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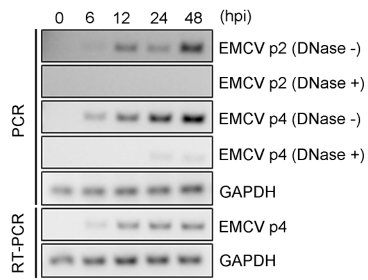

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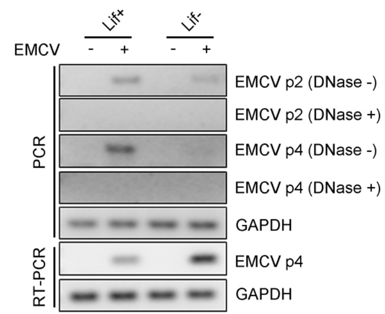

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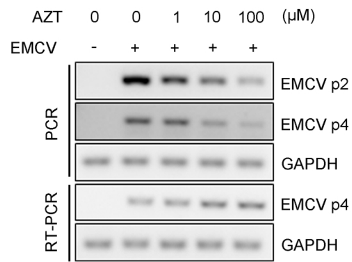

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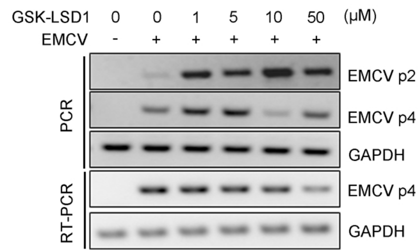

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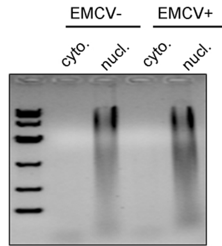

f

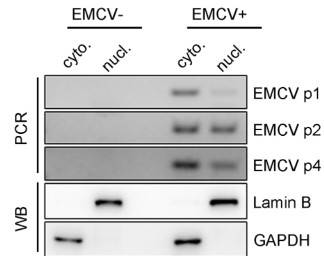

Fig. S9: Generation of viral DNA in the cytoplasm of infected D3 mESCs.

a, Kinetics of the vDNA synthesis in D3 cells. D3 cells were infected with EMCV (moi = 1), followed by extraction of total DNA and RNA at 0-48 hpi. Total DNA was treated with Turbo DNase (DNase +) or without (DNase -). The virus RNA and vDNA levels were analyzed by RT-PCR (lower panel) and PCR (upper panel), respectively. GAPDH was used as loading control. **b**, the vDNA level decreased following the differentiation of mESCs. D3 cells cultured in the medium with or without Lif for 7 days were infected by EMCV. Twenty-four hours later, the virus RNA and vDNA levels were analyzed, respectively. **c**, AZT inhibits vDNA synthesis in D3 cells. D3 cells were infected with EMCV (moi = 1) after treatment with AZT at the indicated concentrations for 6 h. The virus RNA and vDNA levels were analyzed by RT-PCR (lower panel) and PCR (upper panel), respectively. **d**, GSK-LSD1 promotes vDNA production in D3 cells. 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 virus RNA and vDNA levels were analyzed, respectively. **e**, **f**, The vDNA is mainly localized in the cytoplasm. The cytoplasmic and nuclear fractions were separated from D3 cells with or without virus infection. The DNA of each fraction was extracted and analyzed by agarose gel electrophoresis (**e**). The vDNA was detected by PCR with the indicated primers (**f**, upper panel). Lamin B and GAPDH were used as nuclear and cytoplasmic markers by Western blotting, respectively (**f**, lower panel). Data in **a-f** are representative of three independent experiments.