Supplementary information

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Table S1   si-CXCL16 sequences

Name of the gene	Sequence information
si-CXCL16-1	GGAUCACUGUCCUCGGACATT
si-CXCL16-2	CATCGGTGTCTATACTACA

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Table S2   Primer sequences

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Name of the gene	Sequence information	
β-actin	Forword Primer:	CATGTACGTTGCTATCCAGGC
	Reverse Primer:	CTCCTTAATGTCACGCACGAT
CXCL16	Forword Primer:	GACATGCTTACTCGGGGATTG
	Reverse Primer:	GGACAGTGATCCTACTGGGAG
ITGB4	Forword Primer:	GCAGCTTCCAAATCACAGAGG
	Reverse Primer:	CCAGATCATCGGACATGGAGTT
PLAU	Forword Primer:	GGGAATGGTCACTTTTACCGAG
	Reverse Primer:	GGGCATGGTACGTTTGCTG
BSG	Forword Primer:	GAAGTCGTCAGAACACATCAACG
	Reverse Primer:	TTCCGGCGCTTCTCGTAGA
ICAM1	Forword Primer:	ATGCCCAGACATCTGTGTCC
	Reverse Primer:	GGGGTCTCTATGCCCAACAA
AXL	Forword Primer:	GTGGGCAACCCAGGGAATATC
	Reverse Primer:	GTACTGTCCCGTGTCGGAAAG
GPX1	Forword Primer:	CAGTCGGTGTATGCCTTCTCG
	Reverse Primer:	GAGGGACGCCACATTCTCG
POP7	Forword Primer:	CCCGGAGACCCAATGACATTT
	Reverse Primer:	GGGCCTTAAAGTCCGTCTTCA
IDH1	Forword Primer:	TGTGGTAGAGATGCAAGGAGA
	Reverse Primer:	TTGGTGACTTGGTCGTTGGTG
EPHA2	Forword Primer:	TGGCTCACACACCCGTATG
	Reverse Primer:	GTCGCCAGACATCACGTTG
Nfe2l2	Forword Primer:	TCAGCGACGGAAAGAGTATGA
	Reverse Primer:	CCACTGGTTTCTGACTGGATGT
SOD1	Forword Primer:	GGTGGGCCAAAGGATGAAGAG
	Reverse Primer:	CCACAAGCCAAACGACTTCC
SOD2	Forword Primer:	AACCTCACATCAACGCGCAGATC
	Reverse Primer:	CTCCTGGTACTTCTCCTCGGTGAC
GSR	Forword Primer:	CACTTGCGTGAATGTTGGATG
	Reverse Primer:	TGGGATCACTCGTGAAGGCT
NQO1	Forword Primer:	GAAGAGCACTGATCGTACTGGC
	Reverse Primer:	GGATACTGAAAGTTCGCAGGG
GSS	Forword Primer:	GGGAGCCTCTTGCAGGATAAA
	Reverse Primer:	GAATGGGGCATAGCTCACCAC
GCLM	Forword Primer:	TGTCTTGGAATGCACTGTATCTC
	Reverse Primer:	CCCAGTAAGGCTGTAAATGCTC
CXCR6	Forword Primer:	GACTATGGGTTCAGCAGTTTCA
	Reverse Primer:	GGCTCTGCAACTTATGGTAGAAG

Table S3   Clinical characteristics of high and low CXCL16 expression groups

	No. of cases	Low expression	High expression	*P*-value
HPV Status				
Negative	64（75.3%）	32（80.0%）	32（71.1%）	0.34
Positive	21（24.7%）	8（20.0%）	13（28.9%）	
Margin				
Negative	334（85.2%）	162（84.8%）	172（85.6%）	0.83
Positive	58（14.8%）	29（15.2%）	29（14.4%）	
Perineural invasion				
Negative	159（54.1%）	78（53.1%）	81（55.1%）	0.73
Positive	135（45.9%）	69（46.9%）	66（44.9%）	
Drinking				
None or occasionally	50（32.3%）	30（37.5%）	20（26.7%）	0.15
Frequently	105（67.7%）	50（62.5%）	55（73.3%）	
Age				
<60	168（42.9%）	94（49.2%）	74（36.8%）	0.01*
≥60	224（57.1%）	97（50.8%）	127（63.2%）	
Gender				
Female	97（24.7%）	41（21.5%）	56（27.9%）	0.14
Male	295（75.3%）	150（78.5%）	145（72.1%）	
Tumor Stage				
T1/T2	147（39.6%）	75（41.7%）	72（37.7%）	0.44
T3/T4	224（60.4%）	105（58.3%）	119（62.3%）	
TNM Stage				
I/II	79（21.8%）	41（23.2%）	38（20.5%）	0.55
III/IV	283（78.2%）	136（76.8%）	147（79.5%）	
Lymphovascular invasion				
Negative	182（64.3%）	101（71.1%）	81（57.4%）	0.02*
Positive	101（35.7%）	41（28.9%）	60（42.6%）	

