Colosimo et al., 2019, Cell Host & Microbe 26, 273–282
August 14, 2019 © 2019 The Author(s). Published by Elsevier Inc.
https://doi.org/10.1016/j.chom.2019.07.002
Mapping Interactions of Microbial Metabolites with Human G-Protein-Coupled Receptors

Dominic A. Colosimo, Jeffrey A. Kohn, Peter M. Luo, Frank J. Piscotta, Sun M. Han, Amanda J. Pickard, Arka Rao, Justin R. Cross, Louis J. Cohen, and Sean F. Brady

1Laboratory of Genetically Encoded Small Molecules, the Rockefeller University, 1230 York Avenue, New York City, NY 10065, USA
2Donald B. and Catherine C. Marron Cancer Metabolism Center, Memorial Sloan Kettering Cancer Center, New York City, NY 10065, USA
3Division of Gastroenterology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York City, NY 10029, USA
4Lead Contact
*Correspondence: sbrady@rockefeller.edu
https://doi.org/10.1016/j.chom.2019.07.002

SUMMARY

Despite evidence linking the human microbiome to health and disease, how the microbiota affects human physiology remains largely unknown. Microbiota-encoded metabolites are expected to play an integral role in human health. Therefore, assigning function to these metabolites is critical to understanding these complex interactions and developing microbiota-inspired therapies. Here, we use large-scale functional screening of molecules produced by individual members of a simplified human microbiota to identify bacterial metabolites that agonize G-protein-coupled receptors (GPCRs). Multiple metabolites, including phenylpropanoic acid, cadaverine, 9-10-methylenehexadecanoic acid, and 12-methyltetradecanoic acid, were found to interact with GPCRs associated with diverse functions within the nervous and immune systems, among others. Collectively, these metabolite-receptor pairs indicate that diverse aspects of human health are potentially modulated by structurally simple metabolites arising from primary bacterial metabolism.

INTRODUCTION

Human bodies are home to diverse and ever-changing collections of bacteria. The ability of the microbiota to influence human health has been explored extensively (Knight et al., 2017). In addition to hypothesis-driven studies in model organisms, one of the most common methods for studying host-microbe interactions has featured “omics”-based analyses that have examined genomic, transcriptomic, proteomic, or metabolic differences between patient cohorts (Fritz et al., 2013; Gagliani et al., 2014; Hugenholtz and de Vos, 2018; Qin et al., 2012, 2014; Turnbaugh et al., 2009). Although these informatics-based methods have served as powerful tools for uncovering correlations between changes in the microbiota and health and disease, they are somewhat limited in their ability to reveal the mechanistic details of how the microbiota might alter mammalian physiology (Cani, 2018). Much of the influence the microbiota has on its human host is likely encoded in the collection of small molecules it produces or modulates (Brown and Hazen, 2017). The number of well-defined interactions between metabolites produced by human-associated bacteria and discrete human receptors is dwarfed by the number of reports attributing biological phenotypes to the microbiome, highlighting the need for a more systematic characterization of microbiota-encoded bioactive metabolites.

In the case of synthetic small molecules that have proved useful for therapeutically modulating human physiology (i.e., U.S. Food and Drug Administration (FDA) approved drugs) the majority (60%–70%) function through just three classes of receptors: G-protein-coupled receptors (GPCR), ion channels, or nuclear hormone receptors (Santos et al., 2017). Many of these same proteins bind endogenous signaling molecules that regulate a wide range of physiological responses (Rosenbaum et al., 2009). Based on the fact that these receptors play such an important role in how eukaryotic cells have evolved to translate external chemicals into biologic responses, it is likely that the microbiota affects host physiology by modulating these same receptors with secreted metabolites.

Although healthy humans are colonized by hundreds, if not thousands, of different bacterial species, the metabolic diversity they generate is likely limited by a high level of biosynthetic redundancy between bacterial species (Dorrestein et al., 2014). Partly because of this metabolic redundancy, it has been possible to use simplified human microbiomes (SIHUMIs) to model health and disease in murine models (Kovatcheva-Datchary et al., 2019; Subramanian et al., 2014). In lieu of exploring random individual commensal species, we sought to conduct a more in-depth investigation of GPCR-active microbiota-encoded metabolites using bacteria from a model SIHUMI that contained a taxonomically diverse collection of commensal, health-promoting, and pathogenic bacteria. This consortium, which is composed of seven bacteria, assembled as a tool for studying gastrointestinal (GI) inflammation in the context of a healthy bacterial flora fulfills these general criteria and was therefore selected for use in this study (Eun et al., 2014). Bacteria present in this SIHUMI consortium include beneficial bacteria (Lactobacillus plantarum, Bifidobacterium longum, and Faecalibacterium prausnitzii), non-pathogenic bacteria associated with disease (Bacteroides vulgatus and Ruminococcus gnavus), and clinically relevant pathogens (Escherichia coli LF-82 and Enterococcus faecalis).

Cell Host & Microbe 26, 273–282, August 14, 2019 © 2019 The Author(s). Published by Elsevier Inc. 273
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
We screened the metabolites produced by individually grown members of this SIHUMI consortium for agonism against 241 GPCRs. The resulting interaction map provides evidence, at the molecular level, for the existence of multiple potential microbiota metabolite-host interactions, many of which involve receptors that have been modulated therapeutically with synthetic small molecules. Our characterization of interactions predicted by this analysis led to the discovery of both previously unrecognized as well as known microbiota-encoded GPCR agonists. The structures of the active molecules we identified support the growing notion that simple bacterial metabolites arising from primary metabolic processes are likely to broadly impact human physiology.

RESULTS

Culturing Bacteria and GPCR Screening

Bacteria from the SIHUMI consortium were individually fermented under anaerobic conditions in separate large-scale (20 L) culture vessels (Figure 1). After 10 days of static fermentation at 37°C, hydrophobic resin was added directly to each culture. The resulting suspension was mixed to allow organic metabolites present in the fermentation broth to bind to the absorbent resin. Metabolite loaded resin was then collected by filtration, washed, and the bound metabolites were eluted with acetone. Each resulting crude metabolite extract was partitioned into 9 metabolite-rich fractions using reversed-phase flash chromatography. A small aliquot of each fraction, alongside an aliquot of the original crude extract, was arrayed for use in molecule isolation and structure elucidation studies. Although this pre-fractionation process increases the number of samples to be screened, it simplifies the complexity of the crude culture broth extracts, which should improve the signal in the primary screen thereby increasing the diversity of interactions that are identified and facilitating the downstream isolation of bioactive compounds (Butler et al., 2014; Wagenaar, 2008). In addition to the bacterial fermentations, media not inoculated with bacteria were processed under identical conditions to control for the possible bioactivity of small molecules derived directly from the media. The resulting library of bacterial metabolites was then screened with a cell-based assay for fractions that could agonize members of a panel of 241 GPCRs (Table S1). Specifically, a collection of recombinant cell lines engineered to measure β-arrestin recruitment by individual GPCR targets (β-arrestin recruitment assay) was used. For GPCRs with well-characterized endogenous ligands, a maximum value for β-arrestin recruitment (100%) was set by exposing the recombinant cell line to a known agonist (Table S1). In the case of orphan receptors (i.e., receptors without well-characterized endogenous ligands), β-arrestin recruitment was normalized relative to the vehicle control by assigning a 2-fold increase in raw luminescence as 100% activity. Hits were classified as such if a fraction induced a GPCR response to >30% of the control ligand (>50% for orphan GPCRs) and the comparable media control fraction showed <30% activity against the same GPCR (<50% for orphan GPCRs).

The bacterial fraction library induced β-arrestin recruitment above our hit threshold levels for 67 of the 241 individual GPCR reporter cell lines we tested (Figures 2A and 2B; Table S2). Of these 67 GPCRs, 54 did not show a strong background signal from the corresponding media control fraction, suggesting they were responding to bacterially encoded metabolites. Manual review of these 54 hits led us to de-prioritize 15 of these GPCR-fraction pairs because of high background of either the receptor or fraction (Table S3). The remaining 39 GPCRs were re-assayed in replicate; 22 of these GPCRs showed reproducible β-arrestin recruitment in response to 1 or more bacterial fractions (Figure 2C). Of these 22 validated interactions, only 8 reached our hit threshold level in an identical GPCR screen using crude bacterial culture broth extracts (Figure S1), thus supporting our original hypothesis that pre-fractionation methods would enable the discovery of a larger number of GPCR interactions.

