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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

Clinical genomic data sets. TCGA PanCancer Atlas35 https://www.cbioportal.org/study/summary?id=pancan_pcawg_2020, METABRIC https://www.cbioportal.org/study/summary?id=brca_metabric, The Metastatic Breast cancer project (MBP) https://www.cbioportal.org/study/summary?id=brca_mbcproject_2022, and MSK data sets (MSK-IMPACT 2017 https://www.cbioportal.org/study/summary?id=msk_impact_2017, MSK-Cancer Cell 2018, MSK-MET 2021 https://www.cbioportal.org/study/summary?id=msk_met_2021) were obtained from cBioPortal. Additional cohorts of primary breast tumors with known metastatic sites were downloaded from the Gene Expression Omnibus (GEO) repository (accession numbers GSE12276 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse12276 and GSE76714 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76714). Datasets containing expression data of metastasis samples were also downloaded from GEO (accession numbers GSE14017 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14017 and GSE14018 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14018), allowing for the comparison between metastatic sites. A third new cohort containing 6 brain and 26 other metastases was profiled using RNAseq as described below, and is available at GEO (GSE245414). Paired primary breast tumor and BM expression profiles were obtained from GEO (accession number GSE125989 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125989). Expression data from several metastatic sites of a single patient at autopsy was obtained as described below. Cancer cell line genomic data sets. CAL51 TP53-KO RNAseq was taken from Redman-Rivera et al. RNA expression data and metabolomics data65 for 1019 human cancer cell lines was downloaded from the Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle/data). The metastatic potential of 489 human cancer cell lines to several metastatic sites (brain, lung, liver, bone and kidney) in mouse xenografts was taken from the MetMap500 paper. CRISPR (CERES), RNAi (Achille, DRIVE, Marcotte, DEMETER2) dependency scores and drug screen data (GDSC1) were obtained from DepMap 22Q1 release (https://figshare.com/articles/dataset/DepMap_22Q1_Public/19139906). RNA sequencing from patient tumor samples. Tumor material from patient's surgery or biopsy were collected in RNAlater (Thermo Fisher) and cryopreserved in liquid nitrogen for subsequent extraction of nucleic acids (RNA and DNA). RNA was extracted from fresh frozen tissue using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Valencia, CA, USA). The quality of the RNA was assessed with by Bioanalyzer using the Agilent RNA 6000 Nano Kit. Libraries for RNA sequencing were prepared using the TruSeq RNA Exome kit (Illumina) following the

manufacturer's instructions. The quality of the resulting libraries was confirmed by Bioanalyzer (High Sensitivity DNA Kit), the intermediate library prior to exon enrichment was quantified by Bioanalyzer using the Agilent DNA 1000 kit, and the final library was assessed by qPCR. Samples were sequenced in paired-end mode, sequencing 76bp from each side on a NextSeq 500 instrument. RNA-seq data were analyzed with "rnaseq" version 3.9 pipeline of nf-core community RNA sequencing analysis pipeline (https://nf-co.re/rnaseq). The alignment was performed with the STAR algorithm and the gene quantification with the Salmon algorithm. The pipeline was run using the default parameters.
Genome-wide RNAi, CRISPR and drug screens. The differential sensitivities of TP53-WT and TP53-null BC cell lines in genome-wide RNAi screens were compared using gene-set enrichment analysis (GSEA). To this end, the top 500 significant hits were analyzed for enrichment of canonical pathways using the MsigDb database. RNAi and CRISPR sensitivity values for specific genes were correlated with ssGSEA scores for the MSigDB gene signature 'KANNAN_p53_targets_DN as an approximation for p53 pathway activity. Drug sensitivities were similarly compared between TP53-WT and TP53-null human BC cell lines.

