

# Study on *MKNK2* as a potential prognostic and immunological biomarker in pan-cancer

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**Abstract. – OBJECTIVE:** This study aimed to investigate the expression levels of the *MKNK2* gene in pan-cancer, its prognostic significance, and its relationship with the tumor immune microenvironment, as well as to assess its potential as an immunological and prognostic biomarker.

**MATERIALS AND METHODS:** The research utilized data from The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), and Cancer Cell Line Encyclopedia (CCLE), including clinical and mutational information. Bioinformatic tools were employed to analyze the association of *MKNK2* with carcinogenesis, including its links to prognosis, immune cell infiltration, tumor immune microenvironment, gene mutation, and the stemness of various tumor cells. A variety of statistical software and analytical tools were applied, including R software, SPSS 27.0, TIMER, CIBERSORT algorithm, and EPIC algorithm.

**RESULTS:** The study found that *MKNK2* is abnormally expressed in pan-cancer and is associated with a poor prognosis. The levels of *MKNK2* are highly related to immune cell infiltration and tumor stemness. Notably, in liver hepatocellular carcinoma, glioblastoma multiforme, low-grade gliomas, and acute myeloid leukemia, *MKNK2* expression shows a strong correlation with clinical outcomes and immune infiltration. Furthermore, the expression of *MKNK2* shows significant correlations with immune cell infiltration, immune checkpoints, tumor mutational burden (TMB), microsatellite instability (MSI), and stemness scores across various cancers.

**CONCLUSIONS:** The abnormal expression of *MKNK2* is associated with tumor progression, immune checkpoint genes, immune cell infiltration, microsatellite instability (MSI), tumor mutational burden (TMB), and stemness in a variety of tumors, especially in glioblastoma multiforme low-grade gliomas (GBMLGG). Therefore, *MKNK2* may serve as a potent prognostic physiological marker and provide new avenues for the development of tumor mechanisms and therapeutic strategies targeting *MKNK2* to enhance the efficacy of immunotherapy.

*Key Words:*

*MKNK2*, Pan-cancer, Cancer Genome Atlas, Immune analysis, Tumor microenvironment.

## Introduction

Cancer continues to be a significant challenge to global health, with a persistent rise in both its incidence and mortality rates. The World Cancer Report by the World Health Organization (WHO) for the year 2020 underscores the severity of this issue, highlighting that cancer accounts for one in every six deaths annually, with an ever-increasing burden on society and healthcare systems worldwide<sup>1</sup>. The urgency of improving cancer detection and therapeutic strategies is evident, given the high mortality rate associated with the disease.

Despite advances in clinical treatments, the overall prognosis for many cancer patients remains suboptimal due to the development of drug resistance, debilitating side effects, and other treatment-related complications. The quest for novel therapeutic targets and more sensitive physiological biomarkers is, therefore, more critical than ever to enhance early diagnosis and treatment efficacy<sup>2</sup>.

*MKNK2* encodes a protein that is part of the calcium/calmodulin-dependent protein kinases (CAMK) family of Ser/Thr protein kinases, which are members of the broader protein kinase superfamily<sup>3</sup>. Encoded by the *MKNK2* gene, this protein kinase is a crucial downstream effector in the *MAPK* signaling cascade, which is instrumental in various cellular functions, including the promotion of oncogenic transformation, mRNA translation, and cell proliferation upon stimulation by mitogen-activated protein kinases (*MAPK*)<sup>4</sup>. The downstream signaling proteins, *MAPK*-interacting kinases (*MKNK1/2*), have been observed to modulate the synthesis of both non-cancerous and cancerous cell proteins, indicating their potential role in cancer development<sup>5</sup>.

Emerging evidence has demonstrated elevated *MKNK2* expression in a variety of cancers, particularly in drug-resistant ovarian cancer tissues, where it correlates with poor prognosis<sup>6,7</sup>. However, the comprehensive role of *MKNK2* in pan-cancer progression and its underlying mechanisms are not yet fully understood, warranting further investigation.

The nexus between cancer stem cells (CSCs) and the tumor microenvironment (TME) has increasingly become a focal point in cancer research. The TME, which fosters cancer growth and metastasis, is intricately connected with CSCs, emphasizing the importance of understanding the interplay between these components in cancer development and progression<sup>8,9</sup>. *MKNK2*'s association with the maintenance of stemness in multiple cancers suggests a complex interaction within the TME.

The presence of immune cells within the TME, such as B cells, tumor-associated macrophages, regulatory T cells, and natural killer cells, is increasingly recognized for its role in cancer formation and immune evasion<sup>10,11</sup>. Suppressive molecules like programmed cell death ligand-1 and cytotoxic T-lymphocyte associated antigen-4 are pivotal in modulating anti-tumor immunity. Despite the promise of cancer immunotherapy, there is a pressing need to refine existing approaches and explore new targets to enhance the efficacy of immunotherapeutic interventions.

In this study, we delve into the expression patterns of *MKNK2* across diverse non-cancerous and cancerous tissues and cell lines, aiming to systematically elucidate its incidence and prognostic value in various cancer types. By integrating data from authoritative databases, such as The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), and Tumor Immune Estimation Resource (TIMER), we have conducted a comprehensive evaluation of *MKNK2*'s role in immune responses and its prognostic implications in a spectrum of cancers. The potential interplay between *MKNK2*, tumor mutational burden (TMB), microsatellite instability (MSI), immune cell infiltration, stemness, and genetic immune checkpoints was scrutinized. Furthermore, Gene Set Enrichment Analysis (GSEA) was utilized to explore the biological pathways and functions related to *MKNK2*. Our findings reveal a significant association between *MKNK2* expression and patient survival, particularly emphasizing its prognostic value in glioblastoma multiforme low-grade gliomas (GBMLGG), liver hepatocellular carcinoma (LIHC), and acute myeloid leukemia (LAML). These insights underscore the potential

of *MKNK2* as a generalized predictor for cancer diagnosis and prognosis and highlight its therapeutic potential in the realm of immunotherapy.

## Materials and Methods

### Data Collection and Processing

A pan-cancer dataset was downloaded from the TARGET, GTEx, UCSC (<https://xenabrowser.net/>) database, and TCGA Pan-Cancer (PANCAN, N=10,535, G=60,499). Then, the data of *MKNK2* gene levels [*(ENSG00000099875 (MKNK2))*] of each sample, along with the data of primary solid tumor, normal tissue, and primary blood-derived cancer-bone marrow, were extracted. The specimens with 0 expression level were filtered along with studies with <3 samples of single cancer, followed by the determination of  $\log_2(x+0.001)$  transformation per expression value. Finally, the expression data of 26 cancer species from UCSC and TCGA and 34 from TARGET and GTEx were selected. Cell line gene expression matrix of multiple cancer species was obtained from CCLE (<https://portals.broadinstitute.org/ccle/about>) data set and transformed by  $\log_2(\text{TPM} + 1)$  using R package of "rma" in an R environment (R version: 3.6.1).

### Genetic Expression Levels in All Tumor Stages

The R software (version 3.6.4) was utilized to determine the variability in the genetic expression of each sample during different tumor stages. The unpaired Student's *t*-test was performed to evaluate the significant difference of inter-pair analysis. Variance analysis was conducted to determine the difference in multiple sample groups.

### Cox Regression and Kaplan-Meier Survival Analyses

Data from a previous TCGA high-quality prognostic investigations published in the cell and that from the UCSC cancer browser (<https://xenabrowser.net/datapages/>), along with the follow-up data from TARGET (as a supplement) were also obtained<sup>12</sup>. Cox regression analysis was carried out to evaluate *MKNK2* expression association with patients' overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and Progression Free Interval (PFI) in each kind of cancer from GTEx and TCGA samples in the R environment. The Kaplan-Meier assessment was applied to obtain pan-cancer patients' survival curves after separating the patients into

high and low *MKNK2* expression cohorts based on the chief separation strategy.

### ***MKNK2 Expression Association with Immune Cells and Checkpoint***

With the help of TIMER, the correlation of immune cell infiltration with *MKNK2* expression was determined. It provided an important assessment of immune cell integration for TCGA samples' RNA sequencing. These cells involve: B, dendritic, macrophages, CD4+ T, neutrophils, CD8+ T, and cells. More than 60 common immune checkpoint genes were selected, and the link between the expression of these genes and *MKNK2* was evaluated<sup>13</sup>. CIBERSORT algorithm and EPIC algorithm of immunity score were calculated using the `deconvo_CIBERSORT` method and `deconvo_epic` method of R software package IOBR, respectively. Algorithm and EPIC algorithm of immunity score were calculated using the `deconvo_CIBERSORT` method and `deconvo_epic` method of R software package IOBR, respectively. The R software package used in this document can be downloaded from the CRAN package repository (Available at: [https://cran.r-project.org/web/packages/available\\_packages\\_by\\_name.html](https://cran.r-project.org/web/packages/available_packages_by_name.html)).

### ***Function Landscape and Stemness Scores Analyses***

With the help of the R software package `maftools` (version 2.8.05), the function of TMB for each tumor was determined. The MSI scores of each tumor were also evaluated. The MSI and TMB scores were acquired from TCGA. Correlation analysis of TMB/MSI and *MKNK2* expression was carried out using Spearman's protocol. The horizontal axis in the figure depicts the correlation coefficient between TMB/MSI and *MKNK2*; the vertical axis shows various cancer types, and the bar color depth represents the *p*-value significance. The relationship between tumor cell stemness and gene expression was analyzed by methylated signature and mRNA expression<sup>14</sup>. The DNA methylation-based stemness scores (DNAss) and the RNA-based stemness scores (RNAss) reflect epigenetic characteristics and gene expression characteristics of stem cells, respectively.

### ***Gene Set Enrichment Analysis***

The "gmt" file of the hallmark gene set (`h.all.v74.symbols.gmt`), which contains 50 hallmark gene sets, was downloaded from the website of Molecular Signatures Database (MSigDB, Available at: <https://www.gsea-msigdb.org/gsea/index.jsp>) and

used to calculate the normalized enrichment score (NES) and false discovery rate (FDR) of the DEGs between low- and high-*MKNK2* expression cancer groups for each biological process in each cancer type. The GSEA was conducted using the R package "clusterProfiler"<sup>15</sup> (the clusterProfiler package is released under Artistic-2.0 License within Bioconductor project). The source code and vignette are freely available at <http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>, and the results were summarized in the bubble plot depicted by the R package "ggplot2".

### ***Statistical Analysis***

Data analysis was conducted using R (3.6.1 & 3.6.4 version) and SPSS 27.0 (Statistical Package for the Social Sciences; IBM Corp., Armonk, NY, USA); download at: <https://www.ibm.com/cn-zh/spss>. To determine the association of *MKNK2* expression with aimed targets, including immune cell infiltration scores (six aforementioned immune cell types), TMB, MSI, and methylation transferase genes, the Spearman Correlation test was applied. The intergroup comparison of *MKNK2* expression levels or between tumor and normal tissues was determined by the paired or the simple *t*-test, based on whether the samples were paired. Multiple group comparisons were conducted using One-way analysis of variance, while Bonferroni adjustment was used for pairwise comparisons. A *p*-value <0.05 was deemed significant.

## **Results**

### ***Expression Levels of MKNK2 in Pan-Cancer***

By employing the R software, we conducted a meticulous analysis to assess the variability in *MKNK2* expression between tumor and normal samples across a diverse range of cancer types. Through the application of unpaired Wilcoxon Rank Sum and Signed Rank Tests, we meticulously evaluated the statistical significance of observed expression differences. This rigorous approach revealed that *MKNK2* expression significantly diverged in 17 of the 26 examined cancer types. Particularly, we identified substantial upregulation in cancers such as glioblastoma (GBM), glioblastoma multiforme low-grade gliomas (GBMLGG), cervical squamous cell carcinoma (CESC), prostate adenocarcinoma (PRAD), uterine corpus endometrial carcinoma (UCEC), liver hepatocellular carcinoma (LIHC), kidney

chromophobe (KICH), and cholangiocarcinoma (CHOL), along with nine additional tumor categories, including lung adenocarcinoma (LUAD), colon adenocarcinoma (COAD), stomach adenocarcinoma (STAD), colorectal adenocarcinoma with readthrough (COADREAD), esophageal squamous cell carcinoma (STES), lung squamous cell carcinoma (LUSC), head and neck squamous cell carcinoma (HNSC), thyroid carcinoma (THCA), and rectal adenocarcinoma (READ) (Figure 1A).

By expanding our analysis to encompass 34 cancer types, we observed a generally consistent expression pattern. However, certain cancers, including prostate adenocarcinoma (PRAD) and liver hepatocellular carcinoma (LIHC), stood out with significant upregulation, in contrast to the marked downregulation observed in cancers, such as breast invasive carcinoma (BRAC), esophageal carcinoma (ESCA), wild-type skin cutaneous melanoma (WT SKCM), pancreatic adenocarcinoma (PAAD), testicular germ cell tumors (TGCT), and uterine carcinosarcoma (UCS) (Figure 1B). These findings highlight the considerable variability in *MKNK2* expression levels between tumor and non-tumor tissues and underscore the complexity of its role across different cancer types.

Further investigation of *MKNK2* expression across all tumor stages (I-IV) in patients revealed notable differences in 11 tumor types, including cervical squamous cell carcinoma (CESC), esophageal carcinoma (ESCA), stomach squamous cell carcinoma (STES), kidney papillary cell carcinoma (KIPAN), uterine corpus endometrial carcinoma (UCEC), thymoma (THYM), thyroid carcinoma (THCA), testicular germ cell tumors (TGCT), bladder urothelial carcinoma (BLCA), adenocarcinoma of the cervix (ACC), and kidney chromophobe (KICH) (Figure 1C). An analysis of *MKNK2* expression in a variety of cancer cell lines, based on data from the Cancer Cell Line Encyclopedia (CCLE), exposed significant heterogeneity among the cell lines. Advanced tumors, particularly those of the cervical squamous cell carcinoma (CESC), thymoma (THYM), thyroid carcinoma (THCA), and kidney chromophobe (KICH), generally showed upregulation of *MKNK2*, whereas downregulation was observed in advanced tumors of the stomach squamous cell carcinoma (STES), uterine corpus endometrial carcinoma (UCEC), and bladder urothelial carcinoma (BLCA) (Figure 1D). These insights contribute to a nuanced understanding of *MKNK2*'s potential as a biomarker and therapeutic target in cancer progression and heterogeneity.

### ***Prognostic Analysis of MKNK2 in Pan-Cancer***

The existing body of research hints at a connection between *MKNK2* levels and specific cancer types, including gastric and glioma cancers; however, the broader prognostic implications of this gene are not yet fully understood. Our study undertook a comprehensive assessment, evaluating the impact of *MKNK2* on overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and Progression-Free Interval (PFI) across a diverse spectrum of 33 cancer types.

