Supporting Information

Chronic Ethanol Consumption Alters Mammalian Gastrointestinal Content Metabolites

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Table S1. Compositions of modified Lieber-DeCarli control and ethanol liquid diets for step-wise feeding procedure.

	Control	Ethanol	Ethanol	Ethanol	Ethanol
Weeks	1-8	1-2	3-4	5-6	7-8
Ingredients (g/L)					
Casein	36.8	36.8	36.8	36.8	36.8
L-Cystine	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Corn oil	34.8	34.8	34.8	34.8	34.8
Safflower oil	3.0	3.0	3.0	3.0	3.0
Cellulose	10.0	10.0	10.0	10.0	10.0
Choline bitartrate	0.53	0.53	0.53	0.53	0.53
Salt mix	8.75	8.75	8.75	8.75	8.75
Vitamin mix	2.5	2.5	2.5	2.5	2.5
Maltose dextrin	115.2	33.1	30.6	28.1	25.6
Ethanol	-	50.0	51.4	52.9	54.3
Calories (% of total calories)					
Protein	16	16	16	16	16
Fat	34	34	34	34	34
Carbohydrate	50	15	14	13	12
Ethanol	-	35	36	37	38
Total	100	100	100	100	100

Table S2. Metabolites identified in gastrointestinal contents derived from LC-TOFMS analysis.

No.	Compound	Pathway	Library*	Retention time (min)	m/z
1	Acetylcholine	Alcohols and Polyols	Std	3.70	147.1129
2	Choline	Alcohols and Polyols	Std	4.10	105.107
3	5'-Hydroxycotinine	Alkaloids and Derivatives	HMDB	7.27	191.0845
4	Cotinine	Alkaloids and Derivatives	Std	4.15	177.0968
5	3-Dehydroxycarnitine	Alkylamines	HMDB	4.43	146.1167
6	Carnitine	Alkylamines	Std	4.21	162.1095
7	2-Aminomuconic acid semialdehyde	Amino Acids and Derivatives	HMDB	4.16	142.0464
8	2-Methylbutyrylglycine	Amino Acids and Derivatives	HMDB	20.92	158.0827
9	3,4-Dehydro-DL-proline	Amino Acids and Derivatives	Std	4.35	114.0648
10	3,4-dihydroxyphenylalanine	Amino Acids and Derivatives	Std	4.18	198.0848
11	3-Methylhistidine	Amino Acids and Derivatives	HMDB	24.08	170.0962
12	Alanine	Amino Acids and Derivatives	Std	4.15	90.05414
13	Arginine	Amino Acids and Derivatives	Std	4.06	175.1166
14	Argininic acid	Amino Acids and Derivatives	HMDB	4.95	176.1027
15	Aspartic acid	Amino Acids and Derivatives	Std	4.12	134.0421
16	Aspartylysine	Amino Acids and Derivatives	HMDB	20.40	262.1386
17	Citrulline	Amino Acids and Derivatives	Std	3.90	174.0871
18	Cystine	Amino Acids and Derivatives	Std	3.69	239.021
19	Delta-hydroxylysine	Amino Acids and Derivatives	Std	3.94	161.0775
20	Gamma-Aminobutyric acid	Amino Acids and Derivatives	Std	3.91	102.0747
21	Glutamic acid	Amino Acids and Derivatives	Std	4.20	148.0599
22	Hexanoylglycine	Amino Acids and Derivatives	HMDB	3.71	172.1018
23	Homocitrulline	Amino Acids and Derivatives	HMDB	20.26	190.1201
24	L-beta-aspartyl-L-glycine	Amino Acids and Derivatives	HMDB	3.81	191.0667
25	L-beta-aspartyl-L-leucine	Amino Acids and Derivatives	HMDB	7.00	247.1291
26	L-gamma-glutamyl-L-leucine	Amino Acids and Derivatives	HMDB	20.38	261.1421
27	L-gamma-glutamyl-L-valine	Amino Acids and Derivatives	HMDB	20.25	247.1265
28	N6,N6,N6-Trimethyl-L-lysine	Amino Acids and Derivatives	HMDB	18.70	187.1408
29	N-Alpha-acetyllysine	Amino Acids and Derivatives	HMDB	19.96	187.1122
30	Ornithine	Amino Acids and Derivatives	Std	3.77	131.0818
31	Proline	Amino Acids and Derivatives	Std	4.44	116.0705
32	Selenohomocysteine	Amino Acids and Derivatives	HMDB	3.59	180.9678
33	Succinyladenosine	Amino Acids and Derivatives	HMDB	20.03	384.1171
34	Threonine	Amino Acids and Derivatives	Std	4.13	120.0617
35	Imidazoleacetic acid	Azoles	HMDB	18.23	127.049
36	Benzaldehyde	Benzaldehydes	HMDB	20.03	107.0488
37	4-Hydroxy-3-methylbenzoic acid	Benzoic Acid and Derivatives	HMDB	19.50	153.0598
38	5-Hydroxykynurenamine	Benzoic Acid and Derivatives	HMDB	3.93	179.0809
39	Monoisobutyl phthalic acid	Benzoic Acid and Derivatives	HMDB	24.74	221.0822

