Supplementary Material

Supplementary Table 1: Descriptions of the retroviral vectors used.

Vector Plasmid Number	Vector Type	Vector Description	Marker
3637	MMLV-based SIN retroviral vector	Upstream SV40 promoter in front of a multiple cloning site Downstream PGK promoter drives expression of mRFP (empty vector control)	mRFP
3673	MMLV-based SIN retroviral vector	Upstream SV40 promoter in front of a multiple cloning site Downstream PGK promoter drives expression of CFP (empty vector control)	CFP
3628	MMLV-based SIN retroviral vector	Upstream SV40 promoter drives expression of intronic portions of the *BART* locus encoding *BART* 1, 3-20, and 22 Downstream PGK promoter drives expression of mRFP	mRFP
3676	MMLV-based SIN retroviral vector	Upstream SV40 promoter drives expression of intronic portions of the *BART* locus encoding *BART* 1, 3-20, and 22 Downstream PGK promoter drives expression of CFP	CFP

Supplementary Table 2: Primer and probe sequences used.

Gene	Primer	Sequence
BID	Forward PCR	AAACAGTGCAACACTGGTCTGCTG
	Reverse PCR	AATCATGAGTCCGTCAGTGCCCTT
	TaqMan probe	(6 FAM) AACATGAGGCCACTGGAGACGCATAT (3 Iowa Black FQ)
CCR7	Forward PCR	ACACCAGACAGACAACACTGGGAA
	Reverse PCR	ATTTGAGTCTGTGGGAGGCCAGAA
	TaqMan probe	(6 FAM) AAACTGTTCCCACCTGCTGGAGTGAA (3 Iowa Black FQ)
CD2	Forward PCR	TGTGGATTTCTGCCCTCCTGATGT
	Reverse PCR	TGACCACATGGCTGAGTTCGAAGA
	TaqMan probe	(6 FAM) AGAACATTGTCACCTCCTGAGGCTGT (3 Iowa Black FQ)
CGA	Forward PCR	TGTTCTCCATTCCGCTCCTGATGT
	Reverse PCR	AGCAGCCCATGCACTGAAGTATTG
	TaqMan probe	(6 FAM) TTGCCCAGAATGCACGCTACAGGAAA (3 Iowa Black FQ)
CHN1	Forward PCR	TGAAACTACTGCCACCTGCTCACT
	Reverse PCR	TGGGTCCAAAGACGATTCCAAGGT
	TaqMan probe	(6 FAM) TGGCACATCTAAAGAGAGTGACCCTCCA (3 Iowa Black FQ)
HIF1A	Forward PCR	TTGGCAGCAACGACACAGAAACTG
	Reverse PCR	TTGAGTGCAGGGTCAGCACTACTT
	TaqMan probe	(6 TAM) TTTGGCAATGTCTCCATTACCCACCGCT (3 Iowa Black FQ)
GAPDH	Forward PCR	TCAACGACCACTTTGTCAAGCT
	Reverse PCR	CCATGAGGTCCACCACCCT
	TaqMan probe	(6 FAM) TTCCTGGTATGACAACGAATTTGGCTACAGC (TAM)
NEDD4L	Forward PCR	AGGAAGGATGTGGAAAGAGCACCT
	Reverse PCR	AGGACTGGCTGCAGAGAAGCATAA
	TaqMan probe	(6 FAM) TGTGTTGCGGTGTATTCCCAGCTTCA (3 Iowa Black FQ)

Supplementary Figure 1. Ectopic expression of *BART* miRNAs partially complements the loss of EBV in D7-1 cells. (A) The population growth of D7-1 cells was measured over time as EBV was evicted following induction of dominant negative EBNA1 (dnEBNA1) at day 0 with doxycycline. The average of three independent experiments ± SD is shown. * p=0.05, one-sided Wilcoxon signed rank test, comparing total number of cell doublings. (B) Cells were cultured for 20 days with or without induction of dnEBNA1 and then scored for the presence of viral DNA by FISH analysis. For each experiment, at least 200 cells were counted per condition. The average percentage of EBV-negative cells ± SD from two independent experiments is shown. (C) Real-time PCR measurements of the levels of two *BART* miRNAs, *BART* 1-5p (left graph) and *BART* 7 (right graph), were made 12 days after induction of dnEBNA1 in D7-1 cells. The average number of miRNA molecules per 10 picograms of total RNA ± SD is shown from two independent experiments.

