, 9585, 1:1000 dilution
anti Claudin-1 , Cell Signaling Technology, 13255, 1:1000 dilution
anti α-Smooth muscle actin , Cell Signaling Technology, 19245, 1:1000 dilution
anti CHOP , Cell Signaling Technology, 2895, 1:1000 dilution
anti Cleaved PARP,Cell Signaling Technology, 5625,1:1000 dilution
anti FN1 , Sigma-Aldrich, F3648, 1:5000 dilution
anti GAPDH, Arigo Biolaboratories, ARG10112, 1:1000 dilution
anti Tubulin, Biolynx, BX00009-C3, 1:1000 dilution
anti Actin, Arigo Biolaboratories, ARG66382, 1:1000 dilution
anti Lrat, Santa Cruz Biotechnology, sc-101391, 1:500 dilution
anti Cleaved Caspase-1, Cell Signaling Technology, 4199, 1:1000 dilution
anti Cleaved PARP, Cell Signaling Technology, 5625, 1:1000 dilution
anti p-RIPK3, Cell Signaling Technology, 93654, 1:1000 dilution
anti GSDMD, Abcam, ab209845, 1:1000 dilution
2.Flow cytometry:
anti CD24-PE , BD Bioscience, 555428, 1:200 dilution
anti CD44-APC , BD Bioscience, 559942, 1:200 dilution
3.ChIP-Seq:
anti Histone H3 (acetyl K27), Abcam, 4729, approximately 1.5 micrograms of antibodies are added per 4×10^6 cells.
anti ATF4, Cell Signaling Technology, 11815, approximately 1.5 micrograms of antibodies are added per 4×10^6 cells.

Validation

All antibodies are validated by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HMLE and HMLER cells expressing the coding sequence of Twist-ER or Snail were generated from Dr. Robert A. Weinberg's laboratory and maintained in a 1:1 mixture of DMEM + 5% FBS, insulin (10 μg/ml), EGF (10 ng/ml), hydrocortisone (0.5 μg/ml), and Mammary Epithelial Cell Growth Medium (MEGM).
The Twist-ER transgene was activated upon 4-hydroxytamoxifen (4-OHT) treatment. The snail transgene was induced by doxycycline (DOX).
The LX-2 cell line was purchased from Biobank, Xiangya School of Medicine, Central South University, and was cultured in DMEM + 10% FBS.
The AML12 cell line was purchased from National Collection of Authenticated Cell Cultures, and was cultured with specical medium (National Collection of Authenticated Cell Cultures, SCSP-654), including 89% DMEM/F-12 culture medium (1:1) (Gibco, 11330032), 10% Fetal bovine serum (FBS) (Gibco), 1% ITS liquid culture supplement (Sigma, I3146), and adding dexamethasone to the final concentration of 40 ng/ml.

Authentication

The cell authentication for all cell lines was performed by ATCC cell line authentication service using short tandem repeat (STR) profiling in 2022.

Mycoplasma contamination

All cell lines used were regularly tested (every one month and half) and are negative for mycoplasma

Commonly misidentified lines
(See ICLAC register)

no

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

All mice were housed at an ambient temperature around 21-23 C°, humidity of 40-60% and a light dark cycle of 12 hours. Lrat-Cre mice were purchased from GemPharmatech, and cre endonuclease was inserted following the Lrat gene starting code. A male Atf4fl/fl mouse was a gift from Dr. Jinghai Chen's laboratory at Zhejiang University. The conditional HSC-specific Atf4-knockout mice were obtained by crossing Atf4fl/fl mice with Lrat-Cre mice.
We used 8- to 12-week-old age- and male mice for experimental procedures.

Wild animals	no
Reporting on sex	Male mice (aged 8-12 weeks) were used for all experiments.
Field-collected samples	no
Ethics oversight	All mice experiments were approved by the Institutional Animal Care and Use Committee of Zhejiang University School of Medicine.

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	n/a
Study protocol	n/a
Data collection	n/a
Outcomes	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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.*	GSE235786 (https://www.ncbi.nlm.nih.gov/geo/query/ acc.cgi?acc=GSE235786; reviewer token: wzejimounxgjjab)
Files in database submission	all RAW FASTQ, BIGWIG and mcool were uploaded to NCBI GEO.
Genome browser session (e.g. UCSC)	no longer applicable

Methodology

Replicates	each sample we have two replicates
Sequencing depth	Ctrl_ATF4 ,ChIP-seq, ATF4 , 23,759,496 Ctrl_H3K27ac,ChIP-seq, H3K27ac , 23,809,336 Ctrl_input,ChIP-seq,IgG, 23,811,968 TG_ATF4 ,ChIP-seq, ATF4, 23,200,768 TG_H3K27ac,ChIP-seq, H3K27ac, 23,141,527 TG_input,ChIP-seq, IgG, 23,121,626 TGFb_ATF4 ,ChIP-seq, ATF4, 23,714,106 TGFb_H3K27ac,ChIP-seq, H3K27ac, 23,800,293 TGFb_input,ChIP-seq,IgG , 23,761,572
Antibodies	ChIP-seq experiments, The antibodies we used are described in this study, including Histone H3 (acetyl K27) （Abcam, 4729） and ATF4 (Cell Signaling Technology, 11815).

Peak calling parameters	HOMER was used to call ChIP-Seq peaks with default parameter
Data quality	fastqc was used to check raw fastq quality.
Software	Cutadapt, Bowtie2 and HOMER

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	1) HMLE_Twist_ER cell pretreatment: First, HMLE_Twist_ER cells (shControl and shATF4 stable transfection cell lines) are treated with ethanol or 50 nM 4-OHT, until the shControl+4-OHT group of cells shows obvious mesenchymal transformation - the cells become spindle-shaped and are clearly dispersed from each other. 2) Flow cytometry and analysis: After digesting and centrifuging the cells, discard the supernatant and resuspend the cells in 2 ml 1xPBS. After centrifuging again, discard the supernatant and resuspend the cells in 1xPBS containing 2% FBS. Mix well and pipet 100 µl of the cell suspension into an EP tube. Set up four groups: no antibody group, CD24-PE only group, CD44-APC only group, and CD24-PE and CD44-APC together group. Add 5 µl of CD24-PE and 2.5 µl of CD44-APC to the combined group. Mix well and incubate in the dark at 4°C for 30 minutes. After incubation, centrifuge and discard the supernatant. Resuspend the cells in 1 ml 1xPBS and wash them. Centrifuge again and discard the supernatant. Resuspend the cells in 400 µl 1xPBS containing 2% FBS. The cell suspensions were collected and filtered through a 70 micrometer filter (#352350, FALCON).
Instrument	CytoFLEX LX, Beckman Coulter
Software	flowjo v10
Cell population abundance	Minimal cell number in target gate was 10000
Gating strategy	Gating strategy on cells 1. FSC and SSC 2. FSC-H and FSC-A to gate on single cell population 3. We used the fluorescence intensity of APC as the standard for dividing cells into high and low expression of CD44, and the fluorescence intensity of PE as the standard for dividing cells into high and low expression of CD24.

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