"ER stress-induced RAD21 cleavage disrupts Cohesin-dependent conformational control of KSHV latency"

Alessandra De Leo

Dr. Paul Lieberman Lab

THE WISTAR INSTITUTE

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KSHV-associated cancers

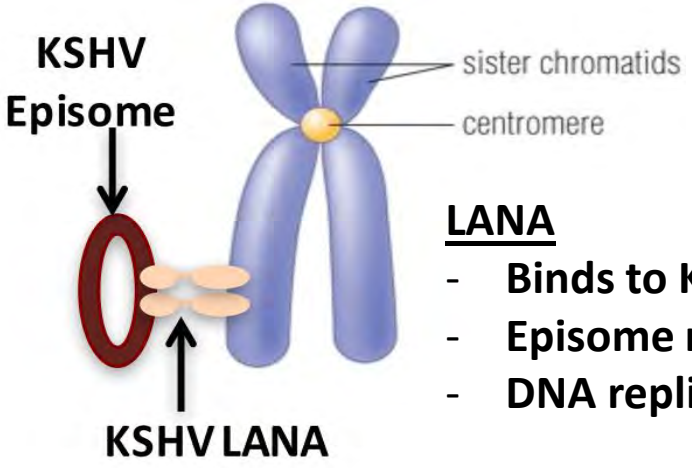
KSHV
(HHV8)

Kaposi's Sarcoma

Kaposi's Sarcoma (KS)

Primary effusion lymphoma (PEL)

