Supplementary information

Sphingomyelin generated by sphingomyelin synthase 1 is involved in attachment and infection with Japanese encephalitis virus

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Supplementary Materials and Methods

Lentivirus infection. Lentivirus particles containing GFP were purchased from Sant Cruz Biothecnology (copGFP Control Lentiviral Particles, sc-108084). WT and SMS DKO tMEFs were seeded on 6 well plate and pre-treated with 1 μg/mL polybrene (Santa Crus Biotechnology) for 20 min at 37°C. Cells were infected for 1 h at 1 MOI, washed with PBS, and cultured. After 48 h infection, GFP-positive cells were observed with fluorescent microscopy BZ-9000 (KEYENCE, Osaka, Japan) and analysed with Gallios (Beckman).

Supplementary Figure Legends

Supplementary Figure S1. Measurement of SM and ceramide after treatment with BSM in WT and SMS DKO tMEFs.

WT and SMS DKO tMEFs were treated with 20 mU/mL BSM for 10 min and then harvested after washing. SM (**a**) and ceramide (**b**) levels were assessed by LC-MS/MS and normalized with total PC amounts. The value presented is the mean ± SD (n = 3). * $P < 0.005$.

Supplementary Figure S2. Lentivirus infection in WT and SMS DKO tMEFs.

WT and SMS DKO tMEFs were infected for 1 h at 1 MOI. After removing unbound lentivirus, cells were cultured for 48 h. (**a**) Cells were observed with fluorescent microscopy, Scale bars, 20 μm. (**b**) Flowcytometry analysis of GFP-positive cells. The value presented is the mean ± SD (n = 3). * $P < 0.005$.

Supplementary Figure S3.

(a) Full-length blots of Figure 2(a). (b) Full-length blots of Figure 2(g). (c) Full-length blots of Figure 2(j). (d) Full-length blots of Figure 2(k).

Supplementary Figure S4.

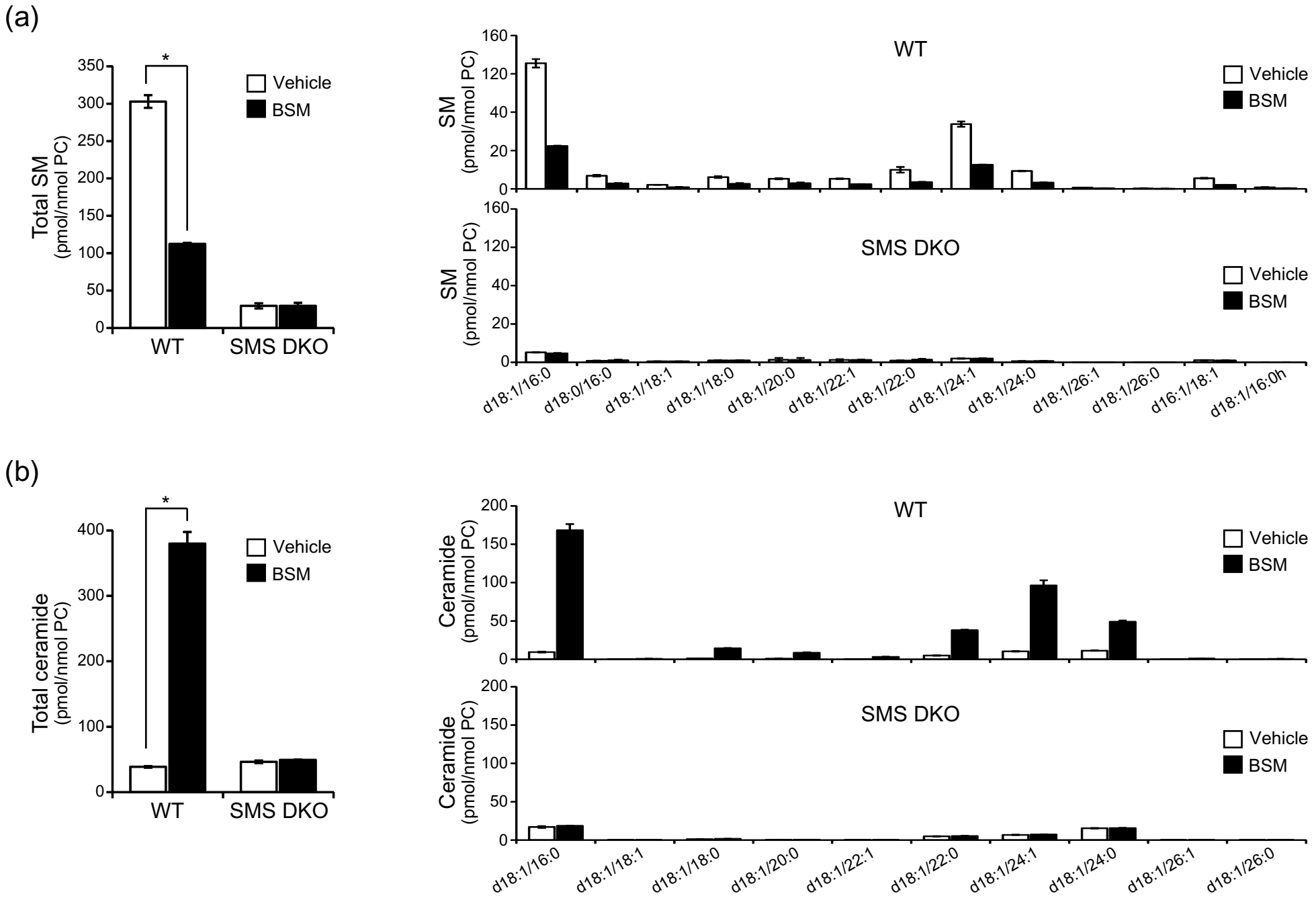
Full-length blots for main figures corresponded to Figure 3(a).