40	3-Hydroxycoumarin	Benzopyrans	HMDB	3.41	161.0189
41	Alpha-CEHC	Benzopyrans	HMDB	20.49	279.1635
42	2,3-Dihydroxysuccinic acid	Carboxylic Acids and Derivatives	Std	4.12	149.0646
43	2-Methylcitric acid	Carboxylic Acids and Derivatives	HMDB	3.84	207.0461
44	Dodecanedioic acid	Carboxylic Acids and Derivatives	Std	20.41	231.1657
45	Malonic semialdehyde	Carboxylic Acids and Derivatives	HMDB	3.61	89.02357
46	Cinnamic acid	Cinnamic Acid Derivatives	Std	20.03	149.059
47	1,3-Dimethyluracil	Diazines	HMDB	4.78	141.0652
48	5-Acetylamino-6-formylamino-3-methyluracil	Diazines	HMDB	3.88	225.0636
49	Dihydrouracil	Diazines	Std	4.41	115.0382
50	Leukotriene E3	Eicosanoids	HMDB	20.44	442.2588
51	3-Methylglutarylcarnitine	Fatty Acid Esters	HMDB	19.99	290.1636
52	(R)-3-Hydroxy-Octadecanoic acid	Fatty Acids and Conjugates	HMDB	23.00	299.2543
53	17-HDoHE	Fatty Acids and Conjugates	HMDB	21.05	345.2386
54	19,20-DiHDPA	Fatty Acids and Conjugates	HMDB	21.20	363.2493
55	2-Ethylacrylic acid	Fatty Acids and Conjugates	HMDB	4.66	101.0603
56	2-Oxo-4-methylthiobutanoic acid	Fatty Acids and Conjugates	HMDB	24.74	149.0231
57	2-Oxoarginine	Fatty Acids and Conjugates	HMDB	4.74	174.0889
58	3-Oxooctadecanoic acid	Fatty Acids and Conjugates	HMDB	23.73	299.2566
59	Bisnorbiotin	Fatty Acids and Conjugates	HMDB	3.86	215.0481
60	Docosahexaenoic acid	Fatty Acids and Conjugates	HMDB	21.77	329.2453
61	Elaidic acid	Fatty Acids and Conjugates	HMDB	23.45	283.262
62	Maleic acid	Fatty Acids and Conjugates	Std	4.64	115.0115
63	Tetracosahexaenoic acid	Fatty Acids and Conjugates	HMDB	24.55	357.2772
64	4-Hydroxynonenal	Fatty Alcohols	HMDB	23.46	157.1223
65	Oleamide	Fatty Amides	HMDB	23.73	282.2771
66	Palmitoylethanolamide	Fatty Amides	HMDB	22.15	300.288
67	N1-Methyl-2-pyridone-5-carboxamide	Hydropyridines	HMDB	11.34	151.0464
68	3-Hydroxypropionic Acid	Hydroxy Acids and Derivatives	Std	6.03	89.03857
69	Hydroxypyruvate	Hydroxy Acids and Derivatives	Std	3.70	102.993
70	Adenine	Imidazopyrimidines	Std	4.66	134.0395
71	Melanin	Indolequinones	HMDB	4.26	317.0608
72	5-Hydroxyindoleacetic acid	Indoles	HMDB	3.62	192.0636
73	Indoleacrylic acid	Indoles	HMDB	20.48	188.0689
74	Melatonin	Indoles	Std	18.61	231.1299
75	Fructosamine	Monosaccharides	HMDB	20.56	180.0915
76	Glucosamine 6-phosphate	Monosaccharides	HMDB	4.39	260.0491
77	N-Acetyl-b-glucosaminylamine	Monosaccharides	HMDB	20.20	221.1115
78	Ribulose	Monosaccharides	HMDB	6.51	151.0611
79	Threonic acid	Monosaccharides	HMDB	8.16	137.0462
80	Phosphoric acid	Non-metal Oxoanionic Compounds	HMDB	3.59	98.98727
81	Glyceraldehyde 3-phosphate	Organic Phosphoric Acids and Derivatives	Std	3.61	171.0056

