

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 data was acquired using the Cytex Aurora. qPCR data was collected using the QuantStudio™ 6 Flex Real-Time PCR System. For 16s rRNA sequencing, amplicons were sequenced on the Illumina MiSeq V3 platform (2x250 bp paired-end reads). For metagenomic sequencing, amplicons were sequenced on the Illumina NovaSeq S4 platform (2x150 bp paired-end reads). Metabolomics data were acquired using a Vanquish UHPLC system coupled to a Bruker Impact II Q-TOF mass spectrometer operated in positive ion mode.
Data analysis	The following software/tools were used for data analysis in this study: GraphPad Prism Pro (v10.6.1), RStudio/R (v4.2.3), MicrobiomeAnalyst (Version 2.0), MetaboAnalyst 6.0, Kraken2, nf-core/amplicon pipeline (v2.14.0), FastQC, MultiQC, Cutadapt, DADA2, bcl2fastq (v2.17), IsoMS Pro, and the R packages vegan, ggplot2, and ggrepel. For 16S rRNA sequencing analysis, reads were quality controlled using FastQC and MultiQC, primer-trimmed using Cutadapt, and processed using DADA2 with taxonomic assignment against the SILVA 138.2 database. Metagenomic sequencing reads were demultiplexed using bcl2fastq and processed using Kraken2. Downstream microbiome analyses were performed using MicrobiomeAnalyst (Version 2.0). Metabolomics raw data were processed using IsoMS Pro for peak detection, alignment, and quantification, and downstream pathway enrichment and multivariate analyses were performed 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](#)

The 16S rRNA gene amplicon sequencing and shotgun metagenomic sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive under BioProject accession PRJNA1468761. For metabolomics data, the final metabolite intensity tables provided by The Metabolomics Innovation Centre (TMIC, University of Alberta, Canada) are included in the Supplementary Information (Supplementary Tables 4-7). All other data are available upon reasonable request.

The MyCompoundID database for metabolomics analysis can be accessed at this link: https://www.mycompoundid.org/mycompoundid_IsoMS/

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	Patient sex can be found in Supplementary Table 3 (NR FMT donor), Supplementary Table 8 (plasma metabolomics), and Supplementary Table 9 (Low/High BMI FMT donors).
Reporting on race, ethnicity, or other socially relevant groupings	We did not collect information on race, ethnicity, or other socially relevant groupings.
Population characteristics	Clinical characteristics collected for the non-responder lung cancer patient donor used for FMT to mice included age, sex, BMI, histology, PD-L1 status, disease stage, line of treatment, and therapy received. Clinical characteristics collected for lung cancer patients included in plasma metabolomics analyses included age, sex, BMI, histology, PD-L1 status, disease stage, line of treatment, therapy received, and oncogenic driver mutation status. Clinical characteristics collected for lung cancer patient FMT donors included in BMI-stratified analyses included age, sex, BMI, histology, PD-L1 status, disease stage, line of treatment, therapy received, and oncogenic driver mutation status.
Recruitment	Patients were recruited to the CR-CHUM lung cancer biobank by a research nurse prior to immunotherapy treatment.
Ethics oversight	The protocol was approved by the Centre Hospitalier de l'Université de Montréal Ethics Review Board: CR-CHUM 17.035, C23046BR, and MP-02-2018-7132/17.035. All patient samples were obtained with informed consent.

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	Statistical methods were not used to predetermine sample size. Our previous work using the subcutaneous HKP1 model (Sorin et al, ref 21), as well as 15 wk Low Fat/High Fat models (McDowell et al, ref 28; Quail et al, ref 27) was used to determine sample size. Pilot studies using the subcutaneous HKP1 model with Low Fat, High Fat, and Western diet were performed (data not included in this manuscript).
Data exclusions	All mice were included in measurements performed prior to tumor injection (including metabolic, gut microbiome, and immune phenotyping). Mice euthanized prematurely due to ulceration were excluded from tumor growth curves and endpoint analyses (flow cytometry and 16s sequencing).
Replication	Experiments were repeated through independent cohorts, independent animal facilities, and independent mouse vendors, as described here and in the corresponding figure legends; all attempts at replication were successful. Longitudinal gut microbiome analysis included fecal samples from 2 separate cages for each diet but no cage effects were observed. Due to the number of experimental conditions for the immunotherapy trials (12 diets x 2 treatment groups), cohorts were staggered, with 2-3 diets enrolled on diet at a given time. For staggered cohorts, all sample collection, metabolic profiling, tumor injection and treatments were performed at the same time of day. Diet switch

