# Identification of SHMT2 as a Potential Prognostic Biomarker and Correlating With Immune Infiltrates in Lung Adenocarcinoma

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## Abstract

**Background:** The role of Serine hydroxymethyltransferase2 (SHMT2) in diverse cancers has attracted increasing attention. However, the prognostic role of SHMT2 in lung adenocarcinoma (LUAD) and its relationship with immune cell infiltration is yet to be studied.

**Methods:** The data of mRNA and clinic in LUAD were respectively downloaded from the GEO and TCGA database. We conducted a biological analysis to select the signature gene SHMT2. Online databases including Oncomine, GEPIA, TISIDB, TIMER, and HPA were applied to analyze the characterization of SHMT2 expression, prognosis and the correlation with immune infiltrates in LUAD.

**Results:** The mRNA expression and protein expression of SHMT2 in LUAD were higher than normal tissue. A Kaplan-Meier analysis showed the lower expression level of SHMT2 had a better overall survival rate. Multivariate analysis and the Cox proportional hazard regression model revealed that SHMT2 expression was an independent prognostic factor for patients with LUAD. Meanwhile, the gene SHMT2 was highly associated with tumor-infiltrating lymphocytes in LUAD.

**Conclusions:** These results suggest that the SHMT2 gene is a promising candidate as a potential prognostic biomarker and highly associated with different types of phenotypes of immune cell infiltration in LUAD.

## Introduction

Lung cancer is the most commonly diagnosed cancer and the leading causes of cancer-related death worldwide as well as a rising concern in society. According to treatment purposes, lung cancer is divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Non-small cell lung cancer accounts for approximately 85% of all lung cancers[1], and has clearly separated two fundamental distinct types, lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD), the latter of which is the most frequently diagnosed histological subtype of NSCLC, followed by squamous cell carcinoma. As the most common histological subtype, lung adenocarcinoma is frequently occurring in women and people without a history of smoking. Especially, there are no obvious clinical symptoms in early stage of disease, but common symptoms of common respiratory diseases which is not easy to be detected by patients. In addition, owing to the presence of metastatic disease at early stages in LUAD, the prognosis of patients is usually poor, with an average 5-year survival rate of less than 20%[2]. Therefore, there is in urgently needed to relieve the problems in early diagnosis and discover new diagnostic and prognostic biomarkers for LUAD.

Serine hydroxy methyl transferase (SHMT) is an essential enzyme for the conversion between serine and glycine as well as one-carbon metabolism, providing the important precursors for protein and nucleic acid synthesis for cancer growth and metastasis. Noteworthily, amino acid and one-carbon metabolism have been regarded as a core feature of the biology of cancer. In addition, through genetic and functional evidence, hyperactivation of one-carbon metabolism has been proved to be a driver of oncogenesis and establishes a link to the epigenetic state of the cell [3]. Several studies has found that SHMT2, a type of SHMT genes, which is shown to encode a protein that localizes to the mitochondria (SHMT2) [4], was identified as a potential diver gene in diverse cancers, playing important roles in cancerous cell growth and aggressiveness [5]. High SHMT2 expression level was correlated with poorer overall survival in patients in intrahepatic cholangiocarcinoma and was proved to be a powerfully and independently prognostic factor as well as a potential therapeutic target for the patients with intrahepatic cholangiocarcinoma [6]. Proteomic profiling of breast cancer metabolism identifies SHMT2 as a prognostic factor [7]. SHMT2 drives glioma cell survival in ischemia depending on glycine clearance [8]. Besides, SHMT2 may serve as a prognostic factor and as a potential therapeutic target for human gliomas in clinic [9]. Therefore, SHMT2 is a very crucial gene in many cancer. However, there is no study linking the expression of SHMT2 in LUAD with immunology and elucidating its prognosis in LUAD.

In recent years, the combination of immunotherapy and high-throughput gene microarray has been widely employed for oncology and other disease areas to analyze deeper correction which is difficult for people to found without enough clinical or experimental data. Immune cells have an intimate connection with the prognosis in various cancers. Mounting evidence supports that the malignant phenotype is not only determined by the intrinsic activities of cancer cells but also by components in the tumor microenvironment, especially tumor infiltrating immune cells [10], which is important determinant of prognosis and immunotherapy response of lung cancer [11]. For example, CD83 + dendritic cells and Foxp3 + regulatory T cells in primary lesions and regional lymph nodes are inversely correlated with prognosis of gastric cancer [12]. Increased infiltration of while TAM is associated with a poor prognosis in NSCLC [13], while DC and T cells are connected with better prognosis[14] [15]. Meanwhile, high-throughput gene microarray makes it accessible for us to further explore the tumors at multiple levels.

In this study, we downloaded the data sets from GEO database (Gene Expression Omnibus) and TCGA (The Cancer Genome Atlas) database, conducted bioinformatics statistical analysis to select different expression genes (DEGs) between normal and tumor tissue. Immediately following, functional analysis and survival analysis were carried out to select and verify signature genes. In addition, we took full advantage of convenient online site tools to explore the relationship between signature and immune cells and verify our suppose at multiple levels specially.

## Methods

# 2.1. Data collection and preprocess

We obtained the LUAD-related microarray profiles (GSE116959 [16], GSE21933 [17], GSE31210 [18]) from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). In this study, the datasets that met the following criteria were selected: (a) studies comparing gene expression between human lung adenocarcinoma cancer samples and corresponding normal tissues; (b) the number of samples in each gene expression profiling dataset shall be more than 30.

The microarray data were normalized and analyzed via the R “limma” package [19]. With the empirically Bayesian method in limma package performed, we set |log2 fold change (FC)|≥1 and an adjusted P value less than 0.01 as the threshold to define important differentially expressed genes (DEGs) which selected for subsequent analysis. We named the DEGs overlapping in the three data matrixes as common DEGs. In addition, if multiple probes corresponded to the same gene in in the annotation file, the average expression of these probes was used as the expression value of the corresponding gene. All data processing and analysis above were accomplished with R language.

Data preprocessing and analysis were accomplished using R language.

Furthermore, we obtained the LUAD RNA seq expression data and clinical data set from the TCGA database (https://cancergenome.nih.gov/) containing 521 tumor samples and 46 normal samples.

## 2.2. Functional Enrichment Analysis of DEGs

### 2.2.1 GO and KEGG pathway analysis

In order to investigate the biological processes and predicted functions of DEGs, we also performed Gene Ontology (GO) and The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on them. The GO analyses classified the common DEGs into three categories, including biological process (BP), cellular component (CC), and molecular function (MF). The KEGG analysis were conducted to determine the pathways in which the DEGs were significantly enriched in P-value < 0.05 as the cutoff significant criteria. Besides, we used the Cytoscape software (version 3.7.2) to selected Hub genes were selected. The GO and KEGG analyses were both based on the online database DAVID (version 6.8) (https://david.ncifcrf.gov) and visually display through R software (version 3.6.1).

### 2.2.2. Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) (http://software.broadinstitute.org/gsea/index.jsp) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states [20] (e.g. phenotypes) (From the official GSEA website). We used this computational method to analyze the function and potential pathway of signature gene. In order to find out how the gene set relates to the function we're interested in, we conducted GSEA analysis based on “C5: GO gene sets” for three groups of GSEs by GSEA software version 4.0.3. And false discovery rate (FDR) < 25% and nominal p < 0.05 were regarded as the cut-off criteria.

## 2.3 Survival Analysis

### 2.3.1 Risk score formula establishment

After screening out the genes, we further investigated the potential roles in clinical outcomes. We used a risk-score formula for LUAD patients’ survival prediction. The risk score formula is as following: Risk score=(1.43 × expression level of AC069513.4) + (0.81 × expression level of AC003092.1) + (1.64 × expression level of RP11-507K2.3) + (-6.56 × expression level of CTC-205M6.2) + (-1.72 × expression level of U91328.21) [21].

### 2.3.2 Risk score formula validation

To validate the gene risk signature in the internal validation data sets, we calculated the risk score for each patient in the complete TCGA cohort. The patients were then divided into a high-risk and a low-risk group based on the corresponding median risk score. The prediction accuracy of this risk model was determined by a time-dependent receiver operating characteristic (ROC) analysis.

### 2.3.3 Statistical Analysis

Statistical analysis and plots were performed using R software (v.3.5.1). Correlations between SHMT2 expression and clinic pathological parameters were determined by chi-squared and Fisher’s exact tests. Prognostic factors were assessed using Cox regression analysis and the Kaplan-Meier method. The survival rates were calculated by Kaplan-Meier method curves and compared using the log-rank test. The significance of prognostic factors was evaluated through a multivariate Cox proportional hazard regression. P-value of less than 0.05 was considered statistically significant.

Then, the Kaplan-Meier plotter was applied to examine the prognostic value of SHMT2. Kaplan-Meier plotter database (http://kmplot.com/analysis/) is an online analysis tool containing microarray profiles and mRNA-seq data with patients’ survival information, including OS and RFS, summarized from TCGA, Gene Expression Omnibus, and the Cancer Biomedical informatics Grid [22]. Kaplan-Meier plotter database analysis the correlation between SHMT2 expression and survival in LUAD was analyzed by Kaplan-Meier plotter. A log-rank P value and a hazard ratio (HR) with confidence intervals of 95% were also calculated.

## 2.4 Signature Gene Online Validation and Analysis

### 2.4.1 Oncomine database Analysis

The signature gene SHMT2 expression level in various types of cancers, especially in lung cancer, was analyzed in the Oncomine database (https://www.oncomine.org/). Oncomine database is an online cancer database with powerful analytical capabilities for computing gene expression signatures, clusters and gene-set modules, automatically extracting biological insights from the data[23]. The mRNA expression difference between tumors and normal tissues were with thresholds as follows: p-value of 0.01, fold change of 2 and gene ranking of all and the data had to be from mRNA.

### 2.4.2 GEPIA database Analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/), an interactive web for analyzing the sequencing expression data of RNA based on 9,736 tumors and 8,587 normal samples from the cancer genome atlas (TCGA) and the GTE projects[24]. In the functional section named as the Survival plot in GEPIA database, we conducted an online Survival analysis of the gene SHMT2. The threshold is determined by the following principles : Gene of SHMT2; Methods of Overall Survival; Group Cutoff of Median; Cutoff-High(%) and Cutoff-Low(%) both are 50; Hazards Ratio (HR) of yes; 95% Confidence Interval of yes; Axis Units of month; Datasets set as LUAD.

## 2.4.3 UALCAN Database Analysis

UALCAN database (http://ualcan.path.uab.edu) is a user-friendly and interactive database, providing easy access to RNA-seq and clinical data of 31 cancer types from The Cancer Genome Atlas(TCGA) [25]. We checked the RNA sequencing expression of SHMT2 again and explore the correlation between SHMT2 protein expression and LUAD in this database.

## 2.4.4 TIMER database Analysis

The correlation between SHMT2 expression and the abundance of immune infiltrates was explored in a Gene module in the TIMER database(https://cistrome.shinyapps.io/timer/), a comprehensive tool established for systematically analyzing immune infiltrates across diverse types of cancer[26]. Meanwhile, we also analyzed the relationship between the expression of SHMT2 and gene markers of tumor-infiltrating immune cells in a Correlation module. Besides, the expression level of SHMT2 in various types of cancers was examined in the TIMER database once more.

## 2.4.5 TISIDB database Analysis

To further investigate the correlations between SHMT2 expression and lymphocytes and immunomodulators, the TISIDB database (http://cis.hku.hk/TISIDB) was applied to analyze. Known as a web portal for tumor and immune system interaction, TISIDB integrates multiple heterogeneous data types, including integrates 988 reported immune-related anti-tumor genes, high-throughput screening techniques, molecular profiling, and para-cancerous multi-omics data, as well as various resources for immunological data retrieved from seven public databases[27]. We used TISIDB database to analyze the link between SHMT2 and immune cell infiltration and learn the GO function in LUAD.

## 2.4.6 Human Protein Atlas Analysis

The protein expression of SHMT2 in both LUAD and normal tissues was retrieved from the Human Protein Atlas database (HPA) (https://www.proteinatlas.org/), a program with the aim to map all the human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology [28] [29]. In this study, we used HPA database to analyze the protein expression and immunohistochemistry (IHC) of SHMT2 in normal tissues and LUAD tissues.

# Results

## 3.1 Identification of common DEGs

The flow chart of the study was generalized in Fig. 1. After performing difference analysis with dataset collection (GSE116959, GSE21933, GSE31210) in GEO database, a total of 356 LUAD samples (304 tumor tissues and 52 normal tissues) in TCGA database were explored. There were 1868 DEGs filtered from the GSE116959 data set, including 624 upregulated and 1244 downregulated genes; 2570 DEGs screened from the GSE21933 data set, including 1193 upregulated and 1377 downregulated genes; 7220 DEGs selected from the GSE31210 data set, including 4107 upregulated and 3013 downregulated genes. Making the results more intuitive, we visualized the results. We displayed the DEGs among each data set via volcano plots (Fig. 2A–2C). What's more, cluster analysis of DEGs showed two evidently different distribution patterns between the tumor and normal samples, suggesting crucial roles of DEGs in tumorigenesis and progression of LUAD (Fig. 2D–2F). Through Venn diagram analysis, 670 common DEGs in the intersection of the three data sets were identified and selected for further analysis.

## 3.2 The Selection of Signature Gene

In order to search for the signature gene, we performed gene set enrichment analysis on GSE21933, GSE31210 and GSE116959. Afterward, with GSEA, we found there were nine sets of results related to immunity closely and all of them were highly expressed gene sets. In particular, GSE21933 was associated with macrophage, which are one of our focuses. The information on GSEA results was listed in Fig. 3A. Next, we selected up-regulated genes for further analysis of the differences between genes and enrichment.

We conducted GO analysis and KEGG pathway enrichment analysis on DEGs from differential analysis results to explore their potential biological functions and pathways associated with LUAD. The results of GO analysis in Fig. 3C showed that DEGs were significantly related to mitotic nuclear division, cell-substrate adhesion, organelle fission, mitotic sister chromatid segregation, nuclear division, regulation of cell-substrate adhesion, regulation of mitotic nuclear division, microtubule cytoskeleton organization involved in mitosis, chromosome segregation, regulation of chromosome segregation, sister chromatid segregation, mitotic spindle organization, extracellular structure organization, urogenital system development, regulation of nuclear division, DNA-dependent DNA replication, cell junction assembly, which were essential for the rapid growth of tumors. Additionally, shown in Fig. 3B, there were 5 hub genes (SHMT2, PYCR1, PSAT1, PC, LDHA) of common DEGs selected from Cytoscape software and, attentionally, the gene SHMT2 was at a specific central position in Fig. 3B, which suggested SHMT2 played an important role in regulating cellular behavior. In a function model of TISIDB, we verified the SHMT2 was involved in Glycine, serine and threonine metabolism, metabolic pathways, carbon metabolism, biosynthesis of amino acids, providing the crucial basis for protein and nucleic acid production for cancer growth and metastasis. Thus, we believe that SHMT2 plays an important role in regulating LUAD growth. Immediately following, we carried out further analysis of SHMT2 in other aspects.

## 3.3 SHMT2 High Expression Level in tumors

The expression level of SHMT2 in tumor and corresponding normal tissues in cancer was verified on Oncomine database. As shown in Fig. 4D, SHMT2 displayed a higher expression level in Bladder cancer, Breast cancer, Colorectal cancer, Kidney cancer, Lung cancer and Lymphoma, while the lower expression level was in Liver cancer and Pancreatic cancer. We also analyzed the mRNA-seq expression data in tumors by UALCAN database and TIMER database (Fig. 4A and 4C). The results both showed the semblable results that SHMT2 display obviously high expression in LUAD. Besides, we explored the protein expression of SHMT2 between LUAD and normal tissues in UALCAN database (Fig. 4B) and conduct immunohistochemistry (IHC) on The Human Protein Atlas (HPA) (Fig. 5A-5F). From above analysis, we summarize the protein expression of SHMT2 was significantly reduced in normal while the protein level significantly elevated in tumors. Higher expression level suggested that SHMT2 possess diverse functions in various tumors, especially in LUAD.

## 3.4 Prognostic Value of SHMT2 in LUAD

We calculated the area under the curve (AUC) of the receiver operating curve (ROC) to evaluate the discriminative ability of prediction rules. And the AUC score for the training dataset was 0.842 (Fig. 6), indicating better survival prediction performance of the training data set. A Kaplan-Meier analysis showed poor overall survival in patients with higher SHMT2 level. Multivariate analysis and the Cox proportional hazard regression model uncovered that the expression of SHMT2 is an independent prognostic indicator for patients with LUAD (Fig. 7A-7B).

