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Analysis and experimental validation of the innate immune gene PSMD1 in liver hepatocellular carcinoma and pan-cancer

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ABSTRACT

This work intends to examine the diagnostic, prognostic, and biological roles of PSMD1 (proteasome 26S subunit, non-ATPase 1) in liver hepatocellular carcinoma (LIHC) and other malignancies, using bioinformatics techniques. PSMD1 is an innate immune gene that has been identified as a biomarker for several cancers. By analyzing TCGA data, we determined that PSMD1 has excellent diagnostic and prognostic value in LIHC. We also examined its correlation with stage-matching clinical features, particularly T staging and stage staging. Independent prognostic analysis, nomogram, and Decision Curve Analysis (DCA) analysis confirmed the predictive ability of PSMD1 on patient clinical outcomes. Our focus was on exploring the biological process, immune infiltration, and genetic variation in which PSMD1 is involved in LIHC. We found a close relationship between PSMD1 and the tumor microenvironment (TME), as well as various immune cell infiltration, immune function, and immune checkpoints. Furthermore, our results suggested that liver cancer patients with low PSMD1 expression were more actively responsive to immunotherapy according to TIDE predictions. Additionally, we observed significant differences in patient survival based on the different immune molecular types of tumors and their correlation with PSMD1 expression. The close relationship between PSMD1 and copy number variation (CNV), tumor mutational burden (TMB), and methylation was also confirmed, showing a significant impact on patient survival. Moreover, the pan-cancer analysis revealed that PSMD1 is closely related to the diagnosis and prognosis of various cancers, as well as immune infiltration across different cancer types. In summary, PSMD1 has the potential to be a useful diagnostic and prognostic biomarker for LIHC and other types of cancers. It is closely associated with indicators such as immune infiltration, CNV, TMB, and methylation. The identification of PSMD1 may offer a potential intervention target for LIHC and various cancers.

1. Introduction

Liver hepatocellular carcinoma (LIHC) is the principal reason for cancer-related deaths, and the prognosis of advanced LIHC patients is very poor. However, due to the diverse etiological sources of hepatocellular carcinoma, our understanding of the biology of this cancer is still limited [1–3]. Numerous prominent factors that contribute to the development of LIHC have been identified, encompassing hepatitis B and C infections, alcoholic hepatitis, as well as exposure to harmful substances like the fungal metabolite

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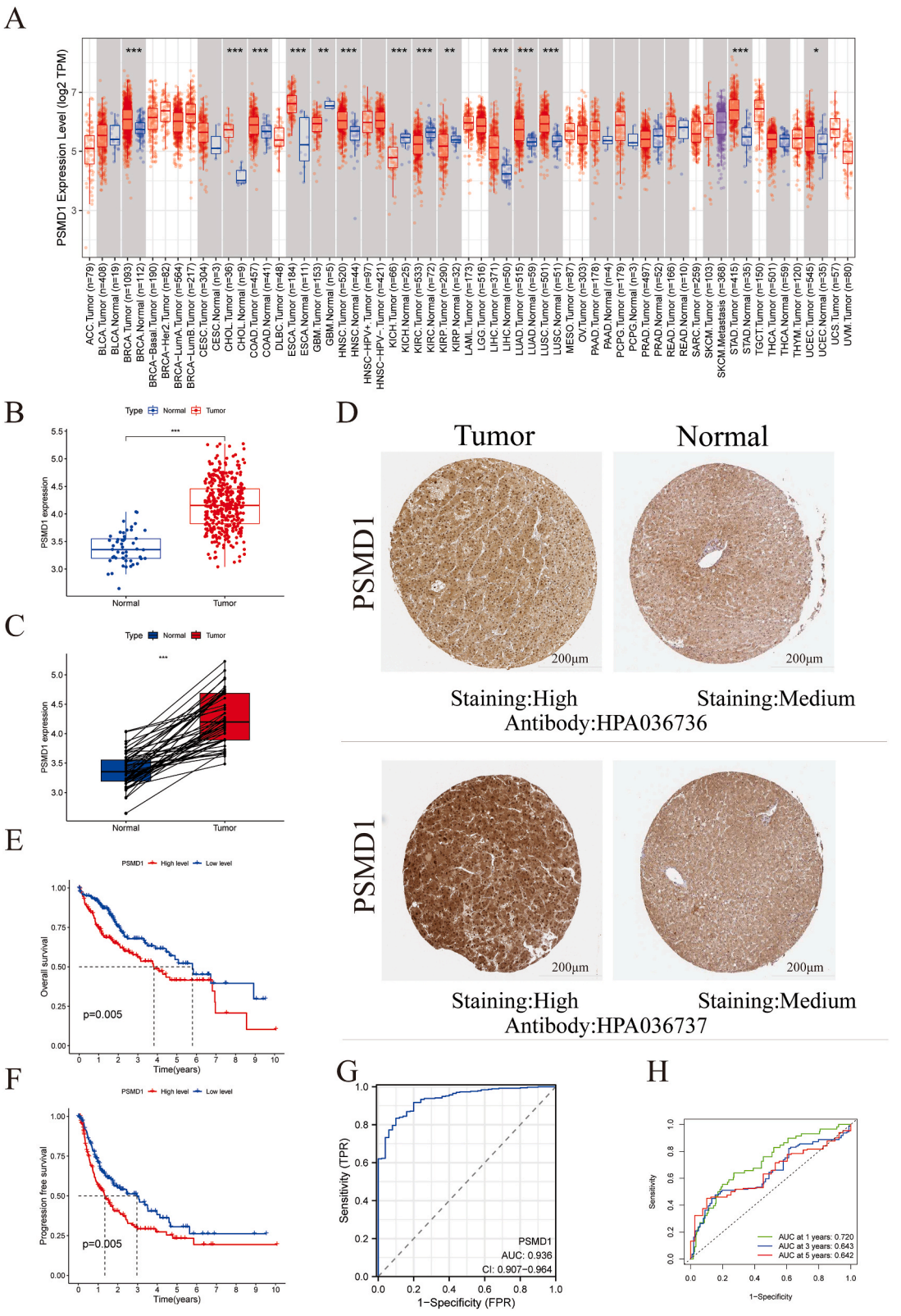

Fig. 1. Diagnostic and prognostic ability of PSMD1 in LIHC. (A). Differential expression of PSMD1 in pan-carcinoma patients. (B). Differences in PSMD1 expression between tissues. (C). Expression differences in paired samples. (D). PSMD1 immunohistochemistry. (E). OS difference curves for different populations. (F). PFS difference curves in different populations. (G). Diagnostic ROC curve. (H). Time-dependent ROC curve.

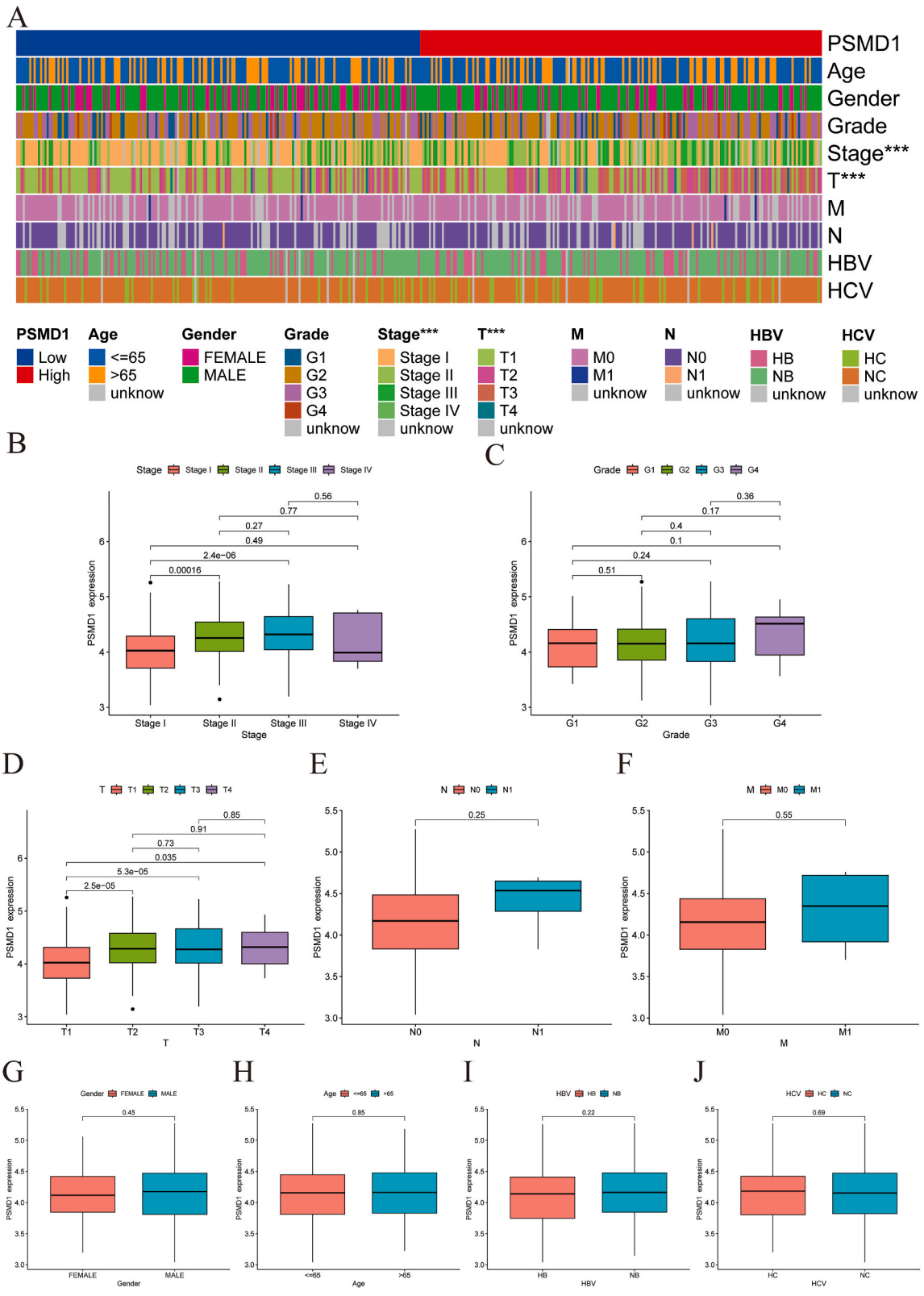

Fig. 2. Clinically matched analysis of PSMD1. (A). Clinical trait classification statistical heat map and correlation. (B–J). Independent matching analysis of clinical traits. (B). Stage staging. (C). Grade grading. (D). T stage. (E). N stage. (F). M stage. (G). Gender. (H). T Age. (I). HBV. (J). HCV.

