Supplemental materials

Supplement Table 1. Bacterial strains used in this study; Related to STAR Methods

Name	**Description**	**Reference/source**
Ligilactobacillus murinus	*L. murinus* isolated from the feces of a male C57BL/N mouse from Charles River	This study
YL27	*Muribaculum intestinale*	DSMZ
YL32	*Clostridium clostridioforme*	DSMZ
B-CC-163-3B	*Clostridium sporogenes*	DSMZ
ERD01G	*Streptococcus daniellae* ERD01G	DSMZ
33-ERD13C	*Staphylococcus xylosus 33-ERD13C*	DSMZ
Mt1b1	*E. coli* isolated from feces of a healthy mouse	DSMZ
L. murinus Pen^R	Penicillin resistant strain of *L. murinus*	This study
313	*Ligilactobacillus murinus*	DSMZ
53103	*Lacticaseibacillus rhamnosus*	ATCC
Scav	*Lactobacillus acidophilus*	ATCC
YL2	*Bifidobacterium longum subsp. animalis*	DSMZ

Supplemental Table 2. Primers for RT-qPCR used in this study; Related to STAR Methods

RT-qPCR			
Organism	Target gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
Mus musculus	*Act2b*	GCTGAGAGGGAAATCGTGCGTG	CCAGGGAGGAAGAGGATGCGG
Mus musculus	*Scd1*	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
Mus musculus	*AboB*	AAGCACCTCCGAAAGTACGTG	CTCCAGCTCTACCTTACAGTTGA
Mus musculus	*Hmgcs2*	GAAGAGAGCGATGCAGGAAAC	GTCCACATATTGGGCTGGAAA
Mus musculus	*Lpl*	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
Mus musculus	*Angptl4*	CATCCTGGGACGAGATGAACT	TGACAAGCGTTACCACAGGC
Mus musculus	*Glut2*	TCAGAAGACAAGATCACCGGA	GCTGGTGTGACTGTAAGTGGG
Mus musculus	*Glut5*	CCAATATGGGTACAACGTAGCTG	GCGTCAAGGTGAAGGACTCAATA
Mus musculus	*Cd36*	TGTGTTTGGAGGCATTCTCA	TGGGTTTTGCACATCAAAGA
Mus musculus	*Fabp1*	ATGAACTTCTCCGGCAAGTACC	CTGACACCCCCTTGATGTCC
Mus musculus	*Fabp2*	GTGGAAAGTAGACCGGAACGA	CCATCCTGTGTGATTGTCAGTT
Mus musculus	*Pparg*	CCAGCATTTCTGCTCCACAC	ATTCTTGGAGCTTCAGGCCA
Mus musculus	*Srebf1*	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Mus musculus	*Fabp4*	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Mus musculus	*Fasn*	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Mus musculus	*Mttp*	CTCTTGGCAGTGCTTTTTCTCT	GAGCTTGTATAGCCGCTCATT
L. murinus	*16S rRNA*	ACTGGCGATGTTACCTTTGG	CAGGCCTTTGTATTGGTGGT
Lactobacillus	*16S rRNA*	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG

Supplemental Table 3. Criteria for blinded scoring of histopathological changes; Related to STAR Methods

Score	Description
0	Normal
1	Mild focal villus atrophy characterized by shortened, blunt villi; mild lymphocyte infiltration
2	Moderate focal to multifocal villus atrophy characterized by shortened, blunt villi; mild lymphocyte infiltration
3	Moderate to severe multifocal to diffuse villus atrophy characterized by shortened, blunt villi; mild to moderate lymphocyte infiltration

Supplemental Table 4. Criteria for blinded scoring of oil red O-stained livers; Related to STAR Methods

Score	Lipid droplets	
	Distribution	Size
0	no droplets	no droplets
1	mild, focal accumulation of lipid droplets in hepatocytes	small lipid droplets (<10% cytoplasm) in hepatocytes
2	moderate, focal to multifocal accumulation of lipid droplets in hepatocytes	medium lipid droplets (10-30% cytoplasm) in hepatocytes
3	severe, diffuse accumulation of lipid droplets in hepatocytes	large lipid droplets (>30% cytoplasm) in hepatocytes

Supplemental Table 5. Criteria for blinded scoring of PPAR-γ immunostaining in the intestinal epithelium; Related to STAR Methods

Score	Description
0	No PPARg staining in intestinal epithelium
1	Mild/Faint PPARg staining in intestinal epithelium
2	Moderate PPARg staining in intestinal epithelium
3	Intense PPARg staining in intestinal epithelium

Supplemental Table 6. Isotopically labeled standards used to measure sample processing and instrument variability; Related to STAR Methods

Isotopically labeled standard	Company	Chemical Formula	Chemical Purity	Sample Processing Assessment	Instrument Variability Assessment	% coefficient of variation (%CV)	% coefficient of variation (%CV)	QA metric (%CV)
Biotin - d2	Cambridge Isotope Laboratories	$C_{10}H_{14}D_2N_2O_3S$	> 97%	x		20.1		< 25%
DL-Phenyl-d5-alanine-2,3,3-d3	CDN Isotopes	$C_9H_3D_8NO_2$	> 98%	x		21.2		< 25%
DL-Tryptophan-2,3,3-d3	CDN Isotopes	$C_{11}H_9D_3N_2O_2$	> 98%		x		1.6	< 10%
Inosine - 4N15	Cambridge Isotope Laboratories	$C_{10}H_{12}{}^{*}N_4O_5$	> 95%		x		1.0	< 10%

