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Pan-cancer analysis identified Tripartite Motif Containing 54 as a potential prognostic and diagnostic biomarker in human cancers

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Abstract

Background

Ubiquitination plays a significant role in biological processes and regulates the tumor microenvironment (TME). Tripartite Motif Containing 54 (TRIM54) is an E3 ubiquitin ligase that, in recent years, has been shown to control the development of certain malignancies. However, the expression and function of TRIM54 across pan-cancer and its role in the TME remain unclear.

Methods

Data from the public databases were utilized to explore the expression, prognostic value, clinical features, enrichment analysis, and genetic alterations of TRIM54 in tumors. Associations between TRIM54 expression and immune cell infiltration (ICI), immunotherapy response, and targeted drugs were analyzed using bioinformatics.

Results

Compared with normal tissues, TRIM54 is aberrantly expressed across a diverse range of malignancies. The expression of TRIM54 could predict diagnosis and prognosis across most cancer types. The enrichment analysis showed that TRIM54 and its related proteins were closely associated with ubiquitination modification and tumor-associated pathways. In addition, TRIM54 was tightly associated with ICI and immunotherapy. Finally, we screened out several targeted drugs that could regulate TRIM54 expression during immunotherapeutic responses.

Conclusion

TRIM54 plays a vital part in pan-cancer development, and its aberrant expression in tumors may be associated with ICI in TME. At the same time, TRIM54 has strong prognostic predictive ability and is a promising predictive biomarker for immunotherapy.

1. Introduction

With about 10.0 million cancer deaths and 19.3 million new cases each year, cancer has been one of the most significant public health challenges in the world [1]. In tumor initiation, progression, metastasis, and response to therapy, the tumor microenvironment (TME), composed of non-cancerous cells and their products in the tumor, plays a crucial role [2, 3]. For TME, the infiltration and activation states of innate and adaptive immune cells can greatly influence its function [4]. And we can classify these immune cells into three types: tumor-antagonizing type mainly including effector T cells (CD8 + cytotoxic T cells and effector CD4 + T cells), natural killer (NK) cells, dendritic cells (DCs), M1-polarized macrophages, and N1-

polarized neutrophils, tumor-promoting type mainly including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and the controversial type with B cells [5].

Ubiquitination, one of the essential post-translational modifications, can play a role in various regulated biological processes via the ubiquitin-proteasome system [6]. The biological processes regulated by ubiquitination include proteasomal protein degradation, receptor endocytosis, DNA repair, gene transcription, kinase activation, protein-protein interactions, and the assembly of signaling complexes, thereby broadly regulating immune signaling [7]. For ubiquitination, the E3 ubiquitin ligases are crucial for ubiquitin modification. It has been found that E3 ubiquitin ligases could mediate the transfer of ubiquitin to substrate proteins, including oncoproteins and tumor suppressor proteins. Therefore, their identification and targeting are a current focus of cancer research [8, 9]. The tripartite motif-containing (TRIM) protein family is classified as one of the largest subfamilies of the E3 ubiquitin ligases with 70 known members [10]. There is growing evidence that TRIM proteins play significant roles, and their dysregulation could contribute to diseases such as cancer, immunological disorders, or developmental disorders [11]. Among TRIMs, TRIM54 is associated with proliferation and migration in hepatocellular carcinoma and gastric cancer [12, 13]. However, its function and regulatory mechanism in immune cells within the TME have rarely been reported.

For cancer treatment, immunotherapy, an advanced strategy, can mobilize the patient's immune system to produce durable anti-tumor effects with fewer side effects, making it a hot research topic [14, 15]. And in immunotherapy, treatments based on immune checkpoints like programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4) function by removing the inhibitory brakes on T cells so that the immune system becomes highly activated and produces effective antitumor immunological responses, which have been applied to treat a variety of cancers and achieved sound curative effects [15, 16]. In addition to targeting T cells, immune checkpoint inhibitors can activate other cells of the innate and adaptive immune systems, working in concert to coordinate an efficient response against cancers [17]. Furthermore, studies on innate immune checkpoint inhibitors are ongoing and may once more revolutionize immune-based cancer therapy [18]. Given the importance of ubiquitination in regulating immune cells, targeting ubiquitination in immune cell activation and checkpoint inhibition could be a new avenue for tumor immunotherapy [19, 20].

In this study, we conducted a comprehensive investigation of TRIM54 across various cancers, encompassing expression analysis, prognostic value, clinical characteristics, enrichment analysis, and genetic alterations. After that, we investigated the relationship between TRIM54 expression and immune response to explore its role in immunotherapy and its potential as a therapeutic target.

2. Materials and methods

2.1. Data collection and processing

The RNA sequence and relevant clinicopathologic data of the samples were obtained from The Cancer Genome Atlas (TCGA, https://www.cancer.gov/tcga) and the Genotype-Tissue Expression (GTEx, https://www.gtexportal.org/) databases. The data were statistically analyzed in the R program after log2 transformation, and the “ggplot2” R package was used to display the results. The Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database was used to generate the body map showing TRIM54 expression in tumor tissues and their corresponding normal tissues [21]. The darker color means higher TRIM54 expression. Single-cell sequencing data and immunofluorescence staining of TRIM54 across pan-cancer were obtained from the Human Protein Atlas (HPA, http://www.proteinatlas.org/) database. The gene expression matrices for cell lines were obtained from the Cancer Cell Line Encyclopedia (CCLE; https://portals.broadinstitute.org/ccle/about) and the HPA database.

2.2. Pathologic stage analysis

We explored the expression trend of TRIM54 across pathologic stages using the Gene Set Cancer Analysis (GSCA) database (http://bioinfo.life.hust.edu.cn/GSCA/#/). The GEPIA database was used to examine the correlation between TRIM54 expression and tumor pathological stage.

2.3. Survival and diagnostic analysis

Related prognostic information for pan-cancer samples was explored by the “survival” R package and visualized by the “survminer” and “ggplot2” R packages [22], including overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI). The minimum P-value was used as a cut-off to classify patients into high- and low-risk groups. Nomogram was utilized to examine the relationship between clinical characteristics and the prognosis of pan-cancer, using “rms” and “survival” R packages [22]. Additionally, a calibration plot curve was used to evaluate the predictive power of the nomogram. Receiver operating characteristic (ROC) curves were used to assess the diagnostic utility of TRIM54 across pan-cancer and selected tumor types, with an AUC above 0.7 used to demonstrate it. The “timeROC” was used for analysis.

2.4. Protein-protein interaction (PPI) network construction

GeneMANIA (http://genemania.org), a flexible and user-friendly website, can be used to prioritize genes for functional experiments, analyze gene lists, and generate hypotheses about gene functions [23]. We explored the PPI network of TRIM54 using it. In addition, the STRING platform (https://string-db.org/) was used to construct interaction networks between known and predicted proteins [24]. We set the following parameters in the STRING platform: the minimum required interaction score [“medium confidence (0.400)”], the maximum number of interactors to show (no more than 20 interactors), and the active interaction sources were set to all. Subsequently, the PPI network was visualized using Cytoscape (version 3.7.1).

