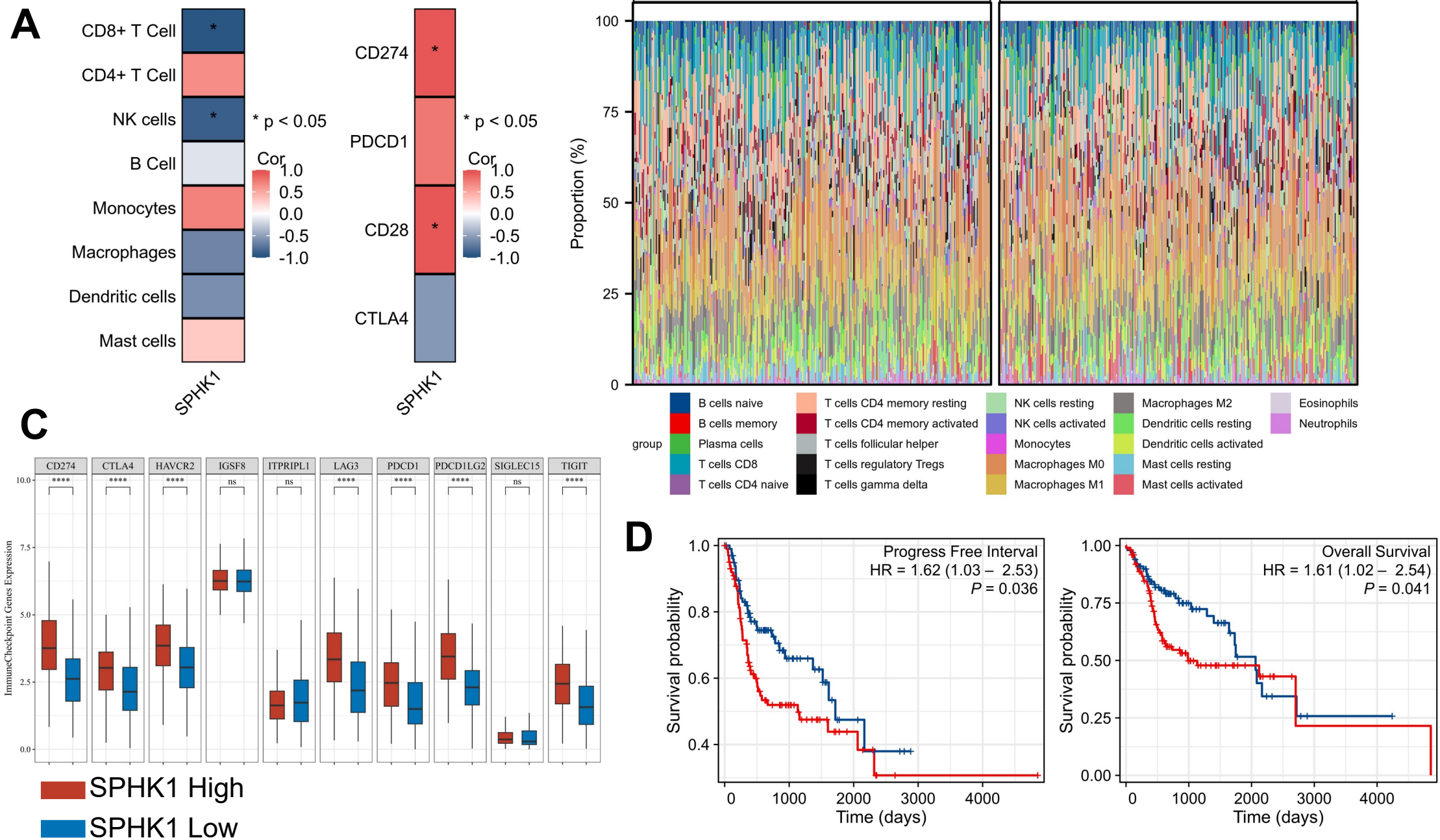