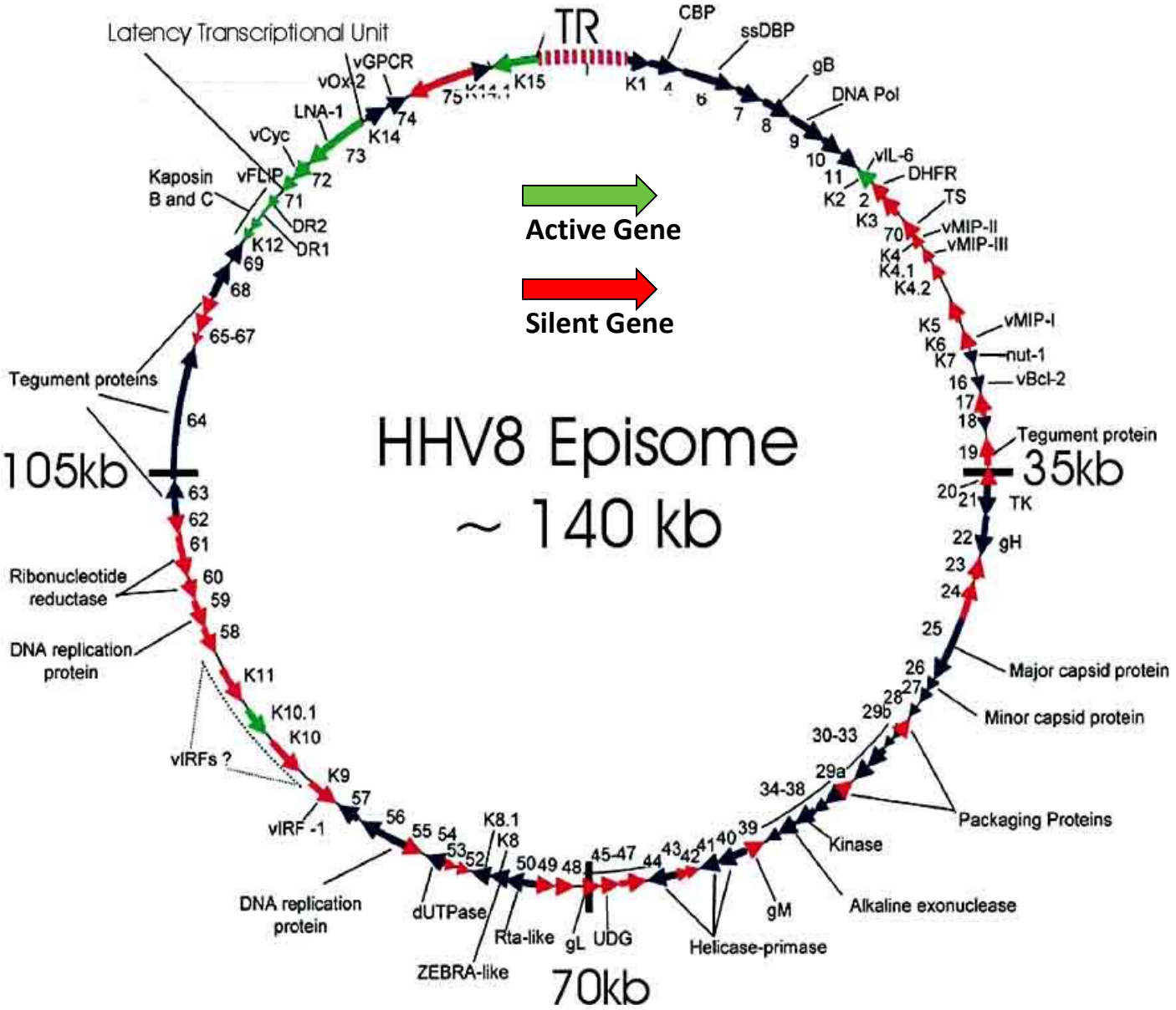
Multicentric Castleman's disease



Episome
- **Circular**
- **Stable**
- **Chromatin-like**

LANA
- **Binds to KSHV TR**
- **Episome maintenance**
- **DNA replication**

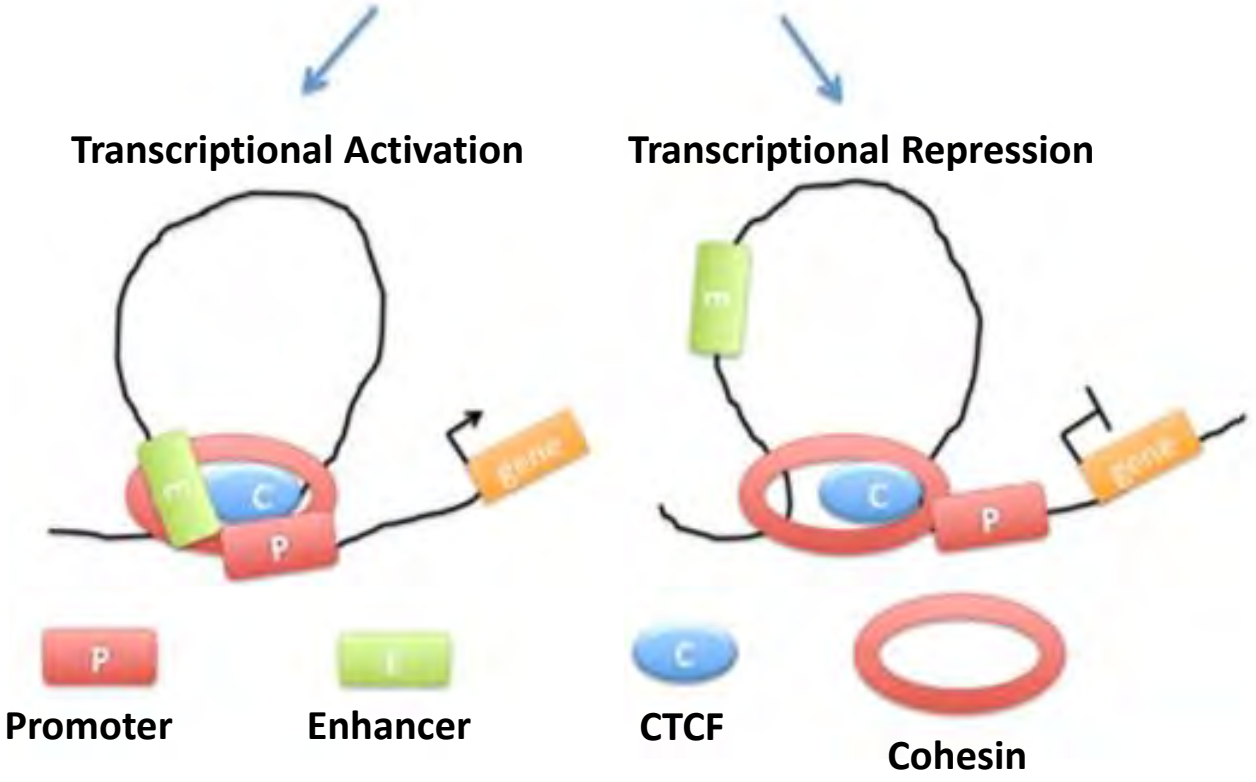
KSHV (HHV8) Latency:
Restricted Viral Gene Expression



Chromatin Organizing Factors: CTCF and Cohesin

-chromatin boundary/insulators
-DNA loop formation for promoter/enhancer interactions
-sister chromatid cohesion

