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	No software was used to collect the data.
Data analysis	Cell Ranger v6, Seurat v4, edgeR v3, fgsea v1.21.2, MSigDB v7.5, and CellPhoneDB v5.0.0 were used to analyze scRNA-seq data. BWA v0.7.12, MACS v2.1.0, DESeq2 v1.24.0, Cistrome Toolkit, and Homer v4.11 were used to analyze ATAC-seq data. GraphPad Prism v9 or v10 were used for statistical analyses.

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 scRNA-seq and ATAC-seq data generated in this study have been deposited in the Gene Expression Omnibus (GEO) under the accession number GSE247454 and are available at the following URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247454. Microarray and RNA-seq gene expression data of human

breast tumor samples were obtained from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) and The Cancer Genome Atlas (TCGA) via cBioPortal (https://www.cbioportal.org/), whereas gene expression data of human breast cancer cell lines were obtained from the Cancer Dependency Map (DepMap) (https://depmap.org/portal/).

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

Life sciences study design

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

Sample size	Sample size was determined based on our previous experience, availability of materials, and cost.
Data exclusions	No data were excluded.
Replication	All experimental findings were consistent across replicates. Number of replicates are described in the figure legends. All in vivo experiments contained >9 biological replicates. All in vitro experiments were replicated at least twice.
Randomization	In tumorigenesis experiments, mice were randomized into experimental groups and different groups were age- and litter-matched for consistency.
Blinding	Investigators were blinded to genotypes and treatment groups in during tumorigenesis experiments. Histological analyses were performed by a pathologist in a blinded fashion.

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

For immunohistochemistry staining, the following antibodies were used: ER (Santa Cruz sc-542, polyclonal, 1:300), PR (CST 8757, clone D8Q2J, 1:200), KRT14 (BioLegend 905301, polyclonal, 1:2,000), KRT8/18 (DSHB, clone TROMA-I, 1:600), ELF5 (Thermo PA5-66521, polyclonal, 1:150 in Pierce Enhancer), JUN (CST 9165, clone 60A8, 1:50), JUNB (CST 3753, clone C37F9, 1:25), JUND (Abcam ab181615, clone EPR17365, 1:50), FOS (Abcam ab222699, clone EPR21930-238, 1:50), FOSB (CST 2251, clone 5G4, 1:50), FOSL1 (Thermo PA5-40361, polyclonal, 1:25), FOSL2 (Sigma HPA004817, polyclonal, 1:50), pSer73-JUN (CST 3270, clone D47G9, 1:25).

For immunofluorescence staining, the following antibodies were used: GFP (CST 2956, clone D5.1, 1:200), KRT8 (Abcam ab192467, clone EP1628Y, 1:400), SMA (Abcam ab202509, clone EPR5368, 1:800), KRT14 (Abcam ab277277, clone EPR17350, 1:300).

For immunoblotting, the following antibodies were used: JUN (Abcam ab40766, clone EP693Y, 1:1,000), HIS tag for JUN-DN (CST 12698, clone D3I1O, 1:1,000), WNT10A (Santa Cruz sc-376029, clone C-9, 1:200), HSP90 (CST 4877, clone C45G5, 1:10,000).

For cell purification by FACS, the following antibodies were used: CD45-FITC (BioLegend 103108, clone 30-F11, 1:200), CD31-FITC (BioLegend 102405, clone 390, 1:50), TER119-FITC (BioLegend 116205, clone TER-119, 1:100), EPCAM-BV605 (BioLegend 118227, clone G8.8, 1:100), CD133-APC (BioLegend 141207, clone 315-2C11, 1:50), CD14-PE (BioLegend 150106, clone M14-23, 1:50).

Validation

The following IHC antibodies have been widely validated as lineage markers in multiple previous studies on mammary tissues. Example publications are listed below:
ER (PMID: 28194015, 28329676, 33378681), PR (PMID: 29795293, 33378681), KRT14 (PMID: 28329676, 33378681), KRT8/18 (PMID: 28329676, 29795293, 33378681).

