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Last updated by author(s): Jan 17, 2026

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	Statistical analyses were performed using GraphPad Prism 10 software. Differences between two groups were analyzed using Welch's t-test for normally distributed data and Man-Whitney non-parametric test for non-normally distributed data. For multiple group comparisons, a one-way ANOVA test was used. Differences were considered statistically significant at $p < 0.05$. For single-cell transcriptomic analysis, we used the following: FASTQ files were processed using the 10X Genomics CellRanger pipeline (version 7.1.0). Read pairs were aligned to the mouse reference genome (mm10, 10X-reference genomes, 2020 version). Computational analysis was performed in R (version 4.4.2 (2024-10-31) "Pile of Leaves" using the Seurat (v4) R package. Additional packages used include Harmony (v1.2.3), CellChat v2, monocle3 (v1.3.7), and UCell (https://github.com/carmonalab/UCell). Information on parameters used are included in the Methods section. The R and Python code used to generate all the plots are available in Github: https://github.com/AthanasiaSt/Notch3-regulates-pericyte-phenotypic-plasticity-in-colorectal-cancer.git

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 in-house scRNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under the accession numbers GSE296872 and GSE296873.

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	We used publicly available datasets of single-cell transcriptomic analyses from colorectal cancer patients(GSE144735, GSE132465, GSE178341, GSE200997). All information about patients are found in the initial publications.
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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 was determined using power analysis. For cell isolation experiments, sample size was determined by the total number of cells needed for downstream experiments.
Data exclusions	No data were excluded from analyses, with the exception of Figure 1K, where only samples that included both states (Normal-Tumor) were used for the pairwise analysis.
Replication	In vitro and in vivo experiments were repeated at least once.
Randomization	In most experiments, animals were allocated in experimental groups based on their genotype. In experiments including treated versus untreated mice, animals were allocated in groups based on their weight and sex to ensure equal representation in both groups.
Blinding	Samples were analyzed in a random order to achieve some level of blinding. However, since the investigator was usually the same one performing both experiments and analysis, this was not entirely possible.

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

A table with all antibodies used is provided in the Methods section. They include the following: anti-CD45-APCCy7 (Clone 30-F11, Biolegend), anti-CD326-APCefluor780 (Clone G8.8, eBioscience), anti-CD31-PercP (Clone 390, Biolegend), anti-CD140a-APC (Clone APA5, Biolegend), anti-CD146-PECy7 (Clone ME-9F1, Biolegend), anti-IL-6-PE (Clone MP5-20F3, Biolegend), anti-Notch3-PE (Clone HMN3-133, Biolegend), anti-aSMA-FITC (Clone 1A4, Sigma), anti-aSMA-efluor660 (Clone 1A4, Invitrogen), anti-CD146 unconjugated (Clone EPR3208, Abcam), anti-CD31 unconjugated (Clone MEC13.3, BD Biosciences), anti-CD326 unconjugated (Clone G8.8, Invitrogen), anti-Notch3 unconjugated (Cat No ab23426, Abcam), anti-Ki67 unconjugated (Cat No ab15580, Abcam), anti-albumin unconjugated (ab8940, Abcam), anti-goat IgG-Alexa555 (Cat No A21432, Invitrogen), anti-rat IgG-Alexa568 (A11077, Invitrogen), anti-rat IgG Alexa594 (Cat No A11007, Invitrogen), anti-rabbit IgG-Alexa488 (Cat No A11008, Invitrogen), anti-rabbit IgG-Alexa647 (Cat No A21244, Invitrogen), anti-sheep-IgG-Alexa647 (A21448, Invitrogen).

Validation

All antibodies were purchased by commercial vendors. Information about antibody validation, applications, and species specificity can be found in the companies' website and in the Antibodies' datasheets.

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

All mice were maintained on the C57BL/6 background. Eight to sixteen-week-old male and female mice were used for all experiments. The following strains were used: PDGFRβ-CreERT2, Notch3-/-, Rosa26-CreERT2. Notch3-CreERT2, Rosa26-N3ICD. References for each strain are provided in the Methods Section. An ARRIVE checklist has been uploaded along with the manuscript.

Wild animals

n/a

Reporting on sex

Both male and female mice were used for all experiments.

Field-collected samples

n/a

Ethics oversight

All animal experiments were approved by the Institutional Committee of Protocol Evaluation in conjunction with the Veterinary Service Management of the Hellenic Republic Prefecture of Attika according to all current European and national legislation.

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

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a

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	The mouse colon or AOM/DSS tumors were dissected and washed with HBSS (Gibco) supplemented with antibiotic antimycotic solution (Gibco). Epithelial cells were removed by incubating the tissue with 5 mM EDTA and 1 mM DTT in HBSS for 25 minutes at 37°C. The colonic tissues were digested in 300 U/mL Collagenase XI (Sigma-Aldrich) and 1 mg/mL Dispase II (Roche) in DMEM for 60 min at 37°C. Tumors were digested in 1000 U/mL Collagenase IV (Sigma-Aldrich, St. Louis, MO, USA), 1 mg/mL Dispase II (Roche, Basel, Switzerland), and 100 U/mL Dnase I (Sigma-Aldrich, St. Louis, MO, USA) in DMEM (BioSera, France) in three serial 20-min digestions. Cell suspensions were centrifuged and washed in FACS buffer (5% FBS (Biowest) in PBS). For stromal cell sorting, 1–2 million cells/100 µL were incubated with anti-CD45 and anti-CD326 antibodies for the exclusion of immune and remaining epithelial cells, respectively, and 1 µg/mL Propidium Iodide (PI) (Sigma) for dead cell exclusion. Cell sorting was performed using the FACSAria III cell sorter (BD) and the FACSDiva (BD) software. For pericyte sorting, cell suspensions were additional stained with an anti-CD146 antibody for positive selection and the Zombie-NIR Fixable Viability Kit (Biolegend) for selection of live cells. For stromal cell analysis, single-cell suspensions were stained with fluorochrome-conjugated antibodies against PDGFRα, MCAM/CD146, and CD31 for 30 minutes at 4°C in the dark. For intracellular stainings, cells were fixed and permeabilized using the Fixation and Permeabilization Buffer set (eBioscience), and subsequently stained with antibodies against αSMA and/or IL-6 (Table 1). Dead cell exclusion was performed using Zombie-NIR Fixable Viability Kit (Biolegend). Data were acquired on a BD Canto flow cytometer (BD Biosciences) and analyzed using the FlowJo software (version 10.9.0, FlowJo, LLC). A list of antibodies is provided in the Method section.
Instrument	BD Canto flow cytometer or FACSAria III cell sorter (BD Biosciences) .
Software	FACSDiva (BD Biosciences) or FlowJo (version 10.9.0, FlowJo, LLC) software.
Cell population abundance	In post-sort fractions the abundance and purity of the sorted population was >90%, as determined by FACS analysis of the fractions.
Gating strategy	Initial cells selected in FSC/SSC plots, followed by doublets exclusion. Dead cells were excluded as PE-Cy5 negative when PI was used. Otherwise dead cells were excluded along with Lineage negative markers (CD45/Epcam) at the APC-Cy7 channel. Fibroblasts were gated as PDGFRa-647 positive cells. Pericytes were gated as MCAM-PECy7 and aSMA-FITC double positive cells. Smotth muscle cells were gated as aSMAhi cells. In lineage tracing analysis, quantifications were performed on GFP +MCAM+ cells.

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