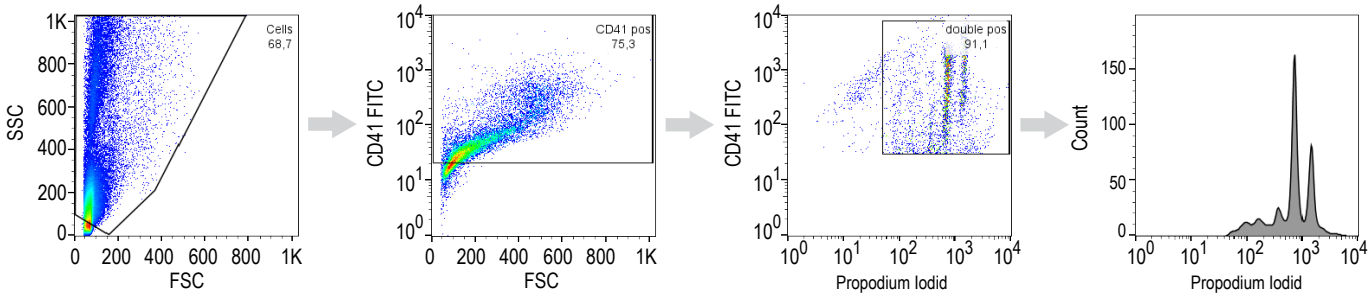

Supplementary Figure 1. Gating strategy MK ploidy

Schematic representation to illustrate the method used to obtain MK ploidy data. FACS plots show the CD41/PI gating strategy. The detectors, dyes and fluorophores are indicated in the X and Y-axes.

Supplementary Table 1. Minimal reporting checklist for targeted lipidomics

General Lipidomics Workflow

Overall study design

Title of the study	Critical shifts in lipid metabolism modulate megakaryocyte differentiation and proplatelet formation		
Principle investigator	Robert Ahrends		
Institution	University of Vienna, Faculty of Chemistry, Institute of Analytical Chemistry		
Corresponding Email	robert.ahrends@univie.ac.at		
Document creation date	11/29/2022	Clinical	No
Is the workflow targeted or untargeted?	Targeted		

Lipid extraction

Extraction method	2-phase system	2-phase system	MTBE
pH adjustment	None	Were internal standards added prior extraction?	Yes

Analytical platform

Number of separation dimensions	One dimension	MS type	QTrap
Separation Type 1	LC	MS vendor	SCIEX
Separation Mode 1	RP	Ion source	ESI
Separation window (1) for lipid analyte selection (±) in minutes	2	MS Level	MS2
RT verified by standard	Yes	Mass window for precursor ion isolation (in Da total isolation window)	unit
CCS verified by standard	No	Mass resolution for detected ion at MS2	Low resolution
Separation of isobaric/isomeric interferece confirmed	Yes	Resolution in Da at MS2	unit
Model for separation prediction	Yes		

Quality control

Blanks	Yes	Quality control	No
Type of Blanks	Injection blank, Extraction blank		

Method qualification and validation

Method validation	Yes	Precison	No
Lipid recovery	Yes	Accuracy	No
Dynamic quantification range	Yes	Guidelines followed	None
Limit of quantitation (LOQ)/Limit of detection (LOD)	Yes		

Reporting

Are reported raw data uploaded into repository?	Yes	Summary data	Quantification and identification data
Are metadata available?	No	Raw data upload	Yes

Sample Descriptions

megakaryocyte differentiation / Mouse / Cells

Provided information	Time to freeze (min)	Storage temperature	-80 °C
Temperature handling original sample	Room temperature	Additives	None
Instant sample preparation	No	Were samples stored under inert gas?	No
Time to freeze (min)	0	Additional preservation methods	No
Snap freezing in liquid N2	Yes	Biobank samples	No

Lipid Class Descriptions

Lipid class Cer[M+H]+ / Lipid identification

Lipid class	Cer	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	- H2O m/z 18		

