Original paper

Single-minded homolog 2 as a potential prognostic signature and assessment of its correlation with immune cell infiltration in pancreatic cancer

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Single-minded homolog 2 (SIM2) has been identified as a potential contributor to the development of solid tumors. Despite this, there is a lack of comprehensive research regarding its biological role and underlying mechanism within pancreatic cancer (PC), as well as its prognostic impact.

This study systematically evaluated the expression level and clinical significance of SIM2 in patients with PC using various databases, including The Cancer Genome Atlas, KM Plotter, and gene expression profiling interactive analysis. To investigate the relationship between SIM2 expression and immune cell infiltration, we conducted ESTIMATE and single-sample gene set enrichment analysis (ssGSEA) analyses.

Single-minded homolog 2 was up-regulated in patients with PC. Pancreatic cancer patients with higher SIM2 expression had poorer overall survival rates. Gene set enrichment analysis results suggested that SIM2 may have a significant impact on the progression of PC and the regulation of immune responses. According to the ssGSEA algorithm, SIM2 has a negative correlation with the levels of infiltrating TFH, mast cells, and pDC.

Our study demonstrated that SIM2 serves as a biomarker, and is associated with both prognosis and immune infiltration in PC. This provides a solid foundation for future investigations into the precise role of SIM2 in the carcinogenesis and progression of PC.

Key words: pancreatic cancer, pancreas, immune cell infiltration, TCGA, prognosis.

Introduction

Pancreatic cancer (PC) is a malignant tumor that spreads rapidly and is associated with a dismal prognosis. Although its occurrence is relatively rare, its mortality rate is alarmingly high, resulting in similar incidence and death rates [1]. Pancreatic cancer is the sixth leading cause of death in China, and because of the insidious nature of the disease in the early stages, over 80% of patients will be diagnosed in the advanced stages, and it is estimated that it will become the second leading cause of death in cancer patients

in the US by 2030 [2]. Despite various therapeutic strategies, including targeted therapies and immunotherapy, being explored to enhance the survival of patients with PC, their impact remains limited [3, 4]. This could be attributed, at least in part, to the intricate nature and heterogeneity of PC. Early diagnosis and more effective treatment of PC are therefore urgently needed.

Single-minded homolog 2 (SIM2) is a neuron-enriched transcription factor that plays a crucial role in the cellular stress responses, homeostasis, and development [5]. It is primarily recognized for its involvement in Down's syndrome [6]. The variability in the occurrence of different malignant conditions in Down syndrome could be attributed to potential alterations in the expression of SIM2 [7]. Single-minded homolog 2 expression continues into adulthood in muscle and kidney, but its precise function in these tissues is still unclear [8]. A previous study showed that SIM2 plays a vital role in regulating the expression of intestinal antimicrobial peptides. This regulation is essential for maintaining the innate immunity of the intestines against microbial threats [9]. Previous studies have reported a correlation between SIM2 and cancer [10], specifically in the context of prostate cancer. Single-minded homolog 2 is involved in key traits of prostate tumor cell biology and may contribute to the onset and progression of prostate cancer [11]. Both isoforms, SIM2-s and SIM2-l, were identified in benign prostate tissue and exhibited significant coexpression. Furthermore, these isoforms were found to be elevated in prostate cancers [12]. However, the role and function of SIM2 in PC, particularly its impact on immune regulation, have not been investigated yet. Our study aimed to examine the variations in SIM2 expression levels among patients with PC and their potential as a prognostic biomarker, considering the significance of SIM2 in tumors.

Material and methods

Data resources and processing

RNA-sequencing and clinicopathological data for PC were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). The cohort consisted of 179 PC tumors and 4 normal samples. We acquired transcripts *per* million (TPM) format RNA-seq data from TCGA. The $\log_2$ (TPM+1) transformed expression data were utilized to generate box plots, enabling us to compare the mRNA expression of SIM2 with clinicopathological details across different groups. In addition, we used the gene expression profiling interactive analysis database (GEPIA) (http://gepia.cancer-pku.cn/) and the TNMplot.com analysis platform (www.tnmplot.com) to further test the expression of SIM2 in PC [13, 14].

Quantitative reverse real-time polymerase chain reaction

We acquired 10 paired tumor and normal renal tissue samples from patients with PC who underwent surgery at West China Hospital. Before the study, we obtained written informed consent from the patients. Total RNA was extracted from the tissues using TRIzol reagent (Takara, Bio, Inc., China), following the manufacturer's protocol. Purified RNA (2 μg) was then used to synthesize cDNA with the cDNA synthesis kit (Takara, Bio, Inc., China), as *per* the manufacturer's instructions. Subsequently, quantitative reverse real-time polymerase chain reaction (qRT-PCR) was performed on the ABI7500 fluorescent quantitative PCR System (Applied Biosystems, USA) using the SYBR GREEN PCR kit. The mRNA expression level of SIM2 was quantified using the $2^{-\Delta\Delta Ct}$ method. The primer sequences can be found in Supplementary File Table S1.

Prognostic and diagnostic value analysis

In this study, PC patients were categorized into two groups based on the median value of SIM2 expression: high and low. The survival analysis was conducted using the Kaplan-Meier method along with the log-rank test. To validate the predictive performance of SIM2, the pROC package in the receiver operating characteristic (ROC) analysis was employed to generate the ROC curve and determine the area under the curve (AUC) [15].

Associating single-minded homolog 2 expression with clinicopathological features in pancreatic cancer

We performed a correlation of SIM2 expression with clinical parameters in PC. These features included pathologic T stage, histologic grade, residual tumor, smoker, overall survival (OS) event, and history of chronic pancreatitis. The Kruskal-Wallis test was used to compare the subgroups. To visualize the statistical data, we utilized the ggplot2 package.

Establishment of nomogram

Proportional hazards hypothesis testing and Cox regression analysis were conducted using the survival package. The nomogram model was constructed and visualized using the rms package.

Functional enrichment analysis of single-minded homolog 2 related differentially expressed genes

In the TCGA dataset, PC patients were categorized into low- and high-SIM2 subgroups using the median value of SIM2 gene expression level. The differentially expressed genes (DEGs) between

these subgroups were identified using the DESeq2 package. The criteria for selection were an absolute fold change (FC) value greater than 1 and an adjusted *p*-value less than 0.05. The ClusterProfiler R package was utilized to conduct the gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses. Significance was determined at an adjusted *p*-value of less than 0.05.

Gene set enrichment analysis

We investigated the potential biological role of SIM2 in PC using gene set enrichment analysis (GSEA). We first identified genes that were co-expressed with SIM2 and then performed GSEA using the ClusterProfiler package to identify the signaling pathways that were significantly enriched in these genes. Significant enrichment was determined based on a *p*-value adjustment (P. adjust) of less than 0.05 and a false discovery rate of less than 0.25. The reference gene collection used was C2.CP.V7.2.symbols.fmt, and the gene set database was obtained from Molecular Signatures Database (www.gsea-msigdb.org/gsea/).

Immunocyte infiltration analysis

The gene set variation analysis package of R was utilized to conduct single-sample gene set enrichment analysis (ssGSEA) and compare the proportions of 24 immune cell types between the SIM2 low and high expression subgroups. The ESTIMATE software was employed to assess the ESTIMATE score, immune score, and stromal score. The correlation between the immune cell infiltrate level and the SIM2 expression was analyzed using Spearman correlation.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of West China Hospital. Written informed consent was obtained from the patients.

All data are available from the corresponding author upon request.

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Results

Expression level of single-minded homolog 2 in human tumors

Expression of SIM2 at the mRNA level was analyzed using the TCGA database (Fig. 1A), which revealed that SIM2 has low expression in head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), and kidney renal papillary cell carcinoma (KIRP) compared to normal samples. Conversely, SIM2 has high expression in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD) (Fig. 1B), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) compared to normal samples. To further validate the expression of SIM2 in PC, we analyzed RNA-seq data from 179 PC tumor tissues and 171 normal tissues using GEPIA. Our analysis confirmed the high expression of SIM2 in PC (Fig. 1C). This finding is consistent with the up-regulation of SIM2 observed in PC, as evidenced by data obtained from the TNMplot.com analysis platform (Fig. 1D) and qRT-PCR analysis (Fig. 1E).

