

SREBP2 regulates Gastric Cancer Progression and Immune Escape through TGF- β pathway–Mediated TAMs Polarization

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Research Article

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Abstract

Background and Objective

SREBP2 has been shown to regulate the progression of gastric cancer (GC). However, there is little knowledge about how SREBP2 induces immune evasion in gastric cancer by regulating TAMs M2 polarization.

Methods

The association of SREBP2 expression with the clinicopathological characteristics and prognosis in patients with GC was assessed by Western blot, Immunohistochemistry and public datasets. Flow cytometry was used to check the correlation between SREBP2 and TAMs, and it induced immune escape.

Results

SREBP2 is significantly upregulated in gastric cancer tissues and positively correlates with advanced disease stage and poor patient survival. Transcriptomic analyses reveal that SREBP2 expression is enriched in tumor-associated macrophages and is associated with an immunosuppressive tumor microenvironment characterized by M2 macrophage infiltration and upregulation of immune checkpoint molecules. Functionally, tumor-derived SREBP2 promotes the polarization of macrophages toward an immunosuppressive M2 phenotype and suppresses CD8⁺ T cell effector function via TGF- β pathway, thereby facilitating immune escape.

Conclusion

SREBP2 orchestrates gastric cancer progression by promoting TGF- β -dependent macrophage M2 polarization and suppressing antitumor T-cell immunity.

Clinical significance of this study

Unmet need. Gastric cancer (GC) is largely refractory to immune checkpoint inhibitors, primarily due to an immunosuppressive microenvironment driven by tumor-associated macrophages (TAMs). The molecular triggers that enable TAM-mediated immune evasion in GC remain elusive.

Key findings: SREBP2 is markedly overexpressed in GC, correlates with advanced stage and poor prognosis, and promotes M2 polarization of TAMs via TGF- β , which suppresses CD8⁺ T-cell function and facilitates immune escape.

Implications: SREBP2 may serve as a prognostic biomarker and a promising therapeutic target. Disrupting SREBP2 or its downstream TGF- β signaling could potentially reverse the immunosuppressive TAM phenotype and enhance the efficacy of immunotherapies in GC patients.

Introduction

Gastric cancer (GC) remains the fifth leading cancer for a major health challenge worldwide, both in terms of mortality and morbidity, accounting for nearly one million new cases diagnosed annually and over 650,000 deaths each year^{1, 2}. *Helicobacter pylori* infection, diet, obesity, smoking, and genetic predisposition are the key risk factors of GC. Minimally invasive surgery is increasingly used in operable gastric cancer, and at the same time, immune checkpoint inhibitors, targeted therapy, and next-generation immunotherapy offer hope for improving survival and quality of life. Although surgical treatment is central, comprehensive treatments including chemotherapy, immunotherapy, and targeted therapy have indeed brought survival benefits to gastric cancer patients, the recurrence rate after adjuvant therapy is high for many patients, and the prognosis for advanced GC patients remains unsatisfactory. Hence, there is an urgent need to search for potential strategies to optimize anti-tumor immunotherapy in GC.

In tumor initiation and growth, the TME acts as an important mediator. By targeting specific biomolecules or processes within the TME, tumor progression can be hindered at critical therapeutic sites³. TAMs are a key component of the TME, accounting for up to 50% of certain solid tumors⁴. TAMs mainly exhibit two phenotypes, M1 and M2. M1 macrophages produce type I pro-inflammatory cytokines (such as IL-1 β , IL-1 α , IL-12, and TNF- α), while M2 macrophages produce type II cytokines (such as IL-4 and IL-10) that promote anti-inflammatory responses and have pro-tumor functions⁵. TAMs mainly exhibit a pro-tumor M2 phenotype in the GC TME, which is a key factor in GC progression⁶, and increased TAM density is closely associated with a higher risk of metastasis and poor prognosis in GC^{7, 8}. TAMs also promote GC progression by secreting extracellular vesicles (EVs), cytokines, chemokines, and proteins⁹. Therefore, in-depth studies on the mechanisms of TAMs in gastric cancer and the improvement of the immune microenvironment in GC are key issues faced in the treatment of gastric cancer.

Sterol regulatory element-binding protein 2 (SREBP2) is a key transcription factor that controls cholesterol synthesis, uptake, and metabolism^{10, 11}. Studies have shown that SREBP2 is highly expressed in various tumors, promoting tumor cell autologous cholesterol synthesis to meet the rapid proliferation needs of tumors¹². SREBP2-mediated cholesterol metabolic reprogramming has become an important driving force for tumor progression. In macrophages, SREBP2 activation can induce cholesterol accumulation, promote M2 phenotype polarization, and suppress anti-tumor immunity¹³. In a colorectal cancer liver metastasis model, SREBP2 inhibitors can reverse the immunosuppressive phenotype of TAMs and enhance T cell infiltration¹⁴. However, the regulatory role of SREBP2 in gastric cancer TAMs and its association with immune evasion has not been systematically studied, and especially lacking is exploration of its upstream regulatory mechanisms.

In this study, we aimed to investigate the role of SREBP2 in the progression of GC and identify new immune checkpoint targets that can be used for TAM-targeted immunotherapy approaches. We found

that SREBP2 promotes M2 polarization of TAMs through the TGF-beta pathway, thereby facilitating immune escape in gastric cancer. These findings highlight this specific cell subtype as a predictive biomarker for metastasis detection and pave the way for the implementation of combined anti-angiogenic and immunotherapy strategies in clinical practice.

Materials and Methods

Cell Culture

Human gastric cancer cell lines HGC-27 and AGS, and the mouse gastric cancer cell line YTN16, were purchased from Prosperity Life Sciences Ltd. (Wuhan, China). HGC-27 cells were maintained in RPMI-1640, AGS cells in DMEM/F12, and YTN16 cells in DMEM (Gibco, China), each supplemented with 10% fetal bovine serum (FBS; BI, Israel) and 1% penicillin–streptomycin (Wisent, Canada). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂. All cell lines were authenticated and confirmed to be mycoplasma-free before use.

Western Blotting

Protein of GC tissue samples and cells using the fast protein extraction kit (WB3050, NCM Biotech, Suzhou, China). The supernatants were resolved in SDS-PAGE and transferred onto Polyvinylidene fluoride (PVDF) membranes (IPVH00010, Millipore, MA, USA). The membranes were probed with anti-SREBP2(1:1000, Cat No. 28212-1-AP, Wuhan, China), anti-CD163(1:1000, Cat No. 16646-1-AP), anti-CD80(1:1000, Cat No. 66406-1-Ig, Wuhan, China) and anti-GAPDH (1:1000, AB-P-R 001, Hangzhou, China) overnight at 4°C. Protein bands were shown by the enhanced chemiluminescence (ECL) method.

Immunohistochemical and Multiple Immunohistochemical

For IHC, the tissue microarray was deparaffinized, rehydrated, and subjected to antigen retrieval by microwave heating in sodium citrate buffer (10 mmol/L, pH 6.0). The slides were then incubated with a mouse anti-SREBP2 monoclonal antibody (1:1000, Cat No. 28212-1-AP, Wuhan, China) using a SABC (mouse IgG) FITC immunohistochemistry kit (Lot No. A0130, Wuhan, China).

For mIHC, SREBP2, and CD163 were performed on specimens from human GA patients.

Cell Proliferation, Migration, and Invasion Assays

These assays were conducted as previously reported¹⁵.

Bioinformatics Data Acquisition and Processing

Pan-cancer RNA-seq (raw counts and TPM), somatic mutation data, and clinical metadata were retrieved from the UCSC Xena Pan-Cancer Atlas. TPM values were normalized and $\log_2$ (TPM + 1) transformed. GTEx was used to obtain expression profiles of normal tissues. Single-cell expression data were visualized using TISCH2.

Differential Expression Analysis

Tumor and normal SREBP2 expression levels were compared using TIMER2.0 and SangerBox. Diagnostic performance was assessed via ROC curves (pROC v1.18.0), and the AUC with 95% CI was derived from 1,000 bootstrap iterations.

