Ingredients	Concentration in Medium
	(mg/L or mL of solution)
Resazurin (0.01% w/v stock)	1 mL
KH <sub>2</sub> PO <sub>4</sub>	2000
K <sub>2</sub> HPO <sub>4</sub>	2000
MgCl <sub>2</sub> · 6H <sub>2</sub> O	200
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5000
L-glycine	75.1
L-valine	117.2
L-leucine	131.2
L-isoleucine	131.2
L-methionine	149.2
L-histidine	155.2
L-arginine	174.2
L-phenylalanine	165.2
L-tyrosine	181.2
L-tryptopnan	204.23
NaHCO <sub>3</sub> (10% W/V stock, pH 7)	25 mL
Cysteine HCI (pH 6, 5% W/V stock)	10 ML
Glucose Stock (500 mM) or ddH20°	40.0 mL
Trace Element Solution (pH 7.0) $(g/L)^b$	10.0 mL
Nitrilotriacetic acid	12.8
FeSO <sub>4</sub> · 7H <sub>2</sub> O	0.1
MnCl <sub>2</sub> · 4H <sub>2</sub> O	0.1
$CoCl_2 \cdot 2H_2O$	0.17
CaCl <sub>2</sub> · 2H <sub>2</sub> O	0.1
ZnCl <sub>2</sub>	0.1
CuCl <sub>2</sub>	0.01
H <sub>3</sub> BO <sub>3</sub>	0.01
NaMoO₄ · 2H₂O	0.01
NaCl	1
NaSeO₃	0.017
NiCl <sub>2</sub> · 6H <sub>2</sub> O	0.026
$NaWO_4 \cdot 2H_2O$	0.1
General Vitamin Solution (mg/L) <sup>b</sup>	10.0 ml
Biotin (B <sub>7</sub> )	2
Folic acid $(B_9)$	2
Pvridoxamine HCI (B <sub>6</sub> )	10
Thiamine HCI (B <sub>1</sub> )	5
Riboflavin HCI (B <sub>2</sub> )	5
Nicotinic acid (B <sub>3</sub> )	5
Pantothenic acid $(B_5)$	5
Cvanocobalamin (B <sub>12</sub> )	- 1
p-amino benzoic acid (PABA)	5
Lipoic acid	5
•	

# Supplementary Table 1. Standard amino acid complete (SACC) medium for culturing *Clostridium sporogenes* ATCC 15579.

<sup>a</sup>Medium was prepared with either sterile water or glucose depending on the experiment.

<sup>b</sup>Masses listed below indicate the amount of each compound per liter of stock solution.