2.5. Gene enrichment analysis

We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of 40 TRIM54-interacting proteins by using “cluster Profiler” and “org. Hs.eg.db” R packages [25]. Then, we utilized the “ggplot2” R package to display the most significant enrichment terms and loop network plots to visualize the genes associated with these significant terms. In addition, we utilized reverse-phase protein array data to calculate pathway activity scores for 10 cancer-related pathways, including TSC/mTOR, RTK, RAS/MAPK, PI3K/AKT, Hormone ER, Hormone AR, EMT, DNA Damage Response, Cell Cycle, and Apoptosis pathways, from 7876 samples of 32 tumor types in the TCGA database. Each cell's number represents the percentage of each cancer type.

2.6. Genomic alteration analysis of TRIM54

cBioPortal (http://www.cbioportal.org) is a database for examining, visualizing, and evaluating multidimensional cancer genomic data [26]. The mutant genetic aspects of TRIM54 were explored using this database. 2922 total samples (including 2583 patients) from ICGC/TCGA (Nature 2020) were evaluated. Gene alterations in TRIM54, including copy number variation (CNV) and DNA methylation, were downloaded from the GSCA database. The hazard ratio indicates the risk of the high-methylation group relative to the low-methylation group. When it is greater than 1, the high-methylation group is considered at a higher risk of death, and vice versa for the low-methylation group.

2.7. Immune cell infiltration and immune modulator genes analysis

The ESTIMATE and immunedeconv algorithms, including TIMER, xCell, MCP-counter, CIBERSORT, EPIC, and quanTIseq, were used to assess immune correlation in TRIM54. The ESTIMATE algorithm was reported to assess the infiltration level of stromal cells and immune cells in tumor tissue. In addition, we downloaded immune cell infiltration data for TRIM54 across pan-cancer from the TIMER2.0 database (http://timer.cistrome.org/). The “ggplot2” R package was used to visualize the heatmaps. The Spearman correlation analysis was performed to examine associations between TRIM54 gene expression and immune-related genes or immune checkpoints across 33 cancer types. The numbers in each cell of the heat map indicate the percentage of each cancer type.

2.8. Immunotherapy and sensitive drug prediction analysis

Using Spearman correlation analysis, the association between TRIM54 and tumor mutational burden (TMB), microsatellite instability (MSI) in pan-cancer was also examined. To investigate the association between TRIM54 and immune checkpoint blockade (ICB) treatment response, we compared gene expression levels before and after ICB treatment and between responders and non-responders across different treatment cohorts in pan-cancer, thereby validating TRIM54's predictive capability for immunotherapy response. The immunotherapy response and biomarker relevance of TRIM54 in immunotherapy cohorts were predicted using the Tumor Immune Syngeneic Mouse (TISMO, http://tismo.cistrome.org) and the Tumor Immune Dysfunction and Exclusion (TIDE, http://tide.dfci.harvard.edu) platforms [27]. TRIM54 mRNA gene expression data and drug sensitivity

data of Genomics of Drug Sensitivity in Cancer (GDSC) and Cancer Therapeutics Response Portal (CTRP) databases were obtained from GSCA and subjected to Pearson correlation analysis.

2.9. Statistical analysis

R (version 3.6.3) and Cytoscape (version 3.7.1) were used to analyze all data. The Wilcoxon rank-sum test was used to assess TRIM54 expression in tumor and normal tissues. The association between TRIM54 expression and clinicopathological traits was assessed using the Kruskal-Wallis Test and Dunn's test. The Kaplan-Meier method and univariate Cox regression were used to plot survival curves. The two-sample t-test was used to identify group differences. To examine the associations between gene expression, Spearman's correlation analysis was used, and statistical significance was assessed. $|R| > 0.1$ were considered as correlations and P value < 0.05 were considered statistically significant.

3. Results

3.1. The mRNA expression map of TRIM54 in tissues and cells

To explore TRIM54 expression, we first analyzed it at the tissue level. Using an interactive bodymap, we found that the median expression of TRIM54 differed between human tumors and standard samples, primarily in the brain, thyroid, lung, liver, and stomach (Fig. 1A). Compared with normal tissues, TRIM54 was markedly downregulated in ACC, BRCA, GBM, HNSC, KICH, LGG, LUSC, OV, PCPG, PRAD, SKCM, TGCT, THYM, and UCEC. In contrast, TRIM54 expression was significantly upregulated in LIHC, LUAD, PAAD, READ, STAD, and THCA tissues (Fig. 1B). TRIM54 expression in paired adjacent non-tumor and pan-cancer tissues was also explored. The findings demonstrated that TRIM54 expression was considerably downregulated in BRCA and KICH relative to paired adjacent non-tumor tissues, but significantly upregulated in COAD, KIRC, LIHC, LUAD, STAD, and THCA (Fig. 1C). Next, by analyzing single-cell RNA sequence (scRNA-seq) data from normal tissues, we found that the top three cell types with the highest TRIM54 mRNA expression were cardiomyocytes, pancreatic endocrine cells, and exocrine glandular cells (Fig. 1D). In cancer cells, data from the CCLE database showed that TRIM54 was expressed in a variety of tumor cell lines, consistent with its expression in tumor tissues (Fig. 1E). And data from the HPA database revealed that the top three TRIM54-enriched cell lines were NTERA-2, HAP-1, and HepG2 (Fig. 1F). To further subdivide the intracellular localization of TRIM54, the subcellular distribution of TRIM54 in A-431, HAP1, and U-2 OS cells was evaluated by immunofluorescence staining. The findings demonstrated that TRIM54 localized to the plasma membrane and cytoplasmic lysate in A-431, HAP1, and U-2 OS cells (Fig. 1G).

3.2. Correlation analysis between TRIM54 expression and pathologic stages in pan-cancer

In addition to the difference between normal and tumor tissues, we also analyzed TRIM54 expression across different pathologic stages, an essential clinical parameter for prognosis. We analyzed TRIM54 mRNA expression across different pathologic stages and displayed the results in a trend plot and a heat map (Fig. 2A&B). We found that only the pathologic stages of CESC, ESCA, KIRC, and UCS were substantially correlated with TRIM54 expression levels (Fig. 2C-F).

3.3. Prognostic and diagnostic value of TRIM54 in pan-cancer

We used the Cox regression analysis to further examine the connection between TRIM54 expression level and survival prognosis in patients with pan-cancer. The results showed that TRIM54 expression significantly predicted OS, DSS, and PFI in multiple tumor types (Fig. 3A-C). We also constructed nomograms to predict the survival risk of clinical patients, using TRIM54 expression and other prognostic factors (Figure S1A-H). And the C-index for OS in BRCA, CESC, KIRC, LUAD, and UCEC was above 0.7, indicating that the nomograms may be better models for predicting survival in these patients than specific prognostic markers. In addition, the calibration curves for the nomograms in CESC, KIRC, LUAD, and UCEC showed strong concordance between predicted and observed 1-, 3-, and 5-year survival (Figure S1M-P). Additionally, we conducted the ROC curve analysis to evaluate the diagnostic utility of TRIM54 in pan-cancer. The findings indicated that TRIM54 mRNA expression might be a useful clinical diagnostic marker for CHOL, ESAD, KICH, LIHC, LUAD, PAAD, READ, and THCA (Figure S2).

