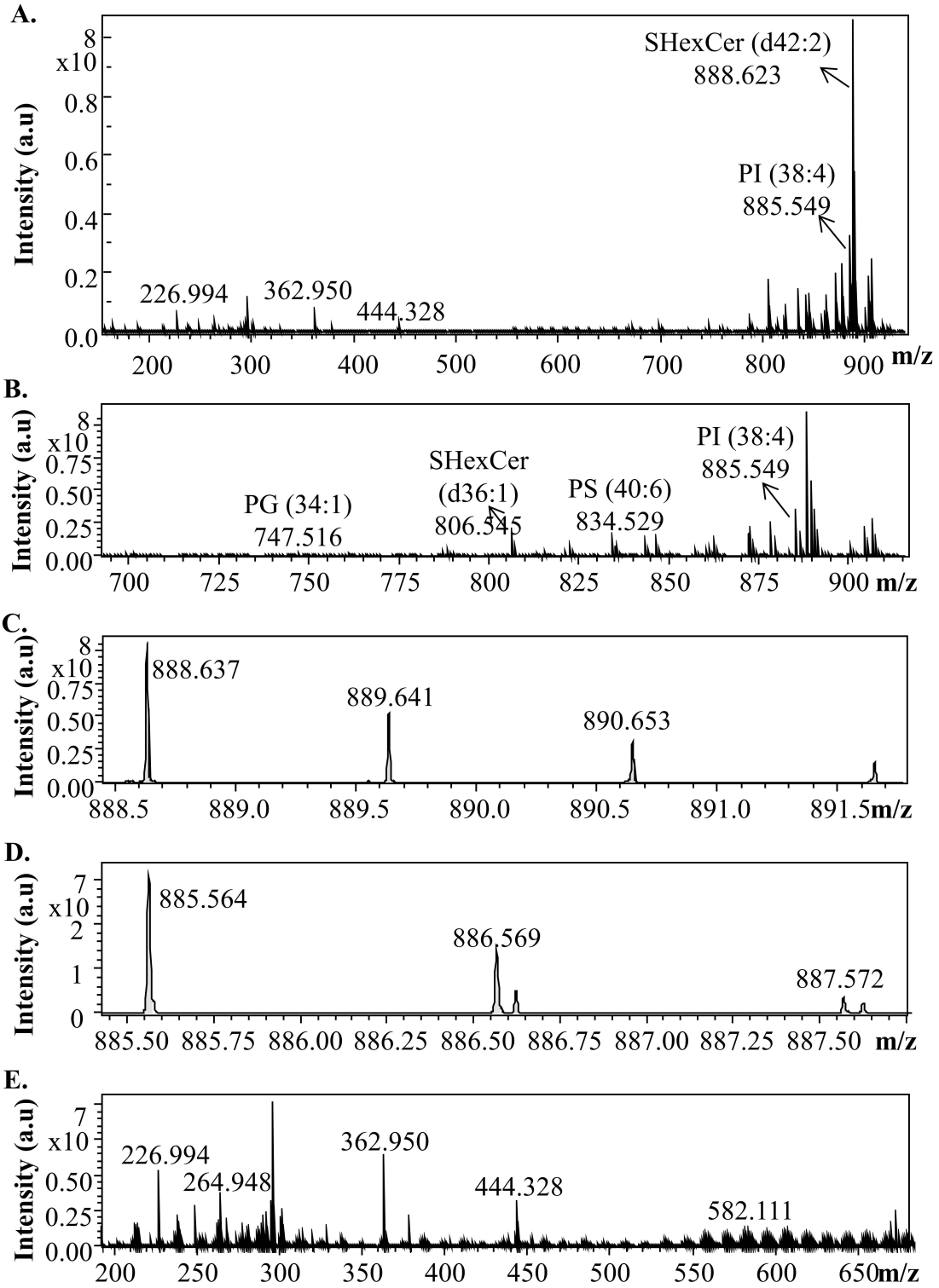
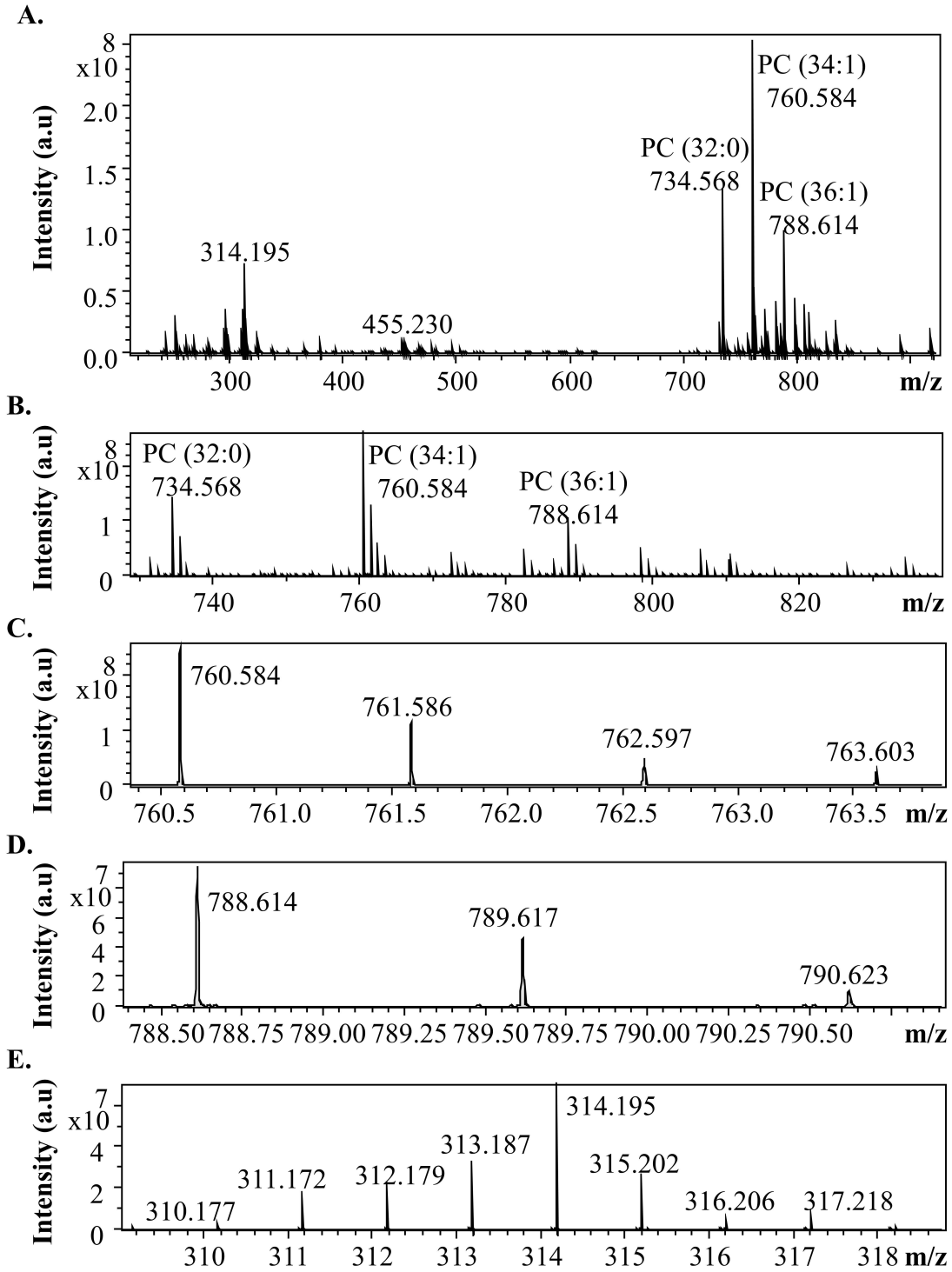


