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Original Article

Decreased APOC1 expression inhibited cancer progression and was associated with better prognosis and immune microenvironment in esophageal cancer

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Abstract: Several studies have demonstrated the involvement of apolipoprotein C1 (APOC1) in multiple cancers. However, the role of APOC1 in esophageal cancer (ESCA) has not been elucidated. Hence, we examined the expression of APOC1 in ESCA tissues acquired from The Cancer Genome Atlas (TCGA) database and clinical samples from our hospital. An investigation of the association of APOC1 with the clinicopathological characteristics, prognosis, and diagnosis of ESCA was carried out on the basis of survival, receiver operating characteristics, and correlation analyses. Gene ontology, KEGG analysis, and protein-protein interaction network showed that co-expressed APOC1 genes were involved in the functions, mechanisms, and action network. The effects of APOC1 expression on ESCA cells were explored using CCK-8, migration and invasion assays. The relationship between APOC1 expression and ESCA immune-infiltrating cells and cell markers were examined using correlation analysis. We found that APOC1 was overexpressed in TCGA ESCA tissues and the same was validated in clinical ESCA tissues, with the area under the curve for APOC1 being 0.887. Overexpression of APOC1 was associated with short overall survival, disease-specific survival, progression-free interval, T stage, pathological stage, body mass index, and histological grade. Inhibition of APOC1 expression significantly reduced the proliferation, migration, and invasion of ESCA cells. Furthermore, APOC1 expression positively correlated with the ESTIMATE, immune, and stromal scores in ESCA. Overexpression of APOC1 correlated with the tumor purity, B cells, T helper cells, natural killer cells, cytotoxic cells, and other immune cells. Moreover, APOC1 was involved in ESCA progression via T cell receptor, B cell receptor, and other immune signaling pathways. Thus, APOC1 overexpression is expected to be a biomarker for dismal prognosis and diagnosis of ESCA. Inhibition of APOC1 expression significantly reduced the proliferation, migration, and invasion of ESCA cells. Overexpression of APOC1 was associated with the immune microenvironment in ESCA. Thus, APOC1 may be an efficient biomarker for proper prognosis and diagnosis of ESCA.

Keywords: APOC1, ESCA, immune microenvironment, prognosis, biomarker

Introduction

Esophageal cancer (ESCA) is one of the most common malignant tumors worldwide [1, 2]. At present, the incidence of ESCA is low in Western countries but high in China. There is a lack of effective techniques for early diagnosis and treatment of ESCA patients. Several patients with ESCA visit the hospital due to eating obstructions. However, by then, the tumor has already reached the middle and advanced stages, therefore, the survival of such patients remains dismal even after surgical treatment. Hence, novel diagnostic techniques and therapies are needed to improve the prognosis and treatment of ESCA patients. Currently, targeted therapy in cancer has shown a great therapeutic value and is expected to improve the prognosis of cancer patients [3-6]. For example, the overall survival (OS) was significantly longer in patients with non-small cell lung cancer (NSCLC) treated with erlotinib than when treated with a placebo [3]. The PD-1/PD-L1 inhibitor improved OS and progression-free survival in North

Table 1. The clinical information of ESCA patients

Baseline	N	High APOC1 expression	Low APOC1 expression	Percentage (%)
Gender	40			
Male	31	27	4	77.5
Female	9	7	2	22.5
Age	40			
≤ 60	25	21	4	62.5
> 60	15	13	2	37.5
Tumor size	40			
T1-2	17	12	5	42.5
T3-4	23	22	1	57.5
Lymph node metastasis	40			
No	25	20	5	62.5
Yes	15	14	1	37.5
Tumor grade	40			
G1-2	30	27	3	75
G3	10	7	3	25

American and European cancer patients. Further, compared to European cancer patients, North American patients benefit more from the PD-1/PD-L1 inhibitors [4]. These results suggest that targeted therapy can potentially improve the prognosis of cancer patients.

Several studies have shown that elevated or inhibited gene expression can delay the progression of ESCA [7-9]. For example, importin 5 (IPO5) expression is significantly higher in tissues of patients with ESCA than in adjacent esophageal tissues. Increased IPO5 expression is associated with late pathological stage and short OS in ESCA patients. Interfering with IPO5 expression decreases ESCA cell proliferation and promotes MMP7 protein expression in ESCA cells. Thus, IPO5 might promote the malignant progression of ESCA by regulating MMP7 expression [7]. SIX homeobox 4 (SIX4) is significantly upregulated in esophageal squamous cell carcinoma (ESCC) tissues and associated with adverse clinical outcomes in ESCC patients. SIX4 knockdown inhibits the proliferation, migration and invasion of ESCC cells, enhances their ability to induce ESCC cell apoptosis, and inhibits epithelial to mesenchymal transition (EMT). Upregulation of SIX4 activates the PI3K/AKT signaling pathway in ESCC cells and promotes tumor growth *in vivo* [9]. Such proteins can act as novel prognostic biomarkers to track ESCA progression and predict the prognosis of patients with ESCA.

In recent years, apolipoprotein C1 (APOC1) has been associated with cancer progression [10-14]. In colorectal cancer (CRC) tissues, APOC1 is significantly overexpressed, and its overexpression is associated with lymph node metastasis, tumor-node-metastasis (TNM) stage, distant metastasis, and poor prognosis in CRC patients. APOC1 overexpression affects the growth and migration of CRC cells through the MAPK signaling pathway [10]. Further, it is an independent risk factor for the OS in CRC patients [10]. In addition, APOC1 concentration in the serum and tissues of patients with gastric cancer is significantly higher than that in the control group. Elevated APOC1 expression significantly correlates with the clinical stage, tumor classification, lymph node metastasis, and poor prognosis in gastric cancer patients and thus has diagnostic value in gastric cancer [11]. Moreover, APOC1 expression is significantly upregulated in renal cell carcinoma (ccRCC). High APOC1 levels are associated with poor survival in ccRCC patients, and APOC1 enhances ccRCC metastasis by promoting STAT3 activation [14]. However, to date, the roles and mechanisms of APOC1 in the development of ESCA have not been reported. Therefore, this study aimed to identify APOC1 expression in ESCA tissues using The Cancer Genome Atlas (TCGA) database and clinical tissues. We also explored the roles and potential mechanisms of APOC1 in ESCA by receiver operating characteristic (ROC) analysis, survival and correlation analysis, gene set enrichment analysis (GSEA), and cell experiments.

Materials and methods

Clinical ESCA tissues

From 2016 to 2021, cancer and adjacent tissues were acquired from 40 ESCA patients in the pathology department of our hospital. The clinical data of ESCA patients are detailed in **Table 1**. Our study was reviewed and approved by the ethics committee of Taihe Hospital. Immunohistochemistry revealed the expression of APOC1 in ESCA tissues. For immunohis-

tochemistry, we performed deparaffinization, antigen retrieval, and blocking according to routine methods, and the primary antibody concentration was 1:100 [15]. The APOC1 protein expression was calculated for normal and ESCA tissues. The results were analyzed using the staining index, which is a combination of the percentage of positive cells (staining range) and the intensity of staining. The staining index was calculated as the staining range multiplied by the staining intensity. The experiments and analyses were performed under double-blind conditions.

Gene expression of ESCA patient from TCGA database

In August 2021, gene expression data of fragments per kilo base of exon per million mapped (FPKM) types in pan-cancer and normal tissues, along with the prognostic data and clinicopathological characteristics of ESCA patients were downloaded from the TCGA database. Gene expression data from pan-cancer tissues were sorted and merged, and the expression of APOC1 in pan-cancer tissues, paired pan-cancer tissues, and normal tissues were calculated using the t-test.

Exploring the relationship between APOC1 expression and the clinicopathological features of ESCA

APOC1 gene expression data and clinicopathological characteristics of ESCA patients were merged. Patients with ESCA were grouped according to the clinicopathological features and the median expression value of APOC1. This grouping explored the relationship between the APOC1 expression and clinicopathological features of ESCA patients.

Diagnostic and prognostic values of APOC1 in ESCA

ROC analysis is often used to evaluate the diagnostic value of genes in cancer. If the area under the ROC curve is close to 1, the diagnostic value is high [15-17]. The diagnostic value of APOC1 in ESCA in normal and cancerous tissues were analyzed using ROC. The relationship between APOC1 expression and OS, disease-specific survival, and progression-free interval was explored in ESCA patients using the Kaplan-Meier survival analysis according to the best cut-off value.

Functions and mechanisms of APOC1 co-expressed genes

We performed a correlation analysis to explore the genes co-expressed with APOC1 in the tissues of ESCA patients obtained from the TCGA database. If the correlation coefficient was close to 1, the two genes were more related [18]. We considered a value of $P < 0.001$, and $r > 0.5$ or $r < -0.5$ of the correlation coefficients as significantly correlated. The functions and signaling mechanisms of the APOC1 co-expressed genes were also explored using gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Protein-protein interaction (PPI) network

The PPI network between APOC1 co-expressed genes was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. In the PPI network, a binding score > 0.4 was used as the screening criterion, and unconnected genes were removed. The PPI network was then imported into the Cytoscape software for network visualization. Enrichment analysis of APOC1 co-expressed genes in the PPI network was performed using the MCODE plugin [19, 20].

Gene set enrichment analysis (GSEA) analysis

Gene expression data of ESCA patients in TCGA database were grouped by the median value of APOC1 expression and were further divided into APOC1 high- and low-phenotype groups. In order to understand the underlying signaling mechanisms by which APOC1 might affect ESCA progression, the effects of APOC1 high- and low-phenotype groups on TCGA gene set were explored using KEGG analysis in GSEA (version: 4.1.0) software [21]. This cycle was performed 1000 times, and nominal (NOM) $P < 0.05$ was the screening criterion for potential signaling mechanisms.

Construction of APOC1 cell model

The ESCA cells were fed with 10% fetal bovine serum (FBS) complete DMEM media. An appropriate amount of ESCA cells was seeded in six-well plates. Transfection was per-

formed according to the manufacturer's instructions of GeneCopoeia (Guangzhou, China) for siRNA. The interfering sequences of APOC1 were 5'-GCAUCAAACAGAGUGAACUTT-3' (siRNA-1), and 5'-GCCGCAUCAAACAGAGU GATT-3' (siRNA-2), and the expression levels of APOC1 in ESCA cell models were tested by the standard PCR procedure [21].

Cell counting kit (CCK-8) assay

Cells from the control group (NC) and APOC1 inhibition group (si-APOC1) were plated on the 96-well plates, and 10 μl of the CCK-8 solution was added after cells adhered, and the time was recorded as 0 h. In addition, 10 μl of the CCK-8 solution was added at 24, 48, and 72 h of cell incubation. Furthermore, the cells were incubated for 2 h and the absorbance was detected.

Cell migration and invasion

Serum-containing complete medium was added to the lower chamber and serum-free cell suspension was added to the upper chamber. Both the chambers were incubated for 24 h. Post incubation, liquid from the upper chamber was discarded. Upper chamber cells on the membrane that had not passed through the membrane were wiped with a wet cotton swab. Crystal violet staining was performed, and after rinsing, slides were mounted, observed, and the cells were counted.

Analysis of the relationship between APOC1 expression and immune cell infiltration

The relationship between APOC1 expression and ESCA immune cell levels was verified using the Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) database [22, 23]. The ESTIMATE, immune and stromal scores, and immune cell levels in the tissues of ESCA patients from TCGA database were calculated using the ESTIMATE and Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) analysis methods. In the ESCA tissues, the association of APOC1 expression with the ESTIMATE, immune and stromal scores, and immune cell levels was explored using Pearson correlation analysis. In addition, the median value of APOC1 expression was divided into high- and low-expression groups. Further, the ESTIMATE, immune and stromal scores, and the immune cell levels in APOC1 high- and low-expression groups were investigated.

Analysis and identification of the relationship between APOC1 and immune cell markers

The relationship between APOC1 expression and ESCA immune infiltrating cell markers was examined using the correlation analysis module of the TIMER database. Under the conditions of tumor purity and non-purity, the relationship between APOC1 and immune cell marker levels was investigated, with $P < 0.05$ as the filter criterion. The relationship between significant cellular markers and APOC1 expression in the TIMER database was identified using the GEPIA database.

Statistical analysis

The expression of APOC1 in pan-cancerous tissues was explored using the t-test. The role of APOC1 in the diagnosis and prognosis of ESCA was analyzed by ROC and survival analysis. Co-expressed genes of APOC1 were screened using the correlation analysis to determine the relationship between APOC1 and the clinicopathological features and immune infiltrating cells of ESCA. Statistical significance was set at $P < 0.05$.