3.4. Protein-protein interaction network and functional enrichment analysis of TRIM54 and its related targets

In this part, we first applied the GeneMANIA database to explore the PPI network of TRIM54 and obtained 20 targets associated with TRIM54 (Fig. 4A). Meanwhile, we identified 20 TRIM54-binding targets using the STRING database and subsequently constructed the PPI network in Cytoscape (Fig. 4B). We discovered 39 TRIM54-related targets in total using the analysis above. Then we performed GO and KEGG functional enrichment analyses of TRIM54 and its associated targets (Fig. 4C-F). For GO analysis, the results revealed that the primary biological process (BP) contained protein modification by small protein conjugation or removal, ubiquitin-dependent protein catabolic process, modification-dependent protein catabolic process, modification-dependent macromolecule catabolic process, and muscle cell development; the cellular component (CC) was mainly enriched in supramolecular fiber, supramolecular polymer, supramolecular complex, myofibril, and contractile fiber; the molecular function (MF) was primarily involved in catalytic activity, acting on a protein, zinc ion binding, cysteine-type peptidase activity, ubiquitin-like protein transferase activity, and thiol-dependent ubiquitinyl hydrolase activity. The KEGG enrichment analysis showed that these genes were mainly associated with ubiquitin-mediated proteolysis, the MAPK signaling pathway, Apoptosis, Protein processing in the endoplasmic reticulum, and Viral carcinogenesis pathways. We further analyzed the potential impact of TRIM54 mRNA expression on the activity of 10 cancer-related pathways [27–31]. The outcomes demonstrated

that TRIM54 might inhibit the activity of the Apoptosis, cell cycle, PI3K/AKT, and TSCmTOR pathways, but have both inhibitory and activating effects on the Hormone AR, RAS/MAPK, EMT, and RTK pathways (Fig. 4G). In addition, we found that TRIM54 affected the activity of the cancer pathways in different cancers to varying degrees (Fig. 4H). Finally, based on GSVA scores, we found that TRIM54 was negatively associated with numerous cancer-relevant pathways across pan-cancers, especially cell cycle, Hormone AR, PI3K/AKT, RAS/MAPK, and RTK (Fig. 4I).

3.5. Genetic alteration analysis of TRIM54 in pan-cancer

To further investigate the regulatory mechanism of TRIM54 expression, we first examined the relationship between gene methylation and TRIM54 expression. We discovered a strong inverse relationship between TRIM54 mRNA expression and gene methylation in 29 of 33 cancer types (Fig. 5A). Next, we further investigated TRIM54 methylation and found that cancer types differed in TRIM54 methylation levels (Fig. 5B). We also performed a prognostic analysis of TRIM54 methylation in pan-cancer. Additionally, the outcomes demonstrated that patients with lower TRIM54 methylation had a higher risk of death than those with higher TRIM54 methylation in LGG and STAD, but the opposite prognostic effect in ACC, LUSC, KIRP, and TGCT (Fig. 5C). We further investigated the association between TRIM54 expression and its CNV in pan-cancer. As shown in Fig. 5D, the proportions of TRIM54 CNVs (including heterozygous amplification, heterozygous deletion, homozygous amplification, and homozygous deletion) varied across cancers. The CNV status of TRIM54 was significantly correlated with its mRNA levels in several tumors (Fig. 5E). CNV heterozygous amplification or deletion occurred commonly in pan-cancer, whereas CNV homozygous amplification occurred in 16 of 33 cancer types, and CNV homozygous deletion occurred in 5 of 33 cancer types (Fig. 5F). Furthermore, we examined genetic alterations in TRIM54 in tumor tissues. For gene alteration, TRIM54 was found to be altered in 2.7% (69/2565) of pan-cancer patients, mainly gene amplification (Figure S3A). Moreover, we investigated how often TRIM54 was altered across different cancer types and found that non-small cell lung cancer (15.22%) had the highest frequency (Figure S3B). To gain insight into the mutational profile of TRIM54 in the protein domain across pan-cancer, our study identified 9 mutant sites, including 7 missense mutations, 1 truncating mutation, and 1 splice-site mutation, located between 0 and 358 (Figure S3C).

3.6. Function of TRIM54 in the tumor microenvironment (TME) and immune checkpoint blockade (ICB) therapy response

To explore the functions of TRIM54 in the TME, we first analyzed the relationship between TRIM54 expression in tumor tissues and immune-related scores in pan-cancer. The findings revealed that, in most malignancies, TRIM54 expression was strongly associated with immune-related scores (Fig. 6A). We further examined the relationship between TRIM54 and immune cell infiltration (ICI) in the TME using six algorithms: XCELL, CIBERSORT, TIMER, QUANTISEQ, EPIC, and MCPCOUNTER. These findings

demonstrated that TRIM54 was strongly associated with ICI across pan-cancer (Fig. 6B and Figure S4). To further clarify TRIM54's role in predicting ICB therapy response, we investigated the association between TRIM54 expression and two well-established immunotherapy predictive biomarkers, TMB and MSI [32, 33]. We found that TRIM54 showed a positive correlation with TMB in ESCA, KIRC, KIRP, LIHC, LUSC, and PAAD, but a negative correlation in ACC, CESC, COAD, DLBC, and HNSC (Fig. 6C). And TRIM54 was positively associated with MSI in ESCA, LGG, LUSC, and TGCT, and negatively associated with MSI in LAML (Fig. 6D). The link between TRIM54 and important immunological checkpoint genes, such as CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT, and SIGLEC1, was also examined. In most cancer types, we found that TRIM54 was strongly associated with immune checkpoint genes, particularly in BRCA, CESC, COAD, KIRC, LIHC, PCPG, STAD, TGCT, THCA, and THYM (Fig. 6E).

3.7. Prediction of immunotherapy response and sensitive drugs based on TRIM54 expression

To comprehensively assess the potential of TRIM54 as a new immunological biomarker, we examined TRIM54 expression in immunotherapy responses and in response to sensitive drugs. The findings demonstrated that, across 3 murine immunotherapy cohorts, TRIM54 could substantially predict immunotherapy response, with responders more likely to have elevated TRIM54 expression (Fig. 7A). The biomarker relevance of TRIM54 was calculated and compared with standardized biomarkers of immunotherapy response. The results showed that in 7 of the 25 immunotherapy cohorts, the AUC of TRIM54 alone exceeded 0.5. And TRIM54 displayed the same predictive value as B. Clonality (Fig. 7B). To identify TRIM54-related drugs, we examined the relationship between TRIM54 mRNA levels and drug sensitivity in the CTPR and GDSC databases. Significantly, JQ-1, belinostat, and dacarbazine were negatively correlated with TRIM54 expression in CTPR (Fig. 7C). Moreover, TRIM54 expression was also negatively associated with Cetuximab, KIN001-055, and KIN001-236 in GDSC (Fig. 7D).

4. Discussion

Expression alterations of some TRIM family members are associated with carcinogenesis, metastasis, resistance, and recurrence in several types of tumors, which has been the focus of current tumor research [34]. TRIM54 is a member of the TRIM family, yet its relationship with tumors has not been well investigated.

In this work, we first examined TRIM54 expression at the tissue and cellular levels in normal and tumor samples. Additionally, information from the TCGA and GTEx databases showed that TRIM54 expression differed significantly across 20 types of malignancy compared with normal tissue. Secondly, we assessed the association between TRIM54 expression and the pathological stage, OS, DSS, and PFI in patients with 10 tumors and found that TRIM54 was substantially correlated with patients' prognoses. Nomograms and calibration curves validated the results, and ROC curves showed that TRIM54 expression had high diagnostic value across eight tumor types. These findings suggest that TRIM54 can efficiently assist doctors in screening high-risk groups and predicting tumor patient prognosis at various

time points, and is anticipated to be a novel prognostic biomarker for patients. However, it needs further verification in more clinical cohorts.

