Propagation of Epstein-Barr Virus in Stratified Epithelium

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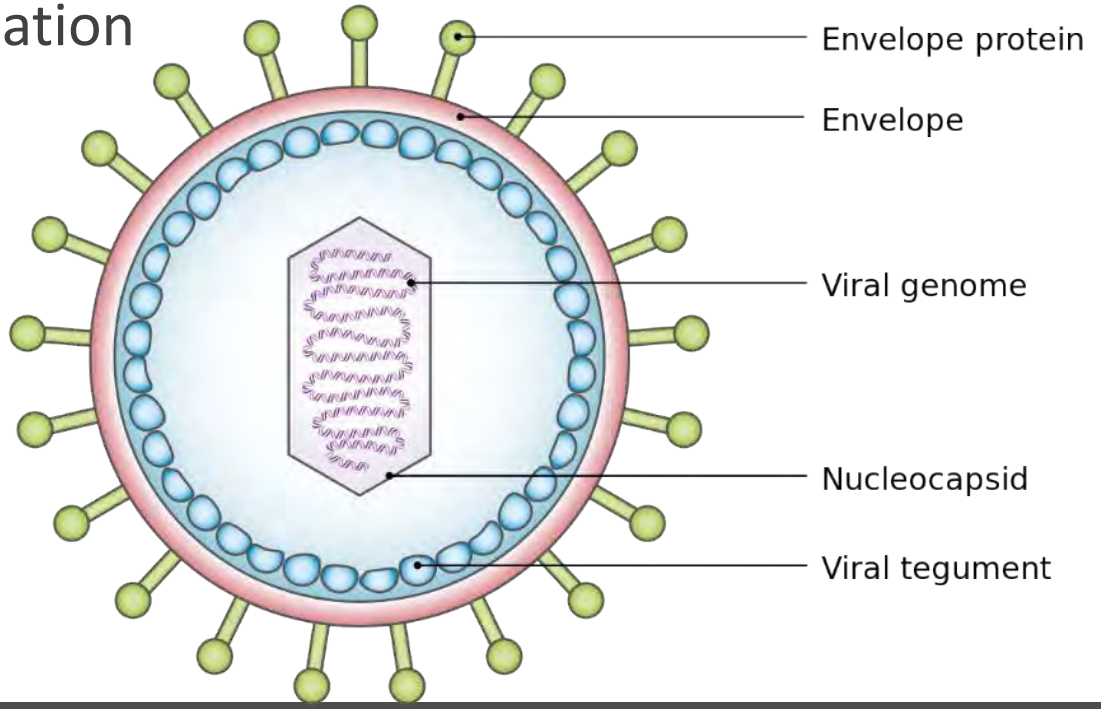
The Epstein-Barr Virus (EBV) – “The First Human Tumor Virus”

Enveloped γ-herpesvirus with 170 kb linear dsDNA genome

Originally discovered in 1964 in Burkitt lymphoma

Primarily infects B and epithelial cells

Approximately 95% prevalence in world’s adult population



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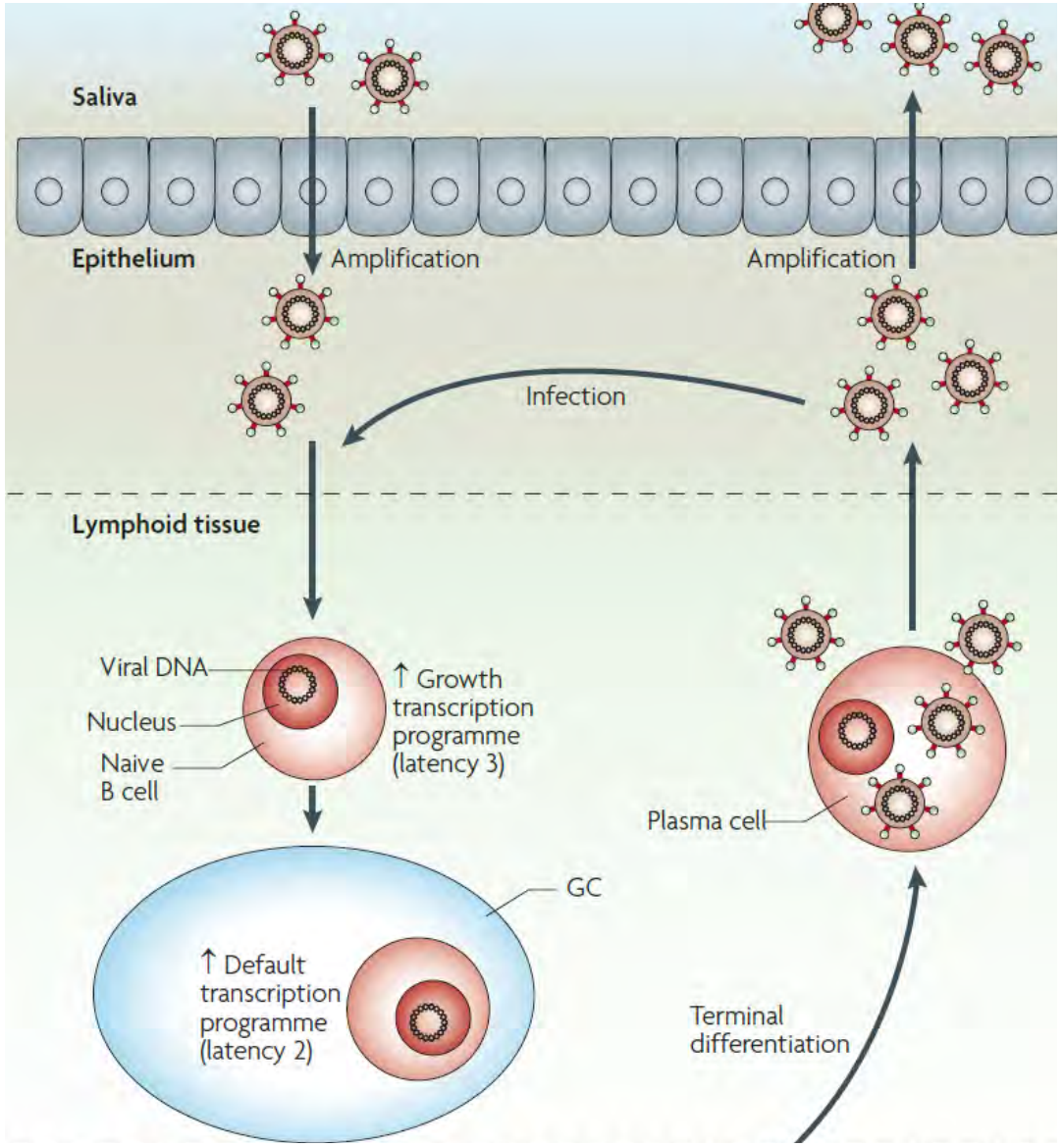
EBV Primarily Infects B Cells and Epithelia



(Thorley-Lawson and Allday, 2008)

