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SUPPLEMENTARY INFORMATION

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GABA-modulating bacteria of the human gut microbiota

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Supplemental Information

Title: GABA Modulating Bacteria of the Human Gut Microbiota

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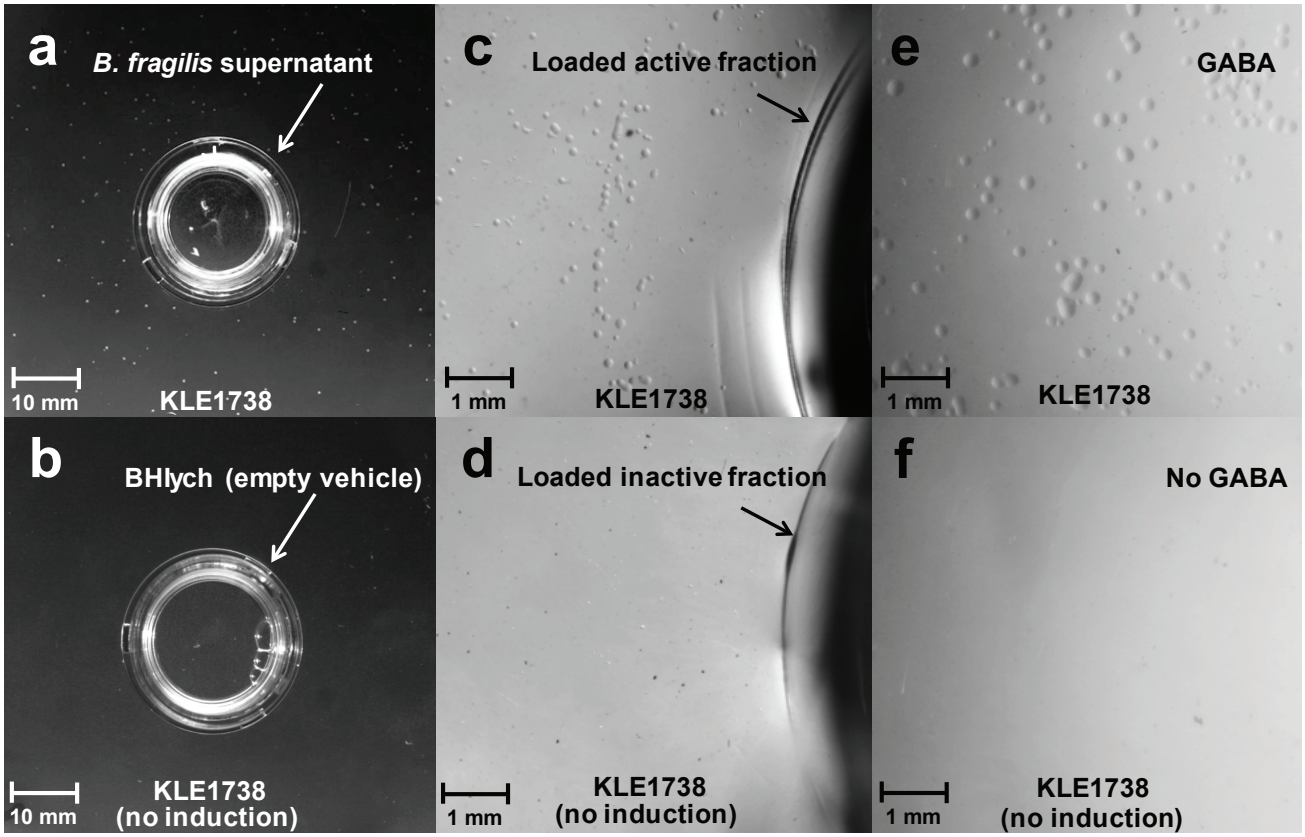
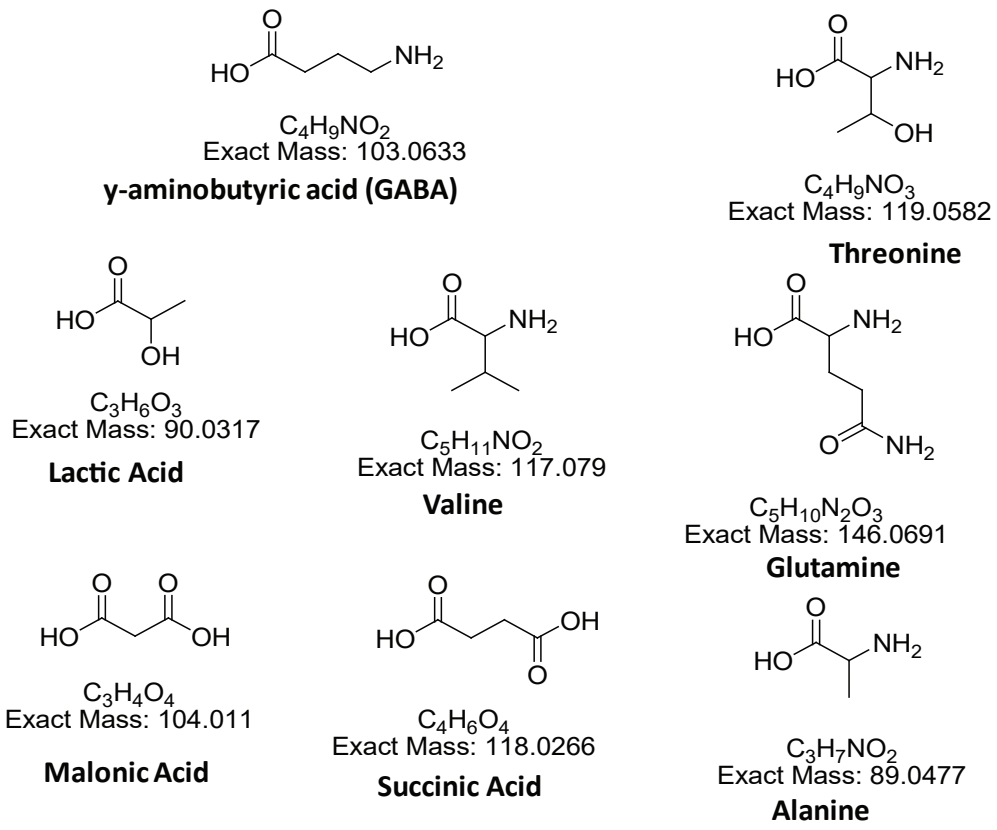
Supplemental Information Fig. 1