^ *calculations based on pooled QC injections*

& *calculations based on all samples injections*

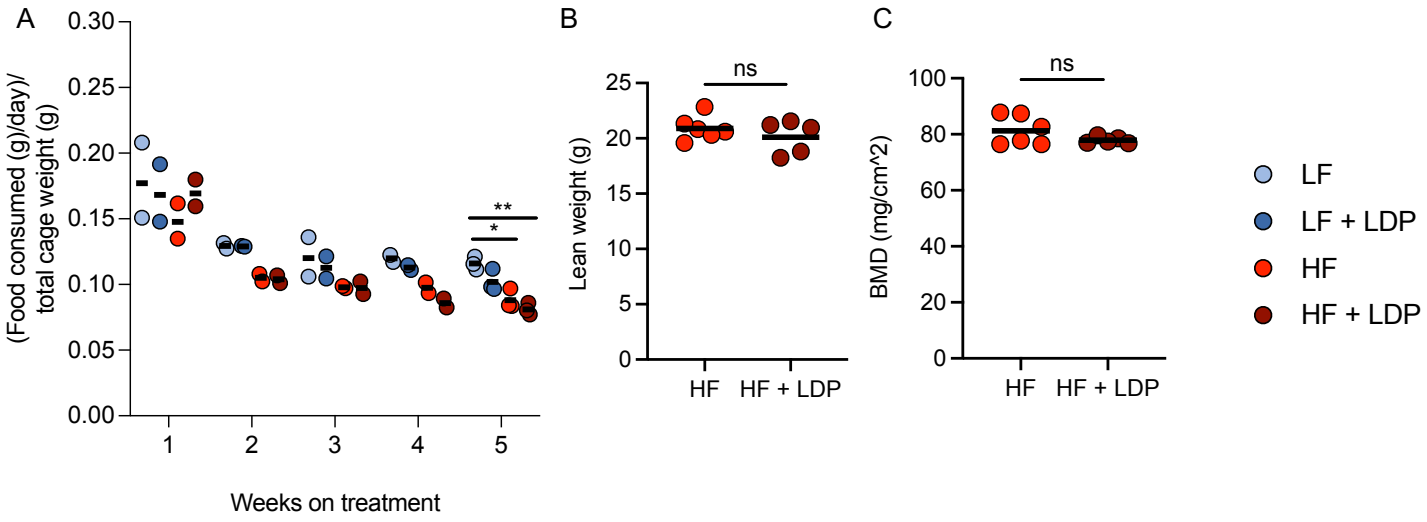
* *location of N15 incorporation*

Supplemental Table 7. Annotations of significantly different compounds in pairwise comparison between HF diet and HF diet and LDP-treated mice; Related to STAR Methods

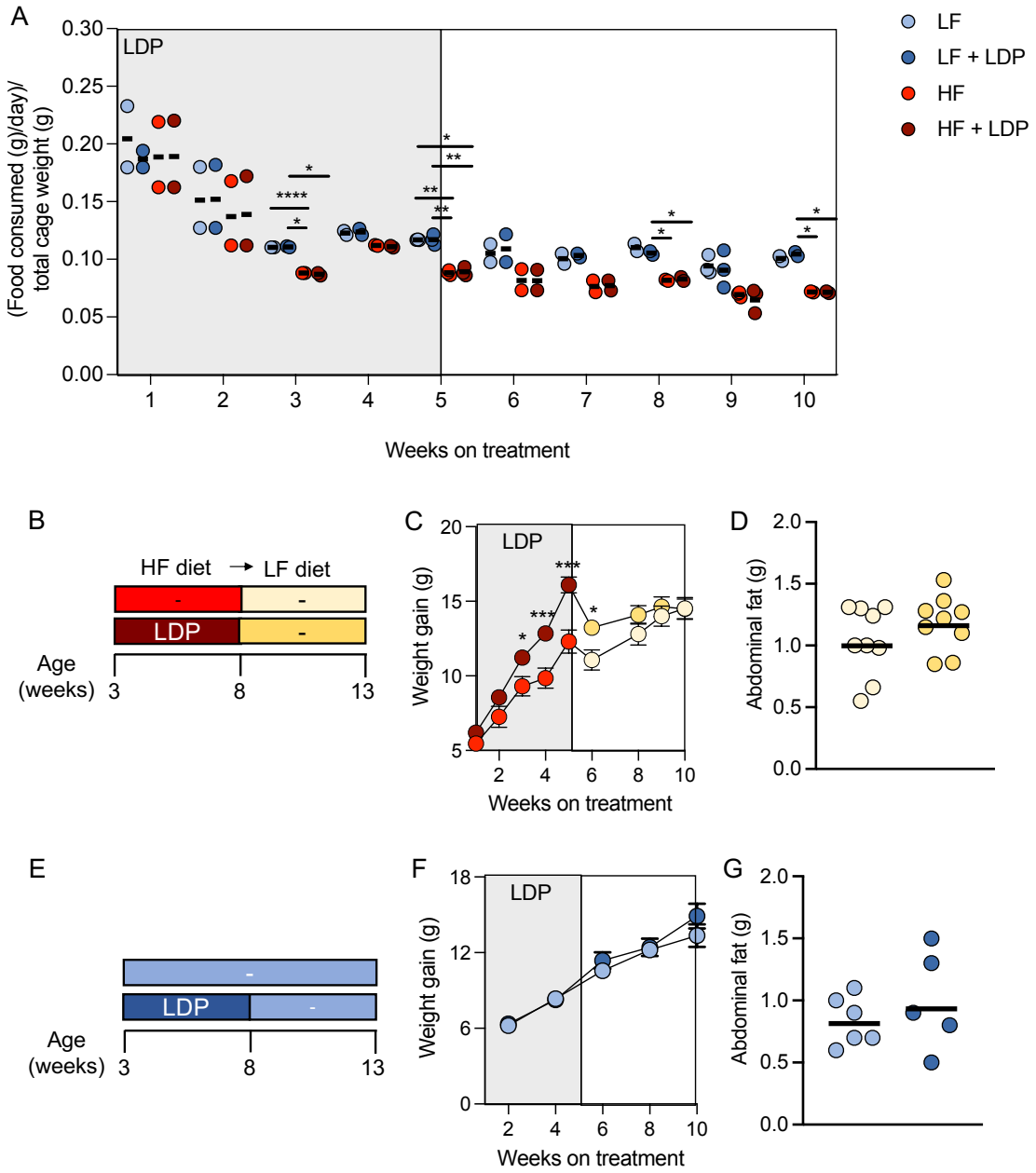
Compound	Description	Metabolite annotation confidence level	Adducts	Formula	Anova (p) HF+ LDP_HF	Max Fold Change HF + LDP_HF
4.54_181.0506m/z	Hydroxyphenyllactic acid	L2	M-H	C9H10O4	0.011535606	-4.635819853
8.85_204.0664m/z	Indolelactic acid	L2	M-H	C11H11NO3	0.00851237	-6.927760766
13.12_487.2374m/z	7-Sulfocholic acid	L2	M-H	C26H34O6	0.024795548	-2.023073492
4.43_204.0300m/z	Xanthurenic acid	L2	M-H	C10H7NO4	0.010801232	-5.688463785
5.45_212.0022m/z	Indoxyl sulfate	L2	M-H	C8H7NO4S	0.003926965	-10.71370615