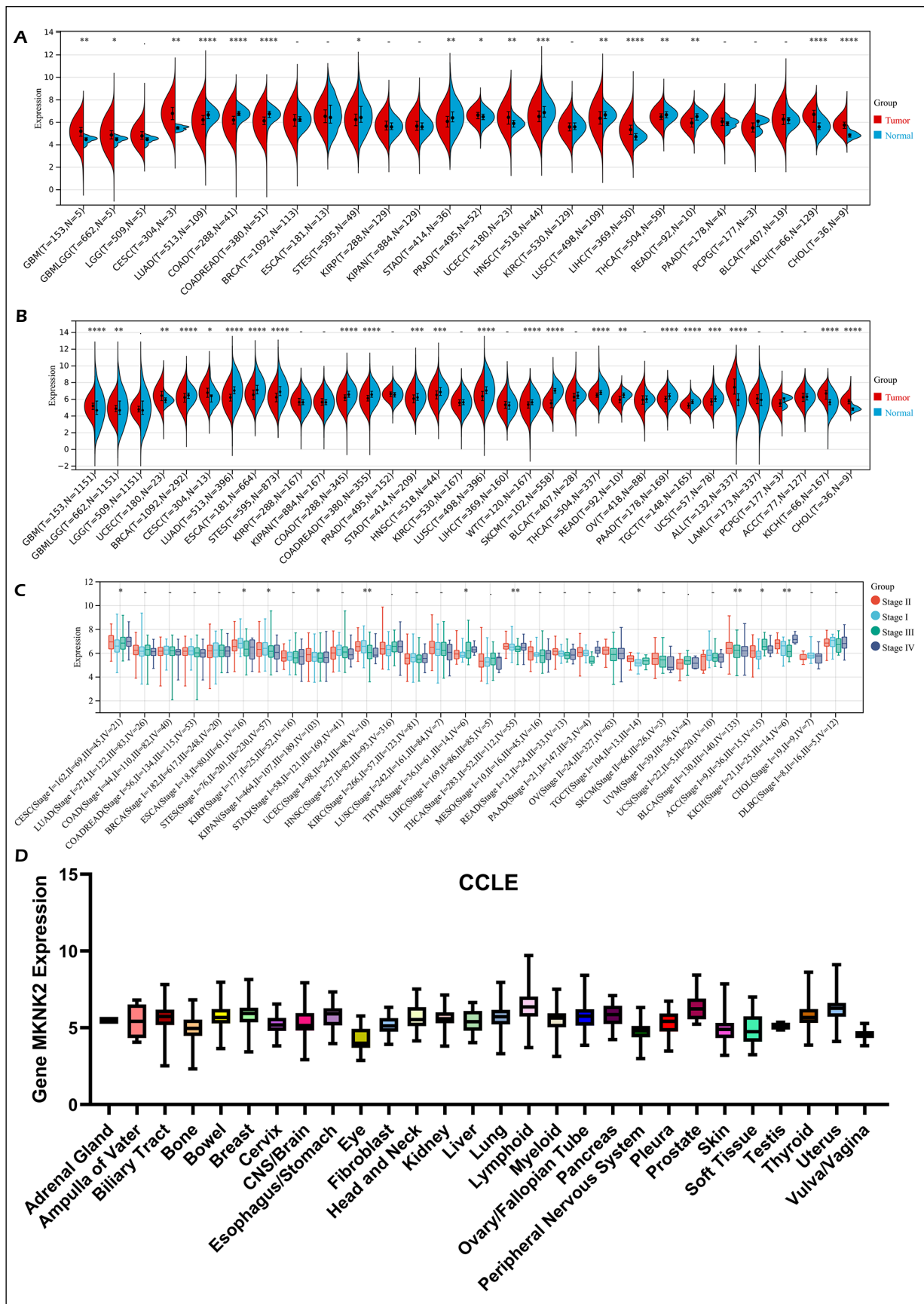
Through rigorous Cox regression analysis, we identified a significant correlation between *MKNK2* expression and OS in eight distinct cancer types, specifically TCGA-designated cancers, such as GBMLGG, LAML, LGG, ACC, LIHC, STES, KIPAN, and STAD. The Kaplan-Meier survival curves provided further validation, illustrating a particularly strong association between elevated *MKNK2* levels and diminished OS in cancers like CGA-GBM, CGA-LGG, TCGA-LGG, TCGA-LIHC, TCGA-LAML, and TCGA-ACC. Conversely, in cancers such as TCGA-STES, TCGA-KIPAN, and TCGA-STAD, lower *MKNK2* levels were associated with poorer outcomes (Figure 2).

Our examination of the relationship between DSS, DFI, PFI, and *MKNK2* expression levels revealed that high expression in cancers like TCGA-GBMLGG, TCGA-LGG, TCGA-PRAD, and TCGA-ACC was linked to a poorer prognosis. Additionally, high *MKNK2* levels in TCGA-ACC and low levels in TCGA-STAD were correlated with adverse DFI outcomes. The PFI analysis mirrored these findings, with high expression in TCGA-GBMLGG, TCGA-LGG, and TCGA-ACC, and low expression in TCGA-BLCA, indicating a poorer prognosis (Figures 3-5).

The synthesis of these analyses reinforces *MKNK2*'s prognostic relevance in a subset of cancers. These findings not only point to *MKNK2*'s potential as an oncogene but also underscore its significance in the prognostic evaluation of cancer, offering valuable insights that may inform future therapeutic strategies and patient management.

### ***Immune Cells Infiltration and Checkpoint Analyses***

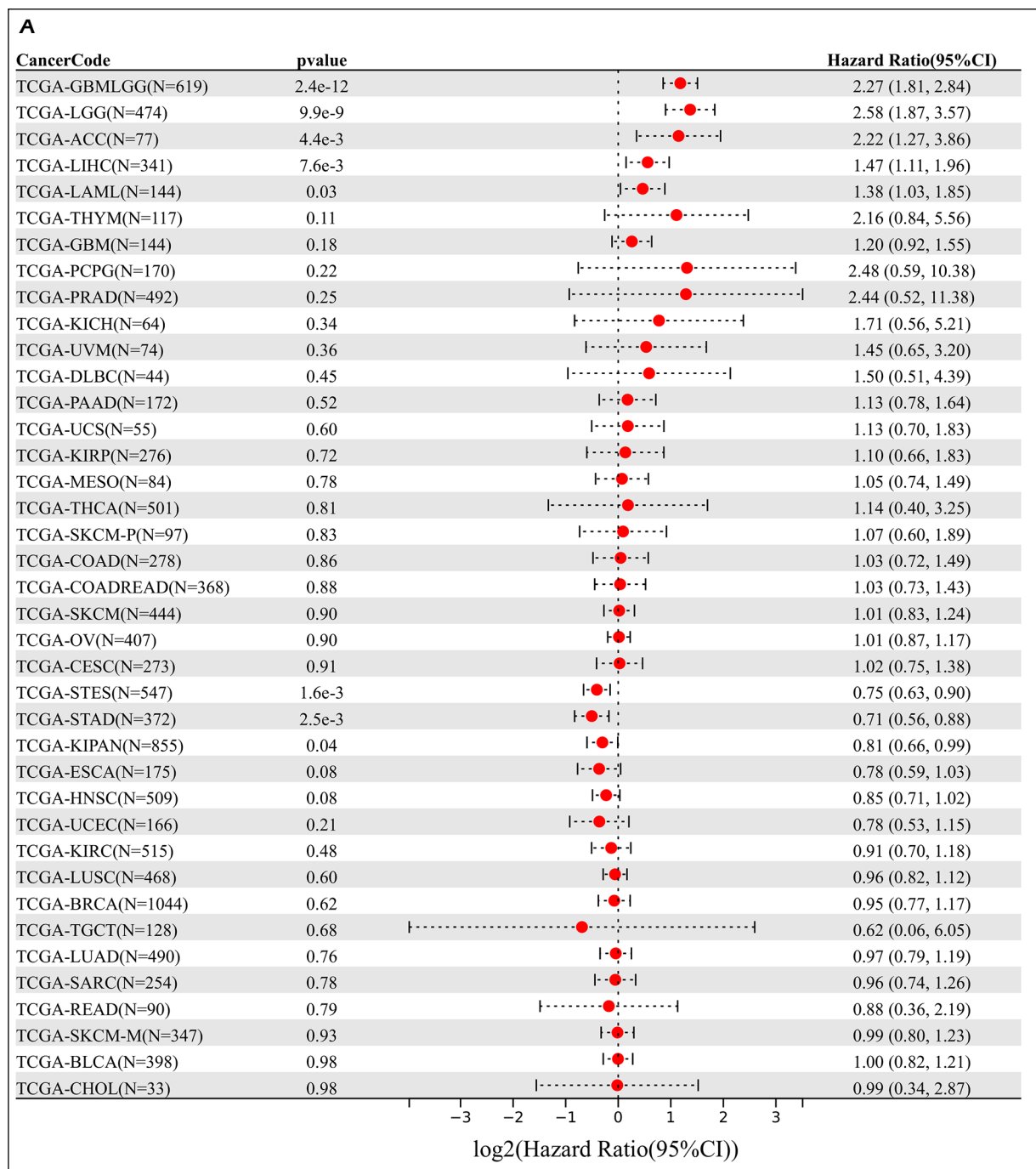
Emerging evidence has underscored the prognostic relevance of *MKNK2* across a spectrum of cancer types, with particular significance in glioblastoma multiforme low-grade gliomas (GBMLGG), liver hepatocellular car-



**Figure 1.** Differential expression of *MKNK2*. **A**, The expression level of *MKNK2* in different cancer types from TCGA. **B**, The expression level of *MKNK2* in different cancer types from TCGA and GTEx. **C**, Correlations between the *MKNK2* expression and the major pathological stages of multiple cancer types were investigated based on the TCGA data. **D**, *MKNK2* expression in various tumor cell lines based on the Cancer Cell Line Encyclopedia (CCLE) database. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . ns, not statistically significant.

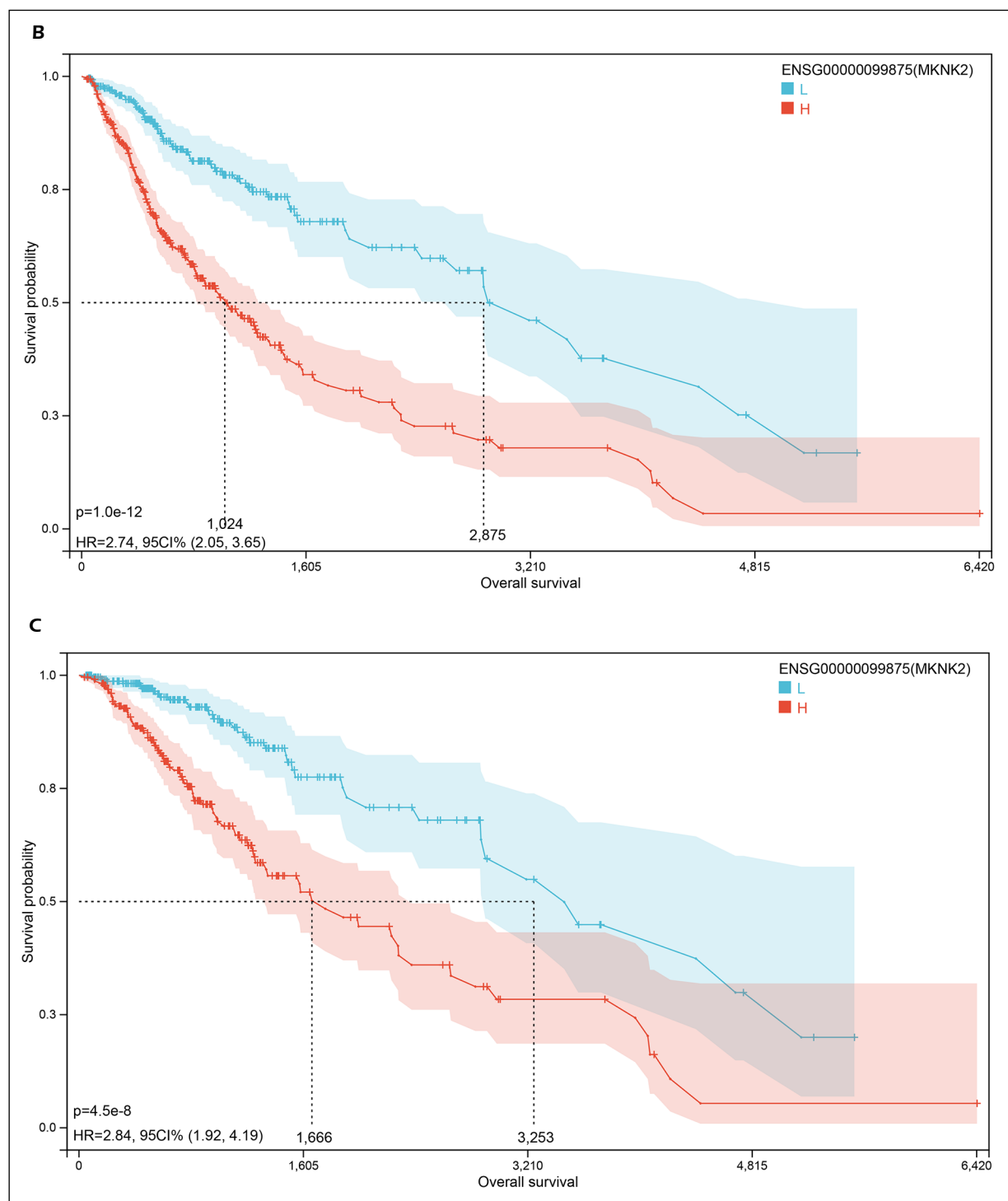
cinoma (LIHC), and adenocarcinoma of the cervix (ACC). The immune microenvironment, modulated by the infiltration of immune cells, is increasingly recognized for its influence on cancer prognosis. Notably, research has established that *MKNK2* plays a regulatory role in

the anti-inflammatory phenotype of macrophages, a key component of the tumor's immune landscape<sup>16</sup>. Despite this, the extent to which *MKNK2* may influence the recruitment of immune cells into the tumor microenvironment remains an open question.