BEI number	Strain name	Genome Assembly <sup>a</sup>	Growth in RCM?
<u>HM-36</u>	Clostridium sp. 7_2_43FAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-79</u>	Ruminococcaceae bacterium D16	Genome sequenced, assembled, and annotated	No
<u>HM-81</u>	Acidaminococcus sp. D21	Genome sequenced, assembled, and annotated	Yes
<u>HM-84</u>	Clostridium sp. D5	Genome sequenced, assembled, and annotated	Yes
<u>HM-85</u>	Coprobacillus sp. D6 (29_1)	Genome sequenced and assembled (no annotation)	Yes
<u>HM-173</u>	Clostridium innocuum 6_1_30	Genome sequenced (no assembly or annotation)	Yes
<u>HM-176</u>	Coprobacillus sp. 8_2_54BFAA	Genome sequenced (no assembly or annotation)	Yes
<u>HM-178</u>	Eubacterium sp. 3_1_31	Genome sequenced, assembled, and annotated	Yes
<u>HM-182</u>	Lachnospiraceae bacterium 6_1_37FAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-184</u>	Propionibacterium sp. 5_U_42AFAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-229</u>	Bacillus smithii 7_3_47FAA	Genome sequenced, assembled, and annotated	No
<u>HM-231</u>	Bacillus sp. 7_6_55CFAA_CT2	Genome sequenced, assembled, and annotated	Yes
<u>HM-287</u>	Clostridium sp. HGF2	Genome sequenced, assembled, and annotated	Yes
<u>HM-303</u>	Clostridium orbiscindens 1_3_50AFAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-304</u>	Collinsella sp. 4_8_47FAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-306</u>	[Clostridium] clostridioforme 2_1_49FAA	Genome sequenced, assembled, and annotated	Yes
<u>HM-307</u>	[Clostridium] aldenense WAL-18727	Genome sequenced (no assembly or annotation)	Yes
<u>HM-308</u>	Hungatella hathewayi WAL-18680	Genome sequenced, assembled, and annotated	Yes
<u>HM-309</u>	Clostridium symbiosum WAL-14163	Genome sequenced, assembled, and annotated	Yes
<u>HM-310</u>	Clostridium perfringens WAL-14572	Genome sequenced, assembled, and annotated	Yes
<u>HM-315</u>	Clostridium citroniae WAL-17108	Genome sequenced, assembled, and annotated	Yes
<u>HM-317</u>	[Clostridium] clostridioforme WAL-7855	Genome sequenced, assembled, and annotated	Yes
<u>HM-318</u>	[Clostridium] bolteae WAL-14578	Genome sequenced, assembled, and annotated	Yes
<u>HM-319</u>	[Clostridium] symbiosum WAL-14673	Genome sequenced, assembled, and annotated	Yes
<u>HM-473</u>	Faecalibacterium cf. prausnitzii KLE1255	Genome sequenced, assembled, and annotated	Yes
<u>HM-634</u>	Clostridium sp. L2-50	Genome sequenced, assembled, and annotated	No
<u>HM-635</u>	Clostridium sp. M62/1	Genome sequenced, assembled, and annotated	Yes
<u>HM-793</u>	Coprococcus sp. HPP0074	Genome sequenced, assembled, and annotated	Yes
<u>HM-794</u>	Coprococcus sp. HPP0048	Genome sequenced, assembled, and annotated	Yes

#### Supplementary Table 2. Culturing HMP reference strains.

<u>HM-1033</u>	Clostridium sp. KLE 1755	Genome sequenced, assembled, and annotated	Yes
<u>HM-1038</u>	Clostridium bolteae CC43_001B	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1039</u>	Clostridium cadaveris CC40_001C	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1040</u>	Clostridium cadaveris CC44_001G	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1041</u>	Clostridium cadaveris CC88A	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1043</u>	Clostridium lentocellum CC70A	Genome sequenced (no assembly or annotation)	No
<u>HM-1044</u>	Clostridium orbiscindens CC43_001K	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1049</u>	Paenibacillus barengoltzii CC33_002B	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1051</u>	Peptostreptococcus anaerobius CC14N	Genome sequenced (no assembly or annotation)	Yes
<u>HM-1056</u>	Ruminococcus gnavus CC55_001C	Genome sequenced, assembled, and annotated	Yes
N/A	Clostridium difficile 630 ∆Erm	Genome sequenced, assembled, and annotated	Yes
N/A	Clostridium sporogenes ATCC 15579	Genome sequenced, assembled, and annotated	Yes

<sup>a</sup> Genome finishing status for relevant HMP reference genomes at the onset of this study.

