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Single-cell sequencing analysis of the pancreatic cancer samples

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Abstract. Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most lethal human malignancies with poor survival outcome. It is characterized by late diagnosis, rapid growth and limited therapeutic choices. Increasing evidence has suggested that gene expression may undergo extensive changes during the process of normal cells to malignant transformation which may play a critical role in the initiation and progression of human PDAC. In this study, we applied single-cell RNA sequencing (scRNA-seq) technology to comprehensively compared the Peripheral Blood Mononuclear Cells (PBMCs) and tissue samples from pancreatic ductal adenocarcinoma patients and normal individuals. We characterized transcriptional heterogeneity and identified differentially expressed genes as well as the changes of immune microenvironment between PDAC and normal individuals through integrative bioinformatic analysis. We believe that our results will provide new insights into the cellular and molecular characterization of PDAC and may provide potential implications for future therapeutic approaches and biomarker discoveries.

Keywords: Pancreatic Ductal Adenocarcinoma (PDAC), single-cell RNA sequencing (scRNA-seq), Peripheral Blood Mononuclear Cells (PBMCs), differential gene expression, differential pathway enrichment, altered peripheral immune states

1. Introduction

Cancer is one of the main public health problems worldwide, leading to millions of deaths every year. Among all kinds of human malignancies, Pancreatic Cancer (PC) is one of the most lethal human malignancies with high morbidity and late diagnosis, causing a large number of deaths in recent years [1, 2]. The pancreas is responsible for endocrine (e.g. secretion of insulin and glucagon) and exocrine (e.g. secretion of digestive enzyme) functions. Pancreatic cancer arises from non-invasive precursor lesions, termed pancreatic ductal adenocarcinoma in situ (PanIN), during which the duct epithelial cells acquire multiple genetic and epigenetic alterations [3, 4]. These alterations disrupt tumor-suppressive programs and drive the gain of aberrant signaling and chronic inflammation, finally presenting nonspecific symptoms, such as anorexia, jaundice and diabetes [5, 6]. Because pancreatic ductal adenocarcinoma presents no apparent symptoms in its early stage, it is usually diagnosed at late stages when curative resection is no longer possible. Currently, systemic chemotherapy is the main treatment for advanced pancreatic ductal adenocarcinoma with poor prognosis [7]. Therefore, more effective strategies for early detection are urgently needed.

Traditional RNA sequencing (bulk RNA-seq) plays an important role in decoding cellular heterogeneity in normal and diseased tissues to address biological questions and build transcriptome-level maps of a single cell. Bulk RNA sequencing averages signals from heterogeneous cell populations, whereas single-cell RNA sequencing (scRNA-seq) can decompose cellular heterogeneity and separate different cell types, states, and dynamic trajectories [8]. The general workflow of scRNA-seq involves tissue dissociation, single-cell isolation, reverse transcription and amplification of mRNA from single cells, library preparation, sequencing, and computational analysis for clustering and annotation [9]. Current transcriptome analysis methods based on traditional technology are unable to capture the diversity of cells. It is challenging to identify rare cell populations or trace lineage hierarchies in tissues such as tumors [10]. In contrast, the high resolution, sensitivity, and scalability of scRNA-seq make it an essential tool in numerous applications, including immunology, oncology, and developmental biology [10, 11]. ScRNA-seq has led to major breakthroughs in the discovery of new biomarkers, the study of cell communication, and the analysis of specific transcriptional programs. The application of scRNA-seq in cancer research has achieved remarkable success [12].

ScRNA-seq has significantly reshaped oncology research by enabling detailed insights into tumor heterogeneity, the tumor microenvironment (TME), and mechanisms of immune evasion. Bernard et al. applied scRNA-seq technology to pancreatic cancer precursor single cells and suggested that epithelial and microenvironmental heterogeneity is an early event in cancer development [13]. Sinjab et al. applied scRNA-seq technology to cells from early lung adenocarcinoma (LUAD) and normal tissues and discovered the basic characteristics of LUAD evolving from the peripheral lung ecosystem. Evidence that normal tissues evolve into LUAD in multiple aspects of cell lineage, state, and transcriptome characteristics was revealed [14]. Wu et al. used scRNA-seq technology to profile advanced non-small cell lung cancer and identified rare cell subtypes with distinct transcriptomic features, highlighting the spatial and functional heterogeneity of the TME [15]. Philip Bischoff et al.'s analysis of LUAD using scRNA-seq technology revealed heterogeneity in cancer cell transcriptomes that reflected histological grade and activity of oncogenic pathways, as well as two distinct microenvironmental patterns [16]. In summary, the high-resolution capability of scRNA-seq enables comprehensive mapping of the tumor ecosystem and facilitates the identification of potential diagnostic and therapeutic targets, thereby advancing our understanding of immunobiological processes in early-stage malignancies and other solid tumors.

The tumor microenvironment of pancreatic cancer displays a highly immunosuppressive environment that makes it challenging to challenge with immunotherapy. Although a variety of immune cell subsets infiltrate PDAC tumors, many exhibit dysfunctional or immunoregulatory phenotypes. Cytotoxic CD8+ T cells (CTLs) often display a exhausted gene expression profile, especially in advanced stages. Notably, NLRP3 mediated IL-18 signaling has been reported to promote T cell exhaustion by activating downstream suppressive pathways [17]. In addition, a negative correlation between tumor infiltrating myeloid cells and CD8+ T cell abundance has been discovered, which suggests a myeloid-dominant suppressive TME [18]. These characteristics present substantial challenges to effective immunotherapy and motivate strategies to modulate the TME and reinvigorate antitumor immunity.

2. Methods

2.1. Data source and software environment

The single-cell RNA sequencing (scRNA-seq) data analyzed in this study were obtained from the Gene Expression Omnibus (GEO) database (accession number: GSE155698), which contains PBMC and tissue samples from 17 Pancreatic Ductal Adenocarcinoma (PDAC) patients and 4 healthy donors. Data analysis was

performed primarily in the R environment (v4.4.3). The Seurat package (v5) was used for data import, quality control, normalization, dimensionality reduction, clustering, and visualization. Azimuth was applied for reference-based cell type annotation. Functional enrichment analyses were conducted using the shinyGO platform (v0.85). The tidyverse package suite was used for data processing and visualization (including volcano plots generated with ggplot2). RColorBrewer and ggrepel were used to enhance the aesthetics and labeling of figures.

2.2. Analysis pipelines

2.2.1. QC and normalization

Expression matrices processed by Cell Ranger were imported using Read10X and converted into Seurat objects. Cells with < 200 or > 5,000 detected genes or with > 30% mitochondrial gene content were excluded. Data were log-normalized (NormalizeData), highly variable genes were identified (FindVariableFeatures), and data were scaled (ScaleData).

2.2.2. Clustering and annotation

Principal Component Analysis (PCA) was performed, followed by construction of a k-nearest neighbor graph (FindNeighbors) and clustering (FindClusters, resolution = 2). UMAP was used for visualization. Cell populations were annotated using Azimuth PBMC or Azimuth Pancreas reference, assigning both major (level 1) and fine-grained (level 2) cell types.

2.2.3. Immune cell extraction of tissue

Based on predicted annotation, immune cells were subsetted and reanalyzed. Standard preprocessing, dimensionality reduction, and clustering were repeated to refine immune cell subsets. Immune subsets were annotated using Azimuth PBMC reference to identify specific T cells, B cells and other immune components.

2.2.4. Differential gene expression and functional analysis

For each major cell type, differential expression analysis between PDAC and healthy groups was performed using Seurat's FindMarkers function. Genes with adjusted p-value < 0.05 and |log2 fold change| > 0.6 were considered significant.

Significant DEGs were visualized by volcano plots using ggplot2, with the top 30 genes highlighted. Gene Ontology (GO) enrichment analysis was conducted via shinyGO, and the top enriched biological processes were visualized as bar plots.

3. Results

3.1. Initial clustering and identification of cell types

First Clustering and Identification of Cell Types. In order to explore the immune microenvironment of pancreatic ductal adenocarcinoma, we collected and analyzed scRNA-seq data of 21 peripheral blood mononuclear cell samples in total, including 17 pancreatic ductal adenocarcinoma samples from patients and 4 samples from healthy individuals.

Data preprocessing and integration were implemented via Seurat v5 pipeline, and Azimuth was used for the automated cell type annotation based on a well-curated human PBMC reference. Since there may be certain batch effects and inter-sample variation exists, we applied Seurat's integration workflow to normalize these inter-sample variations. We evaluated the impact of integration by comparing clustering results before and after integration (Fig. 1A-B). After integration, cells from different samples were more cohesive in the low dimensional UMAP space, and the overall alignment of clusters was significantly improved across conditions.

