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Supplemental Information

Gut Microbiota-Derived Tryptophan Metabolites Modulate Inflammatory Response in Hepatocytes and Macrophages

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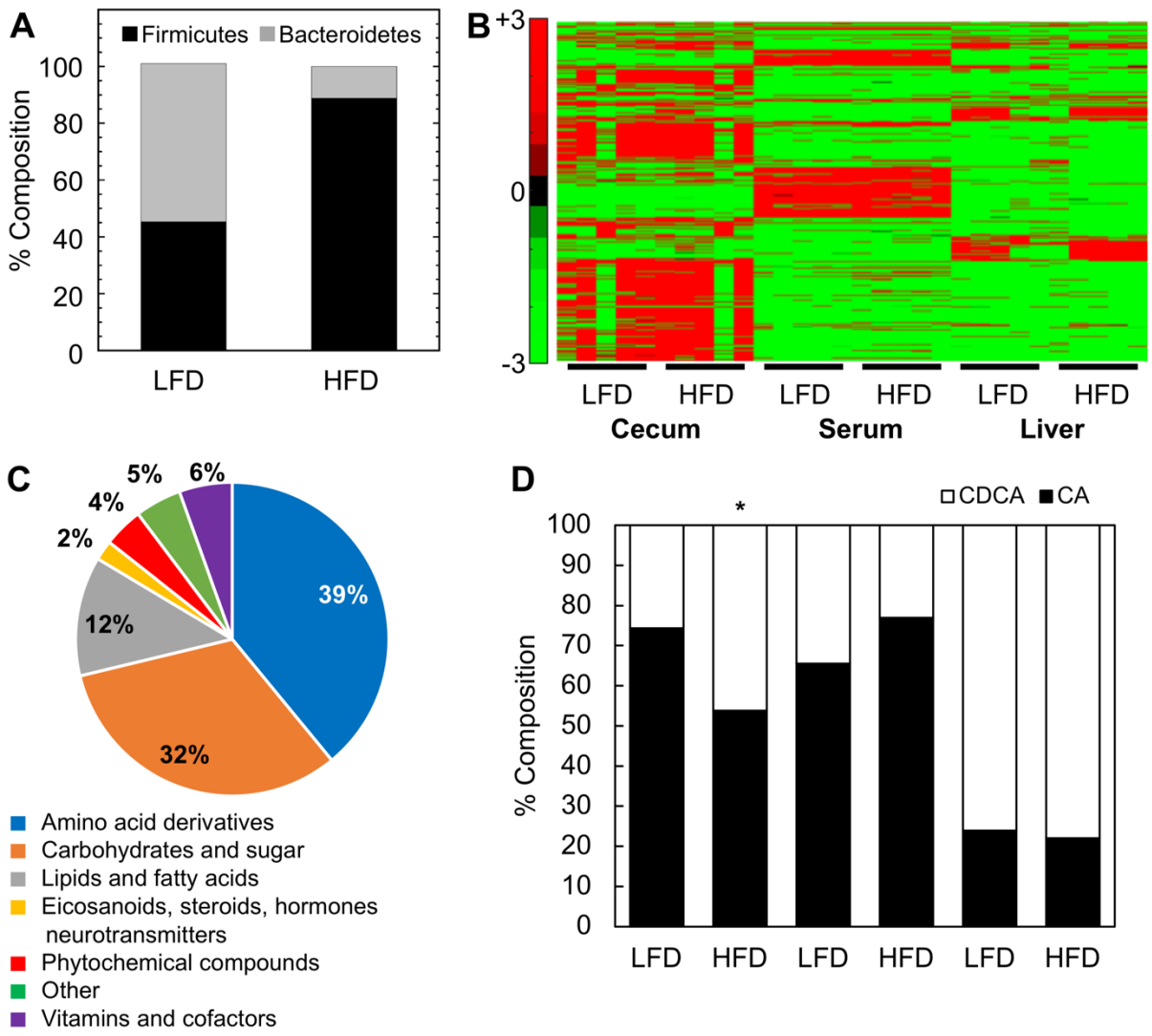

Figure S1, Effect of diet on microbiome and metabolite profile, related to Figure 2. Fecal material from mice fed a high-fat diet (HFD) or low-fat diet (LFD) were assayed for Firmicutes and Bacteroidetes using qRT-PCR (A). Ratios between the two phyla were determined from copy numbers calculated based on a DNA standard curve. Data shown are averages of n = 5 mice. Error bars represent one standard deviation. *: $p<0.05$ compared to LFD using Wilcoxon rank-sum test. Heat map shows hierarchically clustered LC-MS features (i.e., metabolites) detected in cecum, liver, and serum samples (B). Features from cecum, serum, and liver samples were separately scaled prior to clustering using the Pareto method. Red or green color denotes a feature that is elevated or depleted, respectively, relative to the mean value for the feature in the tissue compartment. Features significantly elevated or depleted were annotated and mapped to pathways cataloged in KEGG (C). Primary bile acid composition showing contribution from cholic acid (CA) and chenodeoxycholic acid (CDCA) (D).