Survival Analysis

The relationship between SREBP2 expression and overall survival (OS) as well as disease-free survival (DFS) was assessed using GEPIA2 and KM-Plotter, with hazard ratios (HRs) and 95% confidence intervals (CIs) determined by Cox proportional hazards regression models.

Protein–Protein Interaction Network

A PPI network was generated using STRING (minimum interaction score 0.4), integrating experimental data, text mining, co-expression analyses, and curated databases.

Immune Cell Infiltration

ESTIMATE was used to calculate immune and stromal scores. The relative abundance of 22 immune cell types was quantified with CIBERSORT.

KEGG Enrichment analysis

“clusterProfiler” R package was utilized to annotate the genes with KEGG. Statistical threshold was set as P value < 0.05.

Flow Cytometry

Apoptosis Assay

Apoptosis was measured using an Annexin V–APC/7-AAD Detection Kit (AP107, MultiSciences). Cells were harvested with Accutase, washed, stained with Annexin V–APC and 7-AAD in binding buffer, incubated for 20 min at room temperature in the dark.

Macrophage Polarization: To generate TAMs, THP-1 monocytes were treated with 100 ng/mL PMA (Sigma) for 48 hours to generate M0 macrophages. Conditioned media from AGS or HGC-27 cells transfected with siSREBP2 or control siRNA were then applied for 24 hours to induce TAM-like

polarization. Cells were subsequently stained with antibodies against CD68 (11-0689-42), CD14 (415453), CD163 (12-1639-42), and CD80 (46-0809-42) (all from eBioscience).

CD8⁺ T-Cell Co-culture

Splenic CD8⁺ T cells were isolated from C57BL/6J mice using the EasySep™ CD8⁺ T-Cell Positive Selection Kit II (StemCell, #18953). The cells were then activated with anti-CD3ε (BioLegend, #110340) and anti-CD28 (BioLegend, #102116) for 72 hours, followed by co-culture with TAMs for an additional 24 hours. Subsequently, cells were stained for CD3, CD8, TNF-α, and IFN-γ.

siRNA Transfection

The negative vector and si-NC were indicated as the control group. GC cell lines (AGS and HGC-27) were planted in 6-well plates 24 h before si-SREBP2, or inhibitor transfection with 50–60% confluence, and then mixed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions¹⁵.

RNA Extraction and Quantitative PCR (qPCR)

AGS, HGC-27, and TAMs were seeded in 6-well plates and cultured until they reached 50% confluence after 24 hours. Total RNA was extracted at 4-hour intervals and analyzed by qRT-PCR. Actin was used as the internal reference, and relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Ethical statement

Human Experiment: tumor tissues and matched adjacent non-tumor gastric tissues were collected from 100 patients with pathologically confirmed gastric cancer at the Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangxi Medical University (Nanning, China). Immediately after surgical resection, all samples were snap-frozen in liquid nitrogen and stored at -80°C until further analysis. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Animal Experiments: All animal experiments were conducted in accordance with institutional guidelines and approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University. Male C57BL/6J mice (4 weeks old, Guangxi Medical University, China) were randomly assigned to two groups ($n = 3$ per group) to establish a syngeneic mouse model. Each mouse was inoculated subcutaneously in the right flank with 5×10^6 YTN16 cells stably expressing either SREBP2-targeting shRNA or control shRNA, with or without concurrent administration of SRI-01138124 (30 mg/kg, intraperitoneal injection, once every 2 days for 20 days). Tumour dimensions were measured every 5 days over 20 days. Mice were euthanized when tumour volumes reached $50\text{--}100\text{ mm}^3$. Euthanasia was performed by gradual exposure to 100% CO_2 (introduced at a rate displacing 10–30% of the chamber volume per minute), allowing the animals to lose consciousness painlessly in a hypoxic environment and

subsequently succumb to CO₂ anesthesia and asphyxiation. Excised tumours were weighed, photographed, and processed for subsequent analyses.

The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University.

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Statistical Analysis

In brief, the values are expressed as the mean $\pm$ standard error of the mean (SEM). Students' tests and analysis of variance were used for comparisons between groups. Kaplan–Meier analysis was used to assess the association of SREBP2 with GC prognosis. All data were analyzed using two-tailed tests. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression models. Statistical analyses were performed using R 4.0.1 (R Foundation for Statistical Computing), CaseViewer 2.2 (3DHISTECH Ltd.), QuPath 0.4.3, and GraphPad Prism 8.0 (GraphPad Software). $p < 0.05$ was considered statistically significant.

Results

1. SREBP2 promotes gastric cancer cell proliferation, migration, and survival

In addition to SREBP2's functions, we examined the behavior of SREBP2 in gastric cancer cells (AGS and HGC-27). Immunofluorescence (IF) analyses revealed that the subcellular localization of SREBP2 is predominantly expressed in the nucleoplasm, vesicles, and endoplasmic reticulum of GC cell lines, consistent with its role in GC using the Human Protein Atlas website (<https://www.proteinatlas.org>) (Fig. 1A–B). Subsequently, we transfected siRNA to construct SREBP2-silenced (siSREBP2) AGS (Fig. 1C) and HGC-27 (Fig. 1D) cell lines, as determined by Western blot and quantitative PCR (qPCR). Our results indicated that SREBP2 knockdown significantly suppressed GC cell proliferation, as assessed by Edu (colony-formation) assays (Fig. 1E). Moreover, siSREBP2 markedly impaired GC cells' migration (Fig. 1F) and invasion (Fig. 1G) in Transwell and wound-healing assays. In addition, apoptosis detection results showed that after SREBP2 knockdown, the number of Annexin V/PI positive cells increased, and the expression of pro-apoptotic markers was upregulated (Fig. 1H). In summary, the above findings suggest that SREBP2 exerts a pro-survival function in gastric cancer cells by inhibiting apoptosis, thereby promoting the malignant progression of gastric cancer.

2. SREBP2 is positively associated with poor prognosis in Gastric Cancer

Subsequently, we collected the GC patients' tumor and adjacent tissues, and analysis of immune cell populations revealed that adjacent non-tumor tissues contained higher frequencies of effector CD8⁺ T cells expressing TNF- α , IFN- γ , granzyme B, and Ly-108 (Fig. 2A–D). In contrast, tumor tissues were enriched in immunosuppressive CD163⁺ (Fig. 2E) and CD206⁺ (Fig. 2F) TAMs, which was confirmed with

Gambardella et al.⁶ results that TAMs mainly exhibit a pro-tumor M2 phenotype in GC microenvironment. Hematoxylin and Eosin Staining (HE) and immunohistochemical (IHC) analyses further demonstrated that higher SREBP2 expression was spatially within tumor tissues (Fig. 2G-H) compared with adjacent tissues. Results of Western blot (Fig. 2I), qPCR (Fig. 2J), and TCGA database (Fig. 2K) were confirmed with higher expression of SREBP2 in GC tumor tissues. Furthermore, overall survival (OS) analysis revealed that higher expression of SREBP2 has a poor prognosis in gastric cancer using the TCGA database (Fig. 2L-M). These findings suggested that SREBP2 may promote immune evasion in gastric cancer by fostering an immunosuppressive microenvironment characterized by the enrichment of M2 macrophages and impaired CD8⁺ T cell activity, which induced the poor prognosis of GC. Next, we attempted to elucidate the molecular mechanism of SREBP2-mediated immunosuppression.

3. SREBP2 Is Enriched in Tumor-Associated Macrophages and Correlates with an Immunosuppressive Tumor Microenvironment

To define the cellular source of SREBP2 more precisely, we analyzed single-cell RNA-sequencing datasets from gastric cancer specimens (**GSE167297**) (Fig. 3A). SREBP2 expression was predominantly localized to macrophage populations, particularly tumor-associated macrophages (TAMs), with minimal expression in malignant epithelial cells or lymphocytes (Fig. 3B-C). Consistent with these findings, there were a strong correlation between SREBP2 expression and ImmuneScore (Fig. 3D), StromalScore (Fig. 3E), and ESTIMATEScore (Fig. 3F), which reveals a significant correlation between SREBP2 expression and the non-tumor cellular elements within the GC tumor microenvironment (Fig. 3A-F). Some studies have shown that alterations in the SREBP2 pathway can lead to cholesterol deficiency in T cells, which in turn triggers abnormal metabolic and signaling pathways, promoting T cell exhaustion/dysfunction in the tumor microenvironment¹⁶. We also analyzed the correlation between SREBP2 and immune cells (Fig. 3G) and immune checkpoint genes (Fig. 3G). These genes may interact with SREBP2, triggering abnormal metabolic and signaling pathways and promoting the exhaustion or dysfunction of T cells in the tumor microenvironment.

