Supporting Information

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SI Materials and Methods

Cell Culture. EBV-positive lymphoblastoid cell lines (LCLs) RN (kind gift of W. Stoorvogel, Utrecht, The Netherlands), IM1 (spontaneous LCL), X50-7 (gift of M. Rowe, University of Birmingham, Birmingham, UK), and EBV-negative B-cell line (BJAB) were cultured in RPMI-1640 (BioWitthaker), supplemented with 10% FBS (HyClone; Perbio Sciences), 100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, and 2 mM glutamine. HeLa cells were cultured in DMEM (BioWitthaker), supplemented with 10% FBS (HyClone; Perbio Sciences), 100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, and 2 mM glutamine.

Exosome Isolation and Purification. Exosomes were isolated from the supernatants of B-cell cultures by differential centrifugation as described previously (1). In short, LCLs for exosome culturing were washed in PBS and recultured for 48 h at a density of 0.5×10^6 cells/mL in a total volume of 200 mL RPMI-1640, supplemented with 5% exosome-depleted FBS (1 h centrifugation at $70,000 \times g$ in a SW28 Beckman swing rotor), 100 U/mL penicillin G, 100 μg/mL streptomycin sulfate, and 2 mM glutamine. Cell death was analyzed routinely by trypan-blue exclusion, and cultures with ≤95% viability were not considered for exosome purification. The cell-culture medium was centrifuged at $500 \times g$ for 10 min (2×), $2,000 \times g$ for 15 min (2×), and $10,000 \times g$ for 30 min (2×) to remove cells and cellular debris. The exosomes subsequently were pelleted from the medium by ultracentrifugation at $70,000 \times g$ for 1 h, after which the pellet was washed in 15 mL PBS and pelleted again by ultracentrifugation at $70,000 \times g$ for 1 h. The washed pellet was dissolved in PBS in a total volume of 200 μL and analyzed by EM to confirm purity and the presence of exosomes. The total protein yield for a typical LCL exosome purification protocol was ∼60 μg, as determined by BCA protein assay (Pierce). Further purification for initial EM analysis was performed using a sucrose cushion with a density of 1.13 μg/mL. The exosome pellet was centrifuged overnight at $100,000 \times g$.

Western Blot/Proteomics. Proteomics on B cell-secreted exosomes was performed as previously reported (2). Briefly, bands were washed and dehydrated three times in 50 mM ammonium bicarbonate (ABC) (pH 7.9)/50 mM ABC + 50% acetonitrile (ACN). Subsequently, cysteine bonds were reduced with 10 mM dithiotreitol for 1 h at 56 °C and alkylated with 50 mM iodoacetamide for 45 min at room temperature in the dark. After two subsequent wash/dehydration cycles, the bands were dried 10 min in a vacuum centrifuge and incubated overnight with 0.06 μg/μL trypsin at 25 °C. Peptides were extracted once in 1% formic acid and subsequently two times in 50% ACN in 5% formic acid to enable liquid chromatography-MS/MS analysis. MS/MS spectra were searched against the human IPI database 3.31 using Sequest version 27, and proteins were ranked using Scaffold 2 (Proteome Software). For Western blotting, lysates of purified exosomes were made, and equal amounts of protein were loaded on an SDS/PAGE gel.

RNA Isolation. Total cellular and exosomal RNA was isolated with TRIzol reagent (Invitrogen), according to the manufacturer's protocol. Because low RNA yields were expected from purified exosomes, exosomes were disrupted in 800 μL of TRIzol reagent, and 5 μL glycogen (Roche) was added to the aqueous phase before the isopropanol precipitation step according to the RNA isolation notes of the manufacturer's protocol. The amount, quality, and composition of isolated RNA was analyzed by the NanoDrop 1000 spectrophotometer (Thermo Scientific) and the Agilent 2100 Bioanalyzer for total RNA (RNA 6000 nano kit), and the 2100 small RNA kit were used to assess the large and small RNA profiles in LCL cells and exosomes, respectively. To confirm that isolated RNA was confined within the lumen of exosomes, exosome preparations were treated with final concentrations at 10 ng/μL and 400 ng/μL RNase A (Sigma) for 45 min at 37 °C before TRIzol-mediated RNA extraction. RNA yield from exosomes secreted by 10^8 LCL cells typically was 1–2 μg as determined by the NanoDrop.

