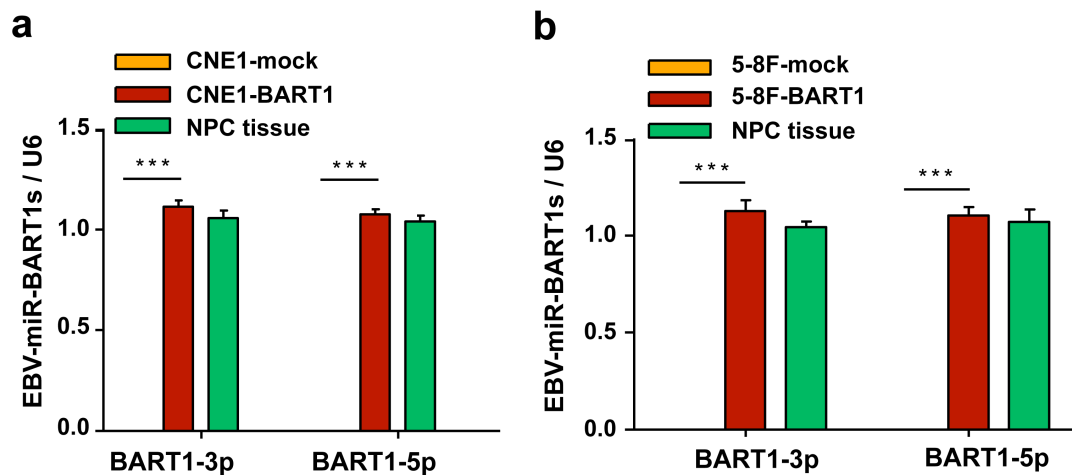


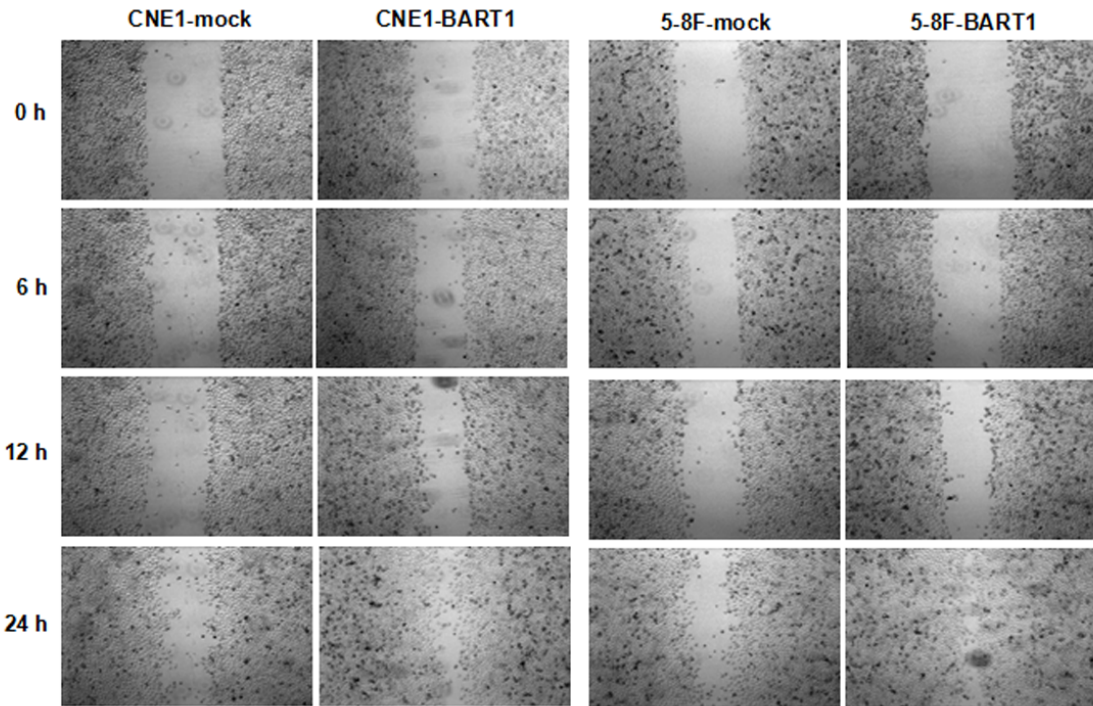
## Supplementary Information

### Supplementary Figures



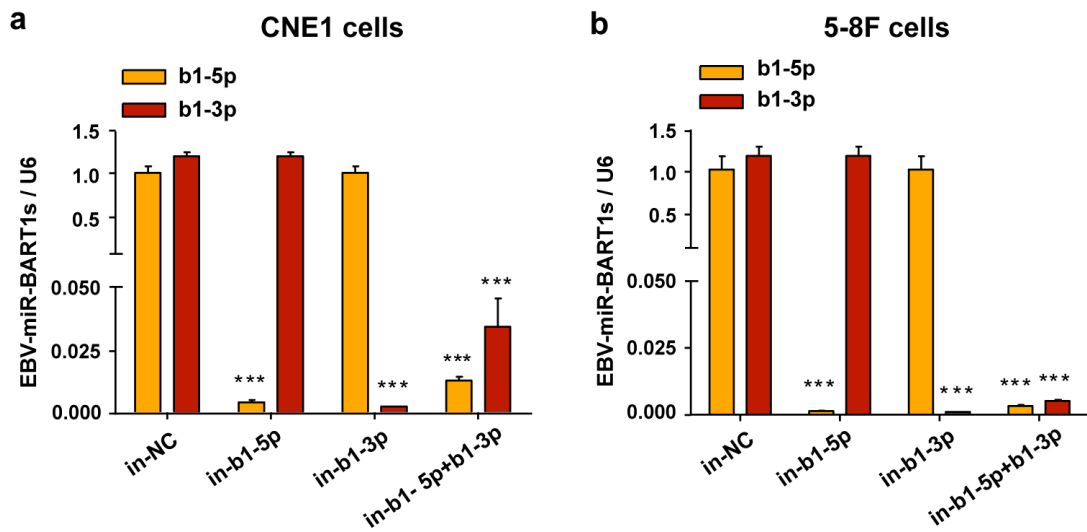
### Supplementary Figure 1. Up-regulation of EBV-miR-BART1 in both CNE1 and 5-8F cells by lentivirus-mediated transduction

