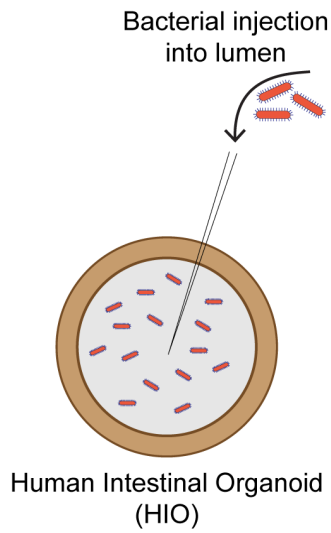
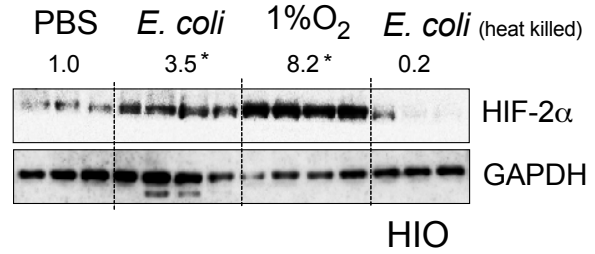
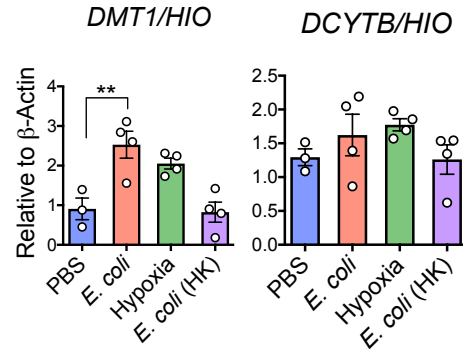
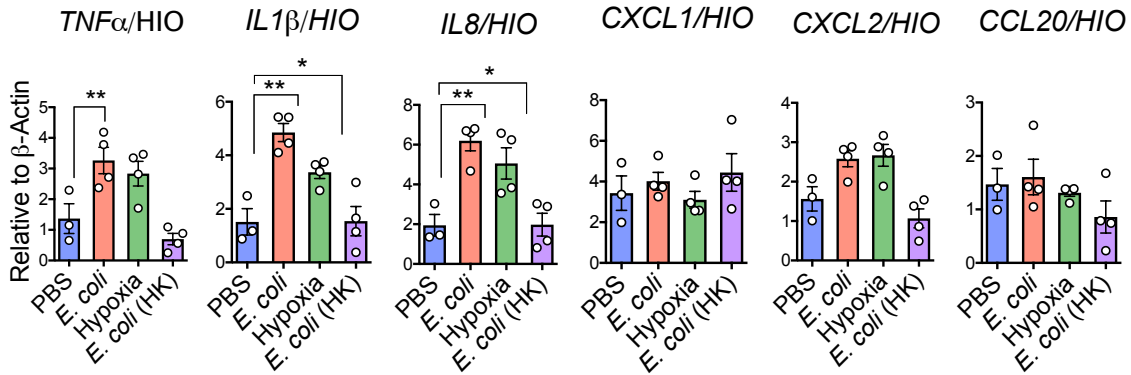
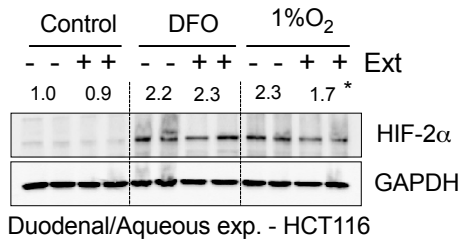
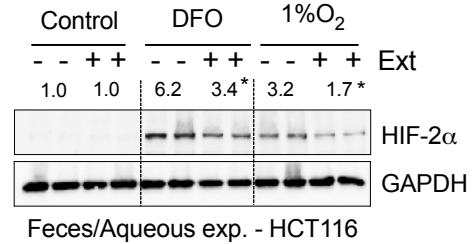
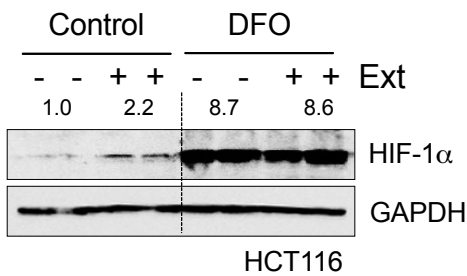
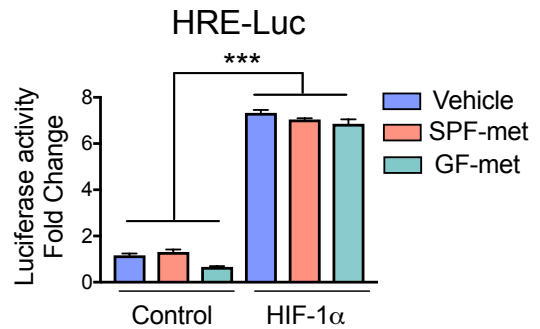
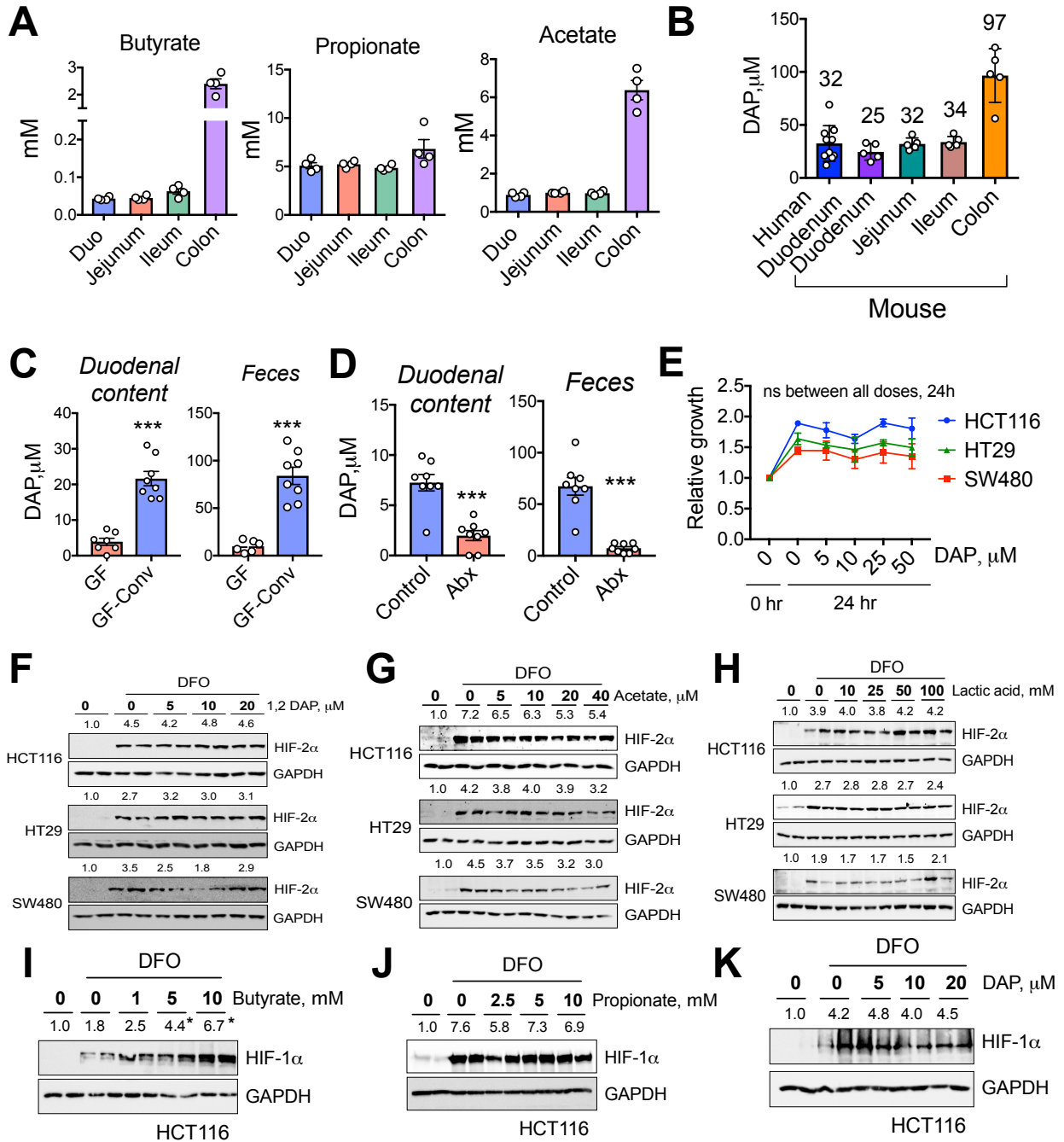