4.18_232.0040n	(4-ethenyl-2,6-dihydroxyphenyl)oxidanesulfonic acid	L3	M-H, M+Na-2H	C8H8O6S	0.03236764	-6.429636526
3.57_382.1004m/z	Succinyladenosine	L2	M-H	C14H17N5O8	0.042868208	2.463034152
15.36_391.2854m/z	Chenodeoxycholic acid	L2	M-H	C24H40O4	0.048809691	-2.131784044
8.15_165.0555m/z	Phenyllactic acid	L3	M-H	C9H10O3	0.010607418	-3.980789046
14.33_369.1554m/z	3,4,5-trihydroxy-6-{[1-(4-methoxyphenyl)pentan-3-yl][44]oxy}oxane-2-carboxylic acid	L3	M-H	C18H26O8	0.014385821	-2.242308977
7.21_131.0713m/z	(5R)-5-Hydroxyhexanoic acid	L3	M-H	C6H12O3	0.003143156	-2.915496425
1.14_243.0621m/z	Uridine	L3	M-H	C12H14O4	0.006708958	-2.684641964
4.67_392.1200m/z	Isradipine	L3	M+Na-2H	C19H21N3O5	0.043456312	-2.259781161
2.11_392.1200m/z	Dide-O-methylsimmondsin	L3	M+FA-H	C14H21NO9	0.036395971	-2.085742975
3.59_194.0457m/z	Salicyluric acid	L3	M-H	C9H9NO4	0.007327095	-3.070700392
1.52_189.0404m/z	Ethyl hydrogen fumarate	L3	M+FA-H	C6H8O4	0.027494472	-2.380472461
13.76_93.0346m/z	Phenol	L3	M-H	C6H6O	0.00000612	2000
1.90_181.0366m/z	1-Methyluric acid	L3	M-H	C6H6N4O3	0.005530456	-3.217937428
1.34_122.0247m/z	Isonicotinic acid	L3	M-H	C6H5NO2	0.000685966	-9.921546424
1.53_115.0036m/z	Maleic acid	L3	M-H	C4H4O4	0.048489209	2.950566638
4.55_324.0724m/z	DHBOA-Glc	L3	M-H2O-H	C14H17NO9	0.020403272	-2.218251385
4.34_324.0724m/z	Dihyroxy-1H-indole glucuronide I	L3	M-H	C14H15NO8	0.03502611	-2.199232618
1.46_187.0723m/z	N-Acetylglutamine	L3	M-H	C7H12N2O4	0.003187344	-2.16013877
1.05_256.0594m/z	3-Oxo-carbofuran	L3	M+Na-2H	C12H13NO4	0.020123888	2.00611216
15.78_400.2163m/z	Pipercide	L3	M+FA-H	C22H29NO3	0.038330336	-2.302125268
10.52_323.1070m/z	Acetohexamide	L3	M-H	C15H20N2O4S	1.26E-11	2000
11.09_323.1070m/z	Dictyoquinazol C	L3	M-H2O-H	C18H18N2O5	6.15E-10	2000
8.07_283.0823m/z	p-Cresol glucuronide	L3	M-H	C13H16O7	0.021570154	-3.905801337
1.06_130.0621m/z	Creatine	L2	M-H	C4H9N3O2	0.031262325	2.009045786
13.76_349.0863m/z	Penicillin V	L3	M-H	C16H18N2O5S	0.00000026	2000
1.40_333.0940m/z	(S)-a-Amino-2,5-dihydro-5-oxo-4-isoxazolepropanoic acid N2-glucoside	L3	M-H	C12H18N2O9	0.009545933	2.447823207
15.15_374.2006m/z	Trilostane	L3	M+FA-H	C20H27NO3	0.010078748	-2.524506159
15.20_398.2007m/z	Quinacrine	L3	M-H	C23H30ClN3O	0.012357295	-2.24340063
13.33_416.2112m/z	Valsartan	L3	M-H2O-H	C24H29N5O3	0.043214627	-19.87137498
1.38_340.0672m/z	4-hydroxymidazolam	L3	M-H	C18H13ClFN3O	0.046901745	-4.87630232