Lentiviral particles carrying 282-nt pri-EBV-miR-BART1 precursor (H1-miRNA-CMV-GFP-BART1) or a BART1 control sequence (H1-miRNA-CMV-GFP-mock) was transduced into EBV-negative NPC cell lines. QRT-PCR confirmed the up-regulation of EBV-miR-BART1 in either CNE1-BART1 **(a)** or 5-8F-BART1 **(b)** cells compared with relative mock control cells. The expression level of EBV-miR-BART1 in either CNE1-BART1 or 5-8F-BART1 cells was very close to the mean level of 20 NPC specimens ( $P > 0.05$ ) that used for the miRNA-microarray profile analysis. Student's t test, mean  $\pm$  SEM,  $N=3$ , \*\*\* $P < 0.001$ .



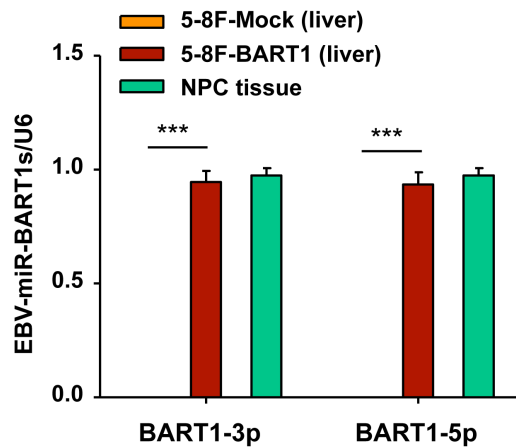
**Supplementary Figure 2. Wound-healing assays evaluating the migration of NPC cells**

Up-regulation of EBV-miR-BART1 increased the migration of both CNE1-BART1 and 5-8F-BART1 cells compared with that of CNE1-mock and 5-8F-mock control cells respectively. Wound-healing pictures were taken at 0, 6, 12, and 24hr after scratch.



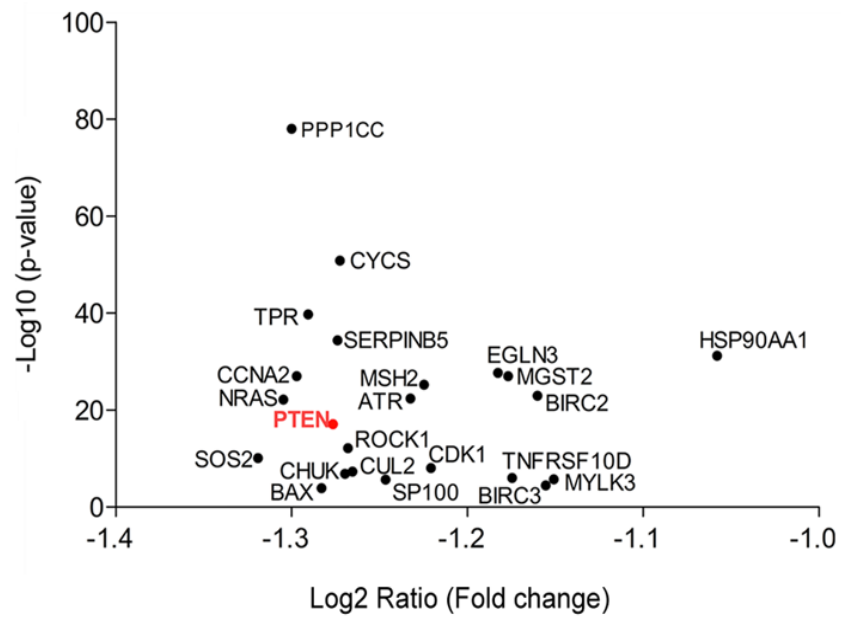
**Supplementary Figure 3. Down-regulation of the expression of EBV-miR-BART1 in both CNE1-BART1 and 5-8F-BART1 cells by transfection of BART1-inhibitory oligonucleotide**

Either CNE1-BART1 cells (a) or 5-8F-BART1 cells (b) was transfected with the BART1-inhibitory oligonucleotide (in-b1-5p for the BART1-5p inhibitor and in-b1-3p for the BART1-3p inhibitor) or the non-specific inhibitor control (in-NC) at 50nM by using Lipofectamine 2000 reagent. The expression of BART1 (either BART1-5p or BART1-3p) was determined by qRT-PCR. Either in-b1-5p or in-b1-3p specifically down-regulated the expression of BART1-5p or BART1-3p respectively. The combination of in-b1-5p and in-b1-3p down-regulated both BART1-5p and BART1-3p. Student's t test, mean  $\pm$  SEM, N=3, \*\*\*P<0.001.

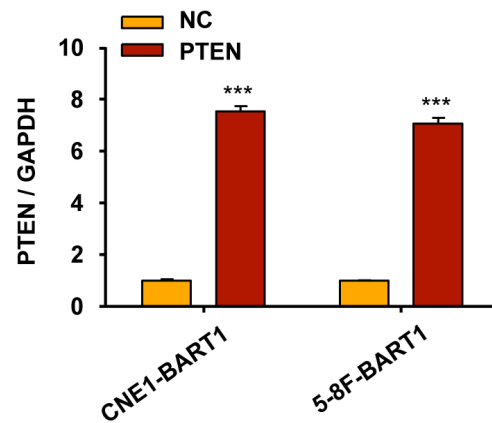


**Supplementary Figure 4. Validation of EBV-miR-BART1 expression in liver-metastasized tumors of mice xenografted with 5-8F NPC cells**

QRT-PCR confirmed the up-regulation of BART1 (either BART1-5p or BART1-3p) in liver-metastasized tumors of mice implanted with 5-8F-BART1 cells compared with that of 5-8F-Mock control. The expression level of either BART1-5p or BART1-3p in liver-metastasized tumors was very close to the mean level of 20 NPC specimens ( $P > 0.05$ ) that used for the miRNA-microarray profile analysis. Student's t test, mean  $\pm$  SEM, N=3, \*\*\* $P < 0.001$ .