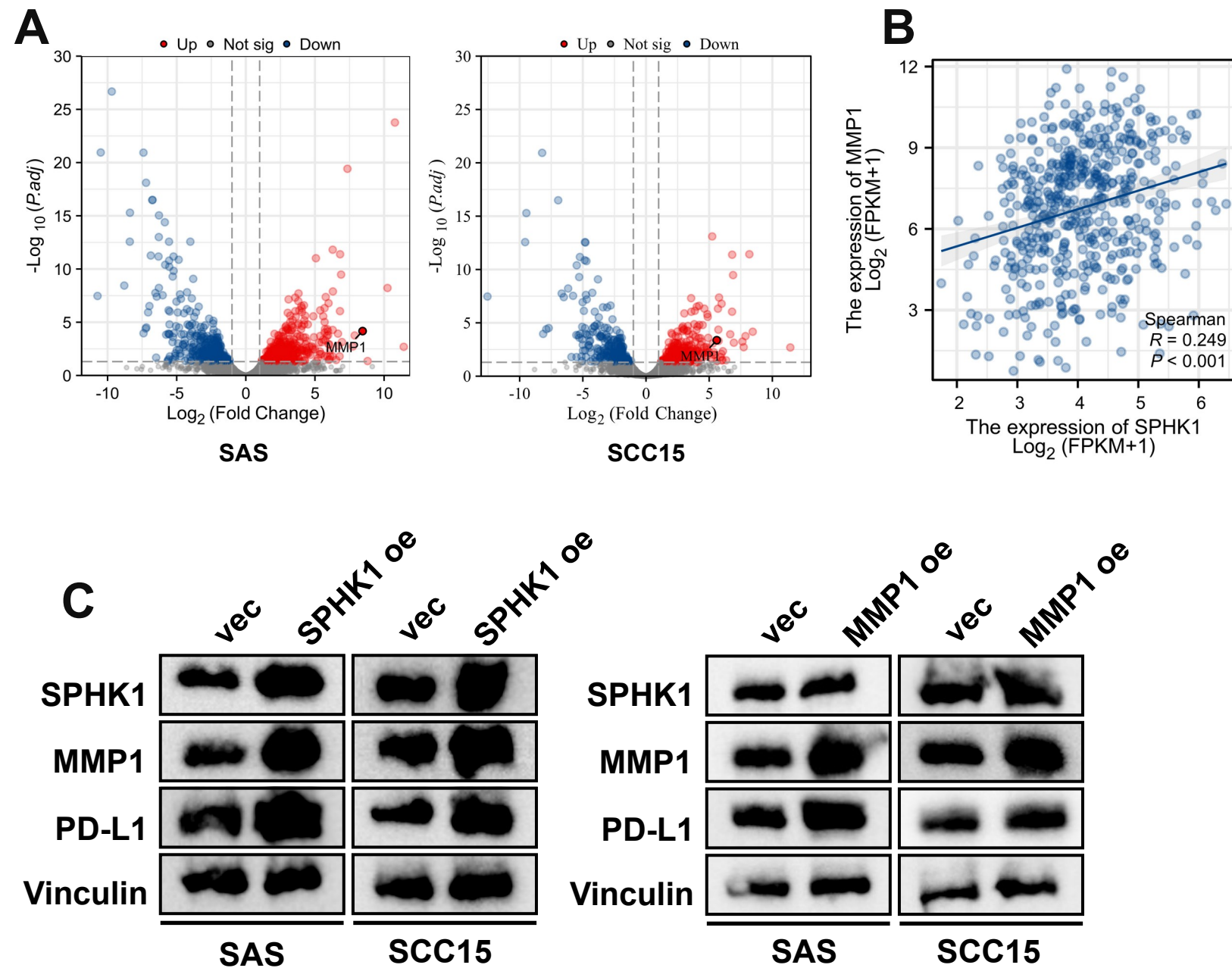