aflatoxin B1 [4]. Regrettably, the clinical prognosis of LIHC remains poor, and conventional treatment options include drug therapy, interventional therapy, surgical treatment, and liver transplantation [5]. In recent years, there has been a shift towards immunotherapy and novel targeted drugs for the treatment of LIHC, with promising evidence of improved prognosis for LIHC patients [6]. This expansion of treatment options highlights the need to identify key biomarkers that can enrich the available treatment options for LIHC and enhance our understanding of the biological processes involved, ultimately providing more opportunities for the effective treatment of LIHC patients.

The ubiquitin-proteasome system (UPS) is a crucial component in maintaining protein quality control and normal cellular processes in the body. It consists of important components such as ubiquitinases, deubiquitinases, and 26S proteasomes. Dysfunction in the UPS can lead to the development of certain diseases, including cancer [7]. The 26S proteasome plays a vital role in regulating ubiquitinated proteins in human cells and is involved in various microenvironmental regulatory mechanisms, such as DNA synthesis, repair, transcription, translation, and cell signaling [8]. PSMD1 is a component of the 26S proteasome and contributes to its function regulation. PSMD1 belongs to the innate immunity genes [9] and has been found to be closely related to a variety of cancers such as lung adenocarcinoma and breast cancer [10–14].

In this study, we investigated the prognosis and function of PSMD1 in LIHC by a thorough, multiscale bioinformatics analysis. Firstly, we verified PSMD1's diagnostic and prognostic utility in LIHC. Then, we performed stage-matched analysis of clinical traits to gain a detailed understanding of the specific processes associated with PSMD1. After conducting independent prognostic analysis, we confirmed the excellent prognostic efficacy of PSMD1 in the clinic using a nomogram model and DCA (Decision Curve Analysis). Through GO (Gene Ontology) function, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, DO (Disease Ontology) disease, and GSEA (Gene Set Enrichment Analysis) enrichment analysis, we identified the biological processes in which PSMD1 may participate in LIHC. Furthermore, we conducted specific analyses focusing on immune-related aspects, including TME (Tumor Microenvironment) analysis, immune cell infiltration, and immune function analysis. These analyses confirmed the presence of immune cell and functional differences in different expression populations. Additionally, we discovered a close association between PSMD1 and immune checkpoints. Differential analysis using TIDE (Tumor Immune Dysfunction and Exclusion) predicted that the low-expression population is more sensitive to immunotherapy, providing a foundation for LIHC immunotherapy. Our study reveals that PSMD1 is not only closely associated with tumor immune molecular typing, but also contributes to various genetic variations such as TMB, CNV, and methylation. These variations are strongly correlated with the abnormal expression of PSMD1 and have a significant impact on prognosis. Our findings demonstrate that PSMD1 is highly sensitive in diagnosing multiple cancers and has a significant negative impact on prognosis. Additionally, we found that PSMD1 contributes to immune cell infiltration in several malignancies. Therefore, we conclude that PSMD1, as an innate immune gene, holds promise as a biomarker for identifying LIHC and gauging its prognosis. Furthermore, it exhibits substantial potential in the diagnosis and prognosis of various types of cancers.

2. Results

2.1. PSMD1 diagnostic and prognostic value in LIHC

The analysis comparing the tumor group and adjacent cancer in PSMD1 in pan-cancer revealed significant expression differences. PSMD1 exhibited high expression in BRCA, CHOL, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, STAD and UCEC, but a negative correlation in GBM, KICH, KIRC and KIRP (Fig. 1A). Furthermore, differential expression analysis in LIHC demonstrated that tumor samples had increased PSMD1 expression compared to the control group ($p < 0.001$) (Fig. 1B). Paired sample analysis yielded the same result ($p < 0.001$) (Fig. 1C), suggesting that PSMD1 could serve as an important diagnostic marker for LIHC. Immunohistochemical analysis using two antibodies showed that LIHC tissue exhibited darker staining and higher protein expression than normal liver tissue (Fig. 1D). Survival difference analysis indicated that a poorer prognosis was related with greater PSMD1 expression($p = 0.005$) (Fig. 1E), which was also supported by progression free survival (PFS) analysis ($p = 0.005$) (Fig. 1F). Diagnostic ROC analysis demonstrated that PSMD1 had excellent diagnostic value for LIHC and accurately identified LIHC patients (AUC = 0.936) (Fig. 1G). The multi-time point time-dependent ROC curve revealed that PSMD1 had high accuracy in predicting patient survival, particularly within the first year (AUC = 0.720) (Fig. 1H). In conclusion, PSMD1 could be an important indicator for distinguishing LIHC patients and predicting clinical outcomes.

2.2. Analysis of clinical characteristics

After analyzing the common clinical characteristics of each sample, including STAGE, GRADE, T, N, M, GENDER, AGE, HBV, and HCV, we created a heat map to visualize the relationship between these traits and the expression of PSMD1. The results indicated that STAGE and T stages significantly differed in PSMD1 expression($P < 0.001$) (Fig. 2A). We used stage matching analysis to look into the relationship between genes and clinical traits.

The results showed a trend toward positive connection between PSMD1 expression and both STAGE and T stage. This shows that PSMD1 is essential for the development of LIHC and may be intimately related to tumor development. (Fig. 2B–J).

2.3. PSMD1 clinical predictive ability

We conducted an independent prognostic analysis on PSMD1, considering its expression level and common clinical traits. Both univariate and multivariate prognostic analyses demonstrated that PSMD1 has a substantial impact on LIHC development. This

proposes that PSMD1 may independently contribute to adverse patient survival as a risk factor (P ≤ 0.001) (Fig. 3A and B). Furthermore, we created a Nomogram model that took PSMD1 expression levels and clinical characteristics into account. Our findings revealed that PSMD1 can serve as a highly sensitive indicator for predicting patient survival time, resulting in excellent overall accuracy of the Nomogram model (P < 0.05) (Fig. 3C). Additionally, the Calibration curves at 1, 3, and 5 years closely aligned with the standard line, demonstrating the exceptional predictive ability of PSMD1 in clinical aspects (C-index = 0.719) (Fig. 3D). Moreover, our

