



Channel catfish virus infects channel catfish ovary cells through clathrin-mediated endocytosis

Hong-Xun Chen, Shu-Xing Li

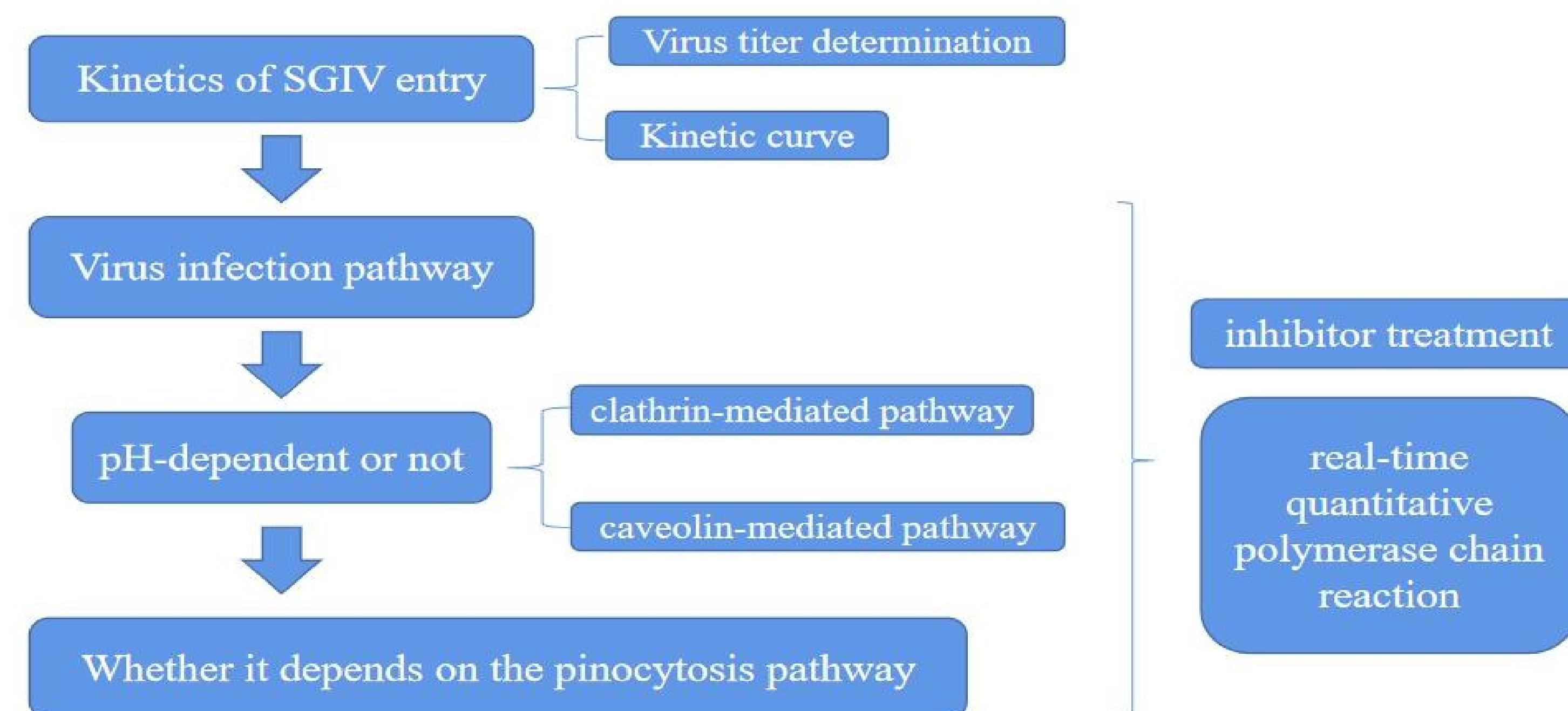
Department of Marine Biology, College of Oceanography, Hohai University, Nanjing, China

Contact: 985530519@qq.com

Background

- Channel catfish virus is an important member of the fish heteroherpesvirus family, which can cause lethal infection to channel catfish. So far, the mechanism by which channel catfish virus enters the host cell is still poorly understood.
- Viruses can infect host cells in many different ways. Some viruses enter by fusing with the plasma membrane of the cell, a few can enter host cells directly from the cell surface, and most viruses infect host cells through endocytosis. The ways to enter host cells through endocytosis mainly include the following: 1) clathrin-mediated pathway; 2) caveolin-mediated pathway; 3) macropinocytosis pathway; 4) phagocytic pathway; 5) pathway independent of the endocytic pathway of clathrin and caveolin.