Su	Supplementary Table 3. Metabolite levels in resting cell suspensions of <i>C. sporogenes</i> WT and mutants.												
							Metaboli	te detecte	ed <sup>a</sup>				
	Substrate						4-OH-	4-0H-	4-0H-				
	Added	Phenyl- alanine	Phenyl-	Phenyl- lactate	Pheny- lacrylate	Tyrosine	phenyl-	phenyl- lactate	phenyl- acrylate	Tryptophan	Indole-	Indole-	Indole- acrylate
	Phenylpyruvate	N.D.	1650 ± 130	4.39 ± 0.16	4.31 ± 0.091	Tyrosine	pyruvate	laolate	dorylate	Tryptophan	pyravate	luolulo	dorylate
	Phenyllactate	55 ± 9.9	40 ± 9.1	672 ± 90	N.D.								
	Phenylacrylate	N.D.	N.D.	N.D.	N.D.								
	Phenylpropionate	1270 ± 290	1080 ± 460	720 ± 320	810 ± 290								
	Phenylacetate	31.7 ± 7.0	93.3 ± 17	N.D.	N.D.								
	4-OH- phenylpyruvate					N.D.	909 ± 200	N.D.	N.D.				
	4-OH- phenyllactate					36.2 ± 10	12.2 ± 1.1	3010 ± 780	N.D.				
۲V	4-OH-					N.D.	N.D.	N.D.	N.D.				
_	4-OH-					492 ±	942 ±	220 ±	579 ±				
	phenylpropionate					203	38	64	2.5				
	4-OH- phenylacetate					18.7 ± 9.2	27.0 ± 11	N.D.	N.D.				
	Indolepyruvate									N.D.	N.D.	N.D.	N.D.
	Indolelactate									11.6 ± 1.6	51.8 ± 4.2	466 ± 33	N.D.
	Indoleacrylate									N.D.	N.D.	N.D.	1020 ± 93
	Indolepropionate									18.1 ± 3.1	19.2 ± 1.3	97.7 ± 5.2	29.9 ± 0.99
	Indoleacetate									N.D.	70.4 ± 1.5	N.D.	N.D.
	Phenylpyruvate	14.1 ± 2.8	1840 ± 270	5.76 ± 0.34	4.20 ± 0.070								
4	Phenyllactate	79.7 ± 24	190 ± 22	303 ± 60	N.D.								
por	Phenylacrylate	N.D.	N.D.	N.D.	N.D.								
	Phenylpropionate	452 ± 280	1390 ± 370	744 ± 180	880 ± 110								
	Phenylacetate	N.D.	N.D.	N.D.	N.D.								

	4-OH- phenylpyruvate 4-OH- phenyllactate 4-OH- phenylacrylate 4-OH- phenylpropionate 4-OH- phenylacetate					9.57 ± 3.4 48 ± 9.7 N.D. 711 ± 230 N.D.	2010 ± 990 60.1 ± 10 N.D. 157 ± 130 3.07 ± 0.86	19.2 ± 6.0 2976 ± 740 3.96 ± 0.65 353 ± 99 N.D.	N.D. N.D. 868 ± 220 N.D.				
	Indolepyruvate									N.D.	N.D.	N.D.	N.D.
	Indolelactate									29.6 ± 5.4	65.3 ± 8.3	498 ± 51	N.D.
	Indoleacrylate									N.D.	N.D.	0.447 ± 0.16	1200 ± 57
	Indolepropionate									18.7 ± 3.5	21.9 ± 1.8	88.8 ± 12	37.0 ± 1.5
	Indoleacetate									N.D.	77.4 ± 7.0	N.D.	N.D.
	Phenylpyruvate	N.D.	2140 ± 140	4.29 ± 0.073	4.24 ± 0.079								
	Phenyllactate	136 ± 23	1330 ± 48	1180 ± 350	2.35 ± 0.99								
	Phenylacrylate	N.D.	N.D.	N.D.	210 ± 40								
	Phenylpropionate	N.D.	N.D.	N.D.	1140 ± 290								
	Phenylacetate	21.1 ± 6.7	91.3 ± 16	N.D.	N.D.								
Ŋ	4-OH- phenylpyruvate					N.D.	1390 ± 490	N.D.	N.D.				
flc	4-OH- phenyllactate					336 ± 94	76.8 ± 16	2110 ± 550	N.D.				
	4-OH- phenylacrylate					N.D.	N.D.	N.D.	372 ± 50				
	4-OH- phenylpropionate					N.D.	N.D.	N.D.	912 ± 150				
	4-OH- phenylacetate					6.48 ± 4.0	9.11 ± 2.3	N.D.	N.D.				
	Indolepyruvate									N.D.	N.D.	N.D.	N.D.
	Indolelactate									46.2 ± 7.3	00.4 ± 7.4	575 ± 19	N.D.

