SUPPLEMENTARY FIGURES

Figure S1. Generation of SMSrdelEx6 mice. (A) Schematic drawing for the generation of SMSrdelEx6 mice by homologous recombination (B) Genotyping PCR for wild type (+/+), heterozygous (+/SMSrdelEx6) and homozygous (SMSrdelEx6) mice; characteristic DNA fragments 386 bp (wild type), 707 bp (delEx6), 451 bp (floxed) (C) Southern blot analysis of SMSrdelEx6 mice. Expected fragment sizes: *Xmn*I restriction: wild type 11.5 kbp, delEx6 2.9 kbp.

Figure S2. LC-MS analysis of main CPE species in testis (A), kidney (B), spleen (C), lung (D), heart (E), muscle (F) and small intestine (G) of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. Data are means ± SEM; *, p<0.05, **, *p<0.005; n=3-4.*

Figure S3. LC-MS analysis of main SM species in testis (A), kidney (B), spleen (C), lung (D), heart (E), muscle (F) and small intestine (G) of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. Data are means ± SEM; *, p<0.05, **, *p<0.005,* ***, *p<0.001; n=3-4.*

Figure S4. MS analysis of main ceramide species in testis (A), kidney (B), spleen (C), lung (D), heart (E), muscle (F) and small intestine (G) of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. Data are means of two independent measurements ± SEM; *, p<0.05, **, *p<0.005,* ***, *p<0.001; n=3-4.*

Figure S5. MS analysis of main hexosylceramide species in testis (A), kidney (B), spleen (C), lung (D), heart (E), muscle (F) and small intestine (G) of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. Data are means of two independent measurements ± SEM; *, p<0.05, **, *p<0.005; n=3-4.*

Figure S6. LC-MS analysis of CPE and SM content in forebrain, cerebellum, liver, testis, kidney, spleen, lung, heart, muscle and small intestine of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. (A-J) Analysis of total CPE in different tissues. (K-T) Analysis of total SM in different tissues.

Data are means ± SEM; *, p<0.05, **, *p<0.005,* ***, *p<0.001; n=3-4.*

Figure S7. MS analysis of ceramide and hexosylceramide content in forebrain, cerebellum, liver, testis, kidney, spleen, lung, heart, muscle and small intestine of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. (A-J) Analysis of total ceramide in different tissues. (K-T) Analysis of total hexosylceramide in different tissues. Data are means of two independent measurements ± SEM; *, p<0.05, **, *p<0.005; n=3-4.*

Figure S8. MS analysis of glycerophospholipid content in forebrain (A), cerebellum (B), liver (C), testis (D), kidney (E), spleen (F), lung (G), heart (H), muscle (I) and small intestine (J) of wild type, SMSrD348E, SMS2gt*X*SMSrD348E and SMS2gt mice. Data are means of two independent measurements ± SEM; *, p<0.05, **, *p<0.005; n=3-4* (PC, phosphatidyl choline, PE, phosphatidyl ethanolamine, PS, phosphatidyl serine, PI, phosphatidyl inositol).

Figure S9. Analysis of serum activities of Alanine transaminase (ALT), Aspartate transaminase (AST) and Creatine phosphokinase (CPK) in wild type (*n=6*) and SMSrD348E (*n*=3) mice. Data are means ± SEM.

