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(54) Title: METHODS AND COMPOSITIONS FOR IMPROVED PHYSICAL AND PSYCHOLOGICAL HEALTH

(57) Abstract: In some aspects, provided herein are methods of improving, increasing, or potentiating vagal nerve activity (e.g., afferent vagal nerve activity) in a subject in need thereof, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.



METHODS AND COMPOSITIONS FOR IMPROVED PHYSICAL AND PSYCHOLOGICAL HEALTH

RELATED APPLICATIONS

This application claims a right of priority to and the benefit of U.S. Provisional Application No. 63/533,797, filed on August 21, 2023, which is hereby incorporated by reference herein in its entirety.

5 GOVERNMENT SUPPORT

This invention was made with government support under W911NF-17-1-0402 awarded by the Army Research Laboratory - Army Research Office. The government has certain rights in the invention.

BACKGROUND

10 The gut microbiota is emerging as a key modulator of brain function and behavior, as several recent studies reveal effects of gut microbes on neurophysiology, complex animal behaviors, and endophenotypes of neurodevelopmental, neurological, and neurodegenerative diseases. Despite these findings supporting a “microbiome-gut-brain axis”, the mechanisms that underlie interactions between gut microbes and the brain remain poorly understood.

15 SUMMARY

In some aspects, provided herein are methods of improving, increasing, or potentiating vagal nerve activity (e.g., afferent vagal nerve activity) in a subject in need thereof, the methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6. In some embodiments, the
20 compositions disclosed herein are useful in simulating the vagus nerve and increasing afferent vagal nerve activity.

In some embodiments, the subject is afflicted with or at risk for a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction. In
25 some aspects, provided herein are methods of treating a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction in a subject, the methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some embodiments, the subject is afflicted with or at risk for epilepsy (e.g., refractory epilepsy), Alzheimer's disease/cognition, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, or visceral pain.

5 In some embodiments, the subject is afflicted with autism spectrum disorder.

In some aspects, provided herein are methods of improving mood or ameliorating a depressive symptom in a subject, the methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6 (e.g., at least one metabolite selected from Table 1). In some embodiments, the depressive symptom
10 may be anxiety, apathy, general discontent, guilt, hopelessness, loss of interest or pleasure in activities, mood swings, sadness, agitation, excessive crying, irritability, restlessness, social isolation, early awakening, excess sleepiness, insomnia, restless sleep, excessive hunger, fatigue, loss of appetite, lack of concentration, slowness in activity, suicidal ideation, weight gain and weight loss.

15 In some embodiments, the methods further comprise administering an antibiotic to the subject prior to administration of the composition to the subject.

In some aspects, provided herein are methods of treating a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction in a subject, the
20 methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some embodiments, the methods comprise administering an additional therapy for a psychiatric or neurologic disorder.

In some aspects, provided herein are methods of treating autism spectrum disorder in
25 a subject, the methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some aspects, provided herein are methods of treating or preventing epilepsy (e.g., refractory epilepsy), Alzheimer's disease/cognition, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and visceral
30 pain in a subject, the methods comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some embodiments, the composition comprises 3-indoxyl sulfate.

In some embodiments, the composition comprises glutamate.

In some embodiments, the composition comprises at least one bile acid (e.g., 7-ketodeoxycholate, beta-muricholate, cholate, chenodeoxycholate, deoxycholate, glycocholate, taurodeoxycholate, tauroolithocholate, taurohyodeoxycholic acid, ursodeoxycholic acid, lithocholate, and/or alpha-muricholate). In some embodiments, the composition comprises at least one short chain fatty acid (e.g., acetate, propionate, and/or butyrate). In some embodiments, the composition comprises at least one tryptophan metabolite (e.g., kynurenate, kynurenine, xanthurenate, dihydrocaffeate, and/or tryptamine). In some embodiments, composition comprises at least one of hippurate, trimethylamine-N-oxide (TMAO), imidazole propionate, phenethylamine, palmitoyl ethanolamide (PEA), oleoyl ethanolamide (OEA), linoleoyl ethanolamide (LEA), a monohydroxy fatty acid (MFA, e.g., 13-sHODE or 9-s-HODE), and/or succinate.

Any one of the methods disclosed herein can comprise administering at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, at least thirty-four, at least thirty-five, at least thirty-six, at least thirty-seven, at least thirty-eight, at least thirty-nine, or at least forty of the metabolites disclosed herein. In some embodiments, the compositions disclosed herein comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, at least thirty-four, at least thirty-five, at least thirty-six, at least thirty-seven, at least thirty-eight, at least thirty-nine, or at least forty of the metabolites disclosed herein.

In some embodiments, the composition is formulated for oral delivery.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows average vagal afferent nerve activity of conventionally colonized (SPF) male (n = 7) and female (n = 5) mice. There are no sex differences in baseline vagal afferent

nerve activity. Normalized to male cohort average vagal tone value. Welch's t-test. All data displayed as mean +/- SEM.

FIG. 2A-I show the gut microbiota and luminal microbial metabolites promote vagal afferent nerve activity. **FIG. 2A** shows a diagram of *in vivo* whole nerve vagal electrophysiology for quantification of vagal tone (left, for data in B-C) or acute vagal afferent nerve response to luminal stimuli (right, for data in D-I). **FIG. 2B** shows

representative electrophysiological recording traces over 10s in conventionally colonized (SPF), germ-free (GF), antibiotic-treated (ABX), and conventionalized GF (CONV) mice.

FIG. 2C shows average vagal afferent tone of SPF, GF, ABX, and CONV mice over 300s of recording. (SPF, n = 11 mice; GF, n = 11 mice; ABX, n = 12 mice; CONV, n = 11 mice).

Normalized to SPF average. One-Way ANOVA + Tukey. **FIG. 2D** shows vagal afferent

nerve response in SPF or GF mice perfused intestinally with non-absorbable antibiotics (ABX, vancomycin/neomycin, 1mg/mL) or vehicle (VEH) (SPF +VEH, n = 12 mice; SPF +ABX, n = 12 mice; GF + ABX, n = 10 mice). Normalized within-subject to the final 60s of the baseline vehicle recording period (t = 540s-600s). **FIG. 2E** shows vagal afferent nerve

firing rates before and after intestinal perfusion of SPF or GF mice with ABX or VEH. (SPF +VEH, n = 12 mice; SPF +ABX, n = 12 mice; GF + ABX, n = 10 mice). Paired t-test. **FIG.**

2F shows the change in vagal afferent nerve activity after intestinal perfusion with ABX or VEH (t=1800) relative to stable baseline (t=600s) (SPF +VEH, n = 12 mice; SPF +ABX, n = 12 mice; GF + ABX, n = 10 mice). One-way ANOVA + Tukey. **FIG. 2G** shows vagal

afferent nerve response in SPF mice intestinally perfused with ABX, followed by re-

perfusion with pooled small intestinal (SI) and cecal contents from SPF mice (+SPF) or GF mice (+GF), sterile filtered SI/cecal contents from SPF mice (+SPF-SF), or VEH (+VEH, n = 10 mice; +SPF, n = 11 mice; +GF, n = 9 mice; +SPF-SF, n = 11 mice). Normalized within-subject to the final 60s of the baseline vehicle recording period (t = 540s-600s). **FIG. 2H**

shows vagal afferent nerve firing rate after intestinal ABX perfusion (t=1800s) and after re-

perfusion with VEH, SI/cecal contents from SPF mice, SI/cecal contents from GF mice, or sterile filtered SI/cecal contents from SPF mice, at the time of maximum mean firing rate for perfusion of SPF SI/Cecal contents (SPF MAX, t = 2760s) (+VEH, n = 10 mice; +SPF, n = 11 mice; +GF, n = 9 mice; +SPF-SF, n = 11 mice). Paired t-test. **FIG. 2I** shows vagal afferent

nerve activity as measured by area under the curve (AUC) (from 1800-3600s) in response to

intestinal perfusion with VEH (n = 10 mice), SPF SI/cecal contents (n = 11 mice), GF

SI/cecal contents (n = 9 mice), and SPF-SF SI/cecal contents (n = 11 mice). Brown-Forsythe

and Welch ANOVA + Games-Howell. All data displayed as mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

FIG. 3 shows levels of 16S rRNA gene in perfusate outflow samples during antibiotic or vehicle luminal perfusion through quantification of 16S rRNA gene in perfusate outflow samples collected from ABX- or VEH-perfused mice at $t = 0$ —120s, $t = 600$ –720s, $t = 1200$ –1320s, and $t = 1800$ –1920s. Welch's t-test. All data displayed as mean $\pm$ SEM, * $p < 0.05$

FIG. 4A-F show the gut microbiome regulates metabolites in the lumen of the small intestine and cecum. **FIG. 4A** shows PCA analysis of metabolomic data from small intestinal (SI, top) or cecal (bottom) luminal contents from SPF, GF, ABX, and CONV mice. (n=6 mice for all groups). **FIG. 4B** shows a Venn diagram of differentially modulated metabolites in SI (top) and cecal (bottom) luminal contents. The top number indicates the total number of differentially regulated metabolites; the second row indicates the number of upregulated metabolites; and the third row indicates the number of downregulated metabolites. (n=6 mice for all groups). **FIG. 4C** shows a random forest (RF) analysis of metabolomic data from SI luminal contents reveals the top 30 metabolites that distinguish GF/ABX from SPF/CONV samples with 100% predictive accuracy. Asterisks indicate metabolites included in experiments in **FIG. 6A-F**. Hash symbols indicate metabolites included in experiments in **FIG. 5A-E** (n=6 mice for all groups). **FIG. 4D** and **FIG. 4E** show luminal levels of microbially modulated bile acids and the short-chain fatty acid butyrate from SI and/or cecum of SPF, GF, ABX, and CONV mice. (n=6 mice for all groups). Welch's t-test. **FIG. 4F** shows luminal levels of microbially modulated metabolites with unknown signaling to vagal neurons from SI or cecum of SPF, GF, ABX, and CONV mice. (n=6 mice for all groups). Welch's t-test. All data displayed as mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

FIG. 5A-E show luminal levels of microbially-modulated cecal metabolites that were screened for effects on vagal afferent nerve activity. **FIG. 5A** shows tryptophan metabolites; **FIG. 5B** shows fatty-acid ethanolamides (FAEs) PEA (palmitoyl ethanolamide); OEA (oleoyl ethanolamide); LEA (linoleoyl ethanolamide); **FIG. 5C** shows monohydroxy fatty acids (MFAs) 9-s- HODE and 13-s- HODE; **FIG. 5D** shows succinate; **FIG. 5E** shows glutamate. Welch's t-test. All data displayed as mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

FIG. 6A-I show select luminal microbial metabolites increase vagal afferent nerve activity with varied response kinetics. **FIG. 6A** shows vagal afferent nerve firing rate in SPF

mice after intestinal perfusion with vehicle (VEH: PBS, n=10 mice) or pooled bile acids (BAs: cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM; deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM; n = 9 mice), with or without pre- and co-perfusion with TGR5 antagonist (SBI-115, 200uM, n = 8 mice). Pure antagonist was infused from 300-600 seconds prior to co-perfusion of antagonist with metabolites. **FIG. 6B** shows normalized vagal afferent nerve firing rate before and during treatment with BAs (left, n = 9 mice), or BAs and SBI-115 (right, n = 8 mice). BA max, t = 1114s. Wilcoxon matched-pairs signed rank test. **FIG. 6C** shows vagal afferent nerve firing rate as measured by area under the curve (AUC) (from 600-2400s) in response to intestinal perfusion with VEH (PBS, n = 10 mice) or pooled BAs (n = 9 mice), with or without pre- and co-perfusion with TGR5 antagonist (SBI-115, 200uM, n = 8 mice). Brown-Forsythe and Welch ANOVA + Games-Howell. **FIG. 6D** shows vagal afferent nerve firing rate in SPF mice after intestinal perfusion with VEH (PBS, n = 10 mice) or pooled short-chain fatty acids (SCFAs: acetate, 80uM; butyrate, 22uM; propionate, 10uM, pooled, n = 10 mice), with or without pre- and co-perfusion with FFAR2 antagonist (GLPG0794, 10uM, n = 8 mice). Pure antagonist was infused from 300-600 seconds prior to co-perfusion of antagonist with metabolites. **FIG. 6E** shows normalized vagal afferent nerve firing rate before and during treatment with SCFAs (left, n = 10 mice), or SCFAs and GLPG0794 (right, n = 8 mice). SCFA max, t = 2400s. Paired t-test. **FIG. 6F** shows vagal afferent nerve firing rate as measured by AUC (from 600-2400s) in response to intestinal perfusion with VEH (PBS, n = 10 mice) or pooled SCFAs (10uM, n = 10 mice), with or without pre- and co-perfusion with FFAR2 antagonist (GLPG0794, 10uM, n = 8 mice). One-way ANOVA + Tukey. **FIG. 6G** shows vagal afferent nerve firing rate in SPF mice after intestinal perfusion with VEH (1uM KCl, n = 7 mice) or 3-indoxyl sulfate (3-IS, 1uM, n = 10 mice), with or without pre- and co-perfusion with TRPA1 antagonist (A967079, 10uM, n = 7 mice). Pure antagonist was infused from 300-600 seconds prior to co-perfusion of antagonist with metabolites. **FIG. 6H** shows normalized vagal afferent nerve firing rate before and during treatment with 3IS (left, n = 10 mice), or 3IS and A967079 (right, n = 7 mice). 3IS max, t = 2400s. Paired t-test. **FIG. 6I** shows vagal afferent activity as measured by AUC (from 600-2400s) in response to intestinal perfusion with VEH (1uM KCl, n=7 mice) or 3-indoxyl sulfate (3-IS, 1uM, n = 10 mice), with or without pre- and co-perfusion with TRPA1 antagonist (A967079, 10uM, n = 7 mice). One-way ANOVA +

Tukey. All time-series data were normalized within-subject to the final 60s of the 600s vehicle recording period (first 300s of recording not shown). All data displayed as mean $\pm$ SEM, * $p < 0.05$.

FIG. 7A-I show luminal perfusion of select microbially-modulated metabolites does not alter vagal afferent nerve activity *in vivo*. Each of **FIG. 7A-I** shows vagal afferent nerve firing rate over time (left), firing rate at time of average maximum increase in vagal afferent nerve response (middle), and area under the curve (AUC) in response to luminal perfusion (right) of VEH (PBS, $n = 10$ mice) or a metabolite of interest. **FIG. 7A** shows tryptophan metabolites (TRPs) (kynurenine 100uM, kynurenic acid 16.1uM, xanthurenate 1uM, dihydrocaffeate 30nm, tryptamine 0.03uM, pooled, $n = 10$ mice); **FIG. 7B** shows fatty-acid ethanolamides (FAEs, oleylethanolamide (OEA), palmitoylethanolamide (PEA), linoleylethanolamide (LEA), 10uM, pooled, $n = 9$ mice); **FIG. 7C** shows monohydroxy fatty acids (MFAs) (9-s- and 13-s- HODE, 1uM, pooled, $n = 7$ mice); **FIG. 7D** shows succinate (2mM, $n = 7$ mice); **FIG. 7E** shows glutamate (50mM, $n = 8$ mice); **FIG. 7F** shows hippurate (2uM, $n = 5$ mice); **FIG. 7G** shows imidazole propionate (ImP, 200nM, $n = 8$ mice); **FIG. 7H** shows phenethylamine (PEA, 100uM, $n = 8$ mice); and **FIG. 7I** shows trimethylamine-N-oxide (TMAO, 3uM, $n = 8$ mice). Welch's t-test for each comparison. All time-series data were normalized within-subject to the final 60s of the 600s vehicle recording period. All data displayed as mean $\pm$ SEM, s, ** $p < 0.01$, *** $p < 0.001$.

FIG. 8 shows mRNA expression of microbial metabolite receptors in the nodose ganglia. Relative expression of TRPA1 ($n = 3$ mice), FFAR3 ($n = 3$ mice), and TGR5 ($n = 3$ mice) in mouse nodose ganglia, All data normalized to GAPDH expression (GOI-GAPDH).

FIG. 9A-C shows *in vitro* calcium imaging of dissociated nodose neurons. **FIG. 9A** shows a heatmaps depicting vagal neuronal calcium activity in response to bath perfusion of high K^+ (60mM), bile acids (BAs: cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM; deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM; pooled), short-chain fatty acids (SCFAs: acetate, 80uM; butyrate, 22uM; propionate, 10uM, pooled), or 3-indoxyl sulfate (3IS, 1uM) ($n = 100$ cells for all conditions). The baseline period for the calcium signal was calculated as the average of the 60 seconds before stimulus onset (F_0). **FIG. 9B** shows the percentage of neuronal responders

to bath application of high K^+ , BAs, SCFAs, and 3-IS. **FIG. 9C** shows the maximal change in fluorescence in response to bath application of high K^+ , BAs, SCFAs, and 3-IS.

FIG. 10A-E show that microbial metabolites activate EECs. **FIG. 10A** shows a diagram of microbial-metabolite activation of vagal afferents via indirect activation of EECs. **FIG. 10B** shows existing single-cell data (Haber et al., 2017) demonstrating EEC enrichment for the microbial metabolite receptors Gpbar (TGR5), FFAR2, and TRPA1. **FIG. 10C** shows qRT-QPCR of intestinal enteroids and STC-1 cells targeting enterocyte (*Ace2*) and enteroendocrine cell (*Chga*) markers, and microbial metabolite receptors (*Gpbar1*, *Ffar2*, *Trpa1*). Welch's t-test. **FIG. 10D** shows the percentage of metabolite-responding STC-1 cells in response to bath application of High K^+ (60mM, n = 4 experiments), D-glucose (20mM, n = 4 experiments), BAs (cholate 2480nM, glycocholate 14nM, chenodeoxycholate 168nM, alpha-muricholate 568nM, beta-muricholate 2480nM, deoxycholate 780nM, taurodeoxycholate 520nM, ursodeoxycholate 148nM, taurohyodeoxycholate 37.6nM, 7-ketodeoxycholate 200nM, lithocholate 780nM, tauroolithocholate 0.66nM, pooled, n = 4 experiments), SCFAs (acetate 160uM, butyrate 44uM, propionate 20uM, pooled, n = 4 experiments) or 3IS (2uM, n = 4 experiments). One-Way ANOVA. **FIG. 10E** shows calcium signal ($\Delta F/F$) for metabolite-responsive STC-1 cells with bath application of High K^+ (60mM), D-glucose 920mM), BAs, SCFAs, or 3IS. All data displayed as mean $\pm$ SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

FIG. 11A-F shows luminal BAs, SCFAs, and 3IS activate distinct subsets of vagal neurons with heterogeneous kinetics and restore vagal afferent nerve tone. **FIG. 11A** shows a diagram of experimental setup for *in vivo* calcium imaging and associated micrograph of the imaging window. **FIG. 11B** shows representative heatmaps for cells responding to only pooled bile acids (BAs: cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM; deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM; n = 4 mice, n = 131 units, right), only pooled short-chain fatty acids (SCFAs: acetate, 80uM; butyrate, 22uM; propionate, 10uM, n = 4 mice, n = 82 cells, middle), or only 3-indoxyl sulfate (3IS, 1uM, n = 4 mice, n = 132 cells, left). Recording duration for all experiments was 10 minutes. Only metabolite-responsive neurons are displayed. Each ROI was normalized to the average of the 120s imaging period prior to stimulus onset. **FIG. 11C** shows the percentage of metabolite-responsive neurons out of total excitable neurons in response to luminal perfusion of BAs (n

= 5 mice), SCFAs (n = 5 mice), or 3IS (n = 4 mice). **FIG. 11D** shows latency to maximum change in fluorescence for metabolite-responding neurons with luminal perfusion of BAs (n = 4 mice), SCFAs (n = 4 mice) or 3IS (n = 4 mice). One-way ANOVA + Tukey. **FIG. 11E** shows the percentage of single- or dual- responding neurons following serial perfusion of BAs (n = 5 mice), SCFAs (n = 5 mice), and 3IS (n = 4 mice). Order of metabolites for perfusion was counterbalanced between experiments. **FIG. 11F** shows vagal afferent nerve activity of GF mice following three-day treatment via oral gavage with VEH (12uM KCl, 1.34mM NaCl, 0.012% DMSO in PBS, n = 7 mice) or pooled microbial metabolites (MMs: cholate, 14.88uM; glycocholate, 42nM; chenodeoxycholate, 504nM; alpha-muricholate, 1.7uM; beta-muricholate, 12.96uM; deoxycholate, 4.68uM; taurodeoxycholate, 3.12uM; ursodeoxycholate, 888nM; taurohyodeoxycholate, 226nM; 7-ketodeoxycholate, 1.2uM; lithocholate, 4.68uM; tauroolithocholate, 3.96nM, acetate, 0.96mM; butyrate, 264uM; propionate, 120uM, 3IS, 12uM, n = 9 mice) . All data displayed as mean +/- SEM, *p < 0.05, ***p < 0.001

FIG. 12A-C show microbial metabolites alter cFos levels in NTS neurons in a receptor-dependent manner. **FIG. 12A** shows representative image of full ROIs in mNTS for cFos quantification (dotted line) and inset images in B (solid line). **FIG. 12B** shows significant increase in the % of cFos+ NTS neurons following luminal perfusion of BAs (cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM; deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM, pooled, n = 3 mice), SCFAs (acetate 80uM, butyrate 22uM, propionate 10uM, pooled, n = 3 mice), and 3-IS (1uM, n = 4 mice) compared to vehicle (VEH, n = 3 mice) or corresponding receptor antagonists alongside each respective metabolite class (SBI-115, 200uM, n = 3 mice, GLPG0794, 10uM, n = 3 mice, A967079, 10uM, n = 3 mice). One-way ANOVA + Tukey. **FIG. 12C** shows a statistical summary of **FIG. 12B**. All data depicted as mean +/- SEM, *p < 0.05, **p < 0.01.

FIG. 13A-F show microbial metabolites modestly alter ARH, but not DMV, cFos levels in a receptor-dependent manner. **FIG. 13A** shows representative image of ROI's in DMV for quantification (dotted line) or representative images (solid line). **FIG. 13B** shows representative images from different conditions. **FIG. 13C** shows the percentage of cFos+ DMV neurons following luminal perfusion of of BAs (cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM;

deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM, pooled, n = 3 mice), SCFAs (acetate 80uM, butyrate 22uM, propionate 10uM, pooled, n = 3 mice), and 3-IS (1uM, n = 3 mice) compared to vehicle (n = 5 3 mice). One-way ANOVA + Tukey. **FIG. 13D** shows representative images of ROI's in ARH (dotted line) or representative images in **FIG. 13E** (solid line). **FIG. 13E** shows representative images from different conditions. **FIG. 13F** shows the percentage of cFos+ neurons in the ARH following luminal perfusion of BAs (cholate, 1240nM; glycocholate, 3.5nM; chenodeoxycholate, 42nM; alpha-muricholate, 142nM; beta-muricholate, 1080nM; 10 deoxycholate, 390nM; taurodeoxycholate, 260nM; ursodeoxycholate, 74nM; taurohyodeoxycholate, 18.8nM; 7-ketodeoxycholate, 100nM; lithocholate, 390nM; tauroolithocholate, 0.33nM, pooled, n = 3 mice), SCFAs (acetate 80uM, butyrate 22uM, propionate 10uM, pooled, n = 4 mice), and 3-IS (1uM, n = 3 mice) compared to vehicle (n = 4 mice) or corresponding receptor antagonists alongside each respective metabolite class 15 (SBI-115, 200uM, n = 3 mice, GLPG0794, 10uM, n = 3 mice, A967079, 10uM, n = 3 mice). One-way ANOVA + Tukey.

FIG. 14A-B show microbially-modulated metabolites in the small-intestine. Metabolites are considered modulated if they are significantly decreased or increased in GF and ABX compared to SPF and CONV samples. In **FIG. 14A**, cells shaded darker grey (GF- 20 SPF and ABX-SPF columns) indicate that the mean values are significantly lower for that comparison; cells shaded lighter grey (CONV-GF and CONV-ABX) have mean values that are significantly higher for that comparison. In **FIG. 14B**, cells shaded lighter grey (GF-SPF and ABX-SPF columns) have mean values that are significantly higher for that comparison; cells shaded darker grey (CONV-GF and CONV-ABX columns) indicate that the mean 25 values are significantly lower for that comparison.

FIG. 15A-I show microbially-modulated metabolites in the cecum. Metabolites are considered modulated if they are significantly decreased or increased in GF and ABX compared to SPF and CONV samples. In **FIG. 15A-F**, cells shaded darker grey (GF-SPF and ABX-SPF columns) indicate that the mean values are significantly lower for that comparison; 30 cells shaded lighter grey (CONV-GF and CONV-ABX) have mean values that are significantly higher for that comparison. In **FIG. 15G-I**, cells shaded lighter grey (GF-SPF and ABX-SPF columns) have mean values that are significantly higher for that comparison;

cells shaded darker grey (CONV-GF and CONV-ABX columns) indicate that the mean values are significantly lower for that comparison.

DETAILED DESCRIPTION

The compositions and methods provided herein are based, in part, on the discovery
5 that modulation of specific metabolites (e.g., any one of the metabolites listed in Tables 1-6)
can affect vagal nerve activity. The vagus nerve represents the main component of the
parasympathetic nervous system, which oversees a vast array of crucial bodily functions,
including control of mood, immune response, digestion, metabolism, lung function, and heart
rate. It establishes one of the connections between the brain and the gastrointestinal tract and
10 sends information about the state of the inner organs to the brain via afferent fibers. Vagal
afferents primarily terminate in the nucleus of solitary tract (NTS) in the brainstem, where
visceral information is further transmitted to higher brain regions such as the locus coeruleus
(LC), the rostral ventrolateral medulla, the dorsal raphe nucleus, the insular cortex, the
ventral tegmental area, the hypothalamus, the hippocampus, the dorsal striatum, and the
15 basolateral amygdala via multi-synaptic connections.

The vagus nerve is proposed to enable communication between the gut microbiome
and brain, but activity-based evidence is lacking. Herein, the extent of gut microbial
influences on afferent vagal activity and metabolite signaling mechanisms involved is
assessed. Mice reared without microbiota (germ-free, GF) exhibit decreased vagal afferent
20 tone relative to conventionally colonized mice (specific pathogen-free, SPF), which is
reversed by colonization with SPF microbiota. Perfusing non-absorbable antibiotics (ABX)
into the small intestine of SPF mice, but not GF mice, acutely decreases vagal activity, which
is restored upon re-perfusion with bulk lumenal contents or sterile filtrates from the small
intestine and cecum of SPF, but not GF, mice. Of several candidates identified by
25 metabolomic profiling, microbiome-dependent short-chain fatty acids, bile acids, and 3-
indoxyl sulfate stimulate vagal activity with varied response kinetics, which is blocked by co-
perfusion of pharmacological antagonists of FFAR2, TGR5, and TRPA1, respectively, into
the small intestine. At the single-unit level, serial perfusion of each metabolite class elicits
more singly responsive neurons than dually responsive neurons, suggesting distinct neuronal
30 detection of different microbiome- and macronutrient- dependent metabolites. Oral
administration of these metabolites into GF mice elevated vagal afferent tone to levels seen in
conventionalized controls. Finally, microbial metabolite-induced increases in vagal activity
correspond with activation of neurons in the nucleus of the solitary tract, which is also

blocked by co-administration of their respective receptor antagonists. Results from this study reveal that the gut microbiome regulates select metabolites in the intestinal lumen that differentially activate chemosensory vagal afferent neurons, thereby enabling microbial modulation of chemosensory signals for gut-brain communication.

5 In some aspects, the present disclosure provides a method of increasing vagal nerve activity in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6. In some embodiments, the vagal nerve activity is afferent vagal nerve activity.

10 In another aspect, the present disclosure provides a method of improving mood or ameliorating a depressive symptom in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6. In some embodiments, the depressive symptom is selected from anxiety, apathy, general discontent, guilt, hopelessness, loss of interest or pleasure in activities, mood swings, sadness, agitation, excessive crying, irritability, restlessness, social isolation, early awakening, excess
15 sleepiness, insomnia, restless sleep, excessive hunger, fatigue, loss of appetite, lack of concentration, slowness in activity, suicidal ideation, weight gain and weight loss.

20 In some embodiments, the subject is afflicted with or at risk for a psychiatric or neurologic disorder. In some embodiments, the subject is afflicted with or is at risk for bipolar depression. In some embodiments, the subject is afflicted with or is at risk for post-partum depression (PPD). In some embodiments, the subject is afflicted with or is at risk for post-traumatic stress disorder (PTSD). In some embodiments, the subject is afflicted with or is at risk for major depressive disorder (MDD). In some embodiments, the subject is afflicted with or is at risk for food addiction.

25 In some embodiments, the method comprises administering an additional therapy for a psychiatric or neurologic disorder.

 In another aspect, the present disclosure provides a method of treating autism spectrum disorder in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

30 In another aspect, the present disclosure provides a method of treating or preventing refractory epilepsy, Alzheimer's disease, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and visceral pain in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6. In another aspect, the present

disclosure provides a method of treating or preventing refractory epilepsy, Alzheimer's disease, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and visceral pain in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6. In another aspect, the present disclosure provides a method of preventing refractory epilepsy, Alzheimer's disease, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and visceral pain in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some embodiments, the subject is afflicted with or is at risk for Parkinson's disease. In some embodiments, the subject is afflicted with or is at risk for Alzheimer's disease/cognition. In some embodiments, the subject is afflicted with or is at risk for obesity and metabolic syndrome. In some embodiments, the subject is afflicted with or is at risk for impaired glucose metabolism. In some embodiments, the subject is afflicted with or is at risk for inflammatory bowel disease. In some embodiments, the subject is afflicted with or is at risk for visceral pain.

In some embodiments, the method further comprises administering an antibiotic to the subject prior to administration of the composition to the subject. In some embodiments, the composition is formulated for oral delivery.

In some embodiments, the composition comprises two or more, three or more, four or more or five or more metabolites selected from the metabolites listed in Tables 1-6. In some embodiments, the method comprises administering two or more, three or more, four or more or five or more compositions comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some preferred embodiments, the composition comprises 3-indoxyl sulfate.

In some preferred embodiments, the composition comprises glutamate.

In some preferred embodiments, the composition comprises a bile acid. In some embodiments, the bile acid is selected from at least one of 7-ketodeoxycholate, beta-muricholate, cholate, chenodeoxycholate, deoxycholate, glycocholate, taurodeoxycholate, tauroolithocholate, taurohyodeoxycholic acid, ursodeoxycholic acid, lithocholate, and alpha-muricholate. In some embodiments, composition comprises 7-ketodeoxycholate. In some embodiments, composition comprises beta-muricholate. In some embodiments, composition comprises cholate. In some embodiments, composition comprises chenodeoxycholate. In

some embodiments, composition comprises deoxycholate. In some embodiments, composition comprises glycocholate. In some embodiments, composition comprises taurodeoxycholate. In some embodiments, composition comprises tauroolithocholate. In some embodiments, composition comprises taurohyodeoxycholic acid. In some embodiments, composition comprises ursodeoxycholic acid. In some embodiments, composition comprises lithocholate. In some embodiments, composition comprises alpha-muricholate. In some embodiments, the composition comprises 7-ketodeoxycholate, beta-muricholate, cholate, chenodeoxycholate, deoxycholate, glycocholate, taurodeoxycholate, tauroolithocholate, taurohyodeoxycholic acid, ursodeoxycholic acid, lithocholate, and alpha-muricholate.

In some preferred embodiments, the composition comprises a short chain fatty acid. In some embodiments, the short chain fatty acid is selected from at least one of acetate, propionate, and butyrate. In some preferred embodiments, composition comprises acetate. In some preferred embodiments, composition comprises propionate. In some preferred embodiments, composition comprises butyrate.

In some preferred embodiments, the composition comprises a tryptophan metabolite. In some embodiments, the tryptophan metabolite is selected from at least one of kynurenate, kynurenine, xanthurenate, dihydrocaffeate, and tryptamine. In some embodiments, the composition comprises kynurenate. In some embodiments, the composition comprises kynurenine. In some embodiments, the composition comprises xanthurenate. In some embodiments, the composition comprises dihydrocaffeate. In some embodiments, the composition comprises tryptamine. In some embodiments, the composition comprises kynurenate, kynurenine, xanthurenate, dihydrocaffeate, and tryptamine.

In some embodiments, composition comprises at least one of hippurate, trimethylamine-N-oxide (TMAO), imidazole propionate, phenethylamine, palmitoyl ethanolamide (PEA), oleoyl ethanolamide (OEA), linoleoyl ethanolamide (LEA), 13-sHODE, 9-s-HODE, and succinate. In some embodiments, the composition comprises hippurate. In some embodiments, the composition comprises trimethylamine-N-oxide (TMAO). In some embodiments, the composition comprises imidazole propionate. In some embodiments, the composition comprises phenethylamine. In some embodiments, the composition comprises palmitoyl ethanolamide (PEA). In some embodiments, the composition comprises oleoyl ethanolamide (OEA). In some embodiments, the composition comprises linoleoyl ethanolamide (LEA). In some embodiments, the composition comprises monohydroxy fatty acids (MFAs). In some embodiments, the monohydroxy fatty acids comprise at least one of

13-sHODE and 9-s-HODE. In some embodiments, the composition comprises 13-sHODE. In some embodiments, the composition comprises 9-s-HODE. In some embodiments, the composition comprises succinate.

Any one of the methods disclosed herein can comprise administering at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, at least thirty-four, at least thirty-five, at least thirty-six, at least thirty-seven, at least thirty-eight, at least thirty-nine, or at least forty of the metabolites disclosed herein. In some embodiments, the compositions disclosed herein comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, at least thirty-four, at least thirty-five, at least thirty-six, at least thirty-seven, at least thirty-eight, at least thirty-nine, or at least forty of the metabolites disclosed herein.

In some embodiments, the composition comprises all of the metabolites in Table 1.

Definitions

As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

As used herein, "*afferent vagal nerve activity*" refers to the transfer of nerve electrical information from the gut to the nervous system (e.g., brain).

In some embodiments, the subject has epilepsy (e.g., pediatric epilepsy; refractory or non-refractory epilepsy). The disclosed epilepsy disorder may be benign Rolandic epilepsy, frontal lobe epilepsy, infantile spasms, juvenile myoclonic epilepsy (JME), juvenile absence epilepsy, childhood absence epilepsy (e.g. pyknolepsy), febrile seizures, progressive

myoclonus epilepsy of Lafora, Lennox-Gastaut syndrome, Landau-Kleffner syndrome, Dravet syndrome, Generalized Epilepsy with Febrile Seizures (GEFS+), Severe Myoclonic Epilepsy of Infancy (SMEI), Benign Neonatal Familial Convulsions (BFNC), West Syndrome, Ohtahara Syndrome, early myoclonic encephalopathies, migrating partial
5 epilepsy, infantile epileptic encephalopathies, Tuberous Sclerosis Complex (TSC), focal cortical dysplasia, Type I Lissencephaly, Miller-Dieker Syndrome, Angelman's syndrome, Fragile X syndrome, epilepsy in autism spectrum disorders, subcortical band heterotopia, Walker-Warburg syndrome, Alzheimer's disease, posttraumatic epilepsy, progressive myoclonus epilepsies, reflex epilepsy, Rasmussen's syndrome, temporal lobe epilepsy, limbic
10 epilepsy, status epilepticus, abdominal epilepsy, massive bilateral myoclonus, catamenial epilepsy, Jacksonian seizure disorder, Unverricht-Lundborg disease, or photosensitive epilepsy. The epilepsy may include generalized seizures or partial (i.e. focal) seizures.

“Epilepsy” or “epilepsy disorder” as disclosed herein, may be Dravet Syndrome, Lennox-Gastaut Syndrome, infantile spasm, or Ohtahara Syndrome. The epilepsy disorder
15 may be Dravet Syndrome, Lennox-Gastaut Syndrome, infantile spasm, or Ohtahara Syndrome, or a pediatric epilepsy disorder. The pediatric epilepsy disorder may be benign childhood epilepsy, Benign Neonatal Familial Convulsions (BFNC), febrile seizures, Dravet Syndrome, Lennox-Gastaut Syndrome, infantile spasm, Ohtahara Syndrome, juvenile myoclonic epilepsy, juvenile absence epilepsy, childhood absence epilepsy (e.g. pyknolepsy),
20 infantile spasms. In embodiments the epilepsy disorder is Dravet Syndrome. According to the disclosure, the epilepsy disorder may be Dravet syndrome or Lennox-Gastaut syndrome. The disclosed pediatric epilepsy disorder may be benign childhood epilepsy. The disclosed pediatric epilepsy disorder may be Benign Neonatal Familial Convulsions (BFNC). The disclosed pediatric epilepsy disorder may be febrile seizures. In embodiments, the pediatric
25 epilepsy disorder is Dravet Syndrome. In embodiments, the pediatric epilepsy disorder is Lennox-Gastaut Syndrome. The disclosed pediatric epilepsy disorder may be infantile spasm. The disclosed pediatric epilepsy disorder may be Ohtahara Syndrome. The disclosed pediatric epilepsy disorder may be juvenile myoclonic epilepsy. The disclosed pediatric epilepsy disorder may be juvenile absence epilepsy. The disclosed pediatric epilepsy disorder may be
30 childhood absence epilepsy (e.g., pyknolepsy). The disclosed pediatric epilepsy disorder may be infantile spasms.

In some embodiments, the epilepsy disorder may be a result of a neurological disease or injury such as, for example, encephalitis, cerebritis, abscess, stroke, tumor, trauma,

genetic, tuberous sclerosis, cerebral dysgenesis, or hypoxic-ischemic encephalopathy. The epilepsy disorder may be associated with a neurodegenerative disease such as, for example, Alzheimer's disease or Parkinson's Disease. The epilepsy disorder may be associated with autism. The epilepsy disorder may be associated with a single gene mutation. The epilepsy disease may be associated with compulsive behaviors or electrographic seizures.

The epilepsy disorder may be an epilepsy disorder which is non-responsive to treatment (e.g., refractory epilepsy), for example, treatment with an antiepileptic drug (AED). The AED may be acetazolamide. The AED may be benzodiazepine. The AED may be cannabidiols. The AED may be carbamazepine. The AED may be clobazam. The AED may be clonazepam. The AED may be eslicarbazepine acetate. The AED may be ethosuximide. The AED may be ethotoin. The AED may be felbamate. The AED may be fenfluramine. The AED may be fosphenytoin. The AED may be gabapentin. The AED may be ganaxolone. The AED may be huperzine A. The AED may be lacosamide. The AED may be lamotrigine. The AED may be levetiracetam. The AED may be nitrazepam. The AED may be oxcarbazepine. The AED may be perampanel. The AED may be piracetam. The AED may be phenobarbital. The AED may be phenytoin. The AED may be potassium bromide. The AED may be pregabalin. The AED may be primidone. The AED may be retigabine. The AED may be rufinamide. The AED may be valproic acid. The AED may be sodium valproate. The AED may be stiripentol. The AED may be tiagabine. The AED may be topiramate. The AED may be vigabatrin. The AED may be zonisamide.

As used herein, "*improving mood*" includes, but is not limited to, improving one or more depressive symptoms disclosed herein, increasing coping strategies such as "acceptance" or "resilience", and/or increasing or improving scores on clinical tests or diagnostic criteria meant to evaluate an individual's mood or mental state. Resilience is defined as the ability to positively adapt in response to significant adversity or stressors. An example of a clinical test to evaluate mental state/health includes Brief Resilience Scale (BRS) resilience scores. The Brief Resilience Scale assesses the perceived ability to bounce back or recover from stress. The scale was developed to assess a unitary construct of resilience, including both positively and negatively worded items. The possible score range on the BRS is from 1 (low resilience) to 5 (high resilience).

As used herein, "*improving a depressive symptom*" includes improvement that is self-reported and that which is reported as improved by a clinician (e.g., though a clinical scoring protocol or test).

The term “*inflammatory bowel disorder*,” “*inflammatory bowel disease*” or “IBD” is used herein in the broadest sense and includes all diseases and pathological conditions the pathogenesis of which involves recurrent inflammation in the intestine, including small intestine and colon. Commonly seen IBD includes ulcerative colitis (UC) and Crohn's disease (CD), but is not limited to UC and CD. The manifestations of the disease include but not limited to inflammation and a decrease in epithelial integrity in the intestine. In some embodiments, inflammatory bowel disease includes autoimmune gastrointestinal dysmotility (AGID), esophagitis, gastritis, duodenitis, ischemic colitis, celiac sprue, Whipple's disease, peptic ulcer disease, pancreatitis, hepatitis including autoimmune hepatitis, non-alcoholic steatohepatitis (NASH), cholecystitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, and Wilson's disease. As used herein, IBD can also refer to a clinical state, wherein an individual is showing signs of pain (such as abdominal pain or cramping), nausea, vomiting, diarrhea and/or constipation.

The phrase “*impaired glucose metabolism*” encompasses any disorder characterized by clinical symptoms or a combination of clinical symptoms with elevated glucose levels and/or elevated insulin levels in subjects. Elevated glucose and/or insulin levels can be manifested in the following diseases, disorders, and conditions: hyperglycemia, type II diabetes, gestational diabetes, type I diabetes, insulin resistance, impaired glucose tolerance, high Insulinemia, abnormal glucose metabolism, diabetic reserves, other metabolic disorders (such as metabolic syndrome, also called syndrome X), and obesity. Individuals having impaired glucose metabolism can have hyperglycemia, hyperinsulinemia, and/or impaired glucose tolerance, as well as many related disorders. For example, insulin resistance, a disorder often associated with abnormal levels of glucose and/or insulin, is characterized by liver, fat, and muscle cells that have lost the ability to respond to normal blood insulin levels. Commonly, obesity and/or metabolic syndrome is often accompanied by various glucose metabolism disorders. Obesity increases the likelihood that an individual will develop various diseases such as diabetes, hypertension, atherosclerosis, coronary artery disease, gout, rheumatism, and arthritis. In addition, mortality risk is directly correlated with obesity, for example, a body mass index greater than 40 results in a decrease in life expectancy of over 10 years.

As used herein, the terms “improve”, “increase”, and grammatical equivalents thereof, indicate qualitative or quantitative difference from a reference. The difference may be at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least

40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 100%, at least 150%, at least 200%, at least 250%, at least 300%, or at least 400% higher compared to the reference.

As used herein, “reference” refers to a standard or control relative to which a comparison is performed. In some embodiments, a reference is a measured value. In some embodiments, a reference is an established standard or expected value. In some embodiments, a reference is a historical reference (*i.e.*, before the compositions disclosed herein are administered to the subject). A reference can be quantitative or qualitative. Typically, as would be understood by those of skill in the art, a reference and the value to which it is compared represent assessments under comparable conditions. Those of skill in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison. In some embodiments, an appropriate reference may be an agent, sample, sequence, subject, animal, or individual, or population thereof, under conditions those of skill in the art will recognize as comparable, *e.g.*, for the purpose of assessing one or more particular variables (*e.g.*, presence or absence of an agent or condition), or a measure or characteristic representative thereof.

The term “*metabolic syndrome*” includes but is not limited to hyperinsulinemia, impaired glucose tolerance, obesity, fat redistribution to the abdomen or upper body, hypertension, dysfibrinolysis, and dyslipidemia (high refers to a cluster of related traits, including triglycerides, low density high density lipoprotein (HDL)-cholesterol, and high low density low density lipoprotein (LDL) particles). A subject suffering from metabolic syndrome is at risk of developing type 2 diabetes and/or other disorders (*e.g.*, atherosclerosis).

As used herein, a “*psychiatric or neurologic disorder*” includes bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD), or food addiction. Food addiction may be diagnosed by a clinician and/or diagnosed based on the Yale Food Addiction Scale (YFAS). The YFAS allows for a systematic examination of a food addiction. The YFAS includes 25 items and translates the diagnostic criteria for substance dependence as stated in the DSM-IV (American Psychiatric Association, 2000) to relate to the consumption of calorie-dense foods (*e.g.*, high in refined carbohydrates and fat). The scale includes items that assess specific criteria, such as diminished control over consumption, a persistent desire or repeated unsuccessful attempts to quit, withdrawal, and clinically significant impairment. The YFAS includes two scoring options: 1) a “symptom count” ranging from 0 to 7 that reflects the number of addiction-like

criteria endorsed and 2) a dichotomous “diagnosis” that indicates whether a threshold of three or more “symptoms” plus clinically significant impairment or distress has been met. For example, a subject may be deemed to be afflicted with a food addiction if they achieve a YFAS symptom count ≥ 3 . Lower numbers may indicate at risk individuals. The term

5 “psychiatric disorder” can also refer to any disease of the mind and includes diseases and disorders listed in the Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition (DSM-IV), published by the American Psychiatric Association, Washington D. C. (1994). Psychiatric disorders include anxiety disorders (e.g., acute stress disorder agoraphobia, generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, posttraumatic
10 stress disorder, separation anxiety disorder, social phobia, and specific phobia), childhood disorders (e.g., attention-deficit/hyperactivity disorder, conduct disorder, and oppositional defiant disorder), eating disorders (e.g., anorexia nervosa and bulimia nervosa), mood disorders (e.g., depression, bipolar disorder, cyclothymic disorder, dysthymic disorder, and major depressive disorder), personality disorders (e.g., antisocial personality disorder,
15 avoidant personality disorder, borderline personality disorder, dependent personality disorder, histrionic personality disorder, narcissistic personality disorder, obsessive-compulsive personality disorder, paranoid personality disorder, schizoid personality disorder, and schizotypal personality disorder), psychotic disorders (e.g., brief psychotic disorder, delusional disorder, schizoaffective disorder, schizophreniform disorder, schizophrenia, and
20 shared psychotic disorder), substance-related disorders (e.g., alcohol dependence, amphetamine dependence, cannabis dependence, cocaine dependence, hallucinogen dependence, inhalant dependence, nicotine dependence, opioid dependence, phencyclidine dependence, and sedative dependence), adjustment disorder, autism, delirium, dementia, multi-infarct dementia, learning and memory disorders (e.g., amnesia and age-related
25 memory loss), and Tourette’s disorder.

The term “*preventing*” is art-recognized, and when used in relation to a condition, such as a local recurrence, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus,
30 prevention of acne includes, for example, reducing the number of detectable acne lesions in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable lesions in a treated population

versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

The term “*prophylactic*” or “*therapeutic*” treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term “*subject*” refers to a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. In some embodiments, the subject is human.

A “*therapeutically effective amount*” of a compound with respect to the subject method of treatment refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or prevented, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

As used herein, the term “*treating*” or “*treatment*” includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in a manner to improve or stabilize a subject's condition.

As used herein, “*visceral pain*” is distinct from somatic pain and is generally described as pain that originates from the body's internal cavities or organs. Visceral pain has five important clinical and sensory characteristics: (1) it is not evoked from all visceral organs (e.g., liver or lung); (2) it is not always elicited by visceral injury (e.g., cutting an intestine does not evoke pain); (3) it is diffuse; (4) it may be referred to other locations; and (5) it may be associated with other autonomic and motor reflexes (e.g., nausea, lower-back muscle tension from renal colic) (Lancet 1999, 353, 2145-48).

Therapeutic Methods

In some aspects, provided herein are methods of improving, potentiating, and/or increasing vagal nerve activity in a subject in need thereof, the method comprising administering a composition comprising at least one metabolite selected from the metabolites

listed in Tables 1-6. In some embodiments, the vagal nerve activity is afferent vagal nerve activity.

In some embodiments, the subject is afflicted with or at risk for a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction. In some aspects, provided herein are methods of treating a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction in a subject.

In some aspects, provided herein are methods of improving mood and/or ameliorating a depressive symptom in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6 (e.g., at least one metabolite selected from Table 1). In some embodiments, the depressive symptom may be the depressive symptom is selected from anxiety, apathy, general discontent, guilt, hopelessness, loss of interest or pleasure in activities, mood swings, sadness, agitation, excessive crying, irritability, restlessness, social isolation, early awakening, excess sleepiness, insomnia, restless sleep, excessive hunger, fatigue, loss of appetite, lack of concentration, slowness in activity, suicidal ideation, weight gain and weight loss.

In some aspects, provided herein are methods of treating a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction. In some aspects, provided herein are methods of treating a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or food addiction in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some aspects, provided herein are methods of treating autism spectrum disorder in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

In some aspects, provided herein are methods of treating or preventing epilepsy (e.g., refractory epilepsy), Alzheimer's disease/cognition, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and/or visceral pain in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.

Any one of the methods disclosed herein can comprise administering at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, at least twenty-five, at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, at least thirty-four, at least thirty-five, at least thirty-six, at least thirty-seven, at least thirty-eight, at least thirty-nine, or at least forty of the metabolites listed in Tables 1-6.

