

Corresponding author(s): Vincenzo Cerullo

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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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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 data was collected using BD FACSDiva Software v 8.0

Data analysis Flow data was analysed using Flowjo v10.10.0. Prism 10 was used to perform statistical tests. Single-cell RNA sequencing data was analyzed with Rstudio using the Seurat v5 package. Metabolomics data was analysed using Metaboanalyst 6.0

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](#)

Data generated will be available in the source data. scRNA sequencing data have been submitted under GSA submission number CRA024974 (<https://ngdc.cncb.ac.cn/gsa/browse/CRA024974>)

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

Six colorectal cancer patients were enrolled in this study: CRC-001, CRC-002, CRC-003, CRC-004, CRC-005 and CRC-006. CRC-001, CRC-006 and CRC-002 subjects are male and CRC-004, CRC-005 and CRC-003 is female; however, our findings are not limited by patient sex, and sex or gender were not used as inclusion or exclusion criteria during study design. Peripheral blood mononuclear cells (PBMCs) and buffy coats obtained from healthy donors via the Finnish Red Cross Blood Service were collected under broad consent without recording donor sex or gender; consequently, these details are not reported in the manuscript.

Reporting on race, ethnicity, or other socially relevant groupings

All three patients were of Finnish descent, and because our findings are not specific to any ethnic group, race and ethnicity were not used as inclusion or exclusion criteria and are therefore not reported. Similarly, PBMCs and buffy coats obtained from healthy donors via the Finnish Red Cross Blood Service were collected without recording donor race or ethnicity.

Population characteristics

CRC-001 was diagnosed with colon cancer, CRC-002 was diagnosed with colon sigmoideum and CRC-003 was diagnosed rectum. All patients had levels of >1ng/ml of CEA in blood.

Recruitment

Patients were recruited under the Umbrella of iCAN cancer precision medicine flagship.

Ethics oversight

Patient's samples were received by the Helsinki University Hospital under the approval of the ethical review board (review number HUS/970/2021) and Helsinki University Hospital institutional review board (IRB) (approval HUS/8/2022).

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 formal sample size calculation was conducted prior to the experiments. Instead, sample sizes were determined based on the research team's extensive experience and established laboratory practices, which have consistently yielded reliable and reproducible results in similar experimental contexts

Data exclusions

Data exclusions were limited to the in vivo experimental component of the study. Animals were excluded based on predefined criteria: (1) failure of tumor engraftment, defined as the absence of measurable tumor growth following tumor cell injection; and (2) early euthanasia due to clinical signs of discomfort or distress in accordance with ethical guidelines. These criteria were applied uniformly across all treatment groups to ensure the integrity and consistency of the data.

Replication

All results were repeated at least three independent times unless otherwise specified. Similar data were obtained in independent experiments.

Randomization

For in vivo experiments, once a visible tumor mass was detected on the flanks of mice following tumor cell injection, all animals within a cohort were transferred to a large holding cage. Mice were then redistributed into smaller cages, ensuring a maximum of five animals per cage. To maintain balanced baseline tumor sizes across treatment groups and prevent clustering of larger tumors within a single group, animals were initially sorted by tumor volume. Mice with the largest tumors were allocated first, followed by those with progressively smaller tumors. After cage assignment, treatment groups were randomly assigned to each cage. At the time of treatment, both the order of groups and individual mice within each group were randomized. Similarly, the order of euthanasia and tissue collection was randomized to prevent procedural bias during downstream analyses. For in vitro experiments, in each experimental setting, all the samples were treated with all the conditions (controls). The order of the groups was randomized either in the placement in a multi well plate or in the acquisition.

Blinding

Blinding was not applied in this study as the experiments were conceptualized, executed and analyzed by the same person.

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

Anti-mouse CD3, clone 145-2C11, BD Biosciences Cat #563123 RRID: AB_2738196

Anti-mouse CD8, clone 53-6.7, BD Biosciences Cat #563068 RRID: AB_2737947

Anti-mouse CD4, clone RM4-5, BD Biosciences Cat #562285 RRID: AB_11153794

Anti-mouse CD62L, clone MEL-14, eBioscience Cat #17-0621-81 RRID: AB_469420

Anti-mouse CD44, clone IM7, BioLegend Cat #103026 RRID: AB_493399

Anti-mouse CCR7, clone 4B12, BD Biosciences Cat #560805 RRID: AB_2034006

Anti-mouse TNF- α , clone MP6-XT22, BioLegend Cat #506333 RRID: AB_2562772

Anti-mouse IL-2, clone JES6-5H4, BioLegend Cat #503832 RRID: AB_315409

Anti-mouse IFN- γ , clone XMG1.2, BioLegend Cat #505808 RRID: AB_315425

Anti-mouse IL-6, clone MP5-20F3, BioLegend Cat #503806 RRID: AB_315390

Anti-mouse CD11b, clone M1/70, BioLegend Cat #101241 RRID: AB_2563882

Anti-mouse CD11c, clone N418, eBioscience Cat #25-0114-82 RRID: AB_469579

Anti-mouse H-2K^d, clone AF6-88.5.5.3, BioLegend Cat #116516 RRID: AB_10564495

Anti-mouse CD103, clone 2E7, BioLegend Cat #121406 RRID: AB_313401

Anti-mouse PDCA-1, clone 927, BioLegend Cat #127015 RRID: AB_1953322

Anti-mouse CD3, clone 17A2, BioLegend Cat #100236 RRID: AB_312663

Anti-mouse CD8 α , clone 53-6.7, BioLegend Cat #100706 RRID: AB_312753

Anti-mouse CD107a (LAMP-1), clone 1D4B, BioLegend Cat #121625 RRID: AB_2158009

Alexa Fluor® 647 anti-Asialo-GM1 Antibody, polyclonal, BioLegend Cat #146003 RRID: AB_2563195

APC/Cyanine7 anti-mouse F4/80 Recombinant Antibody, recombinant, BioLegend Cat #157315 RRID: AB_2894638

Anti-H3K4me3, polyclonal, Diagenode Cat #C15410003 RRID: AB_2615079

Validation

All purchased antibodies were used according to the manufacturer's instructions.

