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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	No software was used for data collection.
Data analysis	1- "Metabolic tracing and analyses": For global steady-state metabolite profiling, data were generated by Metaboanalyst 4.0, median-normalized, log-transformed, mean-centered and divided by the s.d. of each variable. 2- "Analysis of public data": RNA sequencing data of tumors and normal samples from the TCGA and GTEx projects were analyzed on the Gene Expression Profiling Interactive Analysis or GEPIA web server: http://gepia.cancer-pku.cn. 3- "Identification of transcription factors": Transcription factors and their binding sites were predicted using TRANSFAC database (version 1.9) with MATCH tools (FMatch for a set of genes; Match for a single gene). 4- "RNA-Seq": Sequence reads were aligned to a transcriptome reference by STAR aligner (version 2.7.8a) using the GRCh38 reference genome supplemented by read-length-specific exon-exon junction sequences (GENCODE V35 gene annotations) and bam files were generated using Samtools (version 1.11). Differential expression analysis was performed in a pairwise manner across all conditions using DESeq260. To quantify exon and gene expression, reads per kilobase per million mapped reads (RPKM) metrics was calculated using DESeq2 total normalized count. Pathway enrichment analysis was performed using GSEA (version 4.1) and Molecular Signature Database (MSigDB) version 7.4, Gene Ontology (GO) sets c5.all. 5- "ATAC-Seq": Sequence reads were aligned to GRCh38 using Bowtie2 (version 2.4.2) and converted to bam format by Samtools (version 1.11). Sequence reads that aligned to the same genomic coordinate were counted only once in the profile generation. Enriched regions were identified using MACS2 in each data with q-value threshold of 0.01. MACS2 identified enriched regions overlapping with ENCODE blacklist regions were eliminated. The remaining regions across all data were then merged into a single catalogue of open chromatin regions. Bedtool (version 2.30) and DESeq2 were used to calculate the number of reads in each region for each library and determine differentially marked regions ($q < 0.05$) across all samples, respectively. Normalized signal across each region was calculated as reads per kilobase per million mapped reads (RPKM) using DESeq2 normalized total reads. Heatmap of ATACseq signal was generated using Deeptools (version 3.5.1) with

1X normalization.
6- "Flow cytometry": Analysis was performed on a BD Fortessa flow cytometer using FlowJo v10.8 Software (BD Life Sciences)

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

All data supporting this study are available within the Article, its Supplementary Information, and the corresponding author upon request. The RNA-Seq and ATAC-Seq data have been deposited in National Center for Biotechnology Information (NCBI)'s Gene Expression Omnibus and are accessible through GEO Series accession no. GSE193411. Box plots in Extended Data Fig. 4a were generated by GEPIA46 http://gepia.cancer-pku.cn/help.html, which uses a combination of public datasets from both TCGA and GTEx for expression analyses. In Extended Data Fig. 9a, b, bar plots and data were generated using TRANSFAC database (version 1.9) https://genexplain.com/transfac-2-0/.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	No sex/gender specific analysis was performed since there is no known association with prognosis.
Population characteristics	Ages: between 45-80 years old; Genotypic info: not available; Diagnosis: pancreatic ductal adenocarcinoma; Treatment: no neoadjuvent treatment.
Recruitment	Patients seeking treatment at Massachusetts General Hospital (MGH), and having had no neoadjuvent treatment, consented that any resected tissues, which would otherwise be discarded following diagnosis, be used instead for research purposes. Patients were provided this option irrespective of age, sex/gender, race of any other bias that could influence study outcomes.
Ethics oversight	The Institutional Review Board or IRB (Protocol Number 2003P001289) at Massachusetts General Hospital (MGH).

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 following well-established protocols previously performed in our lab and others (Zaytouni et al., Nat. Commun. 2017, PMID: 28808255; Tsai et al., PNAS, 2021, PMID: 33653947) and provide adequate power to detect substantial effects ($p < 0.05$), while the added expense of larger samples would not furnish a meaningful improvement.
Data exclusions	No data were excluded from the analyses.
Replication	Experiments were replicated at least twice and up to 6 times.
Randomization	The experiments were not randomized because the results were not predicted to follow a single model that might lead to bias.
Blinding	The investigators were not blinded to allocation during experiments or outcome assessment, because the findings were not predicted to follow a sole hypothesis that might lead to bias.

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

Methods

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

Antibodies

Antibodies used: All antibodies are described in detail in Supplementary Table 1.

Validation: Primary Antibodies were validated by the respective manufacturers listed in Supplementary Table 1.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): Human pancreatic, breast, colon and prostate cancer cell lines were obtained from the American Type Culture Collection or ATCC (pancreatic: AsPC-1, BxPC-3, HPAC, MIA PaCa-2, PANC-1 and SW1990; breast: BT-474, MCF7, MDA-MB 157, MDA-MB 361, T-47D and ZR-75-1; lung: A549, Calu-1, NCI-H596, NCI-H838, NCI-H1299 and NCI-H1975; colorectal: SW480, SW620; prostate: PC3, DU-145); except for pancreatic cancer cells SUIT-2 which were from the Japanese Collection of Research Bioresources; PA-TU-8902 and PA-TU-8988T were from the German Collection of Microorganisms and Cell Cultures; PI3K-wild-type and mutant isogenic colorectal cancer cells HCT116WT and HCT116PIK3CA as well as DLD-1WT, were kindly provided by Bert Vogelstein (Johns Hopkins University, MD). Non-transformed immortalized cells were obtained as follows: human pancreatic ductal epithelial (HPDE) cells were a gift of Ming-Sound Tsao at University Health Network, Princess Margaret Hospital (Toronto, Canada); mammary epithelial cells (MCF10A) were from the Karmanos Cancer Institute (Michigan, USA); lung bronchial epithelial (BEAS-2B), colonic epithelial (FHC) and prostate epithelial (RWPE-1 and RWPE-2) cells were from ATCC.