Chromatin loop formation by CTCF and Cohesin

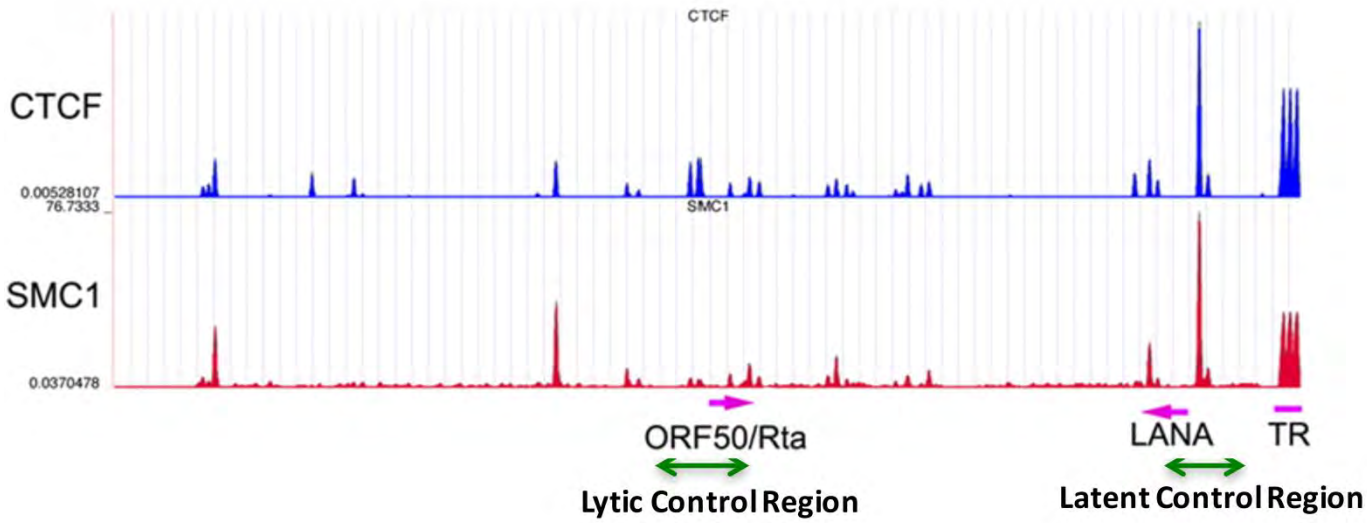


CTCF & Cohesin Binding on KSHV Genome

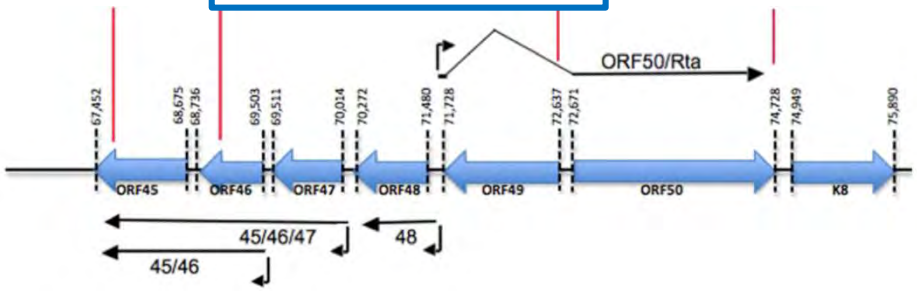
DNA Looping

ChIP-seq Analysis

KSHV (HHV8) latent genome



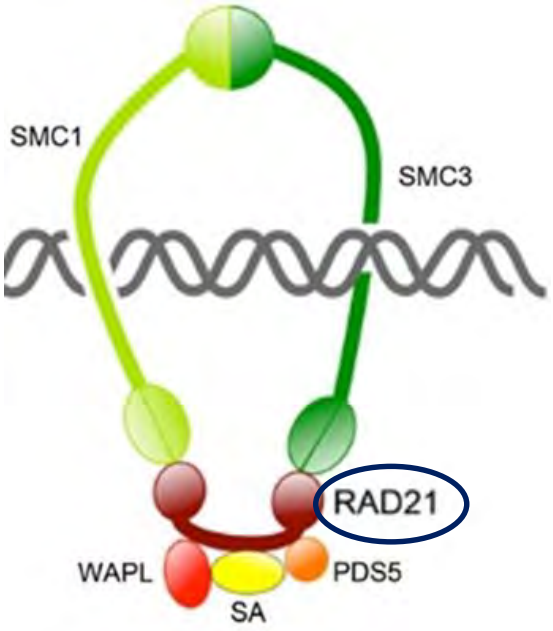
Lytic Control Region



Latency Control Region

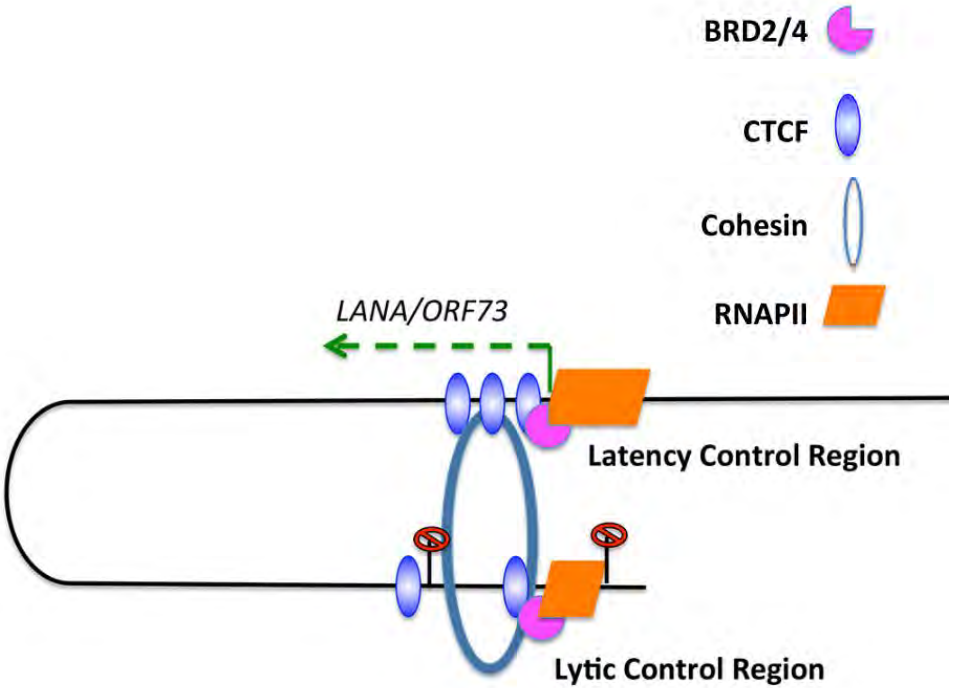
Cohesins (Rad21) Restrict KSHV Lytic Reactivation

Cohesin complex



The cohesin complex consists of four core subunits:
SMC1 and SMC3, RAD21, SA

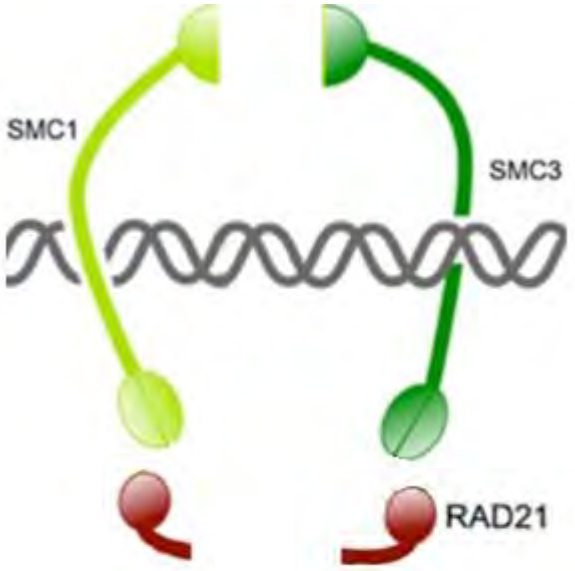
Chromatin fibres are encircled by cohesin but there is no direct binding to DNA



KSHV Latency phase

Caspase proteolysis of the cohesin component RAD21

- Cleavage of Rad21 occurs during apoptosis induced by diverse stimuli.
- Induction of apoptosis results in generation of 64- and 60-kDa RAD21 cleavage products.
- The apoptotic cleavage site is distinct from mitotic cleavage sites.