Eukaryotic cell lines

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

Cell line source(s)

CT26 cells were purchased from ATCC while MC38 were kindly provided by Dr. Cristian Smerdou, Cima Universidad de Navarra. Colon-26 was purchased from Cytion.

Authentication

None of the cells were authenticated

Mycoplasma contamination	All cell lines were tested for mycoplasma contamination using MycoAlert™ Mycoplasma Detection Kit (Lonza) following manufacturer instructions. Murine cells were additionally tested prior in vivo tumor engraftment. All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study were found in the registry of commonly misidentified cell lines (v11)

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 obtained from Envigo and consisted of female BALB/c animals aged 4–6 weeks upon arrival. They were maintained in individually ventilated cages (IVC) at up to five mice per cage, with unlimited access to food and water, and kept on a 12-hour light/12-hour dark cycle.
Wild animals	This study did not use wild animals
Reporting on sex	Findings do not apply to a only one sex.
Field-collected samples	No animals collected from the field were used
Ethics oversight	All in vivo experiments were reviewed and approved by the Experimental Animal Committee of the University of Helsinki and the Provincial Government of Southern Finland (license numbers ESAVI/11895/2019 and ESAVI/12722/2022)

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

Plants

Seed stocks	<i>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.</i>
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>

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	<i>For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.</i>
Files in database submission	<i>Provide a list of all files available in the database submission.</i>
Genome browser session (e.g. UCSC)	<i>Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.</i>

Methodology

Replicates	<i>Describe the experimental replicates, specifying number, type and replicate agreement.</i>
Sequencing depth	<i>Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.</i>
Antibodies	<i>Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	<i>Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.</i>

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

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

Tumor or spleen samples were passed through cell-strainer to make cell suspensions. As for adherent cells, cells were detached using PBS +10mM EDTA. For regular flow cytometry analysis, samples were first blocked with Fc-blocker and then stained with relevant flow cytometry antibodies. As for intracellular staining, splenocytes were blocked with anti-Fc receptor reagent, and surface-stained with lineage and memory markers. Samples were fixed and permeabilized and intracellular cytokines were labeled following the kit protocols. Samples were acquired on a BD LSR Fortessa cytometer and analyzed using FlowJo v10.8.2.

Instrument

BD LSR Fortessa cytometer
BD FACS ARIA Fusion

Software

FlowJo v10.8.2.

Cell population abundance

When sorting was performed, purification was always > 90%

Gating strategy

Gating Strategy for supplementary figure 2 is as following: Extended Figure 2 Gating Strategy for T memory subsets A Singlets were added on a SSC vs FSC and the lymphocyte population was gated on. CD3 cells were then gated on and checked for CD8 and CD4. Both CD4 and CD8 cells were analysed for CD62L and CD44 to determine memory phenotypes. CD44-CD62L- were considered CTLs, CD44-CD62L were considered Naïve memory, CD44+CD62L+ were considered central memory and CD44+CD62L- were considered effector memory cells. CD8+ and CD4 T cells were further subjected for IL-2, IFN- γ , TNF- α and CD40L expression. Baseline expression was done based on expression of each cytokine in unstimulated samples.

Gating strategy for supplementary figure 5 is as following: Extended Figure 5 Gating Strategy for DC populations A Single Cells were gated based on both FSC-H vs FSC-A and SSC-H vs SSC-A. Gated Single cells were then gated for lymphocytes based on FSC-A vs SSC-A. CD11b+ cells were then gated on and analysed for CD11c+ cells. CD11c+ cells were gated and were considered dendritic cells. CD11c+ dendritic cells had PDCA expression analysed. PDCA+ cells were considered pDC. PDCA-cells were then examined for CD103

Gating strategy for supplementary figure 9 is as following: Gating Strategy for macrophage and NK cells gating. Single cells were examined under FSC-A vs SSC-A. Counting beads were gated and immune cells were also gated. Immune cells were then gated on CD11b+ cells. CD11b+ cells were then gated on macrophages based on F4/80 expression while NK cells were gated on Anti-GM1 expression. CD103+ cells were considered cDC1 while CD103- cells were considered cDC2

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)	<i>Specify: functional, structural, diffusion, perfusion.</i>
Field strength	<i>Specify in Tesla</i>
Sequence & imaging parameters	<i>Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.</i>
Area of acquisition	<i>State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.</i>
Diffusion MRI	☐ Used ☐ Not used

Preprocessing

Preprocessing software	<i>Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).</i>
Normalization	<i>If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.</i>
Normalization template	<i>Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.</i>
Noise and artifact removal	<i>Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).</i>
Volume censoring	<i>Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.</i>

Statistical modeling & inference

Model type and settings	<i>Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).</i>
Effect(s) tested	<i>Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.</i>
Specify type of analysis:	☐ Whole brain ☐ ROI-based ☐ Both
Statistic type for inference	<i>Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.</i>
(See Eklund et al. 2016)	
Correction	<i>Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).</i>

Models & analysis

n/a	Involvement in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis
Functional and/or effective connectivity	<i>Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).</i>
Graph analysis	<i>Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).</i>
Multivariate modeling and predictive analysis	<i>Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.</i>