Results

APOC1 was significantly overexpressed in ESCA tissues

Compared to the unpaired normal tissues from the TCGA database, APOC1 was significantly overexpressed in bladder urothelial carcinoma, colon adenocarcinoma, ESCA, breast invasive carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, endocervical adenocarcinoma, kidney chromophobe, kidney renal papillary cell carcinoma, stomach adenocarcinoma, uterine corpus endometrial carcinoma, kidney renal clear cell carcinoma, and thyroid carcinoma tissues. Conversely, APOC1 expression significantly decreased in lung adenocarcinoma, cholangiocarcinoma, and lung squamous cell carcinoma tissues (**Figure 1A**). Compared to paired normal tissues, APOC1 was significantly overexpressed in the paired bladder urothelial carcinoma, colon adenocarcinoma, kidney renal clear cell carcinoma, kid-

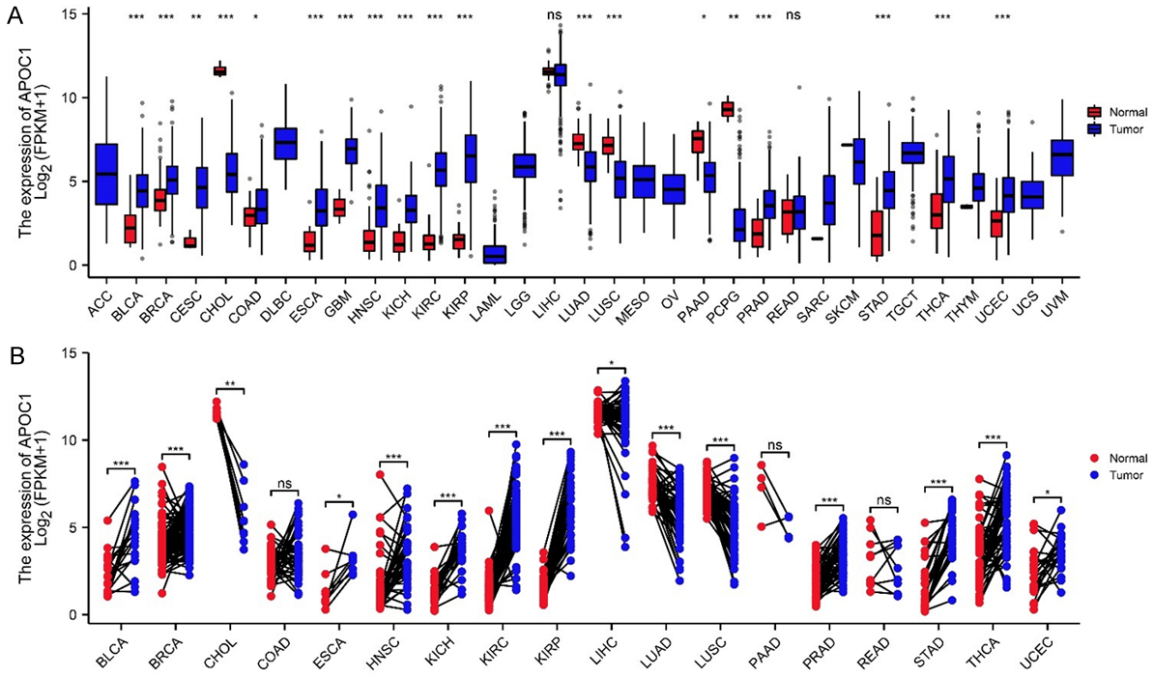


Figure 1. MRNA expression of APOC1 in pan-cancer tissues. A. Unpaired cancer tissues; B. Paired cancer tissues. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not statistically significant.

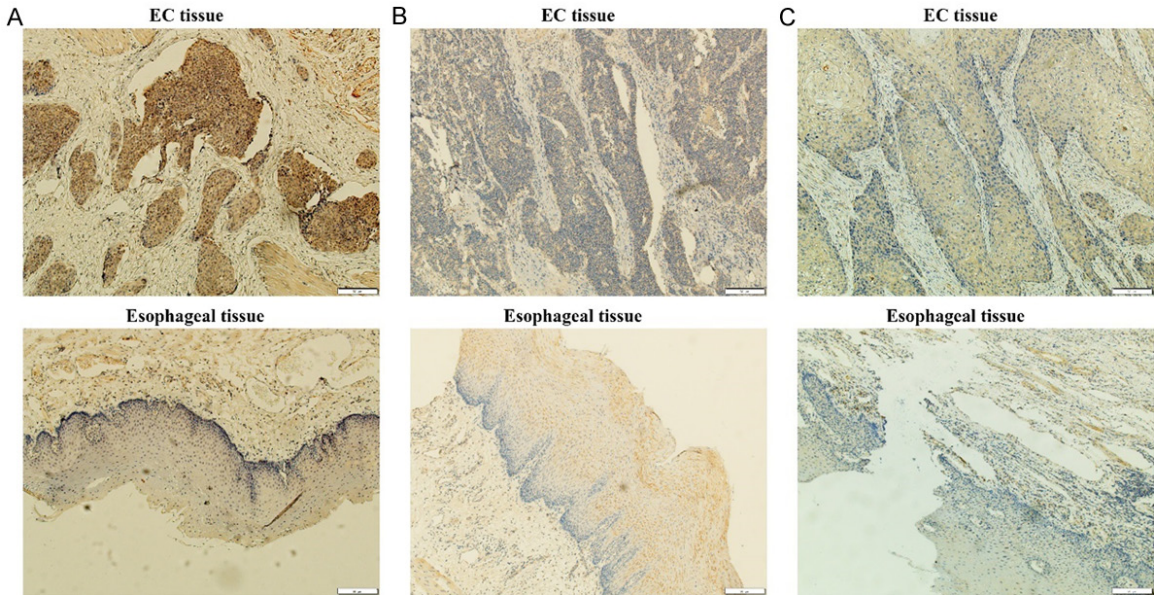


Figure 2. Protein expression of APOC1 in paired EC tissues. EC, Esophageal Cancer; ESCA, Esophageal Cancer.

ney renal papillary cell carcinoma, ESCA, head and neck squamous cell carcinoma, kidney chromophobe, prostate adenocarcinoma, stomach adenocarcinoma, thyroid carcinoma, breast invasive carcinoma, and uterine corpus endometrial carcinoma tissues; whereas APOC1 expression significantly decreased in the cholangiocarcinoma, hepatocellular carcinoma, lung adenocarcinoma, and lung squamous cell carcinoma tissues (**Figure 1B**). Among the 40 ESCA samples obtained from our hospital, APOC1 protein expression was significantly elevated in 34 (85%) patients, which was considered statistically significant (**Figure 2**).

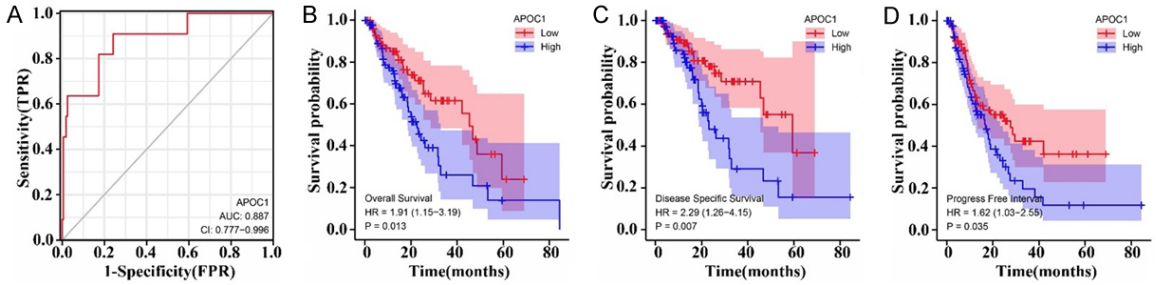


Figure 3. Clinical diagnostic and prognostic roles of APOC1 in ESCA. A. Diagnosis; B. OS; C. DSS; D. PFI. OS, Overall Survival; DSS, Disease-Specific Survival; PFI, Progress-Free Interval; ESCA, Esophageal Cancer.

APOC1 overexpression was associated with the diagnosis and dismal prognosis of ESCA

ROC analysis showed that the Area Under the ROC curve (AUC) of APOC1 was 0.887, which was the diagnostic indicative of ESCA (**Figure 3A**). Kaplan-Meier survival analysis showed a poor prognosis in ESCA patients with APOC1 overexpression (**Figure 3B-D**). APOC1 overexpression was significantly associated with a short OS, disease-specific survival, and progression-free interval in ESCA patients.

APOC1 overexpression was associated with the T stage, pathological stage, body mass index (BMI), and histological grade in ESCA patients

In ESCA tissues obtained from the TCGA database, we found that APOC1 expression was correlated with the T stage (which refers to the size and extent of the main tumor), pathological stage, BMI, and histological grade of ESCA patients grouped by the median expression of APOC1 (**Table 2**). Abnormal APOC1 expression in terms of BMI, T stage, and pathological stage was revealed by grouping according to clinicopathological features of ESCA (**Figure 4**). Logistic regression analysis showed that the expression of APOC1 was abnormal in the T stage (T3-4 versus T1-2), pathological stage (Stage III-IV versus Stage I-II), and BMI (> 25 versus ≤ 25) (**Table 3**).

Functional mechanisms and protein action network of APOC1 co-expressed genes

There were 229 APOC1 co-expressed genes that positively correlated with APOC1 (**Figure 5** and **Table 4**). The top 20 APOC1 positively correlated genes by fold-change were visualized using a heatmap (Figure S1). KEGG analysis showed that APOC1 co-expressed genes were involved in cell adhesion, Th1, Th2, and Th17 cell differentiation, antigen processing and presentation, natural killer (NK) cell-mediated cytotoxicity, T cell receptor signaling pathway, chemokine signaling pathway, primary immunodeficiency, cytokine-cytokine receptor interaction, B cell receptor signaling pathway, PD-L1 expression and PD-1 checkpoint pathway in cancer, Toll-like receptor signaling pathway; leukocyte trans endothelial migration, and others (**Table 5**). Gene ontology annotation analysis showed that APOC1 co-expressed genes were involved in the regulation of leukocyte, lymphocyte, and T-cell activation, positive regulation of cell and leukocyte activation, regulation of lymphocyte and mononuclear cell proliferation, leukocyte cell-cell adhesion, immune response-activating cell surface receptor signaling pathway, leukocyte differentiation, T-cell proliferation, and other functions (Table S1). **Figure 6A** shows the PPI network between genes positively related to APOC1 and the results of enrichment analysis of genes positively associated with APOC1 using the MCODE plugin (**Figure 6B-D**).

Downregulation of APOC1 expression reduced cell growth and metastasis of ESCA

Figure 7A and **7B** show a successful construction of cell models intended to suppress APOC1 expression. Inhibition of APOC1 expression reduced the proliferation of ESCA EC109 and TE-1 cells as analyzed by CCK-8 (**Figure 7C**). Compared with EC109 and TE-1 cells in the control groups, migration and invasion ability of EC109 and TE-1 cells in the downregulation of APOC1 expression groups decreased significantly, and had significant statistical sig-

Table 2. Association of APOC1 overexpression with the clinicopathological features in ESCA patients

Characteristic	Low APOC1 expression	High APOC1 expression	P
T stage			0.012
T1	18 (12.4%)	9 (6.2%)	
T2	21 (14.5%)	16 (11%)	
T3	31 (21.4%)	46 (31.7%)	
T4	0 (0%)	4 (2.8%)	
N stage			0.424
N0	37 (25.7%)	29 (20.1%)	
N1	29 (20.1%)	34 (23.6%)	
N2	3 (2.1%)	6 (4.2%)	
N3	2 (1.4%)	4 (2.8%)	
M stage			1.000
M0	57 (44.2%)	64 (49.6%)	
M1	4 (3.1%)	4 (3.1%)	
Pathologic stage			0.005
Stage I	13 (9.2%)	3 (2.1%)	
Stage II	36 (25.4%)	33 (23.2%)	
Stage III	16 (11.3%)	33 (23.2%)	
Stage IV	4 (2.8%)	4 (2.8%)	
Radiation therapy			0.703
No	55 (38.2%)	52 (36.1%)	
Yes	17 (11.8%)	20 (13.9%)	
Primary therapy outcome			0.175
PD	7 (7.4%)	3 (3.2%)	
SD	3 (3.2%)	4 (4.3%)	
PR	0 (0%)	3 (3.2%)	
CR	42 (44.7%)	32 (34%)	
Gender			0.177
Female	15 (9.3%)	8 (4.9%)	
Male	66 (40.7%)	73 (45.1%)	
Race			0.787
Asian	18 (12.5%)	20 (13.9%)	
Black or African American	4 (2.8%)	2 (1.4%)	
White	49 (34%)	51 (35.4%)	
Age			0.346
≤ 60	45 (27.8%)	38 (23.5%)	
> 60	36 (22.2%)	43 (26.5%)	
Weight			1.000
≤ 70	38 (23.8%)	38 (23.8%)	
> 70	43 (26.9%)	41 (25.6%)	
Height			1.000
< 170	24 (15.7%)	23 (15%)	
≥ 170	54 (35.3%)	52 (34%)	
BMI			0.040
≤ 25	36 (23.5%)	48 (31.4%)	
> 25	42 (27.5%)	27 (17.6%)	

nificance (**Figures 7D-G** and **8A-D**). Preliminary results showed that APOC1 could play a biological role as a carcinogen gene, and down-regulation of APOC1 expression could delay cancer cell progression in ESCA.

APOC1 was involved in the signaling mechanisms of ESCA progression

Grouped by the median value of APOC1 expression, cell adhesion molecules, hematopoietic cell lineage, antigen processing and presentation, NK cell-mediated cytotoxicity, graft versus host disease, cytokine-cytokine receptor interaction, primary immunodeficiency, ABC transporters, extracellular membrane receptor interaction, and several signaling pathways, including the T cell receptor, chemokine, B cell receptor, Toll-like receptor, JAK-STAT, and NOTCH pathways, were significantly enriched in the APOC1 overexpression group by GSEA analysis (Figure S2 and **Table 6**).

APOC1 was associated with ESCA immune cell infiltration

In the TIMER database, Pearson correlation analysis revealed a significant correlation of APOC1 expression with the tumor purity, B cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells (DCs) (Figure S3). Among ESCA tissues from TCGA database, APOC1 expression positively correlated with the ESTIMATE, immune, and stromal scores in ESCA using Pearson correlation analysis (**Figure 9A-C**). Furthermore, APOC1 expression significantly correlated with B cells, eosinophils, pDC, T helper cells, NK cells, Tem, TFH, DC, NK CD56dim cells, $CD8^+$ T cells, aDC, Th1 cells, T cells, TReg, cytotoxic cells, iDC, macrophages, and others in ESCA (**Figures 9D-P** and S4). The ESTIMATE, immune, and stromal scores were abnormal in the high- and low-APOC1 expression groups as grouped by the median value of APOC1 expression (Figure S5). Further, T cells, T helper cells, Tem, TReg,

Histological type			0.157
Adenocarcinoma	35 (21.6%)	45 (27.8%)	
Squamous Cell Carcinoma	46 (28.4%)	36 (22.2%)	
Residual tumor			0.764
R0	62 (46.3%)	59 (44%)	
R1	4 (3%)	7 (5.2%)	
R2	1 (0.7%)	1 (0.7%)	
Histologic grade			0.027
G1	11 (8.7%)	5 (4%)	
G2	36 (28.6%)	30 (23.8%)	
G3	15 (11.9%)	29 (23%)	
Smoker			0.552
No	21 (14.6%)	26 (18.1%)	
Yes	50 (34.7%)	47 (32.6%)	
Alcohol history			0.635
No	21 (13.2%)	25 (15.7%)	
Yes	58 (36.5%)	55 (34.6%)	
Barretts esophagus			0.511
No	51 (38.6%)	55 (41.7%)	
Yes	15 (11.4%)	11 (8.3%)	
Reflux history			0.967
No	42 (30.9%)	42 (30.9%)	
Yes	25 (18.4%)	27 (19.9%)	
Tumor cental location			0.168
Distal	55 (34.2%)	58 (36%)	
Mid	24 (14.9%)	18 (11.2%)	
Proximal	1 (0.6%)	5 (3.1%)	
Columnar mucosa dysplasia			0.363
High grade dysplasia	14 (20.6%)	11 (16.2%)	
Low grade dysplasia	1 (1.5%)	4 (5.9%)	
Negative/no dysplasia	21 (30.9%)	17 (25%)	
Columnar metaplasia			0.543
No	39 (39.8%)	31 (31.6%)	
Yes	13 (13.3%)	15 (15.3%)	
OS event			0.200
Alive	53 (32.7%)	44 (27.2%)	
Dead	28 (17.3%)	37 (22.8%)	
DSS event			0.105
Alive	63 (39.1%)	52 (32.3%)	
Dead	18 (11.2%)	28 (17.4%)	
PFI event			0.209
Alive	46 (28.4%)	37 (22.8%)	
Dead	35 (21.6%)	44 (27.2%)	

ESCA, Esophageal Cancer.