Single-minded homolog 2 is a prognostic marker for pancreatic cancer patients

Analysis of the GEPIA (Fig. 2A) and Kaplan-Meier plotter (Fig. 2B) revealed that high expression of SIM2 in PC was correlated with poorer overall survival. To evaluate SIM2 as a prognostic biomarker for PC, we further analyzed the data from TCGA. Our findings revealed a significant association between high SIM2 expression and unfavorable OS (Fig. 2C), disease-specific survival (Fig. 2D), and progression-free interval (Fig. 2E). Additionally, the multivariate Cox regression analysis indicated that SIM2 was an independent risk factor for PC patients (Table S2).

Relationships between single-minded homolog 2 gene and clinical pathological features in pancreatic cancer

As shown in Figure 3 and Table S3, SIM2 expression was significantly up-regulated in the pathologic T stage (T3 and T4) (Fig. 3A) ($p < 0.01$), histologic grade (G2 and G3 and G4) (Fig. 3B) ($p < 0.05$, $p < 0.01$), residual tumor (R1 and R2) (Fig. 3C) ($p < 0.01$), smoker (yes) (Fig. 3D) ($p < 0.05$), OS event (dead) (Fig. 3E) ($p < 0.05$), and history of chronic pancreatitis (yes) (Fig. 3F) ($p < 0.05$).

In addition, high SIM2 expression was associated with worse prognosis in the G1 and G2 subgroup of histologic grade (Fig. 4A) ($p = 0.008$), M0 and M1 subgroup of pathologic M stage (Fig. 4B) ($p = 0.044$), N0 subgroup of pathologic N stage (Fig. 4C) ($p = 0.018$), R0 and R1 subgroup of residual tumor (Fig. 4D) ($p = 0.001$), stage I and stage II

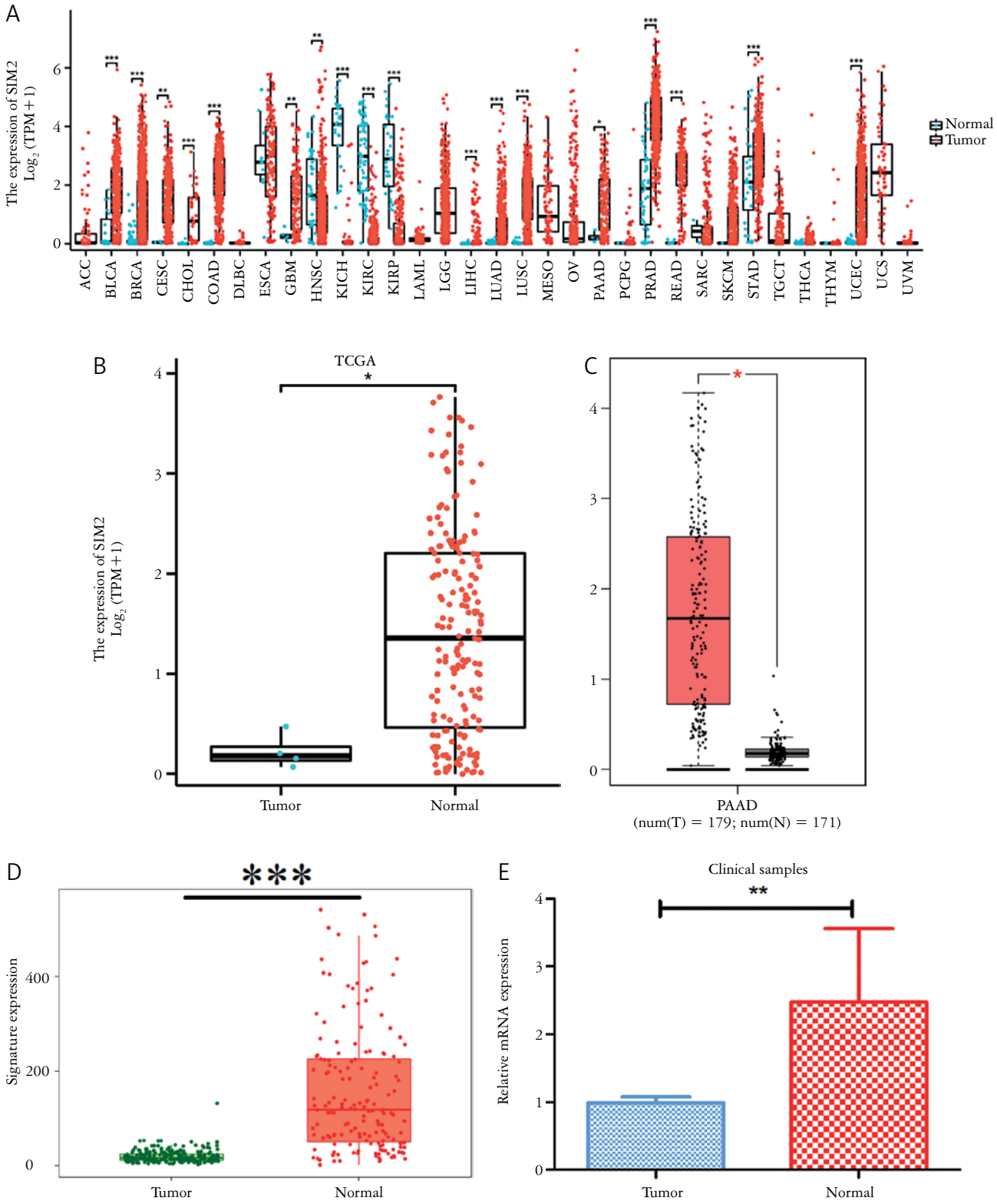

Fig. 1. Expression level of single-minded homolog 2 (SIM2). A) Pan cancer analysis of SIM2 expression by The Cancer Genome Atlas database; B) the level of SIM2 was up-regulated in pancreatic cancer (PC) compared with corresponding normal samples; C) validation of SIM2 mRNA expression in PC samples by gene expression profiling interactive analysis; D) TNMplot.com analysis platform; E) quantitative reverse real-time polymerase chain reaction analysis

** $p < 0.05$*
*** $p < 0.01$*
**** $p < 0.001$*
**PAAD – pancreatic adenocarcinoma, SIM2 – single-minded homolog 2, TCGA – The Cancer Genome Atlas, TPM – transcripts per million*

