TMIC
The Metabolomics Innovation Center

In-depth Global Lipidomics Analysis Report

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The Metabolomics Innovation Centre

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1 Overview

This document reports the lipidomics analysis results for "Lipidomics of 8 Cell Samples". LC-MS and LC-MS/MS data were collected using the In-depth Global Lipidomics approach (Nova Medical Testing Inc.). Data analysis and statistical analysis were performed using LipidScreener 1.0.0 (Nova Medical Testing Inc.).

2 Materials and Methods

2.1 General Information

Sample Type:	Cells
Sample Group:	A total of 8 samples divided into two groups: • 4 samples labeled as "KO" • 4 samples labeled as "WT" Samples were analyzed randomly and blindly without group or background information.
Replicates:	Extraction singlet/sample Injection singlet/sample extraction for each polarity (positive and negative electrospray ionization)
Analysis Method:	Microflow-based lipidomic profiling in positive and negative ionization with MS/MS identification

2.2 Sample Preparation

2.2.1 Lipids Extraction

The extraction was performed strictly following the SOP based on a modified Folch liquid-liquid extraction protocol. Each aliquot of sample was mixed with NovaMT LipidRep Internal Standard Basic Mix for Tissue/Cells (an internal standard mixture composed of 15 deuterated lipids), dichloromethane and methanol. Samples were homogenized with a bead beater homogenizer using ceramic beads. A clean-up step was performed with water. Samples were equilibrated at room temperature for 10 min and centrifuged at 16,000 g for 10 min at 4°C. An aliquot of the organic layer was evaporated to dryness with a nitrogen blowdown evaporator. The residue was immediately re-suspended in NovaMT MixB, vortexed for 1 min, and diluted with NovaMT MixA.

A pooled mixture composed by one aliquot of the organic extract from each sample was prepared for quality control (QC). The pooled mixture was split into multiple aliquots of equal volume, evaporated to dryness with a nitrogen blowdown evaporator, purged with nitrogen and stored at -80°C. One QC aliquot was resuspended with each randomized batch of samples (2 batches of 4 samples). The samples within each batch were injected in between two injection replicates of the corresponding QC aliquot. Multiple QC aliquots were also injected before and after all samples to ensure technical stability.

2.3 LC-MS Analysis Condition

The LC-MS analyses were performed by strictly following the SOP in both positive and negative ionization. Sample extracts were injected between injection replicates of the QC pooled mixture prepared with the same sample batch. A total of 8 sample injections (experimental singlets of 8 samples) and 16 QC injections (8 aliquots of the pooled mixture with singlet extractions and duplicate injections) were performed in each ionization polarity. MS/MS spectra were acquired for all samples for alignment and identification. Parameters used for data acquisition are described below.

Instrument:	Thermo Vanquish UHPLC linked to Bruker Impact II QTOF Mass Spectrometer
Column:	Waters Acquity CSH C18 column, 1.7 µm.
MPA:	NovaMT MixA
MPB:	NovaMT MixB
Gradient:	NovaMT 20-min-gradient
Flow Rate:	210 to 300 µL/min.
Injection Volume:	4.0 µL for positive ionization and 12.0 µL for negative ionization
Column Oven Temperature:	45 °C
Mass Range:	m/z 150-1500
MS/MS Collision Energies:	10-60 eV

2.4 Data Processing

LC-MS data from 24 injections (singlet experiments from 8 samples, *i.e.*, 8 sample injections; and duplicate injections of 8 QC aliquots, *i.e.*, 16 QC injections) were independently processed in positive and negative ionization. Lipid features were extracted and aligned using NovaMT LipidScreener. The data acquired in positive and negative ionization from each sample experiment were combined, *i.e.*, the detected features from all samples were merged into one feature-intensity table. Features not detected for ≥80% of injections within at least one sample group or QCs were filtered out. Parameters used for data processing are below.

Intensity threshold:	3000 cts
Signal-to-Noise ratio (S/N) threshold:	3
Minimum Peak Length:	6 spectra
Retention Time Tolerance	6 seconds for retention time correction; 4 seconds for alignment
m/z Tolerance:	20.0 ppm for peak picking; 20.0 ppm and 5.0 mDa for alignment
Feature Filtering:	Detection for ≥80% of injections in at least one sample group or QCs

Missing values were substituted by (1) the median intensity of the sample group for features detected in at least 75% of injections within the group; (2) the minimum intensity within the group for features detected in at least 50% of injections; or (3) the global minimum for all sample and QC injections for features detected in less than 50% of injections within the group.

2.5 Lipid Identification

A three-tier identification approach based on MS/MS spectral similarity, retention time and accurate mass match was employed for lipid identification. A nine-tier filtering and scoring approach embedded in NovaMT LipidScreener was employed to calculate MS/MS match scores, restrict the number of matches, and select the best identification. The parameters used for identification are described below.

Tier 1 (MS/MS identification):	MS/MS match score ≥500; precursor m/z error ≤20.0 ppm and 5.0 mDa
Tier 2 (MS/MS identification):	MS/MS match score <500; precursor m/z error ≤20.0 ppm and 5.0 mDa
Tier 3 (MS match):	Mass match with m/z error ≤20.0 ppm and 5.0 mDa

Tiers 1 and 2 identifications were determined at the species or molecular species level, *i.e.*, definition of lipid classes and subclasses, composition of fatty acyl/alkyl residues (or summed composition if individual residues are not specified in the source database), and functional groups. The position of double bonds and stereochemistry of compounds were not determined in this report. Common names provided for selected lipids identified in Tiers 1 and 2 were attributed based on biological intelligence rather than analytical evidence. Tier 3 identifications are provided at the species level, *i.e.*, lipid class and subclass, total number of carbon atoms, total number of double bond equivalents, and total number of additional oxygen or other atoms. Further information regarding the assumptions and definitions for lipid classification and nomenclature can be found at Liebisch *et al.* J. Lipid Res. 2020, 61, 1–17 (https://doi.org/10.1194/jlr.S120001025).

All compounds identified in tiers 1, 2 and 3 were combined for normalization and statistical analysis (Supplemental Table 1). Unidentified features were saved for further investigation (Supplemental Table 2) but not employed for statistics.

2.6 Data Normalization

Identified features were normalized by internal standards and the median intensity ratio. First, data normalization of identified features was performed by using a set of 15 deuterated internal standards belonging to different lipid classes (NovaMT LipidRep Internal Standard Basic Mix for Tissue/Cells). The positively and putatively identified lipids were matched to one of the 15 internal standards according to lipid class similarity and expected retention time range for each class. Intensity ratios, *i.e.*, intensity of each lipid divided by intensity of the matched internal standard, were calculated for internal standard normalization. Second, the identified features were median-normalized, *i.e.*, the intensity ratios for each identified feature were divided by the median intensity ratio of all identified features within each sample experiment. The median and internal standard-normalized intensity ratios for the identified compounds are presented in Supplemental Table 1.

Unidentified features could not be matched to the most similar internal standard; hence, they were not normalized by internal standards nor employed for statistics. The detected peak intensities for unidentified features were median-normalized, *i.e.*, the absolute intensity for each feature was divided by the median intensity of each sample experiment. The median-normalized intensities for the unidentified features are presented in Supplemental Table 2.

2.7 Statistical Analysis

Statistical analysis was performed with NovaMT LipidScreener using Supplemental Table 1 formatted to proper .csv file. Supp. Table 1 contains peak intensity ratios for identified compounds, *i.e.*, peak intensities normalized by internal standards and median. Non-informative features (*e.g.*., internal standards, common contaminants and features with low experimental reproducibility) were filtered out during data processing.

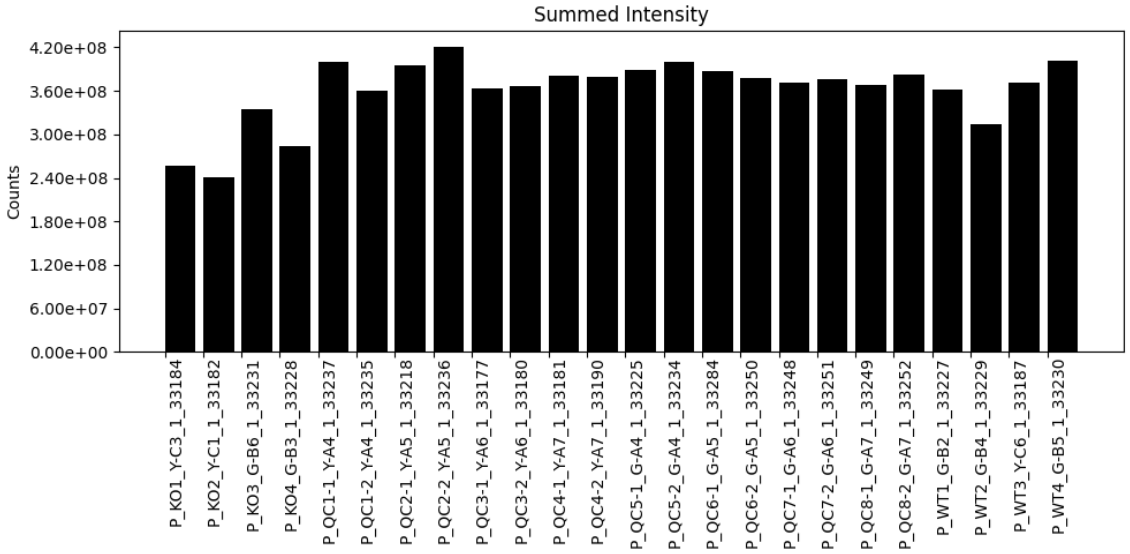
For multivariate statistics, features with near-constant values between the groups (the 30% features with the lowest relative standard deviation for all samples) were filtered out after uploading Supp. Table 1 to NovaMT LipidScreener. The dataset was also auto-scaled. No other filtering, normalization, transformation, or scaling methods were employed before multivariate statistical analysis.

For univariate statistics, no extra filtering or data scaling methods were applied.

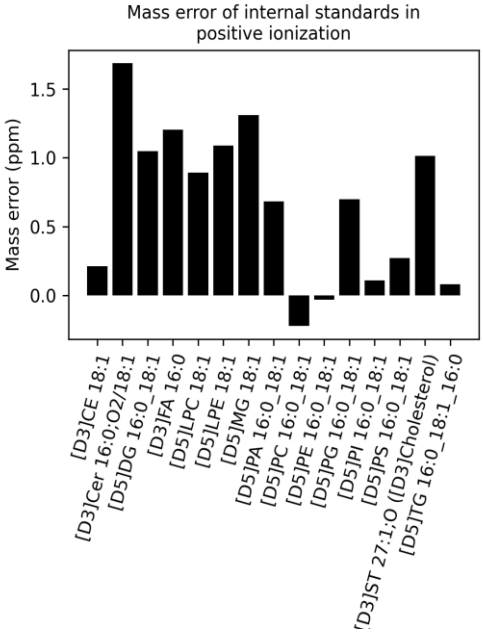
3 Results

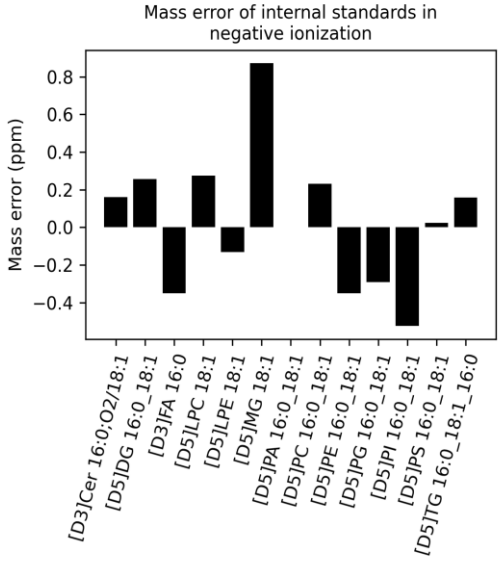
3.1 Data Quality Check

The summed raw peak intensities for all detected features in each sample experiment (*i.e.*, each extraction after combining the features detected with positive and negative ionization) were calculated to gauge the data acquisition quality. The detected peak intensities for each identified feature were further normalized by the internal standards and to the median intensity ratio (Supplemental Table 1) prior to statistical analysis to correct for any variations.



