HSV-1 Activates the DNA Damage Response through ICP4

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Laboratory of Jane Clifford

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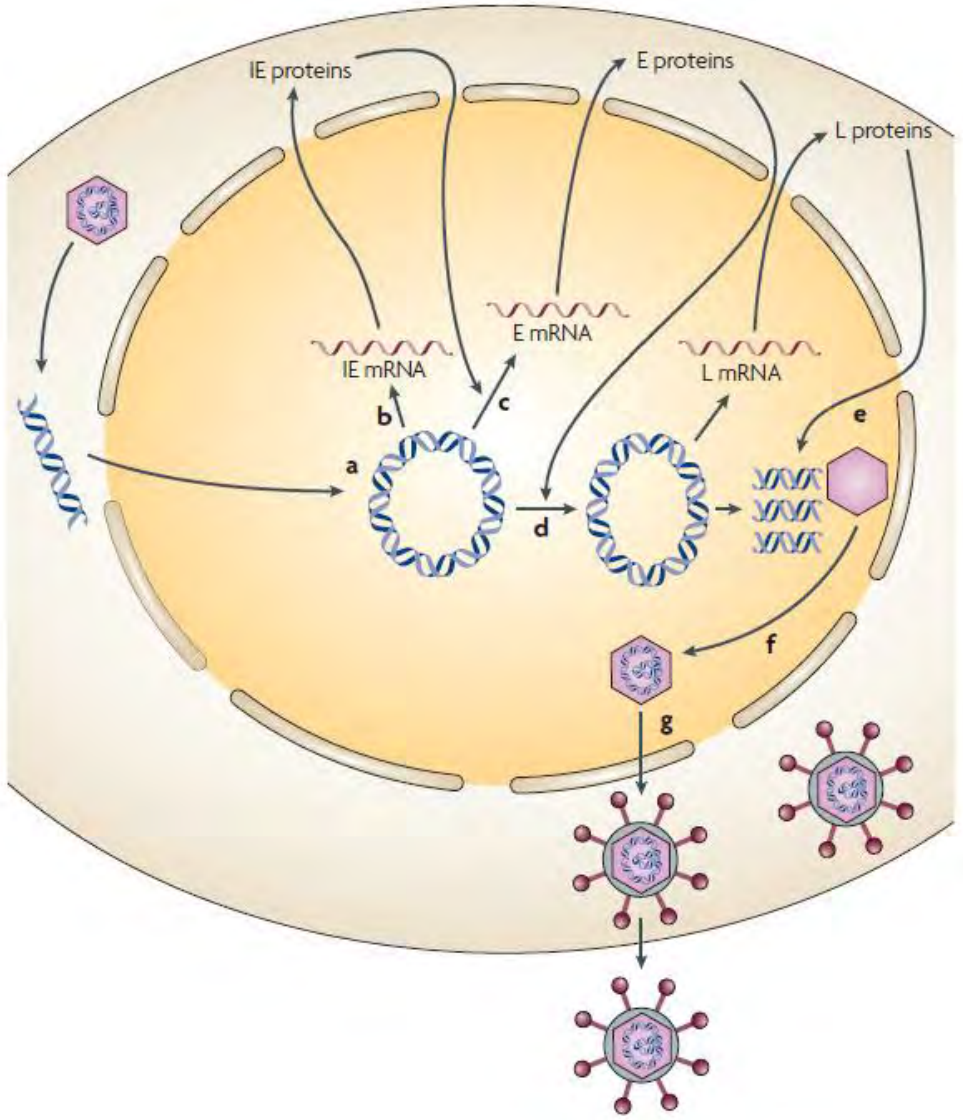
HSV-1 Replication



Knipe D, et al. *Nat Rev Microbiologyr* (2008), 6(3):211-21

DNA Damage Response

Singe Strand DNA Exposure
(Replication Arrest)

ATR

Downstream Functions

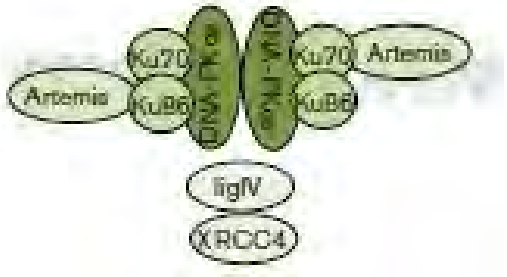
- Stabilization of single strand DNA
- Checkpoint activation/Cell cycle stalling
- Apoptosis

Double Strand Break
(Radiation, Genotoxins)

ATM

- DNA Repair: Homologous Recombination
- Checkpoint activation/Cell cycle stalling
- Apoptosis