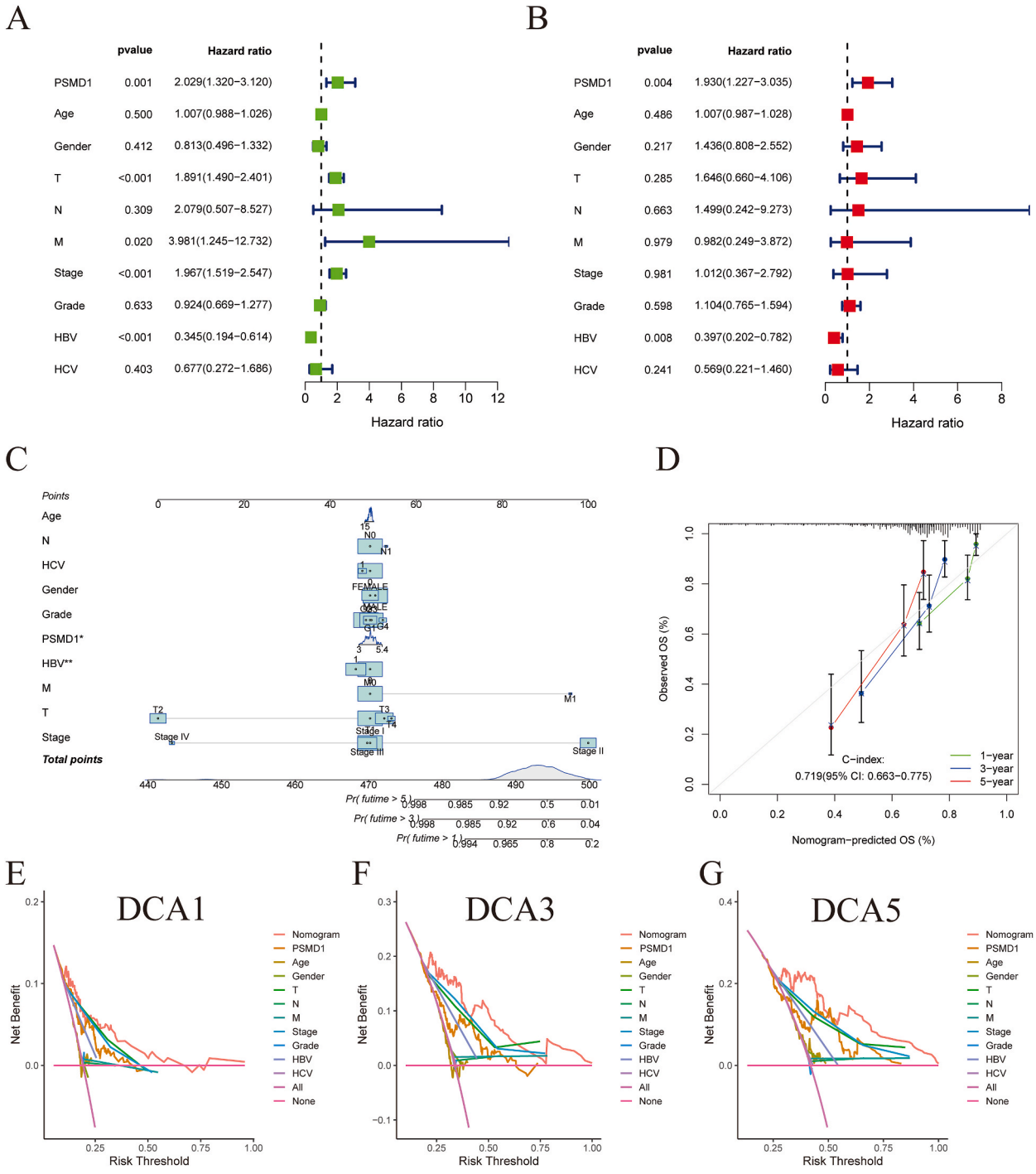

Fig. 3. Prognostic value of PSMD1 in patients with LIHC. (A). Univariate independent prognostic analysis. (B). Multi-factor independent prognostic analysis. (C). Synthetic multi-factor nomogram model. (D). Calibration curve. (E). DCA curve of PSMD1 and clinical traits in first-year. (F). DCA curve of PSMD1 and clinical traits in 3rd year. (G). DCA curve of PSMD1 and clinical traits in 5th year.

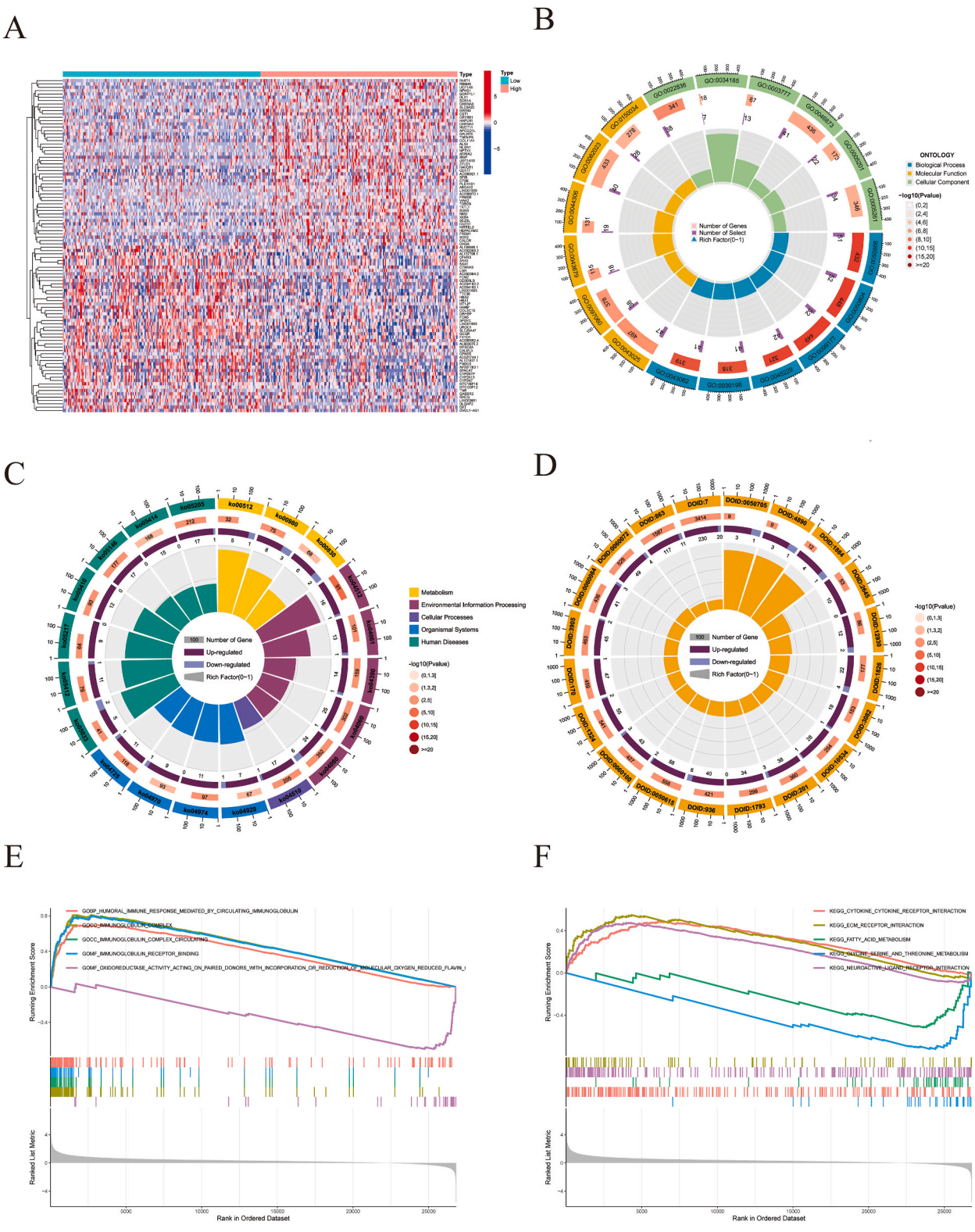

Fig. 4. Biological behavior analysis of PSMD1 in LIHC. (A). Differential gene heat map of different expression populations. (B). GO functional enrichment analysis. (C). KEGG pathway enrichment analysis. (D). DO disease enrichment analysis. (E). GSEA-based GO functional enrichment analysis. (F). GSEA-based KEGG pathway enrichment analysis.

