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SUPPLEMENTAL DATA

RESEARCH ARTICLE

Liu et al: WFDC3 as biomarker in pancreatic cancer

WFDC3 identified as a prognostic and immune biomarker in pancreatic cancer

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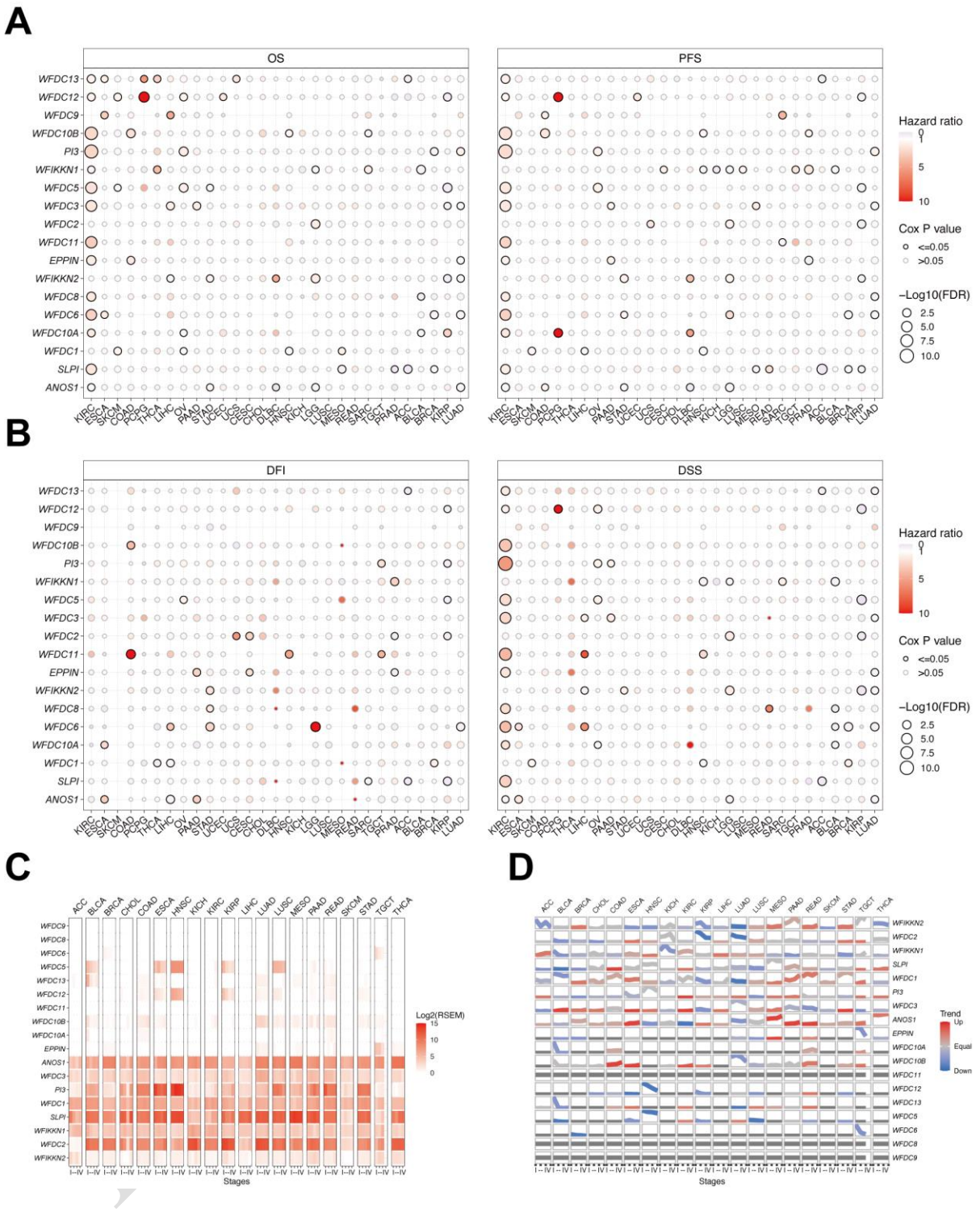


Figure S1. Survival analysis of WFDC family genes in pan-cancer based on GSCA database. (A-B) Bubble plots illustrating the effects of WFDC family genes expression on the prognosis of patients in pan-cancer. (C) Heatmap presenting the WFDC family genes expression profile among stages in multiple cancers. (D) Trend plot summarizing the trend of genes expression from early stage to late stage. FDR, false discovery rate; OS, overall survival;

PFS, progression free survival; DFI, disease free interval; DSS, disease specific survival; GSCA, Gene Set Cancer Analysis.

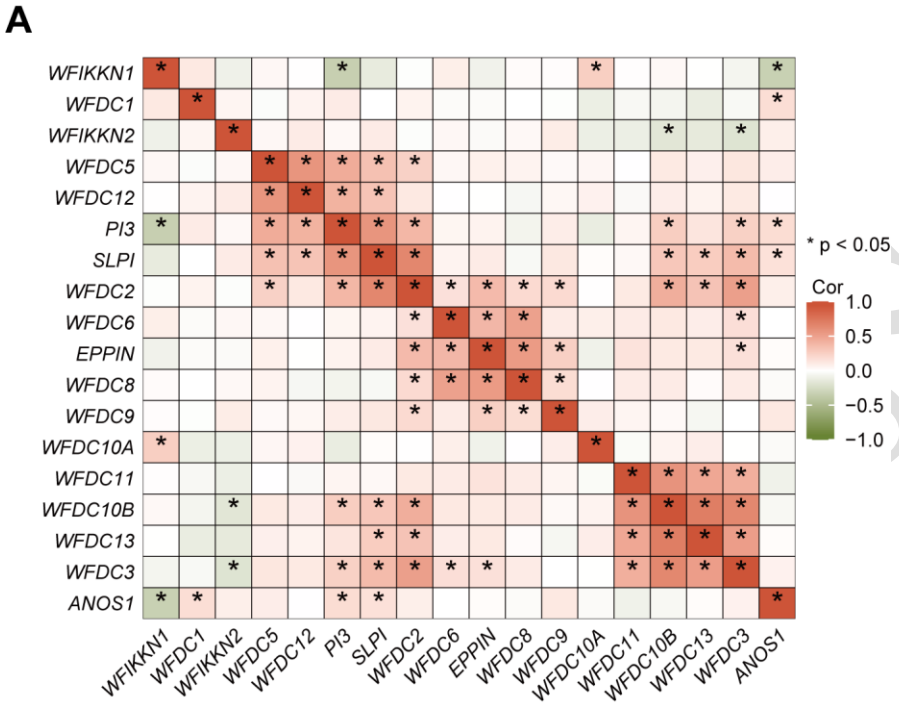


Figure S2. Heat map of Pearson correlation values among WFDC family genes. Significant correlations ($p < 0.05$) marked with an asterisk.

