

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	Standard Visium and Codex methods were used to collect the data with parameters described in the paper. All computer code will be made available upon acceptance of manuscript. Software and coding environment were R language version 4.2.2, R studio 12.0, GraphPad 9, and Seurat 4.3.
Data analysis	All computer code will be made available upon acceptance of manuscript. Software and coding environment were R language version 4.2.2, R studio 12.0, GraphPad 9, monocle3, and Seurat 4.3 and Ingenuity Pathway Analysis. Custom code was written for optimizing cluster qualities, correlation analysis, differential expression visualization, cell type enrichment analysis, and pipelines including Seurat and monocle.

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](#)

Level 3 transcriptomic datasets from Stanford (STPDAC11, STPDAC12, STPDAC13, STPDAC14, STIPMN11, STIPMN12, STIPMN21, STIPMN22, STIPMN31, STIPMN32, STIPMN33, STIPMN34) in this study have been deposited in the Synapse.org database (created by Sage Bionetworks in partnership with scientific organizations) under accession code syn61831984 [<https://doi.org/10.7303/syn61831984>]. Level 3 datasets are a genomic terminology used by HTAN and others and refers to spatial transcriptomic datasets where derived biomolecular data are mapped to image positions and include barcodes, features, matrices, and corresponding histology image file. Access can be obtained by registering as a user at Synapse.org. The processed data are available as corresponding Source Data file. The processed transcriptomic data and CODEX data generated in this study are provided in the Supplementary Information/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	The visium transcriptomic data analyzed here was approximately balanced across gender (14 female and 13 male). This is reported in the caption of the patient table. Sex and/or gender was not considered in the study design and sex and/or gender of participants was determined based on self-report. For the primary analyses performed here (CLDN4 overexpression in PDAC), no gender related differences were found.
Reporting on race, ethnicity, or other socially relevant groupings	We used pre-acquired data or Stanford available surgical samples and race and ethnicity were not available for some patients. Therefore this was not reported and was not a part of the study plan.
Population characteristics	See above
Recruitment	All patients undergoing surgery for pancreatic cancer or IPMN at Stanford University are routinely recruited to provide samples for retrospective studies. These most recent samples were included in this study to ensure high quality RNA and were augmented with all available spatial transcriptomic data from Washington University and MD Anderson. Therefore no patient selection was made based on race or ethnicity.
Ethics oversight	Stanford University IRB approved this study.

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	Our spatial transcriptomic data included 27 patients with ~4000 spatial spots sequenced per patient for a total of 112,000 spots. In some analysis, these were separated according to the diagnosis of PDAC, IPMN or normal surrounding tissue. In other analyses, the spots were combined to assess overall differences. These data were augmented with the single cell data from 346 PDAC patients as stated below. Within each analysis all relevant spots were combined and significance was determined based on the adjusted p value and reported in this fashion.
Data exclusions	No data were excluded unless a gene was not reported in a patient sample (this occurred in one case- this omitted gene was then not included for that patient). This may have resulted from the failure of a single gene within a Visium chip.
Replication	Our spatial transcriptomic data from 27 patients were compared with the Cancer Surfaceome Atlas (which reported on single cell data from 346 PDAC patients - 167 GTEx and 179 TCGA). We compare the results with the conclusions of this work within this paper.
Randomization	Within each analysis all relevant spots were combined and significance was determined based on the adjusted p value and reported in this fashion.
Blinding	Within each analysis all relevant spots were combined and significance was determined based on the adjusted p value and reported in this fashion. The analysis was not blinded to the diagnosis.

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	All antibodies were described.
Validation	Commercial Akoya Bioscience antibody were used and were validated by EnableMedicine based on Akoya's validation scheme

Eukaryotic cell lines

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

Cell line source(s)	The source of the cell lines was described.
Authentication	None of the cell lines were authenticated.
Mycoplasma contamination	All cell lines were routinely tested for mycoplasma and this test was negative in all cases.
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	We studied Kras ^{LSL-G12D/+} ;Rosa26 ^{LSL-tdTomato} /LSL-tdTomato;Ptf1a ^{CreER} ;Trp53 ^{fl/fl} mouse model by activating oncogenic Kras ^{G12D} and deleting Trp53 in mouse pancreatic cells with a tamoxifen-inducible knock-in Ptf1a ^{CreER} allele. Xenograft tumors were established in both hind flanks of NOD/SCID mice (Jackson Laboratory) by subcutaneous injection of Capan-1 cells (1 × 10 ⁶).
Wild animals	None
Reporting on sex	Both genders were studied in both models.
Field-collected samples	None
Ethics oversight	The Stanford IACUC approved the study.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	This was not a clinical trial.
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Plants

Seed stocks	None
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>