Supplementary Figure 2. No correlation between the presence of the *BART* miRNAs and Bim protein levels. Bim_{EL} levels were analyzed by Western blot in Sav-BL S1-1 cells transduced with empty vectors or ones encoding the *BART* miRNAs. Bim_{EL} levels were normalized to alpha-tubulin levels, and then compared to the control cells (cells transduced with empty vectors, dnEBNA1 kept off) whose normalized level was arbitrarily set to one. The average of four independent experiments ± SD is shown.

Supplementary Figure 3. Microarray and realtime PCR analysis reveal several candidate genes for regulation by the *BART* miRNAs.

Seven candidate genes (BID, CCR7, CD2, CGA, CHN1, HIF1A, NEDD4L) for being regulated by the *BART* miRNAs were identified by three independent microarrays and the levels of these genes were then measured by realtime PCR in S1-1 cells 20 days after induction of dnEBNA1. The top graph depicts genes that were confirmed to change as predicted by microarray, the bottom graph depicts

genes that were not. Detected levels of the transcripts were normalized to an internal control transcript (GAPDH) and then compared to wild-type levels (cells transduced with empty vectors in which dnEBNA1 is not induced) which were arbitrarily set to one. The average of three independent experiments ± SD is shown. The differences in the transcript levels between uncomplemented cells (dnEBNA1 on, no ectopic *BART* miRNAs) and complemented cells (dnEBNA1 on, ectopic *BART* miRNAs present) is statistically significant for the confirmed genes (p=0.04 for CCR7, p=0.02 for CD2, p=0.02 for CGA, one-sided Wilcoxon rank sum test, top graph) and not significant for the unconfirmed genes (p=0.14 for HIF1A, p=0.14 for CHN1, p=0.26 for NEDD4L, one-sided Wilcoxon rank sum test, bottom graph). BID was not detected in S1-1 cells, but was efficiently detected (soon after 25 cycles in the reaction) in both the EBV-negative lymphoma cell line BJAB and 293 cells, and is thus likely a false positive.

Supplementary Figure 4. **Ablation of predicted *BART* miRNA target sites prevents *BART* miRNA regulation of a CASP3 reporter.** (A) Reporter assays were conducted with constructs encoding either the wild-type 3`-UTR of *CASP3* or a form with the predicted BART 1-3p and BART 16 binding sites mutated cloned downstream of luciferase. The predicted target sites, mutated sites, and corresponding miRNA seed sequences are shown. (B) The vectors were introduced into EBV-positive or EBV-negative Oku-BL cells, and the normalized luciferase activity in the EBV-negative cells was set to 100%. Data are the average of three independent experiments ± s.d (Wilcoxon rank sum test).

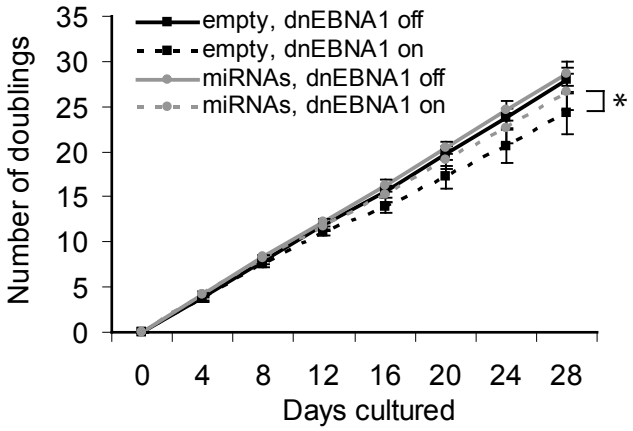
Supplementary Figure 5. Relative importin-7 and caspase-3 protein levels are reduced in EBV-positive and EBV-depleted/BART+ cells. Cell lysates collected from S1-1 cells 20 days after induction of dnEBNA1 were separated by SDS-PAGE and blotted with antibodies detecting importin-7 or caspase-3. Bands were quantified using ImageJ. Measured levels of importin-7 and

caspase-3 were normalized to alpha-tubulin as a loading control. The normalized importin-7 and caspase-3 levels of the EBVdepl sample for each replicate was then set to 100% and the other samples were normalized to this sample. Standards shown are dilutions of a lysate from a BL cell line transduced with a GFP expression vector and were quantified to ensure the detection of each protein was within the linear range of detection. The normalized expression values are reported in Figure 2e.

