

Figure S1, Related to Figure 1

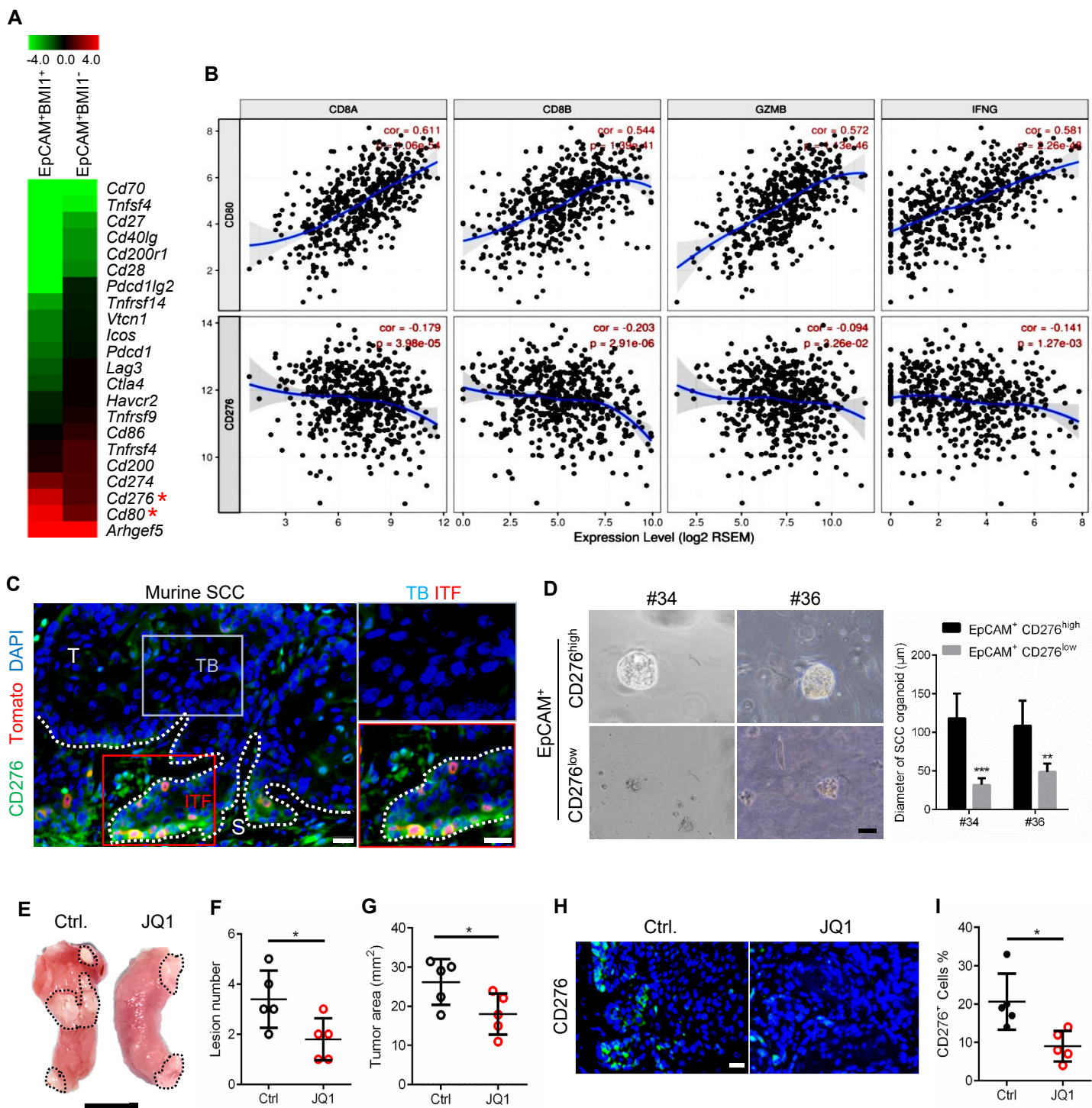


Figure S1, related to Figure 1, CD276 is highly expressed in CSCs of HNSCC

(A) Heatmap showing the expression of 22 immune checkpoints by RNA-seq data in Bmi1⁺ CSCs and Bmi1⁻ non-CSCs. * indicated fold change>2, q value<0.05.

