Suppression of monkeypox virus by downregulation of fatty acid synthase and upregulation of cholesterol-25 hydroxylase

Saba Aliyari R[1], Jean Shanaa[1], Daniel Yu[1], Mari Yamamoto[1], Kaitlyn N. Bateman[1], Lulan Wang[1], and Genhong Cheng[1]

[1]UCLA Department of Microbiology Immunology and Molecular Genetics

June 05, 2024

Abstract

The re-emergence of the monkeypox virus (MPXV) three years after the start of the SARS-CoV-2 epidemic further emphasizes the need to develop broad spectrum antivirals (BSAs) that might control the spread of poorly understood pathogens. The induction of innate immune responses to a viral infection triggers rapid expression of type-I-interferon (IFN-I), which subsequently results in the differential expression of more than 300 genes that foster an antiviral state. Whereas the expression of a majority of these "interferon-stimulated genes" (ISGs) are enhanced, , other ISGs' expression are suppressed, including some involved in lipid metabolism which is hijacked to promote viral growth. Herein, we report that the expression of fatty acid synthase (FASN), an enzyme involved in *de novo* biosynthesis of fatty acids, was significantly reduced upon MPXV infection. Moreover, MPXV infection was impaired in FASN knockout cells, and biological inhibitors of FASN significantly inhibited MPXV. Interestingly, the ISG chosterol-25-hydroxylase was induced in MPXV-infected cells, and its enzymatic product, 25-hydroxychlosterol (25HC), blocked MPXV infection. Overall, this study indicates that 25HC and FASN inhibitors are highly potent BSAs and may have therapeutic applications in combating understudied infectious diseases in early outbreak settings when targeted therapies have not yet been developed.

Hosted file

`MPXV-Main Text.doc` available at https://authorea.com/users/791496/articles/1070783-suppression-of-monkeypox-virus-by-downregulation-of-fatty-acid-synthase-and-upregulation-of-cholesterol-25-hydroxylase

Suppression of monkeypox virus replication by down-regulation of fatty acid synthase and upregulation of Cholesterol-25 hydroxylase