The detected internal standards were used to check mass accuracy. Amongst the 15 deuterated lipids added to each sample and QC before lipid extraction (NovaMT LipidRep Internal Standard Basic Mix for Tissue/Cells), 15 internal standards were detected in positive ionization and 13 internal standards were detected in negative ionization. The maximum mass error was 1.69 ppm or 0.91 mDa for positive ionization, and 0.84 ppm or 0.35 mDa for negative ionization, showing good mass accuracy for data acquisition.





3.2 Feature Detection

LC-MS data from 24 injections (singlet experiments from 8 samples, *i.e.*, 8 sample injections; and duplicate injections from 8 QC aliquots, *i.e.*, 16 QC injections) in positive and negative ionization were processed. Data acquired under positive and negative ionization from each experiment were combined. Features detected in at least 80% of injections in any group were aligned. Less commonly detected features were filtered out to ensure data quality. An average of 5333 ± 202 features per sample analysis were aligned and employed for identification.

Table 1. Number of detected features for each sample and QC experiment.

Experiment Code	Sample Name	Group	Number of Features
1	KO1	KO	5039
2	KO2	KO	4988
3	KO3	KO	5375
4	KO4	KO	5236
5	QC1-1	QC	5373
6	QC1-2	QC	5366
7	QC2-1	QC	5394
8	QC2-2	QC	5389
9	QC3-1	QC	5377
10	QC3-2	QC	5365
11	QC4-1	QC	5365
12	QC4-2	QC	5390
13	QC5-1	QC	5339
14	QC5-2	QC	5374
15	QC6-1	QC	5395
16	QC6-2	QC	5370
17	QC7-1	QC	5375
18	QC7-2	QC	5334
19	QC8-1	QC	5278
20	QC8-2	QC	5381
21	WT1	WT	5380
22	WT2	WT	5356
23	WT3	WT	5373
24	WT4	WT	5377

Two supplemental tables showing the list of features were generated with this report.

Supplemental Table 1 contains the list of identified and normalized features along with the peak intensity ratios for each sample. The features are identified using a three-tier ID approach (see below). The intensities detected for identified lipids were normalized by (1) the intensity of the most similar deuterated internal standard, and (2) the median intensity ratio within each sample experiment. Supplemental Table 1 can be used for further statistical analysis after the application of the data filtering and scaling approaches required by the chosen statistical models (*e.g.*, autoscaling for Principal Component Analysis - PCA).

Supplemental Table 2 contains the list of unidentified features. The intensities detected for unidentified lipids were normalized by the median intensity ratio within each sample experiment. Supplemental Table 2 is typically not employed for statistical analysis but contains valuable information that can be used in the future.

3.3 Lipid Identification and Normalization

A three-tier ID approach was used to perform lipid identification using NovaMT LipidScreener. A nine-tier filtering and scoring approach, including a retention time filter based on lipid classes and fatty acyl groups, was used for features that were matched to more than one lipid to determine the best matching results.

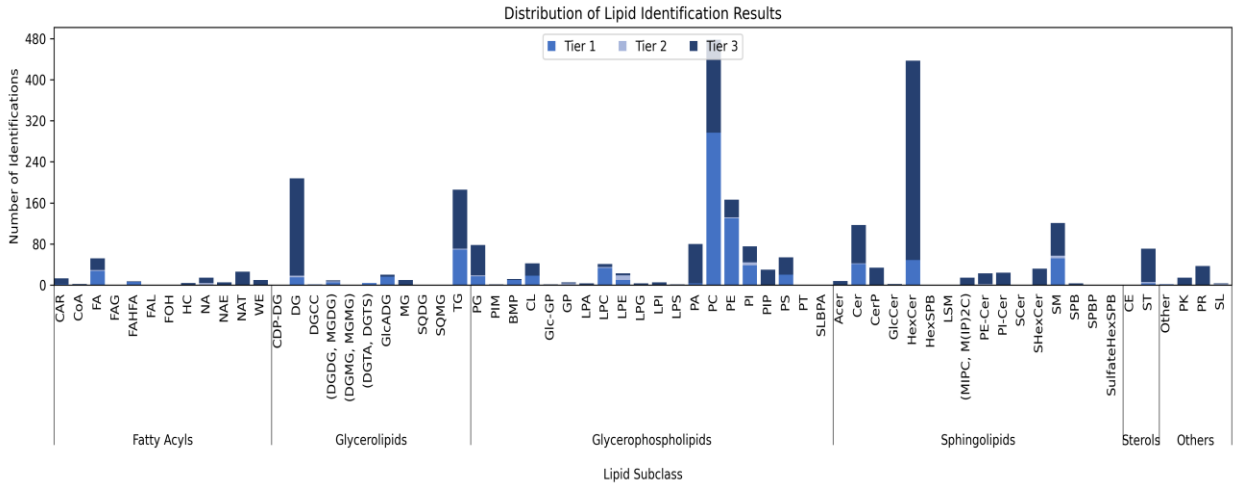
First, features were identified in Tier 1 using MS/MS spectral similarity match (MS/MS similarity score ≥500) and a precursor mass filter (m/z tolerance of 20.0 ppm and 5.0 mDa). Second, the unidentified features were identified in Tier 2 using MS/MS similarity score <500 and mass match (m/z tolerance of 20.0 ppm and 5.0 mDa). Third, the unidentified features were mass-matched to a database of lipids in Tier 3 (m/z tolerance of 20.0 ppm and 5.0 mDa).

Amongst the 5370 unique peaks detected after data processing, 2611 peaks were identified (Supp. Table 1). The identified compounds were normalized by the most similar internal standard (*i.e.*, detected peak intensity divided by the intensity of the most similar internal standard) and the median intensity ratio (*i.e.*, internal standard-normalized intensity ratio divided by the median value within the sample experiment). The internal standard and median-normalized intensity ratios were employed for statistical analysis after application of the appropriate filtering and scaling approaches (Supp. Table 1).

In Tier 1, 867 features were identified with MS/MS match score threshold of 500 and a precursor m/z error threshold of 20.0 ppm and 5.0 mDa (molecular species or species level).

In Tier 2, 51 additional features were identified with MS/MS match score <500 and a precursor m/z error threshold of 20.0 ppm and 5.0 mDa (molecular species or species level).

In Tier 3, the remaining features were searched on NovaMT LipidScreener for putative mass match. 1693 features were putatively identified in Tier 3 at the species level with m/z error threshold of 20.0 ppm and 5.0 mDa.



The abbreviations used for each lipid subclass are described in Table 2. The classification and shorthand notation of lipids followed the guidance of LipidMaps (https://www.lipidmaps.org/shorthand_nomenclature), MSDial (http://prime.psc.riken.jp/compms/msdial/lipidnomenclature.html), and Liebisch *et al*. J. Lipid Res. 2020, 61, 1–17 (DOI: https://doi.org/10.1194/jlr.S120001025).

Table 2. List of lipid subclass abbreviations.

Classification (Category – Main Class – Subclass)	Abbreviation
Sphingolipids - Ceramides - Acylceramides	ACer
Glycerophospholipids - Glycerophosphoglycerols - Monoacylglycerophosphomonoradylglycerols	BMP
Fatty Acyls - Fatty esters - Fatty acyl carnitines	Car
Glycerophospholipids - Cytidine-5'-diphosphate (CDP)-Glycerols - CDP-diacylglycerols	CDP-DG
Sterol Lipids - Sterols - Steryl esters	CE
Sphingolipids - Ceramides - N-acylsphingosines (ceramides)	Cer
Sphingolipids - Ceramides - Ceramide 1-phosphates	CerP
Glycerophospholipids - Glycerophosphoglycerophosphoglycerols - Monoacylglycerophosphoglycerophosphomonoradylglycerols (Cardiolipins)	CL
Fatty Acyls - Fatty esters - Fatty acyl CoAs	CoA
Glycerolipids - Diradylglycerols - Diacylglycerols	DG
Glycerolipids - Other Glycerolipids (hydroxymethyl-choline)	DGCC
Glycerolipids - Glycosyldiradylglycerols – Glycosyldiacylglycerols (Digalactosyldiacylgylcerols)	DGDG
Glycerolipids - Glycosylmonoradylglycerols – Glycosylmonoacylglycerols (Digalactosylmonoacylgylcerols)	DGMG
Glycerolipids - Other Glycerolipids (trimethyl-alanine)	DGTA
Glycerolipids - Other Glycerolipids (trimethyl-homoserine)	DGTS
Fatty Acyls - Fatty Acids and Conjugates - Fatty acids	FA
Fatty Acyls - Fatty acyl glycosides - Fatty acyl glycosides of mono- and disaccharides	FAG
Fatty Acyls - Fatty esters - Fatty acid estolides	FAHFA
Fatty Acyls - Fatty aldehydes	FAL
Fatty Acyls - Fatty alcohols	FOH
Glycerolipids - Glycosyldiradylglycerols - Glycosyldiacylglycerols	GlcADG
Sphingolipids - Acidic glycosphingolipids - Glucuronosphingolipids	GlcCer
Glycerophospholipids - Glycosylglycerophospholipids - Diacylglycosylglycerophospholipids	Glc-GP
Glycerophospholipids - Other Glycerophospholipids	GP
Fatty Acyls - Hydrocarbons	HC
Sphingolipids - Glycosphingolipids - Hexosyl ceramides	HexCer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (2 hexosyl groups)	Hex2Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (3 hexosyl groups)	Hex3Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (4 hexosyl groups)	Hex4Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (5 hexosyl groups)	Hex5Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (6 hexosyl groups)	Hex6Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (7 hexosyl groups)	Hex7Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (8 hexosyl groups)	Hex8Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (9 hexosyl groups)	Hex9Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (10 hexosyl groups)	Hex10Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (11 hexosyl groups)	Hex11Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (12 hexosyl groups)	Hex12Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (13 hexosyl groups)	Hex13Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (14 hexosyl groups)	Hex14Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (15 hexosyl groups)	Hex15Cer
Sphingolipids - Glycosphingolipids - Hexosyl ceramides (20 hexosyl groups)	Hex20Cer
Sphingolipids - Neutral glycosphingolipids - Hexosyl sphingoid bases	HexSPB
Glycerophospholipids - Glycerophosphates - Monoacylglycerophosphates (lysophosphatidic acids)	LPA
Glycerophospholipids - Glycerophosphocholines - Monoacylglycerophosphocholines (lysophosphatidylcholines)	LPC
Glycerophospholipids - Glycerophosphoethanolamines - Monoacylglycerophosphoethanolamines (lysophosphatidylethanolamines)	LPE

