



Sup Fig. 1 - LANA expression after primary infection of differentiated gingival epithelial cells. Primary human oral keratinocyte cultures were induced to differentiate using high calcium for 24 hours. The differentiated cell culture was infected with KSHV and 24 hpi the culture was fixed and stained for KSHV LANA (green) using the LN53 anti-LANA monoclonal antibody. Nuclei were labeled with TO-PRO-3 (blue). Cytoplasmic LANA staining was observed in a small percentage of cells that had a migratory morphology. The intensity of the images was increased to visualize the punctate nuclear staining pattern of LANA in a subset of cells.

A) KSHV LANA: All Modifications

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1  MAPPGMRLRS GRSTGAPLTR GSCRKRNRSP ERCDLGNDLH LQPRRKHVAD SVDGRECGPH
61  TLPIPGSPTV FTSGLPFVSV SPTLPVAPIP SPAPATPLPP PALLSPVTTS SSPIPPSHPV
121 SPGTTDTHSP SPALPPTQSP ESSQRPLSS PTGRPDSSTP MRPPPSQOTT PPHSPTTPPP
181 EPPSKSSPDS LAPSTLRSLR KRRLSSPQGP STLNPICQSP VVSPPRCDFR NRSVYPPWAT
241 ESPIYVGSS DGDTPPRQPP TSPISIGSSS PSEGSWGDDT AMLVLLAEIA EEASKNEKEC
301 SENNQAGEDN GDNEISKESQ VDKDDNDNKD DEEEQETDED DEEDDEEDDE EDDEEDDEED
361 DEEDDEEDDE EDDEEDDEED DEEDDEEED EDEEEEEDE EDDDDDEDNED EEDDEEEDKK
421 EDEEDGGDGN KTLISIQSSQ QQEPQQQEPQ QQEPQQQEPL QEPQQQEPQ QEPQQQEPQ
481 QEPQQQEPQ QEPQQQEPQ REPQQQEPQ REPQQQEPQ REPQQREPQ REPQQREPQ
541 REPQQREPQ QEPQQQEPQ QEPQQQEPQ QEPQQQEPQ QDEQQQDEQ QDEQQQDEQ
601 QDEQQQDEQ QDEQQQDEQ QDEQQQDE QQQQDEQQQ DEQQQEEQEQ QEEQEELEE
661 QEQELEEQEQ ELEEQELEE EQELEELEE QELEEQEQEL EEQEELEE QEELEELEE
721 LEEQEELEE QEELEELEE ELEEQELEE EQELEELEE QELEEQEQEL EEQEELEE
781 EQELEELEE LEEQEELEE QEELEELEE EQELEELEE EQEEEQEELE EEEQEELEE
841 EQEELEE EV EEQEELEE VEEQEELEE EVEEQEQGV EQEETVEE PIILHGSSE
901 DEMEVDPVV STHEQIASSP PGDNTDPPP QPGPSREYRY VLRTSPPHRP GVMRRVPVT
961 HPKKPHRYQ QPPVYRQID DCPAKARPH IFYRRFLGKD GRRDPKCQWK FAVIFWGNP
1021 YGLKKLSQAF QFGGVKAGPV SCLPHLGPDQ SPITYCVYVY CQNKDTSKKV QMARLAWAS
1081 HPLAGNLQSS IVKFKKPLPL TQPGENQGGP DSPQEMT

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B) KSHV LANA – Modifications in isoforms in upper band-gel region 1

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1  MAPPGMRLRS GRSTGAPLTR GSCRKRNRSP ERCDLGNDLH LQPRRKHVAD SVDGRECGPH
61  TLPIPGSPTV FTSGLPFVSV SPTLPVAPIP SPAPATPLPP PALLSPVTTS SSPIPPSHPV
121 SPGTTDTHSP SPALPPTQSP ESSQRPLSS PTGRPDSSTP MRPPPSQOTT PPHSPTTPPP
181 EPPSKSSPDS LAPSTLRSLR KRRLSSPQGP STLNPICQSP VVSPPRCDFR NRSVYPPWAT
241 ESPIYVGSS DGDTPPRQPP TSPISIGSSS PSEGSWGDDT AMLVLLAEIA EEASKNEKEC
301 SENNQAGEDN GDNEISKESQ VDKDDNDNKD DEEEQETDED DEEDDEEDDE EDDEEDDEED
361 DEEDDEEDDE EDDEEDDEED DEEDDEEED EDEEEEEDE EDDDDDEDNED EEDDEEEDKK
421 EDEEDGGDGN KTLISIQSSQ QQEPQQQEPQ QQEPQQQEPL QEPQQQEPQ QEPQQQEPQ
481 QEPQQQEPQ QEPQQQEPQ REPQQQEPQ REPQQQEPQ REPQQREPQ REPQQREPQ
541 REPQQREPQ QEPQQQEPQ QEPQQQEPQ QEPQQQEPQ QDEQQQDEQ QDEQQQDEQ
601 QDEQQQDEQ QDEQQQDEQ QDEQQQDE QQQQDEQQQ DEQQQEEQEQ QEEQEELEE
661 QEQELEEQEQ ELEEQELEE EQELEELEE QELEEQEQEL EEQEELEE QEELEELEE
721 LEEQEELEE QEELEELEE ELEEQELEE EQELEELEE QELEEQEQEL EEQEELEE
781 EQELEELEE LEEQEELEE QEELEELEE EQELEELEE EQEEEQEELE EEEQEELEE
841 EQEELEE EV EEQEELEE VEEQEELEE EVEEQEQGV EQEETVEE PIILHGSSE
901 DEMEVDPVV STHEQIASSP PGDNTDPPP QPGPSREYRY VLRTSPPHRP GVMRRVPVT
961 HPKKPHRYQ QPPVYRQID DCPAKARPH IFYRRFLGKD GRRDPKCQWK FAVIFWGNP
1021 YGLKKLSQAF QFGGVKAGPV SCLPHLGPDQ SPITYCVYVY CQNKDTSKKV QMARLAWAS
1081 HPLAGNLQSS IVKFKKPLPL TQPGENQGGP DSPQEMT

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C) KSHV LANA – Modifications in isoforms in lower band-gel region 2

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1  MAPPGMRLRS GRSTGAPLTR GSCRKRNRSP ERCDLGNDLH LQPRRKHVAD SVDGRECGPH
61  TLPIPGSPTV FTSGLPFVSV SPTLPVAPIP SPAPATPLPP PALLSPVTTT SSPIPPSHPV
121 SPGTTDTHSP SPALPPTQSP ESSQRPLSS PTGRPDSSTP MRPPPSQOTT PPHSPTTPPP
181 EPPSKSSPDS LAPSTLRSLR KRRLSSPQGP STLNPICQSP VVSPPRCDFA NRSVYPPWAT
241 ESPIYVGSSS DGDTPPRQPP TSPISIGSSS PSEGSWGDDT AMLVLLAEIA EEASKNEKEC
301 SENNQAGEDN GDNEISSESQ VDKDDNDNKD DEEEQETDED DEEDDEEDDE EDDEEDDEED
361 DEEDDEEDDE EDDEEDDEED DEEDDEEDEE EDEEEEEDEE EDDDDDEDNEE EEDDEEEDKK
421 EDEEDGGDGN KTLSTIQSSQQ QQEPQQQEPQ QQEPQQQEPQ QEPQQQEPQQ QEPQQQEPQQ
481 QEPQQQEPQQ QEPQQQEPQQ REPQQQEPQQ REPQQQEPQQ REPQQREPPQ REPQQREPPQ
541 REPQQREPPQ QEPQQQEPQQ QEPQQQEPQQ QEPQQQEPQQ QDEQQQDEQQ QDEQQQDEQQ
601 QDEQQQDEQQ QDEQQQDEQE QQDEQQQDE QQQQDEQQQQ DEQQQEEQE QEEQELEE
661 QELEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE
721 LEEQELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE
781 QEELEE QEELEE LEEQELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE QEELEE
841 QEEQELEE QEEQELEE VEEQELEE QEEQELEE QEEQELEE QEEQELEE QEEQELEE QEEQELEE
901 DEMEVDPYVY STHEQIASSP PGDNTDPPP QPGPSREYRY VLRTSPPHRP GVRMRVVPVT
961 HPKKPHPRYQ QPPVPYRQID DCPAKARPOH IFYRRFLGKD GRRDPKCQWK FAVIFWGNDE
1021 YGLKKLSQAF QFGGVKAGPV SCLPHLGPDQ SPITYCVYVY CQNKDTSKKV QMARLAWEAS
1081 HPLAGNLQSS IVKFKKPLPL TQPGENQGGP DSPQEMT

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Supplementary Figure 2: Amino acids associated with post translational modifications of ORF73 LANA mapped onto the sequence of the BCBL-1 LANA isoform (ADQ57959).

Sequence of 1117aa LANA isoform in KSHV strain in BCBL-1 cells with A) all known modifications, B) modifications detected in KSHV isoforms present in upper band in gel region 1, C) modifications detected in KSHV isoforms present in lower band in gel region 2 (see Figure 11).

Legend:

Blue highlight – serine and threonine phosphorylation detected in cytoplasmic LANA (this study)

Black highlight– arginine and lysine methylation detected in cytoplasmic LANA (this study)

Red highlight – arginine methylation detected in recombinant LANA (2) and cytoplasmic LANA (this study)

Green highlight – PIM kinase associated serine phosphorylation (1, 3)

Underlined region – RING3-associated serine/threonine phosphorylation (4)

1. **Bajaj, B. G., S. C. Verma, K. Lan, M. A. Cotter, Z. L. Woodman, and E. S. Robertson.** 2006. KSHV encoded LANA upregulates Pim-1 and is a substrate for its kinase activity. *Virology* **351**:18-28.
2. **Campbell, M., P. C. Chang, S. Huerta, C. Izumiya, R. Davis, C. G. Tepper, K. Y. Kim, B. Shevchenko, D. H. Wang, J. U. Jung, P. A. Luciw, H. J. Kung, and Y. Izumiya.** 2012. Protein arginine methyltransferase 1-directed methylation of Kaposi sarcoma-associated herpesvirus latency-associated nuclear antigen. *J Biol Chem* **287**:5806-5818.
3. **Cheng, F., M. Weidner-Glunde, M. Varjosalo, E. M. Rainio, A. Lehtonen, T. F. Schulz, P. J. Koskinen, J. Taipale, and P. M. Ojala.** 2009. KSHV reactivation from latency requires Pim-1 and Pim-3 kinases to inactivate the latency-associated nuclear antigen LANA. *PLoS pathogens* **5**:e1000324.
4. **Platt, G. M., G. R. Simpson, S. Mittnacht, and T. F. Schulz.** 1999. Latent nuclear antigen of Kaposi's sarcoma-associated herpesvirus interacts with RING3, a homolog of the *Drosophila* female sterile homeotic (*fsh*) gene. *J Virol* **73**:9789-9795.