Supplementary Figure 1. Related to Figure 1. **A)** Serum, liver and spleen iron levels in SPF and GF mice fed with 350-ppm, 35-ppm or < 5-ppm iron diet and **B)** Hepatic hepcidin gene expression analysis in them. **C)** Hepatic hepcidin gene expression analysis in control vs. Abx treated wild type SPF mice. Gene expression analysis of **D)** *Dmt1* and **E)** *Dcytb* in SPF and GF mice fed with 350-ppm or 35-ppm iron diet. **F)** CBC values of GF and GF-conv mice. All data are mean \pm SEM. t-test (A(between corresponding iron diet groups), C, D, E, F) or one way ANOVA with Tukey's multiple comparisons test (B). * P < 0.05, ** P < 0.01, *** P < 0.001.

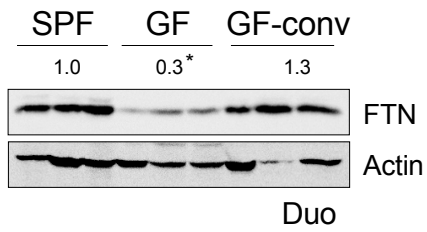
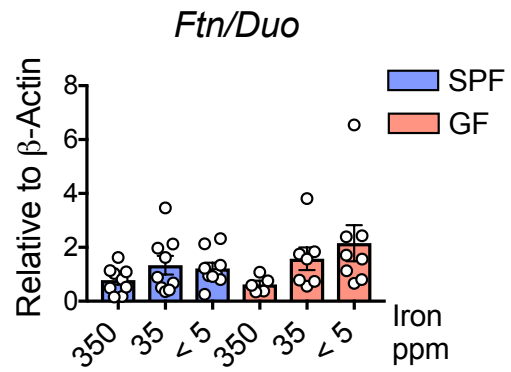
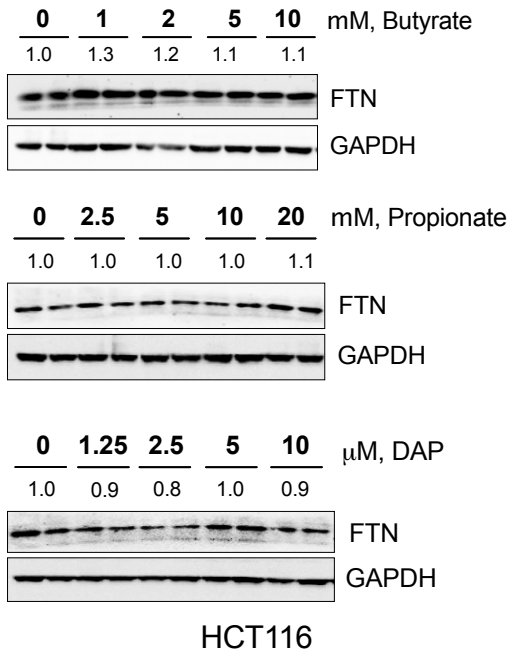
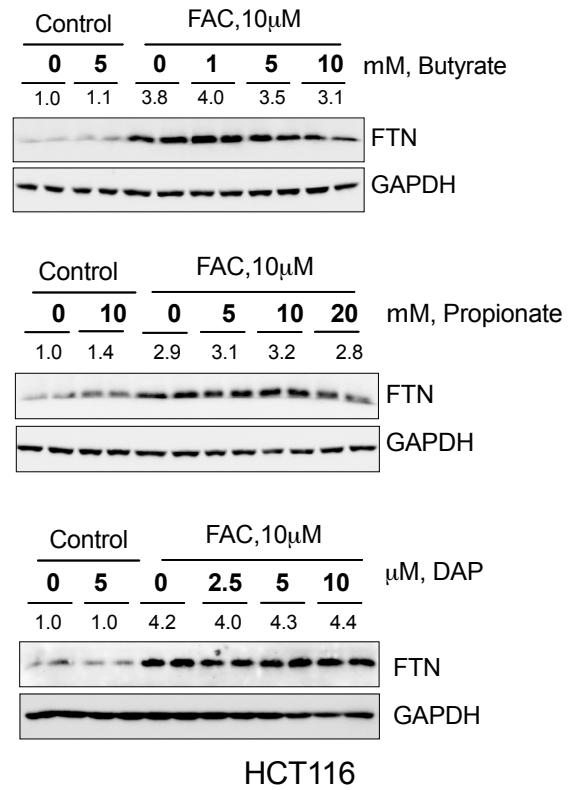
A**B****C****D****E****F****G****H**

