# SULF2 Is a Prognostic Biomarker and Correlated with Tumor Associated Macrophages in Gastric Cancer

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# Abstract

**Background**: Recently, the mutual effects of tumor cells and the tumor immune microenvironment have been identified as key factors in promoting cancer progression. Sulfatase 2 (SULF2) encodes an extracellular endoglucosamine-6-sulfatase, which could remodel the highly sulfated domains of heparan sulfate. The abnormal expression of SULF2 is reported to play an important role in the carcinogenesis of many kinds of cancer. However, the prognostic value of SULF2 and its correlation with immune cell infiltration in gastric cancer (GC) remain unclear.

**Results**: SULF2 expression was significantly increased in GC compared with gastric normal tissue, especially in the advanced stage GC. In addition, high SULF2 expression significantly predicted an unfavorable prognosis in GC patients (overall survival P=0.0074), particularly who had metastatic lymph nodes. Besides, pathway analyses of SULF2 in GC revealed SULF2 may take part in extracellular structure organization, cell-cell adhesion and proteoglycans in cancer, etc. Importantly, the expression level of SULF2 was found to be positively correlated with the infiltration levels of tumor associated macrophages (TAMs). Moreover, SULF2 expression in GC positively correlated with expression of several immune cell markers, including monocyte markers, TAMs markers and programmed cell death 1 ligand 1 (PD-L1), suggesting its role in regulating tumor immunity.

**Conclusion**: This study identified distinct expression and prognostic values of SULF2 in GC using public databases. Significantly, our findings shed light on the role of SULF2 in GC progression and provided an underlying mechanism that SULF2 expression might modulate tumor immunity by regulating the infiltration of TAMs in GC.


# Background

GC is still the fifth most common cancer and fourth leading cause of cancer related death in the world[1]. Although a variety of treatment methods have been used in the past decades, the prognosis of patients with GC remains unsatisfactory, and the 5 year survival rate is about 30% around the world[2]. Unfortunately, there is a lack of clinically useful biomarkers to inform prognosis or aid treatment stratification for GC patients. Therefore, it is necessary to explore novel molecular prognostic factors and potential therapeutic targets for GC.

The tumor microenvironment (TME) consists of immune cells, inflammatory cells, fibroblasts and vascular endothelial cells, which could release various molecules to either directly activate the growth signaling or remodel extracellular matrix, favoring the growth and expansion of cancer cells[3]. The concept of GC treatment is changing with the deepening understanding of TME. Researchers are trying to explore various components and their interactions in the TME of GC and try to block some signal pathways, so as to improve the therapeutic effect of the tumor. Immunotherapy, such as nonspecific immune enhancer[4], cytokines[5], adoptive therapy of immune cells[6] and immune checkpoint inhibitor[7-9], has been associated with improved outcomes among a part of GC patients. However, the benefit

population is too small and needs to be further screened. The biomarkers for immunotherapy are still lacking in GC.

Sulfatase 2 (SULF2) gene at chromosome 20q13 encodes a protein with heparin-degrading endosulfatase activity. SULF2 could modify heparan sulfate glycosaminoglycan (HSGAG) chains by removing 6-O-sulfate groups from heparan sulfate disaccharide units, decreasing the affinity of Heparan sulfate proteoglycans (HSPGs) for heparan-sulfate binding ligands and releasing sequestered factors from storage sites[10]. In this way, SULF2 is involved in the modulation of ligand-receptor interactions and activation of downstream signaling pathways[11]. A detailed investigation of the association between SULF2 and infiltrating immune cells is needed.

In fact, promotion of tumor progression via the increased expression of SULF2 is established in several types of cancers, including hepatocellular carcinoma (HCC) [12], non-small cell lung cancer (NSCLC)[13], colorectal cancer[14], cervical cancer[15], breast cancer[16], pancreatic ductal adenocarcinoma[17], neuroblastoma[18] , and head and neck squamous cell carcinoma (HNSC)[19]. In HCC, SULF2 acts as an oncogenic protein by increasing Wnt3a and glypican-3 (GPC3) expression and activating the Wnt/β-catenin pathway, thus promoting growth of HCC cell lines and xenografts[20]. Besides, SULF2 induces the differentiation of hepatic stellate cells into carcinoma-associated fibroblasts (CAFs) through the TGF-β1/SMAD3 signaling pathway. Then SULF2-induced CAFs attenuated HCC apoptosis by activating the SDF-1/CXCR4/PI3K/AKT signaling pathway and induced epithelial-mesenchymal transition through the SDF-1/CXCR4/OIP5-AS1/miR-153-3p/SNAI1 axis[12]. Aside from that, in NSCLC, SULF2 activated TGF-β1/SMAD signaling pathway, which involved in the induction of migration and epithelial-mesenchymal transition[13]. Moreover, SULF2 expression increases VEGF-A release and activity in TME in colorectal cancer[21]. And SULF2 promotes tumorigenesis through the ERK/AKT signaling pathway in cervical cancer and colorectal cancer[14,15]. Besides, in breast cancer, SULF2 facilitated lymphangiogenesis by regulating VEGF-D and the AKT1-related signaling pathway was involved[16]. Therefore, SULF2 can serve as an independent risk factor and prognostic biomarker for different types of cancer. However, the prognostic and immunological significance of SULF2 in GC has not been elucidated.

In this study, we aimed to integrate a variety of bioinformatics methods to study whether SULF2 is involved in GC progression and immune infiltration. We found that the expression of SULF2 was significantly upregulated in GC tissues compared with normal tissues. The high expression of SULF2 was negatively correlated with the prognosis of GC patients. In addition, there was a significant relationship between the expression of SULF2 and the infiltration levels of tumor-associated macrophages (TAMs) in GC. Importantly, SULF2 seemed to affect the prognosis of GC patients partially through TAMs infiltration.

## Results

# SULF2 expression is increased in stomach adenocarcinoma (STAD) patients

To determine differences of SULF2 expression in various cancer types, we analyzed mRNA expression between different cancers and normal tissues in multiple cancers using Tumor Immune Estimation Resource (TIMER) database. Compared with the corresponding normal tissues, we observed higher expression of SULF2 in breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), HNSC, kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), STAD and thyroid carcinoma (THCA) (Figure 1A). To further verify the conclusion, we also consistently found that higher mRNA of SULF2 was expressed in STAD than in para-carcinoma tissues in the Gene Expression Profiling Interactive Analysis (GEPIA) and UALCAN databases (Figures 1B, C). Furthermore, the protein expression of SULF2 was investigated in STAD by immunohistochemical staining, and we found that the SULF2 protein levels were obviously increased in STAD tissues compared with para-carcinoma tissues (Figures 1D). These findings suggest that SULF2 expression is upregulated in multiple types of cancers, including STAD.