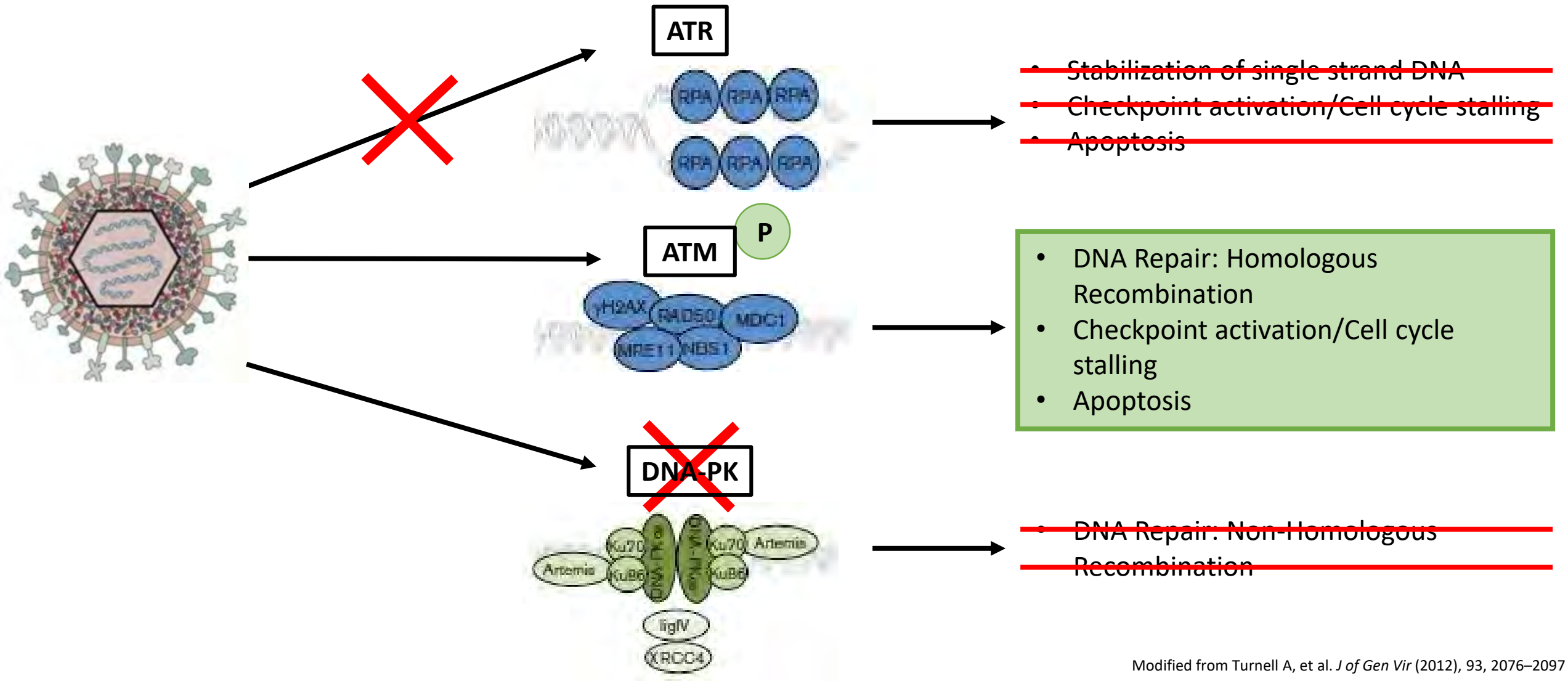
DNA-PK



- DNA Repair: Non-Homologous Recombination

Modified from Turnell A, et al. *J of Gen Vir* (2012), 93, 2076–2097

HSV-1 Infection and the DNA Damage Response

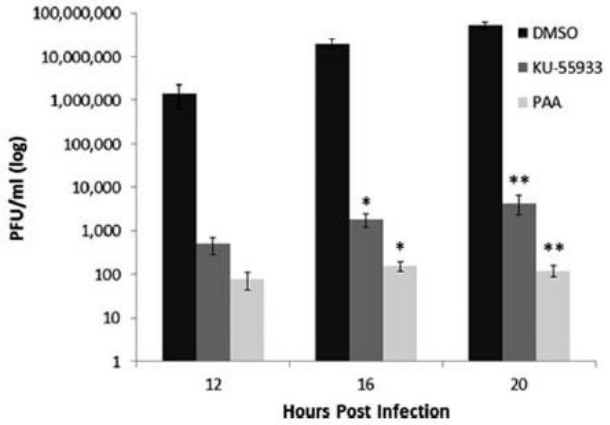


Modified from Turnell A, et al. *J of Gen Vir* (2012), 93, 2076–2097

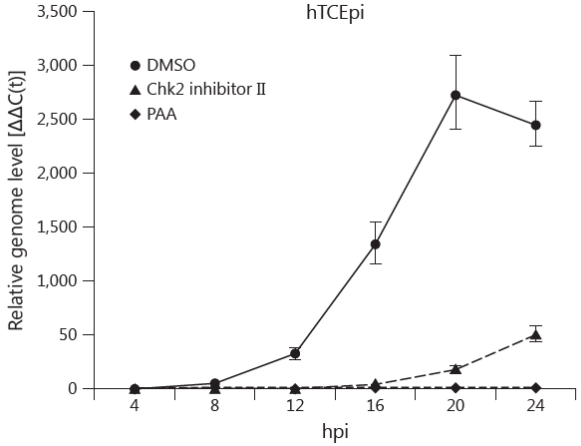
Inhibition of ATM or Chk2 Suppresses HSV-1 in Models of Herpes Simplex Keratitis and Herpes Simplex Labialis

Herpes Simplex Keratitis

In Vitro

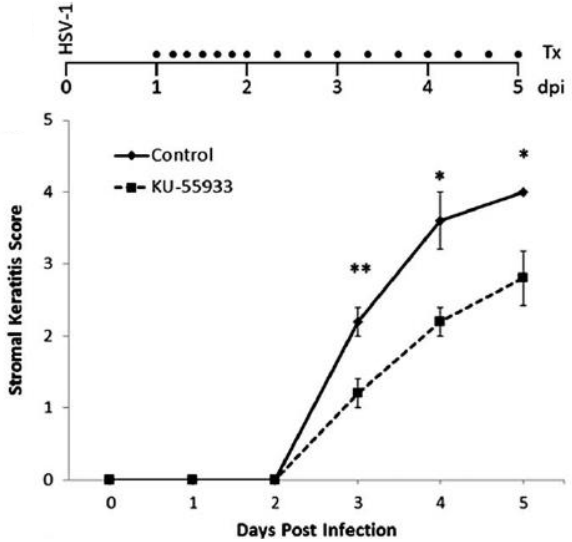


Alekseev et al. 2014 *IOVS*; 55(2):706-15.