Classification (Category – Main Class – Subclass)	Abbreviation
Glycerophospholipids - Glycerophosphoglycerols - Monoacylglycerophosphoglycerol (lysophosphatidylglycerol)	LPG
Glycerophospholipids - Glycerophosphoinositols - Monoacylglycerophosphoinositols (lysophosphatidylinositols)	LPI
Glycerophospholipids - Glycerophosphoinositolglycans - N-acylglycerophosphoinositolglycans	LPIM
Glycerophospholipids - Glycerophosphoserines - Monoacylglycerophosphoserines (lysophosphatidylserines)	LPS
Sphingolipids - Sphingoid bases - Lysosphingomyelins and lysoglycosphingolipids	LSM
Sphingolipids - Phosphosphingolipids - Ceramide phosphoinositols	M(IP)2C
Glycerolipids - Monoradylglycerols - Monoacylglycerols	MG
Glycerolipids - Glycosyldiradylglycerols – Glycosyldiacylglycerols (Monogalactosyldiacylgylcerols)	MGDG
Glycerolipids - Glycosylmonoradylglycerols - Glycosylmonoacylglycerols (Monogalactosylmonoacylgylcerols)	MGMG
Sphingolipids - Phosphosphingolipids - Ceramide phosphoinositols	MIPC
Fatty Acyls - Fatty Acids and Conjugates - Nitrogenated fatty acids (primary amides, N-acyl amides, fatty nitriles and others)	NA
Fatty Acyls - Fatty amides - N-acyl ethanolamines (endocannabinoids)	NAE
Glycerophospholipids - Glycerophosphoethanolamines - Diacylglycerophosphoethanolamines	NAPE
Fatty Acyls - Fatty amides - N-acyl amines (taurines)	NAT
Glycerophospholipids - Glycerophosphates – Diacylglycerophosphates (Phosphatidic Acids)	PA
Glycerophospholipids - Glycerophosphocholines – Diacylglycerophosphocholines (Phosphatidylcholines)	PC
Glycerophospholipids - Glycerophosphoethanolamines – Diacylglycerophosphoethanolamines (Phosphatidylethanolamines)	PE
Sphingolipids - Phosphosphingolipids - Ceramide phosphoethanolamines	PE-Cer
Glycerophospholipids - Other Glycerophospholipids	PE-isoK
Glycerophospholipids - Glycerophosphoethanolamines – Diacylglycerophosphoethanolamines (N-methylethanolamine or N,N-dimethylethanolamine)	PE-NMe
Glycerophospholipids - Glycerophosphoglycerols - Monoacylglycerophosphoglycerols (Phosphatidylglycerols)	PG
Glycerophospholipids - Glycerophosphoglycerophosphates - Diacylglycerophosphoglycerophosphates	PGP
Glycerophospholipids - Other Glycerophospholipids (sulfate)	PGS
Glycerophospholipids - Glycerophosphoinositols – Diacylglycerophosphoinositols (Phosphatidylinositols)	PI
Sphingolipids - Phosphosphingolipids - Ceramide phosphoinositols	PI-Cer
Glycerophospholipids - Glycerophosphoinositolglycans - Diacylglycerophosphoinositolglycans	PIM
Glycerophospholipids - Glycerophosphoinositol monophosphates	PIP
Polyketides	PK
Glycerophospholipids - Glycerophosphonocholines - Diacylglycerophosphonocholines	PnC
Glycerophospholipids - Glycerophosphonoethanolamines - Diacylglycerophosphonoethanolamines	PnE
Glycerophospholipids - Glyceropyrophosphates - Diacylglyceropyrophosphates	PPA
Prenol Lipids	PR
Glycerophospholipids - Glycerophosphoserines – Diacylglycerophosphoserines (Phosphatidylserines)	PS
Glycerophospholipids - Glycerophosphoserines - Triacylglycerophosphoserines	PS-NAc
Glycerophospholipids - Other Glycerophospholipids	PT
Sphingolipids - Other Sphingolipids - Sulfoceramides	SCer
Sphingolipids - Acidic glycosphingolipids - Sulfoglycosphingolipids (sulfatides)	SHexCer
Saccharolipids	SL
Glycerophospholipids - Glycerophosphoglycerols - Diacylglycerophosphomonoradylglycerols	SLBPA
Sphingolipids - Phosphosphingolipids - Ceramide phosphocholines (sphingomyelins)	SM
Sphingolipids - Sphingoid bases	SPB
Sphingolipids - Sphingoid bases - Sphingoid base-1 phosphates	SPBP
Glycerolipids - Glycosyldiradylglycerols – Glycosyldiacylglycerols (Sulfoquinovosyldiacylglycerols)	SQDG
Glycerolipids - Glycosylmonoradylglycerols – Glycosylmonoacylglycerols (Sulfoquinovosylmonoacylglycerols)	SQMG
Sterol Lipids	ST
Sphingolipids - Amphoteric glycosphingolipids - Sulfohexosyl sphingoid bases	SulfateHexSPB
Glycerolipids - Triradylglycerols - Triacylglycerols	TG
Fatty Acyls - Fatty esters - Wax esters and diesters	WE

The combined Tier 1 and Tier 2 identification results are shown below (Table 3). The complete list of identified features is shown in Supplemental Table 1. The list of unidentified features is shown in Supplemental Table 2. The abbreviations used for lipid subclasses are described in Table 2.

Table 3. Lipids identified by MS/MS spectral match (Tiers 1 and 2).

Lipid	Subclass	Lipid	Subclass
LPC 22:6	*LPC*	*HexCer 14:2;O2/28:2*	*HexCer*
LPC 14:0	*LPC*	*TG 12:1_19:2_19:2*	*TG*
LPC 20:4	*LPC*	*PC 21:0_21:0*	*PC*
LPC 22:5	*LPC*	*TG 14:0_16:0_16:1*	*TG*
PC O-14:1_2:0	*PC*	*SM 30:1;O2(FA 28:1)*	*SM*
LPC 18:2	*LPC*	*TG 12:1_19:1_19:1*	*TG*
PC O-18:3_2:0	*PC*	*TG 15:0_18:1_15:0(d7)*	*TG*
LPC 22:4	*LPC*	*TG 16:0_16:0_18:0*	*TG*
LPC 16:0	*LPC*	*DG 13:0_27:0*	*DG*
PC O-14:1_4:0	*PC*	*DG 31:3*	*DG*
LPC O-16:1	*LPC*	*PC O-14:0_18:4*	*PC*
LPC 22:3	*LPC*	*PE 14:0_22:3*	*PE*
LPC O-13:1	*LPC*	*PC 22:5_22:5*	*PC*
LPE O-18:2	*LPE*	*PC 13:0_16:1*	*PC*
LPC O-16:0	*LPC*	*PI 40:4*	*PI*
LPC O-18:1	*LPC*	*PE 12:0_26:4*	*PE*
PC O-14:0_4:0	*PC*	*PC 11:0_26:4*	*PC*
MGDG O-8:0_24:3	*MGDG*	*TG 13:1_18:2_18:2*	*TG*
LPE O-21:1	*LPE*	*TG 12:0_14:0_18:0*	*TG*
LPC O-15:1	*LPC*	*DGTS 5:0_26:1*	*DGTS*
LPC O-18:0	*LPC*	*DGTS 7:0_26:1*	*DGTS*
MGDG O-9:0_24:3	*MGDG*	*TG 16:0_18:0_18:0*	*TG*
LPC O-20:1	*LPC*	*TG 14:1_22:1_22:1*	*TG*
BMP 22:4_22:6	*BMP*	*TG 16:0_18:0_22:1*	*TG*
BMP 18:1_22:6	*BMP*	*TG 18:0_18:0_18:0*	*TG*
BMP 20:2_22:6	*BMP*	*LPE O-21:2*	*LPE*
SM 14:0;O2/18:2	*SM*	*TG 8:0_9:0_16:2*	*TG*
BMP 18:1_20:4	*BMP*	*SM 54:11;2O*	*SM*
BMP 18:1_22:5	*BMP*	*TG 18:2_18:2_18:2*	*TG*
PI 38:6	*PI*	*TG O-8:0_22:1_22:1*	*TG*
LPC O-22:0	*LPC*	*TG O-18:0_18:1_22:4*	*TG*
LPC O-24:1	*LPC*	*TG 14:1_22:4_22:4*	*TG*
PI 40:7	*PI*	*PC O-14:0_16:1*	*PC*
SM 14:1;O2/17:0	*SM*	*Hex2Cer 16:1;O2/25:0*	*HexCer*
PC 22:6_22:6	*PC*	*DG 35:1*	*DG*
PC 18:3_18:3	*PC*	*PI 34:2*	*PI*
PI 36:4	*PI*	*PC 4:0_26:1*	*PC*
TG 12:0_12:3_20:6	*TG*	*PI 36:3*	*PI*
PI 38:5	*PI*	*PC 18:1_26:2*	*PC*
PC 14:0_22:6	*PC*	*PC 24:1_26:4*	*PC*
PI 40:6	*PI*	*DG 18:1_24:1*	*DG*
PC 10:0_22:3	*PC*	*PE O-14:1_4:0*	*PE*
PC 20:4_22:6	*PC*	*LPC 20:1*	*LPC*
PC 16:1_22:6	*PC*	*TG 14:0_16:0_18:1*	*TG*
HexCer 16:3;O2/12:1;O	*HexCer*	*LPE 20:0*	*LPE*
PG 18:1_20:4	*PG*	*PC O-14:0_12:0*	*PC*
PC 22:5_22:6	*PC*	*PE 14:1_26:4*	*PE*
PC 12:0_16:0	*PC*	*GlcADG 27:0_17:1*	*GlcADG*
SM 14:0;O2/18:1	*SM*	*TG 12:0_18:2_18:2*	*TG*
PG 16:0_20:2	*PG*	*TG 12:0_18:0_22:1*	*TG*
PC 14:0_20:4	*PC*	*PG 16:0_18:1*	*PG*
PC 16:4_24:4	*PC*	*DGTS 6:0_27:0*	*DGTS*
SM 17:2;O2/17:0	*SM*	*PG 16:3_24:4*	*PG*
PC 14:0_22:5	*PC*	*SM 14:0;O2/34:1*	*SM*
PC 20:4_22:5	*PC*	*SM 50:1;O2*	*SM*
PC 22:4_22:6	*PC*	*SM 14:0;O2/38:2*	*SM*
PE 20:3_20:3	*PE*	*TG 12:0_21:1_21:1*	*TG*
PC 11:0_22:5	*PC*	*SE 28:2/18:2*	*ST*
PC 17:1_22:6	*PC*	*CAR 15:2*	*CAR*

