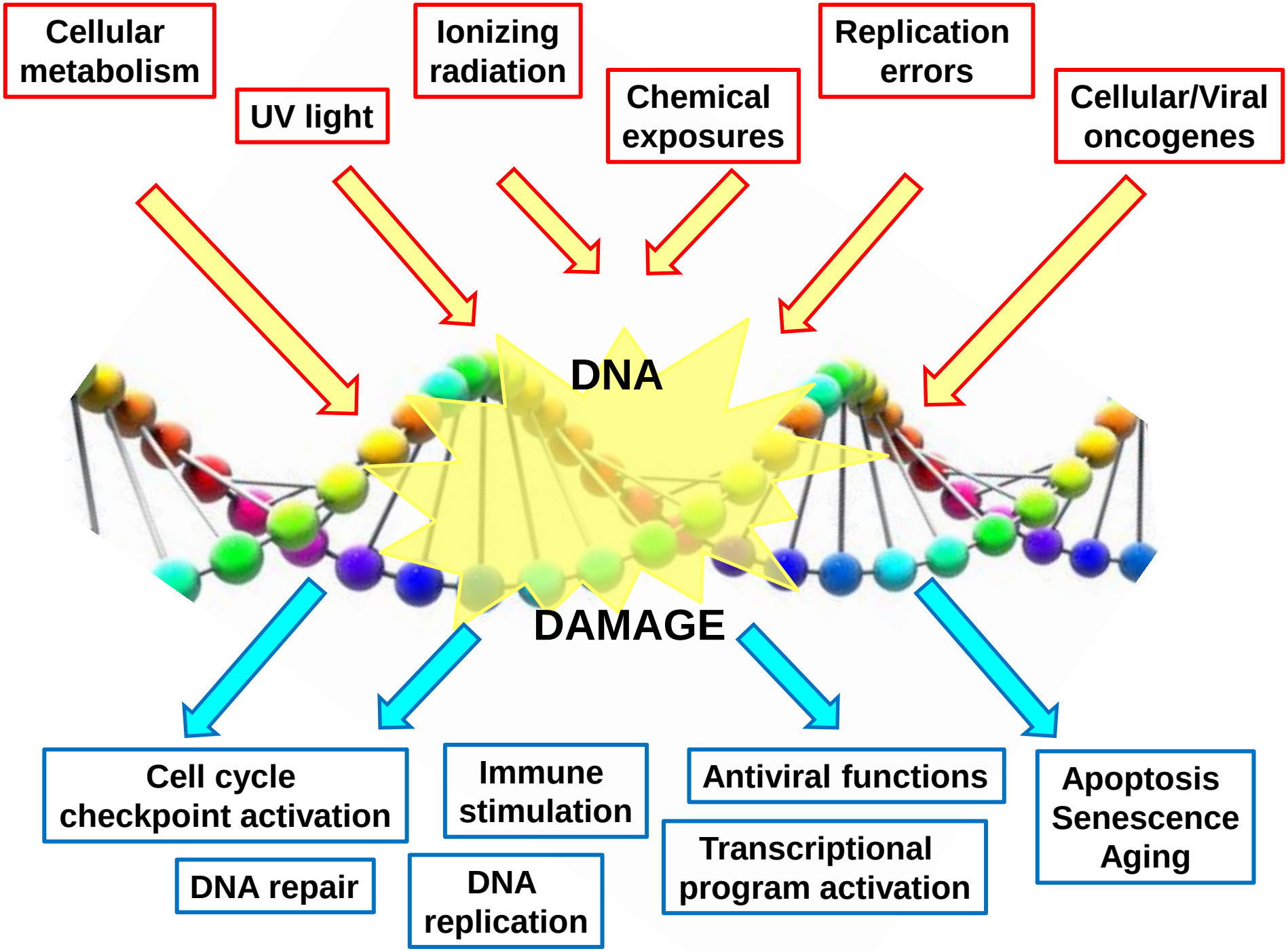
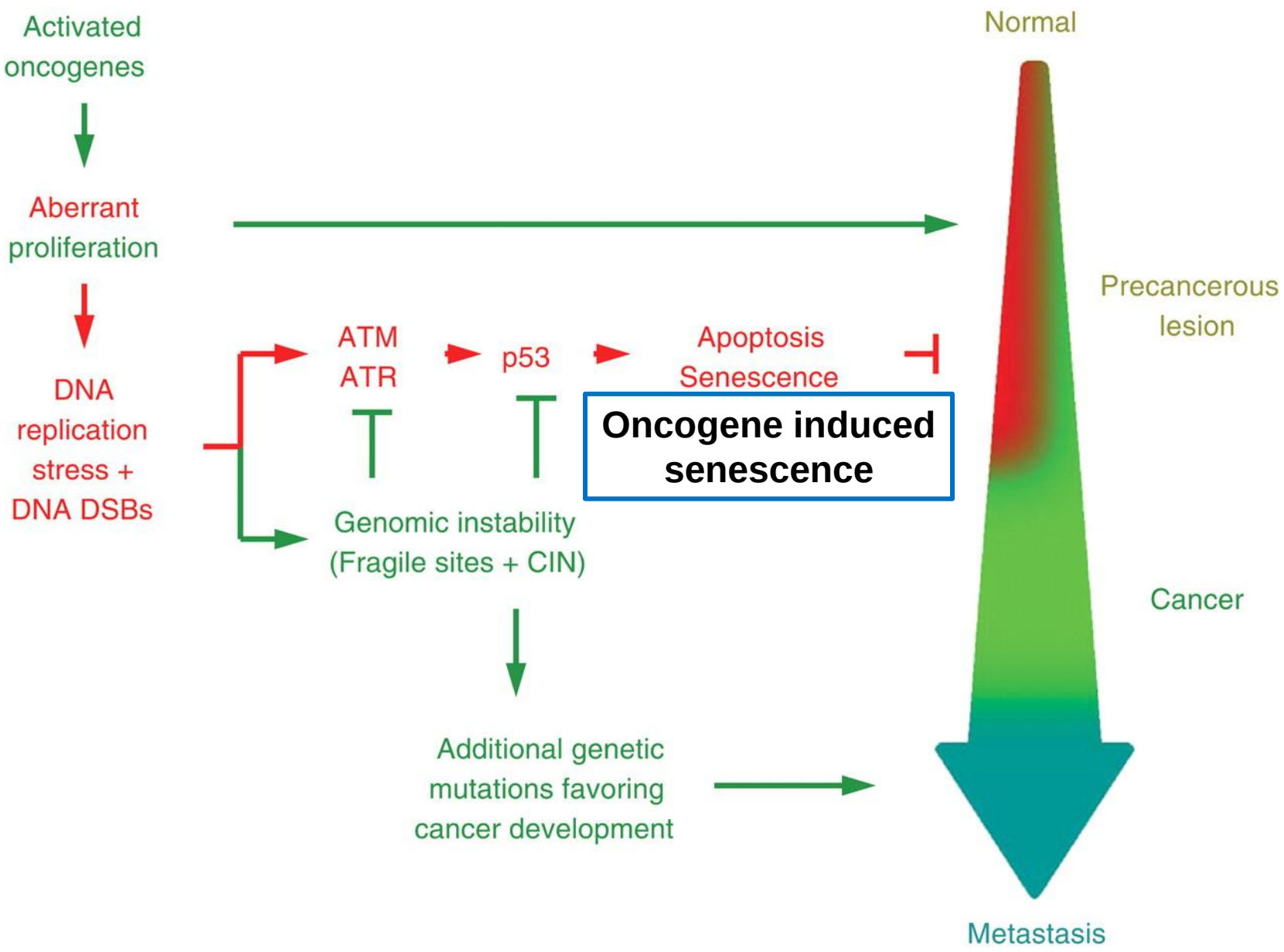
Stony Brook Medicine

Stony Brook Children's

Insights into DNA damage response signaling from an oncovirus

Sumita Bhaduri-McIntosh, M.D., Ph.D.
Pediatric Infectious Diseases &
Molecular Genetics and Microbiology


Cellular metabolism
UV light
Ionizing radiation
Chemical exposures
Replication errors
Cellular/Viral oncogenes
DNA
DAMAGE
Cell cycle checkpoint activation
DNA repair
DNA replication
Immune stimulation
Antiviral functions
Transcriptional program activation
Apoptosis
Senescence
Aging


Activated oncogenes
Aberrant proliferation
DNA replication stress + DNA DSBs
ATM
ATR
p53
Apoptosis
Senescence
Oncogene induced senescence
Genomic instability (Fragile sites + CIN)
Additional genetic mutations favoring cancer development
Normal
Precancerous lesion
Cancer
Metastasis

Circumventing DDR during oncogene-driven cell proliferation

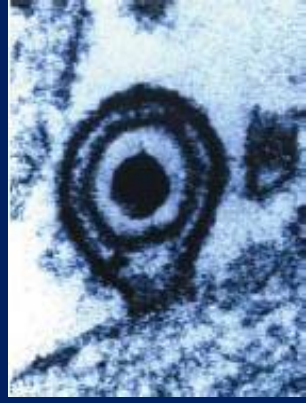
- For oncogenes to successfully drive cell proliferation and cancer, DNA damage checkpoint barriers need to be overridden

- Mechanisms of DDR attenuation: inherited versus sporadic cancers

- STAT3 – transcription factor
  - – frequently activated by growth factors and cytokines
  - – prosurvival, angiogenesis, metastasis
  - – constitutively active in many human cancers
  - – precise contribution to tumorigenesis unknown

Does/Can STAT3 mediate DDR suppression during the initial rounds of oncogene-driven cell proliferation?