A large number of the receptors that were reproducibly agonized by microbiota-encoded metabolites are also targeted by FDA approved drugs, indicating that receptors with proven physiological relevance are potentially modulated by bacterial ligands (Figure 2C). Based on data from the Human Protein Atlas, most of the receptors that reproducibly responded to bacterial metabolites are expressed at body sites regularly exposed to the microbiota (Figure 2C) (Uhlén et al., 2015). We focused on the characterization of agonists for receptors with demonstrated expression in either the GI tract or in immune cells that infiltrate
and survey the GI tract. In both cases, receptors would be activated as a result of metabolites accumulating in proximity to the GI epithelium and not require the metabolite to circulate peripherally in the bloodstream. To identify specific GPCR-active metabolites, we used bioassay-guided isolation to purify metabolites from the large-scale culture broth fractions and de novo structure elucidation methods to determine their structures.

**Bacterial Ligands for Hydroxycarboxylic Acid Receptors**

A number of receptors agonized in our screen are known to respond to bacterial ligands. As an initial validation exercise, we characterized activities expected to arise from well-known bacterial GPCR agonists. The hydroxycarboxylic acid receptors, GPR81, GPR109A, and GPR109B, are agonized by both human and bacterial ligands (Offermanns, 2017). Bioassay-guided fractionation of GPR109A active fractions from cultures of both *L. plantarum* and *R. gravis* yielded nicotinic acid (vitamin B3) as the active metabolite (Figures 3A and S2). Nicotinic acid, an essential nutrient acquired either through diet or gut bacteria, is the most extensively studied non-endogenous ligand for this receptor. Its ability to regulate lipid metabolism in hyperlipidemic patients is well established in the clinic (Garg et al., 2017). The identification of this well characterized and *in vivo* validated ligand-receptor pair suggests that the data generated in our screen have the potential to uncover biologically relevant metabolite GPCR interactions.

Fractons derived from cultures of both *E. coli* LF82 and *L. plantarum* agonized a second hydroxycarboxylic acid receptor, GPR109B. Bioassay-guided fractionation did not identify the endogenous ligand produced in humans, 3-hydroxyoctanoic acid but instead yielded phenylpropanoic acid as the active metabolite (Figures 3B and S2). This previously unknown GPR109B agonist elicited a similar GPCR response to 3-hydroxyoctanoic acid (Figure 3B). While the EC50 values for the known and bacterial ligands (304 and 208 μM, respectively) are higher than is often seen for endogenous GPCR ligands (Table S1), no more potent GPR109B agonists have been identified outside of those derived synthetically (Jung et al., 2007). Whether this is an inherent attribute of the receptor or represents a failure to identify the natural human ligand for this receptor remains to be seen.

Phenylpropanoic acid is not produced by human eukaryotic cells. Its presence in human fecal and sera samples has been attributed to either *de novo* biosynthesis by bacteria or microbial transformation of dietary compounds, most notably by species of *Clostridium* (Gao et al., 2009; Loke et al., 2009; Muñoz-González et al., 2013; Rowland et al., 2018). While we demonstrate that phenylpropanoic acid is a microbiota-derived agonist of GPR109B, in a screen of synthetic molecules, several aromatic D-amino acids were found to be GPR109B agonists that can trigger chemoattraction signaling pathways in leukocytes (Ahmed et al., 2009; Irukayama-Tomobe et al., 2009). In a quantitative analysis of human fecal water, phenylpropanoic acid was reported in healthy patients at an average concentration of 77.30 μg/mL (513 μM) (Gao et al., 2009). At this concentration, production of phenylpropanoic acid by gut bacteria would be high enough to agonize GPR109B.

**Aromatic Amines Agonize Neurotransmitter Receptors**

Our GPCR interaction map revealed numerous bacterial fractions that strongly agonized neurotransmitter receptors, a key component of the gut-brain axis (Figure 1C) (Mittal et al., 2017). Bacterially produced aromatic amines, most notably tryptamine, have recently been reported as agonists of neurotransmitter receptors, particularly serotonergic GPCRs (5-hydroxytryptamine receptors, or HTRs) (Bhattarai et al., 2018). A majority of bacteria in this SIHUMI produced fractions that agonized HTRs (Figure 3C). Isolation of the active metabolite yielded tryptamine, which was produced in varying quantities by members of this SIHUMI (Figure S3). These results agree...
with various reports that HTRs are responsive to a wide array of bacteria because of the generality of tryptamine production across species (Luqman et al., 2018).

In fractions from multiple bacterial species, we observed agonism of the D2-type dopamine receptors (DRDs), DRD2 and DRD3 (Figure 3D). Bioassay-guided isolation led to the aromatic amine tyramine as the major metabolite responsible for DRD agonism in these fractions (Figure S3). Tyramine arises from decarboxylation of tyrosine and differs from dopamine only by the absence of a second hydroxyl on the aromatic ring. It is reported to accumulate to $\mu$M levels in the GI tract, a phenomenon which has been attributed to production by human microbiota (Sridharan et al., 2014). While no biological significance has been assigned to the microbiota-dependent accumulation of tyramine in animal models, it is sufficiently potent that its observed concentration in the GI tract is high enough to agonize D2-subtype DRDs.

**Polyamine Ligand for a Histamine Receptor Family Member**

In contrast to the broad activation seen for DRDs and HTRs across extracts from all of the bacteria in this consortium, a specific response to fractions from *E. coli* LF82 was detected for a member of the histamine receptor (HRH) family, HRH4. Our inability to retain HRH4-activity when using hydrophobic chromatography during the bioassay-guided purification process suggested that the active molecule was highly polar. We did not, however, expect that the activity was due to bacterially produced histamine, as the active fraction did not agonize other HRH family receptors and we could not detect histamine by LC-MS or NMR. We ultimately found the polyamine cadaverine to be the metabolite responsible for HRH4 agonism (Figure 4A). The activity of cadaverine was confirmed using a commercial standard (EC50, 1.1 $\mu$M) (Figure 4B). In addition to cadaverine, bacteria commonly produce a number of other simple polyamines including agmatine, spermidine, and putrescine (Michael, 2018). To explore the promiscuity of HRH4 agonism by polyamines, we tested synthetic standards of these metabolites for the ability to induce $\beta$-arrestin recruitment by each member of the HRH receptor family. Agmatine and putrescine showed limited activity against HRH4 (Figure 4C), while spermidine did not show activity against any receptor in the family. The inability of humans to biosynthesize cadaverine suggests that the influence of cadaverine on histamine signaling pathways is likely specific to bacterial metabolism.

Cardaverine is produced by bacteria through the decarboxylation of lysine (Figure 4A), while agmatine and putrescine are derived from arginine. In a number of bacteria, including many *E. coli* strains and other species associated with the human microbiota (Table S4), cadaverine is encoded by both the constitutive *ldc* gene cluster as well as the *cad* gene cluster, which is induced at low pH (pH < 6.8) (Ma et al., 2017). High-level production of cadaverine by the CadA lysine decarboxylase is known to play a role in protecting against acid stress (Moreau, 2007). To confirm the presence of this acid-stress response in our *E. coli* LF82 strain, we constructed cadA knockout strains and observed cadA-dependent accumulation of extracellular cadaverine in response to growth media acidification (Figure S4). As the
pH of the digestive system varies longitudinally and features multiple acidic sections (e.g., cecum pH \( \approx 5.7 \)), increased production of cadaverine by cad gene cluster containing bacteria is likely to occur at numerous sites in the GI tract. The biological relevance of GI production of polyamines remains unclear; however, host responses to polyamines have been reported in various contexts (Kovács et al., 2019; Paik Jung and Bjeldanes, 1979). Interestingly, although HRH subtypes differ in their associated functions and their distribution throughout the human body, HRH4 is expressed in the GI tract and altered expression levels have been linked to inflammatory responses that are related to inflammatory bowel diseases and cancer (Coruzzi et al., 2012).