EBV-Encoded microRNA Detection Through Multiplex RT-PCR. Quantitative multiplex EBV-microRNA (miRNA) RT-PCR was performed as previously described (3). This technique makes use of a stem-loop primer for RT, followed by TaqMan real-time PCR using miRNA-specific forward primers and probes and a universal reverse primer specific for the constant region of the stem-loop RT primer. Stem-loop RT primers for up to 10 EBV-miRNAs were combined so that the RT reaction contained a final concentration of 12.5 nM of each RT primer. TaqMan MicroRNA RT kit (Applied Biosystems) was used, and the reactions were incubated according to kit instructions. The volume of the RT reaction was adjusted according to the number of PCRs to be performed. All RT reactions, including a no-template control, were performed in duplicate. Real-time PCR primers and probes were designed for each EBV-miRNA as previously described (3). Each 10 μL miRNA PCR included 1 μL of RT product, 5 μL of 2× IQ Supermix (Bio-Rad), 1.5 μM forward primer, 0.7 μM universal reverse primer, and 0.2 μM TaqMan probe (Applied Biosystems). Typically, the final input for the PCR was ∼1–10 ng of exosomal RNA. The samples were incubated on a Bio-Rad MyIQ cycler at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. All real-time PCRs were performed from the same batch of RT product for each sample and were performed in duplicate.

Exosome Transfer Studies. LCLs were labeled with PKH67 green fluorescent membrane linker-dye (Sigma) according to the manufacturer's protocol. Purified exosomes (180 μL) were labeled with PKH67 dye by adding 180 μL Diluent C (Sigma linker kit) and 20 μL diluted PKH67 dye. Labeled purified exosomes were washed in PBS and pelleted (1-h centrifugation at $70,000 \times g$ in a SW28 Beckman swing rotor) and resuspended in a final volume of 200 μL. PKH67-stained LCLs or purified exosomes were seeded into porous 3.0-, 1.0-, or 0.4-μm 24-well transwell devices (Corning Costar) when indicated. Typically, 6-day-old differentiated monocyte-derived (immature) dendritic cells (MoDC) ($CD14^-$, $CD1a^+$ as determined by FACS) were placed in the bottom of a transwell with a 1-μm pore size at a ratio of 5 LCL to 1 MoDC. With a pore size of 0.4 μm, exosome transfer of dye to MoDC was observed only after 48 h of coculture. MoDC and LCL were cocultured for indicated times in Iscove's modified Dulbecco's medium (IMDM) (BioWhittaker). MoDC were analyzed for PKH67 positivity at 0, 6, and 24 h by FACS analysis after EDTA treatment for 10 min at 37 °C to dislodge the adherent MoDC and remove potentially attached noninternalized exosomes. In control experiments we found that either γ-irradiation, UV exposure, or treatment with Fas ligand induced a high percentage of cell death (30%) in PKH67-stained LCL, but induced cell death did not significantly increase PKH67 positivity or the mean fluorescence of cocultured MoDC after 24 h. Total RNA was isolated from a total of 3×10^5 MoDC that were cocultured in separate wells for 24 and 48 h with 5×10^5 (unstained) B95-8 (RN) LCL. EBV-encoded miRNA copy numbers in the cocultured MoDC were quantified by RT-PCR as described. Based on previous results (3) and FACS analysis, a significant contribution to EBV-encoded miRNAs ascribed to the continuous uptake of LCL exosomes by

MoDC through LCL contamination was excluded. Pharmacological stimulation of exosome release from PKH67-labled LCL at 24 h was achieved by addition of the calcium ionophore monensin (4) for the last 3 h of coculture at nontoxic concentrations (10–30 μM).