(B) Analysis of the correlation between CD276 or CD80 and CD8A, CD8B, GZMB or IFNG in HNSCC based on TCGA HNSCC datasets.

(C) BMI1⁺ CSCs highly expressing CD276 in murine HNSCC. Scale bar, 20μm

(D) EpCAM⁺CD276^{high} cells freshly isolated from primary human HNSCC formed larger organoid than EpCAM⁺CD276^{low} cells. **p<0.01 and ***p<0.001 by Student's t test. Scale bar, 100μm. 5 organoids were counted in each group.

(E) Representative image of tongue lesions in 4NQO-induced mouse treated with control (Ctrl) and JQ1. Scale bar, 5mm

(F) Quantification of lesion numbers visible in the mouse tongues. Values are mean ± SD, n = 5, *p<0.05 by Student's t test.

(G) Quantification of lesion areas visible in the mouse tongues. Values are mean ± SD, n=5, *p<0.05 by Student's t test.

(H) Representative images of CD276 immunostaining in mouse HNSCC treated with control (Ctrl) and JQ1. Scale bar, 20μm

(I) Quantification of CD276 expression in mouse HNSCC treated with control (Ctrl) and JQ1. Values are mean ± SD, n=5, *p<0.05, by Student's t test.

Figure S2, related to Figure 5

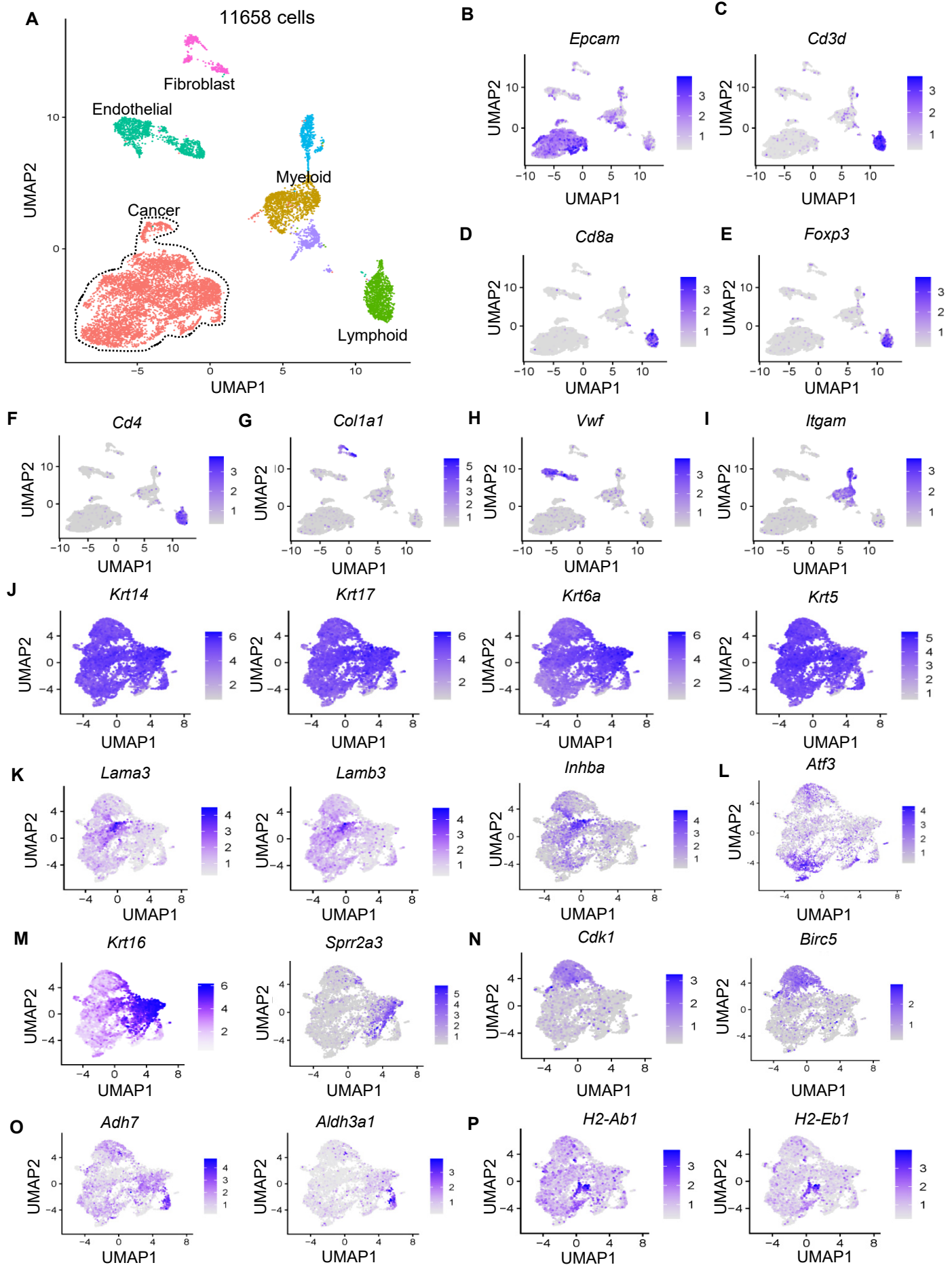


Figure S2, related to Figure 5, scRNA-seq identifies landscape alterations of HNSCC upon anti-CD276 treatment.

(A) UMAP plot showing identified cell populations within whole tumor from two groups merged at 4 weeks after anti-CD276 treatment.

(B-I) UMAP plots of identified cell populations displaying marker gene expression.

(J-P) UMAP plots of cancer cells displaying selected marker gene expression.

