

# DOCK4 Correlated with Immune Infiltration is a Valuable Prognostic Biomarker in Stomach Adenocarcinoma.

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## Research Article

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# Abstract

**Background:** Deducator for cytokinesis 4 (DOCK4), a guanine nucleotide exchange factor (GEF) for the small GTPase Rac1, the mechanisms involved in immune infiltration in STAD (stomach adenocarcinoma) remain unclear.

**Methods:** The UALCAN database was used to analyze the expression of the DOCK family, and the Kaplan-Meier plotter and GEPIA databases were used to assess the prognostic value of the DOCK family in STAD. Furthermore, the correlation between DOCK4 expression with tumor immune infiltration and the expression of related immune marker genes in STAD was explored using TIMER and GEPIA websites. Subsequently, the relationship between DOCK4 expression and clinical characteristics was verified using the UALCAN database. Finally, the mutation of DOCK4 was analyzed via TIMER2.0 and cBioPortal databases. And the protein-protein interaction (PPI) networks of DOCK4 were constructed using GeneMANIA and STRING websites.

**Results:** DOCK4 was finally screened out, and its expression in tumors was significantly evaluated relative to paracancerous tissues and had a negative impact on the prognosis of patients with STAD. DOCK4 was found significantly related to tumor immune infiltration in STAD.

**Conclusions:** In summary, DOCK4 is a potential regulator of the recruitment and regulation of immune infiltrating cells, thus becoming a valuable prognostic biomarker in STAD.

## Background

Gastric cancer is a common malignancy with a complex etiology worldwide, and despite its incidence and mortality rates have a decline, its burden remains high in many countries [1, 2]. The histological classification of gastric cancer mainly includes adenocarcinomas, adenosquamous carcinoma, squamous cell carcinoma, neuroendocrine carcinoma and undifferentiated carcinoma [3]. Adenocarcinomas account for approximately 90% of gastric cancer, and The World Health Organization (WHO) system has classified gastric adenocarcinoma into five histologic subtypes: tubular adenocarcinomas, papillary adenocarcinomas, mucinous adenocarcinomas, poorly cohesive carcinomas and mixed carcinomas [4]. Studies have shown that the histological classification of stomach adenocarcinoma can affect patient's survival outcomes but it remains limitations [5], as well as the American Joint Committee on Cancer (AJCC) TNM staging system [6]. So, we need to discover a new biomarker to improve the prognostic accuracy. However, incorporating the information of tumor-infiltrating neutrophils can improve the prognostic accuracy [7]. The tumor microenvironment (TME) is a complex mixture that contains a variety of cells and molecules, and most of them are reported to be related to tumor immune escape, growth and metastasis [8–10]. The development of immune checkpoint inhibitors (ICIs) has led to improved clinical outcomes in multiple solid tumors [11–13]. For stomach adenocarcinoma, the breakthrough of immune checkpoint inhibitors that block the cytotoxic T-lymphocyte antigen 4 (CTLA4) axis and the programmed death-1 (PD-1) axis provides patients with new treatment strategies [14]. However, immune checkpoint inhibitors such as PD-1 inhibitors are not sensitive to all GC patients [15]. Thus, the role of the tumor microenvironment in STAD is not completely understood. It is necessary to identify valuable prognostic biomarkers and immunotherapeutic targets for STAD.

DOCK family proteins are known to be activators of the Rho GTPases Rac and Cdc42. The DOCK proteins have 11 members, from DOCK1 to DOCK11 [16]. DOCK proteins, because of the similarity and difference of the

sequence and domain, are divided into four subgroups: DOCK-A, DOCK-B, DOCK-C, and DOCK-D[17]. DOCK family proteins are the most researched in cell adhesion and migration. In addition to cell adhesion and migration, interactions between the immune system and DOCK family members have also been reported. DOCK2 and DOCK8 are widely expressed in immune cells, and as activators of Rac and Cdc42 they are necessary for mediating cytotoxic function and the cell-cell adhesion of immune cells, respectively [16, 18, 19]. There are only a few studies on gastric cancer. DOCK1 might be the target of mycophenolic acid (MPA) in modulating gastric cancer cell migration[20]. DOCK5 could also be a chemosensitivity-related gene in gastric cancer [21]. DOCK6 plays a pivotal role in regulating cancer stem cells of gastric cancer[22] and promotes GC metastasis through the Rac1/Cdc42 axis[23]. DOCK4 belongs to the DOCK-B subgroup. It is an atypical guanine nucleotide exchange factor for Rac1, whose encoding-gene is located on chromosome 7[24]. A few studies have demonstrated a carcinogenic role in multiple tumors. Overexpressed DOCK4 promotes proliferation, migration and invasion of liver cancer[25]. lncRNA AC073284.4 suppresses cell invasion, metastasis and EMT of breast cancer via the miR-18b-5p/DOCK4 axis[26]. DOCK4 regulates the migration of breast cancer cells by combining with SH3YL1 to activate Rho GTPase Rac1[27]. Furthermore, DOCK4 promotes lung adenocarcinoma cell extravasation and metastasis through the TGF- $\beta$ /Smad pathway [28]. DOCK4, as a prognostic biomarker in ovarian cancer, has been shown to be associated with immune infiltration [29]. However, the correlation between the expression and prognosis of DOCK4 and stomach adenocarcinoma remains unknown.

In the present study, we comprehensively analyzed the expression and clinical prognosis of the DOCK family in STAD. Then, we screened out DOCK4 and explored the vital correlation of DOCK4 with the tumor microenvironment of STAD. These results revealed the prognostic value of DOCK4 in stomach adenocarcinoma and provided new evidence for the interaction of DOCK4 with tumor immunity.

## Methods

### UALCAN database analysis

UALCAN (<http://ualcan.path.uab.edu>.) aims to analyze the relative expression of genes as well as the correlation with diverse clinicopathologic features from the data of TCGA, MET500 and CPTAC [30]. This database was used to assess the expression level of the DOCK family in tumor tissues of STAD and paracancerous tissues, as well as the relationship between DOCK4 expression and clinical characteristics.

### Kaplan-meier Plotter Database Analysis

The Kaplan-Meier plotter (<http://kmplot.com/analysis/>) was used to evaluate the prognostic impact of 54,675 genes in 10,461 cancer samples from 21 cancer types[31]. This database was first used to explore the influence of DOCK family expression on the OS of patients with STAD, and to analyze the impact of clinicopathological factors and immune cells on the OS of STAD patients.

### Timer Database Analysis

TIMER (<https://cistrome.shinyapps.io/timer/>) comprehensively assesses the correlation between tumor-infiltrating immune cells (TIICs) and important tumoral genomic changes using six functional modules[32]. In

our study, the correlation between the expression of DOCK3 and DOCK4 with the degree of immune infiltration of B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells was analyzed using gene modules. Next, the tumor infiltration levels in different somatic copy number alterations were compared using the SCNA module. The relationship between DOCK4 expression and immune marker genes was explored using a correlation module. In addition, the mutation of DOCK4 in 34 cancer types was analyzed using gene mutation module in the TIMER2.0.

## Gene Correlation Analysis In Gepia

The online database Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/detail.php>) provides RNA sequencing expression data to analyze the expression differences between tumor and control samples in multiple cancer types[33]. This database was used to assess the OS based on the expression of DOCK4, and the link between this gene and the expression of particular immune-related marker genes was verified.

## Cbioportal Database Analysis

The cBioPortal (<http://cbioportal.org>) is a website for analyzing multidimensional cancer genomics data from cancer tissues and cells to providing the genetic, epigenetic, gene expression, and proteomic information for users[34]. The database was used to analyze the alterations of DOCK4 in stomach adenocarcinoma samples.