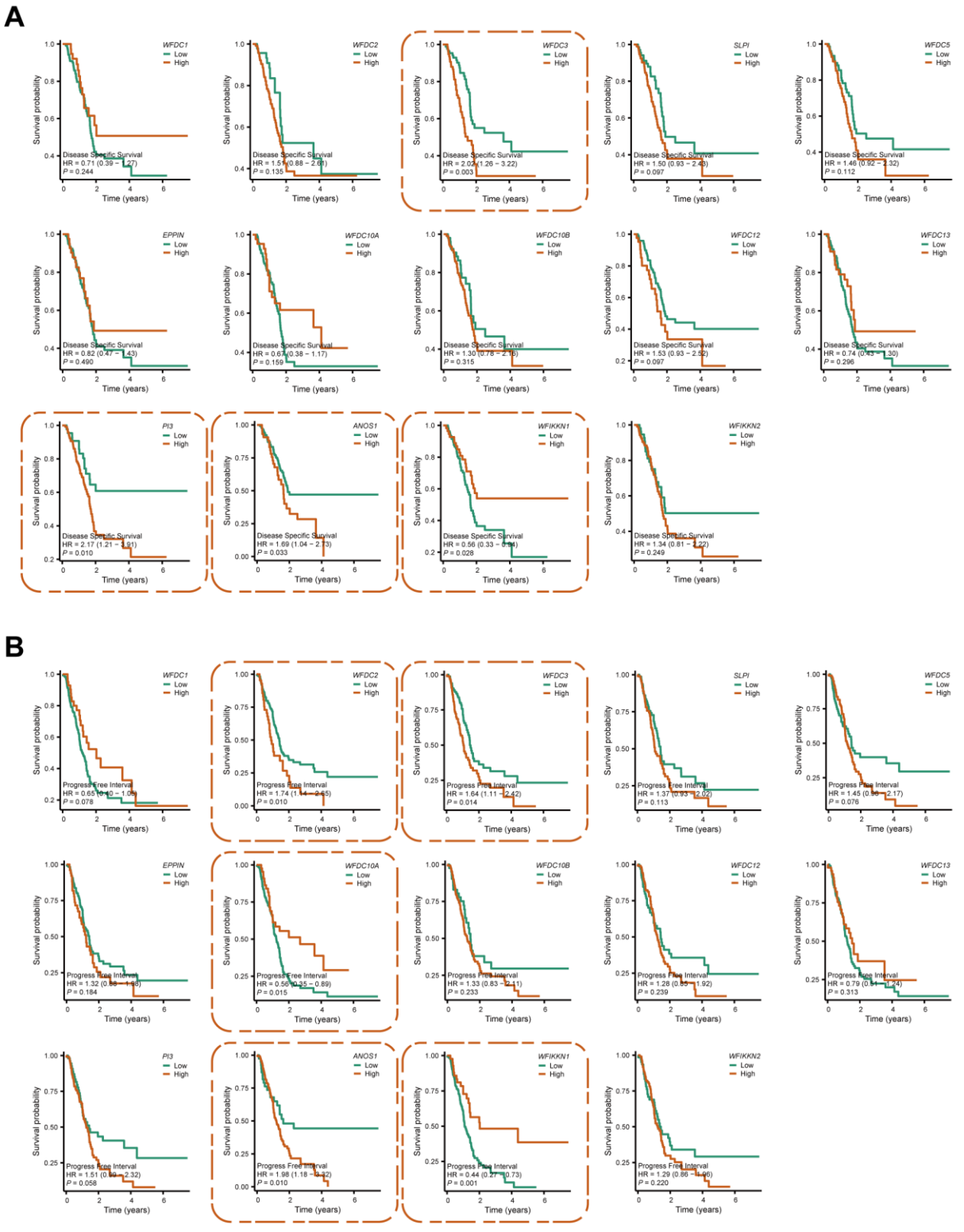


Figure S3. The prognostic value of WFDC family genes in pancreatic cancer. (A) KM curves of DSS between patients with high and low WFDC family genes expression level. (B) KM curves of PFI between patients with high and low WFDC family genes expression level. KM, Kaplan-Meier; DSS, disease specific survival; PFI, progression free interval; HR, hazard ratio; Inside the dashed box are curves with significant *p*-values (<0.05).

