Supporting information

Simultaneous non-polar and polar lipid analysis by on-line combination of HILIC, RP and high resolution MS

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Supporting information

This section contains the extended methods section and additional information on (S1) separation of 5 µM lipid standard mix using HILIC-RP-HRMS, (S2) separation of *Pichia pastoris* yeast extract by HILIC-RP-HRMS, (S3) comparison of peak widths using HILIC and RP separately or coupled, (S4) calibration curves of the exemplary lipids PC 34:2 and HexCer 34:1 in positive and FA 16:0 and Cer 36:1 in negative mode, (S5) Accuracy assessment for SRM 1950 - "Metabolites in Frozen Human Plasma", (Table S1) showing the lipid profiles of human plasma (SRM1950) and yeast (*Pichia pastoris*) identified by HILIC-RP-HRMS (LipidSearch 4.1) and Shotgun MS (LipidXplorer), and (Table S2) comparison of lipid annotations by shotgun MS and HILIC-RP-MS.

Extended method section

Shotgun MS

For the shotgun MS based lipid identifications in human plasma and yeast samples, 50 µL of 2-Prop/MeOH/$CHCl_3$ (4:2:1, v/v/v) with 7.5 mM ammonium formiate were added to the nitrogen dried lipid extracts in a 96 well plate (Eppendorf, Hamburg, Germany) and then infused *via* robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca NY, USA) into a Q Exactive HF instrument (Thermo Fisher Scientific, Bremen, Germany) using chips with spraying nozzles of 4.1 µm. Shotgun parameters were applied as previously described[1,2]. In this work, 17 min runs with polarity switching after 8 min were performed. The ddMS2 mode was recorded at 240 000 MS1 resolution and 30 000 MS2 resolution with a normalized collision energy of 24 (+) and 28 (-), an isolation window of 1 m/z and a dynamic exclusion for each time event. A maximum IT of 150 ms (MS1) and 50 ms (MS2) and an AGC target of 1e6 (MS1) and 1e5 (MS2) were chosen for both polarities and charges of 3 or higher were excluded. All spectra were recorded in centroid and the following source parameters were applied: capillary temperature of 250°C, sheath gas flow rate, auxillary flow rate and sweep gas were turned off, S-lens RF level of 50, auxiliary gas heater temperature of 30 °C and a spray voltage of 3.4 kV in positive mode and 3.3 kV in negative mode. All spectra were imported by LipidXplorer 1.2.7 into a MasterScan database and lipid identification was carried out as previously described[1,3].

Figure S1. **Separation of 5 µM lipid standard mix using HILIC-RP-HRMS**. **A.** High resolution MS1 chromatogram in positive mode of different lipid classes (HexCer, PC, PE, SM, LPC, ST, Cer, DAG, TG, Cer) **B.** High resolution MS1 detection in negative mode of different lipid classes (HexCer, PG, PC, PE, PA, LPC, FA, Cer).

