Inhibition of HSV-1 Replication by a Pin1 inhibitor

Presented by

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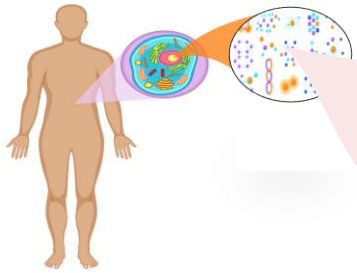
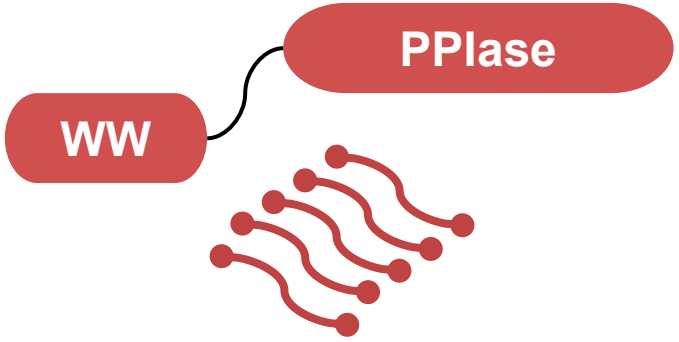
Under the supervision

Prof. Takemasa Sakaguchi

I have no potential conflicts of interest in relation to this presentation



Pin1

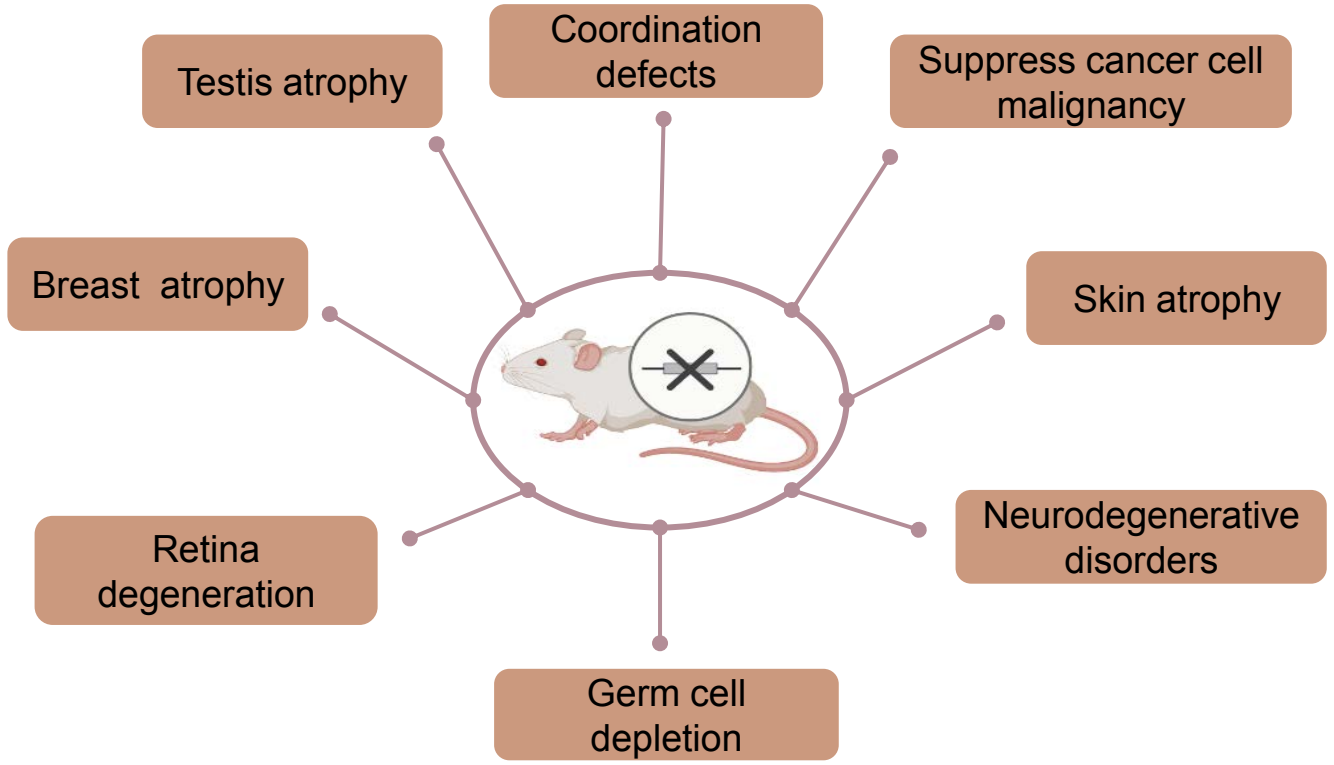


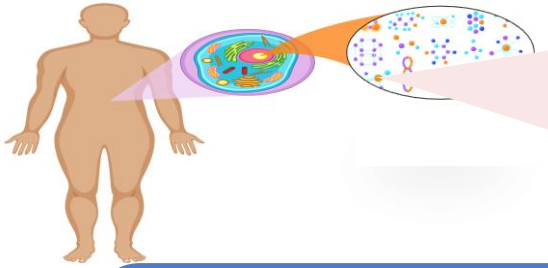
- **P**eptidyl-prolyl *cis-trans* **i**somerase **N**IMA –interacting **1** (**Pin1**) is a regulatory enzyme.
- Pin1 is one of the members of the peptidyl-prolyl isomerase (PPIase) family of proteins, subfamily parvulins.
- Pin1 interacts with phosphorylated Ser/Thr Pro motifs and catalyzes the *cis* and *trans* amide isomers, leading to the conformational changes of its substrate proteins.

Overexpression of Pin1 is correlated to

- **Most of Human cancers**
- **Inflammation**
- **Fibrosis**
- **Obesity**

In vivo studies in Pin1 knockout mice showed some side effects on long term





Pin1

WW — PPIase

- Targeting a host factor like Pin1 by using Pin1 inhibitors may have fewer side effects than a Pin1 knockout.
- Additionally, using host factors as an antiviral treatment will overcome the antiviral drug resistance problem.

We reported that SARS-CoV-2 growth was inhibited by a novel Pin1 inhibitor called H-77 at an EC_{50} below 5 μM, indicating that RNA synthesis of SARS-CoV-2 is likely to be promoted by Pin1.

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Our next target is HSV-1 tested with (H-77)

AIM

Study the mechanism of inhibition of H-77 on the replication cycle and protein synthesis of HSV-1

H-77 experiment with HSV-1

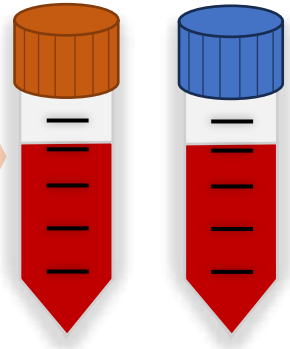
1. VeroE6 cells cultivated in a 6 well plate (1.1×10^6 cells/well).

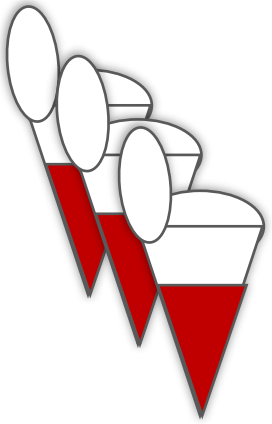
2. After washing the cells, 2ml/well from tube A and 2 ml/well from tube B were added in triplicate.

3. The plate Incubated for 2 hours in a 5% CO_2 incubator at 37°C

A. Serum-free DMEM

B. Serum-free DMEM+H-77, final Conc of H-77:10 μM





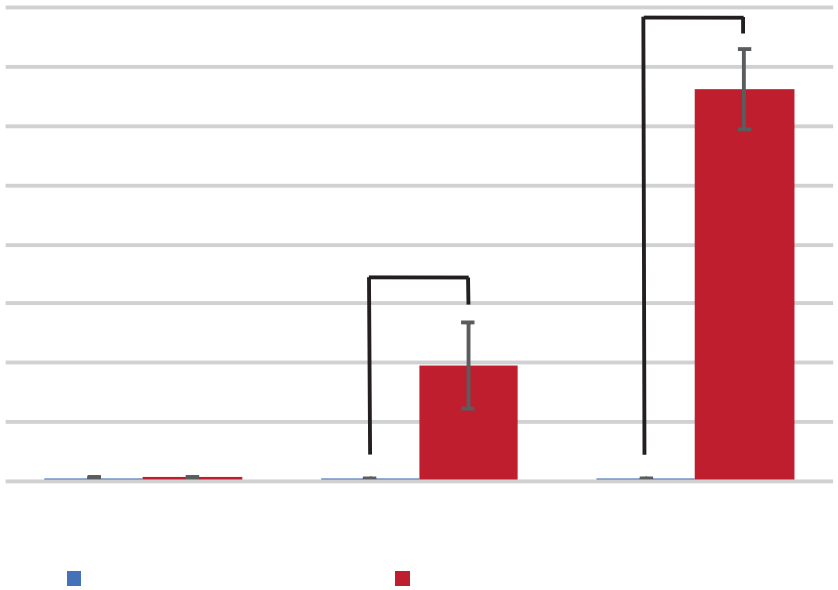
4. After incubation, HSV-1 (MOI = 0.1) was added, 100 μl/well.

