EXTENDED EXPERIMENTAL PROCEDURES

Animals

SPF C57Bl/6J mice and SPF Slc6a4 KO mice (Jackson Laboratories) were bred in Caltech's Broad Animal Facility. GF C57Bl/6J mice (rederived from SPF C57Bl/6J mice from Jackson Laboratories), GF Swiss Webster mice, GF Rag1 KO, *B. fragilis* monoassociated and SFB monoassociated mice were bred in Caltech's Gnotobiotic Animal Facility. GF Slc6a4 KO mice were generated by C-section rederivation, cross-fostering to GF Swiss Webster mice (Taconic Farms) and bred as an independent GF line in Caltech's Gnotobiotic Animal Facility. All animal experiments were approved by the Caltech IACUC.

Microbiota Conventionalization

Fecal samples were freshly collected from adult SPF C57Bl/6J mice and homogenized in pre-reduced PBS at 1 ml per pellet. 100 μl of the settled suspension was administered by oral gavage to postnatal day (P)21 and P42 GF mice. For conventionalization at P0, GF mothers were gavaged with 100 μl of the SPF fecal suspension, and the mother and litter were transferred into a dirty cage, previously housed for 1 week with adult SPF C57Bl/6J mice. For mock treatment, mice were gavaged with pre-reduced PBS.

Antibiotic Treatment

P21 and P42 SPF mice were gavaged with a solution of vancomycin (50mg/kg), neomycin (100 mg/kg), metronidazole (100 mg/kg) and amphotericin-B (1 mg/kg) every 12 hr daily until P56, according to methods described in (Reikvam et al., 2011). Ampicillin (1 mg/ml) was provided *ad libitum* in drinking water. For antibiotic treatment at P0, drinking water was supplemented with ampicillin (1 mg/ml), vancomycin (500 mg/ml) and neomycin (1 mg/ml) until P21, and from P21-P56, mice were gavaged with antibiotics as described above. For mock treatment, P42 mice were gavaged with unsupplemented drinking water every 12 hr daily until P56.

Human Biopsy Sample and Colonization of GF Mice

Archived, de-identified clinical samples of colonic microbiota were provided by Eugene Chang at the University of Chicago and handled as described previously (Ma et al., 2014). Briefly, a sample of mucosal brush and luminal aspirate from the colon of a healthy human subject was placed on ice, transferred into an anaerobic chamber immediately after collection and homogenized in grants buffered saline solution (GBSS) supplemented with 5% DMSO by vortexing for 5 min. Aliquots of the samples were flash frozen with liquid nitrogen and preserved at −80°C. 100 μl of the suspension was used to gavage founder GF mice, housed in a designated gnotobiotic isolator.

Bacterial Treatment

Frozen fecal samples from Sp- and ASF-colonized mice were generously supplied by the laboratory of Cathryn Nagler (University of Chicago). Fecal samples were suspended at 50 mg/ml in pre-reduced PBS, and 100 μl was orally gavaged into adult C57Bl/6J GF mice. These "founder" mice were housed separately in dedicated gnotobiotic isolators and served as repositories for fecal samples used to colonize experimental mice. For generation of "founder" mice colonized with human spore-forming bacteria, fecal pellets were collected from humanized mice, described above, and suspended in a 10X volume of pre-reduced PBS in an anaerobic chamber. Chloroform was added to 3% (vol/vol), the sample was shaken vigorously and incubated at 37°C for 1 hr. Chloroform was removed by percolation with CO_2 from a compressed cylinder, and 200 μl suspension was orally gavaged into adult C57Bl/6J GF mice housed in designated gnotobiotic isolators.

Fecal samples were collected from founder mice and immediately frozen at −80°C for later Sp or ASF colonization. Experimental GF or antibiotic-treated mice were colonized on P42 by oral gavage of 100 μl of 50 mg/ml fecal suspension in pre-reduced PBS. For mock treatment, mice were gavaged with pre-reduced PBS. For the *Bacteroides* (Bd) consortium, feces from adult SPF Swiss Webster mice was suspended at 100 mg/ml in BHI media and serially plated on *Bacteroides* Bile Esculin (BBE) agar (BD Biosciences). 100 μl of a 10^{10} cfu/ ml suspension in PBS was used for colonization of P42 GF mice. Colony PCR and sequencing indicates that among the most abundant species in the Bd consortium are *B. thetaiotaomicron, B. acidifaciens, B. vulgatus* and *B. uniformis*.

Intestinal qRT-PCR

The entire length of the mouse colon, or 1 cm regions of the distal, medial and proximal of the mouse small intestine were washed in PBS, flushed with PBS to remove luminal contents, and homogenized in ice-cold Trizol for RNA isolation using the RNeasy Mini Kit with on-column genomic DNA-digest (QIAGEN) and cDNA synthesis using iScript (Biorad). qRT-PCR was performed on an ABI 7900 thermocycler using SYBR green master mix with Rox passive reference dye (Roche) and validated primer sets obtained from Primerbank (Harvard).

Serotonin Measurements

Blood samples were collected by cardiac puncture and spun through SST vacutainers (Becton Dickinson) for serum separation or PST lithium hepararin vacutainers (Becton Dickinson) for plasma separation. The entire length of the colon or 1 cm regions of the distal, medial and proximal colon of the small intestine were washed in PBS, flushed with PBS to remove luminal contents, and sonicated on ice in 10 s intervals at 20 mV in ELISA standard buffer supplemented with ascorbic acid (Eagle Biosciences). Serotonin

levels were detected in sera and supernatant of tissue homogenates by ELISA according to the manufacturer's instructions (Eagle Biosciences). Readings from tissue samples were normalized to total protein content as detected by BCA assay (Thermo Pierce). Data compiled across multiple experiments are expressed as 5-HT concentrations normalized to SPF controls within each experiment.