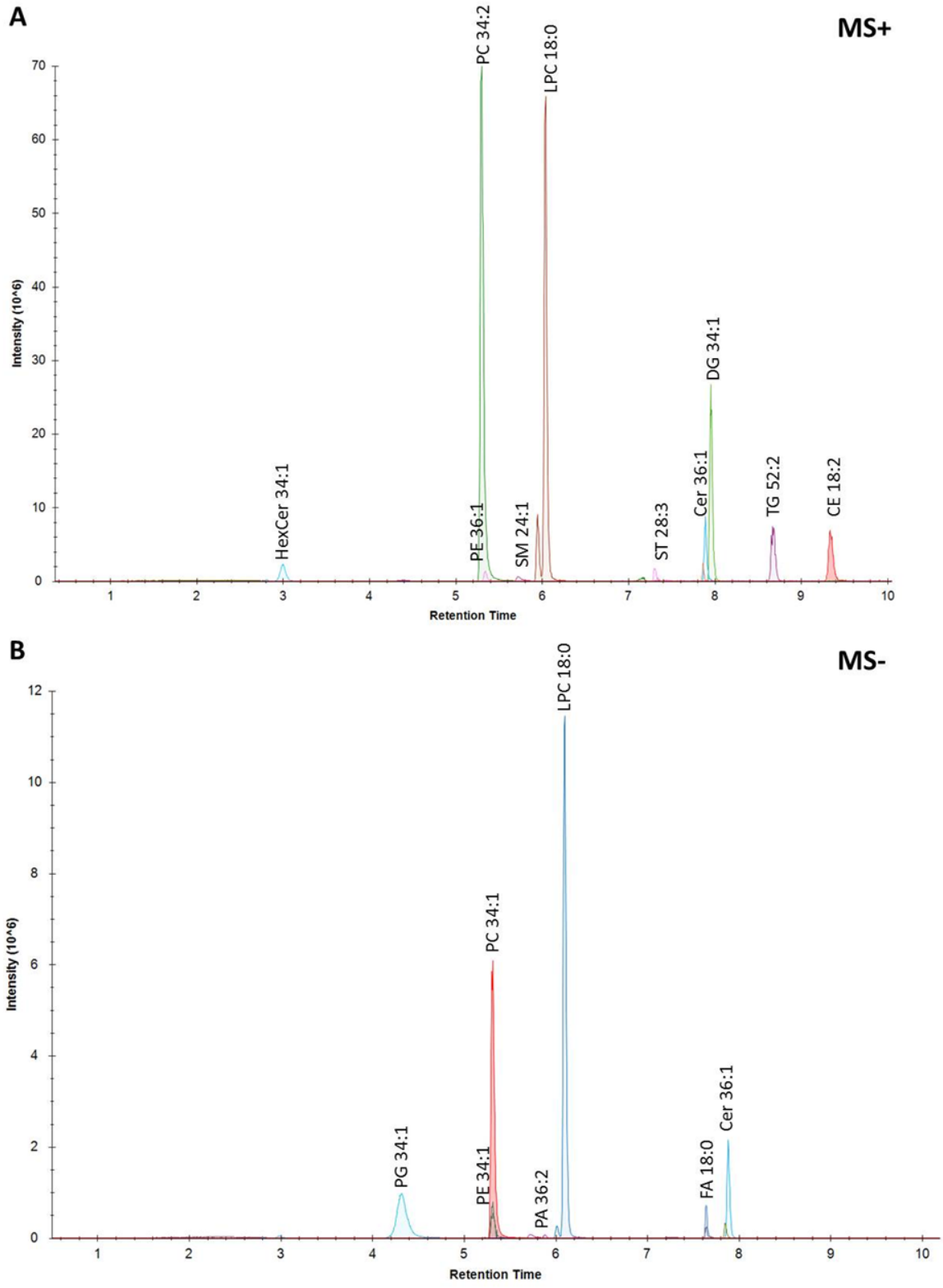