Methods



Result

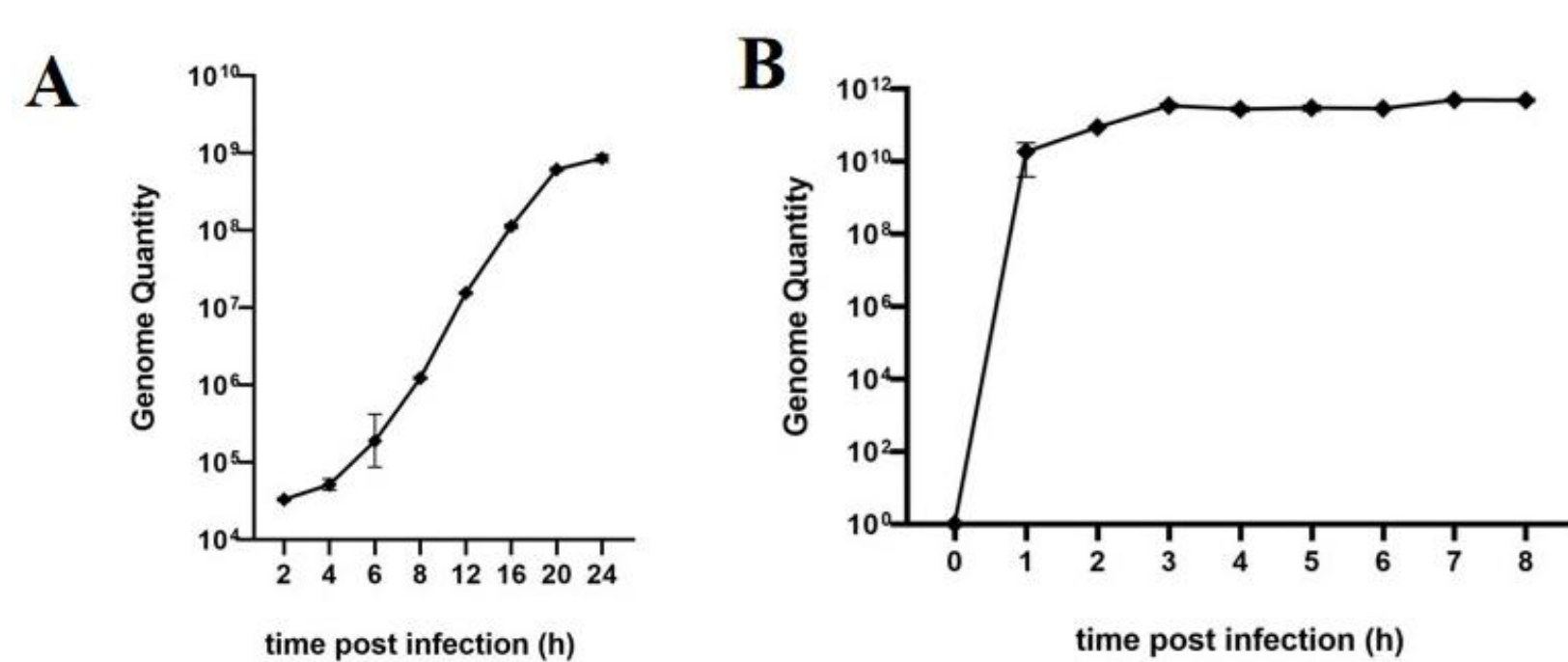


Figure 1. Kinetics of channel catfish virus (CCV) strains in CCO cells.

(A) One-step growth curves of CCV strains in CCO cells. (B) CCO cells were incubated with CCV, and viruses were washed twice with medium to remove unbound viruses at the different time points. The genome quantity was calculated by real-time quantitative polymerase chain reaction.

