

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give <i>P</i> values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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 generated in this study.
Data analysis	GraphPad Prism (v10), IBM SPSS Statistics (v24; RRID: SCR_002865) software, and R (v4.2.2; https://www.r-project.org/), a publicly available statistics analysis tool, were used for data analysis. Statistical analyses were performed using the Student t-test for comparisons between 2 groups, and 1-way or 2-way ANOVA for comparisons among multiple groups. All data represent the mean ($\pm$ standard error of the mean) from at least 3 independent experiments or samples unless otherwise specified. For survival studies, Kaplan-Meier curves were used to compare cohorts, and the log-rank (Mantel-Cox) test was applied to determine differences. Band intensity from western blotting was quantified using ImageJ (RRID: SCR_003070). Specific statistical methods are outlined in the respective figure legends where applicable. The data processing and bioinformatics analyses were performed by the Department of Bioinformatics and Computational Biology at MD Anderson Cancer Center. Briefly, paired-end reads in FASTQ format were checked for read quality using FastQC/v0.11.8 and then aligned to the combined reference genome (GENCODE human GRCh38 and mouse GRCm38 concatenated) using TopHat2/v2.1.1. FeatureCounts (Subread 1.6.3) was used to count reads that are mapped to each feature. The features were annotated using the combined gene transfer format (GTF) file using GENCODE human and mouse reference (gencode.vM20.annotation.gtf for mouse and gencode.v29.annotation.gtf for human). Only the human gene count matrix was retrieved for downstream analysis. The read counts for all samples were then normalized using the trimmed mean of M method implemented in the R Bioconductor package (Bioconductor, RRID:SCR_006442) edgeR (edgeR, RRID:SCR_012802) to generate the abundance for each feature. We applied the edgeR pipeline with default filtering, TMM normalization, dispersion estimation, and quasi-likelihood F-test based on the negative binomial generalized linear model to determine differentially expressed genes (controlled by false discovery rate (FDR) cutoff 5%) between groups. The Benjamini-Hochberg correction was applied to the resulting p-values for multiple testing adjustment.

Functional analysis of the differentially expressed gene sets or pathways was performed using gene set enrichment analysis (GSEA). We conducted pre-ranked GSEA for the comparisons of each resistant cell line vs. the parental cell line to explore enriched pathways. The Hallmark and Reactome gene sets from the Molecular Signatures Database (MSigDB) were used. All the genes tested were annotated in HUGO gene symbols and ranked based on the significance (p-values) in combination with the sign of log2 fold change values resulting from 2-group comparisons. We used the FDR cutoff 5% to select significant gene sets for each comparison.

Single-sample GSEA (ssGSEA) was performed using the GSVA package. This analysis was performed using the log2-transformed data in transcripts per million data, which was generated using RSEM/v1.3.3 based on the FASTQ files with human reads and GENCODE human reference GRCh38. For each individual sample, an enrichment score was calculated by applying a weighted running sum to the normalized ranking scores of genes within the gene set. For generating senescence scores, gene sets were obtained from Reactome_Cellular-Senescence and from Casella, Fridman, Hernandez-Segura, and Purcell and colleagues. For p21/DREAM pathway scores, gene sets were obtained from MSigDB database v7.5.1: FISCHER_DREAM_TARGETS (DREAM-F), FISCHER_DIRECT_P53_TARGETS_META_ANALYSIS, FISCHER_G1_S_CELL_CYCLE, FISCHER_G2_M_CELL_CYCLE, SHEPARD_BMYB_TARGETS. The FOXM1 and E2F-RB target genes were obtained from Fischer et al. A second set of DREAM targets (DREAM-E) was obtained from Engeland. A set of DREAM-dependent p53-targets (DREAM-U) and a set of DREAM-independent p53 target genes were obtained from Uxa et al.

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 publicly available data used in this study are available in the Gene Expression Omnibus (GEO) under accession code GSE282705 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE282705>). The dataset is publicly available without restrictions. Any datasets requiring restricted access are available upon reasonable request by contacting Dr. Khandan Keyomarsi (Email: kkeyomar@mdanderson.org). The remaining data are available within the Article, Supplementary Information, or Source Data file.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](https://www.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

For in vitro experiments, each assay was performed at least three times, with each independent experiment including at least three biological replicates. For in vivo experiments, each condition included a minimum of five mice. Sample sizes were based on our previous work and on standard practice in the field, and were chosen to ensure reproducible effects across independent biological replicates. The number of independent experiments and/or samples (n) for each figure is indicated in the corresponding legend, and was considered sufficient to detect the reported differences given the observed effect sizes and variability. For animal experiments, group sizes were selected based on prior similar studies and ethical considerations to minimize animal use while still allowing robust detection of treatment effects.

