Cancer Medicine

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RESEARCH ARTICLE OPEN ACCESS

Multi-Omics Analysis of TYMS as a Prognostic Biomarker and Therapeutic Target for Lung Adenocarcinoma

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Received: 5 November 2025 | **Revised:** 7 March 2026 | **Accepted:** 2 April 2026

Keywords: bioinformatics | biomarkers | LUAD | multi-omics analysis | prognostic markers | therapeutic targets | tumour immune microenvironment | TYMS

ABSTRACT

Lung adenocarcinoma (LUAD) is a predominant form of lung cancer with poor prognosis due to early diagnosis challenges and treatment resistance. Thymidylate synthetase (TYMS), a key enzyme in DNA synthesis, has been associated with poor prognosis in multiple cancers, yet its multi-omics characteristics in LUAD remain systematically uncharacterized. This study focuses specifically on LUAD, integrating transcriptomic, proteomic, and genomic data to elucidate TYMS expression patterns, prognostic value, immune microenvironment interactions, and therapeutic potential.

Using data from The Cancer Genome Atlas (TCGA), the Clinical Proteomic Tumour Analysis Consortium (CPTAC), the Genome-Tissue Expression Project (GTEx), the Gene Expression Profile Interaction Analysis (GEPIA2), etc., we conducted comprehensive bioinformatics analyses of TYMS in LUAD. TYMS showed significant overexpression at both mRNA and protein levels in LUAD tissue. High TYMS expression correlated with earlier tumour staging, higher histological grade, and poor prognosis. Functional enrichment analysis revealed TYMS involvement in p53/Rb and mammalian Target of Rapamycin (mTOR) signalling pathways. TYMS expression was associated with immune cell infiltration, showing negative correlation with B-cell activity and positive correlation with neutrophil infiltration, as well as significant association with Cytotoxic T Lymphocyte-Associated Protein 4 (CTLA4), Programmed Death-1 (PDCD1, PD-1), and Interleukin-6 (IL-6). Somatic copy number variation (SCNA) analysis indicated that TYMS amplification was linked to poor prognosis and enriched in the folate metabolism pathways. Drug sensitivity analysis demonstrated that high TYMS expression positively correlated with sensitivity to Afatinib and Gefitinib, but negatively correlated with Methotrexate and Vorinostat.

In conclusion, TYMS is upregulated in LUAD and may serve as an independent prognostic biomarker and therapeutic target.

1 | Introduction

LUAD, the most common histological subtype of non-small cell lung cancer (NSCLC), is a leading cause of cancer-related deaths worldwide [1]. Patients with LUAD have a poor prognosis, primarily attributable to difficulties in early diagnosis, limited treatment options, and the development of treatment resistance [2–4]. Despite advances in diagnosis and treatment, the lack of reliable biomarkers for early detection and prognostic prediction remains a significant challenge [5, 6]. Therefore, there is an

urgent need to identify novel biomarkers and therapeutic targets to improve patient outcomes.

Among these, TYMS has gradually attracted attention due to its pivotal role in cell proliferation and survival [7]. As the rate-limiting enzyme in the de novo thymidine synthesis pathway, TYMS catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a critical step in DNA synthesis and repair [8, 9]. Previous studies have demonstrated that TYMS is overexpressed in multiple malignant tumours, including colorectal cancer, gastric cancer, and breast cancer, and is associated with poor clinical outcomes [10–14]. Research has revealed that TYMS expression levels are consistently elevated in patients with lung adenocarcinoma, correlating with enhanced invasive capacity and shorter survival periods. This demonstrates its potential value as a biomarker and therapeutic target [15–17]. Furthermore, high TYMS expression may influence the tumour immune microenvironment by regulating immune cell infiltration and potentially promoting immune escape mechanisms [18, 19]. However, systematic multi-omics studies (such as transcriptomics and proteomics) remain lacking regarding the comprehensive molecular characterisation of TYMS in LUAD, its prognostic implications, interactions with the tumour immune microenvironment, and its potential as a therapeutic target.

This study aims to address this gap by conducting a comprehensive bioinformatics analysis to explore the multifaceted role of TYMS in LUAD. Specifically, we evaluated its expression patterns at the transcriptional and proteomic levels, assessed its potential as a prognostic biomarker and therapeutic target, examined relevant biological pathways and immune infiltration characteristics, and investigated copy number variations and drug sensitivity.

2 | Materials and Methods

2.1 | Data Sources and Gene Screening

This study collected gene expression profiles and clinical data from LUAD patients across multiple public databases, specifically including TCGA (https://portal.gdc.cancer.gov/), GTEx (https://gtexportal.org/home/), and CPTAC (https://pdc.cancer.gov/pdc/). The proteomics data were obtained from the CPTAC LUAD dataset.

To identify potential prognostic genes, we employed GEPIA2 (http://gepia2.cancer-pku.cn), an interactive gene expression analysis platform based on TCGA and GTEx data. This yielded two gene sets: one comprising the top 500 genes significantly correlated with overall survival (OS) in LUAD ($p < 0.05$), and another containing 4235 genes differentially expressed between LUAD and normal tissue (adjusted $p < 0.05$ and $|\log2FC| > 1$). We employed the median expression level as the cut-off for grouping and identified 148 candidate genes through intersection analysis. Subsequently, functional enrichment analysis of these genes was performed using the DAVID online tool (https://davidbioinformatics.nih.gov/), encompassing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses.

2.2 | Analysis of Protein–Protein Interactions

Using the STRING database (https://cn.string-db.org), a protein–protein interaction (PPI) network was constructed for TYMS-related proteins. During the construction process, an interaction score threshold greater than 0.40 was set to screen for biologically significant protein pairs.

2.3 | Expression Level Analysis

The mRNA expression levels of TYMS in LUAD and normal tissue were analysed using GEPIA2 and the University of Alabama at Birmingham Cancer (UALCAN, http://ualcan.path.uab.edu). Concurrently, protein expression levels of TYMS in LUAD and normal tissue were assessed via the UALCAN platform in conjunction with the CPTAC dataset. Furthermore, immunohistochemical (IHC) images of TYMS in LUAD and normal lung tissue were obtained from the Human Protein Atlas (HPA, https://www.proteinatlas.org/) for visualisation.

2.4 | Survival Analysis

Based on the different expression levels of GEPIA2, the relationship between the expression level of TYMS and the OS of LUAD patients was evaluated, and samples were divided into a high expression group and a low expression group with the median expression value as the cut-off point. The hazard ratio (HR) and its 95% confidence interval (CI) were calculated using the Cox proportional hazards model, with statistical significance evaluated via the Log-rank test.

2.5 | Pathway Enrichment and Immune Cell Infiltration Analysis

Based on proteomic data from the CPTAC-LUAD cohort, analyse the association between TYMS expression and pathways related to Wnt, mTOR, receptor tyrosine kinases (RTKs), p53/Rb, the SWI-SNF complex, MYC/MYCN, and chromatin modifications. Classify pathway alteration status as either “altered” or “unaltered”.

The Tumour Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) was employed to analyse the relationship between TYMS expression and immune cell infiltration. Spearman's correlation analysis was employed to evaluate associations between TYMS expression and the abundance of immune cells (including B cells, CD8+ T cells, CD4+ T cells, neutrophils, dendritic cells, etc.) alongside expression levels of immune checkpoint molecules (including CTLA4, PD-1, IL-6, etc.).