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RIN14B In Vitro Culture Experiments

RIN14B cells (ATCC) were seeded at 10^5 cells/cm^2 and cultured for 3 days in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 ug/ml streptomycin according to methods described in Nozawa et al. (2009). Total colonic luminal contents were collected from adult SPF, GF and GF mice colonized with spore-forming bacteria, suspended at 120 μl/mg in HBSS supplemented with 0.1% BSA and 2 uM fluoxetine, and centrifuged at 12,000 xg for 10 min. Supernatants were passed through 0.2 um pore syringe filters. Cultured RIN14B cells were incubated with colonic luminal filtrate at 125 μl/cm^2 for 1 hr at 37°C. Positive controls were incubated with 15 uM ionomycin in vehicle (HBSS). After incubation, supernatant was collected, centrifuged at 6000 xg for 5 min to pellet any residual cells, and frozen for downstream 5-HT assays. Remaining adherent RIN14B cells were lysed in Trizol for downstream RNA isolation, cDNA synthesis and qRT-PCR as described above. For experiments with colonic luminal contents, starting 5-HT levels in filtrate were subtracted from post-assay 5-HT levels, and this difference is reported as "5-HT released."

For metabolite sufficiency assays, cells were incubated with biochemicals in HBSS or 1% DMSO in HBSS at the indicated concentrations. Pilot experiments were conducted to test the ability of physiologically relevant concentrations (as identified in existing scientific literature) of acetate, α-tocopherol, arabinose, azelate, butyrate, cholate, deoxychoate, ferulate, GABA, glycerol, N-methyl proline, oleanolate, p-aminobenzoate (PABA), propionate, taurine, and tyramine to induce 5-HT in RIN14B cultures. 5-HT concentrations were normalized to levels detected in the appropriate RIN14B + vehicle (HBSS or 1% DMSO in HBSS) control. For biochemicals that raised 5-HT levels in culture, additional pilot experiments were conducted to determine the lowest concentrations possible for elevating 5-HT in vitro. These concentrations were further tested in triplicate to generate the data presented in Figure 6D.

Intestinal Histology and Immunofluorescence Staining

Mouse colon was cut into distal, medial and proximal sections, and 1 cm regions of the distal, medial and proximal small intestine were fixed in Bouin's solution (Sigma Aldrich) overnight at 4°C, washed and stored in 70% ethanol. Intestinal samples were then paraffin-embedded and cut into 10 um longitudinal sections by Pacific Pathology, Inc (San Diego, CA). Sections were stained using standard procedures. Briefly, slides were deparaffinized, and antigen retrieval was conducted for 20 min in a 95°C water bath in 10mM sodium citrate, pH 6.0 or DAKO solution (Agilent Technologies), followed by a 15 minute incubation at room temperature. Slides were washed, blocked in 5% normal serum or 5% bovine serum albumin (Sigma Aldrich), and stained using the primary antibodies, rabbit anti-mouse CgA (1:500; Abcam), rat anti-mouse 5-HT (1:50; Abcam), rabbit anti-mouse c-*fos* (1:100; Abcam), goat anti-mouse calretinin (1:1500; Millipore), rabbit anti-5HT4 (1:3000; Abcam), and secondary antibodies conjugated to Alexa fluor 488 or 594 (Molecular Probes). Slides were mounted in Vectashield (Vector Labs), and 3-15 images were taken per slide at 20X or 40X magnification along transections of the intestinal crypts for each biological replicate (EVOS FL System; Life Technologies). Monochrome images were artificially colored, background corrected and merged using Photoshop CS5 (Adobe). For 5-HT and CgA staining, numbers of positively-stained puncta were scored blindly, normalized to total area of intestinal mucosa using ImageJ software (NIH) (Schneider et al., 2012), and then averaged across biological replicates. For calretinin, c-*fos* and 5HT4 staining, fluorescence intensity for individual stains was quantified and normalized to total area of intestinal submucosa and muscularis externa using ImageJ software. Colocalization was measured and analyzed using the Coloc2 plug-in for Fiji software (Schindelin et al., 2012). Representative images are presented in the figures, where Alexa fluor 594 staining is replaced with magenta.

Platelet Activation and Aggregation Assays

Blood samples were collected by cardiac puncture, diluted with a 2x volume of HEPES medium (132 mM NaCl, 6 mM KCl, 1 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM HEPES, 5 mM glucose; pH 7.4) and centrifuged through PST lithium hepararin vacutainers (Becton Dickinson). Expression of platelet activation markers was measured by flow cytometry (Nieswandt et al., 2004; Ziu et al., 2012). Briefly, PRP samples were supplemented with 1 mM CaCl2, and 1 × 10^6 platelets were stimulated with 10 μg/ml type-1 HORM collagen (Chronolog), and stained with anti-JON/A-PE, anti-P-selectin-FITC (Emfret Analytics), anti-CD63-PE (Biolegend), anti-CD41-FITC (BD Biosciences) and anti-CD9-APC (Abcam) for 15 min at room temperature. Samples were then washed in PBS, fixed

with 0.5% formaldehyde and analyzed using a FACSCalibur flow cytometer (BD Biosciences). Platelet aggregation assays were conducted according to methods described in De Cuyper et al. (2013). Briefly, 4×10^6 platelets were stained separately with CD9-APC or CD9-PE (Abcam) for 15 min at room temperature and then washed with HEPES medium. Labeled platelets were mixed 1:1 and incubated for 15 min at 37°C, with shaking at 600 rpm. Platelets were then stimulated with 10 ug/ml type-1 collagen for 2 min and fixed in 0.5% formaldehyde for flow cytometry. Remaining unstained PRP was treated with collagen as described above, and then used to generate PRP smears. Slides were stained with Wright Stain (Camco) according to standard procedures. Platelets were imaged at 200x magnification, and 9 images were taken across each PRP smear, processed using ImageJ software (intensity threshold: 172, size threshold: 500) (Schneider et al., 2012), totaled for each biological replicate, and then averaged across biological replicates. Comprehensive complete blood counts were conducted by Idexx Laboratories using the ProCyte Dx Hematology Analyzer.