Figure S1

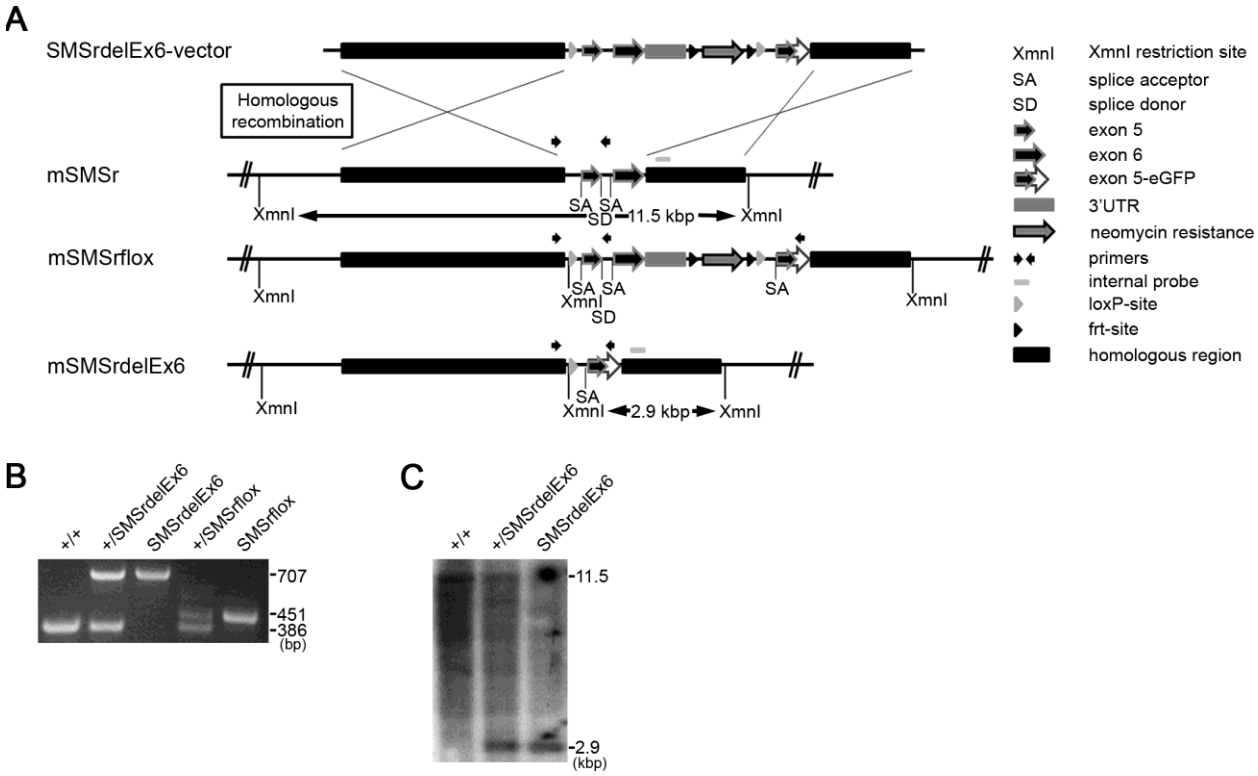

A
SMSrdelEx6-vector
Homologous recombination
mSMSr
XmnI
SA SA
SD
11.5 kbp
XmnI
mSMSrflox
XmnI
SA SA
XmnI
SD
SA
XmnI
mSMSrdelEx6
XmnI
SA
XmnI
2.9 kbp
XmnI
XmnI XmnI restriction site
SA splice acceptor
SD splice donor
exon 5
exon 6
exon 5-eGFP
3'UTR
neomycin resistance
primers
internal probe
loxP-site
frt-site
homologous region
B
+/+
+/SMSrdelEx6
SMSrdelEx6
+/SMSrflox
SMSrflox
707
451
386
(bp)
C
+/+
+/SMSrdelEx6
SMSrdelEx6
11.5
2.9
(kbp)