Figure S3, Related to Figure 6

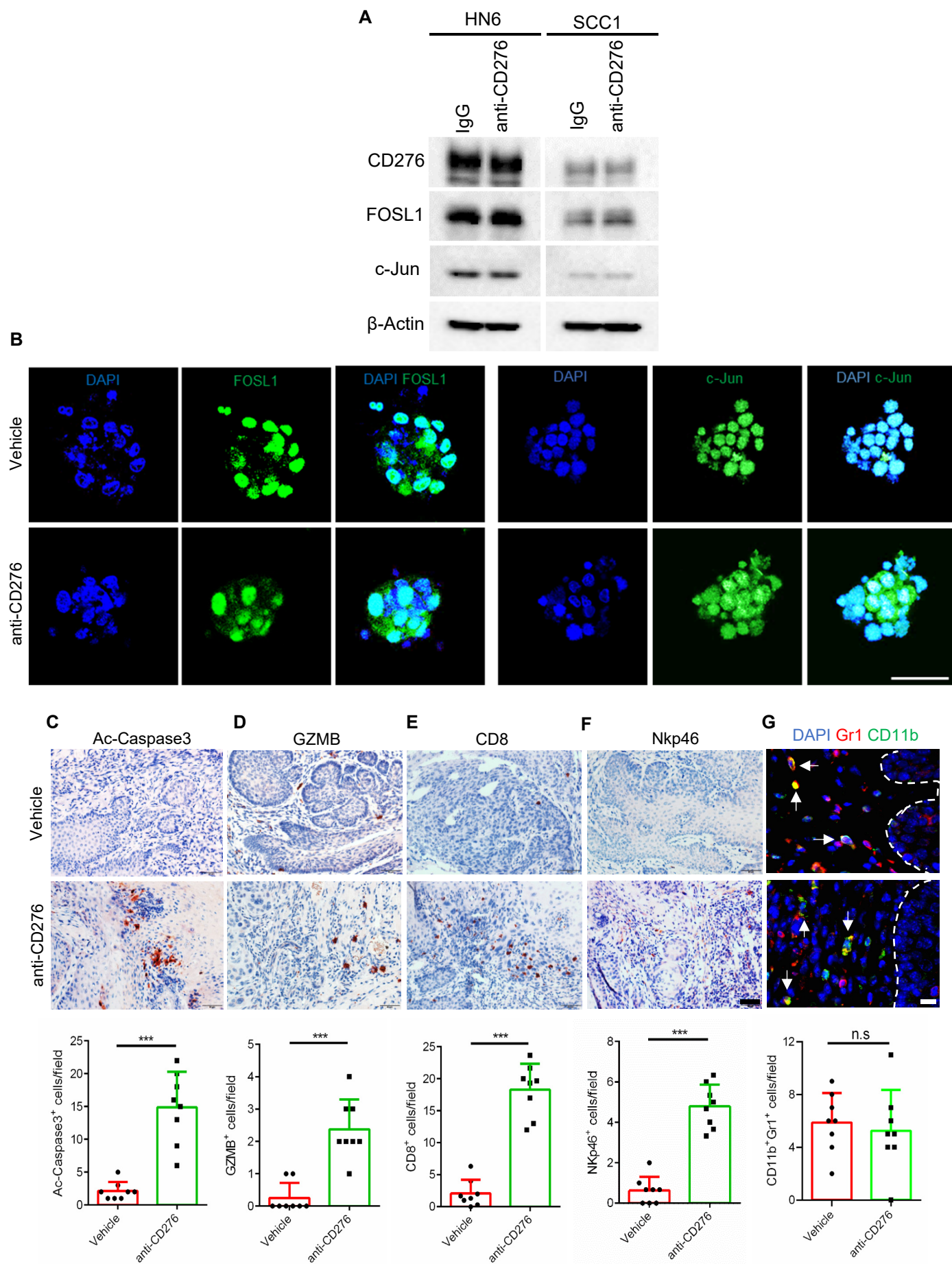


Figure S3, related to Figure 6, Anti-CD276 treatment increases CD8⁺ lymphocytes and induces apoptosis in HNSCC

(A) Anti-CD276 did not inhibit the expression of c-Jun and FOSL1 expression in SCC cells by Western blot analysis.

(B) Anti-CD276 did not inhibit the expression of c-Jun and FOSL1 in human HNSCC organoids by immunostaining.

(C) Representative image and quantification of active-caspase-3⁺ cells in HNSCC from *Bmi1^{CreER};Rosa^{tdTomato}* mice treated with anti-CD276. n=8, ***p<0.001 by Student's t test.

(D) Representative image and quantification of GZMB⁺ cells in HNSCC from *Bmi1^{CreER};Rosa^{tdTomato}* mice treated with anti-CD276. n=8, ***p<0.001 by Student's t test.

(E) Representative image and quantification of CD8⁺ cells in HNSCC from *Bmi1^{CreER};Rosa^{tdTomato}* mice treated with anti-CD276. n=8 ***p<0.001 by Student's t test.

(F) Representative image and quantification of NK cells in HNSCC from *Bmi1^{CreER};Rosa^{tdTomato}* mice treated with anti-CD276. n=8 ***p<0.001 by Student's t test. Scale bar, 50µm

(G) Representative image and quantification of MDSCs in HNSCC from *Bmi1^{CreER};Rosa^{tdTomato}* mice treated with anti-CD276. White arrows indicate Gr1⁺ (red) CD11b⁺ (green) MDSCs. Nuclei were stained with DAPI (blue). White dashed lines demark tumor-stromal junction. Values are mean ± SD; n = 8. Scale bar, 10 µm. ns, not significant by unpaired Student's t test.