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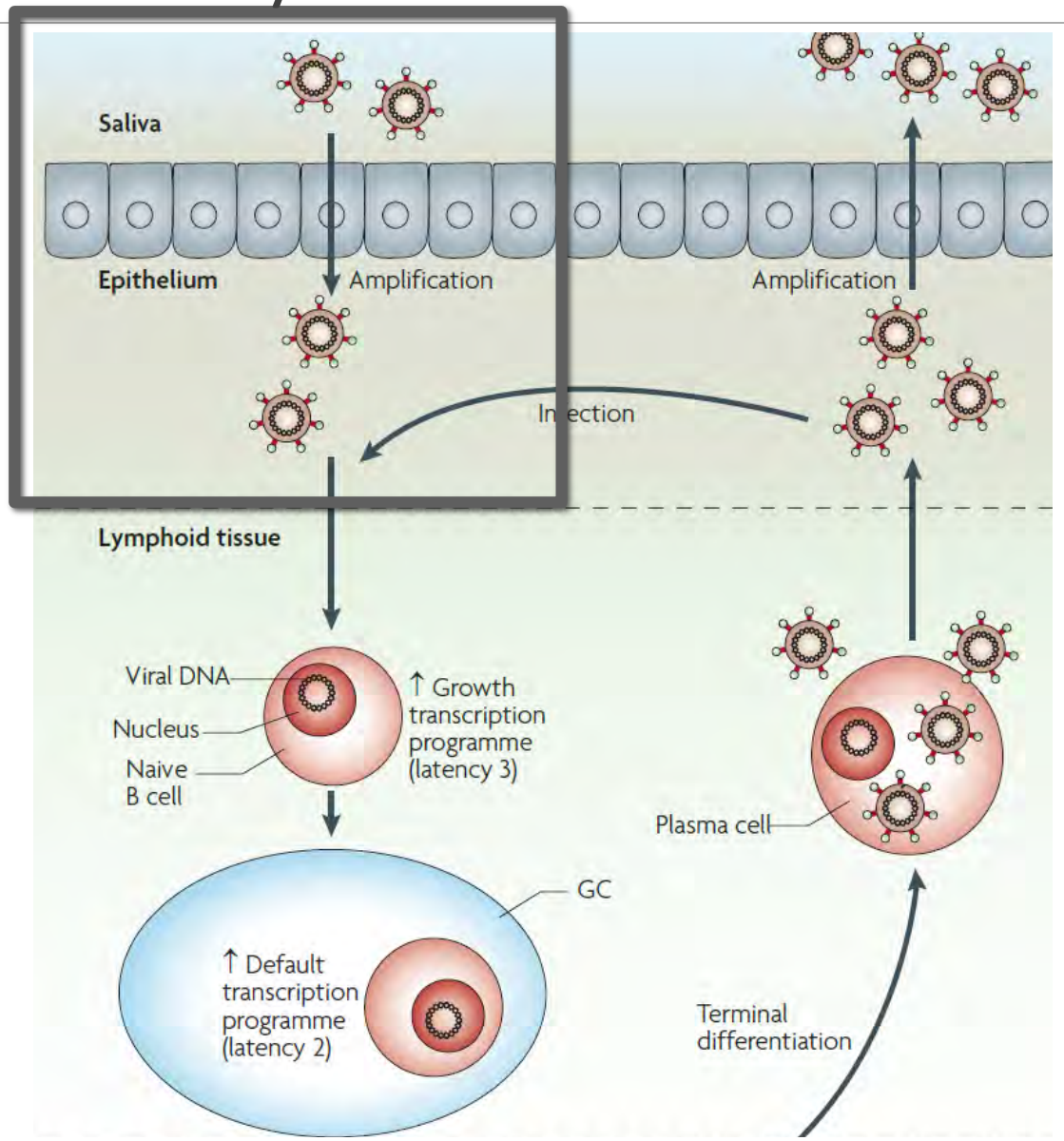

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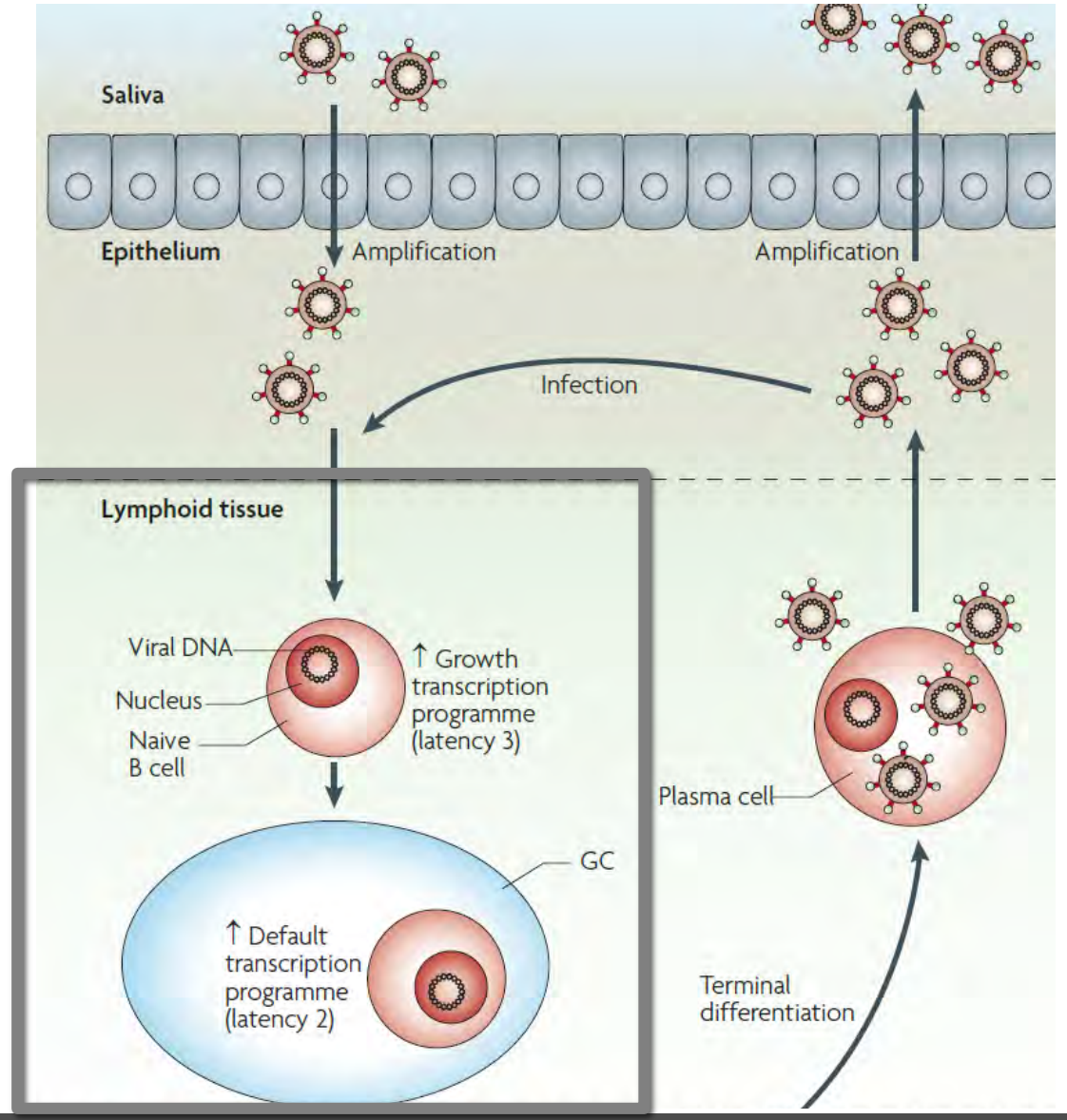

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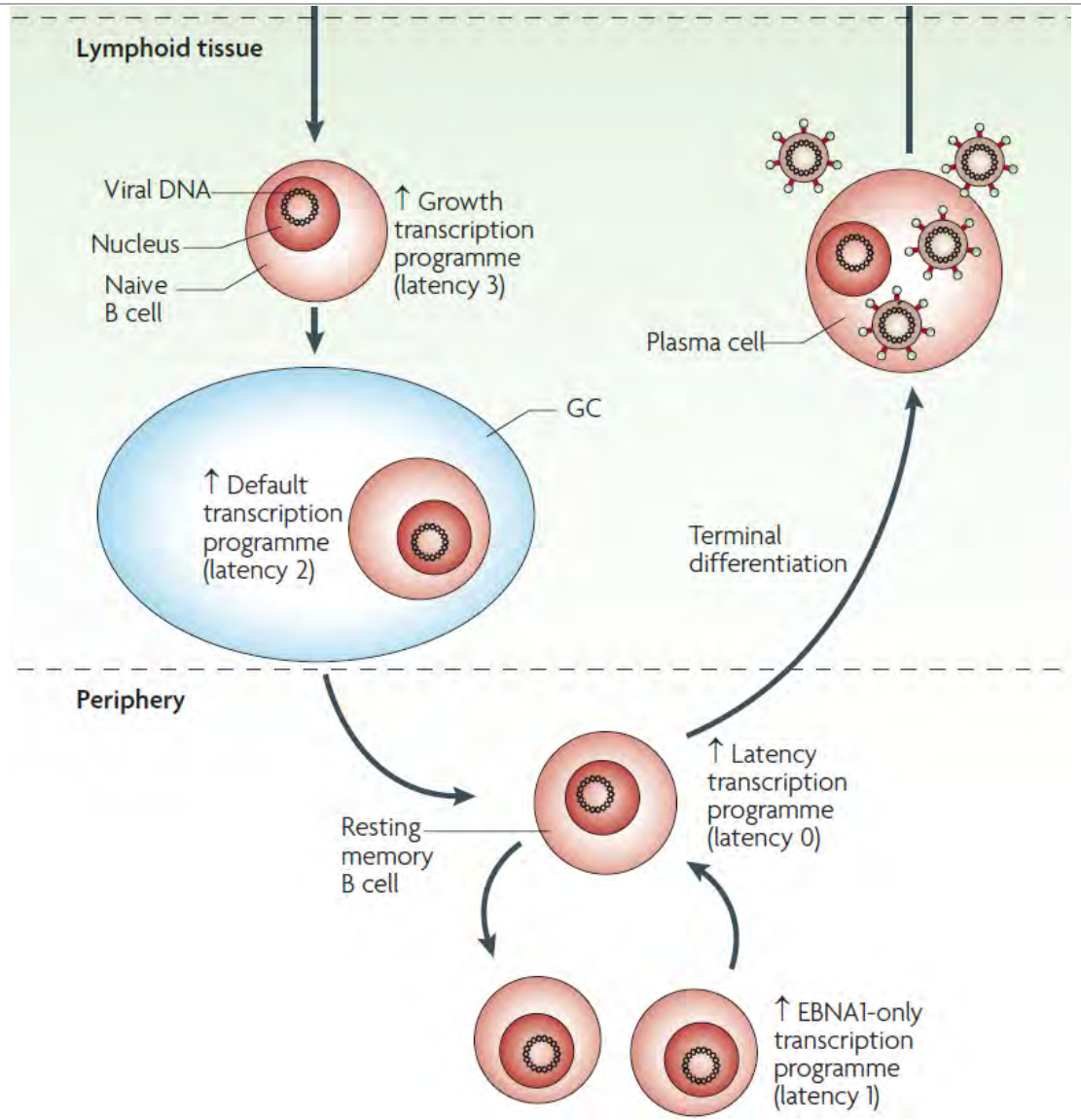

