Table W1. Distribution of Small RNA from Cloned Libraries.

	C666-1	X2117
Human sequences	812	167
Total known hsa-miRNA	277	433
Total known ebv-miRNA*	615	504
BART1-5p (–3p)	25 (9)	6 (2)
BART2-5p	1	0
BART3-3p (–5p)	30 (11)	6 (1)
BART4	11	2
BART5	22	5
BART6-5p (–3p)	18 (17)	0 (0)
BART7	45	64
BART8 (8*)	15 (7)	16 (10)
BART9	179	288
BART10	169	82
BART11-5p (–3p)	7 (4)	0 (0)
BART12	6	1
BART13 (13*)	1 (0)	3 (1)
BART14 (14*)	5 (2)	0 (2)
BART16	7	0
BART17-3p (–5p)	14 (9)	6 (0)
BART18-5p	1	0
BART19-3p (–5p)	0 (0)	8 (1)
EBV fragments (Group 1)[†]	1	0
EBV fragments (Group 2)[‡]	14	1
EBV fragments (Group 3)[§]	17	2
Total number of EBV fragments	32	3
Unknown sequences	77	8
Total clones for analysis	1813	1115

*All miR-BHRF1s, BART15, and BART20 were not cloned.
[†]miR-BART21 clones.
[‡]miR-BART22 clones.
[§]EBV sequences from EBERs.

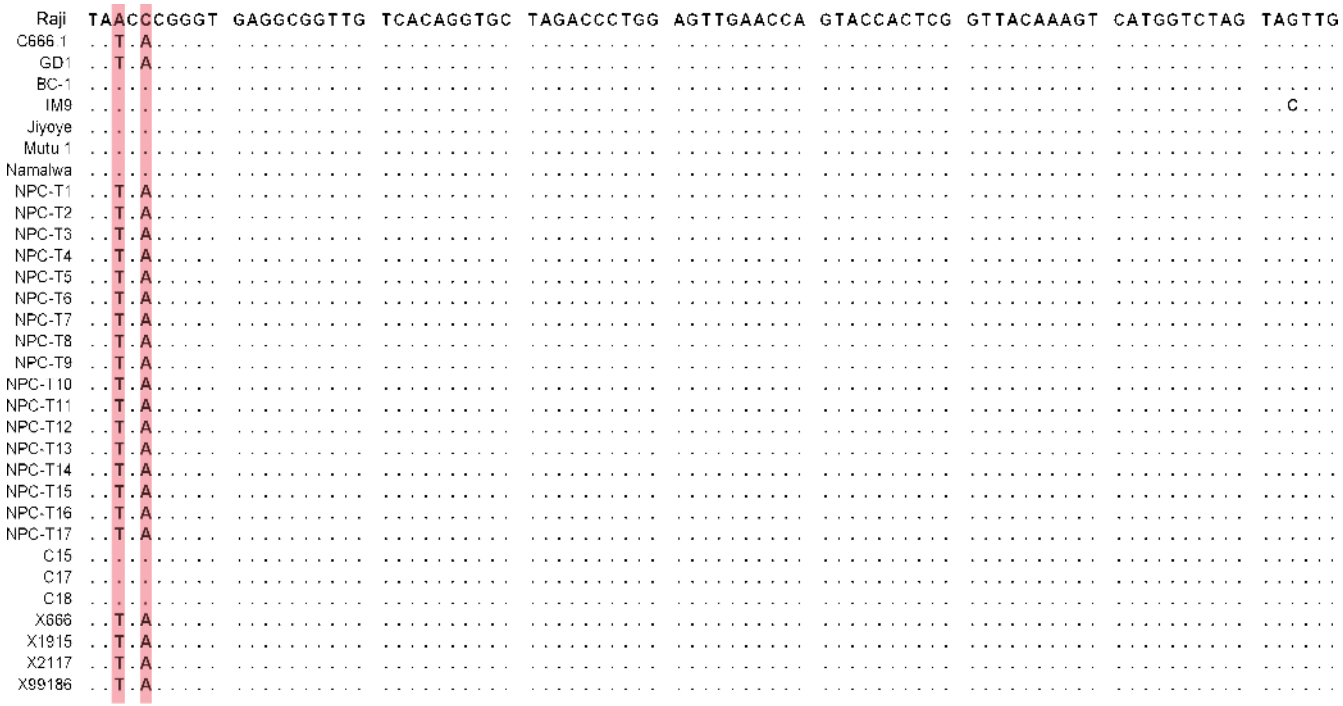

Figure W1. Alignments of pri-miR-BART22 in different EBV strains. Alignment of pri-miR-BART22 (AJ507799, 147142:147227) in 17 NPC samples (NPC-T1 to NPC-T17), EBV-infected cell lines, and NPC xenografts. The sequences of Raji (M35547), IM9 (EU828628), Mutu 1 (EU828632), and NPC samples GD1 (AY961628), and C18 (EU828627) were extracted from GenBank for analysis. The sequences of other samples were directly obtained by sequencing in our laboratory. The two critical nucleotide variations for small side stem-loop formation are shown inside the pink boxes.