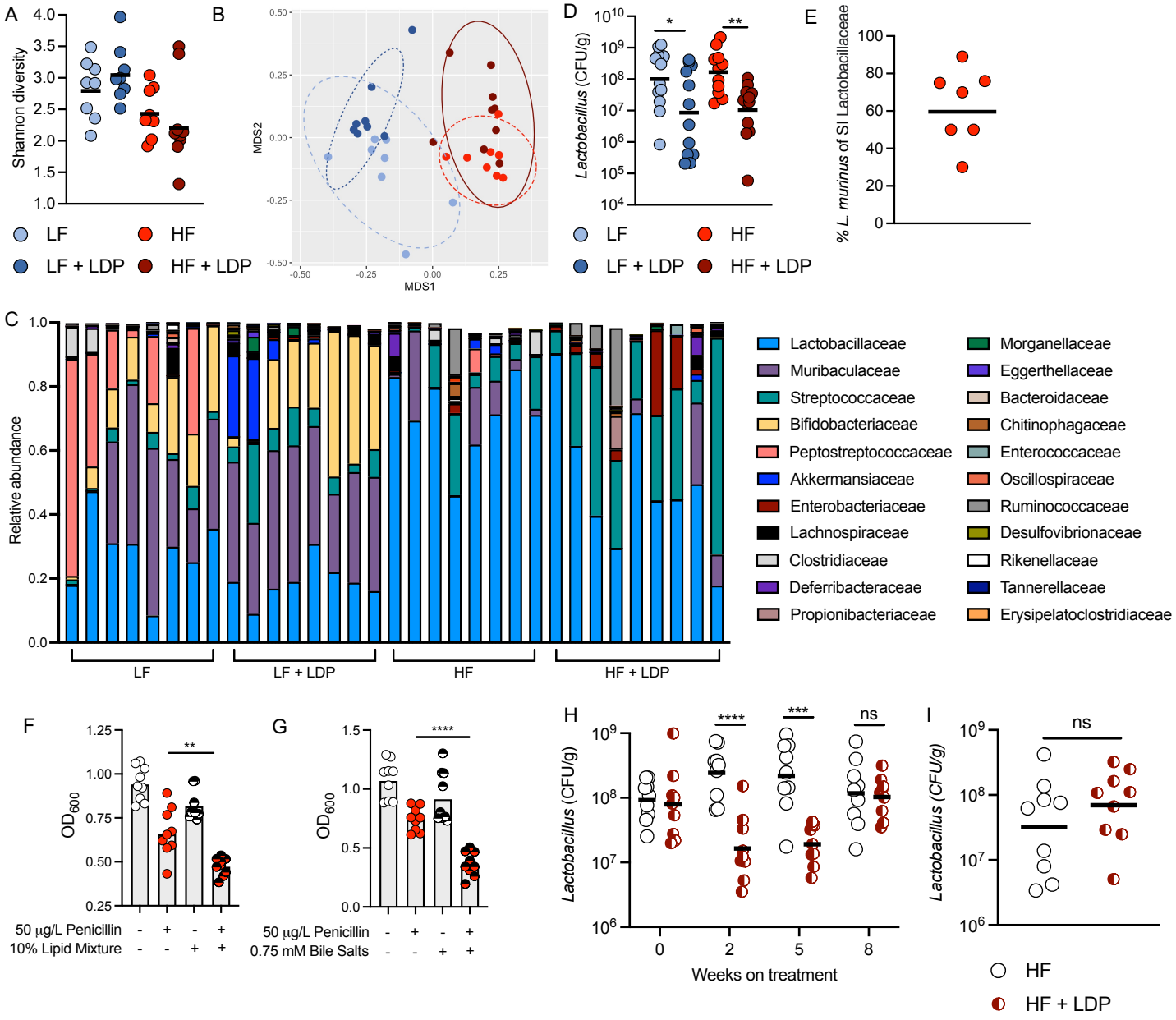
14.99_541.2398m/z	Withaperuvin B	L3	M+Na-2H	C28H40O9	0.030480752	-3.842378385
5.83_308.0776m/z	Indoxyl glucuronide	L3	M-H	C14H15NO7	0.021475029	-2.524691211
14.09_541.2480m/z	Physagulin C	L3	M-H	C30H38O9	0.007918487	-2.674732949
1.11_97.9769n	Phosphoric acid	L3	M-H2O-H, M-H	H3O4P	0.003568168	4.256690097
6.41_196.0073m/z	4-Thiocyanatophenol	L3	M+FA-H	C7H5NOS	0.028474673	-3.946873934
14.99_553.1957m/z	gibberellin A3 O-beta-D-glucoside	L3	M+FA-H	C25H32O11	0.018787005	-6.145634649
3.30_341.1221n	PC(2:0/2:0)	L3	M-H, M+Na-2H	C12H24NO8P	0.043455035	-3.129936677
1.14_224.0233m/z	S-Carboxymethyl-L-cysteine	L3	M+FA-H	C5H9NO4S	0.030206684	-5.331911132
10.86_525.2707m/z	Estrogen E3, tris(trimethylsilyl) ether	L3	M+Na-2H	C27H48O3Si3	0.048006559	-2.197149081
10.71_525.2707m/z	Neuromedin N (1-4)	L3	M+Na-2H	C26H40N4O6	0.023487252	-2.433619455
3.45_710.2929m/z	LPIM5(19:1(9Z)/0:0)	L3	M-2H	C58H103O37P	0.005903512	2.595282357
3.57_450.0880m/z	4-Phenylbutyl glucosinolate	L3	M-H	C17H25NO9S2	0.029360524	2.5205626
1.68_281.9912m/z	Risedronate	L3	M-H	C7H11NO7P2	0.002980041	-4.630879058
1.55_540.1697m/z	Neoacrimarine K	L3	M-H2O-H	C31H29NO9	0.007183516	2.887249951



Supplementary Figure 1. Treatment with LDP does not alter food consumption, lean weight, or bone mineral density (Related to Figure 1). (A) Food consumption (g) (normalized to total cage weight) was measured twice a week over the course of the 5-week experiment. (N = 6 mice/group). (C) Lean weight and (D) bone mineral density were determined by DEXA scanning 5 weeks after exposure to a HF diet or a HF diet and LDP. (A) *, $p < 0.05$; **, $p < 0.01$ using Tukey's multiple comparisons test. (B and C) Differences between groups were not significant (ns) as determined by an unpaired two-tailed Student's t test. N = 5 or 6 mice/ group.



