Supplemental Materials

Table S1. The clinical characteristics of 95 patients with primary NPC diagnosis

Characteristics	No. of patients (%)
Age, year (Range 15-84)	
< 47	46 (48.4)
≥ 47	49 (51.6)
Gender	
Male	75 (78.9)
Female	20 (21.1)
T classification	
T1, T2	12 (12.6)
T3, T4	83 (87.4)
N classification	
N0	5 (5.3)
N1, N2, N3	90 (94.7)
M classification	
M0	88 (92.6)
M1	7 (7.4)
Clinical stage	
Ⅱ	12 (12.6)
Ⅲ	42 (44.2)
Ⅳ	41 (43.2)

WHO histological classification	
NKUC(UD)	88 (92.6)
NKUC(DF)	2 (2.1)
Others	5 (5.3)

3

4 **Table S2. The clinical characteristics of 8 NP and 25 NPC patients**

	Clinical characteristics	No. of patients (%)
NP	**Age, year (Range 30-69)**	
	< 49	3 (37.5)
	≥ 49	5 (62.5)
	Gender	
	Male	5 (62.5)
	Female	3 (37.5)
NPC	**Age, year (Range 35-73)**	
	< 48	9 (36.0)
	≥ 48	16 (64.0)
	Gender	
	Male	16 (64.0)
	Female	9 (36.0)
	WHO histological classification	
	NKUC(UD)	21 (84.0)
	NKUC(DF)	4 (16.0)

Table S3. The clinical characteristics of 30 NP and 92 NPC patients assessed by microarray

	Clinical characteristics	No. of patients (%)
NP	**Age, year (Range 16-72)**	
	< 39	14 (46.7)
	≥ 39	16 (53.3)
	Gender	
	Male	18 (60.0)
	Female	12 (40.0)
NPC	**Age,year (Range 25-68)**	
	< 45	44 (47.8)
	≥ 45	48 (52.2)
	Gender	
	Male	71 (77.2)
	Female	21 (22.8)
	Neck lymph node	
	Negative	37 (40.2)
	Positive	40 (43.5)
	No information	15 (16.3)
	EBER status	
	Negative	0

	Positive	92 (100)

8

9 **Table S4. The clinical characteristics of 129 NPC patients assessed by microarray**

Clinical characteristics	No. of patients (%)
Age, year (Range 20-82)	
≤ 47	67 (51.9)
> 47	62 (48.1)
Gender	
Male	100 (77.5)
Female	29 (22.5)
Neck lymph node	
Negative	35 (27.1)
Positive	94 (72.9)
Recurrence	
Negative	70 (54.3)
Positive	58 (45.0)
No information	1 (0.7)
EBER	
Negative	2 (1.5)
Positive	127 (98.5)
Clinical stage	
I	15 (11.6)

Ⅱ	56 (43.4)
Ⅲ	38 (29.5)
Ⅳ	20 (15.5)

Table S5. SiRNA sequence

siRNA	Sequence
siNOX2 #1	GGCCCAACTGGGATAATGA
siNOX2 #2	GTCCCATGTTTCTGTATCT
siLMP1 #3	CAUUGUUCCUUGGAAUUGUTT
siLMP1 #4	GGUAUCUGGAUCUACUUAUTT

Table S6. Primer sequences used in this study

Gene	Forward Primer sequence	Reverse primer sequence
LMP1	CGTTATGAGTGACTGGACTGGA	TGAACAGCACAATTCCAAGG
BARF1	CAGGTTCATCGCTCAGCTCC	CATGGGAGATGTTGGCAGC
EBER1	AGGACCTACGCTGCCCTAGA	AAAACATGCGGACCACCAGC
BZLF1	CATGTTTCAACCGCTCCGACTGG	GCGCAGCCTGTCATTTTCAGATG
BMRF1	CTAGCCGTCCTGTCCAAGTGC	AGCCAAACGCTCCTTGCCCA
NOX2	CCCTTTGGTACAGCCAGTGAAGAT	CAATCCCAGCTCCCACTAACATCA
Nrf2	TACTCCCAGGTTGCCCACA	CATCTACAAACGGGAATGTCTGC
NQO1	CATTCTGAAAGGCTGGTTTGA	CTAGCTTTGATCTGGTTGTCAG
SOD1	CTGAAGGCCTGCATGGATTC	CCAAGTCTCCAACATGCCTCTC
XDH	AGGTGGACCACTTCAGCAAT	GTTGGAGGGAAGGTTGGTTT

