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SUPPLEMENTAL DATA

WFDC3 identified as a prognostic and immune biomarker in pancreatic cancer

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Table S1. Relevant primer sequences for qRT-PCR

Gene	Primer sequences	
WFDC3	Forward	5′- TCGGATCTGCCGAGACATTCCT -3′
	Reverse	5′- CTACACAGCTCTTGTTGCAGCC -3′
GAPDH	Forward	5′- GTCTCCTCTGACTTCAACAGCG -3′
	Reverse	5′- ACCACCCTGTTGCTGTAGCCAA -3′
Perforin	Forward	5′- GGGATTCCAGAGCCCAAGTG -3′
	Reverse	5′- CAGCAGCAGGAGAAGGATGC -3′
Granzyme B	Forward	5′- TGGGGGACCCAGAGATTAAAA -3′
	Reverse	5′- TTTCGTCCATAGGAGACAATGC -3′
Granulysin	Forward	5′- CAGGCTCCCTGCCCATAAAA -3′
	Reverse	5′- CTCAAGGCCTGGGTTGCC -3′
IFNG	Forward	5′- GGGTTCTCTTGGCTGTTACTG -3′
	Reverse	5′- TTTCTGTCACTCTCCTCTTTCC -3′
IL-2	Forward	5′- AGAACTCAAACCTCTGGAGGAAG -3′
	Reverse	5′- GCTGTCTCATCAGCATATTCACAC -3′

Table S2. Antibodies for Western blot and IF

Antibody	Manufacture	Item number	Species

WFDC3	immunoway, USA	Cat#YN7483	Rabbit
ZO-1	Cell Signaling Technology, USA	Cat#13663S	Rabbit
N-Cadherin	Cell Signaling Technology, USA	Cat#14215S	Mouse
E-Cadherin	Cell Signaling Technology, USA	Cat#14472S	Mouse
Vimentin	Cell Signaling Technology, USA	Cat#5741S	Rabbit
Snail	Cell Signaling Technology, USA	Cat#3879S	Rabbit
GAPDH	Cell Signaling Technology, USA	Cat#2118S	Rabbit
goat anti-mouse IgG	ZSGB-BIO, China	Cat#ZB-2305	Goat
goat anti-rabbit IgG	ZSGB-BIO, China	Cat#ZB-2301	Goat
FITC-goat anti-mouse IgG	ZSGB-BIO, China	Cat#ZF-0312	Goat
TRITC-goat anti-rabbit IgG	ZSGB-BIO, China	Cat#ZF-0316	Goat