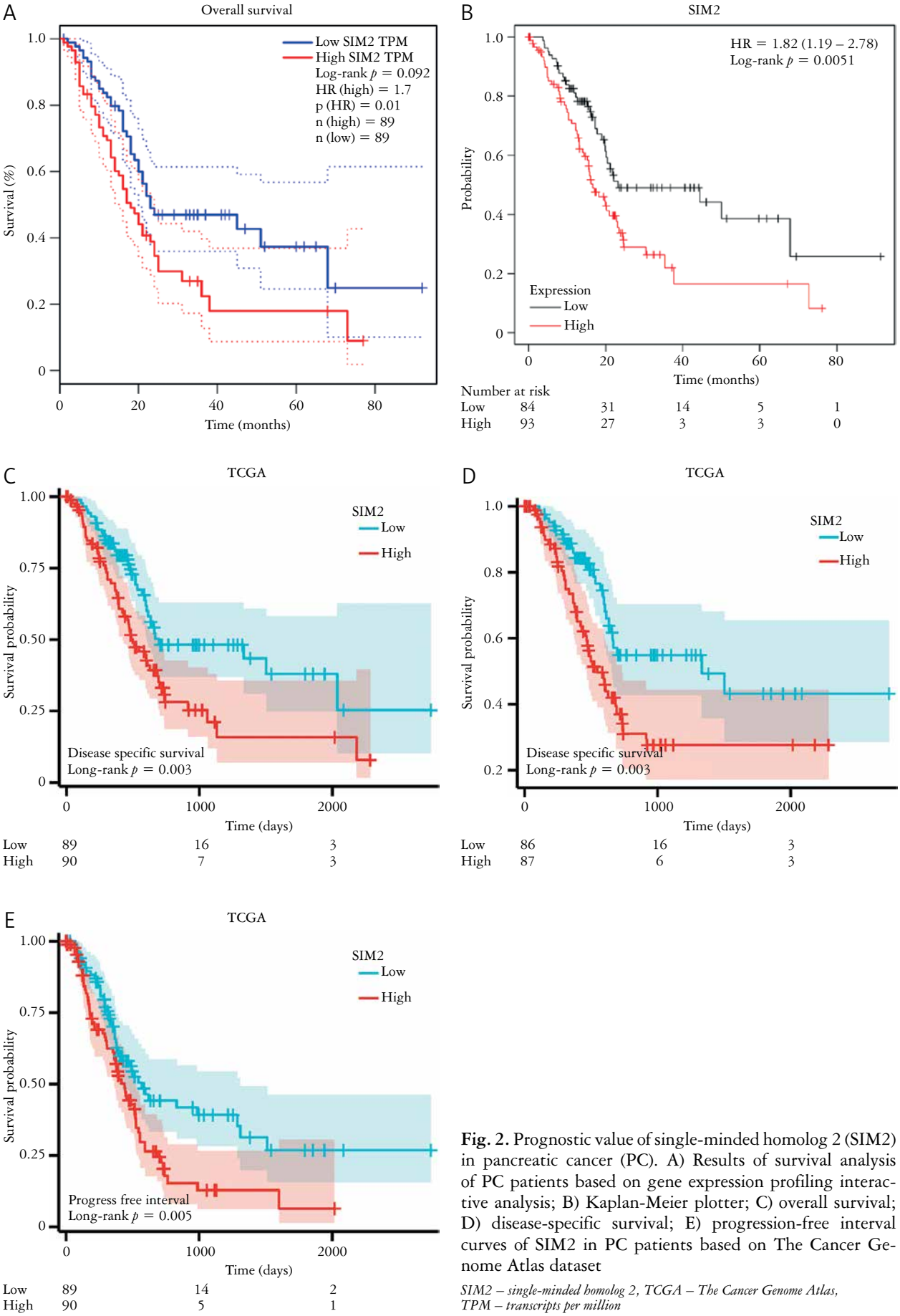

Fig. 2. Prognostic value of single-minded homolog 2 (SIM2) in pancreatic cancer (PC). A) Results of survival analysis of PC patients based on gene expression profiling interactive analysis; B) Kaplan-Meier plotter; C) overall survival; D) disease-specific survival; E) progression-free interval curves of SIM2 in PC patients based on The Cancer Genome Atlas dataset

SIM2 – single-minded homolog 2, TCGA – The Cancer Genome Atlas, TPM – transcripts per million

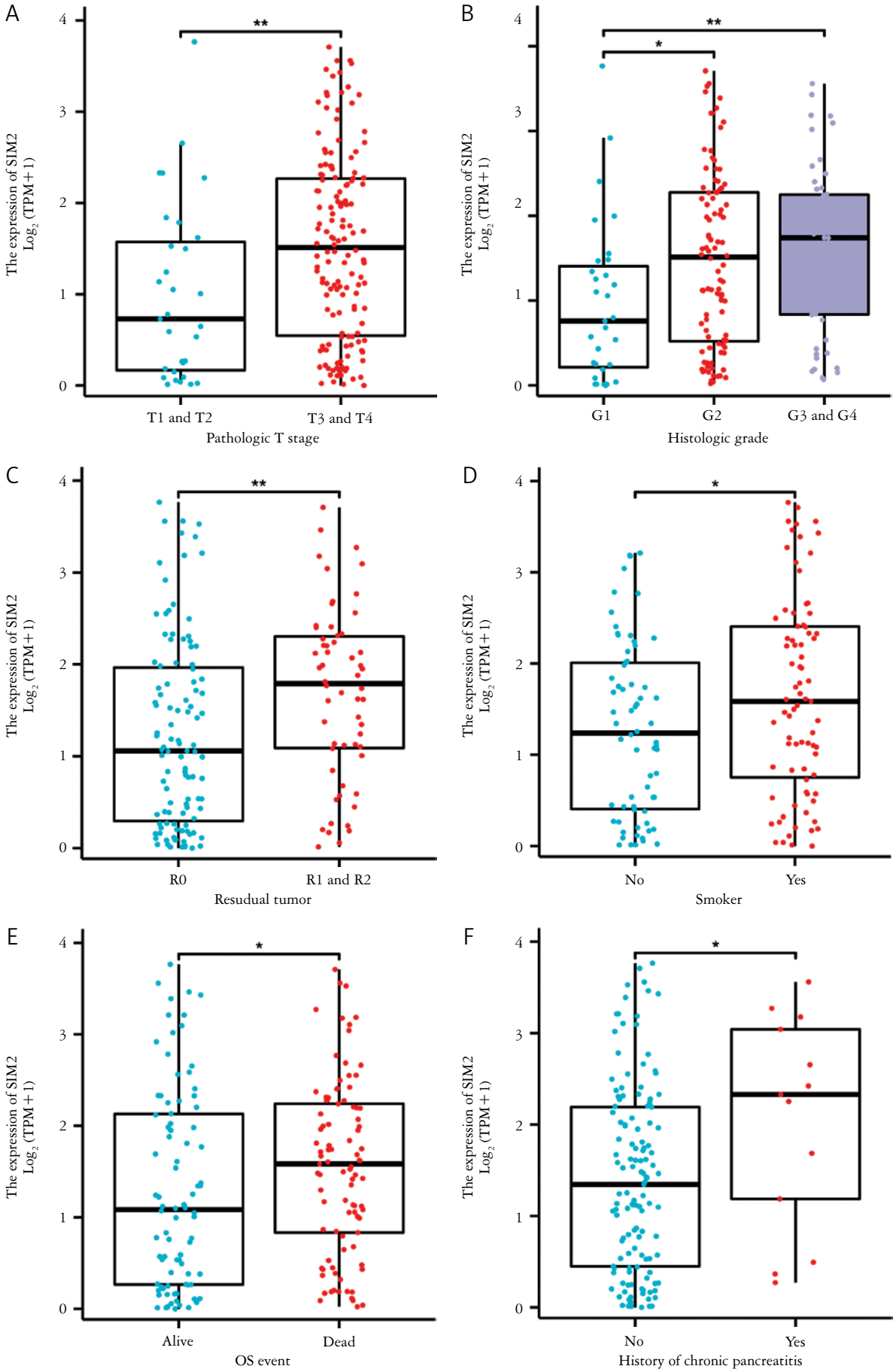

Fig. 3. A) Single-minded homolog 2 (expression level was significantly different in pancreatic cancer patients with different pathologic T stage; B) histologic grade; C) residual tumor; D) history of smoking ; E) overall survival event; F) history of chronic pancreatitis

* $p < 0.05$
** $p < 0.01$
OS – overall survival, SIM2 – single-minded homolog 2, TPM – transcripts per million

