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Research article

TRIM36 serves as a prognostic indicator linked to immune infiltration in KIRC

Jikai Zhang [a,c,2], Botao Zou [a,2], Yunfeng Geng [a,2], Hang Yin [a], Baoying Qin [a], Wanjun Gao [a], Xiaoman Lin [d,**], Nan Sun [a,b,*,1]

[a] Xuzhou Medical University, Xuzhou, China
[b] Jiangsu Center for the Collaboration and Innovation of Cancer Biotherapy, Cancer Institute, Xuzhou Medical University, Xuzhou, China
[c] Department of Pathogen Biology and Immunology, Jiangsu Key Laboratory of Immunity and Metabolism, Xuzhou Medical University, Xuzhou, China
[d] Department of Obstetrics, Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

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ABSTRACT

Renal cell carcinoma (RCC) represents approximately 85 % of all renal malignant tumors, with kidney renal clear cell carcinoma (KIRC) being the most typical subtype. The tripartite motif (TRIM) family is involved in cancer initiation, progression, and therapy resistance. While TRIM36 has exhibited anti-tumor effects in various cancers, its relationship with KIRC remains unclear. In our research, we studied the relationship between TRIM36 and KIRC. Through a combination of bioinformatic analyses and validation experiments, we noted a rise in TRIM36 expression in KIRC, and the upregulation of TRIM36 expression is associated with a poorer prognosis in KIRC. Also, our findings from wound healing assays and transwell migration assays showed that TRIM36 promotes the proliferation and migration of KIRC cells. To understand the underlying mechanisms, we screened relevant genes and conducted enrichment analysis. We identified that TRIM36 may interact with 5 hub genes and involve in the cell cycle and cell division processes in KIRC. Additionally, through immune infiltration analysis, we found that TRIM36 may interact with 6 tumor-infiltrating lymphocytes (TILs) and 6 immune inhibitors. In summary, our research identifies TRIM36 as a promising biomarker and comprehensively explores its promoting effect on the proliferation of KIRC.

1. Introduction

Worldwide, renal cell carcinoma (RCC) contributes to 5 % and 3 % of all oncological diagnoses in men and women, placing it as the sixth most commonly diagnosed cancer in men and the tenth in women [1]. The predominant subtype, kidney renal clear cell carcinoma (KIRC), comprises nearly 80 % of RCC cases [2]. However, due to challenges in early detection and treatment, KIRC is linked to a poor prognosis. Additionally, KIRC exhibits high resistance to chemotherapy and limited response to immunotherapy [3]. Early

* Corresponding author. Xuzhou Medical University, Xuzhou, China.
** Corresponding author.
E-mail addresses: lxm19720@126.com (X. Lin), sunnan312@xzhmu.edu.cn (N. Sun).
[1] Lead contact.
[2] These authors contribute equally.

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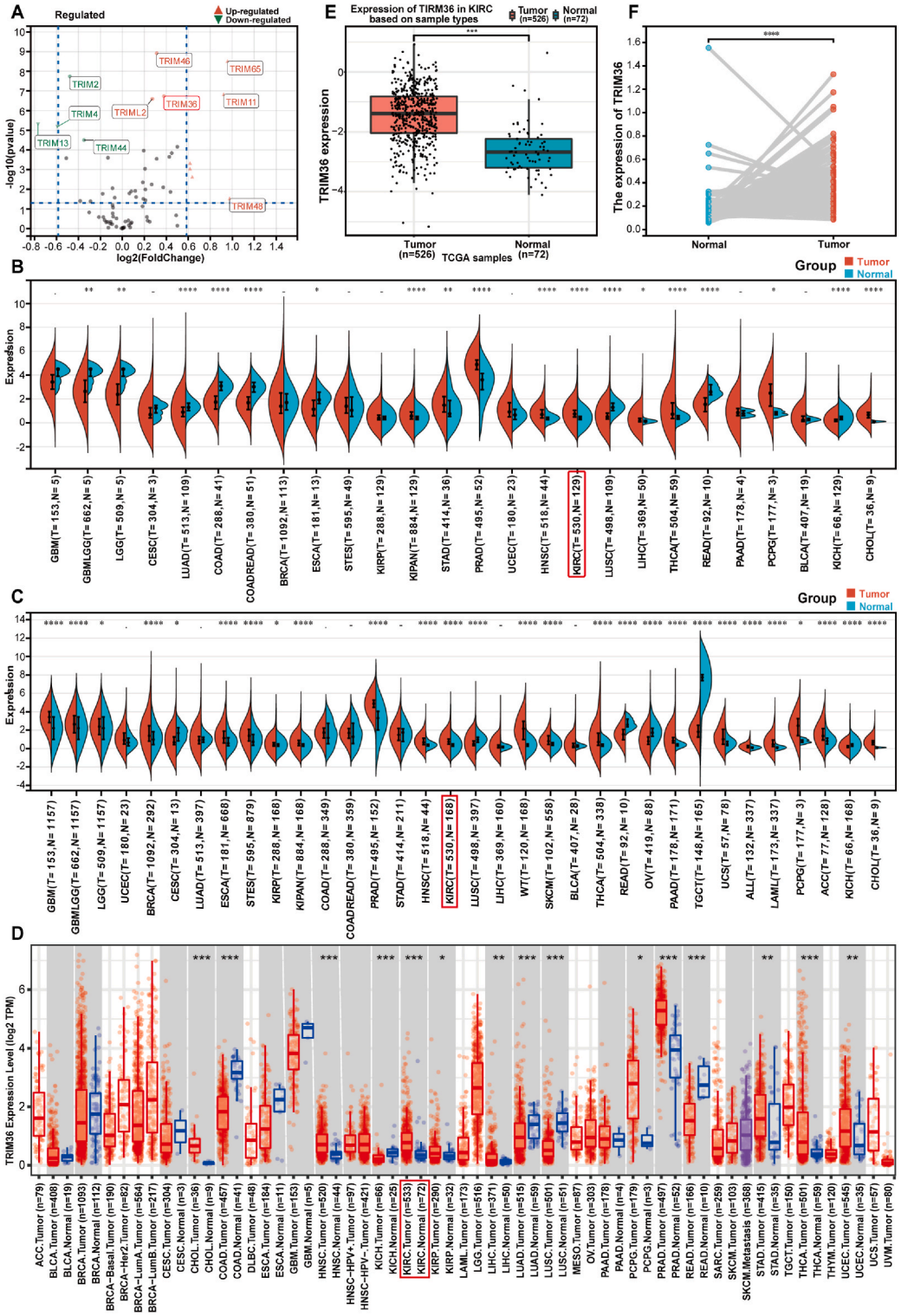

Fig. 1. Features of TRIM family genes in KIRC and pan-cancer analysis of TRIM36. (A) Using the LinkFinder module of LinkedOmics, a volcano plot was drawn to depict the TRIM family genes linked to KIRC prognosis. Genes showing relations to poor prognosis are represented in red, while those exhibiting relations to better prognosis are depicted in green. (B) We employed R software (version 3.6.4) to compute the expression variances between normal and tumor samples in various cancers. Signed Rank Tests and unpaired Wilcoxon Rank Sum were used to evaluate the significance of these differences. This was followed by an assessment of the relative TRIM36 mRNA expression across all TCGA tumors by Sangerbox [20]. (C) Pan-cancer analysis of TRIM36 mRNA expression using GTEx and TCGA databases. (D) Pan-cancer analysis of TRIM36 conducted with TIMER2.0.

(E) The expression levels of TRIM36 were higher in KIRC tissues than those in normal tissues. (F) Examination of TRIM36 expression in 72 paired patient samples revealed differences between KIRC tissues and adjacent normal tissues. $P < 0.0001$ (****); $P < 0.001$ (***); $P < 0.01$(**); and $P < 0.05$ (*). KIRC, kidney renal clear cell carcinoma, TRIM36, tripartite motif containing 36. OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; TCGA, The Cancer Genome Atlas.

identification and diagnosis of KIRC patients are essential for guiding precise clinical treatment. Yet, our understanding of KIRC's pathogenesis remains limited, and sensitive tumor biomarkers have yet to be identified [4]. Recently, PTGER4, PTK6, and members of the MMP family have emerged as potential therapeutic targets or biomarkers for KIRC [5–7]. Developing effective prognostic biomarkers and understanding the underlying molecular mechanisms are crucial for advancing clinical care and enhancing the diagnosis, treatment, and monitoring of KIRC.

The tripartite motif-containing (TRIM) protein family, exhibits a highly conserved and ordered structural composition comprising circular, B-box, and helical domains. The circular finger present in TRIM proteins suggests their involvement as E3 ubiquitin ligases in various cellular processes. Latest research has shown that certain TRIMs have important regulatory roles in metabolic diseases like type 2 diabetes mellitus, obesity, nonalcoholic fatty liver disease, and atherosclerosis [8]. Given the extensive role of the TRIM family in post-translational protein modification, cell signaling pathways, and gene regulation, it is not surprising that various TRIM family members have diverse effects on cancer cell proliferation, migration, and invasion [9]. Former researchers have found that TRIM proteins have roles in a number of cancers, including hepatocellular carcinoma, lung cancer, and breast cancer [10–12].

