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Supplemental Information

Multi-Omic Analyses of Dietary Fatty Acid-Microbe-Host Interactions Reveal Metaorganismal Lipid Metabolic Crosstalk Impacting Cardiometabolic Disease

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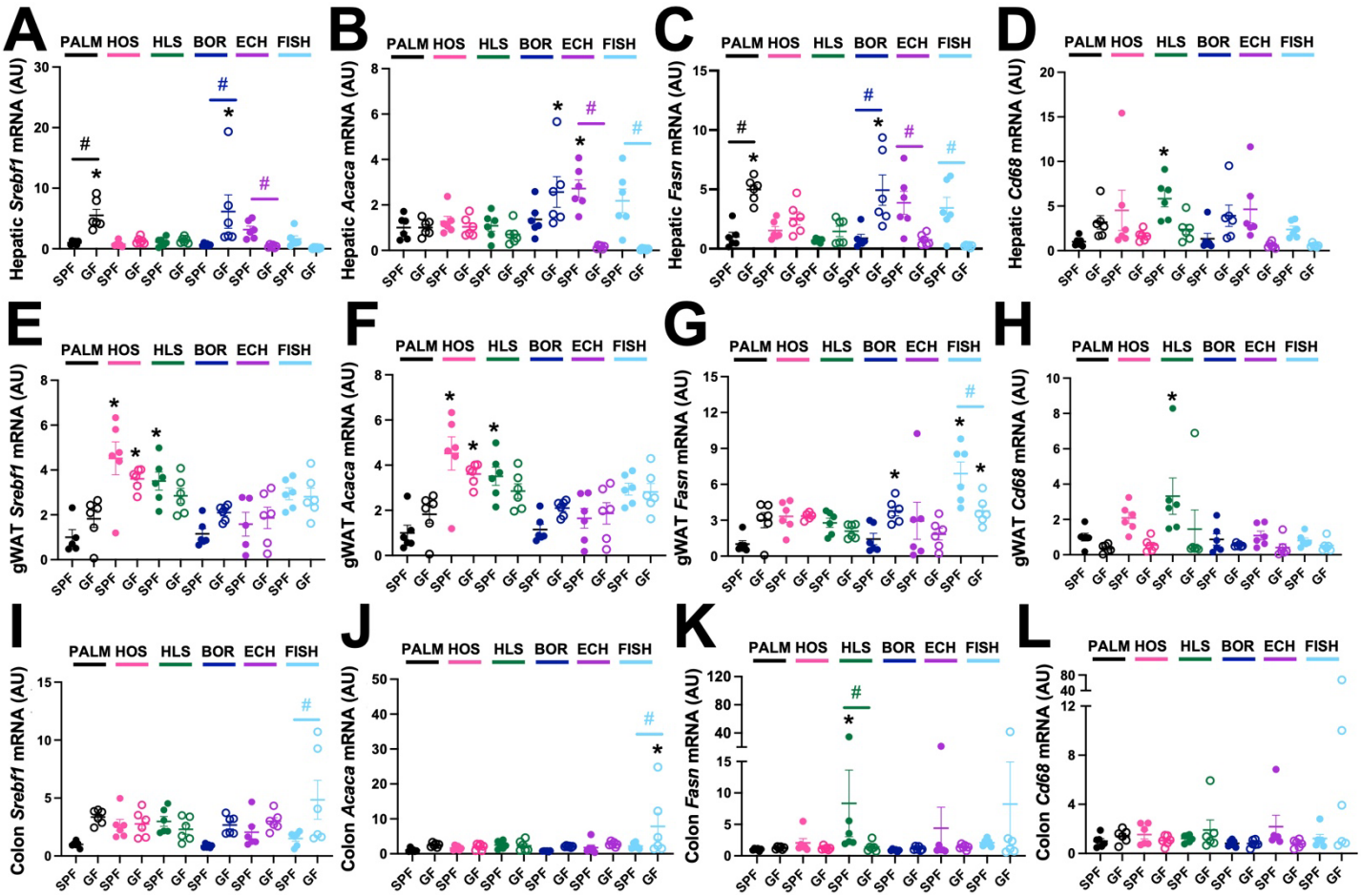

Figure S1. The Ability of Dietary Fatty Acids to Alter Host Metabolic Gene Expression is Shaped by Resident Microbiota (*Related to Figure 1*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, **(A-D)** liver, **(E-H)** gonadal white adipose (gWAT), and **(I-L)** colon tissue was subjected to quantitative real-time PCR (qPCR) to examine the mRNA expression of sterol regulatory element-binding protein 1c (*Srebf1*), acetyl-CoA carboxylase α (*Acaca*), fatty acid synthase (*Fasn*), and cluster of differentiation 68 (*Cd68*). Data represent the mean ± S.E.M. from n=6 per group, and statistically significant differences (p<0.05) were detected using ANOVA with post-hoc Tukey-Kramer HSD for all pairs comparisons. * = significantly different than the SPF palm oil-fed control group; # = significantly different when comparing SPF and GF groups within each dietary condition.

