

Figure S1. Effect of 2'-FL supplementation on glucose metabolism. Blood glucose level (A) and AUC (B) during an oral-glucose-tolerance test (2 mg/kg) in mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks. Two-way analysis of variance (ANOVA) followed by Tukey's or Sidak's post-hoc test was used to determine differences among groups. Repeated measures ANOVA was performed for blood glucose level on variable clusters with a random subject effect whereas variable cluster members, diet and 2'-FL treatment, were used as fixed effects. Values are means \pm SEMs; $n = 8$ /group. Asterisk (*) and sharp (#) denote significant differences between LF/Con vs. HF/Con and LF/2'-FL vs. HF/2'-FL, respectively at * $P < 0.05$, ** or ## $P < 0.01$, *** or ### $P < 0.001$, **** $P < 0.0001$. AUC, area under the curve; HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; 2'-FL, 2-fucosyllactose.

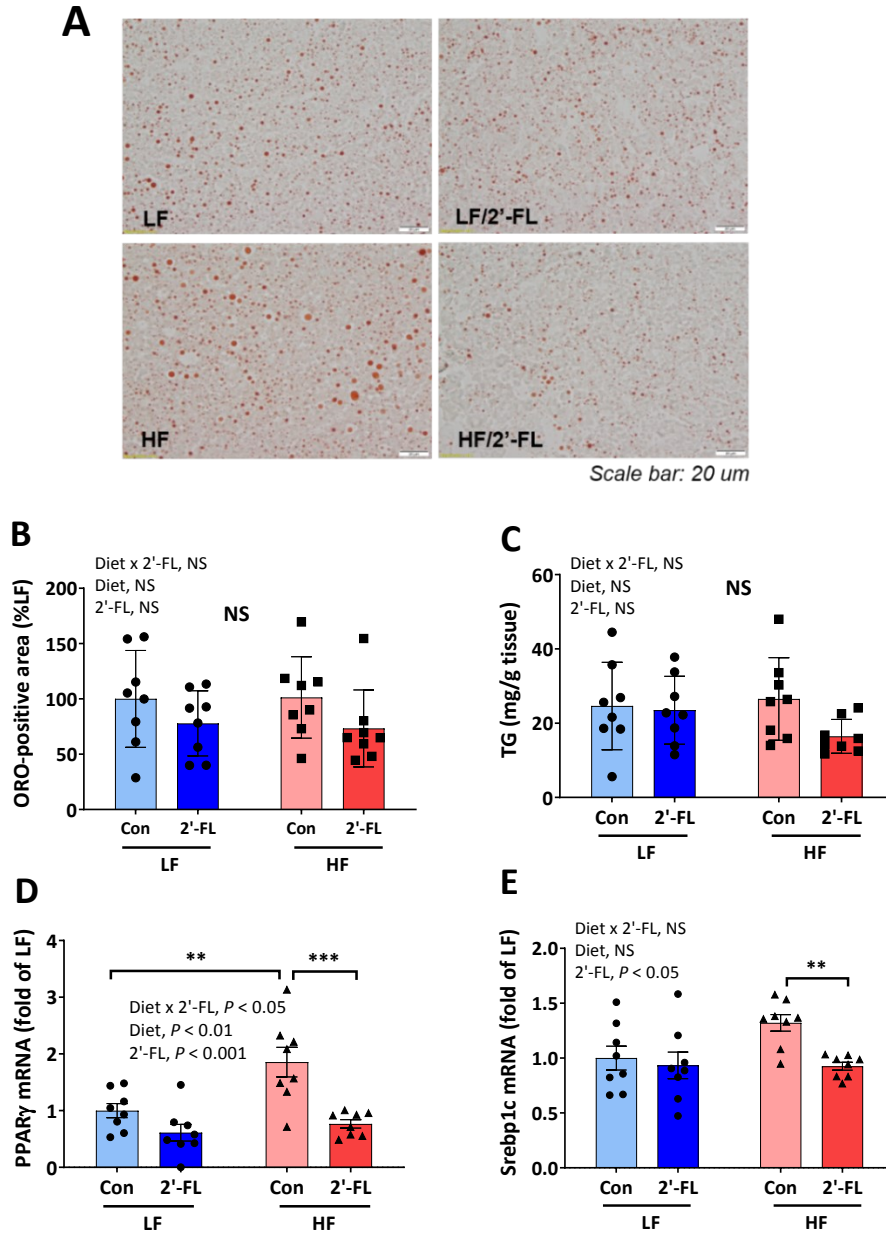
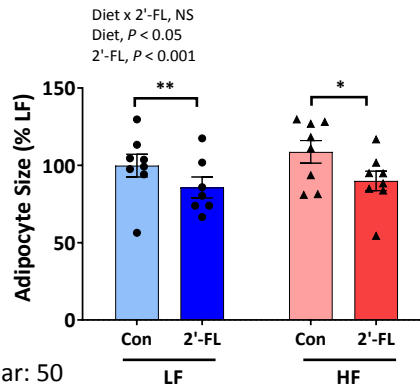
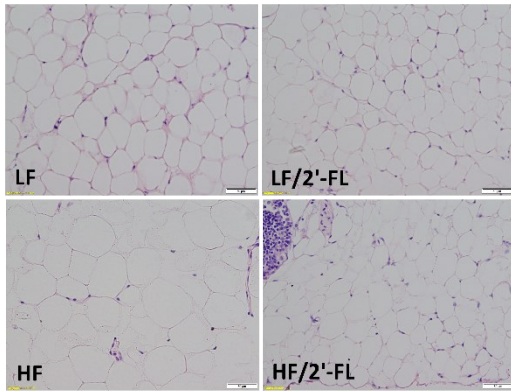