Lipid	Subclass	Lipid	Subclass
SM 14:0;O2/18:0	*SM*	*DG 16:0_16:1*	*DG*
PC 16:3_24:4	*PC*	*TG 16:0_18:1_19:1*	*TG*
PI 40:5	*PI*	*PE O-14:1_26:2*	*PE*
PI 38:4	*PI*	*DGTS 22:1_22:1*	*DGTS*
SM 14:0;O2/19:1	*SM*	*TG 18:1_18:2_18:2*	*TG*
AcylGlcADG 20:3_22:6_22:6	*GlcADG*	*TG 12:0_14:0_14:0*	*TG*
SM 34:1;O3	*SM*	*TG 12:1_21:2_21:2*	*TG*
PI 36:2	*PI*	*GlcADG 27:0_19:2*	*GlcADG*
PC 7:0_26:2	*PC*	*LPE 22:6*	*LPE*
PC 16:0_16:2	*PC*	*LPE 20:4*	*LPE*
PC 18:2_18:2	*PC*	*LPC 16:1*	*LPC*
PC 16:0_22:6	*PC*	*LPE 22:5*	*LPE*
PC 18:1_22:6	*PC*	*LPC 20:3*	*LPC*
PC 18:1_16:2	*PC*	*LPC 18:1*	*LPC*
SM 14:0;O2/20:1	*SM*	*FA 22:6*	*FA*
PC 16:4_26:4	*PC*	*LPE O-16:1*	*LPE*
PC 14:1_24:4	*PC*	*FA 20:4*	*FA*
PC 13:0_22:6	*PC*	*FA 22:5*	*FA*
PC 14:0_16:0	*PC*	*FA 16:1*	*FA*
PC 14:0_18:1	*PC*	*LPC 18:0*	*LPC*
NAGlySer 22:6/19:2	*NA*	*FA 20:3*	*FA*
NAGlySer 26:7/17:2	*NA*	*FA 17:1*	*FA*
GlcADG 14:0_26:4	*GlcADG*	*FA 22:4*	*FA*
PC 16:1_18:1	*PC*	*LPE O-18:1*	*LPE*
PC 16:2_24:4	*PC*	*FA 16:0*	*FA*
PC 18:1_18:4	*PC*	*FA 21:3*	*FA*
PE O-20:5_21:2	*PE*	*FA 18:1*	*FA*
PEtOH 16:1_20:4	*GP*	*FA 24:5*	*FA*
PC 16:0_16:1	*PC*	*FAHFA 18:1/20:3*	*FAHFA*
PC 24:4_18:5	*PC*	*FAHFA 3:0/18:3*	*FAHFA*
PMeOH 16:0_22:5	*GP*	*FA 20:2*	*FA*
PC 15:1_20:4	*PC*	*FA 22:3*	*FA*
PC O-18:2_22:6	*PC*	*FA 19:1*	*FA*
PC 15:0_16:0	*PC*	*FA 24:4*	*FA*
GlcADG 12:0_24:1	*GlcADG*	*FA 18:0*	*FA*
PC 18:1_18:2	*PC*	*FAHFA 18:0/20:2*	*FAHFA*
PI 38:3	*PI*	*FA 20:1*	*FA*
GlcADG 12:0_26:2	*GlcADG*	*FA 22:2*	*FA*
PC O-18:5_17:2	*PC*	*FA 28:7*	*FA*
PC O-16:0_22:6	*PC*	*FA 24:3*	*FA*
SM 14:0;O2/20:0	*SM*	*PI 16:0_22:6*	*PI*
PE O-18:2_22:6	*PE*	*PI 18:1_22:6*	*PI*
PC O-18:1_22:6	*PC*	*FA 22:1*	*FA*
PEtOH 14:0_20:1	*GP*	*PG 18:1_22:6*	*PG*
PC O-14:0_22:5	*PC*	*PI 16:0_20:4*	*PI*
PC 16:0_20:3	*PC*	*FA 24:2*	*FA*
SM 14:0;O2/21:1	*SM*	*PI 18:1_20:4*	*PI*
PC 16:0_17:1	*PC*	*PI 14:1_26:2*	*PI*
PC 18:1_20:3	*PC*	*PI 17:1_17:1*	*PI*
PC 13:1_26:4	*PC*	*PG 18:1_22:4*	*PG*
PC O-14:0_16:0	*PC*	*PI 18:1_22:5*	*PI*
PC 17:1_18:1	*PC*	*PC 18:3_20:4*	*PC*
PC O-18:4_15:1	*PC*	*PG 8:0_26:1*	*PG*
PC O-14:0_22:4	*PC*	*PI 18:1_18:2*	*PI*
PC O-14:0_18:1	*PC*	*PG 18:1_18:1*	*PG*
PC O-18:4_17:2	*PC*	*PE 16:0_18:1;O2*	*PE*
PC O-14:1_24:4	*PC*	*PE 16:1_22:6*	*PE*
PC 18:0_22:6	*PC*	*PC 20:4_20:4*	*PC*
PC 16:0_22:4	*PC*	*PI 14:0_22:3*	*PI*
PC 18:1_22:4	*PC*	*PC 16:1_20:4*	*PC*
PC 15:0_22:6	*PC*	*PI 17:0_20:4*	*PI*
PC O-14:0_20:2	*PC*	*PI 18:1_20:3*	*PI*
PC 5:0_27:0	*PC*	*PI 13:1_26:4*	*PI*
NAGlySer 13:1/26:4	*NA*	*PI O-12:0_26:5*	*PI*
PC 18:0_20:4	*PC*	*PC 16:1_22:5*	*PC*
GlcADG 12:0_24:0	*GlcADG*	*PE 16:1_20:4*	*PE*
PC 18:1_18:1	*PC*	*PC 14:1_18:1*	*PC*
PC 14:1_26:4	*PC*	*PI 18:0_22:6*	*PI*

Lipid	Subclass	Lipid	Subclass
GlcADG 12:0_26:1	*GlcADG*	*Hex3Cer 34:1;O2*	*HexCer*
PE O-18:1_21:2	*PE*	*PI 18:1_22:4*	*PI*
PC 15:0_20:4	*PC*	*PE 18:0_20:3;O2*	*PE*
PC 13:0_18:1	*PC*	*PI 8:0_26:1*	*PI*
PC 15:0_22:5	*PC*	*AcylGlcADG 22:4_22:6_20:5*	*GlcADG*
PC 18:1_20:2	*PC*	*FA 24:1*	*FA*
PC 15:1_18:1	*PC*	*PI 18:1_18:1*	*PI*
PC 17:0_18:1	*PC*	*Hex2Cer 34:1;O2*	*HexCer*
PE O-18:1_22:6	*PE*	*Cer 16:0;O2/16:1;O*	*Cer*
PC O-16:2_24:4	*PC*	*PS 16:0_18:1*	*PS*
PC O-18:3_17:2	*PC*	*PA 21:1_22:6*	*PA*
PE O-18:4_23:0	*PE*	*Cer 18:2;O2/16:0;O*	*Cer*
PE O-14:0_21:1	*PE*	*PS 18:0_22:5*	*PS*
PC O-14:1_26:4	*PC*	*PE 16:0_22:6*	*PE*
PE O-16:2_24:4	*PE*	*PE 16:0_18:0;O2*	*PE*
PC 16:0_17:0	*PC*	*PE 18:1_22:6*	*PE*
PC 16:0_19:1	*PC*	*PS 21:1_18:2*	*PS*
PC 18:0_20:3	*PC*	*PE 18:1_18:0;O2*	*PE*
PE O-16:0_21:2	*PE*	*PI 18:0_20:3*	*PI*
PC 18:1_19:1	*PC*	*PS 21:0_20:4*	*PS*
PC 18:1_22:3	*PC*	*PS 21:0_16:1*	*PS*
PC 13:1_24:4	*PC*	*PC 18:1_22:5*	*PC*
PC O-14:0_22:3	*PC*	*PI 20:2_20:2*	*PI*
PC O-14:0_18:0	*PC*	*PE 16:0_20:4*	*PE*
PC O-14:0_20:1	*PC*	*PE 18:1_20:4*	*PE*
PC O-14:0_24:4	*PC*	*PE 14:0_18:1*	*PE*
PE O-18:0_22:6	*PE*	*PC O-16:1_20:4*	*PC*
PC O-14:0_22:2	*PC*	*PS 18:1_21:1*	*PS*
PC 18:0_22:4	*PC*	*PE 16:1_20:2*	*PE*
PC 18:1_21:2	*PC*	*PS 16:0_20:0*	*PS*
PC 14:1_24:2	*PC*	*PI 18:0_18:1*	*PI*
PC 16:1_26:4	*PC*	*PE O-16:0_18:0;O2*	*PE*
DG 16:1_18:1	*DG*	*PS 19:0_19:1*	*PS*
PE O-14:0_20:1	*PE*	*PS 18:0_22:4*	*PS*
LPC 36:0	*LPC*	*PC O-16:0_20:4*	*PC*
PC 22:3_22:3	*PC*	*PE 18:0_18:0;O*	*PE*
PC 15:0_22:4	*PC*	*PI 19:1_19:1*	*PI*
PC 15:0_20:3	*PC*	*PE O-16:1_22:5*	*PE*
NAGlySer 13:0/26:4	*NA*	*PG 18:0_18:1*	*PG*
GlcADG 12:0_26:0	*GlcADG*	*PC O-16:0_22:5*	*PC*
SM 14:0;O2/26:2	*SM*	*Cer 18:1;O2/16:0;O*	*Cer*
PC 10:0_26:1	*PC*	*PS 18:0_18:1*	*PS*
DGDG 10:0_22:3	*DGDG*	*PE O-18:5_21:2*	*PE*
SM 14:1;O2/28:2	*SM*	*PE 18:0_22:6*	*PE*
PE O-14:0_24:1	*PE*	*PE 16:0_22:4*	*PE*
PC O-14:0_19:0	*PC*	*PE 18:1_22:4*	*PE*
PC O-14:1_24:2	*PC*	*PC 16:1_20:1*	*PC*
PC 15:0_18:1	*PC*	*PC 18:0_22:5*	*PC*
PE O-16:0_22:4	*PE*	*PE 18:0_16:1*	*PE*
PC O-14:0_26:4	*PC*	*PE 38:4*	*PE*
PC O-18:3_19:2	*PC*	*PE 18:0_22:5*	*PE*
PC 11:0_26:1	*PC*	*PE 36:2*	*PE*
ST 24:1;O4;G/14:1	*ST*	*FA 40:5*	*FA*
PE 11:0_27:0	*PE*	*PS 16:0_22:0*	*PS*
PC O-16:2_26:4	*PC*	*LPE 40:7*	*LPE*
PE O-18:0_22:5	*PE*	*PE O-16:1_22:4*	*PE*
Hex3Cer 16:0;O2/26:2	*HexCer*	*PC O-16:0_22:4*	*PC*
PC 21:2_21:2	*PC*	*PC O-18:1_22:4*	*PC*
SM 14:0;O2/26:1	*SM*	*LPE 40:6*	*LPE*
SM 14:1;O2/27:1	*SM*	*PC 16:0_22:3*	*PC*
PC 18:0_22:3	*PC*	*PC O-16:1_18:1*	*PC*
PE 21:1_21:1	*PE*	*Hex3Cer 42:3;O2*	*HexCer*
PC 16:0_26:4	*PC*	*PS 23:0_17:1*	*PS*
PE O-14:0_26:4	*PE*	*PE O-18:0_18:0;O2*	*PE*
PC O-14:0_20:0	*PC*	*PC O-18:1_16:0*	*PC*
PC O-14:0_22:1	*PC*	*PE 18:0_20:3*	*PE*
PC O-14:0_19:2	*PC*	*PE O-18:1_20:4*	*PE*
Hex2Cer 16:0;O2/26:2	*HexCer*	*LPE 34:2*	*LPE*