Data analysis

Cancer cell line genomic data sets. CAL51 TP53-KO RNAseq was taken from Redman-Rivera et al. RNA expression data and metabolomics data65 for 1019 human cancer cell lines was downloaded from the Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle/data). The metastatic potential of 489 human cancer cell lines to several metastatic sites (brain, lung, liver, bone and kidney) in mouse xenografts was taken from the MetMap500 paper14. CRISPR (CERES), RNAi (Achille, DRIVE, Marcotte, DEMETER2) dependency scores and drug screen data (GDSC1) were obtained from DepMap 22Q1 release (https://figshare.com/articles/dataset/DepMap_22Q1_Public/19139906).
Gene set enrichment analysis (GSEA). Gene expression profiles were obtained from the above-mentioned data sets. Normalized matrix files were downloaded, and samples were manually annotated according to the provided sample information. Raw expression matrices were processed following a three-step protocol: (1) The lowest 10-20% expressed genes were defined and set as the expression threshold value. (2) Using this threshold value, genes not expressed in >20% of the samples were also removed. (3) Lastly, genes with lower expression values were raised to the threshold value. The GSEA tool (https://www.gsea-msigdb.org/gsea/index.jsp) was used with the following parameters:: Number of permutations – 1000, collapse/remap to gene symbols – collapse, permutation type – geneset, Chip platform: ftp.broadinstitute.org://pub/gsea/annotations_versioned/Human_Gene_Symbol_with_Remapping_MSigDB.v2023.1.Hs.chip. Single cell gene set enrichment analysis (ssGSEA)
Correlating BM prevalence with TP53 perturbation prevalence. Across carcinomas: BM prevalence in different cancer types were taken from Riihimäki et al. The analysis focused on carcinomas, and other tumor types were excluded. For lung and kidney, the highest-ranking subtypes were considered. TP53 perturbation prevalence in the respective cancer types were obtained from cBioPortal as described above (TCGA data35). Across subtypes: For the analysis of the brain metastatic potential of BC subtypes, BM prevalence was taken from Smid et al, and TP53 perturbation prevalence in the respective molecular subtypes were calculated from METABRIC data6,115,116. was performed using GenePattern (https://www.genepattern.org/).
RNA sequencing analysis of patient autopsy samples. Patient 6071 was diagnosed with invasive ductal carcinoma with micropapillary features. Despite treatments, the patient deceased of advanced disease and metastases from the brain, lung (right and left), spine, lymph node, bone and the left rib were collected shortly after death. The mRNA was extracted from the patient autopsy samples using the miRNEasy kit (Qiagen, #217084) according to the manufacturer's recommendations. Briefly, after being crushed in liquid nitrogen with a mortar and pestle, the samples were resuspended in the lysis buffer and the mRNA was isolated using a guanidinium thiocyanate-phenol-chloroform extraction approach. Remaining genomic DNA was digested by RNase-Free DNase (Qiagen, #79254) on the purification column. The RNA was washed and eluted in water. The quantity and the quality of the isolated mRNA were assessed using the TapeStation (Agilent, 4200 TapeStation System), and 100 ng of the mRNA was used as an input for the library preparation using the TruSeq RNA Library Prep Kit (Illumina) according to the manufacturer's recommendations. The indexed libraries were pooled and diluted to 1.5pM for paired-end sequencing (2 × 81 cycles) on a NextSeq 500 instrument using the v2 150 cycle high output kit (Illumina) as per the manufacturer's instructions.
RNA-seq reads were aligned to the human reference genome GRCh38/hg38 using the Subread aligner129. Read counts were generated for NCBI RefSeq human genes (build 38.2) using the featureCounts program130. Genes were excluded from analysis if they failed to achieve a CPM (counts per million reads) value of 1 in at least one library. Genes with no official symbol were also removed from analysis. Counts were converted to log2-CPM and then quantile-normalized using voom function in limma package131,132. Log2-CPM values were then further transformed to log2-RPKM (log2 reads per kilo bases per million reads) values.
Statistical analyses were performed using Microsoft Excel and GraphPad PRISM® 9.1. Details of each statistical test, the number of analyzed samples and the number of independent experiments are specified in the Figure legends. Significance is indicated as: *p<0.05, ** p<0.01, *** p<0.001, ****<1*10-4. Statistical significance between two groups was calculated by a two-sided or one-sided Student's t-test as appropriate. Paired student's t-test was performed for patient-matched expression analysis and bilateral intracranial injections that contained matched p53-WT and p53-null cells. The Mann-Whitney test (independent samples) and Wilcoxon sign rank test (paired samples) were used to compare two groups when one or both groups showed a data distribution that was not normal. A 2-way ANOVA was used to determine significance for interventions in mice comparing p53-WT and p53-null groups. Two-sided Chi-square or Fisher's Exact test (when sample numbers were limited) were used to determine the significance of differences in the prevalence of events between groups. Significance of GSEA was based on normalized enrichment score (NES) and the false discovery rate q-value (FDR q-value) as calculated by the GSEA software (version 4.03). Significance of ssGSEA was determined through a two-sided or one-sided Student's t-test between two groups as appropriate. Q-values for ssGSEA results were computed using the Benjamini–Hochberg method.
Correlations and linear regression were calculated using GraphPad PRISM 9.1. Survival curves were calculated using the Kaplan Meyer method with GraphPad PRISM 9.1. Significance was determined with the Gehan-Breslow-Wilcoxon test, which prioritizes early events. Generally, a one-sided statistical test was chosen when the directionality of the experiment was predicted, based on previous experimental results with a different data set or cell line.
In each presented box plot, all data points are shown and the internal line represents the median of the distribution. The box extends from the 25th to 75th percentiles. Whiskers extend to the highest and lowest data point

