

# Increased expression of SLC35F2 in non-small cell lung cancer predicts poor prognosis

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## Research article

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# Abstract

**Background:** Although previous studies have documented the expression of Homo sapiens solute carrier family 35 member F2 (SLC35F2) in NSCLC, its prognostic value for NSCLC has not been reported.

**Methods:** We performed Wilcoxon signed-rank test and logistic regression to investigate the correlations between SLC35F2 expression and clinical parameters. A Cox regression was used to investigate the association between clinical features and overall survival (OS). Gene Set Enrichment Analysis (GSEA) was performed to further understand the pathways involved in NSCLC pathogenesis related SLC35F2.

**Results:** In LUAD, increased expression of SLC35F2 was associated with stage (OR = 1.53 for stage III-IV vs. Stage II), N stage (OR = 1.62 for N1-3 vs. N0), and residual tumor (OR = 5.00 for with tumor vs. Tumor free) ( $p < 0.05$ ). The Kaplan-Meier curve showed that the prognosis of SLC35F2-high NSCLC was worse than that of SLC35F2-low. For LUAD, the univariate analysis showed that increased expression of SLC35F2 was associated with a poor overall survival (HR 1.04; 95% CI 1.00-1.08;  $p = 0.04$ ). Multivariate analysis showed that SLC35F2 can be used as an independent prognostic factor for LUAD. GSEA show that P53 signal pathway and apoptosis are significantly enriched in SLC35F2-high in NSCLC.

**Conclusions:** Increased expression of SLC35F2 in non-small cell lung cancer predicts poor prognosis. Moreover, SLC35F2 can be used as an independent prognostic factor for LUAD.

## Introduction

Lung cancer (LC) is considered a life-threatening disease, and its morbidity and mortality ranks second among all tumors(1). Non-small cell lung cancer (NSCLC) accounts for 85% of all diagnosed LC cases. Currently, the main treatment of NSCLC is the combination of surgery and chemotherapy(2). Although early detection, diagnosis and targeted therapy of NSCLC have made great progress, the five-year survival rate is still very low(3). Regional or distant metastasis is a risk factor for relapse and poor prognosis of NSCLC(3). However, there are currently no effective biomarkers to predict poor prognosis in NSCLC(4). Therefore, there is an urgent need to identify new markers to predict poor prognosis in NSCLC(5).

The SLC35 gene, as a member of the solute vector family, is capable of encoding a nucleotide sugar transporter(6). The SLC35 gene has six members: SLC35A-F, in which the SLC35 A-E protein transport nucleotide sugars into the Golgi apparatus(7). Previous studies have shown that this type of nucleotide sugar transporter was associated with tumor metastasis(8). The function of the SLC35F family has not been reported. Bu et al report that SLC35F2 was highly expressed in non-small cell lung cancer (NSCLC) tissues(9). However, its prognostic value for NSCLC has not been reported.

Thus, the aim of this study was to investigate the prognostic value of SLC35F2 in NSCLC. GSEA was performed to understand the pathways associated with SLC35F2.

## Materials And Methods

## Data collection

The Cancer Genome Atlas (TCGA) was used to obtain expression values and corresponding clinical information of SLC35F2 in NSCLC and normal samples.

## Gene set enrichment analysis

GSEA was performed to evaluate the survival differences between the high expression and low expression SLC35F2 phenotype groups. Gene set permutations were performed 1000 times for each analysis. The nominal p value was used to classify the pathways enriched in each phenotype. NOM p-val < 0.05 was considered as significant.

## Statistical analysis

We performed Wilcoxon signed-rank test and logistic regression to investigate the correlations between SLC35F2 expression and clinical parameters. Cox regression was used to evaluate the association between clinicopathologic features and OS. Comparing the effects of SLC35F2 expression on survival together with other clinical features (age, stage, gender, TMN stage, tumor status, residual tumor, race, and history of other malignancy) using Multivariate Cox analysis.

# Results

## Patient characteristics

We downloaded the gene expression dataset and clinical information of NSCLC from the TCGA database. TCGA provided gene expression profiles of 522 LUAD and 504 LUSC samples (Supplement Table 1).

The median age of patients for LUAD and LUSC was 65 years and 67 years, respectively. For tumor status, there were 314 tumor-free (60.15%) and 111 with tumor (21.26%) in LUAD, and 313 tumor-free (62.10%) and 81 (16.07%) with tumor in LUSC. For LUAD, there were 172 patients (32.95%) in stage I, 281 (53.83%) in stage II, 47 (9%) in stage III and 19 (3.6%) in stage IV. For LUSC, there were 245 patients (48.61%) in stage I, 163 (32.34%) in stage II, 85 (16.87%) in stage III and 7 (1.4%) in stage IV. Twenty-Five (4.8%) patients had lymph node metastases in LUAD, and seven (1.4%) in LUSC. For residual tumor, eighteen patients (3.4%) had residual tumor in LUAD, and Sixteen (3.2%) in LUSC. For gender, there included 242 male (46.36%) and 280 (53.64%) female in LUAD, and 373 male (74%) and 131 female (36%) in LUSC. For LUAD, most patients (75.29%, n = 393) were of white, 10.2% (n = 53) were Black or African American, 1.5% (n = 8) were Asian. For LUSC, most patients (69.64%, n = 351) were of white, 6.2% (n = 31) were Black or African American, 1.8% (n = 9) were Asian. For history of other malignancy, 93 patients (17.82%) with LUAD and 70 patients (13.89%) with LUSC had other malignancy.