We then examined the prognostic value of SHMT2 using the Kaplan-Meier plotter and the Gene Expression Profiling Interactive Analysis (GEPIA) database. We calculated the Cox P/log-rank P value and hazard ratio with 95% intervals. We set Cox P/log-rank P = 0.05 as the thresholds. The patients were divided into two groups based on the median level of the SHMT2 expression in each queue. Univariate analysis was carried out through the Kaplan-Mayer plotter database and GEPIA to assess the impact of SHMT2 on various cancer survival rates (Fig. 7C-7D). The results indicated that the expression level of SHMT2 had a significant effect on the prognosis of LUAD. Moreover, low level of SHMT2 evinced a longer survival period for patients with lung adenocarcinoma. Given all that, these results suggested that high expression of SHMT2 was related to the poor prognosis of LUAD.

## 3.5 SHMT2 immune regulation molecules

The result of GESA analysis based on 3 datasets in Fig. 8 showed that the up-regulated gene in GSE21933, GSE31210 was apparently correlated with immune-related biological functions and SHMT2 was proved as a significantly up-regulated gene in 3 datasets, suggesting SHMT2 is possibly connected with immune.

In order to explore whether SHMT2 exert potential biological roles in immune infiltration, we conducted an integrated analysis based on TIMER database and TISIDB database, analyzing the link between SHMT2 and immune cell infiltration as well as the gene markers of immune cell subtypes in LUAD. The results in Fig. 8 suggested high levels of SHMT2 mRNA expression was associated with high immune infiltration in LUAD. SHMT2 mRNA expression level was significantly negatively correlated with infiltrating levels of immune cells, CD4 + T cells (r = -0.055, P = 2.22e-01), macrophages (r = -0.17, P = 1.65e-04) and dendritic cells (DCs) (r = -0.111, P = 1.44e-02) (Fig. 9). Besides, Table 1 also demonstrated the SHMT2 mRNA expression level had significant correlations with immune cells, TAMs, DCs, CD4 + T cells, neutrophils, Th1, Th2, Thf, T Cell exhaustion in LUAD.

Table 1
Correlation analysis between SHMT2 and related gene markers of immune cells

| Description | Gene marker | SHMT2 | | | | Description | Gene marker | SHMT2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | None | | Purity | | | | None | | Purity | |
| | | cor | p | cor | p | | | cor | p | cor | p |
| TAM | VSIG4 | -0.0859 | ** | -0.05576 | 0.079945 | B cell | CD40 | -0.13984 | ** | -0.10275 | * |
| | CSF1R | -0.11735 | ** | -0.07542 | 0.094366 | | CD80 | -0.09015 | * | -0.05303 | 0.239 |
| | FCGR2A | -0.09113 | * | -0.05383 | 0.232868 | | CXCR4 | -0.09868 | * | -0.07199 | 0.110 |
| | FCER2 | -0.19299 | *** | -0.15948 | *** | | CXCR5 | -0.11635 | ** | -0.07293 | 0.105 |
| Treg | FOXP3 | 0.024662 | 0.576457 | 0.085218 | 0.058653 | | TLR4 | -0.13316 | ** | -0.1059 | * |
| | STAT5B | -0.06034 | 0.171461 | -0.05006 | 0.267301 | Dendritic cell | HLA-DPA1 | -0.25847 | *** | -0.23163 | *** |
| | TGFB1 | -0.19353 | *** | -0.16703 | *** | | CCR7 | -0.18066 | *** | -0.05576 | 0.216 |
| | CCR8 | -0.00152 | 0.972596 | 0.049541 | 0.27226 | | ITGAM | -0.17099 | *** | -0.05383 | 0.232 |
| Th1 | STAT4 | -0.18081 | *** | -0.15209 | *** | | CD59 | -0.39605 | *** | 0.085218 | 0.058 |
| | CD4 | -0.16932 | *** | -0.13212 | ** | | HLA-DPB1 | -0.30492 | *** | -0.28417 | *** |
| | STAT1 | 0.161045 | *** | 0.207551 | *** | | HLADQB1 | -0.16634 | *** | -0.136 | ** |
| | IFNG | 0.120743 | ** | 0.165272 | *** | | HLA-DRA | -0.2821 | *** | -0.26451 | *** |
| Th2 | GATA3 | -0.08165 | 0.064121 | -0.03198 | 0.478618 | | ITGAX | -0.09346 | * | -0.05407 | 0.230 |
| | CXCR4 | -0.09868 | ** | -0.07199 | * | | CD1C | -0.3915 | *** | -0.37063 | *** |
| | CCR4 | -0.17256 | *** | -0.1376 | ** | | THBD | -0.1806 | *** | -0.16764 | *** |
| Tfh | IL21 | 0.134901 | ** | 0.174578 | *** | Natural killer cell | KIR2DL1 | 0.071633 | 0.104432 | 0.082454 | 0.067 |
| | BCL6 | -0.12226 | ** | -0.11344 | * | | KIR2DL3 | 0.094744 | * | 0.115068 | * |
| T Cell exhaustion | PDCD1 | 0.103873 | * | 0.167267 | *** | | KIR2DL4 | 0.254623 | *** | 0.284648 | *** |
| | CTLA4 | -0.00659 | 0.881405 | 0.054575 | 0.22644 | | KIR3DL1 | 0.05615 | 0.203316 | 0.080966 | 0.072 |
| | LAG3 | 0.157443 | *** | 0.205703 | *** | | KIR3DL2 | 0.120751 | ** | 0.14666 | ** |
| | GZMB | 0.262616 | *** | 0.323581 | *** | | KIR3DL3 | 0.171332 | *** | 0.193336 | *** |
| Neutrophil | CCR7 | -0.18066 | *** | -0.14587 | *** | M1 Macrophage | IRF5 | -0.15333 | *** | -0.13549 | ** |
| | ITGAM | -0.17099 | *** | -0.13038 | *** | | | | | | |
| | CD59 | -0.39605 | *** | -0.38037 | *** | | | | | | |
| | CCR7 | -0.18066 | *** | -0.14587 | ** | | | | | | |

*P < 0.05, **P < 0.01, ***P < 0.001

For further investigation, we found the expression of SHMT2 was associated with tumor-infiltrating lymphocytes (TILs), including activated Type 1 T helper cell, nature killer cell, T follicular helper cell, active B cell, immature B cell, active CD4 T cell, Type 17 T helper cell, Tem CD8 cell, CD56dim nature killer cell (Fig. 10A-10l). Particularly, the P-value of above-mentioned cells are all less than 0.001. Overall, these results suggested that the SHMT2 and its associated genes were important for immune cells infiltration in LUAD microenvironment and possibly exited a more significant effect on the prognosis of LUAD.

## Discussion

As an important branch of glycolysis and an essential source of one-carbon metabolism [3], serine was found highly supported the proliferation of tumor cell[30]. SHMT, an essential enzyme in the conversion of serine to glycine, not only can regulate serine metabolism but also one-carbon metabolism, providing the important precursors for protein and nucleic acid synthesis for cancer growth and metastasis [3]. SHMT2, a type of SHMT genes in the human genome had been found is associated with the prognosis of various tumors [6]. We know from previous studies that SHMT2 is a key enzyme in serine/glycine synthesis pathway. SHMT2 can catalyze the transformation of serine into glycine in mammalian mitochondria [8]. Clinically, SHMT2 may serve as a prognostic factor and as a potential therapeutic target for human gliomas [9] [31]. However, there is no study about the relationship between SHMT2 and LUAD. Therefore, analyzing the role of SHMT2 in LUAD existed an important meaning.

As the most common NSCL cancer, dense lymphocytic infiltrate is one of the most obvious characteristics of LUAD, indicating the immune system exerts an active role in the development and growth of lung adenocarcinoma. In this study, Oncomine database and TIMER database were used to compare the expression level of SHMT2 among different cancers and verified its increased expression in LUAD. Univariate analyses from this study were carried out to evaluate the effect of SHMT2 expression on the survival rates in LUAD via the R software and Kaplan-Meier plotter database. The high expression level of SHMT2 had a more significant effect on the prognosis of LUAD patients. After screening tumors prognosis related to SHMT2, the relationship between SHMT2 and immune infiltration levels in different tumors was investigated in the TIMER database and TISIDB database. The levels of infiltration for immune cells in LUAD were performed on TIMER database, revealing SHMT2 owned an obvious correlation with immune filtration in this cancer.

Besides,multivariate analysis and the Cox proportional hazard regression model validated that SHMT2 could be an independent prognostic factor for patients with LUAD. The expression level of SHMT2 also had a significantly negative correlation with tumor-infiltrating lymphocytes like immature B cell, active CD4 T cell, Th17, CD56dim nature killer cell (all Cor > 0.2; P < 0 .01). Additionally, the results of correlation between SHMT2 and gene markers of immune cells show the SHMT2 was closely related with T cells (CD8 + T cells, Th1 cells, Th2 cells, Thf cell, general T cells, and exhausted T cells), TAM, NK cells and DCs (Table 1). Tumor-infiltrating lymphocytes (TILs), including T cells, B cells and are another important component of immune cells that exhibit anti-tumoral functions, especially CD8 and CD4 T cells. Some studies revealed that Th1 cells were associated with prolonged survival. SHMT2 regulating immune infiltration may be involved in these immune cells, especially T cell receptor interaction. The analysis mentioned above suggested that SHMT2 could be serve as a potential overall prognostic marker for patient survival, improving the survival and prognosis of LUAD, and SHMT2 may also played an important role in the microenvironment of lung adenocarcinoma via regulating tumor infiltration of immune cells.

At present, according to the known research results, the high expression of SHMT2 could be detected in different types of cancer, as reported, playing pivotal roles in the migration and invasiveness. SHMT2 was validated that reduce cell growth and tumorigenicity in vitro and vivo if knocking out it in hepatocellular cancer cell lines. Gene set enrichment analysis revealed that SHMT2 had a strong correlation with cancer invasion and poor survival among breast cancer patients. Besides, SHMT2 also was reported to control inflammatory cytokine signaling via its interaction with the BRISC deubiquitylase (DUB) and its important catalyst [32]. And SHMT2 impaired T cell survival in culture and antigen-specific T cell abundance in vivo [33]. Overall, these studies provide evidence that SHMT2 participated in different diseases via immune mechanisms.

## Conclusion

In this study, we showed SHMT2 as an independent prognostic factor and found its high expression was associated with poor prognosis of LUAD. And further analysis conjectured that SHMT2 may mediate the immune cell infiltration via regulation of macrophages and T cell in lung adenocarcinoma microenvironment. Although there are many shortcomings in this study, such as our lack of experimental verification, we also exit some highlights which deserve attention. We take full advantage of available public online datasets to verify our conjecture. However, further exploration and research to study the specific mechanism also required. We hope that our article can contribute to the following research.

## Declarations


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### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.


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Not applicable.


### Author contributions

LX Luo conceived the idea; XL Li, LX Luo, YS Zheng, ZP Lin, XD Li, and XL Li contributed to the acquisition, analysis, and interpretation of data. LX Luo and YS Zheng wrote the manuscript; MY Li, H Luo, L Cui, and LX Luo reviewed the paper and provided comments, and all authors reviewed the manuscript.

## References


1. Neal RD, Hamilton W, Rogers TK: **Lung cancer**. *BMJ* 2014, **349**:g6560.
2. Lin JJ, Cardarella S, Lydon CA, Dahlberg SE, Jackman DM, Janne PA, Johnson BE: **Five-Year Survival in EGFR-Mutant Metastatic Lung Adenocarcinoma Treated with EGFR-TKIs**. *J Thorac Oncol* 2016, **11**(4):556-565.
3. Amelio I, Cutruzzola F, Antonov A, Agostini M, Melino G: **Serine and glycine metabolism in cancer**. *Trends Biochem Sci* 2014, **39**(4):191-198.
4. Anderson DD, Stover PJ: **SHMT1 and SHMT2 are functionally redundant in nuclear de novo thymidylate biosynthesis**. *PLoS One* 2009, **4**(6):e5839.
5. Yang X, Wang Z, Li X, Liu B, Liu M, Liu L, Chen S, Ren M, Wang Y, Yu M *et al*: **SHMT2 Desuccinylation by SIRT5 Drives Cancer Cell Proliferation**. *Cancer Res* 2018, **78**(2):372-386.
6. Ning S, Ma S, Saleh AQ, Guo L, Zhao Z, Chen Y: **SHMT2 Overexpression Predicts Poor Prognosis in Intrahepatic Cholangiocarcinoma**. *Gastroenterol Res Pract* 2018, **2018**:4369253.
7. Bernhardt S, Bayerlova M, Vetter M, Wachter A, Mitra D, Hanf V, Lantzsch T, Uleer C, Peschel S, John J *et al*: **Proteomic profiling of breast cancer metabolism identifies SHMT2 and ASCT2 as prognostic factors**. *Breast Cancer Res* 2017, **19**(1):112.
8. Kim D, Fiske BP, Birsoy K, Freinkman E, Kami K, Possemato RL, Chudnovsky Y, Pacold ME, Chen WW, Cantor JR *et al*: **SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance**. *Nature* 2015, **520**(7547):363-367.
9. Wu M, Wanggou S, Li X, Liu Q, Xie Y: **Overexpression of mitochondrial serine hydroxyl-methyltransferase 2 is associated with poor prognosis and promotes cell proliferation and invasion in gliomas**. *Onco Targets Ther* 2017, **10**:3781-3788.
10. Mantovani A, Allavena P, Sica A, Balkwill F: **Cancer-related inflammation**. *Nature* 2008, **454**(7203):436-444.
11. Liu X, Wu S, Yang Y, Zhao M, Zhu G, Hou Z: **The prognostic landscape of tumor-infiltrating immune cell and immunomodulators in lung cancer**. *Biomed Pharmacother* 2017, **95**:55-61.
12. Candido J, Hagemann T: **Cancer-related inflammation**. *J Clin Immunol* 2013, **33 Suppl 1**:S79-84.
13. Raiha MR, Puolakkainen PA: **Tumor-associated macrophages (TAMs) as biomarkers for gastric cancer: A review**. *Chronic Dis Transl Med* 2018, **4**(3):156-163.
14. Inoshima N, Nakanishi Y, Minami T, Izumi M, Takayama K, Yoshino I, Hara N: **The influence of dendritic cell infiltration and vascular endothelial growth factor expression on the prognosis of non-small cell lung cancer**. 2002.
15. Petersen RP, Campa MJ, Sperlazza J, Conlon C, Joshi MB, Harpole Jr DH, Patz Jr EF: **Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients**. *Journal of Thoracic Oncology* 2010.
16. Moreno Leon L, Gautier M, Allan R, Ilie M, Nottet N, Pons N, Paquet A, Lebrigand K, Truchi M, Fassy J *et al*: **The nuclear hypoxia-regulated NLUCAT1 long non-coding RNA contributes to an aggressive phenotype in lung adenocarcinoma through regulation of oxidative stress**. *Oncogene* 2019, **38**(46):7146-7165.
17. Shimizu K, Nakata M, Hirmi Y, Yukawa T, Maeda A, Tanemoto K: **The database of chromosome imbalance regions and genes resided in lung cancer from Asian and Caucasian identified by array-comparative genomic hybridization**. 2012.
18. Okayama H, Kohno T, Ishii Y, Shimada Y, Shiraishi K, Iwakawa R, Furuta K, Tsuta K, Shibata T, Yamamoto S *et al*: **Identification of genes upregulated in ALK-positive and EGFR/KRAS/ALK-negative lung adenocarcinomas**. *Cancer Res* 2012, **72**(1):100-111.
19. Smyth GK, Michaud J, Scott HS: **Use of within-array replicate spots for assessing differential expression in microarray experiments**. *Bioinformatics* 2005, **21**(9):2067-2075.
20. Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP: **GSEA-P: a desktop application for Gene Set Enrichment Analysis**. *Bioinformatics* 2007, **23**(23):3251-3253.
21. Shi D, Qu Q, Chang Q, Wang Y, Gui Y, Dong DJO: **A five-long non-coding RNA signature to improve prognosis prediction of clear cell renal cell carcinoma**. 2017, **8**(35):58699-58708.
22. Lánczky A, Nagy Á, Bottai G, Munkácsy G, Szabó A, Santarpia L, Győrffy B: **miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients**. *Breast Cancer Research and Treatment* 2016, **160**(3):439-446.
23. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincead-Beal C, Kulkarni P *et al*: **Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles**. *Neoplasia* 2007, **9**(2):166-180.
24. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses**. *Nucleic Acids Res* 2017, **45**(W1):W98-W102.
25. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S: **UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses**. *Neoplasia* 2017, **19**(8):649-658.
26. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS: **TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells**. *Cancer Res* 2017, **77**(21):e108-e110.
27. Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, Chu KC, Wong CY, Lau CY, Chen I *et al*: **TISIDB: an integrated repository portal for tumor-immune system interactions**. *Bioinformatics* 2019, **35**(20):4200-4202.
28. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A *et al*: **Proteomics. Tissue-based map of the human proteome**. *Science* 2015, **347**(6220):1260419.
29. Uhlen M, Zhang C, Lee S, Sjostedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F *et al*: **A pathology atlas of the human cancer transcriptome**. *Science* 2017, **357**(6352).