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

RNA sequencing data for the hTERT-HMEC p53 allelic series was deposited to SRA under accession number PRJNA1246204 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1246204).). RNA sequencing data of metastases was deposited to GEO under accession number GSE245414. RNA sequencing data of patient autopsy samples is available upon request to BROCADE. RNA sequencing data of MDA-MB231 cells following siRNA-mediated knockdown of DEPDC1 was deposited to GEO under accession number GSE281411. p53 CUT&tag data was deposited to SRA, under accession number PRJNA1247024.

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	Breast cancer data were obtained from females.
Reporting on race, ethnicity, or other socially relevant groupings	Data were obtained from patients in the respective participating countries: Israel, Italy, Germany and Australia.
Population characteristics	No further information about the population characteristics was disclosed for the purpose of this study.
Recruitment	Recruitment was not performed as part of the current study.
Ethics oversight	Ethics and study approval. Animal studies: All procedures complied with Tel Aviv University guidelines and were approved by the Institutional Animal Care and Use Committee (TAU IACUC, protocol 01-20-074). Human tissue (Sheba Medical Center): FFPE samples from BCBM patients (n = 6; Fig. 5j–l, Supplementary Fig. 11) were collected with informed consent. Manipulation for immunostaining was approved by the ethics committees of Tel Aviv University and Sheba Medical Center (IRB 5727-18-SMC). Human tissue (University of Turin): BCBM specimens (n = 17; Fig. 3s, Supplementary Fig. 9) with histology and follow-up data were retrieved from pathology archives (2014–2017). The study followed the Declaration of Helsinki and University of Turin regulations (approval 00008/2024). Informed consent for surgery and data collection was obtained. RNA sequencing: Human metastasis samples (GSE245414) were collected with written informed consent. The study was approved by the IRB of Regina Elena National Cancer Institute (IFO 1270/19) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (ICH guidelines). Autopsy samples: The BROCADE rapid autopsy program (www.petermac.org/research/research-cohort-studies/brocade) operated under PMCC HREC approval (15-24). Donors provided written consent during life for rapid post-mortem tissue donation, with involvement of next of kin. Organotypic BM culture: Patients provided written informed consent, and the study was approved by the University Cancer Center Frankfurt (UCT) and the Ethical Committee of University Hospital Frankfurt (UCT-12-2022).