With reference-based annotation, we detected 30 distinct immune cell types, including a diverse range of T cells, B cells, monocytes, dendritic cells, NK cells, and other rare immune subsets. Interestingly, annotation also revealed fine-grained T cell subsets, including naive, effector, central memory (TCM), effector memory (TEM), cytotoxic, and unconventional subsets (e.g., MAIT, double-negative T cells). This annotation result facilitated subsequent comparative analysis between PDAC and control groups.

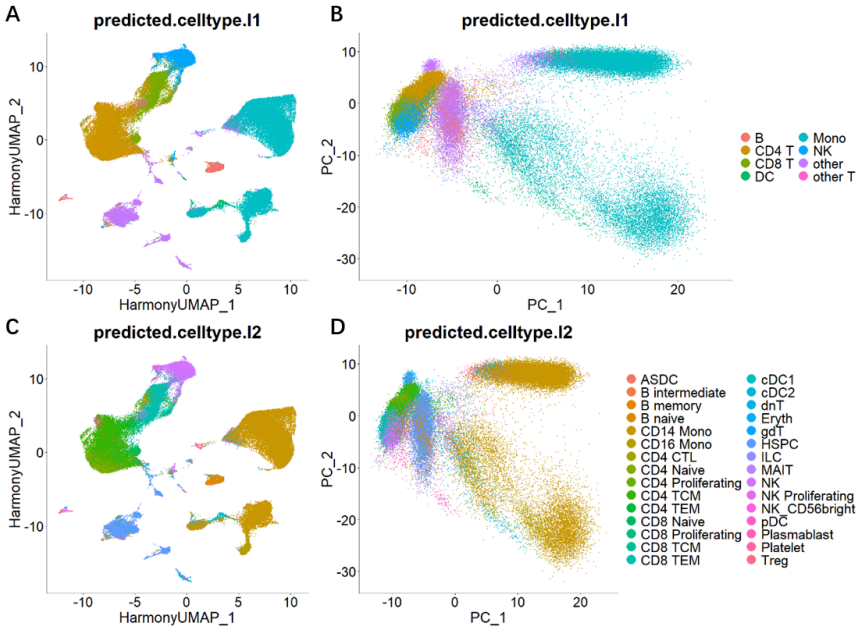

Figure 1. Cell clustering and annotation before and after integration. (A, B) UMAP visualization of single-cell PBMC profiles using cell type classification scheme 1, showing results before (B) and after (A) integration. (C, D) UMAP visualization using cell type classification scheme 2, also shown before (C) and after (D) integration

Integration markedly improved the alignment of clusters across samples, facilitating more accurate identification of immune cell types across PDAC and control groups.

3.2. Neoplastic cell characteristics

To characterize disease-related transcriptional changes, we performed differential expression analysis for each annotated cell type between PDAC and healthy donors. Volcano plots and heatmaps of top Differentially Expressed Genes (DEGs) were generated to highlight gene-level alterations across major immune lineages (Fig. 2, 3).

Volcano plots of DEGs (PDAC vs. healthy) in four representative immune cell subsets from cell type classification scheme 1. The plots share identical analytical settings and visualization parameters, differing only in the analyzed cell type (A, B cell; B, $CD4^+$ T cell; C, $CD8^+$ T cell; D, natural killer cell).

Upregulated and downregulated genes are shown in red and blue respectively.

Volcano plots for 9 representative subsets from cell type classification scheme 2, showing diverse transcriptional responses across cell types. The plots share identical analytical procedures and visualization parameters, differing only in the analyzed cell type (A–I, Naive B cell, CD4+ Cytotoxic T cell, CD8+ Naive T cell, CD8+ Effector Memory T cell, CD4+ Naive T cell, CD16+ Monocyte, Erythroid cell, CD56-dim Natural Killer cell, Plasmablast).

The results reveal strong transcriptional perturbations in T cells and other immune populations, providing insights into PDAC-associated immune remodeling.

From a global perspective, we found that the expression profile of multiple immune cell types was globally reprogrammed in the PDAC group. Many cell types upregulated pro-inflammatory genes and immune checkpoint molecules, as well as genes involved in cellular stress and activation. Some cell types downregulated genes involved in immune surveillance and cytotoxicity, which may represent a state of immune exhaustion or suppression.

Among all subsets, T cells showed especially prominent transcriptional reprogramming. Both $CD4^+$ and $CD8^+$ T cell lineages upregulated or downregulated certain effecter functions [19], memory status, and several signaling pathways. These results suggest that the transcriptional state of T cell functional states are significantly altered in PDAC, potentially contributing to tumor immune escape or shaping the systemic immune contexture. The observed degree and direction of transcriptional changes pointed to T cells as potential biomarkers for PDAC immune contexture and rational targets for treatment.

Given these results, we further sought to dissect T cell subsets to identify their functional heterogeneity and pathway-level alterations that may drive their contributions to PDAC pathogenesis.

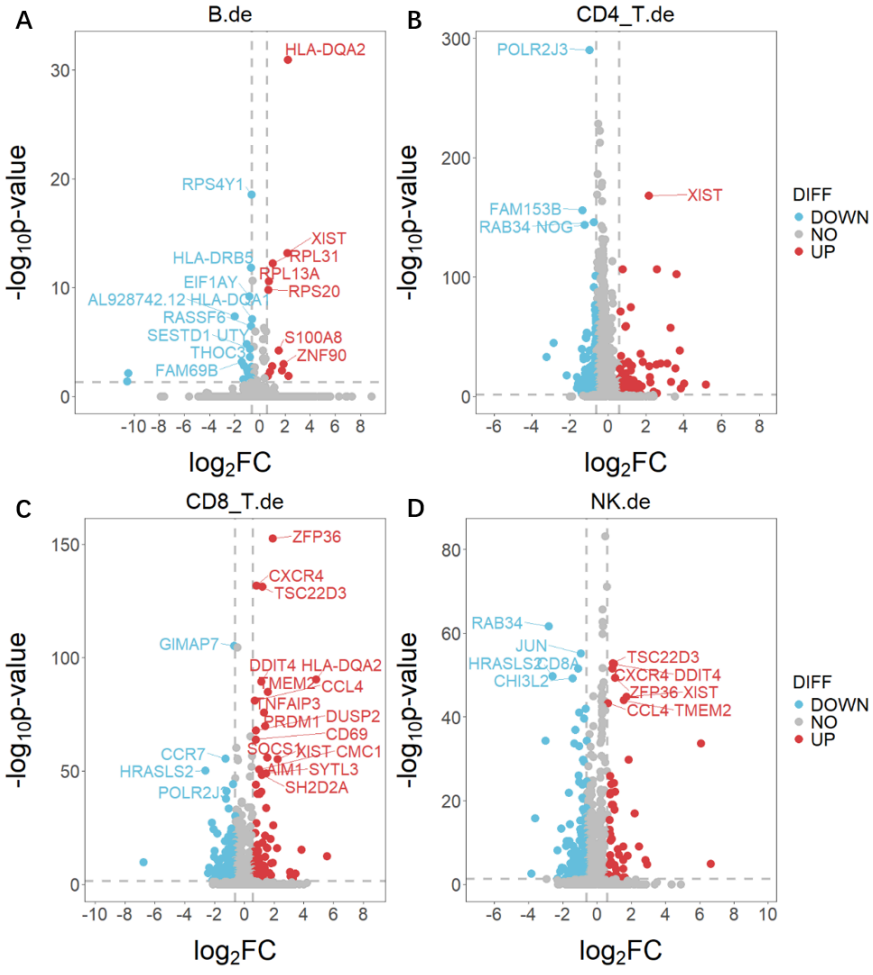

Figure 2. Differential gene expression analysis across representative immune cell types (classification scheme 1)