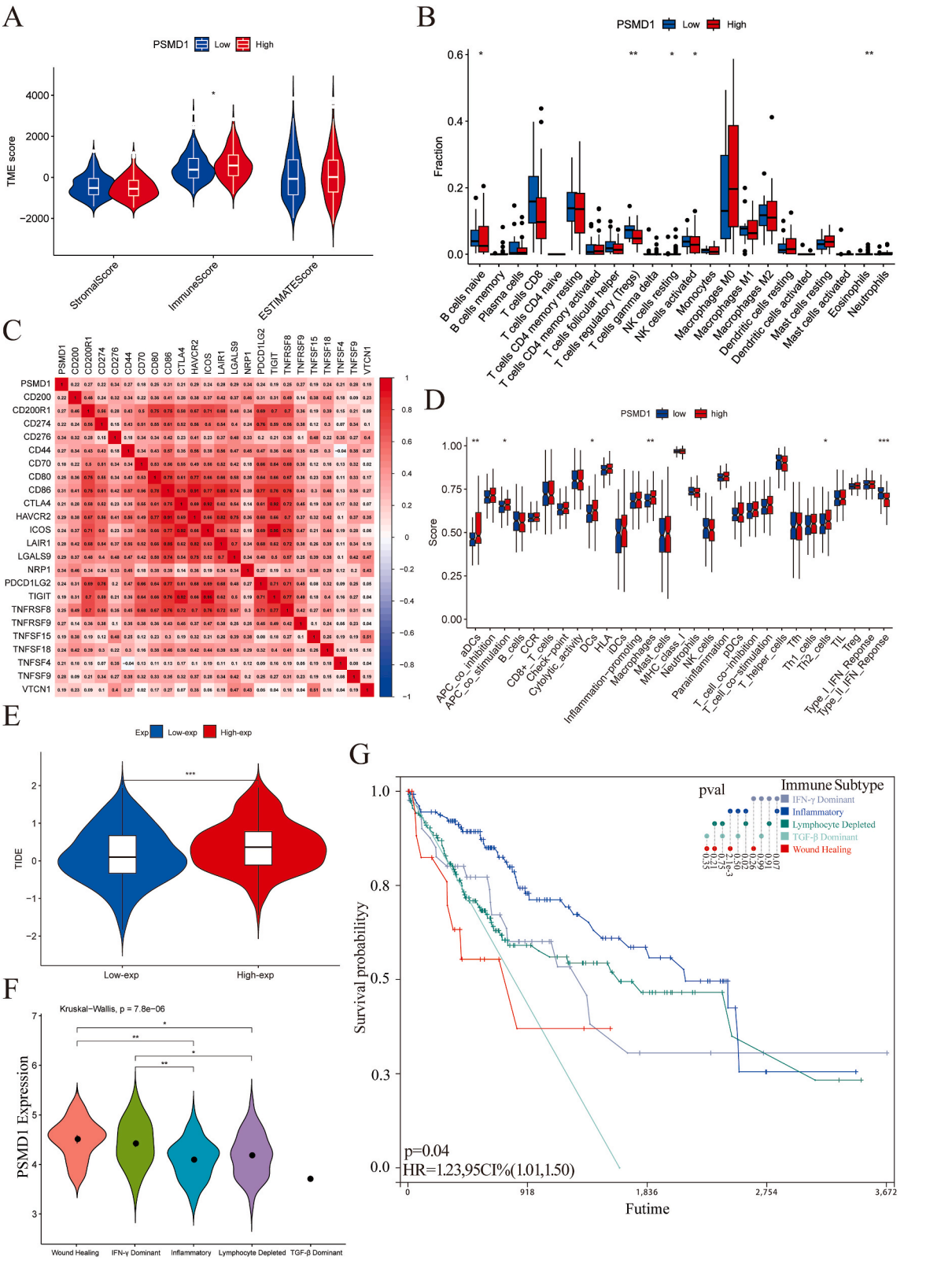

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Fig. 5. Immune-related analysis of PSMD1. (A). Differences in TME in different expression populations. (B). Differences in immune cell infiltration. (C). PSMD1 correlation analysis with immune checkpoints. (D). Differences in immune function in different expressed populations. (E). TIDE differential analysis of PSMD1 expression and immune evasion ability. (F). Differences in PSMD1 expression in tumor immunophenotyping. (G). Survival varies between immunotypes.

1, 3, and 5-year DCA analysis comparing the prognostic ability of PSMD1 indicated that it can be used as a single index with similar performance to the nomogram model in predicting the clinical outcome of patients (Fig. 3E–G).

2.4. Analysis of the potential mechanism of action of PSMD1

In this study, we first identified the differential genes associated with PSMD1 based on their expression levels. We then generated a correlation heat map (Fig. 4A) to visualize the relationship between these genes. Subsequently, we conducted an analysis to explore the biological behaviors associated with these differential genes. Our functional enrichment analysis revealed that molecular functions such as “synapse organization” (GO:0050808), “neuronal cell body” (GO:0043025), and “gated channel activity” (GO:0022836) were particularly important. Additionally, we analyzed the most significant biological processes in terms of cell group using GO terms (Fig. 4B). Furthermore, our KEGG analysis highlighted “ko04512 ECM-receptor interaction” and “Mucin type O-glycan biosynthesis” as the most significant pathway (Fig. 4C). Lastly, we performed an enrichment analysis of diseases and found a significant association between PSMD1 and “DOID:1884 viral hepatitis” based on the Disease Ontology (Fig. 4D).

We investigated PSMD1 functional and pathway variations, using gene set enrichment analysis (GSEA).Our findings reveal that PSMD1 exhibits significant activity in immune-related functions, specifically in the "Immunoglobulin Complex" function. Conversely, it shows significant silencing in functions related to "oxidoreductase activity" (Fig. 4E). Additionally, our analysis suggests that PSMD1 may be involved in positive regulation pathways, such as "ECM Receptor Interaction", while inhibiting pathways like "Glycine Serine and Threonine Metabolism" (Fig. 4F). Our research suggests that PSMD1 may have an impact on tumor development via modifying biological immune response and receptor-ligand interactions.

2.5. Analysis of PSMD1 immunoinfiltration

Tumor formation is significantly influenced by changes in the immunological microenvironment. In our investigation of the variances in the tumor microenvironment comparing LIHC and PSMD1, we observed that the immuneScore exhibited elevated levels in the group with high expression of PSMD1 ($P < 0.05$) (Fig. 5A). We then conducted a detailed analysis of the immune infiltration status of PSMD1 in LIHC. In the PSMD1 high expression group compared to the low expression group, there were considerably more eosinophils and resting NK cells present, but the content of B cell naïve, T cell regulation (Tregs), and activated NK cells exhibited the opposite trend ($P < 0.05$) (Fig. 5B). Furthermore, PSMD1 and immunological checkpoints have a very high positive association, according to the analysis of immune checkpoints. The overexpression or overactivation of immune checkpoint molecules caused by PSMD1 overexpression ultimately hinders immune function, diminishing the body’s immunity. As a result, individuals become prone to LIHC (Fig. 5C). We also analyzed the differences in immune function between different expression groups. The high-expression group exhibited stronger aDCs, APC co-stimulation, DCs, Macrophages, and Th2 cells, while Type_II_IFN_Response was more significant in the low-expression group ($P < 0.05$) (Fig. 5D). In addition, we utilized TIDE to predict the immune escape ability of various expression groups. The TIDE score of the PSMD1 high expression group was significantly higher, indicating that this group was more likely to experience immune escape ($P < 0.001$) (Fig. 5E). When classifying the patients based on immune type, we observed a significant difference in the expression of PSMD1 among the different immune type groups ($P = 7.8e\text{-}06$) (Fig. 5F). Analysis of the subsequent survival among various groups classified by immune type indicated notable variations in survival duration, particularly when comparing the Inflammatory and Wound Healing groups ($P = 0.04$) (Fig. 5G).

2.6. Gene mutation landscape and methylation modifications

To investigate the relationship between PSMD1 and genetic variation, we conducted gene mutation and methylation studies. We generated a mutation waterfall diagram based on the differential expression of PSMD1. The analysis revealed that Missense Mutation was the predominant mutation type across different populations. In our analysis, we successfully identified the top 15 genes exhibiting the greatest frequency of mutations. Notably, TP53 emerged as the gene with the most significant mutations across both groups. Curiously, TP53 displayed a higher frequency of mutations within the population exhibiting high expression levels (Fig. 6A). Additionally, we compared the tumor mutational burden (TMB) between different expression populations and observed a more pronounced TMB in the high-expression group ($P = 0.046$) (Fig. 6B). Survival differential analysis revealed that patients with high TMB tended to have a worse prognosis ($P = 0.035$) (Fig. 6C). To further explore patient survival, we combined PSMD1 expression with TMB and found significant differences in survival among patients with different expression levels and mutations. Notably, patients with both high TMB and high PSMD1 expression had the worst prognosis. Moreover, patients with high PSMD1 expression generally had a poorer prognosis, suggesting that PSMD1 expression might have a detrimental effect on survival ($P < 0.001$) (Fig. 6D).

We conducted an analysis of PSMD1 expression data and copy number variation (CNV) in LIHC. Our findings revealed that among the patients, 13 had copy number gain, 14 had copy number deletion, and there was a positive correlation between PSMD1 expression and mutation type, specifically in the categories of Gain, Neutral, and Loss. Notably, PSMD1 expression differed significantly amongst

