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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	Flow cytometry data was collected by Beckman coulter MoFL XDP, Cytek NL-CLC3000 and Attune NxT v2.6. RT-qPCR was collected by Bio-Rad CFX Manager 3.1. IF images were collected by Nikon Eclipse 80i fluorescence microscope. BLI was measured by Perkin Elmer IVIS Lumina Series III.
Data analysis	GraphPad Prism10, flowjo V10, ImageJ 1.8.0, Cell Ranger (v9.0.1)

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

The human pancreatic ductal adenocarcinoma genomic and survival data were derived from the TCGA Research Network: http://cancergenome.nih.gov/. The RNA-

seq and scRNA-seq datasets generated in this study have been deposited in the Gene Expression Omnibus (GEO),under the accession number GSE 279749 and GSE 309596, respectively. The raw sequence data of WGS dataset for HRD score have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA013700) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. All the data are available from the corresponding authors upon reasonable request. Source data for figures are provided with this paper.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	Sample sizes were chosen based on previous manuscripts, which were sufficient for statistical analysis (Zhu et al., Nat Commun. 2021 Nov 17;12(1):6653;Zeng et al., Nat Cancer. 2022 Sep;3(9):1088-1104.) .
Data exclusions	No samples were excluded from analysis.
Replication	All experiments results were reproduced. The replication numbers were described in the corresponding figure legends.
Randomization	All samples such as cells or animals were randomly allocated into experimental groups.
Blinding	To establish baseline observations in tumor growth experiments, initial measurements were performed by a blinded investigator. For data collection and analysis of immunohistochemistry, no blinding was performed due to machine-based readouts.

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-γH2AX (Millipore, 05-636, WB-1:1000, IF-1:1000, IHC-1:200, mouse)
Anti-MDC1 (Millipore, 05-1572, IF-1:1000, Rabbit)
Anti-BRCA1 (Santa Cruz, sc-6954, WB-1:1000, IF-1:200, mouse)
Anti-FK2 (Sigma-Aldrich, 04-263, IF-1:1000, mouse)
Anti-RAD51 (GENEtex, GTX100469, WB-1:1000, IF-1:500, IHC-1:300, rabbit)
Anti-RPA32 (Santa Cruz, sc56770, WB-1:1000, IF-1:500, mouse)
Anti- PARP (Cell Signaling Technology, 9542, WB-1:1000, rabbit)
Anti- Phospho-Chk2 (Cell Signaling Technology, 2661, WB-1:1000, rabbit)
Anti- Cleaved Caspase-3 (Cell Signaling Technology, 9661, WB-1:1000, IHC-1:1000, rabbit)
Anti- Phospho-Akt(S473) (Cell Signaling Technology, 4060, WB-1:1000, rabbit)
Anti-Phospho-Akt(T308)(Cell Signaling Technology, 9275, WB-1:1000, rabbit)
Anti- Phospho-Erk1/2 (Cell Signaling Technology, 4370, WB-1:1000, rabbit)
Anti- Akt (proteintech, 10176-2-AP, WB-1:1000, rabbit)
Anti- Erk1/2 (proteintech, 51068-1-AP, WB-1:1000, rabbit)
Anti-RAS G12D(Cell Signaling Technology, 14229, WB-1:1000, rabbit)
Anti-KRAS (F234)(Santa Cruz, sc-30, WB-1:1000, mouse)
Anti- CD8 (abcam, ab217344, IHC-1:1000, rabbit)
Anti- Ki67 (abcam, ab15580, IHC-1:1000, rabbit)
Anti-GAPDH (proteintech, 60004-1-Ig, WB-1:1000, rabbit)
Anti-β-tubulin(proteintech, 10094-1-AP, WB-1:1000,rabbit)
Anti-β-actin(proteintech, 66009-1-Ig, WB-1:1000,rabbit)
Anti-mouse CD16/32 Fc block (clone 93, BioLegend, FCS-1:1000)
BD Horizon® Fixable Viability Stain 510(BD Biosciences, 564406, FCS-1:1000)
APC/Cyanine7 anti-mouse CD3ε(BioLegend, 100330, FCS-1:100)
Alexa Fluor® 700 anti-mouse CD45(BioLegend, 103128, FCS-1:100)
PE/Dazzle™ 594 anti-mouse CD4(BioLegend, 100456, FCS-1:100)
PerCP/Cyanine5.5 anti-mouse CD8α(BioLegend, 100734, FCS-1:100)
Brilliant Violet 711m anti-mouse TNF-α(BioLegend, 506349, FCS-1:100)
Brilliant Violet 785 anti-mouse CD279(PD-1)(BioLegend, 135225, FCS-1:100)
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L, 715-585-150) and Alexa Fluor® 594 AffiniPure Donkey Anti-Mouse IgG (H+L, 715-585-150) were purchased from Jackson ImmunoResearch.