Figure S2

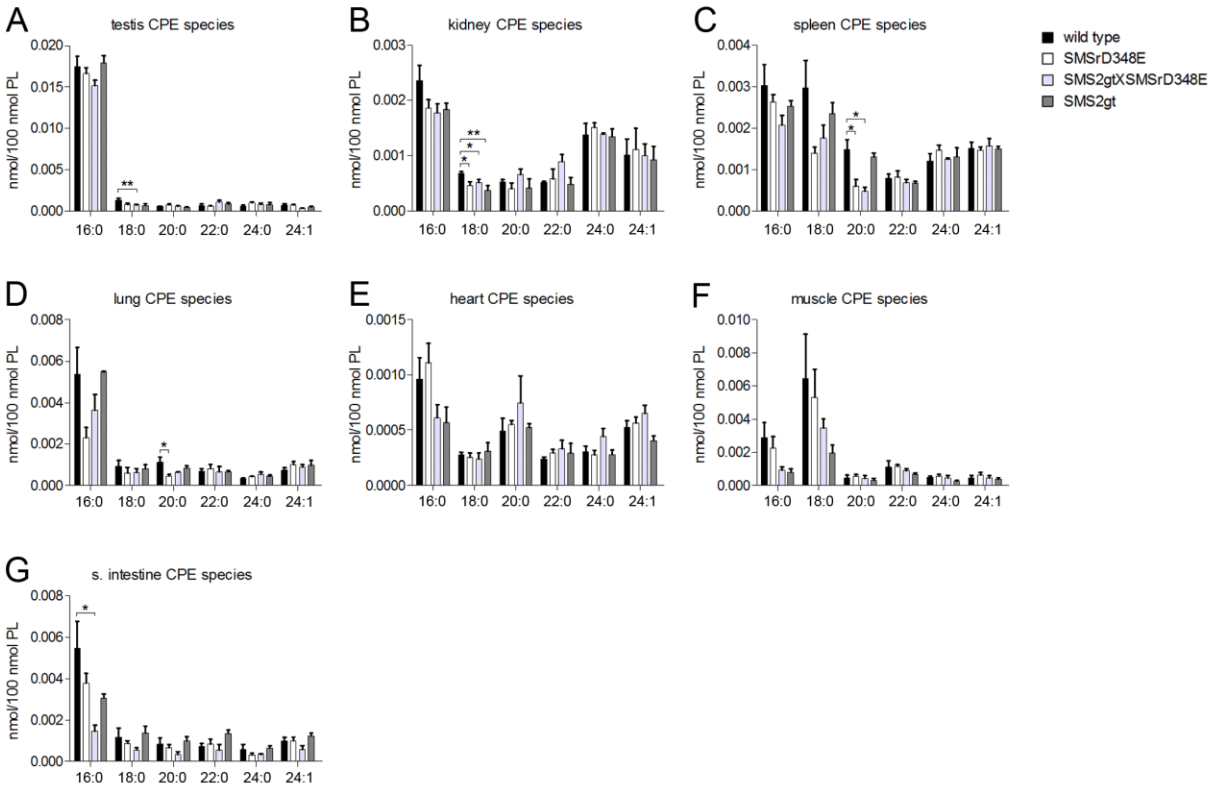

A
testis CPE species
B
kidney CPE species
C
spleen CPE species
D
lung CPE species
E
heart CPE species
F
muscle CPE species
G
s. intestine CPE species
nmol/100 nmol PL
16:0
18:0
20:0
22:0
24:0
24:1
wild type
SMSrD348E
SMS2gtXSMSrD348E
SMS2gt