## Expression and paired expression of SLC35F2 in NSCLC and normal samples.

As is illustrated in Figure 1. Highly expressed of SLC35F2 was found in the LUAD and LUSC groups than in the normal groups and adjacent tissue (all p-values <0.01).

### **Association with SLC35F2 expression and clinicopathologic variables**

For LUAD, increased expression of SLC35F2 was associated with the age (p=0.005), N stage (p=0.023), and residual tumor (p = 0.009) (Figure 2). Univariate analysis showed that SLC35F2 expression was associated with clinicopathological features of poor prognosis. Increased SLC35F2 expression was associated with stage (OR = 1.53 for stage III-IV vs. Stage II), N stage (OR = 1.62 for N1-3 vs. N0), and residual tumor (OR = 5.00 for with tumor vs. Tumor free) (p <0.05) (Supplement Table 2). These results indicate that LUAD with high SLC35F2 expression is more likely to progress to more advanced and lymph node metastasis than those with low SLC35F2 expression in LUAD. For LUSC, increased expression of SLC35F2 was associated with the race (OR=0.11 for white vs. Asian, p=0.04) (Figure 3 and Supplement Table 2).

### **Survival analysis and multivariate analysis**

For LUAD, the Kaplan-Meier curve showed that the prognosis of SLC35F2-high NSCLC was worse than that of SLC35F2-low (p=0.006) (Figure 4a). The univariate analysis revealed that high level of SLC35F2 was associated with a poor OS (HR 1.04; 95% CI 1.00-1.08; p = 0.04). Other variables associated with poor survival include T3-4 stage and stage III-IV, and positive tumor status and lymph nodes (Supplement Table 3). A multivariate cox analysis showed that SLC35F2 was associated with overall survival (HR 1.05; 95% CI 1.00-1.10; p = 0.03), together with T stage and positive tumor status and lymph nodes (Figure 5).

For LUSC, the Kaplan-Meier curve showed that the prognosis of SLC35F2-high NSCLC was worse than that of SLC35F2-low (p=0.017) (Figure 4b). Clinical features associated with poor os include male and positive tumor status (Supplement Table 3). A multivariate analysis indicated that male and positive tumor status were associated with os (all p-values<0.05) (Figure 6).

### **GSEA**

We performed GSEA between low and high expression of SLC35F2 to identify pathways for differential enrichment in NSCLC.

For LUAD, the ubiquitin mediated proteolysis, galactose metabolism, P53 signal pathway and apoptosis were enriched in SLC35F2 high expression phenotype, and type II diabetes mellitus, linoleic acid metabolism, and neuroactive ligand receptor interaction in SLC35F2 low expression phenotype (Figure 7 and Supplement Table 4). For LUSC, the focal adhesion, small cell lung cancer and P53 signal pathway were enriched in SLC35F2 high expression phenotype, and metabolism of xenobiotics by cytochrome P450, drug metabolism cytochrome P450 and retinol metabolism in SLC35F2 low expression phenotype (Figure 8 and Supplement Table 5).

## Discussion

The SLC gene family regulates important physiological functions, and its dysfunction may lead to diseases such as malignant tumors(10). For example, previous studies reported that the SLC12A5 was associated with poor os in colorectal cancer(11). The solute carrier family 39 member 6 gene can promote metastasis of esophageal cancer(12). However, we know very little about the function of members of the SLC35 gene family. The expression of SLC35F2 was initially found in ataxia telangiectasia(13). Subsequently, high expression level of SLC35F2 in salivary glands was detected by RT-PCR(14). Winter et al reported that the SLC35F2 permits YM155-mediated DNA damage toxicity(15).

However, studies on the role of the SLC35F2 gene in NSCLC are currently lacking. Previous study reported that the expression of SLC35F2 was higher in NSCLC samples than in normal lung samples(9). Li et al reported that the decreased expression of SLC35F2 can attenuate the proliferation, migration and invasion of H1299 cells(6). Therefore, we speculate that SLC35F2 may be involved in the development of NSCLC. However, the prognostic value of SLC35F2 for NSCLC has not been reported, so this is the focus of this study. In our study, bioinformatic analysis showed that an increased SLC35F2 in LUAD was associated with advanced clinical features and poor os. we performed GSEA to further investigate the functions of SLC35F2 in NSCLC. The results of GSEA showed that p53 signaling pathway and apoptosis are differentially enriched in SLC35F2 high expression phenotype. Since NSCLC has a high frequency of TP53 mutations, we predict that increased expression of SLC35F2 in this tumor type will be associated with a poor prognosis. Multivariate analysis showed that SLC35F2 was independently associated with overall survival in LUAD. These results indicate that SLC35F2 can be used as a prognostic marker for NSCLC.

In conclusions, increased expression of SLC35F2 in non-small cell lung cancer predicts poor prognosis. Moreover, apoptosis and p53 signaling pathway may enriched in NSCLC regulated by SLC35F2. However, further experiments are needed to verify the role of SLC35F2 in NSCLC.

## Declarations

**Ethics approval and consent to participate**□Not applicable.

**Availability of data and material**□The datasets supporting the conclusions of this article are included within the article and its additional files.

