

# T cell exhaustion-related gene CD79B predicts prognosis, inhibits malignant progression and promotes tumor-associated macrophage M1-like polarization in lung adenocarcinoma

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Received March 23, 2025; Accepted July 18, 2025

DOI: 10.3892/or.2025.8977

**Abstract.** Lung adenocarcinoma (LUAD) is one of the most common malignancies in the lung. T cell exhaustion (TEX) is an important immune escape mechanism that may be targeted to improve tumor immunotherapy. The present study investigated TEX-related genes in the tumor microenvironment to predict the prognosis of patients with LUAD. Data from 516 patients with LUAD were collected from The Cancer Genome Atlas database and classified them into TEX-C1 and TEX-C2 by unsupervised clustering based on the TEX-related genes. Compared with TEX-C1, TEX-C2 cluster had worse prognosis, with shorter overall and progression-free survival. Functional analysis revealed that upregulated genes in the TEX-C2 cluster were significantly enriched in tumor immune- and metabolism-related pathways. TEX-C2 cluster had a poor immune checkpoint blockade (ICB) response and a shorter survival after ICB treatment by Tumor Immune Dysfunction and Exclusion algorithm. A prognostic TEX-related gene signature was constructed for patients with LUAD by Least Absolute Shrinkage and Selection Operator regression analysis. B cell antigen receptor complex-associated protein  $\beta$  chain (CD79B) was considered an independent prognostic indicator; in the clinical correlation analysis, the effect of CD79B on prognosis was associated with advanced lung cancer, advanced age, female sex and patients with history of smoking. The overexpression of CD79B was associated with more infiltration of CD8<sup>+</sup> T cells, M1 macrophages (the classically activated type 1, pro-inflammatory type), and less

infiltration of M0 (undifferentiated) and M2 macrophages (the alternatively activated type 2, anti-inflammatory type) by CIBERSORT algorithm, which was also significantly correlated with gene markers of innate and adaptive immune cells, and higher levels of immune checkpoint genes. Upregulation of CD79B could inhibit the proliferation, migration and invasion capabilities and promote the apoptosis of LUAD cells, and induce M1-like tumor-associated macrophage (TAM) polarization. In conclusion, patients with LUAD in the TEX-C2 cluster had worse prognosis and adverse immune microenvironment. CD79B may be a potential prognostic indicator, which could inhibit malignant progression of LUAD cells and induce M1-like TAM polarization.

## Introduction

Lung adenocarcinoma (LUAD) is the most common primary lung cancer, accounting for ~40% of cases (1). Patients with early-stage LUAD mainly receive surgical therapy, while advanced and metastatic LUAD often require systemic therapy. These treatments improve symptoms and prolong their overall survival to a certain extent (2). However, the anti-tumor effect in patients with LUAD is often limited by chemotherapy or radiotherapy resistance and immune escape, leading to tumor recurrence and poor prognosis (3). For patients with LUAD, individualized and comprehensive treatments should be developed based on tumor progression. Molecular targeted therapy targeting oncogenic driver genes, such as Kirsten rat sarcoma viral oncogene homologue (KRAS), epidermal growth factor receptor (EGFR), v-Raf murine sarcoma viral oncogene homolog B (BRAF) and anaplastic lymphoma kinase (ALK) has greatly advanced the field of treatment for non-small cell lung cancer, especially LUAD (4). Immune checkpoint inhibitors have been approved by the US Food and Drug Administration for monotherapy or combination with other agents in LUAD, which provide hope for patients with negative expression of driver oncogenes or resistance to molecular-targeted therapy (5,6). However, clinical trials have shown that only a small number of patients benefit from immunotherapy (7,8). Therefore, it is necessary to identify

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**Key words:** T cell exhaustion, lung adenocarcinoma, prognosis, tumor-associated macrophage polarization

more biomarkers to ensure that more patients can benefit from them.

The tumor immune microenvironment is widely recognized as the key regulator of tumor progression and clinical response to therapy (9,10). Components of the tumor microenvironment include cancer and inflammatory cells, and suppressive cytokines that contribute to the transition of T cells into 'exhausted' T cells by regulating T cell phenotypes and functions (11,12). T cell exhaustion (TEX) is defined as a decline in proliferation capacity, weakened effector function, loss of cytokine production and sustained high expression of inhibitory receptors (13,14). Exhausted T cells demonstrate upregulation of immune checkpoint proteins such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein-1 (PD-1). Transcription and epigenetic reprogramming lead to mitochondrial dysfunction and decreased metabolic activity, thereby triggering immune evasion (15). Immune checkpoint blockade (ICB) therapy can inhibit negative regulatory signals and release T cells from exhaustion (16), however not all TEX cells respond to ICB therapy. Recent studies have revealed heterogeneous subpopulations within the exhausted T cell lineage including ICB-sensitive and -resistant subpopulations, which lead to differences in treatment responses (17,18). TEX is closely related to tumor-associated macrophage (TAM) polarization. Metabolites secreted by cancer cells promote cell escape from M1 TAM polarization towards M2 TAM, contributing further towards TEX development (19). M2 TAMs promote TEX, resulting in anti-PD-1 blockade resistance (20). Therefore, inducing the switching of TAM from M2 to M1 may reverse TEX and restore T cells from an exhausted state, providing potential targets in tumor immunotherapy.

The present study performed clustering analysis of TEX-related genes in patients with LUAD patients by analyzing a public database; subsequently the present study aimed to construct a prognostic signature and nomogram to predict prognosis for LUAD. Then, the study aimed to explore the potential of B cell antigen receptor complex-associated protein  $\beta$  chain (CD79B) as a prognostic marker and the role of immune function in LUAD, which may play a key role in tumor immunotherapy.

## Materials and methods

**Data collection.** RNA-sequencing expression profiles and corresponding clinical information of 516 LUAD samples and 59 para-tumor tissue samples were downloaded from The Cancer Genome Atlas (TCGA) database ([portal.gdc.cancer.gov/](http://portal.gdc.cancer.gov/); age range: 33-88 year; female: 278, male: 238). STAR-counts data, mutation annotation format (MAF) data, and corresponding clinical information were downloaded from the TCGA database ([portal.gdc.cancer.gov](http://portal.gdc.cancer.gov/)) (TCGA-LUAD dataset). Data was then extracted in TPM format and performed normalization using the log<sub>2</sub> (TPM+1) transformation. After retaining samples that included both RNAseq data and clinical information, 516 LUAD samples and 59 para-tumor tissue samples were selected for further analysis (21). Based on previous research, 12 TEX-related genes that may have prognostic value were obtained (22). The protein-protein interaction network was obtained from the

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database ([cn.string-db.org/](http://cn.string-db.org/)) (23).

**Consensus clustering analysis.** To evaluate consistency in TCGA-LUAD database, R package ConsensusClusterPlus (v1.72.0; [bioconductor.org/packages/release/bioc/html/ConsensusClusterPlus.html](http://bioconductor.org/packages/release/bioc/html/ConsensusClusterPlus.html)) was used (24), with a maximum of 6 clusters and 80% of the samples were extracted 100 times with clusterAlg='hc', innerLinkage='ward.D2'. Patients were clustered according to the optimal k-value based on the cumulative distribution function (CDF) curve and consensus matrix. Principal component analysis (PCA) was used to analyze the distribution differences between clusters.

**Survival analysis and construction of prognostic signature.** Survival analysis was conducted between clusters in TCGA-LUAD database using the 'survival' and the 'survminer' R packages (v0.4.9; [rdocumentation.org/packages/survminer/versions/0.4.9](http://rdocumentation.org/packages/survminer/versions/0.4.9)). The appropriate conditions for the construction of the nomogram prediction model were determined using univariate and multivariate Cox regression analysis. Using the 'forestplot' R package (v3.1.7) (<https://github.com/cran/forestplot>), the P-value and hazard ratio (HR) with 95% confidence interval (CI) of each variable were calculated. To predict the overall recurrence rate over the next x years, a nomogram was created based on the results of multivariate Cox proportional hazards analysis using the 'rms' R package (v8.0.0; [cran.r-project.org/web/packages/rms/index.html](http://cran.r-project.org/web/packages/rms/index.html)); the nomogram represented factors which can be used to determine the likelihood of recurrence for an individual patient (25).

The least absolute shrinkage and selection operator (LASSO) regression used a feature selection algorithm, incorporating 10-fold cross-validation, and was examined using the 'glmnet' R package (v4.1-10; [cran.r-project.org/web/packages/glmnet/index.html](http://cran.r-project.org/web/packages/glmnet/index.html)) (26). Using the 'survival' R package, a novel prognostic model of TEX-related genes was constructed based on multivariate Cox regression analysis. The time-receiver operating characteristic (ROC) (v0.4) ([huppertlab.net/home/training/knowledge-base/nirs\\_toolbox/full demos/receiver-operating-characteristic-roc/](http://huppertlab.net/home/training/knowledge-base/nirs_toolbox/full demos/receiver-operating-characteristic-roc/)) analysis was performed on TEX-related genes to determine the accuracy and risk score (27).

**Functional enrichment analysis.** The 'limma' R package (v3.40.2) ([bioconductor.org/packages/release/bioc/html/limma.html](http://bioconductor.org/packages/release/bioc/html/limma.html)) was used to analyze the differential expression of mRNA between clusters (28). The criteria for statistical significance were adjusted P<0.05 and log<sub>2</sub> (fold-change) >1 or <-1. Using the ClusterProfiler package in R software (v3.18.0; [bioconductor.org/packages/release/bioc/html/clusterProfiler.htm](http://bioconductor.org/packages/release/bioc/html/clusterProfiler.htm)) (29), gene functions of enrichment analysis were investigated in Gene Ontology (GO) ([geneontology.org/](http://geneontology.org/)) (30) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.genome.jp/kegg/>) (31). The enrichment scores were calculated using the Gene Set Variation Analysis package of R software (v1.3.0) (<https://www.gsea-msigdb.org/gsea/index.jsp>) based on the absolute enrichment scores of gene sets in multiple publications and gene signatures validated

by previous experiments (32-36), with the parameter chosen as method='ssGESA' (37). Spearman's correlation was calculated to determine the relationship between genes and pathway scores.

**Tumor immune microenvironment analysis.** The 'immuneconv' R package (v2.0.3; [rdocumentation.org/packages/immunedeconv/versions/2.0.3/](http://rdrr.io/github/immunedeconv/versions/2.0.3/)) was used to predict 22 types of immune infiltrating cell based on the CIBERSORT algorithm (a gene expression-based deconvolution algorithm) (<http://cibersort.stanford.edu/>) (38). The mRNA levels of immune checkpoint-related genes were compared between clusters, including Programmed Cell Death 1 Ligand 1 (PD-L1/CD274), Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA4), Hepatitis A Virus Cellular Receptor 2 (HAVCR2), Lymphocyte Activating 3 (LAG3), Programmed Cell Death 1 (PDCD1), Programmed Cell Death 1 Ligand 2 (PDCD1LG2), T Cell Immunoreceptor With Ig And ITIM Domains (TIGIT), and Sialic Acid Binding Ig Like Lectin 15 (SIGLEC15). To predict the ICB response, the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm (v6.5.1) (<http://tide.dfci.harvard.edu/>) was used to simulate computational approaches to tumor immune evasion, based on two molecular mechanisms, including dysfunction of tumor infiltrating cytotoxic T lymphocytes (CTLs) and rejection of CTLs by immunosuppressive factors (7). The correlation between gene expression and immune score was analyzed by 'ggstatsplot' (v0.13.1; [cran.r-project.org/web/packages/ggstatsplot/index.html](https://cran.r-project.org/web/packages/ggstatsplot/index.html)) and 'pheatmap' R package (v1.0.13) (<https://www.rdocumentation.org/packages/pheatmap/versions/1.0.13/topics/pheatmap>) (39).