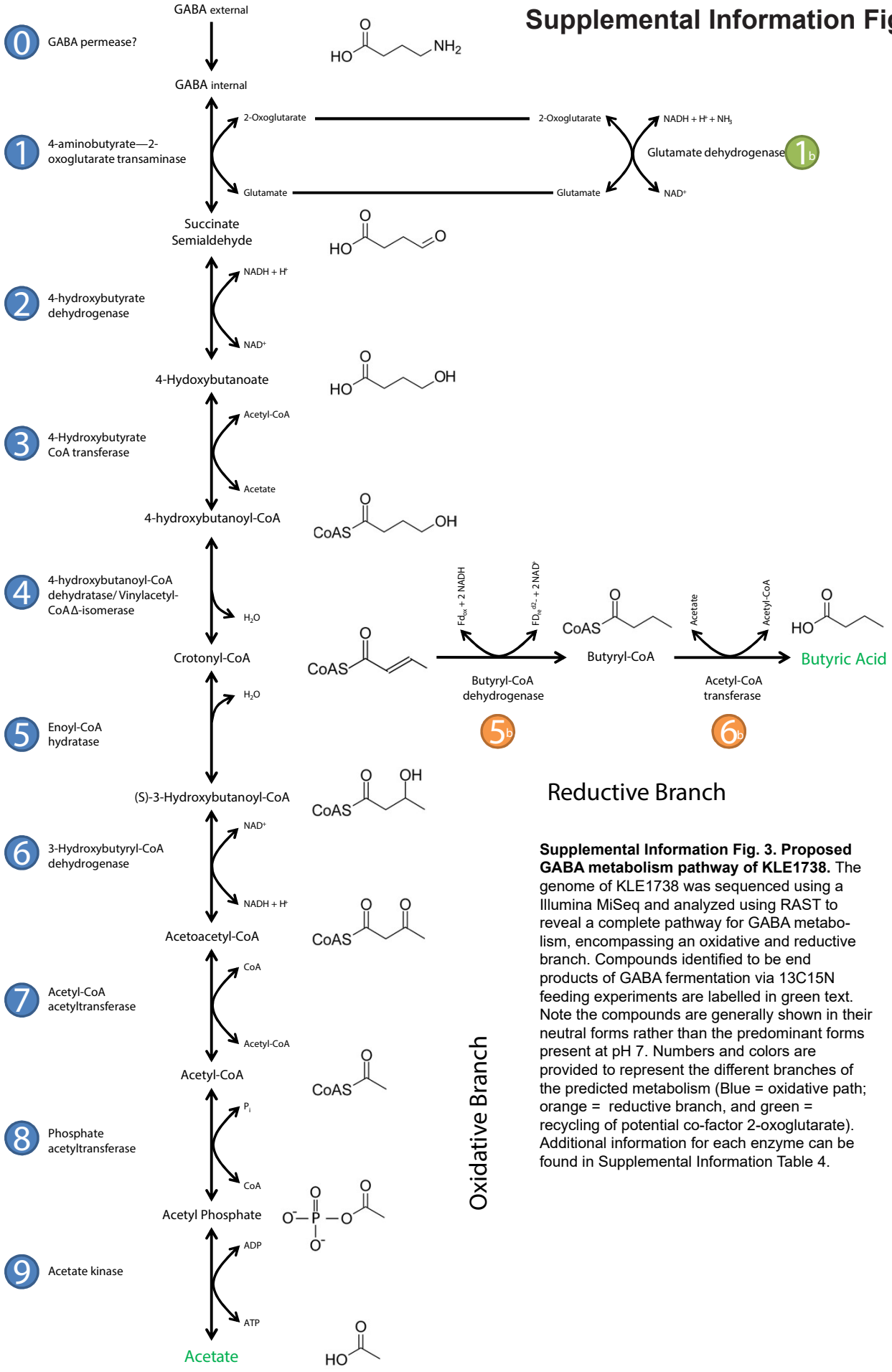
Supplemental Information Fig. 1. Identification of GABA as the *B. fragilis*-secreted growth factor for KLE1738. (A) B. fragilis KLE1758 supernatant, or (B) the base medium, Brain Heart Infusion broth with 2.5 g/L yeast extract, 0.1% cysteine, and 15 mg/L hemin (BHIych) medium, were loaded in cell-culture inserts (0.22µm pore size, Millipore) placed on top of solid BHIych medium with bead-spread KLE1738 cells. Supernatant of *B. fragilis* induced the growth of KLE1738. (C) Bioassay-driven extraction/fractionation with ethyl acetate and methanol led to isolation of an active fraction. (D) All other fractions were inactive, with an example highlighted here. (E) NMR revealed GABA in the inducing fraction (amongst 9 other compounds – Supplementnal Information Fig. 2), and pure, commercially available GABA induced growth of KLE1738. (F) Control without GABA. Growth induction was searched for on the entirety of the Petri plate. All experiments were performed in duplicate.

