

Supplementary Table**Supplementary Table 1. RNA sequence, small interfering RNA target sequence and PCR primer sequence used in this study.**

gene	direction	sequence	company
miR-200a	sense	5'-UAACACUGUCUGGUAACGAUGU-3'	RiboBio (China)
mimics	antisense	5'-UCUUCGUUCCUGUCUGUGUUU-3'	
Mimics NC	sense	5'-UUUGUACUACACAAAAGUACUG-3'	
	antisense	5'-CUGUUCUUUUGUGUUGUUCUUU-3'	
miR-200a inhibitor		5'-UCUUCGUUCCUGUCUGUGUUU-3'	
Inhibitor NC		5'-CUGUUCUUUUGUGUUGUUCUUU-3'	
si-CXCL12-001	Target sequence 5'-AGATGCCCATGCCGATTCT-3'		
si-CXCL12-002	Target sequence 5'-CCATGCCGATTCTTCGAAA-3'		
si-CXCL12-003	Target sequence 5'-GCCGATTCTTCGAAAGCCA-3'		
si-c-JUN-001	Target sequence 5'-CCAAGAACGTGACAGATGA-3'		
si-c-JUN-002	Target sequence 5'-CGCAGCAGTTGCAAACATT-3'		
si-c-JUN-003	Target sequence 5'-GACCTTATGGCTACAGTAA-3'		
GAPDH	Forward	5'-GTCAGCCGCATCTTCTTT-3'	Tianyi Huiyuan (China)
	Reverse	5'-AGGCTGTTGTCATACTTCTC-3'	
c-JUN	Forward	5'-GGAGGAAAAAGTGAAAACCTTGAA-3'	
	Reverse	5'-TTTAAGCTGTGCCACCTGTTCC-3'	
CXCL12	Forward	5'-CAGCCTGAGCTACAGATGCCC-3'	
	Reverse	5'-TTCTTCAGCCGGGCTACAATCT-3'	
TGFβ1	Forward	5'-TCGCCAGAGTGGTTATCTTT-3'	

	Reverse	5'-TGAACCCGTTGATGTCCACT-3'	
SMAD3	Forward	5'-CACCAGGATGCAACCTGAAG-3'	
	Reverse	5'-AACTGGTAGACAGCCTCAAAGC-3'	
miR200a	Forward	5'-CCAGCCTGTGCAGGTGG-3'	GZSCBio (China)
promoter site1	Reverse	5'-GGCCAGACTCAGCTTGGG-3'	
miR-200a	Forward	5'-CCCAAGCTGAGTCTGGCC-3'	
promoter site2	Reverse	5'-TCCGAGAGCCCCACG-3'	
miR200a	Forward	5'-CTGCCACACGGGTCTGTC-3'	
promoter site3	Reverse	5'-CCAGCCTTCTCAGGAACCC-3'	
miR200a	Forward	5'-GGTGGCGAAGGCCTGTG-3'	
promoter site4	Reverse	5'-CAAAGGCCACGTCTCCGAG-3'	
miR200a	Forward	5'-CAGGGCTGCCCACTTCC-3'	
promoter site5	Reverse	5'-CTGCGGTTTGTGTGTCTGC-3'	
miR200a	Forward	5'-GATCTGGGGATTAGGACGCTC-3'	
promoter site6	Reverse	5'-CTCTGTTGGGTCCTGCTCG-3'	
GAPDH	Forward	5'-GGTTTTACGGGCGCACGT-3'	
promoter	Reverse	5'-GCTGACTGTGGAACAGGAGG-3'	
miR200a	The miR200a qPCR primers used in this study were proven primers provided by GeneCopoeia and used in conjunction with the all-in-one miRNA qPCR kit produced by GeneCopoeia. The manufacturer refused to provide the sequence information to the customer due to commercial confidentiality.		
U6	The U6 qPCR primers used in this study were proven primers provided by GeneCopoeia and used in conjunction with the all-in-one miRNA qPCR kit produced by GeneCopoeia. The manufacturer refused to provide the sequence information to the customer due to commercial confidentiality		

Supplementary Table 2. Primary antibodies used in this study.

Antibodies	Company	Cat.No.	Dilution
Anti-Human CD4 (RPA-T4)-FITC	MultiSciences	AH00401	5 μ l/test(FACS)

Anti-Human CD25 (BC96)-APC	MultiSciences	AH02505	5µl/test(FACS)
Anti-Human CD8a (OKT8)-PerCP-Cy5.5	MultiSciences	AH008A0307	5µl/test(FACS)
Anti-Human CD8a (RPA-T8)-PerCP-Cy5.5	MultiSciences	AH008A07	5µl/test(FACS)
Anti-Human CD127 (IL-7Ra) (RDR5)-PE	MultiSciences	AH012704	5µl/test(FACS)
Anti-Human Foxp3 (236A/E7)-PE	MultiSciences	AH0F04	5µl/test(FACS)
Anti-Mouse CD4 (GK1.5)-FITC	MultiSciences	AM00401	5µl/test(FACS)
Anti-Mouse CD25 (PC61.5)-APC	MultiSciences	AM02505	5µl/test(FACS)
Anti-Mouse CD8a (53-6.7)-PerCP-Cy5.5	MultiSciences	AM008A07	5µl/test(FACS)
Anti-Mouse CD127 (IL-7Ra) (A7R34)-PE	MultiSciences	AM012704	5µl/test(FACS)
Anti-Mouse Foxp3 (3G3)-PE	MultiSciences	AM0F04	5µl/test(FACS)
Mouse anti-Human CD184 (CXCR4) Monoclonal Antibody (12G5)- PE-Cyanine7	eBioscience	25-9999-42	5µl/test(FACS)
BV421 Mouse Anti-Human CD184 Clone 12G5(RUO)	BD Horizon	562448	5µl/test(FACS)
BV421 Rat Anti-Mouse CD184 Clone 2B11/CXCR4(RUO)	BD Horizon	562738	5µl/test(FACS)
Asialo GM1	eBioscience	16-6507-39	20-50µg/mouse (neutralising)
EBV EBNA1	SANTA CRUZ	SC-81581	1:200(IHC) 1:500(WB)
FOXP3	Affinity	AF5387	1:100(IHC)
TGFβ1	abcam	Ab92486	1:200(IHC) 1:1000(WB)
TGFβ1	abcam	Ab46780	1:1000(IF)
TGFβ1	abcam	Ab64715	1:1000(WB) 2-5µg/ml(neutralising)
SMAD3	Affinity	BF0378	1:500(IHC) 1:1000(IF) 1:1000(WB)
p-SMAD3	Affinity	AF3365	1:100(IHC) 1:500(WB)
c-JUN	abcam	Ab31419	1:50 (ChIP) 1:50(Co-IP) 1:400(IHC) 1:1000(IF) 1:1000(WB)

