Supplementary Materials and Methods

Cell culture and transfection. BC-3 cells were cultured in RPMI 1640 medium with 15% fetal bovine serum. HeLa cell and 293T cells were maintained in DMEM with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C with 5% CO_2. BC-3 cells (1×10^6 cells/ml) were induced with 40 ng/ml 12-0-tetradecanoylphorbol 13-acetate (TPA) and 3 mM sodium butyrate (Sigma, St. Louis, MO). HeLA and 293T cells were transfected with different plasmid DNAs using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions.

Quantitative real-time PCR analysis. Total RNA was isolated by Trizol (Invitrogen). cDNA for real-time PCR was generated from 5 μg total RNAs with the First Strand cDNA Synthesis Kit (Invitrogen). The primer sequences for qPCR are listed in supplemental table 1.

Detection and quantification of KSHV DNA. Genomic DNA was purified using DNeasy Kit (QIAGEN). Real-time PCR was performed for the quantification of KSHV DNA by ORF26 region.

Northern blotting. Total RNA was isolated from BC-3 and 293T cells with Trizol (Invitrogen) and separated in 14% acrylamide gel with 7.5 M urea. The RNA was

transferred and hybridized with a ^{32}P-labeled, single-stranded antisense sequence of miR-K3.

Immunoblotting. BC-3 cells were lysed in MPER buffer containing protease inhibitor (Roche). Equal total proteins were assessed with primary antibodies to NFIB (Active Motif) and GAPDH (Santa Cruz Biotechnology Inc.) followed by HRP-conjugated secondary antibodies.

FACS analysis. BC-3 cells were treated with DMSO or NaB for 24 hours, fixed, permeabilized and stained with a mouse monoclonal antibody recognizing KSHV ORF59 antibody (Advanced Biotechnologies Inc., Colombia, MD, USA) and a goat anti-mouse antibody conjugated to *Alexa* Fluor® 488 (Invitrogen). The ORF59 expression was quantified by the mean fluorescence intensity (MFI) of *Alexa* Fluor® 488 dye.

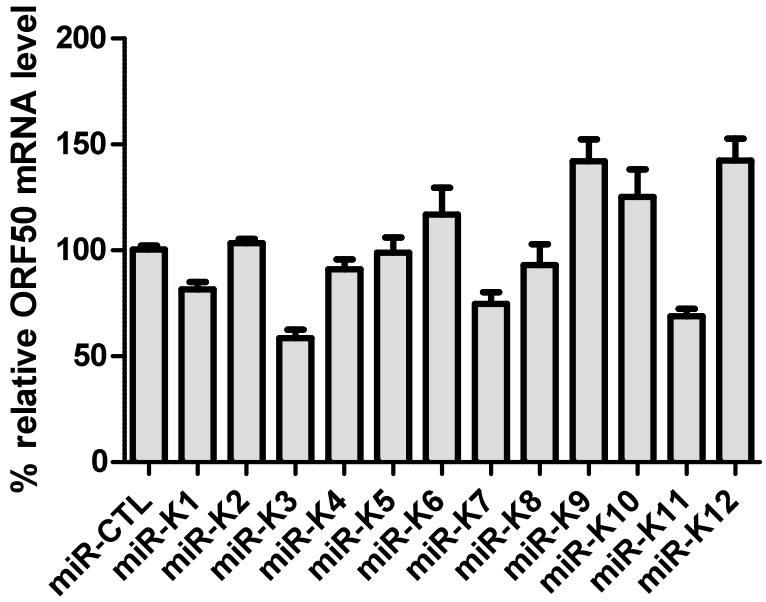
Legends for Supplementary Figures

Supplementary Figure 1 | KSHV miRNAs regulate viral life cycle. BC-3 cells were transduced with lentiviruses expressing various KSHV miRNAs, selected for miRNAs stable clones with zeocin, and analyzed by RT-qPCR analysis for mRNA levels of the viral lytic gene, ORF50. Data are normalized to the mRNA level in cells transduced with control miRNA (miR-CTL). All data are normalized to the internal control GAPDH. GAPDH was used as an internal control. The results represent the mean ± [SEM] of seven independent experiments.

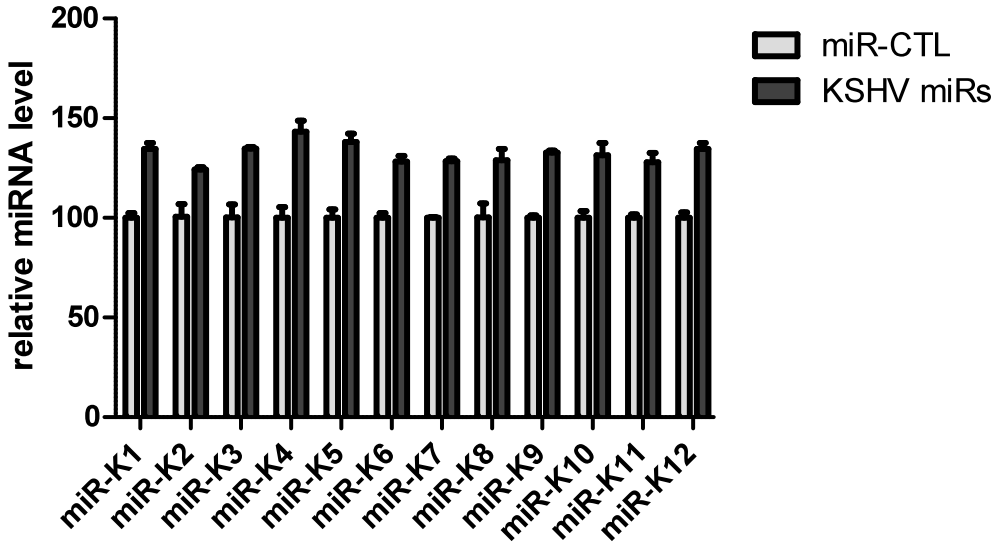
Supplementary Figure 2 | Expression of miRNAs in latently infected cells. BC-3 cells were transduced with lentiviruses expressing various KSHV miRNAs and selected with zeocin. miRNA expression was monitored by a commercially available kit, QuantiMir RT *kit* (System Biosciences, Mountain View, CA).

Supplementary Figure 3 | Potential binding sites of NFIB on ORF50's promoter. TGGC(N5)GCCC site is shown in red. Lower affinity sites are shown in blue (TGGCA) and in grey (TTGGC).

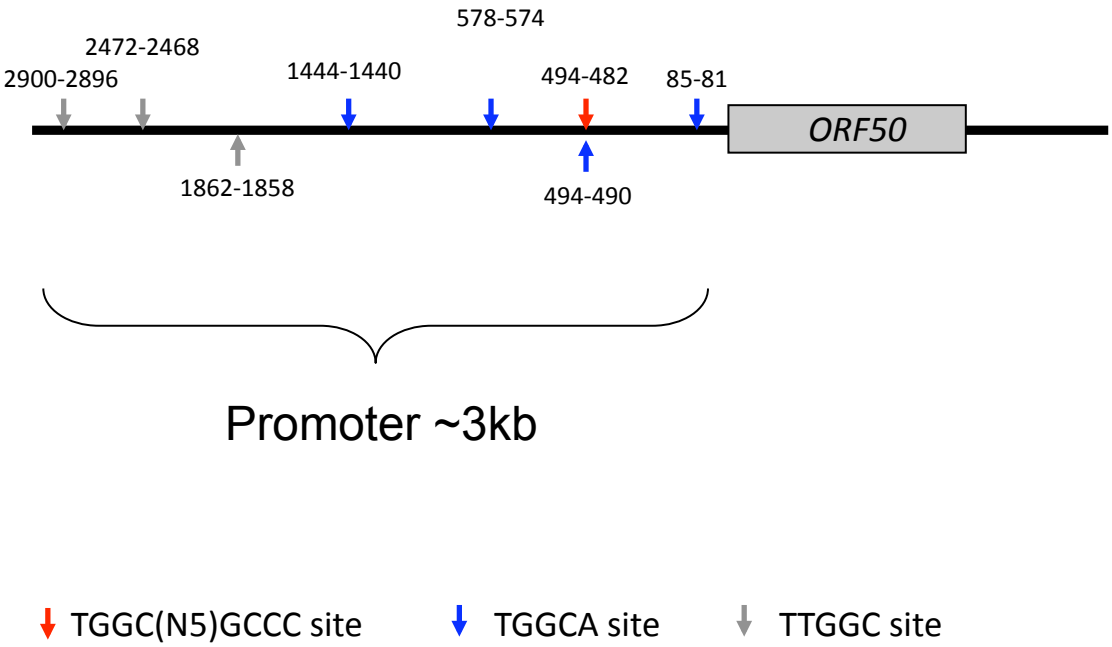
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table 1: Primer Sequences

ORF50	Forward	5′-AGACCCGGCGTTTATTAGTACGT-3′
	Reverse	5′-CAGTAATCACGGCCCCTTGA-3′
ORF59	Forward	5′-TTGGCACTCCAACGAAATATTAGAA-3′
	Reverse	5′-CGGGAACCTTTTGCGAAGA-3′
NFIB	Forward	5’- ATGACCCATCCAGTCCACA-3’
	Reverse	5’-CACTTGGAAAGGAACCAAGC-3’
ORF26 DNA	Forward	5'-AGCCGAAAGGATTCCACCAT-3'
	Reverse	5' TCCGTGTTGTCTACGTCCAG-3'