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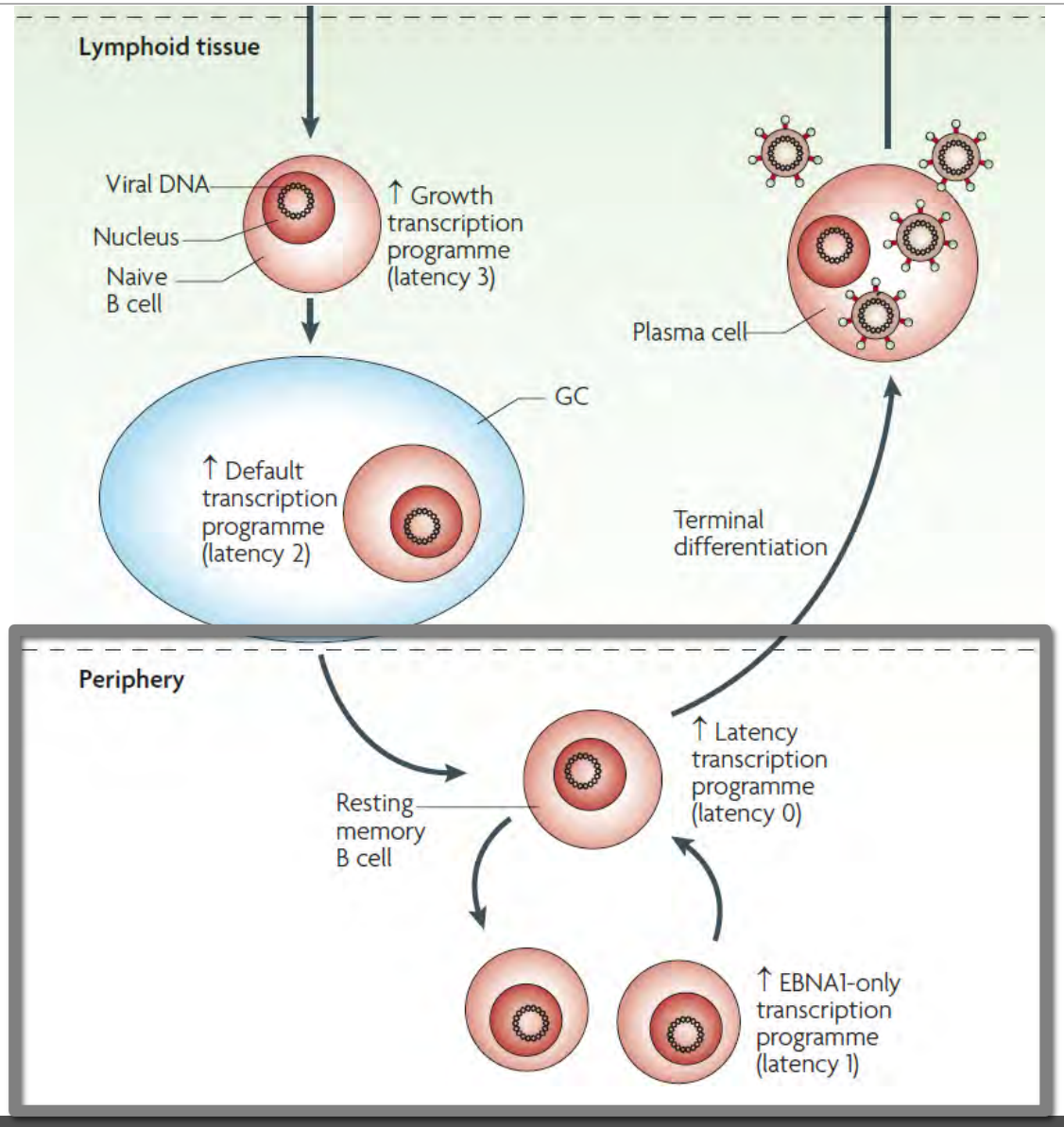

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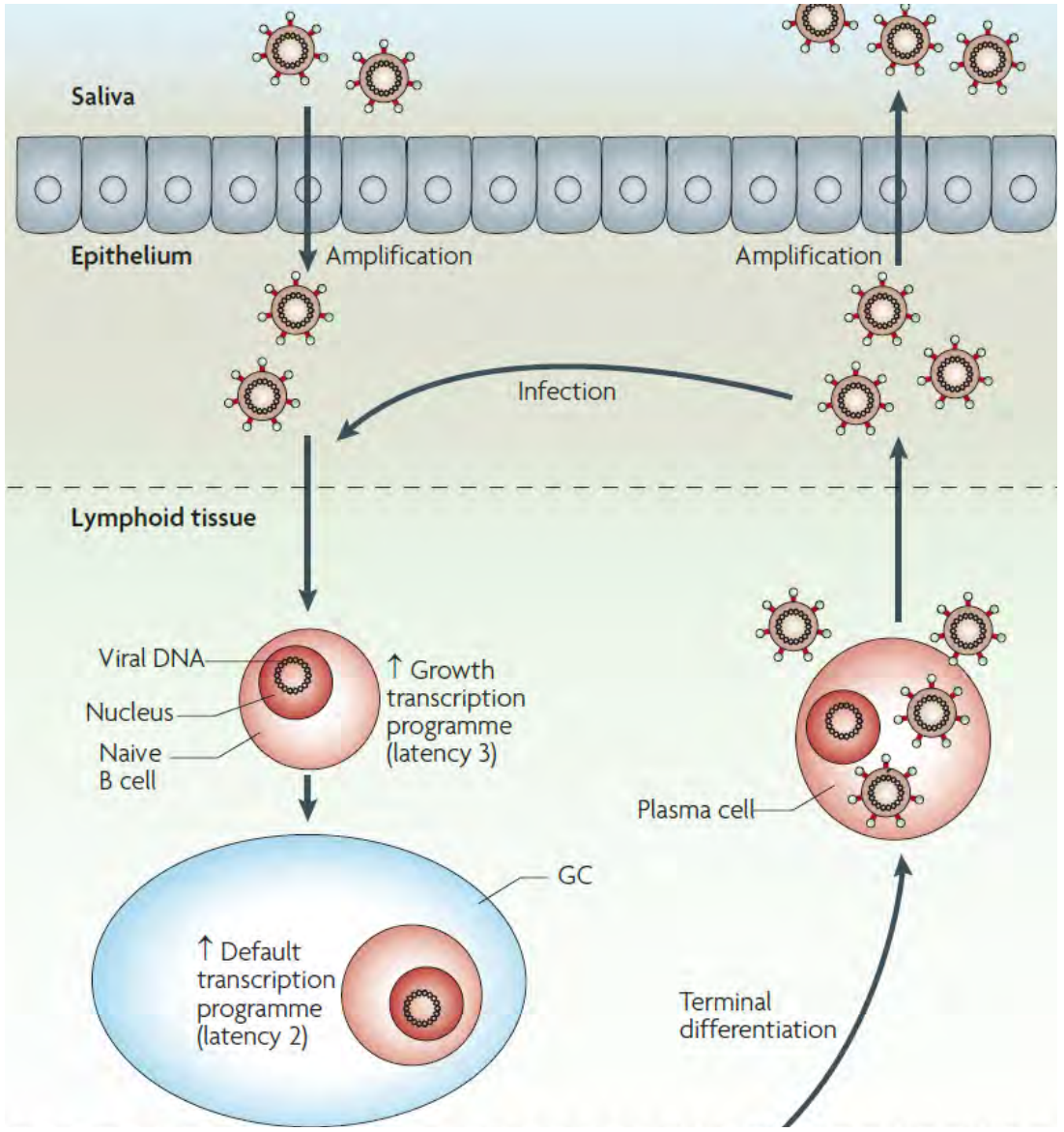