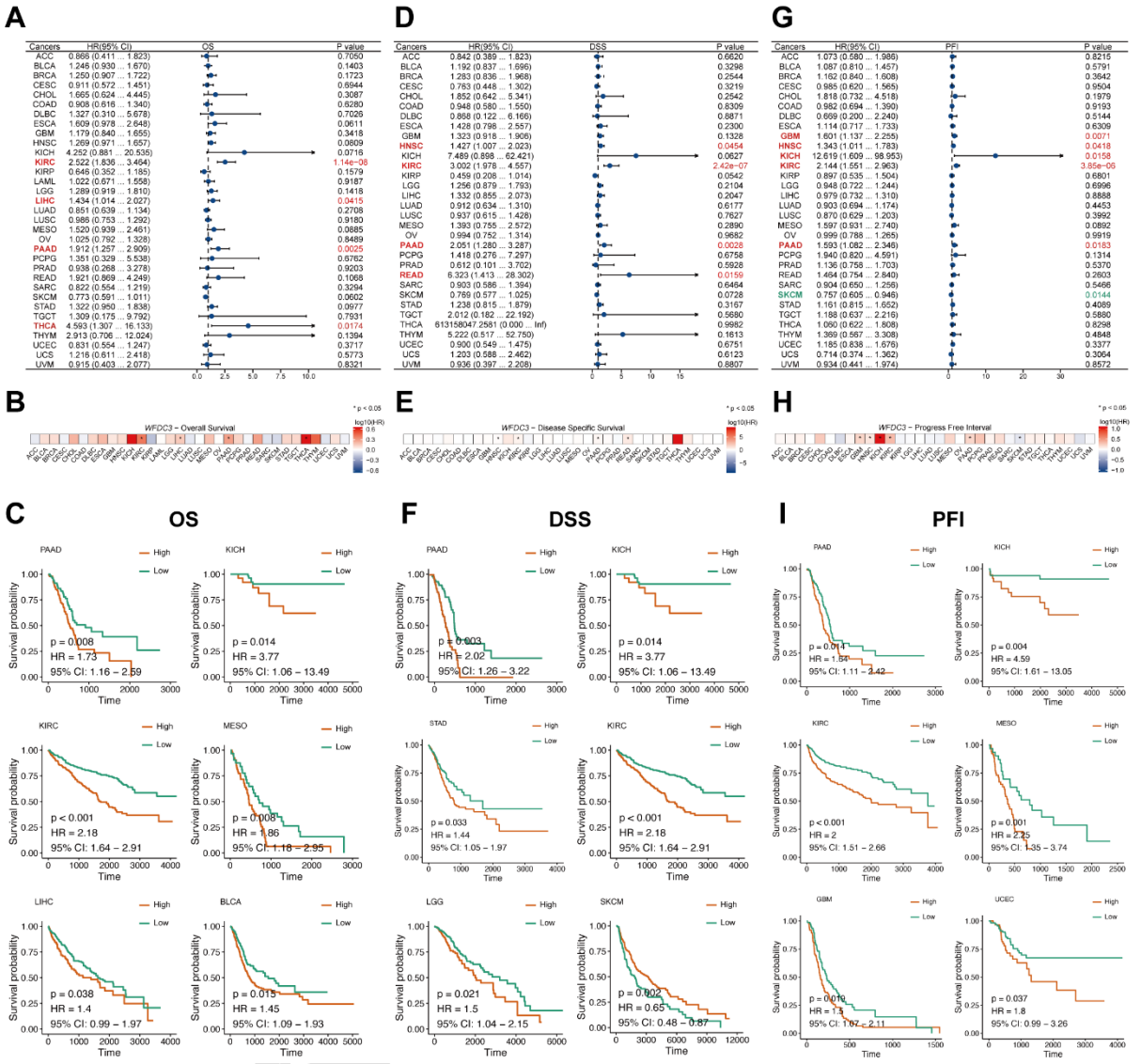


Figure S4. The prognostic value of *WFDC3* in pan-cancer based on TCGA database. (A) Forest map and (B) Survival map of univariate Cox regression analysis of *WFDC3* for OS across cancer types. (C) KM curves of OS between patients with high and low *WFDC3* expression in different cancer types. (D) Forest map and (E) Survival map of univariate Cox regression analysis of *WFDC3* for DSS across cancer types. (F) KM curves of DSS between patients with high and low *WFDC3* expression in different cancer types. (G) Forest map and (H) Survival map of univariate Cox regression analysis of *WFDC3* for PFI. (I) KM curves of the PFI between patients with high and low *WFDC3* expression in different cancer types. HR, hazard ratio; OS, overall survival; DSS, disease specific survival; PFI, progress free interval;

KM, Kaplan-Meier. Significant HR ($p < 0.05$) marked with an asterisk and labeled red (risk factors)/green (protective factors).

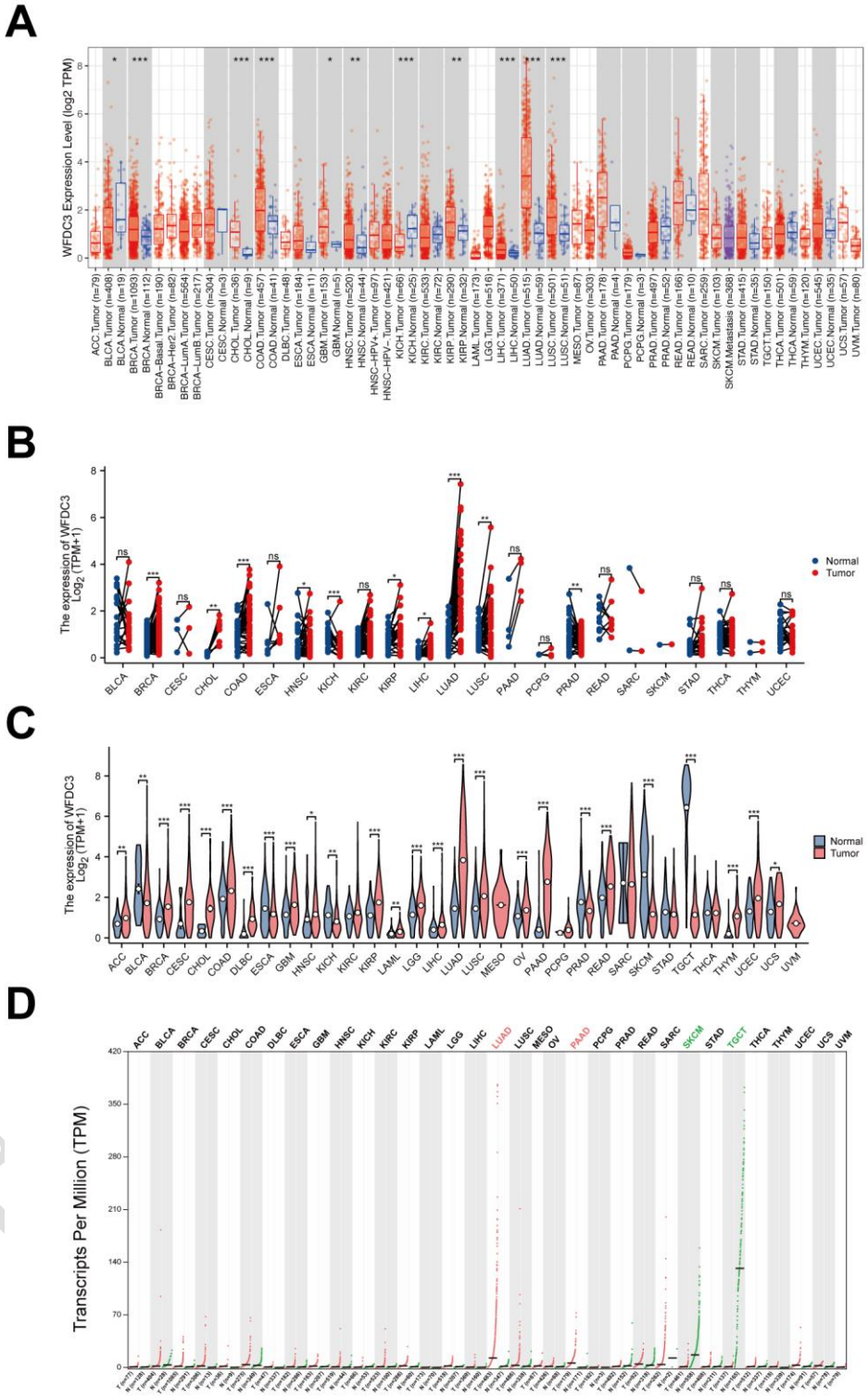


Figure S5. *WFDC3* expression levels in pan-cancer. (A) Expression levels of *WFDC3* in different tumor tissues compared to normal tissues (TIMER). (B) *WFDC3* expression levels in paired adjacent noncancerous tissues and tumor tissues (TCGA). (C) Expression levels of