82	L-a-glutamyl-L-Lysine	Peptides	HMDB	19.28	276.1537
83	L-leucyl-L-proline	Peptides	HMDB	20.37	229.1514
84	L-phenylalanyl-L-proline	Peptides	HMDB	20.49	263.1365
85	Homoanserine	Peptidomimetics	HMDB	19.57	255.1444
86	Phenylephrine	Phenethylamines	HMDB	18.54	166.0869
87	Methylnoradrenaline	Phenols and Derivatives	HMDB	17.77	182.0831
88	Norepinephrine	Phenols and Derivatives	Std	3.90	168.0525
89	Phenol	Phenols and Derivatives	HMDB	3.59	95.04602
90	Tyrosol	Phenols and Derivatives	HMDB	6.15	137.0575
91	Benzeneacetic acid	Phenylacetic Acid Derivatives	Std	19.58	137.0459
92	Scopolamine	Phenylacetic Acid Derivatives	HMDB	19.71	304.1513
93	Ecgonine methyl ester	Piperidines	HMDB	21.32	200.1276
94	Perillic acid	Prenol Lipids	HMDB	18.41	165.0877
95	1-Methylguanosine	Purine Nucleosides and Analogues	HMDB	19.77	298.1158
96	8-Hydroxy-deoxyguanosine	Purine Nucleosides and Analogues	HMDB	19.50	284.1041
97	Guanine	Purines and Purine Derivatives	HMDB	6.47	152.0559
98	3-Pyridylacetic acid	Pyridines and Derivatives	Std	4.42	138.0509
99	Nicotinamide N-oxide	Pyridines and Derivatives	HMDB	6.51	139.0518
100	Cytidine	Pyrimidine Nucleosides and Analogues	Std	3.88	242.074
101	Pyroglutamic acid	Pyrrolidines	Std	4.16	130.0473
102	Phytosphingosine	Sphingolipids	HMDB	21.85	318.2983
103	Sphinganine	Sphingolipids	HMDB	22.39	302.3033
104	Sphingosine	Sphingolipids	HMDB	24.35	300.2876
105	11-Hydroxyprogesterone 11-glucuronide	Steroids and Steroid Derivatives	HMDB	20.47	507.2612
106	11-Oxo-androsterone glucuronide	Steroids and Steroid Derivatives	HMDB	20.47	481.2486
107	12-Ketodeoxycholic acid	Steroids and Steroid Derivatives	HMDB	24.81	391.2825
108	21-Hydroxypregnenolone	Steroids and Steroid Derivatives	Std	21.77	333.2414
109	3,7-Dihydroxy-12-oxocholanoic acid	Steroids and Steroid Derivatives	HMDB	21.15	407.2761
110	3β,16α-Dihydroxyandrostenone sulfate	Steroids and Steroid Derivatives	HMDB	19.28	383.1505
111	3β-Hydroxy-5-cholenoic acid	Steroids and Steroid Derivatives	HMDB	23.33	375.2869
112	3-Oxo-4,6-choladienoic acid	Steroids and Steroid Derivatives	HMDB	21.13	371.2553
113	4-Hydroxyestrone	Steroids and Steroid Derivatives	HMDB	19.72	285.1501
114	5b-Pregnanediol	Steroids and Steroid Derivatives	HMDB	21.58	289.2908
115	6-Ketoestriol	Steroids and Steroid Derivatives	HMDB	19.59	303.1576
116	7α-Hydroxy-3-oxo-5β-cholanoic acid	Steroids and Steroid Derivatives	HMDB	22.89	391.2822
117	7-Hydroxy-3-oxocholanoic acid	Steroids and Steroid Derivatives	HMDB	21.79	391.2823
118	7-Ketodeoxycholic acid	Steroids and Steroid Derivatives	HMDB	22.02	407.2777
119	Glycochenodeoxycholic acid 3-glucuronide	Steroids and Steroid Derivatives	HMDB	20.50	626.3493
120	Nutriacholic acid	Steroids and Steroid Derivatives	HMDB	23.48	391.2824
121	Sulfolithocholic acid	Steroids and Steroid Derivatives	HMDB	20.44	457.2654
122	Sulfolithocholylglycine	Steroids and Steroid Derivatives	HMDB	20.81	514.2827

123	Gluconate	Sugar Acids and Derivatives	Std	3.97	195.053
124	Glucuronic acid	Sugar Acids and Derivatives	Std	3.87	193.0493
125	Neuraminic acid	Sugar Acids and Derivatives	HMDB	19.55	268.1045
126	trans-3-Hydroxycotinine glucuronide	Sugar Acids and Derivatives	HMDB	3.92	367.1115
127	Erythritol	Sugar Alcohols	HMDB	19.95	121.0504
128	Hypotaurine	Sulfinic Acids and Derivatives	Std	3.60	110.0091
129	Taurine	Sulfonic Acids and Derivatives	Std	3.80	124.0259
130	Urobilin	Tetrapyrroles and Derivatives	HMDB	20.51	595.3462
131	Biotin sulfone	Thienoimidazolidines	HMDB	4.65	277.0895

*std, metabolites validated by reference standards; HMDB, annotated with Human Metabolome Database (HMDB)

Table S3. Short chain fatty acids in gastrointestinal contetns of control rats and rats with ethanol consumption for 8 weeks (μmol/g wet wt).