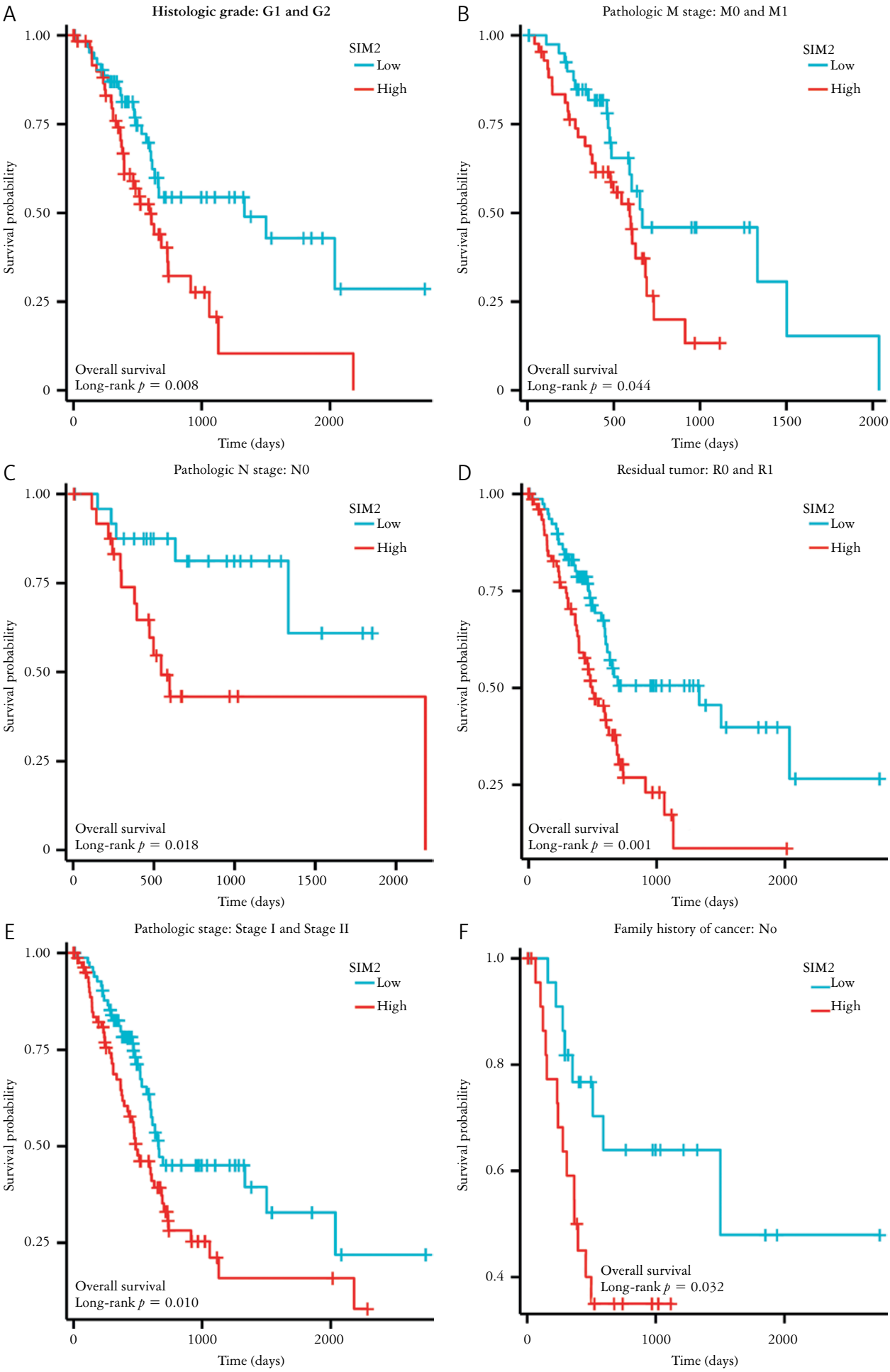

Fig. 4. Prognostic analysis of single-minded homolog 2 expression in the different subgroups. A) Overall survival in pancreatic cancer patients for G1 and G2; B) M0 and M1; C) N0; D) R0 and R1; E) stage I and stage II; F) no family history of cancer

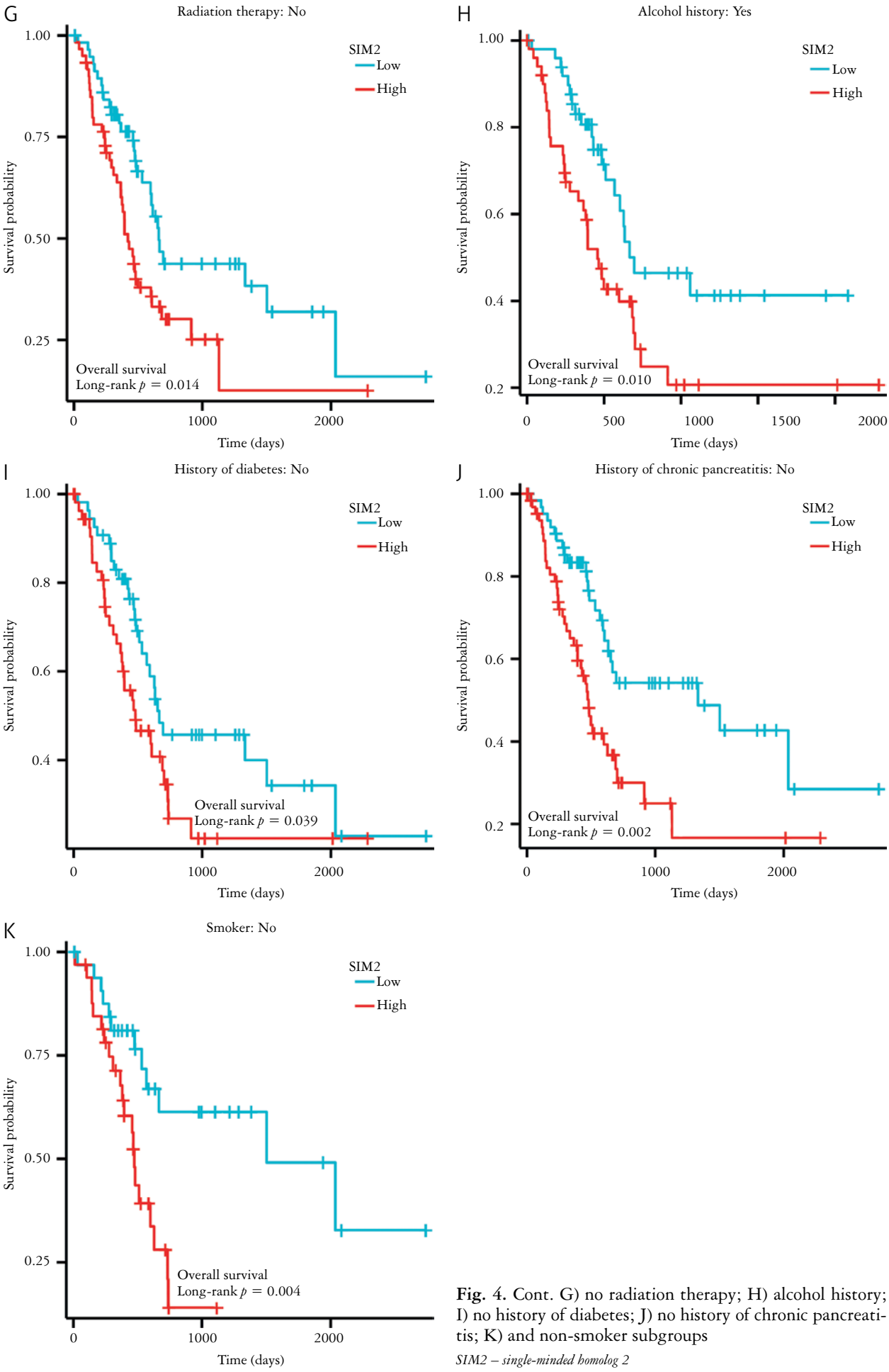

Fig. 4. Cont. G) no radiation therapy; H) alcohol history; I) no history of diabetes; J) no history of chronic pancreatitis; K) and non-smoker subgroups

SIM2 – single-minded homolog 2

subgroup of pathologic stage (Fig. 4E) ($p = 0.01$), no family history of cancer subgroup (Fig. 4F) ($p = 0.032$), no radiation therapy subgroup (Fig. 4G) ($p = 0.014$), alcohol history subgroup (Fig. 4H) ($p = 0.001$), no history of diabetes subgroup (Fig. 4I) ($p = 0.039$), no history of chronic pancreatitis subgroup (Fig. 4J) ($p = 0.002$), and non-smoker subgroup (Fig. 4K) ($p = 0.004$).