2.6 | Somatic Copy Number Variation Analysis

A joint analysis of TYMS-SCNA and RNA-seq datasets from LUAD samples in the TCGA database was conducted using LinkedOmics (https://www.linkedomics.org) to investigate the relationship between TYMS-CNVs and their corresponding

mRNA expression levels. Genes exhibiting statistically significant expression (adjusted p-value < 0.05) and size of CNVs (log2 fold change > 1) were selected.

Pearson correlation analysis was employed to identify genes significantly associated with TYMS copy number variation. Subsequently, these genes underwent GO and KEGG pathway enrichment analysis. Furthermore, gene set enrichment analysis (GSEA) was utilised to identify relevant pathways significantly enriched in TYMS copy number amplification samples.

2.7 | Drug Sensitivity Analysis

Utilising data from the Genomic Database of Cancer Sensitivity (GDSC, https://www.cancerrxgene.org/), we evaluated the correlation between TYMS expression and drug sensitivity. Spearman's correlation analysis was employed to examine the relationship between TYMS mRNA expression levels and the half-maximal inhibitory concentration (IC_{50}) for multiple anticancer drugs, with significant correlations ($p < 0.05$) presented visually.

2.8 | Statistical Analysis

All statistical analyses were performed using R software (version 4.2.1) and corresponding bioinformatics tools. p-value < 0.05 was considered statistically significant.

3 | Result

3.1 | The Screening Process for the Target Gene TYMS

The genes identified through our cross-analysis are not only statistically significant but also biologically likely to be closely associated with the molecular mechanisms of the disease, potentially involving crucial biological processes such as cell proliferation, cell cycle regulation, and cellular metabolism. This study employed two gene sets from GEPIA2 for in-depth analysis. One set comprised 500 genes significantly associated with lung adenocarcinoma prognosis ($p < 0.05$), whilst the other set, comprising 4235 genes ($p < 0.05$), reflected gene expression differences between lung adenocarcinoma and normal tissue. The primary objective of this study is to identify key genes that significantly influence tumour prognosis, playing crucial roles in tumour initiation, development, and therapeutic response.

Through intersection analysis, a total of 148 genes were identified, and a Venn diagram was constructed using the DAVID online tool (Figure 1A). Subsequently, these genes underwent GO analysis (Figure 1B) and KEGG analysis (Figure 1C) to classify their functions and associated pathways.

Subsequently, a cross-comparison was conducted between a set of genes associated with the "deoxyribonucleotide monophosphate biosynthesis pathway" from the GO analysis and a set of genes related to nucleotide metabolism from the KEGG analysis. Through this cross-analysis, the identified genes included TK1 and TYMS. TK1 plays an auxiliary role in cellular DNA synthesis and repair processes, while TYMS is a key enzyme involved in the thymidine synthesis reaction during DNA synthesis. TYMS plays a vital role in biological systems, with its function becoming increasingly critical during cellular proliferation. Furthermore, TYMS exhibits stable expression, and its genetic polymorphisms may influence prognosis. Concurrently, the high expression of TYMS in various tumours is significantly associated with poor prognosis, making it a strong candidate for a prognostic biomarker in lung adenocarcinoma. Consequently, we have selected it as the primary focus of this study.

3.2 | Protein–Protein Interaction Network of TYMS

We constructed a protein–protein interaction (PPI) network centred on TYMS based on the STRING database. As shown in Figure S1, TYMS was predicted to interact directly with ten core proteins, including key enzymes involved in folate metabolism and nucleotide synthesis (DHFR, MTHFR, MTHFD1, SHMT1, DCTD) as well as critical proteins in DNA replication and cell cycle progression (TK1, DTYMK, DUT, MCM2).

3.3 | TYMS Transcription Levels Are Upregulated in LUAD and Associated With Poor Prognosis

We first assessed TYMS transcription levels across 33 distinct cancer types, with particular focus on LUAD, the results of which are presented in Figure 2A. Subsequently, we measured TYMS transcription levels in normal tissue from 59 LUAD patients and 539 primary tumour tissues. Results demonstrated significant differences in mRNA expression between normal and tumour tissues, with markedly elevated TYMS mRNA levels in tumour samples, as shown in Figure 2B.

Moreover, we observed increased TYMS transcriptional levels in tumour tissues across different pathological stages (Figure 2C) and across distinct histological subtypes (Figure 2D), closely associated with tumour development. This finding suggests that elevated TYMS mRNA levels correlate positively with tumour severity. Concurrently, elevated TYMS transcription levels were observed in adeno-alveolar carcinoma of the lung, mixed subtype lung adenocarcinoma, papillary lung adenocarcinoma, and non-mucinous lung bronchoalveolar carcinoma, indicating differential expression across distinct LUAD subtypes. Tumour progression and elevated TYMS transcriptional levels within tumour tissues may correlate with reduced patient survival, suggesting poor prognosis, as depicted in Figure 2E.

3.4 | TYMS Protein Is Significantly Upregulated in LUAD

TYMS protein levels were detected via the CPTAC dataset and the UALCAN data Analysis Portal. Analysis results revealed that data from 111 normal samples and 111 LUAD samples demonstrated a significant positive correlation between TYMS protein expression and LUAD (Figure 3A). Further analysis of TYMS