A growing number of studies have uncovered connections between gut microbiota and the nervous system (Dinan and Cryan, 2017; Sharon et al., 2016). Our exploration of microbiota-encoded neurotransmitter receptor agonists expands the mechanistic evidence for simple biogenic amines serving as potentially widespread modulators of the gut-brain axis (Luqman et al., 2018). These data imply that microbiota-dependent dopaminergic, serotonergic, and histaminergic responses likely represent general signaling events in the GI tract with varying activation profiles, depending on the specific collection of bacteria present in an individual’s microbiome.

**Structurally Distinct Lipids Agonize Diverse GPCRs**

Lipids, which represent diverse GPCR-active ligands (An et al., 2014; Round et al., 2011), predominantly elute very late in our fractionation protocol (Figure 5A). Based on the receptor interaction map, we could initially classify GPCRs as lipid responsive if they were agonized by the late lipid-enriched fractions of the extract library. A subset of receptors, including GPR120, CNR2, GPR171, and GPR132, responded broadly to the lipid fraction from most of the consortium, whereas other responses were specific to particular species (BAI1, NMU1R, and UTR2). HPLC-charged aerosol detection analysis of the lipid fractions indicated they contained not only mixtures of simple, saturated fatty acids but also more complex lipid species (Figure 5B).

Figure 4. Cadaverine Is a Bacterial Ligand for a Specific Histamine Receptor

(A) Top, schematic of cadaverine biosynthesis from L-lysine. Bottom, bacterial enzymes that catalyze this reaction include LydC, which is constitutively expressed and CadA, whose gene expression is induced at low pH.

(B) Dose-response curves for cadaverine against HRH family receptors.

(C) Dose-response curves (bottom) of bacterial polyamines (above) against HRH family receptors.

Receptor symbols are labeled as in (B). All dose-response curves were run in duplicate. Error bars are standard deviation. Error bars that are shorter than the height of the symbol are not shown.

Cell Host & Microbe 26, 273–282, August 14, 2019
The brain angiogenesis factor 1 (BAI1) receptor was agonized by lipid fractions from the Gram-negative bacteria in the consortium: E. coli and B. vulgatus. The E. coli LF82 lipid fraction showed the most potent agonism of BAI1, and therefore it was selected for further analysis. Bioassay-guided fractionation identified the BAI1 agonist as the cyclopropyl-containing lipid 9,10-methylenehexadecanoic acid (EC50, 11 μM). Synthetic 9,10-methylenehexadecanoic acid, but no saturated lipids we tested, agonized BAI1, confirming the specificity of the receptor reflected in the initial GPCR activity map (Figure 5C). The enzyme cyclopropane-fatty-acyl-phospholipid synthase (Cfa) uses the one-carbon donor S-adenosyl-L-methionine to generate cyclopropyl lipids from unsaturated fatty acids (Figure 5G). Cyclopropane-containing fatty acids are important membrane components in Gram-negative as well as mycobacterial bacteria (Table S5) (Wessjohann et al., 2003). Macrophages use BAI1 as a pattern recognition receptor to sense Gram-negative bacteria and to induce selective phagocytosis and antimicrobial responses; 9,10-methylenehexadecanoic acid may represent a previously unrecognized recognition motif for innate immune responses (Billings et al., 2016; Das et al., 2011, 2014; ref14; Lee et al., 2016).

Two peptide receptors, neuromedin receptor 1 (NMU1R), which mediates satiety and peristalsis in the gut (Brighton et al., 2004; Howard et al., 2000), and the vasoconstriction inducing urotensin 2 receptor (UTR2), responded specifically to lipid fractions generated from B. vulgatus. Isolation of the active metabolite yielded the anteiso-methyl branched-chain fatty acid 12-methyltetradecanoic acid (aiC15:0) (Figure 5D). Anteiso-fatty acids (ai) contain an alkyl branch at the ante-penultimate carbon in contrast to iso-fatty acids (i), which branch at the penultimate carbon. Both synthetic and natural aiC15:0, but no simple fatty acids we tested, agonized NMU1R (EC50, 125 μM) and UTR2 (EC50, 191 μM). Lipid sensitivity of NMU1R and UTR2 appears
specific to aiC15:0, as fatty acids with even slightly modified branching patterns (iC15:0) or carbon chain length (aIC17:0) displayed minimal agonist activity (Figures 5E and 5F). Methyl-branched fatty acids arise from the use of a branched primer in place of acetyl CoA in normal fatty acid biosynthesis. In the case of anteiso-methyl-branched fatty acid, 2-methyl-butyryl-CoA, which is derived from isoleucine is used to prime fatty acid biosynthesis (Figure 5G). The selectivity for branched primers lies with the β-ketoacyl acyl carrier protein synthase (KAS III or FABH) that carries out the first condensation in fatty acid biosynthesis.

Anteiso-methyl fatty acids are predominantly produced by Gram-positive FABH enzymes. (Kaneda, 1991; Lu et al., 2004) Roughly 10% of bacteria have lipid pools enriched in branched chain fatty acids (BCFAs) (Kaneda, 1991). B. vulgatus is among those bacteria enriched in BCFAs and maintains aiC15:0 as ~30% of its total fatty acid repertoire (Table S5) (Mayberry et al., 1982).

Bacteria are known to produce diverse and oftentimes taxa-specific collections of lipids. The examples described here from examining even this minimized model microbiome suggest the potential for markedly different receptor activation profiles, and hence biological consequences depending on the specific lipid signature encoded by an individual’s microbiome. For BA11, NMU1R, and UTR2 our data suggest that they differentially respond to lipids produced by largely Gram-positive or Gram-negative bacteria, indicating that their activities will fluctuate with changes in the gross taxonomic composition of a microbiome.

**Analysis of Mice Colonized with the Seven Strain SIHUMI Consortium**

In parallel with our *in vitro* screen studies, we used targeted mass spectrometry-based metabolomics to compare germ-free and SIHUMI-consortium-colonized mice. For this analysis, cultures of individually grown bacteria from the consortium were combined and the mixed sample was gavaged into germ-free C57BL/6 mice. PCR-based species analysis of DNA extracted from the stool of animals 3 days post-inoculation confirmed their colonization by the consortium (Figure S5). Ten days post-colonization the lumen material (cecal stool) were collected from the
DISCUSSION

Phenylpropanoic acid, cadaverine, 9–10-methylenehexadecanoic acid, and 12-methyltetradecanoic acid add to a growing list of structurally simple molecules that are capable of modulating human signaling pathways that underlie diverse clinically relevant areas of physiology, including immune recognition, neurotransmission, and inflammation (Rooks and Garrett, 2016; Sharon et al., 2016). The biosynthetic simplicity of these metabolites combined with their abundant starting materials and demonstrated roles in fundamental bacterial processes likely drives their high titers in the gut and potential broad biological relevance. Expanding functional screening to include not only more bacteria but also additional culture conditions and receptor families will undoubtedly provide additional insight into the biochemical mechanisms and small molecules underlying human-microbiome interactions. For example, a study that was published while the work reported here was under review examined a different collection of bacteria and identified different GPCR-active metabolites (Chen et al., 2019). Advanced methods in laboratory culturing techniques now allow for a majority of gut bacteria to be cultured from fecal samples (Browne et al., 2016; Forster et al., 2019). Systematic functional screening of metabolites produced by this growing collection of bacteria is likely to be a rewarding avenue for developing mechanistic hypotheses that can be tested in specific animal models.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **LEAD CONTACT AND MATERIALS AVAILABILITY**
  - Materials Availability Statement
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Mouse Studies
  - Microbe Strains

- **METHOD DETAILS**
  - Media Construction
  - Cultivation of Bacteria
  - Fractionation of Bacterial Extracts
  - GPCR Assays
  - Biosynthesis Analysis
  - Mutation of cadA in *E. coli* LF82
  - Cadaverine Induction
  - High-Resolution Mass Spectrometry
  - Murine Work
  - Metabolite Quantitation by Mass Spectrometry
  - Method 1: GC-nCl-MS with PFB Derivatization
  - Method 2: LC triple quadrupole with reverse phase chromatography
  - Method 3: LC Q-TOF with HILIC chromatography
  - Tyramine
  - Tryptamine
  - Phenylpropanoic acid
  - Cadaverine
  - 9,10-methylenehexadecanoic acid
  - 12-methyltetradecanoic acid

- **QUANTIFICATION AND STATISTICAL ANALYSIS**

- **DATA AND CODE AVAILABILITY**

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.chom.2019.07.002.