## String Database Analysis

The STRING database (<https://string-db.org/>) aims to collect and integrate the information of protein-protein interaction to constructing a comprehensive and objective global network for users[35]. The database was used to construct the PPI network of DOCK4. The Cytoscape software was used to visualize the results from the STRING database.

## Genemania Database Analysis

The GeneMANIA (<http://genemania.org>) is an open website that can analyze correlated genes and predict gene function and the interactions of them[36]. The database was used to analyze the correlated genes of DOCK4 and construct the PPI network.

## Statistical analysis

The hazard ratio (HR) and P-value of survival curves of OS were computed using the log rank test in the GEPIA and Kaplan-Meier Plotter databases. Spearman correlation was used to analyze the relationship between TIMER and GEPIA. The significance threshold was set at  $P < 0.05$ .

## Results

## Assessment of the expression level of DOCK family in STAD.

The mRNA expression of the DOCK family in STAD was first analyzed using the UALCAN database (Fig. 1). The expression of DOCK1 (Fig. 1A  $p < 0.01$ ), DOCK2 (Fig. 1B  $p < 0.01$ ), DOCK4 (Fig. 1D  $p < 0.001$ ), DOCK5 (Fig. 1E  $p < 0.001$ ), DOCK6 (Fig. 1F  $p < 0.001$ ), DOCK7 (Fig. 1G  $p < 0.001$ ) and DOCK10 (Fig. 1J  $p < 0.001$ ) were higher in tumor tissues than in paracancerous tissues, while DOCK3 (Fig. 1C  $p < 0.01$ ) and DOCK8 (Fig. 1H  $p < 0.01$ ) were lower. There was no statistically significant difference in DOCK9 (Fig. 1I) and DOCK11 (Fig. 1L).

## The Prognostic Value Of Dock Family In Stad

Next, genes that showed no statistical difference in expression was excluded, including DOCK9 and DOCK11. The impact of remaining DOCK family expression on OS in STAD was determined using the Kaplan-Meier plotter database. As shown in figure 2, overexpression of DOCK3 (Fig. 2C, HR=1.95, log rank  $P = 4.5e-05$ ) and DOCK4 (Fig. 2D, HR=1.57, log rank  $P = 0.016$ ) were associated with poor prognosis in patients with STAD. DOCK1 (Fig. 2A, HR=1.28, log rank  $P = 0.15$ ), DOCK2 (Fig. 2B, HR=1.28, log rank  $P = 0.17$ ), DOCK5 (Fig. 2E, HR=0.84, log rank  $P = 0.31$ ), DOCK6 (Fig. 2F, HR=0.81, log rank  $P = 0.26$ ), DOCK7 (Fig. 2G, HR=0.79, log rank  $P = 0.2$ ), DOCK8 (Fig. 2H, HR=0.86, log rank  $P = 0.38$ ) and DOCK10 (Fig. 2I, HR=1.29, log rank  $P = 0.13$ ) showed no statistically significant difference in OS. Furthermore, to further verify the GEPIA website, we reached a consistent conclusion. The details are provided in Supplementary figure 1. Therefore, these results suggest that DOCK3 and DOCK4 showed unfavorable prognosis in patients with STAD.

## Correlation analysis between DOCK3, DOCK4 expression and immune infiltration in STAD

The information reflected by the tumor microenvironment is related to the clinical prognosis [37, 38]. Thus, we used the TIMER database to assess the correlation between the expression of DOCK3 and DOCK4 and the level of immune infiltrates, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, dendritic cells and tumor purity (Fig. 3). DOCK3 expression was significantly related to B cells ( $r = 0.253$ ,  $P = 8.61e-07$ ), CD8+ T cells ( $r = -0.111$ ,  $P = 3.35e-02$ ), CD4+ T cells ( $r = 0.356$ ,  $P = 5.66e-13$ ) and macrophage cells ( $r = 0.308$ ,  $P = 1.45e-09$ ). DOCK4 was significantly positive with CD8+ T cells ( $r = 0.25$ ,  $P = 1.14e-06$ ), CD4+ T cells ( $r = 0.39$ ,  $P = 9.40e-15$ ), macrophage cells ( $r = 0.518$ ,  $P = 8.17e-27$ ), neutrophil cells ( $r = 0.375$ ,  $P = 7.96e-14$ ) and dendritic cells ( $r = 0.458$ ,  $P = 1.11e-20$ ), while it was negative with tumor purity ( $r = -0.143$ ,  $P = 5.33e-03$ ). Copy number variations (CNVA) predict the immune infiltration state and immunotherapy efficacy well [39]. Furthermore, we analyzed the association between copy number variations of DOCK3 and DOCK4 with the above six immune infiltrates (Fig. 4). The results revealed that the levels of B cells ( $p < 0.01$ ), CD8+ T cells ( $p < 0.001$ ), CD4+ T cells ( $p < 0.001$ ), macrophage cells ( $p < 0.001$ ), neutrophil cells ( $p < 0.001$ ) and dendritic cells ( $p < 0.001$ ) were relatively low, as indicated by the deletion of these genes. Above all, these results showed that DOCK4 plays a vital role in immune infiltration.

## Correlation Analysis Between Dock4 With Immune Marker Expression

To further investigate the underlying link between DOCK4 and immune infiltration, we next analyzed the correlation between DOCK4 expression and immune marker genes of numerous immune cells, including CD8+ T

cells, T cells (general), B cells, monocytes, tumor-associated macrophages (TAMs), M1 and M2 macrophages, neutrophils, natural killer cells and dendritic cells (DCs) in STAD using TIMER and GEPIA databases. We additionally analyzed T cells with different subsets, such as T helper 1 cells (Th1), T helper 2 cells (Th2), T helper 17 cells (Th17), follicular helper T cells (Tfh), regulatory T cells (Treg) and exhausted T cells. After adjustment for purity correlation, DOCK4 expression was significantly associated with most immune marker genes in STAD (Table 1). We noticed that the majority of markers of monocytes, TAMs, and M2 macrophages were strongly related to the expression of DOCK4, including monocyte markers (CD86, CD115) ( $r > 0.5$ ,  $P < 0.001$ ), TAM markers (IL10) ( $r > 0.5$ ,  $P < 0.001$ ) and M2 macrophage markers (CD163, VSIG4, MS4A4A) ( $r > 0.5$ ,  $P < 0.001$ ). This suggests that DOCK4 potentially regulates macrophage polarization in STAD. We also found that DC immune marker genes, such as HLA-DPB1, HLA-DRA, HLA-DPA1, BDCA-1, BDCA-4 and CD11c were also significantly related to DOCK4 expression ( $p < 0.001$ ). Strengthening Treg cell function and reducing CD8+T cell cytotoxicity is the main mechanism by which DCs promote the metastatic phenotype of tumors[40], which also reveals the potential function of DOCK4. For Treg cells, there was a significant relationship between FOXP3, TGF $\beta$ , CCR8 and STAT5B with DOCK4 ( $p < 0.001$ ). In addition, DOCK4 was positively related to T cell depletion genes, such as PD-1, CTLA4, LAG3 and TIM-3 ( $p < 0.001$ ).

Table 1  
Correlation analysis between DOCK4 and relate genes and markers of immune cells in TIMER.

