SUPPORTING ONLINE MATERIALS

MATERIALS AND METHODS

vIL-6 Expression and Proliferation Assays: Recombinant GST-vIL-6 was bacterially expressed as a GST-fusion protein and purified as previously described (*S1, S2*). Extensive experiments with the GST-fusion protein before and after thrombin cleavage show no differences in bioactivity or transcriptional activity (*S2*). Recombinant vIL-6 activity was measured through bioassays performed in B9 cells, an IL-6 dependent murine plasmacytoma cell line (*S3*), showing that recombinant vIL-6 has an approximately one log lower activity than hIL-6 (R & D systems) on a (wt/wt) basis. A lower activity of recombinant vIL-6 compared to hIL-6 is also consistent with that seen by others (*S1*). B9 bioassays were performed by plating B9 cells at a 2 x 10^4 cells/ well in 96 well plates and incubating with serial dilutions of hIL-6 (R & D systems, Minneapolis, MN), vIL-6 or GST for 24 hrs. ^{3}H-thymidine (0.5 µCi/well) was added for 8 hrs and cells were harvested and analyzed for ^{3}H-thymidine uptake using a 96-well scintillation counter (Packard Biosciences, Long Island, NY). BCP-1 and Daudi C-11 proliferation assays were similarly performed using 4 x 10^4 cells/well cultured in RPMI medium with 1% FBS (for low serum assays) or 10% FBS and incubated with recombinant cytokines or GST for 48 hrs in the presence of 0.5µCi/well of ^{3}H-thymidine. Each experiment was performed three times with three replicates (BCP-1 cells) or six replicates (C11 Daudi cells) for each condition within a given experiment.

Protein Expression Analyses: Immunoblotting was performed using standard techniques as previously described (*S4*); 50µg of cell lysate was used for each condition and electrophoresed on 8% (gp130), 10% (gp80) or 15% (vIL-6, p21$^{CIP1/WAF1}$) SDS-polyacrylamide gels. Immunoblotting and development used standard protocols with antibodies to gp80 (1:200 dilution), gp130 (1:200 dilution) (both from Santa Cruz Biotechnologies, Santa Cruz, CA), vIL-6 (1:1000) or p21$^{CIP1/WAF1}$ (1:500 dilution; Transduction Labs, San Diego, CA).
Phosphorylation of gp130 was determined by gp130 immunoprecipitation and immunoblotting for phosphotyrosine as described (*S5*). For this purpose untreated and treated BCP-1 cells were lysed in cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1%SDS, and 1% Nonidet P-40) containing protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride and 1.5 ng/ml aprotinin), phosphatase inhibitors (50 mM NaH_2PO_4, 1 mM Na_3VO_4), and 0.01% NaN_3, sonicated and cleared by centrifugation. 700µg of protein was precleared using 50 µl of protein-A sepharose for 1 hr and incubated with 4 µg of anti-gp130 antibody (Santa Cruz Biotechnology) at 4°C overnight. Immune-complexes were collected with 50 µl protein-A sepharose for 2 hrs at 4°C, washed and resolved on 8% SDS-polyacrylamide gel and immunoblotted using anti-phosphotyrosine antibody (1:1000, Upstate Biotechnologies, Lake Placid, NY) or anti-gp130.
mRNA Expression Analyses: Northern blotting for vIL-6 mRNAs was performed using a random-labeled double-stranded DNA probe corresponding to a PCR-derived vIL-6 coding region from BCP-1 DNA using primers: 5' - TCA CGT CGC TCT TTA CTT ATC GTG - 3' and 5'-CGC CCT TCA GTG AGA CTT CGT AAC- 3'. BC-1 and BCP-1 cells in these experiments were cultured in RPMI medium with 1% FBS, total RNA was extracted from these cells using RNAeasy Midi Kit (Qiagen Inc, Chatsworth, CA) and poly-(A) selected using the Oligotex mRNA Kit (Qiagen). Poly-(A) selected RNAs (500 ng/lane) were gel electrophoresed and blotted as previously described (*S3, S6*). The vIL-6 probe hybridizes to a 1.3-kB product. The same blot was stripped and reprobed for GAPDH as a control for loading. Cycloheximide (CHX) inhibition of protein synthesis was performed on PEL cells treated with 50 µg/ml cycloheximide for 30 mins prior to incubation with 500 U/ml IFN-α or 20 ng/ml TPA for 12 hrs, at which point the cells were harvested and northern blotted as described above.

