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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	For Bacterial DNA sequencing and microbiota analysis, DNA were collected from fecal samples with a PowerMag Microbiome RNA/DNA Isolation Kit (Mo Bio Laboratories, Inc.) using an epMotion 5075 liquid handling system.
Data analysis	We used the package mothur (v.1.38.1 and 1.39.1) and the MiSeq SOP(v.2.6.2.1) for 16S rRNA gene sequence data analysis. For FACS data analysis, FlowJo v10.2 were used. GraphPad v7.03 (GraphPad, La Jolla, CA, USA) and Excel2016 to do the graph figures and statistics.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The raw sequencing reads have been deposited at the NCBI Short Read Archive under BioProject ID PRJNA491725. Metabolomic data, mass spectral analytical

parameters and spectral raw data from the study, and metadata have been deposited in the NIH Common Fund's Data Repository and Coordinating Center under Metabolomics Workbench Project ID PR000728

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The samples size of 3-8 per group were used for in vitro studies and 5-10 were used for individual in vivo studies. In some cases, as stated in respective legends- data were combined from all experiments and shown collectively. These numbers were chosen based on hundreds of experiments in our lab (and from other published reports) for a sufficient dynamic range between the positive (allogeneic) and negative (syngeneic) control groups.The Mann-Whitney U test was used for the statistical analysis of in vitro data, and the Wilcoxon rank test was used to analyze survival data. A p value <0.05 was considered to be statistically significant.
Data exclusions	No data were excluded
Replication	All experiments-in vitro and in vivo, except for the microbiome sequencing studies, were done more than twice, in some cases more than 4 times.
Randomization	Samples were randomly allocated into groups based on the controls: naive (control for irradiation) versus syngeneic (control for allogeneic T cells) versus allogeneic animals.
Blinding	The GVHD scoring, the histopathology scoring and sequencing analyses were performed in a blinded manner and the data for respective groups was done after the codes were broken.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used for FACS analysis: FITC-conjugated moAbs to mouse H2Kd (clone SF1-1.1, #116606, 1:200): PerCP-Cy5.5-conjugated moAbs to mouse CD4 (clone GK1.5, #100434, 1:200), CD8 (clone 53-6.7, #100734, 1:200) and CD11b (clone M1/70, #101228, 1:200): PE-conjugated moAbs to mouse CD69 (clone H1.2F3, #104508, 1:200), Ly6C (clone HK1.4, #128008, 1:200), and CD45.1 (clone A20, , #110708, 1:200): APC-conjugated moAbs to mouse Ly6G (clone 1A8, #127614, 1:200), CD45.2 (clone 104, #109814, 1:200), and CD326 (clone G8.8, #118214, 1:200) (Biolegends, San Diego, CA) and FITC-conjugated mouse CD229.1 (clone 30C7, #560201, 1:200, BD Biosciences), PE-conjugated IFN-γ (clone XMG1.2, #505808, 1:200) (Biolegends), anti-Xbox binding protein-1 (XBP-1, M-186, sc-7160, 1:50), anti-aquapollin3 (AQP3, H-80, sc-20811, 1:50), anti-mucin 1(MUC1, F-19, sc-6826, 1:50) and anti-mucin2 (MUC2, R-12, sc-13312, 1:50), donkey anti-goat IgG-FITC (sc-2024, 1:100) or donkey anti-rabbit IgG-FITC (sc-2090, 1:100) (Santa Cruz Biotechnology, Dallas, TX).

For intestinal cell sorting, single cells were stained with 7AAD (#420404, 1:100), PerCP-Cy5.5-anti-CD45.2 (clone 104, #109828, 1:100), APC-anti-CD31 (clone 390, #102410, 1:100), PE-Cy7-anti-CD44 (clone IM7, #10303, 1.5:100), Pacific Blue-anti-CD24 (clone M1/69, #101820, 1.5;100) from Biolegend, PE-anti-CD166 (clone eBioALC48; #17-1161-80, 1.25:100, eBioscience), rabbit anti-GPR78 (clone ET-21; #G9043, 0.5:100, Sigma-Aldrich) and FITC-anti-rabbit IgG (sc-2359, 1:100, Santa Cruz Biotechnology) .

For immunohistochemitry, primary rabbit polyclonal antibody specific for muc2 (GTX100664, 1:1000, GeneTex) and primary anti-NLRP6 antibody (ABF29, 1:1000, Millipore) were used.

Validation

Validation of immunohistochemistry antibodies:
All antibodies used for immunohistochemistry were validated in-house. Positive controls (i.e., tissues that express the antigen of interest) were selected based on validation methods described by the manufacturer and/or through the literature. Isotype controls, no primary controls and negative tissue controls (i.e., tissues that do not express the antigen of interest) were used to determine specificity of the antibody. The antibody against MUC2 was validated in 8 peer-reviewed papers as stated on the manufacture's homepage, see Wang K et al 2014. Antibodies for NLRP6 were stated on the manufacture's homepage and also validated by our Nlrp6-/- mice.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

C57BL/6 (B6, H-2b, CD45.2+), B6 Ly5.2 (H-2b, CD45.1+), and C3H.sw (H-2b, CD229.1) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). BALB/c (H-2d) mice were purchased from Charles River Laboratories (Wilmington, MA, USA). B6-background NLRP6 knock out (Nlrp6-/-) mice (>8 generations) were provided by Dr. Chen Grace at University of Michigan .B6-WT and Nlrp6-/- germ-free (GF) mice were housed and bred at the GF mouse facility, and sterility was regularly verified by aerobic and anaerobic cultures, Gram stains and qPCR. Female mice were used for experiments during 8 to 12 weeks old. These animals were used for both in vitro and in vivo experimental bone marrow transplantation.

Wild animals

No wild animals were used in this study.

Field-collected samples

No Field-collected samples were used in this study.

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

Briefly, to analyze donor T cell expansion and activation markers, splenocytes from transplanted animals were re- suspended in FACS wash buffer (2% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and stained with conjugated monoclonal antibodies (moAbs). Intestinal epithelial cells were re-suspended in FACS buffer and stained with conjugated antibodies.

Instrument

BD Accuri C6 flow cytometer, Attune NxT flow cytometer and Moflo Astrios Cell sorter were used for data collection.

Software

C Flow Plus 1.0.264.15 version and FlowJo v10.2 were used for analyze the flow cytometry data.

Cell population abundance

n/a

Gating strategy

Gating strategy is provided in the Supplementary Information.

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