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Electronic Supplementary Material

Myristoylation of EV71 VP4 is Essential for Infectivity and Interaction with Membrane Structure

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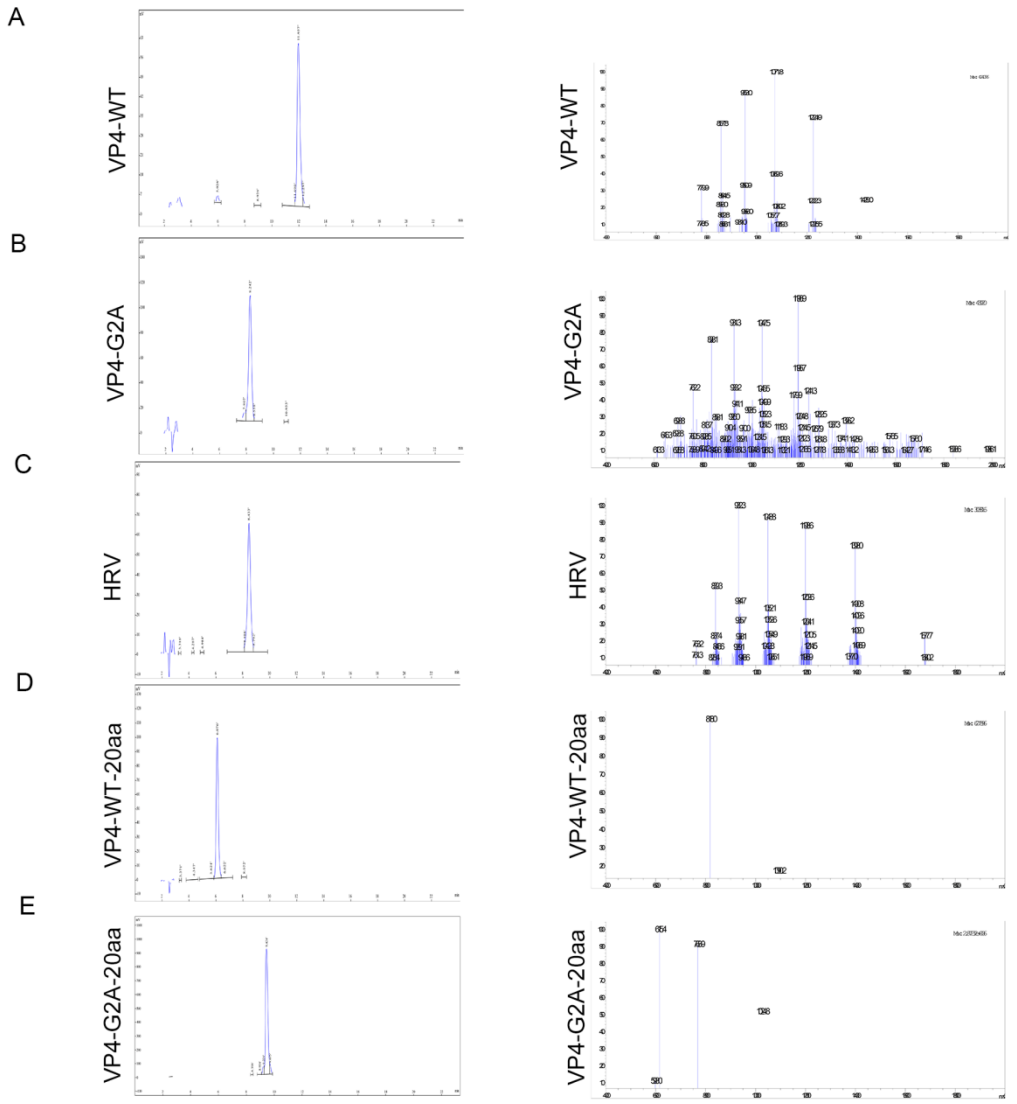

Fig. S1 Analysis of synthetic peptides by HPLC and mass spectrometry (MS). **(A) - (E)** Left pane: Chromatograms of synthetic peptides VP4-WT, VP4-G2A, HRV, VP4-WT-20aa and VP4-G2A-20aa. The significant peaks represent the highest concentration of protein in the sample. **(A) - (E)** Right pane: Mass spectrometry patterns of synthetic peptides VP4-WT, VP4-G2A, HRV, VP4-WT-20aa and VP4-G2A-20aa. The significant peaks in every pattern represent the percentage of proton absorption.