*P<0.05
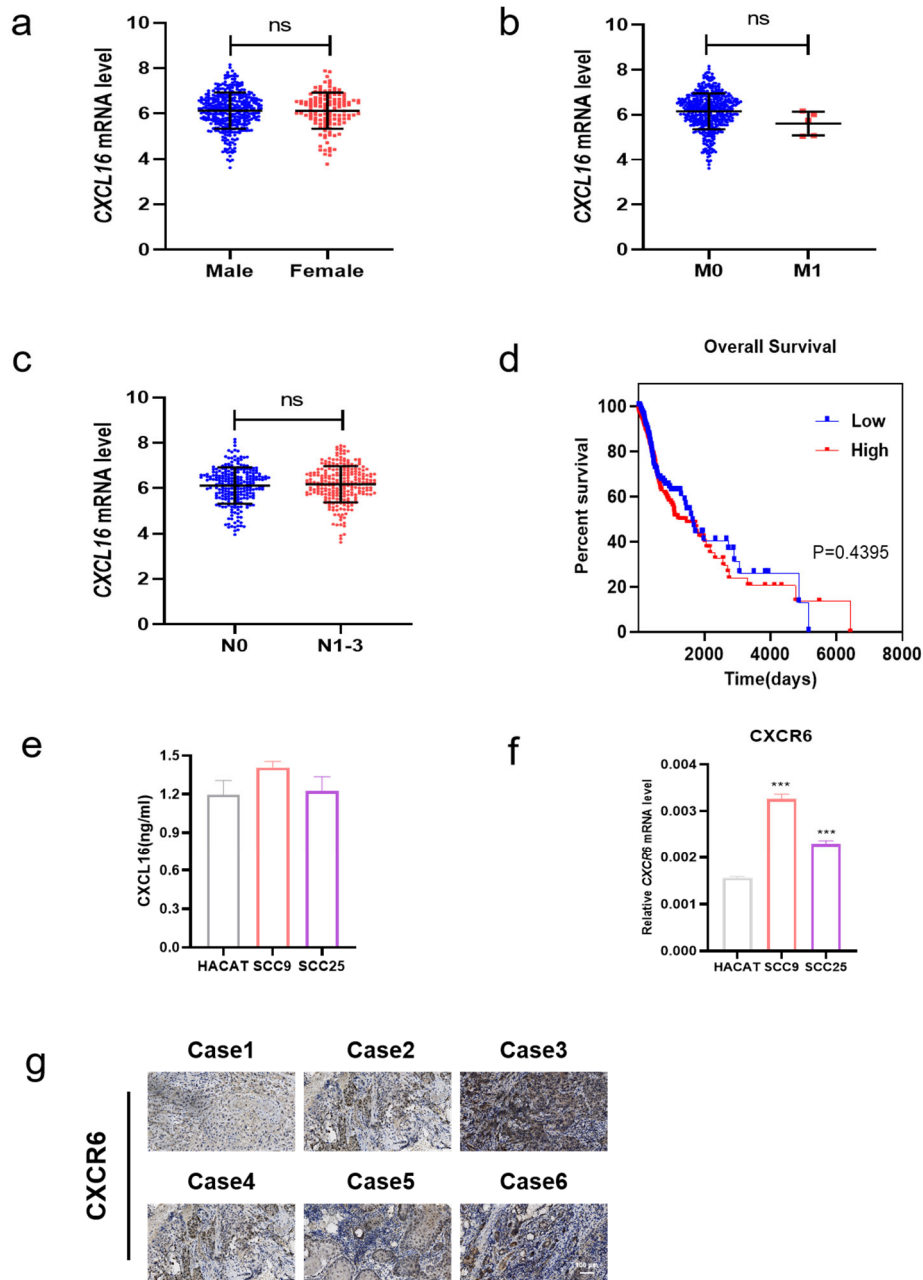
Fig. S1 Differential expression of CXC motif chemokine ligand 16 (CXCL16) in clinical features and expression levels of CXC chemokine receptor 6 (CXCR6 between head and neck squamous carcinoma (HNSCC) cell lines and cancer tissues. (a): Relationship between CXCL16 expression level and gender difference from The Cancer Genome Atlas (TCGA) database. (b): Relationship between CXCL16 expression level and M staging from the TCGA database. (c): Relationship between CXCL16 expression level and N staging from the TCGA database. (d): Association between CXCL16 expression and overall survival in HNSCC