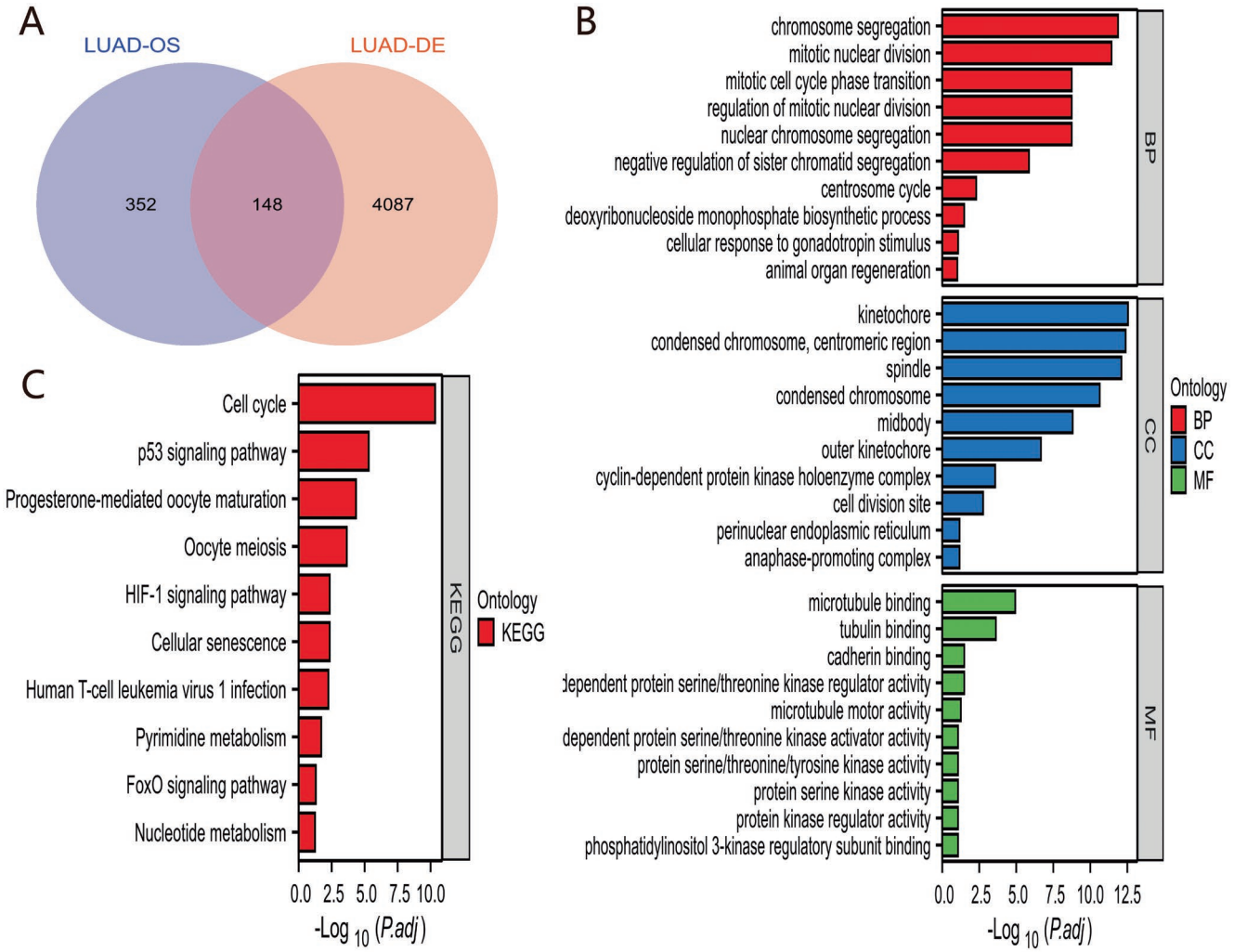

FIGURE 1 | Screening workflow for LUAD-associated target genes and TYMS. (A) shows a Venn diagram of genes associated with survival rates and differential expression. (B, C) GO and KEGG enrichment analyses revealed the functional diversity of 148 intersecting genes. Identify genes associated with the biosynthesis of deoxyribonucleotide monophosphates and nucleotide metabolism.

protein expression across different LUAD stages (Figure 3B), grades (Figure 3C), histological subtypes (Figure 3D), and patient age groups (Figure 3E) revealed that TYMS protein expression was significantly upregulated in early-stage tumors, high-grade tumors, adenocarcinoma histological subtypes, and the 41–60 age cohort.

To enhance visualisation of TYMS protein expression, we collected LUAD IHC images from the HPA database and conducted an in-depth analysis (Figure 3F). IHC results for normal lung tissue in the HPA database showed "not detected". In contrast, tumour tissue expression exhibited considerable variation: seven samples demonstrated moderate expression, three showed weak expression, and only two samples were non-expressing (Figure 3F). Integrating patient information, expression status, and IHC images for both normal and overexpressed samples (Figure 3G) facilitates clearer visualisation of elevated TYMS expression levels in LUAD.

Analysis of protein expression revealed that TYMS exhibited significant elevation in stage 1 and grade 3 lung adenocarcinoma, indicating that its increased expression occurs at early stages of disease progression and in high-grade tumours. Furthermore, in lung adenocarcinoma across different age cohorts, TYMS protein expression was significantly elevated in tissue samples from the 41–60 age group, suggesting a potential association between TYMS expression and patient age, particularly within this demographic. Immunohistochemistry represents a straightforward and convenient diagnostic technique. Consequently, its application for early disease detection and grading assessment holds significant clinical importance.

3.5 | The TYMS Signalling Pathway Is Involved in LUAD

To investigate the role of TYMS in the pathogenesis of LUAD, we analysed potential signalling pathways associated with TYMS using the CPTAC-LUAD dataset. This dataset comprises 109 LUAD cases, which were stratified into a "signalling pathway altered group" and an "other group" based on alterations in signalling pathways. We compared TYMS protein expression levels in these two patient groups with those in 111 cases of normal adjacent tissue.