Epstein-Barr virus (EBV)

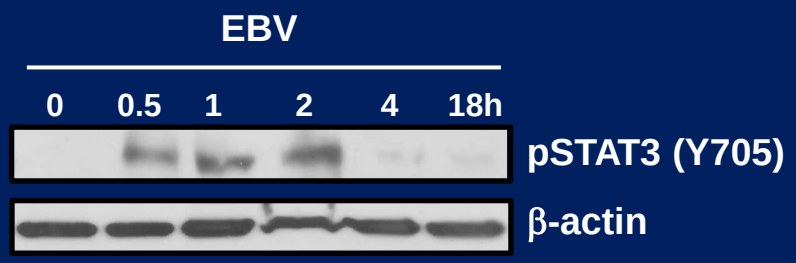


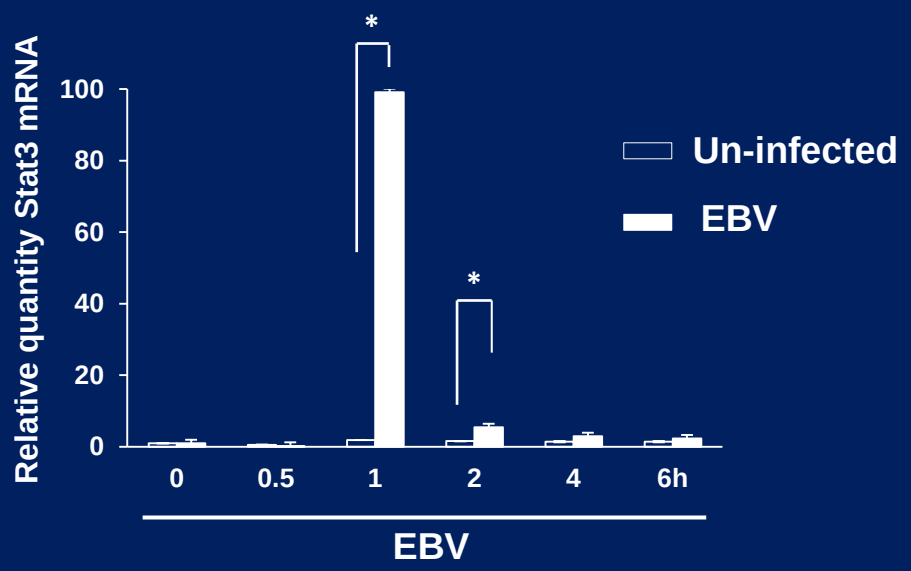
- Post-transplant lymphomas, AIDS-lymphomas, Burkitt lymphoma, NPC, Hodgkin lymphomas, T/NK cell lymphomas, gastric carcinomas

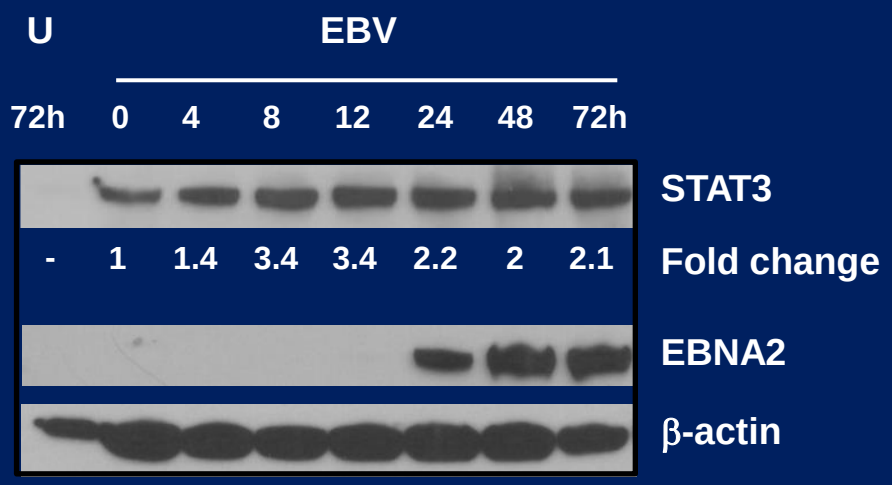
- Oncogenic human gammaherpesvirus
  - – co-evolved with humans, infects nearly everyone
  - – excellent means to uncover fundamental cellular processes
  - – encodes several oncoproteins, drives cellular DNA replication, causes cellular DNA damage
  - – post-transplant lymphomas develop within weeks of infection
  - – primary human B cells + EBV……cell lines in 2 weeks
  - – STAT3 constitutively active in EBV-related cancers

Does STAT3 mediate DDR suppression during the initial rounds of EBV oncogene-driven cell proliferation?