TFH, pDC, Th1, aDC, B cells, NK cells, $CD8^+$ T cells, cytotoxic cells, DCs, eosinophils, and other immune cells were abnormally expressed in the high- and low-APOC1 expression groups (**Figure 10**).

APOC1 overexpression is correlated with the ESCA immune infiltrating cell markers

In the correlation analysis module of the TIMER database, expression of APOC1 was associated with the levels of CD8A, IFNG, CD3D, FOXP3, CD3E, CD2, HLA-DRA, CD19, CD163, CD8B, VSIG4, STAT5A, MS4A4A, ITGAM, HLA-DPB1, PDCD1, HLA-DQB1, CD1C, ITGAX, NRP1, TBX21, STAT1, GATA3, CCR7, CCR8, STAT5B, STAT4, CTLA4, LAG3, HLA-DPA1, HAVCR2, and GZMB, regardless of the tumor purity (**Figure 11** and **Table 7**). The relationship between most of the immune cell markers and APOC1 expression was confirmed using the GEPIA database (**Figures 12** and S6).

Discussion

ESCA is a highly incident malignant tumor that severely affects the long-term quality of life of cancer patients. Therefore, it is crucial to develop new treatment modalities to improve the quality of life and long-term prognosis of ESCA patients. Studies have found that APOC1, a member of the apolipoprotein family, is critical for the progression of several cancers. Thus, inhibiting the expression of APOC1 is expected to delay cancer progression and improve the prognosis of cancer in patients, thereby serving as a potential target for cancer therapy [10-14, 24-27]. For example, APOC1 overexpression in breast cancer tissues is associated with the late TNM stage and lymph node metastasis. Thus, APOC1 enhances the proliferation, invasion, and migration of MDA-MB-231 and MCF-7 breast cancer cells *in vitro* [24]. Likewise, as discussed earlier, APOC1 is highly expressed in CRC tissues, and its downregulation inhibits CRC cell growth and migration, induces cell cycle arrest, and increases apoptosis [10, 13]. Ren et al. preliminarily reported that AOPC1 was overexpressed in ESCA tissues. Overexpression of APOC1 was related to the OS of ESCA patients, and not verified by clinical tis-

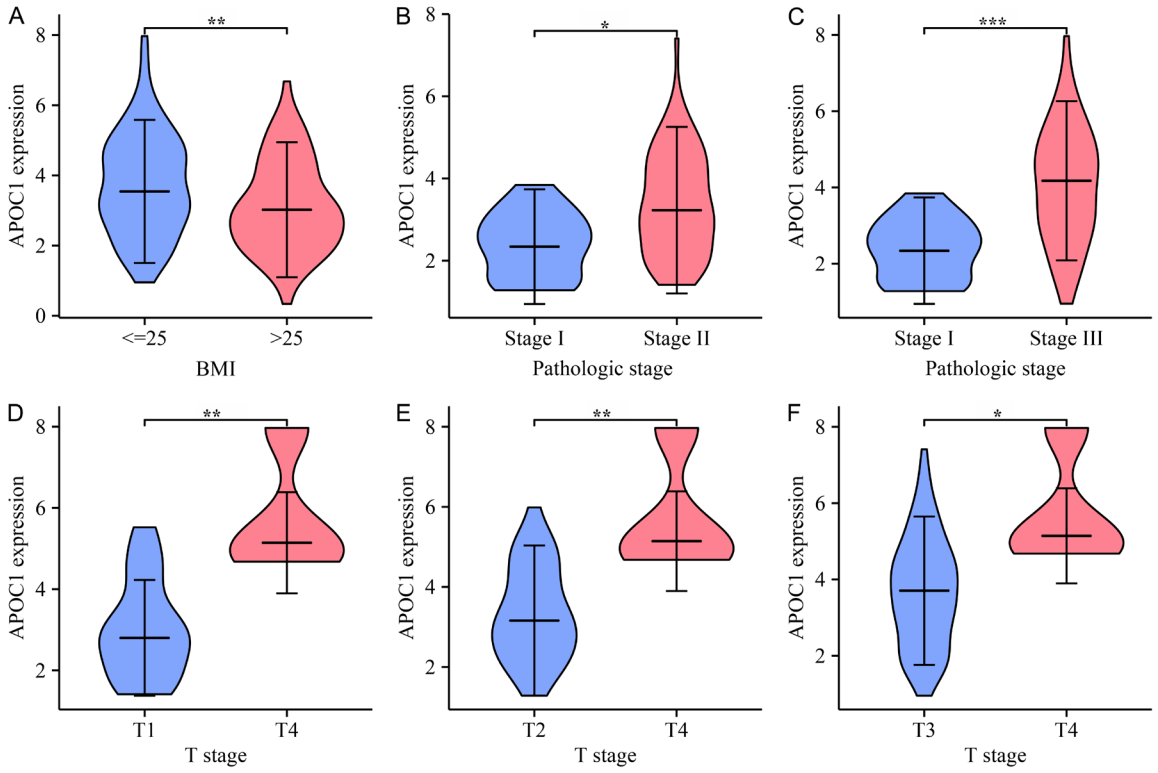


Figure 4. Association of APOC1 overexpression with the BMI, pathological stage, and T stage in ESCA patients. A. BMI; B. Stage I vs II; C. Stage I vs III; D. T1 vs T4; E. T2 vs T4; F. T3 vs T4. ESCA, Esophageal Cancer; BMI, Body Mass Index; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3. Association of APOC1 overexpression associated with the clinicopathological features in ESCA patients using logistics regression analysis

Characteristics	Total (N)	OR	P
T stage (T3-T4 vs T1-2)	145	2.516 (1.293-4.983)	0.007
N stage (N1-3 vs N0)	144	1.651 (0.855-3.217)	0.137
M stage (M1 vs M0)	129	0.891 (0.202-3.923)	0.874
Pathologic stage (Stage III-IV vs Stage I-II)	142	2.518 (1.270-5.105)	0.009
Radiation therapy (Yes vs No)	144	1.244 (0.588-2.655)	0.568
Gender (Male vs Female)	162	2.074 (0.844-5.445)	0.120
Age (> 60 vs ≤ 60)	162	1.414 (0.763-2.636)	0.272
BMI (> 25 vs ≤ 25)	153	0.482 (0.250-0.917)	0.027
Weight (> 70 vs ≤ 70)	160	0.953 (0.512-1.775)	0.880
Height (≥ 170 vs < 170)	153	1.005 (0.505-2.004)	0.989
Smoker (Yes vs No)	144	0.759 (0.375-1.525)	0.440
Histologic grade (G2-3 vs G1)	126	2.545 (0.864-8.527)	0.103
Histological type (SCC vs Adenocarcinoma)	162	0.609 (0.325-1.129)	0.117

SCC, Squamous Cell Carcinoma; OR, Odds Ratio.

sue and cells [27]. Similarly, APOC1 was overexpressed in unpaired and paired ESCA tissues in our study using bioinformatics analysis and clinical tissues, and its overexpression was not conducive to the OS, DSS, and PFI of patients with ESCA. In addition, we found that APOC1 overexpression was also significantly correlated with the T stage, pathological stage, histo-

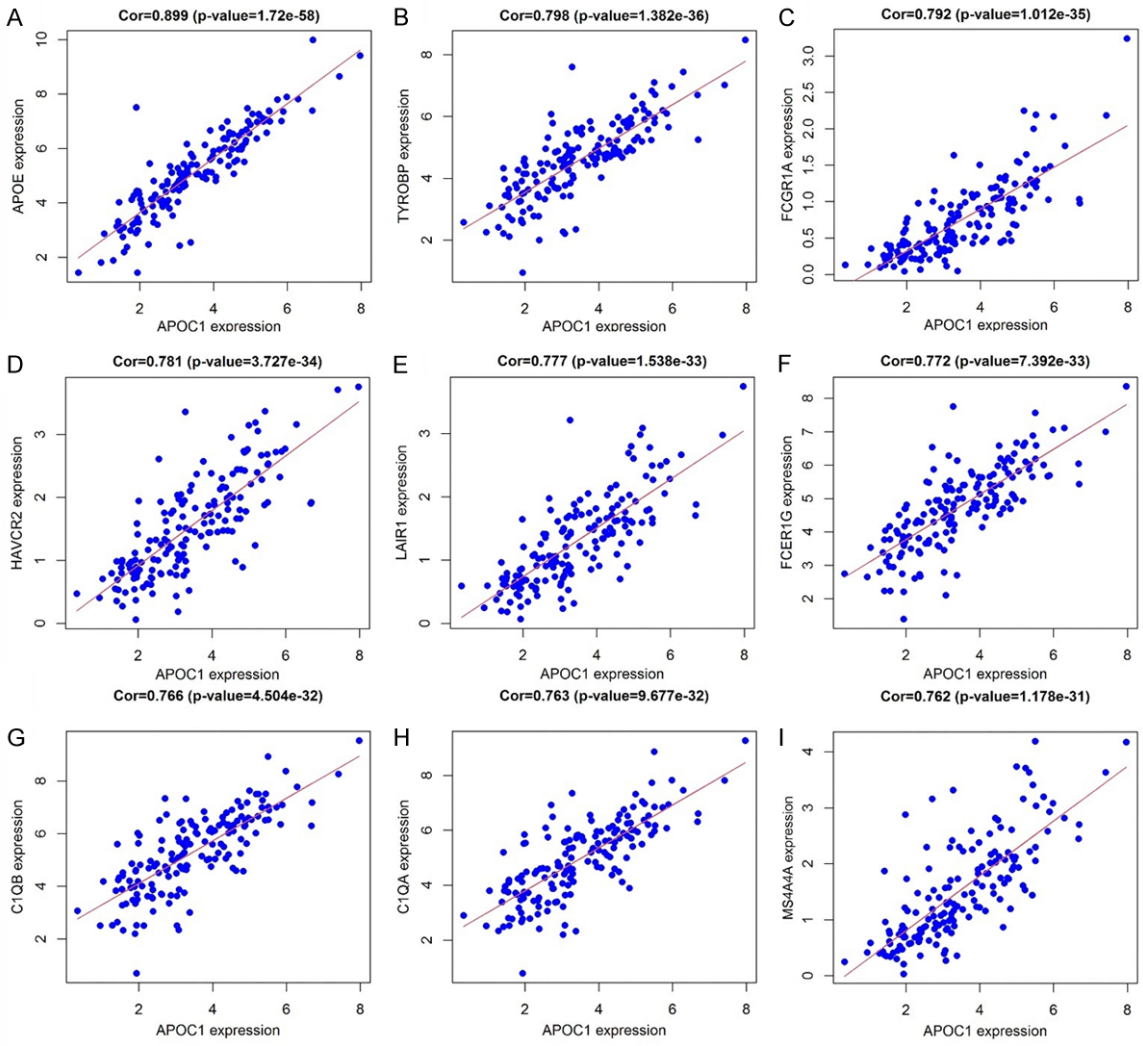


Figure 5. APOC1 co-expressed genes in ESCA tissues. A. APOE; B. TYROBP; C. FCGR1A; D. HAVCR2; E. LAIR1; F. FCER1G; G. C1QB; H. C1QA; I. MS4A4A. ESCA, Esophageal Cancer.