30. Labuschagne CF, van den Broek NJ, Mackay GM, Vousden KH, Maddocks OD: **Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells**. *Cell Rep* 2014, **7**(4):1248-1258.
31. Wang B, Wang W, Zhu Z, Zhang X, Tang F, Wang D, Liu X, Yan X, Zhuang H: **Mitochondrial serine hydroxymethyltransferase 2 is a potential diagnostic and prognostic biomarker for human glioma**. *Clinical Neurology and Neurosurgery* 2017, **154**:28-33.
32. Walden M, Tian L, Ross RL, Sykora UM, Byrne DP, Hesketh EL, Masandi SK, Cassel J, George R, Ault JR *et al*: **Metabolic control of BRISC-SHMT2 assembly regulates immune signalling**. *Nature* 2019, **570**(7760):194-199.
33. Cao J, Sun L, Aramsangtienchai P, Spiegelman NA, Zhang X, Huang W, Seto E, Lin H: **HDAC11 regulates type I interferon signaling through defatty-acylation of SHMT2**. *Proc Natl Acad Sci U S A* 2019, **116**(12):5487-5492.

## Figures

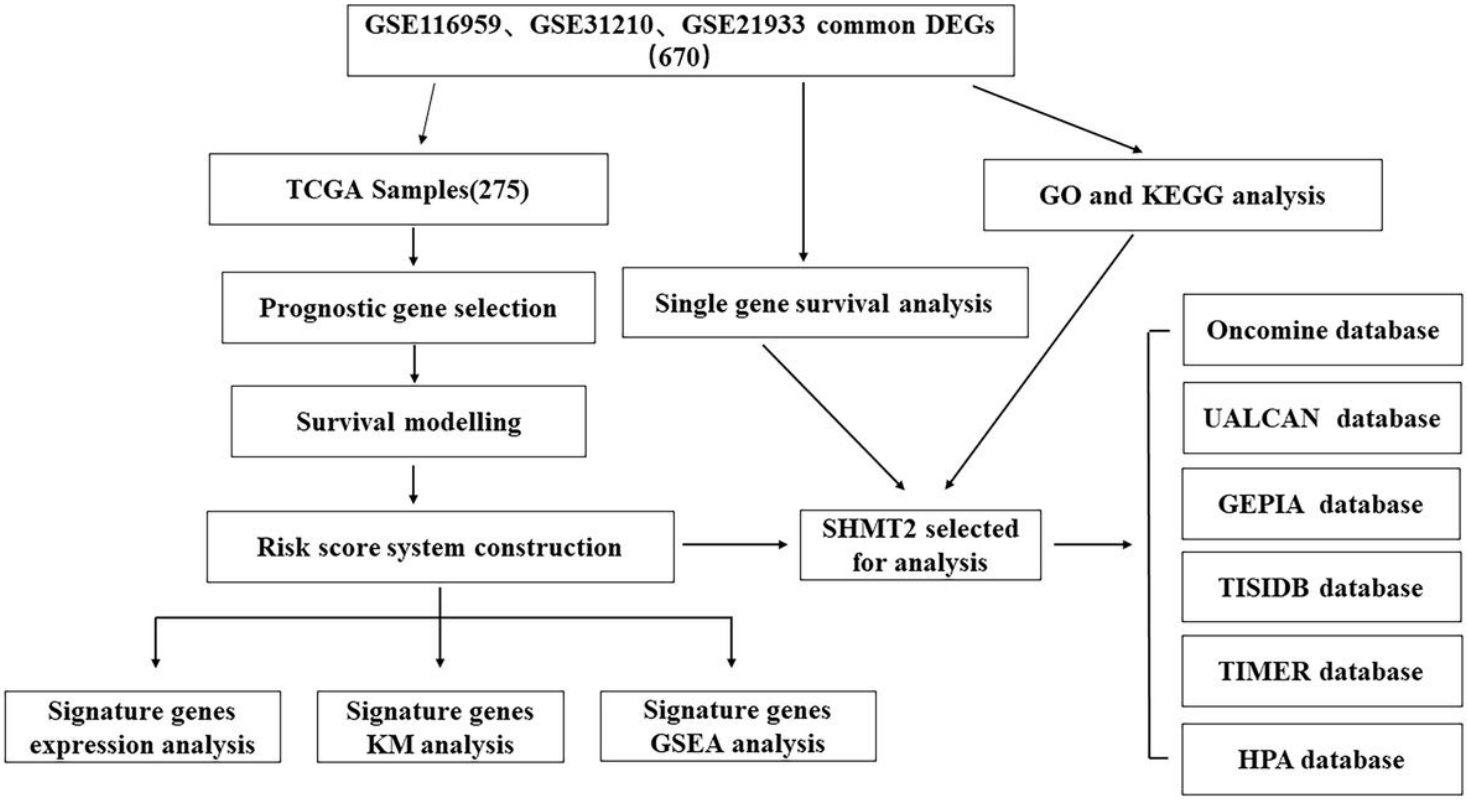
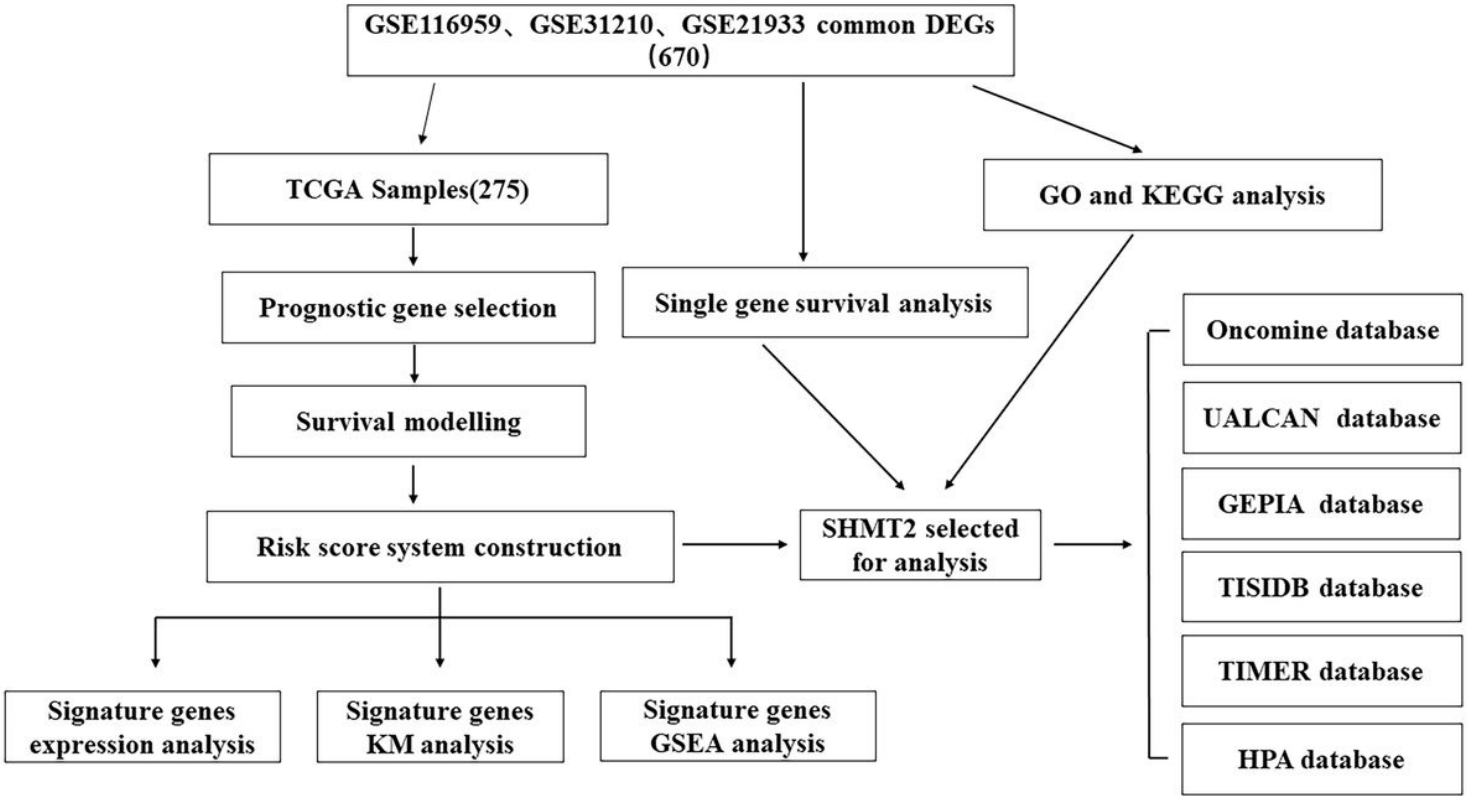



**Figure 1**

Flowchart for this study. DEGs, differential expression genes; GEPIA, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; KM, Kaplan–Meier; TCGA, The Cancer Genome Atlas; TIMER, Tumor Immune Estimation Resource. HPA, the human protein atlas.

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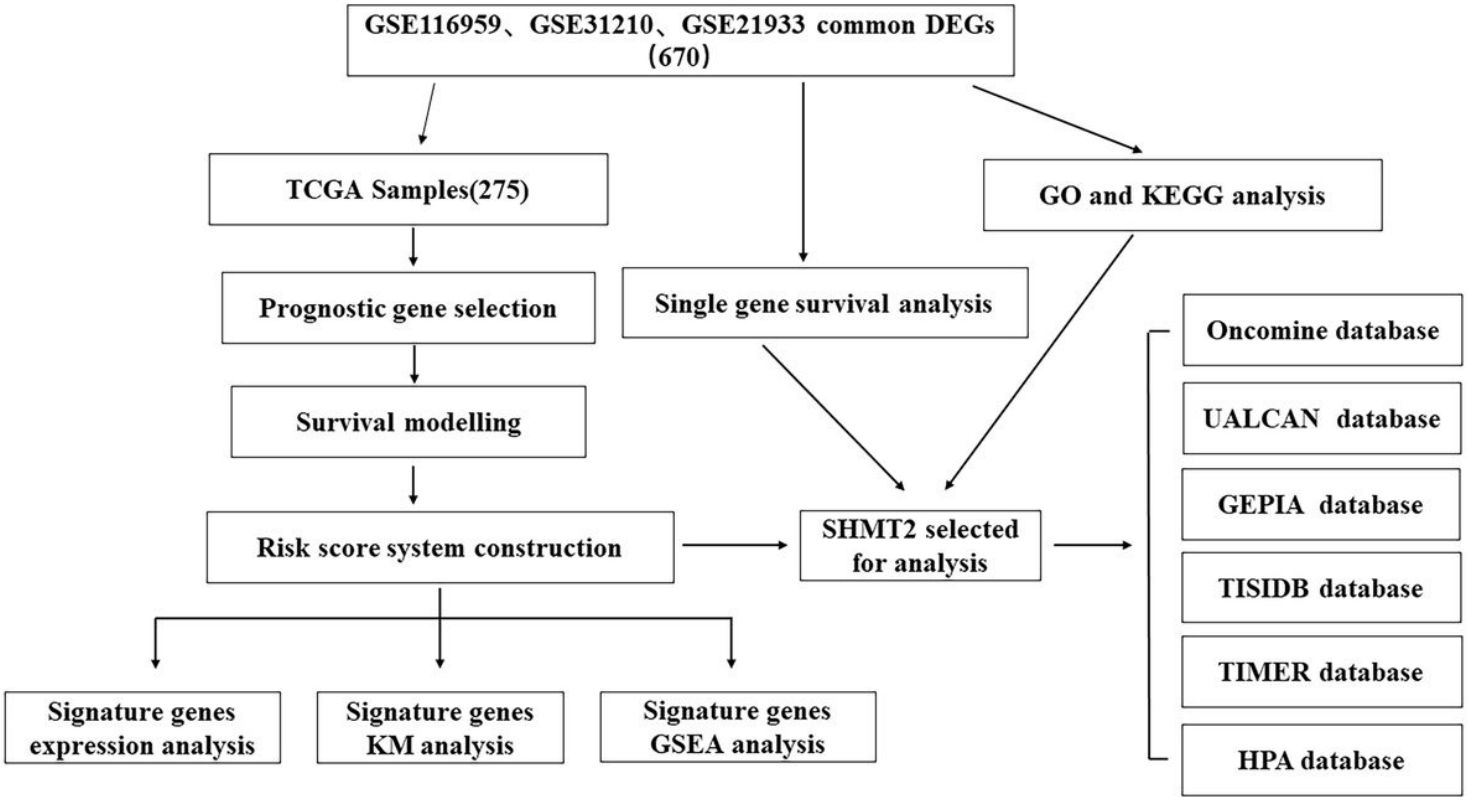