Table W2. Examples of Predicted Cellular mRNA of miR-BART22.

Gene ID*	Gene Function	Alignment (miRanda score and energy)
	Cell cycle	
E2F3 (ENSG00000112242)	Transcription factor E2F3	Query: 3′ TGATGATCTGGT---ACTGAAACATT 5′ Ref: 5′ ACTACTA-ATCAGTTTAGCTTTGTGT 3′ (104, –18.59 kcal/mol)
ATM (ENSG00000149311)	Serine-protein kinase ATM (ataxia telangiectasia mutated)	Query: 3' TGATGATC-TGGTAC-TGAAACATT 5' Ref: 5' ACTGGTGGAAACTTGAACTTTGTGT 3' (100, –18.57 kcal/mol)
	Apoptosis, cell proliferation	
MAKP3K5 (ENSG00000197442)	Mitogen-activated protein kinase kinase kinase 5 (apoptosis signal-regulating kinase 1)	Query: 3′ TGATG-ATCTGGT--ACTGAA-ACATT 5′ Ref: 5′ TCTGCTTAGACCACCTGGTTTATGTGA 3′ (110, –23.12 kcal/mol)
FAS (ENSG000000261033)	Tumor necrosis factor receptor superfamily member 6 precursor	Query: 3' TGATGATCTGGTACTGAAACATT 5' Ref: 5' CCTCCT-CAGCATGGCTTTGTGT 3' (106, –18.4 kcal/mol) Query: 3' TGATGAT--CTG-GTACTGAAACATT 5' Ref: 5' ACTTTTACCCATGCATGATTTTGTAA 3' (124, –18.2 kcal/mol)
CASP3 (ENSG00000164305)	Caspase-3 precursor	Query: 3′ TGATGATCTGG-TACTG-AAACATT 5′ Ref: 5′ CTTACTAGACCTGTAACTTTTGTAA 3′ (119, –23.62 kcal/mol)

*Gene symbol is indicated as recommended by HUGO.

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B95-8       CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
Akata       CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
BC1         CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
C17         CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
C15         CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
X1915       CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
X2117       CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
C666-1      CGTCATCTGG CTCTCCTGTG TGACCCCTCA CTTTGTACAG ACTTTTGGCA
LMP2A-UTR   ---------- ----CCTGTG TGACCCCTCA CTTTGTAC-- ----------
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Figure W2. Conservation of the putative miR-BART22 binding site on the 3′UTR of the *LMP2A* gene in different EBV strains. Direct sequencing results of the 3′UTR of the *LMP2A* gene from different samples are illustrated. B95-8 sequence is extracted from GenBank (accession number: X01995) and is shown as a reference sequence.

