

Reporting Summary

Nature Research 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 Research policies, see [Authors & Referees](#) 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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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 data and normalised RSEM read count tables for pancreatic adenocarcinoma (PAAD) were downloaded from the Broad firehose data repository (<https://gdac.broadinstitute.org/>). The R-packages survival and survminer were used to create the Kaplan-Meier survival plots.

Data analysis

Particle Mapper plugin (<https://bitbucket.org/djpbarray/particletracker/wiki/Particle%20Mapper>), batch colocalisation plugin (https://github.com/djpbarray/CALMScripts/blob/master/CALM_Scripts/Batch_Coloc.ijm), Fiji/ImageJ 1.4 and 1.5, FlowJo 9 or above, MaxQuant v1.3.05, Perseus v1.4.0.2, MANIC v1.0, TraceFinder 4.1 EFS, Xcalibur 4.0.27.19, GraphPad Prism 7 or above, Metaboanalyst 4.0, mbrole 2.0

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Microarray data supporting the findings of this study have been deposited in Gene Expression Omnibus with the accession code GSE121864 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121864>). All other relevant data are available from the corresponding authors.

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	No statistical tests were used to predetermine sample sizes. In the case of animal experiments we struck a balance between reducing the number of animals and using enough animals/primary organoid samples to subject data to two-sided statistical tests, with enough power to draw conclusions based on a standard α cut-off of 0.05.
Data exclusions	No data were excluded.
Replication	All experiments were reproduced at least twice, and number of repeats are indicated in the text and/or figure legends.
Randomization	Animals were not randomised since experiments were dependent on a mouse's given genotype rather than a treatment.
Blinding	The certified pathologist was blinded to the genotype of the mice analysed.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Monoclonal rat anti-mouse CD9 (KMC8), non-conjugated, Santa Cruz sc-18869, 1:500 (FACS)
 Monoclonal rat IgG2a isotype control, Santa Cruz sc-3883, 1:500 (FACS)
 Monoclonal rat anti-mouse CD9 (KMC8), directly conjugated to APC, eBioscience 17-0091-82, 1:500 (FACS)
 Monoclonal rat IgG2a isotype control, directly conjugated to APC, eBioscience 17-4321-81, 1:500 (FACS)
 Polyclonal goat anti-GFP, Abcam ab6673, 1:300 (IF), 1:1000 (WB)
 Monoclonal mouse anti-cMyc (9E10), Santa Cruz sc-40, 1:1000 (WB)
 Polyclonal rabbit anti-mouse ASCT2 (H-52), Santa Cruz sc-99002, 1:1000 (WB), 1:100 (IF), 1 μ g per IP
 Monoclonal rabbit IgG isotype control (EPR25A), Abcam ab172730, 1 μ g per IP
 Monoclonal mouse anti- β -actin (AC-15), directly conjugated to HRP, Abcam ab49900, 1:10,000 (WB)
 Monoclonal rat anti-mouse cytokeratin 19 (Troma III), DSHB AB_2133570, 1:300 (IF/IHC)
 Monoclonal rat anti-mouse CD147/Basigin (RL73), eBioscience 16-1471-82, 1:500 (FACS)
 Monoclonal rat anti-mouse CD98 (RL388), directly conjugated to AF647, BioLegend 128210 AB_2254922, 1:500 (FACS)
 Monoclonal rat anti-mouse EpCAM (G8.8), directly conjugated to APC, eBioscience 17-5791-80, 1:500 (FACS)
 Polyclonal goat anti-rat IgG, directly conjugated to AF647, Invitrogen A21247, 1:500 (FACS)
 Polyclonal rabbit anti-mouse phosphorylated histone H3, Santa Cruz sc-8656-R, 1:100 (IHC)
 Monoclonal rat anti-CD44 (A020), Merck MAB2137, 1:100 (IHC)
 Monoclonal rat anti-mouse CD44 (IM7), directly conjugated to APC, BD Biosciences 559250, 1:500 (FACS)
 Monoclonal rat IgG2b isotype control (A95-1), directly conjugated to APC, BD Biosciences 553991, 1:500 (FACS)
 Polyclonal rabbit anti-CD133, Abcam ab19898, 1:100 (IHC)
 Monoclonal rabbit anti-mouse E-Cadherin (24E10), Cell Signaling Technology #3195, 1:100 (IF)
 Monoclonal mouse anti-vimentin (RV202), Abcam ab8978, 1:100 (IF)
 Monoclonal mouse anti-human CD9 (Alb6), non-conjugated, Santa Cruz sc-59140, 1:100 (IF)
 Monoclonal mouse anti-human CD9 (P1/33/2), non-conjugated, Santa Cruz sc-20048, 1:500 (FACS)
 Monoclonal rabbit anti-human CD9 (EPR2949), non-conjugated, Sigma ab92726, 1:500 (WB)