Table 1: Metabolites Associated with Vagal Nerve Activation

7-ketodeoxycholate
beta-muricholate
cholate
chenodeoxycholate
deoxycholate
glycocholate
taurodeoxycholate
tauroolithocholate
taurohyodeoxycholic acid
ursodeoxycholic acid
lithocholate
alpha-muriculate
acetate
propionate
butyrate
3-indoxyl sulfate
glutamate

Table 2: Small-intestinal metabolites downregulated in GF vs. SPF&CONV

aspartate
glutamate
3-methylhistidine
N-acetylhistamine
N6,N6-dimethyllysine
N6,N6,N6-trimethyllysine
2-aminoadipate
N-acetyl-cadaverine
5-aminovalerate
N,N,N-trimethyl-5-aminovalerate
phenol sulfate
3-indoxyl sulfate
2-hydroxy-4-(methylthio)butanoic acid
N-acetyltaurine
citrulline
N-methylproline

Table 4: Small-intestinal metabolites downregulated in ABX vs. SPF&CONV

N-acetylglutamate
lysine
N6,N6-dimethyllysine
5-aminovalerate
N,N,N-trimethyl-5-aminovalerate
phenol sulfate
N-acetylkynurenine (2)
N-formylanthranilic acid
3-indoxyl sulfate
N,N-dimethylvaline
S-adenosylmethionine (SAM)
S-methylcysteine
citrulline
N-acetyl-isoputrescine
5-methylthioadenosine (MTA)
cysteine-glutathione disulfide

N-acetyl-isoputrescine	cysteinylglycine disulfide*
cysteine-glutathione disulfide	arabinose
prolylglycine	caprylate (8:0)
10-heptadecenoate (17:1n7)	decanoylcarnitine (C10)
oleate/vaccenate (18:1)	laurylcarnitine (C12)
10-nonadecenoate (19:1n9)	arachidoylcarnitine (C20)*
eicosapentaenoate (EPA; 20:5n3)	myristoleoylcarnitine (C14:1)*
heneicosapentaenoate (21:5n3)	eicosenoylcarnitine (C20:1)*
nisinate (24:6n3)	dihomo-linolenoylcarnitine (C20:3n3 or 6)*
docosapentaenoate (n6 DPA; 22:5n6)	3-hydroxybutyrate (BHBA)
(14 or 15)-methylpalmitate (a17:0 or i17:0)	3-hydroxyoctanoate
glutarate (C5-DC)	10-hydroxystearate
octadecenedioate (C18:1-DC)	choline phosphate
10-hydroxystearate	glycerophosphorylcholine (GPC)
19,20-DiHDDPA	glycerophosphoethanolamine
N-oleoylserine	glycerophosphoserine*
trimethylamine N-oxide	glycerophosphoinositol*
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	trimethylamine N-oxide
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	1,2-distearoyl-GPC (18:0/18:0)
1-(1-enyl-oleoyl)-GPE (P-18:1)*	1,2-dipalmitoyl-GPE (16:0/16:0)*
glycerol 3-phosphate	1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*
1-meadoylglycerol (20:3n9)*	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*
1-dihomo-linoleoylglycerol (20:2)	1-(1-enyl-palmitoyl)-GPC (P-16:0)*
1-palmitoyl-galactosylglycerol (16:0)*	1-(1-enyl-oleoyl)-GPE (P-18:1)*
2-palmitoyl-galactosylglycerol (16:0)*	glycerol 3-phosphate
hexadecaspinganine (d16:0)*	glycerophosphoglycerol
myristoyl dihydrosphingomyelin (d18:0/14:0)*	1-palmitoyl-galactosylglycerol (16:0)*
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	sphinganine
behenoyl dihydrosphingomyelin (d18:0/22:0)*	hexadecaspinganine (d16:0)*
sphingomyelin (d18:0/18:0, d19:0/17:0)*	palmitoyl dihydrosphingomyelin (d18:0/16:0)*
sphingomyelin (d18:0/20:0, d16:0/22:0)*	behenoyl dihydrosphingomyelin (d18:0/22:0)*
cholate	sphingomyelin (d18:0/18:0, d19:0/17:0)*
glycocholate	sphingomyelin (d18:0/20:0, d16:0/22:0)*
chenodeoxycholate	stearoyl sphingomyelin (d18:1/18:0)
glycochenodeoxycholate	behenoyl sphingomyelin (d18:1/22:0)*
beta-muricholate	sphingomyelin (d18:1/20:0, d16:1/22:0)*
glyco-beta-muricholate**	mevalonate
cholate sulfate	cholate
deoxycholate	glycocholate
taurodeoxycholate	chenodeoxycholate
tauroolithocholate	beta-muricholate
ursodeoxycholate	glyco-beta-muricholate**
glycoursodeoxycholate	cholate sulfate
7-ketolithocholate	deoxycholate
hyocholate	taurodeoxycholate
taurohyocholate*	tauroolithocholate
taurohyodeoxycholic acid	ursodeoxycholate
7-ketodeoxycholate	glycoursodeoxycholate

1-methyladenine	7-ketolithocholate
N6-succinyladenosine	hyocholate
orotidine	taurohyodeoxycholic acid
5,6-dihydrouridine	7-ketodeoxycholate
cytidine 5'-monophosphate (5'-CMP)	orotidine
thiamin (Vitamin B1)	cytidine 5'-monophosphate (5'-CMP)
thiamin monophosphate	cytosine
retinol (Vitamin A)	(3'-5')-adenylylcytidine
pyridoxamine	(3'-5')-adenylyluridine
hippurate	(3'-5')-uridylylcytidine*
catechol sulfate	(3'-5')-uridylyluridine
cinnamoylglycine	quinolinate
equol	alpha-CMBHC glucuronide
equol glucuronide	dihydrobiopterin
equol sulfate	thiamin (Vitamin B1)
pheophorbide A	thiamin monophosphate
pheophytin A	pyridoxamine
malonylgenistin	pyridoxal
diaminopimelate	hippurate
	benzoate
	catechol sulfate
	cinnamoylglycine
	equol
	equol glucuronide
	equol sulfate
	pheophorbide A
	pheophytin A
	malonylgenistin
	diaminopimelate

Table 3: Cecal metabolites downregulated in GF vs. SPF&CONV

glycine
sarcosine
serine
alanine
N-methylalanine
N,N-dimethylalanine
aspartate
N-acetylaspartate (NAA)
glutamate
glutamine
alpha-ketoglutaramate*
N-acetylglutamate
N-acetylglutamine
glutamate, gamma-methyl ester
carboxyethyl-GABA
N-methyl-GABA
S-1-pyrroline-5-carboxylate
histidine
3-methylhistidine

Table 5: Cecal metabolites downregulated in ABX vs. SPF&CONV

glycine
N-acetylglutamine
sarcosine
dimethylglycine
serine
alanine
N-methylalanine
N,N-dimethylalanine
aspartate
N-acetylaspartate (NAA)
glutamate
alpha-ketoglutaramate*
N-acetylglutamate
N-acetylglutamine
carboxyethyl-GABA
N-methyl-GABA
S-1-pyrroline-5-carboxylate
histidine
trans-uocanate

trans-urocanate	cis-urocanate
cis-urocanate	imidazole propionate
imidazole propionate	formiminoglutamate
formiminoglutamate	1-methylhistamine
imidazole lactate	1-methyl-4-imidazoleacetate
histamine	N-acetylhistamine
1-methylhistamine	pipecolate
1-methyl-4-imidazoleacetate	N-acetyl-cadaverine
N-acetylhistamine	5-aminovalerate
lysine	N,N,N-trimethyl-5-aminovalerate
pipecolate	phenylalanine
N-acetyl-cadaverine	phenylpyruvate
5-aminovalerate	phenyllactate (PLA)
N,N,N-trimethyl-5-aminovalerate	phenethylamine
phenylalanine	phenylacetate
phenylpyruvate	4-hydroxyphenylacetate
phenyllactate (PLA)	tyrosine
phenethylamine	4-hydroxyphenylpyruvate
phenylacetate	tyrosol
4-hydroxyphenylacetate	o-Tyrosine
tyrosine	gentisate
4-hydroxyphenylpyruvate	N-formylphenylalanine
tyrosol	tryptophan
o-Tyrosine	kynurenine
gentisate	kynurenate
N-formylphenylalanine	N-formylanthranilic acid
tryptophan	anthranilate
kynurenine	xanthurenate
kynurenate	picolinate
N-formylanthranilic acid	serotonin
anthranilate	5-hydroxyindoleacetate
xanthurenate	indoleacetate
picolinate	indolepropionate
5-hydroxyindoleacetate	leucine
indoleacetate	4-methyl-2-oxopentanoate
indolepropionate	alpha-hydroxyisocaproate
leucine	isovalerate (i5:0)
4-methyl-2-oxopentanoate	beta-hydroxyisovalerate
alpha-hydroxyisocaproate	beta-hydroxyisovaleroylcarnitine
isovalerate (i5:0)	3-methylglutaconate
beta-hydroxyisovalerate	isoleucine
beta-hydroxyisovaleroylcarnitine	3-methyl-2-oxovalerate
3-methylglutaconate	2-hydroxy-3-methylvalerate
isoleucine	ethylmalonate
3-methyl-2-oxovalerate	methylsuccinate
2-hydroxy-3-methylvalerate	valine
ethylmalonate	3-methyl-2-oxobutyrate
methylsuccinate	alpha-hydroxyisovalerate
valine	N,N-dimethylvaline
3-methyl-2-oxobutyrate	methionine
alpha-hydroxyisovalerate	N-formylmethionine
N,N-dimethylvaline	methionine sulfone

methionine	methionine sulfoxide
N-formylmethionine	4-methylthio-2-oxobutanoate
methionine sulfone	2-hydroxy-4-(methylthio)butanoic acid
methionine sulfoxide	homocysteine
4-methylthio-2-oxobutanoate	alpha-ketobutyrate
2-hydroxy-4-(methylthio)butanoic acid	taurine
homocysteine	N-acetyltaurine
alpha-ketobutyrate	3-sulfo-L-alanine
S-methylcysteine	citrulline
taurine	N-delta-acetylornithine
3-sulfo-L-alanine	N-methylproline
citrulline	diacetylspermidine*
N-delta-acetylornithine	N1,N12-diacetylspermine
N-methylproline	4-acetamidobutanoate
N-carbamoylputrescine	2-hydroxybutyrate/2-hydroxyisobutyrate
diacetylspermidine*	gamma-glutamylleucine
N1,N12-diacetylspermine	alanylleucine
4-acetamidobutanoate	glycylisoleucine
5-oxoproline	glycylleucine
2-hydroxybutyrate/2-hydroxyisobutyrate	glycylvaline
gamma-glutamylleucine	isoleucylglycine
alanylleucine	leucylalanine
glycylisoleucine	leucylglycine
glycylleucine	lysylleucine
glycylvaline	valylglutamine
isoleucylglycine	valylglycine
leucylalanine	valylleucine
leucylglycine	leucylglutamine*
lysylleucine	glucose
phenylalanylglycine	glycerate
threonylphenylalanine	ribose
valylglutamine	ribulose/xylulose
valylglycine	xylose
valylleucine	arabinose
leucylglutamine*	mannose
glucose	N-acetylmuramate
pyruvate	N-acetylglucosamine/N-acetylgalactosamine
glycerate	alpha-ketoglutarate
ribose	succinate
ribulose/xylulose	fumarate
xylose	malate
arabinose	2-methylcitrate/homocitrate
mannose	butyrate/isobutyrate (4:0)
N-acetylmuramate	valerate (5:0)
N-acetylglucosamine/N-acetylgalactosamine	caproate (6:0)
alpha-ketoglutarate	heptanoate (7:0)
succinate	laurate (12:0)
fumarate	5-dodecenoate (12:1n7)
malate	myristate (14:0)
2-methylcitrate/homocitrate	palmitate (16:0)
butyrate/isobutyrate (4:0)	margarate (17:0)
valerate (5:0)	stearate (18:0)

caproate (6:0)	nonadecanoate (19:0)
heptanoate (7:0)	arachidate (20:0)
caprylate (8:0)	myristoleate (14:1n5)
caprate (10:0)	palmitoleate (16:1n7)
laurate (12:0)	10-heptadecenoate (17:1n7)
5-dodecenoate (12:1n7)	oleate/vaccenate (18:1)
myristate (14:0)	10-nonadecenoate (19:1n9)
palmitate (16:0)	eicosenoate (20:1)
margarate (17:0)	erucate (22:1n9)
stearate (18:0)	tetradecadienoate (14:2)*
nonadecanoate (19:0)	hexadecatrienoate (16:3n3)
myristoleate (14:1n5)	stearidonate (18:4n3)
palmitoleate (16:1n7)	eicosapentaenoate (EPA; 20:5n3)
10-heptadecenoate (17:1n7)	heneicosapentaenoate (21:5n3)
oleate/vaccenate (18:1)	docosapentaenoate (n3 DPA; 22:5n3)
10-nonadecenoate (19:1n9)	docosahexaenoate (DHA; 22:6n3)
tetradecadienoate (14:2)*	nisinate (24:6n3)
hexadecatrienoate (16:3n3)	hexadecadienoate (16:2n6)
stearidonate (18:4n3)	linoleate (18:2n6)
eicosapentaenoate (EPA; 20:5n3)	linolenate [α or γ ; (18:3n3 or 6)]
heneicosapentaenoate (21:5n3)	dihomo-linoleate (20:2n6)
docosapentaenoate (n3 DPA; 22:5n3)	dihomo-linolenate (20:3n3 or n6)
docosahexaenoate (DHA; 22:6n3)	arachidonate (20:4n6)
nisinate (24:6n3)	adrenate (22:4n6)
hexadecadienoate (16:2n6)	docosapentaenoate (n6 DPA; 22:5n6)
dihomo-linoleate (20:2n6)	docosadienoate (22:2n6)
dihomo-linolenate (20:3n3 or n6)	mead acid (20:3n9)
arachidonate (20:4n6)	(14 or 15)-methylpalmitate (a17:0 or i17:0)
adrenate (22:4n6)	(16 or 17)-methylstearate (a19:0 or i19:0)
docosapentaenoate (n6 DPA; 22:5n6)	dimethylmalonic acid
docosadienoate (22:2n6)	glutarate (C5-DC)
mead acid (20:3n9)	2-hydroxyglutarate
(14 or 15)-methylpalmitate (a17:0 or i17:0)	2-hydroxyadipate
dimethylmalonic acid	pimelate (C7-DC)
glutarate (C5-DC)	suberate (C8-DC)
2-hydroxyglutarate	azelate (C9-DC)
2-hydroxyadipate	sebacate (C10-DC)
pimelate (C7-DC)	undecanedioate (C11-DC)
suberate (C8-DC)	dodecanedioate (C12-DC)
azelate (C9-DC)	dodecenedioate (C12:1-DC)*
sebacate (C10-DC)	tetradecanedioate (C14-DC)
undecanedioate (C11-DC)	branched chain 14:0 dicarboxylic acid**
dodecanedioate (C12-DC)	hexadecanedioate (C16-DC)
dodecenedioate (C12:1-DC)*	hexadecenedioate (C16:1-DC)*
tetradecanedioate (C14-DC)	octadecenedioate (C18:1-DC)
branched chain 14:0 dicarboxylic acid**	2-hydroxysebacate
hexadecanedioate (C16-DC)	3-hydroxymargaroylglycine
hexadecenedioate (C16:1-DC)*	margaroylcarnitine (C17)*
octadecenedioate (C18:1-DC)	oleoylcarnitine (C18:1)
2-hydroxysebacate	(S)-3-hydroxybutyrylcarnitine
3-hydroxymargaroylglycine	deoxycarnitine
oleoylcarnitine (C18:1)	3-hydroxybutyrate (BHBA)

(S)-3-hydroxybutyrylcarnitine	palmitoylcholine
deoxycarnitine	2-hydroxyheptanoate*
palmitoylcholine	2-hydroxypalmitate
oleoylcholine	2-hydroxystearate
2-hydroxyheptanoate*	2-hydroxyarachidate*
2-hydroxypalmitate	2-hydroxybehenate
2-hydroxystearate	2-hydroxynervonate*
2-hydroxyarachidate*	3-hydroxyhexanoate
2-hydroxybehenate	3-hydroxyoctanoate
2-hydroxynervonate*	3-hydroxydecanoate
3-hydroxyhexanoate	3-hydroxysebacate
3-hydroxyoctanoate	3-hydroxylaurate
3-hydroxydecanoate	3-hydroxypalmitate
3-hydroxysebacate	3-hydroxystearate
3-hydroxylaurate	3-hydroxyoleate*
3-hydroxypalmitate	8-hydroxyoctanoate
3-hydroxystearate	10-hydroxystearate
3-hydroxyoleate*	16-hydroxypalmitate
8-hydroxyoctanoate	13-HODE + 9-HODE
10-hydroxystearate	12,13-DiHOME
16-hydroxypalmitate	9,10-DiHOME
12,13-DiHOME	2S,3R-dihydroxybutyrate
9,10-DiHOME	19,20-DiHDP
2S,3R-dihydroxybutyrate	oleoyl ethanolamide
oleoyl ethanolamide	palmitoyl ethanolamide
palmitoyl ethanolamide	stearoyl ethanolamide
stearoyl ethanolamide	linoleoyl ethanolamide
arachidoyl ethanolamide (20:0)*	arachidoyl ethanolamide (20:0)*
choline phosphate	choline phosphate
glycerophosphoethanolamine	glycerophosphoethanolamine
1,2-dipalmitoyl-GPE (16:0/16:0)*	trimethylamine N-oxide
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	1,2-dipalmitoyl-GPE (16:0/16:0)*
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)
1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	1-palmitoyl-2-oleoyl-GPG (16:0/18:1)
1-palmitoyl-GPA (16:0)	1-palmitoyl-GPA (16:0)
1-stearoyl-GPA (18:0)	1-stearoyl-GPA (18:0)
1-oleoyl-GPA (18:1)	1-oleoyl-GPA (18:1)
1-linoleoyl-GPA (18:2)*	1-linoleoyl-GPA (18:2)*
1-palmitoyl-GPG (16:0)*	1-linoleoyl-GPC (18:2)
1-stearoyl-GPG (18:0)	1-lignoceroyl-GPC (24:0)
1-oleoyl-GPG (18:1)*	1-palmitoyl-GPE (16:0)
glycerol	1-linoleoyl-GPE (18:2)*
glycerol 3-phosphate	1-palmitoyl-GPG (16:0)*
glycerophosphoglycerol	1-stearoyl-GPG (18:0)
1-pentadecanoylglycerol (15:0)	1-oleoyl-GPG (18:1)*
1-palmitoylglycerol (16:0)	glycerol
2-palmitoylglycerol (16:0)	glycerol 3-phosphate
1-palmitoyl-2-linoleoyl-galactosylglycerol (16:0/18:2)*	glycerophosphoglycerol
1-palmitoyl-galactosylglycerol (16:0)*	1-myristoylglycerol (14:0)
2-palmitoyl-galactosylglycerol (16:0)*	1-pentadecanoylglycerol (15:0)

1-palmitoyl-digalactosylglycerol (16:0)*	1-palmitoylglycerol (16:0)
3-ketosphinganine	2-myristoylglycerol (14:0)
sphinganine	1-palmitoyl-2-linoleoyl-digalactosylglycerol (16:0/18:2)*
hexadecasphinganine (d16:0)*	1-palmitoyl-2-linoleoyl-galactosylglycerol (16:0/18:2)*
N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	1-palmitoyl-galactosylglycerol (16:0)*
N-stearoyl-sphingosine (d18:1/18:0)*	2-palmitoyl-galactosylglycerol (16:0)*
N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	1-palmitoyl-digalactosylglycerol (16:0)*
ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*	3-ketosphinganine
hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH))**	sphinganine
stearoyl sphingomyelin (d18:1/18:0)	sphingadienine
sphingomyelin (d18:1/24:1, d18:2/24:0)*	hexadecasphinganine (d16:0)*
sphingomyelin (d18:2/24:1, d18:1/24:2)*	N-stearoyl-sphinganine (d18:0/18:0)*
eicosanoylsphingosine (d20:1)*	N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))
dehydrophytosphingosine*	N-stearoyl-sphingosine (d18:1/18:0)*
3-hydroxy-3-methylglutarate	N-palmitoyl-heptadecasphingosine (d17:1/16:0)*
mevalonate	<i>sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*</i>
4-cholesten-3-one	sphingosine
cholate	heptadecasphingosine (d17:1)
beta-muricholate	eicosanoylsphingosine (d20:1)*
alpha-muricholate	dehydrophytosphingosine*
glyco-beta-muricholate**	3-hydroxy-3-methylglutarate
cholate sulfate	mevalonate
deoxycholate	cholesterol
3-dehydrodeoxycholate	4-cholesten-3-one
taurodeoxycholate	cholate
6-beta-hydroxylithocholate	beta-muricholate
lithocholate	alpha-muricholate
12-ketolithocholate	glyco-beta-muricholate**
dehydrolithocholate	cholate sulfate
6-oxolithocholate	deoxycholate
hyocholate	3-dehydrodeoxycholate
taurohyodeoxycholic acid	taurodeoxycholate
3-dehydrocholate	6-beta-hydroxylithocholate
7-ketodeoxycholate	lithocholate
3b-hydroxy-5-cholenoic acid	12-ketolithocholate
isohyodeoxycholate	dehydrolithocholate
hypoxanthine	6-oxolithocholate
xanthine	hyocholate
2'-deoxyinosine	3-dehydrocholate
adenosine 5'-monophosphate (AMP)	7-ketodeoxycholate
adenosine	3b-hydroxy-5-cholenoic acid
adenine	isohyodeoxycholate
1-methyladenine	hypoxanthine
2'-deoxyadenosine 5'-monophosphate	xanthine

guanosine 5'- monophosphate (5'-GMP)	2'-deoxyinosine
guanosine-2',3'-cyclic monophosphate	adenosine 5'-monophosphate (AMP)
guanine	adenosine
N-carbamoylaspartate	adenine
uridine 5'-monophosphate (UMP)	1-methyladenine
uridine-2',3'-cyclic monophosphate	2'-deoxyadenosine 5'-monophosphate
uracil	guanosine 5'- monophosphate (5'-GMP)
pseudouridine	guanosine-2',3'-cyclic monophosphate
5,6-dihydrouridine	guanine
5-methyluridine (ribothymidine)	N-carbamoylaspartate
2'-deoxyuridine	uridine 5'-monophosphate (UMP)
3-ureidopropionate	uridine-2',3'-cyclic monophosphate
beta-alanine	uridine
cytidine 5'-monophosphate (5'-CMP)	uracil
cytosine	pseudouridine
2'-deoxycytidine 5'-monophosphate	5,6-dihydrouridine
thymine	5-methyluridine (ribothymidine)
3-aminoisobutyrate	2'-deoxyuridine
(3'-5')-adenylylcytidine	3-ureidopropionate
(3'-5')-adenylylguanosine*	beta-alanine
(3'-5')-adenylyluridine	cytidine 5'-monophosphate (5'-CMP)
(3'-5')-adenylyladenosine*	cytosine
(3'-5')-cytidyladenosine	2'-deoxycytidine 5'-monophosphate
(3'-5')-cytidylcytidine*	thymine
(3'-5')-cytidylguanosine	3-aminoisobutyrate
(3'-5')-cytidyluridine*	(3'-5')-adenylylcytidine
(3'-5')-guanylyladenosine*	(3'-5')-adenylylguanosine*
(3'-5')-guanylylcytidine	(3'-5')-adenylyluridine
(3'-5')-guanylyluridine	(3'-5')-adenylyladenosine*
(3'-5')-uridylylcytidine*	(3'-5')-cytidyladenosine
(3'-5')-uridylyluridine	(3'-5')-cytidylcytidine*
nicotinate	(3'-5')-cytidylguanosine
nicotinate ribonucleoside	(3'-5')-cytidyluridine*
nicotinamide riboside	(3'-5')-guanylyladenosine*
riboflavin (Vitamin B2)	(3'-5')-guanylylcytidine
flavin mononucleotide (FMN)	(3'-5')-guanylyluridine
pantothenate	(3'-5')-uridylylcytidine*
pantethine	(3'-5')-uridylyluridine
pantetheine	nicotinate
alpha-tocopherol	nicotinate ribonucleoside
alpha-tocotrienol	riboflavin (Vitamin B2)
gamma-tocotrienol	flavin mononucleotide (FMN)
D-urobilin	pantothenate
thiamin (Vitamin B1)	pantethine
thiamin monophosphate	pantetheine
5-(2-Hydroxyethyl)-4-methylthiazole	alpha-tocopherol
hydroxymethylpyrimidine	delta-tocopherol
retinol (Vitamin A)	alpha-tocotrienol
pyridoxamine	gamma-tocotrienol
pyridoxal	gamma-tocopherol/beta-tocopherol
pyridoxate	bilirubin (Z,Z)
benzoate	D-urobilin

4-hydroxybenzoate	thiamin (Vitamin B1)
2,4,6-trihydroxybenzoate	thiamin monophosphate
p-hydroxybenzaldehyde	5-(2-Hydroxyethyl)-4-methylthiazole
2-(4-hydroxyphenyl)propionate	hydroxymethylpyrimidine
3-(3-hydroxyphenyl)propionate	retinol (Vitamin A)
3-(4-hydroxyphenyl)propionate	carotene diol (1)
3-phenylpropionate (hydrocinnamate)	carotene diol (2)
3,5-dihydroxyphenylpropionate	carotene diol (3)
maltol	beta-cryptoxanthin
piperidine	pyridoxamine
vanillate	pyridoxal
2,3-dihydroxyisovalerate	pyridoxate
dipicolinate	4-hydroxybenzoate
2,8-quinolinediol	2,4,6-trihydroxybenzoate
2-isopropylmalate	p-hydroxybenzaldehyde
2-oxindole-3-acetate	2-(4-hydroxyphenyl)propionate
3-formylindole	3-(3-hydroxyphenyl)propionate
beta-guanidinopropanoate	3-(4-hydroxyphenyl)propionate
dihydroferulate	3-phenylpropionate (hydrocinnamate)
enterodiol	3,5-dihydroxyphenylpropionate
enterolactone	maltol
equol	piperidine
equol sulfate	apigenin
ferulate	vanillate
indolin-2-one	2,3-dihydroxyisovalerate
pheophytin A	dipicolinate
sinapate	2,8-quinolinediol
2-keto-3-deoxy-gluconate	2-isopropylmalate
4-hydroxycinnamate	2-oxindole-3-acetate
3-indoleglyoxylic acid	3-formylindole
2-aminophenol	beta-guanidinopropanoate
diaminopimelate	dihydroferulate
N-methylpipercolate	enterodiol
salicylate	enterolactone
2,6-dihydroxybenzoic acid	equol
1,3-propanediol	equol sulfate
sulfate*	ferulate
ectoine	indolin-2-one
2,4-di-tert-butylphenol	pheophytin A
	sinapate
	2-keto-3-deoxy-gluconate
	4-hydroxycinnamate
	3-indoleglyoxylic acid
	2-aminophenol
	diaminopimelate
	N-methylpipercolate
	salicylate
	2,6-dihydroxybenzoic acid
	1,3-propanediol
	sulfate*
	2,4-di-tert-butylphenol

Table 6: Supplementary Metabolites Associated with Vagal Nerve Activation
Hippurate
trimethylamine-N-oxide (TMAO)
imidazole propionate
phenethylamine
kynurenate
kynurenine
xanthurenate
dihydrocaffeate
tryptamine
palmitoyl ethanolamide (PEA)
oleoyl ethanolamide (OEA)
linoleoyl ethanolamide (LEA)
9-s-HODE
13-s-HODE
succinate

Provided herein are methods of increasing or potentiating vagal nerve activity, improving mood, ameliorating a depressive symptom, or treating or preventing a condition or disorder disclosed herein in a subject by administering to the subject a metabolite disclosed herein.

It should be appreciated that one or more metabolites disclosed herein can be administered conjointly to the subject. In some embodiments, one or more compositions/metabolites and an additional therapy disclosed herein can be administered conjointly to the subject.

As used herein, the phrase “conjoint administration” refers to any form of administration of two or more different agents such that the second agent is administered while the previously administered agent is still effective in the body. For example, the compositions disclosed herein can be administered either in the same formulation or in a separate formulation, either concomitantly or sequentially.

In some embodiments, the method further comprises administering an antibiotic to the subject prior to administration of the one or more metabolites (or compositions comprising the one or more metabolites) to the subject.

In some embodiments, the subject is showing a depressive symptom, such as anxiety, apathy, general discontent, guilt, hopelessness, loss of interest or pleasure in activities, mood swings, sadness, agitation, excessive crying, irritability, restlessness, social isolation, early awakening, excess sleepiness, insomnia, restless sleep, excessive hunger, fatigue, loss of appetite, lack of concentration, slowness in activity, suicidal ideation, weight gain and weight loss.

In some embodiments, the subject is afflicted with or at risk for a psychiatric or neurologic disorder, such as bipolar depression, post-partum depression (PPD), post-traumatic stress disorder (PTSD), major depressive disorder (MDD), or food addiction.

5 In some embodiment, the subject is at risk for or is afflicted with a psychiatric or neurologic disorder disclosed herein. The psychiatric or neurologic disorder may be depression, PPD, PTSD, MDD, food addiction, or bipolar disorder.

10 In some embodiments, the subject is at risk or is afflicted with epilepsy (e.g., refractory epilepsy), Alzheimer's disease/cognition, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and/or visceral pain. In some embodiments, the subject is afflicted with autism spectrum disorder.

15 In some embodiments, the subject has been diagnosed (e.g., by a clinician) prior to administration of a composition disclosed herein. In some embodiments, the subject is at risk for a psychiatric or neurologic disorder disclosed herein. As used herein, an "at risk" subject includes, for example, a subject with a family history of the psychiatric or neurologic disorder or a subject with other symptoms or disorders that are comorbid with the psychiatric or neurologic disorder.

20 In some embodiments, the methods further comprise administering an additional therapy for psychiatric or neurologic disorder, such as an antidepressant medication (e.g., citalopram, fluvoxamine, bupropion, escitalopram, sertraline, mirtazapine, fluoxetine, venlafaxine, amitriptyline, or imipramine). In another aspect, the additional therapy is a cannabinoid, a stimulant, an anti-inflammatory agent, a steroid, a barbiturate, an opioid analgesic, a sleep agent (e.g., melatonin or eszopiclone), an anxiolytic, an antipsychotic, or a combination thereof.

25 The agents and compositions of the present disclosure can be used in combination with at least one medication or therapy useful, e.g., in treating or alleviating symptoms of a psychiatric or a neurological condition. Suitable examples of such medications include levodopa (L-dopa), carbidopa, safinamide, dopamine agonists (e.g., ropinirole, pramipexole, rotigotine), amantadine, trihexyphenidyl, benzotropine, selegiline, rasagiline, tolcapone, and entacapone, or a pharmaceutically acceptable salt thereof. Other examples include
30 antidepressants (e.g., SSRIs, SNRIs, or tricyclic antidepressants) and antipsychotics (e.g., aripiprazole, fluphenazine, haloperidol, paliperidone, or risperidone).

The compositions disclosed herein may be administered to a subject by any means known in the art, for example, the composition may be formulated for oral delivery. The

composition may be in the form of a pill, tablet, or capsule. In some embodiments, the subject may be a mammal (e.g., a human). In some embodiments, the composition is self-administered.

It will be appreciated by a person of skill in the art the “at risk” in the following diagnostic methods may also include determining that the subject being screened is afflicted with a psychiatric or neurologic disorder disclosed herein. The methods may further comprise administration of a composition disclosed herein.

In some aspects, provided herein are methods for determining whether a subject is at risk for a disorder disclosed herein the method comprising: obtaining a sample from the GI tract of the subject, identifying the amount of or activity of at least one metabolite selected from the metabolites listed in Tables 1-6 in the microbiome of the sample, and if the sample comprises a lower level or decreased activity of at least one metabolite selected from the metabolites listed in Tables 1-6 compared to a control level, the subject is considered at risk for the disorder disclosed herein.

In some embodiments, the control level is a level measured in a sample from the GI tract of the subject taken earlier in time. For example, if the sample taken earlier in time comprises at least a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% increase of the level of or activity of at least one metabolite than a sample taken more recently (i.e., the level of the measured metabolite is decreasing over time), the subject is at risk or is afflicted with the condition or disorder disclosed herein.

In other embodiments, the control level is a mean or median level of the metabolite in subjects not afflicted with or at risk for the condition or disorder. For example, if the subject's sample has at least a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%,

79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% decrease in the level of or activity of a metabolite when compared than a mean or median level of the metabolite in a subject population not afflicted with the condition or disorder, the subject is determined to be at risk for or afflicted with the condition or disorder.

In some embodiments, a subject may undergo treatment with antibiotics or a composition comprising antibiotics to target and decrease the prevalence of pathogenic organisms, and subsequently be treated with a composition described herein.

Suitable antimicrobial compounds include capreomycins, including capreomycin IA, capreomycin IB, capreomycin IIA and capreomycin IIB; carbomycins, including carbomycin A; carumonam; cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefbuperazone, cefcapene pivoxil, cefclidin, cefdinir, cefditoren, cefime, ceftamet, cefmenoxime, cefmetazole, cefminox, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotetan, cefotiam, cefoxitin, cefpimizole, cefpiramide, cefpirome, cefprozil, cefroxadine, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftiofur, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephalixin, cephalogycin, cephaloridine, cephalosporin C, cephalothin, cephapirin, cephamycins, such as cephamycin C, cephradine, chlortetracycline; clarithromycin, clindamycin, clometocillin, clomocycline, cloxacillin, cyclacillin, danofloxacin, demeclocyclin, destomycin A, dicloxacillin, dirithromycin, doxycyclin, epicillin, erythromycin A, ethanbutol, fenbenicillin, flomoxef, florfenicol, floxacillin, flumequine, fortimicin A, fortimicin B, forfomycin, foraltadone, fusidic acid, gentamycin, glyconiazide, guamecycline, hetacillin, idarubicin, imipenem, isepamicin, josamycin, kanamycin, leumycins such as leumycin A1, lincomycin, lomefloxacin, loracarbef, lymecycline, meropenam, metampicillin, methacycline, methicillin, mezlocillin, micronomicin, midecamycins such as midecamycin A1, mikamycin, minocycline, mitomycins such as mitomycin C, moxalactam, mupirocin, nafcillin, netilicin, norcardians such as norcardian A, oleandomycin, oxytetracycline, panipenam, pazufloxacin, penamecillin, penicillins (such as penicillin G, penicillin N and penicillin O), penillic acid, pentympenicillin, peplomycin, phenethicillin, pipacyclin, piperacilin, pirlimycin, pivampicillin, pivcefalexin, porfiromycin, propiallin, quinacillin, ribostamycin, rifabutin, rifamide, rifampin, rifamycin SV, rifapentine, rifaximin, ritipenem, rekitamycin, rolitetracycline, rosaramicin, roxithromycin, sancycline, sisomicin, sparfloxacin, spectinomycin, streptozocin, sulbenicillin, sultamicillin, talampicillin, teicoplanin, temocillin,

tetracyclin, thioestrepton, tiamulin, ticarcillin, tigemonam, tilmicosin, tobramycin, tropospectromycin, trovafloxacin, tylosin, and vancomycin, and analogs, derivatives, pharmaceutically acceptable salts, esters, prodrugs, and protected forms thereof.

5 Suitable anti-fungal compounds include ketoconazole, miconazole, fluconazole, clotrimazole, undecylenic acid, sertaconazole, terbinafine, butenafine, clioquinol, haloprogin, nystatin, naftifine, tolnaftate, ciclopirox, amphotericin B, or tea tree oil and analogs, derivatives, pharmaceutically acceptable salts, esters, prodrugs, and protected forms thereof.

10 Suitable antiviral agents include acyclovir, azidouridine, anismoycin, amantadine, bromovinyldeoxusidine, chlorovinyldeoxusidine, cytarabine, delavirdine, didanosine, deoxynojirimycin, dideoxycytidine, dideoxyinosine, dideoxynucleoside, desciclovir, deoxyacyclovir, efavirenz, enviroxime, fiacitabine, foscarnet, fialuridine, fluorothymidine, floxuridine, ganciclovir, hypericin, idoxuridine, interferon, interleukin, isethionate, nevirapine, pentamidine, ribavirin, rimantadine, stavudine, sargramostin, suramin, trichosanthin, tribromothymidine, trichlorothymidine, trifluorothymidine, trisodium
15 phosphomonoformate, vidarabine, zidoviridine, zalcitabine and 3-azido-3-deoxythymidine and analogs, derivatives, pharmaceutically acceptable salts, esters, prodrugs, and protected forms thereof.

Other suitable antiviral agents include 2',3'-dideoxyadenosine (ddA), 2',3'-dideoxyguanosine (ddG), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxythymidine (ddT), 2',3'-
20 dideoxy-dideoxythymidine (d4T), 2'-deoxy-3'-thia-cytosine (3TC or lamivudine), 2',3'-dideoxy-2'-fluoroadenosine, 2',3'-dideoxy-2'-fluoroinosine, 2',3'-dideoxy-2'-fluorothymidine, 2',3'-dideoxy-2'-fluorocytosine, 2',3'-dideoxy-2',3'-didehydro-2'-fluorothymidine (Fd4T), 2',3'-dideoxy-2'-beta-fluoroadenosine (F-ddA), 2',3'-dideoxy-2'-beta-fluoro-inosine (F-ddI), and 2',3'-dideoxy-2'-beta-fluorocytosine (F-ddC). In some embodiments, the antiviral agent is
25 selected from trisodium phosphomonoformate, ganciclovir, trifluorothymidine, acyclovir, 3'-azido-3'-thymidine (AZT), dideoxyinosine (ddI), and idoxuridine and analogs, derivatives, pharmaceutically acceptable salts, esters, prodrugs, and protected forms thereof.

Pharmaceutical Compositions

30 In some aspects, the instant disclosure relates to a composition (e.g., a pharmaceutical composition) comprising a metabolite, and/or composition disclosed herein.

The pharmaceutical compositions disclosed herein may be delivered by any suitable route of administration, including orally, buccally, sublingually, parenterally, and topically, as by powders, ointments, drops, liquids, gels, or creams. In certain embodiments, the

pharmaceutical compositions are delivered generally (e.g., via oral or parenteral administration). In certain other embodiments, the pharmaceutical compositions are delivered locally through injection.

The present application also provides pharmaceutical compositions comprising an effective amount of a compound of the present disclosure disclosed herein, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier. The pharmaceutical composition may also comprise any one of the additional therapeutic agents described herein. In certain embodiments, the application also provides pharmaceutical compositions and dosage forms comprising any one the additional therapeutic agents described herein. The carrier(s) are “acceptable” in the sense of being compatible with the other ingredients of the formulation and, in the case of a pharmaceutically acceptable carrier, not deleterious to the recipient thereof in an amount used in the medicament.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of the present application include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wool fat.

The compositions or dosage forms may contain any one of the compounds and therapeutic agents described herein in the range of 0.005% to 100% with the balance made up from the suitable pharmaceutically acceptable excipients. The contemplated compositions may contain 0.001%-100% of any one of the compounds and therapeutic agents provided herein, in one embodiment 0.1-95%, in another embodiment 75-85%, in a further embodiment 20-80%, wherein the balance may be made up of any pharmaceutically acceptable excipient described herein, or any combination of these excipients.

In certain embodiments, a provided composition comprises one or more disintegrants or solubilizing agents, such as a cyclodextrin, or a carboxymethyl cellulose, calcium carboxymethyl cellulose, low-substituted hydroxypropyl cellulose, sodium croscarmellose, sodium carboxy starch, calcium carbonate, sodium carbonate and the like.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may

be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could prescribe and/or administer doses of the compounds employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

Compositions and formulations described herein may conveniently be presented in a unit dosage form, e.g., tablets, sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. See, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, Baltimore, MD (20th ed. 2000). Such preparative methods include the step of bringing into association with the molecule to be administered ingredients such as the carrier that constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers, liposomes or finely divided solid carriers, or both, and then, if necessary, shaping the product.

In some embodiments, any one of the compounds and therapeutic agents disclosed herein are administered orally. Compositions of the present application suitable for oral administration may be presented as discrete units such as capsules, sachets, granules or tablets each containing a predetermined amount (e.g., effective amount) of the active ingredient; a powder or granules; a solution or a suspension in an aqueous liquid or a non-aqueous liquid; an oil-in-water liquid emulsion; a water-in-oil liquid emulsion; packed in liposomes; or as a bolus, etc. Soft gelatin capsules can be useful for containing such suspensions, which may beneficially increase the rate of compound absorption. In the case of tablets for oral use, carriers that are commonly used include lactose, sucrose, glucose,

mannitol, and silicic acid and starches. Other acceptable excipients may include: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-
5 agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl
10 sulfate, and mixtures thereof. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. Compositions suitable for oral administration include lozenges comprising the ingredients in a flavored basis, usually
15 sucrose and acacia or tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia.

Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions or infusion solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended
20 recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, saline (e.g., 0.9% saline solution) or 5% dextrose solution, immediately
25 prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets. The injection solutions may be in the form, for example, of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution
30 or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this

purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also
5 contain a long-chain alcohol diluent or dispersant.

The pharmaceutical compositions of the present disclosure may be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of the present application with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the
10 rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax, and polyethylene glycols.

The pharmaceutical compositions of the present disclosure may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance
15 bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, U.S. Patent No. 6,803,031. Additional formulations and methods for intranasal administration are found in Ilium, L., J Pharm Pharmacol, 56:3-17, 2004 and Ilium, L., Eur J Pharm Sci 11:1-18, 2000.

20 According to another embodiment, the present disclosure provides an implantable drug release device impregnated with or containing a compound or a therapeutic agent, or a composition comprising a compound of the present application or a therapeutic agent, such that said compound or therapeutic agent is released from said device and is therapeutically active.

25 The foregoing dosages can be administered on a daily basis (e.g., as a single dose or as two or more divided doses, e.g., once daily, twice daily, thrice daily) or non-daily basis (e.g., every other day, every two days, every three days, once weekly, twice weekly, once every two weeks, once a month).

Nutraceutical Composition

30 A nutraceutical composition is a pharmaceutical alternative which may have physiological benefits. In some embodiments, a nutraceutical composition is a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease. See, e.g., Brower (1998) Nat. Biotechnol. 16:728-731; Kalra (2003) AAPS

Pharm Sci. 5(3):25. In other embodiments, a nutraceutical composition is a dietary or nutritional supplement. The compositions described herein may be a nutraceutical composition.

Accordingly, a nutraceutical composition disclosed herein can be a food product, foodstuff, functional food, or a supplement composition for a food product or a foodstuff. As used herein, the term food product refers to any food or feed which provides a nutritional source and is suitable for oral consumption by humans or animals. The food product may be a prepared and packaged food (e.g., mayonnaise, salad dressing, bread, or cheese food) or an animal feed (e.g., extruded and pelleted animal feed, coarse mixed feed or pet food composition). As used herein, the term foodstuff refers to a nutritional source for human or animal oral consumption. Functional foods refer to foods being consumed as part of a usual diet but are demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions.

Food products, foodstuffs, functional foods, or dietary supplements may be beverages such as non-alcoholic and alcoholic drinks as well as liquid preparations to be added to drinking water and liquid food. Non-alcoholic drinks are for instance soft drinks; sport drinks; fruit juices, such as orange juice, apple juice and grapefruit juice; lemonades; teas; near-water drinks; and milk and other dairy drinks such as yogurt drinks, and diet drinks. In other embodiments, food products, foodstuffs, functional foods, or dietary supplements refer to solid or semi-solid foods. These forms can include, but are not limited to, baked goods such as cakes and cookies; puddings; dairy products; confections; snack foods (e.g., chips); or frozen confections or novelties (e.g., ice cream, milk shakes); prepared frozen meals; candy; liquid food such as soups; spreads; sauces; salad dressings; prepared meat products; cheese; yogurt and any other fat or oil containing foods; and food ingredients (e.g., wheat flour). In some embodiments, the food products, foodstuffs, functional foods, or dietary supplements may be in the form of tablets, boluses, powders, granules, pastes, pills or capsules for the ease of ingestion.

It is understood by those of skill in the art that in addition to isolated, and optionally purified and/or sonicated compositions of the present disclosure and other ingredients can be added to food products, foodstuffs, or functional foods described herein, for example, fillers, emulsifiers, preservatives, etc. for the processing or manufacture of the same. Additionally, flavors, coloring agents, spices, nuts and the like may be incorporated into the nutraceutical composition. Flavorings can be in the form of flavored extracts, volatile oils, chocolate

flavorings, peanut butter flavoring, cookie crumbs, crisp rice, vanilla or any commercially available flavoring.

Emulsifiers can also be added for stability of the nutraceutical compositions. Examples of suitable emulsifiers include, but are not limited to, lecithin (e.g., from egg or soy), and/or mono- and di-glycerides. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product. Preservatives can also be added to the nutritional supplement to extend product shelf life. Preferably, preservatives such as potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate or calcium disodium EDTA are used.

In addition, the nutraceutical composition can contain natural or artificial (preferably low calorie) sweeteners, e.g., saccharides, cyclamates, aspartamine, aspartame, acesulfame K, and/or sorbitol. Such artificial sweeteners can be desirable if the nutraceutical composition is intended to be consumed by an overweight or obese individual, or an individual with type II diabetes who is prone to hyperglycemia.

Moreover, a multi-vitamin and mineral supplement can be added to the nutraceutical compositions to obtain an adequate amount of an essential nutrient, which is missing in some diets. The multi-vitamin and mineral supplement can also be useful for disease prevention and protection against nutritional losses and deficiencies due to lifestyle patterns.

Accordingly, particular embodiments provide for the nutritional source of the nutraceutical to modulate endogenous commensal bacterial populations. Such modulation can be achieved by modification of gut pH, consumption of beneficial bacteria (e.g., as in yogurt), by providing nutritional sources (e.g., prebiotics) that select for particular populations of bacteria, or by providing antibacterial compounds. Such modulation can mean an increase or decrease in the gut microbiota populations or ratios. In some embodiments, the absolute or relative numbers of desirable gut microorganisms is increased and/or the absolute or relative numbers of undesirable gut microorganisms is decreased. For example, it is contemplated that there are a variety of nutritional sources exhibiting antibacterial activity that can be used to modulate gut microbiota populations. For example, garlic has been shown to produce the compound allicin (allyl 2-propenethiosulfinate), which exhibits antibacterial activity toward *E. coli* (Fujisawa, et al. (2009) *Biosci. Biotechnol. Biochem.* 73 (9):1948-55; Fujisawa, et al. (2008) *J. Agric. Food Chem.* 56(11):4229-35). Similarly, rosemary extracts and other essential oils have been shown to contain antibacterial activity (Klancnik, et al. (2009) *J. Food Prot.* 72(8):1744-52; Si, et al. (2006) *J. Appl. Microbiol.* 100(2):296-305).

Extracts of the edible basidiomycete, *Lentinus edodes* (Shiitake), have also been shown to possess antibiotic activity (Soboleva, et al. (2006) *Antibiot. Khimioter.* 51(7):3-8; Hirasawa, et al. (1999) *Int. J. Antimicrob. Agents* 11(2):151-7). Moreover, purple and red vegetable and fruit juices exhibit antibacterial activities (Lee, et al. (2003) *Nutrition* 19:994-996).

5 Furthermore, it is contemplated herein that the food products, foodstuffs, functional foods, or dietary supplements may be combined with antibiotics to control the gut microbiota populations.

 The nutraceutical composition can be provided in a commercial package, alone, or with additional components, e.g., other food products, food stuffs, functional foods, dietary
10 supplement. Desirably, the commercial package has instructions for consumption of the instant nutraceutical, including preparation and frequency of consumption, and use in the prevention or treatment of inflammatory diseases, autoimmune diseases and cancer.
 Moreover, in particular embodiments, the commercial package further includes a natural product (e.g., the food, extracts, antibiotics, and oils) that modulates endogenous commensal
15 bacterial populations. A package containing both a nutraceutical of the present disclosure in combination with said natural product can contain instructions for consuming the natural product, e.g., in advance (e.g., 2, 4, 6 or 8 or more hours) of consuming the nutraceutical in order to enhance the activity of the nutraceutical composition.

EXAMPLES

Example 1: Vagal chemosensation of microbial metabolites from the small intestinal lumen.

The gut microbiota is emerging as a key modulator of brain function and behavior, as several recent studies reveal effects of gut microbes on neurophysiology, complex animal behaviors, and endophenotypes of neurodevelopmental, neurological, and neurodegenerative diseases. Despite these findings supporting a “microbiome-gut-brain axis”, the mechanisms that underlie interactions between gut microbes and the brain remain poorly understood. While many studies highlight neuroimmune pathways for microbial influences on the brain, it is also believed that the gut microbiota may directly signal to the brain via gut-innervating vagal neurons. However, existing evidence for the vagal route largely derives from studies wherein subdiaphragmatic vagotomy abrogates behavioral alterations in response to microbiota perturbation in mice. This ablates signaling in both afferent and efferent directions, not only to the intestine, but also with other peripheral organs. While the approach provides an important initial indication that the vagus nerve contributes to behavioral phenotypes that are modified by the microbiome, *in vivo* evidence of microbial signaling through vagal neurons is needed, and fundamental questions remain regarding the nature of microbial effects on vagal activity, the particular molecular constituents involved, and the diversity of neuronal responses elicited.

The gut microbiome is central to dietary metabolism and modulates hundreds of biochemicals in the intestine, as well as the blood and various distal extraintestinal tissues. Biochemical screens of supernatants from cultured human-derived gut microbes find that soluble microbial products (largely uncharacterized) have the capacity to directly bind to numerous G-protein-coupled receptors (GPCRs) that mediate neurotransmitter and neuropeptide signaling, some of which are reportedly expressed by vagal neurons. As such, microbial metabolites generated in the intestinal lumen have the potential to directly or indirectly activate vagal neurons via receptors on mucosal sensory afferents, or on enteroendocrine cells (EECs) that synapse onto the mucosal sensory afferents, as has been described for select lumenal nutrient stimuli and microbial antigens. The data herein study the vagal afferent nerve activity in response to the presence, absence, depletion, and restoration of the gut microbiota was assessed. Further, microbiome-dependent metabolites within the proximal small intestine and cecum, which are poised to signal to gut mucosal vagal afferents, were profiled. Select microbial metabolites that induce vagal afferent neuronal activity with varied kinetics when administered to the lumen of the small intestine and that

elevate vagal tone when orally supplemented to microbiota-deficient mice were identified herein. Finally, pharmacological approaches were applied to probe the potential for direct and/or indirect receptor-mediated signaling of microbial metabolites to vagal neurons. This study provides fundamental functional and mechanistic evaluation of the gut microbiome as a regulator of lumenal metabolites that modulate vagal chemosensory signaling across the gut-brain axis.

Results

The gut microbiome promotes vagal afferent nerve activity: To determine the extent to which the microbiome contributes to gut-brain signaling via the vagus nerve, whole nerve electrophysiology was applied to measure bulk activity of vagal afferents in wildtype C57BL/6J mice reared in the presence vs. absence of microbial colonization (**FIG. 2A, left**). vagal afferent nerve recordings were conducted over a 10-minute period, allowing 5 minutes for signal stabilization, followed by 5 minutes of recording whereby the average spike frequency was quantified. Initial experiments indicated no differences in baseline vagal afferent activity between male and female mice (**FIG. 1**), so all subsequent experiments were performed in males. Germ-free (GF) mice exhibited significantly decreased vagal afferent nerve activity as compared to conventionally colonized (specific pathogen-free, SPF) controls (**FIG. 2B-C**). These reductions were reversed by colonizing GF mice with the SPF microbiota during adulthood (conventionalized, CONV), suggesting active interactions between the microbiome and vagus nerve that occur independently of developmental colonization. Treating adult SPF mice with oral broad-spectrum antibiotics (ABX; ampicillin, neomycin, vancomycin, and metronidazole) for 7 days to reduce bacterial load yielded modest, but not statistically significant, reductions in vagal afferent nerve activity. These data suggest a potential role for the microbiota in mediating various vagally-mediated physiological processes associated with immune, metabolic, and neuronal function that may together influence homeostatic vagal function. It was hypothesized that the inconsistent phenotype between the ABX and GF conditions may be due to incomplete depletion of bacteria by ABX treatment, enrichment of native ABX-resistant bacteria over the one-week treatment period, off-target effects of ABX absorbed into the systemic circulation, metronidazole-induced neurotoxicity, the activity of non-bacterial members of the microbiota, and/or confounding effects of developmental GF rearing. To gain further insight into the acute effects of microbiota depletion on active vagal signaling in response to

interoceptive signals, vagal afferent nerve activity was recorded acutely while introducing a constant flow of the subset of the ABX cocktail that is non-absorbable (i.e., vancomycin and neomycin) directly into the lumen of the duodenum and through the first ~10 cm of the small intestine, a site of dense vagal innervation (**FIG. 2A, right**). In order to address confounding effects of mechanical distention and the surgical procedure, vehicle was perfused into the intestinal lumen for a period of 600s to allow for stabilization of vagal recordings. All experiments were then normalized within-subject to the final 60s of the baseline perfusion period to capture the relative effect sizes of active vagal afferent nerve responses to luminal stimuli. Subsequently perfusing nonabsorbable ABX into the intestinal lumen of SPF mice for 20 minutes decreased vagal afferent nerve activity, as compared to vehicle (VEH)-perfused controls (**FIG. 2D-F**). Intestinal perfusates from ABX-perfused mice exhibited significant decreases in 16S rRNA gene sequence compared to VEH-perfused controls (**FIG. 3**), consistent with reductions in mucosal bacteria. ABX-induced decreases in vagal afferent nerve activity were not seen in GF mice perfused with ABX (**FIG. 2D-F**). These results suggest that intestinal perfusion with non-absorbable ABX decreases vagal afferent nerve activity via the bactericidal actions of ABX on microbes in the small intestine.

