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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 *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	LaVision BioTec Imspector Pro; Zeiss Zen microscope software
Data analysis	Fiji for general image analysis (tiling and 3D rendering); BigStitcher42 (ImageJ plugin) for manual tiles stitching; TeraStitcher plugin for automatic tiles stitching; Imaris x64 9.5.1 (Bitplane – Oxford Instruments)

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

All relevant data is provided in the main figures and supplementary videos of this 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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. Stainings were performed in samples from 2 or more mice. Experiments in the related publications using FLASH for morphometric quantifications were performed in at least 3 biological replicates.
Data exclusions	No data was excluded from the analyses.
Replication	All stainings can be replicated with the antibodies disclosed in this manuscript. Antibodies that do not work for conventional 2D IF often did not work for FLASH.
Randomization	Adult mice and Drosophila were randomly selected for most stainings. Adult virgin or pregnant females were selected for imaging of mammary glands and embryos as indicated. Mouse tumour models were selected at the progression stage/age indicated in the figure. Samples from PDAC patients were stained as indicated (see below).
Blinding	Blinding was not possible as stains and image analysis were performed by the same researchers.

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	Amylase, Polyclonal, Goat, Santa Cruz sc-12821; Neuroendocrine convertase 1, Polyclonal, Rabbit, Millipore SAB1100416; alpha-smooth muscle actin, Monoclonal, Mouse, Sigma-Aldrich A5228; Tyrosine hydroxylase, Polyclonal, Rabbit, Merck AB152; Clara Cell secretory protein, Polyclonal, Goat, Santa Cruz sc-9772; Surfactant Protein C, Polyclonal, Rabbit, Sigma-Aldrich HPA010928; Gastric Intrinsic Factor, Polyclonal, Rabbit, Sigma-Aldrich HPA040774; alpha1 Na/K ATPase, Monoclonal , Mouse, Abcam ab7671; Cadherin 1, Monoclonal, Rat, Thermo Fisher 13-1900; Mucin-1, Polyclonal, Rabbit, Abcam ab15481; Mucin-5AC, Polyclonal, Goat, Santa Cruz sc-16903; Collagen IV, Polyclonal, Goat, Sigma-Aldrich AB769; Mist1, Monoclonal, Mouse, Santa Cruz sc-80984; Cytokeratin 8, Monoclonal, Rat, DSHB TROMA-I; Cytokeratin 5, Monoclonal, Mouse, Biotechne NBP2-22194; Red Fluorescent Protein, Polyclonal, Rabbit, Rockland 600-401-379; Glutamine synthetase, Polyclonal, Rabbit, Abcam ab73593; S100, Polyclonal, Rabbit, Dako Z0311; Keratin 19, Monoclonal, Rat, DSHB TROMA-III; CD44, Monoclonal, Rat, Merck MAB2137; Prospero homeobox protein 1, Polyclonal, Rabbit, Abcam ab101851; Aquaporin 1, Polyclonal, Rabbit, Sigma-Aldrich HPA019206; Vimentin, Polyclonal, Chicken, Sigma-Aldrich AB5733; Keratin 14, Monoclonal , Mouse, Abcam ab9220; Wilms Tumour 1, Polyclonal, Rabbit, Santa Cruz sc-192; CD3, Polyclonal, Rabbit, Abcam ab5690; CD45, Monoclonal, Rat, Biolegend 103202; CD16, Monoclonal, Mouse, Thermo Fisher MA1-7633; Green Fluorescent Protein, Polyclonal, Goat, Abcam ab6673; Proliferation marker Ki67, Monoclonal, Rabbit, Abcam ab16667; Proliferating cell nuclear antigen, Polyclonal, Rabbit, Santa Cruz sc-7907; Aurora B, Monoclonal, Mouse, BD 611082; Podoplanin, Polyclonal, Goat, R&D AF3244; Cleaved caspase 3, Polyclonal, Rabbit, R&D AF835; Tubulin, Monoclonal, Rat, Abcam ab6161; Cytochrome P450, monoclonal, Mouse, Abcam ab22717; Acetylated tubulin, Monoclonal, Mouse, Sigma-Aldrich T7451; Conecting peptide, polyclonal, Rabbit, Cell Signalling Technologies, 4563; Glial Fibrillary Acidic Protein, polyclonal, Rabbit, Abcam ab7260; CD31, polyclonal, Rabbit, Abcam ab28364; FoxP1, polyclonal, Rabbit, Cell Signaling Technologies 2005; Anti-mouse IgG, Alexa Fluor 488, Donkey, Thermo Fisher A-11055; Anti-mouse IgG, Alexa Fluor 546, Donkey, Thermo Fisher A-10036; Anti-mouse IgG, Alexa Fluor 594, Donkey, Thermo Fisher A-21203; Anti-mouse IgG, Alexa Fluor 700, Goat, Thermo Fisher A-21036; Anti-rabbit IgG, Alexa Fluor 546, Donkey, Thermo Fisher A-10040; Anti-rabbit IgG, Alexa Fluor 594, Donkey, Thermo Fisher A10040; Anti-rabbit IgG, Alexa Fluor 647, Donkey, Thermo Fisher A-31573; Anti-rat IgG, Alexa Fluor 488, Donkey, Thermo Fisher A-21208; Anti-rat IgG, Alexa Fluor 594, Donkey, Thermo Fisher A-21209; Anti-rat IgG, Alexa Fluor 647, Donkey, Abcam 150155; Anti-goat IgG, Alexa Fluor 546, Donkey, Thermo Fisher A-11056; Anti-goat IgG, Alexa Fluor 647, Donkey, Thermo Fisher A-21447; Anti-Chicken IgY, FITC, Donkey, Thermo Fisher SA1-72000; GFP sdAb - Fluo Tag-Q, monoclonal, Alpaca, Synaptic Systems N0301-At488-S
Validation	All antibodies used for this protocol were comercially available and validated by the manufacturer. Amylase: information regarding validation of this antibody can be found at https://www.scbt.com/p/amylase-antibody-c-20; Neuroendocrine convertase 1: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/