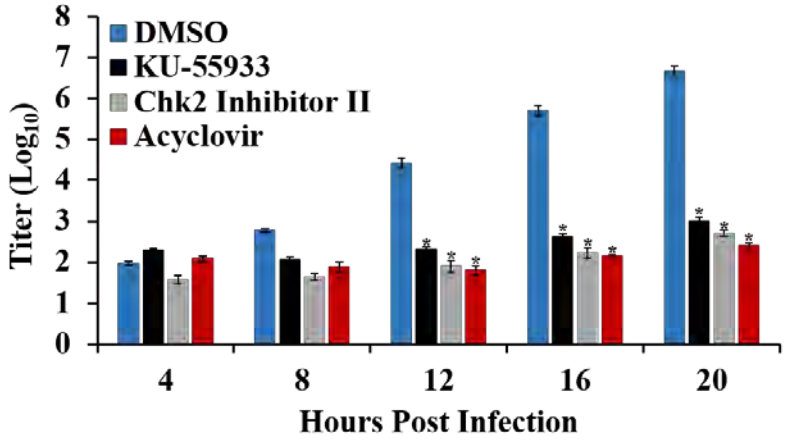


Alekseev et al. 2015 *Opth Res.;* 53(2):55-64

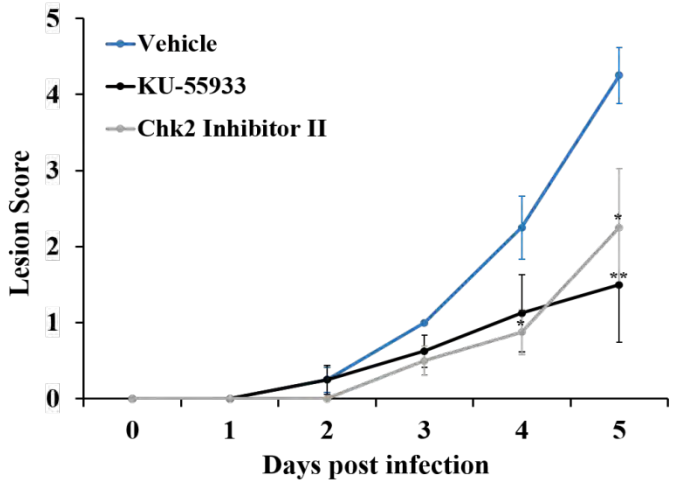
In Vivo



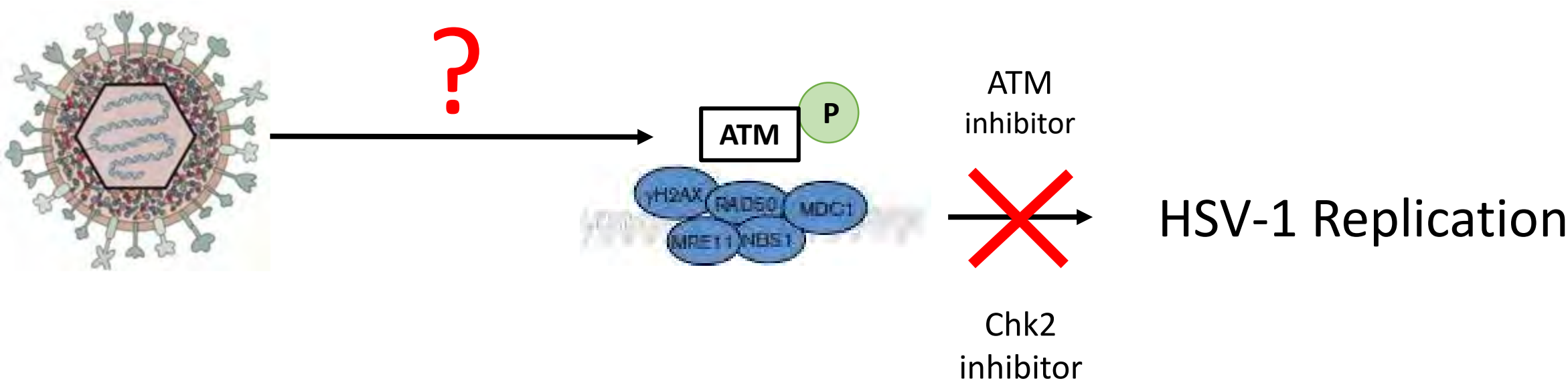
Herpes Simplex Labialis



Donegan et al. 2017 *In Submission*



Research Direction

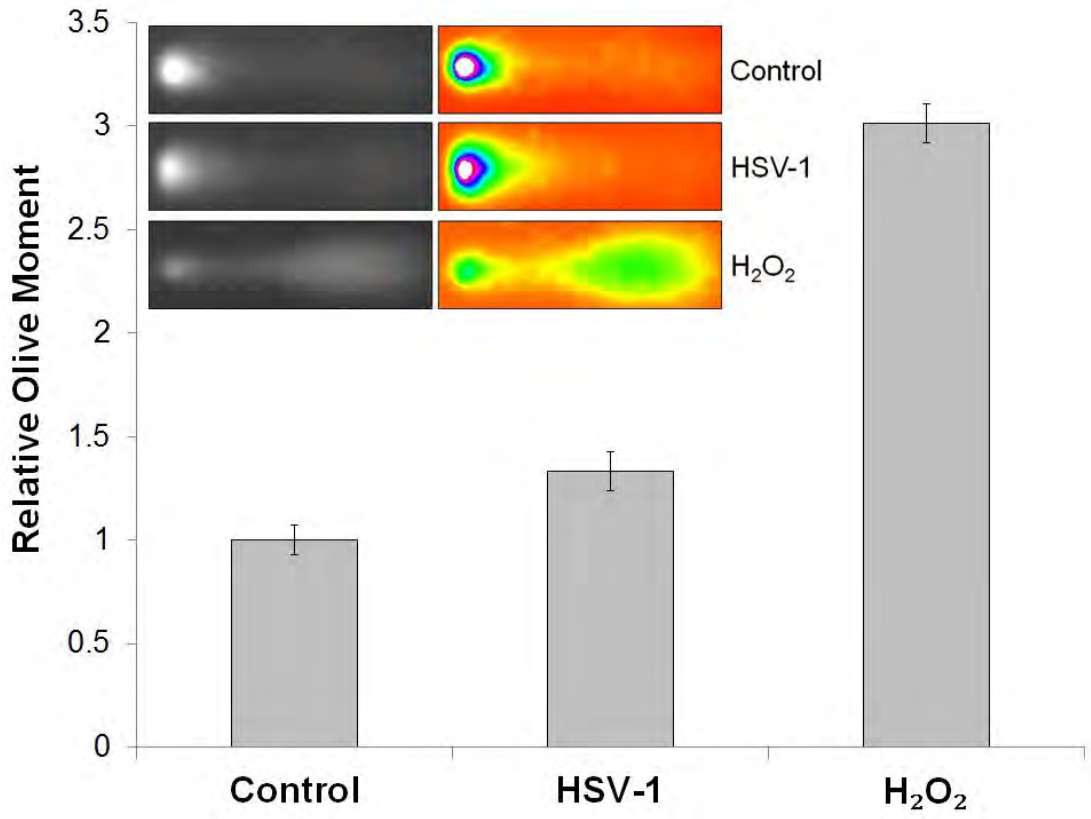


Elucidate the mechanism of ATM activation in response to HSV-1 infection

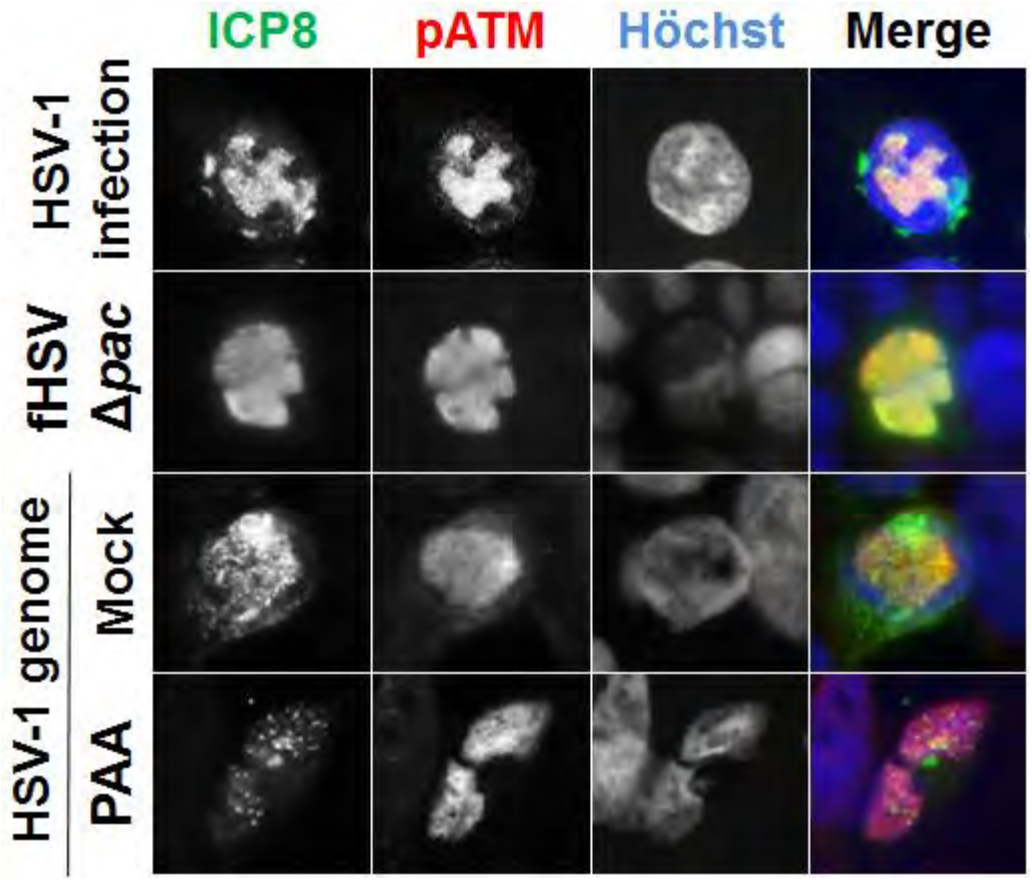
Prevalent Hypotheses

- ATM is activated in response to HSV-1 infection due to damaged DNA.
- Nicks and gaps in the incoming HSV-1 genome activates ATM.
- The linear ends of the incoming HSV-1 genome activates ATM.
- HSV1 activates ATM through the action of a viral gene product (e.g. ICP0)

