

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 <i>P</i> 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	No software was used.
Data analysis	10x Genomics Cell Ranger v7.0.1, SoupX v1.5.258, Seurat v5, scooter 0.0.0.9004, scDblFinder v1.6.0, inferCNV 1.10.0, 10x Genomics SpaceRanger v2.1.1, BayesPrism 2.0, Seq-N-Slide, Trimmomatic v0.36, sambamba v0.6.8, GATK v3.8.1, Control-FREEC v1.6, speckle 0.99.7, singleSeqGset 0.1.2.9000, CellPhoneDB v5.0.0, DESeq2 1.38.3, clusterProfiler 4.6.2, GSEA v4.2.3, spdep v1.3.3, R 4.2.2, phangorn 2.12.1, ape 5.8-1, NICHES 1.1.0

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 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](#)

Processed data from this study are available at Zenodo (scRNA seq: DOI 10.5281/zenodo.20329097; spatial transcriptomics: DOI 10.5281/zenodo.14907947). Raw

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	Sex-based analyses were performed using available annotations, but no significant differences were observed. As a result, the findings were not reported in the main text. Full results are available upon request.
Reporting on race, ethnicity, or other socially relevant groupings	Race-based analyses were not conducted, as the study population wasn't heterogeneous due to the nature of sequential patient enrollment. Since patients were included as they presented to the hospital, the study did not have control over racial diversity in the dataset.
Population characteristics	62 patients with primary pancreatic ductal adenocarcinoma. Samples were collected via surgical resection (n=24) or endoscopic ultrasound-guided core biopsy (n=38). 43 samples were collected prior to chemotherapy, while 19 were from patients who had received chemotherapy before sample collection (FOLFIRINOX-based n=12, gemcitabine/abraxane-based n=6, and mixed chemo-RT n=1). Tumor stages were I (n=12), II (n=13), III (n=12) & IV (n=25). Median age was 69y, while the youngest patient was 49y and the oldest was 88y.
Recruitment	Patients with primary pancreatic ductal adenocarcinoma who presented at NYU Langone Health for treatment between August 2020 and April 2023 were recruited after providing informed consent.
Ethics oversight	The study protocol was approved by the Institutional Review Board (IRB) of NYU Langone Health.

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	The sample size for this study was not predetermined, as patient recruitment was based on sequential enrollment of individuals presenting at NYU Langone Health for treatment. Given the rarity of the disease, and complexity and cost associated with single-cell and spatial transcriptomics, acquiring large cohorts remains challenging. However, with 62 samples, this study represents one of the largest single-cell and spatial transcriptomics dataset for pancreatic adenocarcinoma to date. This sample size provides statistical power to detect cellular interactions and transcriptional programs within the tumor microenvironment. Previous studies with smaller datasets have successfully yielded biologically meaningful insights, further supporting the sufficiency of our cohort for robust spatial and transcriptomic analyses.
Data exclusions	Samples with fewer than 100 epithelial cells were excluded from the clone-specific analysis due to insufficient cell numbers for meaningful analysis. Only resection samples were used for spatial transcriptomics.
Replication	Technical and biological replicates were included where possible to assess consistency across samples. Standardized protocols were followed for tissue processing, library preparation, and sequencing to minimize technical variability. Rigorous quality control (QC) steps were applied, including filtering low-quality cells and spatial spots. Computational analyses were performed using established bioinformatics pipelines with predefined parameters to ensure consistency and reproducibility.
Randomization	Samples were not pre-assigned to experimental groups but were instead allocated based on intrinsic biological characteristics observed post-analysis. Patients were enrolled sequentially as they presented for treatment, without prior stratification. Experimental comparisons (e.g., clone-specific or spatial transcriptomic analyses) were conducted retrospectively based on molecular and spatial features identified in the data rather than predefined groupings.
Blinding	Blinding was not applicable, as samples were not allocated into predefined experimental groups. Instead, patients were enrolled sequentially, and analyses were conducted retrospectively based on molecular and spatial features. However, data processing and computational analyses were performed using standardized pipelines with predefined parameters to minimize potential 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

Methods

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

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

Antibodies

Antibodies used

Myc (Y69) antibody (Abcam; ab168727; 1:1,000 dilution)
 TIGIT (BLR047F) antibody (Abcam; ab243903; 1:400 dilution)
 Nectin-2/CD112 (D8D3F) antibody (Cell Signaling; 95333S; 1:100 dilution)
 CD3 epsilon antibody (SP7) antibody (Abcam; ab16669; 1:500)
 CD8 (4SM16) antibody (Invitrogen; 14-0195-82; 1:50)
 CK19 (Ks19.1) antibody (Biocare; CM242A; 1:400)

Validation

All antibodies are validated by manufacturer to be suitable for WB and IHC/IF at the minimum

Eukaryotic cell lines

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

Cell line source(s)

KMC314J cell lines: Primary PDAC cell line from male Ptf1aCreER; KrasG12D; Rosa26Myc-WT/Myc-WT mouse pancreatic ductal adenocarcinoma generated by Rosalie Sears lab.
 Human cell line: Primary cell lines LW338 and LW90 from male patients with pancreatic ductal adenocarcinoma generated by Diane Simeone lab.

Authentication

We have validated that the KMC314J cell line is mouse derived, able to form tumors by orthotopic transplant, and thus are tumor cells, which express the Rosa-Myc knockin that has an HA tag.
 We have validated that the human PDAC cell lines used in this study are of human origin, are capable of robust proliferation in vitro, and exhibit the expected transcriptional and genomic features of pancreatic ductal adenocarcinoma.

Mycoplasma contamination

Cell lines were tested negative for mycoplasma by PCR.

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

No commonly misidentified cell lines used in the study.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a