Establishment of nomogram

As shown in Figure 5A, the AUC value for the SIM2 gene was 0.859, indicating that it is a diagnostic marker for PC patients. A nomogram was constructed to assess SIM2's predictive ability for the 1- and 3-year OS of patients with PC. The constructed nomogram is shown in Figure 5B. The nomogram calibration curve (Figs. 5C, D) demonstrated a high level of agreement between the predicted outcomes from the nomogram and the observed results of PC patients. In addition, we extracted one-third of the sample size in the TCGA dataset as a validation set, and the results were consistent (Fig. S1).

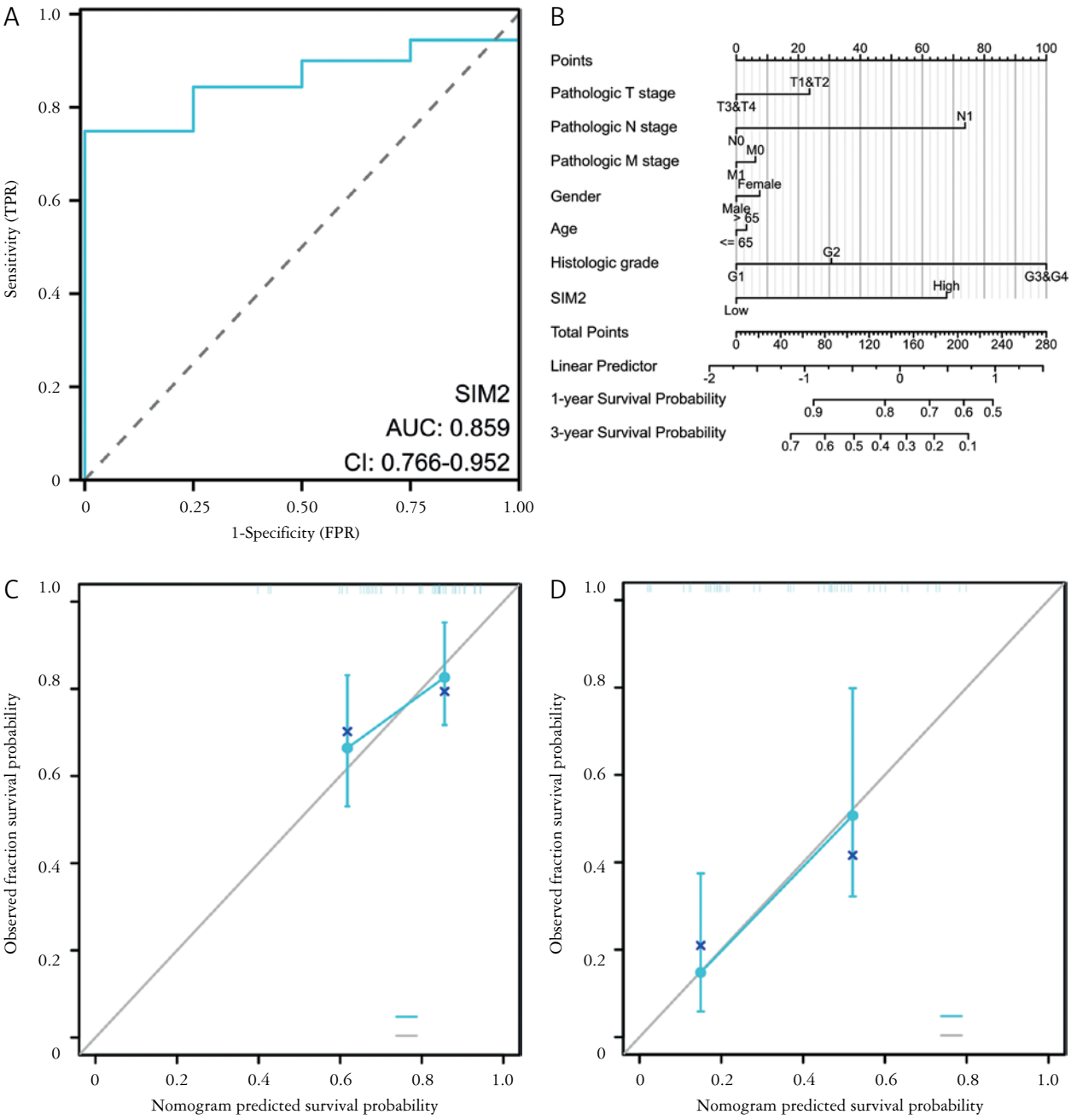

Fig. 5. Development of a nomogram for pancreatic cancer (PC) patients. A) Receiver operating characteristic curve exhibited the diagnostic value of single-minded homolog 2 (SIM2); B) nomogram for assessing the 1-year and 3-year survival probability for PC; C, D) calibration curve of the SIM2 gene

AUC – area under the curve, SIM2 – single-minded homolog 2, TPR – true positive rate

Enrichment analysis of single-minded homolog 2 associated differentially expressed genes

According to Figure 6A, a total of 4107 DEGs were identified between the low- and high-SIM2 subgroups. Among these genes, 1123 were up-regulated and 2984 were down-regulated. Kyoto Encyclopedia of Genes and Genomes enrichment analysis revealed that these DEGs were enriched in neuroactive ligand-receptor interaction, pancreatic secretion, protein digestion and absorption, insulin secretion, and retinol metabolism (Fig. 6B). Gene ontology enrichment analysis indicated that these DEGs were enriched in regulation of membrane potential, regulation of hormone levels, presynapse, T-cell receptor complex, channel activity, and ion channel activity (Fig. 6C). Additionally, GSEA revealed that the SIM2-related DEGs were significantly enriched in immune- and inflammation-related pathways, such as PI3K-AKT signaling pathway, cytokine-cytokine receptor interaction, T-cell receptor signaling pathway, chemokine signaling pathway, CD8 TCR pathway, IL23 pathway, IL12 pathway, IL12 STAT4 pathway, FCGR3A mediated IL10 synthesis, primary immunodeficiency, IL17 pathway, etc. (Fig. 7).

Immunocyte infiltration analysis

We comprehensively assessed the level of immune infiltration between high and low SIM2 subgroups using two algorithms (ESTIMATE and ssGSEA). The ESTIMATE results indicated that the stromal, immune, and ESTIMATE scores were observably higher in the SIM2-low subgroup compared with SIM2-high subgroup (Fig. 8A). The ssGSEA results showed that the score of CD8 T-cells, cytotoxic cells, DC, eosinophils, iDC, mast cells, neutrophils, natural killer (NK) cells, pDC, T-cells, Tem, TFH, Tgd, and Th17 cells was significantly higher in the SIM2-low subgroup compared with the SIM2-high subgroup. In contrast, the score of NK CD56bright cells and Th2 cells was significantly lower in the SIM2-low subgroup compared with the SIM2-high subgroup (Fig. 8B). Additionally, we carried out correlation analysis between SIM2 expression level and immune cell infiltration level (Fig. 9). Expression of SIM2 was negatively correlated with the levels of eosinophils, iDC, neutrophils, T-cells, Th17 cells, NK cells, cytotoxic cells, Tgd, mast cells, pDC, and TFH; SIM2 was positively correlated with the levels of Th2 cells and NK CD56bright cells.

Discussion

Pancreatic cancer is an extremely aggressive tumor characterized by a poor prognosis, with a survival rate of less than 10% over a 5-year period [1]. Despite significant advances in the diagnosis and treatment of malignant tumors, current strategies for the early diagnosis and treatment of PC remain inadequate.