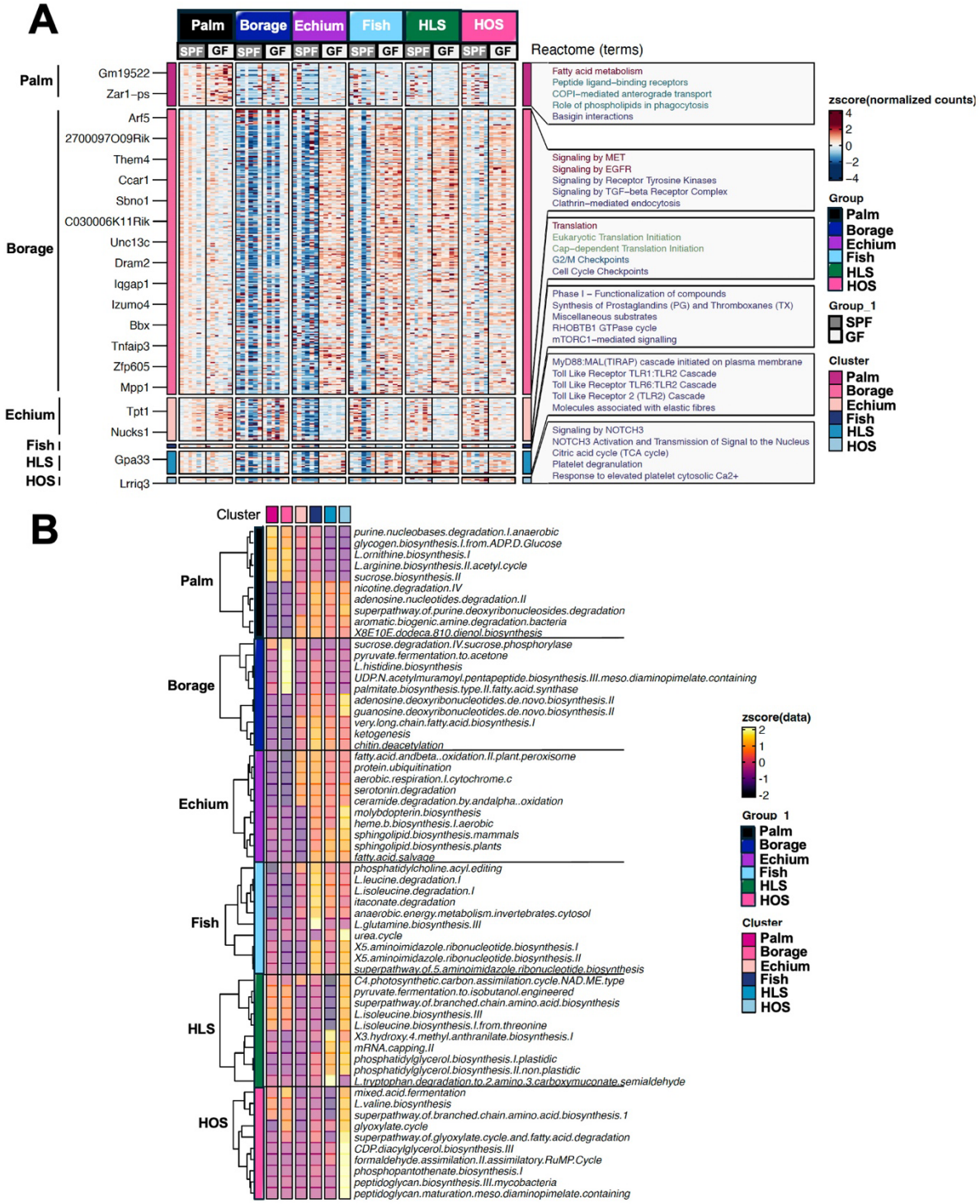

Figure S2. Metatranscriptomic Analyses of Colon Tissue Reveal (*Related to Figures 1 and 2*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, colon tissue was subject to shotgun metatranscriptomic sequencing with data analytic pipelines to examine both host reads (A) and bacterial reads (B). Data represent the mean ± S.E.M. from n=6 per group, and statistically significant differences (p<0.05) were detected using ANOVA with post-hoc Tukey-Kramer HSD for all pairs comparisons. * = significantly different than the SPF palm oil-fed control group; # = significantly different when comparing SPF and GF groups within each dietary condition.