EBV infection results in early STAT3 activation and increased expression



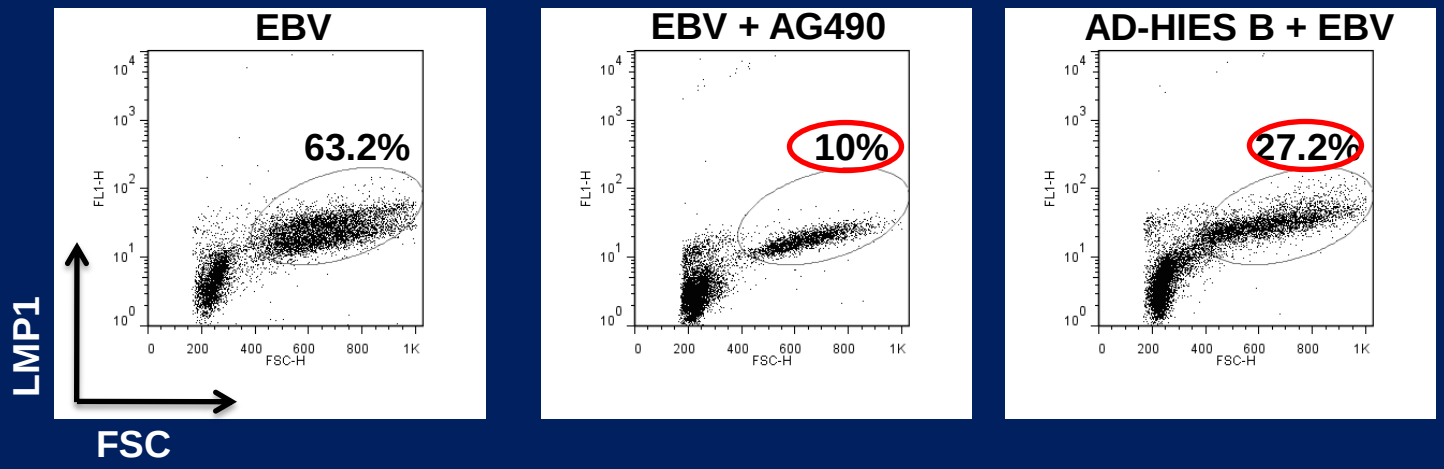


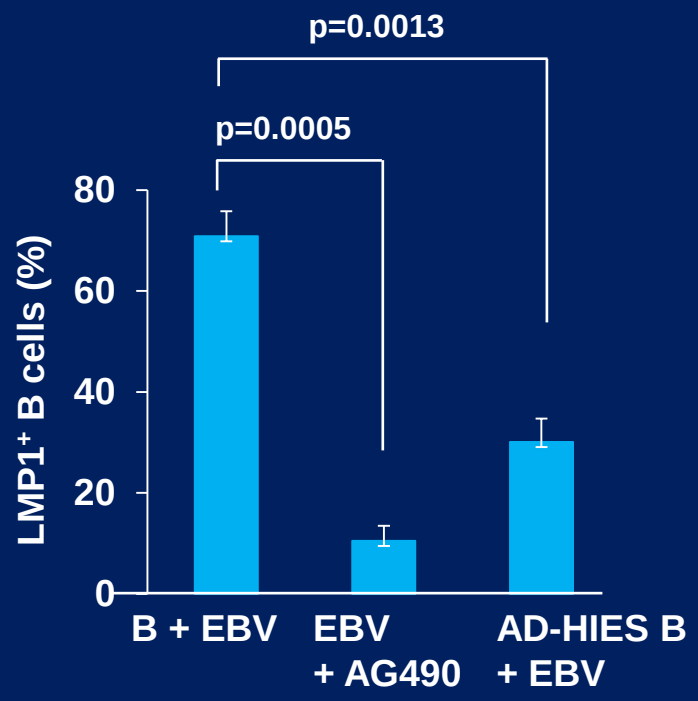


Koganti et al., J. Virology, 2014

Inhibition of STAT3 results in fewer EBV⁺ cells

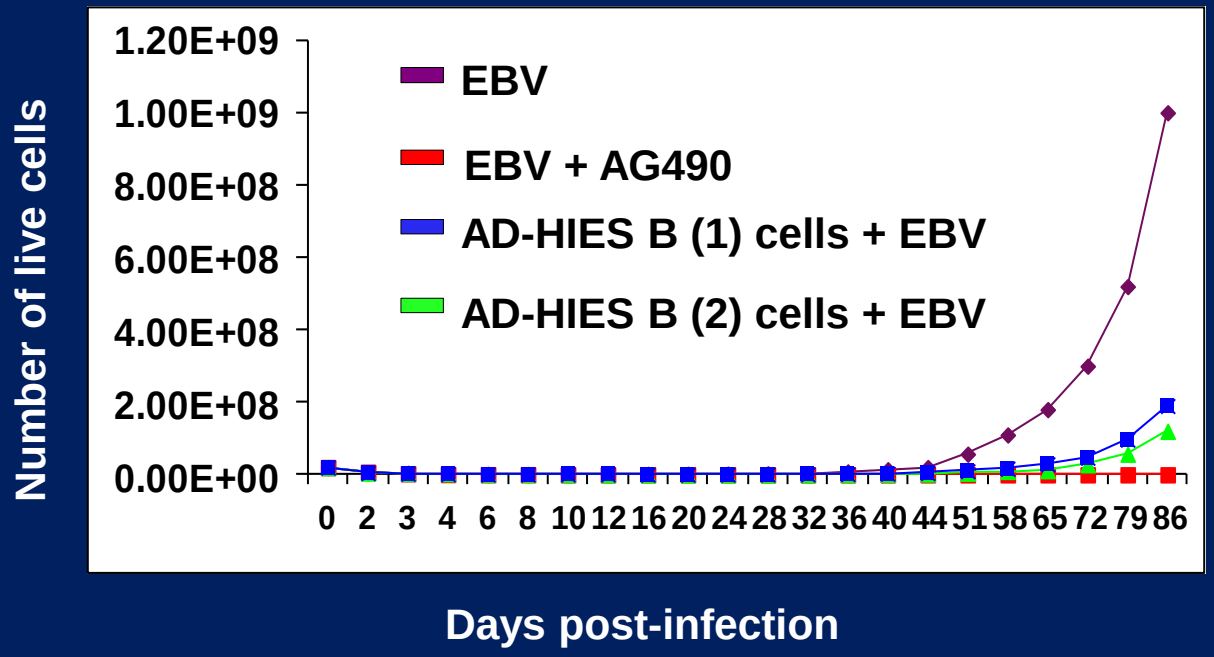
Day 4 Culture





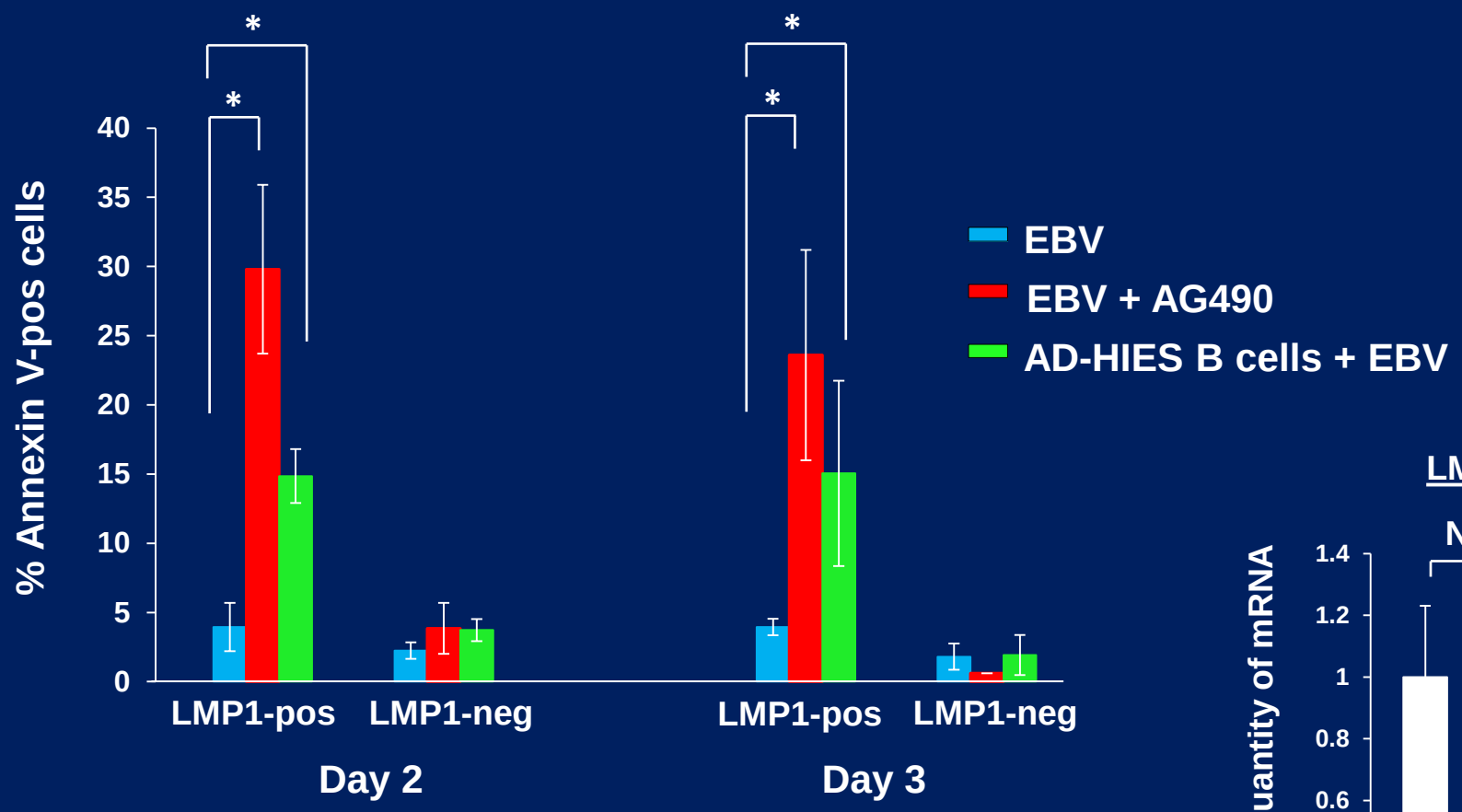
Koganti et al., J. Virology, 2014

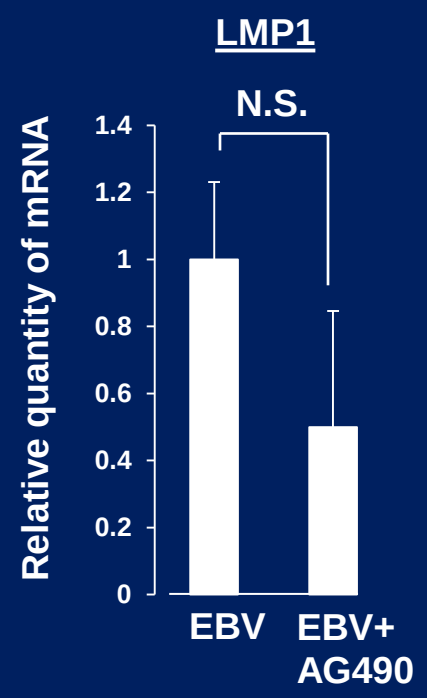
STAT3 is necessary for outgrowth of cell lines



Koganti et al., J. Virology, 2014

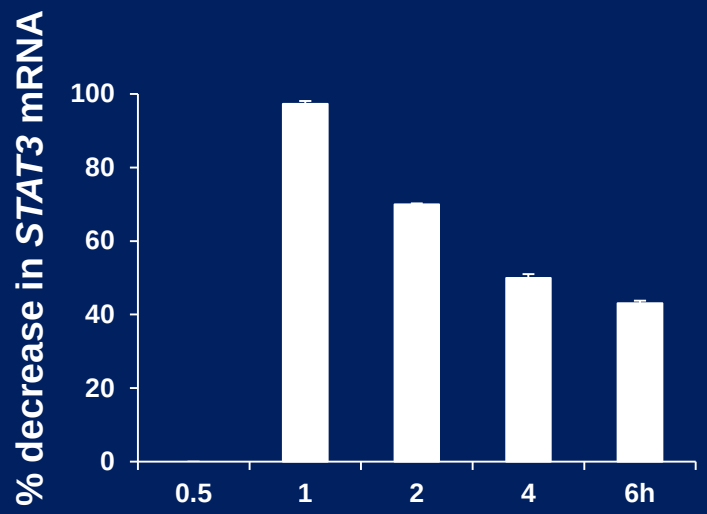
STAT3 is necessary for survival of LMP1^{+} cells