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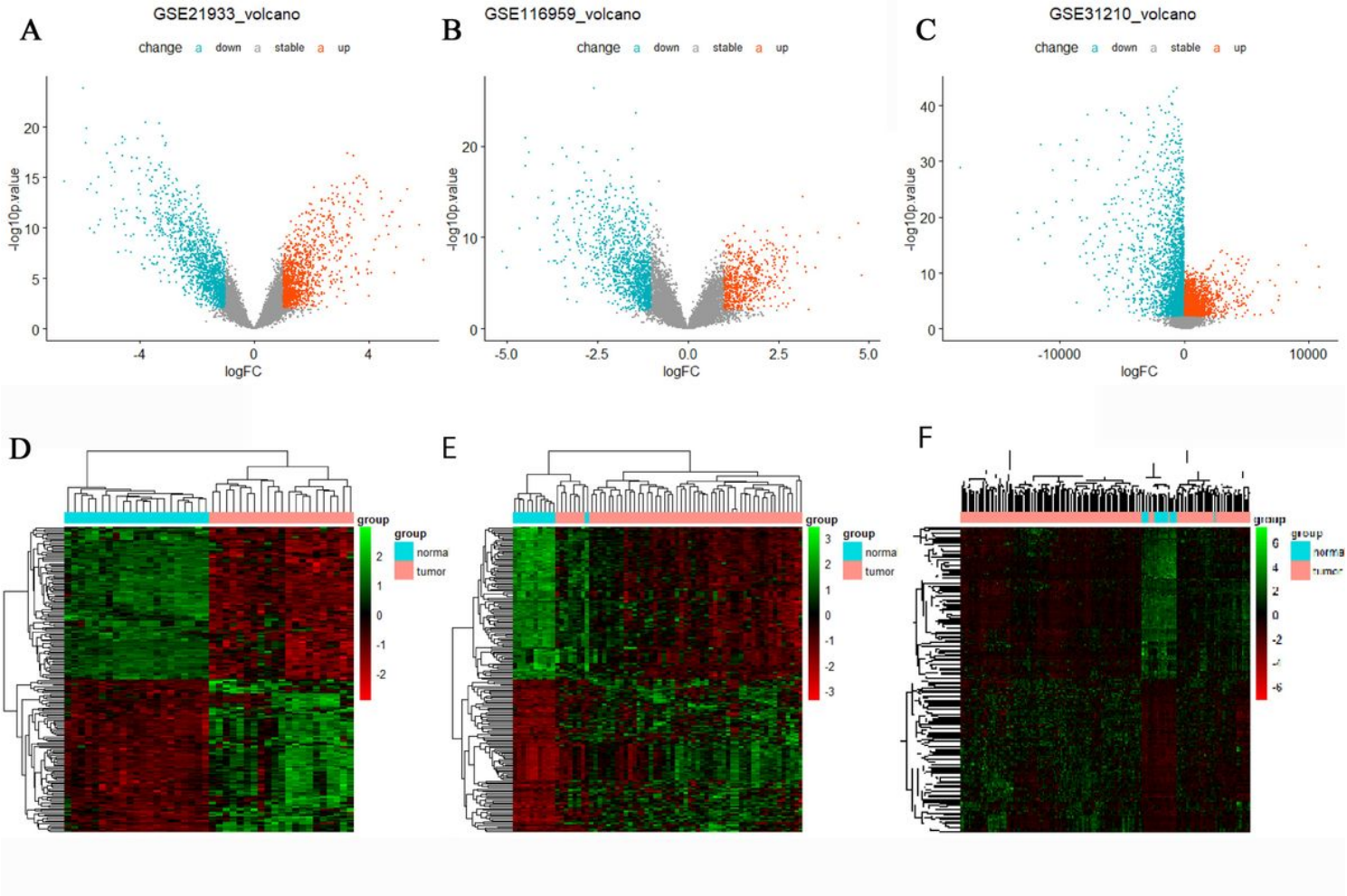
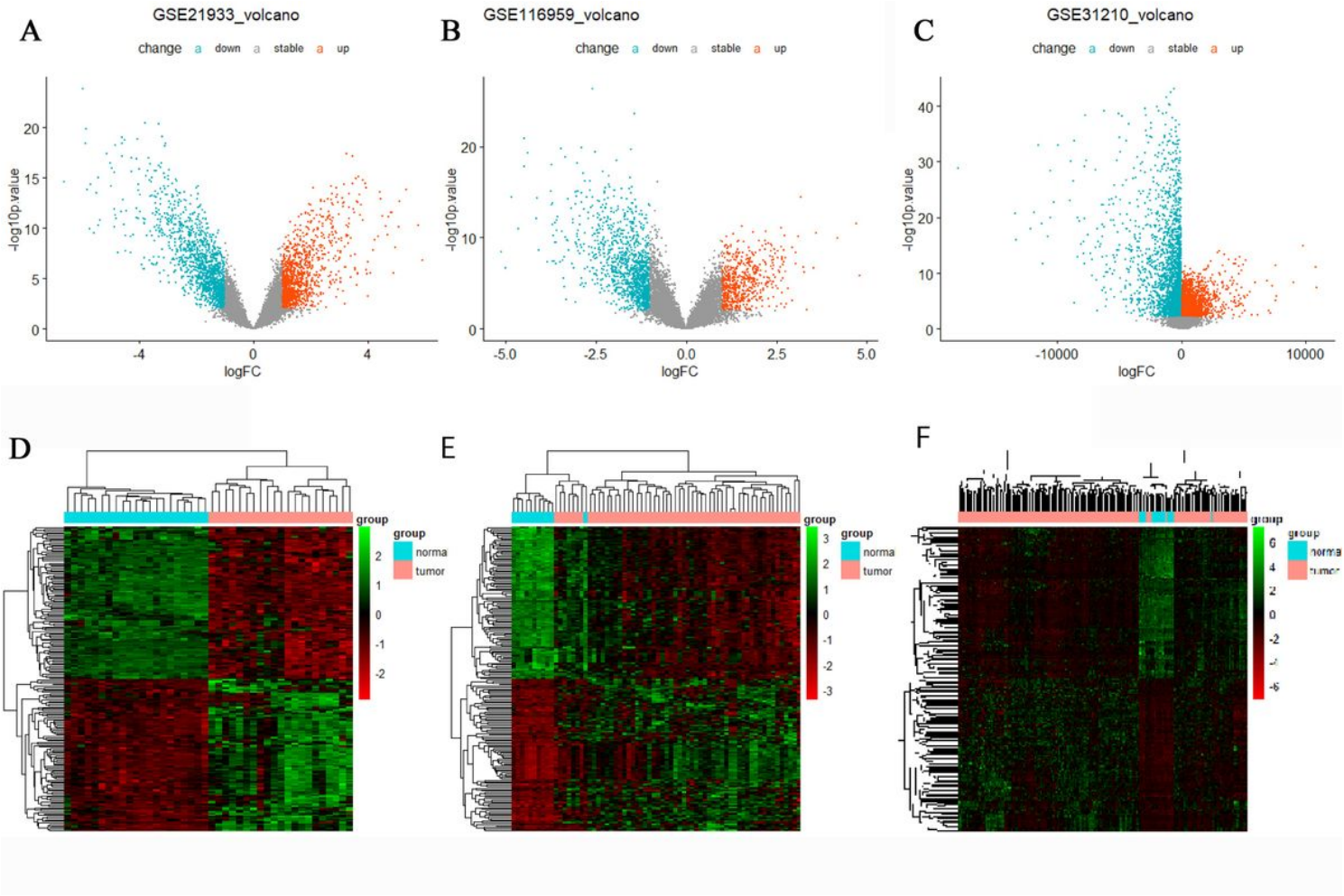



**Figure 2**

DEGs in three data sets. (A–C) The volcano plots visualize the DEGs in GSE116959, GSE21933, GSE31210, respectively. The red nodes represent upregulated genes while the blue nodes represent downregulated genes. (D–F) Heatmap of the top 100 DEGs according to the value of |logFC|> 1 and p<0.01. The green color indicates lower expression and red color indicates high expression.

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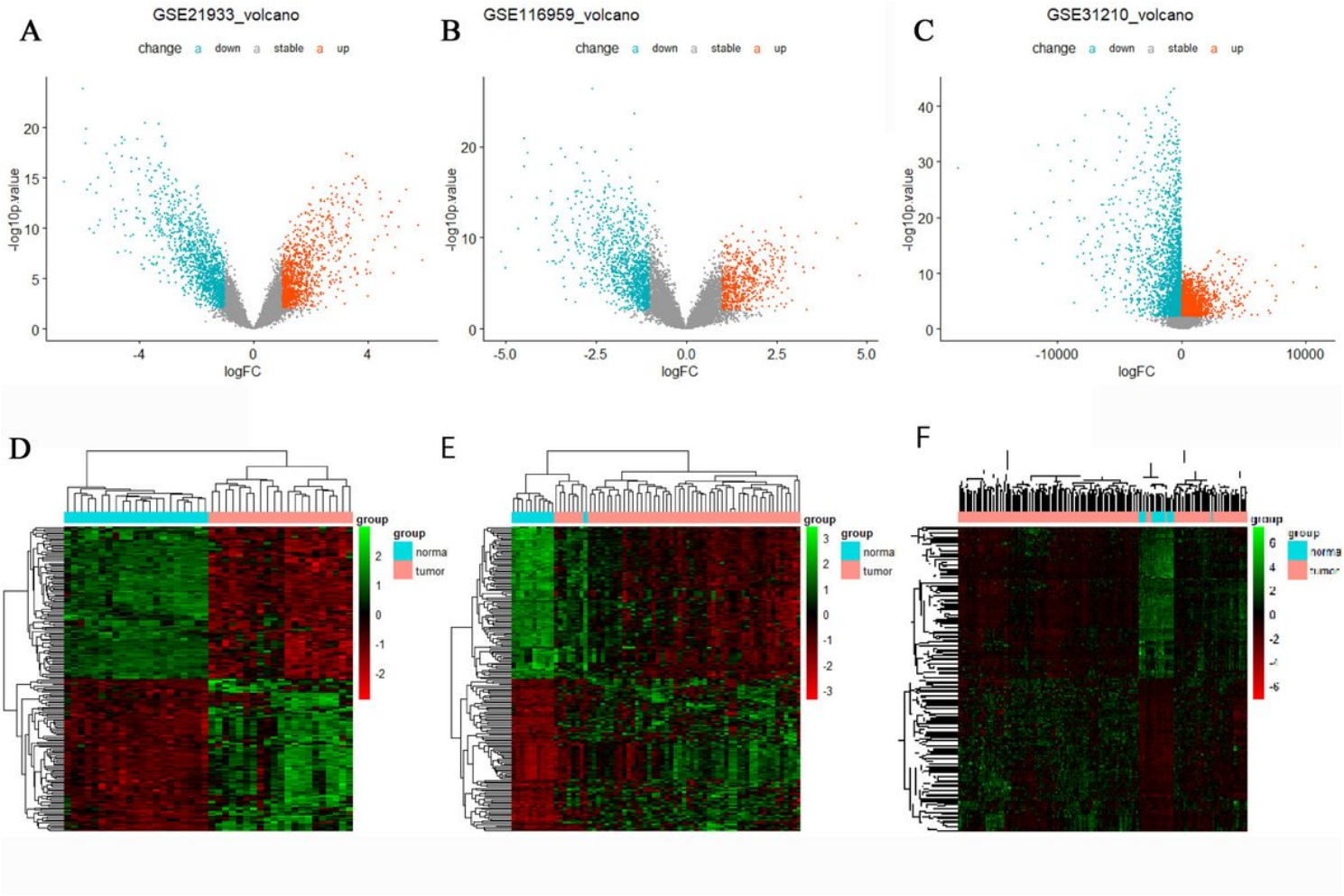


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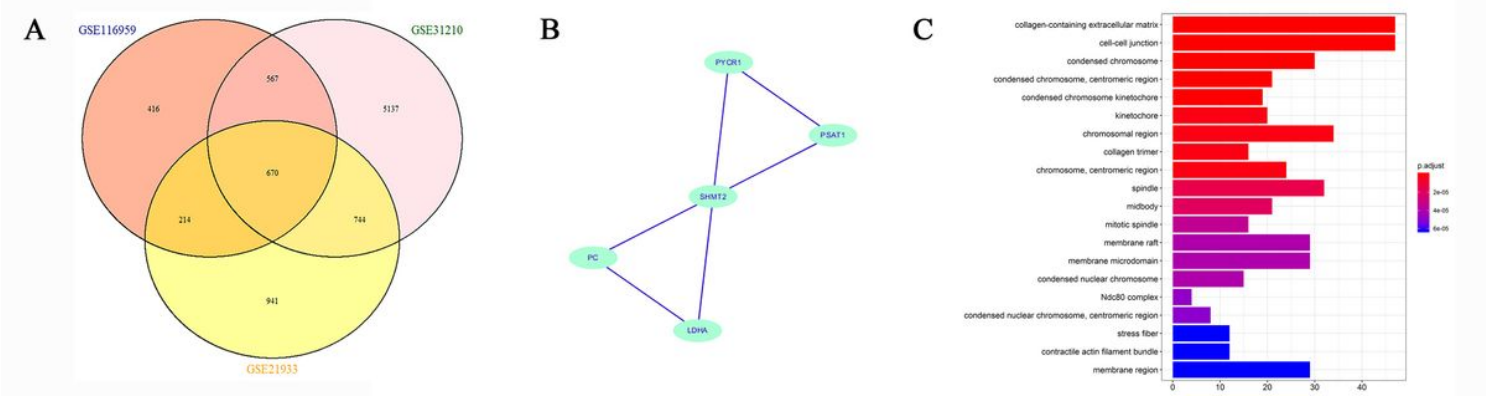


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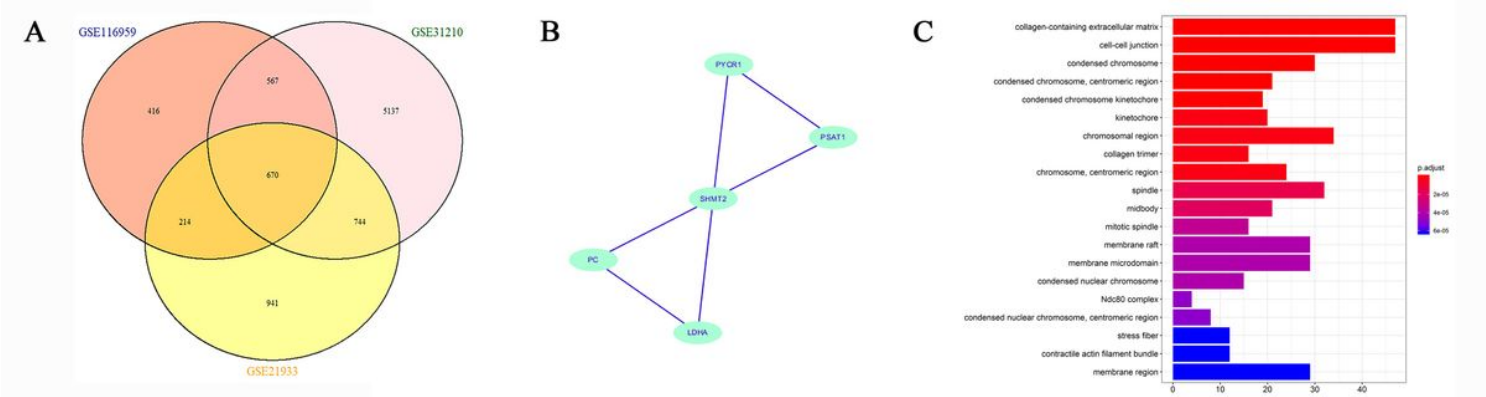
(A) Common DEGs in three data sets. A total of 670 commons in the intersection of three gene sets. (B) Hub gene of common DEGs. There are five hub genes in 670 common genes, including SHMT2, PSAT1, PYCR1, PC, LDHA. (C) GO analysis of common DEGs.

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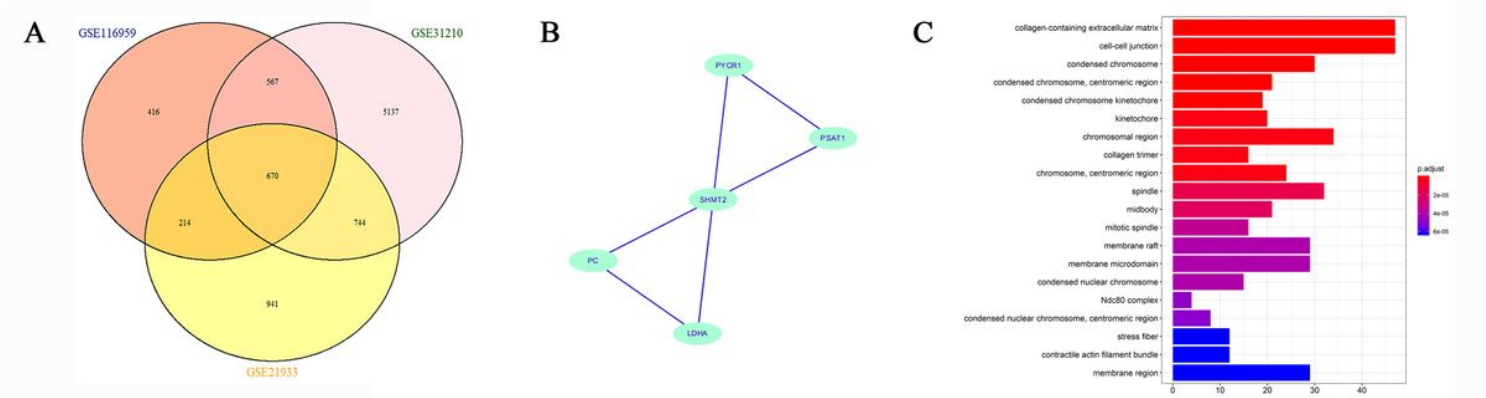