Figure S4, Related to Figure 7

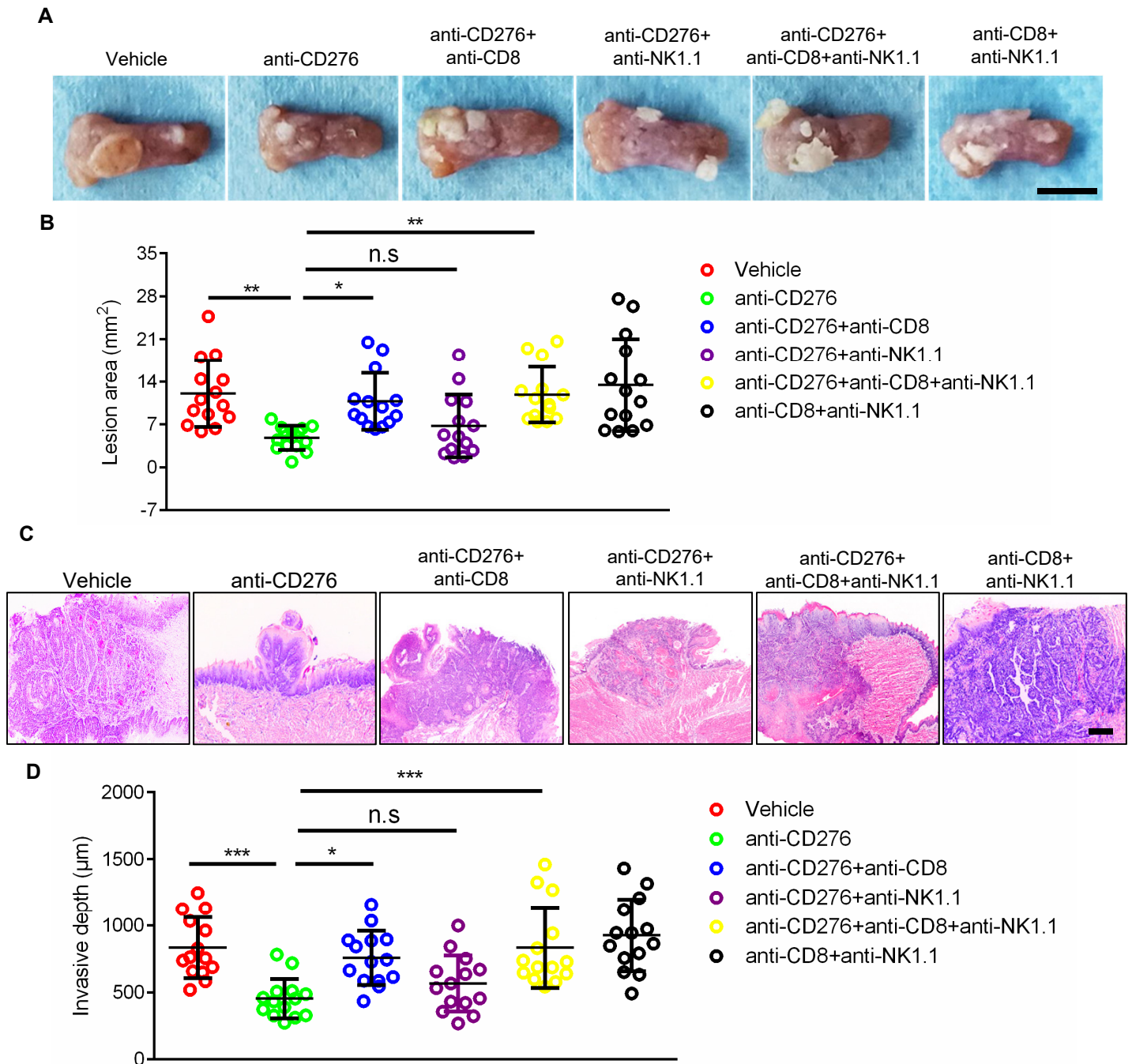


Figure S4, related to Figure 7, The depletion of NK cells does not affect anti-CD276-mediated inhibition of HNSCC.

(A, B) Representative image and quantification of lesion area in 4NQO-induced mice treated with different antibodies. n=14. * $p < 0.05$ and ** $p < 0.01$ by one-way ANOVA; n.s, non-significant. Scale bar, 5mm.

(C) Representative image of H&E staining of HNSCC in 4NQO-induced mice treated with different antibodies. Scale bar, 200µm

(D) Quantification of HNSCC invasive depth in 4NQO-induced mice treated with different antibodies. Vehicle, n=14. * $p < 0.05$ and *** $p < 0.001$ by one-way ANOVA. n.s, non-significant.