Table 4. APOC1 co-expressed genes in ESCA tissues

Gene	cor	Gene	cor	Gene	cor	Gene
LY86	0.618	C3AR1	0.718	LAIR1	0.777	GAB3
TMIGD3	0.687	CXCL9	0.519	FOLR2	0.563	HLA-DRA
SLA	0.604	LAT2	0.517	LPL	0.513	ARHGAP9
ACP5	0.59	LILRB2	0.587	CCL5	0.504	VSIG4
VAV1	0.501	FGR	0.535	CD163	0.63	CD74
FMNL1	0.571	SLAMF8	0.737	GGT5	0.515	CD96
MNDA	0.681	LCP2	0.64	TREM2	0.649	SPN
C1QA	0.763	TFEC	0.664	FPR3	0.723	SIGLEC1
GIMAP7	0.518	CD86	0.667	CYTIP	0.537	CD72
RNASE6	0.696	ABI3	0.648	CMKLR1	0.632	APBB1IP
C1orf54	0.693	CCL4	0.575	LRRC25	0.724	ADA2
LST1	0.686	OSCAR	0.641	PDCD1	0.526	WAS
PLD3	0.505	MILR1	0.584	CD53	0.705	HCLS1

HK3	0.685	CD52	0.674	LAPTM5	0.746	HLA-DPB1
SPI1	0.754	EVI2B	0.626	IL2RB	0.516	ITGAX
GPR183	0.569	MPP1	0.527	SH2D1A	0.539	NCF4
BTK	0.614	EVI2A	0.699	PLEKHO2	0.543	HCK
SAMSN1	0.623	ITGAM	0.533	HLA-DQA2	0.512	APOE
PYHIN1	0.567	APOC1	1	ARHGAP25	0.55	MS4A6A
DOK2	0.696	DOCK2	0.586	CXCR6	0.533	GZMH
RGS18	0.606	HAVCR2	0.781	C5AR1	0.605	SERPING1
FABP3	0.532	LY96	0.593	GPSM3	0.604	ICOS
LYL1	0.526	CD300A	0.659	NFAM1	0.585	CSF1R
STAT4	0.589	RASSF4	0.541	NCKAP1L	0.649	NRROS
TNFSF12	0.507	ZEB2	0.512	NR1H3	0.539	TMEM273
SIGLEC9	0.683	LILRB3	0.581	CCR1	0.665	MRC1
CSF2RA	0.508	CORO1A	0.513	P2RY13	0.502	CD2
SASH3	0.555	MYO1F	0.654	C1orf162	0.723	MMP19
EBI3	0.568	THEMIS2	0.559	GPR34	0.614	CD48
GZMK	0.514	ARHGEF6	0.501	DAB2	0.55	LIPA
FOXP3	0.554	CYTH4	0.611	FLI1	0.504	P2RX7
TNFAIP8L2	0.689	BIN2	0.601	GPR171	0.535	ARHGAP45
IL2RA	0.518	PTPRC	0.572	WIPF1	0.534	LCP1
TNFSF13B	0.621	CLEC4A	0.571	CXorf21	0.599	SDS
IFI30	0.657	RENBP	0.553	C1QC	0.751	HLA-DMB
IL12RB1	0.507	CD3E	0.561	GIMAP4	0.66	PSTPIP1
SEPT6	0.515	CD4	0.725	HLA-DPA1	0.521	RASAL3
LSP1	0.509	PLEK	0.538	CCL18	0.53	OLFML3
CD3G	0.526	HLA-DQA1	0.591	PLXNC1	0.574	CCR5
FCER1G	0.772	CD8B	0.536	MS4A7	0.742	CTSW
GPR65	0.592	LILRB4	0.735	CTSL	0.547	CYBB
KLHL6	0.502	IFFO1	0.526	SNX20	0.533	HSD17B14
CD247	0.524	SELPLG	0.573	VAMP5	0.552	CD300C
ITGB2	0.725	PILRA	0.632	CTLA4	0.516	CALHM6
C1QB	0.766	IGSF6	0.729	TNFRSF4	0.567	CD3D
PIK3R6	0.594	SRGN	0.629	SCIMP	0.574	IL4I1
PIK3R5	0.579	TIGIT	0.582	LPXN	0.533	MSR1
RGS1	0.576	SIGLEC10	0.633	FCGR3A	0.76	CD37
LILRB1	0.627	SEPT4	0.521	IGFLR1	0.505	FCGR2A
SIT1	0.511	FCGR1A	0.792	PLEKHO1	0.516	ITGAL
SLA2	0.521	OLR1	0.596	PLCB2	0.591	ARHGDIB
GLIPR2	0.561	CD8A	0.54	ADAMDEC1	0.66	CD84
AIF1	0.747	DOCK10	0.519	CD14	0.636	TYROBP
CD300LF	0.706	DOK3	0.556	SIRPG	0.553	GMFG
NKG7	0.596	MPEG1	0.568	GNGT2	0.659	GIMAP1
PARVG	0.69	SLC15A3	0.538	IL10RA	0.624	MS4A4A
HCST	0.711	APOBR	0.539	PLA2G7	0.719	LAG3
GIMAP6	0.502					

logical grade, and BMI in ESCA patients. These observations suggest that APOC1 could be used as a promising biomarker for the prognosis and diagnosis of ESCA.

Studies have shown that APOC1 is involved in cancer progression via EMT, MAPK/JNK, STAT3, WNT3A, and other signaling pathways [10-14, 24-27]. Specifically, APOC1 reduces E-cadherin

Table 5. Signaling pathways of APOC1 co-expressed genes using KEGG analysis

ID	Description	FDR
hsa04640	Hematopoietic cell lineage	1.48E-14
hsa05150	Staphylococcus aureus infection	8.46E-14
hsa04514	Cell adhesion molecules (CAMs)	1.27E-13
hsa04145	Phagosome	2.50E-10
hsa04658	Th1 and Th2 cell differentiation	8.16E-10
hsa04380	Osteoclast differentiation	8.84E-10
hsa05152	Tuberculosis	2.63E-09
hsa04659	Th17 cell differentiation	4.75E-09
hsa05140	Leishmaniasis	5.60E-09
hsa05323	Rheumatoid arthritis	5.94E-09
hsa04612	Antigen processing and presentation	8.14E-08
hsa04660	T cell receptor signaling pathway	2.26E-07
hsa04062	Chemokine signaling pathway	1.16E-06
hsa04672	Intestinal immune network for IgA production	1.16E-06
hsa05340	Primary immunodeficiency	1.89E-06
hsa05322	Systemic lupus erythematosus	3.27E-06
hsa05416	Viral myocarditis	5.80E-06
hsa05310	Asthma	5.80E-06
hsa04610	Complement and coagulation cascades	6.24E-06
hsa05321	Inflammatory bowel disease (IBD)	1.01E-05
hsa05320	Autoimmune thyroid disease	1.93E-05
hsa05145	Toxoplasmosis	1.93E-05
hsa05330	Allograft rejection	1.98E-05
hsa05166	Human T-cell leukemia virus 1 infection	2.55E-05
hsa05332	Graft-versus-host disease	3.11E-05
hsa04061	Viral protein interaction with cytokine and cytokine receptor	4.01E-05
hsa04940	Type I diabetes mellitus	4.01E-05
hsa04060	Cytokine-cytokine receptor interaction	4.72E-05
hsa04650	Natural killer cell mediated cytotoxicity	6.76E-05
hsa05133	Pertussis	0.000213265
hsa05142	Chagas disease	0.000276609
hsa05169	Epstein-Barr virus infection	0.00072356
hsa04666	Fc gamma R-mediated phagocytosis	0.000824484
hsa04662	B cell receptor signaling pathway	0.00218021
hsa04611	Platelet activation	0.005440719
hsa05020	Prion diseases	0.012736279
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	0.017381189
hsa05202	Transcriptional misregulation in cancer	0.018176628
hsa05221	Acute myeloid leukemia	0.023013489
hsa04620	Toll-like receptor signaling pathway	0.034245002
hsa04979	Cholesterol metabolism	0.040539463
hsa04670	Leukocyte transendothelial migration	0.046144849

expression and promotes vimentin expression in MDA-MB-231 and MCF-7 breast cancer cell lines. APOC1 is also involved in breast cancer progression through the regulation of the JNK/MAPK signaling mechanism [24]. Downregulation of APOC1 expression reduces the protein expression of N-cadherin, vimentin, Twist, Slug, Snail, and CD44 in cervical cancer cells and

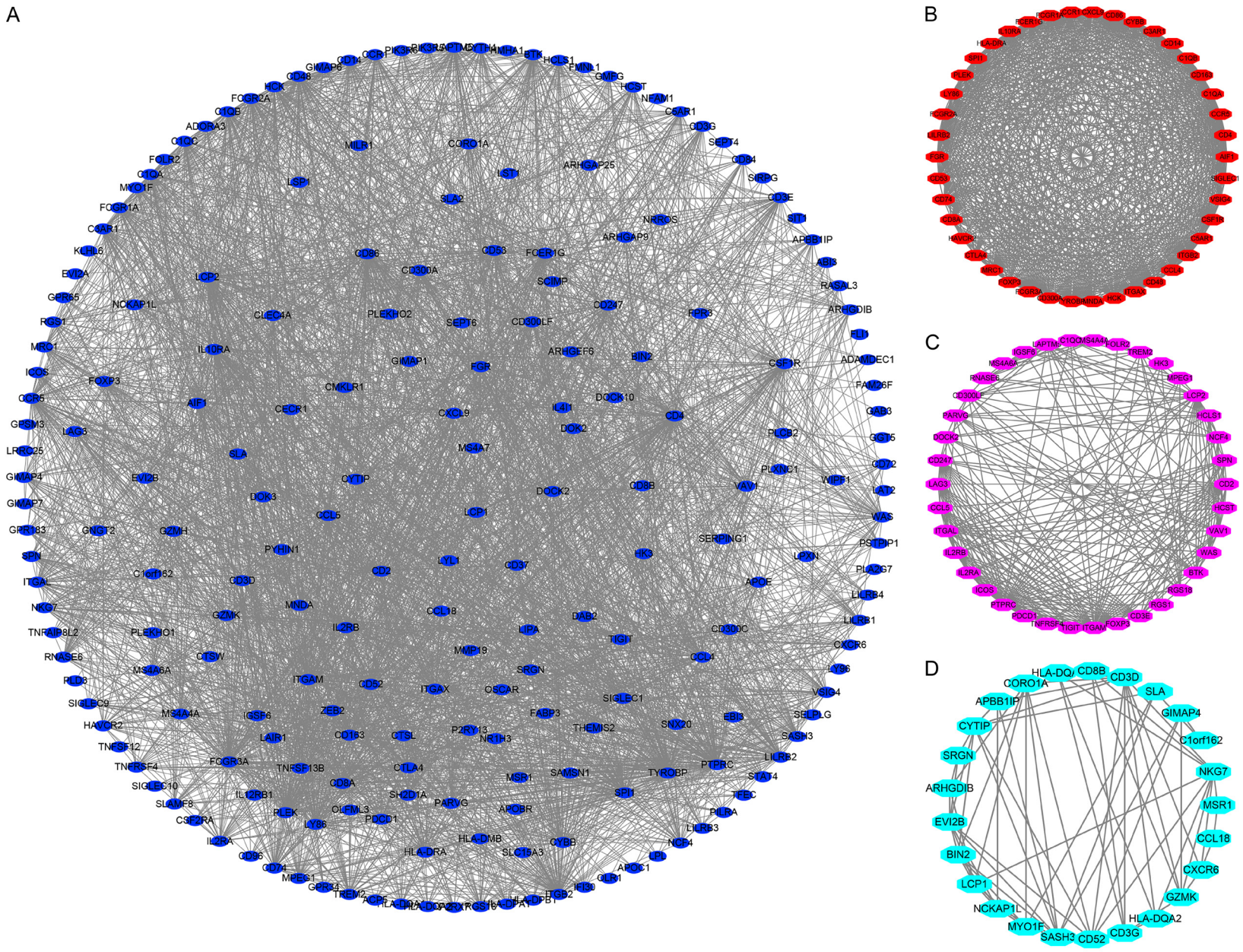


Figure 6. PPI network of APOC1 co-expressed genes. A. PPI network; B-D. Enriched network using MCODE method. PPI, Protein-Protein Interaction.

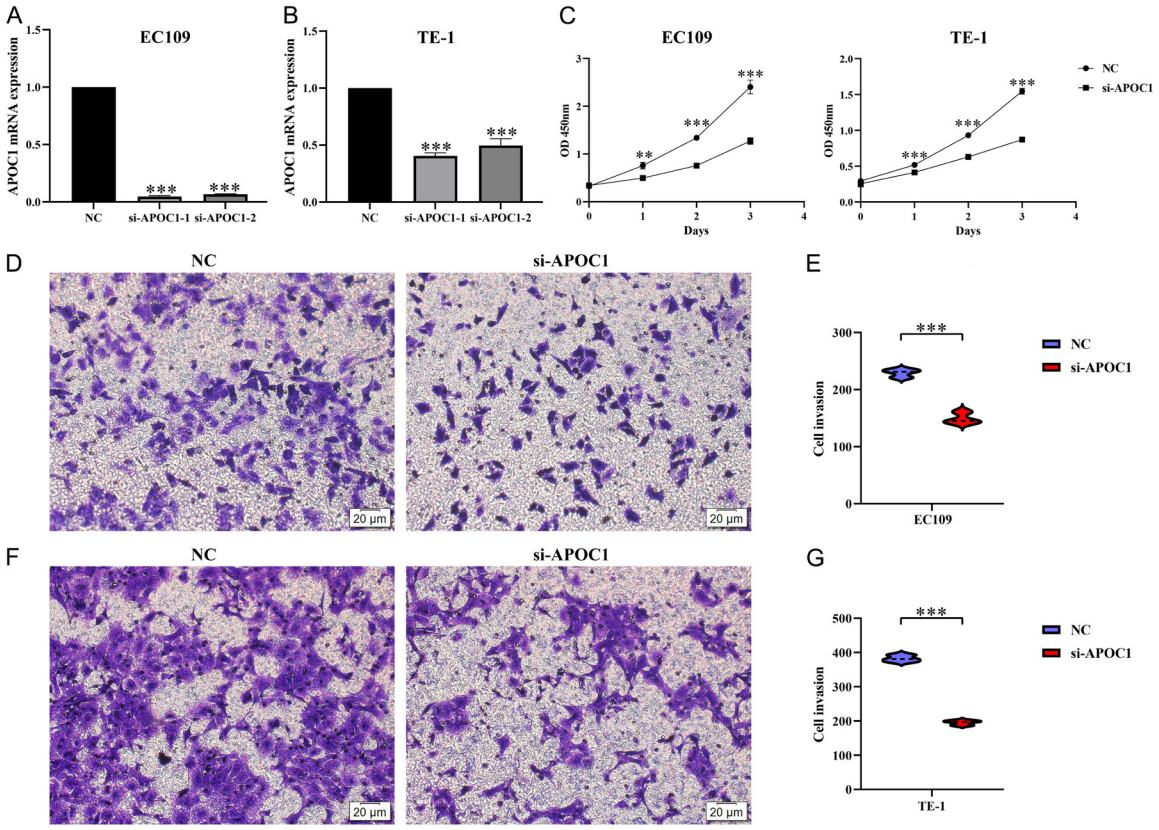


Figure 7. Downregulation of APOC1 expression inhibited ESCA cell proliferation, and invasion. A, B. Cell model; C. Cell proliferation using CCK-8; D-G. Cell invasion using Transwell. ESCA, Esophageal Cancer; **, P < 0.01; ***, P < 0.001.

