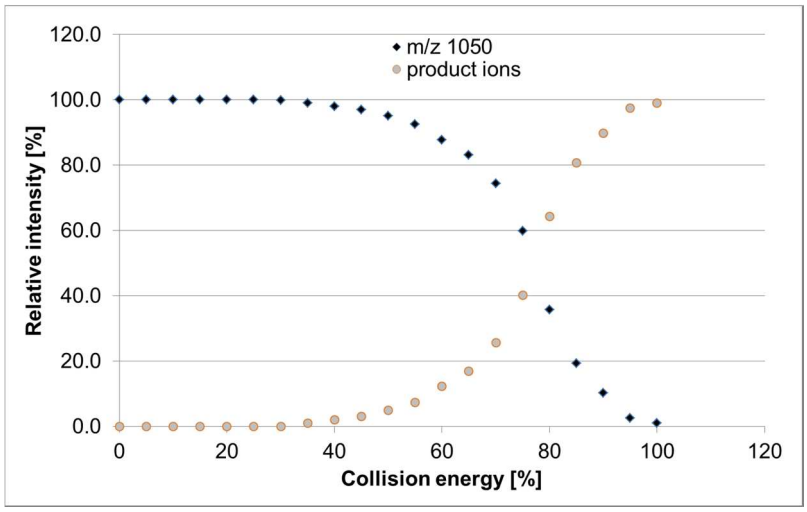
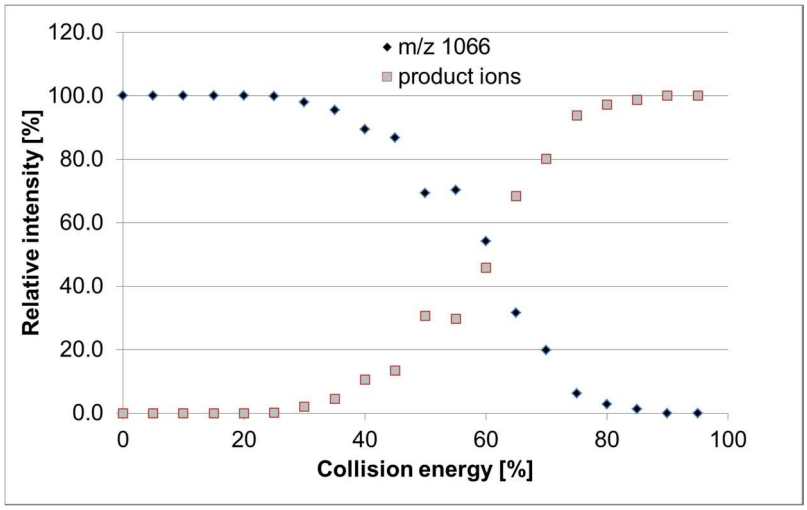
(a)



(b)



(c)

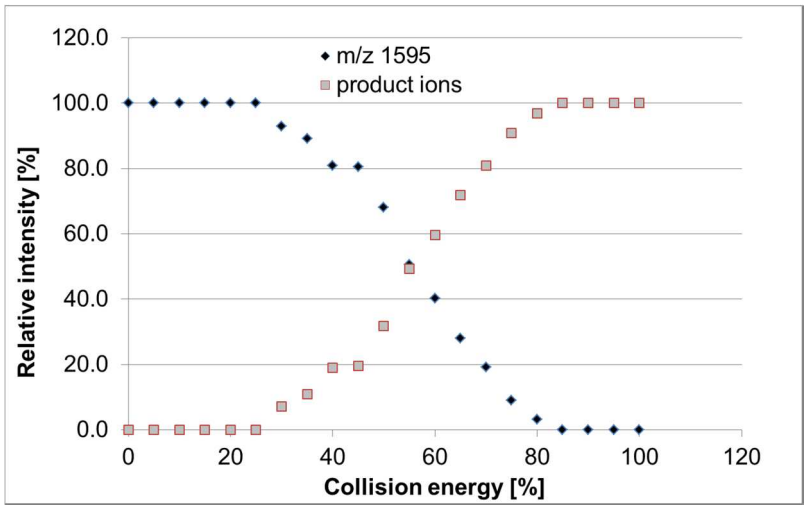


Figure S1. Dependence of fragmentation efficiency on used collision energy of selected sulfoglycosphingolipids: (a) SulfoHex$_2$Cer 42:2 at *m/z* 1050, (b) SulfoHex$_2$Cer 43:1 SulfoHex$_2$Cer 42:2 (OH) at *m/z* 1066, and (c) Sulfo(HexNAc)Hex$_4$Cer 42:1 (OH) at *m/z* 1595

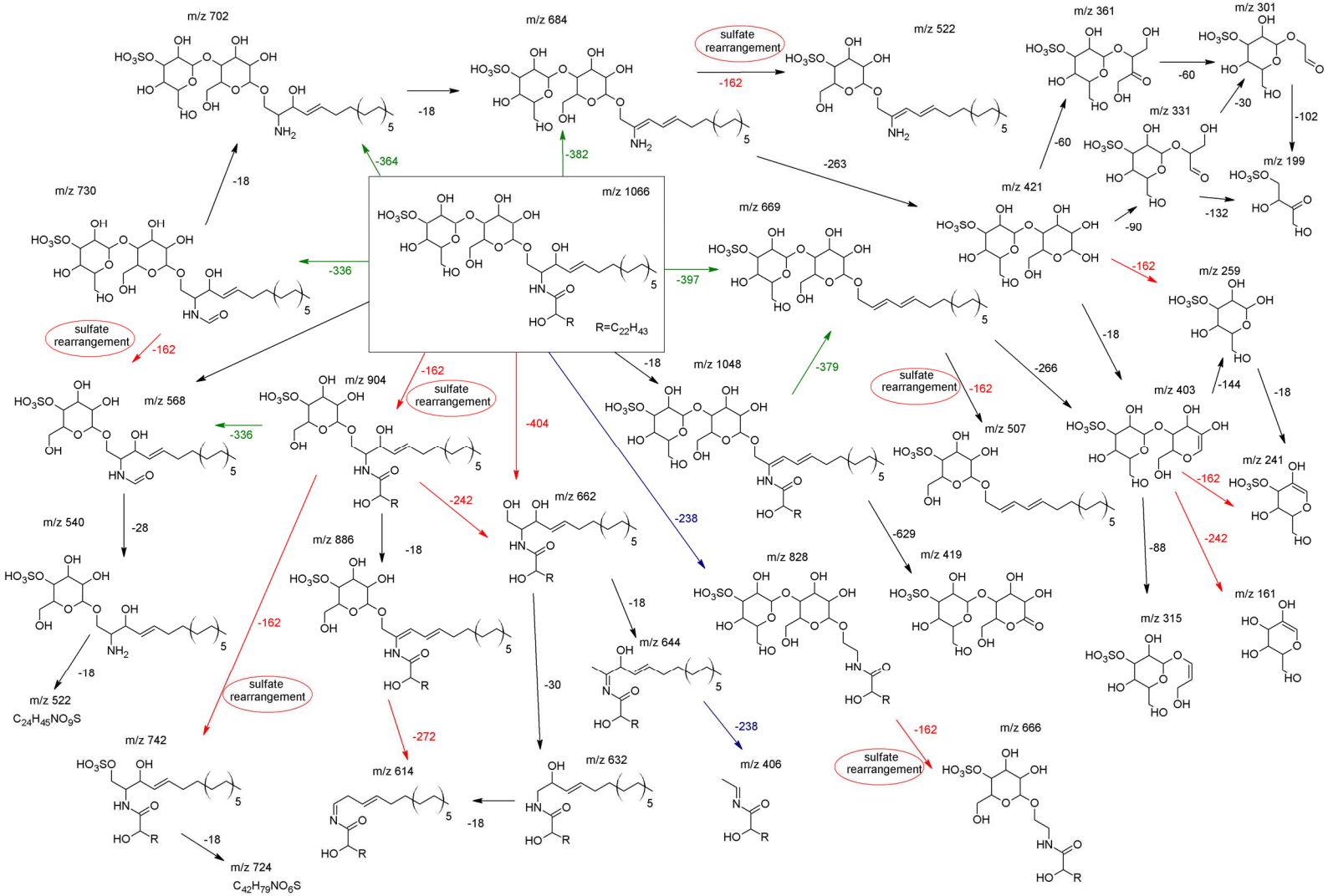


Figure S2. Suggested fragmentation pattern of SulfoHex$_2$Cer d18:1/24:1 (OH) at *m/z* 1066. The color of arrows is associated with NL of particular ion parts, where red color represents NL of saccharide part, green color represents NL of N-acyl part and blue color represents NL of sphingoid base part. Deprotonated molecules are recorded in measured spectra for all shown structures