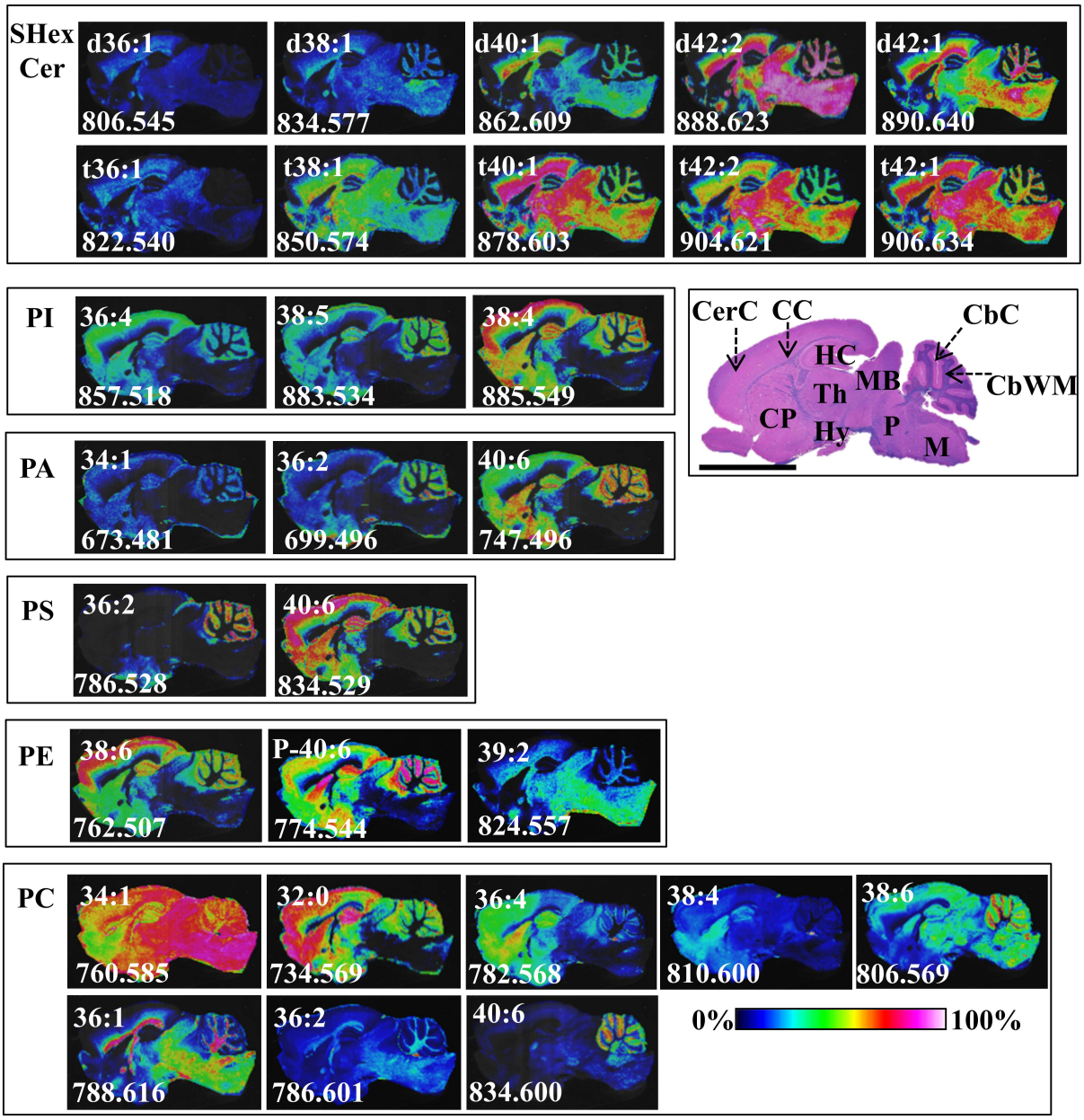
Supplemental Figure 1

Figure S1. A representative average mass spectrum acquired from an entire sagittal section of a normal mouse brain by FTICR based MALDI-IMS in negative mode. (A) Mass (m/z) range 200-900. Lipid ion with m/z 888.6234 assigned to sulfatide, SHexCer (d42:2), was the most intense peak. (B) Magnified view between m/z 700-900 where most lipid associated peaks were detected (C) Isotopic peak pattern of m/z 888.637 that was intensely distributed in the white matter. (D) Isotopic peak pattern of m/z 885.564 assigned to phosphatidylinositol, PI (38:4) that was predominantly detected in the grey matter. (E) Lower m/z range 200-650. Peaks less than 400 m/z have low signal to noise ratios and contains matrix background.