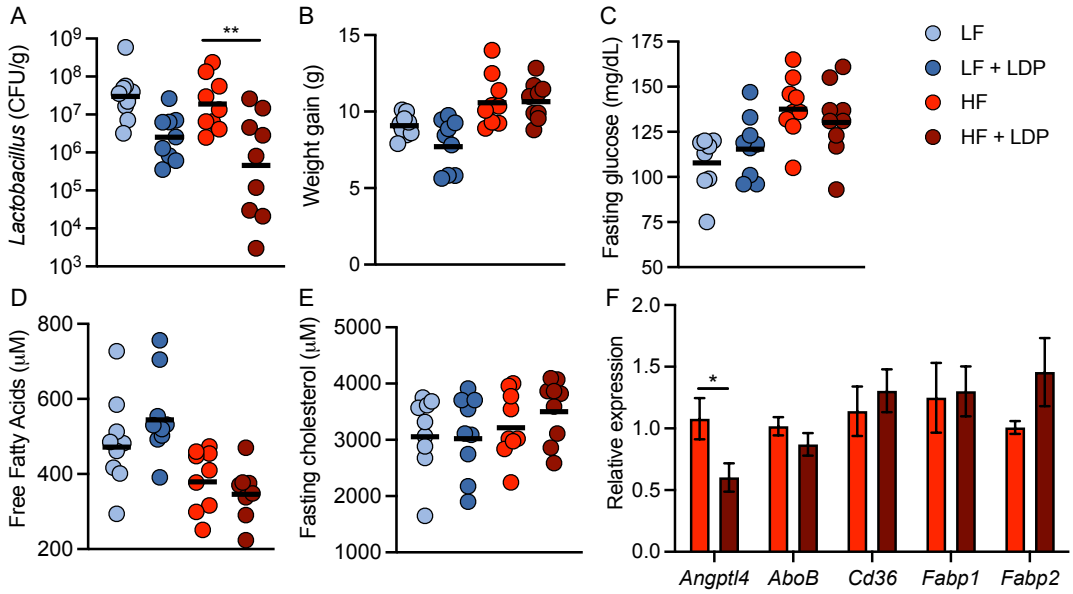
Supplementary Figure 2. Lasting adiposity as a result of LDP treatment requires consumption of HF diet (Related to Figure 1). (A) Food consumption (g) (normalized to total cage weight) was measured twice a week over the course of the 10-week experiment. (B, E) A schematic representation of the 10-week experiment and the groups used. (C, F) The mean weight gain of mice in the different groups over the course of the 10-week experiment. (D, G) Abdominal fat (g) measured after the 10-week diet and antibiotic manipulations. (A, E – G): N = 6 mice/group; (B – D): N = 9 mice/group. (A) *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.001$ using a two-way ANOVA with Tukey's multiple comparisons test. (C) *, $p < 0.05$; ***, $p < 0.005$ using an unpaired two-tailed Student's t test. (C, F) Dots represent mean ± standard error of the mean. (C, G) Each dot represents one animal. Bars represent geometric mean.



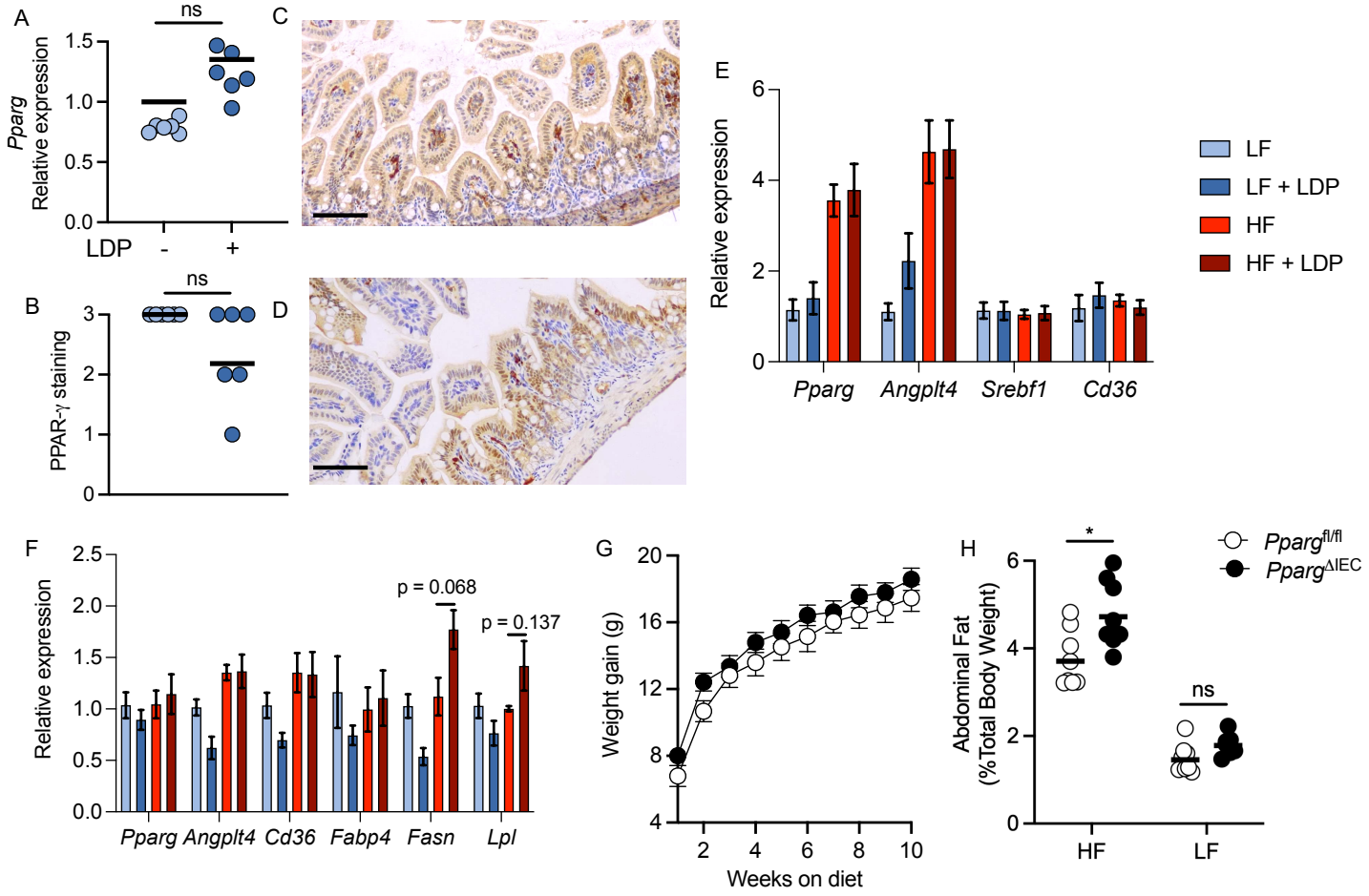
Supplementary Figure 3. *Lactobacillus* species inhibited by combinatorial exposure to penicillin and a HF diet (Related to Figure 2). (A-D) Content from the distal small intestine of mice exposed to one of the four treatments for 5 weeks was collected and subjected to 16S rRNA sequencing and analysis. (A) Shannon diversity scores for mice in each of the four groups. (B) Non-metric multidimensional scaling (MDS) plot displaying 16S rRNA gene-based bacterial community composition in small intestine microbiota. Circles represent 95% confidence intervals. (C) Relative abundance of 22 most abundant families in the small intestine of mice given one of the four treatments (D) *Lactobacillus* abundance (colony forming unit (CFU) / gram) in small intestine (SI) was determined by plating SI material on De Man, Rogosa and Sharpe (MRS) agar. (E) Percent of *L. murinus* 16S rRNA DNA out of all *Lactobacillus* 16S rRNA DNA in the small intestine content of HF diet mice as