**Prediction of therapeutic drug sensitivity.** The prediction of sensitivity to various therapeutic agents were performed by the 'pRRophetic' R package (v4.0.3) ([github.com/paulgeeleher/pRRophetic](https://github.com/paulgeeleher/pRRophetic)), based on the Genomics of Drug Sensitivity in Cancer (GDSC) dataset ([cancerrxgene.org/](https://cancerrxgene.org/)) (40). The ridge regression method was used to evaluate the half-maximal inhibitory concentration (IC<sub>50</sub>) of the samples, and 10-fold cross-validation was performed based on the GDSC training set to evaluate the prediction accuracy (41). The IC<sub>50</sub> for each sample in TCGA-LUAD database was estimated from the predictive model evaluated on GDSC cell line data.

**Cell culture and plasmid transfection.** Human normal lung epithelial HBE, macrophage THP-1 and LUAD A549, H358, PC-9, 95C, 95D cell lines were obtained from the Cell Center of Central South University (Changsha, China). All cells were cultured in RPMI-1640 medium (Gibo; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; HyClone; Cytiva). The pDONR223-CD79B overexpression plasmid (cat. no. G104684) and empty vector control pDONR223 were purchased from Youbio. Lipofectamine™ 3000 Transfection Reagent (cat. no. L3000150, Thermo Fisher Scientific, Inc.) was used to transfect cells with 6 µg plasmid transfection for 24 h at 37°C, followed by 48 h culture before downstream experiments.

**Coculture assay.** THP-1 monocytes (2x10<sup>7</sup> cells) were differentiated into M0 macrophages by treatment with 100 ng/ml phorbol 12-myristate 13-acetate

(PMA; cat. no. P8139, Sigma-Aldrich, Inc.) for 48 h in RPMI-1640 medium containing 10% FBS (37°C). M0 macrophages (5x10<sup>4</sup> cells/insert) were seeded in the upper chamber (0.4 µm pore size; Corning, Inc.) while A549 cells (2x10<sup>5</sup> cells/well) were plated in the lower chamber. Cells were co-cultured for 48 h under standard conditions (37°C, 5% CO<sub>2</sub>). Following coculture, upper-chamber cells were collected for downstream analysis by qPCR.

**Quantitative (q)PCR.** After 48 h co-culture with CD79B-overexpressing A549 cells in a humidified incubator (37°C, 5% CO<sub>2</sub>), total RNA was extracted from M0 macrophage THP-1 cells using the RNA extraction kit (cat. no. 15596026). Reverse transcription kit (cat. no. K1621; both Thermo Fisher Scientific, Inc.) was used to obtain cDNA, according to the manufacturer's instructions. SYBR Green kit (cat. no. 4309155; Thermo Fisher Scientific, Inc.) was used for qPCR analysis by ABI 7500. Thermocycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec, with a melting-curve stage to confirm amplicon specificity. β-actin was used as the reference gene for normalization, and relative gene expression was calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (42). The primers are list in Table S1.

**Western blotting.** The cells (HBE, A549, H358, PC9, 95C, 95D, A549-Vec, A549-OE-CD79B cells) were lysed in immunoprecipitation buffer (cat. no. 87787, Thermo Fisher Scientific, Inc.) containing inhibitors cocktail (cat. no. 4693116001) and phosphatase inhibitor (cat. no. 4906845001, both Roche Diagnostics). The protein concentration was measured with BCA reagent (cat. no. AR0197, Wuhan Boster Biological Technology Ltd.). The samples (50 µg/lane) were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Merck KGaA). The membranes were blocked with 5% non-fat dry milk in TBST (0.1% Tween-20) at room temperature for 1 h, and then incubated at 4°C overnight with rabbit anti-CD79B (1:400, cat. no. ab134147, Abcam) or mouse anti-actin (1:20,000; cat. no. AC026, ABclonal, Biotech Co., Ltd.), followed by incubation with HRP-conjugated goat anti-rabbit secondary antibody (1:6,000; cat. no. AS014, ABclonal Biotech Co., Ltd.) for 2 h at room temperature. Finally, the bands were imaged on a ChemiDoc™ imaging system (Bio-Rad) after detection with an enhanced chemiluminescence detection kit (cat. no. 36208-A, Shanghai Yeasen Biotechnology Co., Ltd.). Densitometric analysis was performed using Image Lab™ 6.1 software (Bio-Rad).

**Cell Counting Kit (CCK)8 assay.** Cell viability was determined by CCK8 assay (cat. no. C0005, TargetMol Chemicals, Inc.) according to the manufacturer's instructions. A549-Vec and A549-OE-CD79B cells were inoculated in 96-well plates, and incubated with CCK8 for 2 h. The optical density at 450 nm was then determined by microplate reader (BioTek ELx800; Agilent Technologies, Inc.).

**Apoptosis assay.** A549-Vec and A549-OE-CD79B cells were collected and stained with annexin V-FITC and propidium iodide using an apoptosis kit (cat. no. KGA103, Nanjing Keygen Biotech Co., Ltd.) according to the manufacturer's instructions. Cells were analyzed on a BD LSRFortessa™ X-20 flow

cytometer (BD Biosciences). Data were processed using FlowJo software (version 10.8.1; BD Biosciences), with apoptotic cells defined as annexin V<sup>+</sup>/PI<sup>-</sup> (early apoptotic) + annexin V<sup>+</sup>/PI<sup>+</sup> (late apoptotic) populations.

**Scratch assay.** A549-Vec and A549-OE-CD79B cells were seeded in 6-well culture plates (2x10<sup>5</sup> cells per well) and incubated overnight to a density of 70-80% confluence (37°C, 5% CO<sub>2</sub>). The medium was replaced with serum-free RPMI-1640, a straight scratch was made with a sterile 100- $\mu$ l pipette tip and floating cells were rinsed away. Plates were incubated at 37°C for 12 h. Phase-contrast images were acquired at 0 and 12 h using an inverted light microscope (AMEX1200, Thermo Fisher Scientific, Inc.; magnification, x100), and wound areas were quantified with ImageJ (v1.53t, NIH, USA) (43).

**Cell migration and invasion assay.** Cell invasion assays were performed using 24-well plates and Transwell invasion chamber (8  $\mu$ m pore size, BD Biosciences). For invasion assays, Matrigel (cat. no. 356234, Corning, Inc.) was added at 37°C for 1 h before cell seeding. Migration assays were performed without matrix gel. A549-Vec and A549-OE-CD79B cells cultured at 37°C with 5% CO<sub>2</sub> were trypsinized, resuspended in serum-free RPMI-1640, and 5x10<sup>4</sup> cells in 200  $\mu$ l were seeded into the upper chamber. The lower chamber received 800  $\mu$ l RPMI-1640 containing 10% FBS as chemoattractant. After 24 h of incubation at 37°C with 5% CO<sub>2</sub>, non-invading/migrating cells on the upper surface were removed with a cotton swab. Cells on the lower surface were fixed at room temperature with 4% paraformaldehyde for 20 min, stained with 0.1% crystal violet at room temperature for 10 min, washed three times with PBS, and air-dried. Invaded/migrated cells in five randomly selected fields per insert were counted manually under a light microscope.

**Statistical analysis.** The bioinformatics data were analyzed using R software v4.1.3 (R Foundation for Statistical Computing, 2022), and the sample test data were processed using SPSS24.0 (IBM Corp.) and GraphPad Prism8.0.1 (Dotmatics) software. Paired Student t-test and Wilcoxon test were applied to normally and non-normally distributed data, respectively. One-way ANOVA followed by Tukey test was used to determine the differences among multiple comparative groups. The sample size for each group was  $\geq 3$ . All data are presented as the mean  $\pm$  SD of three individual experiments. P<0.05 was considered to indicate a statistically significant difference.

## Results

**Cluster and survival analysis of TEX-related genes in patients with LUAD.** A total of 12 TEX-related genes were collected from a previous study (22). To explore the TEX heterogeneity in LUAD, consensus clustering was performed based on the expression of these 12 TEX-related genes in TCGA-LUAD. Based on the consensus CDF curve (Fig. S1A) and relative change in area under the CDF curve (Fig. S1B), k=2 was selected as the best cluster and two clusters (TEX-C1 and -C2) were identified (Fig. 1A and B). There were 242 patients in the TEX-C1 and 274 patients in the TEX-C2 cluster. The

PCA results confirmed significant differences between the two clusters (Fig. S1C). The differences in clinical characteristics between the two clusters are shown in Table I. mRNA expression of eight TEX-related genes was higher in the TEX-C2 than that in the TEX-C1 cluster, including cytoskeleton Associated Protein 4 (CKAP4), Dual Specificity Phosphatase 5 (DUSP5), methylenetetrahydrofolate dehydrogenase (NADP<sup>+</sup> Dependent) 2 (MTHFD2), Opioid Growth Factor Receptor (OGFR), Spondin 2 (SPON2), C-X-C Motif Chemokine ligand 1 (CXCL1), C-C Motif Chemokine ligand 2 (CCL2) and CXCL6. The expression of three TEX-related genes [CD79B, B Cell Scaffold Protein With Ankyrin Repeats 1 (BANK1) and DNA Damage Regulated Autophagy Modulator 1 (DRAM1)] was lower in the TEX-C2 cluster, however, the expression of Lipopolysaccharide Induced TNF Factor (LITAF) was not significantly different between clusters (Fig. 1C). Patients in the TEX-C2 cluster had worse prognosis with shorter overall survival (OS) and progression-free survival (PFS), compared with those in the TEX-C1 cluster (Fig. 1D and E).

**Differential gene expression and gene enrichment analysis of TEX-based clusters in patients with LUAD.** TEX-C1 and TEX-C2 clusters demonstrated 121 differentially expressed genes. Compared with the TEX-C1 cluster, 43 genes were upregulated in the TEX-C2 cluster, such as the pepsinogen C and Surfactant Protein B (SFTB) families. SFTB is a pulmonary surface-active substance synthesized by alveolar epithelial type II cells, which is involved in the development and progression of LUAD by regulating multiple immune-associated pathways (44). In the TEX-C2 cluster, 78 genes were downregulated, including CXCL1 and CXCL8, which are ligands for the chemokine receptor CXCR2 and involved in the recruitment of tumor immune cells such as TAMs and regulatory T cells (Tregs; Fig. 2A and B) (45). Compared with the TEX-C1 cluster, the differentially expressed genes in the TEX-C2 cluster were mainly involved in immune-related pathways. The KEGG analysis of upregulated genes in the TEX-C2 cluster demonstrated enrichment of metabolism-related pathways, while downregulated genes were associated with 'p53 signaling pathway', 'Wnt signaling pathways', 'NF- $\kappa$ B signaling pathways', 'IL-17 signaling pathways' and other signaling pathways (Fig. 2C). In addition, upregulated genes in the TEX-C2 cluster were enriched in metabolism-related pathways according to GO enrichment analysis; downregulated genes were enriched in mitosis (Fig. 2D). Therefore, worse prognosis for patients in the TEX-C2 cluster may be related to tumor immune- and metabolism-associated pathways.