Validation

Anti-γH2AX (Millipore, 05-636, mouse). Reacts with: Vertebrates. Suitable for: ICC, IF, WB, ChIP, IHC. Citation from manufacturer is listed at https://www.emdmillipore.com/US/en/product/Anti-phospho-Histone-H2A.X-Ser139-Antibody-clone-JBW301,MM_NF-05-636#relations.
Anti-MDC1 (Millipore, 05-1572, Rabbit). Reacts with: Mouse, Human, Chimpanzee and Bovine. Suitable for: ICC, WB. Citation from manufacturer is listed at https://www.emdmillipore.com/US/en/product/Anti-MDC1-Antibody-clone-P2B11,MM_NF-05-1572?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1.
Anti-BRCA1 (Santa Cruz, sc-6954, mouse). Reacts with: Mouse, Human, Rat, etc. Suitable for: ICC, WB. Citation from manufacturer is listed at https://www.scbt.com/p/brca1-antibody-d-9.
Anti-FK2 (Sigma-Aldrich, 04-263, mouse). Reacts with: All. Suitable for: ELISA, IF, IP, WB. Citation from manufacturer is listed at https://www.emdmillipore.com/US/en/product/Anti-Ubiquitinylated-proteins-Antibody-clone-FK2,MM_NF-04-263.
Anti-RAD51 (GENEtex, GTX100469, rabbit). Reacts with: Human, Mouse, Rat, Zebrafish. Suitable for: WB, ICC/IF, IHC-P, IP. Citation from manufacturer is listed at https://www.genetex.com/Product/Detail/Rad51-antibody-N1C2/GTX100469#datasheet.
Anti-RPA32 (Santa Cruz, sc56770, mouse). Reacts with: mouse, rat and human. Suitable for: WB, IHC, IF. Citation from manufacturer is listed at https://www.scbt.com/p/rpa-32-kda-subunit-antibody-9h8.
Anti- PARP (Cell Signaling Technology, 9542, rabbit). Reacts with: Human, Mouse, Rat, monkey. Suitable for: WB, simple WesternTM. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/parp-antibody/9542.
Anti- Phospho-Chk2 (Cell Signaling Technology, 2661, rabbit). Reacts with: Human, Monkey. Suitable for: WB, simple WesternTM. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/phospho-chk2-thr68-antibody/2661.
Anti- Cleaved Caspase-3 (Cell Signaling Technology, 9661, rabbit). Reacts with: Human, Mouse, Rat, monkey. Suitable for: WB, simple WesternTM, IP, IHC, IF, F. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661.

Anti- Phospho-Akt (Cell Signaling Technology, 4060, rabbit). Reacts with: Human, Mouse, Rat, Hamster, Monkey, D. melanogaster, Zebrafish, Bovine. Suitable for: WB, IP, IHC, IF, F. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060.

Anti-Phospho-Akt (Cell Signaling Technology, 9275, rabbit). Reacts with: Human, Mouse, Rat. Suitable for: WB, IP. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/phospho-akt-thr308-antibody/9275.

Anti- Phospho-Erk1/2 (Cell Signaling Technology, 4370, rabbit). Reacts with: Human, Mouse, Rat, Hamster, Monkey, Mink, D. melanogaster, Zebrafish, Bovine, Dog, Pig, S. cerevisiae. Suitable for: WB, IP, IHC, IF, F. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370.