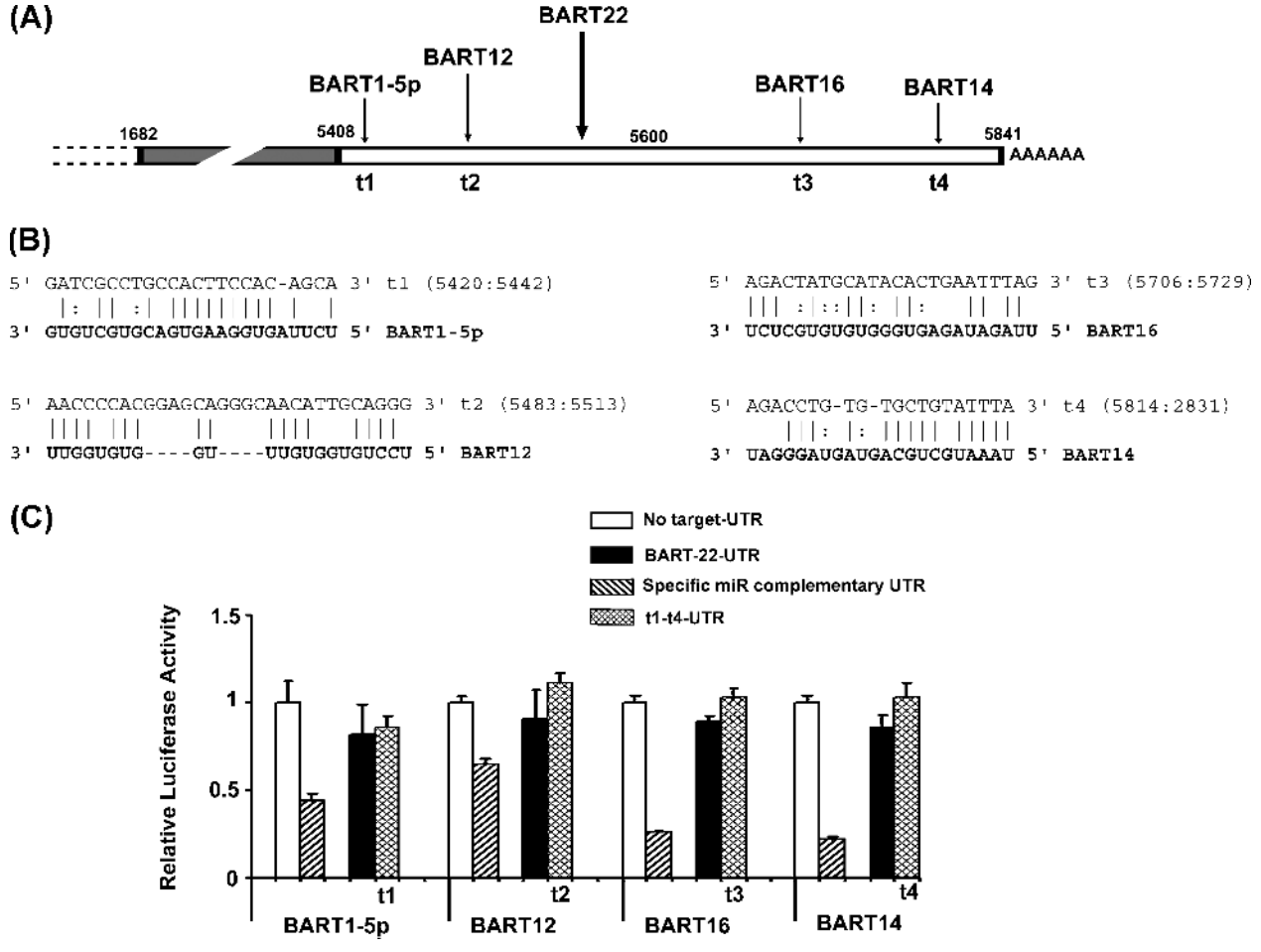

Figure W3. Predicted miR-BARTs binding site on LMP2A 3′UTR. (A) Schematic diagram showing the location of predicted target site (t1-4) on the 3′UTR of the *LMP2A* gene (open bar) according to GenBank accession no. AJ507799. (B) The alignment of the target sites to the corresponding miR-BARTs is shown. The target sites were cloned into luciferase reporters for analysis. (C) Luciferase reporter assays of t1-4 containing constructs in the presence of indicated miR-BARTs were performed in 293FT cells. Reporter activity was normalized to renilla luciferase control. The luciferase activity from construct containing no miRNA target on 3′UTR (white bar) was set at 1. Data shown are the mean ± SD from three independent experiments.