Tail Bleed Assay

Mice were anesthetized with isoflurane and the distal 6mm portion of the tail was transected using a fresh razor blade. The tail was placed immediately at a 2 cm depth into a 50 ml conical tube containing saline pre-warmed to 37°C (Liu et al., 2012). Time to bleeding cessation was recorded, with continued recording if re-bleeding occurred within 15 s of initial cessation and a maximum total bleed time of 5 min.

Metabolomics Screening

Fecal samples were collected from adult mice at 2 weeks post-bacterial treatment, and immediately snap frozen in liquid nitrogen. Each sample consisted of 3-4 fecal pellets freshly collected between 9-11am from mice of the same treatment group co-housed in a single cage. Samples were prepared using the automated MicroLab STAR system (Hamilton Company) and analyzed on GC/MS, 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 Welch's two-sample t test.

Metabolite In Vivo Injection Experiments

Adult GF C57Bl/6 mice were anesthetized with isoflurane, and metabolites were injected intrarectally (α-tocopherol: 2.25 mg/kg, deoxycholate: 125 mg/kg, oleanolate: 0.457 mg/kg) using a sterile 3.5 Fr silicone catheter (Solomon Scientific). Concentrations were based on levels reported in Sayin et al. (2013), Alemi et al. (2013), and Zhao et al. (2010). Mice were suspended by tail for 30 s before return to the home cage. For mock treatment, GF mice were anesthetized and intrarectally injected with vehicle. For experiments evaluating physiological effects of metabolite administration, adult GF mice were injected every 12 hr for 3 days. GI motility assays were initiated at 1 hr after the third injection (day 2). For 5-HT measurements and platelet assays, mice were sacrificed at 1 hr after the final injection. For pilot time course experiments, adult GF Swiss Webster mice were injected once, as described above, and sacrificed at the indicated time points post-injection. Use of the Swiss Webster strain was based on availability and our validation that microbiota effects on colonic and blood 5-HT levels are similarly seen in both the Swiss Webster and C57Bl/6 mouse strains.

16S rRNA Gene Sequencing and Analysis

This experiment evaluates microbes recovered from Sp and hSp-colonized mice, and may not reflect the full microbial diversity within the initial inoculum. Fecal samples were collected at two weeks after orally gavaging GF mice with Sp or hSp. Fecal pellets were beadbeaten in ASL buffer (QIAGEN) with lysing matrix B (MP Biomedicals 6911-500) in a Mini-Beadbeater-16 (BioSpec Products, Inc.) for 1 min. Bacterial genomic DNA was extracted from mouse fecal pellets using the QIAamp DNA Stool Mini Kit (QIAGEN) with InhibitEX tablets. The library was generated according to methods adapted from Caporaso et al. (2011). The V4 regions of the 16S rRNA gene were PCR amplified using individually barcoded universal primers and 30 ng of the extracted genomic DNA. The PCR reaction was set up in triplicate, and the PCR product was purified by Agencourt AmPure XP beads (Beckman Coulter Inc, A63881) followed by Qiaquick PCR purification kit (QIAGEN). The purified PCR product was pooled in equal molar quantified by the Kapa library quantification kit (Kapa Biosystems, KK4824) and sequenced at UCLA's GenoSeq Core Facility using the Illumina MiSeq platform and 2 × 250bp reagent kit. Operational taxonomic units (OTUs) were chosen de novo with UPARSE pipeline (Edgar, 2013). Taxonomy assignment and rarefaction were performed using QIIME1.8.0 (Caporaso et al., 2010).

Phylogenetic trees were built using PhyML (Guindon et al., 2010) (General Time Reversible model, subtree pruning and regrafting method, with ten random start trees) and visualized using iTOL (Letunic and Bork, 2007). The 32 most abundant OTUs in Sp and hSp were included after excluding OTUs that were only present in less than 50% of biological replicates from sequenced fecal samples. Sequenced genomes from JGI's Integrated Microbial Genomes database (Markowitz et al., 2012) were searched for enzymes of interest (EC:1.4.3.4 monoamine oxidase, EC:2.1.1.95 tocopherol o-methyltransferase, EC:1.2.1.68 coniferyl-aldehyde dehydrogenase, EC:4.1.1.25 tyrosine decarboxylase). Hits phylogenetically related to the OTUs from Sp or to sequenced genomes from

B. fragilis, B. uniformis, B. vulgatus, B. thetaiotaomicron, B. acidifaciens and SFB were included. Bacteria with 7α-dehydroxylation activity were identified from previous reports (Hirano et al., 1981; Kitahara et al., 2000, 2001).

Trp/5-HTP Supplementation Experiment

Water was supplemented with Trp, 5-HTP or 5-HT at 1.5 mg/ml (based on calculations from Abdala-Valencia et al. [2012]) and provided *ad libitum* to mice for 2 weeks. Amount of water consumed and mouse weight was measured on days 3, 7, 10 and 14 of treatment. Mice were sacrificed one day after treatment for 5-HT assays.

Slc6a4 Mouse Antibiotic Treatment and Sp Colonization

Adult Slc6a4 mice were gavaged with a solution of vancomycin (50mg/kg), neomycin (100 mg/kg), metronidazole (100 mg/kg) and amphotericin-B (1 mg/kg) every 12 hr daily for 2 weeks, according to methods described in Reikvam et al. (2011). Ampicillin (1 mg/ml) was provided *ad libitum* in drinking water. For Sp colonization, mice were orally gavaged 2 days after the final antibiotic treatment with 100 μl of 50 mg/ml fecal suspension in pre-reduced PBS. For mock treatment, mice were gavaged with pre-reduced PBS. Mice were then tested in 5-HT-related assays 2 weeks after oral gavage.

Statistical Analysis

Statistical analysis was performed using Prism software (Graphpad). Data were assessed for normal distribution and plotted in the figures as mean ± SEM. Differences between two treatment groups were assessed using two-tailed, unpaired Student t test with Welch's correction. Differences among > 2 groups were assessed using one-way ANOVA with Bonferroni post hoc test. Two-way ANOVA with Bonferroni post hoc test was used to assess treatment effects in PCPA experiments involving > 2 experimental groups (e.g., SPF, GF, Sp). Welch's two-sample t test was used for analysis of metabolomic data. 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 near-significant differences ($0.5 < p < 0.1$) are indicated in the figures. Notable non-significant (and non-near significant) differences are indicated in the figures by "n.s.."