All other IHC and IF antibodies have been verified by the manufacturers, with the relevant information available on their websites:
ELF5 (Thermo PA5-66521): https://www.thermofisher.com/antibody/product/ELF5-Antibody-Polyclonal/PA5-66521
JUN (CST 9165): https://www.cellsignal.com/products/primary-antibodies/c-jun-60a8-rabbit-mab/9165
JUNB (CST 3753): https://www.cellsignal.com/products/primary-antibodies/junb-c37f9-rabbit-mab/3753
JUND (Abcam ab181615): https://www.abcam.com/products/primary-antibodies/jund-antibody-epr17365-ab181615.html
FOS (Abcam ab222699): https://www.abcam.com/products/primary-antibodies/c-fos-antibody-epr21930-238-ab222699.html
FOSB (CST 2251): https://www.cellsignal.com/products/primary-antibodies/fosb-5g4-rabbit-mab/2251
FOSL1 (Thermo PA5-40361): https://www.thermofisher.com/antibody/product/Fra1-Antibody-Polyclonal/PA5-40361
FOSL2 (Sigma HPA004817): https://www.sigmaaldrich.com/US/en/product/sigma/hpa004817
pSer73-JUN (CST 3270): https://www.cellsignal.com/products/primary-antibodies/phospho-c-jun-ser73-d47g9-xp-rabbit-mab/3270
GFP (CST 2956): https://www.cellsignal.com/products/primary-antibodies/gfp-d5-1-rabbit-mab/2956
KRT8 (Abcam ab192467): https://www.abcam.com/products/primary-antibodies/alexa-fluor-488-cytokeratin-8-antibody-ep1628y-ab192467.html
SMA (Abcam ab202509): https://www.abcam.com/products/primary-antibodies/alexa-fluor-555-alpha-smooth-muscle-actin-antibody-epr5368-ab202509.html
KRT14 (Abcam ab277277): https://www.abcam.com/products/primary-antibodies/alexa-fluor-488-cytokeratin-14-antibody-epr17350-ab277277.html

All immunoblotting antibodies have been verified by the manufacturers, with the relevant information available on their websites:
JUN (Abcam ab40766): https://www.abcam.com/products/primary-antibodies/c-jun-antibody-ep693y-ab40766.html
HIS tag (used for JUN-DN) (CST 12698): https://www.cellsignal.com/products/primary-antibodies/his-tag-d3i1o-xp-rabbit-mab/12698
WNT10A (Santa Cruz sc-376029): https://www.scbt.com/p/wnt-10a-antibody-c-9
HSP90 (CST 4877): https://www.cellsignal.com/products/primary-antibodies/hsp90-c45g5-rabbit-mab/4877

All FACS antibodies have been verified by the manufacturers, with the relevant information available on their websites:
CD45-FITC (BioLegend 103108): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-antibody-99
CD31-FITC (BioLegend 102405): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd31-antibody-120
TER119-FITC (BioLegend 116205): https://www.biolegend.com/en-us/products/fitc-anti-mouse-ter-119-erythroid-cells-antibody-1865
EPCAM-BV605 (BioLegend 118227): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd326-ep-cam-antibody-9968
CD133-APC (BioLegend 141207): https://www.biolegend.com/de-at/products/apc-anti-mouse-cd133-antibody-7243
CD14-PE (BioLegend 150106): https://www.biolegend.com/en-us/products/pe-anti-mouse-cd14-antibody-15675

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The organoid lines used in this study were developed by the authors.
The 3TZ reporter cell line carrying Cre-inducible GFP was published previously (Sánchez-Rivera et al., Nature 2014; PMID: 25337879).

Authentication

Authentication of the organoid lines was performed by PCR genotyping.
The 3TZ reporter cell line carrying Cre-inducible GFP showed GFP positive signal upon transduction with lentivirus expressing Cre.