**Tumor immune microenvironment analysis of TEX-based clusters in patients with LUAD.** To investigate the heterogeneity of the tumor immune microenvironment between clusters, tumor immune cell infiltration was calculated using the CIBERSORT algorithm (Fig. 3A). Compared with the TEX-C1 cluster, the TEX-C2 cluster had a significantly higher abundance of M0 and M2 macrophage cells, while the abundance of CD4 memory T and B cells was lower. PD-L1 is used as an indicator to predict responses to immunotherapy (46). The present study investigated the association between PD-L1 and TEX in LUAD; expression of PD-L1 in the TEX-C2 cluster was significantly higher than that in the TEX-C1

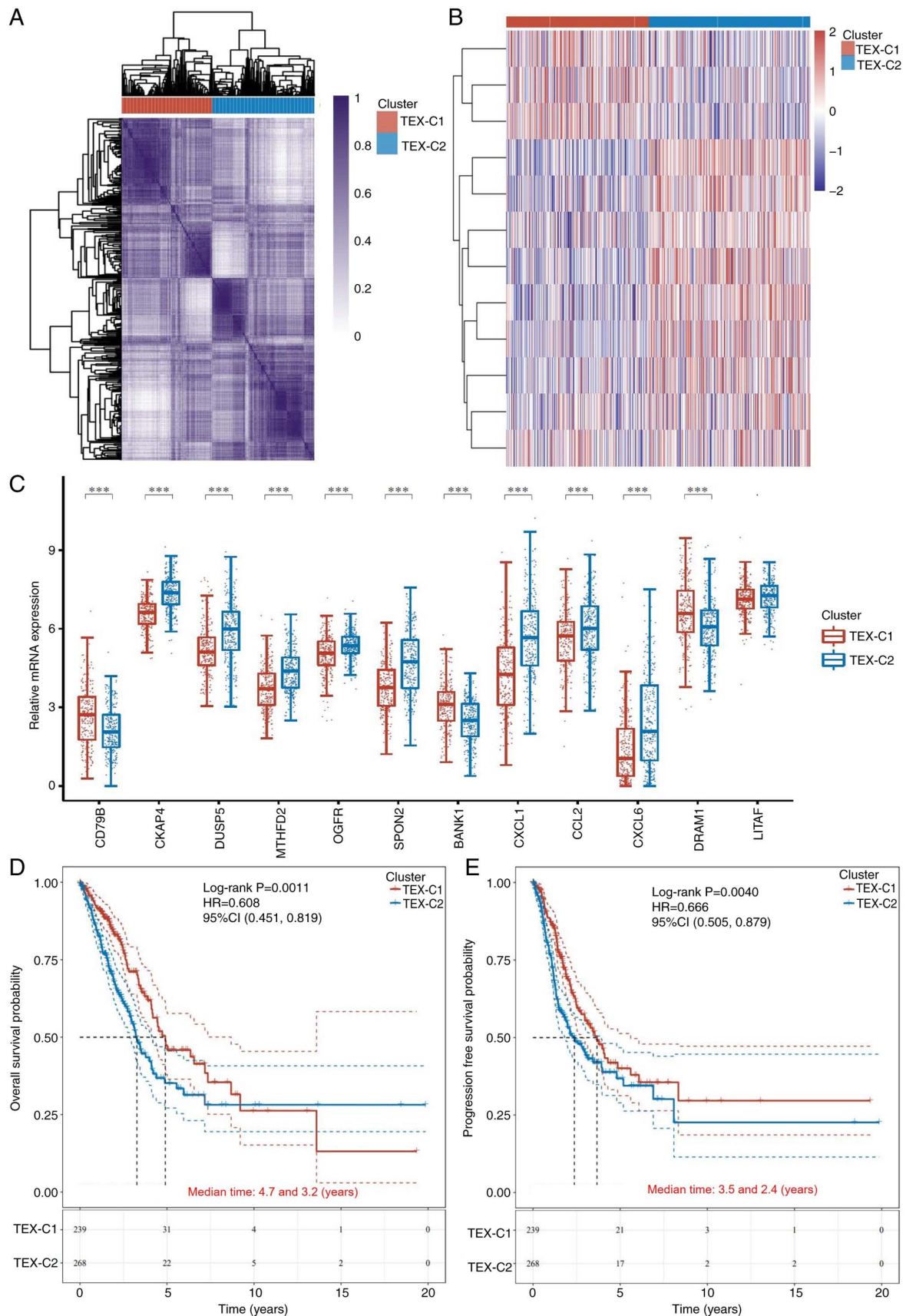


Figure 1. Cluster and survival analysis of TEX-related genes in patients with lung adenocarcinoma. (A) Unsupervised consensus cluster analysis identifying two clusters (k=2). (B) Heatmap of TEX-related genes in the two clusters. (C) mRNA expression of TEX-related genes between clusters. Kaplan-Meier survival analysis of (D) overall and (E) progression-free survival between TEX clusters. \*\*\*P<0.001. TEX-C, T cell exhaustion cluster; CD79B, B cell Antigen Receptor Complex-Associated Protein Beta Chain; DUSP, dual Specificity Phosphatase; MTHFD, Methylenetetrahydrofolate Dehydrogenase; OGFR, Opioid Growth Factor Receptor; SPON, Spondin; BANK, B Cell Scaffold Protein With Ankyrin Repeats; CXCL, C-X-C Motif Chemokine Ligand; CCL, C-C Motif Chemokine Ligand; DRAM, DNA Damage Regulated Autophagy Modulator; LITAF, Lipopolysaccharide Induced TNF Factor; HR, Hazard Ratio; CI, Confidence Interval.

Table I. Clinical characteristics between TEX-C1 and TEX-C2 in lung adenocarcinoma.

| Characteristic            | TEX-C1     | TEX-C2      | P-value |
|---------------------------|------------|-------------|---------|
| Status                    |            |             |         |
| Alive                     | 170        | 159         | 0.005   |
| Dead                      | 72         | 115         |         |
| Mean age, years           | 66.4 (9.8) | 64.3 (10.1) | 0.021   |
| Median age, years (range) | 67 (33-88) | 65 (39-87)  |         |
| Sex                       |            |             | 0.739   |
| Female                    | 128        | 150         |         |
| Male                      | 114        | 124         |         |
| Ethnicity                 |            |             | 0.788   |
| Asian                     | 3          | 5           |         |
| Black                     | 24         | 28          |         |
| White                     | 189        | 200         |         |
| Native American           | 0          | 1           |         |
| pT stage                  |            |             | 0.007   |
| T1                        | 41         | 26          |         |
| T1a                       | 27         | 20          |         |
| T1b                       | 34         | 21          |         |
| T2                        | 74         | 95          |         |
| T2a                       | 32         | 50          |         |
| T2b                       | 10         | 17          |         |
| T3                        | 15         | 32          |         |
| T4                        | 8          | 11          |         |
| TX                        | 1          | 2           |         |
| pN stage                  |            |             | 0.001   |
| N0                        | 173        | 159         |         |
| N1                        | 35         | 61          |         |
| N2                        | 25         | 49          |         |
| NX                        | 8          | 3           |         |
| N3                        | 0          | 2           |         |
| pM stage                  |            |             | 0.343   |
| M0                        | 159        | 188         |         |
| M1                        | 6          | 12          |         |
| M1a                       | 1          | 1           |         |
| M1b                       | 1          | 4           |         |
| MX                        | 73         | 67          |         |
| pTNM stage                |            |             | <0.001  |
| I                         | 1          | 4           |         |
| IA                        | 86         | 45          |         |
| IB                        | 68         | 72          |         |
| II                        | 1          | 0           |         |
| IIA                       | 20         | 30          |         |
| IIB                       | 24         | 47          |         |
| IIIA                      | 25         | 48          |         |
| IIIB                      | 4          | 7           |         |
| IV                        | 8          | 18          |         |
| New tumor event           |            |             |         |
| Metastasis (NOS)          | 24         | 37          |         |
| Metastasis (recurrence)   | 3          | 5           |         |
| Primary                   | 7          | 4           |         |
| Recurrence                | 22         | 26          |         |

Table I. Continued.

| Characteristic         | TEX-C1 | TEX-C2 | P-value |
|------------------------|--------|--------|---------|
| Metastasis (primary)   | 0      | 1      | 0.482   |
| Smoking status         |        |        | 0.255   |
| Non-smoking            | 40     | 35     |         |
| Smoking                | 194    | 233    |         |
| Therapy                |        |        | 0.378   |
| Ancillary chemotherapy | 2      | 1      |         |
| Chemotherapy           | 70     | 85     |         |
| Chemotherapy; targeted | 1      | 5      |         |
| molecular therapy      |        |        |         |
| Targeted molecular     | 2      | 0      |         |
| therapy                |        |        |         |
| Chemotherapy;          | 4      | 0      |         |
| immunotherapy          |        |        |         |
| Immunotherapy          | 1      | 0      |         |
| Vaccine                | 0      | 1      |         |

TEX, T cell exhaustion; C, cluster; X, unidentified; NOS, Not otherwise specified.

cluster (Fig. 3B). Moreover, PD-L1 expression was positively correlated with the expression of TEX-related genes such as BANK1, CCL2, CD79B, CXCL1 and CXCL6 (Fig. 3C). TIDE method was used to predict response to ICB. The TIDE score of the TEX-C2 cluster was significantly higher than that of the TEX-C1 cluster, suggesting this cluster had a poor ICB response and shorter survival time following ICB treatment (Fig. 3D).

*Construction of the prognostic TEX-related gene signature in patients with LUAD.* A risk prediction model for patients with LUAD was constructed based on TEX-related genes and prognosis-related genes were selected using the LASSO algorithm combined with 10-fold cross-validation for further analysis in the best subset regression. A total of six genes (CD79B, CKAP4, DUSP5, MTHFD2, SPON2 and DRAM1) were identified as the best candidates, with a  $\lambda$  value of 0.027. The risk score for prognosis prediction was calculated as follows: Risk score =  $(-0.0863) \times \text{CD79B} + (0.2314) \times \text{CKAP4} + (0.068) \times \text{DUSP5} + (0.0249) \times \text{MTHFD2} + (0.0203) \times \text{SPON2} + (-0.0694) \times \text{DRAM1}$ . The best subset regression model and coefficient and deviance profiles are shown in Figs. 4A and S2A and B. Patients were divided into low- and high-risk groups using median risk score (1.487) as the cutoff value. Kaplan-Meier survival curves indicated that the median OS of the low-risk group was longer than that of the high-risk group (Fig. 4B). ROC analysis showed that the 1-, 3- and 5-year prediction accuracies of the TEX-related gene prediction model were 0.695, 0.668 and 0.645, respectively (Fig. S2C). Cox analysis was performed to analyze the association between CD79B, CKAP4, DUSP5, MTHFD2, SPON2, DRAM1 and clinicopathological factors such as age, sex, ethnicity and grade in the