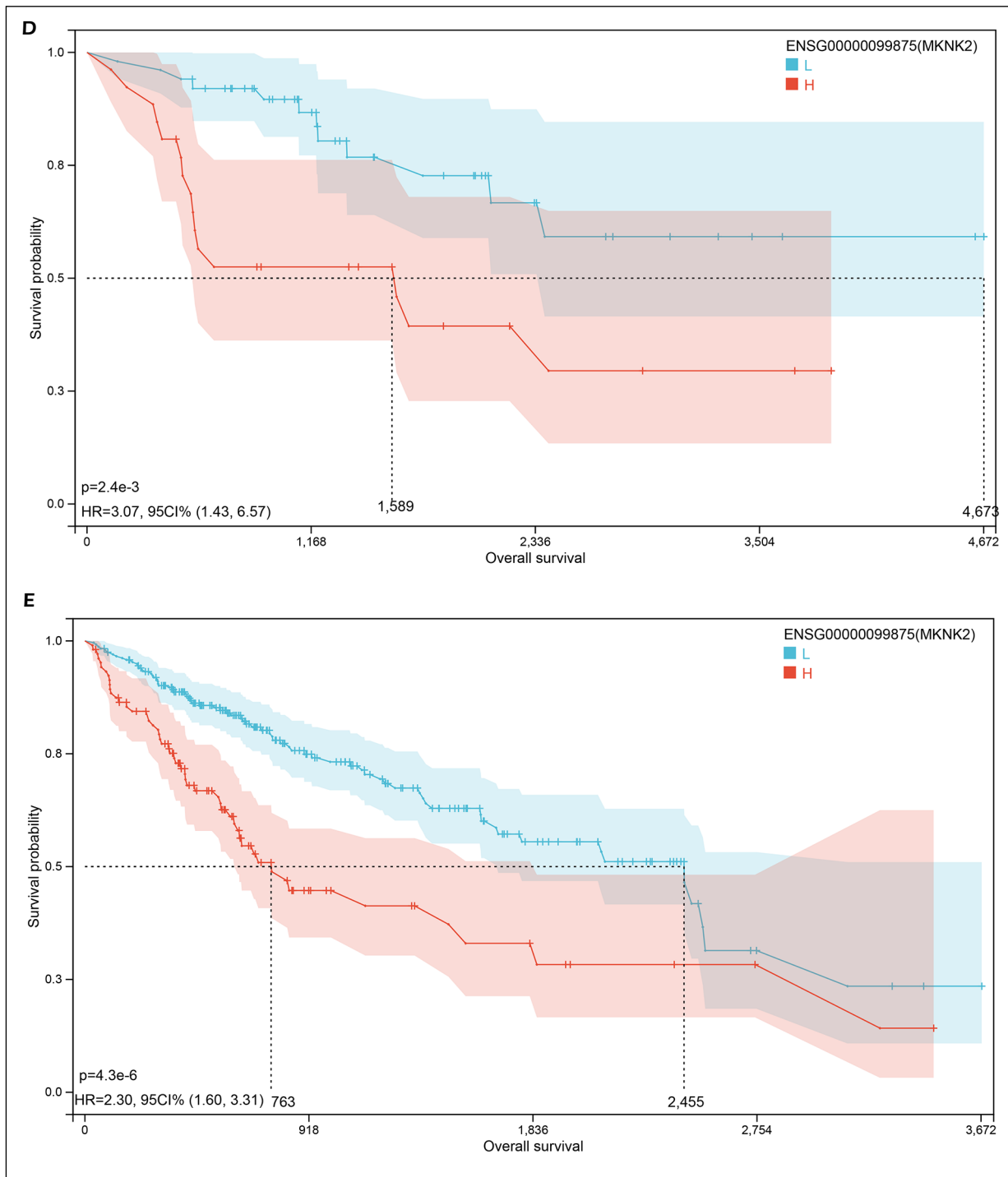
**Figure 2.** Association between the *MKNK2* expression and the OS of cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-I**, Kaplan-Meier survival curves of OS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, LIHC, LAML, STES, STAD, and KIPAN.

Figure continued



**Figure 2 (Continued).** Association between the *MKNK2* expression and the OS of cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-I**, Kaplan-Meier survival curves of OS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, LIHC, LAML, STES, STAD, and KIPAN.

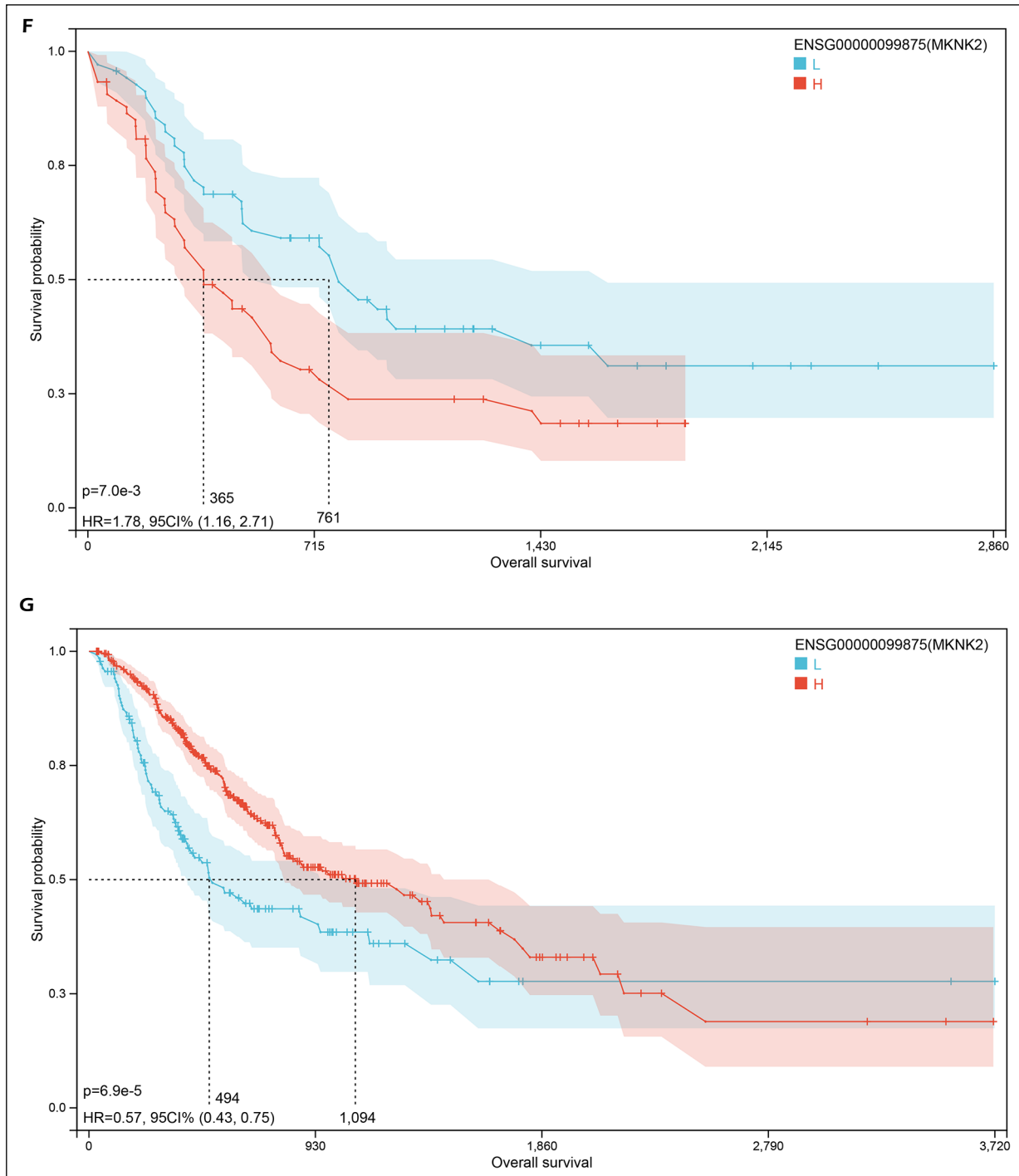
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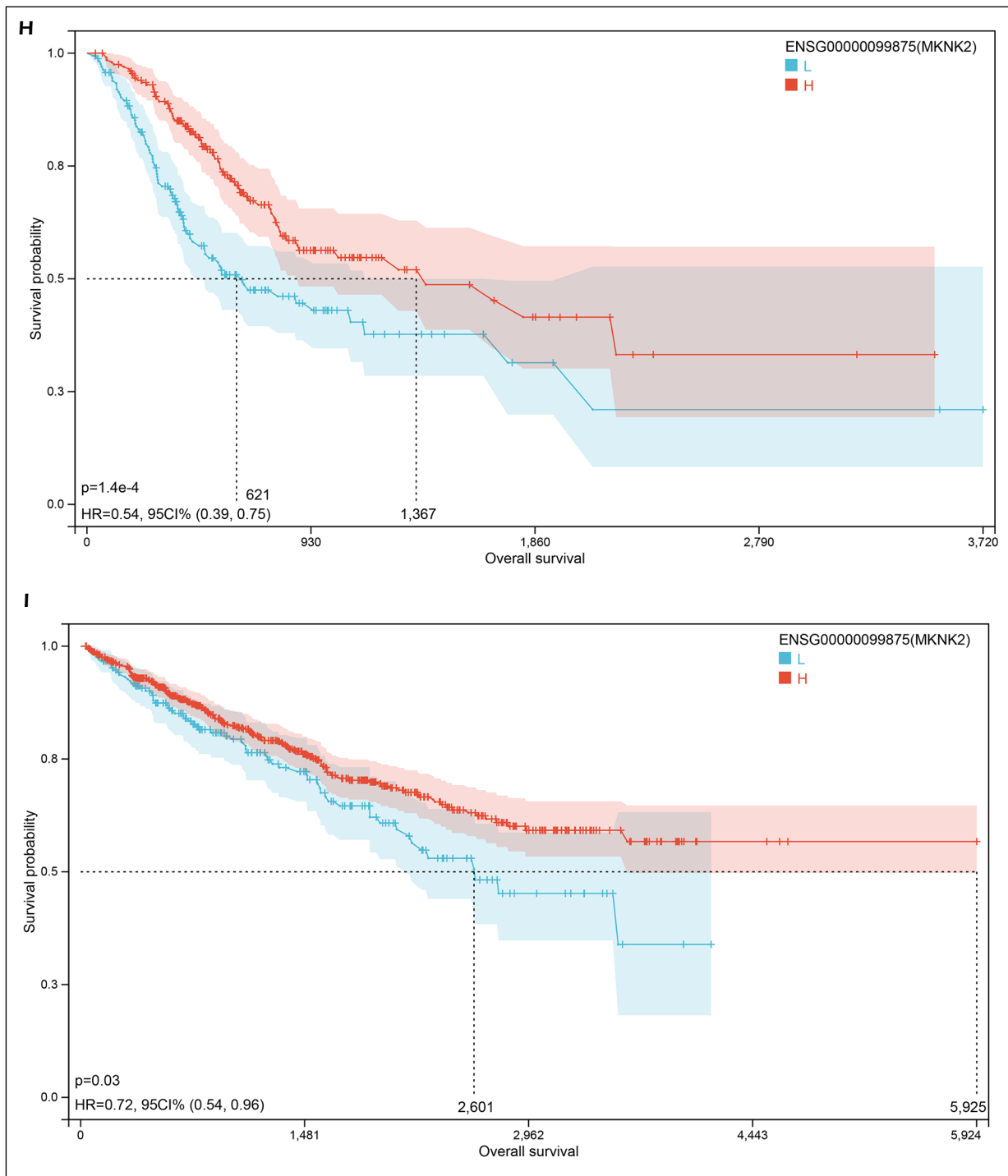
**Figure 2 (Continued).** Association between the *MKNK2* expression and the OS of cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-I**, Kaplan-Meier survival curves of OS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, LIHC, LAML, STES, STAD, and KIPAN.

Figure continued





**Figure 2 (Continued).** Association between the *MKNK2* expression and the OS of cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-I**, Kaplan-Meier survival curves of OS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, LIHC, LAML, STES, STAD, and KIPAN.



**Figure 2 (Continued).** Association between the *MKNK2* expression and the OS of cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-I**, Kaplan-Meier survival curves of OS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, LIHC, LAML, STES, STAD, and KIPAN.

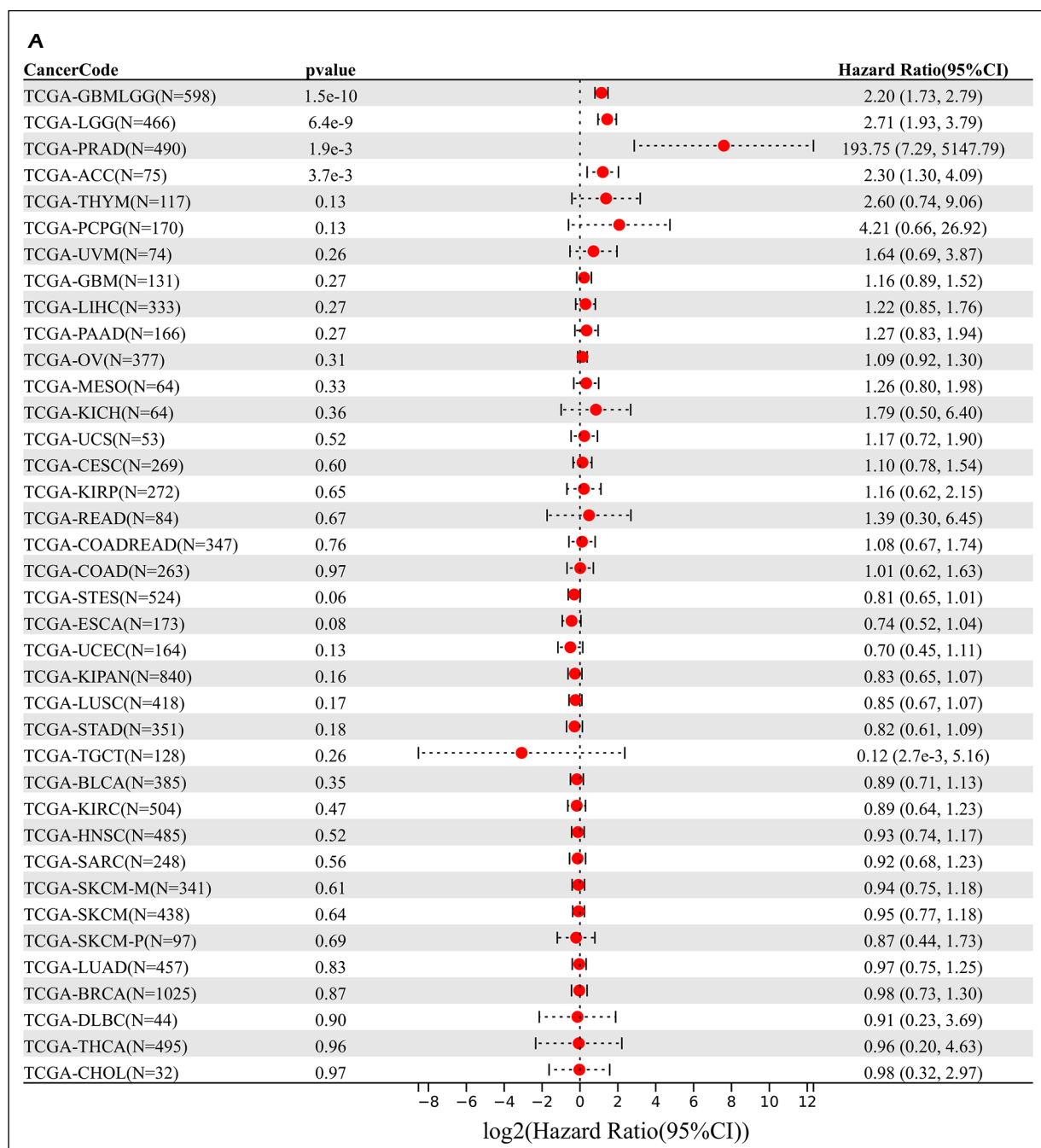
In this study, we meticulously evaluated the relationship between *MKNK2* expression and immune cell infiltration across various cancers,

leveraging data from the TIMER database. The Spearman correlation coefficient was employed to quantify the association between gene expression

and immune cell infiltration scores within each tumor context. Utilizing the R package psych (version 2.1.6) and its corr.test function, we discerned a significant correlation between *MKNK2* levels and immune infiltration in 31 distinct cancer types, including TCGA-designated cancers, such as BLCA, BRCA, CESC, and others. Con-