Authentication: Human PDA cell lines were authenticated by STR profiling at ATCC.

Mycoplasma contamination: All PDA cell lines tested negative for mycoplasma using LookOut Mycoplasma PCR Kit (Sigma, MP0035).

Commonly misidentified lines (See ICLAC register): No misidentified lines were used.

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: 1- For autochthonous models of PDA: the established Dox-inducible TetO-LSL-KrasG12D;ROSA-rtTA;p53fl/fl;p48-Cre or iKrasG12D mouse model was used. iKrasG12D mice and littermates harboring p53fl/fl;p48-Cre alleles but not TetO-LSL-KrasG12D transgene, herein termed iKrasWT were administered 2g. L-1 Dox in the drinking water at 5 weeks of age for 3 weeks, prior to euthanasia and tissue harvest.
2- For human orthotopic xenografts, human PDA cells were injected into the pancreas of 6-7 week-old (or 12-14 week-old for tumors with ARG2 presence/loss) male immune-deficient B6.129S7-Rag1tm1Mom/J mice termed Rag1−/− mice (Jackson Laboratory #002216) and grown for 4-6 weeks.
3- For immune-proficient murine orthotopic transplant models of PDA (except those with or without Arg2 expression), iKras cells previously derived from male iKrasG12D mice were injected into the pancreas of 10-week old male mice of the same strain that however lack p48-Cre. Transplant mice were then administered 2g.L-1 Dox in the drinking water to maintain tumor KrasG12D expression. For murine orthotopic transplants that lack or express Arg2, KPC cell lines previously derived from KPC; Arg2+/+ or KPC; Arg2−/− mice, respectively, were injected into the pancreas of 11–13 week old male C57BL/6J-129 svJae mice and grown for 2 weeks.

Wild animals: The study did not involve wild animals.

Reporting on sex: Although most experiments were performed using male mice, in vivo pharmacological targeting of the identified metabolic pathway was validated in both male and female mice, yielding similar results (n = 9, 10 or 11 mice per group, per gender).

Field-collected samples: The study did not involve samples collected from the field.

Ethics oversight	All mouse studies and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Boston Children's Hospital, as stated in the Methods section "Animal work".

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	Tissues were prepared for flow cytometry analysis using the protocol adapted from previous work (Roehle et al., Sci Transl Med, 2021; PMID: 34011631). Briefly, pancreas was excised, weighed, then pancreatic draining lymph nodes were removed and pancreatic head and tail were separated. Samples were minced then placed in RPMI-1640 containing collagenase type IV and soybean trypsin inhibitor at 37°C for 30 min. Tumors were filtered through a 40-micron cell strainer, washed with PBS and centrifuged at 300g for 5 min. The cell pellet was resuspended in FACS buffer (PBS with 2% fetal calf serum) and stained with a master mix of antibodies purchased from Biolegend, as described in SI Table 1. Draining lymph nodes were crushed through a 40-micron cell strainer and resuspended FACS buffer for staining. Cells from pancreatic draining lymph nodes, pancreas head and pancreas tail were incubated with extracellular staining mix in FACS buffer for 30 min on ice, washed once with PBS and either resuspended in 1% formalin/PBS for extracellular analysis or fixed, permeabilized and stained with intracellular antibodies against specific cytokines (eBioscience Foxp3/Transcription Factor Staining Buffer Set).
Instrument	BD Fortessa flow cytometer.
Software	FlowJo v10.8 Software (BD Life Sciences).
Cell population abundance	n/a (samples were not sorted)
Gating strategy	Analysis of select immune populations gated out of CD45+ live, single cells: CD11b+/- = CD45+; MoMDSCs = CD11b+ Gr1low; GrMDSCs = CD11b+ Gr1high; macrophages = CD11b+ SiglecF- Gr1-; eosinophils = CD11b+ SiglecF+; CD103+/MHCIIhigh; DCs = CD11b- CD4- CD8- CD11c+ MHCII+; CD4 T cells = CD11b- CD4+; CD8 T cells = CD11b- CD8+; Tregs = CD11b- CD4+ FOXP3+ CTLA4+; B cells = CD11b- CD4- CD8- B220+. All positive gates were set based on clearly separated populations except in the case of Gr-1 staining, which was divided into tertiles based on mean fluorescence intensity. Unstained cells were used to define negative gates. Forward scatter and side scatter were used to identify cell-sized objects. Fragments with FSC less than 10% of the sample median were excluded from analysis. Cell doublets were excluded based on deviation from the diagonal of FSC area vs FSC height.

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