nature portfolio

Corresponding author(s): Arielle Elkrief

Last updated by author(s): Dec 2, 2025

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	For shotgun metagenomics, data is publicaly available at the NCBI repository posted in the manuscript. The tumor sizes were measured throughout the experiment using a caliper. Two-dimensional measurements (HxL) were performed. For the flow cytometry, the stained cells were acquired using Cytek Aurora 5-laser spectral analyzer for human PBMCs.
Data analysis	GraphPad Prism (version 10.4.2) was used for the generating Fig(s) 4G-J, 5C-G, Extended Figure(s) 5C-D, 6B-D, 7 E-F, 8A. Microbiome sequencing data were processed and analyzed using the phyloseq package (v.1.50.0). Alpha diversity analysis was performed using the vegan package (v.2.7.1). Principal component analysis was done with the packages prcomp (v.4.4.2), and factoextra (v.1.0.7). PERMANOVA analysis was performed with 999 permutations using the adonis function from the vegan package. Strain sharing between the patients and donors was calculated using StrainPhlAn4 with an in-house database for strain identification as previously published. Bray-Curtis dissimilarity was calculated between the samples and their corresponding donor samples using the distance function from phyloseq. Linear discriminant analysis Effect Size (LEfSe) was performed to identify microbial taxa that differentiate between subject groups according to their response, cohort or toxicity development using the package yingtools2 (v.0.0.1.184). Taxonomic abundance patterns between groups were visualized via heatmaps generated by the ComplexHeatmap package (v.2.22.0). Shotgun metagenomic samples were also processed with the HMP Unified Metabolic Analysis Network (HUMAnN3) pipeline (v.3.8). Differential abundance analysis between baseline and post-FMT was performed using DESeq2 (v.1.46.0).

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

Raw fastq files are publicly available on the NCBI biorepository with accession number PRJNA1289847. Individual patient data will be made available upon request to the corresponding author with data sharing agreement.

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	Biological sex was considered in the study and we had 60% male and 40% female participants. Gender was not considered in the study.
Reporting on race, ethnicity, or other socially relevant groupings	Donor race and ethnicity is included in Extended Data Table 1.
Population characteristics	Population characteristics is included in Extended Data Table 2.
Recruitment	Recruitment: Patients were enrolled and treated at five academic centers in Canada: Centre Hopitalier de l'Université de Montréal (CHUM; Québec), the London Regional Cancer Program (LRCP; Ontario), Centre hospitalier de l'Université de Québec (CHUQ; Québec), McGill University Health Centre (MUHC; Québec), Lakeridge Health (Oshawa; Ontario). Patients were recruited by their treating oncologist or a member of the research unit authorized to review patients with the treating oncologist. Patients were provided with a verbal outline of the trial followed by a copy of the consent dorm to review, Written informed consent was obtained by a co-investigator.
Ethics oversight	The clinical trial and correlative analyses were approved by the CHUM Ethics Review Board and each participating centre, ethics number: MP-02-2022-10121/21.173. For culturomics analysis, this was conducted under project number: 2025-12377 through biobank numbers MP-02-2018-7132/17.035 and 16.161.

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	n=20 NSCLC and 20 melanoma patients. For the NSCLC cohort, assuming that the ORR rate is 39% (null hypothesis), a sample size of 18 patients with has 80% to detect an ORR of 64% (alternative hypothesis) using a one-sided binomial test with 0.10 level of significance. Since the pre-specified primary endpoint of the study was ORR in the NSCLC cohort, there was no pre-specified sample size calculation for the cutaneous melanoma cohort. For mice experiments, groups of 10 mice per condition were used, ensuring statistical power while minimizing animal use.
Data exclusions	Specific sub-group analyses were included in the methods
Replication	Mouse experiments were reproduced in two independent experiments.
Randomization	The clinical trial was not randomized. Mice underwent randomisation as stated in the methods.
Blinding	Blinding is not applicable since the clinical trial was not blinded.

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

Antibodies for flow cytometry including dilution factors were detailed in Methods section and in the provided supplemental tables. For mouse experiments: anti-PD-1 monoclonal antibody (250 µg per mouse; clone RMP1-14, Bio X Cell) or isotype control (clone 2A3, Bio X Cell) were used in this study. The antibodies were purchased from the supplier Cedarlane.