The gut microbiota influences many aspects of host biology, in large part by bacterial metabolism of dietary substrates, synthesis of secondary metabolites, and modification of host-derived molecules in the intestine. To acutely evaluate the effects of luminal microbial molecules on vagal afferent nerve activity, a solution of small intestinal (SI) and cecal contents collected from donor SPF or GF mice were administered into the SI lumen following ABX perfusion in SPF mice. SI perfusion with non-absorbable ABX reduced vagal afferent nerve activity, as reported above, whereas re-perfusion of SI/cecal contents from SPF mice acutely increased activity toward levels seen at pre-ABX baselines (“+ SPF” in **FIG. 2G-I**). No such effect was seen with re-perfusion of SI/cecal contents from GF mice (“+ GF” in **FIG. 2G-I**) or vehicle (“+VEH”, in **FIG. 2G-I**), suggesting that the observed vagal response is due the presence of SI and cecal microbes and/or microbial molecules. To investigate the contribution of microbial small molecules, in particular, the equivalent solution of SPF SI/cecal contents were sterile filtered with a 0.22µm polyethersulfone membrane filter in order to exclude bacteria, fungi, and other larger microorganisms, as well as larger macronutrients such as lipids and proteins and administered it to the SI lumen of ABX-perfused SPF mice. Sterile-filtered SPF SI/cecal contents increased vagal afferent nerve activity to levels similar to SPF on average, albeit with shorter latency and more variability

(“+ SPF-SF” in **FIG. 2G-I**). These differences could be due to differential kinetics of small molecule signaling resulting from a lack of macromolecules present in SPF-SF samples and/or variability in the fidelity of small molecules upon filtration or the distribution of small-molecule activated receptors along the length of the proximal and medial small intestine.

- 5 These data show that active signaling from the gut microbiota modulates vagal afferent activity *in vivo*, and that these effects are mediated, at least in part, by microbial small molecules within the lumen of the small intestine and cecum.

Microbiome-dependent bile acids, short-chain fatty acids, and 3-indoxyl sulfate indirectly stimulate vagal afferent nerve activity in a receptor-dependent manner: The gut

- 10 microbiota regulates numerous metabolites within the host. However, most characterizations to date have profiled metabolites in fecal or serum samples, excluding signaling molecules localized to the small intestine and cecum that are poised to interact with villus-innervating vagal neurons. To identify candidate microbial metabolites in the small intestine and cecum that may modify vagal afferent activity, liquid chromatography-tandem mass spectrometry
- 15 (LC-MS/MS) was performed based on untargeted metabolomic profiling of luminal contents from the duodenum (proximal SI) and cecum of SPF, GF, ABX, and CONV mice. 931 metabolites were identified from mouse proximal SI and cecal contents (**FIG. 14, FIG. 15, and Tables 1-10**). Principal component analysis revealed distinct clustering of GF and ABX samples away from SPF and CONV samples along PC1 (**FIG. 4A**), indicating that acute
- 20 ABX depletion of the gut microbiota yields SI and cecal metabolomic profiles that are similar to those seen with GF rearing and that adult inoculation of GF mice with a conventional microbiota induces SI and cecal metabolomic profiles that are similar to those seen with conventional colonization (SPF). This is consistent with the finding that acute depletion and re-introduction of the microbiota or microbial metabolites alters vagal afferent nerve activity
- 25 (**FIG. 2**). However, there were also notable differences within the cecal datasets in particular, with discrimination of ABX from GF profiles and, to a lesser degree, CONV from SPF profiles, along PC2 (**FIG. 4A, bottom**). These differences highlight potential developmental influences of microbial colonization on host physiology and/or incomplete depletion and/or restoration of microbial communities within the lower GI tract relative to the proximal small
- 30 intestine. Based on the ability of both GF status and acute perfusion of nonabsorbable ABX to decrease vagal afferent nerve activity and of CONV to elevate activity toward levels seen in SPF controls (**FIG. 2A-F**), The datasets were filtered to identify metabolites that were commonly differentially regulated by both microbiota-deficient conditions relative to both

colonized conditions. In SI contents, there were 79 shared metabolites that were significantly modulated by both GF and ABX conditions relative to both SPF and CONV conditions, and in cecal contents, there were 521 (**FIG. 4B, FIG. 14, FIG. 15, and Tables 1-10**). Based on the observation that SI/cecal contents and filtrates from SPF mice stimulate vagal afferent nerve activity compared to filtrates from GF controls (**FIG. 2G-I**), a subset of 49 SI and 335 cecal metabolites were significantly decreased by microbiota deficiency relative to colonized conditions (**FIG. 4B, FIG. 14, FIG. 15, and Tables 1-10**). These included microbial metabolites that were extremely low or undetectable in microbiota-deficient conditions (referred to as “microbiome-dependent”), as well as metabolites that were partially downregulated (but still detectable) in microbiota-deficient conditions (referred to as “microbially modulated”).

To identify the subset of microbial metabolites that have the potential to signal directly to vagal neurons, existing bulk and single-cell RNA sequencing datasets were cross-referenced for reported expression of known or putative receptors for the candidate SI and cecal metabolites. This identified select species of microbiome-dependent bile acids (BAs), a subset of which were identified as key drivers for classifying microbiota status via random-forest analysis in small-intestinal samples (**FIG. 4C-D**). Importantly, reductions in both primary BAs synthesized by the liver and microbially-derived secondary BAs were observed. Absence (GF) or depletion (ABX) of the microbiota results in an ablation of or a reduction in microbial synthesis of microbially-derived secondary BAs in the small intestinal lumen. Moreover, bile acids themselves exert effects on gut microbial composition resulting in altered feedback mechanisms regulating primary bile acid synthesis. Thus, the results reflect alterations in both microbial synthesis of secondary BAs and compositional effects on host BA synthesis. Additional classes of metabolites that were uncovered included the microbiome-dependent short chain fatty acid (SCFA) butyrate (**FIG. 4E**) and microbially modulated tryptophan derivatives (TRPs, **FIG. 5A**), fatty acid ethanolamides (FAEs, **FIG. 5B**), monohydroxy fatty acids (MFAs, **FIG. 5C**), as well as succinate and glutamate (**FIG. 5D-E**). To initially assess the ability of these metabolites to actively modify vagal activity from the SI lumen, vagal afferent nerve activity was recorded while perfusing physiologically relevant concentrations of metabolite pools into the SI. There were no statistically significant changes in vagal afferent nerve activity as measured by AUC over the entire 30-minute stimulus window with SI perfusion of the detected TRPs, FAEs, MFAs, or succinate (**FIG. 7A-D**). Consistent with existing literature demonstrating vagal responses to gastric delivery

of glutamate, SI perfusion of glutamate robustly increased vagal afferent nerve activity (**FIG. 7E**), possibly due to increased recruitment of vagal afferents neurons and/or repeated stimulation of metabotropic glutamate receptors. SI perfusion of select microbiome-dependent BAs elicited more rapid, transient vagal afferent nerve activity that returns to levels similar to baseline (**FIG. 6A-C**) relative to other metabolites tested, which parallels existing literature on systemic administration of select primary and secondary BAs. In contrast, SI perfusion of microbiome-dependent SCFAs (acetate, propionate, and butyrate) led to slower onset and gradual increases in vagal afferent nerve activity (**FIG. 6D-F**). This latency could be due to metabolite-specific differences in the rate of intestinal absorption, differential spatial localization of metabolite absorption and functional activity along the length of the gastrointestinal tract, and/or indirect signaling of the metabolites to non-neuronal mediators or enteric nervous cells.

Microbiome-dependent BAs and SCFAs in the intestinal lumen have the potential to bind to cognate receptors expressed by various cell types in the gastrointestinal tract (e.g., vagal, enteroendocrine, epithelial, immune). To determine the relative contributions of different cognate GPCRs to vagal responses induced by luminal microbial metabolites, select receptor antagonists were perfused immediately before and during administration of their corresponding metabolites into the SI lumen of SPF mice. BAs signal through the membrane-bound Takeda G protein-coupled receptor 5 (TGR5), which is expressed by intestinal epithelial cells, enteric neurons, and various intestinal innate immune cells in mice. Intestinal pre- and co-perfusion of the TGR5 antagonist m-tolyl 5-chloro-2-[ethylsulonyl] pyrimidine-4-carboxylate (SBI-115) prevented the initial rapid, transient increases in vagal afferent nerve activity induced by microbiome-dependent BAs (**FIG. 6A-C**). A difference in total area under the curve (AUC) across the entire stimulus window was not observed, as administration of SBI-115 leads to a delayed rise in vagal activity that was not observed with perfusion of microbiome-dependent BAs alone. This partial ablation suggests that TGR5 antagonism may elicit compensatory vagal responses to microbiome-dependent BAs through farnesoid X receptor (FXR) or other signaling mechanisms independent of local TGR5. SCFAs signal to free fatty acid receptor 2 (FFAR2), which is expressed by intestinal epithelial cells and free fatty acid receptor 3 (FFAR3), which is expressed by gut-innervating vagal neurons. Intestinal pre- and co-perfusion of the FFAR2 antagonist 4-[(R)-1-(benzo[b]thiophene-3-carbonyl)-2-methyl-azetidine-2-carbonyl]-(3-chloro-benzyl)-amino]-butyric acid 99 (GLPG0974) prevented the increase in vagal afferent nerve activity induced

by SCFAs (**FIG. 6D-F**). Mouse vagal afferent neurons do not express appreciable levels of FFAR2 or TGR5 (**FIG. 8**), suggesting that the antagonists prevent metabolite-induced vagal activity by acting through a cellular mediator. To test the potential for direct action of microbial metabolites on vagal neurons, calcium responses in acutely dissociated vagal nodose neurons were measured during bath perfusion of microbial BAs or SCFAs. There was little to no activation in response to BAs and SCFAs, with only 2% and 1% of imaged neurons showing a calcium response, respectively (**FIG. 9A-C**). These results suggest that microbiome-dependent BAs and SCFAs activate vagal afferent neurons via indirect activation of intestinal epithelial cells or other cellular mediators. Small intestinal EECs release neurotransmitters and neuropeptide hormones in response to nutrient- and microbial-derived cues such as glucose, BAs, and SCFAs (**FIG. 10A**). Moreover, EECs are enriched for FFAR2 and TGR5 compared to other epithelial cell subtypes (**FIG. 10B-C**). Therefore the potential for indirect action of microbial metabolites on vagal neurons via EECs was tested. Calcium imaging of intestinal secretin tumor (STC-1) EECs revealed that BAs and SCFAs significantly increase EEC activity compared to PBS alone, with 32% and 26% of sampled EECs eliciting a calcium response, respectively (**FIG. 10D-E**). Taken together, these results demonstrate that microbiome-dependent BAs and SCFAs likely elevate vagal afferent activity via activation of intestinal EECs.

In addition to testing microbial metabolites with reported receptor expression by vagal neurons, effects of select microbiome-dependent metabolites that have as yet unknown signaling mechanisms on vagal activity were evaluated. In particular, focus was given to metabolites that i) are reproducibly dependent upon the microbiome across various studies and biological contexts and ii) have been reportedly linked to brain function and/or behavior. Of these, 3-indoxyl sulfate (3IS), hippurate, and trimethylamine-N-oxide (TMAO) are microbiome-dependent metabolites in SI lumen, imidazole propionate is a microbiome-dependent metabolite in the cecum, and phenethylamine is microbially modulated in the cecum (**FIG. 4F**). These metabolites are also reduced in the serum of microbiome-deficient mice, suggesting that they are typically absorbed from the intestine and poised to interact with mucosal vagal afferents. Perfusing physiologically relevant concentrations of hippurate, TMAO, imidazole propionate, and phenethylamine individually through the small intestine had no measurable effect on vagal afferent nerve activity (**FIG. 7F-I**). In contrast, SI perfusion with 3IS elicited rapid and sustained increases in vagal afferent nerve activity relative to vehicle controls (**FIG. 6G-I**). Microbial indole (the metabolic precursor to 3IS)

and its derivative indole-3-carboxaldehyde are reported to activate vagal afferent neurons in zebrafish via indirect stimulation of colonic EECs in a transient receptor potential ankyrin1 (TRPA1)-dependent manner. To evaluate this possible signaling mechanism for 3IS in the small intestine, the TRPA1 antagonist (1E,3E)-1-(4-Fluorophenyl)-2-methyl-1-penten-3-one oxime (A967079)) was pre- and co-perfused with 3IS into the SI lumen, which completely prevented 3IS-induced vagal afferent nerve activity (**FIG. 6G-I**). TRPA1 is expressed by vagal neurons, (**FIG. 8**) however, applying 3IS directly to dissociated vagal neurons yielded calcium responses in only 8% of imaged neurons (**FIG. 9A-C**). Conversely, TRPA1 is enriched in intestinal EECs (**FIG. 10B-C**) and application of 3IS to STC-1 cells results in activation of 32% of sampled EECs (**FIG. 10D-E**), suggesting an indirect mechanism of metabolite-induced elevation in vagal nerve activity via EEC activation. Overall, these results reveal that microbial BAs, SCFAs, and 3IS promote vagal afferent nerve activity through indirect receptor-dependent signaling from the SI lumen.

Luminal BAs, SCFAs, and 3IS excite both distinct and shared subsets of vagal afferent neurons with varied temporal responses: Different luminal stimuli can activate distinct populations of vagal neurons with differing response kinetics. In particular, recent work has identified populations of vagal afferent neurons that respond exclusively to fats versus sugars. Microbiome-dependent BAs, SCFAs, and 3IS are related to dietary metabolism of fats, complex carbohydrates, and proteins, respectively, raising the question of whether they promote vagal nerve activity via shared vs. distinct vagal afferent neurons. To gain insight, calcium activity of vagal afferent neurons in response to acute SI perfusion of microbiome-dependent metabolites in mice expressing GCaMP6s in Phox2b⁺ sensory neurons was imaged (**FIG. 11A-B**). Microbiome-dependent BAs, SCFAs, and 3IS elicited calcium responses in 57%, 48%, and 58% of detected vagal afferent neurons responsive to electrical stimuli at the end of the imaging session, respectively, on average across independent animals (**FIG. 11C**). The latency to maximum calcium response in metabolite-responsive units varied within each subclass of microbial metabolite, where SCFAs and 3IS similarly elicited primarily delayed calcium responses, while BAs elicited a bimodal distribution of acute and delayed calcium responses (**FIG. 11D**). Upon perfusing pairs of metabolites in counterbalanced sequence, of the metabolite-responsive neurons alone, BAs and SCFAs elicited calcium responses in largely distinct vagal afferent neurons, with 43% responsive to BAs only, 38% responsive to SCFAs, and 19% dually responsive to both BAs and SCFAs (“BAs <> SCFAs” in **FIG. 11E**). In contrast, sequential perfusion of 3IS and

SCFAs yielded many shared neuronal responses, where 40% of responsive vagal afferent neurons were dually activated by 3IS and SCFAs, 27% to SCFAs only, and 33% to 3IS only (“3IS $\leftrightarrow$ SCFAs” in **FIG. 11E**). Similarly, with BAs and 3IS, 43% dual responders, 35% responsive to 3IS only, and 22% responsive to BAs only were observed (“BAs $\leftrightarrow$ 3IS” in **FIG. 11E**). Together, these data reveal distinct and shared neuronal populations for sensing different microbiome- and macronutrient- dependent metabolites, with greater distinct neuronal responses to microbial BAs and SCFAs, than either with 3IS.

Supplementation of microbially-modulated metabolites into GF animals increases homeostatic vagal afferent nerve activity: Diet-induced decreases in gut microbial diversity are associated with alterations in vagal sensitivity to peripheral signals. The initial experiments demonstrated a role for the microbiota in modulating baseline vagal afferent nerve activity in gnotobiotic mice, whereby microbiome-deficient mice exhibited decreased homeostatic vagal nerve activity compared to colonized controls (**FIG. 2C**). Therefore, it was sought to uncover whether oral administration of microbial metabolites could phenocopy the increased vagal activity seen in response to persistent microbial colonization. To address this, GF mice were supplemented via twice-daily oral gavage with a cocktail of microbial metabolites (**MMs**: BAs, SCFAs, and 3IS, pooled) or vehicle solution for three days, followed by whole-nerve vagal electrophysiological recordings. Indeed, supplementation with MMs significantly increased vagal afferent nerve tone compared to vehicle-treated controls (**FIG. 11F**). Thus, microbiome-dependent dietary metabolites are an important link between diet-induced alterations in the gut microbiota and associated vagally-regulated host physiological and behavioral outcomes.

Receptor-mediated signaling of BAs, SCFAs, and 3IS from the small intestinal lumen activate neurons in the NTS: Changes in the gut microbiota have been associated with altered activation of neurons of the nucleus of the solitary tract (NTS), which receives direct visceral afferents from nodose neurons. Consistent with the ability of microbial metabolites in the SI lumen to stimulate vagal afferent neuronal activity (**FIG. 6 and FIG. 11**), acute luminal perfusion of microbiome-dependent BAs, SCFAs, and 3IS each increase neuronal expression of the activation marker cFos in the NTS (**FIG. 12A-B**), to levels similar to those seen with intestinal perfusion of sucrose. As with the vagal afferent nerve and neuronal responses, the microbial metabolite-driven increases in NTS neuronal activation were prevented by pre- and co-administration of antagonists for TGR5, FFAR2, and TRPA1 with their respective metabolite ligands. There were no significant differences in the dorsal motor nucleus of the

vagus (DMV) (**FIG. 13A-C**) suggesting that luminal application of microbial metabolites primarily stimulates ascending vagal pathways. Circulating microbial metabolites regulate satiety via vagal receptor-dependent pathways and alterations in agouti-related protein/neuropeptide Y (AgRP/NPY) and proopiomelanocortin (POMC) neurons of the hypothalamus. It was therefore sought to determine whether luminal application of BAs, SCFAs, and 3IS modulates neuronal activation in the arcuate nucleus of the hypothalamus (ARH) via their cognate receptors. A modest decrease in ARH cFos levels with luminal perfusion of 3IS, and to a lesser degree, BAs, were observed and that these effects were attenuated with pre- and co- perfusion of the selective antagonists for TRPA1 and TGR5, respectively (**FIG. 13D-E**). Conversely, luminal SCFAs had no appreciable effect on ARH cFos levels (**FIG. 13D-E**), suggesting a need for blood-brain barrier (BBB) translocation or engagement of vagal FFAR3 via circulating propionate in order to exert their anorexigenic effects previously described. Together, these data indicate that microbial BAs, SCFAs, and 3IS in the SI lumen may alter brain activity via receptor-mediated modulation of vagal afferent signaling.

Discussion

Results from this study demonstrate that microbial colonization status, as well as acute manipulation of the gut microbiota and microbial metabolites, modulate vagal activity. In particular, this study shows that the gut microbiota regulates numerous small molecules in the small intestine and cecum. Moreover, administering select microbiome-dependent BAs, SCFAs, and 3IS at physiological concentrations and rates of peristalsis into the lumen of the small intestine stimulates vagal afferent neuronal activity. The vagal responses are elicited within relatively short timescales (<~9 min) and are abrogated by pre- and co-administration of select receptor antagonists, suggesting active signaling between the gut microbiome and vagal afferents via excitatory metabolites.

The functional evidence provided in this study show that subdiaphragmatic vagotomy abrogates effects of microbial interventions on behaviors such as anxiety, depression, cognition, feeding, and social behaviors.

Through untargeted metabolomic profiling of SI and cecal contents from conventionally colonized (SPF, CONV) and microbiota deficient (ABX, GF) mice (**FIG. 4**), followed by *in vivo* screening of select microbial metabolites with or without pharmacological antagonists (**FIG. 6**), particular subclasses of microbiome-dependent

molecules that activate vagal afferent neurons in a receptor-dependent manner upon administration to the small intestine were identified. The metabolites (specific microbial BAs, SCFAs, and 3IS) promoted vagal afferent nerve activity with differing response kinetics, which could be due to differences in their physiological concentrations, rate of absorption, spatial localization of cognate receptors, and potential indirect activation via various non-vagal cellular mediators, among other factors. An increase in vagal afferent nerve activity at the time of the average maximal change in frequency with luminal perfusion of TRPs was also observed (**FIG. 7A**), suggesting the possibility that other microbial metabolites may modulate vagal neuronal activity with smaller overall effect sizes. Indeed, neuronal activation via GPCR signaling is dependent upon the concentration of the ligand, whereby differences can induce switching from G-protein coupled to G-protein independent signaling resulting in alterations in the downstream signal transduction pathways engaged during neuronal activation. Additionally, 30 small intestinal and 186 cecal metabolites were increased in GF and ABX mice as compared to SPF and CONV samples (**FIG. 4B**). These metabolites may play a role in microbiome-driven changes in vagal afferent nerve activity that were observed in conditions of microbial colonization.

Microbiome-dependent BAs and SCFAs are reported to be absorbed by the intestinal epithelium, which offers the potential to activate gut-innervating vagal afferents through direct receptor binding. However, the observed lack of vagal neuronal activation during *in vitro* stimulation of primarily dissociated nodose neurons in response to BAs and SCFAs (**FIG. 9A-C**), and the absence of their cognate receptors on mouse vagal neurons (**FIG. 8**), suggests these particular subclasses of microbial metabolites likely act through indirect interactions with diverse EECs, subsets of which can synapse directly onto vagal neurons, or other cellular mediators. Additionally, 3IS has not been shown to be readily re-absorbed following secretion into the intestinal lumen, and was rarely observed to activate vagal neurons *in vitro* (**FIG. 9A-C**), suggesting this metabolite also acts through the indirect pathway in order to activate vagal afferents. It is also possible that select intestinal metabolites may access systemic circulation and act at extra-intestinal sites to modulate vagal activity, presumably with a time delay. In particular, luminal microbiome-dependent BAs more acutely increased vagal afferent nerve activity, primarily via indirect activation of TGR5, which is expressed on various non-vagal cellular mediators along the gastrointestinal tract (**FIG. 6A-C and FIG. 8**). Moreover, direct application of BAs onto TGR5-expressing EECs induced calcium activity (**FIG. 10B-E**). Duodenal and jejunal EECs are enriched for

CCK, suggesting that this may align with prior studies demonstrating that BAs work synergistically with epithelial-derived CCK following absorption from the intestinal lumen, and that CCK signaling is dynamic and rapidly desensitizes. In contrast, perfusion of SCFAs into the SI lumen increased vagal afferent nerve activity following a latency period in an

5 FFAR2-dependent manner (**FIG. 6D-F**). SCFAs also increased activity in EECs expressing FFAR2 (**FIG. 10B-E**), suggesting indirect activation of FFAR2-expressing epithelial cells and subsequent GLP-1 release. Finally, microbiome-dependent 3IS in the small intestine elicited sustained vagal afferent nerve activity in a TRPA1-dependent manner (**FIG. 6G-I**). Further, 3IS activated EECs, but not vagal neurons alone (**FIG. 9 and FIG. 10**). This may

10 align with a previous study wherein indole stimulated TRPA1+ colonic EECs to release serotonin and activate colon-innervating neurons. These observations, considered together with existing vagal single cell transcriptomic data, suggest indirect activation of vagal afferent neurons via EECs and/or other cellular mediators (via FFAR2, TGR5, or TRPA1) by luminal microbial metabolites.

15 The effects of acute luminal perfusion of select microbial BAs, SCFAs, and 3IS (involved in dietary fat, carbohydrate, and protein metabolism, respectively) on vagal afferent neuronal calcium activity *in vivo* were observed. All three classes of microbial metabolites resulted in increased calcium activity in nodose neurons with varied kinetics (**FIG. 11B**). BAs elicited a bimodal distribution of immediate vs. delayed responses, whereas SCFAs and

20 3IS mostly elicited delayed responses (**FIG. 11D**), which aligns with the slow, gradual onset of vagal afferent nerve activity in response to SCFA and sustained onset of afferent nerve activity with 3IS perfusion. When assessing single-unit responses to sequential perfusion of two metabolite classes, microbiome-dependent BAs and SCFAs elicited calcium responses via largely non-overlapping subpopulations of vagal afferent neurons, whereas 3IS and BAs

25 or SCFAs acted primarily via shared subpopulations (**FIG. 11E**). These findings are supported by previous work demonstrating a shared role for both TGR5- and TRPA1-mediated alterations in digestion and satiety via epithelial CCK signaling, as well as TRPA1- and FFAR2-mediated alterations in host metabolism and feeding behaviors that have been reported to act via epithelial secretion of GLP-1. In contrast, previous work demonstrates that

30 BA- and SCFA- mediated alterations in feeding behaviors act via distinct receptor-dependent signaling pathways. More broadly, diet-induced decreases in microbial diversity are associated with alterations in vagal innervation and responses to interoceptive signals. The initial experiments demonstrated that GF mice exhibit decreased baseline vagal activity

compared to colonized controls (**FIG. 2B-C**). Following the identification of macronutrient-derived microbial metabolites that increase vagal activity, it was sought to determine whether oral supplementation of these metabolites can phenocopy increases in baseline vagal activity observed in response to microbial colonization. Indeed, supplementation with MMs increased vagal afferent nerve tone compared to vehicle controls (**FIG. 11F**), providing novel insight into the role of microbiome-dependent dietary metabolites as potential targets.

Despite evidence for vagal chemosensory pathways mediating communication from the intestinal lumen to the brain, effects of specific microbial metabolites and their associated receptors on CNS neuronal activity remain unclear. Therefore, the effects of luminal perfusion of BAs, SCFAs, and 3IS were assessed alongside their respective receptor antagonists for TGR5, FFAR2, and TRPA1 on medial NTS neuronal activation by immunofluorescence detection of the immediate early gene cFos. All metabolite classes significantly increased NTS neuronal activation, which could be prevented by pre- and co-perfusion of antagonists (**FIG. 12**). Sensory information processed in the NTS is directed to several other brain regions, including the DMV and ARH. None of the tested metabolite classes modified DMV neuronal cFos levels compared to VEH perfusion (**FIG. 13A-C**), suggesting that BAs, SCFAs, and 3IS in the SI lumen do not play a predominant role in acute vagovagal circuit modulation. In contrast, luminal BAs and 3IS elicited a modest reduction in neuronal activation in the ARH via TGR5 and TRPA1, respectively, while SCFAs elicited no overt effect (**FIG. 13D-F**). This aligns with reports that microbial metabolites elicit anorexigenic effects via hypothalamic regulation, many of which are vagally mediated. Notably, BAs and 3IS exhibit the highest percentage of dually responsive vagal neurons (**FIG. 11E**), suggesting that these metabolites may engage shared circuitry in order to modulate CNS physiology. Circulating deoxycholic acid promotes satiety synergistically with peripheral CCK via vagal TGR5 and modulation of ARH and POMC neuronal activation. BAs also directly modulate AgRP/NPY neuronal gene expression, suggesting circuit-specific effects of BAs on ARH neuronal activity responsible for driving satiety. Therefore, further investigation of the ARH neuronal subtype-specific effects of vagal afferent signaling and behavioral outcomes in response to luminal BAs is warranted. Previous work has suggested a role for the metabolic precursor to 3IS, indole, in regulating host feeding behaviors via indole-mediated GLP-1 release from enteroendocrine L-cells. Here, it was demonstrated that luminal 3IS functions as a potential modulator of host hypothalamic circuitry via vagal afferent signaling and TRPA1. However, little is known regarding how this signaling

pathway may alter host behaviors. Therefore, future studies aimed at uncovering the precise role for TRPA1-mediated vagal chemosensation of luminal 3IS on host behavioral outcomes is necessary. Together, these data suggest that luminal metabolites modulate CNS neuronal activity via receptor-dependent vagal signaling and may have consequential effects on CNS responses that contribute to behavior.

Findings from this study highlight luminal microbial metabolites derived from various sources of dietary macronutrients-- fats (BAs), complex carbohydrates (SCFAs), and proteins (3IS)—that differentially activate vagal afferent neurons via receptor-mediated signaling to convey information to the brain. Following the ingestion of dietary fats, BAs are released from the liver into the SI lumen where they undergo chemical transformations, such as deconjugation and dehydroxylation, which are carried out by gut microbes. Enzymes capable of catalyzing such reactions are found across all major bacterial phyla, suggesting a broad role for the microbiota in regulating the luminal BA pool. Similarly, SCFAs are derived from microbial fermentation of dietary fibers that are otherwise non-digestible by the host, with differential production by bacterial members of the phyla *Bacteroidetes* (*Bacterioidata*; acetate, propionate) and *Firmicutes* (*Bacillota*; butyrate). Despite being predominantly produced and concentrated in the colon, SCFAs have been shown to be present in the ileum and are able to translocate to more distal body sites via enterohepatic circulation. Tryptophan derivatives, such as indole, are produced by pathobionts, such as *Escherichia coli*, *Enterococcus faecalis*, and *Edwardsiella tarda*, before being hydroxylated and sulfated in the liver and secreted as 3IS into the small intestine.

Table 7. Metabolites of the mouse small-intestine: GF vs. SPF&CONV.

“Decrease in mean value” indicates that the mean values for that comparison are significantly lower; “Increase in mean value” indicates that the mean values for that comparison are significantly higher.

Biochemical Name	GF vs. SPF		CONV vs. GF	
	Decrease in mean value	p-value	Increase in mean value	p-value
7-ketodeoxycholate	0	0.0000	1271.36	0.0002
cholate	0	0.0000	317.1	0.0006
chenodeoxycholate	0	0.0000	98.06	0.0015
cholate sulfate	0.02	0.0000	24.6	0.0058
glycocholate	0.02	0.0000	15.44	0.0000
tauroolithocholate	0.04	0.0000	6.25	0.0001
deoxycholate	0	0.0000	167.83	0.0012

taurodeoxycholate	0	0.0000	287.75	0.0000
taurohyocholate*	0	0.0000	82.45	0.0000
ursodeoxycholate	0	0.0000	706.9	0.0005
beta-muricholate	0.02	0.0000	4.65	0.0261
pheophytin A	0.02	0.0000	33.8	0.0016
diaminopimelate	0.08	0.0000	9.75	0.0000
2-palmitoyl-galactosylglycerol (16:0)*	0.07	0.0000	21.69	0.0017
thiamin (Vitamin B1)	0.27	0.0000	4.23	0.0002
1-(1-enyl-oleoyl)-GPE (P-18:1)*	0.34	0.0000	1.87	0.0074
taurohyodeoxycholic acid	0	0.0000	1060.86	0.0000
equol sulfate	0	0.0000	362.52	0.0001
1-palmitoyl-galactosylglycerol (16:0)*	0.08	0.0000	17.92	0.0006
glycochenodeoxycholate	0.13	0.0000	2.47	0.0410
trimethylamine N-oxide	0.09	0.0000	17.37	0.0003
glyco-beta-muricholate**	0.19	0.0000	1.99	0.0079
glycoursodeoxycholate	0.22	0.0000	2.77	0.0006
equol glucuronide	0.02	0.0001	43.31	0.0000
2-aminoadipate	0.21	0.0001	2.27	0.0135
10-hydroxystearate	0.17	0.0001	4.89	0.0014
N-acetylhistamine	0.09	0.0002	9.51	0.0086
N,N,N-trimethyl-5-aminovalerate	0.3	0.0002	4.7	0.0001
phenol sulfate	0.05	0.0002	14.95	0.0022
7-ketolithocholate	0.02	0.0002	17.62	0.0396
hyocholate	0.02	0.0002	3.4	0.0417
catechol sulfate	0.03	0.0002	23.43	0.0115
N-acetyl-cadaverine	0.08	0.0003	20.06	0.0371
docosapentaenoate (n6 DPA; 22:5n6)	0.66	0.0003	1.33	0.0130
thiamin monophosphate	0.5	0.0003	2.39	0.0001
malonylgenistin	0.03	0.0003	28.48	0.0026
3-indoxyl sulfate	0.09	0.0004	3.59	0.0179
sphingomyelin (d18:0/20:0, d16:0/22:0)*	0.6	0.0004	1.54	0.0107
19,20-DiHDPA	0.44	0.0007	2.62	0.0004
1-methyladenine	0.21	0.0007	4.56	0.0106
retinol (Vitamin A)	0.27	0.0007	5.77	0.0068
cinnamoylglycine	0.12	0.0008	45.33	0.0001
pyridoxamine	0.47	0.0010	2.79	0.0000
behenoyl dihydrosphingomyelin (d18:0/22:0)*	0.51	0.0012	1.76	0.0174
hippurate	0.1	0.0012	20.49	0.0000
10-heptadecenoate (17:1n7)	0.55	0.0014	1.67	0.0042
5,6-dihydrouridine	0.5	0.0018	1.92	0.0259
eicosapentaenoate (EPA; 20:5n3)	0.59	0.0020	1.59	0.0056
myristoyl dihydrosphingomyelin (d18:0/14:0)*	0.45	0.0022	2.74	0.0002
sphingomyelin (d18:0/18:0, d19:0/17:0)*	0.61	0.0028	1.65	0.0342
N-oleoylserine	0.23	0.0036	4.71	0.0028
1-meadoylglycerol (20:3n9)*	0.3	0.0042	4.59	0.0006
aspartate	0.79	0.0051	1.24	0.0371
5-aminovalerate	0.36	0.0054	7.29	0.0091

1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	0.65	0.0055	1.49	0.0276
citrulline	0.34	0.0059	1.56	0.0003
N6-succinyladenosine	0.64	0.0067	1.56	0.0358
orotidine	0.59	0.0073	2.15	0.0077
N-methylproline	0.33	0.0078	3.42	0.0029
3-methylhistidine	0.71	0.0081	1.44	0.0045
cysteine-glutathione disulfide	0.31	0.0088	5.62	0.0064
(14 or 15)-methylpalmitate (a17:0 or i17:0)	0.64	0.0090	1.56	0.0120
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	0.73	0.0092	1.6	0.0032
N-acetyl-isoputrescine	0.6	0.0097	1.9	0.0128
equol	0.24	0.0103	4.96	0.0111
glycerol 3-phosphate	0.5	0.0114	1.99	0.0130
nisinate (24:6n3)	0.62	0.0118	1.49	0.0222
10-nonadecenoate (19:1n9)	0.63	0.0119	1.59	0.0141
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	0.62	0.0119	1.75	0.0001
hexadecasphinganine (d16:0)*	0.27	0.0121	3.56	0.0014
pheophorbide A	0.21	0.0130	4.56	0.0048
glutarate (C5-DC)	0.55	0.0135	2.28	0.0050
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	0.72	0.0135	1.39	0.0325
glutamate	0.83	0.0168	1.15	0.0332
oleate/vaccenate (18:1)	0.74	0.0189	1.34	0.0227
1-dihomo-linoleoylglycerol (20:2)	0.64	0.0215	2.07	0.0043
octadecenedioate (C18:1-DC)	0.64	0.0222	1.75	0.0224
cytidine 5'-monophosphate (5'-CMP)	0.79	0.0244	1.32	0.0303
N-acetyltaurine	0.59	0.0279	1.55	0.0172
prolylglycine	0.79	0.0308	1.2	0.0423
2-hydroxy-4-(methylthio)butanoic acid	0.23	0.0322	5.47	0.0105
N6,N6-dimethyllysine	0.82	0.0362	1.26	0.0279
N6,N6,N6-trimethyllysine	0.79	0.0371	1.41	0.0123
heneicosapentaenoate (21:5n3)	0.65	0.0378	1.67	0.0118

Table 8. Metabolites of the mouse small-intestine: ABX vs. SPF&CONV.

“Decrease in mean value” indicates that the mean values for that comparison are significantly lower; “Increase in mean value” indicates that the mean values for that comparison are significantly higher.

Biochemical Name	ABX vs. SPF		CONV vs. ABX	
	Decrease in mean value	p-value	Increase in mean value	p-value
7-ketodeoxycholate	0	0.0000	1790.29	0.0001
cholate	0	0.0000	177.11	0.0009
chenodeoxycholate	0.01	0.0000	28.99	0.0104
cholate sulfate	0.01	0.0000	39.86	0.0039
glycocholate	0.08	0.0001	4.07	0.0022
tauroolithocholate	0.07	0.0001	3.64	0.0061

deoxycholate	0	0.0000	105.25	0.0013
taurodeoxycholate	0	0.0000	287.75	0.0000
ursodeoxycholate	0	0.0000	706.9	0.0005
beta-muricholate	0	0.0000	24.12	0.0005
pheophytin A	0.02	0.0000	38.09	0.0019
diaminopimelate	0.14	0.0000	5.75	0.0001
thiamin (Vitamin B1)	0.3	0.0000	3.82	0.0004
1-(1-enyl-oleoyl)-GPE (P-18:1)*	0.31	0.0003	2.07	0.0087
taurohyodeoxycholic acid	0.23	0.0245	3.68	0.0033
equol sulfate	0	0.0000	362.52	0.0001
1-palmitoyl-galactosylglycerol (16:0)*	0.42	0.0045	3.27	0.0437
trimethylamine N-oxide	0.11	0.0001	13.2	0.0006
glyco-beta-muricholate**	0.12	0.0000	3.12	0.0000
glycoursodeoxycholate	0.18	0.0000	3.28	0.0001
equol glucuronide	0.02	0.0001	43.31	0.0000
10-hydroxystearate	0.24	0.0001	3.56	0.0020
N,N,N-trimethyl-5-aminovalerate	0.4	0.0002	3.47	0.0002
phenol sulfate	0.05	0.0002	14.95	0.0022
7-ketolithocholate	0.02	0.0002	17.62	0.0396
hyocholate	0.02	0.0002	3.4	0.0417
catechol sulfate	0.03	0.0002	23.43	0.0115
thiamin monophosphate	0.47	0.0000	2.57	0.0000
malonylgenistin	0.06	0.0005	15.29	0.0054
3-indoxyl sulfate	0.09	0.0004	3.59	0.0179
sphingomyelin (d18:0/20:0, d16:0/22:0)*	0.58	0.0009	1.61	0.0083
cinnamoylglycine	0.13	0.0013	41.45	0.0002
sphinganine	0.29	0.0004	1.65	0.0024
pyridoxamine	0.53	0.0025	2.51	0.0000
behenoyl dihydrosphingomyelin (d18:0/22:0)*	0.5	0.0009	1.8	0.0141
hippurate	0.13	0.0025	16.38	0.0000
(3'-5')-adenylylcytidine	0.26	0.0030	6.21	0.0275
sphingomyelin (d18:0/18:0, d19:0/17:0)*	0.53	0.0003	1.89	0.0102
5-aminovalerate	0.5	0.0030	5.19	0.0338
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	0.6	0.0002	1.63	0.0076
citrulline	0.33	0.0060	1.59	0.0001
orotidine	0.63	0.0085	2.01	0.0128
cytosine	0.23	0.0002	4.72	0.0292
cysteine-glutathione disulfide	0.17	0.0024	10.36	0.0014
(3'-5')-uridylylcytidine*	0.28	0.0113	5.19	0.0120
N-acetyl-isoptreanine	0.68	0.0426	1.67	0.0388
equol	0.24	0.0103	4.96	0.0111
glycerol 3-phosphate	0.67	0.0222	1.48	0.0333
(3'-5')-adenylyluridine	0.23	0.0031	8.05	0.0229
hexadecasphinganine (d16:0)*	0.39	0.0430	2.43	0.0054
pheophorbide A	0.25	0.0228	3.79	0.0095
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	0.67	0.0066	1.49	0.0157
alpha-CMBHC glucuronide	0.11	0.0000	5.77	0.0005

cytidine 5'-monophosphate (5'-CMP)	0.74	0.0305	1.39	0.0270
1,2-dipalmitoyl-GPE (16:0/16:0)*	0.33	0.0000	2.13	0.0053
3-hydroxyoctanoate	0.45	0.0000	1.61	0.0135
N6,N6-dimethyllysine	0.78	0.0010	1.32	0.0033
(3'-5')-uridylyluridine	0.28	0.0061	6.72	0.0438
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.51	0.0001	1.78	0.0045
glycerophosphoglycerol	0.5	0.0000	2.11	0.0000
choline phosphate	0.71	0.0121	2.73	0.0037
pyridoxal	0.78	0.0076	1.29	0.0079
1-palmitoyl-2-stearoyl-GPE (16:0/18:0)*	0.23	0.0000	3.44	0.0014
myristoleoylcarnitine (C14:1)*	0.18	0.0011	3.51	0.0480
cysteinylglycine disulfide*	0.34	0.0007	3.27	0.0243
decanoylcarnitine (C10)	0.28	0.0052	2.39	0.0325
stearoyl sphingomyelin (d18:1/18:0)	0.7	0.0019	1.42	0.0032
arabinose	0.42	0.0113	1.76	0.0299
mevalonate	0.67	0.0451	1.77	0.0305
N-acetylkynurenine (2)	0.26	0.0003	3.64	0.0006
N,N-dimethylvaline	0.17	0.0004	4.62	0.0037
glycerophosphoserine*	0.23	0.0001	4.28	0.0001
1,2-distearoyl-GPC (18:0/18:0)	0.23	0.0003	4.53	0.0011
S-methylcysteine	0.66	0.0174	1.56	0.0115
arachidoylcarnitine (C20)*	0.5	0.0018	1.78	0.0037
eicosenoylcarnitine (C20:1)*	0.57	0.0356	1.74	0.0267
dihomo-linolenoylcarnitine (C20:3n3 or 6)*	0.31	0.0084	2.67	0.0246
3-hydroxybutyrate (BHBA)	0.33	0.0000	2.14	0.0011
sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.71	0.0142	1.5	0.0158
glycerophosphorylcholine (GPC)	0.48	0.0000	1.91	0.0000
glycerophosphoethanolamine	0.55	0.0009	1.83	0.0007
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	0.35	0.0086	2.21	0.0138
lysine	0.9	0.0134	1.08	0.0345
N-acetylglutamate	0.79	0.0148	1.42	0.0026
S-adenosylmethionine (SAM)	0.66	0.0380	2.39	0.0004
benzoate	0.5	0.0085	2.36	0.0029
caprylate (8:0)	0.49	0.0021	1.68	0.0068
laurylcarnitine (C12)	0.29	0.0235	2.38	0.0433
behenoyl sphingomyelin (d18:1/22:0)*	0.67	0.0051	1.48	0.0295
5-methylthioadenosine (MTA)	0.33	0.0001	2.76	0.0001
glycerophosphoinositol*	0.32	0.0009	3.67	0.0000
N-formylanthranilic acid	0.63	0.0008	1.23	0.0328
quinolinate	0.54	0.0072	2.16	0.0006
dihydrobiopterin	0.5	0.0001	1.65	0.0028

Table 9. Metabolites of the mouse cecum: GF vs. SPF&CONV.

“Decrease in mean value” indicates that the mean values for that comparison are significantly lower; “Increase in mean value” indicates that the mean values for that comparison are significantly higher.