4. SREBP2 promoted M2-type macrophage polarization in GC

In TME, macrophages could be polarized to M1 or M2-type macrophages¹⁷, change the M2 to M1 polarization could inhibit the progression of GC. To check the role of SREBP2 in TAMs, human THP-1 monocytes were differentiated into M1 and M2 subtypes of macrophages after induction by AGS and HGC-27 cells condition medium. To further explore the mechanism of SREBP2 in macrophages, we constructed cell lines with stable silencing of SREBP2 in THP1 cells (siSREBP2). We used Flow Cytometry, qPCR, and western blot to explore the expression of chemokines and cytokines, which were examined to characterize different macrophage subtypes. Flow cytometry analysis showed that CD163 was decreased and CD80 was increased in macrophages with SREBP2 silencing (Fig. 4A-D). The mRNA level of M2 markers (CD274, IL-10, TGF- β , ARG1, and MRC1) were decreased in M0 macrophages (induced by THP1 cells) with siSREBP2 (Fig. 4E), while the expression of M1 markers (TNF- α , IL-1 β , NOS, iNOS, and IFNG) was increased (Fig. 4F). Furthermore, the protein expression of CD163 was decreased,

and CD80 was increased, respectively. These results indicated that SREBP2 promoted M2-type macrophage polarization.

5. SREBP2 promoter GC immune escape induced by M2 TAMs via TGF- β Signaling in vitro

To explore how SREBP2 regulates TAMs M2 polarization, we analyzed the genes positively (Fig. 5A) and negatively (Fig. 5B) correlated with SREBP2. We also investigated the associated protein-protein interactions (Fig. 5C), cellular functions (Fig. 5D-F), and enriched pathways (Fig. 5G-H) with SREBP2 in GC. These findings suggest that SREBP2 is closely linked to macrophage biology within the GC microenvironment via TGF- β , leading us to investigate whether SREBP2 regulates TGF- β directly. Our results indicated that siSREBP2 reduced the protein expression and mRNA levels of TGF- β in AGS (Fig. 6A) and HGC-27 (Fig. 6B) cells. Then we used the SR1-011381 (an activator of TGF- β ¹⁸) to explore whether it could attenuate the role of siSREBP2. Our qPCR and Western blot results found that SR1-011381 could increase the protein expression and mRNA levels of M2 markers (Fig. 6C) and decrease M1 markers (Fig. 6D) induced by siSREBP2. And Flow cytometry results showed SR1-011381 could increase the % of CD163⁺TAMs (Fig. 6E) and CD206⁺TAMs (Fig. 6F). Finally, we used siSREBP2 TAMs co-culturing with CD8⁺ T cells after being treated with SR1-011381, we found that SR1-011381 could reduce the % of TNF- α ⁺ CD8⁺ T cells (Fig. 6G) and IFN- γ ⁺ CD8⁺ T cells (Fig. 6H). These results indicated that the SREBP2 promoter GC immune escape induced by M2 TAMs was mediated via TGF- β Signaling in vitro.

6. SREBP2 promoter GC immune escape induced by M2 TAMs via TGF- β Signaling in vivo

To validate the function of SREBP2 in vivo, we constructed SREBP2 cells with stable silencing of SREBP2, which were induced into YTN16 cells and subcutaneously injected into mouse to establish a xenograft tumor model (Fig. 7A). The results showed that siSREBP2 significantly decreased both the weight (Fig. 7B) and volume (Fig. 7C) of the tumors, while these effects were attenuated when treated with SR1-011381. To further investigate the role of SREBP2 in the growth of primary gastric cancer tumors, we prepared single-cell suspensions from gastric cancer tumor tissues and examined the functions of TAMs and CD8⁺ T cells. Our Flow cytometry showed that siSREBP2 reduced the % of CD163⁺ TAMs (Fig. 7D) and CD206⁺ T TAMs (Fig. 7E), while SR1-011381 recovered the % of CD163⁺ TAMs and CD206⁺ T TAMs. Interestingly, the opposite result was found in the proportion of TNF- α ⁺ CD8⁺ T cells (Fig. 7F) and IFN- γ ⁺ CD8⁺ T cells (Fig. 7G) in CD8 T cells. Furthermore, multiplex fluorescence immunohistochemistry was used to assess the spatial distribution of SREBP2⁺CD163⁺CD8⁺ in human GC adjacent (Fig. 7H) and tumor tissue (Fig. 7I). Then, we analyzed the positive correlation between the drug sensitivity of Sonidegib (Fig. 8A), Aminoflavone (Fig. 8B), PLX-4720 (Fig. 8C), Oxaliplatin (Fig. 8D), and Enzalutamide (Fig. 8E), and SREBP2 (Fig. 8F) in GC. These findings demonstrate that SREBP2 promotes tumor growth in vivo via TGF- β signaling, suggesting that inhibiting SREBP2 may represent a potential therapeutic target for gastric cancer.

Discussion

Gastric cancer is one of the most aggressive and lethal malignancies in the digestive system, with a higher incidence in males than in females¹⁹. *Helicobacter pylori* infection, unhealthy diet, high alcohol intake, and smoking are risk factors, while the occurrence and progression of GC involve complex interactions among numerous steps and genes. The high mortality rate of GC and its resistance to existing chemotherapeutic drugs highlight the urgent need to develop new anti-tumor drugs aimed at reducing tumor resistance and enhancing the efficacy of chemotherapeutic agents^{20, 21}.

Cholesterol is an important component of the cell membrane and plays a significant role in tumor growth, apoptosis, invasion, adhesion, and migration, highlighting its importance in cancer biology. Dysregulation of cholesterol homeostasis is associated with the occurrence and progression of cancer. The biosynthesis of cholesterol is regulated by major transcription factors known as SREBPs, which control genes involved in cellular cholesterol uptake and biosynthesis. SREBP2 plays a key role in cancer progression through various metabolic pathways, such as skin cancer²², ovarian cancer²³, esophageal cancer²⁴, prostate cancer, breast cancer^{25, 26}, and hepatocellular carcinoma²⁷. Recent studies showed that SREBP2 promotes lymph node metastasis of gastric cancer through lipid synthesis²⁸, and it is involved in enhancing gastric cancer stem cells' evasion of cytotoxicity mediated by immune cells²⁹. SREBP2 activation can induce cholesterol accumulation, promote M2 phenotype polarization, enhance T cell infiltration, and suppress anti-tumor immunity^{13, 14}. However, the regulatory role of SREBP2 in gastric cancer TAMs and its association with immune evasion has not been systematically studied, especially lacking exploration of its upstream regulatory mechanisms.

In this study, we analyzed the role of SREBP2 in GC. Our results found that SREBP2 is mainly expressed in the cytoplasm and cell membrane, consistent with its function in extracellular signaling and immune modulation. This expression pattern differs somewhat from that reported in the HPA database, possibly due to the state of the cells or because SREBP2 has not yet been activated to enter the nucleus. SREBP2 was overexpressed in GC patients, and silencing of SREBP2 inhibited the proliferation, invasion, and migration of AGS and HGC-27 cells, while it promoted the % of apoptosis of GC cells, which was associated with poor outcomes. These findings suggested that SREBP2 may exert oncogenic potential in GC.