ACKNOWLEDGMENTS

All bacterial strains were generously provided by the labs of Daniel Mucida, Howard Hung, and Balfour Sartor. High-resolution mass spectrometry of purified compounds was performed by Caitlin Steckler and Henrik Molina of the Rockefeller University Proteomics Core. Bioinformatics was performed by Thahmina Ali. We are grateful to C. Fermin, E. Vazquez, and G. Escano in the Precision Immunology Institute at the Icahn School of Medicine at Mount Sinai (PriSM) Gnotobiotic facility and Microbiome Translational Center for their help with gnotobiotic experiments. We thank H. Liu for assistance with LC-MS studies. Funding was provided by the Bill and Melinda Gates Foundation (OPP1168674) and the National Institutes of Health (5R01AT009562-02).

AUTHOR CONTRIBUTIONS

D.A.C., L.J.C., and S.F.B. designed the experiments. D.A.C., J.A.K., and P.M.L. performed the analytical chemistry. F.J.P. designed and performed the gene mutation studies. S.M.H. and L.J.C. performed the murine experiments. A.J.P., A.R., and J.R.C. designed, performed, and analyzed the mass spectrometry experiments. D.A.C. and S.F.B. wrote the manuscript.

DECLARATION OF INTERESTS

S.F.B. is the founder of LODO Therapeutics. Patents related to this work are being filed.

Received: May 9, 2019
Revised: June 9, 2019
Accepted: July 11, 2019
Published: August 1, 2019

REFERENCES

Ahmed, K., Tunaru, S., Langhans, C.D., Hanson, J., Michalski, C.W., Köllker, S., Jones, P.M., Okun, J.G., and Offermanns, S. (2009). Deorphinization of
GPR109B as a receptor for the β-oxidation intermediate 3-OH-octanoic acid and its role in the regulation of lipolysis. J. Biol. Chem. 284, 21928–21933.

An, D., Oh, S.F., Olszak, T., Neves, J.F., Avci, F.Y., Erturk-Hasdemir, D., Lu, X., Zeissig, S., Blumberg, R.S., and Kasper, D.L. (2014). Sphingolipids from a symbiotic microbiota drive homeostasis of host intestinal natural killer T cells. Cell 156, 123–133.

Bhattarai, Y., Williams, B.B., Battaglioli, E.J., Whitaker, W.R., Till, L., Grover, M., Linden, D.R., Akiba, Y., Kandimalla, K.K., Zachos, N.C., et al. (2018). Gut microbiota-produced tryptamine activates an epithelial G-protein-coupled receptor to increase colonic secretion. Cell Host Microbe 23, 775–785.

Billings, E.A., Lee, C.S., Owen, K.A., D’Souza, R.S., Ravichandran, K.S., and Casanova, J.E. (2016). The adhesion GPCR BAII1 mediates macrophage ROS production and microbiobial activity against Gram-negative bacteria. Sci. Signal. 9, ra14.

Brighton, P.J., Szekeres, P.G., and Williams, G.B. (2004). Neurelin. J. Biol. Chem. 279, 231–248.

Brown, J.M., and Hazen, S.L. (2017). Targeting of microbiota-derived metabolites to improve human health: the next frontier for drug discovery. J. Biol. Chem. 292, 8560–8568.

Brown, H.P., Forster, S.C., Anonye, B.O., Kumar, N., Neville, B.A., Stares, M.D., Goulding, D., and Lawley, T.D. (2016). Culturing of ‘unculturable’ human microbiota reveals novel taxa and extensive sporeulation. Nature 533, 543–546.

Butler, M.S., Fontaine, F., and Cooper, M.A. (2014). Natural product libraries: assembly, maintenance, and screening. Planta Med. 80, 1161–1170.

Cani, P.D. (2018). Human gut microbiome: hopes, threats and promises. Gut 67, 1716–1725.

Chen, H., Nwe, P.K., Yang, Y., Rosen, C.E., Bielecka, A.A., Kuchroo, M., Cline, G.W., Kruse, A.C., Ring, A.M., Crawford, J.M., et al. (2019). A forward chemical genetic screen reveals gut microbiota metabolites that modulate Host Physiology. Cell 177, 1217–1231.

Coruzzi, G., Adami, M., and Pozzoli, C. (2012). Role of histamine H4 receptors in the gastrointestinal tract. Front. Biosci. (Schol. Ed.) 4, 226–239.

Das, S., Owen, K.A., Ly, K.T., Park, D., Black, S.G., Crawford, J.M., et al. (2019). A forward chemical genetic screen reveals gut microbiota metabolites that modulate Host Physiology. Cell 177, 1217–1231.

Dinan, T.G., and Cryan, J.F. (2017). The microbiome-gut-brain axis in health and disease. Gastroenterol. Clin. North Am. 46, 77–89.

Dorrestein, P.C., Mazmanian, S.K., and Knight, R. (2014). Finding the missing links among metabolites, microbes, and the host. Immun. 40, 824–832.

Eun, C.S., Mishima, Y., Wohlgemuth, S., Liu, B., Bower, M., Carroll, I.M., and Sartor, R.B. (2014). Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-10−/− mice. Infect. Immun. 82, 2239–2246.

Forster, S.C., Kumar, N., Anonye, B.O., Almeida, A., Viciani, E., Stares, M.D., Dunn, M., Mkandawire, T.T., Zhu, A., Shao, Y., et al. (2019). A human gut bacterial genome and culture collection for improved metagenomic analyses. Nat. Biotechnol. 37, 186–192.

Fritz, J.V., Desai, M.S., Shah, P., Schneider, J.G., and Wilmes, P. (2013). From meta-omics to causality: experimental models for human microbiome research. Microbiome 1, 14.

Gagliani, N., Hu, B., Huber, S., Elinav, E., and Flavell, R.A. (2014). The fire within: microbes inflame tumors. Cell 157, 776–783.

Gao, X., Pujos-Guillot, E., Martin, J.F., Galan, P., Juste, C., Jia, W., and Sebedio, J.L. (2009). Metabolite analysis of human fecal water by gas chromatography/mass spectrometry with ethyl chloroformate derivatization. Anal. Biochem. 393, 163–175.
metabolites in human feces after moderate red wine intake. J. Agric. Food Chem. 61, 9470–9479.

Offermanns, S. (2017). Hydroxy-carboxylic acid receptor actions in metabolism. Trends Endocrinol. Metab. 28, 227–236.

Olson, K.R., and Eglen, R.M. (2007). Beta galactosidase complementation: a cell-based luminescent assay platform for drug discovery. Assay Drug Dev. Technol. 5, 137–144.

Paik Jung, H.Y., and Bjeldanes, L.F. (1979). Effects of cadaverine on histamine transport and metabolism in isolated gut sections of the guinea-pig. Food Cosmet. Toxicol. 17, 629–632.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490, 55–60.

Qin, N., Yang, F., Li, A., Prifti, E., Chen, Y., Shao, L., Guo, J., Le Chatelier, E., Yao, J., Wu, L., et al. (2014). Alterations of the human gut microbiome in liver cirrhosis. Nature 513, 59–64.

Rooks, M.G., and Garrett, W.S. (2016). Gut microbiota, metabolites and host immunity. Nat. Rev. Immunol. 16, 341–352.

Rosenbaum, D.M., Rasmussen, S.G.F., and Kobilka, B.K. (2009). The structure and function of G-protein-coupled receptors. Nature 459, 356–363.

Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., Chatila, T.A., and Mazmanian, S.K. (2011). The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974–977.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., and Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. Eur. J. Nutr. 57, 1–24.

Russell, W.R., Duncan, S.H., Scobie, L., Duncan, G., Cantlay, L., Calder, A.G., Anderson, S.E., and Flint, H.J. (2013). Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. Mol. Nutr. Food Res. 57, 523–535.

Santos, R., Ursu, O., Gaulton, A., Bento, A.P., Donadi, R.S., Bologa, C.G., Karlsson, A., Al-Lazikani, B., Hersey, A., Oprea, T.I., et al. (2017). A comprehensive map of molecular drug targets. Nat. Rev. Drug Discov. 16, 19–34.

Sharon, G., Sampson, T.R., Geschwind, D.H., and Mazmanian, S.K. (2016). The central nervous system and the gut microbiome. Cell 167, 915–932.