Figure S3

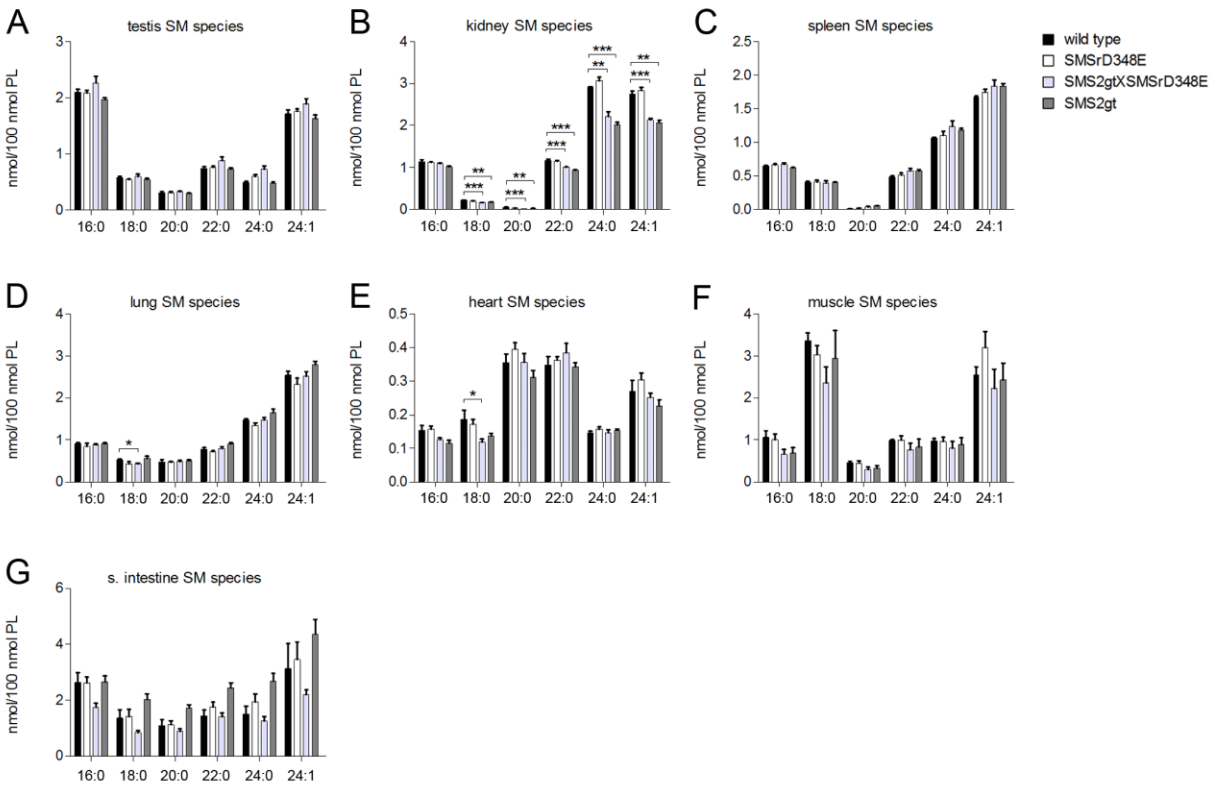

A
testis SM species
B
kidney SM species
C
spleen SM species
D
lung SM species
E
heart SM species
F
muscle SM species
G
s. intestine SM species
nmol/100 nmol PL
16:0
18:0
20:0
22:0
24:0
24:1
wild type
SMSrD348E
SMS2gtXSMSrD348E
SMS2gt