	Indoleacrylate									N.D.	N.D.	N.D.	1230 ± 40
	Indolepropionate									N.D.	N.D.	N.D.	2.91 ± 0.24
	Indoleacetate									N.D.	89.5 ± 2.7	N.D.	N.D.
	Phenylpyruvate	N.D.	1650 ± 140	4.25 ± 0.054	4.06 ± 0.006								
	Phenyllactate	100 ± 15	594 ± 120	870 ± 210	N.D.								
	Phenylacrylate	N.D.	N.D.	N.D.	N.D.								
	Phenylpropionate	48 ± 4.2	80 ± 9.1	N.D.	740 ± 150								
	Phenylacetate	17.7 ± 4.4	72.7 ± 13	N.D.	N.D.								
	4-OH-					N.D.	1590 ±	N.D.	N.D.				
	4-OH-					282 ±	44.5 ±	2460 ±					
A	phenyllactate					46	10	840	N.D.				
acd	phenylacrylate					N.D.	N.D.	N.D.	N.D.				
	4-OH- phenylpropionate					N.D.	N.D.	N.D.	512 ± 140				
	4-OH-					5.43 ±	10.2 ±	N.D.	N.D.				
	Indolepyruvate					1.5	2.0			N.D.	N.D.	N.D.	N.D.
	Indolelactate									34.9 ± 3.2	73.3 ± 0.67	554 ± 31	N.D.
	Indoleacrylate									N.D.	N.D.	N.D.	1230 ± 96
	Indolepropionate									N.D.	N.D.	1.80 ± 0.068	10.6 ± 0.61
	Indoleacetate									N.D.	82.1 ± 5.9	N.D.	N.D.
	Phenylpyruvate	N.D.	1670 ± 60	4.54 ± 0.31	4.38 ± 0.039								
H	Phenyllactate	7.83 ± 2.0	31.6 ± 1.1	358 ± 32	N.D.								
ŧk	Phenylacrylate	N.D.	N.D.	N.D.	193 ± 25								
	Phenylpropionate	68.4 ± 22	173 ± 9.6	331 ± 210	734 ± 120								

Phenylacetate	75.5 ± 12	1010 ± 100	14 ± 1.0	N.D.								
4-OH- phenylpyruvate					N.D.	1460 ± 410	N.D.	N.D.				
4-OH- phenyllactate					N.D.	N.D.	1300 ± 120	N.D.				
4-OH- phenylacrylate					N.D.	N.D.	228 ± 49	N.D.				
4-OH-					N.D.	N.D.	105 ± 15	460 ± 110				
4-OH-					60.2 ±	57.5 ± 12	11.8 ±	N.D.				
Indolepyruvate									N.D.	N.D.	N.D.	N.D.
Indolelactate									N.D.	0.864 ± 0.54	408 ± 19	N.D.
Indoleacrylate									N.D.	N.D.	1.56 ± 0.31	1120 ± 180
Indolepropionate									2.93 ± 0.18	3.86 ± 0.20	162 ± 14	22.0 ± 2.7
Indoleacetate									N.D.	81.3 ± 8.7	N.D.	N.D.

<sup>a</sup> Experiments were performed in triplicate and data are presented as means ± standard errors from the mean.

## **Supplementary Table 4.** Summary of studies identifying AAA oxidative and reductive metabolites in host body fluids

			Metal				
	Alternative						-
Metabolite	name	Wikoff <sup>c</sup>	Aronov <sup>c</sup>	Guo	Spaapen	Loke	Comments
Phenylacetate		PGly	PGIn	P <sub>free</sub> , P <sub>Gln</sub>	n.d.	P <sub>free</sub> , U <sub>free</sub>	
Phenylpyruvate		n.d.	n.d.	n.d.	n.d.	n.d.	Elevated in Phenylketonuria patients
Phenyllactate		n.d.	n.d.	Pfree	U <sub>free</sub>	n.d.	
Phenylacrylate	<i>t</i> -cinnamate	PGly	PGly	P <sub>Gly</sub>	n.d.	U <sub>free</sub>	
Phenylpropionate	hydrocinnamate	PGly	n.d.	Pfree	n.d.	n.d.	
4-hydroxyphenylacetate		n.d.	n.d.	Pfree	n.d.	Ufree	
4-hydroxyphenylpyruvate		n.d.	n.d.	n.d.	n.d.	n.d.	Elevated in Tyrosinemia patients
4-hydroxyphenyllactate		n.d.	n.d.	$P_{free}$	Ufree	n.d.	
4-hydroxyphenylacrylate	<i>p</i> -coumarate	n.d.	n.d.	n.d.	n.d.	Pfree	
4-hydroxyphenylpropionate		n.d.	n.d.	Pfree	n.d.	$P_{free},U_{free}$	
Indoleacetate		n.d.	Pfree	P <sub>free</sub> , P <sub>Gln</sub>	n.d.	n.d.	
Indolepyruvate		n.d.	n.d.	n.d.	n.d.	n.d.	
Indolelactate		n.d.	Pfree	$P_{free}$	n.d.	n.d.	
Indoleacrylate		n.d.	n.d.	$P_{free}$	n.d.	n.d.	
Indolepropionate		Pfree	Pfree	$P_{free}$	n.d.	n.d.	