Numerous studies have demonstrated that the TRIM family influences cancer progression by regulating the cell cycle, apoptosis, EMT, DNA repair, and senescence [35–38]. Given this, we further explored the potential impact of TRIM54 expression on the activity of cancer-related pathways. We found that, across pan-cancer, TRIM54 primarily plays an inhibitory role in apoptosis, cell cycle, DNA damage, Hormone AR, Hormone ER, PI3K/AKT, RAS/MAPK, RTK, and TSC/mTOR pathways. In contrast, it played a more activating role in the EMT pathway. However, the specific regulatory mechanism requires further study in various tumors.

To better understand the potential mechanisms by which TRIM54 contributes to tumors, we also conducted a comprehensive analysis of TRIM54 gene variants. And we found that the differential expression of TRIM54 in non-small cell lung and endometrial cancers was influenced by TRIM54 gene amplification, whereas in BLCA and BRCA, it was also influenced by TRIM54 gene mutations. Abnormal DNA methylation is a central epigenetic regulator driving tumor development, and DNA methylation analysis can be a novel supplement to increase the precision of pathological diagnosis [39, 40]. Our results showed a significantly negative association between TRIM54 expression and gene methylation in 29 cancers. Hypomethylation of the TRIM54 gene was associated with a poor prognosis in LGG and STAD but with a better prognosis in ACC, LUSC, KIRP, and TGCT. Thus, it was tentatively hypothesized that TRIM54 DNA methylation serves a crucial function in pan-cancer diagnosis. Moreover, TRIM54 was mostly positively correlated with CNV across pan-cancers, with homozygous amplification more common than homozygous deletion.

Our enrichment analysis suggested that TRIM54 and its related genes were mainly associated with ubiquitin-mediated protein hydrolysis and cancer-related pathways, consistent with prior findings and indicating their roles in tumors [7, 10]. Whereas ubiquitination could regulate antitumor effects by modulating immune system function in immunotherapies [7, 41], we systematically investigated the mechanism of action of TRIM54 in TME. We found that TRIM54 expression was strongly associated with immune and stromal scores across a range of cancers. In most cancers, TRIM54 expression was not only tightly associated with common immune cell types, including T cells, CD8 + T cells, CD4 + T cells, B cells, neutrophils, DCs, NK cells, and macrophages, but also with immune cell subtypes. These findings indicated that TRIM54 could produce immune-activated TME in most cancers. Moreover, TRIM54 was positively correlated with TMB and MSI across a variety of tumor types. TRIM54 expression was also closely associated with classical immune checkpoint molecules, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT, and SIGLEC1, particularly in BRCA, CESC, COAD, KIRC, LIHC, PCPG, STAD, TGCT, THCA, and THYM.

The TME, which is linked to tumor development and prognosis, comprises tumor cells, tumor-infiltrating immune cells, and stromal components and has been shown to be intimately linked to tumorigenesis and malignant behavior [42, 43]. Related studies suggest that TRIM proteins may play a crucial role in

adaptive immunity by regulating the development and activation of T and B cells through specific antigen-recognition receptors, including T-cell and B-cell receptors [44]. In addition, TRIM family proteins were indispensable regulators of the antiviral effects of neutrophils, macrophages, and DCs in innate immunity [45–47]. The ICB has been a hot research topic in cancer immunotherapy today, essentially involving inhibitors that target CTLA4 and programmed cell death ligand 1 (PD-L1), and activating T cells, thereby restoring the immune system's ability to recognize and attack PD-1 or programmed death ligand 1 (PD-L1) [48]. And TMB and MSI are highly promising predictive biomarkers that interact with PD-L1 [49]. Our results suggested that TRIM54 could effectively drive tumor development and prognosis in the TME and serve as a valid biomarker for predicting response to ICB therapy across various cancers. And we have reason to believe that TRIM54 may be a highly promising biomarker for predicting the response to tumor immunotherapy.

Utilizing open datasets and computational models to identify the most effective, customized treatment medicines has gained popularity [50, 51]. We investigated TRIM54’s potential as a biomarker in the immunotherapy cohort and its association with immunotherapy response and found that TRIM54 has some predictive value. Moreover, our analysis targeting sensitive drugs found that TRIM54 expression was negatively correlated with JQ-1, belinostat, dacarbazine, Cetuximab, KIN001-055, and KIN001-23. JQ-1 could significantly reduce PD-L1 expression in tumor cells and successfully suppress PD-1/PD-L1 signaling [52]. Belinostat, dacarbazine, and Cetuximab were all clinically effective anticancer agents [53–55]. These findings provided a theoretical framework for developing TRIM54-targeting medications.

5. Conclusion

Our study identified that TRIM54 could be a promising prognostic biomarker across pan-cancer and accurately predict response to tumor immunotherapy. These results indicate that TRIM54 is an emerging prognostic biomarker and a potential new immune-related therapeutic target for cancer. We provide additional clues for future exploration of TRIM54's possible function in cancer immunity and therapy.

Abbreviations

TME, tumor microenvironment; NK, natural killer; DCs, dendritic cells; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells; TRIM, tripartite motif-containing; PD-1, death protein 1; CTLA4, cytotoxic T lymphocyte-associated protein 4; TCGA, The Cancer Genome Atlas; GTEx, Genotype Tissue Expression; GEPIA, Gene Expression Profiling Interactive Analysis; HPA, Human Protein Atlas; CCLE, Cancer Cell Line Encyclopedia; GSCA, Gene Set Cancer Analysis; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; ROC, receiver operating characteristic; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CNV, copy number variation; TMB, tumor mutational burden; MSI, microsatellite instability; ICB, immune checkpoint blockade; TISMO, Tumor Immune Syngeneic Mouse; TIDE, Tumor Immune Dysfunction and Exclusion; GDSC, Genomics of Drug Sensitivity in Cancer; CTRP, Cancer of Therapeutics Response Portal; BP, biological process; CC, cellular component; MF, molecular function; ICI, immune cell infiltration; CAF,

cancer-associated fibroblast; Endo, endothelial cell; Eos, eosinophil; HSC, hematopoietic stem cell; Tfh, T cell follicular helper; Tgd, T cell gamma delta; NKT, nature killer T cell.

Declarations

Data Availability Statement

All data generated or analyzed during this study are included in the article and supplementary materials. Further inquiries can be directed to the corresponding author.

Author Contributions

YJL and RJL: Performed the experiments and wrote the manuscript. FWZ and JL: Analyzed the data. YJL and RJL: drew pictures. LHW and XDY: collected the data. YJL and RJL wrote the manuscript. YFY and XBZ: Designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

Competing interests

All authors have no conflict of interest to disclose.

