

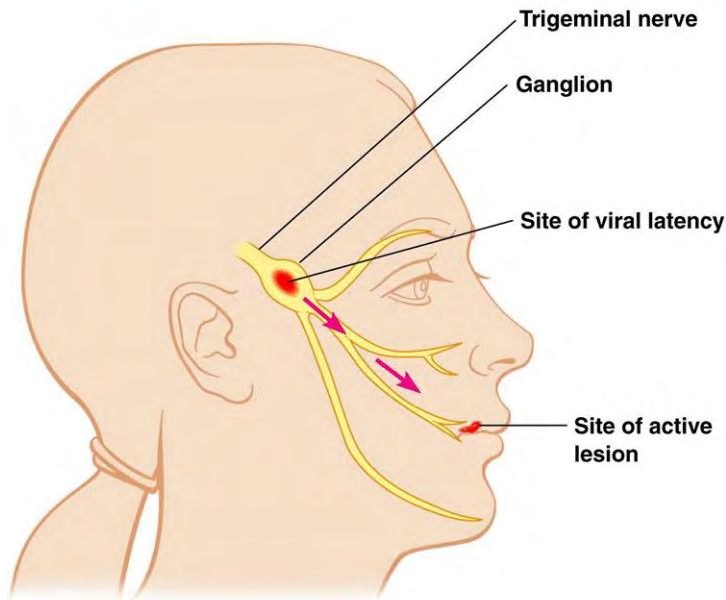
Breaking down the barriers: first steps in herpes simplex virus reactivation

Angus Wilson

Department of Microbiology

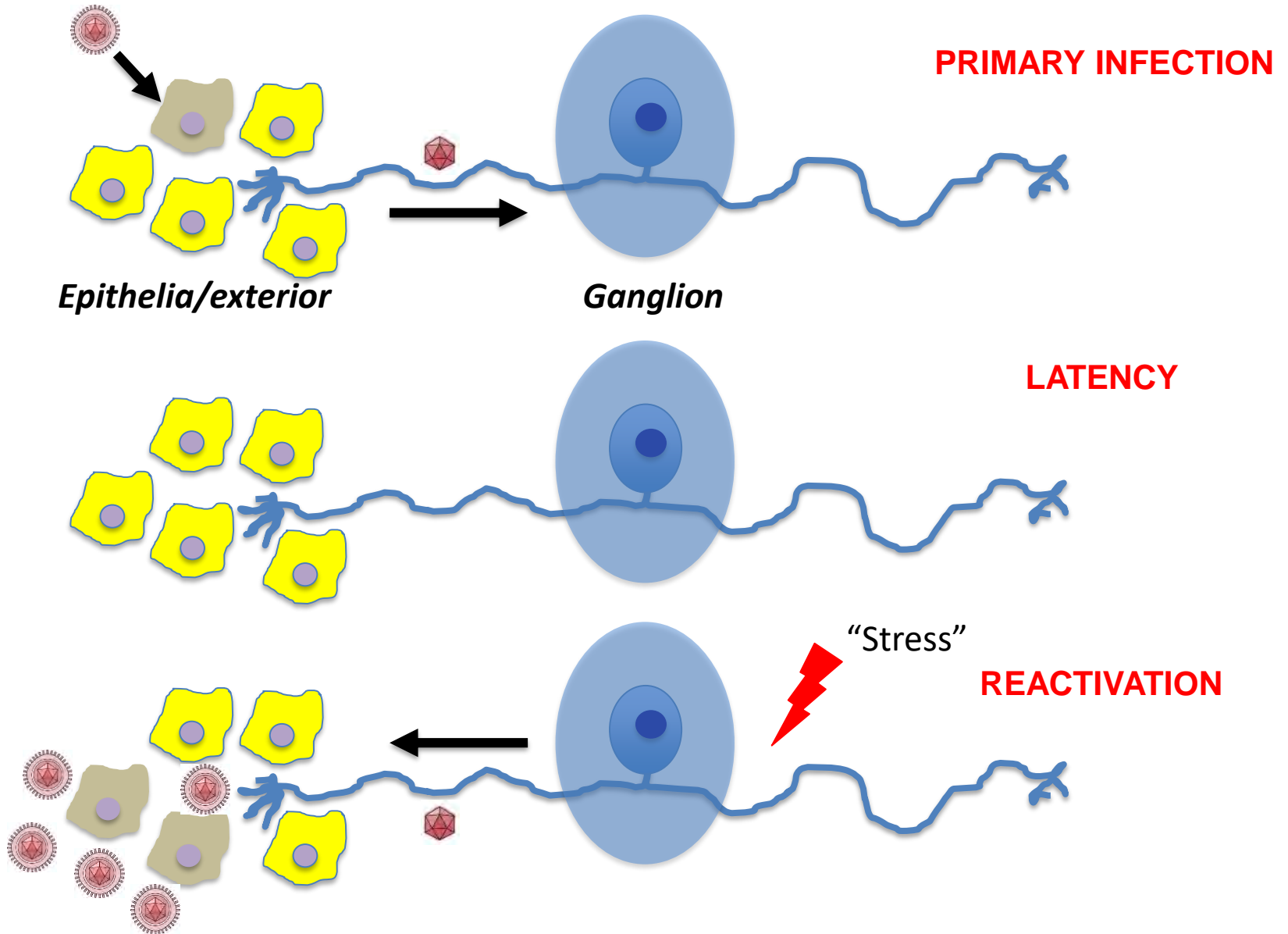
New York University School of Medicine

HSV-1 establishes latency in the human peripheral nervous system

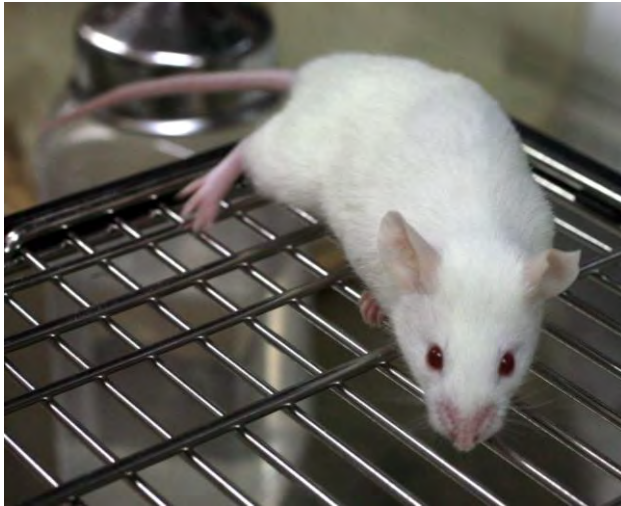


- ***Widespread human pathogen associated with a broad spectrum of disease states***
 - ***Recurrent reactivation of latent virus is a major contributor to persistence and pathogenesis***
 - ***Currently lack effective vaccines or drugs to control latency/reactivation***
-
- ***Latent viral genomes reside in the neuronal nucleus, which in turn is hidden in the nerve ganglion.***
 - ***The latent genome is circularized and associated with chromatin carrying repressive histone modifications and factors that limit viral gene expression.***

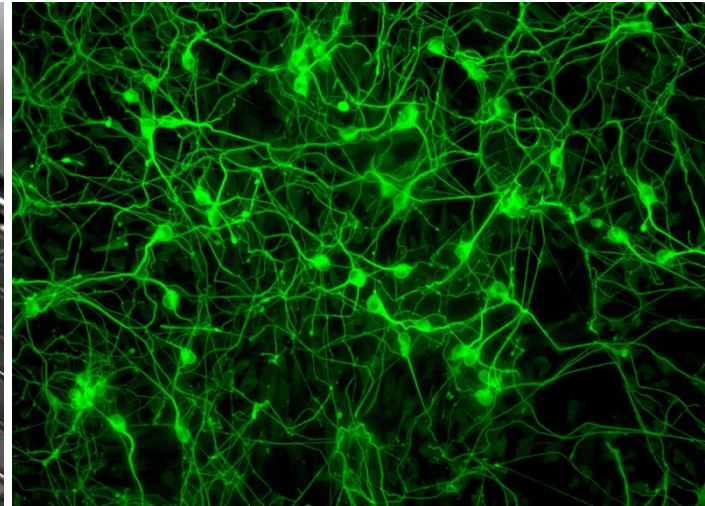
Control of latency is tightly coupled to neuronal physiology



Modeling HSV-1 latency in cultured primary neurons



In vivo infections



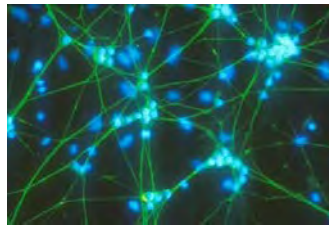
In vitro infections

E21 prenatal rats

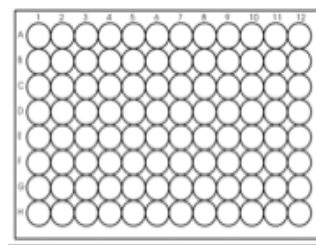
Adaptation of Wilcox & Johnson protocols (1988-90)



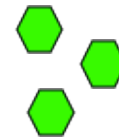
**Isolate superior
cervical ganglia
(SCG)**