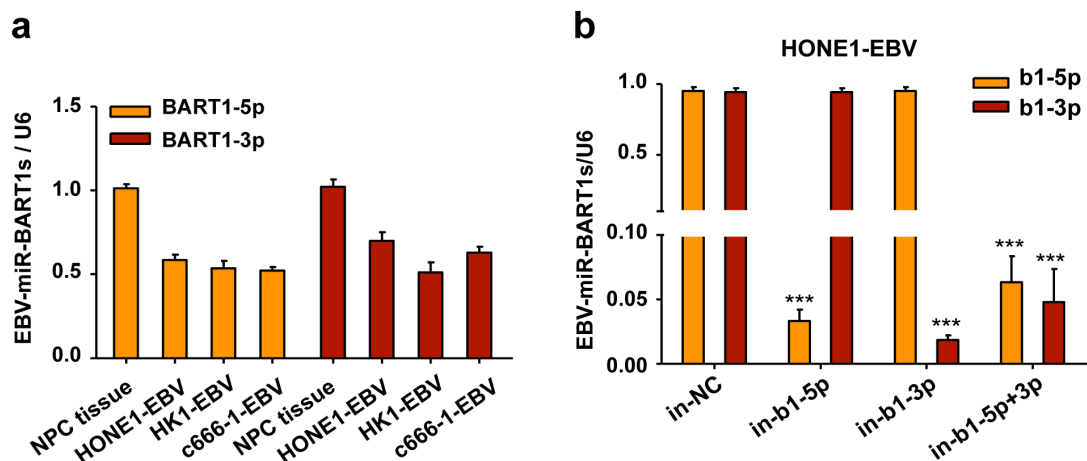


**Supplementary Figure 5. Putative migration/metastasis-associated candidate genes enriched from those EBV-miR-BART1 down-regulated genes obtained from RNA-deep sequencing**



**Supplementary Figure 6. Reconstitution of PTEN expression in CNE1-BART1 and 5-8F-BART1 cells validated by qRT-PCR**

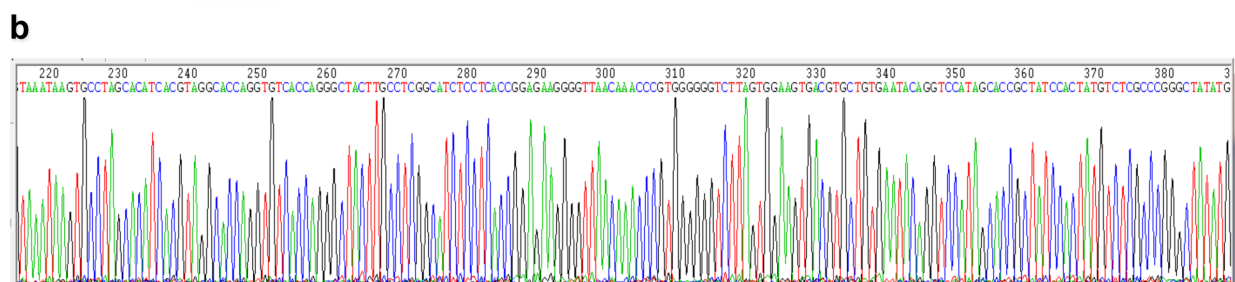
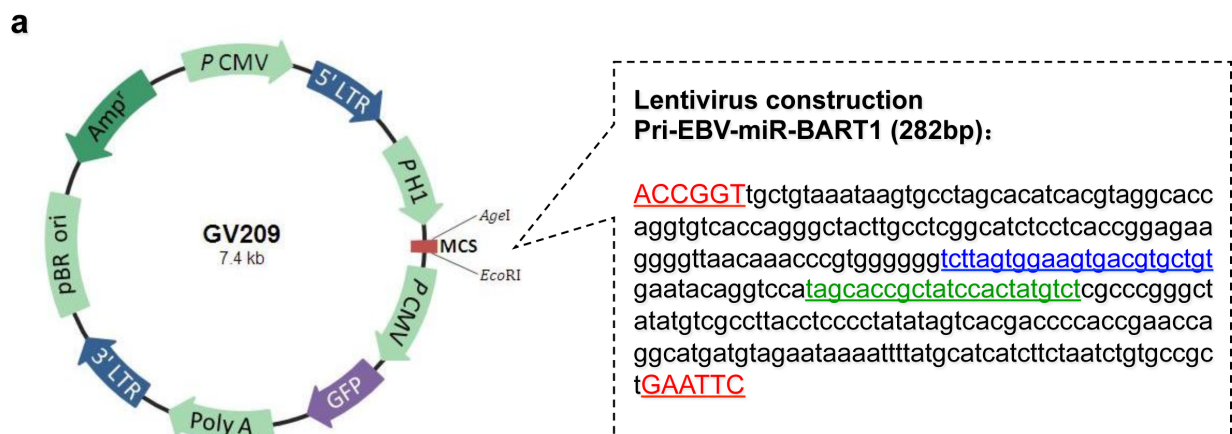
The expression vector GV230 containing whole coding sequence of PTEN and the control vector GV170 (200ng) were transfected into CNE1-BART1 and 5-8F-BART1 cells by using Lipofectamine 2000 reagent. After 48hr, total RNAs were extracted from the transfected cells. QRT-PCR confirmed the restoration of PTEN at mRNA level. Student's t test, mean  $\pm$  SEM, N=3, \*\*\*P<0.001.