Lipid class Cer[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	Cer 18:1;2/12:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class Hex2Cer[M+H]+ / Lipid identification

Lipid class	Hex2Cer	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	No
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	-Hex2 m/z 342		

Lipid class Hex2Cer[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	Hex2Cer 18:1;2/12:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class HexCer[M+H]+ / Lipid identification

Lipid class	HexCer	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	-Hex m/z 180		

Lipid class HexCer[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	HexCer 18:1;2/12:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SHexCer[M+H]+ / Lipid identification

Lipid class	SHexCer	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	- SHex m/z 260		

Lipid class SHexCer[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	HexCer 18:1;2/12:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class LSM[M+H]+ / Lipid identification

Lipid class	LSM	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	PC m/z 184		

Lipid class LSM[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	LSM 18:1;2	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SPBP[M+H]+ / Lipid identification

Lipid class	SPBP	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	- H3PO4 m/z 98		

Lipid class SPBP[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	SPBP 17:1	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SPB[M+H]+ / Lipid identification

Lipid class	SPB	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	LCB (-CH3O2)		

Lipid class SPB[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	SPB 17:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SPB[M+H]+ / Lipid identification

Lipid class	SPB	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	LCB (-CH3O2)		

Lipid class SPB[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	SPB 17:1	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SE[M+NH4]+ / Lipid identi ication

Lipid class	SE	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+NH4]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	1 fragment	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	- FA(+HO) - ST m/z 35	Nomenclature for fragment ions	N/A

Lipid class SE[M+NH4]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	SE 27:1;1/18:1(d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class ST[M+NH4]+ / Lipid identi ication

Lipid class	ST	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+NH4]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	1 fragment	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	- ST m/z 35	Nomenclature for fragment ions	N/A

Lipid class ST[M+NH4]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	ST 27:1;1(d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Lipid class SM[M+H]+ / Lipid identification

Lipid class	SM	Isotope correction at MS2	No
MS Level	MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS2	Yes
Polarity mode	Positive	Check isomer overlap	Yes
Type of positive (precursor)ion	[M+H]+	Lipid Identification Software	LipidCreator
How many fragments used for ID	2 fragments	Nomenclature for intact lipid molecule	Yes
Fragment ion 1	LCB (-H3O2)	Nomenclature for fragment ions	N/A
Fragment ion 2	PC m/z 184		

Lipid class SM[M+H]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	S/N ratio
Internal lipid standard(s)	SM 18:1;2/18:1(d9)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	Skyline
Response correction	No	Batch correction	No
Type I isotope correction	No		

Supplementary Table 2. Minimal reporting checklist for untargeted lipidomics

General Lipidomics Workflow

Overall study design

Title of the study	Critical shifts in lipid metabolism modulate megakaryocyte differentiation and proplatelet formation		
Principle investigator	Robert Ahrends		
Institution	University of Vienna, Faculty of Chemistry, Institute of Analytical Chemistry		
Corresponding Email	robert.ahrends@univie.ac.at		
Document creation date	11/24/2022	Clinical	No
Is the workflow targeted or untargeted?	Untargeted		

Lipid extraction

Extraction method	2-phase system	2-phase system	MTBE
pH adjustment	None	Were internal standards added prior extraction?	Yes

Analytical platform

MS type	Orbitrap	Resolution at m/z 200 at MS1	240000
MS vendor	Thermo	Mass accuracy in ppm at MS1	5
Ion source	nESI	Mass window for precursor ion isolation (in Da total isolation window)	1
Direct type	Chip	Mass resolution for detected ion at MS2	High resolution
MS Level	MS1, MS2	Resolution at m/z 200 at MS2	60000
Mass resolution for detected ion at MS1	High resolution	Mass accuracy in ppm at MS2	10

Quality control

Blanks	Yes	Quality control	No
Type of Blanks	Extraction blank		

Method qualification and validation

Method validation	Yes	Precison	Yes
Lipid recovery	Yes	Accuracy	Yes
Dynamic quantification range	Yes	Guidelines followed	None
Limit of quantitation (LOQ)/Limit of detection (LOD)	Yes		