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Figures

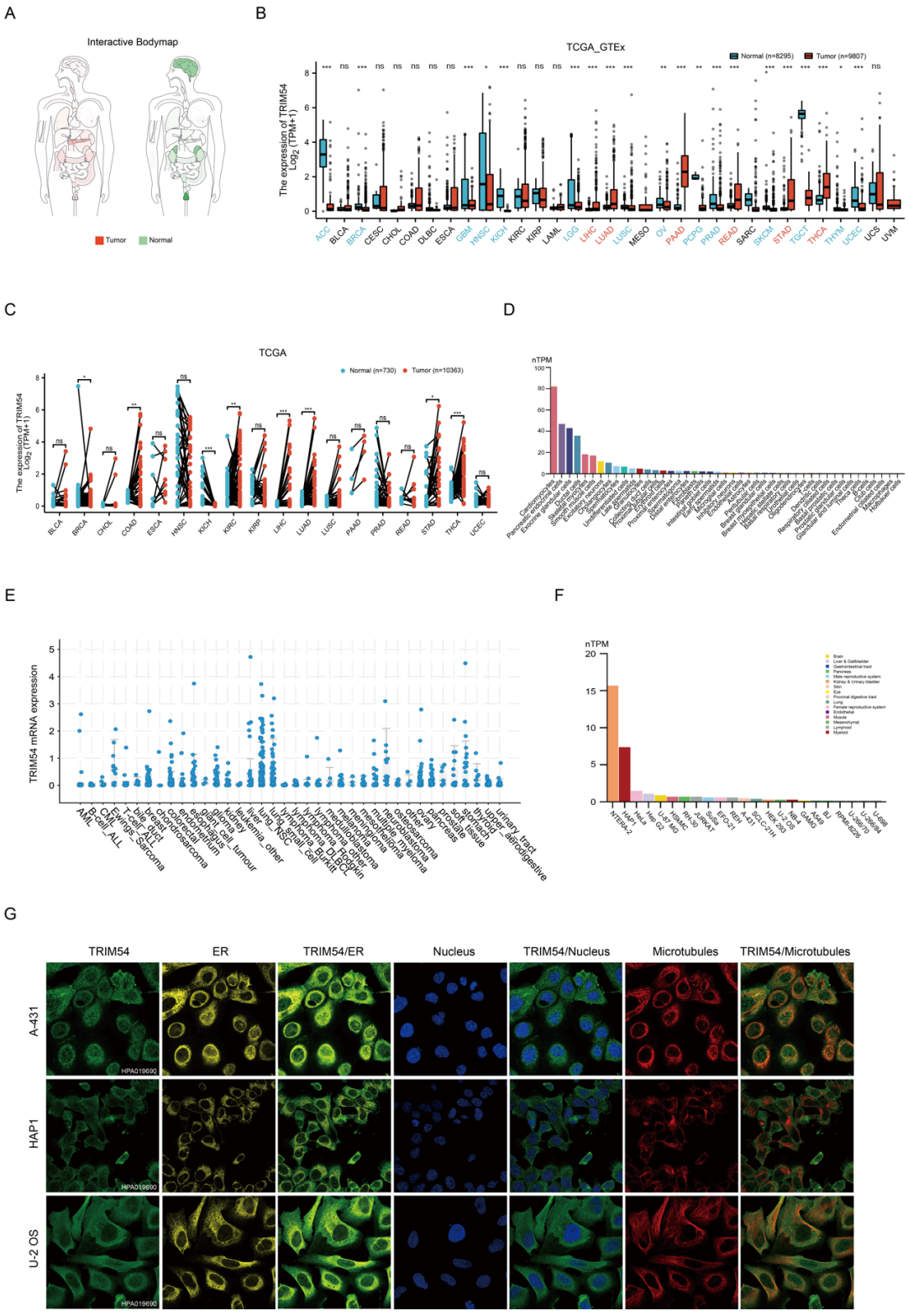


Figure 1

The mRNA expression map of TRIM54 in tissues and cells. **(A)** TRIM54 median expression of tumor and normal tissues in the Interactive BodyMap. **(B)**The TCGA and GETx datasets were used to explore TRIM54 expressions. **(C)**The expression of TRIM54 between pan-cancer and its paired adjacent tissues. **(D)**TRIM54 levels in normal cells based on single-cell RNA sequence data. **(E, F)**TRIM54 levels in tumor cell lines based on the CCLE database **(E)** and the HPA database **(F)**. **(G)** TRIM54 expressions in A-431,

HAP1, and U-2 OS cells were detected using immunofluorescence staining of the microtubules, endoplasmic reticulum, and nucleus through the HPA database. *P < 0.05, **P < 0.01, ***P < 0.001.

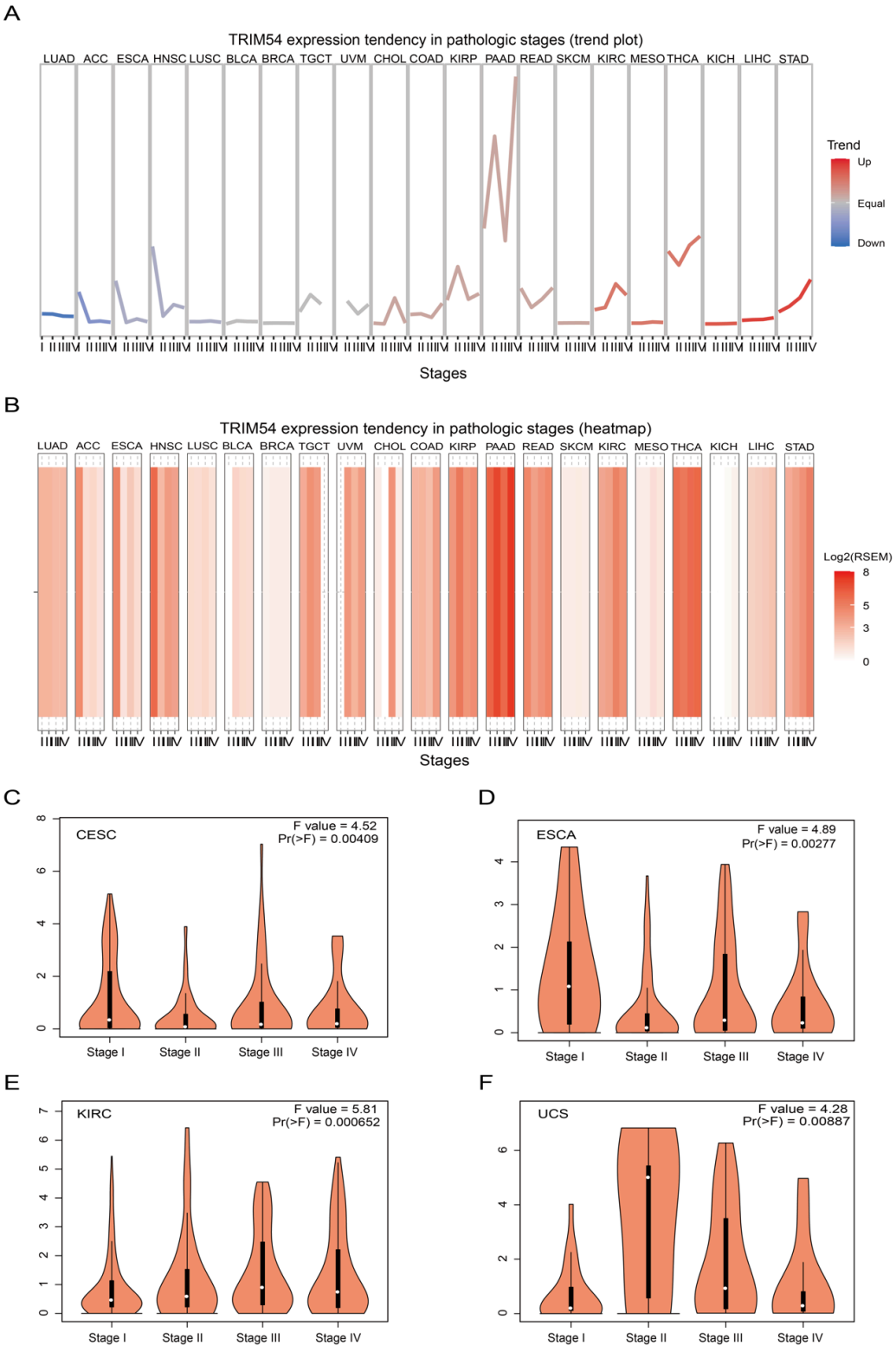


Figure 2

Correlation analysis between TRIM54 expression and pathologic stages in pan-cancer. **(A, B)** TRIM54 expression tendency in pathologic stages was demonstrated by trend **(A)**and heat maps **(B)**. **(C-F)**

Relationships between the TRIM54 expression and pathological stage in CESC **(C)**, ESCA **(D)**, KIRC **(E)**, and UCS **(F)** from GEPIA.