Supplemental Figure 2

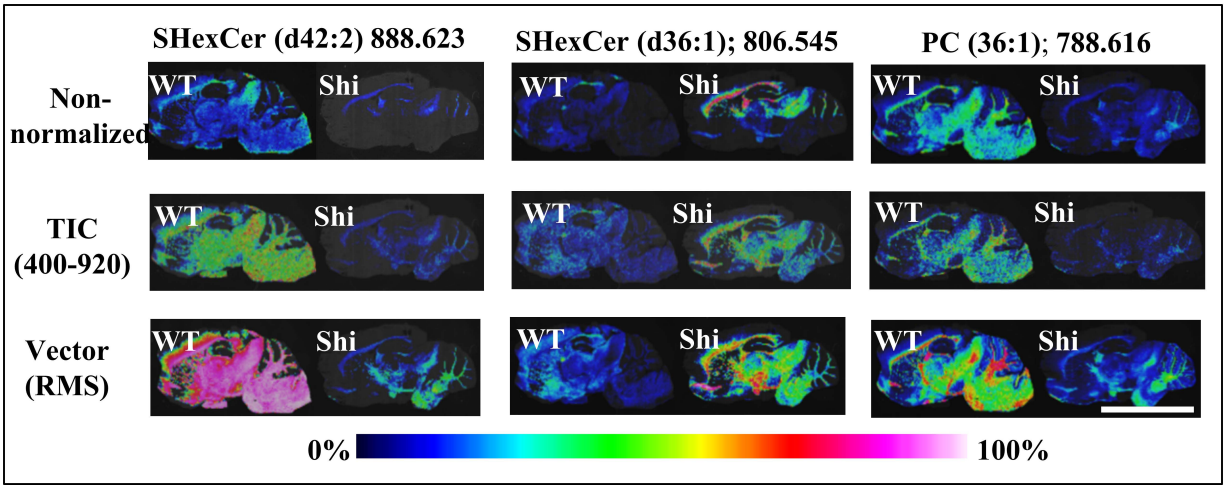
Figure S2. A representative average mass spectrum acquired from an entire sagittal section of a normal mouse brain by FTICR based MALDI-IMS in positive mode. (A) m/z range 200-900. (B) Magnified view of 700-900 m/z range where most lipid associated peaks were detected. Shown are the three predominant phosphatidylcholines (PC) in decreasing order of their intensities: 760.584, PC (34:1); 734.568, PC (32:0); and 788.614, PC (36:1). (C) Isotopic peak pattern of m/z 760.584, a ubiquitous phospholipid distributed in both the grey and white matter. (D) Isotopic peak pattern of m/z 788.614 that was predominantly detected in the white matter. (E) Lower m/z range <400. Peak assignment was not performed due to low signal to noise and presence of large matrix adducts.