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	A minimum of 3 biological replicates (unless stated otherwise) were performed, as indicated in Figure legends. n is specified in figure legends. Sample size was determined based on previous experience in the lab and from relevant papers using similar techniques which allow for statistical comparison.
Data exclusions	Data were excluded from analyses based on pre-determined exclusion criteria (e.g., if control cells did not grow, the entire experiment was excluded).
Replication	All the experiments were performed at least three times (unless otherwise stated).
Randomization	Within each experimental regime, cell populations were assigned randomly to treatment or control conditions.

Blinding	Investigators were not blinded 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

Antibodies used are described in detail in Extended Data Table 19, as following:
Antibody Catalogue number Clone Lot. number Purchased from Dilution

Primary antibodies
a-SCD1 CST-2794S (C12H5) 4 Cell Signaling Technology® (Massachusetts, USA). Rabbit anti-mouse/human 1:1000
a-FASN AB-ab22759 / GR3380499-2 Abcam (Cambridge, United Kingdom). Rabbit anti-human, mouse, rat 1:1000
a-P53 32532s D2H9O 2 Cell Signaling Technology® (Massachusetts, USA). Rabbit anti-mouse, rat 1:1000
a-CD36 antibody AB-ab133625-10 EPR6573 GR3337338-10 Abcam (Cambridge, United Kingdom). Rabbit monoclonal anti-human 1:2500
a-Tubulin T6199 DM1A 048M4751V Sigma-Aldrich Mouse monoclonal antibody 1:2000
a-Ki-67 NB500-170 / G15 Novus (Colorado, USA). Rabbit anti-mouse/human 1:50
a-cleaved caspase-3 9661S D175 45 Cell Signaling Technology® (Massachusetts, USA). Rabbit anti-human, mouse, rat, monkey 1:30
a- Iba1 NBP2-19019 / 41556 Novus (Colorado, USA). Rabbit anti-mouse/human 1:200
a-CD31 550272 / 6273859 BD Biosciences (Franklin Lakes, NJ, USA). Rat anti-mouse 1:25
a-GFAP Z0334 / 20025480 Dako (Denmark). Rabbit anti-mouse/human 1:500
a-SCD1 ab236868 EPR21963 GR3297026-11; Abcam (Cambridge, United Kingdom). Rabbit anti-mouse/rat/human 1:100
a-p53 ab26 PAb240 GR3318620-2 Abcam (Cambridge, United Kingdom). Mouse anti-human 1:100
a-GFAP ab279291 / GR3448894-2 Abcam (Cambridge, United Kingdom). Rat anti-mouse/rat/human 1:100
a-GFP ab13970 / GR3361051-11 Abcam (Cambridge, United Kingdom). Chicken anti-GFP 1:500
a- mCherry ab167453 / GR262983-7 Abcam (Cambridge, United Kingdom). Rabbit anti-mCherry 1:500
a-SCD1 ab19862 CD.E10 GR3451419-1 Abcam; Cambridge, UK Mouse Monoclonal Antibody 1:200
a-p53 800-2912 DO-7 K09838 Ventana, Tucson, AZ, USA); Mouse Monoclonal Antibody prediluted
a-p21WAF1 421M-18 DCS-60.2 V0002590 Cell MarqueTM Tissue Diagnostics (Millipore Sigma), Rocklin, CA, USA); Mouse Monoclonal Antibody prediluted
a-GFAP 258R-18 EP672Y V0003627 Cell MarqueTM Tissue Diagnostics (Millipore Sigma), Rocklin, CA, USA); Rabbit Monoclonal Antibody prediluted
a-CD36 antibody ab133625 EPR6573 LOT 1000523-21 Abcam
a-DEPDC1 (homemade) Fr. 7 N/A Homemade Rabbit Polyclonal Antibody IP: 1mg/IP; WB:1:500
a-SCD1 Ab-19862 CD.E10 GR82172-1 Abcam Mouse Monoclonal Antibody 1:1000
a-SREBP1 Sc-13551 2A4 G2116 Santa Cruz Biotechnology Mouse Monoclonal Antibody IP: 1mg/IP; WB:1:500
a-HA 11867423001 3F10 60789700 Roche Rat Monoclonal Antibody IP: 1mg/IP; WB:1:1000
a-HA Y11 Sc-805 S Y-11 N/A Santa Cruz Biotechnology Rabbit Polyclonal Antibody IP: 1mg/IP
anti-Actin A-9718 N/A 182447 Sigma Rabbit Polyclonal Antibody 1:5000
a-HSP90 Sc-13119 F-8 J0722 Santa Cruz Biotechnology Mouse Monoclonal Antibody 1:5000