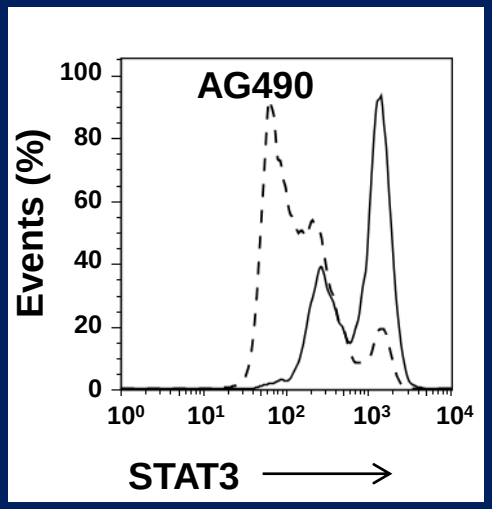


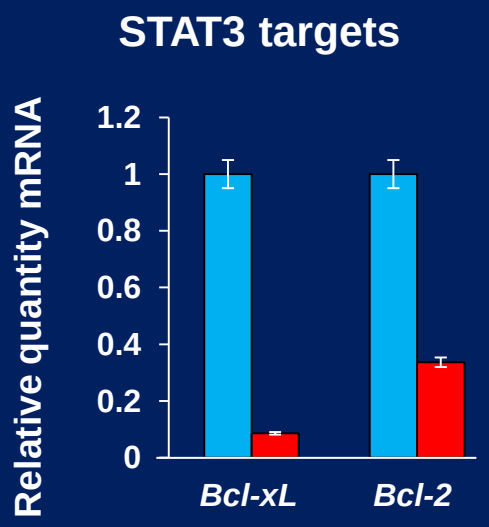


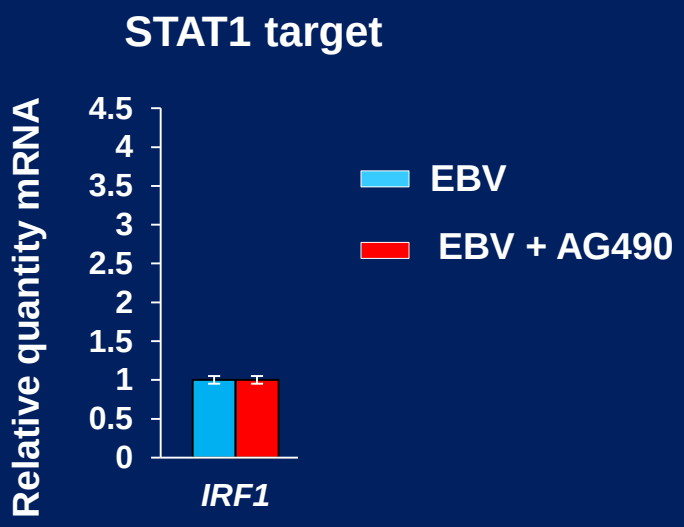
Koganti et al., J. Virology, 2014

AG490 inhibits STAT3 and its targets including pro-survival genes



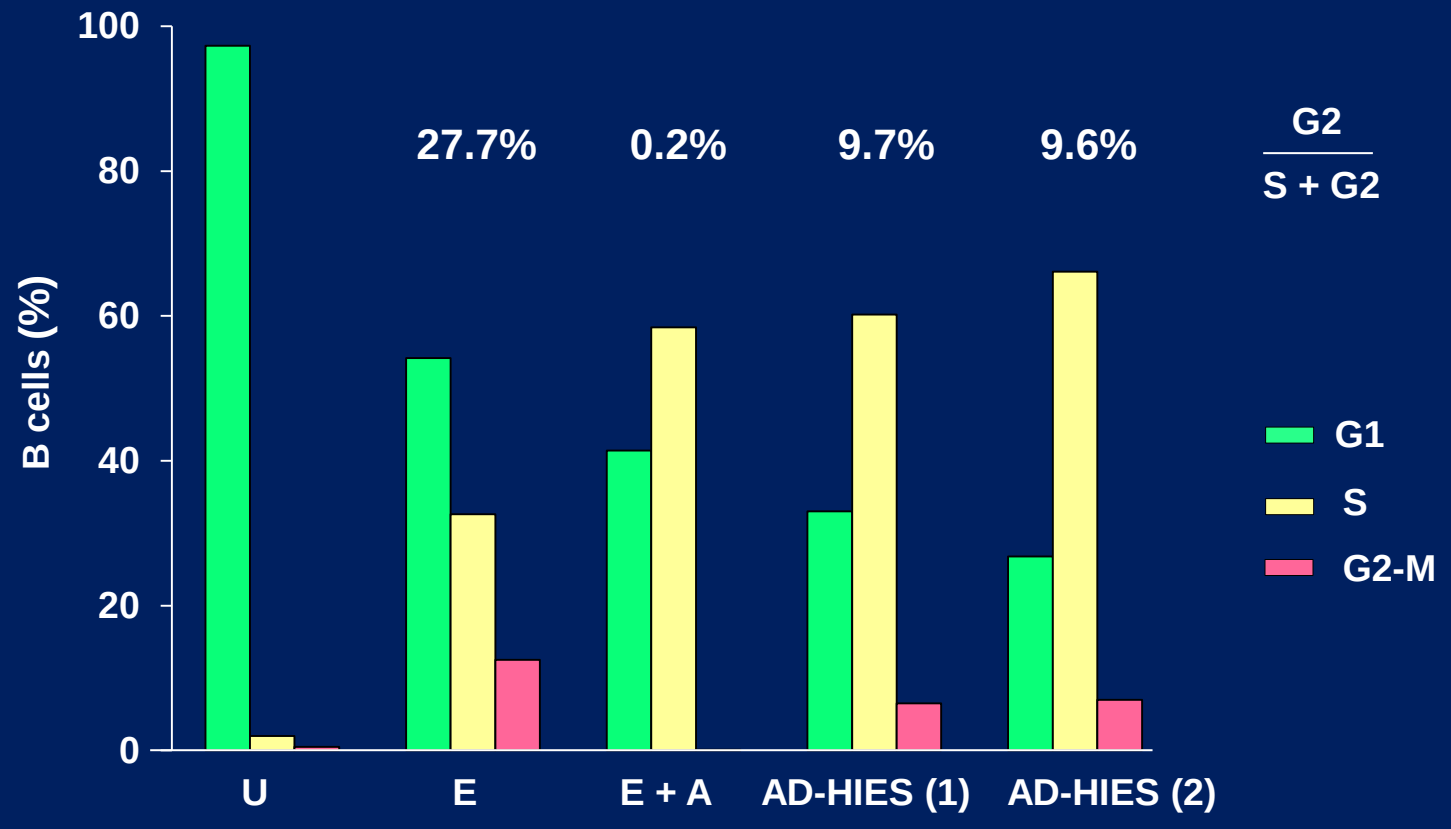






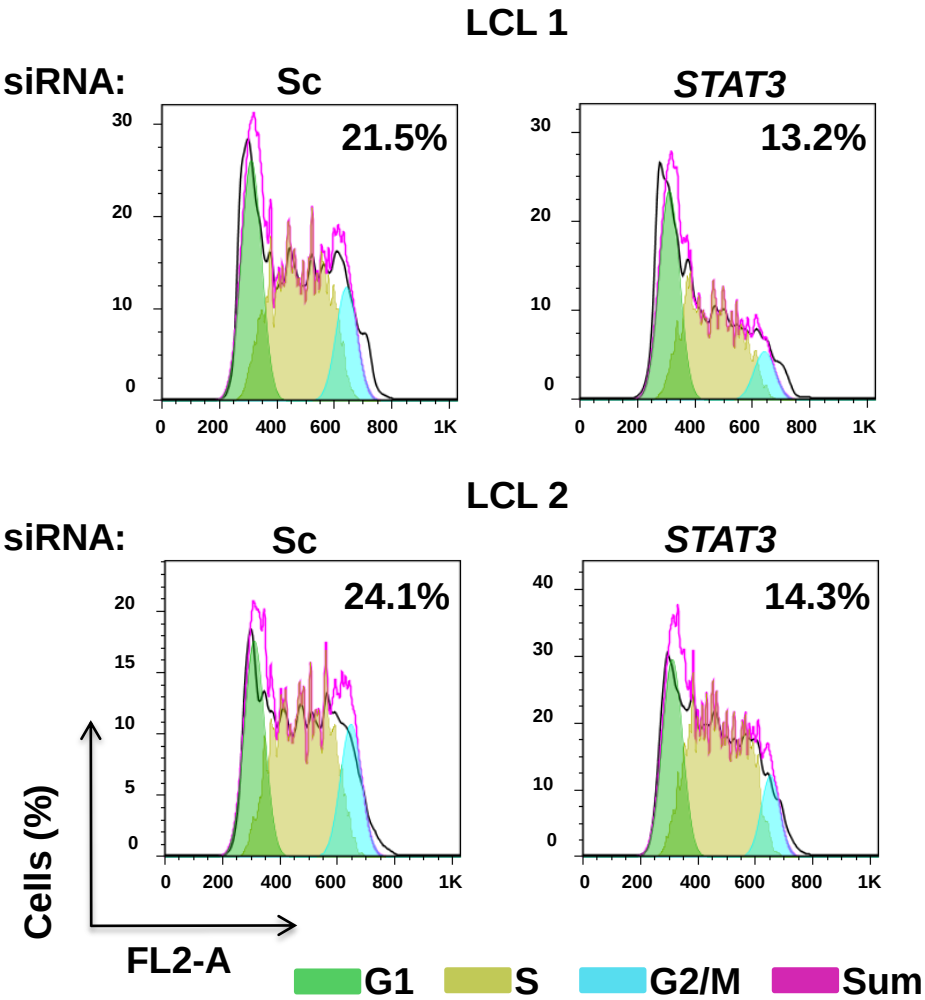
Koganti et al., J. Virology, 2014

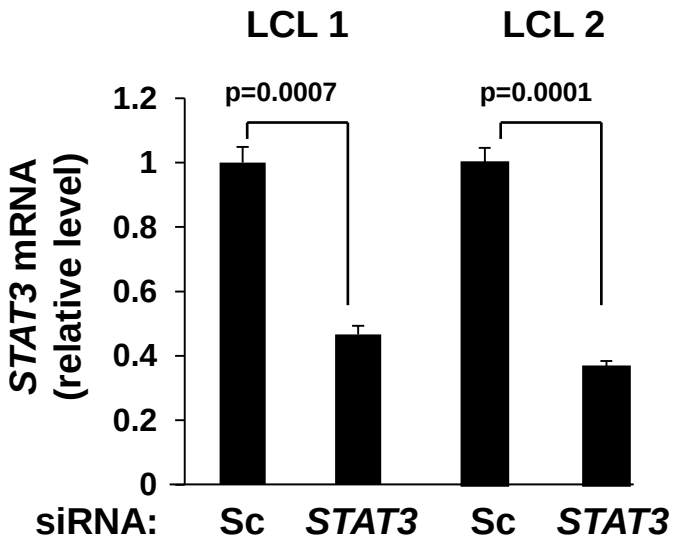
STAT3 is necessary for proliferation past the S phase
……..or impairment of STAT3 causes S phase delay/arrest



Koganti et al., J. Virology, 2014

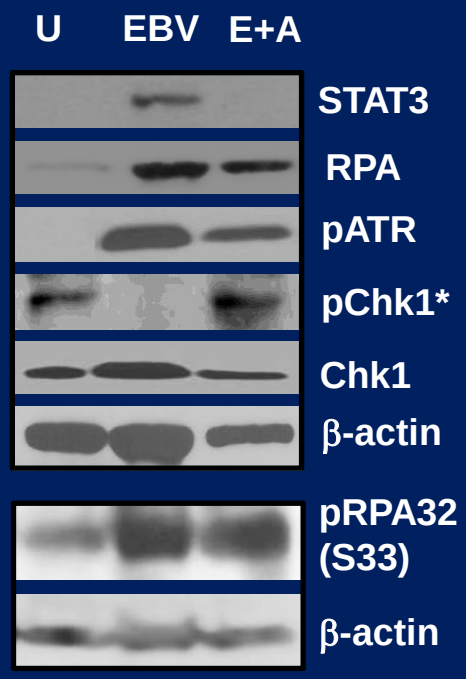