Figure S4

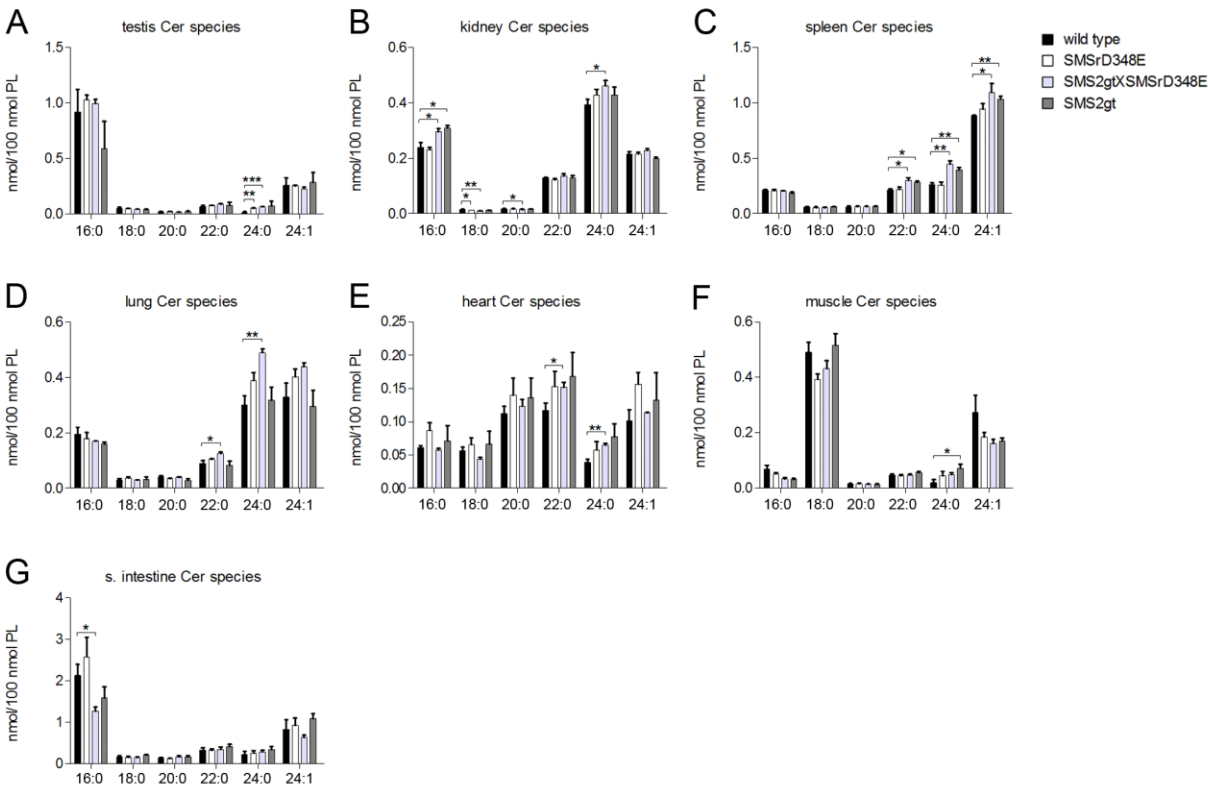

A
testis Cer species
B
kidney Cer species
C
spleen Cer species
D
lung Cer species
E
heart Cer species
F
muscle Cer species
G
s. intestine Cer species
nmol/100 nmol PL
16:0
18:0
20:0
22:0
24:0
24:1
wild type
SMSrD348E
SMS2gtXSMSrD348E
SMS2gt

