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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	Illumina NextSeq 500, StepOnePlus System (Thermo Fischer), PhenoMaster software (TSE Systems Inc.; software version 6.6.9), Tecan Infinite 200 Pro version 2.0, Zeiss ZEN Blue software version 3.5, Prairie View version 5.7.
Data analysis	RStudio (R Consortium; https://www.r-project.org, version 3.1); JMP Pro (JMP from SAS; https://www.jmp.com, version 16); Spike Tailor (Mathworks; Kaelberer et al., Science, 2018); Fiji version 2.9.0. CrunchMaster code is available at https://osf.io/7xgfd/ (see Software policy form), Excel 2024 (Microsoft).

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. The mm10 mouse reference genome is available

from GENCODE vM23/Ensembl 98. RNA-Seq data sets are available on the NIH Gene Expression Omnibus database (GSE288590).
Data from scRNAseq analysis of whole mouse brain (Allen Institute Brain Atlas) can be found at
http://doi:10.1038/s41586-023-06812-z or https://portal.brain-map.org/atlases-and-data/bkp/abc-atlas.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications studying ingestive behavior in mouse models (Han et al., Cell, 2018; Tan et al., Nature, 2020; Sclafani & Ackroff, Physiol. Behav., 2017).
Data exclusions	- For vagal nerve recordings: Throughout experiments, intralipid response was used as a positive control. For all flagellin and laser stimulation conditions, data were excluded if a stable intralipid response was not seen throughout the recording session. - For calcium imaging : Each recording session concluded with 50 mM KCl as an activity control. A response to KCl was defined as a ratio > 10% increase above baseline. Cells that did not reach this KCl threshold were not included in analyses. - For enema experiments, mice that consumed less than 0.01 g/g of food following a PBS enema were excluded from the experiments due to atypical feeding behavior following an overnight fast. -For meal pattern analysis, individual meals that exceeded a rate of 0.25 g/min. or 1 g total volume were excluded for being supraphysiological.
Replication	- For quantitative microscopy experiments, each in-situ labeling technique was performed and quantified in tissue samples from at least 3 individual mice of both sexes. All replication attempts were successful. - For vagal cuff experiments, the response to positive control intralipid was tested and replicated in between treatments in at least three mice per treatment to ensure within subject reproducibility. If the response changed substantially, the inclusion criterion was not met and therefore, the experiment was terminated. - For calcium imaging, the response to stimuli within the same cell was not replicated due to limitations of cell viability with repeated applications. To ensure reproducibility, experiments were conducted across several sessions. - In enema experiments, each experimental condition was replicated in at least 5 mice. Experiments were performed on mice from different litters, ages, and sexes to ensure reproducibility. -For meal pattern analysis, two independent experiments were run to ensure reproducibility.
Randomization	- In all experiments, animal allocation to experimental group was randomized. - All behavioral studies were counterbalanced across age and sex to control for variables including position in cage, order effect, and handedness. - In enema experiments, all included mice received a PBS stimulus, a flagellin stimulus, and in pharmacology experiments, mice received both PBS and flagellin stimuli in combination with pharmacological treatment. The order of stimulus delivery was randomized among different mice. This excludes germ-free mice, in which PBS was delivered first for minimizing bacterial colonization. Each stimulus delivery was separated by at least 48 hours from previous stimulus delivery.
Blinding	Experimenters were not blinded to treatment condition or genotype for ensuring the conduction of proper procedures. Each genotype received identical treatment regimes.

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

- Immunohistochemistry: Rb-Anti-PYY (1:250; Generated in the laboratory of Rodger Liddle - The PYY antibody was raised in rabbits using a synthetic peptide, KPEAPGEDASPEELSRYC, corresponding to amino acids 4–21 of mouse PYY, affinity-purified), Anti-GFP antibody (host = chicken) (1:500, Abcam; Cat#ab13970); Anti-Pgp9.5 (host = rabbit) (1:500; Abcam; CAT#ab27053), Anti-serotonin (host = goat) (1:500; Abcam; CAT#ab66047); Alexa Fluor 488 AffiniPure F(ab') Fragment Donkey Anti-Rabbit IgG (H+L) (1:250, Jackson ImmunoResearch; Cat#711-546-152; RRID#AB_2340619); Cy3 AffiniPure F(ab') Fragment Donkey Anti-Rabbit IgG (H+L) (1:250, Jackson ImmunoResearch;Cat#711-166-152; RRID#AB_2313568); Alexa Fluor 488 AffiniPure F(ab') Fragment Donkey Anti-Chicken IgG (H+L) (1:250, Jackson ImmunoResearch; Cat#703-546-155; RRID#AB_2340376); Dk-Anti-Gt-647 (1:500, Jackson Immunoresearch 705-606-147)