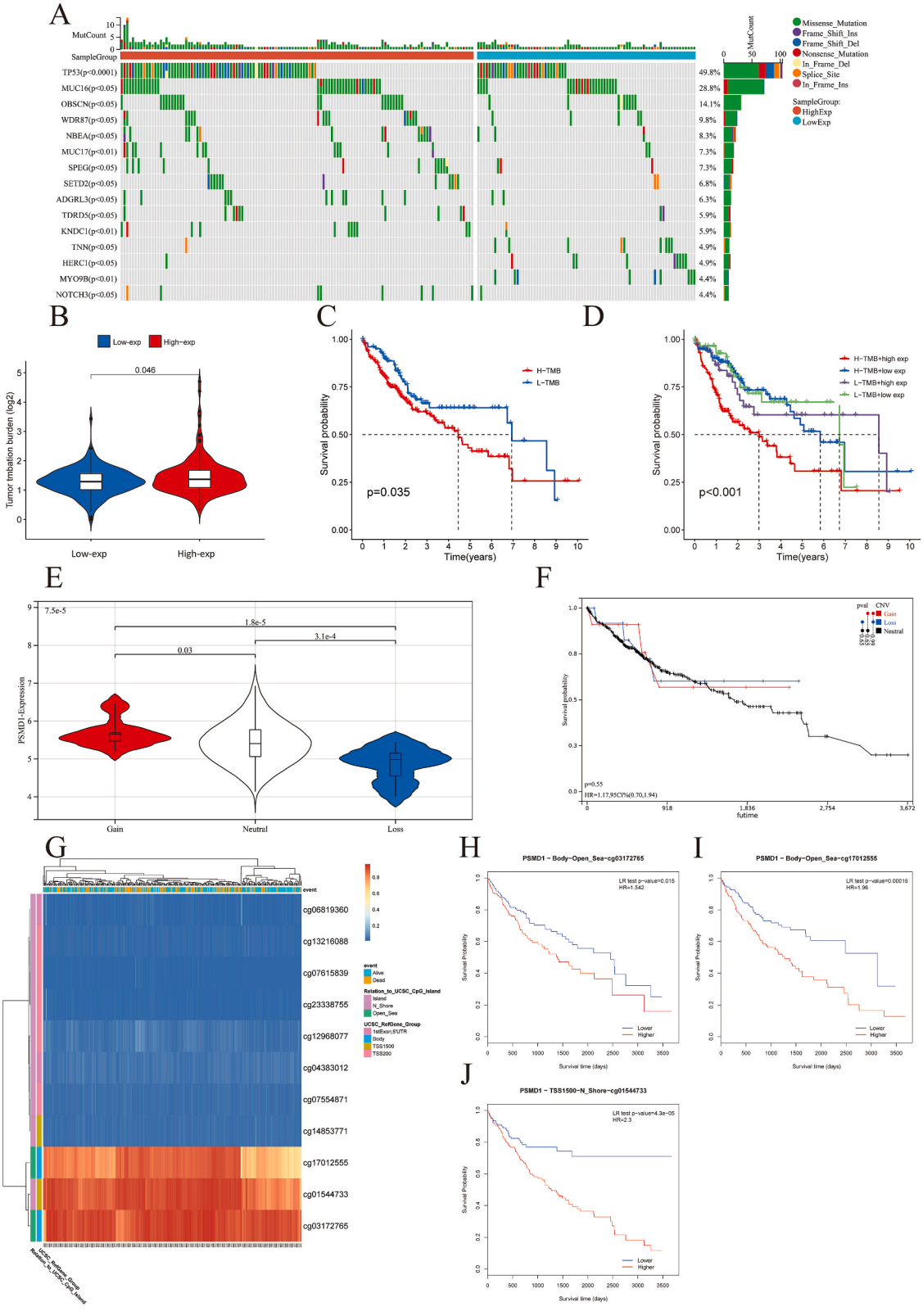

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Fig. 6. PSMD1 mutation and methylation analysis. (A). PSMD1 tumor mutation landscape. (B). TMB difference analysis in different populations. (C). Analysis of survival differences among different TMB populations. (D). Analysis of paired survival differences between TMB and different expression. (E). CNV and expression analysis. (F). CNV and survival analysis. (G). Distribution of methylation between CpG islands in different patients. (H). Survival differences in the cg03172765 region. (I). Survival differences in the cg17012555 region. (J). Survival differences in the cg01544733 region.

these groups. (P = 7.5e-5) (Fig. 6E). However, when we examined the relationship between CNV and survival, we found no significant difference in the survival rates of the various CNV mutation populations (P > 0.05) (Fig. 6F). Furthermore, we analyzed the methylation patterns of PSMD1 in different regions of LIHC. Our results indicated significant methylation in cg03172765, cg17012555, and cg01544733 (Fig. 6G). Subsequently, we performed a survival difference analysis on the groups with methylation in these regions and discovered that the degree of methylation played a detrimental role in patient survival (P < 0.05) (Fig. 6H–J).

2.7. Validation and treatment of PSMD1

We utilized four GEO cohorts (GSE19665, GSE29721, GSE62232, and GSE112790) to investigated the expression of PSMD1 in various tissues. The results indicated a significantly higher expression of PSMD1 in the tumor group compared to normal tissues (P < 0.001) (Fig. 7A–D). Subsequent diagnostic ROC curve analysis demonstrated the effective diagnostic potential of PSMD1 for LIHC (AUC>0.9) (Fig. 7E–H). Thus, we have confirmed that PSMD1, as a prognostic gene, can serve as a sensitive marker for identifying LIHC.

We performed single-cell profiling of PSMD1 in a sample, clustering the cells and annotating them with their corresponding immune cell types (Fig. 7I). We determined the proportion of each immune cell type and observed that CD8T cells were the most abundant, followed by CD4T cells and NK cells (Fig. 7J). The gene scatter plot revealed widespread expression of PSMD1 in various immune cells, including B cells and CD8T cells (Fig. 7K). Moreover, we conducted a comparative analysis of immune cell expression and PSMD1 in various tissue types. Our investigation revealed a significant elevation in PSMD1 expression within tumor tissues compared to that of normal tissues among B cells, CD4conv cells, NK cells, Mono/Macro cells, Tprolif cells, Plasma cells, and Treg cells. However, DC cells and Mast cells exhibited the opposite trend (Fig. 7L). In conclusion, Our data indicate that PSMD1 is intimately related to immune cells in multiple tissues, particularly in LIHC tissues. This supports the hypothesis that PSMD1 may impact the development of LIHC by influencing the immune cell environment.

To investigate the targeted treatment of PSMD1 in a clinical setting, we conducted a drug sensitivity analysis and identified drugs that demonstrate sensitivity in different populations. Notably, drugs such as Foretinib and Imatinib exhibited lower drug levels required to reach IC50 in the high PSMD1 expression group (P < 0.001) (Fig. 7M − N). Additionally, potential drugs suitable for the low expression group include Erlotinib and Vorinostat (P < 0.001) (Fig. 7O–P).

2.8. Prognostic value of PSMD1 in pan-cancer

First, we conducted an analysis on the expression of PSMD1 in various types of cancers using the TCGA and GTEx datasets. The findings revealed that out of the 34 tumors examined, PSMD1 was significantly up-regulated in 27 tumors and significantly down-regulated in 4 tumors (P < 0.05) (Fig. 8A). Subsequently, we looked into the relationship between PSMD1 expression and general prognosis in pan-cancer cases. The findings showed that a trend toward a poor prognosis was associated with high expression of PSMD1 in OS, PFI, and DSS. (P < 0.001) (Fig. 8B–D). Moving forward, we further explored the relationship between PSMD1 and prognosis in common cancers. By conducting COX regression analysis on 44 tumors, we observed that elevated PSMD1 expression was linked to shortened OS in 12 tumors (P < 0.05) (Fig. 8E). In the PFI study which encompassed 38 tumors, the COX regression analysis demonstrated that an elevated expression of PSMD1 acted as a risk factor in 10 malignancies (P < 0.05) (Fig. 8F). Additionally, in the DSS analysis of the same 38 tumors, the COX regression analysis revealed that PSMD1 posed as a risk factor in 13 tumors (P < 0.05) (Fig. 8G).

2.9. The value of immune infiltration in pan-cancer

Using various analysis methods, we extensively investigated the connection between immune cell infiltration and PSMD1 in different types of malignancies. Our research findings strongly suggest a significant association between PSMD1 and immune cells. By applying the MCPCOUNTER algorithm, we discovered a favorable regulatory link between PSMD1 and immune cells specifically in KIRP, whereas the analysis of STES and STAD demonstrated an inverse correlation (P < 0.05) (Fig. 9A). Additionally, the TIMER method analysis demonstrated a strong positive regulatory relationship between PSMD1 and immune cells in COADREAD, KIPAN, and KIRC, while a negative regulation was observed in STAD (P < 0.05) (Fig. 9B). Moreover, the EPIC algorithm results revealed a significant association between PSMD1 and common immune cells in STES, THCA, and UVM (P < 0.05) (Fig. 9C). Furthermore, the QUANTISSEQ analysis showed a close relationship between PSMD1 and LAML, KIPAN, and STES, with a significant positive regulation on Macrophages M1 in various cancers (P < 0.05) (Fig. 9D). Lastly, the xCELL analysis displayed a significant correlation between PSMD1 and immune cells in STES and BLCA, a less pronounced association with CD4+T cells, and a positive relationship with ImmuneScore of LIHC (P < 0.05) (Fig. 9E). In conclusion, the examination of immune cell infiltration across different cancers revealed a robust connection between the levels of PSMD1 and immune cells, specifically in LIHC, KIPAN, and THCA.