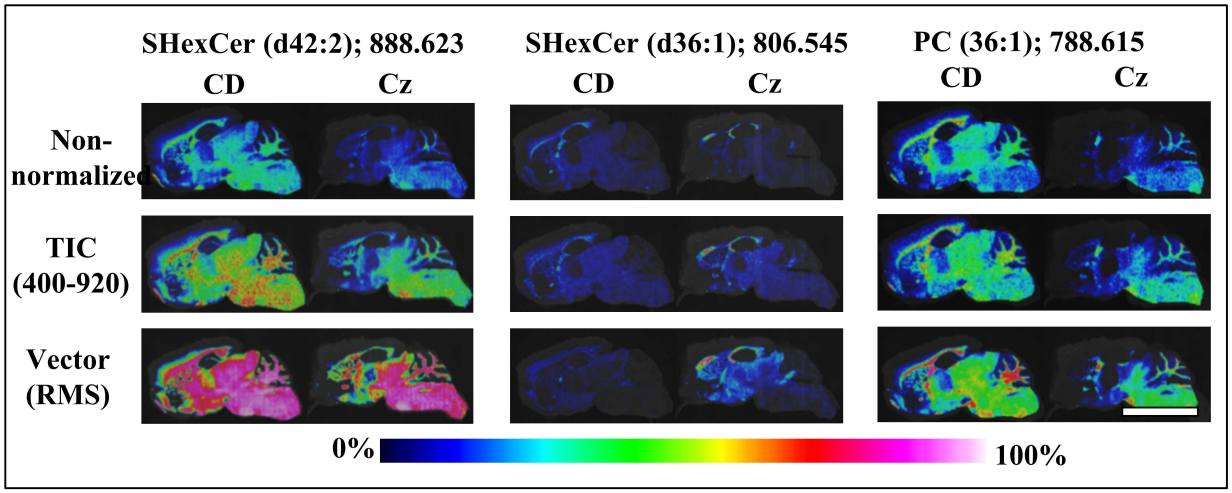
Supplemental Figure 3

Figure S3. FTICR based MALDI-IMS derived spatial distribution pattern of lipids revealed the histological features of the normal mouse brain. Schematic of a sagittal mouse brain section co-stained with LFB and H&E is shown. The following anatomic zones are labeled: cerebral cortex (CerC), corpus callosum (CC), hippocampus (HC), caudate putamen (CP), thalamus (Th), hypothalamus (Hy), midbrain (MB), cerebellar cortex (CbC), cerebellar white matter (CbWM), pons (P) and medulla (M). The outlined image panels display distribution of molecular species that belong to the respective lipid sub-group. The m/z and annotations are noted on individual images of each panel. The annotation represents the total number of carbon atoms present in the head group and fatty acid chain of the respective lipid. The number of double bonds is listed after the colon punctuation. The prefixes 'd' and 't' indicates the presence of non-hydroxylated and hydroxylated fatty acid chain linked to the sulfated ceramide backbone of SHexCer. The SHexCer particularly those with very long chain fatty acids (greater than 40 carbons combined) were distributed in the myelin-enriched regions of the white matter such as the CC, CbWM, MB, Th, P and M. Other lipid ions including PI, PC, PA, PS and PE exhibit a heterogenous distribution pattern in the grey matter regions. The PI (38:4) was intensely detected in CerC, HC and CbC. PA (36:2), PS (36:2) and PC (40:6) were localized in the CbC. PE (39:2) was distributed mainly in the CC, Th, MB, P and M. PC (34:1) was the most intense positive ion and distributed ubiquitously across the various grey matter and white matter regions. PC (36:1) and PC (36:2) show similar distribution patterns and were localized in the CC, CbWM, MB, P and M. PC (32:0) was localized in CerC, CP, HC and CbC and PC (38:4) was confined to CP and HC. Spatial resolution, 80μm; scale bar, 5mm.

A.



B.

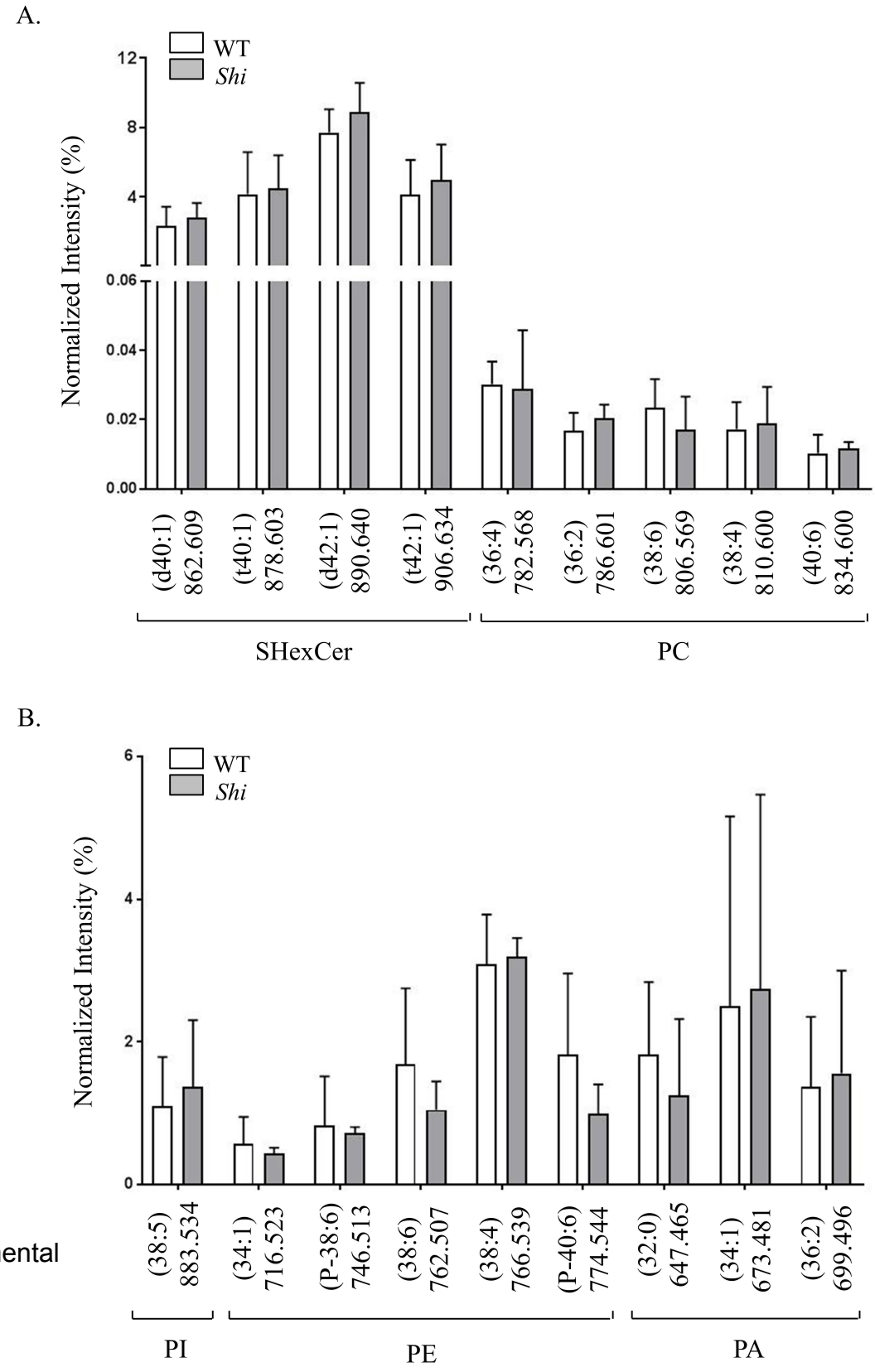


Supplemental Figure 4

Figure S4. Observed lipid distribution pattern in the Shi mouse brain was independent of normalization procedure. The Bruker Daltonics flexImaging software supports Total Ion Current (TIC) and Root-Mean-Square of all peak (RMS) normalization procedures. Mass spectrometric images in columns are the ion distribution maps of SHexCer (d42:2), SHexCer (d36:1) and PC (36:1). Shown in rows are non-normalized, total ion current (TIC; between m/z range 400-920)-normalized and vector (root mean squared, RMS)-normalized MS images.