sigma/sab1100416?lang=en&region=GB; alpha-smooth muscle actin: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/sigma/a5228?lang=en&region=US; Tyrosine hydroxylase: information regarding validation of this antibody can be found at https://www.merckmillipore.com/GB/en/product/Anti-Tyrosine-Hydroxylase-Antibody,MM_NF-AB152; Clara Cell secretory protein: information regarding validation of this antibody can be found at https://www.scbt.com/p/cc10-antibody-t-18; Surfactant Protein C: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/sigma/hpa010928?lang=en&region=US; Gastric Intrinsic Factor: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/sigma/hpa040774?lang=en&region=US; alpha1 Na/K ATPase: information regarding validation of this antibody can be found at https://www.abcam.com/alpha-1-sodium-potassium-atpase-antibody-4646-ab7671.html; Cadherin 1: information regarding validation of this antibody can be found at https://www.thermofisher.com/antibody/product/E-cadherin-Antibody-clone-ECCD-2-Monoclonal/13-1900; Mucin-1: information regarding validation of this antibody can be found at https://www.abcam.com/muc1-antibody-ab15481.html; Mucin-5AC: information regarding validation of this antibody can be found at https://www.scbt.com/p/mucin-5ac-antibody-k-20; Collagen IV: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/mm/ab769?lang=en&region=US; Mist1: information regarding validation of this antibody can be found at https://www.scbt.com/p/mist1-antibody-6e8; Cytokeratin 8: information regarding validation of this antibody can be found at https://dshb.biology.uiowa.edu/TROMA-I; Cytokeratin 5: information regarding validation of this antibody can be found at https://www.novusbio.com/products/cytokeratin-5-antibody-2c2_nbp2-22194; Red Fluorescent Protein: information regarding validation of this antibody can be found at https://rockland-inc.com/store/Antibodies-to-GFP-and-Antibodies-to-RFP-600-401-379-O4L_24299.aspx; Glutamine synthetase: information regarding validation of this antibody can be found at https://www.abcam.com/glutamine-synthetase-antibody-ab73593.html; S100: information regarding validation of this antibody can be found at https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/s100-(dako-omnis)-76198; Keratin 19: information regarding validation of this antibody can be found at https://dshb.biology.uiowa.edu/TROMA-III; CD44: information regarding validation of this antibody can be found at https://www.merckmillipore.com/GB/en/product/msds/MM_NF-MAB2137; Prospero homeobox protein 1: information regarding validation of this antibody can be found at https://www.abcam.com/prox1-antibody-ab101851.html; Aquaporin 1: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/sigma/hpa019206?lang=en&region=GB; Vimentin: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/mm/ab5733?lang=en&region=US; Keratin 14: information regarding validation of this antibody can be found at https://www.abcam.com/cytokeratin-14-antibody-rck107-ab9220.html; Wilms Tumour 1: information regarding validation of this antibody can be found at https://www.scbt.com/p/wt1-antibody-c-19; CD3: information regarding validation of this antibody can be found at https://www.abcam.com/cd3-antibody-ab5690.html; CD45: information regarding validation of this antibody can be found at