Lipid	Subclass	Lipid	Subclass
PC 13:1_24:2	PC	PE 17:0_18:1	PE
PC O-14:0_24:2	PC	PC O-18:1_18:1	PC
DG 14:0_18:0	DG	PE 18:1_22:3	PE
PC O-16:1_26:4	PC	PE O-18:2_18:1	PE
DG 18:0_16:1	DG	PC 20:1_22:4	PC
AHexCer (O-30:3)17:1;O2/24:0;O	HexCer	PE O-18:1_16:0	PE
DG 16:1_20:1	DG	Cer 18:1;O2/18:0;O	Cer
TG O-8:0_8:0_20:2	TG	PE O-18:3_21:2	PE
PC O-14:1_26:2	PC	PE 18:0_22:4	PE
Cer 14:3;O2/28:2;O	Cer	PC 16:0_18:0	PC
PC O-14:0_21:0	PC	PC 16:0_20:1	PC
PC 12:0_26:1	PC	PS 21:0_20:1	PS
PC 9:0_27:0	PC	PE O-18:1_22:5	PE
SM 8:0;O2/34:2	SM	PE 16:0_18:0	PE
HexCer 14:0;O2/28:2	HexCer	PE 18:1_20:1	PE
PC O-16:0_26:4	PC	PE O-18:2_22:3	PE
PC 14:0_26:2	PC	PC 18:1_22:2	PC
HexCer 22:0;O2/18:1	HexCer	PE-Cer 12:1;O2/25:1;O	PE-Cer
PC 16:1_26:2	PC	PS 16:0_24:0	PS
SM 14:1;O2/30:2	SM	FA 42:5	FA
PC 9:0_26:1	PC	PE-Cer 12:0;2O/21:2	PE-Cer
HexCer 16:0;O2/23:0;O	HexCer	Cer 15:3;O2/26:2;O	Cer
PC 11:0_26:2	PC	LPE 40:5	LPE
PE O-18:0_22:4	PE	Hex3Cer 42:2;O2	HexCer
PC O-20:3_21:1	PC	PC O-18:0_22:4	PC
TG O-8:0_8:0_22:3	TG	Hex3Cer 40:1;O2	HexCer
DG 37:7	DG	PS 21:0_21:1	PS
DG 16:0_19:1	DG	HexCer 15:0;O2/26:2	HexCer
PC 22:2_22:2	PC	PS 23:0_22:3	PS
PC 13:1_26:2	PC	PE 21:2_21:2	PE
ST 24:1;O4;G/14:0	ST	PC O-18:0_16:0	PC
SM 14:1;O2/29:1	SM	PC O-16:0_20:1	PC
PC O-20:3_26:4	PC	LPE 36:2	LPE
PC O-18:2_26:4	PC	SL 13:0;O/26:0;O	SL
SM 14:0;O2/28:1	SM	Hex2Cer 42:2;O2	HexCer
Hex3Cer 16:0;O2/26:1	HexCer	PE 18:0_22:3	PE
SM 14:0;O2/26:0	SM	PE O-18:2_20:1	PE
TG O-8:0_10:0_22:4	TG	Hex3Cer 42:1;O2	HexCer
PE 22:1_22:1	PE	PC O-16:0_22:2	PC
SM 14:0;O2/27:1	SM	PE O-16:0_20:1	PE
Cer 12:1;O2/44:12	Cer	PE O-18:2_22:2	PE
HexCer 14:0;O2/27:1	HexCer	Cer 16:0;O2/24:2;O	Cer
PC O-14:0_24:1	PC	Cer 18:1;O2/20:0;O	Cer
PC O-14:0_22:0	PC	PC 16:0_22:1	PC
PC O-14:0_26:2	PC	Hex3Cer 41:1;O2	HexCer
PE O-18:0_20:2	PE	PC 16:0_20:0	PC
Hex2Cer 16:0;O2/26:1	HexCer	SM 14:0;O2/28:2	SM
DG 16:0_18:0	DG	HexCer 14:0;O2/26:1	HexCer
DG 18:0_18:1	DG	PE 16:0_22:1	PE
DG 16:1_22:1	DG	PE 18:1_22:1	PE
Cer 18:1;O2/24:1;O	Cer	PE O-18:1_22:3	PE
PC 27:0_13:1	PC	Hex2Cer 41:1;O2	HexCer
PC 16:0_26:2	PC	Cer 14:0;O2/26:1;O	Cer
SM 14:0;O2/30:2	SM	PE 18:1_24:2	PE
SM 8:0;O2/34:1	SM	PE O-18:0_22:3	PE
HexCer 24:0;O2/18:1	HexCer	Hex3Cer 44:2;O2	HexCer
PC 18:1_21:1	PC	Cer 14:1;O2/27:1;O	Cer
Cer 18:0;O2/24:1;O	Cer	HexCer 14:0;O2/28:1	HexCer
PC O-20:3_26:2	PC	HexCer 14:2;O2/32:2	HexCer
PC O-18:0_26:4	PC	HexCer 15:0;O2/28:2	HexCer
SM 14:0;O2/28:0	SM	PE O-16:1_22:1	PE
Cer 18:1;O2/23:0;O	Cer	Cer 17:3;O2/36:7;O	Cer
PC O-14:0_26:1	PC	PE O-18:2_22:1	PE
PC O-16:0_26:2	PC	Hex2Cer 42:1;O2	HexCer
PC O-14:0_24:0	PC	PC O-18:1_22:1	PC
DG 16:0_22:1	DG	PE 27:0_18:3	PE
PC 27:0_15:1	PC	Hex3Cer 42:0;2O	HexCer
PC 20:0_20:0	PC	PE O-18:0_20:1	PE

Lipid	Subclass	Lipid	Subclass
PC 27:0_17:2	PC	PC 18:1_24:1	PC
Cer 18:1;O2/24:0;O	Cer	PC 16:0_24:1	PC
PC 21:0_18:1	PC	HexCer 18:1;O2/24:0	HexCer
PC 15:0_26:2	PC	PE 18:1_24:1	PE
PC O-16:1_25:0	PC	PE 22:0_18:1	PE
Cer 18:0;O2/24:0;O	Cer	Cer 17:0;O2/26:2;O	Cer
PC O-16:0_26:1	PC	Cer 14:0;O2/26:0;O	Cer
PC O-14:0_26:0	PC	HexCer 14:0;O2/28:0	HexCer
PC 27:0_17:1	PC	FA 18:1-ACer 14:0;O2/12:0	FA
CL 16:0_18:2_16:0_18:2	CL	PC O-16:0_24:1	PC
CL 14:0_22:3_16:0_18:2	CL	PC O-16:0_22:0	PC
CL 14:0_22:3_14:0_22:3	CL	PE O-18:1_22:1	PE
MGDG 15:1_26:4	MGDG	PC O-18:1_24:1	PC
CL 16:0_18:2_18:0_18:2	CL	PE O-18:0_22:1	PE
CL 14:0_22:3_18:0_18:2	CL	PC 16:0_26:1	PC
DG O-28:7_28:7	DG	Cer 18:1;O2/26:1;O	Cer
SM 30:1;O2(FA 18:3)	SM	PC 18:1_26:1	PC
SM 14:0;O2/25:0	SM	PE 24:0_18:1	PE
TG 12:0_20:0_22:6	TG	PE 18:1_26:1	PE
TG 12:0_20:2_20:2	TG	HBMP 18:0_18:1_18:1	BMP
TG 12:0_22:0_22:7	TG	Cer 14:0;O2/29:1;O	Cer
TG 14:0_22:1_22:7	TG	PC O-18:1_24:0	PC
TG 16:0_16:1_18:1	TG	CL 16:0_16:0_16:2_18:2	CL
TG 16:0_18:1_18:2	TG	CL 16:0_16:0_16:0_20:5	CL
TG 16:0_18:1_22:4	TG	CL 16:0_16:0_16:0_22:6	CL
TG 14:0_22:3_22:3	TG	PC 26:0_18:1	PC
TG 18:1_18:2_22:3	TG	PE 26:0_18:1	PE
TG 16:1_18:0_20:2	TG	Cer 14:0;O2/30:1;O	Cer
TG 16:0_16:1_20:1	TG	CL 16:1_18:1_16:1_18:1	CL
TG 18:0_18:1_22:4	TG	CL 16:0_16:0_16:0_22:5	CL
TG 16:0_22:2_22:4	TG	CL 14:0_18:1_18:1_18:1	CL
TG 16:0_18:1_20:1	TG	CL 16:1_18:1_18:1_18:1	CL
TG 16:0_18:0_18:1	TG	CL 18:1_18:1_18:1_18:2	CL
SE 28:1_18:1	ST	CL 16:0_18:1_16:0_18:1	CL
MGDG 27:0_21:1	MGDG	CL 18:1_18:1_18:1_18:1	CL
TG 16:0_18:0_20:1	TG	CL 16:0_16:0_16:0_22:3	CL
TG 18:0_18:1_20:1	TG	HexCer 17:0;O2/38:1;O	HexCer
TG O-16:0_18:1_22:3	TG	FA 18:2	FA
TG 16:1_20:1_22:1	TG	ST 27:1;O;S	ST
TG O-18:1_16:0_18:0	TG	LPE O-20:1	LPE
TG 18:0_18:1_22:1	TG	PI 16:0_20:3	PI
TG 18:1_20:1_22:1	TG	PE O-18:3_19:2	PE
LDGCC 12:0	DGCC	PC O-18:0_22:6	PC
LPC 17:1	LPC	PE O-18:2_20:2	PE
CAR 17:3	CAR	LPE 38:4	LPE
BMP 18:1_18:2	BMP	PC O-16:0_22:3	PC
PC 14:0_16:1	PC	PE O-16:1_22:2	PE
PC 16:1_18:2	PC	PS 22:1_20:3	PS
PC 14:0_15:0	PC	PE 20:0_18:1	PE
PC 5:0_26:1	PC	HexCer 14:0;O2/26:0	HexCer
PC 15:1_22:6	PC	HexCer 16:0;O2/26:0;O	HexCer
DGDG 2:0_24:1	DGDG	PE O-19:2_17:1	PE
Cer 16:0;O2/20:1;O	Cer	PE 27:0_16:1	PE
PC 18:1_20:1	PC	FAHFA 20:1/20:2	FAHFA
DGDG 4:0_26:2	DGDG	PA 3:0_26:1	PA
PC 14:1_26:2	PC	PE O-18:2_22:5	PE
Cer 20:1;O2/20:1;O	Cer	PE 14:0_18:0	PE
Cer 18:2;O2/24:1;O	Cer	PC O-18:1_20:4	PC
PE O-18:1_24:4	PE	Hex2Cer 42:3;O2	HexCer
Cer 11:1;O2/44:12	Cer	PC 18:1_22:1	PC
Cer 14:3;O2/44:12	Cer	PC 18:1_24:2	PC
PE 27:0_15:1	PE	Cer 14:1;O2/28:0	Cer
SM 14:3;O2/29:1	SM	PE O-14:0_22:2	PE
DG 16:0_24:2	DG	LPE 18:1	LPE
PC O-20:3_26:1	PC	FA 24:6	FA
PC O-18:0_26:2	PC	FAHFA 22:6/3:0	FAHFA
TG 12:0_13:0_16:0	TG	PG 6:0_26:1	PG
CL 16:0_18:1_16:0_18:2	CL	PG 17:1_17:1	PG

