

# Comprehensive Analysis of Expression, Prognosis and Immune Infiltrates for Kinesin Superfamily Members in Human Pancreatic Adenocarcinoma

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## Research

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# Abstract

## Background

Kinesin superfamily (KIFs) has a long-reported significant influence on the initiation, development, and progress of pancreatic adenocarcinoma (PAAD). However, the expression level of different KIFs in PAAD and its relationship with the prognosis and immune infiltration in patients with PAAD have not been fully elucidated.

## Methods

Comprehensive bioinformatics analyses were done using data from UCSC XENA data hubs, TCGA database, Oncomine databases, GEO datasets, GEPIA, GTEx, The human protein atlas, Kaplan Meier plotter, cBioPortal, STRING and KEGG database. Then, the relationship between KIFs expression and tumor immune infiltrates was studied by using the TIMER database.

## Results

A total of 24 differentially expressed KIFs at transcriptional levels were identified between tumor tissues and normal tissues with 1 (KIF1A) downregulated and 23 (KIF2A, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF5B, KIF7, KIF9, KIF10, KIF11, KIF13A, KIF13B, KIF15, KIF16B, KIF18B, KIF20A, KIF20B, KIF21B, KIF22, KIF23, KIF26B, KIFC1) overexpressed by GEPIA. The protein expression level of KIF3A, KIF3B, KIF4A, KIF5B, KIF7, KIF9, CENPE, KIF11, KIF13A, KIF13B, KIF15, KIF16B, KIF18B, KIF20A, KIF20B, KIF21B, KIF22, KIF23, KIF26B, KIFC1 are higher in PAAD tissues than in the adjacent tissues, which is almost the same as the results of KIFs at transcriptional levels in PAAD. In addition, the expression levels of KIF1A, KIF1C, KIF3A, KIF5A, KIF5C, KIF6, KIF9, KIF13B, KIF19A, KIF21A, KIF24, KIF26B, KIFC1 are significantly correlated with the tumor stage of PAAD patients. As for prognosis value, the abnormal expression of KIF2C, KIF4A, KIF11, KIF15, KIF18B, KIF20A, KIF20B, KIF21B and KIF23 in PAAD patients is significantly related to the worse overall survival (OS) and relapse-free survival (RFS), and based on this, a 5-KIFs-based (KIF15, KIF20A, KIF20B, KIF21B, KIF23) risk score was generated by LASSO regression with a nomogram validated an accurate predictive efficacy. GO and KEGG enrichments revealed functions and pathways affected in PAAD. We also found that the expression of the 5-KIFs was significantly correlated with immune infiltrates, including B cells, CD8+ T cells, CD4+T cells, neutrophils, macrophages, and dendritic cells.

## Conclusion

Our study may provide new insights into the choice of prognostic biomarkers and immunotherapy targets in PAAD patients.

## Background

Pancreatic adenocarcinoma (PAAD) and its variants account for 90% of all pancreatic carcinomas[1]. PAAD is a kind of extremely malignant tumor of the digestive system that is difficult to diagnose and treat[2]. Due to the difficulty of early diagnosis, high surgical mortality, poor treatment outcome, the 5-year survival rate of PAAD patients is only 5% [3]. Although PAAD treatment has been significantly improved in recent decades, most patients are diagnosed with cancer only in the middle and late stages because the early symptoms of PAAD patients are not obvious, and there is a lack of biomarkers for early diagnosis. It is not effective for surgical resection and ultimately leads to high mortality in patients with PAAD[4]. Therefore, it is urgent to find effective biomarkers for early diagnosis and prognosis of PAAD and develop new strategies for targeted therapies for PAAD.

Kinesin superfamily proteins (KIFs) were first identified by Vale et al. in 1985[5]. KIFs are a group of proteins featured to be microtubule-based motors and functioned as intracellular transporters that directionally transport various cargos, including organelles, protein complexes and mRNAs, along microtubules in an adenosine triphosphate (ATP)-dependent way and played crucial roles in cellular morphogenesis and fundamental biology, like mitosis and meiosis [6–8]. There are 45 KIFs discovered and identified in human, among which several family members were demonstrated varied functions in tumor pathobiology [9]. KIF11 was identified as a molecular target that shuttles between the proliferation and invasion of glioblastoma [10]. KIF20A peptide-based immunotherapy for cancer treatment was demonstrated availability and putative efficacy with promiscuous T-H-cell epitopes derived from KIF20A identified in solid tumor tissue and distinguished KIF20A-specific TH1-cell responses were found in patients with HNMT receiving immunotherapy[11]. Microarray data analyses revealed the highly transactivated status of KIF4A in non-small cell lung cancer and targeting KIF4A might hold a promise for the development of anticancer drugs and cancer vaccines as well as a prognostic biomarker in the clinic[12]. Many researches were done highlighting the importance of KIFs in various aspects of breast cancer [13]. Given the essential roles of KIFs reported in cancer, KIF-targeting cancer therapies were highly expected to be of great efficacy. It is worth noting that several KIF-inhibitors were invented and tested in clinical trials. Ispinesib, a KIF11-targeted inhibitor, was the first KIF-inhibitor that was evaluated both safety and efficacy in breast cancer in phase I clinical study[14]. Other KIF-targeted drugs further tested in various cancers by clinical trials including KIF11 inhibitors (Iitronesib[15, 16], filanesib[17–19], SB-743921[20], AZD4877[21]), KIF5C inhibitors (Lidocaine and Tetracaine[22]) and KIFC1 inhibitors (AZ82 and SR31527[23, 24]). However, despite numerous researches done, the prognostic and therapeutic value of all KIFs remains uncorroborated. Considering the intricate functions of KIFs in mitosis, singling out any particular KIFs may not be an efficient way to fulfill the therapeutic capacity of KIFs. At the same time, common regulatory network of all KIFs is little known, which may give new insight into the limited therapeutic efficacy shown in clinical trials and provide putative drug target by mutually regulating KIFs in cancer.

## Results

### Expression profile of KIFs in PAAD

The TCGA expression profile of all KIFs was shown in the heatmap by a dataset from UCSC XENA data hubs (Fig. 1). Distinct expressions profiles were seen between normal and tumor tissue. Clustering analysis found most KIFs overexpressed in tumor tissue. Then, comparisons were done between TCGA tumor samples and matched TCGA and GTEx normal samples in GEPIA dataset, a total of 24 differentially expressed KIFs were found. Among which, only 1 KIFs (KIF1A) showed decreased expression in tumor samples while 23 KIFs (KIF2A, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF5B, KIF7, KIF9, KIF10, KIF11, KIF13A, KIF13B, KIF15, KIF16B, KIF18B, KIF20A, KIF20B, KIF21B, KIF22, KIF23, KIF26B, KIFC1) significantly overexpressed in PAAD (Fig. 2, 3). Then we study the expression profile of KIFs in PAAD tissues and normal tissues in Oncomine database (Table 1), which is almost correspond with the results in GEPIA dataset.

Figure 2 Expression boxplot of each kinesin superfamily members (KIF1A, KIF1B, KIF1C, KIF2A, KIF2B, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF4B, KIF5A, KIF5B, KIF5C, KIF6, KIF7, KIF11, KIF9, KIF10, CENPE, KIF12, KIF13A, KIF13B, KIF14, KIF15, STARD9, KIF16B, KIF17, KIF18A, KIF18B, KIF19A, KIF20A, KIF20B, KIF21A, KIF21B, KIF22, KIF23, KIF24, KIF25, KIF26A, KIF26B, KIF27, KIFC1, KIFC2, KIFC3) using both TCGA-PAAD and matched GTEx-normal data (Tumor = 179; Normal = 171); \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

Table 1 Expression profile of KIFs in PAAD and normal tissues (Oncomine database).

