Supporting Information for:

Unequivocal Mapping of Molecular Ether Lipid Species by LC-MS/MS in Plasmalogen-Deficient Mice

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Content

Supporting Methods:

Breeding of Tmem189-deficient mice and harvest of mouse tissues

PEDS-deficient mice enzyme (Tmem189tm1a(KOMP)Wtsi mice, Wellcome Sanger Institute, Hinxton, Cambridge, UK, ref 13) and wild-type controls (C57bl/6N) were bred according to ref 5. Mice were housed in individual ventilated cages with nesting material, in a 12 h/12 h light/dark cycle with standard chow (Sniff Spezialdiäten GmbH, standard chow V1534-300, autoclaved at site) and water ad libitum. They were maintained on C57bl/6N genetic background and genotyped using primers Tmem189_35635F (GCGTGTCCTGCTGAGACTTG) and CAS_R1_Term (TCGTGGTATCGTTATGCGCC) for the transgenic allele and Tmem189_35635F and Tmem189-35635R (CATCCCACCTATCCCACCTG) for the wildtype allele. Tissues were harvested at 8 weeks of age from female and male homozygous Peds-deficient mice and their wild type littermates after sacrificing the animals by cervical dislocation. Tissues were snap frozen in liquid nitrogen and stored at -80 °C until further analysis. Experiments were approved by the Austrian Federal Ministry of Education, Science and Research (BMBWF-66.011/0100-V/3b/2019).

Sample extraction and preparation

Sample preparation was performed as previously described in ref 14. Briefly tissues were homogenized in PBS with an Ultra-Turrax (T10, IKA, Staufen, Germany). The protein content was then measured with a standard Bradford assay depending on a BSA serial dilution for quantification. For lipid extraction per tissue 500 µg total protein aliquots were extracted following the Folch method. Dried lipid extracts were finally dissolved in 100 µl 1/1 (v/v) acetonitrile/ethanol and stored at -20 °C. Aliquots with lipids respective to 200 µg (and for brain tissues 150 µg) protein content were dried under N_2-flow, dissolved in 100 µl HPLC starting conditions and subsequently measured.

LC-MS/MS analysis

The PC/PE analysis was performed as described in ref 15, with the modifications described in ref 16. 10 µl of the samples were injected by a Dionex Ultimate 3000 HPLC (Thermofisher Scientific Inc, Waltham, USA) with a flowrate of 0.4 ml/min and a column oven temperature of 50 °C. The HPLC was operated using a reversed phase gradient with an Agilent Poroshell 120 EC-C8 2.7mm 2.1x100mm column (Agilent Technologies, Santa Clara, USA). For gradient elution running Solvent A (4/6 H_2O/ammonium format, 0.2% formic acid) and running Solvent B (9/1 isopropanol/acetonitrile, 10 mM ammonium formate, 0.2% formic acid) were used. First, from 0 to 2 minutes, an isocratic flow with 54% Solvent A was applied, followed by a linear decrease from 54% to 28% Solvent A from minute 2 to minute 22. Next, the column was washed and equilibrated at starting conditions of 54% Solvent A. Further, lipids were ionized through negative electrospray ionization (275 °C capillary temperature, with mass range of 460-1650 m/z) in a LTQ Velos MS (Thermo Fisher Scientific Inc., Waltham, USA), whereas MS2 settings were variable depending on the expected PL. For general LC-MS parameters see Tables S1 and S2.

Supporting Dataset

Dataset S1

Contains additional information and data for all 11 different mouse tissues analyzed. *For all tissues you will find there a PC and PE lipid profiles plot, a radyl and acyl chain plot for PE lipid species, and the data used for most of the analyses presented in this work. A detailed description file is included in the Repository (Readme.html) See Mendeley Data at http://dx.doi.org/10.17632/r5twyf6bnn.1*

Supporting Tables

Table S1

HPLC method details. *Giving details about the different components and their respective settings regarding the used instrument Dionex Ultimate 3000 HPLC (Thermofisher Scientific Inc, Waltham, USA).*