Subsequently, we constructed an in vivo mouse model to investigate the role of SREBP2 in GC, and the in vivo study further confirmed the correlation of SREBP2 with its pro-cancer function. In the YTN16 mouse gastric cancer xenograft model, knocking down SREBP2 inhibited tumor growth, significantly reducing tumor volume and weight, and decreased infiltration of M2 tumor-associated macrophages (CD163⁺ and CD206⁺) while promoting CD8⁺ T cell activity (TNF- α ⁺ and IFN- γ ⁺), reinforcing the role of SREBP2 in the pro-tumor immune microenvironment. Analysis of patient tumor specimens showed that regions with high SREBP2 expression had elevated M2 markers and reduced effector CD8⁺ T cell activity. Our mIHC results demonstrated high SREBP2 expression in GC tumor tissues, which was positively correlated with CD163 and negatively correlated with CD8, whereas the opposite was observed in adjacent non-tumor tissues. These results link SREBP2 expression to immune evasion in human gastric cancer.

GO analysis and KEGG enrichment analysis showed that cytokine–cytokine receptor interaction is the pathway most significantly associated with SREBP2. In AGS and HGC-27 cells, knockdown of SREBP2 reduced TGF- β expression at both the mRNA and protein levels; thus, we mechanistically identified transforming growth factor- β (TGF- β) as a downstream gene of SREBP2 and explored the key role of TGF- β in SREBP2-driven immunosuppression. To determine whether TGF- β is a key factor for SREBP2 function, we investigated SREBP cells treated with the agonist SR1-011381 (an activator of TGF- β). Activation of TGF- β signaling reversed the effects of SREBP2 silencing, suppressing M1 markers (TNF- α , IL-1 β , iNOS, IFNG) while restoring the protein expression and mRNA levels of M2 macrophage polarization markers (CD274, IL-10, ARG1, MRC1). Flow cytometry results showed that SR1-011381 reversed the siSREBP2-induced proportion of CD163⁺TAMs and CD206⁺TAMs. Subsequently, after co-culturing TAMs with CD8 T cells, we found that TGF- β activation weakened the enhancement of CD8⁺ T cell effector function induced by siSREBP2. In vivo, we found the same results, which confirms that SREBP2 promotes immune evasion through a TGF- β -dependent mechanism. These findings are consistent with previous studies reporting the immunosuppressive role of TGF- β in tumors²⁵, including the inhibition of NK cell function.

In summary, SREBP2-mediated TGF- β promotes M2 polarization of TAMs and the CD8 T cell function suppression induced by them, and facilitates immune evasion in gastric cancer. Therefore, the SREBP2/TGF- β signaling pathway may be a potential target for gastric cancer.

Abbreviations

Extracellular vesicles, **EVs**; Gastric Cancer, **GC**; Immunohistochemistry, **IHC**; Immunofluorescence, **IF**; Sterol regulatory element-binding protein 2, **SREBP2**; Tumor-Associated Macrophages, **TAMs**; Tumor Microenvironment, **TME**; Transforming Growth Factor-beta, **TGF- β** .

Declarations

Ethics considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki. All experimental protocols involving human participants and all animal experiments were reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University, with separate approval numbers for human and animal studies. All animal work was carried out in compliance with the institutional guidelines for the care and use of laboratory animals.

Informed consent statement

Written informed consent was obtained from all individual participants included in the study before sample collection. All participants were informed of the study objectives, procedures, potential risks, and their right to withdraw at any time without consequence. Tumor tissues and matched adjacent non-tumor gastric tissues were collected from 100 patients with pathologically confirmed gastric cancer

who underwent surgical resection at the Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangxi Medical University (Nanning, China). All personal identifiers were removed from the datasets before analysis to ensure participant confidentiality.

Data Availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to restrictions imposed by the ethics committee to protect patient privacy and confidentiality but are available from the corresponding author upon reasonable request. The single-cell RNA-sequencing dataset GSE167297 analyzed in this study is publicly available in the Gene Expression Omnibus (GEO) repository (<https://www.ncbi.nlm.nih.gov/geo/>). The TCGA-STAD data used for survival and differential expression analyses are accessible through the UCSC Xena Pan-Cancer Atlas (<https://xena.ucsc.edu/>) and GEPIA2 (<http://gepia2.cancer-pku.cn/>). Additional public databases utilized include TIMER2.0 (<http://timer.cistrome.org/>), KM-Plotter (<https://kmplot.com/analysis/>), and the Human Protein Atlas (<https://www.proteinatlas.org/>). All other data supporting the findings of this study are available within the paper and its supplementary materials.

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Author contributions

TNN designed the research and conducted the experiments. TNN and LCM drafted and revised the manuscript. TNN, LCM, and LLW collected the data. LLW provided the statistical software and performed the data analysis. All authors read and approved the final manuscript.

Disclosure Statement

The authors declare no conflict of interest in this study.

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Figures

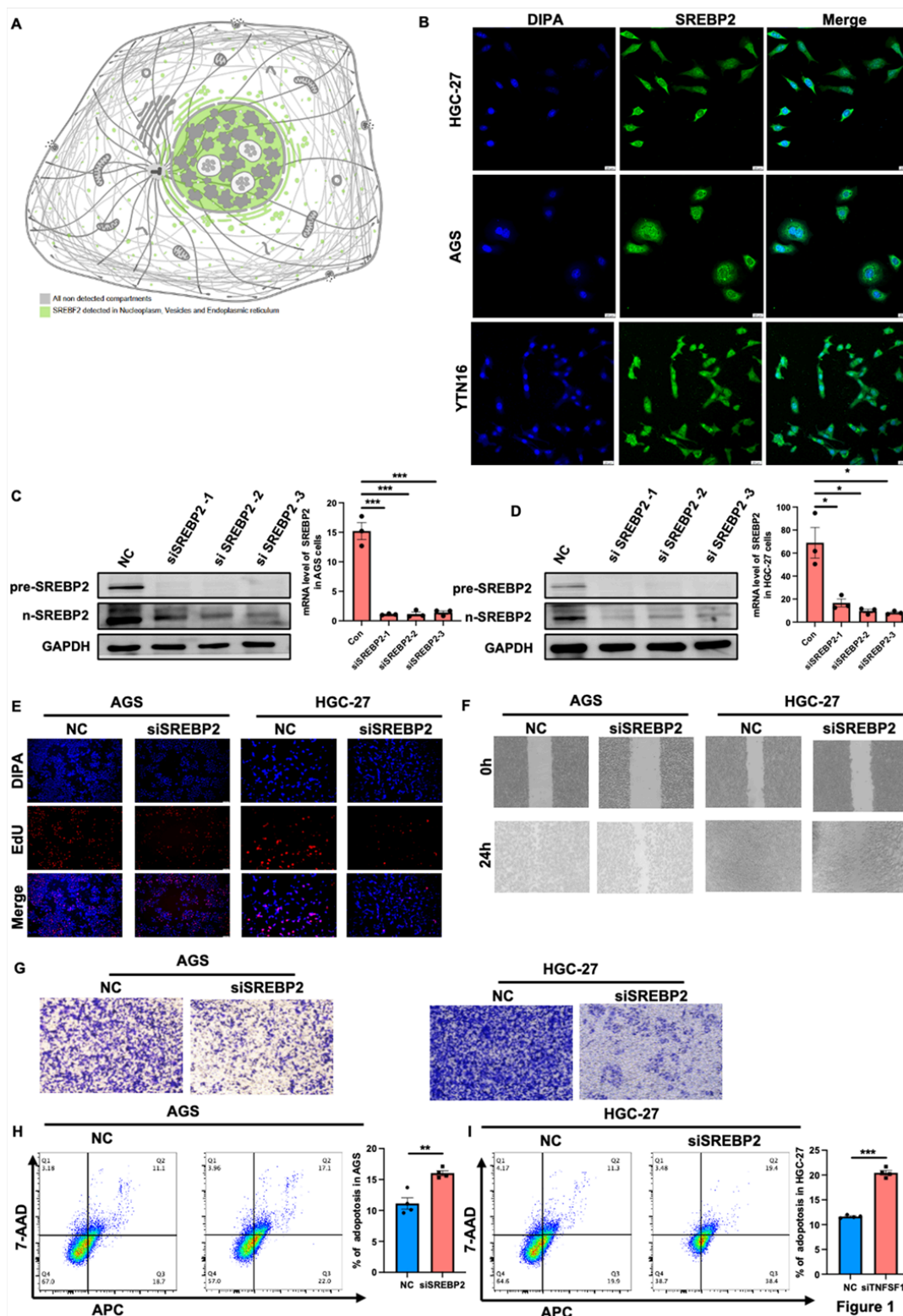


Figure 1

SREBP2 knockdown inhibits the proliferation, invasion, and migration of gastric cancer cells.