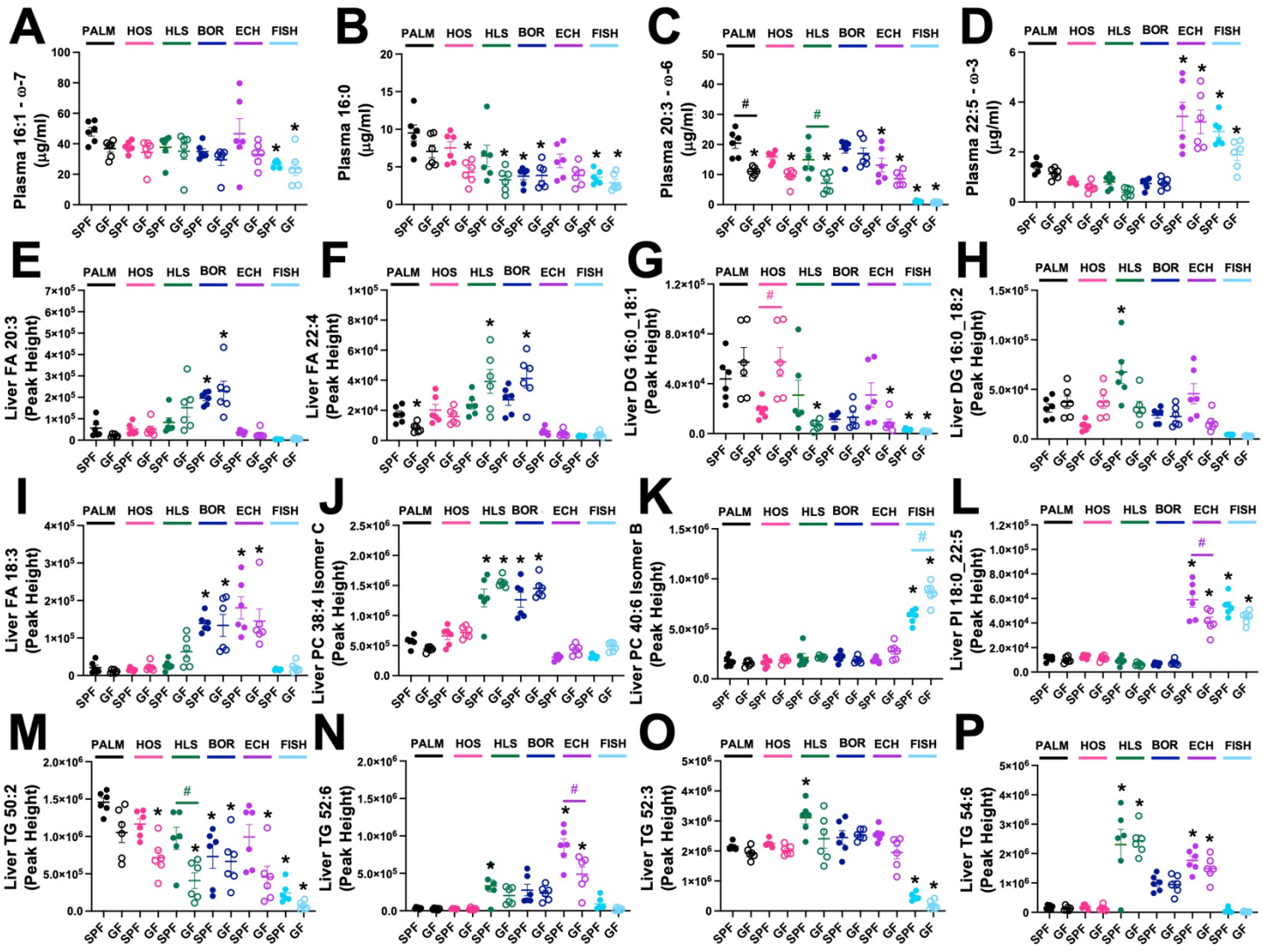

Figure S3. Diet-Microbe-Host Interactions Influence the Systemic Lipidome Within the Mouse Metaorganism (*Related to Figure 3*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, plasma and liver samples were subjected to lipidomic analyses to examine the levels of total fatty acids in the circulation as well as diverse complex lipids in the liver. **(A-D)** The total levels of plasma fatty acids including **(A)** palmitoleic acid (16:1, ω-7), **(B)** palmitic acid (16:0) **(C)** di-homo-γ-linolenic acid (20:3, ω-6), and **(D)** docosapentaenoic acid (22:5, ω-3) was quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS). **(E-P)** Hepatic levels of select molecular species of unesterified fatty acids (FA) and esterified lipids including phosphatidylcholines (PC), phosphatidylinositols (PI), diacylglycerols (DAG), and triacylglycerols (TG) were quantified using reverse phase liquid chromatography – high resolution tandem mass spectrometry (RPLC-MS/MS). Data represent the mean ± S.E.M. from n=6 per group, and statistically significant differences (p<0.05) were detected using ANOVA with post-hoc Tukey-Kramer HSD for all pairs comparisons. * = significantly different than the SPF palm oil-fed control group; # = significantly different when comparing SPF and GF groups within each dietary condition.