Figure S5, Related to Figure 7

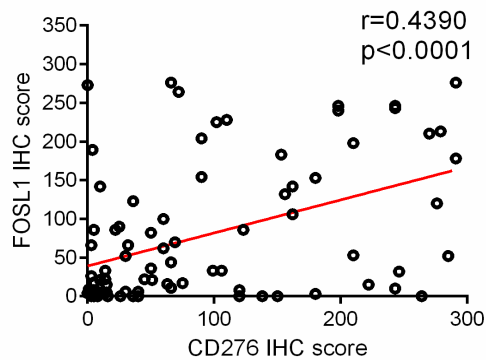
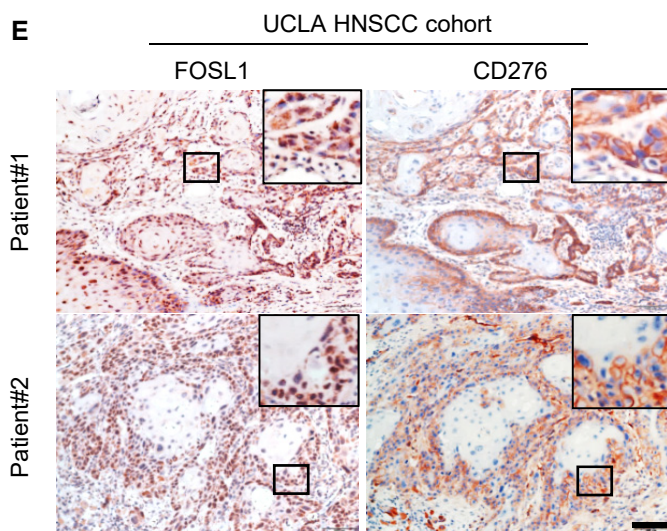
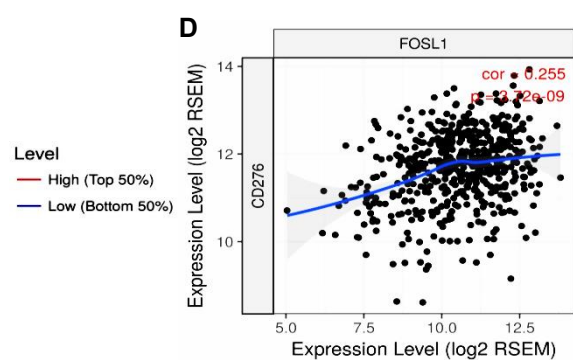