<sup>a</sup> Letters indicate whether compounds were detected in plasma (P) or urine (U), and subscripts indicate whether they were detected as free acids (free), glycine conjugates (Gly), or glutamine conjugates (Gln).

<sup>b</sup> References are as follows: Wikoff<sup>27</sup>, Aronov<sup>28</sup>, Guo<sup>29</sup>, Spaapen<sup>30</sup>, Loke<sup>31</sup>.

<sup>c</sup> Compounds indicated in bold font were elevated in conventional vs. germ free mice (Wikoff), or in humans with intact colons vs. colectomy patients (Aronov).

### Supplementary Table 5. Intron retargeting and sequencing primers for ClosTron disruptions in *Clostridium sporogenes* ATCC 15579.

Gene (Locus ID)	Targeted Region <sup>a</sup>	Sequencing Primers <sup>b</sup>
fldC (CLOSPO_00311)	561a	311F: TGGGGAATATGATATGTTGTCTGGCATGATG
		311R: TGTTCAGCTAATCTATCCATTGGTGTATTCGC
acdA (CLOSPO_00312)	775s	312F: TCGCAAAAACAGATATGAGCA
		312R: ACCACCATGAATTTGTAGTGC
fldH (CLOSPO_00316)	736s	316F: TAGGAGTTATAGGAACTGGAA
		316R: TTGGTTTTTACAAGTTCCTTT
porA (CLOSPO_00147)	1001a	147F: TGGCTAAAGAAGAAGGTATGA
		147R: TCAAACATATCACAGGCAAAA
fldZ (CLOSPO_02780)	1546s	2780F: TTGGCGGAAATATTGTACCAG
		2780R: TTTAGCTACTTCATATGCACT

<sup>a</sup> Targeting regions were designed using the Intron design tool on the ClosTron website

(<u>http://www.clostron.com/clostron2.php</u>). The regions listed indicate the nucleotide position within the gene where the intron is inserted and the a/s designation indicates whether the intron integrates in the antisense or sense strand.

<sup>b</sup> Primers were designed to amplify the region containing the expected intron after integration. Primers were synthesized by Integrated DNA Technologies (Coralville, IA).

Compound	Precursor	Product	Fragmentor	Collision Energy
Compound	( <i>m/z</i> )	( <i>m/z</i> )	(V)	(eV)
Phenylalanine	• •			
Phenylpyruvate	163	91.1	65	5
Phenyllactate	165.1	147.1	90	9
Phenylacrylate	147	103	70	9
Phenylpropionate	149.1	105.1	80	9
Phenylacetate	135	91.1	45	5
Tyrosine				
4-OH-Phenylpyruvate	179	107	69	5
4-OH-Phenyllactate	181.1	162.9	90	9
4-OH-Phenylacrylate	163	119	76	12
4-OH-Phenylpropionate	165.1	59.1	96	9
4-OH-Phenylacetate	151	107	45	5
Tryptophan				
Indolepyruvate	202.1	174	66	1
Indolelactate	204.1	158	90	13
Indoleacrylate	186.1	142	74	13
Indolepropionate	188.1	59.1	90	13
Indoleacetate	174.1	130	74	5