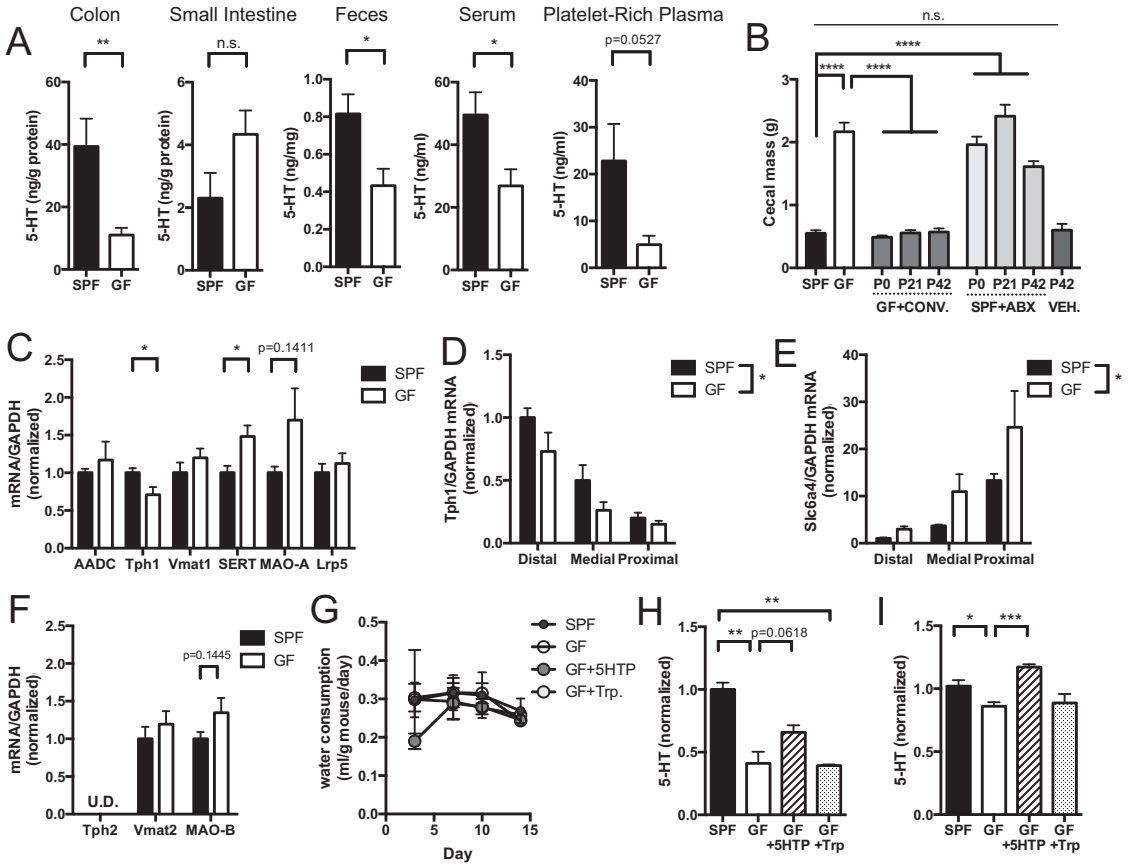

Figure S1. Characterization of Microbiota-Dependent Effects on Serotonin Metabolism, Related to Figure 1

(A) Levels of 5-HT in adult SPF vs. GF mice. Data from colon and small intestine are normalized to total protein content. Colon: n=29-33, small intestine: n=6, feces: n=4, serum: n=12, platelet-rich plasma: n=6.

(B) Cecal weight after conventionalization of GF mice on postnatal day (P) 0, P21 and P42, and after antibiotic treatment of SPF mice on P0, P21 and P42. n=8-13.

(C) Expression of genes involved in 5-HT metabolism relative to GAPDH in colons of adult SPF and GF mice. Data for each gene are normalized to expression levels in SPF mice. n=5.

(D) Expression of *TPH1* relative to *GAPDH* in distal, medial and proximal colons of adult SPF and GF mice. Data are normalized to expression levels in distal colon of SPF mice. n=5.

(E) Expression of *SLC6A4* relative to *GAPDH* in distal, medial and proximal colons of adult SPF and GF mice. Data are normalized to expression levels in distal colon of SPF mice. n=5.

(F) Expression of neural-specific isoforms of genes involved in 5-HT metabolism relative to *GAPDH* in colons of adult SPF and GF mice. Data for each gene are normalized to expression levels in SPF mice. n=5.

(G) Mouse consumption of water supplemented with Trp (1.5 mg/ml) or 5-HTP (1.5 mg/ml). n=4.

(H) Levels of colon 5-HT relative to total protein content two weeks after Trp or 5-HTP supplementation. Data are normalized to 5-HT levels in SPF mice. n=4-7.

(I) Levels of serum 5-HT two weeks after Trp or 5-HTP supplementation. Data are normalized to 5-HT levels in SPF mice. n=4-7.

Data are presented as mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, n.s.=not statistically significant. SPF=specific pathogen-free (conventionally-colonized), GF=germ-free, CONV.=SPF conventionalized, ABX=antibiotic-treated, AADC=aromatic amino acid decarboxylase, Tph=tryptophan hydroxylase, Vmat=vesicular monoamine transporter, SERT=serotonin transporter (Slc6a4), MAO=monoamine oxidase, Lrp=lipoprotein receptor related protein, 5-HTP=5-hydroxytryptophan, Trp=tryptophan.