**Competing interests**□The authors declare that they have no competing interests.

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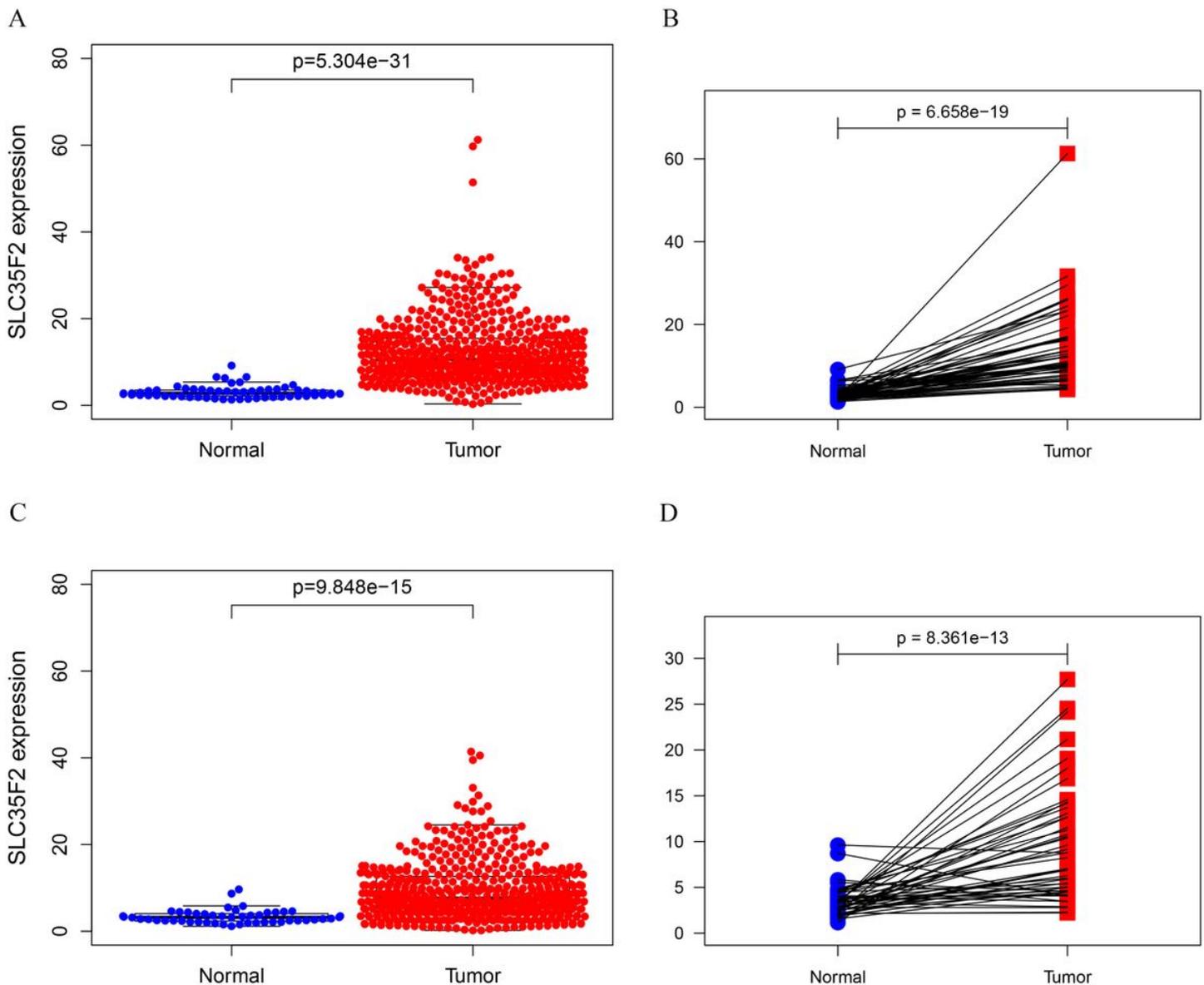
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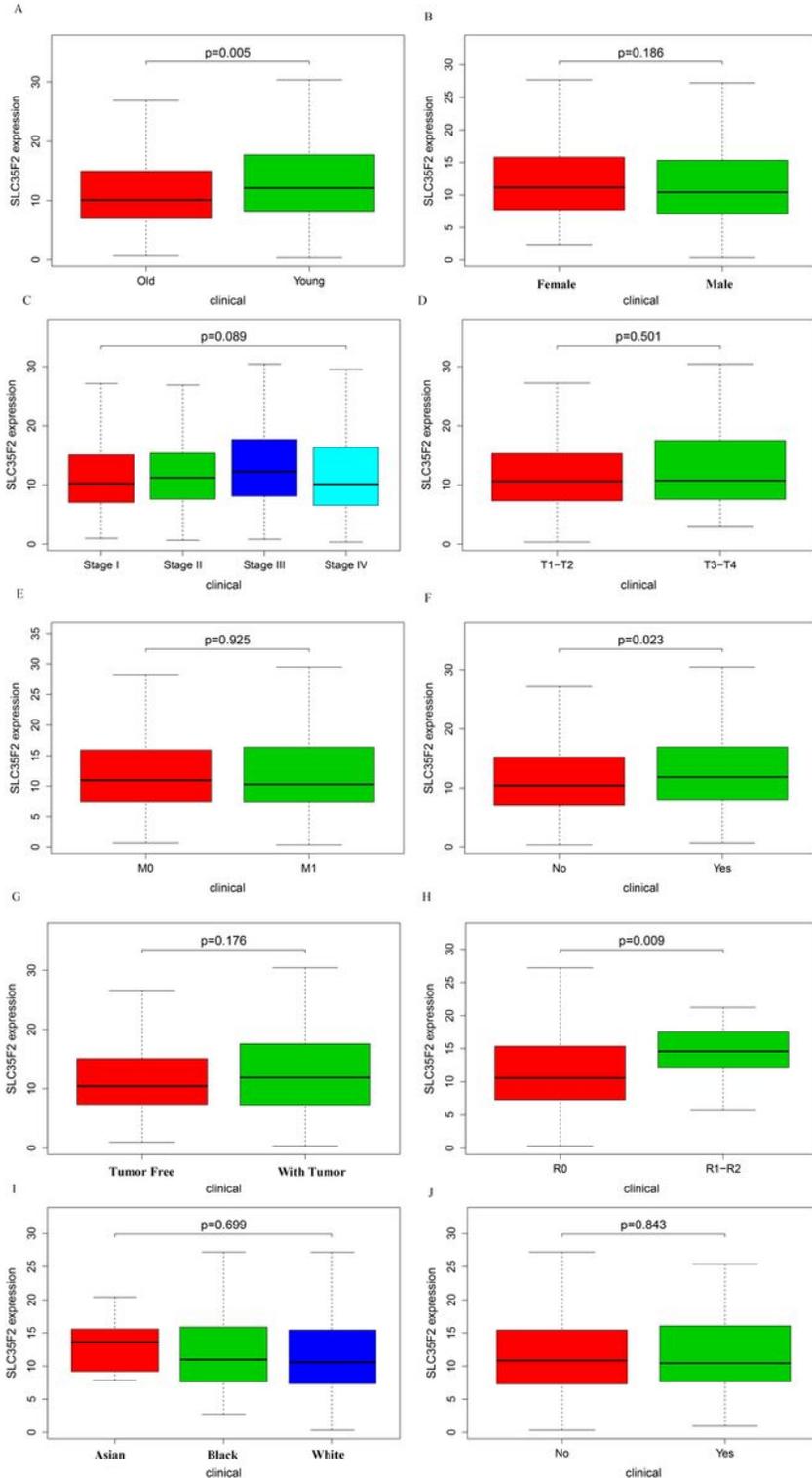
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## Figures