	<i>Types of PAAD vs. normal</i>	<i>Fold Change</i>	<i>p Value</i>	<i>t Test</i>	<i>Source and/or Reference</i>
KIF2A	pancreatic ductal adenocarcinoma	1.600	1.69E-05	4.547	Badea Pancreas Statistics
KIF2C	pancreatic ductal adenocarcinoma	2.558	2.00E-03	4.092	Logsdon Pancreas Statistics
KIF3A	pancreatic ductal adenocarcinoma	1.985	1.10E-02	2.500	Grutzmann Pancreas Statistics
KIF3B	pancreatic ductal adenocarcinoma	1.892	7.29E-12	8.096	Badea Pancreas Statistics
KIF3C	pancreatic ductal adenocarcinoma	1.037	7.00E-03	2.770	TCGA Pancreas Statistics
KIF4A	pancreatic ductal adenocarcinoma	1.038	2.00E-03	3.128	TCGA Pancreas Statistics
KIF5B	pancreatic ductal adenocarcinoma	1.407	3.64E-09	6.619	Badea Pancreas Statistics
KIF7	pancreatic ductal adenocarcinoma	7.872	9.26E-04	3.666	Grutzmann Pancreas Statistics
KIF9	pancreatic carcinoma	2.015	8.00E-03	2.848	Iacobuzio-Donahue Pancreas 2 Statistics
CENPE	pancreatic ductal adenocarcinoma	1.417	3.72E-05	4.358	Badea Pancreas Statistics
KIF11	pancreatic ductal adenocarcinoma	1.024	3.80E-02	1.897	TCGA Pancreas Statistics
KIF13A	pancreatic ductal adenocarcinoma	1.210	1.80E-02	2.193	Pei Pancreas Statistics
KIF13B	pancreatic ductal adenocarcinoma	1.837	1.06E-06	5.145	Badea Pancreas Statistics
KIF15	pancreatic ductal adenocarcinoma	2.778	4.00E-03	2.992	Grutzmann Pancreas Statistics
KIF16B	pancreatic ductal adenocarcinoma	2.302	1.60E-02	2.582	Logsdon Pancreas Statistics
KIF18B	pancreatic ductal adenocarcinoma	1.481	3.76E-04	3.513	Badea Pancreas Statistics
KIF20A	pancreatic ductal adenocarcinoma	2.338	4.59E-04	4.284	Iacobuzio-Donahue Pancreas 2 Statistics
KIF20B	pancreatic ductal adenocarcinoma	1.438	2.38E-06	4.932	Badea Pancreas Statistics
KIF21B	pancreatic ductal adenocarcinoma	1.804	2.00E-03	3.357	Grutzmann Pancreas Statistics
KIF22	pancreatic carcinoma	1.385	4.36E-06	4.886	Badea Pancreas Statistics
KIF23	pancreatic ductal adenocarcinoma	1.347	6.00E-03	2.599	Badea Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	6.294	1.60E-02	2.316	Grutzmann Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.747	4.50E-02	1.857	Iacobuzio-Donahue Pancreas 2 Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.224	2.10E-02	2.275	Iacobuzio-Donahue Pancreas 2 Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.137	6.00E-03	2.570	Badea Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.055	9.72E-06	5.033	TCGA Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.026	2.60E-02	2.105	TCGA Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.645	4.76E-05	4.466	Pei Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.748	1.63E-05	4.429	Badea Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.056	4.87E-06	5.272	TCGA Pancreas Statistics
KIF26B	pancreatic ductal adenocarcinoma	1.216	1.00E-03	3.140	Badea Pancreas Statistics
KIFC1	pancreatic carcinoma	1.534	2.05E-04	3.896	Pei Pancreas Statistics

## Relationship Between the mRNA expression levels of KIFs and the tumor stage of PAAD Patients

We also studied the relationship between mRNA expression levels of KIFs and tumor stage in PAAD patients by using the GEPIA dataset. In comparison of 178 PAAD patient samples and 171 normal samples, it showed that the expression levels of KIF1A, KIF1C, KIF3A, KIF5A, KIF5C, KIF6, KIF9, KIF13B,

KIF19A, KIF21A, KIF24, KIF26B, KIFC1 are significantly correlated with the tumor stage of PAAD patients (Fig. 4), while the expression levels of the other KIFs are not correlated with the tumor stage of PAAD patients.

Figure 4 Correlation between KIFs Expression and Tumor Stage in PAAD Patients (GEPIA)

## The Protein Expression Level Of Kifs In Paad

In The human protein atlas we found that the protein levels of KIF3A, KIF3B, KIF4A, KIF5B, KIF7, KIF9, CENPE, KIF11, KIF13A, KIF13B, KIF15, KIF16B, KIF18B, KIF20A, KIF20B, KIF21B, KIF22, KIF23, KIF26B, KIFC1 are higher in PAAD tissues than in the adjacent tissues (Fig. 5), which is almost the same as the transcriptional levels between TCGA tumor samples and matched TCGA and GTEx normal samples.

Figure 5 The Expression of KIFs in PAAD (HPA)

## The Prognostic Value Of Kifs In Paad Patients

To evaluate the value of KIFs at different transcription levels in the progression of PAAD, we evaluated the correlation between KIFs at different transcription levels and clinical outcome using Kaplan–Meier plotter analysis. In all 24 differentially expressed KIFs, after compared 89 high expression level patients with 89 low expression level patients, PAAD patients with high transcription levels of KIF21B were significantly associated with longer RFS and OS, in contrast, PAAD patients with high transcription levels of KIF2C, KIF4A, KIF11, KIF15, KIF18B, KIF20A, KIF20B, KIF23 were significantly related with worsen OS and RFS (Fig. 6,7).

Figure 6 The prognostic value of different expressed KIFs in PAAD patients in the RFS curve (Kaplan–Meier plotter).

## Construction Of 5-kifs-based Risk Score

Given the expression profile and survival analyses of all KIFs, 9 abnormal expression KIFs (KIF2C, KIF4A, KIF11, KIF15, KIF18B, KIF20A, KIF20B, KIF23, KIF21B) that demonstrated significant prognostic value in PAAD were enrolled for LASSO regression to construct a KIFs-based risk score for prediction of OS in PAAD. According to the cvfit plot, the minimal of lambda value was seen in 5, indicating a 5-KIFs-based risk score model the best for both accuracy and simplicity (Fig. 8a). Therefore, only KIF15, KIF20A, KIF20B, KIF21BA, KIF23 were included (Fig. 8b) and a 5-KIFs-based risk score was generated as below:

$$Rs = 0.12796255\text{Exp}(\text{KIF20A}) - 0.48974996\text{Exp}(\text{KIF15}) + 0.49730528\text{Exp}(\text{KIF20B}) + 0.21624084\text{Exp}(\text{KIF21B}) - 0.07107506\text{Exp}(\text{KIF23})$$

Rs: risk score; Exp(X): the expression level of gene X

Validations of the 5-KIFs-based risk score done by Kaplan–Meier plots demonstrated good prognostic value in predicting OS of PAAD, with higher risk score significantly correlated with worse outcomes in both TCGA-PAAD data ( $P = 0.00076$ ) and dataset (GSE62452) from GEO ( $P = 0.031$ ) (Fig. 8c).

## Multivariate Analysis And Nomogram

To evaluate the prognostic value of the risk score, we performed multivariate analyses on all demographic and clinical parameters (age, family history of cancer, history of chronic pancreatitis, history of diabetes, maximum tumor dimension, tumor grade, number of positive lymphnodes, radiation therapy, TNM stage and risk score) in TCGA dataset. Then we selected several indicators (age, number of positive lymphnodes, risk score) with significant differences for next step. A nomogram predicting 1-year and 3-year OS of PAAD patients was constructed combining age, number of positive lymphnodes and LASSO regression generated 5-KIFs risk score using R package “rms”[46, 47]. Assessment of predictive accuracy was done by calibration plot[48].

### Genetic Alteration, Enrichment of co-expression genes, Strings, GO and KEGG Analysis

The genetic alteration was analyzed by cBioPortal ([www.cbioportal.org](http://www.cbioportal.org))[49]. The genomic map includes data for putative copy-number alterations (CNA) from GISTIC, mRNA expression z-scores and mutations. Co-expression genes correlated with KIFs were enriched using GEPIA by TCGA-PAAD data. STRINGS ([www.string-db.org](http://www.string-db.org)) is an online analysis tool that collects, scores and integrates all publicly available sources of protein–protein interaction (PPI) data and supplements it with calculations and predictions[50]. In this study, we performed a PPI network analysis on co-expression genes of KIFs to explore their interactions. The intersection of co-expressed genes was put into GO, KEGG and transcription factors enrichment using R package “ClusterProfiler”[51]. Enrichment results were represented as bubble plot using R package “ggplot”[52].

The functions of the 5-KIFs and the genes significantly associated with alterations were predicted by analyzing gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) in the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov/>). GO enrichment analysis predicted the functional roles of target host genes based on three aspects, including biological processes, cellular components, and molecular functions. We found that GO:0140014 (Mitotic Nuclear Division), GO:0048285 (Organelle Fission), GO: 0000070 (Mitotic Sister Chromatid Segregation), and GO: 0000280 (Nuclear Division) were significantly regulated by the KIFs alterations in PAAD (Figure 11A, B, C).

KEGG analysis can define the pathways related to the functions of KIFs alterations and the frequently altered neighbor genes; 5 pathways significantly related to the functions of KIFs alterations in PAAD were found through KEGG analysis (Figure 11D). Among these pathways, hsa04110: Cell cycle, hsa04114: Oocyte Meiosis, hsa04914: Progesterone–Mediated Oocyte Maturation, has04115: P53 Signaling

Pathway and has04218: Cellular Senescence were involved in the tumorigenesis and pathogenesis of PAAD.

