

Supplementary Tables

Supplementary Table S1 Growth parameters of C57BL/6J mice fed experimental diets

	Normal	Control	SCG treated
Food intake (g per d)	2.84 ± 0.24	2.97 ± 0.18	2.92 ± 0.19
Initial body weight (g)	28.36 ± 0.45	27.87 ± 0.39	27.96 ± 0.25
Eventual body weight (g)	32.91 ± 0.73	33.87 ± 0.66	32.47 ± 0.52

Note: Results are expressed as means ± SEM; n = 6 mice per group.

Supplementary Table S2 Standard curve, intra- and inter-day RE and RSD, recovery of sphingolipid standards

Sphingolipid	Linear equation	R	precision, accuracy							Recovery (%)
			(nmol/mL)	Intra-day			Inter-day			
				means±SD (nmol/mL)	RE (%)	RSD (%)	means±SD (nmol/mL)	RE (%)	RSD (%)	
Cer(d18:1-C18:0)	y=0.0054x+0.0625	0.998	0.4	0.41±0.05	4.3	4.8	0.42±0.10	3.2	4.3	94.6
			1.6	1.66±0.14	6.2	5.9	1.63±0.24	4.5	6.5	95.3
GalCer(d18:1-C24:1)	y=0.0042x-0.0908	0.997	4	3.94 ± 0.34	7.9	6.3	3.92 ± 0.15	2.9	3.9	98.9
			16	16.74 ± 2.02	7.8	4.7	15.81 ± 2.01	6.4	5.2	97.8
ST(d18:1-C24:1)	y=0.4178x+0.0164	0.999	2	2.13 ± 0.04	3.7	4.5	2.12 ± 0.97	5.6	3.7	91.1
			8	8.23 ± 0.15	5.7	6.3	8.29 ± 1.43	3.0	5.3	89.9
SM(d18:1-C16:0)	y=0.0518x+0.0037	0.998	4	3.96 ± 0.36	4.7	7.6	4.12 ± 0.34	7.8	4.2	95.1
			16	15.94 ± 0.38	5.3	5.9	16.94 ± 0.26	6.2	3.5	95.4

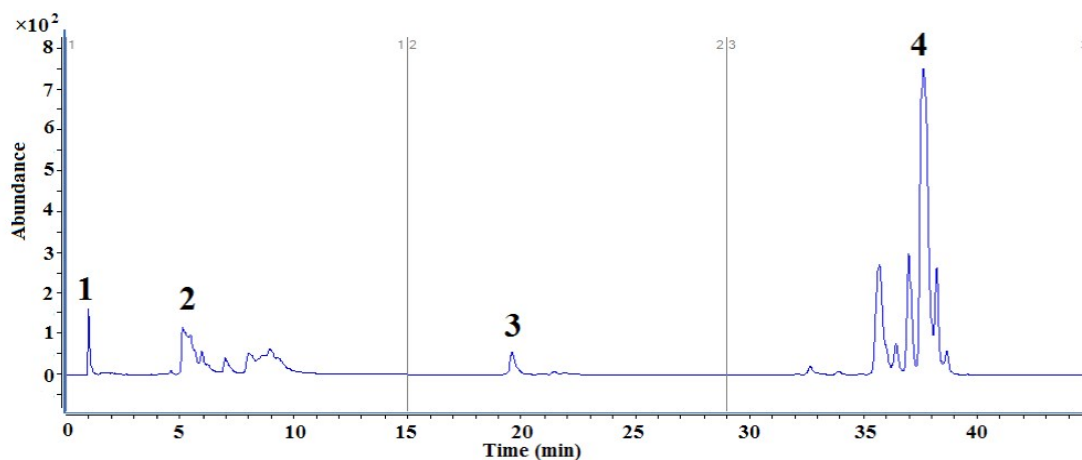
Supplementary Table S3. Identification of Cer, ST and GalCer species in normal mice brain by RPLC-MS/MS