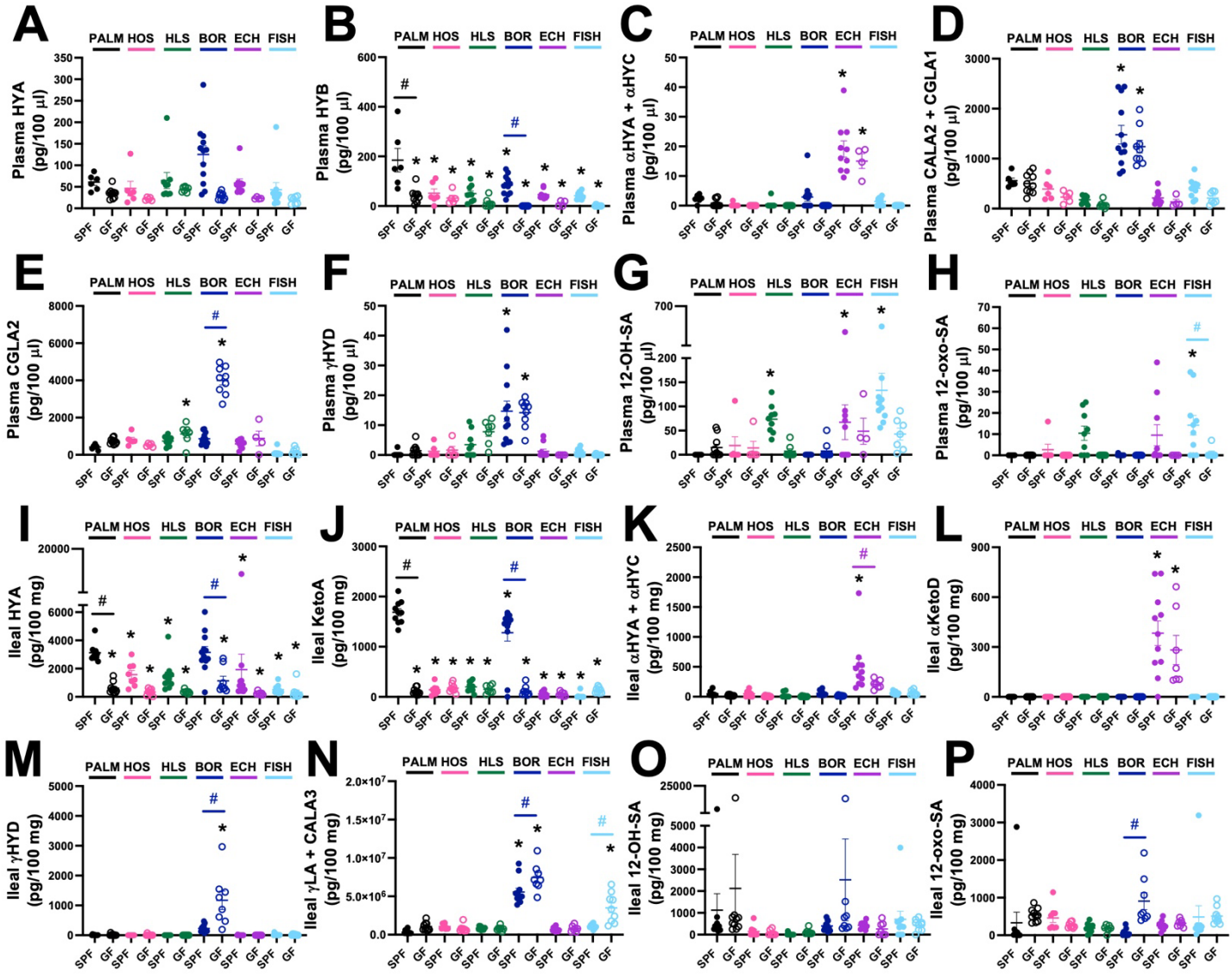

Figure S4. Gut Microbe-Derived Polyunsaturated Fatty Acid (PUFA) Metabolites are Altered in a Dietary Substrate-Specific Manner (*Related to Figure 4*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, either plasma (panels **A-H**) or ileum tissue (panels **I-P**) were extracted to quantify bacterially-derived lipid species originating from polyunsaturated fatty acid (PUFA) metabolism.