Figure S2. Effect of 2'-FL supplementation on lipid metabolism in the liver. Lipid accumulation (A, B), triglyceride (TG, C), and gene expression of lipogenesis transcription factors, peroxisome proliferator-activated receptor gamma (PPAR γ , D) and sterol regulatory element binding protein-1c (SREBP-1c, E) in the liver of mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks. Two-way analysis of variance followed by Tukey's or Sidak's post-hoc test was used to determine differences among groups. Values are means \pm SEMs; $n = 8$ /group. Asterisk (*) denotes significant differences



Scale bar: 50
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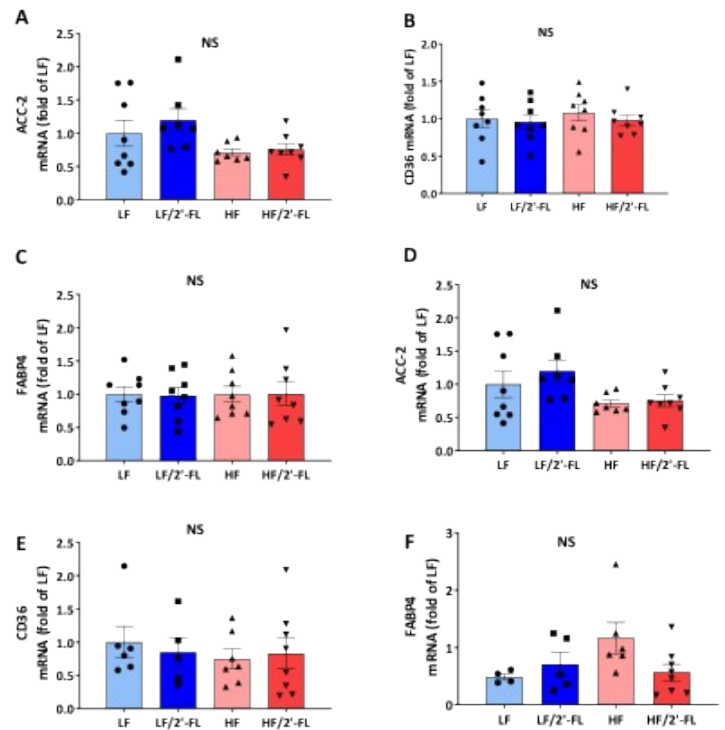


Figure S3. Effect of 2'-FL supplementation on lipid metabolism in adipose tissue. Adipocyte size in visceral adipose tissue of mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks. Gene expression of acetyl-CoA carboxylase-2 (ACC-2; A, D), cluster of differentiation 36 (CD36; B, E), and fatty acid-binding protein 4 (FABP 4; C, F) in the liver and visceral adipose tissues of mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks. Two-way analysis of variance followed by Tukey's or Sidak's post-hoc test was used to determine differences among groups. Values are means \pm SEMs; $n = 8$ /group. Asterisk (*) denotes significant differences among groups at $*P < 0.05$, $**P < 0.01$. ACC-2, acetyl-CoA carboxylase 2; CD36, cluster of differentiation 36; FABP4, fatty acid binding protein 4; HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; 2'-FL, fucosyllactose.