GPX7	CCCACCACTTTAACGTGCTC	GGCAAAGCTCTCAATCTCCTT
GPX8	CCGCCCAAGCAAGGAAGTAG	TCTAACCAGAGCTGC TATGTCAG
β-actin	CCAAGGCCAACCGCGAGAAGATGAC	AGGGTACATGGTGGTGCCGCC AGAC
BamHI-W region DNA	GGGTGCAGTAACAGGTAATC	ATTCGCCTCTAAAGTTTTGA
β-globin DNA	CTGGCAAATTGGATAAGGAGTCA	TCCATCCTTTTATTTCGAGCC

Table S7. Correlation between 8-OHdG and EAD in 129 NPC patients

			EAD	
Biopsies	Spearman's rho	8-OHdG	Correlation coefficient	.725
			Significance (2-tailed)	.000
			N	129

Table S8. Clinical characteristics and 8-OHdG level in 129 non-keratinizing undifferentiated NPC patients who received radiation therapy

Clinical characteristics	**8-OHdG Low level**	**8-OHdG High level**	***p*-value**
Age (yr), mean±S.D.	49.33±10.95	46.94±11.83	0.374[a]
Gender			0.87[b]
Male (n)	51	49	
Female (n)	12	17	
Neck lymph node			0.349[b]
Negative (n)	20	15	
Positive (n)	43	51	

Recurrence			0.000[b]
Negative (n)	49	21	
Positive (n)	14	44	
No information (n)	0	1	
Clinical Stage			0.028[b]
Ⅰ / Ⅱ	42	21	
Ⅲ/Ⅳ	29	47	

[a] Welch's test

[b] Pearson's χ^2 text

Table S9. Clinical characteristics and EAD level in 129 non-keratinizing undifferentiated NPC patients who received radiation therapy

Clinical characteristics	**EAD Low level**	**EAD High level**	***p*-value**
Age (yr), mean±S.D.	49.53±11.25	46.71±11.51	0.326[a]
Gender			0.558[b]
Male (n)	49	51	
Female (n)	15	14	
Neck lymph node			0.589[b]
Negative (n)	17	18	0.589[b]
Positive (n)	47	47	
Recurrence			0.000[b]

Negative (n)	45	25	
Positive (n)	19	39	
No information (n)	0	1	
Clinical Stage			0.011[b]
Ⅰ / Ⅱ	42	29	
Ⅲ/Ⅳ	22	36	

[a] Welch's test

[b] Pearson's χ^2 text

Table S10. Clinical characteristics and 8-OHdG level in 92 non-keratinizing undifferentiated NPC patients who received radiation therapy

Clinical characteristics	**8-OHdG Low level**	**8-OHdG High level**	***p*-value**
Age (yr), mean±S.D.	44.52±10.60	45.09±8.69	0.780[a]
Gender			
Male (n)	35	36	0.804[b]
Female (n)	11	10	
Neck lymph node			
Negative (n)	21	16	0.303[b]
Positive (n)	18	22	
No information (n)	7	8	

[a] Welch's test

[b] Pearson's χ^2 text

Table S11. Clinical characteristics and EAD level in 92 non-keratinizing undifferentiated NPC patients who received radiation therapy

Clinical characteristics	EAD Low level	EAD High level	*p*-value
Age (yr), mean±S.D.	44.28±10.17	45.33±9.17	0.607[a]
Gender			
Male (n)	35	36	0.804[b]
Female (n)	11	10	
Neck lymph node			
Negative (n)	21	16	0.417[b]
Positive (n)	19	21	
No information (n)	6	9	