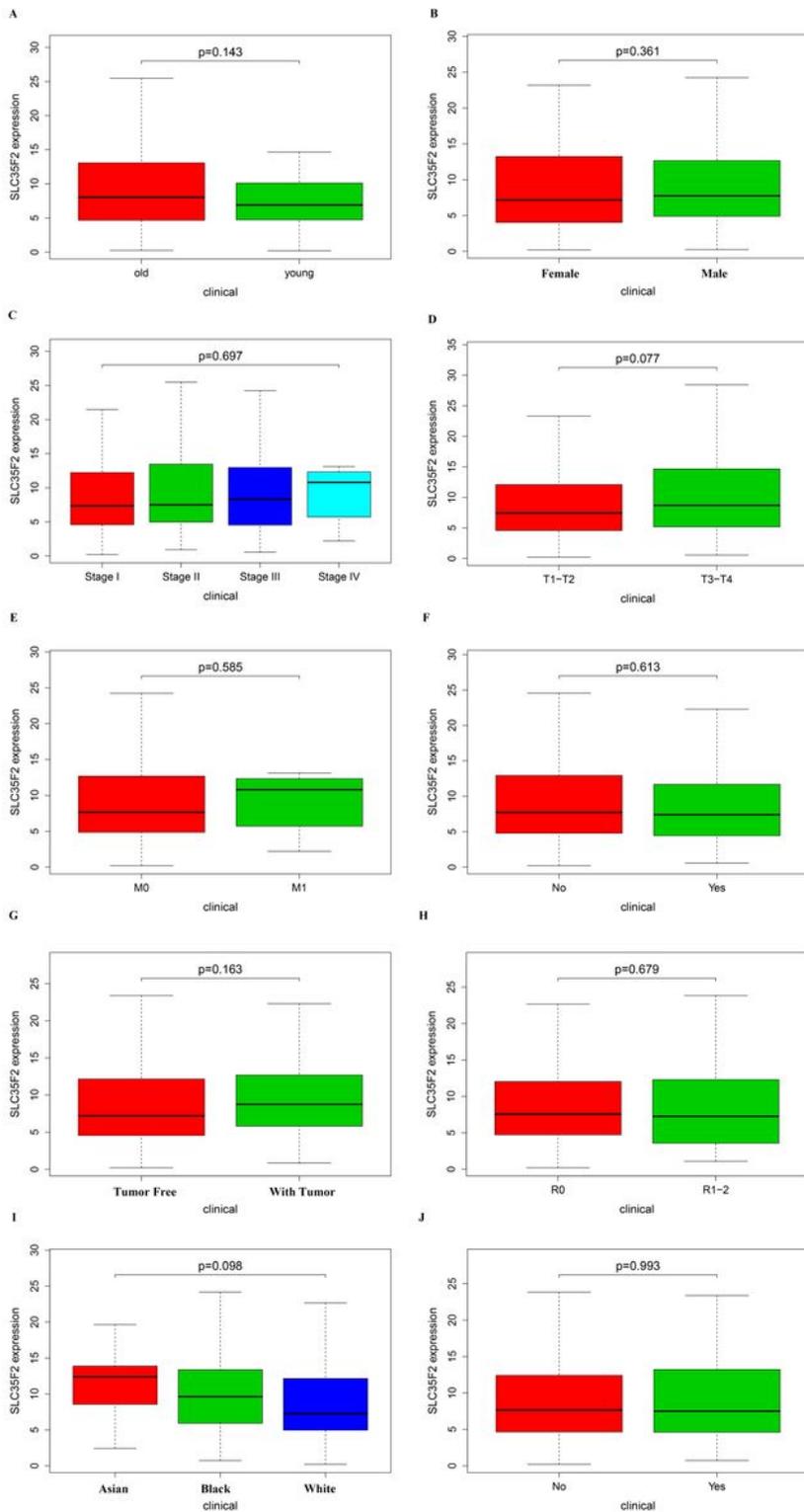
**Figure 1**

. Expression and paired expression plot of SLC35F2 in NSCLC and normal samples. A: The expression of SLC35F2 in LUAD and normal samples; B: The paired expression plot of SLC35F2 in LUAD and adjacent tissue; C: The expression of SLC35F2 in LUSC and normal samples; D: The paired expression plot of SLC35F2 in LUSC and adjacent tissue.



**Figure 2**

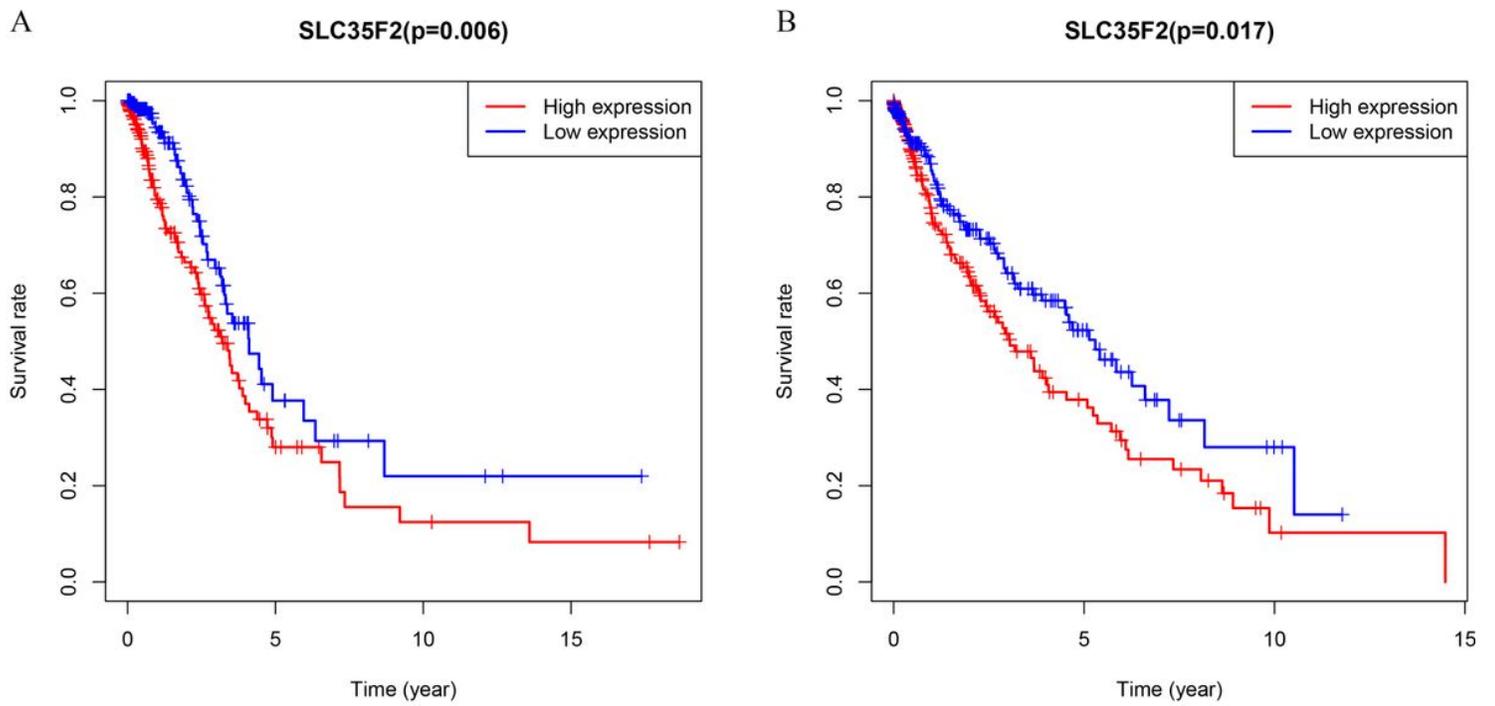
Association with SLC35F2 expression and clinicopathologic characteristics in LUAD. A: age, B: stage, C: gender, D: T stage, E: M stage, F: N stage, G: tumor status, H: residual tumor, I: race, J: history of other malignancy.