Supplementary Figure 2. Related to Figure 2. **A)** Schematic showing bacterial injection into a human intestinal organoid (HIO). HIOs were treated with PBS, 1% O₂, live or heat-killed *E. coli*. HIF-2 α Western analysis (**B**); gene expression analyses of intestinal iron transporters (**C**) and HIF-dependent inflammatory markers (**D**). **E) and F)** HIF-2 α Western analyses of DFO- or 1% O₂ treated HCT116 cells followed by treatment with duodenal and fecal aqueous extracts (Ext), respectively. **G)** HIF-1 α Western analysis of DFO-treated HCT116 cells followed by treatment with fecal organic extract (Ext). **H)** HIF response element (HRE) luciferase assay in HCT116 cells transfected with empty vector (control) or HIF-1 α , followed by treatment with vehicle (DMSO) or fecal organic extracts from SPF- (SPF-met) and GF (GF-met) mice (n=3). All data are mean \pm SEM. One way ANOVA with Tukey's multiple comparisons test (C, D and H). Western analyses : Images were analyzed by Image J software from three independent experiments, representative image shown. Statistical significance compared with PBS group (B) and DFO-only treatment group (E, F and G). * P < 0.05, ** P < 0.01, *** P < 0.001.



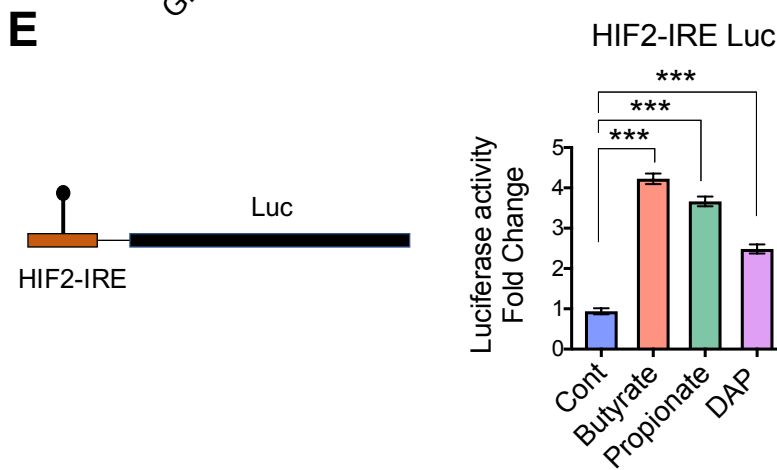
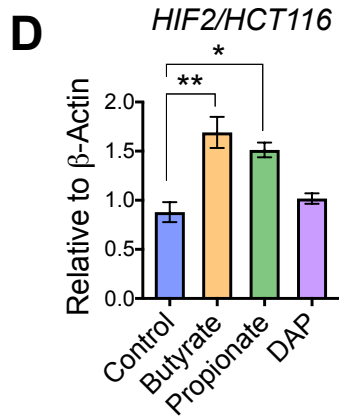
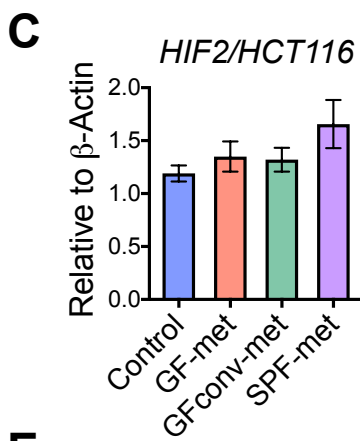
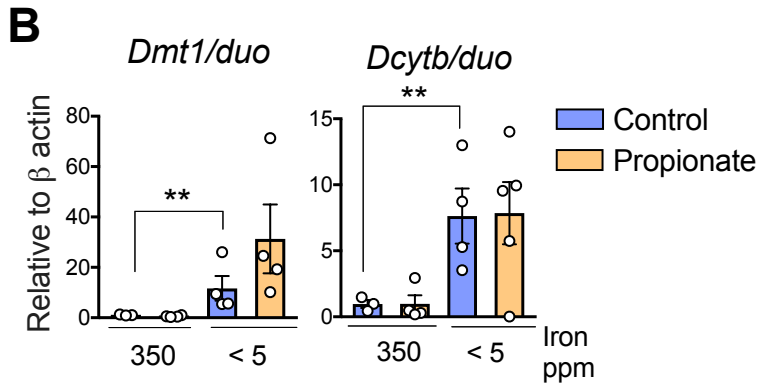
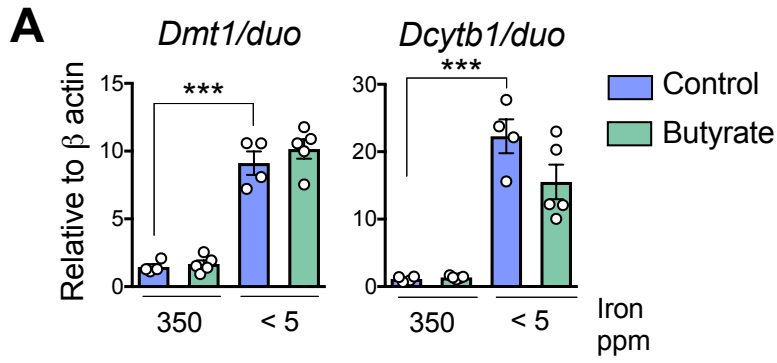
Supplementary Figure 3. Related to Figure 2. **A)** Physiological concentrations of butyrate, propionate and acetate in the mouse intestine. **B)** Mean physiological DAP concentrations in human duodenum and mouse intestine. **C)** DAP concentrations in GF and GF-conv duodenum and colon. **D)** DAP concentrations in wild type Abx-treated or –untreated (control) duodenum and colon. **E)** Cell growth assay of intestinal cell lines following DAP treatment (n=3). HIF-2 α Western analysis of DFO-treated HCT116, HT29 and SW480 cells followed by dose-dependent treatment of 1,2 diaminopropane (1,2 DAP) **(F)**, acetate **(G)** and lactic acid **(H)**. HIF-1 α Western analysis of DFO-treated HCT116 and HT29 cells followed by dose-dependent treatment of butyrate **(I)**, propionate **(J)** and DAP **(K)**.