WFDC3 in different tumor tissues compared to normal tissues (TCGA+GTEx). (D)Expression levels of *WFDC3* in different tumor tissues compared to normal tissues (GEPIA). *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

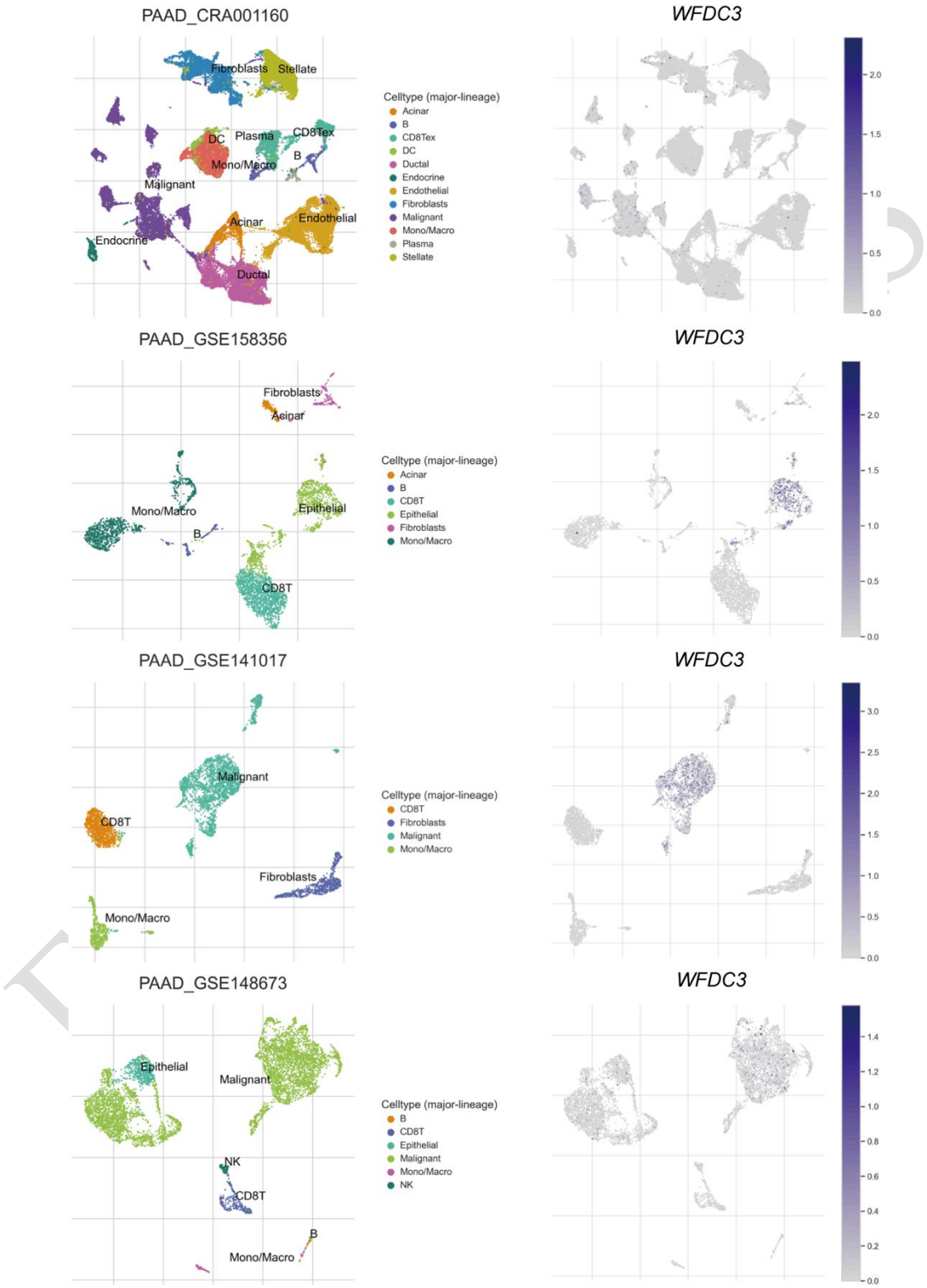


Figure S6. UMAP plots showing the unsupervised clustering of tumor and immune cells from several PAAD patient cohorts. The right panels showing the expression of *WFDC3* across single cells. Single-cell RNA-seq (scRNA-seq) data were downloaded from the TISCH database (Tumor Immune Single-cell Hub; http://tisch.comp-genomics.org/home/).

