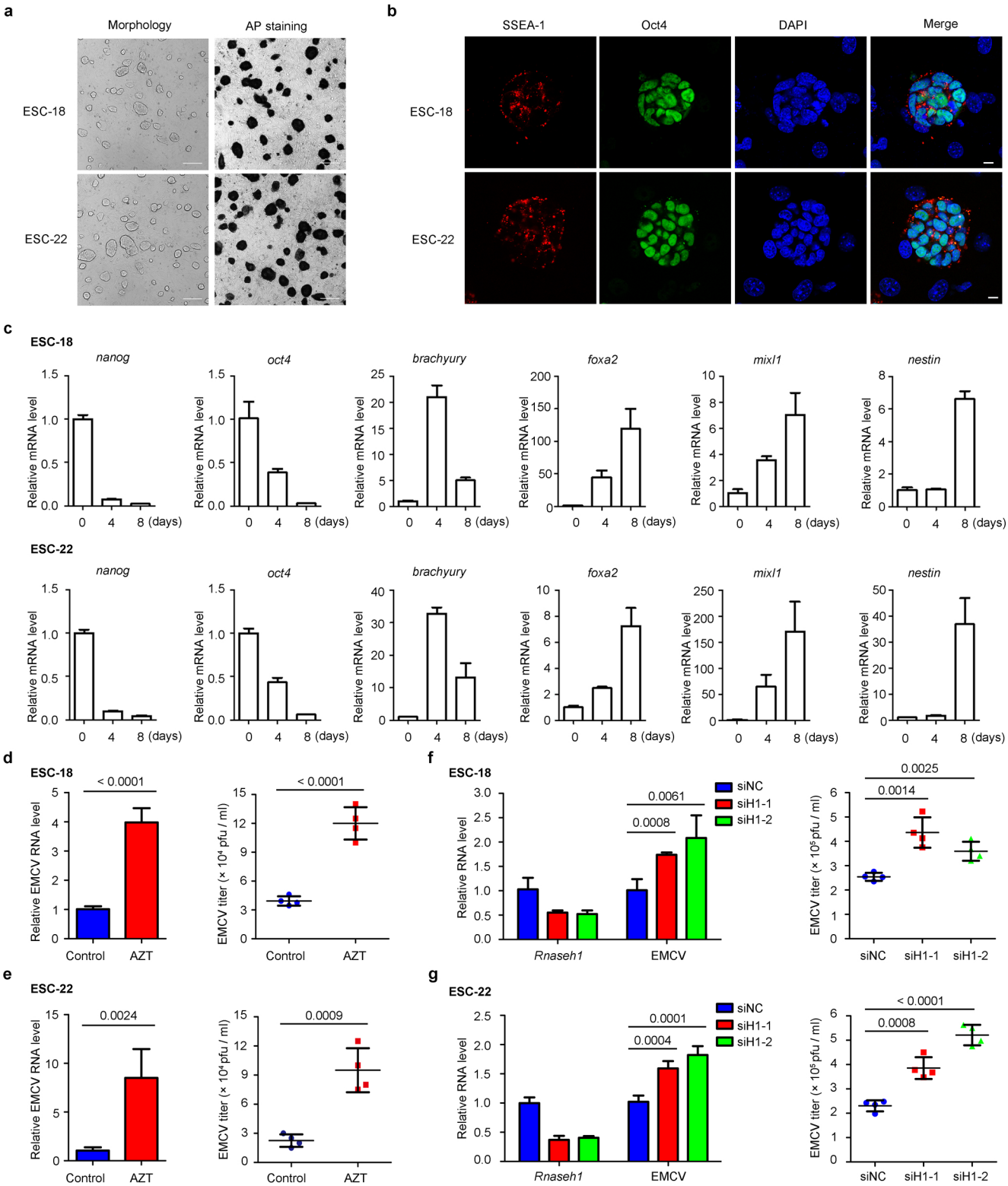
a
Morphology
AP staining
ESC-18
ESC-22
b
SSEA-1
Oct4
DAPI
Merge
ESC-18
ESC-22
c
ESC-18
nanog
oct4
brachyury
foxa2
mixl1
nestin
Relative mRNA level
0 4 8 (days)
ESC-22
nanog
oct4
brachyury
foxa2
mixl1
nestin
Relative mRNA level
0 4 8 (days)
d
ESC-18
< 0.0001
Relative EMCV RNA level
Control
AZT
< 0.0001
EMCV titer (× 10⁴ pfu / ml)
Control
AZT
e
ESC-22
0.0024
Relative EMCV RNA level
Control
AZT
0.0009
EMCV titer (× 10⁴ pfu / ml)
Control
AZT
f
ESC-18
siNC
siH1-1
siH1-2
0.0061
0.0008
Relative RNA level
Rnaseh1
EMCV
0.0025
0.0014
EMCV titer (× 10⁵ pfu / ml)
siNC
siH1-1
siH1-2
g
ESC-22
siNC
siH1-1
siH1-2
0.0001
0.0004
Relative RNA level
Rnaseh1
EMCV
< 0.0001
0.0008
EMCV titer (× 10⁵ pfu / ml)
siNC
siH1-1
siH1-2

Fig. S4: Role of endogenous RTase and RNase H1 in antiviral responses in primary mESCs.

a-c, The derivation and characterization of primary mESCs. **a,** Phase contract images of the two primary mESCs: ESC-18 and ESC-22 (left panel) and alkaline phosphatase staining (AP staining) of the primary ESCs (right panel). **b**, The ESC-18 and ESC-22 cells were fixed and stained with pluripotent markers: SSEA-1 (red) and Oct4 (green). Nuclei were stained with DAPI (blue). Scale bar, 10 μm. **c**, Spontaneous differentiation of the primary mESCs through EB formation. The expression levels of the pluripotent markers (*nanog* and *oct4*) and the germ layer markers (*brachyury*, mesendoderm; *foxa2*, endoderm; *mixl*, mesoderm; *nestin*, ectoderm) were measured by qRT-PCR. **d, e**, Inhibition of endogenous RTase activity by AZT promoted virus infection in the primary ESCs. ESC-18 (**d**) and ESC-22 (**e**) cells were pre-treated with 100 μM AZT for 6 h and then were infected with EMCV (moi=1) for another 24 h. The RNA level of EMCV was determined by qRT-PCR (left panel) and the viral titers were examined by plaque assay (right panel). **f**, **g**, RNase H1 restricted virus infection in the primary mESCs. ESC-18 (**f**) and ESC-22 (**g**) cells were infected with EMCV (moi = 1) after transfected with siRNAs for 36 h. Twenty-four hours later, the RNA level of RNaseH1 and EMCV were determined by qRT-PCR (left panel) and viral titers were valued by plaque assay (right panel). Data in **a**, **b** are representative of two independent experiments. The graphs represent means ± SD from three (**c**) or four (**d-g**) independent replicates measured in triplicate. Statistics were calculated by the two-tailed unpaired Student's *t*-test.