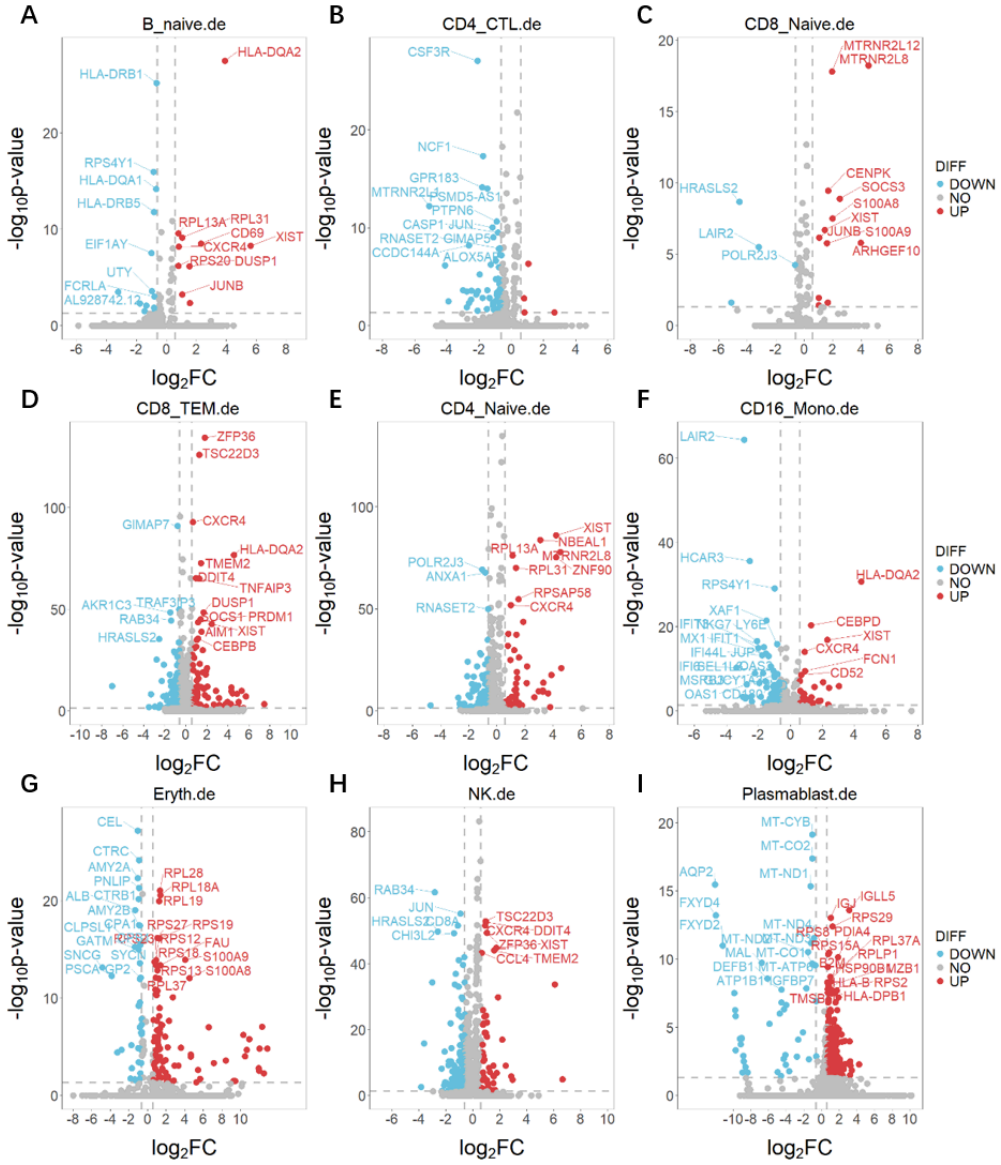

Figure 3. Differential gene expression analysis across representative immune cell types (classification scheme 2)

3.3. Functional alterations of T cell subpopulations in PDAC

3.3.1. Overview of T cell subtypes in PBMC

To explore the immune landscape alterations in Pancreatic Ductal Adenocarcinoma (PDAC), we profiled the Peripheral Blood Mononuclear Cells (PBMCs) from PDAC patients and healthy donors. By using reference-based annotation strategy, we identified 13 types of T cell subtypes, including naïve, memory, cytotoxic, proliferating, regulatory and non-conventional T cells (γδ T, MAIT, double negative T cells). These subtypes were further classified into four functional modules, i.e. activation, effector response, immune regulation and innate-like behavior.

3.3.2. Activation and differentiation changes in naive T cells

In comparison with healthy donors, both $CD4^+$ and $CD8^+$ Naive T cells displayed transcriptional alterations in PDAC samples.

Enrichment analysis of upregulated genes in CD4⁺ Naive T cells revealed significantly enriched activation of multiple inflammatory and metabolic pathways, including the IL-17 signaling pathway [20, 21] and TNF signaling pathway [22, 23] as well as adipocytokine signaling pathway. Chronic inflammation and metabolic stress have been shown to shape the tumor microenvironment in cancer [24]. Additionally, enrichment in Type II diabetes mellitus and insulin resistance signaling pathways may reflect system-wide metabolic dysregulation in PDAC, which has been reported to impair T cell metabolism and function. Notably, the ribosome pathway was also upregulated, suggesting an increase in translational activity, potentially as part of early activation or stress response. The presence of COVID-19-related signaling and osteoclast differentiation pathways may reflect shared cytokine profiles and systemic inflammation (Fig 4-A).

In contrast, CD8⁺ Naive T cells showed broader enrichment across both upregulated and downregulated DEGs, with overlap in inflammatory pathways such as IL-17 and TNF signaling, as well as prolactin signaling, growth hormone signaling, and non-alcoholic fatty liver disease (NAFLD) pathways. These results suggest that CD8⁺ Naive T cells in PDAC may be subjected to hormonal and metabolic reprogramming. The consistent enrichment of osteoclast differentiation in both CD4⁺ and CD8⁺ Naive T cells implies a common axis of cytokine-induced transcriptional regulation (Fig 4-B).

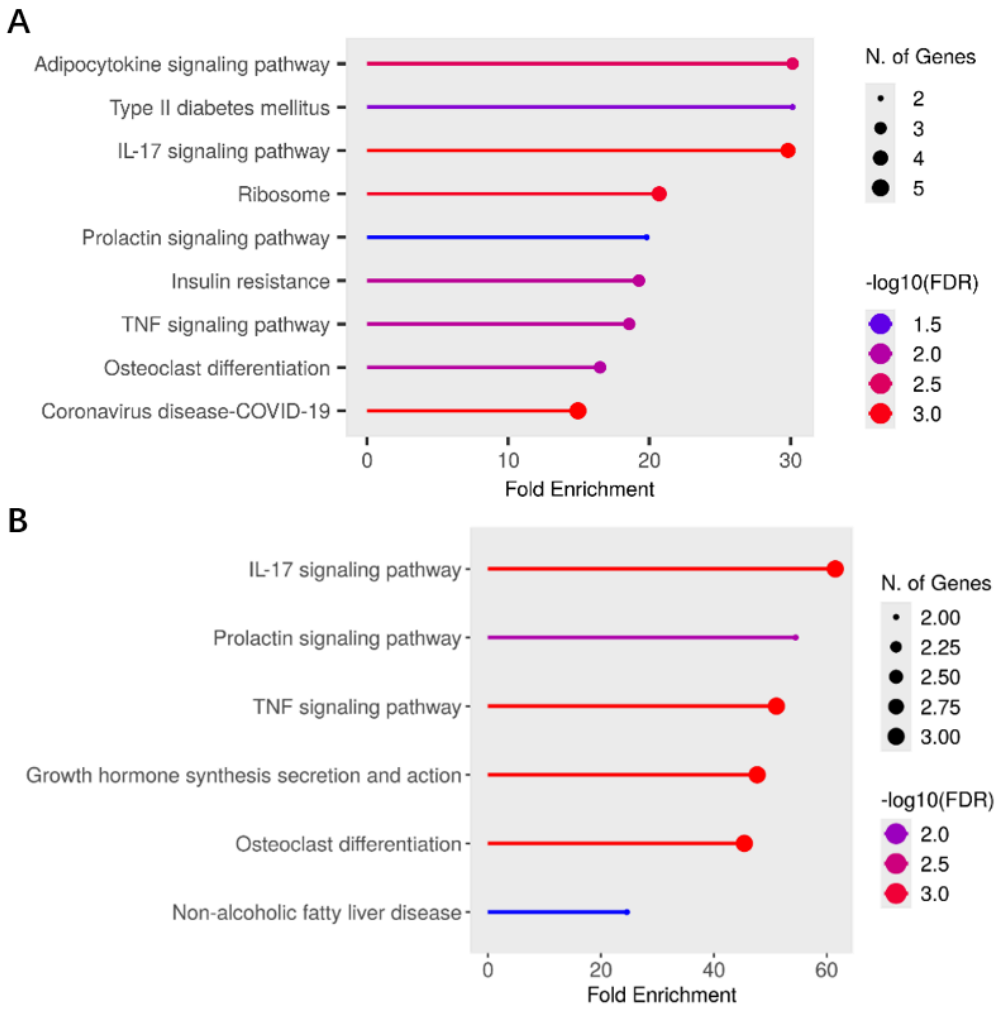

Figure 4. KEGG pathway enrichment analysis of naive T cells. (A) KEGG pathway enrichment of upregulated genes in CD4⁺ naive T cells from PDAC compared with healthy controls. (B) KEGG pathway enrichment of significantly altered genes (both upregulated and downregulated) in CD8⁺ naive T cells

In contrast, $CD8^+$ Naive T cells showed broader enrichment across both upregulated and downregulated DEGs, with overlap in inflammatory pathways such as IL-17 and TNF signaling, as well as prolactin signaling, growth hormone signaling, and Non-Alcoholic Fatty Liver Disease (NAFLD) pathways. These results suggest that $CD8^+$ Naive T cells in PDAC may be subjected to hormonal and metabolic reprogramming. The consistent enrichment of osteoclast differentiation in both $CD4^+$ and $CD8^+$ Naive T cells implies a common axis of cytokine-induced transcriptional regulation (Fig 4-B).