All data are mean \pm SEM. t-test (C and D); one way ANOVA with Tukey's multiple comparisons test (E). Western analyses (F-K): Images were analyzed by Image J software from three independent experiments, representative image shown. Statistical significance compared with DFO-only treatment group. * P < 0.05, *** P < 0.001.

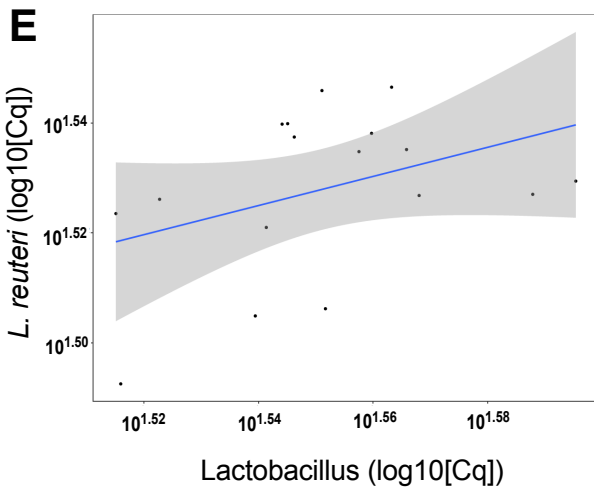
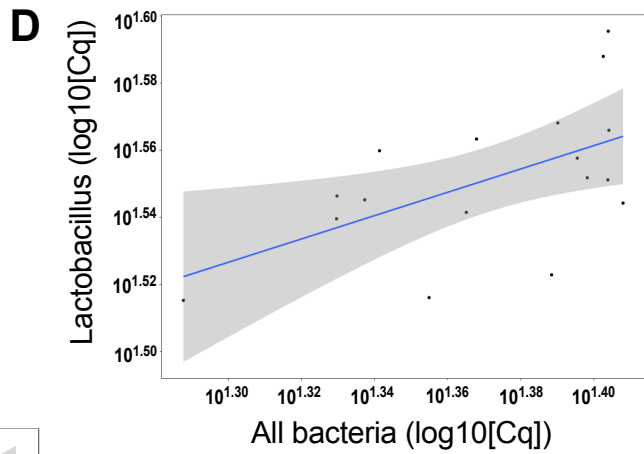
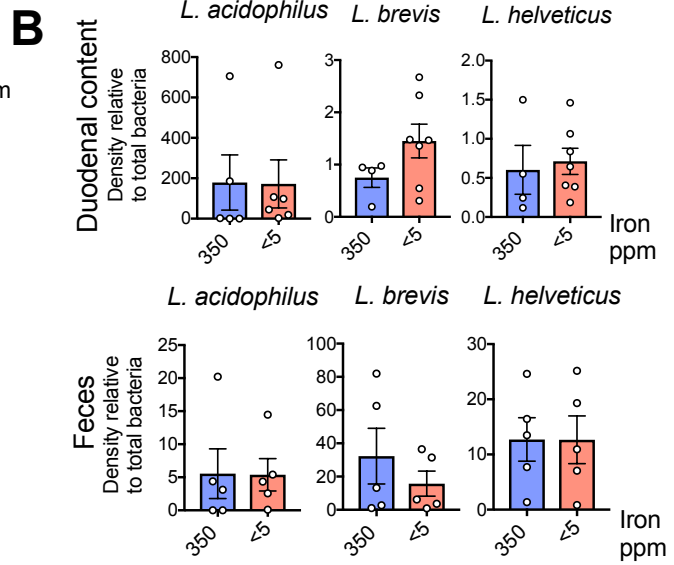
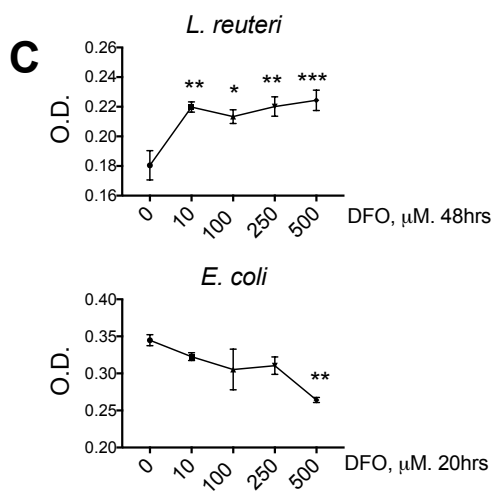
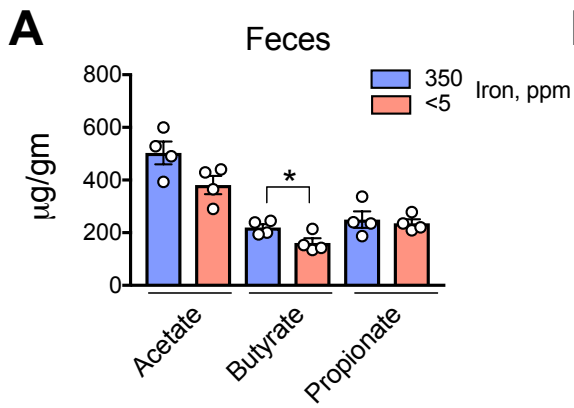
A**B****C****D**

Supplementary Figure 4. Related to Figure 3. **A)** Comparative duodenal FTN Western analysis in SPF, GF and GF-conv mice. **B)** *Ftn* gene expression analysis in SPF and GF mice fed with 350-ppm, 35-ppm or <5-ppm iron diet. **C)** and **D)** FTN Western analysis in HCT116 cell with dose-dependent butyrate, propionate or DAP without or with FAC (10 μ M) pretreatment respectively.