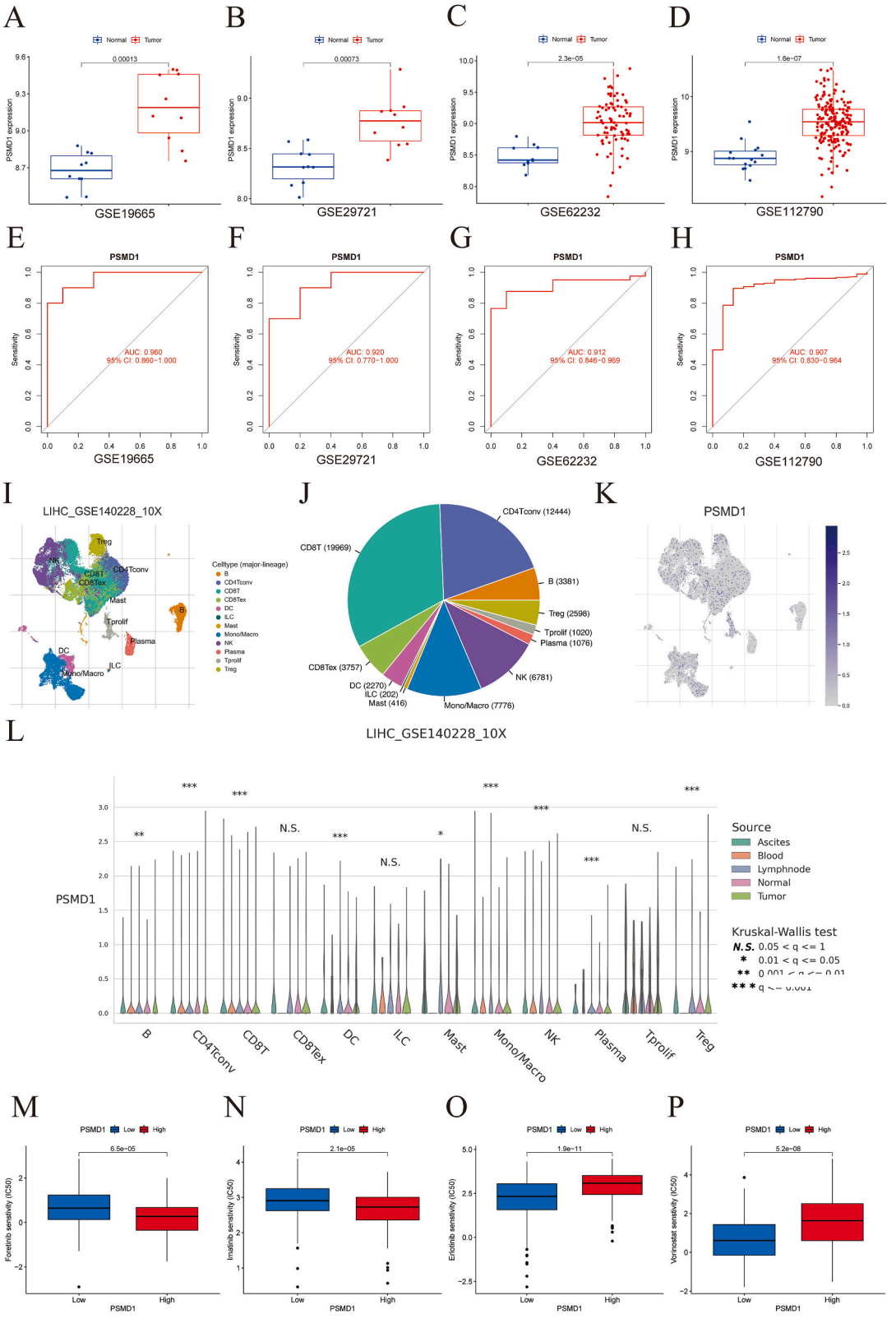

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Fig. 7. Validation, immune evasion and drug sensitivity. (A–D). Multi-dataset expression difference analysis.
(E–H). Multi-dataset diagnostic ROC analysis. (I). Immune cell clustering and annotation. (J). Percentage of immune cell content. (K). Distribution of PSMD1 in different immune cells. (L). Content of PSMD1 in immune cells of different tissues. (M – N). Sensitive drugs for people with high expression volumes. (O–P). Sensitive drugs for people with low expression volumes.

3. Discussion

Liver hepatocellular carcinoma exhibits significant heterogeneity and is generally known to be resistant to chemotherapy [15]. Although chemotherapy can be effective in treating early-stage hepatocellular carcinoma, the progression of the disease and development of chemotherapy resistance often leads to suboptimal outcomes [16]. However, it is important to consider that immunotherapy-based combination therapy or monotherapy with immune checkpoint inhibitors (ICI) shows promise in the treatment of liver cell carcinoma [17]. Current studies have explored the use of atezolizumab and bevacizumab for treating hepatocellular carcinoma, and their effectiveness has been confirmed [18]. Therefore, finding fresh and trustworthy targets is essential in this field. The UPS, an extensively regulated complex of multiple enzymes, plays a crucial role in maintaining protein homeostasis. It has garnered significant attention as a promising therapeutic target against tumors [19]. It functions through the 19S regulatory complex and the 20S core complex. The 26S proteasome is responsible for protein degradation [20]. PSMD1, a member of the 19S complex, has been implicated in the progression and chemotherapy resistance of diseases like breast cancer and CML [14,21]. However, its role in LIHC is not well-studied. Our analysis of the TCGA dataset reveals that PSMD1 is frequently overexpressed in various cancers, including LIHC. This overexpression is frequently linked to poor patient survival and can serve as a sensitive factor for diagnosing and predicting the prognosis of LIHC patients, consistent with previous research findings [22]. Further analysis of clinical traits demonstrates that the T stage is where PSMD1 has the greatest effect on LIHC, suggesting its potential to promote tumor growth and progression to higher stages. Using nomogram model, DCA model, and independent prognostic analysis, we demonstrate that PSMD1, as a single gene, can accurately predict patient survival as an independent risk factor, adversely affecting prognosis. This may be attributed to PSMD1's ability to regulate p38-JNK and AKT signaling, which in turn affects the expression of genes involved in de novo lipid synthesis, promoting the accumulation of cellular lipid droplets and the progression of LIHC [23].

Biological behavior enrichment analysis revealed a close association between PSMD1 and functions such as 'gated channel activity', which has been confirmed to have a causal relationship with the progression of diseases, including cancer [24]. The 'ECM--receptor interaction' pathway is considered a crucial cellular process in cancer development, as it regulates multiple pathways in cancer cells and is crucial to the tumor microenvironment [25,26]. Additionally, proteoglycans, which are essential components of the extracellular matrix, have the ability to regulate cancer cell invasion [27]. Long-term viral hepatitis is regarded as a fundamental and significant stage in the development of LIHC, and we hypothesize that PSMD1 may promote hepatitis progression, ultimately leading to LIHC [28]. GSEA analysis specifically demonstrated that PSMD1 positively regulates immune-related functions, such as 'Immunoglobulin Complex' and 'ECM Receptor Interaction'. This suggests that PSMD1 may enhance immune function stimulation in LIHC patients, and since cell survival relies on extracellular matrix (ECM) attachment, metastatic tumor cells tend to adapt to an environment without ECM [29]. Therefore, we speculate that PSMD1 may regulate ECM-receptor interaction, influencing the metastasis of LIHC cells.

TME consists of various infiltrating immune and stromal cells and is crucial for the initiation and development of cancer [30]. A higher immuneScore indicates that the TME of individuals with high expression of PSMD1 contains more immune cells, which typically suppress tumor growth in the early stages. However, as the tumor progresses, immune escape occurs, which is a major characteristic of tumors [31]. Our research findings on immune checkpoints reveal the intrinsic connection between them. Overexpression of immune checkpoint molecules prevents effective anti-tumor immune responses by the body's immune cells, facilitating immune escape by tumors [32]. We utilized TIDE to analyze the PSMD1-mediated immune escape and found that, as anticipated, PSMD1 enhances LIHC's ability to evade immune responses. This suggests that individuals with low PSMD1 expression may benefit more from immunotherapy. However, previous studies have shown that approximately 70 % of advanced LIHC patients receiving ICI therapy do not experience the benefits of immunotherapy, posing a significant challenge for the treatment of LIHC [33]. Recent research has made new attempts in immunotherapy for LIHC, specifically focusing on the use of cytokines and monoclonal antibodies in conjunction with radiotherapy for the treatment of LIHC [18,34]. The findings indicate that combining immunotherapy with other drugs, particularly two immune checkpoint inhibitors (ICIs), can greatly enhance the likelihood of achieving complete remission in cancer patients. This discovery opens up a promising avenue for clinical treatment of LIHC and carries significant implications [6]. The variation in immune typing plays a crucial role in the treatment and prognosis of LIHC. Patients with different immune typing experience distinct outcomes when undergoing immunotherapy [35]. Our study reveals a significant relationship between the expression of PSMD1 and the diverse immune molecular phenotypes of LIHC, particularly in individuals with Wound Healing typing. Additionally, this subgroup of patients often exhibits a poor prognosis. Hence, we suggest that PSMD1 can be a useful marker for determining patients' immunological profiles, and its overexpression may impact patient survival by altering the tumor's immune environment.

In recent years, there has been widespread acceptance of the significant roles that genetic or epigenetic alterations play in various transcriptional and nontranscriptional biological processes [36]. Reports indicate that abnormal DNA molecular changes serve as the initiators of tumors, appearing in the early stages of tumor development and persisting throughout the entire process. These changes are closely linked to tumor prognosis [37]. Analysis of TMB reveals a frequent occurrence of Missense Mutation in PSMD1. A high TMB corresponds to a high-expression population and is associated with a worse prognosis. Therefore, in line with other research, we