KSHV micoarray: To generate KSHV gene microarrays, 3'-end 300 bp regions of 87 known KSHV open reading frames (ORFs and control genes were PCR amplified using standard protocols. PCR products were purified and concentrated using the Unifilter 96-well PCR purification system (Whatmann Inc, Ann Arbor, Michigan), resuspended in 200 µl of distilled water, quantitated and then normalized to equal concentrations by spectrophotometry (assistance kindly provided by Sergei Kalachikov, Columbia University Microarray facility). Purified DNAs were stamped at a concentration of 10 ng/spot in triplicate on nitrocellulose membranes using a robotic 96-well pin, replicating arm (Beckman Coulter, Carlsbad, CA). The stamped membranes were denatured (0.8 M NaOH, 2.0 M NaCl), UV- crosslinked, washed in sterile water for 2 mins., prehybridized overnight (5 X SSC, 5 X Denhardt's, 50% formamide, 1% SDS). 1µg of mRNA obtained from BCP-1 cells treated as described, was converted to first-strand cDNA, body-labeled using [α^{32}P] dCTP using HotScribe cDNA synthesis kit (Amersham-Pharmacia Biotech, Piscataway, NJ). Membranes were hybridized overnight in hybridization buffer (5x SSC, 5x Denhardt's, 50% formamide, 1% SDS, 10% dextran sulfate). Membranes were washed (0.2 X SSC, 0.5% SDS) and hybridization measured by phosphorimager using ImageQuant software (Amersham-Pharmacia Biotech, Piscataway, NJ). The sensitivity of this array was determined to be equivalent to detecting 50-100 gene copies per cell using serial dilutions of KSHV cosmid DNA in BJAB cell DNA (data not shown). Validation was also performed by qualitative comparisons to previously published KSHV microarray data (S7) and demonstrated similar patterns of viral gene expression after 48 hour TPA induction (data not shown). Sequences used to generate PCR products are shown in Table S1. Results shown in Fig. 3 represent averages of three independent experiments involving separate BCP-1 mRNA collections and cDNA hybridizations.

Electrophoretic Mobility Shift Assays (EMSA): EMSA were performed as previously described (*S8-S11*). Nuclear extracts and [^{32}P]-labeled 2'-5' oligoadenylate synthetase (OAS) promoter interferon-stimulated response element (ISRE) probe were incubated for 15 min at room temperature in 10 mM Tris (pH 7.9), 50 mM NaCl, 1 mM EDTA, 5% glycerol, 2 mM DTT, 20 mg BSA, and 0.3% NP-40. Protein–DNA complexes were resolved by electrophoresis through 5% acrylamide: bisacrylamide (29:1) gel in 0.5x TBE (40 mM Tris, 45 mM boric acid, and 1 mM EDTA) at 200 V for 3 hr. ISGF3 detection was performed by premixing extracts with anti-STAT-1 and anti-STAT-2 antibodies (generous gifts from Chris Schindler; Columbia University). Cold competition was performed by incubating extracts with 100-fold excess unlabeled OAS probe.

vIL-6 Promoter Analysis: The vIL-6 promoter, a region 761 bp upstream of the vIL-6 gene was PCR amplified using primers bearing *Nco* I sites at their 5' ends and ligated into the vector-reporter backbone (pGL3-basic; Promega, Madison, WI). This was repeated using *Mlu* I, *Bgl*II (to delete ISRE-1), *Nhe*I (to delete ISRE-2), *Sma*I, *Xho*I and *Sac*I, generating promoter deletion constructs. Site-directed mutations were made at ISRE-1 and ISRE-2 within the *Mlu*I-luc reporter by PCR mutagenesis. *ISG54* -luc (a gift from David Levy, New York University) was used as a positive control for IFN-α induction during experiments (not shown). Luciferase promoter-reporter assays were performed in BCP-1 cells by transfecting reporter constructs using the *Geneporter* I transfection reagent (Gene Therapy Systems; San Diego, CA) for 10 hrs as per manufacturer's protocol. Cells were then washed to remove the transfection reagent and resuspended in complete medium with or without 500 U/ml IFN-α for 48 hrs. Cells were harvested and luciferase activity was assessed, normalizing transfection efficiency by cotransfection of a *lac Z* expression vector and determining β-galactosidase activity. In each experiment, total DNA was kept a constant. Each value represents three independent experiments with three replicates per experiment.