[a] Welch's test

[b] Pearson's χ^2 text

Supplemental Figures and Figure Legends

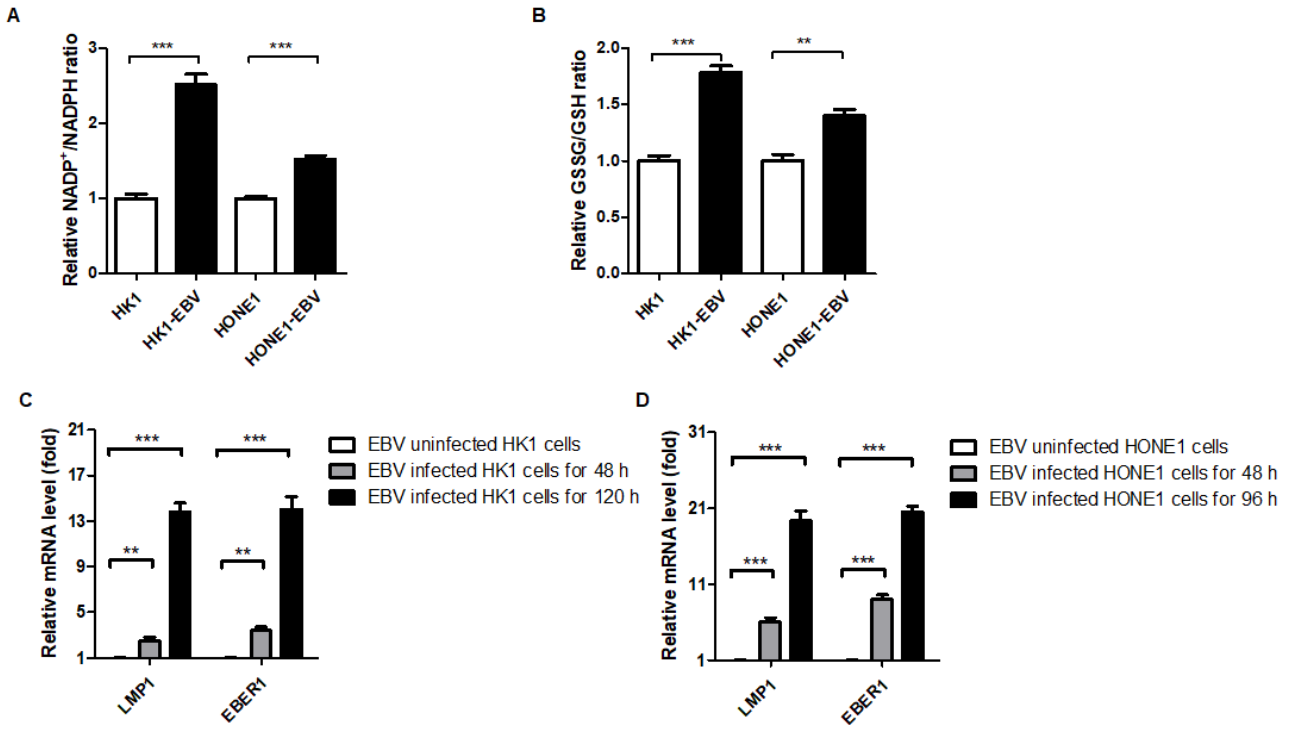


Figure S1. EBV induces high oxidative stress in NPC cells and patients. (A) Intracellular $NADP^+$/NADPH levels were assayed using an $NADP^+$/NADPH assay kit. Data are shown as means ± S.D.; n = 3; ***p < 0.001. **(B)** Total glutathione (GSH) and oxidized glutathione (GSSG) were measured using a GSH/GSSG assay kit. Data are shown as means ± S.D.; n = 3; **p < 0.01, ***p < 0.001. **(C)** The infection in HK1 cells was assessed by the detection of EBV genes (LMP1 and EBER1) by qPCR. Data are shown as means ± S.D.; n = 3; **p < 0.01, ***p < 0.001. **(D)** The infection in HONE1 cells was assessed by the detection of EBV genes (LMP1 and EBER1) by qPCR. Data are shown as means ± S.D.; n = 3; **p < 0.01, ***p < 0.001.

