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	Hematoxylin and Eosin, multiplex Immunohistochemistry and in situ hybridization slides were scanned and digitalised using the Aperio ScanScope XT Slide Scanner (Aperio Technologies). Immunofluorescence images were acquired using EVOS Cell Imaging System (Thermo Fisher Scientific). Multipex immunofluorescence slides were acquired with Leica TCS SP5 laser scanning confocal (Leica) and digitalized by the Leica Application Suite X (LAS X) software or scanned and digitalized by Zeiss Axio Scanner Z.1 (Carl Zeiss AG, Germany).
Data analysis	For images analysis: FIJI (ImageJ2 version 2.9.0/1.53t), Aperio ImageScope (version 12.3.3), HALO (Indica Labs). For RNAseq analysis, salmon (v1.4.0), STAR (2.7), RSEM (1.3.3). R (4.3.1) with packages tximport (1.28.0), dplyr (1.1.2), ggplot (3.4.2), tidyr (1.3.0), ggrepel (0.9.3), GenomicRanges (1.46.1), IRanges (2.28.0), EnsDb.Hsapiens.v86 (2.99.0), biomaRt (2.56.0), msigdbr (7.5.1) ggpubr (0.6.0), survminer (0.4.9), survival (3.5-5), ggbio (1.42.0), circlize (0.4.15), DESeq2 (1.34.0), GSVA (1.42.0), and fgsea (1.20.0).For sc-RNAseq analysis: Cell Ranger (4.0.0), R (v4.3.2) with packages Seurat (4.3.0), DropletUtils (1.20.0), SingleR(2.2.0), infercnv(1.16.0), AUCell (1.22.0), velociraptor (1.10.0), biomaRt (2.56.0), fgsea (1.20.0), Cell Chat (v1.6.1), decoupleR (v2.5.2). For statistical analysis: R (v4.3.2) or GraphPadPrism (v9.5.1). For FACS analisys: FlowJo software v10.10 (BD Biosciences). For spatial analisys: Space Ranger (v3.0.0), Seurat (v5)

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

RNA-seq data generated in this study have been deposited in the GEO database under accession code: GSE246457 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE246457]. scRNA-seq data generated in this study have been deposited in the GEO database under accession code: GSE246458 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE246458]. Spatial transcriptomics data generated in this study have been deposited in the GEO database under accession code: GSE274665 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274665].

The publicly available RNA-seq data from CCLE used in this study were downloaded from DepMap portal[https://depmap.org/portal/]. The publicly available RNA-seq data from TCGA used in this study were downloaded from firebrowse [http://firebrowse.org/?cohort=PAAD]. The publicly available RNA-seq data from ICGC used in this study were downloaded from ICGC data portal, now migrated in ICGC 25K data. The publicly available RNA-seq data from PanCuRx used in this study are available upon request in the EGA database under accession code EGAS00001002543 [https://ega-archive.org/studies/EGAS00001002543]. The publicly available RNA-seq data from ImVigor trial used in this study are available within easierData R package. The publicly available mouse sc-RNA-seq data used in this study are available in the GEO database under accession code GSE129455 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE129455].