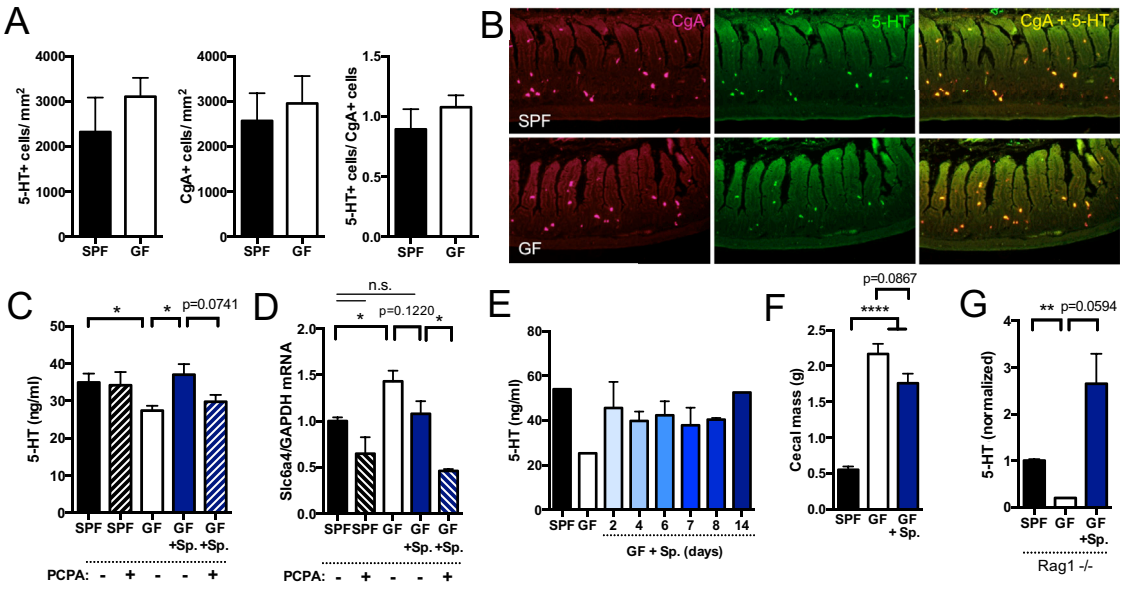

Figure S2. Characterization of Serotonin Modulation by Spore-Forming Bacteria, Related to Figure 2

(A) Quantitation of 5-HT+ (left), CgA+ (center) and ratio of 5-HT+ cells/CgA+ cells per area of small intestinal epithelial tissue. n=3 mice/group.

(B) Representative images of CgA (left), 5-HT+ (center), and merged (right) immunofluorescence staining in small intestines from SPF and GF mice. n=3 mice/group.

(C) Levels of serum 5-HT after intrarectal administration of PCPA or vehicle. n=4-7.

(D) Expression of *SLC6A4* relative to *GAPDH* in colons SPF, GF and Sp-colonized mice after treatment with PCPA or vehicle. Data are normalized to expression levels in SPF mice. n=3.

(E) Levels of serum 5-HT at 2-14 days post treatment with mouse chloroform-resistant bacteria (spores, Sp). SPF: pooled from n=6, GF: pooled from n=6, GF+Sp: n=3-6.

(F) Cecal weight in SPF, GF, and P42 Sp-colonized mice. n=9-10.

(G) Levels of colon 5-HT in SPF, GF and P42 Sp-colonized Rag1 KO mice. Data are normalized to levels in SPF mice. n=3.

Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. SPF=specific pathogen-free (conventionally-colonized), GF=germ-free, Sp=spore-forming bacteria, Rag=recombination activating gene, PCPA=para-chlorophenylalanine.

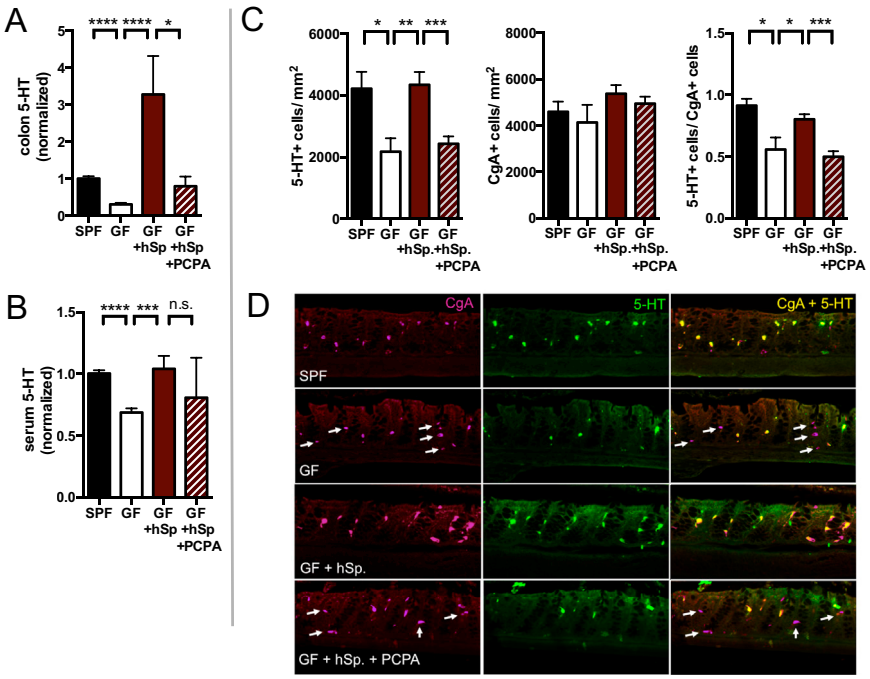

Figure S3. Spore-Forming Bacteria from the Healthy Human Gut Microbiota Promote Colon 5-HT Biosynthesis and Systemic 5-HT Bioavailability, Related to Figure 3

(A) Levels of 5-HT relative to total protein content in colons from P56 SPF, GF, conventionalized GF and antibiotic-treated SPF mice. Data are normalized to colon 5-HT levels relative to total protein content in SPF mice. n=3-8.