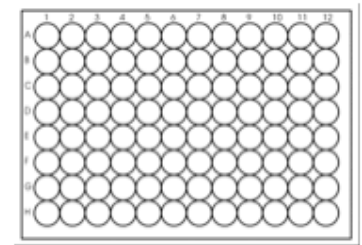
**Dissociate
ganglia**



Establish neuron cultures
Nerve growth factor (NGF)
+ 5-FU & aphidicolin



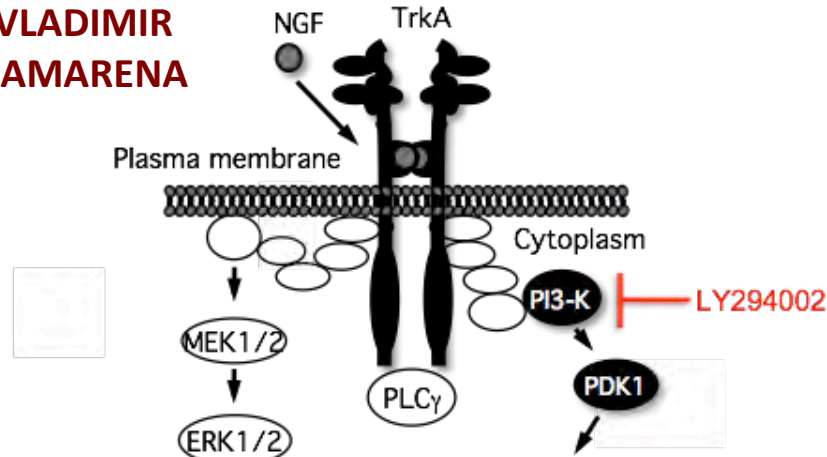
HSV-1



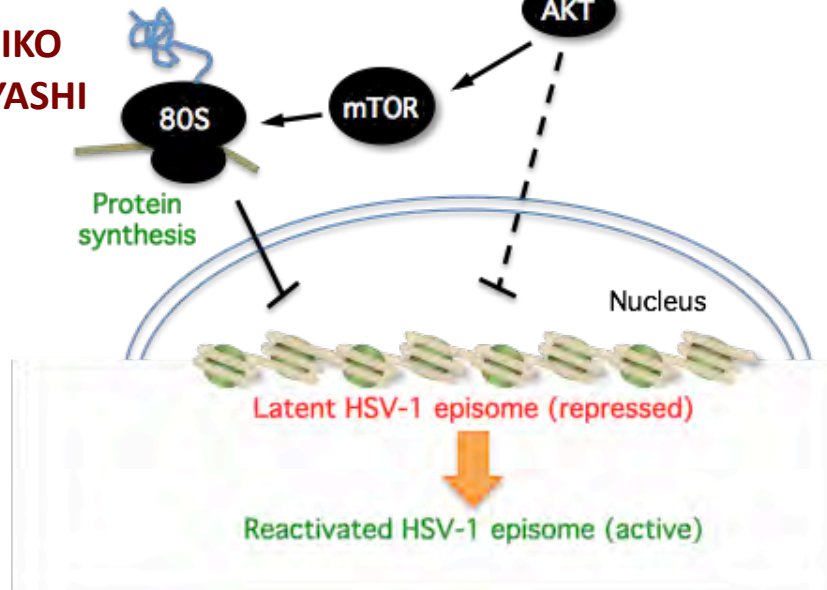
**Infect with HSV-1
+ acyclovir (ACV)**

Sustained cap-dependent translation governs HSV-1 latency

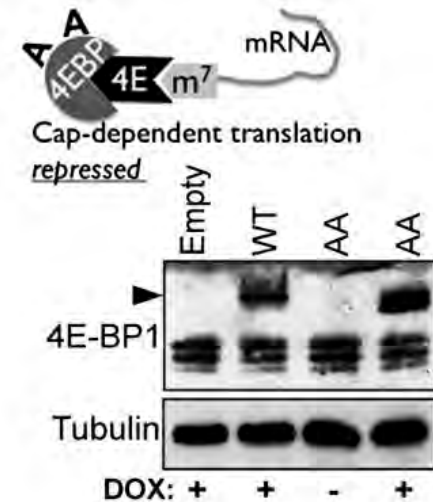
**VLADIMIR
CAMARENA**



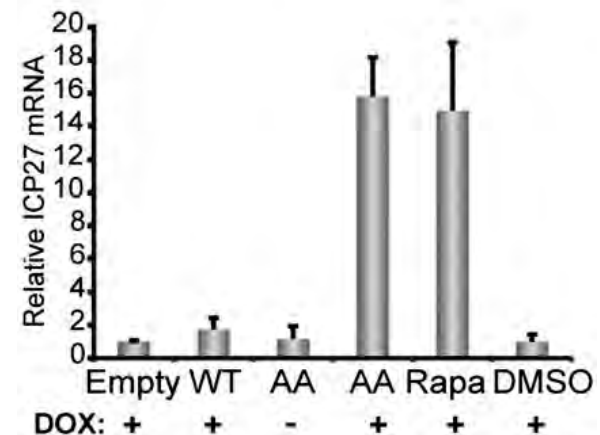
**MARIKO
KOBAYASHI**



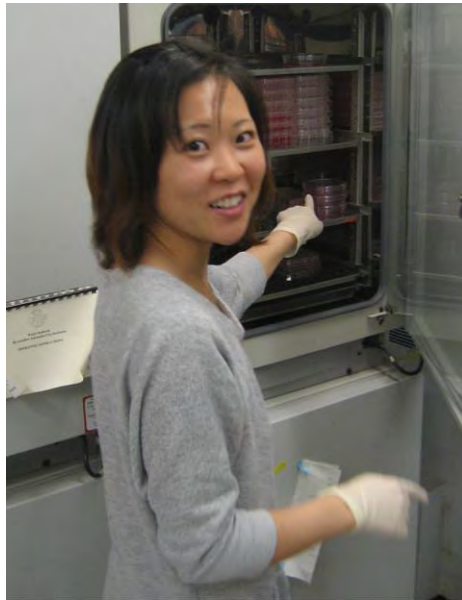
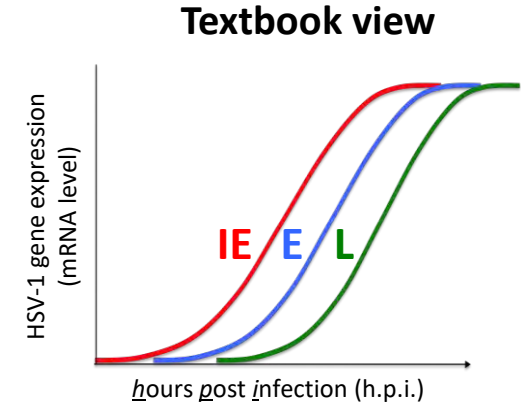
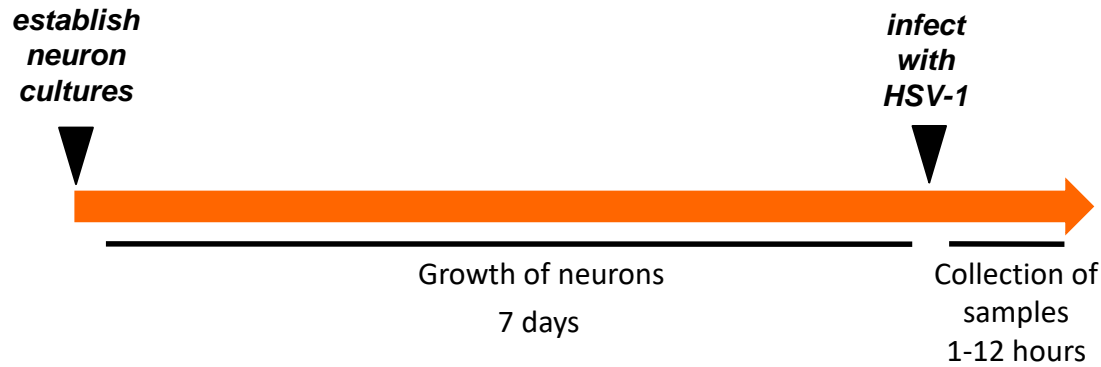
Inhibition of cap-dependent translation using constitutive 4EBP1 repressor



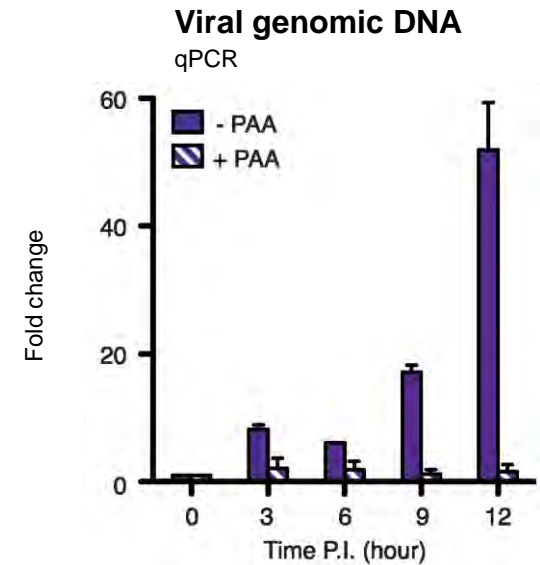
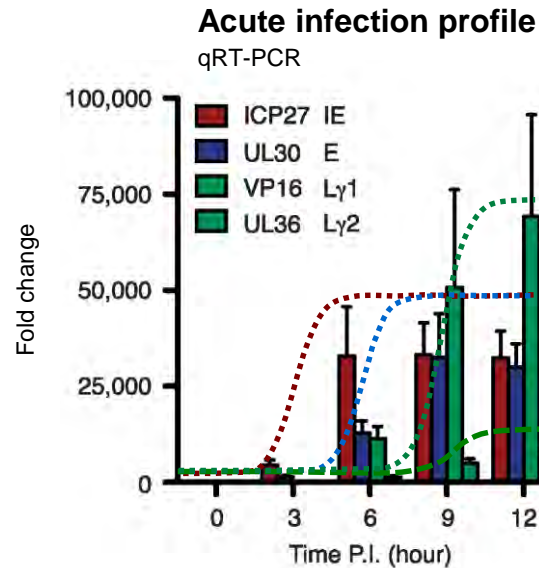
**MARIKO
KOBAYASHI**



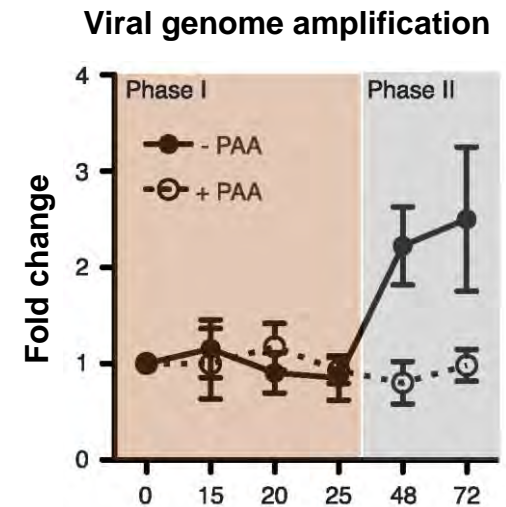
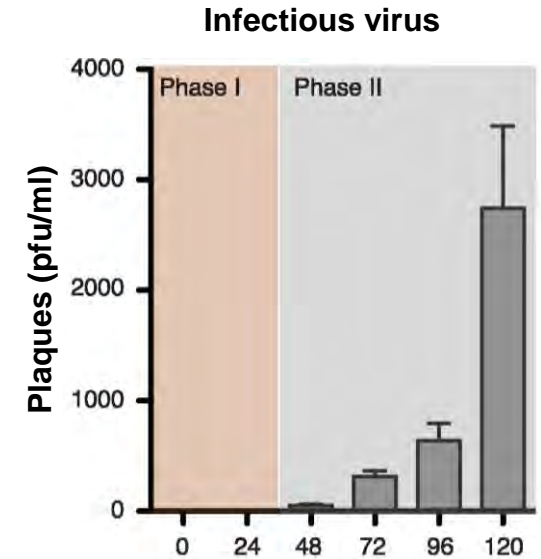
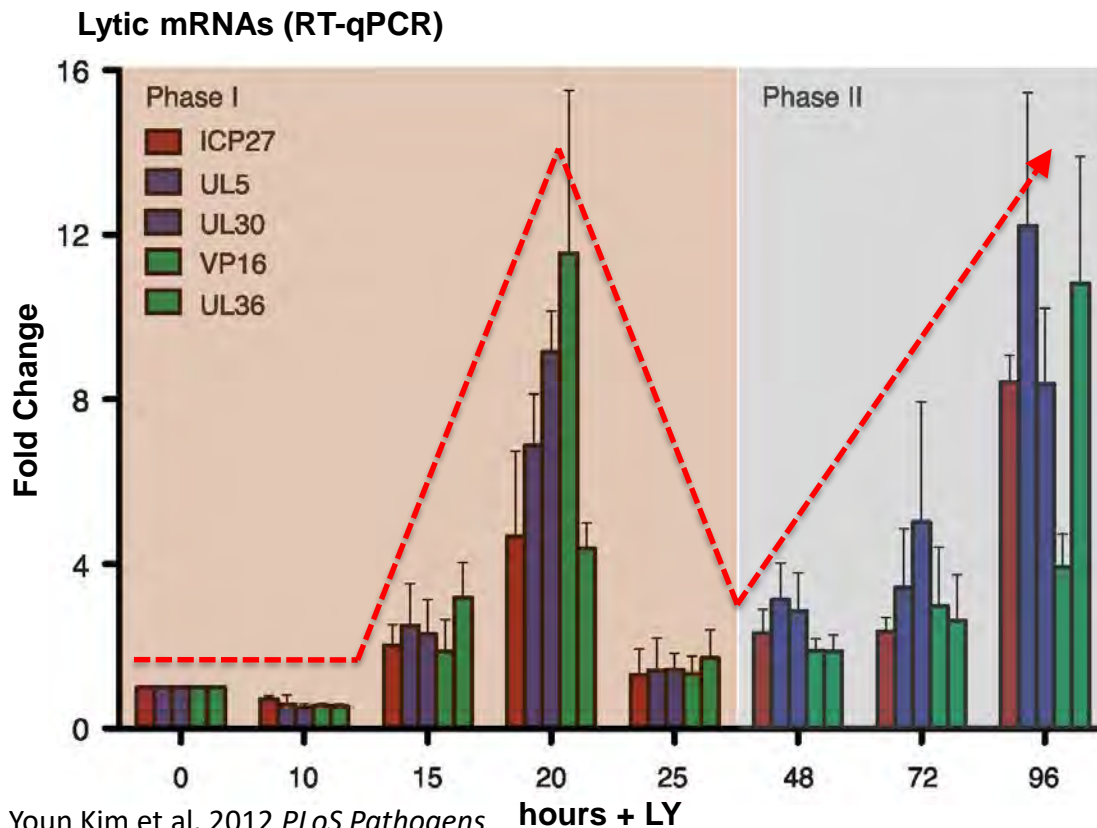
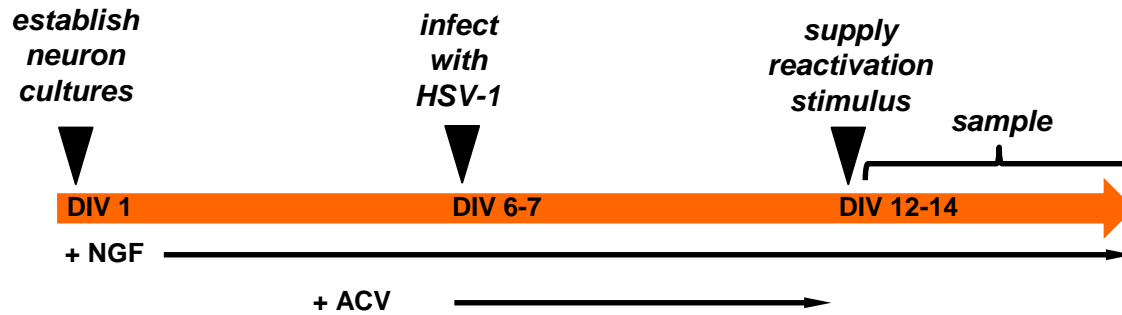
Acute infections of SCG neurons follow the canonical cascade