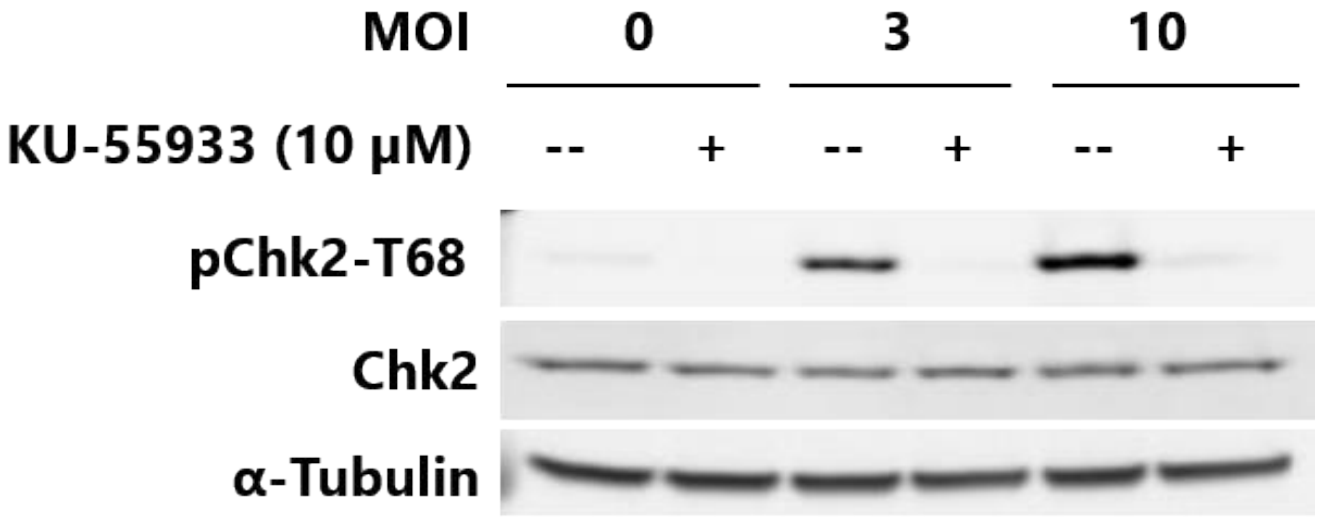
ATM Activation is not Caused by Damaged DNA



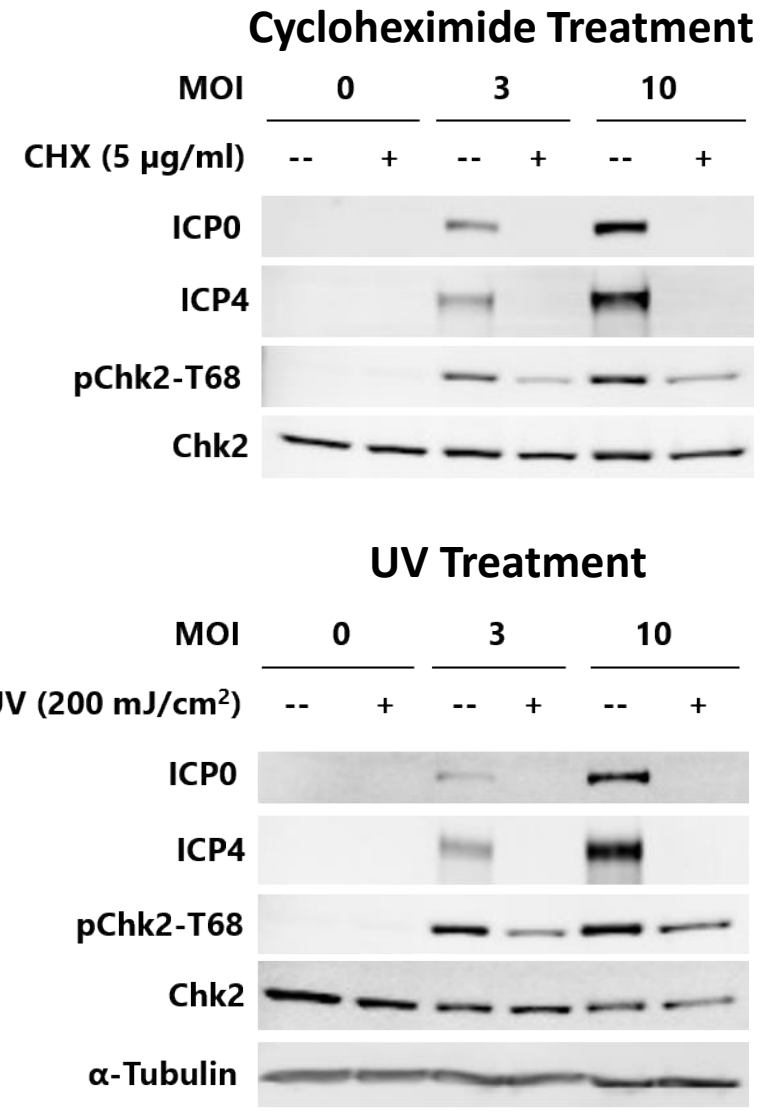
Neither Linear Ends nor Nicks/Gaps in the Viral Genome are Required for ATM Activation