Fig.1

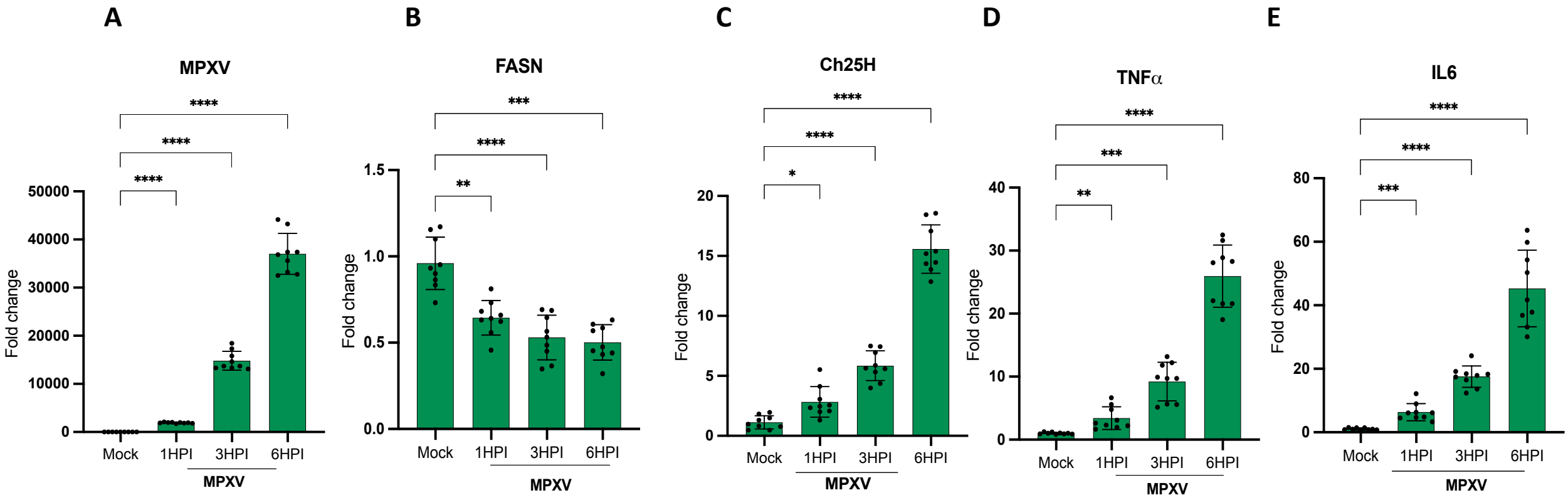

Figure 1. IFN-I-regulated genes control MPXV infection. A) Huh 7.5 cells were infected with MPXV (MOI=0.1) and harvested at 1, 3, and 6 HPI. The expression level of (A) MPXV, (B) FASN, (C) CH25H, (D) TNFα, (E) IL6, (F) IFIT1, (G) IFITM1, (H) IRF1, and (K) DDX58 was quantified by qRT-PCR.

Fig.1

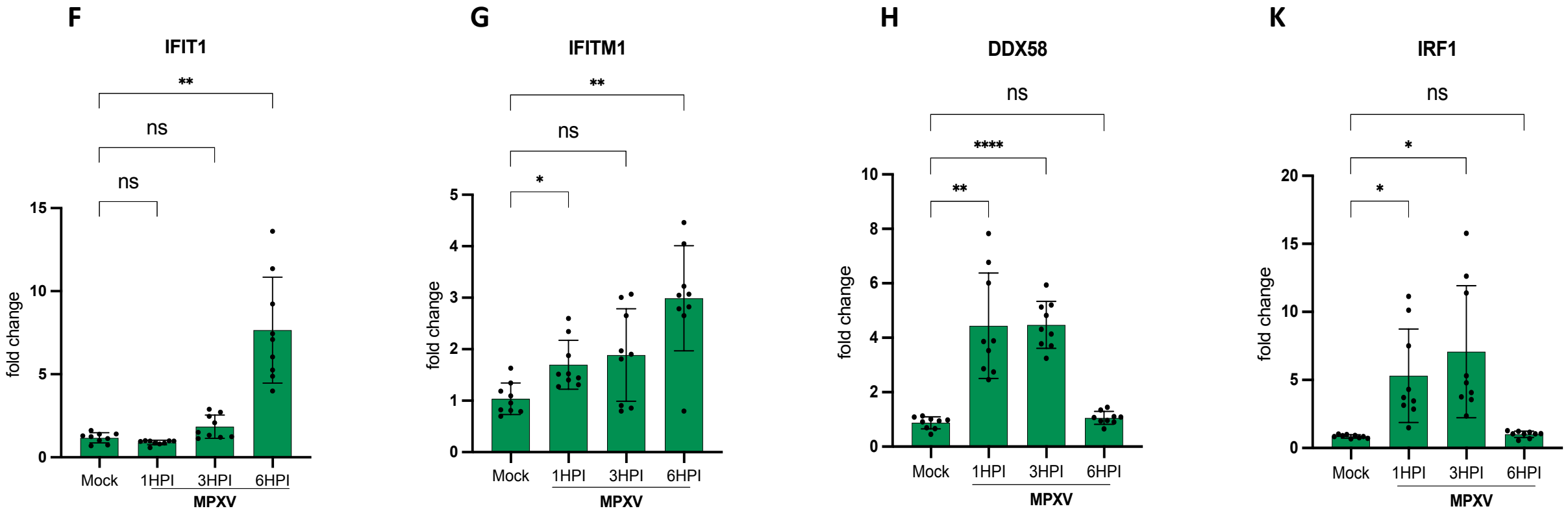

Figure 1. IFN-I-regulated genes control MPXV infection. A) Huh 7.5 cells were infected with MPXV (MOI=0.1) and harvested at 1, 3, and 6 HPI. The expression level of (A) MPXV, (B) FASN, (C) CH25H, (D) TNFα, (E)IL6, (F) IFIT1, (G) IFITM1, (H) IRF1, and (K) DDX58 was quantified by qRT-PCR.