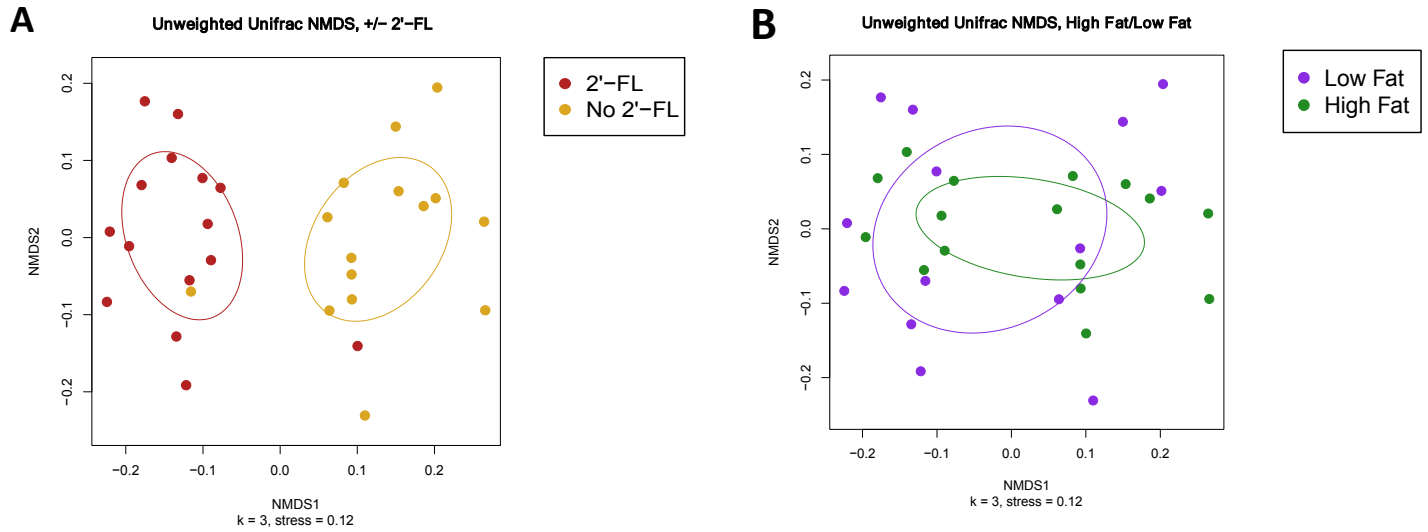


Figure S7. Effect of 10% 2'-FL supplementation on the composition of the gut microbiota. Unweighted UniFrac NMDS with points colored by 2'-FL treatment (A) and by diet (B) in mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks, ellipses are drawn based on the standard deviation of the points. Statistically significant differences by 2'-FL supplementation were calculated based on PERMANOVA testing of unweighted UniFrac distances ($P = 0.001$). $n = 14\sim 16$ /group. HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; 2'-FL, 2-fucosyllactose.