Data exclusions	No data were excluded from the analyses. All data points collected were included in the final results, and no exclusion criteria were applied.
Replication	For in vitro experiments, each assay was performed at least three times, with each independent experiment including at least three biological replicates. In the treatment responsive curve, each independent experiment included eight technical replicates. All attempts at replication were successful, and all experimental findings were reproducible across independent biological replicates.
Randomization	For animal experiments, subjects were randomly assigned to treatment or control groups using simple randomization to minimize allocation bias. Animals were enrolled as cohorts when their tumors reached ~100 mm ³ volume and randomized into treatment arms.
Blinding	Blinding was not applicable as animals needed to be identified for each treatment condition.

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	The following primary antibodies were used in this study. Cyclin E1 (clone HE12; Santa Cruz Biotechnology, sc-247; dilution 1:1000; mouse monoclonal; reactive with human). Cyclin D1 (clone H-295; Santa Cruz Biotechnology, sc-753; dilution 1:1000; rabbit polyclonal; reactive with human). Rb (clone 4H1; Cell Signaling Technology, 9309; dilution 1:1000; mouse monoclonal; reactive with human). Phospho-Rb (Ser807/811) (Cell Signaling Technology, 9308; dilution 1:1000; rabbit polyclonal; reactive with human). CDK4 (clone C-22; Santa Cruz Biotechnology, sc-260-G; dilution 1:1000; goat polyclonal; reactive with human). CDK6 (clone C-21; Santa Cruz Biotechnology, sc-177-G; dilution 1:1000; goat polyclonal; reactive with human). CDK2 (clone D-12; Santa Cruz Biotechnology, sc-6248; dilution 1:1000; mouse monoclonal; reactive with human). Phospho-CDK2 (Thr160) (Cell Signaling Technology, 2561; dilution 1:1000; rabbit monoclonal; reactive with human). γ-H2AX (Ser139) (Millipore, 05-636; dilution 1:1000; mouse monoclonal; reactive with human). p21 (clone 12D1; Cell Signaling Technology, 2947S; dilution 1:1000; rabbit monoclonal; reactive with human). p27 (clone D69C12; Cell Signaling Technology, 3686S; dilution 1:1000; rabbit monoclonal; reactive with human). Vinculin (clone hVIN-1; Sigma-Aldrich, V9131; dilution 1:2000; mouse monoclonal; reactive with human). Actin (clone C4; Millipore, MAB1501; dilution 1:5000; mouse monoclonal; reactive with human). For immunoprecipitation, 1000 µg of protein extracted from total-cell lysates for each immunoprecipitation was used with polyclonal antibodies against CDK2, CDK4, or normal mouse immunoglobulin G (IgG) (SC-2025; Santa Cruz Biotechnology) and incubated with protein A-coated or protein G-coated beads (Calbiochem). After being washed, the immunoprecipitants were subjected to Western blot analysis with the antibodies listed above.
Validation	All primary antibodies were validated by the manufacturers and further confirmed in our laboratory by expected band size on Western blot and known specificity patterns. Validation approaches are detailed in the Methods section of the manuscript. p21 and p27 antibodies were validated for western blot by detecting loss of proteins with CRISPR knockout of target genes. CDK2, Rb, and cyclin E1 antibodies were validated for western blot by detecting loss of proteins with shRNA mediated knockdown of target genes. Vinculin and actin antibodies are standard loading control that are well established by the vendors and many published studies.

Eukaryotic cell lines

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

Cell line source(s)	All cell lines used in this study were obtained from ATCC. MCF7 and T47D palbociclib-resistant (PR) cells were generated as previously described. Similarly, the MDAMB231 PR and BT20 PR cells were generated in medium supplemented with increasing concentrations of palbociclib in a stepwise manner. All PR cells were maintained with corresponding dose of palbociclib used to develop resistance. The cells were maintained in Eagle minimum essential medium, alpha modification, supplemented with 10% fetal bovine serum, 10 mM HEPES, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1 mg/mL insulin, 25 mg/mL epidermal growth factor, and 1 mg/mL hydrocortisone. All cell lines were maintained in a humidified tissue culture incubator at 37°C and in a 5% CO₂ atmosphere and were routinely tested for
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mycoplasma infection and authenticated every 6 months by karyotype and short tandem repeat analysis at the MD Anderson Cancer Center Characterized Cell Line Core Facility. Each vial of frozen cells was thawed and maintained in culture for a maximum of 8 weeks.

Authentication

All cell lines were authenticated using STR DNA profiling.

Mycoplasma contamination

Cells were routinely found to have no mycoplasma contamination using Lonza MycoAlert PLUS Mycoplasma Detection Kit.