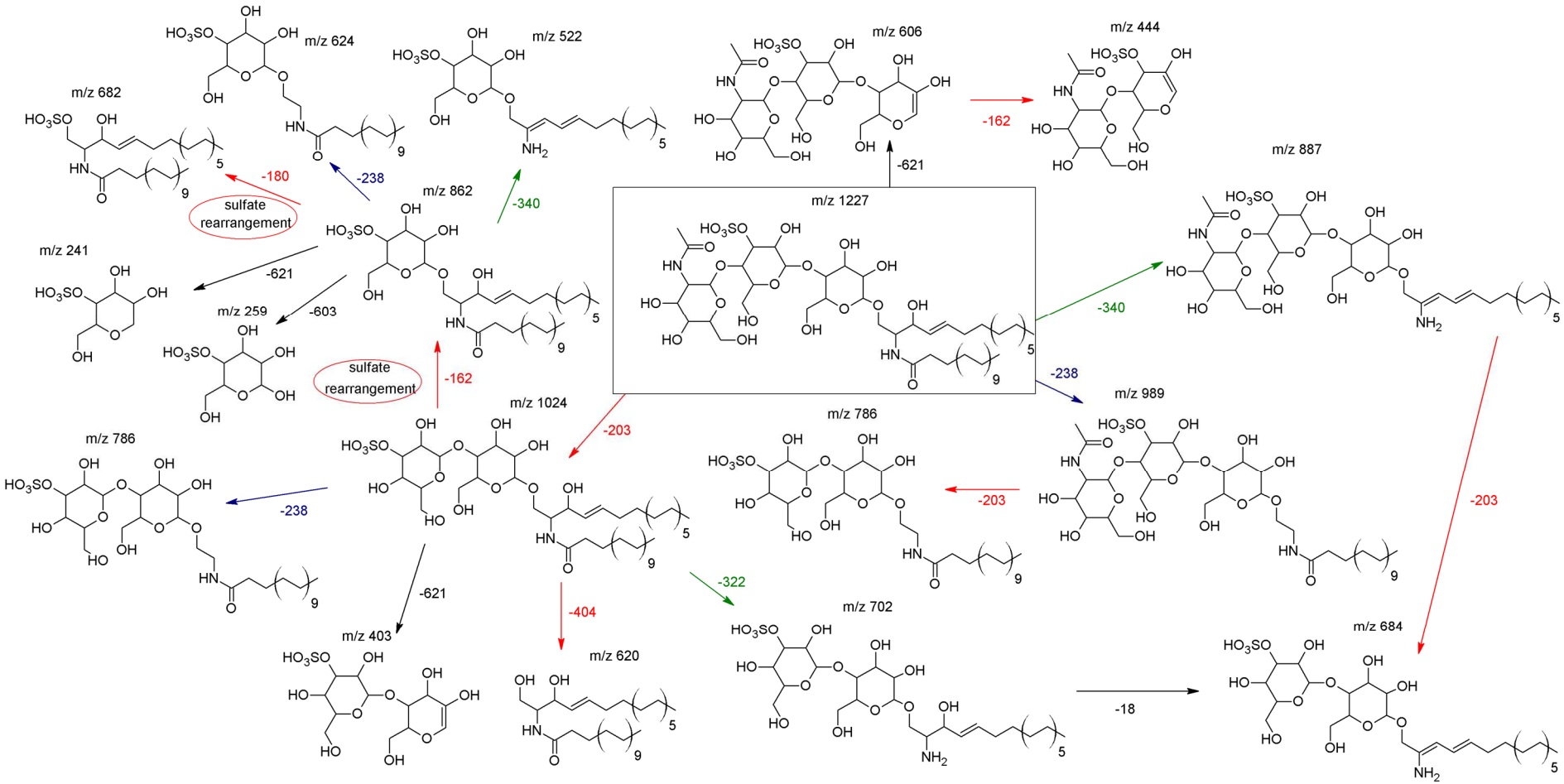


Figure S3. Suggested fragmentation pattern of Sulfo(HexNAc)Hex$_2$Cer d18:1/22:0 at *m/z* 1227. The color of arrows is associated with the NL of particular ion parts, where red color represents NL of saccharide part, green color represents NL of N-acyl part and blue color represents NL of sphingoid base part. Deprotonated molecules are recorded in measured spectra for all shown structures

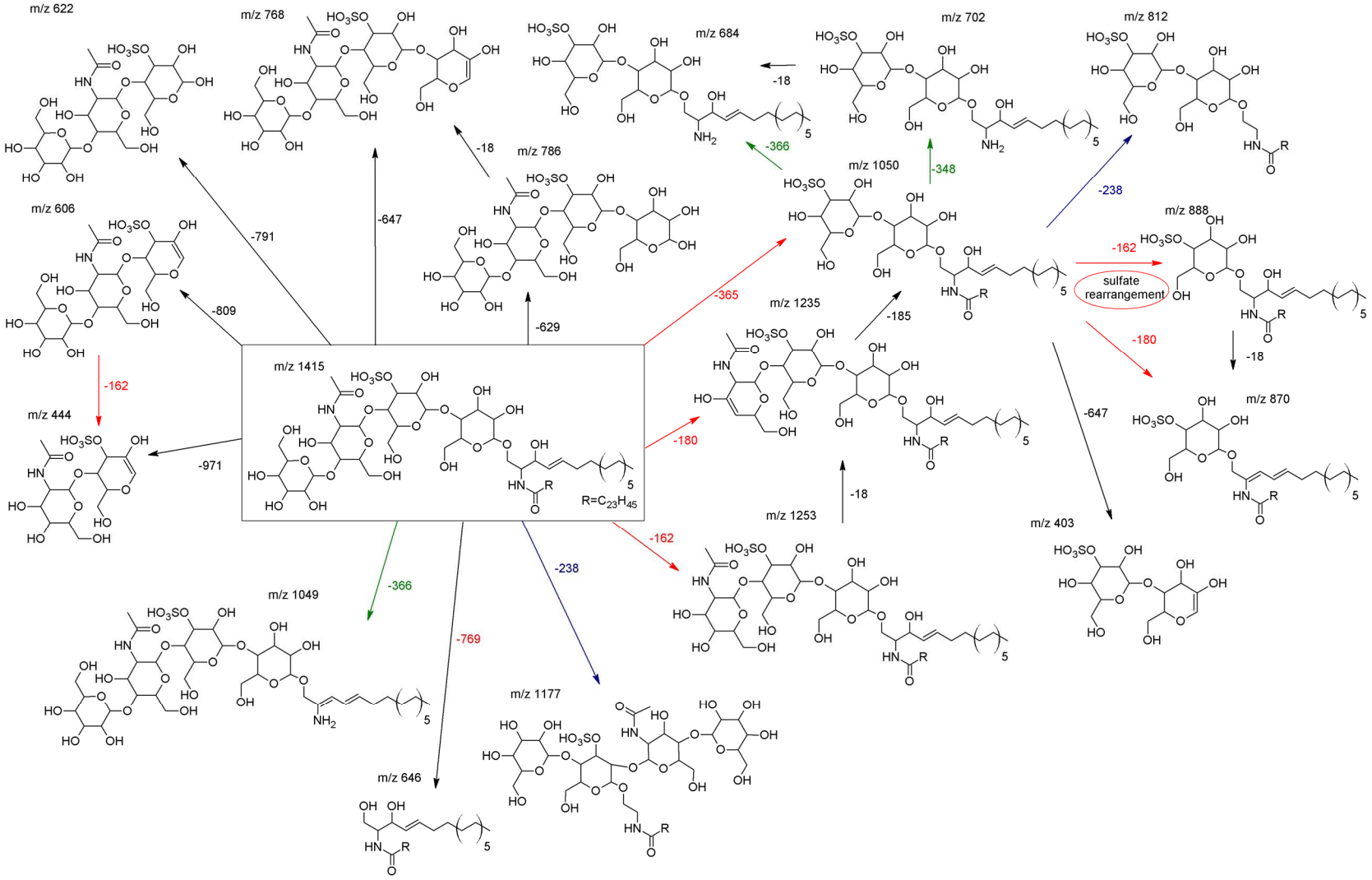


Figure S4. Suggested fragmentation pattern of Sulfo(HexNAc)Hex$_3$Cer d18:1/24:1 at *m/z* 1415. The color of arrows is associated with NL of particular ion parts, where red color represents NL of saccharide part, green color represents NL of N-acyl part and blue color represents NL of sphingoid base part. Deprotonated molecules are recorded in measured spectra for all shown structures

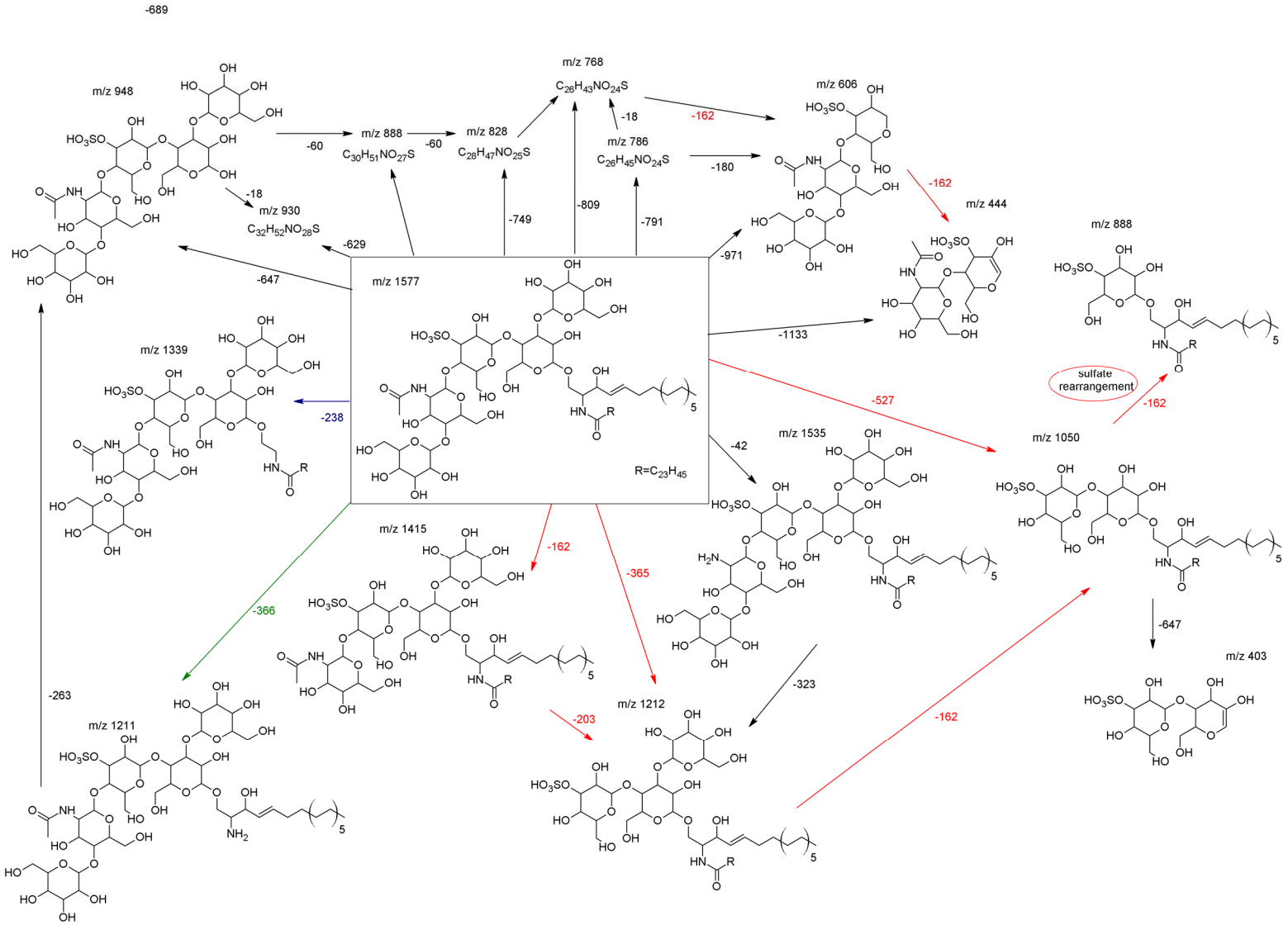


Figure S5. Suggested fragmentation pattern of Sulfo(HexNAc)Hex$_4$Cer d18:1/24:1 at *m/z* 1577. The color of arrows is associated with NL of particular ion parts, where red color represents NL of saccharide part, green color represents NL of N-acyl part and blue color represents NL of sphingoid base part. Deprotonated molecules are recorded in measured spectra for all shown structures