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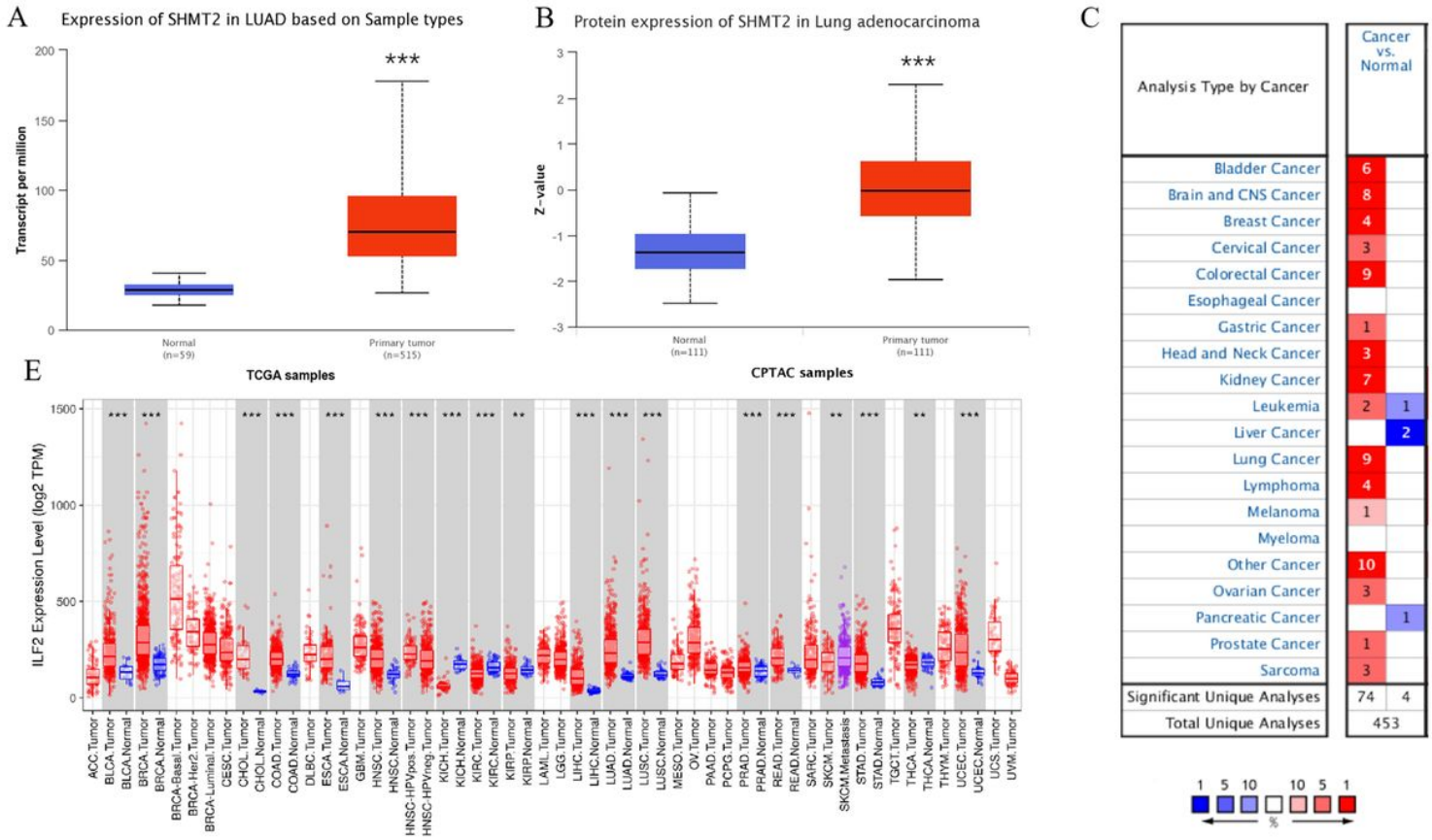
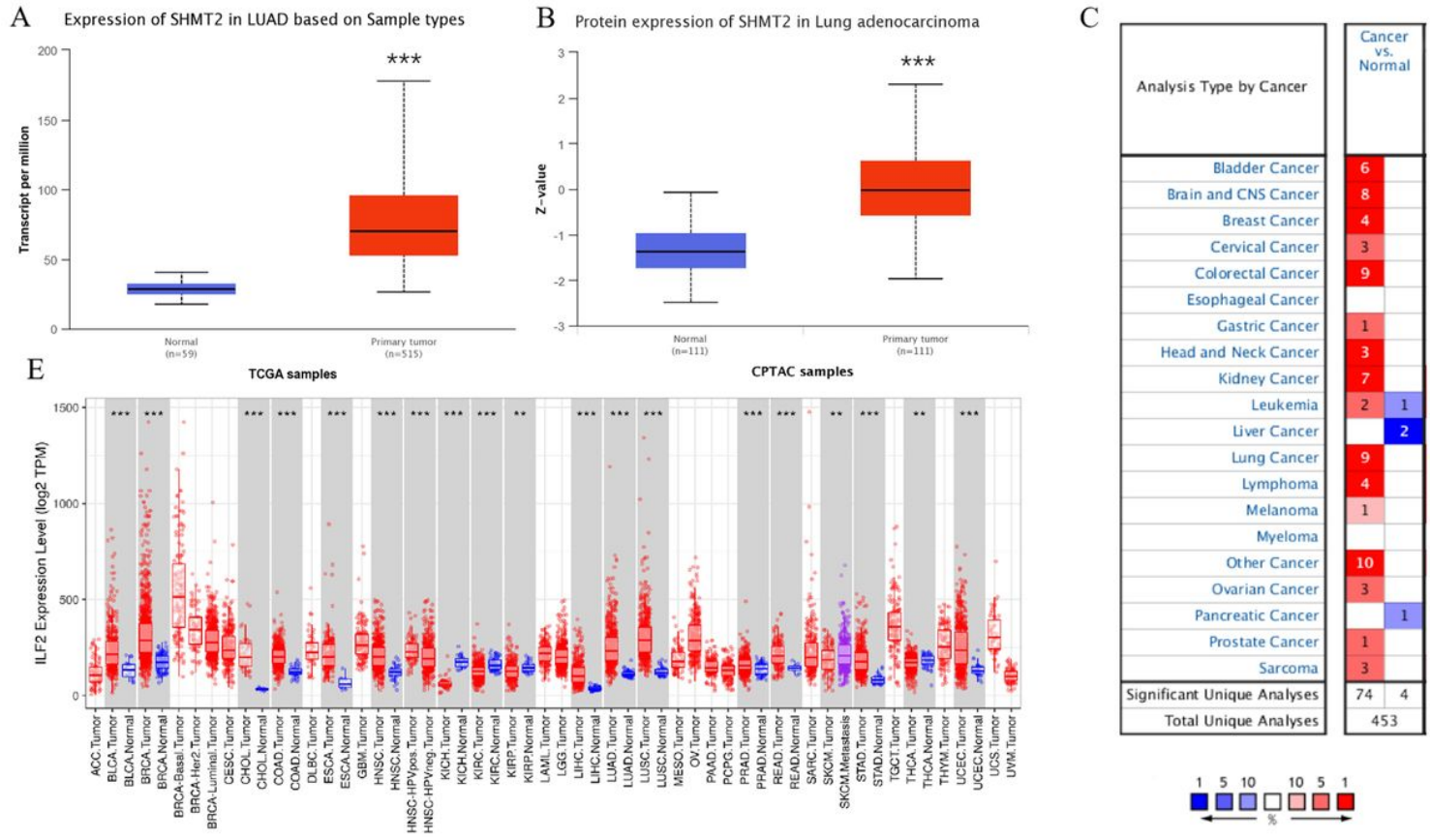



**Figure 4**

SHMT2 expression level (A) mRNA expression of SHMT2 in LUAD. The mRNA expression of SHMT2 is higher in tumor but lower in normal based on TCGA samples; (B) Protein expression of SHMT2 in LUAD. The protein expression of SHMT2 is higher in tumor while lower in normal based on CPTAC samples; (C) The mRNA expression level of SHMT2 in various cancer. Color images are available online. fold change = 2 and P-value = 0.01; (D) SHMT2 different expression between tumor and adjacent normal tissue. *P<0.05, **P<0.01, ***P<0.001

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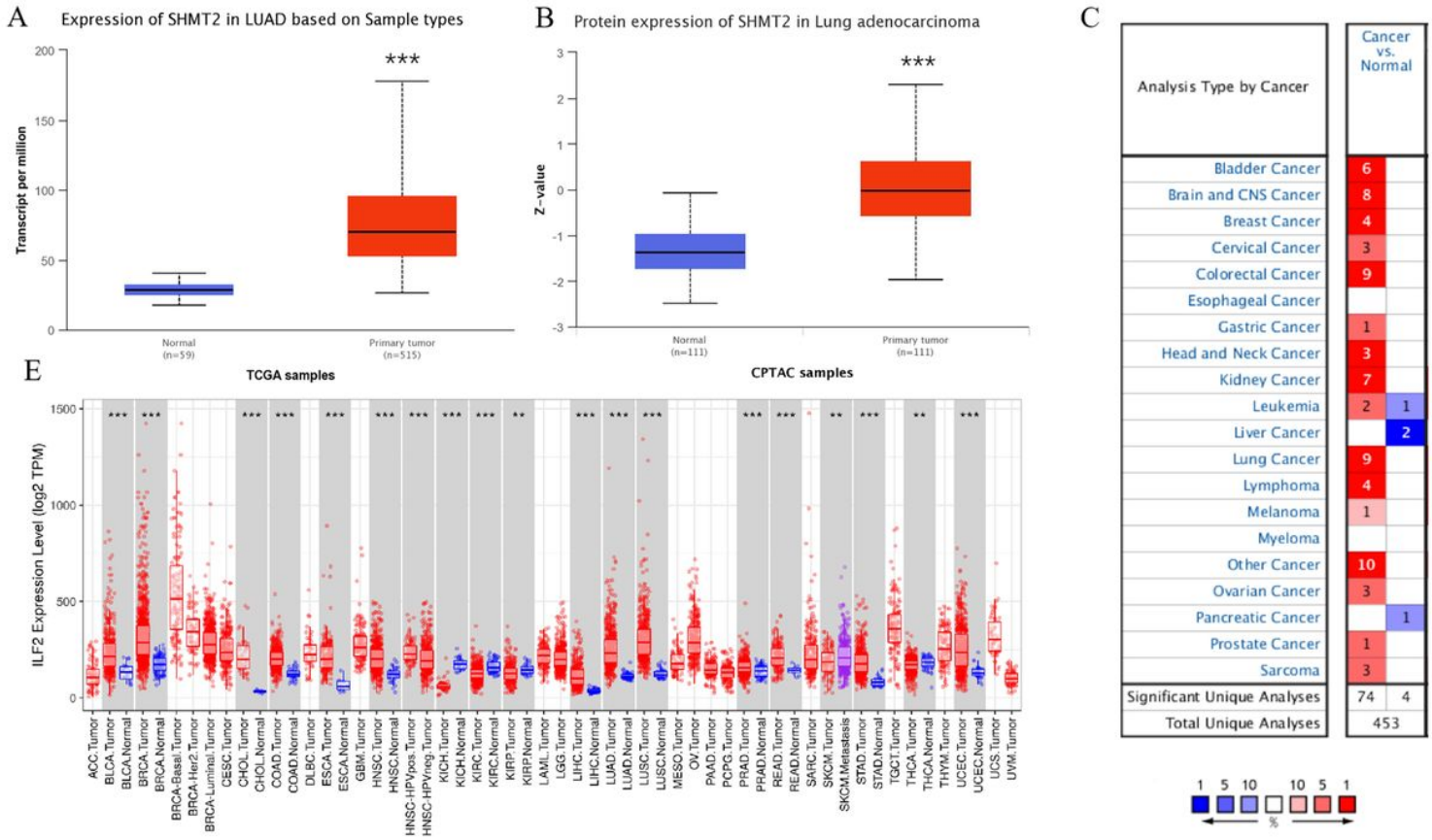


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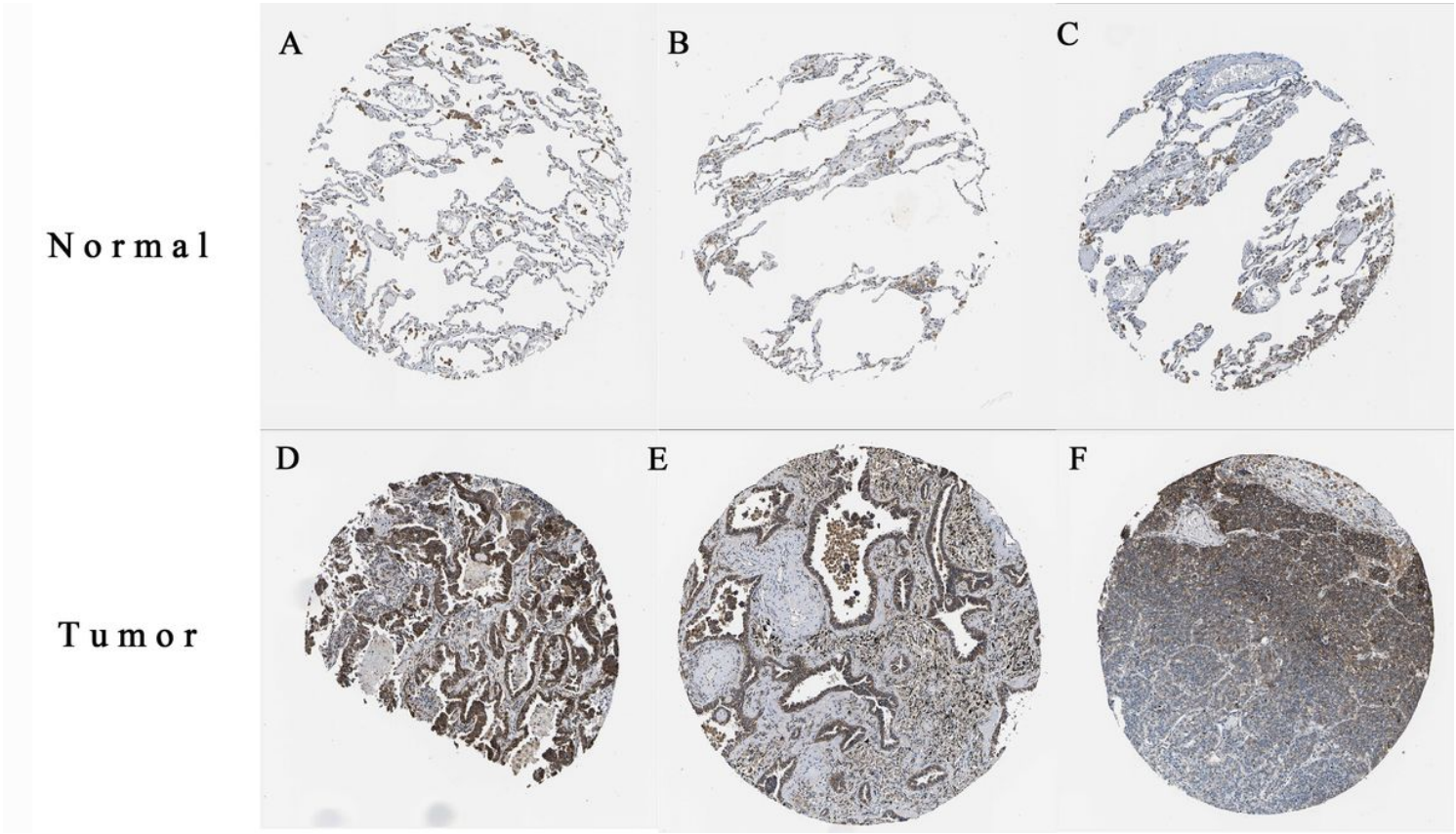
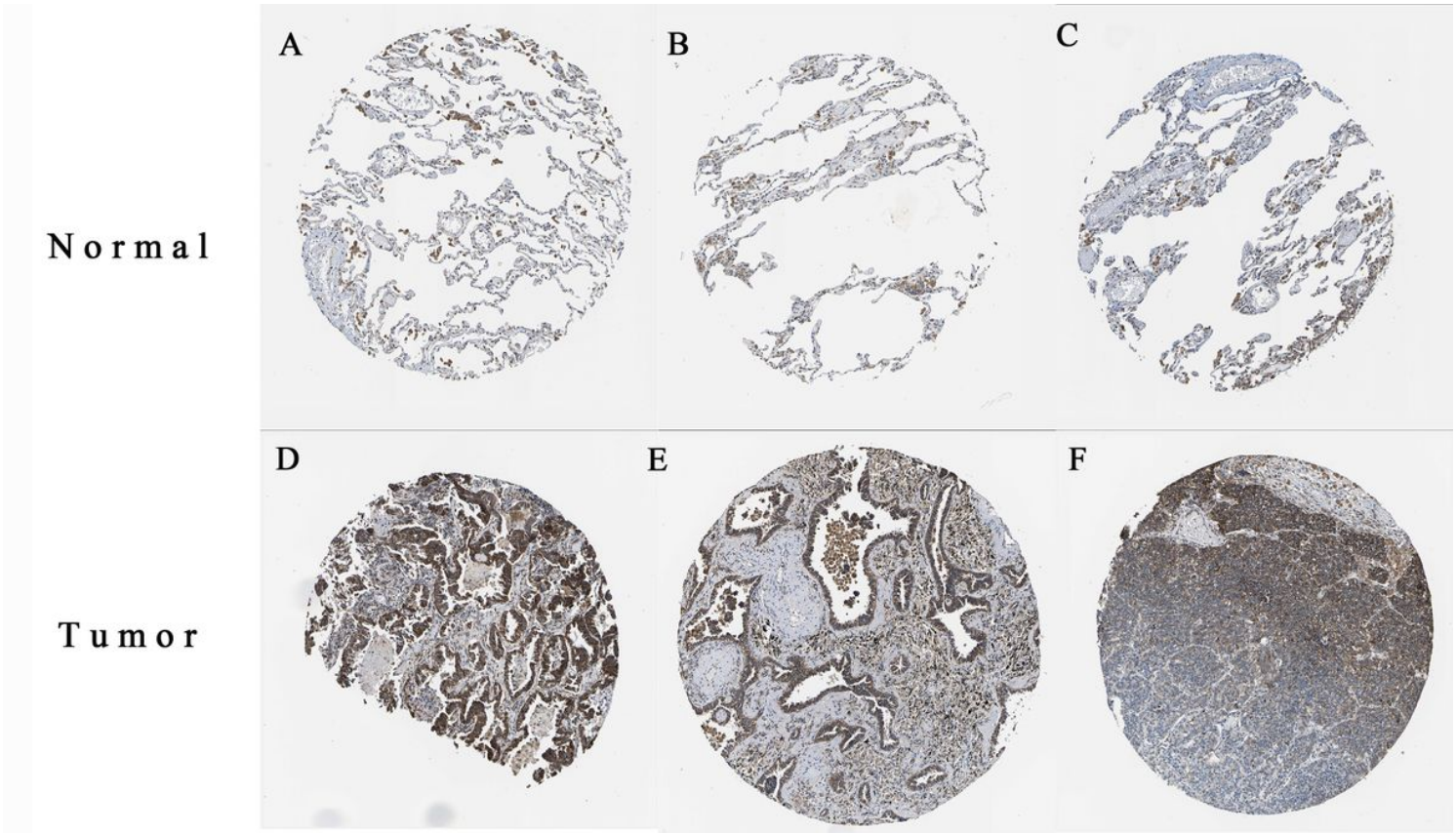