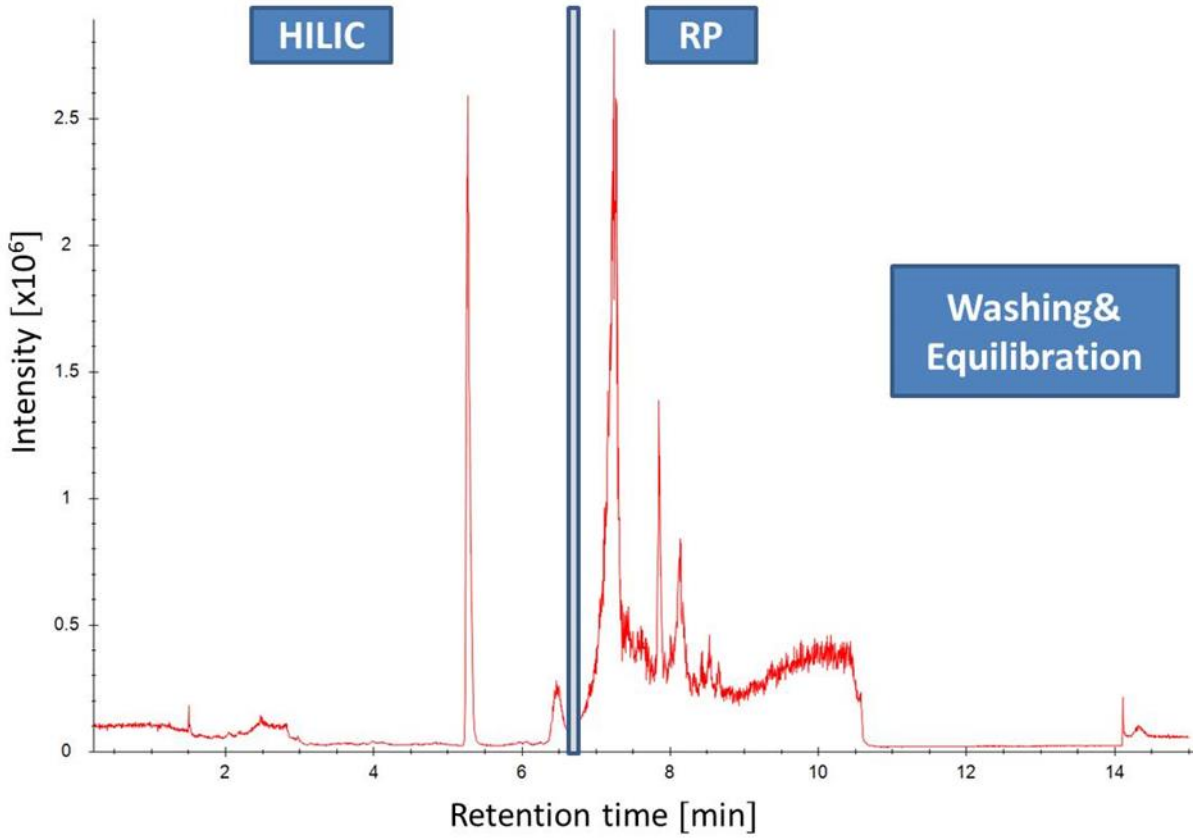