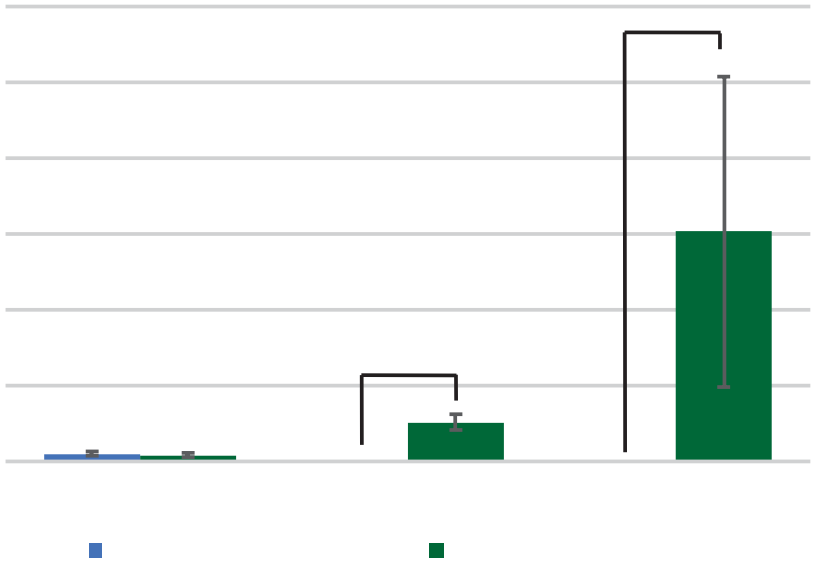
5. 100 μl of supernatant was taken from each well at day 0, day 1, and day 2.

The virus titer and infectivity of the collected samples were measured by qRT-PCR and TCID50