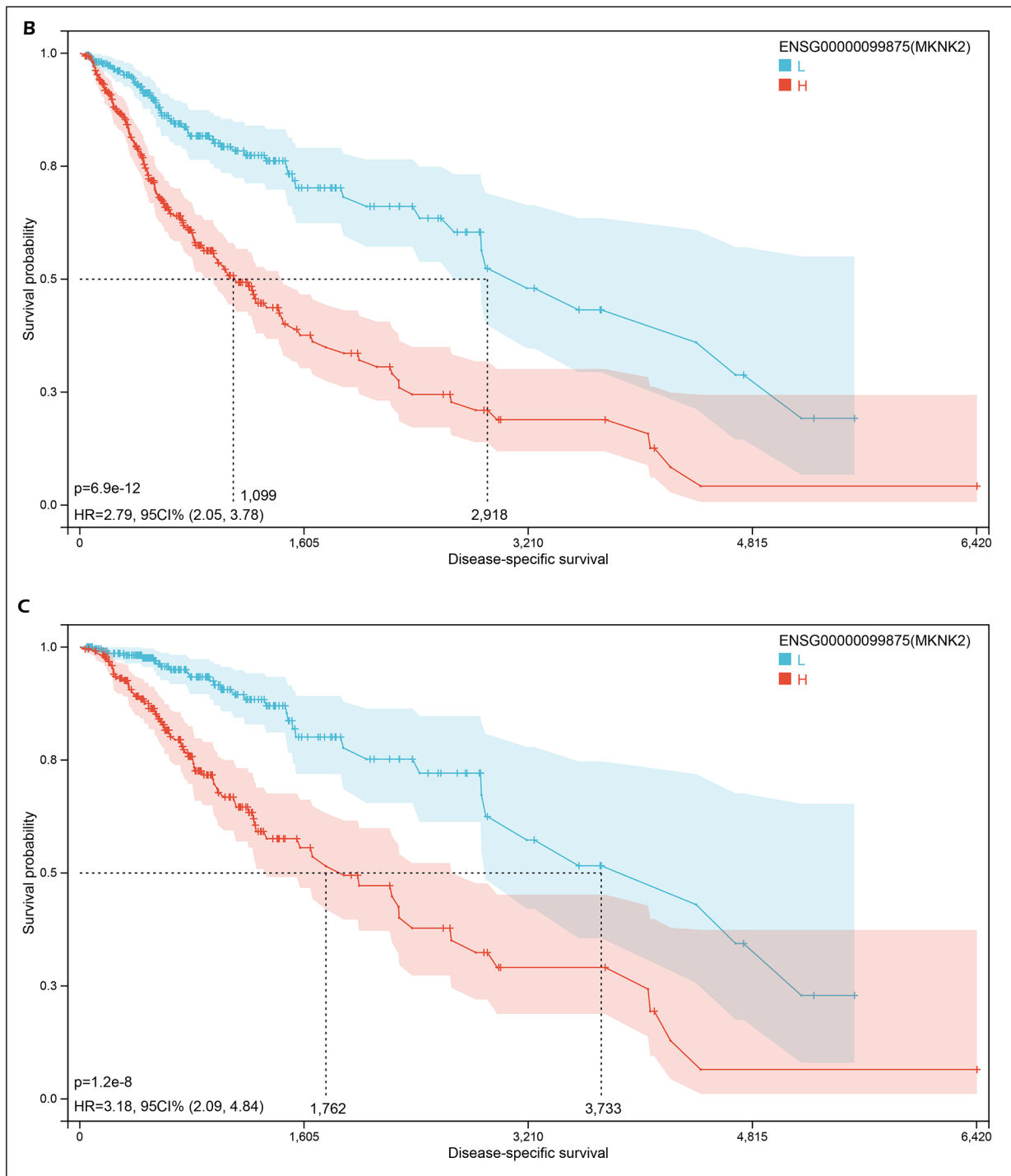
versely, no significant correlation was detected in cancers like MESO, DLBC, KICH, PAAD, and ACC (Figure 6A).

The advent of cancer immunotherapy has brought forth a new era in cancer treatment, with immune checkpoints emerging as pivotal regulators of the tumor-immune interface.



**Figure 3.** Association between the *MKNK2* expression and DSS in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-E**, Kaplan-Meier survival curves of DSS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, and PRAD.

Figure continued



**Figure 3 (Continued).** Association between the *MKNK2* expression and DSS in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-E**, Kaplan-Meier survival curves of DSS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, and PRAD.

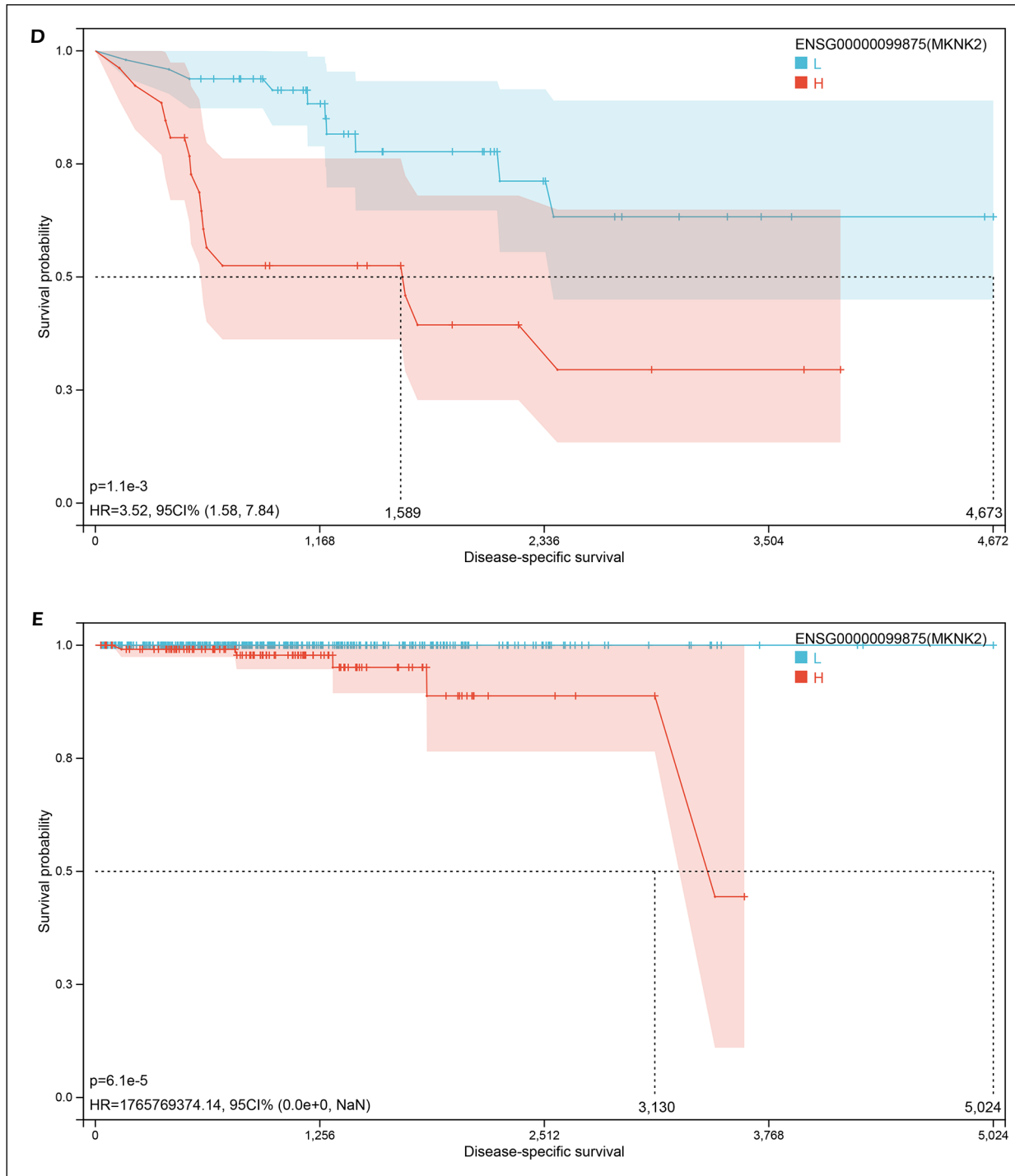
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Our analysis revealed a broad association between *MKNK2* expression and immune checkpoint status across all pan-cancers (Figure 6B). These findings are corroborated by the results

obtained from the CIBERSORT (Figure 6C) and EPIC (Figure 6D) algorithms, which estimate the immune score based on gene expression profiles.

Moreover, recent studies have suggested that the pharmacological inhibition of *MKNK2* can impede *eIF4E*-regulated translation, thereby suppressing the growth of stem cells and decelerat-

ing tumor growth *in vivo*<sup>17-19</sup>. These observations collectively point towards a potential role for *MKNK2* in modulating the density of immune cells within tumors, offering a promising avenue



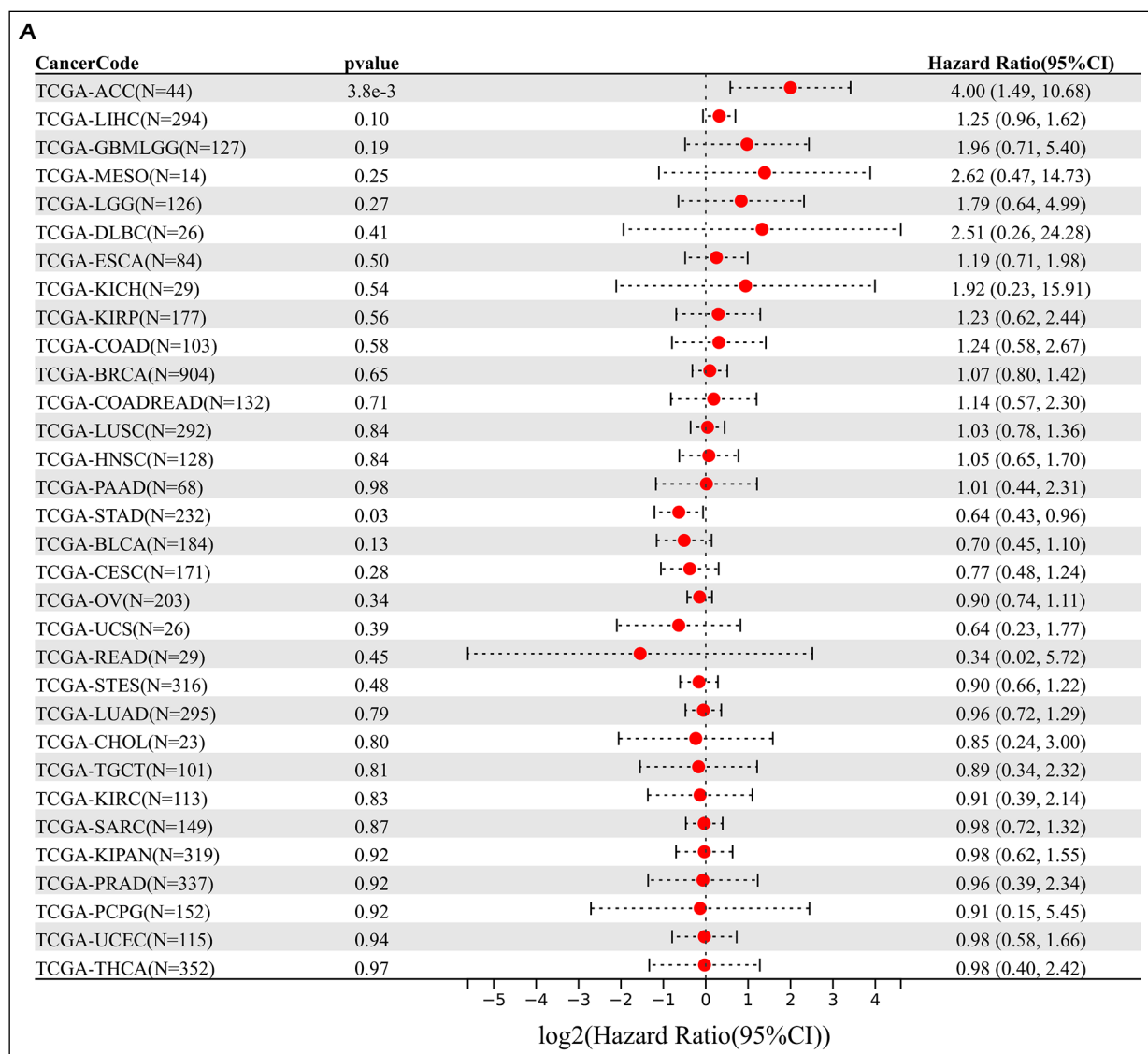
**Figure 3 (Continued).** Association between the *MKNK2* expression and DSS in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-E**, Kaplan-Meier survival curves of DSS for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, and PRAD.

for the development of therapies targeting refractory tumors (Figure 6).

**Tumor Mutation Burden and Microsatellite Instability Analyses**

Microsatellite instability (MSI), associated with defects in DNA mismatch repair, stands as a pivotal clinical marker in oncology. Both tumor mutational burden (TMB) and MSI have emerged as crucial factors that influence the genesis and progression of tumors. Their significance extends to the realm of immunotherapy, where they are recognized as burgeoning biomarkers that predict patient responses to immune checkpoint inhibitors.