(A-H) Plasma levels of select bacterially-derived lipid mediators known to originate from gut microbiota-driven PUFA metabolism including **(A)** 10-hydroxy-cis12-octadecenoic acid (HYA), **(B)** 10-hydroxyoctadecanoic acid (HYB), **(C)** the sum of 10-hydroxy-cis12, cis15-octadecadienoic acid (αHYA) + 10-hydroxy-trans11, cis15-octadecadienoic acid (αHYC), **(D)** The sum of *trans*-9,*trans*-11,*cis*-15-octadecatrienoic acid (CALA2) + cis-6,cis-9,trans-11-octadecatrienoic acid (CGLA1), **(E)** cis-6,trans-9,trans-11-octadecatrienoic acid (CGLA2), **(F)** 13-hydroxy-cis6, cis9-octadecadienoic acid (γHYD), **(G)** 12-hydroxystearic acid (12-OH-SA), and **(H)** 12-oxo-stearic acid (12-oxo-SA).

(I-P) Ileal levels of select bacterially-derived lipid mediators known to originate from gut microbiota-driven PUFA metabolism including **(I)** 10-hydroxy-cis12-octadecenoic acid (HYA), **(J)** 10-oxo-cis12-octadecenoic acid (KetoA), **(K)** the sum of 10-hydroxy-cis12, cis15-octadecadienoic acid (αHYA) + 10-hydroxy-trans11, cis15-octadecadienoic acid (αHYC), **(L)** 13-oxo-cis9, cis15-octadecadienoic acid (αKetoD), **(M)** 13-hydroxy-cis6, cis9-octadecadienoic acid (γHYD), **(N)** The sum of γ-linolenic acid (γLA) and *trans*-10,*trans*-12,*cis*-15-octadecatrienoic (CALA3), **(O)** 12-hydroxystearic acid (12-OH-SA), and **(P)** 12-oxo-stearic acid (12-oxo-SA).

Data represent the mean ± S.E.M. from n=6-12 per group, and statistically significant differences (p<0.05) were detected using ANOVA with post-hoc Tukey-Kramer HSD for all pairs comparisons. * = significantly different than the SPF palm oil-fed control group; # = significantly different when comparing SPF and GF groups within each dietary condition.