Gene Cassette Cloning: vIL-6 expressing C11 Daudi cells were established using a modified pcDNA3 vector to replace the CMV promoter with the vIL-6 promoter+gene. The full length wildtype vIL-6 gene cassette (vIL6WT) was constructed by PCR amplification of a 1543 bp product (corresponding to nucleotide residues 18663 – 17121 of the KSHV genome) containing the vIL-6 coding region and promoter using primers vIL6#1: 5'-CCC AGG ATC AAC ACT TCG TCC C-3' and vIL6#2 (*Kpn*I) 5'-GGG GTA CCC CAT GGG CGT GAT TAG G-3'. The vector backbone pcDNA3 (Invitrogen, Carlsbad, CA) was digested with *Nru*I (blunt cutter) and *Kpn*I to excise its CMV promoter, and the vIL-6 PCR product (insert) was digested with *Kpn*I, and the vector and insert were ligated. Gene cassettes with mutations in ISRE-1 or ISRE-2 where made using the Quik-Change Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and using primers that carried mutations within the GAA core sequence of both ISREs. Wildtype and mutant gene cassettes and pcDNA3 empty vector were transfected into C11-Daudi cells by electroporation at 220V, 975µF and selected for 5-6 weeks using 400µg/ml G418. Basal expression of vIL-6 was comparable among cell lines (except empty vector) indicating similar gene dosages. Dose response curves for ^{3}H-thymidine uptake of were performed by cultivating 1 x 10^5 cells/well of each emergent cell line in the presence of increasing concentration of IFN-α (0-250 U/ml) for 48 hrs as described above.

Flow Cytometry: Surface expression of gp80 and gp130 was measured by flow cytometry using 1x 10^6 unstimulated or IFN-α treated BCP-1 cells cultivated in low serum. These cells were resuspended in 100 µl of wash buffer (PBS with 1% BSA), incubated with either 25 ng/µl anti- gp80 or 20 ng/µl anti-gp130 monoclonal antibody (Biosource International, Camarillo, CA) for 1 hr at 4C. Subsequently cells were washed in wash buffer, and incubated sequentially with 10ng/µl biotinylated goat-anti-mouse IgG antibody (BD Pharmingen) and 1µg of streptavidin-phycoerythrin-Cy5 (Invitrogen, CA) and fixed using 1% paraformaldehyde. Cells were analyzed using a FACScalibur flow-cytometer (Becton-Dickenson). FACS analysis was repeated three times with similar results; a representative example is shown in Fig. 4.

SUPPORTING FIGURES

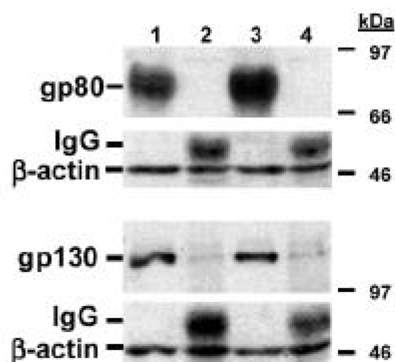

Fig. S1: Both gp80 (IL6Rα, upper panel) and gp130 (lower panel) receptors are expressed in BCP - 1 cells by immunoblotting. Lane 1: C-11 Daudi extracts (positive control), lane 2: C-11 Daudi extracts immunodepleted for gp80 or gp130 (negative control), lane 3: BCP -1 extracts, lane 4: BCP-1 extracts immunodepleted for gp80 or gp130. Blots were stripped and reblotted for β-actin to control for loading.

