

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	BD FACSDiva (v9.0 and v9.2) for flow cytometry; NIS Elements (v5.0.0 and v6.10.01) for microscopy; Incucyte SX5 (v2023A) for cell growth assays.
Data analysis	Flow cytometry data was analyzed with FlowJo (v10.10). Cell growth and incucyte data was imaged and quantified using the Incucyte SX5 (v2023A) software. Fluorescence images were analyzed using Huygen's Professional Software (v26.9), Imaris (v10.1), QuPath (v0.6.0). Histology was analyzed using ImageJ (v2.9.0). Mitochondrial functions were analyzed using the online Seahorse Analytics platform. Single cell RNA-seq was analyzed as described in the methods section using scanpy (v1.9.5), scVI (v1.0.2), CellAssign, and nferCNVpy (v0.4.2). Differential gene expression from TCGA data was analyzed as described in the methods section using the R package TCGABiolinks (v2.25.3), EdgeR (v3.40.0), ActivePathways (v2.0.4), g:Profiler, Cytoscape (v3.10.1) and the EnrichmentMap app (v3.3.6). MoCaSeq analysis was run in a container using Singularity (v3.7.1-5.1.ohpc.2.1) as described in the methods section which contained the following tools: Bcftools (v1.10.2), Bedtools (v2.28.0), Bwa (v0.7.17), Bwa-kit (v0.7.15), ClinEff (v4.3T), CNVKit (v0.9.6), CopywriteR (v2.15.2), Delly (v0.8.3), DISCVRSeq (v1.07), DNACopy (v1.57.0), FastQC (v0.11.8), GATK (v4.1.7.0), GetBaseCounts (v1.2.2), HmmerCopy (v1.25.0), hmmercopy_utils, Htslib (v1.10.2), Manta (v1.6.0), Msisensor (v0.5), MultiQC (v1.9), Picard (v2.20.0), R (v3.6.3), Sambamba (v0.7.0), Samtools (v1.10.0), SnpEff (v4.3T), Strelka (v2.9.10), TitanCNA (v1.21.2), trimmomatic (v0.39), VariantQC (v1.07), Vcf2maf (v1.6.17), VcfTools (v0.1.16) and Vep (v96). CRISPR deep sequencing was analyzed using bowtie (v1.3.1) and MAGeCK (v0.5.9.2). Mouse bulk RNA sequencing was pseudoaligned using kallisto (v0.46.2) and analyzed using DESeq2 (1.42.1). Mass spec was analyzed using Spectronaut (v19.0). Statistical analysis was performed using Prism (v10.3.0) or in R Studio with ggplot2 (v4.0.0).

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 METABRIC and TCGA firehouse legacy datasets were obtained through cBioPortal between April 1 2019 and September 2 2019 from cBioportal. Copy number arm-level correlations were accessed from the USCS Xena web platform on May 15 2023. Patient RNA-sequencing data was accessed from the TCGA PanCan Atlas project on May 3 2024 and Gene Ontology and molecular pathways from reactome was downloaded from the g:Profiler web server on June 26 2024. These can be found available at: <https://www.cbioportal.org/datasets> and <https://xenabrowser.net/datapages/>. The OncoKB cancer gene list was last downloaded from OncoKB on May 12 2024 from <https://oncokb.org/cancer-genes>. The RNA-sequencing data generated in this study is available at the Gene Expression Omnibus under the accession number GSE274219. The whole genome sequencing data generated in this study is available at the Sequence Read Archive under the accession number PRJNA1146341 and gene-level copy number calls are provided at Figshare: <https://doi.org/10.6084/m9.figshare.32144932>. Human single-cell RNA-sequencing data were collected on August 14 2023 and are available at the Gene Expression Omnibus under accession number GSE176078 (doi: 10.1038/s41588-021-00911-1) and GSE161529 (doi: 10.15252/embj.2020107333) and at <https://lambrechtslab.sites.vib.be/en/datasaccess> (doi: 10.1038/s41591-021-01323-8) (doi: 10.1038/s41422-020-0355-0).

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

We calculated mouse sample size for our screens based on the following parameters: the mammary epithelium consists of approximately 350,000 cells; an average transduction rate of 15% results in a minimal double infection rate (less than one in every ten infected cells); and at a 15% transduction rate, every gland would have 50,000 infected cells for a total of 200,000 cells in all four injected glands of a single mouse. These estimates were corroborated using a lentiviral unique molecular identifier library with a complexity of over 1,000,000 unique barcodes, which when injected into the mouse mammary gland resulted in a median of 21,490 unique infections per gland. To ensure that sufficient screening coverage, individual cells were transduced with a given gRNA, our largest library of 4,160 gRNAs requires ~4,200,000 transduced cells or ~20 mice. Therefore, our minimum coverage achieved per gRNA ranges from 413 to 1,313x from our largest to smallest libraries. For the remaining validation mouse experiments, a minimum of five independent mice were injected per condition which based on our preliminary work was sufficient to identify significant differences between cancer driver gene injected mice and controls. Taking into account ethical and reductionist use of animals, we aimed for this sample size to use the minimum number of mice that would recover sufficient biological material for all down stream molecular and histological analyses. For cell culture experiments, all experiments were performed in at least three independent biological replicates.

Data exclusions

No data was excluded from the analysis, except in the case of (1) scratches or artifacts within individual technical replicate wells of the incucyte experiments precluding the reliable quantification of those technical replicates and (2) in the STED microscopy for outlier points determined following the 1.5 x IQR rule, where IQR is the interquartile range defined as the difference between Quartile 3 (Q3) and Quartile 1 (Q1). Points were determined to be outliers if they fell below Q1 - (1.5 x IQR) or above Q3 + (1.5 x IQR). Please note these points are still shown within the graph for transparency but have been removed from the analysis.

Replication	CRISPR hits from the screening were all validated with at least two gRNAs to rule out possible off-target effects and these conditions were treated as equivalent. Importantly, the top scoring guide from the screen and a completely independent guide which was not present in the initial screen was used. Replicates were successful for all of the genes tested and behaved consistently. Additionally, for all CRISPRa experiments, potential offtarget effects of the gRNAs were ruled out by cDNA overexpression constructs. Experiments were repeated in independent cohorts of mice and no full cohort of mice was injected in one sitting. All other cell culture-based assays were repeated in at least three biological replicates (exact numbers per experiment are indicated directly within the appropriate Figure legend). All attempts at replicates were successful.
Randomization	For all mouse experiments, female mice of the appropriate experimental genotype were randomized at weaning and pre-assigned to a chromosome arm library, gRNA, ORF, or vehicle/drug treatment cohort. For cell culture based experiments, cells were split into plates or wells that were pre-assigned an experimental condition or treatment.
Blinding	Blinding was used in all tumor monitoring experiments and analysis of all histological samples. All sequencing libraries for RNA and WGS were prepared by researchers blinded to the genotype of the samples. All histological, live cell imaging, qRT-PCR, and immunofluorescence data acquisition and analysis was performed by researchers blinded to the experimental groups. For metabolic assays, growth curves, western blotting, STED microscopy, and CRISPR library preparation and deconvolution data was collected and analyzed on equipment objectively without bias, therefore investigators were not blinded for the data collection in these experiments.

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

anti-PLGRKT, Thermo Fisher Scientific (PA5-98932), Western Blotting, 1/1000 dilution, RRID: AB_2813545
 anti-PLGRKT, Sigma-Aldrich (HPA011144), Immunofluorescence, 1/200 dilution, RRID: AB_1078364
 anti-Pan-Cytokeratin, Sigma-Aldrich (MAB3412), Clone AE1/AE3, Immunofluorescence, 1/200 dilution, RRID: AB_1078364
 anti-HA, Roche (11867423001), Clone 3F10, STED Microscopy, 1/500 dilution, RRID: AB_390918
 anti-TIMM23, Abcam (ab230253), STED Microscopy, 1/200 dilution, RRID: AB_3697281
 anti-TOMM20, Cell Signaling Technology (42406), Clone D8T4N, STED Microscopy, 1/200 dilution, RRID: AB_2687663
 anti-TOMM20-AF647, Abcam (ab209606), Clone EPR15581-54, Immunofluorescence, 1/200 dilution, RRID: AB_2934123
 anti-ERBB2, Cell Signaling Technology (2165), Clone 29D8, Immunohistochemistry, 1/200 dilution, RRID: AB_10692490
 anti-CSNK1A1, Abcam (ab196684), Western Blotting, 1/1000 dilution
 anti-METTL14, Novus (NBP1-81392), Western Blotting, 1/1000 dilution, RRID: AB_11021780
 anti-Beta-Actin, Sigma-Aldrich (A1978), Clone AC-15, Western Blotting, 1/20000 dilution, RRID: AB_476692
 anti-GAPDH, BioLegend (607904), Clone W17079A, Western Blotting, 1/3000 dilution, RRID: AB_2734505
 anti-Collagen IV, Abcam (ab6586), Immunohistochemistry, 1/400 dilution, RRID: AB_305584
 anti-Rabbit IgG, Bio-Rad (170-6515), Western Blotting, 1/10000 dilution, RRID: AB_11125142
 anti-Mouse IgG, Bio-Rad (170-6516), Western Blotting, 1/10000 dilution, RRID: AB_11125547
 anti-Rabbit IgG, Vector Laboratories (MP-7401), Immunohistochemistry, Neat, RRID: AB_2336529
 anti-Rabbit IgG, Invitrogen (A-21249), Immunofluorescence, 1/500 dilution, RRID: AB_2535850
 anti-Rat IgG, Invitrogen (A-21209), STED Microscopy, 1/500 dilution, RRID: AB_2535795
 anti-Rabbit IgG, Abberior (STP635P-1002-500UG), 1/500 dilution