# Correlation of SULF2 expression and clinical parameters of STAD patients

By using the UALCAN online tool, we then investigated SULF2 expression among groups of patients according to different clinical parameters. SULF2 expression was significantly upregulated in STAD samples from all races including Caucasian, African-American and Asian, compared to the corresponding normal controls (Figure 2A). According to gender, SULF2 expression was significantly upregulated in STAD samples from both males and females (Figure 2B). In terms of age, the expression of SULF2 was dramatically elevated in the STAD tissues of patients from different age groups (41-60 years, 61-80 years and 81-100 years) (Figure 2C). Regarding cancer stage, a significant increase in SULF2 expression was observed in STAD patients in stage 2, 3 and 4, compared to the corresponding normal controls or STAD patients in stage 1 (Figure 2D). Based on nodal metastasis status, SULF2 expression was higher in patients with STAD classified as N0, N1 or N2 and N3 (Figure 2E). Upregulation of SULF2 expression was observed in the STAD tissues of patients from different differentiation status, including well differentiation, moderate differentiation and poor differentiation (Figure 2F). These results suggest that SULF2 expression may related to STAD progression.

# Increased SULF2 expression correlates with poor prognosis in STAD patients

Because the expression levels of SULF2 are closely related to the progression of STAD, we further examined the prognostic value of the SULF2 gene. STAD patients with higher expression of SULF2 gene exhibited faster first progression (FP) (Figure 3A), poorer overall survival (OS) (Figure 3B) and poorer post

progression survival (PPS) (Figure 3C) according to the Kaplan-Meier plotter database. These results indicate that SULF2 is significantly associated with the prognosis of STAD patients.

# Validation of the prognostic value of SULF2 based on various clinicopathological features

In order to better understand the prognostic value and potential mechanism of SULF2 expression in STAD, we used the Kaplan-Meier plotter database to explore the relationship between SULF2 mRNA expression and OS/FP according to clinical characteristics. High SULF2 expression was significantly correlated with poor OS and FP in male but not female STAD patients (Figure 3D). Regarding different cancer stages, high SULF2 expression was associated with poor OS and poor FP only in stage 3 STAD patients (Figure 3D). A significant correlation between SULF2 expression and poor OS was observed in American Joint Committee on Cancer (AJCC) T3 STAD patients (Figure 3D). Besides, high expression of SULF2 was associated with fast FP in T3 and T4 STAD patients (Figure 3D). In addition, high SULF2 expression was associated with poor OS and FP in STAD patients with N1, N2, N1-3 nodal metastasis status (Figure 3D). Upregulated SULF2 levels corresponded with poor OS and FP in M0 patients (Figure 3D). Based on Lauren classification, SULF2 upregulation was correlated with poor OS and poor FP in diffuse type of STAD patients, and correlated with poor FP in intestinal type (Figure 3D). Moreover, we found an association between SULF2 expression and unfavorable OS in poorly differentiated STAD patients (Figure 3D). These results imply that SULF2 mRNA expression possesses prognostic value in STAD.

# Identification of SULF2-interacting genes and proteins

We used the LinkedOmics database to identify RNA-seq genes co-expressed with SULF2 in STAD. We recognized the top 50 genes that positively or negatively interacted with SULF2 (Figure 4A, B). And we used GeneMania to construct a SULF2 correlation network to determine the potential mutual effects between SULF2 and cancer-related targets. The results showed that the 20 most frequently altered genes were closely correlated with SULF2, including SULF1, NOTCH1, and TNIP1 (Figure 4C). Functional analysis suggested that these genes were significantly associated with the sulfuric ester hydrolase activity (Figure 4C). In addition, a protein-protein interaction network of SULF2 was generated using the STRING database. There were 35 edges and 11 nodes, including GPC3, HS2ST1 and GLCE (Figure 4D). The results revealed that multiple differentially expressed genes were correlated with SULF2 expression.

# Enrichment analysis of SULF2 functional networks in STAD

Three independent ontologies (biological process, cellular component, and molecular function) were analyzed by gene set enrichment analysis. The results indicated that SULF2-related differentially expressed genes were involved in a variety of biological processes (extracellular structure organization,

cell-cell adhesion via plasma-membrane adhesion molecules, cell junction organization, etc.), cellular components (extracellular matrix, cell-cell junction, receptor complex, etc.), and molecular functions (extracellular matrix structural constituent, glycosaminoglycan binding, protein tyrosine kinase activity, etc.).We then used KEGG pathway analyses to evaluate the differentially expressed genes associated with SULF2 for potential functional pathways (focal adhesion, cell adhesion molecules, axon guidance, proteoglycans in cancer, etc.) (Figure 5). The enrichment analysis showed that the most important functional network of SULF2 is associated with extracellular matrix and intercellular interaction, which have been proved to play a critical role in tumorigenesis and cancer progression[22].

# Correlation analysis between SULF2 expression and infiltrating immune cells

To comprehensively investigate the role of SULF2 in STAD TME, we analyzed the correlation between SULF2 expression and six types of infiltrating immune cells, including B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells. The results showed that SULF2 expression levels had a significant positive correlation with the infiltration of CD4+ T cells, CD8+ T cells, macrophages and neutrophils, and no significant correlations with B cells and dendritic cells in STAD (Figure 6A).

# Correlation between SULF2 expression and various immune markers

To deepen our understanding of SULF2 crosstalk with the immune response, we used the TIMER database to verify the correlations between SULF2 expression and different immune signatures in STAD. The genes listed in Table 1 were used to characterize immune cells, including B cells, T cells, CD8+ T cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, natural killer (NK) cells and dendritic cells. In clinical cancer biopsies, tumor purity is an important aspect affecting the analysis of immune infiltration. After adjusting for tumor purity, SULF2 expression was significantly correlated with some immune markers of monocyte and macrophage in STAD (Table 1). We further investigated the interrelationship between SULF2 expression and famous T cell checkpoints, such as PD-L1, PD-1 and CTLA-4, in the GEPIA database. SULF2 expression was significantly associated with the expression of PD-L1 in STAD (Figures 6B). These findings further supported that SULF2 expression was significantly related to immune infiltration and indicated that SULF2 played a key role in immune escape in STAD.