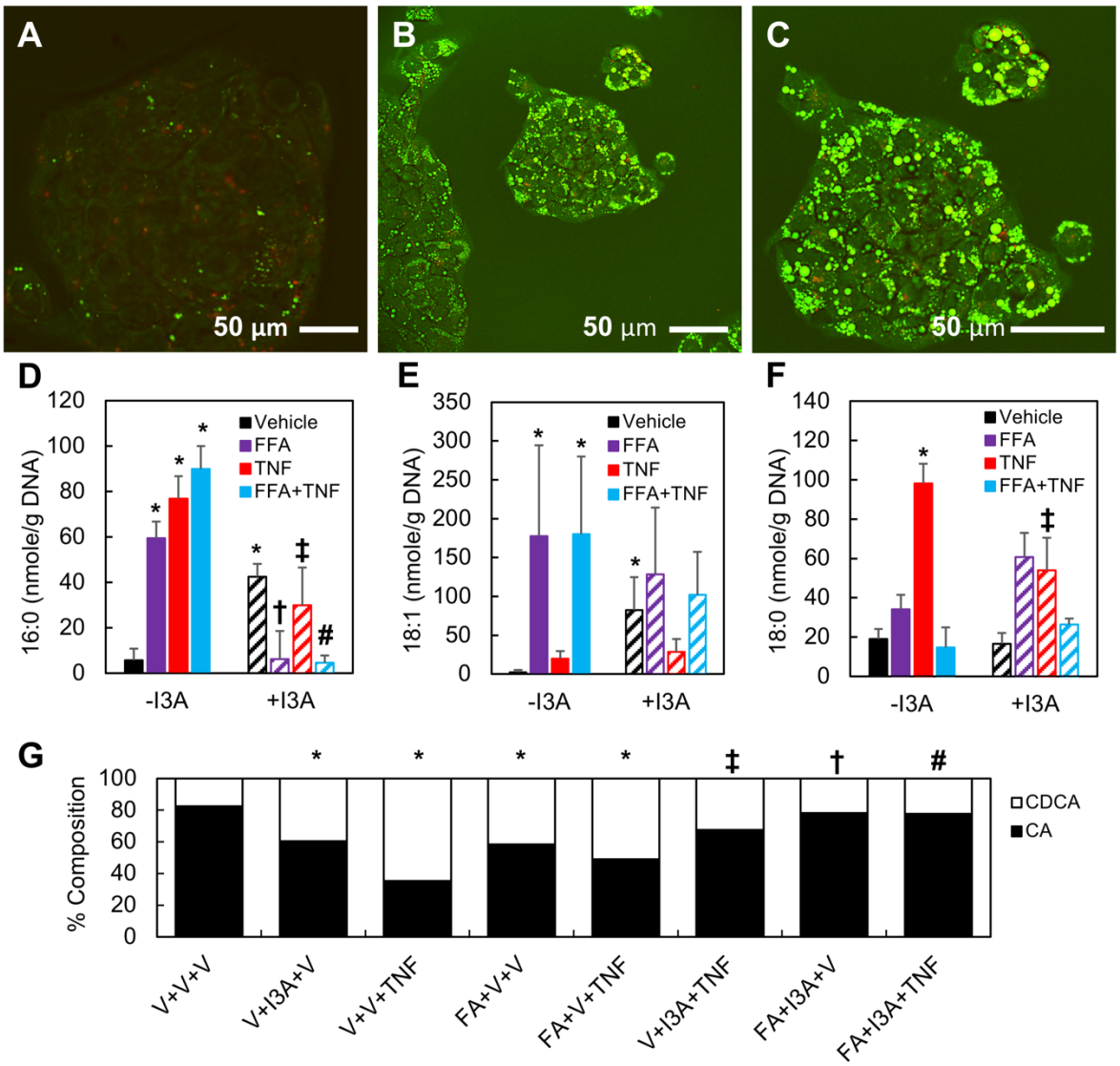

Figure S2, I3A attenuates TNFα-induced alterations in free fatty acid (FA) and bile acid (BA) metabolism in cultured HepG2 cells, related to Figures 5 and 6. Micrographs of HepG2 cells obtained using Coherent anti-Stokes Raman Scattering (CARS) microscopy (A-C). The cells were treated with either (A) vehicle or (B) a combination of palmitic (500 µM) and oleic acids (500 µM) for 48 hours. The microscope was tuned to detected aliphatic C-H vibrations. Lipid inclusion bodies show as green circles. Metabolites were extracted from cell cultures using a solvent-based method and analyzed using a product ion scan experiment for FFAs and BAs. The three most abundant FFAs were palmitic (D), oleic (E), and stearic (F) acids. Composition of primary BAs shown as a ratio of cholic acid (CA) to chenodeoxycholic acid (CDCA) (G). Data shown are averages of 4 independent experiments, each with 6 biological