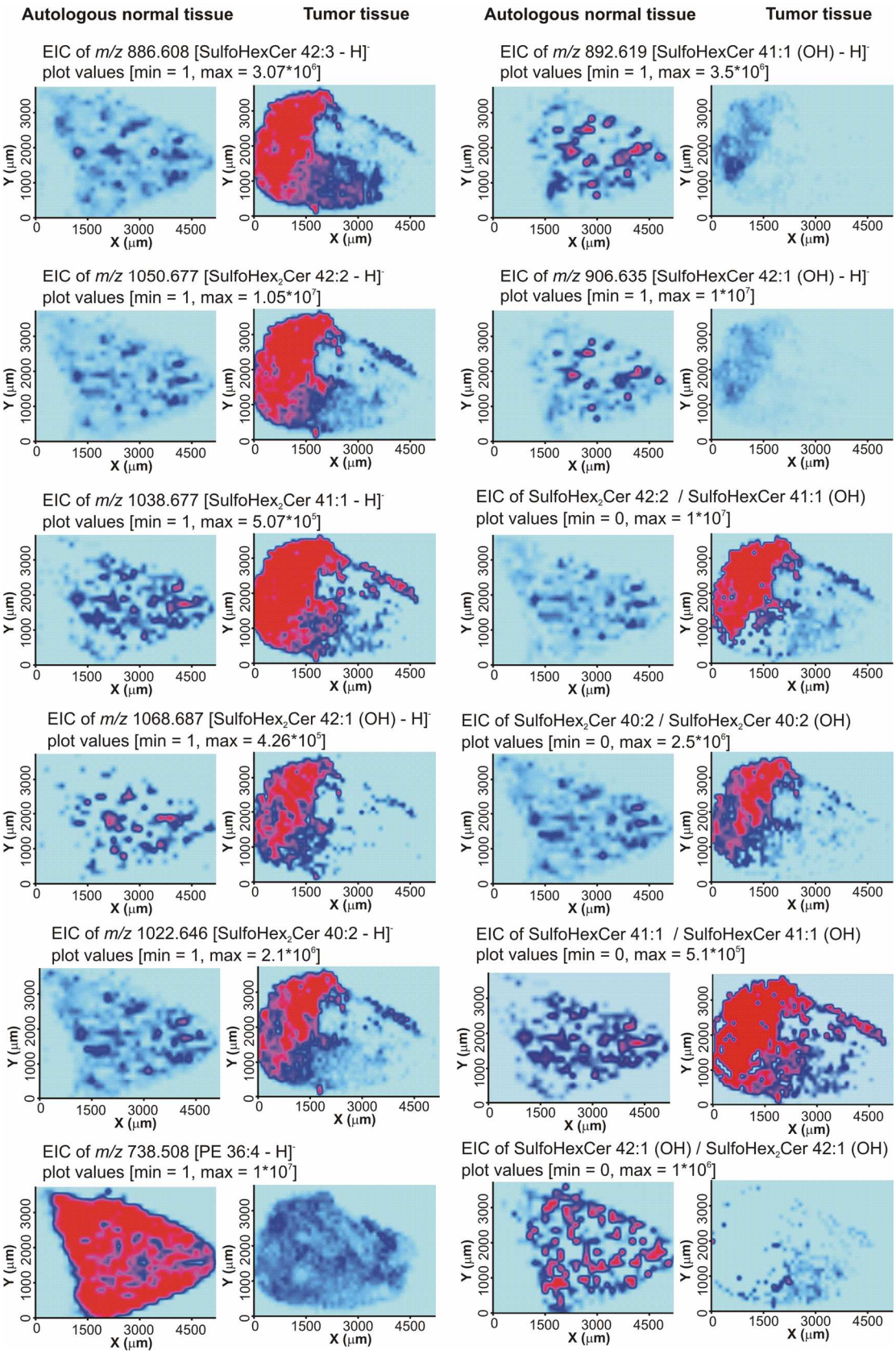


Figure S6. Comparison of EICs of selected lipids or their ratios in separated surgically removed tumor and adjacent normal tissue parts of selected RCC patient. Blue to red style color vizualization was used and intensity values at minimum and maximum are written above particular images

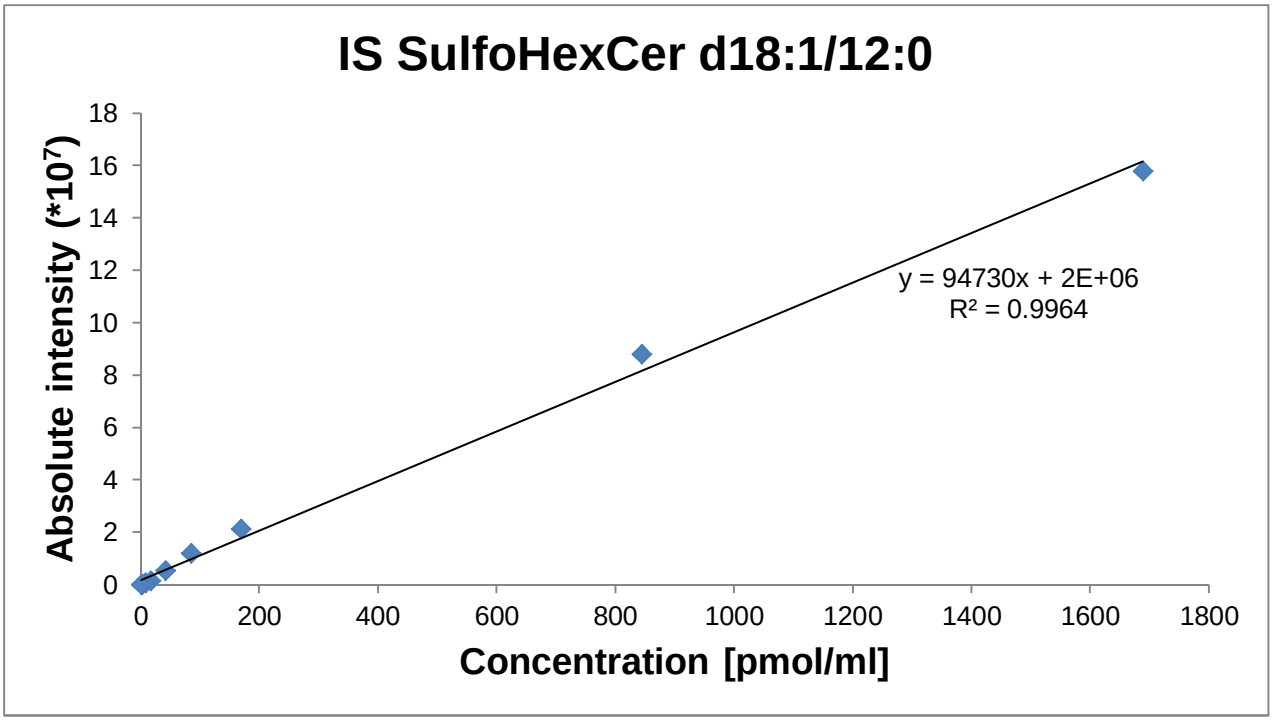


Figure S7. Calibration curve of IS SulfoHexCer d18:1/12:0. Detector saturation was observed for the calibration solution of the concentration above 1688 pmol/ml

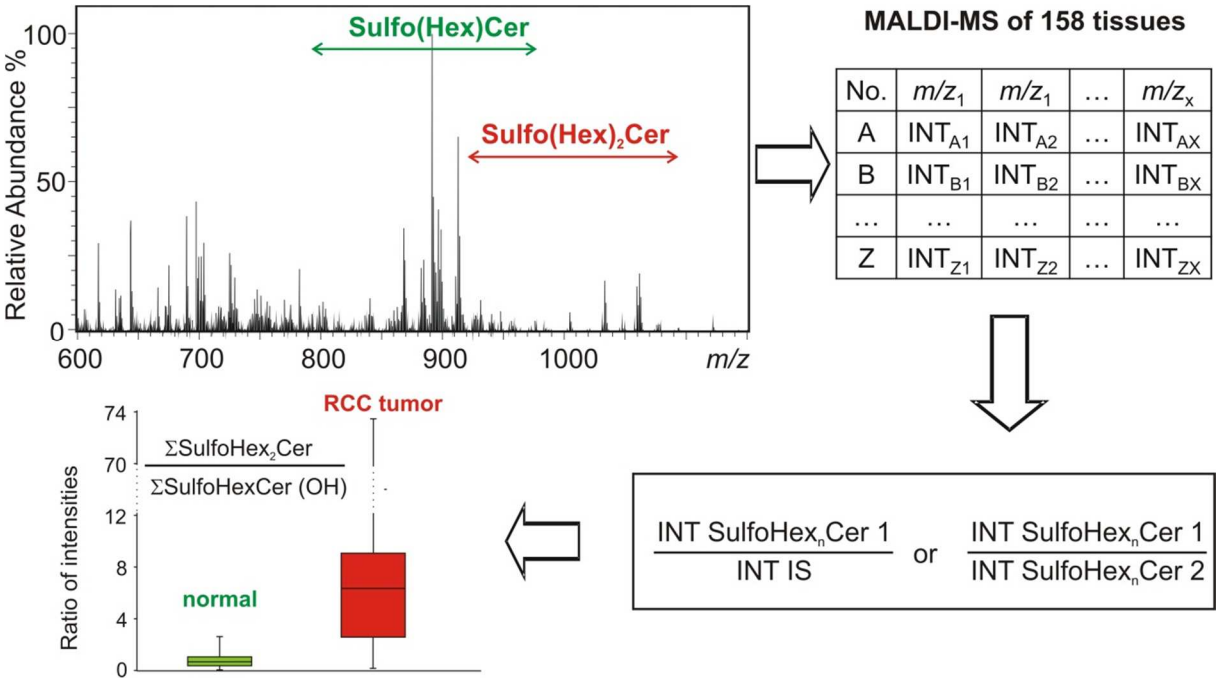


Figure S8. Scheme of MALDI-MS of studied tissues, where the number of tissues is represented by capitals, compared ions are represented by arabic numerals and INT means intensity of ions in averaged full scan mass spectrum