All data are mean \pm SEM. Western analyses : Images were analyzed by Image J software from three independent experiments, representative image shown. Statistical significance compared with SPF group (A), no treatment group (C and D). * P < 0.05



Supplementary Figure 5. Related to Figure 4. **A)** SPF mice were fed with 350-ppm and <5-ppm iron diet with or without butyrate supplementation in drinking water. Duodenal *Dmt1* and *Dcytb* gene expression analysis performed. **B)** SPF mice were fed with 350-ppm and <5-ppm iron diet with or without propionate supplementation in drinking water. Duodenal *Dmt1* and *Dcytb* gene expression analysis performed. **C)** *HIF-2 α* gene expression analysis in HCT116 cells treated with GF-met, GF-conv-met or SPF-met (n=3). **D)** *HIF-2 α* gene expression analysis in HCT116 cells treated with Butyrate (10mM), propionate (5mM) or DAP (10 μ M) (n=3). **E)** Schematic showing HIF2-IRE luciferase construct (upper panel) and HIF2-IRE luciferase assay in HCT116 cells treated with Butyrate (10mM), propionate (5mM) or DAP (10 μ M) (n=3). All data are mean \pm SEM. One way ANOVA with Tukey's multiple comparisons test (A-E). ** P < 0.01, *** P < 0.001.

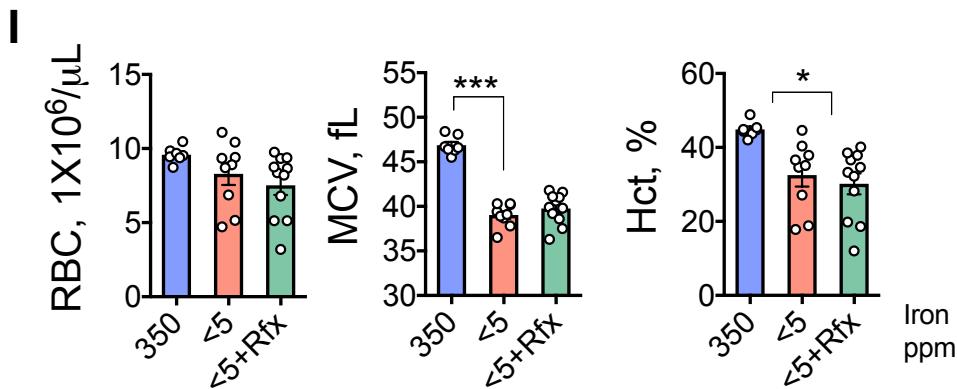
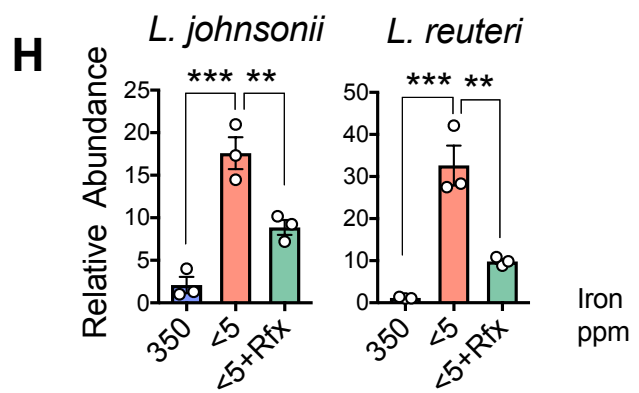
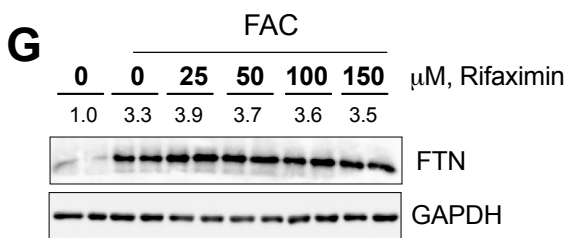
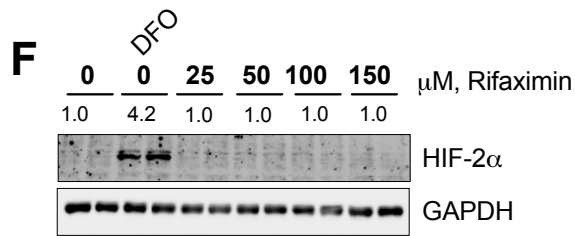
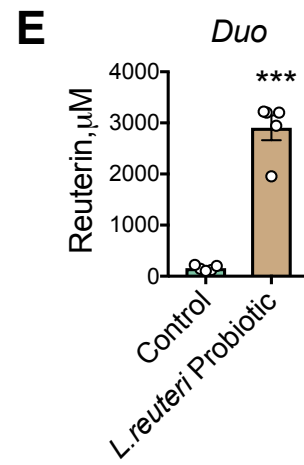
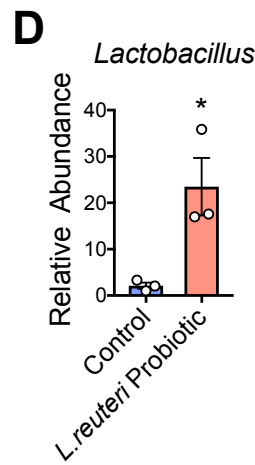
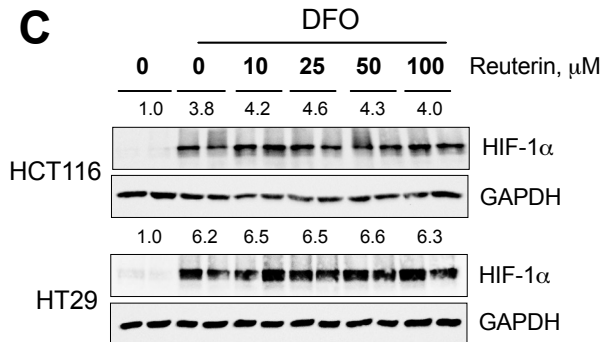
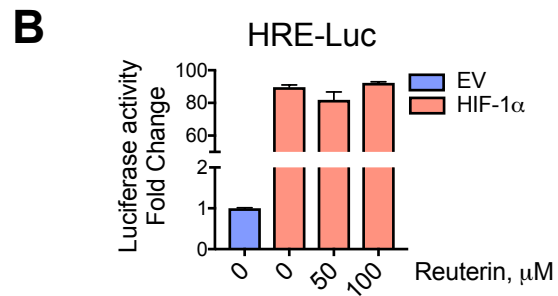
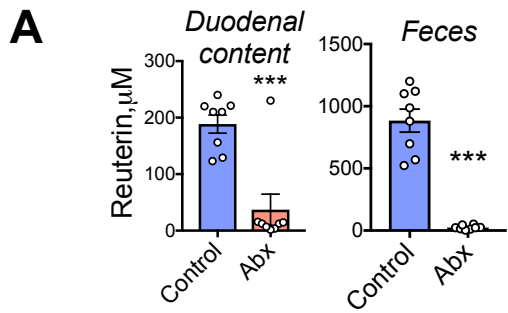