Anti- Akt (proteintech, 10176-2-AP, rabbit). Reacts with: human, mouse, rat, chicken, zebrafish, hamster, sheep, goat, fish, zebra finches. Suitable for: WB, IHC, IF-P, IP, CoIP, ELISA. Citation from manufacturer is listed at https://www.ptglab.com/products/AKT-Antibody-10176-2-AP.htm#product-information.

Anti- Erk1/2 (proteintech, 51068-1-AP, rabbit). Reacts with: human, mouse, rat. Suitable for: WB, IHC, IF/ICC, ELISA. Citation from manufacturer is listed athttps://www.ptglab.com/products/ERK2-Antibody-51068-1-AP.htm.

Anti-RAS G12D(Cell Signaling Technology, 14429, rabbit). Reacts with: Human, Mouse, Rat. Suitable for: WB. Citation from manufacturer is listed at https://www.cellsignal.com/products/primary-antibodies/ras-g12d-mutant-specific-d8h7-rabbit-mab/14429.

Anti-KRAS(F234)(Santa Cruz, sc-30, WB-1:500, mouse).Reacts with: Human, Mouse, Rat. Suitable for: WB, immunoprecipitation and immunofluorescence. Citation from manufacturer is listed at https://www.scbt.com/p/k-ras-antibody-f234.

Anti- CD8 (abcam, ab217344, rabbit). Reacts with: Mouse. Suitable for: IP, Flow Cyt, WB, IHC. Citation from manufacturer is listed at https://www.abcam.com/en-us/products/primary-antibodies/cd8-alpha-antibody-epr21769-ab217344.

Anti- Ki67 (abcam, ab15580, rabbit). Reacts with: Human, Mouse. Suitable for: ICC, IHC. Citation from manufacturer is listed at https://www.abcam.com/en-us/products/primary-antibodies/ki67-antibody-ab15580.

Anti-GAPDH (proteintech, 60004-1-Ig, rabbit). Reacts with: human, mouse, rat, pig, zebrafish, yeast, plant. Suitable for: WB, IHC, IF/ICC, FC (Intra), IP, CoIP, ELISA. Citation from manufacturer is listed at https://www.ptglab.com/products/GAPDH-Antibody-60004-1-Ig.htm.

Anti-β-tubulin(proteintech, 10094-1-AP, rabbit).Reacts with: human, mouse, rat, rabbit, chicken, zebrafish, hamster, sheep, goat, fish. Suitable for: WB, IHC, IF, IP, CoIP. Citation from manufacturer is listed at https://www.ptgcn.com/products/TUBB-Antibody-10094-1-AP.htm.

Anti-β-actin(proteintech, 66009-1-Ig, rabbit).Reacts with: human, mouse, rat, pig, rabbit, canine, monkey, chicken, zebrafish, hamster. Suitable for: WB, IHC, IF, IP, CoIP. Citation from manufacturer is listed at https://www.ptgcn.com/products/Pan-Actin-Antibody-66009-1-Ig.htm.

Anti-mouse CD16/32 Fc block (clone 93, BioLegend, FCS-1:1000). Citation from manufacturer is listed at https://www.biolegend.com/en-gb/products/trustain-fcx-anti-mouse-cd16-32-antibody-5683?GroupID=BLG9237

BD Horizon® Fixable Viability Stain 510(BD Biosciences, 564406, FCS-1:1000). Citation from manufacturer is listed at https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fixable-viability-stain-510.564406?tab=product_details#:~:text=BD%20Horizon%E2%84%A2%20Fixable%20Viability%20Stain%20510%20%28FVS510%29%20is,non-viable%20mammalian%20cells%20in%20multicolor%20flow%20cytometric%20applications.

APC/Cyanine7 anti-mouse CD3ε(BioLegend, 100330, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/en-gb/products/apc-cyanine7-anti-mouse-cd3epsilon-antibody-6070?GroupID=BLG6746.

Alexa Fluor® 700 anti-mouse CD45(BioLegend, 103128, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/nl-be/products/alexa-fluor-700-anti-mouse-cd45-antibody-3407?GroupID=BLG6833.