determined by qPCR. Each dot represents one animal. Bars represent geometric mean. (F) MRS broth containing either 50 µg/L penicillin, 10% lipids, or a combination of both was inoculated with *Ligilactobacillus murinus* (*L. murinus*) and grown anaerobically for 18 hours. OD_{600} of *L. murinus* was measured after 18 hours of anaerobic growth. (G) MRS broth containing either 50 µg/L penicillin, 0.75 mM bile salts, or a combination of both was inoculated with *Ligilactobacillus murinus* (*L. murinus*) and grown anaerobically for 18 hours. OD_{600} of *L. murinus* was measured after 18 hours of anaerobic growth. (H) *Lactobacillus* abundance (colony forming unit (CFU) / gram) in the feces was determined by plating feces on De Man, Rogosa and Sharpe (MRS) agar over the 8 weeks of treatment (see **Fig. 1G** for experimental design). (I) *Lactobacillus* abundance (colony forming unit (CFU) / gram) in small intestine (SI) was determined by plating SI material on De Man, Rogosa and Sharpe (MRS) agar after 10 weeks on a HF diet with or without antibiotics during the first 5 weeks. (A – C) N = 9 mice/group; (D) N = 12 mice/group; (E) N = 7 mice/group. (F and G) Data represents three independent experiments consisting of 3 replicates. (H and I) N = 9 mice/group. (A, B, D, E, H, I) Each dot represents one animal. (C) Each bar represents one animal.

Bars represent the geometric mean. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$; ****, $p < 0.0001$ using an unpaired two-tailed Student's t test.