Molecular species	Precursor ion (<i>m/z</i>)	RT (min)	Molecular species	Precursor ion (<i>m/z</i>)	RT (min)
Cer(d18:1-C16:0)+H	538.5	9.19	GalCer(d18:1-C16:0)+H	700.6	7.60
Cer(d18:1-C18:0h)+H	582.5	9.59	GalCer(d18:1-C18:1h)+H	742.6	7.34
Cer(d18:1-C18:1)+H	564.5	9.60	GalCer(d18:1-C18:1)+H	726.6	8.44
Cer(d18:1-C17:0)+H	552.6	11.54	GalCer(d18:1-C18:0h)+H	744.6	10.32
Cer(d18:1-C18:0)+H	566.5	14.45	GalCer(d18:1-C18:0)+H	728.6	11.67
Cer(d18:1-C20:1)+H	592.6	15.13	GalCer(d18:1-C20:1h)+H	770.7	11.18
Cer(d18:1-C20:0)+H	594.6	22.99	GalCer(d18:1-C20:1)+H	754.7	12.51
Cer(d18:1-C22:1)+H	620.6	23.85	GalCer(d18:1-C20:0h)+H	772.7	16.16
Cer(d18:1-C23:1)+H	634.6	29.78	GalCer(d18:1-C22:1h)+H	798.8	17.13
Cer(d18:1-C24:1h)+H	664.6	32.06	GalCer(d18:1-C20:0)+H	756.7	18.32
Cer(d18:1-C22:0)+H	622.6	37.27	GalCer(d18:1-C22:1)+H	782.7	19.48
Cer(d18:1-C24:0h)+H	666.6	37.91	GalCer(d18:1-C23:1h)+H	812.8	21.87
Cer(d18:1-C24:1)+H	648.6	39.33	GalCer(d18:1-C23:1)+H	796.7	24.32
Cer(d18:1-C23:0)+H	636.6	47.44	GalCer(d18:1-C22:0h)+H	800.8	25.55
Cer(d18:1-C24:0)+H	650.6	58.36	GalCer(d18:1-C24:1h)+H	826.8	26.80
ST(d18:1-C16:0)-H	778.2	2.37	GalCer(d18:1-C22:0)+H	784.7	29.28
ST(d18:1-C18:0h)-H	822.6	2.85	GalCer(d18:1-C24:1)+H	810.8	30.65
ST(d18:1-C18:0)-H	806.6	3.17	GalCer(d18:1-C23:0h)+H	814.8	32.46
ST(d18:1-C20:0h)-H	850.6	3.98	GalCer(d18:1-C25:1h)+H	840.8	33.85
ST(d18:1-C20:0)-H	834.6	4.58	GalCer(d18:1-C23:0)+H	798.8	37.14
ST(d18:1-C22:0h)-H	878.7	5.76	GalCer(d18:1-C25:1)+H	824.8	38.81
ST(d18:1-C24:1h)-H	904.8	5.95	GalCer(d18:1-C24:0h)+H	828.8	41.27
ST(d18:1-C22:0)-H	862.6	6.29	GalCer(d18:1-C26:1h)+H	854.8	42.38
ST(d18:1-C24:1)-H	888.8	6.42	GalCer(d18:1-C24:0)+H	812.8	47.28
ST(d18:1-C24:0h)-H	906.8	8.47	GalCer(d18:1-C26:1)+H	838.8	49.30
ST(d18:1-C26:1h)-H	932.6	8.99	GalCer(d18:1-C25:0h)+H	842.8	52.58
ST(d18:1-C24:0)-H	890.8	9.32	GalCer(d18:1-C25:0)+H	826.8	59.03
ST(d18:1-C26:1)-H	916.8	9.796	GalCer(d18:1-C26:0)+H	840.8	73.26
ST(d18:1-C26:0h)-H	934.8	13.05	GalCer(d18:1-C26:0h)+H	856.8	67.37
ST(d18:1-C26:0)-H	918.6	13.11			