**Figure 3**

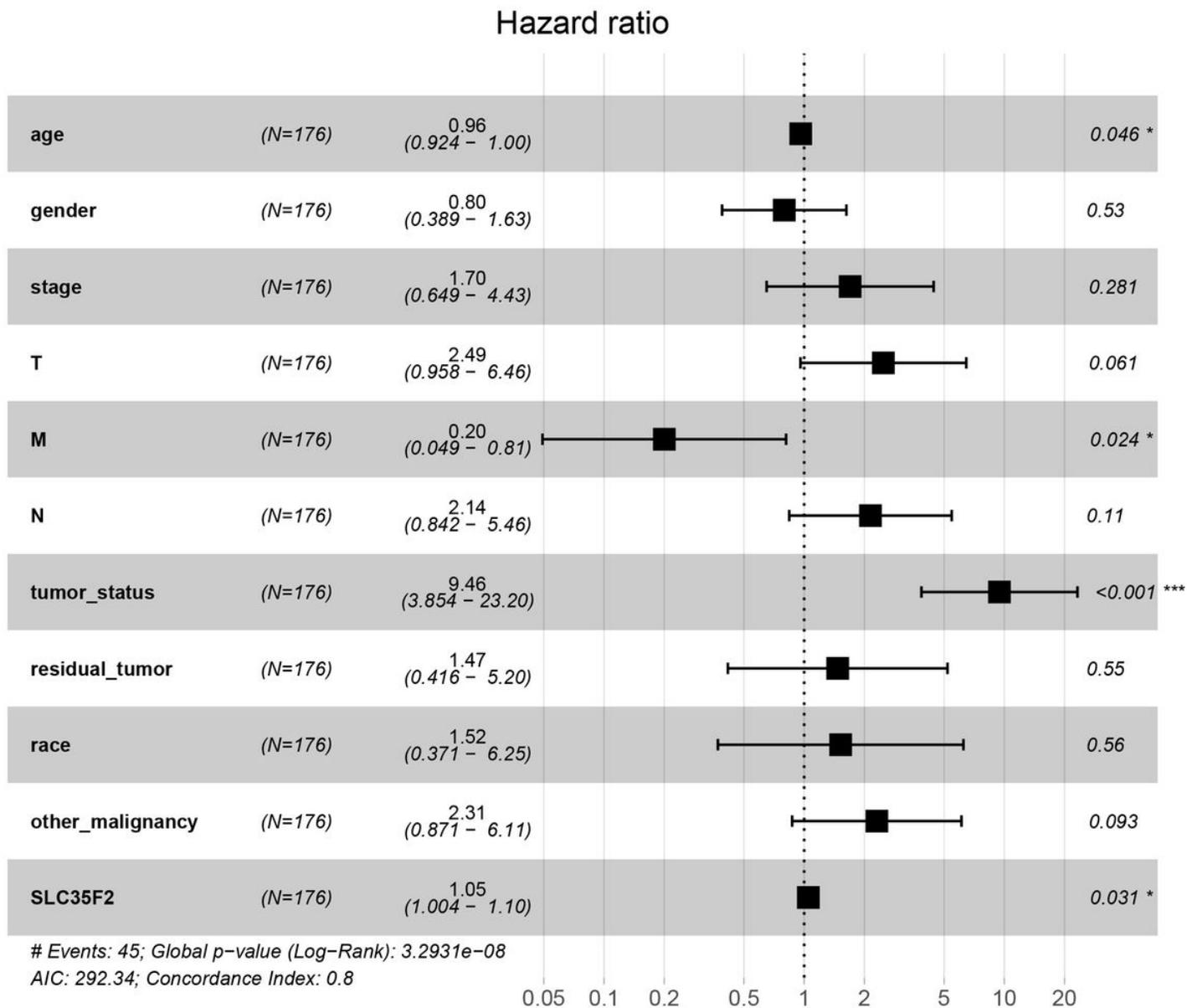
Association with SLC35F2 expression and clinicopathologic characteristics in LUAD. A: age, B: stage, C: gender, D: T stage, E: M stage, F: N stage, G: tumor status, H: residual tumor, I: race, J: history of other malignancy.

malignancy.



