nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Mutation and CNV calling from the WES was performed using MuTect and ABSOLUTE algorithm. RNAseq, miRNA and proteomics were analysed using DESeq2 algorithm and GSEA sofware. Aneuploidy scores for DepMap analysis were obtained using our extension code (https://github.com/BenDavidLab/Ploidy_And_AS_Zerbib-et-al_2024).
Data analysis	For clinical datasets, RNA-based inference of gene level CNV was performed using CNVkit and CAFE algorithms, for RNAseq and microarray data. Aneuploidy scores were determined by counting the number of chromosome arms that deviate from the basal ploidy inferred from gene level CNV using ASCETS. Statistics were performed using GraphPad PRISM 9.1

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

Low-pass whole-genome sequencing, Whole Exome Sequencing and raw RNAseq data are available in the SRA database (https://www.ncbi.nlm.nih.gov/sra) under accession numbers PRJNA672256 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA672256), PRJNA1144469 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1144469) and PRJNA889550, (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA889550) respectively.
Genome-wide CRISPR/Cas9 screening data of RPE1-hTERT clones are available in the DepMap database 21Q3 release (https://figshare.com/articles/dataset/DepMap_21Q3_Public/15160110).
miRNA expression raw data are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE247267 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247267).
Protein expression raw data are available in the PRIDE database under the accession number PXD048833 (http://central.proteomexchange.org/cgi/GetDataset?ID=PXD048833).
Drug screening data are available in the Drug Repurposing Hub (https://repo-hub.broadinstitute.org/repurposing#home).
Cancer cell line expression, CRISPR/Cas9 and RNAi data are available in the DepMap database 22Q1 release (https://figshare.com/articles/dataset/DepMap_22Q1_Public/19139906). All of them are publicly available as of the date of publication.

All output tables are available as Supplementary Tables within the Source Data files (Supp. Data 2-3, 6-9).

All previously published clinical datasets are available as following: Pediatric PDXs (EGAS00001002528, https://doi.org/10.1038/nature23647), PDAC PDXs (GSE235843, https://doi.org/10.1158/2159-8290.CD-22-0412), Breast tumors (GSE173839, https://doi.org/10.1016/j.ccell.2021.05.009).

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	No sample size calculation was pre-determined. A minimum of 3 biological replicates 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	No data were excluded from the analyses.
Replication	All experiments were conducted within 10 passages following thawing to ensure reproducibility of the results.
Randomization	Experimental groups were defiined according to the aneuploidy status of the studied cell lines: isogenic RPE1-hTERT model based on their published karyotype, comparison of top (high AS) and bottom (low AS) quartiles for DepMap analyses.
Blinding	NA

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
	[x] Antibodies
	[x] Eukaryotic cell lines
[x]	Palaeontology and archaeology
[x]	Animals and other organisms
[x]	Clinical data
[x]	Dual use research of concern
[x]	Plants

Methods

n/a	Involved in the study
[x]	ChIP-seq
	[x] Flow cytometry
[x]	MRI-based neuroimaging