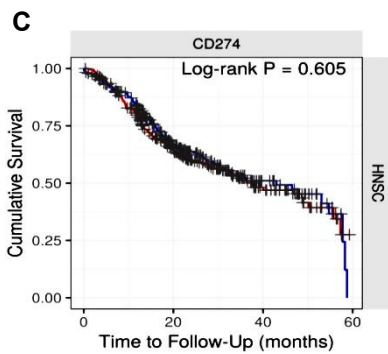
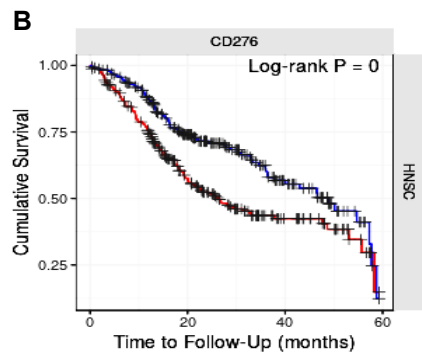
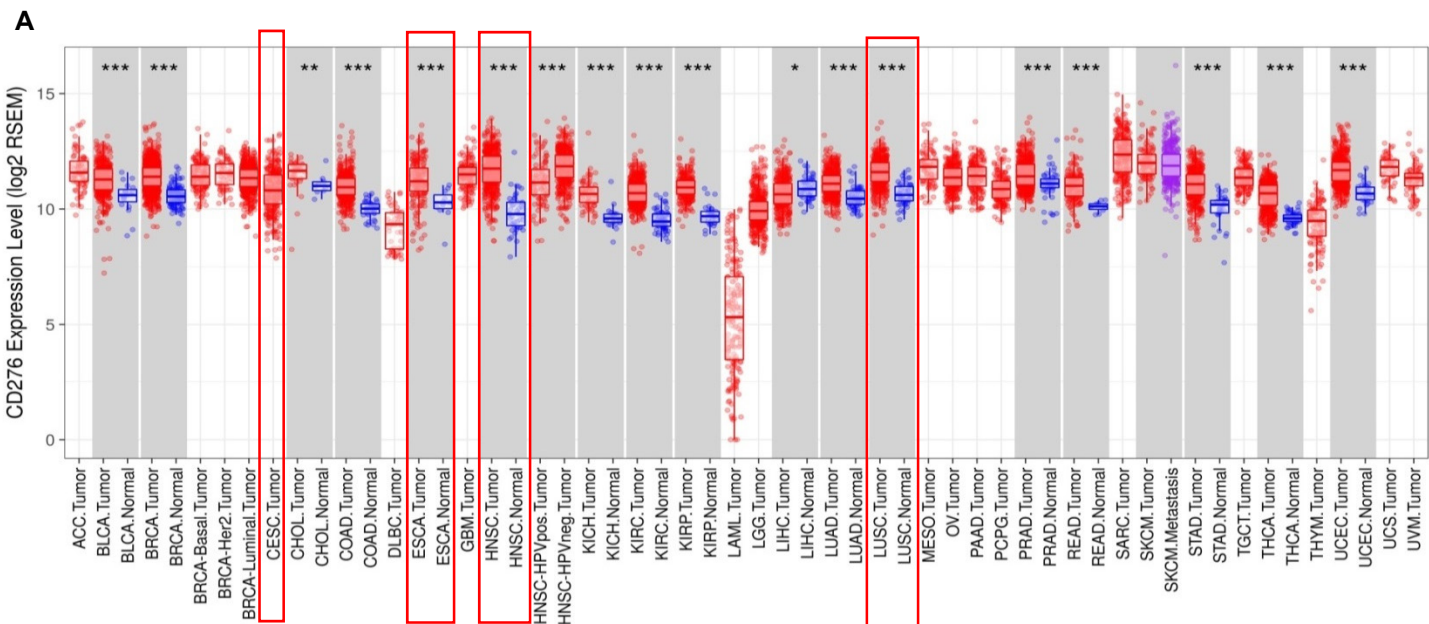