Altogether, our results suggested that in PDAC, compared with healthy donors, naïve T cell subtypes displayed early transcriptional reprogramming and enrichment in activation of inflammatory signaling and metabolic pathways, potentially priming them toward differentiation toward dysfunctional phenotypes or functional exhaustion [25, 26].

3.3.3. Memory and cytotoxic T cells exhibit functional activation and dysregulation in PDAC

To investigate how memory and cytotoxic T cells are involved in immune dysregulation of PDAC, we further conducted KEGG pathway enrichment analysis for DEGs in these T cell subsets.

Among $CD4^+$ TCM, the up - regulated genes are significantly enriched in the ribosome pathway, which means that the translation activity is enhanced and it is well - prepared for coping with chronic antigen exposure. In addition, the enrichment of IL - 17 signaling pathway and miRNAs in cancer shows that there are transcription programs related to sustained inflammation and early cancer recurrence regulation, which may help to construct an immune - permissive environment (Fig 5-A) [27, 28].

$CD8^+$ Effector Memory T cells (CD8 TEM) demonstrated the most extensive enrichment pattern. As expected, multiple immune-related pathways were enriched in CD8 TEM, including T Cell Receptor (TCR) signaling, IL-17 and TNF signaling, and Th1/Th2 differentiation [23, 29]. Meanwhile, the enrichment of PD-1/PD-L1 checkpoint pathways [30, 31], apoptosis, and cytokine-cytokine receptor interaction were also observed, indicating the appearance of exhausted signature and potential immunosuppressive regulation (Fig 5-B). These two signatures might represent a dysfunctional state where $CD8^+$ TEM cells can initiate anti-tumor response but are later impaired in their effector function.

In $CD4^+$ cytotoxic T cells (CD4 CTL), a wide range of signaling pathways were enriched, including TCR signaling [32, 33], JAK-STAT, TNF signaling, and apoptosis, indicating active immune engagement. Notably, the PD-L1 expression and PD-1 checkpoint pathway and chemical carcinogenesis–Reactive Oxygen Species (ROS) pathway were also enriched, highlighting immune checkpoint activation and oxidative stress, two hallmarks of the immunosuppressive PDAC environment. Additionally, enrichment in estrogen signaling, lipid metabolism, and cAMP signaling pathways implies that these CTLs might be influenced by systemic metabolic and hormonal alterations commonly seen in PDAC patients (Fig 4-C).

Together, our results show that memory and cytotoxic T cell subsets in PDAC undergo extensive functional reprogramming with signatures of both activation/effector differentiation as well as exhaustion and regulatory suppression programs that characterize the suppressive context of the tumor. These findings suggest that activation as well as inhibitory signals should be considered when assessing the peripheral T cell response in PDAC.

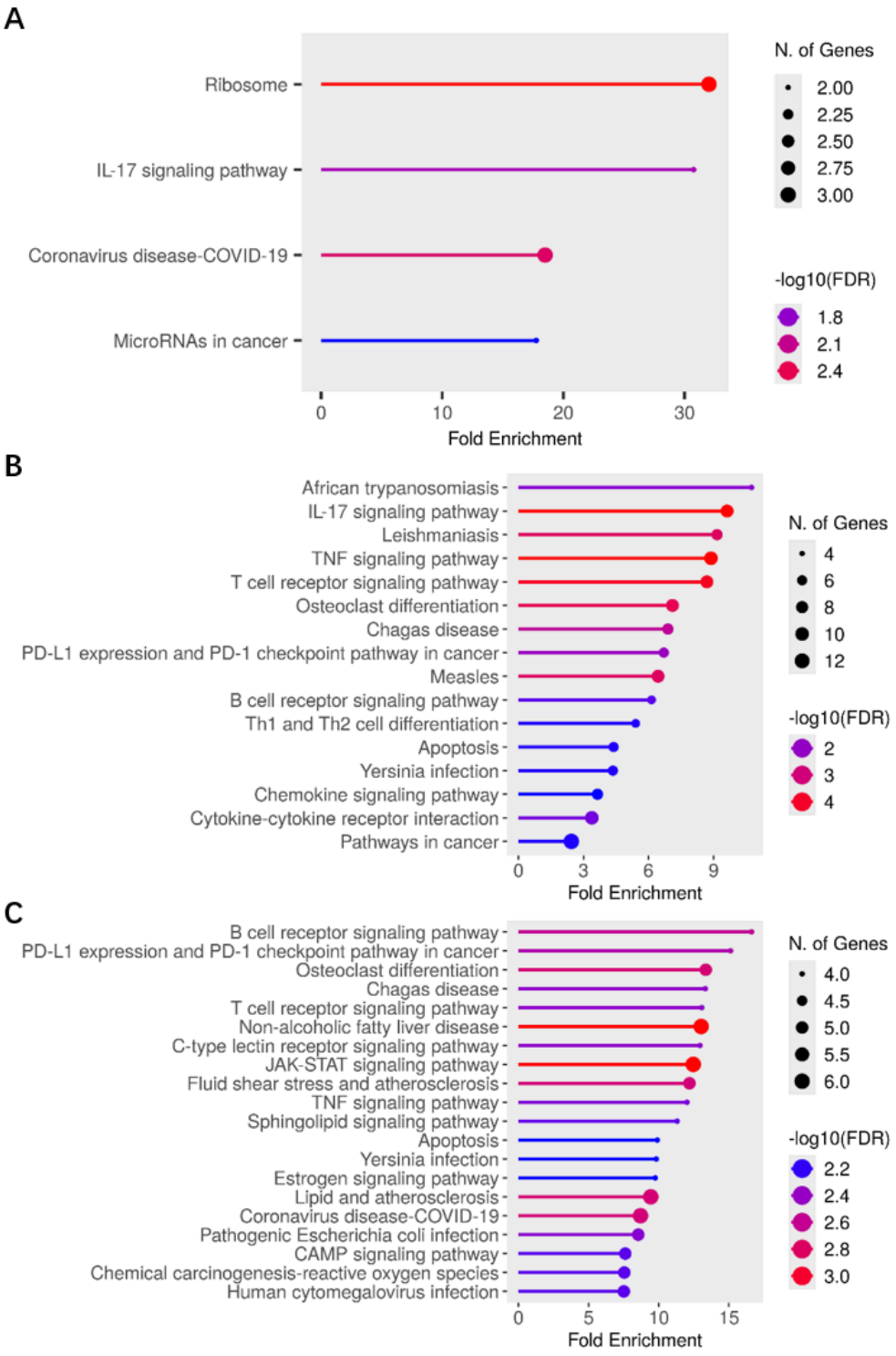

Figure 5. KEGG pathway enrichment analysis of memory and cytotoxic T cell subsets. (A) KEGG pathway enrichment of upregulated genes in $CD4^+$ central memory T cells (CD4 TCM) from PDAC compared with healthy controls. (B) KEGG pathway enrichment of significantly altered genes (both upregulated and downregulated) in $CD8^+$ effector memory T cells (CD8 TEM). (C) KEGG pathway enrichment of significantly altered genes (both upregulated and downregulated) in $CD4^+$ cytotoxic T cells (CD4 CTL)

3.3.4. Non-conventional T cell expansion and immune suppression

To investigate the potential role of non-conventional T cell subsets in PDAC, we further conducted KEGG pathway enrichment analysis for differentially expressed genes in Double-Negative T (DNT) cells and Mucosal-Associated Invariant T (MAIT) cells. Γδ T cells were excluded due to lack of differential genes.