Biochemical Name	GF vs. SPF		CONV vs. GF	
	Decrease in mean value	p-value	Increase in mean value	p-value
glycine	0.46	0.0000	2.2	0.0003
sarcosine	0.05	0.0057	53.3	0.0098
serine	0.38	0.0000	2.45	0.0000
alanine	0.19	0.0000	4.78	0.0000
N-methylalanine	0.29	0.0063	7.43	0.0063
N,N-dimethylalanine	0.03	0.0000	80.86	0.0000
aspartate	0.19	0.0000	4.72	0.0000
N-acetylaspartate (NAA)	0.03	0.0000	9.38	0.0004
glutamate	0.25	0.0000	3.7	0.0000
glutamine	0.57	0.0004	1.72	0.0063
alpha-ketoglutaramate*	0.09	0.0000	10.42	0.0005
N-acetylglutamate	0.01	0.0000	113.32	0.0000
N-acetylglutamine	0.19	0.0000	4.39	0.0076
glutamate, gamma-methyl ester	0.43	0.0000	1.93	0.0028
carboxyethyl-GABA	0.54	0.0002	1.24	0.0214
N-methyl-GABA	0.09	0.0000	7.27	0.0000
S-1-pyrroline-5-carboxylate	0.41	0.0029	2.62	0.0003
histidine	0.4	0.0000	2.49	0.0000
3-methylhistidine	0.44	0.0088	2.35	0.0086
trans-uocanate	0.1	0.0000	7.87	0.0000
cis-uocanate	0.17	0.0000	6.48	0.0000
imidazole propionate	0.06	0.0000	73.07	0.0006
formiminoglutamate	0.03	0.0000	23.3	0.0001
imidazole lactate	0.16	0.0001	10.94	0.0171
histamine	0.06	0.0000	12.71	0.0000
1-methylhistamine	0.2	0.0000	3.92	0.0002
1-methyl-4-imidazoleacetate	0.19	0.0000	3.38	0.0000
N-acetylhistamine	0	0.0000	200.13	0.0000
lysine	0.6	0.0000	1.49	0.0068
pipecolate	0.02	0.0000	42.64	0.0000
N-acetyl-cadaverine	0.02	0.0000	57.61	0.0000
5-aminovalerate	0.03	0.0000	97.28	0.0001
N,N,N-trimethyl-5-aminovalerate	0.01	0.0000	95.38	0.0000
phenylalanine	0.23	0.0000	4.03	0.0000
phenylpyruvate	0.03	0.0000	59.53	0.0000
phenyllactate (PLA)	0.14	0.0009	11.57	0.0003
phenethylamine	0.75	0.0433	1.46	0.0057
phenylacetate	0.01	0.0000	13.77	0.0001
4-hydroxyphenylacetate	0.01	0.0001	4.65	0.0006
tyrosine	0.22	0.0000	3.98	0.0000
4-hydroxyphenylpyruvate	0.02	0.0000	57.54	0.0000
tyrosol	0.21	0.0004	4.1	0.0048
o-Tyrosine	0.55	0.0139	1.68	0.0064
gentisate	0.01	0.0000	143.95	0.0000
N-formylphenylalanine	0.16	0.0001	7.43	0.0000
tryptophan	0.29	0.0000	3.13	0.0000
kynurenine	0.65	0.0001	1.41	0.0155
kynurenate	0.19	0.0000	13.01	0.0000

N-formylanthranilic acid	0.35	0.0037	2.26	0.0001
anthranilate	0.54	0.0117	1.96	0.0000
xanthurenate	0.16	0.0000	13.17	0.0000
picolinate	0.11	0.0000	9.52	0.0000
5-hydroxyindoleacetate	0.47	0.0055	2	0.0056
indoleacetate	0.13	0.0000	9.2	0.0000
indolepropionate	0.32	0.0192	5.64	0.0007
leucine	0.26	0.0000	3.72	0.0000
4-methyl-2-oxopentanoate	0	0.0000	400.36	0.0000
alpha-hydroxyisocaproate	0.14	0.0006	67.45	0.0004
isovalerate (i5:0)	0.01	0.0000	136.89	0.0000
beta-hydroxyisovalerate	0.23	0.0000	4.94	0.0072
beta-hydroxyisovaleroylcarnitine	0.13	0.0000	19.89	0.0017
3-methylglutaconate	0.25	0.0008	5.13	0.0001
isoleucine	0.27	0.0000	3.6	0.0000
3-methyl-2-oxovalerate	0	0.0000	458.87	0.0000
2-hydroxy-3-methylvalerate	0.23	0.0001	61.9	0.0001
ethylmalonate	0.08	0.0000	16.08	0.0000
methylsuccinate	0.04	0.0000	18.58	0.0000
valine	0.2	0.0000	4.99	0.0000
3-methyl-2-oxobutyrate	0.01	0.0000	127.06	0.0000
alpha-hydroxyisovalerate	0.11	0.0000	59.98	0.0000
N,N-dimethylvaline	0.01	0.0000	71.85	0.0000
methionine	0.1	0.0000	8.74	0.0000
N-formylmethionine	0.11	0.0000	7.31	0.0000
methionine sulfone	0.15	0.0000	8.35	0.0000
methionine sulfoxide	0.41	0.0005	2.46	0.0003
4-methylthio-2-oxobutanoate	0.02	0.0000	58.93	0.0000
2-hydroxy-4-(methylthio)butanoic acid	0.09	0.0000	87.77	0.0000
homocysteine	0.03	0.0000	43.96	0.0000
alpha-ketobutyrate	0.01	0.0000	212.06	0.0002
S-methylcysteine	0.27	0.0000	3.88	0.0000
taurine	0.01	0.0000	18.37	0.0000
3-sulfo-L-alanine	0.18	0.0000	3.71	0.0008
citrulline	0.01	0.0000	43.15	0.0010
N-delta-acetylornithine	0.18	0.0000	8.02	0.0000
N-methylproline	0	0.0000	579.27	0.0000
N-carbamoylputrescine	0.15	0.0032	8.68	0.0031
diacetylspermidine*	0.06	0.0000	35.18	0.0005
N1,N12-diacetylspermine	0.15	0.0361	24.22	0.0001
4-acetamidobutanoate	0.17	0.0000	7.04	0.0000
5-oxoproline	0.71	0.0032	1.44	0.0373
2-hydroxybutyrate/2-hydroxyisobutyrate	0.15	0.0001	8.25	0.0000
gamma-glutamylleucine	0.16	0.0000	3.91	0.0000
alanylleucine	0.07	0.0004	5.83	0.0000
glycylisoleucine	0.07	0.0000	11.23	0.0000
glycylleucine	0.04	0.0000	17.37	0.0000
glycylvaline	0.08	0.0000	9.3	0.0000
isoleucylglycine	0.04	0.0001	23.54	0.0001
leucylalanine	0.18	0.0054	2.84	0.0028
leucylglycine	0.16	0.0000	3.63	0.0000
lysylleucine	0.15	0.0000	4.65	0.0000

phenylalanylglycine	0.18	0.0002	3.12	0.0000
threonylphenylalanine	0.28	0.0326	1.47	0.0400
valylglutamine	0.04	0.0000	14.12	0.0000
valylglycine	0.03	0.0000	28.43	0.0000
valylleucine	0.06	0.0000	8.93	0.0000
leucylglutamine*	0.06	0.0002	8.01	0.0001
glucose	0.04	0.0000	26.19	0.0000
pyruvate	0.52	0.0094	2.01	0.0342
glycerate	0.24	0.0000	4.32	0.0000
ribose	0.11	0.0000	11.14	0.0000
ribulose/xylulose	0.08	0.0000	10	0.0000
xylose	0.04	0.0002	17.07	0.0005
arabinose	0.16	0.0001	4.81	0.0017
mannose	0.38	0.0026	2.75	0.0043
N-acetylmuramate	0	0.0000	158.43	0.0000
N-acetylglucosamine/N-acetylgalactosamine	0.12	0.0000	8.92	0.0001
alpha-ketoglutarate	0.02	0.0000	52.13	0.0000
succinate	0.02	0.0000	50.81	0.0000
fumarate	0.01	0.0000	181.96	0.0000
malate	0.07	0.0002	9.76	0.0001
2-methylcitrate/homocitrate	0.11	0.0000	12.26	0.0000
butyrate/isobutyrate (4:0)	0.01	0.0001	136.4	0.0001
valerate (5:0)	0.01	0.0000	151.89	0.0000
caproate (6:0)	0.21	0.0000	4.48	0.0000
heptanoate (7:0)	0.53	0.0089	1.73	0.0149
caprylate (8:0)	0.6	0.0014	2.13	0.0005
caprate (10:0)	0.65	0.0024	1.71	0.0001
laurate (12:0)	0.53	0.0096	1.64	0.0000
5-dodecenoate (12:1n7)	0.16	0.0006	4.61	0.0012
myristate (14:0)	0.25	0.0041	3.75	0.0000
palmitate (16:0)	0.41	0.0286	2.24	0.0020
margarate (17:0)	0.26	0.0003	4.34	0.0000
stearate (18:0)	0.46	0.0138	2.28	0.0044
nonadecanoate (19:0)	0.34	0.0129	2.71	0.0074
myristoleate (14:1n5)	0.18	0.0004	3.68	0.0002
palmitoleate (16:1n7)	0.16	0.0067	4.51	0.0000
10-heptadecenoate (17:1n7)	0.17	0.0005	5.06	0.0000
oleate/vaccenate (18:1)	0.27	0.0082	2.94	0.0006
10-nonadecenoate (19:1n9)	0.21	0.0166	3.36	0.0003
tetradecadienoate (14:2)*	0.39	0.0000	2.22	0.0022
hexadecatrienoate (16:3n3)	0.55	0.0089	2.43	0.0002
stearidonate (18:4n3)	0.42	0.0071	3.09	0.0000
eicosapentaenoate (EPA; 20:5n3)	0.56	0.0188	2.77	0.0001
heneicosapentaenoate (21:5n3)	0.26	0.0097	3.83	0.0001
docosapentaenoate (n3 DPA; 22:5n3)	0.28	0.0271	3.38	0.0003
docosahexaenoate (DHA; 22:6n3)	0.36	0.0367	3.01	0.0001
nisinate (24:6n3)	0.14	0.0027	4.68	0.0002
hexadecadienoate (16:2n6)	0.35	0.0067	2.87	0.0001
dihomo-linoleate (20:2n6)	0.15	0.0000	5.09	0.0001
dihomo-linolenate (20:3n3 or n6)	0.43	0.0425	2.65	0.0005
arachidonate (20:4n6)	0.51	0.0228	2.62	0.0001

adrenate (22:4n6)	0.21	0.0005	3.76	0.0001
docosapentaenoate (n6 DPA; 22:5n6)	0.1	0.0000	9.3	0.0001
docosadienoate (22:2n6)	0.29	0.0007	3.39	0.0018
mead acid (20:3n9)	0.06	0.0000	10.36	0.0046
(14 or 15)-methylpalmitate (a17:0 or i17:0)	0.27	0.0026	2.87	0.0001
dimethylmalonic acid	0.29	0.0000	3.18	0.0000
glutarate (C5-DC)	0.02	0.0001	21.45	0.0000
2-hydroxyglutarate	0.03	0.0000	26.76	0.0000
2-hydroxyadipate	0.17	0.0000	4.15	0.0011
pimelate (C7-DC)	0.41	0.0014	2.28	0.0004
suberate (C8-DC)	0.37	0.0000	3.39	0.0000
azelate (C9-DC)	0.58	0.0489	2.49	0.0042
sebacate (C10-DC)	0.14	0.0000	9.24	0.0000
undecanedioate (C11-DC)	0.13	0.0000	10.55	0.0000
dodecanedioate (C12-DC)	0.11	0.0000	16.82	0.0000
dodecenedioate (C12:1-DC)*	0.22	0.0010	6.13	0.0001
tetradecanedioate (C14-DC)	0.24	0.0010	5.78	0.0004
branched chain 14:0 dicarboxylic acid**	0.02	0.0000	59.38	0.0049
hexadecanedioate (C16-DC)	0.26	0.0000	5.32	0.0000
hexadecenedioate (C16:1-DC)*	0.24	0.0000	4.76	0.0000
octadecenedioate (C18:1-DC)	0.15	0.0000	3.59	0.0000
2-hydroxysebacate	0.66	0.0117	1.97	0.0011
3-hydroxymargaroylglycine	0.09	0.0000	14.31	0.0000
oleoylcarnitine (C18:1)	0.63	0.0005	2.65	0.0421
(S)-3-hydroxybutyrylcarnitine	0.18	0.0027	5.12	0.0031
deoxycarnitine	0.01	0.0000	48.09	0.0000
palmitoylcholine	0.66	0.0107	1.85	0.0004
oleoylcholine	0.34	0.0002	2.86	0.0196
2-hydroxyheptanoate*	0.22	0.0001	21.55	0.0000
2-hydroxypalmitate	0.18	0.0000	5.09	0.0128
2-hydroxystearate	0.24	0.0000	5.82	0.0078
2-hydroxyarachidate*	0.24	0.0000	4.9	0.0094
2-hydroxybehenate	0.17	0.0000	4.61	0.0032
2-hydroxynervonate*	0.18	0.0000	4.24	0.0012
3-hydroxyhexanoate	0.16	0.0002	11.06	0.0000
3-hydroxyoctanoate	0.35	0.0000	3.29	0.0001
3-hydroxydecanoate	0.32	0.0004	3.27	0.0003
3-hydroxysebacate	0.29	0.0000	3.67	0.0000
3-hydroxylaurate	0.26	0.0000	4.86	0.0000
3-hydroxypalmitate	0.05	0.0003	25.32	0.0000
3-hydroxystearate	0.27	0.0004	5.34	0.0000
3-hydroxyoleate*	0.22	0.0000	8.43	0.0000
8-hydroxyoctanoate	0.46	0.0004	2.46	0.0002
10-hydroxystearate	0.02	0.0000	57.76	0.0001
16-hydroxypalmitate	0.29	0.0002	5.23	0.0000
12,13-DiHOME	0.44	0.0000	3.05	0.0002
9,10-DiHOME	0.47	0.0004	3.63	0.0001
2S,3R-dihydroxybutyrate	0.33	0.0001	3.27	0.0007
oleoyl ethanolamide	0.16	0.0000	7.37	0.0000
palmitoyl ethanolamide	0.4	0.0058	3.7	0.0066
stearoyl ethanolamide	0.18	0.0001	5.01	0.0010

arachidoyl ethanolamide (20:0)*	0.15	0.0000	5.2	0.0050
choline phosphate	0.01	0.0005	132.53	0.0000
glycerophosphoethanolamine	0.41	0.0002	2.97	0.0001
1,2-dipalmitoyl-GPE (16:0/16:0)*	0.28	0.0001	2.14	0.0021
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.1	0.0000	4.49	0.0000
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.38	0.0002	3.52	0.0000
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	0.31	0.0005	4.75	0.0001
1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	0.01	0.0000	68.12	0.0000
1-palmitoyl-GPA (16:0)	0.11	0.0001	43.36	0.0001
1-stearoyl-GPA (18:0)	0.16	0.0001	20.2	0.0000
1-oleoyl-GPA (18:1)	0.04	0.0014	60.06	0.0000
1-linoleoyl-GPA (18:2)*	0.16	0.0011	14.13	0.0000
1-palmitoyl-GPG (16:0)*	0.02	0.0000	58.57	0.0000
1-stearoyl-GPG (18:0)	0.01	0.0000	96.55	0.0000
1-oleoyl-GPG (18:1)*	0.26	0.0002	2.32	0.0092
glycerol	0.41	0.0190	1.7	0.0066
glycerol 3-phosphate	0.07	0.0000	7.31	0.0000
glycerophosphoglycerol	0.11	0.0000	6.58	0.0000
1-pentadecanoylglycerol (15:0)	0.15	0.0000	7.87	0.0001
1-palmitoylglycerol (16:0)	0.38	0.0186	3.36	0.0003
2-palmitoylglycerol (16:0)	0.4	0.0066	2.96	0.0008
1-palmitoyl-2-linoleoyl-galactosylglycerol (16:0/18:2)*	0.18	0.0001	10.26	0.0000
1-palmitoyl-galactosylglycerol (16:0)*	0.01	0.0000	114.61	0.0000
2-palmitoyl-galactosylglycerol (16:0)*	0.02	0.0000	88.36	0.0000
1-palmitoyl-digalactosylglycerol (16:0)*	0.39	0.0222	3.75	0.0044
3-ketosphinganine	0.07	0.0000	13.13	0.0000
sphinganine	0.25	0.0001	2.41	0.0194
hexadecasphinganine (d16:0)*	0.05	0.0000	13.34	0.0000
N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.1	0.0000	3.32	0.0161
N-stearoyl-sphingosine (d18:1/18:0)*	0.37	0.0003	1.91	0.0171
N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	0.22	0.0007	2.8	0.0021
ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*	0.27	0.0022	2.19	0.0479
hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH))**	0.5	0.0380	2.01	0.0064
stearoyl sphingomyelin (d18:1/18:0)	0.2	0.0001	2.97	0.0274
sphingomyelin (d18:1/24:1, d18:2/24:0)*	0.26	0.0010	1.67	0.0233
sphingomyelin (d18:2/24:1, d18:1/24:2)*	0.24	0.0090	2.13	0.0395
eicosanoylsphingosine (d20:1)*	0.27	0.0000	2.24	0.0106
dehydrophytosphingosine*	0.39	0.0095	1.91	0.0430
3-hydroxy-3-methylglutarate	0.09	0.0000	8.92	0.0000
mevalonate	0.11	0.0000	29.63	0.0000
4-cholesten-3-one	0.22	0.0000	2.21	0.0002
cholate	0.01	0.0000	504.22	0.0000
beta-muricholate	0	0.0000	1285.61	0.0000
alpha-muricholate	0.04	0.0061	32.78	0.0060

glyco-beta-muricholate**	0.58	0.0469	2.46	0.0185
cholate sulfate	0.03	0.0003	243.84	0.0028
deoxycholate	0	0.0000	2997.53	0.0000
3-dehydrodeoxycholate	0.5	0.0083	1.29	0.0335
taurodeoxycholate	0.56	0.0036	8.13	0.0235
6-beta-hydroxylithocholate	0.06	0.0001	12.85	0.0001
lithocholate	0	0.0000	139.8	0.0000
12-ketolithocholate	0.01	0.0000	101.47	0.0000
dehydrolithocholate	0.04	0.0000	5.78	0.0028
6-oxolithocholate	0	0.0000	87.08	0.0002
hyocholate	0.03	0.0000	35.77	0.0000
taurohyodeoxycholic acid	0.37	0.0050	23.68	0.0432
3-dehydrocholate	0.09	0.0003	33.96	0.0001
7-ketodeoxycholate	0	0.0000	1302.35	0.0000
3b-hydroxy-5-cholenoic acid	0.36	0.0037	2.1	0.0344
isohyodeoxycholate	0.01	0.0000	34.14	0.0003
hypoxanthine	0.03	0.0000	40.36	0.0000
xanthine	0.62	0.0001	1.88	0.0006
2'-deoxyinosine	0.41	0.0000	1.98	0.0002
adenosine 5'-monophosphate (AMP)	0.03	0.0000	17.9	0.0001
adenosine	0.2	0.0002	4.26	0.0100
adenine	0.32	0.0025	2.34	0.0408
1-methyladenine	0.09	0.0000	13.48	0.0000
2'-deoxyadenosine 5'-monophosphate	0.05	0.0001	4.6	0.0083
guanosine 5'- monophosphate (5'-GMP)	0.11	0.0049	3.08	0.0152
guanosine-2',3'-cyclic monophosphate	0.02	0.0000	83.35	0.0036
guanine	0.07	0.0000	12.52	0.0000
N-carbamoylaspartate	0.05	0.0000	10.33	0.0001
uridine 5'-monophosphate (UMP)	0.15	0.0151	7.02	0.0421
uridine-2',3'-cyclic monophosphate	0.03	0.0006	101.76	0.0162
uracil	0.02	0.0000	64.23	0.0000
pseudouridine	0.49	0.0319	1.95	0.0019
5,6-dihydrouridine	0.13	0.0000	9.15	0.0000
5-methyluridine (ribothymidine)	0.1	0.0000	6.73	0.0066
2'-deoxyuridine	0.2	0.0000	3.58	0.0000
3-ureidopropionate	0.24	0.0000	15.11	0.0008
beta-alanine	0.26	0.0000	3.74	0.0000
cytidine 5'-monophosphate (5'-CMP)	0.01	0.0000	64.06	0.0000
cytosine	0.06	0.0000	53.45	0.0012
2'-deoxycytidine 5'-monophosphate	0	0.0000	50.57	0.0000
thymine	0.01	0.0000	124.09	0.0000
3-aminoisobutyrate	0.14	0.0000	18.13	0.0000
(3'-5')-adenylylcytidine	0.09	0.0003	6.03	0.0135
(3'-5')-adenylylguanosine*	0.04	0.0000	14.42	0.0110
(3'-5')-adenylyluridine	0.06	0.0001	6.86	0.0065
(3'-5')-adenylyladenosine*	0.07	0.0001	6.91	0.0168
(3'-5')-cytidylyladenosine	0.06	0.0003	9.18	0.0412
(3'-5')-cytidylylcytidine*	0.06	0.0003	6.45	0.0492
(3'-5')-cytidylylguanosine	0.04	0.0001	13.27	0.0451
(3'-5')-cytidylyluridine*	0.03	0.0002	12.03	0.0020
(3'-5')-guanylyladenosine*	0.05	0.0004	9.74	0.0408
(3'-5')-guanylylcytidine	0.06	0.0004	9.18	0.0108

(3'-5')-guanylyluridine	0.07	0.0010	8	0.0209
(3'-5')-uridylylcytidine*	0.03	0.0000	15.74	0.0009
(3'-5')-uridylyluridine	0.06	0.0003	6.3	0.0057
nicotinate	0.01	0.0000	184.17	0.0000
nicotinate ribonucleoside	0.14	0.0000	5.39	0.0365
nicotinamide riboside	0.41	0.0000	2.19	0.0012
riboflavin (Vitamin B2)	0.61	0.0011	1.46	0.0119
flavin mononucleotide (FMN)	0.26	0.0001	3.06	0.0003
pantothenate	0.03	0.0000	36.51	0.0000
pantethine	0.19	0.0060	11.93	0.0000
pantetheine	0.03	0.0000	37.33	0.0000
alpha-tocopherol	0.46	0.0004	1.73	0.0001
alpha-tocotrienol	0.45	0.0057	2.1	0.0166
gamma-tocotrienol	0.54	0.0100	1.81	0.0395
D-urobilin	0.07	0.0002	12.29	0.0081
thiamin (Vitamin B1)	0.11	0.0000	8.69	0.0000
thiamin monophosphate	0.05	0.0000	14.64	0.0000
5-(2-Hydroxyethyl)-4-methylthiazole	0.05	0.0000	28.21	0.0000
hydroxymethylpyrimidine	0.12	0.0009	14.35	0.0000
retinol (Vitamin A)	0.22	0.0015	6.47	0.0000
pyridoxamine	0.03	0.0000	25.46	0.0000
pyridoxal	0.04	0.0001	21.5	0.0001
pyridoxate	0.19	0.0000	7.09	0.0000
benzoate	0.61	0.0102	2.43	0.0001
4-hydroxybenzoate	0.6	0.0042	11.13	0.0368
2,4,6-trihydroxybenzoate	0.45	0.0389	9.04	0.0000
p-hydroxybenzaldehyde	0.19	0.0000	6.03	0.0000
2-(4-hydroxyphenyl)propionate	0	0.0000	290.21	0.0005
3-(3-hydroxyphenyl)propionate	0	0.0093	195.05	0.0032
3-(4-hydroxyphenyl)propionate	0	0.0115	30.69	0.0011
3-phenylpropionate (hydrocinnamate)	0.01	0.0228	601.56	0.0000
3,5-dihydroxyphenylpropionate	0.04	0.0051	304.42	0.0002
maltol	0.04	0.0000	49.95	0.0000
piperidine	0.05	0.0000	18.77	0.0000
vanillate	0.52	0.0007	3.1	0.0017
2,3-dihydroxyisovalerate	0.02	0.0000	56.75	0.0000
dipicolinate	0.01	0.0123	8.07	0.0001
2,8-quinolinediol	0	0.0000	1220.24	0.0169
2-isopropylmalate	0	0.0000	299.54	0.0000
2-oxindole-3-acetate	0.06	0.0000	28.53	0.0000
3-formylindole	0.14	0.0000	8.8	0.0000
beta-guanidinopropanoate	0.3	0.0000	1.75	0.0466
dihydroferulate	0.69	0.0405	1.58	0.0189
enterodiol	0.68	0.0240	3.61	0.0002
enterolactone	0.02	0.0000	182.91	0.0005
equol	0.01	0.0000	178.61	0.0000
equol sulfate	0.05	0.0000	33.31	0.0006
ferulate	0.09	0.0000	9.58	0.0001
indolin-2-one	0	0.0002	56.24	0.0008
pheophytin A	0.05	0.0000	16.38	0.0000
sinapate	0.27	0.0002	4.45	0.0000
2-keto-3-deoxy-gluconate	0.4	0.0073	2.1	0.0177

4-hydroxycinnamate	0.13	0.0000	6.12	0.0001
3-indoleglyoxylic acid	0.44	0.0002	3.27	0.0001
2-aminophenol	0.35	0.0006	3.85	0.0072
diaminopimelate	0.02	0.0000	60.51	0.0000
N-methylpipecolate	0.06	0.0000	19.28	0.0000
salicylate	0.05	0.0001	19.88	0.0000
2,6-dihydroxybenzoic acid	0.01	0.0000	148.09	0.0000
1,3-propanediol	0.4	0.0087	2.88	0.0116
sulfate*	0.18	0.0000	5.44	0.0000
ectoine	0.2	0.0000	3.88	0.0002
2,4-di-tert-butylphenol	0.41	0.0008	1.78	0.0001

Table 10. Metabolites of the mouse cecum: ABX vs. SPF&CONV.

“Decrease in mean value” indicates that the mean values for that comparison are significantly lower; “Increase in mean value” indicates that the mean values for that comparison are significantly higher.

Biochemical Name	ABX vs. SPF		CONV vs. ABX	
	Decrease in mean value	p-value	Increase in mean value	p-value
glycine	0.66	0.0151	1.54	0.0262
N-acetylglycine	0.54	0.0075	2.02	0.0113
sarcosine	0.06	0.0057	49.1	0.0102
dimethylglycine	0.25	0.0234	5.78	0.0048
serine	0.63	0.0137	1.47	0.0360
alanine	0.31	0.0002	2.98	0.0001
N-methylalanine	0.29	0.0063	7.43	0.0063
N,N-dimethylalanine	0.03	0.0000	80.86	0.0000
aspartate	0.36	0.0000	2.48	0.0000
N-acetylaspartate (NAA)	0.04	0.0000	6.51	0.0011
glutamate	0.36	0.0000	2.55	0.0000
alpha-ketoglutaramate*	0.09	0.0000	9.6	0.0005
N-acetylglutamate	0.01	0.0000	64.75	0.0000
N-acetylglutamine	0.23	0.0000	3.62	0.0105
carboxyethyl-GABA	0.54	0.0005	1.23	0.0242
N-methyl-GABA	0.21	0.0000	3.2	0.0004
S-1-pyrroline-5-carboxylate	0.58	0.0397	1.83	0.0064
histidine	0.59	0.0104	1.7	0.0096
trans-uocanate	0.34	0.0004	2.26	0.0096
cis-uocanate	0.19	0.0000	5.91	0.0001
imidazole propionate	0.07	0.0000	57.81	0.0009
formiminoglutamate	0.07	0.0001	9.23	0.0021
1-methylhistamine	0.41	0.0009	1.94	0.0094
1-methyl-4-imidazoleacetate	0.26	0.0000	2.49	0.0001
N-acetylhistamine	0	0.0000	154.27	0.0000
pipecolate	0.02	0.0000	30.38	0.0000
N-acetyl-cadaverine	0.03	0.0000	28.39	0.0000
5-aminovalerate	0.05	0.0000	47.89	0.0001
N,N,N-trimethyl-5-aminovalerate	0.02	0.0000	80.76	0.0000

phenylalanine	0.39	0.0002	2.3	0.0002
phenylpyruvate	0.04	0.0000	43.11	0.0000
phenyllactate (PLA)	0.14	0.0009	11.57	0.0003
phenethylamine	0.74	0.0172	1.5	0.0003
phenylacetate	0.01	0.0000	13.77	0.0001
4-hydroxyphenylacetate	0.01	0.0001	4.65	0.0006
tyrosine	0.32	0.0000	2.76	0.0001
4-hydroxyphenylpyruvate	0.04	0.0000	35.96	0.0000
tyrosol	0.21	0.0004	4.1	0.0048
o-Tyrosine	0.58	0.0390	1.59	0.0378
gentisate	0.01	0.0000	143.95	0.0000
N-formylphenylalanine	0.15	0.0000	7.91	0.0000
tryptophan	0.53	0.0025	1.72	0.0084
kynurenine	0.62	0.0063	1.48	0.0228
kynurenate	0.17	0.0000	14.72	0.0000
N-formylanthranilic acid	0.35	0.0037	2.26	0.0001
anthranilate	0.28	0.0001	3.77	0.0000
xanthurenate	0.18	0.0001	11.47	0.0000
picolinate	0.17	0.0000	6.37	0.0000
serotonin	0.22	0.0126	6.96	0.0005
5-hydroxyindoleacetate	0.47	0.0055	2	0.0056
indoleacetate	0.11	0.0000	10.36	0.0000
indolepropionate	0.32	0.0192	5.64	0.0007
leucine	0.49	0.0010	1.95	0.0010
4-methyl-2-oxopentanoate	0.01	0.0000	247.73	0.0000
alpha-hydroxyisocaproate	0.14	0.0006	67.45	0.0004
isovalerate (i5:0)	0	0.0000	351.02	0.0000
beta-hydroxyisovalerate	0.25	0.0000	4.45	0.0088
beta-hydroxyisovaleroylcarnitine	0.12	0.0000	20.92	0.0014
3-methylglutaconate	0.25	0.0008	5.13	0.0001
isoleucine	0.44	0.0001	2.22	0.0000
3-methyl-2-oxovalerate	0	0.0000	383.84	0.0000
2-hydroxy-3-methylvalerate	0.23	0.0001	61.9	0.0001
ethylmalonate	0.06	0.0000	20.83	0.0000
methylsuccinate	0.05	0.0000	15.59	0.0000
valine	0.42	0.0014	2.34	0.0018
3-methyl-2-oxobutyrate	0.01	0.0000	104.11	0.0000
alpha-hydroxyisovalerate	0.17	0.0017	39.2	0.0000
N,N-dimethylvaline	0.01	0.0000	95.03	0.0000
methionine	0.19	0.0000	4.48	0.0000
N-formylmethionine	0.08	0.0000	10.33	0.0000
methionine sulfone	0.11	0.0001	11.99	0.0001
methionine sulfoxide	0.33	0.0001	3.02	0.0000
4-methylthio-2-oxobutanoate	0.02	0.0000	58.93	0.0000
2-hydroxy-4-(methylthio)butanoic acid	0.09	0.0000	87.77	0.0000
homocysteine	0.03	0.0000	43.96	0.0000
alpha-ketobutyrate	0.02	0.0000	183.51	0.0002
taurine	0.04	0.0000	6.74	0.0002
N-acetyltaurine	0.28	0.0000	1.66	0.0000
3-sulfo-L-alanine	0.21	0.0000	3.22	0.0020
citrulline	0.02	0.0000	22.06	0.0025
N-delta-acetylorithine	0.27	0.0000	5.19	0.0001

N-methylproline	0	0.0000	400.25	0.0000
diacetylspermidine*	0.07	0.0000	30.99	0.0011
N1,N12-diacetylspermine	0.15	0.0361	24.22	0.0001
4-acetamidobutanoate	0.14	0.0000	8.56	0.0000
2-hydroxybutyrate/2-hydroxyisobutyrate	0.17	0.0001	7.35	0.0000
gamma-glutamylleucine	0.31	0.0009	1.98	0.0155
alanylleucine	0.07	0.0003	6.63	0.0000
glycylisoleucine	0.13	0.0000	6.22	0.0000
glycylleucine	0.1	0.0000	7.13	0.0000
glycylvaline	0.19	0.0000	3.63	0.0001
isoleucylglycine	0.08	0.0002	13.16	0.0001
leucylalanine	0.14	0.0022	3.59	0.0005
leucylglycine	0.21	0.0001	2.79	0.0015
lysylleucine	0.28	0.0001	2.47	0.0001
valylglutamine	0.04	0.0000	15.35	0.0000
valylglycine	0.04	0.0000	23.76	0.0000
valylleucine	0.06	0.0000	9.66	0.0000
leucylglutamine*	0.07	0.0003	6.9	0.0001
glucose	0.05	0.0000	20.64	0.0000
glycerate	0.2	0.0000	5.27	0.0000
ribose	0.04	0.0000	28.71	0.0000
ribulose/xylulose	0.08	0.0000	9.55	0.0000
xylose	0.04	0.0002	17.07	0.0005
arabinose	0.15	0.0001	5	0.0017
mannose	0.2	0.0001	5.1	0.0002
N-acetylmuramate	0.01	0.0000	137.77	0.0000
N-acetylglucosamine/N-acetylgalactosamine	0.07	0.0000	15.25	0.0000
alpha-ketoglutarate	0.04	0.0000	27.1	0.0000
succinate	0.04	0.0000	25.53	0.0000
fumarate	0.02	0.0000	48.95	0.0000
malate	0.11	0.0005	6.26	0.0001
2-methylcitrate/homocitrate	0.14	0.0000	9.8	0.0000
butyrate/isobutyrate (4:0)	0	0.0000	559.4	0.0000
valerate (5:0)	0	0.0000	328.54	0.0000
caproate (6:0)	0.19	0.0000	4.94	0.0000
heptanoate (7:0)	0.46	0.0357	2.02	0.0440
laurate (12:0)	0.5	0.0053	1.74	0.0000
5-dodecenoate (12:1n7)	0.12	0.0004	5.91	0.0003
myristate (14:0)	0.15	0.0005	6.17	0.0000
palmitate (16:0)	0.2	0.0011	4.54	0.0000
margarate (17:0)	0.17	0.0000	6.6	0.0000
stearate (18:0)	0.34	0.0022	3.08	0.0005
nonadecanoate (19:0)	0.22	0.0016	4.33	0.0006
arachidate (20:0)	0.28	0.0027	3.47	0.0011
myristoleate (14:1n5)	0.18	0.0004	3.74	0.0002
palmitoleate (16:1n7)	0.09	0.0012	8.04	0.0000
10-heptadecenoate (17:1n7)	0.11	0.0001	7.52	0.0000
oleate/vaccenate (18:1)	0.12	0.0004	6.32	0.0000
10-nonadecenoate (19:1n9)	0.15	0.0045	4.77	0.0001
eicosenoate (20:1)	0.15	0.0059	4.47	0.0004
erucate (22:1n9)	0.23	0.0039	3.79	0.0041

tetradecadienoate (14:2)*	0.19	0.0000	4.61	0.0000
hexadecatrienoate (16:3n3)	0.24	0.0000	5.44	0.0000
stearidonate (18:4n3)	0.21	0.0001	6.31	0.0000
eicosapentaenoate (EPA; 20:5n3)	0.27	0.0001	5.69	0.0000
heneicosapentaenoate (21:5n3)	0.09	0.0002	11.38	0.0000
docosapentaenoate (n3 DPA; 22:5n3)	0.13	0.0017	7.28	0.0001
docosahexaenoate (DHA; 22:6n3)	0.16	0.0014	6.72	0.0000
nisinate (24:6n3)	0.09	0.0017	7.38	0.0000
hexadecadienoate (16:2n6)	0.13	0.0001	7.65	0.0000
linoleate (18:2n6)	0.15	0.0008	7.13	0.0000
linolenate [alpha or gamma; (18:3n3 or 6)]	0.16	0.0004	8.47	0.0000
dihomo-linoleate (20:2n6)	0.1	0.0000	7.43	0.0000
dihomo-linolenate (20:3n3 or n6)	0.25	0.0030	4.6	0.0002
arachidonate (20:4n6)	0.23	0.0003	5.72	0.0000
adrenate (22:4n6)	0.14	0.0004	5.74	0.0001
docosapentaenoate (n6 DPA; 22:5n6)	0.05	0.0000	20.77	0.0000
docosadienoate (22:2n6)	0.13	0.0000	7.4	0.0001
mead acid (20:3n9)	0.06	0.0000	10.36	0.0046
(14 or 15)-methylpalmitate (a17:0 or i17:0)	0.21	0.0012	3.67	0.0000
(16 or 17)-methylstearate (a19:0 or i19:0)	0.21	0.0176	3	0.0386
dimethylmalonic acid	0.26	0.0000	3.47	0.0000
glutarate (C5-DC)	0.02	0.0000	22.51	0.0000
2-hydroxyglutarate	0.07	0.0000	12.51	0.0000
2-hydroxyadipate	0.18	0.0001	4.01	0.0013
pimelate (C7-DC)	0.41	0.0014	2.28	0.0004
suberate (C8-DC)	0.24	0.0001	5.29	0.0000
azelate (C9-DC)	0.23	0.0002	6.39	0.0000
sebacate (C10-DC)	0.09	0.0000	14.24	0.0000
undecanedioate (C11-DC)	0.1	0.0000	13.98	0.0000
dodecanedioate (C12-DC)	0.09	0.0000	22.06	0.0000
dodecenedioate (C12:1-DC)*	0.16	0.0002	8.6	0.0000
tetradecanedioate (C14-DC)	0.18	0.0000	7.56	0.0000
branched chain 14:0 dicarboxylic acid**	0.02	0.0000	59.38	0.0049
hexadecanedioate (C16-DC)	0.15	0.0000	9.02	0.0000
hexadecenedioate (C16:1-DC)*	0.24	0.0000	4.76	0.0000
octadecenedioate (C18:1-DC)	0.05	0.0000	10.59	0.0000
2-hydroxysebacate	0.32	0.0000	4.03	0.0000
3-hydroxymargaroylglycine	0.09	0.0000	14.31	0.0000
margaroylcarnitine (C17)*	0.26	0.0211	3.54	0.0031
oleoylcarnitine (C18:1)	0.46	0.0037	3.63	0.0083
(S)-3-hydroxybutyrylcarnitine	0.26	0.0054	3.46	0.0074
deoxycarnitine	0.02	0.0000	30.56	0.0000
3-hydroxybutyrate (BHBA)	0.52	0.0087	7.1	0.0313
palmitoylcholine	0.51	0.0016	2.4	0.0002
2-hydroxyheptanoate*	0.18	0.0000	25.88	0.0000
2-hydroxypalmitate	0.09	0.0000	9.91	0.0020
2-hydroxystearate	0.12	0.0000	11.03	0.0012
2-hydroxyarachidate*	0.12	0.0000	10.22	0.0007
2-hydroxybehenate	0.12	0.0000	6.66	0.0007

2-hydroxynervonate*	0.15	0.0000	5.14	0.0005
3-hydroxyhexanoate	0.16	0.0002	11.06	0.0000
3-hydroxyoctanoate	0.4	0.0000	2.93	0.0000
3-hydroxydecanoate	0.38	0.0010	2.71	0.0007
3-hydroxysebacate	0.34	0.0000	3.07	0.0000
3-hydroxylaurate	0.23	0.0000	5.42	0.0000
3-hydroxypalmitate	0.04	0.0001	31.57	0.0000
3-hydroxystearate	0.22	0.0001	6.55	0.0000
3-hydroxyoleate*	0.23	0.0000	7.89	0.0000
8-hydroxyoctanoate	0.39	0.0002	2.85	0.0002
10-hydroxystearate	0.02	0.0000	57.76	0.0001
16-hydroxypalmitate	0.18	0.0000	8.18	0.0000
13-HODE + 9-HODE	0.24	0.0004	5.78	0.0000
12,13-DiHOME	0.23	0.0000	5.97	0.0000
9,10-DiHOME	0.26	0.0000	6.63	0.0000
2S,3R-dihydroxybutyrate	0.56	0.0029	1.9	0.0231
19,20-DiHDPA	0.16	0.0004	8.54	0.0000
oleoyl ethanolamide	0.09	0.0000	13.31	0.0000
palmitoyl ethanolamide	0.15	0.0001	10.2	0.0001
stearoyl ethanolamide	0.08	0.0000	11.06	0.0000
linoleoyl ethanolamide	0.09	0.0000	17.34	0.0000
arachidoyl ethanolamide (20:0)*	0.15	0.0000	5.2	0.0050
choline phosphate	0.12	0.0163	15.18	0.0000
glycerophosphoethanolamine	0.39	0.0003	3.12	0.0002
trimethylamine N-oxide	0.56	0.0415	2.03	0.0266
1,2-dipalmitoyl-GPE (16:0/16:0)*	0.28	0.0001	2.14	0.0021
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.31	0.0005	1.51	0.0461
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.36	0.0002	3.78	0.0000
1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	0.03	0.0000	31.02	0.0000
1-palmitoyl-GPA (16:0)	0.16	0.0001	29.64	0.0000
1-stearoyl-GPA (18:0)	0.15	0.0000	21.18	0.0000
1-oleoyl-GPA (18:1)	0.09	0.0036	24.34	0.0000
1-linoleoyl-GPA (18:2)*	0.39	0.0337	6	0.0001
1-linoleoyl-GPC (18:2)	0.43	0.0444	3.96	0.0103
1-lignoceroyl-GPC (24:0)	0.26	0.0000	2.65	0.0011
1-palmitoyl-GPE (16:0)	0.5	0.0001	2.01	0.0089
1-linoleoyl-GPE (18:2)*	0.28	0.0031	4.67	0.0008
1-palmitoyl-GPG (16:0)*	0.02	0.0000	76.7	0.0000
1-stearoyl-GPG (18:0)	0.01	0.0000	107.02	0.0000
1-oleoyl-GPG (18:1)*	0.26	0.0002	2.32	0.0092
glycerol	0.17	0.0004	4.03	0.0000
glycerol 3-phosphate	0.09	0.0000	5.69	0.0000
glycerophosphoglycerol	0.12	0.0000	5.71	0.0000
1-myristoylglycerol (14:0)	0.5	0.0221	2.72	0.0028
1-pentadecanoylglycerol (15:0)	0.12	0.0000	9.83	0.0000
1-palmitoylglycerol (16:0)	0.42	0.0339	2.99	0.0007
2-myristoylglycerol (14:0)	0.47	0.0075	2.92	0.0013
1-palmitoyl-2-linoleoyl-digalactosylglycerol (16:0/18:2)*	0.22	0.0002	5.09	0.0001
1-palmitoyl-2-linoleoyl-galactosylglycerol (16:0/18:2)*	0.05	0.0000	36.35	0.0000
1-palmitoyl-galactosylglycerol (16:0)*	0.01	0.0000	119.37	0.0000

2-palmitoyl-galactosylglycerol (16:0)*	0.02	0.0000	88.36	0.0000
1-palmitoyl-digalactosylglycerol (16:0)*	0.07	0.0000	22.46	0.0000
3-ketosphinganine	0.07	0.0000	13.11	0.0000
sphinganine	0.09	0.0000	6.71	0.0001
sphingadienine	0.2	0.0000	3.59	0.0030
hexadecasphinganine (d16:0)*	0.05	0.0000	14.58	0.0000
N-stearoyl-sphinganine (d18:0/18:0)*	0.23	0.0193	3.11	0.0320
N-(2-hydroxypalmitoyl)-sphingosine (d18:1/16:0(2OH))	0.08	0.0000	3.85	0.0069
N-stearoyl-sphingosine (d18:1/18:0)*	0.28	0.0050	2.46	0.0202
N-palmitoyl-heptadecasphingosine (d17:1/16:0)*	0.28	0.0016	2.22	0.0105
sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*	0.18	0.0000	1.74	0.0168
sphingosine	0.16	0.0000	5.06	0.0007
heptadecasphingosine (d17:1)	0.31	0.0001	2.57	0.0327
eicosanoylsphingosine (d20:1)*	0.16	0.0000	3.9	0.0004
dehydrophytosphingosine*	0.26	0.0013	2.86	0.0043
3-hydroxy-3-methylglutarate	0.12	0.0000	6.59	0.0000
mevalonate	0.11	0.0000	29.02	0.0000
cholesterol	0.65	0.0013	1.4	0.0161
4-cholesten-3-one	0.37	0.0000	1.27	0.0466
cholate	0.01	0.0000	444.8	0.0000
beta-muricholate	0	0.0000	8677.9	0.0000
alpha-muricholate	0.04	0.0061	32.78	0.0060
glyco-beta-muricholate**	0.58	0.0469	2.46	0.0185
cholate sulfate	0.03	0.0003	234.13	0.0038
deoxycholate	0	0.0000	3378.2	0.0000
3-dehydrodeoxycholate	0.5	0.0083	1.29	0.0335
taurodeoxycholate	0.56	0.0036	8.13	0.0235
6-beta-hydroxylithocholate	0.06	0.0001	12.85	0.0001
lithocholate	0	0.0000	139.8	0.0000
12-ketolithocholate	0.01	0.0000	101.47	0.0000
dehydrolithocholate	0.04	0.0000	5.78	0.0028
6-oxolithocholate	0	0.0000	87.08	0.0002
hyocholate	0.03	0.0000	35.77	0.0000
3-dehydrocholate	0.09	0.0003	33.96	0.0001
7-ketodeoxycholate	0	0.0000	1302.35	0.0000
3b-hydroxy-5-cholenoic acid	0.36	0.0037	2.1	0.0344
isohyodeoxycholate	0.01	0.0000	34.14	0.0003
hypoxanthine	0.02	0.0000	58.62	0.0000
xanthine	0.34	0.0000	3.4	0.0000
2'-deoxyinosine	0.34	0.0000	2.38	0.0001
adenosine 5'-monophosphate (AMP)	0.05	0.0001	11.55	0.0002
adenosine	0.23	0.0009	3.6	0.0224
adenine	0.3	0.0021	2.48	0.0316
1-methyladenine	0.09	0.0000	12.37	0.0000
2'-deoxyadenosine 5'-monophosphate	0.06	0.0000	3.58	0.0123
guanosine 5'- monophosphate (5'-GMP)	0.11	0.0049	3.08	0.0152
guanosine-2',3'-cyclic monophosphate	0.02	0.0000	83.35	0.0036
guanine	0.03	0.0000	27.5	0.0000
N-carbamoylaspartate	0.05	0.0000	10.33	0.0001

uridine 5'-monophosphate (UMP)	0.15	0.0151	7.02	0.0421
uridine-2',3'-cyclic monophosphate	0.03	0.0006	101.76	0.0162
uridine	0.15	0.0000	6.35	0.0045
uracil	0.01	0.0000	75.43	0.0000
pseudouridine	0.41	0.0122	2.34	0.0006
5,6-dihydrouridine	0.08	0.0000	14.25	0.0000
5-methyluridine (ribothymidine)	0.03	0.0000	20.47	0.0005
2'-deoxyuridine	0.06	0.0000	11.13	0.0000
3-ureidopropionate	0.24	0.0000	15.42	0.0010
beta-alanine	0.42	0.0000	2.34	0.0011
cytidine 5'-monophosphate (5'-CMP)	0.04	0.0000	12.84	0.0000
cytosine	0.08	0.0000	38.21	0.0017
2'-deoxycytidine 5'-monophosphate	0.03	0.0002	7.42	0.0030
thymine	0.01	0.0000	124.09	0.0000
3-aminoisobutyrate	0.29	0.0011	8.95	0.0001
(3'-5')-adenylylcytidine	0.09	0.0003	6.03	0.0135
(3'-5')-adenylylguanosine*	0.04	0.0000	14.42	0.0110
(3'-5')-adenylyluridine	0.06	0.0001	6.86	0.0065
(3'-5')-adenylyladenosine*	0.07	0.0001	6.91	0.0168
(3'-5')-cytidylyladenosine	0.06	0.0003	9.18	0.0412
(3'-5')-cytidylylcytidine*	0.06	0.0003	6.45	0.0492
(3'-5')-cytidylylguanosine	0.04	0.0001	13.27	0.0451
(3'-5')-cytidylyluridine*	0.03	0.0002	12.03	0.0020
(3'-5')-guanylyladenosine*	0.05	0.0004	9.74	0.0408
(3'-5')-guanylylcytidine	0.06	0.0004	9.18	0.0108
(3'-5')-guanylyluridine	0.07	0.0010	8	0.0209
(3'-5')-uridylylcytidine*	0.03	0.0000	15.74	0.0009
(3'-5')-uridylyluridine	0.06	0.0003	6.3	0.0057
nicotinate	0	0.0000	261.31	0.0000
nicotinate ribonucleoside	0.08	0.0000	9.49	0.0090
riboflavin (Vitamin B2)	0.61	0.0011	1.46	0.0119
flavin mononucleotide (FMN)	0.26	0.0001	3.06	0.0003
pantothenate	0.02	0.0000	62.19	0.0000
pantethine	0.19	0.0060	11.93	0.0000
pantetheine	0.03	0.0000	31.27	0.0000
alpha-tocopherol	0.61	0.0036	1.32	0.0089
delta-tocopherol	0.39	0.0004	2.51	0.0004
alpha-tocotrienol	0.25	0.0005	3.75	0.0010
gamma-tocotrienol	0.2	0.0000	4.92	0.0001
gamma-tocopherol/beta-tocopherol	0.52	0.0021	1.9	0.0005
bilirubin (Z,Z)	0.25	0.0000	3.87	0.0258
D-urobilin	0.07	0.0002	12.29	0.0081
thiamin (Vitamin B1)	0.04	0.0000	24.39	0.0000
thiamin monophosphate	0.05	0.0000	14.64	0.0000
5-(2-Hydroxyethyl)-4-methylthiazole	0.04	0.0000	36.72	0.0000
hydroxymethylpyrimidine	0.15	0.0019	11.47	0.0000
retinol (Vitamin A)	0.18	0.0018	7.79	0.0000
carotene diol (1)	0.66	0.0005	1.43	0.0000
carotene diol (2)	0.59	0.0030	1.43	0.0259
carotene diol (3)	0.6	0.0020	1.35	0.0017
beta-cryptoxanthin	0.52	0.0001	1.35	0.0136
pyridoxamine	0.03	0.0000	23.15	0.0000

pyridoxal	0.02	0.0000	45.06	0.0000
pyridoxate	0.28	0.0000	4.77	0.0000
4-hydroxybenzoate	0.63	0.0064	10.69	0.0388
2,4,6-trihydroxybenzoate	0.43	0.0321	9.47	0.0000
p-hydroxybenzaldehyde	0.26	0.0002	4.45	0.0000
2-(4-hydroxyphenyl)propionate	0	0.0000	290.21	0.0005
3-(3-hydroxyphenyl)propionate	0	0.0093	195.05	0.0032
3-(4-hydroxyphenyl)propionate	0	0.0115	30.69	0.0011
3-phenylpropionate (hydrocinnamate)	0.01	0.0228	601.56	0.0000
3,5-dihydroxyphenylpropionate	0.04	0.0051	304.42	0.0002
maltol	0.05	0.0000	48.4	0.0000
piperidine	0.04	0.0000	24.88	0.0000
apigenin	0.28	0.0112	6.06	0.0022
vanillate	0.58	0.0046	2.79	0.0022
2,3-dihydroxyisovalerate	0.01	0.0000	79.55	0.0000
dipicolinate	0.02	0.0153	6.23	0.0002
2,8-quinolinediol	0	0.0000	1220.24	0.0169
2-isopropylmalate	0	0.0000	299.54	0.0000
2-oxindole-3-acetate	0.05	0.0000	35.06	0.0000
3-formylindole	0.15	0.0000	8.8	0.0000
beta-guanidinopropanoate	0.22	0.0001	2.33	0.0084
dihydroferulate	0.69	0.0405	1.58	0.0189
enterodiol	0.68	0.0240	3.61	0.0002
enterolactone	0.02	0.0000	182.91	0.0005
equol	0.01	0.0000	178.61	0.0000
equol sulfate	0.03	0.0000	58.25	0.0006
ferulate	0.07	0.0000	12.14	0.0000
indolin-2-one	0	0.0001	40.46	0.0005
pheophytin A	0.02	0.0000	36.26	0.0000
sinapate	0.28	0.0001	4.29	0.0000
2-keto-3-deoxy-gluconate	0.48	0.0020	1.77	0.0071
4-hydroxycinnamate	0.18	0.0000	4.29	0.0000
3-indoleglyoxylic acid	0.34	0.0000	4.21	0.0000
2-aminophenol	0.21	0.0000	6.45	0.0017
diaminopimelate	0.02	0.0000	60.51	0.0000
N-methylpipecolate	0.11	0.0000	9.9	0.0000
salicylate	0.1	0.0004	10.99	0.0000
2,6-dihydroxybenzoic acid	0.02	0.0000	76.05	0.0000
1,3-propanediol	0.4	0.0087	2.88	0.0116
sulfate*	0.12	0.0000	8.13	0.0000
2,4-di-tert-butylphenol	0.31	0.0004	2.35	0.0000

Materials and Methods

Mice: All experimental procedures were carried out in accordance with US NIH guidelines for the care and use of laboratory animals and approved by the UCLA Institutional Animal Care and Use Committees. Mice used for data collection were males, at least 6 weeks of age. C57BL/6J mice were purchased from Jackson laboratories (stock no. 000664), reared as SPF or rederived as GF and bred in flexible film isolators at the UCLA Center for Health

Sciences Barrier Facility. Ai96 (JAX stock no. 028866), *Phox2b-cre* (JAX stock no.016223), were obtained from Jackson laboratories and bred at the UCLA Biomedical Sciences Research Building barrier facility. Mice were housed on a 12-h light-dark schedule in a temperature-controlled (22-25°C) and humidity-controlled environment with *ad libitum* access to water and standard chow (Lab Diet 5010).

Antibiotic treatment and conventionalization: Adult SPF mice were gavaged twice daily for 1 week with a cocktail of vancomycin (50mg/kg), neomycin (100mg/kg) and metronidazole (100mg/kg) every 12 hours daily for 7 days. Ampicillin (1mg/mL) was provided *ad libitum* in drinking water. For conventionalization, fecal samples were collected from adult SPF C57BL/6J mice and homogenized in 1mL pre-reduced PBS per pellet. 100uL of the homogenate was administered by oral gavage to recipient GF mice.

Preparation of antibiotics, small-intestinal, and cecal contents: Vancomycin and neomycin were diluted in water to a final concentration of 1mg/mL for all antibiotic perfusion experiments, then sterile filtered. Vehicle for all antibiotic perfusion experiments was water. For SPF SI and cecal content preparations adult SPF male mice were euthanized, and SI and cecal luminal contents were snap-frozen in liquid nitrogen. Equal weights of frozen SI and cecal content were then combined and diluted to a concentration of 0.1g/mL wet weight in sterile-filtered PBS. Samples were then centrifuged at 500g for 5 minutes to pellet out any large dietary components, and supernatants were used for luminal perfusion. GF SI/cecal contents were collected in the same way from donor GF adult male mice. Sterile-filtered SI/cecal contents were prepared by vacuum-filtering SPF SI/cecal content supernatants through a 0.2µm filter. Sterile-filtered PBS was used as vehicle in all SI/cecal perfusion experiments.

In vivo vagal electrophysiology: Baseline vagal tone recordings: Adult SPF male and female (**FIG. 1**), Adult SPF, GF, ABX, or CONV male (**FIG. 2C**), or adult GF male (**FIG. 11F**) mice were anesthetized with isoflurane (5%) and maintained at 1.8% throughout the experiment. The left cervical vagus nerve was exposed, transected inferior to the nodose ganglion and placed across two platinum iridium wires (insulated except for a short segment in contact with the nerve) for recording of baseline vagal tone. Recordings were conducted for 10 minutes. Vagal recordings with luminal perfusion: the cervical vagus nerve was prepared as above in adult male SPF mice. Additionally, a 20-gauge gavage needle attached to a peristaltic pump (Cole Parmer) with separate tubing for each infusion solution was inserted into the duodenal lumen and secured with sutures. An outflow port was generated by

transecting the small intestine ~10cm distal to the inflow site. During recording, vehicle was first perfused through the lumen at a constant rate for 10 minutes to establish a baseline following surgery at a flow rate of 250 ul/minute. Following the baseline period, stimuli were introduced into the small-intestinal lumen and perfused at the same rate for the remainder of the experiment. Data Acquisition: a differential amplifier was used (A-M Systems LLC). The gain was set to 1000x and a bandpass filter was applied (300Hz-5kHz). The signal was digitized at 20kHz using a data acquisition board (National Instruments) under the control of LabView software Data Analysis: Spikes were detected using an SO-CFAR threshold (window duration of 1501/8000, guard duration 10/8000) to generate an adaptive threshold 4SD above RMS noise. Firing rates were calculated by generating 10s (baseline vagal tone) or 30s (perfusion experiments) bins and then applying a Savitzsky-Golay filter (OriginPro) of 10 points. Baseline vagal tone was defined as the average of the final 300s of recording. For experiments assessing sex differences all raw values were normalized to the SPF male cohort average for the rig on which the recordings took place. For comparison between SPF, GF, ABX, and CONV mice, all raw values were normalized to the SPF cohort average. For metabolite supplementation experiments, all raw values were normalized to the VEH cohort average. Perfusion experiments: baseline values were defined as the average frequency of the final 60s of recording in the initial baseline period (F_0). Frequency of the recording was then normalized to the baseline value within-subject (F/F_0). Area under the curve was calculated for each stimulus window and defined as the integral of frequencies over the stimulus window.

16s rRNA gene qPCR for intestinal perfusate samples: To quantify bacterial loads in perfusate outflow samples, 500uL of perfusate outflow contents were collected over the course of 2 minutes directly from the small-intestinal outflow opening at $t = 0-120s$, $t = 600-720s$, $t = 1200-1320s$, and $t = 1800-1920s$. DNA was then isolated using QIAGEN DNeasy Kit according to the manufacturers protocol and the concentration of each pellet was quantified via spectrophotometry (BioTek Synergy H1 Multimode Reader). qPCR was performed using PowerUp SYBR Green Master Mix, according to the manufacturer's protocol (Applied Biosystems, Carlsbad, USA) with universal primer sets targeting the bacterial 16S rRNA gene.