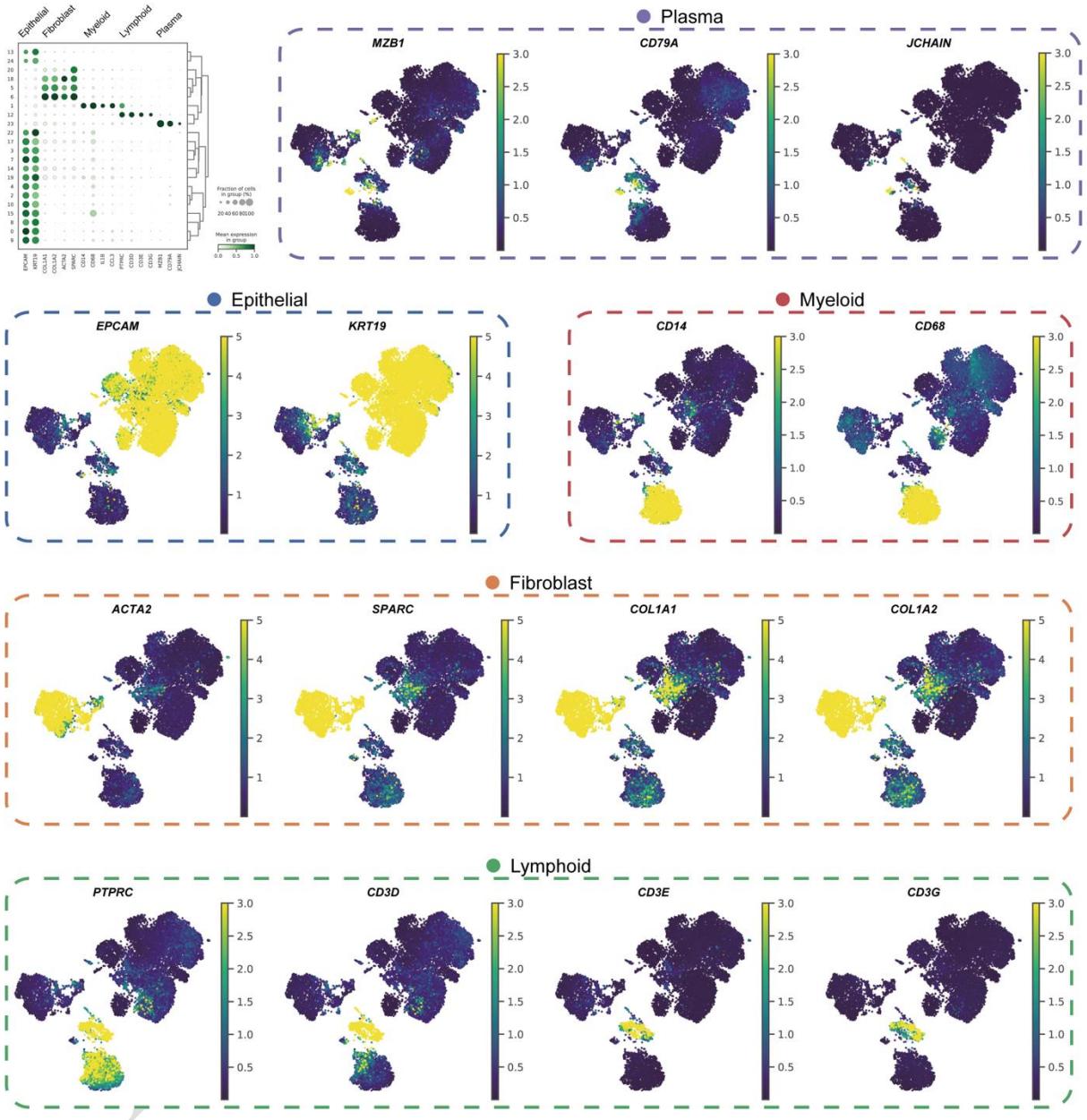


Figure S7. The expression level of markers of cell type annotations across clusters. Bubble plots and UMAP plots illustrated the expression levels of marker genes for the five identified cell types. Inside the purple dashed box was the distribution of plasma cell markers (*MZB1*, *CD79A* and *JCHAIN*). Similarly, the following colored dashed box were used to represent different cell types: blue indicates epithelial cells (*EPCAM* and *KRT19*); red represents myeloid

cells (*CD14* and *CD68*); orange denotes fibroblasts (*ACTA2*, *SPARC*, *COL1A1* and *COL1A2*); and green corresponds to lymphoid cells (*PTPRC*, *CD3D*, *CD3E* and *CD3G*).

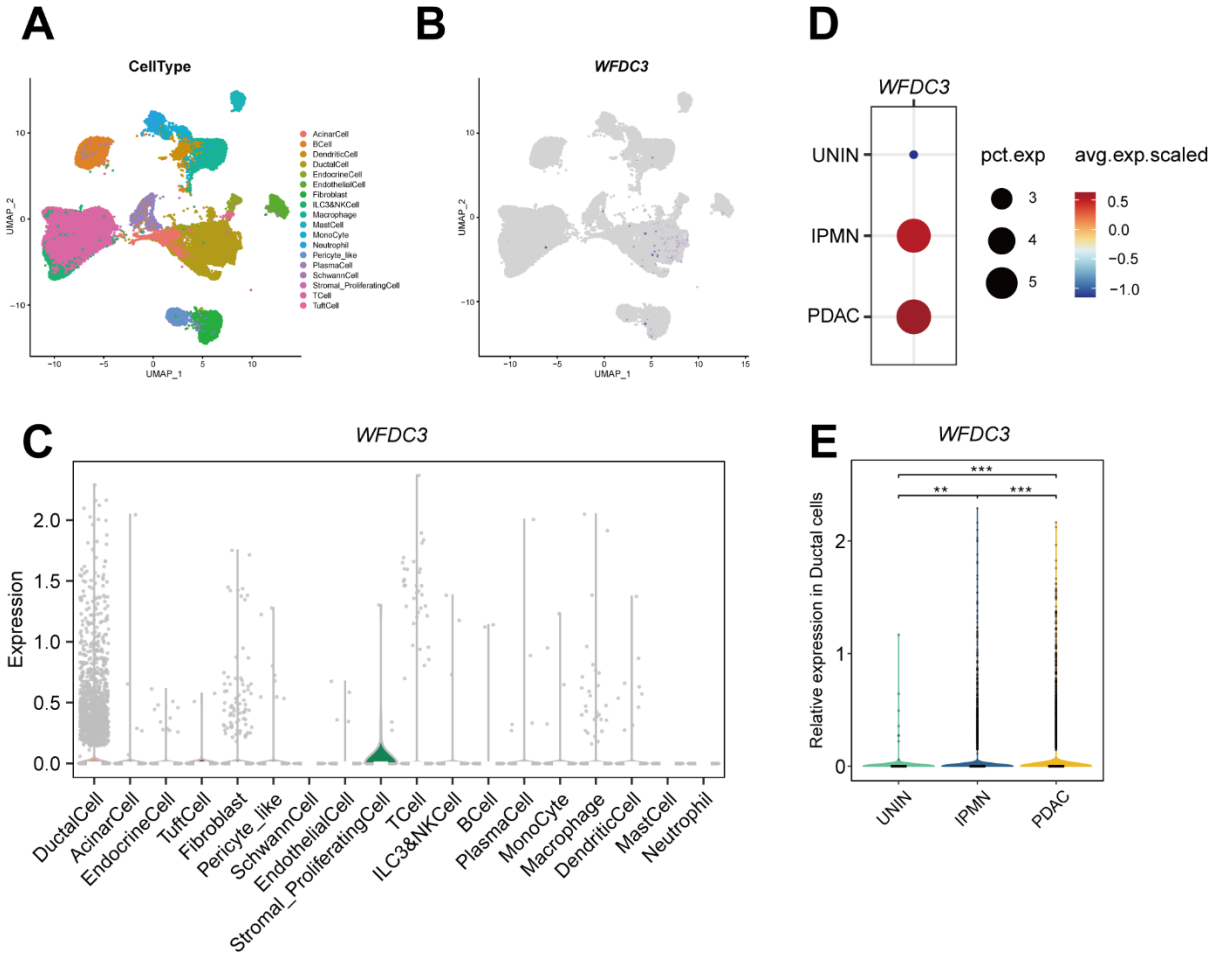


Figure S8. *WFDC3* correlates with malignant evolution of ductal cells in PAAD. (A) UMAP dimensionality reduction was used to show the distribution and dissimilarity of the major cell types. (B) *WFDC3* expression level across all populations. (C) Violin plots visualizing the normalized expression levels of *WFDC3* in the major cell types. (D) Bubble plots and (E) Violin plots of the average expression levels of *WFDC3* in ductal cells from different tissue sources. UNIN, uninvolved pancreatic tissue; IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