Our investigation reveals a significant correlation between *MKNK2* expression levels and TMB across a wide array of tumor types. This association is evident in malignancies such as glioblastoma (GBM), glioblastoma multiforme low-grade gliomas (GBMLGG), cervical squamous cell carcinoma (CESC), colorectal adenocarcinoma with readthrough (COADREAD), colorectal adenocarcinoma (COAD), sarcoma (SARC), uterine corpus endometrial carcinoma (UCEC), stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), thymoma (THYM), kidney chromophobe (KICH), breast invasive carcinoma

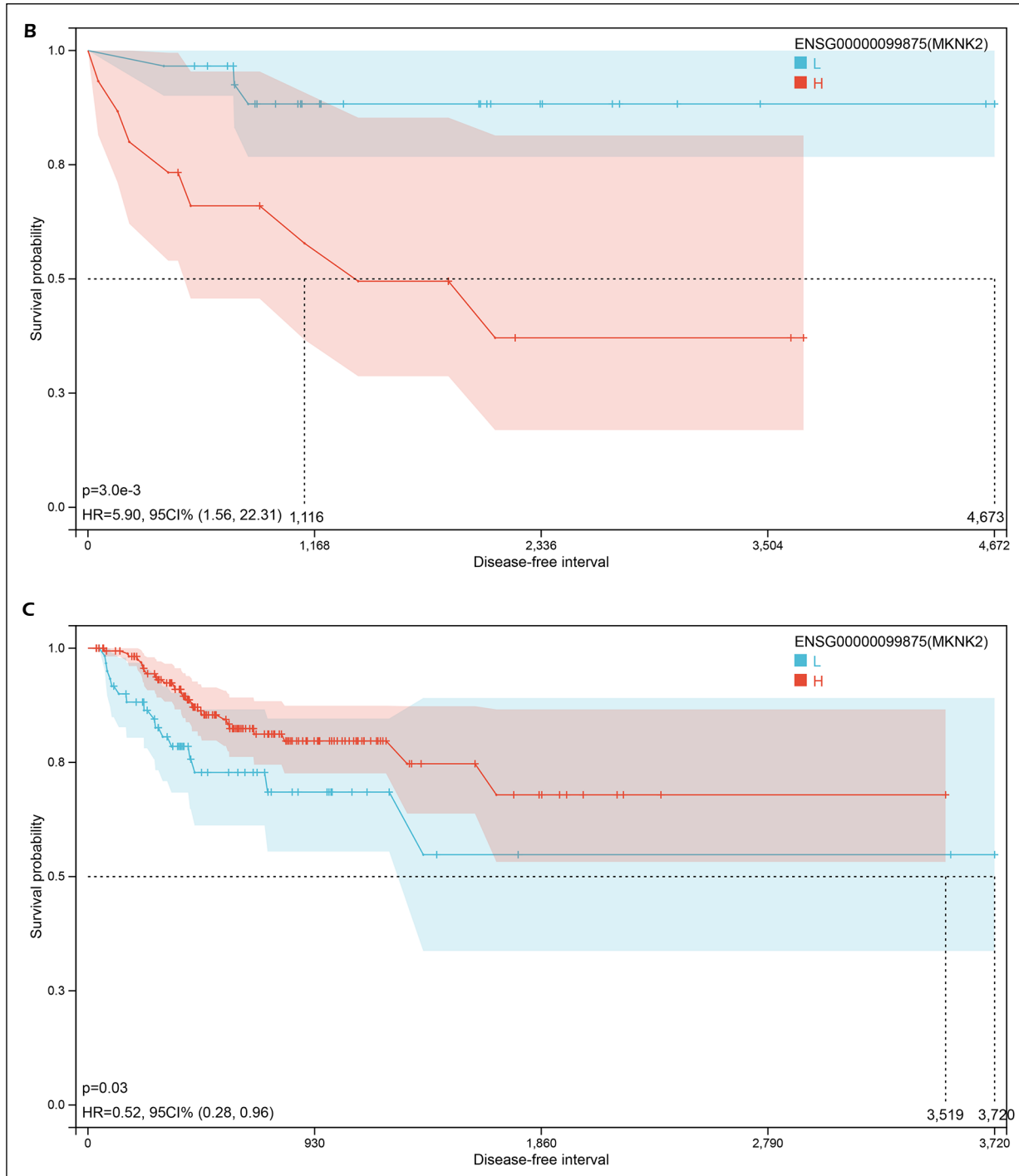


**Figure 4.** Association between the *MKNK2* expression and DFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-C**, Kaplan-Meier survival curves of DFI for patients stratified by the different expressions of *MKNK2* in ACC and STAD.

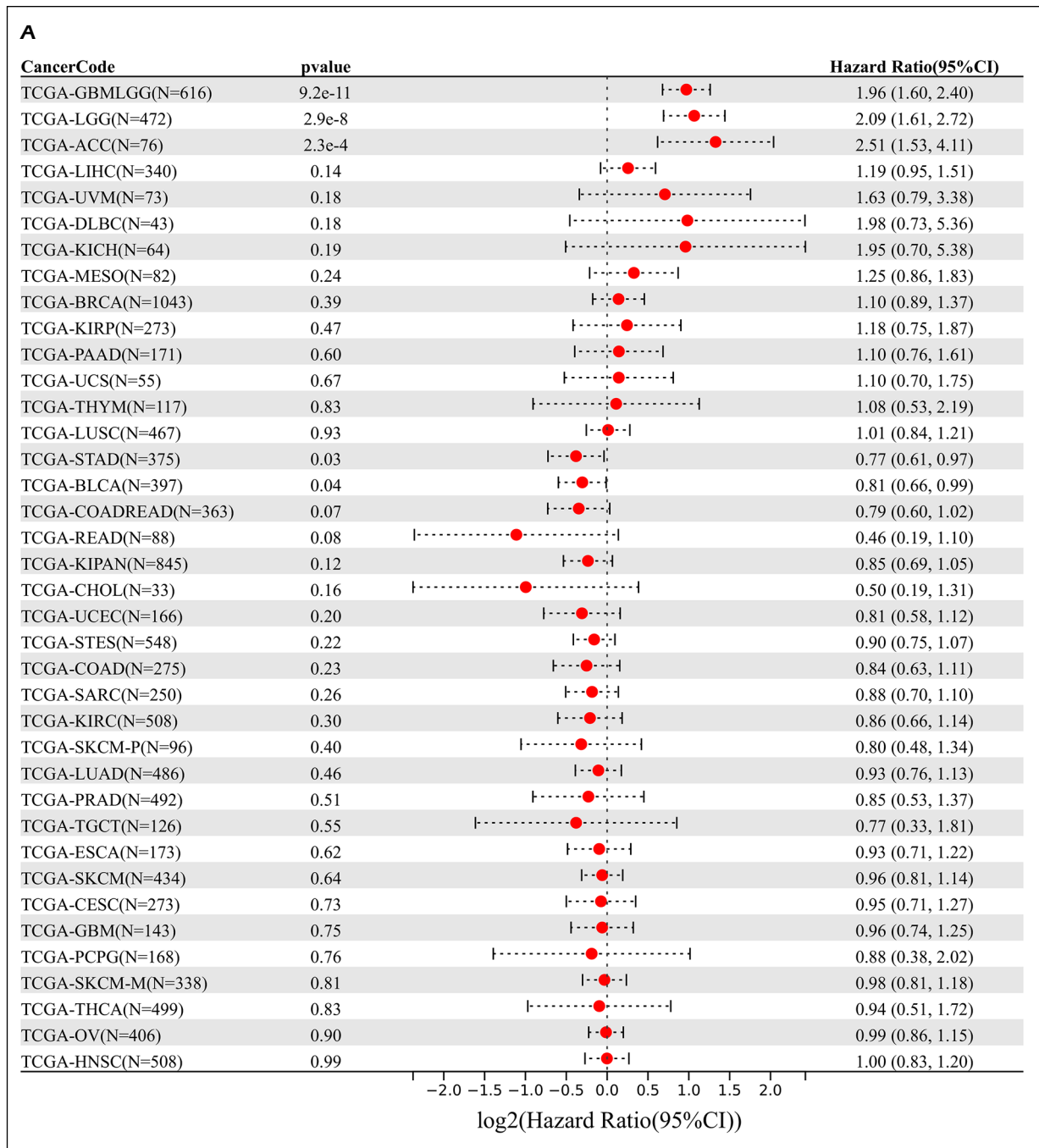
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(BRCA), brain lower grade glioma (LGG), and kidney renal papillary cell carcinoma (KIRP), as well as in kidney renal clear cell carcinoma (KIPAN) (Figure 7A).

Furthermore, an assessment of the relationship between *MKNK2* expression and MSI across 11 cancer types uncovered a markedly positive correlation in tumors such as lung adenocarcinoma



**Figure 4 (Continued).** Association between the *MKNK2* expression and DFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-C**, Kaplan-Meier survival curves of DFI for patients stratified by the different expressions of *MKNK2* in ACC and STAD.



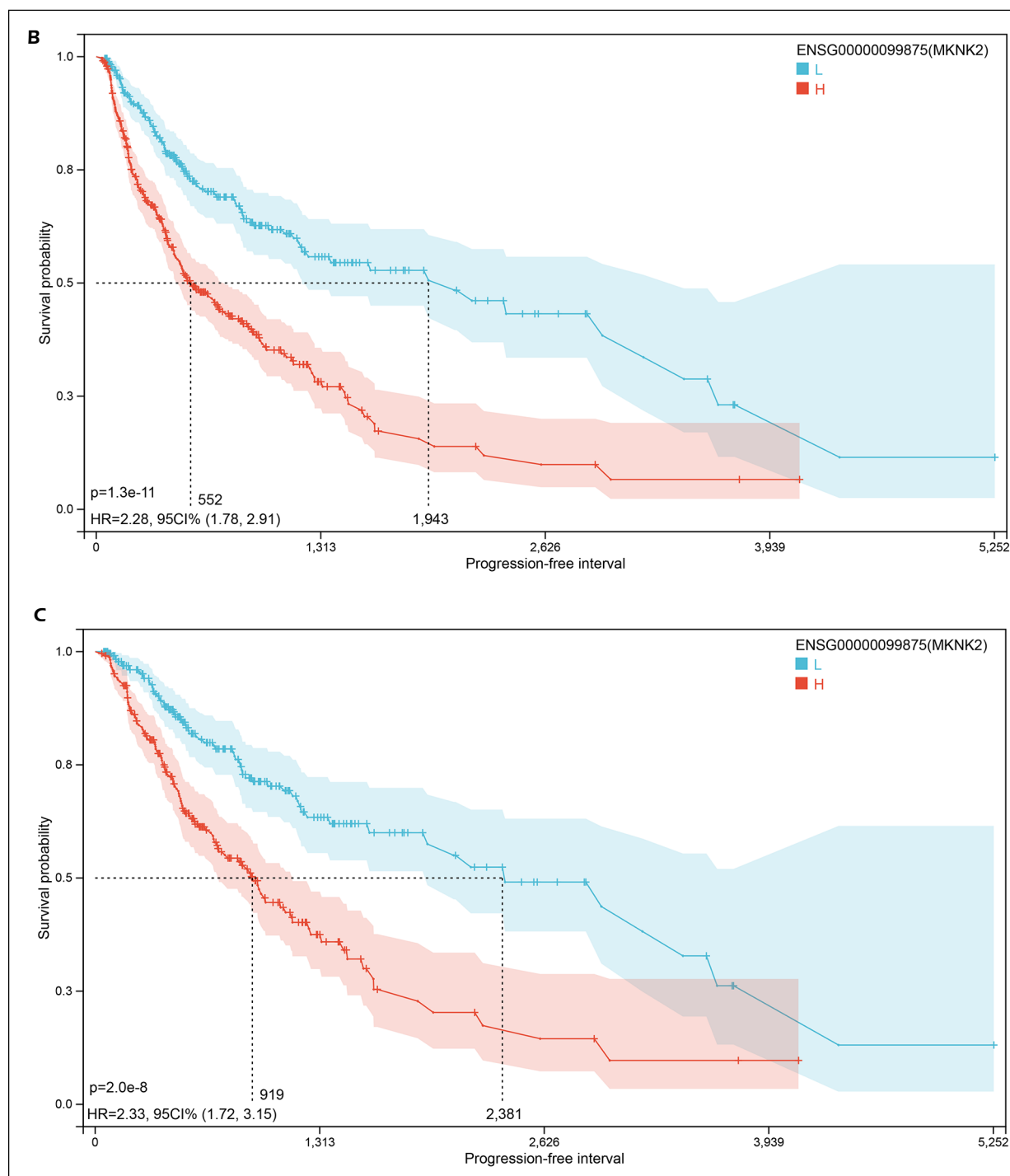
**Figure 5.** Association between the *MKNK2* expression and PFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-F**, Kaplan-Meier survival curves of PFI for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, STAD, and BLCA.

Figure continued

(LUAD), kidney renal papillary cell carcinoma (KIPAN), stomach adenocarcinoma (STAD), uterine corpus endometrial carcinoma (UCEC), lung squamous cell carcinoma (LUSC), liver he-

patocellular carcinoma (LIHC), testicular germ cell tumors (TGCT), and kidney chromophobe (KICH). In contrast, a negative correlation was observed in glioblastoma multiforme low-grade



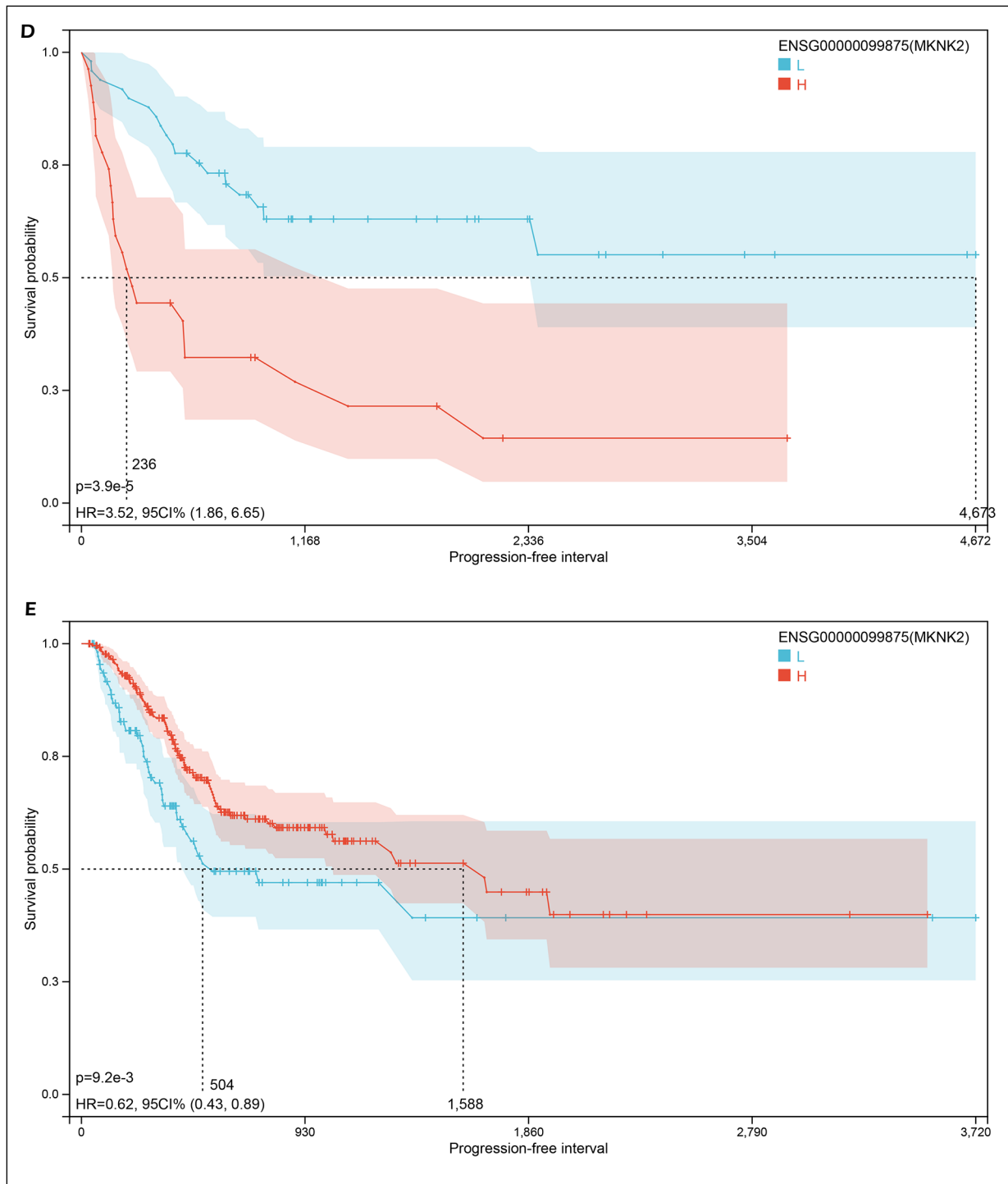


**Figure 5 (Continued).** Association between the *MKNK2* expression and PFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-F**, Kaplan-Meier survival curves of PFI for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, STAD, and BLCA.