**TABLE 1**

Correlation analysis between SULF2 and gene markers of immune cells in TIMER.

| Description | Gene markers | STAD | | | |
|---|---|---|---|---|---|
| | | NONE | | Purity | |
| | | Cor | p | Cor | p |
| **B cell** | CD19 | 0.052 | 0.29 | 0.046 | 0.37 |
| | CD79A | 0.032 | 0.52 | 0.01 | 0.85 |
| **T cell（general）** | CD3D | -0.007 | 0.89 | -0.042 | 0.41 |
| | CD3E | 0.041 | 0.41 | 0.007 | 0.89 |
| | CD2 | 0.058 | 0.24 | 0.025 | 0.63 |
| **CD8+ T cell** | CD8A | 0.057 | 0.25 | 0.017 | 0.74 |
| | CD8B | 0.04 | 0.42 | 0.021 | 0.69 |
| **Monocyte** | CD86 | 0.143 | ** | 0.11 | * |
| | CSF1R | 0.203 | *** | 0.183 | *** |
| **TAMs** | CCL2 | 0.162 | *** | 0.121 | * |
| | CD68 | 0.184 | *** | 0.165 | ** |
| | IL10 | 0.155 | ** | 0.127 | * |
| **M1** | IRF5 | 0.177 | *** | 0.168 | ** |
| | PTGS2 | 0.227 | *** | 0.205 | *** |
| | NOS2 | 0.007 | 0.90 | 0.012 | 0.82 |
| **M2** | CD163 | 0.24 | *** | 0.206 | *** |
| | VSIG4 | 0.22 | *** | 0.197 | *** |
| | MS4A4A | 0.166 | *** | 0.137 | ** |
| **Neutrophils** | CEACAM8 | 0.075 | 0.13 | 0.087 | 0.09 |
| | ITGAM | 0.287 | *** | 0.276 | *** |
| | CCR7 | 0.075 | 0.13 | 0.051 | 0.32 |
| **Natural killer cell** | KIR2DL1 | 0.017 | 0.74 | -0.012 | 0.82 |
| | KIR2DL3 | 0.021 | 0.67 | -0.008 | 0.88 |
| | KIR2DL4 | 0.021 | 0.67 | -0.022 | 0.67 |

| | | | | | |
|---|---|---|---|---|---|
| | KIR3DL1 | 0.08 | 0.10 | 0.053 | 0.30 |
| | KIR3DL2 | 0.023 | 0.64 | 0.005 | 0.94 |
| | KIR3DL3 | -0.005 | 0.92 | 0.007 | 0.90 |
| | KIR2DS4 | -0.015 | 0.76 | -0.05 | 0.33 |
| **Dendritic cell** | HLA-DPB1 | -0.01 | 0.84 | -0.044 | 0.40 |
| | HLA-DQB1 | 0.004 | 0.94 | -0.035 | 0.49 |
| | HLA-DRA | 0.007 | 0.89 | -0.022 | 0.67 |
| | HLA-DPA1 | -0.008 | 0.87 | -0.036 | 0.49 |
| | CD1C | 0.048 | 0.33 | 0.039 | 0.45 |
| | NRP1 | 0.35 | *** | 0.328 | *** |
| | ITGAX | 0.244 | *** | 0.218 | *** |

TAMs: tumor associated macrophages; M1: macrophages with M1 phenotype; M2: macrophages with M2 phenotype. *p < 0.05, **p < 0.01, ***p < 0.001.

# Discussion

GC is thought to be caused by the interaction of host genetic factors and complex environmental factors, and the importance of local tumor-host cell interactions in cancer biology is increasingly recognized[23]. Importantly, SULF2 is at the interface between the cancer cell and the tumor microenvironment. SULF2 performs post-synthetic editing of 6-O-sulfation on HSGAG chains, which liberate the HS-binding proteins, including VEGF, FGF, or Wnt, therefore modifying the interactions between signaling ligands and their cognate receptors[21]. Several pro-angiogenic factors like VEGFB, MMP-2, MMP-9, PDGFA and PDGFB were also overexpressed in SULF2-expressing cells[24]. SULF2 has been shown to have a cancer promoting effect in a variety of tumors, especially in hepatocellular carcinoma, but its role in gastric cancer has not been fully clarified. In GC, Hur et al[25] have previously reported that GC tissues showed higher expression of SULF2 (p = 0.001) compared to normal gastric mucosa with a small sample size, which was correlated with its promoter hypomethylation. And mice injected with SULF2-bearing cell lines showed a significantly higher tumor volume compared with controls at 10 weeks post-injection. However, the sample size is small and they didn't validate whether the expression of SULF2 is a prognostic in human GC patients. Besides, SULF2 CpG island methylation status may influence GC sensitivities to some chemotherapeutics, such as platinum and irinotecan regimen [26,27]. Our study further verified that SULF2 was highly expressed in gastric cancer, and showed that the expression levels of SULF2 were related to GC patient prognosis. Recruiting TAMs to escape immune regulation may be one of the mechanisms.

In the present study, we showed that the expression of SULF2 in GC was higher than that in normal gastric tissue in multiple databases (Figure 1A, B, C). These findings were consistent with the previous report and suggested that SULF2 may act as an oncogene by promoting the development and progression of GC[25]. And we further verified that the protein expression levels of SULF2 in tumor tissue were higher than that in adjacent normal tissues (Figure 1D, E). Subsequently, the clinical characteristics of SULF2 in GC patients was investigated. The results indicated that no matter which race, gender, age, lymph nodal metastasis status and histological grade are, SULF2 is highly expressed in GC compared to corresponding normal tissue (Figure 2). The expression levels of SULF2 in tumor tissues of stage 1 patients and that in normal tissues have no statistical significance, but different from that in advanced tumor tissues, suggesting that SULF2 plays a critical role in tumor progression instead of tumorigenesis. Furthermore, Kaplan-Meier survival analyses showed that GC patients with high SULF2 expression exhibited a markedly worse survival rate than those with low expression (Figure 3A, B, C). With high SULF2 expression, GC patients are more likely to have advanced stage, and the prognosis is worse after progression, especially in those GC patients with local lymph node metastasis but without distant metastasis (Figure 3D). These results indicated that SULF2 may be a prognostic biomarker in GC and may facilitate the development of targeted precision oncology.

We also identified top 50 genes co-expressed with SULF2 (Figure 4A). Several genes of them take part in GC progression, such as LATS2, GLI2 and SULF1. As the Hippo pathway transducer, LATS2, which is located in the centrosome and works for accumulation of γ-tubulin and formation of mitotic spindle, was reported to play a pivotal role in the promotion of GC cell migration [28]. The transcription factor GLI2, as a member of the Hedgehog signaling pathway, modulated several cytokine genes in the TME and also promoted GC tumorigenesis and progression[29,30]. Zheng et al have found that SULF2 Inhibitor 2,4-Disulfonylphenyl-tert-Butylnitrone has an antitumor effect via suppression of Hedgehog/GLI1 Signaling in HCC[31]. Besides, SULF1 was elucidated as a novel prognostic and lymph nodal metastasis predictive marker, and played an oncogenic role in GC[25]. A SULF2 correlation network was then constructed to identify potential interactions between SULF2 and cancer-related gene targets (Figure 4C). The most important function of the identified gene was sulfuric ester hydrolase activity, which corresponded to the function of SULF2. Due to the fact that SULF2 performs post-synthetic editing of HSGAG, PPI network may be more valuable than gene network (Figure 4D). In the PPI network, we can see GPC3, which was the most highly expressed HSPGs in HCC[32]. GPC3, released from HSPGs by SULF2, could exerts inflammatory activity by increasing the expression of NF-κB, CRP, TNF-α, IL-1β and IL-6. SULF2 expression, which was induced by p53, then promoted IL-6 expression by stabilizing β-catenin, followed by stimulation of the STAT3/Bcl-XL pathway[33]. Though SULF2 was confirmed to be a direct transcription target of p53[34], SULF2 might also regulate prion-like behavior of p53 through remodeling HSGAG S-domains in cancer[35]. This interaction between SULF2 and p53 may promote the progression of GC. In GC, IL-6 could increase the density of TAMs in TME[36], which was further clarified in the following text. Consistently, the results of enrichment analysis showed that SULF2 played an important role in tumor-host cell interaction in extracellular matrix (Figure 5).