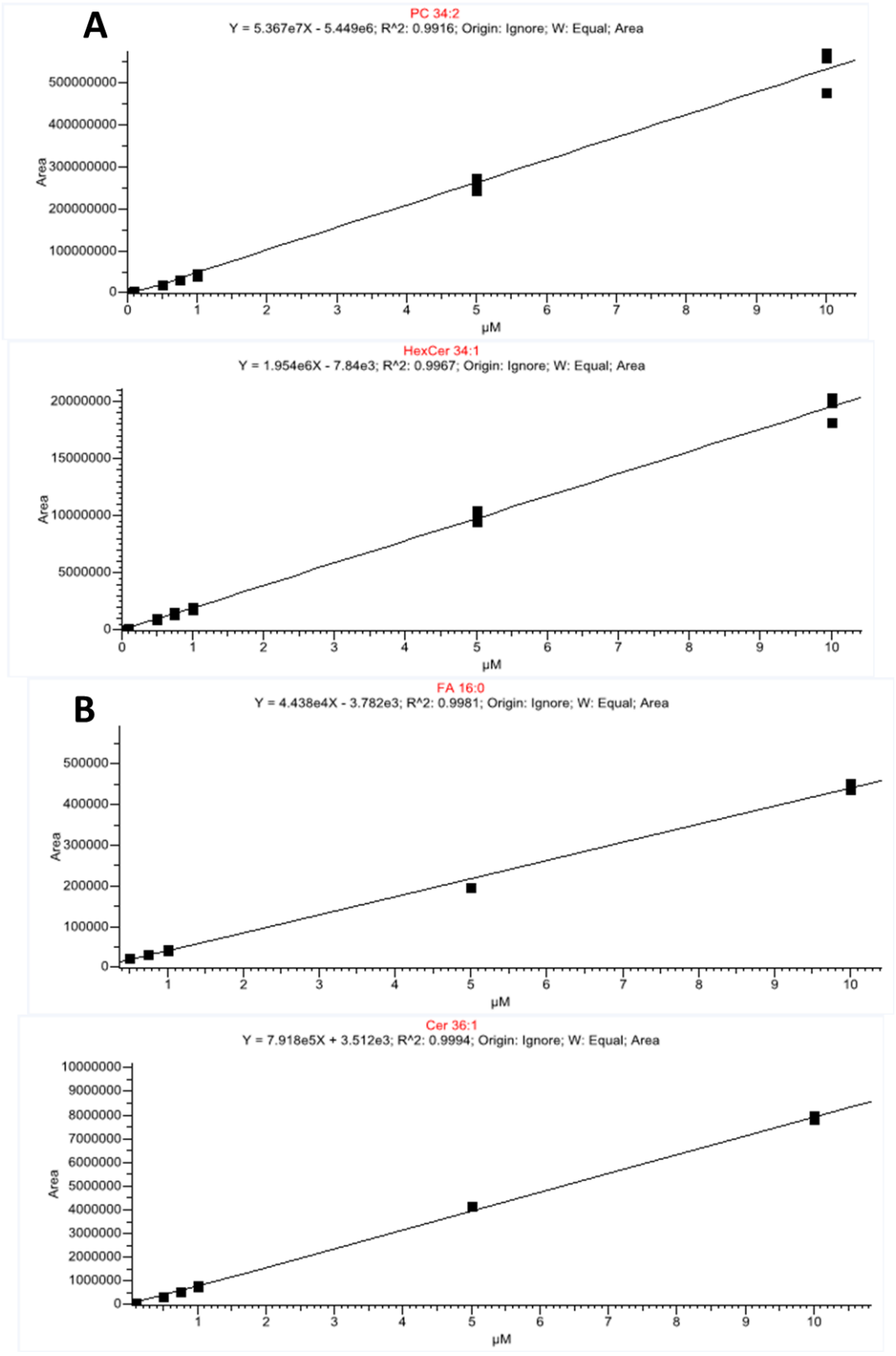