# Figure S1



**Figure S1.** **A.** Correlation between the proportion of immune-infiltrating cells and immune checkpoint inhibitor levels with the level of SPHK1 in six patients with oral squamous carcinoma. **B-C.** Correlation between SPHK1 and immune infiltrating cells in HNSCC patients analyzed by TCGA data. **C.** Correlation between SPHK1 and immune checkpoint levels in HNSCC patients analyzed by TCGA data. **D.** Overall survival, progression-free survival Kaplan-Meier analysis based on SPHK1 expression level in HNSCC in TCGA database. Statistical analysis was performed using the log-rank test.

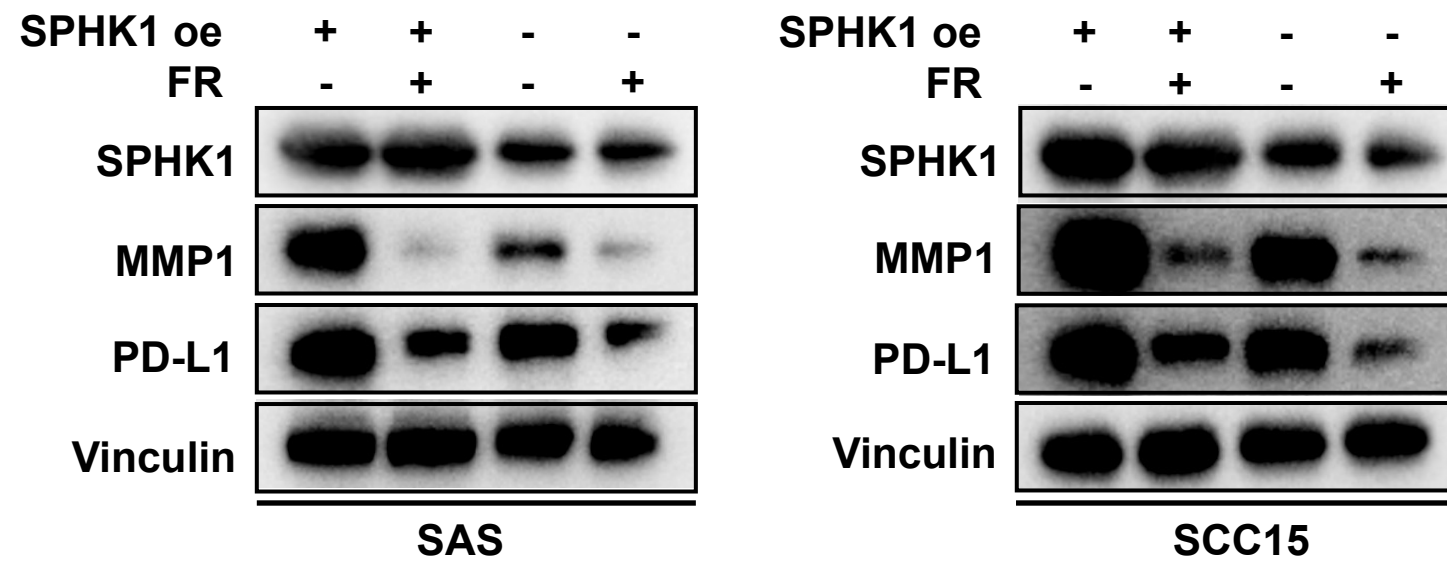
# Figure S2



**Figure S2.** A. RNA-seq analysis and differential analysis were performed on WT and SPHK1-depleted SAS and SCC15 cells. B. Correlation analysis of SPHK1 levels with MMP1 in HNSCC patients in the TCGA database. C. Western blotting showed the effect of SPHK1/MMP1 overexpression in the HNSCC cell lines SAS and SCC15 on MMP1 and PD-L1 protein levels.

# Figure S3

A



**Figure S3.** Western blotting showed the effect of ERK1/2 inhibitor FR 180204 on SPHK1 overexpression.

Supplementary Table 1. Baseline table of 117 HNSCC patients.

<b>Characteristic</b>	<b>Low expression group (SPHK1) n = 59 (100%)</b>	<b>High expression group (SPHK1) n = 58 (100%)</b>
Age		
≤47	6	3
>47	53	55
Gender		
Male	42	47
Female	17	11
TNM stage		
I-II	31	17
III	8	19
IV	20	22

Supplementary Table 2. List of Oligonucleotides used in this study.

<b>Gene</b>	<b>Sense</b>	<b>Antisense</b>
<b>RT-qPCR primers</b>		
SPHK1	TCTATAGTGCTGTTGTACACACC	CAAGCTCCTCCGGCTACTGAGTC
GAPDH	GCGAACTCTGACTCTATCAGCG	ACCACCCTGTTGCTGTAGCCAA
MMP1	GCCTCTATTCATAGAGCGAAGC	TGCATCCCAGCGGCTGACGTGC
PD-L1	AGTAATCTATCAGCCTCTCAGT	CCTGTTCGATGGCGAGCTTCTAGG
<b>siRNA targeting sequence</b>		
siSPHK1	GAGGCUGAAAUCUCCUUCATT	UGAAGGAGAUUUCAGCCUCTT
siMMP1	GCAGCUUCCUUGAACCAUUTT	AAUGGUUCAAGGAAGCUGCTT
siPD-L1	GUGCACCCAAACUACUUCUTT	AGAAGUAGUUUGGGUGCACTT

Supplementary Table 3. List of antibodies used in this study.

<b>Antibodies used for western blot (WB), Immunofluorescence (IF) and immunohistochemistry (IHC)</b>						
<b>anti-SPHK1</b>	<b>Supplier</b>	<b>Catalogue No.</b>	<b>Application</b>	<b>Host species</b>	<b>Species activity</b>	<b>Dilution</b>
anti-SPHK1	Abcam	ab26269	WB, IF, IHC	Rabbit	Hu, Mo	1:2000 for WB, 1:200 for IF, 1:200 for IHC
anti-SPHK1	Affinity Biosciences	DF6005-50	WB, IF	Mouse	Hu, Mo	1:1000 for WB
anti-MMP1	Abcam	ab52631-100ul	IF, IHC	Rabbit	Hu, Mo	1:100 for IF, 1:200 for IHC
anti-MMP1	Affinity Biosciences	DF6325-50	WB	Mouse	Hu	1:2000 for WB
anti-MMP1	ABclonal	A22080-50 µL	WB	Rabbit	Hu, Mo	1:10000 for WB
anti-CD274	Proteintech	28076-1-AP-50ul	IF, IHC	Rabbit	Hu, Mo	1:100 for IF, 1:200 for IHC
anti-CD274	ABclonal	A18103-50 µL	WB	Rabbit	Hu, Mo	1:3000 for WB
anti-CD274	ABclonal	A21443-50 µL	WB	Rabbit	Hu, Mo	1:2000 for WB
anti-CD8a	Servicebio	GB15068-100	IF	Mouse	Mo	1:100 for IF, 1:200 for IHC
anti-CD8a	Proteintech	CL594-66868	IF, IHC	Mouse	Hu	1:100 for IF
anti-GZMB	Proteintech	13588-1-AP-50ul	IF, IHC	Rabbit	Hu, Mo	1:100 for IF, 1:200 for IHC
anti-pERK1/2	Proteintech	51068-1-AP-50ul	IF, IHC	Rabbit	Hu, Mo	1:100 for IF, 1:200 for IHC