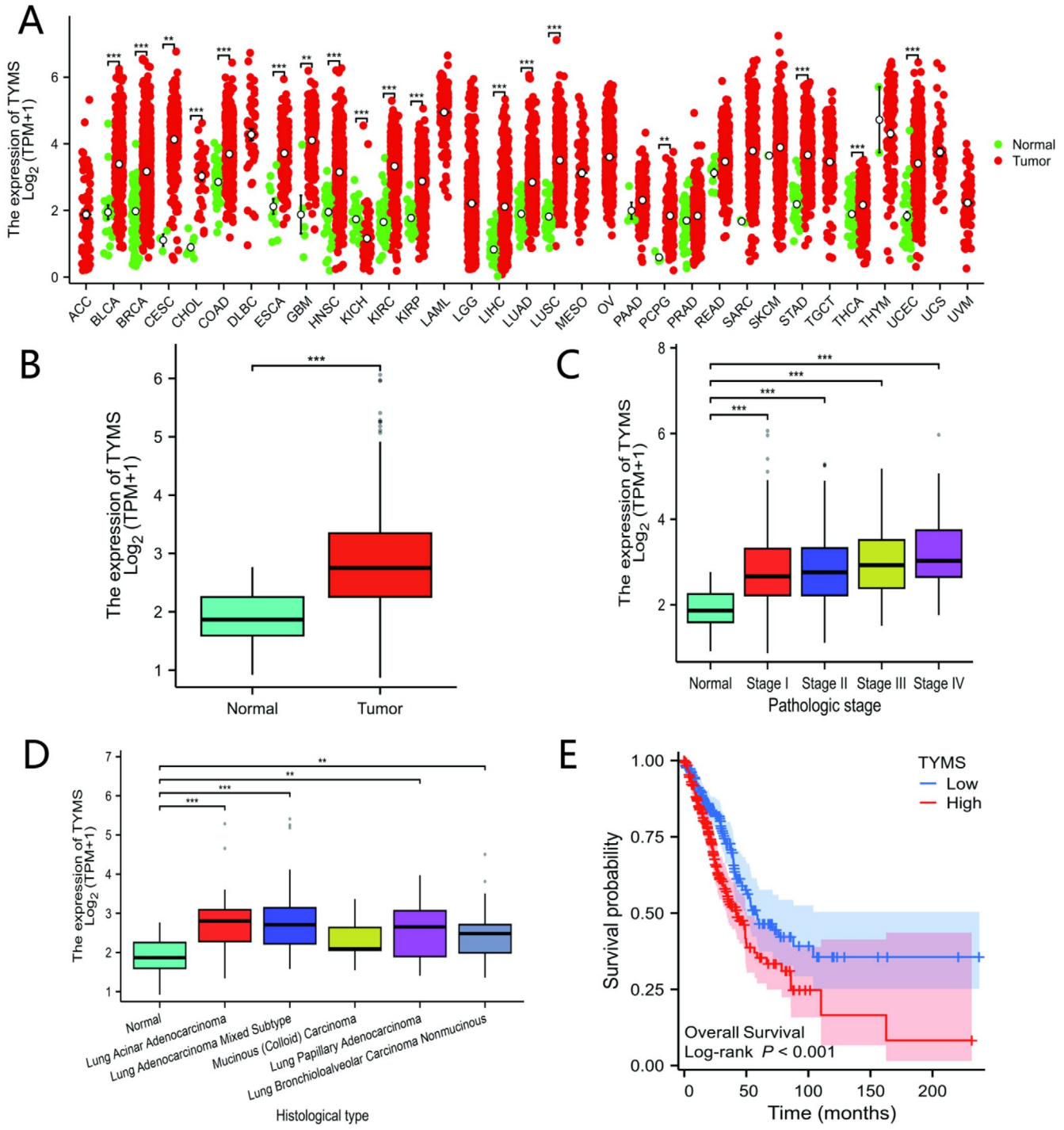

FIGURE 2 | Demonstrates the upregulation of TYMS expression in LUAD patients, and this expression is significantly associated with poor prognosis. (A) shows the overall expression of TYMS across different cancer types, where green indicates normal tissue and red denotes tumour tissue. (B) The expression of TYMS was compared between normal tissue and primary tumour tissue. (C) demonstrates the expression changes of TYMS across different pathological stages. (D) then examined the expression of TYMS across different histological subtypes. (E) Further analysis of overall survival rates between the low-expression and high-expression groups of TYMS was conducted based on 265 tumour samples from the TCGA. Log-rank $p < 0.001$. The 95% confidence interval is represented by a band. Note: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Research findings indicate that TYMS protein levels exhibit significant correlations with multiple signalling pathways, including those related to Wnt, mTOR, RTK, p53/Rb, the SWI-SNF complex, MYC/MYCN, and chromatin modification. (Figure 4). Notably, TYMS protein expression correlates with the inflammation-associated mTOR (Figure 4B) and the p53/Rb pathway (Figure 4D), suggesting that TYMS may play a role in p53 pathway regulation and tumour immunity. The mTOR pathway exerts a central function in immune and inflammatory responses, primarily through regulating cytokine production, T-cell activation, and macrophage polarisation processes. It is closely associated with various inflammatory diseases and the

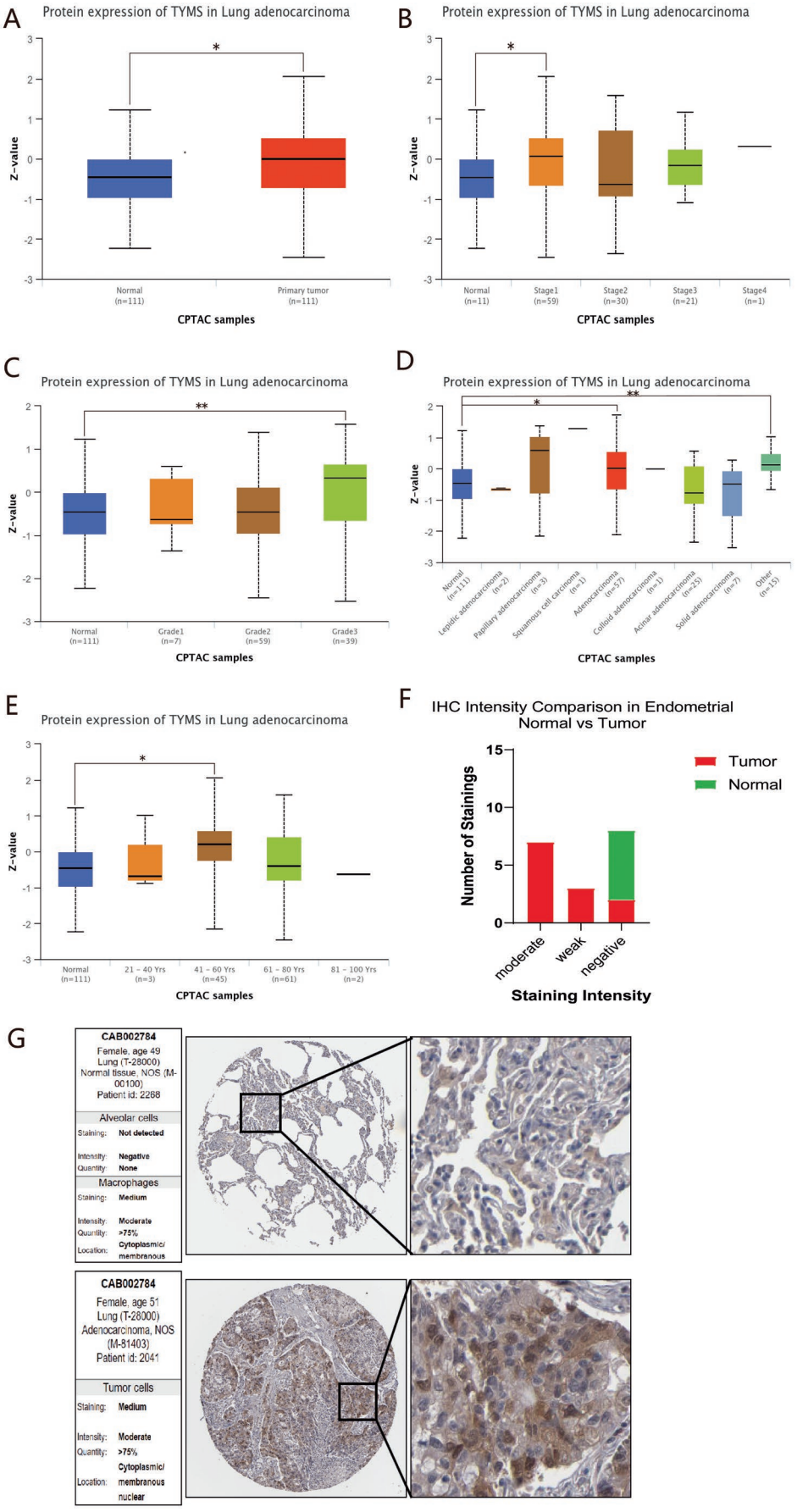

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FIGURE 3 | Demonstrates the upregulation of TYMS protein expression in LUAD patients. (A) In LUAD samples, TYMS protein expression was significantly elevated. The Z-value represents the standard deviation of the sample median. Expression levels of TYMS protein across different LUAD (B) stages, (C) grades, (D) histological subtypes, and (E) age groups. (F) Statistical analysis was performed on all TYMS IHC images derived from the Human Proteome Atlas. (G) shows immunohistochemical images of TYMS in normal tissue and LUAD tissue. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

tumour microenvironment. The p53 pathway is crucial for cell cycle regulation and apoptosis, with its abnormal activation or suppression closely associated with the initiation and progression of numerous tumours. Consequently, elevated TYMS protein expression may promote tumour cell proliferation and survival by modulating the p53/Rb and mTOR pathways while simultaneously influencing immune responses within the tumour microenvironment.

3.6 | TYMS Interacts With Immunotherapy in LUAD

It was observed that TYMS protein expression correlates with alterations in the p53/Rb and mTOR inflammation-related signalling pathways (Figure 4B,D). Given the close association between the p53 and mTOR pathways and immunotherapy, we further investigated the relationship between TYMS and immunotherapy.

To investigate the relationship between TYMS expression and immune cell populations in LUAD patients, we analysed the TCGA-LUAD dataset ($n = 516$) using TIMER. Results revealed a significant negative correlation between TYMS expression and B-cell activity ($p = 8.94e\text{-}03$), whilst a positive correlation was observed with neutrophil activity ($p = 1.34e\text{-}04$) (Figure 5A). TYMS expression levels also demonstrated a significant correlation with survival rates in LUAD patients ($p = 0.003$) (Figure 5B). Among different immune cell types, B cells (Log-rank $p = 2.68e\text{-}04$) and dendritic cells (Log-rank $p = 0.048$) were associated with LUAD patient survival (Figure 5B). Furthermore, we explored the relationship between TYMS and IL-2, *Cluster of Differentiation 19* (CD19), CTLA4, *Forkhead Box P3* (FOXP3), *Granzyme B* (GZMB), PD-1, IL-6, and *C-X-C Motif Chemokine Ligand 8* (CXCL8) (Figure 5C–J). Results revealed negative correlations between TYMS expression and IL-2 and CD19, with correlation coefficients of −0.115 ($p = 0.007$) and −0.116 ($p = 0.007$) respectively. Conversely, it showed positive correlations with CTLA4, FOXP3, GZMB, PD-1, IL-6, and CXCL8, with correlation coefficients of 0.133 ($p = 0.002$), 0.110 ($p = 0.011$), 0.277 ($p < 0.001$), 0.139 ($p = 0.001$), 0.189 ($p < 0.001$), and 0.284 ($p < 0.001$).

