Supporting Information

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SI Materials and Methods

Cell Lines and Cell Culture. The EBV-positive Burkitt's lymphoma cell line Raji, HEK293-based EBV producer cell lines (36), infected human primary B cells, and isolated T cells were maintained in RPMI medium 1640 (Life Technologies). HEK293T cells were maintained in DMEM. All media were supplemented with 10% (vol/vol) FBS (Life Technologies), penicillin (100 U/mL; Life Technologies), and streptomycin (100 mg/mL; Life Technologies). Cells were cultivated at 37 °C in a 5% (vol/vol) CO_2 incubator.

Separation of Human Primary Lymphocytes. Human primary B and T cells were prepared from adenoidal MNCs or PBMCs by Ficoll-Hypaque gradient centrifugation. B cells and $CD8^+$ T cells were isolated using MACS separation columns (Miltenyi Biotec) with CD19 or CD8 MicroBeads, respectively.

Preparation of EBV Stocks. The recombinant EBV genome designated WT/B95-8 in this study is identical to plasmid 2089, which contains the complete EBV strain B95-8 genome, the F factor origin of replication, the chloramphenicol resistance gene, the gene for the green fluorescent protein (GFP) under the control of the cytomegalovirus (CMV) promoter, and the hygromycin resistance gene as a selectable marker in eukaryotic cells (69). Inactivation of all miRNA genes from this construct resulted in plasmid 4027, also called ΔmirALL or ΔmiR (36). Infectious virus was produced by lytic induction of producer cell lines stably carrying these recombinant EBV genomes in episomal form.

To induce EBV's lytic cycle in the ΔmiR (4027) or WT/B95-8 (2089) HEK 293 producer cell lines, plasmids coding for BZLF1 and BALF4 were transiently transfected. Supernatants were collected after 3 d. Virus stocks were titrated using Raji cells as described in detail recently (65). Isolated primary B cells were infected with the virus stocks at a multiplicity of infection of 0.1 GRU (Green Raji Units, see ref. 65). Eighteen hours later, the infected B cells were cultivated in fresh medium at an initial density of 5×10^5 cells per milliliter.

Luciferase Reporter Assays. The 3′UTRs of *TAP2* (ENST00000374897), *TAP1* (ENST00000354258), *IPO7* (ENST00000379719), and *HLA-B7/B8* (ENST00000412585/ENST00000425848) were cloned downstream of Renilla luciferase (*Rluc*) into the expression plasmid psiCHECK-2 (Promega). The pCDH vectors expressing single viral miRNA were used as previously described (35). The psiCHECK-2 reporter and pCDH-EF1-MCS plasmid DNAs (System Biosciences) with a viral miRNA of interest were cotransfected into HEK293T cells using Metafectene Pro (Biontex). miR-BHRF1–3 was expressed from a modified pLSP plasmid vector (70). It was digested with BamHI and EcoRI and ligated with miR-BHRF1–3 sequences obtained from p2089 (36). The resulting pLSP-BHRF1–3 plasmid was digested with SfiI and XbaI, and the *Cerulean* gene was inserted as a phenotypic marker. Twenty-four hours after DNA transfection, we measured luciferase activities with the Dual-Luciferase Assay Kit (Promega) and the Orion II Microplate Luminometer (Titertek-Berthold). The activity of Rluc was normalized to the activity of Firefly luciferase (Fluc) encoded by the psiCHECK-2 reporter. Site-specific mutagenesis was performed as previously described (35). We performed in silico prediction of EBV miRNA binding sites on 3′UTRs with TargetScan (www.targetscan.org) (41).

Quantitative RT-PCR. RNA was isolated using the Direct-zol RNA MiniPrep columns (Zymo Research). RNA was treated with DNase I (Thermo Fisher Scientific) and reverse transcribed with SuperScript III Reverse Transcriptase (Thermo Fisher Scientific), and quantitative PCR was performed using the LightCycler 480 SYBR Green I Mix (Roche) and the LightCycler 480 Instrument II (Roche) according to the manufacturer's instructions. The following primers were used:

HPRT1 for 5′-tgaccttgatttattttgcatacc-3′ and rev 5′-cgagcaagacgttcagtcct-3′,

HMBS for 5′-ctgaaagggccttcctgag-3′ and rev 5′-cagactcctccagtcaggtaca-3′,

GUSB for 5′-cgccctgcctatctgtattcattggaggtg-3′ and rev 5′-gatgaggaactcttggtgacagcc-3′,

IPO7 for 5′-tcgccattgtattcgagaaa-3′ and rev 5′-gaatgcatgtagtaagctgtaccc-3′,

IL12B for 5′-ccctgacattctgcgttca-3′ and rev 5′-aggtcttgtccgtgaagactcta-3′,

TAP1 for 5′-agtgccctggatgcaaac-3′ and rev 5′-agaaagaggatgtggtcacg-3′,

TAP2 for 5′-tgcgggacagaaacaacgtc-3′ and rev 5′-agcctgtgagcaatcaccag-3′,

EBNA1 for 5′-aagcatcgtggtcaaggagg-3′ and rev 5′-gcgaccaagttccttcgtc-3′,

LMP1 for 5′-aggctaggaagaaggccaaa-3′ and rev 5′-ctgttcatcttcgggtgctt-3′.3gtt

LMP2 for 5′-atcgctggtggcagtatttt-3′ and rev 5′-gagtatgccagcgacaatca-3′.

Western Blotting. We lysed cells with RIPA buffer [50 mM Tris·HCl (pH 8), 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% DOC] and boiled the extracts with Laemmli buffer. Proteins were separated on 10% (vol/vol) SDS/PAGE gels (Carl Roth) and transferred to nitrocellulose membranes (GE Healthcare Life Science) using the Mini-PROTEAN Tetra Cell apparatus (Bio-Rad). Membranes were blocked for 30 min with Roti-Block (Carl Roth) followed by antibody incubation. Secondary antibodies conjugated with horseradish peroxidase were used (Cell Signaling) and exposed to CEA films (Agfa HealthCare). Protein levels were quantified with the software ImageJ. The following primary antibodies reactive to human proteins were used: anti-human Tubulin (B-5–1-2; Santa Cruz), anti-human Actin (AC-74; Sigma), anti-human IPO7 (ab88339; Abcam), anti-human TAP1 (1.28; Acris), and anti-human TAP2 (2.17, Acris). Elisabeth Kremmer, Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Molecular Immunology, Munich, provided the antibodies specific for the EBV proteins LMP2, LMP1, and EBNA1.

Flow Cytometry and Antibodies. Stained cell suspensions were measured with the LSRFortessa or FACSCanto (BD Biosciences) flow cytometers and the FACSDiva software (BD Biosciences). Acquired data were analyzed with FlowJo software Ver. 9.8 (FlowJo). The following human-specific antibodies were used:

anti–HLA-ABC APC (W6/32, IgG2a; BioLegend),

anti–HLA-Bw6 PE (REA143, IgG1; Miltenyi Biotec),

anti–HLA-A2 PE (BB7.2, IgG2b; BioLegend),
anti–HLA-B7 PE (BB7.2, IgG2b; Santa Cruz),
anti–HLA-B8 unlabeled mAb (8.L.215; USbiological),
anti-mouse IgG PE (poly4053; BioLegend),
isotype IgG1 PE (MOPC-21; BioLegend),
isotype IgG2b PE (MPC-11; BioLegend),
isotype IgG2a APC (MOPC-173; BioLegend),
anti-CD8 Pacific Blue (RPA-T8; BioLegend),
anti-CD8 PerCP/Cy5.5 (RPA-T8; BioLegend),
anti-CD4 PE (RPA-T4; BioLegend),
anti-CD3 APC (HIT3a; BioLegend),
anti-CD3 APC/Cy7 (HIT3a; BioLegend),
anti-CD19 FITC (HIB19; BioLegend),
HLA-A*0201/CLG pentamer (CLGGLLTMV, LMP2; Proimmune),
HLA-B*0702/RPP pentamer (RPPIFIRRL, EBNA3A; Proimmune), and
HLA-B*0801/QAK pentamer (QAKWRLQTL, EBNA3A; Proimmune).

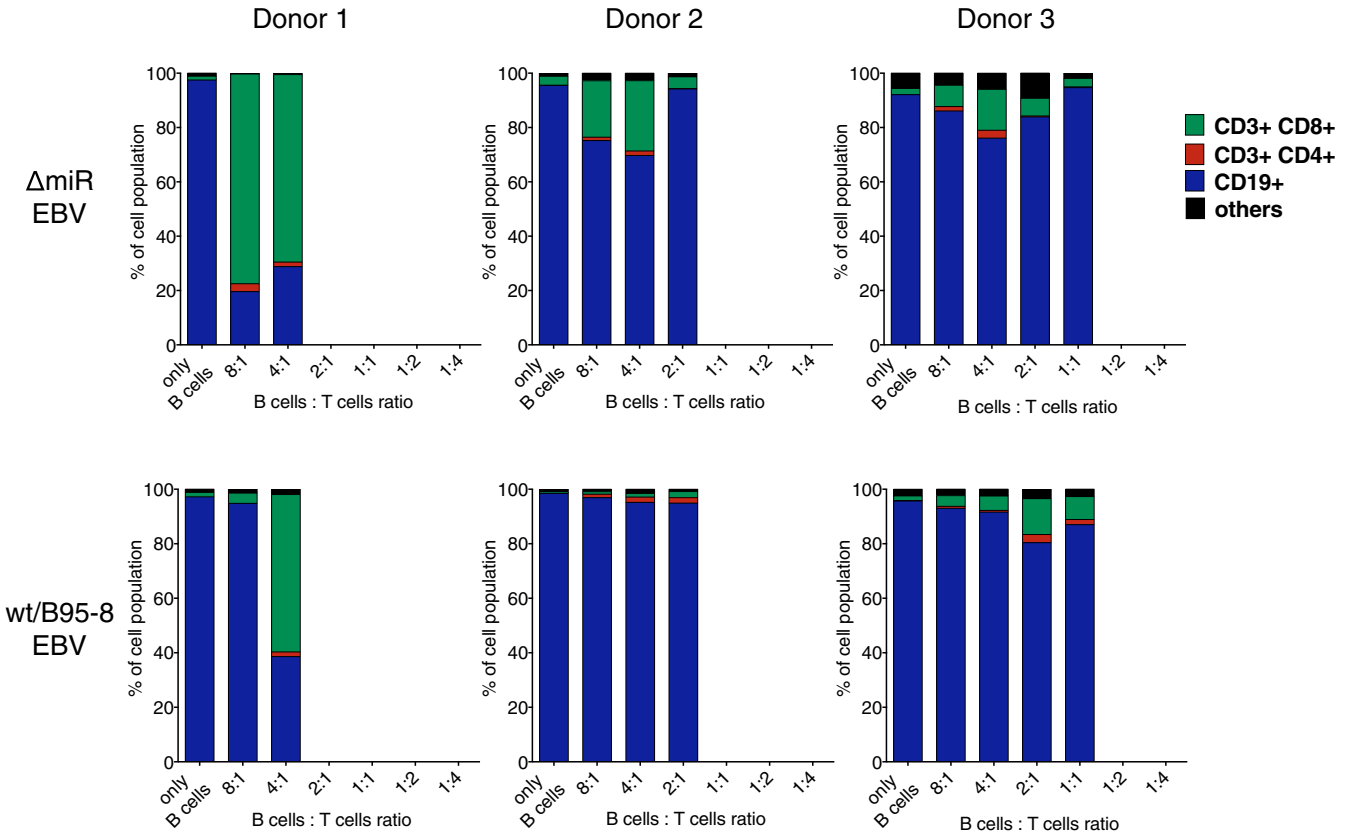

Fig. S1. Composition of long-term cocultures of WT/B95-8 or ΔmiR EBV-infected B cells with autologous $CD8^+$ T cells from three different EBV-positive donors. Coculture experiments of $CD19^+$ B cells infected with WT/B95-8 EBV or ΔmiR EBV stocks and autologous $CD8^+$ T cells were performed as shown in Fig. 1. After 4 wk, the compositions of the outgrowing cells were analyzed by FACS: B cells ($CD19^+$), $CD8^+$ T cells ($CD8^+/CD3^+$), $CD4^+$ T cells ($CD4^+/CD3^+$), or cells negative for all of the four markers are indicated.

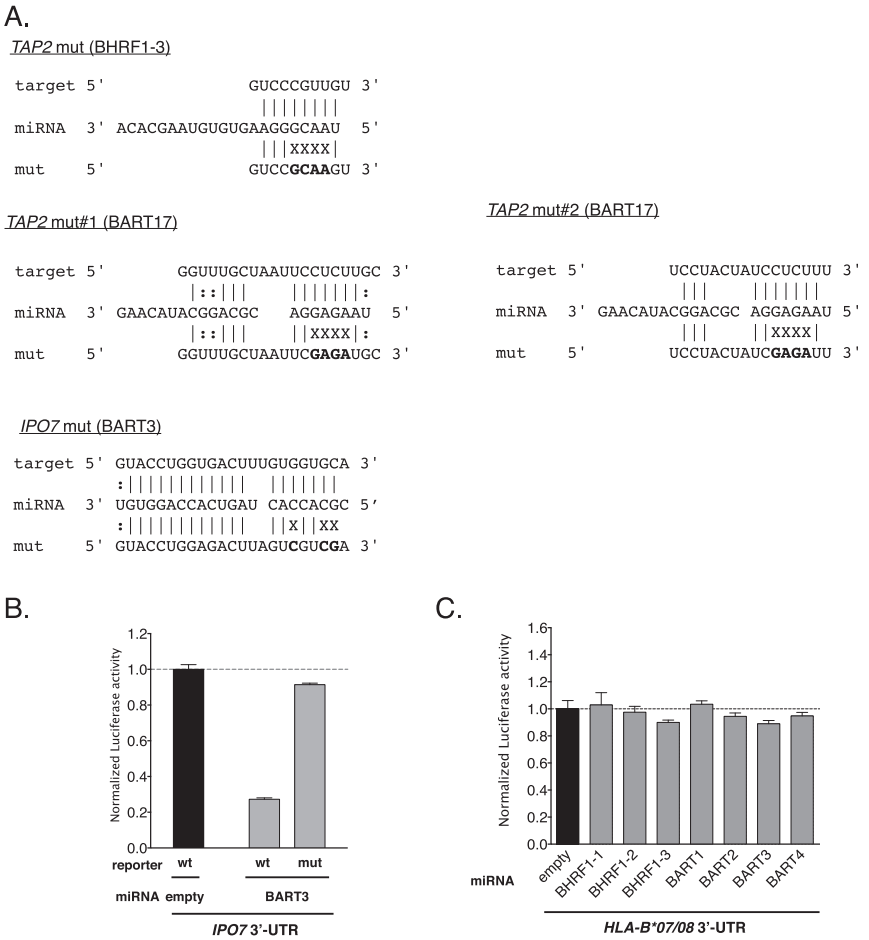

Fig. S2. Predicted miRNA target sites, their mutations, and luciferase assays of selected targets. (*A*) Partial sequences of 3′UTRs of selected transcripts analyzed in Fig. 3*C* and in *B* below are shown with corresponding miRNAs and mutations within the 3′UTRs in the reporter vectors. Complementarities are based on in silico predictions according to the RNAhybrid algorithm and depicted as Watson–Crick ('|') or G:U (':') pairs. Nonmatching nucleotide residues are indicated (X). (*B* and *C*) HEK293T cells were cotransfected with miRNA expression plasmids and luciferase reporter plasmids carrying either a wild-type or mutated 3′UTR of *IPO7* (*B*) or the 3′UTR of HLA-B*07/B*08 (*C*). The luciferase activities were normalized to lysates from cells cotransfected with the wild-type 3′UTR reporter and an empty plasmid in place of a miRNA plasmid. Data are shown as mean values and SD of three replicates. Mut, mutated 3′UTR; WT, wild-type 3′UTR; ∅, empty plasmid.

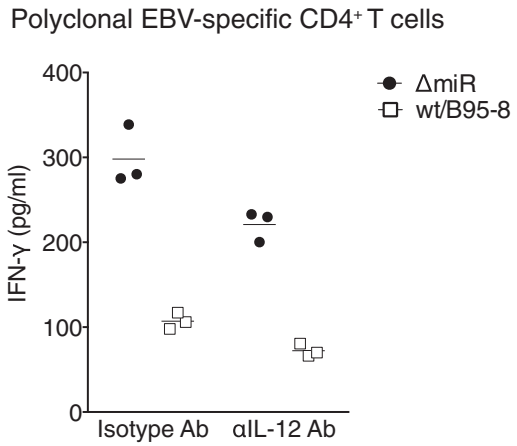

Fig. S3. IL-12 neutralization barely reduces the activation of EBV-specific $CD4^+$ T cells. Equal numbers of infected B cells (5 dpi) were cocultured with polyclonal EBV-specific $CD4^+$ T cells together with an anti–IL-12B antibody or a control antibody of the same isotype (2.5 μg/mL). After 16 h, IFN-γ release was measured by ELISA. One representative experiment out of four with different donors is shown.

Table S1. HLA class I types of the donors

Donor	*HLA-A*	HLA-B	HLA-C
32	*0201, *0301	*3501, *1501	*0102, *0401
59	*11, *24	*35, *40	n.a.
115	*02, *23	*49, *40	n.a.