Metabolomics: Samples were collected from adult SPF, GF, ABX, or CONV mice. Luminal contents were collected from the first 3cm of small intestine and the entirety of the cecum, then snap frozen in liquid nitrogen and stored at $-80^{\circ}C$. Samples were prepared using

the automated MicroLab STAR system (Hamilton Company) and analyzed on gas chromatography (GC)-mass spectrometry (MS), liquid chromatography (LC)-MS and LC-MS/MS platforms by Metabolon, inc. Protein fractions were removed by serial extractions with organic aqueous solvents, concentrated using a TurboVap system (Zymark) and vacuum dried. For LC/MS and LC-MS/MS, samples were reconstituted in acidic or basic LC-compatible solvents containing > 11 injection standards and run on a Waters ACQUITY UPLC and Thermo-Finnigan LTQ mass spectrometer, with a linear ion-trap front-end and a Fourier transform ion cyclotron resonance mass spectrometer back-end. For GC/MS, samples were derivatized under dried nitrogen using bistrimethyl-silyl-trifluoroacetamide and analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. Chemical entities were identified by comparison to metabolomic library entries of purified standards. Following log transformation and imputation with minimum observed values for each compound, data were analyzed using one-way ANOVA to test for group effects. P and q-values were calculated based on two-way ANOVA contrasts. Principal components analysis was used to visualize variance distributions. Supervised Random Forest analysis was conducted to identify metabolomics prediction accuracies.

Preparation of metabolite pools, single metabolites, and receptor antagonists: All working metabolite solutions were made up in PBS and up to 0.05% DMSO, brought to a pH of 7.3, and sterile filtered. The concentrations for each metabolite pool, individual metabolites, and receptor antagonists were determined from serum metabolomics data in the lab (data not shown) and existing literature quantifying candidate metabolites in murine fecal, serum, and luminal content samples or based on dose response curves assessing metabolite-induced effects on the candidate receptors and are as follows: Tryptophan metabolites (kynurenine 100uM, kynurenic acid 16.1uM, xanthurenate 1uM, dihydrocaffeate 30nm, tryptamine 0.03uM, pooled), FAEs (oleylethanolamide (OEA), palmitoylethanolamide (PEA), linoleylethanolamide (LEA) 10uM, pooled), HODEs (9-s- and 13-s- HODE, 1uM, pooled), succinate (2mM), glutamate (50mM), bile acids (BAs, cholate 1240nM, glycocholate 3.5nM, chenodeoxycholate 42nM, alpha-muricholate 142nM, beta-muricholate 1080nM, deoxycholate 390nM, taurodeoxycholate 260nM, ursodeoxycholate 74nM, taurohyodeoxycholate 18.8nM, 7-ketodeoxycholate 100nM, lithocholate 390nM, taurolithocholate 0.33nM, pooled), m-tolyl 5-chloro-2-[ethylsulonyl] pyrimidine-4-carboxylate (SBI-115, 200uM), short-chain fatty acids (SCFAs, acetate 80uM, butyrate

22uM, propionate 10uM, pooled), 4-[[*(R)*]-1-(benzo[*b*]thiophene-3-carbonyl)-2-methyl-azetidine-2-carbonyl]-(3-chloro-benzyl)-amino]-butyric acid 99 (GLPG0974, 10uM), hippurate (2uM), Trimethylamine-N-oxide (TMAO, 3uM), imidazole propionate (200nM), phenethylamine (PEA, 100uM), 3-indoxyl sulfate (3IS, 1uM), or (1*E*,3*E*)-1-(4-

5 Fluorophenyl)-2-methyl-1-penten-3-one oxime (A967079, 10uM). Vehicle for tryptophan metabolites, FAEs, MFAs, succinate, glutamate, SCFAs, TMAO, hippurate, imidazole propionate, and phenethylamine was sterile-filtered PBS. Vehicle for BAs was 0.05% DMSO in PBS, Vehicle for 3-IS was 1uM KCl in PBS.

In vitro calcium imaging: Adult male SPF mice were euthanized and both left and
10 right vagal ganglia were quickly excised and immersed in ice-cold HBSS, then mechanically desheathed with a razor blade. Ganglia were then digested for 1 hour at 37C and 5% CO₂ in HBSS with 1mg/ml collagenase and dispase II. Following enzymatic digestion, the neurons were pelleted via centrifugation at 500g for 2 minutes and the solution was replaced with HDMEM. Neurons were then manually triturated, and placed onto poly-L-lysine coated
15 coverslips. Coverslips were then incubated for 2 hours at 37C and 5% CO₂. The HDMEM was then carefully pipetted off of the coverslips and replaced with a solution of the calcium indicator Fluo-4 and probenecid to prevent extrusion (Life Technologies). Neurons were then incubated for 30 minutes at 37C and 5% CO₂, followed by an additional 15-minute incubation at room temperature. Following loading with fluo-4, coverslips were loaded into a
20 perfusion system and imaged with a Leica Dmi8 Fluorescence microscope. Neurons were initially bath perfused over the course of 10 minutes with live-cell imaging solution with 2% glucose to allow for acclimation. Cells were then imaged for 2 minutes to establish a baseline signal before a 2 minute bath perfusion of High K⁺ (60mM), tryptophan metabolites (kynurenine 100uM, kynurenic acid 16.1uM, xanthurenate 1uM, dihydrocaffeate 30nm,
25 tryptamine 0.03uM, pooled), endocannabinoids (oleylethanolamide (OEA), palmitoylethanolamide (PEA), linoleylethanolamide (LEA) 10uM, pooled), HODEs (9-s- and 13-s- HODE, 1uM, pooled), succinate (2mM), glutamate (50mM), bile acids (cholate 1240nM, glycocholate 3.5nM, chenodeoxycholate 42nM, alpha-muricholate 142nM, beta-muricholate 1080nM, deoxycholate 390nM, taurodeoxycholate 260nM, ursodeoxycholate
30 74nM, taurohyodeoxycholate 18.8nM, 7-ketodeoxycholate 100nM, lithocholate 390nM, tauroolithocholate .33nM, pooled), short-chain fatty acids (acetate 80uM, butyrate 22uM, propionate 10uM, pooled), or 3-indoxyl sulfate (1uM), followed by 30 minutes of bath perfusion of LCIS with 2% glucose to allow for response recovery. Data Analysis: individual

neuronal ROI's were drawn and fluorescence signal was quantified using LASX software (Leica). The baseline period for the calcium signal was calculated as the average of the 60 seconds before stimulus onset (F_o). Responses were reported in units of baseline fluorescence ($\Delta F/F$). Cells were considered responsive to stimuli if the maximal $\Delta F/F$ signal following stimulus onset was >50% of the baseline value.

STC-1 and enteroid cell culture, qPCR, and calcium imaging: STC-1 cells (ATCC #CRL-3254) were thawed and passaged until >70% confluence for maintenance in DMEM with GlutaMAX (Gibco #10569010), 1% Penicillin-Streptomycin (Gibco #15140122), and 10% FBS at 37C and 5% CO₂. qRT-PCR experiments: STC-1 cells were collected 1 day after passaging, lysed, and processed with RNeasy Plus Mini kit (QIAGEN #74134) per the manufacturers protocol for RNA isolation. Enteroids were grown as previously described (Miyoshi 2017). Briefly, stem cell-enriched mouse duodenal spheroids were maintained in 50% L-WRN conditioned medium, dissociated, and grown in differentiation medium for 48h prior to RNA isolation, followed by processing with RNeasy Plus Mini kit. qRT-PCR was carried out with iTaq Universal SYBR Green Supermix (Bio-Rad) and primers targeting enterocyte (*Ace2*) and enteroendocrine cell (*Chga*) markers, and microbial metabolite receptors (*Gpbar1*, *Ffar2*, *Trpa1*). Transcript levels were normalized to $\beta 2$ microglobulin and relative expression values were calculated using the $\Delta\Delta C_t$ method. Calcium Imaging: STC-1 cells were re-suspended in culture media and incubated overnight at 37C and 5% CO₂ in poly-D-lysine coated wells. On the day of imaging, culture media was replaced with Fluo-8 in PBS with Ca²⁺ and Mg²⁺ and incubated at 37C for 1 hour prior to imaging. During imaging, Fluo-8 solution was replaced with PBS and imaged for 60 seconds to establish a baseline signal, followed by addition of 2x microbial metabolites (bile acids: cholate 2480nM, glycocholate 14nM, chenodeoxycholate 168nM, alpha-muricholate 568nM, beta-muricholate 2480nM, deoxycholate 780nM, taurodeoxycholate 520nM, ursodeoxycholate 148nM, taurohyodeoxycholate 37.6nM, 7-ketodeoxycholate 200nM, lithocholate 780nM, tauroolithocholate 0.66nM, pooled, short-chain fatty acids: acetate 160uM, butyrate 44uM, propionate 20uM, pooled, or 3-indoxyl sulfate: 2uM) for a final 1x working concentration, High K⁺ (60mM), or D-glucose (20mM). Cells were continually imaged throughout the experiment for 180s at 1Hz on a Leica Dmi8 fluorescence microscope. Data Analysis: 100 randomly sampled ROIs were drawn and fluorescence signal was quantified using LASX software (Leica). The baseline period for the calcium signal was calculated as the average of the 60s prior to stimulus onset (F_o). Responses were reported in units of baseline fluorescence

($\Delta F/F$). Graphs are displayed beginning at experimental $t = 20s$ for visualization purposes. Cells were considered responsive to stimuli if the maximal $\Delta F/F$ signal following stimulus onset was $>50\%$ of the baseline value.

qPCR of vagal nodose neurons: Vagal nodose ganglia were bilaterally harvested, pooled ($n = 2$ per subject), and snap-frozen in RNAlater (ThermoFisher #AM7020). RNA was then isolated using the RNeasy Micro Kit (QUIAGEN #74004) per the manufacturers protocol. qRT-PCR was carried out using PowerUp SYBR Green Master Mix (ThermoFisher #A25742) and primers targeting microbial metabolite receptors (*Gpbar*, *Ffar2*, *Trpa1*). Transcripts were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and relative expression values were calculated using the ΔC_t method.

In vivo calcium imaging: Vagal afferent neuron imaging was performed as previously described. In brief, SPF mice were anesthetized with a cocktail of ketamine and xylazine (100 mg/kg and 10 mg/kg, intraperitoneal), tracheotomized, and maintained on 1.5% isoflurane for the remainder of the surgery and imaging session via ventilator (Kent Scientific). Body temperature was maintained at $37^\circ C$ using an electrical heating pad and rectal temperature probe (Kent Scientific). The vagus nerve was transected superior to the jugular ganglion and vagal ganglia were then embedded between two 5mm-diameter glass coverslips (neuVibro) with silicone adhesive (KWIK-SIL, World Precision Instruments). Imaging was conducted on a Zeiss LSM 780 confocal microscope at a frame rate of 1Hz. Analysis of imaging data:

Imaging data were analyzed using custom Python scripts based on the Minian pipeline. Imaging data was registered to correct for motion artifacts and denoised using a median filter. A constrained non-negative matrix factorization (CNMF) algorithm was used to identify single cells and extract calcium activity. CNMF output regions of interest were then manually inspected to remove non-neuronal signals. Metabolite stimuli order was randomized to account for order-specific effects on neuronal activity. For single-unit analysis in response to sequential metabolite application, neuronal regions of interest in metabolite-responsive neurons were cross-registered across stimulus runs and compared to determine single- or dual- responsivity to metabolite classes. The baseline period for the calcium signal was calculated as the average of the last 120 seconds before stimulus onset (F_o). Responses were reported in units of baseline fluorescence. Cells were considered responsive to stimuli if the maximal $\Delta F/F$ signal following stimulus onset was i) greater than 2 standard deviations above the baseline fluorescence, and ii) the mean $\Delta F/F$ signal over a 20s window around peak response was $>50\%$ of the baseline value. Only units that displayed a $\Delta F/F$ signal >4 standard

deviations over the baseline fluorescence in response to an electrical stimulus were included in analysis. For visualization, metabolite-responding units were hierarchically clustered over the entire time-course of each experiment.

Immunohistochemistry for cFos: Luminal perfusion of stimuli: Mice were

5 anesthetized with 5% isoflurane and maintained at 2% for the entirety of the surgery. To begin intraluminal perfusion, the small intestine was transected at the pyloric sphincter, the junction between the stomach and duodenum, to create an inflow port. Subsequently, a gavage needle attached to a peristaltic pump was inserted into the duodenal lumen. An outflow port was made by transecting the small intestine 3 centimeters distal to the inflow
10 port. All luminal contents were flushed from the small intestine with PBS. Animals were then continually perfused with PBS at 250uL/min flow rate for a 10-minute baseline period, followed by perfusion of stimuli for 30 minutes at a constant flow rate to account for any mechanical distension. Following stimulus perfusion, mice remained under anesthesia for one hour to allow for cFos induction before tissue harvesting. Following the one-hour rest period,
15 animals were sacrificed, and tissues were harvested and fixed via intracardial perfusion of ice-cold PBS followed by 4% paraformaldehyde (PFA). Brains were then post-fixed in 4% PFA at 4°C for 3 hours followed by an overnight incubation in 30% sucrose at 4°C for cryoprotection. Ganglia were positioned bulb-side down in optimal cutting temperature compound (OCT compound) and frozen for cryostat sectioning. Tissues were then sectioned
20 at 30µm and mounted on a microscope slide for immunohistochemical processing (IHC). Slides were thawed for 10 minutes at room temperature in a humidified chamber to prevent the tissue from drying and then permeabilized in 0.5% Triton/0.05% tween-20 in PBS (PBS-TT). Blocking solution consisting of 5% normal goat serum (NGS) in PBS-TT was applied to the tissue and allowed to incubate at room temperature for two hours to prevent non-specific
25 antibody binding and reduce background staining. The tissue sections were then incubated overnight at 4°C with primary antibodies (rabbit anti-cFos 1:500, and guinea pig anti-NeuN, 1:500) in blocking solution (5% NGS + PBS-TT). The following day, slides were washed three times, five minutes per wash, with PBS-TT before incubating at room temperature for two hours with secondary antibodies (goat anti-rabbit 488 1:1000, goat anti-guinea pig 568
30 1:1000, and DAPI 1:1000) in blocking solution (5% NGS + PBS-TT). Confocal Imaging and Quantification Analysis: Images were obtained using a 20x air objective (NA 0.8) on an upright Zeiss LSM 780 confocal microscope. Z-stacks were acquired for three technical replicates of NTS brain tissue or two technical replicates of DMV and ARH tissue and

maximum-intensity projections were generated for subsequent analysis in ImageJ. NTS, DMV, and ARH sections were selected and ROI's were drawn based off of the Allen Mouse Brain Atlas. First, NeuN positive (NeuN+) cells that were each confirmed to colocalize with a DAPI nucleus were counted using the multi-point tool to obtain the total number of neurons.

5 Subsequently, cFos+ cells were counted using the multi-point tool by confirming colocalization of a cFos immunofluorescence signal with NeuN and DAPI. For each image, the total number of cFos+ neurons were divided by the total number of NeuN+ cells to obtain the percentage of cFos+ neurons. Finally, the percentage of cFos+ neurons for all technical replicates of NTS slices per animal were averaged to obtain a biological n=1 and find the
10 overall percentage of cFos+ neurons.

Metabolite supplementation: Adult male GF C57BL/6J mice were treated twice-daily via oral gavage with 200uL of either vehicle (VEH: 12uM KCl, 1.34mM NaCl, 0.012% DMSO in PBS), or a cocktail of microbial metabolites (MMs: cholate, 14.88uM; glycocholate, 42nM; chenodeoxycholate, 504nM; alpha-muricholate, 1.7uM; beta-
15 muricholate, 12.96uM; deoxycholate, 4.68uM; taurodeoxycholate, 3.12uM; ursodeoxycholate, 888nM; taurohyodeoxycholate, 226nM; 7-ketodeoxycholate, 1.2uM; lithocholate, 4.68uM; tauroolithocholate, 3.96nM, acetate, 0.96mM; butyrate, 264uM; propionate, 120uM, 3IS, 12uM). Concentrations of metabolites were determined in order to account for average total fluid intake over a 24 hour period. On the day of each experiment,
20 mice were additionally given a single 200uL oral gavage of VEH or MMs 1 hour prior to *in vivo* vagal whole-nerve electrophysiological recordings.

Quantification and Statistical Analysis: Statistical analysis was performed using Prism software version 8.2.1 (GraphPad). Data were assessed for normal distribution and plotted in the figures as mean $\pm$ SEM. For each figure, n = the number of independent
25 biological replicates. Outliers were identified with ROUT using a threshold of $q = 2\%$ for all electrophysiological recordings. Differences between two treatment groups were assessed using two-tailed, unpaired Student t test with Welch's correction. Differences among >2 groups with only one variable were assessed using one-way ANOVA. If groups were determined to have significantly different variances, groups were assessed with Brown-
30 Forsythe and Welch ANOVA + Games-Howell testing or Wilcoxon matched-pairs signed rank test. Significant differences emerging from the above tests are indicated in the figures by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Notable non-significant (and non-near significant) differences are indicated in the figures by "n.s.".

Table 11. Reagents and Resources

<i>REAGENT or RESOURCE</i>	<i>SOURCE</i>	<i>IDENTIFIER</i>
Antibodies		
Guinea pig anti-NeuN	Sigma	RRID: AB_11205592
Rabbit anti-cFos	Abcam	RRID: AB_2905616
Prolong Gold Antifade Mountant with DAPI	ThermoFisher	Cat# P36931
Chemicals and Peptides and Recombinant Proteins		
Ampicillin	Sigma	Cat# A9518
Metronidazole	Sigma	Cat# M1547
Neomycin	Sigma	Cat# N1876
Vancomycin	Chem-Impex International	Cat# 00315
7-ketodeoxycholic Acid	Sigma	Cat# SMB00806
Alpha-muricholic Acid	Cayman Chemical	Cat# 20291
Beta-muricholic Acid	Sigma	Cat# ML2372
Sodium Cholate Hydrate	Sigma	Cat# C9282
Sodium Chenodeoxycholate	Sigma	Cat# C8261
Deoxycholate	Sigma	Cat# D6750
Sodium Glycocholate Hydrate	Sigma	Cat# G7132
Lithocholic Acid	Sigma	Cat# L6250
Sodium Taurodeoxycholate Hydrate	Sigma	Cat# T0557
Sodium Taurohyodeoxycholate hydrate	Sigma	Cat# T0682
Sodium Tauroolithocholate	Sigma	Cat# T7515
Ursodeoxycholic Acid	Sigma	Cat# U5127
SBI-115	MedChem Express	Cat# HY-111534
Sodium Acetate	Sigma	Cat# S2889
Sodium Butyrate	Sigma	Cat# B5887
Sodium Propionate	Sigma	Cat# P1880
GLPG0974	MedChem Express	Cat# HY-12940
Indoxyl Sulfate Potassium Salt	Sigma	Cat# I3875
A967079	MedChem Express	Cat# HY-108463
L-Kynurenine	Sigma	Cat# K8625
Kynurenic Acid	Sigma	Cat# K3375
Xanthurenic Acid	Sigma	Cat# D120804
Dihydrocaffeic Acid	Sigma	Cat# 102601
Tryptamine	Sigma	Cat# 193747
Oleylethanolamide	Sigma	Cat# O0383
Palmitoylethanolamide	Sigma	Cat# P0359
Linoleylethanolamide	Sigma	Cat# L1164
9-s-HODE	Sigma	Cat# SML0503
13-s-HODE	Sigma	Cat# H9146
Succinic Acid	Sigma	Cat# S3674
L-Glutamic Acid	Sigma	Cat# G1251
Hippurate	Sigma	Cat# H9380
Imidazole Propionic acid	Sigma	Cat# 77951
Phenethylamine	Sigma	Cat# 241008
Trimethylamine-N-Oxide	Sigma	Cat# 317594
D-glucose	Sigma	Cat# G5767
DMEM + GlutaMAX	Gibco	Cat# 10569010
100x Penicillin/Streptomycin	Gibco	Cat# 15140122
FBS Certified One Shot	Gibco	Cat# A3160401
PBS without Ca/Mg	Gibco	Cat# 10010023
0.5M EDTA	Invitrogen	Cat# AM9260G
0.25% Trypsin-EDTA	Gibco	Cat# 25200056
Poly-D-lysine	Gibco	Cat# A3890401
Matrigel Matrix	Corning	Cat# 354234

L-WRN conditioned medium	ATCC	Cat# CRL-3276
Advanced DMEM/F12	Gibco	Cat# 12634010
EGF	Preprotech	Cat# 315-09
Y-27632	R&D Systems	Cat# 1254
EP4 inhibitor	R&D Systems	Cat# 2514
Collagenase 1A	Sigma-Aldrich	Cat# C96891
Dispase II	Sigma-Aldrich	Cat# D4693
Fluo-8 AM	AAT Bioquest	Cat# 21080
Fluo-4 AM	ThermoFisher	Cat# F14217
Critical Commercial Assays		
RNeasy Micro Kit	QIAGEN	Cat# 74004
RNeasy Plus Mini Kit	QIAGEN	Cat# 74134
Dneasy PowerSoil Kit	QIAGEN	Cat# 47014
Deposited Data		
Experimental Models: Organisms/Strains		
Mouse: C57BL/6J	Jackson Laboratories	RRID:IMSR_JAX:000664
Mouse: Phox2b-cre	Jackson Laboratories	RRID:IMSR_JAX:016223
Mouse: Ai96	Jackson Laboratories	RRID:IMSR_JAX:028866
Experimental Models: Cell Lines		
STC-1	ATCC	#CRL-3254

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments are described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A method of increasing vagal nerve activity in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.
- 5 2. The method of claim 1, wherein the vagal nerve activity is afferent vagal nerve activity.
3. A method of improving mood or ameliorating a depressive symptom in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites listed in Tables 1-6.
- 10 4. The method of claim 3, wherein the depressive symptom is selected from anxiety, apathy, general discontent, guilt, hopelessness, loss of interest or pleasure in activities, mood swings, sadness, agitation, excessive crying, irritability, restlessness, social isolation, early awakening, excess sleepiness, insomnia, restless sleep, excessive hunger, fatigue, loss of appetite, lack of concentration, slowness in activity, suicidal ideation, weight gain and weight
15 loss.
5. The method of any one of the preceding claims, wherein the subject is afflicted with or at risk for a psychiatric or neurologic disorder.
6. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for bipolar depression.
- 20 7. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for post-partum depression (PPD).
8. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for post-traumatic stress disorder (PTSD).
9. The method of any one of the preceding claims, wherein the subject is afflicted with
25 or is at risk for major depressive disorder (MDD).
10. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for food addiction.

11. The method of any one of the preceding claims, wherein the method comprises administering an additional therapy for a psychiatric or neurologic disorder.
12. A method of treating autism spectrum disorder in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites
5 listed in Tables 1-6.
13. A method of treating or preventing refractory epilepsy, Alzheimer's disease, Parkinson's disease, obesity and metabolic syndrome, impaired glucose metabolism, inflammatory bowel disease, and visceral pain in a subject, the method comprising administering a composition comprising at least one metabolite selected from the metabolites
10 listed in Tables 1-6.
14. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for Parkinson's disease.
15. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for Alzheimer's disease/cognition.
- 15 16. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for obesity and metabolic syndrome.
17. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for impaired glucose metabolism.
18. The method of any one of the preceding claims, wherein the subject is afflicted with
20 or is at risk for inflammatory bowel disease.
19. The method of any one of the preceding claims, wherein the subject is afflicted with or is at risk for visceral pain.
20. The method of any one of the preceding claims, wherein the method further comprises administering an antibiotic to the subject prior to administration of the composition to the
25 subject.
21. The method of any one of the preceding claims, wherein the composition is formulated for oral delivery.

22. The method of any one of the preceding claims, wherein the composition comprises two or more, three or more, four or more or five or more metabolites selected from the metabolites listed in Tables 1-6.
23. The method of any one of the preceding claims, wherein the method comprises
5 administering two or more, three or more, four or more or five or more compositions comprising at least one metabolite selected from the metabolites listed in Tables 1-6.
24. The method of any one of the preceding claims, wherein the composition comprises 3-indoxyl sulfate.
25. The method of any one of the preceding claims, wherein the composition comprises
10 glutamate.
26. The method of any one of the preceding claims, wherein the composition comprises a bile acid.
27. The method of claim 26, wherein the bile acid is selected from at least one of 7-ketodeoxycholate, beta-muricholate, cholate, chenodeoxycholate, deoxycholate,
15 glycocholate, taurodeoxycholate, tauroolithocholate, taurohyodeoxycholic acid, ursodeoxycholic acid, lithocholate, and alpha-muricholate.
28. The method of any one of the preceding claims, wherein composition comprises 7-ketodeoxycholate.
29. The method of any one of the preceding claims, wherein composition comprises beta-
20 muricholate.
30. The method of any one of the preceding claims, wherein composition comprises cholate.
31. The method of any one of the preceding claims, wherein composition comprises chenodeoxycholate.
- 25 32. The method of any one of the preceding claims, wherein composition comprises deoxycholate.

33. The method of any one of the preceding claims, wherein composition comprises glycocholate.
34. The method of any one of the preceding claims, wherein composition comprises taurodeoxycholate.
- 5 35. The method of any one of the preceding claims, wherein composition comprises tauroolithocholate.
36. The method of any one of the preceding claims, wherein composition comprises taurohyodeoxycholic acid.
- 10 37. The method of any one of the preceding claims, wherein composition comprises ursodeoxycholic acid.
38. The method of any one of the preceding claims, wherein composition comprises lithocholate.
39. The method of any one of the preceding claims, wherein composition comprises alpha-muricholate.
- 15 40. The method of any one of the preceding claims, wherein the composition comprises a short chain fatty acid.
41. The method of claim 40, wherein the short chain fatty acid is selected from at least one of acetate, propionate, and butyrate.
- 20 42. The method of any one of the preceding claims, wherein composition comprises acetate.
43. The method of any one of the preceding claims, wherein composition comprises propionate.
44. The method of any one of the preceding claims, wherein composition comprises butyrate.
- 25 45. The method of any one of the preceding claims, wherein the composition comprises a tryptophan metabolite.

46. The method of claim 45, wherein the tryptophan metabolite is selected from at least one of kynurenate, kynurenine, xanthurenate, dihydrocaffeate, and tryptamine.

47. The method of any one of the preceding claims, wherein the composition comprises kynurenate.

5 48. The method of any one of the preceding claims, wherein the composition comprises kynurenine.

49. The method of any one of the preceding claims, wherein the composition comprises xanthurenate.

10 50. The method of any one of the preceding claims, wherein the composition comprises dihydrocaffeate.

51. The method of any one of the preceding claims, wherein the composition comprises tryptamine.

15 52. The method of any one of the preceding claims, wherein composition comprises at least one of hippurate, trimethylamine-N-oxide (TMAO), imidazole propionate, phenethylamine, palmitoyl ethanolamide (PEA), oleoyl ethanolamide (OEA), linoleoyl ethanolamide (LEA), 13-sHODE, 9-s-HODE, and succinate.

53. The method of any one of the preceding claims, wherein the composition comprises hippurate.

20 54. The method of any one of the preceding claims, wherein the composition comprises trimethylamine-N-oxide (TMAO).

55. The method of any one of the preceding claims, wherein the composition comprises imidazole propionate.

56. The method of any one of the preceding claims, wherein the composition comprises phenethylamine.

57. The method of any one of the preceding claims, wherein the composition comprises palmitoyl ethanolamide (PEA).
58. The method of any one of the preceding claims, wherein the composition comprises oleoyl ethanolamide (OEA).
- 5 59. The method of any one of the preceding claims, wherein the composition comprises linoleoyl ethanolamide (LEA).
60. The method of any one of the preceding claims, wherein the composition comprises monohydroxy fatty acids (MFAs).
61. The method of claim 60, wherein the monohydroxy fatty acids comprise at least one
10 of 13-sHODE and 9-s-HODE.
62. The method of any one of the preceding claims, wherein the composition comprises 13-sHODE.
63. The method of any one of the preceding claims, wherein the composition comprises 9-s-HODE.
- 15 64. The method of any one of the preceding claims, wherein the composition comprises succinate.

FIG. 1

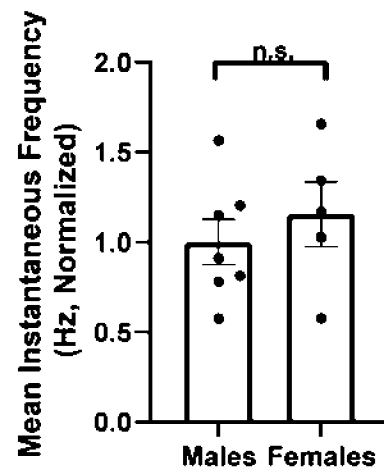


FIG. 2A

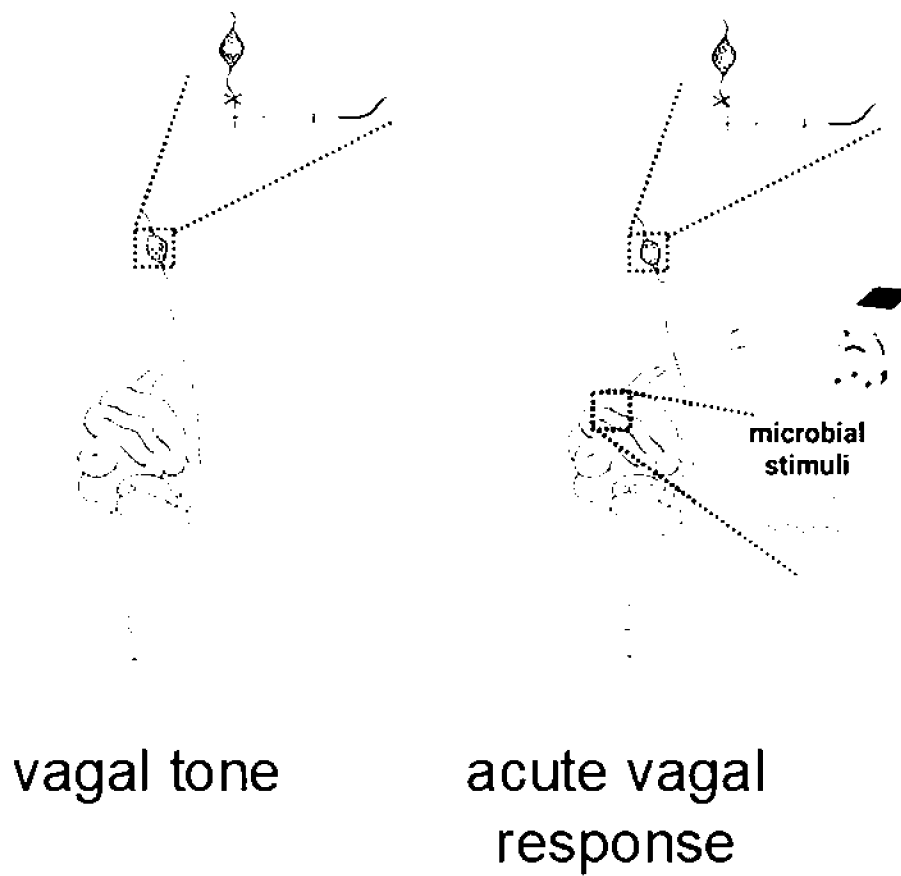


FIG. 2B

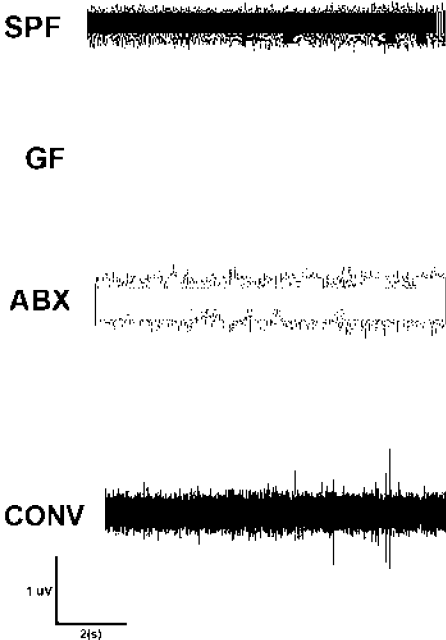


FIG. 2C

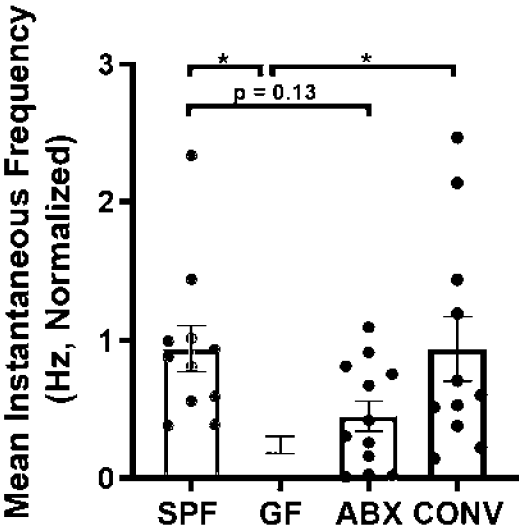


FIG. 2D

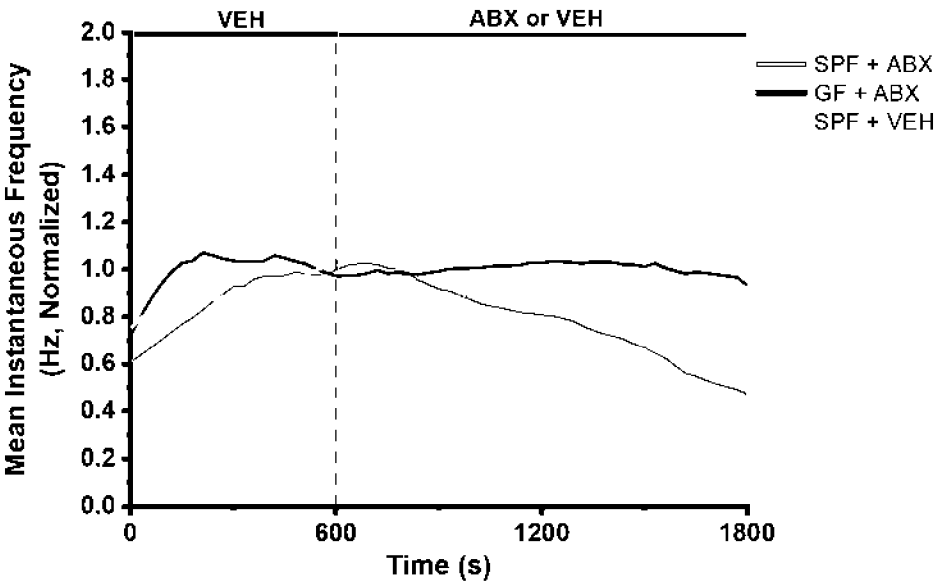


FIG. 2E

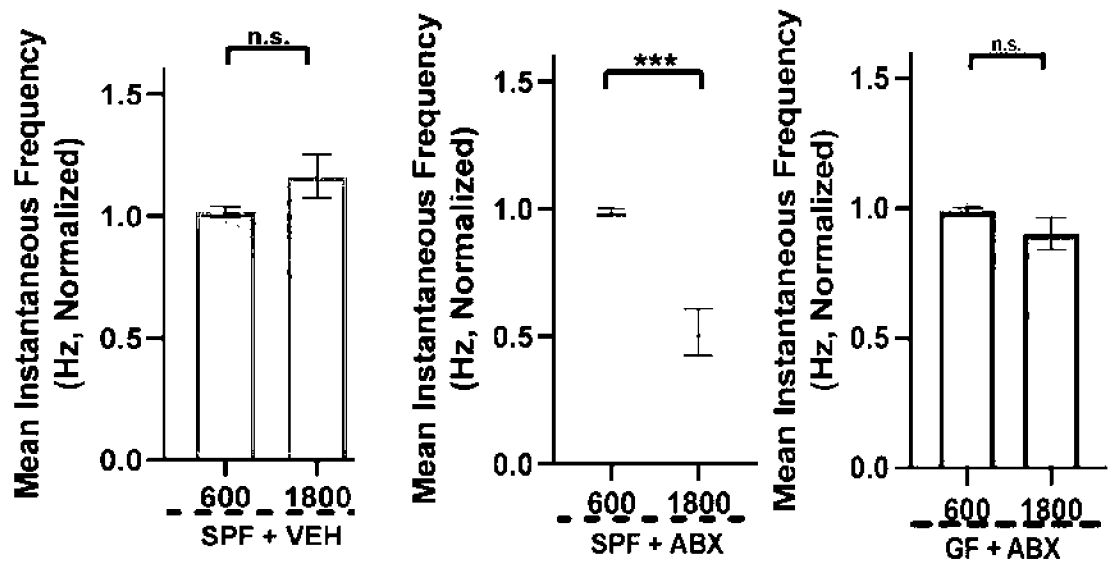


FIG. 2F

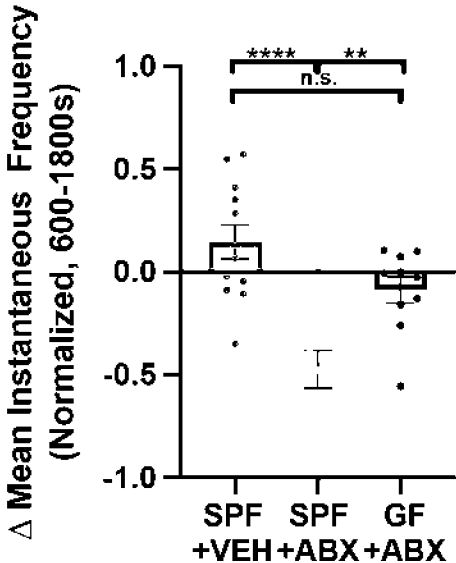


FIG. 2G

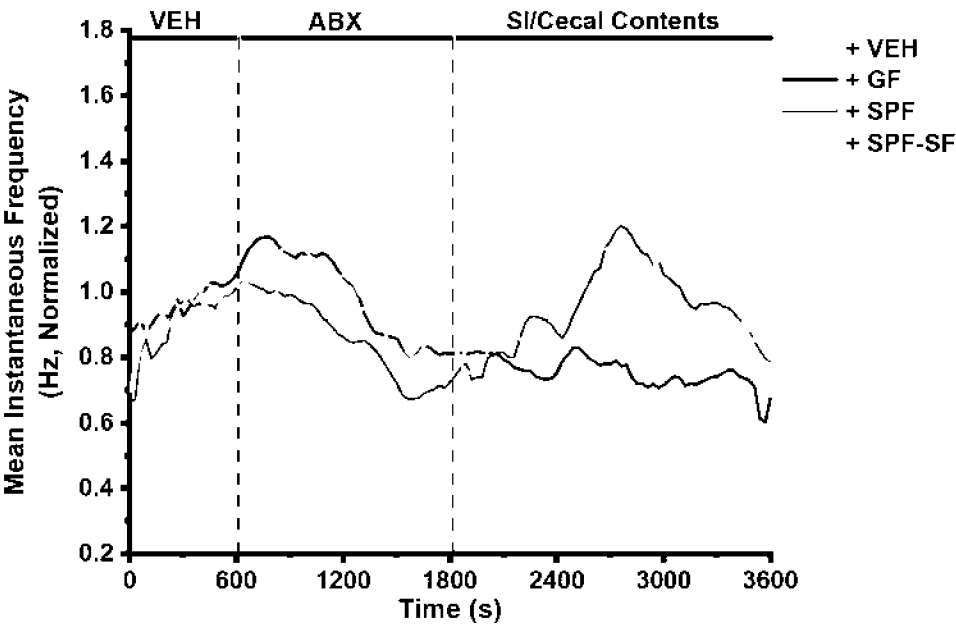


FIG. 2H

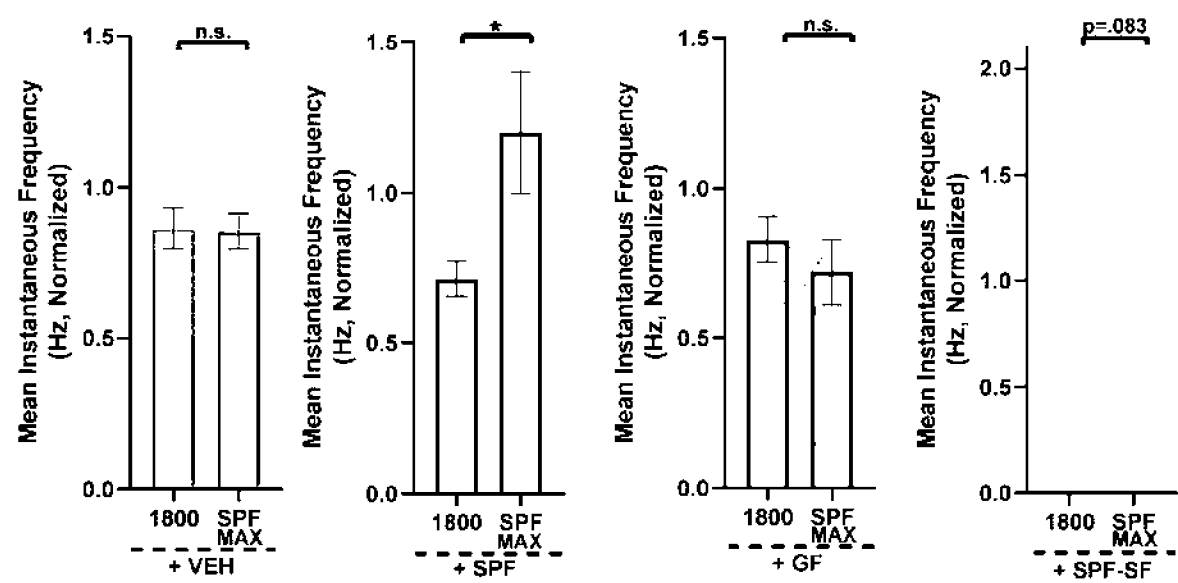


FIG. 2I

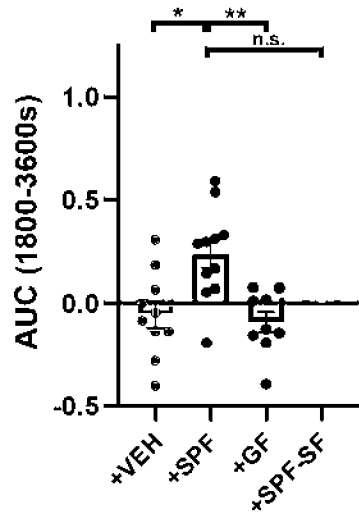


FIG. 3

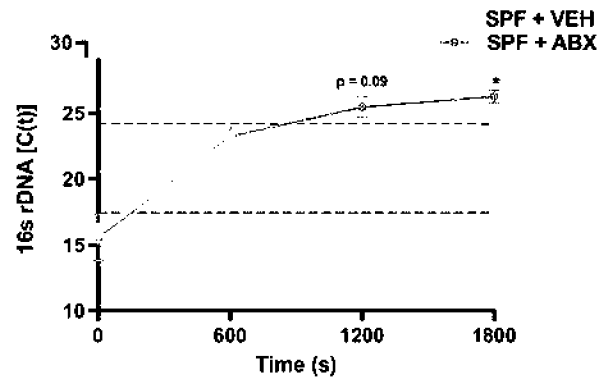


FIG. 4A

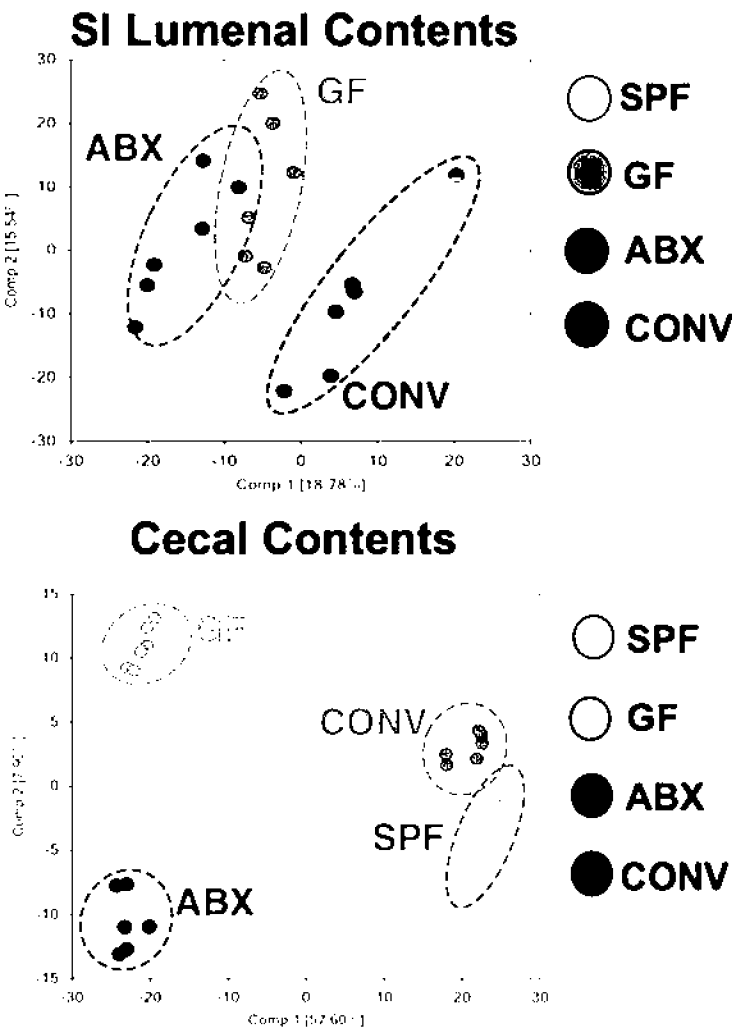
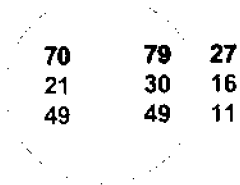