Fig.2

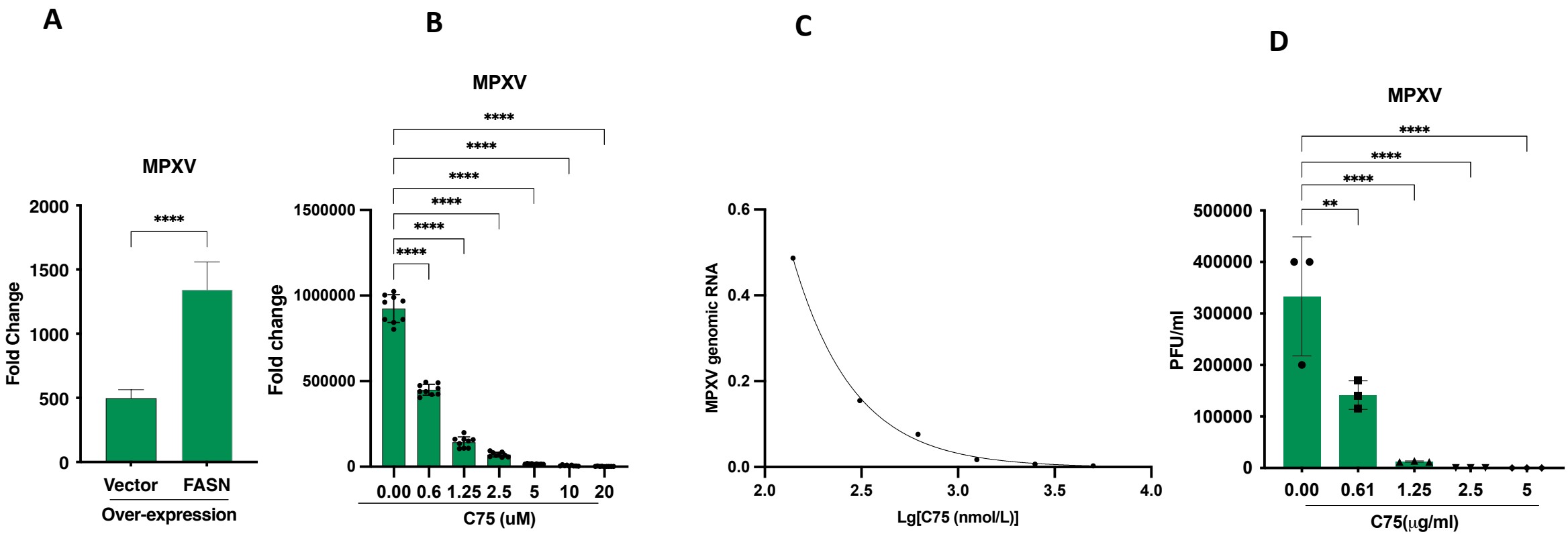

Figure 2. The impact of FASN on MPXV infection. (A) Huh 7.5 cells were transfected with control plasmid (vector only) or expression plasmid, FASN for 24 hours prior to MPXV infection (MOI=0.1) and cells were harvested to assess the rate of viral replication at 24HPI. (B) The rate of MPXV infection in the presence of C75 measured by qRT-PCR. (C) identification of C75 IC50. (D) MPXV titration in cells treated with different concentration of C75 by plaque assay.