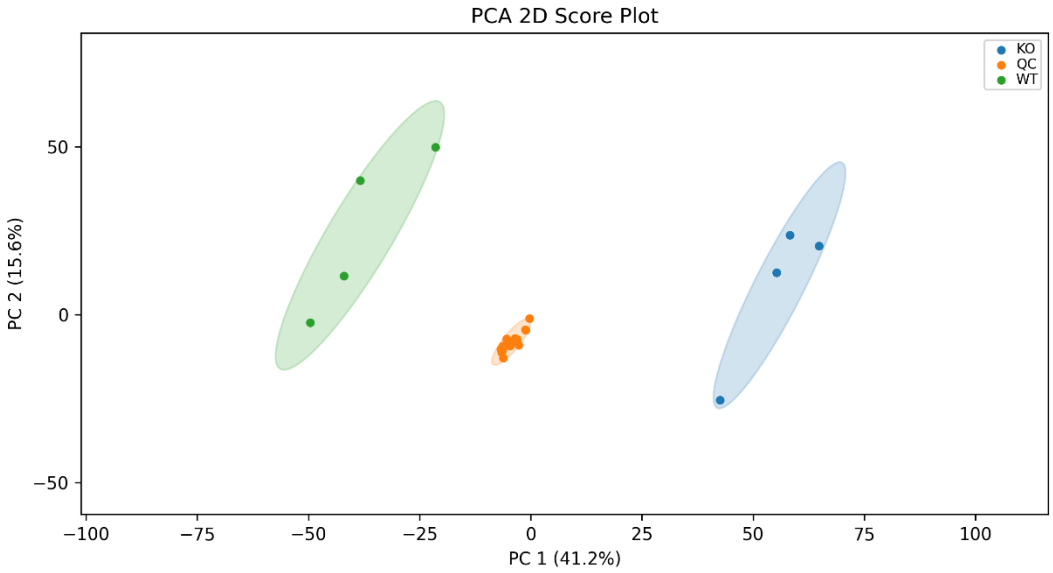
Lipid	Subclass	Lipid	Subclass
TG 16:1_18:1_18:2	*TG*	*PG 18:1_20:2*	*PG*
TG 16:0_18:1_20:3	*TG*	*Cer 14:0;O2/20:0;O*	*Cer*
TG O-12:0_22:2_22:2	*TG*	*PE 18:1_20:2*	*PE*
TG O-14:0_22:0_22:5	*TG*	*PC 15:0_22:1*	*PC*
TG 12:0_16:5_16:5	*TG*	*Cer 14:1;O2/28:1*	*Cer*
PC 16:1_16:1	*PC*	*PE O-16:2_26:4*	*PE*
Hex3Cer 16:0;O2/18:1	*HexCer*	*Cer 22:0;O2/18:2;O*	*Cer*
PC O-14:0_21:2	*PC*	*PI 18:0_22:4*	*PI*
TG O-8:0_8:0_18:2	*TG*	*PC O-18:0_20:4*	*PC*
GlcADG 12:0_25:0	*GlcADG*	*PS 20:1_20:1*	*PS*
Cer 14:2;O2/28:2;O	*Cer*	*PE O-18:3_24:4*	*PE*
PC O-18:1_26:4	*PC*	*PE O-16:0_22:2*	*PE*
PC O-16:0_23:0	*PC*	*PC 2:0_27:0*	*PC*
TG 16:0_16:1_18:2	*TG*	*PI 20:1_20:4*	*PI*
PC O-14:0_23:0	*PC*	*PS 20:3_20:3*	*PS*
GlcADG 23:0_23:0	*GlcADG*	*PC O-16:1_22:6*	*PC*
TG 20:2_20:2_20:2	*TG*	*PE O-18:0_16:0*	*PE*
TG O-18:1_16:0_18:1	*TG*	*PG O-15:0_26:0*	*PG*
TG O-10:0_22:1_22:1	*TG*	*FAHFA 20:5/27:0*	*FAHFA*
SL 12:1;O/12:1;O	*SL*	*Hex3Cer 44:1;O2*	*HexCer*
PC O-14:1_8:0	*PC*	*HexCer 14:0;O2/29:1*	*HexCer*
TG 14:3_14:3_14:3	*TG*	*PG O-17:0_26:1*	*PG*
PC 12:0_22:3	*PC*	*PC O-16:0_16:1*	*PC*
Hex2Cer 16:0;O2/18:1	*HexCer*	*PA 20:1_26:4*	*PA*
PE 18:0_18:0	*PE*	*PE O-18:1_20:3*	*PE*
PE O-16:0_18:2	*PE*	*HexCer 16:0;O2/26:1;O*	*HexCer*
PE O-14:0_24:4	*PE*	*PC O-16:0_20:3*	*PC*
PE 18:1_26:4	*PE*	*PE O-14:0_26:2*	*PE*
Cer 18:1;O2/22:0;O	*Cer*	*PC O-16:0_24:4*	*PC*
PE 27:0_17:1	*PE*	*PE 24:1_22:4*	*PE*
PE O-18:0_22:2	*PE*	*FA 26:5*	*FA*
PC O-18:1_26:2	*PC*	*HexCer 14:0;O2/20:1*	*HexCer*
ST 24:1;O4;G/20:1	*ST*	*PE O-18:1_22:2*	*PE*
TG 8:0_24:4_24:4	*TG*	*PE 18:1_26:2*	*PE*
GlcADG 21:0_21:0	*GlcADG*	*PE 24:0_22:4*	*PE*
TG 16:0_18:1_22:5	*TG*	*Cer 14:0;O2/21:1;O*	*Cer*
TG 18:1_18:1_18:1	*TG*	*HBMP 18:1_18:1_18:1*	*BMP*
GlcADG 24:0_24:0	*GlcADG*	*HexCer 14:2;O2/25:1*	*HexCer*
TG 18:1_18:1_20:1	*TG*	*PC 16:0_18:2*	*PC*
TG 18:1_21:2_21:2	*TG*	*PI 13:1_26:2*	*PI*
TG 14:0_22:2_22:2	*TG*	*PE O-16:1_20:3*	*PE*
LPC 16:2	*LPC*	*PE O-16:0_22:3*	*PE*
LPC 19:1	*LPC*	*PC 12:0_20:3*	*PC*
PE O-14:0_4:0	*PE*	*Hex2Cer 40:1;O2*	*HexCer*
PC O-14:1_10:0	*PC*	*PE 27:0_14:1*	*PE*
PG 18:1_20:3	*PG*	*HBMP 12:0_20:1_20:1*	*BMP*
GlcADG 14:1_26:4	*GlcADG*	*PE 18:1_19:1*	*PE*
PC O-14:1_19:2	*PC*	*PC 18:1_20:5*	*PC*
DG 17:1_18:1	*DG*		

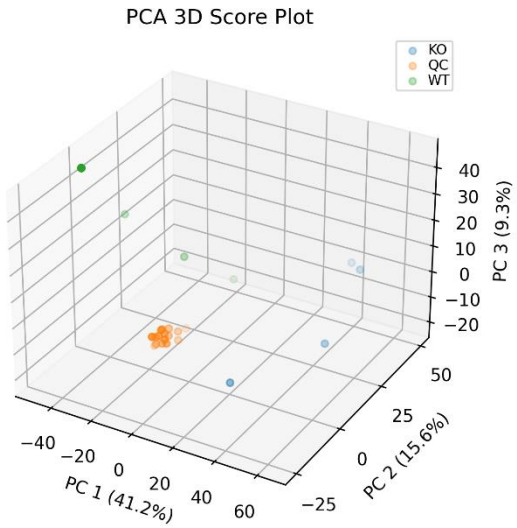
The positions of double bonds and stereochemistry of compounds were not determined in this report. When provided, common names shown for selected lipids in Tiers 1 and 2 were attributed based on biological intelligence, *i.e.*, the most common form of the molecule found in nature. Lipids can have many isomeric forms with identical chemical formulas, masses, and MS/MS fragmentation patterns. The compounds may differ only in the position of double bonds, functional groups, or stereochemistry. These lipids cannot be distinguished by the employed untargeted LC-MS/MS approach, requiring sophisticated targeted methods. Multiple peaks are often annotated as the same lipid at the molecular species level (most identifications for Tiers 1 and 2) or at the species level (Tier 3), corresponding to similar compounds with minor differences in their structures (positions of double bonds, positions of modifications, stereochemistry, etc.). Hence, it is possible to observe more than one peak with identical identifications in Supplemental Table 1. We suggest combining the normalized intensities provided in Supplemental Table 1 for lipids with identical identifications when performing biological evaluations or pathway analysis.

3.4 Quality Control Check

Quality Control Check was performed on NovaMT LipidScreener with Supp. Table 1 converted to the required CSV format (Statistical Analysis module). Supp. Table 1 contains peak intensities normalized by internal standards and median (*i.e.*, intensity ratios). Non-informative features (*i.e.*, internal standards, common contaminants and features with low experimental reproducibility) were filtered out during data processing. After uploading Supp. Table 1 to NovaMT LipidScreener, features with near-constant values between the groups (the 30% features with the lowest relative standard deviation for all samples) were filtered out. The dataset was also auto-scaled. No other filtering, normalization, transformation, or scaling methods were employed before multivariate statistical analysis.

Principal Component Analysis (PCA) 2-dimensional (2D) and 3-dimensional (3D) scores plots with quality control injections (QC, orange) are shown below. The 16 QC injections (injection duplicates of 8 aliquots of a pooled mixture of all samples) are clustered, displaying the reproducibility of the employed methods.



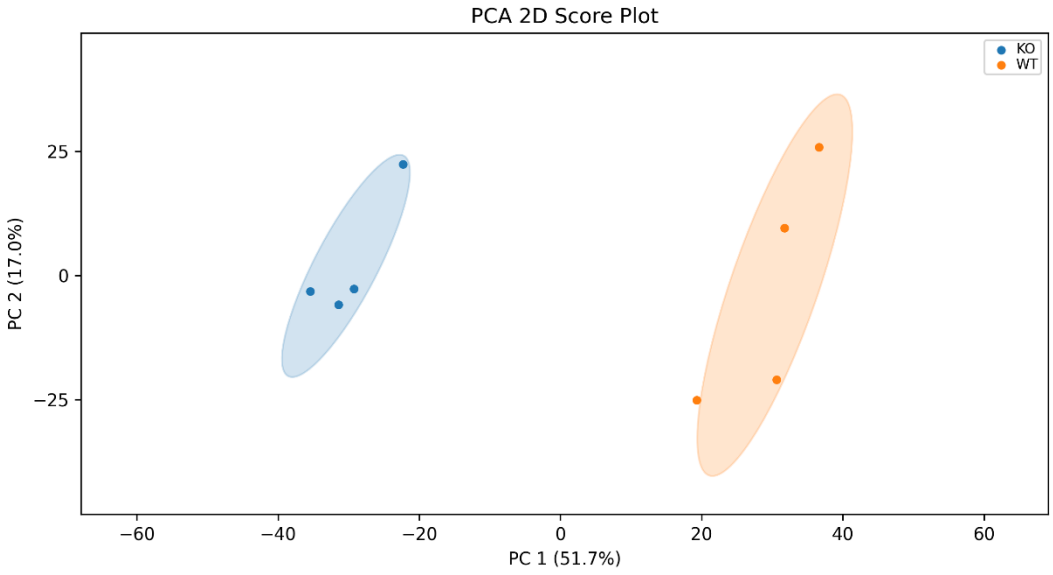


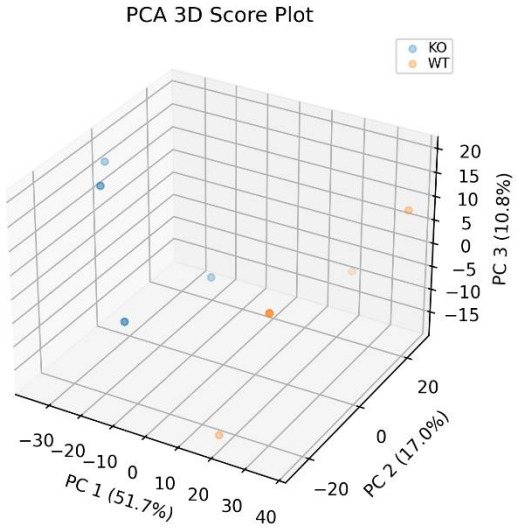
3.5 Multivariate Analysis

Multivariate statistical analysis was performed on NovaMT LipidScreener with Supp. Table 1 converted to the required CSV format (Statistical Analysis module). Supp. Table 1 contains peak intensities normalized by internal standards and median (*i.e.*, intensity ratios). Non-informative features (*i.e.*, internal standards, common contaminants and features with low reproducibility) were filtered out during data processing. After uploading Supp. Table 1 to NovaMT LipidScreener, features with near-constant values between the groups (the 30% features with the lowest relative standard deviation for all samples) were filtered out. The dataset was also auto-scaled. No other filtering, normalization, transformation, or scaling methods were employed before multivariate statistical analysis.