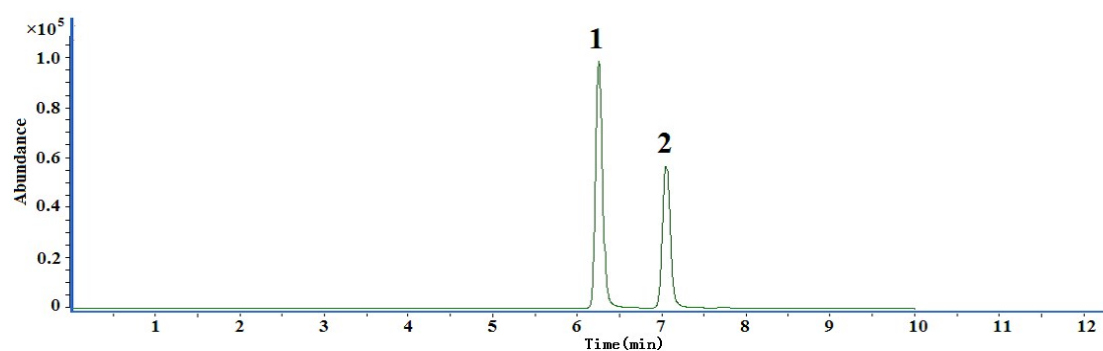
Supplementary Table S4 Standard curve, LOD, LOQ, intra- and inter-day RE and RSD, recovery of GluCer(d18:1-C12:0) and GalCer(d18:1-C12:0)

Sphingolipid	Linear equation	<i>R</i>	LOD (S/N = 3) (pmol/mL)	LOQ (S/N = 10) (pmol/mL)	Precision, accuracy						Recovery (%)	
					(pmol/mL)	Intra-day			Inter-day			
						means±SD (pmol/mL)	RE (%)	RSD (%)	means±SD (pmol/mL)	RE (%)		RSD (%)
GluCer (d18:1-C12:0)	y=7.875x-1.0908	0.999	10	20	40	41.2±3.2	4.4	4.4	39.4±2.1	3.2	4.3	83.2
					160	162.3±5.8	6.0	6.9	163.1±7.4	4.5	6.5	82.9
GalCer (d18:1-C12:0)	y=4.328x-0.5349	0.999	10	20	40	39.7±2.3	3.0	4.7	39.1±3.9	4.7	5.5	85.0
					160	155.1±4.2	2.5	3.3	158.6±6.4	6.8	7.7	87.5