Supplementary Figure 6. Related to Figure 6. A) SPF mice were fed with 350-ppm and <5-ppm iron diet for 2 weeks, SCFA (acetate, butyrate and propionate) analysis in the feces.

B) Comparative analysis of *L.acidophilus*, *L. brevis* and *L.brevis* density by species specific PCR from duodenal content and feces. **C)** *L. reuteri* and *E. coli* growth *in vitro* with dose-dependent DFO treatment (n=3). DNA were extracted from duodenal aspirates of 17 healthy human subjects; **D)** lactobacillus abundance (relative to all bacteria) and **E)** *L. reuteri* abundance (relative to total lactobacilli) shown by Cq values of bacteria-specific PCR.

All data are mean \pm SEM. t-test (A, corresponding diet-groups with respects to SCFA; B); one way ANOVA with Tukey's multiple comparisons test (C).

* P < 0.05, ** P < 0.01, *** P < 0.001.



Supplementary Figure 7. Related to Figure 7. **A)** Reuterin concentrations in wild type Abx-treated or –untreated (control) duodenum and colon. **B)** HRE luciferase assay in HCT116 cells transfected with empty vector (control) or HIF-2 α followed by dose-dependent reuterin treatment (n=3). **C)** HIF-1 α Western analysis of DFO-treated HCT116 and HT29 cells followed by dose-dependent treatment of reuterin. **D)** Analysis of *Lactobacillus* growth in fecal DNA and **E)** Duodenal reuterin production in *L. reuteri* probiotic treated mice. **F)** HIF-2 α Western analysis of HCT116 cells with dose-dependent treatment of Rifaximin. DFO (200 μ M) used as positive control for HIF-2 α induction. **G)** FTN Western analysis of FAC (10 μ M) treated HCT116 cells followed by dose dependent treatment of Rifaximin. **H) & I)** Wild type SPF mice were fed with 350-ppm or <5-ppm iron diet for 1 week followed by 350-ppm, <5-ppm or rifaximin (Rfx) (20 mg/kg/day)-blended <5-ppm diet for another 2 weeks; *L. johnsonii* and *L. reuteri* growth analysis from fecal DNA (**H**) and hematological (RBC, MCV and Hct) analysis (**I**) performed.

All data are mean \pm SEM. t-test (A and D) or one way ANOVA with Tukey's multiple comparisons test (B, H and I). Western analyses: Images were analyzed by Image J software from three independent experiments, representative image shown. Statistical significance compared with DFO-only group (C), no treatment group (F) or FAC-only group (G). * P < 0.05, ** P < 0.01, *** P < 0.001

Supplementary Table 1. Related to Figure 1

	SPF			GF		
	A	B	C	D	E	F
Duodenum	350-ppm n=10	35-ppm n=9	<5-ppm n=8	350-ppm n=5	35-ppm n=7	<5-ppm n=8
<i>Ankrd37</i>	25.58	32.40	519.63	75.55	128.75	1338.11 vs C: ****
<i>Steap4</i>	2.7	2.26	6.26	4.2	5.2	8.45
<i>Arnt</i>	1.13	0.84	1.23	1.71	2.07 vs B: ****	2.15 vs C: **
<i>TNF-α</i>	0.35	0.18	0.55	0.77	0.47	0.48
<i>CxCl1</i>	0.53	0.61	0.62	0.63	0.74	1.22
<i>CxCl2</i>	0.64	0.37	1.62	0.17	.18	0.89
<i>CCL20</i>	0.74	0.65	0.97	0.18	0.72	0.81
<i>Il-1β</i>	1.1	0.47	0.95	0.05	0.05	0.97
<i>Tfr1</i>	1.09	2.28	5.24	0.25	3.18	4.71
<i>Heph</i>	0.72	0.76	0.87	0.27	0.27	1.34
<i>Lcn2</i>	0.42	0.68	0.42	0.22	0.42	0.67
Liver	350-ppm n=10	35-ppm n=9	<5-ppm n=8	350-ppm n=5	35-ppm n=7	<5-ppm n=8
<i>Dmt1</i>	0.54	0.56	0.89	0.22	0.55	0.78
<i>Dcytb</i>	0.86	0.75	1.07	0.24	0.9	1.43
<i>Fpn</i>	0.72	0.45	0.45	0.26	0.58	0.38
<i>FtnH</i>	1.00	0.82	0.95	0.56	1.29	1.01
<i>Tfr1</i>	0.37	0.39	2.89	0.29	0.82	1.91
<i>Tfr2</i>	1.1	1.18	1.19	0.59	1.12	1.08
<i>Hfe</i>	0.61	0.84	0.76	0.45	0.59	0.79
<i>Ankrd37</i>	0.74	0.67	1.06	0.78	2.29	1.35
<i>Vegf</i>	1.23	1.45	0.69	0.81	0.88	0.87
<i>Pdk1</i>	2.01	1.28	0.91	1.2	2.71	1.7
<i>Pgk1</i>	0.58	0.33	0.32	0.18	0.4	0.34
<i>Glut1</i>	0.62	0.59	0.37	0.19	0.48	0.42
Spleen	350-ppm n=10	35-ppm n=9	<5-ppm n=8	350-ppm n=5	35-ppm n=7	<5-ppm n=8
<i>Dmt1</i>	0.73	0.94	0.91	0.84	0.83	1.09
<i>Tfr1</i>	0.51	0.87	0.71	0.45	0.31	1.24
<i>Hmox</i>	0.35	0.36	0.43	0.30	0.19	0.23
<i>Ankrd37</i>	0.53	0.48	0.79	0.71	0.5	0.54
<i>Dcytb</i>	0.68	0.52	0.48	0.79	1.03	0.74
<i>Fpn</i>	0.4	0.39	0.2	0.54	0.3	0.35
<i>Pdk1</i>	0.73	0.85	0.71	0.61	0.76	0.6
<i>Pgk1</i>	0.49	0.49	0.52	0.43	0.32	0.52
<i>Il-1β</i>	0.41	0.19	0.54	0.38	0.17	0.4
<i>Fam132b</i>	0.96	1.26	2.52	0.70	1.04	3.17