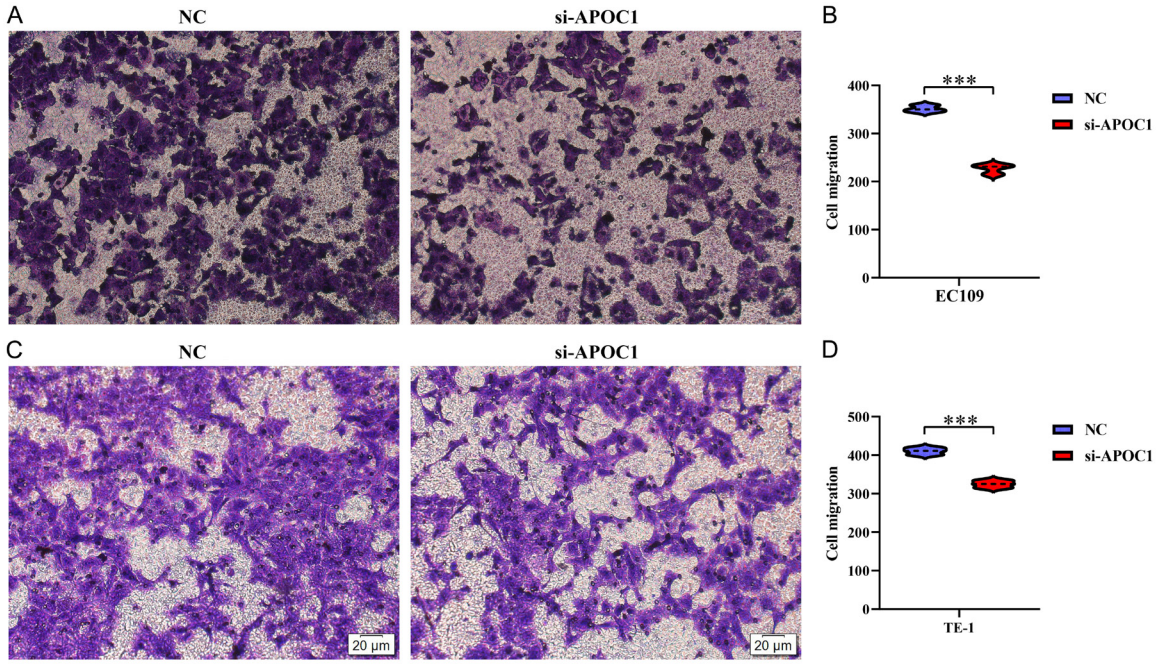


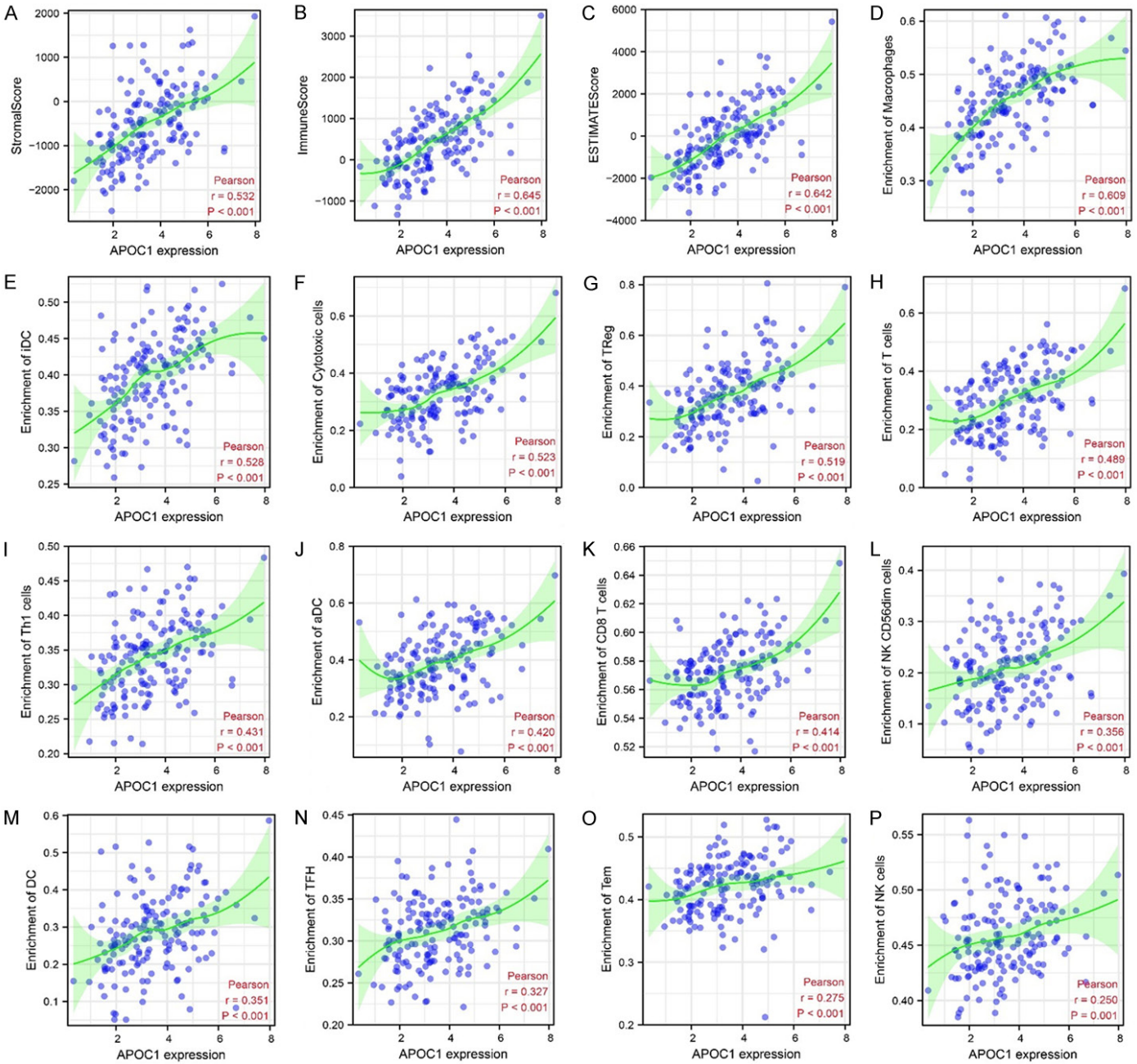
Figure 8. Downregulation of APOC1 expression inhibited ESCA cell migration. ESCA, Esophageal Cancer; ***, P < 0.001.

Table 6. Signaling mechanisms involved in APOC1 overexpression using GSEA analysis

Name	Size	NES	NOM p
Cell adhesion molecules cams	131	2.2515292	0
Hematopoietic cell lineage	85	2.2381618	0
Leishmania infection	69	2.2281723	0
Antigen processing and presentation	80	2.2142258	0
Systemic lupus erythematosus	54	2.1937685	0
Viral myocarditis	68	2.1700046	0
Lysosome	121	2.165117	0
Natural killer cell mediated cytotoxicity	131	2.1341667	0
Intestinal immune network for iga production	46	2.1176693	0
Graft versus host disease	37	2.0940528	0
Type i diabetes mellitus	41	2.0691876	0
Cytokine-cytokine receptor interaction	263	2.0405216	0
Leukocyte transendothelial migration	116	2.0341792	0
T cell receptor signaling pathway	108	2.0321288	0
Asthma	28	2.0263588	0
Allograft rejection	35	2.0152922	0
Chemokine signaling pathway	187	1.9651372	0
Autoimmune thyroid disease	50	1.9487576	0
Primary immunodeficiency	35	1.8914527	0.004106776
Toll like receptor signaling pathway	102	1.7821109	0.006198347
Complement and coagulation cascades	69	1.7474798	0.005870841
ECM receptor interaction	84	1.7368731	0.026748972
ABC transporters	44	1.7211503	0.00984252
JAK stat signaling pathway	155	1.7083862	0.012244898
Fc gamma r mediated phagocytosis	95	1.6750118	0.015841585
B cell receptor signaling pathway	75	1.6455605	0.032
Hypertrophic cardiomyopathy hcm	83	1.6157279	0.030991735
Glycosaminoglycan degradation	21	1.5970777	0.045908183
Renin angiotensin system	17	1.5964756	0.029880479
Neuroactive ligand receptor interaction	271	1.5705323	0.010309278
Fc epsilon ri signaling pathway	79	1.550188	0.01953125
Regulation of actin cytoskeleton	212	1.5455457	0.022727273
Notch signaling pathway	47	1.4989547	0.04496788

Note: GSEA, Gene Set Enrichment Analysis.

increases the protein expression of E-cadherin, facilitating its participation in the EMT process [12]. Furthermore, APOC1 overexpression is positively correlated with the progression of ccRCC. The EMT mediated metastasis of ccRCC is promoted by APOC1, whereas its downregulation inhibits EMT. Moreover, APOC1, a novel pro-transfer factor, activates STAT3 to enhance the metastasis of ccRCC cells [14]. In our study, APOC1 overexpression was involved in the T cell receptor, chemokine, Toll-like receptor, B cell receptor, JAK-STAT, and NOTCH signaling pathways and the pathways involving ABC transporters. Furthermore, STAT3 is one of the hub members of the JAK-STAT signaling pathway, which indicates that APOC1 might be involved in the JAK-STAT pathway to regulate ESCA progression. The T cell receptor, chemokine, Toll-like receptor, ABC transporters, B cell receptor, and NOTCH signaling pathways play crucial roles in cancer progression [28-33]. In our cell models, we confirmed for the first time that inhibition of APOC1 expression significantly reduced proliferation, migration, and inva-


A
StromalScore
APOC1 expression
Pearson
r = 0.532
P < 0.001
B
ImmuneScore
APOC1 expression
Pearson
r = 0.645
P < 0.001
C
ESTIMATEScore
APOC1 expression
Pearson
r = 0.642
P < 0.001
D
Enrichment of Macrophages
APOC1 expression
Pearson
r = 0.609
P < 0.001
E
Enrichment of iDC
APOC1 expression
Pearson
r = 0.528
P < 0.001
F
Enrichment of Cytotoxic cells
APOC1 expression
Pearson
r = 0.523
P < 0.001
G
Enrichment of TReg
APOC1 expression
Pearson
r = 0.519
P < 0.001
H
Enrichment of T cells
APOC1 expression
Pearson
r = 0.489
P < 0.001
I
Enrichment of Th1 cells
APOC1 expression
Pearson
r = 0.431
P < 0.001
J
Enrichment of aDC
APOC1 expression
Pearson
r = 0.420
P < 0.001
K
Enrichment of CD8 T cells
APOC1 expression
Pearson
r = 0.414
P < 0.001
L
Enrichment of NK CD56dim cells
APOC1 expression
Pearson
r = 0.356
P < 0.001
M
Enrichment of DC
APOC1 expression
Pearson
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P < 0.001
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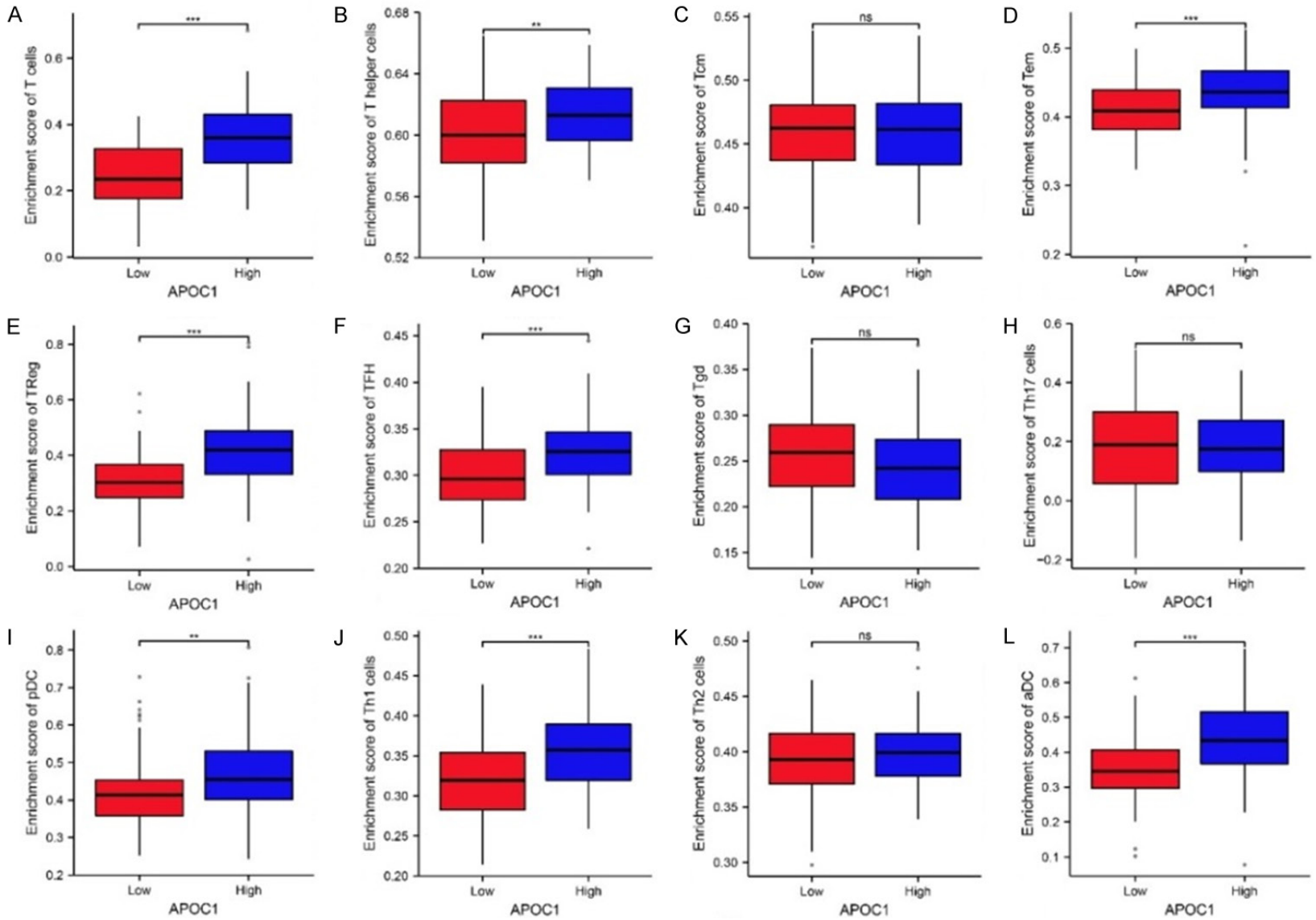
APOC1 expression
Pearson
r = 0.327
P < 0.001
O
Enrichment of Tem
APOC1 expression
Pearson
r = 0.275
P < 0.001
P
Enrichment of NK cells
APOC1 expression
Pearson
r = 0.250
P = 0.001

Figure 9. Correlation of APOC1 expression with the immune cells in ESCA. A. Stromal score; B. Immune score; C. ESTIMATE score; D. Macrophages; E. iDC; F. Cytotoxic cells; G. TReg; H. T cells; I. Th1 cells; J. aDC; K. CD8 T cells; L. NK CD56dim cells; M. DC; N. TFH; O. Tem; P. NK cells. ESCA, Esophageal Cancer.