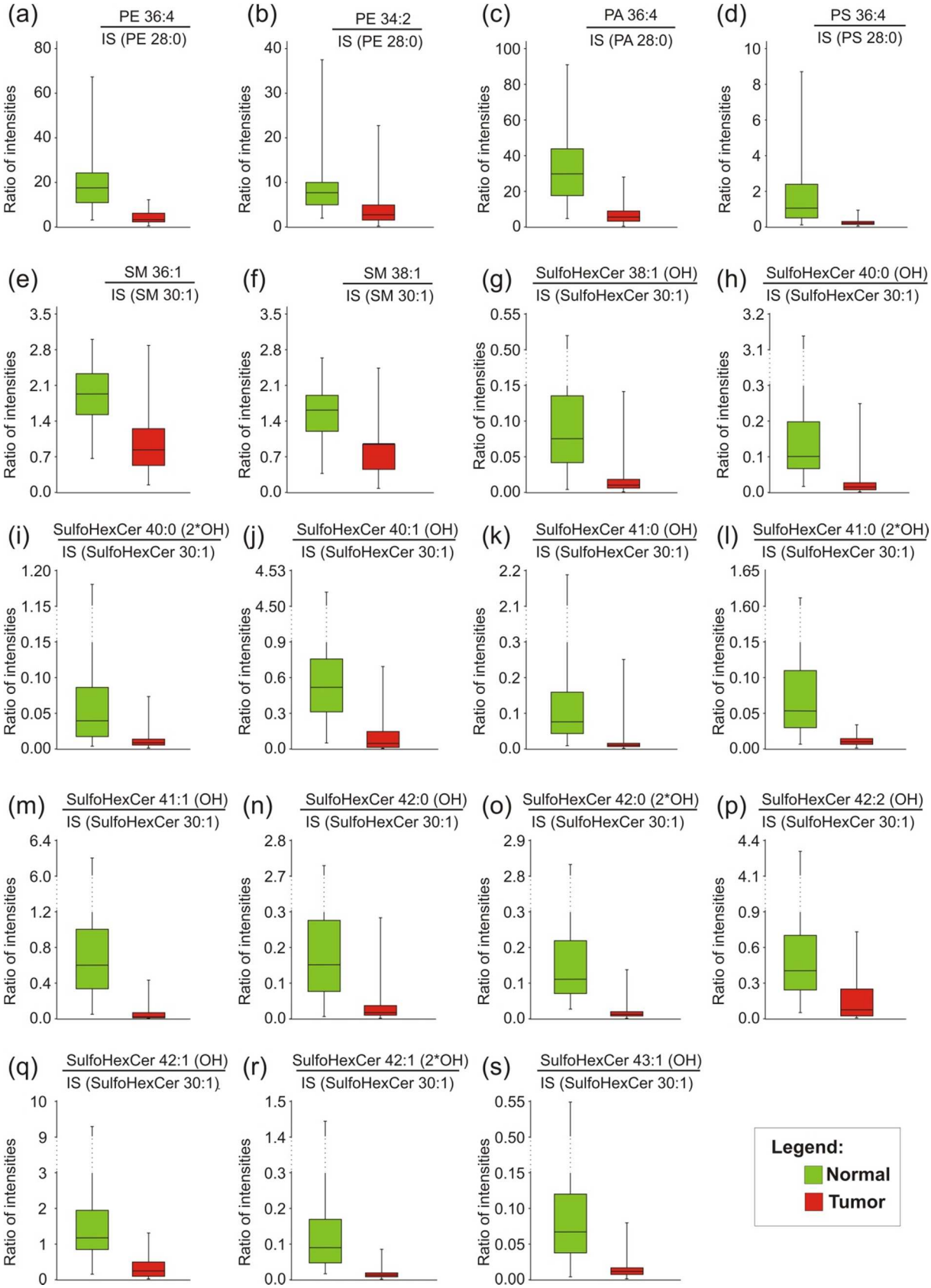


Figure S9. Box plots of selected most important downregulated sulfoglycosphingolipids in normal and tumor tissues of RCC patients

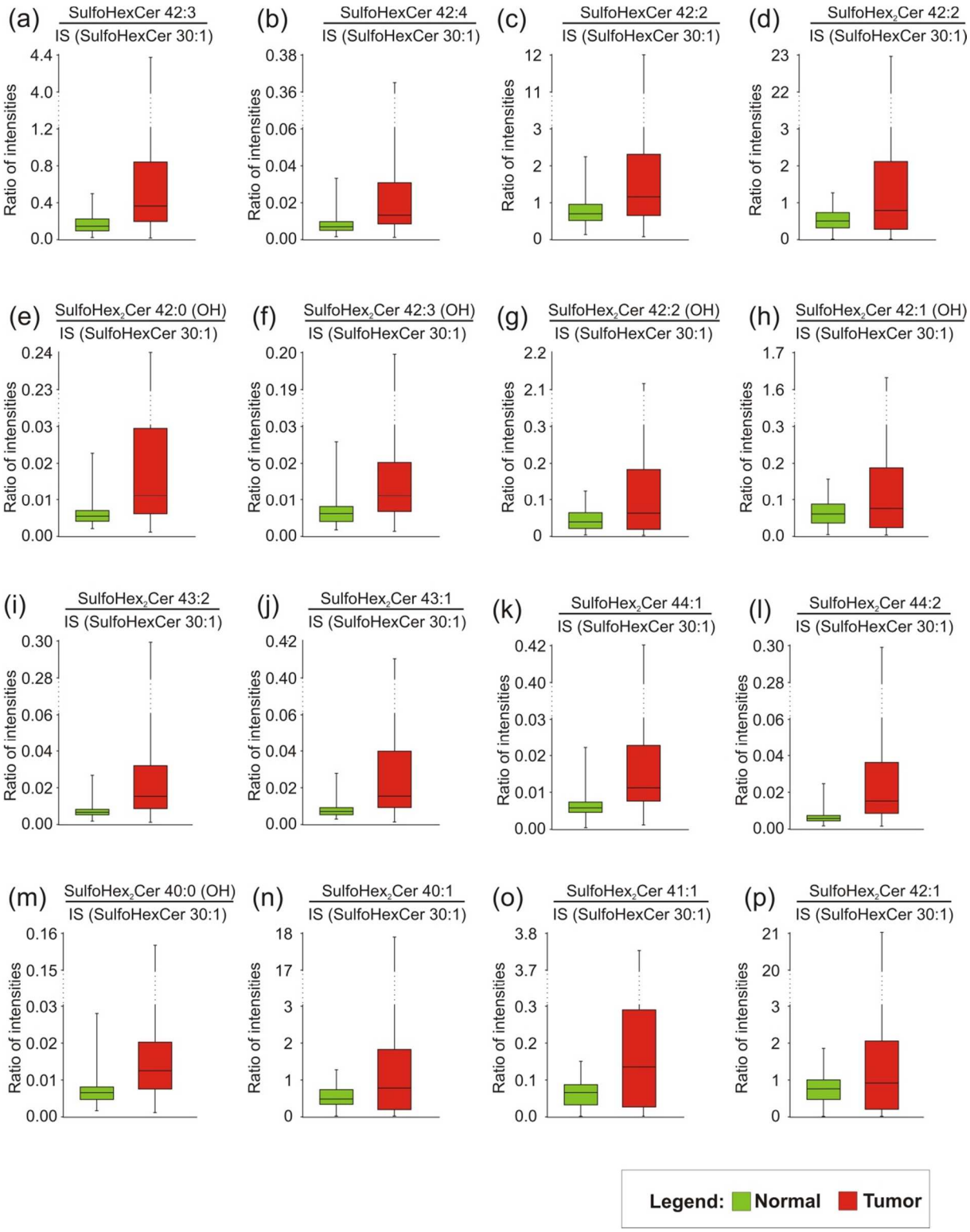


Figure S10. Box plots of selected most important upregulated sulfoglycosphingolipids in normal and tumor tissues of RCC patients