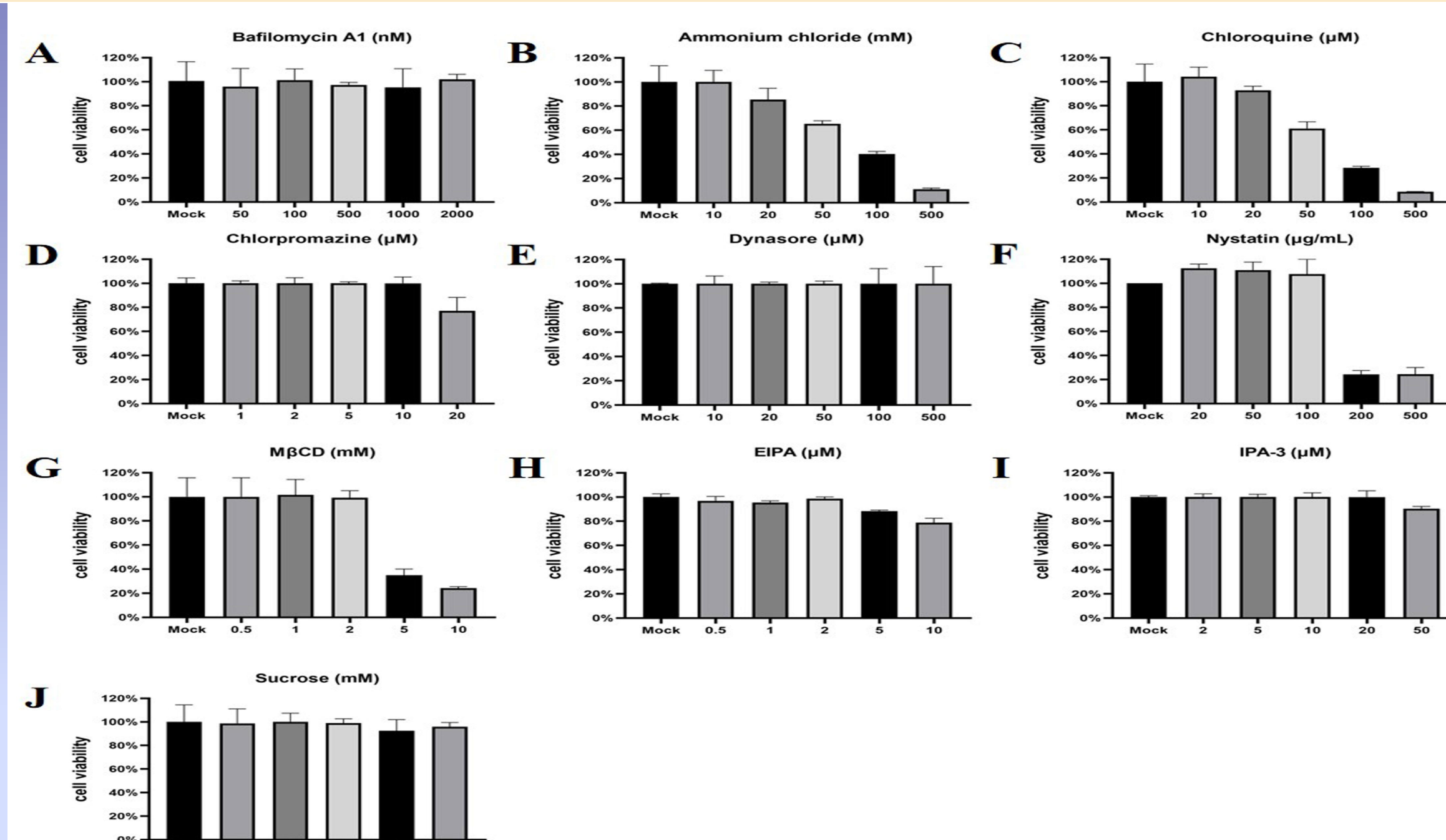


Figure 2. Viability of channel catfish orian (CCO) cells exposed to inhibitors. (A) Bafilomycin A1. (B) Ammonium chloride. (C) Chloroquine. (D) Chlorpromazine. (E) Dynasore. (F) Nystatin. (G) Methyl-β-cyclodextrin (MβCD). (H) 5-(N-ethyl-N-isopropyl) amiloride (EIPA). (I) IPA-3. (J) Sucrose.