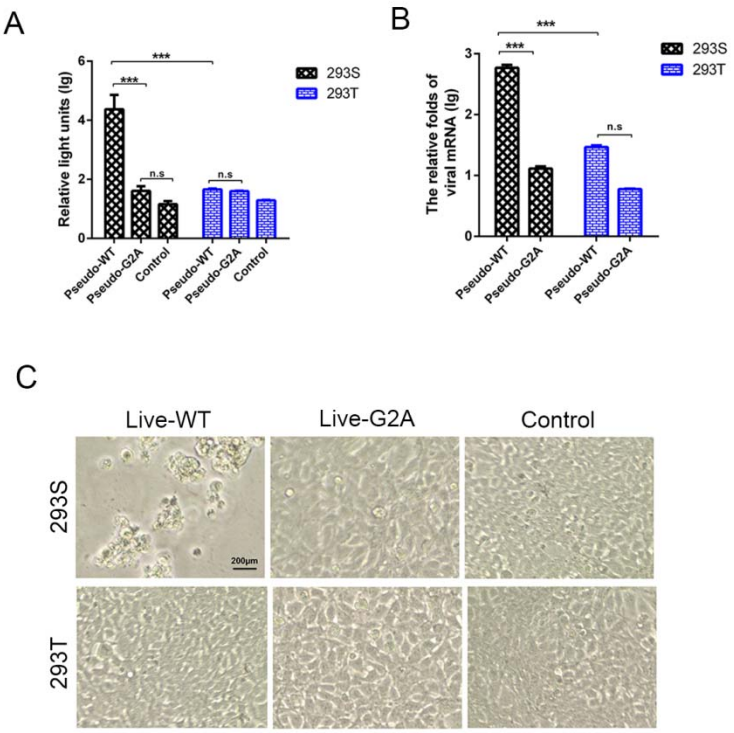

Fig.S2. Comparison of viral infectivity on 293T and 293S cells. **(A)** Luciferase activity analysis of 293S and 293T cells infected with Pseudo-WT and Pseudo-G2A virus. Myristoylation and myristoylation-deficient (G2A) reporter pseudoviruses with the equal amount viral RNA infected 293S and 293T cells, luciferase activity was detected 18 h after infection. Control represents empty cells that were not infected. Data presented as mean ± SD of three independent experiments. ***$P<0.001$. n.s: non-significant. **(B)** RNA content analysis of 293S and 293T cells infected with Pseudo-WT and Pseudo-G2A virus. Myristoylation and myristoylation-deficient (G2A) reporter pseudoviruses with the equal amount viral RNA infected 293S cells. At 24 hpi, total RNA was extracted from infected cells, and then the viral RNA was quantified with real-time PCR. The mRNA levels of viruses were normalized to GAPDH. Data presented as mean ± SD of three independent experiments. ****P <0.001. n.s: non-significant.* **(C)** Representative photography of cytopathic effects (CPE) analysis of 293S and 293T cells infected with WT and G2A virus. Myristoylation and myristoylation-deficient (G2A) viruses with the equal amount viral RNA infected 293S and 293T cells, and after 1 h infection, the supernatant was discarded, and fresh medium was added into the cell culture. Continuous observation of cells was used to evaluate CPE. Scale bar = 200 μm. Live-WT: live myristoylation virus; Live-G2A: live myristoylation-deficient viruses.

Table S1. Amino acid sequences of VP4 peptide

Peptides	Amino acid sequences
WT-VP4	#Myr-GSQVSTQRSGSHENSNSATEGSTIDYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDIFTEMAAPLK
G2A-VP4	ASQVSTQRSGSHENSNSATEGSTIDYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDIFTEMAAPLK
WT-VP4-20aa	#Myr-GSQVSTQRSGSHENSNSATE
G2A-VP4-20aa	ASQVSTQRSGSHENSNSATE
HRV-WT-VP4	#Myr-GAQVSTQKSGSHENQNILTNGSNQTFTVINYYKDAASTSSAGQSLSMDPSKFTEPVKDLMLKGAPALN

#: Myristoyl modification