replicates. Treatment group means were compared using two-tailed Student *t*-test. *: $p<0.05$ compared to negative vehicle control (V+V+V), †: $p<0.05$ compared to the corresponding FFA supplementation group (FA+V+V), ‡: $p<0.05$ compared to the corresponding TNFα treatment group (V+V+TNF), #: $p<0.05$ compared to combined FFA supplementation and TNFα treatment (FA+V+TNF).

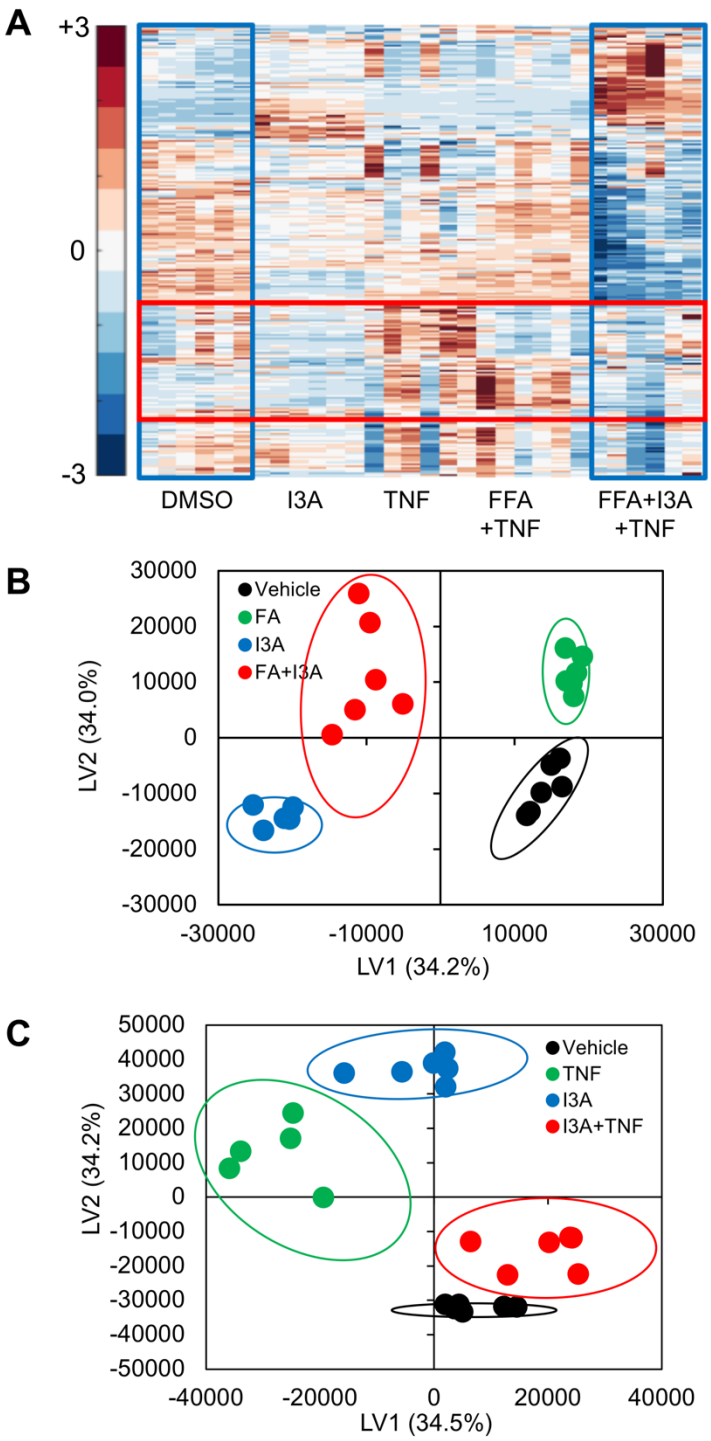

Figure S3, Multivariate analysis of untargeted metabolomics data from AML12 cultures, related to Figure 6. LC-MS data features were pareto-scaled and hierarchically clustered (A) for qualitative comparisons between the effects of TA and I3A on global metabolite profile. The similarity of the metabolite profiles was assessed based on group centroid distances using PLS-DA (B-C). Ellipses show 95% confidence intervals for each group. Numbers in parentheses show % of variance in the data explained by the latent variable (LV).