experiments and mouse to mouse FMT experiments were replicated in two separate rooms within one animal facility at McGill University and yielded similar results. Three week Psyllium and High Fat ICI trials were performed in 3 cell lines and yielded similar results. Single-strain supplementation experiments were performed at a different institution (CR-CHUM), using two different cell lines, based on data from the experiments performed at McGill University. In addition, the functional monocolonization experiments used mice of a different age, sex, and supplier, further emphasizing the reproducibility of our findings.

Randomization Diets and treatments were assigned to each cage at random. All mice in one cage received the same treatment.

Blinding Blinding was not possible for this study due to differences in animal body weight and stool colour, which is indicative of the assigned diet.

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

Flow cytometry antibodies (antibody, fluorophore, clone, dilution, vendor, catalogue number)

CD45, APC Fire 810, 30-F11, 1:200, BioLegend, 103174
 CD3, Spark YG 570, 17A2, 1:400, BioLegend, 100266
 CD3, FITC, 17A2, 1:200, BioLegend, 100203
 CD3, Brilliant Ultra Violet 805, 17A2, 1:100, eBioscience, 368-0032-82
 CD4, PE/Fire 700, GK1.5, 1:200, BioLegend, 100484
 CD8, PE/Fire 640, 53-6.7, 1:200, BioLegend, 100790
 PD-1, APC/Cy7, 29F.1A12, 1:200, BioLegend, 135224
 CD19, PE-Cy5, 6D5, 1:100, BioLegend, 115510
 CD11a, Percp-Cy5.5, M17/4, BioLegend, 101123
 CD11b, BV570, M1/70, 1:100, BioLegend, 101233
 CD11c, BV750, N418, 1:200, BioLegend, 117357
 CD44, APC, IM7, 1:100, BioLegend, 103011
 MHC II, APC, M5/114.15.2, 1:100, BioLegend, 107614
 Ly6G, AF700, IA8, 1:200, BioLegend, 127622
 Ly6C, BV421, HK1.4, 1:200, BioLegend, 128031
 Foxp3, Pacific Blue, MF-14, 1:400, BioLegend, 126410
 Ki67, PE dazzle 594, 16A8, 1:100, BioLegend, 652428
 IFNg, BV711, XMG1.2, 1:50, BioLegend, 505835
 TNFa, AF700, MPX6-X722, BioLegend, 506338
 Isotype for Ki67, PE dazzle 594, RTK2758, 1:100, BioLegend, 400558

Ex vivo T cell activation

Ultra-LEAF anti-mouse CD3, BioLegend, 100238. Final concentration 3 µg/mL
 Ultra-LEAF anti-mouse CD28, BioLegend, 102116. Final concentration 3 µg/mL

in vivo Antibodies (anti-PD-1 treatment)

InVivoPlus anti-mouse PD-1 (CD279), clone:J43, BioXcell, BP0033-2, dose: 10 mg/kg
 InVivoMAb anti-mouse PD-1 (CD279), clone: RMPI-14, BioXcell, BE0146, dose: 250 µg per mouse
 InVivoPlus polyclonal Armenian hamster IgG, BioXcell, BP0091, dose: 10 mg/kg
 InVivoMAb Rat IgG2a, BioXcell, BE0089, dose: 250 µg per mouse

Validation

All flow cytometry antibodies were individually titrated prior to use to identify optimal concentration using splenocytes or tumour-infiltrating immune cells. For titration of anti-IFNg and anti-TNFa, titration was performed using T cells isolated from mouse spleen, and stimulated with anti-CD3/anti-CD28. Flow cytometry antibodies and corresponding gating strategies were further validated using fluorescence minus one (FMO) controls or Isotype controls. The flow cytometry panel was tested and validated on mouse blood and immune cells isolated from HKP1 tumours.