Figure S5

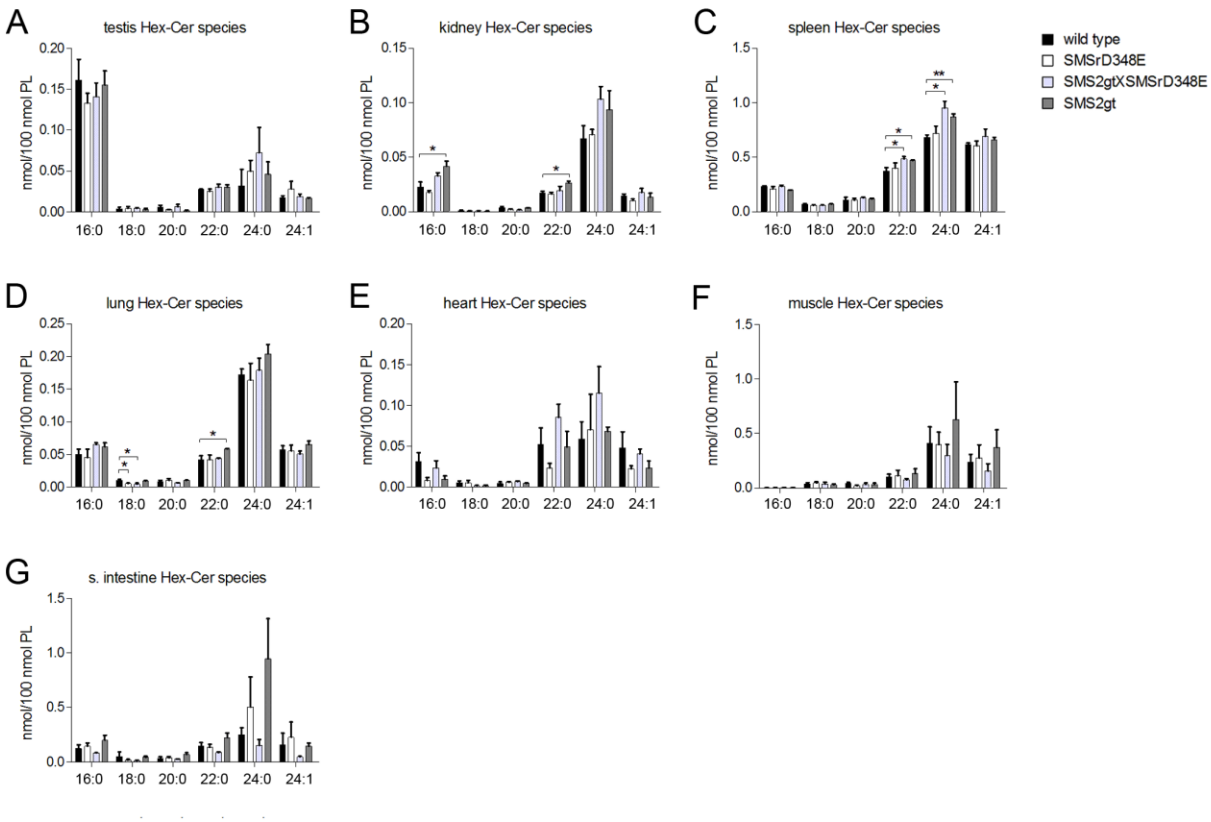

A
testis Hex-Cer species
B
kidney Hex-Cer species
C
spleen Hex-Cer species
D
lung Hex-Cer species
E
heart Hex-Cer species
F
muscle Hex-Cer species
G
s. intestine Hex-Cer species
nmol/100 nmol PL
16:0
18:0
20:0
22:0
24:0
24:1
wild type
SMSrD348E
SMS2gtXSMSrD348E
SMS2gt

Figure S6

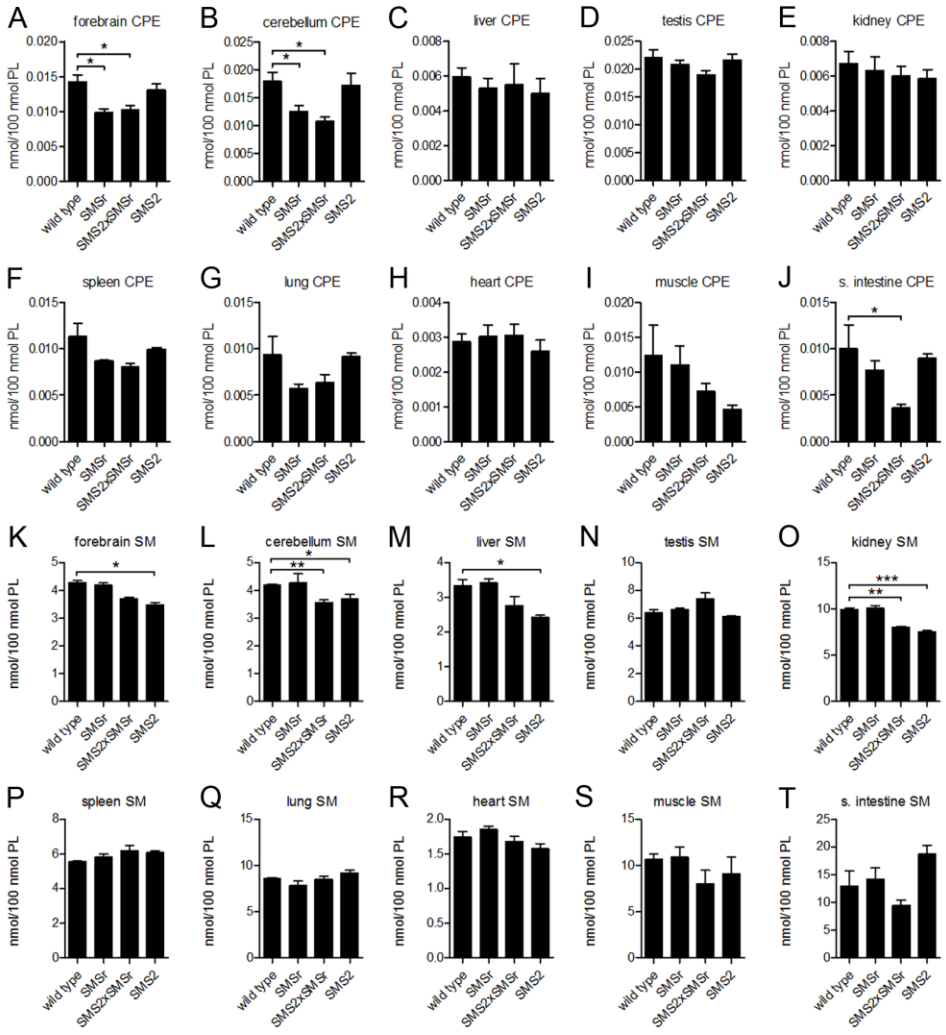

A
forebrain CPE
B
cerebellum CPE
C
liver CPE
D
testis CPE
E
kidney CPE
F
spleen CPE
G
lung CPE
H
heart CPE
I
muscle CPE
J
s. intestine CPE
K
forebrain SM
L
cerebellum SM
M
liver SM
N
testis SM
O
kidney SM
P
spleen SM
Q
lung SM
R
heart SM
S
muscle SM
T
s. intestine SM
nmol/100 nmol PL
wild type
SMSr
SMS2xSMSr
SMS2