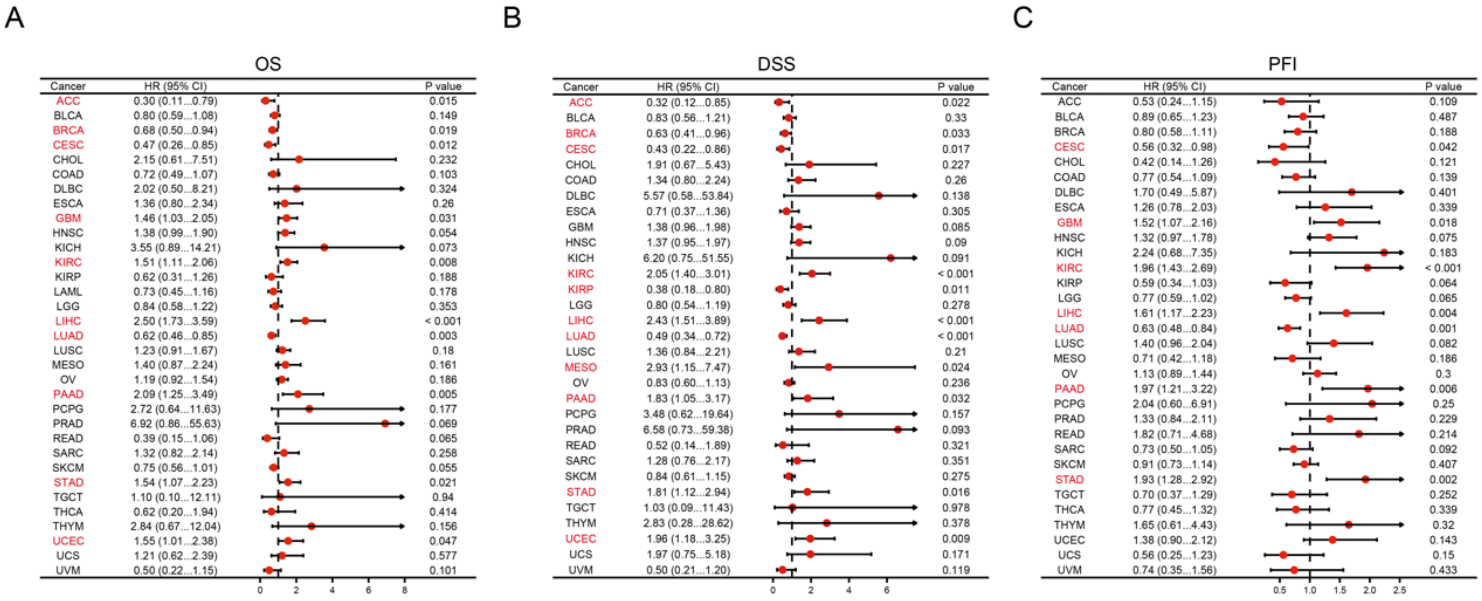


Figure 3

Prognostic value of TRIM54 in pan-cancer. **(A-C)** Prognostic analysis of TRIM54 on OS **(A)**, DSS **(B),** and PFI **(C)** across pan-cancer was displayed in a forest plot.