Figure S5, related to Figure 7, High expression of *CD276* is associated with a poor prognosis in human HNSCC and is correlated with *FOSL1* expression.

(A) *CD276* was highly expressed in human HNSCC (HNSC) compared with normal tissues in TCGA database. Similar results were also observed in lung SCC (LUSC), esophageal carcinoma (ESCA) as compared to the normal tissue in TCGA database.

(B) Kaplan–Meier curves for survival of HNSCC patients in TCGA database with high and low expression levels of *CD276*.

(C) Kaplan–Meier curves for survival of HNSCC patients in TCGA database with high and low expression levels of *CD274*.

(D) The positive correlation between *FOSL* and *CD276* expression in TCGA database.

(E) Immunostaining showed that *CD276* protein expression levels were positively correlated with *FOSL1* expression levels in human HNSCC samples. Scale bar, 100 μm .

Figure S6, Related to Figure 7

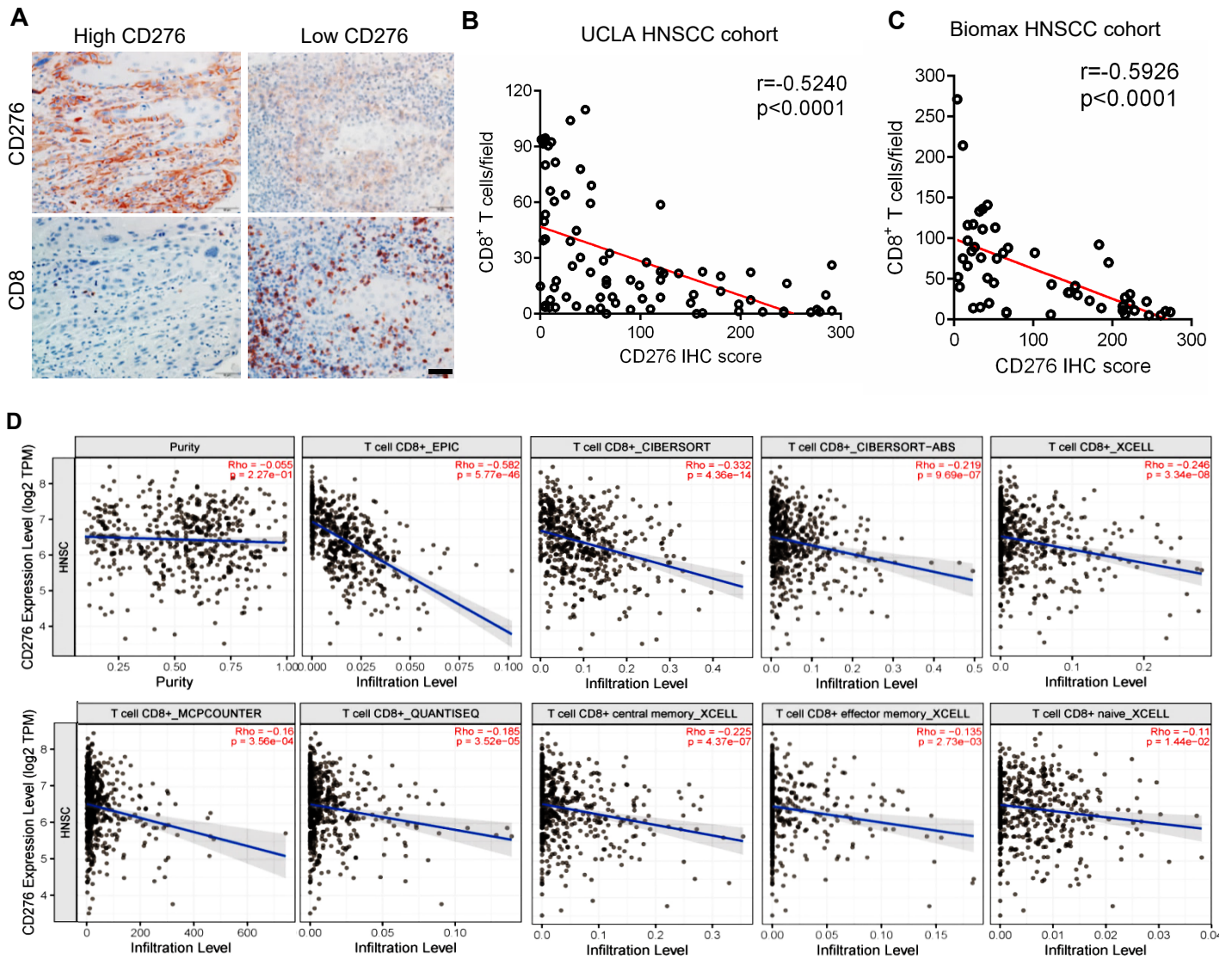


Figure S6, related to Figure 7, CD276 expression is inversely correlated with infiltrated CD8⁺ T cells in human HNSCC.

(A-C) Immunostaining showed that CD276 protein expression levels were inversely correlated with infiltration of CD8⁺ T cells in human HNSCC samples from two independent patient cohorts. Scale bar, 50µm