FIG. 4B

GF&ABX v. SPF GF&ABX v. CONV



GF&ABX v. SPF GF&ABX v. CONV

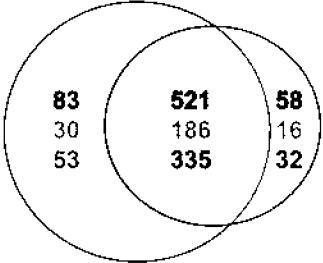


FIG. 4C

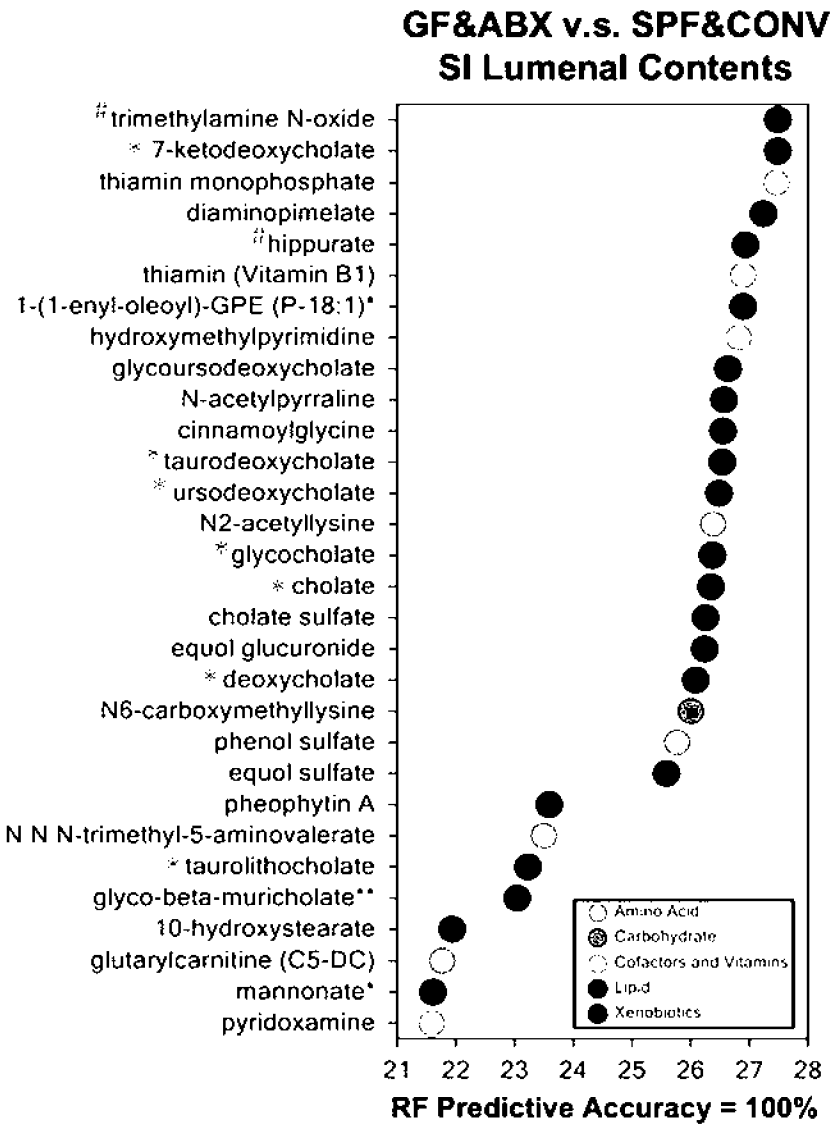


FIG. 4D

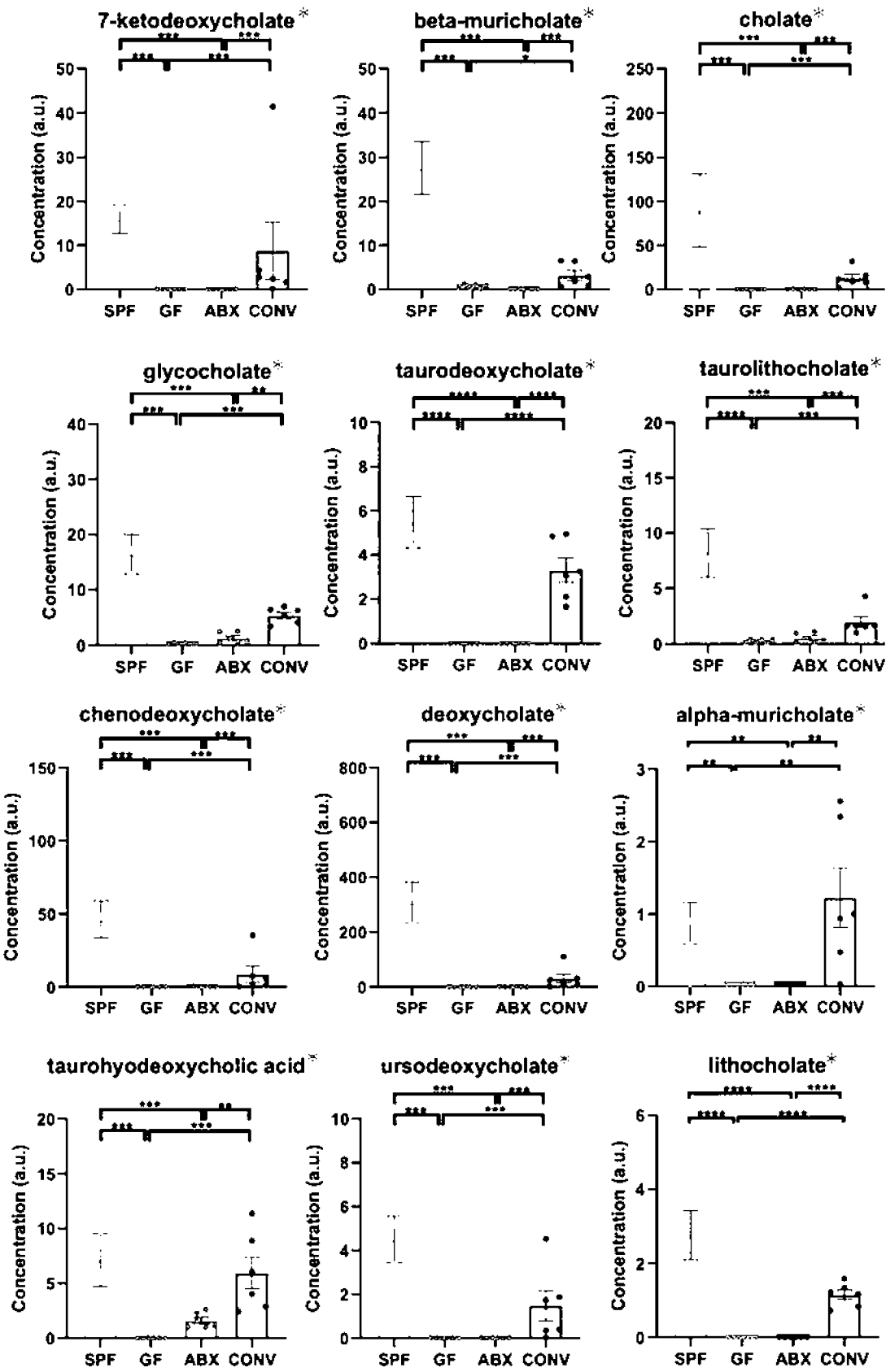


FIG. 4E

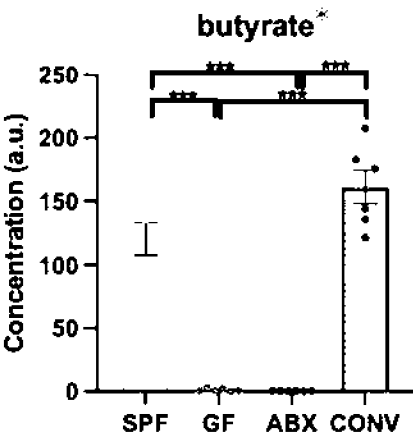


FIG. 4F

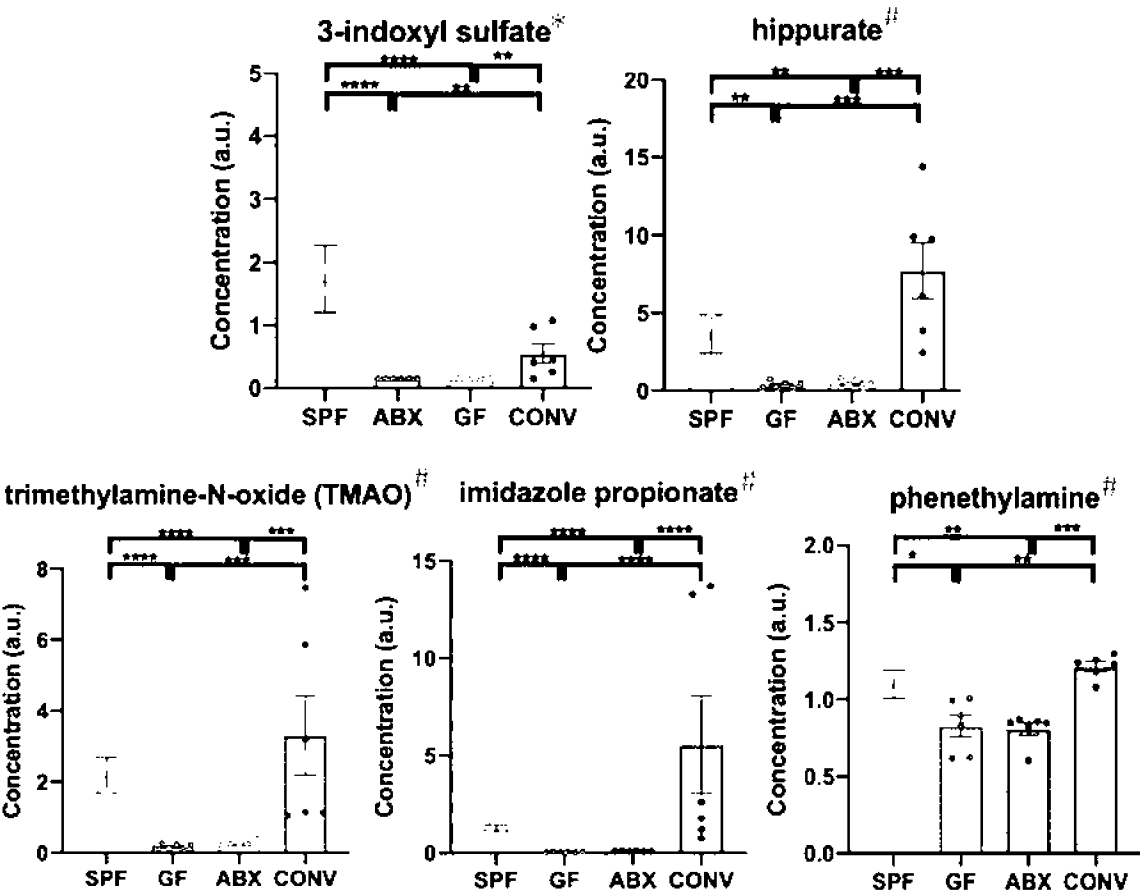


FIG. 5A

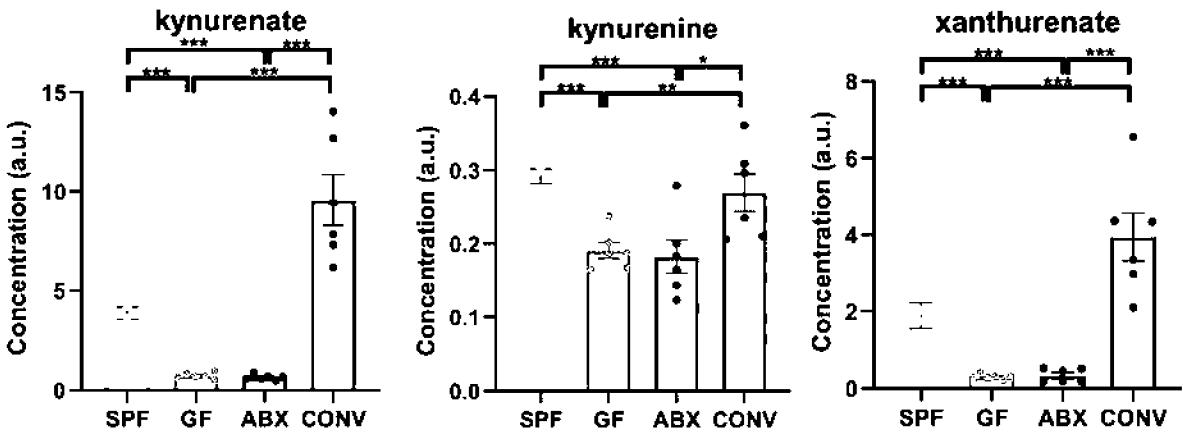


FIG. 5B

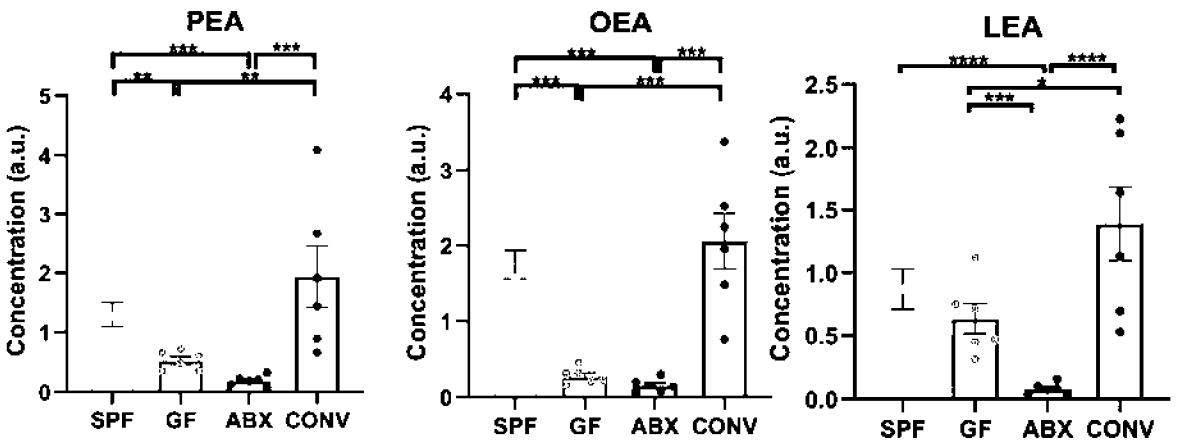


FIG. 5C



FIG. 5D

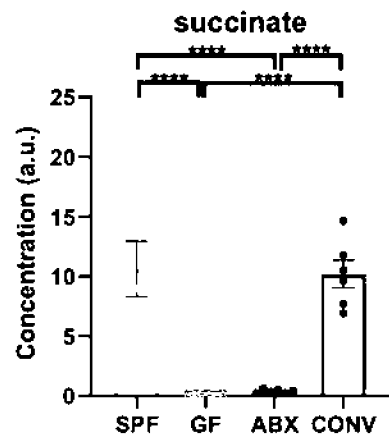


FIG. 5E

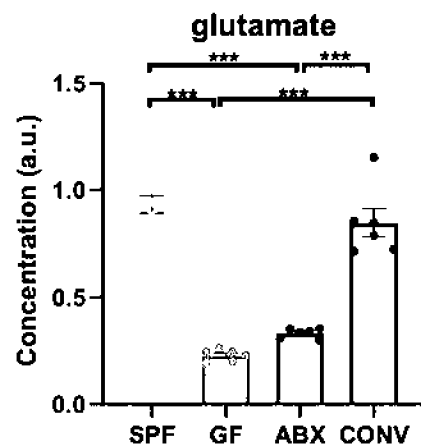


FIG. 6A

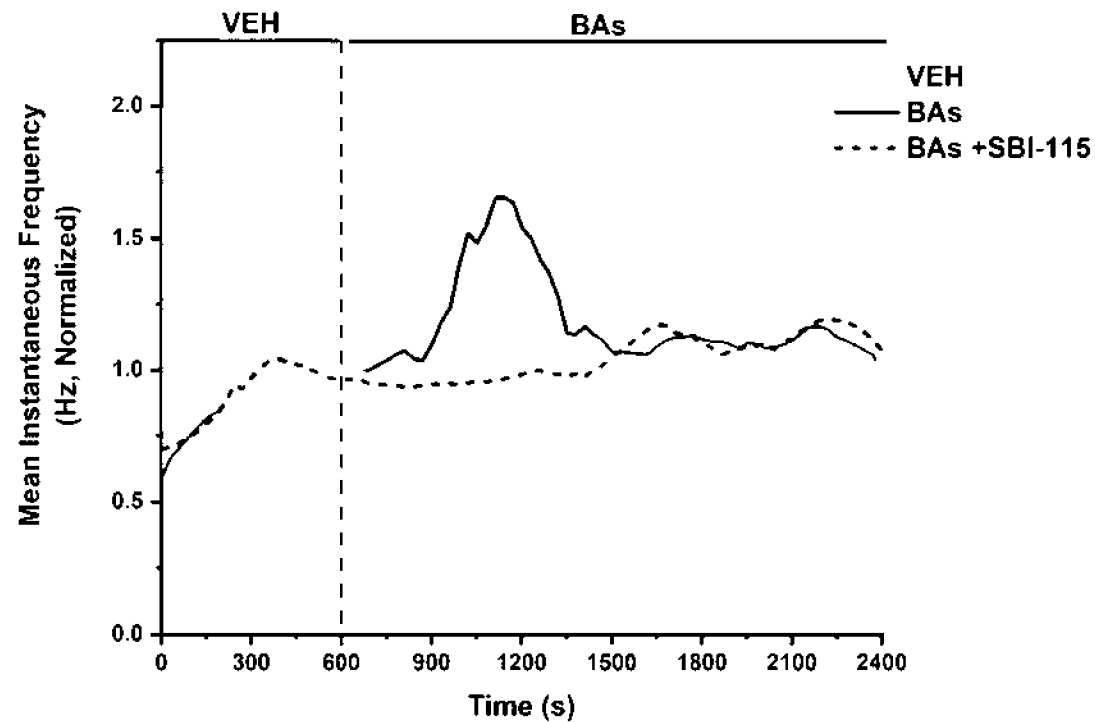


FIG. 6B

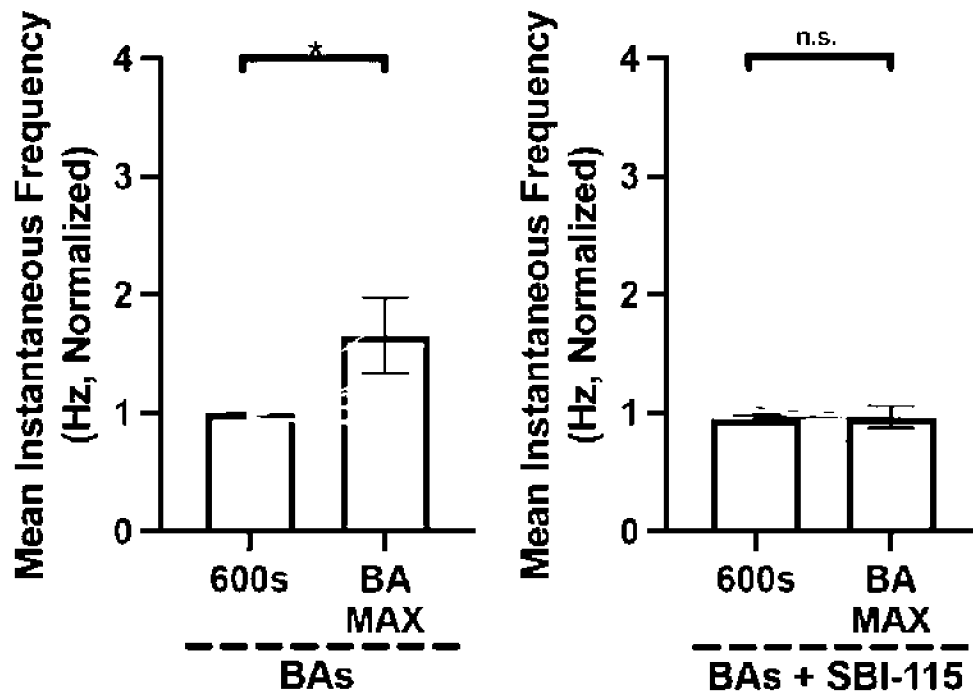


FIG. 6C

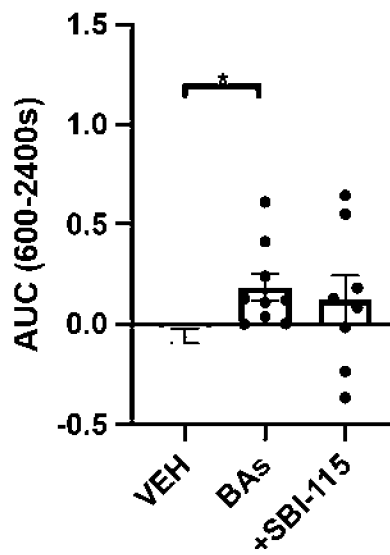


FIG. 6D

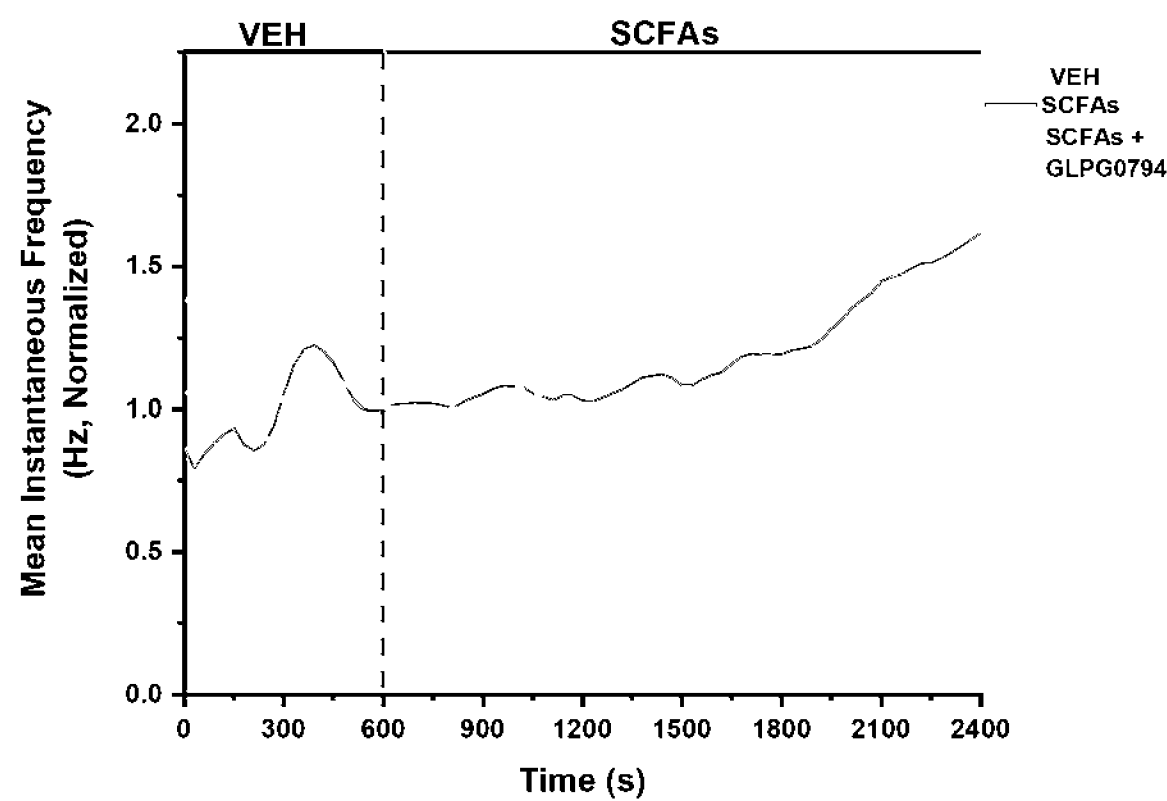


FIG. 6E

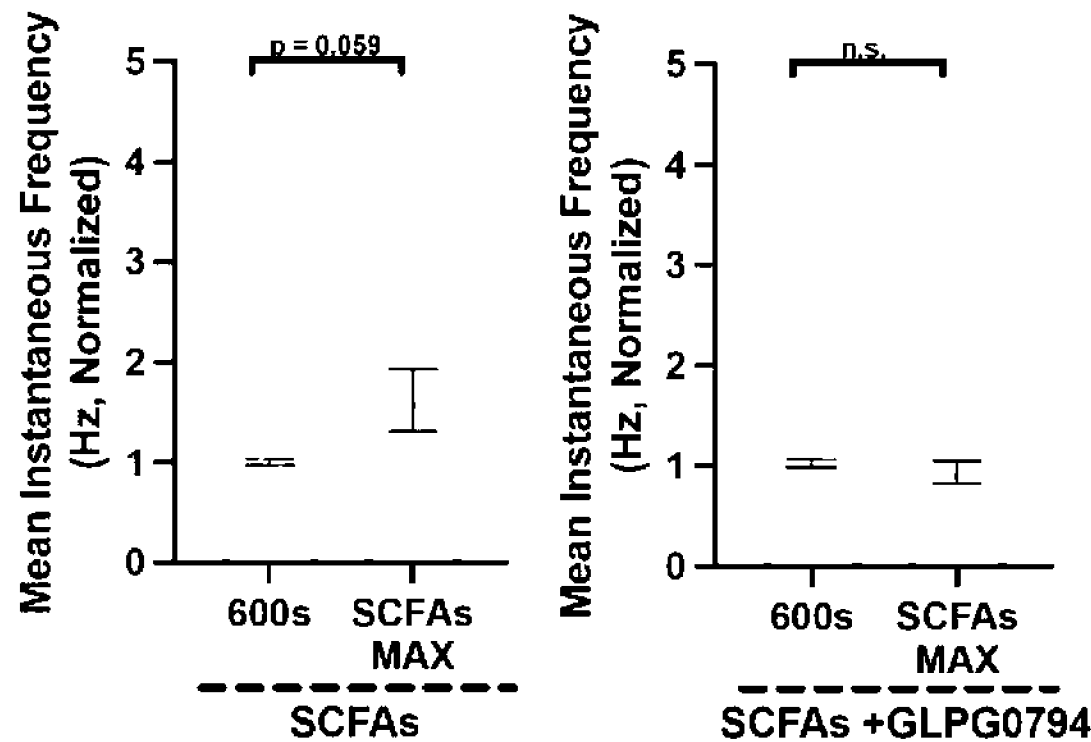


FIG. 6F

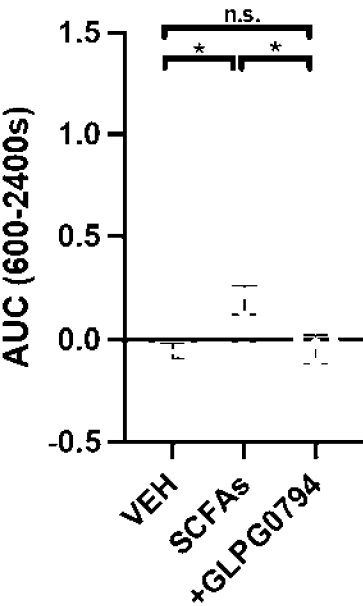


FIG. 6G

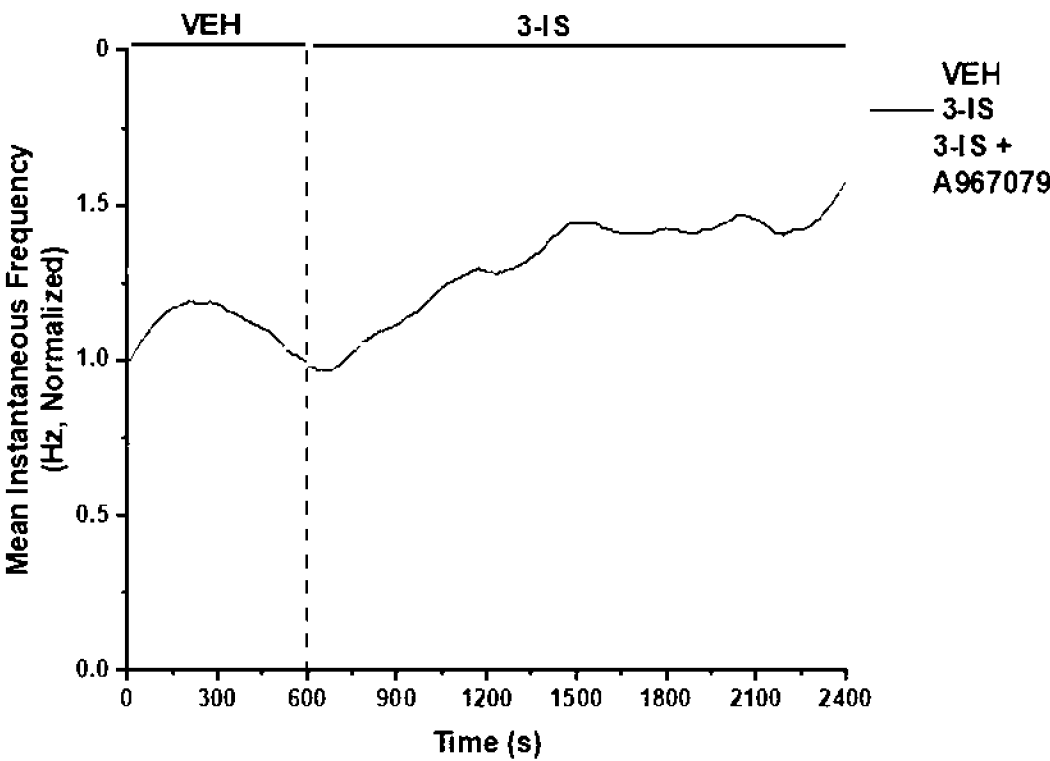


FIG. 6H

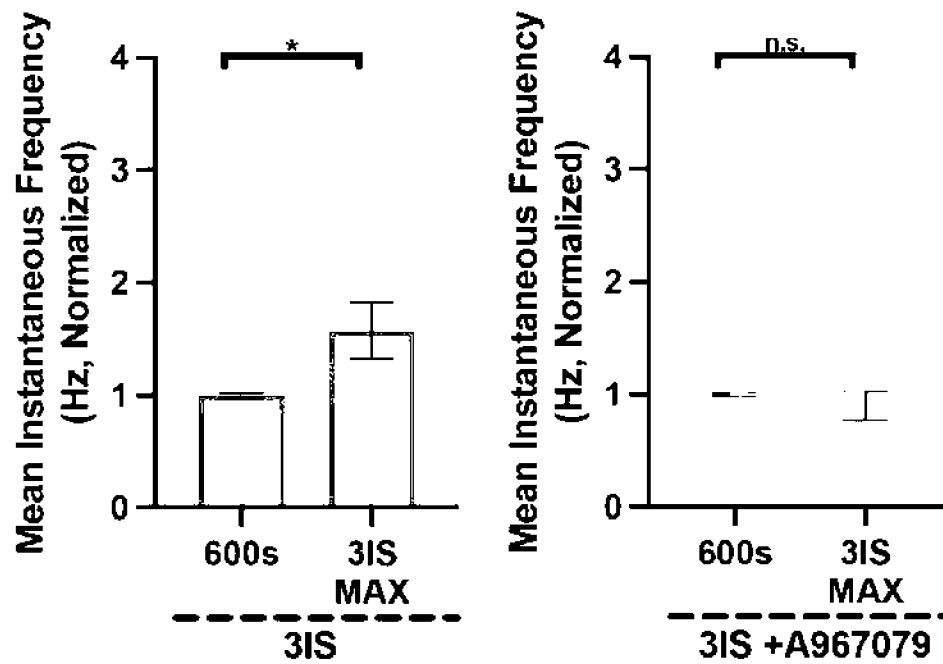


FIG. 6I

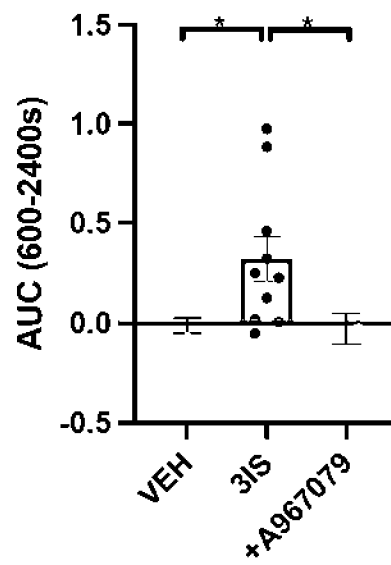


FIG. 7A

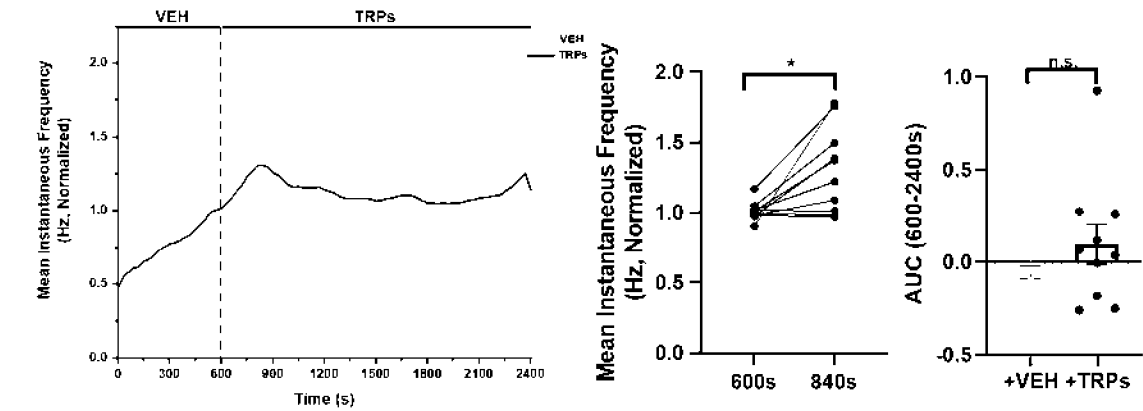


FIG. 7B

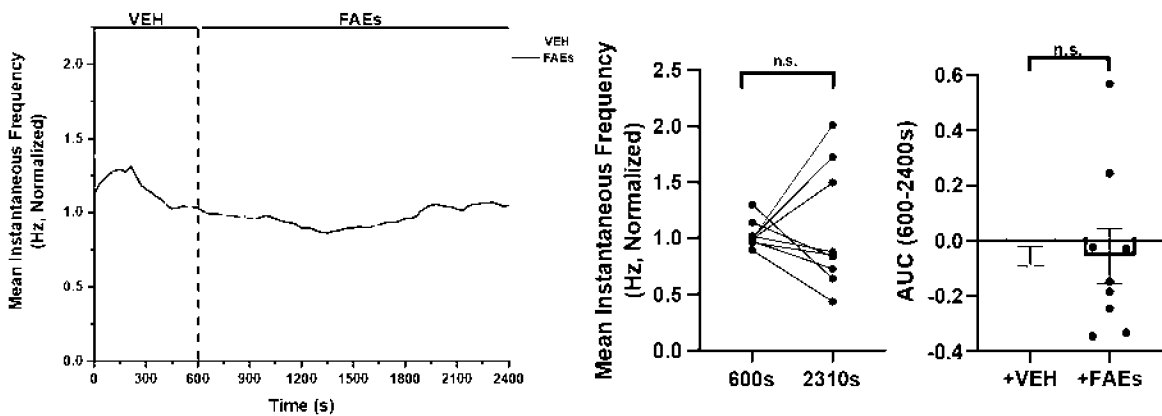


FIG. 7C

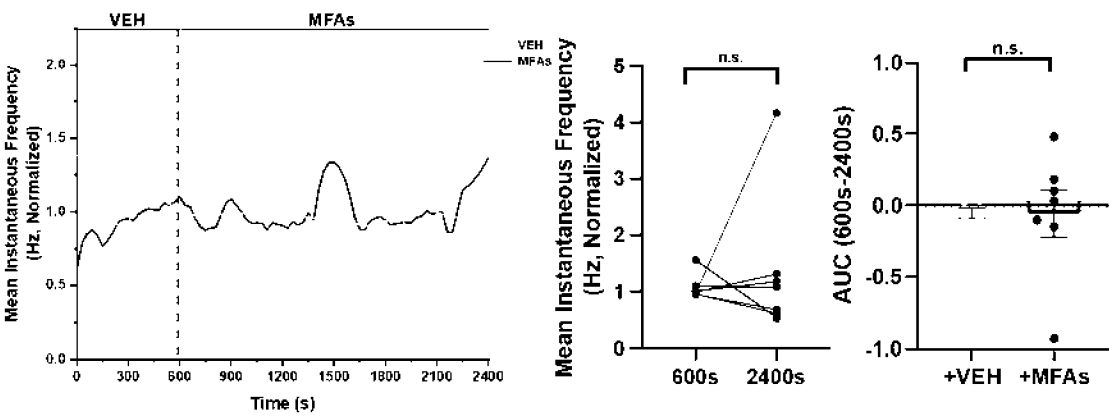


FIG. 7D

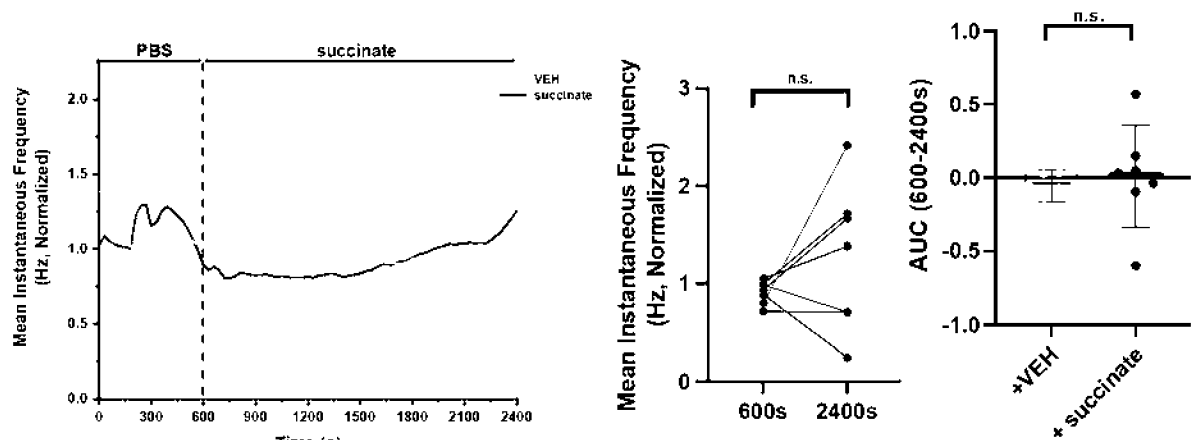


FIG. 7E

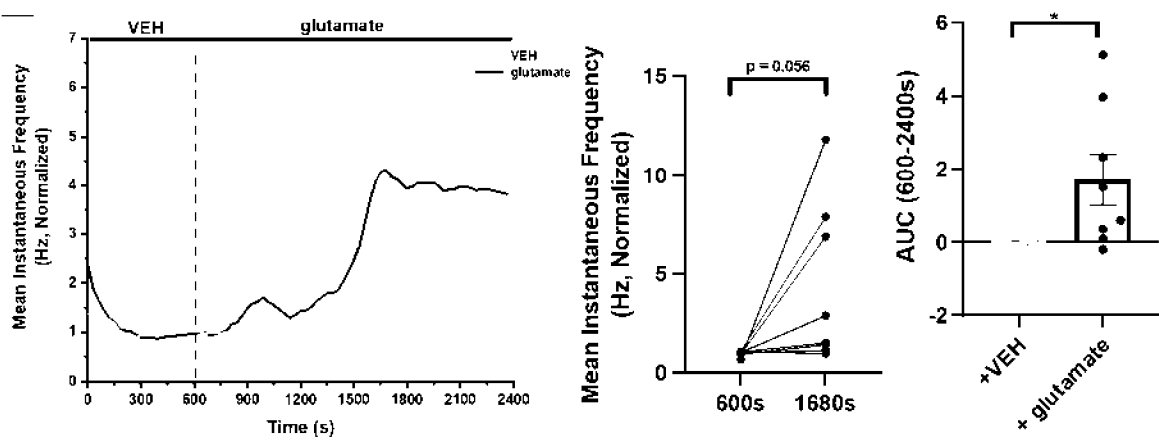


FIG. 7F

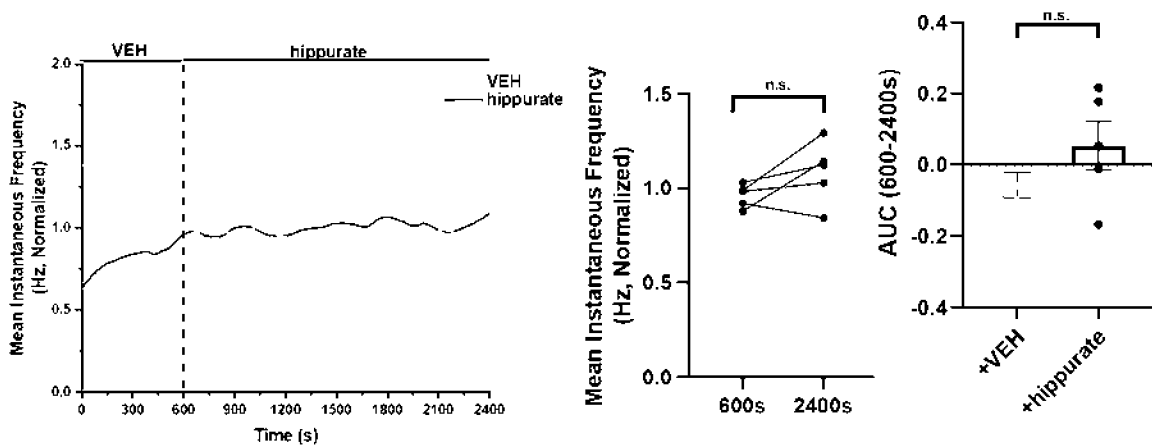


FIG. 7G

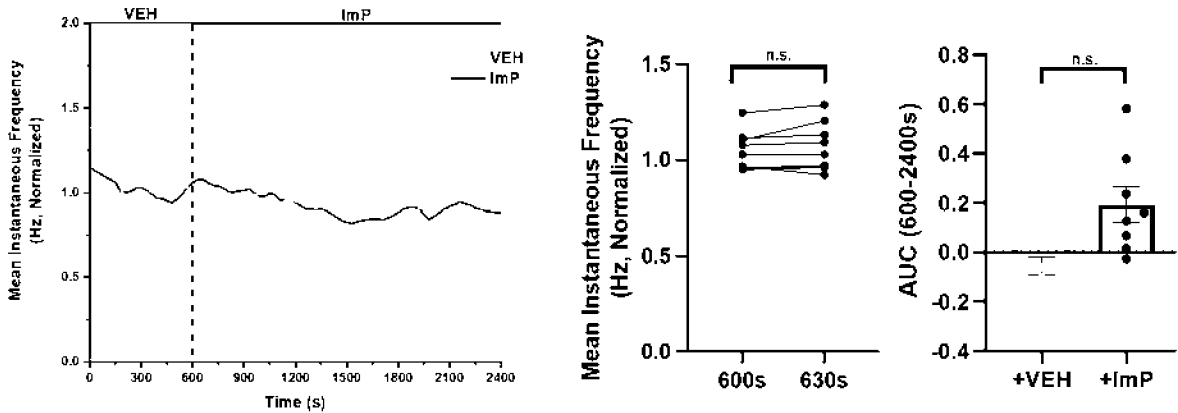


FIG. 7H

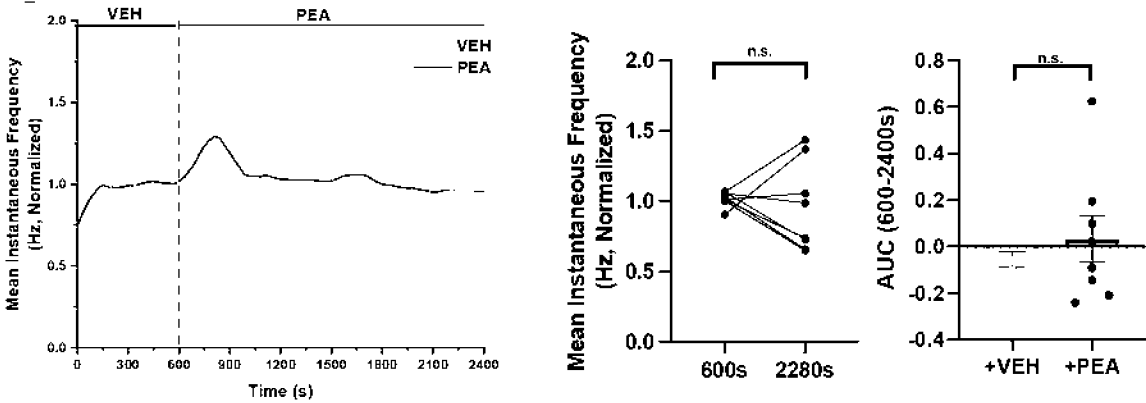


FIG. 7I

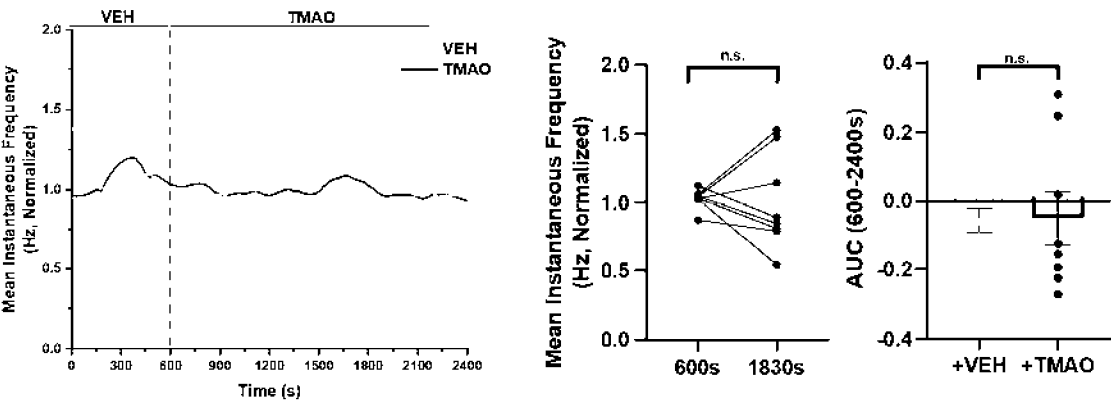


FIG. 8

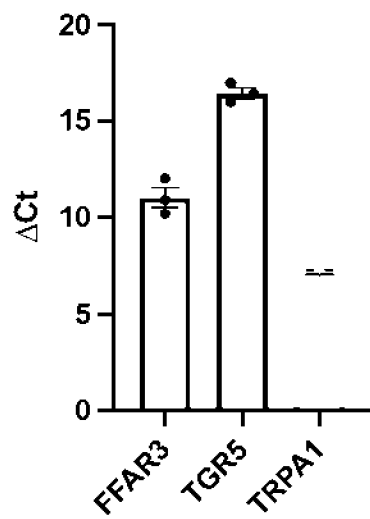


FIG. 9A

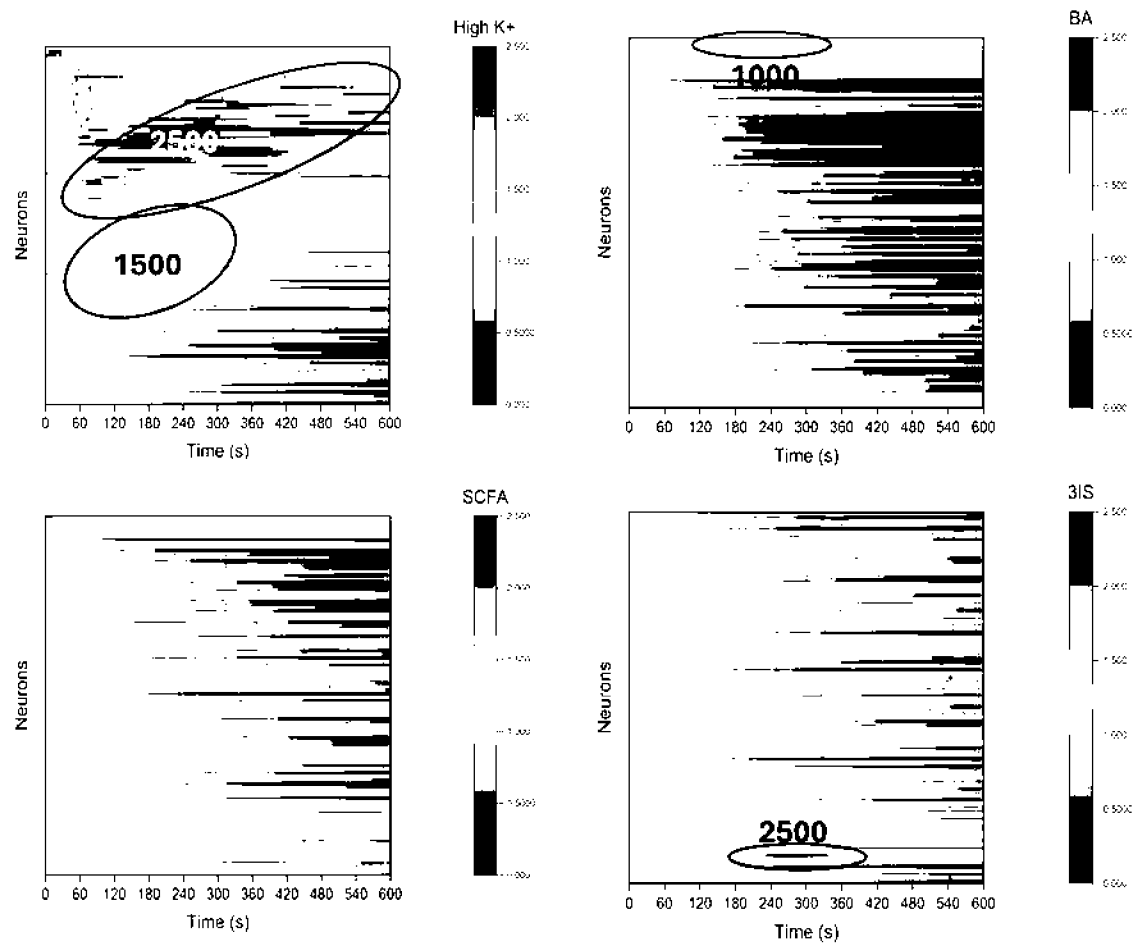


FIG. 9B



FIG. 9C

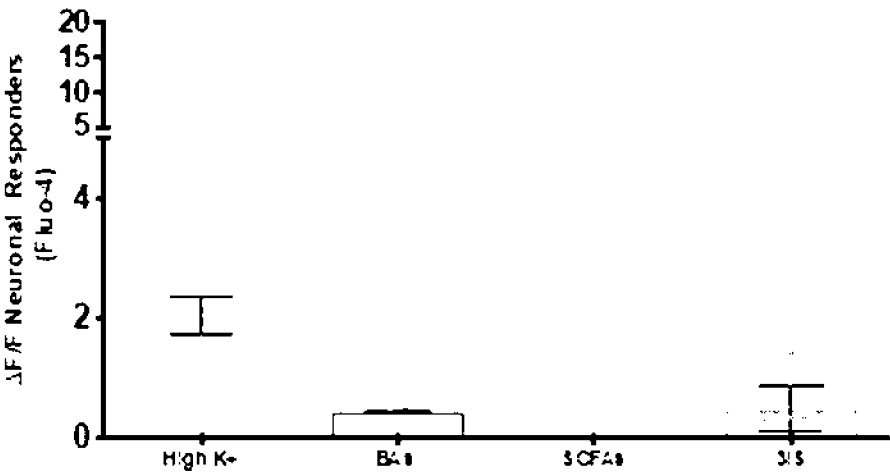


FIG. 10A

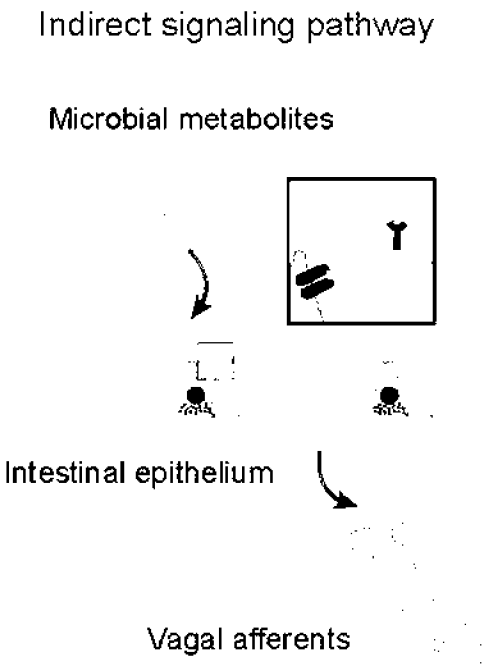


FIG. 10B

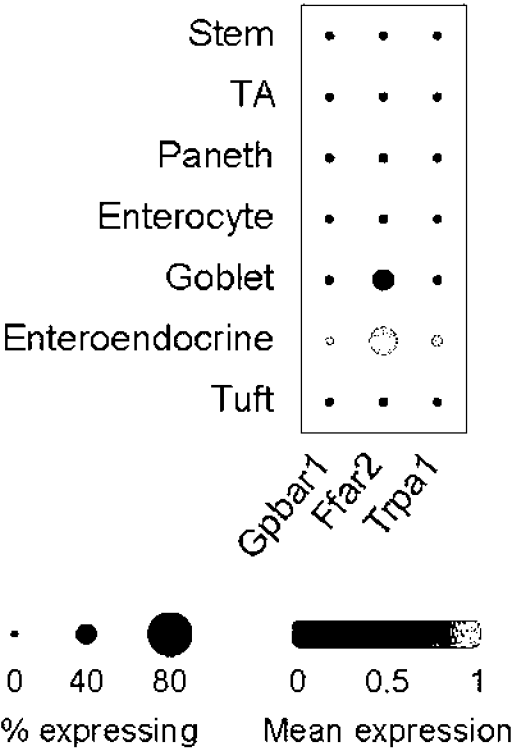


FIG. 10C

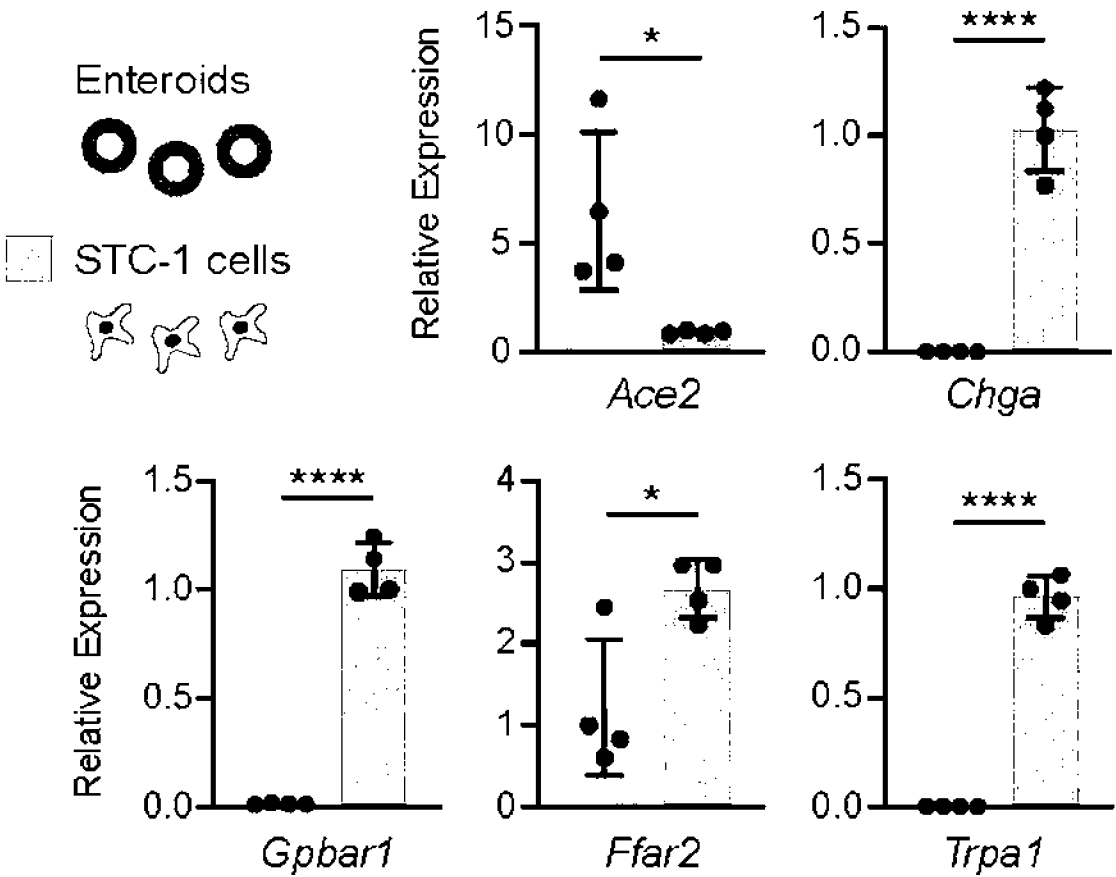


FIG. 10D

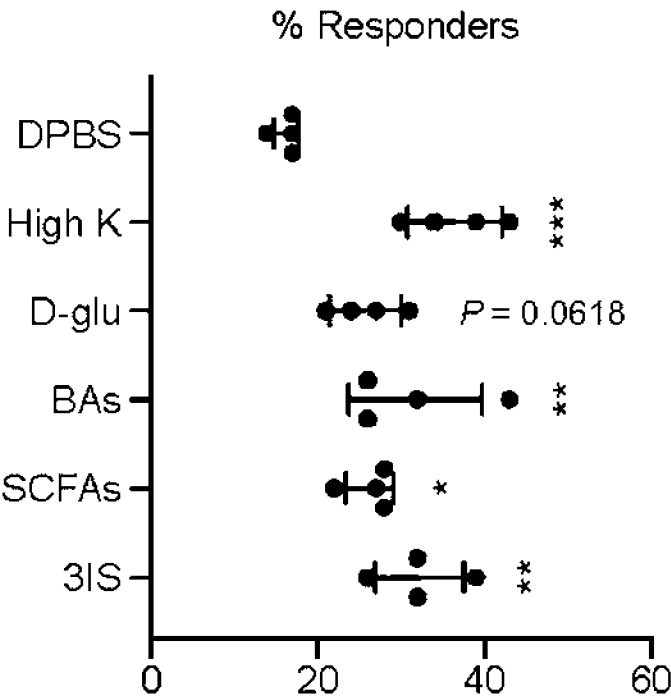


FIG. 10E

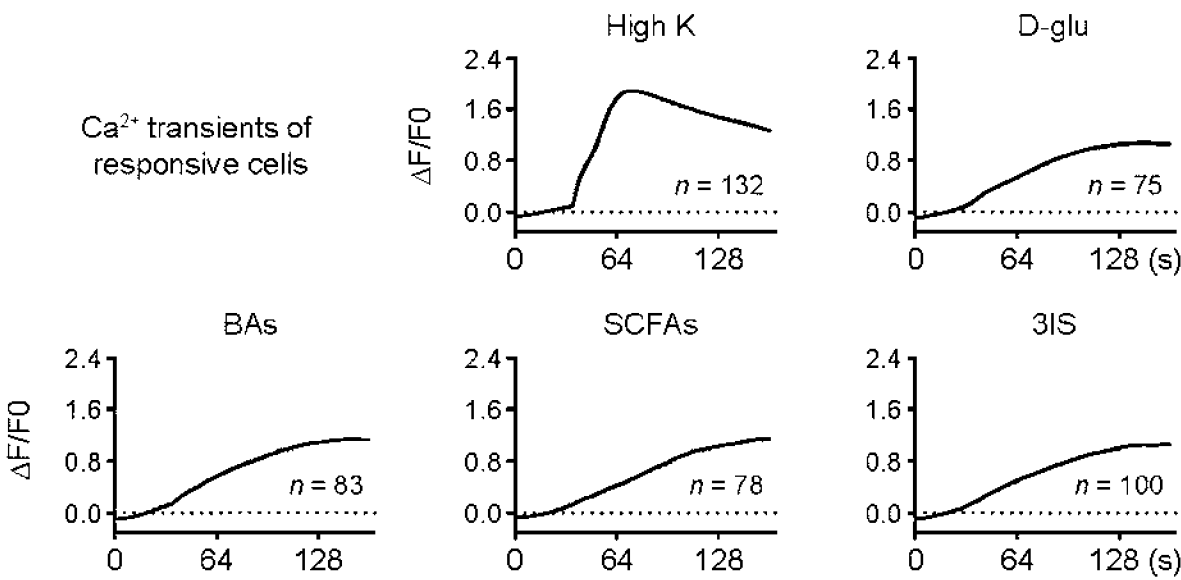


FIG. 11A

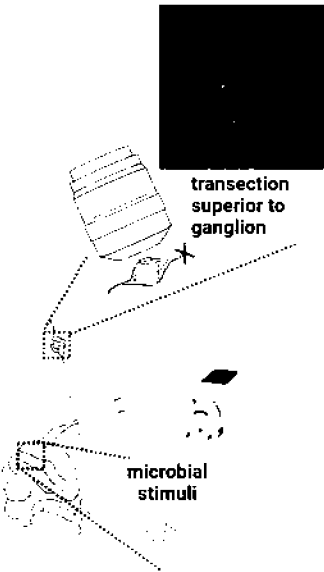


FIG. 11B

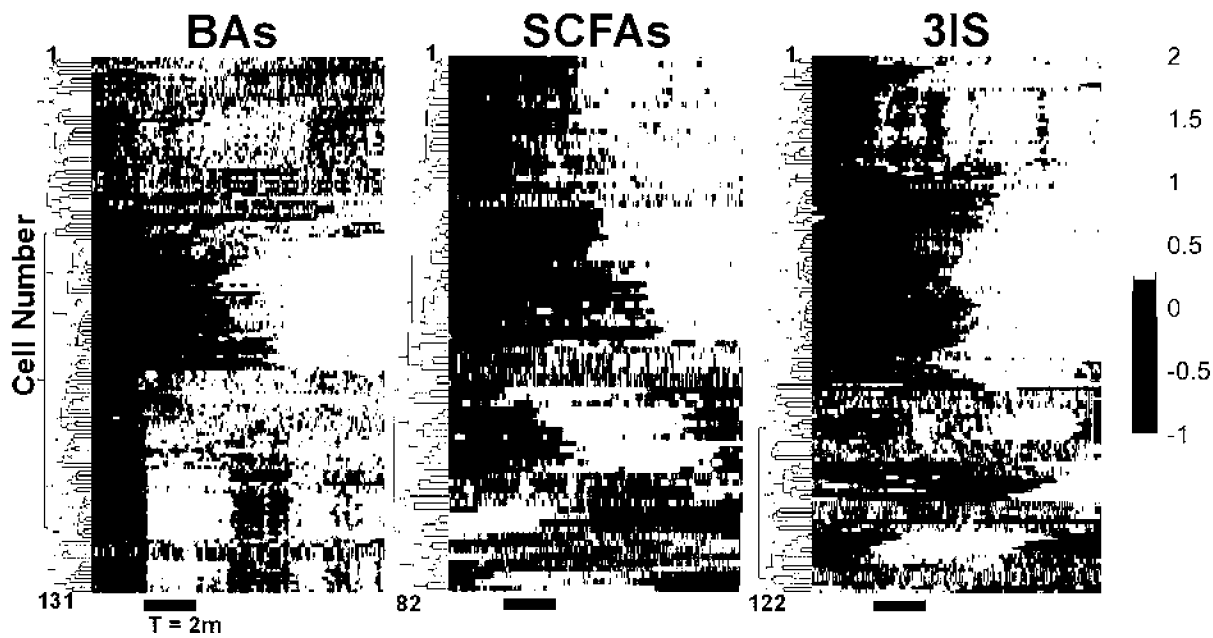


FIG. 11C

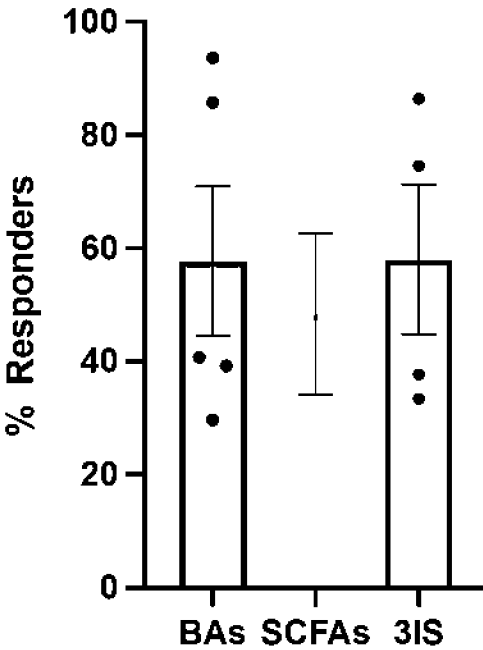


FIG. 11D

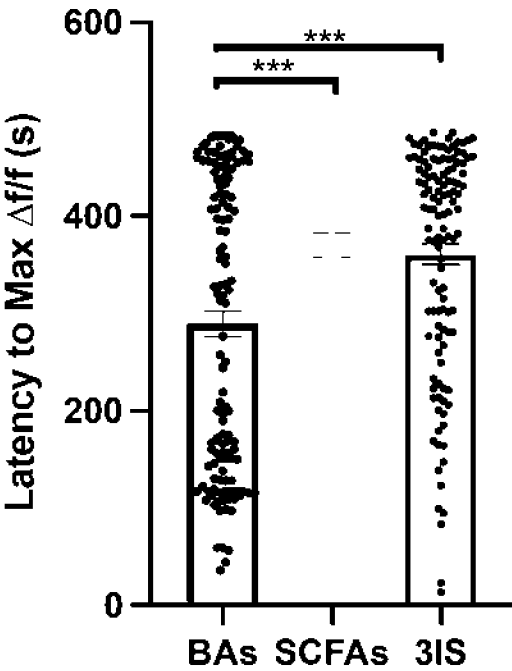


FIG. 11E

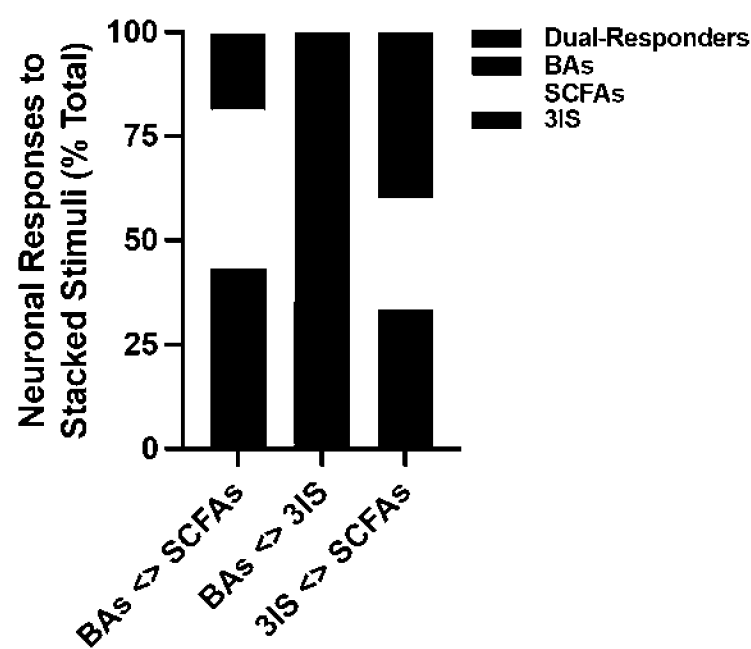


FIG. 11F

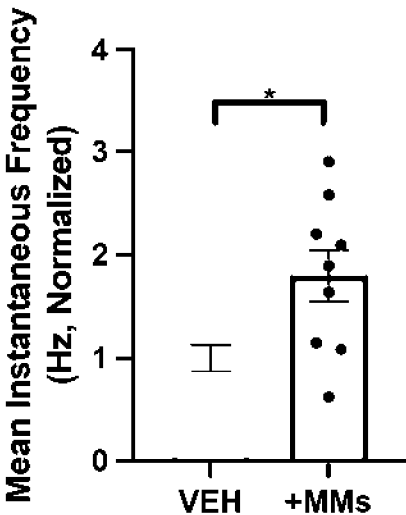


FIG. 12A

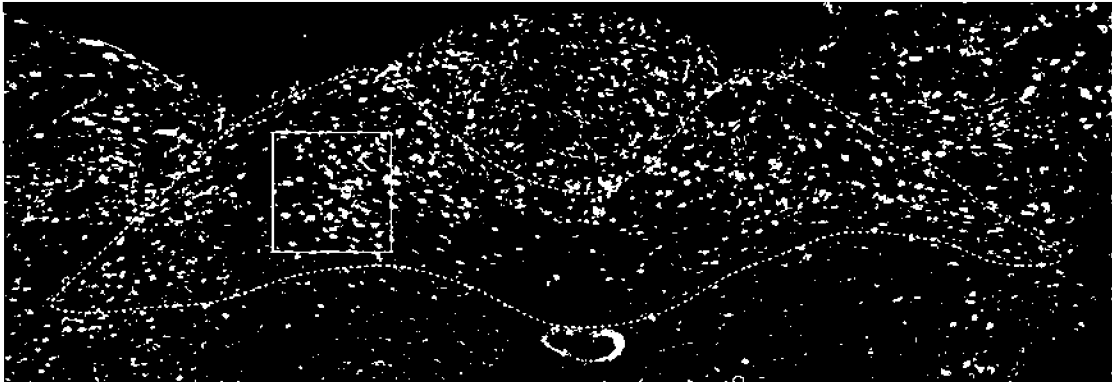


FIG. 12B

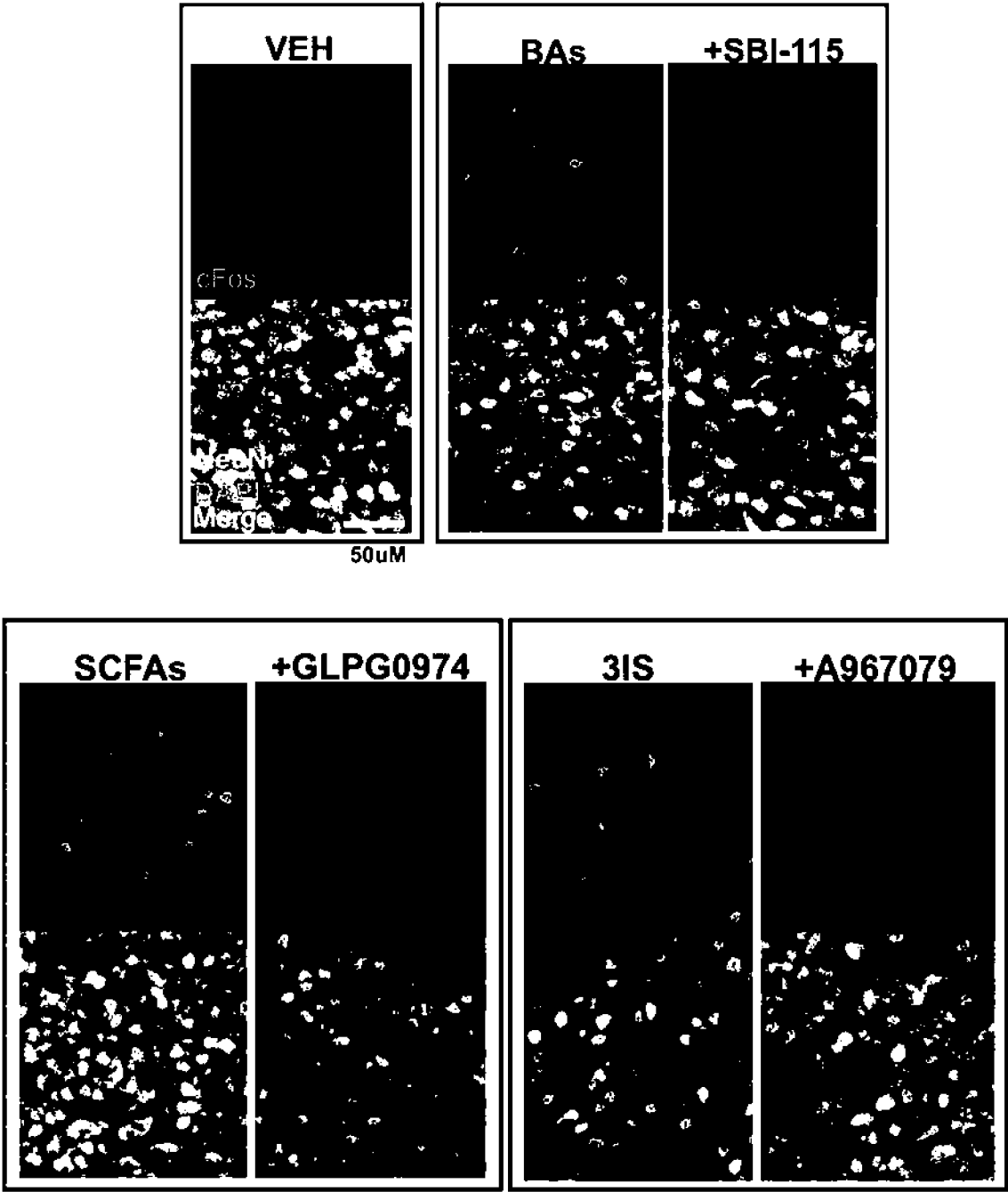


FIG. 12C

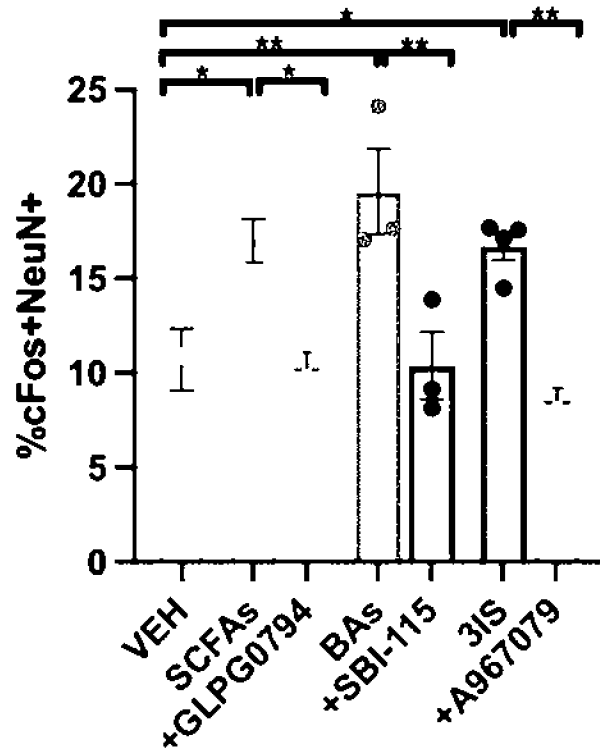


FIG. 13A

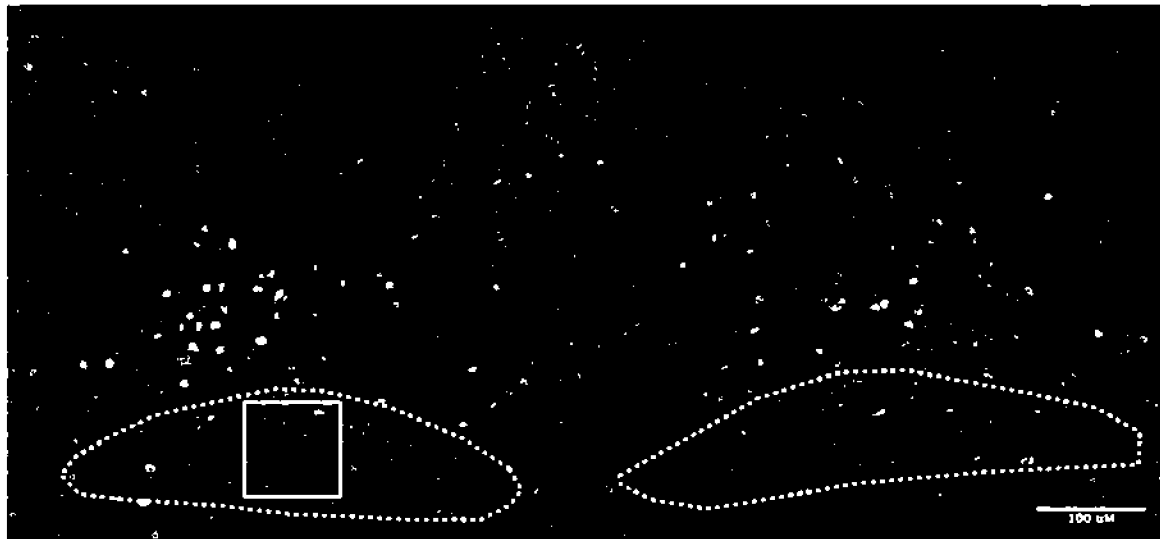


FIG. 13B

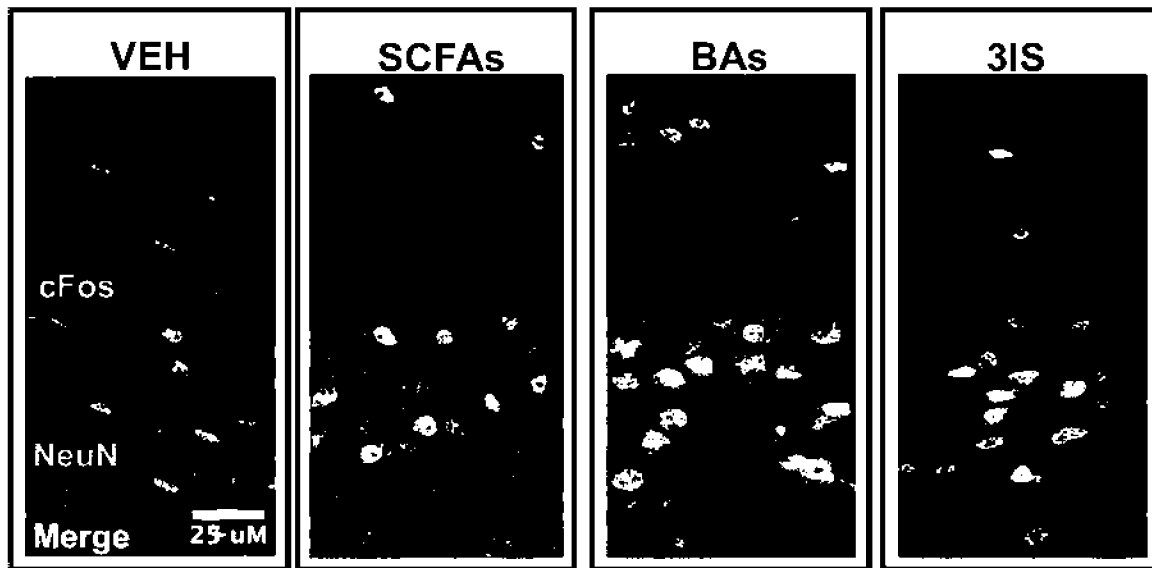


FIG. 13C

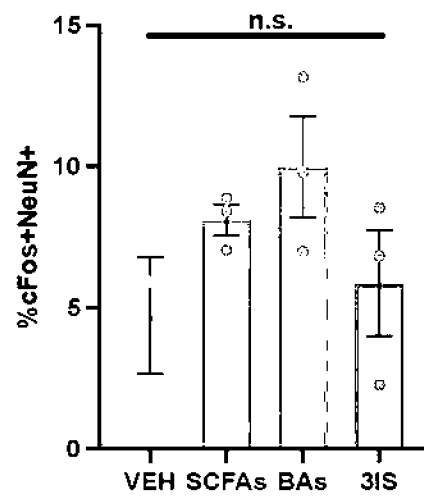


FIG. 13D

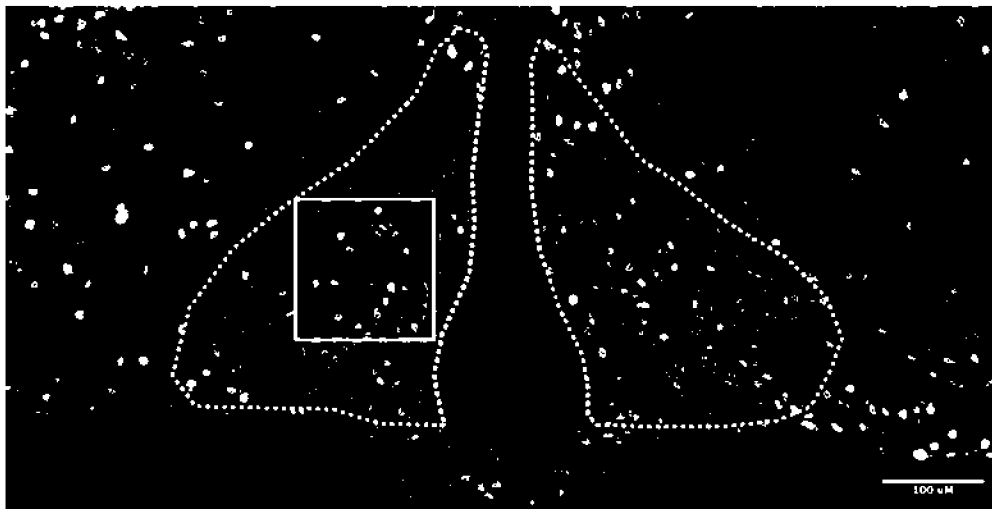


FIG. 13E

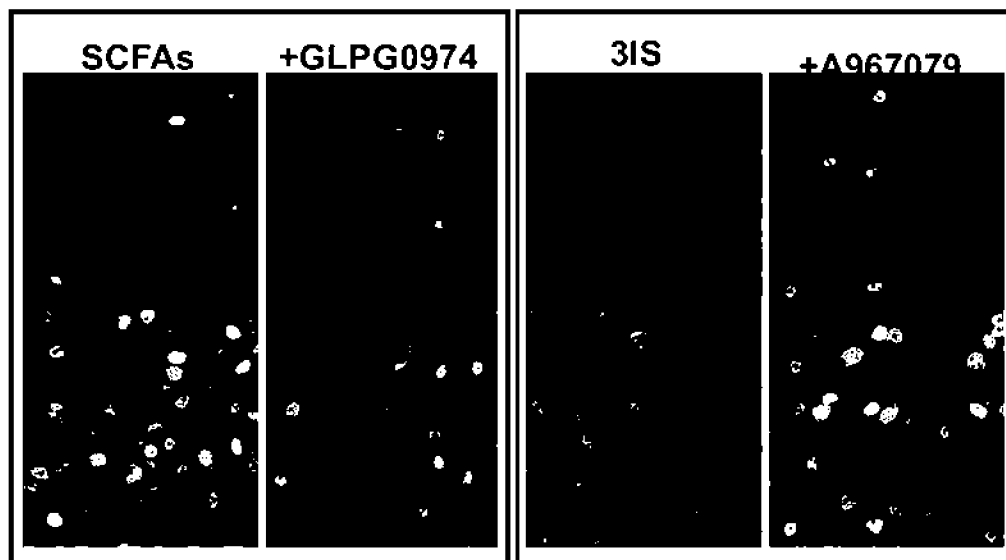
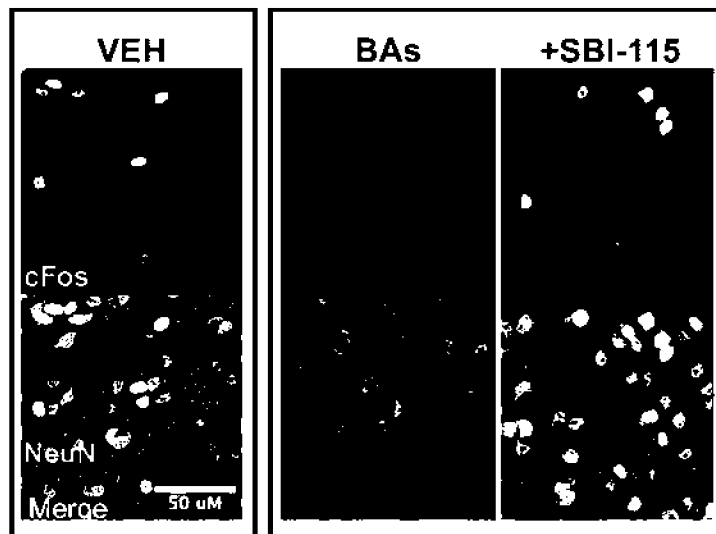


FIG. 13F

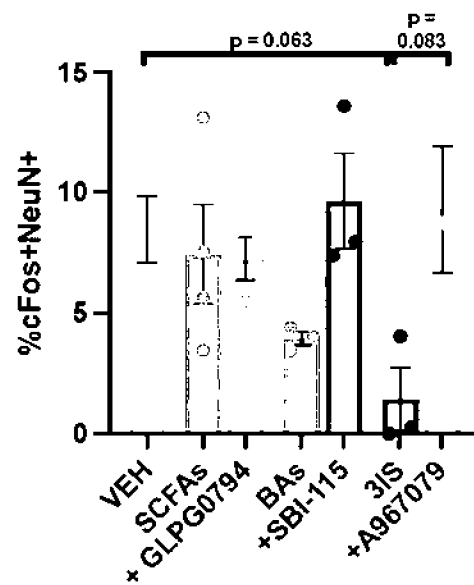


FIG. 14A

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Small Intestine				Small Intestine			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
thiamin monophosphate	0.90	0.47	2.39	2.57	0.0053	0.0000	0.0001	0.0000
taurodeoxycholate	0.00	0.09	287.75	287.75	0.0000	0.0000	0.0000	0.0000
glyco-beta-muricholate**	0.18	0.12	1.89	3.12	0.0000	0.0000	0.0079	0.0000
equol glucuronide	0.02	0.02	43.31	43.31	0.0001	0.0001	0.0000	0.0000
equol	0.10	0.13	20.49	16.38	0.0012	0.0000	0.0000	0.0000
hippurate	0.47	0.63	2.79	2.51	0.0010	0.0000	0.0000	0.0000
pyridoxamine	0.22	0.18	2.77	3.28	0.0000	0.0000	0.0006	0.0001
cyclo-salicylic acid	0.34	0.33	1.56	1.59	0.0000	0.0000	0.0000	0.0001
citrulline	0.00	0.00	362.52	362.52	0.0000	0.0000	0.0001	0.0001
equol sulfate	0.08	0.14	9.75	5.75	0.0000	0.0000	0.0000	0.0001
draminopimolate	0.00	0.00	1271.36	1700.29	0.0000	0.0000	0.0000	0.0001
7-ketodeoxycholate	0.30	0.40	4.70	3.47	0.0002	0.0002	0.0001	0.0002
N,N,N-trimethyl-5-aminovaleate	0.12	0.13	45.33	41.45	0.0000	0.0000	0.0001	0.0000
cinnamoylglycine	0.27	0.30	4.23	3.82	0.0000	0.0000	0.0000	0.0001
thiamin (Vitamin B1)	0.00	0.00	706.90	706.90	0.0000	0.0000	0.0000	0.0000
ursodeoxycholate	0.02	0.00	4.65	24.12	0.0000	0.0000	0.0001	0.0000
beta-muricholate	0.09	0.11	17.37	13.20	0.0000	0.0000	0.0000	0.0000
trimethylamine N-oxide	0.00	0.00	317.10	177.11	0.0000	0.0000	0.0000	0.0000
cholate	0.00	0.00	167.83	105.25	0.0000	0.0000	0.0000	0.0000
deoxycholate	0.31	0.17	5.62	10.36	0.0000	0.0000	0.0000	0.0000
cysteine-glutathione disulfide	0.02	0.02	33.80	38.09	0.0000	0.0000	0.0000	0.0000
pheophytin A	0.17	0.24	4.89	3.56	0.0001	0.0000	0.0000	0.0000
10-hydroxystearate	0.02	0.08	15.44	4.07	0.0000	0.0000	0.0000	0.0000
glycocholate	0.05	0.05	14.95	14.95	0.0000	0.0000	0.0000	0.0000
phenol sulfate	0.00	0.23	1060.86	3.68	0.0000	0.0000	0.0000	0.0000
taurohydrodeoxycholic acid	0.82	0.76	1.26	1.32	0.0000	0.0000	0.0000	0.0000
N6,N6-dimethyllysine	0.02	0.01	24.60	39.86	0.0000	0.0000	0.0000	0.0000
cholate sulfate	0.27	0.39	3.58	2.43	0.0001	0.0000	0.0000	0.0000
hexadecaphinganine (d16:0)*	0.03	0.06	28.48	15.29	0.0000	0.0000	0.0000	0.0000
malonylglycerol	0.04	0.07	6.25	3.64	0.0000	0.0000	0.0000	0.0000
lauro-phosphate	0.65	0.60	1.49	1.63	0.0000	0.0000	0.0000	0.0000
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	0.60	0.58	1.54	1.61	0.0000	0.0000	0.0000	0.0000
sphingomyelin (d18:0/20:0, d16:0/22:0)*	0.34	0.31	1.87	2.07	0.0000	0.0000	0.0000	0.0000
1-(1-enyl-oleoyl)-GPE (P-18:1)*	0.21	0.25	4.56	3.79	0.0000	0.0000	0.0000	0.0000
pheophorbide A	0.01	0.03	1.85	1.89	0.0000	0.0000	0.0000	0.0000
sphingomyelin (d18:0/18:0, d19:0/17:0)*	0.60	0.01	98.06	28.99	0.0000	0.0000	0.0000	0.0000
chenodeoxycholate	0.24	0.24	4.96	4.96	0.0000	0.0000	0.0000	0.0000
equol	0.83	0.03	23.43	23.43	0.0000	0.0000	0.0000	0.0000
catechol sulfate	0.59	0.63	2.15	2.01	0.0000	0.0000	0.0000	0.0000
orotidine	0.51	0.60	1.76	1.80	0.0000	0.0000	0.0000	0.0000
behenoyl dihydrophosphatidylcholine (d18:0/22:0)*	0.72	0.67	1.39	1.49	0.0000	0.0000	0.0000	0.0000
palmitoyl dihydrophosphatidylcholine (d16:0/16:0)*	0.69	0.69	3.59	3.59	0.0000	0.0000	0.0000	0.0000
3-indoxyl sulfate	0.79	0.74	1.32	1.39	0.0000	0.0000	0.0000	0.0000
cytidine 5'-monophosphate (5'-CMP)	0.50	0.67	1.99	1.48	0.0000	0.0000	0.0000	0.0000
glycerol 3-phosphate	0.36	0.60	7.29	5.19	0.0000	0.0000	0.0000	0.0000
5-aminovaleate	0.60	0.58	1.90	1.67	0.0000	0.0000	0.0000	0.0000
N-acetyl-aspartate	0.62	0.02	17.62	17.62	0.0000	0.0000	0.0000	0.0000
7-ketolithocholate	0.62	0.02	3.40	3.40	0.0000	0.0000	0.0000	0.0000
hyocholate	0.66	0.42	17.92	3.27	0.0000	0.0000	0.0000	0.0000

FIG. 14B

	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Small Intestine				Small Intestine			
	GF	ABX	CONV	CONV	GF	ABX	CONV	CONV
	SPF	SPF	GF	ABX	SPF	SPF	GF	ABX
	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value
1-palmitoyl-galactosylglycerol (16:0)	9.75	12.02	0.13	0.10	0.0000	0.0001	0.0000	0.0000
hydroxymethylpyrimidine	8.55	7.59	0.07	0.08	0.0026	0.0011	0.0015	0.0000
N-acetylpyrrolidine	1.62	2.08	0.70	0.55	0.0069	0.0005	0.0213	0.0000
sphingomyelin (d18:1-14:0-d18:1-16:0)	2.36	10.69	0.44	0.10	0.0421	0.0000	0.0450	0.0000
pyrrolidine	2.08	4.08	0.43	0.22	0.0070	0.0000	0.0069	0.0000
mononucleotides	2.48	2.28	0.31	0.34	0.0002	0.0010	0.0004	0.0005
N2-acetyllysine	6.50	5.27	0.28	0.32	0.0000	0.0000	0.0004	0.0005
N6-carboxymethyllysine	2.00	3.27	0.41	0.25	0.0144	0.0013	0.0054	0.0005
homocysteine	2.54	3.26	0.35	0.27	0.0017	0.0011	0.0012	0.0007
6-triisobutyl-guanosine	1.58	1.51	0.89	0.71	0.0008	0.0005	0.0022	0.0010
sphingomyelin (d18:2-16:0-d16:1-16:1)	1.44	1.55	0.58	0.52	0.0202	0.0044	0.0000	0.0010
2-oxodipyrromethane	2.00	2.56	0.57	0.46	0.0029	0.0000	0.0176	0.0011
sphingomyelin (d17:1-16:0-d16:1-16:0-d16:1-17:0)	2.60	3.06	0.44	0.38	0.0007	0.0004	0.0028	0.0014
triisobutyl-GPS (18:2)	4.15	3.19	0.21	0.28	0.0054	0.0044	0.0000	0.0015
valine-lysine-glycine	1.47	2.26	0.57	0.37	0.0070	0.0011	0.0224	0.0000
Glutathionylhomocysteine-SAc	7.35	14.55	0.14	0.07	0.0053	0.0041	0.0053	0.0041
5-hydroxyproline acid	1.59	1.90	0.67	0.58	0.0137	0.0031	0.0229	0.0050
arabonate-xylofuranose	1.67	1.44	0.60	0.70	0.0053	0.0042	0.0058	0.0052
succinylserine (d4-DC)	2.74	6.49	0.34	0.14	0.0128	0.0075	0.0075	0.0063
3-hydroxyproline sulfate	2.25	2.41	0.42	0.39	0.0135	0.0105	0.0107	0.0062
1-palmitoyl-GP (16:0)	2.33	1.73	0.48	0.65	0.0007	0.0048	0.0000	0.0162
glycosyl-N-palmitoyl-sphingosine (d18:1-16:0)	2.18	2.48	0.65	0.57	0.0005	0.0002	0.0054	0.0109
N-palmitoyl-heptacosasphingosine (d17:1-16:0)	1.86	1.70	0.48	0.52	0.0022	0.0061	0.0077	0.0131
N-acetylhistidine	2.22	2.88	0.45	0.35	0.0283	0.0102	0.0117	0.0135
N,N,N-trimethylalanineproline-lysine (15MAP)	3.46	3.53	0.27	0.27	0.0280	0.0246	0.0191	0.0168
d-glucosyl-galactose	2.27	1.68	0.38	0.40	0.0448	0.0406	0.0199	0.0253
alpha-ketoglutarate	2.13	1.69	0.47	0.59	0.0012	0.0290	0.0012	0.0260
galactosylglycerol	2.85	3.03	0.35	0.33	0.0360	0.0301	0.0375	0.0324
glutamate	2.86	1.67	0.35	0.60	0.0327	0.0296	0.0324	0.0383