Ju Youn Kim

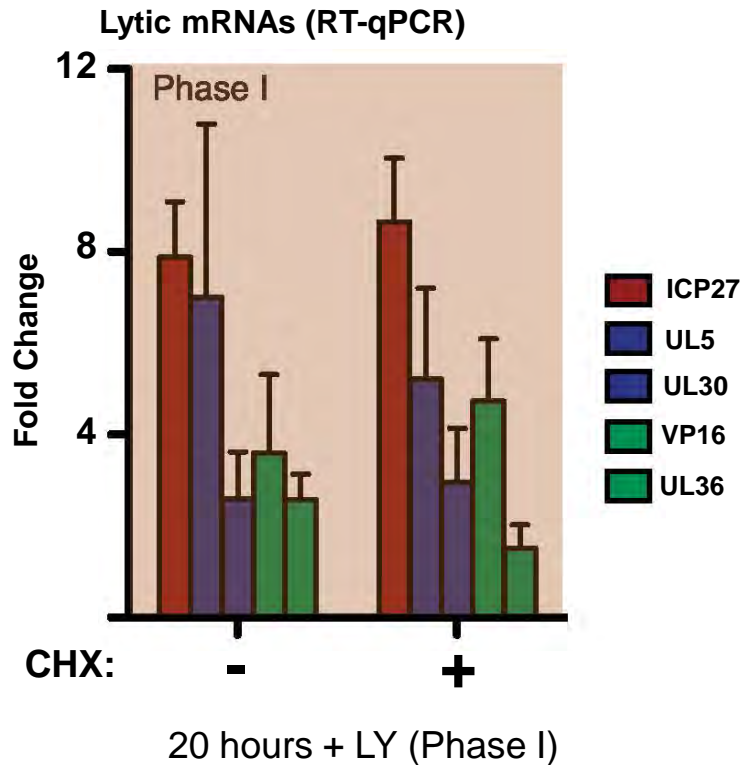


Inducing reactivation results in two waves of viral gene transcription

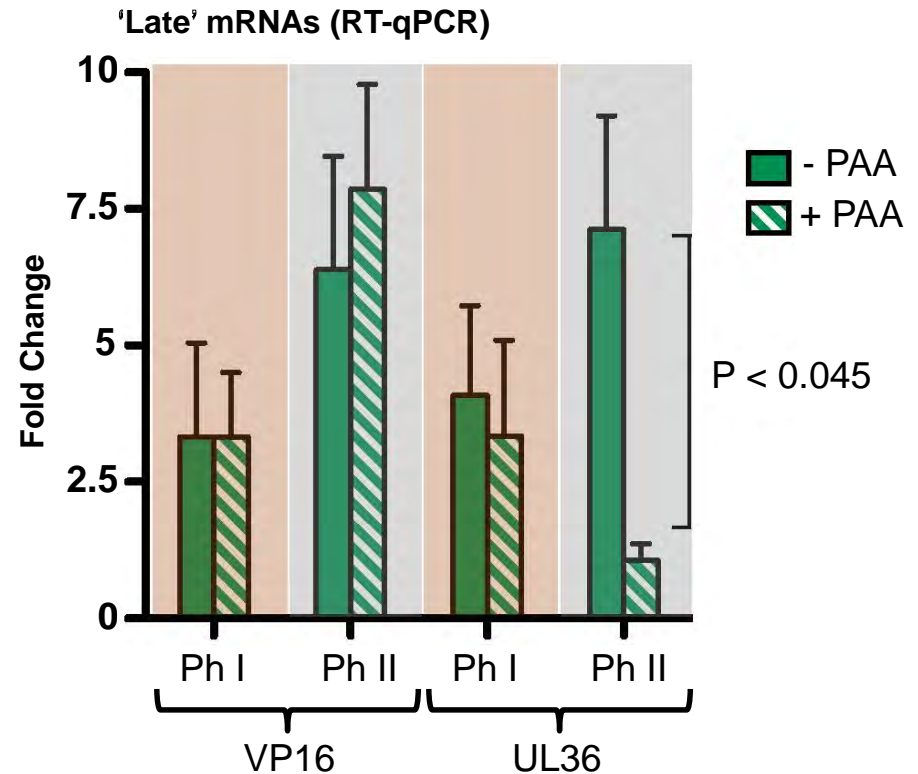


Fundamental differences between Phase I & Phase II

E & L gene transcription independent of protein synthesis



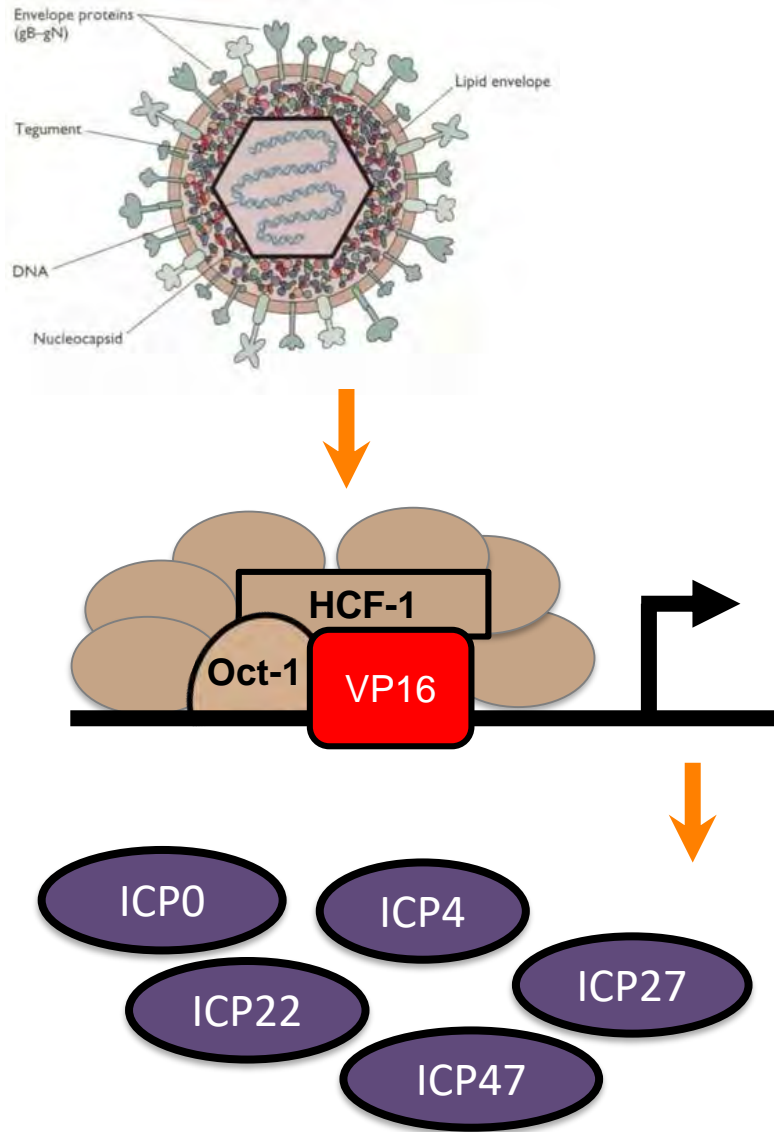
L gene transcription independent of DNA replication



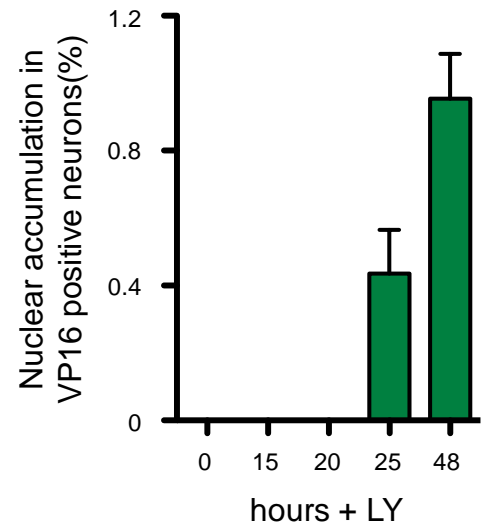
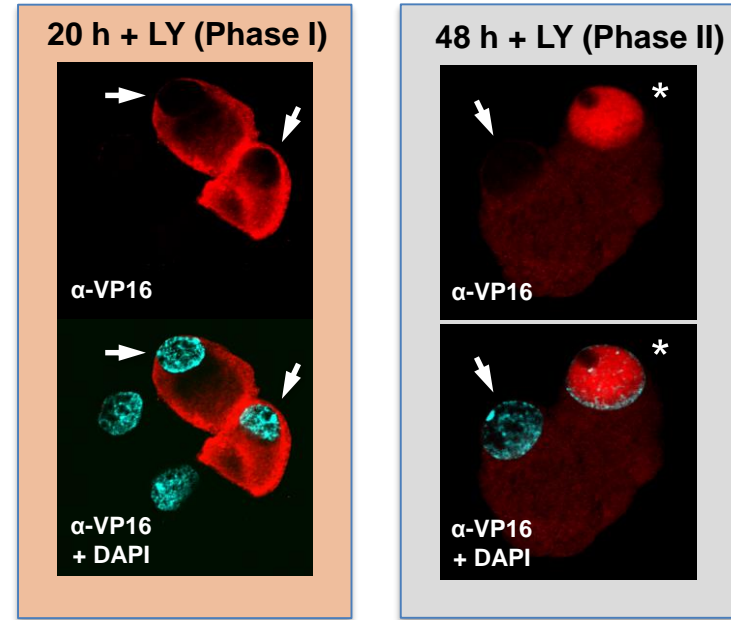
Phase II not due to spread

- Biphasic profile unaffected by DNA encapsidation inhibitor (WAY150138)

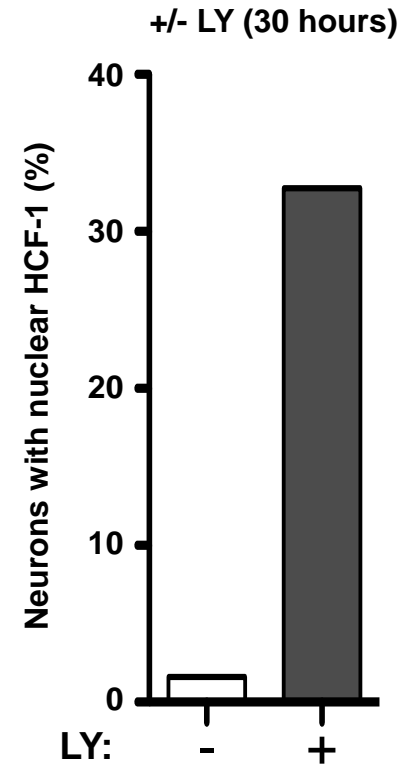
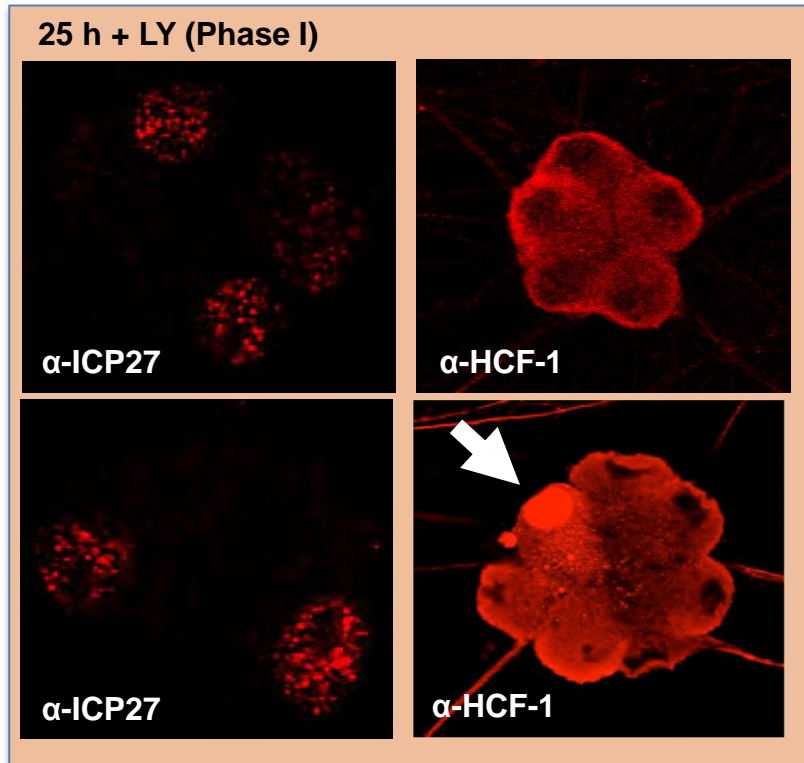
VP16-HCF complex drives IE gene transcription



