Supporting Information

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SI Materials and Methods

Primers. Primers for KSHV ORF49 (Forward: ACAAAATGGGAGAGGCACCA; Reverse: GCGGCCCTGGAATCAGA) and ORF57 (Forward: TGGACATTATGAAGGGCATCCTA; Reverse: CGGGTTCGGACAATTGCT). All activation increases were normalized to glyceraldehyde-3-phosphate-dehydrogenous (GAPDH) (Forward: GAAGGTGAAGGTCGGAGT; Reverse: GAAGATGGTGATGGGATTTC) expression. IFN and NF-κB gene transcription was determined using primers IFN-α (Forward: GTGAGGAAATACTTCCAAAGAATCAC; Reverse:TCTCATGATTTCTGCTCTGACAA), IFN-β (Forward: CAGCAATTTTCAGTGTCAGAAGC; Reverse: TCATCCTGTCCTTGAGGCAGT), NF-kB1 (Forward: GGTGAAGGGAGACCTGGCTT; Reverse: GTGCCTCAGCAATTTCTGGC), NF-kB2 (Forward: TTCTGAAGGCTGGTGCTGAC; Reverse: AGTGAGGTCAAGAGGCGTGT), IFN-γ (Forward: TCAGCTCTGCATCGTTTTGG; Reverse: GTTCCATTATCCGCTACATCTGAA).

Western Blot Procedure. Cell lysates were prepared in RIPA lysis buffer. Proteins were resolved on either 8% or 10% SDS-PAGE gels for TLR or vIL-6 expression, respectively, and transferred to nitrocellulose membranes. Membranes were incubated in either 5% nonfat milk (NFDM) or bovine-serum albumin (BSA) for 1 h at RT, followed by 3× washes in 1× TBS, 0.1% Tween buffer. TLR7 and TLR8 (Abcam) antibodies were incubated overnight at 4 °C in 5% NFDM. vIL-6 (ABI) and β-tubulin (Sigma) antibodies were incubated at 1:2,000 overnight in 5% NFDM. IRF-7 (Cell Signaling) antibody was incubated at 1:50 overnight in 5% BSA. The blots were probed with either anti-mouse (Jackson Laboratories) or anti-rabbit (Cell Signaling) horseradish-peroxidase conjugated secondary antibodies at 1:2,500 and-1:2,000 in 5% NFDM or BSA. IκB-α (Cell Signaling) was used at 1:1,000 in 5% BSA overnight at 4 °C. Flag-tagged IRF-7DN was detected with an anti-flag M2 antibody (Sigma) at 1:10,000 in 1× TBS, 0.1% Tween buffer.

Immunofluorescence Assay. BCBL-1 cells were stimulated with 50 μg/mL ss-PolyU or TPA (20 ng/mL) for 24 h followed by fixation onto microscope slides with 1% paraformaldehyde for 30 min. Next, fixed cells were washed with 1% BSA, 1× PBS and permeabilized with a 0.1% Triton-X 100, 1× PBS solution for 20 min at room temperature. Slides were washed twice in 1× PBS and then KSHV ORF59 antibody was added at a dilution of 1:50 in 1% BSA, 1× PBS overnight at 4 °C in a humidified chamber. Next, slides were washed 2× in PBS and FITC-conjugated anti-mouse (Sigma) was added at 1:50 in 1%BSA, 1× PBS for 1 h at RT. Slides were washed 2× and stained with DAPI (4,6-diamidino-2-phenylindole) nuclear stain (0.5 μg/mL in water) for 5 min at RT, followed by one wash. Coverslips were affixed with Vectorshield (Vector Laboratories). ORF59 and DAPI staining was visualized using Nikon Microphoto FXA upright fluorescence microscope.

Promoter Reporter Assays. 5×10^6 BCBL-1 cells were nucleofected using the T-01 Amaxa program and B-cell specific nucleofection kit (Amaxa) with either 200 ng pGL3-ELAM-luc NF-κB promoter luciferase reporter plasmid (Addgene) or 5 μg KSHV ORF50 promoter luciferase reporter plasmid. Forty-eight hours postnucleofection, cells were pooled and stimulated with TLR agonists as described above. Sixteen hours post-TLR stimulation, total cell protein was harvested and luciferase expression quantified as previously described (1). Fold relative luciferase units were calculated over mock treated samples.

1. West J. Damania B. (2008) Upregulation of the TLR3 pathway by Kaposi's sarcomaassociated herpesvirus during primary infection. *J Virol* 82:5440–5449.

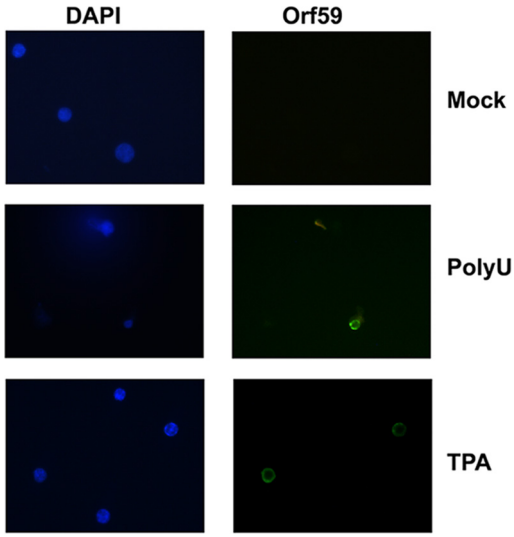

Fig. S1. ssPoly-U treatment induces the expression of KSHV lytic proteins. Immunofluorescence of KSHV ORF59 was performed 48 h after ssPoly-U treatment. Nuclei were stained with DAPI. Pictures are depicted at 60× magnification.

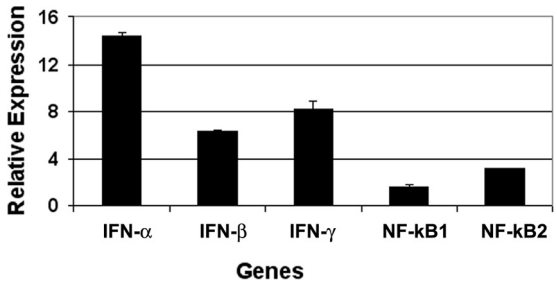

Fig. S2. VSV infection of PEL activates IFN and NF-kB gene transcription. BCBL-1 cells were infected with VSV (MOI of 1). Cells were harvested 24 h postinfection and mRNA levels of IFN-α, β, γ, NF-kB1, and NF-kB2 were determined by qRT-PCR. All values were normalized to GAPDH as the endogenous control.