Supplementary Figure S5.

(a) Full-length blots of Figure 4(e). (b) Full-length blots of Figure 4(f).

Supplementary Figure S6.

Full-length blots for main figures corresponded to Figure 5(b).


(a)
Total SM
(pmol/nmol PC)
350
300
250
200
150
100
50
0
*
Vehicle
BSM
WT
SMS DKO
SM
(pmol/nmol PC)
160
120
40
20
0
WT
Vehicle
BSM
SMS DKO
d18:1/16:0
d18:0/16:0
d18:1/18:1
d18:1/18:0
d18:1/20:0
d18:1/22:1
d18:1/22:0
d18:1/24:1
d18:1/24:0
d18:1/26:1
d18:1/26:0
d16:1/18:1
d18:1/16:0h
(b)
Total ceramide
(pmol/nmol PC)
400
300
200
100
0
Ceramide
(pmol/nmol PC)
150
50
d18:1/18:1
d18:1/20:0
d18:1/22:1
d18:1/22:0
d18:1/24:1
d18:1/24:0
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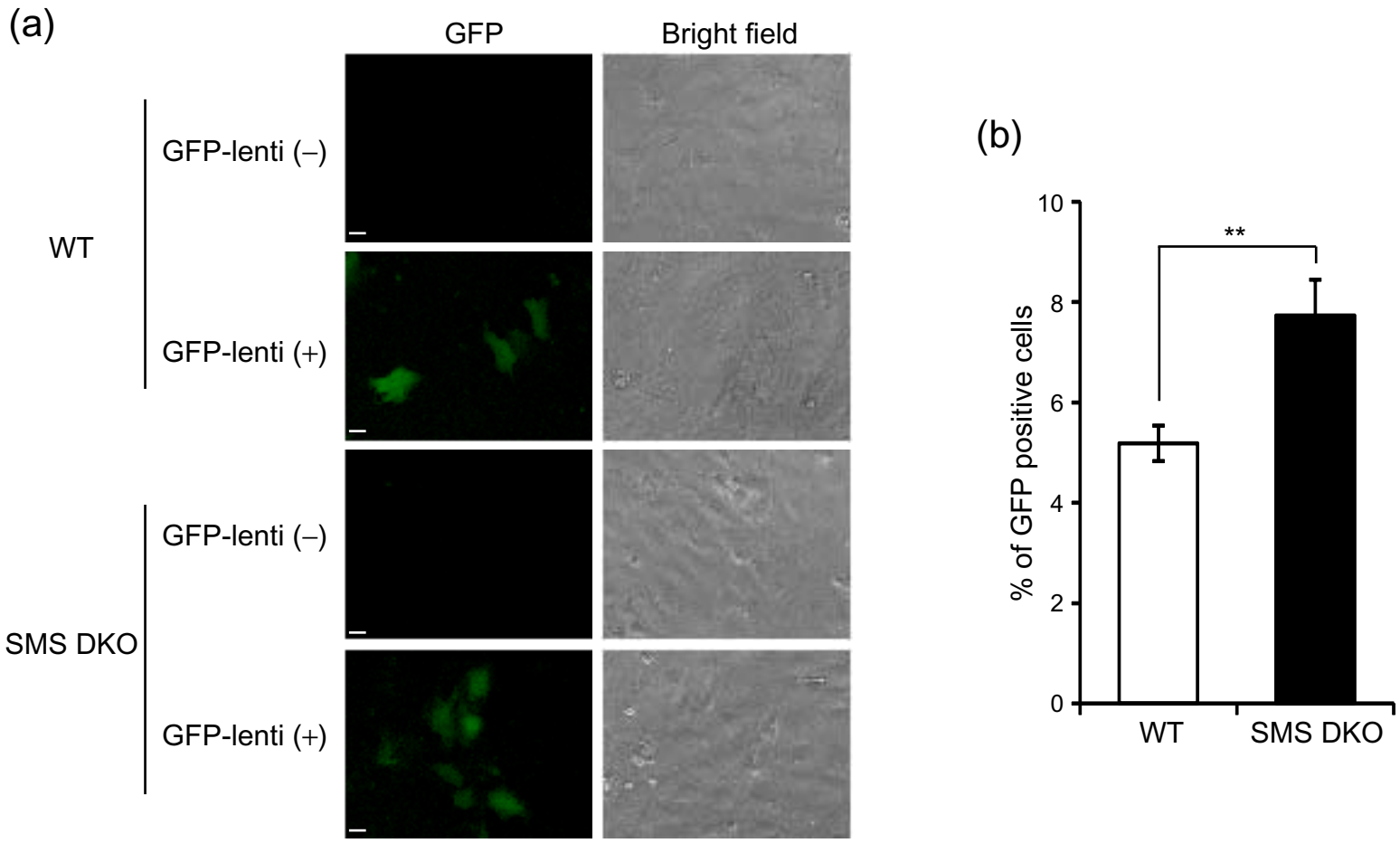
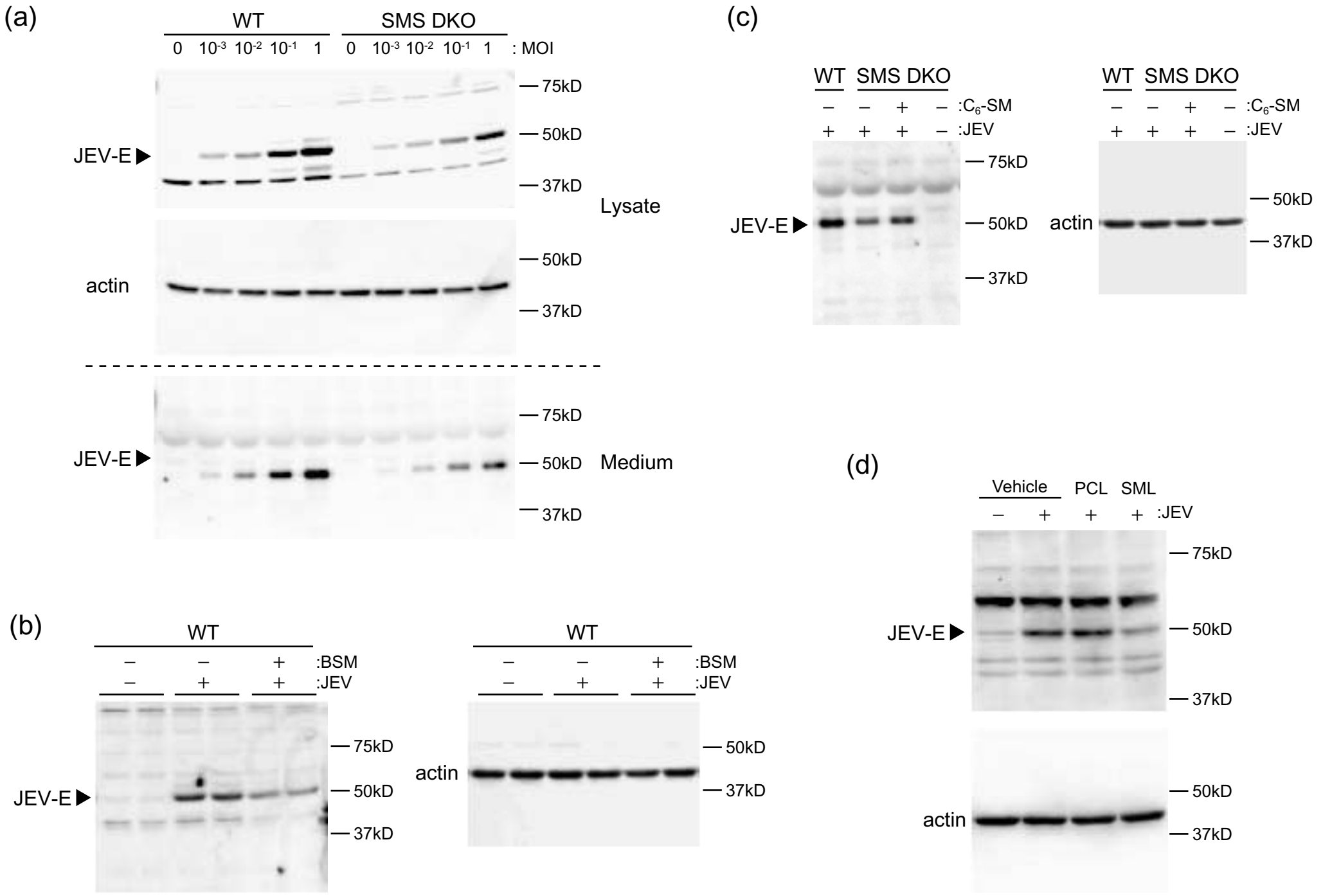
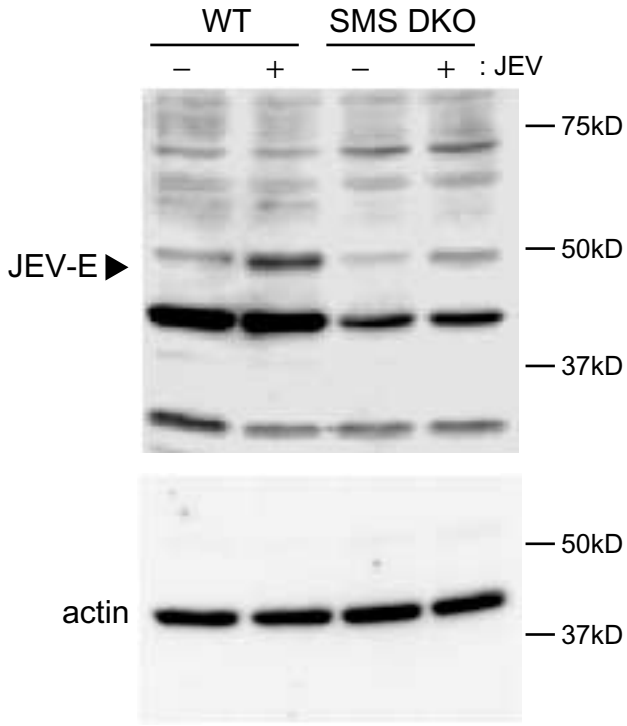
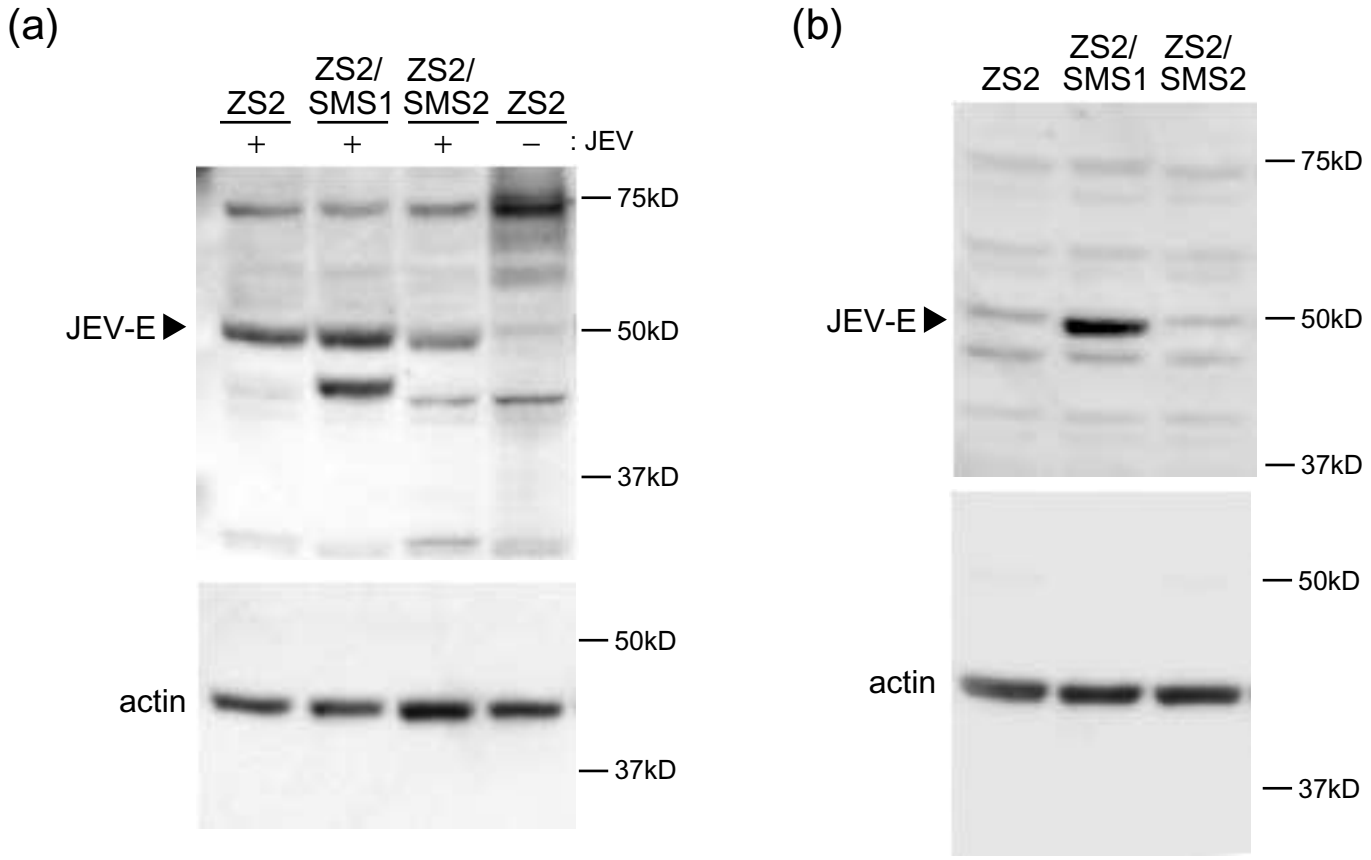
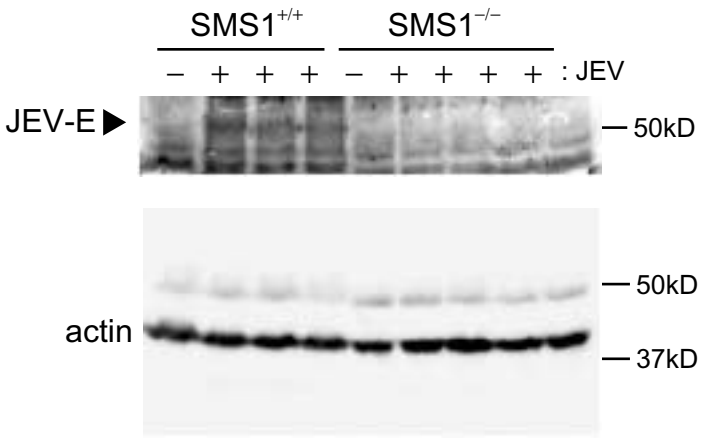
d18:1/26:0