25-30% neurons VP16⁺

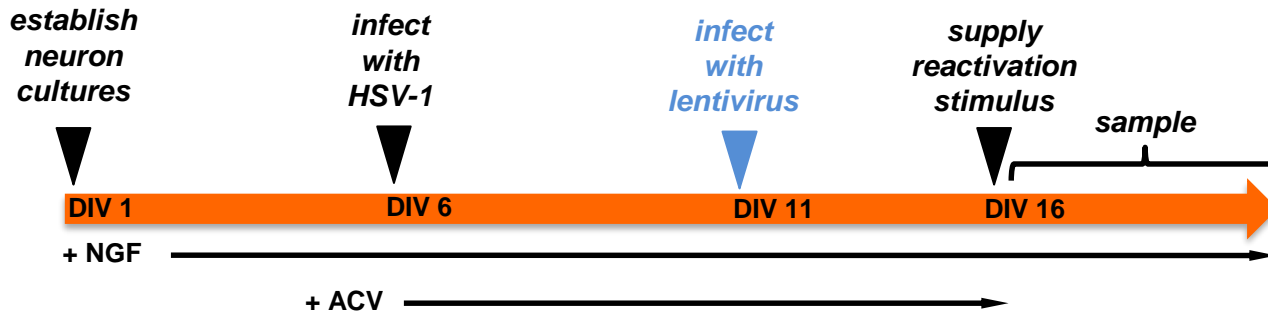


LY treatment promotes HCF-1 nuclear accumulation

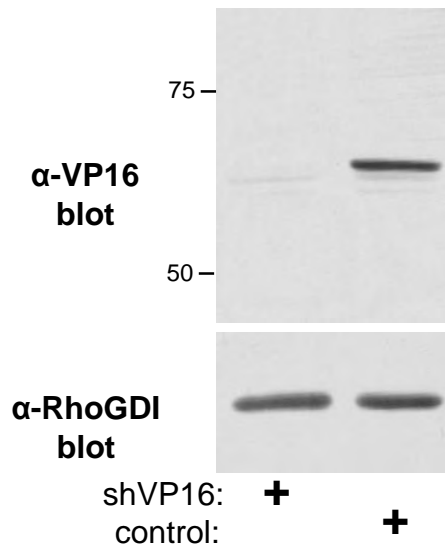


Kristie TM, Vogel JL, Sears AE (1999) Nuclear localization of the C1 factor (host cell factor) in sensory neurons correlates with reactivation of herpes simplex virus from latency. *PNAS* 96: 1229–1233.

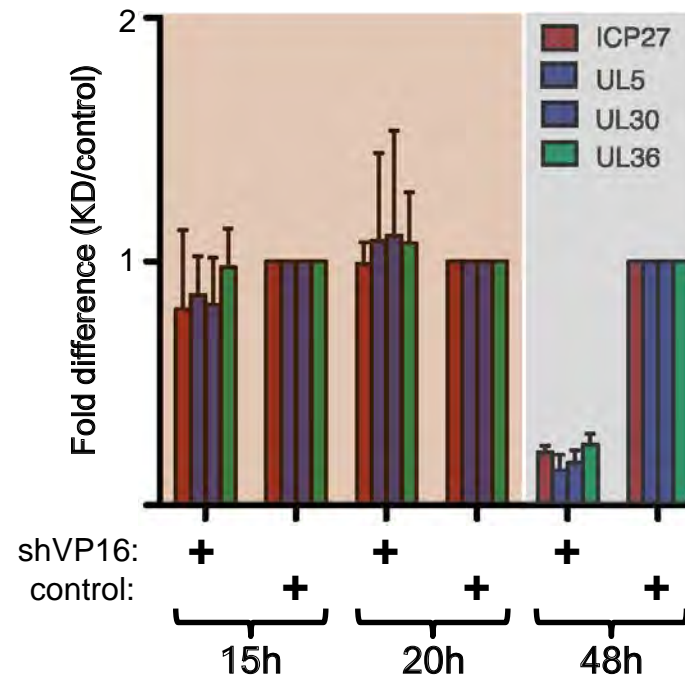
shRNA depletion of VP16 results in reduced Phase II lytic mRNA levels



VP16 knockdown



mRNA levels (RT-qPCR)



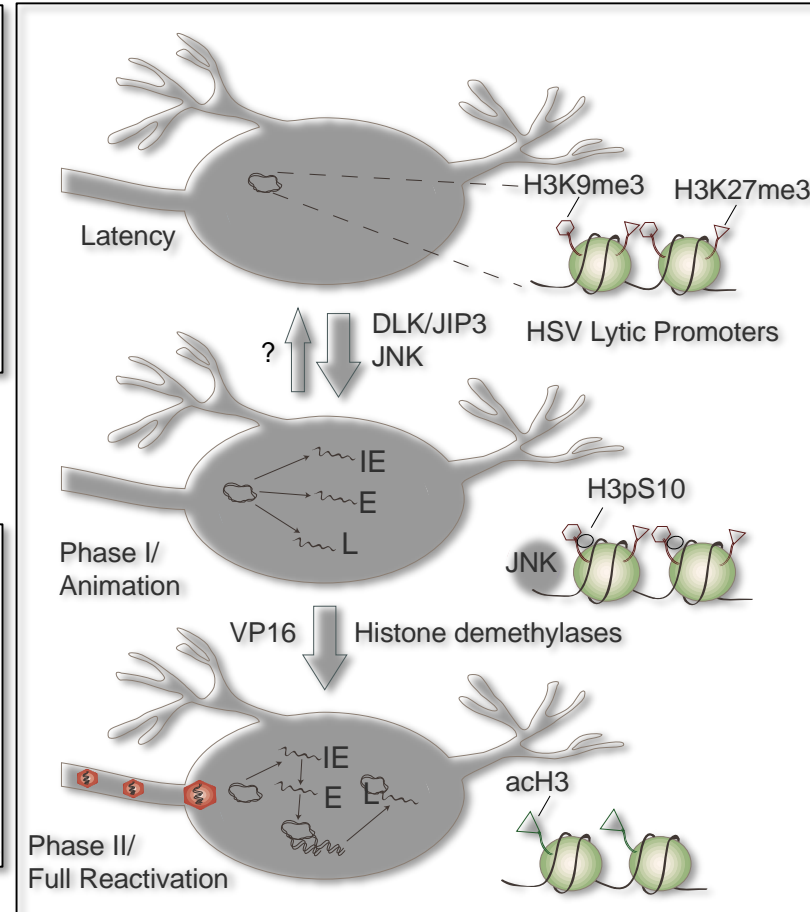
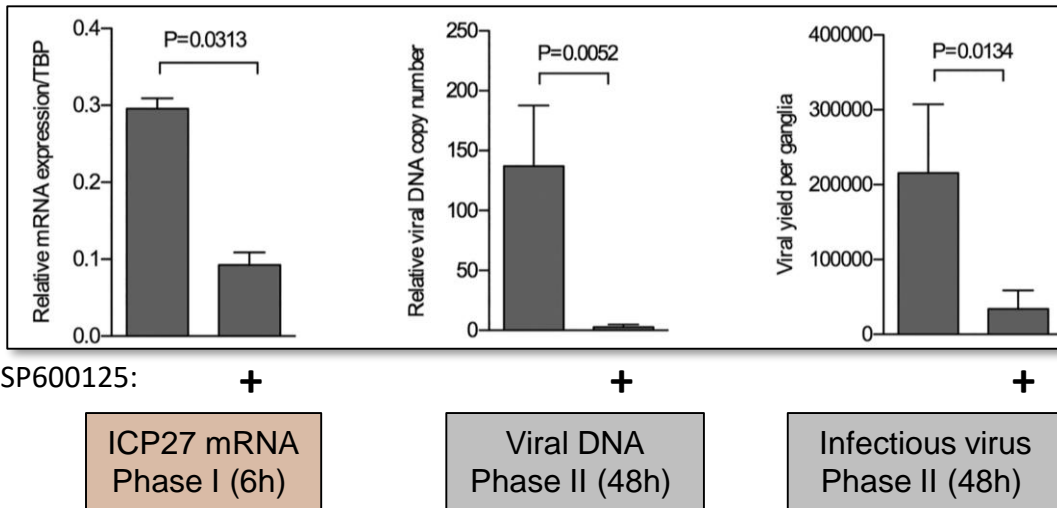
JNK activation connects Phase I to neuronal stress response pathways

Cell Host & Microbe
Article

Neuronal Stress Pathway Mediating a Histone Methyl/Phospho Switch Is Required for Herpes Simplex Virus Reactivation

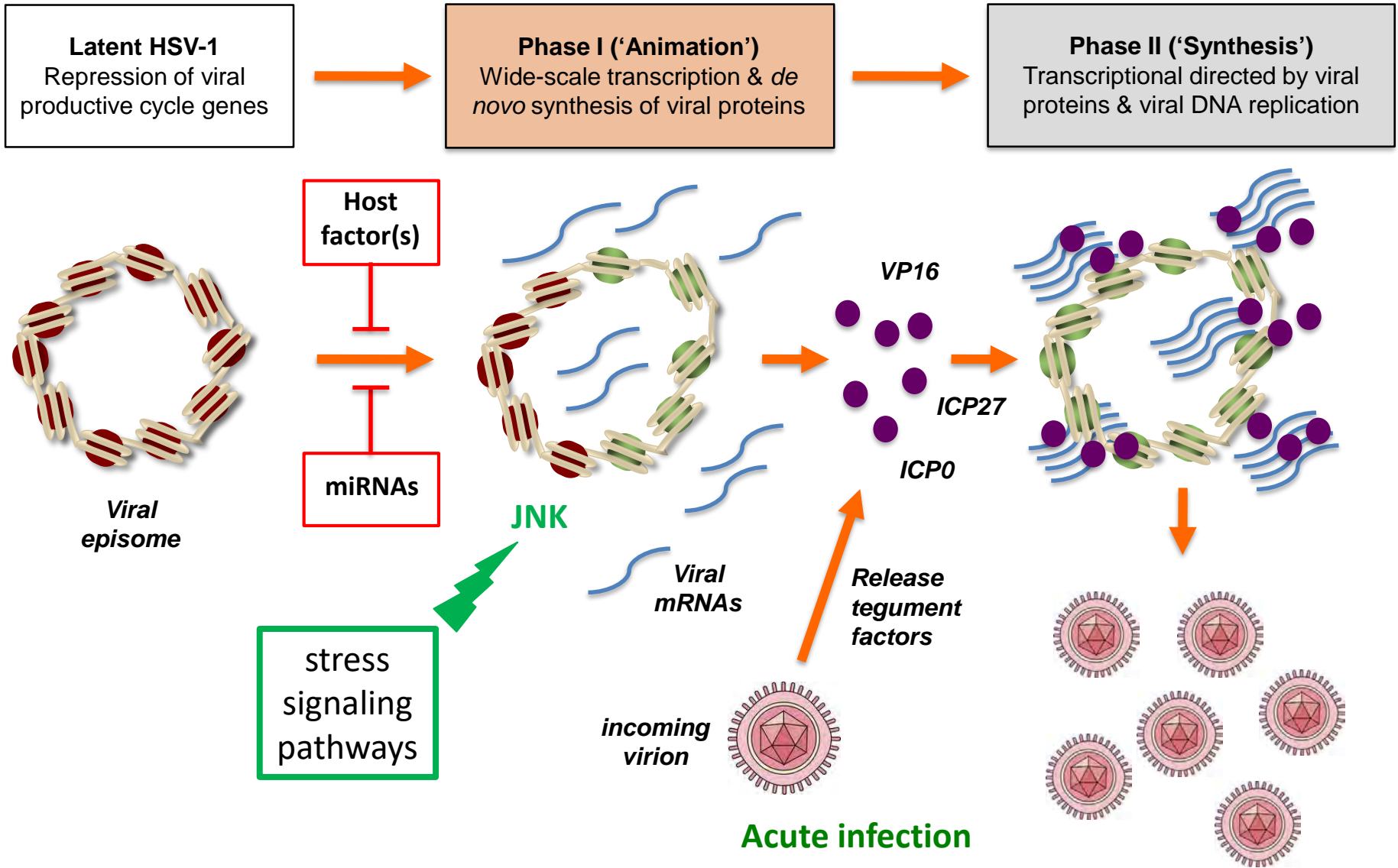
Anna R. Cliffe,^{1,2,*} Jesse H. Ar buckle,³ Jodi L. Vogel,³ Matthew J. Geden,^{1,2} Scott B. Rothbart,^{4,7} Corey L. Cusack,^{2,5} Brian D. Strahl,^{4,6} Thomas M. Kristie,³ and Mohanish Deshmukh^{1,2,6,*}