HPLC Component	Applied settings
Column temperature	50°C
Autosampler temperature	10°C
Running solvent A	10 mM ammonium formate and 0.2% formic acid in 6/4 (v/v) acetonitrile/H2O
Running solvent B	10 mM ammonium formate and 0.2% formic acid in 9/1 (v/v) isopropanol/acetonitrile
Flow rate	0.4 ml/min from 0 to 22 minutes 0.5 ml/min from 22 to 23 minutes 0.6 ml/min from 23 to 28 minutes 0.5 ml/min from 28 to 29 minutes 0.4 ml/min from 29 to 30 minutes
Gradient	46% B for 2 min 46-72% B in 20 min 72-99% B in 1 min 99% B for 5 min 99-50% B in 1 min 46% B for 1 min
Sample amount	10 µl
Washing volume	200 µl

Table S2

Mass spectrometric method details. *Giving details about the different components and their respective settings regarding the used instrument LTQ Velos MS (Thermo Fisher Scientific Inc., Waltham, USA).*

MS component	Setting
Source	ESI negative mode
Capillary temperature	275 °C
Source voltage	3.8 kV
Scan rate	Normal
Activation type	CID
Full mass range	460-1650 m/z
Isolation width	1.5
Normalized collision energy	30/38
Default charge state	1
Activation time	10ms
MS2 mass range	5-105% relative of parent mass
Number of micro scans Full MS	3
Number of micro scans MS2	5
Dynamic exclusion	Enabled
Exclusion duration	6 s

Table S3

Peaklist for the integration of glycerophosphatidylcholines and glycerophosphatidyl-ethanolamines. *Column 1: Lipid identifier (nomenclature described in ref 46); Column 2: SubID for distinguishing between different peaks of the same isomeric species resulting from differing radyl combinations; Column 3: Respective retention times (in minutes); Column 4: Mass to charge ratios (m/z) of the lipids; Column 5: base peak width (in minutes) calculated as $t_{max} - t_{min}$.*

Lipid species	SubID	Retention time (min)	m/z	BPW (min)
PC(32:0)	1	4.51	778.729	0.166597
PC(32:1)	1	4.23	776.645	0.237063
PC(32:1)	2	3.88	776.620	0.299037
PC(32:1)	3	4.05	776.620	0.34059
PC(32:1)	4	3.69	776.586	0.158732
PC(32:2)	1	2.99	774.561	0.195799
PC(33:2)	1	3.40	788.565	0.169899
PC(34:0)	1	5.78	806.561	0.196704
PC(34:1)	1	4.72	804.658	0.204728
PC(34:2)	1	3.86	802.511	0.142141
PC(34:3)	1	3.16	800.610	0.174693
PC(34:3)	2	3.40	800.565	0.235651
PC(36:0)	1	7.31	834.658	0.342768
PC(36:1)	1	6.03	832.606	0.258207
PC(36:2)	1	5.01	830.584	0.182691
PC(36:3)	1	4.11	828.547	0.209035
PC(36:4)	1	3.73	826.479	0.147172
PC(36:4)	2	3.30	826.511	0.150288
PC(36:5)	1	3.08	824.494	0.195811
PC(36:6)	1	2.62	822.496	0.187365
PC(36:7)	1	2.61	820.513	0.181965
PC(37:2)	1	5.69	844.607	0.230799
PC(37:3)	1	4.72	842.591	0.19747
PC(38:2)	1	6.51	858.779	0.260506
PC(38:2)	2	6.16	858.722	0.309771
PC(38:3)	1	5.61	856.607	0.174773
PC(38:3)	2	5.39	856.607	0.175781
PC(38:4)	1	4.91	854.618	0.159161
PC(38:4)	2	4.55	854.583	0.207107
PC(38:4)	3	4.35	854.548	0.244778
PC(38:5)	1	4.18	852.591	0.274464
PC(38:5)	2	3.97	852.574	0.184996
PC(38:5)	3	3.76	852.591	0.207392
PC(38:6)	1	3.47	850.478	0.153984
PC(38:6)	2	3.18	850.495	0.162775
PC(38:7)	1	2.84	848.536	0.238436