Figure S7

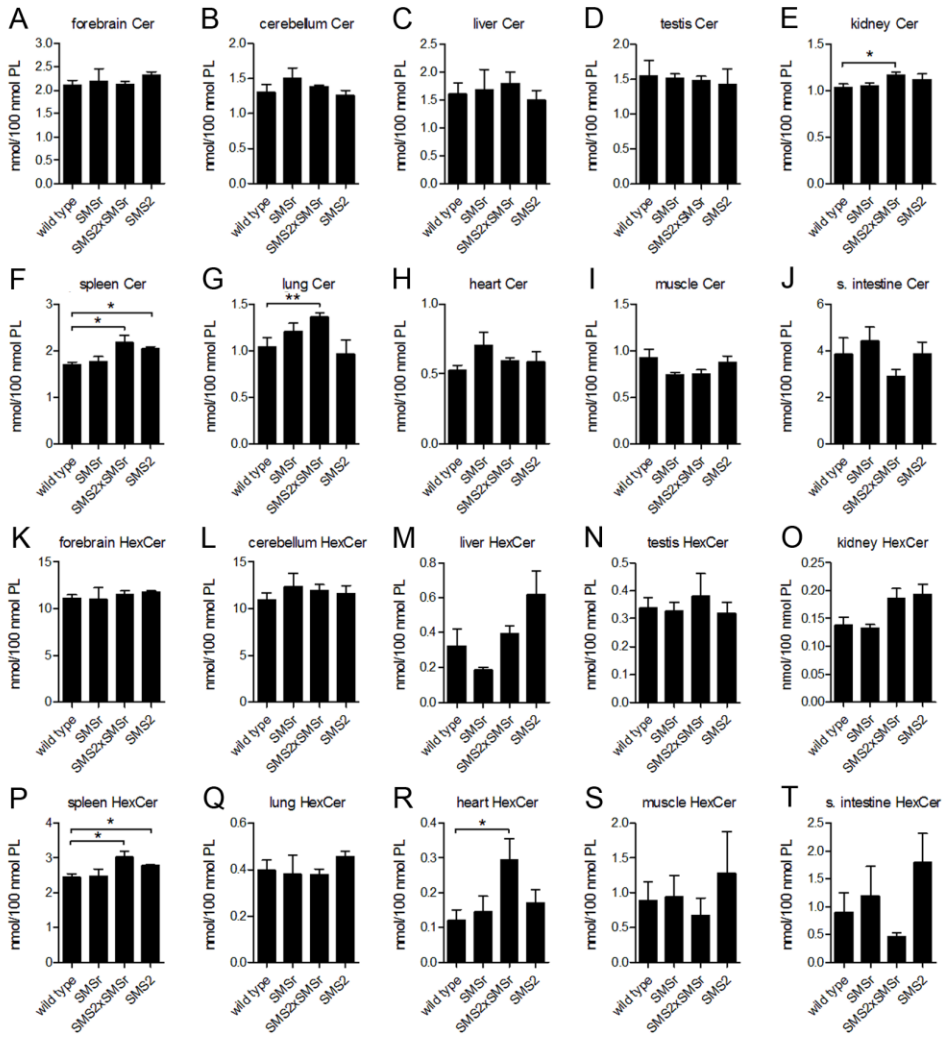

A forebrain Cer
B cerebellum Cer
C liver Cer
D testis Cer
E kidney Cer
F spleen Cer
G lung Cer
H heart Cer
I muscle Cer
J s. intestine Cer
K forebrain HexCer
L cerebellum HexCer
M liver HexCer
N testis HexCer
O kidney HexCer
P spleen HexCer
Q lung HexCer
R heart HexCer
S muscle HexCer
T s. intestine HexCer
nmol/100 nmol PL
wild type
SMSr
SMS2xSMSr
SMS2