Explant/axotomy of latently infected murine trigeminal ganglia

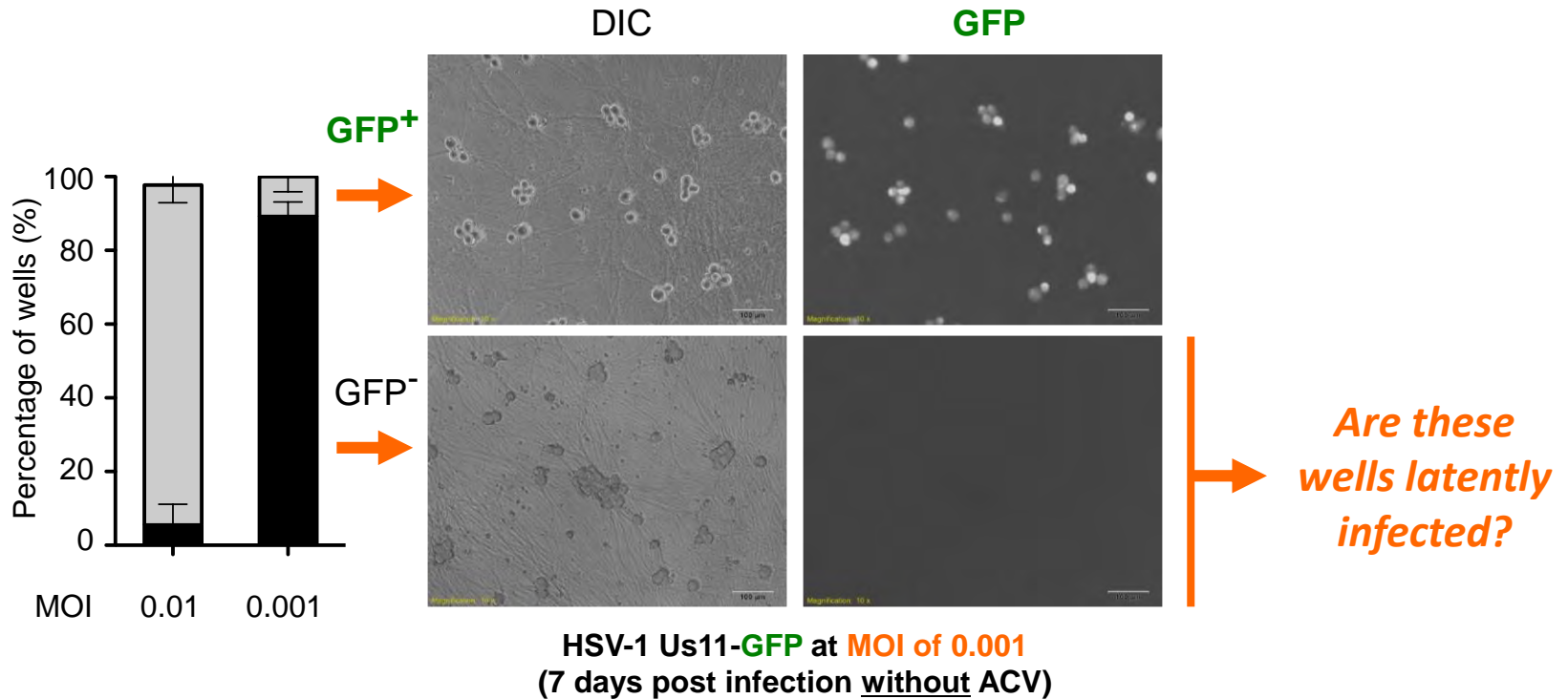
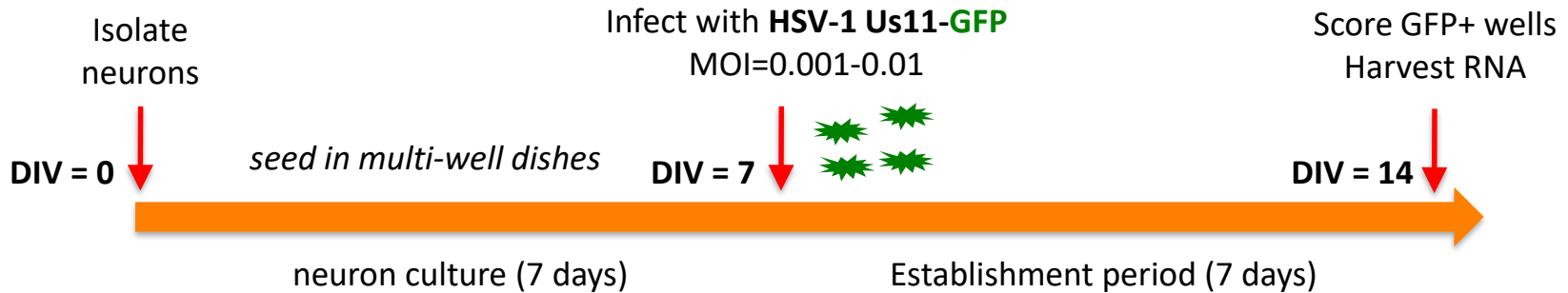


Histone methyl/phospho switch allows derepression of viral genes without removal of repressive lysine marks

Phase I provides latent HSV-1 with missing tegument factors

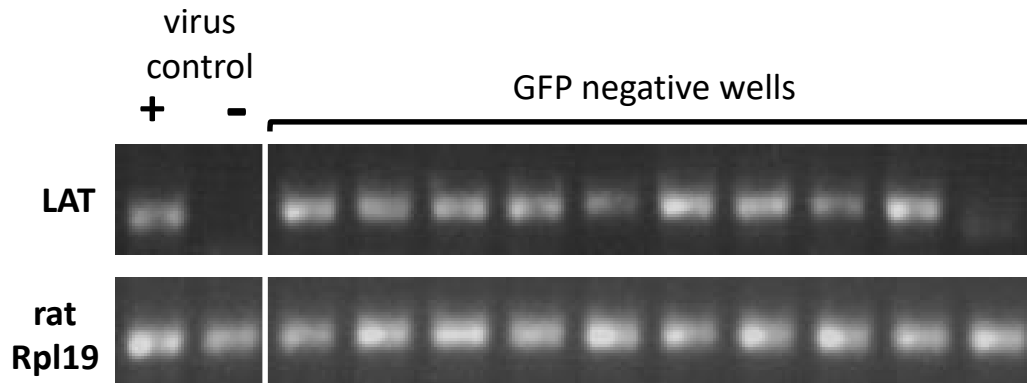


Latency can be established without ACV by infection at low MOI



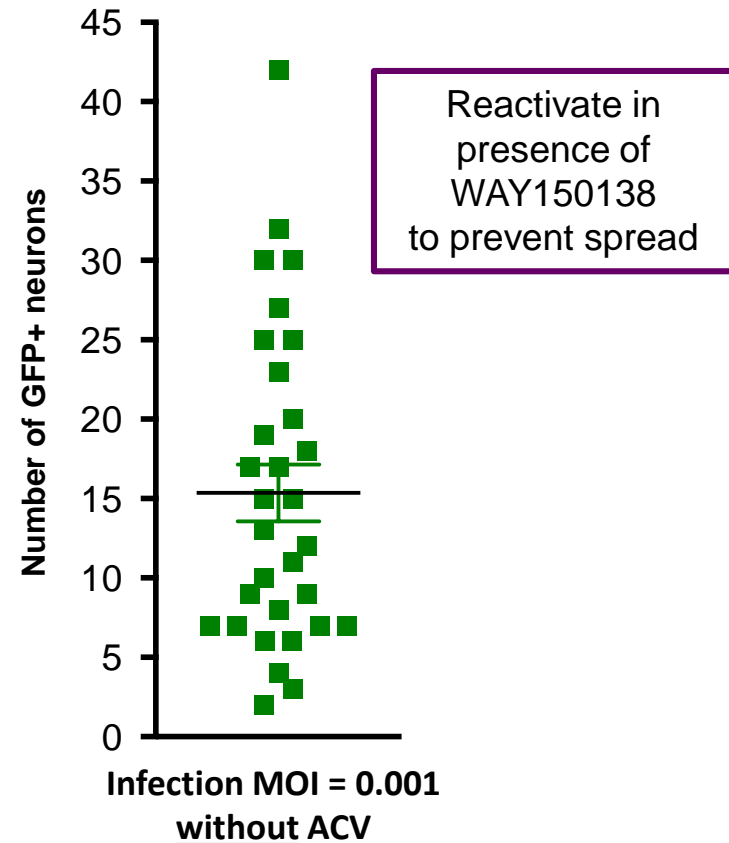
Efficient establishment of latency at low MOI

Detection of LAT ncRNA in GFP negative wells 7 days after infection

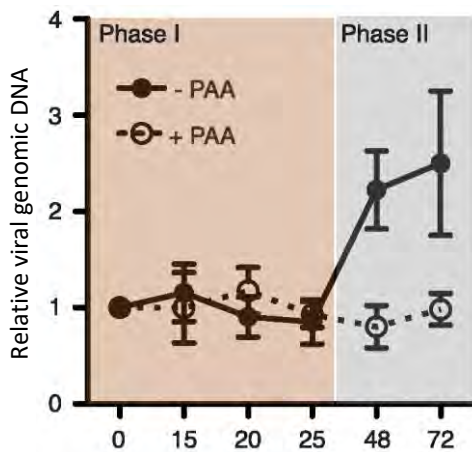
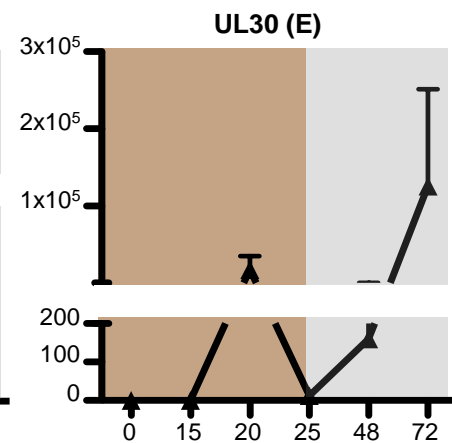
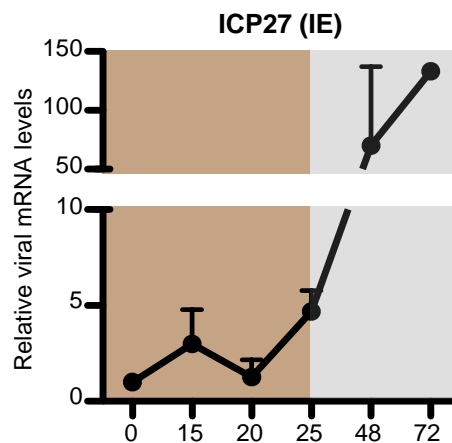
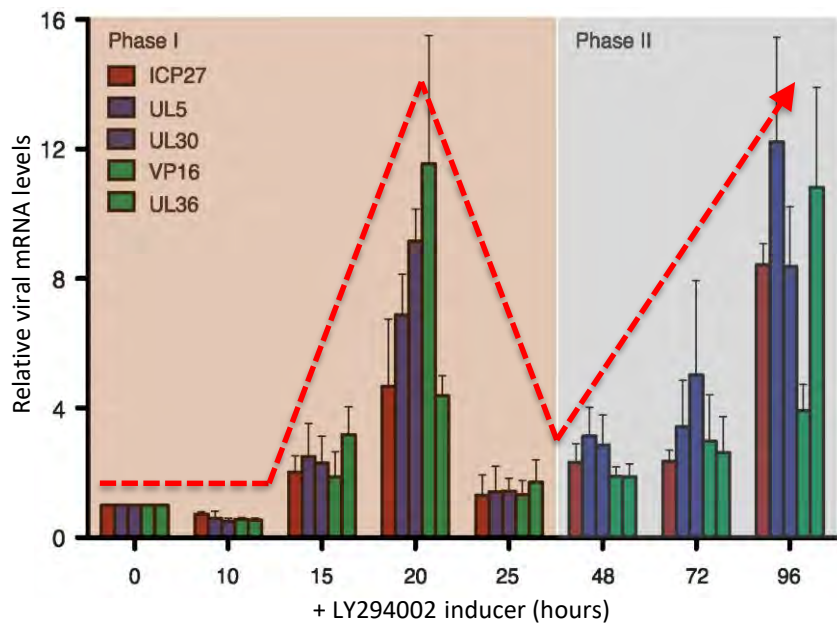


MOI = 0.001 (50 pfu/well) without ACV

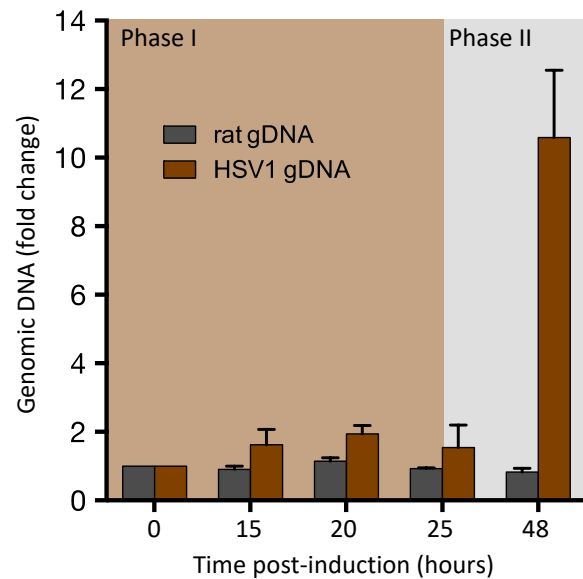
Concordance between input virus and # of neurons supporting reactivation



