Supplementary Materials

Supplementary Table S1: Analytical Conditions for LC/TQ.

Parameter	Agilent 6490 LC/TQ with Dual Agilent Jet Stream Source
Instrument mode	2 GHz, extended dynamic range, m/z 1,700
Polarity	Positive and Negative
Gas temperature	210 ºC
Drying gas (nitrogen)	13 L/min
Nebulizer gas	45 psi
Sheath gas	250 °C @ 12 L/min
Capillary voltage	3,500 V (+), 3,000 V (-)
Nozzle voltage	500 V
Fragmentor	160 V
Oct 1 Rf Vpp	750 V
Acquisition Conditions	See Supplementary Table S1b
	Agilent 1290 Infinity II LC
Analytical column	Agilent InfinityLab Poroshell EC-C18 2.0x100 mm, 1.9 µm (p/n 695675-902)
Column temperature	60 ºC
Injection volume	1 µL
Autosampler temperature	5 ºC
Needle wash	15 seconds in wash port (50:50 MeOH/IPA)
Mobile phase	A) 10mM Ammonium Acetate, 10 uM Medronic Acid in 9:1 H_2O/MeOH B) 10mM Ammonium Acetate in 3:2:5 ACN/MeOH/IPA
Flow rate	0.4 mL/min
Gradient program	Time (min) %B 0 62 2.0 84 8.0 86 8.1 93 10.0 100 12.9 100 13 62
Stop time	20 minutes
Post time	0 minutes

Supplementary Table S3: Analytical Conditions for LC/QTOF

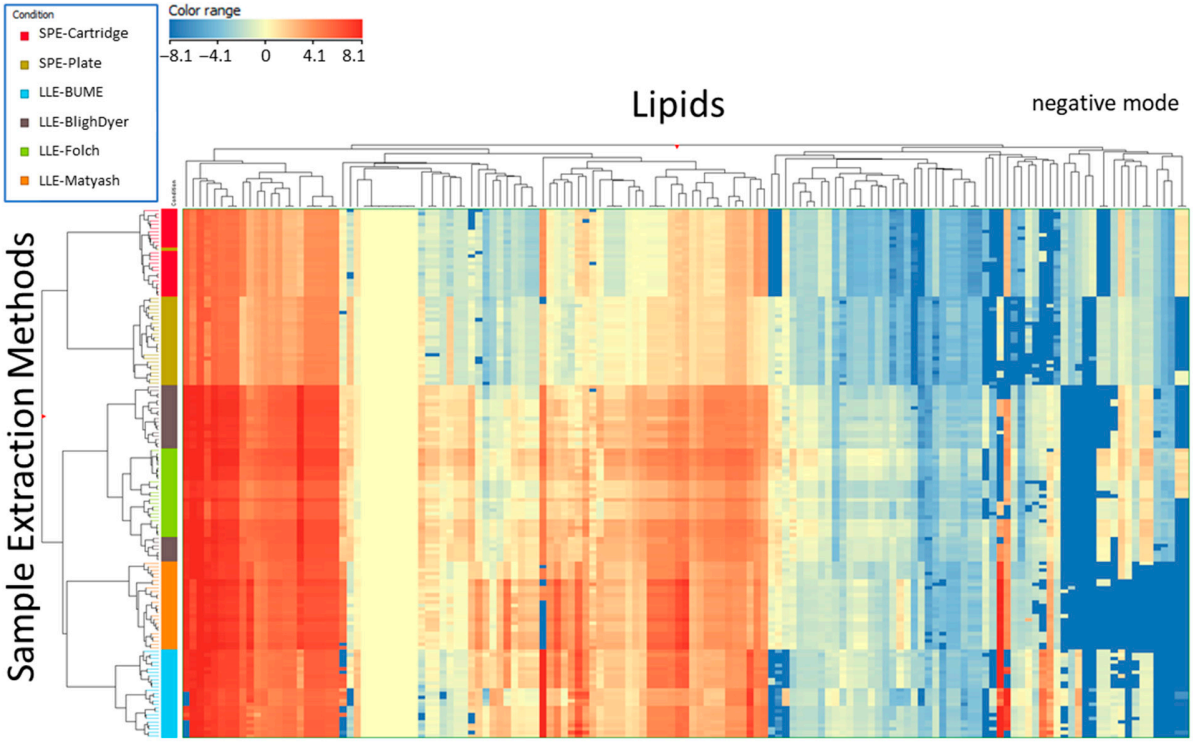
Parameter	Agilent 6545 LC/QTOF with Dual Agilent Jet Stream Source