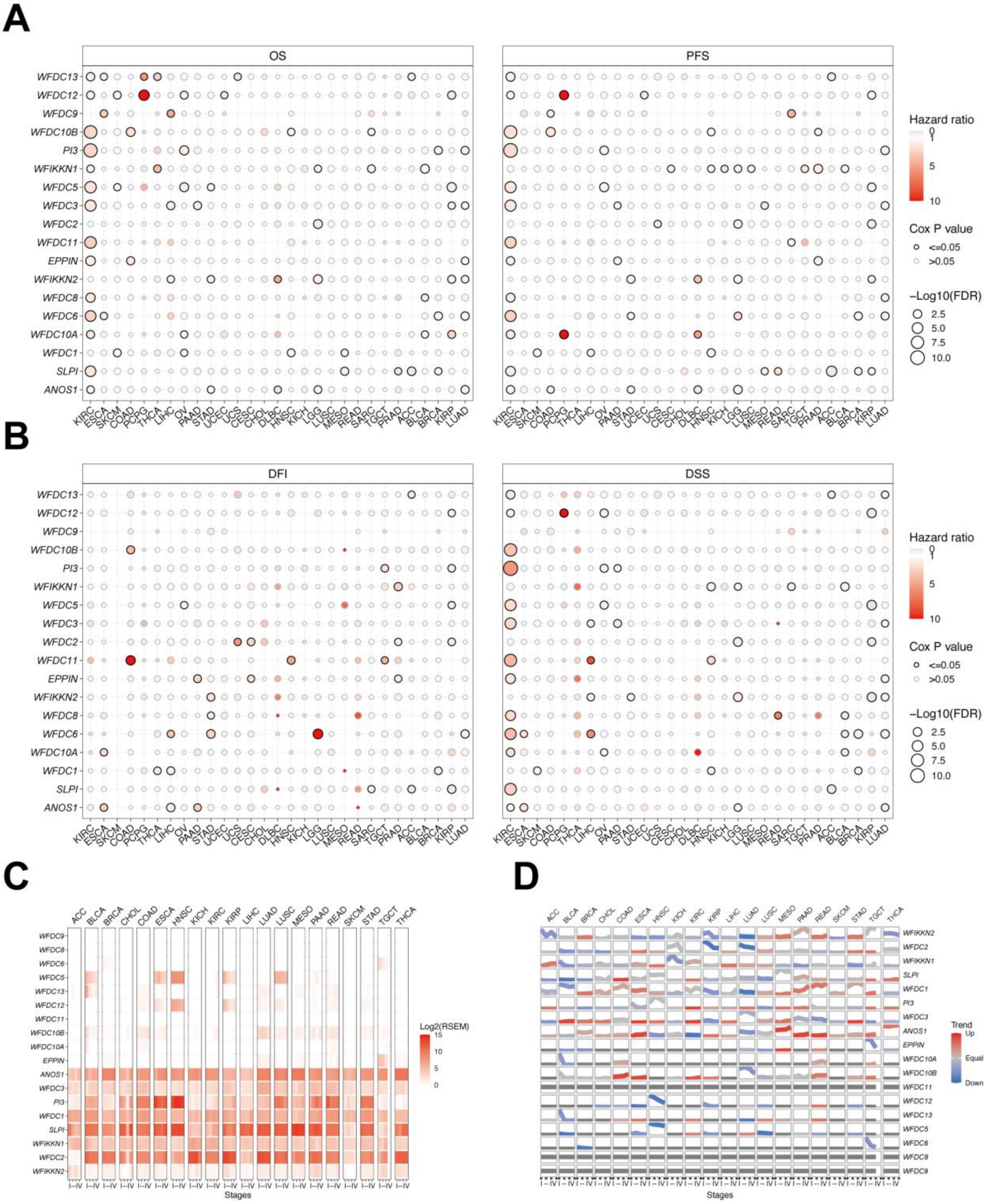

Figure S1. Survival analysis of WFDC family genes in pan-cancer based on GSCA database. (A-B) Bubble plots illustrating the effects of WFDC family genes expression on the prognosis of patients in pan-cancer. (C) Heatmap presenting the WFDC family genes expression profile among stages in multiple cancers. (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.

A

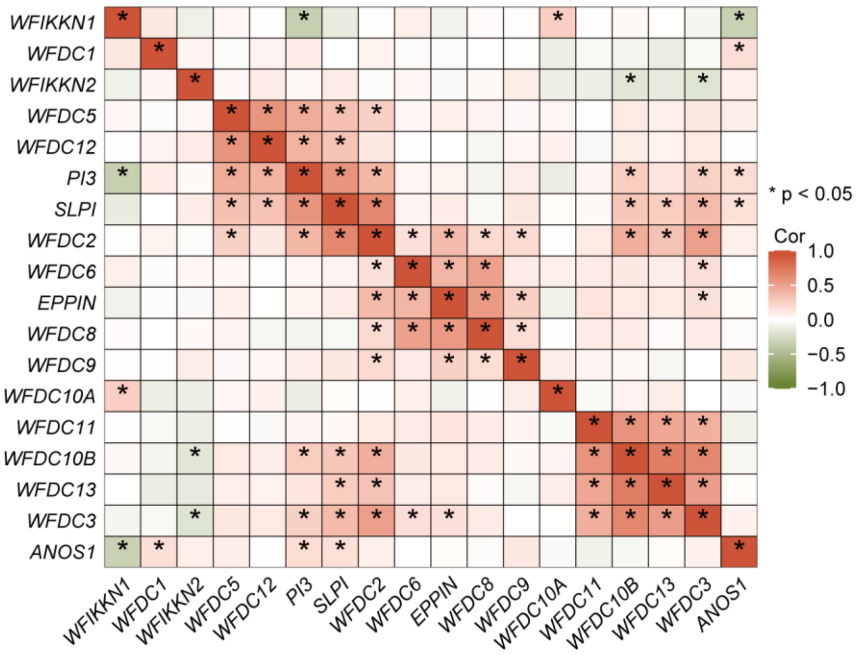

Figure S2. Heat map of Pearson correlation values among WFDC family genes. Significant correlations ($p < 0.05$) marked with an asterisk.

