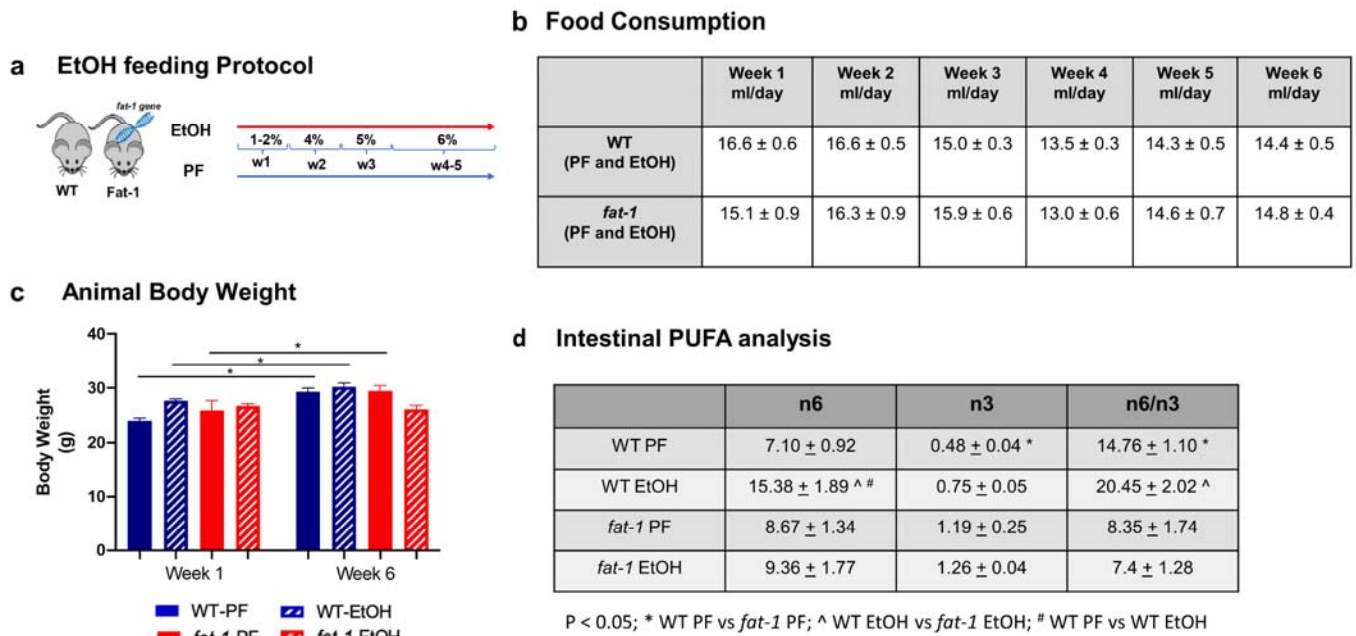


## Supplemental Information

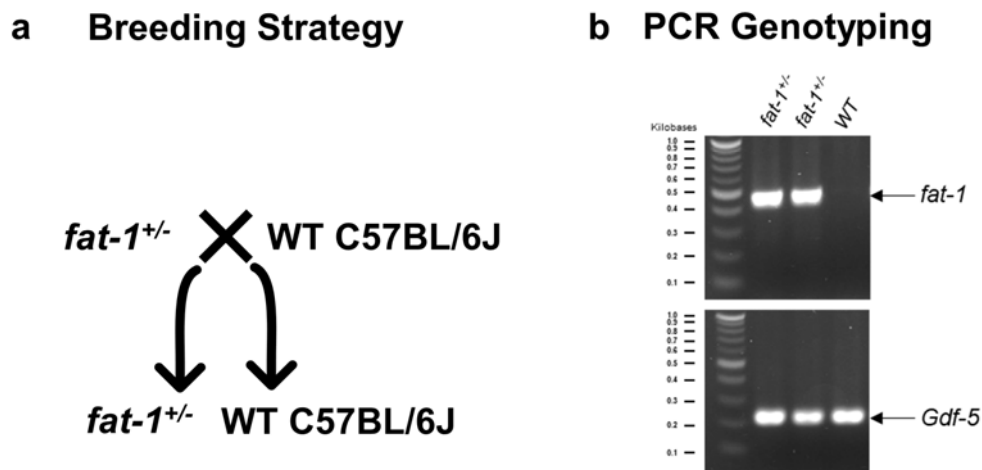
### **Transcriptional signatures of the small intestinal mucosa in response to ethanol in transgenic mice rich in endogenous n3 fatty acids**