Figure 5

Immunohistochemistry (IHC) of SHMT2 expression in LUAD tissues and corresponding normal tissues based on The Human Protein Atlas (HPA). (A-C) Normal lung(T-28000) tissue; (D-F) Lung(T-28000) tumor tissue

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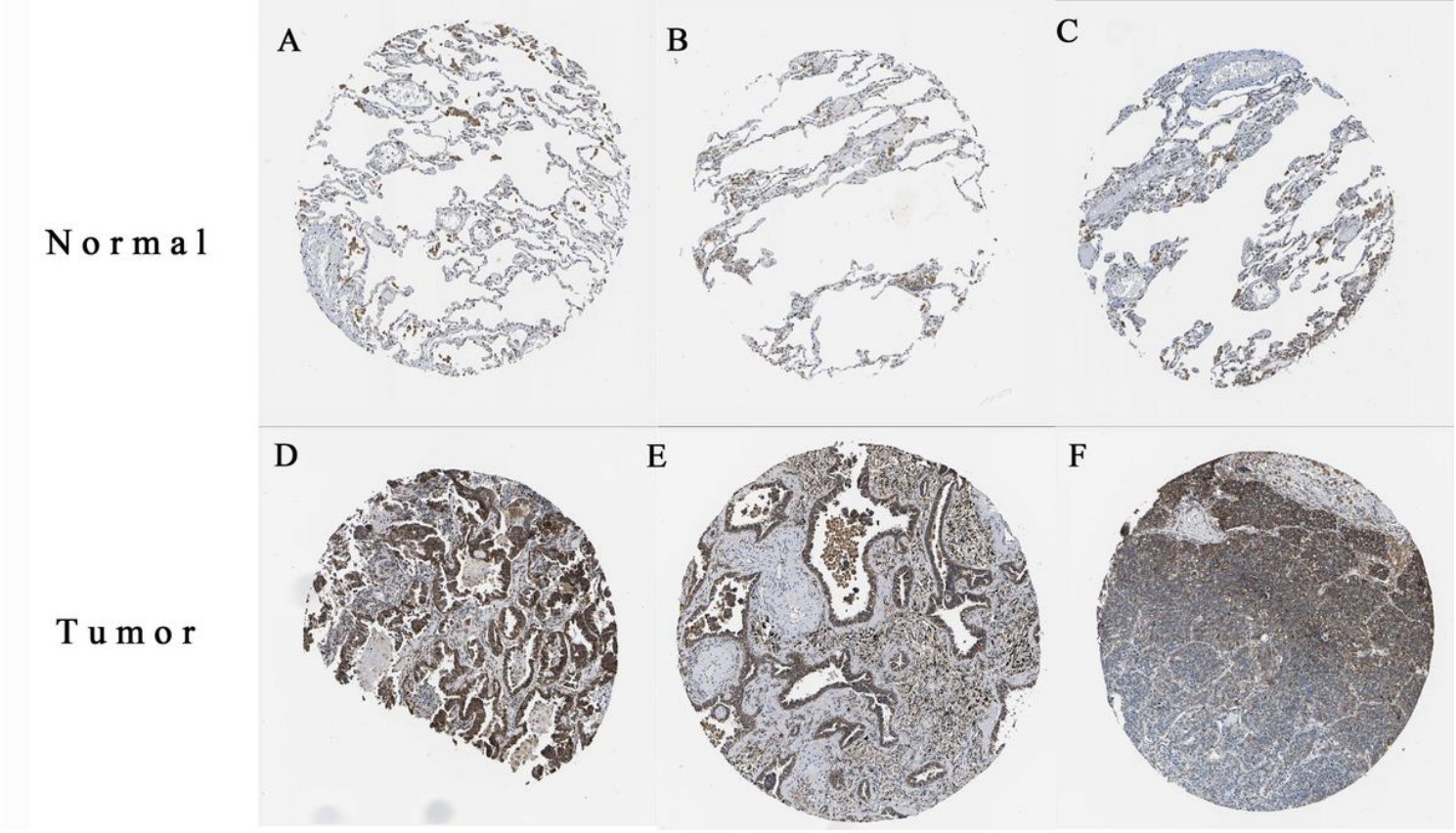


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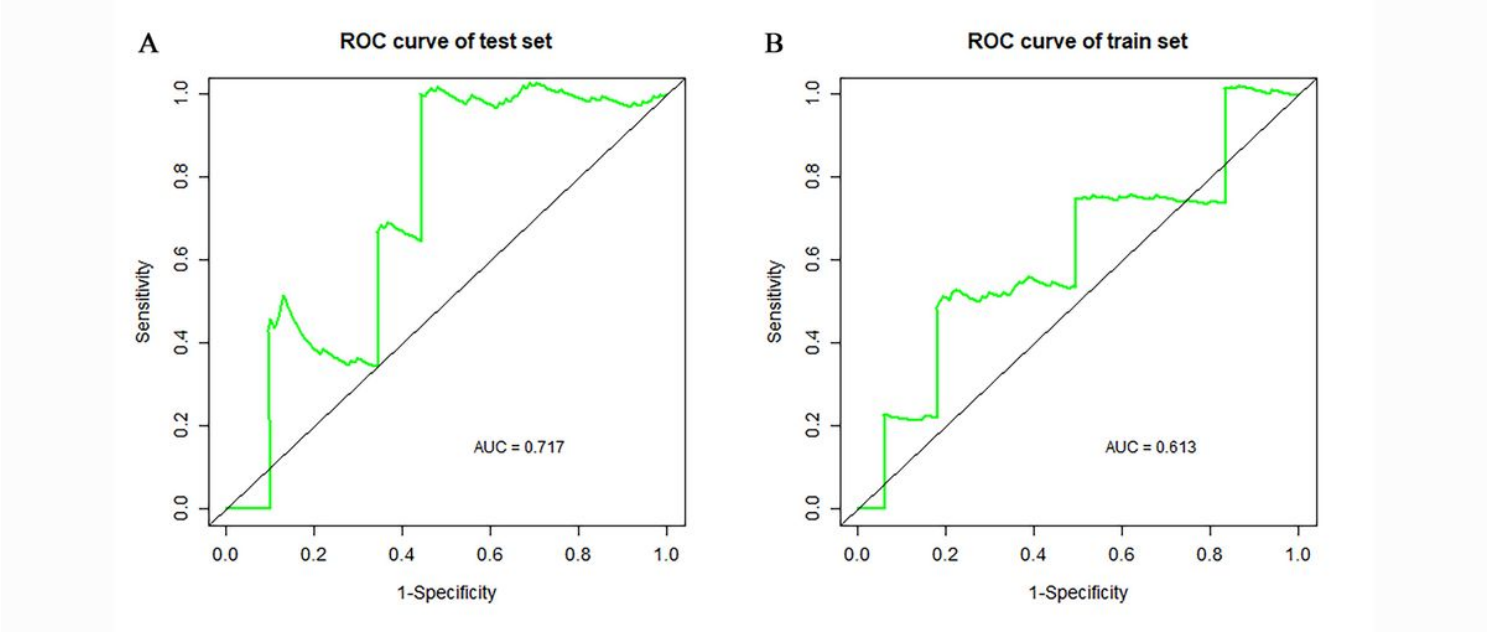
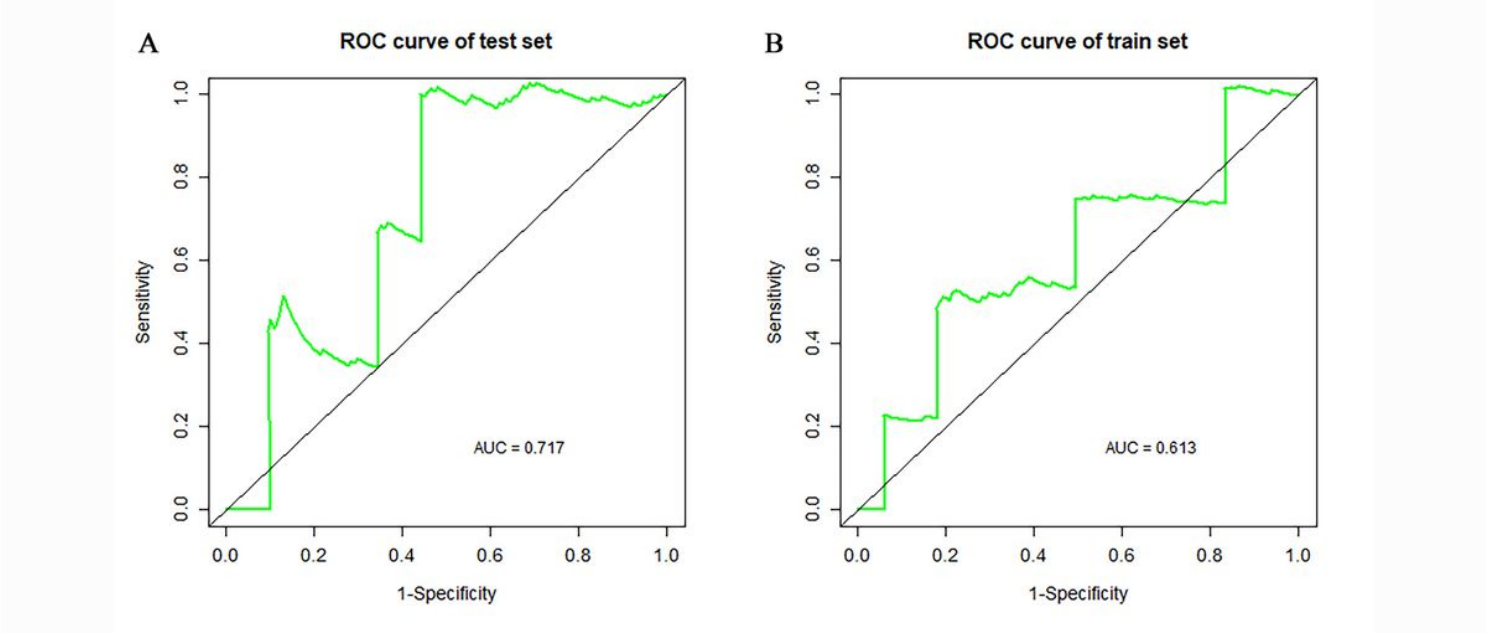



**Figure 6**

The time-dependent receiver operating characteristic (ROC) analysis. The AUC (Area under ROC) score for the training dataset was 0.842, indicating the better performance of survival prediction in the training dataset.

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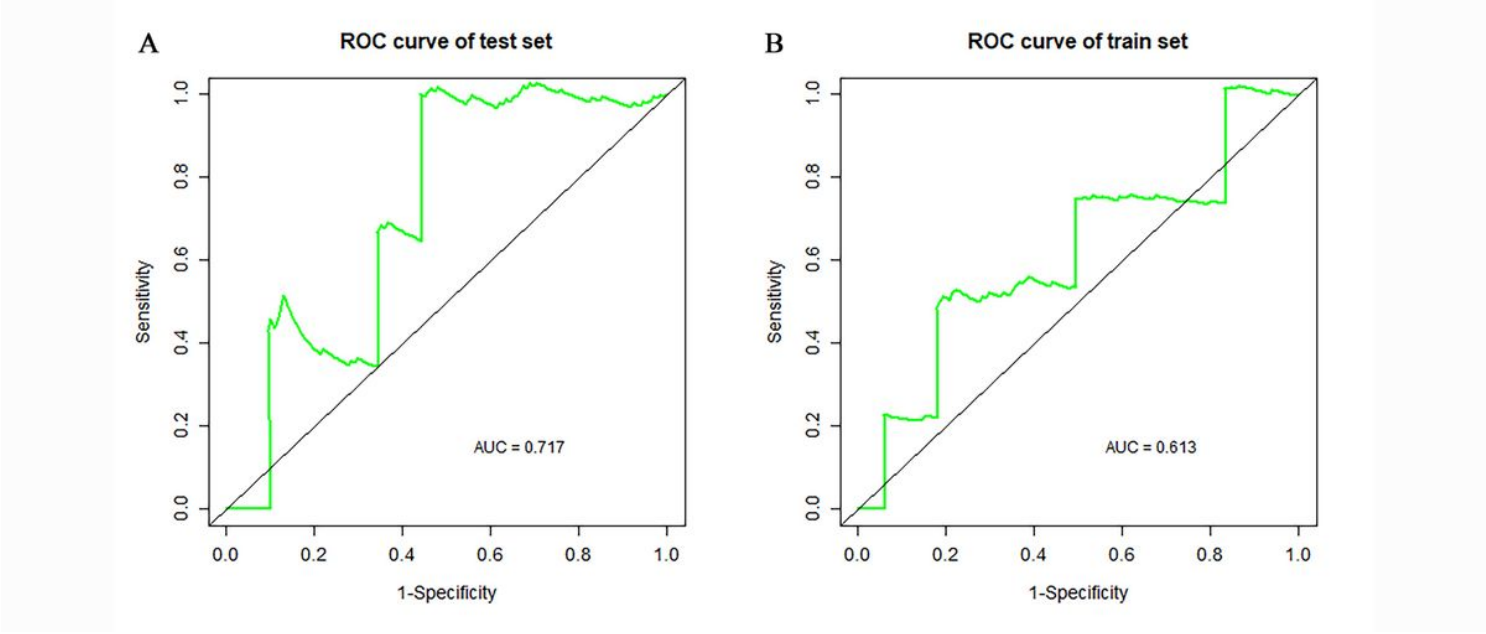