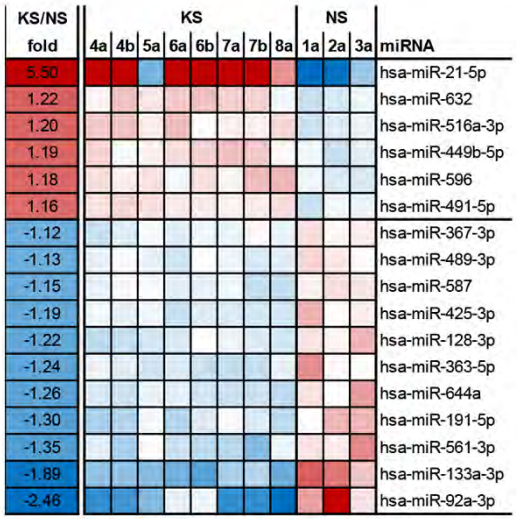
KSHV Lytic Reactivation?

ER stress/Unfolded Protein Response (UPR) signal pathways are altered in KSHV KS

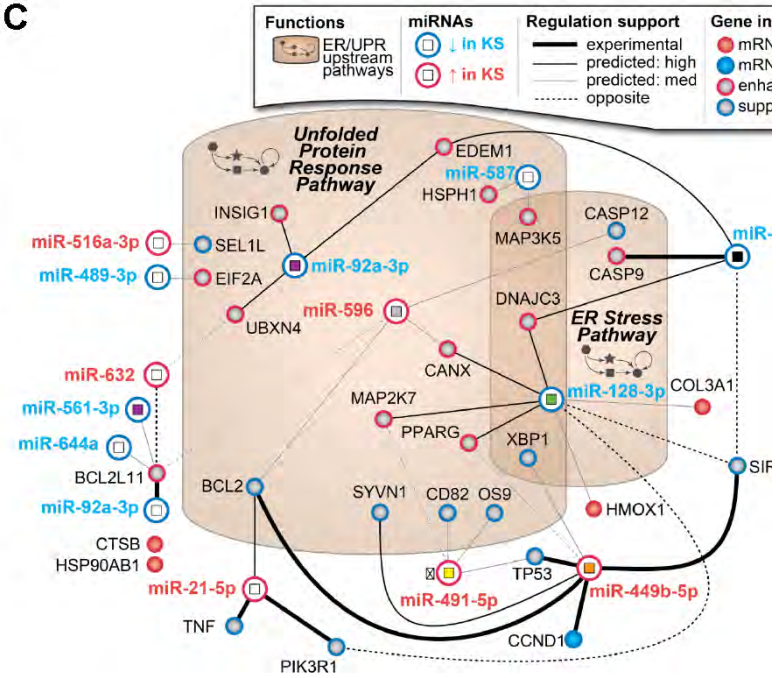
A

KS/NS fold	KS expr	KS a	KS b	NS a	Symbol
117	117				COL4A1
40	40				C1QA
34	171				HMOX1
31	31				TIMP1
28	28				COMP
22	22				CYBA
22	22				ATOX1
19	19				IFITM2
18	18				PLTP
18	18				HSP90AB1