GI tract	Group	Acetic acid	Propionic Acid	Isobutyric Acid (2-methlpropanoic acid)	Butyric Acid	2-methlbutanoic acid	Isovaleric Acid (3-methlbutanoic acid)	Valeric Acid (pentanoic acid)	Caproic Acid (Hexanoic acid)	Heptanoic Acid
Stomach	Control	0.053 ± 0.025	0.211 ± 0.167	0.058 ± 0.027	0.093 ± 0.038	0.081 ± 0.052	0.005 ± 0.004	0.027 ± 0.113	0.005 ± 0.003	0.003 ± 0.001
	Ethanol	0.323 ±0.093**	0.299 ± 0.108	0.036 ± 0.007	0.372 ± 0.201	0.103 ± 0.051	0.004 ± 0.001	0.022 ± 0.006	0.008 ± 0.007	0.003 ± 0.002
Duodenum	Control	0.003 ± 0.003	0.010 ± 0.007	ND	ND	ND	ND	ND	ND	ND
	Ethanol	0.003 ± 0.002	0.005 ± 0.001	ND	ND	ND	ND	ND	ND	ND
Jejunum	Control	0.059 ± 0.111	0.052 ± 0.011	ND	ND	ND	ND	ND	ND	ND
	Ethanol	0.068 ± 0.015	0.059 ± 0.016	ND	ND	ND	ND	ND	ND	ND
Ileum	Control	2.439 ± 0.537	4.559 ± 0.670	0.082 ± 0.014	0.256 ± 0.035	0.254 ± 0.037	0.862 ± 0.127	0.183 ± 0.062	0.004 ± 0.001	0.023 ± 0.003
	Ethanol	6.176±1.532***	2.768±0.679**	0.057 ± 0.012*	0.250 ± 0.047	0.169 ± 0.029**	0.565±0.077***	0.101 ± 0.011*	0.004 ± 0.002	0.015 ± 0.002**
Cecum	Control	68.030 ± 4.689	9.071 ± 0.912	0.631 ± 0.064	11.679 ± 2.497	0.343 ± 0.095	0.678 ± 0.116	0.596 ± 0.109	0.103 ± 0.113	0.026 ± 0.015
	Ethanol	98.507±23.149*	5.660±0.582***	0.579 ± 0.097	3.917±1.057***	0.334 ± 0.089	0.702 ± 0.144	0.666 ± 0.139	0.497±0.084***	0.133 ± 0.045**
Colon	Control	3.302 ± 0.713	3.965 ± 0.832	0.261 ± 0.029	8.406 ± 1.918	0.508 ± 0.078	0.058 ± 0.017	0.070 ± 0.015	0.059 ± 0.055	0.012 ± 0.002
	Ethanol	2.383 ± 0.348*	1.378±0.259***	0.128±0.016***	1.408±0.403***	0.108±0.033***	0.019±0.008***	0.068 ± 0.053	0.040 ± 0.015	0.009 ± 0.003
Rectum	Control	2.175 ± 0.416	2.578 ± 0.633	0.171 ± 0.025	5.335 ± 1.966	0.439 ± 0.080	0.049 ± 0.008	0.053 ± 0.011	0.150 ± 0.085	0.030 ± 0.030
	Ethanol	1.858 ± 0.406	1.419±0.295**	0.112±0.012***	1.579±0.333***	0.140±0.039***	0.019±0.003***	0.068 ± 0.044	0.049 ± 0.021**	0.009 ± 0.003

ND, Non-detectable. Values are presented as mean ± SD (μmol/g wet wt).

* Significantly different from control group. * P<0.05; ** P<0.01; *** P<0.001.

Table S4. Branched chain amino acids in gastrointestinal contents of control rats and rats with ethanol consumption for 8 weeks (μmol/g wet wt).

GI tract	Group	Valine	Leucine	Isoleucine
Stomach	Control	0.016 ± 0.003	0.046 ± 0.011	0.011 ± 0.003
	Ethanol	0.046 ± 0.015*	0.057 ± 0.019	0.053 ± 0.021*
Duodenum	Control	6.023 ± 6.725	3.283 ± 3.429	7.297 ± 8.562
	Ethanol	1.407 ± 0.196	0.846 ± 0.190	1.417 ± 0.168**
Jejunum	Control	5.087 ± 0.689	1.906 ± 0.268	6.686 ± 0.633
	Ethanol	3.954 ± 1.361	1.334 ± 0.368*	4.149 ± 1.239***
Ileum	Control	15.266 ± 3.705	5.272 ± 1.527	13.821 ± 3.210
	Ethanol	4.456 ± 2.952***	1.467 ± 1.121**	4.200 ± 2.789**
Cecum	Control	3.076 ± 1.342	0.845 ± 0.391	2.986 ± 1.406
	Ethanol	0.816 ± 0.279**	0.256 ± 0.083**	0.846 ± 0.336**
Colon	Control	0.324 ± 0.145	0.162 ± 0.051	0.375 ± 0.145
	Ethanol	0.093 ± 0.027**	0.049 ± 0.015***	0.120 ± 0.028**
Rectum	Control	0.264 ± 0.046	0.156 ± 0.031	0.332 ± 0.081
	Ethanol	0.093 ± 0.032***	0.053 ± 0.018***	0.120 ± 0.032**

Values are presented as mean ± SD (μmol/g wet wt).