TRIM36, a member of the tripartite motif-containing (TRIM) protein family, has been identified as an androgen-responsive gene and exerts tumor-suppressive effects in various cancers, including colorectal cancer, hepatocellular carcinoma, human esophageal squamous cell carcinoma, and prostate cancer [13–16]. Notably, TRIM36 is mapped to chromosome 5q22.3, a region associated with tumor suppression [17]. However, despite its tumor-suppressive role documented in previous studies, our present research indicates that TRIM36 exerts a significant promoting effect on KIRC. This finding emphasizes the complex and context-dependent nature of TRIM36's role in cancers, highlighting the necessity for additional research into its mechanisms of action in different cancer types.

Throughout this investigation, we evaluated the prognosis-related value of TRIM36 differential expression in KIRC patients. Our findings indicate that TRIM36 may develop into an innovative diagnostic and prognostic marker for KIRC, as demonstrated through analysis of public sequencing data and validation experiments. Moreover, through gene screening, enrichment analysis, and Immune infiltration analysis, our research emphasized that TRIM36 likely promotes the proliferation of KIRC by interacting with 5 hub genes, thereby contributing to the processes of cell division and cell cycle, and influencing the tumor immune environment.

2. Materials and methods

2.1. Cell culture and transfection

Clear cell renal cell carcinoma cells 786-O and human renal embryo cells 293 were obtained from the American Type Culture Collection (ATCC, USA). The 786-O cells were cultured in RPMI-1640 medium (Gibco, USA) and the 293 cells were cultured in DMEM medium (Biosharp, China). The aforementioned media were supplemented with 10 % fetal bovine serum (FBS; Gibco, USA). The cells were stored under stable, humidified conditions with 5 % CO2 at 37 °C to maintain their viability. The TRIM36 plasmids underwent transfection using M5 HiPer Lipo2000 Transfection Reagent (Mei5 Biotechnology Co.,Ltd, Shanghai, China) according to the manufacturer's instructions.

2.2. Cell proliferation experiments

The wound-healing assay was adopted with transfected cells. The cells were seeded into a 6-well plate at a density of 1×10^5 cells/well, and then we made a straight scratch with a pipette tip in each well as soon as cell concentration reached nearly 100 %. The inverted microscope (Olympus, Japan) was used to record the scratch at 0 h and 24 h.

2.3. Transwell investigations

Migration research was aided by transwell tests conducted in particular chambers (Corning, New York, USA). We resuspended 1×10^5 cells and 2×10^5 cells in 200 μl of serum-free RPMI-1640 medium and placed them in the upper chamber. The lower chamber was then filled with 600 μl of RPMI-1640 containing 10 % FBS. After that, they were kept in a 5 % CO2 incubator at 37 °C for 48h. After being fixed with 4 % paraformaldehyde, the cells that were migrating to the bottom chamber were stained with 0.1 % crystal violet. Lastly, the number of migrating cells was counted.

2.4. Gene expression and pan-cancer analysis

RNA sequencing data from the Cancer Genome Atlas (TCGA) [18] database (https://tcga-data.nci.nih.gov/tcga/) and Genotype-Tissue Expression (GTEx) [19] database were used to measure TRIM36 mRNA expression levels in tumor and normal tissues of KIRC patients. Single-cell RNA-seq data for the 3 KIRC samples were obtained from single-cell RNA-seq data from GEO (GSE242299, GSE171306 and GSE152938). The clinical data were downloaded from the TCGA database (n = 541). For the pan-cancer analysis, an

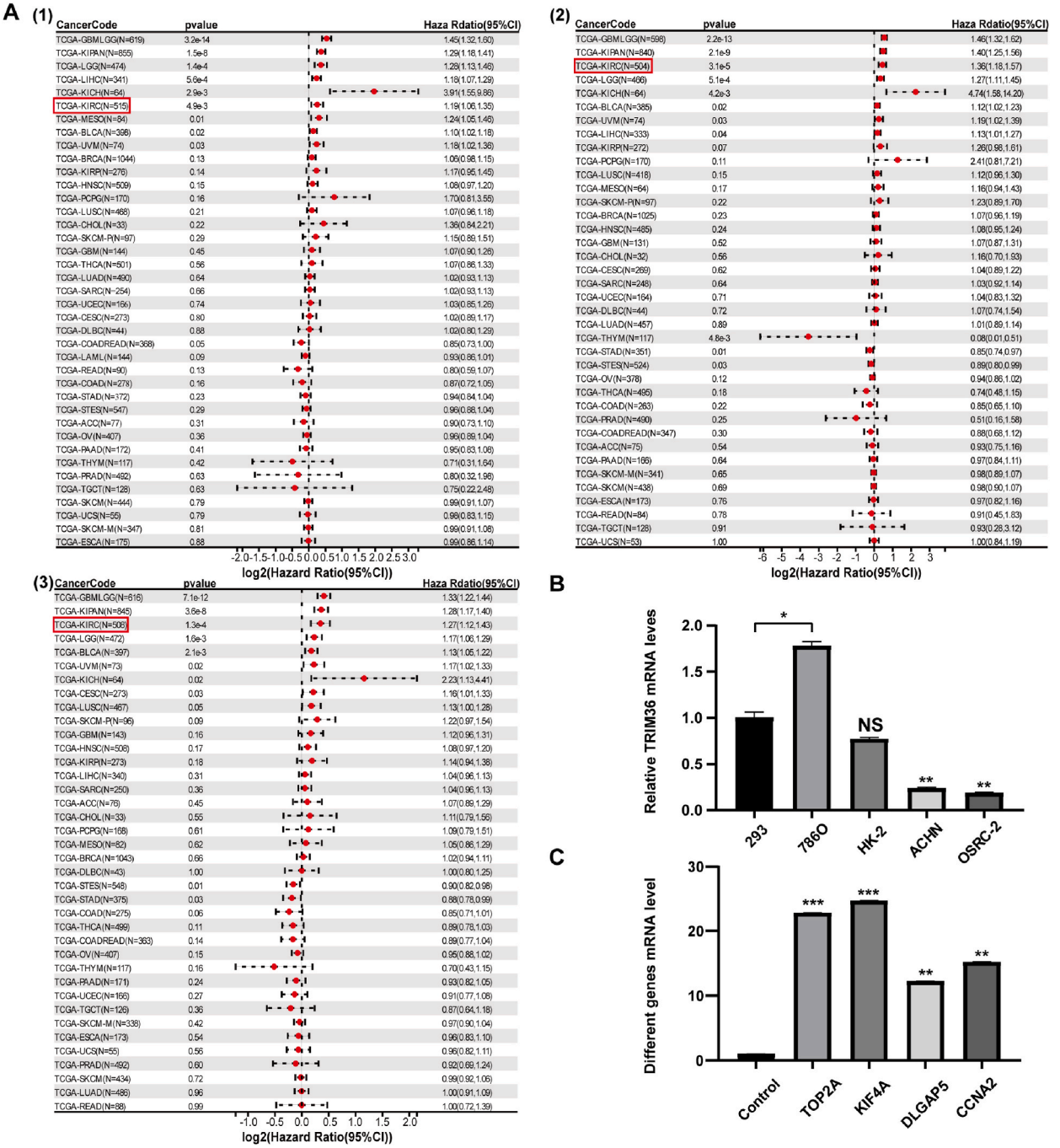

Fig. 2. The impact of TRIM36 expression on key clinical outcomes and real-time polymerase chain reaction (RT-qPCR) experiments. (A) Using the coxph function in the R package "survival'' to establish a Cox proportional hazards regression model to evaluate the association between TRIM36 expression levels and prognosis in every tumor, then utilizing the Logrank test for statistical analysis to determine the significance of prognosis. Specifically examining the impact of TRIM36 upregulation on (1) overall survival (OS), (2) disease-specific survival (DSS), and (3) progression-free interval (PFI) in human cancer patients. (B) Expression levels of TRIM36 were significantly higher in 786O cells in comparison to 293 cells. (C) Expression levels of TRIM36 associated genes were significantly higher in 786O cells in comparison to control cells (293 cells). $P < 0.0001$ (****); $P < 0.001$ (***); $P < 0.01$(**); and $P < 0.05$ (*).