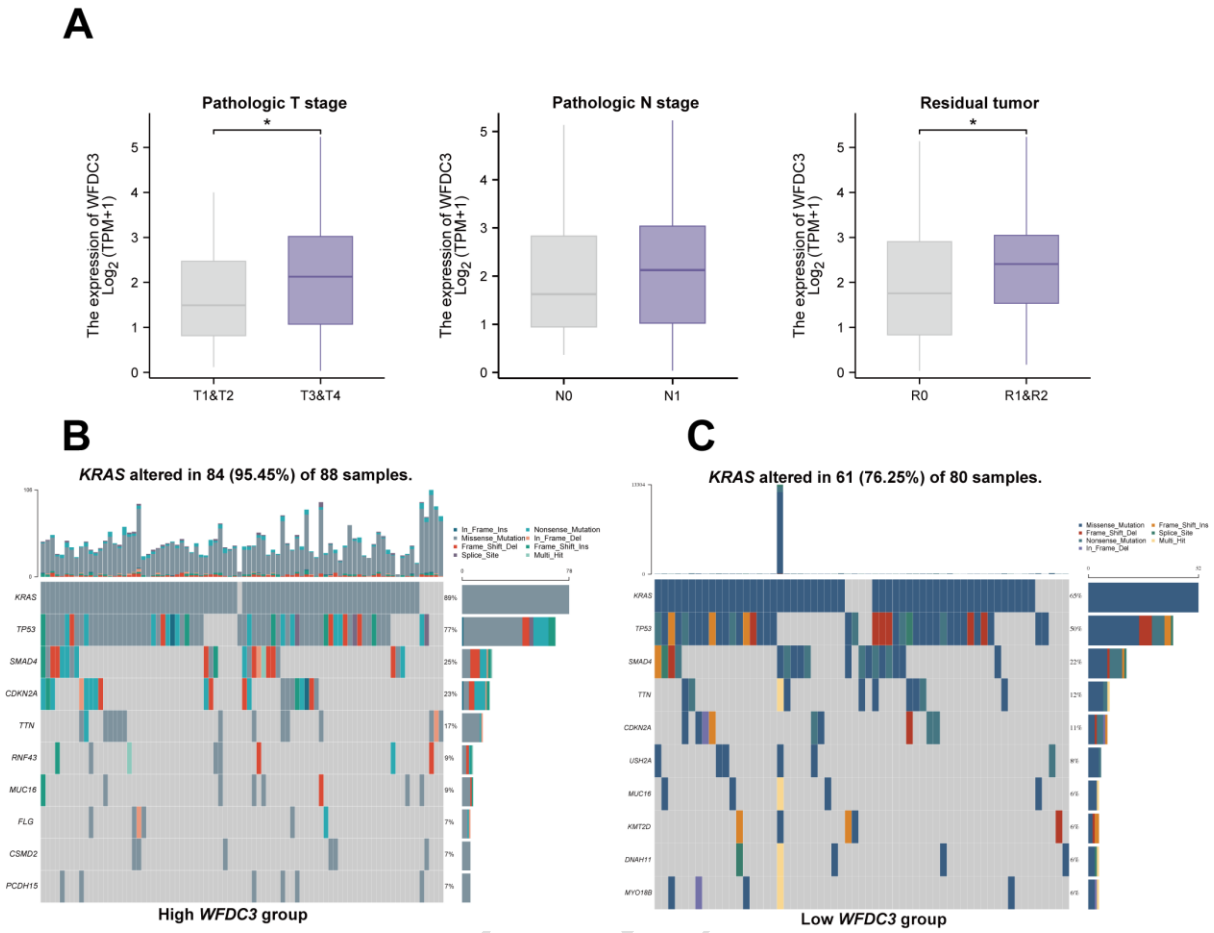


Figure S9. Correlation between *WFDC3* expression and clinical characteristics in PAAD and comprehensive analysis of genomic mutation. (A)Correlation between *WFDC3* and certain clinical characteristics including T stage, N stage and residue tumor. Mutational landscape of (B) the high *WFDC3* expression and (C) the low *WFDC3* expression patients. * $p < 0.05$.