Description	Gene markers	STAD			
		None		Purity	
		Cor	P	Cor	P
B cell	CD19	0.265	***	0.247	***
	CD79A	0.236	***	0.204	***
Tcell(general)	CD3D	0.330	***	0.314	***
	CD3E	0.341	***	0.329	***
	CD2	0.425	***	0.420	***
CD8+T cell	CD8A	0.371	***	0.356	***
	CD8B	0.271	***	0.262	***
Monocyte	CD86	0.534	***	0.526	***
	CD115(CSF1R)	0.607	***	0.596	***
TAM	CCL2	0.387	***	0.361	***
	CD68	0.275	***	0.234	***
	IL10	0.561	***	0.548	***
M1 Macrophage	INOS(NOS2)	0.092	0.061	0.083	0.109
	IRF5	0.241	***	0.225	***
	COX2(PTGS2)	0.305	***	0.293	***
M2 Macrophage	CD163	0.621	***	0.604	***
	VSIG4	0.508	***	0.502	***
	MS4A4A	0.577	***	0.567	***
Neutrophils	CD66b(CEACAM8)	0.166	***	0.183	***
	CD11b(ITGAM)	0.523	***	0.509	***
	CCR7	0.461	***	0.456	***
Natural killer cell	KIR2DL4	0.122	*	0.094	0.068
	KIR2DL3	0.262	***	0.252	***
	KIR3DL3	0.065	0.183	0.078	0.128

STAD, stomach adenocarcinoma; Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell; TAM, tumor associated macrophage; NK, natural killer cell; DC, dendritic cell; None, correlation without adjustment; Purity, correlation adjusted for tumor purity; Cor, R value of Spearman's correlation.

\*P<0.05 \*\*P<0.01 \*\*\*P<0.001

Description	Gene markers	STAD			
	KIR3DL2	0.285	***	0.285	***
	KIR2DS4	0.187	***	0.179	***
	KIR2DL1	0.292	***	0.301	***
	KIR3DL1	0.253	***	0.252	***
Dendritic cell	HLA-DPB1	0.319	***	0.292	***
	HLA-DQB1	0.199	***	0.161	**
	HLA-DRA	0.304	***	0.285	***
	HLA-DPA1	0.290	***	0.263	***
	BDCA-1(CD1C)	0.394	***	0.379	***
	BDCA-4(NRP1)	0.704	***	0.693	***
	CD11c(ITGAX)	0.587	***	0.566	***
Th1	T-bet (TBX21)	0.396	***	0.396	***
	STAT4	0.563	***	0.572	***
	STAT1	0.324	***	0.315	***
	IFN- $\gamma$ (IFNG)	0.214	***	0.209	***
	TNF- $\alpha$ (TNF)	0.196	***	0.168	**
Th2	GATA3	0.332	***	0.329	***
	STAT6	0.211	***	0.204	***
	IL13	0.092	0.061	0.108	*
	STAT5A	0.519	***	0.518	***
Tfh	BCL6	0.490	***	0.472	***
	IL21	0.267	***	0.256	***
Th17	STAT3	0.574	***	0.562	***
	IL17A	0.000	0.995	-0.001	0.978
Treg	FOXP3	0.390	***	0.362	***
	CCR8	0.501	***	0.497	***
	TGF $\beta$ (TGFB1)	0.440	***	0.425	***

STAD, stomach adenocarcinoma; Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell; TAM, tumor associated macrophage; NK, natural killer cell; DC, dendritic cell; None, correlation without adjustment; Purity, correlation adjusted for tumor purity; Cor, R value of Spearman's correlation.

\*P<0.05 \*\*P<0.01 \*\*\*P<0.001

Description	Gene markers	STAD			
	STAT5B	0.616	***	0.613	***
T cell exhaustion	PD-1(PDCD1)	0.319	***	0.312	***
	CTLA4	0.468	***	0.466	***
	LAG3	0.257	***	0.229	***
	TIM-3(HAVCR2)	0.527	***	0.510	***
	GZMB	0.203	***	0.165	**
STAD, stomach adenocarcinoma; Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell; TAM, tumor associated macrophage; NK, natural killer cell; DC, dendritic cell; None, correlation without adjustment; Purity, correlation adjusted for tumor purity; Cor, R value of Spearman's correlation.					
*P<0.05 **P<0.01 ***P<0.001					

A consistent conclusion was shown in the GEPIA database after analyzing the above markers. These immune marker genes, including monocyte markers (CD86,CD11) ( $r>0.5$ ,  $P<0.001$ )(Fig. 5A), TAM marker(IL10)( $r>0.5$ ,  $P<0.001$ )(Fig. 5B), M2 macrophage marker(MS4A4A)( $r>0.5$ ,  $P<0.001$ )(Fig. 5C), dendritic cell markers(BDCA-4, CD11c)( $r>0.5$ ,  $P<0.001$ )(Fig. 5D), Treg marker(STAT5B)( $r>0.5$ ,  $P<0.001$ ) (Fig. 5E), T cell exhaustion marker(TIM-3)( $r>0.5$ ,  $P<0.001$ ) (Fig. 5F), neutrophils cell marker(CD11b)( $r>0.5$ ,  $P<0.001$ )(Fig. 5G), Th1 marker(STAT5A) ( $r>0.5$ ,  $P<0.001$ )(Fig. 5H) and Th17 marker(STAT3)( $r>0.5$ ,  $P<0.001$ )(Fig. 5I), were strongly correlated to DOCK4 expression. These results indicate that DOCK4 plays a key factor in the immune escape of the tumor microenvironment in STAD.

### Prognostic analysis of DOCK4 expression in STAD based on immune cells

We previously analyzed the association between DOCK4 expression and the tumor microenvironment of STAD. We continued to explore the prognosis of STAD based on immune cells, including basophils, B cells, CD4+ memory T-cells, CD8+ T-cells, eosinophils, macrophages, mesenchymal stem cells, natural killer T-cells, regulatory T-cells, type 1 T-helper cells and type 2 T-helper cells, using the Kaplan-Meier plotter website(Table 2).The results revealed that high expression of DOCK4 in decreased status of basophils(HR=1.68,  $P=0.021$ ), CD8+ T-cells(HR=1.96,  $P=0.022$ ), macrophages(HR=1.95,  $P=0.013$ ), mesenchymal stem cells(HR=1.9,  $P=0.013$ ), natural killer T-cells(HR=2.29,  $P=0.0018$ ), type 1 T-helper cells(HR=1.89,  $P=0.0038$ ) played a poor prognostic value. Furthermore, the overexpression of DOCK4 also had an unfavorable impact on the OS in enriched immune cell subtypes including B-cells(HR=1.89,  $P=0.0042$ ), CD4+ memory T-cells(HR=1.84,  $P=0.0069$ ), CD8+ T-cells(HR=1.62,  $P=0.033$ ), eosinophils (HR=1.78,  $P=0.021$ ), macrophages(HR=1.73,  $P=0.021$ ), regulatory T-cells(HR=1.81,  $P=0.015$ ) and type 2 T-helper cells(HR=1.51,  $P=0.033$ ).

Table 2  
Kaplan-Meier plotter to analyze the overall survival on the expression of DOCK4 based on immune cell subgroups in STAD.