The expression level of SULF2 was positively correlated with the infiltration of CD4 + T cells, CD8 + T cells, macrophages and neutrophils in GC (Figure 6A). And the correlations between SULF2 and immune markers of different immune cells were further validated (Table 1). The results indicated that TAMs were significantly related to SULF2, which tallied with the recognition that TAMs might predict a poor OS in GC[37]. As previously mentioned, we hypothesized that SULF2 could release some inflammatory cytokines, like IL-6, in the extracellular space by a direct or indirect means to recruit TAMs. And SULF2-mediated up-regulation of STAT3 may promotes TAMs polarization to the M2 phenotype[38,39], which has been reported to have ability to promote GC invasion, migration and angiogenesis[40-43]. According to the results of GO and KEGG, SULF2 is significantly related to the structure and function of extracellular matrix. The reasons might be as following: (1) The desulfation function of SULF2 directly regulates the components and functions of extracellular matrix. (2) TAMs recruited by SULF2 secrete matrix metalloproteinase[37], thus acting on extracellular matrix indirectly. Besides, the expression of PDL1 was associated with SULF2 (Figure 6B). And TAMs have been reported that it could increase the expression of PDL1 to help GC escape immune regulation[44]. These findings confirmed that SULF2 may help GC cell escape immune regulation via TAMs in TME, so as to promote tumor progression.

Chrysin was reported to have the ability to inhibit GC cells invasion[15]. There is a study suggested that chrysin has the antiproliferative activity against HCC through the suppression of SULF2[45]. So chrysin antiproliferative action against GC cells might also be attributed to the suppression of SULF2. Inhibitors of SULFs are under study[12], and, once these have been developed to the stage where they are suitable for cell-based and preferably in vivo studies, the therapeutic potential of SULF2 inhibition can be explored. It is possible that such compounds could counteract the effect of SULF2 by sequestering growth factors released in the extracellular and pericellular space[18]. At present, some studies have tried to improve the prognosis of GC patients by inhibiting TAMs infiltration, such as pexidatinib[46] and sophoridine[47]. We found a positive correlation between SULF2 and TAMs infiltration, so SULF2 may be used as a biomarker for targeted TAMs treatment in the future.

# Conclusions

Our study found the high expression of SULF2 in GC compared to normal tissue. And its high expression was related to the poor prognosis of GC. The potential mechanism may be direct or indirect releasing inflammatory factors in TME upon the desulfation function of SULF2, and then recruit TAMs, which leads to tumor progression. Therefore, these findings revealed that SULF2 may serve as a candidate prognostic for determining the prognosis of GC associated with immune infiltration.

# Methods

# TIMER

TIMER (http://timer.cistrome.org/) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types[48]. In the present study, we used the “Gene_DE” module to analyze SULF2 expression in multiple types of cancer. In STAD, the correlation of SULF2 and immune cell infiltration (B cells, CD8+ T cells, CD4+ T cells, neutrophils, macrophages, and dendritic cells) was evaluated through the “Gene” module. We also applied TIMER to investigate the relationship between SULF2 expression and different gene marker sets of immune cells by using the “GENE_Corr” module. The correlations of SULF2 expression with immune infiltration were evaluated by purity-correlated partial Spearman’s correlation and statistical significance.

# GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is a user friendly web portal for gene expression analysis based on TCGA and GTEx data[49]. In the current study, we used the module “Expression DIY” of GEPIA to investigate the expression of SULF2 between STAD and normal adjacent stomach tissue samples. Additionally, the relationships between SULF2 and PD-L1, PD-1 and CTLA-4 were determined using Spearman’s correlation coefficient in “correlation analysis”.

# UALCAN

UALCAN (http://ualcan.path.uab.edu/) is a an online site that provides in-depth analyses of levels of gene expression from TCGA database[50]. UALCAN was used to investigate SULF2 expression and the association between SULF2 and various clinicopathological parameters (race, gender, age, cancer stages, nodal metastasis status, tumor grade) of STAD.

# The Human Protein Atlas Database (HPA) Analysis

The HPA database (https://www.proteinatlas.org/) provides information on the distribution and expression of each protein in 48 normal human tissues and 20 tumor tissues through special antibodies and immunohistochemical techniques[51]. In the present study, immunohistochemical images of the SULF2 protein expression in clinical samples of patients with STAD and normal tissues were obtained from the HPA database.

# Kaplan-Meier Plotter Database Analysis

The Kaplan–Meier Plotter (http://kmplot.com/analysis/) was used to analyze the prognostic value of SULF2 in STAD[52]. The patient samples were divided into high and low groups by median expression to analyze FP, OS and PPS with hazard ratios, 95% confidence intervals and logrank p-values.

# LinkedOmics

The “LinkFinder” module of LinkedOmics (http://www.linkedomics.org/login.php) was used to identify differentially expressed genes related to SULF2 (N=415) in the TCGA STAD section[53]. The search and target datasets were obtained by RNA-seq, and the results were analyzed with the Pearson correlation coefficient. Enrichment analysis was performed for Gene Ontology and KEGG analyses through “LinkInterpreter” module.

# Analysis of SULF2-Interacting Genes and Proteins

We used the GeneMANIA database (http://www.genemania.org)[54] to construct the SULF2 interaction network and applied the STRING online database (https://string-db.org/)[55] to construct a protein-protein interaction network of SULF2.