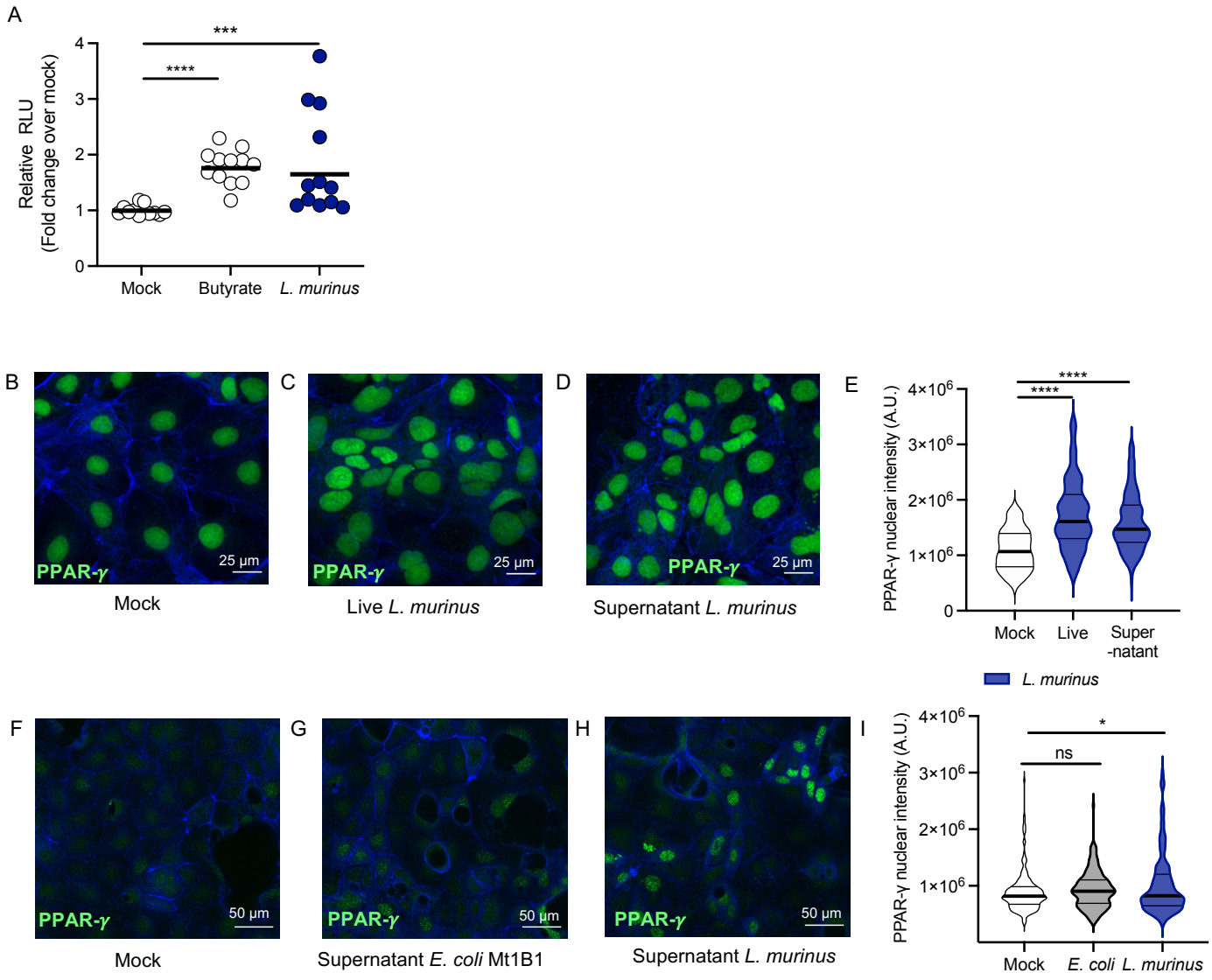


Supplementary Figure 4. Disruption to the small intestinal microbiota and epithelium from exposure to low dose penicillin and a high fat (HF) diet occurs after only two weeks of treatment (Related to Figure 4). (A) *Lactobacillus* abundance (colony forming unit (CFU) / gram) in small intestine (SI) was determined by plating SI material on De Man, Rogosa and Sharpe (MRS) agar. (B) Weight gain of mice in the 4 different groups after 2 weeks. (C) Glucose levels were measured after mice were fasted overnight for approximately 6 hours at 2 weeks. Measurement of (D) free fatty acids and (E) cholesterol in the serum of fasted mice after two weeks exposure to the diet and antibiotic treatments. (F) Expression of genes related to lipid metabolism, as determined by qPCR, in the ileum epithelium of mice fed a HF diet or exposed to a HF diet and LDP for two weeks. (A – E) Each dot represents one animal. Bars represent geometric mean. (F) Bars represent mean ± standard error of the mean (SEM). N = 9 mice/group. *, $p < 0.05$; ***, $p < 0.01$; ****, $p < 0.001$ using an unpaired two-tailed Student's t test.



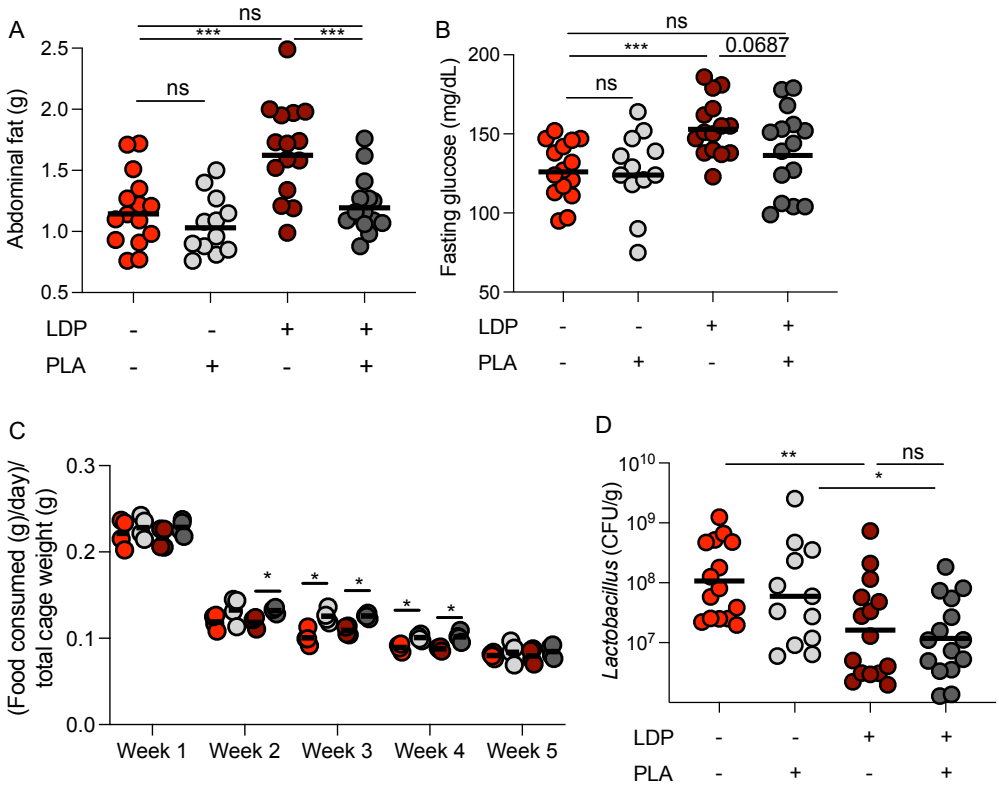
Supplementary Figure 5. Intestinal PPAR-γ is specifically depleted by exposure to both a HF diet and LDP, promoting increased adiposity during consumption of a HF diet (Related to Figure 5). (A) Expression of *Pparg* in the ileum epithelium of LF diet or LF diet and LDP-treated mice measured by qPCR. (B – D) Sections from the ileum of mice exposed to a LF diet or a LF diet and LDP were stained with a primary PPAR-γ antibody and binding visualized with a horseradish-peroxidase conjugated secondary antibody. (B) PPAR-γ abundance in the epithelium was quantified by scoring blinded sections of the ileum. (C – D) Representative images of PPAR-γ staining in LF diet fed mice (C) and HF diet fed and LDP treated mice (D). Scale bar represents 200 μm. (E – F) Transcripts of the indicated genes involved in lipid metabolism measured by quantitative real-time PCR (qPCR) in the liver (E) and adipose tissue (F) of mice given one of the 4 indicated diet and/or antibiotic combinations. (G) 3-week-old *Pparg*$^{fl/fl}$*Villin*$^{cre/-}$ mice (*Pparg*$^{\Delta IEC}$), which lack PPAR-γ in epithelial cells, and littermate control *Pparg*$^{fl/fl}$*Villin*$^{-/-}$ mice (*Pparg*$^{fl/fl}$) were fed a LF diet for 10 weeks and weight gain was determined weekly. (H) Abdominal fat (% total body weight) of *Pparg*$^{\Delta IEC}$ and *Pparg*$^{fl/fl}$ measured after 10-week exposure to a HF or LF diet. (A, B, and H) Each dot represents one animal. Bars represent geometric mean (N = 6 – 7 mice/group). (E and F) Bars represent mean +/- standard error of the mean (N = 6 – 7 mice/group). (G) Dots

represent mean +/- SEM (N = 8 – 9/ genotype). (A) ns using an unpaired two-tailed Student's t test. (B) ns using a two-tailed Mann-Whitney test. (E – F) No significant differences were found between LF and LF + LDP or HF and HF + LDP using an unpaired two-tailed Student's t test. (G) No significant differences determined between genotype by two-way ANOVA with Šídák's multiple comparisons test. (H) *, $p < 0.05$ using a two-way ANOVA with Tukey's multiple comparisons test.