Table S1, Parameters for product ion scan and IDA experiments, related to Figures 1, 2, 3, 6 and 7.

	Bile Acid Method	**Free Fatty Acid Method**	**Hydrophilic Interaction Liquid Chromatography (HILIC)**
Column	Kinetex® 5 µm EVO C18 100 Å, 50 x 2.1 mm	Luna® 5 µm C8(2) 100 Å, 150 x 4.6 mm	Luna® 5 µm NH2 100 Å, 250 x 2 mm
Solvent A	50:50 v/v water:methanol with 0.1% w/v NH_4OH and 10 mM ammonium acetate	97:3 v/v water:methanol with 10 mM tributylamine and 15 mM acetic acid	95:5 v/v water:acetonitrile with 20 mM ammonium acetate and 20 mM NH_4OH
Solvent B	Methanol with 0.1% w/v NH_4OH and 10 mM ammonium acetate	Methanol	Acetonitrile
pH Solvent A	9	4.5	9.45
pH Solvent B	9	N/A	N/A
Column Temperature (°C)	50	25	25
Injection volume (µL); Draw speed (µL/min); Flowrate (µL/min)	10; 200; 200	10; 300; 300	10; 300; 300
MS Method	Product Ion Scan	Product Ion Scan	IDA
Source (Ionization Mode)	ESI (-)	ESI (-)	ESI (+)
Calibration Solution	1 µM cholate, chenodeoxycholate, taurocholate, and glycocholate in 50:50 v/v water:methanol	0.01 mg/mL palmitic, oleic, stearic, palmitoleic, linoleic, and myristic acid in 0.01% v/v chloroform in methanol	Pos PPG Solution (AB Sciex)
Source Gas 1	40	60	35
Source Gas 2	35	60	45
Curtain Gas	25	25	25
Source Temperature (°C)	450	550	450
TOF MS Collison Energy (V)	-10	-5	+5
Declustering Potential (V)	-100	-100	80

Table S2, Collision energies (CE) and expected retention times (RT) for target product ions, related to Figures 2, 3, 6, and 7.

Metabolite	LC/MS Method	Collision Energy (V)	Expected RT (min)
Cholic acid	Bile Acid	-30	0.6
Chenodeoxycholic acid	Bile Acid	-30	0.41
Palmitic Acid	Free Fatty Acid	-25	25.2
Oleic Acid	Free Fatty Acid	-25	26.02
Stearic Acid	Free Fatty Acid	-25	27.74
Palmitoleic Acid	Free Fatty Acid	-25	23.44
Indole-3-acetate	HILIC	+25 (IDA)	16.7
Tryptamine	HILIC	+25 (IDA)	3.4

Table S3, LC method for bile acid assay, related to Figure 6.

Time (min)	Flowrate (uL/min)	%A	%B
0.50	500	100	0
4.50	500	50	50
5.50	500	0	100
6.50	500	100	0

Table S4, LC method for free fatty acid assay, related to Figures 3, 6, and 7.

Time (min)	Flowrate (uL/min)	%A	%B
0	300	20	80
20	300	1	99
40	300	1	99
41	300	20	80
50	300	20	80

Table S5, LC method for IDA experiments, related to Figures 1 and 2.

Time (min)	Flowrate (uL/min)	%A	%B
0	300	15	85
15	300	100	0
28	300	100	0
30	300	15	85
60	300	15	85

Table S6, Primer sequences for qRT-PCR analysis, related to Figures 4 and 7.

Gene	Forward Primer Sequence	Reverse Primer Sequence
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
TNFα	TCTCATGCACCACCATCAAGGACT	TGACCACTCTCCCTTTGCAGAACT
MCP-1	CTCTCTTCCTCCACCACCAT	ACTGCATCTGGCTGAGCCA
IL-1β	TCCAGGATGAGGACATGAGCAC	GAACGTCACACACCAGCAGGTTA
Fas	TACCAGTGCCACAGGAGTCTCA	TAAACACCTCGTCGATTTCGTTC
SREBP-1c	CTCCAAGGTTTCGTCTGACG	TCCAGTGGCAAAGAAACACC
Ahr	CGCGGGCACCATGAGCAG	GAGACTCAGCTCCTGGATGG
Cyp7a1	CCTTGGACGTTTTCTCGCT	GCGCTCTTTGATTTAGGAAG
Cyp27a1	TGCCTGGGTCGGAGGAT	GAGCCAGGGCAATCTCATACTT
GAPDH	GAAGGTCGGTGTGAACGGATTTGGC	TGTTGGGGGCCGAGTTGGGATA
Bacteroidetes	ACGCTAGCTACAGGCTTAACA	ACGCTACTTGGCTGGTTCA
Firmicutes	GCGTGAGTGAAGAAGT	CTACGCTCCCTTTACAC