Polyclonal donkey anti-mouse IgG, directly conjugated to AF488, Invitrogen A32766, 1:100 (IF)
 Monoclonal rat anti-mouse Ki67 (16A8), directly conjugated to AF647, BioLegend AB_2562138, 1:100 (FACS)
 Polyclonal goat anti-tdTomato, biorbyt orb182397, 1:100 (IF)
 Monoclonal rat anti-mouse CD44 (IM7), directly conjugated to eFluor 450, eBioscience AB_1272246, 1:100 (FACS)
 Monoclonal mouse anti-human EpCAM (1B7), directly conjugated to PE, Invitrogen AB_11044497, 1:100 (FACS)
 Monoclonal mouse anti-human CD133/1 (AC133), directly conjugated to APC, Miltenyi Biotec 130-113, 1:100 (FACS)

Validation

The antibodies used were validated according to manufacturer's instructions. Mouse primary PDAC organoids were used to validate FACS antibodies. Human or mouse pancreas sections were used to validate the antibodies for IF or IHC staining.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Cell Services of The Francis Crick Institute for HEK293T and PANC-1 cells.

Authentication

HEK293T and PANC-1 cells were authenticated using STR profiling and results checked against any available STR data for the parent line using commercial banks.

Mycoplasma contamination

All primary mouse and human organoid cells, as well as HEK293T and PANC-1 cells, used in this manuscript tested negative for mycoplasma infection.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Species: *Mus musculus*, strain: C57BL/6; mice of both sexes were used, the number of animals is indicated in the figures or figure legends, tumour mice were weighed twice a week to monitor overall health. KFCY mice were injected at 8 weeks of age and analysed 2 to 4 weeks later; KPC mice had a median survival of 6.2 weeks (maximum survival 10 weeks); KP(het)C mice had a median survival of 17.4 weeks (maximum survival 35 weeks)

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All experiments performed in animals were approved by the Francis Crick Institute's Animal Welfare and Ethical Review Body, and conformed to UK Home Office regulations under the Animals (Scientific Procedures) Act 1986 including Amendment Regulations 2012.
 The collection of human samples was approved by the NHS Health Research Authority following assessment by a Research Ethics Committee (HSC REC B; reference 16/NI/0119).

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

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

Murine cells were isolated either from fresh tissue according to established protocols (Huch et al, 2013; Boj et al, 2015), or from organoid cultures growing in Matrigel using TrypLE.
 Primary human cells were isolated from organoid cultures growing in Matrigel using TrypLE.
 Single-cell suspensions in PBS with 2% FCS (v/v; FACS buffer) were stained with control or experimental antibodies for at least 30 min on ice and washed three times in FACS buffer. Cells were stained with fluorescent secondary antibodies for at least 30 min on ice, in the dark, and washed three times in FACS buffer before filtering and addition of 3 µM DAPI. FACS buffer for primary human cells contained 10 µM ROCK inhibitor Y27632 (Tocris).

Instrument

Sorting: BD Influx, BD Fusion, BD ARIAIII
 Analysis: BD Fortessa, BD LSR II

Software

Data acquisition: BD FACSDiva version 7 or above (for BD Fusion, BD ARIAIII and BD Fortessa), BD FACSSoftware version 1.2 (for

Software	BD Influx) Data analysis: FlowJo version 9 or above
Cell population abundance	To ensure purity of the sorted cells, the sorters were set to “single cell” mode. To increase viability, cells were sorted using a 100 µm nozzle and a low sheath pressure of 20 p.s.i. Purity was determined after sorting by re-running a fraction of the sorted sample directly after the sort, using the same machine, voltages and gates. Percentages of cell populations are indicated in the plots.
Gating strategy	First, dead/DAPI-positive cells were excluded. Second, one to two gates were used to gate on single cells (e.g. FSC-H vs FSC-A, and SSC-W vs FSC-A). Third, if there was still cellular debris in the SSC-A vs FSC-A this was gated out as well. Fourth, where YFP was used as a tracer, we set a gate on those YFP-positive cells. Lastly, populations of interest were gated against a fluorophore-conjugated secondary antibody alone, or an isotype control directly conjugated to a fluorophore. Single-colour and fluorescence minus one controls were used as necessary.

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