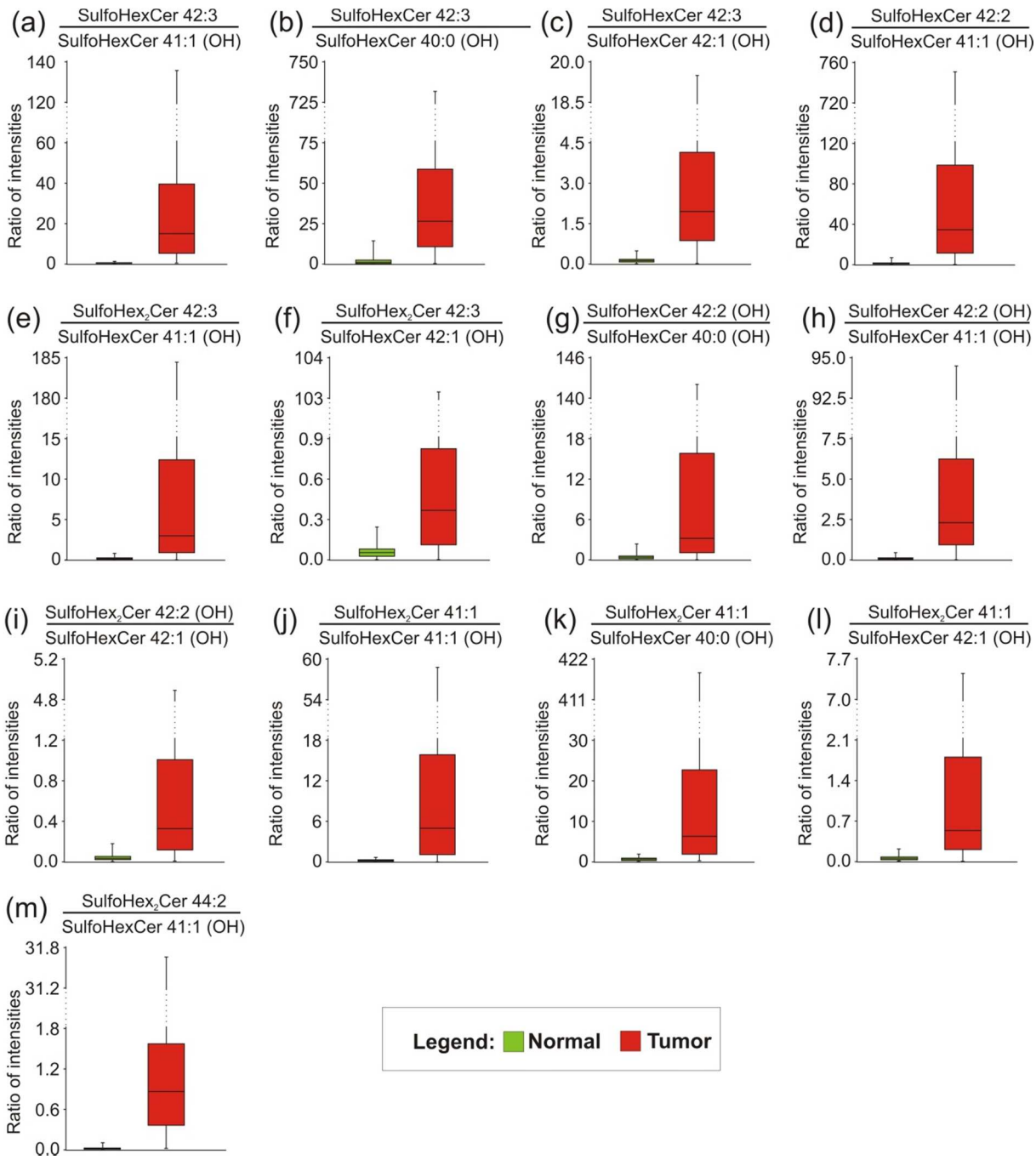


Figure S11. Box plots of selected ratios of intensities of sulfoglycosphingolipids in normal and tumor tissues of RCC patients

Table S1. List of used internal standards, their elemental composition, molecular weights, concentration of particular stock solution, and ratio of volumes used for the preparation of IS mixture 2

Lipid	**Elemental composition**	**MW**	**Concentration of stock solution [mg/ml]**	**Added volume [µl]**
PA 14:0/14:0 (sodium salt)	$C_{31}H_{60}O_8PNa$	614.3924	2	1
PC 14:0/14:0	$C_{36}H_{72}NO_8P$	677.4996	2	21
PE 14:0/14:0	$C_{33}H_{66}NO_8P$	635.4526	2	2.5
PG 14:0/14:0 (sodium salt)	$C_{34}H_{66}O_{10}PNa$	688.4291	2	1.6
PI 8:0/8:0 (ammonium salt)	$C_{25}H_{50}NO_{13}P$	603.3020	2.5	40
PS 14:0/14:0 (sodium salt)	$C_{34}H_{65}NO_{10}NPNa$	701.4244	2	3.6
SM 14:0/14:0	$C_{35}H_{71}N_2O_6P$	646.5050	2	17
Sulfatide 18:1/12:0 (ammonium salt)	$C_{36}H_{72}N_2O_{11}S$	740.4857	1	7
Chloroform/2-propanol (1:4, v/v)				211.5
Total volume of IS mixture 2				305.2

Table S2. List of final concentrations of internal standard SulfoHexCer d18:1/12:0 in particular calibration solution

Dilution	IS mixture 1* [µL]	Pooled extract (dissolved in $CHCl_3$ - 2-PrOH, 1:1, v/v) [µL]	CH_3OH [µL]	Final dilution after addition of matrix (1:1, v/v)	Final concentration of SulfoHexCer d18:1/12:0 [pmol/ml]
5x	4	5	11	10x	8441
12.5x	1.6	5	13.4	25x	3376
25x	8 of 10x diluted	5	7	50x	1688
50x	4 of 10x diluted	5	11	100x	844
100x	2 of 10x diluted	5	13	200x	422
250x	8 of 100x diluted	5	7	500x	169
500x	4 of 100x diluted	5	11	1000x	84
1000x	2 of 100x diluted	5	13	2000x	42
2500x	8 of 1000x diluted	5	7	5000x	17
5000x	4 of 1000x diluted	5	11	10000x	8.4
12500x	1.6 of 1000x diluted	5	13.4	25000x	3.4
25000x	8 of 10000x diluted	5	7	50000x	1.7
50000x	4 of 10000x diluted	5	11	100000x	0.84

*IS mixture 1 was prepared by the mixing of 8 µl of each IS (1:1, v/v).

Table S3. Selected theoretical lipid overlaps in the full scan negative-ion mass spectra

Selectes lipid overlaps of $[M-H]^-$ ions	Selected theoretical example	Difference in number of carbon atoms (CH_2) and double bonds (DB) of present fatty acids	$\Delta m/z$	Possible distinquishing	Impact on MALDI-Orbitrap-MS
PE and M+1 isotope of PA	*m/z* 712.5287 = $[PE\ O\text{-}35:3 - H]^-$ and *m/z* 712.5368 = M+1 of $[PA\ O\text{-}38:3 - H]^-$ *m/z* 688.4923 = $[PE\ 32:1 - H]^-$ and *m/z* 688.5004 = M+1 of $[PA\ 35:1 - H]^-$	PE = PA - $3*CH_2$	0.0081	R>88,000; MS/MS (combination of fatty acids)	no impact, resolved
PA and M+1 isotope of PE	*m/z* 715.5283 = $[PA\ 37:1 - H]^-$ and *m/z* 715.5113 = M+1 of $[PE\ 34:2 - H]^-$	PA = PE + $3*CH_2$ -DB	0.017	R>42,000; MS/MS (combination of fatty acids)	no impact, resolved
PS and M+1 isotope of PI	*m/z* 858.5220 = M+1 of $[PI\ 36:4 - H]^-$ and *m/z* 858.5291 = $[PS\ 42:8 - H]^-$	PS = PI + $6*CH_2$ + 4*DB	0.0071	R>120,000; MS/MS (PS - NL of 87, PI - NL of 162+FA)	double peak (peak widening), manual control required
PI and M+1 isotope of PS	*m/z* 807.5011 = M+1 of $[PS\ 38:6 - H]^-$ and *m/z* 807.5029 = $[PI\ 32:1 - H]^-$ *m/z* 821.5549 = $[PI\ O\text{-}34:1 - H]^-$ and *m/z* 821.5532 = M+1 of $[PS\ O\text{-}40:6 - H]^-$	PI = PS - $6*CH_2$ - 5*DB	0.0018	R>448,000; MS/MS (PS - NL of 87, PI - NL of 162+FA)	mass shift
PG and M+1 isotope of PE (O)	*m/z* 773.5320 = M+1 of $[PE\ O\text{-}40:8 - H]^-$ and *m/z* 773.5338 = $[PG\ 36:2\text{-}H]^-$	PG = PE (O) - $4*CH_2$ -6*DB	0.0018	R>448,000; MS/MS (combination of fatty acids)	mass shift
PE (O) and M+1 isotope of PG	*m/z* 774.5443 = $[PE\ O\text{-}40:7 - H]^-$ and *m/z* 774.5372 = M+1 of $[PG\ 36:2 - H]^-$	PE (O) = PG + $4*CH_2$ + 5*DB	0.0071	R>120,000; MS/MS (combination of fatty acids)	double peak (peak widening), manual control required
PG (O) and M+1 isotope of SulfoHexCer	*m/z* 779.5232 = $[PG\ O\text{-}38:6 - H]^-$ and *m/z* 779.5177 = M+1 of $[SulfoHexCer\ 34:1 - H]^-$	PG (O) = SulfoHexCer + $4*CH_2$ + 5*DB	0.0055	R>140,000; MS/MS (PG - combination of fatty acids, product ions m/z 97 and 241)	double peak (peak widening), manual control required
M+1 isotope of SulfoHexCer (OH) and PG	*m/z* 877.5964 = $[PG\ 44:6 - H]^-$ and *m/z* 877.5909 = M+1 of $[SulfoHexCer\ 40:2\ (OH) - H]^-$	PG = SulfoHexCer (OH) + $4*CH_2$ + 4*DB	0.0055	R>140,000; MS/MS (PG - combination of fatty acids, S - product ions m/z 97 and 241)	double peak (peak widening), manual control required
PS (O) and SulfoHexCer and SulfoHexCer(OH)	*m/z* 792.5185 = $[PS\ O\text{-}38:6 - H]^-$ and *m/z* 792.4937 = $[SulfoHexCer\ 34:2\ (OH) - H]^-$ and *m/z* 792.5301 = $[SulfoHexCer\ 35:1 - H]^-$		0.0116	R>68,000; MS/MS (PS - NL of 87, SulfoHexCer - m/z 97 and 241); R>21,000 (for resolution of individual sulfatides)	no impact, resolved
PS and SulfoHexCer	*m/z* 834.5291 = $[PS\ 40:6 - H]^-$ and *m/z* 834.5771 = $[SulfoHexCer\ 38:1 - H]^-$	PS = SulfoHexCer + $2*CH_2$ + 5*DB	0.048	R>17,000; MS/MS (PS - NL of 87, SulfoHexCer - m/z 97 and 241)	no impact, resolved
PS and M+2 isotope of SulfoHexCer	*m/z* 834.5291 = $[PS\ 40:6 - H]^-$ and *m/z* 834.5652 = $[SulfoHexCer\ 38:2 - H]^-$	PS = SulfoHexCer + $2*CH_2$ + 4*DB	0.0361	R>23,000; MS/MS (PS - NL of 87, S - PQD 97 and CID 241)	no impact, resolved
PI and M+1 isotope of SulfoHexCer	*m/z* 833.5186 = $[PI\ 34:2 - H]^-$ and *m/z* 833.5647 = M+1 of $[SulfoHexCer\ 38:2 - H]^-$	PI = SulfoHexCer - $4*CH_2$	0.0461	R>18,000; MS/MS (S - PQD 97 and CID 241, PI - NL of 162+FA)	
SulfoHexCer (OH) and PS	*m/z* 850.572 = $[SulfoHexCer\ 38:1\ (OH)]^-$ and *m/z* 850.5604 = $[PS\ 41:5 - H]^-$	PS = SulfoHexCer (OH)+ $3*CH_2$ + 4*DB	0.0116	R>73,000	no impact, resolved
DG and PA	*m/z* 687.4970 = $[DG\ 42:10 - H]^-$ and *m/z* 687.4994 = $[PA\ 35:1 - H]^-$	DG = PA + $7*CH_2$ + 9*DB	0.0024	DG do not provide signal in negative-ion mode	no impact
SulfoHexCer and M+2 isotope of previous SulfoHexCer	*m/z* 834.5771 = $[SulfoHexCer\ 38:1 - H]^-$ and *m/z* 834.5652 = M+2 of $[SulfoHexCer\ 38:2 - H]^-$	-	0.0119	R>70,000	no impact, resolved
PI and M+2 isotope of previous PI	*m/z* 835.5342 = $[PI\ 34:1 - H]^-$ and *m/z* 835.5249 = M+2 of $[SulfoHexCer\ 34:2 - H]^-$	-	0.0093	R>89,000	double peak (peak widening), manual control required
PE and demethylated PC	*m/z* 770.5705 = $[PE\ 38:2 - H]^-$ and *m/z* 770.5705 = $[PE\ 38:2 - CH_3]^-$	PE = PC - $2*CH_2$	0	MS/MS, combination of fatty acids, positive-ion mode	not resolved
PS and M+2 isotope of SulfoHexCer (OH)	*m/z* 852.576 = $[PS\ 41:4 - H]^-$ and *m/z* 852.5757 = M+2 of $[SulfoHexCer\ 38:1\ (OH) - H]^-$	PS = SulfoHexCer (OH)+ $3*CH_2$ + 3*DB	0.0003	R>2,840,000 or (detail control of isotopic peaks)	not resolved
CerPE and demethylated SM	*m/z* 689.5617 = $[CerPE\ 36:0 - H]^-$ and *m/z* 689.5617 = $[SM\ 34:0 - CH_3]^-$	CerPE = SM + $2*CH_2$	0	PECer are not present in studied samples	not resolved