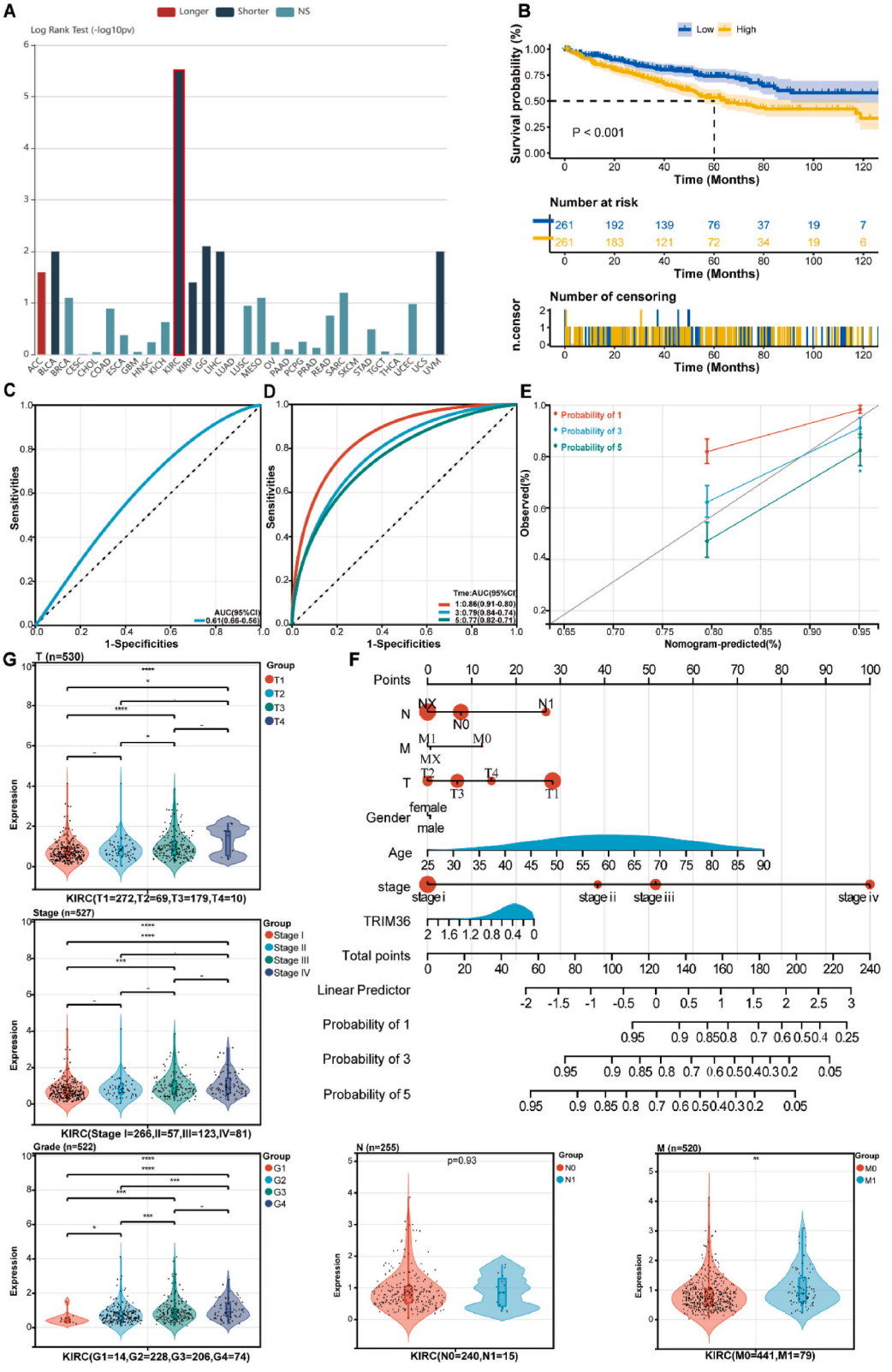

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Fig. 3. The prognostic value of TRIM36 in KIRC and the correlation between TRIM36 expression and KIRC subtypes. (A) Examining the correlation between TRIM36 and clinical outcomes across all TCGA tumors. The KIRC is surrounded by a red box. (B) Presenting Kaplan-Meier curves derived from the TCGA database, stratified by TRIM36 mRNA expression. The p-value was calculated by logrank test. (C) Constructing the ROC curve to assess TRIM36 expression in KIRC patients, yielding an AUC of 0.61. (D) Time-dependent ROC curves for TRIM36 expression in KIRC patients, with AUCs at 1 year (0.86), 3 years (0.79), and 5 years (0.77). (E) Utilizing a calibration curve to analyze the consistency of the Nomogram. (F) Introducing a Nomogram for predicting disease-specific survival (DSS) probabilities at 1-, 3-, and 5- years for individual KIRC patients. (G) High TRIM36 expression was observed in patients with T (n = 530), N (n = 255), M (n = 520), Stage (n = 527), and Grade (n = 522). $P < 0.0001$ (****); $P < 0.001$ (***); $P < 0.01$(**); and $P < 0.05$ (*). T, tumor; N, node; M, metastasis.

interactive tool called Sangerbox [20] (http://www.sangerbox.com/) was employed with datasets sourced from the TCGA database and the GTEx database (https://www.gtexportal.org/). Additionally, datasets from the TIMER2.0 database (http://timer.cistrome.org/) were used to show expression of TRIM36 in different cancers. Datasets in pan - cancer analysis were transformed by log2 (x + 1). The RNA sequencing dataset was in FPKM format, and patients' tumor (T), node (N), metastasis (M), and stage data were extracted from the clinical data for subgroup survival analysis.

2.5. Survival analysis

Based on data from the TCGA database, we generated Kaplan-Meier survival curves, for which the p-value was calculated using the log-rank test, to evaluate the prognostic value of TRIM36 expression in KIRC. Univariate and multivariate COX analyses were conducted to further evaluate the prognostic significance of TRIM36. A nomogram was constructed, and the concordance index (c-index) was calculated using R software along with the packages "rms'', "foreign'' and "survival''. ROC curve and Time ROC curves were plotted with the R packages "survivalROC" and "timeROC". Furthermore, we analyzed the effect of TRIM36 expression on overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) across various cancers using Sangerbox [20]. The statistical significance was assessed by p-values and Hazard Ratios (HR). We.

2.6. RNA isolation and RT-qPCR

Total RNA was extracted from 293 cells and 786-O cells using TRIzol Reagent (Thermo Fisher Scientific, Inc., USA). HiScript II Q RT SuperMix (Vazyme Biotech Co.,Ltd., China)was used to prepare the cDNA for qPCR. The concentration and purity of RNA were measured using NanoDrop One spectrophotometer (Thermo Fisher Scientific). Primers were customized at Sangon (Sangon, Shanghai, China) and β-actin served as an internal control. The relative expression levels of TRIM36 were calculated using the 2-ΔΔCt method. Every step was taken in accordance with the manufacturer's guidelines.

2.7. LinkedOmics database

LinkedOmics database (http://www.linkedomics.org/) serves as an accessible cancer data analysis platform, encompassing multi-omics datasets across 32 TCGA cancer types and 10 Clinical Proteomics Tumor Analysis Consortium (CPTAC) cancer cohorts. We utilized data from both the TCGA and LinkedOmics databases to identify human genes significantly correlated with TRIM36, genes associated with poor prognosis in KIRC, and differentially expressed genes in KIRC. The LinkFinder module allowed us to screen potential genes that might affect KIRC's overall survival.

2.8. Target gene screening and enrichment analysis

STRING [21] database (https://cn.string-db.org/) and Cytoscape were used to construct Protein-Protein Interaction (PPI) networks and evaluate the relationships among the target genes. Undefined Ontology (GO) analysis, KEGG pathway analysis, and Gene Set Enrichment Analysis (GSEA) were performed using the target genes that exhibited high relevance to TRIM36 in KIRC.We used R packages "clusterProfiler'' and "org.Hs.eg.db'' for GO and KEGG analysis.

2.9. TRIM36 and tumor immunity

Based on the "Lymphocyte" and "immunomodulator" modules of TISIDB website (http://cis.hku.hk/TISIDB/), TRIM36 was investigated in connection with tumor immunity, focusing on tumor-infiltrating cells and immune checkpoint inhibitors. The "Correlation" module of TIMER [22] (https://cistrome.shinyapps.io/timer/), and the "Immune" module of TIMER2 [23] (http://timer.cistrome.org/) were employed as valuable resources for the comprehensive evaluation of immune infiltrates in various cancer types. For a more detailed analysis, the correlation of immune cells and with TRIM36 expression was assessed with the CIBERSORT algorithm.

2.10. Single cell sequencing analysis

Three KIRC single-cell RNA-seq data samples from GSE242299, GSE171306 and GSE152938 were obtained from GEO. 3 KIRC samples were combined for further analysis using the R package "Seurat". 38 cell clusters were manually annotated based on known