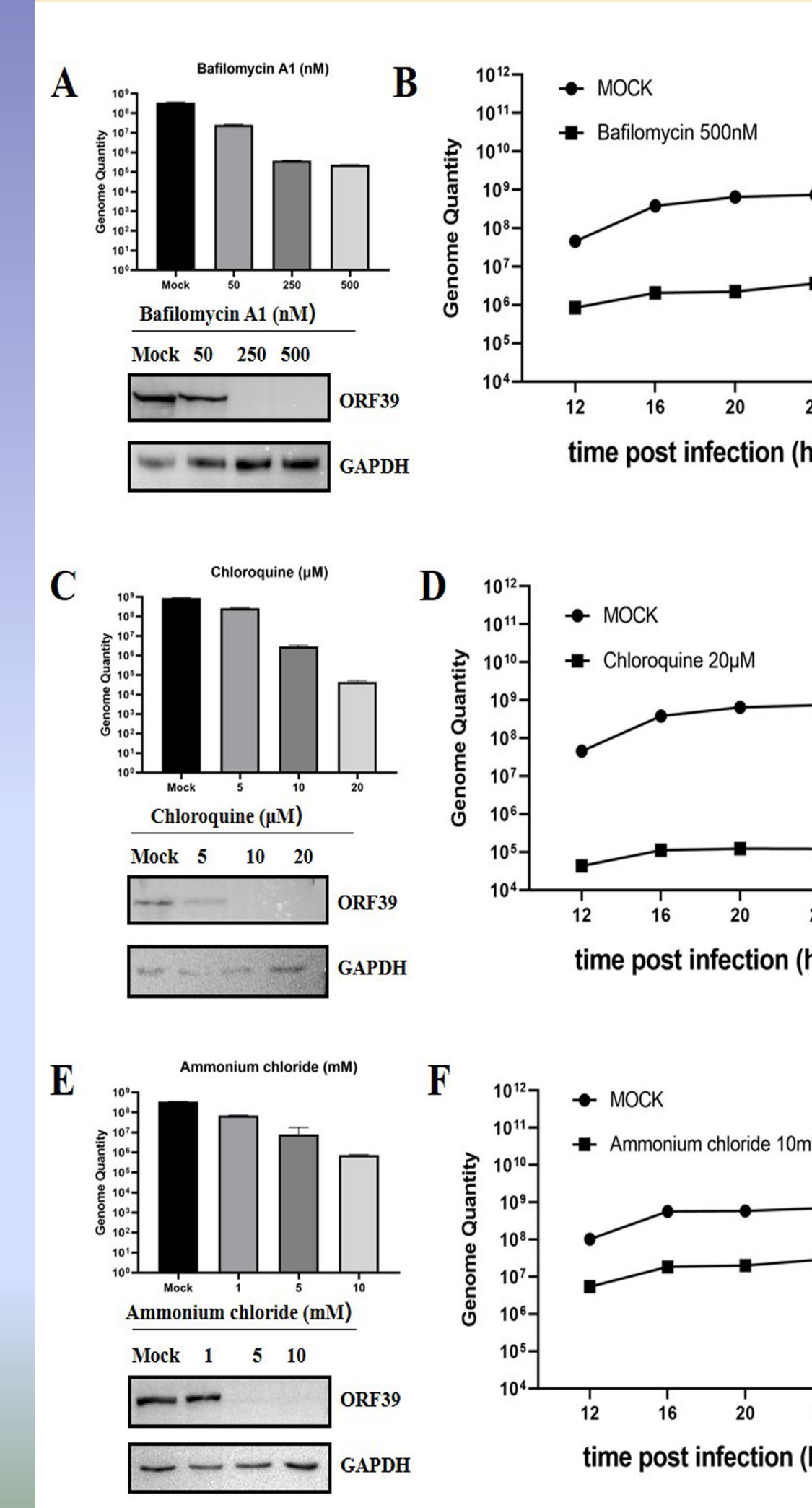


Figure 3. CCV entry is pH dependent.