Sridharan, G.V., Choi, K., Klemashevich, C., Wu, C., Prabakaran, D., Pan, L.B., Steinmeyer, S., Mueller, C., Yousofshahi, M., Alanz, R.C., et al. (2014). Prediction and quantification of bioactive microbiota metabolites in the mouse gut. Nat. Commun. 5, 5492.

Sriram, K., and Insel, P.A. (2018). G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? Mol. Pharmacol. 93, 251–258.

Subramanian, S., Huq, S., Yatsunenko, T., Haque, R., Mathuz, M., Alam, M.A., Beneza, A., DeStefano, J., Meier, M.F., Muegge, B.D., et al. (2014). Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 510, 417–421.

The Human Microbiome Project, C., Huttenhower, C., Gevers, D., Knight. R., Abubucker, S., Badger, J.H., Chinwalla, A.T., Creasy, H.H., Earl, A.M., FitzGerald, M.G., et al. (2012). Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214.

Tumbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., et al. (2009). A core gut microbiome in obese and lean twins. Nature 457, 480–484.

Uhlén, M., Fagerberg, L., Hallström, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., et al. (2015). Proteomics. tissue-based map of the human proteome. Science 347, 1260419.

Wagenaar, M.M. (2008). Pre-fractionated microbial samples—the second generation natural products library at Wyeth. Molecules 13, 1406–1426.

Wessjohann, L.A., Brandt, W., and Thiemann, T. (2003). Biosynthesis and metabolism of cyclopropane rings in natural compounds. Chem. Rev. 103, 1625–1648.
## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and Virus Strains** | | |
| Escherichia coli LF82 | Gift of Daniel Mucida | N/A |
| Enterococcus faecalis OG1RF | Gift of Daniel Mucida | N/A |
| Lactobacillus plantarum WCFS1 | Gift of Daniel Mucida | N/A |
| Faecalibacterium prausnitzii A2-165 | Gift of Daniel Mucida | N/A |
| Bifidobacterium longum ATCC 15707 | Gift of Daniel Mucida | N/A |
| Bacteroides vulgatus ATCC 8482 | Gift of Daniel Mucida | N/A |
| Ruminococcus gnavus ATCC 29149 | Gift of Daniel Mucida | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| tyramine | Alfa Aesar | CAT#: A12220 |
| tryptamine | Alfa Aesar | CAT#: A11030 |
| 12-methyltetradecanoic acid | ChemCruz | CAT#: sc-213601 |
| cis-9,10-methylenehexadecanoic acid | Avanti Polar Lipids, Inc. | CAT#: 857500C |
| cadaverine | Sigma Aldrich | CAT#: C8561 |
| nicotinic acid | Sigma Aldrich | CAT#: N4126 |
| hydrocinnamic acid | Sigma Aldrich | CAT#: 135232 |
| phenol-D6 | Cambridge Isotope Laboratories | CAT#: DLM-370-5 |
| palmitic acid-d31 | Cambridge Isotope Laboratories | CAT#: DLM-215 |
| **Critical Commercial Assays** | | |
| ZymoBIOMICS DNA/RNA Miniprep Kit | ZYMO Research | CAT#: R2002 |
| PathHunter screen | Eurofins DiscoverX | N/A |
| **Deposited Data** | | |
| Protein expression data | Human Protein Atlas (Uhelen et al., 2015) | https://www.proteinatlas.org |
| Human Microbiome Project Genome Database | Human Microbiome Project Consortium, 2012 | https://hmpdacc.org |
| **Experimental Models: Organisms/Strains** | | |
| Germ free C57BL/6 mice | Gnotobiotic facility and Microbiome Translational Center | N/A |
| **Oligonucleotides** | | |
| 27F: AGAGTTTGATCMTGGCTCAG | Integrated DNA Technologies | N/A |
| 1492R: GGTTACCTTGTTACGACTT | Integrated DNA Technologies | N/A |
| Escherichia coli LF82 FWD: GTTAAATACCTTTGCTCATTGA | Integrated DNA Technologies | N/A |
| Escherichia coli LF82 REV: ACCAGGGTATATAAATCCGTT | Integrated DNA Technologies | N/A |
| Enterococcus faecalis OG1RF FWD: CCCTATTGATTTGCCATCATATT | Integrated DNA Technologies | N/A |
| Enterococcus faecalis OG1RF REV: ACTCTTGTAGTCCTCATTGT | Integrated DNA Technologies | N/A |
| Lactobacillus plantarum WCFS1 FWD: AGCAGTAGGGAATTTCTCCA | Integrated DNA Technologies | N/A |
| Lactobacillus plantarum WCFS1 REV: CACCGCTAGACATGGGAG | Integrated DNA Technologies | N/A |
| Faecalibacterium prausnitzii A2-165 FWD: CCCCTATGAGGCCGACTG | Integrated DNA Technologies | N/A |
| Faecalibacterium prausnitzii A2-165 REV: GTGCAGAGATGCTACAGAC | Integrated DNA Technologies | N/A |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for reagents may be directed to and will be fulfilled by the Lead Contact, Sean F. Brady (sbrady@rockefeller.edu).

Materials Availability Statement
This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mouse Studies
In-house bred germ free C57BL/6 mice were maintained in sterile isolators with autoclaved food and water in the Gnotobiotic Facility of the Faith Lab at Mount Sinai. 6-week-old mice were used for all experiments (3M and 3F in the treatment group, 5M and 1F in the control group). All experimental procedures were approved by the Animal Care and Use Committee of The Icahn School of Medicine at Mount Sinai (PI Cohen IACUC-2016-0491).

Microbe Strains
Details of SIHUMI microbe strains can be found in the Key Resources Table. Anaerobic bacteria were cultured in an incubator set to 37° C placed inside of vinyl anaerobic chamber (Coy) with a gas mix of 5% CO₂, 5% H₂, and 90% N₂. The cadA-KO strain of E. coli LF82 was grown aerobically at 37° C in LB with indicated antibiotics during genetic manipulation for practicality.

METHOD DETAILS

Media Construction
LBM media: LBM media was derivative of a media recipe previously utilized in our laboratory. For 1 L media: Bring 17 g/L brain heart infusion, 5 g/L yeast extract, 200 mg MgSO₄-7H₂O, 100 mg MnCl₂-4H₂O up in 800 mL deionized water and autoclave for 30 minutes liquid cycle. After coming to room temp, add supplements (final concentrations: 5 μg/L hemin, 1 g/L maltose, 1 g/L cellobiose, and
Fractionation of Bacterial Extracts

Crude extracts (~1-3 g/12L) were re-suspended in ~300 mL methanol and the soluble material was decanted into a 500 mL round bottom flask (rfb). Free C18 resin (2-3 g) was added and the slurry was evaporated under reduced pressure using a rotary evaporator with temperature set to 25°C (Buchi). The dry material was collected from the rfb, packed semi-tightly into a 50 g cartridge, and capped with a passive frit (Teledyne). This material was chromatographed over a 150 g C18 Gold (Teledyne ISCO) using a solvent system of water (Solvent A) and methanol (Solvent B), with no acid added, with the following conditions. 5 column volumes (CV) of 5% B, 5% C and L-cysteine which should be made fresh. Use autoclaved deionized water to bring final volume to 1 L. For culturing anaerobes: place media in anaerobic chambers for at least 48 hrs to allow diffusion with anaerobic gas. When assessing cadaverine induction, media was acidified with HCl then sterile filtered.

GPCR Assays

GPCR assays were measured by Eurofins DiscoverX using the PathHunter® β-Arrestin assay (Olson and Egle, 2007). This assay uses β-galactosidase (β-Gal) that has been split into two inactive portions as a reporter to measure the activation of a GPCR. The β-Gal fragments are called EA for Enzyme Acceptor and ED for Enzyme Donor.(US Patent: US20090098588A1) Using these fragments, a unique reported cell line was created for each GPCR of interest. In each unique cell line the EA fragment is fused to β-Arrestin and the ED fragment is fused to the GPCR of interest. Upon GPCR activation, β-Arrestin recruitment to the receptor physically co-localizes the ED and EA fragments thereby restoring β-Gal activity. β-Gal complementation is measured using chemiluminescent PathHunter® Detection Reagents. For our initial screen (Figure 2) all 80 culture broth extract fractions were screened in singleton against the Eurofins DiscoverX gpcrMAX (168 GPCRs, Table S2) and orphanMAX (73 GPCRs, Table S2) panels. All subsequent validation and bioassay guided fraction studies were run in at least duplicate using individual reporter cell lines for specific GPCRs of interest.