Josiah E. Hardesty, Jeffrey B. Warner, Ying L. Song, Eric C. Rouchka, Chih-Yu Chen, Jing X. Kang, Craig J. McClain, Dennis R. Warner, and Irina A. Kirpich

## Supplemental Figures:



**Supplemental Figure S1. Experimental Design and Metabolic Parameters.** (a) EtOH feeding model. (b) Average food consumption by experimental animals. (c) Beginning and ending body weights of PF and EtOH-fed WT and *fat-1* mice. (d) Ileum n3 and n6 PUFA levels demonstrating that *fat-1* mice have elevated n3 PUFAs relative to WT mice. Data are presented as mean ± SEM. \* p < 0.05. n=3-5 mice per group.



**Supplemental Figure S2. Description of the *fat-1* mice.** (a) Breeding strategy to develop *fat-1*<sup>+/-</sup> heterozygous mice and WT littermates. (b) Genotype confirmation by PCR and gel electrophoresis. The *fat-1* PCR product is only present in samples from *fat-1* mice whereas *Gdf-5* is present in all samples (positive control) in the representative gel.



**Supplemental Figure S3. Mouse ileum isolation.** Layout of mouse gastrointestinal tract identifying the segment of intestine isolated for ileum RNASeq.

**Supplemental Tables:**

**Supplementary Table S1.** List of the top 10 up-regulated and down-regulated genes (by fold-change) in the intestinal mucosa in *fat-1* compared to WT pair-fed control mice (selected from 420 up- and 210 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000048473	<i>Gm766</i>	40.6	0.00005	0.0022175
ENSMUSG00000045991	<i>Onecut2</i>	32.47	0.00005	0.0022175
ENSMUSG00000059654	<i>Reg1</i>	20.7	0.00005	0.0022175
ENSMUSG00000076540	<i>Igkv4-80</i>	16.8	0.00005	0.0022175
ENSMUSG00000046213	<i>Cym</i>	15.2	0.00005	0.0022175
ENSMUSG00000095429	<i>Ighv5-12</i>	12.0	0.0006	0.016949
ENSMUSG00000054932	<i>Afp</i>	10.2	0.00005	0.0022175
ENSMUSG00000076536	<i>Igkv4-86</i>	9.3	0.00005	0.0022175
ENSMUSG00000036853	<i>Mcoln3</i>	9.0	0.00005	0.0022175
ENSMUSG00000067235	<i>H2-Q10</i>	8.8	0.00005	0.0022175
ENSMUSG00000074442	<i>Defa-rs7</i>	-26.4	0.00005	0.0022175
ENSMUSG00000094662	<i>Gm21498</i>	-19.1	0.00005	0.0022175
ENSMUSG00000094993	<i>Igkv4-51</i>	-15.0	0.00035	0.011114
ENSMUSG00000031089	<i>Slc6a14</i>	-11.9	0.00005	0.0022175
ENSMUSG00000104452	<i>Ighv8-8</i>	-11.4	0.00005	0.0022175
ENSMUSG00000096844	<i>Igkv6-14</i>	-8.6	0.00085	0.0222623
ENSMUSG00000026109	<i>Tmeff2</i>	-6.9	0.0005	0.0147833
ENSMUSG00000033847	<i>Pla2g4c</i>	-6.4	0.00005	0.0022175
ENSMUSG00000078706	<i>Gm53</i>	-5.7	0.00005	0.0022175
ENSMUSG00000094198	<i>Ighv1-50</i>	-5.6	0.00005	0.0022175