The publicly available human sc-RNA-seq data used in this study are available in: GEO database under accession codes GSE154778[https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154778], GSE155698 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155698], GSE210347 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE210347]; in GSA database under accession code CRA001160 [https://ngdc.cncb.ac.cn/gsa/browse/CRA001160] and in EGA database upon request under accession code EGAS00001002543[https://ega-archive.org/studies/EGAS00001002543]. 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	Data on sex and gender of patients were not collected for this study.
Reporting on race, ethnicity, or other socially relevant groupings	Data on race and ethnicity of patients were not collected.
Population characteristics	In house primary cell lines and human tissues, were derived from patients with histologically verified pancreatic cancer who underwent surgical resection. Samples have been classified based on molecular subtypes through multiplex immunofluorescence, immunohistochemical stainings or RNA-seq.
Recruitment	Patients undergoing pancreatic cancer surgery resection were recruited by referring physician. No self-selection biases have been identified.
Ethics oversight	Human PDAC tissues used in this study were obtained from surgical resections of patients treated at the University and Hospital Trust of Verona (Azienda Ospedaliera Universitaria Integrata, AOUI). Written informed consent was acquired from patients before specimens' acquisition. The FFPE samples used for staining were retrieved from the ARC-Net Biobank and were collected under the protocol number 1885 approved by the local Ethics Committee (Comitato Etico Azienda Ospedaliera Universitaria Integrata) to A.S. (Prot. 52070, Prog. 1885). Tissues from surgical resection used for the generation of primary cultures were collected under the protocol number 1911 approved by the local Ethics Committee (Comitato Etico Azienda Ospedaliera Universitaria Integrata) to V.C. (Prot. n 61413, Prog 1911 on 19/09/2018). All experiments were conducted in accordance with relevant guidelines and regulations. The Essen cohort is a retrospective study carried out according to the recommendations of the local ethics committee of the Medical Faculty of the University of Duisburg-Essen. Patients who had undergone pancreatic resection with a final histopathologic diagnosis of human PDAC between March 2006 and February 2016 was used (Approval no: 17-7340-BO).

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 size represents number of cell lines, tumor cells, human or mice tissues. The sample size was not predetermined using a statistical method; instead, it was determined based on our experience with the relevant experiments and similar published studies. Sample sizes for each experiment are provided in the Figure or in the Figure legends.
Data exclusions	In the sc-RNAseq analysis, three non-CAF subclusters were excluded from further analysis following the subclustering process due to their ambiguous classification.
Replication	The number of biological or technical replicas for each experiment is indicated in the figure legends or in material and methods section.
Randomization	For animal studies, mice were randomized for tumor volume before treatment.
Blinding	Blinding was not applicable as all conditions were labelled for data collection. The entire experimental process and subsequent data analysis were conducted using standardized protocols, objective quantitative methods or have been validated with orthogonal techniques.

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 antibodies were used: p-ERK (Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) #9101, 1:200 IF-IHC, 1:2000 WB, polyclonal, lot. 32, Cell Signaling Technology p-ERK (Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) #4376, 1:200, cl. 20G11, lot. 21, Cell Signaling Technology GATA6 (#AF1700, Bio-techne, lot. KWT0523031 GATA6 #ab175349, polyclonal, lot. GR3447918-1, Abcam) KRT81 (sc-100929, Santa Cruz Biotechnology Inc. cl. 36-Z, lot. I3021), S100A2 (#109494 [EPR5392], Abcam), pan-Keratin (#4279, C11, Cell Signaling Technology; #ab6401, Abcam, cl. C11), α-SMA (#ab5694, Abcam, 1:200, polyclonal, lot.GR3183259-39, GR3263275-13), PDPN (ab236529, Abcam, 1:200, cl. EPR22182, lot. GR3330154-1), CD34 (NCL-L-END, Leica, 1:200, cl. QBEND/10, lot. 6088521), Ly6C (#ab15627, Abcam, 1:100, cl. ER-MP20, lot. GR3261661-18), CD8 (CD8a (4SM15) 14-0808-82, Invitrogen; #ab101500, Abcam, 1:200, cl. SP16, lot. 1036318-32; C8/144B, lot. 20042547, DAKO), total ERK (#9102, Cell Signaling Technology, 1:1000, lot. 26), p-AKT (#4060, (Ser473) (D9E) XP®, Cell Signaling Technology, lot. 2), total AKT (#9272, Cell Signaling Technology, lot. 28), p-S6 (#D57.2.2E/ #4858, Cell Signaling Technology, lot. 11), total S6 (#2317, 54D2, Cell Signaling Technology, lot. 4), Vinculin (#4650, Cell Signaling Technology, lot. 5), GADPH (#5174, (D16H11) XP®, 1:3000, Cell Signaling Technology, lot. 9), CD45 BV421 (#563890, BD Biosciences, 0.2mg/ml, cl. 30-F11, lot. 4036259), CD31 PE-Cy7 (#561410, BD Biosciences, 0.2mg/ml, cl. 390, lot. 3327027), PDPN AF488 #127406, Biolegend, 0.2mg/ml, cl. 8.1.1, lot. B355066), Ly6C APC (#128016, Biolegend, 0.2mg/ml, cl. HK1.4, lot. 3115596).
Validation	p-ERK #9101 https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-antibody/9101?srsltid=AfmBOopsqvmx3edTOG_lZQNw5xe22eH-BhlUUM-GdtbVEh0-WyfnMyei