Fig.3

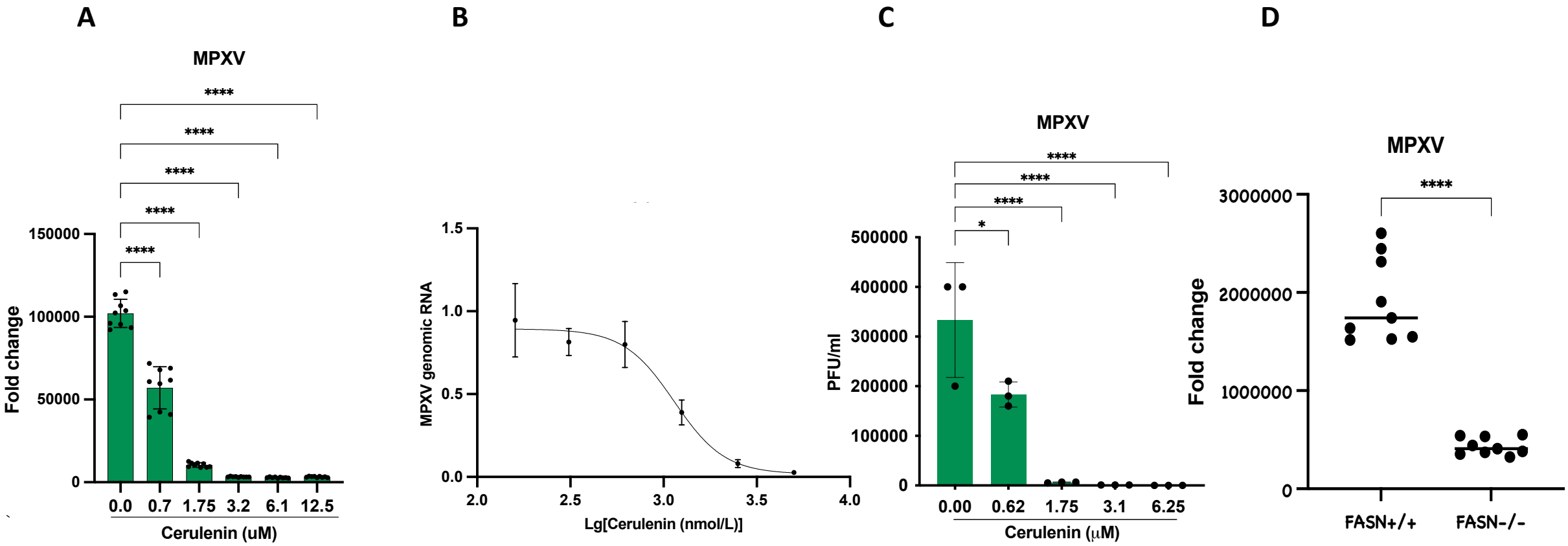

Figure 3. Blocking the MPXV replication by Cerulenin. (A) Huh-7.5 cells were treated with indicated dose of Cerulenin one-hour post-MPXV infection. The cell lysate was subjected to (B) RNA extraction to evaluate the level of viral RNA transcripts (C) and identification of Cerulenin IC_{50} by using RT-qPCR. (D) The supernatant was used for quantification of infectious viral particles by plaque assay.