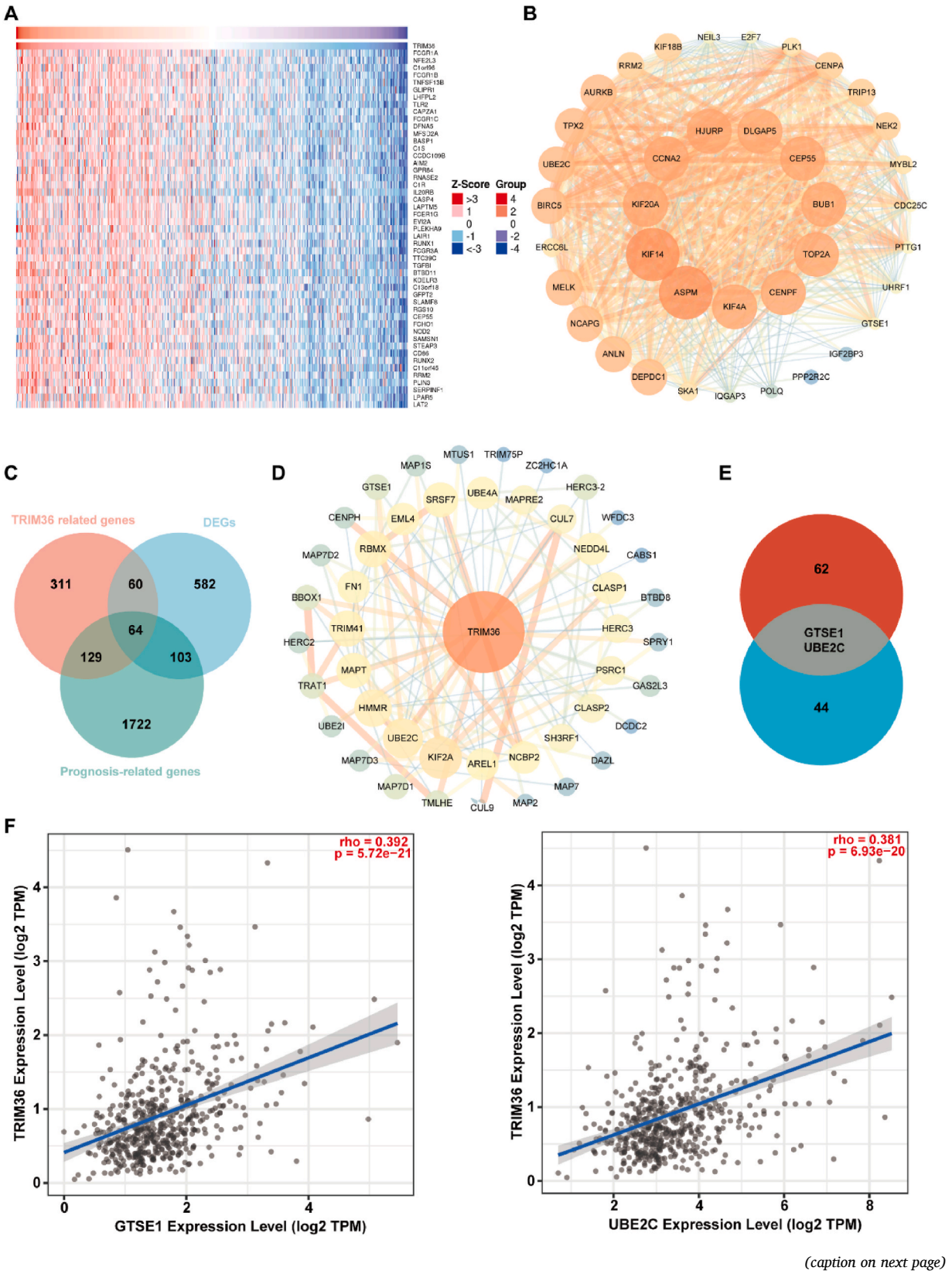

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Fig. 4. Kaplan-Meier curves analysis and gene screening of genes associated with TRIM36. (A) Heatmaps illustrating genes significantly correlated with TRIM36 in KIRC (TOP 50), where blue turquoise indicates low adjacency and red indicates high adjacency. (B) Constructing the interaction network of the 64 target genes associated with TRIM36 via STRING. Orange indicates a strong correlation, While Blue indicates a weak correlation. (C) Conducting an intersection analysis of TRIM36-related genes, prognosis-related genes, and differential expression genes (DEG) presented through a Venn Diagram. (D) A TRIM36 protein-protein interaction (PPI) network was constructed utilizing the STRING database and Cytoscape. Orange represents Strong Correlation, While Blue represents Weak Correlation. (E) Identifying the intersection of the 64 target genes and 46 proteins demonstrated through a Venn Diagram. (F) Investigating the correlation between TRIM36 expression and 2 specific genes.

cell identity markers. 38 cell clusters were manually annotated based on known cell identity markers. Marker genes were obtained from CellMarker database (http://biocc.hrbmu.edu.cn/CellMarker/) [24].

2.11. Statistical analysis

We used R (version 3.6.4) and R packages "rms'', "foreign'', "survival'', "survivalROC'', "SingleR", "limma'', "timeROC'' and "ESTIMATE" for statistical analysis. For paired data, paired *t*-test was used, while for unpaired data, Wilcoxon Rank Sum and Signed Rank Tests,one - way ANOVA or Student's t - test were used. Spearman correlation analysis was employed to evaluate the relationship between TRIM36 expression levels and the presence of tumor-infiltrating immune cells in KIRC tissues. $P < 0.05$ was considered statistically significant.

3. Results

3.1. TRIM36 expression pan-cancer analysis and its upregulation in KIRC correlate with poor prognosis

In our study investigating the involvement of TRIM (Tripartite Motif) family members in KIRC, we utilized LinkedOmics, a tool contains multi-omics data and clinical data for 32 cancer types. Using the LinkFinder module, we could screen candidate genes having influence on the overall survival of KIRC. Our analysis revealed notable associations: increased expressions of TRIM46, TRIML2, TRIM11, TRIM48, TRIM65, and TRIM36 were significantly correlated with an unfavorable prognosis, while TRIM2, TRIM4, TRIM13, and TRIM44 showed significant correlations with a favorable prognosis (Fig. 1A).

While the expression profiles and prognosis of TRIM genes in KIRC have been largely studied, the role of TRIM36 in KIRC remains unknown [25–27]. Focusing on TRIM36, we sought to investigate its specific role in KIRC further. Utilizing information from the extensive TCGA Pan-Cancer data (PANCAN,N = 10535,G = 60499) bases, we assessed TRIM36 mRNA levels in both tumor and normal tissues (Fig. 1B). Results show that TRIM36 is significantly differentially expressed in various cancers, including KIRC ($P < 0.001$). To improve the credibility of our results, we downloaded the standardized pan cancer dataset (PANCAN, N = 19131, G = 60499) from UCSC, which merged datasets from TCGA and GTEx (Genotype-Tissue Expression) (Fig. 1C). Additionally, we consulted public datasets from TIMER2, a comprehensive resource for tumor-immune system interactions (Fig. 1D). The data analysis above consistently indicates a significant increase in TRIM36 expression across various tumors, including KIRC. Furthermore, the upregulation of TRIM36 in renal cancer has been validated in a larger sample size sourced from the TCGA database (Fig. 1E), with similar findings observed in 72 paired patient samples (Fig. 1F).

We then investigated the impact of TRIM36 expression on vital clinical metrics, encompassing disease-specific survival (DSS), progression-free interval (PFI), as well as overall survival (OS) in KIRC patients. Forest plots generated from Sangerbox [20] tool depicted a compelling association between elevated TRIM36 expression levels and poorer prognoses in individuals with KIRC (Fig. 2A). To validate and reinforce our findings, RT-qPCR experiments were performed by us. These experiments aimed to quantify TRIM36 mRNA levels in both kidney normal kidney cells (293) and renal clear cell carcinoma cells (786-O). The results from our experiments confirmed our initial observations, revealing a marked elevation of TRIM36 mRNA levels in 786-O cells compared to 293 cells (Fig. 2B). In addition, we assessed the expression levels of TRIM36 in HK-2, ACHN, and OSRC-2 cells to verify the accuracy of the results. By employing a comprehensive approach that combines bioinformatics analysis with experimental validation, we proposed that TRIM36 could function as an adverse prognostic indicator for KIRC and underscored its promise as a therapeutic target warranting additional exploration.

3.2. The prognostic value of TRIM36 in KIRC and clinicopathologic features associated with TRIM36 expression in KIRC

Initially, TISIDB was employed to investigate the association between TRIM36 mRNA levels and overall survival across various human cancers. The results corroborated previous findings, highlighting a notable correlation between TRIM36 levels and poorer prognosis in KIRC patients (Fig. 3A). Subsequently, to evaluate the diagnostic value of TRIM36 in KIRC, datasets from the TCGA were utilized for ROC curve analysis, Time ROC curve analysis, as well as Kaplan-Meier survival analysis. (Fig. 3B–D). An unfavorable prognostic in KIRC patients was observed to be significantly related to elevated TRIM36 expression, according to the Kaplan-Meier survival analysis. Additionally, the AUC value of the ROC curve for TRIM36 in all patients was 0.61 (Fig. 3C). The time-dependent ROC curves indicated that TRIM36 has a high prognostic value in the early time of KIRC (1-year) with an AUC value of 0.86, and the prognostic value might decrease slightly over time but remain at a relatively high level. The model's AUC values for the 1-, 3-, and 5-year survival rates are 0.86, 0.79, and 0.77, revealing an overall predictive accuracy of significance for KIRC patient survival rates over a range of periods (Fig. 3D). This suggests that TRIM36 holds better prognostic value, especially in distinguishing survival