p-ERK #4376 https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-20g11-rabbit-mab/4376. IF PMID: 30887098
GATA6 #AF1700 https://www.bio-techne.com/p/antibodies/human-gata-6-antibody_af1700
GATA6 #ab175349 https://www.abcam.com/en-us/products/primary-antibodies/gata6-antibody-ab175349
KRT81 sc-100929 https://www.scbt.com/p/keratin-81-antibody-36-z?srsltid=AfmBOoqVIgc0XM2GaC-Ly1Y_MVROukdewuoeYDwzBz9jRL7xABBFB1IM
S100A2 #109494 https://www.abcam.com/en-us/products/primary-antibodies/s100-alpha-2-s100a2-antibody-epr5392-ab109494
pan-Keratin #4279 https://www.cellsignal.com/products/antibody-conjugates/pan-keratin-c11-mouse-mab-biotinylated/4279
pan-Keratin #ab6401 https://www.abcam.com/en-us/products/primary-antibodies/pan-cytokeratin-antibody-pck-26-ab6401
α-SMA #ab5694 https://www.abcam.com/en-us/products/primary-antibodies/alpha-smooth-muscle-actin-antibody-ab5694
PDPN ab236529 https://www.abcam.com/en-us/products/primary-antibodies/podoplanin-antibody-epr22182-ab236529
CD34 NCL-L-END https://shop.leicabiosystems.com/en-de/ihc-ish/ihc-primary-antibodies/pid-cd34
CD8 (CD8a (4SM15) https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-4SM15-Monoclonal/14-0808-82
CD8 #ab101500 https://www.abcam.com/en-us/products/primary-antibodies/cd8-alpha-antibody-sp16-ab101500
CD8 C8/144B https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/cd8-(dako-omnis)-76236
total ERK #9102 https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-antibody/9102
p-AKT #4060 https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060
total AKT #9272 https://www.cellsignal.com/products/primary-antibodies/akt-antibody/9272
p-S6 #D57.2.2E/ #4858 https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-d57-2-2e-xp-rabbit-mab/4858?srsltid=AfmBOoqIIkXS6HdK6Q0TrhKvELT3_DKLaVkP5phwAOYttxTQmS-UBAYF
total S6 #2317 https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-54d2-mouse-mab/2317
Vinculin #4650 https://www.cellsignal.com/products/primary-antibodies/vinculin-antibody/4650
GADPH #5174 https://www.cellsignal.com/products/primary-antibodies/gapdh-d16h11-xp-rabbit-mab/5174
CD45 BV421 #563890 https://www.bdbiosciences.com/en-de/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-rat-anti-mouse-cd45.563890
CD31 PE-Cy7 #561410 https://www.bdbiosciences.com/en-de/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-cd31.561410
PDPN AF488 #127406 https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-podoplanin-antibody-4751?GroupID=BLG5772
Ly6C APC #128016 https://www.biolegend.com/en-gb/products/apc-anti-mouse-ly-6c-antibody-6047