Primary Monocyte Isolation and Dendritic Cell Generation. Peripheral blood mononuclear cells (PBMCs) from various donors were isolated by density centrifugation (Lymphoprep; Nycomed AS) from buffy coats obtained from healthy blood donors (Sanquin). The $CD14^+$ monocytes were isolated from PBMC using magnetic microbeads (Miltenyi Biotec). $CD14^+$ cells were cultured at a density of 4.0×10^5 cells/mL for 6 days in the following culture medium: IMDM (BioWhittaker) supplemented with 10% FCS (HyClone), 50 U/mL penicillin-streptomycin (Invitrogen Life Technologies), 1.6 mM L-glutamine (Invitrogen Life Technologies), and 0.01 mM β-mercaptoethanol (2-ME; Merck) (complete medium) supplemented with 100 ng/mL GM-CSF (Schering-Plough) and 10 ng/mL IL-4 (R&D Systems) at 37 °C, 95% humidity, and 5% CO_2. To induce dendritic cell (DC) maturation, medium of both conditions was enriched at day 6 with a maturation mixture (monocyte-conditioned medium mimic) containing 10 ng/mL recombinant human TNF-α (R&D Systems) and 1,000 U/mL recombinant human IL-6 (R&D Systems). For all experiments, immature MoDCs were used unless indicated.

Flow Cytometry. Immunophenotypic analysis of LCLs and MoDC differentiation and maturation was performed using FACS analysis (FACSCalibur; BD Biosciences). Cells were incubated at 4 °C for 30 min in PBS with 0.1% BSA and 0.01% NaN_3, in the presence of appropriate dilutions of FITC- or PE-labeled mouse mAbs to CD83 (Coulter Immunotech), CD86, HLA-DR (BD Biosciences), CD1a, CD14, CD40, CD80, CD19 (BD Biosciences), and DC-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN; PharMingen). The cells were measured subsequently using the FACSCalibur and analyzed with CellQuest software (BD Biosciences). Results were expressed either as (geometric) mean fluorescence index (MFI) or as the percentage of positive cells.

Confocal and Electron Microscopy. For conventional EM, LCL were fixed with a mixture of 2% paraformaldehyde and 1% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4), postfixed with 1% OsO4, dehydrated in ethanol, and embedded in Araldite 6005 (Canemco Inc.). Ultrathin sections were viewed with a JEOL 1010 electron microscope after counterstaining with uranyl acetate. Purified exosomes (10 μL) were spotted on a grid before fixation. For immunofluorescent staining and confocal microscopy, MoDC that had internalized fluorescent PKH67-labeled exosomes were placed on glass cover slides by cytospin, fixed in 4% paraformaldehyde in PBS for 15 min at room temperature, permeabilized with 0.1% Triton X-100 for 10 min at 4 °C, and blocked with 2% BSA for 1 h at room temperature. Nuclei were stained with TO PRO-3, and finally a coverslip was mounted with VECTASHIELD mounting medium (Vector Labs). Slides were analyzed by laser-scanning microscopy with a Leica TCS SP2 using Leica software.

Cloning and Luciferase Reporter Assays. Total cDNA from RN and BJAB cells was used to isolate the 3′UTRs of *LMP1* and *CXCL11*, respectively, by PCR. Forward primer 5′-ACGTACTAGTGCCTTCTAGGCATTACCATGTC-3′ and reverse primer 5′-ACGTAAGCTTGCTGCATCACAAGTCACATCAA-3′ were used for the 3′UTR of LMP1 (GenBank accession number X01995) and forward primer 5′-ACGTACTAGTGCATATGAAGTCCTGGAAAAGG-3′ and reverse primer 5′-ACGTAAGCTTGCGAAAGGTTGTGGTAGTTTAT-3′ were used for the 3′UTR of CXCL11 (GenBank accession number NM_005409). PCR products were cloned into the SpeI and HindIII sites of pMir-REPORT vector (Applied Biosystems). The mutated 3′UTR of CXCL11 was purchased from Geneart.

HeLa cells were cotransfected with pMir-3′UTR-LMP1 and a pCMV4 vector containing EBV BART cluster 1 or cluster 2 (kind gift from D. Hayward, Johns Hopkins School of Medicine, Baltimore) using Lipofectamine 2000 (Invitrogen). Cells were cotransfected with a plasmid containing an expression cassette for Gaussia luciferase for normalization. After 24 h, cells were lysed (Reporter Lysis Buffer; Promega), and luciferase activity was measured using a luminometer (Lumat LB9507; EG&G Berthold). Immature 6-day-old differentiated MoDC and HeLa were transfected with pMIR-3′UTR-LMP1 or pMIR-3′UTR-CXCL11 using an Amaxa Nucleofector according to the manufacturer's protocol. Cells were cotransfected with a plasmid containing an expression cassette for Gaussia luciferase, CSCW-Gluc (5) to normalize the Firefly luciferase levels expressed from the pMir-Report constructs, and/or a GFP control vector to determine transfection efficiency (typically ∼50%). For longer-duration experiments, 25–100 μL of purified RN or BJAB exosomes were added 5 h after transfection. We added similar and 2× more BJAB exosomes to ensure that the amount of BJAB exosomes was not limiting and was comparable to RN exosomes, because we noticed that BJAB cultures secrete slightly less (∼20%) exosomes in a 24- to 48-h period than RN cultures as measured by protein content. Internalization of exosomes by transfected MoDC was confirmed by addition of fluorescent purified PKH67-labeled RN exosomes and subsequent FACS analysis. After 16 h of incubation, an equal amount of RN or BJAB exosomes was added and incubated for an additional 3 h. Ultimately MoDC were lysed (Reporter Lysis Buffer; Promega), and luciferase activity was measured using a luminometer (Lumat LB9507; EG&G Berthold). Experiments were repeated three times; the data shown are representative of one experiment. In shorter time-point experiments (4–8 h), much smaller amounts (25–40 μL) of purified exosomes were added once, directly upon transfection, and luciferase activity was measured as described.

Patients and Clinical Specimens. Whole blood (≈10 mL) was collected for routine diagnostic testing for plasma HIV-RNA load and CD4 T-cell counts from random asymptomatic HIV carriers visiting the Slotervaart Hospital (Amsterdam, the Netherlands) for routine check-up between 2004 and 2006. Blood not used for these purposes was used for EBV research purposes described herein. PBMCs were isolated using Ficoll and stored in liquid nitrogen until use. Approval for this study was obtained from the Medical Ethical Board of Slotervaart Hospital (Approval U/1859/0402). Only clinical specimens from adults who gave written informed consent were included in the study. B lymphocytes were isolated from PBMCs using Dynabeads M-450 CD19 (Dynal Biotech) according to the manufacturer's protocol. We designated the remaining cells as the non–B-cell fraction. When sufficient PBMCs were available, the non-B cells were separated further into $CD3^+$ T cells and non-B/non-T cells by FACS sorting. We determined the purity of isolated fractions by standard flow cytometry on a FacsCalibur Instrument (BD Biosciences). B-cell populations typically were ∼99% $CD19^+$, and non–B-cell fractions typically were ∼3% $CD19^+$ compared with isotype controls.

1. Wubbolts R, et al. (2003) Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. *J Biol Chem* 278:10963–10972.
2. Piersma SR, et al. (2009) Proteomics of the TRAP-induced platelet releasate. *J Proteomics* 72:91–109.
3. Cosmopoulos K, et al. (2009) Comprehensive profiling of Epstein-Barr virus microRNAs in nasopharyngeal carcinoma. *J Virol* 83:2357–2367.
4. Savina A, Furlán M, Vidal M, Colombo MI (2003) Exosome release is regulated by a calcium-dependent mechanism in K562 cells. *J Biol Chem* 278:20083–20090.
5. Wurdinger T, et al. (2008) A secreted luciferase for ex vivo monitoring of in vivo processes. *Nat Methods* 5:171–173.

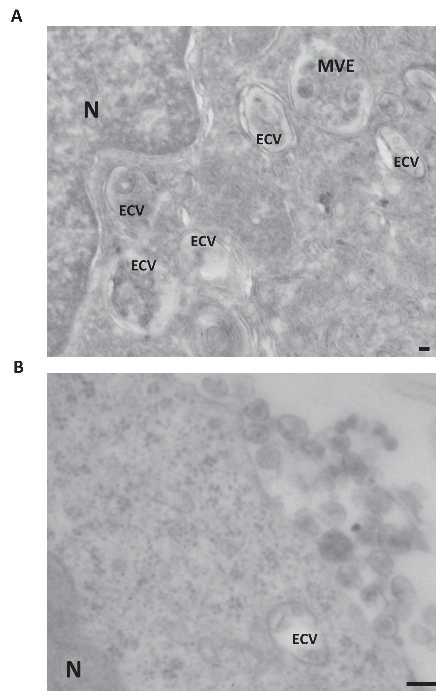

Fig. S1. Early and late endosomes in LCL cells and exosomes in BJAB cells. (*A*) EM image of early and late endosomal structures present in a typical LCL cell, including multivesicular endosomal intermediates (ECV) and a multivesicular endosome (MVE) that holds exosomal precursors known as "intraluminal vesicles" (ILV). N, nucleus. (*B*) ILVs are released as exosomes from BJAB cells when the limiting membrane of the MVE fuses with the plasma membrane. (Scale bar, 100 nm.)

A

Total RNA profile in LCL cells

B

Total RNA profile of purified LCL exosomes

C

Total RNA profile of exosomal RNA (RNAse treated exosomes)

D

Exosomal RNA profile after degradation by RNAse

E

Standard curve for BHRF1-3 miRNA

F

Standard curve for BART1-5p miRNA

Fig. S2. Total RNA profile of LCL cells and exosomes. (*A*) Bioanalyzer graphic showing the typical large and dominant 18s and 28s ribosomal RNA peaks of intact cellular RNA of LCL cells (arrow indicates small RNA region). (*B*) Bioanalyzer results for RNA extracted from a typical purified LCL exosomal fraction before RNase treatment. Exosomes are enriched for small RNAs (arrow) compared with total cellular LCL RNA. Low contaminating ribosomal peaks are present on the outside of the exosomes. (*C*) Exosomes treated for 1 h with exogenous RNase A (10 μg/mL) still contain intact RNA although the degraded ribosomal RNA cannot be detected. (*D*) Exosomal RNA is completely degradable by RNase A (10 μg/mL) treatment. (*E* and *F*) Typical RT-PCR result of a BHRF1 and a BART miRNA standard curve generated from serially diluted synthetic oligonucleotides ranging from 10^2–10^7 copies.

A EBV miRNAs in spontaneous LCL (IM) exosomes with wild type EBV

B Cluster 1

C Cluster 2

Fig. S3. LCLs with wild-type EBV secrete exosomes containing cluster 1 and cluster 2 BARTs although X50-7 LCL cells secrete a limited set. (*A*) Quantitative multiplex RT-PCR using the stem-loop primer method to determine copy numbers was performed in exosomes purified from a spontaneous LCL carrying wild-type EBV. (*B* and *C*) Quantitative multiplex RT-PCR from a common laboratory wild-type LCL X50-7. Shown are the PCR results for 14 individual EBV-encoded miRNAs presented in two clusters based on their location in the genome, cluster 1 (*B*) and cluster 2 (*C*). Note that all cluster 2 BART miRNAs we analyzed were underrepresented in exosomes compared with most cluster 1 BART miRNAs.

Fig. S4. Transfer of fluorescent LCL exosomes to immature MoDC is an active process. (*A*) Surface marker analysis indicating the immature state of monocyte-derived dendritic cells at day 6. (*B* and *C*) Mature MoDC at day 10 endocytose fluorescent exosomes (30 μL) less efficiently than immature MoDC as quantitated by flow cytometry. (Scale bar, 2 μm.) (*D*) Decrease in mean PKH67-mediated fluorescence (MFI) of immature MoDC (300.000) after 3 h of incubation with 100 μL purified PKH67-labeled exosomes at 37 °C (standard conditions) compared with incubation at 4 °C and/or in the presence of 10 μM cytochalasin B to inhibit actin-mediated endocytosis. Conditioned medium from labeled LCL cells was added to MoDC, indicating that simple diffusion of dye from the labeled LCL to MoDC does not occur via the medium.

Fig. S5. Exosome transfer to MoDC during 24 h of coculture with PKH67-labeled LCL in a transwell system. (*A* and *B*) Immature $CD86^{+}/CD19^{-}$ MoDC were cocultured for 24 h in a transwell with PKH67-labeled $CD19^{+}$ LCL cells, and exosome-mediated transfer of dye was analyzed by flow cytometry. (*C*) Treatment with 10 μM monensin (a known chemical stimulator of exosome secretion) for the last 3 h of coculture increased the percentage of PKH67 positivity and mean fluorescence in the recipient $CD86^{+}/CD19^{-}$ MoDC population.

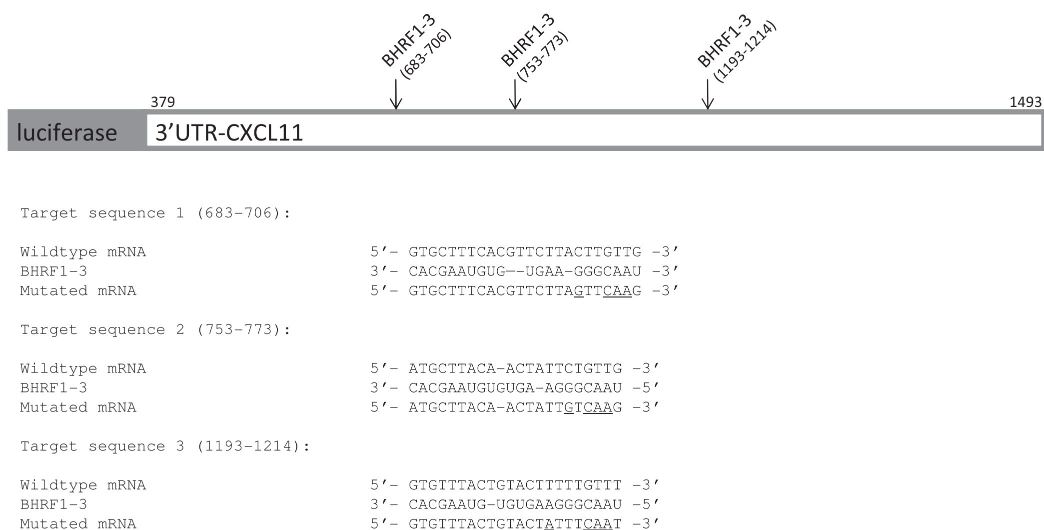

Fig. S6. Schematic representation of the wild-type CXCL11-luciferase reporter construct with BHRF1-3 EBV-miRNA target sites. Detailed below are the published sequences of target sites. Point mutations (underlined) were introduced in the mutated CXCL11-luciferase reporter construct to disrupt BHRF1-3 binding sites.

Table S1. Enrichment of exosomal proteins is similar in exosomes from EBV-infected (LCL) and noninfected cells (BJAB)

Function	MS/MS view: identified proteins (904)	Accession number	Molecular Mass	BJAB cell lysate	BJAB (EBV$^-$) exosome	LCL (EBV$^+$) exosome
Raft protein	Raftlin (RFTN1)	IPI00749454	63 kDa	0	11	2
Raft protein	Flotillin-1 (FLOT1)	IPI00027438	47 kDa	0	7	5
Raft protein	Lactadherin (MFGE8)	IPI00002236	43 kDa	0	2	6
Exosome receptor	Intercellular adhesion molecule 1 (ICAM1)	IPI00008494	58 kDa	56% (1)	11	10
Tetraspanin	CD81 antigen (CD81)	IPI00000190 (+1)	26 kDa	1	3	2
Endosomal GTPase	Ras-related protein Rab-21 (RAB21)	IPI00007755	24 kDa	0	3	3
Endosomal GTPase	Ras-related protein Rab-10 (RAB10)	IPI00016513	23 kDa	3	5	4
Endosomal GTPase	Ras-related protein Rab-7a (RAB7A)	IPI00016342	23 kDa	5	6	9
Endosomal GTPase	Ras-related protein Rab-11B (RAB11B)	IPI00020436	24 kDa	2	7	8
Antigen presentation	HLA class I histocompatibility antigen	IPI00026569	41 kDa	60% (1)	7	9
Antigen presentation	MHC class II antigen(HLA-DQA1)	IPI00719648	29 kDa	60% (1)	4	13
Antigen presentation	HLA class II histocompatibility antigen (DQB1)	IPI00640031	30 kDa	0	3	17
Antigen presentation	HLA-DRA	IPI00005171 (+1)	29 kDa	2	2	5
Mitochondrial	Leucine-rich PPR motif-containing protein, mitochondrial	IPI00783271	158 kDa	27	0	0
DNA repair	DNA mismatch repair protein Msh2 (MSH2)	IPI00017303 (+1)	105 kDa	6	0	0
DNA repair	DNA damage-binding protein 1 (DDB1)	IPI00293464	127 kDa	13	0	0

Table exported from Scaffold 2 software showing LC-MS/MS data. The numbers indicate the amount of unique peptide spectra that were identified. The percentage indicates the certainty that the protein has been identified correctly (in all cases 100%) except when indicated (for full details, see *SI Materials and Methods*).

Table S2. Sequences of primers and probes used in this study

EBV-miRNA	Oligonucleotide	Sequence
BHRF1-1	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG AACTCCGG
	Forward	ACACTCCAGCTGGGUAACCUGAUCAGCCCC
	Probe	TTCAGTTGAG AACTCCGG
BHRF1-2	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTCAATTT
	Forward	ACACTCCAGCTGGGUAUCUUUUGCGGCAGA
	Probe	TTCAGTTGAG TTCAATTT
BHRF1-2*	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCTATCTG
	Forward	ACACTCCAGCTGGGAAATTCTGTTGCAGCA
	Probe	TTCAGTTGAGGCTATCTG
BHRF1-3	RT	CAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGTGTGCTT
	Forward	ACACTCCAGCTGGGUAACGGGAAGUGUGUA
	Probe	TTCAGTTGAG GTGTGCTT
BART1-5p	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACAGCACG
	Forward	ACACTCCAGCTGGGTCTTAGTGGAAGTGAC
	Probe	TTCAGTTGAG ACAGCACG
BART1-3p	RT	CTCAACTGGTGTCGTGGAGT CGGCAATTCA GTTGAG AGACATAG
	Forward	ACACTCCAGCTGGGTAGCACCGCTATCCAC
	Probe	TTCAGTTGAG AGACATAG
BART2-5p	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCAAGGGC
	Forward	ACACTCCAGCTGGGTATTTTCTGCATTCGC
	Probe	TTCAGTTGAG GCAAGGGC
BART2-3p	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG TTTATTTT
	Forward	ACACTCCAGC TGGG AAGGAGCGATTTGGAG
	Probe	TTCAGTTGAGTTTATTTT
BART3	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCACAAC
	Forward	ACACTCCAGCTGGGAACCTAGTGTTAGTGT
	Probe	TTCAGTTGAG AGCACAAC
BART3*	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACACCTGG
	Forward	ACACTCCAGCTGGGCGCACCACTAGTCACC
	Probe	TTCAGTTGAG ACACCTGG
BART4	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCACACC
	Forward	ACACTCCAGCTGGGGACCTGATGCTGCTGG
	Probe	TTCAGTTGAG AGCACACC
BART5	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGATGGGC
	Forward	ACACTCCAGCTGGGCAAGGTGAATATAGCT
	Probe	TTCAGTTGAG CGATGGGC
BART6-5p	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG CCTATGGA
	Forward	ACACTCCAGC TGGG UAAGGUUGGUCCAAUC
	Probe	TTCAGTTGAG CCTATGGA
BART6-3p	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTAAGGC
	Forward	ACACTCCAGCTGGGCGGGGATCGGACTAGC
	Probe	TTCAGTTGAG TCTAAGGC
BART7	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCCTGGAC
	Forward	ACACTCCAGCTGGGCATCATAGTCCAGTGT
	Probe	TTCAGTTGAG CCCTGGAC
BART8	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTGTACAA
	Forward	ACACTCCAGCTGGGTACGGTTTCCTAGATT
	Probe	TTCAGTTGAG CTGTACAA
BART8*	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTACGACC
	Forward	ACACTCCAGCTGGGGTCACAATCTATGGGG
	Probe	TTCAGTTGAG CTACGACC
BART9	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTACGGGA
	Forward	ACACTCCAGCTGGGTAACACTTCATGGGTC
	Probe	TTCAGTTGAG CTACGGGA
BART10	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACAGCCAA
	Forward	ACACTCCAGCTGGGACATAACCATGGAGTT
	Probe	TTCAGTTGAG ACAGCCAA
BART1-5p	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG CAACTAGC
	Forward	ACACTCCAGC TGGG UCAGACAGUUUGGUGC
	Probe	TTCAGTTGAG CAACTAGC
BART12	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAG AAACCACA
	Forward	ACACTCCAGCTGGGTCCTGTGGTGTTTGGT
	Probe	TTCAGTTGAG AAACCACA

Table S2. Cont.

EBV-miRNA	Oligonucleotide	Sequence
BART13	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGCCGT
	Forward	ACACTCCAGCTGGGTGTAACTTGCCAGGGA
	Probe	TTCAGTTGAG TCAGCCGT
BART1-5	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG CTTGTATG
	Forward	ACACTCCAGC TGGG UAAGAGGACGCAGGCA
	Probe	TTCAGTTGAG CTTGTATG
BART1-5p	RT	CTCAACTGGT GTCGTGGAGT CGGCAATTCA GTTGAG TGTATAGG
	Forward	ACACTCCAGC TGGG UCAAGUUCGCACUUCC
	Probe	TTCAGTTGAG TGTATAGG
Quantitative EBV-DNA PCR		
EBNA	Forward	GCCGGTGTGTTCGTATATGG
	Reverse	CAAAACCTCAGCAAATATATGAG
	Probe 1	TCTCCCCTTTGGAATGGCCCTG-fluorescein
	Probe 2	LCRed640-ACCGGCCCACAACCTG
Semiquantitative RT-PCR for BAMHI-A (BART) transcripts and CXCL11 mRNA		
BAR	Forward	AGAGACCAGGCTGCTAAACA
	Reverse	AACCAGCTTTCCTTTCCGAG
CXCL1	Forward	GCTATAGCCTTGGCTGTGATAT
	Reverse	GCCTTGCTTGCTTCGATTTGGG