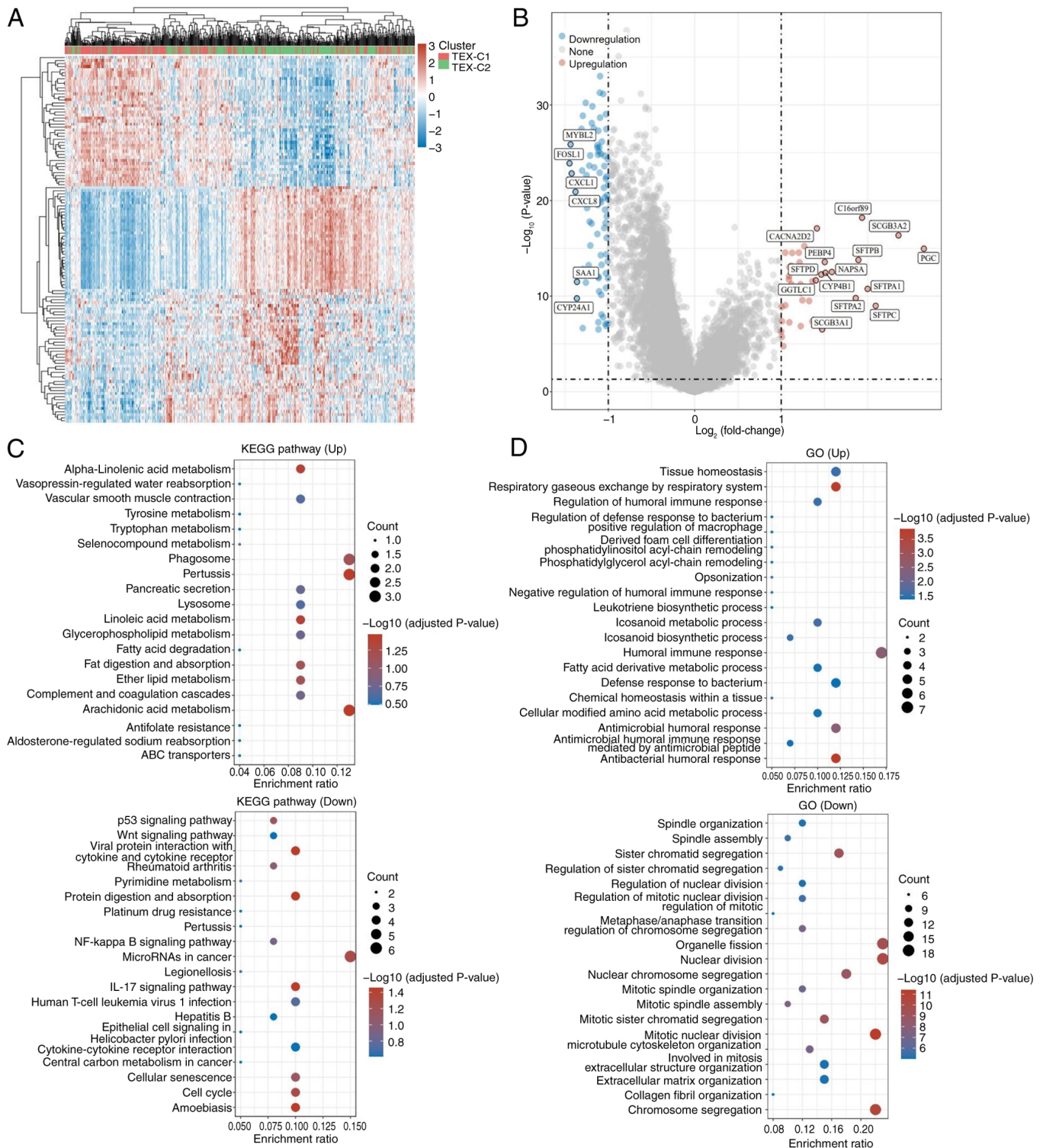


Figure 2. Differential gene expression and gene enrichment analysis of TEX-based clusters in patients with lung adenocarcinoma. (A) Heatmap of differentially expressed genes between TEX clusters, (B) Volcano plot displays the magnitude of gene expression changes (fold change) and the significance of changes (P-value). Functional enrichment analysis includes the KEGG pathway enrichment results (C) and GO term enrichment results (D) for differentially up- or downregulated genes between the two TEX clusters. TEX-C, T cell exhaustion cluster; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

OS of patients with LUAD. Univariate prognostic analysis indicated that CD79B, CKAP4, DUSP5, DRAM1, MTHFD2 and p-TNM grades were associated with OS in patients with LUAD (Fig. 4C). Multivariate prognostic analysis further demonstrated that CD79B and pTNM grade were reliable

independent prognostic factors for predicting the prognosis of patients with LUAD. In survival analysis, CD79B indicated a decreased risk while pTNM grade indicated an increased risk (Fig. 4D). CD79B was marked with a scale indicating the value range; the length of the line reflected

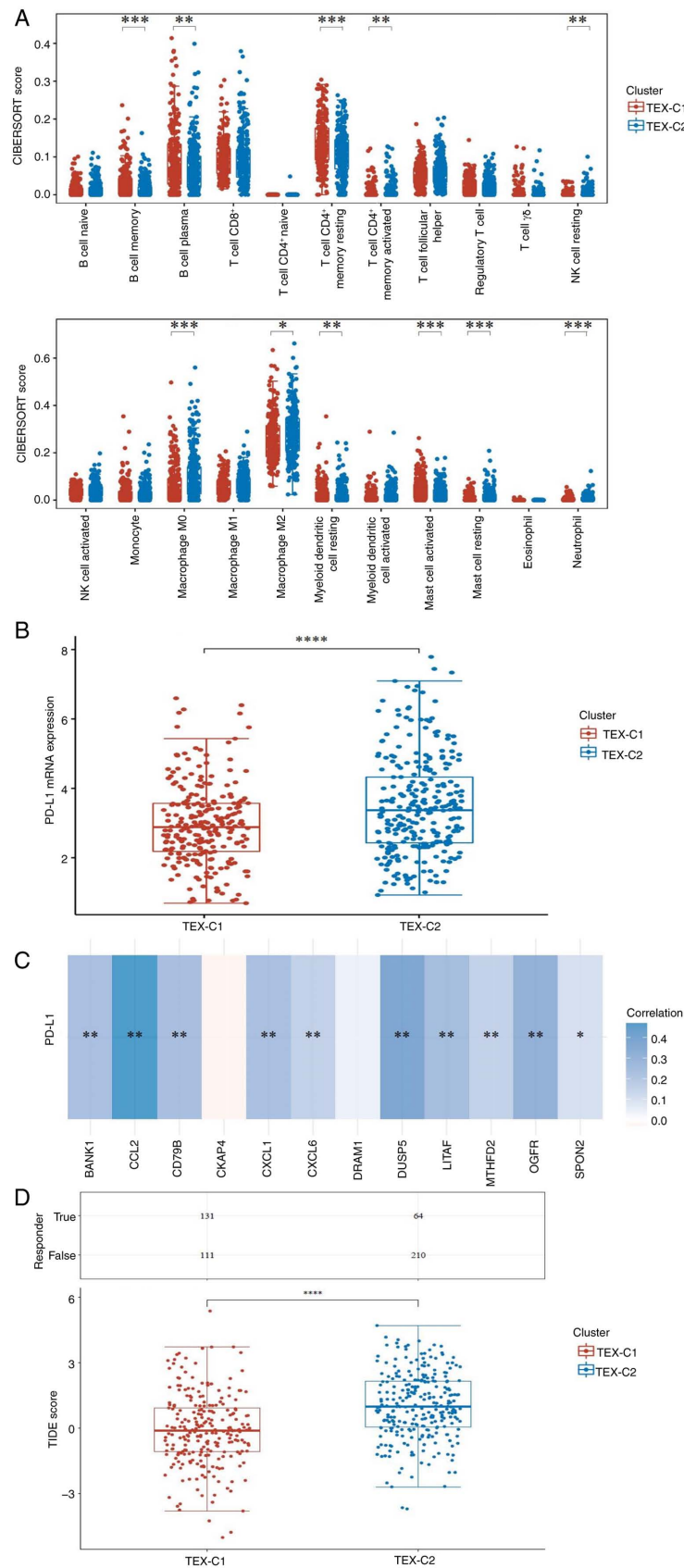


Figure 3. Tumor immune microenvironment analysis of TEX-based clusters in patients with lung adenocarcinoma. (A) Infiltration of immune cells between TEX clusters was analyzed using the CIBERSORT algorithm. (B) mRNA expression of PD-L1 between TEX clusters. (C) Heatmap of correlation between PD-L1 and TEX-related genes. Blue, positive; red, negative; the darker the color, the stronger the association. (D) TIDE scores in TEX clusters. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$ . TEX-C, T cell exhaustion cluster; TIDE, Tumor Immune Dysfunction and Exclusion; CD79B, B cell Antigen Receptor Complex-Associated Protein Beta Chain; DUSP, Dual Specificity Phosphatase; MTHFD, Methylene tetrahydrofolate Dehydrogenase; OGFR, Opioid Growth Factor Receptor; SPON, Spondin; BANK, B Cell Scaffold Protein With Ankyrin Repeats; CXCL, C-X-C Motif Chemokine Ligand; CCL, C-C motif chemokine ligand; DRAM, DNA Damage Regulated Autophagy Modulator; LITAF, Lipopolysaccharide Induced TNF Factor; PD-L1, Programmed Cell Death 1 Ligand 1; NK, natural killer cell.

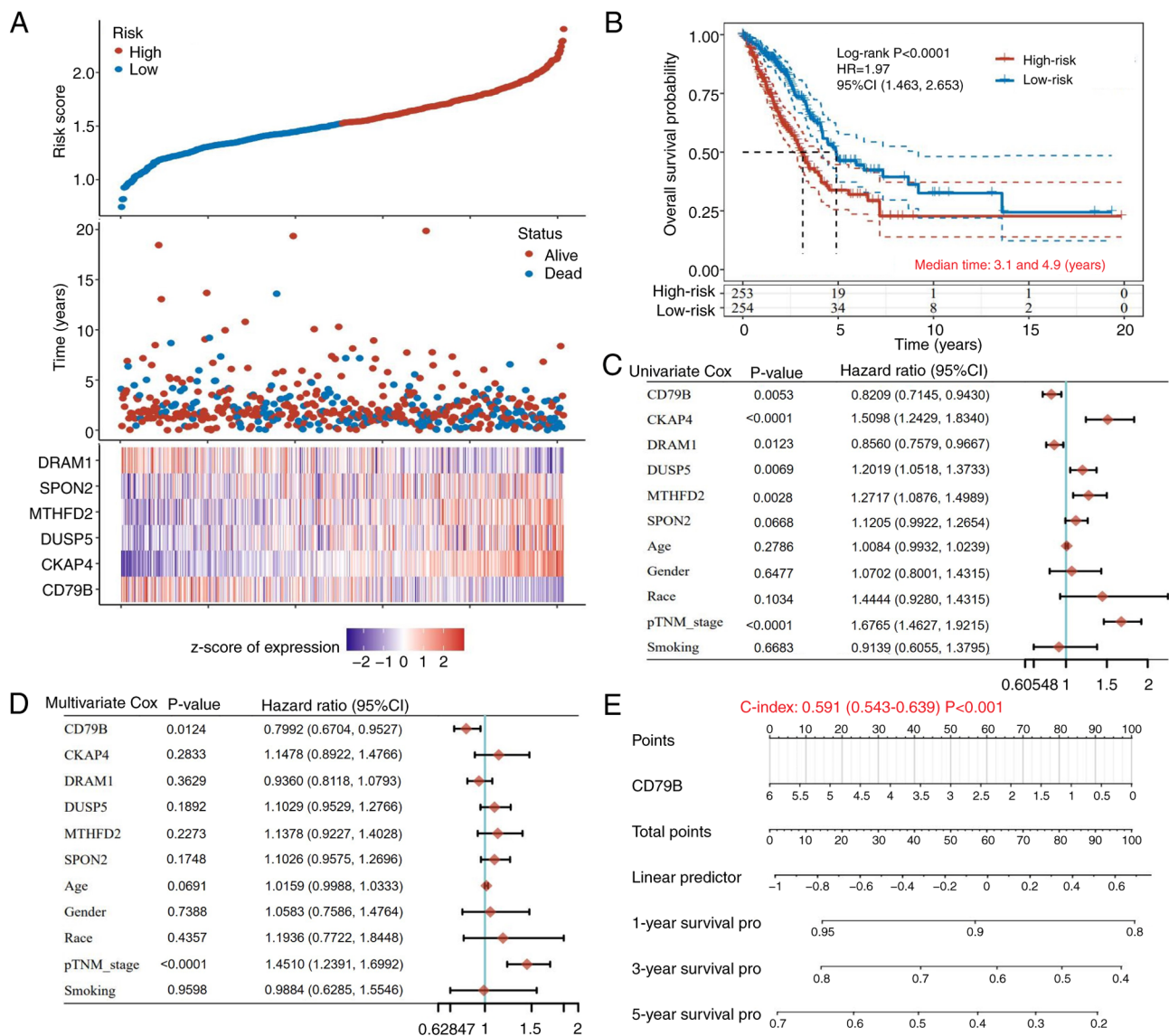


Figure 4. Construction of the prognostic TEX-related gene signature in patients with LUAD. (A) Prognostic analysis of the TEX-related gene signature in patients with LUAD. The patients were divided into low- and high-risk groups using median risk score (1.487) as the cutoff value. (B) Kaplan-Meier analysis of overall survival between low- and high-risk groups. (C) Uni- and (D) multivariate Cox overall survival analyses of the prognostic TEX-related genes and other clinicopathological factors in patients with LUAD. (E) Nomogram based on the results of the multivariate analysis to predict the 1-, 3- and 5-year overall survival of patients with LUAD. TEX, T cell exhaustion; LUAD, lung adenocarcinoma; CD79B, B cell Antigen Receptor Complex-Associated Protein Beta Chain; DRAM, DNA damage Regulated Autophagy Modulator; DUSP, Dual Specificity Phosphatase; MTHFD, Methylenetetrahydrofolate Dehydrogenase; SPON, Spindin; pro, probability.

contribution to the prognostic analysis; consistency between the prognosis predicted by CD79B and the actual occurrence was statistically significant. (Fig. 4E). Based on the score of these factors, the nomogram predicted the 1-, 3- and 5-year OS in patients with LUAD (Fig. S2D).