* Significantly different from control group. * P<0.05; ** P<0.01; *** P<0.001.

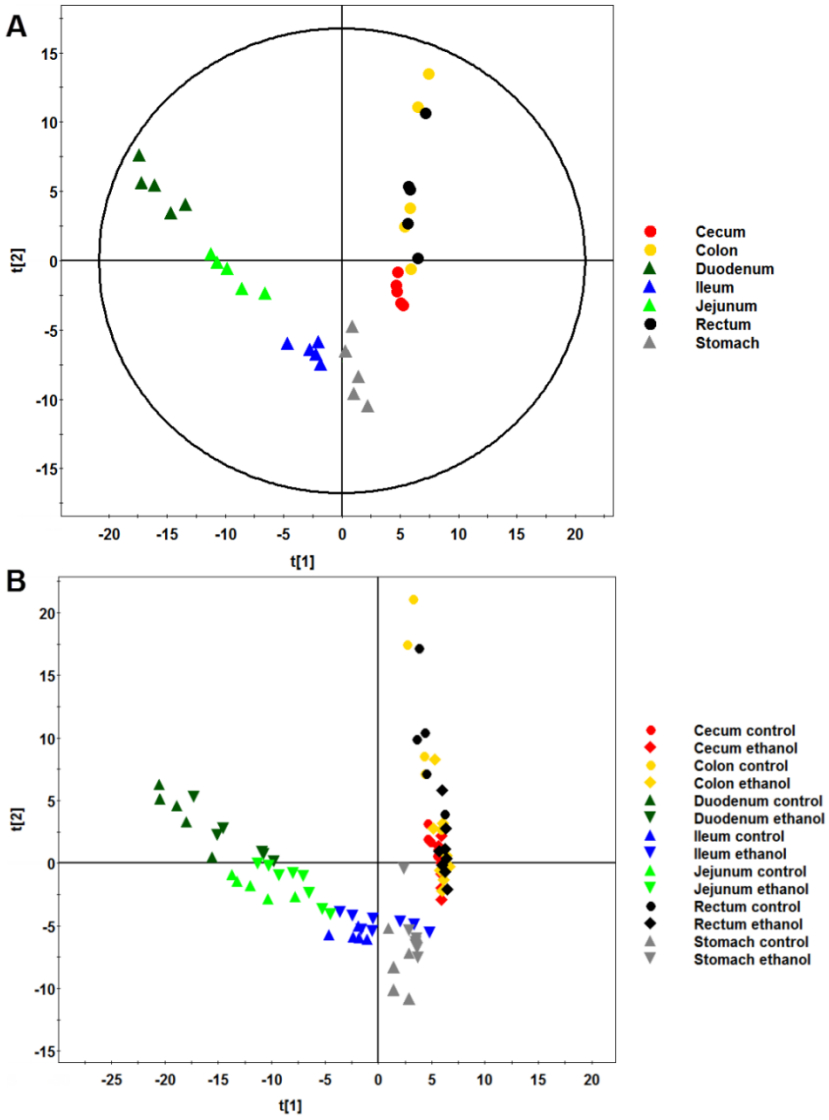

Figure S1. PCA scores plot obtained by analysis of contents from 7 gut compartments including stomach, Duodenum, Jejunum, Ileum, Cecum, Colon, and Rectum of control rats (A) and rats under chronic ethanol consumption (B).