**Supplementary Figure 7. Basal expression of EBV-miR-BART1 in three EBV-positive NPC cell lines and down-regulation of endogenous BART1 expression in HONE1-EBV cells**

**(a)** Expression of BART1 (either BART1-5p or BART1-3p) in three EBV-positive cell lines and 20 NPC clinical samples as quantified by qRT-PCR.

**(b)** Expression of BART1 (either BART1-5p or BART1-3p) was significantly decreased by the BART1 inhibitory oligonucleotides (in-b1-5p, in-b1-3p or both) but not by the control oligonucleotide (in-NC). Mean  $\pm$  SEM, N=3, \*\*\*P < 0.001.



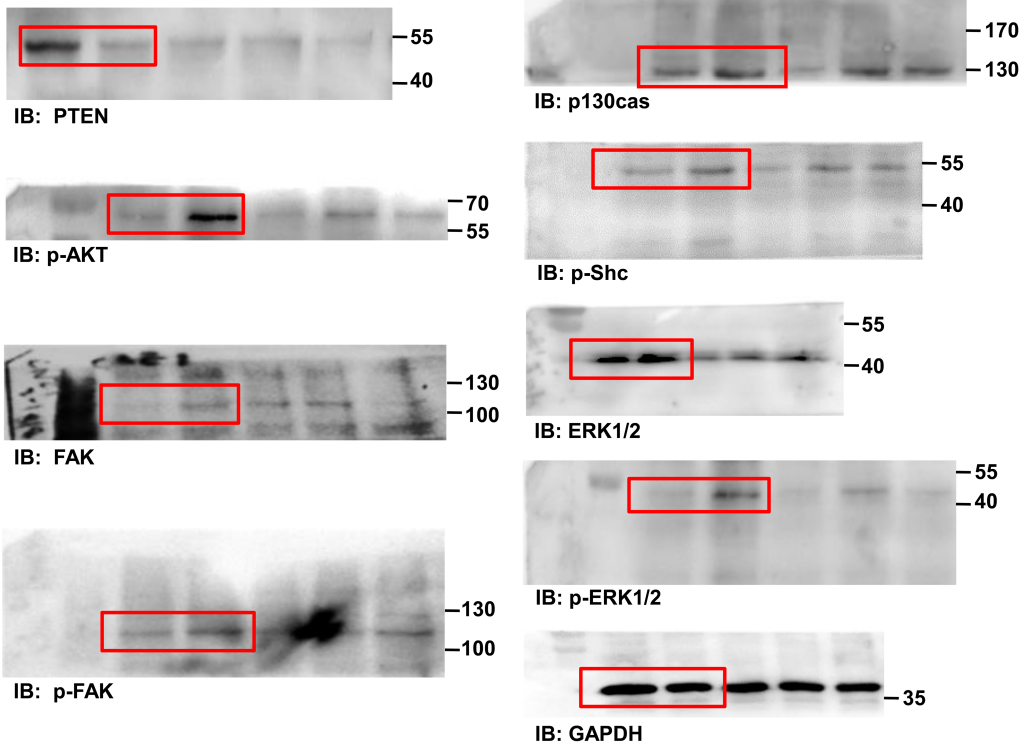
**Supplementary Figure 8. Construction and sequence validation of the lentiviral expression vector used for up-regulation of EBV-miR-BART1**

(a) The GV209 lentiviral vector (H1-miRNA-CMV-EGFP-BART1) containing 282-nt of pri-EBV-miR-BART1 precursor. The BART1-5p and BART1-3p sequences are underlined by blue and green color respectively. *AgeI* (ACCGGT) and *EcoRI* (GAATTC) restriction sites of the insert are capitalized in red color.

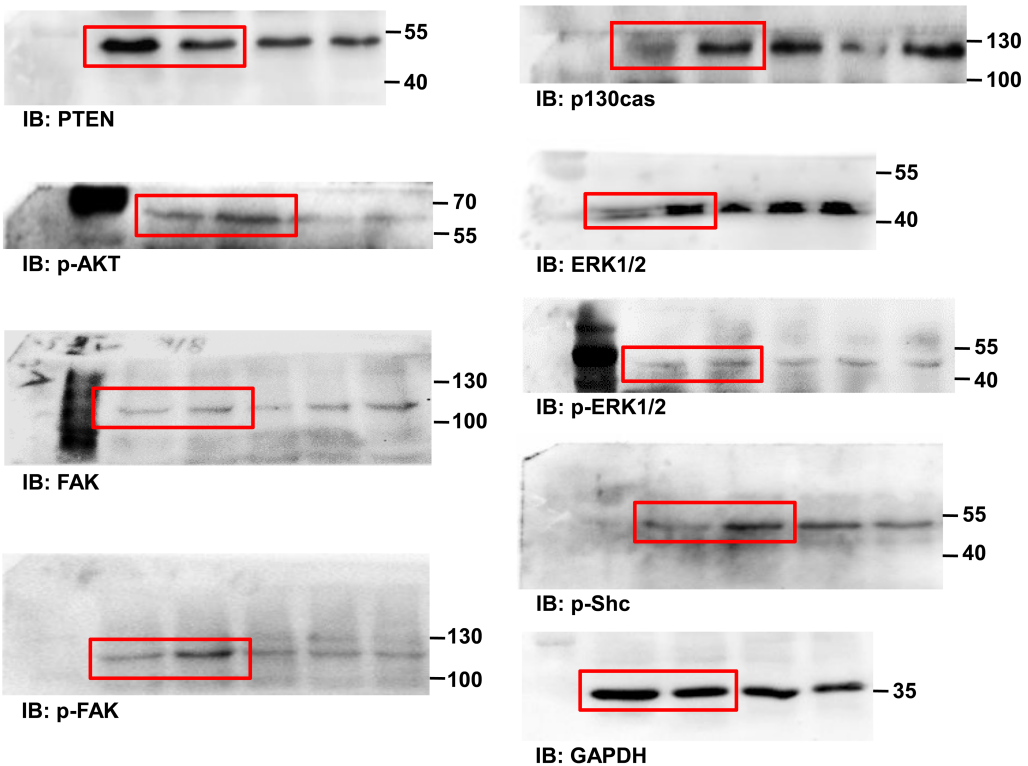
(b) Sequence validation of EBV-miR-BART1 by sequencing the insert.