Eurofins DiscoverX Generic Agonist Protocol

1. Sample is added to individual GPCR reporter cell lines grown in microtiter plates. Cells are incubated at 37°C or room temperature for 90 or 180 minutes. 2. Assay signal is generated through addition of 12.5 or 15 μL (50% v/v) of PathHunter Detection reagent

Cultivation of Bacteria

Bacterial strains of the SIHUMI consortium used listed in the Key Resources Table. Cultures of <1 L: anaerobic bacteria were cultured in an incubator set to 37°C placed inside of vinyl anaerobic chamber (Coy) with a gas mix of 5% CO₂, 5% H₂, and 90% N₂. Cultures of >1 L: When cultivating bacteria for construction of bacterial extract library, bacteria were inoculated in 1 L or 2 L media bottles (Chemglass) inside anaerobic chamber, then sealed with anaerobic septa (Chemglass) and moved into large walk-in 37°C incubator constructed from a 6x6x12 light protective tent (APOLLO® HORTICULTURE) outfitted with a regulator (INKBIRD®), heat source (VORNADO®), and ventilation system (IPOWER®). Freezer stocks of the SIHUMI cohort were generously donated by the Muuida laboratory (Rockefeller University). Freezer stocks were thawed and bacteria were cultivated overnight in LBM media until turbid. These bacteria were streaked onto LBM agar plates and upon growth, single colonies were picked, cultivated overnight, and genotyped (GeneWiz). Upon confirmation of genetic identity, these same cultures were used to generate colony 20% glycerol stocks that would be used for the entirety of the study. Strain specific primers were used to allow PCR-based identification of each strain and are listed in the Key Resources Table. For large scale fermentations the following protocol was used: Bacterial stocks were thawed and used to inoculate 5 mL LBM liquid cultures that were cultivated overnight. The next day, species specific primers were used to amplify (GeneWiz). Upon confirmation of genetic identity, the 500 mL culture was used to inoculate 12 L of LBM media at a 1:100 inoculation ratio. After turbidity was reached, an aliquot of the 500 mL culture was removed and PCR was performed with universal 16s rRNA primers 27F and 1492R (sequences in Key Resources Table). The PCR product was subject to Sanger sequencing (GeneWiz) and upon passing inspection for the correct species, the 500 mL culture was used to inoculate 12 L of LBM media at a 1:100 inoculation ratio. The 20 L cultures were cultivated, protected from light at 37°C, for 10 days without shaking. Amerlite XAD-7HP (Sigma Aldrich) was aliquoted in 20 g increments and activated by soaking in methanol for 10 minutes, followed by 5 washes with deionized water to remove excess methanol. After 10 days, activated Amerlite XAD-7HP was added to the cultures (20 g dry weight/L) and the slurries were gently shaken (90 rpm) on a tabletop shaker for 4 hrs. After incubation with the cultures, the resin was removed via cheese-cloth filtration and the collected resin, alongside the cheese-cloth, was placed inside a 1 L Fernbach flask to which 5 L acetone was added. This acetone elution was allowed to occur for 2 hrs with shaking (150 rpm), after which the organic solvent was collected and fresh acetone, of equivalent volume, was added. This second elution was allowed to occur overnight with light shaking at 22°C. Both elutions were added together and solvent was removed via rotary evaporation (Buchi) at 25°C to afford the dry crude extract, which was stored at -20°C until fractionated, as detailed below.

GPCR Assays

GPCR assays were measured by Eurofins DiscoverX using the PathHunter® β-Arrestin assay (Olson and Egle, 2007). This assay uses β-galactosidase (β-Gal) that has been split into two inactive portions as a reporter to measure the activation of a GPCR. The β-Gal fragments are called EA for Enzyme Acceptor and ED for Enzyme Donor.(US Patent: US20090098588A1) Using these fragments, a unique reported cell line was created for each GPCR of interest. In each unique cell line the EA fragment is fused to β-Arrestin and the ED fragment is fused to the GPCR of interest. Upon GPCR activation, β-Arrestin recruitment to the receptor physically co-localizes the ED and EA fragments thereby restoring β-Gal activity. β-Gal complementation is measured using chemiluminescent PathHunter® Detection Reagents. For our initial screen (Figure 2) all 80 culture broth extract fractions were screened in singleton against the Eurofins DiscoverX gpcrMAX (168 GPCRs, Table S2) and orphanMAX (73 GPCRs, Table S2) panels. All subsequent validation and bioassay guided fraction studies were run in at least duplicate using individual reporter cell lines for specific GPCRs of interest.

Eurofins DiscoverX Generic Agonist Protocol

1. Sample is added to individual GPCR reporter cell lines grown in microtiter plates. Cells are incubated at 37°C or room temperature for 90 or 180 minutes. 2. Assay signal is generated through addition of 12.5 or 15 μL (50% v/v) of PathHunter Detection reagent
Mutation of \( \text{cadA} \) in \( E. \text{coli} \) LF82

Deletion of the \( \text{cadA} \) gene was performed by Red/ET recombination. \( E. \text{coli} \) LF82 cells were transformed with the pRedET plasmid (Genebridges) and grown overnight at 30°C on LB agar plates supplemented with 3 \( \mu \)g/mL tetracycline. A single colony was picked and grown overnight at 30°C in 5 mL LB, followed by 100-fold dilution in 50 mL fresh LB. This culture was grown at 30°C to OD600 0.3, at which point L-arabinose was added to a final concentration of 0.4 wt% to induce recombination-mediating proteins. The culture was grown for 1 hr at 37°C before making the cells electrocompetent. These cells were transformed with 200 ng of a linear piece of DNA bearing an apramycin resistance cassette flanked by 250 bp regions upstream and downstream of the \( \text{cadA} \) gene. Recombination was allowed to occur for 3 hr at 37°C before plating the cells on LB agar supplemented with 50 \( \mu \)g/mL apramycin and growing overnight at 37°C. Colony PCR was used to check for the appropriate gene deletion and apramycin cassette insertion.

Cadaverine Induction

LB was acidified to indicated pH using 1N HCl then sterile filtered. In duplicate, 5 mL of media was inoculated by pipetting 50 \( \mu \)L of turbid liquid cultures of either wild-type \( E. \text{coli} \) LF82 or \( \text{cadA-KO} \) \( E. \text{coli} \) LF82. Cultures were allowed to grow overnight at 37°C. Turbid cultures were centrifuged at 4,500 rpm for 10 minutes to pellet cells, the dry weight of which was used for normalization between samples. The supernatant was moved to a 16 mL glass tube and the pH was raised to ~11 with 1N sodium hydroxide. Basified supernatants were then extracted 1:1 with ethyl acetate one time and the organic layer was dried under nitrogen gas. To each vial was added 100 \( \mu \)L of 50% acetonitrile in water and samples were run and analyzed using HR-MS techniques described below. A synthetic standard of cadaverine was used to set analysis parameters including retention time and accurate mass.

High-Resolution Mass Spectrometry

High Resolution Mass Spectrometry was used in structure elucidation of pure, unknown compounds as well as relative quantification between samples. HRMS was acquired on a C18 column (Thermo Acclaim 120 C18, 2.1 \( \times \) 150 mm) using a Dionex U-3000 HPLC system connected to an LTQ-Orbitrap Mass Spectrometer (Thermo-Fisher). Analysis was performed using Thermo Xcalibur.