Validation

All antibodies used are commercially available. All primary antibodies were validated by the manufacturer for antigen specificity using knockout cell lines for western blotting, immunofluorescence, immunohistochemistry, or flow cytometry and/or western blotting against recombinant protein for each specific antigen. Validation statements are available on the manufacturer's website for each individual catalogue number above.

Eukaryotic cell lines

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

Cell line source(s)

ATCC (293T, 4T1, MCF10A); this study (LSL-tdTom MEFs [both male and female MEFs were isolated], 2409 mammary epithelial cells [epithelial tumor cells derived from a female mouse mammary tumor]); MCF10A cell lines sensitized towards

oncogenic transformation with PIK3CA-H1047R and MYC were maintained under the same conditions and were received as a kind gift from Dr. Linda Penn.

Authentication

ATCC-derived and MCF10A sensitized towards oncogenic transformation with PIK3CAH1047R and/or MYC cell lines were not independently authenticated. LSL-tdTom MEFs - Mouse embryonic fibroblasts were established from homozygous B6.Cg-Gt(ROSA)26Sortm(CAG-tdTomato)Hze/J E14.5 embryos (Strain #007914, The Jackson Laboratory) and authenticated with in vitro treatment of Cre-expressing viral and non-viral constructs resulting in the induction of tomato fluorescent protein. 2409 cells - A mouse BLBC cell line syngeneic on a C57BL/6 background was generated from Pten^{-/-};Trp53^{-/-};Cas9 mammary tumors at endpoint. These tumors were initiated from Ptenfl/fl;Trp53fl/fl;Cas9 that were intraductal injected with Cre adenovirus. This line was authenticated by shallow whole genome sequence and recombination PCR confirming the loss of both Pten and Trp53 floxed cassettes. Further authentication of Cas9 or EGFP was confirmed using positive control gRNAs or FACS and immunofluorescence, respectively.

Mycoplasma contamination

All cell lines tested negative for mycoplasma.

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

None of these 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

Mus musculus was used in this study with all experimental animals ranging between 8 weeks and 114 weeks of age. The specific strains used were LSL-Cas9-EGFP (Gt(ROSA)26Sortm1(CAG-xstpx-cas9,-EGFP)Fehz/J, Strain #: 026175, The Jackson Laboratory), Ptenfl/fl (B6.129S4-Ptentm1Hwu/J, Strain #: 006440, The Jackson Laboratory), Rb1fl/fl (Rb1tm2Brn/J, Strain #: 026563, The Jackson Laboratory), Trp53fl/fl (B6.129P2-Trp53tm1Brn/J, Strain #: 008462, The Jackson Laboratory), Mettl14fl/fl (Mettl14tm1.1Chhe, a kind gift from Dr. Chuan He, Howard Hughes Medical Institute)⁸⁹ and were all maintained in a C57BL/6 background. The experimental mouse lines Trp53fl/fl;LSL-Cas9-EGFP, Ptenfl/fl;Trp53fl/fl;LSL-Cas9-EGFP, Rb1fl/fl;Trp53fl/fl;LSL-Cas9-EGFP, Mettl14fl/fl;Trp53fl/fl;LSL-Cas9-EGFP and Mettl14fl/fl;Ptenfl/fl;Trp53fl/fl;LSL-Cas9-EGFP were all generated from crossing the stock strains listed above and maintained as homozygous mating pairs. Mettl14fl/+;Trp53fl/fl;LSL-Cas9-EGFP and Mettl14fl/+;Ptenfl/fl;Trp53fl/fl;LSL-Cas9-EGFP experimental animals were generated by crossing Mettl14fl/fl;Trp53fl/fl;LSL-Cas9-EGFP with Trp53fl/fl;LSL-Cas9-EGFP or Mettl14fl/fl;Ptenfl/fl;Trp53fl/fl;LSL-Cas9-EGFP with Ptenfl/fl;Trp53fl/fl;LSL-Cas9-EGFP, respectively. LSL-Pik3caH1047R/+ (Gt(ROSA)26Sortm1(Pik3ca*H1047R)Egan) were maintained in a clean FVB/N background. For experimental LSL-Pik3caH1047R/+;LSL-Cas9-EGFP cohorts mice were crossed and the F1 FVB/N;C57BL/6 generation was used. Nod scid gamma/J (Strain #: 005557, The Jackson Laboratory) and BALBc/J (Strain #: 000651, The Jackson Laboratory) were used for orthotopic transplantation experiments. Mice were housed in a 12-hour dark/light cycle facility with continuous access to water and chow. The room temperature was maintained at 20-22°C and humidity was maintained at 40-60%. All animals were supplied with 100% fresh air and supply and exhaust ventilation exchange rates were set at 10-15 air changes per hour.