Instrument mode	2 GHz, extended dynamic range, m/z 1,700
Polarity	Positive and Negative
Gas temperature	210 ºC
Drying gas (nitrogen)	13 L/min
Nebulizer gas	45 psi
Sheath gas	250 °C @ 12 L/min
Capillary voltage	3,500 V (+), 3,000 V (-)
Nozzle voltage	0 V
Fragmentor	160 V
Oct 1 Rf Vpp	750 V
Acquisition speed	MS-Only: 3 spectra/second (MS) Auto MS/MS: 3 spectra/second (MS), 4 spectra/second (MS/MS)
Auto MS/MS parameters	Isolation width: Narrow (~1.3 amu) Collision energy: 20, 35 eV
Reference correction	2 points at m/z 121.050873(+), 922.009798(+) 2 points at m/z 119.036320(-), 980.016375(-)
	Agilent 1290 Infinity II LC
Analytical column	Agilent InfinityLab Poroshell EC-C18 2.0x100 mm, 1.9 µm (695675-902)
Column temperature	60 ºC
Injection volume	1 µL
Autosampler temperature	5 ºC
Needle wash	15 seconds in wash port (50:50 MeOH/IPA)
Mobile phase	A) 10 mM Ammonium Acetate, 10 µM Medronic Acid 9:1 H_2O/MeOH B) 10 mM Ammonium Acetate, 3:2:5 ACN/MeOH/IPA
Flow rate	0.3 mL/min
Gradient program	Time(min) %B 0 60 0.5 60 3.0 57 9.5 87 10.5 95 14.0 96 14.5 100 20.0 100 20.1 60
Stop time	22 minutes
Post time	5 minutes