**Prognosis analysis between CD79B expression and clinical data in patients with LUAD.** To confirm the prognostic role of CD79B in LUAD, TCGA-LUAD patients were divided into a CD79B-low and a -high group based on the median CD79B mRNA expression (2.391). Low CD79B expression in patients with LUAD was associated with shorter OS and PFS, compared with those with high expression of CD79B (Fig. 5A and B). Survival analysis showed that patients with higher CD79B expression had better prognosis in advanced

LUAD (stage III; Fig. 5C), while there was no significant difference in the prognostic effect of CD79B in early-stage lung cancer (stages I and II; Fig. S3A). Similarly, elderly patients (aged  $\geq 65$  years) with high CD79B expression had longer OS, compared with low CD79B expression (Fig. 5D), while there was no significant difference in the prognostic role of CD79B for patients  $< 65$  years (Fig. S3B). Female patients with high CD79B expression had better prognosis than male patients, however, there was no significant difference in prognosis among male patients based on CD79B levels ( $P=0.109$ ; Fig. S3C). High expression of CD79B in smokers indicated better prognosis (Fig. 5F), however, there was no significant difference in prognosis for non-smokers based on CD79B levels (Fig. S3D). These results suggested that CD79B can be a potential prognostic biomarker for LUAD, especially in

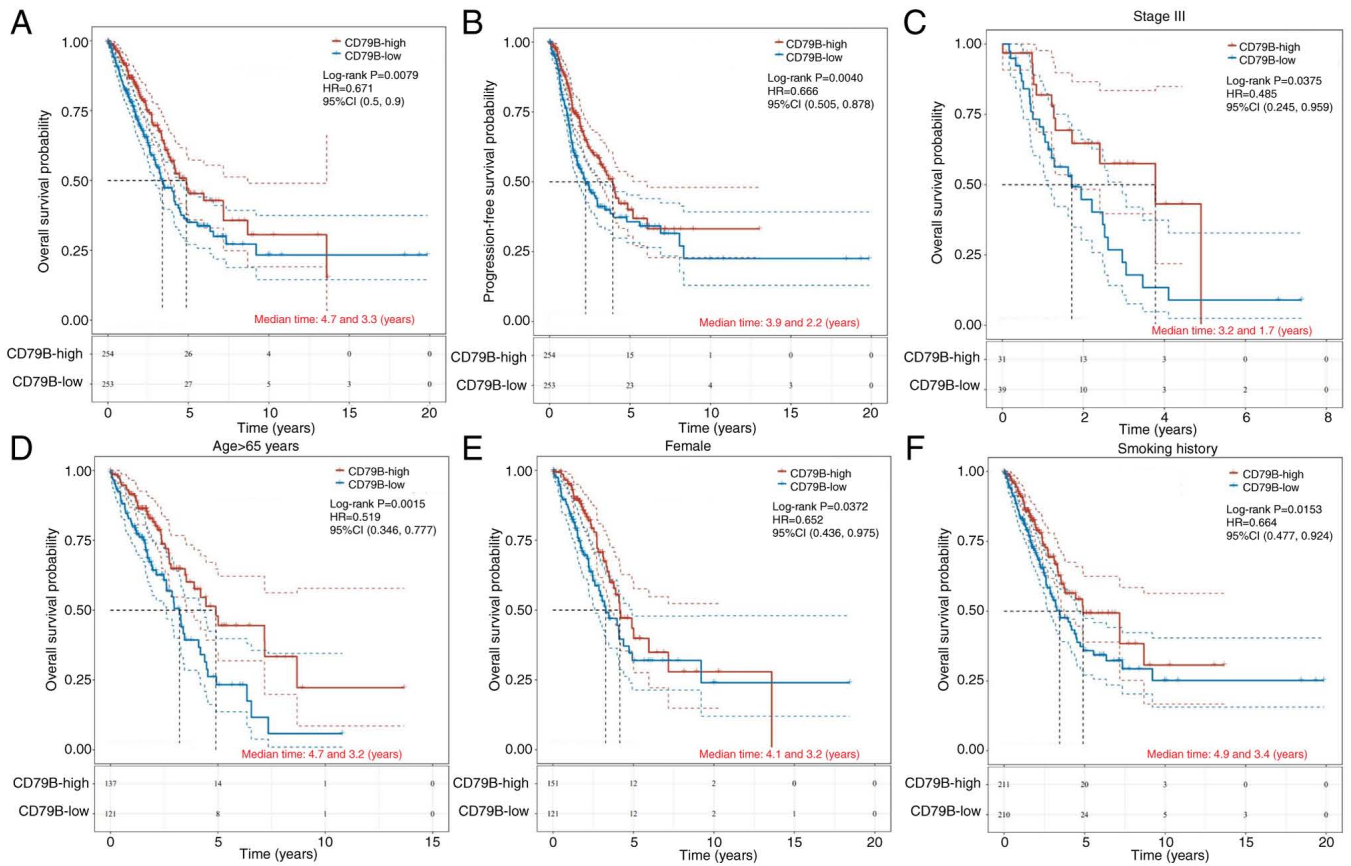


Figure 5. Prognosis analysis between CD79B expression and clinical data in patients with LUAD. The patients were divided into CD79B-low and -high groups. Kaplan-Meier survival analysis of (A) overall and (B) progression-free survival between CD79B-low and -high groups. Association between CD79B expression and prognosis in patients with LUAD and (C) stage III, (D) age >65 years, (E) female sex and (F) smoking history. CD79B, B cell antigen receptor complex-associated protein Beta Chain; LUAD, lung adenocarcinoma; HR, Hazard Ratio; CI, Confidence Interval.

advanced cases, elderly individuals, female patients and those with a history of smoking.

**Bioinformatics and tumor immune microenvironment analysis of CD79B expression in patients with LUAD.** Tumor proliferation signature and G2M checkpoint were negatively associated with CD79B expression by Spearman's correlation analysis based on the Gene Set Variation Analysis (Fig. S4A). However, the p53 pathway, apoptosis, inflammatory response and IL-10 anti-inflammatory signaling pathway were positively correlated with CD79B expression (Fig. S4A). The IL-10 signaling pathway inhibits activation of pro-inflammatory macrophages by downregulating mTOR and promoting mitochondrial autophagy (47). IL-10 is secreted by Tregs, which synergistically promote TEX in tumors by regulating the expression of numerous inhibitory receptors (48). In KEGG enrichment analysis, genes upregulated by CD79B expression were associated with immune-related pathways such as 'T cell receptor signaling pathway', 'B cell receptor signaling pathway', 'Th1 and Th2 cell differentiation', 'Th17 cell differentiation', 'PD-L1 expression and PD-1' and 'primary immunodeficiency' (Fig. S4B). GO analysis of CD79B expression demonstrated enrichment of innate and adaptive immune-related pathways, including 'mononuclear cell proliferation' and 'leukocyte proliferation' as well as 'T cell activation', 'T cell proliferation' and 'B cell activation'

(Fig. S4C). The protein-protein interaction network of CD79B was obtained from the STRING database. Most of the proteins interacting with CD79B were B cell markers (CD19 and CD79A) as well as components of B cell receptor (BCR)-dependent kinase signaling pathways (Spleen Associated Tyrosine Kinase and Bruton Tyrosine Kinase, BTK), which are common in diffuse large B cell lymphoma (DLBCL; Fig. S4D) (49). These results indicated that CD79B may affect prognosis in patients with LUAD patients through immune-related pathways.

The correlation between CD79B expression and immune cell levels in patients with LUAD was analyzed; six types of immune cells (B and CD4<sup>+</sup> and CD8<sup>+</sup> T cells, neutrophils, macrophages and dendritic cells) were all positively correlated with CD79B expression (Fig. 6A). CIBERSORT algorithm was used to investigate the association between CD79B expression and selected immune cells to determine their effect on the tumor microenvironment. Compared with the CD79B-low group, there was more infiltration of CD8<sup>+</sup> T cells and M1 macrophages (classically activated type 1, pro-inflammatory type), but less infiltration of M0 (the undifferentiated) and M2 macrophages (the alternatively activated type 2, anti-inflammatory type), which may be relevant to the inhibition of TEX (Fig. 6B). In addition, the association between CD79B and immune markers of infiltrating immune cells in LUAD was investigated. The expression of CD79B in LUAD was strongly correlated with