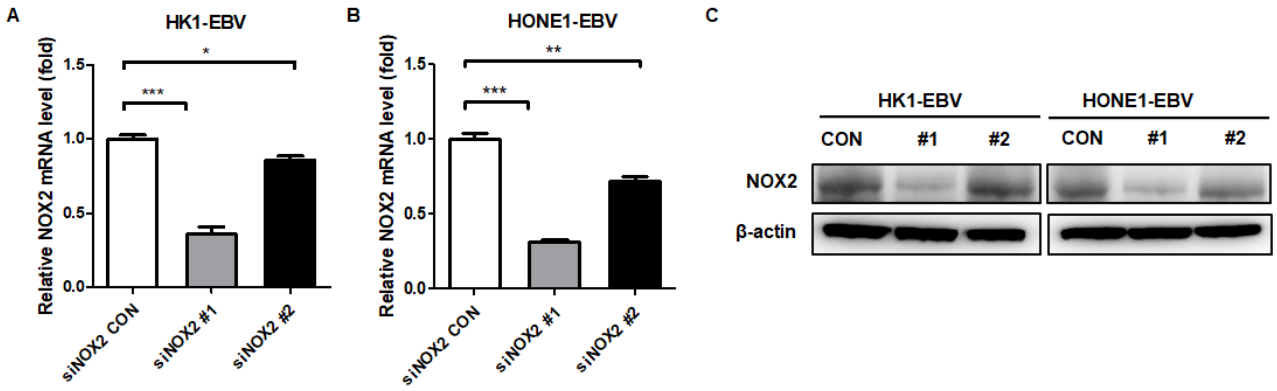


Figure S2. The NOX2 knockdown efficiency by siRNA transfection was detected by qPCR and Western blot. (A-B) The mRNA levels of *NOX2* after siRNA transfection were detected by RT-PCR. Data are shown as means ± S.D.; n = 3; **p* < 0.05, ***p* < 0.01, ****p* < 0.001. **(C)** NOX2 protein expression was detected by Western blot analysis and β-actin was used as an internal control.

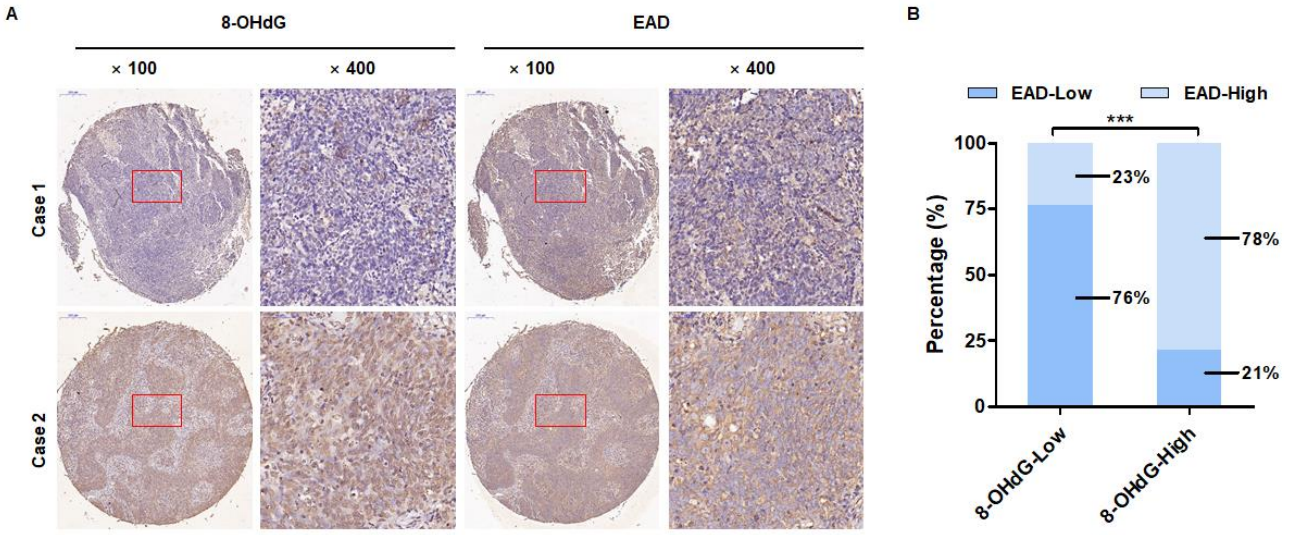


Figure S3. 8-OHdG expression was positively correlated with EAD in human NPC patients. (A) Representative IHC photographs showing the expression of 8-OHdG and EAD in consecutive sections of NPC microarrays (HNasN129Su01, Shanghai, China). **(B)** EAD level is calculated based on 8-OHdG expression in NPC microarrays (****p* < 0.001).