qRT-PCR

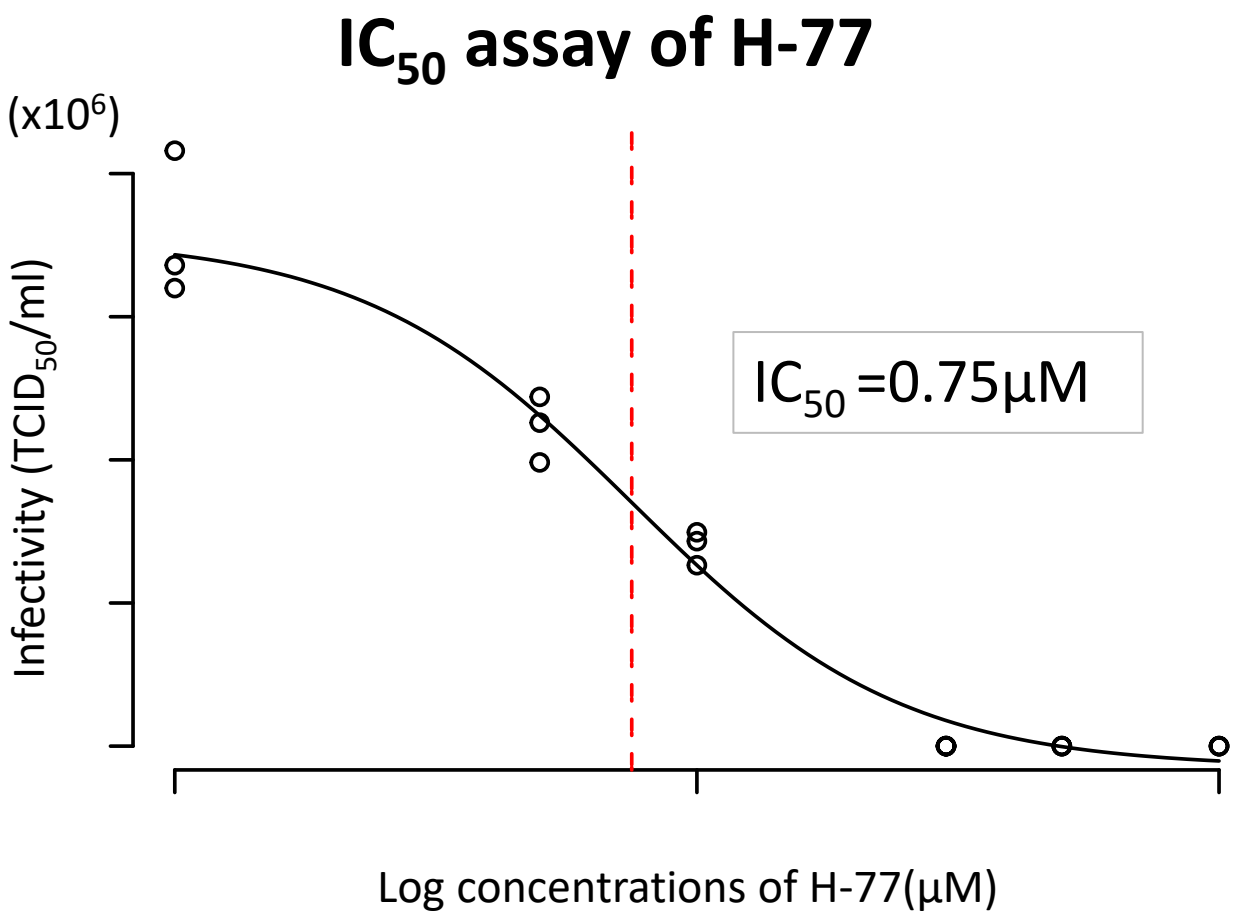
$TCID_{50}$

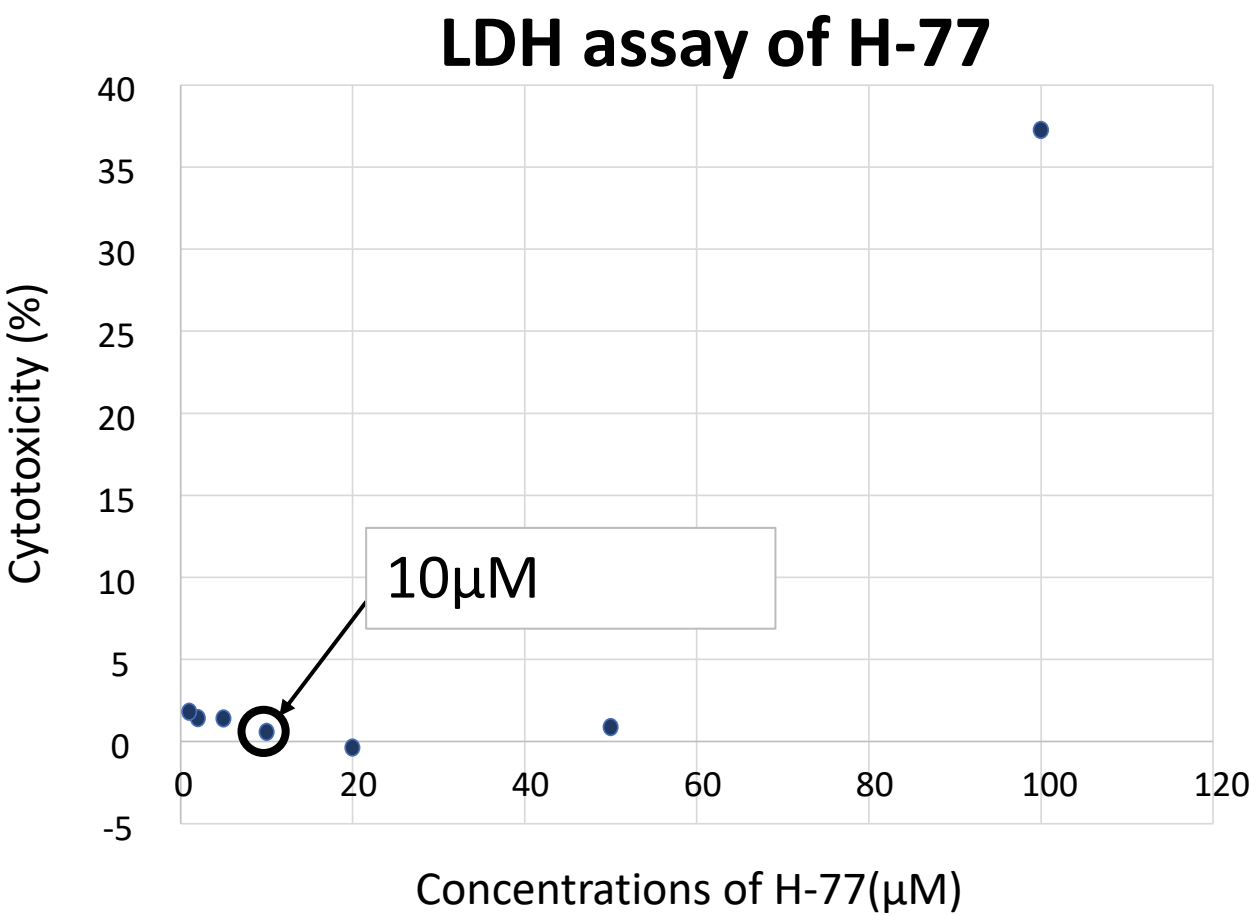




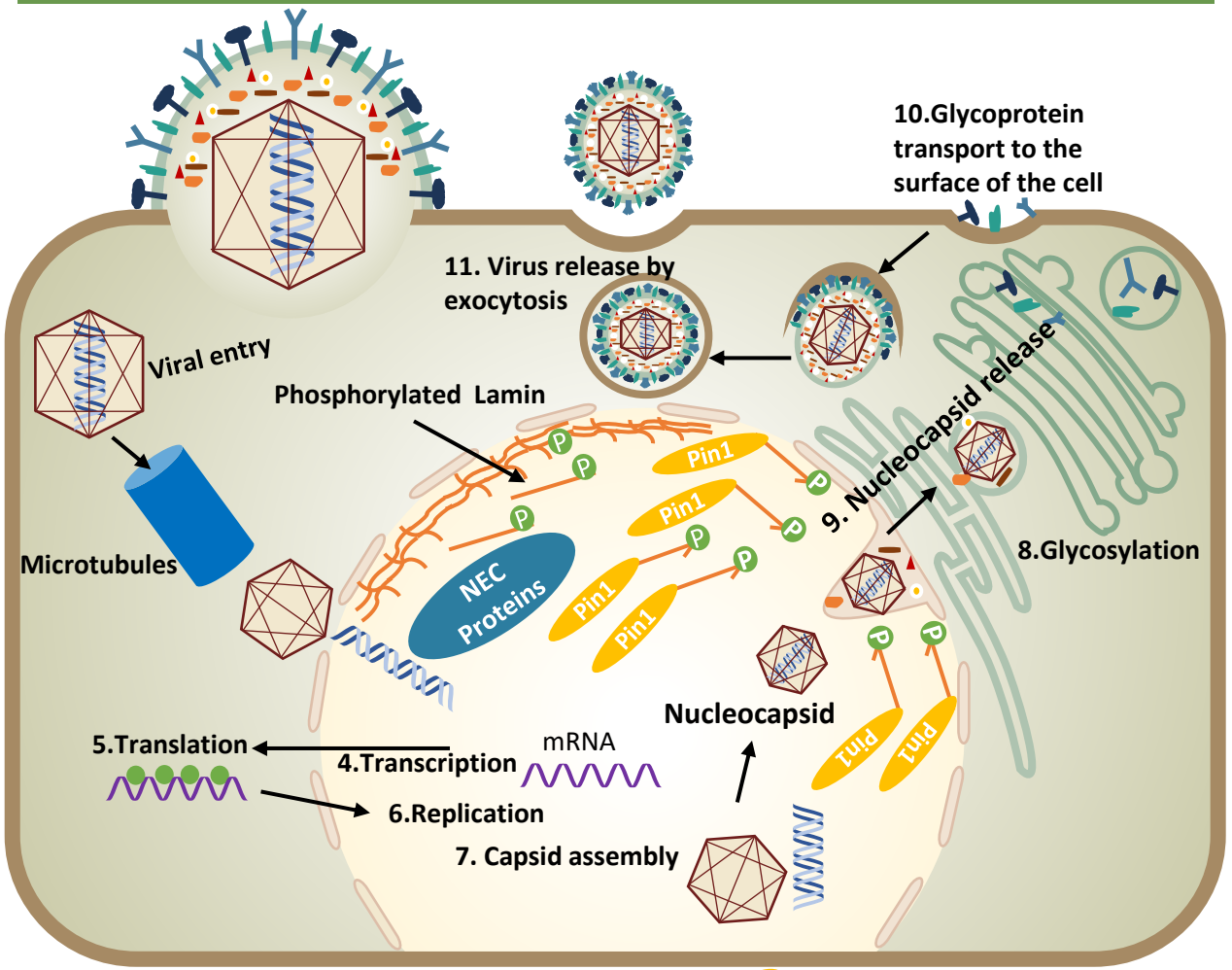
IC_{50} assay