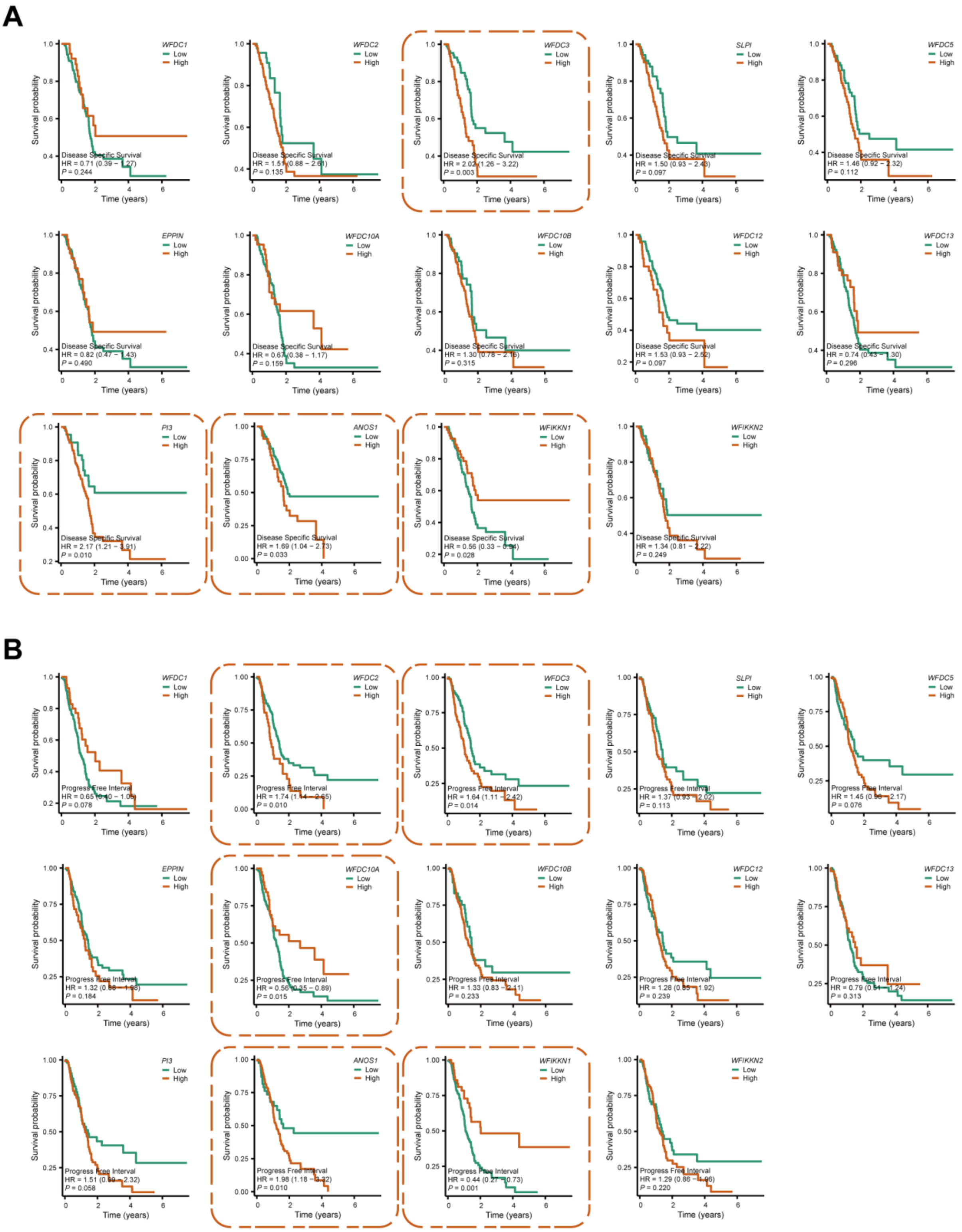

Figure S3. The prognostic value of WFDC family genes in pancreatic cancer. (A) KM curves of DSS between patients with high and low WFDC family genes expression level. (B) KM curves of PFI between patients with high and low WFDC family genes expression level. KM, Kaplan-Meier; DSS, disease specific survival;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

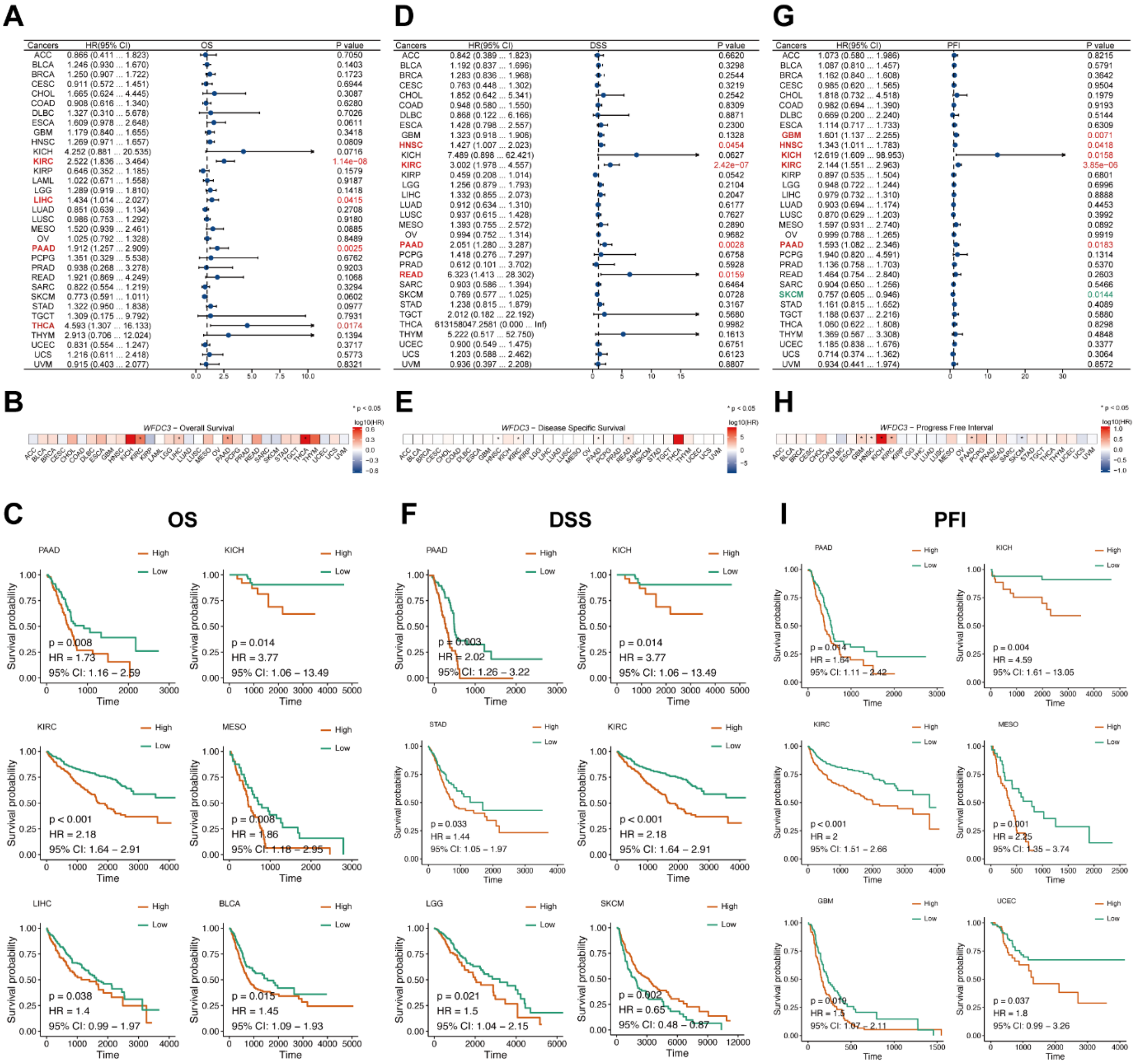

Figure S4. The prognostic value of *WFDC3* in pan-cancer based on TCGA database. (A) Forest map and (B) Survival map of univariate Cox regression analysis of *WFDC3* for OS across cancer types. (C) KM curves of OS between patients with high and low *WFDC3* expression in different cancer types. (D) Forest 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).