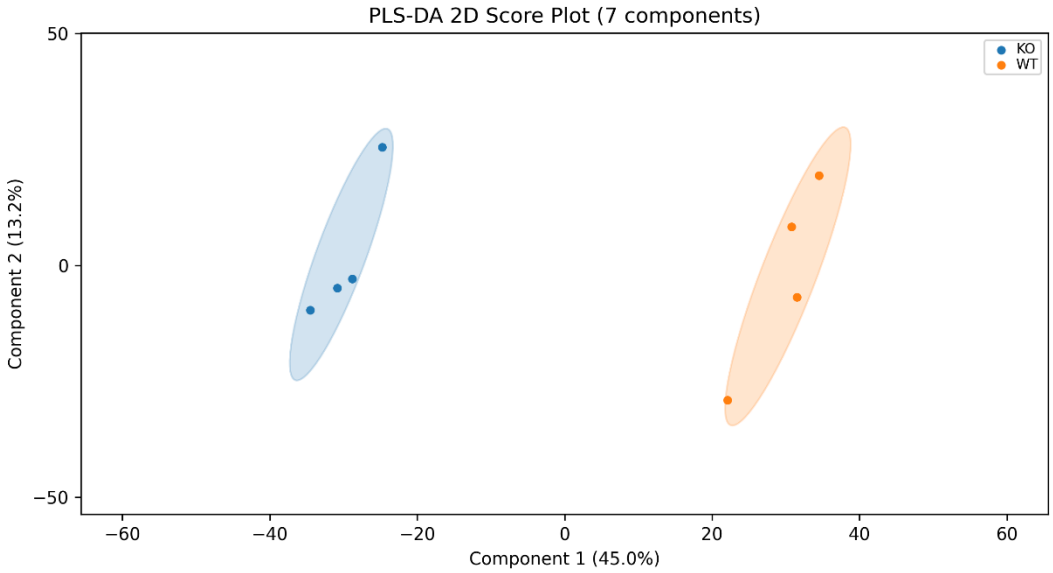
3.5.1 Comparison between groups "KO" group and "WT" group

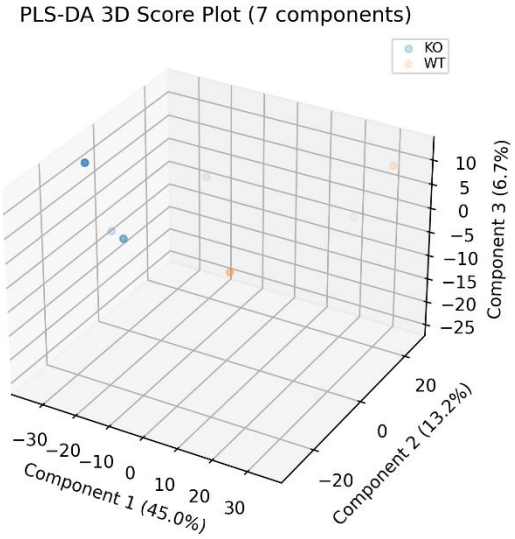
PCA 2D and 3D scores plots without QC injections are shown below.



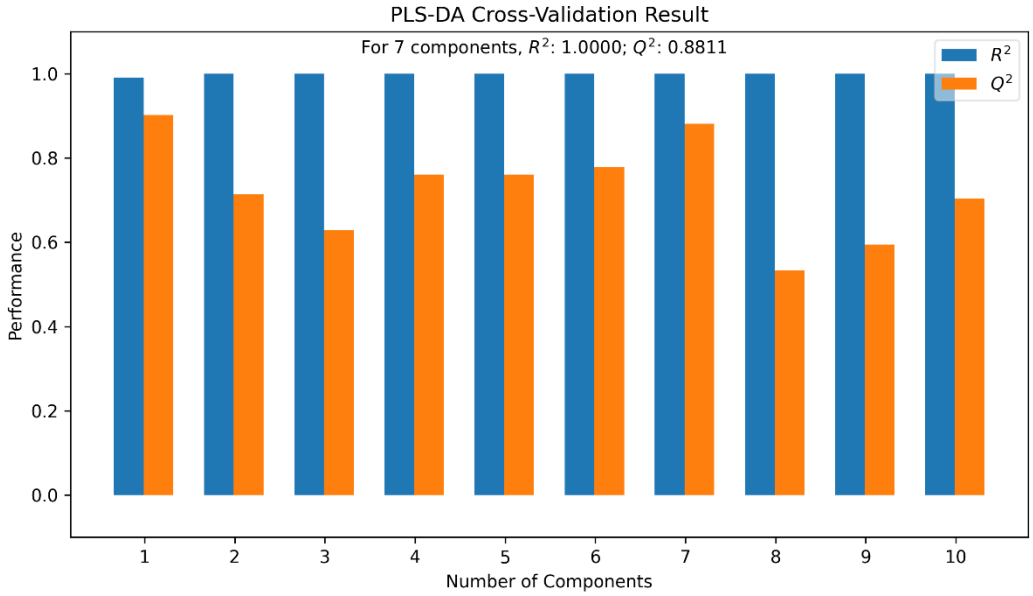


Partial Least Squares-Discriminant Analysis (PLS-DA) 2D and 3D scores plots are shown below.

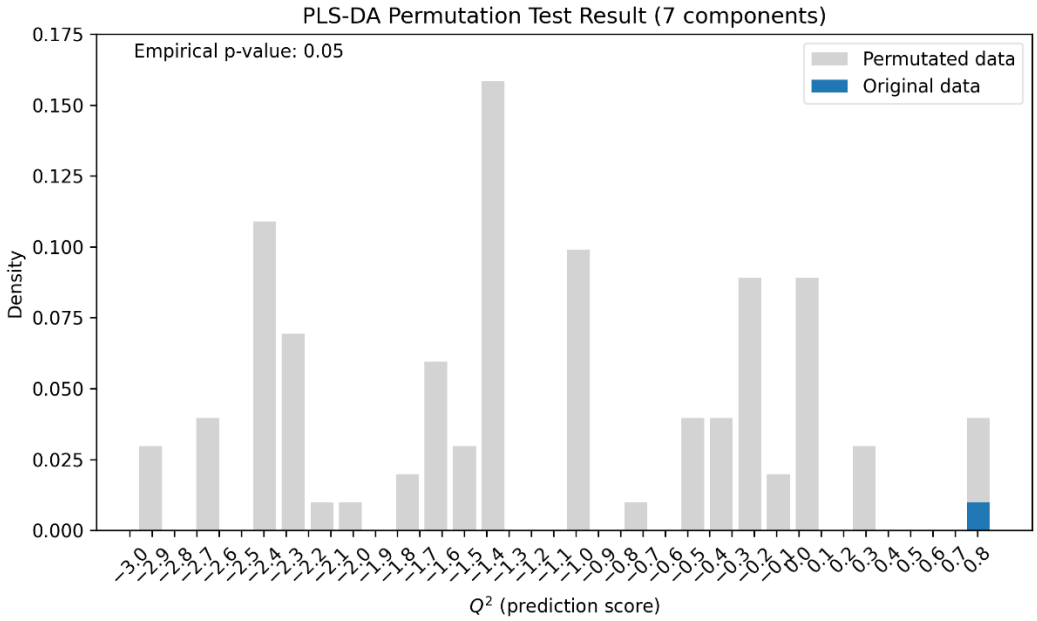




PLS-DA 10-fold cross-validation (10-fold CV) results are shown below (R^2 = 1.000, Q^2 = 0.8811, 7 components).

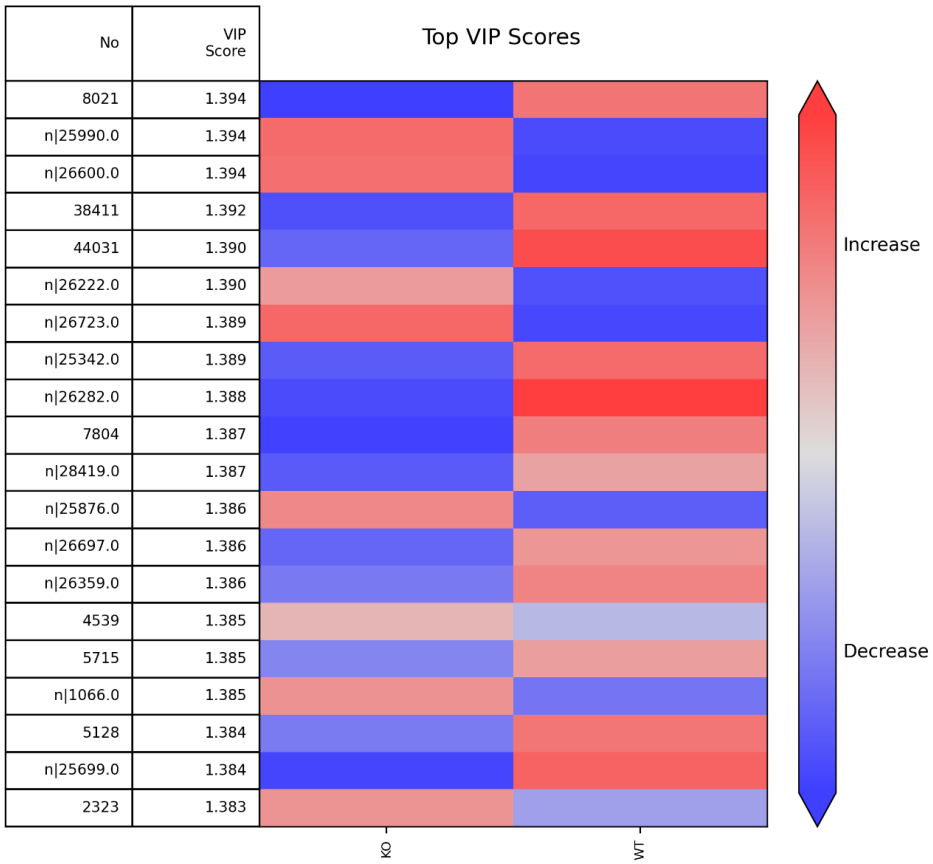


PLS-DA permutation test is shown below (empirical p-value of 0.05 for 100 permutations, 7 components).