https://www.biolegend.com/en-us/search-results/purified-anti-mouse-human-cd45r-b220-antibody-449; CD16: information regarding validation of this antibody can be found at https://www.thermofisher.com/antibody/product/CD16-Antibody-clone-ASH-1975-Monoclonal/MA1-7633; Green Fluorescent Protein: information regarding validation of this antibody can be found at https://www.abcam.com/gfp-antibody-ab6673.html; Proliferation marker Ki67: information regarding validation of this antibody can be found at https://www.abcam.com/ki67-antibody-sp6-ab16667.html; Proliferating cell nuclear antigen: information regarding validation of this antibody can be found at https://www.scbt.com/p/pcna-antibody-fl-261; Aurora B: information regarding validation of this antibody can be found at https://www.bdbiosciences.com/ptProduct.jsp?ccn=611082; Podoplanin: information regarding validation of this antibody can be found at https://www.rndsystems.com/products/mouse-podoplanin-antibody_af3244; Cleaved caspase 3: information regarding validation of this antibody can be found at https://www.rndsystems.com/products/human-mouse-active-caspase-3-antibody_af835; Tubulin: information regarding validation of this antibody can be found at https://www.abcam.com/tubulin-antibody-yol134-microtubule-marker-ab6161.html; Cytochrome P450: information regarding validation of this antibody can be found at https://www.abcam.com/cytochrome-p450-1a2-antibody-d15-16vii-f10f12-ab22717.html#description_images_2; Acetylated tubulin: information regarding validation of this antibody can be found at https://www.sigmaaldrich.com/catalog/product/sigma/t7451?lang=en&region=GB; Conecting peptide: information regarding validation of this antibody can be found at https://www.cellsignal.com/products/primary-antibodies/c-peptide-antibody/4593; Glial Fibrillary Acidic Protein: information regarding validation of this antibody can be found at https://www.abcam.com/gfap-antibody-ab7260.html; CD31: information regarding validation of this antibody can be found at https://www.abcam.com/cd31-antibody-ab28364.html#description_images_1; Forkhead box protein P1: information regarding validation of this antibody can be found at https://www.cellsignal.com/products/primary-antibodies/foxp1-antibody/2005; GFP sdAb - FluoTaq-Q: information regarding validation of this antibody can be found at https://sysy.com/product/N0301-At488-S

Animals and other organisms

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

Laboratory animals	Mus musculus, C57BL/6 and FvB strains, males and females, E13.5 embryos to 6 months of age. Adult Drosophila melanogaster males and females.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected in the field.
Ethics oversight	All our animal experiments are approved by the London Research Institute Animal Ethics Committee or the Animal Welfare and Ethical Review Body of the Francis Crick Institute and conformed to UK Home Office regulations under the Animals (Scientific Procedures) Act 1986 including Amendment Regulations 2012.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Patients diagnosed with pancreatic ductal adenocarcinoma (PDAC).
Recruitment	Patients of the King's College Hospital were recruited before undergoing the Whipple surgical procedure.
Ethics oversight	All our experiments with human material are 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.