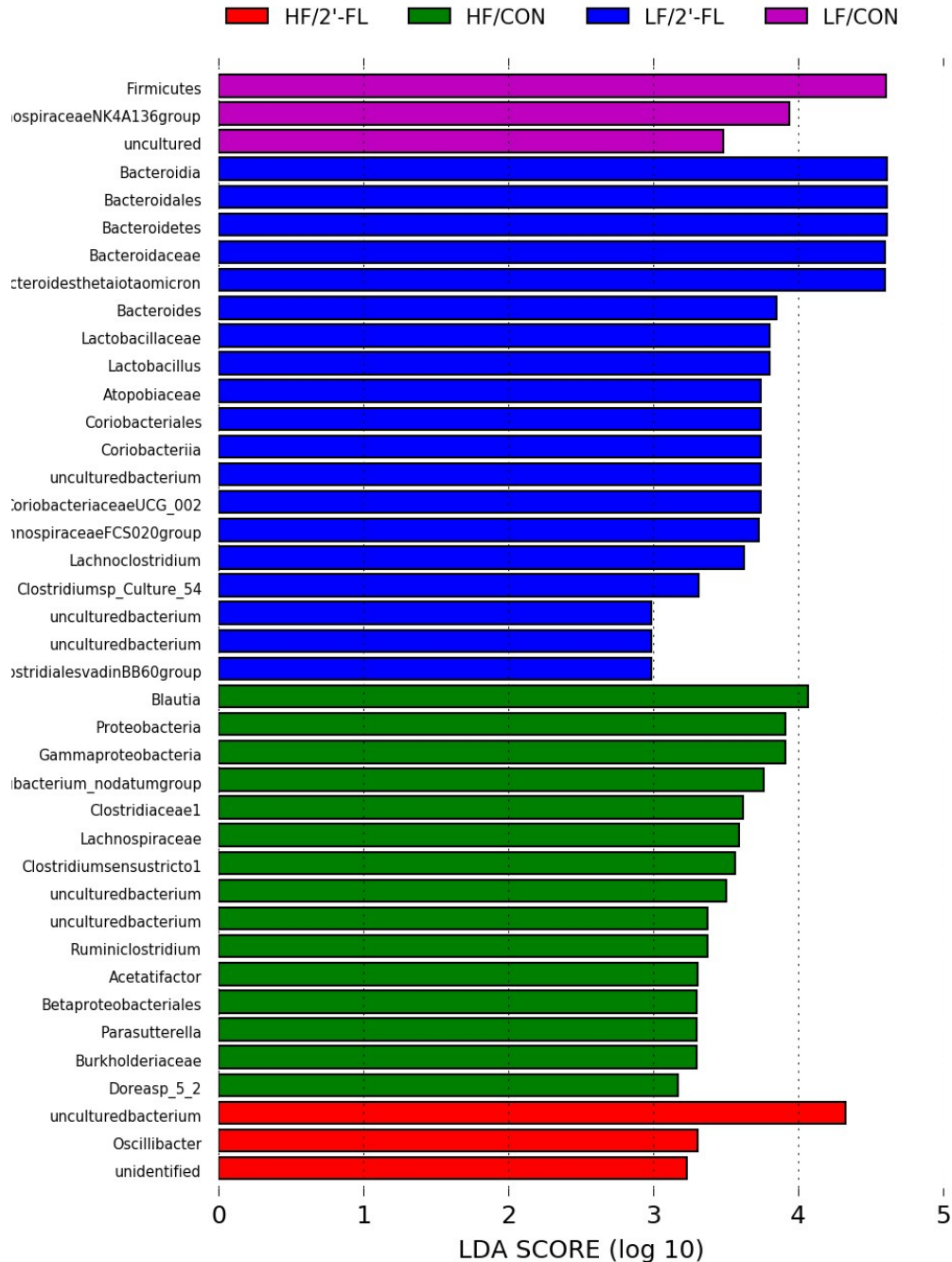


Figure S8. Histogram of the LDA scores from LEfSe analysis, showing the most differentially abundant taxa at all taxonomic levels enriched in microbiota from mice fed an LF or HF with or without 10% 2'-FL supplementation diet for 8 weeks. $n = 7\sim 8$ /group. The LEfSe method was used to identify taxa that were significantly differentially abundant for each group. The threshold of the logarithmic linear discriminant analysis score was 4.0. HF, high fat; HF/CON, HF without 2'-FL; HF/2'-FL, HF with 2'-FL (w/w) in diet; LEfSe, linear discriminant analysis effect size; LF, low fat; LF/CON, LF without 2'-FL; LF/2'-FL, LF with 2'-FL (w/w) in diet; 2'-FL, 2-fucosyllactose.