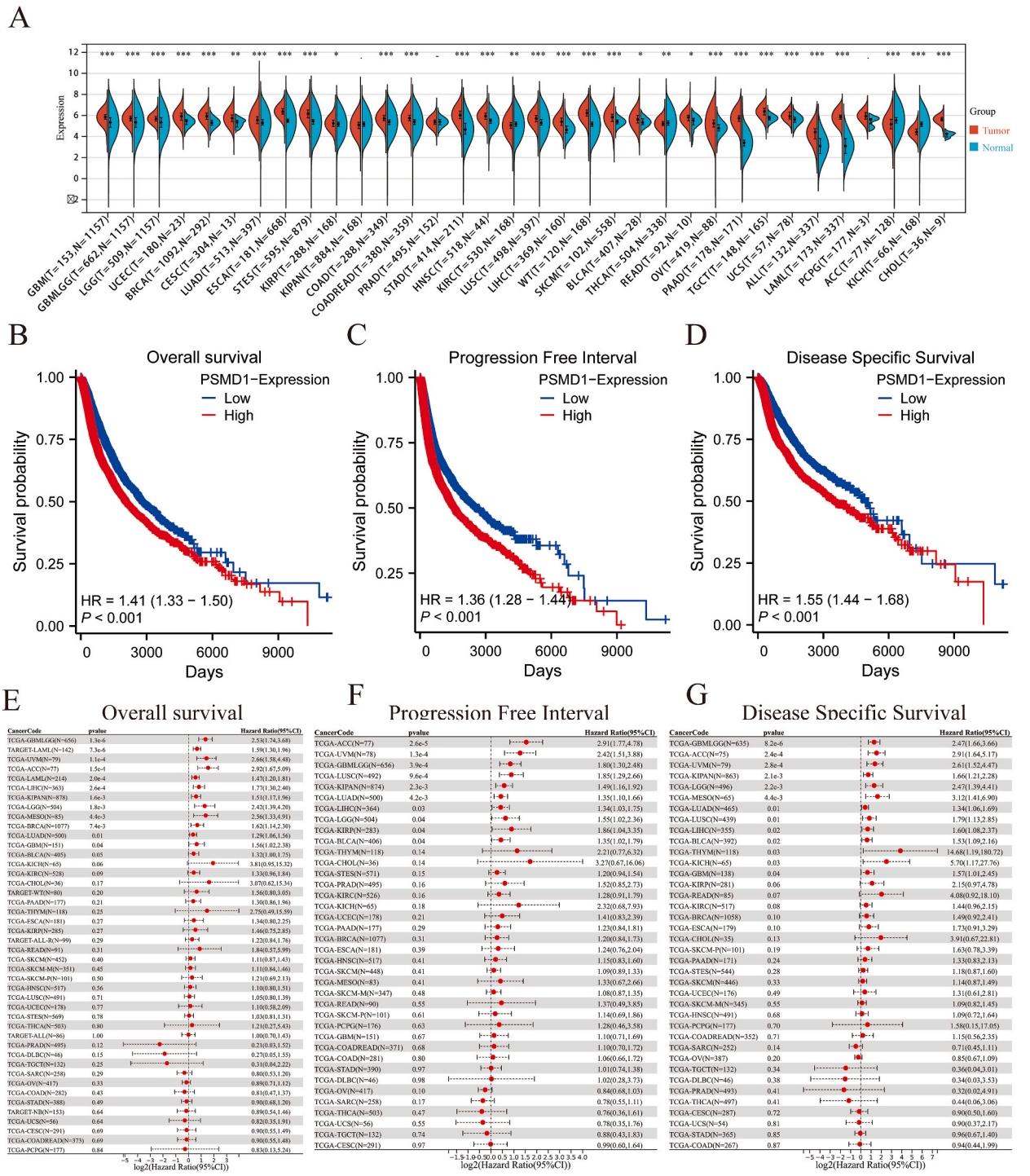

Fig. 8. Diagnosis and Prognosis related to PSMD1 in pan-carcinoma. (A). Comparison of PSMD1 expression in cancerous and non-cancerous samples across various types of cancer. (B). Correlation between PSMD1 expression and OS. (C). Correlation between PSMD1 expression and PFI. (D). Correlation between PSMD1 expression and DSS. (E). Cox regression analysis between PSMD1 expression and pan-carcinogenic OS. (F). Cox regression analysis between PSMD1 expression and pan-carcinogenic PFI. (G). Cox regression analysis between PSMD1 expression and pan-carcinogenic DSS.

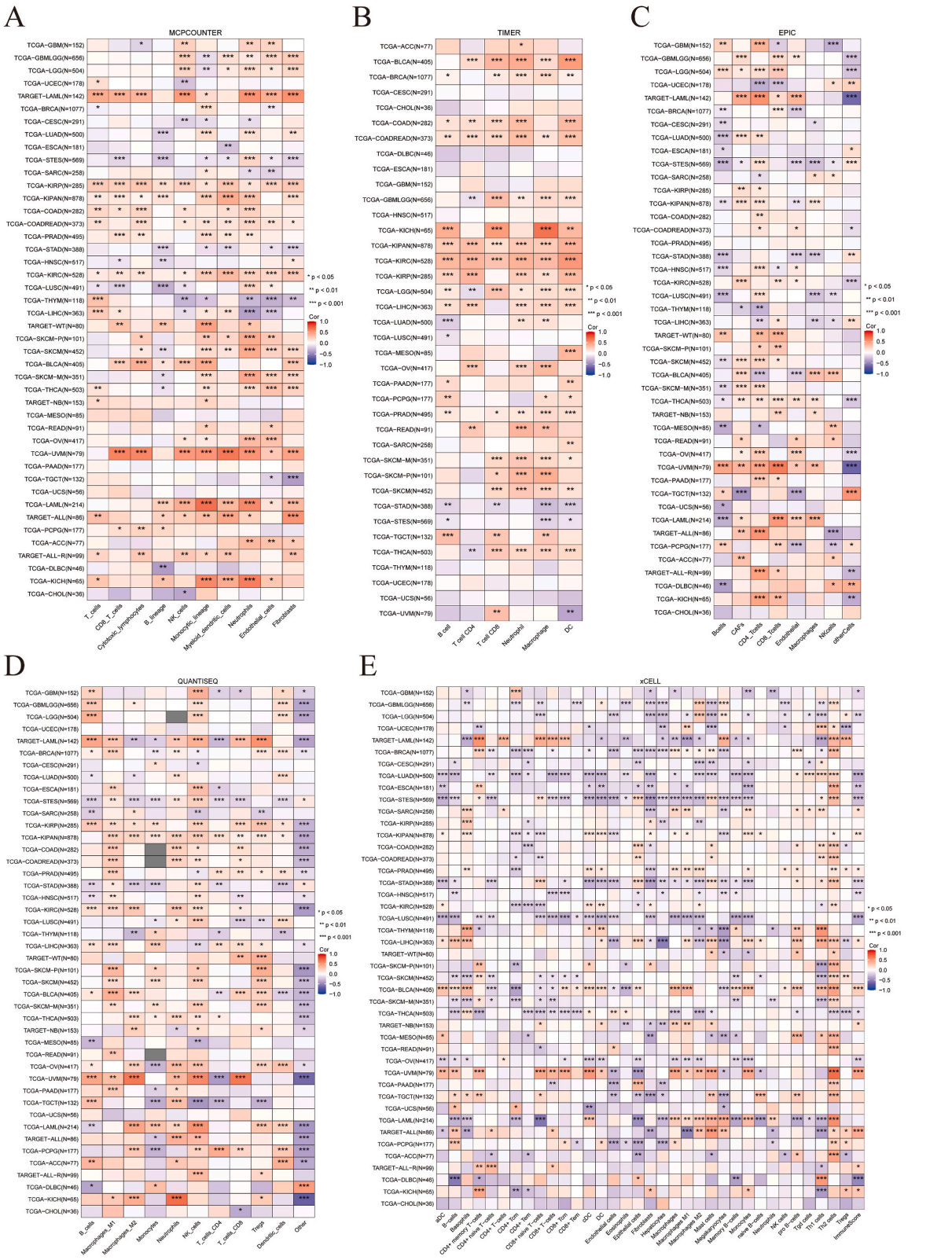

Fig. 9. PSMD1 in pan-carcinogenic immune cell infiltration. (A). MCPCOUNTER Immunoassay. (B). TIMER Immunoassay. (C). EPIC Immunoassay. (D). QUANTISSEQ Immunoassay. (E). XCELL Immunoassay.

postulate that high TMB is a significant factor in the poor prognosis of the high-expression group [38]. Further investigation is warranted to explore the impact of PSMD1 on tumor mutation. CNV is commonly considered a characteristic feature of human cancer [39]. Our study revealed an association between PSMD1 and CNV mutation patterns. Overexpression of PSMD1 is more likely to lead to CNV acquisition, while low expression results in CNV loss. Interestingly, We found no discernible variations in survival across the various mutant populations. Previous studies have demonstrated that abnormally methylated genes can be used as early LIHC diagnostic and prognostic indicators [40]. Therefore, we investigated the relationship between PSMD1 and methylation. Our findings showed that individuals with high levels of methylation in any region or site of PSMD1 tend to have a worse prognosis. Based on our analysis, we concluded that PSMD1 is involved in various genetic variations that closely interact with the transcriptome. Abnormal overexpression of PSMD1 has a profound impact on the prognosis of LIHC patients.

Several studies have established the significance of PSMD1 as a diagnostic biomarker for LUAD [12], BRCA [10], CML [14], GC [11] and THCA [13]. We carried out a pan-cancer investigation to learn more about PSMD1's clinical impact. The results showed that PSMD1 is not only crucial for the diagnosis and prognosis of the aforementioned tumors but also significantly worsens the prognosis of GBM, LAML, UVM, ACC, KIPAN, LGG, MESO and overall cancer. Since PSMD1 is an innate immune molecule and plays a crucial role in the immunity of LIHC, we extensively analyzed the immune infiltration of PSMD1 in various cancers. We found a close relationship between PSMD1 and immune cells in most cancers. However, interestingly, we observed that immune cells in CHOL adjacent to LIHC have a very low correlation with PSMD1. Exploring the difference in immune cells between CHOL and LIHC may become an important direction for distinguishing their source and identifying the pathogenesis. In summary, PSMD1, as an innate immune gene, has the potential to serve as a biomarker for the diagnosis and prognosis of LIHC. It primarily promotes the progression of LIHC by regulating the immune environment and various genetic variations. Additionally, PSMD1 shows high potential for diagnosing pan-cancer and predicting prognosis. Further research on PSMD1 will provide new perspectives on the clinical treatment and pathogenesis of LIHC and pan-cancer, ultimately advancing our knowledge of how to face and combat cancer.