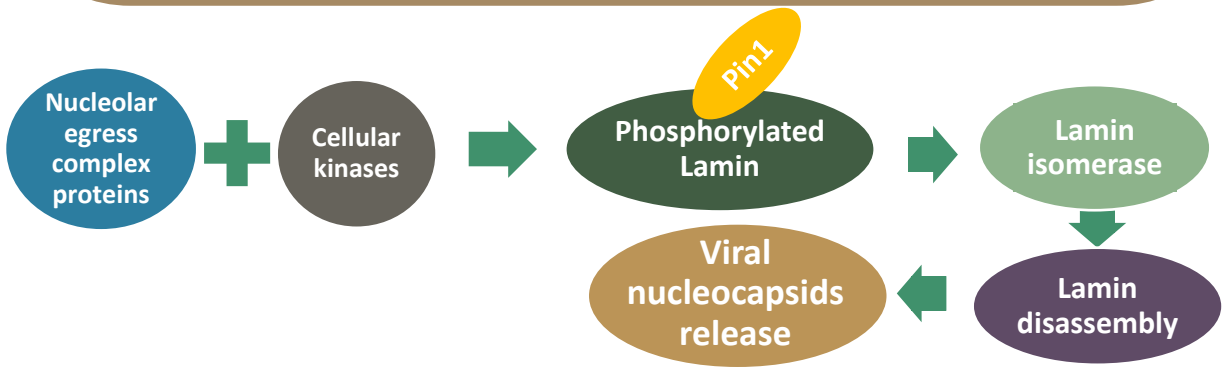
Cytotoxicity assay



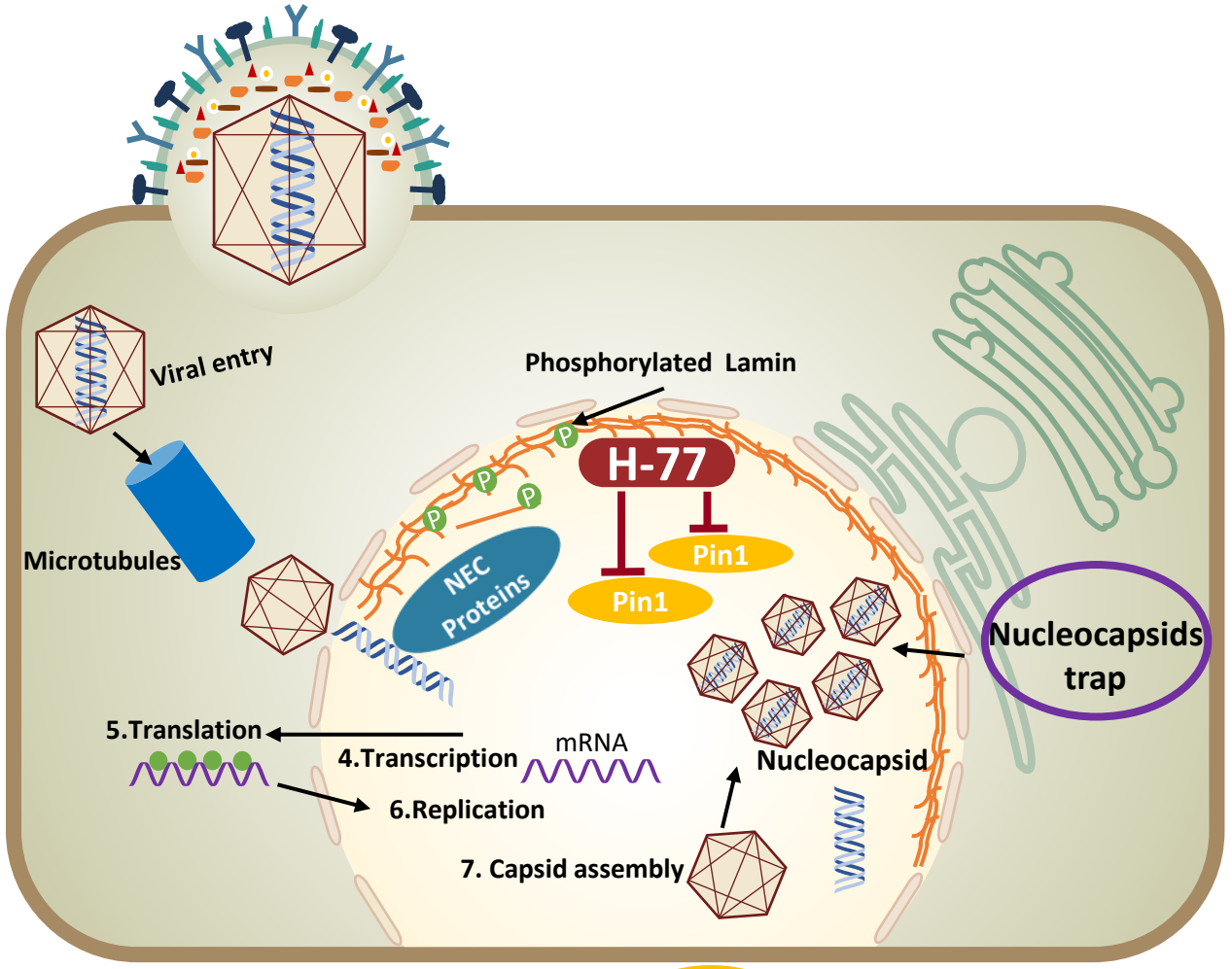


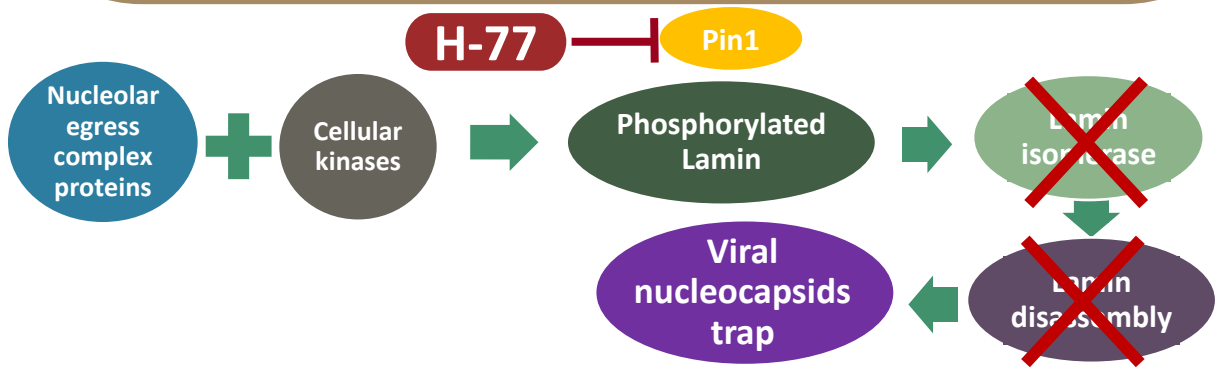
Mechanism of Pin1 in HSV-1 nuclear egress





