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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 data for Flow cytometry was acquired using BO FACSDiva Software V9.0 (RRID:SCR_001456) Single cell RNA-seq sample preparation and data collection Freshly dissected tissue was micro-dissected into small chunks and processed as above for flow cytometry. Following tissue dissection, single cells were filtered using a 40 µm filter to remove residual clumps. 10 million cells were taken and dead cells were removed using the dead cell removal kit (Miltenyi Biotec; #130-090-101). Cells were pooled from 2 tumor samples for control and FOXM1 KO 1 tumors; we used one sample for FOXM1 KO 3 tumors due to limited numbers of samples. Cells were then counted and loaded onto the 10X Chromium controller, targeting 10,000 cells for capture per well. Single-cell RNA sequencing libraries were generated using the Chromium Single Cell 3' Library & Gel Bead Kit V3.1 kit and Chromium Single Cell 3' Chips according to the manufacturer's instructions (10X Genomics, Pleasanton, CA). In brief, all single-cell samples and required reagents were loaded on a 10X Chromium controller for droplet generation, followed by reverse transcription in the droplets, cDNA amplification, fragmentation, adapter, and index addition following the manufacturer's instructions. Barcoded single-cell transcriptome libraries were sequenced with 100 bp paired-end reads on a NovaSeq S4 platform.
Data analysis	Single-cell RNA sequencing analysis Raw sequencing reads were aligned to the GRCm38 genome (mouse) using Cell Ranger (v5; RRID:SCR_017344) software from 10X Genomics with default parameters. Subsequently, genes were quantified as UMI counts using Cell Ranger (v5;RRID:SCR_017344). Doublets were removed using DoubletFinder (v2.0.3; RRID:SCR_018771) with an expectation of 7.5% doublet rate assuming Poisson statistics. Downstream analysis was performed on filtered feature counts generated by Cell Ranger, and low-quality single cells containing <200 expressed genes or >15% mitochondrial transcripts were removed. Single cells were then normalized and clustered using Seurat 46(v4.9.9.9060; RRID:SCR_016341).

Two complementary methods were used to differentiate between normal epithelial cells and cancer cells. First, inferCNV package(v3.9; https://github.com/broadinstitute/infercnv) was utilized to differentiate between malignant and normal cells, based on Copy Number Variations. Tumor cells show significantly higher proportion CNV in most chromosomes compared to normal epithelial cells. Second, tumor specific genes from bulk RNAseq data performed on control tissue cultured E0771 cells (#GSE139239). The top marker genes from this sample were utilized as E0771 specific markers. Single-cell gene expression counts were normalized to the library size and log2-transformed. Seurat 46 was used to perform PCA reduction using the top 2000 most variable genes in the dataset. Computed PCAs were batch-corrected for variations between samples using Harmony (v1.1.0; RRID:SCR_018809). Batch-corrected PCs (1:40 for all clusters and 1:50 for T cell de novo analyses) were used as input for Louvain-based graphing with a resolution of 1 for all clusters and 0.5 for T cells de novo clustering. Cluster-specific marker genes were identified using the fast Wilcoxon rank-sum test wilcoxauc() function from the presto R package (V1.0.0 "github.com/immunogenomics/presto") and used to identify the cell types. We used ggplot2 (v3.3.3; RRID:SCR_014601) and ComplexHeatmap (v2.16.0; RRID:SCR_017270) packages in R to produce boxplots and heatmaps.

Pathway analysis

Gene Set Enrichment Analysis (GSEA): We used the fGSEA (v1.26.0 -RRID:SCR_020938) R package to test for enrichment of hallmark gene sets downloaded from MsigDB (RRID:SCR_016863, msigdbr R package v7.5.1). For input, we used pre-ranked gene lists generated using a fast Wilcoxon rank-sum test (scRNAseq) (presto R package v1.0.0 "github.com/immunogenomics/presto").

Cell-cell communication analysis

The CellChat R package (v1.6.1;RRID:SCR_021946) was used to assess cell-cell interaction between the cell types in each sample. The Seurat object was subsetted by sample to provide separate inputs for the createCellChat() function. CellChat's internal literature validated ligand-receptor databases containing secreted, extracellular rmatrix-receptor, and cell-cell contact signaling were then used to construct cell-cell communication networks using standard scripts. CellChat's suite of visualization tools were used to plot significant interactions through chord plots, heatmaps, and bubble plots.