(A) Subcellular localization of SREBP2.

(B) Immunofluorescence (IF) showing SREBP2 localization in AGS, HGC-27, and YTN16 cells.

(C-D) RT-qPCR **(C)** and Western blot **(D)** confirming siRNA-mediated SREBP2 knockdown (n=3 biological replicates).

(E) Edu assay assessing proliferation, Transwell assays measuring invasion (F) and migration (G) after SREBP2 knockdown.

(H-I) Flow cytometry quantifying the proportion of apoptosis in AGS (H) and HGC-27 (I) cells following siSREBP2. *Data are mean $\pm$ SE from three independent experiments (n=3). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

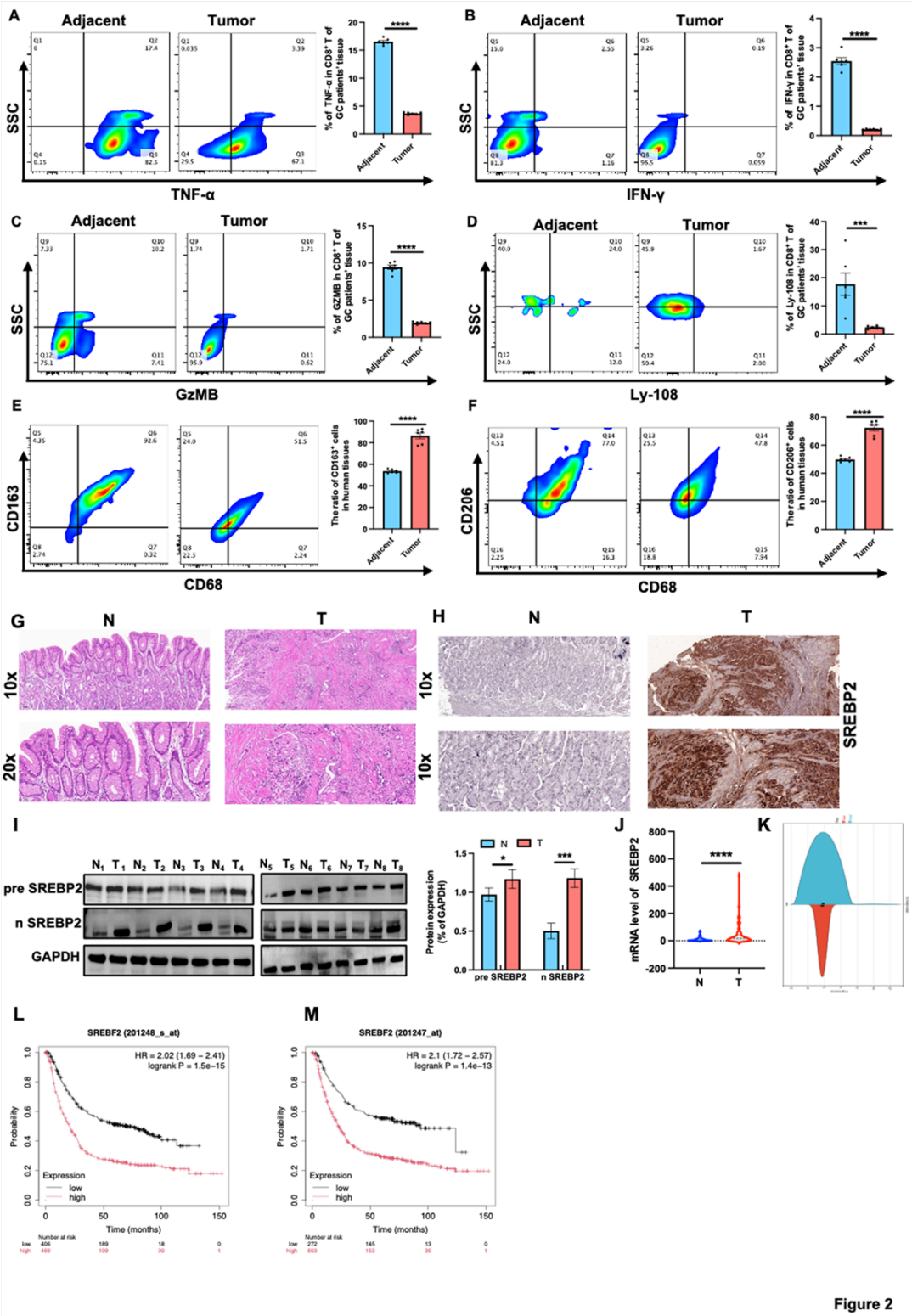


Figure 2

Figure 2

SREBP2 promotes tumor growth and correlates with M2 TAM infiltration in vivo.

(A-D) Percentages of TNF- α ⁺ CD8⁺T cells **(A)**, IFN- γ ⁺CD8⁺ T cells **(B)**, GZMB⁺CD8⁺T cells **(C)**, and Ly108⁺CD8⁺ T cells **(D)** in patient's tumor and adjacent tissues.

(E-F) Percentages of CD163⁺**(E)** and CD206⁺**(F)** TAMs in patient tissues.

(G-H) Representative HE **(G)** and IHC **(H)** staining for SREBP2 in tumor tissues.

(I-J) Representative Western blot **(I)** and qPCR **(J)** assay for protein expression and mRNA levels of SREBP2 in patient tumor and adjacent tissues.

(K) Representative qPCR assay for mRNA levels of SREBP2 in patient tumor and adjacent tissues using the TCGA database.

(L-M) The overall survival of gastric cancer patients in differential expression of SREBP2. *Data are mean $\pm$ SE from three independent experiments (n=3). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