Fig.4

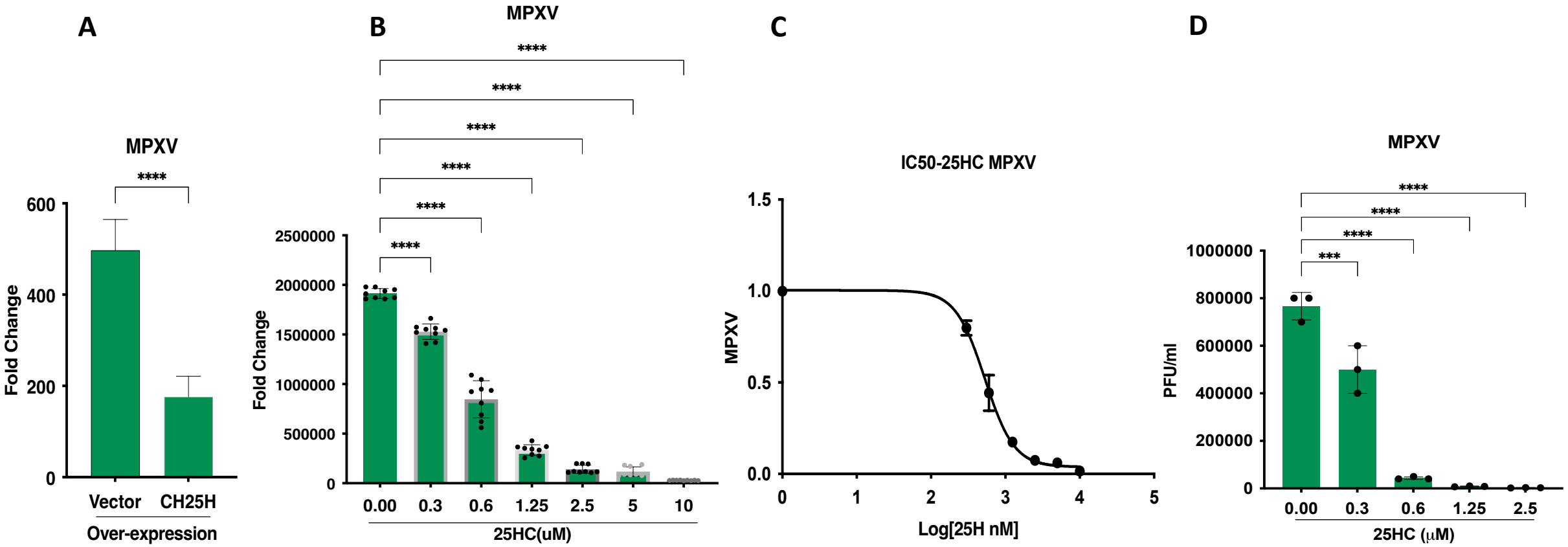

Figure 4. Over-expression of IFN-I regulated gene, CH25H or treating cells with 25HC can inhibit MPXV infection. (A) Huh 7.5 cells were transfected with expression plasmids, Vector only or the vector expressing CH25H for 24 hours prior to MPXV infection (MOI=0.1) and cells were harvested to assess the rate of viral replication at 24HPI. (B) The Huh 7.5 cells were infected with MPXV and treated with the indicated dose of 25HC one hour-post infection. The cell lysate was subjected to RNA extraction to evaluate the level of viral RNA transcripts (C) and identification of 25HC IC50 by using RT-qPCR. (D) The supernatant was used for titration of infectious MPXV particles by plaque assay.