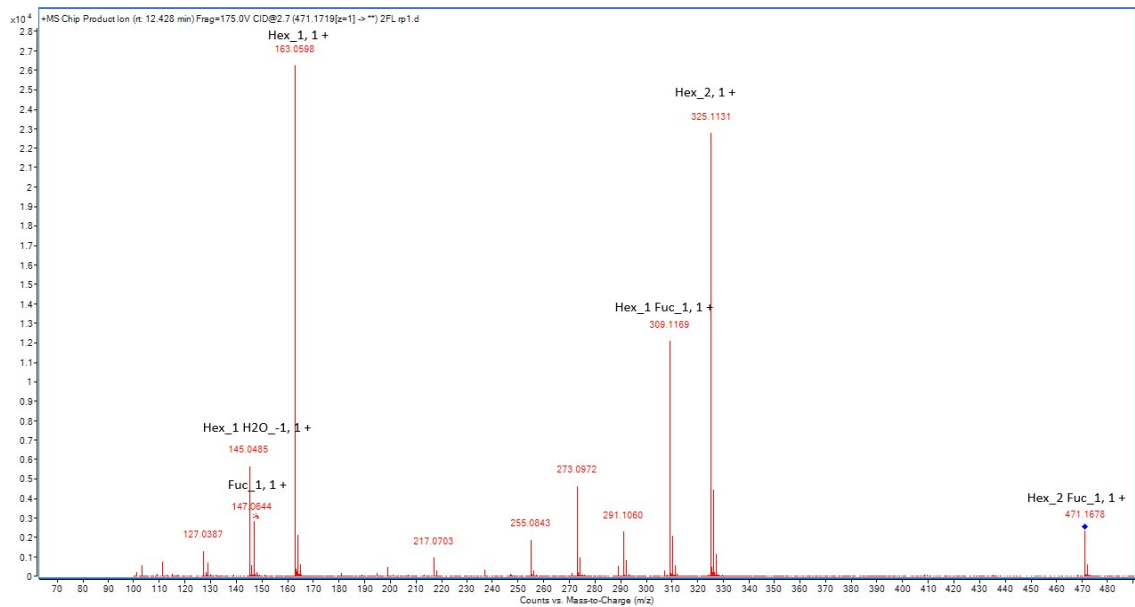
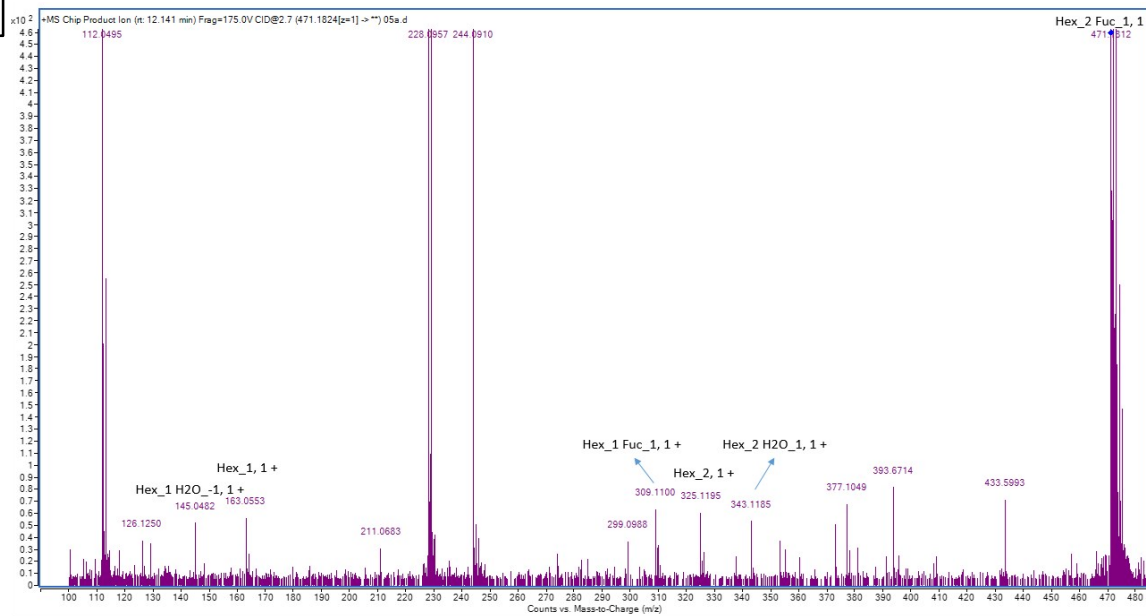
A**B**

Figure S9. LC-QToF MS/MS fragmentation confirming presence of 2' FL in the reference standard (Panel A), blood plasma of mice fed on high fat diet with 2' FL supplementation (Panel B). A blue diamond denotes the precursor ion, 471.17 m/z (derived from ion 489.18 m/z minus one water molecule). The composition of oligosaccharides is reported in the order of composing monosaccharides Hex_HexNAc_Fuc_NeuAc_NeuGc, where Hex = hexose, HexNAc = N-acetylhexosamine, Fuc = fucose, NeuAc = N-acetylneuraminic acid (sialic acid) and NeuGc = N-glycolylneuraminic acid (sialic acid).