Supplementary Figure 6. The *BART* miRNAs promote the transformation of primary human B cells. Primary human B cells isolated from adenoids (B-C) or PBMCs (D) (1.5×10^5 per ml) were infected with either 2089 or 4080 at a multiplicity of infection of 0.01, incubated for 18 hours, and seeded in fresh medium (at a density of 1.5×10^5 cells per ml). At day 5 (6 in D), 9, and 12 p.i., cells were harvested and their proliferation analyzed by FACS. To determine the absolute number of cells counted, a volume standard was added prior to FACS analysis as described (26). (A) A schematic of the viruses used. 4080 contains a cassette with 22 pre-miRNAs derived from the Jijoye strain of EBV expressed from the HCMV immediate early promoter and introduced by recombination into the *BALF1* gene of 2089. (B) Representative results of FACS analysis. EBV-infected cells with forward (FSC) and sideward (SSC) scatter characteristics indicative of activated lymphoblast cells ("transformed" cells) were gated as indicated and the number of cells in this gate was recorded. (C) The number of transformed cells at each time point was measured. To correct for variation between donors, the raw cell counts were normalized to those infected by 2089 at day 5 (whose value was arbitrarily set to one). The average from four different donors ± SD is shown. p=0.05 for all time points (one-sided Wilcoxon signed rank test). (D) Primary B cells (1.5×10^5 per ml) purified from PBMCs were infected and analyzed as in C.

Supplementary Fig. 1

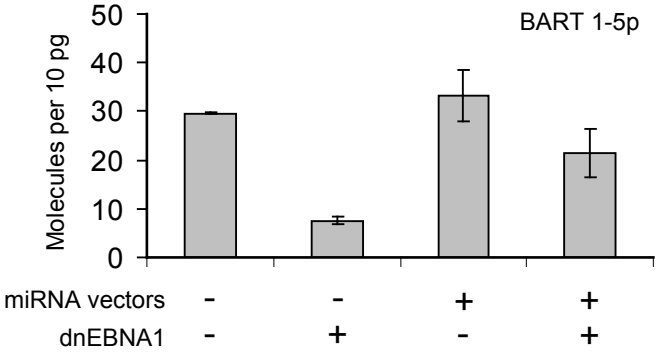
a

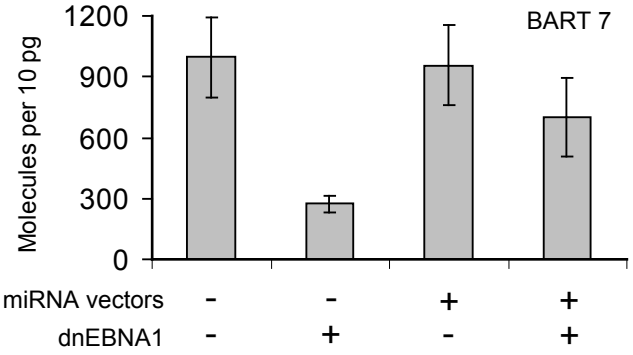


b

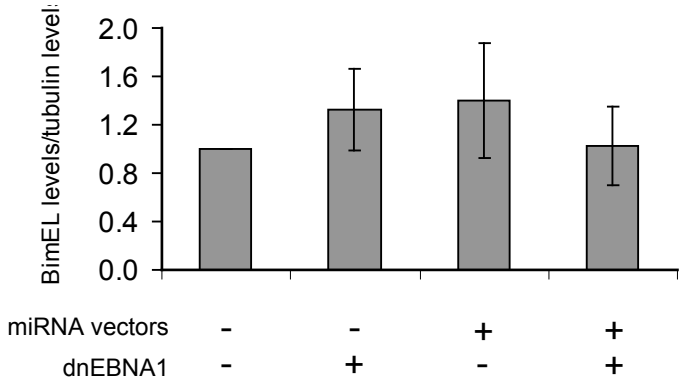
Vector	dnEBNA1	Percentage EBV-negative
Empty	Off	1 ± 1
	On	30 ± 7
miRNAs	Off	<1
	On	27 ± 7