Table S4. List of sulfatides identified in RCC pooled sample, their elemental composition and molecular weights. All ions were verified by the PQD fragmentation based on the presence of three typical product ions at *m/z* 259, *m/z* 241, and *m/z* 97

Lipid name (abb.)	Elemental composition	*m/z* of precursor $[M-H]^-$
SulfoHexCer 34:2	$C_{40}H_{75}NO_{11}S$	776.4988
SulfoHexCer 34:1	$C_{40}H_{77}NO_{11}S$	778.5145
SulfoHexCer 34:2 (OH)	$C_{40}H_{75}NO_{12}S$	792.4937
SulfoHexCer 35:1	$C_{41}H_{79}NO_{11}S$	792.5301
SulfoHexCer 34:1 (OH)	$C_{40}H_{77}NO_{12}S$	794.5094
SulfoHexCer 35:0	$C_{41}H_{81}NO_{11}S$	794.5458
SulfoHexCer 34:0 (OH)	$C_{40}H_{79}NO_{12}S$	796.5250
SulfoHexCer 36:2	$C_{42}H_{79}NO_{11}S$	804.5301
SulfoHexCer 36:1	$C_{42}H_{81}NO_{11}S$	806.5458
SulfoHexCer 36:2 (OH)	$C_{42}H_{79}NO_{12}S$	820.5250
SulfoHexCer 37:1	$C_{43}H_{83}NO_{11}S$	820.5614
SulfoHexCer 36:1 (OH)	$C_{42}H_{81}NO_{12}S$	822.5407
SulfoHexCer 38:2	$C_{44}H_{83}NO_{11}S$	832.5614
SulfoHexCer 38:1	$C_{44}H_{85}NO_{11}S$	834.5771
SulfoHexCer 39:1	$C_{45}H_{87}NO_{11}S$	848.5927
SulfoHexCer 38:1 (OH)	$C_{44}H_{85}NO_{12}S$	850.5720
SulfoHexCer 40:2	$C_{46}H_{87}NO_{11}S$	860.5927
SulfoHexCer 40:1	$C_{46}H_{89}NO_{11}S$	862.6084
SulfoHexCer 39:1 (OH)	$C_{45}H_{87}NO_{12}S$	864.5876
SulfoHexCer 41:2	$C_{47}H_{89}NO_{11}S$	874.6084
SulfoHexCer 40:2 (OH)	$C_{46}H_{87}NO_{12}S$	876.5876
SulfoHexCer 41:1	$C_{47}H_{91}NO_{11}S$	876.6240
SulfoHexCer 40:1 (OH)	$C_{46}H_{89}NO_{12}S$	878.6033
SulfoHexCer 40:0 (OH)	$C_{46}H_{91}NO_{12}S$	880.6189
SulfoHexCer 42:4	$C_{48}H_{87}NO_{11}S$	884.5927
SulfoHexCer 42:3	$C_{48}H_{89}NO_{11}S$	886.6084
SulfoHexCer 42:2	$C_{48}H_{91}NO_{11}S$	888.6240
SulfoHexCer 41:2 (OH)	$C_{47}H_{89}NO_{12}S$	890.6033
SulfoHexCer 42:1	$C_{48}H_{93}NO_{11}S$	890.6397
SulfoHexCer 41:1 (OH)	$C_{47}H_{91}NO_{12}S$	892.6189
SulfoHexCer 41:0 (OH)	$C_{47}H_{93}NO_{12}S$	894.6346
SulfoHexCer 40:0 (2OH)	$C_{46}H_{91}NO_{13}S$	896.6138
SulfoHexCer 42:3 (OH)	$C_{48}H_{89}NO_{12}S$	902.6033
SulfoHexCer 43:2	$C_{49}H_{93}NO_{11}S$	902.6397
SulfoHexCer 42:2 (OH)	$C_{48}H_{91}NO_{12}S$	904.6189
SulfoHexCer 43:1	$C_{49}H_{95}NO_{11}S$	904.6553
SulfoHexCer 42:1 (OH)	$C_{48}H_{93}NO_{12}S$	906.6346
SulfoHexCer 42:0 (OH)	$C_{48}H_{95}NO_{12}S$	908.6502
SulfoHexCer 41:0 (2OH)	$C_{47}H_{93}NO_{13}S$	910.6295
SulfoHexCer 44:3	$C_{50}H_{93}NO_{11}S$	914.6397
SulfoHexCer 44:2	$C_{50}H_{95}NO_{11}S$	916.6553
SulfoHexCer 43:2 (OH)	$C_{49}H_{93}NO_{12}S$	918.6346
SulfoHexCer 44:1	$C_{50}H_{97}NO_{11}S$	918.6710
SulfoHexCer 43:1 (OH)	$C_{49}H_{95}NO_{12}S$	920.6502
SulfoHexCer 42:1 (2OH)	$C_{48}H_{93}NO_{13}S$	922.6295
SulfoHexCer 43:0 (OH)	$C_{49}H_{97}NO_{12}S$	922.6659
SulfoHexCer 42:0 (2OH)	$C_{48}H_{95}NO_{13}S$	924.6451
SulfoHexCer 44:2(OH)	$C_{50}H_{95}NO_{12}S$	932.6502
SulfoHexCer 44:1(OH)	$C_{50}H_{97}NO_{12}S$	934.6659
SulfoHexCer 43:1 (2OH)	$C_{49}H_{95}NO_{13}S$	936.6451
SulfoHexCer 44:0 (OH)	$C_{50}H_{99}NO_{12}S$	936.6815
SulfoHexCer 43:0 (2OH)	$C_{49}H_{97}NO_{13}S$	938.6608

Table S5. List of CID important product ions used for the structural elucidation of SulfoHex$_2$Cer species in studied RCC samples