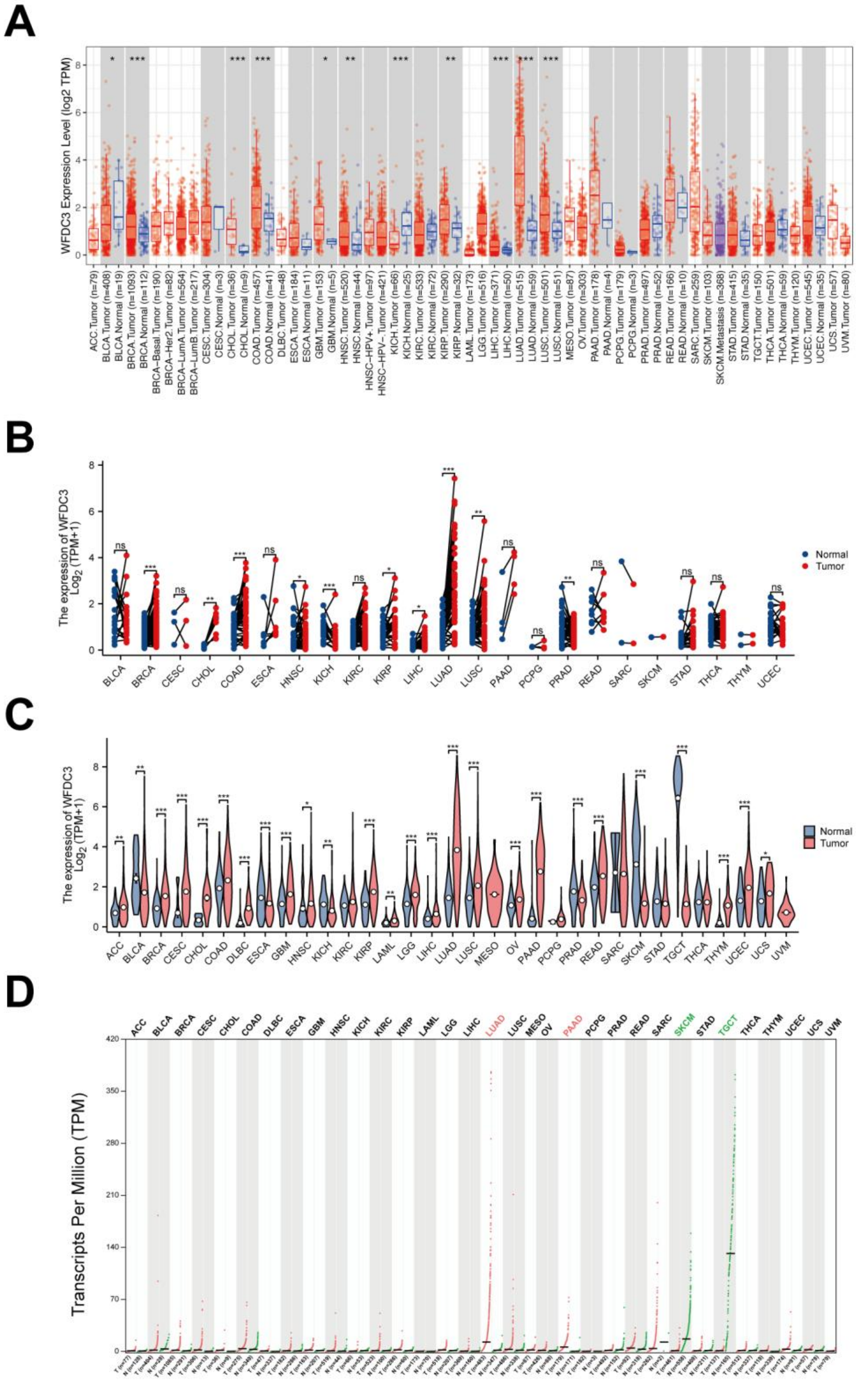

Figure S5. *WFDC3* expression levels in pan-cancer. (A) Expression levels of *WFDC3* in different tumor tissues compared to normal tissues (TIMER). (B) *WFDC3* expression levels in paired adjacent noncancerous tissues and tumor tissues (TCGA). (C) Expression levels of *WFDC3* in different tumor tissues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