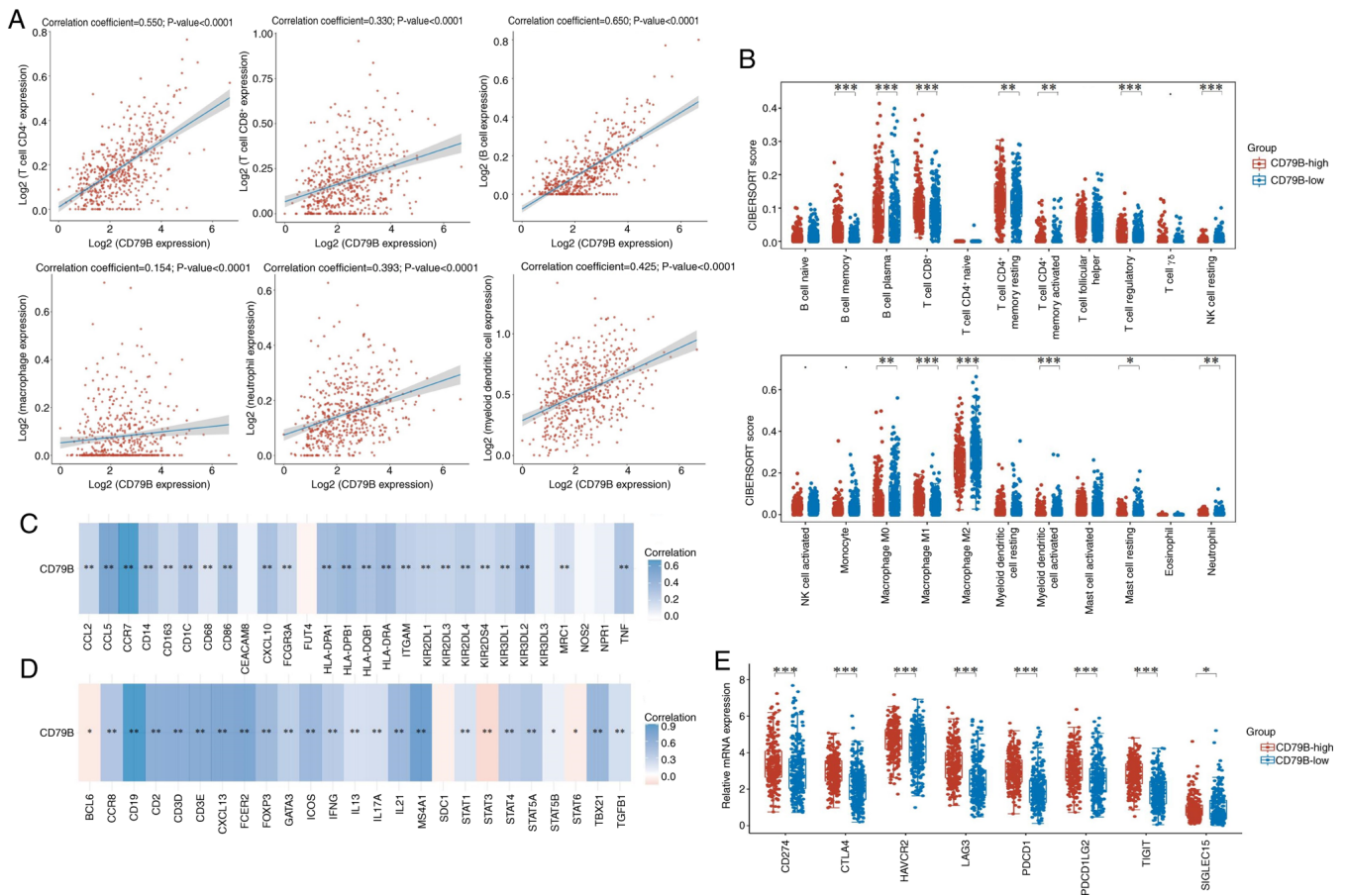


Figure 6. Tumor immune microenvironment analysis of CD79B expression in patients with lung adenocarcinoma. (A) Correlation between CD79B expression and immune cell levels was analyzed using Spearman's correlation. (B) Infiltration of immune cells between CD79B-low and -high groups was analyzed using the CIBERSORT algorithm. Correlation between CD79B and markers of (C) innate and (D) adaptive immune cells. (E) mRNA expression levels of immune checkpoint genes between CD79B-low and -high groups. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ . CD79B, B cell Antigen Receptor Complex-Associated Protein Beta Chain; NK, natural killer cell; CTLA, Cytotoxic T-Lymphocyte Associated Protein 4; HAVCR, Hepatitis A Virus cellular receptor 1; LAG, Lymphocyte Activating 3; PDCD1LG2, Programmed Cell Death 1 Ligand 2; TIGIT, T Cell Immunoreceptor with Ig and ITIM Domains; SIGLEC, Sialic Acid Binding Ig Like Lectin.

innate immune cell markers, including monocyte [CD14, CD86 and Fc  $\gamma$  receptor IIIa (FCGR3A)], TAM (CD68, CCL2 and CCL5), M1 [CXCL10 and Tumor Necrosis Factor (TNF)] and M2 macrophage [Mannose Receptor C-Type 1 (MRC1) and CD163], neutrophil [Integrin Subunit Alpha M (ITGAM) and C-C Motif Chemokine Receptor 7 (CCR7)] and natural killer [Killer Cell Immunoglobulin Like Receptor, Two Ig Domains And long cytoplasmic tail 1 (KIR2DL1), KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2 and KIR2DS4] and dendritic cell markers [Major Histocompatibility Complex, Class II, DP Beta 1 (HLA-DPB1), HLA-DQB1, HLA-DRA, HLA-DPA1 and CD1C; Table II]. The CD79B expression was significantly correlated with adaptive immunity cell markers, including CD3D, CD3E and CD2 of T cells, CD19, Membrane Spanning 4-Domains A1 (MS4A1) and Fc Epsilon Receptor 2 (FCER2) of B cells, T-Box Transcription Factor 21 (TBX21), Signal Transducer And Activator Of Transcription 4 (STAT4), STAT1 and Interferon Gamma (IFNG) of T helper 1 (Th1) cells, Trans-Acting T-Cell-Specific Transcription Factor GATA-3 (GATA3), STAT6, STAT5A and Interleukin 13 (IL13) of Th2 cells, B Cell CLL/Lymphoma 6 (BCL6), IL21, Inducible T Cell Costimulator (ICOS) and CXCL13 of T follicular helper (Tfh) cells, STAT3 and IL17A of Th17 cells, as well as Forkhead

Box P3 (FOXP3), CCR8, STAT5B, Transforming Growth Factor Beta 1 (TGFB1) and Interleukin 2 Receptor Subunit Alpha (IL2RA) of Tregs (Table III). The expression of CD79B was significantly correlated with gene markers for both innate and adaptive immune cells; most correlations were positive, CD79B negatively correlated with BCL6, STAT6 and STAT3 (Fig. 6C and D). mRNA expression of eight immune checkpoint genes was significantly increased in the CD79B-high group (Fig. 6E). Based on these results, the high expression of CD79B was closely associated with immune cell infiltration in LUAD, which may serve a key role in reprogramming T cells from TEX states.

*Survival analysis between CD79B expression and driver gene mutations in patients with LUAD.* MAF files from TCGA database were used to show the mutation rate in LUAD patients, and the top 20 gene landscape of driver genes with mutation frequency is illustrated in Fig. S5. Patients with LUAD with high CD79B expression and wild-type KRAS, EGFR, BRAF, ALK or TP53 had better OS compared with the CD79B-low group (Fig. 7). However, there was no significant difference in OS between patients with KRAS, EGFR, BRAF, ALK or TP53 mutations.

Table II. Correlation analysis between B cell Antigen Receptor Complex-Associated Protein Beta Chain and markers of innate immunity cells.

| Cell           | Gene markers | Cor    | P-value |
|----------------|--------------|--------|---------|
| Monocyte       | CD14         | 0.306  | <0.001  |
|                | CD86         | 0.313  | <0.001  |
|                | FCGR3A       | 0.192  | <0.001  |
|                |              |        |         |
| TAM            | CD68         | 0.124  | 0.005   |
|                | CCL2         | 0.200  | <0.001  |
|                | CCL5         | 0.544  | <0.001  |
|                |              |        |         |
| M1 macrophage  | NOS2         | 0.029  | 0.515   |
|                | CXCL10       | 0.319  | <0.001  |
|                | TNF          | 0.300  | <0.001  |
|                |              |        |         |
| M2 macrophage  | MRC1         | 0.145  | <0.001  |
|                | CD163        | 0.192  | <0.001  |
| Neutrophils    | CEACAM8      | 0.042  | 0.338   |
|                | ITGAM        | 0.240  | <0.001  |
|                | CCR7         | 0.666  | <0.001  |
|                | FUT4         | -0.342 | 0.438   |
| Natural killer | KIR2DL1      | 0.214  | <0.001  |
|                | KIR2DL3      | 0.209  | <0.001  |
|                | KIR2DL4      | 0.250  | <0.001  |
|                | KIR3DL1      | 0.232  | <0.001  |
|                | KIR3DL2      | 0.353  | <0.001  |
|                | KIR3DL3      | 0.063  | 0.154   |
|                | KIR2DS4      | 0.185  | <0.001  |
|                |              |        |         |
| Dendritic      | HLA-DPB1     | 0.394  | <0.001  |
|                | HLA-DQB1     | 0.324  | <0.001  |
|                | HLA-DRA      | 0.367  | <0.001  |
|                | HLA-DPA1     | 0.349  | <0.001  |
|                | CD1C         | 0.298  | <0.001  |
|                | NPR1         | 0.068  | 0.123   |
|                |              |        |         |

TAM, tumor-associated macrophage; cor, correlation coefficient.

*Prediction of therapeutic drug sensitivity based on CD79B expression in patients with LUAD.* To explore the drug sensitivity between CD79B expression subgroups, IC50 values of common chemotherapy and molecular targeted therapeutic drugs for LUAD were calculated. The CD79B-low group had higher sensitivity to chemotherapeutic drugs such as docetaxel and vinorelbine, compared with CD79B-high group. However, patients in the CD79B-high group had higher sensitivity to targeted agents such as gefitinib, crizotinib and dabrafenib. Additionally, there was no significant difference in the sensitivity to paclitaxel, gemcitabine, cisplatin and erlotinib (Fig. 8). Given the extensive crosstalk between tumor and immune cells within the tumor microenvironment (50), TEX-related gene CD79B may be a potential target for combination therapy to improve tumor immunotherapy.

*CD79B inhibits proliferation, migration and invasion and promotes apoptosis of LUAD cells, and induces M1-like TAM polarization in vitro.* qPCR and western blotting showed

Table III. Correlation analysis between CD79B and markers of adaptive immunity cells.

| Cell         | Gene markers | Cor    | P-value |
|--------------|--------------|--------|---------|
| T            | CD3D         | 0.641  | <0.001  |
|              | CD3E         | 0.672  | <0.001  |
|              | CD2          | 0.631  | <0.001  |
| B            | CD19         | 0.902  | <0.001  |
|              | MS4A1        | 0.816  | <0.001  |
|              | SDC1         | -0.075 | 0.088   |
|              | FCER2        | 0.722  | <0.001  |
| Th1          | TBX21        | 0.541  | <0.001  |
|              | STAT4        | 0.346  | <0.001  |
|              | STAT1        | 0.187  | <0.001  |
|              | IFNG         | 0.357  | <0.001  |
| Th2          | GATA3        | 0.334  | <0.001  |
|              | STAT6        | -0.090 | 0.041   |
|              | STAT5A       | 0.378  | <0.001  |
|              | IL13         | 0.197  | <0.001  |
| Tfh          | BCL6         | -0.098 | 0.026   |
|              | IL21         | 0.516  | <0.001  |
|              | ICOS         | 0.521  | <0.001  |
|              | CXCL13       | 0.701  | <0.001  |
| Th17         | STAT3        | -0.179 | <0.001  |
|              | IL17A        | 0.208  | <0.001  |
| Regulatory T | FOXP3        | 0.515  | <0.001  |
|              | CCR8         | 0.397  | <0.001  |
|              | STAT5B       | 0.095  | 0.031   |
|              | TGFB1        | 0.212  |         |
|              | IL2RA        | 0.369  | <0.001  |

Th, T helper; Tfh, T follicular helper; cor, correlation coefficient.

that the expression of CD79B in LUAD cells was lower than that in normal lung epithelial cells (Fig. 9A and B). To investigate the role of CD79B in LUAD cells, A549 cells were transfected with the CD79B-overexpression plasmid and verified by qPCR and western blotting (Fig. 9C and D). Compared with the control group, the viability of A549 cells overexpressing CD79B was significantly inhibited (Fig. 9E). Flow cytometry revealed that overexpression of CD79B could induce apoptosis in A549 cells (Fig. 9F). Scratch and Transwell assay results indicated that the migration and invasion ability of A549 cells overexpressing CD79B significantly weakened (Fig. 9G and H). M0 macrophage THP-1 cells were co-culture with A549 cells overexpressing CD79B. Compared with the vector group, macrophages co-cultured with A549 cells overexpressing CD79B demonstrated upregulation of M1 marker genes such as CD86, TNF- $\alpha$  and iNOS, while expression of M2 macrophage marker genes such as CD206 and IL-10 was significantly decreased (Fig. 9I). Thus, the TEX-related gene CD79B is not only involved in proliferation, invasion, migration and apoptosis of cancer cells but also contributed to M1-like TAM polarization in LUAD.