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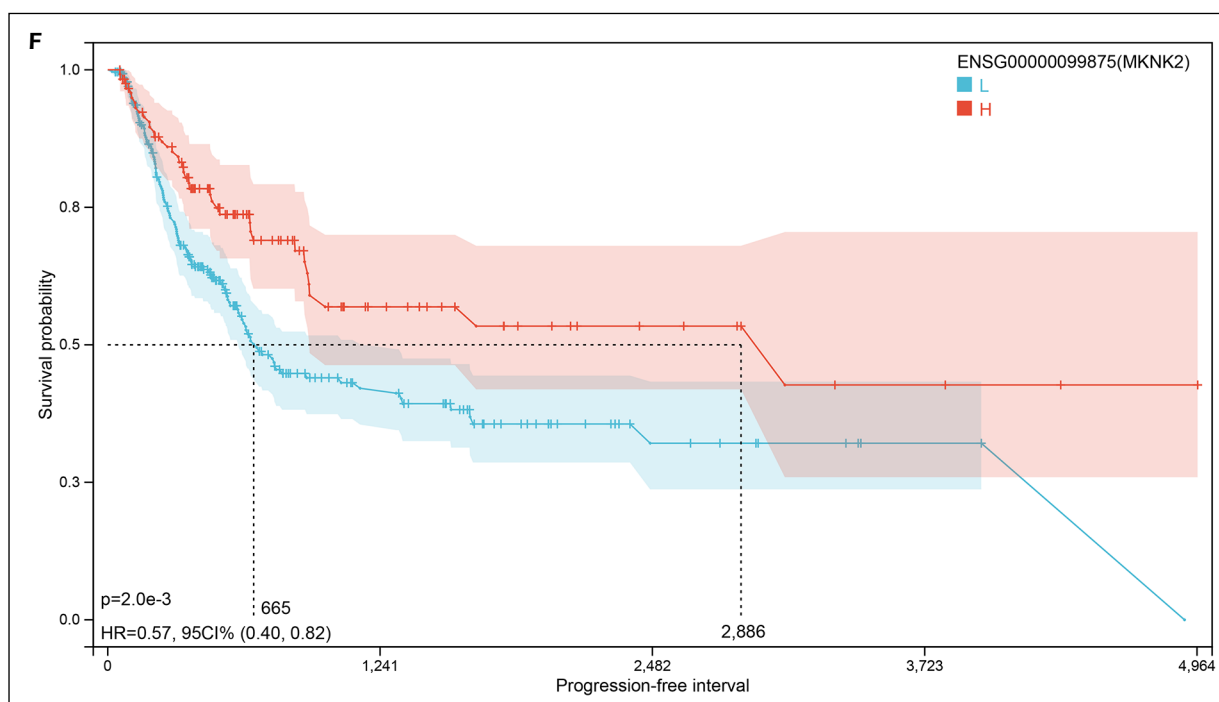
gliomas (GBMLGG), breast invasive carcinoma (BRCA), and diffuse large B-cell lymphoma (DLBC) (Figure 7B).

These findings not only highlight the intricate relationship between *MKNK2*, TMB, and MSI but also emphasize the potential of *MKNK2* as a bio-



**Figure 5 (Continued).** Association between the *MKNK2* expression and PFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-F**, Kaplan-Meier survival curves of PFI for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, STAD, and BLCA.

Figure continued



**Figure 5 (Continued).** Association between the *MKNK2* expression and PFI in cancer patients. **A**, A forest plot of hazard ratios of *MKNK2* in different cancer types from TCGA. **B-F**, Kaplan-Meier survival curves of PFI for patients stratified by the different expressions of *MKNK2* in GBMLGG, LGG, ACC, STAD, and BLCA.

marker that could inform therapeutic strategies, particularly in the context of immunotherapy.

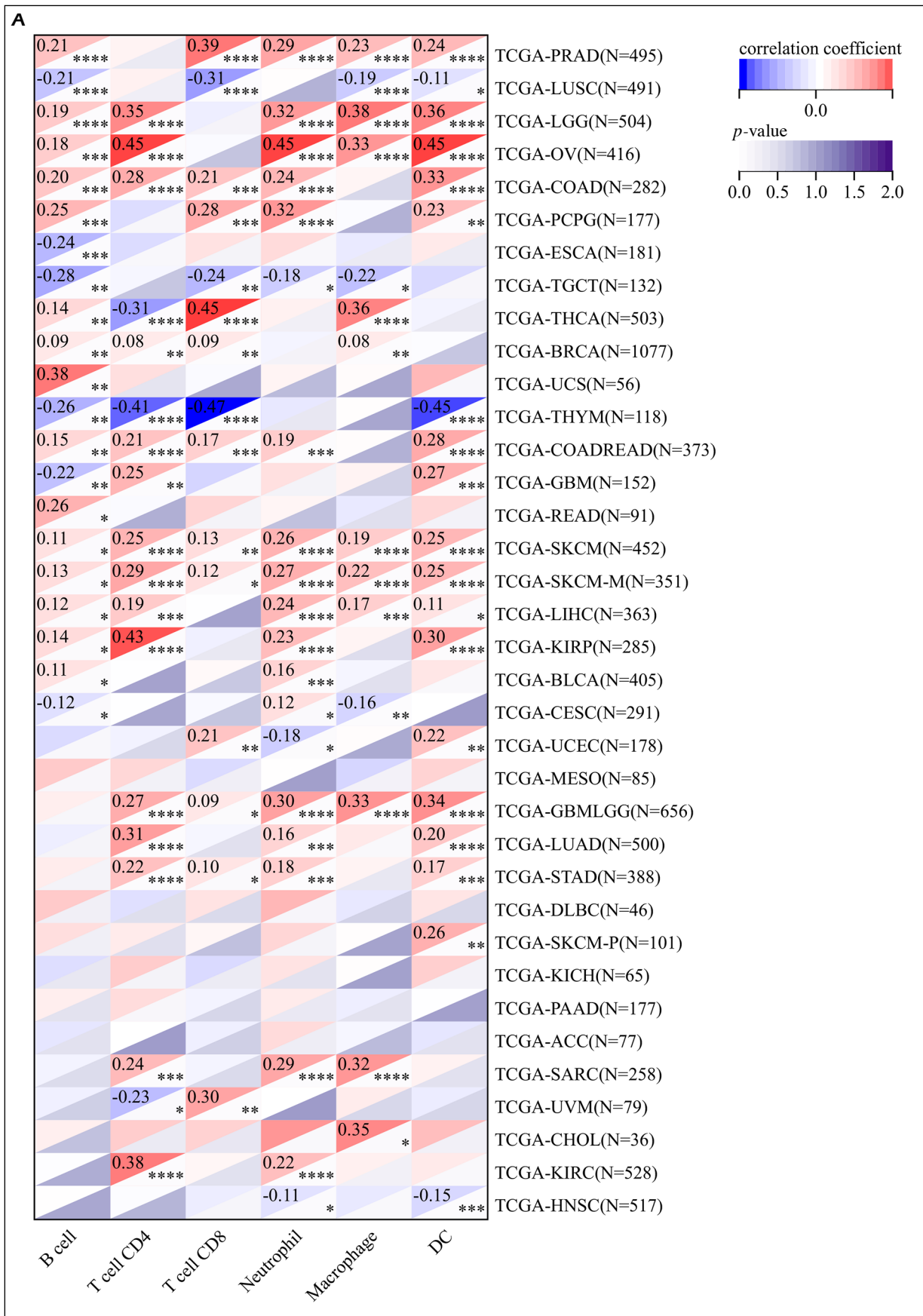
### **The DNA Methylation-Based and the RNA-Based Stemness Scores**

It is widely recognized that tumor stem cells play a pivotal role in the initiation of tumors and the establishment of the tumor microenvironment (TME). In our analysis, we calculated stemness scores and determined the Spearman correlation for each type of tumor, uncovering a significant association in 16 different malignancies. Notably, we identified a robust positive correlation in nine of these tumors, including glioblastoma multiforme low-grade gliomas (GBMLGG), lower-grade glioma (LGG), cervical squamous cell carcinoma (CESC), kidney renal papillary cell carcinoma (KIRP), kidney pancreatic cancer (KIPAN), thymoma (THYM), penile cancer (PCPG), uveal melanoma (UVM), and cholangiocarcinoma (CHOL). Conversely, a significant negative correlation was observed in seven tumors, comprising lung adenocarcinoma (LUAD), colorectal adenocarcinoma (COAD), colorectal adenocarcinoma with readthrough (COADREAD), breast invasive carcinoma (BRCA), thymic carcinoma (THCA), rectal ad-

enocarcinoma (READ), and testicular germ cell tumors (TGCT) (Figure 7C).

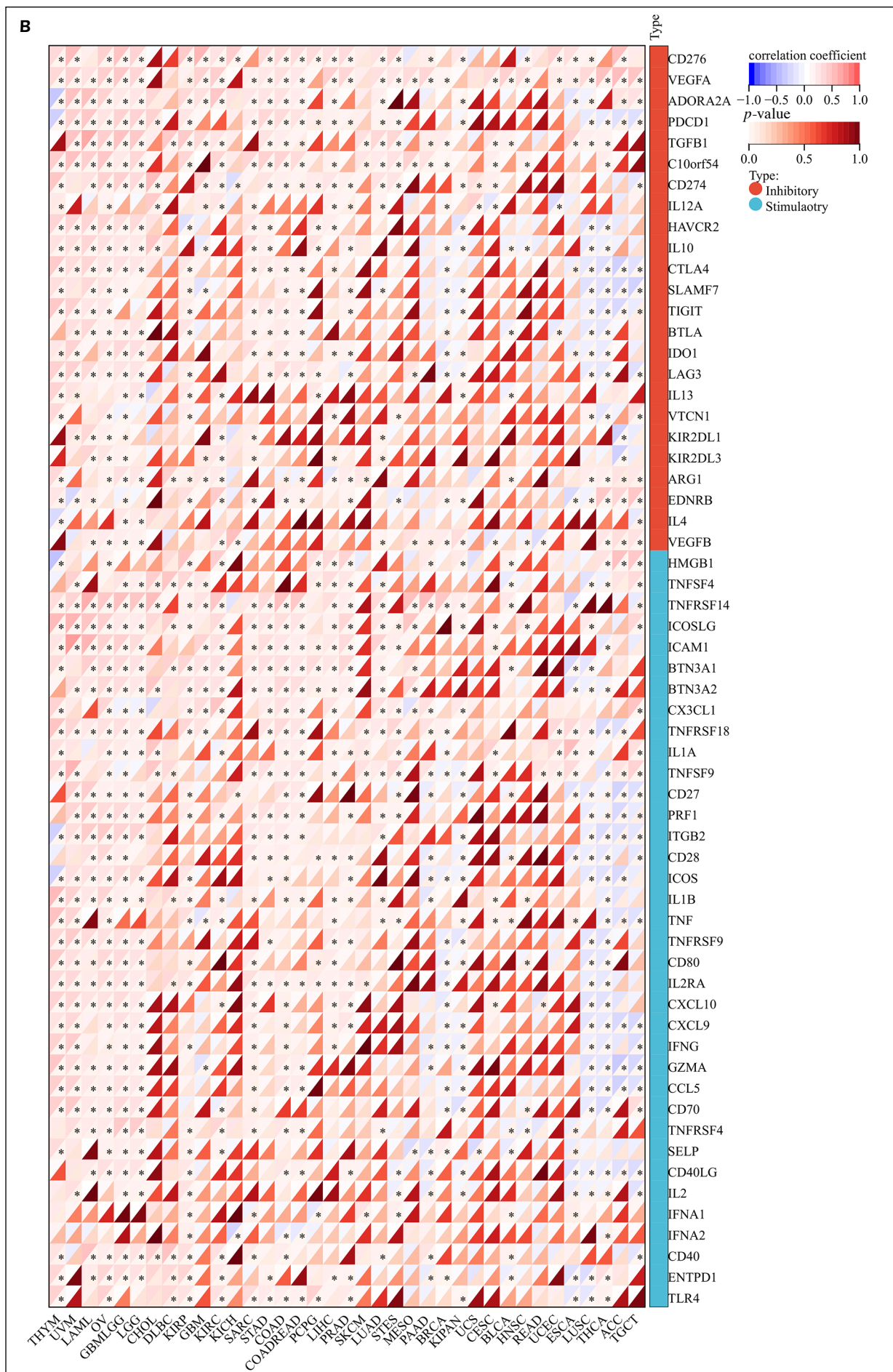
The *MNK* signaling pathway has been identified as essential for the preservation of stem cell populations across various cancers (Figure 7C-D). Specifically, in blast crisis chronic myeloid leukemia, the *MNK-eIF4E* signaling cascade is known to sustain the leukemic precursors<sup>19</sup>. Additionally, *MKNK1* has been reported to positively regulate the levels of *Sema3C* and growth-supporting factors such as *TGF- $\beta$* , which are implicated in the maintenance and progression of glioma stem cells<sup>20,21</sup>. The *TGF- $\beta$*  signaling pathway is also recognized for its role in promoting the growth, invasion, and immune evasion of mesenchymal stem cells within tumors.