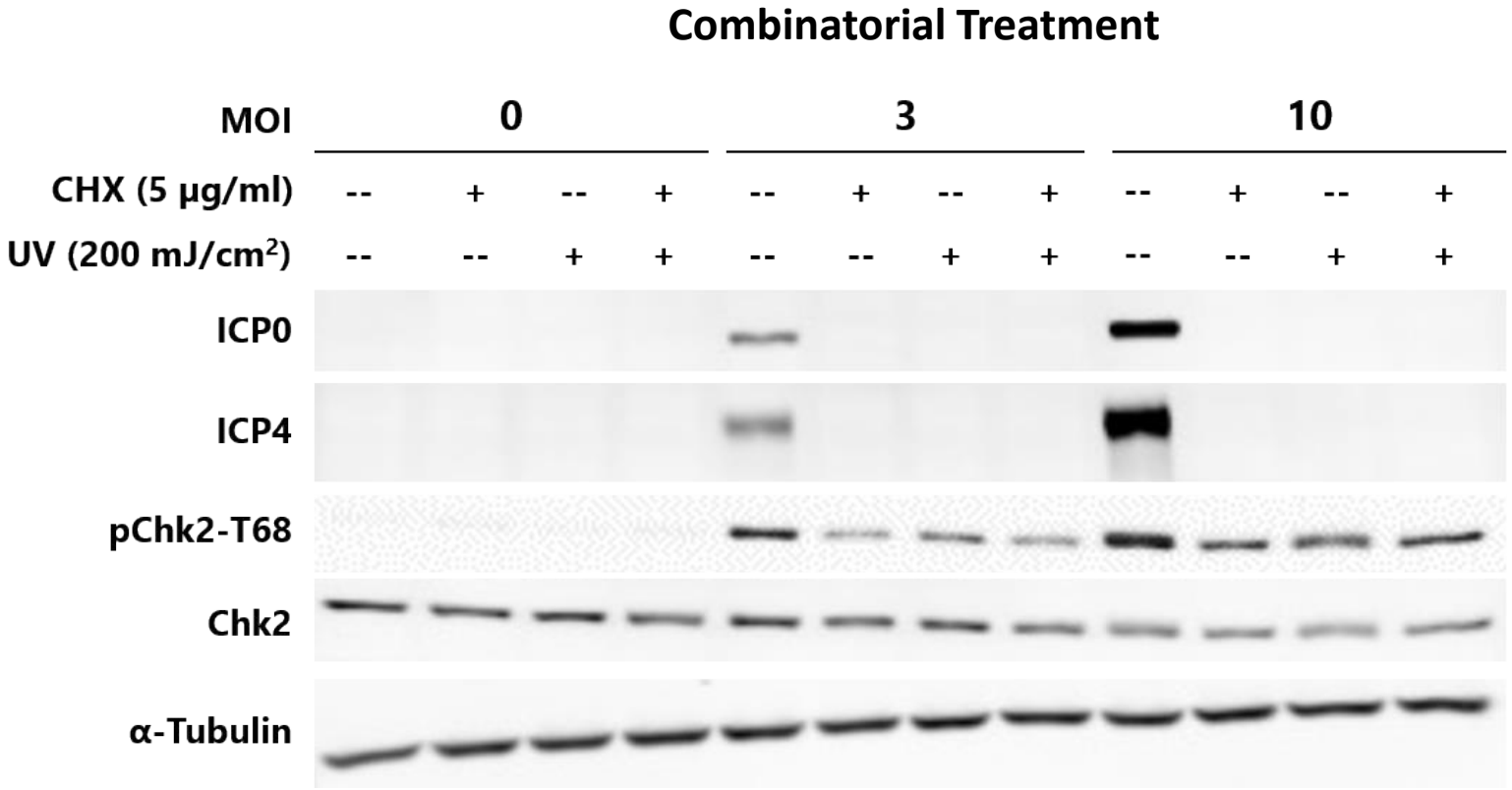


ATM Activation is Reliably Measured Through Chk2 Phosphorylation at 1 hpi



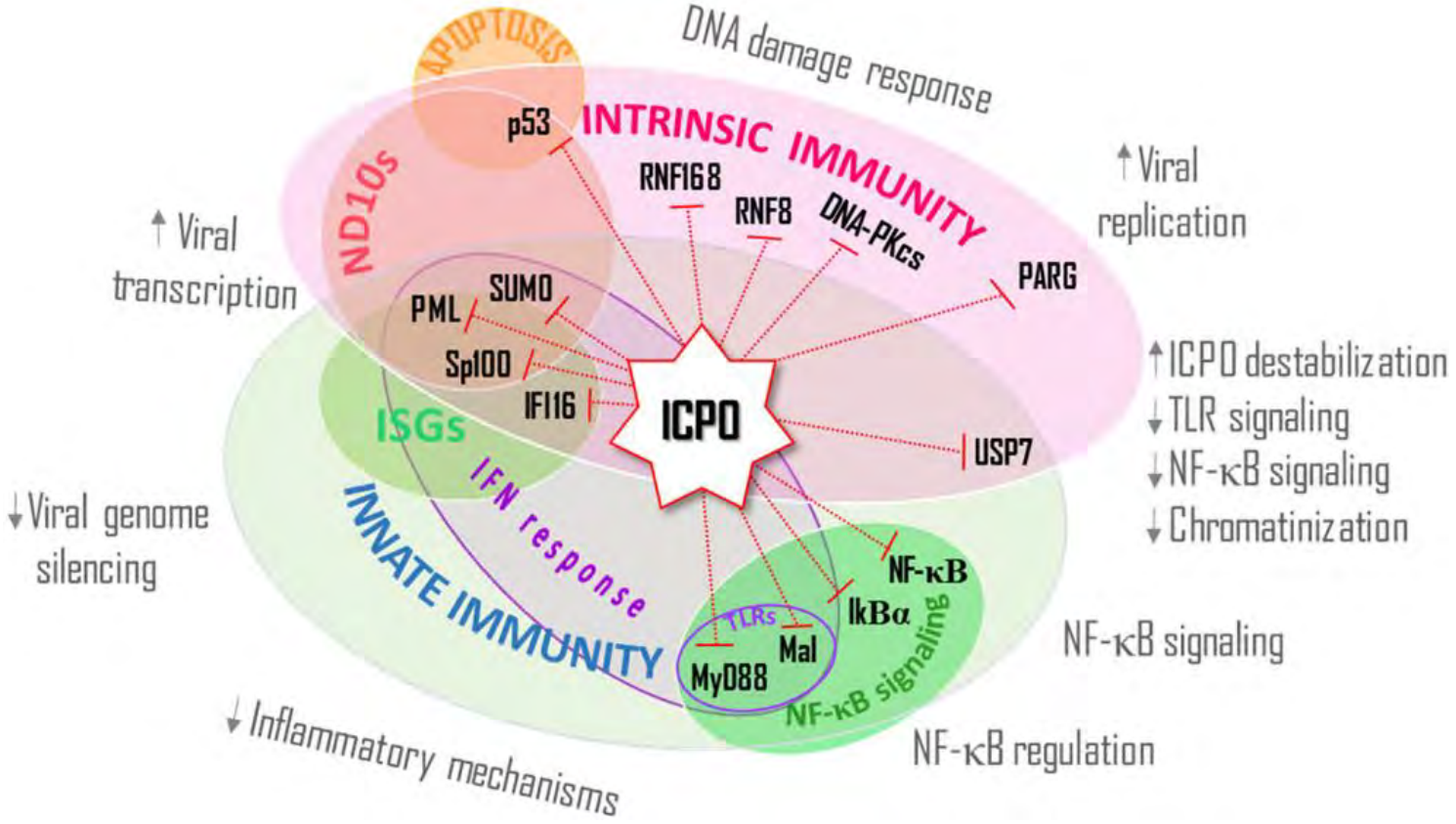
De *novo* Synthesis of HSV-1 Factors is Required to Achieve Full Levels of ATM Activation





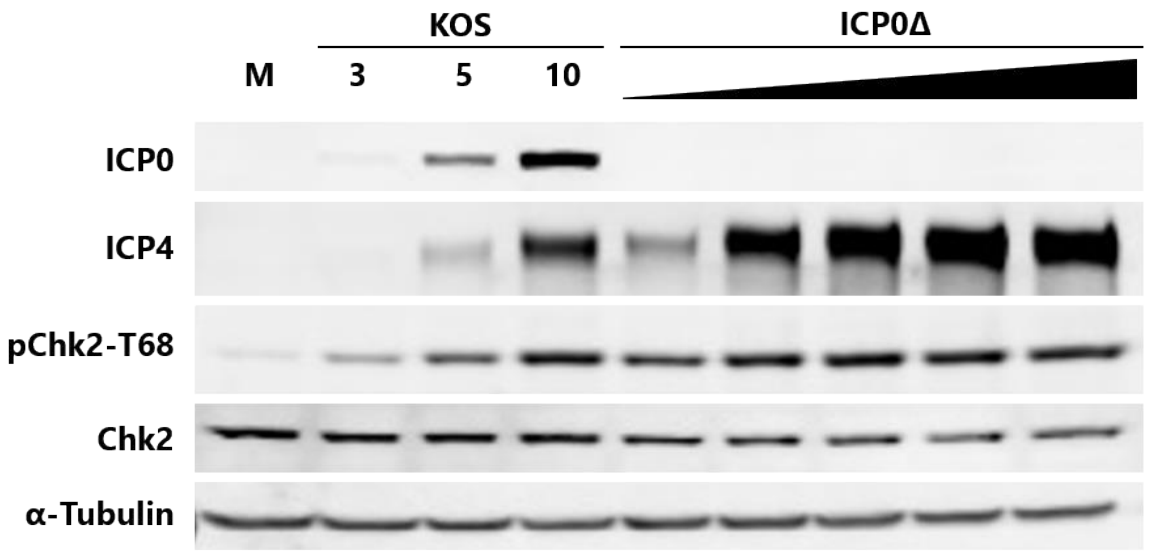
Manipulation of the Cellular Environment by ICP0