c-JUN	Affinity	AF6089	1:500(WB)
p-c-JUN	Affinity	AF3093	1:200(IHC) 1:500(WB)
PI3K	Proteintech	60225-1-Ig	1:1000(WB)
p-PI3K	Affinity	AF3241	1:500(WB)
AKT	Proteintech	10176-2-AP	1:1000(WB)
p-AKT	Affinity	AF0016	1:500(WB)
CXCL12	abcam	Ab155090	1:200(IHC) 1:500(WB)
CXCL12	Proteintech	17402-1-AP	1:200(IHC)
Goat Anti Chicken IgY H&L(Alexa Fluor 647) preadsorbed	abcam	Ab150175	1:1000(IF)
Alexa Fluor488 AffiniPure Donkey Anti-Rabbit IgG(H+L)	Yeasen	34206ES60	1:1000(IF)
Alexa Fluor594 AffiniPure Goat Anti-Mouse IgG(H+L)	Yeasen	33212ES60	1:1000(IF)

Supplementary Table 3. Correlation between EBNA1 expression and clinicopathological characteristics in 105 NPC.

Clinical characteristics	EBNA1 expression			p value	pearson correlation value
	negative (n=3)	positive(n=102)			
		low(n=33)	high(n=69)		
Mean age(years)	35	45	47	0.306	0.101
Gender				0.908	-0.011
Male	3	23	46		
Female	0	10	23		
EBV-encoded RNA (EBER)				0.000	—
positive	3	33	69		
negative	0	0	0		
Clinical stage				0.000	0.389
I	0	5	4		
II	0	13	7		
III	1	8	24		
IV	2	7	34		
T classification				0.003	0.290
T1	0	9	13		
T2	0	12	13		
T3	1	8	22		
T4	2	4	21		
N classification				0.000	0.430
N0	0	11	6		
N1	2	12	20		

N2	1	7	26		
N3	0	3	17		
Distant metastasis				0.959	0.005
M0	3	33	67		
M1	0	0	2		
Recurrence				0.000	0.499
Yes	1	3	23		
No	2	30	46		
Death				0.000	0.414
Yes	0	4	22		
No	3	29	47		
Mean survival time (month)	110.6667	116.1818	103.4783	0.000	-0.352
Mean Treg infiltration number	19	44	172	0.000	0.694
TGFβ1 expression				0.000	0.630
low or negative expression	2	11	2		
high expression	1	22	67		
p-SMAD3 expression				0.000	0.709
low or negative expression	2	18	11		
high expression	1	15	58		
CXCL12 expression				0.000	0.628
low or negative expression	0	5	1		
high expression	3	28	68		
c-JUN expression				0.000	0.647
low or negative expression	2	13	4		
high expression	1	20	65		
p-c-JUN expression				0.000	0.627
low or negative expression	3	17	8		
high expression	0	16	61		

Supplementary Table 4. Correlation between Treg infiltration and clinicopathological characteristics on 105 NPC.

Clinical characteristics	Treg infiltration level		p value	pearson correlation value
	Low(n=61)	High(n=44)		
Mean age(years)	46	47	0.399	0.025
Gender			0.281	-0.057
Male	23	34		
Female	38	10		
EBV-encoded RNA (EBER)			0.000	—
positive	61	44		
negative	0	0		
Clinical stage			0.000	0.373

I	9	0		
II	15	5		
III	21	12		
IV	16	27		
T classification			0.020	0.202
T1	15	7		
T2	17	8		
T3	20	11		
T4	9	18		
N classification			0.000	0.472
N0	17	0		
N1	23	11		
N2	15	19		
N3	6	14		
Distant metastasis			0.486	0.003
M0	60	43		
M1	1	1		
Recurrence			0.000	0.325
Yes	9	18		
No	52	26		
Death			0.002	0.285
Yes	9	17		
No	52	27		
Mean survival time (month)	111.7541	102.0227	0.019	-0.203
EBNA1 expression			0.000	0.694
low or negative expression	34	2		
high expression	27	42		
TGF β 1 expression			0.000	0.583
low or negative expression	15	0		
high expression	46	44		
p-SMAD3 expression			0.000	0.643
low or negative expression	27	4		
high expression	34	40		
CXCL12 expression			0.000	0.568
low or negative expression	6	0		
high expression	55	44		
c-JUN expression			0.000	0.593
low or negative expression	17	2		
high expression	44	42		
p-c-JUN expression			0.000	0.557
low or negative expression	25	3		
high expression	36	41		