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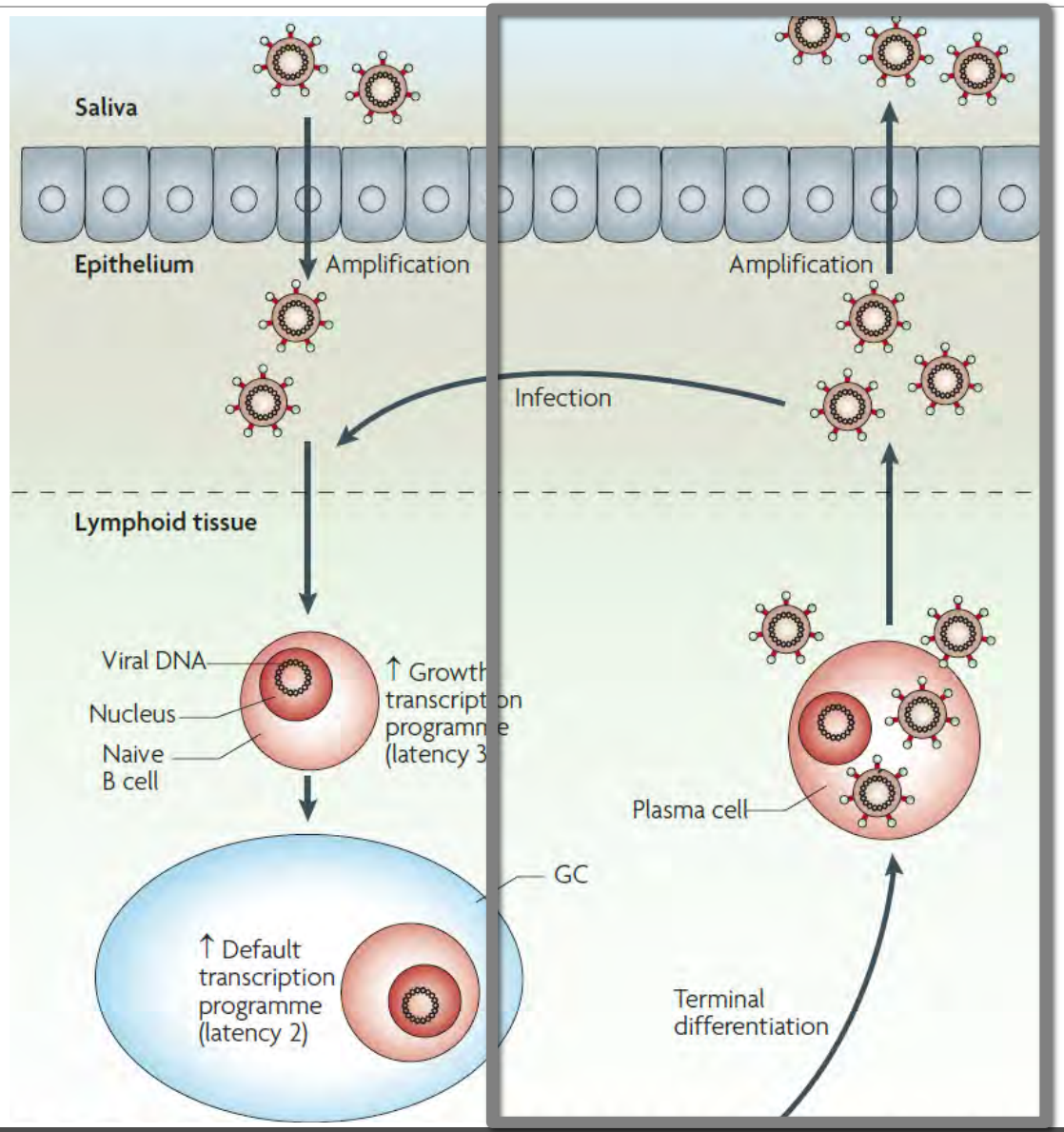


(Thorley-Lawson and Allday, 2008)

EBV is Associated with Lymphocyte and Epithelial Malignancies

Table1. EBV-associated diseases

Disease	At risk population	EBV association
Lymphocyte origin		
Infectious mononucleosis (IM)	Adolescents/young adults from western societies/ high socioeconomic groups	Majority. IM-like syndromes also occur in cytomegalovirus, HIV primary infection
X-linked lymphoproliferative syndrome (XLPS)	Male offspring of female carriers of XLPS mutation	Majority. A few non-EBV-associated lymphomas occur in children with the mutation
B lymphoproliferative disease (BLPD)	Post-transplant lymphoproliferative disease HIV infection—primary central nervous system lymphoma —peripheral lymphoma	^90% <100% ^50%
Burkitt's lymphoma (BL)	African children—endemic BL HIV infection—sporadic BL	97–100% ^25%
Hodgkin's disease	Children—developing countries Young adults—high socioeconomic groups —history of IM	Overall ^65% Mixed cellularity type 80% Childhood ^80%
T/NK cell lymphoma	Chronic active EBV HIV infection	10–100%, depending on histological type
Primary effusion lymphoma	HIV infection	
Epithelial cell origin		
Oral hairy leukoplakia	HIV infection Other immunodeficiencies	100%
Nasopharyngeal carcinoma	S Chinese and Inuit races—high incidence Mayaks, Dyaks, Indonesians, Filipinos, Vietnamese—moderate incidence	Non-keratinised 100% Keratinised 30–100%
Gastric carcinoma	Not identified	Undifferentiated carcinoma of naso-pharyngeal type 100% Adenocarcinoma 5–15%

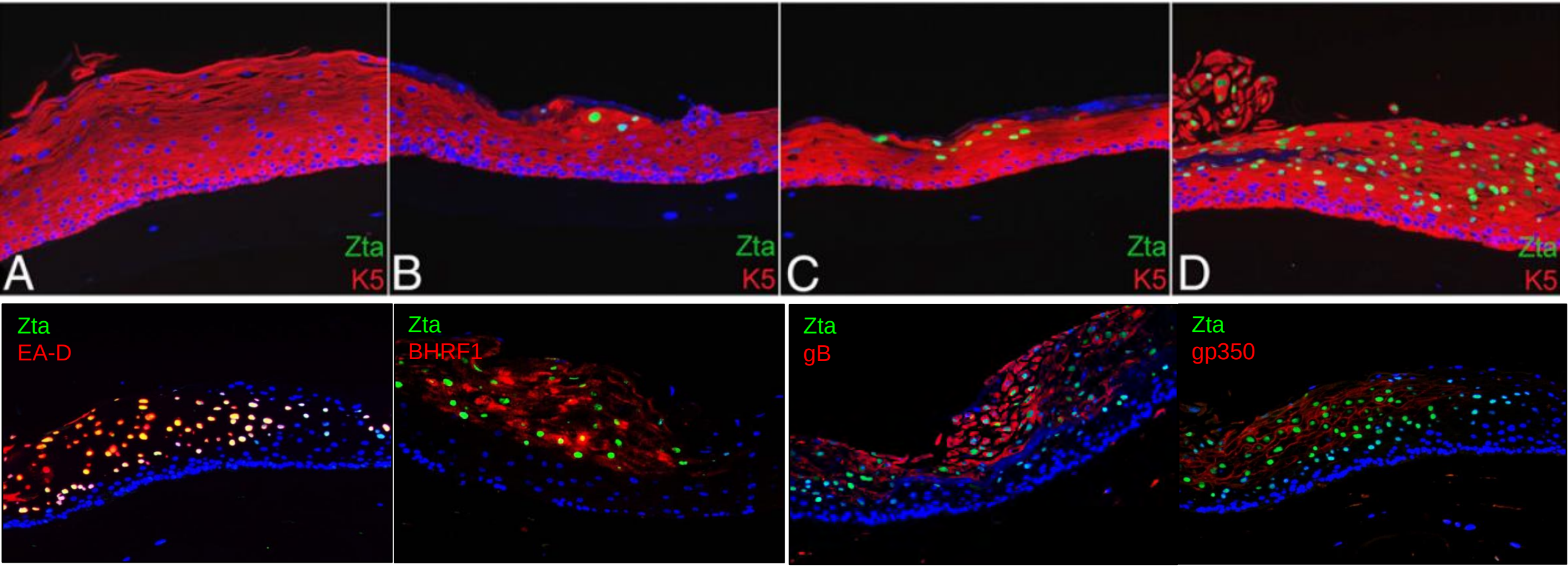
Several big questions remain in the field:

1. How does EBV infect epithelia and through what receptors?

2. How does productive replication proceed in epithelia?

(Macsween and Crawford, 2003)

EBV Productively Replicates in Raft Culture



Early Genes

Late Genes

Magnification = 10X

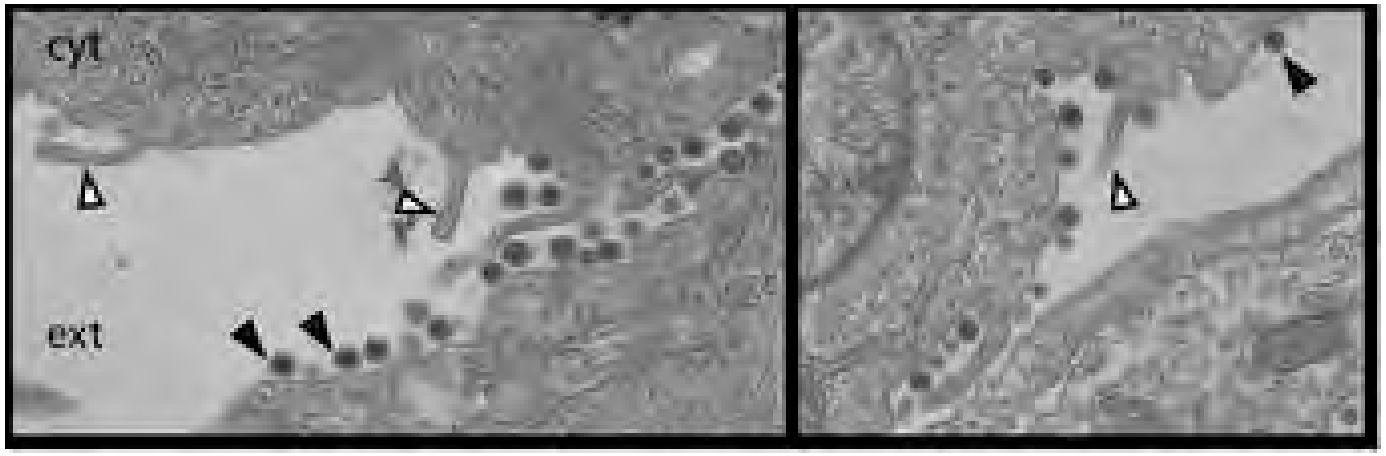
(Temple, 2014)

MHV-68 Encodes a Glycoprotein for Viral Spread

A Gamma-Herpesvirus Glycoprotein Complex Manipulates Actin to Promote Viral Spread

Michael B. Gill, Rachel Edgar, Janet S. May, Philip G. Stevenson*

Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom



Cos7 cells infected with MHV68

(Dai et al., 2008)
(Gill et al., 2008)

A (New) Glycoprotein Discovered: BDLF2

The BDLF2 protein of Epstein–Barr virus is a type II glycosylated envelope protein whose processing is dependent on coexpression with the BMRF2 protein

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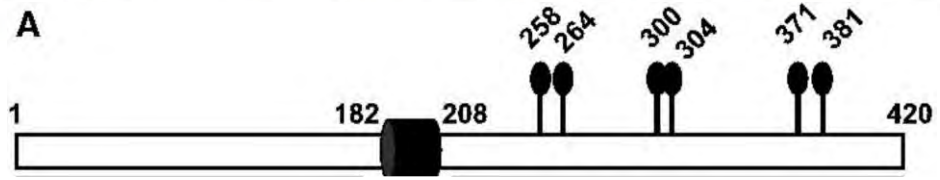
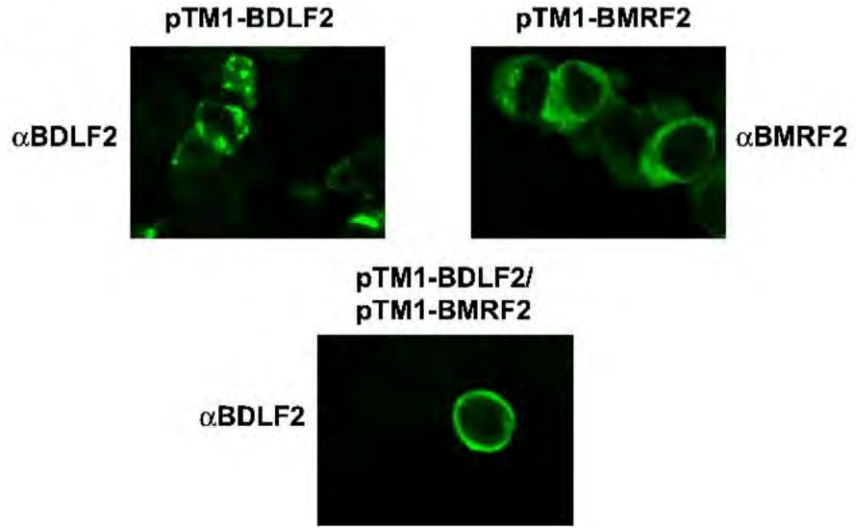
Department of Microbiology and Immunology, Center for Molecular and Tumor Virology and Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, USA





Previously believed to be a tegument protein*

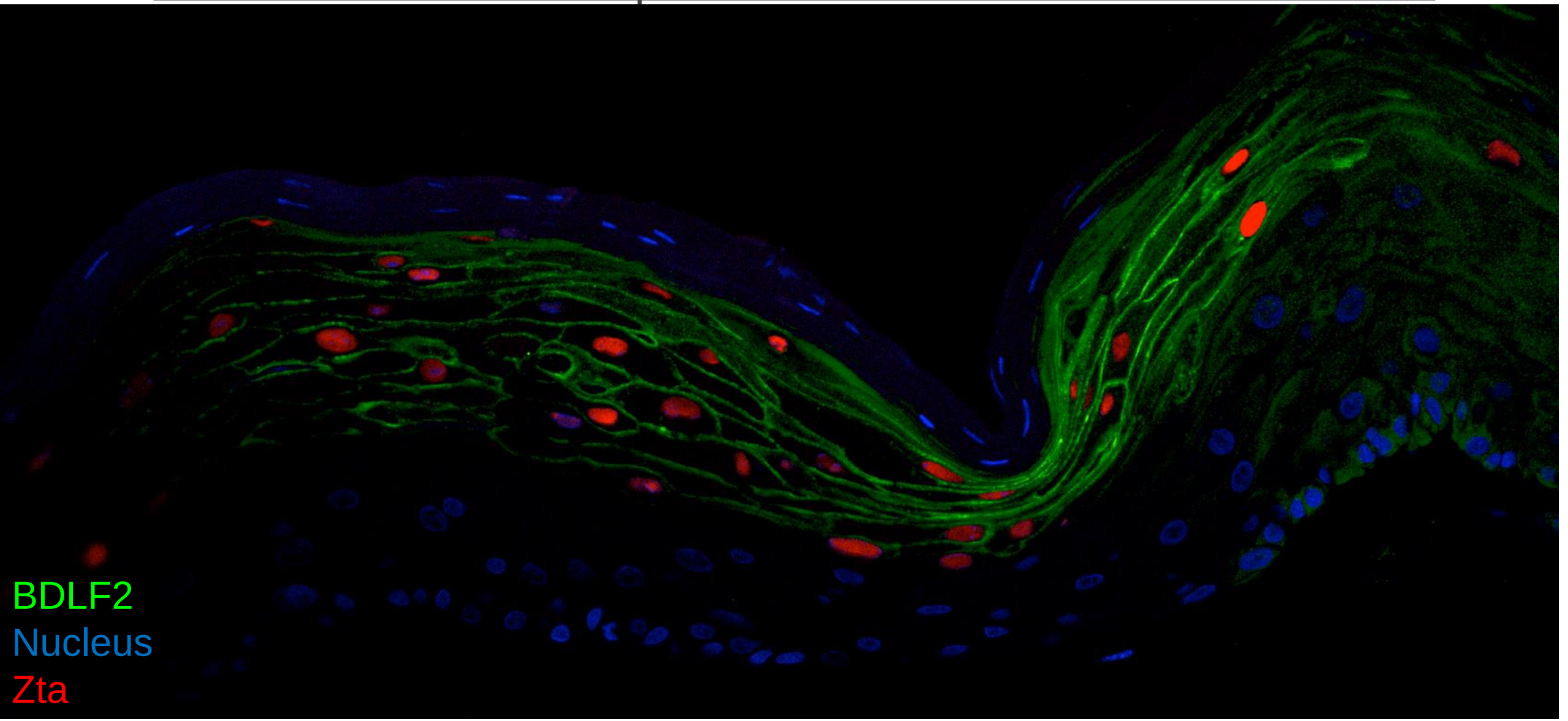
(Gore and Hutt-Fletcher, 2009)