Reporting

Are reported raw data uploaded into repository?	Yes	Summary data	Quantification and identification data
Are metadata available?	Yes	Raw data upload	Yes

Sample Descriptions

megakaryocyte differentiation + PL biosynthesis inhibitors / Mouse / Cells

Provided information	Time to freeze (min)	Storage temperature	-80 °C
Temperature handling original sample	Room temperature	Additives	None
Instant sample preparation	No	Were samples stored under inert gas?	No
Time to freeze (min)	0	Additional preservation methods	No
Snap freezing in liquid N2	Yes	Biobank samples	No

megakaryocyte differentiation / Mouse / Cells

Provided information	Time to freeze (min)	Storage temperature	-80 °C
Temperature handling original sample	Room temperature	Additives	None
Instant sample preparation	No	Were samples stored under inert gas?	No
Time to freeze (min)	0	Additional preservation methods	No
Snap freezing in liquid N2	Yes	Biobank samples	No

Lipid Class Descriptions

Lipid class CL[M-H]- / Lipid identification

Lipid class	CL	MS1 verified by standard	Yes
MS Level	MS1	Background check at MS1	Yes
Identification level	Species level	Check isomer overlap	Yes
Polarity mode	Negative	Lipid Identification Software	LipidXplorer
Type of negative (precursor)ion	[M-H]-	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS1	Type 2	Nomenclature for intact lipid molecule	Yes

Lipid class CL[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PG 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class DG[M+NH4]+ / Lipid identification

Lipid class	DG	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Positive	Background check at MS2	Yes
Type of positive (precursor)ion	[M+NH4]+	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	-FA1 (-H) -DG m/z 35	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	-FA2 (-H) -DG m/z 35	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class DG[M+NH4]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	DG 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPA[M-H]- / Lipid identification

Lipid class	LPA	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	1 fragment	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS1	Type 2	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS2	Type 2	Nomenclature for fragment ions	Yes

Lipid class LPA[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPE 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPC[M+CH3COO]- / Lipid identification

Lipid class	LPC	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M+CH3COO]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	PC m/z 74	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class LPC[M+CH3COO]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPC 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPE[M-H]- / Lipid identification

Lipid class	LPE	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	1 fragment	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS1	Type 2	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS2	Type 2	Nomenclature for fragment ions	Yes

Lipid class LPE[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPE 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPG[M-H]- / Lipid identification

Lipid class	LPG	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	1 fragment	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS1	Type 2	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS2	Type 2	Nomenclature for fragment ions	Yes

Lipid class LPG[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPE 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPI[M-H]- / Lipid identification

Lipid class	LPI	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	PI m/z 241	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class LPI[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPE 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class LPS[M-H]- / Lipid identification

Lipid class	LPS	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	PS m/z 87	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class LPS[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	LPE 18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PA[M-H]- / Lipid identification

Lipid class	PA	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	FA2 (+O)	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class PA[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PA 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PC O-a[M+CH3COO]- / Lipid identification

Lipid class	PC O-a	Isotope correction at MS2	Type 2
MS Level	MS1, MS2	MS1 verified by standard	Yes
Identification level	Molecular species level	MS2 verified by standard	Yes
Polarity mode	Negative	Background check at MS1	Yes
Type of negative (precursor)ion	[M+CH3COO]-	Background check at MS2	Yes
How many fragments used for ID	3 fragments	Check isomer overlap	Yes
Fragment ion 1	- FA2 (-H)	Lipid Identification Software	LipidXplorer
Fragment ion 2	FA2 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 3	PC m/z 74	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes

Lipid class PC O-a[M+CH3COO]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PC O-p18:0/18:1 (d9)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PC[M+CH3COO]- / Lipid identification