Biphasic reactivation from latency established without ACV

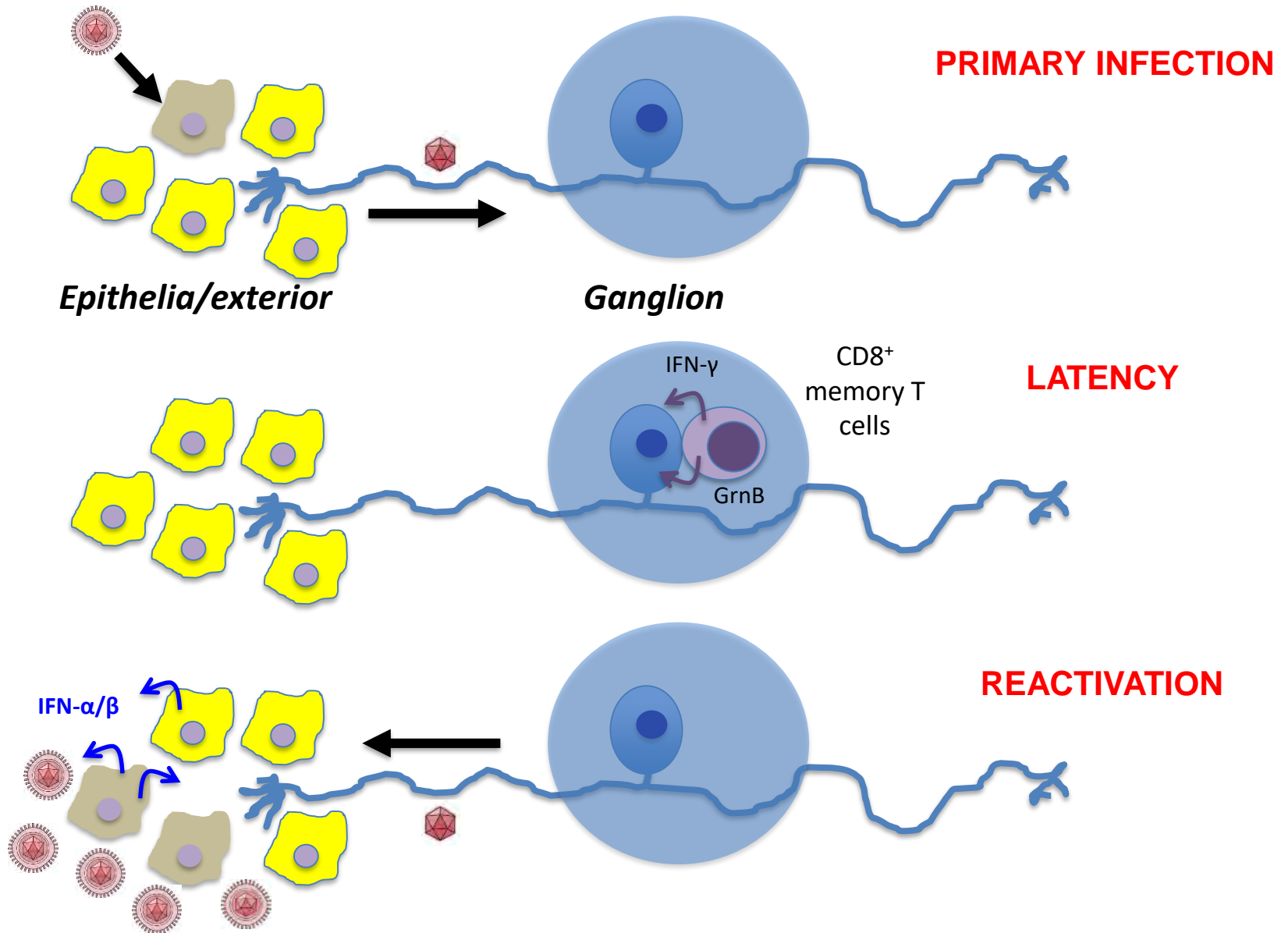


MOI=0.01
without ACV



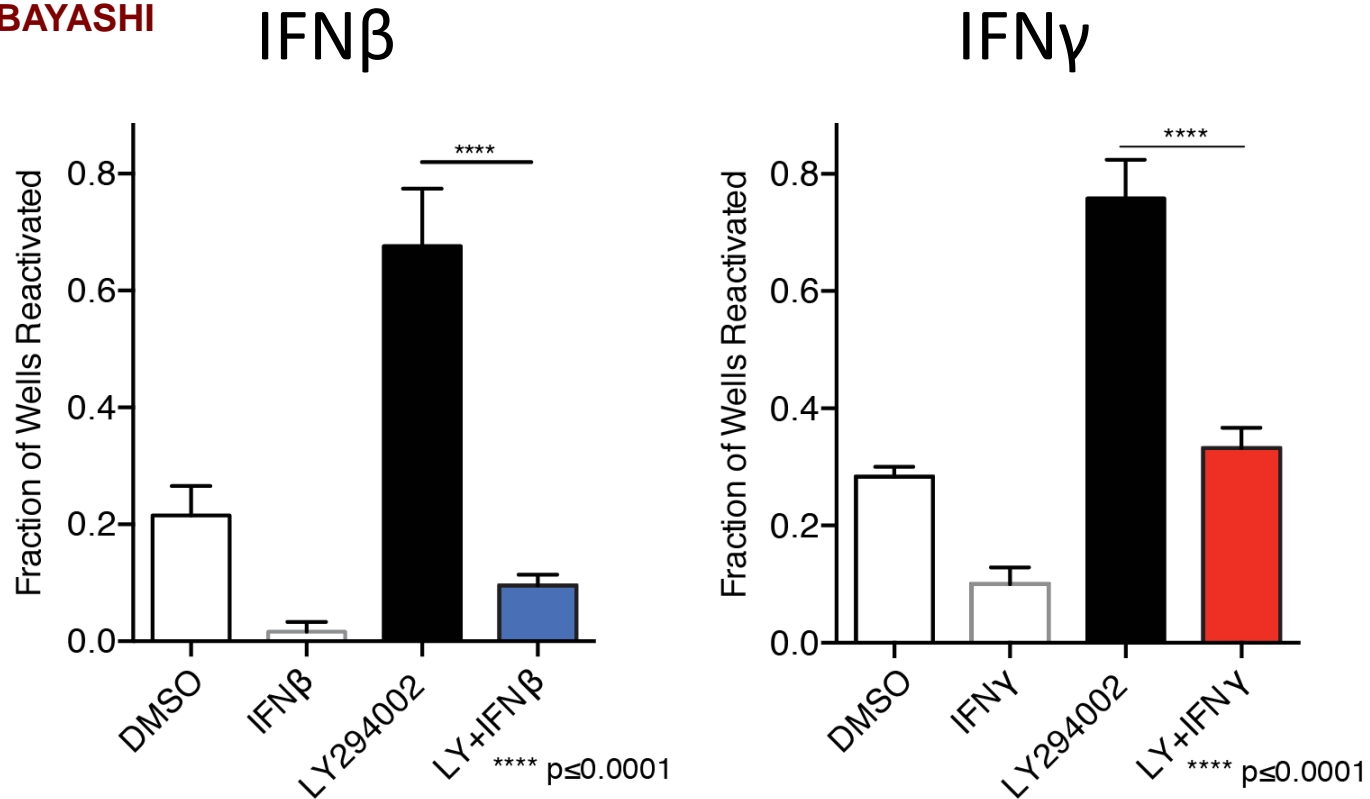
MOI=1
+ 100 μ m ACV

HSV-specific T-cells infiltrate ganglia and prevent reactivation without destroying the neurons



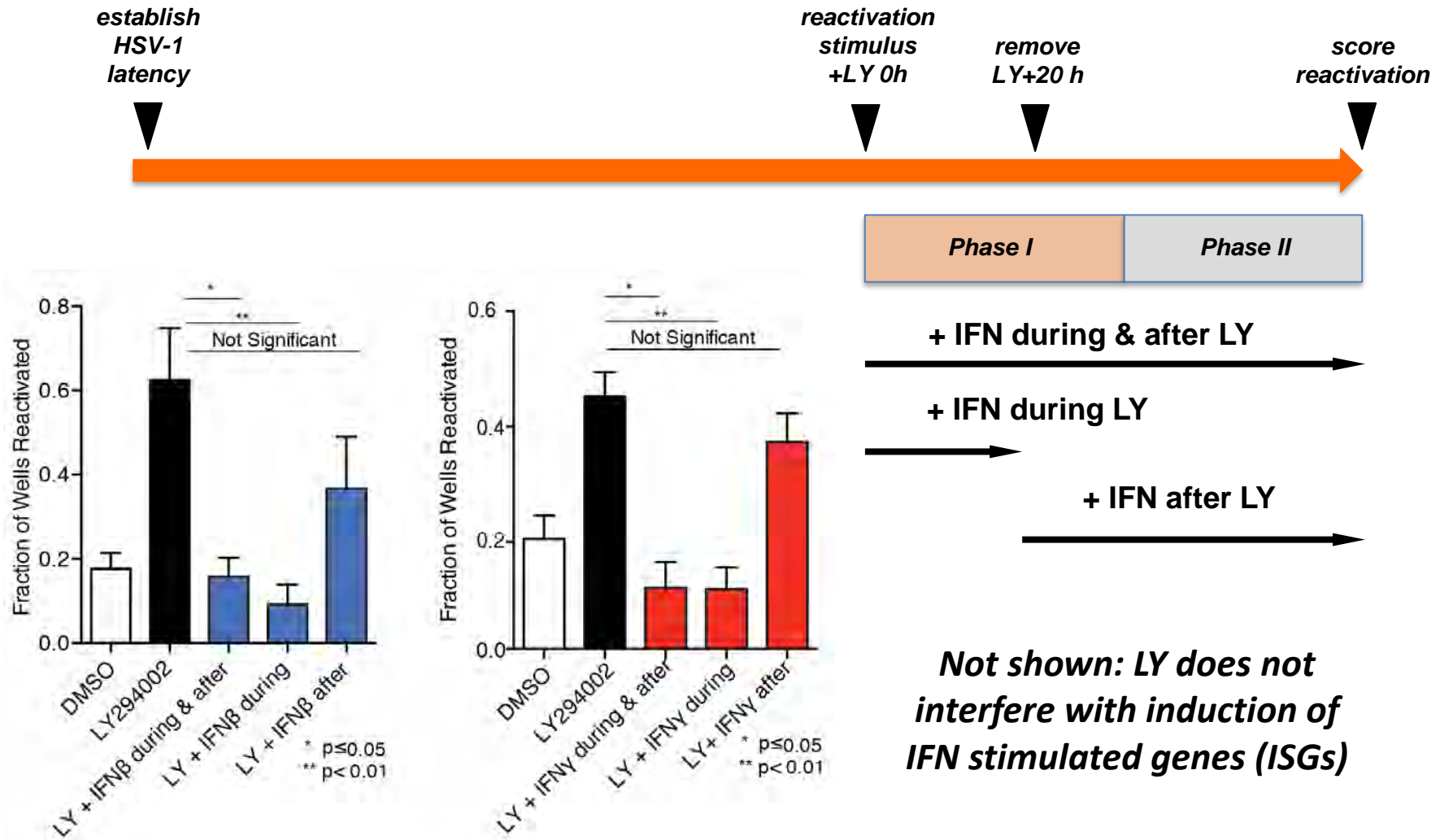
Exogenous interferon inhibits reactivation in neuron-only cultures

JESSICA LINDERMAN
MARIKO KOBAYASHI



Not shown: Reactivation signal not impaired by IFN

Interferon is less effective after Phase I



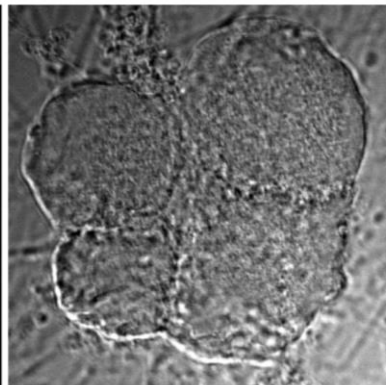
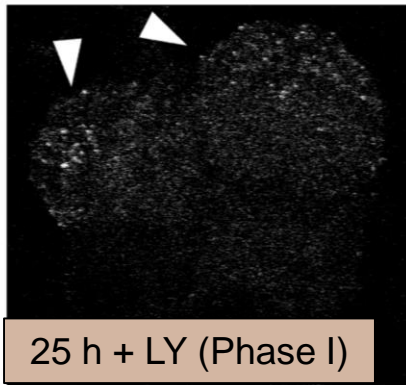
HSV-1 encodes multiple IFN antagonists

Okay immune system: bring it on!