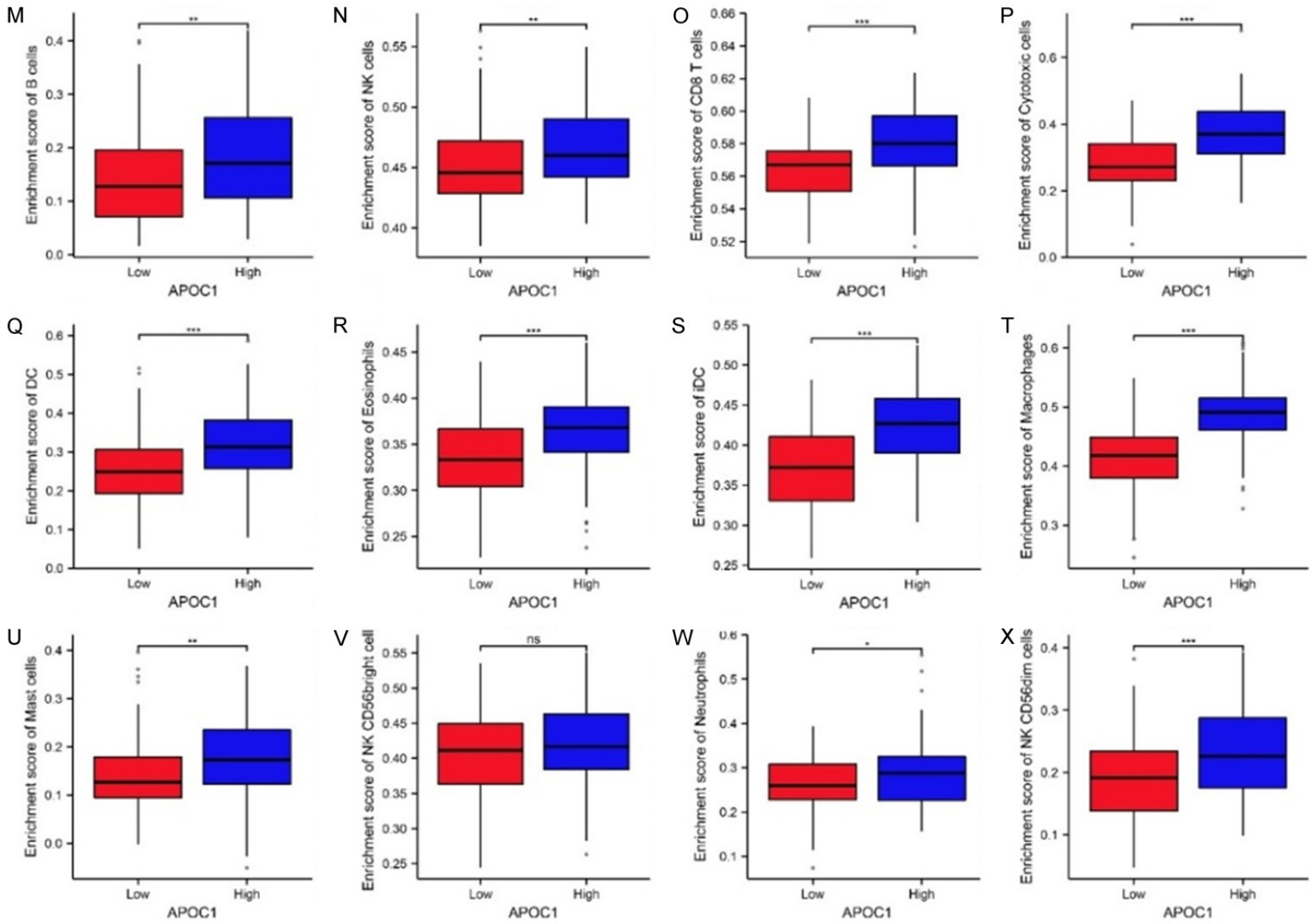


Figure 10. Correlation of APOC1 expression with the immune infiltration in ESCA. A. T cells; B. T helper cells; C. Tcm; D. Tem; E. TReg; F. TFH; G. Tgd; H. Th17 cells; I. pDC; J. Th1 cells; K. Th2 cells; L. aDC; M. B cells; N. NK cells; O. CD8 T cells; P. Cytotoxic cells; Q. DC; R. Eosinophils; S. iDC; T. Macrophages; U. Mast cells; V. NK CD56bright cell; W. Neutrophils; X. NK CD56dim cells. ESCA, Esophageal Cancer; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, Not statistically significant.

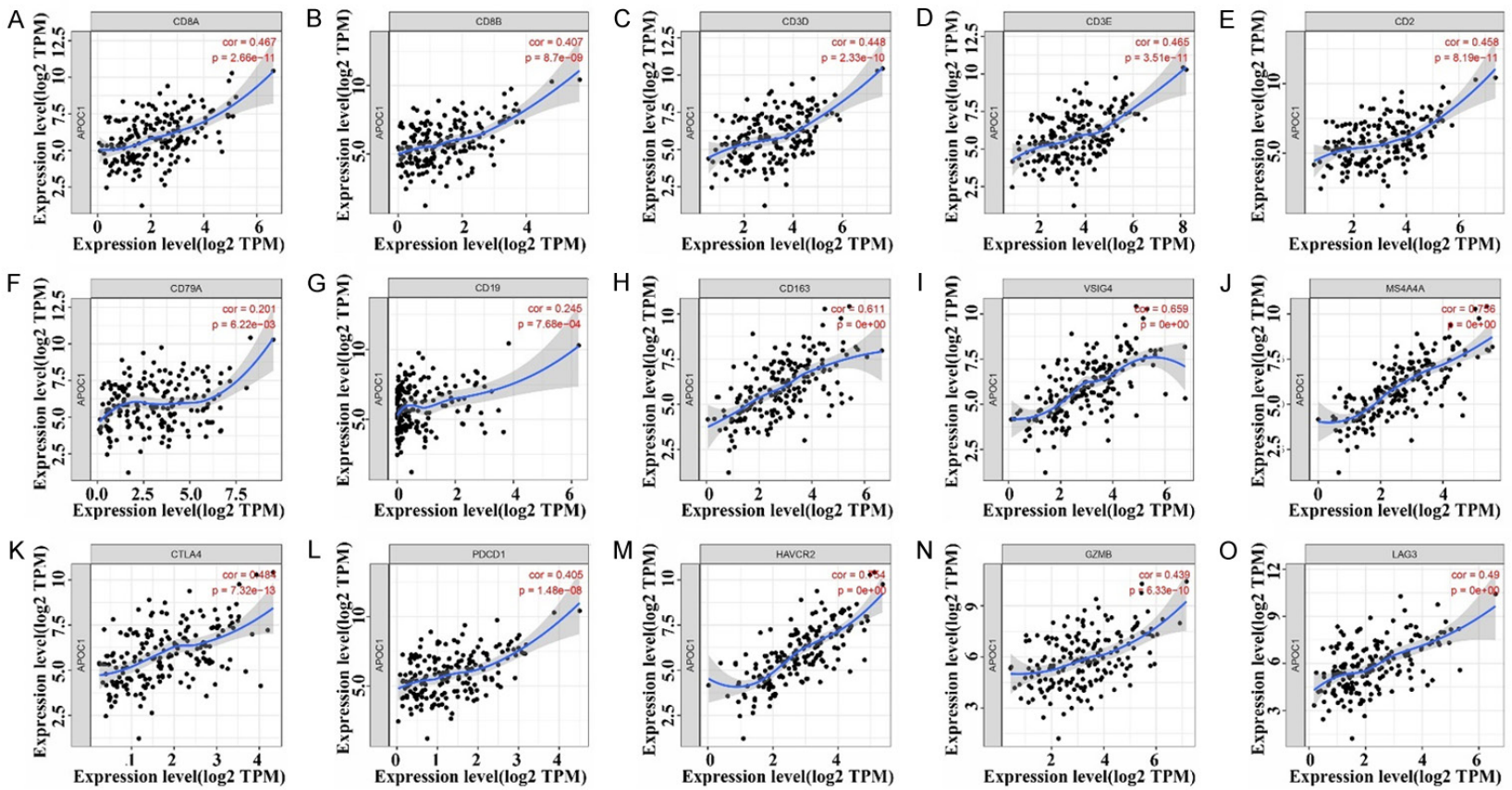
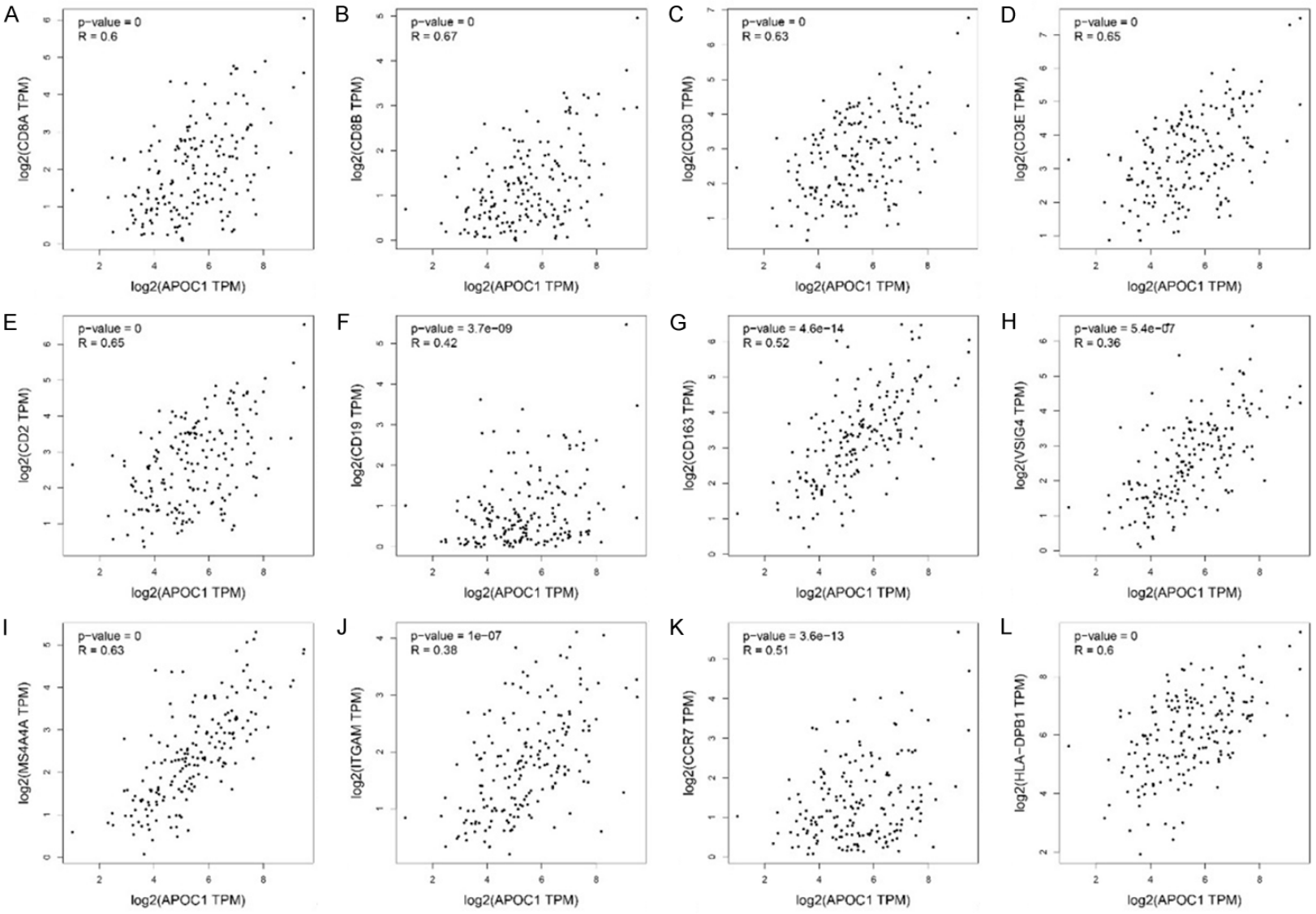


Figure 11. Association of APOC1 with the levels of immune cell markers under the conditions of non-tumor purity in TIMER database. A. CD8A; B. CD8B; C. CD3D; D. CD3E; E. CD2; F. CD79A; G. CD19; H. CD163; I. VSIG4; J. MS4A4A; K. CTLA4; L. PDCD1; M. HAVCR2; N. GZMB; O. LAG3.