(A) Analysis of the cells treated with bafilomycin A1. (B) Cells were treated with maximum concentration of bafilomycin A1. (C) Analysis of the cells treated with chloroquine. (D) Cells were treated with maximum concentration of chloroquine. (E) Analysis of the cells treated with ammonium chloride. (F) Cells were treated with maximum concentration of ammonium chloride.

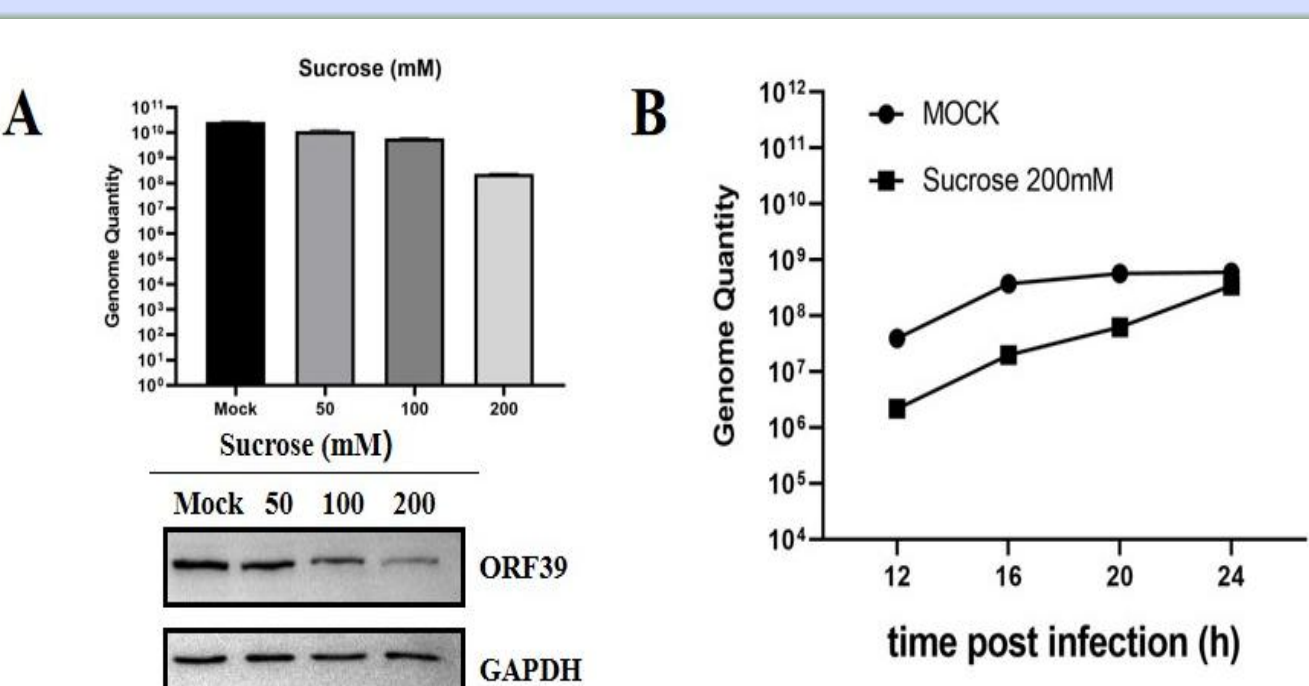


Figure 4. CCV entry is depend on endocytic pathway.

CCO cells were treated with sucrose 1 h prior to with CCV. (A) RT-qPCR and Western blot analysis of the cells treated with sucrose. (B) Cells were treated with maximum concentration of sucrose.