Supplemental Information Fig. 2



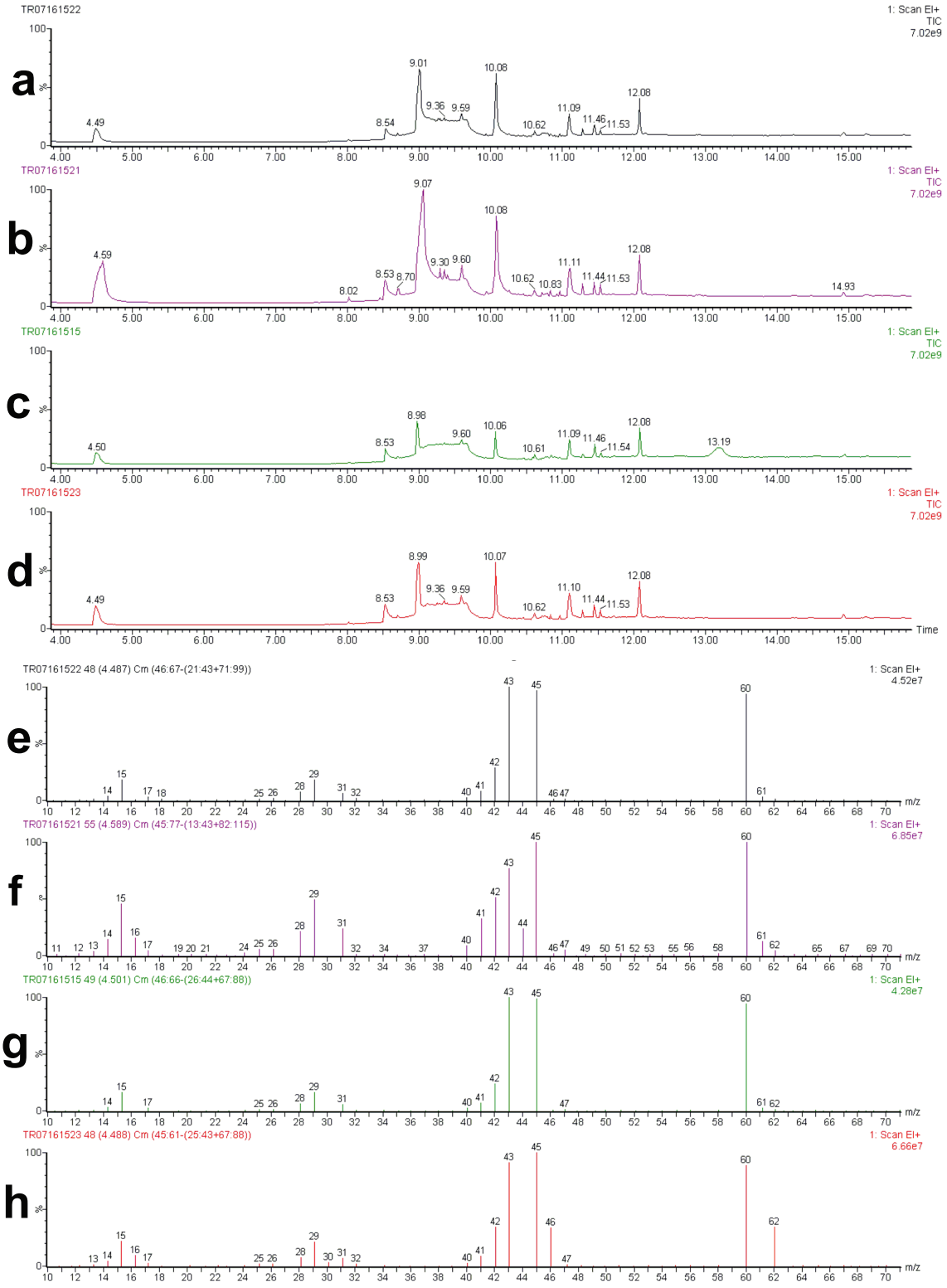
Supplemental Information Fig. 2. Compounds in the final active fraction of *B. fragilis* KLE1758 supernatant which induced growth of KLE1738. A final active fraction of *Bacteroides fragilis* KLE1758 supernatant was directly applied to NMR analysis including 1H, 13C, 1H-1H COSY, TOCSY, HSQC, and HMBC NMR experiments to identify its constituents. Ten compounds were identified, including GABA, threonine, lysine, arginine, lactic acid, valine, glutamine, malonic acid, succinic acid, and alanine. All NMR experiments were carried out on a Varian INOVA 600 MHz NMR spectrometer equipped with an indirect detection probe.

Supplemental Information Fig. 3



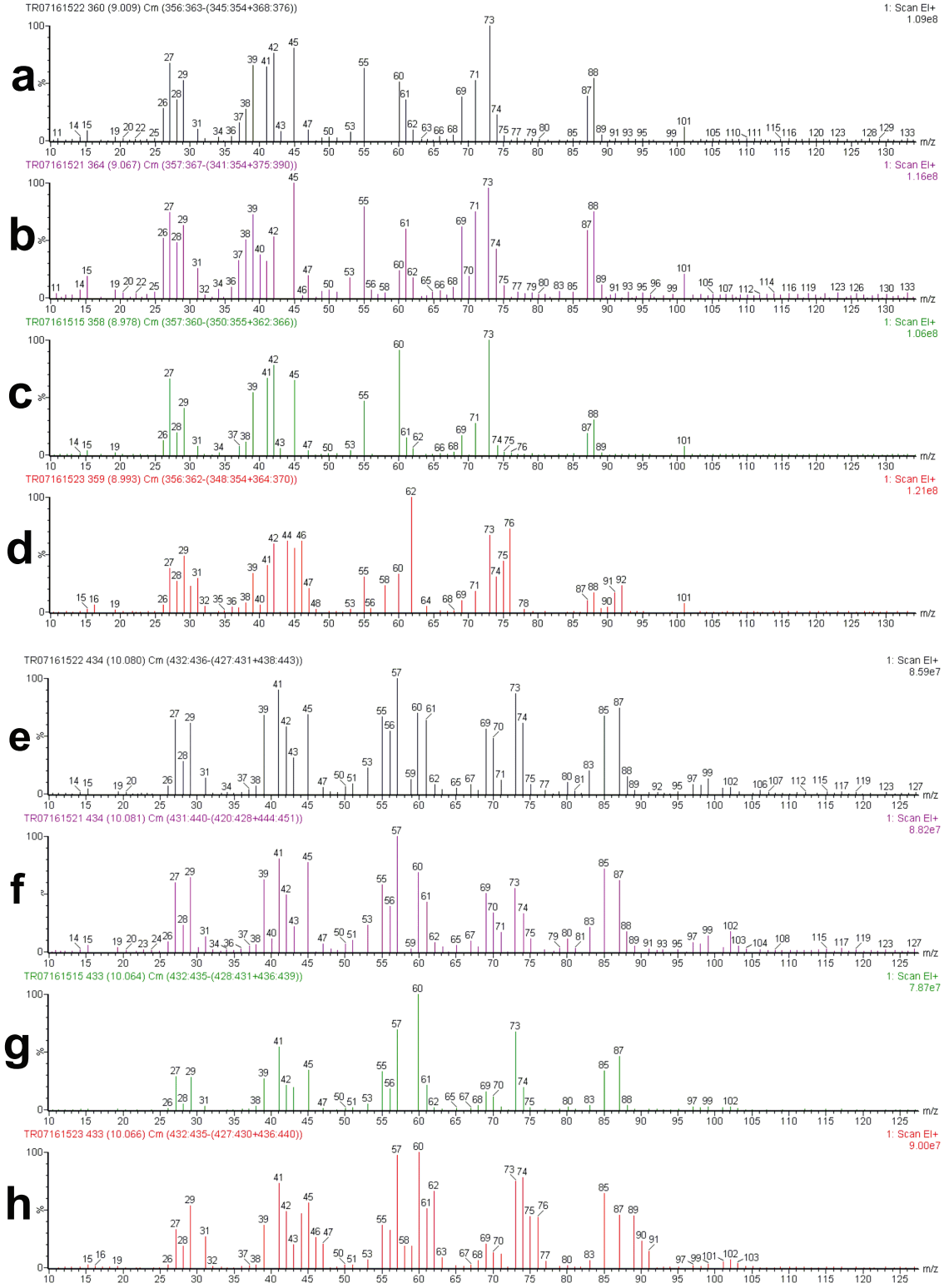
Supplemental Information Fig. 3. Proposed GABA metabolism pathway of KLE1738. The genome of KLE1738 was sequenced using a Illumina MiSeq and analyzed using RAST to reveal a complete pathway for GABA metabolism, encompassing an oxidative and reductive branch. Compounds identified to be end products of GABA fermentation via 13C15N feeding experiments are labelled in green text. Note the compounds are generally shown in their neutral forms rather than the predominant forms present at pH 7. Numbers and colors are provided to represent the different branches of the predicted metabolism (Blue = oxidative path; orange = reductive branch, and green = recycling of potential co-factor 2-oxoglutarate). Additional information for each enzyme can be found in Supplemental Information Table 4.

Supplemental Information Fig. 4



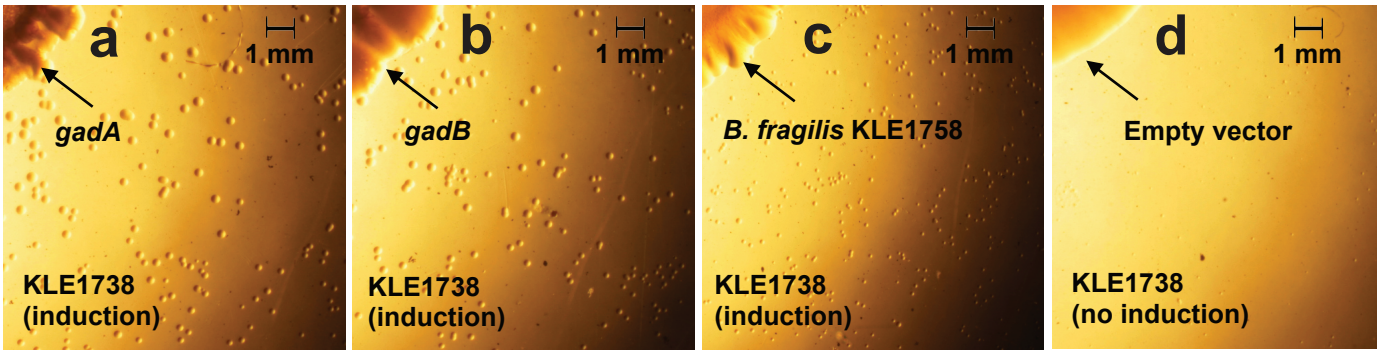
Supplemental Information Fig. 4. GC trace patterns for butyric acid and hexanoic acid; EI-MS fragmentation pattern for acetic acid of KLE1738 cell mass post-feeding commercial, synthesized, and synthesized $^{13}C,^{15}N$-labeled GABA. GC trace for agar extract samples where KLE1738 was grown on media containing A) and B) unlabeled GABA (two different commercial sources), C) unlabeled GABA biosynthesized from glutamic acid, and D) $^{13}C,^{15}N$-labeled GABA biosynthesized from $^{13}C,^{15}N$-labeled glutamic acid. Acetic acid retention time (tR) = 4.49 min, butyric acid tR = 8.99 min, hexanoic acid tR = 10.08 min. EI-MS fragmentation pattern for acetic acid (GC tR = 4.49 min) from agar extract samples where KLE1738 was grown on media containing E) and F) unlabeled GABA (two different commercial sources), G) unlabeled GABA biosynthesized from glutamic acid, and H) $^{13}C,^{15}N$-labeled GABA biosynthesized from $^{13}C,^{15}N$-labeled glutamic acid. MW of acetic acid = 60.05 g/mol. Data represent a single biological experiment.

Supplemental Information Fig. 5



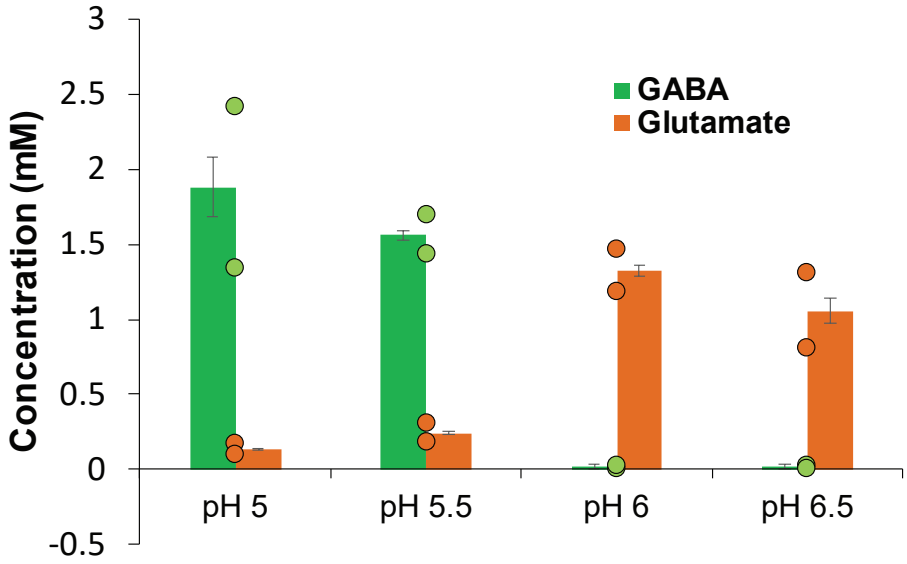
Supplemental Information Fig. 5. EI-MS fragmentation patterns for butyric acid and hexanoic acid of KLE1738 cell mass post-feeding commercial, synthesized, and synthesized $^{13}C,^{15}N$-labeled GABA. EI-MS fragmentation pattern for butyric acid (GC tR = 8.99 min) from agar extract samples where KLE1738 was grown on media containing A) and B) unlabeled GABA (two different commercial sources), C) unlabeled GABA biosynthesized from glutamic acid, and D) $^{13}C,^{15}N$-labeled GABA biosynthesized from $^{13}C,^{15}N$-labeled glutamic acid. MW of butyric acid = 88.11 g/mol. EI-MS fragmentation pattern for hexanoic acid (GC tR = 10.08 min) from agar extract samples where KLE1738 was grown on media containing E) and F) unlabeled GABA (two different commercial sources), G) unlabeled GABA biosynthesized from glutamic acid, and H) $^{13}C,^{15}N$-labeled GABA biosynthesized from $^{13}C,^{15}N$-labeled glutamic acid. Hexanoic acid ionizes easily under the conditions in this experiment, explaining why minimal peaks were observed at MW 116.1 g/mnol. Data represent a single biological experiment.

Supplemental Information Fig. 6



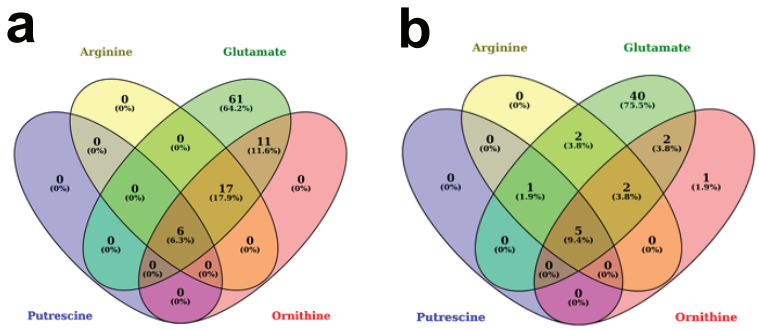
Supplemental Information Fig. 6. Overexpression of glutamate decarboxylase A or glutamate decarboxylase B of *Escherichia coli* K12 induces the growth of KLE1738. *E. coli* clones harboring native glutamate decarboxylases (gadA, gadB) in the pCA24N IPTG inducible high-copy number vector, were tested for GABA production via co-cultivation assay with KLE1738 on FAAy (described in Fig 1). (A) Overexpression of (A) gadA or (B) gadB induced KLE1738 to similarly observed levels as seen with (C) *B. fragilis* KLE1758, while overexpression of (D) empty vector did not. E. coli strains were taken from the ASKA library, and grown in the presence of 1mM IPTG. Experiments were performed in duplicate.

Supplemental Information Fig. 7



Supplemental Information Fig. 7. GABA production of *Bacteroides fragilis KLE1758* at multiple pH. GABA production of *B. fragilis* KLE1758 was only observed at a pH of 5.5 and below. GABA and glutamate levels of KLE1758 were measured by LC/MS and error bars represent one standard deviation. The experiment was performed in duplicate, and colored circles indicate measured values with the bars representing mean values.

Supplemental Information Fig. 8



Supplemental Information Fig. 8. Predicted precursors of GABA production in KBase models. (A) Venn diagram of the overlap in a model's capability to produce GABA using glutamate, arginine, putrescine, or ornithine as a single carbon source, if it was available internally. (B) Venn diagram of the overlap in a model's capability to produce GABA using glutamate, arginine, putrescine, or ornithine as a single carbon source, if it was available externally (Table 2).

Supplemental Information Table 1

Supplemental Information Table 1. Compounds tested for growth induction of KLE1738. For spotted compounds (top table), 5 µL of the stocks, prepared in water unless otherwise indicated, were spotted on FAAy plates spread with KLE1738, and incubated in the anaerobic chamber at 37°C for a week. No compounds showed growth induction besides GABA. A Biolog An Plate (Biolog, Hayward, CA), a commercially available 96-well plate loaded with multiple carbon and nitrogen sources, was also tested by following manufacturer's instructions. None of the conditions in any of the wells (compounds listed in bottom table) supported growth of KLE1738. All samples were tested in duplicate experiments.

Compound	Spotted Conc.	Compound	Spotted Conc.	Compound	Spotted Conc.
y-amino Butyric Acid (GABA)	100 mg/mL*	Fructose	100 mg/mL	L-Alanine	50 mg/mL
y-Butyrolactone	200 mg/mL	Malate	100 mg/mL	L-Asparagine monohydrate	20 mg/mL
y-Hydroxybutryic Acid	100 mg/mL	Sodium Nitrate	100 mg/mL	L-Aspartic Acid	5 mg/mL
Putrescine Dihydrochloride	100 mg/mL	Sodium Pyruvate	100 mg/mL	L-Cysteine HCl	50 mg/mL
Spermidine Trihydrocholride	100 mg/mL	Sodium Succinate	100 mg/mL	L-Glutamic Acid	5 mg/mL
Spermine Tetrahydrochloride	100 mg/mL	Sodium Acetate	100 mg/mL	L-Glutamine	20 mg/mL
Succinate Semialdehyde	15% w/v	Sodium Butyrate	100 mg/mL	L-Phenylalanine	20 mg/mL
Butyric Acid	100% soln	Sodium Propionate	100 mg/mL	L-Proline	50 mg/mL
Lactic Acid	100 mg/mL	L-Histidine	40 mg/mL	L-Threonine	50 mg/mL
Malonic Acid	100 mg/mL	L-Isoleucine	40 mg/mL	L-Glycine	20 mg/mL
Succinic Acid	100 mg/mL	L-Leucine	20 mg/mL	Malic Acid	100 mg/mL
Monosodium Glutamate	100 mg/mL	L-Lysine	100 mg/mL	ATCC Mineral Mix	100% solution
Glycerol	99.9% solution	L-Methionine	50 mg/mL	ATCC Vitamin Mix	100% solution
Glucose	100 mg/mL	L-Tryptophan	10 mg/mL	Induction	
Fructose	100 mg/mL	L-Valine	50 mg/mL	No induction	

*Induction also seen with as low as 10 mg/mL

Biolog An Plate					
Water	D-Galactose	D-Mannitol	Sucrose	D-Malic Acid	L-Alanyl-L-Threonine
N-Acetyl-D-Glucosamine	D-Galacturonic Acid	D-Mannose	D-Trehalose	L-Malic Acid	L-Asparagine
Uridine	Gentiobiose	D-Melezitose	Turanose	Propionic Acid	L-Glutamic Acid
Adonitol	D-Gluconic Acid	D-Melibiose	Acetic Acid	Pyruvic Acid	L-Glutamine
Amygdalin	D-Glucosaminic Acid	3-Methyl-D-Glucose	Formic Acid	Pyruvic Acid Methyl Ester	Glycyl-L-Aspartic Acid
D-Arabitol	a-D-Glucose	a-Methyl-D-Galactoside	Fumaric Acid	D-Saccharic Acid	Glycyl-L-Glutamine
Arbutin	a-D-Glucose-1-Phosphate	b-Methyl-D-Galactoside	Glyoxylic Acid	Succinamic Acid	Glycyl-L-Methionine
D-Cellobiose	D-Glucose-6-Phosphate	a-Methyl-D-Glucoside	a-Hydroxybutyric Acid	Succinic Acid	Glycyl-L-Proline
a-Cyclodextrin	Glycerol	b-Methyl-D-Glucoside	B-Hydroxybutyric Acid	Thymidine	L-Methionine
B-Cyclodextrin	D,L-a-Glycerol Phosphate	Palatinose	Itaconic Acid	m-Tartaric Acid	L-Phenylalanine
Dextin	m-Inositol	D-Raffinose	a-Ketobutyric Acid	Urocanic Acid	L-Serine
Dulcitol	a-D-Lactose	L-Rhamnose	D,L-Lactic Acid	Alaninamide	L-Threonine
i-Erythritol	Lactulose	Salicin	L-Lactic Acid	L-Alanine	L-Valine
D-Fructose	Maltose	D-Sorbitol	D-Lactic Acid Methyl Ester	L-Alanyl-L-Glutamine	L-Valine + Aspartic Acid
L-Fucose	Maltotriose	Stachyose	Uridine-5'-Monophosphate	L-Alanyl-L-Histidine	L-Aspartic Acid
Succinic Acid Mono-Methyl Ester	Thymidine-5'-Mono-phosphate	N-Acetyl-B-D-Mannosamine		Inosine	2'-Deoxy Adenosine

Supplemental Information Table 2

Supplemental Information Table 2. Minimal growth requirements of KLE1738. Individual components of FAA were removed and growth of KLE1738 was tested. Growth was not observed without GABA, and no component of FAA was essential for growth besides peptone. Green boxes indicate growth on that medium, and pink indicates no growth. Results are from a single experiment.

Medium	
FAA	FAA + GABA - L-Cysteine
FAA + GABA	FAA + GABA - Sodium Pyrophosphate
FAA + GABA - Vitamin K	FAA + GABA - L-Arginine
FAA + GABA - Sodium Chloride	FAA + GABA - Sodium Succinate
FAA + GABA - Soluble Starch	FAA + GABA - Hemin
FAA + GABA - Sodium Bicarbonate	FAA + GABA - Peptone
FAA + GABA - Glucose	GABA + Peptone
FAA + GABA - Sodium Pyruvate	GABA

Supplemental Information Table 3

Supplemental Information Table 3. Predicted transport systems in KLE1738. The presence of transport systems in the genome of KLE1738 was analyzed using RAST.

Category	Subsystem	Role
Amino Acids and Derivatives	Polyamine Metabolism	ABC transporter, periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)
Amino Acids and Derivatives	Polyamine Metabolism	Spermidine Putrescine ABC transporter permease component PotB (TC 3.A.1.11.1)
Amino Acids and Derivatives	Polyamine Metabolism	Spermidine Putrescine ABC transporter permease component potC (TC 3.A.1.11.1)
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter ATP-binding protein
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter permease protein
Amino Acids and Derivatives	Methionine Biosynthesis	Methionine ABC transporter substrate-binding protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter ATP-binding protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter permease protein
Amino Acids and Derivatives	Methionine Degradation	Methionine ABC transporter substrate-binding protein
Clustering-based subsystems	PhoR-PhoB two-component regulatory system	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Membrane Transport	ABC transporter dipeptide (TC 3.A.1.5.2)	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)
Membrane Transport	ABC transporter oligopeptide (TC 3.A.1.5.1)	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA (TC 3.A.1.5.1)
Membrane Transport	ABC transporter branched-chain amino acid (TC 3.A.1.4.1)	Branched-chain amino acid ABC transporter, amino acid-binding protein (TC 3.A.1.4.1)
Phosphorus Metabolism	High affinity phosphate transporter and control of PHO regulon	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Phosphorus Metabolism	Phosphate metabolism	Phosphate ABC transporter, periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	Glycine betaine ABC transport system, glycine betaine-binding protein OpuAC
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	Glycine betaine ABC transport system, permease protein OpuAB
Stress Response	Choline and Betaine Uptake and Betaine Biosynthesis	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1)
Sulfur Metabolism	Alkanesulfonate assimilation	ABC-type nitrate/sulfonate/bicarbonate transport system, ATPase component
Sulfur Metabolism	Alkanesulfonate assimilation	Alkanesulfonates ABC transporter ATP-binding protein

Supplemental Information Table 4

Supplemental Information Table 4. Enzymes of the KLE1738 GABA metabolism pathway predicted by RAST. Matching colors indicate that the enzymes were found in close proximity to each other on the genome. Enzymes present in multiple copies are shown in bold.