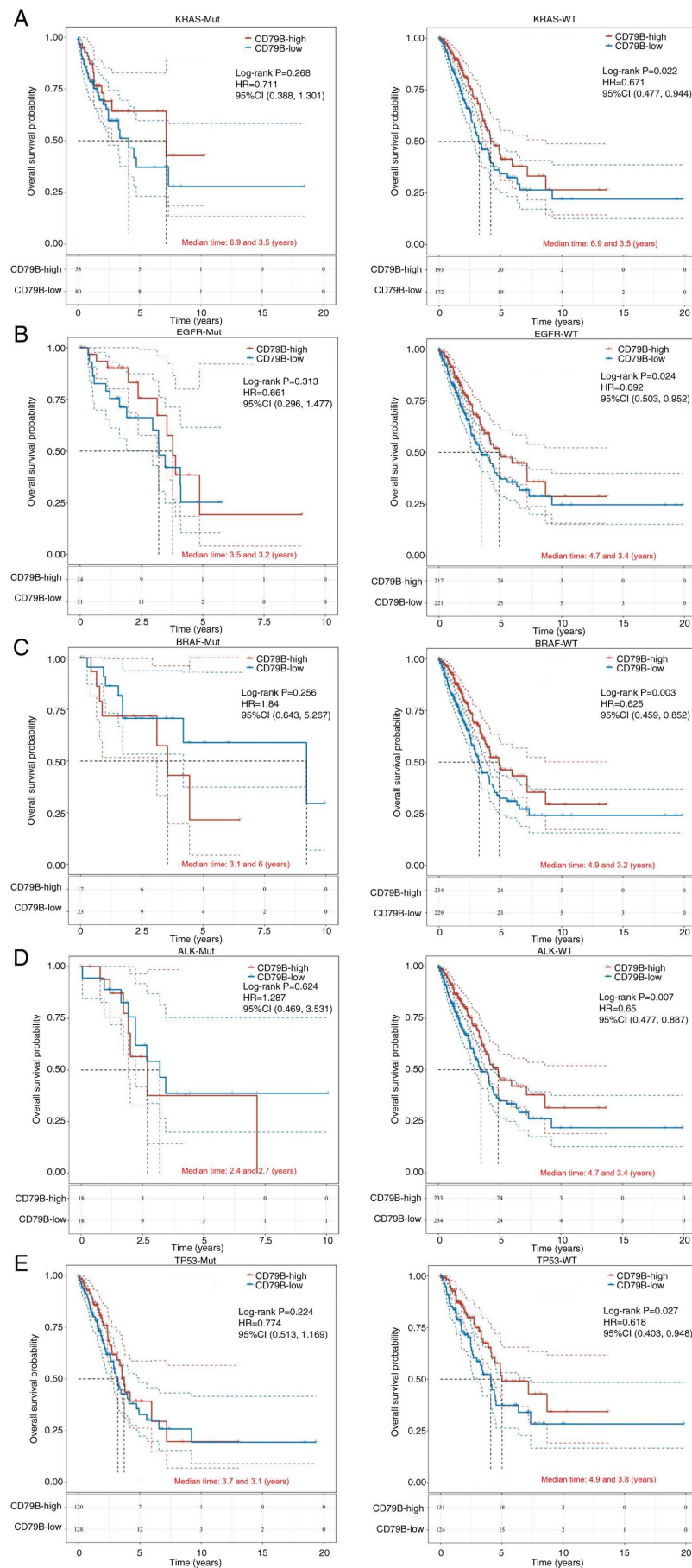


Figure 7. Survival analysis between CD79B expression and driver gene mutations in patients with lung adenocarcinoma. Overall survival of patients with mut or WT (A) KRAS, (B) EGFR, (C) BRAF, (D) ALK and (E) TP53 between CD79B-low and -high groups. CD79B, B-Cell Antigen Receptor Complex-Associated Protein Beta Chain; ALK, Anaplasticlymphoma Kinase; mut, mutant; HR, Hazard Ratio; CI, Confidence Interval; WT, wildtype.

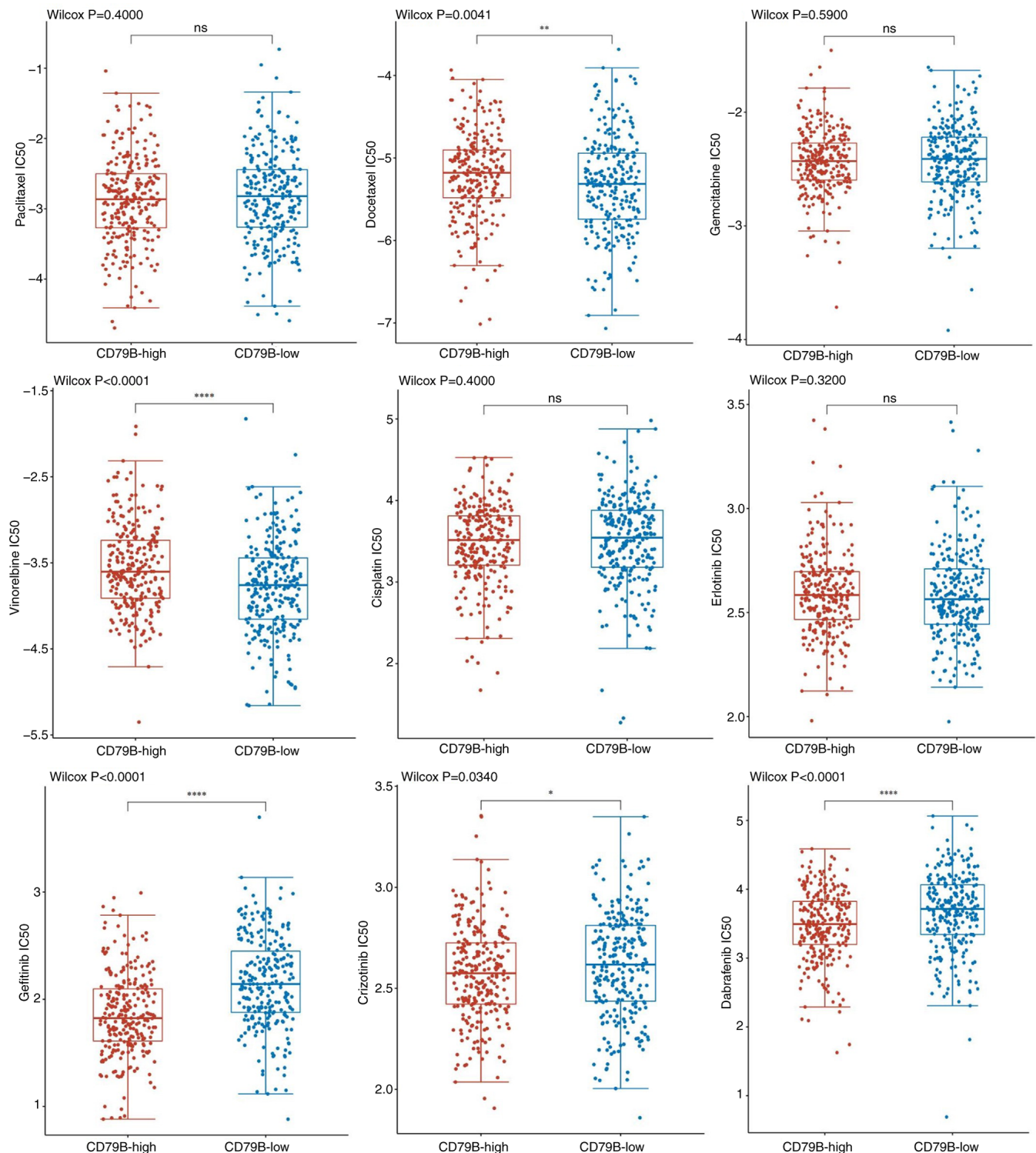


Figure 8. Prediction of therapeutic drug sensitivity based on CD79B expression in patients with LUAD. The distribution of IC50 scores of LUAD chemotherapy and targeted drugs between CD79B- and CD79B-high groups. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\*\* $P < 0.0001$ . CD79B, B-Cell Antigen Receptor Complex-Associated Protein Beta Chain; LUAD, lung adenocarcinoma; ns, not significant; IC50, half-maximal inhibitory concentration.

## Discussion

TEX, a state of effector T cell dysfunction, is a notable obstacle to developing effective cancer immunotherapy (51). Therefore, exploring the mechanism of TEX and the effect of immunotherapy has become a target in anti-tumor research (17,52). The present study performed consensus clustering analysis

of TCGA-LUAD patients based on the expression of 12 TEX-related genes, and the patients were divided into two clusters. Patients in the TEX-C2 cluster had worse OS and PFS compared with those in the TEX-C1 cluster. Upregulated genes in the TEX-C2 cluster were enriched in metabolism-related pathways, while downregulated genes were enriched in p53, NF- $\kappa$ B and IL-17 signaling pathways. TEX-C2 cluster had

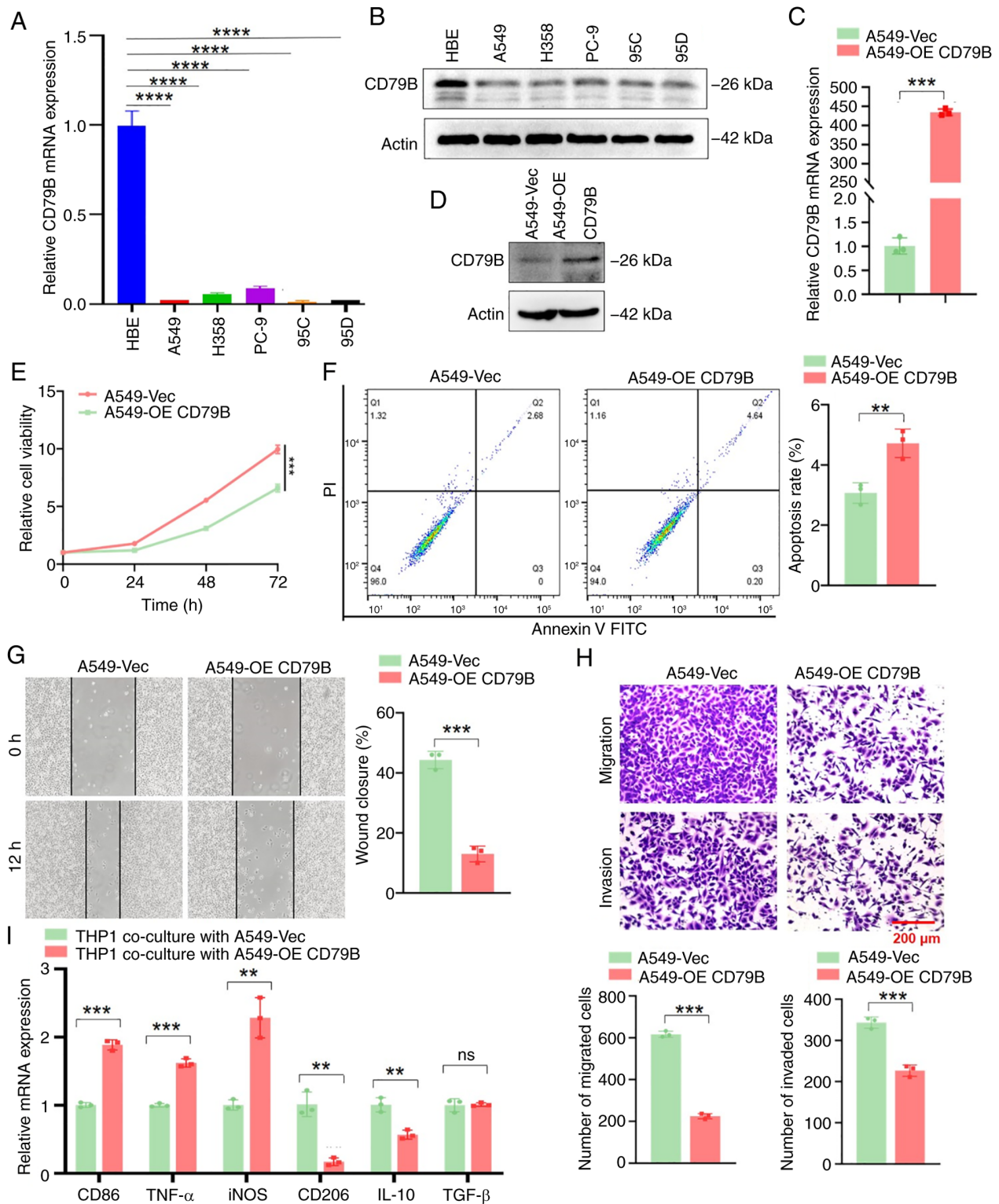


Figure 9. CD79B inhibits proliferation, migration and invasion and promotes apoptosis of LUAD cells, and induces M1-like tumor-associated macrophage polarization *in vitro*. mRNA and protein expression of CD79B were detected by (A) qPCR and (B) western blotting in normal lung epithelial HBE and LUAD cells. A549 cells were transfected with the CD79B-OE plasmid and mRNA and protein expression of CD79B were detected by (C) qPCR and (D) western blotting. (E) Cell viability was detected by Cell Counting Kit 8 assay. (F) Flow cytometry was used to analyze cell apoptosis. (G) Scratch assay was used to detect cell migration. (H) Transwell assay was used to analyze cell migration and invasion ability. (I) M0 macrophage THP-1 cells were co-culture with A549 cells, and the mRNA levels of M1/M2 marker genes were detected by qPCR after 48 h. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$ . CD79B, B-Cell Antigen Receptor Complex-Associated Protein Beta Chain; LUAD, lung adenocarcinoma; q, quantitative; OE, Over-Expression; ns, not significant; Vec, Vector; iNOS, Nitric Oxide Synthase, Inducible.