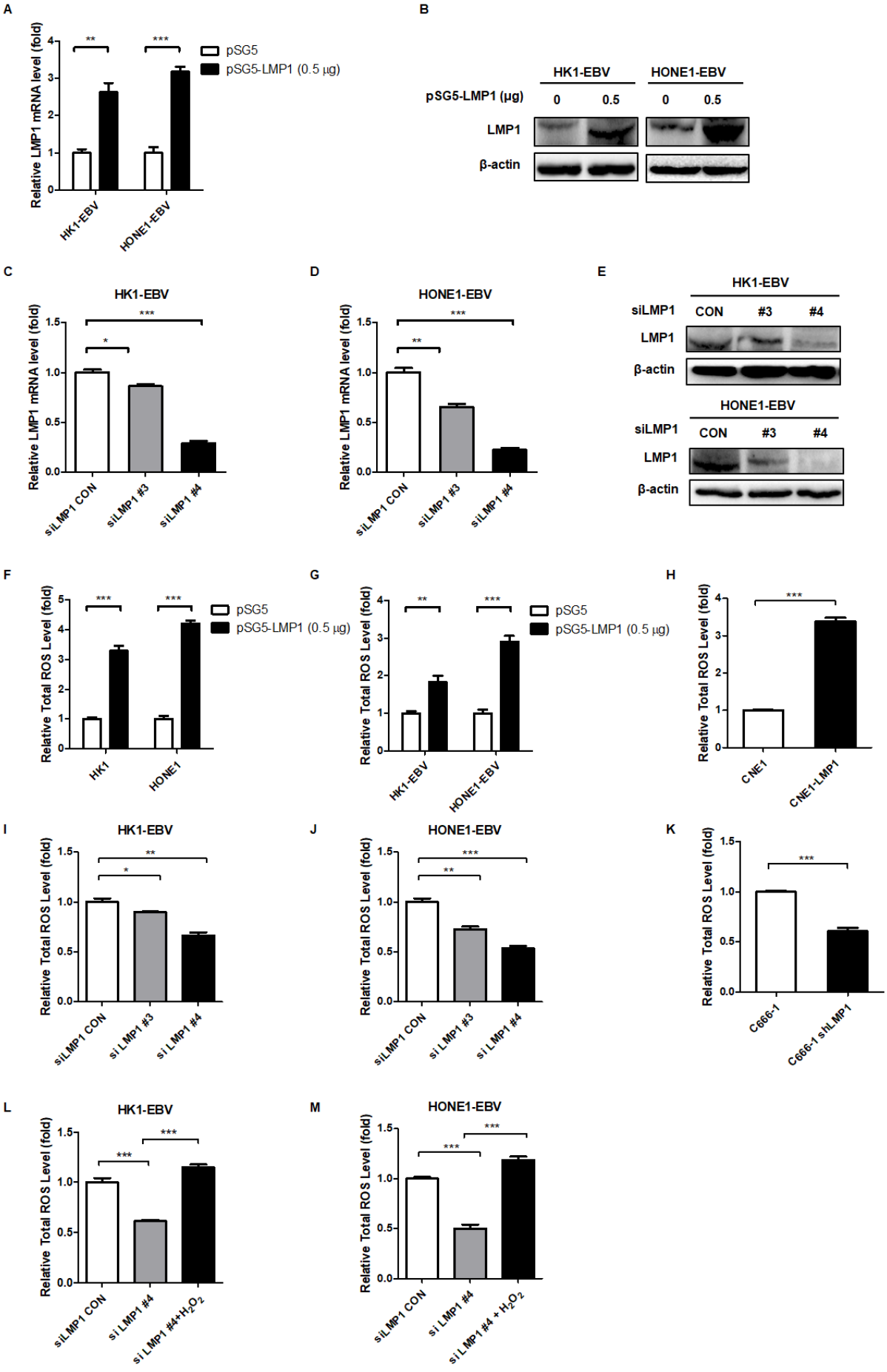

A
Relative LMP1 mRNA level (fold)
pSG5
pSG5-LMP1 (0.5 μg)
HK1-EBV
HONE1-EBV
B
HK1-EBV
HONE1-EBV
pSG5-LMP1 (μg)
0
0.5
LMP1
β-actin
C
HK1-EBV
Relative LMP1 mRNA level (fold)
siLMP1 CON
siLMP1 #3
siLMP1 #4
D
HONE1-EBV
E
HK1-EBV
siLMP1
CON
#3
#4
LMP1
β-actin
HONE1-EBV
F
Relative Total ROS Level (fold)
HK1
HONE1
G
H
CNE1
CNE1-LMP1
I
J
K
C666-1
C666-1 shLMP1
L
siLMP1 #4+H2O2
M
siLMP1 #4 + H2O2

Figure S4. LMP1 promotes the production of ROS. The LMP1 overexpression and knockdown efficiency was detected by qPCR and Western blot. **(A)** The mRNA levels of *LMP1* after pSG5-LMP1 plasmid transfection were detected by qPCR. Data are shown as means ± S.D.; n = 3; $^{**}p < 0.01$, $^{***}p < 0.001$. **(B)** LMP1 protein expression after pSG5-LMP1 plasmid transfection was detected by Western blot analysis and β-actin was used as an internal control. **(C-D)** The mRNA levels of *LMP1* after siLMP1 transfection were detected by qPCR. Data are shown as means ± S.D.; n = 3; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. **(E)** LMP1 protein expression was detected by Western blot analysis and β-actin was used as an internal control. **(F-G)** LMP1 induces ROS generation. EBV cells were transfected with pSG5 and pSG5-LMP1 plasmids and total ROS levels were detected. Data are shown as means ± S.D.; n = 3; $^{**}p < 0.01$, $^{***}p < 0.001$. **(H)** The total ROS levels in CNE1 and LMP1 stable overexpression cell line CNE1-LMP1 were detected. Data are shown as means ± S.D.; n = 3; $^{***}p < 0.001$. **(I-J)** LMP1 knockdown decreases ROS generation. HK1-EBV and HONE1-EBV cells were transfected with LMP1 siRNA pool and total ROS levels were detected. Data are shown as means ± S.D.; n = 3; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. **(K)** The total ROS levels in C666-1 and LMP1 stable cell line C666-1 shLMP1 were detected. Data are shown as means ± S.D.; n = 3; $^{***}p < 0.001$. **(L-M)** HK1-EBV and HONE1-EBV cells were transfected with LMP1 siRNA pool or negative siRNA, then H_2O_2 was added to recover the ROS levels and the ROS levels were detected. Data are shown as means ± S.D.; n = 3; $^{***}p < 0.001$.