PC(38:7)	2	2.63	848.532	0.198988
PC(40:0)	1	10.83	890.685	0.167431
PC(40:1)	1	9.12	888.647	0.233164
PC(40:2)	1	7.84	886.654	0.357445
PC(40:3)	1	6.92	884.638	0.334337
PC(40:3)	2	6.62	884.651	0.271277
PC(40:4)	1	6.32	882.610	0.231211
PC(40:4)	2	5.82	882.641	0.179022
PC(40:5)	1	5.41	880.691	0.172851
PC(40:5)	2	5.03	880.630	0.184235
PC(40:5)	3	4.81	880.630	0.25063
PC(40:5)	4	4.14	880.630	0.17417
PC(40:6)	1	4.53	878.712	0.166068
PC(40:6)	2	4.13	878.527	0.170882
PC(40:7)	1	3.62	876.610	0.16709
PC(40:7)	2	3.36	876.610	0.203261
PC(40:7)	3	3.18	876.610	0.310579
PC(40:8)	1	2.91	874.569	0.178516
PC(40:9)	1	2.44	872.526	0.224422
PC(41:4)	1	5.60	868.615	0.202395
PC(O-32:0)	1	5.30	764.664	0.209075
PC(O-32:1)	1	4.35	762.629	0.196252
PC(O-34:2)	1	4.64	788.599	0.167752
PC(O-36:0)	1	6.25	820.576	0.295241
PC(O-36:0)	2	5.06	820.614	0.193469
PC(O-36:1)	1	7.23	818.532	0.404213
PC(O-36:2)	1	5.94	816.612	0.308341
PC(O-36:3)	1	4.84	814.623	0.212182
PC(O-36:4)	1	4.50	812.583	0.183634
PC(O-38:1)	1	7.59	846.662	0.367895
PC(O-38:2)	1	7.52	844.677	0.250078
PC(O-38:4)	1	5.75	840.634	0.216293
PC(O-38:4)	2	4.36	840.634	0.16429
PC(O-38:5)	1	4.63	838.586	0.183154
PC(O-38:5)	2	4.40	838.681	0.313252
PC(O-38:6)	1	4.08	836.609	0.157464
PC(O-38:7)	1	3.25	834.584	0.274347
PC(O-40:6)	1	5.41	864.632	0.245683
PC(O-40:6)	2	5.26	864.637	0.232174
PC(O-40:7)	1	4.26	862.611	0.197312
PC(O-40:8)	1	3.39	860.591	0.18608
PC(P-32:0)	1	5.18	762.592	0.163605
PC(P-34:1)	1	5.49	788.522	0.339217
PC(P-36:1)	1	6.97	816.612	0.330522
PC(P-36:2)	1	5.73	814.596	0.285785
PC(P-36:3)	1	5.28	812.581	0.225074
PC(P-36:4)	1	4.36	810.490	0.300679
PC(P-36:5)	1	3.09	808.448	0.194987

PC(P-36:6)	1	2.50	806.504	0.213078
PC(P-38:1)	1	7.81	844.671	0.376745
PC(P-38:2)	1	5.53	842.645	0.200175
PC(P-38:4)	1	5.54	838.662	0.206863
PC(P-38:5)	1	4.75	836.581	0.209468
PC(P-38:6)	1	4.02	834.608	0.386986
PC(P-38:7)	1	3.34	832.549	0.253262
PC(P-40:6)	1	5.11	862.566	0.206162
PC(P-40:7)	1	4.12	860.618	0.181354
PC(P-40:8)	1	3.29	858.664	0.351065
PE(32:0)	1	4.83	690.540	0.15962
PE(32:1)	1	3.98	688.510	0.151085
PE(32:2)	1	3.19	686.460	0.171965
PE(34:0)	1	6.17	718.517	0.161493
PE(34:1)	1	5.11	716.764	0.178694
PE(34:2)	1	4.17	714.621	0.141498
PE(34:3)	1	3.38	712.576	0.16101
PE(34:3)	2	3.65	712.478	0.227163
PE(34:4)	1	3.09	710.627	0.178863
PE(35:2)	1	4.76	728.608	0.160035
PE(36:1)	1	6.53	744.540	0.223292
PE(36:2)	1	5.45	742.507	0.173246
PE(36:3)	1	4.42	740.478	0.175127
PE(36:3)	2	4.81	740.559	0.390935
PE(36:4)	1	4.07	738.482	0.138872
PE(36:4)	2	3.58	738.450	0.149085
PE(36:5)	1	3.25	736.479	0.159366
PE(36:5)	2	2.89	736.519	0.202354
PE(37:1)	1	7.23	758.576	0.251049
PE(37:2)	1	6.13	756.629	0.202137
PE(38:1)	1	8.17	772.621	0.206407
PE(38:1)	2	7.97	772.657	0.244338
PE(38:2)	1	6.95	770.564	0.217973
PE(38:2)	2	6.67	770.617	0.263121
PE(38:3)	1	6.28	768.533	0.205781
PE(38:3)	2	5.97	768.533	0.16667
PE(38:3)	3	5.78	768.560	0.176434
PE(38:4)	1	5.27	766.503	0.177145
PE(38:4)	2	4.88	766.476	0.176051
PE(38:4)	3	4.73	766.503	0.184579
PE(38:5)	1	4.27	764.532	0.156345
PE(38:5)	2	3.97	764.583	0.221705
PE(38:5)	3	4.56	764.532	0.330263
PE(38:6)	1	3.70	762.509	0.147468
PE(38:6)	2	3.42	762.476	0.233517
PE(38:7)	1	3.97	760.458	0.184217
PE(38:7)	2	3.65	760.501	0.239579
PE(38:7)	3	3.49	760.487	0.182575