Eukaryotic cell lines

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

Cell line source(s)	The HKP1 cell line was provided by Dr. Vivek Mittal (Weill Cornell Medical College, NY, USA). The cell line was derived from a Kras G12D/+ Trp53-/- mouse (PMID: 25704820). The MCA-205 cell line was provided by Dr. John Stagg (CR-CHUM, Montreal, QC, Canada). The YUMM1.7 cell line was provided by Dr. Ian Watson (McGill University, Montreal, QC, Canada) who purchased directly from the American Type Culture Collection (ATCC). The YUMMER1.7 cell line was provided by Dr. Ian Watson, from Sonia del Rincon (McGill University, Montreal, QC, Canada) who received it from Marcus Bosenburg (Yale University, CT, USA).
Authentication	Cell lines were validated based on morphology via light microscopy. No additional authentication was performed.
Mycoplasma contamination	All cell lines tested negative for mycoplasma by PCR.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	For the experiments performed at McGill University, C57BL6/J male mice were purchased from Jackson Laboratory. For 15-week diet enrollment experiments, mice were purchased at 4 weeks of age and enrolled at 5 weeks of age. For 3-week diet enrollment experiments, mice were purchased at age 12-15 weeks. The DAT-supplemented water experiment was performed on 11-week old C57BL6/J male mice from the in-house colony at McGill University. For experiments performed at the Centre de recherche du Centre hospitalier de l'Université de Montréal, female SPF C57BL6/J mice were purchased from Charles River at 6 weeks of age, and female germ-free C57BL6/J mice were purchased from the International Microbiome Centre Germ-Free Facility (University of Calgary) at 7-10 weeks of age.
Wild animals	This study did not involve wild animals.
Reporting on sex	Male mice were used for all experiments at McGill University. Female mice were used for all experiments at the CR-CHUM.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All procedures were approved by institutional animal care committees, including the Institutional Animal Care committee (CRCHUM) or the Comparative Medicine and Animal Resources Centre (McGill). All experiments complied with the Canadian Council on Animal Care guidelines. Unless indicated otherwise, tumour assays were monitored for 15-24 days, with early humane endpoints including development of tumour ulceration, total volume exceeding 2.5 cm ³ , or onset of adverse clinical signs

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>

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

Detailed sample preparation is included in the methods. Briefly, red blood cell lysis was performed on both tumor and blood samples (BD Biosciences). Tumor samples were enriched using Percoll (Sigma-Aldrich). Cells were incubated with Zombie viability dye, followed by Fc block (BD Biosciences), followed surface antibody cocktail (BioLegend), followed by fixation buffer (ThermoFisher Scientific), followed by intracellular antibody cocktail, with appropriate washing after each step.

Instrument

Samples were acquired on the CYTEK Aurora 5 laser full spectrum analyzer (355/405/488/561/633 nm)

Software

During acquisition, the SpectroFlo software was used. All subsequent analysis was performed using FlowJo V 10.8.0.

Cell population abundance

No cell sorting was performed.

Gating strategy

Debris was excluded using SSC-A and FSC-A gates. Doublets were removed using FSC-H and FSC-A gate. Dead cells were excluded using Zombie Aqua Viability stain. Leukocytes were identified as CD45+. Lymphoid and myeloid cells were identified as CD11b- and CD11b+, respectively. B cells were identified as CD19+. T cells were identified as CD3+ with subsequent gating on CD4+ and CD8+ subgroups. PD-1+, CD44+, CD11a+ and Granzyme B+ T cells were identified using an FMO. Ki67+ and - T cells were determined using an isotype control. IFN γ + and TNF α + production from T cells was determined using an FMO and/or unstimulated control. All cells that were CD3- were then identified as dendritic cells (MHC II+ CD11c+), or other. The negative population expressing CD11b was then divided into 3 populations: high SSC myeloid cells - eosinophils, neutrophils (Ly6G+) or other myeloid cells (monocytes; Ly6G-). Monocytes were divided into gates based on Ly6C expression (-/low, high).

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