**Supplementary Table S2.** List of up-regulated and down-regulated genes in the intestinal mucosa in WT EtOH compared to WT pair-fed control mice. The top 10 up-regulated and down-regulated genes (by fold-change) are shown in a list (selected from 481 up- and 663 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000032122	<i>Slc37a2</i>	47.2	0.00005	0.00128109
ENSMUSG00000073608	<i>Gm6086</i>	39.1	0.00005	0.00128109
ENSMUSG00000094559	<i>Cyp2d34</i>	29.5	0.0001	0.00236173
ENSMUSG00000057074	<i>Ces1g</i>	21.1	0.00005	0.00128109
ENSMUSG00000001943	<i>Vsig2</i>	20.4	0.00155	0.0230212
ENSMUSG00000033847	<i>Pla2g4c</i>	19.6	0.00005	0.00128109
ENSMUSG00000022650	<i>Retnlb</i>	18.2	0.00005	0.00128109
ENSMUSG00000068893	<i>Sprr2a2</i>	17.2	0.00005	0.00128109
ENSMUSG00000076666	<i>Ighv14-4</i>	15.2	0.00005	0.00128109
ENSMUSG00000096672	<i>Ighv1-63</i>	14.1	0.0009	0.015004
ENSMUSG00000026354	<i>Lct</i>	-1791.5	0.00005	0.00128109
ENSMUSG00000021944	<i>Gata4</i>	-135.6	0.00005	0.00128109
ENSMUSG00000029134	<i>Plb1</i>	-130.9	0.00005	0.00128109
ENSMUSG00000024354	<i>Slc23a1</i>	-93.3	0.0022	0.0307049
ENSMUSG00000035780	<i>Ugt2a3</i>	-79.9	0.00005	0.00128109
ENSMUSG00000054422	<i>Fabp1</i>	-69.8	0.00005	0.00128109
ENSMUSG00000027690	<i>Slc2a2</i>	-43.2	0.00005	0.00128109
ENSMUSG00000030483	<i>Cyp2b10</i>	-40.5	0.00005	0.00128109
ENSMUSG00000076655	<i>Ighv4-1</i>	-35.3	0.00005	0.00128109
ENSMUSG00000005980	<i>Dnase1</i>	-33.7	0.00005	0.00128109

**Supplementary Table S3.** List of up-regulated and down-regulated genes in the intestinal mucosa in *fat-1* EtOH compared to *fat-1* pair-fed control mice. The top 10 up-regulated and down-regulated genes are shown in a list (by fold-change) (selected from 1045 up- and 1062 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000027556	<i>Car1</i>	1454.1	0.00345	0.027756
ENSMUSG00000094559	<i>Cyp2d34</i>	258.4	0.00315	0.0258838
ENSMUSG00000026390	<i>Marco</i>	151.1	0.00135	0.0136846
ENSMUSG00000022650	<i>Retnlb</i>	131.0	0.0014	0.0140118
ENSMUSG00000031089	<i>Slc6a14</i>	105.5	0.00005	0.00092057
ENSMUSG00000037362	<i>Nov</i>	96.6	0.00005	0.00092057
ENSMUSG00000033579	<i>Fa2h</i>	87.0	0.00005	0.00092057
ENSMUSG00000013653	<i>1810065E05Rik</i>	71.2	0.00005	0.00092057
ENSMUSG00000096001	<i>2610528A11Rik</i>	47.3	0.00005	0.00092057
ENSMUSG00000024039	<i>Cbs</i>	42.6	0.00005	0.00092057
ENSMUSG00000026354	<i>Lct</i>	-1668.9	0.00005	0.00092057
ENSMUSG00000030483	<i>Cyp2b10</i>	-274.6	0.00005	0.00092057
ENSMUSG00000035780	<i>Ugt2a3</i>	-217.1	0.00005	0.00092057
ENSMUSG00000021944	<i>Gata4</i>	-191.2	0.00005	0.0210196
ENSMUSG00000029134	<i>Plb1</i>	-166.9	0.00005	0.00092057
ENSMUSG00000002992	<i>Apoc2</i>	-151.5	0.00005	0.00092057
ENSMUSG00000074373	<i>Gm10680</i>	-120.0	0.00045	0.0058381
ENSMUSG00000036216	<i>Leap2</i>	-115.0	0.00005	0.00092057
ENSMUSG00000032080	<i>Apoa4</i>	-106.5	0.00335	0.0270893
ENSMUSG00000019989	<i>Enpp3</i>	-106.3	0.00005	0.00092057

**Supplementary Table S4.** List of commonly up-regulated and down-regulated genes in the intestinal mucosa in *fat-1* and WT mice in response to EtOH. The top 10 up-regulated and down-regulated genes (by fold-change) are shown in a list (selected from 310 up- and 525 down-regulated genes).