Cell Types	STAD					
	Enriched			Decreased		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
Basophils	n=86	1.61(0.63-4.11)	0.32	n=303	1.68(1.07-2.62)	0.021
B-cells	n=204	1.89(1.21-2.93)	0.0042	n=165	1.61(0.86-3.02)	0.14
CD4+ memory T-cells	n=222	1.84(1.17-2.88)	0.0069	n=147	1.48(0.91-2.4)	0.12
CD8+ T-cells	n=186	1.62(1.04-2.55)	0.033	n=183	1.96(1.09-3.54)	0.022
Eosinophils	n=277	1.78(1.09-2.92)	0.021	n=92	1.6(0.8-3.18)	0.18
Macrophages	n=193	1.73(1.08-2.76)	0.021	n=176	1.95(1.14-3.34)	0.013
Mesenchymal stem cells	n=150	1.45(0.78-2.73)	0.24	n=219	1.9(1.13-3.17)	0.013
Natural killer T-cells	n=237	1.28 (0.81-2)	0.29	n=132	2.29(1.34-3.91)	0.0018
Regulatory T-cells	n=272	1.81(1.11-2.93)	0.015	n=97	1.52(0.78-2.96)	0.21
Type1T-helper cells	n=155	1.6 (0.95-2.68)	0.074	n=214	1.89(1.22-2.93)	0.0038
Type 2 T-helper cells	n=344	1.51(1.03-2.22)	0.033	n=25	2.98(0.62-14.39)	0.15

## Correlation Between Dock4 Expression And Clinical Characteristics Of Stad

Based on the prognostic value of DOCK4 in STAD, we further analyzed the relationship between DOCK4 expression and clinical characteristics in the UALCAN database. It is known that DOCK4 expression is higher in tumor tissues than in paracancerous tissues. The results showed that DOCK4 expression was elevated in cancer stage 3 relative to stage 2 ( $p < 0.01$ ) and stage 1 ( $p < 0.05$ ) (Fig. 6A). DOCK4 expression was lower in the 81-100 years age group than 61-80 years age group (Fig. 6D,  $p < 0.05$ ). Based on tumor grade, the expression of this gene in grade 3 was excessive to grade 2, as well as grade 3 to grade 1 (Fig. 6E,  $p < 0.001$ ). For TP53 mutation status, the DOCK4 expression of the TP-53 non-mutated subtype was higher than that of the TP53-mutated subtype (Fig. 6G,  $p < 0.05$ ). There was no statistical significance in the subgroups of race, gender, H. pylori infection status

and nodal metastasis status of STAD patients. Next, in the stratified prognostic analysis of DOCK4 expression in STAD ,the upregulated DOCK4 had a negative impact on OS in clinical stage 3 (OS,P-VALUE=0.025) and clinical stage 4 (OS,P-VALUE=0.036) using the Kaplan-Meier database(Table 3).Furthermore, DOCK4 also played an unfavorable role in the following clinicopathological characteristics: gender of female (OS, P-value =0.00035), race of white (OS, P =0.01) and mutation burden (OS, P =0.046)(Table 3).There was no statistically significant difference between the subgroups of the other clinicopathological characteristics(p>0.05)(Table 3).These results suggest that DOCK4 expression potentially influences the prognosis of STAD patients with advanced clinical stages.

Table 3  
Kaplan-Meier plotter to determine the effect of different clinicopathological factors on the expression of DOCK4 gene and clinical prognosis in STAD.

Clinicopathological characteristics	Overall survival (n = 371)			Clinicopathological characteristics	Overall survival (n = 371)		
	N	Hazard ratio	P-value		N	Hazard ratio	P-value
Gener				Mutation burden			
female	n=133	2.82(1.56-5.09)	0.00035	high	n=186	1.45(0.87-2.41)	0.15
male	n=238	1.3 (0.84-2.01)	0.24	low	n=182	1.85 (1-3.43)	0.046
Stage				Grade			
1	n=50	2.37 (0.51-11.05)	0.26	1	/		
2	n=111	1.44 (0.68-3.01)	0.34	2	n=134	1.79(0.95-3.39)	0.069
3	n=149	2.12 (1.08-4.15)	0.025	3	n=218	1.45(0.95-2.21)	0.08
4	n=38	2.58 (1.03-6.47)	0.036	4	/		
Race							
white	n=237	1.99 (1.16-3.4)	0.01				
asian	n=73	2.33 (0.9-6.03)	0.072				

## Mutation Analysis Of Dock4

The mutation profile of DOCK4 was analyzed using the TIMER2.0 and cBioPortal databases. The mutation of DOCK4 was rare in most cancers from 34 TCGA cancer types, which was ranging from 0.02% (1 of 500 THCA patients) to 11% (60 of 531 UCEC patients) (Fig. 7A) in the TIMER2.0 database. And the mutation rate of DOCK4 in STAD patients was 7% (31 of 439), which was similar to the result from the cBioPortal database that was ranging from 5.44% (8 of 147) to 6.36% (28 of 440) (Fig. 7B) (Table S1). In addition, the amplification rate of DOCK4 was ranging from 1.36% (4/295) to 2.72% (4/147), and the rate of deep deletion was from 0.23% (1/440) to 0.68% (1/447) (Fig. 7B) (Table S1).

## Ppi Networks Construction Analysis Of Dock4

The PPI networks of DOCK4 was constructed using the GeneMANIA website. The results revealed a list of correlated genes for DOCK4. The correlated genes were enriched in the pathways as follows: Rho GTPase binding, regulation of smooth muscle cell migration, smooth muscle cell migration, positive regulation of smooth muscle cell migration, regulation of vascular associated smooth muscle cell migration, vascular associated smooth muscle cell migration and muscle cell migration (Fig. 8A). In addition, the data of PPI network of DOCK4 from STRING website was visualized in the Cytoscape software (Fig. 8B). DOCK4 was interacted with ribosomal proteins such as RPL6, RPL24 and RPL18, and ribosomal protein subunit proteins such as RPS27A, RPS25, RPS29 and RPS15.

## Discussion

Stomach adenocarcinoma (STAD) is the fifth most common malignant tumor and ranks third in cancer mortality worldwide[1]. In recent years, with the improvement of treatment methods of stomach adenocarcinoma including surgical treatment, neoadjuvant radiotherapy, chemotherapy and targeted therapy, the 5-year survival rate of patients with early diagnosis and treatment has increased, but the therapeutic effect in advanced/metastatic patients remains unsatisfactory [41].The possible development mechanism of stomach adenocarcinoma involves the malignancy of cancer cells and various immune cells in the tumor-related microenvironment [42]. The development of immune checkpoint inhibitors is beneficial for patients with advanced/metastatic stomach adenocarcinoma. For example, pembrolizumab prolongs the overall survival (OS) and reduces adverse effects of treatment in patients with advanced stomach adenocarcinoma[43]. The combination of nivolumab and ipilimumab is effective for chemotherapy-refractory gastroesophageal adenocarcinoma[14]. However, the incomplete validity of immune checkpoint inhibitors in GC patients has become a new challenge[15, 44]. Thus, in order to optimize individualized treatment strategies, we aimed to explore new immunotherapeutic-related molecules for STAD. There are 20 Rho GTPases in the human genome, especially Rac1 and Cdc42, which are reported more frequently and have an important influence on cell proliferation, differentiation, motility, adhesion, survival and secretion[45]. As Rho GTPase activators, the DOCK family was first identified as the exchange factor of Rac(1/2/3) and Cdc42 more than ten years ago[16].Thus, the DOCK family plays an important role in carcinogenesis ,central nervous and immune system diseases through the activation of Rho GTPases [46, 47].DOCK4,as a member of the DOCK family, is mostly a qualified atypical Rho GTPase GEF, which leads to the development of physiological processes and diseases that act as activators of Rca1. DOCK4 plays an important role in neurite differentiation, erythropoiesis and vascular smooth muscle cell (VSMC) migration[48–51]. For tumors, DOCK4 mostly promotes the migration, invasion and EMT of cancer cells[25–27]. However, no study has reported the interaction between DOCK4 and the tumor

microenvironment of STAD. In this study, we found that high DOCK4 expression was a poor prognostic factor for STAD. DOCK4 expression was significantly related to the expression of different immune-related markers, highlighting its possible role in STAD immunity, making it a valuable biomarker for this cancer.