## Abbreviations

SULF2: Sulfatase 2; GC: gastric cancer; TAMs: tumor associated macrophages; PD-L1: programmed cell death 1 ligand 1; TME: tumor microenvironment; HSGAG: heparan sulfate glycosaminoglycan; HSPGs: Heparan sulfate proteoglycans; HCC: hepatocellular carcinoma; NSCLC: non-small cell lung cancer; HNSC: head and neck squamous cell carcinoma; GPC3: glypican-3; CAFs: carcinoma-associated fibroblasts; STAD: stomach adenocarcinoma; TIMER: Tumor Immune Estimation Resource; BRCA: breast invasive carcinoma; CHOL: cholangiocarcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; READ: rectum adenocarcinoma; THCA: thyroid carcinoma; GEPIA: Gene Expression Profiling Interactive Analysis; FP: first progression; OS: poorer overall survival; PPS: poorer post progression survival; AJCC: American Joint Committee on Cancer; NK: natural killer; HPA: The Human Protein Atlas Database

## Declarations


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# Competing interests

The authors declare that they have no competing interests.

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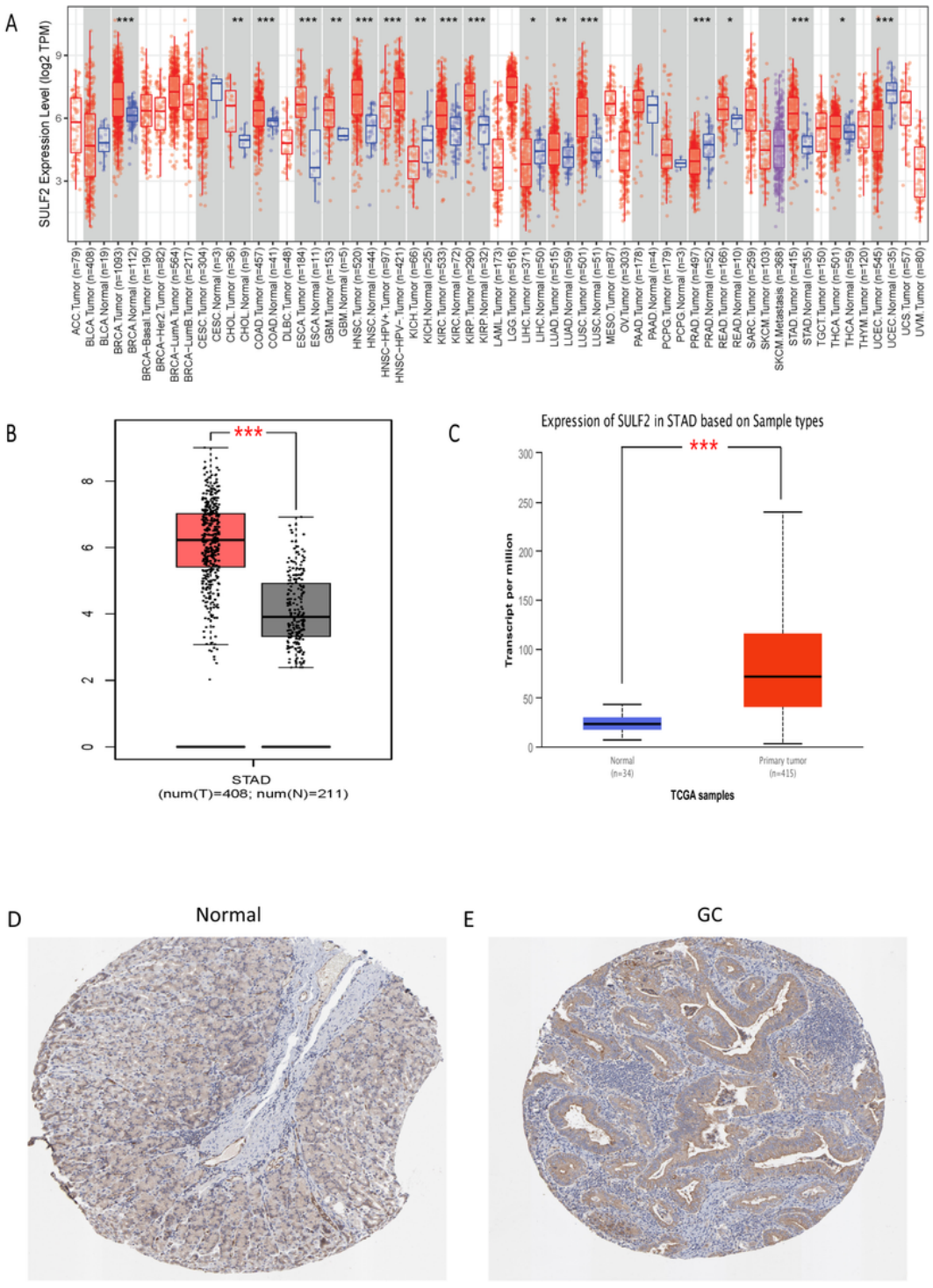
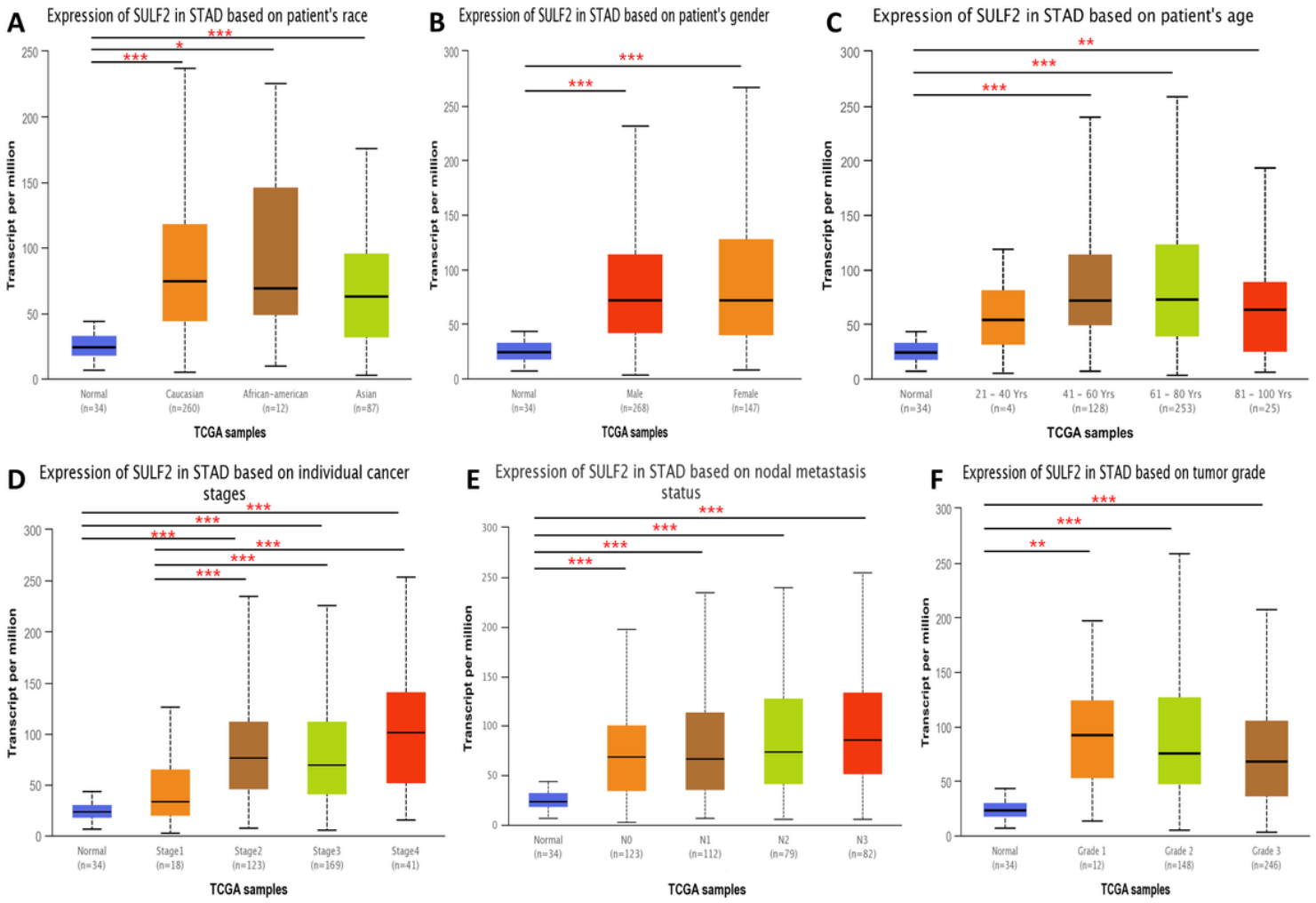
# Figures