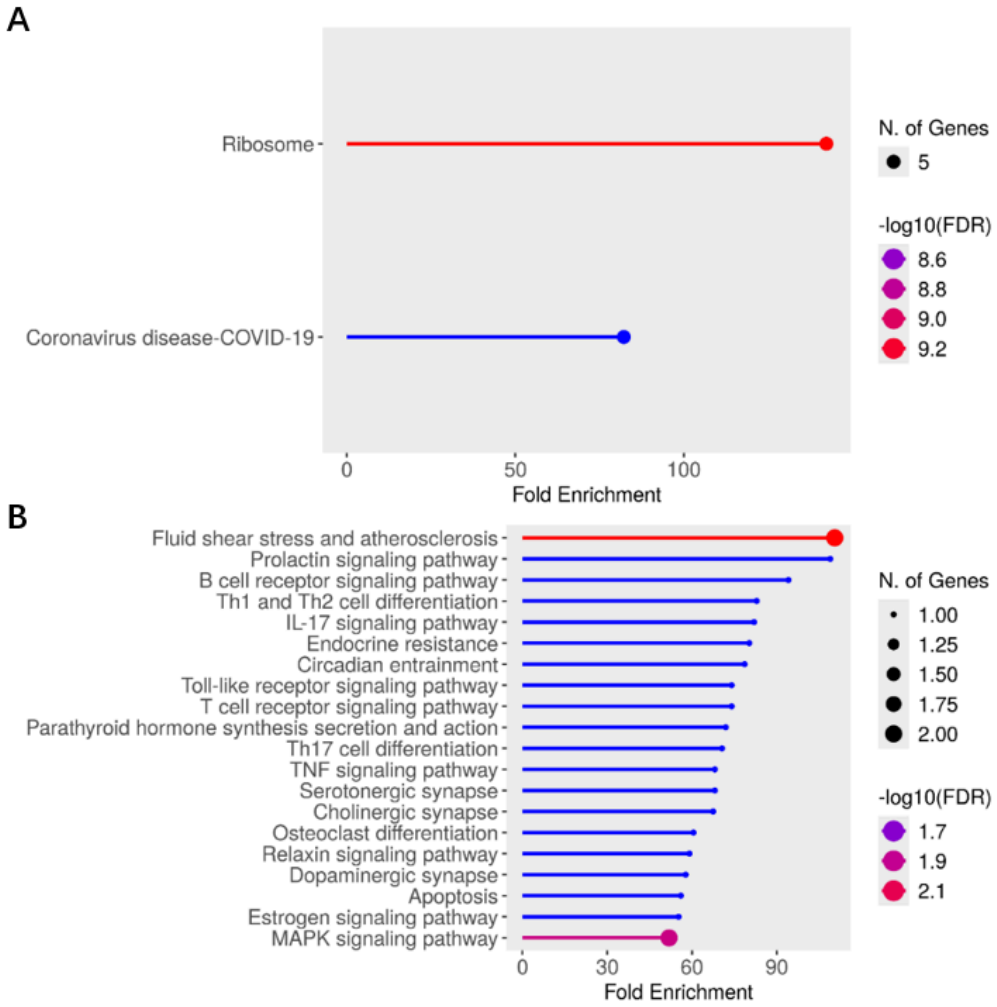

Figure 6. KEGG pathway enrichment analysis of Non-conventional T Cell subsets. (A) KEGG pathway enrichment of significantly altered genes (both upregulated and downregulated) in DNT cells. (B) KEGG pathway enrichment of significantly altered genes (both upregulated and downregulated) in MAIT cells

DNT cells showed enrichment in the ribosome pathway, indicating increased translational activity that may reflect a primed or activated state in the PDAC environment. Although the enrichment of the Coronavirus disease–COVID-19 pathway is not directly related to PDAC, it shares components with interferon and inflammatory signaling, suggesting that DNT cells may be engaged in nonspecific inflammatory responses. Overall, DNT cells may be transcriptionally active in PDAC, but their precise functional contribution remains unclear due to limited pathway specificity (Fig 6-A).

MAIT cells enriched in diverse pathways, with the most significant enrichment pathway pointing toward inflammatory activation and immune modulation. Enriched pathways for MAIT cells included IL-17 signaling, Th1/Th2 and Th17 cell differentiation, T Cell Receptor (TCR) and Toll like receptor signaling, and TNF signaling (Fig 6-B). Pathways such as apoptosis and MAPK signaling were also enriched, which may

reflect immune stress responses or regulatory feedback in these cells [34]. These suggest that MAIT cells may contribute to shaping the inflammatory milieu and possibly promoting chronic immune activation.

Although several neuro–immune signaling pathways were enriched (e.g., dopaminergic, serotonergic, and circadian entrainment), these findings were not specific and may reflect more general transcriptional changes rather than functional specialization. In summary, MAIT cells may be transcriptionally active in PDAC and highly responsive, and may participate in both inflammation and immunosuppression on a systemic scale.

3.3.5. Functional remodeling of non-T cell immune subsets in PBMC

In addition to T cells, various non-T immune cell subsets in PBMCs also exhibited altered functional states in PDAC patients compared to healthy controls. These included B cells, Natural Killer (NK) cells, monocytes, and Dendritic Cells (DCs), among others.

In PDAC PBMCs, monocyte subsets ($CD14^+$ and $CD16^+$) showed enrichment of several immune-related signaling pathways that are closely associated with tumor progression and the inflammatory microenvironment. Notably, $CD14^+$ monocytes enriched in NF-κB signaling pathway, TNF signaling pathway, and cytokine–cytokine receptor interaction, which are well-known positive regulators of inflammatory response and frequently implicated in enrichment of PDAC progression and therapy resistance [22, 35]. Similarly, $CD16^+$ monocytes enriched in IL-17 signaling pathway and viral infection-related pathways (e.g., Influenza A, Epstein–Barr virus infection) further support that PDAC-associated monocytes may acquire a pro-inflammatory and immunomodulatory phenotype (Fig 7-A).

Natural Killer (NK) cells in PDAC patients exhibited significant enrichment of immune-related pathways, including the TNF signaling pathway, IL-17 signaling pathway, and NF-κB signaling pathway, all of which are strongly linked to tumor-associated immune regulation. The presence of chemical carcinogenesis and metabolic reprogramming pathways further suggest that NK cells may have functional adaptations in the tumor context, which may reflect both their anti-tumor phenotype and tumor-induced dysfunction (Fig 7-B) [36].

In contrast, memory B cells/plasmablasts and Dendritic Cells (DCs) did not display any PDAC-specific transcriptional alterations in this analysis. The lack of distinct enrichment pathways may reflect the limited remodeling of these subsets in PBMCs, or alternatively, may suggest that their functional changes occur primarily within the tumor tissue microenvironment rather than in peripheral circulation. Further studies with higher sample depth or tissue-resident analyses will be required to clarify the role of these subsets in PDAC.