ICP0

DIC



ICP0

- SUMO-dependent Ub E3 ligase
- Disrupts ND10 bodies
- Impairs activation of sensors
- Blocks induction of ISGs

ICP34.5

- Inhibits IFN-mediated eIF-2 α phosphorylation

ICP27

- Inhibits IFN & ISG expression

Us11

- Inhibits 2'-5 OAS synthesis & eIF-2 α phosphorylation

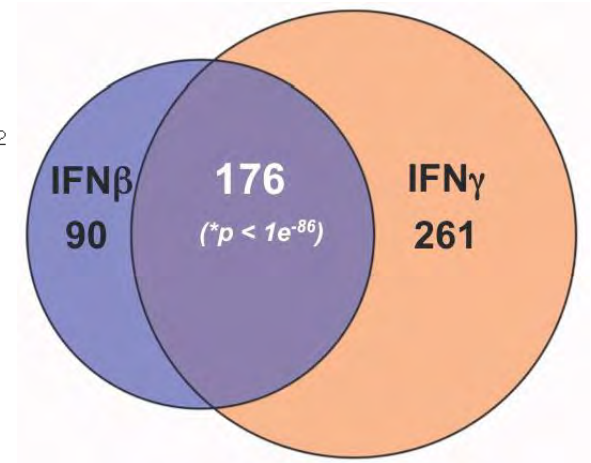
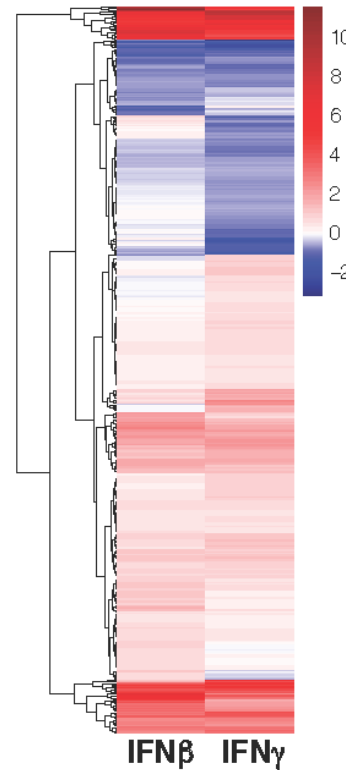
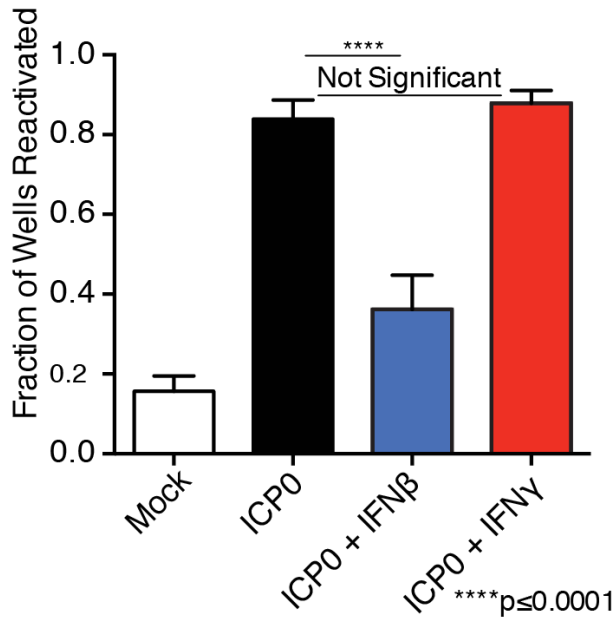
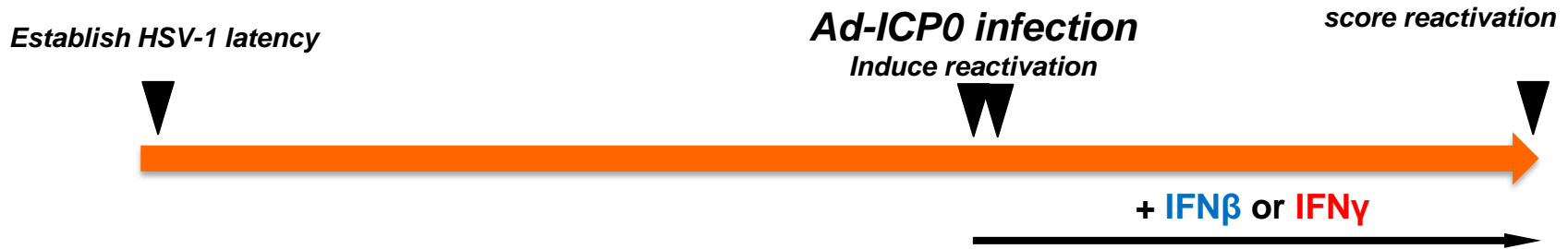
Vhs

- Inhibits STAT signaling & eIF-2 α phosphorylation

Us3

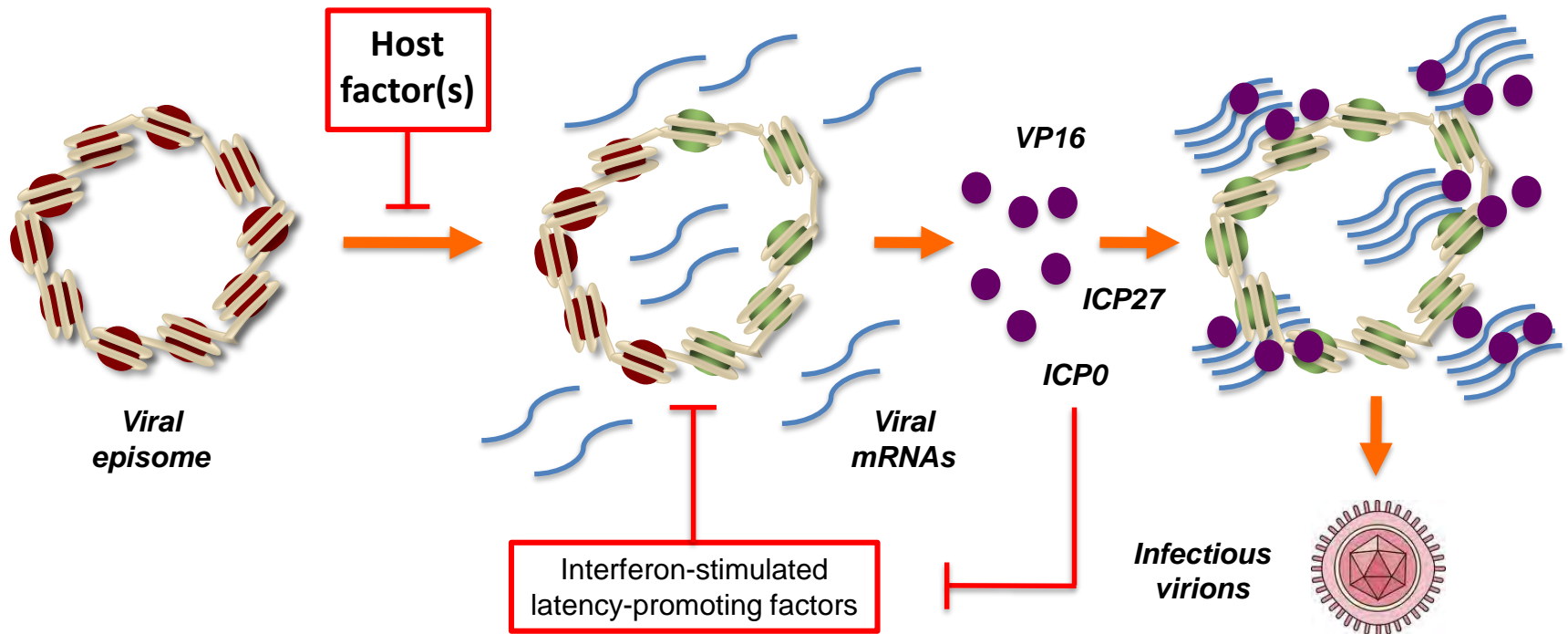
- Inhibits IFN receptor signaling & ISG synthesis

Ectopic ICP0 allows HSV-1 reactivation in the presence of IFN- γ



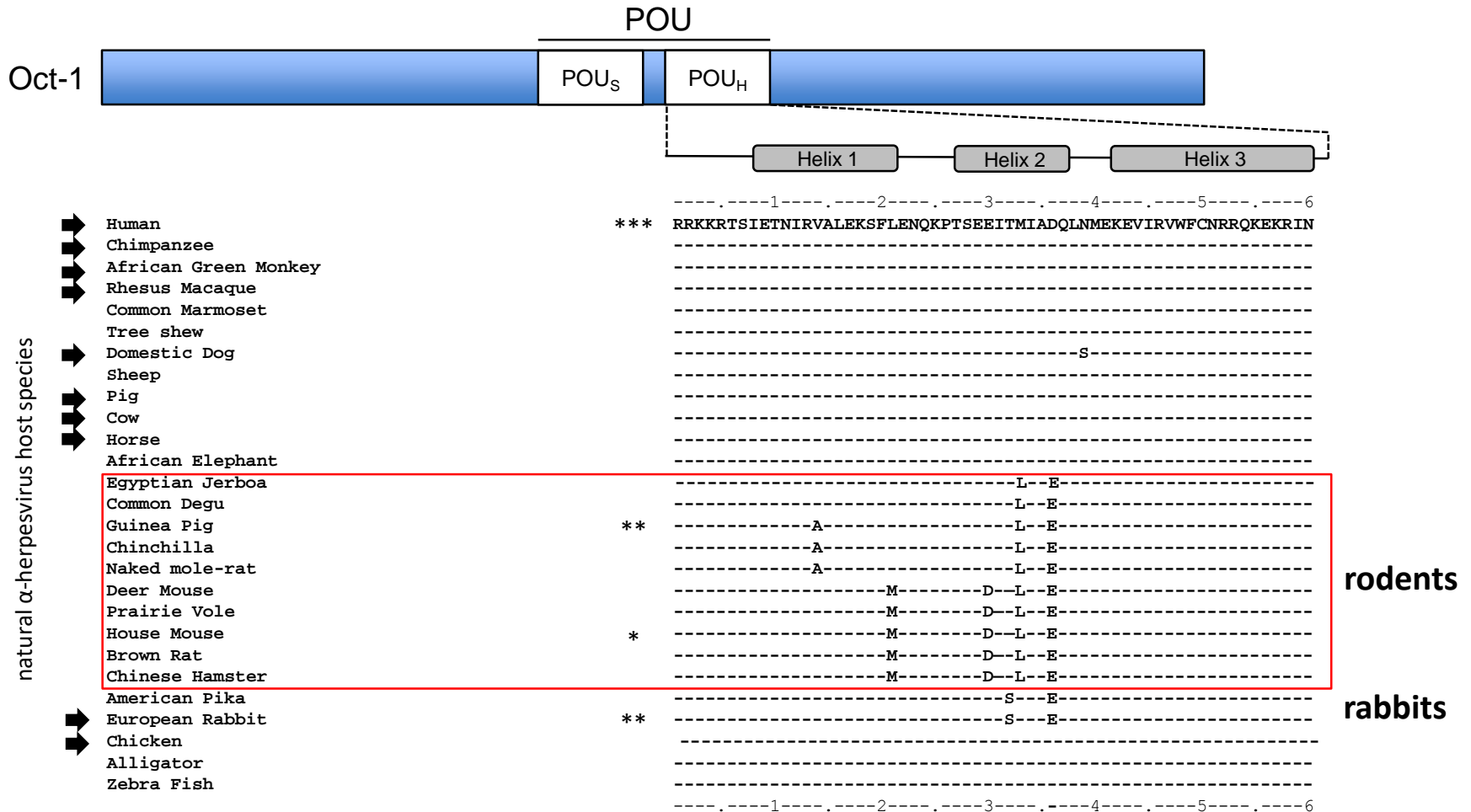
IFN β and IFN γ induce unique profiles of ISG expression in SCG neurons

