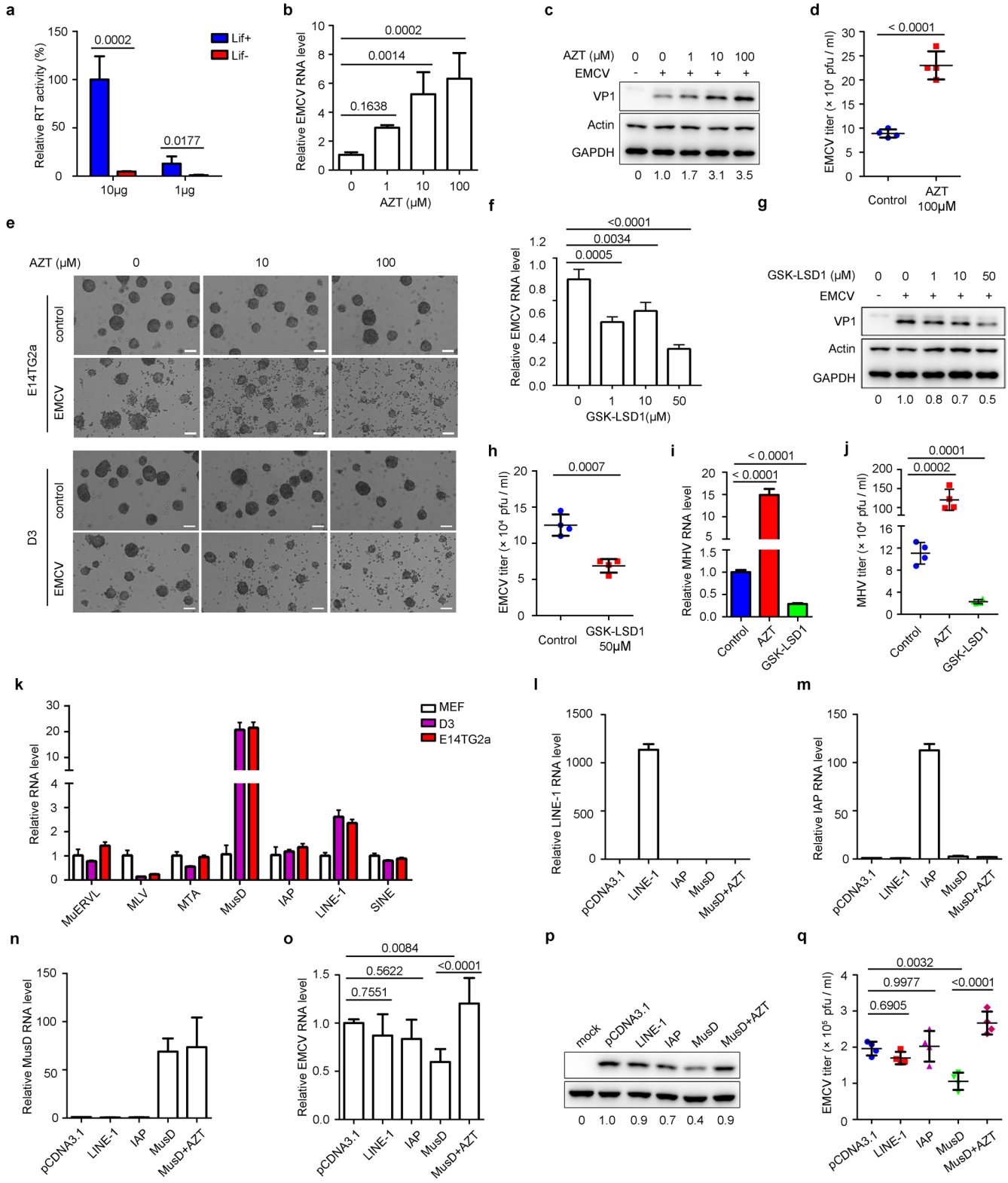
a
Relative RT activity (%)
Lif+
Lif-
0.0002
0.0177
10μg
1μg
b
Relative EMCV RNA level
0.0002
0.0014
0.1638
AZT (μM)
c
AZT (μM) 0 0 1 10 100
EMCV - + + + +
VP1
Actin
GAPDH
0 1.0 1.7 3.1 3.5
d
EMCV titer (× 10⁴ pfu / ml)
< 0.0001
Control
AZT 100μM
e
AZT (μM) 0 10 100
E14TG2a
D3
control
EMCV
f
Relative EMCV RNA level
<0.0001
0.0034
0.0005
GSK-LSD1(μM)
g
GSK-LSD1 (μM) 0 0 1 10 50
EMCV - + + + +
VP1
Actin
GAPDH
0 1.0 0.8 0.7 0.5
h
EMCV titer (× 10⁴ pfu / ml)
0.0007
Control
GSK-LSD1 50μM
i
Relative MHV RNA level
< 0.0001
< 0.0001
Control
AZT
GSK-LSD1
j
MHV titer (× 10⁴ pfu / ml)
0.0001
0.0002
Control
AZT
GSK-LSD1
k
Relative RNA level
MEF
D3
E14TG2a
MuERVL
MLV
MTA
MusD
IAP
LINE-1
SINE
l
Relative LINE-1 RNA level
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT
m
Relative IAP RNA level
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT
n
Relative MusD RNA level
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT
o
Relative EMCV RNA level
0.0084
0.5622
<0.0001
0.7551
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT
p
mock
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT
0 1.0 0.9 0.7 0.4 0.9
q
EMCV titer (× 10⁵ pfu / ml)
0.0032
0.9977
<0.0001
0.6905
pCDNA3.1
LINE-1
IAP
MusD
MusD+AZT