## The Relationship Between KIFs Expression Levels and Immune Infiltration Levels in PAAD

TIMER online analysis tool is used to evaluate the relationship between the transcription level of KIFs and the level of immune infiltration in PAAD. It was found that KIFs are involved in immune cell infiltration, which affect the clinical outcome of PAAD patients. The analysis results are shown in Figure 12. These studies indicate that the level of KIFs expression is associated to the level of immune infiltration in PAAD. Among the 5-KIFs, KIF15 expression was positively correlated with infiltration of B cells. KIF20A expression was positively correlated with the infiltration of B cells, neutrophil and dendritic cells while it was negatively correlated with infiltration of CD4+ T cells. KIF20B expression was positively correlated with the infiltration of CD8+ T cells, B cells, macrophage, neutrophil and dendritic cells while it was negatively correlated with infiltration of CD4+ T cells. KIF21B expression was positively correlated with infiltration of CD4+ T cells, CD8+ T cells, B cells, neutrophil and dendritic cells. KIF23 expression was negatively correlated with infiltration of CD4+ T cells, while it was positively correlated with infiltration of B cells, neutrophil and dendritic cells. These studies indicate that the level of KIFs expression is associated to the level of immune infiltration in PAAD.

Based on above results, we selected immune B cell to construct a Cox regression with KIF15, KIF20A, KIF20B, KIF21BA and KIF23. Therefore, TIMER online tool was used to proofread B cell, the 5-KIFs covariate factors and automatically output Cox regression results. These studies indicate that the KIF15 (P = 0.027), KIF21B (P=0.039) and KIF23 (P=0.024) was significantly correlated with the clinical outcomes of patients with PAAD (Table 2).

Table 2 The cox proportional hazard model of the 5-KIFs and B immune cells in PAAD (TIMER).

	coef	HR	95%CI_l	95%CI_u	P-value	sig
B cell	-0.007	0.993	0.091	10.820	0.995	
KIF15	-0.642	0.526	0.299	0.928	0.027	*
KIF21B	-0.413	0.661	0.447	0.979	0.039	*
KIF20A	0.216	1.241	0.687	2.243	0.474	
KIF20B	0.289	1.335	0.738	2.416	0.339	
KIF23	0.714	2.043	1.098	3.800	0.024	*

## Discussion

The KIF proteins participate in multiple normal cellular biological activities including mitosis and intracellular transport of vesicles and organelles[6]. Increasing evidence indicates kinesin proteins play critical roles in the genesis and development of human cancers. Some kinesin proteins are associated with malignancy as well as drug resistance of solid tumor. Thus, targeting KIF therapy seems to be a

promising anticancer strategy. Therefore, comprehensive bioinformatics analyses were done in our study using data from multi-dataset to explore the expression, prognosis as well as Immune infiltrates of kinesin superfamily in PAAD patients. A total of 24 differentially expressed KIFs were identified between tumor tissue and normal tissue with 1(KIF1A) downregulated and 23 (KIF2A, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF5B, KIF7, KIF9, KIF10, KIF11, KIF13A, KIF13B, KIF15, KIF16B, KIF18B, KIF20A, KIF20B, KIF21B, KIF22, KIF23, KIF26B) overexpressed. Survival analyses revealed 9 abnormal expressed KIFs (KIF2C, KIF4A, KIF11, KIF15, KIF18B, KIF20A, KIF20B, KIF21B, KIF23) significantly correlated with worse OS and RFS of PAAD patients, indicating efficient biomarkers for predicting the prognosis of PAAD. Further analyses were done with a 5-KIFs-based risk score generated by LASSO regression, a nomogram was constructed with elements selected by multivariate survival analysis and an accurate predictive efficacy was validated by another dataset.

KIF15 plays an important role in several kind of tumors. As reported, KIF15 is required for maintenance of spindle bipolarity and is the breast cancer tumor antigen[25]. In our group's early study, we reported for the first time that KIF15 promotes pancreatic cancer cell proliferation by promoting G1/S phase transition via regulating the MEK–ERK signaling pathway[26].

KIF20A has been documented to accumulate in the midzone of the spindle during anaphase and to the cleavage furrow and midbody during telophase[27]. It has been reported that KIF20A is overexpressed in human pancreatic ductal adenocarcinoma (PDAC) based on cDNA microarray analyses and that KIF20A and DLG5, which collaborate in the cytoplasm but not in the midzone of the spindle, are likely to be involved in pancreatic carcinogenesis[28]. KIF20A functions as part of the intracellular trafficking machinery for DLG5, transporting it to membrane sites in PDAC cells in which DLG5 can interact with other proteins including  $\beta$ -catenin[28]. Therefore, it is possible that the intracellular trafficking function of KIF20A is highly important for pancreatic carcinogenesis.

KIF20B is strongly overexpressed in bladder cancer tissues and downregulation of endogenous KIF20B leads to cytokinesis defect[29]. It's also reported that in multiple cancer cells, knockdown of KIF20B not only dramatically inhibits tumor cell growth, but also causes mitotic arrest, senescence and postmitotic apoptosis[30].

KIF21B is not only a classic kinesin protein but also a regulator of microtubule dynamics[31]. In vitro reconstitution studies show that KIF21B increases the microtubule growth rate and catastrophe frequency. Moreover, the purified protein surprisingly associates primarily with depolymerizing microtubule and microtubule ends[32]. A study published in 2017 confirmed that KIF21B is a potential microtubule-pausing factor[33]. Microtubules and kinesins play roles in intercellular signal transduction, transport, malignant tumorigenesis, and tumor progression, invasion, and metastasis[34, 35].

KIF23 is essential for the completion of cytokinesis in midbody formation and for recruitment to the spindle midzone/midbody by a chromosomal passenger protein[36]. In a study, it discovered the high expression of KIF23 was associated with poor prognosis by immunohistochemical analysis of tumor sections and clinical data from patients with PDAC[37]. They also found that knockdown of KIF23

decreased proliferation in PANC-1 and BxPC-3 PDAC cells, and suppression of KIF23 in PANC-1 cells inhibited tumor growth in nude mice[37].

Furthermore, the mutation rate (6%) of the 5-KIFs in PAAD is low, which indicates the expression of the 5-KIFs is stable relatively. Then we analyzed the function of the 5-KIFs and its neighboring proteins by GO enrichment analysis and KEGG pathway enrichment. Studies have found that the functions of these proteins are mainly related to the Cell cycle, Oocyte Meiosis, Progesterone – Mediated Oocyte Maturation, P53 Signaling Pathway and Cellular Senescence, and some of these pathways are significantly associated with the occurrence and development of PAAD. These data indicate that differentially expressed KIFs in PAAD are potential targets for drug therapy.

Another important result of this study is that the transcription levels of the 5-KIFs is closely correlated with various levels of immune infiltration in PAAD. The correlation between the expression of the 5-KIFs and the marker genes of PAAD immune cells indicates that KIFs may be involved in the regulation of PAAD tumor immunity.

In conclusion, KIFs not only can be used as a prognostic indicator of patients with PAAD, but also reflect their immune status. These findings may help better study the molecular basis of PAAD and may help create more appropriate prognostic tools for PAAD and facilitate the development of new immunotherapeutic. However, more experimental studies are needed to confirm our results, thereby promoting the clinical application of KIFs as a prognostic indicator or immunotherapy target in PAAD.

## **Conclusion**

Our research comprehensively illustrates the expression of KIFs in PAAD. We also built a risk score model of PAAD patients based on the TCGA database and it can still accurately predict the prognosis of PAAD patients in independent GEO data set. And we found that KIFs are involved in immune cell infiltration. The cox regression of several KIFs and immune cell can also affect the clinical outcome of PAAD patients. In summary, our study may provide new choices of prognostic biomarkers and immunotherapy targets in PAAD patients.

## **Materials And Methods**

### **TCGA Data and UCSC XENA data hubs Analysis**

KIFs RNA sequencing expression data of PAAD from the cancer genome atlas (TCGA, <http://cancergenome.nih.gov/>) were downloaded using UCSC XENA data hubs (<https://tcga.xenahubs.net>), the heatmap was drawn using R(4.0.2) package “pheatmap” with clinical parameters from TCGA-PAAD dataset[38].

### **Gepia Dataset And Oncomine Databases Analysis**

Gene Expression Profiling Interactive Analysis (GEPIA, [www.gepia.cancer-pku.cn](http://www.gepia.cancer-pku.cn)) is a newly developed interactive web server that analyzes RNA sequencing expression data from 9,736 tumors and 8,587 normal samples from the Cancer Genome Atlas (TCGA) and Genotypic Tissue Expression (GTEx) projects using standard processing pipelines ([www.gepia.cancer-pku.cn/](http://www.gepia.cancer-pku.cn/)). GEPIA can provide customizable functions, including differential expression analysis, patient survival analysis, cancer type staging, cancer pathological staging, correlation analysis, similar gene detection and dimensionality reduction analysis[39]. Oncomine gene expression array dataset ([www.oncomine.org](http://www.oncomine.org)) is a publicly accessible online cancer microarray database for analyzing the KIFs transcription levels in different cancers[40]. Student's t test was used to compare the transcription levels of KIFs in clinical cancer specimens with that in normal controls. The cut-off of P-value was defined as 0.05.

## The Human Protein Atlas Analysis

The Human Protein Atlas(HPA, <https://www.proteinatlas.org/>) is a Swedish-based program initiated in 2003 with the aim to map all the human proteins in cells, tissues and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology[41]. We can find the expression levels of KIFs in PAAD tissues and its adjacent tissues.