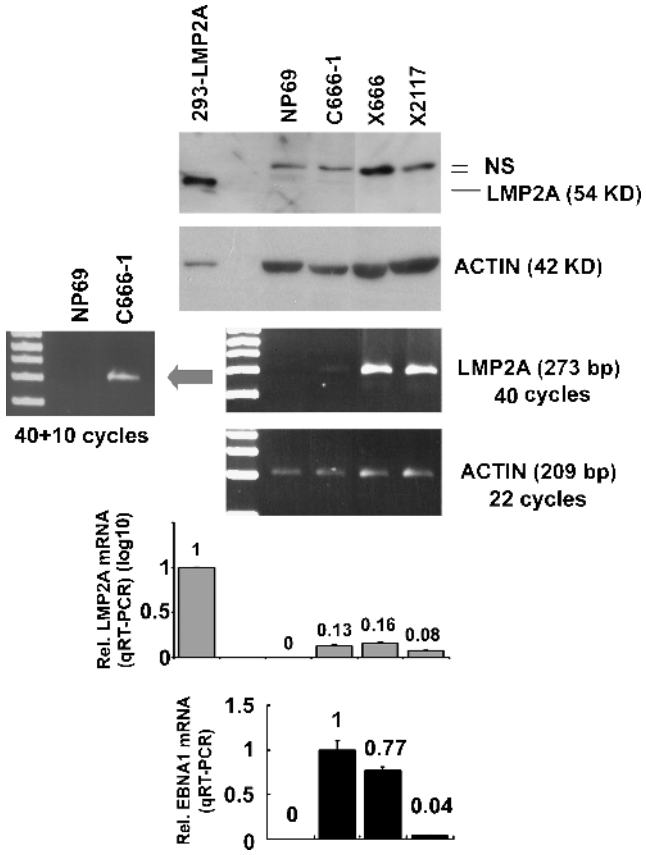

Figure W4. Western blot analysis of LMP2A expression in NPC samples. One NPC cell line (C666-1) and two NPC xenografts (X666 and X2117) were analyzed. Protein samples from LMP2A-transfected 293FT cells (293-LMP2A) and an EBV-negative epithelial cell line, NP69, were included as the positive and negative controls, respectively. The nonspecific bands (NS) are labeled. The LMP2A and EBNA1 mRNA expression levels in the same sample were confirmed by RT-PCR and QRT-PCR, respectively. The QRT-PCR results were normalized to GAPDH and are shown as mean ± SD from at least three independent experiments. The expression levels of LMP2A in 293-LMP2A and EBNA1 in C666-1 were set at 1.

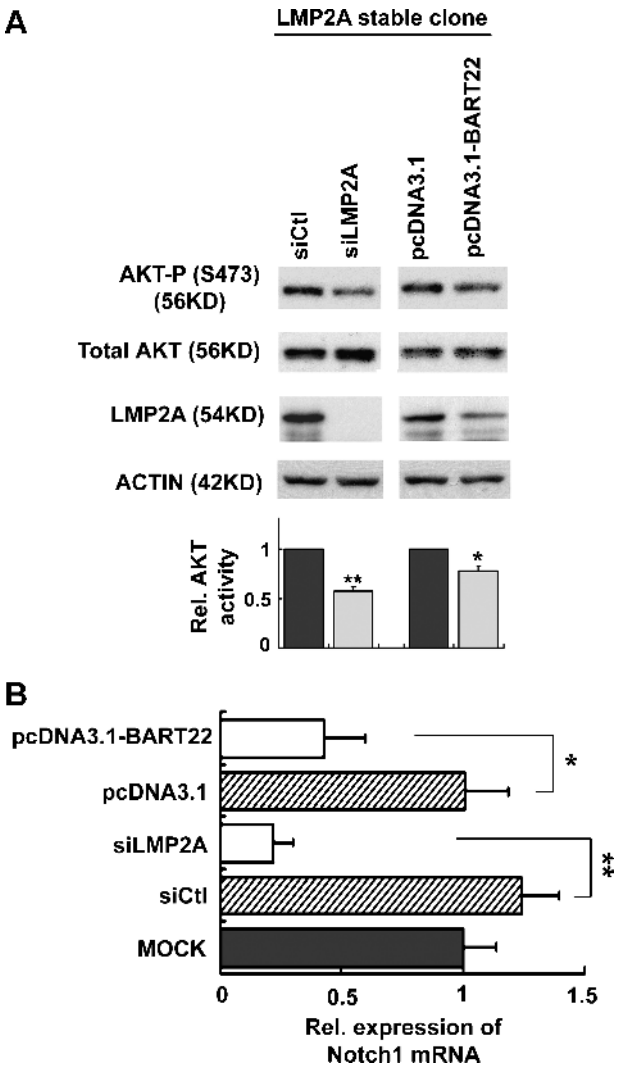

Figure W5. Inhibition of LMP2A downstream effectors by miR-BART22. (A) Inhibition of LMP2A-mediated AKT activity by miR-BART22 was demonstrated using Western blot. HEK293-LMP2A stable cells transfected with siRNA control (siCtl) and LMP2A specific siRNA (siLMP2A) were included as controls. The representative blot from three independent experiments is shown in panel A. The AKT activity was calculated by the expression level of phosphorylated AKT over total AKT and relative to control transfection (set at 1) is shown. (B) The relative expressions of Notch-1 of these transfected cells were analyzed by QRT-PCR. Notch-1 expression levels were normalized to actin and were compared with the mock transfection (set at 1). All data shown in the figure are the mean ± SD from three independent transfection experiments. Statistical analysis by Student's *t* test was used and compared with the control transfection. $*P < .05$; $**P < .001$.