STAT3 is necessary for proliferation past the S phase
……..or impairment of STAT3 causes S phase delay/arrest

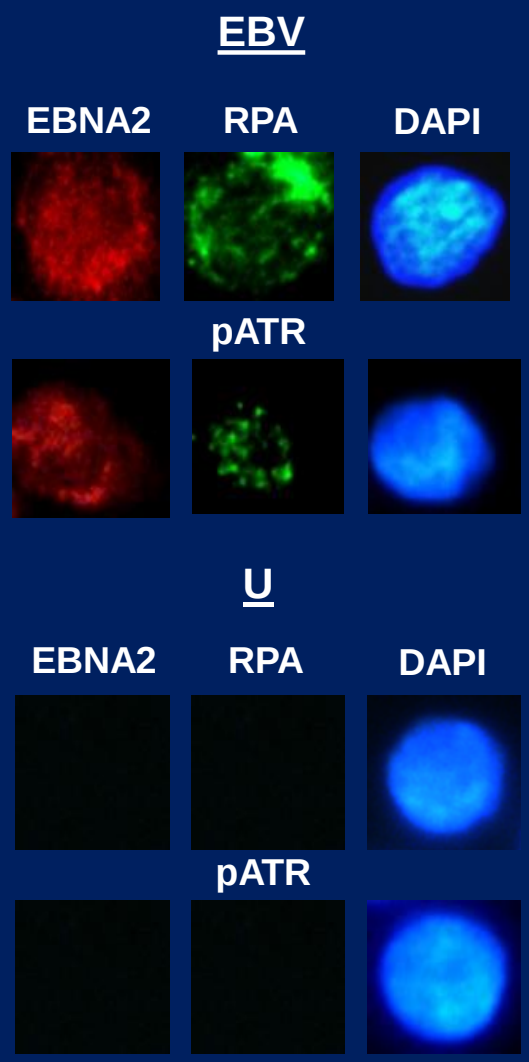


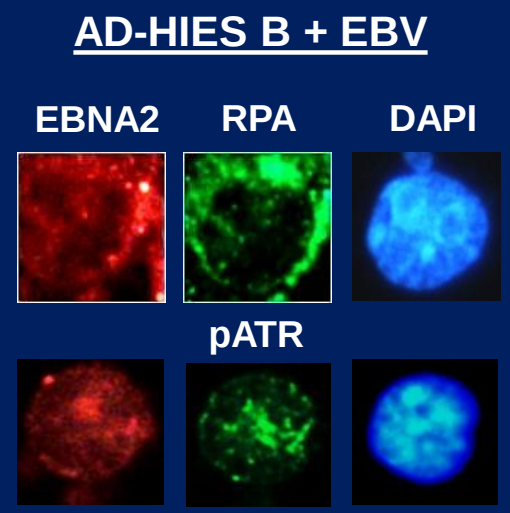


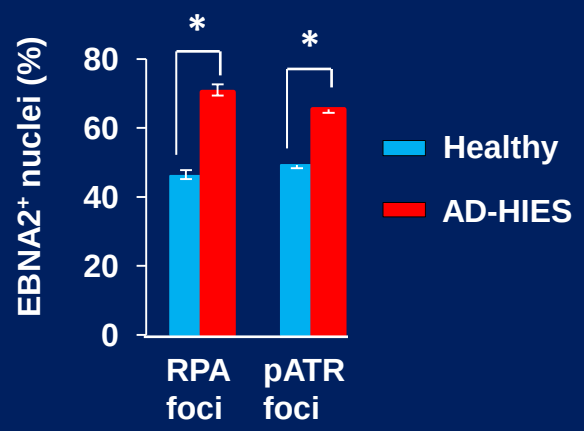
Koganti et al., PNAS, 2014

STAT3 blocks signaling downstream of ATR





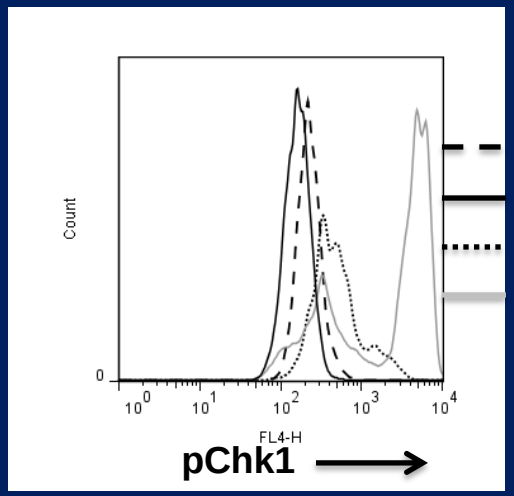




Koganti et al., PNAS, 2014

STAT3 suppresses pChk1 levels

Day 4 Culture



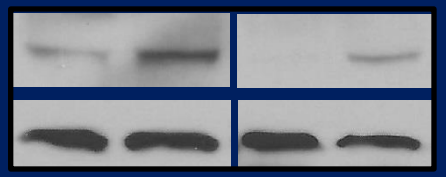
Un-infected healthy B cells
Healthy B + EBV, LMP1 pos
Un-infected AD-HIES B cells
AD-HIES B + EBV, LMP1-pos