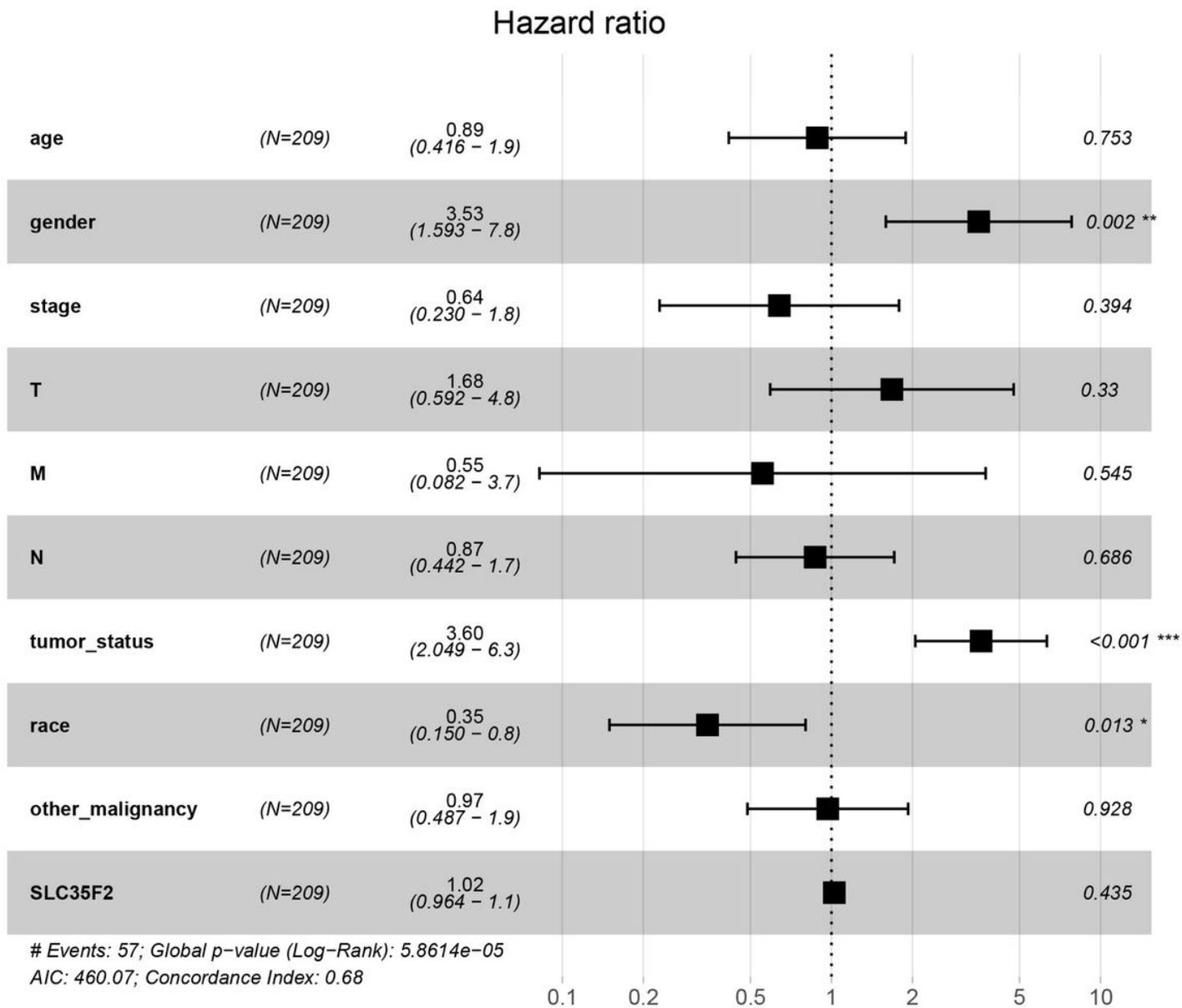
**Figure 4**

Impact of SLC35F2 expression on overall survival in NSCLC patients. A: Impact of SLC35F2 expression on overall survival in LUAD, B: Impact of SLC35F2 expression on overall survival in LUSC.



