PLAGL2 Is a Prognostic Biomarker in Stomach cancer：A Comprehensive Study Based on Bioinformatics and Experiments

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Abstract

Background

Polymorphic adenoma-like protein 2 (PLAGL2), a zinc finger protein, has been linked to the advancement of serval-type malignancies. However, the relevance of PLAGL2 to the prognosis and regulatory networks of different cancers remains unclear.

Methods

The expression of PLAGL2 was explored through Oncomine, TIMER and SangerBox websites. The relationship between PLAGL2 expression and prognosis in various cancers was analyzed through the Kaplan-Meier Plotter database and the PrognoScan databases. The expression of PLAGL2 in several immunological and molecular subtypes of human cancer was evaluated through the TISIDB database. The differentially expressed genes associated with PLAGL2 was explored through LinkedOmics database. The relationship between PLAGL2 expression and clinicopathological features in STAD was explored through the UALCAN database. The expression of PLAGL2 in STAD specimens was analyzed through western blot and IHC. The function of PLAGL2 in STAD was explored through the CCK8 test and the colony formation test.

Results

In this study we found that PLAGL2 was overexpressed in most types of cancer and overexpression of PLAGL2 might predicted a poor prognosis in STAD. Next, we investigated PLAGL2 expression in several immunological and molecular subtypes and found that PLAGL2 expression differs considerably across immunological subtypes and molecular subtypes of most cancer types. Our research also shows that the expression of PLAGL2 is correlated with various immunostimulatory and immunosuppressive cytokines. We also analyzed the PLAGL2 co-expression network, and evaluated the prognostic potential of genes positive co-expression with PLAGL2 in STAD through Kaplan-Meier plotter. The results show that most of co-expression genes have a significant effect on the prognosis. We further designed experiments to explore the function of PLAGL2 in STAD. Consistent with previous studies, PLAGL2 was significantly overexpressed in STAD tissues compared with that of adjacent normal tissues. And PLAGL2 can promote the proliferation of STAD cells both in vivo and in vitro.

Conclusions

Our findings showed that data mining successfully identifies PLAGL2 expression and putative regulatory networks in STAD, laying the groundwork for additional research into the function of PLAGL2 in carcinogenesis.

Background

Polymorphic adenoma-like protein 2 (PLAGL2), a zinc finger protein, is upregulated in several malignancies[1–5]. Like its related gene PLAGL1, PLAGL2 functions as an oncogene in a variety of malignancies. For example, PLAGL2 could promote the development of lung adenocarcinoma [6, 7]. What's more, acting as a transcription factor, PLAGL2 could promote the development of hepatocellular carcinoma through HIF-1alpha signaling pathway[5]. PLAGL2 can also promote the proliferation and migration of colorectal cancer[8–12]. At the same time, our studies showed that PLAGL2 suppresses cell migration and proliferation in Hirschsprung's disease[13], and it also increases colon cancer growth via binding to the MYH9 promoter[14]. PLAGL2 could also promote the development stomach cancer by promoting the deubiquitination of Snail1 protein[15]. According to previous research, we can make a conclusion that PLAGL2 is a unique proto-oncogene in cancer growth, invasion, and metastasis.

Here we explored the expression and prognosis of PLAGL2 in various cancers through different databases. We also analyzed the functional network related to PLAGL2 in STAD. In addition, we also evaluated the association of PLAGL2 with tumor infiltrating immune cells. In this study we revealed for the first time the relationship between PLAG2 and tumor immune interaction. Finally, we further explored the function of PLAGL2 in STAD cells, and we found that PLAGL2 could promote the proliferation of STAD cells. In conclusion, our findings might lead to the development of new targets and techniques for the diagnosis and treatment of STAD.

Materials And Method

The Oncomine database

We analyzed the expression levels of PLAGL2 in tumors and normal tissues of various cancer types thorough the Oncomine database (https://www.oncomine.org/resource/login.html)[16].

The TIMER database

We analyzed the expression of PLAGL2 from various malignancies in TCGA through the TIMER database (https://cistrome.shinyapps.io/timer/)[17]. The association between PLAGL2 expression and tumor infiltrating immune cell gene markers was also investigated through the TIMER database[18-20]. Gene expression levels were visualized using log2 RSEM.

The SangerBox website

We analyzed the expression of PLAGL2 in TCGA and GTEx through the SangerBox website (http://sangerbox.com/Tool). The association between PLAGL2 expression and immune checkpoint genes or infiltrating immune cells were also investigated through the SangerBox website[21].