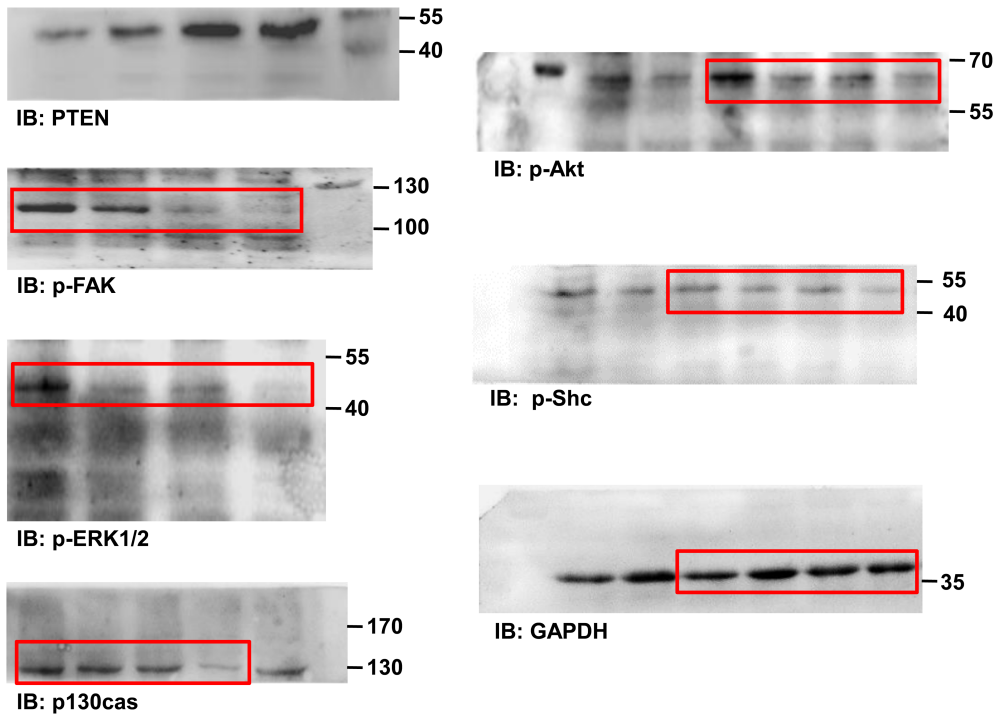
**For Figure 4a CNE1 (Mock BART1)**



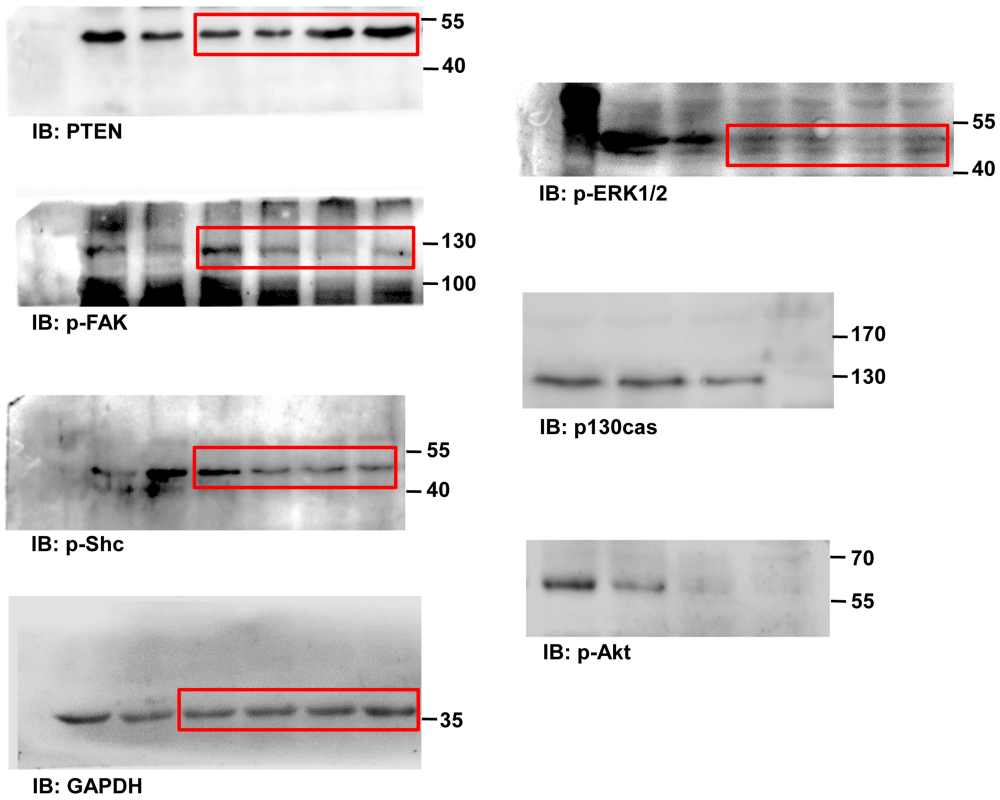
**For Figure 4a 5-8F (Mock BART1)**

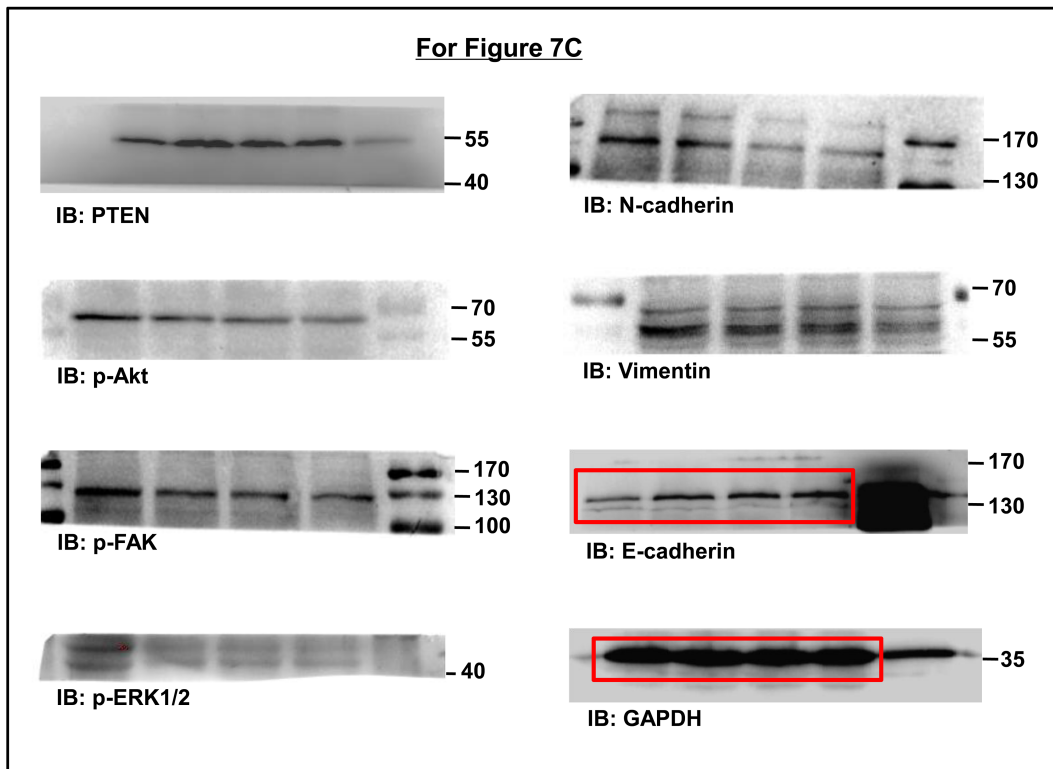


**For Figure 4b CNE1 (inhibition)**



**For Figure 4b 5-8F (inhibition)**





**Supplementary Figure 9. Original Western blotting images of the key components of PTEN-dependent pathway and EMT**

## Supplementary Tables

**Supplementary Table 1.** A cohort of clinical tissue samples for miRNA-microarray analysis and qRT-PCR validation (\*Independent t test; <sup>§</sup>Chi-square test. SCC, squamous cell carcinoma)

Characteristics	Clinical tissue samples		
	NP (n=20)	NPC (n=20)	P-value
Age	40.10	43.35	0.45*
Gender, male	9 (45%)	14 (70%)	0.11 <sup>§</sup>
Poorly differentiated SCC		3 (15%)	
Undifferentiated cancer		17 (85%)	
Differentiated SCC		0	