(D) CD276 expression was inversely correlated with infiltration of CD8⁺ T cells in TCGA HNSCC datasets based on 6 different algorithms from TIMER 2.0

Supplemental Tables

Table S1. Related to STAR Methods, Primer sequence.

Gene	Assay	Forward (5'-3')	Reverse (5'-3')
<i>Bmi1^{CreER}</i>	genotyping (wildtype)	ACCAGCAACAGCCCCAGTGC	TAGGCATTAATTGAGATTAACAAACTA
	Genotyping (CreER)	ACCAGCAACAGCCCCAGTGC	AAAGACCCCTAGGAATGCTC
<i>tdTomato</i>	genotyping (wildtype)	AAGGGAGCTGCAGTGGAGTA	CCGAAAATCTGTGGGAAGTC
	Genotyping (tdTomato)	GGCATTAAAGCAGCGTATCC	CTGTTCCCTGTACGGCATGG
<i>GAPDH</i>	qRT-PCR	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
<i>CD276</i>	qRT-PCR	CTGGCTTTCGTGTGCTGGAGAA	GCTGTCAGAGTGTTCAGAGGC
<i>FOSL1</i>	qRT-PCR	GGAGGAAGGAACTGACCGACTT	CTCTAGGCGCTCCTTCTGCTTC
<i>CD276</i>	ChIP-qPCR	TGTTCTGCCGCCAGTCGATGACC	CCCACCC CGTCCCACACTTCCA
<i>CD276</i> -Negative	ChIP-qPCR	ACCCTTAGTTGTT CTGGTCAGTG	CCTTCAGTTTTGCATCATAGGTGC
<i>CD276 shRNA1</i>	Knockdown	AGAAGATGATGGACAAGAA	
<i>CD276 shRNA2</i>	Knockdown	TGAAACACTCTGACAGCAA	
<i>FOSL1</i> siRNA1	Knockdown	GCUCAUCGCAAGAGUAGCA	
<i>FOSL1</i> siRNA2	Knockdown	GAGCUGCAGUGGAUGGUAC	