Duodenal, Hepatic and Splenic gene expression analyses of HIF-dependent inflammatory and HIF-independent iron genes in SPF and GF mice fed with 350-ppm-, 35-ppm or < 5-ppm iron diet. Mean values presented. One way ANOVA with Tukey's multiple comparisons test. Significance (if any) shown only for SPF vs GF between corresponding diet groups. ** P < 0.01, *** P < 0.001.

Supplementary Table 2. Related to Figure 2

Name	Cat # and Company	Number	Fold change HRE-Luc
LPS/ATP	ATP: A2383 SIGMA-ALDRICH	1	-0.607
Acetate	W302406 SIGMA-ALDRICH	2	-0.177
Propionate	P1880 SIGMA	3	-0.500
Succinic acid	398055 SIGMA-ALDRICH	4	-0.185
Formate	247596 SIGMA-ALDRICH	5	0.116
Fumaric acid	240745 SIGMA-ALDRICH	6	-0.155
3-Phenylpropionic acid	W288918 ALDRICH	7	0.025
Valeric acid	240370 ALDRICH	8	0.121
2'-Deoxycytidine 5'-monophosphate	D7625 SIGMA	9	-0.170
m-Toluic acid	T36609 ALDRICH	10	-0.241
p-Toluic acid	T36803 ALDRICH	11	-0.108
4-Hydroxyphenylacetic acid	H50004 ALDRICH	12	0.100
N-Methyl-DL-alanine	02676 SIGMA-ALDRICH	13	-0.138
Pyridoxamine dihydrochloride	287091 ALDRICH	14	0.224
2'-Deoxyadenosine 5'-monophosphate	D6375 SIGMA	15	-0.718
Indole-3-acetamide	286281 SIGMA-ALDRICH	16	-0.233
N-Acetyl-L-aspartic acid	00920 SIGMA-ALDRICH	17	0.056
N-Acetyl-L-glutamic acid	855642 ALDRICH	18	0.160
N-Acetylneuraminic acid	A0812 SIGMA	19	0.187
4-Pyridoxic acid	P9630 ALDRICH	20	0.194
D-Pantothenic acid hemicalcium salt	21210 SIGMA	21	-0.325
α -Ketoglutaric acid	75890 SIGMA-ALDRICH	22	0.141
Cytidine 5'-monophosphate	C1131 SIGMA	23	-0.636
3-(4-Hydroxyphenyl)propionic acid	H52406 ALDRICH	24	0.205
γ -Aminobutyric acid	A2129 SIGMA	25	-0.155
5-Aminovaleric acid	123188 ALDRICH	26	0.072
L-Homoserine	H6515 SIGMA	27	-0.145
L-Pipecolic acid	P2519 SIGMA	28	-0.286
L-Glyceric acid sodium salt	51738 SIGMA	29	-0.059
L-Tyrosine methyl ester	T90808 ALDRICH	30	0.345
Piperidine	104094 SIGMA-ALDRICH	31	0.036
Taurine	T0625 SIGMA	32	-0.018
Tyramine	T90344 ALDRICH	33	0.075
Urocanic acid	859796 ALDRICH	34	-0.295
(3-Carboxypropyl)trimethylammonium chloride	403245 ALDRICH	35	-0.148
N-Acetyl-D-glucosamine	A8625 SIGMA	36	0.098
Nicotinic acid	N4126 SIGMA-ALDRICH	37	-0.186

Name	Cat # and Company	Number	Fold change HRE-Luc
Pyridoxal hydrochloride	P9130 SIGMA	38	-0.659
Pyridoxine	P5669 SIGMA	39	-0.086
Sarcosine	131776 ALDRICH	40	-0.013
N,N-Dimethylglycine	D1156 SIGMA	41	0.132
Cytosine	C3506 SIGMA	42	0.234
Uracil	U0750 SIGMA	43	-0.164
Adenine	A2786 SIGMA	44	-0.126
Guanine	G11950 ALDRICH	45	-0.193
Cytidine	C122106 ALDRICH	46	0.199
1,3-Diaminopropane	D23602 ALDRICH	47	-0.797
Amino-2-propanol	110248 ALDRICH	48	-0.151
Putrescine	P7505 SIGMA	49	0.003
Cadaverine	33220 ALDRICH	50	-0.104
Glutaric acid	G3407 ALDRICH	51	0.026
5-Hydroxyindole-3-acetic acid	H8876 SIGMA	52	-0.171
Ornithine	O2375 SIGMA	53	0.290
Beta-alanine	146064 ALDRICH	54	-0.265
DL-3-Aminoisobutyric acid	217794 ALDRICH	55	0.071
Isobutyric acid	I1754 SIGMA	56	-0.129
Lactic acid	W261114 SIGMA-ALDRICH	57	0.098
Urea	U5378 SIGMA-ALDRICH	58	0.020
DL-Malic acid	240176 SIGMA-ALDRICH	59	-0.102
Xanthine	X0626 SIGMA-ALDRICH	60	-0.477
L-Citrulline	C7629 SIGMA-ALDRICH	61	-0.457
Hypoxanthine	H9377 SIGMA-ALDRICH	62	-0.166
L-Methionine	M9625 SIGMA-ALDRICH	63	-0.391
Spermidine	S2626 SIGMA-ALDRICH	64	-0.302
LPS	L2630 SIGMA-ALDRICH	65	-0.463
Butyrate	303410 SIGMA-ALDRICH	66	-0.839
<i>E. coli</i> (live)		67	-0.461
<i>E. coli</i> (heat-killed)		68	0.022