3.7 | Correlation Between Somatic Copy Number Alterations and Prognosis in LUAD

SCNA refers to the phenomenon of genomic region copy number alterations in tumour cells, encompassing both amplifications and deletions, which influence the initiation, progression, and prognosis of cancer. DNA copy number variation (CNV) refers to genomic structural variations involving alterations in DNA sequence copy numbers, which are commonly observed in cancers and other diseases. These changes lead to abnormal gene expression and promote tumour progression. In LUAD, copy number alterations significantly impact prognosis.

Within LinkedOmics, by integrating TYMS-SCNA data with RNA-seq data from the TCGA-LUAD dataset ($n = 516$), we conducted volcano plot analysis, heatmap analysis, and enrichment analysis. The volcano plot (Figure 6A) revealed genes significantly positively and negatively correlated with TYMS-SCNA, with red dots on the right indicating positive correlation and green dots on the left indicating negative correlation. These expression changes were further visualised via heatmaps, displaying the expression patterns of the top 50 genes significantly correlated with TYMS-SCNA (Figure 6B,C).

Furthermore, GO enrichment analysis of these genes (Figure 6E–G) indicates their primary involvement in biological functions such as one-carbon metabolism, interconversion of nucleobase-containing small molecules, and binding to G protein β/γ subunit complexes. Conversely, proteins exhibiting taste receptor activity were suppressed (Figure 6G).

KEGG pathway analysis (Figure 6D) revealed enrichment in the one-carbon pool by the folate pathway, which may influence the development and progression of LUAD. Notably (Figure 6H,I), the genes NDC80 (*Nuclear division cycle 80*), TYMS, *spindle and kinetochore-associated complex subunit 3* (SKA3), and *gap junction protein beta 2* (GJB2) exhibited high hazard ratios (HR) with *p*-values below 0.05, suggesting they may serve as high-risk markers for LUAD. The elevated expression of these genes correlates significantly with poor prognosis in LUAD patients, offering novel potential biomarkers for LUAD diagnosis and treatment.

3.8 | Drug Sensitivity Analysis

To further evaluate the potential therapeutic value of TYMS-SCNA in LUAD treatment, we retrieved TYMS-related drug sensitivity data from the GDSC database to investigate the relationship between TYMS and various drugs (Figure 7). The GDSC database collates IC_{50} values for 265 small molecules across 860 cell lines alongside corresponding mRNA gene expression data. This repository integrates mRNA expression information with drug sensitivity data. Subsequently, researchers employed Pearson correlation analysis to assess the relationship between gene mRNA expression and drug IC_{50} values.

Therefore, through Spearman's correlation analysis, we found that TYMS was significantly correlated with sensitivity to multiple drugs (Figure 7A). TYMS showed significant positive correlations with *Afatinib*, *Gefitinib*, and *Trametinib*. Conversely, TYMS exhibited significant negative correlations with *BX-912, FK866, GSK1070916, Methotrexate*, *Navitoclax, NPK76-II-72-1*, and *Vorinostat*.

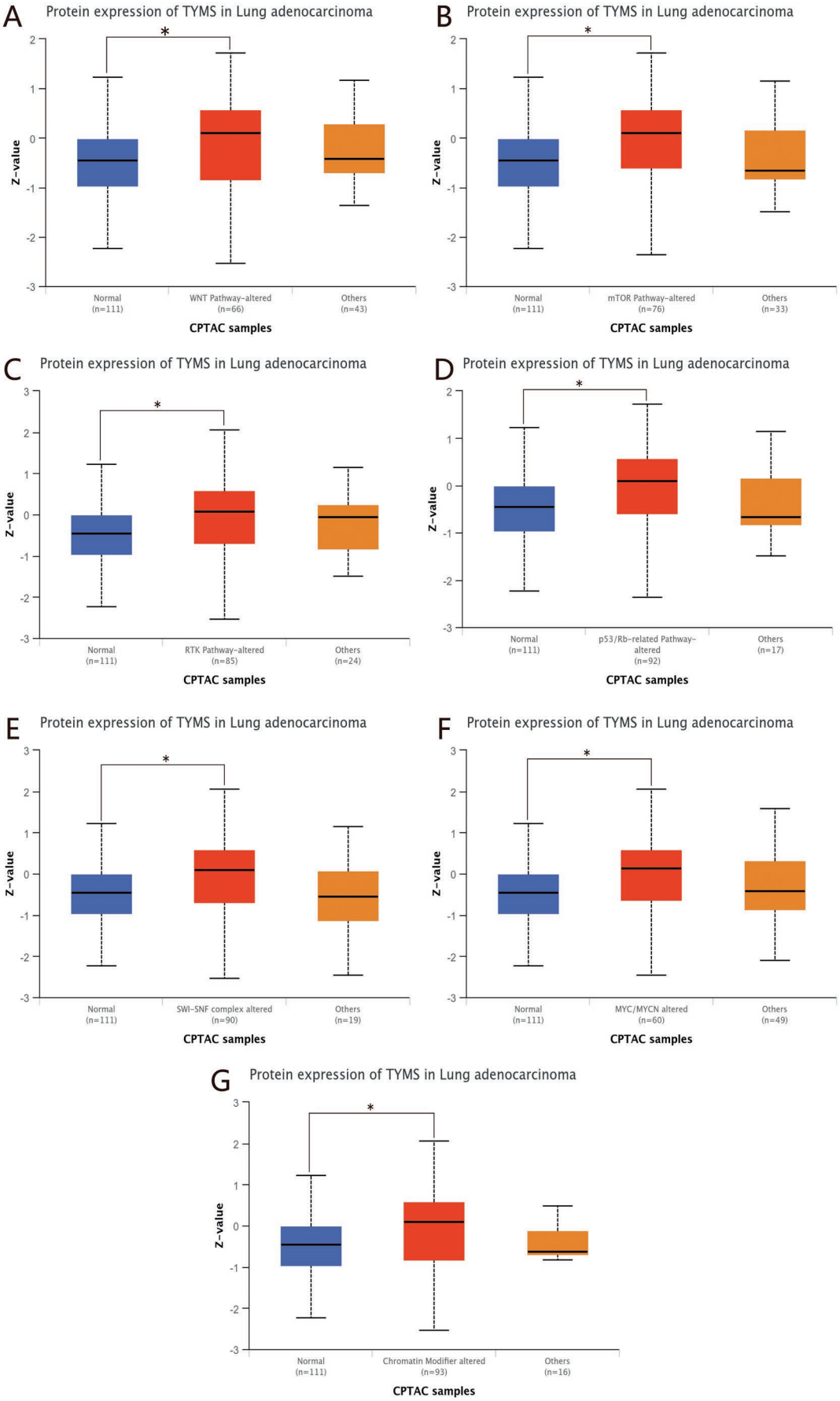

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FIGURE 4 | illustrates alterations in pathways such as p53/Rb and mTOR, etc., associated with TYMS protein expression. This analysis involved an omics study of TYMS protein across Wnt, mTOR, RTK, p53/Rb, MYC/MYCN, SWI/SNF complex, and chromatin modifiers. Mass spectrometry was employed to detect TYMS protein expression levels. The z-value represents the standard deviation of LUAD sample medians. This study included 109 CPTAC-LUAD cases. Statistical significance is denoted as: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