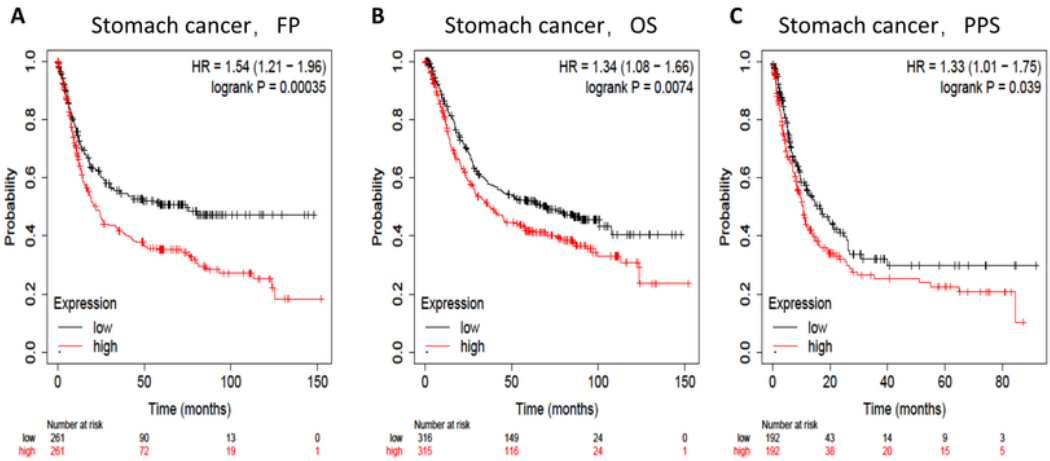

## Figure 1

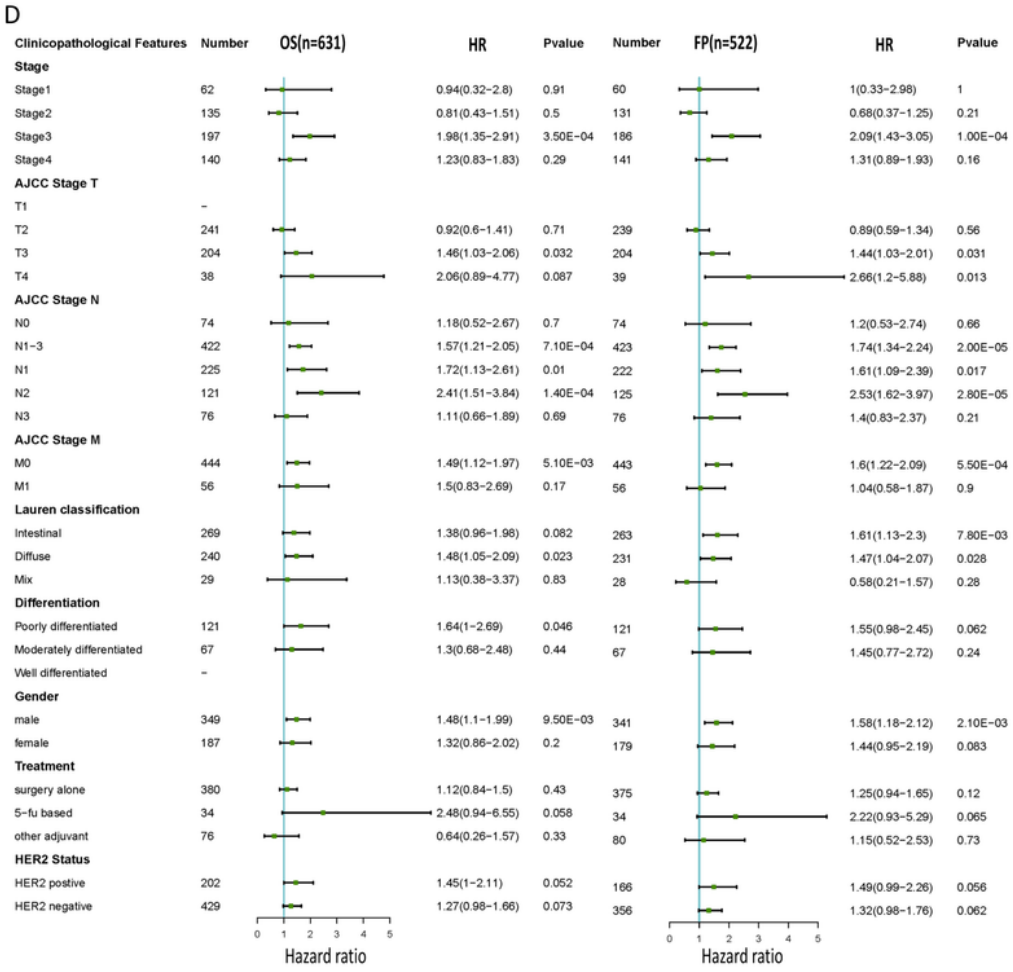
Expression of SULF2 in STAD. (A) SULF2 expression in different types of cancer was investigated with the TIMER database. (B) Increased expression of SULF2 in STAD was identified in the GEPIA database. (C) SULF2 expression in STAD was examined by using the UALCAN database. *p < 0.05, **p<0.01, ***p < 0.001. Immunohistochemical analysis of protein expression in normal tissues (D) and STAD (E) (The Human Protein Atlas Database).