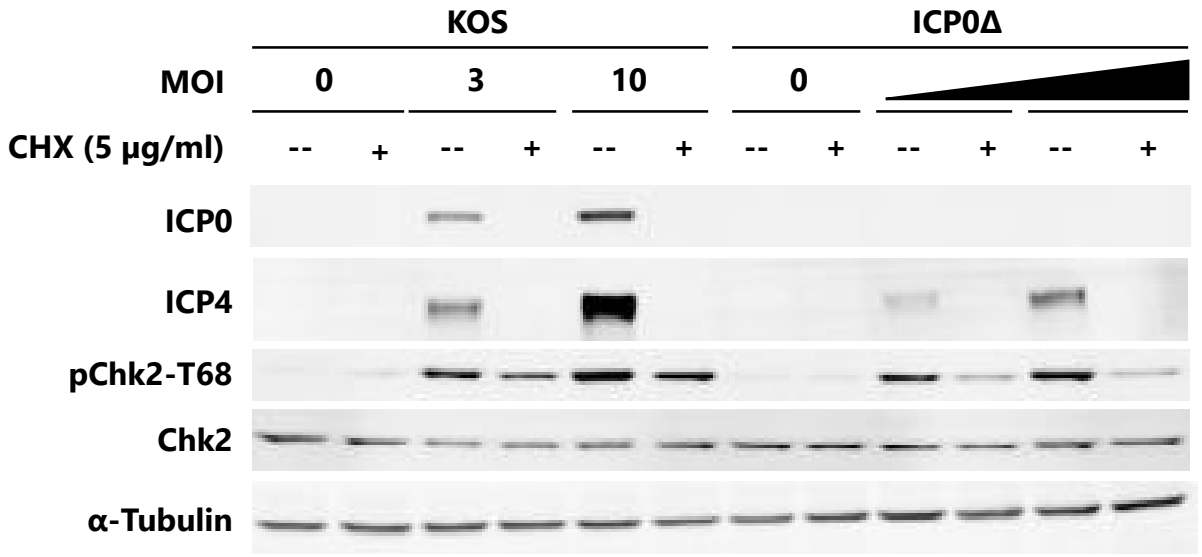
Ubiquitin ligase that targets cellular factors for degradation



Lanfranca *Cells* 2014, *3*(2), 438-454

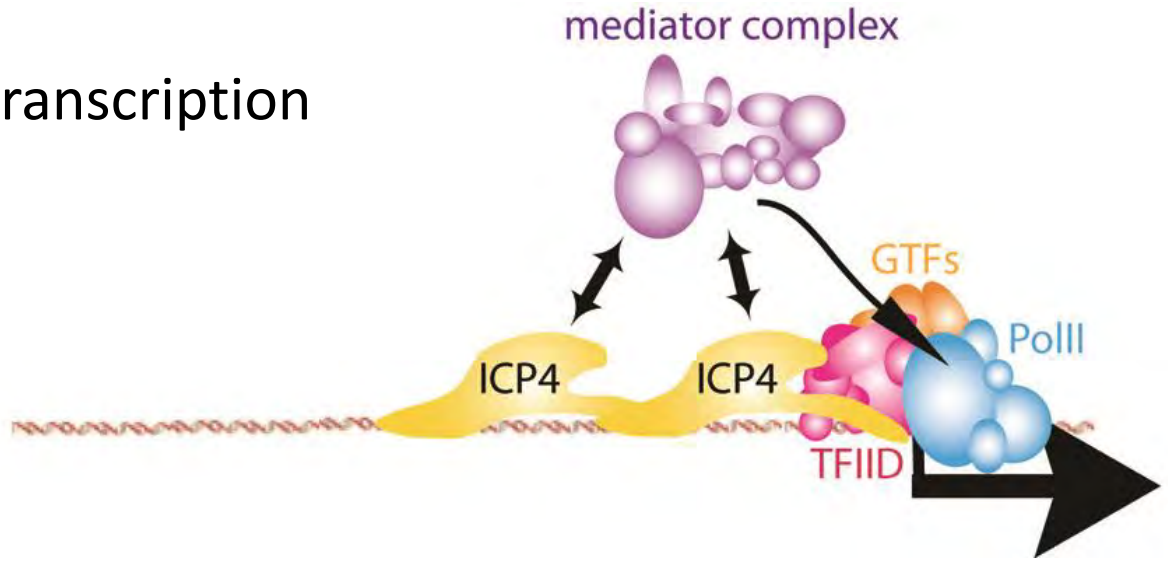
ICP0 is not Required for ATM Activation





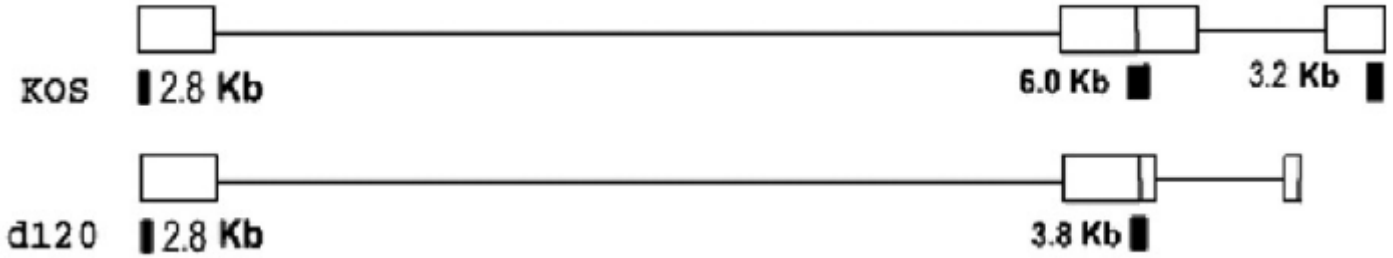
ICP4 and the DDR

- Multiple functions
  - Transactivates Early and Late gene transcription
  - Multimerizes on the HSV-1 genome
- Required for Circularization