a poor ICB response and shorter survival following ICB treatment. T cells are prone to exhaustion in an environment

characterized by mitochondrial capacity loss, glucose metabolism defects and exposure to hypoxia, particularly intolerable

levels of reactive oxygen species (53). TEX may further reduce metabolic fitness, resulting in anti-tumor immune dysfunction and suppression (54). In acute myeloid leukemia with TP53 mutation, Tregs show gene expression signatures suggestive of metabolic adaptation to their environment, whereas CTLs exhibit features of exhaustion/dysfunction with stronger expression of T cell Immunoglobulin and mucin domain 3 (55). Inhibition of NF- $\kappa$ B activity due to loss of Interleukin 1 Receptor Associated Kinase 4 leads to T cell dysfunction through production of immunosuppressive factors and checkpoint ligands in pancreatic ductal adenocarcinoma (56). CD4<sup>+</sup> T cells trigger the recruitment of CD11b+Gr-1+ Myeloid-derived suppressor cells (MDSCs) to promote terminal exhaustion of CD8<sup>+</sup> T cells and tumor progression *in vivo* by secreting IL-17 (57). ICB therapy is used to inhibit TEX by blocking antibodies against immune checkpoints, including CTLA4, PD-1 and its ligand PD-L1. However, the efficacy of ICB therapy depends on the stage of T cell differentiation and is most successful when antigen-specific T cells are functional, proliferate and produce effective responses to maintain anti-tumor function (58). Additionally, ICB therapy alone does not reprogram T cells out of their exhausted state (59). Current studies aim to develop new strategies to target terminally exhausted T cells to reactivate and restore their immune function for immunotherapy (60,61). The present results indicate that the poor prognosis of TEX-C2 cluster patients may be associated with higher susceptibility to TEX due to tumor immune metabolism-related pathways, which makes it more difficult to benefit from ICB therapy.

A prognostic TEX-related gene signature model was constructed to predict the prognosis of patients with LUAD. Among the prognosis-related genes, CD79B was considered an independent prognostic biomarker. Survival analysis showed that the high expression of CD79B in patients with LUAD was associated with a good prognosis, especially in advanced lung cancer, the elderly, female patients and patients with a history of smoking. The results of qPCR and western blot showed that CD79B was expressed at low levels in LUAD cells. Previous studies have shown that CD79B expression is also downregulated in cervical cancer tissues and hepatocellular carcinoma cell lines (62,63). CD79B, as a component of the B cell antigen receptor, has a notable impact on immune synapse formation, antigen affinity and cell migration and serves a crucial role in the maturation and maintenance of B cells (64). CD79B is highly expressed on the surface of cells in almost all patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia (65). There generally exists a positive correlation between the intensity of BCR signaling and the expression of CD79B (66). Another mechanism through which mutated CD79B increases BCR signaling is its inability to properly activate the Src-family kinase Lyn, which triggers a negative feedback loop-based inhibition of BCR signaling (67). However, in tumor cells from classical Hodgkin lymphoma, many B cell lineage-specific genes are downregulated, including CD79B. Silencing of CD79B is associated with its promoter methylation (68). In CD30-positive diffuse large B cell lymphoma, low CD79B expression is related to paired box 5 interacting with complexes of histones that modify enhancers and promoters for target genes, thereby influencing transcriptional activation (69). In Epstein-Barr

Virus-negative Burkitt lymphoma cells, Epstein-Barr nuclear antigen 2 inhibits the expression of CD79B by binding its promoter and interfering with the binding and transcriptional activation of Early B cell factor 1 (70). However, the molecular mechanisms underlying downregulation of CD79B expression remain to be elucidated in LUAD. These may involve complex alterations in gene regulatory networks, abnormal signaling pathways and other factors such as promoter methylation.

In the present study, high CD79B expression was associated with immune-related pathways. Moreover, CD79B was strongly associated with a large number of immune cell populations and most immune genetic markers, including immune checkpoint-related genes, which also supported the poor OS of the CD79B-low group. In addition, CD79B was positively correlated with infiltrating CD8<sup>+</sup> T cells and M1 macrophages and negatively correlated with M0 and M2 macrophages. Regardless of the expression of CD79B, targeted therapy could affect the prognosis of LUAD patients with driver gene mutations. EGFR, ALK, BRAF, KRAS and TP53 can provide targeted therapy for eligible patients with LUAD (71). *In vitro*, upregulation of CD79B could inhibit proliferation, migration and invasion while promoting apoptosis of LUAD cells and inducing M1-like TAM polarization. Expansion of IFN gene numbers can be found at the CD79B locus (72). IFN is associated with numerous B cell receptor pathway genes and genes involved in adaptive immune responses. Among them, CD79B has also been validated as an IFN- $\gamma$  response gene in multiple sclerosis (73). CD79B lymphocyte cells produce notable amounts of IL-2, granulocyte-macrophage colony-stimulating factor (CSF), IFN- $\gamma$ , and IL-17A in response to tumor cells in B cell lymphomas (74). M1 macrophages are generated following stimulation by lipopolysaccharide and IFN- $\gamma$  while M2 macrophages are generated by IL-4, IL-10 and IL-13 stimulation (75). Therefore, it was hypothesized that the upregulation of CD79B in tumor cells may promote the production of cytokines such as IFN- $\gamma$ , thereby facilitating the polarization of M0 to M1 macrophages and reprogramming TEX to inhibit malignant progression of LUAD cells. Exhausted T cells could contribute to the recruitment and polarization of macrophages by secreting CSF1 (76). TAMs present cancer cell antigens to CD8<sup>+</sup> T cells via interferon regulatory factor 8, leading to the induction of PD-1 expression, a decrease in effector cytokines and TEX (77). M0 TAMs significantly increase the expression of PD-L2 via the PD-1 signaling pathway, which promotes the polarization of immunosuppressive M2 type macrophages and decreases anti-tumor effector T cells, leading to immune evasion and tumor promotion (78). Xanthine oxidoreductase loss in TAMs promotes M2-like polarization by increasing Isocitrate Dehydrogenase [NADP(+)] 3 activity and  $\alpha$ -ketoglutarate production. It can also promote CD8<sup>+</sup> TEX by upregulating immunosuppressive metabolites, thereby exacerbating hepatocellular carcinoma (HCC) progression (79). Inhibition of 3-Oxoacid CoA-Transferase 1 (OXCT1) expression in TAMs promotes reprogramming to an M1 phenotype via the succinyl-H3K4me3-Arg1 axis, which may also decrease CD8<sup>+</sup> TEX and enhance the anti-tumor effect of T cells (80). In breast cancer model mice, Interleukin 1 Receptor Type 2 (IL1R2) blockade could decrease tumor burden and prolong survival by decreasing PD-L1 transcriptional activators and macrophage recruitment while inhibiting TAM polarization

and CD8<sup>+</sup> TEX (81). Hence, TAM M2 polarization results in CD8<sup>+</sup> TEX, whereas TAM M1 polarization restores migration and infiltration of CD8<sup>+</sup> T cells. This suggests that TAM M1 polarization may be an immunotherapeutic target for reprogramming TEX.

TAM serves key functions in promoting a suppressive tumor immune microenvironment and immune evasion, which limits the treatment effects of ICB therapy in different types of cancer. By using TAM modulators, the suppressive tumor immune microenvironment may be reversed, thereby polarizing M2 into M1 TAMs releasing IL-12, IL-6 and TNF- $\alpha$ , enhancing the activation of CD8<sup>+</sup> T and natural killer cells, and possibly synergizing with PD-1/PD-L1 checkpoint inhibition to enhance anti-cancer immune responses (82). In glioblastoma multiforme, combination therapy with rapamycin and hydroxychloroquine downregulates the CD47-signal regulatory protein  $\alpha$  axis in tumor cells, promoting the polarization of TAMs to a pro-inflammatory M1-like phenotype and enhancing the therapeutic response to anti-PD-1 antibody (83). Humanized modified macrophage-derived microparticles loaded with methionine induce M2-like TAMs to repolarize into the M1 phenotype, promote the infiltration of CD8<sup>+</sup> T cells into tumor tissue, and thereby enhance the anti-cancer activity of anti-PD-1 antibody (84). In the treatment of oral cancer, the combination of selective receptor tyrosine kinase inhibitor and ICB immunotherapy increases the ratio of M1 to M2 macrophages in the tumor microenvironment while promoting polarization of TAMs towards an immunostimulatory state, thereby prolonging survival time (85). The aforementioned studies indicate that TAMs may serve as a potential target for combination therapy to enhance the efficacy of tumor immunotherapy. Patients in CD79B-high group had higher sensitivity to targeted agents such as gefitinib, crizotinib and dabrafenib. The upregulation of CD79B induced M1-like TAM polarization to inhibit malignant progression of LUAD cells *in vitro*. It was hypothesized that patients with high levels of CD79B may exhibit a better response to combined use of immune checkpoint inhibitors and TAM polarizing agents.

However, there are certain limitations in the present study. Firstly, the present retrospective study was based on TCGA database, which may have potential selection bias. Therefore, it is necessary to conduct prospective studies to verify the conclusions. Secondly, there was a lack of molecular mechanism studies to investigate the functional role of CD79B in TAM polarization toward the M1 phenotype. Further *in vitro* and *in vivo* experiments are required to elucidate the potential regulatory mechanisms. Thirdly, given the absence of relevant data from patients with LUAD, the results require further validation through clinical studies involving larger sample sizes.

In summary, the present study performed clustering analysis using TEX-related genes and developed a prognostic TEX-related gene signature model to predict the prognosis of patients with LUAD. Among the prognosis-related genes, CD79B was considered an independent prognostic marker. Upregulation of CD79B inhibited proliferation, migration and invasion while promoting apoptosis in LUAD cells and inducing M1-like TAM polarization. These results demonstrated the clinical value of TEX-related genes, suggesting CD79B may be a potential prognostic indicator and therapeutic target for improving tumor immunotherapy.

## Acknowledgements

Not applicable.

## Funding

The present study was supported by the Natural Science Foundation of Hunan Province, China (grant no. 2025JJ50551) and the Fundamental Research Funds for the Central Universities of Central South University (grant no. 2024ZZTS0912).

## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

XW constructed figures, analyzed data, performed experiments and wrote the manuscript. CQ performed experiments. YO designed and performed the experiments. LY analyzed data, designed and performed the experiments and edited the manuscript. WJ conceived the study, designed and performed the experiments, analyzed data and edited the manuscript. XW and CQ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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