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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	No specific software was used for the data collection.
Data analysis	All analyses in this study were conducted using tools that are publicly accessible. Specifically, GraphPad Prism (version 10.1.0) was utilized for both graphical representation and statistical evaluation of data derived from animal experiments. For the Small-Angle X-ray Scattering data reduction, PRIMUSqt from the ATSAS package (version 2.8.32) was used. For processing RNA sequencing data, a suite of software including SOAPnuke (version 1.5.2), HISAT2 (version 2.0.4), Bowtie2 (version 2.2.5), and RSEM (version 1.2.12) was employed. Additionally, analysis was performed with the use of R (version 4.3.0) and several of its packages: ggplot2 (version 3.4.4) for data visualization, dplyr (version 1.1.4) and tidyr (version 1.3.0) for data processing, along with DESeq2 (version 1.40.2) for differential expression analysis.

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

Anonymized clinical data of participants included in this study are provided in Supplementary Tables 6 and 7. Raw proteomics data that support the findings of this study have been deposited to the ProteomeXchange Consortium with the dataset identifier PXD057146 (human plasma) and MassIVE database (massive.ucsd.edu) with identifiers MSV000093785 (bacterial supernatants) and MSV000097951 (rat liver). The raw RNA-seq reads of rat liver tissue are available at the European Nucleotide Archive with accession number PRJEB89704. The predicted protein structure of RUMTOR_00181 is available at AlphaFold Protein Structure Database via identifier A5KIY5. Source data are provided with this paper.

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	This study includes two cohorts of human participants. Cohort 1: For quantification of RUMTOR_00181-carrying strains in human stools and estimation of the abundance of RORDEP1 and 2 in human plasma, respectively, 59 healthy adult men were recruited. Cohort 2: For the assessment of a potential impact of dietary habits on abundance of RT strains releasing RORDEPs in stool samples, the absolute cell counts of RUMTOR_00181-carrying strains were measured in 54 healthy adults (35 females and 19 males) following vegan or omnivorous diet.
Reporting on race, ethnicity, or other socially relevant groupings	For Cohorts 1 and 2, participants are Danish Caucasian.
Population characteristics	In Cohort 1, all participants are males (age 25 ± 4.0 years, body mass index (BMI) 23.8 ± 2.0 kg/m2) In Cohort 2, participants following vegan diet are aged 29.7 ± 1.7 years with BMI of 20.7 ± 5.3 kg/m2; participants following omnivorous diet are aged 30.4 ± 6.9 years with BMI of 21.6 ± 2.6 kg/m2.
Recruitment	For Cohort 1, participants were recruited in Denmark by advertisement in local newspapers, social media, and other online resources between 2016 and 2017. Inclusion criteria for the study were being male of about 18 to 35 years in good health, with stable weight, normal glucose metabolism, normal kidney and liver functions and normal blood pressure. Exclusion criteria from the study included all the people allergic to glucocorticoids, smokers, people that where prescribed oral medication in the previous 4 months, that consumed probiotics daily for the previous 4 months, with relevant dietary changes in the previous 2 months, that suffered either chronic or acute illness the previous 2 months as well, previous GI operation, mental disorders or individuals who were unable to give informed consent. Finally, all the individuals that required medical treatment during the study were also excluded from the study. For Cohort 2, participants were recruited from urban areas in Denmark by advertisement in local newspapers, social media, and other online resources from November 2013 to November 2014. Volunteers were eligible for inclusion if they were between 18 – 65 years of age and weight-stable (±1 kg, assessed by interview) for a minimum of 2 months. Vegan volunteers were eligible for inclusion in the study if they had been adherent to a vegan diet for a minimum of 1 year. Volunteers who received antibiotic treatment within 3 months, had known gastrointestinal disease or reported gastrointestinal symptoms at the time of the study, or followed a medically prescribed diet were ineligible for inclusion. Pregnant and lactating women were also ineligible. For both cohorts, there were no specific inclusion criteria that would introduce self-selection bias beyond standard voluntary participation in observational studies.
Ethics oversight	For Cohort 1, the study was approved by the Ethical Committees of the Capital Region of Denmark (H-16021787). For Cohort 2, the study was approved by the Ethical Committee of the Capital Region of Denmark (H 3 2012-145).