## Figure 2

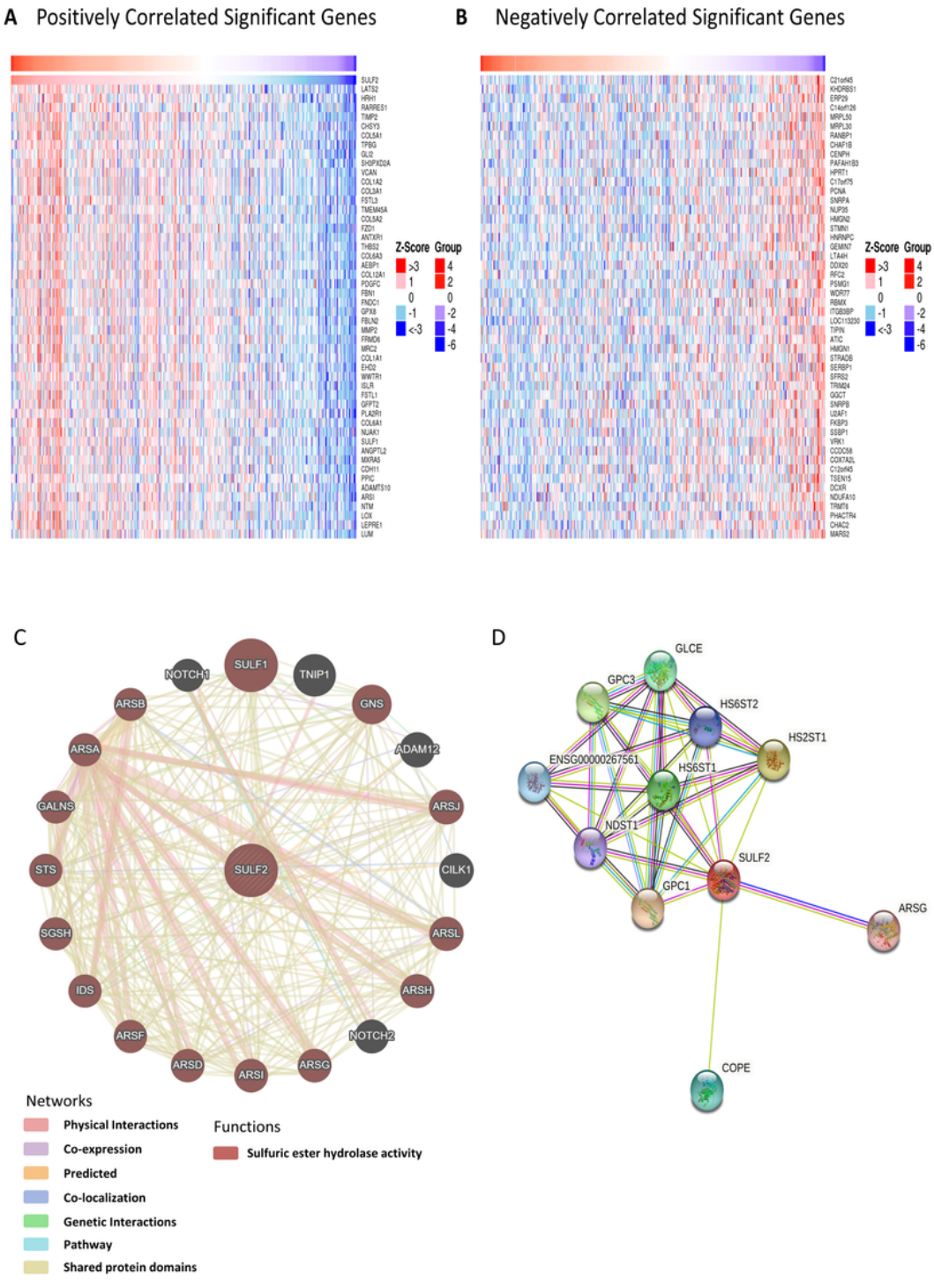
Box plots evaluating SULF2 expression among different groups of patients based on clinical parameters using the UALCAN database. Analysis is shown for race (A), gender (B), age (C), stages(D), nodal metastasis status(E), and tumor grade(F). N0: no regional lymph node metastasis; N1: metastases in 1 to 3 axillary lymph nodes; N2: metastases in 4 to 9 axillary lymph nodes; N3: metastases in 10 or more axillary lymph nodes. Grade 1: Well differentiated; Grade 2: Moderately differentiated; Grade 3: Poorly differentiated. *p < 0.05, **p < 0.01, ***p < 0.001.