patients. (e): The expression level of soluble CXCL16 was detected by taking supernatants from HACAT, SCC9 and SCC25 cells after 24 h of culture (*n*=3). (f): Expression levels of CXCR6 messenger RNA (mRNA) in normal epidermal cell lines (HACAT) and HNSCC cell lines (SCC9, SCC25) (*n*=3). (g): Immunohistochemical results of CXCR6 in tumor tissues and adjacent normal tissues of HNSCC patients from our central hospital (20×). Scale bar=100 μm. The data were expressed as mean±SEM, n=3. M: distant metastasis; N: lymph node metastasis; ns: no significant; * *P*<0.001; mRNA: messenger RNA; TCGA: The Cancer Genome Atlas; CXCR6: CXC chemokine receptor 6; SEM: standard error of mean.**
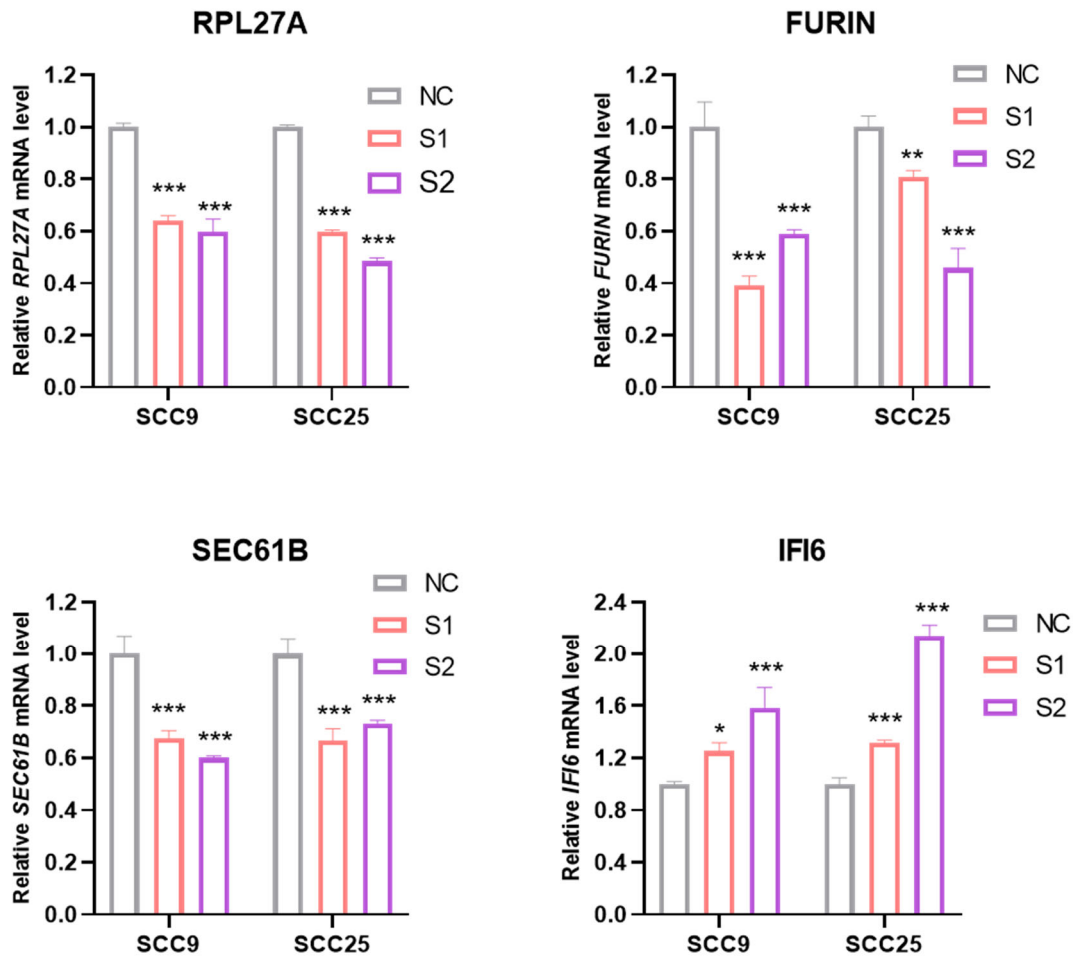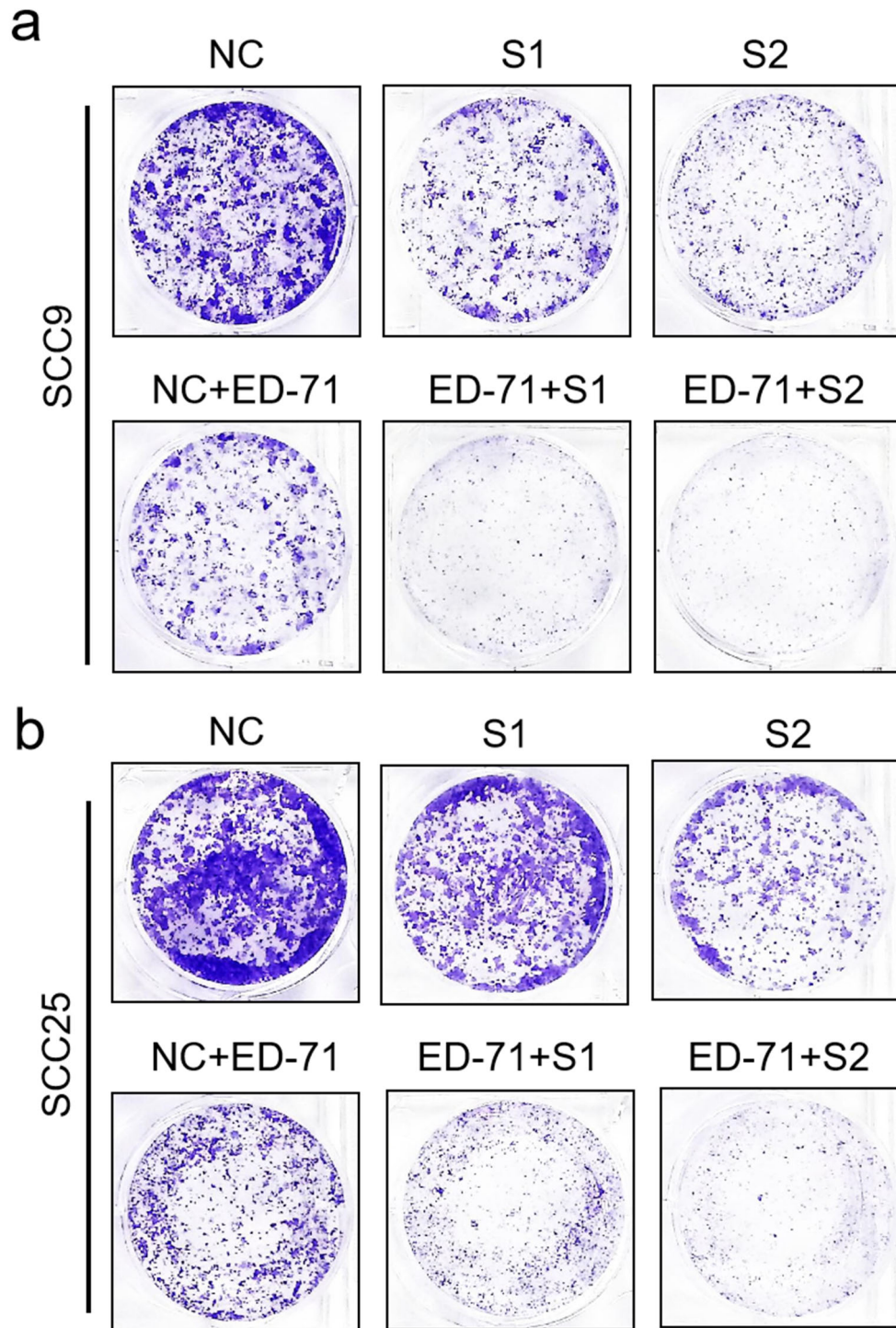
Fig. S2   Validation of mRNA levels of unregulated genes in the transcriptome results (*n*=3). The data were expressed as mean±SEM, *n*=3. * *P*<0.001; ** *P*<0.01; * *P*<0.05. RPL27A: ribosomal Protein L27A; SEM: standard error of mean.**

Fig. S3　Results of clone formation experiments of SCC9 and SCC25 cells after the combination of ED-71 and si-CXCL16. (a): ED-71 and si-CXCL16 inhibited the clone formation of SCC9 cells after co-action. (b): ED-71 and si-CXCL16 inhibited the clone formation of SCC25 cells after co-action.