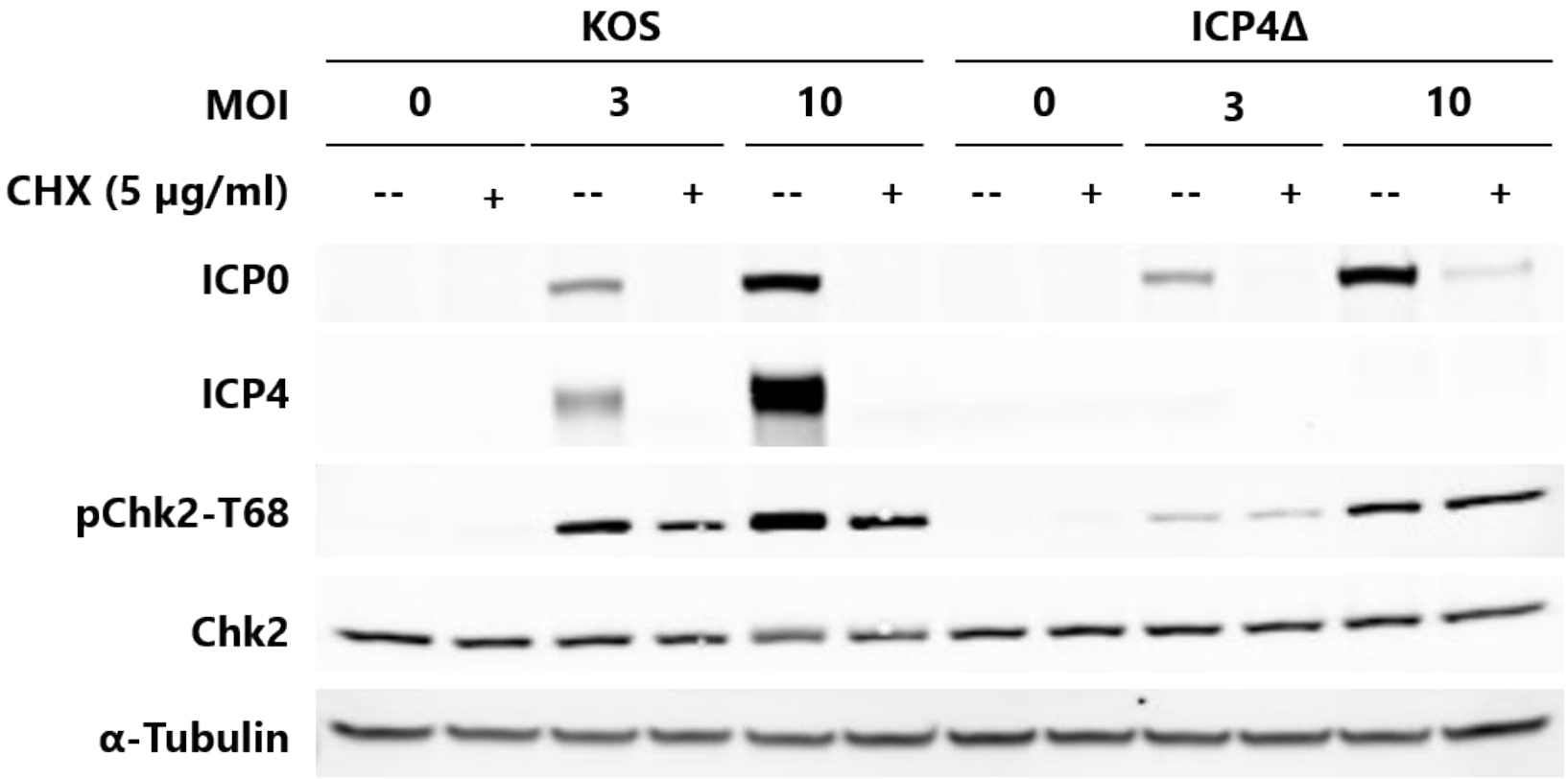
Neal DeLuca, University of Pittsburgh

ICP4Δ Mutant Virus

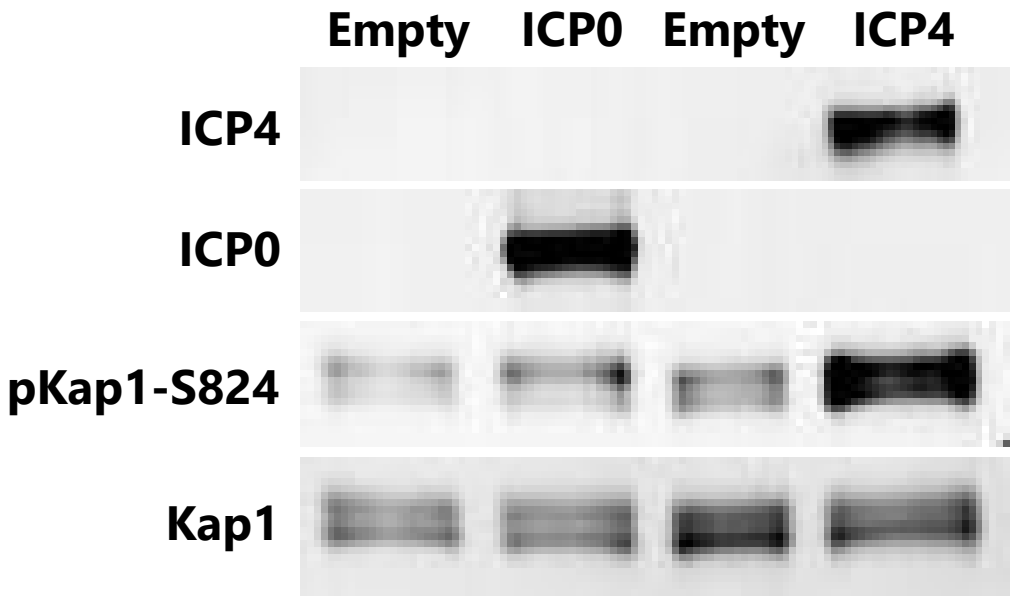


4.1-kb deletion in both ICP4 loci

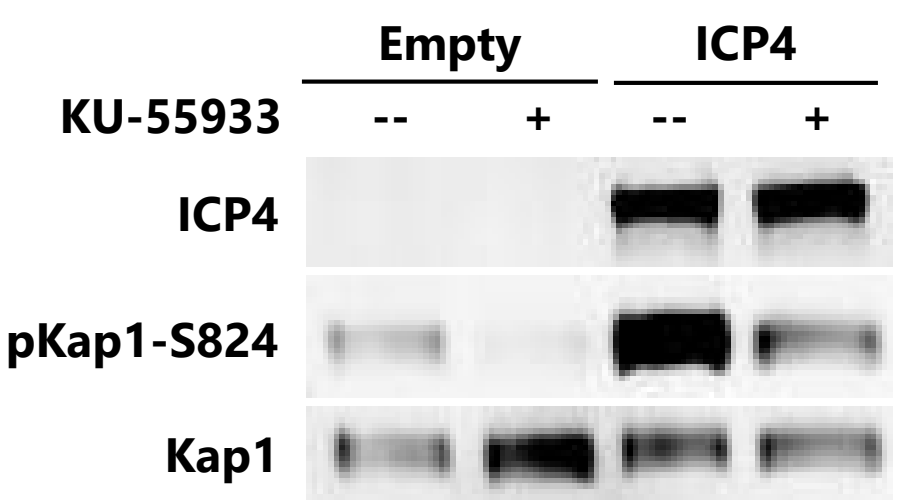
Full Activation of ATM Through *De Novo* Synthesis Requires ICP4

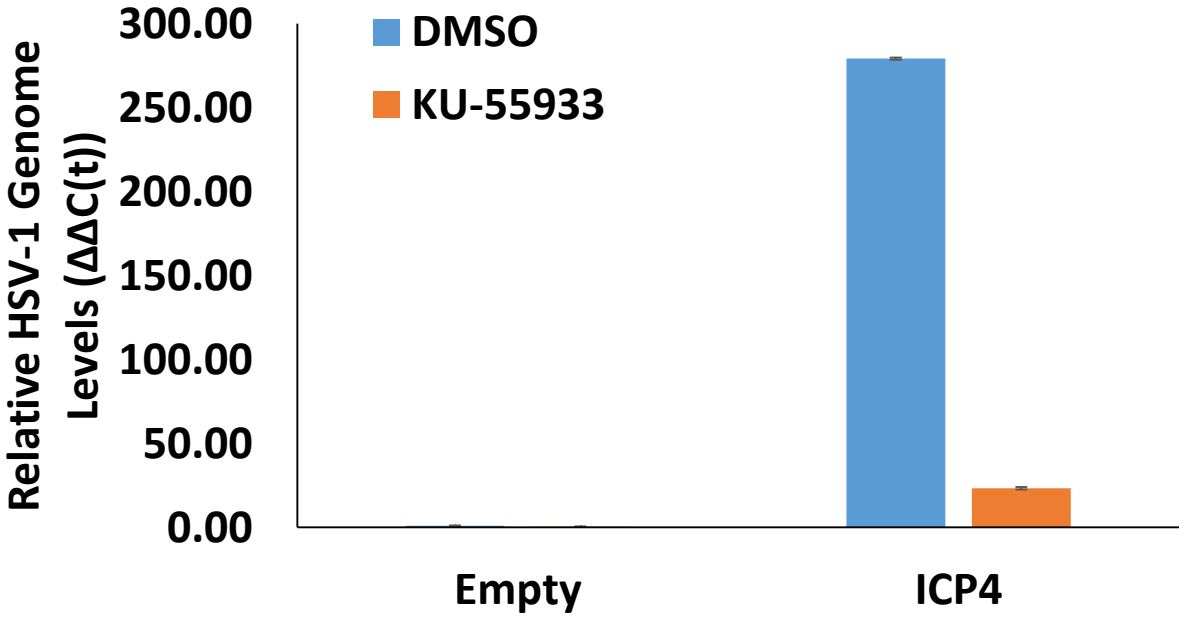


ICP4 Activates ATM



ICP4 Rescue of ICP4Δ Virus is dependent on ATM Activation





DREXEL UNIVERSITY College of Medicine

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- Rebecca Yao
- Alexandra Hunt