(A) comparison of lipid species between WT and Shi mice. SHexCer (d42:2) and PC (36:1) have decreased spatial intensities in the Shi mouse; whereas, spatial intensity of SHexCer (d36:1) was increased. Spatial resolution, 80μm; scale bar, 5mm.

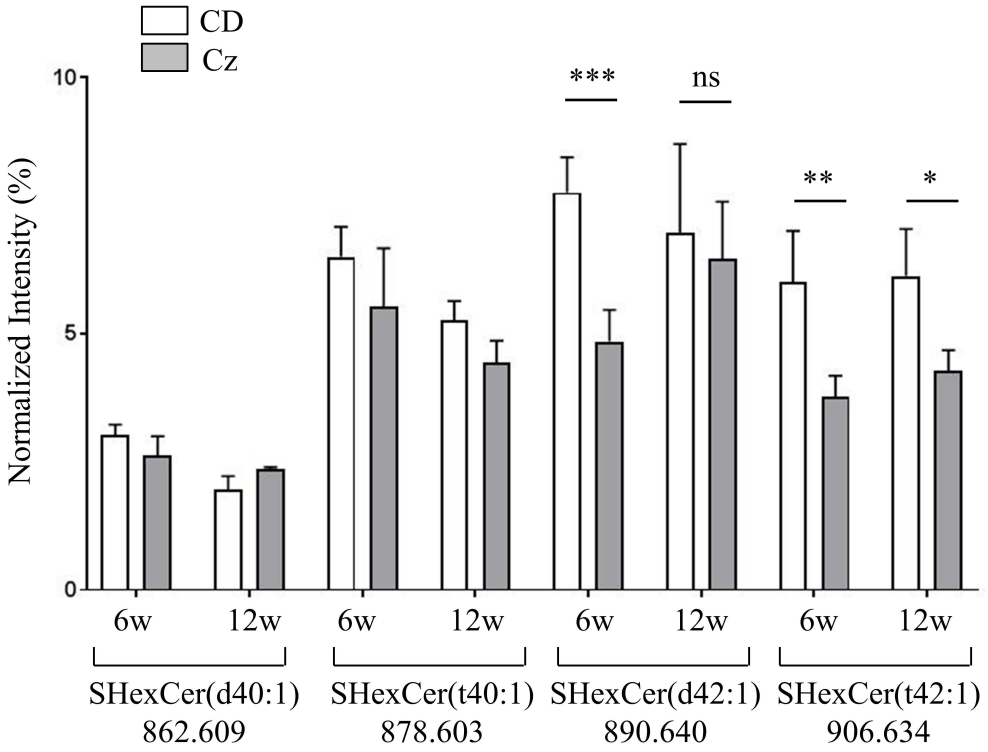
(B) Comparison of WT (chow diet) and cuprizone-fed (at 6w) mice. SHexCer (d42:2) and PC (36:1) have decreased spatial intensities in the Cz-fed mouse; whereas, spatial intensity of SHexCer (d36:1) was increased. Spatial resolution, 80μm; scale bar, 5mm.



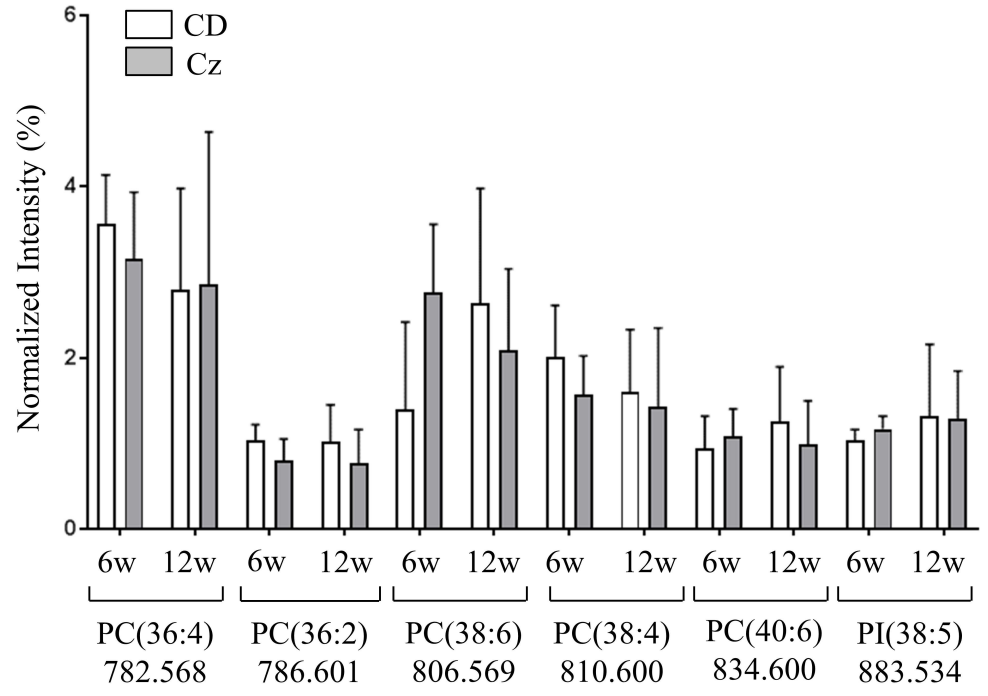
Supplemental Figure 5

Figure S5. Normalized intensity charts of additional sulfatide and phospholipid species that did not differ between the Shi and WT mouse (A-B). There was no significant difference in the normalized intensities of the SHexCer and PC species listed here as well as other phospholipid species that belong to PI, PA and PE between the Shi and WT mouse. SHexCer, sulfatide; PI, phosphatidylinositol; PA, phosphatidic acid; and PE, phosphatidylethanolamine; two-way ANOVA; differences were considered as significant when $p < 0.05$; $n = 5$.

A.