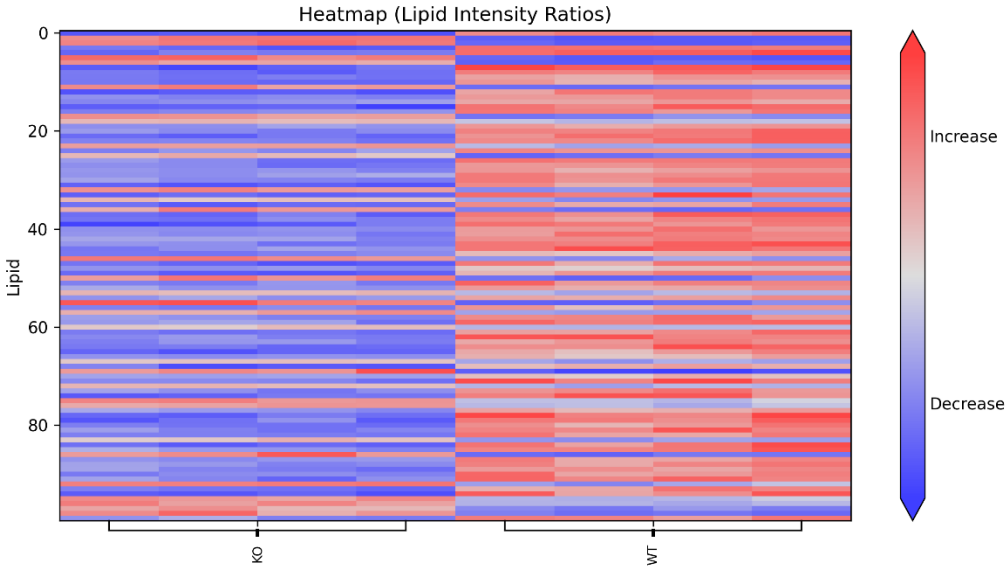


PLS-DA Variable Importance in the Prediction (VIP) scores are shown below for the 20 most important lipids. Their identifications are provided in Supp. Table 1.

No	VIP Score
8021	1.394
n|25990.0	1.394
n|26600.0	1.394
38411	1.392
44031	1.390
n|26222.0	1.390
n|26723.0	1.389
n|25342.0	1.389
n|26282.0	1.388
7804	1.387
n|28419.0	1.387
n|25876.0	1.386
n|26697.0	1.386
n|26359.0	1.386
4539	1.385
5715	1.385
n|1066.0	1.385
5128	1.384
n|25699.0	1.384
2323	1.383



Heatmap is shown below for all samples with the top 100 lipids ranked by p-value.



3.6 Univariate Analysis

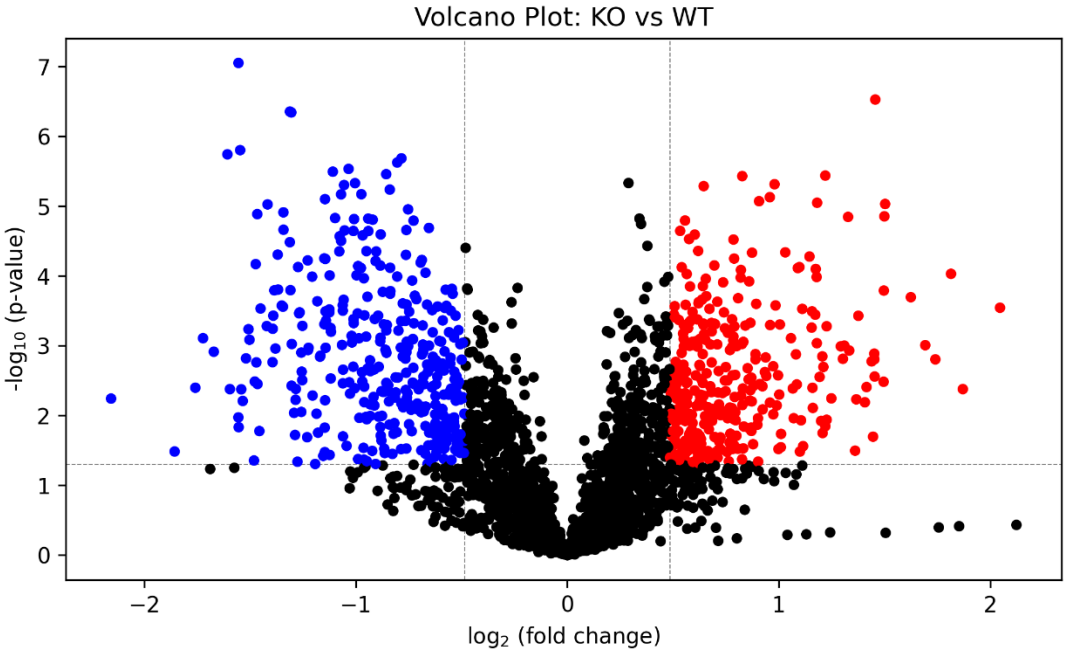
Univariate statistical analysis was performed on NovaMT LipidScreener with Supp. Table 1 converted to the required CSV format. Supp. Table 1 contains peak intensities normalized by internal standards and median (*i.e.*, intensity ratios). Non-informative features (*i.e.*, internal standards, common contaminants and features with low reproducibility) were filtered out during data processing. After uploading Supp. Table 1 to NovaMT LipidScreener, no other filtering, normalization, transformation, or scaling methods were employed before statistical analysis.

3.6.1 Comparison between "KO" and "WT"

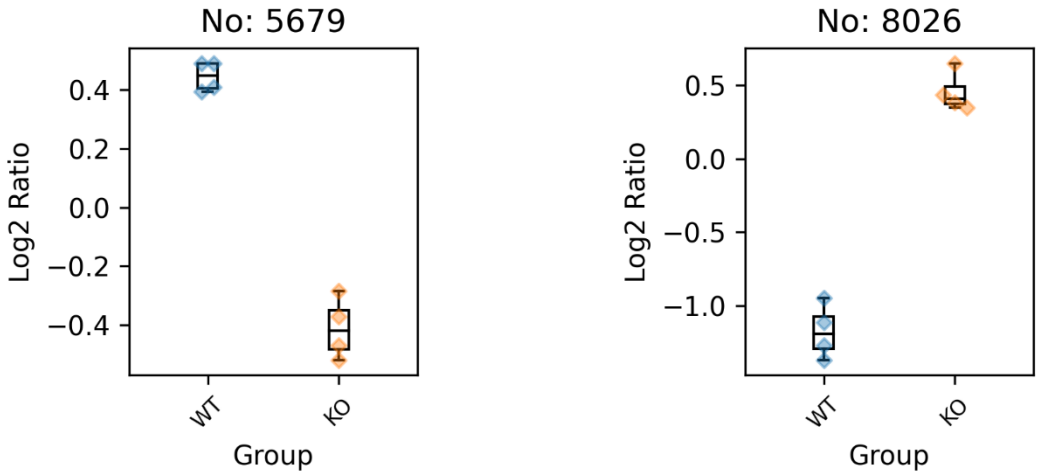
The volcano plot was constructed by plotting the fold change (FC) of each lipid, calculated as Mean(KO) / Mean(WT), against the p-value for each compound (Supplemental Table 3).

When using FC >1.40 or <0.71, p-value <0.05, and p-value adjusted for false-discovery rate (q-value) <0.25 as the criteria for significance, the analysis resulted in 347 significantly altered lipids with FC >1.40; and 370 significantly altered lipids with FC <0.71. The p-value threshold of 0.05 corresponded to a maximum q-value of 0.0356.

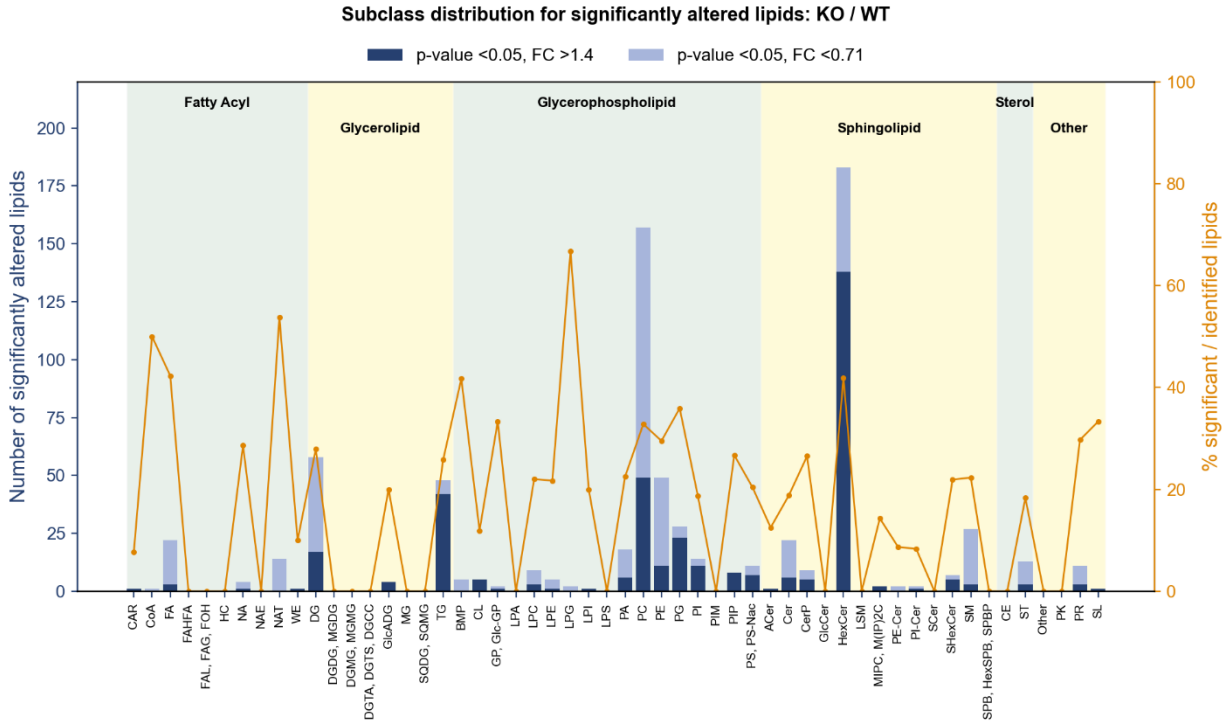
Without considering FDR adjustment and using FC >1.40 or <0.71 and p-value <0.05 as the criteria for significance (disregarding q-value), the analysis resulted in 347 significantly altered lipids with FC >1.40 (red); and 370 significantly altered lipids with FC <0.71 (blue). The Volcano plot is shown below.



The box plots of two significantly changed lipids are shown below with median and internal standard-normalized intensity ratios, working as examples to show the lipid amount changes. Their identifications are provided in Supp. Table 1.



The lipid class distribution for the significantly altered lipids when using FC >1.40 or <0.71 and p-value <0.05 as the criteria for significance is shown below. The abbreviations for lipid subclasses are described in Table 2. The FC, p-values and q-values for all compounds employed for univariate statistical analysis are provided in Supp. Table 3.



4 Conclusions

1) LC-MS data from 8 samples were processed. All data passed quality checks.

2) An average 5333 ± 202 features per experiment were detected.

3) A three-tier ID approach was used to perform lipid identification: 867 features were identified by MS/MS match in Tier 1 (MS/MS match score ≥500 and precursor mass tolerance of 20 ppm and 5.0 mDa), 51 features were identified by MS/MS match in Tier 2 (MS/MS match score <500 and precursor mass tolerance of 20 ppm and 5.0 mDa) and 1693 lipids were identified in Tier 3 using MS match (mass tolerance of 5.0 mDa and 20.0 ppm).

4) PCA was used to confirm data quality through clustering of quality control injections.

5) Volcano plots, PCA, PLS-DA, and Heatmaps were used to analyze and view the dataset.