**Virologica Sinica**

**Supplementary Data**

**Enterovirus 71 3C proteolytically processes the histone H3 N-terminal tail during infection**

Meng Miaoa, b\*\*\*, Gang Deng a, Xiaobei Xiongc, Yang Qiu c, Wenda Huang a, Meng Yuan a, Fei Yu a, Shimei Baib, Xi Zhouc\*\*, Xiaolu Zhaoa\*

a Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University, Wuhan 430072, China

b Institute of Biochemistry, College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, 310018, China.

c Laboratory of RNA Virology, Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, China

\*Corresponding author. Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University, Wuhan 430072, China

\*\* Corresponding author. Laboratory of RNA Virology, Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, China

\*\*\* Corresponding author. Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University, Wuhan 430072, China; Institute of Biochemistry, College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, 310018, China.

Email addresses: zhaoxiaolu@whu.edu.cn (X. Zhao), zhouxi@whu.edu.cn (X. Zhou), miaom@whu.edu.cn (M. Miao)

**Supplementary Materials and Methods**

**Cells and viruses**

Human rhabdomyosarcoma cells (RD) cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Amarillo, TX, USA) supplemented with 10% fetalbovine serum (FBS; Gibco BRL, Grand Island, NY, USA), 1 mmol/L sodium pyruvate, 100 μg/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO2 incubator. Wild-type EV71 was rescued from infectious cDNA clone pEV-HeN09. The viruses were amplified using RD cells.

**Plasmid construction**

The EV71 3C gene was amplified from the cDNA clone pEV-HeN09 and then inserted into the pRK vector with an N-terminal FLAG tag. EV71 3C mutants C147S, and 3×KKKRK NLS tag fusion plasmid were generated using standard site-directed mutagenesis. The primers used for mutation in this study are shown in Supplementary Table S1.

**Histone purification**

Histones were acid extracted from nuclei as described (Guo et al., 2018).

**Western blot analysis and antibodies**

Cell lysate preparation, SDS-PAGE, and western blot was performed as previously

described (Miao M et al., 2019). The following antibodies were used: HIST3H3 Polyclonal Antibody (ABclonal), Anti-Histone H3 antibody-C-terminal (ABclonal), DiMethyl-Histone H3-K4 Polyclonal Antibody (ABclonal), DiMethyl-Histone H3-K14 Polyclonal Antibody (ABclonal), Acetyl-Histone H3-K18 Polycolonal Antibody (ABclonal), TriMethyl-Histone H3-K27 Polyclonal Antibody (ABclonal), Histone H4 Polyclonal Antibody (ABclonal), Enterovirus 71 3C antibody (Gene Tex), anti-GAPDH antibody (Abcam), [Anti-Lamin B1 antibody](https://www.abcam.cn/lamin-b1-antibody-epr8985b-ab133741.html) (Abcam), Anti-β-actin antibody (Abcam).

**Immunofluorescence microscopy**

RD cells (20%–50% confluent) seeded onto coverslips in 6-well plates were infected as indicated. At 8, 24, and 36 h post-infection, the cells were fixed with 2% formaldehyde, permeabilized with 0.25% Triton X-100, blocked in 10% serum, and incubated with Enterovirus 71 3C antibody (Gene Tex) diluted 1:1000 for 2 h. They were then stained with FITC-conjugated goat anti-mouse IgG (Invitrogen) for 1 h. The coverslips were then washed once with PBS; treated with nuclear stain DAPI (diluted 1:500) for 15 min; washed three times with PBS, and mounted on glass slides with mounting fluid (75% glycerol in PBS). The images were obtained under a confocal laser-scanning microscope (Zeiss; LSM 510 NLO).

**Preparation and purification of recombinant protein**

To produce recombinant 3C and 3C-C147S proteins, plasmid pMAL-EV71-3C and pMAL-EV71-m3C-C147S, were introduced into competent E. coli BL21 (DE3 pLysS) and protein expression was induced using 40 mmol/L isopropyl b-Dthiogalactopyranoside. 3C-MBP, 3C-C147-MBP fusion proteins and MBP alone were purified using amylose affinity chromatography (New England BioLabs, Ipswich, MA) according to the manufacturer′s protocol and then concentrated using Amicon Ultra-15 filters (Millipore, Schwalbach, Germany). All purified proteins were quantified with a bicinchoninic acid (BCA) protein assay Kit (CWBIO, China) and stored at −80 °C in aliquots. Proteins were separated on 10% SDS-PAGE and visualized by Coomassie blue.

***In vitro* protease cleavage assay**

Purified recombinant 3C and 3C-C147S proteins were incubated with 0.5 μg histones samples (purified from nuclear extracts) in buffer (50 mmol/L HEPES-KOH at pH=8.0) with total volume of 10 μL at 37°C for two hours and analyzed by immunoblotting with Histone H3 C-terminal antibody and/or H3K18ac, H3K27me3 antibodys. MBP protein was used as a negative control.

Table S1 Primers used for EV71 3C protein mutation.

|  |  |
| --- | --- |
| **Primers** | **Sequence (5**′ **to 3**′**)** |
| 3C-F | AAATATGCGGCCGCGGGGACCGAGCTTGGACTTCGCCTTATCTC |
| 3C-R | CCGCTCGAGTTATTGTTCACTGCAAAAGTATCCTCTCTTCAGAGC |
| 3×NLS-3C-F | GCGTCGACGGATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGTAGCGGCCGCAA |
| 3×NLS-3C-R | TTGCGGCCGCTACCTTTCTCTTCTTTTTTGGATCTACCTTTCTCTTCTTTTTTGGATCTACCTTTCTCTTCTTTTTTGGATCCGTCGACGC |
| 3×NLS-3C-C147S-F | GCAGGACAGAGCGGTGGTGTTG |
| 3×NLS-3C-C147S-R | CAACACCACCGCTCTGTCCTGC |

**References:**

Guo Q, Sidoli S, Garcia BA, Zhao X, 2018. Assessment of Quantification Precision of Histone Post-Translational Modifications by Using an Ion Trap and down To 50 000 Cells as Starting Material. J Proteome Res. 17, 234-242.

Miao M, Yu F, Wang D, Tong Y, Yang L, Xu J, Qiu Y, Zhou X, Zhao X. 2019. Proteomics Profiling of Host Cell Response via Protein Expression and Phosphorylation upon Dengue Virus Infection. Virol Sin. 34, 549-562.