Fig. S-1

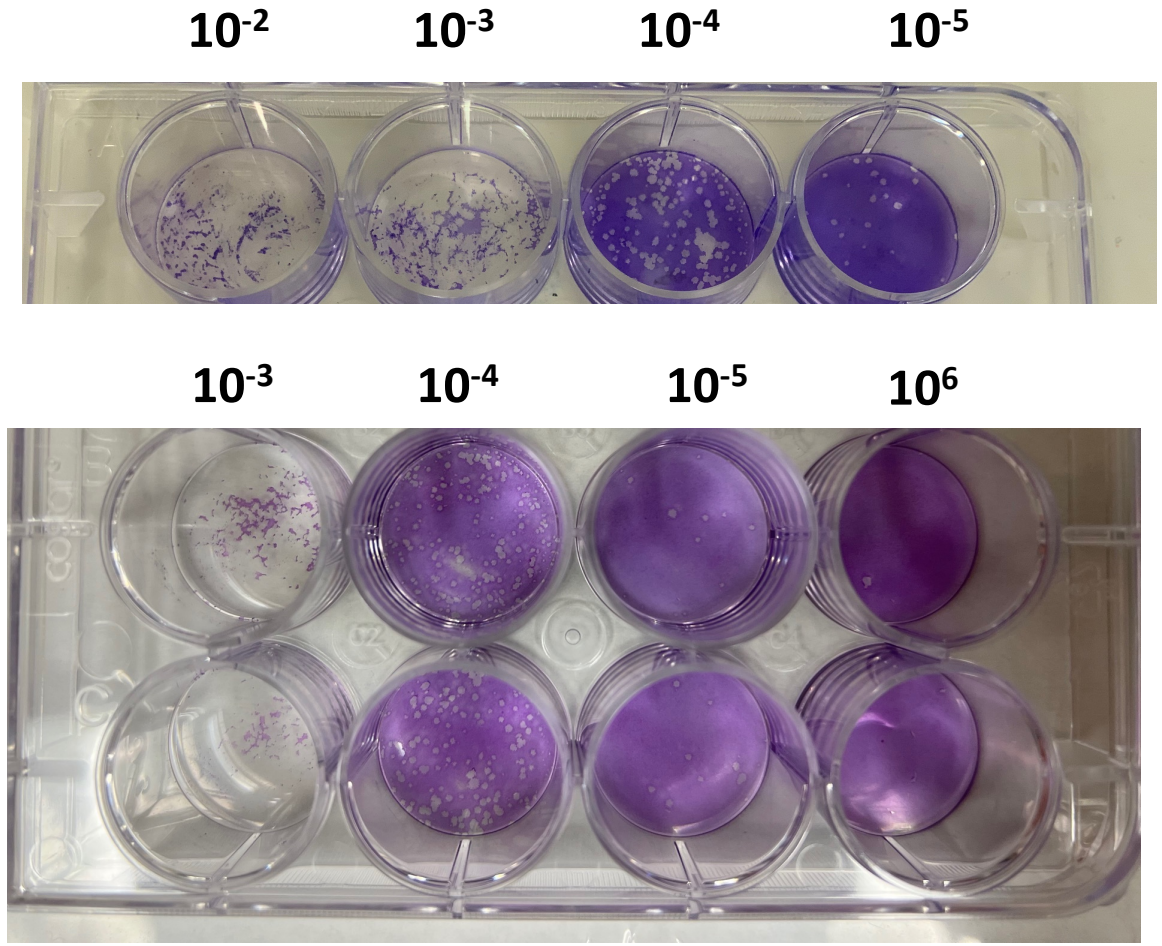

Figure S1. Expansion and titration of MPXV. A T75 flask of Vero or Hela cells at 80% confluency was infected with MPXV (MOI=0.1) and harvested at 60 hours. After three times freeze and thaw, samples were centrifuged to remove the cell debris. MPXV was serially diluted and titrated on a mono-layer of Vero cells. The results of two trial has been shown in upper and lower panel.

Fig. S-2

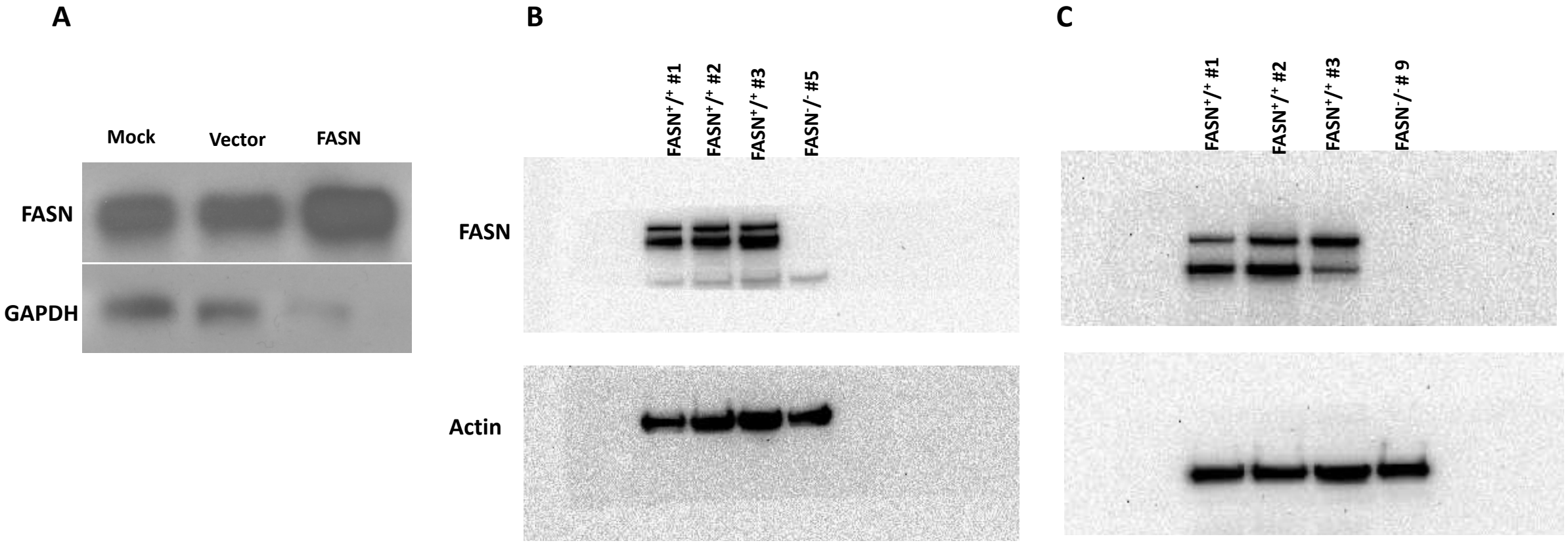

Figure S2. Generating the FASN Knockout and overexpression of FASN in in Huh7.5 cells. (A) Huh 7.5 cells were transfected with expression plasmids, FASN or control for 24 hours prior to MPXV infection. Only 10% of total protein was loaded in FASN-over-expressed cells. (B-C) FASN knockout cells were generated by using CRISR/CAS9 technology. The quality of two FASN Knockout single cell colony #5 and #9 was investigated by Western blotting as indicated in panel A and B. Actin was used as loading control.