We first found that the expression of most DOCK family members was higher than that of control samples using the UALCAN database (Fig. 1). Based on their prognostic value in the Kaplan-Meier Plotter and GEPIA databases, we selected DOCK3 and DOCK4, both of which had an unfavorable impact on the OS of STAD (Fig. 2). DOCK3 is specifically expressed in neurons and participates in the development of Alzheimer's disease[52]. Furthermore, Twist1 promotes EMT through the NEDD9/DOCK3/Rac axis in head and neck squamous cell carcinoma[53]. DOCK3 drives tumor cell adhesion, migration, and invasion by regulating Rac1 activity in lung cancer [54]. Besides metastasis, the relationship between immune infiltration and the two genes in STAD was explored via TIMER and GEPIA websites. There was only a weak relationship between DOCK3 and B cells, CD8+ T cells, CD4+ T cells and macrophages (Fig. 3A). However, DOCK4 was positively correlated with CD8+ T cells, CD4+ T cells, macrophage cells, neutrophil cells, and dendritic cells, while it was negatively correlated with tumor purity (Fig. 3B). In addition to copy number variations, deletion of DOCK4 was significantly associated with lower levels of six immune cells (Fig. 4). Thus, DOCK4 is closely related to the tumor microenvironment of STAD. Although the structure of DOCK4 has high homology with DOCK3, it can also be detected as a secreted protein in peripheral blood [29]. The function of chemokines in the migration of lymphocytes depends on the activation of DOCK2 and the mutations in DOCK8 also cause functional defects of the immune system, which proves that DOCK2 and DOCK8 are necessary regulators of the immune system[55, 56]. Therefore, this suggests that DOCK4 might have a particular correlation with immune infiltration, similar to DOCK2 and DOCK8. Thus, we further verified the link between the expression of immune-related gene markers and this gene. There was a significant correlation between DOCK4 and diverse marker genes of immune infiltrating cells, including monocytes, TAMs, M2 macrophages, DCs, Tregs, and exhausted T cells in the TIMER database. M1 (anti-tumor) and M2 (pro-tumor) phenotypes represent different functional states[57]. DOCK4 was weakly associated with M1 macrophage markers (IRF5, COX2) and strongly associated with M2 macrophage markers (CD163, VSIG4, MS4A4A) (Table 1). These results revealed that DOCK4 participates in macrophage polarization. The high level of immune infiltration of Treg cells is related to the poor prognosis of cancer patients, and FOXP3 participates in the regulation of differentiation of regulatory T cells, thus suppressing the anti-tumor immune response[58]. PD-1, CTLA4, Tim-3, and LAG3 are vital markers of exhausted T cells, which represent a poor response to antigen-mediated TCR stimulation[59]. There was a significant correlation between DOCK4 expression and Treg cell markers (FOXP3, TGF $\beta$ , CCR8, STAT5B) and T cell exhaustion markers (PD-1, CTLA4, TIM-3, LAG3) (Table 1). These results provided evidence that elevated DOCK4 levels suppressed the anti-tumor immune response of clinical patients with STAD. NRP1(DC marker) was strongly related to DOCK4, which was determined to be a prognostic factor for STAD[60]. Furthermore, the T helper cells infiltration is conducive to prognostic prediction[61]. And the significant link between DOCK4 expression and several markers of T helper cells (Th1, Th2, Tfh, Th17) increased the accuracy of survival prognosis prediction of STAD patients (Table 1). Based on the homologous data from TCGA, we analyzed the above markers in the GEPIA database and obtained similar results (Fig. 5). Furthermore, we revealed that DOCK4 expression had an impact on OS in different stages of immune infiltration (Table 2). The decreased status of basophils, CD8+ T-cells, macrophages, mesenchymal stem cells, natural killer T-cells and type 1 T-helper cells, which is consistent with our conclusion. However, overexpressed DOCK4 also showed poor prognosis in enriched status of B-cells, CD4+ memory T-cells, CD8+ T-cells, eosinophils, macrophages, regulatory T-cells and type 2 T-helper cells, which revealed the complexity of the

immune system. The environment of immune infiltration, possibly as an upstream regulator of DOCK4, participates in the development process of STAD. Furthermore, according to the analysis of The Cancer Genome Atlas (TCGA) genomic subtypes, the EBV-positive stomach adenocarcinoma is rich in the infiltration of CD8+ T-cells [14] and DOCK4 is related with the OS of STAD patients with enriched status of CD8+ T-cells, thus decreasing DOCK4 expression improves individualized treatment of stomach adenocarcinoma. The prognosis of patients with STAD in the same classification and staging remains significant difference [4], and the molecular subtypes that are divided by TCGA can well distinguish patients and provide personalized therapy. DOCK4 expression might enhance the prognostic accuracy of STAD to well predict the survival outcomes of patients. Immunotherapy appears to prolong the life of patients with multiple cancers [62, 63]. DOCK4 is not only a therapeutic target. Decreasing DOCK4 expression improves patient prognosis of STAD, and the combination with other immune checkpoint inhibitors may be a new treatment strategy for partial patients, such as those with poor treatment effects of PD-1 inhibitors.

Finally, we analyzed the correlation between DOCK4 levels and the clinical characteristics of STAD (Fig. 6). Combining the current research, we noticed some significant characteristics. The TP53-mutated subtype had a lower degree of immune infiltration than the TP53-wildtype subtype in GC [64], and DOCK4 expression was low in the TP53-mutated subtype. The high expression of DOCK4 was associated with poor prognosis in the low mutation burden of STAD. Although a high mutation burden may indicate a good treatment response to cancers [65, 66], kidney renal clear cell carcinoma is another immunotherapeutically responsive tumor with moderate mutation burden [67]. In our study, the prognostic significance of low mutation burden is possibly related to the regulation of TP53 mutations. The most common genetic alteration in cancer is the DNA copy number alterations (CNAs). The results of genetic alterations in the DOCK4 showed that the copy gain frequency of DOCK4 accounts for 2.7% of STAD samples, which may explain the significant upregulation of DOCK4 in STAD tissues from UALCAN. DOCK4 interacted with the ribosomal related proteins (Fig. 8B). The ribosomal proteins are reported to participate in the development of cancers and the mutation of them is related with the deregulation of the p53 tumor suppressor network [68]. Thus, it provides further evidence for the correlation between DOCK4 and p53 in STAD. We also observed that DOCK4 expression was higher in advanced subtypes of cancer stages and grades. Both the TGF- $\beta$ /Smad pathway, which is an important signaling pathway of the metastatic phenotype and the transcription factor c-MAF, which is the key factor of bone metastasis, are related to DOCK4 expression [28, 69]. And the correlated genes of DOCK4 are enriched in regulating cell migration pathways (Fig. 8A). This might be the potential mechanism by which DOCK4 participates in metastasis in STAD, but it was necessary for experimental verification. Reducing the high mortality of advanced/metastatic patients with STAD is a complicated problem [70]. However, DOCK4 expression had an impact on the prognosis of OS in STAD patients with advanced clinical stages (Table 3). Systemic chemotherapy is an effective method for treating advanced gastric cancers [71]. DOCK4 is associated with immune infiltration as a platinum-chemosensitive gene in ovarian cancer [29]. In addition, DOCK4 plays an unfavorable prognosis by regulating the immune escape of the tumor microenvironment in STAD, which potentially as a target of platinum chemosensitivity, provided new treatment strategies for advanced/metastatic patients.