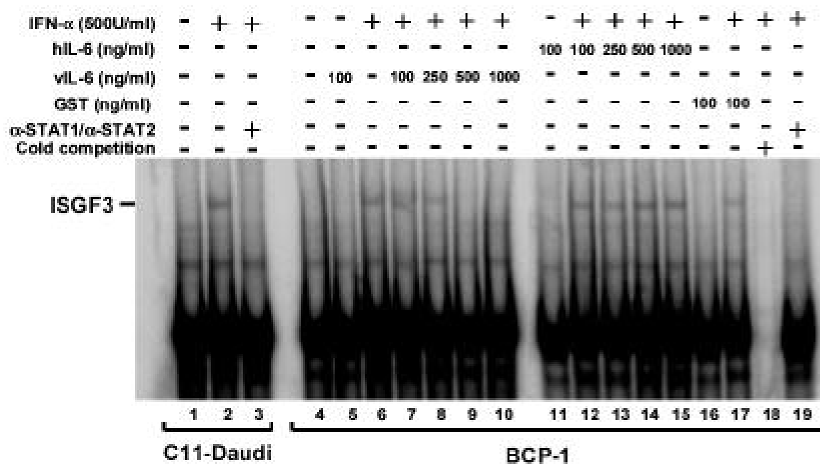

Fig. S2. vIL-6 inhibits IFN-α signaling as measured by ISGF3 binding to an OAS-ISRE probe. EMSA were performed using nuclear extracts from C11-Daudi cells (lane 1, negative control; lanes 2 and 3, 500 U/ml IFN-α positive control) and BCP-1 cells (lanes 4-19). IFN-α (500 U/ml) also activates formation, in PEL cells, of ISGF3 transcription factor composed of phosphorylated STAT-1, STAT-2 and p48/IRF-9 (lane 6). vIL-6 inhibits ISGF3 binding to OAS element in a dose-dependent (lanes 7-10). hIL-6 (lanes 12-15) has no effect on IFN-α activation of ISGF3 (comparable GST control for only the lowest concentration of vIL6, 100 ng/ml, is shown). Antibodies to STAT-1 and STAT-2 (lanes 3, 19) and competition using unlabeled OAS element (lane 18) confirm the specificity of the assay.

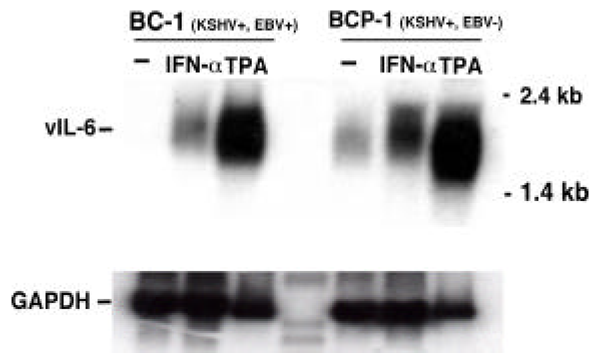

Fig. S3. Transcription of the *ORFK2* gene encoding vIL-6 is induced by IFN-α and the lytic replication-inducing chemical agent, TPA, in both KSHV+/EBV- (BCP-1) and KSHV+/EBV+ (BC-1) cell lines. Northern blots were performed on poly(A)-selected mRNA from uninduced BC-1 (lane 1) and BCP-1 cells (lane 4), cells treated with 500 U/ml IFN-α (lane 2 and 5) or 20 ng/ml TPA for 48 hrs (lanes 3 and 6). Reprobing with a GAPDH probe (lower panel) was performed as control for loading.

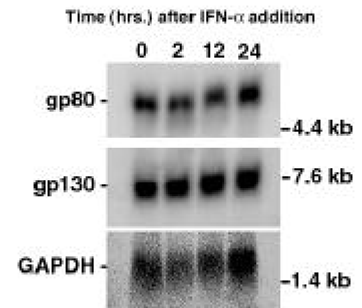

Fig. S4. IFN-α does not inhibit gp80 transcription in BCP-1 cells. Northern blots for gp80 (upper panel) performed using total RNA (20μg) from BCP-1 cells treated with 1000 U/ml IFN-α for different time periods. Lack of change in gp80 or gp130 messages after IFN-α treatment suggests that downregulation of gp80 receptor occurs through post-transcriptional mechanisms. The blot was stripped and reprobed for GAPDH (lower panel) as a RNA loading control.

TABLE S1: PCR products used for KSHV cDNA microarray

ORF	Forward Primer	Position*	Reverse Primer	Position*	Size (bp)
ORF K1	CGCGTTGTGCCAATATAACT	622 - 641	ACCAATCCACTGGTTGCGTAT	969 - 949	348
ORF 4	TCAGAAAAGGCCACTTCCTC	2429 - 2448	GCGACAGCGGTTAGTAGTCC	2745 - 2726	317
ORF 6	AAACCTGCCGAAGACAGTTG	6251 - 6270	GACGGAAAAAGCTCCTCCAC	6538 - 6519	288
ORF 7	CATAGAGCCGAAGGACTGGA	8361 - 8380	CTAGACCTGGGAGTCATTGTG	8715 - 8695	355
ORF 8	CCTAGGTGGCATGCTGATG	10894 - 10912	TCACTCCCCCGTTTCCGGACTG	11236 - 11215	343
ORF 9	CGCAGCGTACCAGTAGACAA	14030 - 14049	CTAGGGCGTGGGAAAAGTC	14401 - 14383	372
ORF 10	AAACCACCACCCTGAGGATT	15384 - 15403	CTACGTGGCAAGGTGGTGTA	15734 - 15715	351
ORF 11	CCAGGTAGTCTGGCACAACA	16668 - 16687	GGTGGCAACATACATTTTTCTG	17001 - 16980	334
ORF K2	GAGTCACGTCTGGGATAGAGTCC	17281 - 17303	TGATCACTGCGGGTTAATAGG	17636 - 17616	356
ORF 2	AATCAGGCAACGCCGTATC	17968 - 17986	GCTCACTTTTTGGCGAGTTC	18298 - 18279	331
ORF K3	TTAATGAAACATAAGGGCAGACGA	18608 - 18631	GCATCGCGACTCAGAAGAAT	18941 - 18922	334
ORF 70	CTATACTGCCATTTCCATACGAA	20091 - 20113	TCCGCCCTGTCACTTGTTAT	20457 - 20438	367
ORF K4	CAGCGAGCAGTGACTGGTAA	21549 - 21568	TGACTGCCTTGCTTTGTTTG	21798 - 21779	250
ORF K4.1	CGCTTCGCGAGAGGTATC	22222 - 22239	TGGCCGTGATTGAGCTATGT	22477 - 22458	256
ORF K4.2	ACCCCCGAAAACAAAACAAC	22790 - 22809	TCAGTCACCTTGCCACCTC	23050 - 23032	261
ORF K5	TAGGTCGGTACGCGCTGTC	25767 - 25785	GTGCTCGGTTTTCTCTGCTT	26114 - 26095	348
ORF K6	GCTGCATCAGCTGCCTAA	27157 - 27174	CTGCGTTAGCGTACTGCTTG	27401 - 27382	245
ORF K7	ATAAAAGGGGCCAGCTTGAG	28637 - 28656	CTACAACTGGCCTGGAGATTGA	29002 - 28981	366
ORF 16	GAGGCCATGTTGGCTAATGT	30298 - 30317	AGTGGCCAATAATGCAATGCT	30639 - 30619	342
ORF 17	GCTCCTCGCAGAACATCTTG	30838 - 30857	TGTCAATTCCCGACAATGAA	31186 - 31167	349
ORF 18	AAACCTGGTGGACGGAAACT	32843 - 32862	CGTTGTTGTTAAACGCACCAA	33189 - 33169	347
ORF 19	GGCAGCCAAGAACCATAA	33220 - 33237	CACACTGCAGGCTAATCCAA	33566 - 33547	347
ORF 20	GTCCAGCTCTAGGGAAAGCA	34631 - 34650	CACGCCCCTGCTGATATTTA	34971 - 34954	342
ORF 21	TATTCCGGAAATCTGCTTCC	36780 - 36799	CTAGACCCTGCATGTCTCCTCT	37125 - 37104	346
ORF 22	TGCTCCGGCTTTAACTTTTC	38937 - 38956	TAACCCCCGAGAACCCAATA	39267 - 39248	331
ORF 23	TTAGACGGTCAATAAAGCGTAGATT	39302 - 39326	GCGCTCTGCTAGAAAGACTCA	39621 - 39601	320
ORF 24	CGGTGTCTGCTCGTTGGA	40562 - 40579	CCTGGAGTGTCTGGTGGAAT	40913 - 40894	352
ORF 25	GTCCTTGTCGGCAGTTCTTC	46567 - 46586	ACCACCTTGTTTCCGAGCTT	46900 - 46881	334
ORF 26	GGATTACTCCATGGCCCTTA	48370 - 48389	GGAGGGGTGGATAGATTGG	48723 - 48705	354
ORF 27	CCTTAGAAGGGCTTTTGCAG	48385 - 48404	AATCAAGGGAGGGGTGGAT	48730 - 48712	346
ORF 28	AAAATTCGCAGGGAGACAAG	48915 - 48934	AGGGTGCGAAGGACCTGATA	49214 - 49195	300
ORF 29b	AAAATGGGCCATGACAACTG	49410 - 49429	CCGCTCCCCATACATTTTCT	49718 - 49699	309
ORF 30	ATCGTATGGGTGAGCCAGTG	50618 - 50637	TTTCGCACCGGTGTCTAGG	50853 - 50835	236
ORF 31	GGTTGACAGGGACGCATATC	51074 - 51093	GCATGCGCATCCATTTTGT	51417 - 51399	344
ORF 32	GAGGCGTGCCTAGTGTTACG	52409 - 52428	GCCTCGAATGAACACCAGAT	52756 - 52737	348
ORF 33	TGGACTACATGGCGTTCTGT	53296 - 53315	GTCGTCGAGCACTCCTGTTA	53645 - 53626	350
ORF 29a	CTGGGCTTACGTTCTTATTGA	53746 - 53766	ACGCACCAGTTGTTTGGATT	54115 - 54096	370
ORF 34	CATGCCCTGAAGTACACCAA	55290 - 55309	TTAGAGTTGGTTGAGTCCATTCTCCT	55658 - 55633	369
ORF 35	GCCGTGTTTGAGAAGCAGTT	55744 - 55763	TTAGGGAGTTTCAGGGCACAC	56091 - 56071	348
ORF 36	ATCTCGATCTGCCGCTAAAC	56931 - 56950	CCTCCATGGTTGAAAAAGTCT	57279 - 57259	349
ORF 37	CTTCAAAGCCAACCTCTTCG	58352 - 58371	TGTGAGGGACGTTTGCAGAT	58725- 58706	374
ORF 38	CTATCTGCAAACGTCCCTCAC	58704 - 58724	AATGCGGGATATGGGGATT	58836 - 58818	133
ORF 39	TGAATATCATTTGCGTTTCGTC	58978 - 58999	GTTCGTGCAGGCTAATCTCC	59294 - 59275	317
ORF 40	GCCATTTCAAGGGAATGTGA	61304 - 61323	TCAAGCAGGGACAGTAGGTCTC	61681 - 61660	378
ORF 41	TAAACATGCCTCCCGACACT	62086 - 62105	TCAAAATAAAGATAAAAGCCTGGT	62444 - 62421	359

ORF 42	ACTTCATTGTTCTCCAGGTGCTT	62500 - 62522	GTCGAGACATGTCACCAACG	62869 - 62850	370
ORF 43	CTATGCACTTCCAGGACAAGG	63136 - 63156	GGCTCAGCGTCACCTATTCT	63488 - 63469	353
ORF 44	AGGACTCATCGGGGTTCATT	66897 - 66916	AGAGTAGTCTTGGGGTTGCAT	67248 - 67228	352
ORF 45	AGCCACGGCCAGTTATATGC	67360 - 67379	GAGTCAGAGAATCGGGCTCA	67722 - 67703	363
ORF 46	GTGGCGAGTCAAATAGTCGTT	68676 - 68696	GCCAGGGTGTGTTGCTACTAA	68989 - 68969	314
ORF 47	TCTCCGGCTGCTGCTTTTA	69439 - 69457	CTTTGCGGTCCACTCTATCC	69796 - 69777	358
ORF 48	CATACTCATCGTCGGAGCTGT	70178 - 70198	AAACCGGTTCCTGTGGAAAT	70526 - 70507	349
ORF 49	TTGTATACTGAACAATGCGTGTTTAC	71633 - 71658	CTCCCTCCTTTCAGTGGATG	72015 - 71996	383
ORF 50	ACCCCCGCTACTGTCGTTAC	74275 - 74294	GGAAGTAATTACGCCATTGGT	74621 - 74601	347
ORF K8	ACAGCTTCCAACTCGCAGAT	75209 - 75228	CTATACCTGCTGCAGCTGTCTTGT	75569 - 75546	361
ORF K8.1a	CCAAATGTCTCCGTATCTGTTG	76164 - 76185	CATCGGTCAGTTCTGTGGTG	76317 - 76298	154
ORF K8.1a/b	ACGGCAGAAATAGTGGTGCT	76496 - 76515	CGTGGAACGCACAGGTAA	76664 - 76647	169
ORF K8.1m	CAATACTGACGGTGGGGAAA	74966 - 74985	GCGAAATGTGTGGTCCTCTT	75207 - 75188	242
ORF 52	GTGTTGTCGAGGCTCCTCTT	76822 - 76841	CTGTGGAGAATCGGGAGCTT	77124 - 77105	303
ORF 53	CTATGCATGGACCACCTCGT	77333 - 77352	CTAGGTCACTGCTGGGTTACG	77620 - 77600	288
ORF 54	CACGTGGCTCTAGCATTGG	78246 - 78264	CCCTTGGTTCCACGAATGT	78601 - 78583	356
ORF 55	GTCGAACCTATCGCGCTTT	78769 - 78787	GTCGTGTGGAACACAATGCT	79121 - 79102	352
ORF 56	CCCTATCCGGTCGTTCAATA	81612 - 81631	AGTCCCACTGGTACCACAAA	81957 - 81938	346
ORF 57	TTGACAAGCAGAGCGAGCTA	83180 - 83199	GTGGATAAAAGAATAAACCCTTGTT	83536 - 83512	357
ORF K9	CATAACGGCTTCGGCATTA	83876 - 83894	GCAAAAGGCATTCTGCTGAC	84228 - 84209	353
ORF K10	TCAATGTAGACTATCCCAAATGGAG	86074 - 86098	CCGAGCAAATGGAGATTTTG	86438 - 86419	365
ORF K10.5	GTCACCTCCGATGAACACAGTTG	89678 - 89700	GCAATGTCTTTGGAAGTGGAACG	90073 - 90051	396
ORF K10.6	TCGAGCCGTACACTGTGTTG	90961 - 90980	AAGAGGGAATGCCTATCGAA	91226 - 91207	266
ORF K11	GGTAAAATGGGGCAAGGTAAA	91975 - 91995	ACAGACGCTGGACAGGTTTT	92276 - 92257	302
ORF 58	TTAGCCAACAACTTTATTTATTACCG	94471 - 94496	TCCTGATTGGCCTGGATAAG	94820 - 94801	350
ORF 59	TCAAATCAGGGGGTTAAATGTG	95549 - 95570	TGCCAATCAGGTGACGTAAA	95884 - 95865	336
ORF 60	TCACAAATCGTCAGTCACACAC	96870 - 96891	TGCCAACGATTACATTTCCA	97242 - 97223	373
ORF 61	CTACTGACAGACCAGGCACTCG	97816 - 97837	CCAAGCATCACAGGGAAAGA	98195 - 98176	380
ORF 62	CTCAAAGTTCGCAAACACCA	100232 - 100251	GATGAGTCATGTCCCATGGTC	100573 - 100553	342
ORF 63	GGACGCAGCAAACACTTGTA	103568 - 103587	AGCGTCAGTGGGACAAAA	103963 - 103946	396
ORF 64	AGTCATCCCCACCGACTTC	111549 - 111567	TCACAAGTACCACTTCTTTATTCTGTC	111907 - 111881	359
ORF 65	CGTTTCCGTCGTGGATGA	111985 - 112002	ATATGTCGCAGGCCGAATAC	112340 - 112321	356
ORF 66	GAGGAACACTTCCCGCAAC	112474 - 112492	GTCAATTTTCCTCCCACCAA	112826 - 112807	353
ORF 67	CCAAACCAAACCAAGACACA	113708 - 113727	GGACTACAGGCAGGTTTTGC	114032 - 114013	325
ORF 68	CGCCATGCCATATGTATCAG	116024 - 116043	CAGGTCTAGGCGGTGTCTTT	116369 - 116350	346
ORF 69	CCTGCAGCCTGGTCTACTGT	116992 - 117011	TTATAGGGCGTTGACAAGTGC	117346 - 117326	355
ORF K12	GTTGCAACTCGTGTCCTGA	117932 - 117950	AGGCTTAACGGTGTTTGTGG	118093 - 118074	162
ORF K13	CTATGGTGTATGGCGATAGTGTTG	122145 - 122168	TTCACTTAGACCCGCGTTTT	122494 - 122475	350
ORF 72	GCTGTCCAGAATGCGCAGAT	122800 - 122819	GGCGAACAGAGGCAGTCTTA	123137 - 123118	338
ORF 73	TTATGTCATTTCCTGTGGAGAGT	123809 - 123831	GGATGGAAGACGAGATCCAA	124166 - 124147	358
ORF K14	GGAACAACGCCTCTTCTACC	128605 - 128624	GGTGGATAGGGGGTCCAT	128924 - 128907	320
ORF 74	CCTGGCCCTCCTTATTCTGT	130040 - 130059	GGACATGAAAGACTGCCTGA	130385 - 130366	346
ORF 75	TTAGTGGTGGTCGTTGATCTTC	130551 - 130572	AGGACGCCCTCGAGTACATA	130858 - 130839	308
ORF K15	CAGACATAATTGCATAGCAGTAAAA	135989 - 136013	TCAGCCATTCTATCACTTGGTG	136271 - 136250	283

*BC-1 strain position (*S12*)

Control Genes	Forward Primer	Position	Reverse Primer	Position	Size	Template	Gene ID
EBNA	TCATTGGTGCCACTGGTCTC ATCC	101037 -101060	TTTCTTCTACTCGAGGCCTT TTTGG	101283 - 101259	247	EBV (Strain B95-8)	V01555
BHRF1	TGCGTTATCATGTGTTGCTT GAG	54518 - 54540	TAATGTAGACCAGCCGCCCT G	54834 - 54814	317	EBV (Strain B95-8)	V01555
LacZ	GGCGATTACCGTTGATGTTG AAG	3542 - 3564	AGATGGCGATGGCTGGTTTC	3874 - 3855	333	pcDNA3 lacZ	Invitrogen
Ampicillin	AACTTTATCCGCCTCCATCC AG	4669 - 4690	GTCGCCGCATACACTATTCT CAG	5042 - 5020	374	pCDNA3	Invitrogen
Cylophilin	TCTGCTGACAAAACTTAGTG CCC	14177 - 14199	ATCTTCTGGTGCTATCCCCT GAG	14562 - 14540	386	Human cyclophilin 40	D63861
ß-actin	TGAAGGTGACAGCAGTCGG TTG	1325 - 1346	TCAAGTTGGGGGACAAAAAG G	1662 - 1642	338	Human b-actin	X00351
Glyceraldehyde 3-phoshate dehydrogenase	ATCACTGCCACCCAGAAGAC TG	601 - 622	TGTAGCCCAGGATGCCTTTG	889 - 870	289	G3PD, EC 1.2.1.12	X01677

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