The code to replicate the main and supplementary scRNA-seq figures in this paper is available through gitfront. The links are as follows:
1) https://gitfront.io/r/nourhanemad/nkZwBQVsds3y/Timilsina-FOXM1-Manuscript-Figs/blob/MainFigures.md
2)

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 data for single-cell sequencing are publicly available without restrictions through GEO series GSE266617 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE266617).

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	No sex/gender analysis was performed in this study for the meta-analysis of patient samples because the majority of breast cancer(>99%) occurs in females.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings were not used in the study.
Population characteristics	See above.
Recruitment	De-identified archival breast cancer patient samples were obtained from Mays Cancer Center histology core at UT Health San Antonio. There was no selection criteria for age or ethnicity.
Ethics oversight	Though we have IRB approval(IRB#20120041HR) to collect patient samples, this is considered a non-human research as de-identified archival breast cancer patient samples were used.

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

Life sciences study design

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

Sample size	For in vivo mouse studies, using a Chi-squared test with 1 degree of freedom from a Generalized Estimating Equations (GEE) analysis, we estimated that at least four mice (each for Ctrl and KO groups) were required to achieve a statistical power of ~90% with significance at 0.05.
Data exclusions	For single cell RNA seq analysis, cells with high ribosomal and mitochondrial RNA were excluded in order to remove dead cells, based on previously established methods: https://pubmed.ncbi.nlm.nih.gov/30827681/ "Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity".
Replication	All in vitro experiments were performed with at least 3 technical replicates and repeated at least twice. The mouse experiments were performed several times, with similar findings. A representative figure is shown in the figures. For single cell RNA-seq studies, 10k cells from two tumor samples were pooled together for each group, except KO sg3 group, where one tumor sample was used, due to limited number of samples.
Randomization	The mice were randomized before tumor implantation and treatment.
Blinding	Blinding was performed for the counting of micronuclei, Immunofluorescence analysis of immunological markers and ULBP1 in patient and mouse tumor samples.

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

FOXM1(C-20) (Cat #SC-502) (Santa Cruz Biotechnology) 1:500
STING (D2P2F) Rabbit mAb 13647 (Cat #13647) (Cell signaling) 1:1000
"Phospho-STING (Ser366) (E9A9K)Rabbit mAb 50907" (Cat #50907) (Cell signaling) 1:1000
TBK1/NAK (D1B4) Rabbit mAb 3504 (Cat #3504) (Cell signaling) 1:1000
"Phospho-TBK1/NAK (Ser172) (D52C2)XP® Rabbit mAb 5483" (Cat #5483) (Cell signaling) 1:1000
STING (D1V5L) Rabbit mAb (Rodent Preferred) 50494 (Cat #50494) (Cell signaling) 1:1000
"Phospho-STING (Ser365) (D8F4W)Rabbit mAb 72971" (Cat #72971) (Cell signaling) 1:1000
ATF-6 (D4Z8V) Rabbit mAb #65880 (Cat #65880S) (Cell signaling) 1:1000
IRE1α (14C10) Rabbit mAb #3294 (Cat #3294S) (Cell signaling) 1:1000
CHOP (L63F7) Mouse mAb 2895 (Cat #2895S) (Cell signaling) 1:1000
DNMT1 (D63A6) XP® Rabbit mAb (Cat #5032S) (Cell Signaling) 1:1000
Normal Rabbit IgG (Cat #PP64) (Millipore)
mouse anti-rabbit IgG-HRP (Cat #sc-2357) "(Santa CruzBiotechnology)" 1:5000
m-IgGκ BP-HRP (Cat #sc-516102) "(Santa CruzBiotechnology)" 1:5000
β-actin-HRP (Cat #A3854) (Sigma) 1:25000
BV711 Rat Anti-Mouse I-A/I-E (Cat #563414) (BD Biosciences) 1:100
PE Rat Anti-CD11b (Cat #557397) (BD Biosciences) 1:100
APC Hamster Anti-Mouse CD11c (Cat #550261) (BD Biosciences) 1:50
"APC/Cyanine7 anti-mouse CD45.2Antibody" (Cat #560694) (BD Biosciences) 1:100
APC Hamster Anti-Mouse CD3e (Cat #553066) (BD Biosciences) 1:33
PE Rat Anti-Mouse CD4 (Cat #561837) (BD Biosciences) 1:67
FOXP3-FITC "(Cat #35-5773-U025)" "(CytekBiosciences)" 1:67
CD8-V500 "(Cat #85-1886-U025)" "(CytekBiosciences)" 1:40