## Conclusions

Our study revealed the crucial role of DOCK4 in tumorigenesis and the development of STAD. Above all, as a new prognostic factor of STAD, DOCK4 plays a vital role in immune infiltration and is a valuable molecule for

immunotherapy.

## Abbreviations

DOCK4: Deducator for cytokinesis 4

STAD: stomach adenocarcinoma

TME: tumor microenvironment

HR: hazard ratio

OS: overall survival

ICI: immune checkpoint inhibitors

TIMER: Tumor Immunoassay Resource

GEPIA: Gene Expression Profiling Interactive Analysis

PPI: protein-protein interaction

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

All data supporting the findings of this study are available within the article. These data come from public databases.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

LZ and LY designed and performed the research, analyzed data, and wrote the manuscript; YJX, DQP and DY participated in data preparation, analysis, and figure preparation. All authors have read and approved the

manuscript for publication.

## Acknowledgements

Not applicable.

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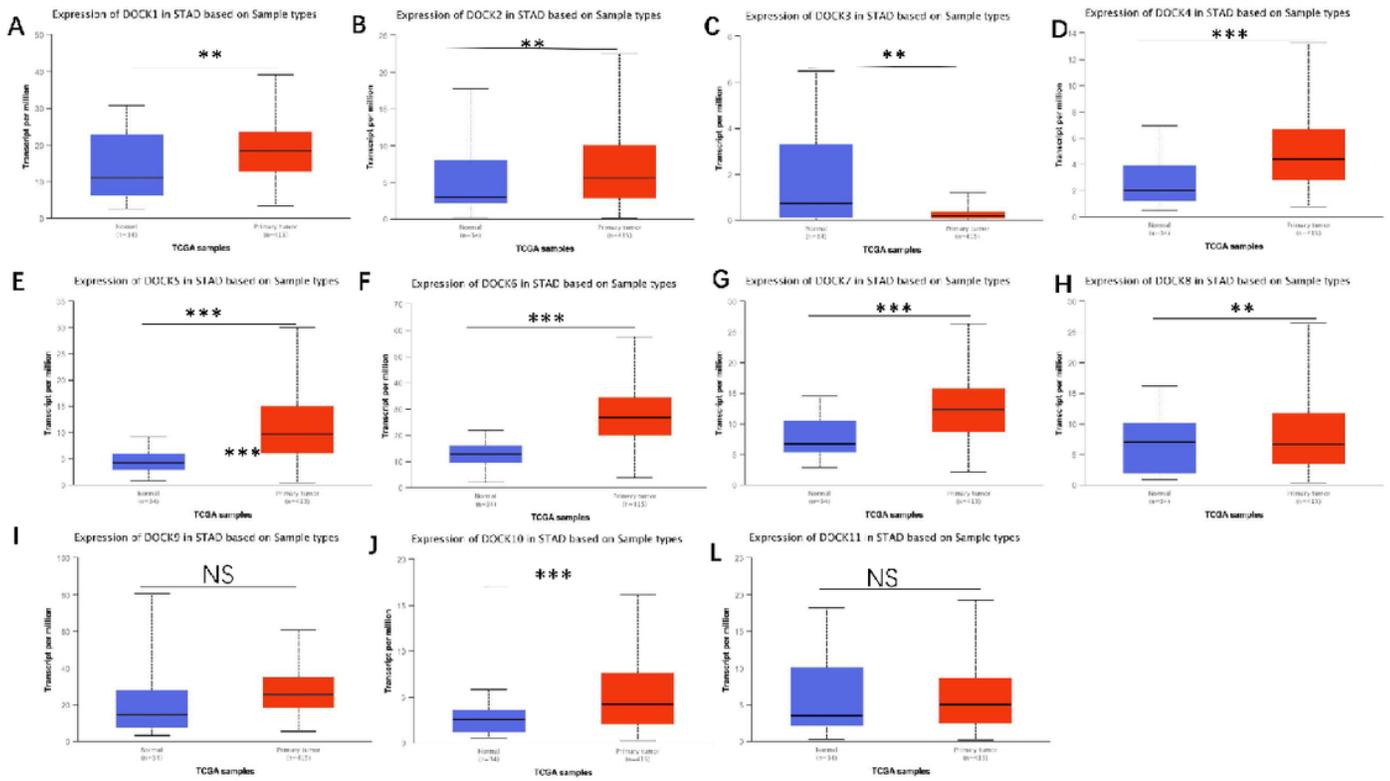
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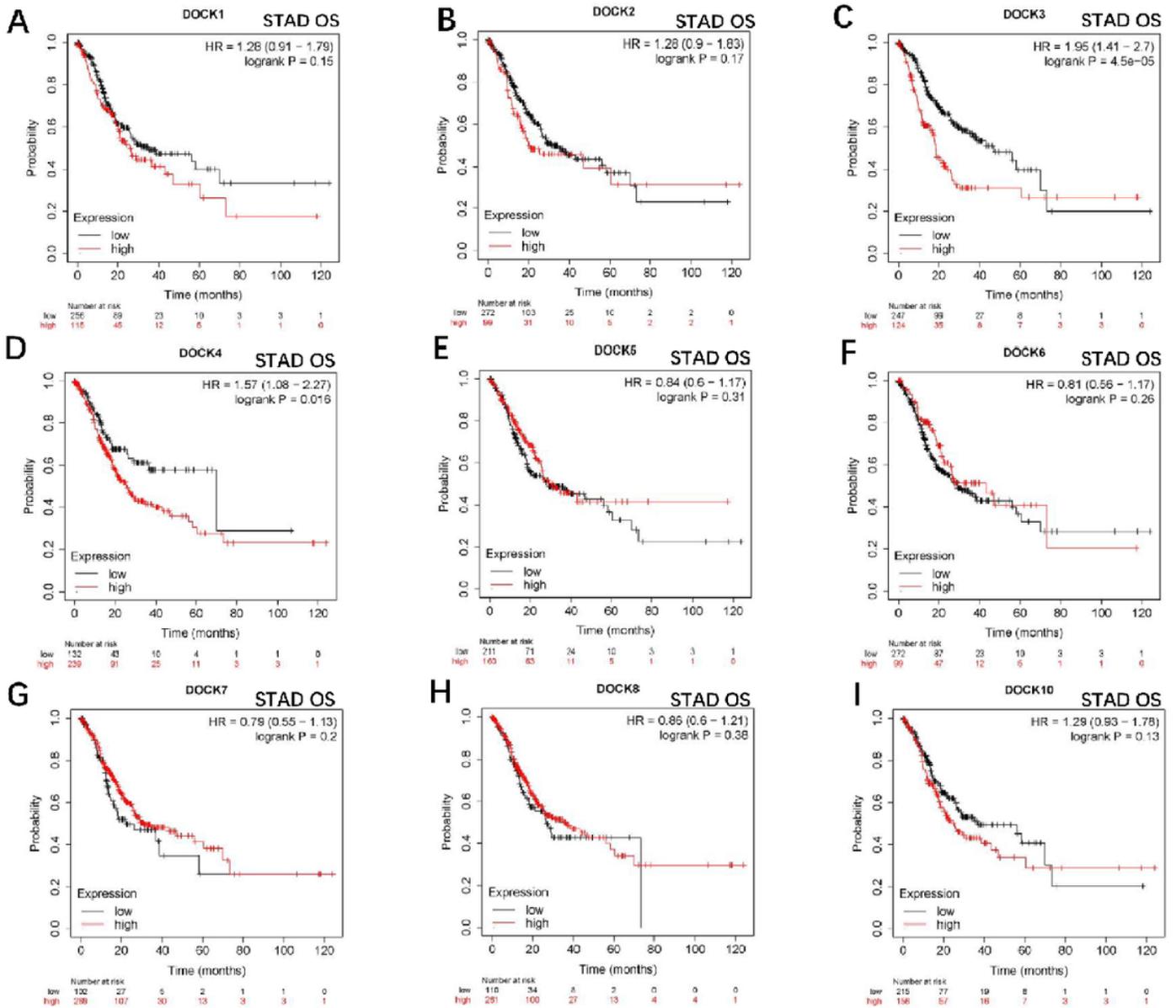
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## Figures