## The Kaplan–meier Plotter Analysis

The Kaplan–Meier plotter is an online database ([www.kmplot.com](http://www.kmplot.com)) that contains gene expression data and survival information for clinical cancer patients[42, 43]. We used this online tool to assess the prognostic value of KIFs mRNA expression in PAAD patients and analyzed the OS and RFS of patients with PAAD. The database divides patient samples into high expression groups and low expression groups according to the median values of KIFs mRNA expression and validates them by Kaplan–Meier survival curve. Information on number of patients, median values of mRNA expression, 95% confidence interval (CI), hazard ratio (HR), and P-value can be found on the Kaplan–Meier plotter web page. P-value < 0.05 was considered as statistically significant.

## Lasso Regression

LASSO Cox regression is a widely used method for high-dimensional predictors selection[44]. In this study, TCGA-PAAD data were used to construct a risk score of KIFs for the prediction of OS. R package “glmnet” was used to execute LASSO Cox regression model analysis[45]. Cvfit plot was drawn, and the minimum lambda value was used as cutoff. The predictive model was validated in TCGA-PAAD and GEO datasets using KM plotter as previously described.

# Timer Analysis

TIMER ([www.cistrome.shinyapps.io](http://www.cistrome.shinyapps.io)) is a convenient and accurate online analysis tool that can infer the abundance of tumor-infiltrating immune cells from gene expression profiles and evaluate their clinical impact[53]. In this study, we used TIMER to assess the correlation between KIFs expression levels and immune cell infiltration. And we also assess the correlation between clinical outcomes and immune cell infiltration and KIFs expression.

## Statistics

For all the analyses done above, a P-value < 0.05 was considered statistically significant except for specifically mentioned.

## Declarations

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**Consent for publication:** Not applicable.

**Author contributions** Zehao Chen conceived the project and wrote the manuscript. Jie Wang, Jian Xu and Wenjie Zhu participated in data analysis. Jie Wang, Jian Xu and Wenjie Zhu participated in discussion and language editing. Jianxin Jiang reviewed the manuscript. All authors contributed to the article and approved the submitted version.

**Competing interests:** The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that they have no competing interests.

**Availability of data and materials:** The datasets generated and/or analyzed during the current study are available in the UCSC XENA repository(<https://tcga.xenahubs.net>), Cancer Genome Atlas (TCGA, <http://can-cergenome.nih.gov/>), the GTEx projects and Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>).

**Ethics approval and consent to participate:** Not applicable.

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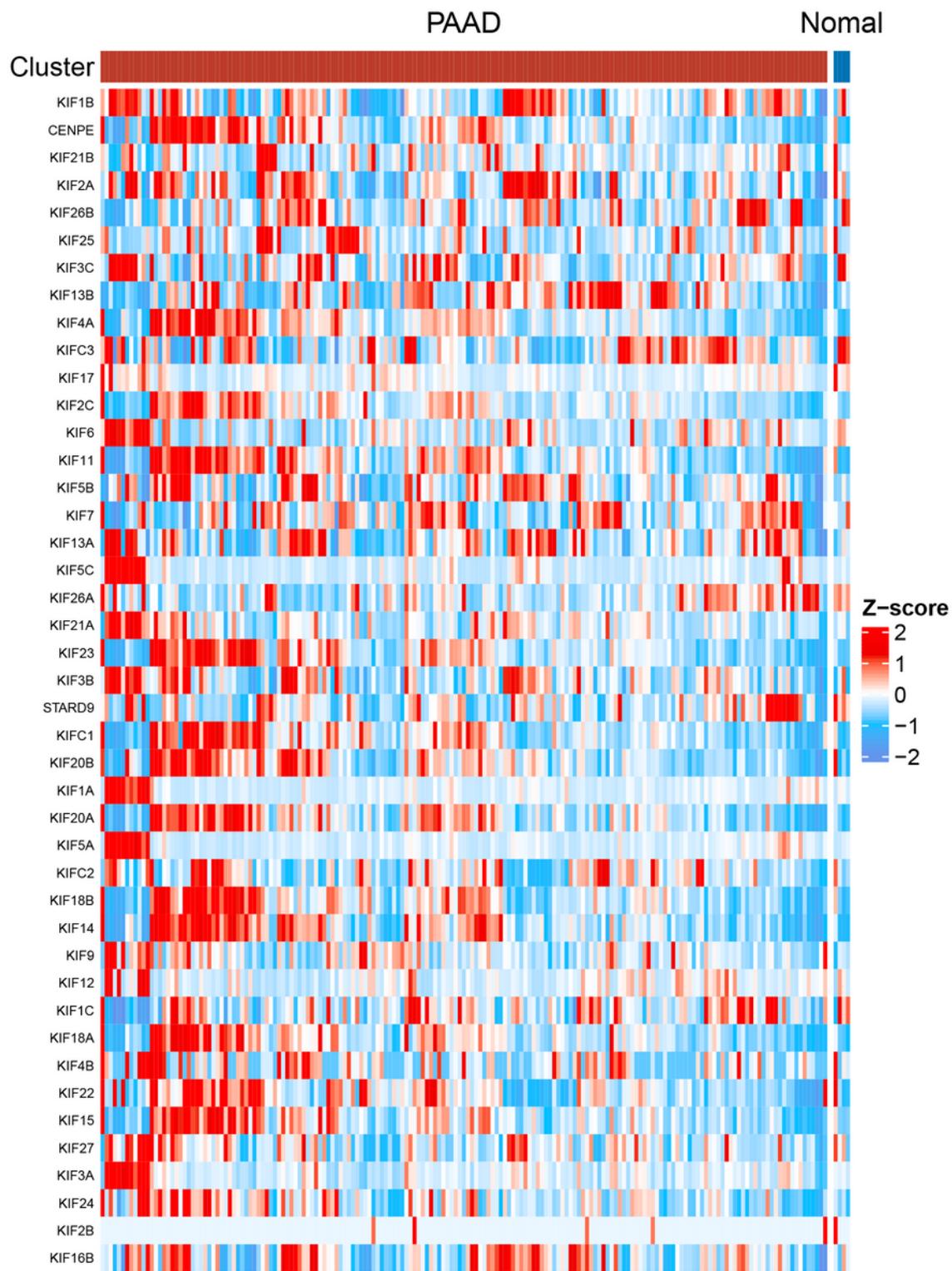
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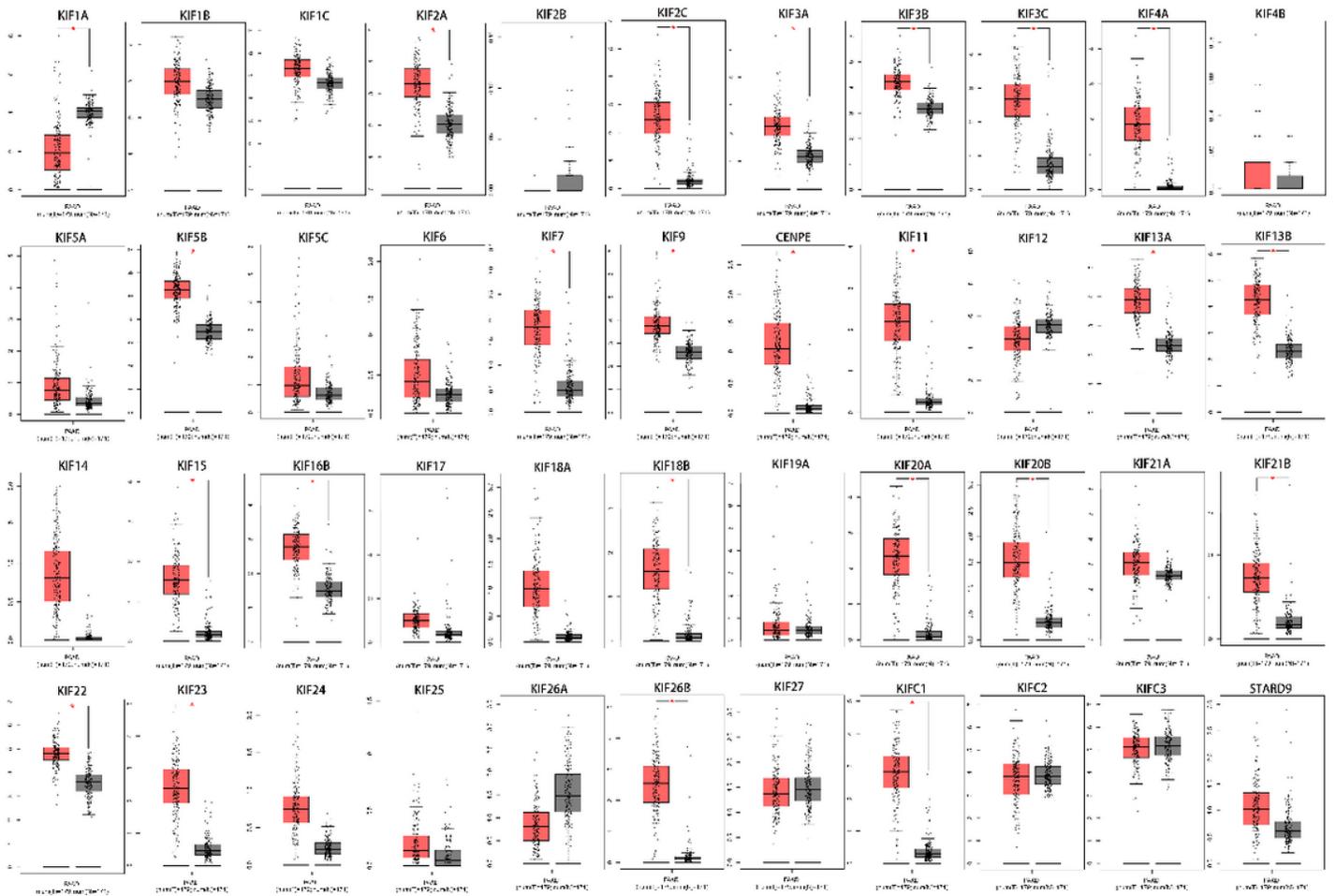
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## Figures