Single-minded homolog 2 plays a vital role in cellular stress responses, homeostasis, and development. There is a growing body of literature suggesting abnormal expression of SIM2 in different types of cancers, which is also believed to contribute to the initiation and progression of these cancers [10–12]. The ectopic expression of SIM2 was able to reverse the inhibitory effects on cell invasion, migration, and proliferation in colorectal cancer caused by TMEM75 depletion [16]. In the present study, the findings from multiple databases revealed that SIM2 is upregulated in PC, whereas patients with an unfavorable prognosis generally exhibit high expression levels of SIM2. The Kaplan-Meier survival curve, depicting overall survival, disease-specific survival, and progression-free interval, demonstrated that PC patients with elevated levels of SIM2 experience shorter OS times. These results align with the impact of SIM2 on prognosis in esophageal squamous cell carcinoma and uterine cervical squamous cell carcinoma [17, 18]. These findings indicated that SIM2 may have a tumor-promoting role, and that high expression of SIM2 is closely associated with a poorer prognosis for patients with PC.

How SIM2 is involved in the development and progression of PC remains poorly understood. To further investigate the biological function of SIM2 in PC, we divided PC patients into two subgroups – high and low SIM2 expression – and performed DEG analysis. Gene set enrichment analysis results from this study showed that these DEGs were mainly enriched in immune and inflammation-related pathways, including the cytokine-cytokine receptor signaling pathway, CD8 TCR pathway, primary immunodeficiency, etc. Inflammation and immunity are closely linked, with the same immune cell populations playing a role in both processes. Chronic inflammation, whether systemic or localized, can increase the risk of developing PC. Additionally, the inflammatory infiltrate present in the tumor microenvironment (TME) of PC contributes to tumor growth and metastasis [19, 20]. During postpartum mammary involution, SIM2 plays a crucial role in the negative regulation of the NF-κB signaling pathway in normal mammary tissues. This regulatory mechanism has been recognized as a significant contributor to the progression and spread of tumors [21]. A previous study provided evidence supporting the involvement of SIM2 in inhibiting the progression of breast cancer. This is achieved by suppressing the expression of PTGS2 through regulation of the NF-κB signaling pathway [22]. Our GSEA results consistently demonstrated a strong correlation between SIM2 and inflammation. These findings strongly indicated that SIM2 could potentially have a crucial function in cancer-related inflammation.

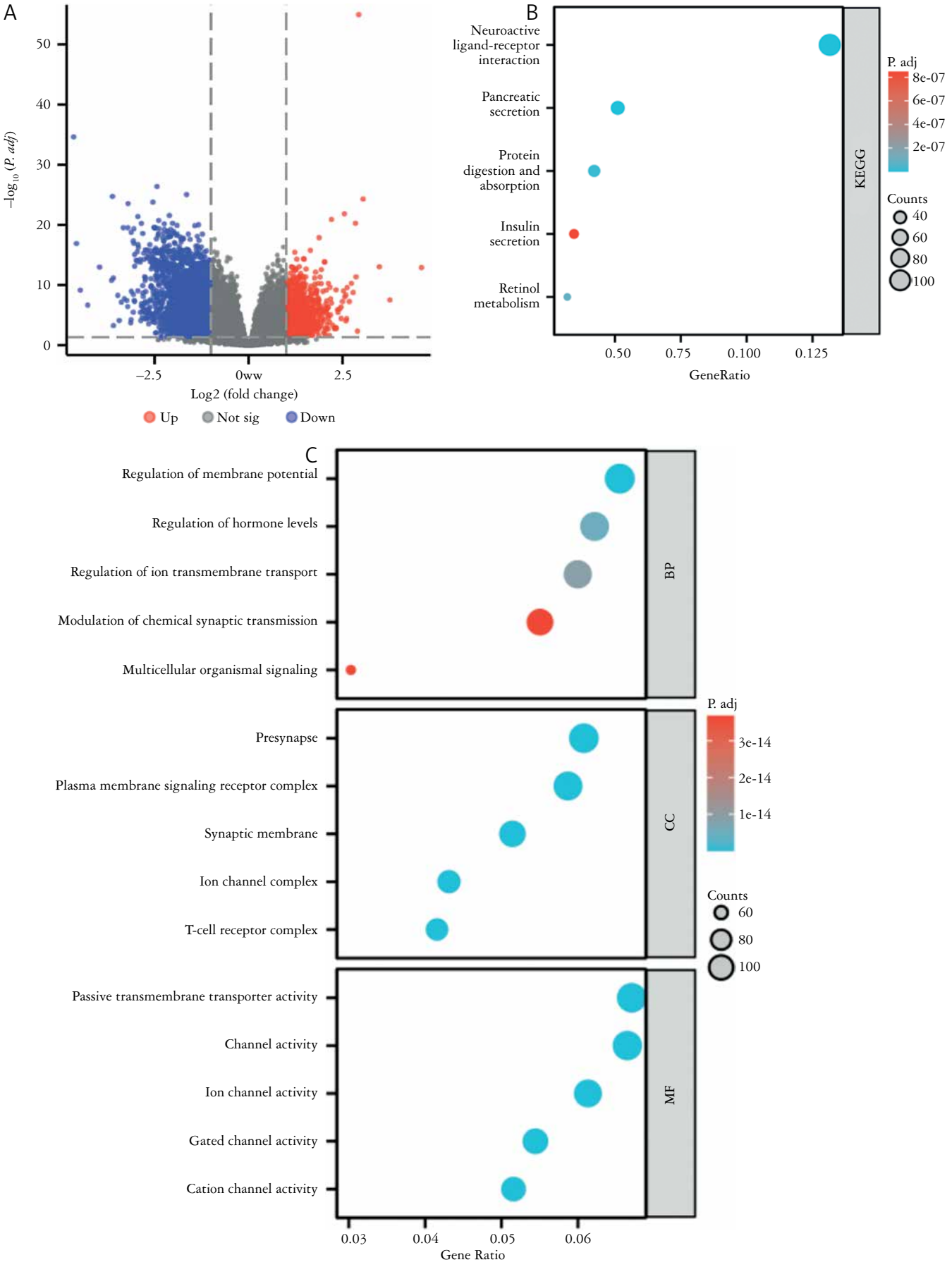

Fig. 6. Enrichment analysis of single-minded homolog 2 (SIM2)-related differentially expressed genes (DEGs). A) Volcano plots exhibiting the DEGs between the low- and high-SIM2 subgroups; B) Kyoto Encyclopedia of Genes and Genomes; C) gene ontology enrichment analyses of SIM2-related DEGs

BP – biological processes, CC – cellular components, KEGG – Kyoto Encyclopedia of Genes and Genomes, MF – molecular functions