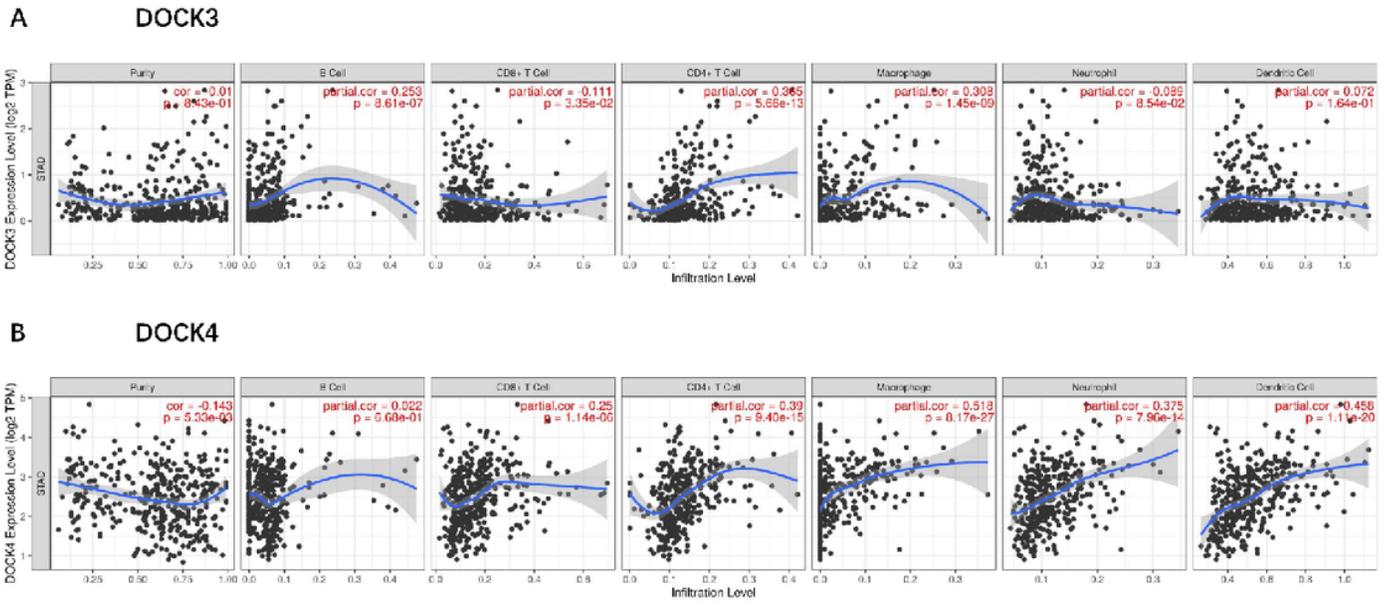
**Figure 1**

The expression of DOCK family in stomach adenocarcinoma (STAD) and paracancerous tissues via UALCAN database. (A) DOCK1, (B) DOCK2, (C) DOCK3, (D) DOCK4, (E) DOCK5, (F) DOCK6, (G) DOCK7, (H) DOCK8, (I) DOCK9, (J) DOCK10, (K) DOCK11 (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) NS, not statistically significant.



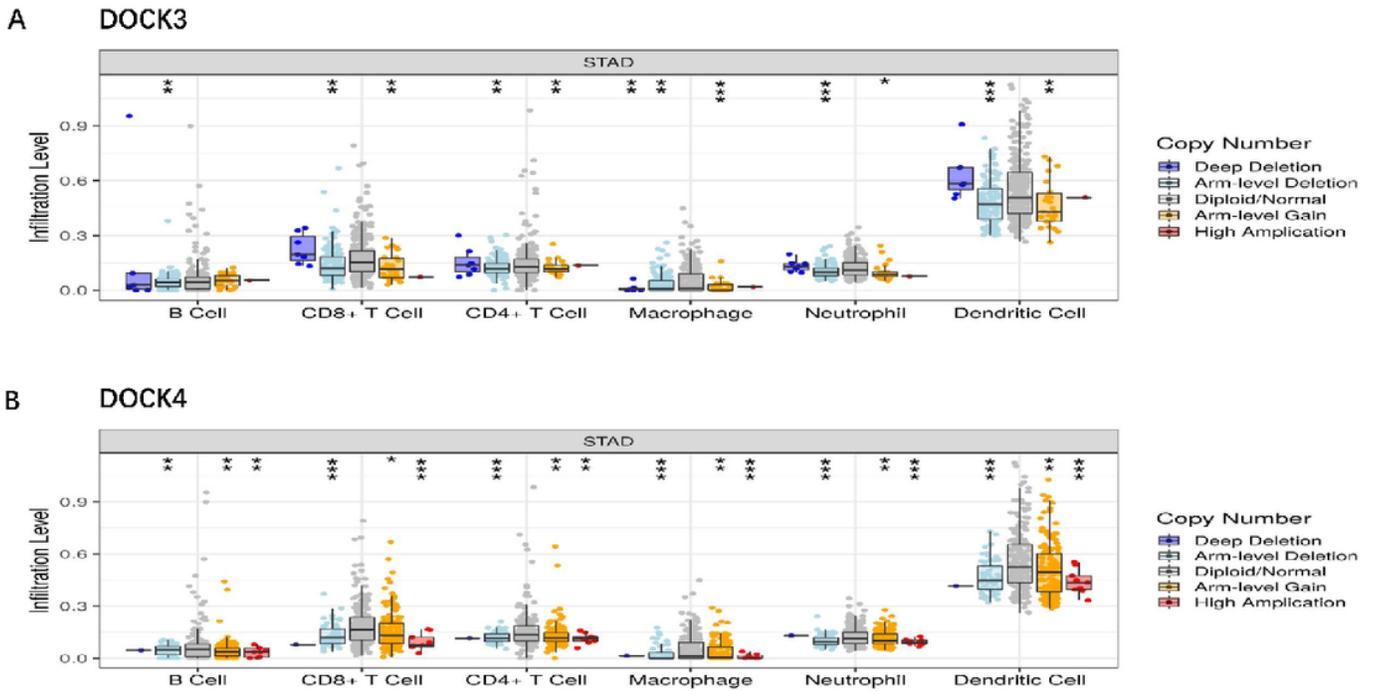
**Figure 2**

Comparison of Kaplan-Meier survival curves of DOCK family in STAD. High DOCK3 expression(C) and DOCK4 expression(D) had unfavorable OS in STAD(n=371). The expression of DOCK1(A), DOCK2(B), DOCK5(E), DOCK6(F), DOCK7(G), DOCK8(H), DOCK10(I) had no significance with OS. HR, hazard ratio; OS, overall survival.