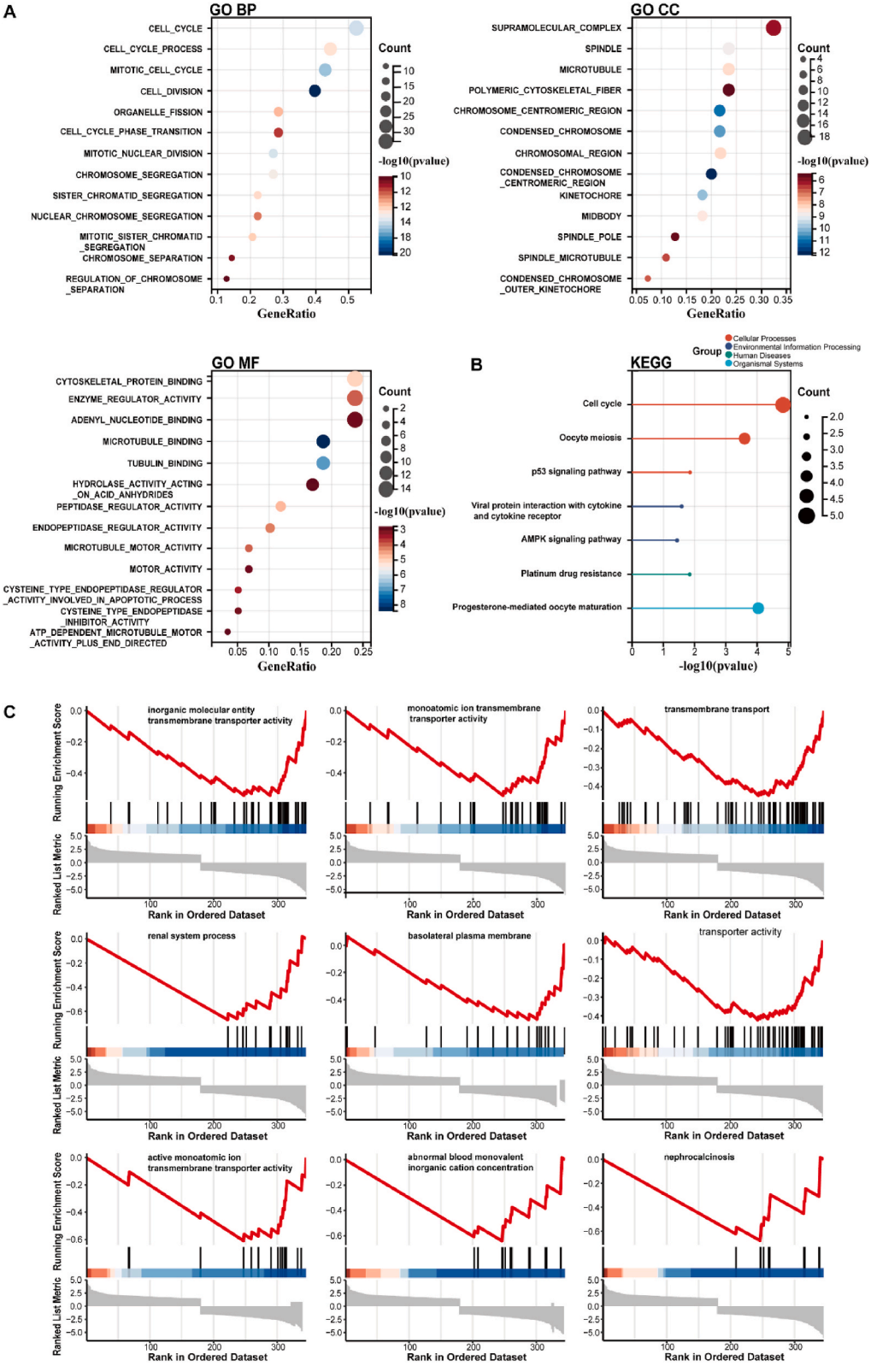

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Fig. 5. GO Functional Annotation and Pathway Enrichment of TRIM36 correlative genes. (A) GO analysis to explore biological processes, molecular functions, and cellular components related to TRIM36. (B) Performing KEGG pathway analysis to investigate pathways linked to TRIM36-related genes. (C) Presenting enrichment plots from Gene Set Enrichment Analysis (GSEA) for various terms including inorganic molecular entity transmembrane transporter activity, monoatomic ion transmembrane transporter activity, transmembrane transport, renal system process, basolateral plasma membrane, transporter activity, active monoatomic ion transmembrane transporter activity, abnormal blood monovalent inorganic cation concentration, nephrocalcinosis, and inorganic anion transmembrane transporter activity.

outcomes among KIRC patients. Evaluations of univariate as well as multivariate Cox regression were carried out. Additionally, a nomogram was created for forecasting KIRC patients' disease-specific survival across 1, 3, and 5 years. (Fig. 3F). The C-index was calculated to be 0.768 (0.732–0.804), and a calibration plot was utilized to demonstrate the association between the forecasted and observed values (Fig. 3E). The above data collectively indicate the prognostic value of TRIM36 in KIRC, as well as its association with TRIM36 expression and clinicopathologic features in KIRC.

Furthermore, to explore the trend of changes in TRIM36 expression levels in KIRC with the progression of stage and grade, we utilized the TCGA databases. Violin diagrams were employed to elucidate the clinical implications of TRIM36 in KIRC, confirming previous research findings that TRIM36 overexpression is significantly correlated with T (n = 530), N (n = 255), M (n = 520), stage (n = 527), and grade (n = 522) (Fig. 3G). We observed that patients with more advanced tumor stages had higher TRIM36 levels compared to individuals at earlier stages, for instance, stage I compared to stage IV, and T1 compared to T3/T4. The results indicate that TRIM36 expression gradually increases in KIRC as the stage and grade advance.

3.3. Potential mRNA regulated by TRIM36

After a meticulous analysis of data from both the TCGA and LinkedOmics databases, we identified 582 genes that exhibit significant differential expression between KIRC tumor tissues and normal tissues, alongside 1722 genes demonstrating prognostic relevance. Notably, 311 genes exhibited a notable positive correlation with TRIM36 expression, with the top 50 molecules depicted via a heat map (Fig. 4A). Noteworthy correlations include FCGR1A (r = 0.603, P = 3.88E-54), NFE2L3 (r = 0.596, P = 1.23E-52), C1orf96 (r = 0.591, P = 1.70E-51), and FCGR1B (r = 0.588, P = 6.98E-51).

Subsequently, a Venn diagram was employed to facilitate the identification of overlapping genes among these three cohorts (Fig. 4B), leading to further investigation on 64 target genes. Drawing upon the STRING data base, we created a network of protein-protein interactions, which Cytoscape was then used to visualize (Fig. 4C). Central genes were identified according to their significance within the network. For the related mRNAs (DLGAP5, CEP55, BUB1, TOP2A, CENPF, KIF4A, ASPM, KIF14, KIF20A, CCNA2, and HJURP), further Kaplan-Meier survival analysis and ROC curve analysis revealed their influence on the overall survival of KIRC patients as well as their diagnostic utility; these images have been added to the Appendix. Intriguingly, the aforementioned mRNAs exhibited a pronounced association with poor prognosis, with their upregulation significantly correlating with decreased overall survival time ($P < 0.05$). Additionally, they all displayed an area under the curve (AUC) > 0.5, underscoring their diagnostic utility in KIRC. Notably, these mRNAs appear to be potentially regulated by and closely related to TRIM36.

Subsequent proteome analysis revealed 46 proteins interacting with TRIM36, visualized similarly to the previously examined PPI network (Fig. 4D). Beyond expectations, the intersection of the 64 target genes and 46 proteins revealed two genes, namely UBE2C and GTSE1 (Fig. 4E). Their significant correlation with TRIM36 expression ($P < 0.001$) further suggests their research prospects (Fig. 4F). Based on the aforementioned research screening, we've identified mRNA candidates potentially linked to TRIM36 in KIRC, paving the way for further targeted investigation.

3.4. Exploring the potential mechanisms and route enrichment evaluations of TRIM36 inside KIRC

Using the 108 genes found through the previously described screening, we conducted Genomes pathway enrichment, GO, and KEGG evaluation to deeper investigate the possible involvement of TRIM36 in KIRC. Notably, GO classification results revealed significant enrichment in cell division-related functions among the top-ranked biological processes (BPs). These comprised essential functions like cell division, mitotic cell cycle, cell cycle regulation, mitotic nuclear division, chromosome segregation, sister chromatid segregation, organelle fission, nuclear chromosome segregation, and cell cycle phase transition. Additionally, we observed noteworthy enrichment in functions associated with microtubule binding, tubulin binding, cytoskeletal protein binding, peptidase regulator activity, endopeptidase regulator activity, and microtubule motor activity. The cellular component was also elucidated via a bubble diagram (Fig. 5A). Furthermore, the KEGG pathways analysis highlighted prominent involvement in key pathways including the Cell cycle, Progesterone-mediated oocyte maturation, and Oocyte meiosis pathways (Fig. 5B). According to the above discoveries, TRIM36 might have a pivotal function in governing the cell cycle and related pathways involved in KIRC advancement, potentially exerting control over cellular operations and biological mechanisms.