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 formal sample size calculation was performed, as this is an observational study. The sample sizes were determined by the number of eligible participants available during the study period. The sample sizes are considered as sufficient for exploratory analysis and to detect relevant associations, in line with similar observational studies (PMID: 30425247 and 30397356).
Data exclusions	No data was excluded.
Replication	As a hypothesis generating study, no explicit replication attempts were made. As for the mouse study, all experiments were at least duplicated and all attempts at replication were successful and supported the conclusions in the manuscript.
Randomization	As the studies in humans were observational, there was no allocation or randomization. The experiments in rodents employed a methodological approach where animals were assigned to groups in a random manner, with assignments based on their body weights prior to the initiation of the experimental interventions.
Blinding	Blinding was not performed in the human sample analysis; however, all human samples were labeled with unique, non-identifying codes and were not linked to any personal participant information, minimizing potential bias. For all other experiments, investigators were blinded to group allocation during both data collection and analysis to ensure objectivity and reduce potential observer bias.

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	Polycolonal: Rabbit anti-UCP1 (Abcam, ab10983) Rabbit anti-His-Tag Antibody (Cell Signal Technology, #2365) Monoclonal: Rabbit anti-beta-actin antibodies (Abcam, ab115777)[SP124]
Validation	Rabbit anti-UCP1 (Abcam, ab10983) has been validated in previous report PMID: 26772600. Rabbit anti-His-Tag Antibody (Cell Signal Technology, #2365) has been validated in PMID: 37674080. Rabbit anti-beta-actin antibodies (Abcam, ab115777) has been validated in PMID: 30649474.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human white preadipocytes (PromCell, #C-12732, Lot #456Z005.1, isolated from human omentum) Human osteoblasts (Promocell, #C-12720, Lot #445Z012.2, isolated from human femoral head) Human skeletal myoblasts (PromoCell, #C-12530, Lot #451Z031.15, isolated from human musculus pectoralis major) GLP-1 secreting human cell line NCI-H716 (ATCC, CCL-251) Rat insulin-secreting INS-1E 832/13 cells (a kind gift from Dr. Brice Emanuelli, Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen) Human epithelial Caco-2 cell line (ATCC, #HTB-37)
Authentication	Cell lines obtained from publicly available cell banks were not re-authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.

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	Male mice with a C57BL/6 background (specific pathogen-free grade) and with varying ages as described in detail in the manuscript were purchased from Janvier Labs (Le Genest-Saint-Isle, France). Male Sprague-Dawley (SD) rats, nine weeks old and weighing around 300 grams, were purchased from the Experimental Animal Center of Chongqing Medical University, China, or from Janvier Labs (France) with ages specified in the manuscript. Male BKS-Leprdb/db/JOrlRj mice (Mus musculus) were obtained from Janvier Laboratories (France) at eight weeks of age, with initial body weights ranging from 30 to 45 grams. In the comparative study of the effects of r-RORDEP1 and scrambled r-RORDEP1 on glucose tolerance, 45 male C57BL/6J mice at eight weeks of age were obtained from Gempharmatech (Nanjing, China).
Wild animals	No wild animals were used.
Reporting on sex	Male animals were used in this study.
Field-collected samples	This work does not utilize field-collected samples.
Ethics oversight	All protocols for mice experiments were approved by the Danish Animal Experiments Inspectorate (license numbers: 2018-15-0201-01491 for the intervention study with RT strain and 2020-15-0201-00568 for the GMO1 study), and the University of Copenhagen (project numbers: P20-392 for the intervention study with RT strain and P23-145 for the GMO1 study). The comparative study between r-RORDEP1 and scrambled r-RORDEP1 in mice was conducted with ethical approval ID: GP01-QD112-2024v1.2 at WUXI (https://www.wuxiapptec.com). The rat research protocols were reviewed and approved by the Danish Animal Experimentation Council for the intravenous infusion (approval ID: 2023-15-0201-01508) and assessment of r-RORDEP1 effects on incretin release (approval ID: 2023-15-0201-01393), and by the Animal Ethics Committee of Chongqing Medical University, China (approval ID: IACUC-CQMU-2024-0036) for the intraducdenal infusion study.

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA