

1 **Supplementary Table 1: Patient characteristics Stratified by Trajectory of**
 2 **Respiratory Failure**

	HFNC Success	HFNC Failure	p
n	30	20	
Baseline Characteristics			
Race (%)			0.543
Asian/Mideast Indian	1 (3.3)	0 (0.0)	
Black/African-American	20 (66.7)	13 (65.0)	
Hispanic	1 (3.3)	3 (15.0)	
More than one Race	1 (3.3)	0 (0.0)	
Native Hawaiian/Pacific Islander	1 (3.3)	0 (0.0)	
White	6 (20.0)	4 (20.0)	
Male (%)	15 (50.0)	12 (60.0)	0.685
Age (median [IQR])	59.47 [54.31, 67.43]	67.96 [59.28, 76.10]	0.113
Body Mass Index (median [IQR])	31.05 [27.30, 38.80]	30.73 [26.95, 33.77]	0.759
Hypertension (%)	20 (66.7)	12 (60.0)	0.857
Hyperlipidemia (%)	8 (26.7)	9 (45.0)	0.3
Diabetes (%)	11 (36.7)	9 (45.0)	0.768
Cancer (%)	6 (20.0)	3 (15.0)	0.94
Chronic Kidney Disease (%)	1 (3.3)	4 (20.0)	0.149
Clinical Characteristics			
SOFA Score (median [IQR])	4.00 [4.00, 5.75]	9.00 [8.75, 9.00]	0.001
APACHE Score (median [IQR])	18.00 [13.00, 21.25]	21.00 [16.75, 29.50]	0.033
Charlson Comorbidity Index (median [IQR])	3.00 [2.00, 4.75]	4.00 [2.00, 6.00]	0.361
Days from Symptom Onset (median [IQR])	5.00 [3.00, 6.75]	5.00 [2.00, 8.00]	0.905
Admission (%)			0.495
Emergency Department	22 (73.3)	12 (60.0)	
Hospital Medicine	6 (20.0)	7 (35.0)	
Outside Hospital	2 (6.7)	1 (5.0)	
Medication Administration			
Antivirals (%)			
Steroid Treatment	22 (73.3)	18 (90.0)	0.279
Remdesivir Treatment	24 (80.0)	18 (90.0)	0.581
Antibiotics (%)			
Betalactams	5 (16.7)	4 (20.0)	1
Levofloxacin	1 (3.3)	0 (0.0)	1
Vancomycin	6 (20.0)	6 (30.0)	0.636
Metronidazole	1 (3.3)	1 (5.0)	1
Macrolides	7 (23.3)	7 (35.0)	0.563
Doxycycline	4 (13.3)	0 (0.0)	0.242
Trimethoprim-Sulfamethoxazole	4 (13.3)	2 (10.0)	1

3 Supplementary Table 1: Univariable analysis comparing patient characteristics and medication
 4 administration for high flow nasal cannula (HFNC) failure, defined as need for endotracheal
 5 intubation versus HFNC success, defined as transition to low flow nasal cannula. Categorical
 6 variables absolute number (percent within group). Categorical variables were compared using a
 7 two-tailed, chi-squared test, while continuous variables were compared using the Wilcoxon
 8 rank-sum, two-tailed test (n = 71 independent samples from patients). Unadjusted p-values are
 9 presented as exact values. Adequate treatment with COVID-19 specific therapy included at
 10 least three consecutive days of therapy during index hospitalization. Sufficient total dose of
 11 dexamethasone was 18mg and Remdesivir was 400mg. Only antibiotics received 72 hours prior
 12 to fecal specimen collection are represented.

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14**Supplementary Table 2: Assay 1 Analyzed Compounds – PFBBr Derivatization**

Common name	Mass to Charge m/z [M-H] ⁻ or [M+PFBB-H] ⁻	Retention time (min)
2-hydroxy-3-methylbutyrate	117	6.39
2-hydroxyhexanoate	131	7.03
2-hydroxyisocaproate	131	6.80
2-methylbutyrate	101	5.69
3-aminoisobutyrate	282	8.85
4-methylvalerate	115	6.31
5-aminovalerate	476	11.07
acetate	59	4.44
alanine	268	8.36
aspartate	492	11.10
benzoate	121	7.53
butyrate	87	5.48
catechol	269	9.25
crotonate	85	5.79
cysteine	480	11.21
desaminotyrosine	345	11.02
dopamine	512	12.81
glutamate	506	11.48
glycine	434	10.05
hexanoate	115	6.49
histamine	430	11.76
hydrocinnamate	149	8.20
indole-3-acetate	174	10.19
indole-3-carboxaldehyde	144	9.95
indole-3-propionate	188	10.52
isobutyrate	87	5.20
isoleucine	310	8.97
isovaleric acid	101	5.74
leucine	310	8.91
lysine	505	12.01
methionine	328	9.97
palmitate	255	10.51
p-cresol	107	7.09
phenol	93	6.61
phenylacetate	135	7.69
phenylalanine	344	10.37
proline	294	9.19
propionate	73	4.98
p-toluate	135	8.076

serine	464	10.69
succinate	297	9.26
synephrine	346	11.27
threonine	478	10.52
trans-indole-3-acrylate	186	11.93
tryptamine	500	11.69
tryptophan	383	12.74
tyramine	316	10.71
tyrosine	373	11.42
valerate	101	6.00
valine	296	8.67

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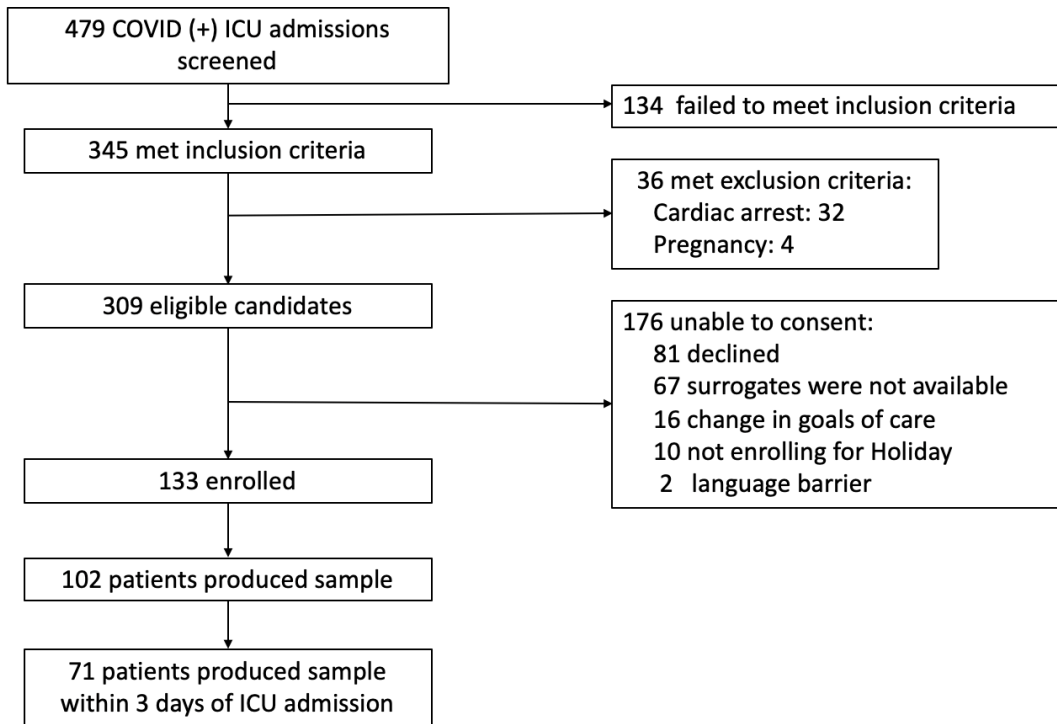
Supplementary Table 3: Assay 2 Analyzed Compounds – Bile Acids

Formal Name	Molecular Formula	Neutral Mass	Common name	Mass to Charge m/z [M-H] ⁻	Retention Time (+/- 0.15 min within run, +/- 0.40 min across batches)
5 β -Cholanic acid-3 α , 7 α , 12 α -triol	C24H40O5	408.2876	Cholic acid	407.2798	11.75
5 β -Cholanic acid-3 α , 12 α -diol	C24H40O4	392.2927	Deoxycholic acid	391.2849	15.27
5 β -Cholanic acid-3 α -ol	C24H40O3	376.2977	Lithocholic acid	375.2899	18.06
5 β -Cholanic acid-3 α , 7 α , 12 α -triol N-(carboxymethyl)-amide	C26H43NO6	465.309	Glycocholic acid	464.3012	8.73
5 β -Cholanic acid-3 α , 7 α , 12 α -triol N-(2-sulphoethyl)-amide	C26H45NO7S	515.2917	Taurocholic acid	514.2839	4.97
5 β -Cholanic acid-3 β , 12 α -diol	C24H40O4	392.2927	Isodeoxycholic acid	391.2849	13.24
5 α -Cholanic acid-3 β -ol	C24H40O3	376.2978	Alloisolithocholic acid	375.2900	16.46
3-Oxo-5 β -cholanoic acid	C24H38O3	374.2821	3-oxolithocholic acid	373.2743	18.62
5 β -Cholanic acid-3 α , 6 β , 7 α -triol-N-(2-sulphoethyl)-amide	C26H45NO7S	515.2917	Tauro- α -muricholic acid	514.2839	2.35
5 β -Cholanic acid-3 α , 6 β , 7 β -triol N-(2-sulphoethyl)-amide	C26H45NO7S	515.2917	Tauro- β -muricholic acid	514.2839	2.35
5 β -Cholanic acid-3, 7, 12-trione-N-(carboxymethyl)-amide	C26H37NO6	459.2621	Glycodehydrocholic acid	458.2543	1.90
5 β -Cholanic acid-3 α , 7 α -diol N-(carboxymethyl)-amide	C26H43NO5	449.3141	Glycochenodeoxycholic acid	448.3063	11.97
5 β -Cholanic acid-3 α , 12 α -diol N-(carboxymethyl)-amide	C26H43NO5	449.3141	Glycodeoxycholic acid	448.3063	12.56
5 β -Cholanic acid-3 α , 7 β -diol N-(carboxymethyl)-amide	C26H43NO5	449.3141	Glycoursodeoxycholic acid	448.3063	8.26
5 β -Cholanic acid-3 α -ol N-(carboxymethyl)-amide	C26H43NO4	433.3192	Glycolithocholic acid	432.3114	15.23

5β-Cholanic acid-3α,6α-diol N-(carboxymethyl)-amide	C26H43NO5	449.3100	Glycohyodeoxycholic acid	448.3022	8.70
5β-Cholanic acid-3α,6α,7β-triol	C24H40O5	408.2876	ω-muricholic acid	407.2798	9.07
5β-Cholanic acid-3α,6β,7β-triol	C24H40O5	408.2876	β-Muricholic acid	407.2798	10.22
5β-Cholanic acid-3α,6α,7α-triol	C24H40O5	408.2876	γ-Muricholic acid	407.2798	11.10
5β-Cholanic acid-3α,6β,7α-triol	C24H40O5	408.2876	α-Muricholic acid	407.2798	9.90
3α,7α,12α-Trihydroxy-5α-cholan-24-oic acid	C24H40O5	408.2876	Allocholic acid	407.2798	11.62
5β-Cholanic acid-3α,7α-diol	C24H40O4	392.2927	Chenodeoxycholic acid	391.2849	14.87
5α-Cholanic acid-3α-ol	C24H40O3	376.2978	Allolithocholic acid	375.2900	18.20
5β-Cholanic acid-3α,7β-diol	C24H40O4	392.2927	Ursodeoxycholic acid	391.2849	11.88
3α,6α-Dihydroxy-5β-cholan-24-oic acid	C24H40O4	392.2927	Hyodeoxycholic acid	391.2849	12.23
5β-Cholanic acid-7α,12α-diol	C24H40O4	392.2927	3-Deoxycholic acid	391.2849	17.35
5β-Cholanic acid-3β-ol	C24H40O3	376.2977	Isolithocholic acid	375.2899	17.01
5β-Cholanic acid-3α,7α-diol-12-one	C24H38O5	406.2719	12-Oxocholedeoxycholic acid	405.2641	8.61
5β-Cholanic acid-3α,12α-diol-7-one	C24H38O5	406.2719	7-Oxodeoxycholic acid	405.2641	8.96
5β-Cholanic acid-7α,12α-diol-3-one	C24H38O5	406.2719	3-Oxocholeic acid	405.2641	10.70
5β-Cholanic acid-12α-ol-3-one	C24H38O4	390.277	3-Oxodeoxycholic acid	389.2692	14.76
5β-Cholanic acid-7α-ol-3-one	C24H38O4	390.277	3-Oxocholedeoxycholic acid	389.2692	14.76
5β-Cholanic acid-3α-ol-7-one	C24H38O4	390.277	7-Oxolithocholic acid	389.2692	12.37
5β-Cholanic acid-3α-ol-6-one	C24H38O4	390.277	6-Oxolithocholic acid	389.2692	12.37
5β-Cholanic acid-3α-ol-12-one	C24H38O4	390.277	12-Oxolithocholic acid	389.2692	12.67
5β-Cholanic acid-3α,12α-diol N-(2-sulphoethyl)-amide	C26H45NO6S	499.2968	Taurodeoxycholic acid	498.289	9.35
5β-Cholanic acid-3α-ol N-(2-sulphoethyl)-amide	C26H45NO5S	483.3018	Tauroolithocholic acid	482.294	11.80
5β-Cholanic acid-3α,7α-diol N-(2-sulphoethyl)-amide	C26H45NO6S	499.2968	Taurocholedeoxycholic acid	498.289	8.70

3 α ,7 β -Dihydroxy-5 β -cholan-24-oic acid N-(2-sulfoethyl)amide	C26H45NO6S	499.2968	Tauroursodeoxycholic acid	498.289	4.30
5 β -Cholanic acid-3 α ,6 α -diol-N-(2-sulphoethyl)-amide	C26H45NO6S	499.2968	Taurohyodeoxycholic acid	498.289	4.60
5 β -Cholanic acid-3 α -ol-7,12-dione	C24H36O5	404.2563	3-Hydroxy-7,12-diketolithocholic acid	403.2485	5.00
3 α ,7 β ,12 α -Trihydroxy-5 β -cholan-24-oic acid	C24H40O5	408.2876	Ursocholic acid	407.2798	6.75
D4-Cholic acid	C24H36D4O5	412.3126	D4-Cholic acid	411.3048	11.76
D4-Deoxycholic acid	C24H36D4O4	396.3177	D4-Deoxycholic acid	395.3099	15.20
D4-Glycocholic acid	C26H39D4NO6	469.3341	D4-Glycocholic acid	468.3263	8.73
D4-Glycodeoxycholic acid	C26H39D4NO5	453.3392	D4-Glycodeoxycholic acid	452.3314	12.50
D4-Lithocholic acid	C24H36D4O3	380.3228	D4-Litchocholic acid	379.3150	18.00
D4-Taurocholic acid	C26H41D4NO7S	519.3167	D4-Taurocholic acid	518.3089	5.01
D4-Taurodeoxycholic acid	C26H41D4NO6S	503.3218	D4-Taurodeoxycholic acid	502.3140	9.40
D5-Alpha-muricholic acid	C24H35D5O5	413.3189	D5-Alpha-muricholic acid	412.3111	9.85
D4-Taurochenodeoxycholic acid	C26H41D4NO6S	503.3218	D4-Taurochenodeoxycholic acid	502.3140	8.70

51 **Supplementary Figure 1: Consort Diagram**



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53 Supplementary Figure 1 Legend: Consort diagram for study enrollment.

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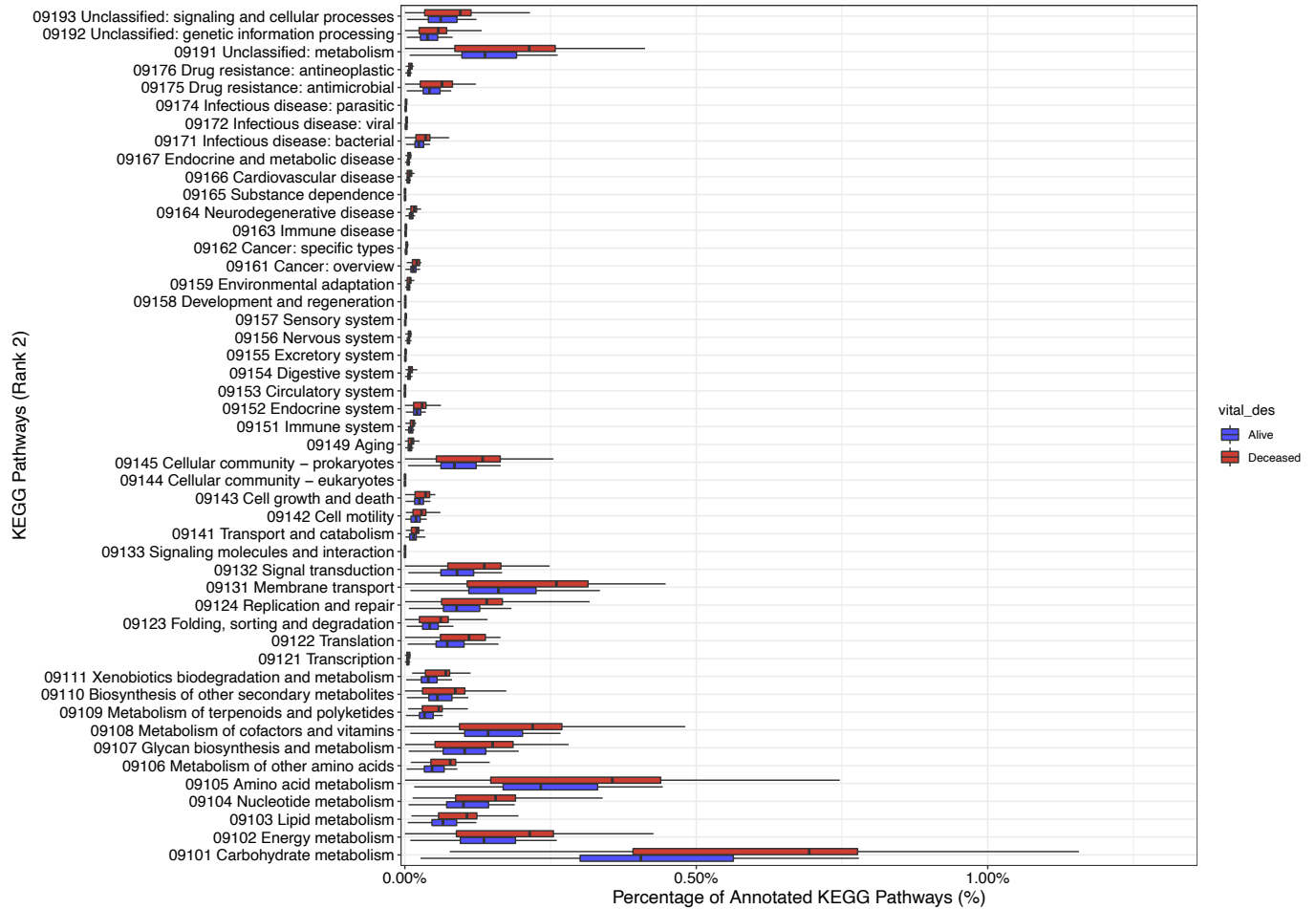
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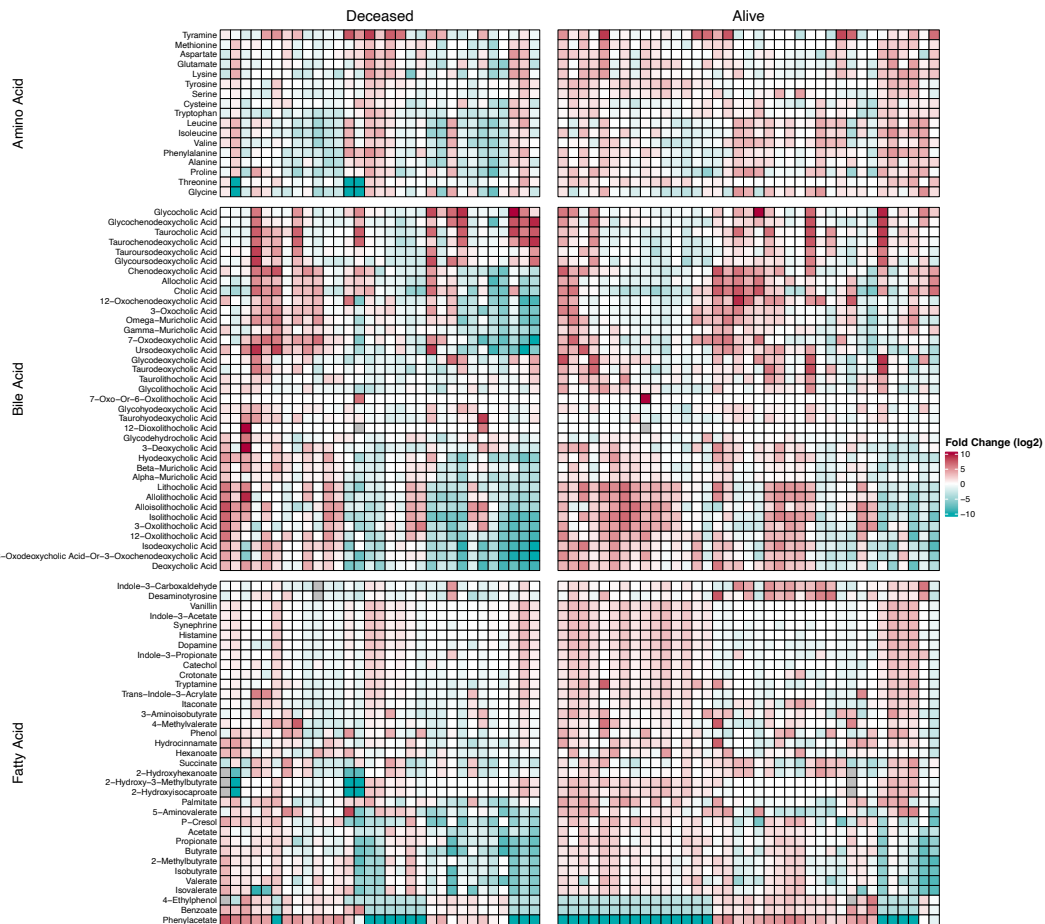
Supplementary Figure 2



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Supplementary Figure 2 Legend: KEGG pathway analysis stratified by survival outcome (blue box: alive and red box: deceased). KEGG pathways (Rank 2) were calculated as a total percent of all pathways encoded and plotted as boxplots with values per each rank 2 category. After performing a two-tailed, Wilcoxon rank-sum test, there were no statistical differences across all KEGG pathways shown (n = 71 independent samples from patients). Boxes show interquartile ranges (IQR) where the center black line represents the median and the whiskers (vertical black lines) extend to 1.5 x IQR or to the minimum and maximum value, whichever is closest to the median

97 **Supplementary Figure 3**
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 102 Supplementary Figure 3 Legend: Heatmap of 89 relatively quantified fecal metabolites. Dark
 103 cyan colors correlate to low log₂ fold-change values relative to the median value per compound,
 104 while dark red colors correlate to high log₂ fold-change values relative to the median value per
 105 compound. Missing values are shown in gray. n = 71 independent samples from patients.

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116 Supplementary Methods:

117 **Supplementary Metabolomic Methods (MetaboLights study MTBLS5288)**

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119 **Stool Collection and Extraction for All Metabolomic Assays**

120 **Collection:** Freshly obtained stool samples were collected from patients in a sterile specimen vessel
121 (University of Chicago, IRB 20-1102). Samples were immediately placed on ice or at 4 °C and then
122 immediately aliquoted for downstream analyses. **Stool Splitting and Storage:** Stool samples were then
123 split into aliquots for downstream analyses (metabolomics, genomics). For each analysis, stool was split
124 into a separate 1.5 ml Eppendorf tube. Mass requirements for analyses were: metabolomics, ~100 mg;
125 genomics, ~250 mg (16S and shotgun sequencing). Minimum requirements for metabolomic analyses in
126 this study was 20 mg/assay. The bulk stool sample and split Eppendorf tubes were kept on ice and the
127 aliquots were immediately stored at -80 °C. **Extraction:** Extraction solvent (80% methanol spiked with
128 internal standards and stored at -80 °C) was added to pre-weighed fecal samples at a ratio of 100 mg of
129 material/ml of extraction solvent in beadruptor tubes (Fisherbrand; 15-340-154). Samples were
130 homogenized at 4 °C on a Bead Mill 24 Homogenizer (Fisher; 15-340-163), set at 1.6 m/s with 6 x 30s
131 cycles, 5 s off per cycle. Samples were then centrifuged at -10 °C, 20,000 x g for 15 min and the
132 supernatant was used for subsequent metabolomic analysis

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134 **Assay 1 (CI-GC-MS): Pentafluorobenzyl bromide (PFBBr) derivatization for targeted**

135 **metabolomics with (-)CI-GCMS (quantitative and normalized relative abundance)**

136 **Preparation for Pentafluorobenzyl Bromide (PFBBr) Derivatization:** Samples were derivatized as
137 previously described with the following modifications [1]. Briefly, the metabolite extract (100 µl) was
138 added to 100 µl of 100 mM borate buffer (pH 10) (Thermo Fisher, 28341), 400 µl of 100 mM
139 pentafluorobenzyl bromide (Millipore Sigma; 90257) in acetonitrile (Fisher; A955-4), and 400 µl of n-
140 hexane (Acros Organics; 160780010) in a capped mass spectrometer autosampler vial (Microliter; 09-
141 1200). Samples were heated in a thermomixer C (Eppendorf) to 65 °C for 1 h while shaking at 1300 rpm.
142 After cooling to RT, samples were centrifuged at 4 °C, 2000 x g for 5 min, allowing phase separation.
143 The hexanes phase (100 µl) (top layer) was transferred to an autosampler vial containing a glass insert
144 and the vial was sealed. Another 100 µl of the hexanes phase was diluted with 900 µl of n-hexane in an
145 autosampler vial. **Chromatography:** Concentrated and dilute samples were analyzed using a GC-MS
146 (Agilent 8890/7890B GC system, Agilent 5977B MSD) with a 1 µl split injection (1:10 split ratio). Oven
147 ramp parameters: 1 min hold at 60 °C, 25 °C/min up to 300 °C with a 2.5 min hold at 300 °C. Inlet
148 temperature was 280 °C and transfer line was 310 °C. Using a HP-5ms ultra inert column (30 m x 0.25
149 mm, 0.25 µm; Agilent Technologies 19091S-433UI), methane as the reagent gas. **Instrument Settings:**
150 The instrument was in Scan acquisition mode (m/z 50-600) with a solvent delay of 4.2 with Normal
151 Scanning and operating in negative chemical ionization mode with methane used as the reagent gas
152 (99.999% pure). **Data Transformation:** A 10-point calibration curve was prepared with acetate (100
153 mM), propionate (25 mM), butyrate (12.5 mM), and succinate (50 mM), with 9 subsequent 2x serial
154 dilutions. Data analysis was performed using **MassHunter Quantitative Analysis software (version**
155 **B.10, Agilent Technologies)** and confirmed by comparison to retention time, nominal mass, and
156 fragmentation patterns of authentic standards (**Supplementary Table 2**). Normalized peak areas were
157 calculated by dividing raw peak areas of targeted analytes by raw peak areas of spiked internal standards.
158 Internal standards used for quantitation (Cambridge Isotope Laboratories, D3-acetate DLM-3126-25; D5-
159 propionate DLM-1601-1; D7-butyrate DLM-7616-PK; D9-valerate DLM-572-5 DLM-572-5; D8-valine
160 DLM-488-0.25; D6-succinate DLM-831-PK; D6-phenol DLM-370-PK). Relative abundance data were
161 normalized to the average of D8-valine/D6-valerate or D7-proline/D6-phenol. **Metabolite Identification:**
162 Authentic standards were purchased for all targeted compounds and prepared at 1 mg/ml in methanol. All
163 metabolite identifications are level 1 according to the CAWG standard initiative [2].

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166 **Assay 2 (UPLC-QTOF-MS): Bile acid targeted metabolomics (-)ESI-LC-QTOF (quantitative and**
167 **normalized relative abundance)**

168 **Extraction:** For bile acid analysis, 75 μ l of supernatant was added to pre-labeled microcentrifuge tubes
169 and dried down completely under a nitrogen stream at 30 l/min (top) 1 l/min (bottom) at 30 °C (Biotage
170 SPE Dry 96 Dual; 3579M). Samples were reconstituted in 50:50 water:methanol (750 μ l) and added to a
171 thermomixer C (Eppendorf) to resuspend analytes at 4 °C, 1000 rpm for 15 min with an infinite hold at 4
172 °C. Samples were then centrifuged at 4 °C, 20,000 x g for 15 min to remove insoluble debris, and the
173 supernatant (700 μ l) was transferred to fresh, pre-labeled mass spectrometry autosampler vials
174 (Microliter; 09-1200) for LCMS analysis. **Chromatography:** Samples were analyzed using a liquid
175 chromatography system (Agilent 1290 Infinity II). Each sample (5 μ l) was injected onto an XBridge BEH
176 C18 Column (3.5 μ m, 2.1 x 100 mm; Waters Corporation, PN) fitted with an XBridge BEH C18 guard
177 (Waters Corporation, PN) at 45 °C. Elution started with 72% A (Water, 0.1% formic acid) and 28% B
178 (Acetone, 0.1% formic acid) with a flow rate of 0.4 ml/min for 1 min and linearly increased to 33% B
179 over 5 min, and then linearly increased to 65% B over 14 min. The flow rate was then increased to 0.6
180 ml/min and B was increased to 98% over 0.5 min, and these conditions were held constant for 3.5 min.
181 Finally, re-equilibration at a flow rate of 0.4 ml/min of 28% B was performed for 3 min. **Instrument**
182 **Settings:** Samples were analyzed using a quadrupole time-of-flight (QTOF) mass spectrometer (Agilent
183 6546) (coupled with Agilent 1290 Infinity II) in negative mode, equipped with an Agilent Jet Stream
184 Electrospray Ionization source. The electrospray ionization conditions were set with the capillary voltage
185 at 3.5 kV, nozzle voltage at 2 kV, gas temperature 325 °C, gas flow 10 l/min, nebulizer 20 psig, sheath
186 gas temperature 275 °C, and sheath gas flow 11 l/min. Data was acquired at a scan rate of 2 spectra/s,
187 MS1 mode, and detection window set to 100-1700 m/z with continuous infusion of a reference mass
188 (Agilent ESI TOF Biopolymer Analysis Reference Mix) for mass calibration. **Data Transformation:** A
189 10-point calibration curve was used for quantitation of 8 bile acids (cholic acid, glycocholic acid,
190 taurocholic acid, deoxycholic acid, lithocholic acid, isodeoxycholic acid, alloisolithocholic acid and 3-
191 oxolithocholic acid). Bile acid data analysis was performed using **MassHunter Profinder Analysis**
192 **software (version B.10, Agilent Technologies)**. Identification of endogenous compounds was confirmed
193 by comparison of m/z and retention time to authentic standards (**Supplementary Table 3**). Normalized
194 peak areas were calculated by dividing raw peak areas of targeted analytes by averaged raw peak areas of
195 spiked internal standards. **Metabolite Identification:** Authentic bile acid standards were prepared at 1
196 mg/ml in methanol and analyzed as pure standards as well as spiked into a pooled biological sample from
197 each batch. (**Avanti Polar Lipids and Sigma Aldrich**). All metabolite identifications are level 1
198 according to the CAWG standard initiative [2]. Additionally, ions with epimer/isomers were purchased
199 and analyzed individually and as mixtures to ensure accurate identification in biological samples.
200 Compounds that were not resolvable with clear overlapping epimer/isomers were removed from analyses
201 (if applicable). All identified compounds are reported at < 5 ppm difference between the known m/z and
202 the detected m/z with a mass resolution of 50,000.