Mycoplasma contamination

Organoid lines have been tested negative for mycoplasma contamination.
The 3TZ reporter cell line carrying Cre-inducible GFP was not tested for mycoplasma.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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 animal work was performed in accordance with protocol (IS00000990) approved by the Institutional Animal Care and Use Committee at Harvard University. The following mice were used for tumorigenesis experiments, scRNA-seq, and ATAC-seq: Mice of the Brca1/p53 model, including: - Brca1(F/F); Trp53(F/F) mice (WT) - Brca1(F/–); Trp53(F/F) mice (Brca1-HET) Mice of the Brca1/p53/Pten model, including: - Brca1(F/F); Trp53(F/F); Rosa26(LSL-Cas9-2a-EGFP/+) mice (WT) - Brca1(F/–); Trp53(F/F); Rosa26(LSL-Cas9-2a-EGFP/+) mice (Brca1-HET) These mice were maintained in a mixed FVB, 129, C57BL/6J background. For scRNA-seq analysis, mice of 3-4 months (young age) and 13-14 months (older age) were used. For ATAC-seq analysis, mice of 3-4 months were used. For tumorigenesis experiments in the Brca1/p53 model with Lenti-CMV-Cre, mice of 7 months of age (range 5-9 months) were used. For tumorigenesis experiments in the Brca1/p53/Pten model with Lenti-PGK-Cre-U6-sgPten, mice of 6 months of age (range 4-8 months) were used. To test the effect of JunDN on tumorigenesis in the Brca1/p53/Pten model with Lenti-PGK-Cre-U6-sgPten-EF1a-JunDN, mice of 4 months of age (range 3-6 months) were used. To test the effect of Wnt10a on tumorigenesis in the Brca1/p53/Pten model with Lenti-PGK-Cre-U6-sgPten-EF1a-Wnt10a, mice of 3 months of age (range 2-5 months) were used. For each tumorigenesis experiment, female mouse cohorts were age- and litter-matched for consistency. Mice used for fat pad transplantation were female SCID mice (Taconic ICRSC-F) at 5 weeks of age. Mice were housed with a 12-h light/12-h dark cycle with temperatures in the range 20–24°C and 30–70% humidity.
Wild animals	This study did not involve wild animals.
Reporting on sex	Only female mice were used in this study based on the substantially higher prevalence of breast cancer in females than males.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal work was performed in accordance to protocols approved by the Institutional Animal Care and Use Committee at Harvard University.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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) Mammary glands with lymph nodes resected or (2) tumors with normal tissues removed were finely minced and then incubated in a digestion buffer containing DMEM/F12 (GIBCO 11330), 10% heat-inactivated fetal bovine serum, 2 mg/mL collagenase XI (Sigma C9407), and 0.1 mg/mL hyaluronidase (Sigma H3506) for 1.5 hours at 37°C with constant shaking at 220 rpm. The dissociated cells were then subjected to red blood cell lysis (Biolegend 420301), a 5-minute treatment with 1 U/mL dispase (Stem Cell Technologies 07913) and 0.1 mg/mL DNase (Stem Cell Technologies 07470), a 5-minute treatment with TrypLE (GIBCO 12605010), and finally filtered through a 40-um cell strainer to obtain single cells. Samples were then purified by FACS. To isolate AV cells, samples were incubated with CD16/32 antibody (eBioscience 16-0161) to block Fc receptor and with rat serum (eBioscience 24-5555) to block non-specific binding, and then stained for 30 minutes with DAPI (1 mg/mL) and the following antibodies: CD45-FITC (BioLegend 103108, 1:200), CD31-FITC (BioLegend 102405, 1:50), TER119-FITC (BioLegend 116205, 1:100), EPCAM-BV605 (BioLegend 118227, 1:100), CD133-APC (BioLegend 141207, 1:50), CD14-PE (BioLegend 150106, 1:50). To isolate GFP+ tumor epithelial cells, samples were incubated with CD16/32 antibody, rat serum, DAPI, and EPCAM antibody (as listed above). (3) To detect Brca1 floxed and null alleles in cultured mammary epithelial cells derived from WT and HET mice before and after infection with a lentiviral vector expressing the Cre recombinase, GFP+ cells were purified by FACS on Day 4, 7 and 11 after Cre infection.
Instrument	BD FACSAria II
Software	BD FACSDiva 8.0.1
Cell population abundance	(1) AV cells: ~30% of EPCAM-high luminal epithelial cells. (2) GFP+ tumor epithelial cells: ~80% of DAPI- live cells. (3) Cre-infected, GFP-positive cells: ~40% of singlets. Samples with sufficient cell number was re-sorted to check purity.
Gating strategy	(1) To isolate AV cells: SSC-H/SSC-W singlets, FSC-H/FSC-W singlets, DAPI- live cells, CD45-/CD31-/TER119- Lin- cells, EPCAM-high luminal epithelial cells, CD133-/CD14+ AV cells. (2) To isolate GFP+ tumor epithelial cells: SSC-H/SSC-W singlets, FSC-H/FSC-W singlets, DAPI- live cells, EPCAM+/GFP+ tumor epithelial cells. (3) To isolate Cre-infected, GFP-positive cells to test Cre deletion efficiency: SSC-H/SSC-W singlets, FSC-H/FSC-W singlets, GFP + cells.

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