Wild animals

No wild animals were used.

Reporting on sex

All experimental animals used in this study were female.

Field-collected samples

No field work was performed in this study.

Ethics oversight

Animal husbandry, ethical handling of mice, and all approved animal work was carried out according to guidelines approved by the Canadian Council on Animal Care and under protocols approved by the Centre for Phenogenomics Animal Care Committee (26-0272H).

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

At humane tumor endpoint mice were euthanized and tumors were immediately dissected and placed on ice. Tumors were minced to a fine paste and digested in DMEM supplemented with 0.25% (w/v) of collagenase type I (17100017, ThermoFisher Scientific) for forty minutes at 37°C on a shaker set to 100 rpm. Tumors were then washed once in flow buffer (Hank's Basic salt Solution [HBSS] with 4% fetal bovine serum and 10 mM HEPES) and digested with trypsin at 37°C on a shaker set to 100 rpm for five minutes. Single cell suspensions were then washed once in flow buffer and passed through a 40 µm strainer. Red blood cells were lysed in an ammonium chloride solution (07850, STEMCELL Technologies) for five minutes. After three washes, cells were either processed for fluorescence-activated cell sorting (FACS) or viably frozen in 90% fetal bovine serum and 10% DMSO at -80°C. Viably frozen tumor cells were thawed briefly in a 37°C water bath and washed three times in flow buffer prior to FACS.

For lentiviral titring, following transduction, cells were washed once in PBS, trypsinized and resuspended in flow buffer (Hank's Basic salt Solution [HBSS] with 4% fetal bovine serum and 10 mM HEPES) with 0.1 µg/mL of 4',6-diamidino-2-phenylindole (DAPI).

To measure mitochondrial superoxide and total cellular ROS, 4T1 mammary epithelial cells were grown in 6-well plates and treated with DMSO or 200 nM oligomycin for up to 48 hours. Cells were collected at the indicated timepoint washed three times in PBS and 1x10⁶ cells was resuspended in 1 mL of PBS containing either 5 µM MitoSOX Red (M36008, ThermoScientific) or 1 µM H2DCFDA (D399, ThermoScientific), to measure mitochondrial superoxide or total cellular ROS, respectively. Cells were then incubated for 45 minutes at 37°C incubator with controlled 5% CO₂ levels. Cells were then washed three times and single cell suspensions were incubated in flow buffer containing 0.1 µg/mL of 4',6-diamidino-2-phenylindole (DAPI).

Instrument

Tumor cells were sorted on either the MA900 (Sony Biotechnology) or MoFlo Astrios EQ (Beckman Coulter) cell sorters. Samples for analysis were acquired on an LSR Fortessa.

Software

Data acquisition was done with BD FACS Diva software. Data analysis was performed using FlowJo V10.10.

Cell population abundance

Following sorting, an aliquot of the sorted fraction was re-acquired to ensure that the sample purity was over 98%.

Gating strategy

For FACS of viable and nucleated cells, single cell suspensions were incubated in flow buffer containing 0.1 µg/mL of 4',6-diamidino-2-phenylindole (DAPI) and 0.5 mM DRAQ5 (62251, ThermoFisher Scientific). As all the tumors in this experiment are either EGFP or H2BmRFP positive, we gated live, nucleated tumor epithelial cells as the DAPI negative, DRAQ5 positive, EGFP or RFP positive population.

For cell lines, GFP, RFP, FITC, or PE mean fluorescence intensity was gated on the DAPI-negative, live cell population. An example of this for RFP-positive cell lines is shown in the Supplementary Information.

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