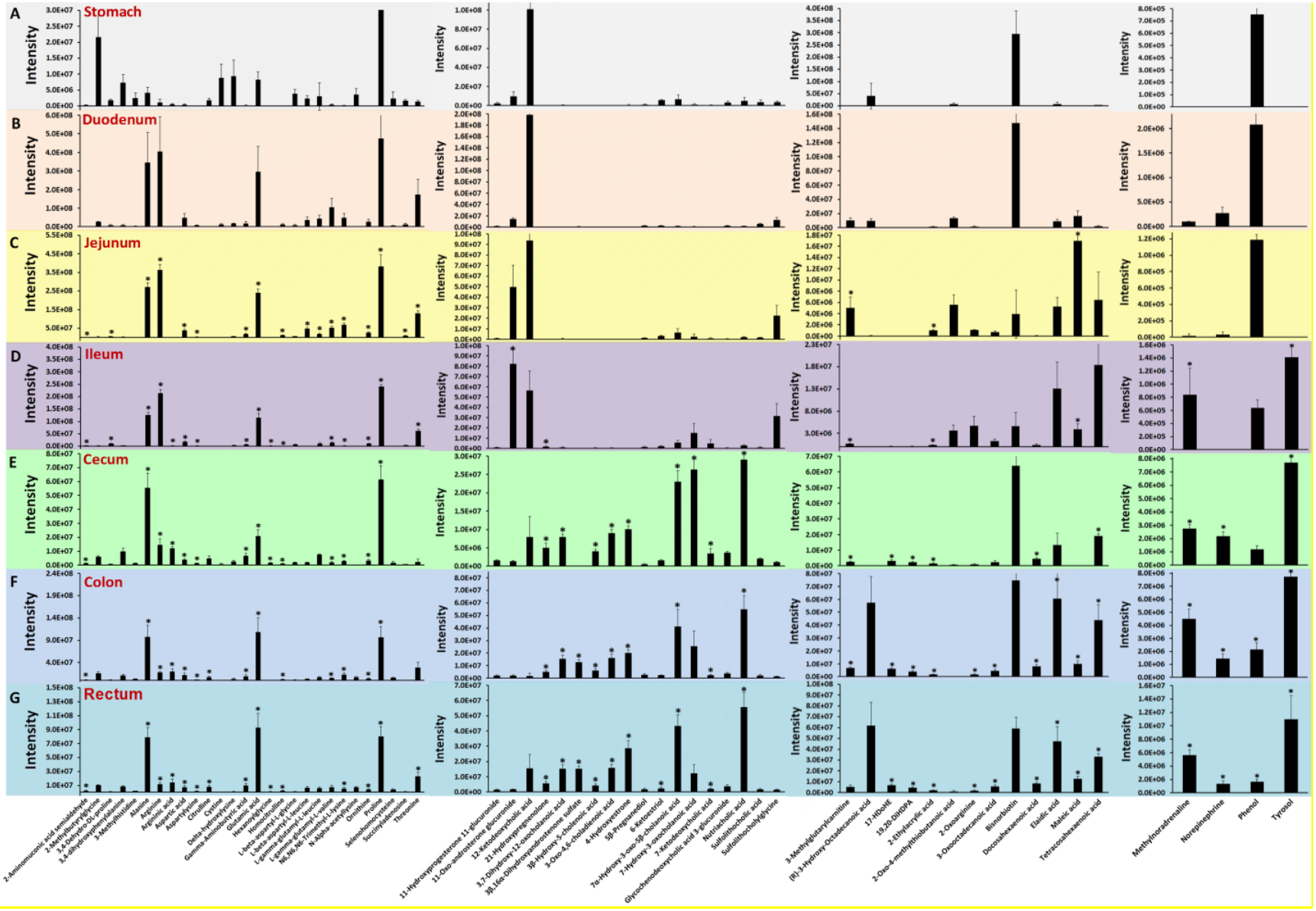
A
Stomach
B
Duodenum
C
Jejunum
D
Ileum
E
Cecum
F
Colon
G
Rectum
Intensity
2-Aminomuconic acid semialdehyde
2-Methylbutyrylglycine
3,4-Dehydro-DL-proline
3,4-dihydroxyphenylalanine
3-Methylhistidine
Alanine
Arginine
Arginic acid
Aspartic acid
Asparthyine
Citrulline
Cystine
Delta-hydroxylysine
Gamma-Aminobutyric acid
Glutamic acid
Homoarginine
Hexanoylglycine
Homocitrulline
L-beta-aspartyl-L-alysine
L-beta-aspartyl-L-leucine
L-gamma-glutamyl-L-leucine
L-gamma-glutamyl-L-lysine
N6,N6,N6-Trimethyl-L-lysine
N-Alpha-acetyllysine
Ornithine
Proline
Selenohomocysteine
Succinyladenosine
Threonine
11-Hydroxyprogesterone 11-glucuronide
11-Oxo-androsterone glucuronide
12-Ketodeoxycholic acid
21-Hydroxypregnenolone
3,7-Dihydroxy-12-oxocholanoic acid
3β,16α-Dihydroxyandrostenone sulfate
3β-Hydroxy-5-cholenoic acid
3-Oxo-4,6-choladienoic acid
4-Hydroxyestrone
5β-Pregnanediol
6-Ketocastisol
7α-Hydroxy-3-oxo-5β-cholanoic acid
7-Hydroxy-3-oxocholanoic acid
7-Ketodeoxycholic acid
Glycochenodeoxycholic acid 3-glucuronide
Nutriacholic acid
Sulfolithocholic acid
Sulfolithocholylglycine
3-Methylglutarylcarnitine
(R)-3-Hydroxy-Octadecamoic acid
17-HDoHE
19,20-DiHDPA
2-Ethylacrylic acid
2-Oxo-4-methylthiobutanoic acid
2-Oxoarginine
3-Oxooctadecanoic acid
Biorobiotin
Docosahexaenoic acid
Elaidic acid
Malaic acid
Tetracosahexaenoic acid
Methylnoradrenaline
Norepinephrine
Phenol
Tyrosol

Figure S2. Metabolic profiles (A-G) in GI contents (A, stomach; B, Duodenum; C, Jejunum; D, Ileum; E, Cecum; F, Colon; G, Rectum) of control rats. Values are mean intensities ± SEM measured using LC-MS. * $P < 0.05$ different from stomach concentration of the same metabolite.