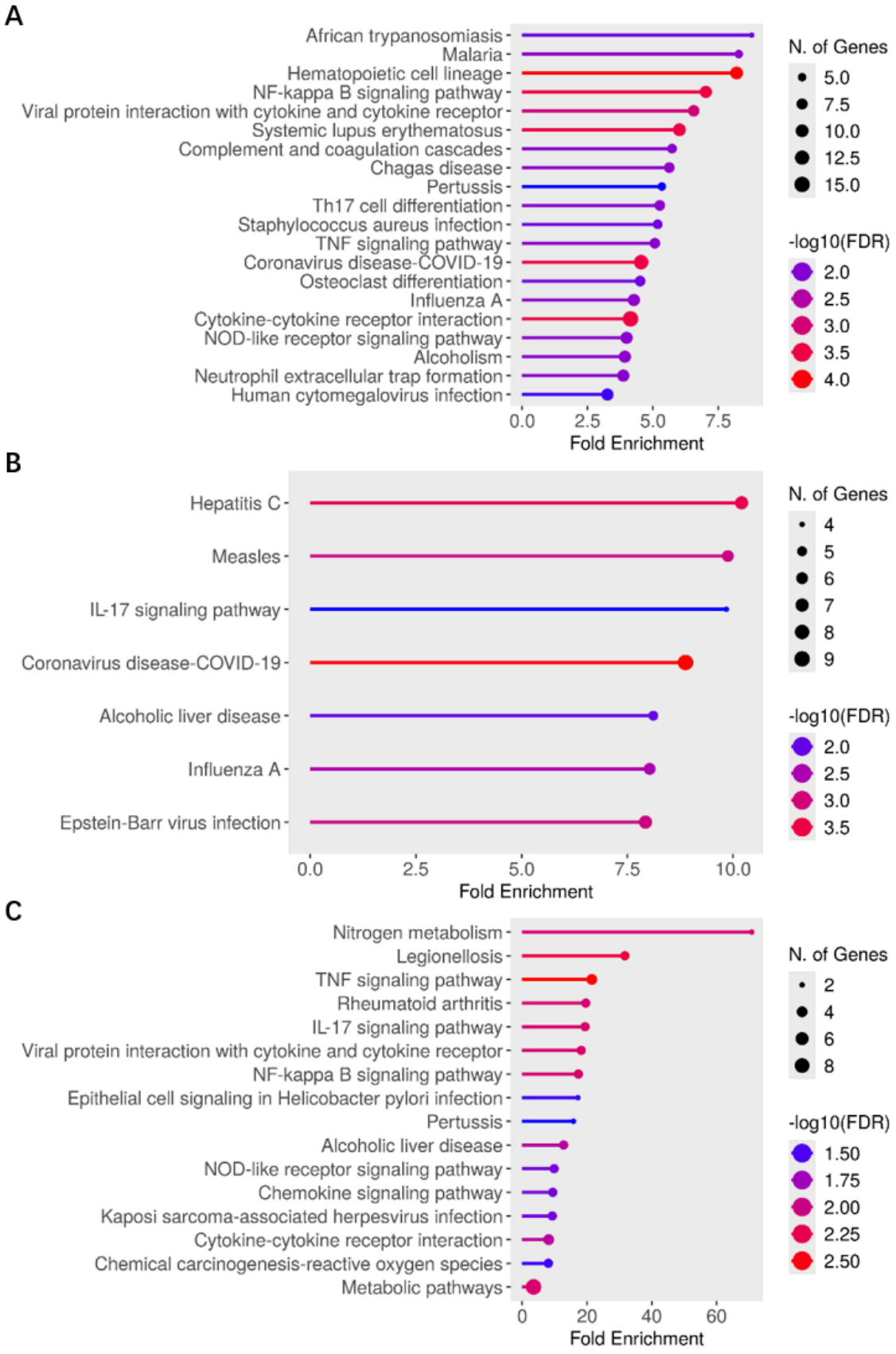
A
African trypanosomiasis
Malaria
Hematopoietic cell lineage
NF-kappa B signaling pathway
Viral protein interaction with cytokine and cytokine receptor
Systemic lupus erythematosus
Complement and coagulation cascades
Chagas disease
Pertussis
Th17 cell differentiation
Staphylococcus aureus infection
TNF signaling pathway
Coronavirus disease-COVID-19
Osteoclast differentiation
Influenza A
Cytokine-cytokine receptor interaction
NOD-like receptor signaling pathway
Alcoholism
Neutrophil extracellular trap formation
Human cytomegalovirus infection
0.0
2.5
5.0
7.5
Fold Enrichment
N. of Genes
5.0
7.5
10.0
12.5
15.0
-log10(FDR)
2.0
2.5
3.0
3.5
4.0
B
Hepatitis C
Measles
IL-17 signaling pathway
Coronavirus disease-COVID-19
Alcoholic liver disease
Influenza A
Epstein-Barr virus infection
0.0
2.5
5.0
7.5
10.0
Fold Enrichment
N. of Genes
4
5
6
7
8
9
-log10(FDR)
2.0
2.5
3.0
3.5
C
Nitrogen metabolism
Legionellosis
TNF signaling pathway
Rheumatoid arthritis
IL-17 signaling pathway
Viral protein interaction with cytokine and cytokine receptor
NF-kappa B signaling pathway
Epithelial cell signaling in Helicobacter pylori infection
Pertussis
Alcoholic liver disease
NOD-like receptor signaling pathway
Chemokine signaling pathway
Kaposi sarcoma-associated herpesvirus infection
Cytokine-cytokine receptor interaction
Chemical carcinogenesis-reactive oxygen species
Metabolic pathways
0
20
40
60
Fold Enrichment
N. of Genes
2
4
6
8
-log10(FDR)
1.50
1.75
2.00
2.25
2.50

Figure 7. KEGG pathway enrichment analysis of non-T cell immune subsets. (A) KEGG pathway enrichment of significantly altered genes in $CD14^+$ cells (up). (B) KEGG pathway enrichment of significantly altered genes in $CD16^+$ cells (down). (C) KEGG pathway enrichment of significantly altered genes in NK-Proliferating cells

3.4. Immune cell landscape and functional state in PDAC tumor microenvironment

To better understand the immune landscape at the tumor site, we analyzed single-cell transcriptomic data from tumor tissues and adjacent normal tissues from PDAC patients and healthy controls. We first extracted immune cells based on canonical markers and performed clustering and annotation (Fig. 8 A-C).

Compared to PBMCs, the tumor-resident immune cell population exhibited distinct composition and functional states. Notably, $CD4^+$ cytotoxic T cells (CD4 CTL) and $CD4^+$ naïve T cells were relatively rare in the dataset, which may reflect either technical limitations of sampling or true biological depletion within the tumor microenvironment, as suggested by previous studies [37, 38].

In summary, the results show strong transcriptional perturbations across all immune populations, and tissue-resident immune cells display markedly lower transcriptional activity compared to PBMCs, likely reflecting a state of immune exhaustion that may contribute to PDAC-associated immune evasion (Fig. 9, 10).

Volcano plots of DEGs (PDAC vs. healthy) in four representative immune cell subsets from cell type classification scheme 1, highlighting both upregulated (red) and downregulated (blue) genes. The plots share identical analytical settings and visualization parameters, differing only in the analyzed cell type (A, B cell; B, $CD4^+$ T cell; C, $CD8^+$ T cell; D, natural killer cell).

Volcano plots for nine representative subsets from cell type classification scheme 2, illustrating diverse transcriptional responses across distinct immune populations. The plots share identical analytical procedures and visualization parameters, differing only in the analyzed cell type (A–G, Naive B cell, CD8+ Naive T cell, CD8+ Effector Memory T cell, CD16+ Monocyte, Erythroid cell, CD56-dim Natural Killer cell, Plasmablast).

3.4.1. Activation and differentiation changes in tissue of PDAC

KEGG pathway enrichment analysis of tissue-derived immune cells revealed the unexpected enrichment of pathways typically associated with pancreatic exocrine and digestive functions, including Pancreatic secretion, Protein digestion and absorption, and Fat digestion and absorption, across multiple immune cell subsets. This enrichment pattern was recurrently observed across multiple immune cell subsets and may reflect extensive crosstalk between the immune compartment and the tumor-associated pancreatic microenvironment, which may either represent transcriptional imprinting by the surrounding exocrine tissue or functional adaptation of the immune compartment to the metabolic and proteolytic environment of PDAC. Additionally, $CD8^+$ T cells and $CD4^+$ T cells significantly enriched pathways involved in differentiation of Th17, Th1, and Th2 cells [39]. These results suggest transcriptional plasticity of these two T cell subsets and potential lineage skewing due to the influence of the tumor microenvironment. Thus, PDAC appears to induce both microenvironment-driven transcriptional reprogramming and functional remodeling of the transcriptional program of immune cells in both the peripheral and tumor-resident compartments (Fig 11).

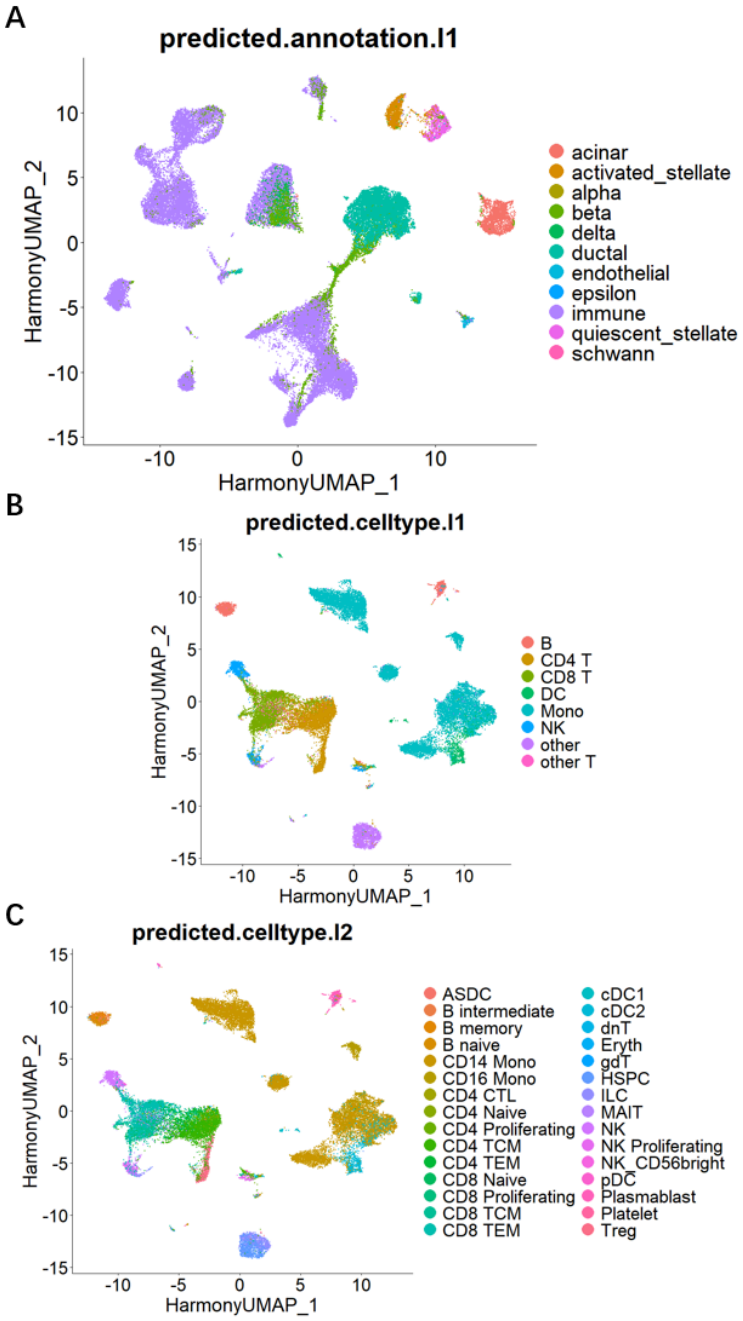

Figure 8. Cell clustering and annotation of tissue. (A) Annotation results of all tissue-derived cells. (B) Annotation of immune cell populations subsetted from tissue samples using PBMC reference (using cell type classification scheme 1). (C) Annotation of immune cell populations subsetted from tissue samples using PBMC reference (using cell type classification scheme 2)