By conducting GSEA analysis using TRIM36-low and TRIM36-high datasets from the TCGA transcriptome dataset, we identified signaling pathways influenced by TRIM36 expression in KIRC. Remarkably, gene sets related to inorganic molecular entity transmembrane transporter activity, monoatomic ion transmembrane transporter activity, transmembrane transport, renal system processes, basolateral plasma membrane, transporter activity, active monoatomic ion transmembrane transporter activity, abnormal blood monovalent inorganic cation concentration, nephrocalcinosis, and inorganic anion transmembrane transporter activity was notably enriched in the TRIM36-low expression cohort (Fig. 5C). These analyses reveal that TRIM36 expression levels in KIRC may

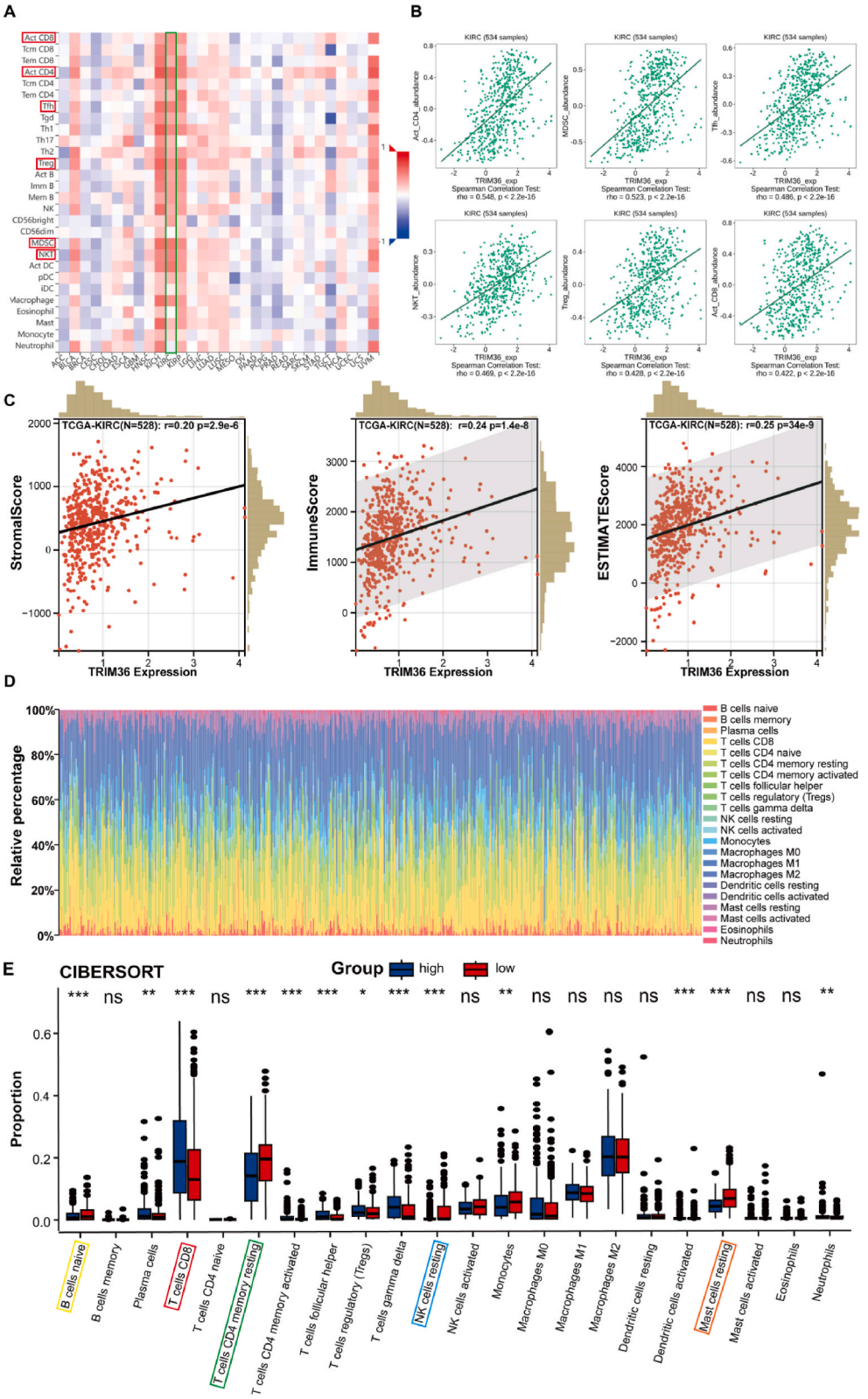

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Fig. 6. Connectivity between TRIM36 and immune cell infiltration. (A) Presenting a heatmap demonstrating the close relationship between Tumor-Infiltrating Lymphocytes (TILs) and TRIM36 in KIRC among all TCGA tumors. (B) Analyzing the relationship between TRIM36 expression and the enrichment of immune cells (Activated CD4 T cells, Myeloid-Derived Suppressor Cells (MDSC), T follicular helper cells (Tfh), Natural Killer T cells (NKT), Regulatory T cells (Treg), and Activated CD8 T cells) in KIRC. (C) Assessing the Stromal, Immune, and ESTIMATE scores associated with TRIM36 expression in KIRC. (D) Using CIBERSORT, displaying the percentage of immune subsets that infiltrate tumors by analyzing 21 different immune cell profiles in KIRC. (E) Comparing the infiltration of multiple TILs identified by CIBERSORT between the high and low TRIM36 expression groups based on the TCGA dataset. $P < 0.0001$ (****); $P < 0.001$ (***); $P < 0.01$(**); and $P < 0.05$ (*).

impact various signaling pathways and biological processes, especially those linked to membrane transport and gene sets related to renal function. These findings underscore the significance of TRIM36 in modulating KIRC development and offer valuable insights for further investigating its role in KIRC pathophysiology.

In summary, TRIM36 significantly influences the progression of KIRC and impacts cellular functions. It is associated with transport and cell proliferation within KIRC, potentially affecting ion transport and renal system functionality. The expression levels of TRIM36 in KIRC may influence signaling pathways, highlighting its importance in disease development. These findings underscore the significance of TRIM36 in KIRC and provide insights for further research.

3.5. The associations between TRIM36 levels and immune cells' ability to infiltrate cancers

Utilizing the TISIDB website, we explored the associations between the tumor immunological environment and TRIM36 expression in KIRC. The heatmap suggested significant correlations between TRIM36 and tumor-infiltrating lymphocytes (TILs) in KIRC compared to other TCGA tumors (Fig. 6A). Notably, there were substantial links identified between TRIM36 expression levels and the presence of various TILs, including Active $CD4^+$ T cells ($r = 0.548$, $P < 0.001$), Myeloid-derived suppressor cells (MDSCs; $r = 0.523$, $P < 0.001$), T follicular helper cells (Tfh; $r = 0.486$, $P < 0.001$), Natural killer T cells (NKT; $r = 0.469$, $P < 0.001$), Regulatory T cells (Tregs; $r = 0.428$, $P < 0.001$), and Active $CD8^+$ T cells ($r = 0.422$, $P < 0.0001$) (Fig. 6B). Given its association with cell types directly implicated in immunological responses, which include Active $CD4^+$ T cells and Active $CD8^+$ T cells, data that was presented suggest that TRIM36 probably strengthens immune defense. This implies that TRIM36 might have a pivotal function in modulating the immune system's reaction to tumors.

Additionally, we specifically analyzed the Stromal, Immune, and ESTIMATE scores associated with TRIM36 expression in KIRC. We analyzed associations between genes and immune infiltration scores across various tumors to pinpoint noteworthy connections with immune infiltration scores. Estimating stromal and immune cell populations within malignant tumor tissues via expression data enabled us to derive stromal ($P < 0.001$), immune ($P < 0.001$), and ESTIMATE scores ($P < 0.001$) (Fig. 6C). These results aid in assessing the significance of TRIM36 inside the stromal and immunological cells in the tumor immunological milieu, and their potential impact on tumor growth and progression. Typically, high stromal and immune scores indicate abundant immune cell presence in tumor tissue, which could affect tumor proliferation, dissemination, and response to treatment. These findings contribute to a deeper comprehension of TRIM36's involvement in tumor progression and in formulating possible therapeutic approaches.

Through previous studies, Active $CD8^+$ T cells in KIRC have been identified as a highly exhausted subtype of intratumoral $CD8^+$ T cells, characterized by impaired immune function and the presence of exhausted markers. This suggests an association with immunoreactivity and poor prognosis. Additionally, leveraging the CIBERSORT algorithm and TCGA database, the distribution of tumor-infiltrating immune subsets was elucidated through 21 immune cell profiles in KIRC samples (Fig. 6D). Furthermore, the degree of TRIM36 mRNA expression in tumors was evaluated, and expression disparities between tumor and normal tissues were discerned. This confirmed the differing status of the tumor immune microenvironment in KIRC patients using CIBERSORT (Figs. 6E and 7A). Intriguingly, by comparing the immune cells in the TRIM36 expression difference groups with that in the tumor and normal cohorts, we observed that nearly all immune cells associated with high TRIM36 expression were linked to the tumor group. Consequently, the pro-cancerous impact of TRIM36 in KIRC could be significantly intertwined with tumor-infiltrating immune cells.

