

SUPPLEMENTLA MATERIALS

FIGURE S1. Measurement Km and Vmas using NBD-C6-Ceramide as the substrate. Panel A and B, HeLa cells overexpressing SMS1 were treated with SMS2 and SMSr siRNA. Panel C and D, HeLa cells overexpressing SMS2 were treated with SMS1 and SMSr siRNA. Panel E and F, HeLa cells overexpressing SMSr were treated with SMS1 and SMS2 siRNA. CPE synthase activity were measured as described in the „Materials and Methods“. Velocity of the reaction was expressed as pmol/mg/min (A, C and E). Km and Vmax were calculated by Lineweaver Burk’s plot (B, D and F).

FIGURE S2. Measurement Km and Vmas using NBD-C12-Ceramide as the substrate. Panel A and B, HeLa cells overexpressing SMS1 were treated with SMS2 and SMSr siRNA. Panel C and D, HeLa cells overexpressing SMS2 were treated with SMS1 and SMSr siRNA. Panel E and F, HeLa cells overexpressing SMSr were treated with SMS1 and SMS2 siRNA. CPE synthase activity were measured as described in the „Materials and Methods“. Velocity of the reaction was expressed as pmol/mg/min (A, C and E). Km and Vmax were calculated by Lineweaver Burk’s plot (B, D and F).

Table S1. Measurement of CPE in tissues from *Smsr* KO and control mice by LC/MS/MS.

	C16	C18	C20	C24	C24:1
Plasma (ng/ml)					
WT	17.5±2.8	7.5±2.1	6.1±1.0	9.7±2.9	18.5±1.5
<i>Smsr</i> KO	15.1±3.0	6.2±1.9	5.1±0.8	8.7±3.2	16.2±2.2
WT	17.9±1.6	8.1±1.3	6.6±1.2	9.8±1.7	19.3±1.0
<i>Sms2</i> KO	14.7±2.2*	5.5±0.6*	5.0±0.7	8.0±1.2	16.9±1.1*
Liver (ng/mg protein)					
WT	3.3±0.5	1.2±0.2	1.4±0.3	2.3±0.7	4.6±0.5
<i>Smsr</i> KO	3.1±0.3	1.0±0.1	1.2±0.2	1.9±0.5	4.1±0.3
WT	3.6±0.2	1.0±0.1	1.6±0.2	2.1±0.5	4.2±0.3
<i>Sms2</i> KO	3.0±0.3*	0.9±0.2	1.3±0.1	1.8±0.2	3.6±0.2*
Macrophages (ng/mg protein)					
WT	4.8±0.6	2.9±0.3	2.7±0.2	5.1±1.1	9.6±1.4
<i>Smsr</i> KO	4.4±0.3	2.4±0.4	2.3±0.2	4.4±1.5	9.1±0.9
WT	5.0±0.3	3.1±0.4	2.9±0.4	5.5±0.6	10.2±1.0
<i>Sms2</i> KO	4.8±0.4	2.8±0.3	2.7±0.3	4.9±0.8	9.8±0.7

Values are the mean ± standard deviation of five mice. *P<0.05.

Table S2. Measurement of sphingomyelin in tissues from *Smsr* KO and control mice by LC/MS/MS.

	C16	C18	C22	C22:1	C24	C24:1
<u>Plasma (µg/ml)</u>						
WT	32.2±4.1	3.3±0.3	10.5±1.2	9.1±1.3	13.9±2.1	23.7±2.6
<i>Smsr</i> KO	31.5±3.2	3.0±0.4	11.8±1.9	9.3±1.7	12.4±2.8	23.2±4.9
<u>Liver (µg/mg protein)</u>						
WT	2.4±0.4	0.5±0.1	2.1±0.2	1.2±0.2	1.6±0.2	1.7±0.1
<i>Smsr</i> KO	2.2±0.3	0.6±0.1	1.9±0.3	1.1±0.1	1.7±0.3	1.8±0.2
<u>Macrophages (µg/mg protein)</u>						
WT	8.8±1.0	0.8±0.1	1.5±0.2	2.1±0.3	3.8±0.5	7.3±1.2
<i>Smsr</i> KO	9.0±0.9	0.9±0.2	1.7±0.3	2.3±0.1	3.9±0.2	7.7±1.6

Values are the mean ± standard deviation of five mice.

Table S3. Measurement of ceramide in tissues from *Smsr* KO and control mice by LC/MS/MS.