The Kaplan-Meier Plotter database

We investigated the prognostic value of PLAGL2 in human cancers and the genes positive co-expression of PLAGL2 in STAD through the Kaplan-Meier Plotter database (http://kmplot.com/analysis/)[22].

The PrognoScan databases

We investigated the prognostic value of PLAGL2 in human cancers through the PrognoScan databases (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html)[23].

The TISIDB database

We investigated the association between PLAGL2 expression and immunological or molecular subtypes of different cancer types through the TISIDB database (http://cis.hku.hk/TISIDB/index.php)[24].

The LinkedOmics database

The differentially expressed genes associated with PLAGL2 was explored through LinkedOmics database (http://www.linkedomics.org/login.php). The results were statistically analyzed using Pearson correlation coefficients. Then, the analysis of GO (CC, BPand MF), KEGG pathway was measured through the WebGestalt[25].

The UALCAN database

The relationship between PLAGL2 expression and clinicopathological features in STAD was explored through the UALCAN database (http://ualcan.path.uab.edu)[26].

Cell culture

MKN45 and 7901 cells were cultured in RPMI-1640 supplemented with 10% FBS. All cells were maintained in a 5% CO2 humidified atmosphere at 37°C.

Western blotting

The western blotting was conducted as we have published elsewhere. The antibodies usedincludedPLAGL2 (1:1000, Proteintech), GAPDH (1:1000, Proteintech)[14].

Cell proliferation assay

The cell proliferation assay was conducted as we have published elsewhere[14].

Colony-formation assay

The colony-formation assay was conducted as we have published elsewhere[14].

Immunohistochemical (IHC) staining

The IHC staining was conducted as we have published elsewhere[14].

Xenograft subcutaneous implantation model

Forthexenograft subcutaneous implantation model, 7901 cells were subcutaneously injected into nude mice. After 25 days of normal feeding, all the mice were sacrificed and the tumor volume was measured every 3 days.

Statistics

Statistical analysis was conducted using SPSS and GraphPad. The data were expressed as means ± standard deviation. All the experiments were repeated at least three times. A P value less than 0.05 was considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001).

Results

The expression of PLAGL2 in human cancers

First of all, in order to analyze the expression level of PLAGL2 in various types of cancer, the Oncomine database was applied (Figure.1A). The results indicate that PLAGL2 is significantly overexpressed in various type cancers including stomach cancer. Then, we also evaluated the expression of PLAGL2 through the TIMER database which include RNA-sep data from various malignant tumors in TCGA (Figure.1B). We can find that PLAGL2 was overexpressed in most malignant tumors. Finally, the SangerBox website was used to analyzed the expression of PLAGL2 in TCGA and GTEx (Figure.1C). We can find that PLAGL2 was obviously overexpressed in most types of cancer. The above data indicate that PLAGL2 is overexpressed in most cancer types and may play an important role in the development of various types of cancer.

Prognostic potential of PLAGL2 in cancers

Firstly, we investigated the prognostic potential of PLAGL2 in cancers through the PrognoScan. The results indicated that PLAGL2 might participate in the prognosis of breast cancer and colorectal cancer. However, as shown in the results, high PLAGL2 expression is slightly related to a better prognosis in colorectal cancer (Figure.2A-2C), and the prognosis of PLAGL2 in breast cancer is not consistent (Figure.2D-2F).

So, we further explore the prognostic value of PLAGL2 through the Kaplan-Meier plotter. We can find that high PLAGL2 expression is slightly related to a poor prognosis in stomach cancer (Figure.2G-2I). However, high PLAGL2 expression is probably related to a better prognosis in ovarian (Figure.2J-2L) and breast (Figure.2M-2O) cancer. The results above indicated that PLAGL2 might be a prognostic biomarker in stomach cancer. Then we further explored the association between the expression of PLAGL2 and the clinical characteristics of STAD through the Kaplan-Meier plotter (Table 1). We can find that high expression of PLAGL2 related to both poorer OS and PFS in female, male, HER2 negative and HER2 positive in STAD. Specifically, high expression of PLAGL2 related to both poorer OS and PFS in STAD patients belonging to stages 2, stage N1+2+3, stage N1 and stage M0.