To further demonstrate that KIRC has a high level of immune infiltration, we extracted ccRCC samples from single-cell RNA-seq data (GSE242299, GSE171306 and GSE152938) for different cellular annotations. We first used the "SingleR" package to automatically annotate the single-cell mRNA sequencing data, and the results showed that KIRC had a high level of immune infiltration (Fig. S2A). Using the obtained marker genes [28], we manually annotated the single-cell data and showed the expression of each type of marker gene (Figs. S2B–C). According to previous studies, the PTPRC gene is a marker gene for immune cell populations [28], and single-cell mRNA sequencing data labeling of TRIM36 revealed that its expression was highly correlated with immune-infiltrating cells, especially $CD8^+$ T cells, a finding that corroborates with previous (Fig. S2D).

Meanwhile, utilizing the resources available on the TISIDB platform, we constructed a heatmap to visualize the association between immune inhibitors linked to TRIM36 across diverse cancer types, while concurrently examining the relationship between immunology-associated genes in KIRC and TRIM36 levels (Fig. 8A). It is noteworthy that Programmed cell death protein 1 (PD-1/PDCD1), Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T cell immunoreceptor with Ig and ITIM domains (TIGIT), T cell-activated increased late expression (CD96/TACTILE), Lymphocyte activation gene 3 (LAG3), and B and T lymphocyte attenuator (BTLA) represent indispensable immune checkpoints that orchestrate tumor immune evasion mechanisms. Furthermore, these checkpoints serve as prognostic indicators for the therapeutic effectiveness of ICIs.

Particularly, our focus centered on PDCD1 (PD-1), CTLA-4, TIGIT, CD96, LAG3, and BTLA, recognized as predictive indicators for the therapeutic effectiveness of ICIs (Fig. 8B). Insights gleaned from the TIMER and TIMER2 databases revealed a positive correlation between TRIM36 expression and PDCD1 (PD-1), CTLA-4, TIGIT, CD96, LAG3, and BTLA in KIRC, persisting even after adjusting for

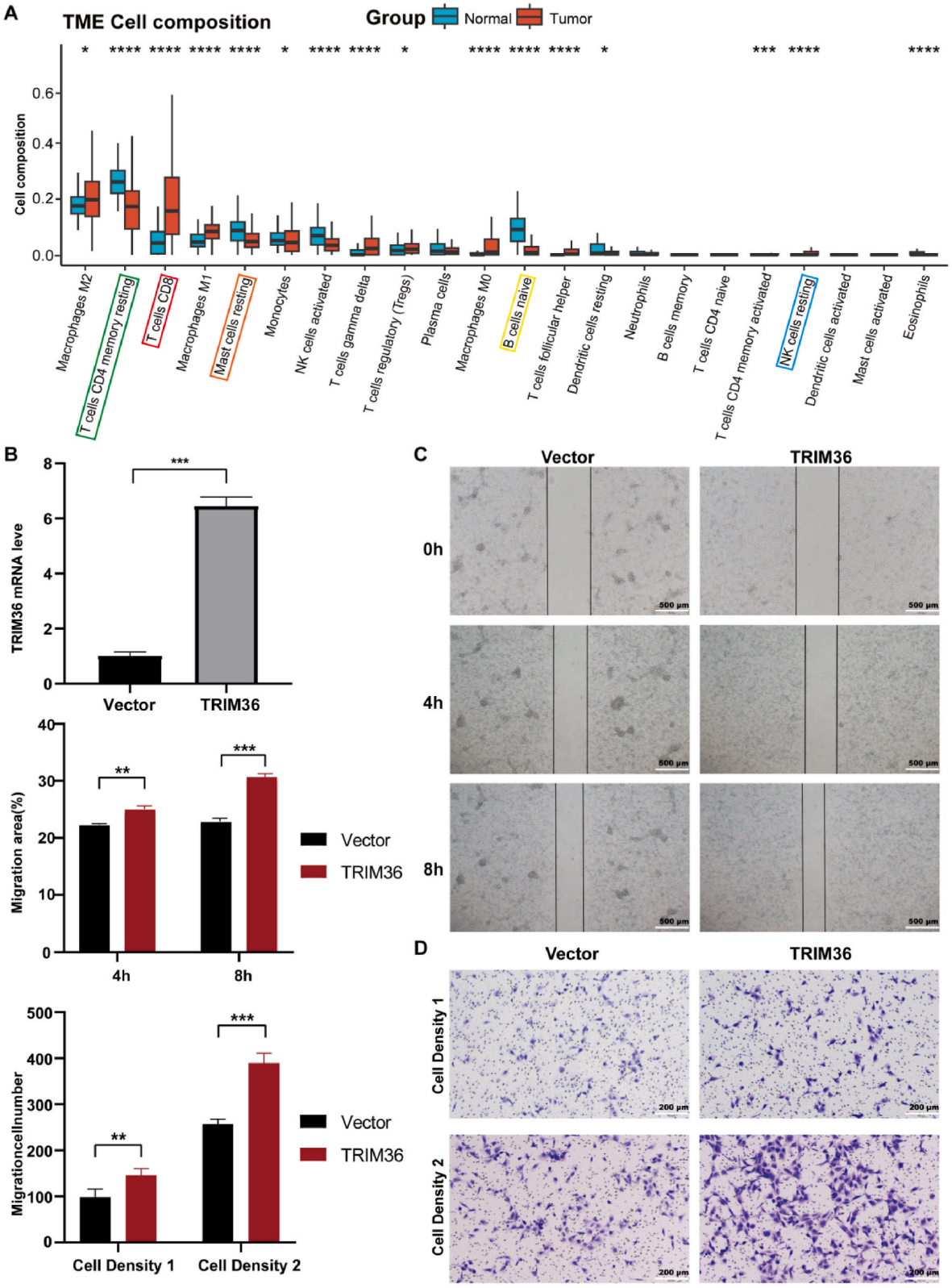

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Fig. 7. TRIM36 promotes the proliferation and migration of 7860 cells. (A) Examining the infiltration of multiple TILs identified by CIBERSORT in the TCGA dataset's tumor and normal groups. (B) The relative expression of TRIM36 was determined by qRT-PCR in 786O cells. (C) Wound healing assays was performed to demonstrate that overexpression of TRIM36 can promote the proliferation of 786-O cells. (D) Transwell assays without Matrigel indicated that overexpression of TRIM36 can promote the migration of 786-O cells at different cell densities. $P < 0.0001$ (****); $P < 0.001$ (***); $P < 0.01$(**); and $P < 0.05$ (*).

tumor purity (Fig. 8C). Additionally, our analysis showed an intensely favorable association between TRIM36 levels and PD-1 ($r = 0.328$, $P < 0.001$), CTLA-4 ($r = 0.354$, $P < 0.001$), TIGIT ($r = 0.397$, $P < 0.001$), CD96 ($r = 0.427$, $P < 0.001$), LAG3 ($r = 0.379$, $P < 0.001$), and BTLA ($r = 0.383$, $P < 0.001$) in KIRC (Fig. 8C). In summary, these observations imply that TRIM36 potentially influences tumorigenesis and KIRC development by modulating mechanisms associated with tumor immune evasion.

3.6. TRIM36 promotes cell proliferation and migration in KIRC

To investigate the impact of TRIM36 on the invasive and migratory capabilities of KIRC cells, we overexpressed TRIM36 and GFP in 786O cells and the expression levels of TRIM36 were quantified using qPCR (Fig. 7B). Next, we assessed the invasive and migratory ability of TRIM36-overexpressing KIRC cells through Transwell assays (Fig. 7C). We found that TRIM36-overexpressing KIRC cells exhibited a marked enhancement in invasion and migration compared to the control group (overexpressing GFP). Consistent with these results, wound healing experiments showed that the migratory ability of 786O cells overexpressing TRIM36 was significantly upregulated (Fig. 7D). Therefore, overexpression of TRIM36 may promote the invasion and migration of KIRC cells.

4. Discussion

Although there has been significant advancement in diagnosis, screening, surgery, and therapy, clinical results for KIRC remain unsatisfying [29]. As a result, more promising biomarkers or therapeutic targets are in urgent need of identification for faster diagnosis and more accurate prognosis. Currently, the correlation between TRIM36 and tumors remains largely unexplored. However, existing knowledge indicates that TRIM36 prolongs recurrence-free survival and inhibits cell cycle progression [30]. Many former studies concentrated on the role of TRIM36 in inhibiting tumorigenesis and found that TRIM36 is tumor-suppressive in multiple cancers [13–16]. However, the role of TRIM36 in KIRC has not been investigated. Our study shows that TRIM36 significantly promotes the development of KIRC.

Through integrative bioinformatics analysis and real-time qPCR, we were the first to observe a notable uptick in TRIM36 expression in KIRC tissues. Moreover, elevated TRIM36 expression is usually accompanied by a poorer prognosis. The findings from wound healing assays and transwell assays also collectively suggested that TRIM36 can promote the invasion and migration of KIRC cells. These results all indicated that TRIM36 played a role in the progression of KIRC. Additionally, a nomogram was used to forecast individual survival time, emphasizing the potential of TRIM36 as a biomarker in KIRC.