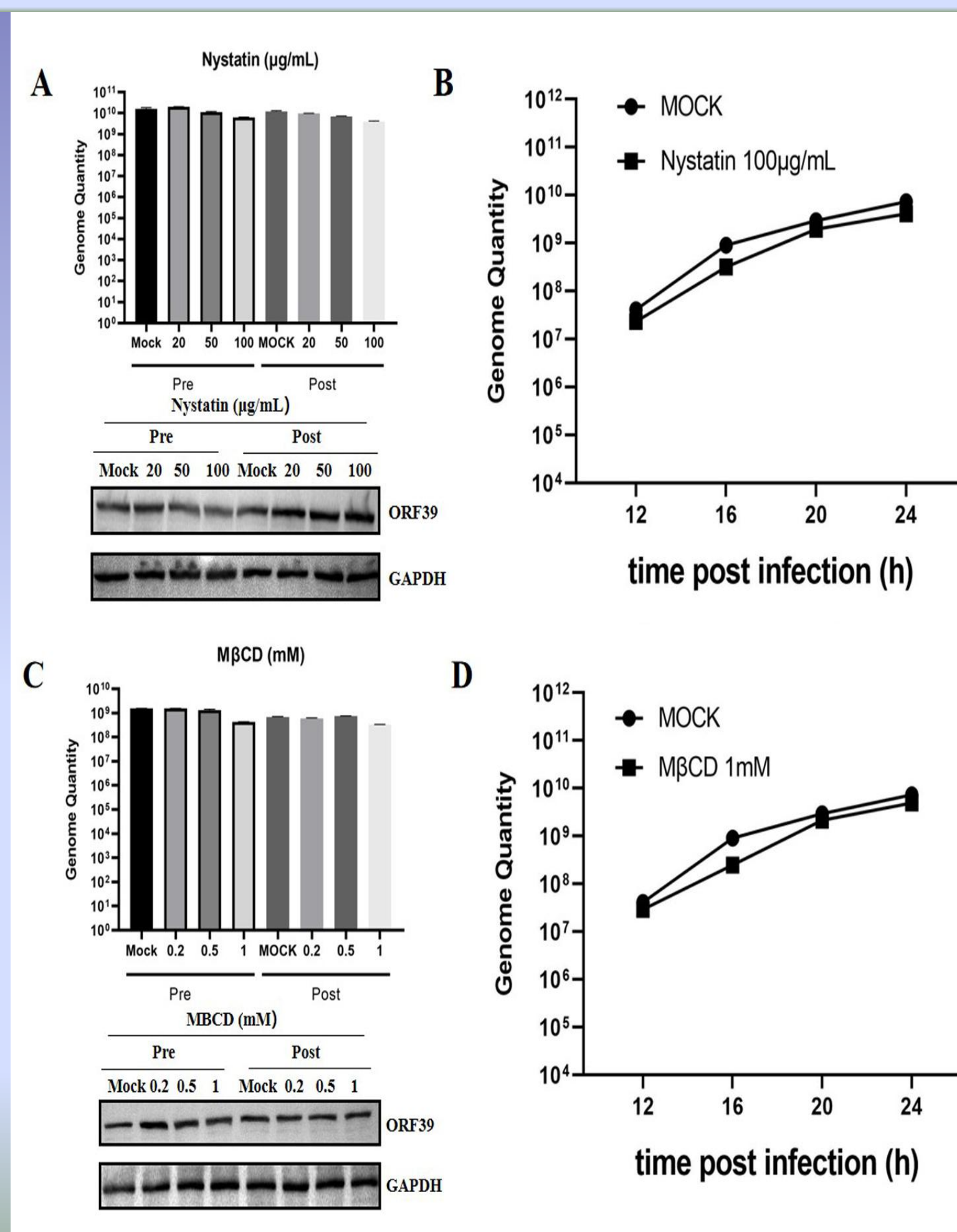


Figure 5. CCV entry is independ on caveolin-mediated endocytic pathway. Cells were treated with nystatin or MβCD 1 h prior to (pre-inoculation) or post-inoculation with CCV.

(A) Analysis of the cells treated with nystatin. (B) Cells were treated with maximum concentration of nystatin. (C) Analysis of the cells treated with MβCD. (D) Cells were treated with maximum concentration of MβCD.

