Supporting information for

A facile method for monitoring sphingomyelin synthase activity in HeLa cells using liquid chromatography/mass spectrometry

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Table S1. Method validation for C6-Cer and C6-SM lipid species using cell lysates (n=5). LOD: limit of detection, LOQ: limit of quantification, RSD: relative standard deviation. Low concentration (Low): 5 fmol and High concentration (High): 50 fmol.

Lipids	**Detection limits**		**Extraction recovery (%)**		**Matrix effect (%)**		**Intra-day (RSD, %)**		**Inter-day (RSD, %)**	
	LOD (fmol)	LOQ (fmol)	Low	High	Low	High	Low	High	Low	High
C6-Cer	0.05	0.5	105.8	103.7	68.7	73.2	11.4	4.8	5.2	2.4
C6-SM	0.05	0.5	120.5	116.5	72.2	71.1	12.3	5.0	5.4	1.9

Figure S1: Production of C6-SM in control (HeLa), HeLa/SMS1, and HeLa/SMS2 over different incubation time at a protein concentration 0.1 µg/ µL (n=3).

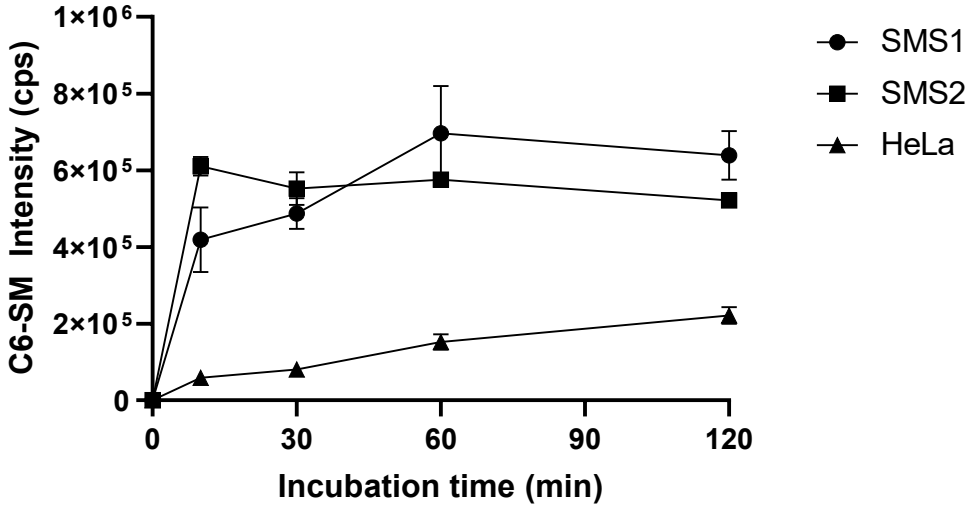

Figure S2: (A) Comparison of sequences of SMS1 and SMS2 (B) Predicted Local Distance Difference Test (p lDDT) score of SMS1 and SMS2.

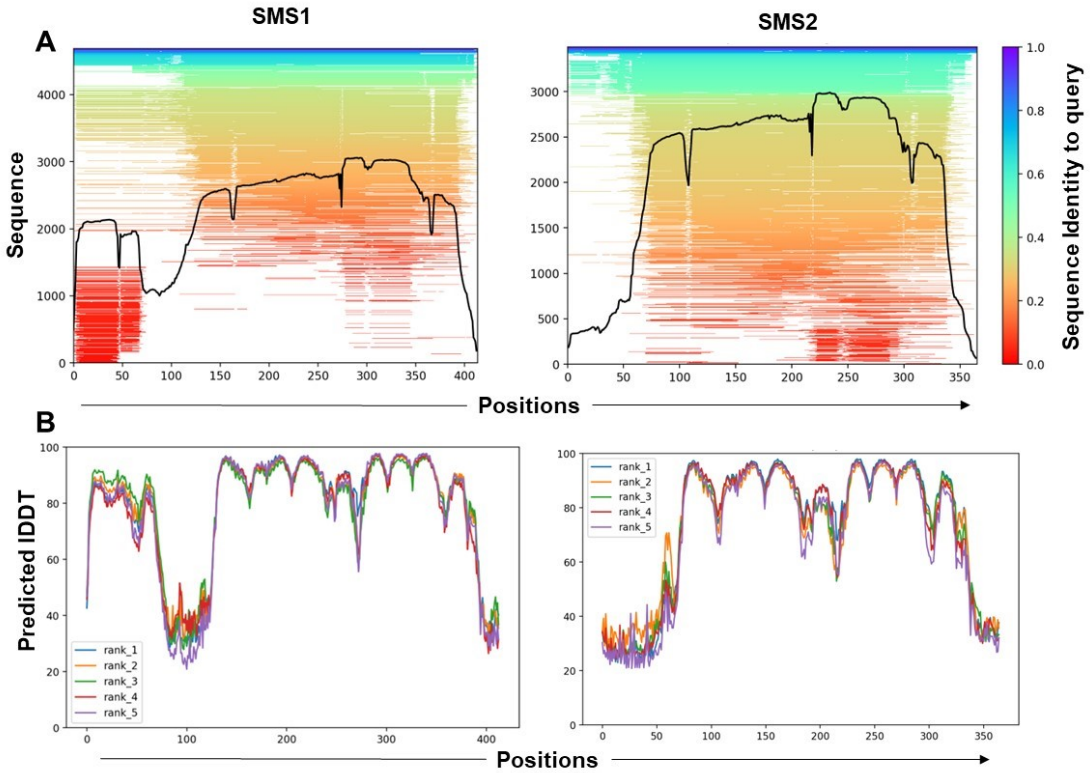

Figure S3: Root Mean Square Deviation (RMSD) of protein-ligand Complex of SMS1.