pyruvate	2.15	2.01	0.47	0.51	0.0009	0.0369	0.0017	0.0417

FIG. 15A

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
N-methylproline	0.00	0.00	579.27	400.25	0.0000	0.0000	0.0000	0.0000
pheophytin A	0.05	0.02	16.38	35.26	0.0000	0.0000	0.0000	0.0000
N-acetylhistamine	0.00	0.00	200.13	154.27	0.0000	0.0000	0.0000	0.0000
beta-muricholate	0.00	0.00	1285.61	8677.90	0.0000	0.0000	0.0000	0.0000
doxycholate	0.00	0.00	2997.53	3378.20	0.0000	0.0000	0.0000	0.0000
hydroxymethylmucine	0.12	0.15	14.35	11.47	0.0009	0.0019	0.0000	0.0000
uracil	0.02	0.01	64.23	75.43	0.0000	0.0000	0.0000	0.0000
Butyrate: isobutyrate (4:0)	0.01	0.00	136.40	559.40	0.0001	0.0000	0.0001	0.0000
isobutyrate	0.01	0.00	184.17	261.31	0.0000	0.0000	0.0000	0.0000
doxycycline	0.01	0.02	48.09	30.56	0.0000	0.0000	0.0000	0.0000
2-oxo-3-oxoacetate	0.06	0.05	28.53	35.06	0.0000	0.0000	0.0000	0.0000
thiamin (Vitamin B1)	0.11	0.04	8.69	24.39	0.0000	0.0000	0.0000	0.0000
sialic acid	0.05	0.10	19.88	10.99	0.0001	0.0000	0.0000	0.0000
sialic acid	0.06	0.06	8.93	9.66	0.0000	0.0000	0.0000	0.0000
pyoxanthine	0.03	0.02	40.36	56.62	0.0000	0.0000	0.0000	0.0000
3-methyl-2-oxovalerate	0.00	0.00	458.87	383.84	0.0000	0.0000	0.0000	0.0000
3-methyl-2-oxobutyrate	0.01	0.01	127.06	104.11	0.0000	0.0000	0.0000	0.0000
5-(2-hydroxyethyl)-4-methylthiazole	0.05	0.04	28.21	36.72	0.0000	0.0000	0.0000	0.0000
N-acetylglutamate	0.01	0.01	113.32	64.75	0.0000	0.0000	0.0000	0.0000
4-methyl-2-oxopentanoate	0.00	0.01	400.36	247.73	0.0000	0.0000	0.0000	0.0000
3-hydroxyisovalerate	0.05	0.04	25.32	31.57	0.0003	0.0001	0.0000	0.0000
N,N,N-trimethyl-5-aminovalerate	0.01	0.02	95.38	80.76	0.0000	0.0000	0.0000	0.0000
2'-deoxyuridine	0.20	0.06	3.58	11.11	0.0000	0.0000	0.0000	0.0000
hexadecatrienoic acid (16:3n3)	0.55	0.24	2.43	5.44	0.0009	0.0000	0.0002	0.0000
ribose	0.11	0.04	11.14	28.71	0.0000	0.0000	0.0000	0.0000
octadecanedioate (C18:4-DC)	0.16	0.05	3.59	10.59	0.0000	0.0000	0.0000	0.0000
isocaproate	0.02	0.02	42.64	30.38	0.0000	0.0000	0.0000	0.0000
sulfate*	0.18	0.12	5.44	8.13	0.0000	0.0000	0.0000	0.0000
pyridoxal	0.04	0.02	21.50	45.06	0.0001	0.0000	0.0001	0.0000
hexadecanedioate (C16:2n3)	0.35	0.13	2.87	7.65	0.0067	0.0001	0.0001	0.0000
glycyl-L-histidine	0.04	0.10	17.37	7.13	0.0000	0.0000	0.0000	0.0000
phenyl-L-histidine	0.07	0.07	5.83	6.63	0.0004	0.0000	0.0000	0.0000
isovalerate (5:0)	0.01	0.00	136.89	351.02	0.0000	0.0000	0.0000	0.0000
valerate (5:0)	0.01	0.00	151.89	328.54	0.0000	0.0000	0.0000	0.0000
2-hydroxyglutamate	0.03	0.07	26.76	12.51	0.0000	0.0000	0.0000	0.0000
lysine	0.19	0.17	13.01	14.72	0.0000	0.0000	0.0000	0.0000
N-methylisocaproate	0.06	0.11	19.28	9.90	0.0000	0.0000	0.0000	0.0000
succinate	0.02	0.04	50.81	25.53	0.0000	0.0000	0.0000	0.0000
5,6-dihydrocandine	0.13	0.08	9.15	14.25	0.0000	0.0000	0.0000	0.0000
1-palmitoyl-galactosylglycerol (16:0)*	0.01	0.01	114.81	119.37	0.0000	0.0000	0.0000	0.0000
anthranilate	0.54	0.28	1.95	3.77	0.0117	0.0001	0.0000	0.0000
glycerolphosphoglycerol	0.11	0.12	6.58	5.71	0.0000	0.0000	0.0000	0.0000
oleic acid (18:1n3)	0.42	0.21	3.09	6.31	0.0071	0.0001	0.0000	0.0000
4-acetamidobenzoate	0.17	0.14	7.04	8.56	0.0000	0.0000	0.0000	0.0000
linoleic acid	0.00	0.00	139.80	139.80	0.0000	0.0000	0.0000	0.0000
phenanthrene	0.03	0.03	37.33	31.27	0.0000	0.0000	0.0000	0.0000
2-methyl-3-oxohexanoate	0.11	0.14	12.26	9.80	0.0000	0.0000	0.0000	0.0000
fumarate	0.01	0.02	191.96	48.95	0.0000	0.0000	0.0000	0.0000
1-oalmitoyl-GPG (16:0)*	0.02	0.02	58.57	76.70	0.0000	0.0000	0.0000	0.0000
phenylpyruvate	0.03	0.04	59.53	43.11	0.0000	0.0000	0.0000	0.0000
pyridoxamine	0.03	0.03	25.46	23.15	0.0000	0.0000	0.0000	0.0000
hexadecanedioate (C16:2-DC)	0.26	0.16	5.32	9.02	0.0000	0.0000	0.0000	0.0000
sebacate (C10:2-DC)	0.14	0.09	9.24	14.24	0.0000	0.0000	0.0000	0.0000
2-oalmitoyl-galactosylglycerol (16:0)*	0.02	0.02	88.36	88.36	0.0000	0.0000	0.0000	0.0000
3-hydroxyisovalerate	0.27	0.22	5.34	6.55	0.0004	0.0001	0.0000	0.0000
3-ketosphingamine	0.07	0.07	13.13	13.11	0.0000	0.0000	0.0000	0.0000
*2-ketosphingamine	0.01	0.01	101.47	101.47	0.0000	0.0000	0.0000	0.0000
3-hydroxysebacate	0.29	0.34	3.67	3.07	0.0000	0.0000	0.0000	0.0000
2-hydroxysebacate	0.06	0.32	1.97	4.03	0.0117	0.0000	0.0011	0.0000
2,6-dihydroxybenzoic acid	0.01	0.02	148.09	76.05	0.0000	0.0000	0.0000	0.0000
3-phenylpropionate:hydroxymethylol	0.01	0.01	601.56	601.56	0.0228	0.0228	0.0000	0.0000
cytidine 5'-monophosphate (5'-CMP)	0.01	0.04	64.06	12.84	0.0000	0.0000	0.0000	0.0000

FIG. 15B

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
2-hydroxyheptanoate ⁺	0.22	0.18	21.55	25.88	0.0001	0.0000	0.0000	0.0000
4-hydroxyphenyl pyruvate	0.02	0.04	57.54	35.96	0.0003	0.0000	0.0000	0.0000
glycerol 3-phosphate	0.07	0.09	7.31	5.69	0.0003	0.0000	0.0000	0.0000
methylsuccinate	0.04	0.05	18.58	15.59	0.0003	0.0000	0.0000	0.0000
thiamin monophosphate	0.05	0.05	14.64	14.64	0.0003	0.0000	0.0000	0.0000
2-isobutyrate	0.00	0.00	299.54	299.54	0.0003	0.0000	0.0000	0.0000
alpha-ketoglutarate	0.02	0.04	62.13	27.10	0.0003	0.0000	0.0000	0.0000
valylglycine	0.03	0.04	28.43	23.76	0.0003	0.0000	0.0000	0.0000
coconanedioate :C12:0C:	0.11	0.09	16.82	22.06	0.0003	0.0000	0.0000	0.0000
malol	0.04	0.05	49.95	48.40	0.0003	0.0000	0.0000	0.0000
ethylmalonate	0.08	0.08	16.08	20.83	0.0003	0.0000	0.0000	0.0000
picric acid	0.11	0.17	9.52	6.37	0.0003	0.0000	0.0000	0.0000
glucose	0.04	0.05	26.19	20.64	0.0003	0.0000	0.0000	0.0000
heptacosapentaenoate (21:5n3)	0.26	0.09	3.83	11.38	0.0007	0.0002	0.0021	0.0000
pantothenate	0.03	0.02	36.51	52.19	0.0003	0.0000	0.0000	0.0000
indoleacetate	0.13	0.11	9.20	10.36	0.0003	0.0000	0.0000	0.0000
methionine	0.10	0.19	8.74	4.48	0.0003	0.0000	0.0000	0.0000
16-hydroxypalmitate	0.29	0.18	5.23	8.18	0.0002	0.0000	0.0000	0.0000
N,N-dimethylamine	0.01	0.01	71.85	95.03	0.0003	0.0000	0.0000	0.0000
1-palmitoyl-2:1-trioleoyl-galactosyl-glycerol (18:0+18:2)	0.18	0.05	10.25	36.35	0.0001	0.0000	0.0000	0.0000
1-stearoyl-GPA (18:0)	0.16	0.15	20.20	21.18	0.0001	0.0000	0.0000	0.0000
palmitoleate (18:1n7)	0.16	0.09	4.51	8.04	0.0007	0.0012	0.0000	0.0000
N-acetyl-muramate	0.00	0.01	158.43	137.77	0.0003	0.0000	0.0000	0.0000
hexadecanedioate :C16:1+DC:	0.24	0.24	4.76	4.76	0.0003	0.0000	0.0000	0.0000
linoleate	0.27	0.28	4.45	4.29	0.0002	0.0001	0.0000	0.0000
10-heptadecenoate (17:1n7)	0.17	0.11	5.06	7.52	0.0005	0.0001	0.0000	0.0000
diaminopimelate	0.02	0.02	60.51	60.51	0.0002	0.0000	0.0000	0.0000
1-palmitoyl-2-oleoyl-GPG (16:0+18:1)	0.01	0.03	68.12	31.02	0.0005	0.0000	0.0000	0.0000
2,3-dihydroxyvalerate	0.02	0.01	56.75	79.55	0.0003	0.0000	0.0000	0.0000
pyruvate (14:0)	0.25	0.15	3.75	6.17	0.0001	0.0005	0.0000	0.0000
coconanedioate :C12:1+DC:	0.22	0.16	6.13	8.60	0.0003	0.0002	0.0001	0.0000
D-bulose xylulose	0.08	0.08	10.00	9.55	0.0003	0.0000	0.0000	0.0000
3-hydroxystearate ⁺	0.22	0.23	8.43	7.89	0.0003	0.0000	0.0000	0.0000
equol	0.01	0.01	178.61	178.61	0.0003	0.0000	0.0000	0.0000
docosahexaenoate (DHA 22:6n3)	0.36	0.16	3.01	6.72	0.0007	0.0014	0.0001	0.0000
N-acetylglucosamine N-acetyl galactosamine glycerol	0.12	0.07	8.92	15.25	0.0003	0.0000	0.0001	0.0000
	0.41	0.17	1.70	4.03	0.0163	0.0004	0.0008	0.0000
N-oleoylphenylalanine	0.18	0.15	7.43	7.91	0.0001	0.0000	0.0000	0.0000
2,4,6-trihydroxybenzoate	0.45	0.43	9.04	9.47	0.0009	0.0021	0.0000	0.0000
2,4-dichlorobenzophenol	0.41	0.31	1.78	2.35	0.0008	0.0004	0.0001	0.0000
pyruvate	0.19	0.28	7.09	4.77	0.0003	0.0000	0.0000	0.0000
undecanedioate :C11:0C:	0.13	0.10	10.55	13.98	0.0003	0.0000	0.0000	0.0000
malonate (17:0)	0.26	0.17	4.34	6.60	0.0003	0.0000	0.0000	0.0000
valylglutamine	0.04	0.04	14.12	15.35	0.0003	0.0000	0.0000	0.0000
glycylsarcosine	0.07	0.13	11.23	6.22	0.0003	0.0000	0.0000	0.0000
(14 or 16-methyl palmitate (a17:0 or 17:0)	0.27	0.21	2.87	3.67	0.0026	0.0012	0.0001	0.0000
chloride phosphate	0.01	0.12	132.53	15.18	0.0025	0.0163	0.0000	0.0000
eicosapentaenoate (EPA 20:5n3)	0.56	0.27	2.77	5.69	0.0198	0.0001	0.0001	0.0000
3-hydroxy-3-methylglutarate	0.09	0.12	8.92	6.59	0.0003	0.0000	0.0000	0.0000
cholate	0.01	0.01	504.22	444.80	0.0003	0.0000	0.0000	0.0000
3-formyl-nucle	0.14	0.15	8.80	8.80	0.0003	0.0000	0.0000	0.0000
7-ketodeoxycholate	0.00	0.00	1302.35	1302.35	0.0003	0.0000	0.0000	0.0000
hexadecaphinganine (d16:0) ⁺	0.05	0.05	13.34	14.58	0.0003	0.0000	0.0000	0.0000
1-stearoyl-GPG (18:0)	0.01	0.01	96.55	107.02	0.0003	0.0000	0.0000	0.0000
xanthine	0.62	0.34	1.88	3.40	0.0001	0.0000	0.0000	0.0000
peppidine	0.05	0.04	18.77	24.88	0.0003	0.0000	0.0000	0.0000
thymine	0.01	0.01	124.09	124.09	0.0003	0.0000	0.0000	0.0000
xanthuronate	0.16	0.18	13.17	11.47	0.0003	0.0001	0.0000	0.0000
1-palmitoyl-2-galactosyl-glycerol (16:0)	0.38	0.07	3.75	22.46	0.0022	0.0000	0.0004	0.0000
glutarate :C5:0C:	0.02	0.02	21.45	22.51	0.0001	0.0000	0.0000	0.0000
glutamate	0.25	0.36	3.70	2.55	0.0003	0.0000	0.0000	0.0000