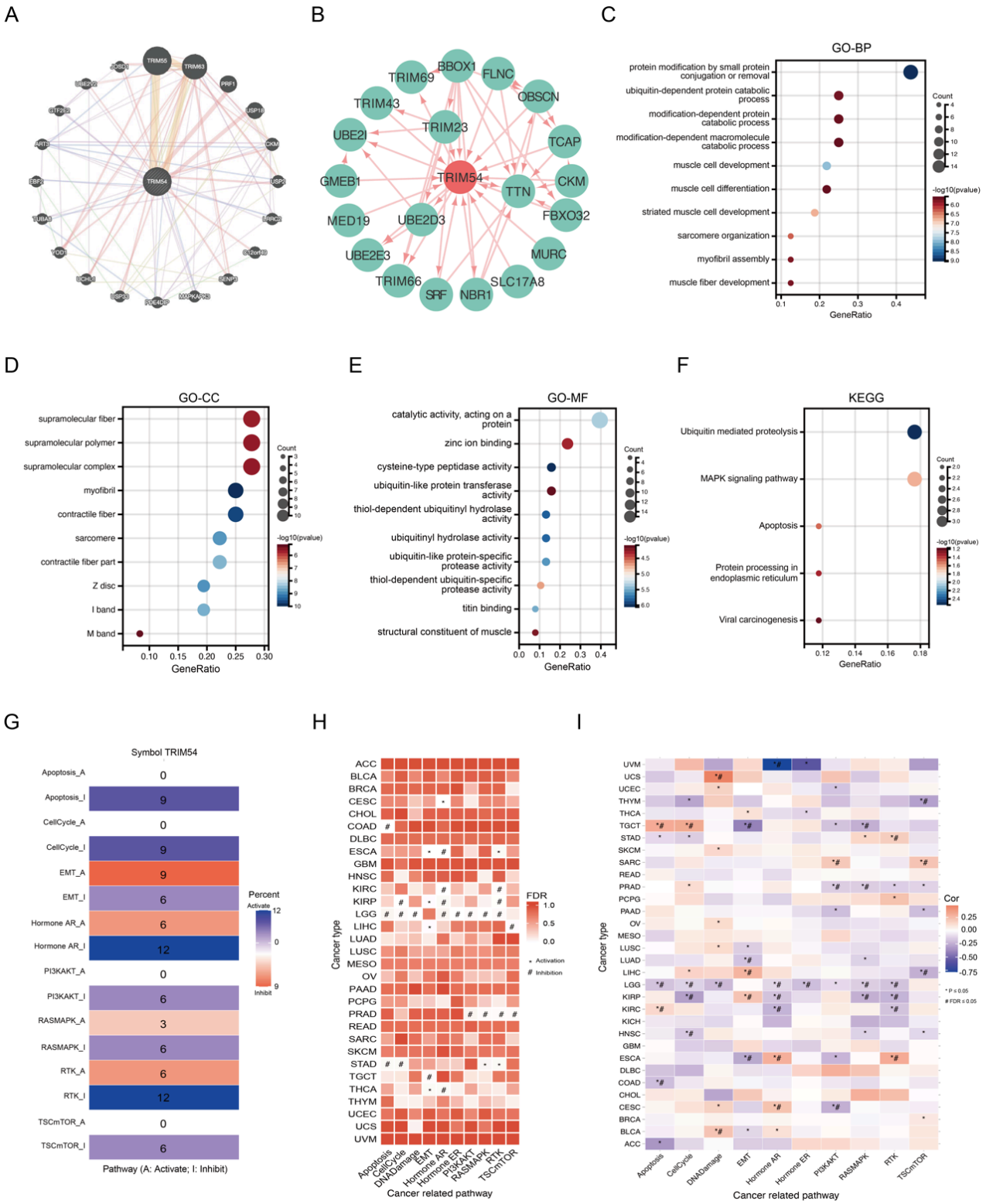


Figure 4

Protein-protein interaction (PPI) network and functional enrichment analysis of TRIM54.**(A-B)** The PPI networks of TRIM54 and its associated proteins were constructed with the GeneMANIA **(A)** and STRING **(B)** databases. **(C-F)**GO-BP **(C)**, GO-CC **(D)**, GO-MF **(E)**, and KEGG **(F)**enrichment analysis of TRIM54. **(G-H)** The potential effect of TRIM54 mRNA expression on the activity of 10 cancer-related pathways was

demonstrated by percentages **(G)** and heat maps **(H)**. **(I)** Heatmap showing the relationship between 10 cancer-related pathways and TRIM54 GSVA scores across pan-cancer. *P ≤ 0.05, #FDR ≤ 0.05.

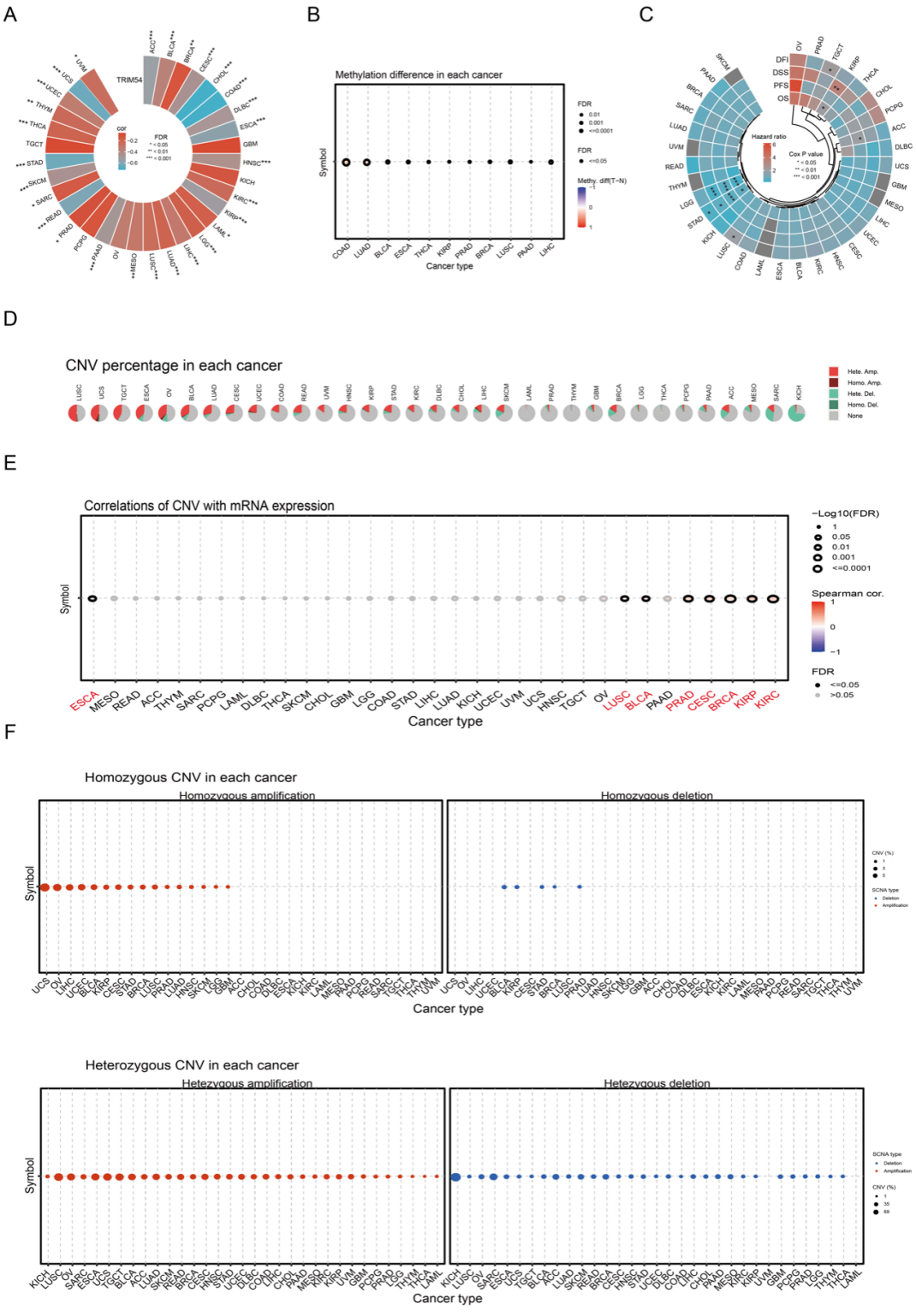


Figure 5

Relationships between TRIM54 mRNA expression, methylation, and gene copy number variation across pan-cancer. **(A)** The correlation between methylation and the mRNA level of TRIM54. *FDR < 0.05, **FDR

< 0.01, ***FDR < 0.001. **(B)**Comparison of the methylation levels between tumor and normal samples of the specified tumor type. **(C)** Summarized the prognostic differences between higher and lower methylation groups in specified sorts of tumors. **(D)**The CNV of TRIM54 in the specified tumor types was depicted as a pie plot. **(E)** The correlation between CNV and TRIM54 expression. **(F)** The homozygous and heterozygous CNV profiles showed the percentage of TRIM54 amplification or deletion in each tumor. *P < 0.05, **P < 0.01, ***P < 0.001.