To further investigate the possible functions of TRIM36 in KIRC, we filtered out 11 hub genes (DLGAP5, CEP55, BUB1, TOP2A, CENPF, KIF4A, ASPM, KIF14, KIF20A, CCNA2, and HJURP) that are closely related to both TRIM36 and KIRC. Surprisingly, the upregulation of corresponding mRNAs of these hub genes all lowers the overall survival time. Intriguingly, former researches have shown each of these genes can contribute to the progression of multiple cancers. Previous studies indicated that TOP2A is thought to be a therapeutic target in a number of cancers and is an indicator of prognosis in advanced renal cell carcinoma [31,32]. Moreover, according to former research, KIF4A, DLGAP5, HJURP, and CCNA2 might become KIRC diagnostic and therapeutic potential biomarkers [33–35]. In light of this research, we conducted real-time qPCR analysis using the aforementioned 5 genes (TOP2A, KIF4A, DLGAP5, HJURP, and CCNA2). Notably, we observed an upregulation in 4 out of the 5 genes in KIRC tumor tissues (786-O cells) when compared to normal tissues (293 cells), which was expected (Fig. 2C). This gave us ample reason to believe TRIM35 may regulate the expression of these hub genes, therefore affecting the biological activity of KIRC.

A previous study has demonstrated that TRIM36 is capable of delaying cell cycle progression, suggesting its potential to induce chromosomal instability [30]. Using screened genes to carry on GO and KEGG analysis, we reached a similar conclusion that TRIM36 correlated genes were significantly enriched in cell cycle and cell division functions among the top-ranked BPs. This led us to infer that overexpression of TRIM36 could disrupt the cell cycle process, potentially resulting in uncontrolled cell growth and an increased likelihood of transformation into tumor cells.

Membrane proteins called transporters let organic and inorganic solutes move selectively across the plasma membrane and intracellular organelle membranes. In cancer cells, there is a substantial need for nutrients, leading to an increase in transporters for these nutrients to meet this need [36]. Through the use of GSEA, we discovered that gene sets associated with various transporter activities, such as inorganic molecular entity transmembrane transporter activity, monoatomic ion transmembrane transporter activity, and active monoatomic ion transmembrane transporter activity, were considerably more abundant in the group that had lower TRIM36 expression. This finding provides insight into why TRIM36 exhibits tumor-suppressive effects in various cancers, although its promoting effects on KIRC still require further investigation.

KIRC has been known as an immunological cancer and multiple immune cells can be involved in mediating the anti-tumor immune response. Past studies have demonstrated that immune infiltrations have been associated with patient prognosis [37]. Through immune cell infiltration analysis, we found that a majority of tumor-infiltrating lymphocytes (TILs) are closely correlated with TRIM36 in KIRC. We thus inferred that the tumor-infiltrating immune cells may play a major role in the pro-oncogenic action of TRIM36 on KIRC.

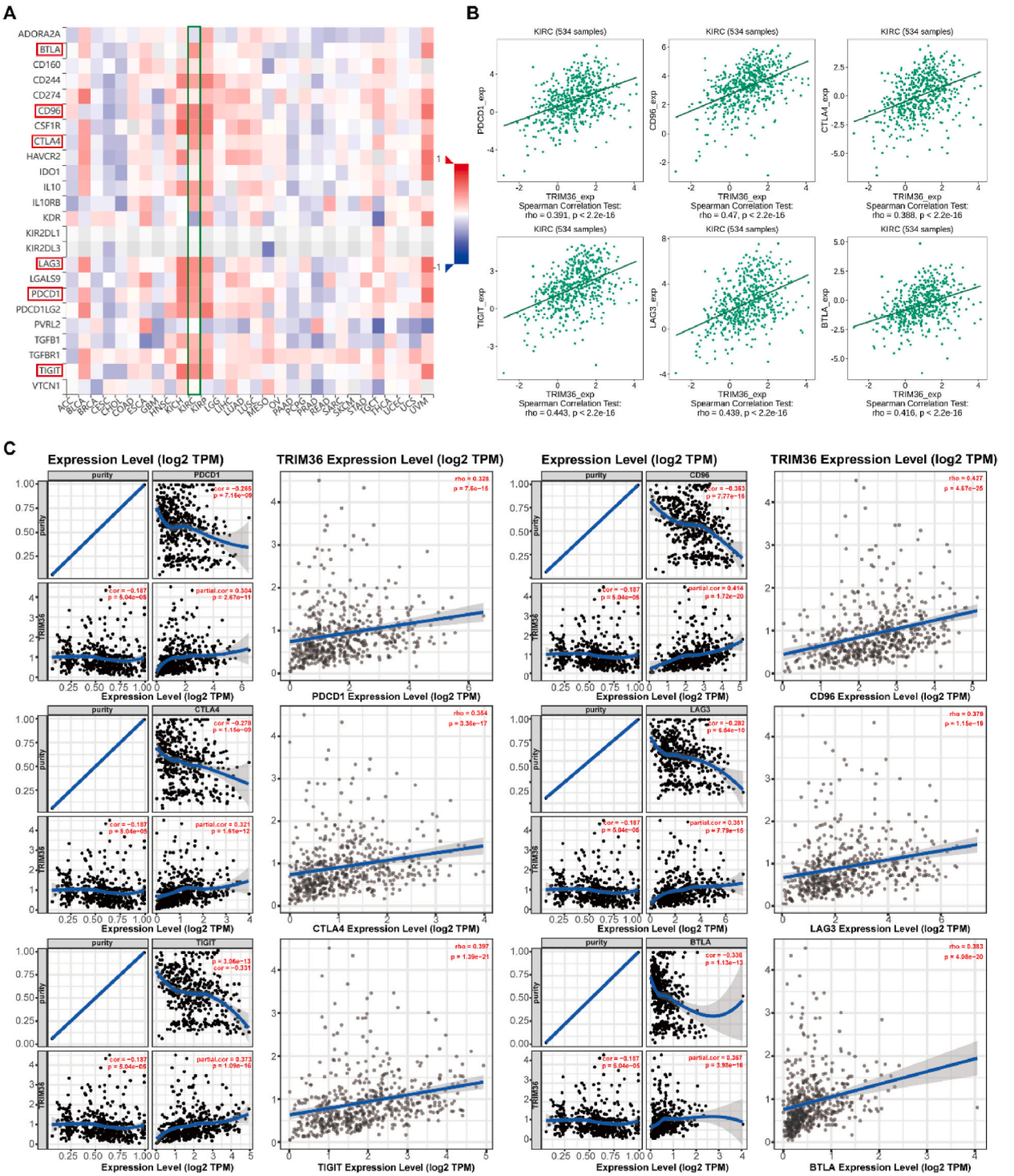

Fig. 8. The correlation between the expression of TRIM36 and immune-related genes in KIRC. (A) Presenting a heatmap demonstrating the close relationship between Immunoinhibitors and TRIM36 in KIRC across human cancers. (B) Analyzing the correlation between TRIM36 expression and PDCD1, CTLA-4, TIGIT, CD96, LAG3, and BTLA immunomodulators. (C) Examining the correlation of TRIM36 expression with immune checkpoint genes (PDCD1, CTLA-4, TIGIT, CD96, LAG3, and BTLA) in KIRC which was adjusted by tumor purity using the TIMER database.

Among these immune cells, active $CD4^+$ T cells and myeloid-derived suppressor cells (MDSCs) showed the strongest correlation with TRIM36. The extensive expansion of MDSCs during tumor progression is well-established, and it is well-known that they play a vital role in the growth and metastasis of tumors. The mounting evidence suggests that MDSCs represent a fundamental characteristic of malignant tumors [38]. We inferred that TRIM36 may regulate MDSCs to promote the stemness of KIRC.

Many cancer types have been clinically treated with immune checkpoint inhibitors, which have been extensively studied while being utilized in this way [39,40]. The immuno-inhibitors PDCD1 (PD-1), CTLA-4, TIGIT, CD96, LAG3, and BTLA have garnered significant attention as they serve as crucial immune checkpoint targets across various cancers. By effectively blocking immunoinhibitory signals, they facilitate enhanced antitumor responses [41]. Our study has unveiled a positive correlation between TRIM36 expression and these pivotal immune checkpoints. This correlation underscores the potential central role of TRIM36 in modulating immune checkpoint expression and thereby influencing the efficacy of immunotherapy. Further investigation into TRIM36 holds promise for substantially improving therapeutic outcomes in KIRC while concurrently deepening our comprehension of immunotherapy mechanisms.

Collectively, our research established TRIM36 as a novel tumor-promoting factor and diagnostic and prognostic marker for KIRC. However, it should be noted that our study has several limitations. This study mainly utilized public databases and did not include validation from clinical samples collected at our study center. Moreover, the potential clinical applicability of TRIM36 as a biomarker for KIRC patients, including its sensitivity and specificity, will require validation in future clinical studies.

CRediT authorship contribution statement

Jikai Zhang: Writing – review & editing, Funding acquisition. **Botao Zou:** Writing – original draft, Software, Data curation, Funding acquisition. **Yunfeng Geng:** Investigation, Data curation, Funding acquisition. **Hang Yin:** Investigation, Formal analysis. **Baoying Qin:** Investigation, Formal analysis. **Wanjun Gao:** Investigation, Formal analysis. **Xiaoman Lin:** Writing – review & editing. **Nan Sun:** Writing – review & editing, Funding acquisition, Conceptualization.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42540.

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