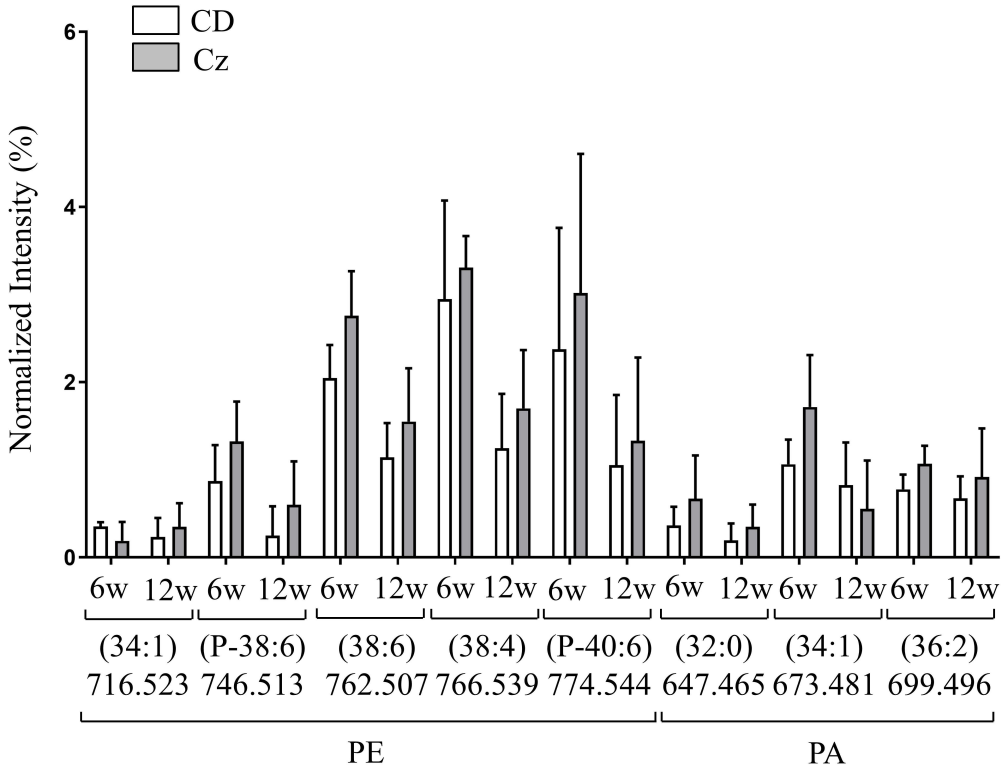


B.



Supplemental Figure 6AB

C.



Supplemental Figure 6C

Figure S6. Normalized intensity charts of additional sulfatide species and phospholipid in the Cz-fed and CD-fed mouse (A-C). A, there was no significant difference in the normalized intensities of SHexCer (d40:1) and SHexCer (t40:1) between the Cz-fed and CD-fed mouse at 6w and 12w. Normalized intensities of SHerCer (d42:1) and SHerCer (t42:1) were significantly decreased at 6w in Cz-mice. By 12w, the intensities of both lipid species restored. However, the intensity of SHexCer (t42:1) remained significantly lower compared to the CD-fed mouse. B, there was no significant difference in the normalized intensities of PC and PI species listed here at both 6w and 12w points between the Cz-fed and CD-fed mouse. C, Normalized intensities of PE and PA also do not significantly differ nor there was a particular trend in the changes between the Cz-fed and Cd-fed mouse at both 6w and 12w. Two-way ANOVA; differences were considered as significant when $p < 0.05$.

m/z (Avg)	RT (Avg)	Matched Mass	Delta	Formula	Ion	**CD-6W (Avg)**	CD-6W (SD)	**Cz-6W (Avg)**	Cz-6W (SD)	Ratio Cz/CD	P value
369.352	11.522	369.3516	-0.00042	C27H45	[M+H-H2O]+	2.86E+08	8.83E+07	2.73E+08	3.92E+07	9.55E-01	0.73
387.363	11.5469	387.3627	-0.00031	C27H47O	[M+H]+	2.79E+06	1.37E+06	2.02E+06	1.11E+06	7.25E-01	0.12
404.389	11.5211	404.3892	0.000239	C27H50ON	[M+NH4]+	2.41E+08	5.42E+07	1.99E+08	3.13E+07	8.28E-01	0.11
409.345	11.6108	409.3446	-0.00037	C27H46ONa	[M+Na]+	3.85E+06	2.43E+06	1.61E+06	6.10E+05	4.19E-01	0.10

m/z = mass; RT = retention time; CD = chow diet (control); Cz = cuprizone diet; Avg = average; SD = standard deviation. Highlighted are average total ion currents.

Supplemental Figure 7

Figure S7. Cholesterol ions were detected with LC-ESI-MS. There was a small reduction in the ion intensities of 6w Cz-fed mouse compared to CD-fed mouse (highlighted columns in the table below). However, there were no statistically significant difference in the cholesterol levels between CD-fed versus 6w Cz-fed mouse.

Table S1. In situ identification of lipids in sagittal mouse brain sections by FTICR based MALDI-IMS in the negative and positive modes using 1,5-DAN as a matrix for sublimation.