	C16	C18	C22	C22:1	C24	C24:1
<u>Plasma (ng/ml)</u>						
WT	397±42	88±19	51±4	19±2	208±39	352±55
<i>Smsr</i> KO	371±62	70±9	39±2	15±3	189±28	318±33
<u>Liver (ng/mg protein)</u>						
WT	89±10	37±5	19±2	7±3	66±8	75±7
<i>Smsr</i> KO	86±6	35±4	6±1	16±2	60±9	71±10
<u>Macrophages (ng/mg protein)</u>						
WT	272±36	130±28	45±7	18±3	191±32	202±41
<i>Smsr</i> KO	260±40	112±21	40±4	15±2	178±25	199±30

Values are the mean ± standard deviation of five mice.

Table S4. Measurement of dihydroceramide in tissues from *Smsr* KO and control mice by LC/MS/MS.

	C16	C18	C24	C24:1
<u>Plasma (ng/ml)</u>				
WT	61±8	9±1	35±4	70±2
<i>Smsr</i> KO	65±6	12±2	39±4	77±5
<u>Liver (ng/mg protein)</u>				
WT	18±2	6±1	8±2	23±3
<i>Smsr</i> KO	17±1	5±2	6±1	20±2
<u>Macrophages (ng/mg protein)</u>				
WT	46±5	20±3	13±3	53±6
<i>Smsr</i> KO	50±10	22±3	15±2	56±9

Values are the mean ± standard deviation of five mice.

Table S5. Measurement of glucosylceramide in tissues from *Smsr* KO and control mice by LC/MS/MS.

	C16	C18	C18:1	C24:1
<u>Plasma (ng/ml)</u>				
WT	750±81	858±105	268±38	804±76
<i>Smsr</i> KO	731±62	821±90	235±35	772±60
<u>Liver (ng/mg protein)</u>				
WT	38±5	29±3	21±2	49±7
<i>Smsr</i> KO	35±6	25±2	18±3	44±4
<u>Macrophages (ng/mg protein)</u>				
WT	64±9	20±3	25±4	59±7
<i>Smsr</i> KO	60±4	18±2	21±2	55±5

Values are the mean ± standard deviation of five mice.

Table S6. Measurement of plasma lipids in *Smsr* KO, *Smsr/Sms2* double KO and WT mice

Mice	PL(mg/dl)	Chol(mg/dl)	TG(mg/dl)
WT	155±26	96±15	66±17
<i>Smsr</i> KO	138±29	90±9	55±13
WT	166±21	101±12	71±6
<i>Smsr/Sms2</i> KO	151±18	96±14	64±4