B

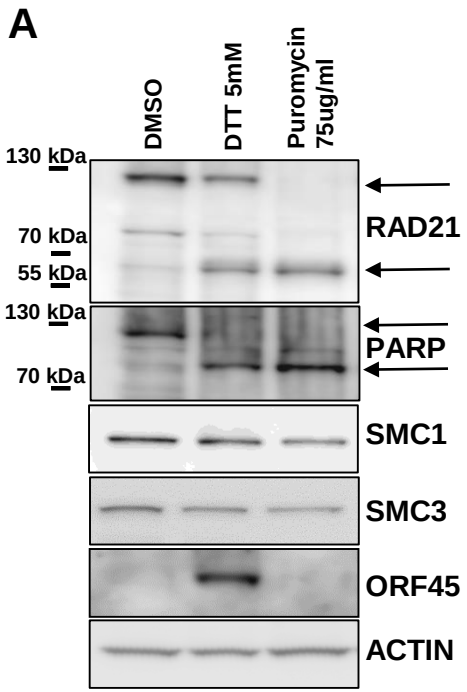


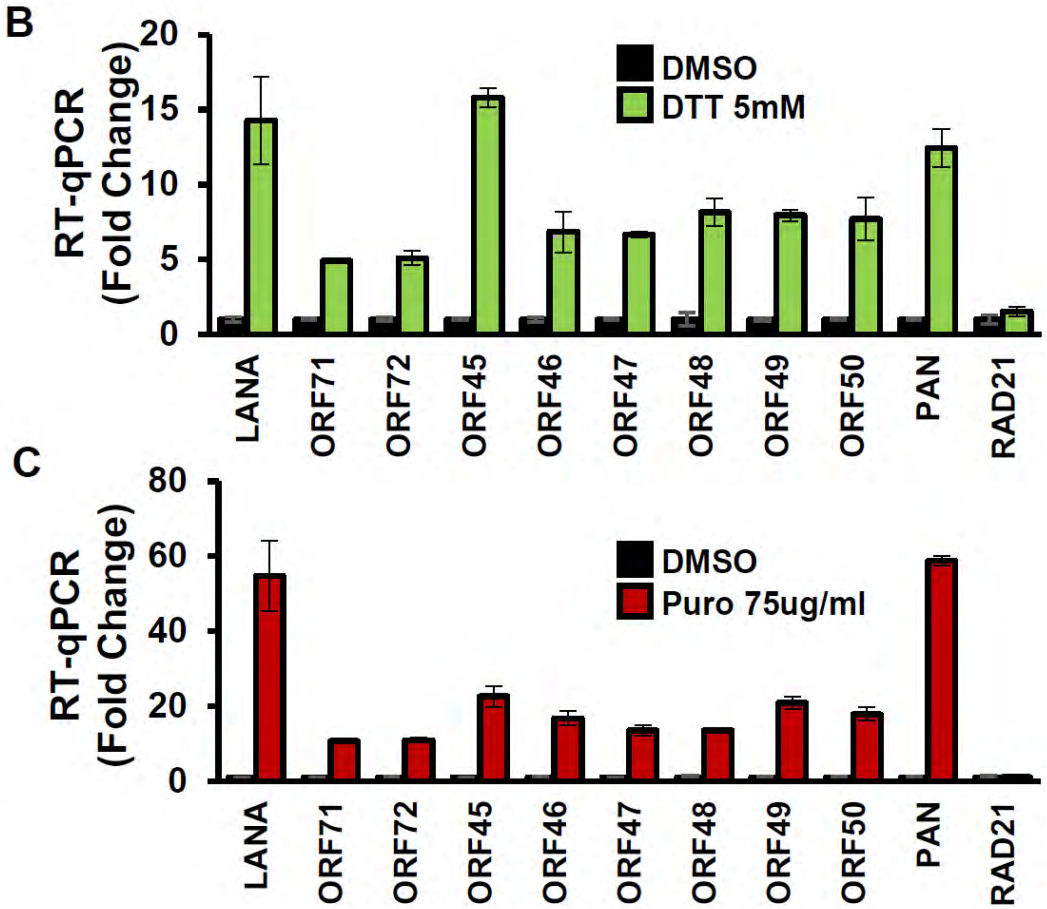
C



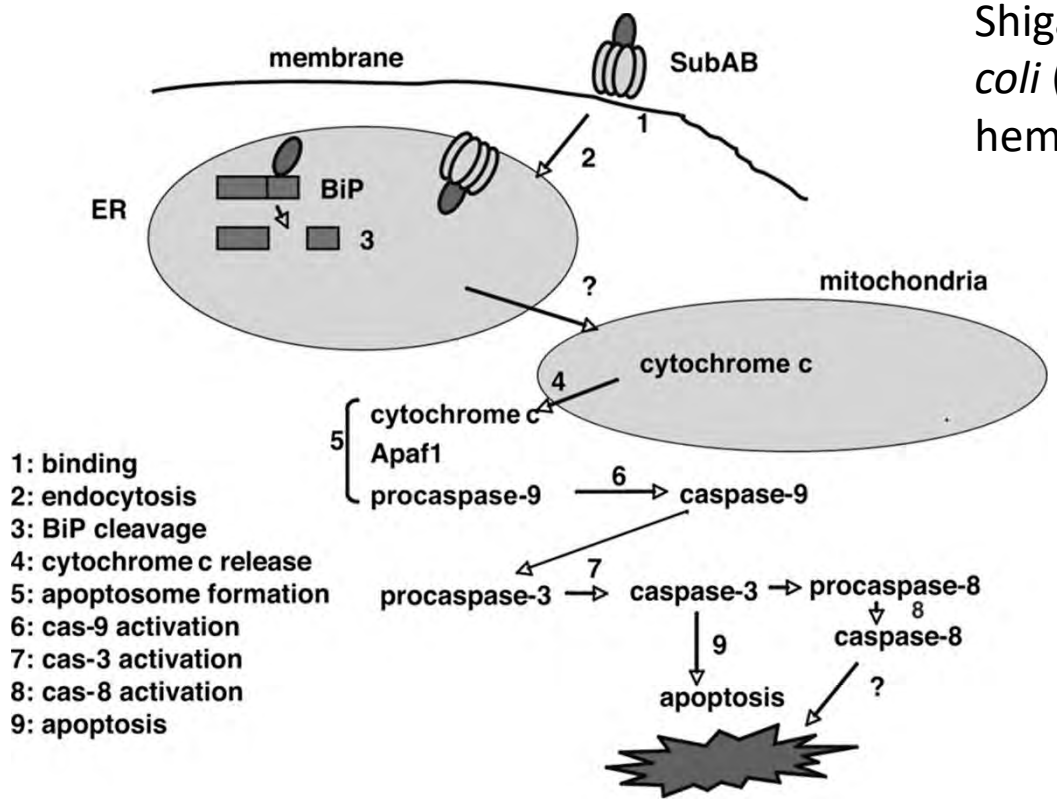
Metadata Analysis of mRNA and miRNA expression in KS compared to normal skin reveals role of ER stress /UPR pathways!

ER stress inducers promote Rad21 cleavage and KSHV reactivation





Subtilase Cytotoxin (SubAB) Produced by Shiga-Toxigenic *Escherichia coli* Induces ER stress and Apoptosis