Lipid Class	Measured m/z	Calculated m/z	Error (ppm)	Lipid Annotation	Molecular Formula	Ion form	Distribution in Brain
SHexCer	806.545	806.545	0.62	SHexCer(d36:1)	C42H80NO11S	[M-H]-	CC
	822.540	822.540	0.61	SHexCer(t36:1)	C42H80NO12S	[M-H]-	CC
	834.577	834.577	0.72	SHexCer(d38:1)	C44H84NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	850.574	850.572	2.35	SHexCer(t38:1)	C44H84NO12S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	860.593	860.592	1.28	SHexCer(d40:2)	C46H86NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	862.609	862.608	1.97	SHexCer(d40:1)	C46H88NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	864.623	864.624	0.93	SHexCer(d40:0)	C46H90NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	874.608	874.608	0.57	SHexCer(d41:2)	C47H88NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	876.626	876.624	2.97	SHexCer(d41:1)	C47H90NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	878.603	878.603	0.11	SHexCer(t40:1)	C46H88NO12S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	880.619	880.618	0.91	SHexCer(t40:0)	C46H90NO12S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	888.623	888.624	0.68	SHexCer(d42:2)	C48H90NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	890.640	890.639	0.67	SHexCer(d42:1)	C48H92NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	902.641	902.639	1.88	SHexCer(d43:2)	C49H92NO11S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	904.621	904.618	2.54	SHexCer(t42:2)	C48H90NO12S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	906.634	906.634	0.11	SHexCer(t42:1)	C48H92NO12S	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
PI	599.320	599.320	0.00	LPI(18:0)	C27H52O12P	[M-H]-	CerC, HC, CP, CbC
	857.518	857.518	0.00	PI(36:4)	C45H78O13P	[M-H]-	CerC, HC, CP, CbC
	881.519	881.518	1.36	PI(38:6)	C47H78O13P	[M-H]-	CbC, CerC
	883.534	883.534	0.00	PI(38:5)	C47H80O13P	[M-H]-	CerC, HC, CP, CbC
	885.549	885.549	0.00	PI(38:4)	C47H82O13P	[M-H]-	CerC, HC, CP, CbC

SHexCer Sulfatide, PI Phosphatidyl Inositol, CC Corpus Callosum, CerC Cerebral Cortex, HC Hippocampus, CbC Cerebellar Cortex, CbWM Cerebellar White Matter, Th Thalamus, Hy Hypothalamus, CP Caudate Putamen, MB Mid-Brain, P Pons, M medulla

Lipid Class	Measured m/z	Calculated m/z	Error (ppm)	Lipid Annotation	Molecular Formula	Ion form	Distribution in Brain
PG	747.516	747.518	2.54	PG(34:1)	C40H76O10P	[M-H]-	CerC, HC, CP, CbC
PS	788.544	788.544	0.25	PS(36:1)	C42H79NO10P	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	786.528	786.529	0.51	PS(36:2)	C42H77NO10P	[M-H]-	CbC
	810.530	810.529	1.23	PS(38:4)	C44H77NO10P	[M-H]-	MB, Th
	834.529	834.529	0.24	PS(40:6)	C46H77NO10P	[M-H]-	CerC, HC, CbC, CP
PA	647.465	647.465	0.15	PA(32:0)	C35H68O8P	[M-H]-	CerC, HC, CbC
	673.481	673.481	0.74	PA(34:1)	C37H70O8P	[M-H]-	CerC, HC, CbC
	699.496	699.497	0.57	PA(36:2)	C39H72O8P	[M-H]-	CerC, HC, CbC
	701.512	701.512	0.29	PA(36:1)	C39H74O8P	[M-H]-	CC, CbWM, MB, P, M
	747.496	747.497	1.07	PA(40:6)	C43H72O8P	[M-H]-	CerC, HC, CbC
PE	716.523	716.523	0.00	PE(34:1)	C39H75NO8P	[M-H]-	CerC, HC, CbC
	726.544	726.544	0.00	PE(P-36:2)	C41H77NO7P	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	728.559	728.559	0.00	PE(P-36:1)	C41H79NO7P	[M-H]-	CC, CbWM, Th, Hy, MB, P, M,
	742.539	742.539	0.00	PE(36:2)	C41H77NO8P	[M-H]-	CbC, P, M, MB, Th, Hy
	744.554	744.554	0.00	PE(36:1)	C41H79NO8P	[M-H]-	CerC, HC, CbC
	746.513	746.513	0.54	PE(P-38:6)	C43H73NO7P	[M-H]-	CerC, HC, CP, CbC
	750.544	750.544	0.80	PE(P-38:4)	C43H77NO7P	[M-H]-	CerC, HC, CP, CbC
	762.507	762.507	0.00	PE(38:6)	C43H73NO8P	[M-H]-	CerC, HC, CP
	764.523	764.523	0.00	PE(38:5)	C43H75NO8P	[M-H]-	CerC, HC, CP, CbC
	766.539	766.539	0.00	PE(38:4)	C43H77NO8P	[M-H]-	CerC, HC, CP, CbC
	774.544	774.544	0.00	PE(P-40:6)	C45H77NO7P	[M-H]-	CerC, HC, CP, CbC
	776.560	776.559	0.90	PE(P-40:5)	C45H79NO7P	[M-H]-	CerC, HC, CP, CbC
	790.539	790.539	0.00	PE(40:6)	C45H77NO8P	[M-H]-	CerC, HC, CP, CbC
	794.570	794.570	0.00	PE(40:4)	C45H81NO8P	[M-H]-	CerC, HC, CP, CbC
	824.557	824.556	0.85	PE(39:2)	C44H84NO8PK	[M+K]+	CC, CbWM, Th, Hy, MB,

PG Phosphatidyl Glycerol, PS Phosphatidyl Serine, PA Phosphatidic Acid, PE Phosphatidyl- Ethanolamine, CC Corpus Callosum, CerC Cerebral Cortex, HC Hippocampus, CbC, Cerebellar Cortex, CbWM Cerebellar White Matter, Th Thalamus, Hy Hypothalamus, CP Caudate Putamen, MB Mid-Brain, P, Pons; M, medulla