Mechanism of H-77 in HSV-1 nuclear egress





Immunofluorescence experiment

Green: anti-laminB2 mouse MoAb

Blue stain: cell nucleus using DAPI

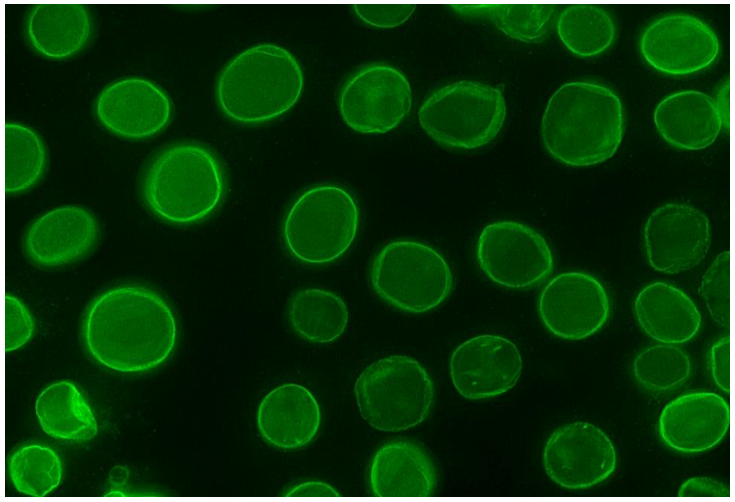
anti-HSV-1 rabbit Ab solution

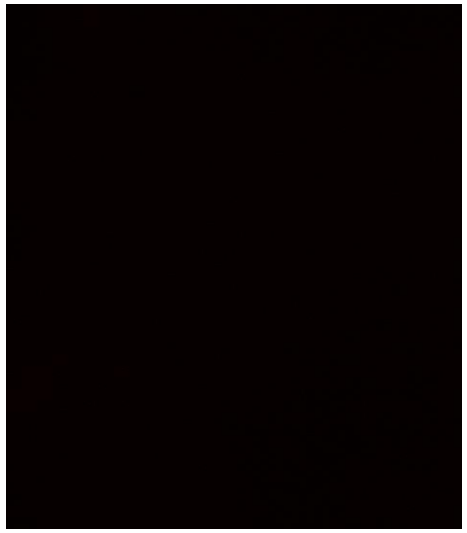
Mock with H-77

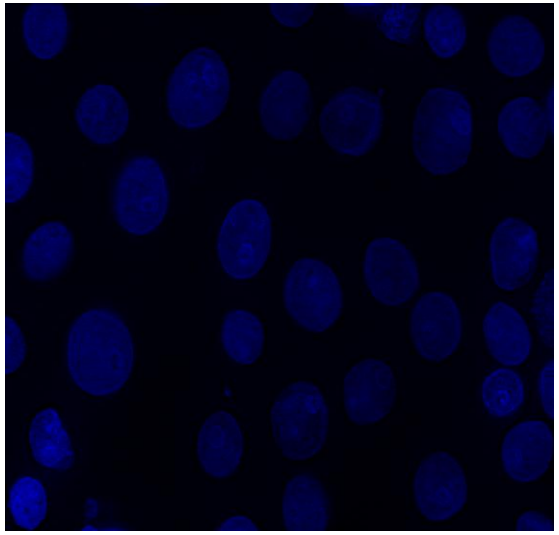
No viral protein

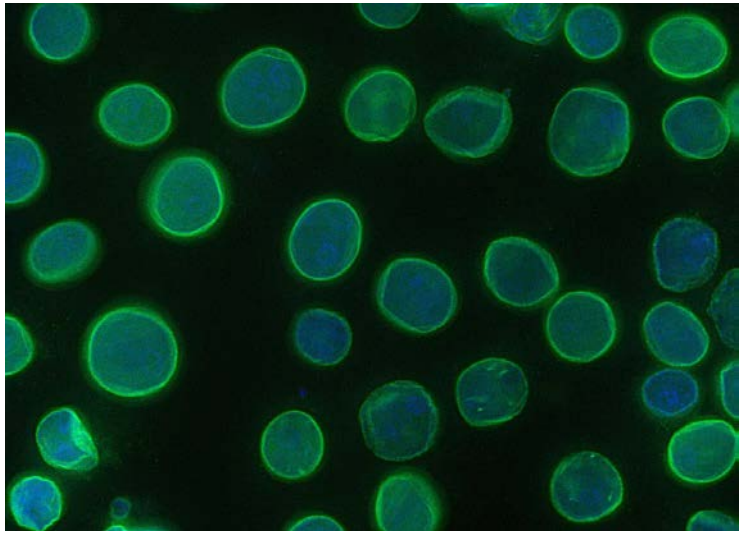
DAPI stained cell nucleus

Merged images

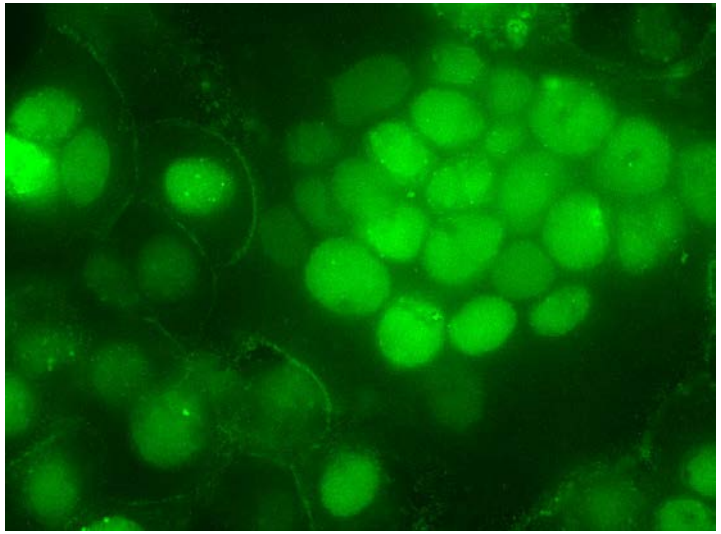


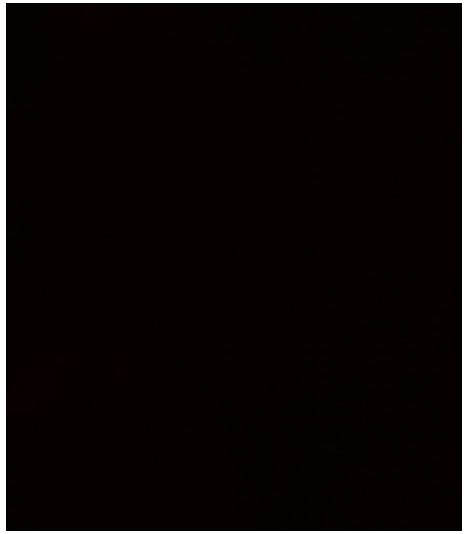


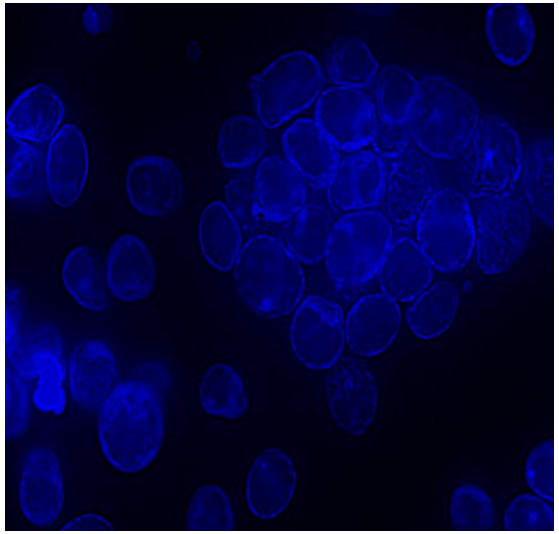


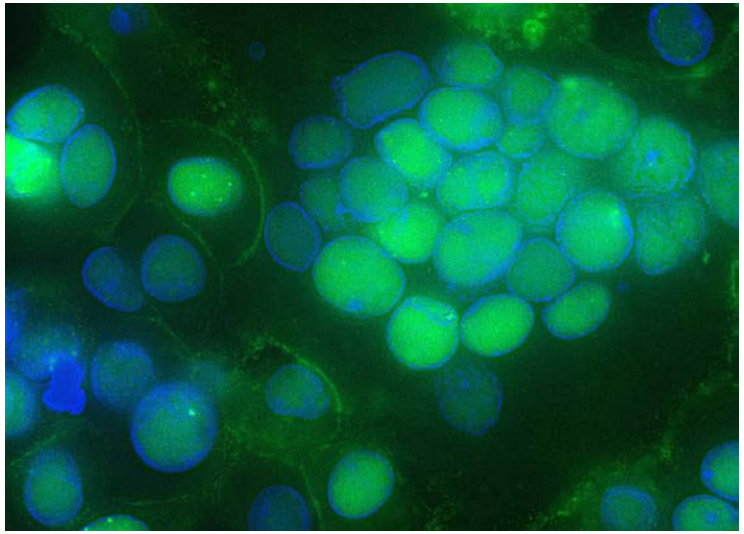


Mock without H-77

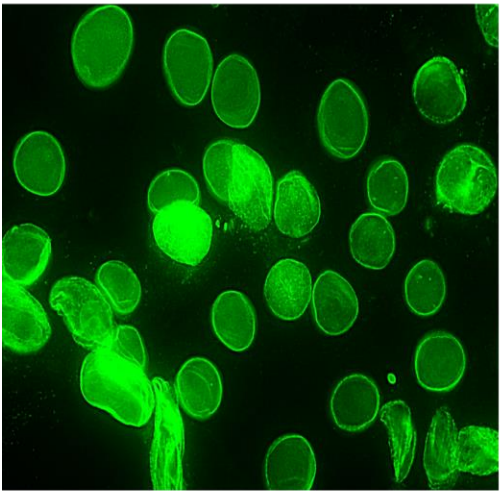


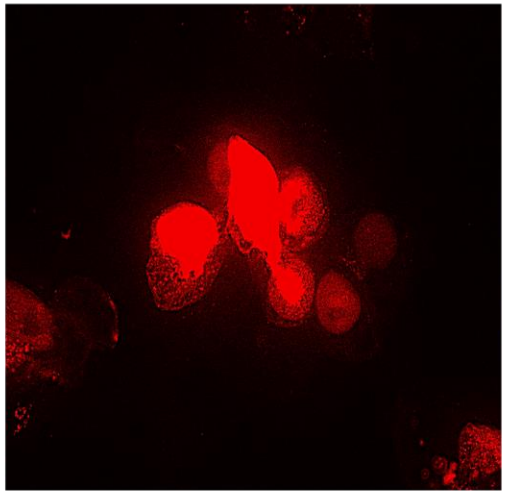


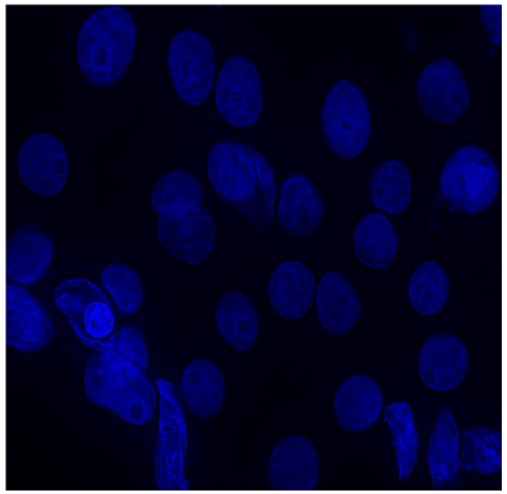


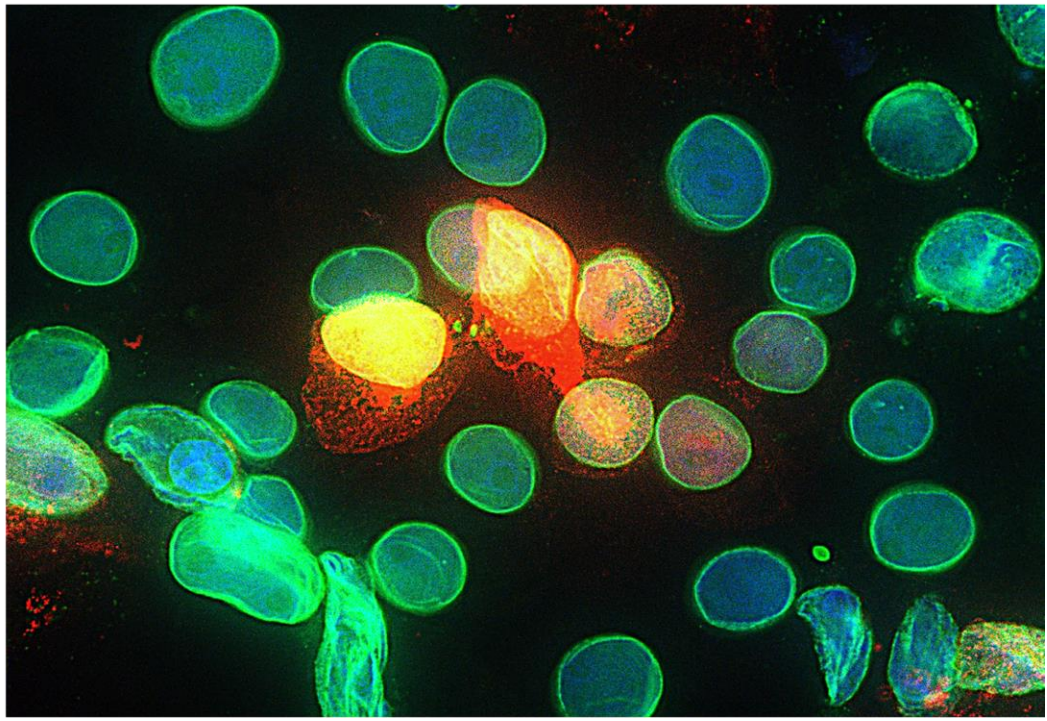