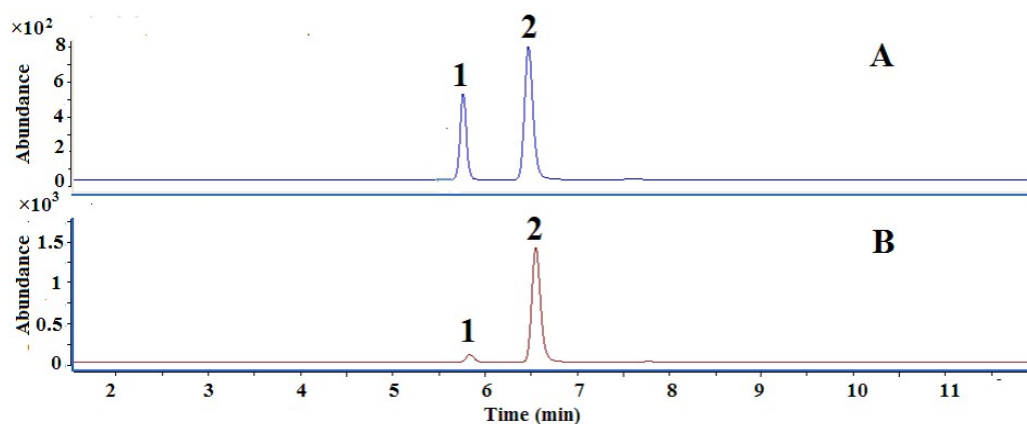
Supplementary Figures



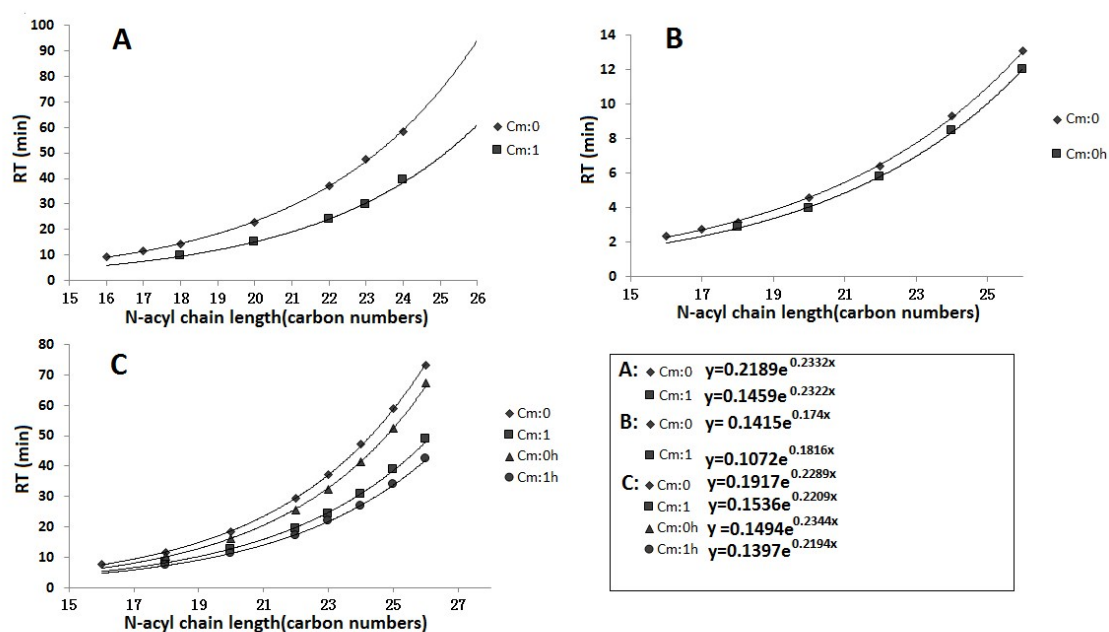
Supplementary Fig. S1 LC ESI-MS/MS elution profiles for the sphingolipids in mice brains of normal group on normal phase liquid chromatography. Elution of complex sphingolipids in the extract of normal mice brain in positive ion mode for Cer, GalCer and SM then negative ion mode for ST. Peak 1: Cer; Peak 2: GalCer; Peak 3: ST; Peak 4: SM



Supplementary Fig. S2 Separation of GluCer(d18:1-12:0) and GalCer(d18:1-12:0) standards by HILIC. Separation of GluCer(d18:1-12:0) and GalCer(d18:1-12:0) standards using a Waters Atlantis HILIC Silica (4.6 mm \times 150 mm, 5 μ m) column with the elution system acetonitrile-methanol- H_2O (95/2.5/2.5, v/v) including 2% formic acid and 2 mM ammonium formate. 1: GluCer(d18:1-C12:0) 6.26 min; 2: GalCer(d18:1-C12:0) 7.13 min



Supplementary Figure S3 Extracted ion chromatograms of GluCer(d18:1-C18:0) and GalCer(d18:1-C18:0) in standard mixtures and mice brain. GluCer and GalCer with the same mass (m/z 728.6) in cerebroside standard mixtures (A) and mice brain (B) were eluted at 5.83 and 6.56 min using MRM mode. 1: GluCer(d18:1-C18:0); 2: GalCer(d18:1-C18:0) (m/z 728.6)



Supplementary Figure S4 Linear relation between N-acyl chain length (carbon numbers) of Cer (A), ST (B) and CBS (C) molecular species and retention time. Cm:0 means sphingolipid with a sphingosine backbone with no double bond, and Cm:1 means a m-carbon N-acyl chain with one double bond; Cm:0h and Cm:1h mean HFA-GalCer.