SUPPLEMENTARY DATA

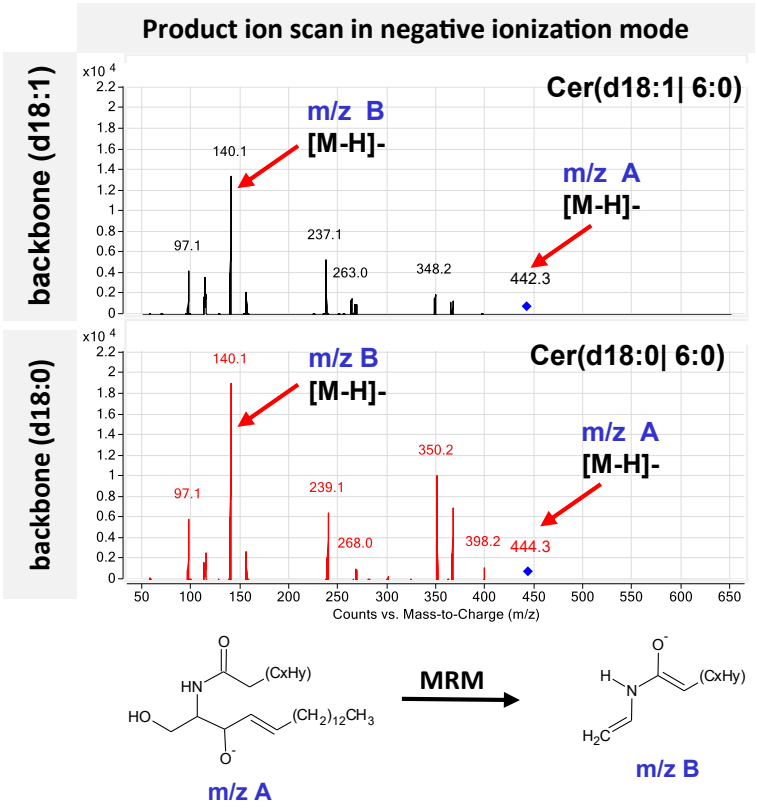
Supplement Table S1. Inbred strains used in this study

Strain	Female (n)	Male (n)
129X1/SvJ	2	3
A/J	3	3
AKR/J	2	3
AXB-10/PgnJ	3	3
AXB-12/PgnJ	2	2
AXB-13/PgnJ	3	3
AXB-15/PgnJ	3	3
AXB-18/PgnJ	3	2
AXB-19/PgnJ	3	3
AXB-20/PgnJ	2	3
AXB5/PgnJ	2	2
BALB/cJ	3	3
BTBRT<+>tf/J	3	3
BUB/BnJ	3	3
BXA-1/PgnJ	3	3
BXA-11/PgnJ	3	3
BXA-14/PgnJ	3	3
BXA-16/PgnJ	3	3
BXA-2/PgnJ	2	3
BXA-24/PgnJ	3	3
BXA-4/PgnJ	3	2
BXA-7/PgnJ	3	2
BXA-8/PgnJ	3	2
BXD-12/TyJ	3	3
BXD-13/TyJ	3	2
BXD-14/TyJ	3	1
BXD-15/TyJ	2	2
BXD-19/TyJ	3	3
BXD-20/TyJ	3	3
BXD-21/TyJ	3	3
BXD-24/TyJ	2	3
BXD-31/TyJ	2	2
BXD-32/TyJ	3	3
BXD-34/TyJ	2	2
BXD-38/TyJ	3	3
BXD-40/TyJ	3	3
BXD43	3	3

BXD44	3	3
BXD45	3	3
BXD48	2	2
BXD49	2	3
BXD-5/TyJ	2	2
BXD50	3	3
BXD51	3	3
BXD55	3	3
BXD56	3	3
BXD-6/TyJ	3	2
BXD60	3	2
BXD61	3	3
BXD62	3	1
BXD64	3	3
BXD66	2	3
BXD68	3	3
BXD70	3	2
BXD71	4	4
BXD73	1	4
BXD74	3	2
BXD75	3	3
BXD79	3	4
BXD84	2	3
BXD85	3	2
BXD86	2	3
BXD87	3	3
BXD-9/TyJ	3	3
BXH-19/TyJ	3	1
BXH-6/TyJ	3	3
BXH-8/TyJ	3	3
BXH-9/TyJ	3	3
BXHA1	3	3
BXHB2	3	3
C3H/HeJ	4	2
C57BL/6J	3	3
C57BLKS/J	2	3
C58/J	2	3
CBA/J	3	2
CE/J	2	3
CXB-11/HiAJ	3	2
CXB-12/HiAJ	2	3
CXB-13/HiAJ	2	3
CXB-3/ByJ	2	2

CXB-6/ByJ	2	3
CXB-7/ByJ	3	2
CXBH	3	3
DBA/2J	3	3
FVB/NJ	3	3
I/LnJ	3	3
KK/HIJ	3	3
MA/MyJ	3	3
NOD/LtJ	3	2
NON/LtJ	3	3
NZB/BINJ	3	3
NZW/LacJ	3	3
PL/J	3	3
RIIIS/J	3	3
SEA/GnJ	3	3
SJL/J	3	3
SM/J	3	3
SWR/J	3	3

Supplement Figure S1. Identification of specific MRM transitions for ceramide standards. Product ion scans of two ceramide model standards, Cer(d18:1/6:0) and Cer(d18:0/6:0), performed in negative ionization mode. The main fragment (m/z B) found for the two standards investigated was m/z 140.1.