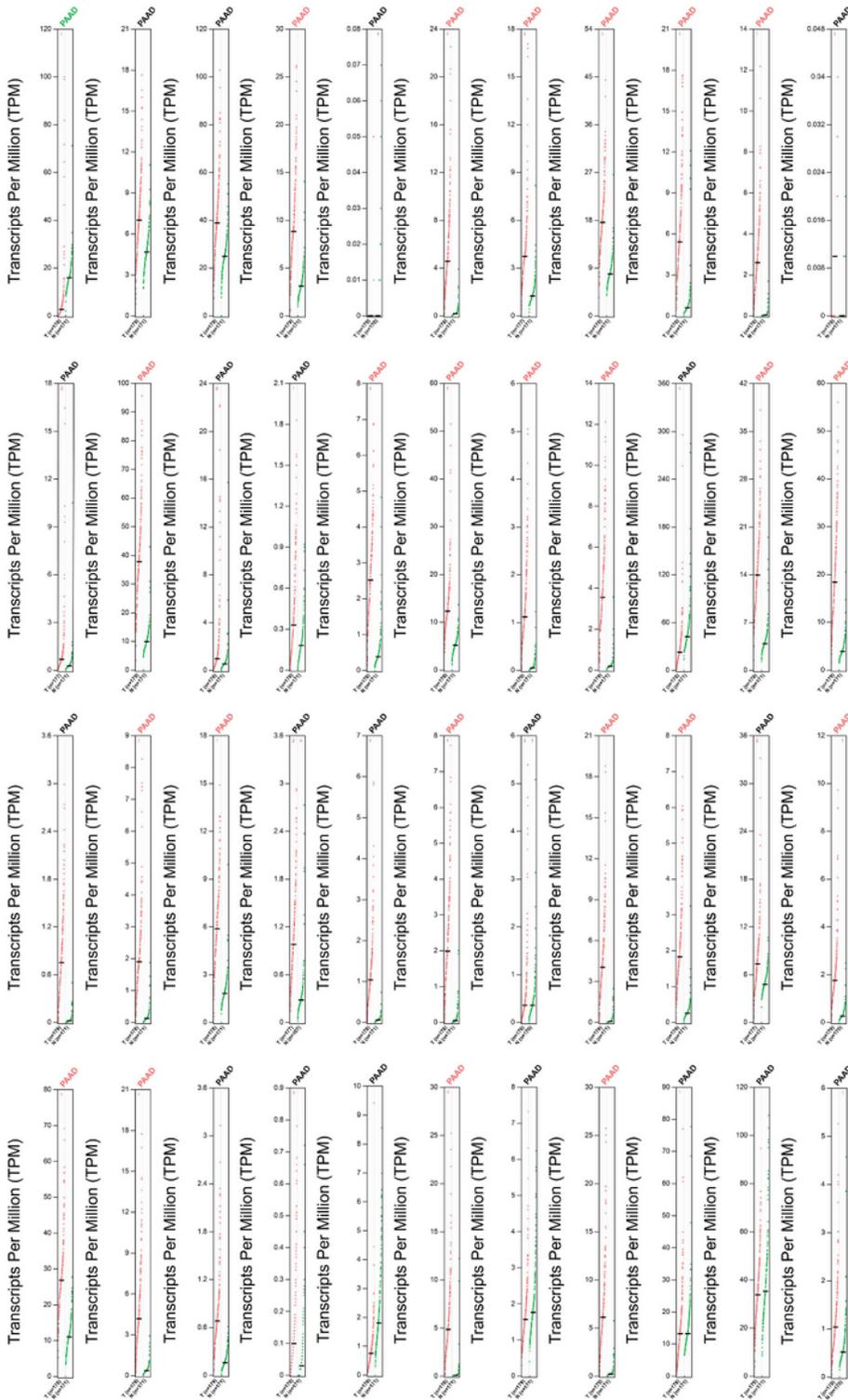
**Figure 1**

Expression profiles of kinesin superfamily in Pancreatic Adenocarcinoma.



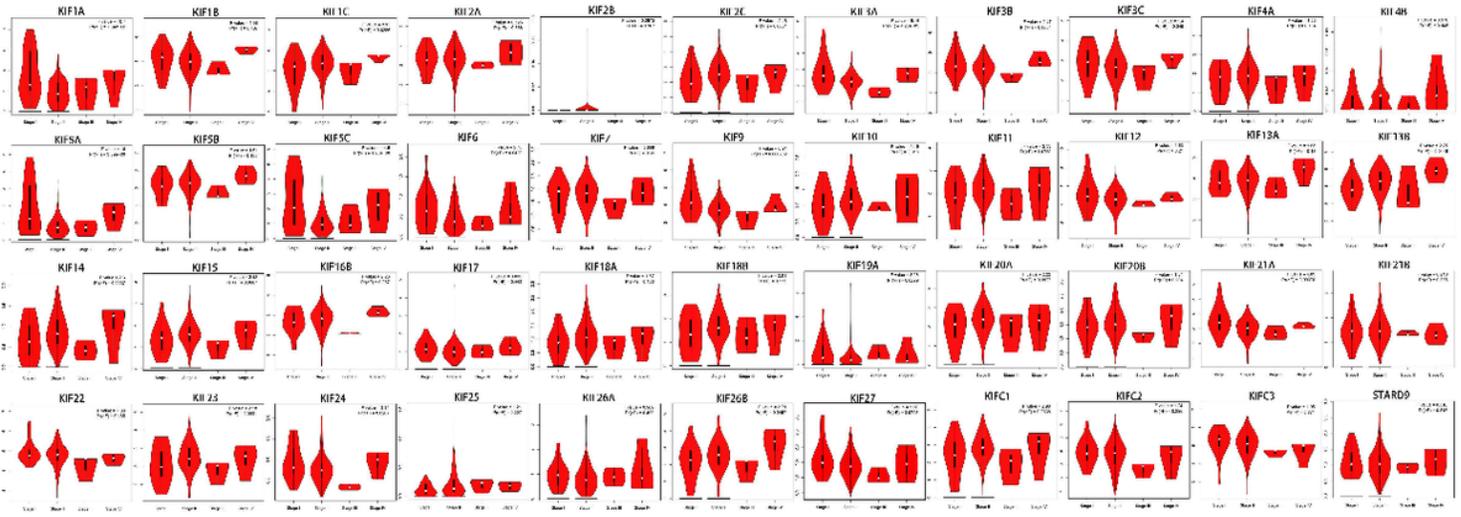
**Figure 2**

Expression boxplot of each kinesin superfamily members (KIF1A, KIF1B, KIF1C, KIF2A, KIF2B, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF4B, KIF5A, KIF5B, KIF5C, KIF6, KIF7, KIF11, KIF9, KIF10, CENPE, KIF12, KIF13A, KIF13B, KIF14, KIF15, STARD9, KIF16B, KIF17, KIF18A, KIF18B, KIF19A, KIF20A, KIF20B, KIF21A, KIF21B, KIF22, KIF23, KIF24, KIF25, KIF26A, KIF26B, KIF27, KIFC1, KIFC2, KIFC3) using both TCGA-PAAD and matched GTEx-normal data (Tumor = 179; Normal = 171); \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001



