

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 P 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	QuantStudio Design & Analysis Software 2.8.0 (ThermoFisher Scientific, RT-PCR/ChIP-PCR), Gen5 3.03 software (BioTek, AlamarBlue assays), FCS Express 7 flow cytometry software (DeNovo Software, flow cytometry), inForm® Software v2.6.0 (AKOYA Biosciences, multispectral immunofluorescence), Phenoptr software (AKOYA Biosciences, multispectral immunofluorescence), Image Studio 5.2 (LICORbio, western blots)
Data analysis	QuantStudio Design & Analysis Software 2.8.0 (ThermoFisher Scientific, RT-PCR/ChIP-PCR), Gen5 3.03 software (BioTek, AlamarBlue assays), FCS Express 7 flow cytometry software (DeNovo Software, flow cytometry), inForm® Software v2.6.0 (AKOYA Biosciences, multispectral immunofluorescence), Phenoptr software (AKOYA Biosciences, multispectral immunofluorescence), Image Studio 5.2 (LICORbio, western blots), DESeq2 (RNA-seq), fgsea (v1.8.0) package in R (GSEA), Excel (Microsoft), and GraphPad Prism 10 (statistics).

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 datasets generated and/or analyzed during the current study are available in the GEO repository (GSE253260; <https://dx.doi.org/10.1016/j.ebiom.2024.105373>) or are available from the corresponding author on reasonable request.

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	This information was not collected.
Reporting on race, ethnicity, or other socially relevant groupings	This information was not collected.
Population characteristics	This information was not collected.
Recruitment	This information was not collected.
Ethics oversight	TMA cores were obtained from PDAC patients who had been treated at Thomas Jefferson University Hospitals between the years 2002 and 2010 Institutional Review Board approval.

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	Mouse numbers were determined using a priori power analysis using a 95% confidence interval based on previous published experiments (PMID: 40731412, PMID: 38294344, PMID: 30074477, PMID: 31048544, PMID: 24647545, and PMID: 23543055).
Data exclusions	No data were excluded from analysis.
Replication	All in vitro studies were done in experimental triplicates. All replications were successful and the data represents the means and standard errors of these replicates.
Randomization	Mice were randomly assigned (by cage) to their respective cohorts, although all cohorts contained equal numbers of males and females.
Blinding	Staining and data collection/analysis of multispectral immunofluorescence was done in a blinded manner by individuals with no data regarding the tissue microarray cores. In vitro and in vivo studies were not performed in a blinded manner as they are set up and analyzed by the individuals collecting the data.

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

Mouse anti-human EPCAM (CD326) clone HEA-125 (RRID: AB_2751108, flow cytometry) was purchased from Miltenyi Biotec (San Diego, CA). Mouse anti-human CD44 clone G44-26 and CD24 clone ML5 purchased from BD Biosciences (San Jose, CA, flow cytometry). For multispectral immunofluorescent: HNF1A (GT4110, 1:150, Opal 620; RRID: AB_2538735) from ThermoFisher, BRD4 (E2A7X, 1:1000, Opal 780; RRID: AB_2687578), ERBB3 (D22C5, 1:75, Opal 570; RRID: AB_2721919), and phospho-ERBB3 Y1289 (D1B5, 1:200, Opal 690; RRID: AB_11178795) from Cell Signaling, FGFR4 (sc-136988, 1:75, Opal 520; RRID: AB_2103663) from Santa Cruz Biotechnology, and Cytokeratin AE1/AE3 (M3515, 1:150, Opal 480; RRID: AB_2132885) from Agilent Dako were used for staining. For western blots: β -Actin (AC-15; RRID: AB_10950489) antibody was purchased from Thermo Fisher Scientific. HNF1A (D7Z2Q; RRID: AB_2728751), HNF4A (C11F12; AB_2295208), HNF4G (E4V2B), MYC (D3N8F; RRID: AB_2631168), BRD2 (D89B4; RRID: AB_10835146), BRD4 (E1Y1P), phospho-RB (S807/811) (D20B12; RRID: AB_111786578), phospho-Histone H3 S10 (D7N8E; RRID: AB_2799431), and GFP (D5.1; RRID: AB_1196615) were purchased from Cell Signaling. BRD3 (2088C3a; RRID: AB_868478) antibody was purchased from Abcam (Cambridge, MA). ZsGreen1 (RRID: AB_2622267) was purchased from Origene. Ki-67 (ab15580, Abcam, 1:2500, RRID: AB_443209) was used for IHC.

Validation

RRIDs are provided for the above antibodies. Additionally, most antibodies were previously validated in the following studies: PMID: 40731412, PMID: 38294344, PMID: 30074477, PMID: 31048544, PMID: 24647545, and PMID: 23543055. Antibodies in these studies were validated by knockdown/overexpression of target proteins and/or use of relevant inhibitors (e.g., CDK4/6 or EGFR/ERBB inhibitors).

Eukaryotic cell lines

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

Cell line source(s)

AsPC-1 (ATCC), HPAF-II (ATCC), UM5 (patient derived xenograft line, University of Michigan), UM15 (patient derived xenograft line, University of Michigan), UM53 (patient derived xenograft line, University of Michigan).

Authentication

Authenticated by STR profiling (Roswell Park Comprehensive Cancer Center Genomics Shared Resource).

Mycoplasma contamination

All cells tested negative for mycoplasma contamination.

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

NOD/SCID/IL2 γ R-/- (NSG) mice.

Wild animals

No wild animals were used.

Reporting on sex

Sex-based data was not collected. Equal numbers of male and female mice were used.

Field-collected samples

Field-collected samples were not used.

Ethics oversight

All animal protocols were approved by The Institute Animal Care and Use Committee (IACUC) at Roswell Park Comprehensive Cancer Center. The animal welfare assurance number for this study is A3143-01.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks No plants were used.

Novel plant genotypes No plants were used.

Authentication No plants were used.

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 analysis of PCSC markers, cells were trypsinized, washed with HBSS/2% FBS twice, and resuspended in HBSS/2% FBS at a concentration of 1 million cells/100 μ L. Primary antibodies were diluted 1:40 in cell suspensions and incubated for 30 minutes on ice with vortexing every 5 minutes. Cells were washed twice with HBSS/2% FBS and resuspended in this solution. For PI staining, cells were trypsinized, washed in PBS and fixed in ice-cold 70% ethanol by drop-wise addition to cell suspensions and fixed at 4°C for 30 mins. Cells were then washed twice with PBS following removal of ethanol and RNA digestion was performed using 200 μ g/mL RNAase for 30 mins at 37°C. Cells were then stained with 167 μ g/mL propidium iodide for 30 mins.

Instrument

LSRII B analyzer (BD Biosciences, San Jose, CA; RRID: SCR_002159)

Software

FCS Express 7 flow cytometry software (DeNovo Software, Pasadena, CA; RRID: SCR_016431).

Cell population abundance

Cell sorting was not performed, only analysis.

Gating strategy

Side scatter and forward scatter profiles were used to eliminate cell doublets.

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