**Supplementary Table 2.** Identification of 69 miRNAs that are differentially expressed between NPC and NP

ID of miRNA	Fold-change (NPC/NP)	Parametric P-value*	FDR	Permutation P-value	Geom mean of ratios in NP	Geom mean of ratios in NPC
EBV-mir-BART1	19.27250161	< 1e-07	< 1e-07	< 1e-07	0.8125691	15.660236
EBV-mir-BART7	18.07791945	< 1e-07	< 1e-07	< 1e-07	0.9346132	16.8958559
EBV-mir-BART3	17.58748896	< 1e-07	< 1e-07	< 1e-07	0.9132633	16.0620225
EBV-miR-BART10	14.69989431	< 1e-07	< 1e-07	< 1e-07	1.1458645	16.8440805
EBV-mir-BART8	9.488377212	< 1e-07	< 1e-07	< 1e-07	1.1705958	11.1070561
EBV-mir-BART9	8.929823198	< 1e-07	< 1e-07	< 1e-07	1.0091342	9.0113889
EBV-mir-BART5	7.846405052	< 1e-07	< 1e-07	< 1e-07	0.9187861	7.2091699
EBV-mir-BART6	7.118709463	1.00E-07	7.40E-06	< 1e-07	0.7954561	5.6626205
EBV-miR-BART2	6.916700789	< 1e-07	< 1e-07	< 1e-07	1.3506757	9.3422202
EBV-mir-BART14	4.411855538	3.00E-07	1.92E-05	< 1e-07	1.2572036	5.5466018
EBV-mir-BART16	3.987080265	2.50E-06	0.0001412	< 1e-07	1.0048799	4.0065376
hcmv-miR-UL22A-1	3.885736046	< 1e-07	< 1e-07	< 1e-07	0.7876988	3.0607896
EBV-mir-BART4	3.787084074	7.00E-07	4.20E-05	< 1e-07	0.9391749	3.5567349
hsa-mir-643	3.265885184	0.0004774	0.0100596	4.00E-04	0.7455299	2.4348153
hsa-miR-205	3.248118933	6.92E-05	0.002076	3.00E-04	6.728439	21.8547711
hsa-mir-660	3.147664166	0.0002148	0.0054265	7.00E-04	1.6899949	5.3195365
hsa-mir-651	2.851257861	5.44E-05	0.0018651	4.00E-04	0.8795513	2.5078271
EBV-mir-BART12	2.823659898	2.38E-05	0.000952	< 1e-07	0.8353809	2.3588313
hsa-mir-613	2.792151931	5.84E-05	0.0019264	3.00E-04	0.7482905	2.0893406
hsa-mir-581	2.696646908	0.00028	0.0064	8.00E-04	1.0545058	2.8436301
EBV-mir-BART17	2.290131754	0.0025353	0.0325018	0.0043	1.2044927	2.7584471
EBV-mir-BART20	1.963470804	0.001917	0.0259484	0.002	1.0313375	2.025001
hcmv-miR-US25-2-5p	1.751267173	0.0004925	0.0100596	5.00E-04	0.7969985	1.3957573
hsa-mir-604	1.707947764	0.0002276	0.0055488	3.00E-04	0.8396544	1.4340858
hsa-miR-191	0.675653489	0.0024665	0.0324362	0.0012	1.2266716	0.8288049
hsa-miR-199a	0.667823292	0.0016459	0.0232362	0.001	1.0138932	0.6771015
<a href="#">hsa-let-7i</a>	0.624801665	0.0032592	0.0391692	5.00E-04	1.6666703	1.0413384
<a href="#">hsa-miR-26a</a>	0.583824466	0.002897	0.0361184	0.0014	3.2261411	1.8835
<a href="#">hsa-let-7c</a>	0.573459207	0.0009439	0.0158973	5.00E-04	0.9209456	0.5281247
hsa-miR-221	0.563403611	0.0019191	0.0259484	0.0022	0.956716	0.5390172
<a href="#">hsa-let-7d</a>	0.562094115	0.0014468	0.021702	0.0012	0.9135164	0.5134822
hsa-miR-375	0.553670369	0.0028318	0.0357701	0.0026	1.056692	0.585059
hsa-miR-136	0.55210868	0.0013857	0.0211154	0.0016	1.8847563	1.0405903
hsa-miR-10b	0.548276244	0.0007865	0.014246	0.0012	0.5592348	0.3066151
hsa-miR-199b	0.536033283	0.001025	0.0169655	0.0017	1.8175629	0.9742742