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	We used 5 mouse PDAC cell lines, 1 mouse pancreatic stellate cell line, and 17 human PDAC cell lines. The mouse PDAC cell line FC1199 was generated from tumour of KPC mice (KrasG12D/+; p53R172H/+; Pdx1-Cre). FC1199 were provided by the Tuveson laboratory (Cold Spring Harbor Laboratory, NY, USA). Primary murine PDAC cell lines 60400, 60590, 511892, and 110299 were derived from corresponding tumour pieces of KPC mice (60400, 60590, and 511892: Ptf1awt/Cre;Kraswt/LSL-G12D;p53fl/fl 48; 110299: Ptf1awt/Cre;Kraswt/LSL-G12D;p53LSL-R172H/fl 82). The mouse pancreatic stellate cell line (mPSC4) has been established from WT C57BL/6J mice and was provided by the Tuveson laboratory (Cold Spring Harbor Laboratory, NY, USA). The human PDAC cell lines HPAF-II, PANC-1, and AsPC1 were obtained from ATCC (catalog numbers CRL-1997, CRL-1469, CRL-1682). The Suit-2, Hs766T, Colo 357, and BxPC3 cell lines were generously supplied by Prof. Aldo Scarpa from the University of Verona. The MIA PaCa-2 line was provided by Prof. Vincenzo Bronte, also at the University of Verona. hF2, hT1, and hM1 were kindly provided by Dr. David A. Tuveson from Cold Spring Harbor Laboratory (USA). Patu 8988S were kindly provided by Dr. Francisco X. Real (CNIO, Madrid). Human primary PDAC monolayer cell lines (VR2-2D, VR6-2D, VR9-2D, VR20-2D, and VR23-2D) were established by digesting tissue samples and directly plating them onto tissue culture vessels to initiate monolayer cultures.
Authentication	Commercially available cell lines are authenticated. In house cell lines were sequenced along with patient-derived material to confirm their origin.
Mycoplasma contamination	Cell lines were routinely screened for Mycoplasma contamination using MycoAlert Mycoplasma Detection Kit (Lonza). All cell lines were negative.
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	In this study were used six- to eight- weeks oldC57Bl/6J (B6J) Strain #:000664 and NSG (NOD.Cg-Prkdcscid;Il2rgtm1Wjl) Strain #:005557
Wild animals	No wild animals have been used in this study.
Reporting on sex	We provide information on the sex of the mice but did not consider it as a variable in our analysis, as there is no evidence to suggest

	that sex would impact the results of this study. Only female C57BL/6J mice were used for experiments with FC1199 derived grafts. Both male and female C57BL/6J mice were used for generating grafts from the other KPC cell lines.
Field-collected samples	Field-collected samples were not used.
Ethics oversight	All animal experiments regarding transplanted mice were conducted in accordance with procedures approved by CIRSAL at University of Verona (approved project 655/2017-PR) or were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) under the license number 81-02.04.2020.A316. Animal care procedures and protocols were as prescribed in the national (Tierschutzgesetz) and European (Directive 2010/63/EU) laws and regulations as well as European Federation of Animal Science Associations (FELASA) http://www.felasa.eu.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Freshly isolated cells from CKP-derived orthotopic tumors (1x10^6) resuspended in 1 mL DPBS were first stained with BD Horizon™ Fixable Viability Stain 440UV (#566332, BD Biosciences), incubated at room temperature in the dark for 15 minutes and washed twice with FACS buffer (DPBS with 2% FBS). Upon the addition of 50 µL of BD Horizon Brilliant Stain Buffer (#563794, BD Biosciences), the cells were incubated with the antibodies at 4°C in the dark for 45 minutes in a final volume of 100 µL.
Instrument	BD FACSDiscover™ S8 Cell Sorter (BD Biosciences)
Software	Data analysis was conducted with the FlowJo software v10.10 (BD Biosciences).
Cell population abundance	Not applicable
Gating strategy	Based on cell size (Forward scatter, FSC, and light loss) and granularity (Side scatter, SSC), cell debris were excluded from cells. Cell doublets based on light loss and SSC (Height vs width) were then excluded from cells. Cell debris and doublets were excluded from all flow cytometric analysis.

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