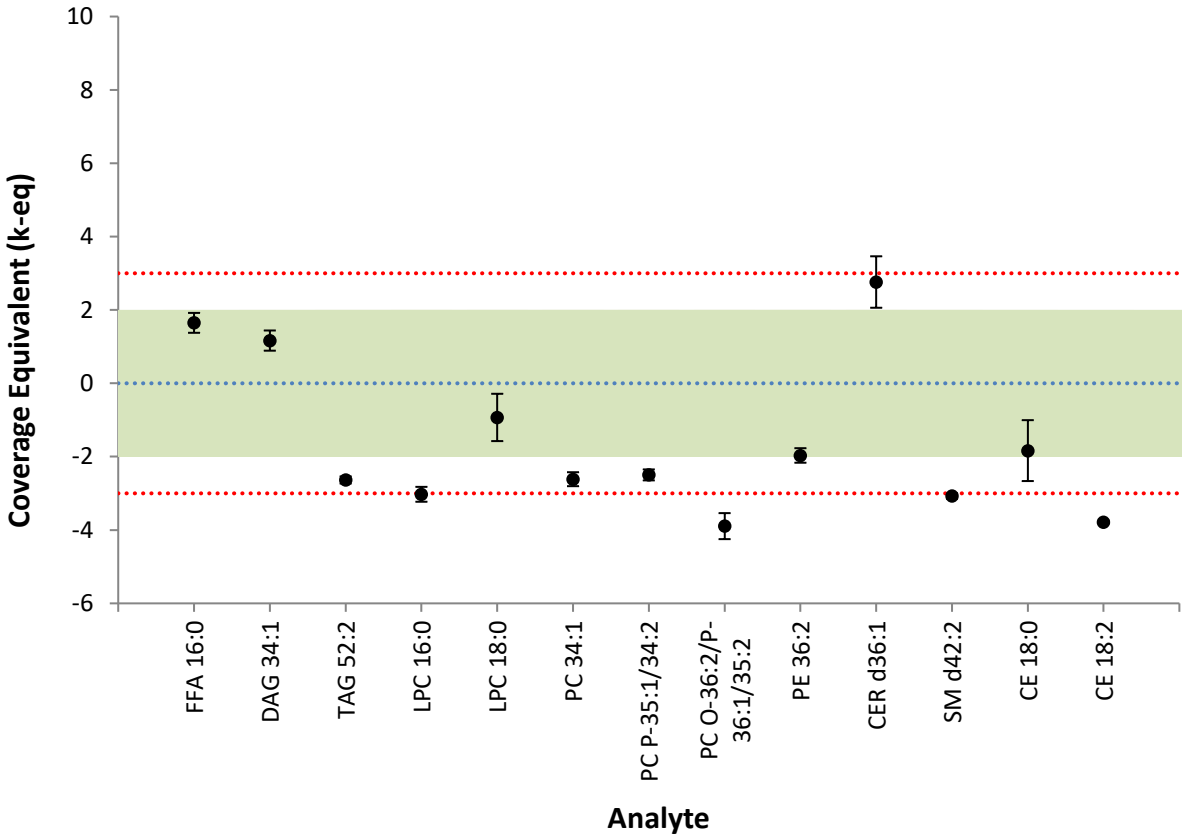
Figure S2. **Separation of *Pichia pastoris* yeast extract by HILIC-RP-HRMS**. High resolution MS1 TIC in positive mode of different lipid classes: From 0-6.5 min lipid class separation of the HILIC column (Acquity UHPLC BEH Amide, 2.1 x 100 mm, 1.7 µm) is performed prior to RP (C18 Acquity UHPLC HSS T3, 2.1 mm x 150 mm, 1.8 µm) chromatography for non-polar lipid elution from 6.5-11 min followed by washing and equilibration step of both columns.