- In-situ hybridization: Mm-Tlr5 (cat#759 468888), Mm-Pyy-C3 (420681-C3), and Mm-Npy2r (cat# 515431) probes were purchased from ACD.

Validation

Rb-Anti-PYY antibody was extensively validated in colonic epithelial cells by Bohórquez et al., J Mol Hist, 2011; Ck-Anti-GFP was commercially validated for immunocytochemistry by the manufacturer (Abcam) and further validated for immunohistochemistry by PMID:29895301, Rb-Anti-Pgp9.5 was commercially KO validated and validated for immunohistochemistry by the manufacturer (Abcam), Gt-Anti-serotonin was commercially validated for immunocytochemistry by the manufacturer (Abcam) and further validated for immunohistochemistry by PMID: 25420775).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK-Blue™-mTLR5 cells were purchased from a commercial supplier (Invivogen). These cells were obtained by co-transfection of the murine TLR5 gene and an inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene into HEK293 cells.

Authentication

HEK-Blue™-mTLR5 cells were authenticated by the manufacturer, including validation for mTLR5 expression by RT-PCR and guaranteed stability for the up to 20 passages (cells were passaged in our lab up to 10 times per experiment).

Mycoplasma contamination

HEK-Blue™-mTLR5 cells were guaranteed mycoplasma-free by the supplier (Invivogen).

Commonly misidentified lines
(See ICLAC register)

HEK-293 cells are a commonly misidentified cell line. These cells were used as a reported cell line following their transfection with the murine TLR5 gene and an inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene for quantifying the levels of flagellin in mouse stool samples. Authentication of cells was performed by the supplier (Invivogen).

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

Male and female adult mice aged 6-20 weeks were used in all experiments. Mice were group housed in Duke University's Division of Laboratory Animal Resources, where they were kept on a 12-hour light-dark cycle (0700-1900) at with access to water and standard mouse chow (Purina 5001) ad-libitum, unless otherwise indicated in the manuscript Methods. The facility maintained an ambient temperature of 18-23°C and humidity of 40-60%. Mice used were: C57BL/6J (wild-type) (Jackson Lab; Stock #000664); PyyGFP (background = Swiss Webster) (Rodger Liddle, M.D.; Bohórquez et al., 2011); PyyCre ((background = C57BL/6J) (Jackson Lab; Stock #012706); CckGFP (background = Swiss Webster) (Rodger Liddle, M.D.; Wang et al., 2010); Neurod1Cre (background = C57BL/6J) (Jackson Lab; Stock #028364); LSL_tdTomato (background = C57BL/6J) (Jackson Lab; Stock #007914); LSL_Halo-YFP (background = C57BL/6J) (Jackson Lab; Stock #014539); LSL_ChR2-tdTomato (background = C57BL/6J) (Jackson Lab; Stock #012567); LSL_Salsa6f

	(background = C57BL/6J) (Jackson Lab; Stock #031968); Tlr5-KO (background = C57BL/6J) (Jackson Lab; Stock #028599); Myd88-KO (background = C57BL/6J) (Jackson Lab; Stock #00888); B6.Cg-Snap25tm3.1Hze/J (Jackson Lab; Stock #025111; B6.129(Cg)-Fostm2.1(cre/ERT2)Luo/J (Jackson Lab; Stock #030323).
Wild animals	No wild animals were used in this study.
Reporting on sex	Sex-related findings and differences are detailed in the manuscript with sample sizes indicated.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All experiments on mice were performed following approval by the Institutional Animal Care and Use Committee at Duke University Medical Center under the protocol A212-21-10. All collaborating laboratories followed the guidelines of this animal protocol.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A