PD-1-PE (Cat #50-9985- U025) "(CytekBiosciences)" 1:100
CD4, APC-Cy7, clone GK1.5 (Cat #25-0041-U025) "(CytekBiosciences)" 1:100
CD4,FITC, clone GK1.5 "(Cat #35-0041-U025)" "(CytekBiosciences)" 1:100
CD4, PE, clone GK1.5 (Cat #50-0041- U025) "(CytekBiosciences)" 1:100
CD4, APC, clone GK1.5 "(Cat #20-0041-U025)" "(CytekBiosciences)" 1:100
CD4, Violet Fluor 500, clone GK1.5 "(Cat #85-0041-U025)" "(CytekBiosciences)" 1:100
Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) (Cat #553141) (BD Biosciences) 1:200

Validation

1) FOXM1(C-20) Cat # SC-502 (Santa Cruz Biotechnology) https://datasheets.scbt.com/sc-502.pdf
2) STING (D2P2F) Rabbit mAb 13647 Cat # 13647 (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/sting-d2p2f-rabbit-mab/13647
3) "Phospho-STING (Ser366) (E9A9K)Rabbit mAb 50907" Cat # 50907 (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/phospho-sting-ser366-e9a9k-rabbit-mab/50907
4) STING (D1V5L) Rabbit mAb (Rodent Preferred) 50494 Cat # 50494 (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/sting-d1v5l-rabbit-mab/50494
5) "Phospho-STING (Ser365) (D8F4W)Rabbit mAb 72971" Cat # 72971 (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/phospho-sting-ser365-d8f4w-rabbit-mab/72971
6) ATF-6 (D4Z8V) Rabbit mAb #65880 Cat # 65880S (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/atf-6-d4z8v-rabbit-mab/65880
7) IRE1α (14C10) Rabbit mAb #3294 Cat # 3294S (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/ire1a-14c10-rabbit-mab/3294
8) CHOP (L63F7) Mouse mAb 2895 Cat # 2895S (Cell signaling) https://www.cellsignal.com/products/primary-antibodies/chop-l63f7-mouse-mab/2895
9) Normal Rabbit IgG Cat # PP64 (Millipore) https://www.merckmillipore.com/INTL/en/product/IgG-Rabbit,MM_NF-PP64
10) mouse anti-rabbit IgG-HRP sc-2357 "(Santa CruzBiotechnology)" https://www.scbt.com/p/mouse-anti-rabbit-igg-hrp
11) m-IgGκ BP-HRP sc-516102 "(Santa CruzBiotechnology)" https://www.scbt.com/p/m-igg-kappa-bp-hrp
12) β-actin-HRP A3854 (Sigma) https://www.sigmaaldrich.com/US/en/product/sigma/a3854
13) BV711 Rat Anti-Mouse I-A/I-E 563414 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-rat-anti-mouse-i-a-i-e.563414
14) PE Rat Anti-CD11b 557397 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-cd11b.557397
15) APC Hamster Anti-Mouse CD11c 550261 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-hamster-anti-mouse-cd11c.550261
16) "APC/Cyanine7 anti-mouse CD45.2Antibody" 560694 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-mouse-anti-mouse-cd45-2.560694
17) APC Hamster Anti-Mouse CD3e 553066 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-hamster-anti-mouse-cd3e.553066
18) PE Rat Anti-Mouse CD4 561837 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-rat-anti-mouse-cd4.561837
19) FOXP3-FITC "35-5773-U025" "(CytekBiosciences)" https://cytekbio.com/products/fitc-anti-mouse-foxp3-3g3
20) CD4, APC-Cy7, clone GK1.5 (Cat #25-0041-U025) "(CytekBiosciences)" https://cytekbio.com/products/apc-cyanine7-anti-mouse-cd4-gk1-5
21) CD8-V500 "85-1886-U025" "(CytekBiosciences)" https://cytekbio.com/products/violetfluor-500-anti-mouse-cd8a-2-43
22) PD-1-PE 50-9985- U025 "(CytekBiosciences)" https://cytekbio.com/products/pe-anti-mouse-cd279-pd-1-j43-1
23) CD4,FITC, clone GK1.5 "35-0041-U025" "(CytekBiosciences)" https://cytekbio.com/products/fitc-anti-mouse-cd4-gk1-5
24) CD4, PE, clone GK1.5 50-0041- U025 "(CytekBiosciences)" https://cytekbio.com/products/pe-anti-mouse-cd4-gk1-5
25) CD4, APC, clone GK1.5 "20-0041-U025" "(CytekBiosciences)" https://cytekbio.com/products/apc-anti-mouse-cd4-gk1-5
26) CD4, Violet Fluor 500, clone GK1.5 "85-0041-U025" "(CytekBiosciences)" https://cytekbio.com/products/violetfluor-500-anti-mouse-cd4-gk1-5
27) Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) 553141 (BD Biosciences) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd16-cd32-mouse-bd-fc-block.553141