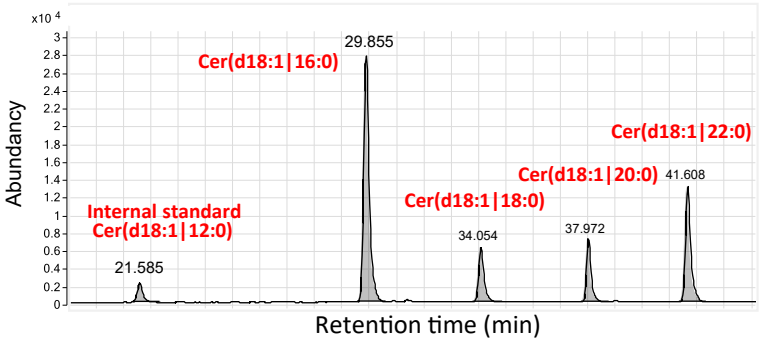


Supplement Table S2. Eight specific MRM transitions used to verify and quantify the ceramides

Number	Ceramide	MRM transition
1	Cer(d18:1/16:0)	582.5 – 280.2
2	Cer(d18:0/16:1)	582.5 – 278.2
3	Cer(d18:1/18:0)	610.5 – 308.2
4	Cer(d18:0/18:1)	610.5 – 306.2
5	Cer(d18:1/20:0)	638.5 – 336.2
6	Cer(d18:0/20:1)	638.5 – 334.2
7	Cer(d18:1/22:0)	666.5 – 364.3
8	Cer(d18:0/22:1)	666.5 – 362.3

Supplement Figure S2. Identification of hepatic Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0) and Cer(d18:1/22:0) ceramides. A representative total ion chromatogram showing four identified ceramides with d18:1 backbone in a hepatic lipid extract (A). Ceramides with d18:0 backbone were not detected in any of the samples analyzed. The specific multiple reaction monitoring mode transitions for the detected hepatic Cer(C18:1/16:0), Cer(C18:1/18:0), Cer(C18:1/20:0) and Cer(18:1/22:0) ceramides (B).

A



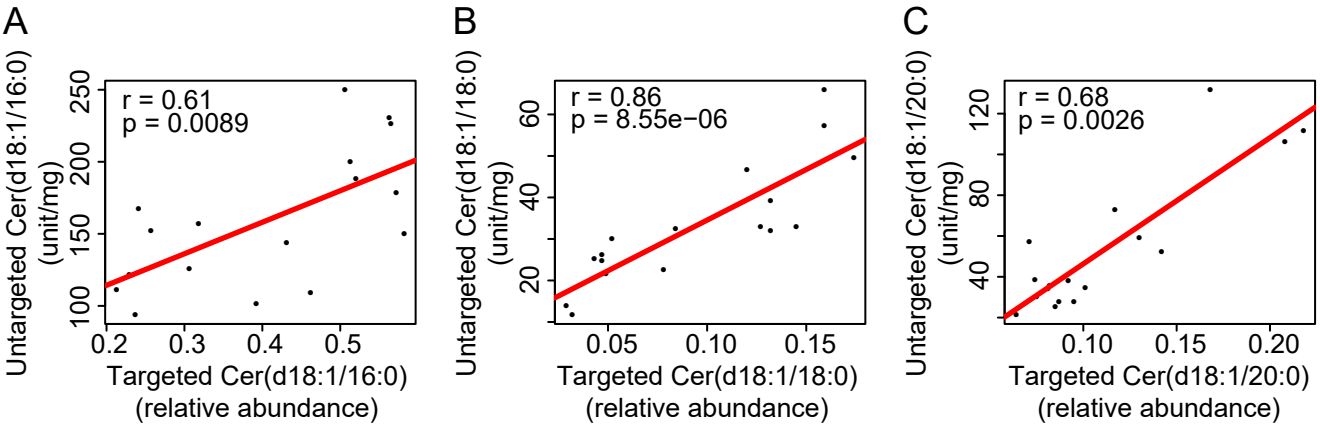
B

Ceramide with d18:1 backbone

Type	m/z (MS1)	m/z (MS2)
Cer(d18:1|16:0) - Ceramide C16:0	582.5	280.2
Cer(d18:1|18:0) - Ceramide C18:0	610.5	308.2
Cer(d18:1|20:0) - Ceramide C20:0	638.5	336.2
Cer(d18:1|22:0) - Ceramide C22:0	666.5	364.3

Supplement Figure S3. Correlations between hepatic ceramides measured with targeted and untargeted lipidomics.

Correlation between of hepatic Cer(d18:1/16:0) (A), Cer(d18:1/C18:0) (B), and Cer(d18:1/C20:0) (C) ceramides measured with LC-MS/MS (targeted) and HPLC-TOF-MS (untargeted) lipidomics.



Supplement Figure S4. Sex differences in hepatic Cer(d18:1/16:0), Cer(d18:1/18:0) and Cer(18:1/20:0) ceramides. Hepatic Cer(d18:1/16:0) (A), Cer(d18:1/18:0) (B) and Cer(d18:1/20:0) (C) ceramides in individual female (red) and male (blue) mice from five different strains (n = 1-3 per sex and strain). Each bar represents values from individual mice. The hepatic ceramide levels were measured with LC-MS/MS.