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

All animal experiments were conducted in compliance with institutional and national guidelines and were approved by the MD Anderson Institutional Animal Care and Use Committee. Procedures adhered to the standards specified by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). Breast tumor tissues used to establish patient-derived xenograft (PDX) models were obtained from patients during surgery, after written informed consent was obtained under protocols approved by the MD Anderson Cancer Center Institutional Review Board and in accordance with recognized ethical guidelines. Only female mice were used in this study, consistent with established breast cancer xenograft and PDX models. Sex was therefore not included as a variable in the statistical analyses. The species, strain (including substrain), sex, number, and age of animals used in each experiment are reported in the Methods file. Generally, PDX tumours revived from liquid nitrogen were transplanted into the T4 mammary fat pad of 8-week-old female NSG mice (Jackson Laboratory, strain #005557). For expansion and passaging in study animals, PDX tumour fragments (approximately 4 × 4 mm) were transplanted into the T4 mammary fat pad of 10-week-old female NCR-NU-F (athymic nude, sp/sp) mice obtained from Taconic Biosciences. The TNBC PDX models BCX017, BCX070 and XC5172013 were previously developed. The transgenic LMW-E overexpression T1p model was previously developed. The HR+ breast cancer PDX model PR4 (originally named T141-004), which progressed on palbociclib, was provided by Dr. Funda Meric-Bernstam's laboratory at MD Anderson. The generation of the 3 PDX models PR1, PR2, and PR3, originally named CTG-3277, CTG-3266, and CTG-2308, respectively, were described previously. All PDX tumor fragments (4×4 mm) were transplanted into the T4 mammary fat pad of female NCR-NU-F sp/sp nude mice from Taconic Biosciences.

Wild animals

No wild animals were used in this study.

Reporting on sex

All xenografts were completed in female nuliparous mice.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All mouse work was done in compliance with the Institutional Animal Care and Use Committee (IACUC) at the University of Texas MD Anderson Cancer Center.

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

Cell cycle analysis: Cell cycle analysis was performed as previously described. Cells were plated at 3-5×10⁵ cells on 10-cm petri dish and grown in medium containing 0.3 uM BLU-222 and/or 0.3 uM of palbociclib for 3 days. The medium was replaced every other day with or without drug. At the end of the treatment, cells were trypsinized, washed with 1x phosphate-buffered saline (PBS), and fixed with 3.5 mL of ice-cold 1x PBS and 1.5 mL of 95% ethanol at 4°C overnight. Cells were then stained with propidium iodide (1mg/mL) and RNaseA (1mg/mL) in 1x PBS without Ca/Mg containing 0.5% (v/v) Tween 20 (Sigma-Aldrich) and 0.5% bovine serum albumin at 4°C overnight protected from light. Samples were read using an Attune Flow Cytometer (Thermo Fisher Scientific), and data were analyzed with FlowJo v10.10.0 software. Each condition included 2 technical replicates and was repeated in at least 3 independent experiments.

Apoptosis analysis: Apoptosis analysis was performed as previously described (29,31). Cells were plated at 3-5×10⁵ cells on 10-cm petri dish and grown in medium containing 0.3 uM BLU-222 and/or 0.3 uM of palbociclib for 3 days. At the end of the treatment, cells were trypsinized, centrifuged and washed with 1x PBS. The apoptotic cells were labeled with Alexa Fluor 488 Annexin V according to the manufacturer's protocol (Invitrogen, #V13241).

Instrument

Samples were read using an Attune Flow Cytometer (Thermo Fisher Scientific).

Software

Data were analyzed with FlowJo v10.10.0 software

Cell population abundance

There is no sorting data.

Gating strategy

For cell cycle analysis, gating begins with forward scatter (FSC) vs. side scatter (SSC) to identify and exclude debris, ensuring only intact live cells are analyzed. Next, doublets and aggregates are removed by gating on FSC-H vs. FSC-A (or FSC-W) and SSC-H vs. SSC-A to isolate single cells. DNA content is analyzed using a DNA-binding dye such as propidium iodide (PI) and plotted on a histogram. Cells are separated into G0/G1 phase (2N DNA content, lowest fluorescence), S phase (intermediate fluorescence due to DNA synthesis), and G2/M phase (4N DNA content, highest fluorescence). Gates are drawn to quantify the percentage of cells in each phase of the cell cycle.

For apoptosis analysis, the gating strategy starts similarly with FSC vs. SSC to identify the live cell population while excluding debris. A viability stain (PI) is used to distinguish live cells (viability dye-negative) from dead cells (viability dye-positive). Apoptotic cells are identified using Annexin V staining, which detects phosphatidylserine externalization on the cell surface. Cells are plotted on a bivariate dot plot of Annexin V vs. the viability dye to differentiate between live cells (Annexin V-negative, viability dye-negative), early apoptotic cells (Annexin V-positive, viability dye-negative), late apoptotic/necrotic cells (Annexin V-positive, viability dye-positive), and necrotic cells (Annexin V-negative, viability dye-positive).

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