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	All cell lines were obtained from ATCC.
Authentication	Cell lines were authenticated using STR analysis.
Mycoplasma contamination	All cell lines were tested for mycoplasma using the Universal Mycoplasma Detection Kit (ATCC # 30-1013K). All cell lines were negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were utilized in the 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	Several strains of female mice were used for the study including NOD-SCID-GAMMA(NSG), Rag1 Knockout mice, and C57BL6/J. The mice were injected(mammary fat pad) with E0771 mouse breast cancer cells at 6-7 weeks of age.). All the experimental procedures were approved by the Institutional Animal Care and Use Committee(IACUC). Age matched mice were used for all experiments. Mice were euthanized upon signs of morbidity or at the end of the study before tumors reached 2cm3, using CO2 inhalation.
Wild animals	No wild animals were used in the study.
Reporting on sex	All the in vivo experiments were done in female mice (no sex or gender analysis was performed) because breast cancer predominantly occurs in females (99%).
Field-collected samples	No field collected samples were used for the study.
Ethics oversight	The study was approved by UTHCSA IACUC committee.

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 ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	No clinical trials were performed in this study.
Study protocol	See above. Not applicable.
Data collection	See above. Not applicable.
Outcomes	See above. Not applicable.

Plants

Seed stocks	No plants were used in the study.
Novel plant genotypes	See above.
Authentication	See above.

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	E0771 tumors were weighed and cut into small pieces then digested with RPMI media containing 2% FBS, 1mg/ml Collagenase IV(Worthington, Cat #LS004188), and 20µg/ml DNAse I(Sigma, Cat #4536282001) at 37 degrees for 1 hr with shaking. Samples were mashed on top of a 70µM strainer in RPMI Media then filtered into a 50ml tube. RBC lysis was performed before cells were counted and 1 million cells were taken per panel. Cells were centrifuged and then washed one more time before incubation with CD16/32 Fc block(1:200, BD Biosciences, Cat #553142) and Zombie Violet fixable viability dye(Biolegend, Cat # 423114, 1:500) for 30 min at 4 degrees. Cells were washed then stained with surface antibodies for 30

	min, based on the panel. The samples were washed once before fixation and staining for intracellular proteins. Once staining was finished, the cells were fixed with 2% PFA at 37°C for 10min before washing with FACS buffer and storage at 4°C. Spleens stained with various CD4 conjugated fluorophores were used as single-color antibody controls for the T cell panel. For the myeloid panel, single color staining with spleen samples with each antibody was used for compensation. The data was acquired on LSR II Fortessa X-20 cytometer.
Instrument	BD LSRII Fortessa X-20 Flow cytometer
Software	The data was acquired using BO FACSDiva Software V9.0 (RRID:SCR_001456). The compensation and data analysis was performed on Flowjo v10 software(RRID:SCR_008520)
Cell population abundance	The immune cells represented at least 50-90% of the tumors, depending on the sample.
Gating strategy	The samples were divided into 3 panels- CD4, CD8, and Myeloid. The gating strategy for the CD4 and CD8 panel was as follows: Selection of live T cells using live/dead and forward scatter-area-> removal of debris using FSC-A/SSC-A-> Singlet selection using FSC-A/FSC-H followed by SSC-A/SSC-H-> CD45+ selection-> CD3+. For CD4 panel, CD3+ cells were further divided into CD4+/FSC-A --> FOXP3+/FSC-A and/or CD4+/FOXP3+ cells. For CD8 panel, CD3+ cells were further divided into CD8+, FSC-A and CD8+, PD-1 cells. For the myeloid panel, cells were first gated to remove debris using FSC-A/SSC-A-> singlet selection with FSC-A/FSC-H and SSC-A/SSC-H -> Live/dead cell selection with zombie violet. The live cells were then divided into CD11b+ CD11c+, CD11b+ MHC-II+, and CD11c+ MHC-II+ populations.

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