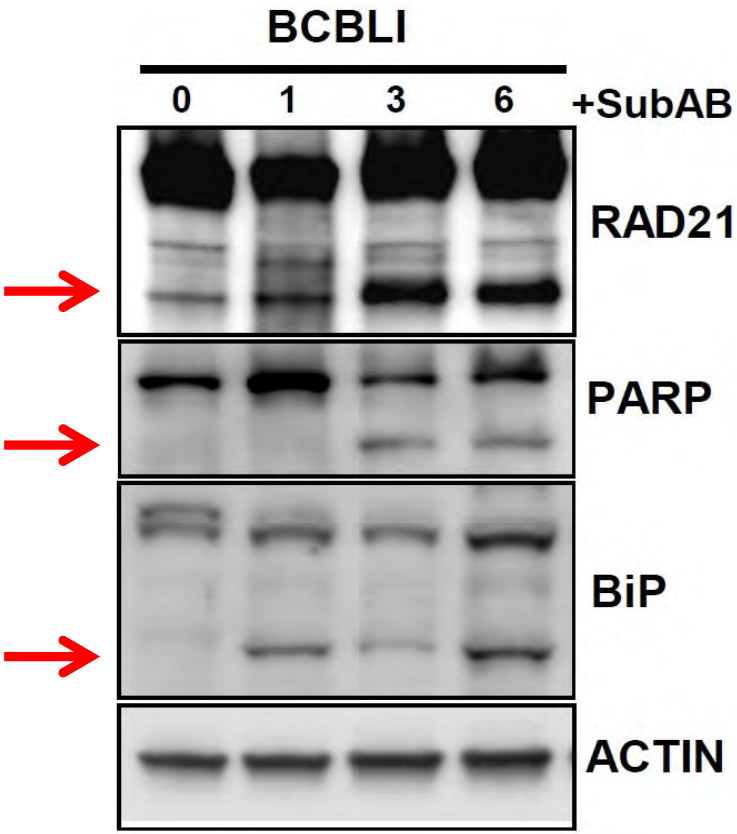


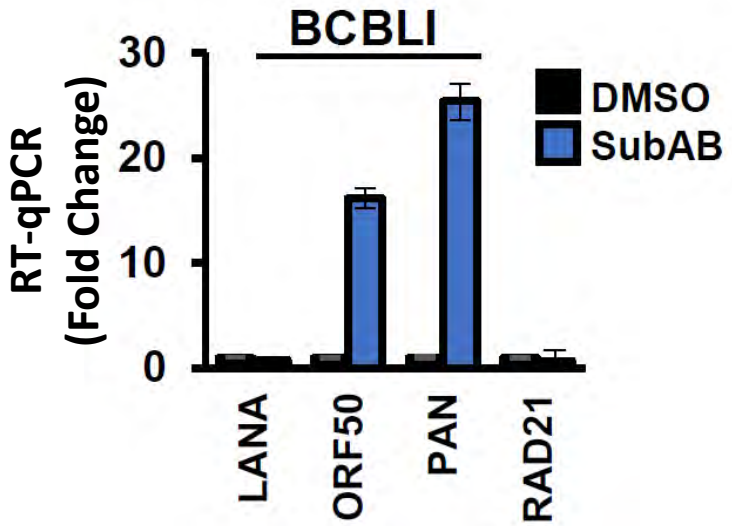
Shiga-toxigenic *Escherichia coli* (STEC) is an etiologic agent of hemorrhagic colitis.

Infection is primarily acquired through ingestion of contaminated food or water or by transmission from animal carriers or infected humans

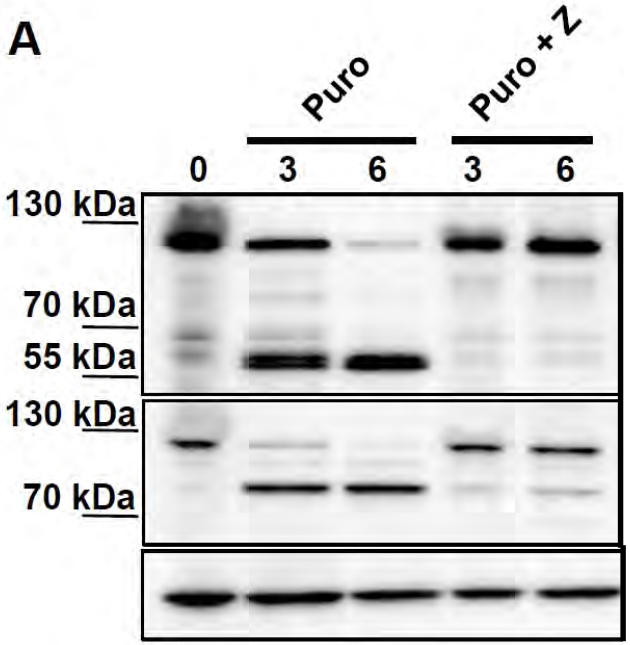
Infect. Immun. **July 2009** vol. 77 no. 7 **2919-2924**

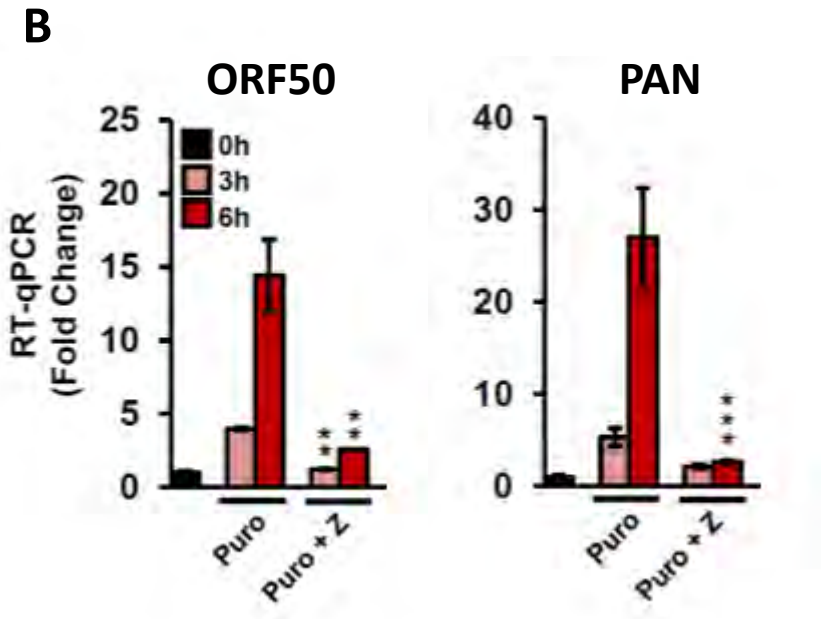
SubAB induces RAD21 cleavage and KSHV lytic genes transcription in PEL cells