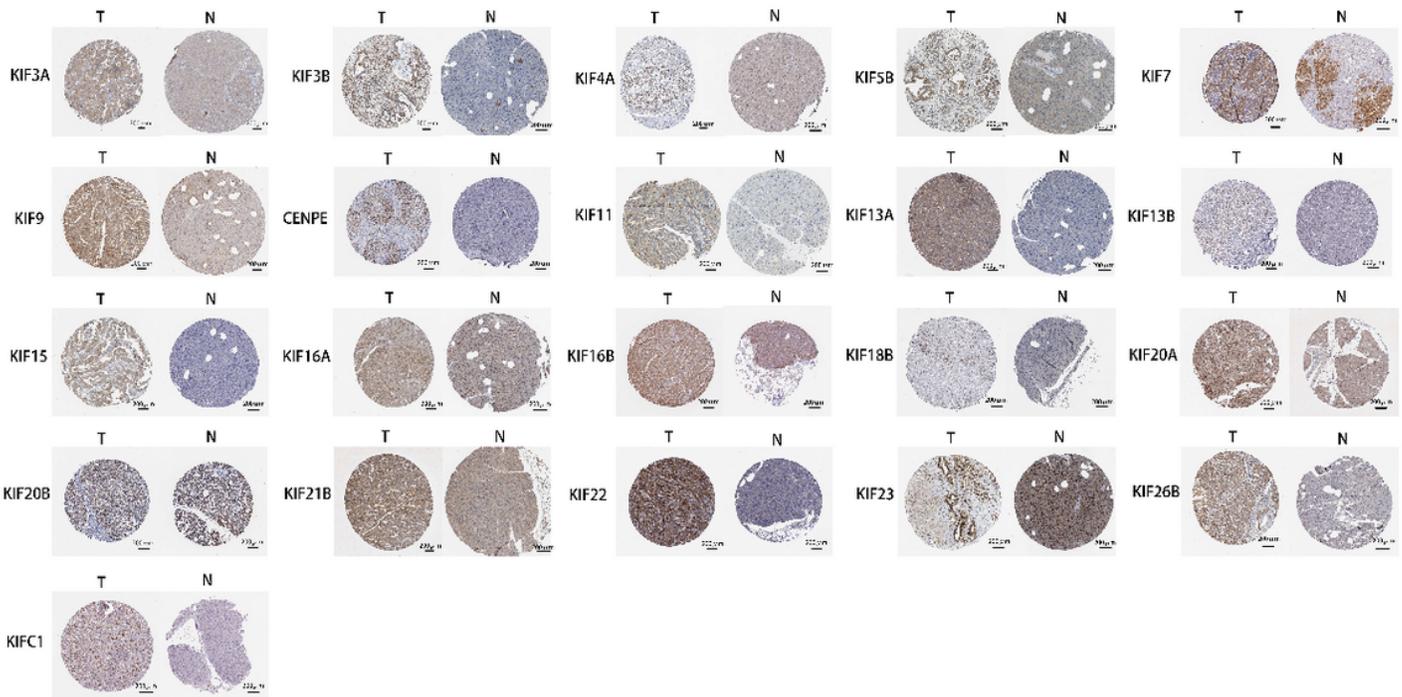
**Figure 3**

Expression profile of each kinesin superfamily members (KIF1A, KIF1B, KIF1C, KIF2A, KIF2B, KIF2C, KIF3A, KIF3B, KIF3C, KIF4A, KIF4B, KIF5A, KIF5B, KIF5C, KIF6, KIF7, KIF11, KIF9, KIF10, CENPE, KIF12, KIF13A, KIF13B, KIF14, KIF15, STARD9, KIF16B, KIF17, KIF18A, KIF18B, KIF19A, KIF20A, KIF20B, KIF21A, KIF21B, KIF22, KIF23, KIF24, KIF25, KIF26A, KIF26B, KIF27, KIFC1, KIFC2, KIFC3) using both TCGA-PAAD and matched GTEx-normal data (Tumor = 179; Normal = 171)



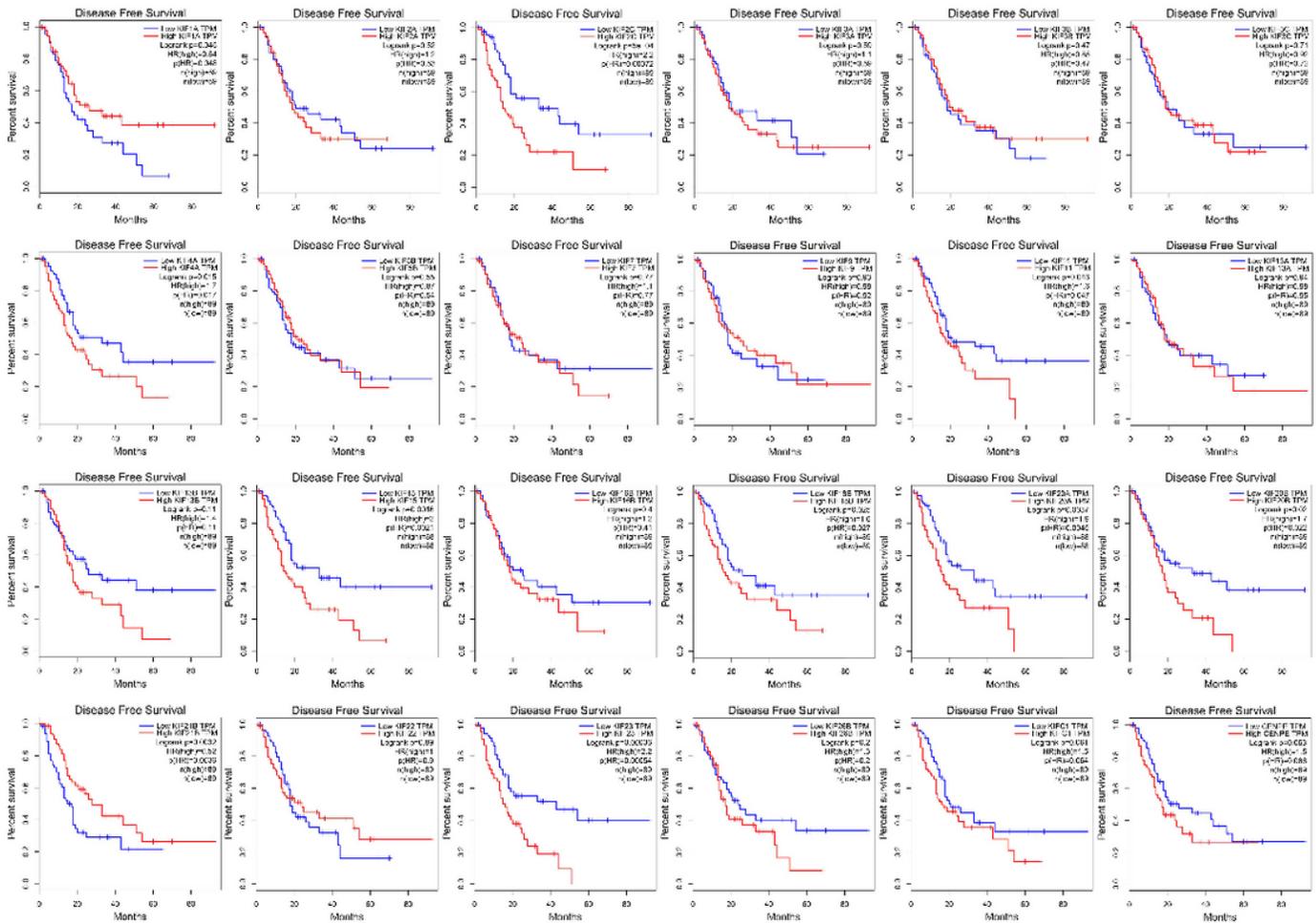
**Figure 4**

Correlation between KIFs Expression and Tumor Stage in PAAD Patients (GEPIA)



**Figure 5**

The Expression of KIFs in PAAD (HPA)



**Figure 6**

The prognostic value of different expressed KIFs in PAAD patients in the RFS curve (Kaplan–Meier plotter).