Fig. S-3

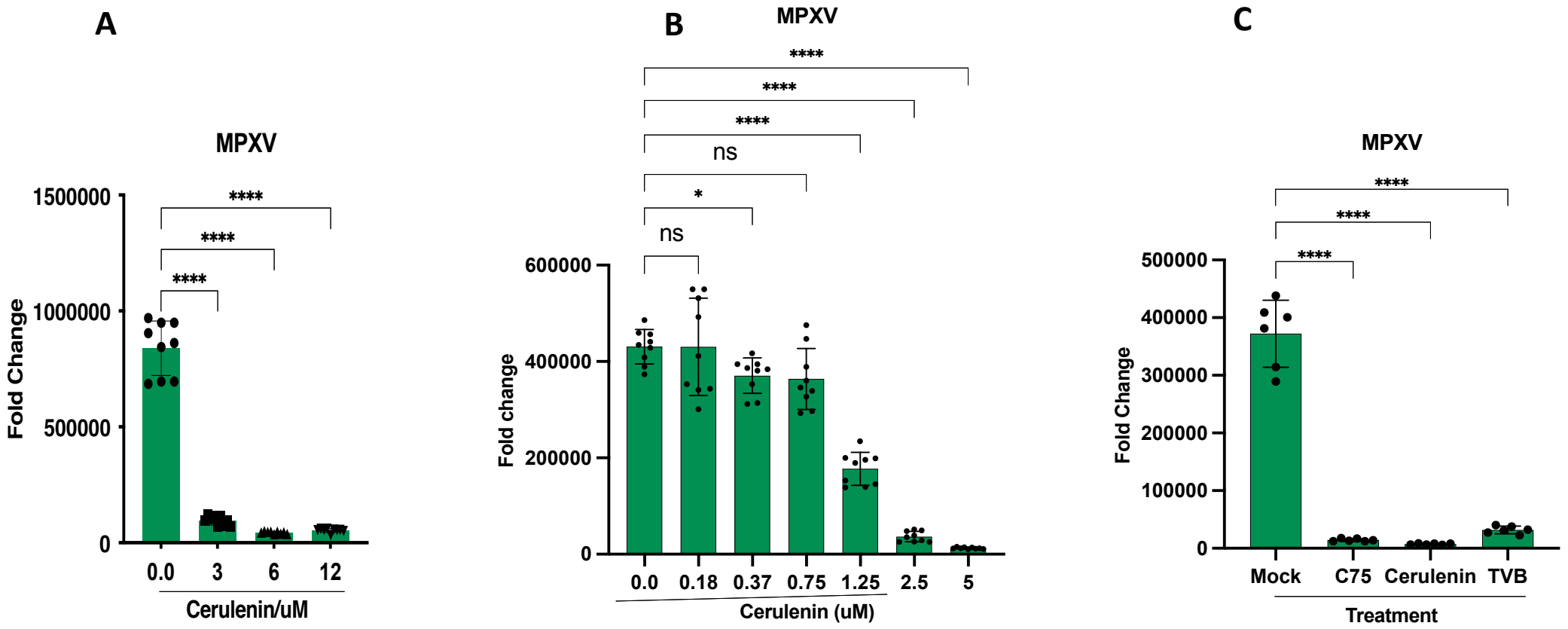

Figure S3. The pharmacological inhibitor of FASN, Cerulenin inhibits MPXV infection. (A-B). Hela cells were treated with indicated dose of Cerulenin one-hour post-MPXV infection. The rate of MPXV infection were measure by RT-qPCR.