### Supplementary Table 6. Ion transitions and instrument parameters for AAA metabolite detection.

	<i>C. bolteae</i> CC43_001B	<i>C. cadaveris</i> CC40 _001C	<i>C. cadaveris</i> CC44_001G	C. cadaveris CC88A	<i>P. anaerobius</i> CC14N
BioProject Accession Number	PRJNA71569	PRJNA71541	PRJNA71543	PRJNA71545	PRJNA71563
Combined length	6,094,255 bp	3,754,827 bp	3,562,150 bp	3,556,482 bp	2,043,901 bp
Percent GC content	49.18%	31.03%	30.97%	31.51%	30.51%
Number of sequences	102	221	142	78	18
Mean sequence length	59,748 bp	16,990 bp	25,086 bp	45,596 bp	113,550 bp
Sequence n50	1,681,030 bp	159,835 bp	307,414 bp	367,796 bp	1,583,849 bp
Sequence n90	298,438 bp	33,731 bp	58,107 bp	55,850 bp	275,990 bp
Longest sequence	2,058,090 bp	371,381 bp	485,405 bp	631,552 bp	1,583,849 bp
Average coverage depth	256X	461X	556X	569X	366X
% Corrected Reads aligned	95.5%	95.0%	95.4%	95.9%	94.2%
Phenyllactate gene cluster average coverage depth	NA	425X	629X	607X	461X
<i>rpoB</i> average coverage depth	290X	445X	684X	642X	496X
Phenyllactate dehydratase cluster GenBank accession number	N/A	BK010268	BK010335	BK010336	BK010337
RpoB GenBank accession number	BK010331	BK010269	BK010332	BK010333	BK010334

#### Supplementary Table 7. Summary of genome assemblies

### Supplementary Table 8. Oligonucleotide primers for qPCR analysis of bacterial community membership.

Organism	Genome Region <sup>a</sup>	qPCR Primers
Bacteroides vulgatus ATCC 8482	1252631 - 1252765	Bvu_qPCR_F: TCCGGCTCTGCTCGTCAAGA
		Bvu_qPCR_R: GCTTCCACTGCCTGCAACCA
Bacteroides thetaiotaomicron VPI-5482	16052 - 16154	Bth_qPCR_F: CGGGATTGTTGCGGCAGACA
		Bth_qPCR_R: CGCGTTCATCCGTGCCTTCA
Parabacteroides distasonis ATCC 8503	375224 - 375316	Pdi_qPCR_F: CGGTTTCGTGCGGGTGATGA
		Pdi_qPCR_R: CGGAAGGTGGCGTTCGTGTT
Edwardsiella tarda ATCC 23685	526079 - 526227	Eta_qPCR_F: ACGCCCACTCACGGATCTGA
		Eta_qPCR_R: ACTCCCGCTCGCTCAGGTTA
Eubacterium rectale ATCC 33656	1863134 - 1863277	Ere_qPCR_F: GGTGCAGGCGAGTTTCCCTT
		Ere_qPCR_R: GCCAAACAGCCCCGACATCA
Bifidobacterium breve UCC2003	1357556 - 1357701	Bbr_qPCR_F: ACCCCACGCACACCAATCAC
		Bbr_qPCR_R: GACCGCGCCGATGAAGTTGA
Clostridium scindens ATCC 35704	863 - 978	Csc_qPCR_F: TTCAGGAGAAATGCCAGCCAGT
		Csc_qPCR_R: GTTCTGCCACGGTCGTTAGC
Clostridium sporogenes ATCC 15579	197533 - 197672	Csp_qPCR_F: TTGGCTCTGCACCGGGAATC
		Csp dPCR R: CTGCAAACGCCGTCCCTCTT

<sup>a</sup> qPCR primers were designed using Geneious v10.1.3 with the integrated Primer3 tool. The regions were designed to represent single copy genes and to avoid dynamic regions of the genome (e.g., not within a mobile genetic element). The primers were then used to BLAST against the other organisms in the community to ensure that no cross-priming would occur.

#### **Supplemental References**

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