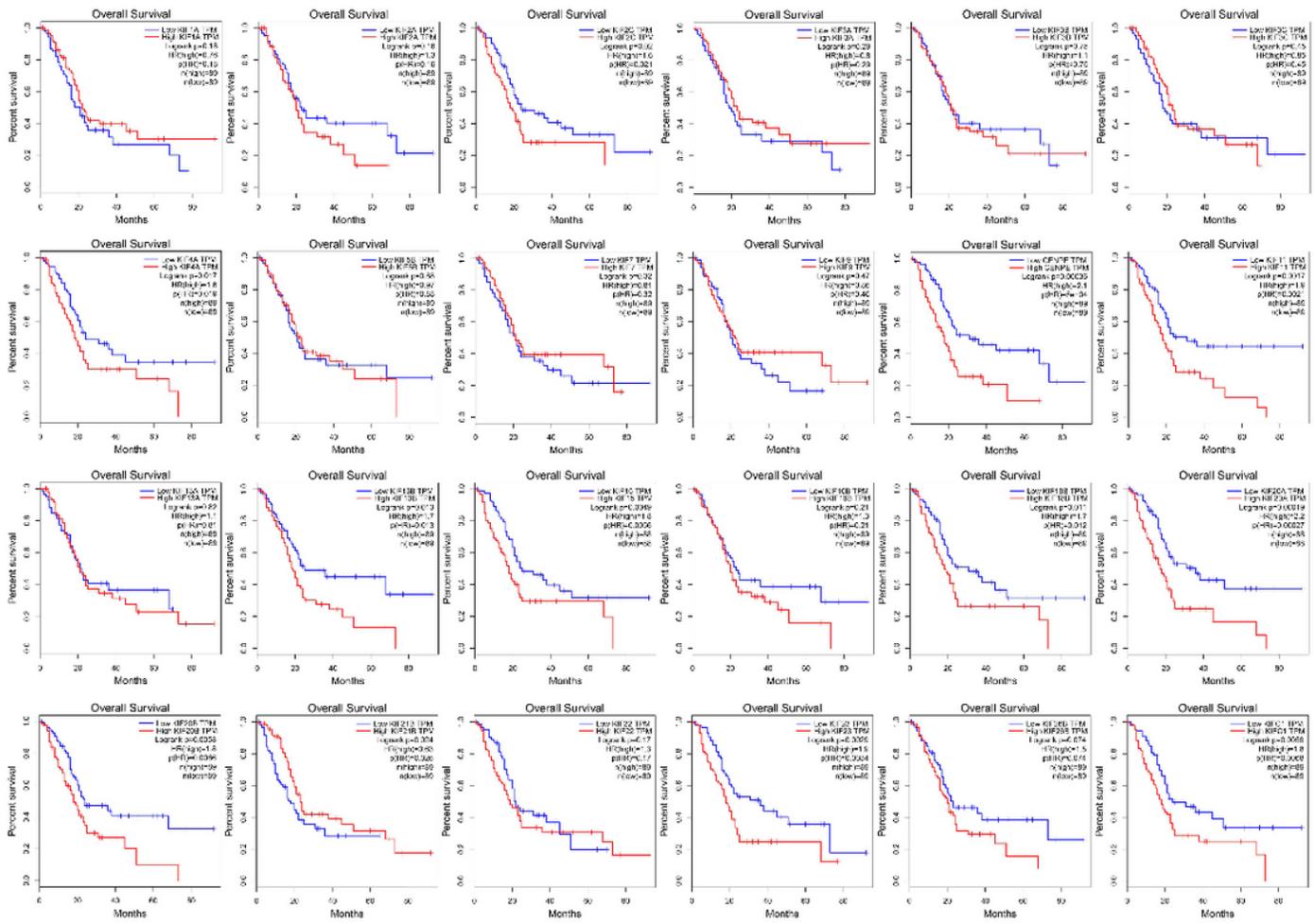
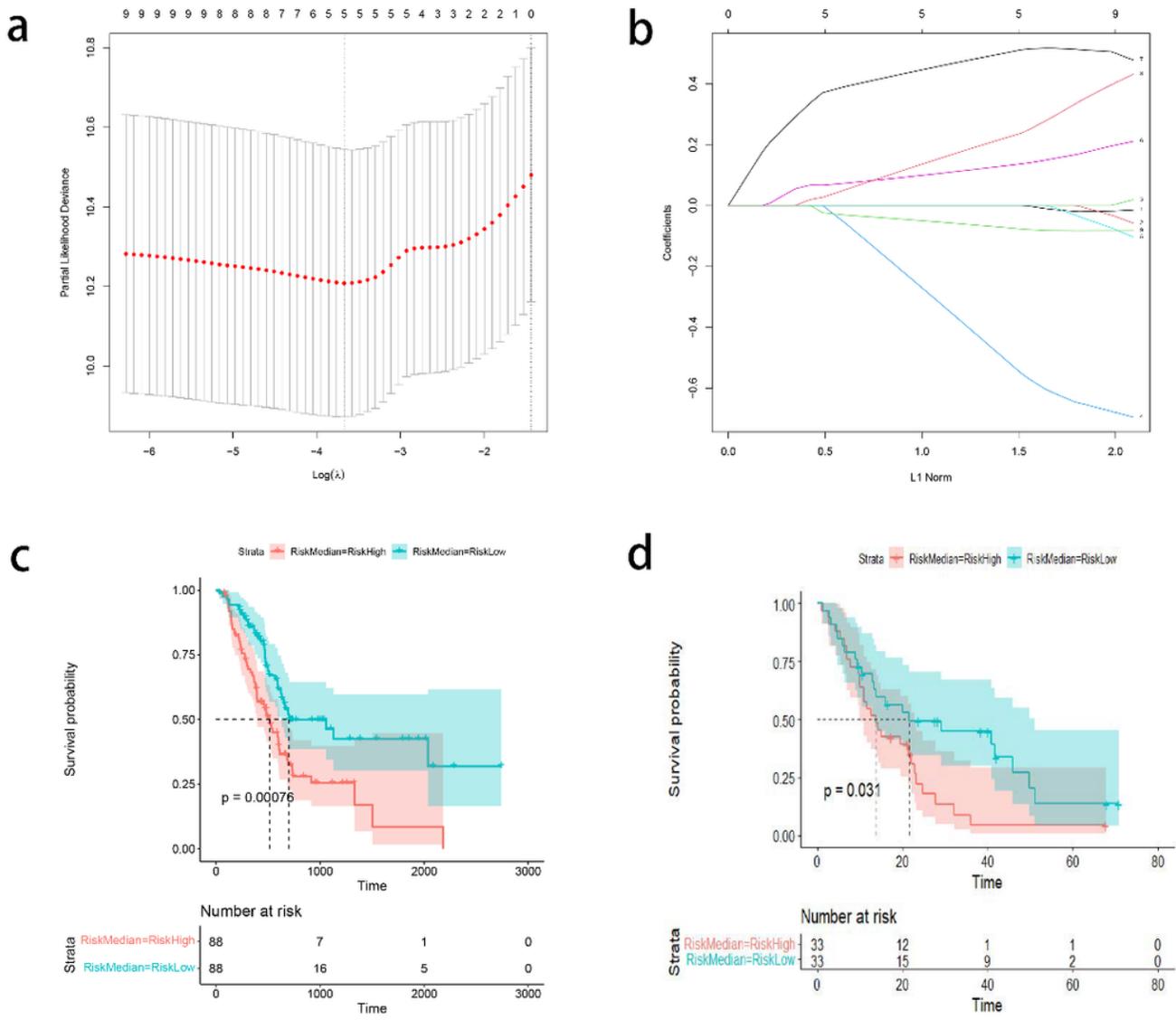


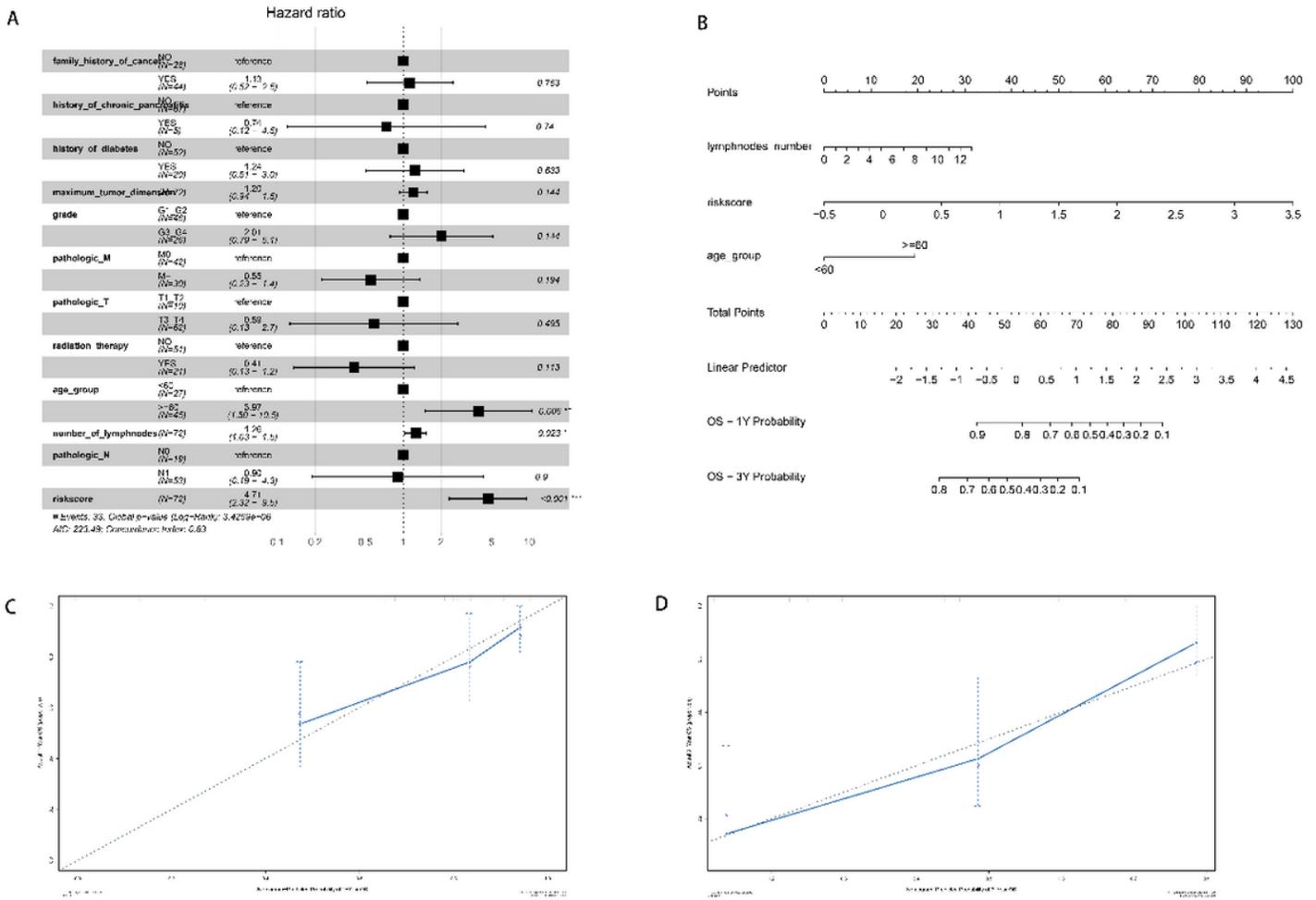
Figure 7

The prognostic value of different expressed KIFs in PAAD patients in the OS curve (Kaplan–Meier plotter).