Lipid	Composition of fatty acids	Elemental composition	*m/z* of [M-H]$^-$	Important product ions														
				-H_2O	-SO_3	Rearrangement - $C_6H_{10}O_5$	Rearrangement - $C_6H_{12}O_6$	d16:1 base part (-210)	d18:1 base part (-238)	t18:0 base part (-256)	d20:1 base part (-266)	t20:0 base part (-284)	N-acyl part (-R)	N-acyl part (-RCO)	N-acyl part (-RCO - H2O)	-404 ($C_{12}H_{20}O_{13}S$)	[Hex_2SO_3+H_2O]$^-$	[Hex_2SO_3]$^-$
SulfoHex$_2$Cer 44:2 (OH)	-	$C_{56}H_{105}NO_{17}S$	1094.703		-	-	-	-	-	-	-	-	-	-	-	-	421.1	403.1
SulfoHex$_2$Cer 42:1 (2*OH)	t18:0/24:1_OH	$C_{54}H_{103}NO_{18}S$	1084.6823	1066.7	-	922.6	-	-	-	828.4	-	-	748.3	720.3	702.3	-	421.1	403.1
SulfoHex$_2$Cer 43:2 (OH)	t18:0/25:2 and t20:0/23:2	$C_{55}H_{103}NO_{17}S$	1080.6874	1062.7	1000.7	918.7	900.6	-	-	824.5	-	796.5	-	-	702 (25:2) 730.4 (23:2)	676.7	421.1	403.1
SulfoHex$_2$Cer 44:1	d18:1/26:0 and d20:1/24:0	$C_{56}H_{107}NO_{16}S$	1080.7238	1062.7	1000.7	918.7	900.6	-	842.5	-	814.5	-	-	730.4	684.3 (26:0), 712.3 (24:0)	676.7	421.1	403.1
SulfoHex$_2$Cer 44:2	d20:1/24:1 and d18:1/26:1	$C_{56}H_{105}NO_{16}S$	1078.7081	1060.7	998.7	916.7	898.6	-	840.5	-	812.4	-	-	-	712.3 (24:1), 684.3 (26:1)	674.6	421.1	403.1
SulfoHex$_2$Cer 44:3	d18:1/26:2	$C_{56}H_{103}NO_{16}S$	1076.6925	1058.7	-	914.6	896.6	-	838.5	-	-	-	-	-	684.3	672.6	421.1	403.1
SulfoHex$_2$Cer 44:4	d18:1/26:3	$C_{56}H_{101}NO_{16}S$	1074.677	1056.7	-	912.6	894.6	-	-	-	-	-	-	-	684.3	670.6	421.1	403.1
SulfoHex$_2$Cer 42:0 (OH)	t18:0/24:0	$C_{54}H_{105}NO_{17}S$	1070.703	1052.7	-	908.7	890.6	-	-	814.5	-	-	-	720.3	702.3	666.6	421.1	403.1
SulfoHex$_2$Cer 42:1 (OH)	d18:1/24:0_OH and t18:0/24:1	$C_{54}H_{103}NO_{17}S$	1068.6874	1050.7	-	906.6	888.6	-	830.5	812.4	-	-	730.3	702.3	702.3, 684.3	664.6	421.1	403.1
SulfoHex$_2$Cer 43:1	d20:1/23:0 and d18:1/25:0	$C_{55}H_{105}NO_{16}S$	1066.7081	1048.7	986.7	904.6	886.6	-	-	-	800.4	-	-	730.3 (23:0), 702.3 (25:0)	712.3 (23:0), 684.3 (25:0)	662.6	421.1	403.1
SulfoHex$_2$Cer 42:2 (OH)	d18:1/24:1_OH and t18:0/24:2	$C_{54}H_{101}NO_{17}S$	1066.6717	1048.7	986.7	904.6	886.6	-	828.4	810.4	-	-	730.3	702.3	684.3	662.6	421.1	403.1
SulfoHex$_2$Cer 43:2	d18:1/25:1	$C_{55}H_{103}NO_{16}S$	1064.6925	1046.7	984.7	902.6	884.6	-	826.5	-	-	-	-	702.3	684.3	660.6	421.1	403.1
SulfoHex$_2$Cer 42:3 (OH)	d18:1/24:2_OH	$C_{54}H_{99}NO_{17}S$	1064.6561	1046.7	984.7	902.6	884.6	-	826.5	-	-	-	730.3	702.3	684.3	660.6	421.1	403.1
SulfoHex$_2$Cer 42:1	d18:1/24:0	$C_{54}H_{103}NO_{16}S$	1052.6925	1034.7	972.7	890.6	872.6	-	814.5	-	-	-	-	702.3	684.3	648.6	421.1	403.1
SulfoHex$_2$Cer 42:2	d18:1/24:1	$C_{54}H_{101}NO_{16}S$	1050.6768	1032.7	970.7	888.6	870.6	-	812.5	-	-	-	-	702.3	684.3	646.6	421.1	403.1
SulfoHex$_2$Cer 42:3	d18:1/24:2	$C_{54}H_{99}NO_{16}S$	1048.6612	1030.7	-	886.6	868.6	-	810.5	-	-	-	-	702.3	684.3	644.6	421.1	403.1
SulfoHex$_2$Cer 42:3	d18:1/24:3	$C_{54}H_{97}NO_{16}S$	1046.6455	1028.6	-	884.6	-	-	808.4	-	-	-	-	-	684.3	642.6	421.1	403.1
SulfoHex$_2$Cer 40:0 (OH)	t18:0/22:0	$C_{52}H_{101}NO_{17}S$	1042.6717	1024.6	-	880.6	862.6	-	-	786.4	-	-	-	720.3	702.3	638.6	421.1	403.1
SulfoHex$_2$Cer 40:1 (OH)	d18:1/22:0_OH	$C_{52}H_{99}NO_{17}S$	1040.6561	1022.6	-	878.6	860.6	-	802.4	-	-	-	730.3	702.3	684.3	636.6	421.1	403.1
SulfoHex$_2$Cer 41:1	d18:1/23:0	$C_{53}H_{101}NO_{16}S$	1038.6768	1020.6	958.7	876.6	858.6	-	800.4	-	-	-	-	702.3	684.3	634.6	421.1	403.1
SulfoHex$_2$Cer 40:2 (OH)	d18:1/22:1_OH	$C_{52}H_{97}NO_{17}S$	1038.6404	1020.6	-	876.6	858.6	-	800.4	-	-	-	730.3	702.3	684.3	634.6	421.1	403.1
SulfoHex$_2$Cer 41:2	d18:1/23:1	$C_{53}H_{99}NO_{16}S$	1036.6612	1018.6	-	874.6	856.6	-	798.4	-	-	-	-	-	682.3	632.6	421.1	403.1
SulfoHex$_2$Cer 41:3	d18:1/23:2	$C_{53}H_{97}NO_{16}S$	1034.6455	1016.6	-	872.6	854.6	-	796.4	-	-	-	-	-	-	-	421.1	403.1
SulfoHex$_2$Cer 40:1	d18:1/22:0	$C_{52}H_{99}NO_{16}S$	1024.6612	1006.6	944.7	862.6	844.6	-	786.4	-	-	-	-	702.3	684.3	620.6	421.1	403.1
SulfoHex$_2$Cer 40:2	d18:1/22:1 and d16:1/24:1	$C_{52}H_{97}NO_{16}S$	1022.6455	1004.6	-	860.6	842.6	812.4	784.4	-	-	-	-	-	684.3, 656.3	618.6	421.1	403.1
SulfoHex$_2$Cer 40:3	d18:1/22:2 and d16:1/24:2	$C_{52}H_{95}NO_{16}S$	1020.6299	1002.6	-	858.6	-	810.4	782.4	-		-	-	-	684.3, 656.3	616.6	421.1	403.1
SulfoHex$_2$Cer 38:1 (OH)	d18:1/20:0_OH	$C_{50}H_{95}NO_{17}S$	1012.6248	994.6	-	850.6	-	-	774.4	-	-	-	730.3	702.3	684.3	-	421.1	403.1
SulfoHex$_2$Cer 39:1	d18:1/21:0	$C_{51}H_{97}NO_{16}S$	1010.6455	992.6	-	848.6	830.6	-	772.4	-	-	-	-	-	684.3	606.6	421.1	403.1
SulfoHex$_2$Cer 38:1	d18:1/20:0	$C_{50}H_{95}NO_{16}S$	996.6299	978.6	-	834.6	816.6	-	758.4	-	-	-	-	-	684.3, 656.3	592.6	421.1	403.1
SulfoHex$_2$Cer 38:2	-	$C_{50}H_{93}NO_{16}S$	994.6142	976.6		832.6	-	-	-	-	-	-	-	-	682.3	590.6	421.1	403.1
SulfoHex$_2$Cer 36:1 (OH)	d18:1/18:0_OH	$C_{48}H_{91}NO_{17}S$	984.5935	966.6	-	822.5	-	-	-	-	-	-	730.3	702.3	-	-	421.1	403.1
SulfoHex$_2$Cer 37:1	d18:1/19:0	$C_{49}H_{93}NO_{16}S$	982.6142	964.6	-	820.6	802.6	-	744.4	-	-	-	-	-	684.3	578.6	421.1	403.1
SulfoHex$_2$Cer 36:1	d18:1/18:0 and d16:1/20:0	$C_{48}H_{91}NO_{16}S$	968.5986	950.6	-	806.5	788.5	758.4	730.4	-	-	-	-	-	684.3, 656.3	564.5	421.1	403.1
SulfoHex$_2$Cer 36:2	d18:1/18:1	$C_{48}H_{89}NO_{16}S$	966.5829	948.6	-	804.5	-	-	728.4	-	-	-	-	702.3	684.3	-	-	403.1
SulfoHex$_2$Cer 34:1 (OH)	d18:1/16:0_OH	$C_{46}H_{87}NO_{17}S$	956.5622	938.6	-	794.5	-	-	-	-	-	-	730.3	702.3	684.3	-	421.1	403.1
SulfoHex$_2$Cer 35:1	d18:1/17:0	$C_{47}H_{89}NO_{16}S$	954.5829	936.6	-	792.5	-		716.4	-	-	-	-	-	684.3	-	421.1	403.1
SulfoHex$_2$Cer 34:1	d18:1/16:0	$C_{46}H_{87}NO_{16}S$	940.5673	922.6	860.6	778.5	760.5	-	702.3	-	-	-	-	-	684.3	536.5	421.1	403.1
SulfoHex$_2$Cer 34:2	-	$C_{46}H_{85}NO_{16}S$	938.5516	920.5	-	776.5	758.5	-	-	-	-	-	-	-	682.3, 656.5	534.5	421.1	403.1
SulfoHex$_2$Cer 33:1	-	$C_{45}H_{85}NO_{16}S$	926.5516	908.5	846.6	764.5	-	-	-	-	-	-	-	-	-	764.5	-	403.1
SulfoHex$_2$Cer 32:1	d16:1/16:0	$C_{44}H_{83}NO_{16}S$	912.536	894.5	-	750.5	-	702.3	-	-	-	-	-	-	656.3	508.5	-	403.1

Table S6. List of important product ions used for the structural elucidation of studied Sulfo(HexNAc)Hex_2Cer, Sulfo(HexNAc)Hex_3Cer, and Sulfo(HexNAc)Hex_4Cer species in studied RCC samples