Supplementary Figure S1a: Recovery of stable labeled lipids internal standards based for individual lipids classes. Each bar represents the average of 5 preparation replicates x 5 MS replicates. recovery of stable labeled lipids internal standards based on lipids classes based on targeted LC-TQ workflow. In the box plots, the box represents the 1st to 3rd quartile to data with the central line denoting the median. The small square represents the mean and the whiskers are set at 5th and 95th percentile. Cholesterol esters(CE), ceramides(Cer), diacylglycerides (DG), lysophosphocholine(LPC), lysophosphoethanola-mine(LPE), lysophosphoglycerol(LPG), lysophosphoinosotol(LPI), lysophosphoserine(LPS), sphingolmyelin(SM).

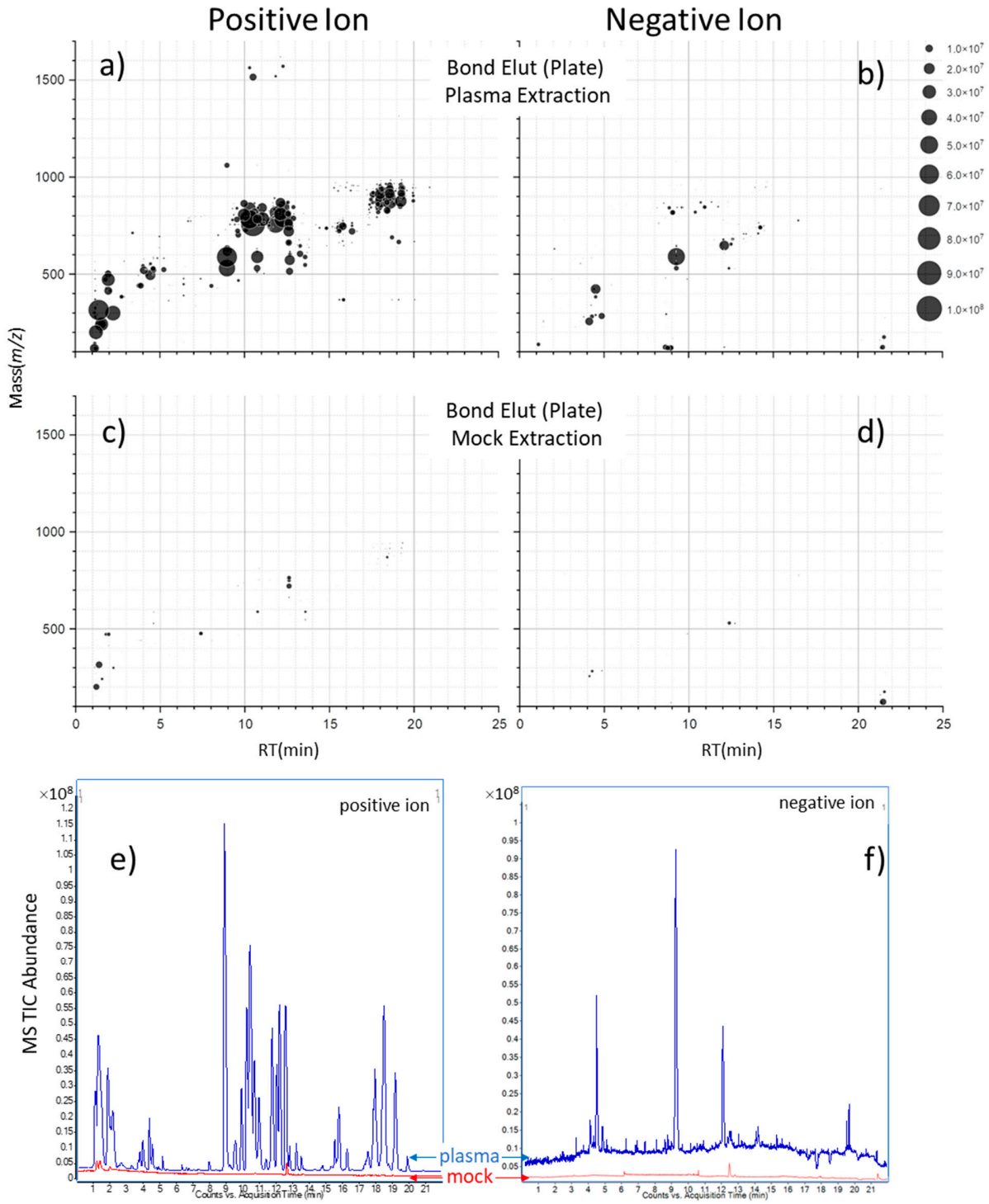
Supplementary Figure S1b: Recovery of stable labeled lipids internal standards based for individual lipids classes. Each bar rep-

resents the average of 5 preparation replicates x 5 MS replicates. recovery of stable labeled lipids internal standards based on lipids classes based on targeted LC-TQ workflow. In the box plots, the box represents the 1st to 3rd quartile to data with the central line denoting the median. The small square represents the mean and the whiskers are set at 5th and 95th percentile. Phosphatidylcholine(PC), phosphatidylethanolamine(PE), phosphatidylgycerol(PG), phosphatidylinosi-tol(PI), phosphatidylserine(PS), triacylglycerol(TG).