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205 **Assay 3 (UPLC-TQ-MS): Desaminotyrosine (DAT) and indole-3-carboxaldehyde (I3C) targeted**
206 **quantitation by (-)ESI-LC-QQQ (quantitative)**

207 **Extraction:** For analysis of desaminotyrosine and indole-3-carboxyaldehyde, 200 μ l of supernatant was
208 added to pre-labeled microcentrifuge tubes and dried down completely under a nitrogen stream at 30
209 l/min (top) 1 l/min (bottom) at 30 °C (Biotage SPE Dry 96 Dual; 3579M). Samples were reconstituted in
210 50:50 water:methanol (50 μ l) and added to a thermomixer C (Eppendorf) to resuspend analytes at 4 °C,
211 1000 rpm for 15 min with an infinite hold at 4 °C. Samples were then centrifuged at 4 °C, 20,000 x g for
212 15 min to remove insoluble debris, and supernatant (40 μ l) from each sample was transferred to a 96 well
213 plate (Agilent; 5065-4402) for LCMS analysis. **Chromatography:** Samples were analyzed on an Agilent
214 1290 infinity II liquid chromatography system. Each sample (2 μ l) was injected into an Acquity UPLC
215 HSS PFP column, 1.8 μ m, 2.1 x 100 mm (Waters; 186005967) equipped with an Acquity UPLC HSS
216 PFP VanGuard Pre-column, 100 Å, 1.8 μ m, 2.1 mm x 5 mm (Waters; 186005974) at 45 °C. Mobile phase

217 A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in 95:5 acetonitrile:water.
218 The flow rate was set to 0.5 ml/min starting at 0% B held constant for 3 min, then linearly increased to
219 50% over 5 min, then linearly increased to 95% B over 1 min, and held at 100% B for the next 3 min.
220 Mobile phase B was then brought back down to 0% over 0.5 min and held at 0% for re-equilibration for
221 2.5 min. **Instrument Settings:** Samples were analyzed using an Agilent 6470 triple quadrupole mass
222 spectrometer (coupled with Agilent 1290 Infinity II) in negative mode, equipped with an Agilent Jet
223 Stream Electrospray Ionization source. The electrospray conditions were set with capillary voltage at 4
224 kV and nozzle voltage at 500 V, and Dynamic MRM was used with a cycle time of 500 ms. Sheath gas
225 flow and temperature were 11 l/min and 275 °C, respectively. Transitions were monitored in negative
226 mode, and the transitions for desaminotyrosine and indole-3-carboxaldehyde were 165.17 m/z to m/z 93.1
227 and 144.16 m/z to 116 m/z, respectively. DAT acquisition: Fragmentor (70), Collision Energy (32), Cell
228 Accelerator Voltage (5). I3C acquisition: Fragmentor (90), Collision Energy (16), Cell Accelerator
229 Voltage (5). **Data Transformation:** An 11-point calibration curve (ranging from 0.98 nM to 1 mM) was
230 prepared using desaminotyrosine and indole-3-carboxaldehyde standards. Data analysis was performed
231 using **MassHunter Quant software (version B.10, Agilent Technologies)** and confirmed by comparison
232 of retention time, nominal mass, and qualifying fragment ion to authentic standards. Normalized peak
233 areas were calculated by dividing raw peak areas of targeted analytes by averaged raw peak areas of
234 spiked internal standards. **Metabolite Identification:** Stock solutions of desaminotyrosine and indole-3-
235 carboxaldehyde (1 mg/ml in 50:50 water:methanol) were prepared using standards purchased from **Sigma**
236 **Aldrich**. All metabolite identifications are level 1 according to the CAWG standard initiative [2].

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