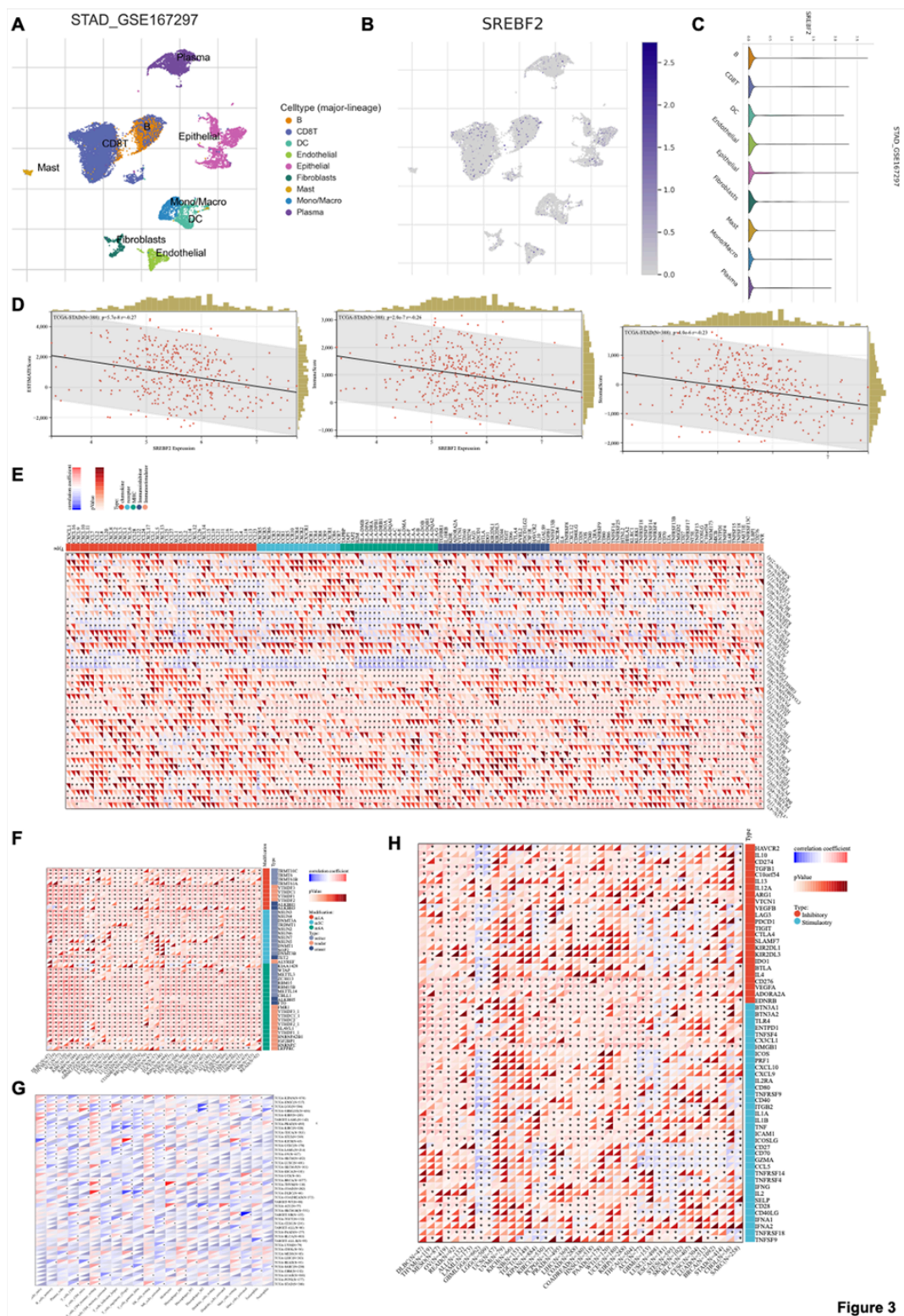


Figure 3

Figure 3

SREBP2 correlates positively with immune features across cancer types.

(A) Single-cell analysis of SREBP2 in STAD (Single Cell Portal database).

(B)UMAP visualization of SREBP2 expression across clusters and cell types.

(C) Violin plots showing SREBP2 expression in different cell populations.

(D-F) Relationship between SREBP2 expression and ESTIMATE scores (Left), immune scores (middle),

and stromal scores (**Right**) in STAD.
(**G-J**) Correlation between SREBP2 and immune regulatory genes (**G**) and immune checkpoint genes (**H**).

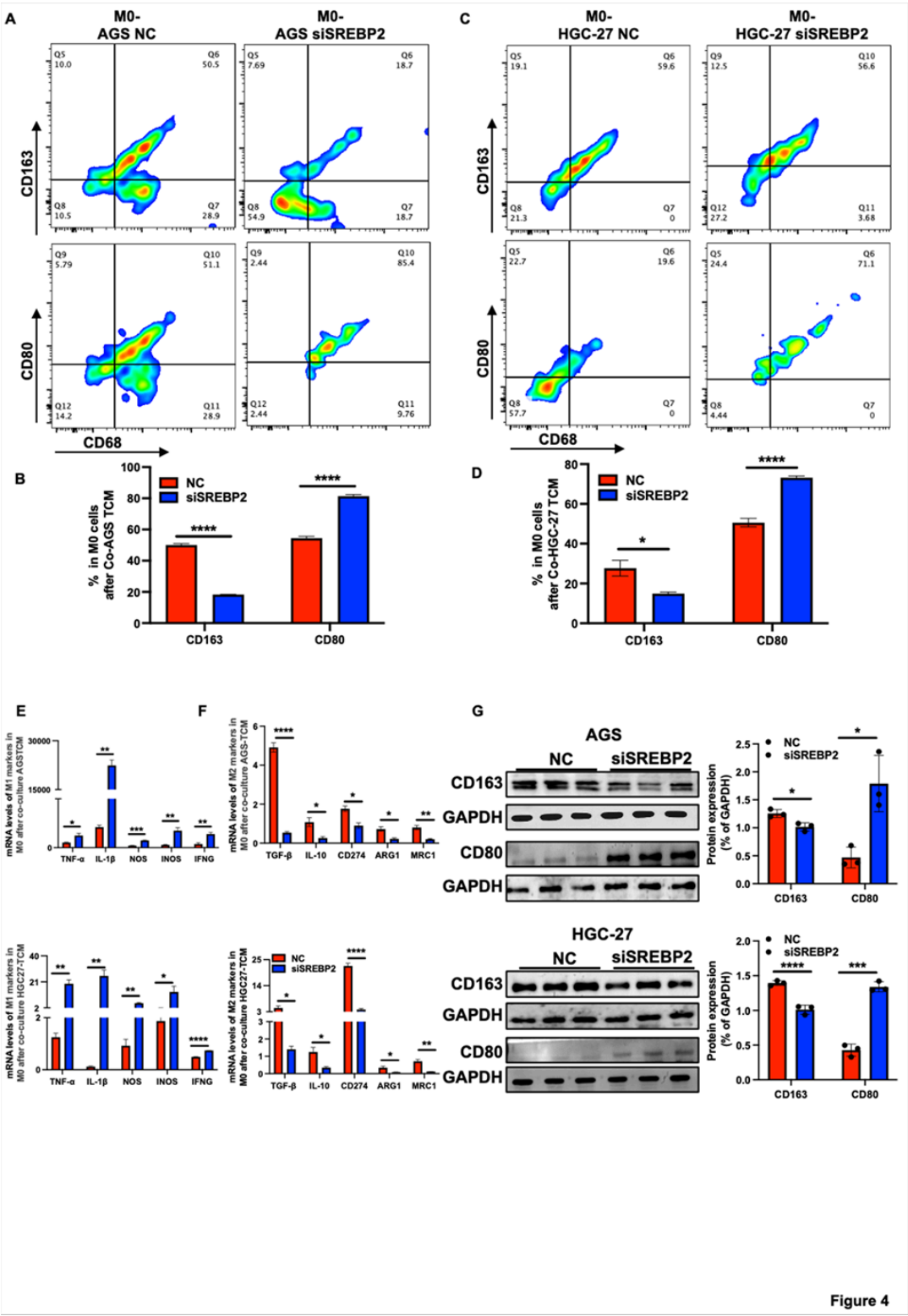


Figure 4

siSREBP2 reduces M2 polarization of tumor-associated macrophages (TAMs)

(**A-D**) Percentages of CD163⁺ and CD206⁺ in TAMs after co-culturing with AGS (**A-B**) or HGC-27 (**C-D**) NC

or siSREBP2 condition medium.

(E-G) qPCR (E-F) and Western blotting (G) analysis of M1 and M2 markers in different TAMs. *Data are mean ± SE from three independent experiments (n=3). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

