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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<br>*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<br>*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

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| Data collection | Behavioral studies were analyzed with Ethovision XT15 (Noldus). Microbiome and metabolomic analyses were performed using MicrobiomeAnalyst 2.0 and MetaboAnalyst 6.0 and WebIDQ (Biocrates), respectively. For metabolite prediction model, gapseq (2024. 10. 30. committed version 8536a73, https://github.com/jotech/gapseq/tree/8536a73383bb902a25af6be7cf5f9f15a8e237c5) and sybil R package (v. 2.2.0) was used. Specific coding regarding IMB015 metabolite prediction model is deposited to Zenodo respository (https://doi.org/https://doi.org/10.5281/zenodo.15478642). |
| Data analysis | All software programs utilized for this study are available free or commercially. Statistical analyses were performed using GraphPad Prism 10 (Domatics). Flow cytometry analyses were performed using FlowJo 10 (Tree Star). Zeiss Zen 3.8 and ImageJ v1.54 (NIH) was used for imaging processing. Excel v1808 (Microsoft) and Word v1808 (Microsoft) were used for data and word processing, respectively. Illustrator v25.1 (Adobe) was used for generation of figures. |

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 authors declare that all data supporting this study are available within the article, source data, and supplementary information files. The data produced and used to generate the figures in this study are provided as source data in the Source Data file. Gating strategy for flow cytometry is available in the Supplementary Information file. The 16S rRNA sequencing, fecal metabolomic analyses, and metabolic flux prediction data for L. reuteri IMB015 are available at: https://doi.org/10.6084/m9.figshare.29109347. Raw 16S rRNA sequence data are deposited in the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA1268144. Custom coding used for metabolite prediction model is available at https://doi.org/10.5281/zenodo.15478642.

## 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.

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| 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.*<br>*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.*<br>*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).*<br>*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.)*<br>*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.

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| Sample size | Sample sizes were determined according to previously published literature in similar fields, as well as based on our experience in the relevant studies. Sample size calculations were not made prior to the study. Flow cytometry of immune populations in prior publications generally utilize three to five mice per group, with at least two experimental replicates. Individual experiment replicates were used to represent flow cytometry data, consisting of a minimum of 3 to 5 mice per group, without pooling of replicates, unless otherwise stated in the figure legends. To determine an appropriate sample size for behavioral experiments, prior studies conducting the same protocols were referenced. In prior publications, a range from minimum 10 (10.1038/s41586-019-1843-6) to 32 (10.1016/j.cell.2021.02.009) are used the three-chamber sociability and novelty tests. For the open field and marble burying tests, prior studies used 8 (10.1016/j.cell.2019.05.004) to 24 (10.1038/s41586-022-04396-8) mice per group. Each mice may display slightly varying behavioral tendencies during each experiment set, so in studies using behavioral experiments, a larger sample size (consisting of pooled replicates) is used to mitigate artifacts from outliers and more consistently demonstrate the overall behavior of each group. For 16S rRNA and metabolomic sequencing studies, 5-6 mice per group were sequenced to more robustly delineate the general effect of the gut microbiota and minimize any potential impact from fecal handling and |

processing procedures. For immunofluorescence imaging studies, a minimum of 3 mice and up to 5 mice were analyzed per each group, as previously demonstrated (10.1038/s41467-024-52974-3).

Data exclusions

Animals exhibiting disruptive behavior (such as jumping out of the behavior test arenas) or complete lack of participation during behavior tests (including completely standstill movement throughout the entire duration) were excluded prior to data analyses and these mice were not used for any other experiments.

Replication

Independent replicates were conducted. At least two replicates were completed for all experiments, and specific details to replicates are avilable in the figure legends.

Randomization

For all experiments, only male mice were used to alleviate any sex-based influence on behavior experiments, and mice were age-matched. Whenever possible, littermates were used for experiments. Social targets in the three-chamber tests were age- and weight- matched and randomly assigned to each tests.

Blinding

Flow cytometry and immunofluoresence imaging experiments were conducted in a non-blinded fashion, as the same gating and quantifiation strategies were used across all groups. The open field maze and three-chamber sociability tests were conducted in a non-blinded fashion, as the data was automatically recorded by EthoVision software. Scoring of the marble burying test was conducted in blinded fashion, with investigators blinded to group allocations during data analysis.