Primary Hypothesis

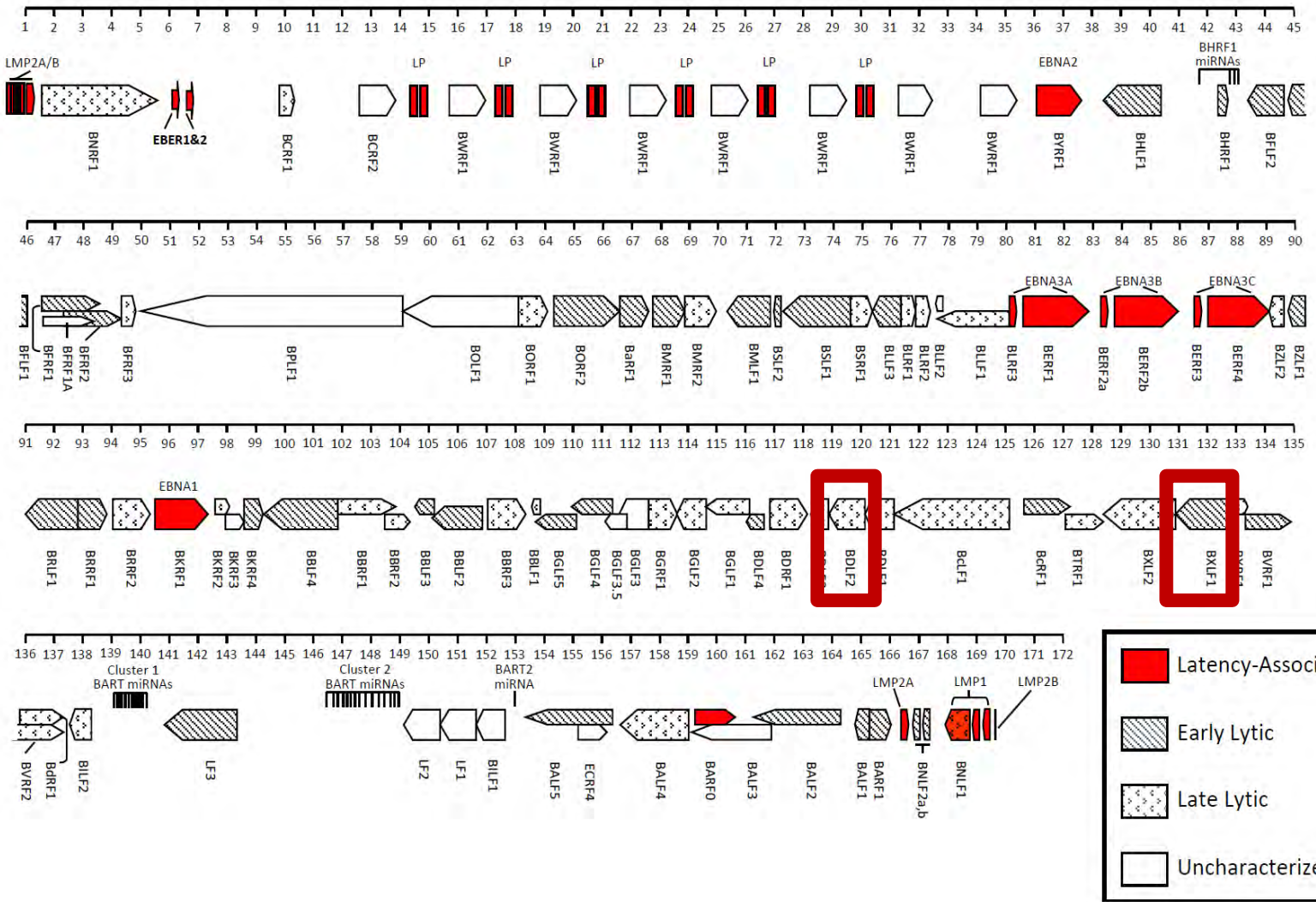
- EBV glycoprotein BDLF2 plays an important role in intercellular trafficking during EBV infection

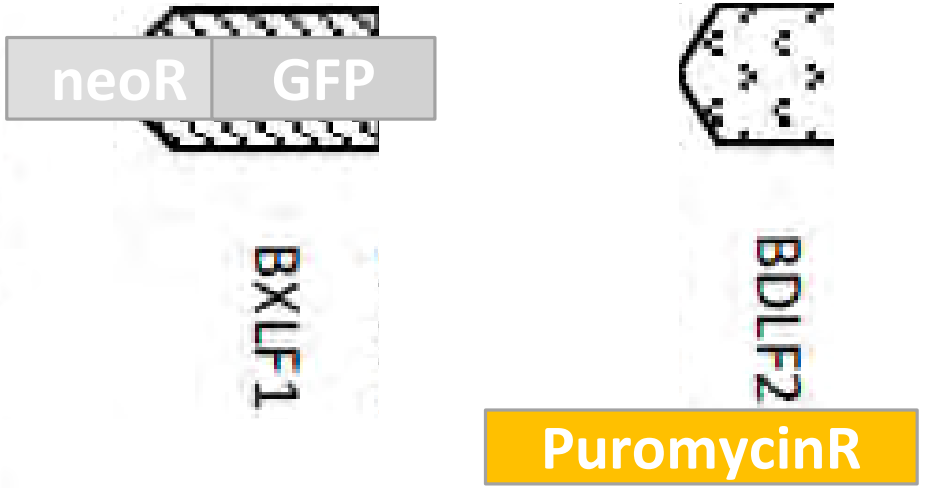
- **BDLF2(BMRF2) may modulate the actin cytoskeleton of its host cell in order to facilitate infection of nearby susceptible cells.**