PE(39:4)	1	5.97	780.604	0.18974
PE(40:1)	1	9.99	800.586	0.239802
PE(40:1)	2	10.46	800.567	0.183937
PE(40:2)	1	8.36	798.594	0.272478
PE(40:3)	1	7.35	796.720	0.221665
PE(40:4)	1	6.76	794.631	0.27859
PE(40:4)	2	6.21	794.559	0.175672
PE(40:5)	1	5.82	792.594	0.18124
PE(40:5)	2	5.42	792.671	0.248407
PE(40:5)	3	5.10	792.550	0.23279
PE(40:6)	1	4.86	790.583	0.151854
PE(40:6)	2	4.21	790.625	0.19154
PE(40:6)	3	3.51	790.550	0.17454
PE(40:7)	1	3.90	788.509	0.152111
PE(40:8)	1	3.11	786.512	0.169751
PE(40:9)	1	2.60	784.543	0.193763
PE(42:2)	1	10.01	826.608	0.198146
PE(42:2)	2	9.67	826.608	0.209406
PE(42:3)	1	8.69	824.720	0.227781
PE(42:4)	1	7.50	822.705	0.245516
PE(42:5)	1	6.34	820.631	0.302828
PE(42:6)	1	5.37	818.660	0.240952
PE(42:7)	1	4.46	816.680	0.162783
PE(O-34:0)	1	7.30	704.577	0.225921
PE(O-34:1)	1	6.03	702.662	0.214186
PE(O-34:2)	1	4.99	700.679	0.179768
PE(O-34:3)	1	4.04	698.658	0.162711
PE(O-34:3)	2	4.37	698.568	0.158151
PE(O-34:4)	1	3.69	696.591	0.161079
PE(O-35:1)	1	6.75	716.764	0.187278
PE(O-35:2)	1	5.63	714.621	0.178
PE(O-35:4)	1	4.24	710.579	0.152908
PE(O-36:1)	1	7.71	730.631	0.250061
PE(O-36:1)	2	7.46	730.562	0.38152
PE(O-36:2)	1	6.37	728.621	0.285603
PE(O-36:3)	1	5.83	726.594	0.204733
PE(O-36:3)	2	5.62	726.533	0.312631
PE(O-36:3)	3	5.36	726.594	0.19575
PE(O-36:3)	4	5.12	726.628	0.202648
PE(O-36:4)	1	4.83	724.550	0.151973
PE(O-36:5)	1	4.11	722.444	0.202695
PE(O-36:5)	2	3.88	722.479	0.151394
PE(O-36:6)	1	3.33	720.446	0.187549
PE(O-37:4)	1	5.51	738.570	0.164309
PE(O-37:4)	2	5.37	738.570	0.196999
PE(O-37:6)	1	3.78	734.522	0.165923
PE(O-38:0)	1	10.14	760.658	0.211456
PE(O-38:0)	2	10.63	760.597	0.177666