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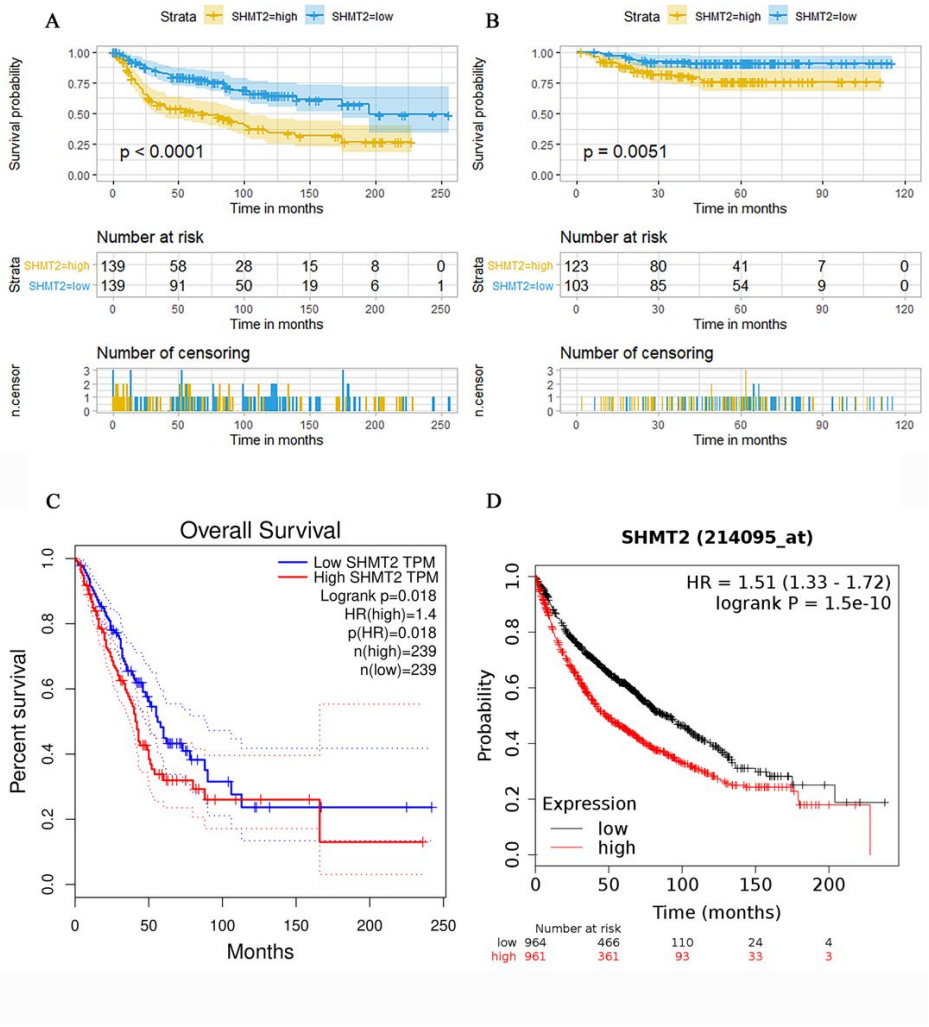
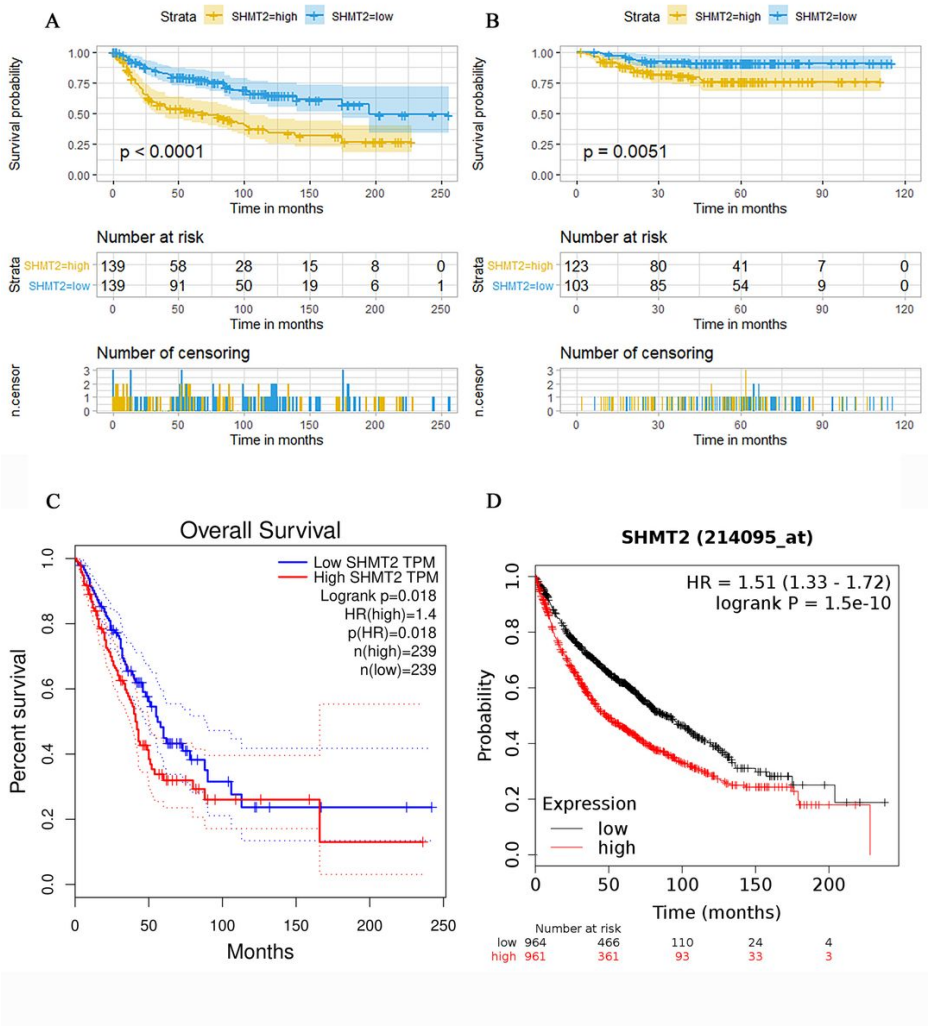



**Figure 7**

Kaplan-Meier survival analysis. (A) OS (Overall survival) of SHMT2 in GSE21933. (B) OS (Overall survival) of SHMT2 in GSE31210. The numbers below the figures represent the number of patients at risk in each group. (C and D) Kaplan-Meier survival curves comparing the high and low expression of SHMT2 in lung adenocarcinoma in the Kaplan-Meier plotter database and GEPIA database.

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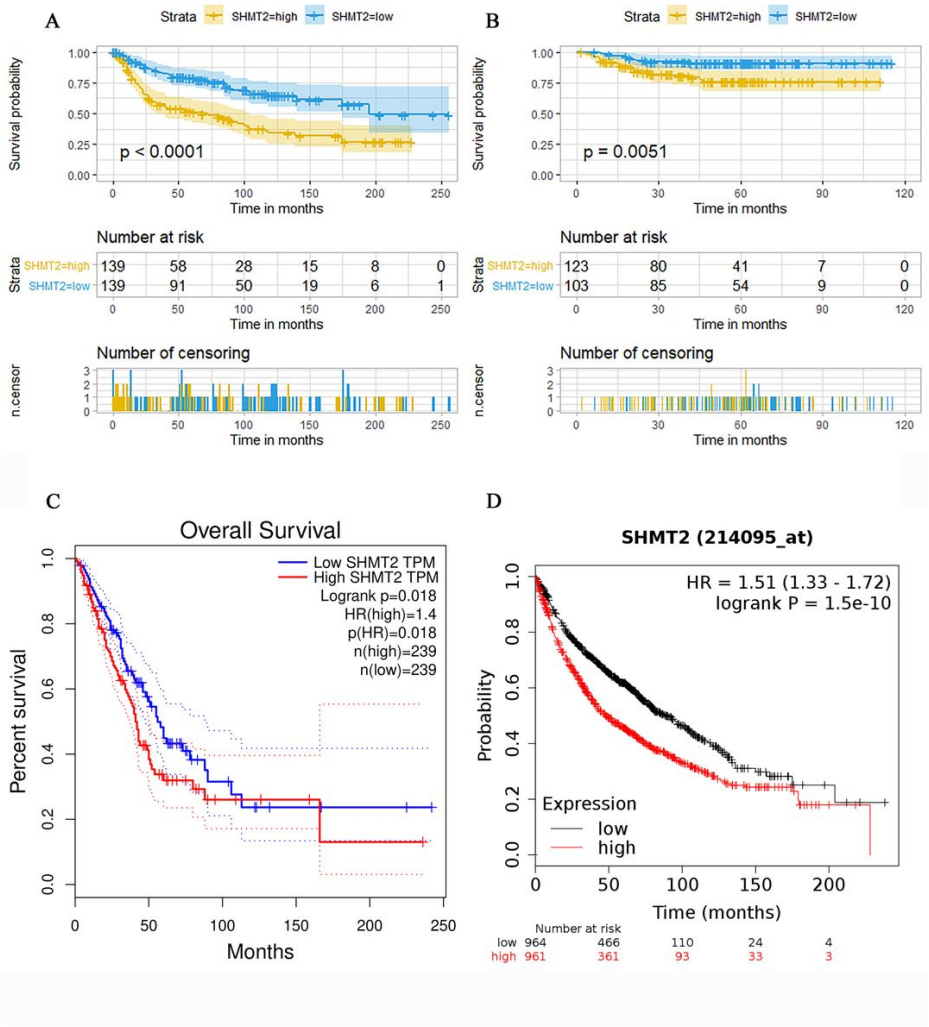


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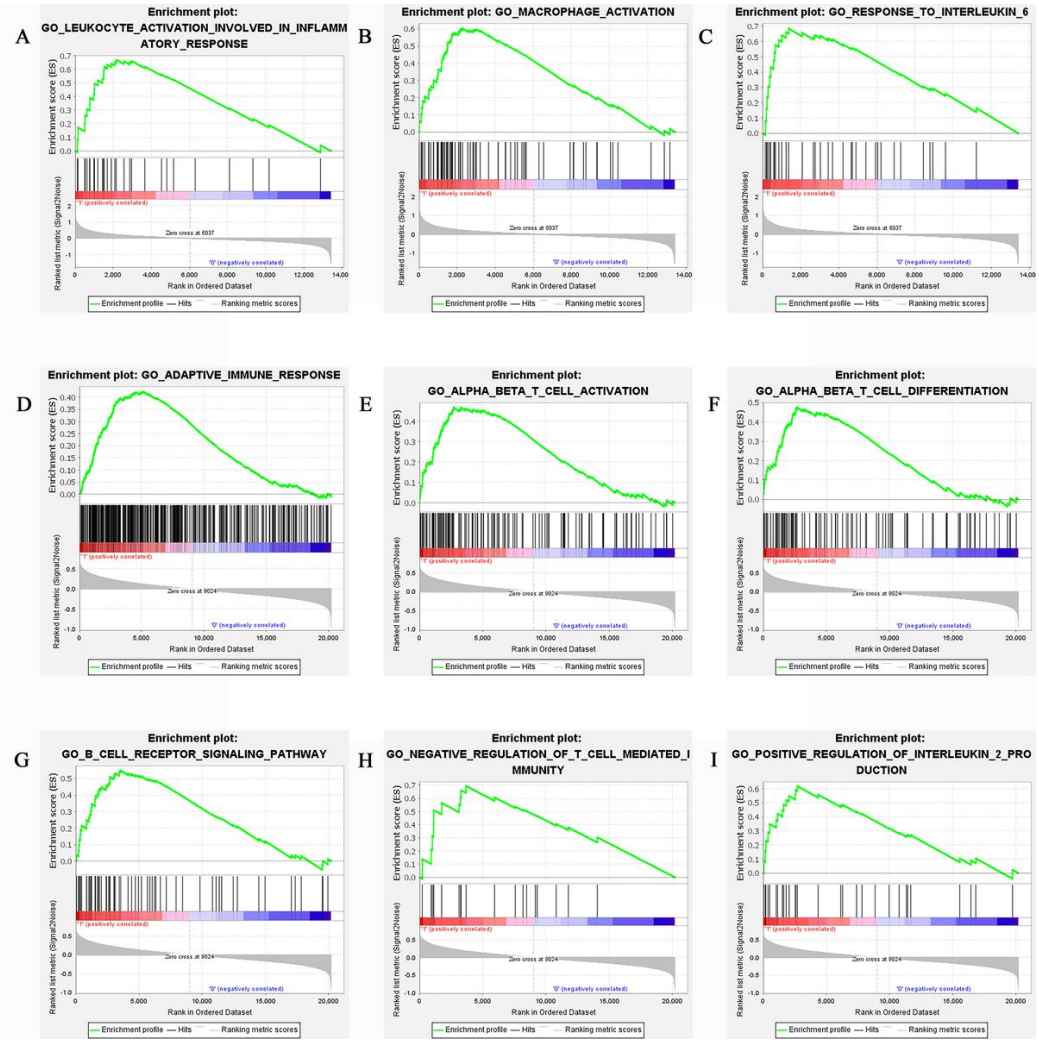
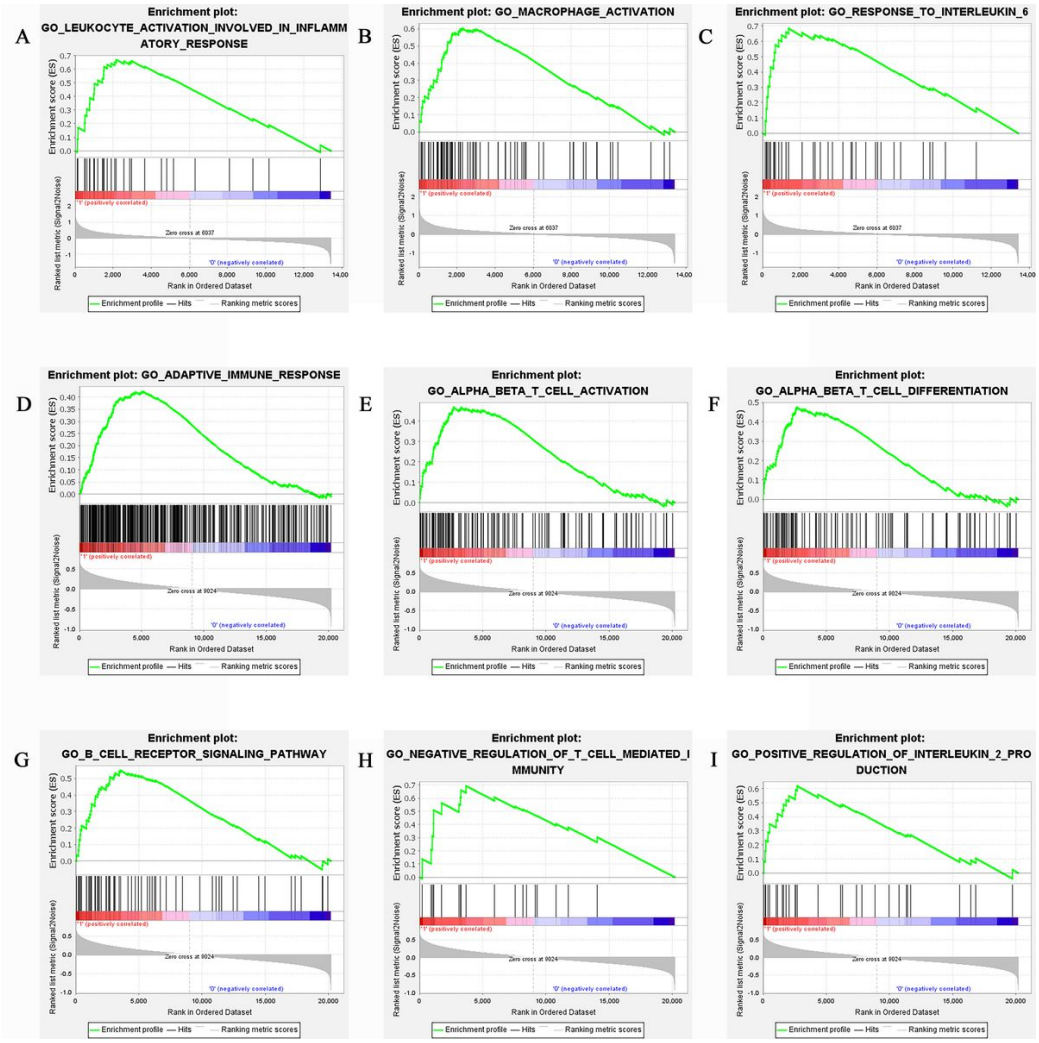



## Figure 8

Up-regulated gene expression was associated with an immunologic process and was validated by GSEA of GO gene sets analysis of high expression of SHMT2 in GSE21933, GSE31210 and GSE116959. (A) Leukocyte activation involved in inflammatory response; (B) Macrophage activation; (C) Response to interleukin 6; (D) adaptive immune response; (E) Alpha beta T cell activation; (F) Alpha beta T cell differentiation; (G) B cell receptor signaling pathway; (H) Negative regulation of T cell mediated immunity; (I) Positive regulation of interleukin 2 production.