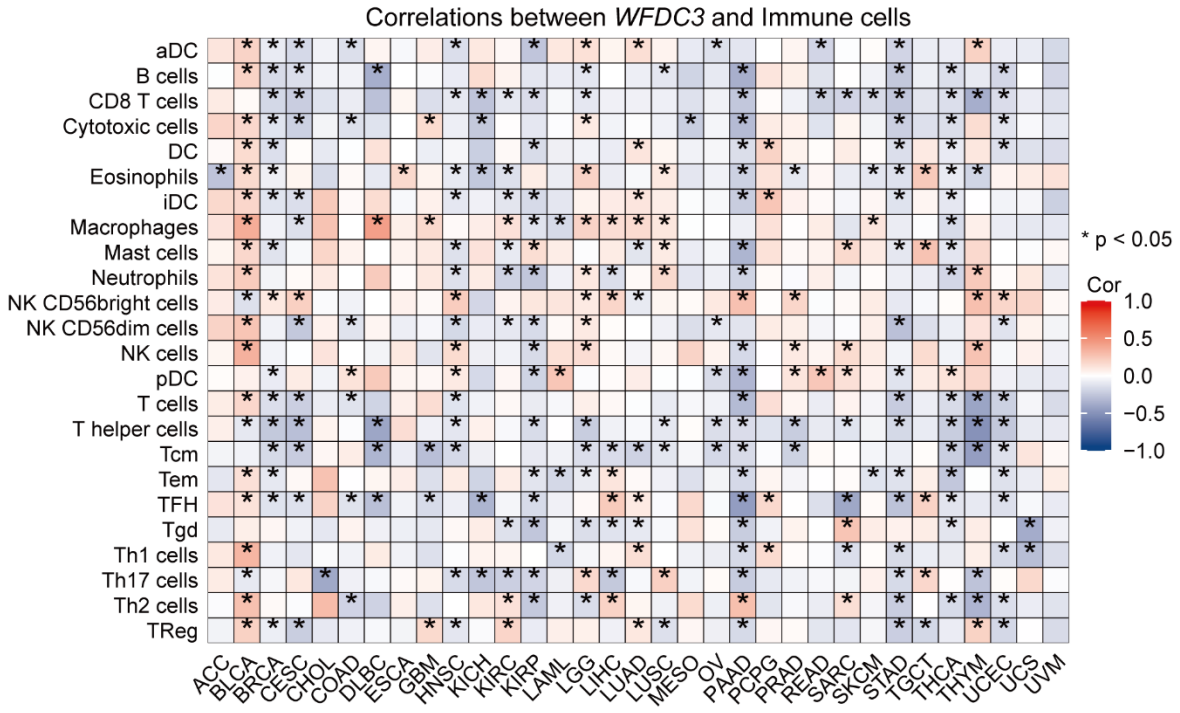


Figure S10. Correlation between *WFDC3* expression and the relative abundances of 24 immune cell types in pan-cancer. * $p < 0.05$.

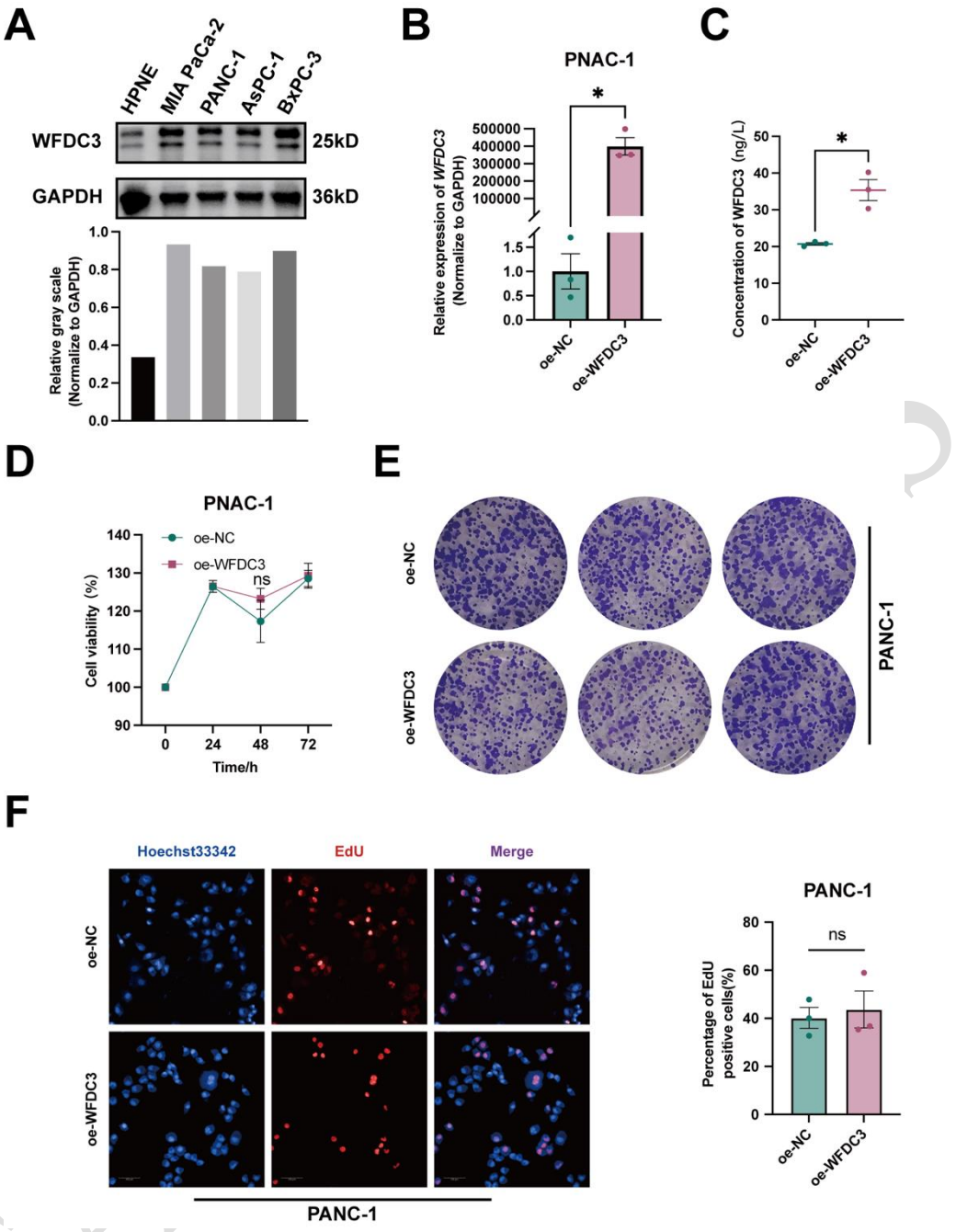


Figure S11. Expression level and proliferative function of WFDC3 in pancreatic cell lines. (A) Western blot and gray values of WFDC3 protein expression in normal human pancreatic cell line (HPNE) and pancreatic cancer cell lines (MIA PaCa-2, PANC-1, AsPC-1 and BxPC-3). (B) The mRNA expression level of *WFDC3* was evaluated using qRT-PCR in PANC-1 transfected with *WFDC3* overexpression plasmids. (C) The concentration of secreted WFDC3 in the supernatant of WFDC3-overexpressing PANC-1 cells was measured via ELISA. (D) CCK-8 assays and (E) colony formation assays were conducted to explore the proliferative function of WFDC3-overexpressing PANC-1 cells. (F) Representative image and

quantification of the EdU proliferation assays evaluating the proliferation of WFDC3-overexpressing cell, scale bar: 100µm. * $p < 0.05$.