Identifier	Gene title	<i>Fat-1</i> EtOH vs <i>fat-1</i> PF			WT EtOH vs WT PF		
		fold	p-value	q-value	fold	p-value	q-value
ENSMUSG00000094559	<i>Cyp2d34</i>	258.4	0.00315	0.0258838	29.5	0.0001	0.00236173
ENSMUSG00000022650	<i>Retnlb</i>	131.0	0.0014	0.0140118	18.2	0.00005	0.00128109
ENSMUSG00000031089	<i>Slc6a14</i>	105.5	0.00005	0.00092057	6.7	0.00005	0.00128109
ENSMUSG00000033579	<i>Fa2h</i>	87.0	0.00005	0.00092057	8.3	0.00005	0.00128109
ENSMUSG00000013653	<i>1810065E05Rik</i>	71.2	0.00005	0.00092057	8.3	0.00005	0.00128109
ENSMUSG00000096001	<i>2610528A11Rik</i>	47.3	0.00005	0.00092057	3.8	0.00005	0.00128109
ENSMUSG00000024039	<i>Cbs</i>	42.6	0.00005	0.00092057	4.6	0.00005	0.00128109
ENSMUSG00000027870	<i>Hao2</i>	38.9	0.00005	0.00092057	8.4	0.00005	0.00128109
ENSMUSG00000027875	<i>Hmgcs2</i>	35.2	0.00005	0.00092057	9.1	0.00005	0.00128109
ENSMUSG00000026811	<i>St6galnac6</i>	32.3	0.00005	0.00092057	4.5	0.00005	0.00128109
ENSMUSG00000026354	<i>Lct</i>	-1668.9	0.00005	0.00092057	-1791.6	0.00005	0.00128109
ENSMUSG00000030483	<i>Cyp2b10</i>	-274.6	0.00005	0.00092057	-40.5	0.00005	0.00128109
ENSMUSG00000035780	<i>Ugt2a3</i>	-217.1	0.00005	0.00092057	-79.9	0.00005	0.00128109
ENSMUSG00000021944	<i>Gata4</i>	-191.2	0.0024	0.0210196	-135.6	0.00005	0.00128109
ENSMUSG00000029134	<i>Plb1</i>	-166.9	0.00005	0.00092057	-130.9	0.00005	0.00128109
ENSMUSG00000002992	<i>Apoc2</i>	-151.5	0.00005	0.00092057	-19.9	0.00005	0.00128109
ENSMUSG00000074373	<i>Gm10680</i>	-120.0	0.00045	0.0058381	-20.6	0.00025	0.00525227
ENSMUSG00000036216	<i>Leap2</i>	-115.0	0.00005	0.00092057	-11.2	0.00005	0.00128109
ENSMUSG00000032080	<i>Apoa4</i>	-106.5	0.00335	0.0270893	-20.5	0.00005	0.00128109
ENSMUSG00000019989	<i>Enpp3</i>	-106.3	0.00005	0.00092057	-25.2	0.00005	0.00128109

**Supplementary Table S5.** List of genes exclusively up-regulated and down-regulated in the intestinal mucosa in WT mice in response to EtOH. The top 10 up-regulated and down-regulated genes (by fold-change) are shown in a list (selected from 171 up- and 138 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000032122	<i>Slc37a2</i>	47.2	0.00005	0.00128109
ENSMUSG00000076666	<i>Ighv14-4</i>	15.2	0.00005	0.00128109
ENSMUSG00000096672	<i>Ighv1-63</i>	14.1	0.0009	0.015004
ENSMUSG00000050296	<i>Abca12</i>	13.2	0.00005	0.00128109
ENSMUSG00000086474	<i>9130204K15Rik</i>	11.6	0.0003	0.00615377
ENSMUSG00000065037	<i>Rn7sk</i>	11.0	0.00025	0.00525227
ENSMUSG00000098814	<i>Igkv19-93</i>	6.7	0.00005	0.00128109
ENSMUSG00000079174	<i>Gm3054</i>	6.1	0.00005	0.00128109
ENSMUSG00000095204	<i>Ighv1-52</i>	5.7	0.00005	0.00128109
ENSMUSG00000086389	<i>Gm15998</i>	5.2	0.0015	0.0224225
ENSMUSG00000054422	<i>Fabp1</i>	-69.8	0.00005	0.00128109
ENSMUSG00000094164	<i>Ighv2-3</i>	-16.1	0.00005	0.00128109
ENSMUSG00000094993	<i>Igkv4-51</i>	-15.1	0.00075	0.0130517
ENSMUSG00000014351	<i>Gip</i>	-14.3	0.00005	0.00128109
ENSMUSG00000104452	<i>Ighv8-8</i>	-8.2	0.00005	0.00128109
ENSMUSG00000032454	<i>Rbp2</i>	-7.7	0.00005	0.00128109
ENSMUSG00000102888	<i>Ighv1-11</i>	-7.5	0.00035	0.00701364
ENSMUSG00000076538	<i>Igkv13-84</i>	-7.4	0.0005	0.00940854
ENSMUSG00000076532	<i>Igkv4-91</i>	-6.9	0.00005	0.00128109
ENSMUSG00000029082	<i>Bst1</i>	-6.5	0.00005	0.00128109