Enzyme #	Contig	Start	Stop	Length (bp)	Function
1	KLE1738_5	26729	28084	1356	Gamma-aminobutyrate:alpha-ketoglutarate aminotransferase (EC 2.6.1.19)
2	KLE1738_5	25576	26688	1113	NAD-dependent 4-hydroxybutyrate dehydrogenase (EC 1.1.1.61)
3	**KLE1738_2**	**195690**	**197033**	**1344**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
3	**KLE1738_28**	**9343**	**10665**	**1323**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
3	**KLE1738_5**	**24249**	**25547**	**1299**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
3	**KLE1738_7**	**87690**	**86401**	**1290**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
3	**KLE1738_4**	**195133**	**196542**	**1410**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
3	**KLE1738_8**	**81438**	**80098**	**1341**	**4-hydroxybutyrate:acetyl-CoA CoA transferase (EC 2.3.1.-)**
4	KLE1738_5	22352	23902	1551	4-hydroxybutanoyl-CoA dehydratase (EC 4.2.1.-) / Vinylacetyl-CoA Delta-isomerase (EC 5.3.3.3)
5	**KLE1738_6**	**2958**	**2092**	**867**	**Enoyl-CoA hydratase (EC 4.2.1.17)**
5	**KLE1738_11**	**1132**	**356**	**777**	**Enoyl-CoA hydratase (EC 4.2.1.17)**
5	**KLE1738_2**	**157073**	**157849**	**441**	**3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55)**
5	**KLE1738_2**	**195253**	**195693**	**777**	**3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55)**
5	**KLE1738_8**	**87054**	**87830**	**777**	**3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55)**
6	**KLE1738_6**	**4710**	**5762**	**1053**	**3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.35)**
6	**KLE1738_8**	**87921**	**88769**	**849**	**3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.35)**
7	**KLE1738_14**	**4910**	**6139**	**1230**	**Acetyl-CoA acetyltransferase (EC 2.3.1.9)**
7	**KLE1738_19**	**28638**	**27382**	**1257**	**Acetyl-CoA acetyltransferase (EC 2.3.1.9)**
8	KLE1738_13	26709	25738	972	Phosphate acetyltransferase (EC 2.3.1.8)
9	KLE1738_1	119924	121141	1218	Acetate kinase (EC 2.7.2.1)
1b	KLE1738_30	8650	9999	1350	NADP-specific glutamate dehydrogenase (EC 1.4.1.4)
5b	**KLE1738_6**	**4303**	**3083**	**1221**	**Butyryl-CoA dehydrogenase (EC 1.3.8.1)**
5b	**KLE1738_4**	**117577**	**116435**	**1143**	**Butyryl-CoA dehydrogenase (EC 1.3.99.2)**
5b	**KLE1738_4**	**140200**	**141366**	**1167**	**Butyryl-CoA dehydrogenase (EC 1.3.99.2)**
5b	**KLE1738_1**	**140091**	**141236**	**1146**	**Butyryl-CoA dehydrogenase (EC 1.3.99.2)**
6b	KLE1738_1	158124	159767	1644	Acetyl-CoA:acetoacetyl-CoA transferase (EC 2.8.3.8)

Supplemental Information Table 6

Supplemental Information Table 6. Identified transcripts in human stool transcriptome that mapped to the GABA producing gene glutamate decarboxylase. BLASTN results for five active gad transcripts as retrieved from human transcriptome data (Ni et al., 2016).

gad transcript	Transcript length (in bp)	Best blast hit	%identity	E-value
Transcript1	519	*Escherichia coli* strain AR_0058	100	0
Transcript2	234	*Bacteroides vulgatus* strain mpk	99	1.00E-96
Transcript3	231	*Bacteroides vulgatus* strain mpk	100	5.00E-116
Transcript4	276	*Escherichia coli* strain AR_0058	100	6.00E-141
Transcript5	286	*Escherichia coli* strain AR_0058	98	3.00E-142

Supplemental Information Table 7

Supplemental Information Table 7. Mapping results of the two gad transcripts on genome sequences of closest references strains to cultured panel isolates. The rest of the three transcripts with best blast hit from *E. coli* were not mapped on any of the genomes

Reference strain for cultured panel	Copy No. (transcript2)	Copy No. (transcript3)	Total copies
Bacteroides caccae ATCC 43185	1	0	1
Bacteroides dorei DSM 17855	1	1	2
Bacteroides ovatus ATCC8483	1	0	1
Bacteroides uniformis ATCC8492	1	0	1
Bacteroides vulgatus ATCC8482	1	1	2
Bifidobacterium adolescentis ATCC15703	0	0	0
Eubacterium rectale ATCC 33656	0	0	0
Parabacteroides merdae ATCC43184	1	1	2

Supplemental Information Table 8

Supplemental Information Table 8. Medications taken by individuals in the Major Depressive Disorder Cohort.

American Gut ID	Medications at Time of Stool Collection
17358	None
17359	Baclofen 10mg daily, Duloxetine 120mg daily, aspirin 81mg daily, Pantoprazole 40mg daily, Chantix 2mg daily, klonopin 3mg daily, oxycodone 30mg daily, seroquel 100mg daily, simvastatin 20mg daily, trazadone 100mg daily, docolace 200mg daily, oxycontin 60mg daily, gabapentin 1800mg daily, senna 8mg daily
17361	None
17362	Venlafaxine 37.5mg Daily
17393	None
17394	None
17395	None
17399	Klonopin 0.5mg daily
17368	None
17407	Nexium 40mg daily, Zovia oral contraceptive 1tab daily, lithium 1500mg daily, synthroid 50mcg daily, prazosin 2mg bedtime, temazepam 30mg bedtime, topiramate 75mg q12h
17376	Venlafaxine 75mg Daily, adderall xr 20mg daily
17366	Lamictal 25mg daily, seroquel 50mg bedtime
17377	Wellbutrin 150mg daily
17363	Abilify 5mg daily, Wellbutrin (dose unknown), Estradiol 6mg daly, spironolactone 200mg daily
17374	Citalopram 20mg daily, finasteride 1mg daily, trazadone 25mg bedtime, truvada 1tab daily
17389	Abilify 4mg daily, aspirin 81mg daily
17388	Melatonin 3mg bedtime
17365	Duloxetine 60mg daily, sertraline 100mg daily
17406	Seroquel 300mg bedtime, nortriptyline 25mg bedtime, gabapentin 300mg 3x/day, klonopin 0.25mg daily as needed, imitrex 100mg 3x/month as needed for migraine
17403	None
17367	Metformin (dose unknown), lovastatin (dose unknown), aspirin 81mg daily
66721	Unknown
66723	None