

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 Data was collected using 10x Genomics analysis pipelines which are all publicly available, including: Space Ranger v3.0., Cell Ranger v8.0.0, Xenium Onboard Analysis pipeline v2.0.0, Loupe Browser v8.0

Data analysis Data was analysed using 10x Genomics analysis pipelines which are all publicly available, including: Space Ranger v3.0, Cell Ranger v8.0.0, Xenium Onboard Analysis pipeline v2.0.0. Software (R or Python packages): Seurat v5, spacr v2.2.1, stardist v0.9.0 and LIANA v0.1.12 were used for downstream analysis.
Custom scripts used for this paper are available on GitHub at: https://github.com/10XGenomics/HumanColonCancer_VisiumHD
All code has also been deposited at Zenodo <https://zenodo.org/records/15042463>
All details are outlined in the Methods section and third party tools are referenced.

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 datasets generated in this manuscript are available for download here: <https://www.10xgenomics.com/products/visium-hd-spatial-gene-expression/dataset-human-crc>

For enrichment analysis we used the MSigDB_Hallmark_2020: <https://www.gsea-msigdb.org/gsea/msigdb/human/genesets.jsp?collection=H>

We have uploaded the data to Gene Expression Omnibus (GEO) under accession number GSE280318

Data deposited at GEO contains raw data (fastq files) and processed data for the 8 micron bin size. Data available for download at the 10x Genomics website includes the all outputs from spaceranger, including the results for all bin sizes.

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 considered in study design due to limited sample number.
Reporting on race, ethnicity, or other socially relevant groupings	Not considered in study design as this information was not available.
Population characteristics	Not considered in study design, information is included in Table 1 but was not considered in the analysis.
Recruitment	Samples were obtained via a commercial vendor (Discovery Life Sciences).
Ethics oversight	Human biological samples were obtained from Discovery Life Sciences. The research use of these specimens was approved by the corresponding Institutional Review Boards (IRB) under protocol numbers DLS-BB044-v.1 and SB-GP_v1. All patients provided written informed consent to Discovery Life Sciences for the research use of their biological samples.

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	No statistical methods were used to predetermine sample size. We selected samples that were all classified as colorectal adenocarcinoma, pre-treatment, on the basis of availability. We included samples from 5 patients, for 3 of these patients we were able to obtain samples from tumor, normal adjacent tissue (NAT), and dissociated tumor cells (DTC).
Data exclusions	None
Replication	Visium HD experiments were performed on one replicate per patient sample included (as described in Methods and Figure legends). Findings were validated using an orthogonal spatial platform (Xenium in situ) for selected samples (as described in Methods and Figure legends).
Randomization	Not relevant due to limited sample number.
Blinding	Not relevant due to limited sample number.

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

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	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.
Authentication	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.