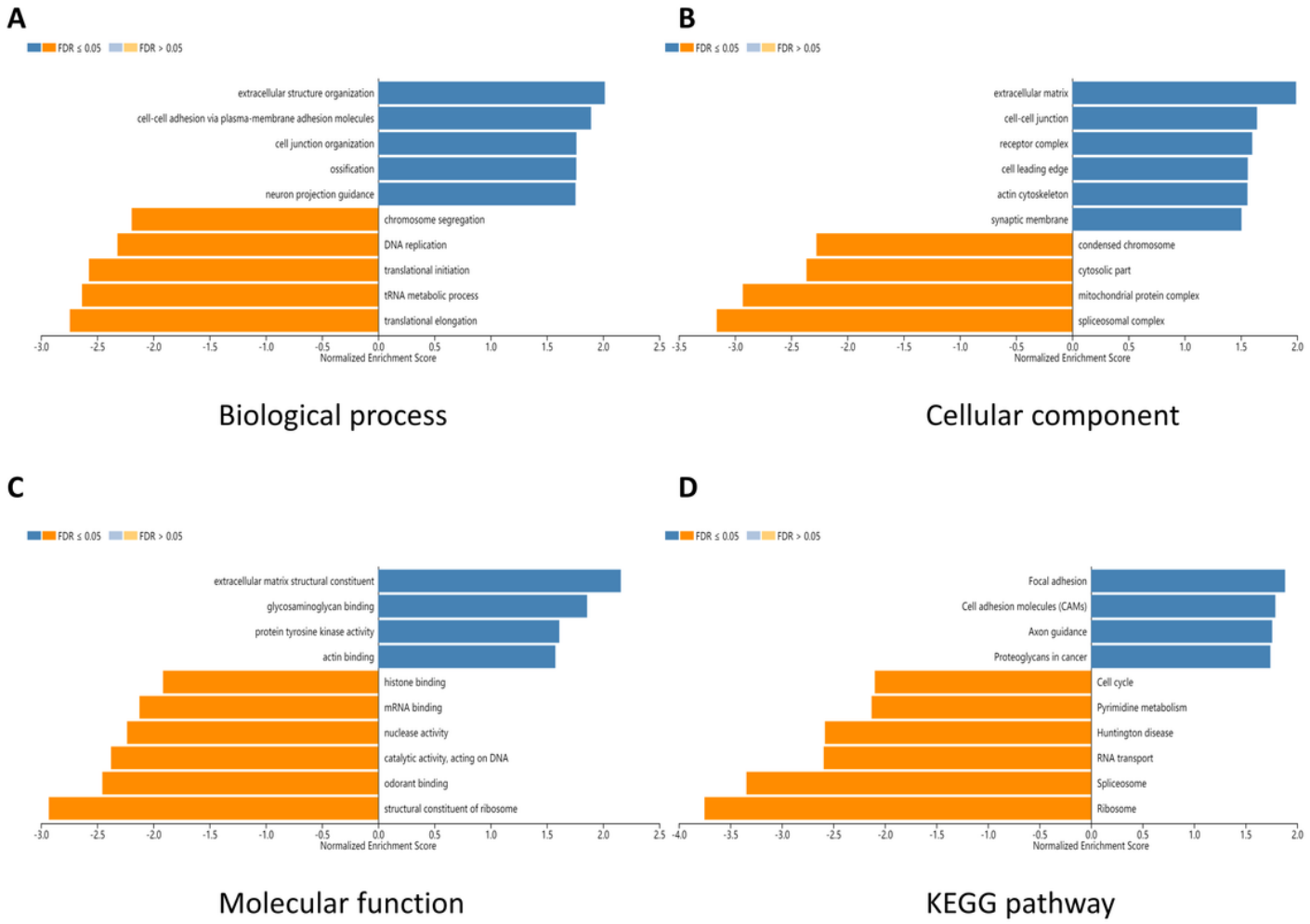

## Figure 3

Survival curve evaluating the prognostic value of SULF2. Survival curves using the Kaplan-Meier plotter are shown for FP (A), OS (B)and PPS (C). A forest plot shows the correlation between SULF2 expression and prognosis of STAD patients according to different clinicopathological parameters (D). FP: first progression; OS: overall survival; PPS: post progression survival.




## Figure 4

Genes associated with SULF2 expression (LinkedOmics). (A)Heat map of genes positively correlated with SULF2 in STAD. (B)Heat map of genes negatively correlated with SULF2 in STAD. (C) The gene-gene interaction network of SULF2 was constructed using GeneMania. (D) The PPI network of SULF2 was generated using STRING.




## Figure 5

Enrichment analysis of SULF2 functional networks in STAD (LinkedOmics). Enriched Gene Ontology annotations of SULF2-correlated genes in STAD: (A) Biological process. (B) Cellular component. (C) Molecular function. Enrichment pathway analysis of SULF2-correlated genes in STAD: (D) KEGG pathway.

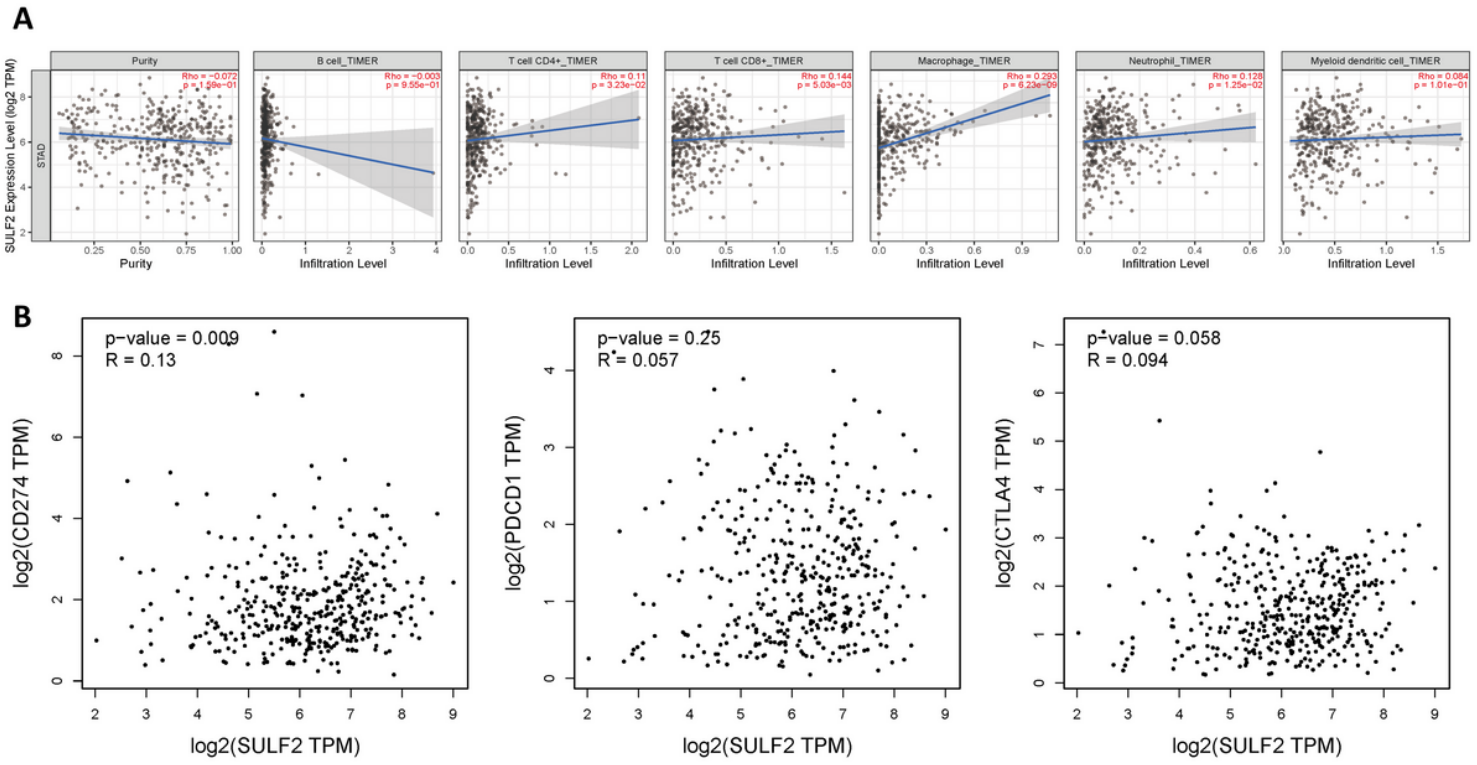



**Figure 6**

Correlation of SULF2 expression with immune infiltration level. (A) SULF2 is positively correlated with the infiltration of different immune cells using the TIMER database. (B) Scatterplots of the correlations between SULF2 expression and PD-L1, PD-1 and CTLA-4 in STAD using the GEPIA database.

# Supplementary Files

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