<a href="#">hsa-miR-30a-5p</a>	0.520525329	2.35E-05	0.000952	< 1e-07	4.7382753	2.4663923
<a href="#">hsa-miR-30d</a>	0.520322198	0.0002312	0.0055488	< 1e-07	4.3641825	2.2707811
<a href="#">hsa-miR-152</a>	0.51214102	0.0005287	0.010574	6.00E-04	2.6944112	1.3799185
<a href="#">hsa-miR-145</a>	0.500895953	0.0043537	0.0491712	0.0062	17.6262937	8.8289392

hsa-mir-624	0.48726506	0.0032641	0.0391692	0.0032	1.3162108	0.6413435
hsa-miR-365	0.485030362	0.0016908	0.0235242	0.0028	0.8250951	0.4001962
<a href="#">hsa-let-7g</a>	0.480341011	0.0004898	0.0100596	< 1e-07	1.7995287	0.8643874
<a href="#">hsa-miR-31</a>	0.477838587	0.0006132	0.0115426	6.00E-04	0.4731818	0.2261045
hsa-miR-199a*	0.473994909	0.0036812	0.0425777	0.0051	2.6174524	1.2406591
hsa-miR-196b	0.471017271	0.003782	0.0432229	0.0063	1.5101803	0.711321
<a href="#">hsa-miR-30e-5p</a>	0.458486626	2.80E-06	0.0001493	< 1e-07	6.0942278	2.7941219
<a href="#">hsa-miR-195</a>	0.447332403	0.0008874	0.0154892	0.0015	1.99611	0.8929247
<a href="#">hsa-miR-29a</a>	0.437574812	2.33E-05	0.000952	< 1e-07	4.1010735	1.7945265
<a href="#">hsa-miR-16</a>	0.435157393	0.0045243	0.0505038	0.0051	1.8793853	0.8178284
<a href="#">hsa-miR-29b</a>	0.431569238	0.0001835	0.0047611	1.00E-04	1.7628566	0.7607947
hsa-miR-346	0.42473145	0.0005502	0.0107794	0.0016	4.9578175	2.1057411
<a href="#">hsa-miR-146a</a>	0.418978959	2.77E-05	0.0010228	< 1e-07	10.756221	4.5066303
<a href="#">hsa-miR-30b</a>	0.406976952	0.0003932	0.0085789	2.00E-04	4.5581951	1.8550803
hsa-miR-342	0.397489125	2.77E-05	0.0010228	< 1e-07	8.6870346	3.4530018
hsa-miR-449	0.396329922	0.0035867	0.0419906	0.0079	6.6484496	2.6349795
<a href="#">hsa-miR-143</a>	0.379372457	0.000574	0.0110208	8.00E-04	43.9659697	16.6794781
hsa-miR-144	0.37808031	0.0006346	0.0117157	0.0014	47.9652601	18.1347207
<a href="#">hsa-miR-29c</a>	0.36916765	8.40E-06	0.0004244	< 1e-07	4.5125747	1.6658966
hsa-miR-126*	0.362801182	0.000942	0.0158973	0.0028	9.3399427	3.3885423
hsa-miR-140	0.331890429	0.0008268	0.0146987	0.0017	2.2027896	0.7310848
<a href="#">hsa-miR-30a-3p</a>	0.322618985	2.15E-05	0.000952	< 1e-07	4.5292535	1.4612231
hsa-miR-485-3p	0.317850375	0.0011268	0.0180288	0.0021	13.4029323	4.2601271
<a href="#">hsa-miR-26b</a>	0.312392088	4.29E-05	0.0015253	< 1e-07	4.4792998	1.3992978
hsa-miR-105	0.304738919	0.0001367	0.0037495	6.00E-04	2.615638	0.7970867
hsa-miR-32	0.282737728	0.0021969	0.029292	0.0049	2.6416395	0.7468911
<a href="#">hsa-miR-101</a>	0.26323514	8.98E-05	0.0025355	3.00E-04	2.5906225	0.6819429
<a href="#">hsa-miR-150</a>	0.253107259	6.02E-05	0.0019264	3.00E-04	20.454355	5.1771457
<a href="#">hsa-miR-34b</a>	0.173349588	3.00E-07	1.92E-05	< 1e-07	3.0396284	0.5269183
<a href="#">hsa-miR-34c</a>	0.126005664	< 1e-07	< 1e-07	< 1e-07	1.614109	0.2033869

**Notes:**

Normalization: Normalize (center) each array using lowess smoother.