Healthy LCL **AD-HIES LCL**

pChk1
β-Actin

Healthy LCL

LCL 1 **LCL 2**

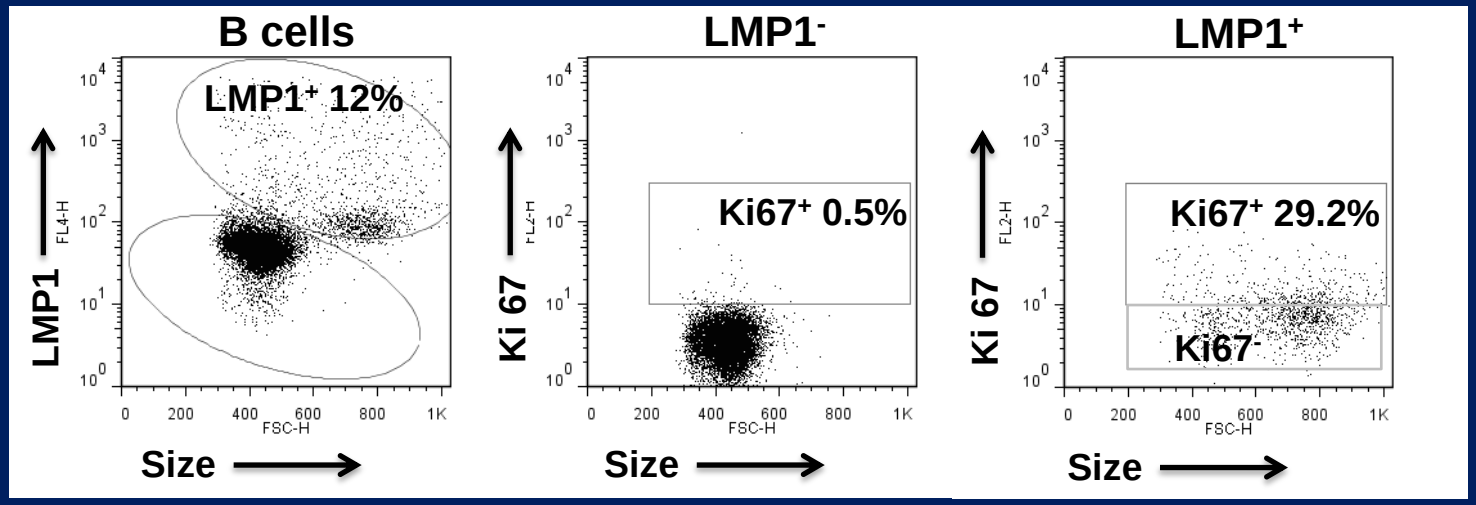


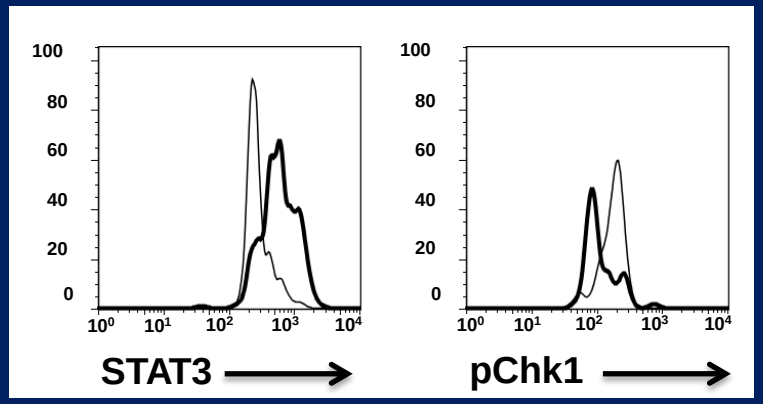
pChk1
β-Actin

siRNA Sc *STAT3* Sc *STAT3*

Koganti et al., PNAS, 2014

Proliferating EBV-infected cells in vivo show high STAT3 but low pChk1

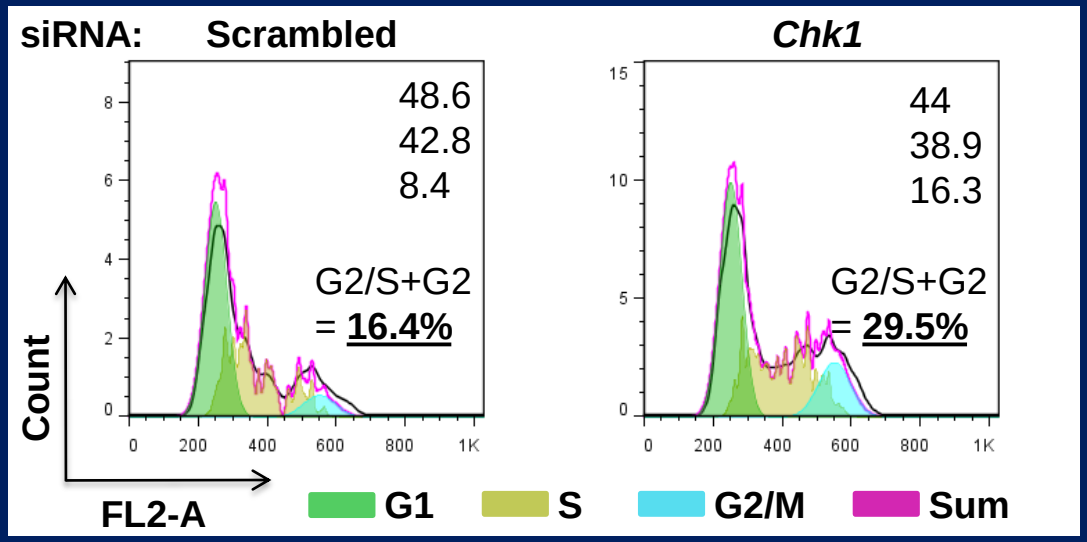


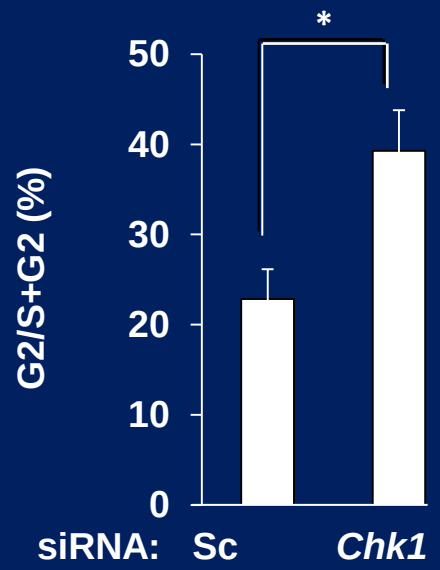


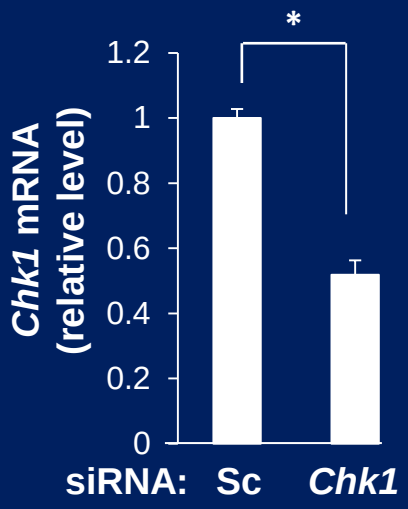
Koganti et al., PNAS, 2014

STAT3 functions via Chk1 to promote progression of EBV-infected cells past the S phase