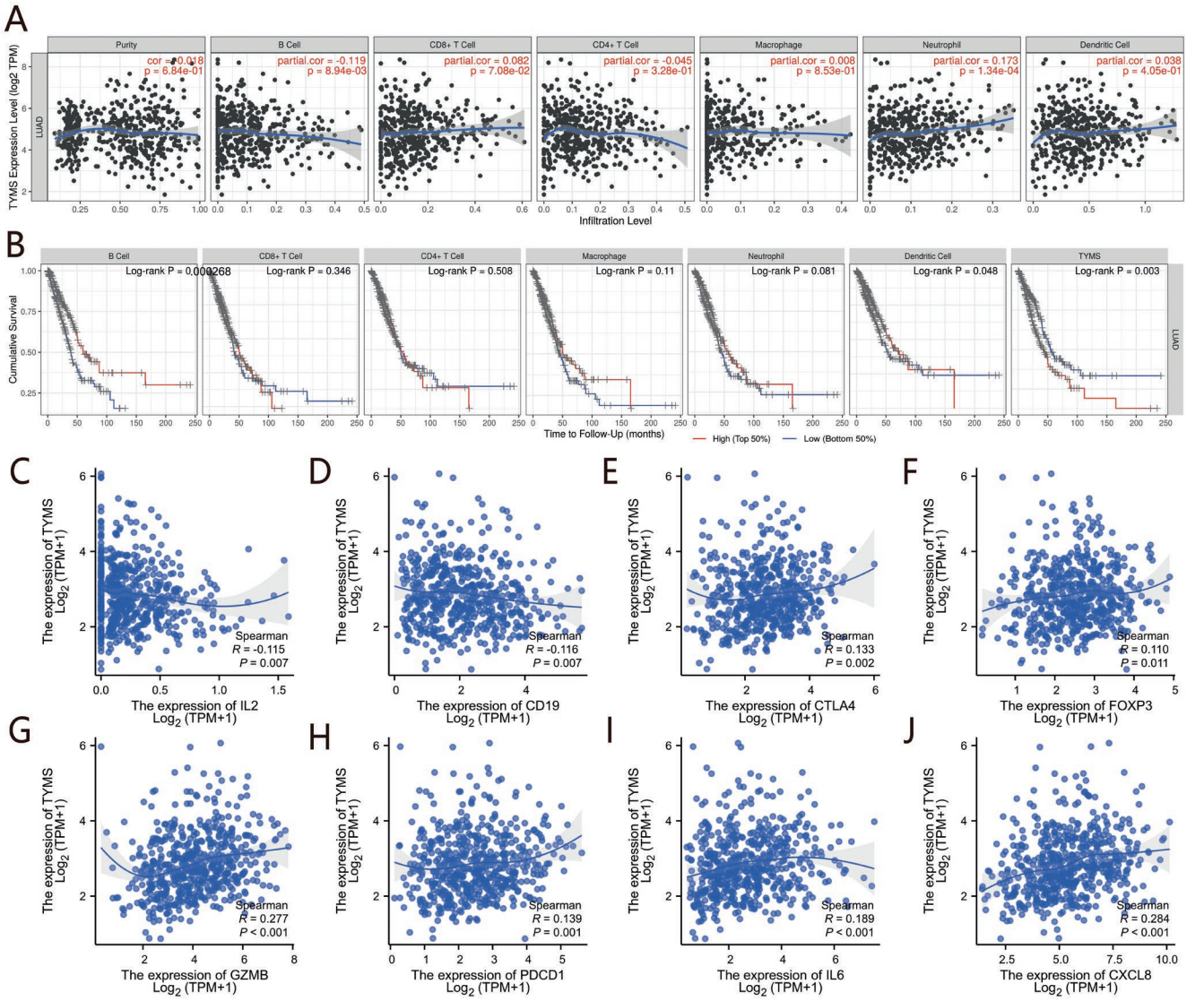

FIGURE 5 | Interaction between TYMS and LUAD immunotherapy. (A) Within the immune system, following purity correction, the correlation between TYMS and immune cells was measured using Spearman's rho coefficient and was found to be statistically significant. Log2 (TPM) denotes the logarithmic value of the number of transcripts per million. (B) Clinical correlation analysis between B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, dendritic cells, and TYMS expression in TCGA LUAD samples. The cut-off value for each group was set at the median. (C–J) Analysed the correlations between IL-2, CD19, CTLA-4, FOXP3, GZMB, PD-1, IL-6, and CXCL8. Included 539 TCGA-LUAD cases.

4 | Discussion

The TYMS gene is located on human chromosome 18p11.32 and plays a crucial role in DNA synthesis and cell cycle regulation within cells [20]. As a key enzyme, TYMS catalyses the conversion of dUMP to dTMP, a process that constitutes the rate-limiting step in thymidine synthesis [9]. The activity of TYMS is not only directly linked to cell proliferation but also crucial for cellular function during DNA replication and repair processes [21, 22]. Research has revealed that overexpression of TYMS is closely associated with the progression of multiple cancers. Particularly in the context of malignant tumours such as colorectal cancer and gastric cancer, the expression levels of TYMS can serve as a prognostic indicator [13, 14]. During cell division, TYMS activity and its protein expression levels are significantly influenced by cell cycle changes, which are crucial for maintaining cell proliferation capacity and genomic stability [23–25]. Research has revealed a close association between the expression levels of TYMS and distinct phases of the cell cycle [26]. In human lymphoblasts, TYMS enzyme activity is extremely low

during the G0 and G1 phases [26]. It peaks during the S phase to meet the cell's demand for nucleotides [26]. Consequently, the pivotal role of TYMS in nucleic acid synthesis and cell cycle regulation renders it a significant therapeutic target in cancer treatment.

Given the critical role of TYMS in cancer, this study adopted a multi-omics integrated analysis strategy to systematically elucidate its multi-omics characteristics and clinical significance in LUAD [27]. To this end, we integrated transcriptomic, proteomic, and genomic data from databases such as TCGA and