Fig. S-4

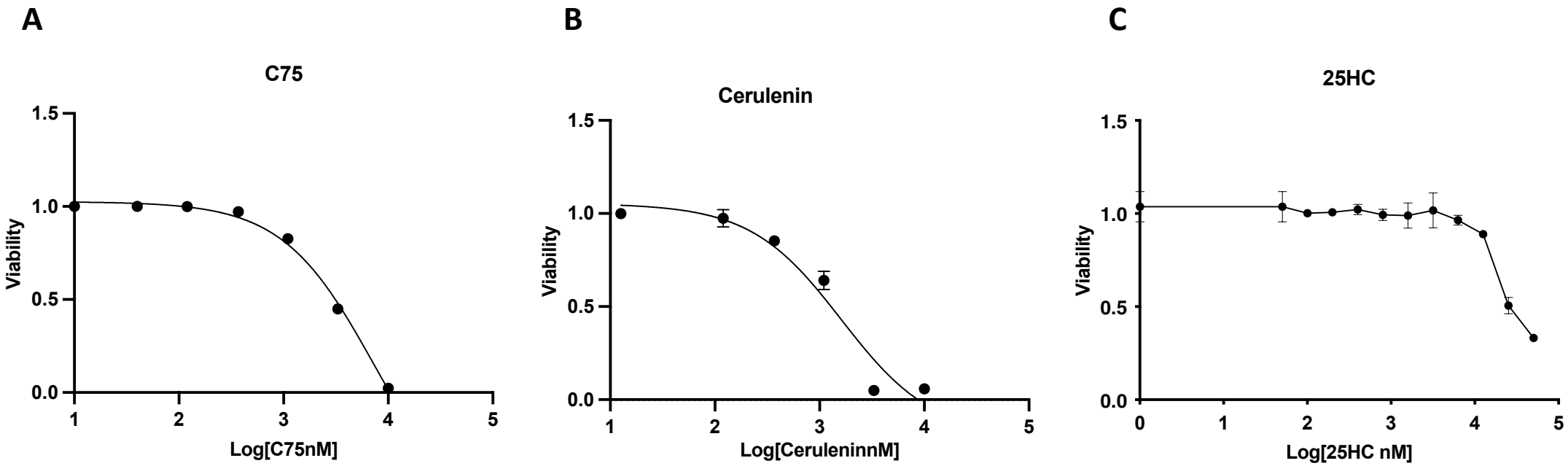

Figure S4. Identification of IC50. 50% cytotoxic concentration (CC50) was determined for Cerulenin, C75 and 25HC 24 hours post-treatment by using Cell Titer-Glo® Luminescent cell viability assay.

Fig. S-5

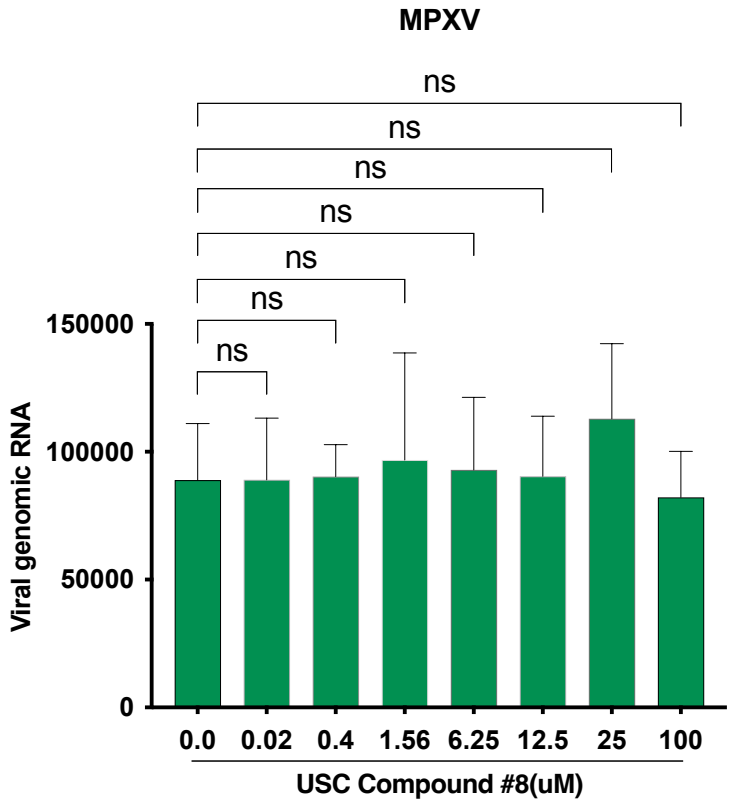

Figure S5. The effect of PAXLOVID™ on MPXV. Different doses of the nirmatrelvir [PF-07321332] drug authorized for the emergency use against COVID-19 was used to investigate its efficacy against MPXV infection.