Secondary antibodies
goat anti-mouse Alexa Fluor® 647 ab15115 GR309891-3 Abcam (Cambridge, United Kingdom). goat anti-mouse 1:300
goat anti-rabbit Alexa Fluor® 488 ab150077 GR315933-2 Abcam (Cambridge, United Kingdom). goat anti-rabbit 1:300
goat anti-rabbit Alexa Fluor® 647 Ab150079 GR3176223-2 Abcam (Cambridge, United Kingdom). goat anti-rabbit 1:300
goat anti-rabbit Alexa Fluor® 594 ab150080 GR3398865-1 Abcam (Cambridge, United Kingdom). goat anti-rabbit 1:300
goat anti-rat Alexa Fluor® 488 112-545-068 143654 Jackson ImmunoResearch Laboratories, Inc. (West Grove, Pennsylvania, USA) goat anti-rat 1:300
goat anti-chichen Alexa Fluor® 488 ab150173 GR3358442-3 Abcam (Cambridge, United Kingdom). goat anti-chichen 1:300

Validation

All antibodies have been validated by the manufacturers for the applications performed in this study. No additional validation was carried out. Data are available on the manufacturer's websites. Antibodies were subject to quality control testing by the manufacturers and validation data are available on the vendor website for each product number listed.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	BC cell lines: EMT6, 4T1, MC7, MCF10A, MDA-MB-231, MDA-MB-468 and SKBR3 cells were obtained from ATCC and CAL51 cells were obtained from the Broad Institute cell line repository. Human and Murine Astrocytes: Human astrocytes (ScienceCell Research Laboratory, Carlsbad, CA, USA, # 1800) and freshly (in-house) isolated murine astrocytes.
Authentication	Authentication was performed by comparing the copy number landscapes of the cell lines to the published copy number landscapes.
Mycoplasma contamination	Breast cancer cell lines were tested free for mycoplasma contamination using Myco Alert (Lonza) according to manufacturer's instructions. Cells were also re-tested by PCR analysis in house.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified 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	Astrocyte isolation from mice. Brains from 8-10 week-old C57BL/6 mice were isolated, cut into small pieces and digested with Collagenase III/ Dispase solution (Worthington Biochemical Corporation, LS02104/ LS004186) for 50 min at 37°C under rotation. Myelin was removed using a percoll gradient and red blood cells were lysed. Microglia and endothelial cells were then removed from the cell suspension using CD11b and CD31 microbeads. Following negative selection, the remaining cell population was highly enriched for astrocytes (98% purity by FACS using anti GFAP antibody) and was plated in a 10 cm2 dish and grown in astrocyte medium, as previously described99. Intra-cardiac injection experiments. To assess the metastatic potential of Trp53-WT / Trp53-null isogenic cells, 0.2*106 GFP-labeled EMT6 BC cells were injected into the left ventricle of immunocompetent BALB/c mice under guidance of an ultrasound device (2 cohorts, 15 females/group). Ten days after injection, mice were euthanized, and their organs (brain, liver, spleen, femoral bone, and lungs) were harvested and imaged for GFP fluorescence using the Cambridge Research & Instrumentation, Inc. (Cri)-MAESTRO device (The University of Texas Southwestern Medical Center, Dallas, Texas, USA). Multispectral image-cubes were obtained through 550–800 nm spectral range in 10 nm steps using excitation (575–605 nm) and emission (645 nm