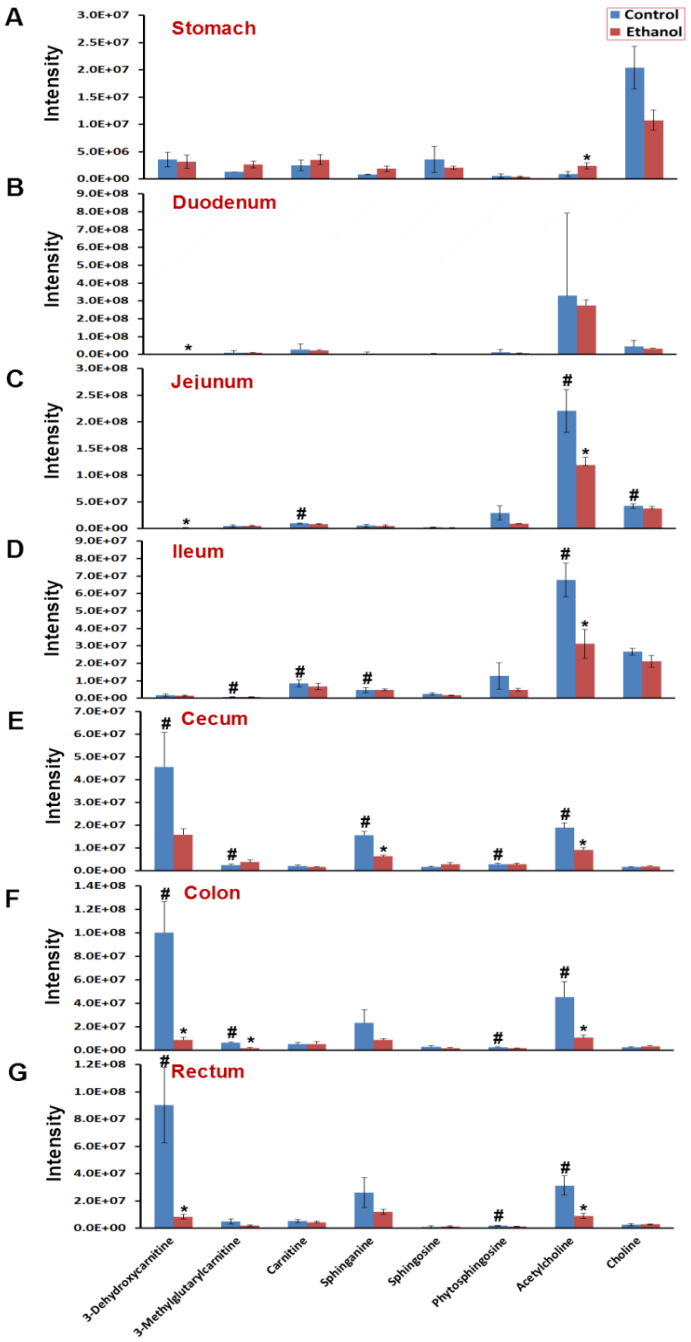

Figure S3. Metabolic profiles (A-G) in GI contents (A, stomach; B, Duodenum; C, Jejunum; D, Ileum; E, Cecum; F, Colon; G, Rectum) of control rats and rats with chronic ethanol consumption. Values are mean intensities ± SEM measured using LC-MS. # $P < 0.05$ different from stomach concentration of the same metabolite. * $p < 0.05$ vs. Control group.