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

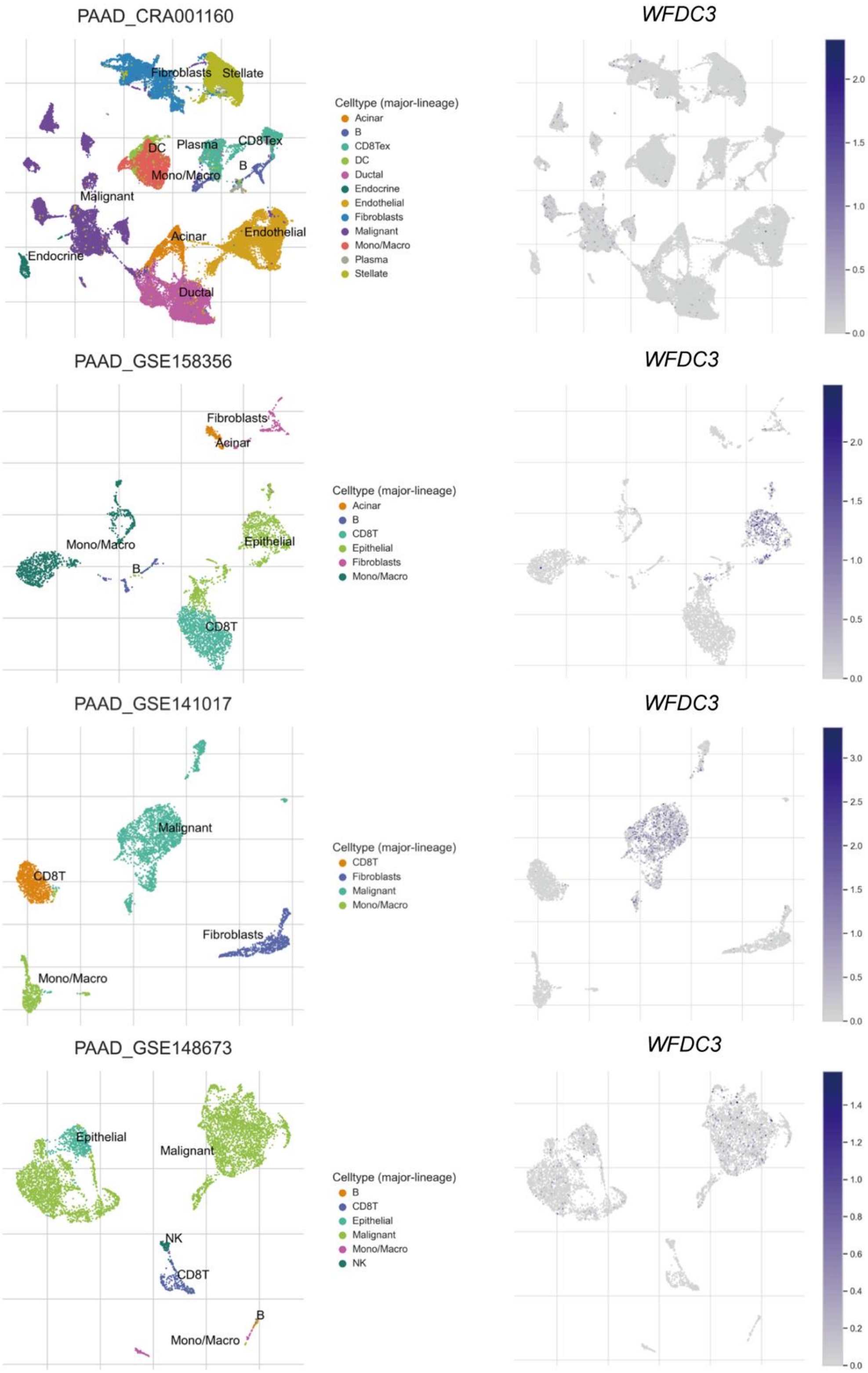

Figure S6. UMAP plots showing the unsupervised clustering of tumor and immune cells from several PAAD patient cohorts. The right panels showing the expression of *WFDC3* across single cells. Single-cell RNA-seq (scRNA-seq) data were downloaded from the TISCH database (Tumor Immune Single-cell Hub; http://tisch.comp-genomics.org/home/).

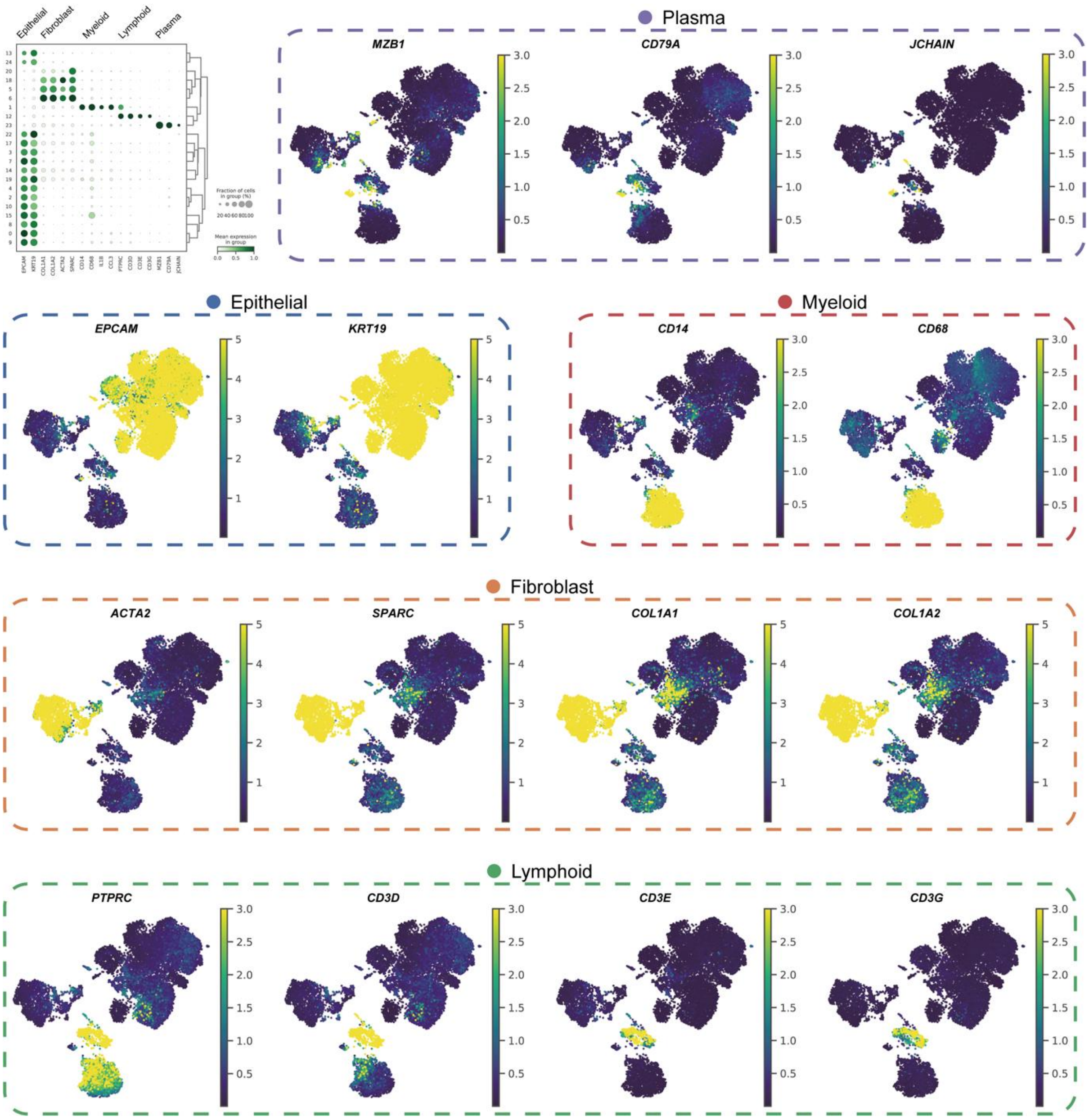

Figure S7. The expression level of markers of cell type annotations across clusters. Bubble plots and UMAP plots illustrated the expression levels of marker genes for the five identified cell types. Inside the purple dashed box was the distribution of plasma cell markers (*MZB1,* *CD79A* and *JCHAIN*). 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*).