Table 7. Association of APOC1 expression with the immune cell marker expressions under the conditions of non-tumor or tumor purity

Cell markers	Non-tumor purity		Tumor purity	
	Cor	P	Cor	P
CD8A	0.46667867	2.66E-11	0.419671663	4.51E-09
CD8B	0.407329331	8.70E-09	0.364980379	4.72E-07
CD3D	0.448152079	2.33E-10	0.387981979	7.39E-08
CD3E	0.464554035	3.51E-11	0.405422768	1.64E-08
CD2	0.457573633	8.19E-11	0.400739732	2.48E-08
CD19	0.245201454	0.000768217	0.164961092	0.026901111
CD79A	0.200742959	0.006224018	0.121163445	0.105183281
NOS2	-0.136789735	0.063402652	-0.121679666	0.103698276
IRF5	0.154035101	0.036401056	0.111076524	0.137686046
PTGS2	-0.012450248	0.86630636	-0.040969374	0.585023433
CD163	0.611417308	0	0.578344015	1.86E-17
VSIG4	0.658862439	0	0.629127277	3.15E-21
MS4A4A	0.755558925	0	0.735734442	6.07E-32
CEACAM8	-0.043029998	0.560853773	-0.068040549	0.36411476
ITGAM	0.545017247	0	0.503922599	5.55E-13
CCR7	0.227589932	0.001879205	0.149260216	0.045521706
HLA-DPB1	0.526187408	0	0.480887822	8.34E-12
HLA-DQB1	0.328050491	5.83E-06	0.273737912	0.000200593
HLA-DRA	0.463475607	4.02E-11	0.419042704	4.78E-09
HLA-DPA1	0.489373034	0	0.44607355	3.48E-10
CD1C	0.36399182	3.52E-07	0.293353256	6.43E-05
NRP1	0.433423676	1.05E-09	0.394195146	4.37E-08
ITGAX	0.679964747	0	0.659768538	7.34E-24
TBX21	0.479165837	5.21E-12	0.423403412	3.18E-09
STAT4	0.491499564	0	0.437745899	8.00E-10
STAT1	0.310558735	1.86E-05	0.266484101	0.000299236
IFNG	0.426015304	1.49E-09	0.373271249	2.46E-07
TNF	0.06080323	0.410632226	0.002225182	0.976349379
GATA3	0.333340283	4.04E-06	0.284327919	0.000109629
STAT6	-0.029121337	0.693694062	-0.005441508	0.942206058
STAT5A	0.197742694	0.007055398	0.165760518	0.026160254
IL13	0.179041415	0.014750095	0.126007961	0.091888006
BCL6	0.125580911	0.088515862	0.115121387	0.12383454
IL21	0.160170311	0.029415997	0.111592651	0.13585537
STAT3	-0.07916872	0.283797218	-0.110320996	0.140399792
IL17A	-0.08746033	0.236493776	-0.085289793	0.254959189
FOXP3	0.507260907	0	0.45905102	9.10E-11
CCR8	0.47297766	1.06E-11	0.428596632	1.94E-09
STAT5B	0.161839961	0.02784297	0.170284702	0.02228878
TGFB1	0.193707593	0.008329308	0.131337886	0.078844978
PDCD1	0.404876616	1.48E-08	0.34857135	1.62E-06
CTLA4	0.483819795	7.32E-13	0.431513348	1.47E-09
LAG3	0.490447671	0	0.442887002	4.80E-10
HAVCR2	0.75387021	0	0.733515964	1.14E-31
GZMB	0.438501194	6.33E-10	0.385415792	9.16E-08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log2(APOC1 TPM)
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R = 0.67
log2(CD8B TPM)
log2(APOC1 TPM)
C
p-value = 0
R = 0.63
log2(CD3D TPM)
log2(APOC1 TPM)
D
p-value = 0
R = 0.65
log2(CD3E TPM)
log2(APOC1 TPM)
E
p-value = 0
R = 0.65
log2(CD2 TPM)
log2(APOC1 TPM)
F
p-value = 3.7e-09
R = 0.42
log2(CD19 TPM)
log2(APOC1 TPM)
G
p-value = 4.6e-14
R = 0.52
log2(CD163 TPM)
log2(APOC1 TPM)
H
p-value = 5.4e-07
R = 0.36
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log2(APOC1 TPM)
I
p-value = 0
R = 0.63
log2(MS4A4A TPM)
log2(APOC1 TPM)
J
p-value = 1e-07
R = 0.38
log2(ITGAM TPM)
log2(APOC1 TPM)
K
p-value = 3.6e-13
R = 0.51
log2(CCR7 TPM)
log2(APOC1 TPM)
L
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R = 0.6
log2(HLA-DPB1 TPM)
log2(APOC1 TPM)

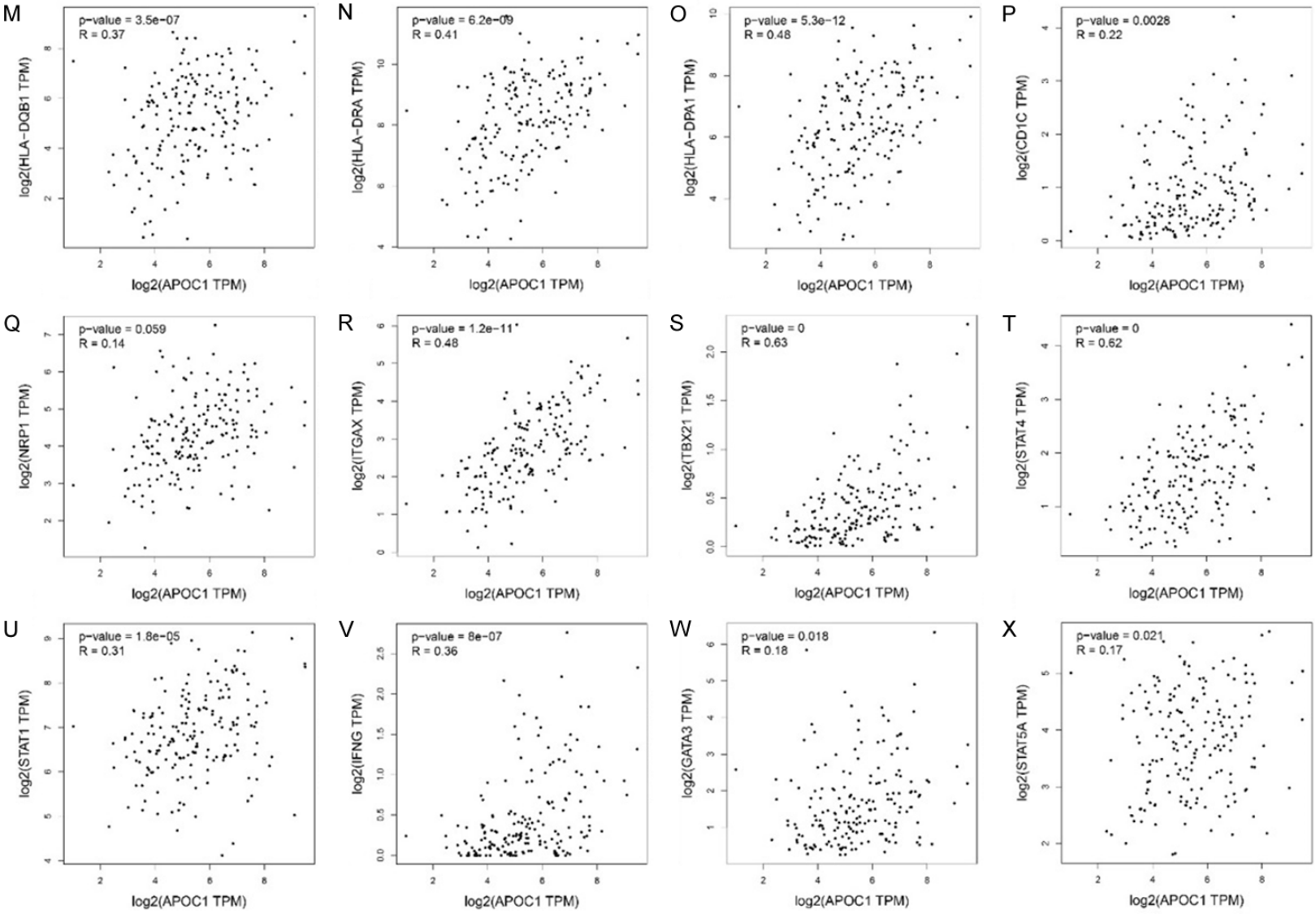

Figure 12. Association of APOC1 with the expression of immune cell markers in GEPIA database. A. CD8A; B. CD8B; C. CD3D; D. CD3E; E. CD2; F. CD19; G. CD163; H. VSIG4; I. MS4A4A; J. ITGAM; K. CCR7; L. HLA-DPB1; M. HLA-DQB1; N. HLA-DRA; O. HLA-DPA1; P. CD1C; Q. NRP1; R. ITGAX; S. TBX21; T. STAT4; U. STAT1; V. IFNG; W. GATA3; X. STAT5A.

sion of ESCA cells. However, the roles of APOC1 in these mechanisms need to be further validated by western blotting of ESCA cells.

The tumor immune microenvironment plays a pivotal role in cancer progression [34-37], and the immune microenvironment of ESCA is no exception [38-40]. For example, L1CAM expression was significantly elevated in ESCC tissues and correlated with poor prognosis in patients with ESCC. Downregulation of L1CAM expression in ESCC cells inhibits tumor growth and migration and increases tumor cell apoptosis. In the tumor microenvironment, L1CAM expression affects CCL22 secretion and is correlated with Treg cell infiltration in ESCC. L1CAM promotes CCL22 expression and Treg cell recruitment by activating the PI3K/AKT/NF-κB signaling pathway, which, in turn, secretes TGF-β to positively regulate L1CAM expression [39]. Increased CCL2 expression correlates with tumor-associated macrophage accumulation during the ESCA development. Studies have shown that blocking the CCL2-CCR2 signaling axis can reduce tumor incidence by impeding tumor-associated macrophage recruitment *in vivo*, thereby enhancing the antitumor efficacy of $CD8^+$ T cells in the tumor microenvironment. M2 cell polarization increases PD-L2 expression in tumor-associated macrophages, leading to immune evasion and tumor promotion through the PD-1 signaling pathway [40]. In this study, we found that APOC1 was associated with tumor purity, B cells, $CD4^+$ T cells, macrophages, neutrophils, DCs, T cells, T helper cells, Tem, TReg, TFH, pDC, Th1, aDC, B cells, NK cells, $CD8^+$ T cells, cytotoxic cells, and eosinophil levels in ESCA. This result corroborates previous studies showing an association of APOC1 with the ESCA immune microenvironment. In the TIMER and GEPIA databases, we found that APOC1 expression correlated with the expression of immune cell markers CD8A, IFNG, CD3D, FOXP3, CD3E, CD2, HLA-DRA, CD19, CD163, CD8B, VSIG4, STAT5A, MS4A4A, ITGAM, HLA-DPB1, PDCD1, HLA-DQB1, CD1C, ITGAX, NRP1, TBX21, STAT1, GATA3, CCR7, CCR8, STAT5B, STAT4, CTLA4, LAG3, HLA-DPA1, HAVCR2, and GZMB. These markers are closely associated with cancer progression [22, 41-44]. For instance, circUHRF1 is associated with poor prognosis and NK cell dysfunction in patients with hepatocellular carcinoma (HCC). The secretion of IFN-γ and TNF-α derived from NK cells is inhibited by circUHRF1 which upregulates the expression of TIM-3 by degrading the expression of miR-449c-5p. As a result, the function of NK cells inhibited. Resistance against the anti-target PD1 therapy in patients with HCC may be caused by circUHRF1 [41]. However, the relationship between APOC1 and ESCA immune cell markers in the immune microenvironment needs to be further investigated.

In conclusion, APOC1 mRNA and protein expression were significantly elevated in ESCA tissues, as analyzed using the TCGA database and clinical tissues. Downregulation of APOC1 significantly inhibited the ESCA progression. However, the limitations of our study included lack of experimental validation *in vivo*, and more tissue samples and patient information to evaluate the values of APOC1 in patients with ESCA, which we plan to explore in future studies. The expression of APOC1 might be critical for the diagnosis of ESCA, since it was associated with the T stage, pathological stage, BMI, histological grade, and dismal prognosis of ESCA patients. Additionally, APOC1 was also associated with ESCA immune cell infiltration and thus might be involved in the ESCA progression by participating in cytokine-cytokine receptor interactions, T cell receptor, chemokine, B cell receptor, and other immune mechanisms.

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Disclosure of conflict of interest

None.

Abbreviations

APOC1, Apolipoprotein C1; EMT, Epithelial To Mesenchymal Transition; NK, Natural Killer; CRC, Colorectal Cancer; ccRCC, Renal Cell Carcinoma; DCs, Dendritic Cells; ESCA, Esophageal Cancer; ESCC, Esophageal Squamous Cell Carcinoma; ROC, Receiver Operating Characteristic; GSEA, Gene Set Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; OS, Overall Survival; TCGA, The Cancer Genome Atlas.

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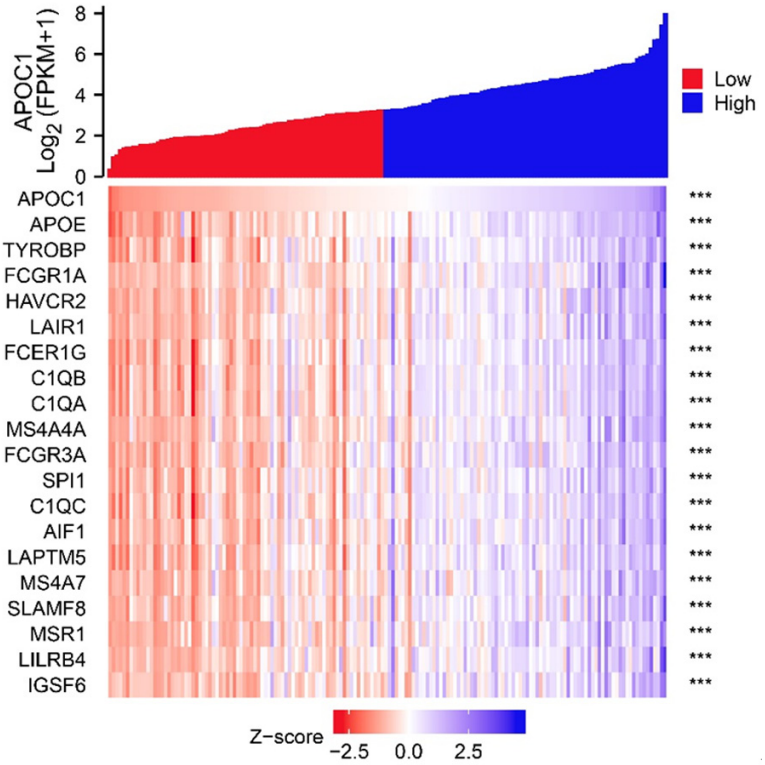

Figure S1. APOC1 positively correlated genes were visualized using a heatmap.