PE(O-38:1)	1	9.20	758.587	0.306851
PE(O-38:2)	1	8.13	756.600	0.252232
PE(O-38:2)	2	7.80	756.608	0.266428
PE(O-38:3)	1	7.44	754.593	0.284787
PE(O-38:3)	2	6.83	754.610	0.252814
PE(O-38:4)	1	6.20	752.569	0.182063
PE(O-38:4)	2	5.73	752.587	0.177284
PE(O-38:5)	1	5.37	750.615	0.306834
PE(O-38:5)	2	5.10	750.625	0.236244
PE(O-38:5)	3	4.80	750.605	0.233243
PE(O-38:6)	1	4.42	748.574	0.166432
PE(O-38:7)	1	3.56	746.549	0.221824
PE(O-39:5)	1	7.06	766.619	0.236816
PE(O-39:7)	1	5.01	762.604	0.194583
PE(O-40:1)	1	11.13	786.805	0.229395
PE(O-40:2)	1	9.96	784.664	0.255863
PE(O-40:3)	1	8.56	782.576	0.22972
PE(O-40:3)	2	8.34	782.584	0.218451
PE(O-40:4)	1	7.95	780.588	0.297793
PE(O-40:4)	2	7.31	780.633	0.230334
PE(O-40:5)	1	6.81	778.663	0.242573
PE(O-40:5)	2	6.36	778.618	0.244077
PE(O-40:5)	3	6.13	778.618	0.226285
PE(O-40:6)	1	5.69	776.668	0.206162
PE(O-40:7)	1	4.59	774.612	0.22179
PE(O-40:8)	1	3.69	772.544	0.180811
PE(O-40:9)	1	2.99	770.544	0.174586
PE(O-42:10)	1	4.80	796.600	0.169393
PE(O-42:12)	1	4.44	792.600	0.313333
PE(O-42:6)	1	7.30	804.703	0.305466
PE(O-42:7)	1	5.86	802.612	0.243888
PE(P-34:0)	1	7.14	702.454	0.220687
PE(P-34:1)	1	5.88	700.599	0.177374
PE(P-34:2)	1	4.84	698.570	0.160928
PE(P-34:3)	1	4.21	696.398	0.15869
PE(P-34:3)	2	3.99	696.528	0.179597
PE(P-34:4)	1	3.57	694.335	0.16005
PE(P-35:1)	1	6.59	714.329	0.197703
PE(P-35:2)	1	5.49	712.673	0.166445
PE(P-35:4)	1	4.12	708.436	0.158825
PE(P-36:1)	1	7.38	728.620	0.334584
PE(P-36:2)	1	6.20	726.500	0.247797
PE(P-36:3)	1	5.63	724.515	0.18147
PE(P-36:3)	2	5.41	724.515	0.350202
PE(P-36:3)	3	5.13	724.635	0.258507
PE(P-36:4)	1	4.67	722.479	0.145428
PE(P-36:5)	1	3.82	720.529	0.15205
PE(P-36:6)	1	3.25	718.536	0.167316

PE(P-37:4)	1	5.35	736.525	0.168873
PE(P-38:1)	1	9.02	756.602	0.232903
PE(P-38:2)	1	7.93	754.512	0.256397
PE(P-38:2)	2	7.61	754.646	0.251603
PE(P-38:3)	1	7.16	752.578	0.363642
PE(P-38:3)	2	6.62	752.467	0.333731
PE(P-38:4)	1	6.01	750.615	0.167144
PE(P-38:4)	2	5.59	750.605	0.203945
PE(P-38:5)	1	5.17	748.502	0.25912
PE(P-38:5)	2	4.95	748.553	0.222963
PE(P-38:5)	3	4.76	748.584	0.28209
PE(P-38:6)	1	4.25	746.474	0.151824
PE(P-38:6)	2	3.97	746.535	0.174431
PE(P-38:7)	1	3.52	744.498	0.170424
PE(P-39:5)	1	6.80	764.569	0.231956
PE(P-39:7)	1	4.83	760.487	0.155761
PE(P-40:1)	1	10.91	784.727	0.188197
PE(P-40:2)	1	9.19	782.690	0.253743
PE(P-40:3)	1	8.33	780.556	0.211495
PE(P-40:4)	1	7.73	778.648	0.262058
PE(P-40:4)	2	7.17	778.633	0.210306
PE(P-40:5)	1	6.55	776.628	0.245419
PE(P-40:5)	2	6.20	776.655	0.368812
PE(P-40:5)	3	5.98	776.664	0.213967
PE(P-40:6)	1	5.53	774.629	0.165019
PE(P-40:7)	1	4.49	772.499	0.181515
PE(P-40:8)	1	3.60	770.620	0.159735
PE(P-40:9)	1	2.93	768.510	0.179776
PE(P-42:10)	1	4.24	794.528	0.168127
PE(P-42:6)	1	7.09	802.578	0.284406
PE(P-42:7)	1	5.67	800.618	0.165514
PE(P-42:8)	1	4.60	798.526	0.177889