(a)
GFP
Bright field
WT
GFP-lenti (–)
GFP-lenti (+)
SMS DKO
GFP-lenti (–)
GFP-lenti (+)
(b)
% of GFP positive cells
10
8
6
4
2
0
**
WT
SMS DKO


(a)
WT
SMS DKO
0 10-3 10-2 10-1 1 0 10-3 10-2 10-1 1 : MOI
JEV-E
75kD
50kD
37kD
Lysate
actin
50kD
37kD
JEV-E
75kD
50kD
37kD
Medium
(b)
WT
– – + :BSM
– + + :JEV
JEV-E
75kD
50kD
37kD
WT
– – + :BSM
– + + :JEV
actin
50kD
37kD
(c)
WT SMS DKO
– – + – :C6-SM
+ + + – :JEV
JEV-E
75kD
50kD
37kD
WT SMS DKO
– – + – :C6-SM
+ + + – :JEV
actin
50kD
37kD
(d)
Vehicle PCL SML
– + + + :JEV
JEV-E
75kD
50kD
37kD
actin
50kD
37kD


WT
SMS DKO
–
+
–
+
: JEV
75kD
JEV-E
50kD
37kD
50kD
actin
37kD


(a)
ZS2
ZS2/ SMS1
ZS2/ SMS2
ZS2
+
+
+
–
: JEV
JEV-E
75kD
50kD
37kD
actin
50kD
37kD
(b)
ZS2
ZS2/ SMS1
ZS2/ SMS2
JEV-E
75kD
50kD
37kD
actin
50kD
37kD


SMS1+/+
SMS1−/−
− + + + − + + + + : JEV
JEV-E ▶
50kD
actin
50kD
37kD