Supplementary Table 3: Primers. Related to STAR Methods
A. qPCR Primers

Mouse genes	Forward (5'--3')	Reverse (5'--3')
<i>β-actin</i>	TATTGGCAACGAGCGGTTCC	GGCATAGAGGTCTTTACGGATGT
<i>Dmt1</i>	TGTTTGATTGCATTGGGTCTG	CGCTCAGCAGGACTTTCGAG
<i>DcytB</i>	CATCCTCGCCATCATCTC	GGCATTGCCTCCATTTAGCTG
<i>Hepcidin</i>	CTATCTCCATCAACAGATGAGACAGA	AACAGATAACCACACTGGGAA
<i>Ankrd37</i>	CGGCCTTGCGTGCTTT	TGGTTGAGGTCAGCACCTGTT
<i>STEAP4</i>	GGAAACTCATCTGCATGTGCT	CTAGAAGGCAGAGCCCACC
<i>ARNT</i>	CAAGCCATCTTTCCTCACTGATC	ACACCACCCGTCAGTCTCA
<i>TNF-α</i>	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
<i>CXCL1</i>	TCTCCGTTACTTGGGGACAC	CCCACTCAAGAATGGTCGC
<i>CXCL2</i>	TCCAGGTCAGTTAGCCTTGC	CGGTCAAAAAGTTTGCCTTG
<i>CCL20</i>	TGTACGAGAGGCAACAGTCG	TCTGCTCTTCCTTGCTTTGG
<i>IL-1β</i>	AAGAGCTTCAGGCAGGCAGTATCA	TGCAGCTGTCTAGGAACGTCA
<i>TfR1</i>	GGAAGACTCTGCTTTGCAGCTAT	GCCCAGGTAGCCCATCATGA
<i>Heph</i>	FTGGGCTTCCTAGGACCACTGT	RGCAAAATTCTTCAGGTGAATCAAG
<i>Lipocalin2</i>	CACCACGGACTACAACCAGTTCGC	TCAGTTGTCAATGCATTGGTCGGTG
<i>TfR2</i>	TAGCCCTCCAACCACTCTGT	CTCTTCCATGGTCAGCAATG
<i>HFE</i>	CACCGCGTTCACATTCTCTAA	CTGGCTTGAGGTTTGCTCC
<i>VEGF</i>	CCACGTCAGAGAGCAACATCA	TCATTCTCTCTATGTGCTGGCTTT
<i>PDK1</i>	TTACTCAGTGGAACACCGCC	GTTTATCCCCGATTCAGGT
<i>PGK1</i>	CAAATTTGATGAGAATGCCAAGACT	TTCTTGCTGCTCTCAGTACCACA
<i>Glut1</i>	CAAGTCTGCATTGCCCATGAT	CCAGCTGGGAATCGTCGTT

<i>Hmox1</i>	AGGTACACATCCAAGCCGAGA	CATCACCAGCTTAAAGCCTTCT
<i>Fam132b</i>	ATGGGGCTGGAGAACAGC	TGGCATTGTCCAAGAAGACA
<i>Ftnh1</i>	GGCAAAGTTCTTCAGAGCCA	CATCAACCGCCAGATCAAC
Human genes	Forward (5'--3')	Reverse (5'--3')
<i>β-actin</i>	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT
<i>DMT1</i>	GCTCTCATACCCATCCTCACATT	TCCATTGGCAAAGTCACTCATT
<i>DCYTB</i>	CATGGTCACCGGCTTCGT	CAG GTCCACGGCAGTCTGTA
<i>TNF-α</i>	AGATGATCTGACTGCCTGGG	CTGCTGCACTTTGGAGTGAT
<i>IL-1β</i>	AAGCCCTTGCTGTAGTGGTG	GAAGCTGATGGCCCTAAACA
<i>IL8</i>	AGCACTCCTTGCCAAAAGT	CGGAAGGAACCATCTCACTG
<i>CXCL1</i>	AACAGCCACCAGTGAGCTTC	GAAAGCTTGCCTCAATCCTG
<i>CXCL2</i>	CTTCAGGAACAGCCACCAAT	CACACTCAAGAATGGGCAGA
<i>CCL20</i>	CGTGTGAAGCCCACAATAAA	GTGCTGCTACTCCACCTCTG
<i>HIF-2α</i>	CATCCCGGGACTTCTCCT	GTCTGAACGTCTCAAAGGGC

B. Cloning Primers

	Forward (5'--3')	Reverse (5'--3')
Mouse Hif-2α G324E	TGTAGATGACCGTCTCCTGGGTCTCCAGC	GCTGGAGACCCAGGAGACGGTCATCTACA
Mouse Hif-2α S305M	CCGGTACTGGCCATTACCACCTGCCCTTGGTG	CACCAAGGGGCAGGTGGTAATGGGCCAGTACCGG

C. Bacterial Primers

	Forward (5'--3')	Reverse (5'--3')
All Bacteria	ACTCCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG
<i>L. johnsonii</i>	TCTTCCAATTTTCGGCAGT	CAGTGGGAGCTACAGAAGCA
<i>L. reuteri</i>	ACCGAGAACACCGCGTTATTT	CATAACTTAACCTAAACAATCAAAGATTGTCT
<i>L. acidophilus</i>	GAAAGAGCCCAAACCAAGTGATT	CTCCCAGATAATTCAACTATCGCTTA
<i>L. brevis</i>	GCACAAGATGGCTCATGACGTTAAGACTAAGG	GTCTAAGCTCGTATCAACCCACGGG
<i>L. helveticus</i>	GTCTAAGCTCGTATCAACCCACGGG	GATCAACAATGACTTGCCTTGTTGAACAATTC

D. CRISPR guide RNA for mouse NCOA4, exon 3

5'-AGAGGTGTGGCTCAATGAAC-3'