Vero cells infected with HSV-1 MOI (0.05), treated with H-77

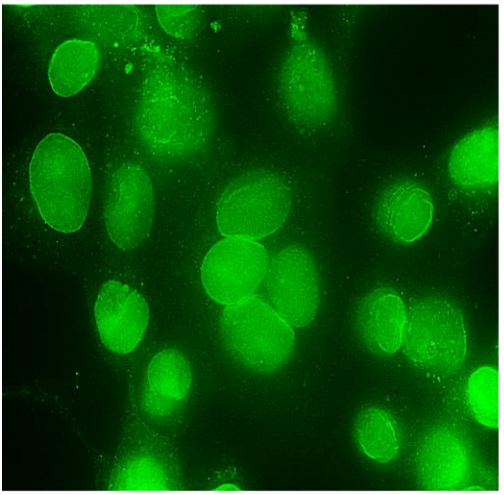


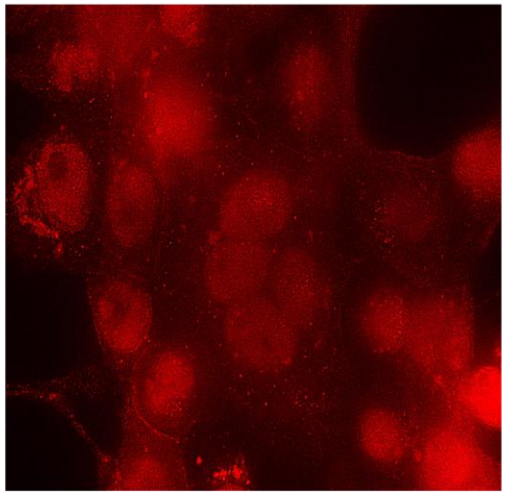


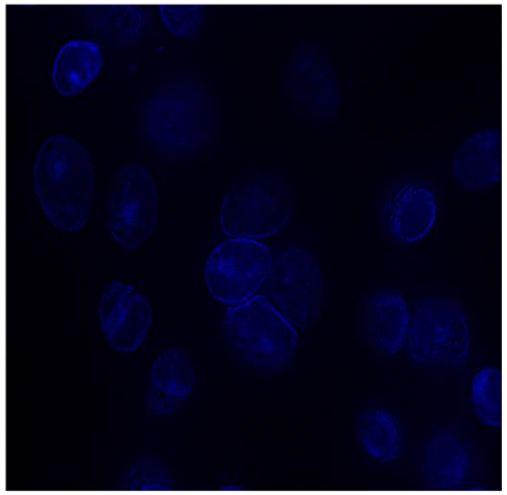


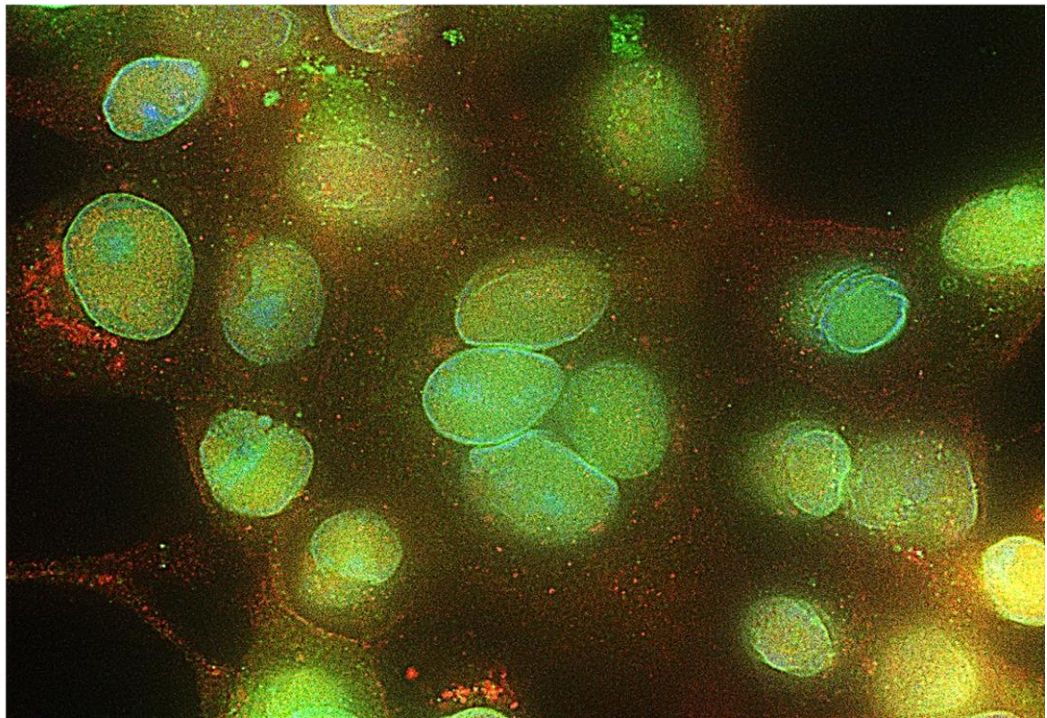


Vero cells infected with HSV-1 MOI (0.05), Untreated with H-77

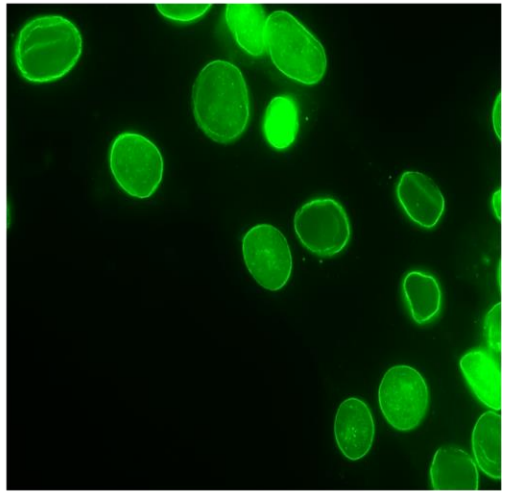


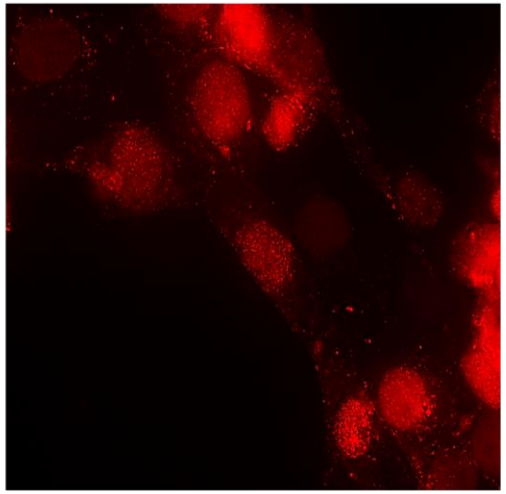


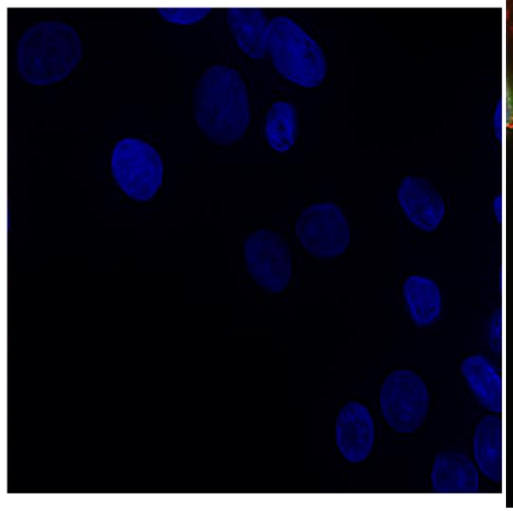


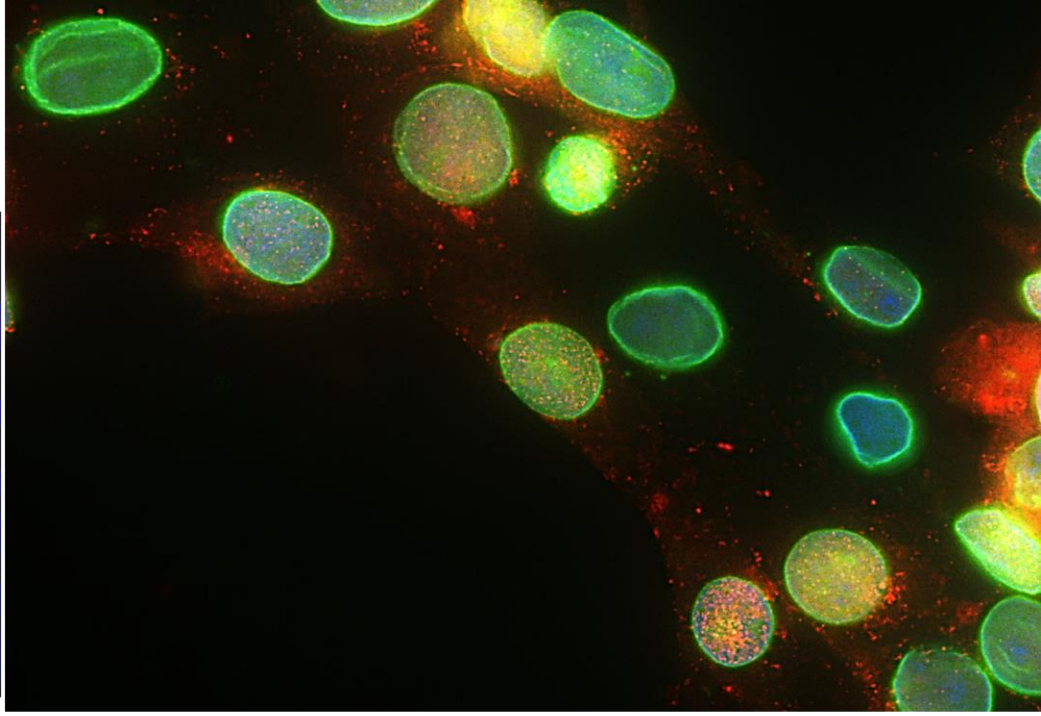


Vero cells infected with HSV-1 MOI (10), treated with H-77

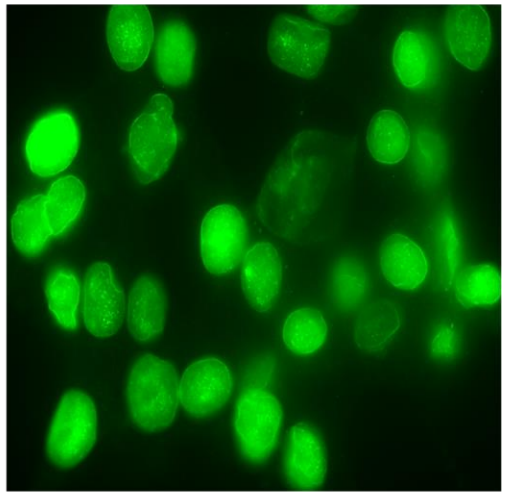


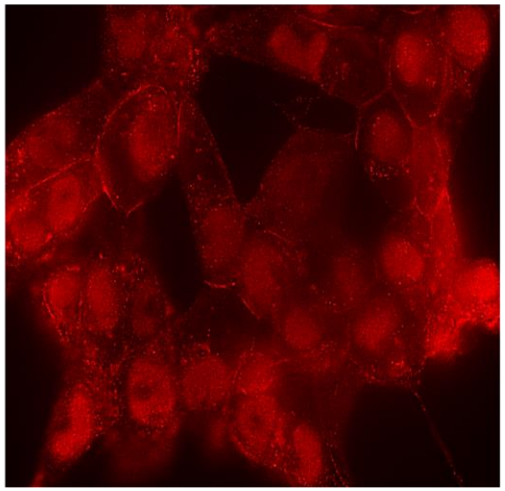


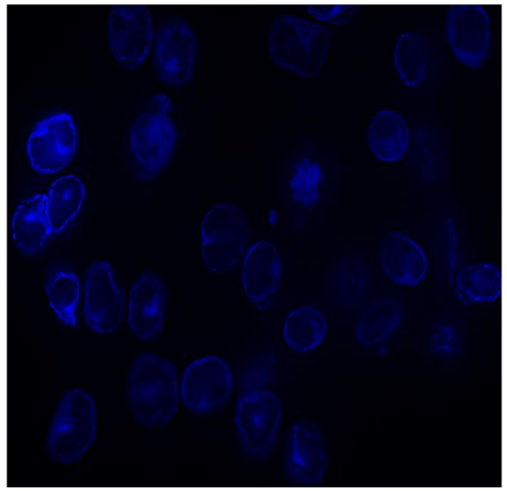


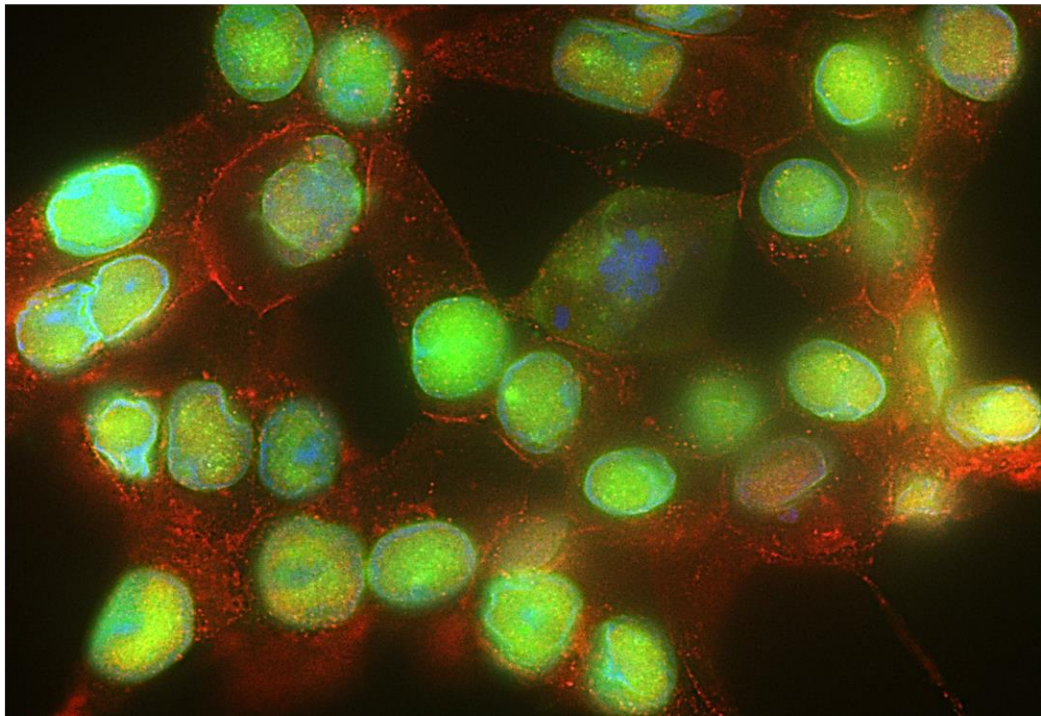


Vero cells infected with HSV-1 MOI (10), Untreated with H-77



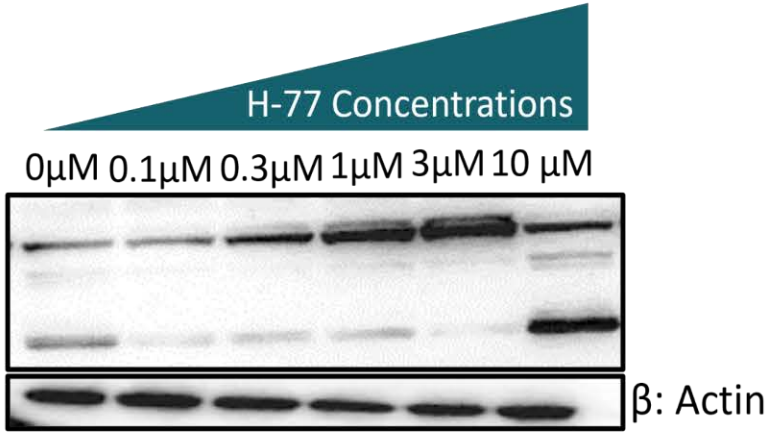




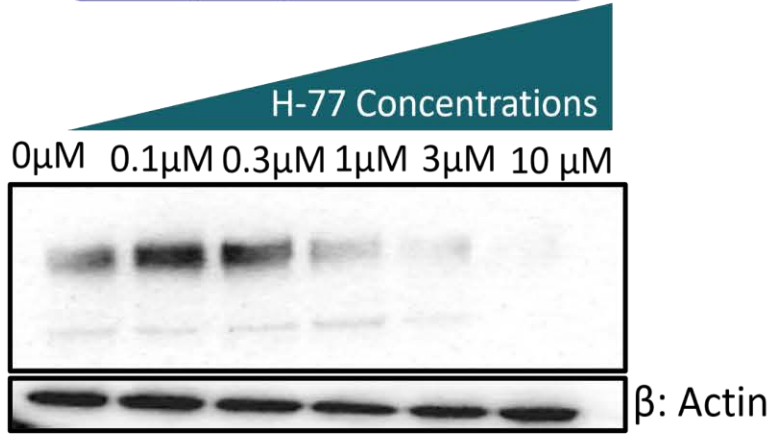


