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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 *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	Trimmomatic (0.38) for data filter, HISHAT2(2.1.0) for mapping, featureCounts (2.0.0) for quantification, Illumina Novaseq 600 platform for 16S Metagenomic Sequencing Library Preparation, QIIME2 DADA2 plugin (v2020.11) for sequencing raw reads denoising, SILVA (v132) databases, miRDB
Data analysis	R (v3.3.1) for PCoA/PLS-DA analysis, clusterProfiler (3.14.3) for GO/KEGG enrichment, STAMP software (v2.1.3) for welch's t-test, DESeq2 for relative log expressionMiRDeep2

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 data supporting the findings of this study are available within the paper and its Supplementary Information.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	The sample size for this study was determined based on previous studies examining similar experimental endpoints and microbiota variability. A minimum of six biological replicates per group was chosen to ensure sufficient statistical power and to detect meaningful differences in microbiota composition and host intestinal responses. This sample size is consistent with standards for microbiome and intestinal function research.
Data exclusions	No data were excluded from the analysis. All collected data were processed and included in the results unless technical errors (e.g., sequencing failures) were identified.
Replication	All experiments were repeated at least three times, and all attempts at replication were successful. Consistent trends were observed across independent biological replicates.
Randomization	Samples and animals were randomly allocated to experimental groups to minimize bias. Allocation was performed using a random number generator, ensuring balanced distribution across treatment groups.
Blinding	Investigators were blinded to the group allocation during data collection and analysis to reduce potential bias. Group labels were anonymized during experimental procedures and data processing.

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	ZO-1 (ab221547/ab96587, Abcam, Bristol, UK), MUC13 (bs-10074R, Bioss, Boston, Massachusetts, USA), AB-PAS staining (Abcam, Bristol, UK), beta-actin (SC-47778, Santa Cruz, Dallas, Texas, USA), OCC (ab216327, Abcam, Bristol, UK), GAPDH (GTX100118, GENETEX, Texas, USA)
Validation	The primary antibodies used in this study were validated by the manufacturers for the specific species and applications indicated, such as Western blotting, immunohistochemistry, and immunocytochemistry. Validation information, including specificity and sensitivity data, is available on the manufacturers' websites and in their respective datasheets. Additionally, the antibodies used in this study are well-cited in published literature for similar experimental applications. For further confirmation, control experiments, such as omitting primary antibodies and comparing with housekeeping proteins (e.g., beta-actin), were performed to validate the results and ensure specificity.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Caco-2 cells (BCRC 60182) and LS174T cells (BCRC 60053) were obtained from the Bioresource Collection and Research Center (BCRC), Taiwan. Both cell lines were derived from human sources and are widely used for intestinal research.
Authentication	The cell lines were authenticated by the Bioresource Collection and Research Center (BCRC) using standard procedures, including DNA fingerprinting and species confirmation. No additional authentication was performed by the authors.
Mycoplasma contamination	All cell lines used in this study were tested for mycoplasma contamination using a PCR-based detection assay, and all results were confirmed negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines, as listed in 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	The study used C57BL/6 male mice (6-week-old) purchased from the Laboratory Animal Center of National Cheng Kung University, Taiwan. The mice were kept under specific-pathogen-free conditions in a controlled environment (12-hour light/dark cycle, temperature 22 ± 2°C, and humidity 50–60%). Nonfluorescent 57W5 feed (TestDiet, Saint Louis, MO, USA) was provided throughout the study.
Wild animals	This study did not involve wild animals.
Reporting on sex	The study exclusively involved male mice to control for potential sex-based variability in microbiota and intestinal barrier responses. The choice of using male mice was based on standard practice in similar studies, and no sex-based analysis was performed.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of National Cheng Kung University under approval numbers 108247 and 109330. All procedures were conducted in compliance with the ARRIVE guidelines and institutional policies for the ethical treatment of laboratory animals.

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

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	Mice were orally administered NP (100 nm, Fluoresbrite YG carboxylate microspheres) (Polysciences, Warrington, PA, USA). After 2 hours, fecal samples were collected and homogenized in 1 mL PBS, followed by centrifugation at 100 ×g for 10 minutes to remove debris. The supernatant was filtered through a 100 µm-mesh sieve and centrifuged at 8,000 ×g for 10 minutes to collect bacterial pellets.
Instrument	Flow cytometric sorting was performed using a FACSAria IIIu (Becton Dickinson, Bergen County, NJ, USA).
Software	The data were collected and analyzed using BD FACSDiva software (Becton Dickinson, NJ, USA). Post-analysis and visualizations were performed using FlowJo software (Tree Star, Ashland, OR, USA).
Cell population abundance	The abundance of bacteria positive for NP uptake was determined by fluorescence signal intensities, with percentages calculated relative to the total bacterial population. Post-sort purity was assessed, and results were validated through fluorescence microscopy (FV3000, Olympus, Shinjuku City, Japan) and 16S rRNA sequencing.
Gating strategy	Initial gates were set using forward scatter (FSC) and side scatter (SSC) to exclude debris and isolate the bacterial population. Positive gates for NP uptake were determined based on fluorescence intensity of the labeled microspheres. Gating boundaries were established using unstained bacterial controls and single-stain controls to define positive and negative populations.

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