Phase I also provides viral factors that block innate defenses



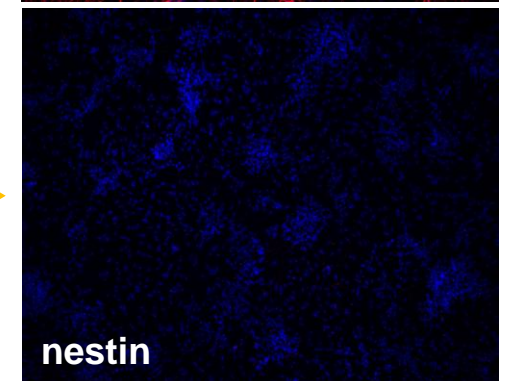
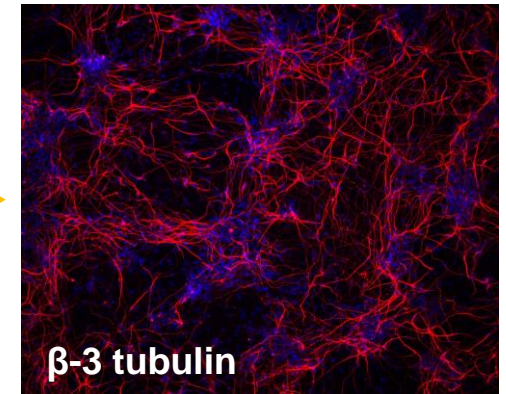
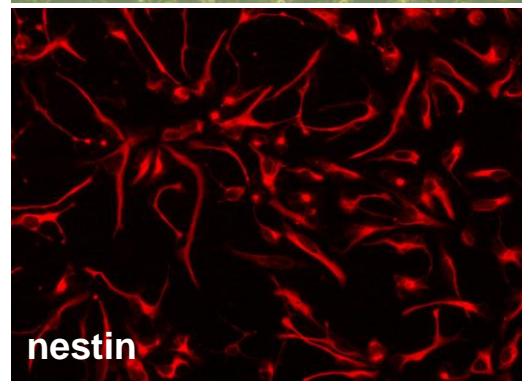
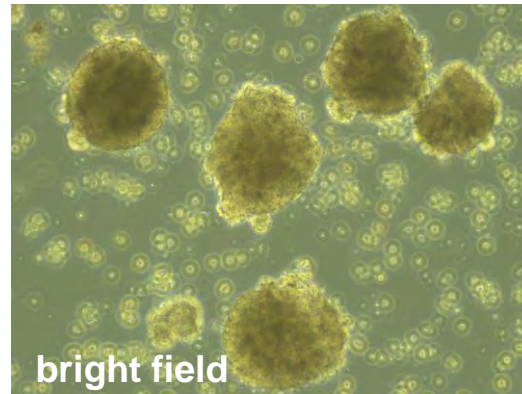
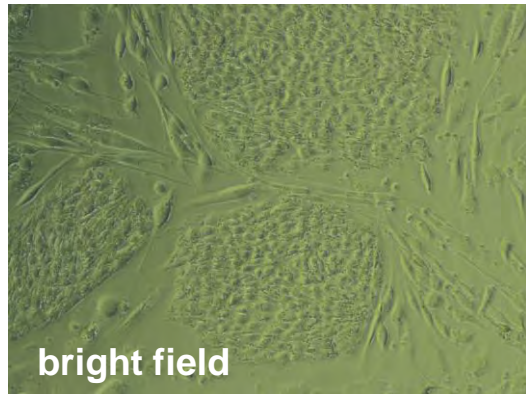
Limitations of the Rat Model

- Few molecular reagents, neurons expensive
- Molecular mismatches: human virus in non-human cells.



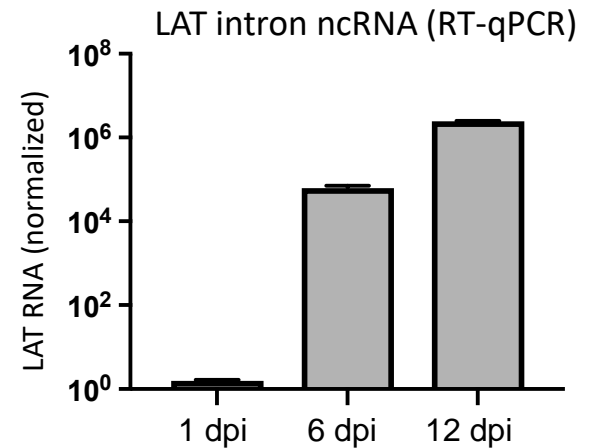
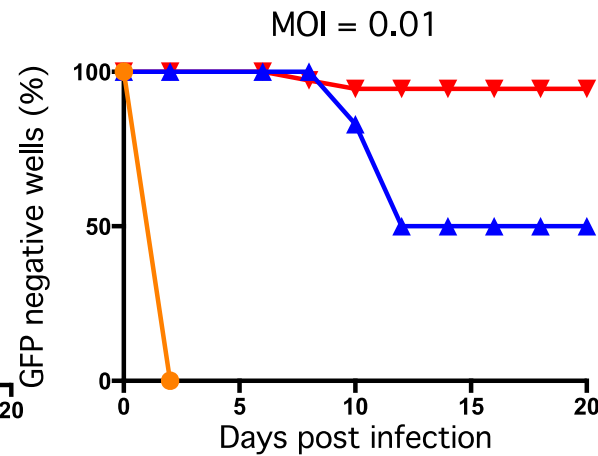
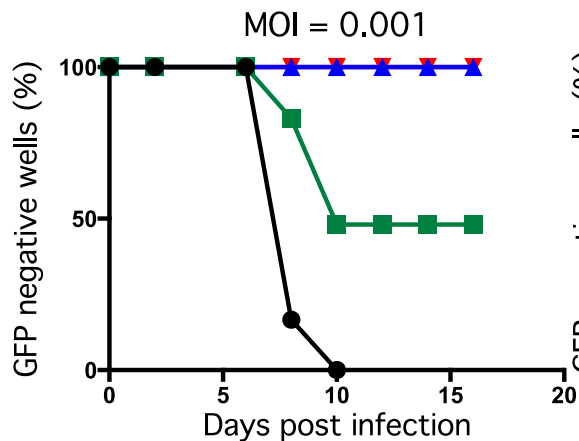
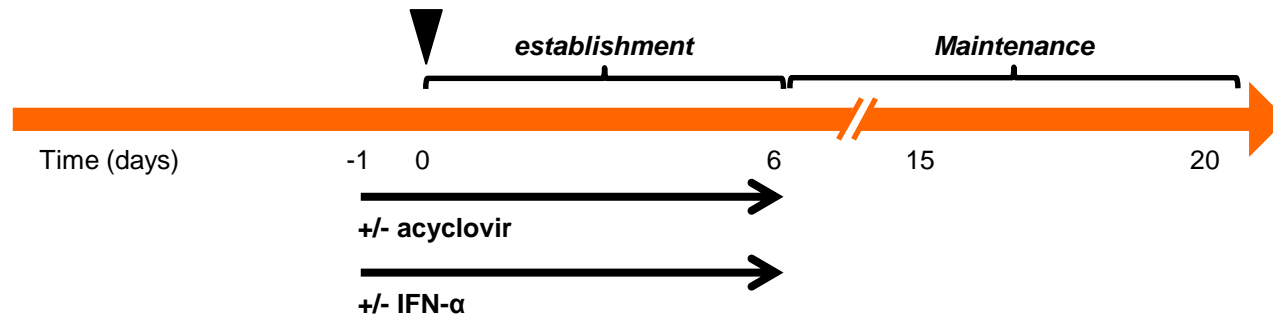
*** recurrent shedding, ** some shedding, * no unassisted shedding

Differentiation of human embryonic stem cells into neurons



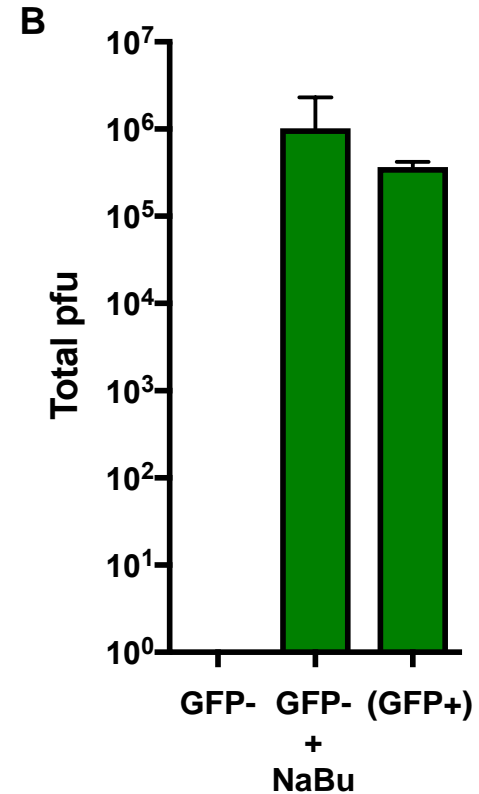
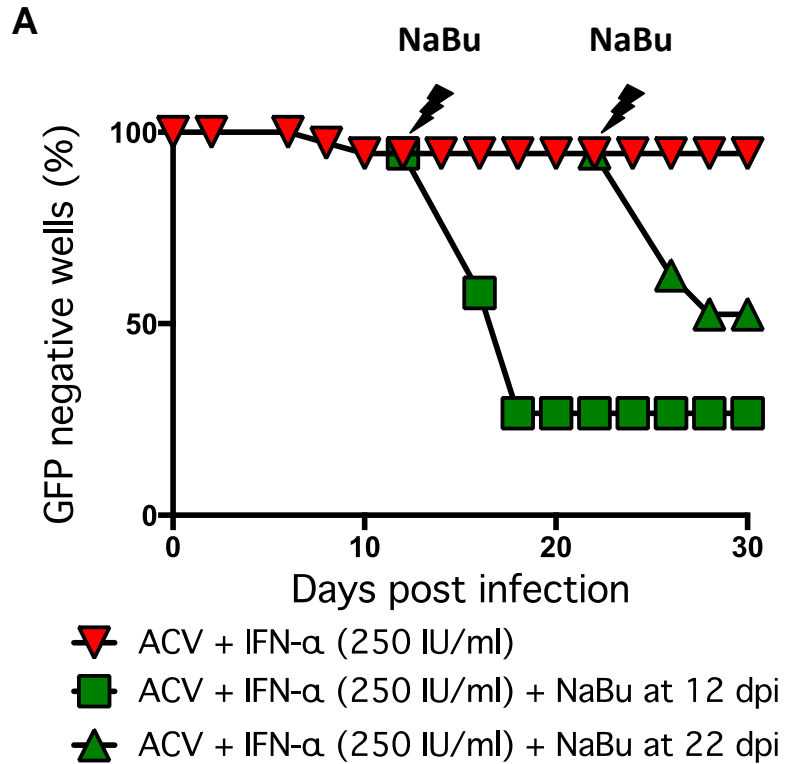
Establishment of non-productive infections in human neurons using IFN- α in combination with ACV and low pfu/neuron ratio

Infect with HSV-1 GFP-Us11



- ACV
- ACV + IFN- α (50 IU/ml)
- ▲ ACV + IFN- α (125 IU/ml)
- ▼ ACV + IFN- α (250 IU/ml)
- IFN- α (250 IU/ml)
- ▲ ACV + IFN- α (125 IU/ml)
- ▼ ACV + IFN- α (250 IU/ml)

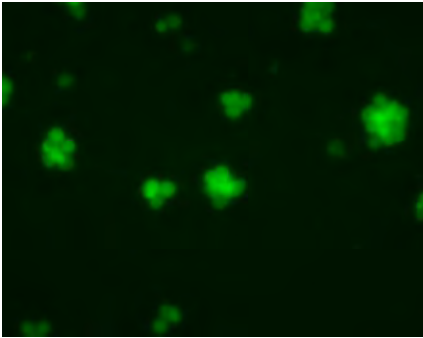
Non-productive infections reactivate to produce infectious virus



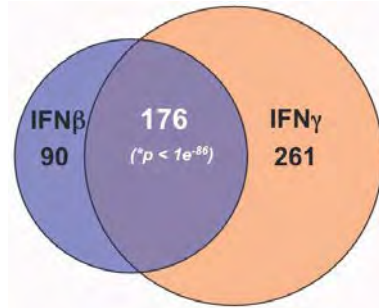
Take Home Lessons

- **HSV latency in cultured neurons requires sustained NGF signaling and cap-dependent protein synthesis.**
- **Reactivation involves 2 mechanistically distinct steps, initiated by activation of JNK and release of epigenetic suppression.**
- **Requirement for Phase I ('animation') makes sense for HSV in light of the minimal viral protein expression during latency.**
- **Controlled localization of VP16 and HCF-1 provides another additional host control.**
- **IFN suppresses reactivation in a neurons by blocking Phase I transcription.**
- **Phase I products such as ICP0 antagonize IFN, allowing reactivation.**
- **Latency can now be modeled in human neurons, the appropriate species matched environment for this human virus.**

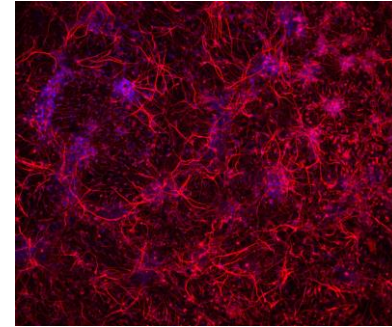
Acknowledgements



Ju Youn Kim



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Aram Modrek
Dimitris Placantonakis**



Ian Mohr



Moses Chao