Antibodies

Antibodies used

anti-Phospho-c-Raf (Ser338) (56A6), Cell Signaling Technology (1:1000, Cat#9427 RRID:AB_2067317)
anti-c-Raf, BD Biotechnologies (1:1000 Cat#610152 RRID:AB_397553) or Cell Signaling Technology (1:1000 Cat#9422 RRID:AB_390808)
anti-Phospho-MEK1/2 (Ser217/221), Cell Signaling Technology (1:1000 Cat#9121 RRID:AB_331648)
anti- MEK1/2, Cell Signaling Technology (1:1000 Cat#9122 RRID:AB_823567)
anti-Phospho-ERK1/2 (Thr202/Tyr204) XP®, Cell Signaling Technology (1:1000 Cat#4370 RRID:AB_2315112)
anti- ERK1/2, Santa-Cruz Biotechnologies (1:1000 Cat#sc-514302 RRID:AB_2571739)
anti-Phospho-Histone H2A.X (Ser139), Cell Signaling Technology (1:1000 Cat#9718 RRID:AB_2118009) or Millipore (1:1000 Cat#05-636 RRID:AB_309864)
anti-53BP1, Abcam (1:1000 Cat#ab175933 RRID:AB_2890610)
anti-p53, Cell Signaling Technology (1:1000 Cat#9282 RRID:AB_331476) or Santa-Cruz (1:1000 Cat#sc-393031 RRID:AB_3083496)
anti-p21, Cell Signaling Technology (1:1000 Cat#2947 RRID :AB_823586)
anti-GAPDH, Cell Signaling Technology (1:1000 Cat#2118 RRID:AB_561053)
anti-Vinculin, Sigma-Aldrich (1:2000 Cat#V9131 RRID:AB_477629)
anti-Tubulin, Sigma-Aldrich (1:2000 Cat#T9026 RRID:AB_477593)
HRP-conjugated Goat anti-mouse IgG, Jackson ImmunoResearch Labs (1:10000 Cat#115-035-003 RRID:AB_10015289)
HRP-conjugated Goat anti-rabbit, IgG Jackson ImmunoResearch Labs (1:10000 Cat#111-035-003 RRID:AB_2313567)
Alexa Fluor® 488 conjugated IgG, Cell Signaling Technology (1:1000 Cat#4408 RRID:AB_10694704) or Jackson ImmunoResearch Labs (1:400 Cat#711-545-152 RRID:AB_2313584)
Alexa Fluor® 555 conjugated IgG, Cell Signaling Technology (1:1000 Cat#4409 RRID:AB_1904022)
Alexa-Cy3 conjugated IgG, Jackson ImmunoResearch Labs (1:400 Cat#715-165-150 RRID:AB_2340813)

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)

hTERT RPE-1 cells (ATCC) and derivative isogenic aneuploid SS clones (generated by Dr Stefano Santaguida) and isogenic RPTs (RPE1 Post Tetraploid, generated by Dr Zuzana Storchova), IMR90 (ATCC), hTERT-BJ (N/A), SW48 (ATCC), CAL51 (DSMZ)

Authentication

None of the cell lines was authenticated by the authors.

Mycoplasma contamination

All 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.

Plants

Seed stocks: NA

Novel plant genotypes: NA

Authentication: NA

Flow Cytometry

Plots

Confirm that:

- [x] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- [x] 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).
- [x] All plots are contour plots with outliers or pseudocolor plots.
- [x] A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation:

For cell cycle analysis, 70% confluent RPE1-hTERT clones were collected and fixed with ice-cold 70% ethanol for 2hrs on ice. Ethanol was then washed and cells were stained with 50µg/mL Propidium Iodine (BioLegend) and 0.1mg/mL RNAse A (Invitrogen) in PBS for 10min RT. Flow cytometry acquisition was performed on CytoFLEX® (Beckman Coulter) and data analysis was performed using CytExpert v2.4 analysis software (Beckman Coulter). Gating of living cells and singlet was common in all the analyzed samples, gating of cell cycle phase was specific to each sample.

For cell death analysis, 100,000 cells were seeded in a 6-well plate and treated for 72hr with 10µM of 8-Br-cAMP, and with Etoposide 2.5µM for 72hrs as a positive control. Cells were stained with SYTOX™ Green Ready Flow™ Reagent (Invitrogen), following the manufacturer's protocol. Flow cytometry acquisition was performed using CytoFLEX® (Beckman Coulter) and data analysis was performed using Kaluza Analysis software 2.1 (Beckman Coulter). The gating of living cells and singlets was common in all the analyzed samples, per experiment. Gating of positive cells (defined as upper half of the pick in etoposide-treated cells) was defined per cell line.

Instrument: CytoFLEX (Beckman Coulter)

Software: CytEXPERT v2.4 (Beckman Coulter)

Cell population abundance: NA

Gating strategy: For PI and cell death stainings, the gating strategy consisted only of gating for singlets. Cells were gated for singlets and alive cells and then FSC-A, SSC-A, and PI (filter B-585) or FITC. CytEXPERT was used to perform data analysis and to generate the plots.

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