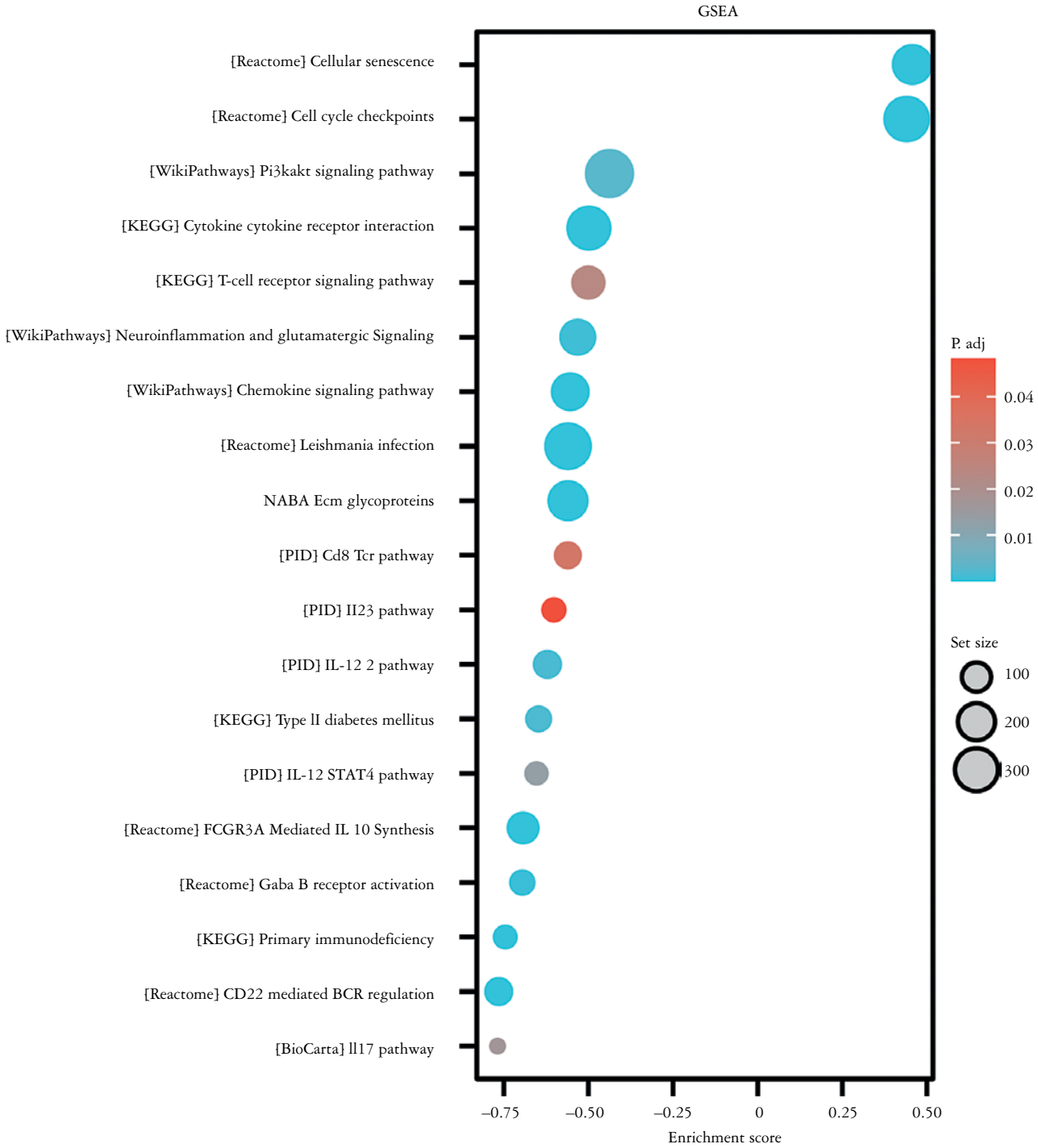

Fig. 7. Gene set enrichment analysis results indicating the changed signaling pathways based on single-minded homolog 2 related differentially expressed genes

BCR – B-cell antigen receptor, GSEA – gene set enrichment analysis, KEGG – Kyoto Encyclopedia of Genes and Genomes

The tumor microenvironment is composed of immune cells, endothelial cells, and stromal cells, and it plays a crucial role in determining the effectiveness of tumor immunotherapy and patients' prognosis [23, 24]. Numerous studies have shown that TME influences cancer prognosis through various pathways. The microenvironment of PC plays a role in metastasis and could be targeted for combination therapy to improve OS [25]. In multivariate analysis, the immune cell score, along with the histological grade of the tumor and perineural invasion, was identified as an independent prognostic factor for better disease-specific survival and OS in PC [26]. In the present study, we found that the immune score, stromal score, and ESTIMATE score were significantly higher in the SIM2-low subgroup. The results, from another perspective, implied the association between SIM2 and a poorer prognosis in patients

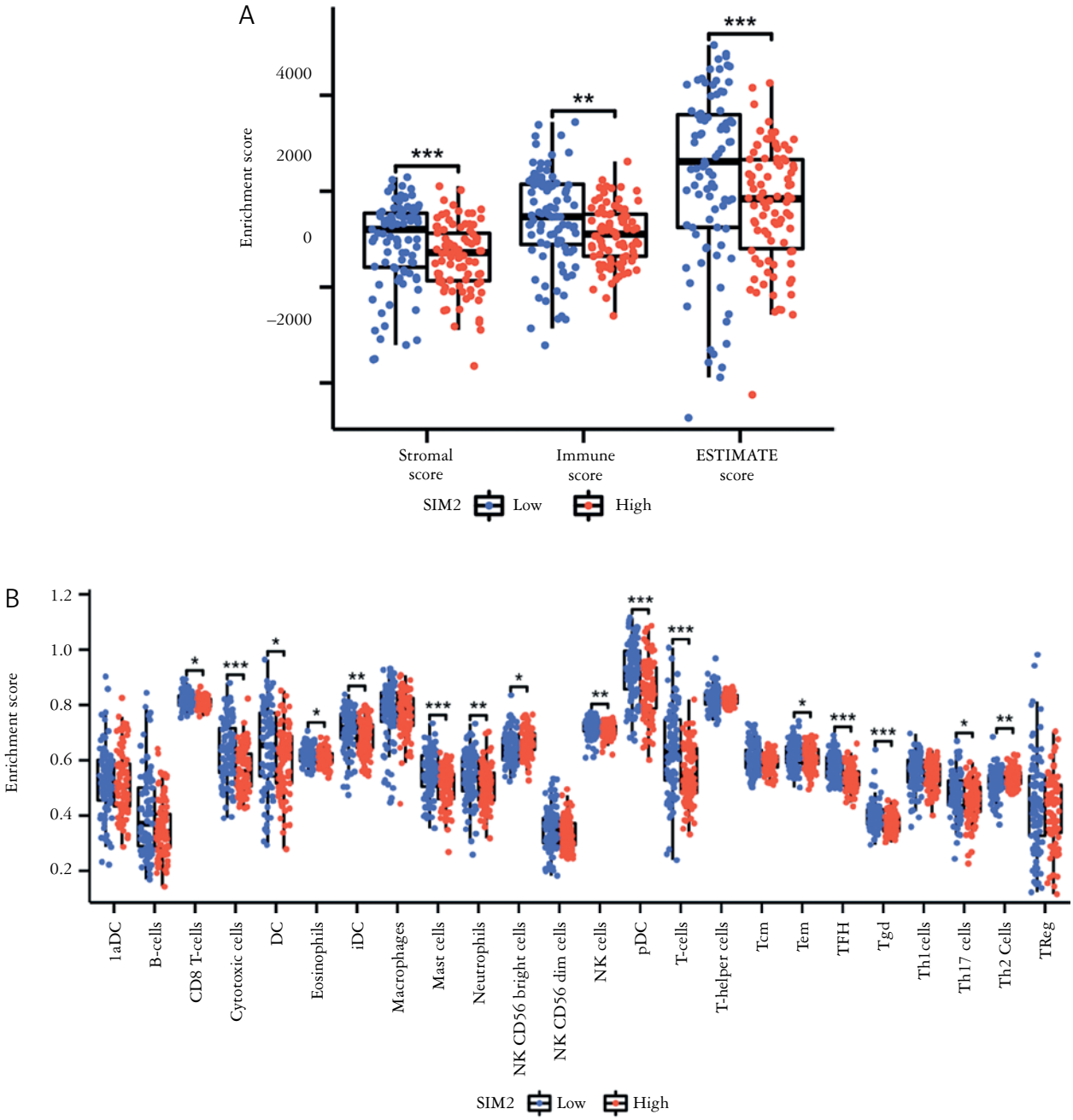

Fig. 8. A) Characterization of immune cell infiltration landscape between single-minded homolog 2 (SIM2) subgroups demonstrating differential levels of immune cell infiltration between SIM2 subgroups using ESTIMATE; B) and single-sample gene set enrichment analysis algorithms