Figure S8

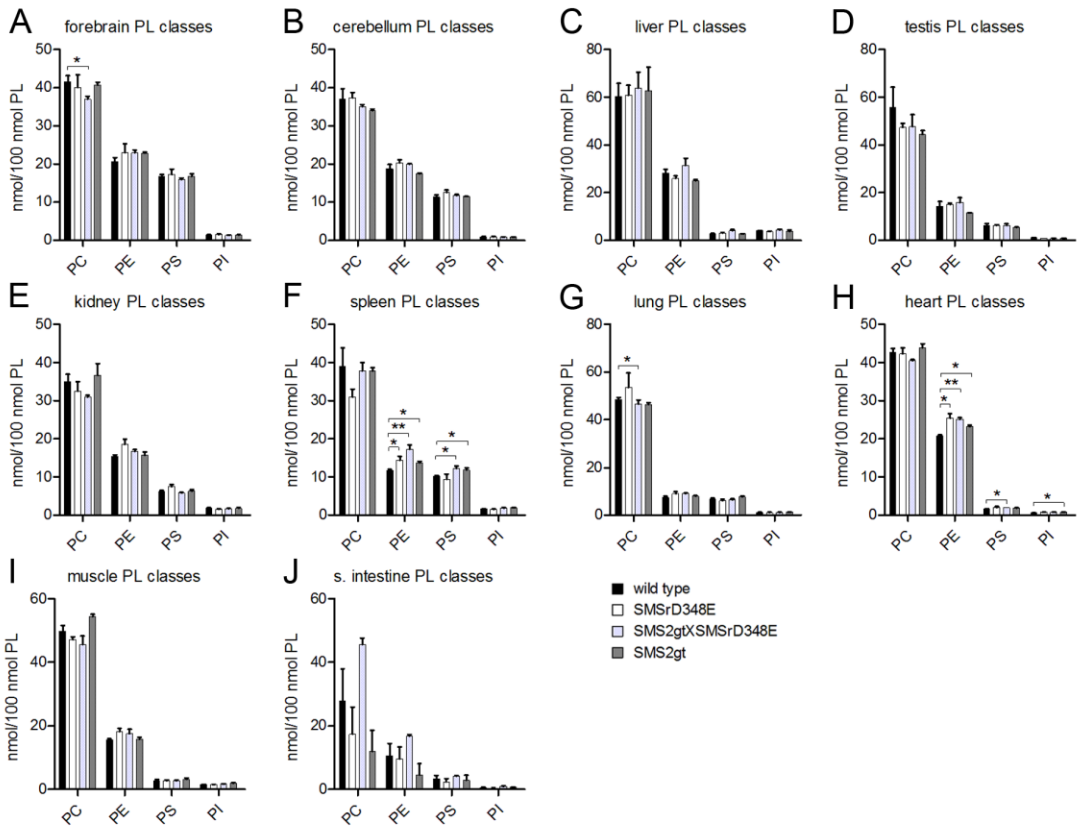
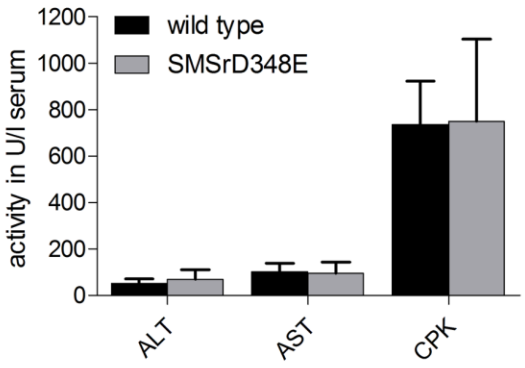

A
forebrain PL classes
B
cerebellum PL classes
C
liver PL classes
D
testis PL classes
E
kidney PL classes
F
spleen PL classes
G
lung PL classes
H
heart PL classes
I
muscle PL classes
J
s. intestine PL classes
nmol/100 nmol PL
PC
PE
PS
PI
wild type
SMSrD348E
SMS2gtXSMSrD348E
SMS2gt

Figure S9


1200
1000
800
600
400
200
0
activity in U/l serum
wild type
SMSrD348E
ALT
AST
CPK