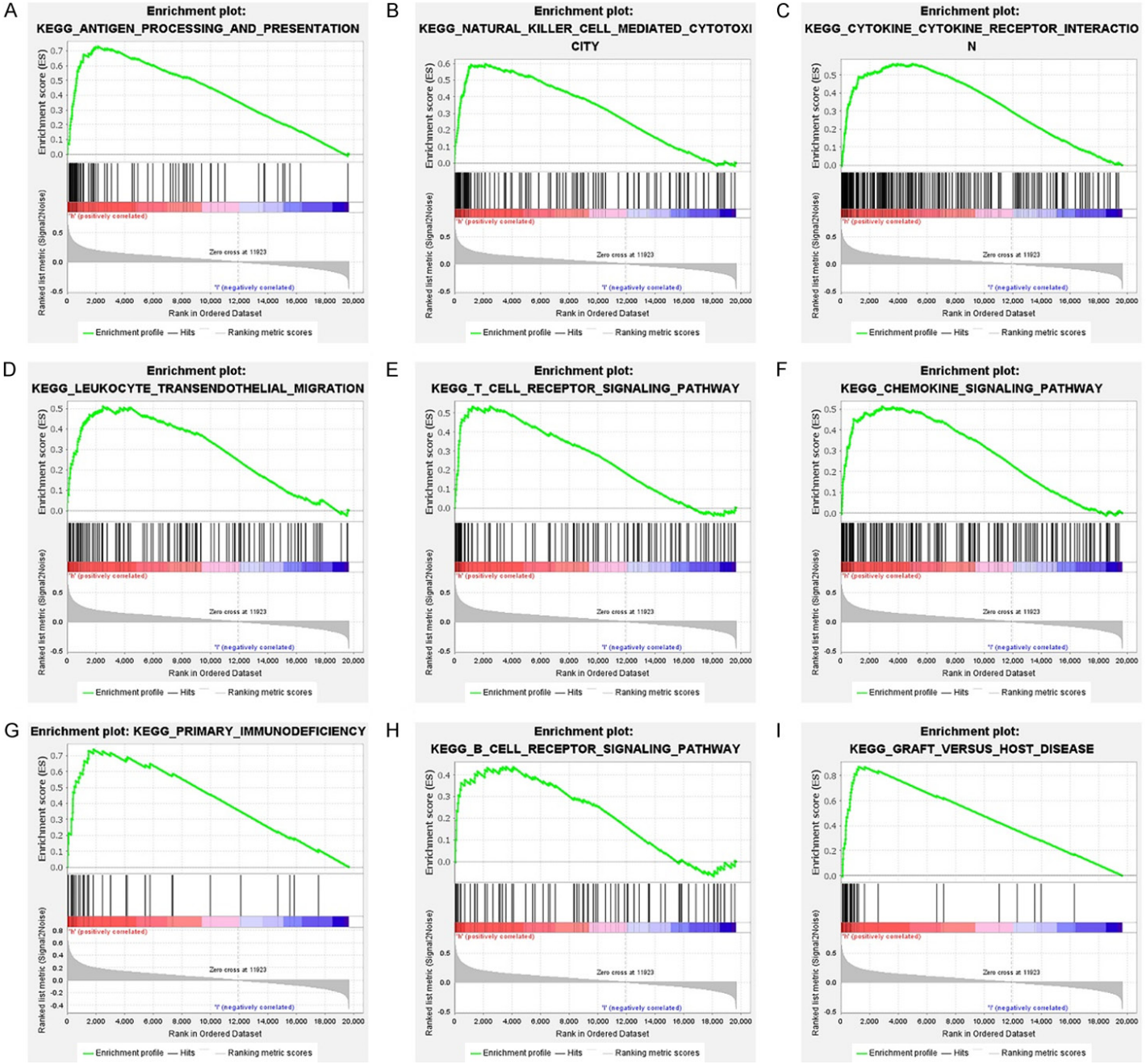
A
Enrichment plot:
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
B
Enrichment plot:
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY
C
Enrichment plot:
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
D
Enrichment plot:
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
E
Enrichment plot:
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY
F
Enrichment plot:
KEGG_CHEMOKINE_SIGNALING_PATHWAY
G
Enrichment plot: KEGG_PRIMARY_IMMUNODEFICIENCY
H
Enrichment plot:
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY
I
Enrichment plot:
KEGG_GRAFT_VERSUS_HOST_DISEASE
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'h' (positively correlated)
Zero cross at 11923
't' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S2. Signaling pathways of APOC1 via the GSEA analysis. A. Antigen processing and presentation; B. Natural killer cell mediated cytotoxicity; C. Cytokine-cytokine receptor interaction; D. Leukocyte transendothelial migration; E. T cell receptor signaling pathway; F. Chemokine signaling pathway; G. Primary immunodeficiency; H. B cell receptor signaling pathway; I. Graft versus host disease. GSEA, Gene Set Enrichment Analysis.

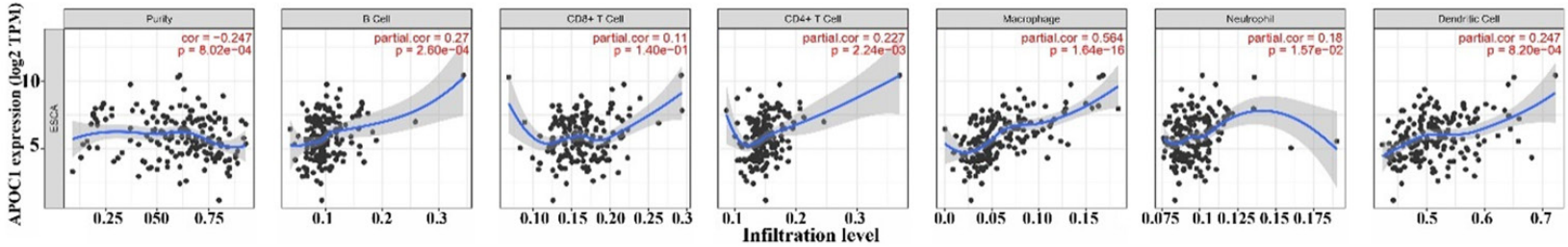

Figure S3. APOC1 expression level was significantly correlated with tumor purity, B cells, $CD4^+$ T cells, macrophages, neutrophil, and Dendritic cells in the TIMER database.

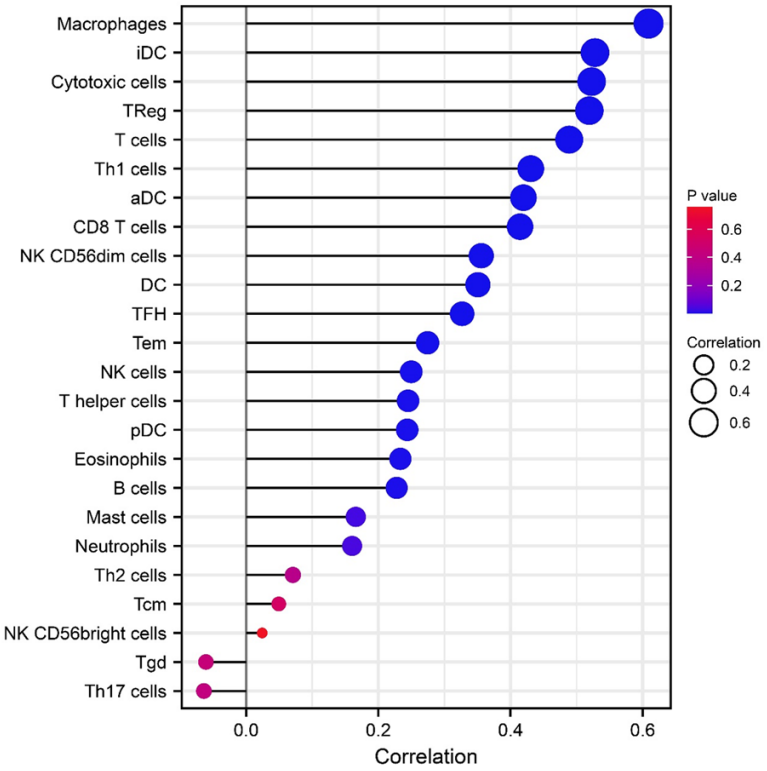

Figure S4. APOC1 expression level was significantly correlated with the immune cells in the TCGA database.

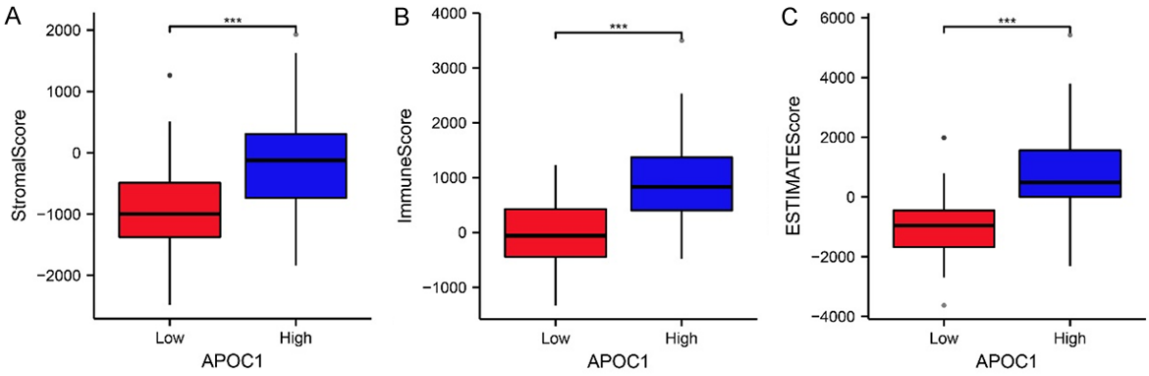

Figure S5. High and low expression of APOC1 was significantly correlated with the immune infiltration in ESCA. A. Stromalscore; B. Immunescore; C. Estimatescore; ***, P < 0.001.

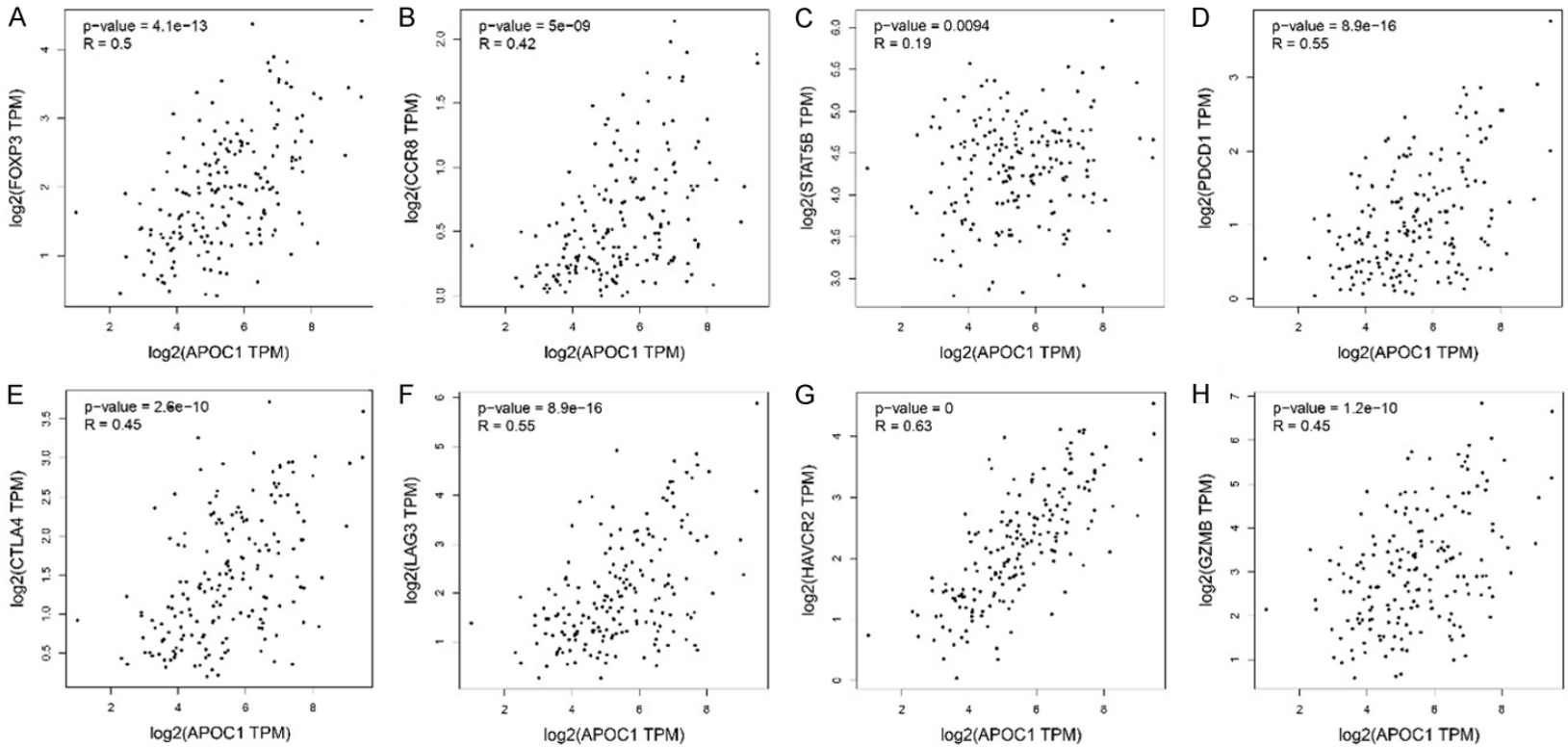

Figure S6. APOC1 was associated with levels of the immune cell markers in GEPIA database. A. FOXP3; B. CCR8; C. STAT5B; D. PDCD1; E. CTLA4; F. LAG3; G. HAVCR2; H. GZMB.