Supporting Figure S3. Calibration curves of the exemplary lipids PC 34:2 and HexCer 34:1 in positive and FA 16:0 and Cer 36:1 in negative mode using external calibration with endogenous standards.



Supporting Figure S4. Accuracy assessment for SRM 1950 - "Metabolites in Frozen Human Plasma" comparing lipids measured by HILIC-RP-MS and a recent interlaboratory study by the NIST[4,5]. Values are presented as normalized coverage equivalents at the mean (dots) and stdev (error bars) of measurements, overlaid onto the consensus mean value (blue line) and uncertainty (95% coverage-green region, 99% coverage-red region). The figure was prepared using LipidQC[5].



*FFA=free fatty acid (FA), DAG=D diacyl glyceride (DG), TAG= triacyl gylceride (TG), LPC= lysophosphatidylcholine, PC= phosphatidylcholine, PE= phosphatidylethanolamine, SM= spinghomyelin, CE= cholesteryl ester

Supporting Table S2. Comparison of lipid annotations by shotgun MS and HILIC-RP-MS. Number of lipids identified the different lipid classes of human plasma SRM 1950 and *Pichia pastoris* yeast samples using direct infusion MS and HILIC-RP-MS.

	ID Human plasma		ID yeast	
Lipid class	**Shotgun MS**	**HILIC-RP-MS**	**Shotgun MS**	**HILIC-RP-MS**
Ceramide (Cer)	2	23	-	5
Cholesteryl ester (CE)	11	9	-	-
Diacylglycerol (DG)	6	17	9	13
Triacylglycerol (TG)	23	111	16	40
Monoacylglycerol (MG)	-	2	-	1
Dihexosylceramide (Hex_2Cer)	-	-	-	-
Hexosyl ceramide (HexCer)	-	6	-	-
Lysophosphatidylcholine (LPC)	4	35	2	6
Lysophosphatidyethanolamine (LPE)	9	9	4	3
Lysophosphatidylserine (LPS)	7	-	8	-
Lysophosphatidylglycerol (LPG)	1	-	1	-
Phosphatidic acid (PA)	-	-	4	-
Phosphatidylcholine (PC)	81	82	29	28
Phosphatidylethanolamine (PE)	13	12	14	14
Phosphatidylglycerol (PG)	2	-	5	2
Phosphatidylserine (PS)	-	-	1	6
Phosphatidylinositol (PI)	2	1	1	4
Dimethyl-phosphatidylethanolamine (DMPE)	-	-	-	4
Sphingomyelin (SM)	25	52	-	-
Acyl carnitine (AcCa)		31	-	-
Coenzyme (Co)	-	1	-	3
Sterol (ST)	1	-	-	-
Sum	**187**	**391**	**94**	**129**

References

1 R. Herzog, K. Schuhmann, D. Schwudke, J. L. Sampaio, S. R. Bornstein, M. Schroeder and A. Shevchenko, *PLoS One*, 2012, **7**, 15–20.

2 C. Coman, F. A. Solari, A. Hentschel, A. Sickmann, R. P. Zahedi and R. Ahrends, *Mol. Cell. Proteomics*, 2016, **15**, 1453–1466.

3 K. Schuhmann, R. Almeida, M. Baumert, R. Herzog, S. R. Bornstein and A. Shevchenko, *J. Mass Spectrom.*, 2012, **47**, 96–104.

4 J. A. Bowden, A. Heckert, C. Z. Ulmer, C. M. Jones, J. P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A. Armando, J. M. Asara, T. Bamba, J. R. Barr, J. Bergquist, C. H. Borchers, J. Brandsma, S. B. Breitkopf, T. Cajka, A. Cazenave-Gassiot, A. Checa, M. A. Cinel, R. A. Colas, S. Cremers, E. A. Dennis, J. E. Evans, A. Fauland, O. Fiehn, M. S. Gardner, T. J. Garrett, K. H. Gotlinger, J. Han, Y. Huang, A. Huipeng Neo, T. Hyötyläinen, Y. Izumi, H. Jiang, H. Jiang, J. Jiang, M. Kachmann, R. Kiyonami, K. Klavins, C. Klose, H. C. Köfeler, J. Kolmert, T. Koal, G. Koster, Z. Kuklenyik, I. J. Kurland, M. Leadley, K. Lin, K. R. Maddipati, M. Danielle, P. J. Meikle, N. A. Mellett, C. Monnin, M. A. Moseley, R. Nandakumar, M. Oresic, R. Patterson, D. Peake, J. S. Pierce, M. Post, P. A. D., R. Pugh, Y. Qiu, O. Quehenberger, P. Ramrup, J. Rees, B. Rembiesa, D. Reynaud, M. R. Roth, S. Sales, K. Schuhmann, M. L. Schwartzman, C. N. Serhan, A. Shevchenko, S. E. Sommerville, L. St. John-Williams, M. A. Surma, H. Takeda, R. Thakare, J. W. Thompson, F. Torta, A. Triebl, M. Trötzmüller, K. Ubhayasekera, D. Vuckovic, J. M. Weir, R. Welti, M. R. Wenk, C. E. Wheelock, L. Yao, M. Yuan, X. H. Zhao and S. Zhou, *J. Lipid Res.*, 2017, accepted.

5 C. Z. Ulmer, J. M. Ragland, J. P. Koelmel, A. Heckert, C. M. Jones, T. Garrett, R. A. Yost and J. A. Bowden, *Anal. Chem.*, 2017, acs.analchem.7b04042.