**Figure 8**

LASSO regression of overall survival related KIFs. a Cvfit plot for the selection of LASSO regression lambda value as best cutoff. b LASSO regression of overall survival related KIFs (KIF2C, KIF4A, KIF11, KIF15, KIF18B, KIF20A, KIF20B, KIF23, KIF21B).c Validation of prognostic value of the 5-KIFs-based risk score generated by LASSO regression using survival analyses with TCGA-PAAD data. d Validation of prognostic value of the 5-KIFs-based risk score generated by LASSO regression using survival analyses with GEO dataset (GSE62452).



**Figure 9**

A. Multivariate survival analyses of clinical and pathologic information of PAAD patients. B. Nomogram constructed with pathologic information, clinical information and 5-KIFs-based risk score for prediction of 1/3-year OS of PAAD patients. C. Calibration plot done to self-validate the 1-year survival prediction efficacy of nomogram constructed. D. Calibration plot done to self-validate the 3-year survival prediction efficacy of nomogram constructed

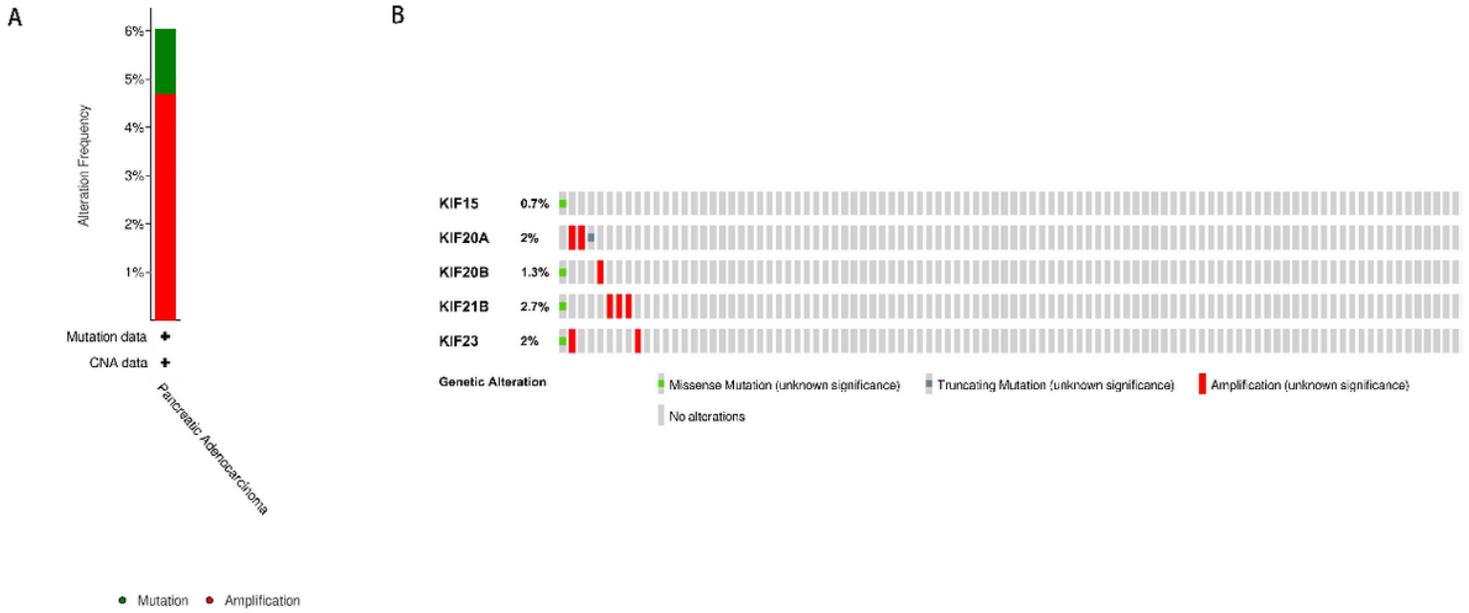


Figure 10

Summary of alterations in the 5-KIFs expressed in PAAD(A). OncoPrint visual summary of alteration on a query of the 5-KIFs family members(B).

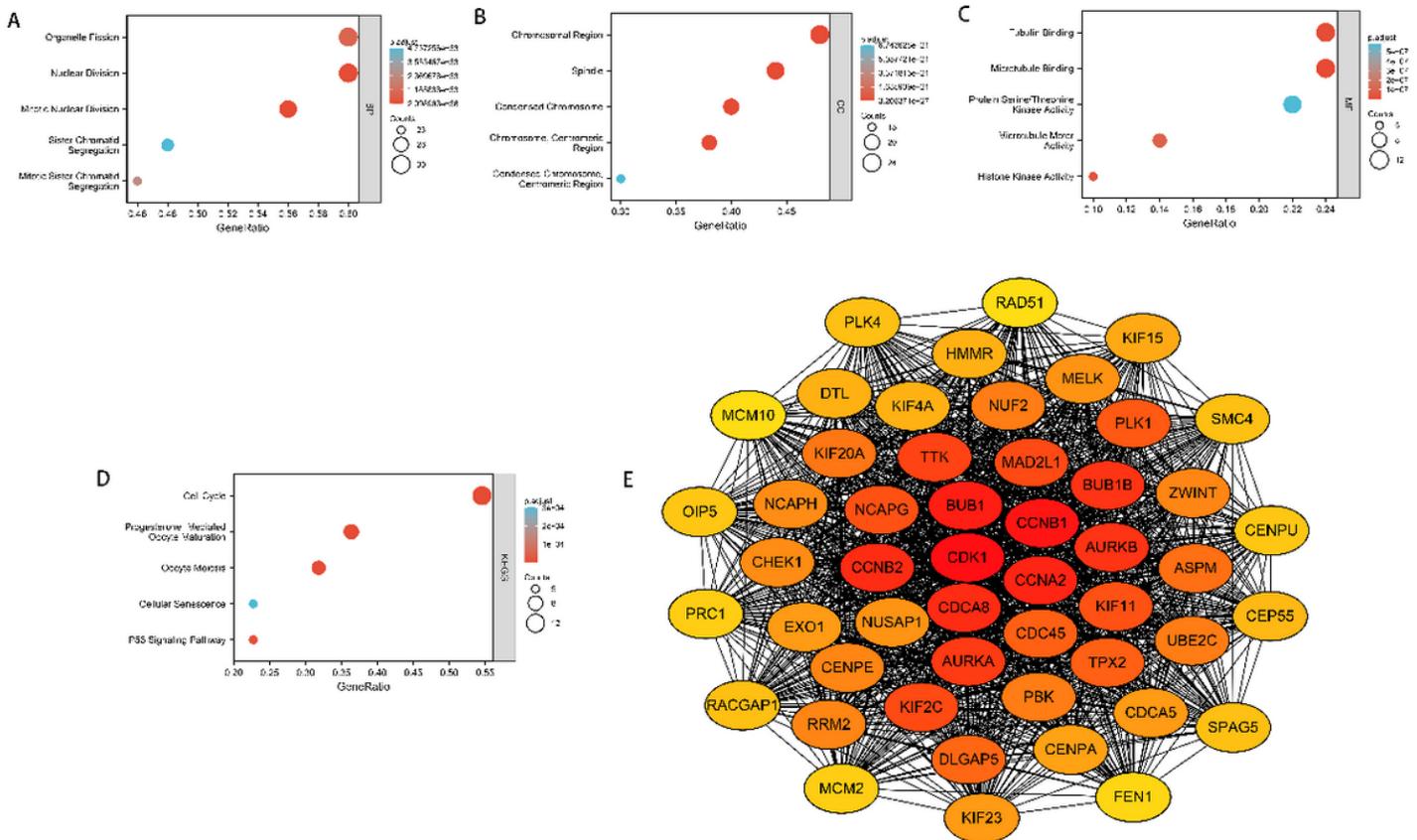


Figure 11