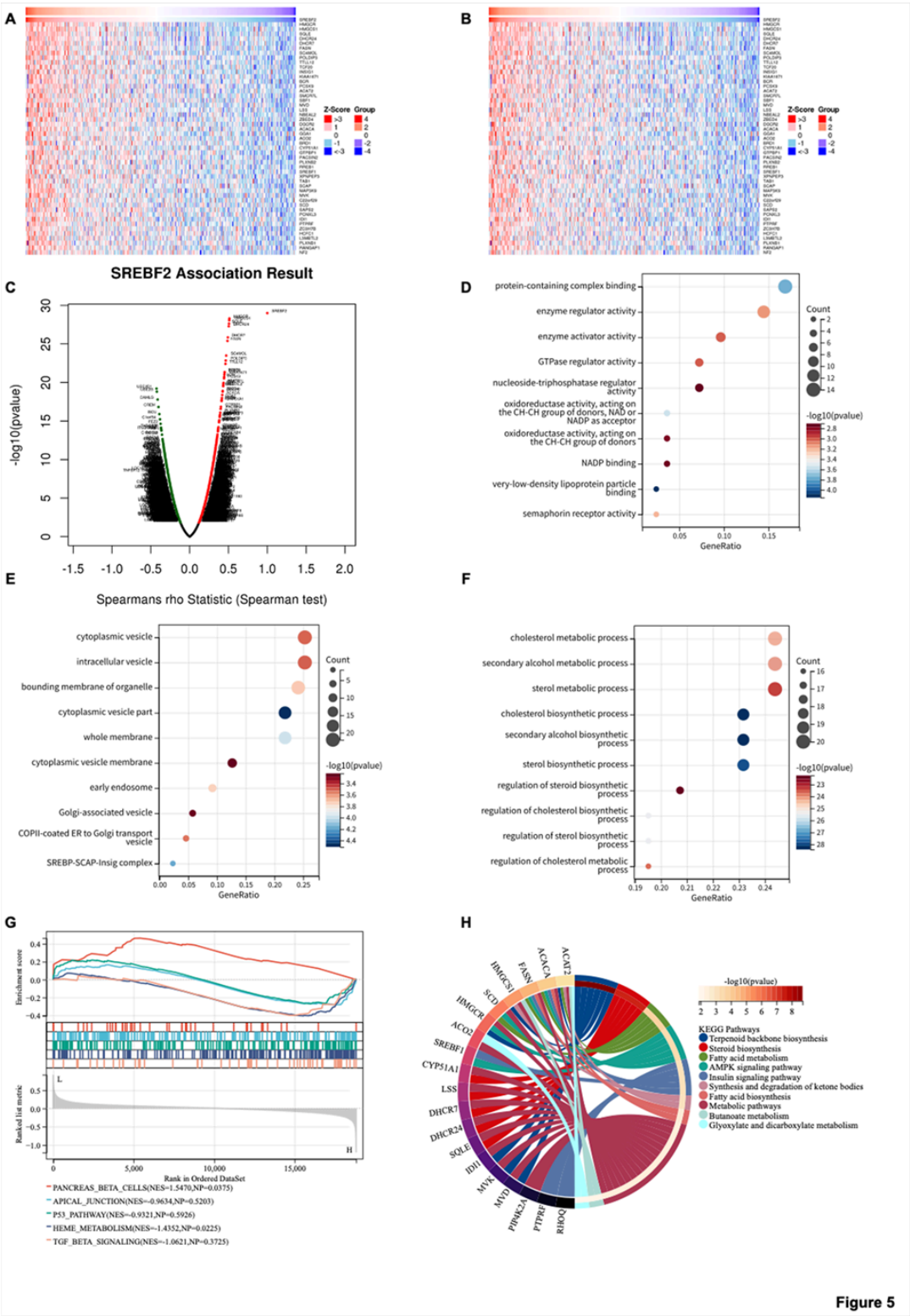


Figure 5

Figure 5

Gene function enrichment analysis of SREBP2 in STAD.

(A-B) Heatmap analysis shows the top genes positively (A) and negatively (B) correlated with SREBP2.
(C) Correlation strength of SREBP2 with representative genes using a scatter plot.
(D-F) Correlation between GO pathway enrichment analysis and SREBP2 in STAD.
(G-H) KEGG pathway shows the enrichment pathway of SREBP2-associated genes.

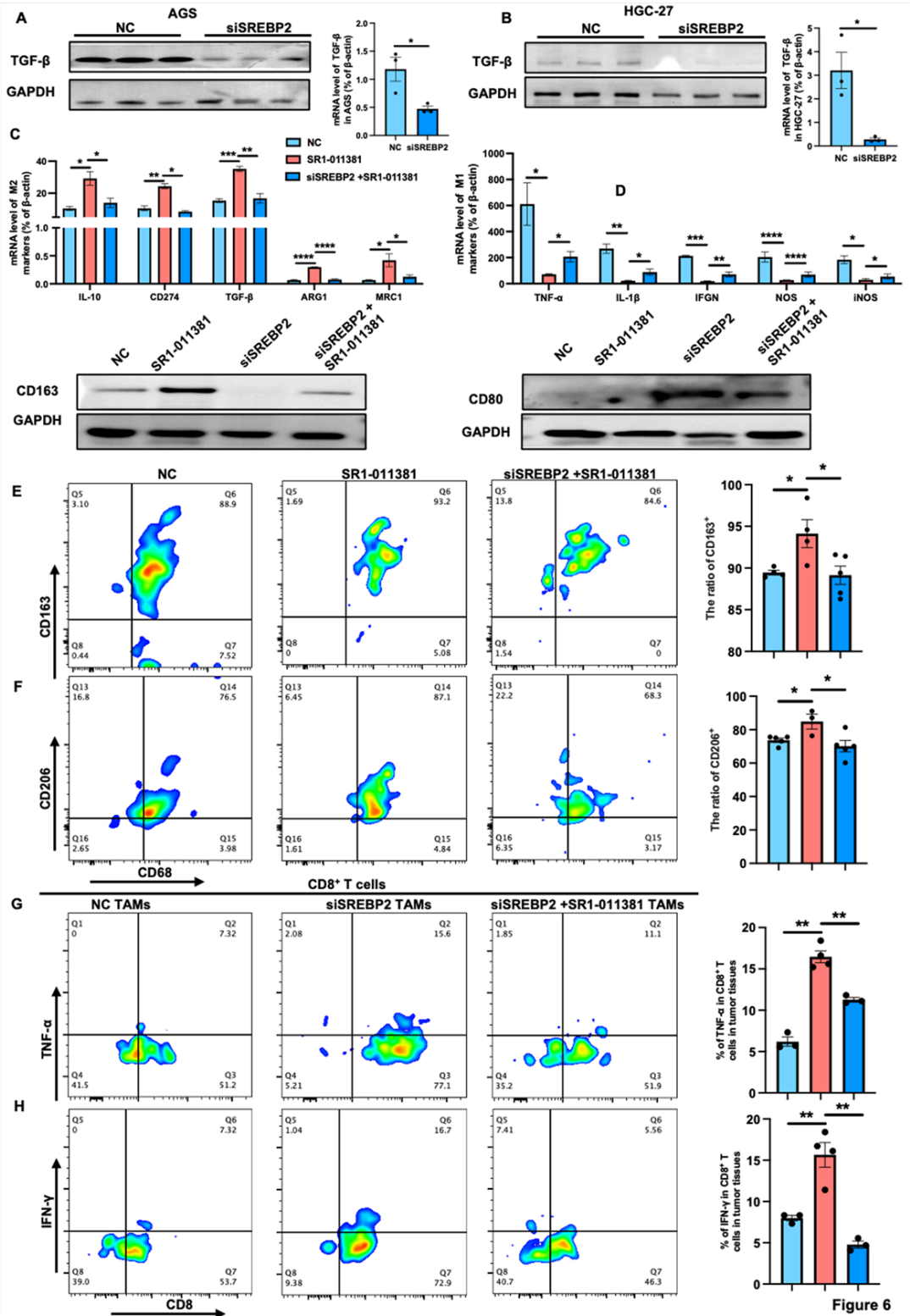


Figure 6

SREBP2 knockdown reduces M2 polarization of TAMs via the TGF- β signal pathway.

(**A-B**) Western blotting and qPCR analysis of protein expression and mRNA levels of TGF- β in AGS (**A**) and HGC-27 (**B**) after knockdown of SREBP2.

(**C-D**) mRNA of M1 and M2 markers in M0 macrophages after co-culture with control or SREBP2-silenced tumor-conditioned medium (TCM) $\pm$ SR1-011381 (an activator of TGF- β) using qPCR analysis.

(**E-F**) Flow cytometry analysis showed % of CD163⁺ and CD80⁺ populations in M0 macrophages after co-culture with AGS (**E**) or HGC-27 (**F**) TCM.