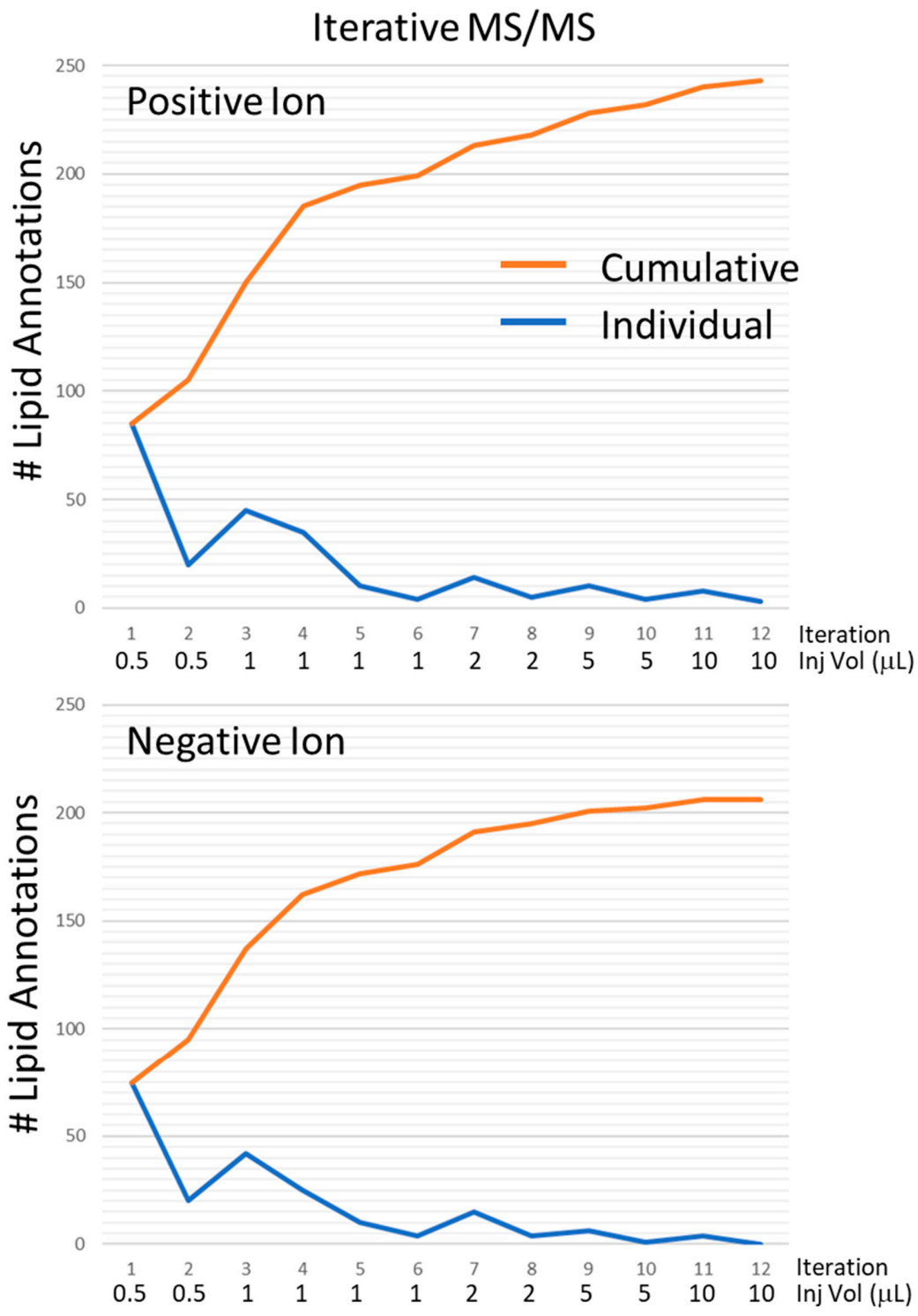
Supplementary Figure S2: Unsupervised hierarchical lipid clustering by different sample preparation



methods for negative ion mode LC-HRMS data. Each extraction method is represented by 5 preparation replicates x 5 analytical replicates. Log2 transformed abundance were normalized with respect to deuterium labeled lipid class standards (EquiSplash™).



Supplementary Figure S3. Comparison of plasma and mock extractions with Bond Elut Lipid Extraction Plates. RT(min) vs Mass(m/z) for plasma extract in positive (a) and negative modes(b). RT(min) vs Mass(m/z) for mock extract in positive (c) and negative modes(d). Bubble size in a-d represent peak area as indicated in legend. Corresponding MS Total Ion Chromatograms are shown in positive ion (e) and (f), with TIC chromatograms from plasma sample prepared by SPE (red) and those from reagent blank prepared through muck SPE procedure (blue).



Supplementary Figure S4 . Iterative MSMS for pooled plasma samples. In the course of this study, the effect of # of iterations vs injection volume was evaluated. It was found that 12 iterations was more than necessary. As the # of iterations increase, the cumulative # annotated lipids (red line) increases. Considering the number of lipid annotations for each individual data file (blue line), at each point that the injection volume is increased, the # of new annotated lipids increases, but then drops.