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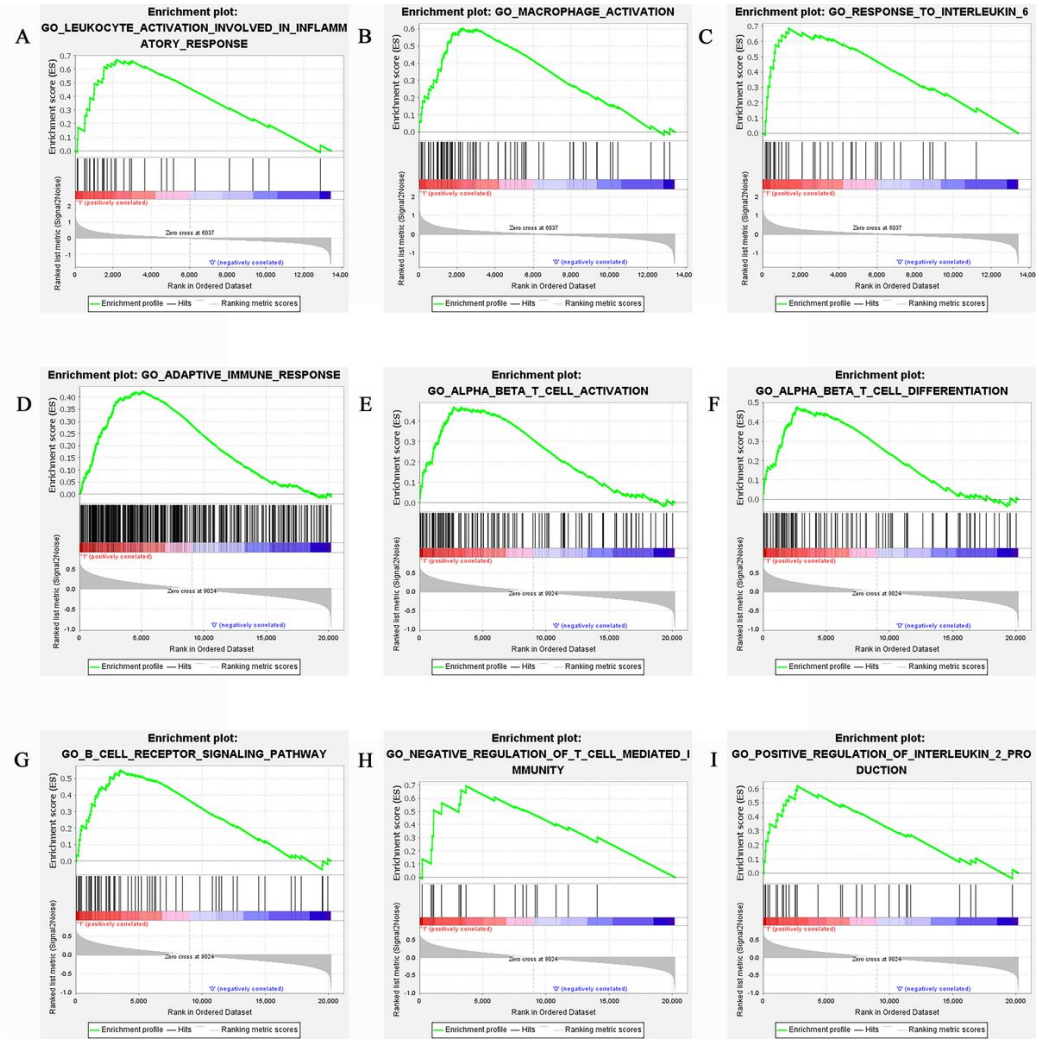


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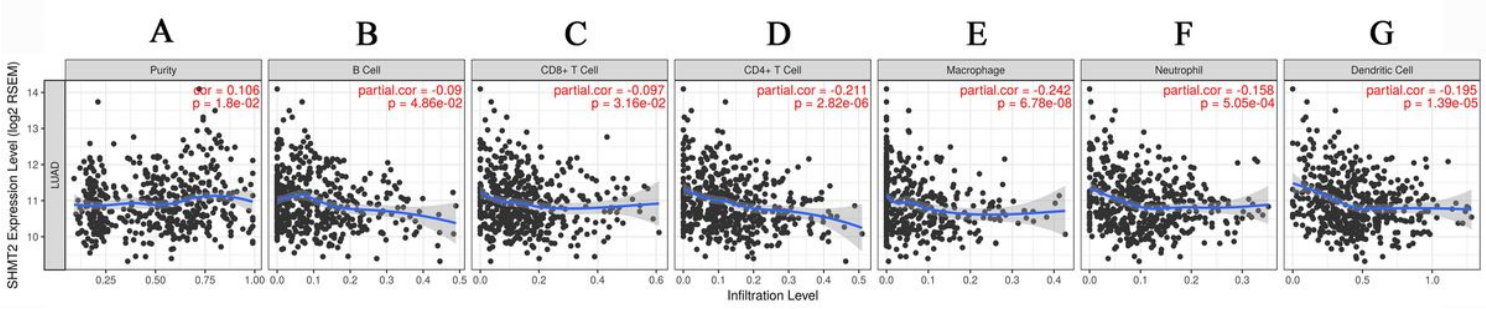


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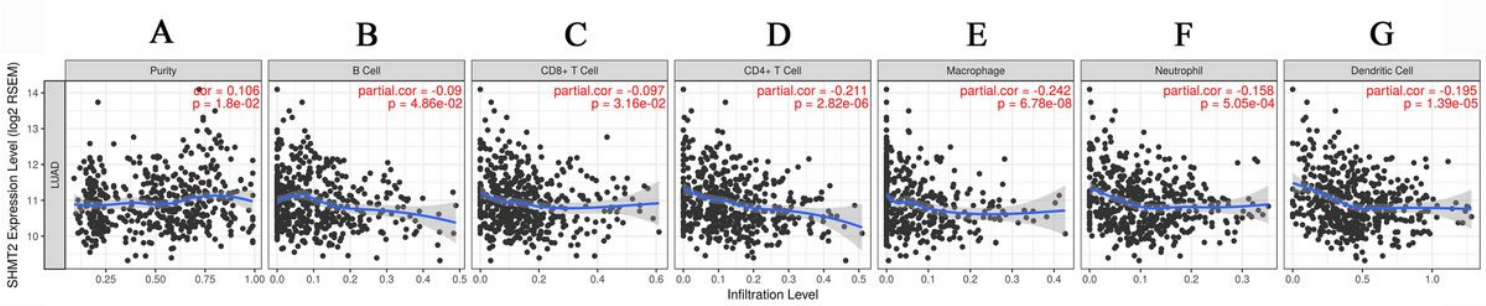


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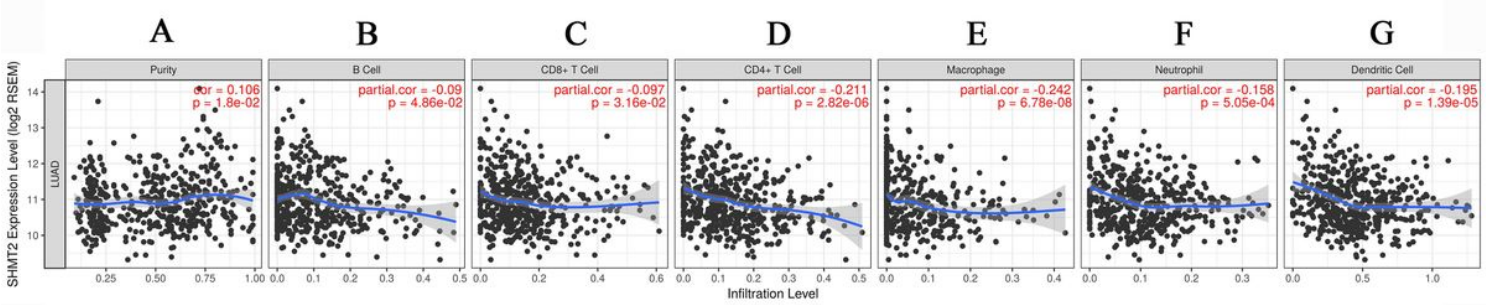


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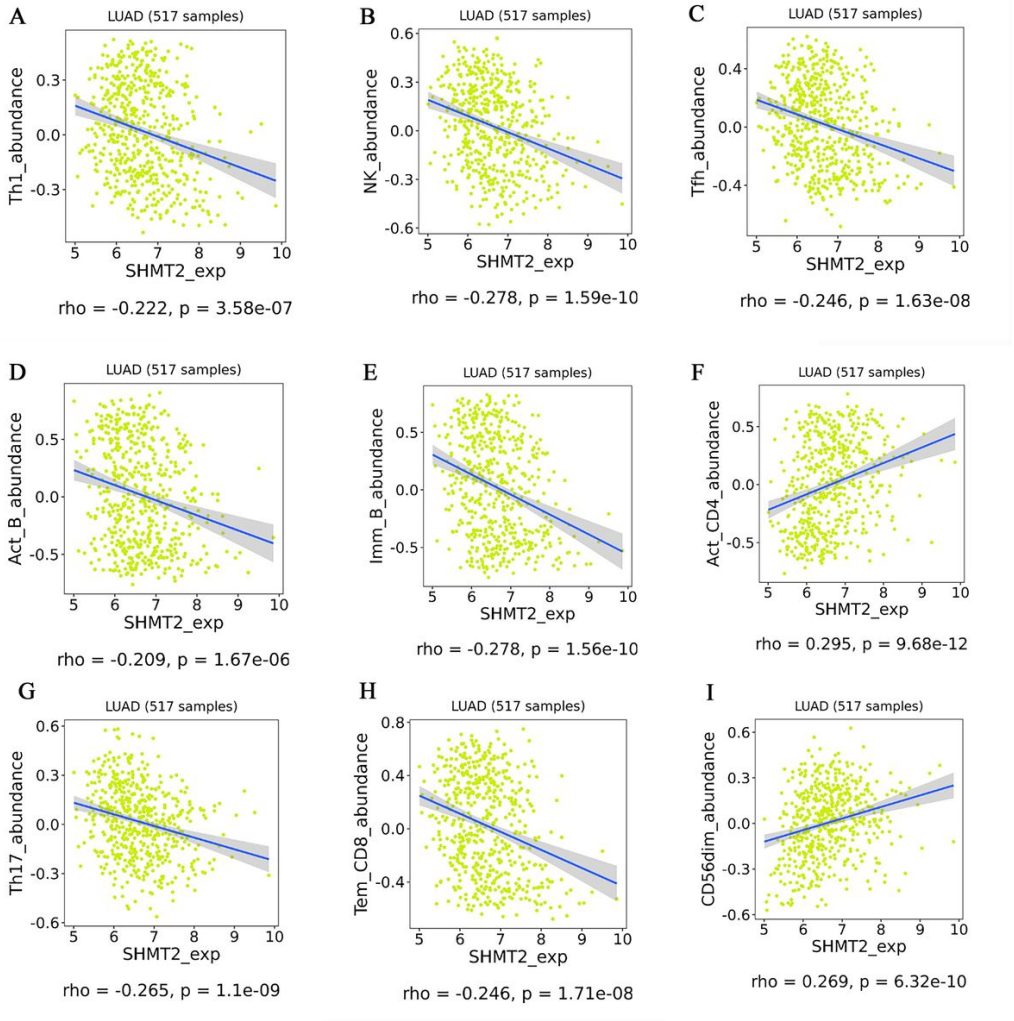
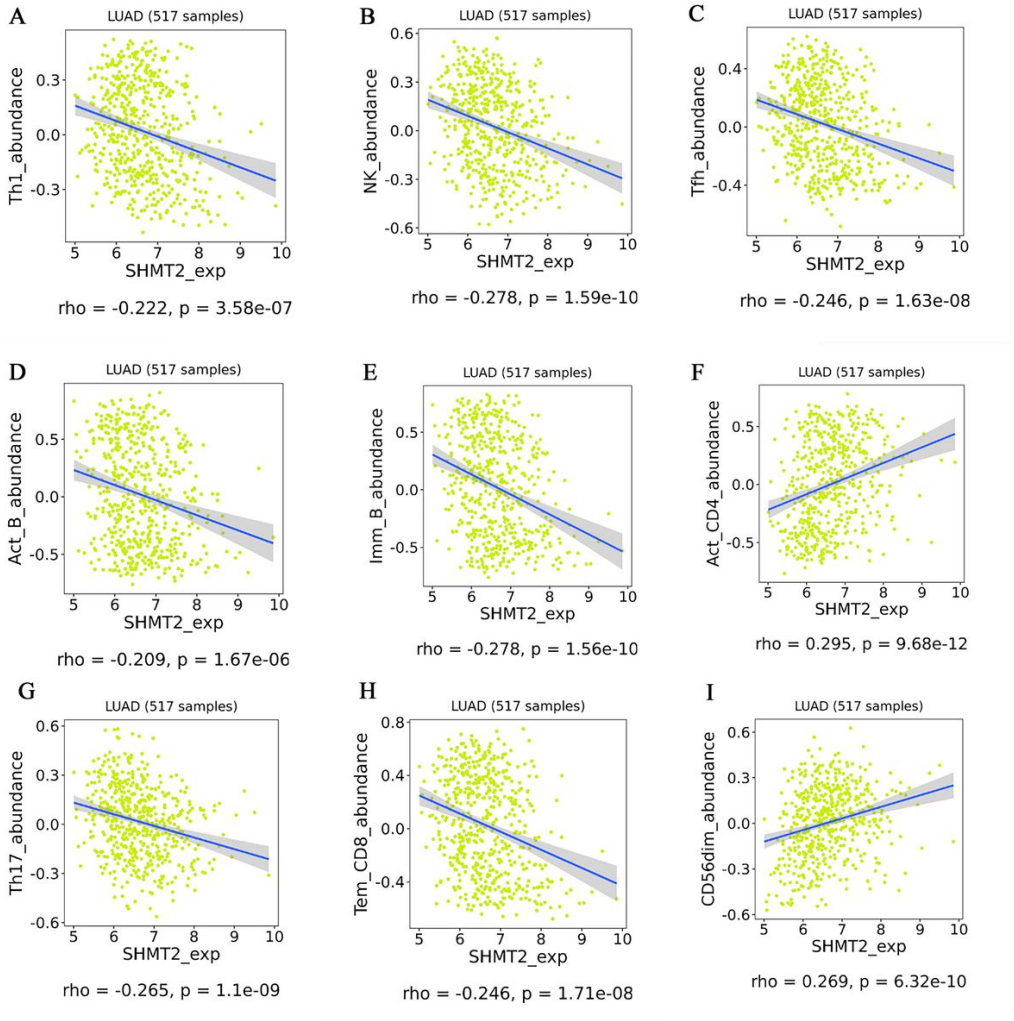



**Figure 10**

Relations between the abundance of tumor-infiltrating lymphocytes (TILs) and SHMT2 expression, including (A) Type 1 T helper cell; (B) Nature killer cell; (C) T follicular helper cell; (D) Active B cell; (E) immature B cell ; (F) Active CD4 T cell; (G) Type 17 T helper cell; (H) Tem CD8; (I) CD56 dim nature killer cell

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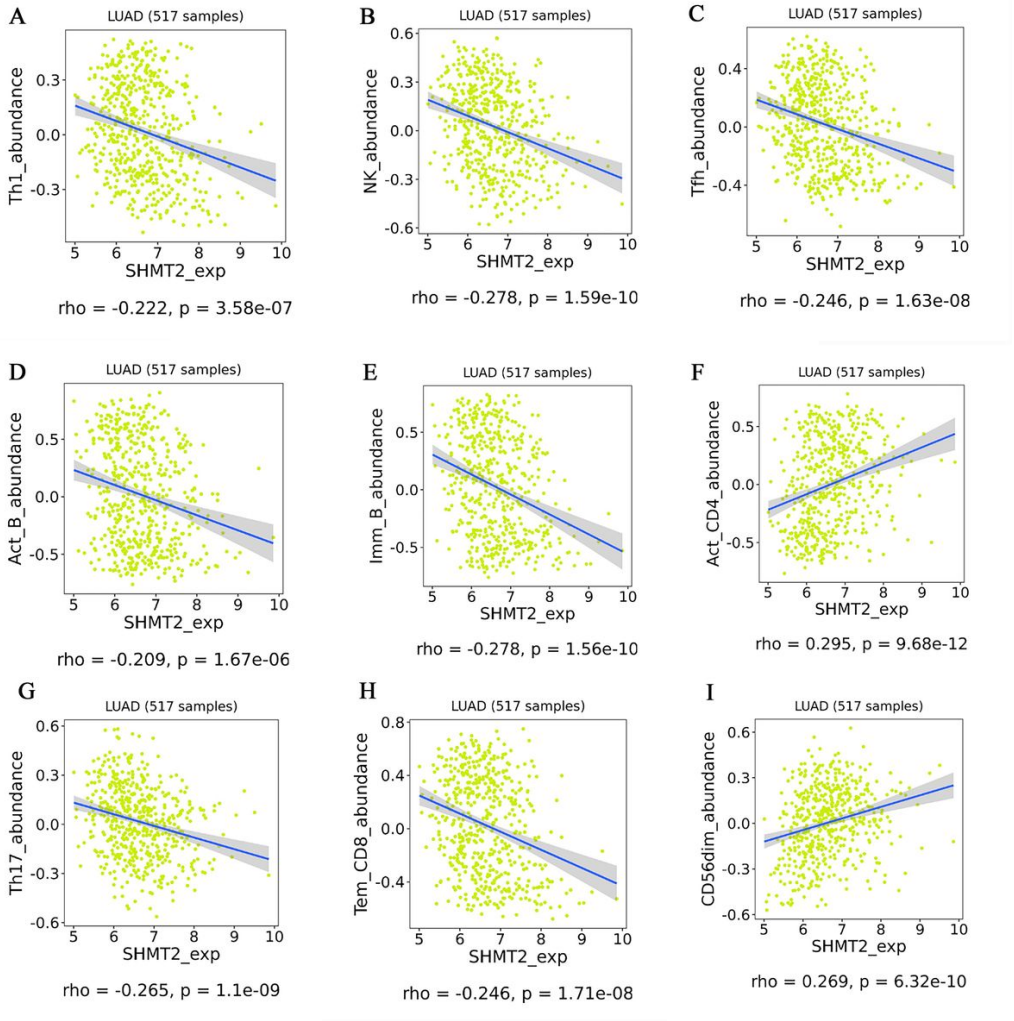


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