4. Conclusion

PSMD1, an innate immune gene, has been the subject of extensive research due to its ability to accurately predict the diagnosis and prognosis of LIHC. This gene holds significant importance in the advancement of tumor staging and T-stage in LIHC patients. We conducted multiple validation methods to confirm the sensitivity of PSMD1 as an independent risk factor for LIHC. Studies investigating the function and pathway of PSMD1 in LIHC have revealed its impact on LIHC progression through modulation of immune complex function, involvement in peripheral changes of the ECM, and induction of hepatitis. Our findings indicate that PSMD1 is implicated in alterations of the TME and immune cells, and the study of immune checkpoints and TIDE provides valuable insights for targeted clinical treatments. Furthermore, we identified a close association between PSMD1 and various immune types. Additionally, we observed that PSMD1 is involved in multiple genetic variations, including TMB, CNV, and methylation. These variations are closely linked to the abnormal overexpression of PSMD1 and significantly impact patient prognosis. Pan-cancer analysis suggests that PSMD1 holds great potential as a therapeutic target in 12 different tumor types, including LIHC, ACC, UVM, KIPAN, and LUAD, thereby highlighting its importance in immune research.

5. Materials and methods

5.1. Materials

We obtained the TCGA, TARGET, and GTEx pan-cancer expression and clinical information datasets from the University of California at Santa Cruz (UCSC) database (PANCAN, N = 19131, G = 60499) (https://xenabrowser.net/). Specifically, the LIHC section comprised of 374 tumor samples and 50 normal liver tissue samples. Additionally, we acquired 357 TMB data and 379 CNV data of LIHC patients. The expression of PSMD1 was normalized using log2(x+1) transformation. To validate our results, we downloaded the GSE19665, GSE29721, GSE62232, and GSE112790 datasets from the Gene Expression Omnibus (GEO) database. Immunophenotyping data from 362 samples were utilized to determine the expression levels related to immunophenotype and conduct survival analysis in patients with LIHC.

5.2. Analysis of diagnostic and prognostic capacity of PSMD1 in LIHC

The TIMER database was utilized to analyze the expression differences of PSMD1 in pan-cancer within the TCGA dataset, with a focus on LIHC as the subject of research. The gene expression files of TCGA LIHC were examined to analyze the expression differences in individual and paired samples across various tissues. By dividing the samples into high and low expression groups based on the median expression value, we utilized clinically relevant information to investigate the differences in OS and PFS among these groups. To evaluate the association between PSMD1 expression and the disease, we compared the expression of PSMD1 in both the normal population and the LIHC population, and generated a diagnostic ROC curve. Additionally, we sorted the combined expression of survival time and survival state to create time-dependent ROC curves for 1, 3, and 5-year periods. The expression of PSMD1 in tumor tissues and normal tissues was further identified using two immunohistochemical stains, HPA036736 and HPA036737, available in The Human Protein Atlas database (HPA)(https://v15.proteinatlas.org/).

5.3. Clinical trait and stage matching analysis

We collected common clinical data from the TCGA LIHC dataset, which included Age, Gender, Grade, Stage, T, N, M, HBV, and HCV. The patients were divided into different groups based on the median value of expression. A correlation heat map was generated to observe the differences in traits among these expression groups as a whole. Furthermore, the patients were grouped based on different clinical stages to analyze the expression differences of PSMD1 in relation to these stages. This analysis aimed to provide insights into the clinical process involving PSMD1 and its impact at different stages.

5.4. Clinical predictive power

The nine clinical traits and expression levels of PSMD1 were analyzed to determine if PSMD1 has adverse effects on the survival of LIHC patients as an independent prognostic factor. Univariate and multivariate prognostic analyses were conducted. A Nomogram model was then constructed using the above indicators to predict patient survival time. A calibration curve was drawn to assess the model's clinical predictive ability, and the c-index value was calculated to determine the model's credibility. The Nomogram score, expression level, and other clinical traits were considered, and decision curves were drawn for 1, 3, and 5-year periods to evaluate the predictive sensitivity of PSMD1 expression level on clinical prognosis.

5.5. Biological behavior analysis of PSMD1 in LIHC

We initially screened the TCGA dataset for differential genes related to PSMD1 (logFCfilter = 1, fdrFilter = 0.05). Subsequently, we conducted GO function, KEGG pathway, and DO disease enrichment analyses on the differential genes to identify functions associated with these genes. To visualize the most significantly different items (pvalueFilter = 0.05), we set the minimum gene set to 15 and the maximum to 500. We used the (c5.go.symbols.gmt) and (c2.cp.kegg.symbols.gmt) files to analyze the regulatory relationship between the differential genes and different functions and pathways. Finally, we identified the top 5 functions and pathways with the most significant differences (pvalueFilter = 0.05).

5.6. Immunoinfiltration analysis of PSMD1 in LIHC

Firstly, based on the median PSMD1 expression level, the patients were classified into various expression groups. The estimation algorithm was used to determine the StromalScore and ImmuneScore for each LIHC sample. These scores were then summed up to obtain the ESTIMATEScore. Differences in TME scores among different expression groups were analyzed. CIBERSORT was used to determine the content of immune cells in each sample, and differential analysis was performed to observe differences in immune cells between high and low expression groups. The expression level of immune checkpoint genes in the TCGA LIHC data set was examined, and a circular correlation analysis was conducted to identify immune checkpoints related to PSMD1 (pFilter = 0.001). Additionally, ssGSEA analysis was employed to score the immune-related functions of LIHC patients, and differences in immune functions between different expression groups were analyzed. The TIDE website (http://tide.dfci.harvard.edu/) was utilized to score LIHC patients and assess the immune escape ability of different expression groups based on the differences in TIDE scores. Furthermore, the expression of PSMD1 in different immune populations was analyzed, and the survival time and status of these populations were examined. The logrank test method was performed to assess the importance of the differences in prognosis between different immune populations.

5.7. Analysis of genetic variation and transcriptional variation involved in PSMD1

Based on the median expression of PSMD1, the patients were divided into high and low expression groups. The mutation data of different expression groups were analyzed to determine the gene mutation information. A waterfall diagram was created using the top 15 genes with the highest mutation frequency, and the mutation patterns of genes in different expression groups were observed. The tumor mutation burden of different expression populations was calculated, and a differential analysis was performed. The survival differences between populations with different mutation burdens were compared by considering survival information. Additionally, the expression level and tumor mutation burden were combined for joint survival differential analysis to observe their internal correlations. The occurrence of CNV in PSMD1 in LIHC patients was recorded, and the expression level differences of PSMD1 among different cases were analyzed. Patients were divided into different groups based on the CNV mode, and the survival time and status of each group were sorted for survival analysis. The MethSurv database (https://biit.cs.ut.ee/methsurv/) was used to visualize the regions and sites of PSMD1 methylation in LIHC. Patients were divided into high and low methylation groups based on the degree of methylation, and the survival differences between groups with different degrees of methylation were calculated.

5.8. Validation of PSMD1 diagnostic capabilities

GSE19665, GSE29721, GSE62232, and GSE112790 datasets were downloaded from the GEO database. The datasets were processed by converting gene symbols and cleaning the data. Rows and columns with missing values greater than 50 % were deleted, and the expression levels were transformed using log2(X+1). The samples were then divided into normal and tumor groups. The expression levels of PSMD1 in the two groups were analyzed for differences. Additionally, the expression levels of PSMD1 were combined with clinical outcomes, and ROC curves were used to evaluate the diagnostic accuracy of PSMD1 in each independent cohort of the

verification group for LIHC.

5.9. Single cell analysis

We utilized the LIHC_GSE140228_10X sample from the Tumor Immune Single-cell Hub 2 (TISCH2) database (http://tisch.comp-genomics.org/home/) for conducting single-cell studies. Initially, we performed unsupervised clustering to categorize the cells into distinct groups. Then, we employed immune cells to annotate these clusters and calculated the proportion of various immune cells present in the sample. Additionally, we examined the distribution of PSMD1 across different immune cells and conducted a comparative analysis of PSMD1 expression levels in immune cells from different tissues.

5.10. Drug sensitivity analysis

Tumor samples were divided into two groups, namely the high-expression group and the low-expression group, using the median truncation method. We used a predictive software package to analyze the difference in drug sensitivity between these two groups. A significance level of pFilter = 0.001 was set to identify drugs that exhibited higher sensitivity in the different expression groups. The results were then visualized.

5.11. Prognostic analysis of PSMD1 in pan-cancer

The pan-cancer PSMD1 expression data from TCGA, TARGET, and GTEx were log2(x+1) transformed. Each cancer was divided into corresponding tumor and control groups, and the difference in expression between these groups was analyzed. All the samples were combined into the pan-cancer tumor and control groups. Patients were then divided into high and low expression groups based on the median value of PSMD1, and the differences in prognosis for Overall Survival, Progression Free Interval, and Disease Specific Survival were analyzed at the overall level. Subsequently, the Logrank test was used to statistically analyze the above analysis for each common cancer separately, and the impact of PSMD1 in each cancer was assessed.

5.12. Immune invasion of PSMD1 in pan-cancer

The expression data of PSMD1 in pan-cancer samples from TCGA, TARGET, and GTEx were transformed using log2(x+1). To evaluate the immune cell infiltration scores in each patient based on the expression of PSMD1, we employed the Timer, deconvo epic, deconvo mcpcounter, deconvo quantiseq, and deconvo xCell methods available in the 'IOBR' package. Furthermore, we used the corr. test function to calculate the correlation and significance between PSMD1 and immune cell infiltration scores in each tumor. Finally, a correlation heatmap was plotted.

5.13. Statistical analysis and visualization

Differences in parameters were analyzed using the chi-square test.The Spearman test was used to analyze correlations. The prognostic significance was tested by Log-rank. For the operations and statistical processing, R (v4.2.3) software was employed. Images were visualized and optimized using Adobe Illustrator 2021 software and R data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

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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