# 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

The following antibodies were used for immunofluorescence imaging: 2% goat serum (Cat. 005-000-121, Lot: 146807, Jackson ImmunoResearch), rabbit anti-c-Fos 1:1000 (Cat. 226 008, Lot: 1-17, Clone Rb108B5, Synaptic Systems), guinea pig anti-NeuN 1:1000 (Cat. ABN90P, Lot: 4209899, Clone A60, Millipore), goat anti-rabbit Alexa Fluor 488 1:500 (Cat. 111-545-003, Lot: 164290, Polyclonal, Jackson ImmunoResearch), and donkey anti-guinea pig Cy3 1:500 (Cat. 706-165-148, Lot: 127715, Polyclonal, Jackson ImmunoResearch). The antibodies and their clones used for flow cytometry are as follows: Fixable Viability Dye 1:1000 (Cat. 65-0866-18, Lot: 2010926, Invitrogen), CD4 – BUV395; 1:400 (Cat. 563790, Lot: 3184615, Clone GK1.5, BD), CD8a – BV605; 1:400 (Cat. 100744, Lot: B336700, Clone 53-6.7, Biolegend), CD45 – PerCP/Cy5.5; 1:400 (Cat. 103132, Lot: B295198, Clone 30-F11, Biolegend), TCRβ – APC/eF780; 1:200 (Cat. 47-5961-82, Lot: 2481347, Clone H57-597, Invitrogen), CD11b – A488; 1:400 (Cat. 101217, Lot: B307666, Clone M1/70, Biolegend), CD86 – BV605; 1:200 (Cat. 105037, Lot: B373866, Clone GL-1, Biolegend), CD206 – APC; 1:200 (Cat. 141708, Lot: B218214, Clone C068C2, Biolegend), Foxp3 – FITC; 1:200 (Cat. 11-5773-82, Lot: 2731698, Clone FJK-16a, Invitrogen), IFN-γ – APC; 1:100 (Cat. 17-7311-82, Lot: 2524225, Clone XMG1.2, Invitrogen), IL-1β – PE/Cy7; 1:100 (Cat. 25-7114-80, Lot: 2885871, Clone NJTEN3, Invitrogen), IL-10 – BV421; 1:100 (Cat. 505022, Lot: B375635, Clone JES5-16E3, Biolegend), IL-6 – PerCP/eF710; 1:100 (Cat. 46-7061-82, Lot: 2117496, Clone MP5-20F3, Invitrogen), TNFα – PE; 1:100 (Cat. 12-7321-82, Lot: 2473693, Clone MPX-XT22, Invitrogen). Anti-CD4 (Cat. BP0003-1, Lot: 798421M1, Clone GK1.5, BioXCell) was used for CD4 T cell depletion study.

Validation

c-Fos and Neun antibodies are extensively utilized in prior publications for immunofluorescence imaging of neurons within the brain. Antibodies used for flow cytometry are based from prior publications from both our and other groups. All antibodies have been documented in prior studies, and specific details to their validations can be found on the manufacturers' websites, as below:
https://www.bdbiosciences.com/en-gb/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-rat-anti-mouse-cd4.563790?tab=product_details
https://www.thermofisher.com/order/catalog/product/kr/en/65-0866-18
https://www.biolegend.com/nl-nl/products/brilliant-violet-605-anti-mouse-cd8a-antibody-7636
https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-mouse-cd45-antibody-4264?GroupID=BLG6829
https://www.thermofisher.com/antibody/product/TCR-beta-Antibody-clone-H57-597-Monoclonal/47-5961-82
https://www.biolegend.com/fr-fr/products/alexa-fluor-488-anti-mouse-human-cd11b-antibody-2700
https://www.biolegend.com/de-at/products/brilliant-violet-605-anti-mouse-cd86-antibody-7798
https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd206-mmr-antibody-7425?GroupID=BLG9506
https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/11-5773-82
https://www.thermofisher.com/antibody/product/IFN-gamma-Antibody-clone-XMG1-2-Monoclonal/17-7311-82
https://www.thermofisher.com/antibody/product/IL-1-beta-Pro-form-Antibody-clone-NJTEN3-Monoclonal/25-7114-80
https://www.biolegend.com/fr-fr/products/brilliant-violet-421-anti-mouse-il-10-antibody-7190

https://www.thermofisher.com/antibody/product/IL-6-Antibody-clone-MP5-20F3-Monoclonal/46-7061-82
https://www.thermofisher.com/antibody/product/TNF-alpha-Antibody-clone-MP6-XT22-Monoclonal/12-7321-82
https://www.sysy.com/product/226008
https://www.sigmaaldrich.com/US/en/product/mm/abn90p
https://www.jacksonimmuno.com/catalog/products/111-545-003
https://www.jacksonimmuno.com/catalog/products/706-165-148
https://bioxcell.com/invivoplus-anti-mouse-cd4-bp0003-1

## Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

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|---|---|
| Laboratory animals | All mouse experiments were approved by the POSTECH Institutional Animal Care and Use Committee (POSTECH-2020-0110-R4). Mice were maintained under SPF or GF conditions in sterile flexible film isolators (Class Biological Clean Ltd., USA) at the POSTECH Biotech Center animal facility. C57BL/6 (JAX: 000664; RRID:IMSR_JAX:000664) mice were purchased from Hyochang Science (Daegu, Korea) and maintained at POSTECH. BTBR T+ Itpr3tf/J (BTBR) (JAX: 002282; RRID:IMSR_JAX:002282) mice were obtained from The Jackson Laboratory and maintained at POSTECH. GF BTBR mice were derived embryonic transfer and GF status was monitored monthly by culture of cecal and fecal contents, as well as PCR of bacterial 16S rRNA using universal primers56. Mice were housed under a 12-h light/dark cycle and food and water were available ad libitum. Diets were composed of Purina Lab Rodent Diet (100000 95135, Cargill Agri Purina) for SPF and Teklad Global 18% Protein Rodent Diet (2018S, Inotiv) for GF mice. Autoclaved aspen shaving bedding (L) (KOATECH) was used for all animals. Cages and water were changed on a weekly basis, unless otherwise specified for treatment groups. Ambient temperature and humidity was maintained at 20 °C and 60% humidity, respectively. All behavior, flow cytometry, imaging, and sequencing experiments were performed with male mice between 7-8 weeks old. |
| Wild animals | No wild animals were used in this study. |
| Reporting on sex | Only male mice were used in this study primarily for two reasons. Firstly, clinical evidence indicate autism spectrum disorder has a higher prevalence among males. We chose to use male mice to bridge more translational relevance in our study. Secondly, only one mouse sex was used for behavior studies to prevent any sexual bias that could alter mouse behaviors, particularly during social tests. Female mice were not tested in this study, and no data were collected using them. |
| Field-collected samples | No field-collected samples were used in this study. |
| Ethics oversight | All mouse experiments were approved by the POSTECH Institutional Animal Care and Use Committee (POSTECH-2020-0110-R4). Mice were euthanized through the use of CO2 and cervical dislocation. For analyses of the brain, mice were administered with mixtures of ketamine and xylazine (80mg/kg i.p. and 10mg/kg i.p., respectively) 20 minutes prior to perfusion of the heart with PBS and/or and 4% paraformaldehyde. |

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

## Plants

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| 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

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| Sample preparation | Immune cells were harvested from the spleen, lymph nodes, small intestines, colon, and the brain of mice. Mice were |

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| Sample preparation | anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) prior to perfusion with 50ml of cold 1x PBS. The spleen was dissociated with 70 µm strainers and red blood cells were lysed with 1x RBC lysis buffer (eBioscience). For the small intestines and colon, tissues were processed as previously described59. Briefly, the intestines were longitudinally cut, washed with PBS, and then cut into 0.5 cm pieces. Tissue were incubated in FACS buffer containing PBS with 10 mM EDTA, 20 mM HEPES, 1 mM sodium pyruvate, and 3% FBS while being stirred for 20 min at 37 °C. Intestinal and brain tissues were minced and incubated with RPMI 1640 media with 3% FBS, 20 mM HEPES, 1 mM sodium pyruvate, 0.5 mg/ml of Collagenase D (Roche), and DNase I (Sigma-Aldrich) for 45 min at 37 °C. Immune cells were isolated using a 30% and 70% PercollTM (GE Healthcare) gradient and then washed with FACS buffer. |
| Instrument | Cell acquisition was performed on the Beckman Coulter CytoFLEX S. |
| Software | Flow optometry analyses was performed on FlowJo (Tree Star) and statistical analyses were performed with GraphPad Prism. |
| Cell population abundance | In the brain parenchyma, approximately a total of 300,000 to 1,000,000 cells were detected during flow cytometry, depending on the strain of mouse. Among them, approximately 20,000 to 40,000 cells were identified as microglia, while 150 to 2,000 cells were identified as either CD4 or CD8 T cells. In the spleen, approximately 2x10^6 to 1x10^7 CD4 or CD8 T cells were detected. The purity of these cells were greater than 96% from flow cytometry. |
| Gating strategy | Target immune cell population was gated based on their specific FSC and SSC profile. Live cells were gated from fixable viability dye (Invitrogen) negative populations. Specific gating strategy for the brain is available in the supplementary information. |

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