nature portfolio

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Last updated by author(s): July 9th, 2024

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	n/a
Data analysis	n/a

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 sequencing data generated in this study was deposited under the accession code GSE236384. Reviewer can use the following token to access the data: uxexusyyjpktnmn

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 report sex as self-reported attribute in the Supplementary Table 1 for each participant. We did not perform sex-based analyses due to small sample sizes.
Reporting on race, ethnicity, or other socially relevant groupings	We did not apply socially relevant grouping on participants.
Population characteristics	Male and female adults with MSS treatment naiive adenocarcinoma of the colon or rectum (see Supplementary Table 1).
Recruitment	Patients of the Division of Visceral Surgery, Department of General Surgery, Medical University of Vienna, scheduled for surgical resection for adenocarcinoma of the colon or rectum with no history of radiation therapy or cytoablative treatment were enrolled into the study after submitting written informed consent.
Ethics oversight	Local Ethics Committee of the Medical University of Vienna, Vienna, Austria

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	Due to the explorative character of the study the sample size was not pre-calculated. Wherever applicable, two-sided t-tests yielded 80% power at alpha = 0.05.
Data exclusions	Samples were excluded if history revealed radio or chemotherapy, inflammatory bowel diseases and/or microsatellite instability.
Replication	All attempts of replication were successful.
Randomization	Not relevant, no randomized grouping was performed for this study.
Blinding	Investigators were blinded for data analysis. Codes were allocated to patient samples instead of phenotype.

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

CD3 APC SK7 BD Bioscience 345767
CD3 BV786 UCHT1 BD Bioscience 565491
CD27 BV421 M-T271 BD Bioscience 562513
CD31 APC-Cy7 WM59 BioLegend 303119
CD45 PE/CF594 HI30 BD Bioscience 562279
CD45RA APC-Cy7 HI100 BioLegend 304127
CD90 PE-Cy7 5,00E+10 BioLegend 328124
CD107a PE H4A3 BioLegend 328607
CD160 PerCp-Cy5.5 BY55 BioLegend 341210
CD244 PerCp-Cy5.5 C1.7 BioLegend 329516
CTLA-4 BV421 BNI3 BioLegend 369605
EpCAM BV421 CO17-1A BioLegend 369821
FasL PE-Vio770 NOK-1 Miltenyi Biotec 130-104-270
GranzymeB BV421 GB11 BD Bioscience 563389
IFNγ V450 B27 BD Bioscience 560372
Ki-67 PE-Cy7 20Raj1 BD Bioscience 561283
LAG-3 PE-Cy7 11G3C65 BioLegend 369310
PD1 APC EH12.2H7 BioLegend 329908
PD1 FITC EH12.2H7 BioLegend 329903
Perforin PerCp-Cy5.5 δG9 BD Bioscience 563762
TCRγδ PE B1 BioLegend 331210
TCR Vδ1 APC REA173 Miltenyi Biotec 130-118-968
TCR Vδ2 FITC B6 BD Bioscience 555738
TIGIT APC-Cy7 A15153G BioLegend 372707
TIGIT Blocking A15153A BioLegend 613703
Tim3 APC-Cy7 F38-2EZ BioLegend 345026
TNF-α PE-Cy7 MAb11 BD Bioscience 560678
TRAIL Biotin RIK-2 BD Bioscience 550431

Validation

For each entibody signals were compared to isotype and unstained controls to estimate nonspecific binding of primary antibodies.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HT29, SW480

Authentication

We purchased the cell lines from American Type Culture Collection (ATCC) and used cells in low passages.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination using PCR Mycoplasma test kit (Biological Industries; 2070020).

Commonly misidentified lines
(See ICLAC register)

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

Human colon tissue, HC and CRC, was cut into small pieces using a sterile scalpel. Phosphate buffered saline (PBS, Gibco) with 10% fetal bovine serum (FBS, Gibco) was used to rinse the tissue. Human colon samples were digested with medium, consisting of plain RPMI 1640 medium (Gibco) supplemented with collagenase IV (10 U/ml, Sigma-Aldrich) and deoxyribonuclease I (10mg/ml, DNAse, Sigma-Aldrich). Digestion was performed using a digestion machine (gentleMACS Octo dissociator with heaters, Miltenyi) with the preset 37°C_h_TDK1 protocol. The cell suspension was passed through a 70µm cell strainer using the backside of a syringe. RPMI 1640 medium supplemented with 10% FBS was used to rinse the strainers.

Instrument	Sample acquisition and cell sorting was performed on a FACSAria III (BD Biosciences) with FACSDiva Software.
Software	The acquired samples were analyzed with FlowJo software (v10.8).
Cell population abundance	Sorting purity was determined using BD FacsDiva software (Accudrop application); using 4-way purity sorting strategy, cell purity was determined at >99%;
Gating strategy	Lymphocyte polulation was gaten in SSCA/FSCA, single cells were gated i) in FSC-W/FSC-H and ii) in SSC-W/SSC-H; single cells were gated on live, CD45+; from this gate, T lymphocytes were determined as SSCA lo, CD3+ cells. Further T cell subpopulations were gated from this (e.g. cytokines). Generally, populations >10^3 were considered positive; n-1 control stainings showed <0.1% positive cells above this cutoff.

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