Exclude a gene under any of the following conditions: <20% of expression data have  $\geq 1.5$ -fold change in either direction from gene's median value. Percent of data missing or filtered out exceeds 50%.

\*Global test: probability of getting at least 69 genes significant by chance (at the 0.005 level).

FDR, False discovery rate.

Tumor suppressor miRNAs are highlighted with colors.

**Supplementary Table 3.** A cohort of clinical samples used for the association analysis with pathological and clinical data

	Clinical tissue samples		P-value
	NP (n=32)	NPC (n=82)	
Age, years	43.18	54.65	0.076*
Gender, male	18 (65.63%)	54 (65.85%)	0.390 <sup>§</sup>
T stage			
T1		18 (21.95%)	
T2		22 (26.83%)	
T3		20 (24.39%)	
T4		22 (26.83%)	
N stage			
N0		7 (8.54%)	
N1		26 (31.71%)	
N2		33 (40.24%)	
N3		16 (19.51%)	
M stage			
M0		77 (93.90%)	
M1		5 (6.10%)	
M2		0	
M3		0	
TNM stage			
I		15 (18.29%)	
II		22 (26.83%)	
III		25 (30.49%)	
IV		20 (24.39%)	

\*Independent t test. <sup>§</sup>Chi-square test.

**Supplementary Table 4.** Candidate genes and their associated pathways

Gene symbol	Fold-change	P-value	Pathways*
PTEN	0.422484	8.35E-18	<b>5</b>
NRAS	0.470386	6.71E-23	2
CHUK	0.411017	1.31E-07	3
SOS2	0.495144	7.63E-11	3
CYCS	0.415773	1.51E-51	3
BAX	0.433427	0.000129	1
TNFRSF10D	0.257243	1.05E-06	1
CDK1	0.33074	8.87E-09	1
ROCK1	0.408148	6.83E-13	1
ATR	0.34964	4.08E-23	1
SERPINB5	0.418178	4.31E-35	1
HSP90AA1	0.081898	5.94E-32	2
BIRC3	0.227606	3.61E-05	3
BIRC2	0.234874	1.05E-23	3
MGST2	0.260917	1.02E-27	2
EGLN3	0.26978	2.00E-28	1
MSH2	0.336967	6.10E-26	1
CUL2	0.403895	4.71E-08	1
SP100	0.372665	2.11E-06	1
TPR	0.446203	1.88E-40	1
CCNA2	0.457381	9.58E-28	1
MYLK3	0.220494	1.84E-06	1
PPP1CC	0.462373	9.43E-79	1

\*Metastasis/invasion-associated pathways in which candidate genes were involved. These pathways are p53 signaling pathway, pathway in cancer, prostate cancer, focal adhesion and small cell lung cancer.



**Supplementary Table 5. Primer sequences**

Gene	Primer	Sequence
PTEN	Forward	5'-TGCAGAAGAAGCCCCGCCA-3'
	Reverse	5'-ACGCCTTCAAGTCTTTCTGCAGG-3'
mut1*	Forward	5'-AACGCAAGGTTTCCGAAGGGTTTTGC-3'
	Reverse	5'-CAACTGCAAACCTTATCTGTTGCCAC-3'
mut2 <sup>§</sup>	Forward	5'-CGAAGAAAATTCAGATGCTGTTAG-3'
	Reverse	5'-TGGCCTTGATTACACTGGAGATGG-3'
GAPDH	Forward	5'-GCCACATCGCTCAGACACCA-3'
	Reverse	5'-CTCAGCCTTGACGGTGCCAT-3'

\*Mut1, PTEN 3'-UTR containing mutant EBV-miR-BART1-5p binding site;

§Mut2, PTEN 3'-UTR containing mutant EBV-miR-BART1-3p binding site

**Supplementary Table 6. A list of antibodies used for Western blotting and IHC staining**

Name of antibody	Cat. No	Company	Mol weight	Dilution (WB/IHC)
PTEN (D375) pAb	BS1305	Bioworld	54kDa	1:800/1:200
FAK phospho (pY576)	2103-1	Epitomics	125kDa	1:5000
Anti-FAK antibody	BM0503	ABZOOM	119kDa	1:1000
Anti-ERK1/2 antibody	BM0431	ABZOOM	43kDa	1:1000
pErk1 (pT202)/Erk2 (pT185)	1481-1	Epitomics	42/44kDa	1:3000/1:100
p-Shc (Tyr239/240)	2434	CST	52kDa	1:800/1:200
p-Paxillin (Y31) pAb	BS4721	Bioworld	68kDa	1:800
H/K/N-Ras (H27) pAb	BS1309	Bioworld	25kDa	1:800/1:200
p130 <sup>Cas</sup> (v404) pAb	BS1416	Bioworld	130kDa	1:800/1:200
Anti-AKT (p-Ser473)	AM1006	ABZOOM	60kDa	1:1000/1:200
E-cadherin (24E10) mAb	3195	CST	135kDa	1:500/1:100
N-Cadherin (C-term)	2019-1	Epitomics	140kDa	1:1000
Vimentin	BS1776	Bioworld	57kDa	1:500/1:100
GAPDH	P30008	Abmart	37kDa	1:1000