(**G-H**) Flow cytometry analysis showed % of TNF- α ⁺ (**G**) and IFN- γ ⁺ (**H**) in CD8⁺ T cells after co-culture with TAMs (Figure 6E and 6F). Data are mean $\pm$ SE from three independent experiments (n=3). * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

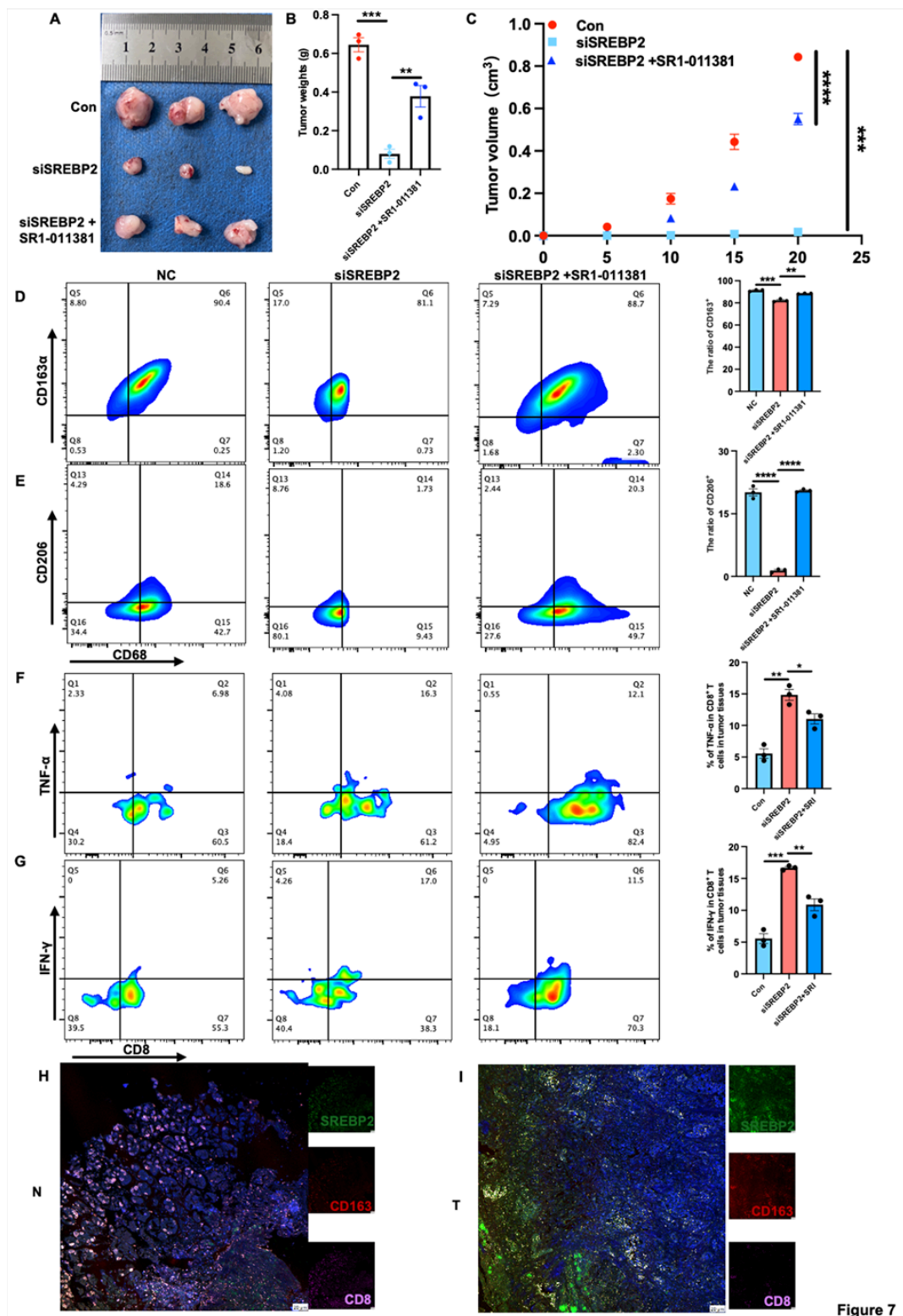


Figure 7

Figure 7

SREBP2 knockdown reduces immune escape of Gastric cancer induced by M2 polarization of TAMs via the TGF- β signal pathway.

(A-C) Xenograft tumors formed by NC/siSREBP2 YTN16 cells in C57BL/6J mice (A). Tumor volume (B) of xenografts and quantitative analysis of tumor weight (C).

(D-G) Flow cytometry analysis of the % of CD163⁺ TAMs (D), CD80⁺ TAMs (E), TNF- α ⁺ CD8⁺ T cells (F), and IFN- γ ⁺CD8⁺ T cells (G) in the tumor tissues.

(H-I) Representative pictures of mIHC analysis for SREBP2, CD163, and CD8 markers in human adjacent (H) and tumor tissues (I). *Data are mean $\pm$ SE from three independent experiments (n=3). * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

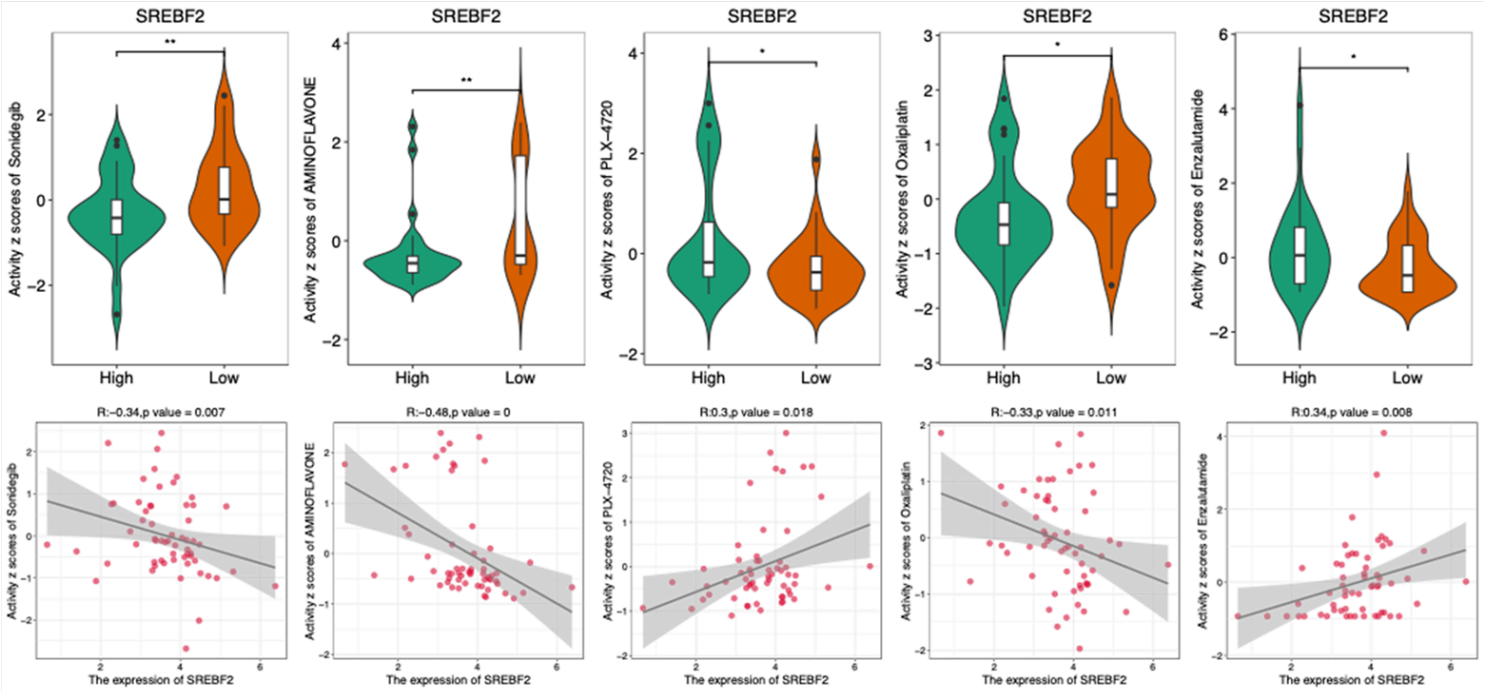


Figure 8

Figure 8

Drug sensitivity analysis of SREBP2 in gastric cancer.

Correlation of SREBP2 expression with Z-scores for Sonidegib(A), AMINOFLAVONE (B), PLX-4720 (C), Oxaliplatin (D), and Enzalutamide (E).