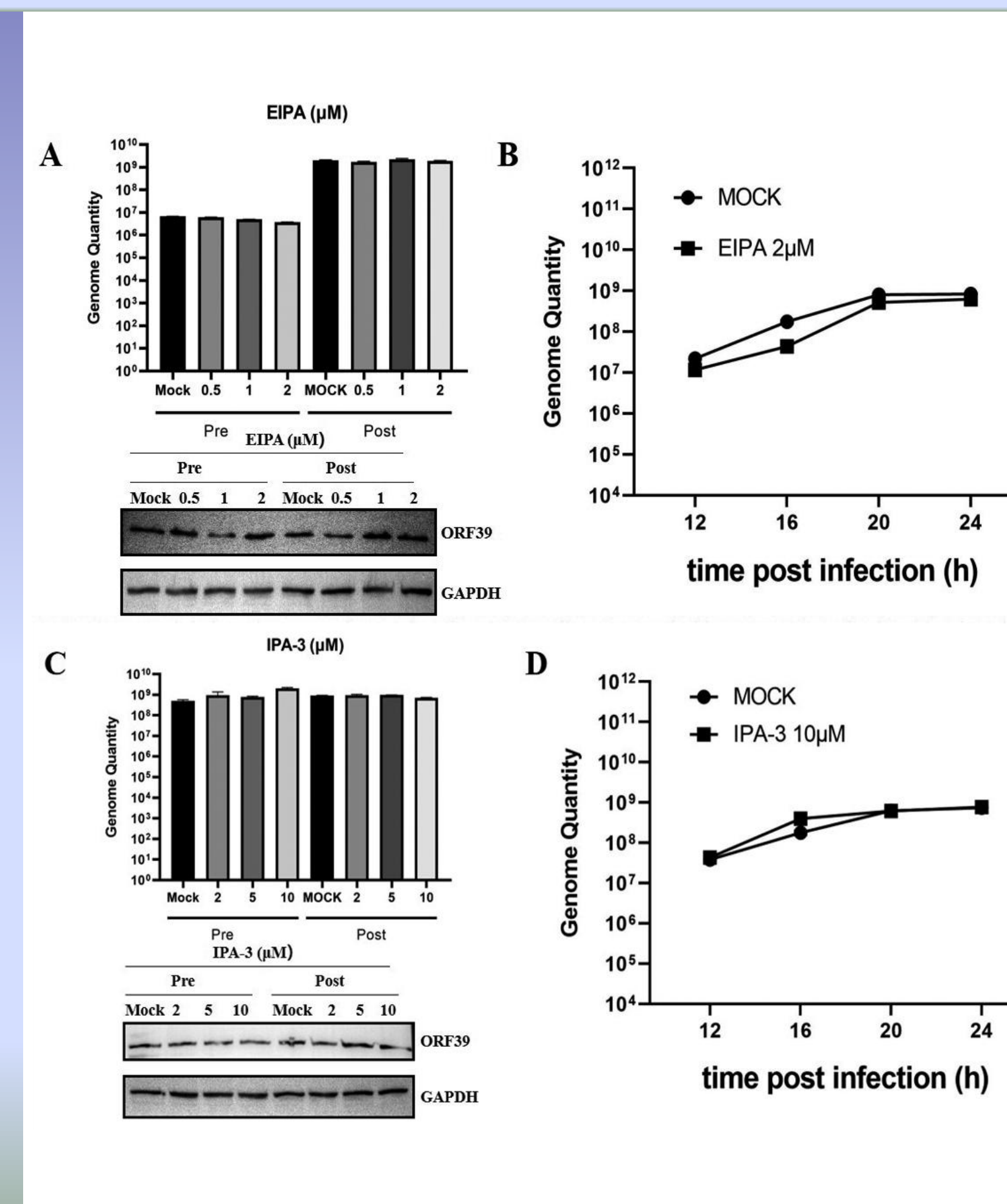


Figure 6. CCV entry is independ on macropinocytosis pathway.

Cells were treated with EIPA or IPA-3 1 h prior to (pre-inoculation) or post-inoculation with CCV.