Lipid Class	Measured m/z	Calculated m/z	Error (ppm)	Lipid Annotation	Molecular Formula	Ion form	Distribution in Brain
PC	734.569	734.569	0.14	PC(32:0)	C40H81NO8P	[M+H]+	CerC, HC, CbC
	756.552	756.551	1.06		C40H80NO8PNa	[M+Na]+	
	772.526	772.525	0.91		C40H80NO8PK	[M+K]+	
	732.554	732.553	0.96	PC(32:1)	C40H79NO8P	[M+H]+	CC, CbWM
	770.510	770.509	1.04		C40H78NO8PK	[M+K]+	
	760.585	760.585	0.00	PC(34:1)	C42H83NO8P	[M+H]+	Ubiquitous
	798.541	798.541	0.50		C42H82NO8PK	[M+K]+	
	758.570	758.569	1.05	PC(34:2)	C42H81NO8P	[M+H]+	CC, CbWM
	796.525	796.525	0.50		C42H80NO8PK	[M+K]+	
	788.616	788.616	0.13	PC(36:1)	C44H87NO8P	[M+H]+	CC, CbWM, Th, Hy, MB, P, M
	826.572	826.572	0.48		C44H86NO8PK	[M+K]+	
	786.601	786.600	0.38	PC(36:2)	C44H85NO8P	[M+H]+	CC, CbWM, Th, MB, P, M
	824.557	824.556	0.85		C44H84NO8PK	[M+K]+	
	782.568	782.569	1.28	PC(36:4)	C44H81NO8P	[M+H]+	HC, CP, Th
	804.552	804.551	0.87		C44H80NO8PNa	[M+Na]+	
	820.525	820.525	0.61	PC(36:4)	C44H80NO8PK	[M+K]+	
	810.600	810.600	0.12	PC(38:4)	C46H85NO8P	[M+H]+	HC, CP
	848.556	848.556	0.59		C46H84NO8PK	[M+K]+	
	846.540	846.541	0.24	PC(38:5)	C46H82NO8PK	[M+K]+	HC, CP
	872.556	872.556	0.57		C48H84NO8PK	[M+K]+	
	870.540	870.541	0.69	PC(40:7)	C48H82NO8PK	[M+K]+	CC, CbWM, MB, P, M
	806.569	806.569	0.12	PC(38:6)	C46H81NO8P	[M+H]+	CbC, CerC, Th, Hy, MB
	828.551	828.551	0.00		C46H80NO8PNa	[M+Na]+	
	844.525	844.525	0.36		C46H80NO8PK	[M+K]+	
	834.600	834.600	0.12	PC(40:6)	C48H85NO8P	[M+H]+	CbC, MB, P, M
	856.582	856.582	0.12		C48H84NO8PNa	[M+Na]+	

PC Phosphatidyl Choline, CC Corpus Callosum, CerC Cerebral Cortex, HC Hippocampus, CbC Cerebellar Cortex, CbWM Cerebellar White Matter, Th Thalamus, Hy Hypothalamus, CP Caudate Putamen, MB Mid-Brain, P Pons, M medulla

Table S2. Lipid identification by tandem MS/MS.

Parent ion (m/z)	Ion form	Fragment ion	Lipid Annotation	Tentative Molecular Formula
888.625	$(M-H)^-$		SHexCer (C18:1/ 24:1)	$C_{48}H_{91}O_{11}NS$
		241.002		$C_6H_9O_8S$
860.592	$(M-H)^-$		SHexCer (C18:1/22:1)	$C_{46}H_{87}O_{11}NS$
		241.002		$C_6H_9O_8S$
862.608	$(M-H)^-$		SHexCer (C18:1/ 22:0)	$C_{46}H_{89}O_{11}NS$
		241.002		$C_6H_9O_8S$
806.54520	$(M-H)^-$		SHexCer (d18:0 18:0)	$C_{42}H_{80}O_{11}NS$
		241.002		$C_6H_9O_8S$
		255.233		$C_{16}H_{31}O_2$
904.619	$(M-H)^-$		SHexCer (C18:1/ h24:1)	$C_{48}H_{91}O_{12}NS$
		241.002		$C_6H_9O_8S$
		522.275		$C_{24}H_{44}O_9NS$
		554.300		$C_{25}H_{48}O_{10}NS$
		568.280		$C_{25}H_{47}O_{11}NS$
906.6351	$(M-H)^-$		SHexCer (C18:1/ h24:0)	$C_{48}H_{93}O_{12}NS$
		241.002		$C_6H_9O_8S$
		522.275		$C_{24}H_{44}O_9NS$
		554.300		$C_{25}H_{48}O_{10}NS$
		568.280		$C_{25}H_{47}O_{11}NS$
834.53	$(M-H)^-$		PS (40:6)	$C_{46}H_{77}NO_{10}P$
		283.264		$C_{18}H_{35}O2$
716.5242	$(M-H)^-$		PE (34:1)	$C_{39}H_{75}NO_8P$
		255.233		$C_{16}H_{31}O_2$
		281.248		$C_{18}H_{33}O_2$
744.5555	$(M-H)^-$		PE (36:1)	$C_{41}H_{79}NO_8P$
		281.248		$C_{18}H_{33}O_2$
		283.264		$C_{18}H_{35}O2$
742.5414	$(M-H)^-$		PE (36:2)	$C_{41}H_{77}NO_8P$
		281.248		$C_{18}H_{33}O_2$
774.545	$(M-H)^-$		PE (O-40:6)	$C_{45}H_{77}NO_7P$
		196.038		$C_5H_{11}O_5PN$
		255.233		$C_{16}H_{31}O_2$
		267.269		$C_{18}H_{35}O$
747.5191	$(M-H)^-$		PG (34:1)	$C_{40}H_{76}O_{10}P$
		255.233		$C_{16}H_{31}O_2$
		281.248		$C_{18}H_{33}O_2$
885.55130	$(M-H)^-$		PI (O-38:4(OH))	$C_{47}H_{82}O_{13}P$
		223.001		$C_6H_8O_7P$
		241.012		$C_6H_{10}O_8P$
		259.243		$C_{12}O_3H_{36}P$
		283.264		$C_{18}H_{35}O_2$
		297.038		$C_9H_{14}O_9P$
		303.233		$C_{13}O_5H_{36}P$

SHexCer Sulfatide, PS Phosphatidyl Serine, PE Phosphatidyl Ethanolamine, PG Phosphatidyl Glycerol, PS Phosphatidyl Inositol