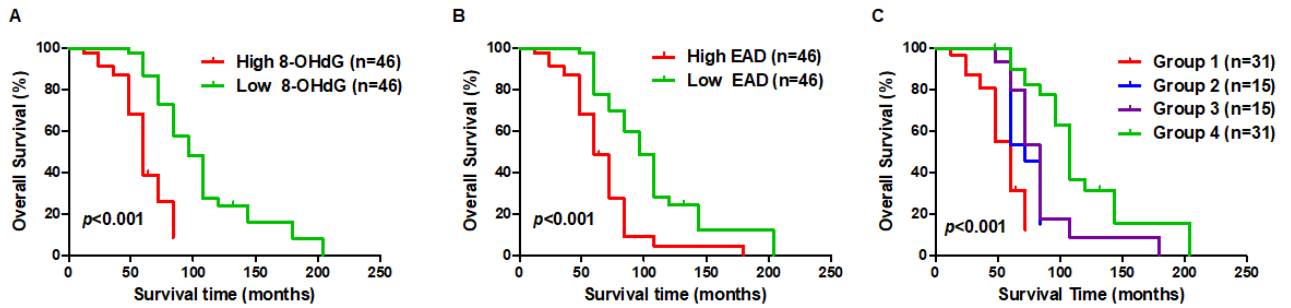
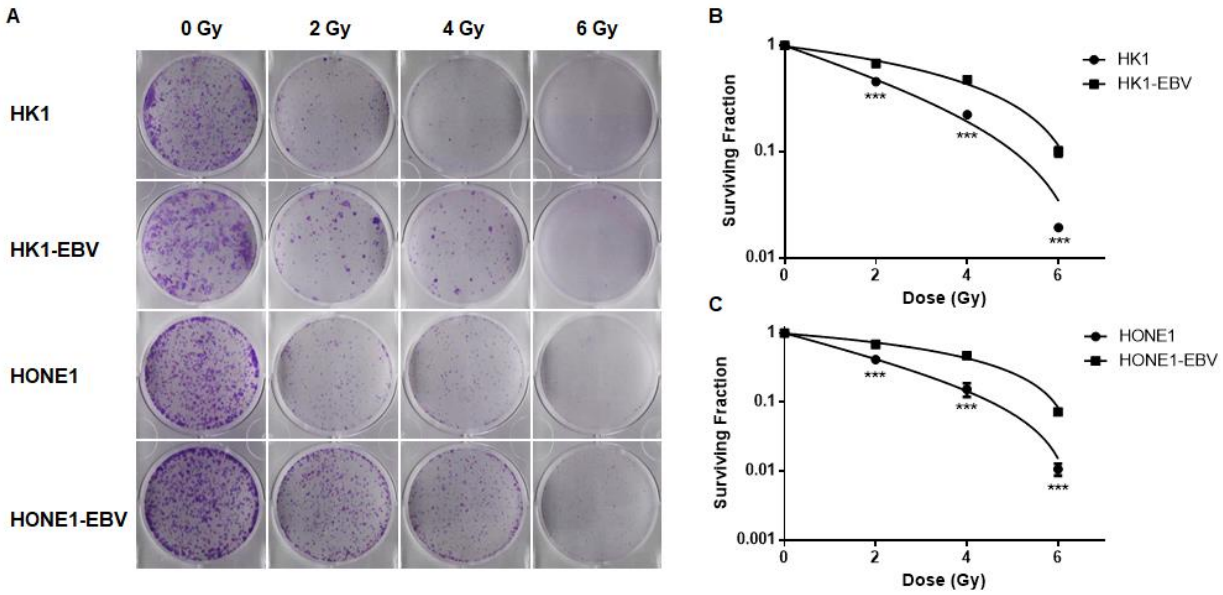


Figure S5. High oxidative stress and high EBV lytic reactivation are positively associated with poor survival in NPC patients. (A) A high expression level of 8-OHdG is associated with poor overall survival in NPC after radiation therapy. Overall survival rates of NPC patients after radiation therapy with low (n = 46) or high (n = 46) expression levels of 8-OHdG were estimated using the Kaplan–Meier method by log-rank test ($p < 0.001$). **(B)** A high expression level of EAD is associated with poor overall survival in NPC after radiation therapy. Overall survival rates of NPC patients after radiation therapy with low (n = 46) or high (n = 46) expression levels of EAD were estimated using the Kaplan–Meier method by log-rank test ($p < 0.001$). **(C)** Cumulative overall survival curves of the combination of 8-OHdG and EAD. NPC patients were classified into 4 groups based on 8-OHdG and EAD expression: Group 1 (n = 31): high 8-OHdG and EAD expression; Group 2 (n = 15): high 8-OHdG but low EAD expression; Group 3 (n = 15): low 8-OHdG but high EAD expression; Group 4 (n = 31): low 8-OHdG and EAD expression ($p < 0.001$).



106

107 **Figure S6. EBV positive cells were resistant to radiation therapy than EBV negative**
108 **cells. (A)** Colony formation of EBV negative and EBV-positive cells with a single dose of 0,
109 2, 4 or 6 Gy irradiation. **(B-C)** Survival curve of EBV negative and EBV positive cells after a
110 single dose of 0, 2, 4 or 6 Gy irradiation. Surviving fractions were calculated by comparing
111 the colony number of each treatment group with untreated groups (0 Gy). Results are plotted
112 as the mean surviving fraction ± S.D. The survival curves were drawn using the GraphPad
113 Prism 5 software program, ***$p < 0.001$.

114