**Supplementary Table S6.** List of genes exclusively up-regulated and down-regulated in the intestinal mucosa in *fat-1* mice in response to EtOH. The top 10 up-regulated and down-regulated genes (by fold-change) are shown in a list (selected from 735 up- and 537 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000027556	<i>Car1</i>	1454.1	0.00345	0.027756
ENSMUSG00000026390	<i>Marco</i>	151.1	0.00135	0.0136846
ENSMUSG00000037362	<i>Nov</i>	96.6	0.00005	0.00092057
ENSMUSG00000032068	<i>Plet1</i>	30.9	0.00005	0.00092057
ENSMUSG00000021803	<i>Cdhr1</i>	29.8	0.00125	0.0128913
ENSMUSG00000048337	<i>Npy4r</i>	26.6	0.0009	0.0100225
ENSMUSG00000046589	<i>Lrrc8e</i>	17.8	0.0009	0.0100225
ENSMUSG00000032028	<i>Nxpe2</i>	14.9	0.00005	0.00092057
ENSMUSG00000017723	<i>Wfdc2</i>	14.8	0.00005	0.00092057
ENSMUSG00000029088	<i>Kcnp4</i>	14.5	0.00035	0.00481741
ENSMUSG00000041660	<i>Bbox1</i>	-58.5	0.00005	0.00092057
ENSMUSG00000068323	<i>Slc4a5</i>	-25.4	0.00005	0.00092057
ENSMUSG00000005237	<i>Dnah2</i>	-19.8	0.00005	0.00092057
ENSMUSG00000002324	<i>Rec8</i>	-18.5	0.00005	0.00092057
ENSMUSG000000054932	<i>Afp</i>	-15.5	0.0027	0.0230512
ENSMUSG000000095981	<i>Ighv10-1</i>	-14.4	0.00005	0.00092057
ENSMUSG00000038496	<i>Slc19a3</i>	-14.3	0.0001	0.00170036
ENSMUSG00000076665	<i>Ighv7-1</i>	-13.7	0.00005	0.00092057
ENSMUSG00000030402	<i>Ppm1n</i>	-10.9	0.0013	0.0132723
ENSMUSG00000070332	<i>Trim80</i>	-9.8	0.0006	0.00729846

**Supplementary Table S7.** List of top 10 up-regulated and down-regulated genes (by fold-change) in the intestinal mucosa in *fat-1* EtOH compared to WT EtOH littermates (selected from 80 up- and 14 down-regulated genes).

Identifier	Gene title	Fold change	p-value	q-value
ENSMUSG00000037362	<i>Nov</i>	60.6	0.00005	0.00367576
ENSMUSG00000032068	<i>Plet1</i>	46.3	0.00005	0.00367576
ENSMUSG00000065987	<i>Cd209b</i>	19.5	0.00005	0.00367576
ENSMUSG00000076674	<i>Ighv1-85</i>	15.7	0.00005	0.00367576
ENSMUSG00000039579	<i>Grin3a</i>	13.6	0.00005	0.00367576
ENSMUSG00000032028	<i>Nxpe2</i>	10	0.00005	0.00367576
ENSMUSG000000094806	<i>Cyp2d10</i>	9.6	0.00015	0.0282662
ENSMUSG00000055210	<i>Foxd2</i>	8.9	0.0002	0.0341412
ENSMUSG00000095457	<i>Gm8989</i>	7.7	0.00005	0.0117016
ENSMUSG00000019775	<i>Rgs17</i>	6.9	0.0002	0.0341412
ENSMUSG000000096150	<i>Ighv3-8</i>	-18.9	0.00005	0.00367576
ENSMUSG00000076666	<i>Igkv2-109</i>	-10.8	0.00005	0.00367576
ENSMUSG00000076563	<i>Igkv5-48</i>	-7.5	0.00005	0.0117016
ENSMUSG00000033847	<i>Pla2g4c</i>	-7.0	0.00005	0.0117016
ENSMUSG00000031073	<i>Fgf15</i>	-6.8	0.00005	0.0117016
ENSMUSG00000096833	<i>Igkv4-55</i>	-5.9	0.0002	0.0341412
ENSMUSG00000076586	<i>Igkv8-21</i>	-4.7	0.00005	0.0117016
ENSMUSG00000095571	<i>Ighv5-17</i>	-4.5	0.00005	0.0117016
ENSMUSG00000094433	<i>Igkv5-43</i>	-3.1	0.00005	0.0117016
ENSMUSG00000020826	<i>Nos2</i>	-2.9	0.00005	0.0117016