(B) Levels of 5-HT in sera from P56 SPF, GF, conventionalized GF and antibiotic-treated SPF mice. Data are normalized to serum 5-HT concentrations in SPF mice. n=3-8.

(C) Quantitation of 5-HT+ (left), CgA+ (center) and ratio of 5-HT+ to CgA+ cell number per area of colonic epithelial tissue. n=3-7 mice/group.

(D) Representative images of chromagranin A (CgA) (left), 5-HT (center), and merged (right) immunofluorescence staining in colons from SPF, GF, P42 human spore-forming bacteria-colonized mice. Arrows indicate CgA-positive cells that lack 5-HT staining, n=3-7 mice/group.

Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n.s.=not statistically significant. SPF=specific pathogen-free (conventionally-colonized), GF=germ-free, hSp=human-derived spore-forming bacteria, PCPA=para-chlorophenylalanine.

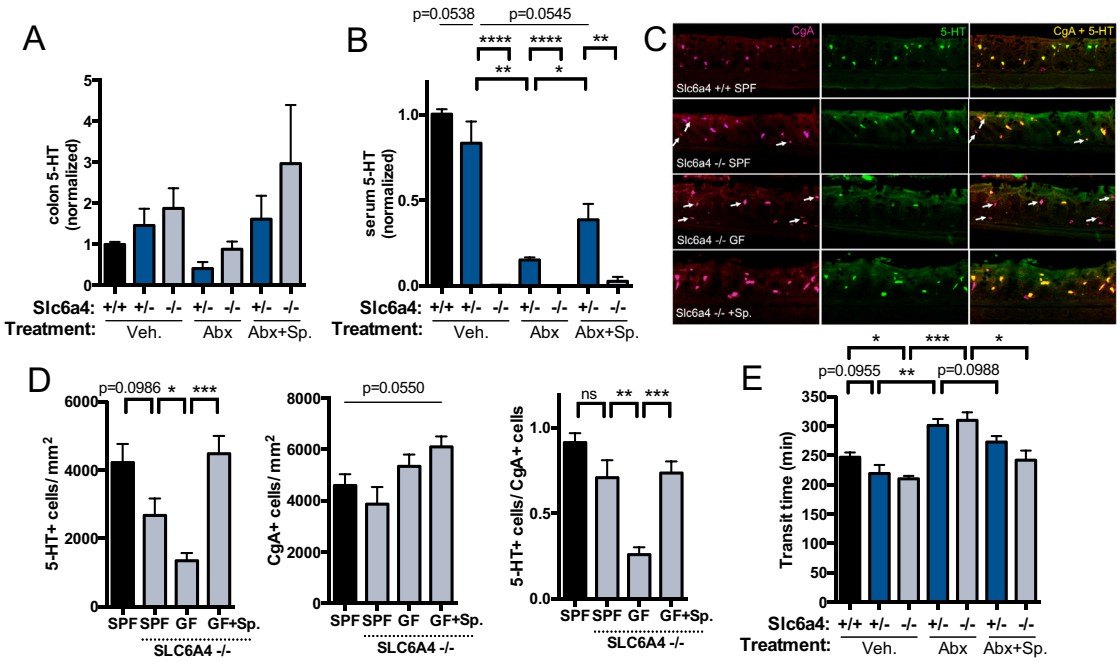

Figure S4. The Microbiota Modulates Gastrointestinal 5-HT in the Context of Serotonin Transporter Gene Deficiency, Related to Figure 4

(A) Levels of 5-HT relative to total protein content in colons from *SLC6A4* wildtype (+/+), heterozygous (+/-) and knockout (-/-) mice, treated with vehicle (water), antibiotics (Abx), or Abx+colonization with spore-forming bacteria (Sp). Data are normalized to colon 5-HT levels relative to total protein content in vehicle-treated (SPF) *SLC6A4* +/+ mice. n=5-8.

(B) Levels of 5-HT in sera from *SLC6A4* +/+, +/- or -/- mice, treated with vehicle, Abx, or Abx and Sp. Data are normalized to serum 5-HT concentrations in vehicle-treated *SLC6A4* +/+ mice. n=5-8.

(C) Representative images of chromagranin A (CgA) (left), 5-HT (center), and merged (right) immunofluorescence staining in colons from *SLC6A4* -/- mice, treated with vehicle, Abx, or Abx and Sp, relative to *SLC6A4* +/+ SPF controls. Arrows indicate CgA-positive cells that lack 5-HT staining, n=3-7 mice/group.

(D) Quantitation of 5-HT+ (left), CgA+ (center) and ratio of 5-HT+ to CgA+ cell number per area of colonic epithelial tissue from *SLC6A4* -/- mice, treated with vehicle, Abx, or Abx and Sp, relative to *SLC6A4* +/+ SPF controls. n=3-7 mice/group.

(E) Total time for transit of orally administered carmine red solution through the GI tract. n=5-8.

Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, n.s.=not statistically significant. SPF=specific pathogen-free (conventionally-colonized), Veh=vehicle (water), Abx=antibiotics, Sp=spore-forming bacteria.