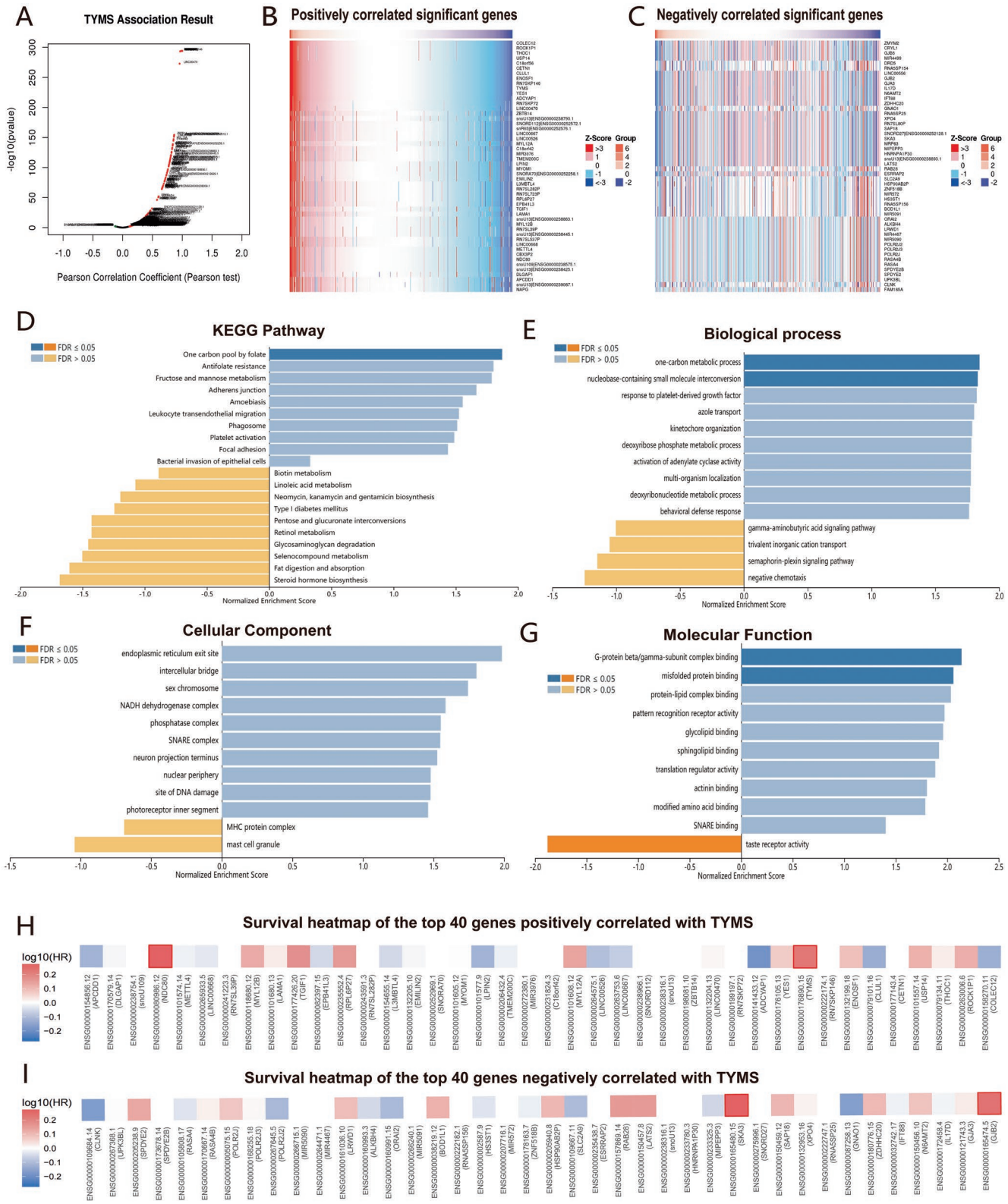

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FIGURE 6 | Changes in somatic copy number variations in TYMS and co-expressed genes in LUAD (LinkedOmics). (A) RNA sequencing data for differentially expressed genes extracted from the TCGA-LUAD project, alongside SCNA data associated with TYMS mutations, presenting the results for differentially expressed genes. The *p*-value was calculated using Pearson's correlation coefficient. Red and green dots denote genes positively and negatively correlated with TYMS, respectively. (B and C) Heatmaps display the expression patterns of genes positively and negatively correlated with TYMS in LUAD. (D and E) present the enrichment results for KEGG pathways and biological processes among the differentially expressed genes. (F and G) present enrichment results for Cellular Component and Molecular Function among the differentially expressed genes. (H and I) Survival heatmaps displaying the top 40 genes positively and negatively correlated with TYMS in LUAD. The survival heatmap displays the log-risk ratios for different genes (log10), with red and blue blocks representing higher and lower risks, respectively. Rectangular boxes denote outcomes that were significantly adverse or favourable in the prognostic analysis ($p < 0.05$). FDR denotes the false discovery rate.

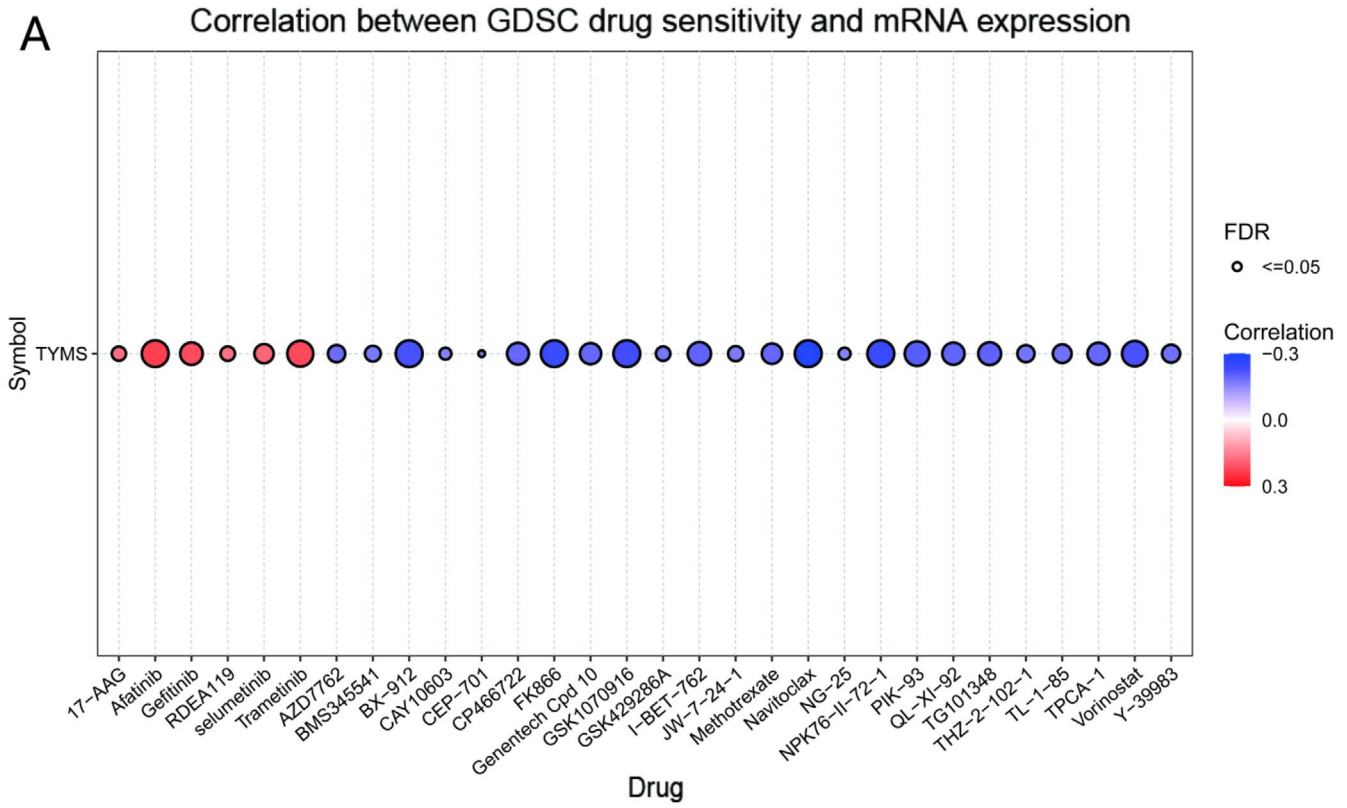

FIGURE 7 | Drug metabolism in TYMS-SCNA and LUAD. (A) Correlation analysis between TYMS gene expression and GDSC drug sensitivity in pan-cancer patients (top 30). Red indicates a positive correlation, while blue indicates a negative correlation.

CPTAC and employed bioinformatics tools to construct a multi-level, integrative analytical framework focusing on the expression patterns of TYMS in LUAD, its prognostic associations, interactions with the immune microenvironment, and therapeutic target potential.