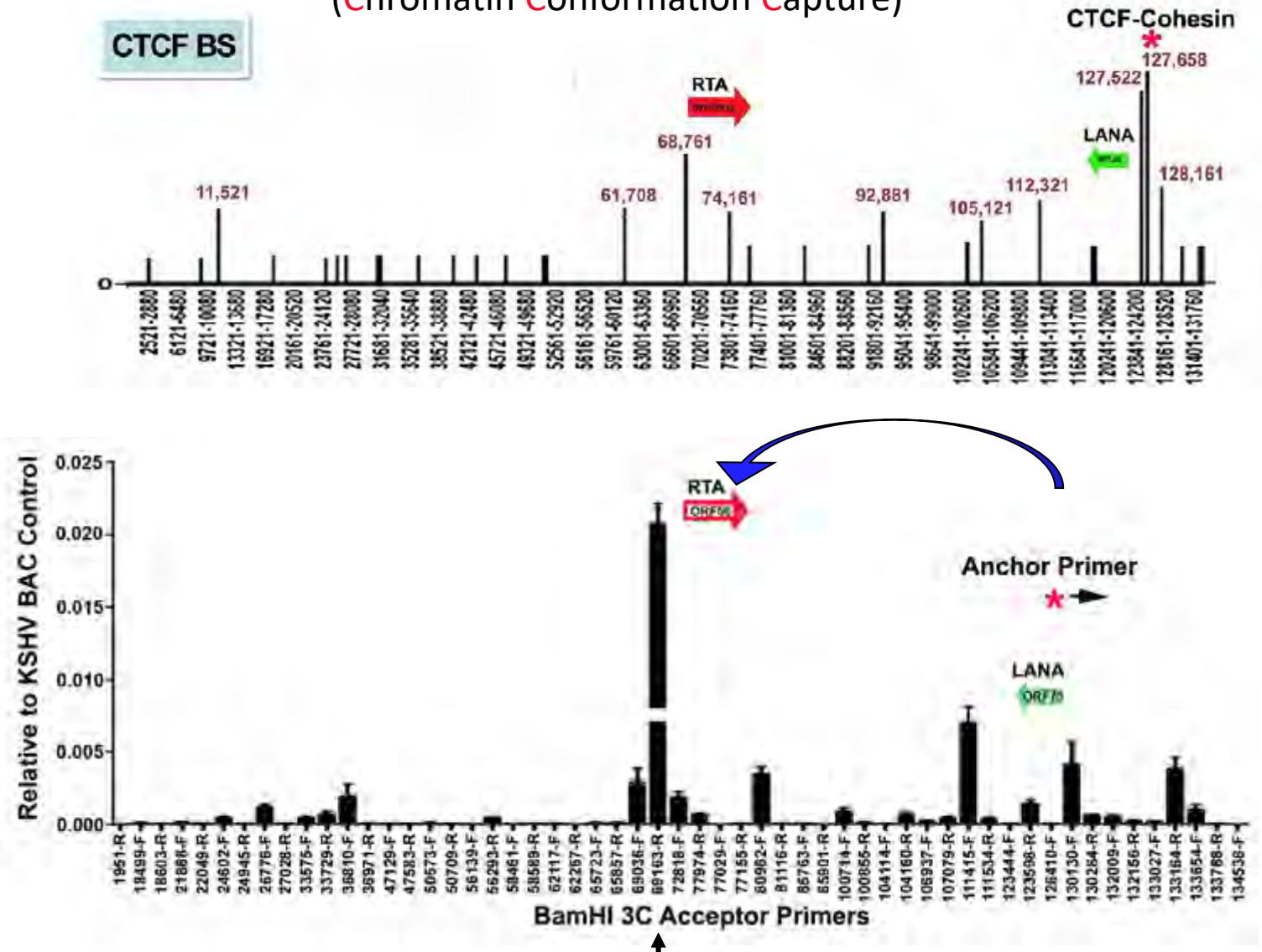
Effects of caspase inhibitors on Rad21 cleavage and KSHV replication





KSHV Genome-Wide 3C

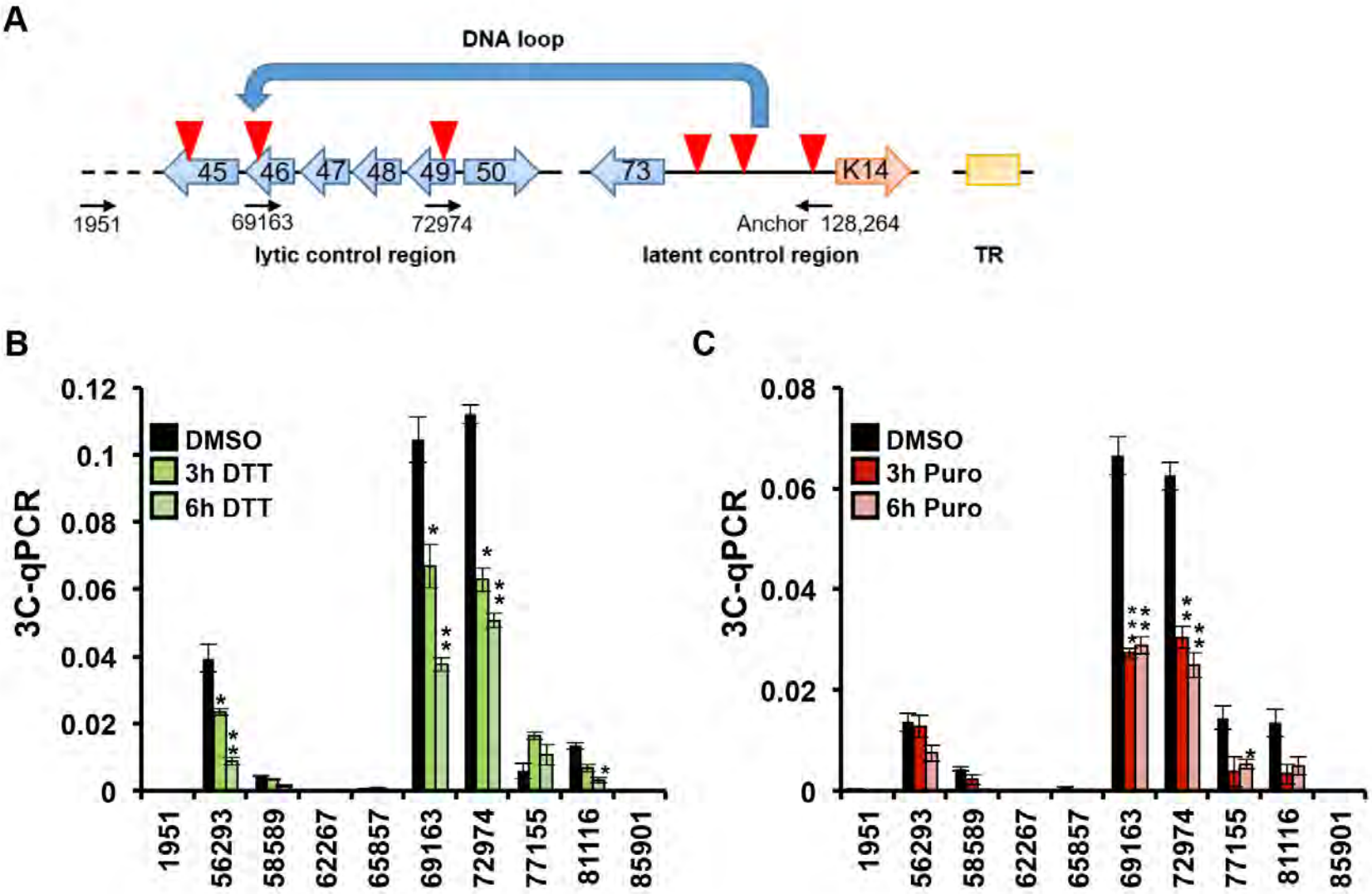
(Chromatin Conformation Capture)



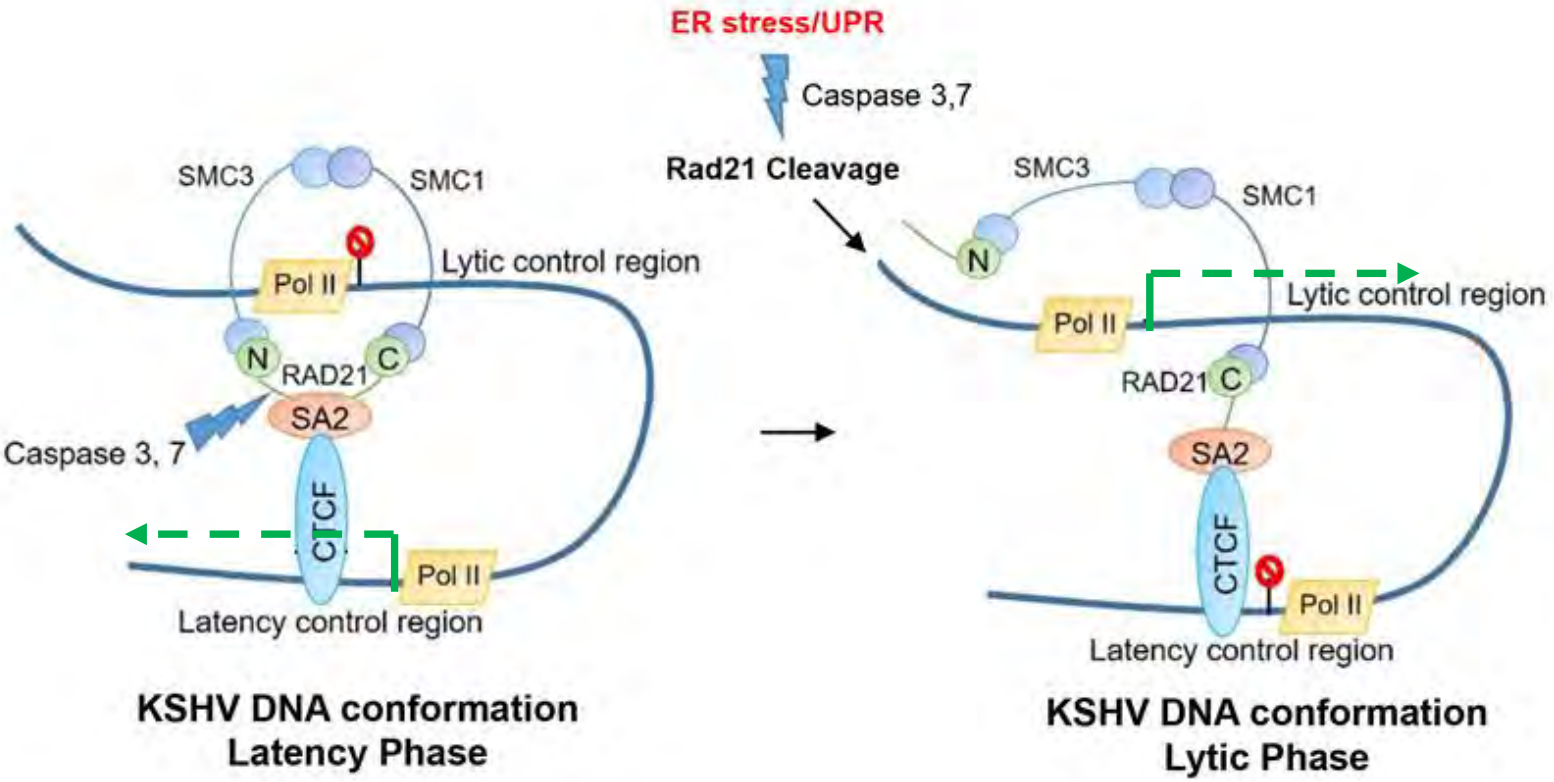
Looping Target Site

Kang et al., 2011 PLoS Path

Rad21 cleavage promotes DNA-loop disruption between KSHV latent and lytic control regions



Model of Rad21 cleavage and KSHV lytic induction by ER stress inducers



Conclusions

- **ER stress/UPR inducers lead to rapid degradation of RAD21 and KSHV lytic reactivation in PEL cells**
- **The extent of Rad21 cleavage correlates with the amplitude of KSHV reactivation**
- **Pan-caspase inhibitor prevents RAD21 cleavage and inhibits viral reactivation**
- **ER stress-induced Rad21 cleavage causes the opening of the cohesin ring**
- **An open cohesin ring structure fails to preserve the linkage between the KSHV latency and lytic control regions essential to keep the latency status**

Acknowledgments

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Wistar Flow Cytometry facility

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