Table S4

Table detailing the systematic analysis of relative retention time differences (rRT). We analyzed relative RT changes for acyl chain elongation (+2 CH2), an additional acyl chain double bond (+1 DB (>Δ4)), additional vinyl ether double bonds (+1 DB (Δ1)), and between 1-O-alk-1'-enyl and 1-O-alkyl lipids in 6-10 relevant lipid species, which allowed us to number the impact of double bond positions and chain lengths on lipid retardation. Relative RT (rRT) was calculated as $rRT = RT_{Lipid\ 1}[s]\ /\ RT_{Lipid\ 2}[s]$. Values were first summarized per mouse (mean) and then summarized by calculation of mean and standard deviation of the twelve biological replicates.

	Lipid 1	Lipid 2	Mean (rRT)	SD (rRT)
+2 CH2	PC(34:0)	PC(32:0)	1.291	0.013
	PC(34:1)	PC(32:1)	1.131	0.017
	PC(34:2)	PC(32:2)	1.310	0.012
	PC(36:1)	PC(34:1)	1.283	0.011
	PC(36:2)	PC(34:2)	1.304	0.006
	PC(38:3)	PC(36:3)	1.324	0.039
	PE(34:0)	PE(32:0)	1.294	0.039
	PE(34:1)	PE(32:1)	1.284	0.009
	PE(34:2)	PE(32:2)	1.334	0.053
	PE(36:1)	PE(34:1)	1.285	0.023
	PE(36:2)	PE(34:2)	1.301	0.011
	PE(38:3)	PE(36:3)	1.331	0.041
+1 DB (>Δ4)	PC(32:1)	PC(32:0)	0.837	0.008
	PC(32:2)	PC(32:1)	0.703	0.015
	PC(34:1)	PC(34:0)	0.821	0.008
	PC(34:2)	PC(34:1)	0.814	0.004
	PC(34:3)	PC(34:2)	0.810	0.005
	PC(36:2)	PC(36:1)	0.827	0.008
	PC(36:3)	PC(36:2)	0.820	0.004
	PE(32:1)	PE(32:0)	0.826	0.005
	PE(32:2)	PE(32:1)	0.788	0.031
	PE(34:1)	PE(34:0)	0.820	0.023
	PE(34:2)	PE(34:1)	0.817	0.003
	PE(34:3)	PE(34:2)	0.804	0.006
	PE(36:2)	PE(36:1)	0.827	0.010
	PE(36:3)	PE(36:2)	0.810	0.007
	PE(P-34:1)	PE(P-34:0)	0.828	0.014
	PE(P-34:2)	PE(P-34:1)	0.817	0.012
	PE(P-34:3)	PE(P-34:2)	0.863	0.016
+1 DB (Δ1)	PC(P-36:1)	PC(O-36:1)	0.977	0.026
	PC(P-36:2)	PC(O-36:2)	0.955	0.021
	PC(P-36:4)	PC(O-36:4)	0.955	0.012
	PC(P-38:4)	PC(O-38:4)	0.949	0.018
	PC(P-38:6)	PC(O-38:6)	0.962	0.005