c

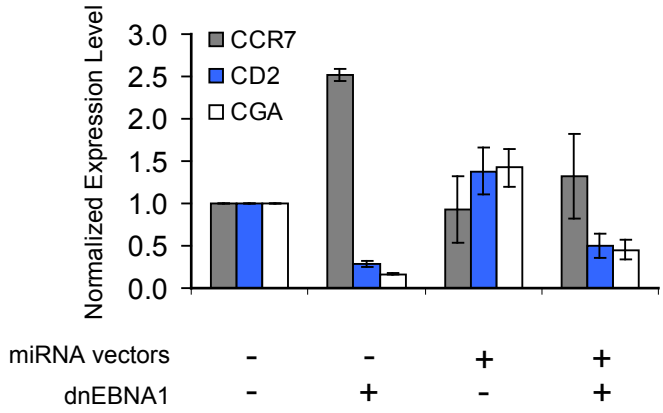


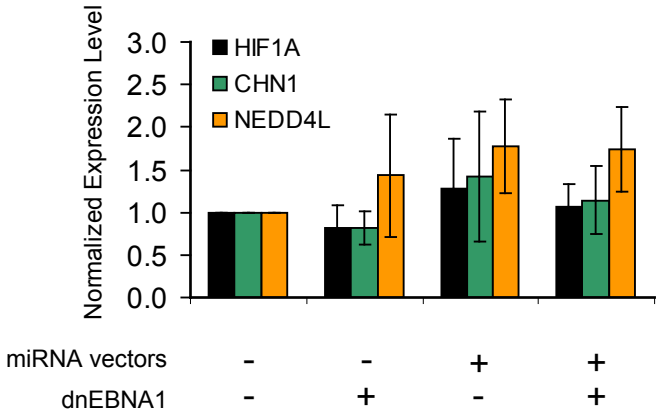


Supplementary Fig. 2



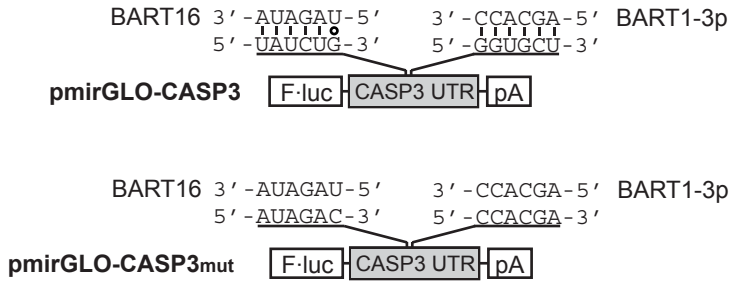
Supplementary Fig. 3





Supplementary Figure 4

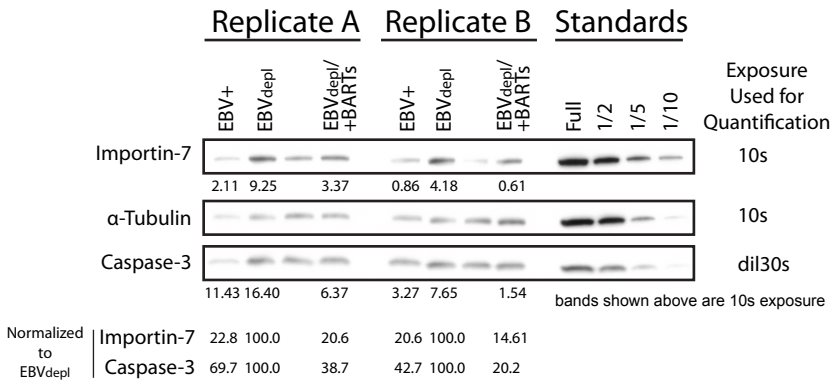
a



b

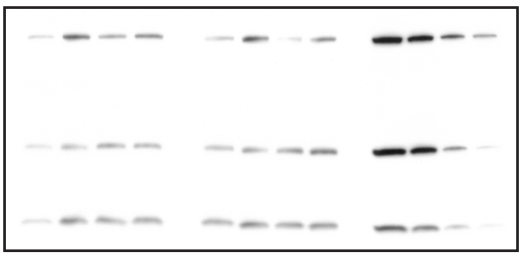
	Mean	Std. Dev	p-value
CASP3 UTR	86%	8%	<0.05
CASP3 UTR$_{mut}$	94%	29%	>0.50

Supplementary Fig. 5



10s exposure with undiluted substrate (10s)

30s exposure with diluted substrate (dil30s)



Supplementary Fig. 6