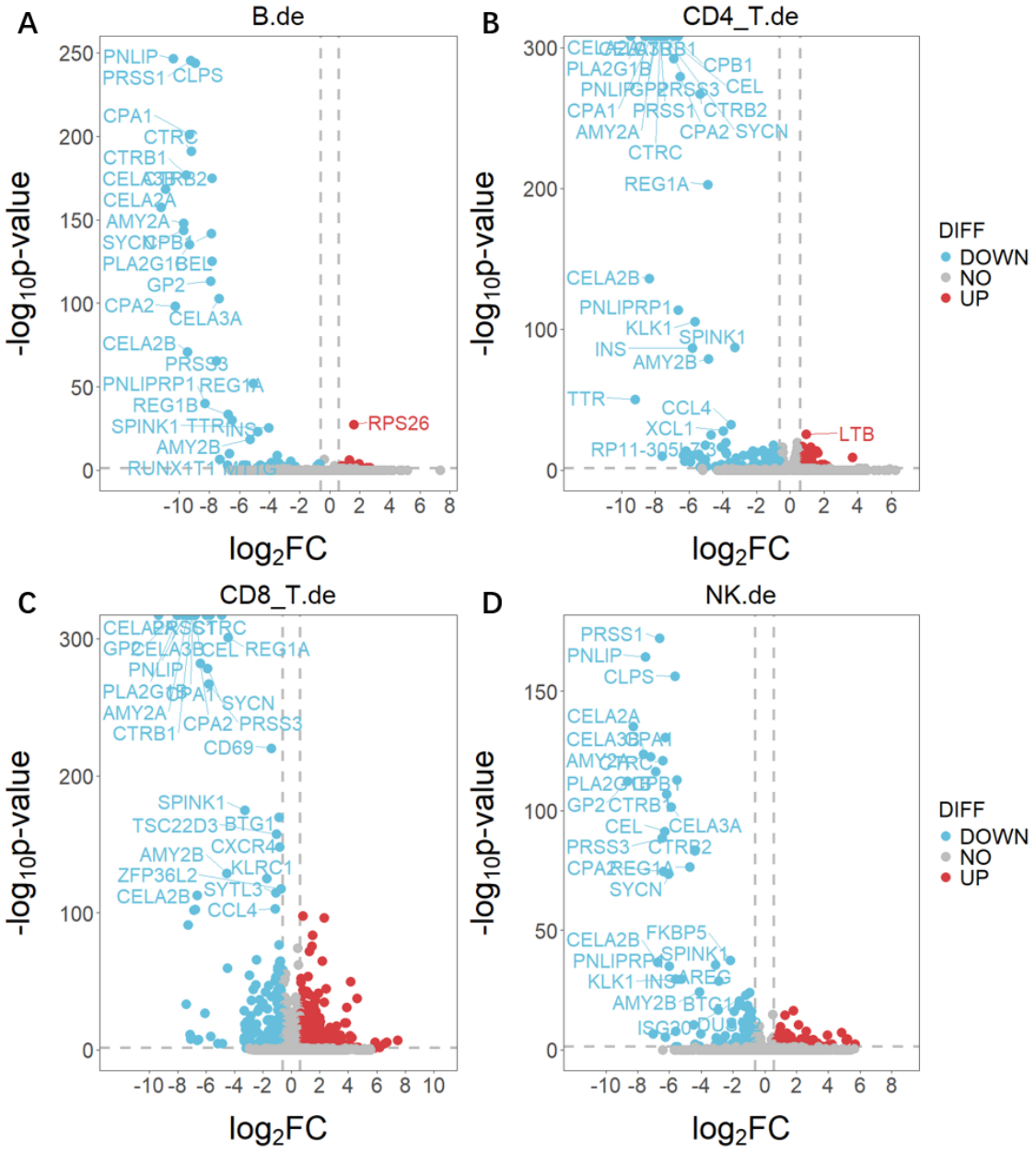

Figure 9. Differential gene expression analysis across representative immune cell types in tissue (classification scheme 1)

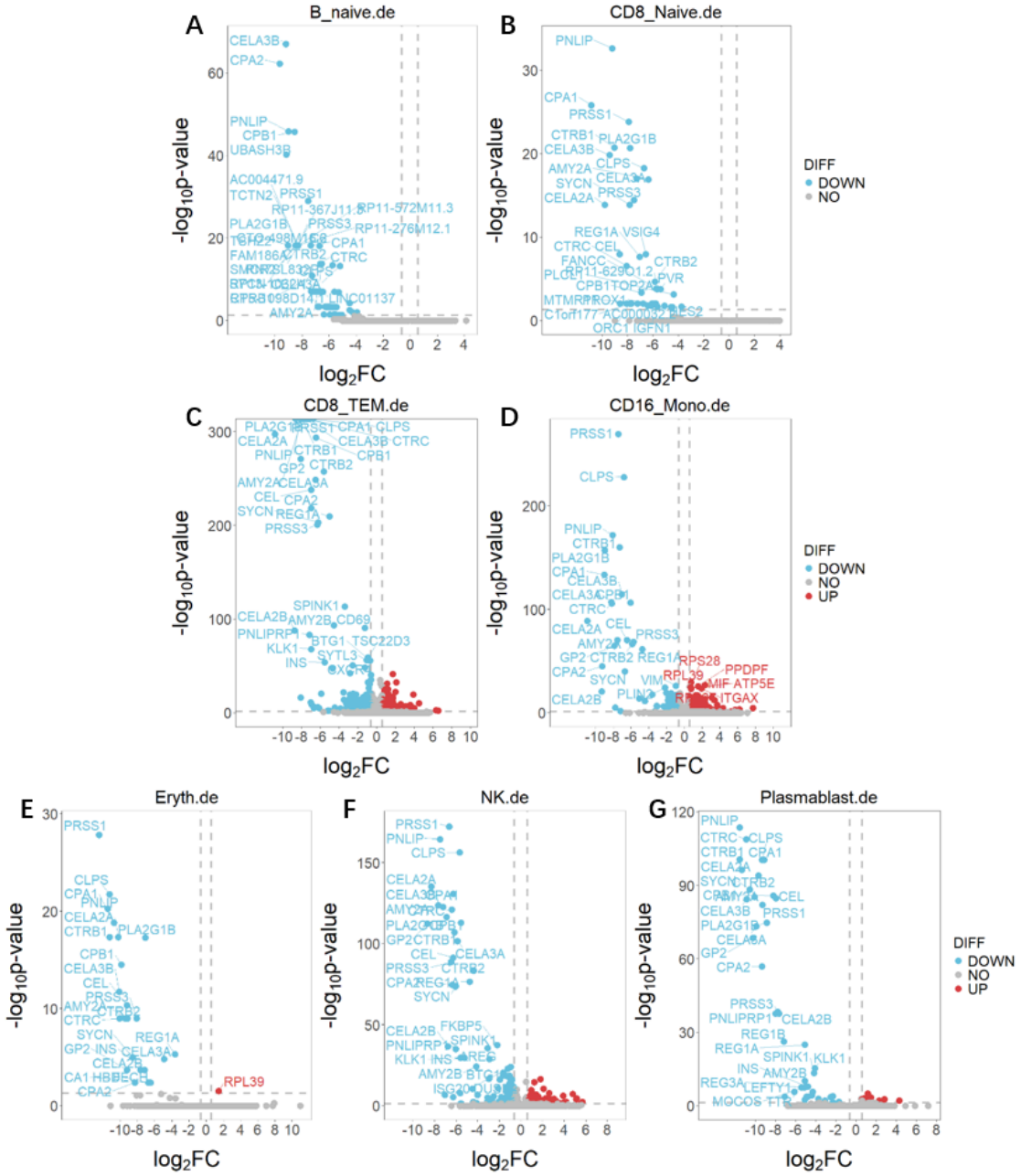

Figure 10. Differential gene expression analysis across representative immune cell types in tissue (classification scheme 2)

