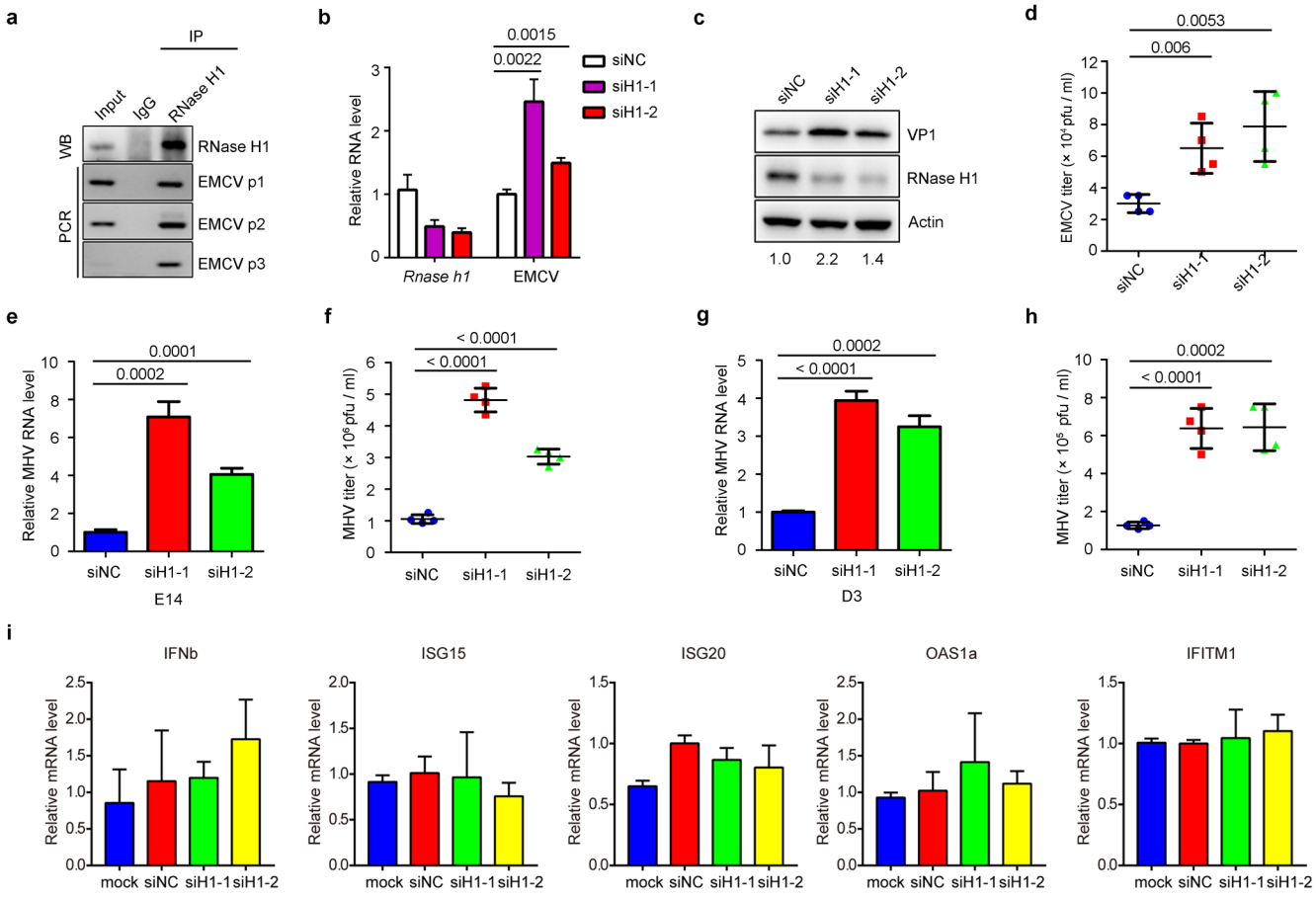
a
IP
Input
IgG
RNase H1
WB
PCR
RNase H1
EMCV p1
EMCV p2
EMCV p3
b
0.0015
0.0022
siNC
siH1-1
siH1-2
Relative RNA level
Rnase h1
EMCV
c
siNC
siH1-1
siH1-2
VP1
RNase H1
Actin
1.0
2.2
1.4
d
0.0053
0.006
EMCV titer (× 10⁶ pfu / ml)
siNC
siH1-1
siH1-2
e
0.0001
0.0002
Relative MHV RNA level
siNC
siH1-1
siH1-2
E14
f
< 0.0001
< 0.0001
MHV titer (× 10⁶ pfu / ml)
siNC
siH1-1
siH1-2
g
0.0002
< 0.0001
Relative MHV RNA level
siNC
siH1-1
siH1-2
D3
h
0.0002
< 0.0001
MHV titer (× 10⁵ pfu / ml)
siNC
siH1-1
siH1-2
i
IFNb
ISG15
ISG20
OAS1a
IFITM1
Relative mRNA level
mock
siNC
siH1-1
siH1-2

Fig. S11: RNase H1 is essential for the antiviral function of vcDNA in undifferentiated mESCs.

a, RNase H1 binds to vcDNA. EMCV-infected D3 cell lysates were immunoprecipitated by anti-RNase H1 antibody. The pull-down efficiency was validated by Western blotting with anti-RNase H1 antibody (upper panel). The enrichment of vcDNA was analyzed by PCR with the indicated primers (lower panel). **b**-**d**, Depletion of RNase H1 promoted EMCV infection in mESCs. D3 cells were infected with EMCV (moi = 1) after transfection with siRNAs for 36 h. The RNA level of RNaseH1 and EMCV was determined by qRT-PCR (**b**), the protein level of VP1 was analyzed by immunoblotting (**c**), and EMCV viral titers were valued by plaque assay (**d**). **e**-**h**, Depletion of RNase H1 promoted MHV infection in mESCs. After siRNA transfection for 36 h, E14TG2a (**e**, **f**) and D3 cells (**g**, **h**) were infected with MHV (moi = 1). Twenty-four hours later, the RNA level of MHV was determined by qRT-PCR (**e**, **g**) and MHV viral titers were valued by plaque assay (**f**, **h**). **i**, The influence of RNase H1 on the expression of ISGs. E14TG2a cells were transfected with siRNAs for 36h and then were infected with EMCV (moi=1) for 24h. The mRNA levels of IFNβ and the indicated ISGs were analyzed by qRT-PCR. Data in **a**, **c** are representative of three independent experiments. The graphs represent means ± SD from three (**b**, **i**) or four (**d**-**h**) independent biological replicates measured in triplicate. Statistics were calculated by the two-tailed unpaired Student's t-test.