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- Thomas Kristie
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- Yosef Shiloh
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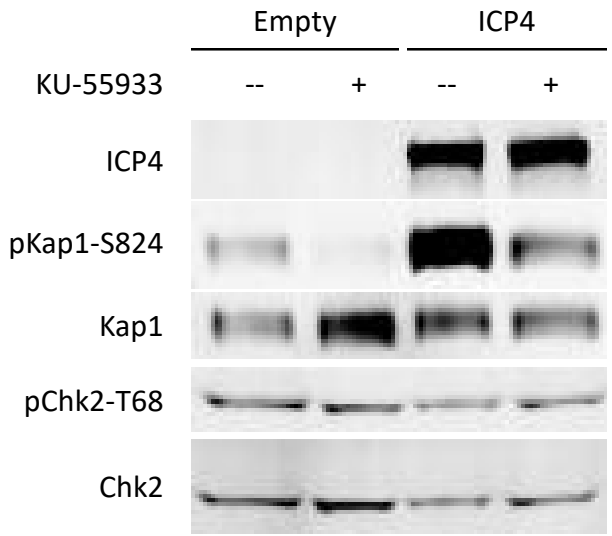
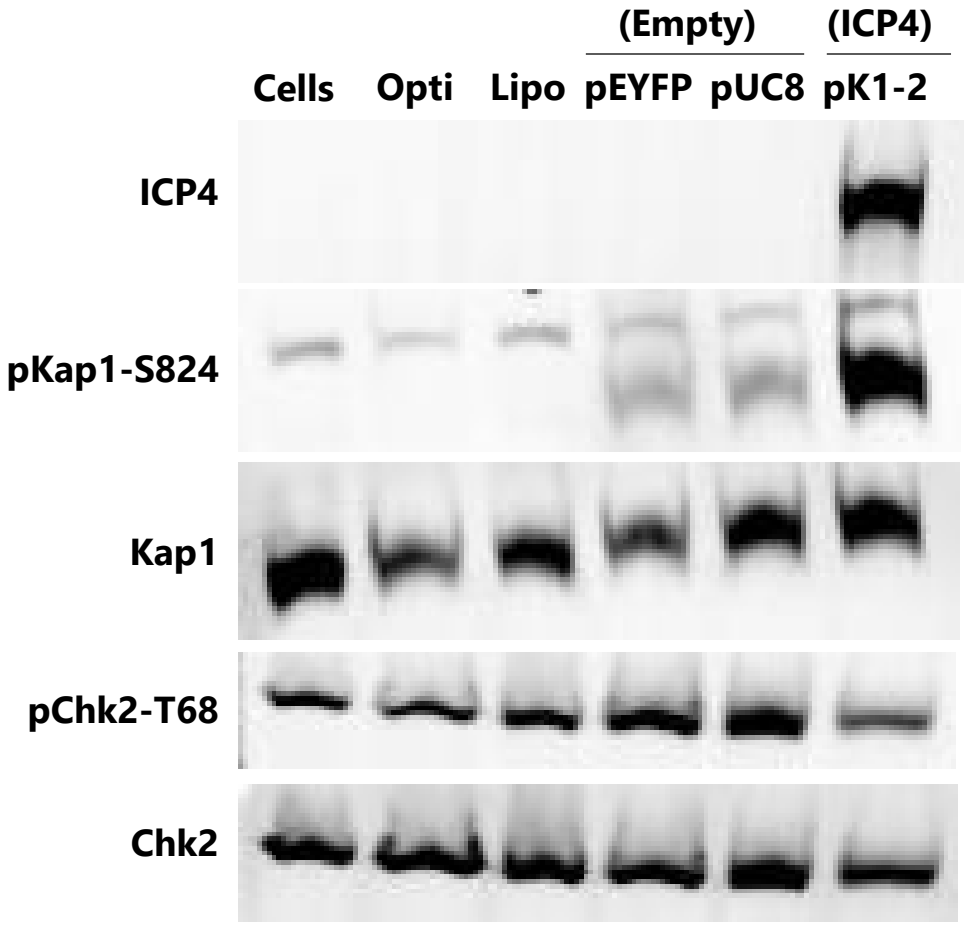
Extra Slides

5-10-17 HEK293 Transfection with KU-55933

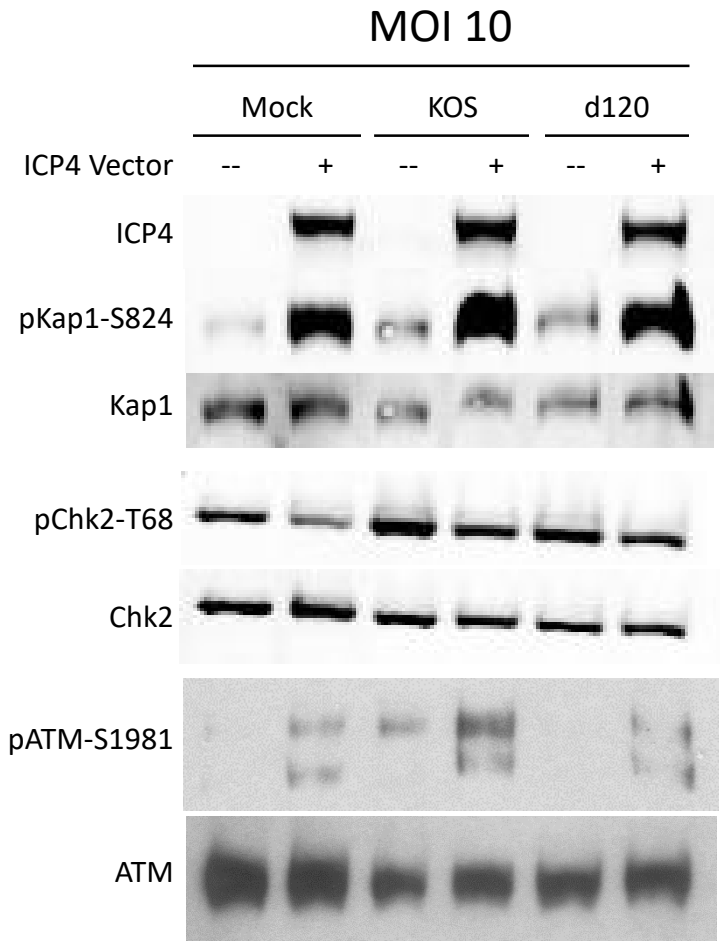


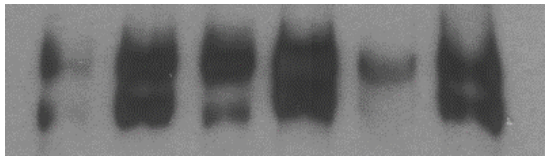
Measuring the effect of different transfection reagents on ATM activation



- High levels of basal pChk2-T68
- Adding Optimem or Lipofectamine
  - No impact on pKap1 or pChk2
- Addition of DNA (empty vectors)
  - Low levels of pKap1
  - Increased levels of pChk2
- ICP4 Expression
  - Large increase in pKap1
  - Decrease in pChk2

Autophosphorylation of ATM Follows pKap1 Pattern





(Longer exposure)

HEK293 Cells require ATM activation in a similar manner as hTCEpi and OKF6 cells