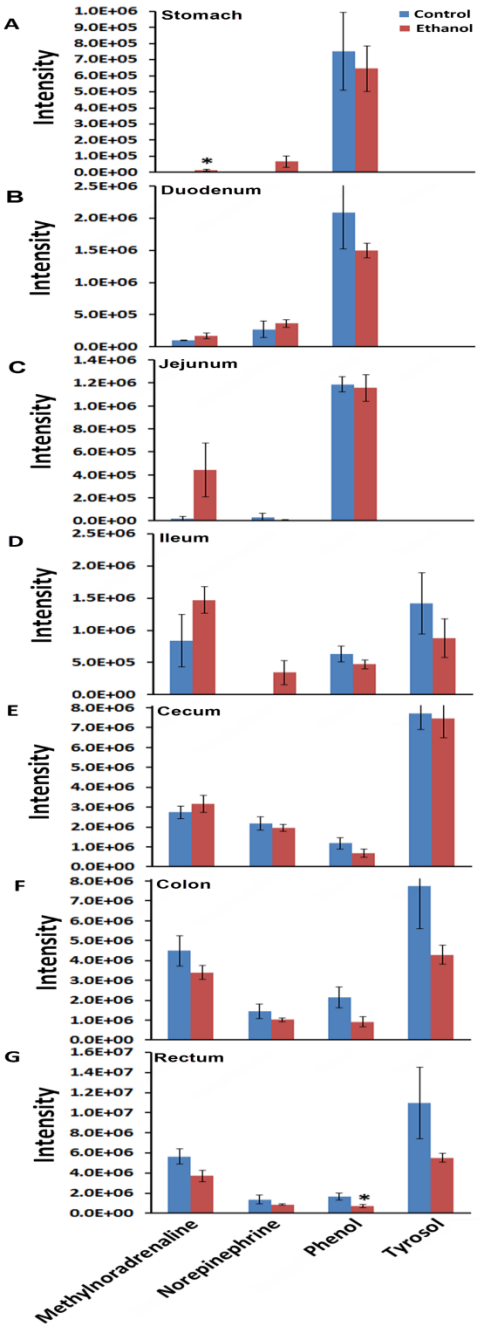

Figure S4. Alteration of phenol and derivatives profiles (A-G) in GI contents (A, stomach; B, Duodenum; C, Jejunum; D, Ileum; E, Cecum; F, Colon; G, Rectum) due to ethanol consumption. Values are mean intensities ± SEM measured using LC-MS. *P < 0.05 vs. Control group.

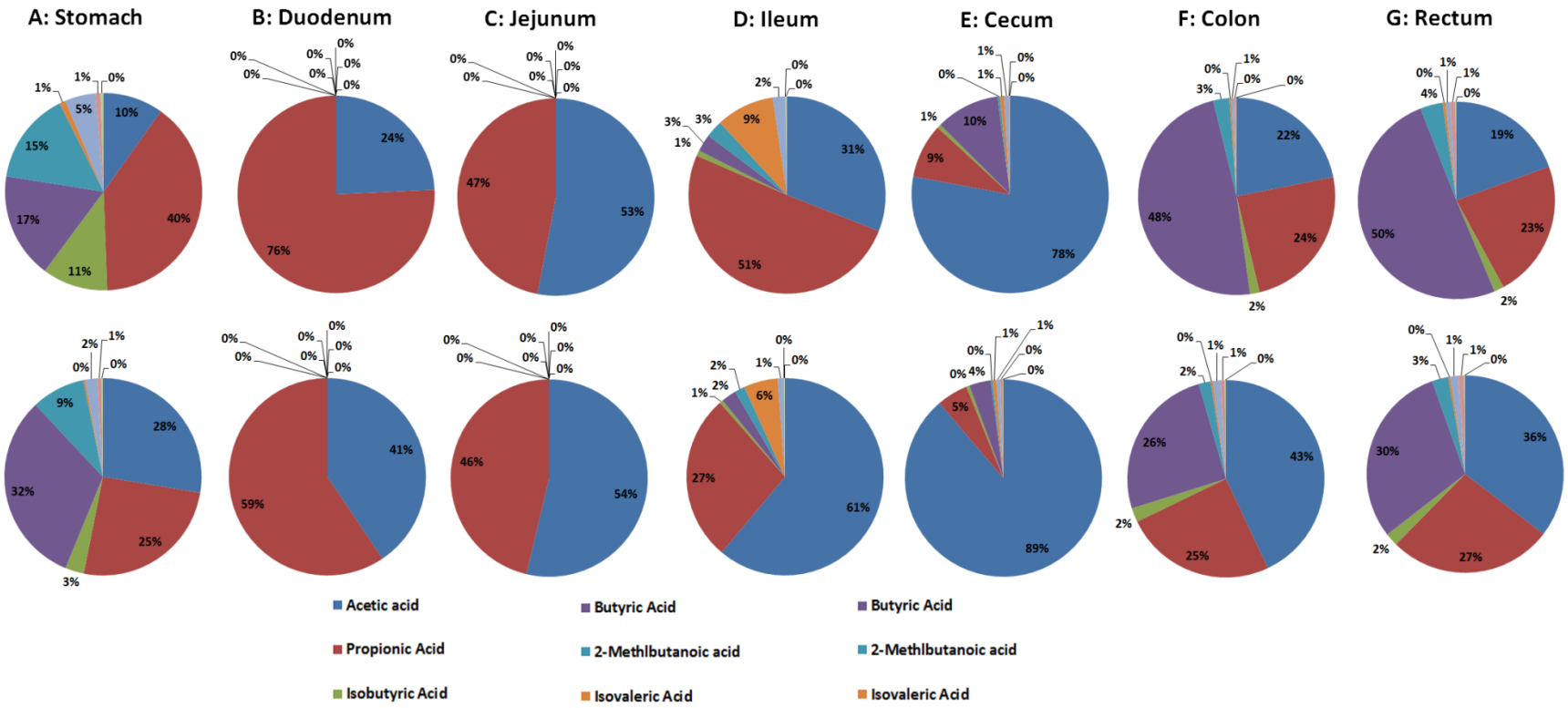

Figure S5. The composition of short chain fatty acids in gastrointestinal contents of control rats (upper panel) and ethanol treated rats (under panel). A, stomach; B, Duodenum; C, Jejunum; D, Ileum; E, Cecum; F, Colon; G, Rectum.