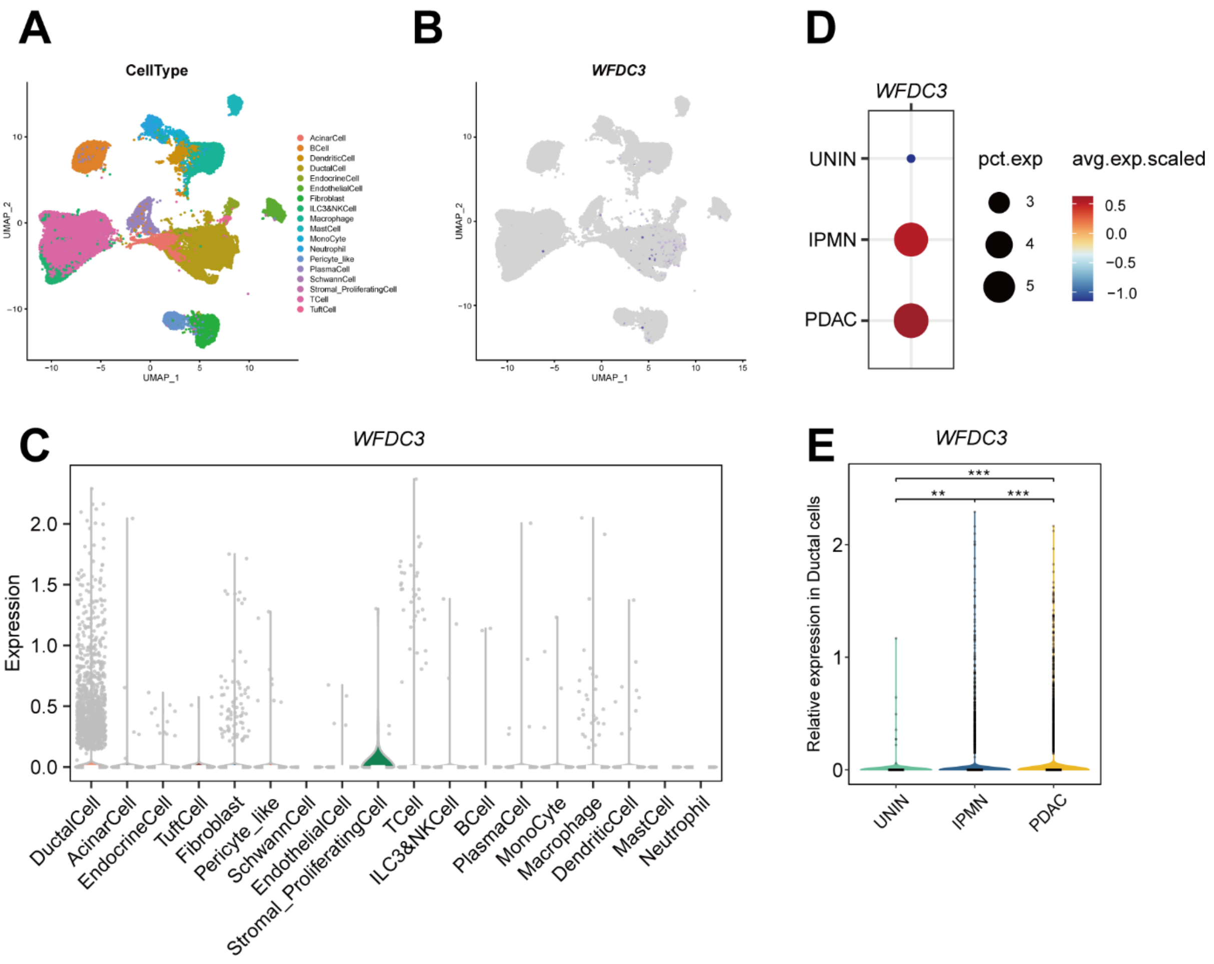

Figure S8. *WFDC3* correlates with malignant evolution of ductal cells in PAAD. (A) UMAP dimensionality reduction was used to show the distribution and dissimilarity of the major cell types. (B) *WFDC3* expression level across all populations. (C) Violin plots visualizing the normalized expression levels 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. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