Through the integrated analysis of multi-omics data, this study transcends single-level observations and systematically constructs a multidimensional functional map of TYMS in LUAD. This integrative strategy not only cross-validated the consistency of its expression using transcriptomic and proteomic data, but also, more importantly, linked genomic variations (copy number amplification), protein functions (pathway association), drug response phenotypes, and immune microenvironment characteristics. This provides a coherent evidence framework for understanding how TYMS transitions from gene drive to functional realisation, ultimately influencing clinical prognosis and therapeutic response.

The following findings of this study exemplify this framework. The results demonstrate that TYMS exhibits significantly elevated levels at both the transcriptional and protein levels in LUAD compared to normal tissue, and this increase is closely associated with reduced patient survival. This discovery aligns with multiple prior studies, such as those demonstrating that high TYMS expression in colorectal cancer, hepatocellular carcinoma, and pancreatic cancer correlates with poor prognosis [28–30].

Moreover, its high expression is closely associated with tumour staging, grading, and histological subtypes, suggesting that TYMS may play a significant role in the development and progression of LUAD. Notably, (Figure 3B) TYMS protein exhibits significant upregulation in the early disease stage (Stage 1), suggesting its potential as a valuable biomarker for early diagnosis and prognosis of LUAD. According to immunohistochemical statistics from HPA, TYMS protein expression levels in LUAD were markedly higher than in normal lung tissue.

In our proteomics study, pathway enrichment analysis revealed that TYMS is significantly enriched in key signalling pathways such as p53/Rb and mTOR. The p53 pathway plays a central role in cell cycle regulation, DNA repair, and apoptosis [31]. As p53 is a tumour suppressor gene, its inactivation can lead to uncontrolled cell proliferation and immune evasion, thereby promoting tumour invasion and metastasis [32, 33]. Furthermore, the mTOR pathway is closely associated with immune inflammatory responses and the tumour microenvironment [34, 35]. TYMS is implicated in the mTOR pathway, suggesting it may promote tumour growth by regulating metabolism and the immune microenvironment.

This study is the first to reveal, at the multi-omics level, the correlation between TYMS expression and the LUAD immune microenvironment. High TYMS expression was negatively correlated with B-cell activity and positively correlated with neutrophil infiltration, while also showing significant associations with multiple immune regulatory molecules (such as CTLA4, PD-1, IL-6, etc.) (Figure 5C–J). These findings suggest that TYMS may indirectly promote tumour immune escape by suppressing antitumour immune responses—such as diminishing cytotoxic T cell function—and fostering the development of an immunosuppressive microenvironment [36–38]. Notably, the positive correlation between TYMS and immune checkpoint molecules such as CTLA-4 and PD-1 suggests that patients with high expression may exhibit greater sensitivity to immune checkpoint inhibitor therapy. This finding plays a significant role in the development of tumour immunotherapy for LUAD [39].

Subsequently, we utilised TCGA LUAD data to integrate TYMS SCNA data with RNA-seq data. We then performed GSEA on differentially expressed genes. Results indicated that TYMS copy number amplification was significantly associated with poor LUAD prognosis and may impact key biochemical processes such as folate metabolism. Moreover, folate metabolism serves as a target for multiple chemotherapy drugs (such as *Methotrexate*, *5-fluorouracil* and *Pemetrexed*, etc.), and TYMS amplification may contribute to the development of chemotherapy resistance [12, 40–43].

Multi-omics integration analysis has unique advantages in predicting drug sensitivity [27]. Therefore, we evaluated the relationship between TYMS and drug sensitivity across multiple cell lines. We observed that *Afatinib* and *Gefitinib* act as epidermal growth factor receptor (EGFR) inhibitors, whereas *Trametinib* functions as a MEK (Mitogen-Activated Protein Kinase Kinase) inhibitor [44]. TYMS may influence the activity of these drug targets by modulating intracellular metabolism or signalling pathways, thereby enhancing drug sensitivity [45–52]. TYMS expression was negatively correlated with drugs such as *BX-912, FK866, GSK1070916, Methotrexate, Navitoclax, NPK76-II-72-1*, and *Vorinostat*, suggesting that its elevated levels may inhibit the therapeutic efficacy of these agents. This may stem from an antagonistic relationship between TYMS activity and the mechanisms of action of these drugs [53–55]. For instance, *Methotrexate* functions as a folate antagonist [56], whilst TYMS plays a pivotal role in folate metabolism [57]. Consequently, elevated TYMS expression may induce alterations in folate metabolism, thereby diminishing the therapeutic efficacy of *Methotrexate* [58–61]. Similarly, the efficacy of other drugs (such as *BX-912* and *Navitoclax*, etc.) may also be influenced by TYMS expression levels, ultimately inhibiting the action of these drugs [59, 60, 62].

Although this study has identified the significance of TYMS in LUAD through a multi-omics approach, several limitations remain. Firstly, the study lacks clinical samples. Consequently, future research should validate the expression patterns and prognostic value of TYMS using clinical specimens. Secondly, the specific molecular mechanisms by which TYMS influences the immune microenvironment require further experimental validation. Furthermore, the potential of TYMS as a prognostic biomarker warrants additional investigation.

5 | Conclusion

This study, based on GTEx LUAD data, identified 148 genes significantly associated with prognosis through cross-analysis of the top 500 survival-related genes and differentially expressed genes. Findings revealed that TYMS transcription and protein levels were markedly elevated in LUAD patients, with its overexpression closely linked to poor patient outcomes. Furthermore, fluctuations in TYMS protein levels were significantly correlated with alterations in signalling pathways such as p53/Rb and mTOR, etc. Additionally, TYMS expression demonstrated associations with the LUAD immune microenvironment, encompassing B-cell activity, neutrophil infiltration, and the expression of multiple immunosuppressive molecules (e.g., CTLA4, PD-1, IL-6, etc.). These findings suggest TYMS may be implicated in immunotherapy responses.

Moreover, somatic copy number amplification of TYMS is significantly associated with poor prognosis in LUAD and is predominantly enriched in the folate metabolism pathway, potentially conferring resistance to multiple chemotherapeutic agents. Drug sensitivity analysis indicates that TYMS expression levels correlate with sensitivity to various targeted therapies (such as *Afatinib* and *Gefitinib*) and chemotherapeutic drugs, offering novel perspectives for combination therapies. In summary, TYMS not only represents a potential prognostic biomarker but may also serve as a novel therapeutic target. Consequently, further research is warranted to elucidate its underlying mechanisms.

Author Contributions

Hansen Shi: writing – original draft, software. **Peijun Zhang:** copy editor, methodology. **Jiayi Chen:** investigation, formal analysis. **Hua Li:** software, visualisation. **Xiaohuai Zhang:** investigation, software. **Biyun Zeng:** investigation, validation. **Tiancai Liu:** project administration, methodology, conceptualization. **Tao Zeng:** conceptualization, supervision.

Acknowledgements

We would like to express our gratitude to Professor Xiaocong Lin from the Institute of Biochemistry and Molecular Biology at Guangdong Medical University for providing valuable discussions on various aspects of the manuscript. We thank all individuals who contributed to this study.

Funding

Guangdong Basic and Applied Basic Research Foundation (2023A1515010235, 2025A1515011140); Start-up Fund for High-level Talents in Affiliated Hospital of Guangdong Medical University (51301Z20200007), Medical Science and Technology Research Project of Guangdong Province (A2023168, B2021180), Discipline construction project of Guangdong Medical University (4SG21266P), National Natural Science Foundation of China (82372344), and Natural Science Foundation of Guangdong Province (2023A1515011925, 2024A1515011850).

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Protein–Protein Interaction Network of TYMS.