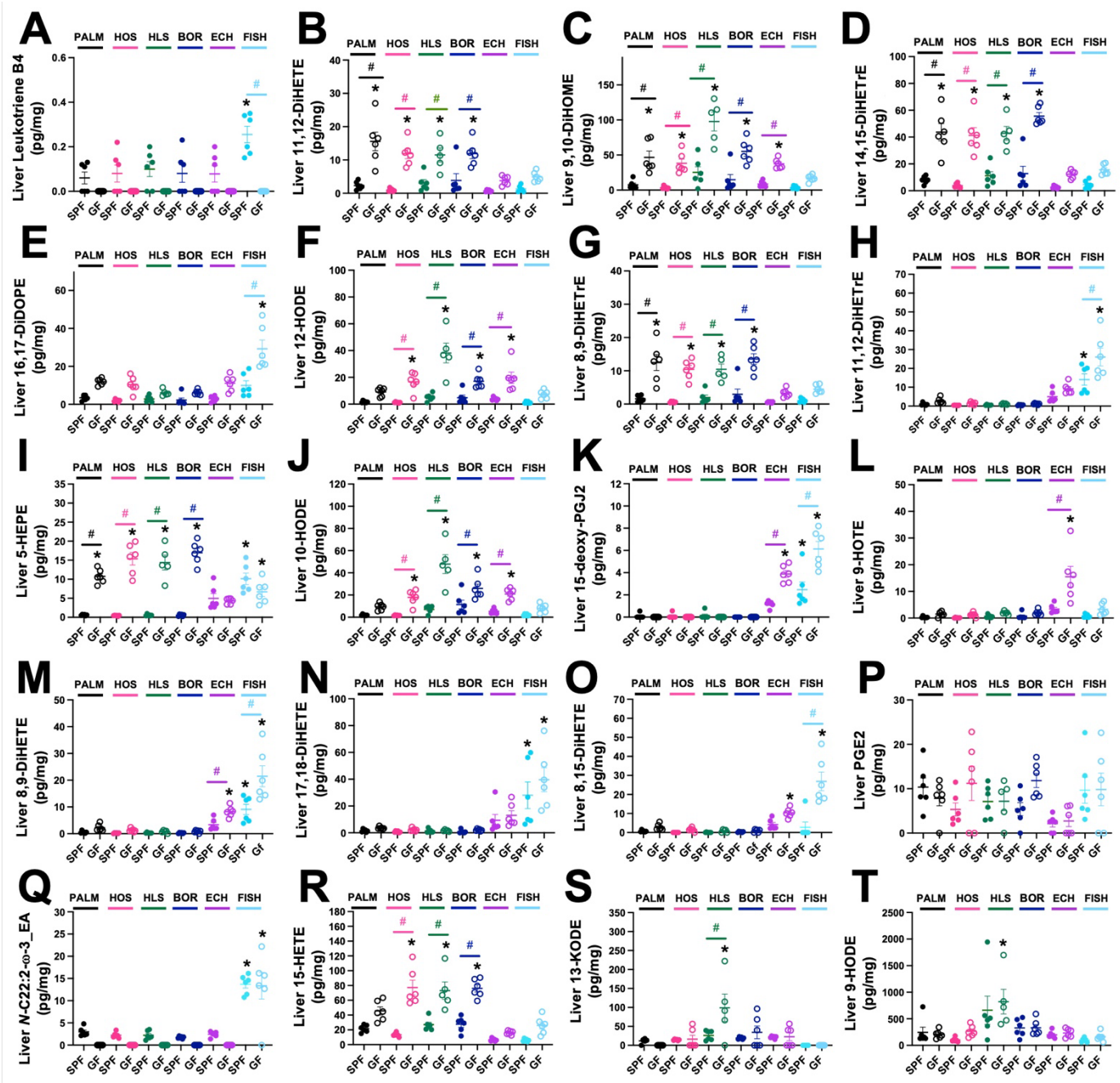

Figure S5. Hepatic Oxylipin Levels are Powerfully Shaped by Diet-Microbe-Host Interactions in the Mouse Metaorganism (*Related to Figure 4*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, liver tissue was extracted to quantify many diverse molecular species of oxylipin-related lipids known to originate from polyunsaturated fatty acid (PUFA) substrates including: **(A)** leukotriene B4 (LTB4), **(B)** 11,12-dihydroxyicosatetraenoic acid (11,12-DiHETE), **(C)** 9,10-dihydroxy-12Z-octadecenoic acid (9,10-DiHOME), **(D)** 14,15-dihydroxyeicosatrienoic acid (14,15-DiHETrE), **(E)** 16,17-dihydroxy-docosapentaenoic acid (16,17-DiDOPE), **(F)** 12-hydroxyoctadecadienoic acid (12-HODE), **(G)** 8,9-Dihydroxy-eicosatrienoic acid. (8,9-DiHETrE), **(H)** 11,12-dihydroxyeicosatrienoic acid (11,12-DiHETrE), **(I)** 5-Hydroxyeicosapentaenoic acid (5-HEPE), **(J)** 10-Hydroxyoctadecadienoic acid (10-HODE), **(K)** 15-deoxy-prostaglandin J2 (15-deoxy-PGJ2), **(L)** 9-hydroxyoctadecatrienoic acid (9-HOTE), **(M)** 8,9-dihydroxy-eicosatetraenoic acid (8,9-DiHETE), **(N)** 17,18-dihydroxy-eicosatetraenoic acid (17,18-diHETE), **(O)** 8,15-dihydroxyeicosatetraenoic acid (8,15-DiHETE), **(P)** prostaglandin E2 (PGE2), **(Q)** *N*-docosadienoic acid-ethanolamide (*N*-C22:2-ω-3_EA), **(R)** 15-Hydroxyeicosatetraenoic acid (15-HETE), **(S)** 13-oxooctadeca-9,11-dienoic acid (13-KODE), and **(T)** 9-hydroxy-octadecadienoic acid (9-HODE). Data represent the mean ± S.E.M. from n=6 per group, and statistically significant differences (p<0.05) were detected using ANOVA with post-hoc Tukey-Kramer HSD for all pairs comparisons. * = significantly different than the SPF palm oil-fed control group; # = significantly different when comparing SPF and GF groups within each dietary condition.