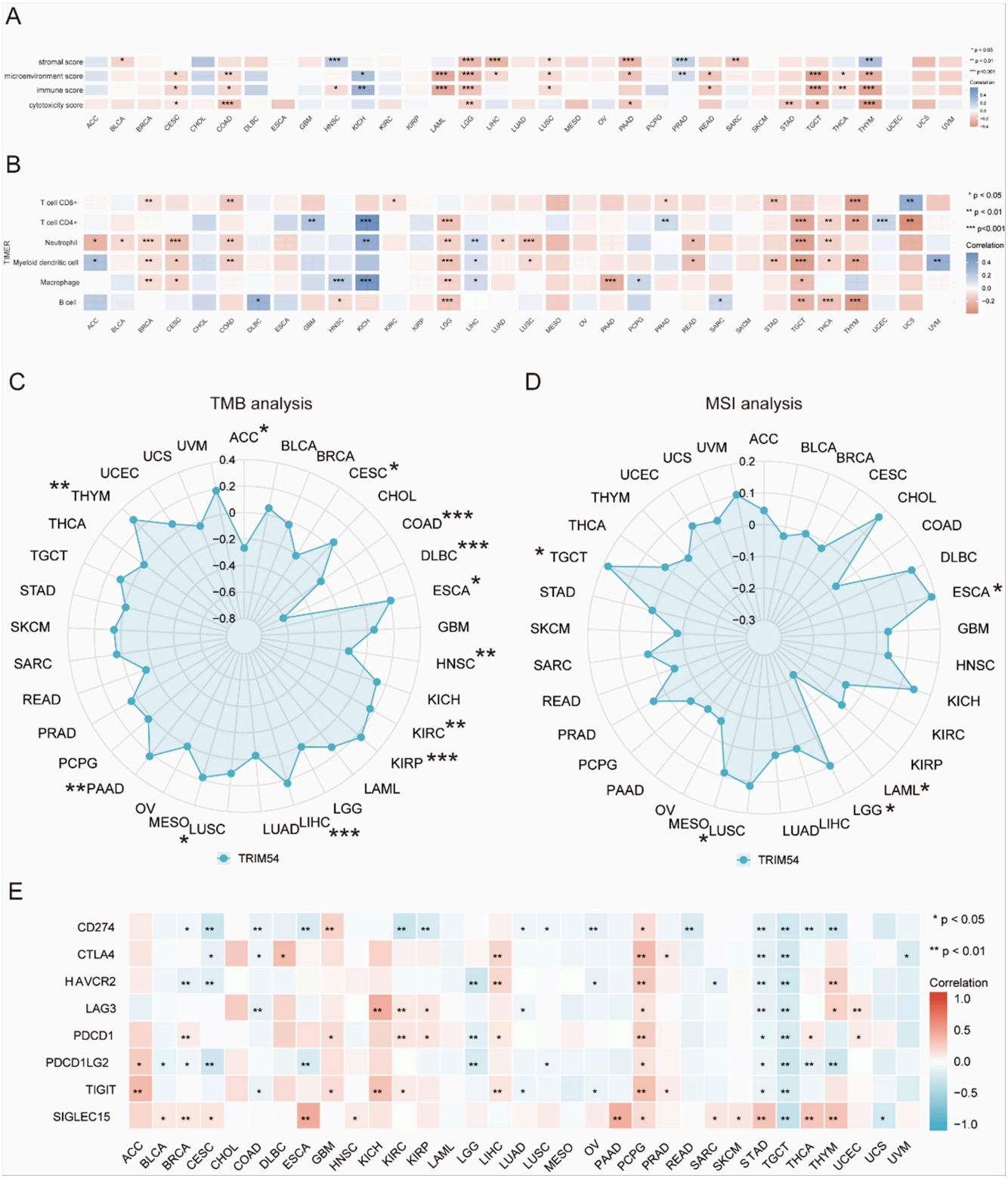


Figure 6

Correlations between TRIM54 expression and TMB, MSI, and immune checkpoints in pan-cancer. **(C, D)** Correlations between TRIM54 expression and TMB **(C)** or MSI **(D)**are shown as radar plots. **(E)** Correlations between TRIM54 and immune checkpoint genes. *P < 0.05, **P < 0.01, ***P < 0.001.

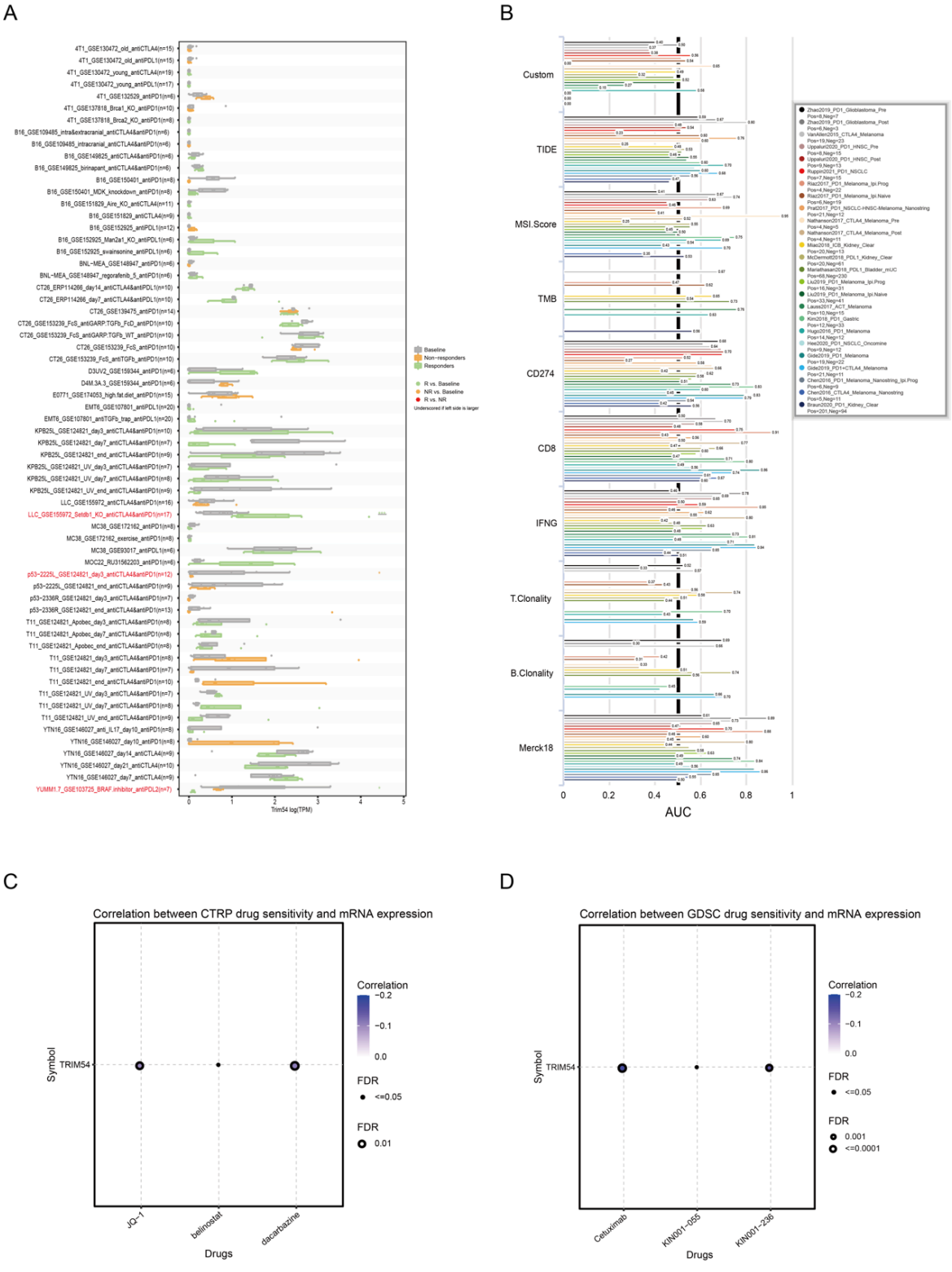


Figure 7

Immunotherapy response, biomarker relevance, and sensitive drug prediction of TRIM54 in pan-cancer. **(A, B)** Immunotherapy response **(A)** and biomarker relevance **(B)** of TRIM54 in immunotherapy cohorts. **(C, D)** Sensitive drug prediction of TRIM54 in pan-cancer based on the CTRP **(C)** and GDSC **(D)** datasets.

Supplementary Files

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- SupplementaryFigures.docx