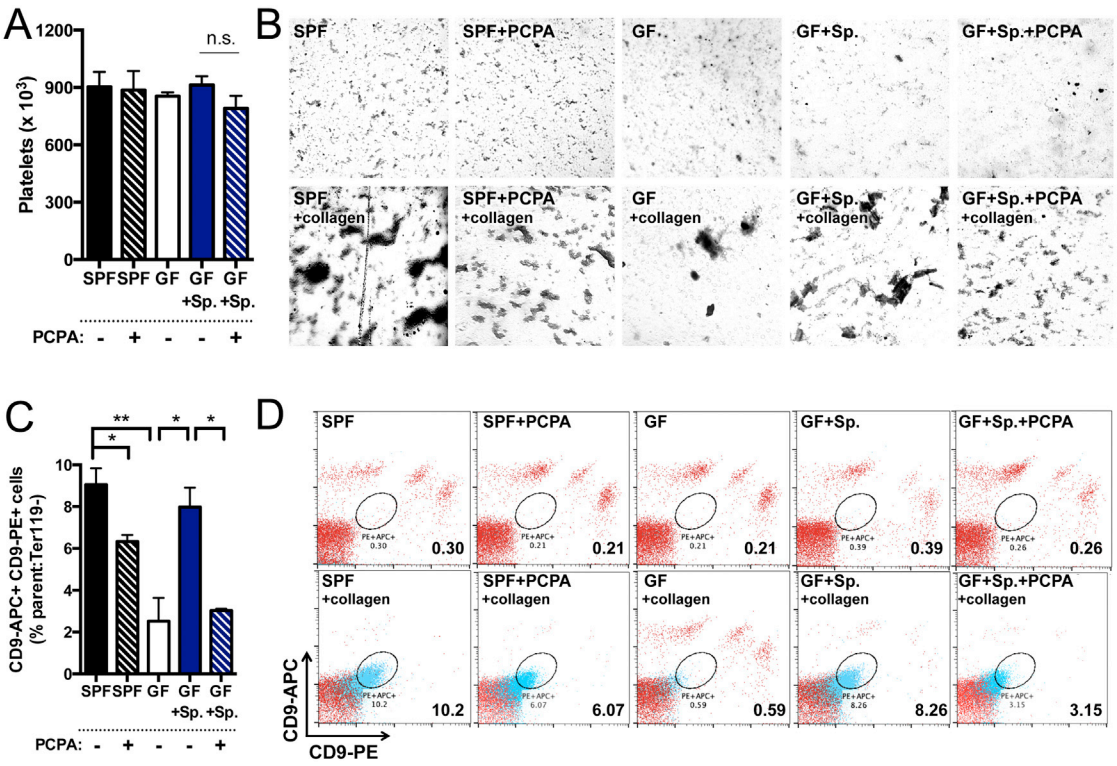

Figure S5. Microbiota Effects on Platelet Aggregation, Related to Figure 5

(A) Platelet counts from SPF, GF and Sp-colonized mice after treatment with PCPA or vehicle. n=3-7.

(B) Representative images of platelets after treatment with collagen (bottom) or vehicle (top). n=3.

(C) Platelet aggregation, as measured by percentage of large, high granularity CD9-APC^{mid}, CD9-Pe^{mid} Ter119- events, after collagen stimulation. Relative flow cytometry plots are shown in panel E. n=3.

(D) Representative flow cytometry plots of large, high granularity (FSC^{high}, SSC^{high}; events colored as blue) CD9-APC^{mid}, CD9-PE^{mid} aggregated platelets after collagen stimulation (bottom), as compared to unstimulated controls (top). n=3.

Data for platelet activation and aggregation assays are representative of three independent trials with at least three mice in each group. Data are presented as mean ± SEM. n.s.=not statistically significant. SPF=specific pathogen-free (conventionally-colonized), GF=germ-free, Sp=spore-forming bacteria, PCPA=para-chlorophenylalanine.

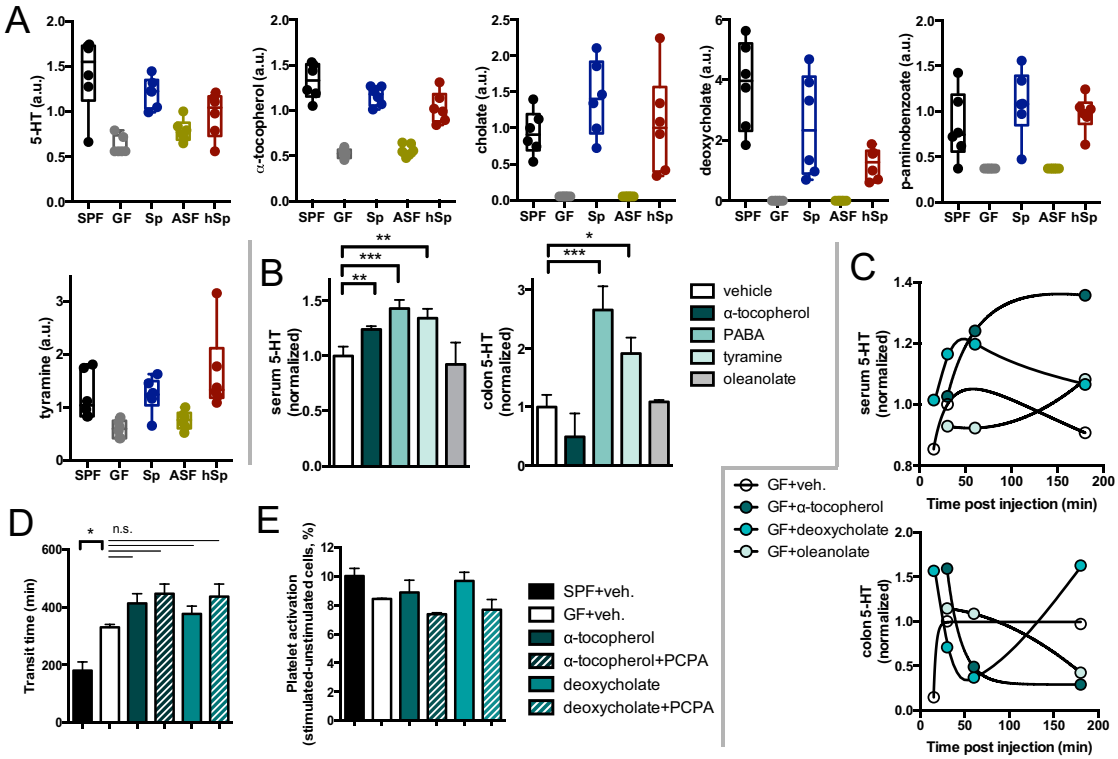

Figure S6. Metabolite Effects on Host 5-HT-Related Phenotypes, Related to Figure 6

(A) Relative levels of 5-HT and additional metabolites that co-vary with 5-HT in colonic luminal contents from SPF, GF, Sp, ASF and hSp-colonized mice. a.u.=arbitrary units. n=6.

