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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	Flow Cytometry and cell sorting: LSRFortessa Cell Analyzer (BD Bioscences); CytoFLEX SRT (Beckman Coulter). NovaSeq 6000 and NextSeq2000 were used for Bulk RNA-seq, Bulk ATAC-seq, Single cell RNA-seq and Single cell Multiome.
Data analysis	Limma v3.50.l; FastQC v0.11.8; Trim Galore! v0.6.6; STAR aligner v2.7.5b; Salmon vl.2.1; DESeq2 vl.28.1; clusterProfiler v4.2.2; gProfiler; Bowtie2 v2.2.8; SAMtools v.1.11; Picard v2.2.4; Deeptools v3.2.1; Seurat package v4.0.3; TOBIAS v0.13.2 ;HOMER v4.10; RunHarmony v0.1.0; scanpy vl.9.3;Muscat vl.12.1; Cell Ranger ARC v2.0.0; chromVAR Signac vl.3.0; rtracklayer vl.54.0; Gviz vl.38.4; Prism 9.5.1, FlowJo vl0.7.1

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

RNA-seq, ATAC-seq, scRNA-seq, scATAC-seq data supporting the findings of this study have been deposited into the National Center for Biotechnology Information

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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	No predetermined sample size calculations were used. Animal/tumor numbers were based on the variability in tumor take rate and growth; our sample sizes align with those in previous studies (PMID: 28358093 and PMID: 28355176). For in vitro experiments, sample sizes (n=2-7) were based on previous experience and publications with similar experiments (PMID: 28358093, PMID: 35773527 and PMID: 28355176).
Data exclusions	Exclusion criteria included death or humane endpoints (e.g., ≥20% weight loss, respiratory distress, persistent hypothermia, or tumor size >1cm3).
Replication	All data were replicated in at least 2 independent experiments with consistent results. Multiple members of the research project independently validated results.
Randomization	Mice were randomized between treatment groups. Mathematically equivalent grouping distributions were confirmed (tumor volumes). If equivalence was not achieved, random shuffling was done again to ensure approximately equal initial mean tumor volume per condition before the start of treatment.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment. Data collection and analysis were not performed blind to the conditions of the experiments. Researchers involved in experimental allocation and analyses were commonly the same researchers performing the experiment - as such, blinding was not conducted.

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	mCherry (Rockland #600-401-379) 1:500. GFP (abcam #ab183734); dilution 1:100.
Validation	These antibodies were used for immunohistochemistry on mouse tumors according to the manufacturer's recommendations and optimized/validated on negative and positive controls form mouse origin by HistoWiz, Inc

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Mouse: All organoids and tumoroids were derived from genetic mouse models described in the methods section. Patient-derived organoids were obtained from the Human Cancer Models Initiative(HCMI) https://ocg.cancer.gov/programs/HCMI; dbGaP accession number phs001486, these lines were de-identified and deposited at ATCC. HEK-293T cells were obtained from ATCC.
Authentication	Mouse organoids and the source mouse models were genotyped by PCR. Human organoids underwent whole-genome sequencing. HEK293T were authenticated by ATCC and are widely used in the field.
Mycoplasma contamination	All cell lines were tested and remained mycoplasma-free.
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	Lgr5-2A-CreERT2 (Leushacke et al., 2017) mice were generated through homologous recombination in embryonic stem cells, specifically targeting the insertion of the 2A-CreERT2 cassette at the stop codon of the Lgr5-ORF. To obtain the experimental mice, Lgr5-2A-CreERT2 mice were crossbred with Rosa26-tdTomato (Ai14) (Madisen et al., 2010), LSL-KrasG12D (Johnson et al., 2001), Apc-loxP flanked (Shibata et al., 1997), Trp53-loxP flanked (Jonkers et al., 2001) and Smad4-loxP flanked (Yang et al., 2002) mice, which were acquired from the Jackson Laboratory. All mice were bred onto the C57Black6/J background and were 6-8week-old on average. Experimental mice were at least 8-weeks-old. To ensure pathogen-free conditions, all mice were bred and housed in ventilated cages. Intracolonic induction in a 12-week-old male mouse villinCreER Apcfl/+ KrasG12D/+ Trp53fl/fl Smad4fl/fl on a C57BL/6 background was performed under general anesthesia. A single 70μl l00uM dose of 4-hydroxy tamoxifen (H7904-5MG from Sigma) was injected into the colonic sub-mucosa via a colonoscope (Karl Storz TELE PACK VET X LED endoscopic video unit). At clinical endpoint (weight loss with or without the presentation of hunching) colonic tumor tissue was collected, and organoid cell lines were generated as previously described (Jackstadt et al., 2019). 7-8 week old female NSG mice (Strain #:005557, Jackson Laboratory) were used for subcutaneous transplantation experiments. Mice were closely monitored by the authors, facility technicians and by a veterinary scientist responsible for animal welfare. They were euthanized when they reached a humane endpoint as determined by the Institutional Animal Care and Use Committee (IACUC). Some of these criteria include clinical signs of persistent distress or pain, significant loss of body weight(> 20%), and tumor size exceeding 1000mm3.
Wild animals	The study did not involve wild animals.
Reporting on sex	Female mice were used for the subcutaneous transplantation experiments.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All mouse studies were approved by the Icahn School of Medicine at Mount Sinai (ISMMS) Institutional Animal Care and Use Committee (protocol number 2018-0013).

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

Plants

Seed stocks	Non-applicable.
Novel plant genotypes	Non-applicable.
Authentication	Non-applicable.

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	Matrigel drops containing organoids/tumoroids were collected in cold basal media. Following centrifugation (500g for 5 mins at 4"C), cells were incubated in TrypLE at 37 C and then mechanically dissociated by pipetting. After washing with 5 ml of cold basal medium (500g for 5 minutes at 4 C), samples were resuspended in 1ml of FACS buffer (PBS with 2% FBS and lmM EDTA) containing a live/dead dye diluted 1:2000 (Live/Dead Fixable Aqua, lnvitrogen #L34957) and incubated for 10 min at RT. In some experiments, cells were resuspended in 1ml of PBS and live/dead dye used at 1:500 (Zombie NIR, Biolegend #423106). Cells were washed with 5ml of FACS buffer, passed through a 40uM cell strainer into a new 15ml conical tube, and centrifugated (500g for 5 minutes at 4 C). Pellets were resuspended in 200ul of FACS buffer, transferred to 5ml polystyrene FACS tube, and immediately acquired on a BD LSRFortessa Cell Analyzer (BD Biosciences). For sorting experiments, an additional filtering step (35 m) was added. Cells were sorted on a CytoFLEX SRT (Beckman Coulter). In all experiments, unstained cells and FMO controls were included to determine basal levels of fluorescence intensity. Data were analyzed using FlowJo software (version 10.7.1, Tree Star, Ashland, OR, USA).
Instrument	CytoFLEX SRT (Beckman Coulter) and BD LSRFortessa Cell Analyzer (BD Biosciences)
Software	FlowJo software (version 10.7.1, Tree Star, Ashland, OR, USA).
Cell population abundance	The enrichment of GFP+ cells in the sorted samples was validated by analysis of GFP expression in the RNA-sequencing.
Gating strategy	The first gates were based on FSC-A/SSC-A live cells» SSC-W, SSC-H singlets»FSC-H, FSC-W singlets» then GFP+ and mCherry+ cells were gated based on an unstained (not transduced parental line) and FMO controls.

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