Fig. S2: Role of endogenous RTase in antiviral responses in D3 mESCs.

a, The endogenous RTase activity decreased following the differentiation of mESCs. D3 cells were cultured in the medium with or without Lif for 7 days. Cells were lysed and the RTase activity was measured as described above. The relative RTase activity was presented by setting the Lif+ (10 μg) group as 100%. **b-d**, Inhibition of endogenous RTase activity by AZT promoted virus infection in ESCs. D3 cells were infected with EMCV (moi=1) after pre-treated with AZT at the indicated concentrations for 6 h. The RNA level of EMCV was determined by qRT-PCR (**b**) and the protein level of EMCV VP1 was analyzed by immunoblotting (**c**). Actin and GAPDH were used as loading control. Intensity of VP1 bands was quantitated by ImageJ and normalized to intensity of actin bands. The result is shown at the bottom. The viral titers were examined by plaque assay (**d**). **e**, AZT treatment increased the cytopathic effects of EMCV to mESCs. E14TG2a and D3 cells were treated with AZT and infected by EMCV as indicated above. Three days later, cells were imaged with Zeiss microscope. Scale bar, 100 μm. **f-h**, GSK-LSD1 inhibited virus infection in mESCs. D3 cells were pre-treated by GSK-LSD1 at the indicated concentrations for 24 h and were infected with EMCV (moi=1) for another 24 h. The RNA level of EMCV was determined by qRT-PCR (**f**). The protein level of VP1 and the level of histone H3K4 dimethylation were analyzed by immunoblotting (**g**). Viral titers were measured by plaque assay (**h**). **i**, **j**, D3 cells were pre-treated with 100 μM AZT or 50 μM GSK-LSD1 and infected with MHV (moi=1) for 24 h. The RNA level of MHV was determined by qRT-PCR (**i**) and MHV viral titers were measured by plaque assay (**j**). **k**, The relative mRNA level of representative retroelements in E14TG2a, D3 and MEF cells were analyzed by qRT-PCR. **l-q**, MusD may be one source of RTase activity to protect the mESCs from virus infection. D3 cells were transfected with plasmids containing LINE-1, IAP or MusD for 36h before infected with EMCV (moi=1) for 24 h. For one of the MusD overexpression group, cells were pretreated with 100 μM AZT for 6 h before virus infection. The expression level of LINE-1 (**l**), IAP (**m**) and MuSD (**n**) were evaluated by qRT-PCR with specific primers. The RNA level of EMCV (**o**) were valued by qRT-PCR and the protein level of VP1 was analyzed by immunoblotting (**p**). The viral titers were examined by plaque assay (**q**). Data in **c**, **e**, **g**, **p** are representative of three independent experiments. The graphs represent means ± SD from three (**b**, **f**, **k-n**), four (**a, d, h-j, q**) or six (**o**) independent replicates measured in triplicate. Statistics

were calculated by the two-tailed unpaired Student's *t*-test (**a**, **d**, **h-j**) or one-way ANOVA with Tukey's post hoc tests (**b**, **f**, **o**, **q**).