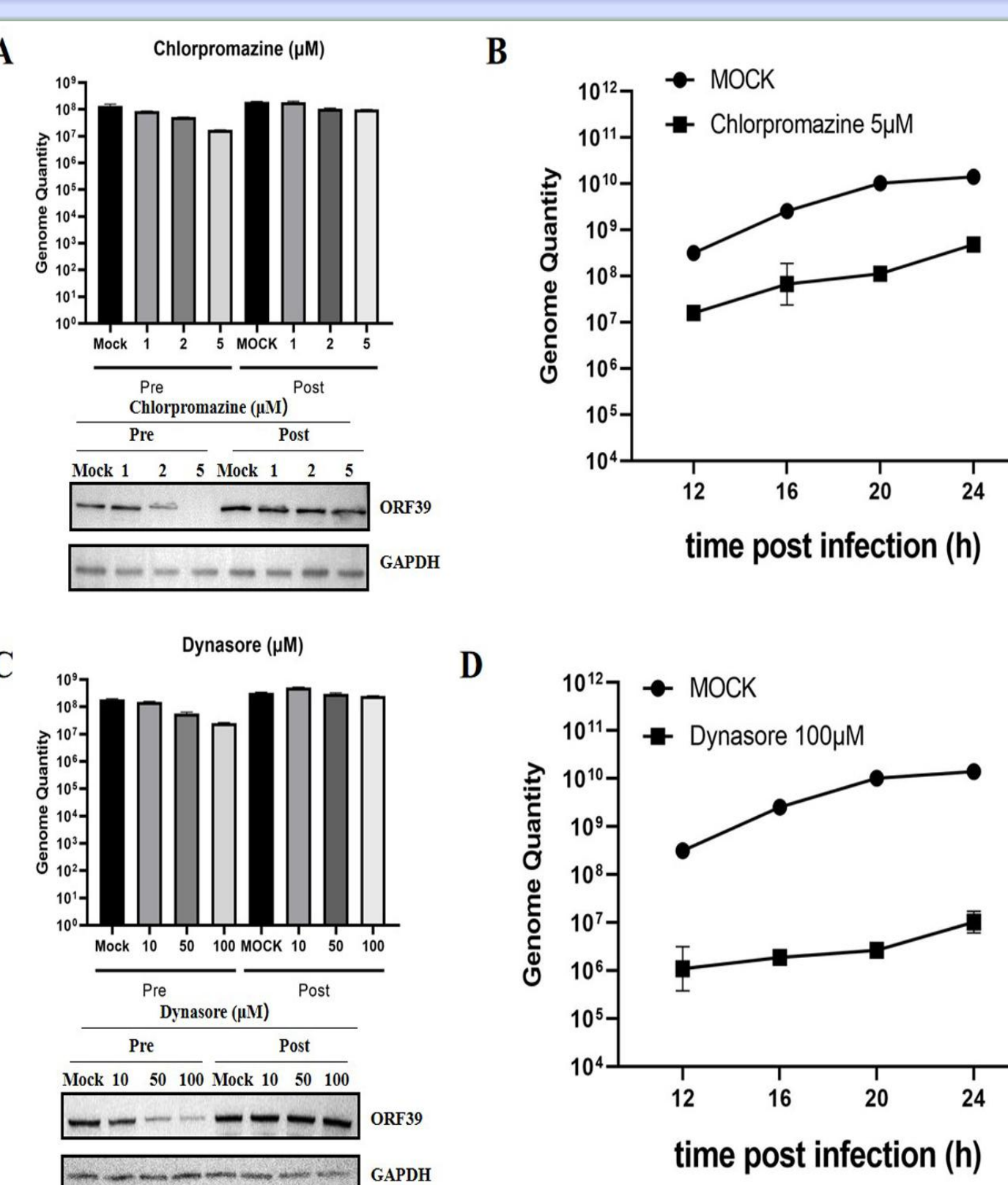


Figure 7. CCV entry is depend on clathrin-mediated endocytic pathway. Cells were treated with chlorpromazine or dynasore 1 h prior to (pre-inoculation) or post-inoculation with CCV.

(A) Analysis of the cells treated with chlorpromazine. (B) Cells were treated with maximum concentration of chlorpromazine. (C) Analysis of the cells treated with dynasore. (D) Cells were treated with maximum concentration of dynasore.

Conclusion

- When the cells were pretreated with inhibitors that block clathrin-mediated endocytosis, the infection in the host cells was inhibited.
- In contrast, the destruction of cellular cholesterol by methyl-β-cyclodextrin and nystatin has no effect on viral infection, which indicates that the virus enters the channel catfish ovary cells was not through caveola-dependent endocytosis.
- In addition, endosomal acidification inhibitors can block viral infections.
- The research results show that the entry of channel catfish virus involves not only a pH-dependent pathway, but also a clathrin-mediated endocytic pathway.

Acknowledgements

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