FIG. 15C

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
1-oleoyl-GPA (18:1)	0.04	0.09	60.06	24.34	0.0014	0.0036	0.0001	0.0009
hydrocholate	0.03	0.03	35.77	35.77	0.0003	0.0006	0.0000	0.0006
oleoyl ethanolamide	0.16	0.08	7.37	13.31	0.0003	0.0000	0.0000	0.0003
tetradecanoate (C14:0)	0.24	0.18	5.78	7.56	0.0013	0.0000	0.0014	0.0006
9,10-D HOME	0.47	0.26	3.63	6.63	0.0004	0.0003	0.0001	0.0003
mevalonate	0.11	0.11	29.63	29.02	0.0003	0.0003	0.0000	0.0003
leucate	0.08	0.07	9.58	12.14	0.0003	0.0003	0.0001	0.0003
caproate (C6:0)	0.21	0.19	4.48	4.94	0.0003	0.0003	0.0000	0.0003
N-acetyl-ketolavone	0.02	0.03	57.61	28.39	0.0003	0.0003	0.0000	0.0003
docosapentaenoate (n3 DPA, 22:5n3)	0.10	0.05	9.30	20.77	0.0003	0.0003	0.0001	0.0000
3-hydroxyoctanoate	0.35	0.40	3.29	2.93	0.0003	0.0003	0.0001	0.0000
glycerate	0.24	0.20	4.32	5.27	0.0003	0.0003	0.0000	0.0000
3-hydroxy-margaricoyl-glycerol	0.08	0.08	14.31	14.31	0.0003	0.0003	0.0003	0.0000
3-hydroxyhexanoate	0.16	0.16	11.08	11.08	0.0003	0.0003	0.0003	0.0000
3-hydroxy-succate	0.26	0.23	4.86	5.42	0.0003	0.0003	0.0003	0.0000
laurate (C12:0)	0.53	0.50	1.64	1.74	0.0006	0.0003	0.0003	0.0000
palmitate (C16:0)	0.41	0.20	2.24	4.54	0.0286	0.0011	0.0020	0.0000
N-formylmethionine	0.11	0.08	7.31	10.33	0.0003	0.0003	0.0003	0.0000
12,13-D HOME	0.44	0.23	3.05	5.97	0.0003	0.0003	0.0002	0.0003
homocysteine	0.03	0.03	43.96	43.96	0.0003	0.0003	0.0000	0.0003
gentisate	0.01	0.01	143.95	143.95	0.0003	0.0003	0.0000	0.0003
suberate (C8:0)	0.37	0.24	3.39	5.29	0.0003	0.0003	0.0000	0.0003
d-homo-glycerate (20:2n6)	0.15	0.10	5.08	7.43	0.0003	0.0003	0.0001	0.0003
6-methylvaleric acid	0.29	0.26	3.18	3.47	0.0003	0.0003	0.0000	0.0000
1-palmitoyl-GPA (18:0)	0.11	0.16	43.36	29.64	0.0001	0.0001	0.0001	0.0000
3-hydroxyglyoxylic acid	0.44	0.34	3.27	4.21	0.0002	0.0003	0.0001	0.0000
8-pharmylinoxyisovalerate	0.11	0.17	59.98	39.20	0.0003	0.0017	0.0000	0.0000
tetradecanoate (C14:2)	0.39	0.19	2.22	4.61	0.0003	0.0003	0.0022	0.0000
1-methyladenine	0.08	0.08	13.48	12.37	0.0003	0.0003	0.0003	0.0000
2-hydroxybutyrate/2-hydroxyisobutyrate	0.15	0.17	8.25	7.35	0.0001	0.0001	0.0003	0.0000
isoleucine	0.27	0.44	3.60	2.22	0.0003	0.0003	0.0003	0.0000
panthoic	0.19	0.19	11.93	11.93	0.0003	0.0003	0.0003	0.0000
aspartate	0.19	0.36	4.72	2.48	0.0003	0.0003	0.0003	0.0000
methionine sulfoxide	0.41	0.33	2.46	3.02	0.0005	0.0001	0.0003	0.0003
stearoyl ethanolamide	0.18	0.06	5.01	11.06	0.0001	0.0003	0.0010	0.0003
4-hydroxyphenamate	0.13	0.16	6.12	4.29	0.0003	0.0003	0.0001	0.0003
6-hydroxybenzaldehyde	0.19	0.26	6.03	4.45	0.0003	0.0002	0.0000	0.0003
1-palmitoyl-2-oleoyl-GPE (16:0/18:2)	0.38	0.36	3.52	3.78	0.0002	0.0002	0.0000	0.0003
2-hydroxy-4-methyl-3-oxobutanoic acid	0.08	0.08	87.77	87.77	0.0003	0.0003	0.0000	0.0003
N,N-dimethylalanine	0.03	0.03	80.86	80.86	0.0003	0.0003	0.0000	0.0000
1-pentadecanoyl-glycerol (15:0)	0.15	0.12	7.87	9.83	0.0003	0.0003	0.0001	0.0000
β-alanine	0.07	0.03	12.52	27.50	0.0003	0.0003	0.0000	0.0000
azelate (C8:0)	0.58	0.23	2.49	6.39	0.0489	0.0002	0.0042	0.0003
retinol (Vitamin A)	0.22	0.16	6.47	7.79	0.0015	0.0018	0.0000	0.0003
aspartate (24:0n3)	0.14	0.08	4.68	7.38	0.0027	0.0017	0.0002	0.0003
4-methylthio-2-oxobutanoate	0.02	0.02	58.93	58.93	0.0000	0.0003	0.0000	0.0003
arachidonate (20:4n6)	0.51	0.23	2.62	5.72	0.0228	0.0003	0.0001	0.0003
oleate xacetonate (18:1)	0.27	0.12	2.94	6.32	0.0062	0.0004	0.0006	0.0003
docosadecanoate (22:0n6)	0.28	0.13	3.39	7.40	0.0007	0.0003	0.0018	0.0001
10-hydroxystearate	0.02	0.02	57.76	57.76	0.0003	0.0003	0.0001	0.0001
docosapentaenoate (n3 DPA, 22:5n3)	0.28	0.13	3.38	7.28	0.0271	0.0017	0.0003	0.0001
methionine sulfone	0.15	0.11	8.35	11.99	0.0003	0.0001	0.0000	0.0001
3-aminobutyrate	0.14	0.29	18.13	8.95	0.0003	0.0011	0.0000	0.0001
glyoxyalate	0.08	0.19	9.30	3.63	0.0003	0.0003	0.0000	0.0001
lysine	0.22	0.32	3.98	2.76	0.0003	0.0003	0.0000	0.0001
N-acetyl-acetylamine	0.18	0.27	8.02	5.19	0.0003	0.0003	0.0000	0.0001
cis-urocanate	0.17	0.19	6.48	5.91	0.0003	0.0003	0.0000	0.0001
N1,N2-diacyl spermine	0.15	0.15	24.22	24.22	0.0061	0.0061	0.0001	0.0001
3-dehydrocholate	0.08	0.08	33.96	33.96	0.0003	0.0003	0.0001	0.0001
5-aminovalerate	0.03	0.05	97.28	47.89	0.0000	0.0003	0.0001	0.0001
2-hydroxy-3-methyl valerate	0.23	0.23	61.90	61.90	0.0001	0.0001	0.0001	0.0001
6-beta-hydroxythiothioate	0.06	0.06	12.85	12.85	0.0001	0.0001	0.0001	0.0001

FIG. 15D

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
phenylacetate	0.01	0.01	13.77	13.77	0.0000	0.0000	0.0001	0.0001
N-carbamoyl aspartate	0.05	0.05	10.33	10.33	0.0000	0.0000	0.0001	0.0001
3-methylglutamate	0.25	0.25	5.13	5.13	0.0008	0.0008	0.0001	0.0001
1-methyl-4-micazoleacetate	0.19	0.26	3.38	2.49	0.0001	0.0000	0.0000	0.0001
alanine	0.19	0.31	4.78	2.98	0.0000	0.0002	0.0000	0.0001
lysine	0.15	0.28	4.65	2.47	0.0000	0.0001	0.0000	0.0001
1-Indo-3,4-GPA (16:2)	0.16	0.39	14.13	6.00	0.0001	0.0000	0.0000	0.0001
valate	0.07	0.11	9.76	6.26	0.0000	0.0000	0.0001	0.0001
hydroxyglycine	0.04	0.06	23.54	13.16	0.0001	0.0002	0.0001	0.0001
gamma-glutamine	0.06	0.07	8.01	6.90	0.0002	0.0003	0.0001	0.0001
adrenate (22:4n6)	0.21	0.14	3.76	5.74	0.0005	0.0004	0.0001	0.0001
N-formyl anthranilic acid	0.35	0.35	2.26	2.26	0.0000	0.0000	0.0001	0.0001
Zinc-oxy-nosine	0.41	0.34	1.98	2.38	0.0000	0.0000	0.0002	0.0001
10-nonadecanoate (18:1n9)	0.21	0.15	3.36	4.77	0.0166	0.0045	0.0003	0.0001
palmitoyl ethanolamide	0.40	0.15	3.70	10.20	0.0000	0.0001	0.0000	0.0001
sphinganine	0.25	0.09	2.41	6.71	0.0001	0.0000	0.0194	0.0001
gamma-aminobutyrate	0.54	0.20	1.81	4.92	0.0100	0.0000	0.0000	0.0001
phenylalanine	0.23	0.39	4.03	2.30	0.0000	0.0000	0.0000	0.0000
taurine	0.01	0.04	18.37	6.74	0.0000	0.0000	0.0000	0.0000
crotonate	0.01	0.02	8.07	6.23	0.0123	0.0153	0.0001	0.0002
adenosine 5'-monophosphate (AMP)	0.03	0.05	17.90	11.55	0.0000	0.0001	0.0001	0.0002
glutathionyl-epinephrine	0.41	0.39	2.97	3.12	0.0000	0.0003	0.0001	0.0000
6-oxolithocholate	0.09	0.00	87.08	87.08	0.0000	0.0000	0.0002	0.0002
alpha-ketobutyrate	0.01	0.02	212.06	183.51	0.0000	0.0000	0.0002	0.0002
6-hydroxyoctanoate	0.46	0.39	2.46	2.85	0.0004	0.0002	0.0002	0.0002
myristate (14:1n6)	0.16	0.18	3.68	3.74	0.0004	0.0004	0.0002	0.0002
3,5-dihydroxyphenyl propionate	0.04	0.04	304.42	304.42	0.0001	0.0001	0.0002	0.0002
enterolactone	0.06	0.06	3.61	3.61	0.0000	0.0000	0.0000	0.0000
dimethylcholine	0.06	0.01	1.85	2.40	0.0100	0.0000	0.0000	0.0000
decanoic acid (10:0n0)	0.43	0.25	2.65	4.60	0.0000	0.0000	0.0000	0.0000
fructose	0.36	0.20	2.75	5.10	0.0000	0.0001	0.0000	0.0000
isohydroxycholesterol	0.01	0.01	34.14	34.14	0.0000	0.0000	0.0000	0.0000
flavin mononucleotide (FMN)	0.26	0.26	3.06	3.06	0.0001	0.0001	0.0000	0.0000
phenylacetate (PAA)	0.14	0.14	11.57	11.57	0.0000	0.0000	0.0000	0.0000
5-oxodecanoate (12:1n7)	0.16	0.12	4.61	5.91	0.0000	0.0004	0.0001	0.0000
phenethylamine	0.75	0.74	1.46	1.50	0.0000	0.0172	0.0000	0.0000
N-methyl GABA	0.09	0.21	7.27	3.20	0.0000	0.0000	0.0000	0.0000
alpha-hydroxy succinate	0.14	0.14	67.45	67.45	0.0000	0.0000	0.0000	0.0000
melatonin (5-HT)	0.41	0.41	2.28	2.28	0.0000	0.0000	0.0000	0.0000
epicardiolipid (18:1n7)	0.27	0.16	2.24	3.90	0.0000	0.0000	0.0000	0.0000
2,4-dihydroxyphenylpropionate	0.09	0.00	290.21	290.21	0.0000	0.0000	0.0000	0.0000
enterolactone	0.02	0.02	182.91	182.91	0.0000	0.0000	0.0000	0.0000
alpha-ketoglutarate	0.09	0.09	10.42	9.60	0.0000	0.0000	0.0000	0.0000
lysine	0.04	0.04	17.07	17.07	0.0000	0.0000	0.0000	0.0000
hydroxy-2-oxo	0.06	0.00	56.24	40.46	0.0000	0.0000	0.0000	0.0000
2-hydroxyphenylacetate	0.16	0.15	4.24	5.14	0.0000	0.0000	0.0000	0.0000
gamma-aminobutyrate	0.16	0.14	2.84	3.59	0.0000	0.0000	0.0000	0.0000
stearate (18:0)	0.46	0.34	2.28	3.08	0.0138	0.0000	0.0000	0.0000
5-methyluridine (methyluridine)	0.16	0.03	6.73	20.47	0.0000	0.0000	0.0000	0.0000
equol sulfate	0.05	0.03	33.31	58.25	0.0000	0.0000	0.0000	0.0000
4-hydroxyphenylacetate	0.01	0.01	4.65	4.65	0.0000	0.0000	0.0000	0.0000
phenylalanine	0.49	0.41	1.95	2.34	0.0000	0.0122	0.0000	0.0000
nonadecanoate (19:0)	0.34	0.22	2.71	4.33	0.0129	0.0000	0.0000	0.0000
3-hydroxydecanoate	0.32	0.38	3.27	2.71	0.0000	0.0000	0.0000	0.0000
1-palmitoylglycerol (16:0)	0.36	0.42	3.36	2.99	0.0183	0.0000	0.0000	0.0000
hydroxyphenylacetate	0.32	0.32	5.64	5.64	0.0152	0.0192	0.0000	0.0000
2-hydroxybutyrate	0.17	0.12	4.61	6.66	0.0000	0.0000	0.0000	0.0000

FIG. 15E

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
2-hydroxyarachidate ⁺	0.24	0.12	4.90	10.22	0.0000	0.0000	0.0004	0.0007
imidazole propionate	0.06	0.07	73.07	57.81	0.0000	0.0000	0.0006	0.0008
(3'-5'-nucleosyl)uridine ⁺	0.03	0.03	15.74	15.74	0.0000	0.0000	0.0006	0.0008
leucine	0.26	0.48	3.72	1.85	0.0000	0.0100	0.0000	0.0010
3-ureidopropionate	0.24	0.24	15.11	15.42	0.0000	0.0000	0.0002	0.0010
alpha-tocopherol	0.45	0.25	2.10	3.75	0.0057	0.0065	0.0168	0.0010
beta-alanine	0.26	0.42	3.74	2.34	0.0000	0.0000	0.0000	0.0011
N-acetylaspartate + NAA	0.03	0.04	9.38	6.51	0.0000	0.0000	0.0004	0.0011
6-acetyl spermidine ⁺	0.06	0.07	35.18	30.99	0.0000	0.0000	0.0000	0.0011
3'-4'-hydroxyphenylsuccinate	0.00	0.00	30.69	30.69	0.0115	0.0115	0.0011	0.0011
2-hydroxysebacate	0.24	0.12	5.82	11.03	0.0000	0.0000	0.0078	0.0012
2-hydroxyadipate	0.17	0.18	4.15	4.01	0.0000	0.0001	0.0011	0.0013
betamyoxyisovalerylcarbamidine	0.13	0.12	19.89	20.92	0.0000	0.0000	0.0017	0.0014
leucylglycine	0.16	0.21	3.63	2.79	0.0000	0.0001	0.0000	0.0015
cycloserine	0.06	0.06	53.45	38.21	0.0000	0.0000	0.0012	0.0017
arabinose	0.16	0.15	4.81	5.00	0.0001	0.0001	0.0017	0.0017
2-aminophospho	0.35	0.21	3.85	6.45	0.0008	0.0000	0.0072	0.0017
sucrose	0.20	0.42	4.99	2.34	0.0000	0.0014	0.0000	0.0018
3-sulfotl-valanine	0.18	0.21	3.71	3.22	0.0000	0.0000	0.0004	0.0020
(3'-5'-nucleosyl)uridine ⁺	0.03	0.03	12.03	12.03	0.0002	0.0002	0.0020	0.0020
2-hydroxygalactate	0.18	0.09	5.09	9.91	0.0000	0.0000	0.0128	0.0026
formiminoglutamate	0.03	0.07	23.30	9.23	0.0000	0.0001	0.0001	0.0021
1'-2'-palmitoyl-GPE (16:2, 16:0) ⁺	0.28	0.28	2.14	2.14	0.0001	0.0001	0.0021	0.0021
vanillate	0.52	0.58	3.10	2.78	0.0007	0.0046	0.0017	0.0022
citrulline	0.01	0.02	43.15	22.06	0.0000	0.0000	0.0010	0.0025
dehydrochondroate	0.04	0.04	5.78	5.78	0.0000	0.0000	0.0008	0.0028
2-deoxycytidine 5-monoisophosphate	0.00	0.03	50.57	7.42	0.0000	0.0002	0.0000	0.0030
3'-3'-hydroxyphenylpropionate	0.00	0.00	195.05	195.05	0.0000	0.0000	0.0002	0.0032
guanosine 2'-3'-cyclic monochosphate	0.02	0.02	83.35	83.35	0.0000	0.0000	0.0006	0.0036
cholate sulfate	0.03	0.03	243.84	234.13	0.0000	0.0000	0.0028	0.0038
dehydrophytylsphingosine ⁺	0.39	0.26	1.91	2.86	0.0005	0.0015	0.0430	0.0043
mead acid (20:3n9)	0.06	0.06	10.36	10.36	0.0000	0.0000	0.0048	0.0046
buprolol	0.21	0.21	4.10	4.10	0.0001	0.0001	0.0048	0.0045
branched chain 14:0 dicarboxylic acid ⁺	0.02	0.02	59.38	59.38	0.0000	0.0000	0.0045	0.0049
arachidic stearic amide (20:0) ⁺	0.15	0.15	5.20	5.20	0.0000	0.0000	0.0050	0.0050
5-hydroxyisobutyrate	0.47	0.47	2.00	2.00	0.0005	0.0005	0.0058	0.0056
(3'-5'-nucleosyl)uridine	0.06	0.06	6.30	6.30	0.0003	0.0003	0.0057	0.0057
alpha-methochate	0.04	0.04	32.78	32.78	0.0001	0.0001	0.0060	0.0060
N-methyl alanine	0.29	0.29	7.43	7.43	0.0003	0.0003	0.0063	0.0063
S ⁺ pyroglutamic carboxylate	0.41	0.58	2.62	1.83	0.0029	0.0097	0.0003	0.0064
(3'-5'-adenosyl)uridine	0.06	0.06	6.86	6.86	0.0001	0.0001	0.0066	0.0065
N ⁺ 2-hydroxyglutamicyl sphingosine (d18:1) (6:0)OH ⁺	0.10	0.06	3.32	3.85	0.0000	0.0000	0.0161	0.0069
2-oxo-3-oxoethyl-gluconate	0.40	0.45	2.10	1.77	0.0073	0.0020	0.0177	0.0071
(5S)-3-hydroxybutylcarbamidine	0.18	0.26	5.12	3.46	0.0027	0.0004	0.0031	0.0074
D-glucose	0.07	0.07	12.29	12.29	0.0002	0.0002	0.0081	0.0081
6-deoxy carnitine (C18:1)	0.63	0.46	2.65	3.63	0.0005	0.0007	0.0421	0.0093
tryptophan	0.29	0.53	3.13	1.72	0.0000	0.0025	0.0000	0.0084
beta-guanidinopropionate	0.30	0.22	1.75	2.33	0.0000	0.0001	0.0488	0.0084
betamyoxyisovalerate	0.23	0.25	4.94	4.45	0.0000	0.0000	0.0072	0.0086
alpha-tocopherol	0.46	0.61	1.73	1.32	0.0004	0.0006	0.0001	0.0089
inosinate + benucleoside	0.14	0.06	5.39	9.49	0.0000	0.0000	0.0365	0.0090
1'-3'-oxyl GPG (18:1) ⁺	0.26	0.26	2.32	2.32	0.0002	0.0002	0.0092	0.0092
1-methylhistamine	0.20	0.41	3.92	1.94	0.0000	0.0009	0.0002	0.0094
histidine	0.40	0.59	2.49	1.70	0.0000	0.0004	0.0000	0.0093
trans-ubiquinate	0.10	0.34	7.87	2.26	0.0000	0.0004	0.0000	0.0098
serosone	0.05	0.06	53.30	49.10	0.0007	0.0007	0.0098	0.0102
N-acetyl-L-threidodecylsphingosine (d17:1) (6:0) ⁺	0.22	0.26	2.80	2.22	0.0007	0.0016	0.0021	0.0105
N-acetyl-L-threonine	0.19	0.23	4.39	3.62	0.0000	0.0000	0.0076	0.0105
(3'-5'-guanosyl)uridine	0.06	0.06	9.18	9.18	0.0001	0.0001	0.0108	0.0108
(3'-5'-adenosyl)guanosine ⁺	0.04	0.04	14.42	14.42	0.0000	0.0000	0.0110	0.0110
1'-3'-propanediol	0.40	0.40	2.88	2.88	0.0087	0.0087	0.0116	0.0116
propanolol (Vitamin B2)	0.61	0.61	1.46	1.46	0.0011	0.0011	0.0119	0.0119

FIG. 15F

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
2-deoxyadenosine 5'-monophosphate	0.05	0.06	4.60	3.58	0.0001	0.0003	0.0063	0.0123
(3'-5'-adenyloctidine	0.09	0.09	6.03	6.03	0.0003	0.0003	0.0135	0.0135
guanosine 5'-monophosphate (5'-GMP)	0.11	0.11	3.08	3.08	0.0049	0.0049	0.0152	0.0152
gamma-glutamylglutamate	0.16	0.31	3.91	1.98	0.0160	0.0009	0.0000	0.0155
guanosine 2'-3'-cyclic monophosphate	0.03	0.03	101.76	101.76	0.0000	0.0000	0.0182	0.0182
(3'-5'-adenylyladenosine*	0.07	0.07	6.91	6.91	0.0000	0.0000	0.0188	0.0188
2-8-cyclinucleoside	0.00	0.00	1220.24	1220.24	0.0000	0.0000	0.0189	0.0189
glyco-beta-muricholate**	0.08	0.08	2.46	2.46	0.0468	0.0468	0.0185	0.0185
hydroxylate	0.09	0.09	1.58	1.58	0.0465	0.0465	0.0189	0.0189
N-sialoyl-sphingosine (d16:1-18:0)	0.37	0.28	1.91	2.46	0.0003	0.0050	0.0171	0.0202
(3'-5'-guanylyluridine	0.07	0.07	8.00	8.00	0.0000	0.0000	0.0208	0.0208
adenosine	0.20	0.23	4.26	3.60	0.0002	0.0009	0.0100	0.0274
kyurenine	0.05	0.02	1.41	1.48	0.0001	0.0083	0.0155	0.0229
2S-3R-2-hydroxybutyrate	0.33	0.08	3.27	1.90	0.0001	0.0029	0.0037	0.0231
taurodeoxycholate	0.06	0.06	8.13	8.13	0.0036	0.0036	0.0235	0.0235
carboxyethyl-GABA	0.04	0.04	1.24	1.23	0.0002	0.0005	0.0214	0.0242
glycine	0.46	0.08	2.20	1.54	0.0000	0.0151	0.0000	0.0262
adenine	0.32	0.30	2.34	2.48	0.0025	0.0021	0.0406	0.0016
3-oxohydroxycholelate	0.00	0.00	1.29	1.29	0.0083	0.0083	0.0335	0.0335
3b-hydroxy-5-cholesterol acid	0.06	0.06	2.10	2.10	0.0037	0.0037	0.0344	0.0344
serine	0.38	0.03	2.45	1.47	0.0000	0.0137	0.0000	0.0360
D-Tyrosine	0.06	0.06	1.68	1.59	0.0138	0.0090	0.0084	0.0378
4-hydroxybenzoate	0.00	0.03	11.13	10.69	0.0042	0.0064	0.0068	0.0386
(3'-5'-guanylyladenosine*	0.05	0.05	9.74	9.74	0.0004	0.0004	0.0409	0.0408
(3'-5'-guanylyladenosine	0.06	0.06	9.18	9.18	0.0003	0.0003	0.0412	0.0412
uridine 5'-monophosphate (UMP)	0.15	0.15	7.02	7.02	0.0151	0.0151	0.0421	0.0421
heptanoate (7:0)	0.03	0.46	1.73	2.02	0.0088	0.0057	0.0149	0.0440
(3'-5'-guanylylglutamate	0.04	0.04	13.27	13.27	0.0001	0.0001	0.0451	0.0451
1-palmitoyl-2-oleoyl-GPE (16:0-18:1)	0.10	0.31	4.49	1.51	0.0000	0.0005	0.0000	0.0461
cholesterol 3-one	0.22	0.37	2.21	1.27	0.0000	0.0000	0.0002	0.0468
(3'-5'-guanylylcytosine*	0.06	0.06	6.45	6.45	0.0003	0.0003	0.0497	0.0492

FIG. 15G

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
N5-succinyladenosine	11.35	12.63	0.10	0.09	0.0003	0.0000	0.0003	0.0000
galactinol	210.55	372.72	0.00	0.00	0.0003	0.0000	0.0003	0.0000
caixazin sulfate (2)	2102.76	3009.63	0.00	0.00	0.0003	0.0000	0.0003	0.0000
apigenin glucuronide (2)	68.55	89.13	0.01	0.01	0.0003	0.0000	0.0003	0.0000
N2-N2-dimethylguanosine	241.63	375.19	0.00	0.00	0.0003	0.0000	0.0003	0.0000
carbinol-7-glucuronide	23.29	51.87	0.04	0.02	0.0000	0.0000	0.0000	0.0000
β-galactosylglycerol	1448.51	846.51	0.00	0.00	0.0000	0.0000	0.0003	0.0000
gerstenin glucuronide*	25.51	77.90	0.04	0.01	0.0000	0.0000	0.0003	0.0000
flavone derivative C26H28O14 (2)	14.25	31.04	0.07	0.03	0.0003	0.0000	0.0003	0.0000
Flavone derivative C26H28O14 (1)	7.28	14.51	0.14	0.07	0.0003	0.0000	0.0003	0.0000
glycerol glucuronide (2)	43.95	140.16	0.02	0.01	0.0000	0.0000	0.0003	0.0000
gamma-glutamyl-L-lysine	22.01	20.93	0.03	0.03	0.0000	0.0000	0.0003	0.0000
flavone derivative C26H28O14 (5)	5.37	15.32	0.19	0.07	0.0000	0.0000	0.0003	0.0000
nicotinamide N-oxide	32.82	30.92	0.03	0.03	0.0000	0.0000	0.0003	0.0000
glutamate	170.19	196.28	0.00	0.00	0.0000	0.0000	0.0003	0.0000
1'-ketotetraose	84.27	40.89	0.01	0.02	0.0000	0.0000	0.0003	0.0000
doxorubicin-7-O-glucuronide	25.71	76.74	0.04	0.01	0.0000	0.0000	0.0003	0.0000
anthraquinone	65.87	97.11	0.02	0.01	0.0000	0.0000	0.0003	0.0000
transferrin*	150.40	263.52	0.00	0.00	0.0000	0.0000	0.0003	0.0000
verbascoside	45.59	53.43	0.02	0.02	0.0000	0.0000	0.0003	0.0000
ferulic acid-4-sulfate	35.41	38.02	0.03	0.03	0.0000	0.0000	0.0003	0.0000
glucuronate	27.79	19.19	0.04	0.06	0.0000	0.0000	0.0003	0.0000
fructofuranose	222.12	397.11	0.01	0.00	0.0000	0.0000	0.0003	0.0000
glycine sulfate (2)	23.40	44.36	0.04	0.02	0.0000	0.0000	0.0003	0.0000
glucuronate	19.98	39.81	0.02	0.01	0.0000	0.0000	0.0003	0.0000
mannitol sorbitol	28.42	37.15	0.04	0.03	0.0001	0.0001	0.0003	0.0000
5-hydroxyphenylacetyl glutamine	74.53	73.16	0.01	0.01	0.0000	0.0000	0.0003	0.0000
4-hydroxyphenylacetate sulfate	43.08	37.69	0.02	0.03	0.0000	0.0000	0.0003	0.0000
5-methyl-2'-deoxycytidine	6.36	7.36	0.18	0.16	0.0000	0.0000	0.0003	0.0000
sodium acetate	256.04	735.46	0.00	0.00	0.0000	0.0000	0.0003	0.0000
auricularin (C12)	53.08	12.78	0.02	0.00	0.0000	0.0000	0.0003	0.0000
glutamine	245.80	286.78	0.00	0.00	0.0000	0.0000	0.0003	0.0000
sucrose	26.25	22.20	0.08	0.10	0.0000	0.0000	0.0003	0.0000
5-methylcytosine	2.33	3.53	0.43	0.28	0.0000	0.0000	0.0003	0.0000
tris-4-decylolamine (C10-1)	40.00	12.94	0.02	0.00	0.0000	0.0000	0.0003	0.0000
galactosylglycerol	72.09	27.77	0.01	0.04	0.0000	0.0000	0.0003	0.0000
alpha-Glucuronide	40.80	40.33	0.02	0.02	0.0000	0.0000	0.0003	0.0000
sulfonic acid sulfate	63.74	62.77	0.02	0.02	0.0000	0.0000	0.0003	0.0000
5-O-galactosylhydroxy-L-lysine	53.52	56.54	0.07	0.06	0.0000	0.0000	0.0003	0.0000
viscose kinase	5.06	4.12	0.25	0.30	0.0000	0.0000	0.0003	0.0000
ascorbate (vitamin C)	11.61	13.46	0.09	0.07	0.0000	0.0000	0.0003	0.0000
nicotinamide	2.50	8.92	0.31	0.09	0.0001	0.0000	0.0003	0.0000
erythritol	6.50	10.10	0.15	0.10	0.0000	0.0000	0.0003	0.0000
octadecyl carnitine (C8)	13.81	5.70	0.07	0.18	0.0000	0.0000	0.0003	0.0000
succinyl taurine	1.63	1.74	0.28	0.24	0.0000	0.0002	0.0003	0.0000
octadecyl carnitine (C8)	15.21	13.27	0.07	0.08	0.0000	0.0000	0.0003	0.0000
5-hydroxyguanine	7.75	4.98	0.10	0.16	0.0000	0.0001	0.0003	0.0000
pentose acid*	141.70	218.78	0.01	0.01	0.0000	0.0000	0.0003	0.0000
gerstenin sulfate*	5437.21	8103.16	0.00	0.00	0.0003	0.0000	0.0000	0.0000
urea	1.79	3.81	0.56	0.25	0.0001	0.0000	0.0001	0.0000
amino acid glycine	61.79	62.12	0.02	0.02	0.0000	0.0000	0.0000	0.0000
Flavone derivative C26H28O14 (5)	1.84	4.84	0.54	0.21	0.0001	0.0000	0.0001	0.0000
N5-carbamoylthreosyladenosine	10.75	11.24	0.14	0.13	0.0003	0.0000	0.0000	0.0000
xylofuranose	9.36	4.21	0.10	0.23	0.0003	0.0003	0.0000	0.0000
hexanoyl carnitine (C6)	13.02	5.90	0.08	0.17	0.0003	0.0003	0.0000	0.0000
alpha-oligomannose	83.99	151.77	0.01	0.01	0.0003	0.0003	0.0000	0.0000
5-methylthioribose**	4.26	3.50	0.23	0.29	0.0003	0.0003	0.0000	0.0000
N-acetylalanine	4.35	6.11	0.20	0.14	0.0003	0.0003	0.0001	0.0000
fructosyl lysine	6.01	10.47	0.17	0.10	0.0003	0.0003	0.0000	0.0000
N1-N10-dicarbonyl spermidine	62.08	67.26	0.02	0.02	0.0003	0.0003	0.0000	0.0000
guanine	167.75	72.10	0.01	0.02	0.0003	0.0003	0.0000	0.0000

FIG. 15H

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
L-tyrosine	76.19	44.05	0.01	0.02	0.0000	0.0000	0.0000	0.0000
soyababonin I	169.05	184.09	0.03	0.02	0.0000	0.0000	0.0000	0.0000
ascorbic acid 2-sulfate	11.52	8.45	0.09	0.12	0.0001	0.0000	0.0001	0.0000
histidine/leucine (C14 : 1 : 1)	17.57	5.74	0.07	0.22	0.0000	0.0000	0.0000	0.0000
N6,N6,N6-trimethyl-lysine	2.28	2.03	0.44	0.49	0.0001	0.0001	0.0000	0.0000
1-((1-ethyl-palmitoyl)-2-iodo-ethyl-GPC (P-16,0:20:4))	5.55	3.46	0.14	0.23	0.0001	0.0003	0.0000	0.0000
1-((1-ethyl-palmitoyl)-2-iodo-ethyl-GPC (P-16:0:18:2))	2.20	2.76	0.18	0.14	0.0074	0.0186	0.0000	0.0000
1-carboxyethyl-phenylalanine	1.52	2.25	0.66	0.44	0.0185	0.0000	0.0185	0.0000
soyababonin I	123.76	152.17	0.03	0.03	0.0000	0.0000	0.0000	0.0000
2-deoxyglucose	3.28	4.49	0.30	0.22	0.0075	0.0032	0.0001	0.0000
cellulose	11528.50	17427.00	0.00	0.00	0.0000	0.0000	0.0000	0.0000
decanal/dodecanal 7-O-glucoside	2.33	8.10	0.43	0.12	0.0003	0.0000	0.0003	0.0000
anserine	2.33	3.50	0.43	0.28	0.0014	0.0001	0.0000	0.0000
ascorbic acid	72.07	68.54	0.02	0.02	0.0000	0.0000	0.0000	0.0000
N-acetylvaline	4.15	4.22	0.24	0.24	0.0000	0.0000	0.0001	0.0000
indoleacetylserine (C11 : 1)	28.43	11.68	0.04	0.09	0.0000	0.0001	0.0000	0.0000
orotidine	5.54	3.89	0.18	0.28	0.0000	0.0000	0.0000	0.0000
3-hydroxy-2-norbornene	2.31	2.93	0.63	0.50	0.0077	0.0000	0.0412	0.0000
dodecyl sulfate (C12 : 1)	271.54	458.25	0.01	0.00	0.0000	0.0000	0.0000	0.0000
threonate	4.51	5.84	0.18	0.14	0.0004	0.0001	0.0001	0.0000
indoleacetylserinate	30.53	32.52	0.05	0.05	0.0000	0.0000	0.0000	0.0000
glutamate, glutamate	7.26	9.56	0.16	0.12	0.0000	0.0001	0.0000	0.0000
N-acetyl methionine	3.08	4.02	0.28	0.20	0.0000	0.0000	0.0001	0.0000
pro-hydroxy-pro	1001.07	1036.69	0.00	0.00	0.0000	0.0000	0.0000	0.0000
orotate	2.01	2.77	0.23	0.17	0.0170	0.0000	0.0001	0.0000
peracetylcarnitine (C10 : 1)	15.79	5.54	0.06	0.18	0.0000	0.0000	0.0000	0.0000
1-((1-ethyl-palmitoyl)-2-iodo-ethyl-GPC (P-16:0:18:2))	2.94	4.83	0.19	0.12	0.0042	0.0005	0.0002	0.0000
N-acetyl threonine	2.22	2.45	0.43	0.39	0.0002	0.0000	0.0002	0.0000
bacterin	4.10	2.36	0.17	0.30	0.0010	0.0078	0.0000	0.0000
histidine, betaine, threonine	247.71	462.37	0.02	0.01	0.0000	0.0000	0.0001	0.0000
argininate	3.64	5.18	0.16	0.11	0.0000	0.0000	0.0002	0.0001
4-galactonolactate	15.74	13.91	0.10	0.12	0.0000	0.0000	0.0001	0.0001
1-stearoyl-2-iodo-ethyl-GPC (P-18:0:16:2)	1.47	2.67	0.57	0.31	0.0482	0.0002	0.0079	0.0001
serine, threonine	4.31	3.62	0.24	0.28	0.0000	0.0000	0.0000	0.0001
azobonate, pyruvate	68.63	85.71	0.03	0.03	0.0000	0.0000	0.0001	0.0001
histidine	3.52	4.61	0.48	0.36	0.0000	0.0000	0.0005	0.0001
cysteine	1.49	1.48	0.64	0.55	0.0000	0.0001	0.0001	0.0001
gamma-glutamyl-histidine	4.23	5.85	0.20	0.14	0.0000	0.0000	0.0003	0.0001
6-methylthioadenosine (MTA)	3.17	4.48	0.41	0.29	0.0000	0.0000	0.0006	0.0001
protonylcarnitine (C3 : 1)	4.33	5.46	0.17	0.14	0.0000	0.0014	0.0002	0.0001
hydroxy-N6,N6,N6-trimethyl-lysine	2.43	4.12	0.47	0.28	0.0000	0.0000	0.0014	0.0001
malonylglycine	16.30	22.57	0.10	0.07	0.0000	0.0000	0.0002	0.0001
pyruvate	3.79	6.07	0.31	0.19	0.0001	0.0000	0.0001	0.0001
N1-methyl-nosine	95.00	103.22	0.03	0.02	0.0000	0.0000	0.0001	0.0001
N-acetyl methionine sulfide	7.23	4.79	0.16	0.22	0.0001	0.0005	0.0000	0.0001
1-palmitoyl-GPC (P-16:0)	3.03	2.02	0.30	0.45	0.0002	0.0019	0.0000	0.0001
N-acetyl histidine	4.87	5.41	0.16	0.12	0.0000	0.0000	0.0002	0.0001
glutathione, hydroxyproline	10.78	10.91	0.11	0.11	0.0001	0.0000	0.0002	0.0001
2-deoxyglucose	3.06	3.30	0.33	0.30	0.0002	0.0001	0.0002	0.0001
N-acetyl arginine	3.50	4.45	0.14	0.11	0.0000	0.0002	0.0002	0.0001
2-methylbutylcarnitine (C5)	13.29	5.16	0.08	0.19	0.0000	0.0002	0.0000	0.0002
N6-formyl-lysine	2.02	2.06	0.46	0.45	0.0000	0.0006	0.0000	0.0002
N-acetyl cysteine	2.07	2.18	0.54	0.51	0.0000	0.0001	0.0001	0.0002
urate	6.31	4.87	0.11	0.14	0.0003	0.0001	0.0001	0.0002
gamma-glutamyl-glycine	7.88	8.56	0.10	0.09	0.0003	0.0000	0.0003	0.0002
1-stearoyl-2-arachidonyl-GPC (P-18:0:20:4)	2.11	4.07	0.49	0.25	0.0073	0.0000	0.0007	0.0002
ascorbic acid 3-sulfate	117.41	99.63	0.02	0.03	0.0000	0.0000	0.0002	0.0000
isoleucine, hydroxyproline	12.09	10.58	0.10	0.11	0.0003	0.0005	0.0002	0.0000
6-acetylglucose	16.83	14.45	0.07	0.08	0.0000	0.0000	0.0003	0.0003
bilirubin degradation product (C17H12N2O4H12H)	3.72	3.88	0.35	0.33	0.0000	0.0001	0.0005	0.0000
glycerolphosphoserine	4.35	5.08	0.44	0.38	0.0000	0.0000	0.0005	0.0003
Flavone derivative (C26+28O14H4)	32.65	67.13	0.05	0.02	0.0001	0.0000	0.0007	0.0000

FIG. 15I

Biochemical Name	Fold of Change				Statistical Values			
	Welch's Two-Sample t-Test				Welch's Two-Sample t-Test			
	Cecum				Cecum			
	GF SPF	ABX SPF	CONV GF	CONV ABX	GF SPF p-value	ABX SPF p-value	CONV GF p-value	CONV ABX p-value
cystine-5-sulfate	3.22	5.32	0.25	0.15	0.0066	0.0001	0.0011	0.0003
1-stearoyl-2-oleoyl-GPC (18:0:18:1)	2.07	4.39	0.49	0.23	0.0117	0.0024	0.0262	0.0003
1-stearoyl-2-oleoyl-GPI (18:0:18:2)	5.65	5.59	0.24	0.25	0.0000	0.0006	0.0000	0.0004
octadecanoyl carnitine (C18:0-DC)	3.45	3.60	0.06	0.08	0.0015	0.0006	0.0005	0.0004
N-acetylserine	2.77	2.53	0.44	0.48	0.0013	0.0013	0.0003	0.0005
homocysteine	2.21	2.17	0.32	0.32	0.0012	0.0002	0.0004	0.0005
ethylenediamine	7.49	8.19	0.23	0.21	0.0000	0.0000	0.0005	0.0005
1-stearoyl-2-arachidonyl-GPI (18:0:20:4)	4.04	6.06	0.42	0.28	0.0011	0.0001	0.0007	0.0005
NNN-trimethyl-L-lysine betaine (TMAP)	14.44	14.42	0.03	0.03	0.0010	0.0010	0.0006	0.0008
N1-methylhistidine	4.08	8.80	0.27	0.13	0.0003	0.0001	0.0003	0.0006
gamma-glutamylserine	1.96	3.32	0.44	0.26	0.0007	0.0004	0.0006	0.0007
gamma-glutamyltryptophan	1.53	4.02	0.50	0.18	0.0003	0.0010	0.0047	0.0007
1-stearoyl-2-docosahexaenoyl-GPI (18:0:22:6)	2.09	4.19	0.56	0.28	0.0052	0.0006	0.0006	0.0007
gamma-glutamylproline	3.88	2.99	0.21	0.28	0.0000	0.0002	0.0004	0.0008
cystathionine	2.23	4.60	0.54	0.26	0.0011	0.0000	0.0017	0.0009
1-methyl nicotinamide	219.97	487.51	0.11	0.05	0.0000	0.0000	0.0022	0.0010
7-alpha-hydroxy-3-oxo-4-cholestenate (7-keto)	2.92	2.66	0.20	0.22	0.0019	0.0018	0.0000	0.0011
N1-methylhistidine	12.03	12.55	0.11	0.11	0.0000	0.0000	0.0015	0.0011
acetyl carnitine (C2)	2.94	3.46	0.35	0.30	0.0041	0.0022	0.0022	0.0012
N6-carboxymethyllysine	9.25	13.98	0.25	0.16	0.0000	0.0000	0.0037	0.0013
gamma-glutamylglutamine	2.11	3.55	0.39	0.23	0.0024	0.0017	0.0087	0.0013
N-acetyl-aspartyl-glutamate (NAAG)	6.27	6.44	0.23	0.23	0.0000	0.0000	0.0016	0.0016
ributamate-xylitol-phosphate-lysine	132.09	137.35	0.06	0.06	0.0000	0.0000	0.0021	0.0019
creatine	5.53	9.36	0.40	0.22	0.0002	0.0000	0.0140	0.0023
ribonate	26.38	33.50	0.19	0.15	0.0000	0.0000	0.0040	0.0025
cystine	5.21	7.16	0.23	0.16	0.0020	0.0010	0.0068	0.0026
1-stearoyl-GPE (18:0)	2.60	2.26	0.46	0.53	0.0003	0.0006	0.0015	0.0009
1-oleoyl-GPC (18:1)	2.38	1.58	0.42	0.65	0.0005	0.0006	0.0000	0.0006
creatine	66.81	96.36	0.24	0.17	0.0000	0.0000	0.0084	0.0004
prolylglycine	2.73	4.89	0.44	0.24	0.0111	0.0019	0.0009	0.0005
2-O-methylglycine	5.72	3.41	0.25	0.43	0.0000	0.0002	0.0007	0.0001
bi-rubin degradation product: C17H ₃₂ N ₂ O ₄ (5:1)	4.56	5.23	0.35	0.31	0.0007	0.0001	0.0104	0.0006
flavone derivative C28H ₂₈ O ₁₄ (3:1)	3.84	6.74	0.27	0.15	0.0043	0.0007	0.0043	0.0007
lactonate (hydroxymalonate)	13.02	15.07	0.27	0.23	0.0001	0.0001	0.0006	0.0009
1-ribosyl-midazoleacetate	10.74	19.21	0.32	0.18	0.0000	0.0000	0.0185	0.0076
1-stearoyl-GPC (18:0)	2.47	1.81	0.48	0.82	0.0006	0.0040	0.0007	0.0079
bi-rubin degradation product: C17H ₃₂ N ₂ O ₄ (2:1)	3.81	3.93	0.31	0.30	0.0006	0.0004	0.0081	0.0081
N-acetyl-isoputrescine	1.86	1.84	0.65	0.65	0.0007	0.0006	0.0103	0.0007
1-carboxymethylvaline	2.28	1.57	0.44	0.64	0.0005	0.0002	0.0005	0.0002
choline	2.47	1.59	0.43	0.87	0.0000	0.0000	0.0001	0.0006
N7-acetyllysine	1.38	1.34	0.51	0.82	0.0074	0.0060	0.0081	0.0087
succinyl carnitine (C4:DC)	2.99	2.56	0.52	0.60	0.0012	0.0001	0.0035	0.0111
4-hydroxyphenolate	3.35	2.78	0.30	0.36	0.0020	0.0112	0.0020	0.0112
octadecanoyl carnitine (C18:0-DC)	11.71	8.50	0.25	0.34	0.0000	0.0000	0.0053	0.0128
gamma-glutamylvaline	2.66	2.74	0.21	0.20	0.0000	0.0000	0.0133	0.0179
N2-N6-diacyllysine	1.64	1.93	0.87	0.57	0.0052	0.0049	0.0074	0.0134
benzylglycine (2)	10.65	2.44	0.09	0.41	0.0123	0.0139	0.0123	0.0139
N1-Methyl-2-pyridone-5-carboxamide	6.91	7.47	0.40	0.37	0.0000	0.0000	0.0175	0.0140
cystinylglycine	2.49	2.84	0.62	0.55	0.0000	0.0000	0.0060	0.0154
trans-5-hydroxyproline	3.18	1.63	0.28	0.57	0.0000	0.0003	0.0006	0.0155
2-O-methylascorbic acid	13.39	13.17	0.32	0.33	0.0000	0.0000	0.0185	0.0151
xyrisoyl carnitine (C14)	33.99	10.55	0.12	0.38	0.0000	0.0000	0.0010	0.0192
citrate	1.75	1.93	0.62	0.55	0.0118	0.0062	0.0344	0.0200
D-sulfate-L-tyrosine	1.57	1.42	0.40	0.45	0.0110	0.0362	0.0148	0.0212
S-carboxymethyl-L-cysteine	1.84	2.12	0.58	0.51	0.0050	0.0022	0.0414	0.0253
proline	1.59	1.23	0.60	0.78	0.0000	0.0025	0.0014	0.0280
1-carboxymethylleucine	2.56	1.62	0.38	0.62	0.0005	0.0284	0.0055	0.0284
1-methyl-phenethyl-GPC (1P:1P:1P)	3.95	2.05	0.25	0.48	0.0013	0.0027	0.0013	0.0291
malate	1.88	2.42	0.60	0.47	0.0003	0.0150	0.0037	0.0290
N-acetylserine	6.60	3.18	0.17	0.36	0.0001	0.0224	0.0000	0.0311
N-palmitoyl-phosphatidylglycerol (18:0:18:0)	1.93	1.50	0.52	0.87	0.0000	0.0020	0.0000	0.0320
2-O-methyluridine	4.71	2.62	0.37	0.66	0.0000	0.0006	0.0010	0.0377
N-acetylserine	6.43	6.04	0.48	0.62	0.0000	0.0000	0.0015	0.0396