Murine Work

All experimental procedures were approved by the Animal Care and Use Committee of The Icahn School of Medicine at Mount Sinai (PI Cohen IACUC-2016-0491). Germ free C57BL/6 mice were maintained in sterile isolators with autoclaved food and water in the Gnotobiotic Facility of the Faith Lab at Mount Sinai. 6-week-old mice were used for all experiments (3M and 3F in the treatment group, 5M and 1F in the control group). The treatment group was colonized with the SIHUMI whereas the control group was left germ free. For colonization studies 5 ml of an overnight culture in LBM media of the SIHUMI (treatment group) was centrifuged at 500 \( \times \) g for 2 minutes, the supernatant was decanted and the cells were resuspended in 2 ml of sterile PBS. Germ free mice were gavaged with 100 \( \mu \)L of bacterial culture immediately upon removal from sterile isolators. Colonization was confirmed by collection of fecal pellets after 3 and 10 days. Crude DNA was extracted from fecal pellets per protocol (ZymoBIOMICS DNA/RNA Miniprep Kit) and colonization confirmed by targeted PCR of each strain using specific primers as detailed above (Figure S5). After colonization mice were housed in specific-pathogen-free conditions and fed with autoclaved water and food. After colonization for 10 days the mice were euthanized and samples were collected for analysis. 200 mg of cecal contents were collected from each mouse and placed immediately at -80°C. The animal experiments were not randomized and the investigators were not blinded to the allocation during experiments and outcome assessment. No statistical methods were used to predetermine sample size. All mice which completed the experiments were analyzed.
Metabolite Quantitation by Mass Spectrometry

Cecal samples were weighed into 2 mL microtubes containing 2.8 mm ceramic beads (Omni International) and resuspended to a final concentration of 100 mg/mL using 80:20 methanol:water containing phenol-d6, palmitic acid-d31 and 13C,15N-amino acid internal standards (Cambridge Isotope Laboratories). Homogenization was using a Bead Ruptor (Omni International) at 6 m/s for 50 s for 6 cycles, at 4°C. Samples were centrifuged for 20 minutes at 20,000 x g at 4°C and then divided for 3 analytical methods.

Estimated concentrations of all metabolites (Table S6) were determined by preparing a calibration curve of pure standards in 80% MeOH and processed as described below for Methods 1-3. Calibration curves were 1/x2-weighted and absolute ranges (μM) were defined where the accuracy of the calibrators were calculated to be within +/- 30% of the nominal value.

Method 1: GC-nCl-MS with PFB Derivatization

100 μL of cecal extract was added to 100 μL of 100 mM borate Buffer (pH 10), 400 μL of 100 mM pentafluorobenzyl bromide (Thermo Scientific) in acetone (Fisher), and 400 μL of cyclohexane (Acros Organics) in a sealed autosampler vial. Samples were heated to 65°C for 1 hour with shaking. After cooling to room temperature and allowing the layers to separate, 100 μL of the cyclohexane upper phase was transferred to an autosampler vial containing a glass insert and sealed. Samples were analyzed using a GC-MS (Agilent 7890A GC system, Agilent 5975C MS detector) operating in negative chemical ionization mode, using a DB-5MS column (30 m x 0.25 mm, 0.25 μm; Agilent Technologies), methane as the reagent gas and 1 μL split injection (1:5 split ratio). Raw peak areas for aromatic analytes (tyramine and phenylpropanoic acid) were normalized to phenol-d7 internal standard and lipid analytes (9,10-methylenehexadecanoic acid and 12-methylene-tetradecanoic acid) were normalized to palmitic acid-d31 internal standard. Data analysis was performed using MassHunter Quantitative Analysis software (version B.09, Agilent Technologies) and confirmed by comparison with authentic standards.

Method 2: LC triple quadrupole with reverse phase chromatography

200 μL of extract was dried using a vacuum concentrator (Genevac) and resuspended in 200 μL 50:50 methanol:water, clarified by centrifugation and analyzed using reverse phase chromatography coupled to TSQ Vantage triple quadrupole mass spectrometer with HESI II source. LC separation was using an HSS T3 column (100 x 2.1 mm, 1.8 μm particle size, Waters) and Agilent 1260 binary pump. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The gradient was 0 min, 0% B; 2 min, 0% B; 5 min, 12% B; 7 min, 70% B; 8.5 min, 97% B with 3.5 min of re-equilibration time. LC parameters were: flow rate 300 μL/min, injection volume 15 μL and column temperature 35°C. The mass spectrometer was operated in positive ionization with transitions for tyramine (m/z 161.1 → 115.1, CE 30 V*; 161.1 → 144.1, CE 4 V) and nicotinic acid (m/z 124.1 → 80.1, CE 18 V*; 124.1 → 78.1, CE 19 V), with * indicating the primary transition used for quantitation. MS parameters were: capillary temp: 300°C; vaporizer temp: 350°C; sheath gas: 50; aux gas: 30; spray voltage 4000 V. Data was acquired and analyzed using TraceFinder software (version 4.1, Thermo Scientific) confirmed by comparison with authentic standards.

Method 3: LC Q-TOF with HILIC chromatography

Samples were prepared as for Method 2, with 100 μL of the 50:50 methanol:water extract added to an additional 100 μL 60:40 acetonitrile:water and analyzed by hydrophilic interaction chromatography (HILIC) coupled to the 6545 Q-TOF mass spectrometer with Dual JetStream source (Agilent). The LC separation was using an Acquity UPLC BEH Amide column (150 mm x 2.1 mm, 1.7 μm particle size, Waters) and Agilent 1290 Infinity II binary pump. Mobile phase A was 90:10 water:acetonitrile with 10 mM ammonium acetate and 0.2% acetic acid, and mobile phase B was 90:10 water:acetonitrile with 10 mM ammonium acetate and 0.2% acetic acid. The gradient was 0 min, 95% B; 9 min, 70% B; 10 min, 40% B; 13 min, 30% B; 15 min, 95% B. LC parameters were: flow rate 400 μL/min, column temperature 40°C, and injection volume 5 μL. The mass spectrometer was operated in positive ionization mode. MS parameters were: gas temp: 350°C; gas flow: 10 L/min; nebulizer pressure: 35 psig; sheath gas: 400°C; sheath gas flow: 12 L/min; VCap: 4,000 V; fragmentor: 125 V. Active reference mass correction was done through a second nebulizer using masses with m/z: 121.050873 and 922.009798. Data were acquired over m/z range 50–1700 and analyzed using MassHunter Profinder software (version B.09, Agilent) and confirmed by comparison with a cadaverine authentic standard. Compiling these data sets in GraphPad Prizm was then used to derive p-values. Unpaired t-test (two-tailed) were used.

Tyramine

Fraction 3 from E. coli LF82 was chosen as the pilot fraction for the dopamine receptors. 1 mL of Fraction 3 (100 mg/mL in DMSO) was dried down resuspended in 1 mL 50/50 MeOH:H2O and injected in 50 μL increments onto a semi-preparative 250 x 10 mm Luna® C18 (2 μm, 100 Å) column. A single resulting fraction retained activity and this compounds was identified as tyramine by NMR and HRMS (LC-HRMS-ESI (m/z): [M+H]⁺ calcd for C9H7NO, 138.0841; found 138.0911). Tyramine: 1H NMR (DMSO-d6, 600 MHz): δH 7.02 (2H, d, J = 8.5 Hz), 6.70 (2H, d, J = 8.5 Hz), 2.892 (t, J = 7.6 Hz), 2.71 (2H, t, J = 7.6 Hz). 13C NMR (DMSO-d6, 151 MHz): δC 156.1 (1C, s), 129.5 (2C, s), 115.3 (2C, s), 40.8 (1C, s), 33.45 (1C, s).

Cell Host & Microbe 26, 273–282.e1–e7, August 14, 2019 e5
Tryptamine
A single fraction from the fermentation of *Ruminococcus gnavus* was chosen as a pilot fraction to find serotonin active compounds which could then be assessed in other bacteria. 1 mL of Fraction 5 solution (100 mg/mL in DMSO) was dried down, resuspended in 1 mL 50/50 MeOH:H2O, and injected in 50 μL increments onto a semi-preparative 250×10 mm Luna® Omega 2.6 μM Polar C18 LC column on an Agilent 1100 HPLC with a solvent system where Solvent A was H2O + 0.1% formic acid and Solvent B was CH3CN + 0.1% formic acid. The chromatographic method was as follows: 0% B for 5 CV, then up to 90% B over 15 CV, with a 5 CV hold at 90% B. Peak detection and fraction collection was driven by UV absorbance at 210 nm, 254 nm, 280 nm, and 300 nm. Fractions were collected and re-assayed against HTRSA to guide further purification. The active fraction (41 mg) was ~90% tryptamine as evident by NMR and HRMS (LC-HRMS-ESI (m/z): [M+H]+ calcd for C9H10N2O, 151.0786; found 151.0779). Tryptamine: 1H NMR (DMSO-δ6, 600 MHz): δH 7.54 (1H, d, J = 7.9 Hz), 7.20 (1H, d, 2.0 Hz), 7.08 (1H, t, 7.6 Hz), 7.00 (1H, t, 7.6 Hz), 3.01 (2H, dd, 8.5 Hz, 7.1 Hz), 2.93 (2H, dd, 8.8, 6.2 Hz). 13C NMR (DMSO-δ6, 151 MHz): δC 136.3 (1C, s), 126.9 (1C, s), 123.2 (1C, s), 121.1 (1C, s), 118.4 (1C, s), 118.1 (1C, s), 111.5 (1C, s), 110.1 (1C, s), 40.0 (1C, s), 24.6 (1C, s).

Phenylpropanoic acid
Due to its relative simplicity in composition, Fraction 2 was chosen for further study. 40 mg of Fraction 2 was injected in two equal increments onto a semi-preparative 150 × 10 mm XBridge® 5 μm C18 column an Agilent 1100 HPLC with a solvent system where Solvent A was H2O + 0.1% formic acid and Solvent B was CH3CN + 0.1% formic acid. The chromatographic method was as follows: flow rate 4 mL/min; 2.5% B for 5 min, then increased to 35% B over 25 min, then flushed at 99% B for 5 min. Peak detection and fraction collection was driven by UV absorbance at 210 nm, 254 nm, 280 nm, and 300 nm. Fractions were collected per minute and re-assayed against GPR109B. All activity was found in subfractions 30 (8.8 mg) and 31 (0.1 mg), which were identified as pure phenylpropanoic acid by NMR and MS. Significant quantities of phenylpropanoic acid was also subsequently detected in Fraction 7 from all bacterial extracts. LC-HRMS-ESI (m/z): [M+H]+ calcd for C9H9O2, 149.0602; found 149.0599. Phenylpropanoic acid: 1H NMR (DMSO-d6, 600 MHz): δH 7.54 (1H, d, J = 7.9 Hz), 7.20 (1H, d, 2.0 Hz), 7.08 (1H, t, 7.6 Hz), 7.00 (1H, t, 7.6 Hz), 3.01 (2H, dd, 8.5 Hz, 7.1 Hz), 2.93 (2H, dd, 8.8, 6.2 Hz). 13C NMR (DMSO-δ6, 151 MHz): δC 136.3 (1C, s), 126.9 (1C, s), 123.2 (1C, s), 121.1 (1C, s), 118.4 (1C, s), 118.1 (1C, s), 111.5 (1C, s), 110.1 (1C, s), 40.0 (1C, s), 24.6 (1C, s).

Cadaverine
Fraction 4 from *E. coli* LF82 was chosen as the pilot fraction for the histamine receptors. 1 mL of Fraction 4 (100 mg/mL in DMSO) was dried down resuspended in 1 mL H2O and injected in 50-μL increments onto a semi-preparative 250 × 10 mm Luna® Omega 2.6 μM Polar C18 LC column on an Agilent 1100 HPLC with a solvent system where Solvent A was H2O + 0.1% formic acid and Solvent B was CH3CN + 0.1% formic acid. The chromatographic method was as follows: flow rate 4 mL/min; 2.5% B for 5 min, then increased to 35% B over 25 min, then flushed at 99% B for 5 min. Peak detection and fraction collection was driven by UV absorbance at 210 nm, 254 nm, 280 nm, and 300 nm. Fractions were collected per minute and re-assayed against HRH4 to guide further purification. The active fraction was further purified two more times using the same Polar C18 column with extended flushes at 0% B, as the activity was constantly eluting in the void. A HILIC method proved to be less effective. A single resulting fraction retained activity and this compound was identified as cadaverine by NMR and HRMS (LC-HRMS-ESI (m/z): [M+H]+ calcd for C5H14N2, 102.1157; found 102.1157). Co-eluted in this fraction was the compound agmatine (LC-HRMS-ESI (m/z): [M+H]+ calcd for C5H14N4, 120.1060; found 120.1060). Cadaverine: 1H NMR (D2O, 600 MHz): δH 3.04 (4H, t, J = 7.6 Hz), 1.74 (4H, p, J = 7.7 Hz), 1.49 (2H, p, J = 7.7 Hz). 13C NMR (D2O, 151 MHz): δC 39.2 (2C, s), 26.2 (2C, s), 22.7 (1C, s).

9,10-methylenedecanoic acid
Fraction 9 of *E. coli* LF-82 was injected in DMSO onto a semi-preparative 150 × 10 mm XBridge® 5 μm C18 column with a solvent system where Solvent A was H2O + 0.1% formic acid and Solvent B was CH3CN + 0.1% formic acid. The chromatographic method was as follows: 30% B for 3 column CV then up to 99% B over 5 CV, with a 15 CV hold at 99% B. Peak detection and fraction collection was driven by charged aerosol detection using a Corona Veo (Thermo Fisher Scientific) after UV proved to not be useful. Fractions were collected and re-assayed against BAI1 to guide further purification. A single resulting fraction retained activity and this compound was identified as 9,10-methylenedecanoic acid by NMR and HRMS (LC-HRMS-ESI (m/z): [M+H]+ calcd for C17H32O2, 267.2393; found 267.2385). 9,10-methylenedecanoic acid: 1H NMR (CDCl3, 600 MHz): δH 2.35 (2H, t, J = 7.4 Hz), 1.64 (2H, p, J = 7.4 Hz), 1.37 (16H, m), 1.32 (2H, m), 1.14 (2H, m), 0.89 (3H, t, J = 6.6 Hz), 0.65 (2H, m), 0.57 (1H, td, J = 8.2 Hz, 4.2 Hz), -0.33 (1H, q, J = 5.2, 4.4 Hz). 13C NMR (CDCl3, 151 MHz): δC 177.7 (1C, s), 33.8 (1C, s), 32.2 (1C, s), 30.4 (1C, s), 29.7 (1C, s), 29.6 (1C, s), 29.5 (1C, s), 29.3 (1C, s), 29.0 (1C, s), 28.9 (1C, s), 25.0 (1C, s), 23.0 (1C, s), 16.0 (1C, s), 16.0 (1C, s), 14.4 (1C, s), 11.2 (1C, s).

12-methyltetradecanoic acid
Fraction 9 of *B. vulgatus* was injected in DMSO onto a semi-preparative 150 × 10 mm XBridge® 5μm C18 column with a solvent system where Solvent A was H2O + 0.1% formic acid and Solvent B was CH3CN + 0.1% formic acid. The chromatographic method was as follows: 30% B for 3 column CV then up to 99% B over 5 CV, with a 15 CV hold at 99% B. Peak detection and fraction collection was driven by charged aerosol detection using a Corona Veo (Thermo Fisher Scientific) after UV proved to not be useful. Fractions were collected and re-assayed against BAI1 to guide further purification. A single resulting fraction retained activity and...
this compound was identified as 9,10-methylenehexadecanoic acid by NMR and HRMS. (LC-HRMS-ESI (m/z): [M-H]- calcd for C$_{15}$H$_{30}$O$_2$, 241.2245; found 241.2178) 12-methylmyristic acid: $^1$H NMR (CDCl$_3$, 600 MHz): $\delta$H 2.35 (2H, t, 7.5 Hz), 1.64 (2H, p, 7.5 Hz), 1.26 (16H, m), 1.12 (1H, m, 6.9 Hz), 1.08 (2H, m), 0.85 (3H, t, 7.4 Hz), 0.84 (3H, d, 5.1 Hz). $^{13}$C NMR (CDCl$_3$, 151 MHz): $\delta$C 178.2 (1C, s), 36.6 (1C, s), 34.3 (1C, s), 33.7 (1C, s), 30.0 (1C, s), 29.5 (1C, s), 29.4 (1C, s), 29.2 (1C, s), 29.0 (1C, s), 27.0 (1C, s), 24.6 (1C, s), 19.2 (1C, s), 11.4 (1C, s).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using Prism (Graphpad). Statistical parameters including precision measures (mean ± SD) and statistical significance are reported in the Main text, Figures and Figure Legends. Data was judged to be statistically significant when p < 0.05 by unpaired t test (two-tailed).

DATA AND CODE AVAILABILITY

All raw screening data is included in Table S2. Screenshots of raw NMR data for isolated compounds are included in Figures S6–S11.