BDLF2 is Expressed in Raft Culture

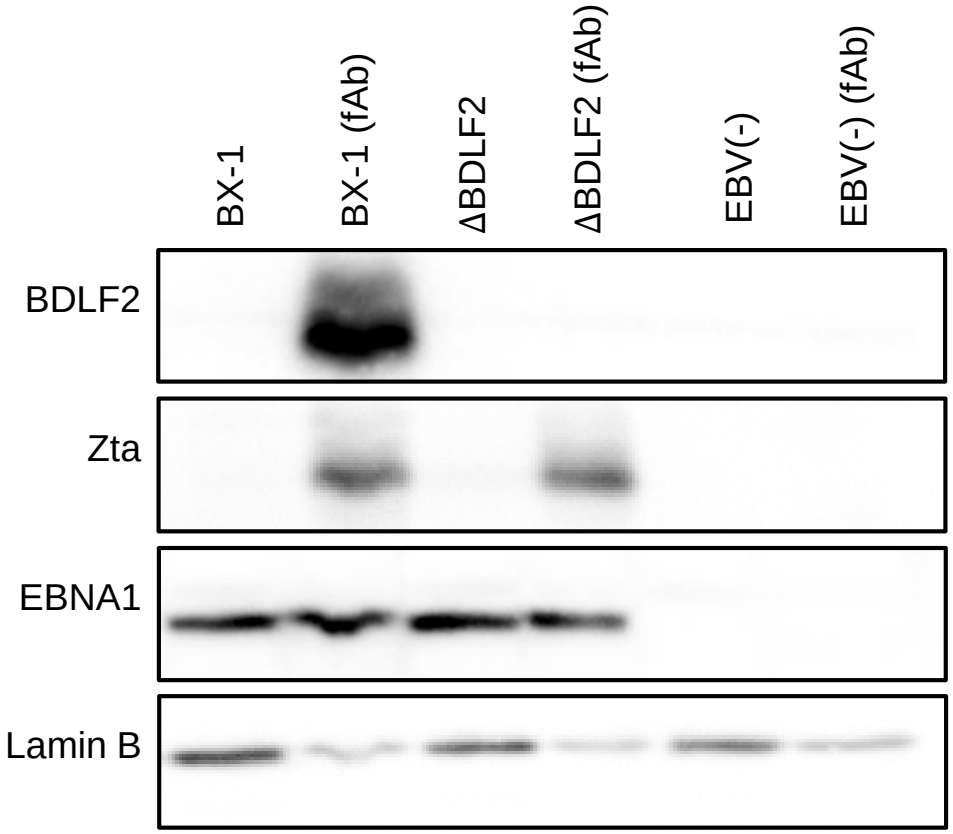


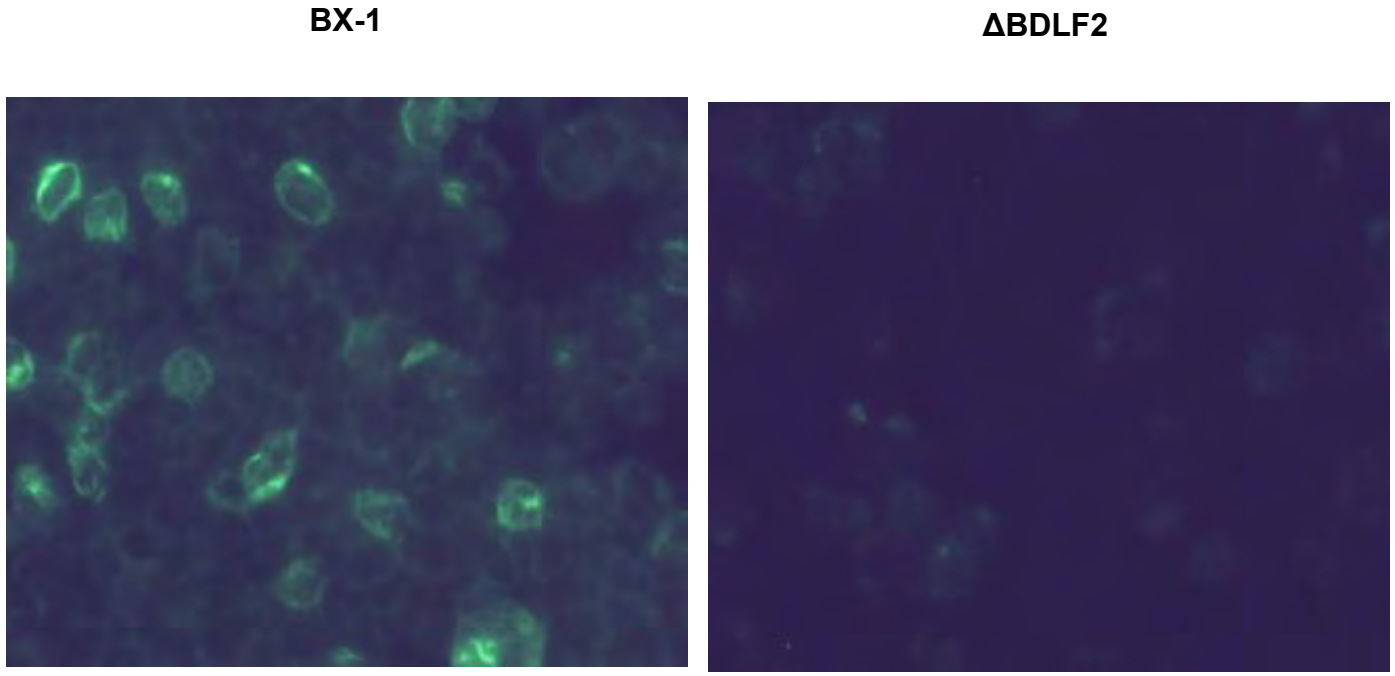
BX-1 and BDLF2 - Knockout Viruses



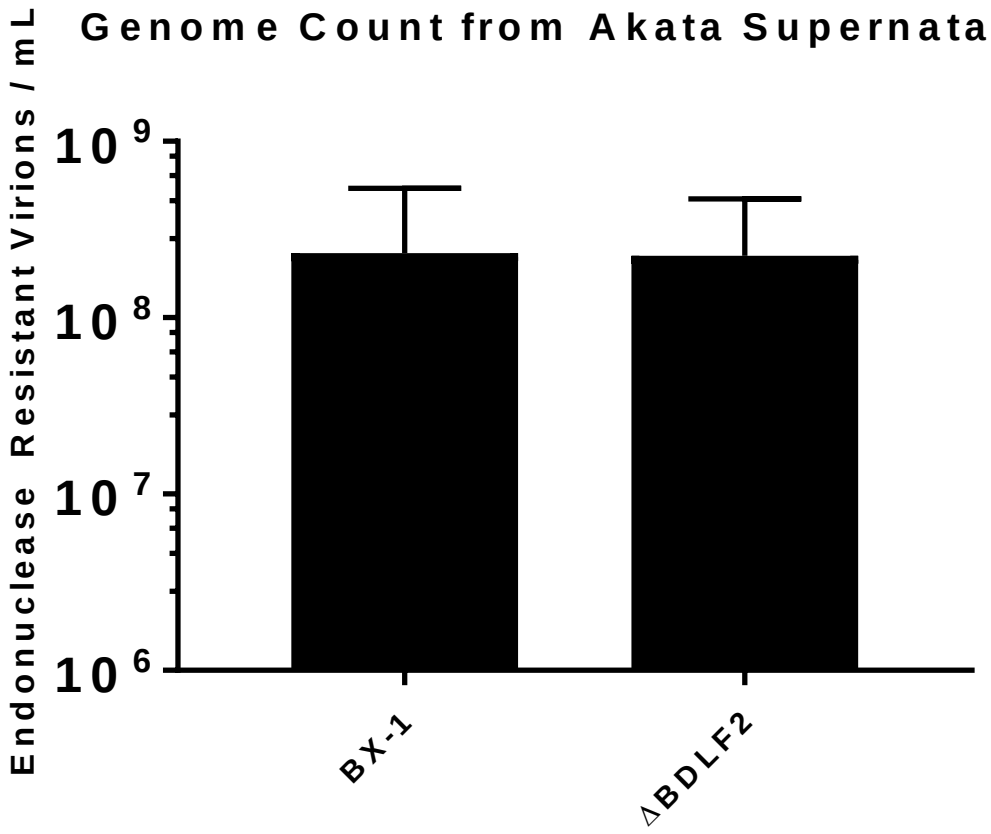


Confirmation of Loss of BDLF2 in rEBV

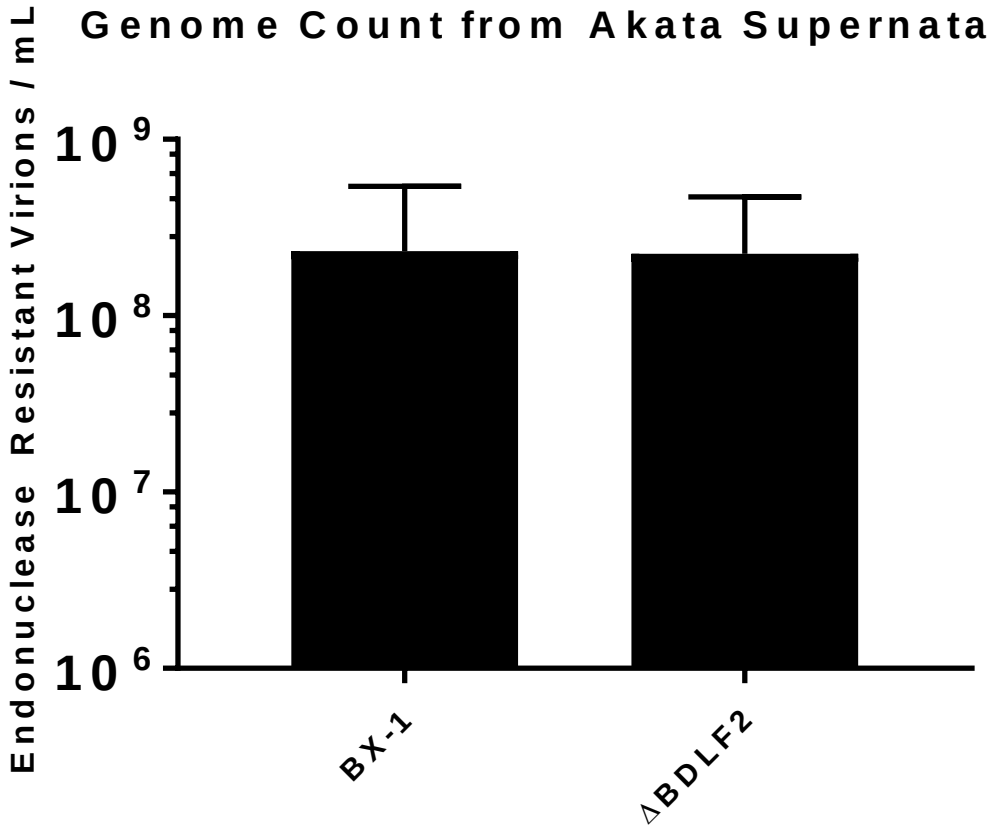


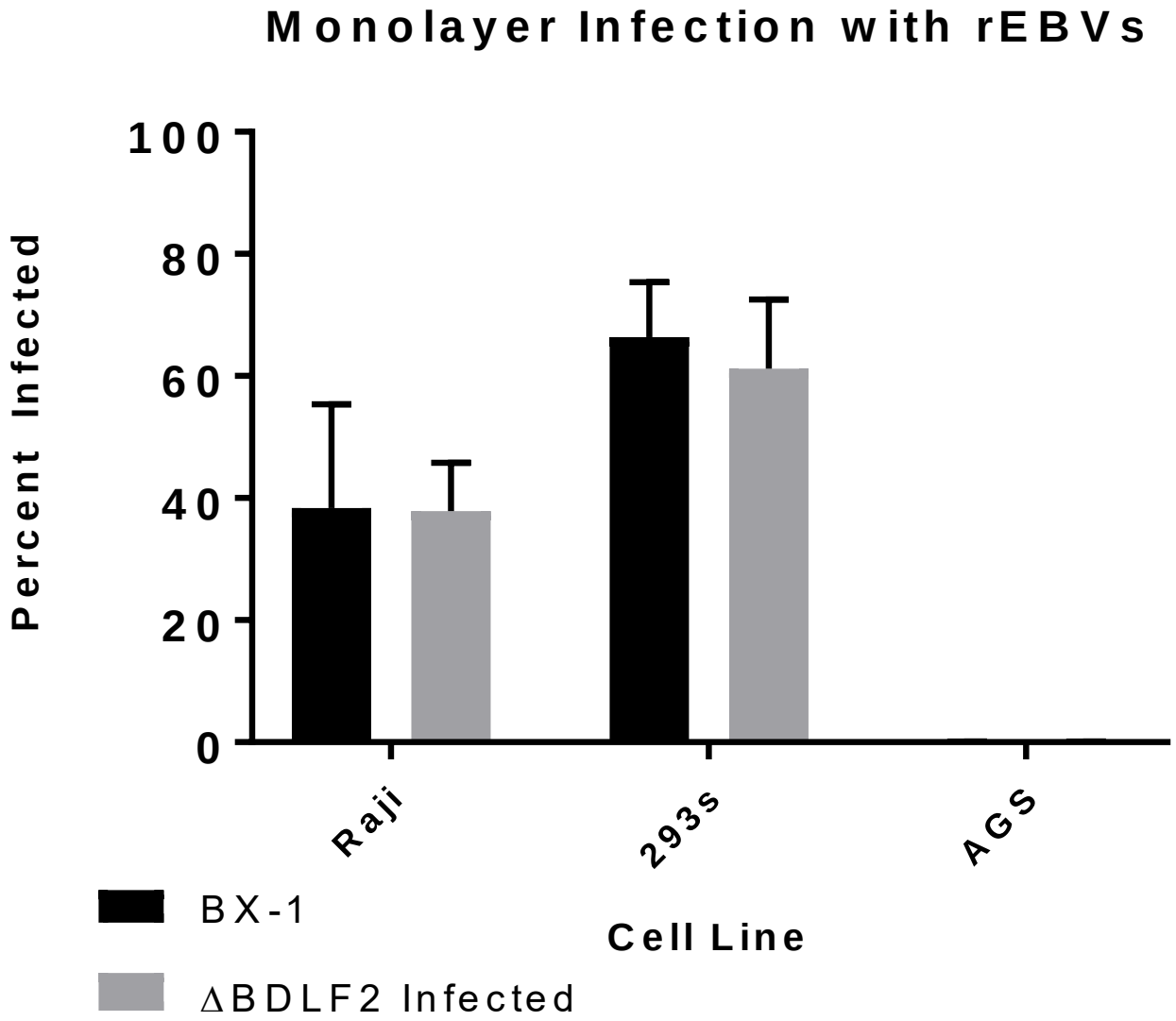


BX-1 and ΔBDLF2 Akatas Produce Similar Titers of Virus

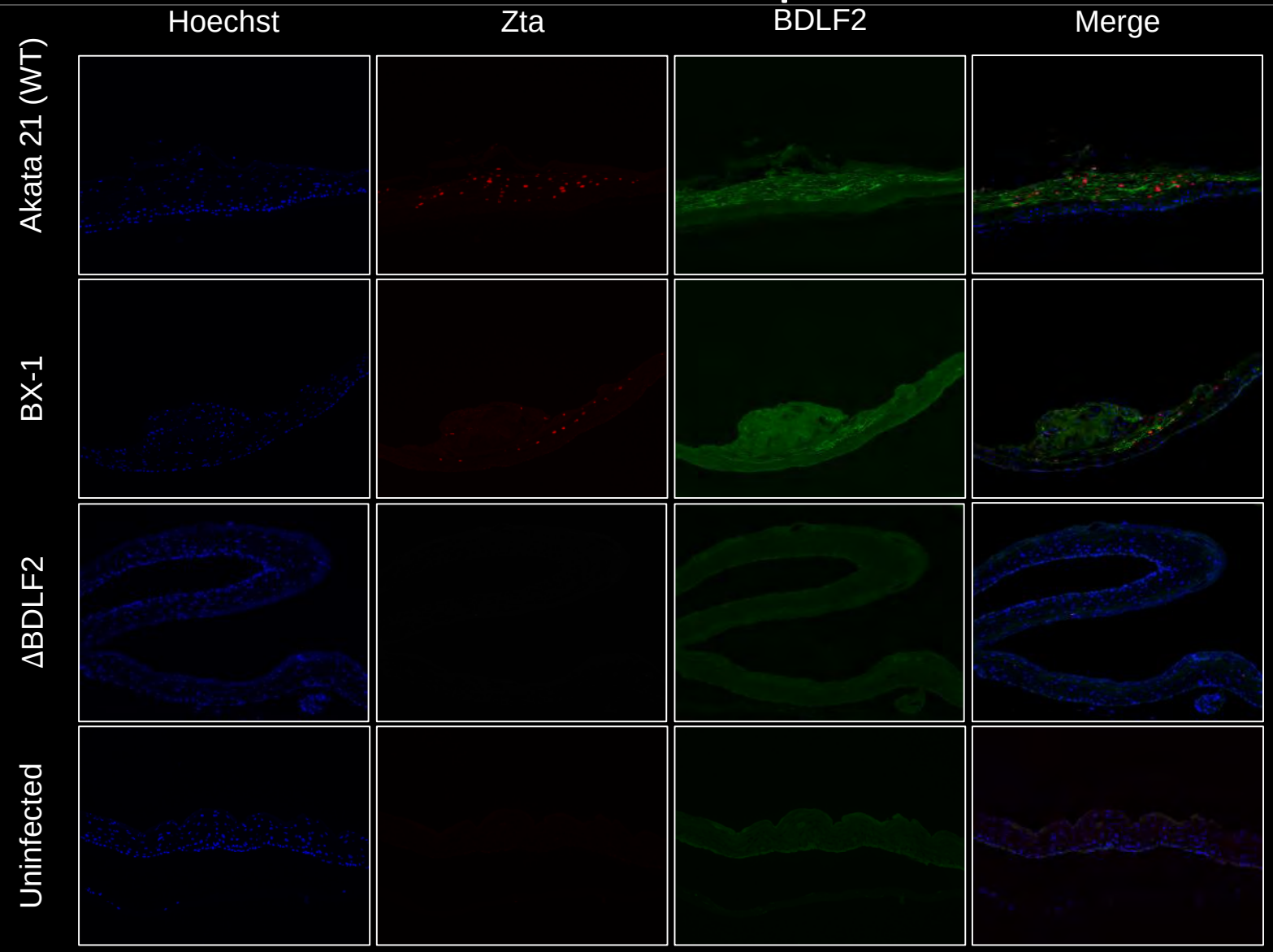


BX-1 and ΔBDLF2 Viruses Infect Similarly in Monolayer





Infection with ΔBDLF2 is Impaired in Raft Culture



Primary Conclusions Thus Far

- BDLF2 does not appear necessary for monolayer / suspension infection
- BX-1 and ΔBDLF2 Akata strains produce similar titers of virus
- EBV BDLF2 appears to play role in viral spread in differentiated epithelium

Acknowledgements

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Questions?