longpass) filter sets. Mice autofluorescence and background signals were eliminated by spectral analysis and linear un-mixing algorithm. Tumor characteristics were quantified using image J. In a modified version of this experimental setup (competition assay), one group of mice (10 females) was similarly injected with both Trp53-WT (labeled with mCherry) and Trp53-null cells (labeled with eGFP), 0.5*106 cells per genotype. After 8 days the animals were imaged by MRI, then sacrificed and their organs were analyzed for fluorescence using a Maestro device as described above. Intra-cranial injection experiments. For brain growth adaptation experiments, EMT6 or CAL51 isogenic Trp53/TP53-WT/null cells with and without functional SCD1 (1.5*104 and 5*104 cells/2µl, respectively) were inoculated stereo-tactically to the striatum (2 mm left and right from the Bregma (Trp53/TP53-null) and 3.5 mm depth (Trp53/TP53-WT)) of 8 to 10-week-old BALB/c or SCID female mice, respectively. Injections were either performed bilaterally into the two brain hemispheres, to compare tumor growth of Trp53/TP53-WT/null isogenic cells within the same brains (Cohort size: 10-15 mice/experiment), or unilaterally, allowing for the assessment of survival and the isolation of tumor cells from the brain. Mice body weight was monitored twice a week, and tumor growth was followed using a 4.7T/1H MRI. For EMT6 experiments, MRIs were taken every 3 days, usually starting on day 4. At endpoint, mice were euthanized and immediately perfused with 4% PFA in PBS and brains were harvested for immunohistochemistry (day 6 and 8). For CAL51, tumors were growing considerably slower and tumor growth was monitored by MRI on day 7, 14, 22, 29 and 36. In vivo SCD1 inhibitor treatments. EMT6 Trp53-WT and Trp53 null cells (1.5x104 cells/2 µl) were bilaterally injected into the left and right brain hemispheres of 10-week-old mice (BALB/C females, n=10) as detailed above. SCDi treatment was started 24hrs later: five mice received vehicle (10% DMSO+ 10% Kolliphor (Sigma Aldrich) + 80% lactic acid pH 5.5) and the other five mice received SCD1 inhibitor SW203668 (Cayman Chemical) at 40 mg/kg, twice daily through intraperitoneal injections for 10 days. Body weight was monitored every two days, and tumor growth was measured on day 4, 7, 9 and 11 using 3.7T/1H MRI.
Wild animals	N/A
Reporting on sex	Female mice were used.
Field-collected samples	N/A
Ethics oversight	All animal procedures were performed in compliance with Tel Aviv University and approved by the Institutional Animal Care and Use Committee (TAU IACUC protocol no. 01-20-074).

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.*

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.

p53 CUT&tag data was deposited to SRA, under accession number PRJNA1247024.

Files in database submission

barcode_P53_KO_R1.fq
barcode_P53_KO_R1_2.fq
barcode_P53_KO_R2.fq
barcode_P53_KO_R2_2.fq
barcode_P53_WT_R1.fq
barcode_P53_WT_R1_2.fq
barcode_P53_WT_R2.fq
barcode_P53_WT_R2_2.fq

Genome browser session
(e.g. UCSC)

https://genome-euro.ucsc.edu/s/shaiovadia/Uri_paper

Methodology

Replicates — CUT&tag of endogenous p53 was performed in p53-WT cells and isogenic p53-KO cells, lacking detectable p53 protein as controls. n=1

Sequencing depth — Libraries were sequenced in Nextseq 2000 sequencer with 75bp paired-end reads.