PE/Dazzle™ 594 anti-mouse CD4(BioLegend, 100456, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/de-at/products/alexa-fluor-594-anti-mouse-cd4-antibody-9412?GroupID=BLG4745.

PerCP/Cyanine5.5 anti-mouse CD8α(BioLegend, 100734, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/de-at/products/percp-cyanine5-5-anti-mouse-cd8a-antibody-4255.

Brilliant Violet 711m anti-mouse TNF-α(BioLegend, 506349, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/de-at/products/brilliant-violet-711-anti-mouse-tnf-alpha-antibody-13622.

Brilliant Violet 785 anti-mouse CD279(PD-1)(BioLegend, 135225, FCS-1:100). Citation from manufacturer is listed at https://www.biolegend.com/de-at/products/brilliant-violet-785-anti-mouse-cd279-pd-1-antibody-9874.

The commercial secondary antibodies were validated by manufactures.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	AsPC-1 (Cell Resource Center, Peking Union Medical College) BxPC-3 (Cell Resource Center, Peking Union Medical College) CFPAC-1 (Cell Resource Center, Peking Union Medical College) Mia PaCa-2 (Cell Resource Center, Peking Union Medical College) PANC-1 (Cell Resource Center, Peking Union Medical College) SW1990 (Cell Resource Center, Peking Union Medical College) BEAS-2B(Cell Resource Center, Peking Union Medical College) hTERT-HPNE(Cell Resource Center, Peking Union Medical College) KPC1199 and KPC1242 were kindly provided by Dr. Mingyang Liu from Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China)
Authentication	The Cell lines have been authenticated based on morphological criteria.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	None.

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	Balb/c-nude and C57BL/6j mice were purchased from Beijing Huafukang Biological Technology Co. Ltd. and used for indicated tumor cells implantation. All in vivo experiments were performed in according to the guidelines established by the Institutional Animal Care and Use Committee of the Cancer Hospital, Chinese Academy of Medical Sciences. Experiments were conducted using only 6-8 weeks old female mice. Mice were housed in a controlled environment with a 12-hour light and dark cycle, a temperature range of 20–25°C, and humidity maintained between 30–70%.
Wild animals	No wild animals were used in this study.
Reporting on sex	Experiments were conducted using only 6-8 weeks old female mice. The study did not include sex-based comparisons, as the focus was not on sex-specific effects. To avoid additional variables from mixed sex populations, all female mice were used in this study.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	All experimental procedures were approved by the Animal Ethics Committee of the Tumor Hospital of the Chinese Academy of Medical Sciences (NCC2024A149) and conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

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	1. For HR efficiency analysis, cells were transfected with m-cherry/ GFP expression plasmid, trypsinized and suspended in PBS. Further details are provided in the materials and methods. 2.For preparation of single-cell suspensions,all fresh tumor tissues were cut into small piece by a scissor. The tumors were mechanically dissociated by being pushed through a 70-μm cell strainer placed in a 25-mm dish using the plunger of a 5 ml syringe. The cell suspension was then collected and centrifuged at 500 g for 10 minutes at room temperature. The cell pellet was resuspended with the ACK Lysis buffer for 5 minutes at room temperature. Single-cell suspensions were stained with indicated antibodies for 30 min at 4°C in the dark. The samples were detected by flow cytometry.
Instrument	Beckman coulter MoFL XDP, Cytek NL-CLC3000 and Attune NxT v2.6.
Software	FlowJo-V10.8.1 software.
Cell population abundance	Cell sorting was not performed in this study.
Gating strategy	1. For HR efficiency analysis, after FCS/SSC gating, GFP and m-cherry were gated for GFP-positive cells and RFP/GFP double-positive cells. 2.Cells were first gated by FSC-A and FSC-H to eliminate non-singlet cells and gated by FSC-A/comp-FVS510-A to eliminate dead cells. TNF-α and PD-1 expression were detected in the populations of CD45+CD3+CD8+ cells. Gates were set based on

no stain control, isotype control, and density distributions based on best practices.

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