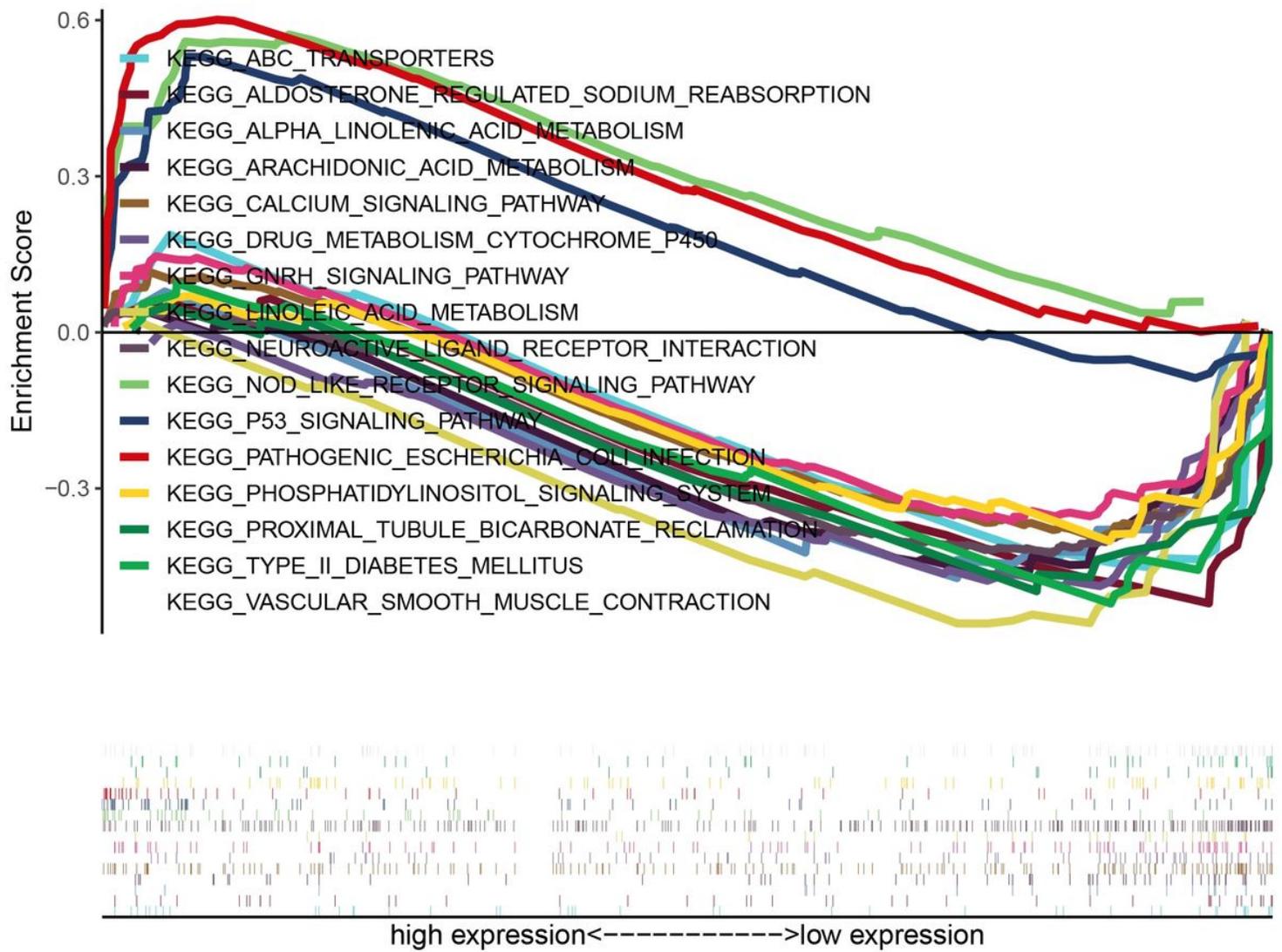
**Figure 5**

Associations with overall survival and clinicopathologic characteristics in TCGA patients using Multivariate analysis in LUAD.



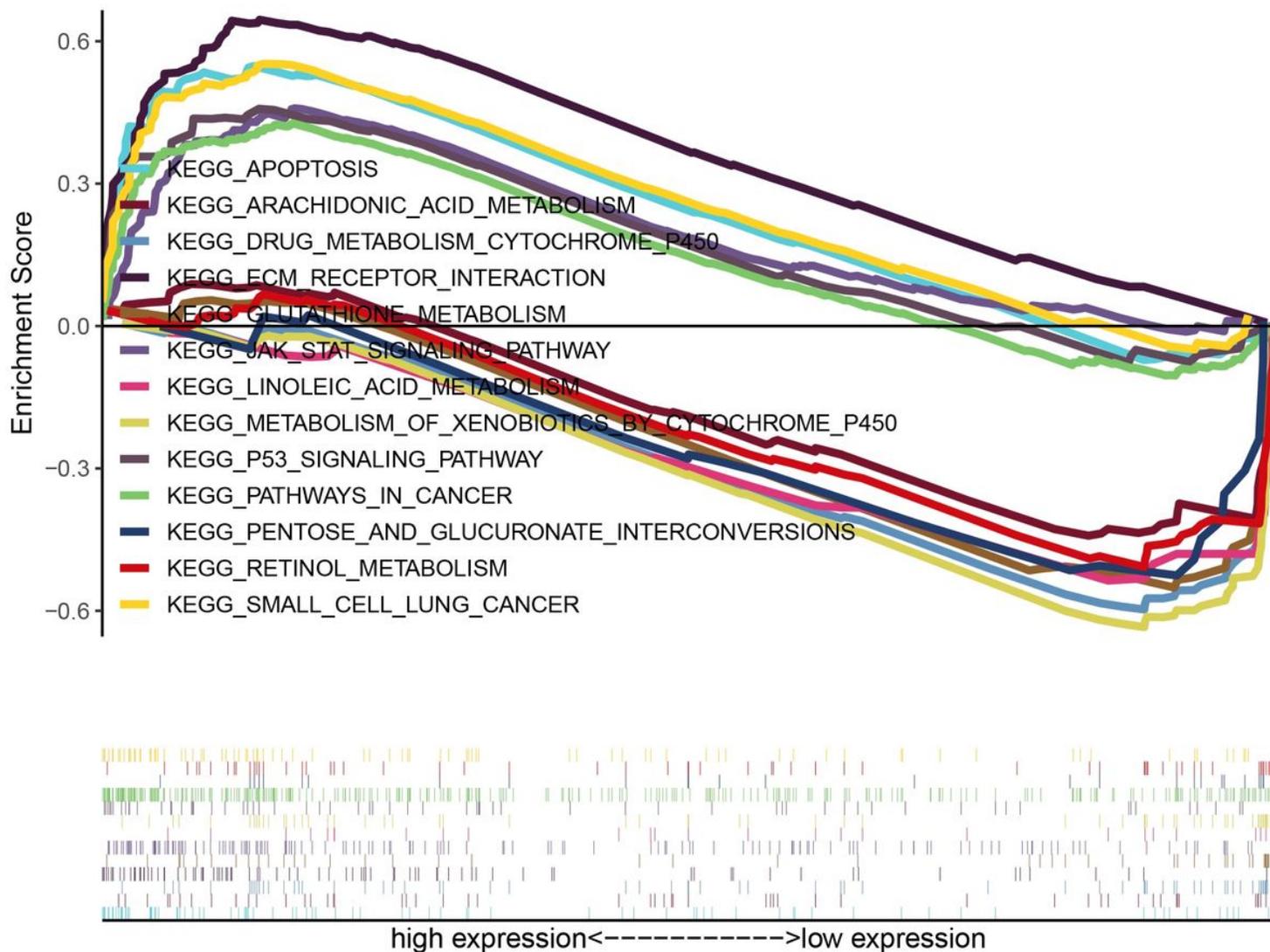
**Figure 6**

Associations with overall survival and clinicopathologic characteristics in TCGA patients using Multivariate analysis in LUSC.



**Figure 7**

Gene Set Enrichment Analysis results in LUAD patients.



**Figure 8**

Gene Set Enrichment Analysis results in LUSC patients.

## Supplementary Files

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