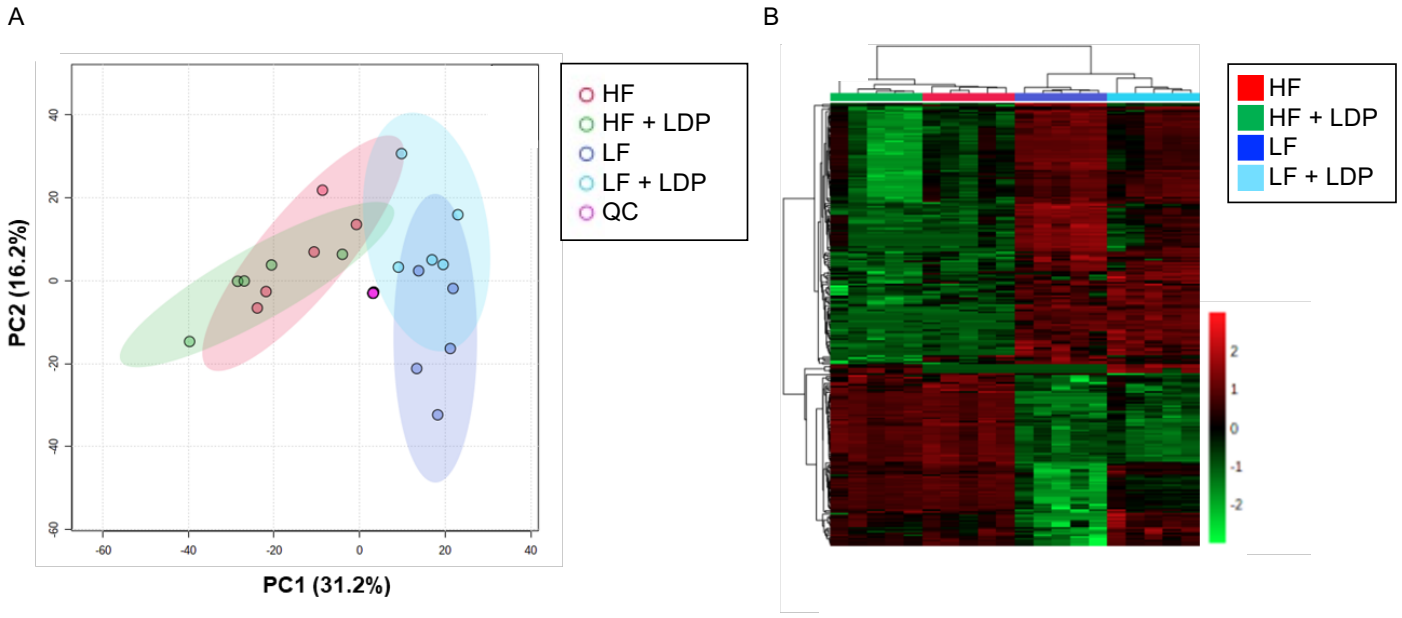


Supplementary Figure 6. *L. murinus* increases the transcriptional activity and nuclear abundance of PPAR- γ in Caco-2 cells (Related to Figure 5). (A) Caco-2 cells were transfected with a PPAR-γ reporter plasmid (PPRE-x3) and 24 hours later transfected cells were treated with 8 mM butyrate, live *L. murinus*, or left untreated. After five hours of treatment, luminescence was measured using Promega Luciferase Assay System. (B – E) Caco-2 cells were infected with *Ligilactobacillus murinus* (*L. murinus)* at an MOI of 100. Undifferentiated Caco-2 cells were treated with *L. murinus* (C), treated with *L. murinus* supernatant (D), or left untreated (B) and incubated for 16 hours. Cells were subsequently stained with anti-PPAR-γ and phalloidin to stain for F-actin. (E) PPAR-γ nuclear intensity was quantified using ImageJ; 25 cells were selected from 2 images from 3 independent experiments (F – I) Caco-2 cells were either mock-treated (F) or treated with supernatants from *E. coli* Mt1B1 (G) or *L. murinus* (H) for 16 hours. Cells were then stained for PPAR- γ as described above. (H) PPAR-γ nuclear

intensity was quantified using ImageJ; 30 cells were selected from 3 images from 2 independent experiments. (A) Dots represent one technical replicate. Data shown from 3 biological replicates. Bars represent geometric mean. ***, $p < 0.005$; ****, $p < 0.0001$ using an unpaired two-tailed Student's t test. (E, I) Violin plot showing the distribution of PPAR-γ nuclear intensity (n = 150 cells (D) or 180 cells (H)). *, $p < 0.05$; ****, $p < 0.0001$ an unpaired two-tailed Student's t test.



Supplementary Figure 7. Increased intestinal phenyllactic acid protects against HF + LDP induced metabolic dysfunction (Related to Figure 7). (A) Abdominal fat (g) measured at the end of the 5-week experiment. (B) Glucose levels were measured after mice were fasted overnight for approximately 6 hours at the end of the 5-week treatment. (D) Food consumption of mice in each group per day (relative to total weight of mice in each group) (dots represent two independent experiments, N = 6 mice/ group). (E) *Lactobacillus* abundance (Colony forming unit (CFU) / gram of small intestinal (SI) count was determined by plating SI material on MRS agar. (A – C, E) Each dot represents one animal. N = 12 mice/group. (A-C, E) Lines represent geometric mean. (D) Dots represent group food consumption (g)/day/total group weight. Line represents geometric mean. (A - C, E) *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ using an unpaired two-tailed Student's t test. (D) *, $p < 0.05$ using a two-way ANOVA with Tukey's multiple comparison test.



Supplementary Figure 8. Untargeted Metabolomics of ileum contents; Related to STAR Methods. (A) Principal component analysis (PCA) illustrates distinct metabolomic profiles for the experimental groups and pooled quality control (QC) samples. (B) Heat Map clustering of the experimental sample groups and compounds showing significant differences based on compound abundances across the groups. (A – B) N = 5 mice/group.