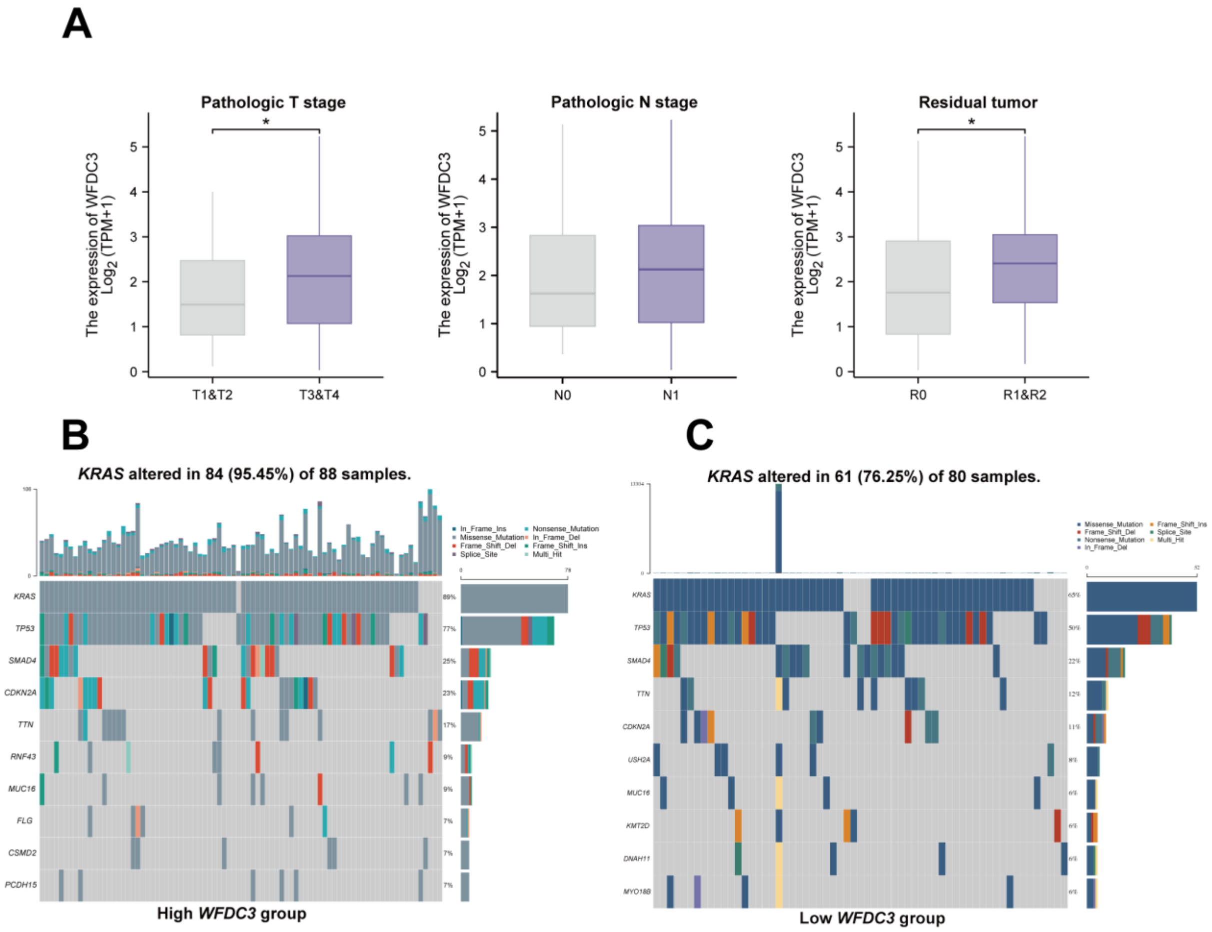

Figure S9. Correlation between *WFDC3* expression and clinical characteristics in PAAD and comprehensive analysis of genomic mutation. (A)Correlation between *WFDC3* and certain clinical characteristics including T stage, N stage and residue tumor. Mutational landscape of (B) the high *WFDC3* expression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

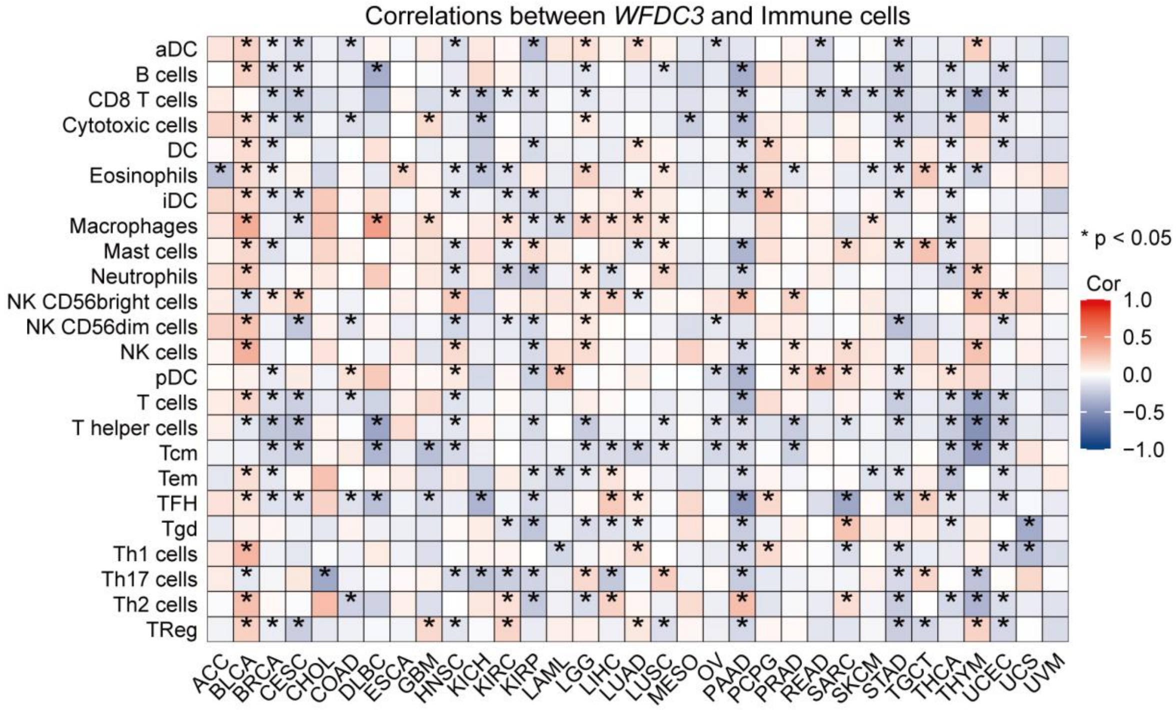

Figure S10. Correlation between *WFDC3* expression and the relative abundances of 24 immune cell types in pan-cancer. * $p < 0.05$.