Validation

For flow cytometry antibodies (Human experiments):
https://www.thermofisher.com/order/catalog/product/L23105
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/buffers-and-supporting-reagents-ruo/brilliant-stain-buffer-plus.566385?tab=product_details
https://www.biolegend.com/de-de/products/brilliant-violet-510-streptavidin-8140
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-mouse-anti-human-cd194.562906?tab=product_details
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv661-mouse-anti-human-cd196-ccr6.569509?tab=product_details
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv737-mouse-anti-human-ccr7-cd197.749676?tab=product_details
https://www.biolegend.com/de-de/products/pe-dazzle-594-anti-human-cd199-ccr9-antibody-14763
https://www.biolegend.com/de-de/products/brilliant-violet-570-anti-mouse-human-cd11b-antibody-7380
https://www.biolegend.com/de-de/products/alexa-fluor-647-anti-human-cd123-antibody-8525
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/r718-mouse-anti-human-cd127.566967?tab=product_details
https://www.biolegend.com/de-de/products/spark-blue-550-anti-human-cd14-antibody-18496
https://www.biolegend.com/de-de/products/brilliant-violet-421-anti-human-cd141-thrombomodulin-antibody-9130
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv805-mouse-anti-human-cd16.569165?tab=product_details
https://www.biolegend.com/de-de/products/apc-anti-human-cd163-antibody-6276
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb515-mouse-anti-human-siglec-1-cd169.565353?tab=product_details
https://www.biolegend.com/de-de/products/pefire-640-anti-human-cd19-antibody-19533
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv480-mouse-anti-human-cd1c.746677?tab=product_details
https://www.biolegend.com/de-de/products/percp-cyanine5-5-anti-human-cd206-mmr-antibody-7404
https://www.biolegend.com/de-de/products/pefire-700-anti-human-cd25-antibody-19788
https://www.biolegend.com/de-de/products/brilliant-violet-650-anti-human-cd3-antibody-13475
https://www.biolegend.com/de-de/products/pe-anti-human-cd301-clec10a-antibody-7914
https://cytekbio.com/products/cfluor-568-anti-human-cd4?variant=32351881986084
https://www.biolegend.com/de-de/products/brilliant-violet-650-anti-human-cd40-antibody-12322
https://www.biolegend.com/de-de/products/percp-anti-human-cd45-antibody-12393
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-human-cd45ra.740298?tab=product_details
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv563-mouse-anti-human-cd5.741354?tab=product_details
https://www.biolegend.com/de-de/products/spark-nir-685-anti-human-cd69-antibody-20086
https://www.biolegend.com/de-de/products/apc-fire-810-anti-human-cd8-antibody-19536
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-mouse-anti-human-cd86.740990?tab=product_details
https://www.biolegend.com/de-de/products/apc-fire-750-anti-human-cd88-c5ar-antibody-16464
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv496-mouse-anti-human-cd89.750617?tab=product_details
https://www.thermofisher.com/antibody/product/CD152-CTLA-4-Antibody-clone-14D3-Monoclonal/46-1529-42
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-mouse-anti-human-cd183.560831?tab=product_details
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv615-rat-anti-human-cxcr5-cd185.751293?tab=product_details
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-mouse-anti-human-fc-r1.747783?tab=product_details

https://www.biolegend.com/de-de/products/pe-fire-810-anti-human-hla-dr-antibody-21104
https://www.rndsystems.com/products/human-integrin-alpha4beta7-lpam-1-research-grade-vedolizumab-biosimilar-alexa-fluor-405-conjugated-antibody-hu117_fab10078v
https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv750-mouse-anti-human-cd279-pd-1.747446?tab=product_details
https://www.thermofisher.com/antibody/product/CD274-PD-L1-B7-H1-Antibody-clone-MIH1-Monoclonal/15-5983-42
https://www.miltenyibiotec.com/CA-en/products/slan-m-dc8-antibody-anti-human-dd-1.html

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	MCA-205: a mouse fibrosarcoma cell line synergenic to C57BL/6 mice. Provided by Dr. John Stagg from CRCHUM Montreal
Authentication	The cell lines were authenticated by IDEXX BioResearch through PCR assay.
Mycoplasma contamination	Cell lines were checked for mycoplasma using PlasmoTest Mycoplasma Detection Kit (InvivoGen).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines from the ICLAC register were used in this study.

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	Murine experiments were conducted using seven-week-old female wild type C57BL/6 mice, obtained from Charles River and maintained at the Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM) GF Facility. All mice were 6-8 weeks old for this study.
Wild animals	Not applicable.
Reporting on sex	Female mice were used.
Field-collected samples	Not applicable.
Ethics oversight	All animal studies were approved by the Institutional Animal Care Comimittee (CIPA) and carried out in compliance with the Canadian Council on Animal Care guidelines. Ethical number: C22017Br.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT04951583
Study protocol	The study protocol is included in the submission.
Data collection	Medidata Rave EDC. Samples were collected from patient enrolled in the trial from six centres across Canada.
Outcomes	Primary and secondary outcomes are stated in the methods. For the NSCLC cohort, assuming that the ORR rate is 39% (null hypothesis), a sample size of 18 patients with has 80% to detect an ORR of 64% (alternative hypothesis) using a one-sided binomial test with 0.10 level of significance. Since the pre-specified primary endpoint of the study was ORR in the NSCLC cohort, there was no pre-specified sample size calculation for the cutaneous melanoma cohort.

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, mosiacism, off-target gene editing) were examined.*

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 — Cryopreserved peripheral blood mononuclear cells (PBMCs) from NSCLC patients undergoing combined anti-PD-1 and FMT and melanoma patients undergoing combined anti-PD-1+anti-CTLA-4 and FMT were thawed, washed and resuspended in complete RPMI medium. One million cells were labelled for viability with LIVE/DEAD™ Fixable Blue Dead Cell Stain (Invitrogen) (30min, room temperature). Fc blocking antibody was added to cells to decrease non-specific binding and background fluroscence (10min, 4 deg C) and intracellular markers (30mins, room temperature).

Instrument — Flow Cytometry data was acquired using a Cytek Aurora 5-laser spectral analyzer

Software — BD FACSDiva Software was used during the stained cells in the cytometer.
Flow cytometry raw data anlysis were performed with FlowJo v10.8.1

Cell population abundance — One million cells were labelled for viability with LIVE/DEAD™ Fixable Blue Dead Cell Stain (Invitrogen) (30min, room temperature). Fc blocking antibody was added to cells to decrease non-specific binding and background fluroscence (10min, 4 deg C) and intracellular markers (30mins, room temperature) (Extended Data Table).

Gating strategy — Gating strategy: The gating strategy for the human PBMC staining are provided in Extended Figure 8.

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