AD-HIES LCL



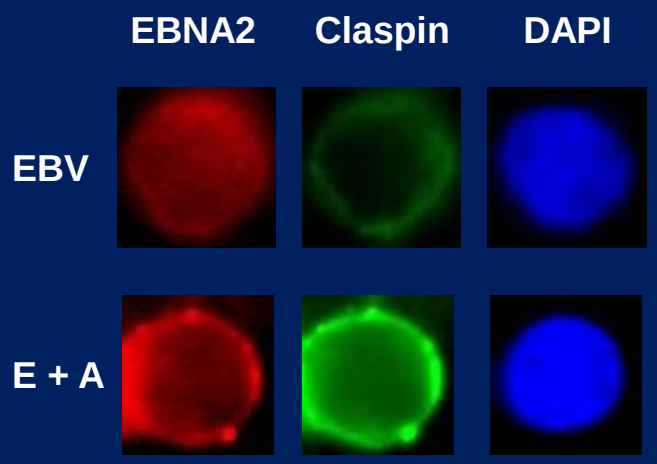


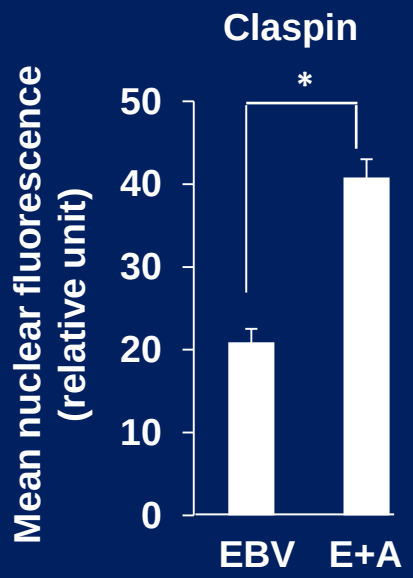


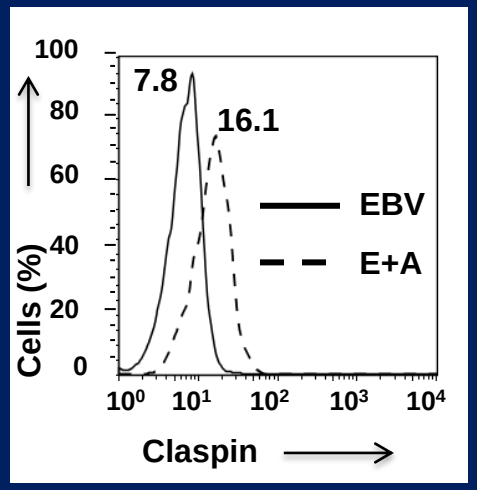
Koganti et al., PNAS, 2014

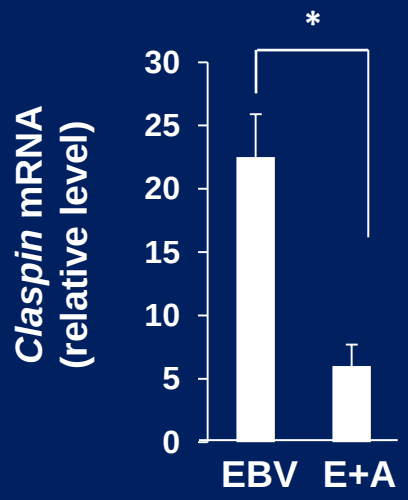
Cells with functional STAT3 demonstrate loss of nuclear Claspin