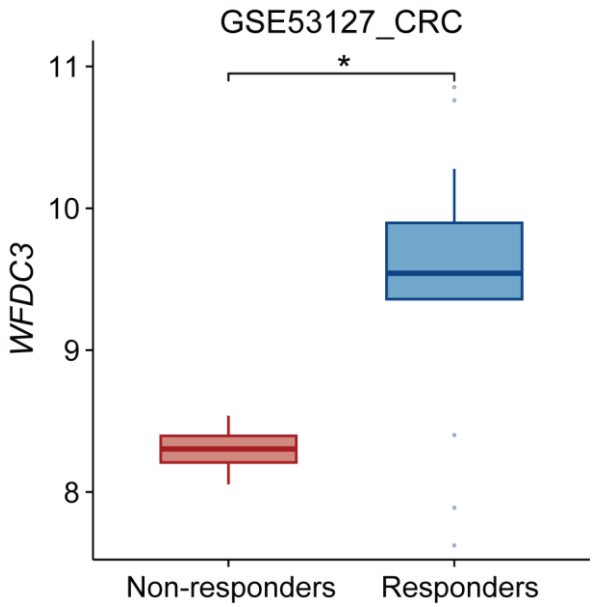
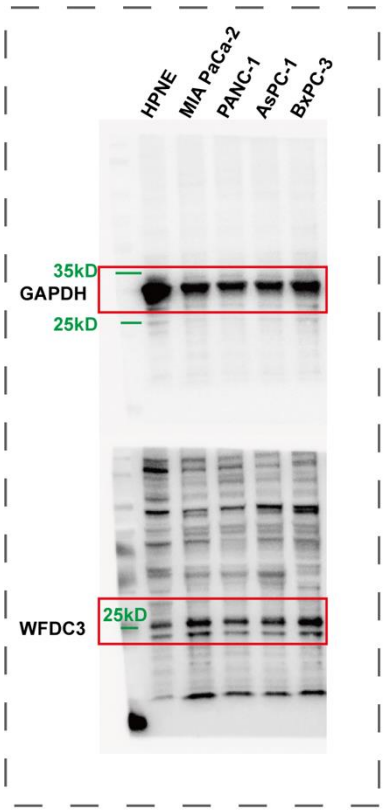
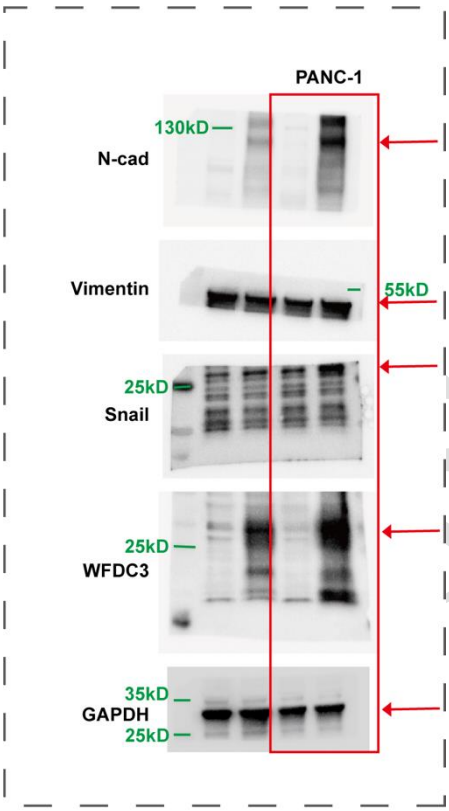


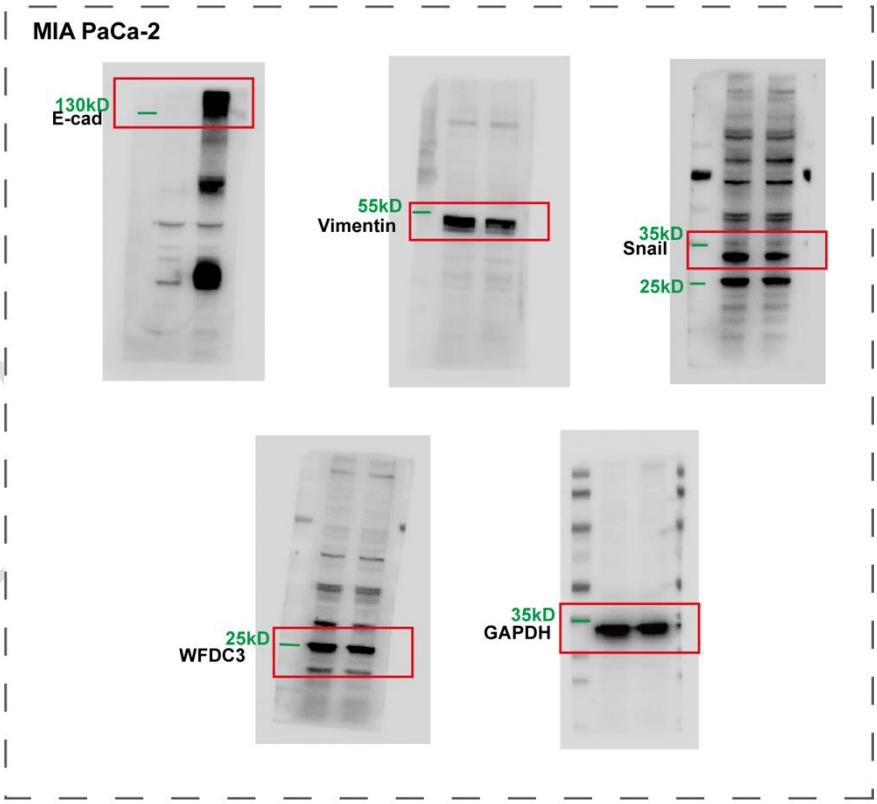
Figure S12. *WFDC3* expression levels in responders vs non-responders in the GSE53127 immunotherapy-treated colorectal cancer cohort. Statistical significance was assessed using a Wilcoxon rank-sum test. * $p < 0.05$.



Original to Fig.S11 (A)



Original to Fig.7 (C)



Original to Fig.7 (D)

Original uncropped Western blot images corresponding to Fig. S11(A), Fig. 7(C), and Fig. 7(D).

Upper left panel (original to Fig. S11A): Raw Western blot bands of WFDC3 and GAPDH across 4 pancreatic cancer cell lines (MIA PaCa-2, PANC-1, AsPC-1, BxPC-3) and normal pancreatic ductal epithelial cells (HPNE).

Upper right panel (original to Fig. 7C): Protein expression of N-cadherin, Vimentin, Snail, WFDC3, and GAPDH in WFDC3-overexpressing PANC-1 cells.

Lower panel (original to Fig. 7D): Expression of E-cadherin, Vimentin, Snail, WFDC3, and GAPDH in WFDC3-knockdown MIA PaCa-2 cells.

GAPDH serves as a loading control. Red boxes indicate the bands shown in the main figures. Green labels indicate protein ladders for reference.