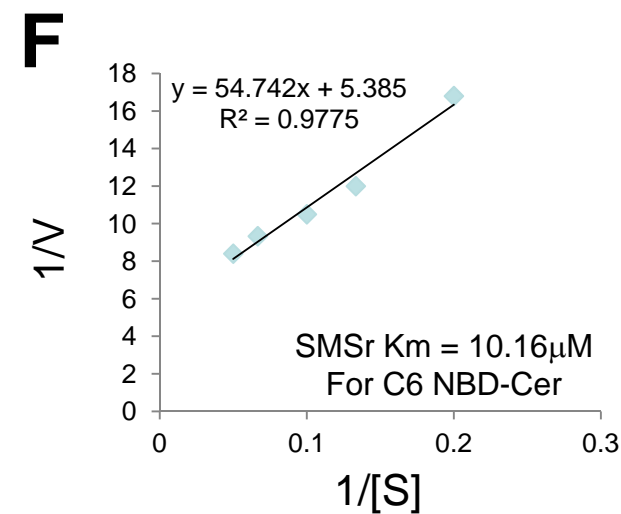
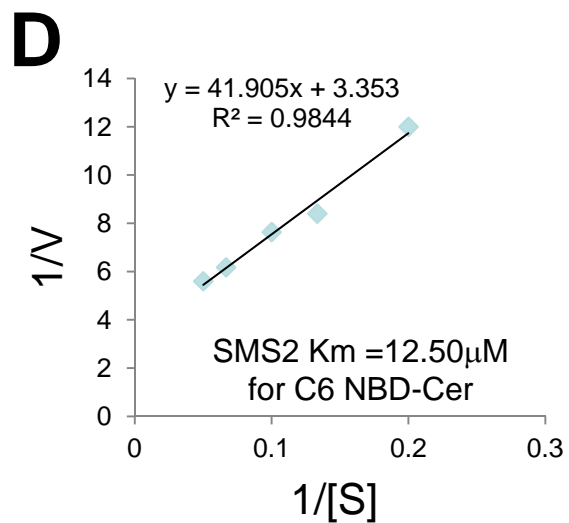
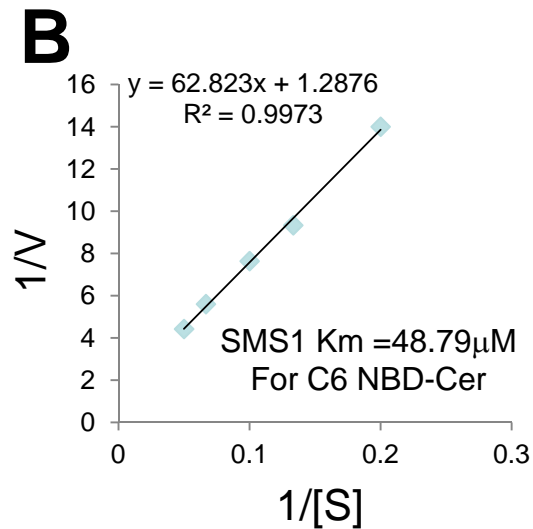
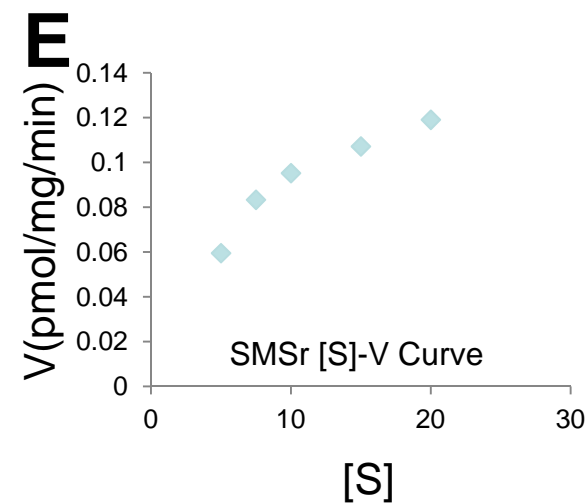
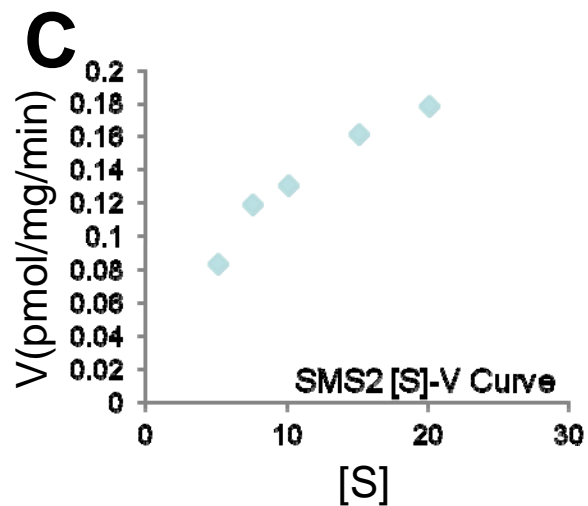
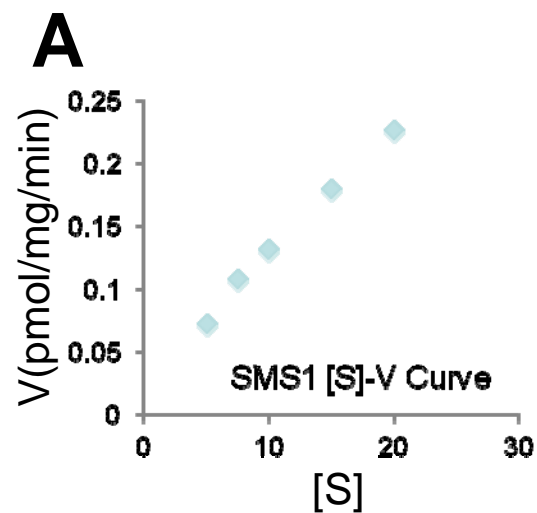
Values are the mean ± standard deviation of five mice. PL, choline-containing phospholipids; Chol, total cholesterol; TG, triglyceride.

Table S7. Measurement of CPE in the plasma of *Sms1* KO and control mice by LC/MS/MS.

	C16	C18	C20	C24	C24:1
WT	19.1±1.6	7.9±0.9	6.2±0.7	10.8±1.3	19.5±2.2
<i>Sms1</i> KO	15.6±2.1*	6.2±0.6	5.2±1.0	8.5±0.8*	15.9±1.6*

Values are the mean ± standard deviation of five mice. *P<0.05.

Supplemental Fig. S1. Ding et al.



Supplemental Fig. S2. Ding et al.