Lipid class	PC	Isotope correction at MS2	Type 2
MS Level	MS1, MS2	MS1 verified by standard	Yes
Identification level	Molecular species level	MS2 verified by standard	Yes
Polarity mode	Negative	Background check at MS1	Yes
Type of negative (precursor)ion	[M+CH3COO]-	Background check at MS2	Yes
How many fragments used for ID	3 fragments	Check isomer overlap	Yes
Fragment ion 1	FA1 (+O)	Lipid Identification Software	LipidXplorer
Fragment ion 2	FA2 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 3	PC m/z 74	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes

Lipid class PC[M+CH3COO]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PC 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PE O-a[M-H]- / Lipid identification

Lipid class	PE O-a	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	- FA2 (-H)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	FA2 (+O)	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class PE O-a[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PE O-p18:1/18:1 (d9)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PE[M-H]- / Lipid identification

Lipid class	PE	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	FA2 (+O)	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class PE[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PE 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PG[M-H]- / Lipid identification

Lipid class	PG	MS1 verified by standard	Yes
MS Level	MS1, MS2	MS2 verified by standard	Yes
Identification level	Molecular species level	Background check at MS1	Yes
Polarity mode	Negative	Background check at MS2	Yes
Type of negative (precursor)ion	[M-H]-	Check isomer overlap	Yes
How many fragments used for ID	2 fragments	Lipid Identification Software	LipidXplorer
Fragment ion 1	FA1 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 2	FA2 (+O)	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes
Isotope correction at MS2	Type 2		

Lipid class PG[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PG 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PI[M-H]- / Lipid identification

Lipid class	PI	Isotope correction at MS2	Type 2
MS Level	MS1, MS2	MS1 verified by standard	Yes
Identification level	Molecular species level	MS2 verified by standard	Yes
Polarity mode	Negative	Background check at MS1	Yes
Type of negative (precursor)ion	[M-H]-	Background check at MS2	Yes
How many fragments used for ID	3 fragments	Check isomer overlap	Yes
Fragment ion 1	FA1 (+O)	Lipid Identification Software	LipidXplorer
Fragment ion 2	FA2 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 3	PI m/z 241	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes

Lipid class PI[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PI 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class PS[M-H]- / Lipid identification

Lipid class	PS	Isotope correction at MS2	Type 2
MS Level	MS1, MS2	MS1 verified by standard	Yes
Identification level	Molecular species level	MS2 verified by standard	Yes
Polarity mode	Negative	Background check at MS1	Yes
Type of negative (precursor)ion	[M-H]-	Background check at MS2	Yes
How many fragments used for ID	3 fragments	Check isomer overlap	Yes
Fragment ion 1	FA1 (+O)	Lipid Identification Software	LipidXplorer
Fragment ion 2	FA2 (+O)	Data manipulation	Centroiding, Lock mass correction
Fragment ion 3	PS m/z 87	Nomenclature for intact lipid molecule	Yes
Isotope correction at MS1	Type 2	Nomenclature for fragment ions	Yes

Lipid class PS[M-H]- / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	PS 15:0-18:1 (d7)	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		

Lipid class TG[M+NH4]+ / Lipid identification

Lipid class	TG	MS1 verified by standard	Yes
MS Level	MS1	Background check at MS1	Yes
Identification level	Species level	Check isomer overlap	Yes
Polarity mode	Positive	Lipid Identification Software	LipidXplorer
Type of positive (precursor)ion	[M+NH4]+	Data manipulation	Centroiding, Lock mass correction
Isotope correction at MS1	Type 2	Nomenclature for intact lipid molecule	Yes

Lipid class TG[M+NH4]+ / For additional quantification methods

Quantitative	Yes	Limit of quantification	Signal theshold
Internal lipid standard(s)	TG 15:0-18:1(d7)-15:0	Normalization to reference	No
Type of quantification	Internal standard amount	Lipid Quantification Software	LipidXplorer
Response correction	No	Batch correction	No
Type I isotope correction	Yes		