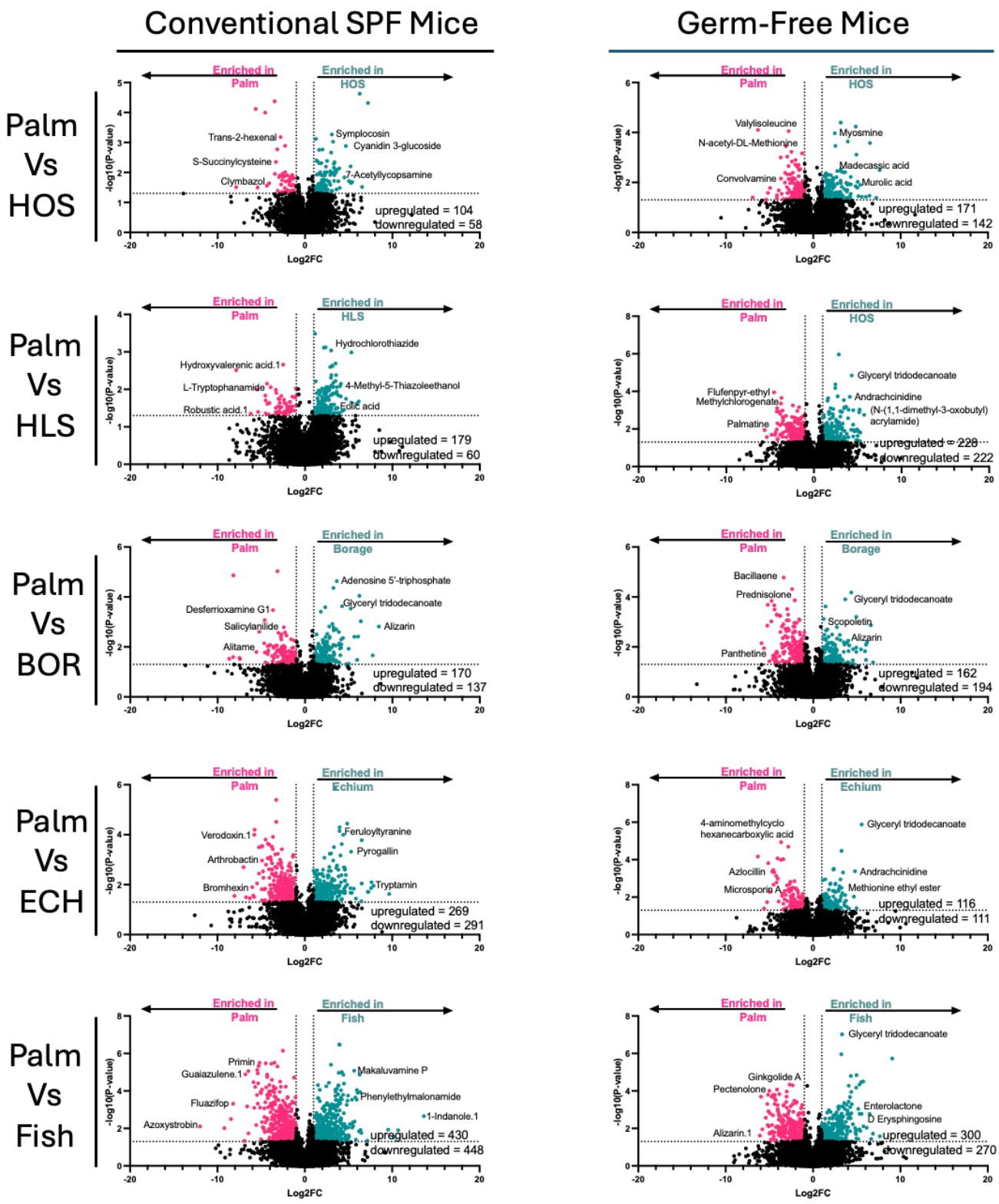

Figure S6. The Ability of Dietary Fatty Acids to Reorganize the Hepatic Metabolome is Altered in Germ-Free Mice (*Related to Figures 6*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, liver tissue from n=6 mice per group was extracted to perform untargeted metabolomics using a hydrophilic interaction liquid chromatography (HILIC) column coupled to high resolution tandem mass spectrometry. Volcano plots are shown to compare and contrast each experimental diet group to the control palm oil-fed group either in SPF or GF mice. Significantly altered metabolites (p<0.05) are shown in red (decreased) or blue (increased) when compared to controls within each plots.