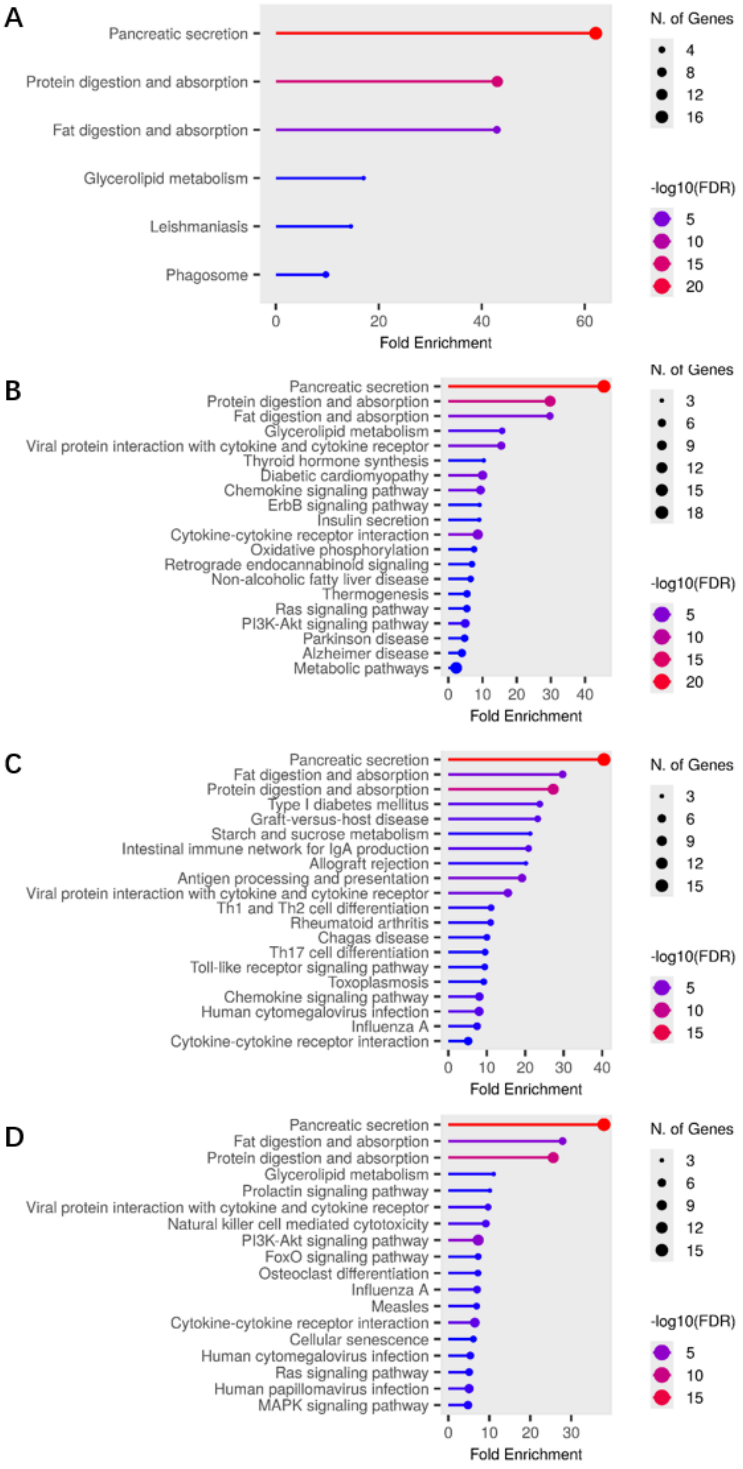

Figure 11. KEGG pathway enrichment analysis of differentially expressed genes across major immune subsets in PDAC tissue. (A) KEGG pathway enrichment of all significant DEGs in B cells. (B) Enrichment analysis of DEGs in $CD4^+$ T cells. (C) KEGG enrichment of DEGs in $CD8^+$ T cells. (D) Enrichment analysis of DEGs in NK cells

4. Conclusion

4.1. PDAC-associated immune alterations in the peripheral and tumor-resident compartments

Our analysis reveals that both the peripheral and tumor-resident compartments are profoundly remodeled in PDAC. All examined lineages exhibited changes, with the most prominent ones observed in T cells. Phenotypic changes included shifts from naïve to effector and memory states, upregulation of exhaustion-associated markers, and alterations in activity of different pathways. Among PBMCs, subsets including $CD8^+$ TEM, $CD4^+$ CTL, and MAIT cells exhibited transcriptional signatures associated with system-wide immune activation; however, this activation was insufficient to control tumor growth likely due to the accompanying functional dysregulation and restraint. In contrast, examined tumor-resident immune cell subsets displayed hallmarks of suppression, including enrichment of regulatory T cells and checkpoint-related pathways, suggesting establishment of a locally immunosuppressive TME. These findings support the notion that PDAC may employ a two-pronged immune remodeling program, including both system-wide activation and concurrent restraint.

4.2. Immune pathway rewiring and tumor immune evasion

At the level of pathways, consistent rewiring of signaling circuits was observed among all PDAC-associated immune subsets. Two central axes around IL-17 and TNF signaling, reflecting their well-characterized dual roles in promoting inflammation and enabling immunoregulation that favors tumor survival. TNF signaling can drive tumoricidal activity at high concentrations; however, in chronic settings TNF can promote tumor progression by inducing NF-κB/MAPK activation, PD-L1 upregulation, and recruitment of suppressive myeloid cells [22].

Activation of TCR signaling in PDAC immune subsets was induced by persistent antigen recognition likely reflecting tumor antigen recognition. However, chronic stimulation resulted in exhaustion signatures characterized by checkpoint upregulation and loss of effector function. Interestingly, consistent enrichment of PD-1/PD-L1 signaling was detected, supporting the notion that checkpoint pathway represents a major mechanism of immune escape in PDAC. In summary, our results suggest that PDAC maintains an immune landscape characterized by both activation and suppression, which might be adaptive for tumor elimination while promoting inflammation.

4.3. Implications for therapy

The altered immune pathways we identified in our study reveal potential therapeutic targets. The consistent enrichment of IL-17, TNF and checkpoint signaling were highly enriched and may represent rational targets for intervention. Chronic stimulation of exhausted T cells might be re-engaged by blocking the expression of checkpoint proteins, however, the efficiency of checkpoint blockade therapies in PDAC has been limited possibly due to the existence of additional immunosuppressive programs in PDAC. Thus, combination therapies targeting both inflammatory (IL-17/TNF) and checkpoint pathways may be more efficacious. Furthermore, subsets such as $CD8^+$ TEM and $CD4^+$ CTL exhibited exhaustion but retained partial effector programs. It has been proposed that therapeutic reactivation may rescue the effector program in select patients [40]. Our findings therefore support the exploration of multi-target immunotherapies that simultaneously relieve suppression and enhance effector activity.

4.4. Implications for noninvasive immune-based diagnosis in PDAC PBMC

Based on the reproducible immune alterations identified in PBMC, we propose that these peripheral immune perturbations may represent potential candidates for development of noninvasive immune-based diagnosis in PDAC. The circulating immune signature may be incorporated into liquid biopsy strategies to monitor disease progression and response to therapy without the need for invasive sampling. It should be noted that the peripheral tumor-induced immune program in PDAC patients likely reflects reprogramming of the system immune contexture and may therefore possess potential utility for early diagnosis in high-risk individuals. The existence of residual immune programs in the TME and primary tumors will require validation in larger, independent test sets to identify candidate biomarkers and to define robust immune-based panels [41, 42].

4.5. Residual immune programs and tumor microenvironmental adaptation

Although the overwhelming majority of immune signatures indicated exhaustion programs, we observed residual effector and memory programs in tumor-infiltrating T cells, especially $CD8^+$ TEM and $CD4^+$ CTL subsets. The coexistence of effector and inhibitory programs may reflect the failure of tumor microenvironment to completely extinguish cytotoxic function but instead imposes a restrained functional equilibrium [43, 44]. In addition, the enrichment of exocrine- and metabolism-related pathways in different immune subsets may represent transcriptional imprinting from the pancreatic microenvironment, further supporting the notion that tissue context exerts a major impact on immune states.

Together, our results support a model in which PDAC modulates both systemic and local immunity in an activating and suppressing program, respectively, to facilitate immune evasion. By untangling these two programs, our work facilitates potential therapeutic strategies and suggests that residual immune relevance in the TME may be exploited for more effective treatment.

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