Antibodies — p53 antibody (CST#9282) or H3K4me3 (positive control, Cell Signaling catalog 9751), CUT&Tag-IT® Assay Kit, Anti-Rabbit, catalog number 53160

Peak calling parameters — Sequenced reads were aligned to the human reference genome version 38 (hg38) using the Bowtie2 algorithm153 with the following parameters: --end-to-end, --very-sensitive, --no-mixed, --no-discordant, --phred33, -I 10, and -X 700. Subsequently, peaks were called using MACS2154 with the parameters: -g 1.3e+8, -f BAMPE, -p 0.01, and --keep-dup all. Peaks were compared between p53-WT and p53-KO cells.

Data quality — CUT&Tag was performed in p53-WT cells, and isogenic cells lacking p53 as control. To evaluate the specificity of our data, we used HOMER to perform motif analysis on the identified peaks.

Software — For visualization, we utilized bamCoverage155 to create CPM-normalized signal files, applying the options --binSize 10, --normalizeUsing CPM, and --ignoreForNormalization chrM. Finally, genomic tracks were generated using the Signac package156

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	For cell death analysis by flow cytometry, EMT6 cells were cultured with either ACM or with SFM for a period of 48h, collected and stained with antibodies for Annexin-V and Propidium Iodide (PI). Briefly, cells were washed twice with cell staining buffer (Biolegend; #420201) before being resuspended in cell binding buffer (Biolegend; 422201). Cells were incubated with 2.5µl Annexin-V (Biolegend; #640920; 12µg/ml) and 1µl PI (Biolegend; #421301; 0.5mg/ml) for 15 mins in the dark at room temperature. CAL51 cells were similarly treated with ACM or SFM for 72h, stained with Sytox green (S34860, Invitrogen, 1:1000) and Pacific Blue-AnnexinV Biolegend; #640918; 60µg/ml) for 15 min at room temperature.
Instrument	FACSAria Fusion flow cytometer (BD) ; Cytoflex4L (Belkman Coulter)
Software	FlowJo 10.7.2 ; Kaluza software 2.1 (Belkman Coulter)
Cell population abundance	single cells: >90%
Gating strategy	Gating strategy consisted on the identification of the singlets based on FSC-A and SSC-A, then gated cells were counted for fluorophore signal. Positive and negative gates were determined by the no-stained controls and adjusted per cell line.

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

Magnetic resonance imaging

Experimental design

Design type	MRI was performed to follow tumor growth in the brain. Mice were imaged at resting state.
Design specifications	Mice were scanned with a conventional T1 or T2 protocol in the same scanning session on 4.7T MRI or 7 T MRI (MR Solutions, UK) using 32-channel head-coil, followed gadolinium (Soreq, Israel) intraperitoneal injection. T1-tomography data were analyzed using RadiAnt DICOM Viewer or MRIcro software, the tumor area was calculated per each axial scan and the tumor's volume was obtained by summing the tumor's areas of all slices.
Behavioral performance measures	No behavioral performace measures were performed. Mice were anesthetizes and respiratory functions were monitored during the scan.

Acquisition

Imaging type(s)	Structural T1 or T2 weighted images were obtained.
Field strength	4.7 Tesla
Sequence & imaging parameters	T1 weighted, average-4, RF pules-1, TR-1150, Echo spacing-11, TE-11, Echo train-4, PE order-1, total slices-17, thickness-1mm, field of view-25, orientation-coronal. T2 weighted, average-2, RF pules-1, TR-4000, Echo spacing-17, TE-51, Echo train-7, PE order-0, total slices-17, thickness-1mm, field of view-25, orientation-coronel.
Area of acquisition	Whole brain scans were performed. Area was defined by a "scout" scanning prior to T1 or T2 sequences.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	1-tomography data were analyzed using RadiAnt DICOM Viewer or MRIcro software, the tumor area was calculated per each axial scan and the tumor's volume was obtained by summing the tumor's areas of all slices.
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	Respiratory gating was performed.
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
Effect(s) tested	The effect of p53 status on tumor growth was tested. Student's t-test and Anova were used to determine statistical significance.

Specify type of analysis: ☒ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

N/A

Correction

N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis