Supplementary Methods

Antibodies

Blimp-1/PRDM1 rabbit monoclonal antibody (C14A4) was purchased from Cell Signaling (Danvers, MA) and used at 1:1000 to detect the total *PRDM1* protein level in all cell lines by Western blotting. Blimp-1/PRDM1 mouse monoclonal antibody (3H2E8) for immunohistochemistry was purchased from Santa Cruz (Dallas, Texas) and used at 1:25 dilution. Antibody against Lamin B was purchased from Santa Cruz Biotechnology Inc (Dallas, TX), and used at 1:500 dilutions. EBNA2 mouse monoclonal antibody (PE1) was purchased from DAKO North American, Inc (Carpinteria, CA) and used at 1:50 dilution. Fluorescence labeled goat anti-rabbit, goat anti-mouse and Donkey anti-goat from Li-Cor (Lincoln, NE) were used as secondary antibodies at 1:10000 dilutions.

miRNA inhibitor and siRNA

EBV-miR-BHRF1-2-3p miRNA inhibitor (mature EBV-miR-BHRF1-2-3p sequence: UAUCUUUUGCGGCAGAAAUUGA) was purchased from Applied Biosystems, Grand Island, NY (Cat. No. 4464084-Assay ID: MH13071). Smartpool-On-TARGET plus PRDM1 siRNA was purchased from GE HEALTHCARE DHARMACON (Pittsburgh, PA, Cat No. L-009322-00-0005). ON-TARGET plus Non-targeting control Pool was purchased from GE HEALTHCARE DHARMACON (Cat No. D-001810-10-05). SiGLO Green Transfection Indicator was purchased from GE HEALTHCARE DHARMACON (Cat No. D-001630-01-20).

Plasmid construction

The PMSCV-PRDM1 expression plasmid was first generated by PCR amplification using PRDM1-f: 5'-AAGGTCGACATGTTGGATATTTGCTTGGAA-3' as forward primer, and PRDM-r: 5'-GCCGAATTCTTAAGGATCCATTGGTTCA-3' as reverse primer, with PRDM1α cDNA as the

template. The DNA fragment was then gel purified and double digested with XhoI and EcoRI prior to sub-cloning into the PSMCV-PIG vector (Addgene, Cambridge, MA). Construction of pSIC.PRDM1.3 UTR.538–2419 was as follows: A genomic fragment containing nucleotides 1 to 2419 of the PRDM1 3 UTR was PCR-amplified using 5'-CTCGAGGATTTTCAGAAAACACTTATTTTGTTTC-3' as forward primer, and 5'-GCGGCCGCACATTTTGACAATTTGCACATAAATAAC-3' as reverse primer, the insert was gel purified and cloned into psiCHECK-2 (Promega, Madison, WI) down-stream of the Renilla luciferase coding region between the XhoI and NotI sites. The mutant reporter constructs were generated by using pSIC.PRDM1.3′UTR.538-2419 as a template and mutating the third and fourth positions of the seed region of EBV-miR-BHRF1-2 binding site using the GeneEditor *in vitro* Site-Directed Mutagenesis System (Promega). The sequence of EBV-miR-BHRF1-2 mut is: 5'-GTGAGCCAAGCCATGTAATTGATCTACTTTTTCTAAGGGC-3'. Sequence of both wild type and the mutant reporter constructs were confirmed by double-stranded sequencing. For construction of the pcDNA3.1-SCARNA20 plasmid, PCR amplification was first performed using CCL156 genomic DNA as template, and SCARNA20-100-F-NHEI: 5'-CTGGCTAGCGAGAACTATGAAGGAGCAGCTATTAC-3' as forward primer, SCARNA20+100-R-APAI: 5'-ACCGGGCCCTACAGCTTTGGGCAATTATAAAACCT-3' as reverse primer. The DNA fragment was then gel purified and double digested with NHEI and APAI prior to sub-cloning into the pcDNA3.1 vector (Addgene, Cambridge, MA).

Western blotting and Immunohistochemistry

Immunoblotting and Immunoperoxidase staining were performed according to previously described methods.[9, 21] Intensity of PRDM1 protein was measured by grayscale using Image J software, and normalized to Lamin B expression.

Immunohistochemistry double staining of Blimp1/EBNA2 was accomplished by staining the two antibodies sequentially, using Bond III Autostainer (Leica Microsystems). Sections were first baked and deparaffinized. They were then subjected to appropriate antigen retrieval and incubation procedures. For PRDM1, antigen retrieval was performed using Bond Epitope Retrieval Solution2 (ER2) at 99-100°C for 20 minutes (Leica Microsystems). Endogenous alkaline phosphatase was inactivated using Dako Dual Endogenous Block (Dako) for 5 minutes. Then sections were incubated with primary antibody, post primary AP, polymer AP (Bond Polymer Refine Red Detection, Leica Microsystems) and vector blue chromogen (Vector Laboratories, Inc.) for 15, 20, 30, and 20 minutes, respectively. For EBNA2. antigen retrieval was achieved by using Bond Epitope Retrieval Solution2 (ER2) at 99-100°C for 20 minutes (Leica Microsystems). Sections were then incubated sequentially with Dako Dual Endogenous Block (Dako) for 5 minutes, primary antibody for 15 minutes, post primary AP for 20minutes, polymer AP for 30 minutes and refine red for 15 minutes (Bond Polymer Refine Red Detection, Leica Microsystems). Finally slides were washed in H2O, then dehydrated in 100% Ethanol, and mounted using Leica CV5030 Glass Coverslipper.

Gene expression assay probes

PRDM1 gene expression assay (Cat No. 4331182-Assay ID: Hs01068507_m1) that detects the total PRDM1 mRNA level was purchased from Applied Biosystems (Grand Island, NY). Gene expression assays for qPCR validation including: *KRTAP5-6*, *PTGER3*, *SLFNL1*, *SNORD92*, *SNORD7*1, *BCYRN1*, *BTCC1*, *LOC100505978*, *SCARNA11*, *MIR4659A*, *SNOARD80*, *GPN1*, *SCARNA20*, and *GAPDH* gene were also purchased from Applied Biosystems.

MicroRNA Quantitation

EBV-miR-BHRF1-2 expression levels in a panel of LCLs and FFPE samples were determined using EBV-miR-BHRF1-2 TaqMan® MicroRNA Assay (Cat No. 4427975-Assay ID: 197239_mat) (Applied Biosystems, Grand Island, NY) according to manufacturer's instructions. The expression levels of EBV-miR-BHRF1-2 were normalized to endogenous control UN48 and the relative levels were calculated using the ΔΔCt method.

Firefly luciferase assay

To assess the effect of EBV-miR-BHRF1-2 binding site on luciferase activity, 20 nM of EBV-miR-BHRF1-2 precursor molecule (Ambion, Austin, TX) was co-transfected with either 20 nM of pSIC.PRDM1.3'UTR 538 to 2419 wild type or mutant plasmids in 293T cells, respectively. Firefly luciferase assay was performed as previously described. [9]

Cell cycle analysis and apoptosis assay

For the miRNA/siRNA study, $0.5x10^6$ CCL159 and CCL156 cells were transfected either with 1μg pMSCV-PIG vector or pMSCV-PRDM1 plasmids, or co-transfected with one of the four combinations: miRNA inhibitor negative control plus siRNA scramble control; EBV-miR-BHRF1-2 inhibitor plus scramble control; miRNA inhibitor negative control plus *PRDM1* siRNA; EBV-miR-BHRF1-2 inhibitor plus PRDM1 siRNA. For the SCARNA20 study, $0.5x10^6$ LCL cells were co-transfected with one of the four combinations: pcDNA3.1 plus pMSCV-PIG; pcDNA3.1-SCARNA20 plus pMSCV-PIG; pMSCV-PRDM1 plus pcDNA3.1; pcDNA3.1-SCARNA20 plus pMSCV-PRDM1 at 48 h time point. Cells were labeled with bromodeoxyuridine (BD Biosciences, San Jose, CA) for 2 h prior to stain with V450 conjugated anti-Brdu antibody (BD Biosciences) according to manufacturer's instruction. The percentage of cell cycle distribution was measured by flowJo (Tree Star Inc. Ashland, OR). Cell viability was determined at 48 h time point by PE annexin V Apoptosis Detection Kit I (BD Biosciences) using LSRII flow cytometer.

Whole transcriptome sequencing (RNA-seq)

1.5 x10^7 of JY25 and CCL159 cells were transfected with 30 μg of one of the following: PRDM1-PIG, PMSCV-PRDM1 plasmid, 2 μM EBV-miR-BHRF1-2 inhibitor or miRNA inhibitor negative control. Total RNA was extracted from cells at 48 h time point using Qiagen RNasey mini kit (Valencia, CA) upon transfection, and transfection efficiency was determined by flow cytometry. The *PRDM1* mRNA expression was also quantified using qPCR assay prior to the RNA seq analyses. The integrity of RNA samples were checked on the 2100 Electrophoresis Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA) with RNA integrity number of greater than 9. In addition, RNA concentrations were measured on Qubit (life technologies, Grand Island, NY). 500 ng of total RNA was reverse transcribed into single stranded cDNA using Truseq RNA sample preparation kit v2 (illumina, San Diego, CA), DNA library was then validated using the 2100 Electrophoresis Bioanalyzer instrument, and sequence was performed on HiSeq 2500/1500 with 4 pooled libraries per lane.

Total and miRNA extraction from formalin-fixed paraffin-embedded (FFPE) samples

Total RNA, including the small RNA fraction was extracted from eight FFPE samples using miRNeasy kit according to the instruction manual (Qiagen, Valencia, CA). EBV-miR-BHRF1-2 and *PRDM1* mRNA expression were determined by qPCR using cDNA reverse transcribed from either 10 ng or 80 ng of RNA inputs as described previously.[6]