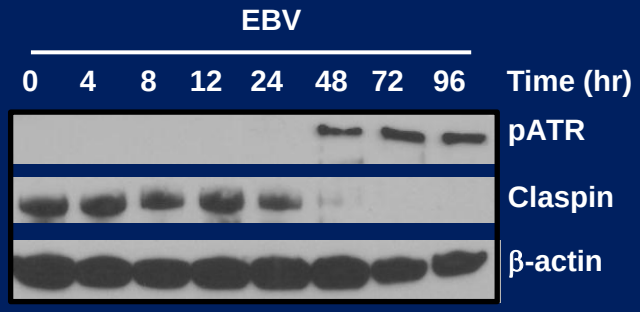
Day 4





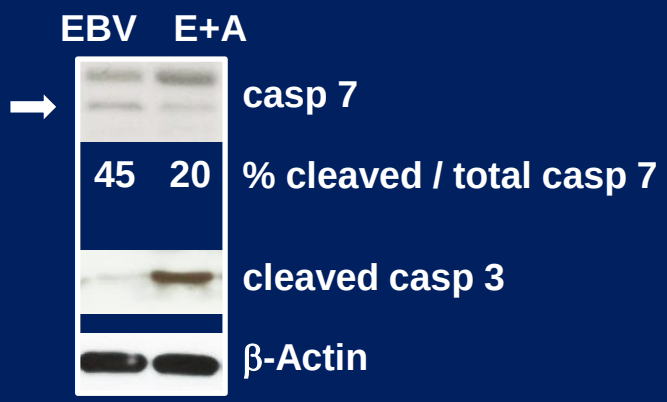


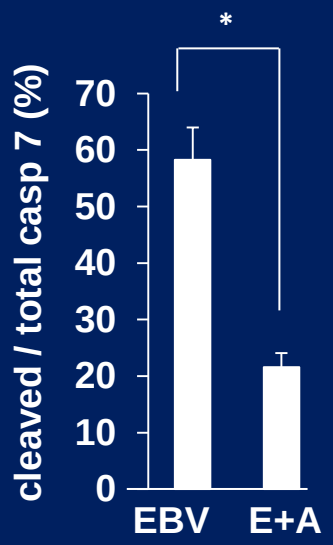


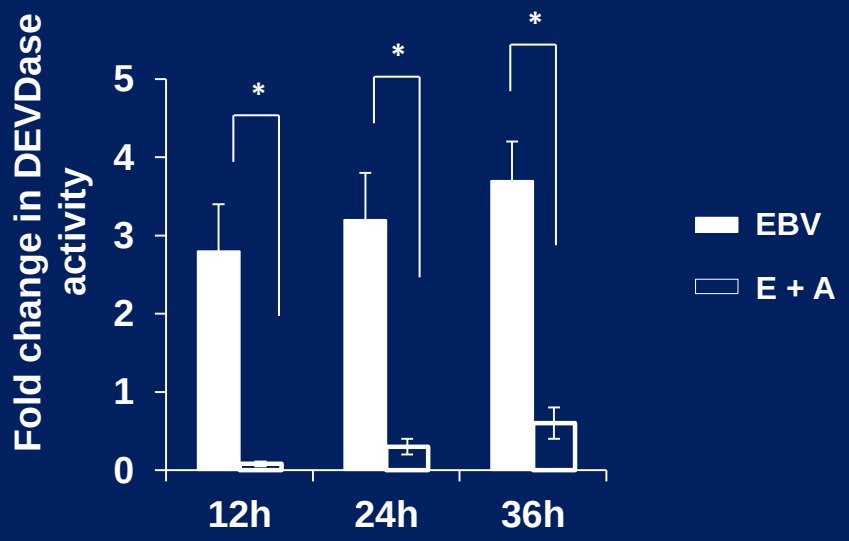


Koganti et al., PNAS, 2014

EBV infection in the presence of STAT3 results in caspase 7 activation

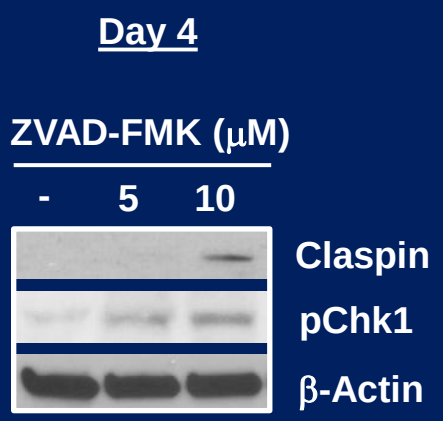


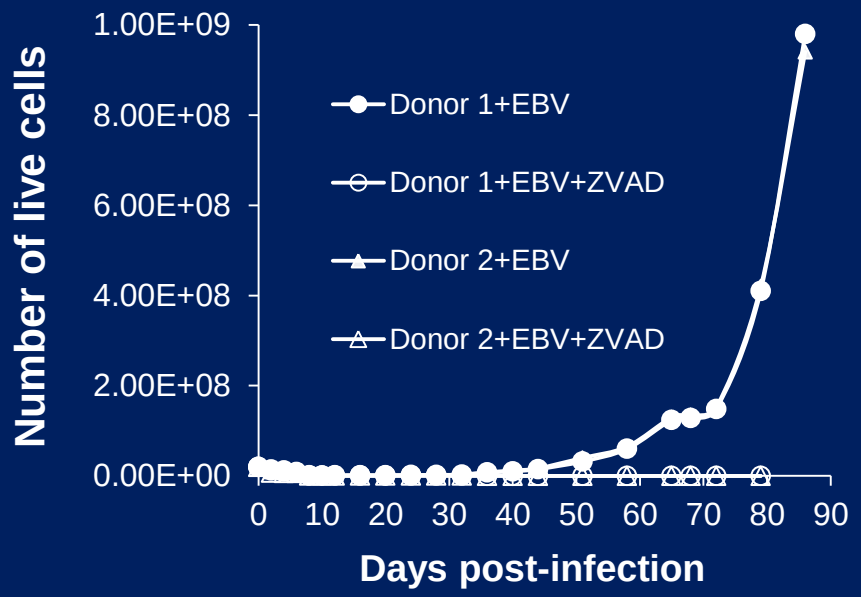




Koganti et al., PNAS, 2014

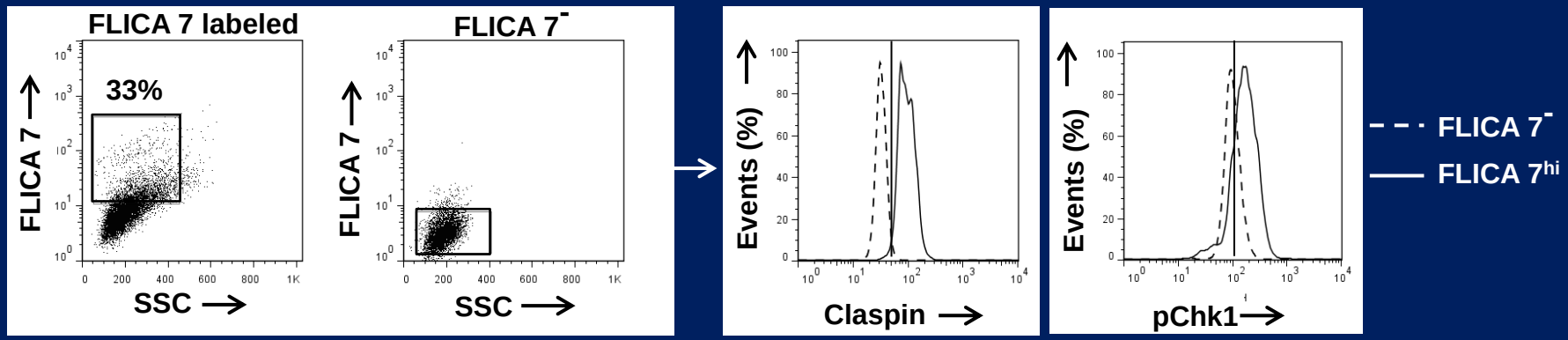
Caspase inhibition causes Claspin recovery and failure of EBV-mediated growth transformation

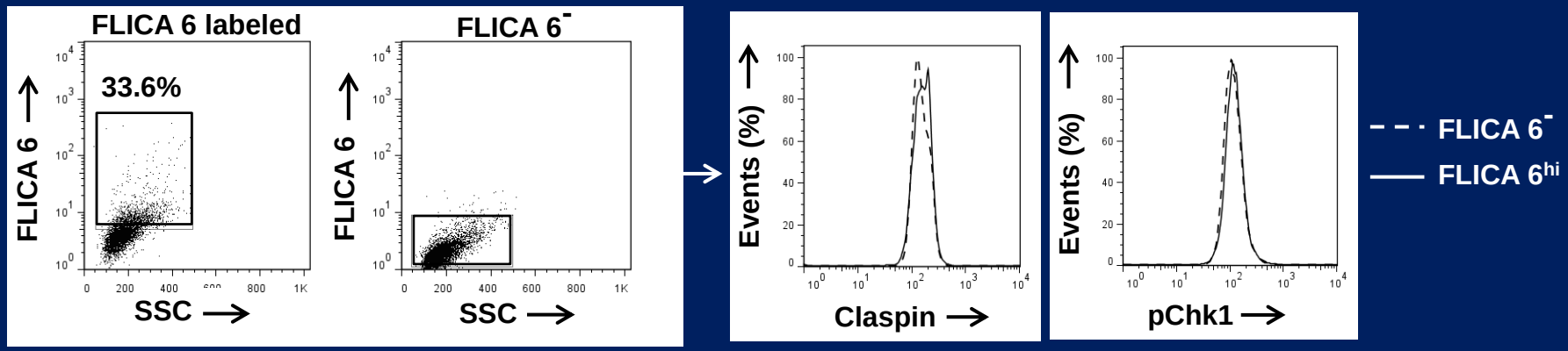




Koganti et al., PNAS, 2014

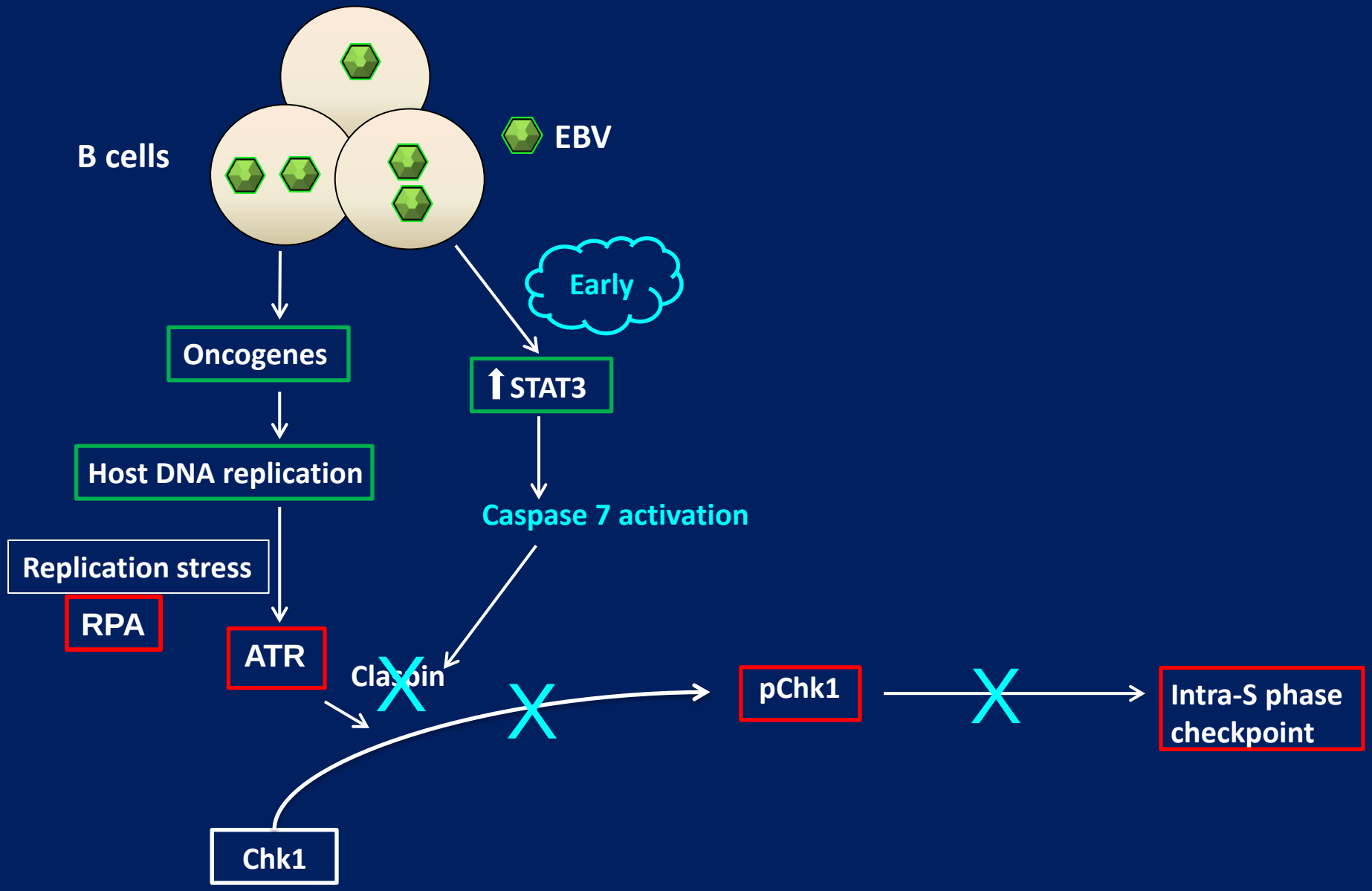
Inhibition of Caspase 7 (but not Caspase 6) causes recovery of Claspin and pChk1





Koganti et al., PNAS, 2014

Model for DDR suppression during the initial rounds of EBV-oncogene-driven cell proliferation



Summary

- **Link STAT3 to DDR <u>suppression</u> during oncogene-driven cell proliferation**
- **A newly discovered function for STAT3 (constitutively active in most cancers)**
- **STAT3 mediated relaxation of the intra-S phase checkpoint is a previously unknown mechanism for genomic instability**
- **Caspase 7 in a non-apoptotic role**
- **STAT3, a major transcription factor, is central to cell proliferation – implications beyond EBV infection and tumorigenesis**

Contributions

Siva Koganti, Ph.D.
Xiaofan Li, Ph.D.
Eric Burton, B.S.
Sal Spadaro, B.S., B.A.
Erik Hill, Ph.D.
Shane McAllister, M.D., Ph.D.
Joyce Hui-Yuen, M.D., M.S.
Sameer Lapsia, M.D.
Cynthia Megyola, M.S.

Funding:

NIAID
NICHD
Charles Hood Foundation
Yale Center for Clinical Investigation
American Academy of Pediatrics
Stony Brook Research Foundation

Collaborators:

Alexandra Freeman, M.D.
Steven Holland, M.D.
NIAID, NIH, USA

Jizu Zhi, Ph.D.
Stony Brook University, USA

Paul Farrell, Ph.D.
Imperial College, UK

Benjamin Gardner, M.D.
Yale University, USA

Stuart Tangye, Ph.D.
Garvan Institute, Australia

Friedrich Grässer, Ph.D.
Universitätsklinkum des Saarlandes, Germany