**Figure 3**

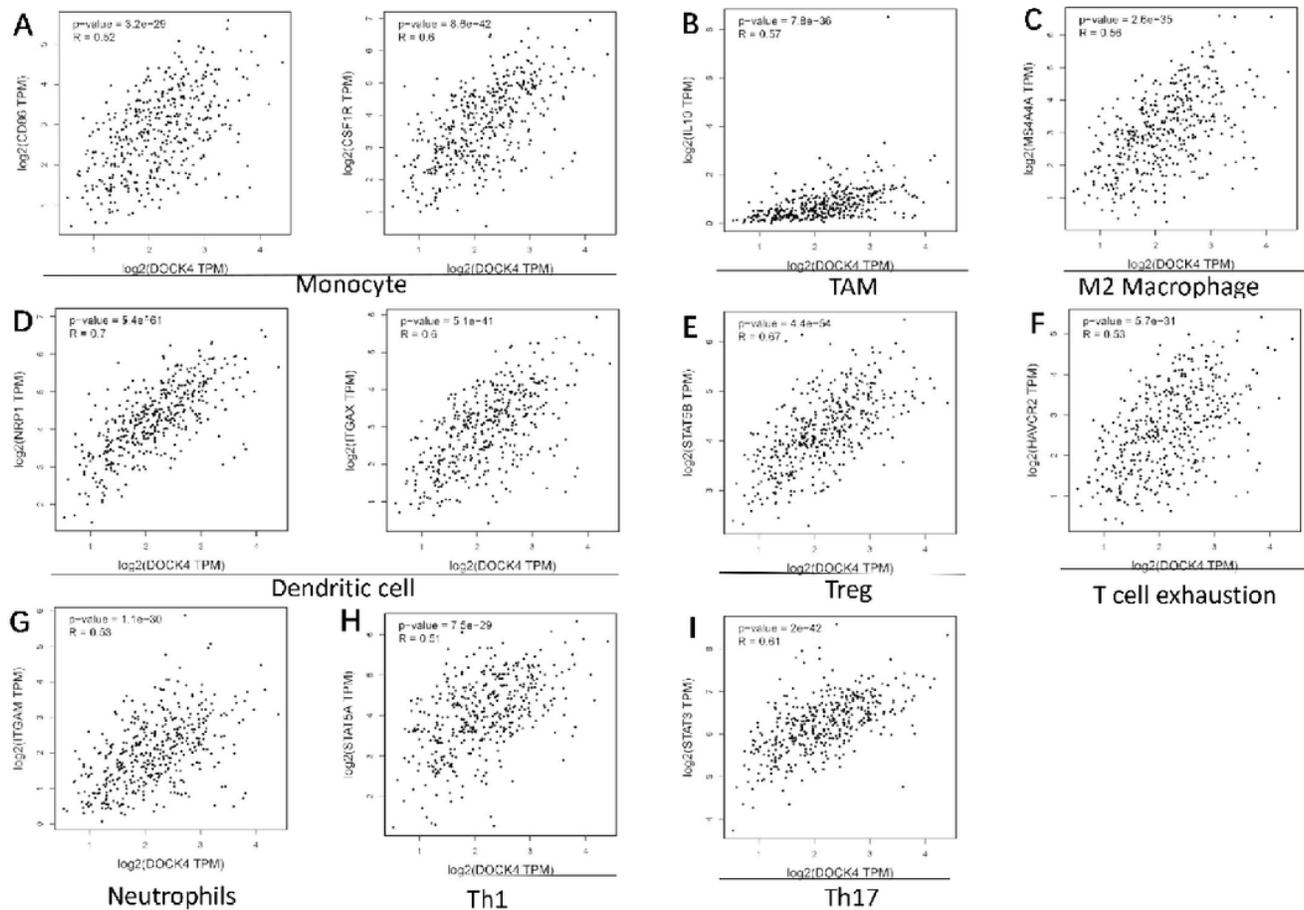
Correlation of DOCK4 and DOCK3 expression with immune infiltration level in STAD. The correlations between DOCK3 (A) and DOCK4 (B) expression with tumor purity and infiltrating levels of B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil and dendritic cell in STAD.



**Figure 4**

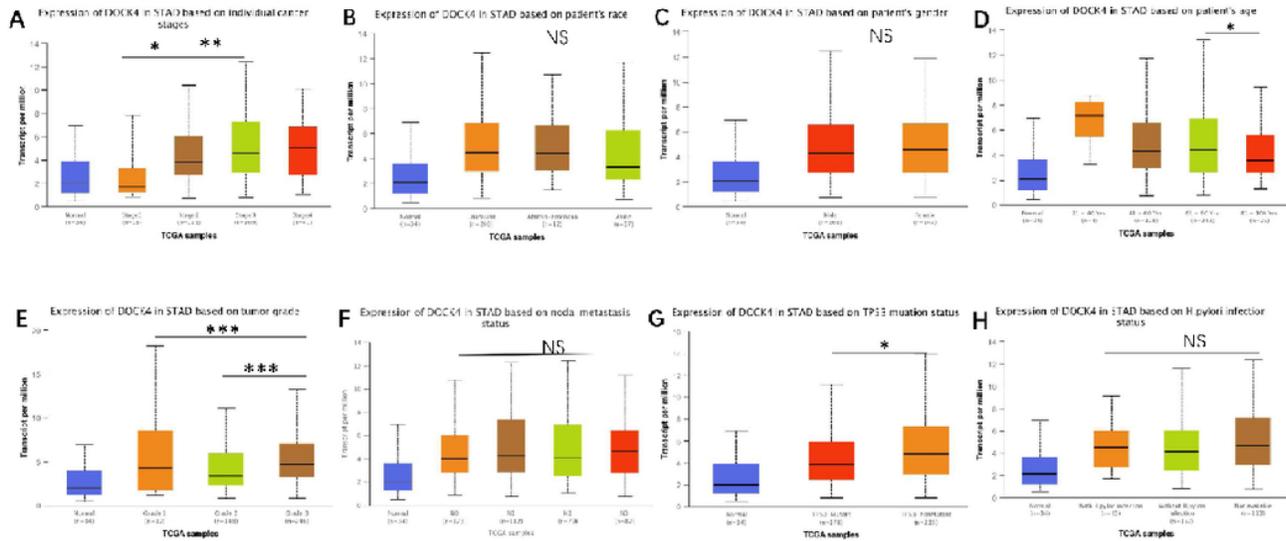
Correlation of somatic copy number variations of DOCK3 and DOCK4 with immune infiltration level in STAD. The correlations between the somatic CNA of DOCK3 (A) and DOCK4 (B) with six immune infiltrating levels of B cell,

CD8+ T cell, CD4+ T cell, macrophage, neutrophil and dendritic cell in STAD.



**Figure 5**

DOCK4 expression correlated with partial immune marker genes in STAD. Markers include (A) CD86 and CD115 of Monocyte; (B) IL10 of TAM; (C) MS4A4A of M2 macrophages; (D) BDCA-4(NRP1) and CD11c(ITGAX) of DCs; (E) STAT5B of Treg; (F) TIM-3(HAVCR2) of T cell exhaustion; (G) CD11b(ITGAM) of Neutrophils; (H) STAT5A of Th1; (G) STAT3 of Th17.



**Figure 6**

Correlations between DOCK4 expression with clinical characteristics in STAD.(A) DOCK4 expression in individual cancer stages of STAD; (B) DOCK4 expression in the race of STAD patients; (C) DOCK4 expression in the gender of STAD patients; (D) DOCK4 expression in the age of STAD patients; (E) DOCK4 expression in tumor grade of STAD; (F) DOCK4 expression in nodal metastasis status of STAD; (G) DOCK4 expression in TP-53 mutation status of STAD; (H) DOCK4 expression in H.pylori infection status of STAD. (\* $P < 0.05$  \*\* $P < 0.01$  \*\*\* $P < 0.001$ ) NS, not statistically significant.



## Supplementary Files

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