(B) Levels of serum 5-HT (left) and colon 5-HT (right) in adult GF Swiss Webster mice at 1 hour after intrarectal injection with a-tocopherol (2.25 mg/kg), p-aminobenzoate (PABA; 1.37 ug/kg), tyramine (0.137 mg/kg), oleanolate (0.46 mg/kg) or vehicle. Data are normalized to 5-HT levels from GF mice injected with vehicle. n=5-8.

(C) Levels of serum 5-HT (left) and colon 5-HT (right) in GF Swiss Webster mice intrarectally injected with α-tocopherol (2.25 mg/kg), deoxycholate (125 mg/kg), oleanolate (0.457 mg/kg) or vehicle. Data are normalized to serum 5-HT levels at 30 min after injection of GF mice with vehicle. n=2-5.

(D) Total time for transit of orally administered carmine red solution through the GI tract in GF C57Bl/6 mice intrarectally injected with a-tocopherol (2.25 mg/kg) or deoxycholate (125 mg/kg) and co-injection of PCPA or vehicle. n=3.

(E) Platelet activation, as measured by percentage of large, high granularity (FSC^{high}, SSC^{high}) events after collagen stimulation relative to unstimulated controls. n=3.

Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n.s.=not statistically significant. SPF=specific pathogen-free (conventionally-colonized), GF=germ-free, PCPA=parachlorophenylalanine

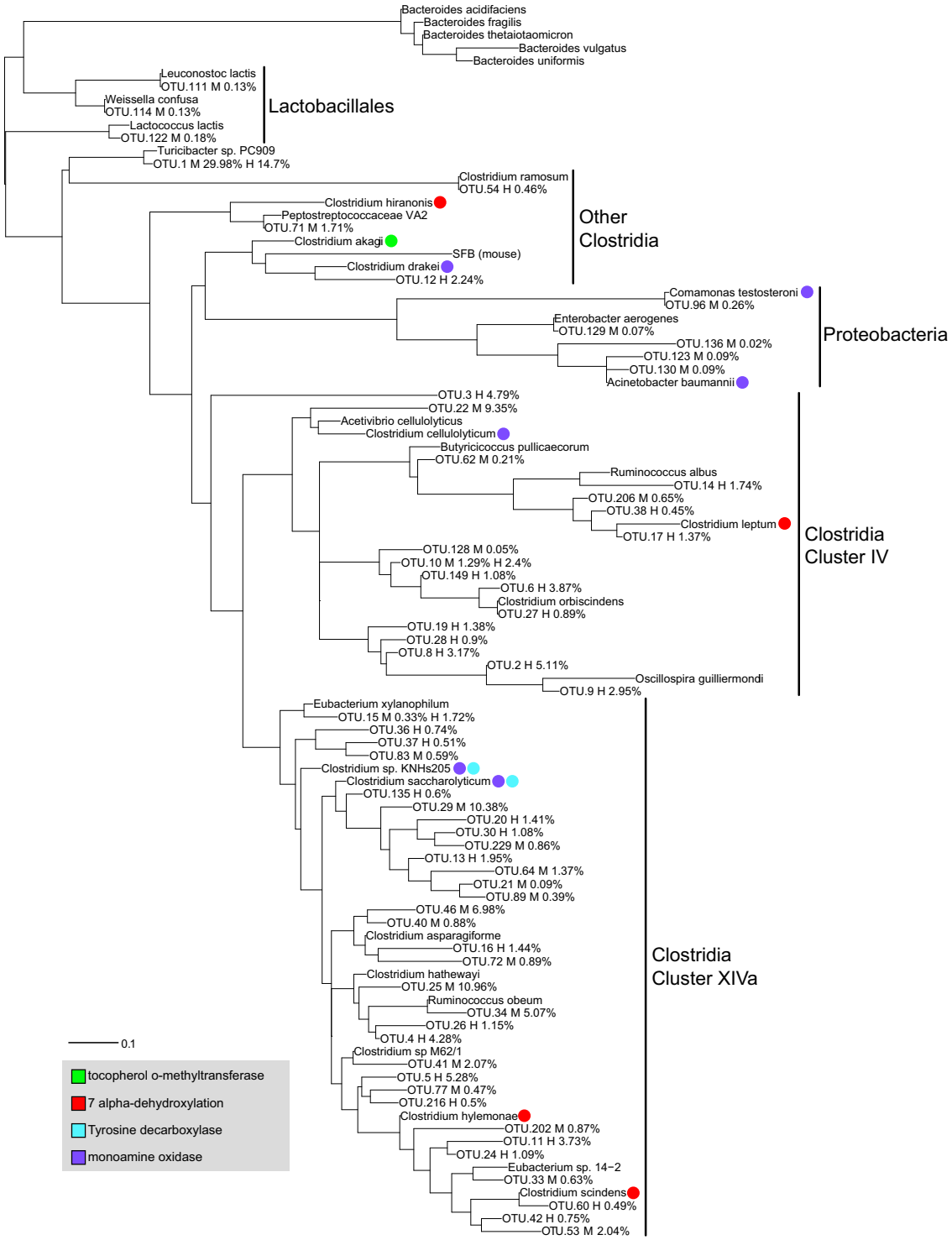

Figure S7. Phylogenetic Analysis of OTUs from Feces of Mice Colonized with Indigenous Spore-Forming Bacteria, Related to Figure 6

Phylogenetic tree, based on nearest-neighbor analysis of 16S rRNA gene sequences from fecal samples of mice colonized with Sp (M, n=3) or hSp (H, n=4), displaying Sp and hSp operational taxonomic units (OTUs) relative to reference species with reported 7α-dehydroxylation activity (red circles) or gene homology to enyzmes involved in metabolism of α-tocopherol (tocopherol o-methyltransferase), tyramine (tyrosine decarboxylase) and serotonin (among other monomines, monoamine oxidase). Relative abundances of OTUs are indicated in parentheses. Select *Bacteroides* species found to have no effect on colon and serum 5-HT levels (Figure 3A) are included.