Lipid	Composition of fatty acid	Elemental composition	*m/z* of $[M-H]^-$	Important product ions (neutral losses)																		
				-18 (H_2O)	-80 (SO_3)	-162 ($C_6H_{10}O_5$)	-180 ($C_6H_{12}O_6$)	-203 ($C_8H_{13}NO_5$)	-R	-RCO	- RCO - H2O	d18:1 base part (-238)	-365 (203+162)	-527 (203+2*162)	$[Hex_4(HexNAc)SO_3+H_2O]^-$	$[Hex_4(HexNAc)SO_3]^-$	948-$C_2H_4O_2$	$[Hex_3(HexNAc)SO_3+H_2O]^-$	$[Hex_3(HexNAc)SO_3]^-$	$[Hex_2(HexNAc)SO_3]^-$	$[Hex(HexNAc)SO_3]^-$	$[Hex_2SO_3]^-$
Sulfo(HexNAc)Hex_4Cer 42:1 (OH)	t18:0/24:1, t20:0/22:1	$C_{74}H_{136}N_2O_{22}S$	1595.8724	1577.9	-	1433.8	-	-	-	-	1229.5, 1257.5	-	1230	1068.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 42:1 (OH)	t18:0/24:2, t20:0/22:2	$C_{74}H_{134}N_2O_{22}S$	1593.8568	1575.9	-	1431.8	-	-	-	-	1229.5, 1257.5	-	1228	-	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 42:1	d18:1/24:0	$C_{74}H_{136}N_2O_{21}S$	1579.8775	1561.9	-	1417.8	-	-	-	1229.5	1211.5	-	1214.7	1052.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 42:2	d18:1/24:1	$C_{74}H_{134}N_2O_{21}S$	1577.8618	1559.9	-	1415.8	-	-	-	1229.5	1211.5	1339.6	1212.9	1050.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 42:3	d18:1/24:2	$C_{74}H_{132}N_2O_{21}S$	1575.8462	1557.8	1495.9	1413.8	-	-	-	-	-	-	1210.7	1048.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 41:1	d18:1/23:0	$C_{73}H_{134}N_2O_{21}S$	1565.8619	1547.9	-	1403.8	-	-	-	-	1211.5	-	1200.7	1038.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 41:2	-	$C_{73}H_{132}N_2O_{21}S$	1563.8462	1545.8	-	1401.79	-	-	-	-	-	-	1198.7	-	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 40:1	d18:1/22:0	$C_{72}H_{132}N_2O_{21}S$	1551.8462	1533.8	-	1389.8	-	-	-	-	1211.5	-	1186.7	1024.7	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 40:2	-	$C_{72}H_{130}N_2O_{21}S$	1549.8306	1531.8	-	-	-	-	-	-	-	-	1184.7	1022.6	948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_4Cer 38:1	d18:1/20:0	$C_{70}H_{128}N_2O_{21}S$	1523.8149	1505.8	-	1361.8	-	-	-	-	1211.5	-	1158.7		948.3	930.3	888.3	786.2	768.2	606.1	444.1	cut off effect
Sulfo(HexNAc)Hex_3Cer 42:2 (OH)	d18:1/24:0_OH	$C_{68}H_{126}N_2O_{22}S$	1433.8196	1415.8	-	1271.8	1253.8	-	1095.5	1067.5	1049.5	-	1068.7	-	-	-	-	786.2	768.2	606.1	444.1	403.1
Sulfo(HexNAc)Hex_3Cer 42:2 (OH)	d18:1/24:1_OH	$C_{68}H_{124}N_2O_{22}S$	1431.8039	1413.8	-	1269.8	1251.7	-	1095.5	1067.5	1049.5	-	1066.7	-	-	-	-	786.2	768.2	606.1	444.1	403.1
Sulfo(HexNAc)Hex_3Cer 42:1	d18:1/24:0	$C_{68}H_{126}N_2O_{21}S$	1417.8246	1399.8	-	1255.8	1237.8	-	-	-	-	-	1052.7	-	-	-	-	786.2	768.2	606.1	444.1	403.1
Sulfo(HexNAc)Hex_3Cer 42:2	d18:1/24:1	$C_{68}H_{124}N_2O_{21}S$	1415.809	1397.8	-	1253.8	1235.7	-	-	-	-	1177.6	1050.7	-	-	-	-	786.2	768.2	606.1	444.1	403.1
Sulfo(HexNAc)Hex_3Cer 40:1	d18:1/22:0	$C_{68}H_{126}N_2O_{21}S$	1389.7934	1371.8	-	1227.7	1209.7	-	-	-	-	-	1024.7	-	-	-	-	786.2	768.2	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 44:2	d18:1/26:1	$C_{64}H_{118}N_2O_{21}S$	1281.7875	1263.8	-	-	-	1078.7	-	-	887.4	1043.6	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 44:3	-	$C_{64}H_{116}N_2O_{21}S$	1279.7719	1261.8	-	-	-	1076.7	-	-	-	-	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 42:2	d18:1/24:0	$C_{62}H_{116}N_2O_{21}S$	1255.7719	1237.8	1175.8	-	-	1052.7	-	-	887.4	1017.5	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 42:2	d18:1/24:1	$C_{62}H_{114}N_2O_{21}S$	1253.7562	1235.8	1173.8	-	-	1050.7	-	-	887.4	1015.5	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 42:2	d18:1/24:2	$C_{62}H_{112}N_2O_{21}S$	1251.7406	1233.8	-	-	-	1048.7	-	-	-	-	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 42:2	d18:1/24:3	$C_{62}H_{110}N_2O_{21}S$	1249.7249	1231.8	-	-	-	1046.7	-	-	-	-	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 41:1	-	$C_{61}H_{114}N_2O_{21}S$	1241.7562	1223.7	-	-	-	1038.7	-	-	-	-	-	-	-	-	-	-	-	606.1	-	403.1
Sulfo(HexNAc)Hex_2Cer 41:2	-	$C_{61}H_{112}N_2O_{21}S$	1239.7406	1221.7	-	-	-	1036.7	-	-	-	-	-	-	-	-	-	-	-	606.1	-	-
Sulfo(HexNAc)Hex_2Cer 40:1	d18:1/22:0	$C_{60}H_{112}N_2O_{21}S$	1227.7406	1209.7	1147.8	-	-	1024.7	-	-	887.4	989.5	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 40:2	-	$C_{60}H_{110}N_2O_{21}S$	1225.7249	1207.7	-	-	-	1022.6	-	-	-	-	-	-	-	-	-	-	-	606.1	444.1	403.1
Sulfo(HexNAc)Hex_2Cer 38:1	d18:1/20:0	$C_{58}H_{108}N_2O_{21}S$	1199.7093	1181.7	1119.8	-	-	996.7	-	-	-	-	-	-	-	-	-	-	-	606.1	-	403.1
Sulfo(HexNAc)Hex_2Cer 34:1	d18:1/16:0	$C_{54}H_{100}N_2O_{21}S$	1143.6467	1125.6	1063.7	-	-	940.6	-	-	887.4	-	-	-	-	-	-	-	-	606.1	444.1	403.1

Table S7. Within-day and between-day precision for SulfoHexCer d18:1/12:0 at high concentration level of IS together with within-day precision for low concentratin level of IS

Within-day precision, 1st day (6 HL extraction)	
Extraction	Absolute signal of IS (RSD of 5 spots)
$1^{st}dayHL_1$	7038195 (9%)
$1^{st}dayHL_2$	6450001 (10%)
$1^{st}dayHL_3$	11116704 (8%)
$1^{st}dayHL_4$	7421920 (8%)
$1^{st}dayHL_5$	6854202 (8%)
$1^{st}dayHL_6$	11808094 (4%)
Median	7230058
standard deviation	2159660
RSD [%]	**30**
Within-day precision, 2nd day (3 HL extraction)	
Extraction	Absolute signal of IS (RSD of 5 spots)
$1^{st}dayHL_1$	9905999 (14%)
$1^{st}dayHL_2$	8530094 (8%)
$1^{st}dayHL_3$	11171955 (6%)
Median	9905999
standard deviation	1078847
RSD [%]	**11**
Between-day precision, (9 HL extraction)	
Median of absolute signal	7421920
standard deviation	1913431
RSD [%]	**27**
Within-day precision, 1st day (3 LL extraction)	
Extraction	Absolute signal of IS (RSD of 5 spots)
5 spots of $1^{st}dayLL_1$	606657 (8%)
5 spots of $1^{st}dayLL_2$	545721 (10%)
5 spots of $1^{st}dayLL_3$	400272 (9%)
Median	545721
standard deviation	86579
RSD [%]	**16**

Table S8. Freeze/thaw stability of SulfoHexCer d18:1/12:0

Freeze/thaw stability HL_1	
Extraction	Absolute signal of IS (RSD of 5 spots)
5 spots of 1stday HL_1	7038195 (9%)
5 spots of 1st freeze/thaw cycle HL_1	6854202 (11%)
5 spots of 2nd freeze/thaw cycle HL_1	9905999 (14%)
Median	7038195
standard deviation	1397284
RSD [%]	**20**
Freeze/thaw stability LL_1	
Extraction	Absolute signal of IS (RSD of 5 spots)
5 spots of 1stday LL_1	606657 (8%)
5 spots of 1st freeze/thaw cycle LL_1	549757 (5%)
5 spots of 2nd freeze/thaw cycle LL_1	494668 (6%)
Median	549757
standard deviation	45722
RSD [%]	**8**

Table S9. Values of averaged absolute signals of deprotonated molecules of IS SulfoHexCer d18:1/12:0 in selected RCC tissues, their median, standard deviation and RSD represented matrix effect

Patient and type of tissue extract	Absolute signal of IS
PAC08T tumor tissue	854748
PAC08N normal tissue	2503911
PAC17T tumor tissue	1148039
PAC17N normal tissue	1371283
PAC25T tumor tissue	1692350
PAC25N normal tissue	2050883
PAC35T tumor tissue	1072511
PAC35N normal tissue	680295
PAC49T tumor tissue	1463089
PAC49N normal tissue	2066004
PAC58T tumor tissue	1072376
PAC58N normal tissue	1267579
PAC65T tumor tissue	904812
PAC65N normal tissue	1574813
PAC74T tumor tissue	709546
PAC74N normal tissue	1582483
PAC86T tumor tissue	736878
PAC86N normal tissue	1044382
PAC93T tumor tissue	680946
PAC93N normal tissue	913734
Median	1110275
Standard deviation	491654
RSD [%]	44

Table S10. Reproducibility of relative signal for selected sulfoglycosphingolipids. The RSD value in the brackets behind the average value and standard deviation inside of particular columns under the each day represents signal fluctuation within three positions (start, middle, and end) of MALDI plate (except 1st day run of sample 10T for which only one position was measured). Total RSD expresses total signal variation including plate positions and particular days of all three runs

Ratio of lipid absolute signal	**Patient**	**1st day run**	**2nd day run**	**3rd day run**	**Total RSD [%]**
$\frac{\sum \text{SulfoHex}_2\text{Cer}}{\sum \text{SulfoHexCer(OH)}}$	10T	8.3	7.81 ± 0.43 (6%)	8.43 ± 0.1 (1%)	4
	01N	0.32 ± 0.001 (4%)	-	-	-
$\frac{\text{SulfoHex}_2\text{Cer 41:1}}{\text{SulfoHexCer 41:1 (OH)}}$	10T	5.36	5.24 ± 0.59 (11%)	5.36 ± 0.24 (4%)	7
	01N	0.05 ± 0.007 (14%)	-	-	-
$\frac{\text{SulfoHexCer 41:1 (OH)}}{\text{SulfoHexCer 41:2}}$	10T	1.34	1.14 ± 0.2 (18%)	1.42 ± 0.19 (13%)	13
	01N	5.65 ± 0.35 (6%)	-	-	-
$\frac{\sum \text{SulfoHex}_2\text{Cer}}{\text{IS}}$	10T	23.4	21.9 ± 0.42 (2%)	29.4 ± 4.89 (17%)	17
	01N	0.75 ± 0.03 (4%)	-	-	-
$\frac{\text{SulfoHex}_2\text{Cer 42:2 (OH)}}{\text{IS}}$	10T	1.57	1.56 ± 0.06 (4%)	2.06 ± 0.34 (17%)	17
	01N	0.01 ± 0.003 (30%)	-	-	-
$\frac{\text{SulfoHexCer 42:1 (OH)}}{\text{IS}}$	10T	0.91	0.84 ± 0.05 (6%)	1.08 ± 0.16 (15%)	14
	01N	0.72 ± 0.03 (4%)	-	-	-