Given these insights, further research is both necessary and promising, potentially leading to significant clinical applications in tumor therapy. To gain a comprehensive understanding of the molecular processes regulated by *MKNK2* in different types of cancer, we conducted Gene Set Enrichment Analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The collective results from these analyses suggest that elevated *MKNK2* expression is predominantly associated with immune response and stemness in cancer, offering valuable clues for the



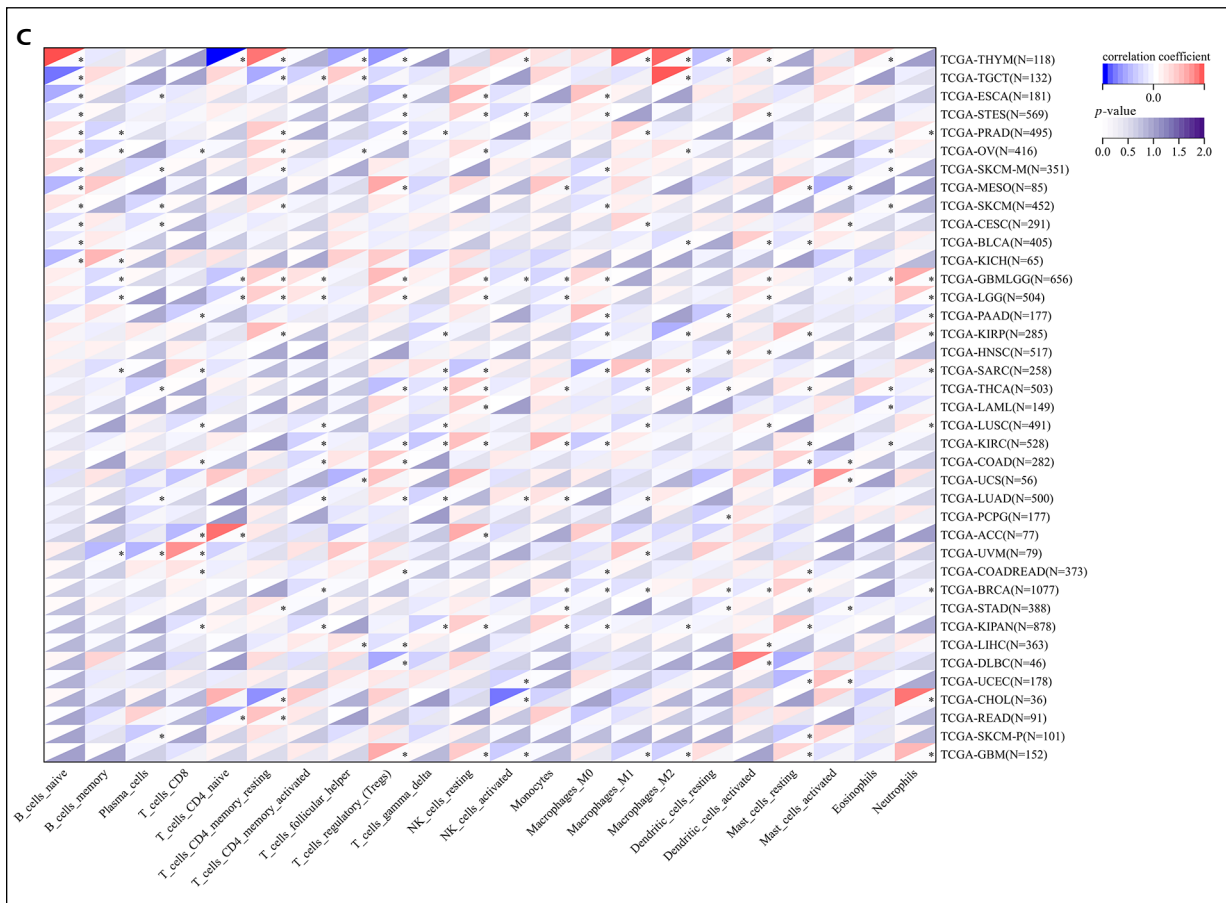
**Figure 6.** Different algorithms were used to explore the potential correlation between the expression level of the *MKNK2* gene and the level of immune cell infiltration in all types of cancer in TCGA. **A**, The *MKNK2* expression significantly correlated with the infiltration levels of various immune cells in the TIMER database. **B**, Correlation between *MKNK2* expression and immune checkpoint. **C**, CIBERSORT predicts that *MKNK2* expression is correlated with immunocytes. **D**, EPIC predicts that *MKNK2* expression is correlated with immunocytes. \*Indicates statistical significance, where \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Figure continued



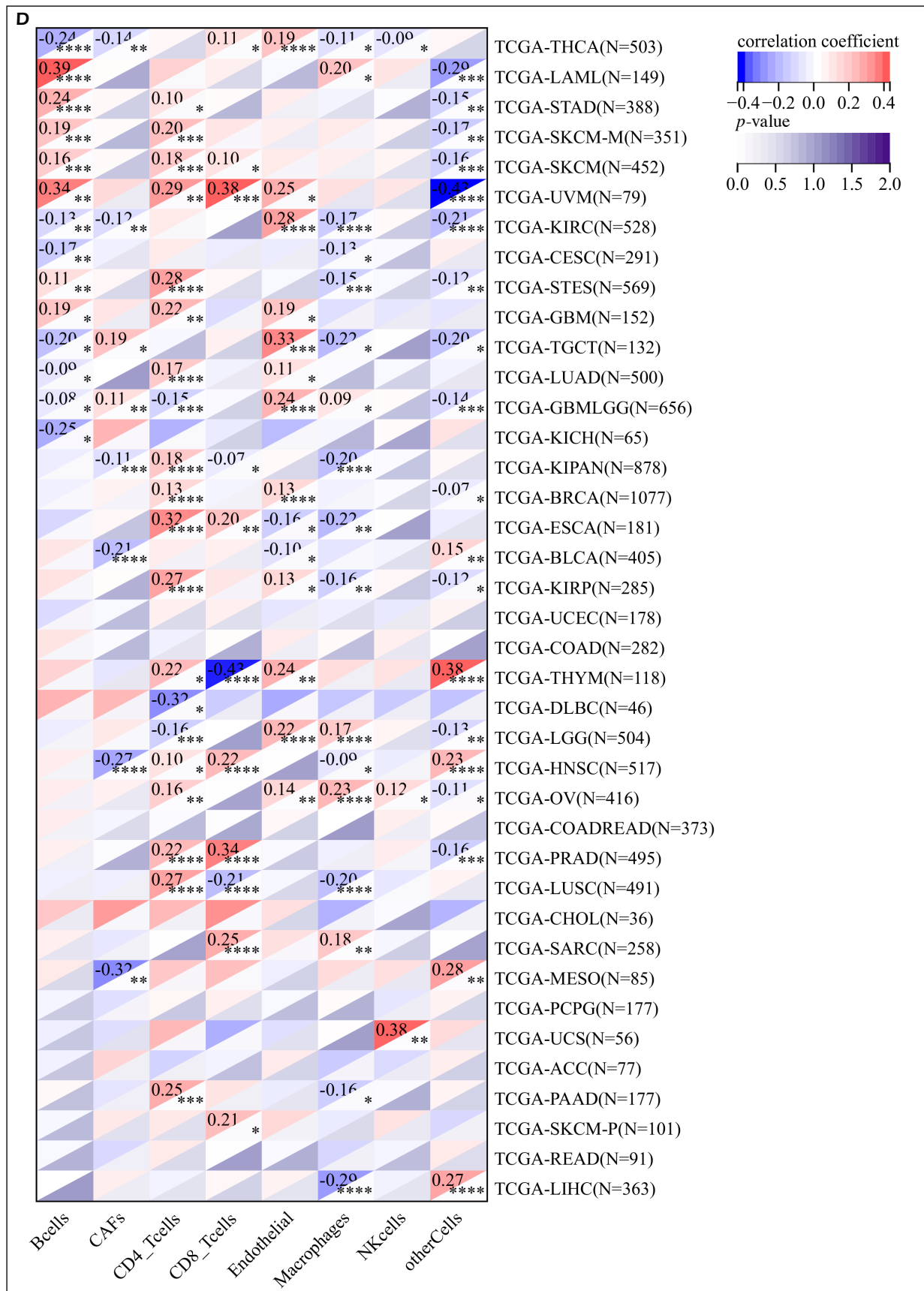
**Figure 6 (Continued).** Different algorithms were used to explore the potential correlation between the expression level of the *MKNK2* gene and the level of immune cell infiltration in all types of cancer in TCGA. **A**, The *MKNK2* expression significantly correlated with the infiltration levels of various immune cells in the TIMER database. **B**, Correlation between *MKNK2* expression and immune checkpoint. **C**, CIBERSORT predicts that *MKNK2* expression is correlated with immunocytes. **D**, EPIC predicts that *MKNK2* expression is correlated with immunocytes. \*Indicates statistical significance, where \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Figure continued

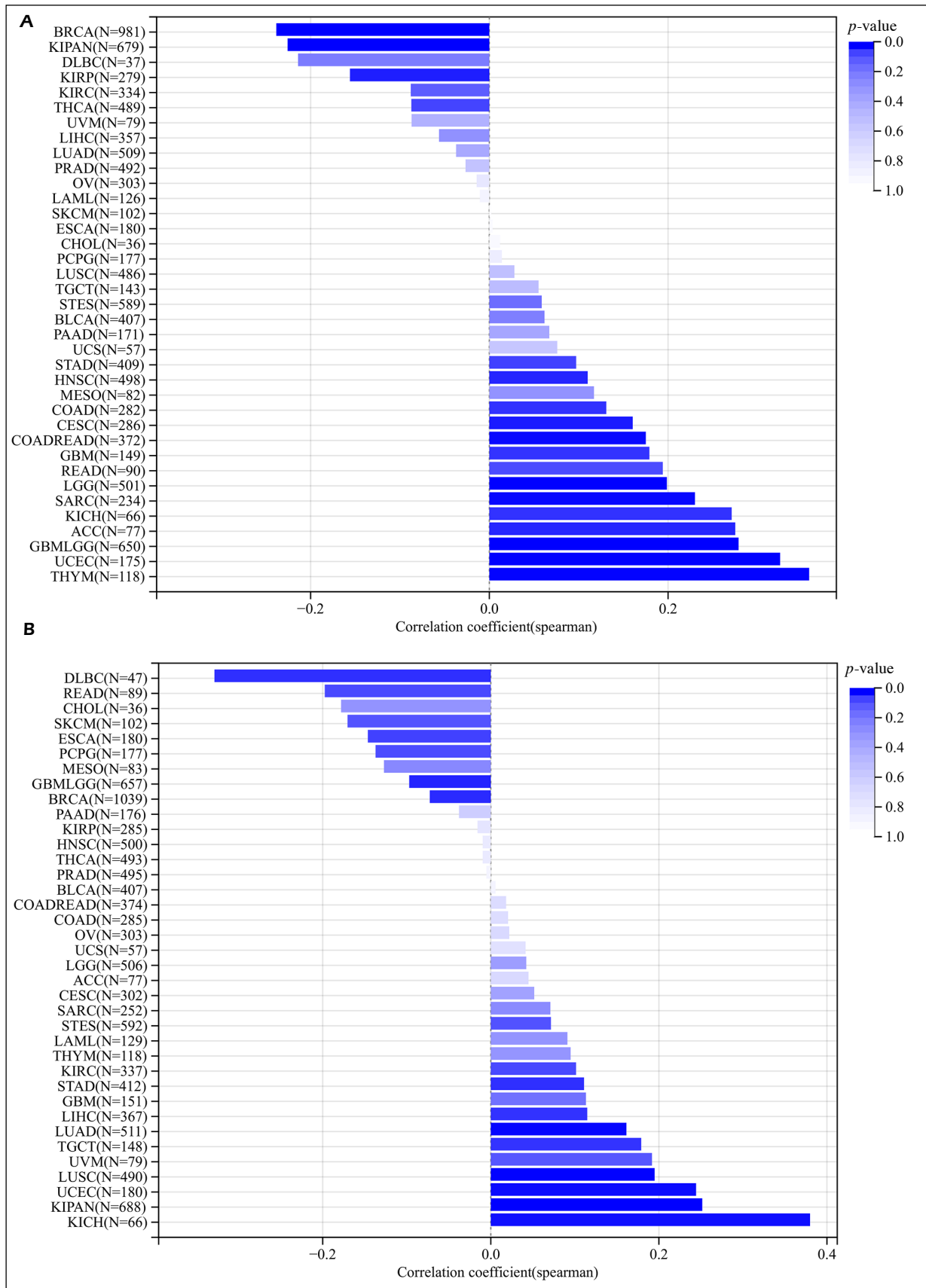


**Figure 6 (Continued).** Different algorithms were used to explore the potential correlation between the expression level of the *MKNK2* gene and the level of immune cell infiltration in all types of cancer in TCGA. **A**, The *MKNK2* expression significantly correlated with the infiltration levels of various immune cells in the TIMER database. **B**, Correlation between *MKNK2* expression and immune checkpoint. **C**, CIBERSORT predicts that *MKNK2* expression is correlated with immunocytes. **D**, EPIC predicts that *MKNK2* expression is correlated with immunocytes. \*Indicates statistical significance, where  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ .

Figure continued



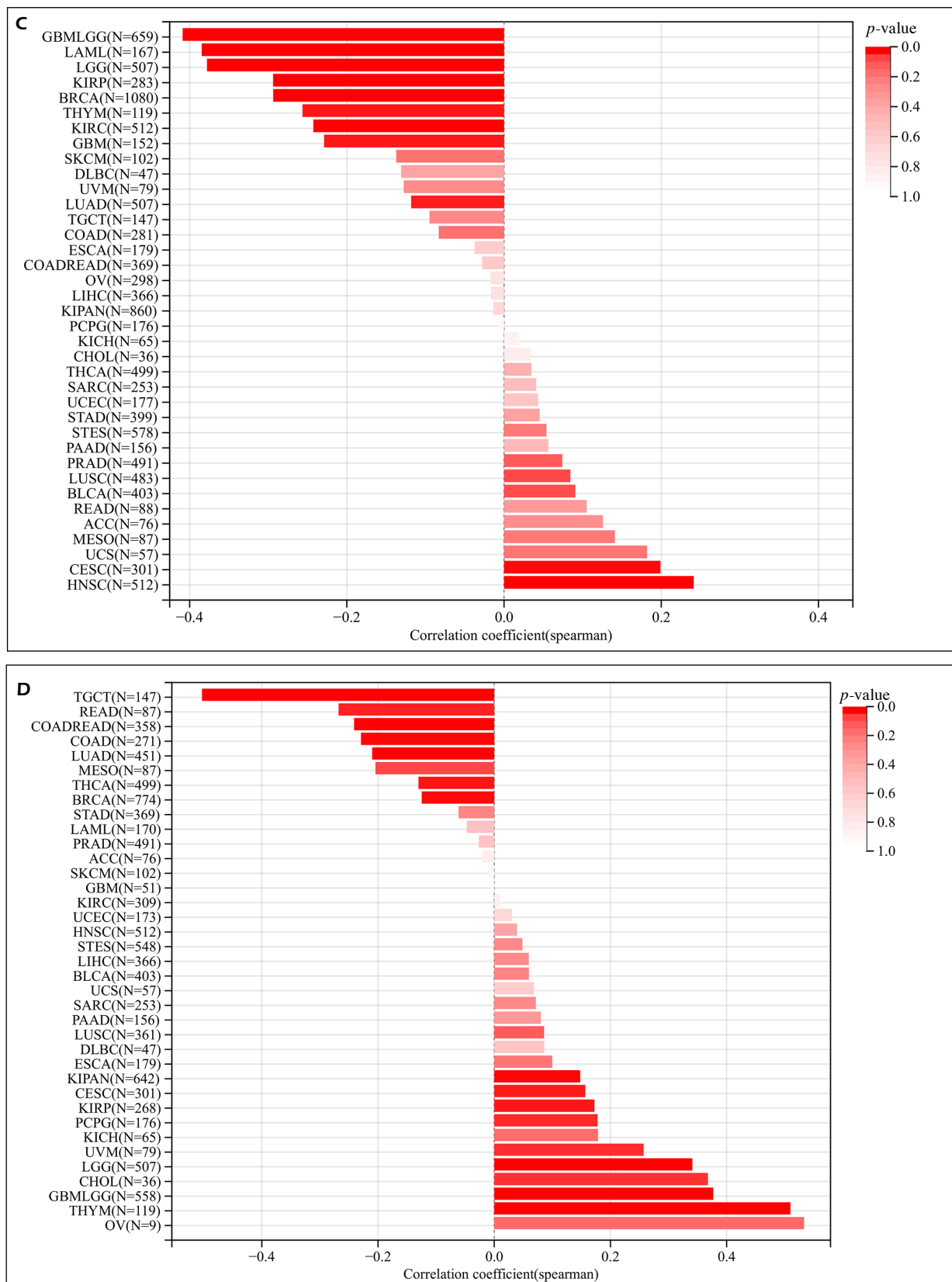
**Figure 6 (Continued).** Different algorithms were used to explore the potential correlation between the expression level of the *MKNK2* gene and the level of immune cell infiltration in all types of cancer in TCGA. **A**, The *MKNK2* expression significantly correlated with the infiltration levels of various immune cells in the TIMER database. **B**, Correlation between *MKNK2* expression and immune checkpoint. **C**, CIBERSORT predicts that *MKNK2* expression is correlated with immunocytes. **D**, EPIC predicts that *MKNK2* expression is correlated with immunocytes. \*Indicates statistical significance, where  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ .



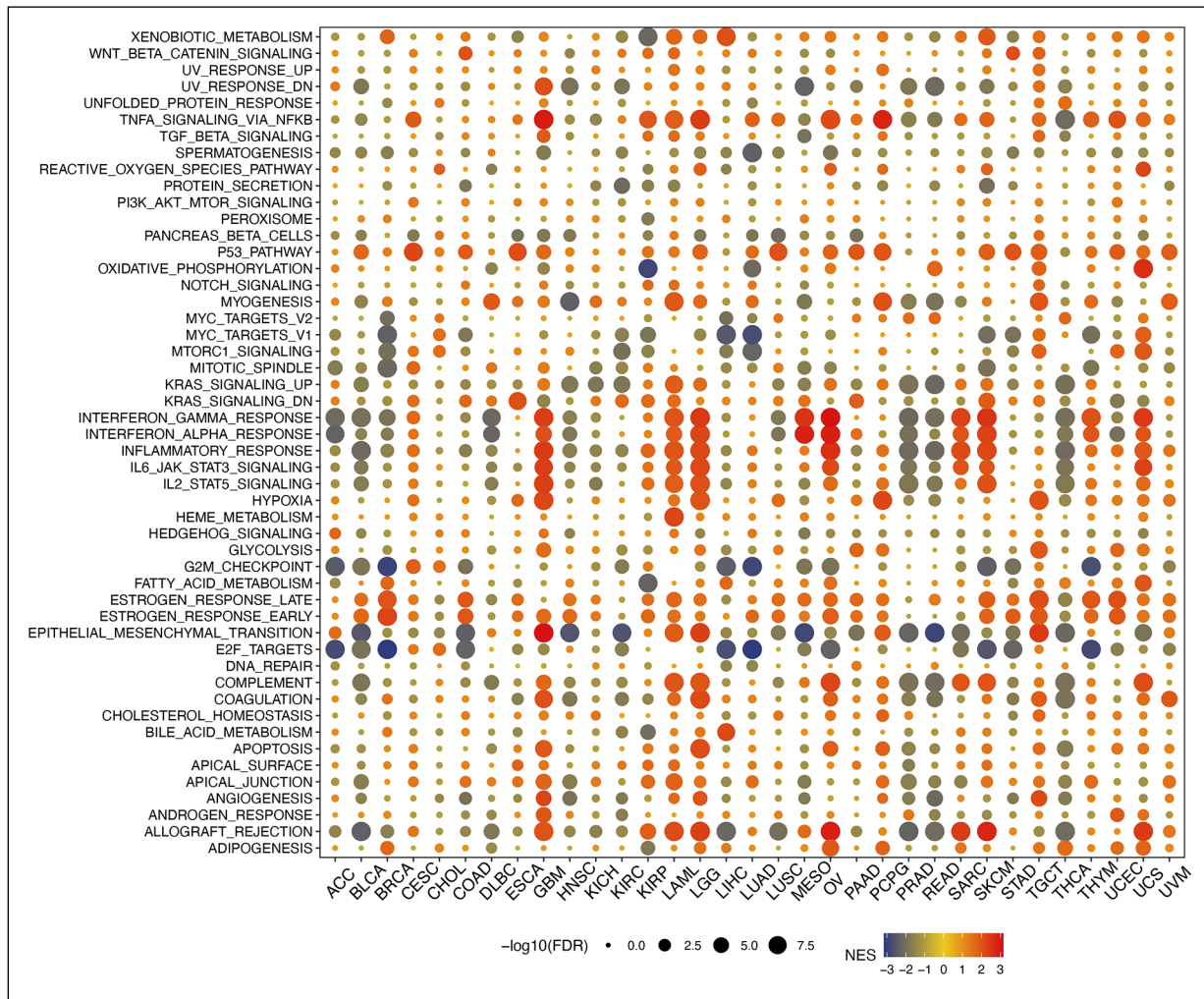
**Figure 7.** Relationship between MKNK2 expression and tumor mutation burden (A), microsatellite Instability (B), and Stemness Scores DNAss (C), RNAss (D) in pan-cancer.

Figure continued





**Figure 7 (Continued).** Relationship between *MKNK2* expression and tumor mutation burden (A), microsatellite Instability (B), and Stemness Scores DNAss (C), RNAss (D) in pan-cancer.



**Figure 8.** The hallmarks gene set enrichment analysis of *MKNK2* in pan-cancer. The size of the circle represents the FDR value of the enriched term in each cancer, and the color indicates the normalized enrichment score (NES) of each term.

development of targeted therapies that leverage the immunological and stemness-related roles of *MKNK2*.

### GSEA of *MKNK2* in Pan-Cancer

To delve deeper into the molecular underpinnings of *MKNK2*'s role in cancer, we conducted Gene Set Enrichment Analysis (GSEA) utilizing the differentially expressed genes (DEGs) between subgroups characterized by low and high *MKNK2* expression within each cancer type. This approach was instrumental in identifying cancer hallmarks associated with *MKNK2*. Our analysis revealed a striking correlation between *MKNK2* expression and a spectrum of immune-related pathways. Notably, these included *TNF-α* signaling via NF-κB, IFN-α response, IFN-γ response, inflammatory

response, *IL6-JAK-STAT3* signaling, *IL-2-STAT5* signaling, and allograft-rejection pathways, with particularly strong associations observed in cancers such as ESCA, KIRP, LAML, MESO, SARC, and UCEC (Figure 8). The pathways *TNF-α* signaling via NF-κB and JAK-STAT3 signaling, which are implicated in our findings, have been well-documented in the literature for their critical role in sustaining the stemness of tumor stem cells<sup>22</sup>.

Moreover, our study also identified a tight association between *MKNK2* expression and key cancer-related processes such as epithelial-mesenchymal transition (EMT) and the *p53* pathway. EMT is recognized for its contribution to cancer metastasis, while the *p53* pathway is a key regulator of cellular responses to DNA damage and a well-known tumor suppressor.

In conclusion, the collective results of our analyses suggest that elevated *MKNK2* expression is intricately linked to the immune-activation status of various cancers. These findings not only shed light on the multifaceted roles of *MKNK2* in the immune landscape of cancers but also provide a foundation for further research into the functions and roles of *MKNK2* in the initiation and progression of cancer. Understanding these mechanisms could pave the way for novel therapeutic strategies that leverage the immunomodulatory effects of *MKNK2*.

## Discussion

Multiple investigations prove the association of *MKNK2* with tumor progression, prognosis, and treatment response<sup>4,23</sup>. The *MKNK2* kinase, which is encoded by the gene *MKNK2*, is important for *MAPK* signaling and is linked with oncogenesis<sup>20,24</sup>. Many well-known kinase networks, such as *EGFR*, *MAPKs*, and *c-Src* also take part in tumor pathogenesis<sup>25-28</sup>. The MAP kinase-interacting serine/threonine-protein kinases (*MNKS*) is a downstream protein of *MAPKs* that can phosphorylate *eIF4E* and increase oncogenic mRNA translation<sup>23,29</sup>. The literature suggests that *MKNK2* not only modulates some physiological processes but also regulates tumor growth and immune infiltration (Figure 6), but studies reporting its importance in the cancer field are still missing<sup>27</sup>. Recently, a relationship between *MKNK2* expression and tumor cell progression (mainly in GBM and Gastric Cancer) was acknowledged<sup>5,27</sup>. Therefore, it can be speculated that *MKNK2* expression may affect a patient's survival *via* tumor cell progression (Figures 2 and 3). Consistent with previous research, this study also noted that *MKNK2* has diverse activity in pan-cancers<sup>28-30</sup>. The differential *MKNK2* expression in pan-cancers and their matched para-cancer normal tissues was evaluated. Results may differ because of the variability in data sources, collection approach, and the number of cancers in the investigation cohort. Nonetheless, the three databases revealed consistently poor prognostic value with *MKNK2* expression in GBMLGG, ACC, and STAD (Figures 2 and 3).

In association with *MKNK2*'s influence on tumor immunosuppressive microenvironment and antitumor immune response *via* the *MNK1/2-eIF4E* axis, the systematic analysis further correlated *MKNK2* and tumor immunity<sup>31</sup>. The relationship between *MKNK2* expression and tumor immune

cell infiltration levels is mostly positively associated. Based on TIMER analysis, it was found that *MKNK2* levels were substantially correlated with the degree of CD4+ T, CD8+ T, B, neutrophils, macrophages, and dendritic cell infiltration (Figure 6A). Tumor-associated macrophages (TAMs) are the main part of the immune microenvironment of many tumors, which is closely linked with tumor growth, invasion, and drug resistance<sup>32</sup>. The available research results show that *MKNK2* can govern the macrophage anti-inflammatory phenotype<sup>16</sup>. Analysis results of immune cell infiltration show that *MKNK2* is significantly associated with macrophages in many tumors, suggesting that it induces metabolic reprogramming and regulates the function of TAMs. Although the high *MKNK2* expression is associated with a substandard prognosis of ACC, the *MKNK2* expression correlation with immune cell infiltration in ACC is very low, suggesting that *MKNK2* may affect the prognosis of ACC through non-immune pathways. *MKNK2* expression was also widely associated with immune checkpoints. *MNK1/2-eIF4E* axis is considered a potential therapeutic index in melanoma<sup>33</sup>. Dysregulated mRNA translation is crucial for tumorigenesis. *MAPK* interacting kinases (*MNK1/2*) are key mRNA translation regulators, integrating oncogenic and immune pathways signals *via* phosphorylation of *eIF4E* and other mRNA binding proteins<sup>34</sup>. Although the expression of *MKNK2* is linked with immune infiltration and patient survival, it has not been demonstrated that *MKNK2* affects patient's survival through immune infiltration.

The correlation of *MKNK2* with TMB, MSI, and Stemness in various cancer types was also investigated in our study (Figure 7). It was reported that inhibition of *MNKs* with small molecule inhibitors or knockdown of *MKNK1* and *MKNK2* disrupts the growth of tumor cells and prevents tumor growth *in vivo*<sup>35</sup>. TMB levels affect immunogenic peptide generation, thus affecting the patient's response to immune checkpoint inhibitors<sup>36</sup>. MSI is a vital index for predicting tumorigenesis and development<sup>37</sup>. We found that *MKNK2* expression is highly related to TMB, MSI, and Stemness in most cancer types. GBMLGG, KIPAN, STAD, UCEC, BRCA, and other cancer species showed a significant correlation in TMB and MSI scores (Figure 7A-B).

The GSEA result suggests that *PDIA3* is closely associated with immune-activated processes, such as *TNF- $\alpha$*  signaling *via* NF- $\kappa$ B, IFN- $\alpha$  response, IFN- $\gamma$  response, inflammatory-response,

*IL6-JAK-STAT3* signaling, *IL-2-STAT5* signaling, and allograft-rejection pathways, but completely opposite results were observed in distinct cancer types. For example, these processes were mostly significantly enriched in high-*MKNK2* cancer subgroups, but reversed results were found in ACC, BLCA, PCPG, PRAD, and TGCT (Figure 8). This indicated that *PDIA3* might play different roles in distinct cancer types.

Recent studies demonstrated that pharmacological inhibition of *MNKs* could block *eIF4E*-mediated translation and thus suppress tumor cell proliferation and slow down tumor growth *in vivo*<sup>27,31</sup>. *eIF4E*-mediated mRNA translation plays an important role in oncogenic transformation, and an elevated level of *eIF4E* expression/activity has been observed in many tumors<sup>17</sup>. *MNK* activity also plays an important role in regulating the innate and adaptive immune systems<sup>38,39</sup>. Interference with the reprogramming of immune cells in the immunosuppressive microenvironment and the stemness maintenance of tumor stem cells could be an underlying regulatory mechanism associated with *MKNK2* and bad prognosis<sup>16,27</sup>.

The present study unveiled a complicated role of *MKNK2* aberrant expression in cancer progression and patient outcome that warrants further investigation. Since our study was based on bioinformatics and relies on public databases, there are major limitations. First, the quality of data collection and the method used to generate the data could be inconsistent depending on the sources. This could affect the results of different analyses. Secondly, the results and the conclusions are not experimentally or prospectively confirmed in the laboratory or clinic. A future investigation is required to validate *MKNK2* expression and function *in vivo* and *in vitro*.

Altogether, this pan-cancer analysis indicated the clinical importance of *MKNK2* in prognosis, stemness, immune cell infiltration, and immunity indices such as TMB and MSI in understanding its activity in tumorigenesis.

## Conclusions

This investigation comprehensively evaluated the prognostic significance and immunological aspects of *MKNK2* in pan-cancer. *MKNK2* is differentially expressed in many tumors, and its abnormal expression is linked with tumor progression, immune checkpoint genes, immune cell infiltration, MSI, TMB, and stemness, especially

in GBMLGG. Therefore, it may be a potent prognostic physiological marker.

This article only describes the correlation between *MKNK2* expression and tumor immunity from the perspective of bioinformatic analysis. Future prospective and experimental studies on *MKNK2* expression and immune cell infiltration in different cancer populations may provide more ideas and channels for the development of tumor mechanisms and therapeutic strategies targeting *MKNK2* to improve the therapeutic effect of immunotherapy.

## Conflicts of Interest

All authors declare no conflict of interest.

## Ethics Approval and Informed Consent

The data used in this article belongs to public databases. The patients involved in the databases have received ethical approval with informed consent, which could be downloaded for free to conduct research and publish relevant articles. This study is based on open resource data, so there are no ethical issues or other conflicts of interest.

## Data Availability

The data that support the findings of this study are openly available in <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga> and <https://portal.gdc.cancer.gov/>. Stemness indices across all tumor samples/types can be accessed at [https://bioinformaticsfmrp.github.io/Pan-CanStem\\_Web](https://bioinformaticsfmrp.github.io/Pan-CanStem_Web). TCGA, GTEx, and CCLE belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles.

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## Authors' Contributions

Yiming Zhang: writing original draft editing, original draft, methodology, validation, and visualization. Jikang Fan: original draft, methodology, validation. Xuya Wang: original draft, methodology, validation. Jie Liu: methodology, validation, and visualization. Xisen Wang and Tao Li: methodology and visualization. Xuejun Yang: original draft and supervision.

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### AI Disclosure

In conducting this study, we have employed artificial intelligence tools for data analysis and visualization where appropriate. The use of AI has been confined to tasks that enhance the efficiency and accuracy of our research without replacing the intellectual input and decision-making processes of the research team. The authors thoroughly reviewed and validated all AI-generated outputs to ensure the integrity and reliability of the study's findings.

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