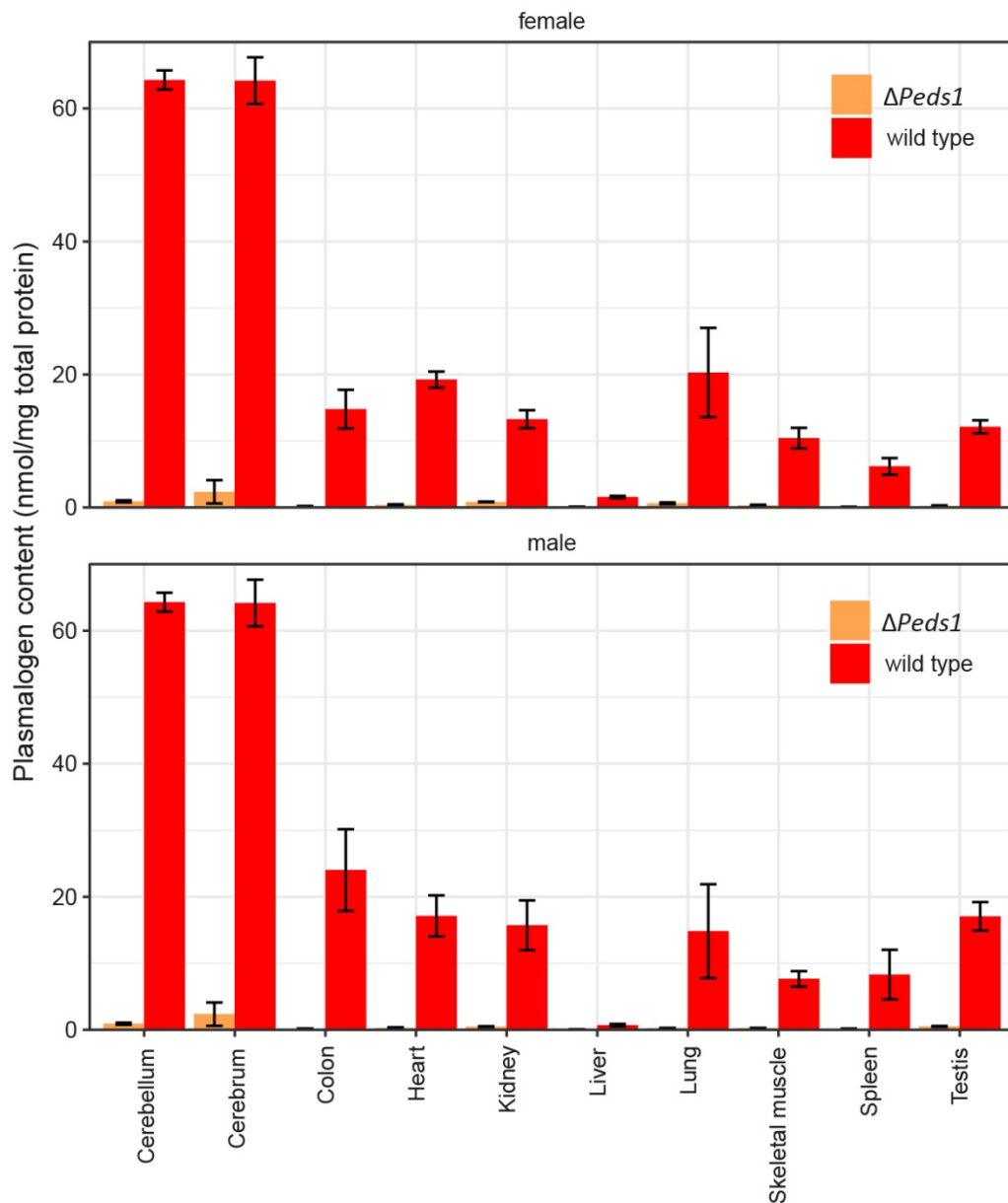|	PC(P-40:6)	PC(O-40:6)	0.957	0.012
	PC(P-40:8)	PC(O-40:8)	0.966	0.031
	PE(P-34:0)	PE(O-34:0)	0.977	0.013
	PE(P-34:1)	PE(O-34:1)	0.987	0.010
	PE(P-34:2)	PE(O-34:2)	0.974	0.008
	PE(P-36:1)	PE(O-36:1)	0.984	0.017
	PE(P-36:2)	PE(O-36:2)	0.981	0.014
	PE(P-36:5)	PE(O-36:5)	0.942	0.017
1-O-alk-1´-enyl/1-O-alkyl	PC(P-34:1)	PC(O-34:2)	1.188	0.018
	PC(P-36:1)	PC(O-36:2)	1.172	0.025
	PC(P-36:2)	PC(O-36:3)	1.181	0.026
	PC(P-36:3)	PC(O-36:4)	1.185	0.027
	PC(P-38:1)	PC(O-38:2)	1.053	0.024
	PC(P-38:5)	PC(O-38:6)	1.140	0.012
	PC(P-38:6)	PC(O-38:7)	1.206	0.014
	PC(P-40:7)	PC(O-40:8)	1.226	0.008
	PE(P-34:0)	PE(O-34:1)	1.193	0.024
	PE(P-34:1)	PE(O-34:2)	1.192	0.018
	PE(P-34:2)	PE(O-34:3)	1.201	0.023
	PE(P-35:1)	PE(O-35:2)	1.177	0.024
	PE(P-36:1)	PE(O-36:2)	1.162	0.031
	PE(P-36:4)	PE(O-36:5)	1.151	0.021
	PE(P-38:1)	PE(O-38:2)	1.117	0.014