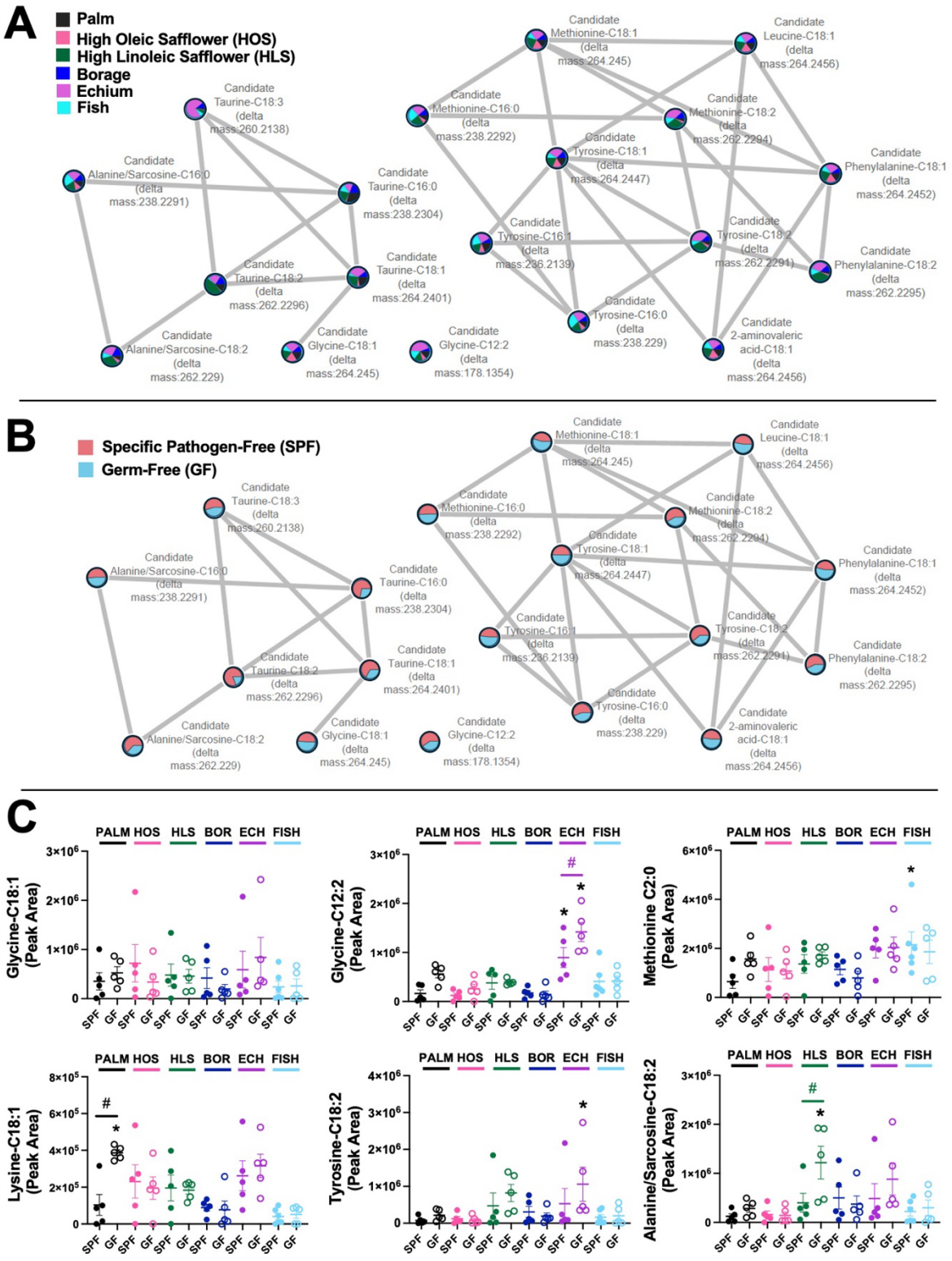

Figure S7. Both Dietary Fatty Acid Substrate and Resident Microbiota Impact Hepatic Levels of Diverse N-Acyl Lipids. (*Related to Figures 6 and Figure S6*). Starting at 6 weeks of age, either conventionally-raised specific pathogen-free (SPF) or germ-free (GF) cohorts of male C57BL/6N mice were maintained on sterile fatty acid-defined diets containing saturated (Palm), high oleic safflower (HOS), high linoleic safflower (HLS), borage (BOR), echium (ECH), or fish oils for 18 weeks. Thereafter, liver tissue from n=6 mice per group was extracted to perform untargeted metabolomics using a C18 column coupled to high resolution tandem mass spectrometry. The raw data generated were mined to identify diverse *N*-acyl lipid species. Molecular networks obtained for the N-acyl-lipids comparing across all 6 diet groups **(A)** or comparing SPF to GF cohorts **(B)**. The molecular networks were created using the feature-based molecular networking workflow within the GNPS2 environment. The nodes are annotated based on spectral similarity matches with the N-acyl lipids library. The nodes represent each MS/MS spectrum, while the edges represent their spectral similarity. The delta mass is the mass difference between the feature and Amino-containing compound. Pie charts indicate the relative abundance of ion features in each group. **(C)** The relative abundance of select N-acyl lipids show diet-microbe-host interactions.

ADDITIONAL ONLINE SUPPLEMENT FILES INCLUDED (All provided as accessory excel data tables):

1. **Supplemental Table 1.** Diet information sheet including ingredient list.
2. **Supplemental Table 2.** Untargeted lipidomics analyses of mouse liver using reverse phase liquid chromatography high resolution tandem mass spectrometry (RPLC- MS/MS).
3. **Supplemental Table 3.** Targeted lipidomic analysis of gut bacterially-produced fatty acid metabolites in the plasma, liver, and distal small intestine (ileum) via liquid chromatography tandem mass spectrometry (LC-MS/MS).
4. **Supplemental Table 4.** Targeted lipidomic analysis of diverse oxylipin species in mouse liver was performed using ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS).
5. **Supplemental Table 5.** Quantitative global proteomics in mouse liver was performed using Data Independent Acquisition (DIA) liquid chromatography tandem mass spectrometry (LC-MS/MS).
6. **Supplemental Table 6.** Untargeted metabolomics in mouse liver data using C18 column separation coupled to high resolution mass spectrometry.
7. **Supplemental Table 7.** Untargeted metabolomics in mouse liver data using HILIC column separation coupled to high resolution mass spectrometry.