Western blotting

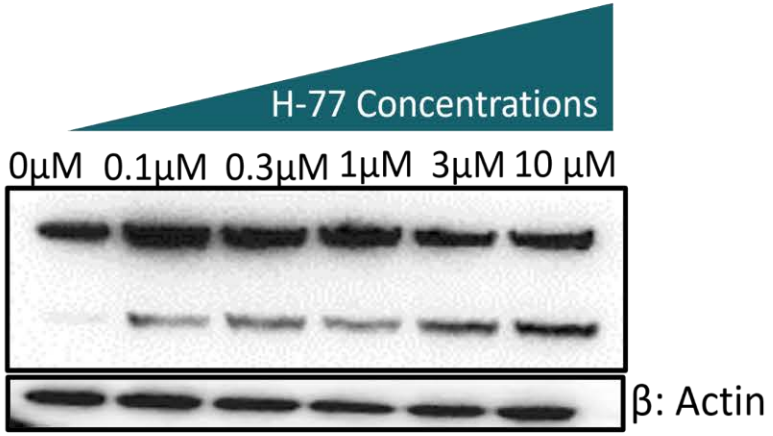
Lamin B2

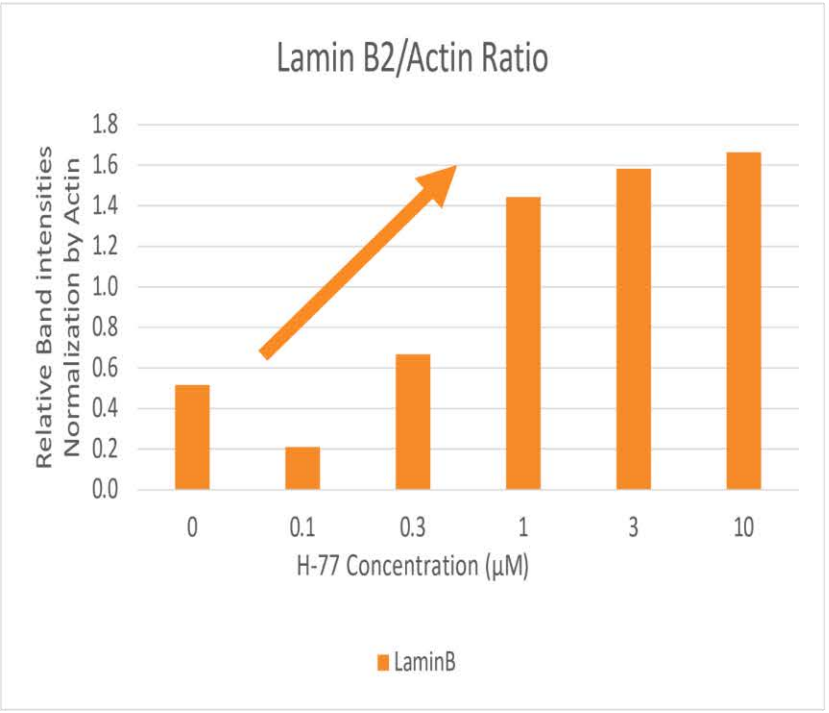


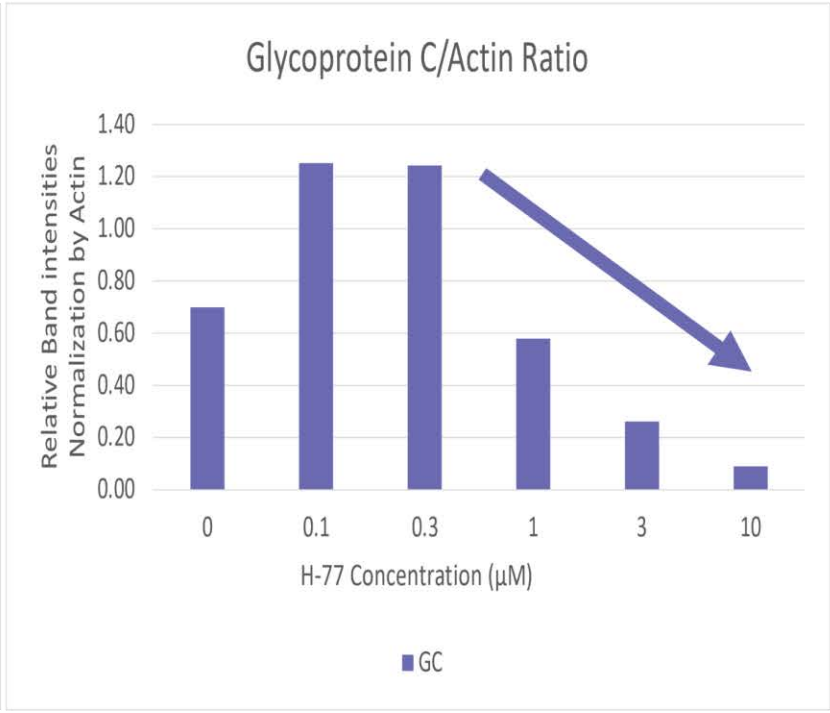
Glycoprotein C

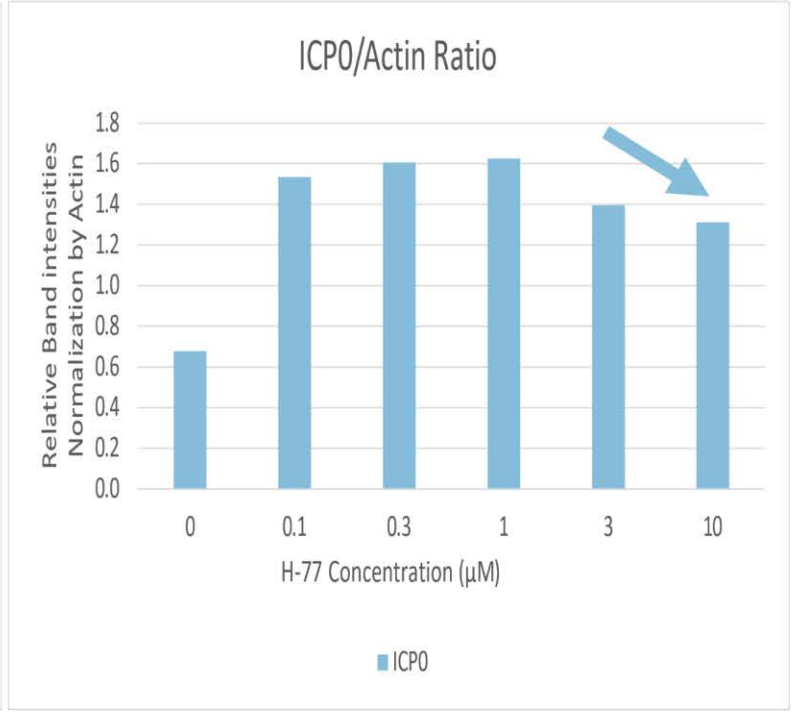


Infected Cell Protein 0









Summary

1. H-77-treated cells had significantly lower HSV-1 copy numbers and infectivity than untreated cells.
2. H-77 inhibited HSV-1 with an IC_{50} of 0.75 μM.
3. H-77 can block the nuclear lamina disassembly of HSV-1, which results in the trapping of the virus nucleocapsids inside the nucleus.
4. H-77 can inhibit the synthesis of glycoprotein C while enhancing LaminB2, as shown by western blotting

Future Experiments

1. Study the effect of H-77 on HSV-1 using other primary cell lines
2. Study the Pin1 function on HSV-1 proliferation by using siRNA-mediated silencing
3. Conduct more research on the effects of other novel Pin1 inhibitors
4. In vivo studies of H-77 and other Pin1 inhibitors to elucidate the in vivo effects of the novel Pin1 inhibitors
5. Continuation of the H-77 experiments on other DNA and RNA viruses, for example, SeV, influenza A virus, coxsackievirus, poliovirus, and other viruses

Take Home Message

H-77 significantly inhibited the replication of HSV-1.
Our immunofluorescence study suggested that H-77 can prevent nuclear egress. As a result, H-77 can prevent the spreading of the virus to nearby cells.

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  Department of Virology
  Hiroshima University, Graduate School of Biomedical and Health Sciences

Thank you!

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