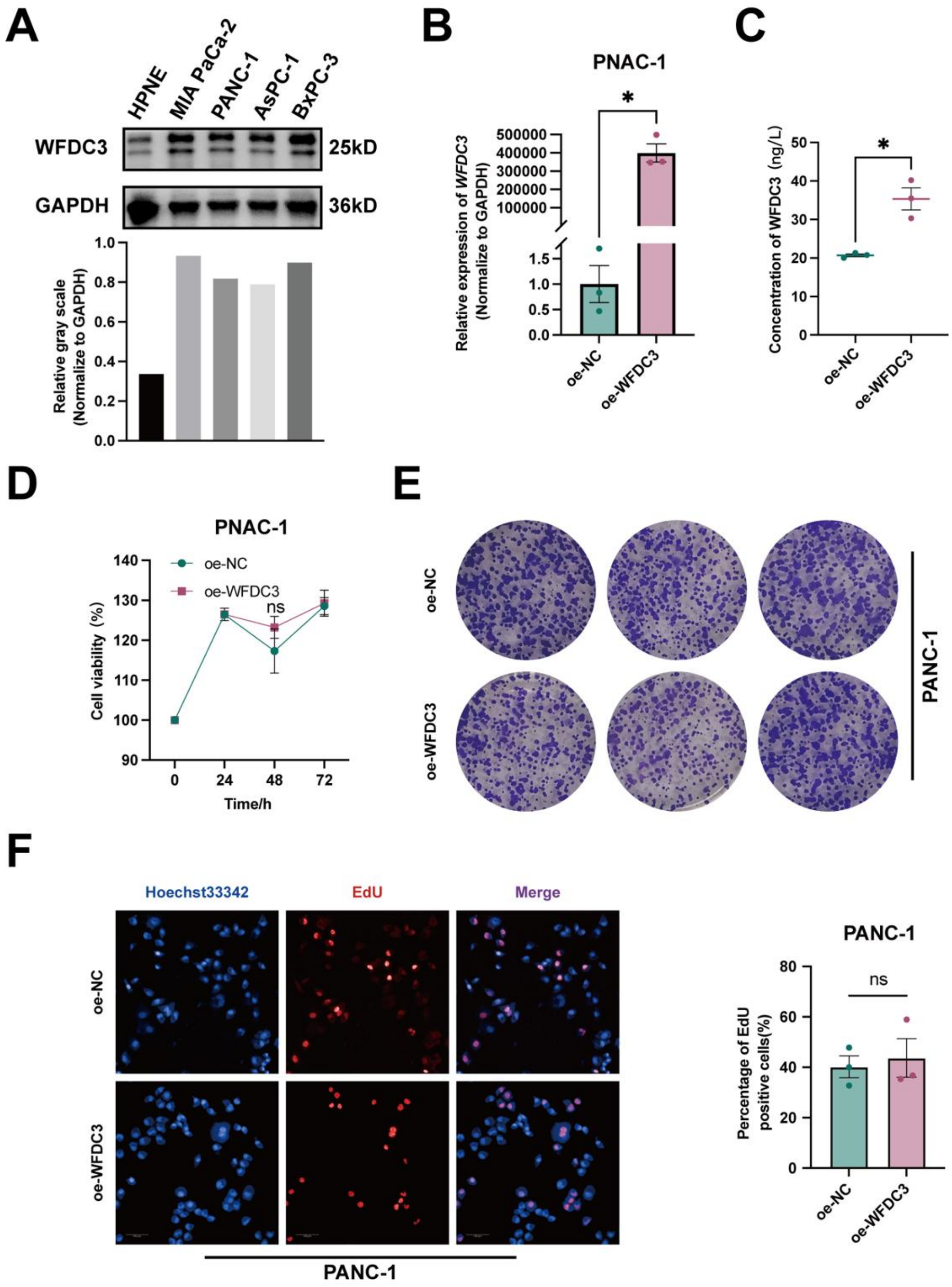

Figure S11. Expression level and proliferative function of WFDC3 in pancreatic cell lines. (A) Western blot and gray values of WFDC3 protein expression in normal human pancreatic cell line (HPNE) and pancreatic cancer cell lines (MIA PaCa-2, PANC-1, AsPC-1 and BxPC-3). (B) The mRNA expression level of *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. * $p < 0.05$.

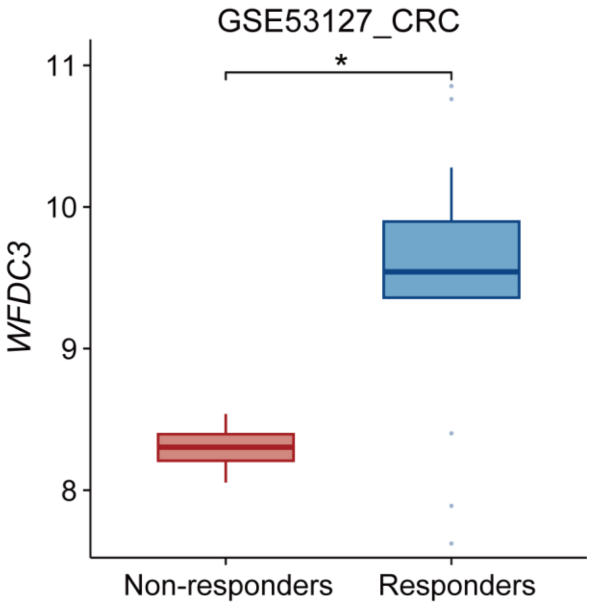

Figure S12. *WFDC3* expression levels in responders vs non-responders in the GSE53127 immunotherapy-treated colorectal cancer cohort. Statistical significance was assessed using a Wilcoxon rank-sum test. * $p < 0.05$.