Supporting Figures

Figure S1

Figure S1: Plasmalogen content per mg total protein. *Measured using the plasmalogen analysis method reported in ref 14. Data visualized from values in ref 5.*
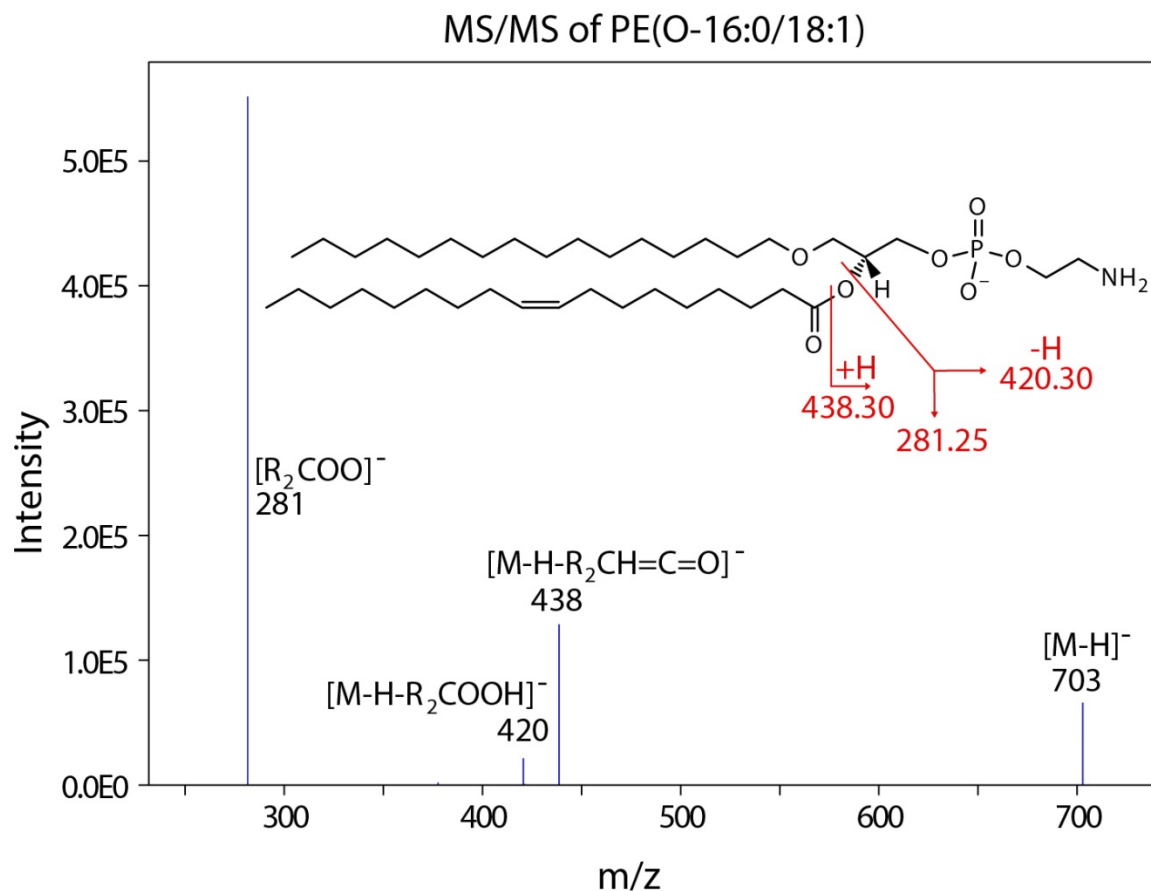
Figure S2

Figure S2: Fragment spectrum of PE(O-16:0/18:1) single lipid standard. Typical fragmentation behavior of plasmanyl and plasmenyl lipids are i) a hydrogen rearrangement and simultaneous neutral loss leading to either a M-H-R$_2$CH=C=O$^-$ (438 m/z) or a M-H-R$_2$-COOH$^-$ (420 m/z) fragment and ii) breakage at the sn-2 position producing a side chain fragment (R$_2$COO$^-$, 281 m/z). R$_2$: sn-2 residue. No fragmentation of the sn-1 position was observed (expected major fragment would be at 255 m/z).