** $p < 0.05$*
*** $p < 0.01$*
**** $p < 0.001$*
SIM2 – single-minded homolog 2

with PC. In the SIM2-low expression group, we observed a significant increase in NK cells, DC, neutrophils and eosinophils. As a result, we put forward the hypothesis that reduced levels of SIM2 expression may enhance the infiltration of immune cells and hinder the progression of tumors in PC. This finding is in line with the results of other studies, as alterations in immune infiltration are also strongly linked to the development of PC [27–30]. In addition, previous studies have indicated that the infiltration of these cells into tumors is correlated with the prognosis for PC patients [31–34]. Our findings collectively suggest that high expression of SIM2 can inhibit immune cell infiltration, which is associated with a poor prognosis in patients with PC. This finding contributes to a better understanding of the re-

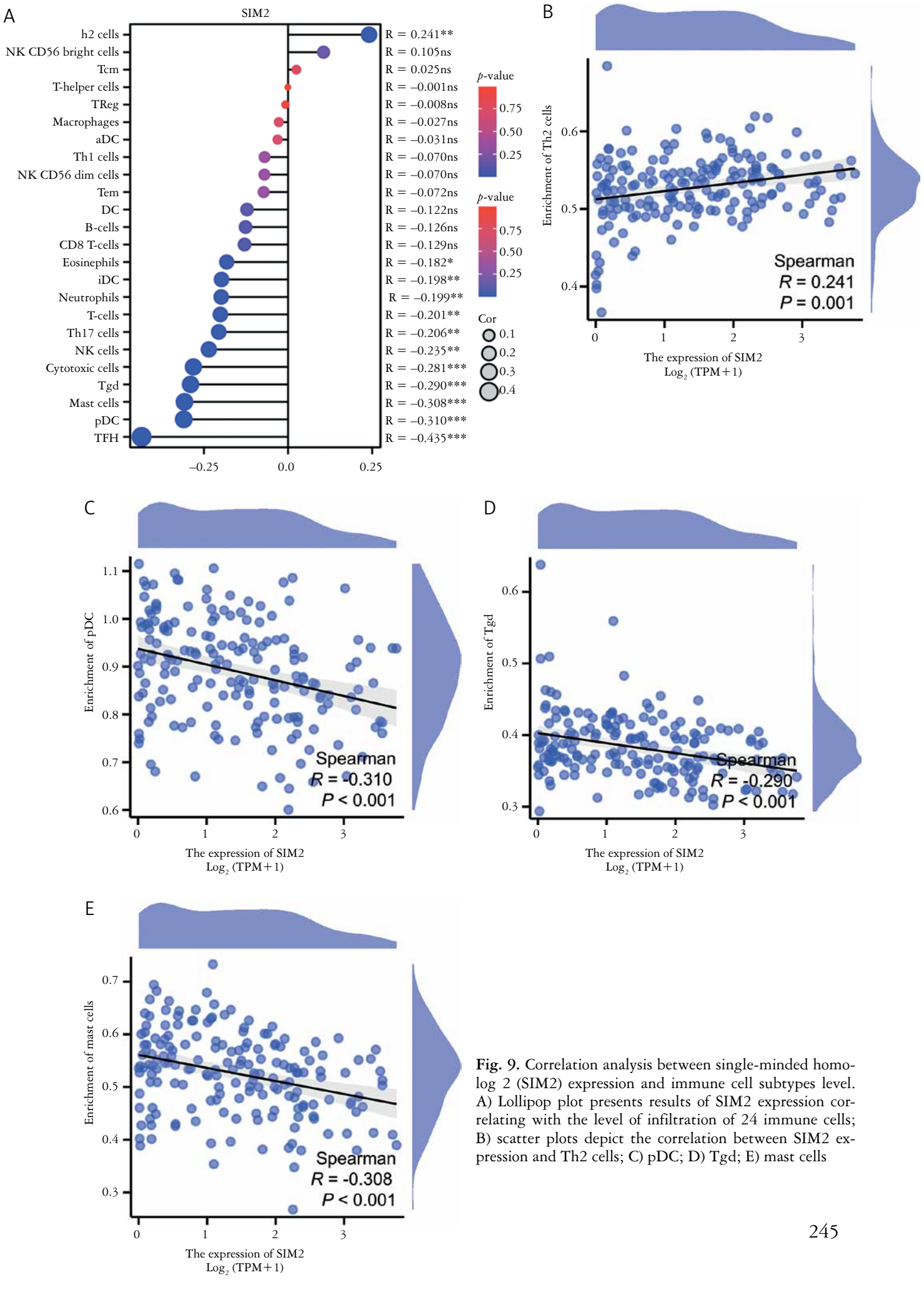

Fig. 9. Correlation analysis between single-minded homolog 2 (SIM2) expression and immune cell subtypes level. A) Lollipop plot presents results of SIM2 expression correlating with the level of infiltration of 24 immune cells; B) scatter plots depict the correlation between SIM2 expression and Th2 cells; C) pDC; D) Tgd; E) mast cells

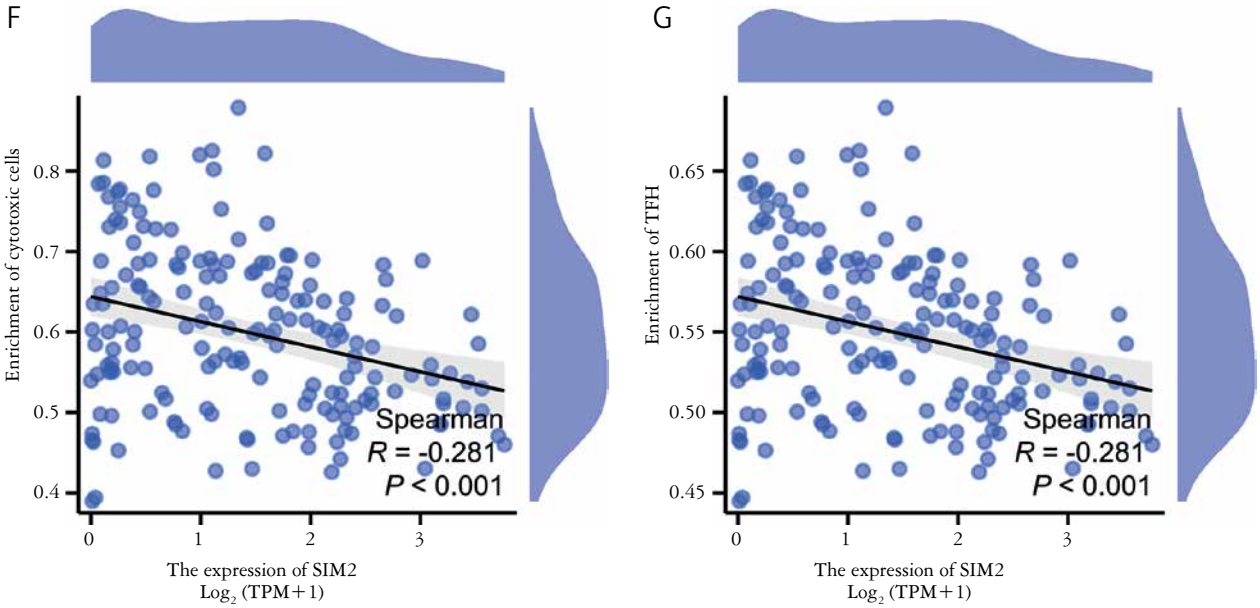

Fig. 9. Cont. F) cytotoxic cells; G) TFH

** $p < 0.05$*
*** $p < 0.01$*
**** $p < 0.001$*
SIM2 – single-minded homolog 2, TFH – T follicular helper, TPM – transcripts per million

lationship between SIM2 expression and prognosis in PC patients.

Conclusions

In conclusion, our results show that SIM2 may serve as a prognostic biomarker and is associated with immune cell tumor infiltration in PC. This provides a basis for future investigations into the role of SIM2 in the development and progression of PC.

The authors declare no conflict of interest.

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