Relationship between PLAGL2 expression and immune and molecular subtypes in human cancers

Next, we further analyzed the relationship between PLAGL2 expression and immune and molecular subtypes in human cancers through the TISIDB website. We can find that there was a clearly relationship between PLAGL2 and different subtypes of UCEC, BRCA, CESC, COAD, HNSC, KIRC, KIRP, LGG, LUSC, TGCT and LIHC (Figure.3). In addition, the expression of PLAGL2 is also related to different cancer molecular subtypes in various cancers (Figure.3S). We may deduce from the aforementioned findings that PLAGL2 may play an important role in the immunological and molecular subtypes of many malignancies.

Relationship between PLAGL2 expression and immune checkpoint (ICP) genes in human cancers

Immune cell infiltration and immunotherapy have both been shown to be significantly influenced by ICP genes[27]. The relationship between PLAGL2 expression and ICP genes in human malignancies was then investigated (Figure.4). The results indicated that PLAGL2 expression is associated to immune checkpoint genes in a range of malignancies.

Relationship between PLAGL2 and immune cell infiltration in human cancers

We further investigated the possible association between PLAGL2 and immune cell infiltration, we can find that there is a substantial correlation in numerous cancer types (Figure.5). PLAGL2 expression is related to dendritic cells in 19 cancers, macrophages in 14 cancers, neutrophils in 23 cancers, CD8+ T cells in 14 cancers, and B cells in 20 cancers. In 15 cancers, there is a strong correlation between CD4+ T cells. Then, we also explored the relationship between the expression of PLAGL2 and different immune marker genes in STAD (Table 2). We can find that there exist a significantly relationship between the expression level of PLAGL2 and most immune markers in various immune cells in STAD.

The regulation network of PLAGL2 in stomach cancer

Previous results in this study we have demonstrated that PLAGL2 was overexpressed in STAD, and predicted a poor prognosis. So, we further explored the regulation network of PLAGL2 in stomach cancer. Firstly, As shown in the volcano map (Figure.6A-6C), we explored the co-expression genes of PLAGL2 in STAD through LinkedOmics. The differentially expressed genes associated to PLAGL2 are mostly involved in cell cycle control, according to the results of GO term analysis (Figure.6E-6G). KEGG pathway analysis revealed the enrichment of cell cycle, Staphylococcus aureus infection, basic transcription factors, complement and coagulation cascade(Figure.6H).

Prognostic potential of PLAGL2 co-expression genes in stomach cancers

Then, we further evaluated the prognostic potential of the genes positive co-expression with PLAGL2 in STAD through Kaplan-Meier plotter (Figure.7, Figure.7S). The results showed that among the top 40 genes co-expressed with PLAGL2 in STAD, most of the genes were related to the prognosis of STAD.

Relationship between PLAGL2 and different clinical subgroups in STAD.

Next, the expression of PLAGL2 in STAD with different clinical characteristics was explored through UALCAN database. The results indicated that there existed an significantly difference between the expression of PLAGL2 in different STAD patients' gender (Figure.8A), age(Figure.8B), tumor grade(Figure.8C), lymph node metastasis status(Figure.8D), cancer stage(Figure.8E), and Helicobacter pylori infection status(Figure.8F), indicating that PLAGL2 may act as an important oncogene in the progress of STAD.

The function of PLAGL2 in STAD cells

Finally, to verify the association between PLAGL2 and STAD clinicopathological characteristics, we performed Western blot and IHC to detect PLAGL2 expression in 57 paraffin-embedded STAD specimens(Figure.9A-9C). we can find that the PLAGL2 was overexpressed in STAD tissues. More importantly, our findings reveal that the PLAGL2 is associated to lymph node metastasis and tumor size in STAD(Table 3). Then, we used a lentivirus-based system to establish stable PLAGL2 knockdown 7901 and MKN-45 cell lines (Figure.9D-9F). Next, the results of CCK8 test(Figure.9G) and the colony formation test(Figure.9H-9I) revealed that PLAGL2 could promote the proliferation ability of STAD cells. Finally, the results of the xenograft subcutaneous transplantation model showed that PLAGL2 could promote the growth of STAD cells in vivo (Figure.9J-9L). In summary, the data above supports the conclusion that PLAGL2 is an oncogene in STAD and promotes the proliferation of STAD cells in vitro and in vivo.

Discussion

Previous studies have demonstrated that PLAGL2, acts as a transcription factor, might participate in the progression of various cancers[8, 9, 11, 12, 28]. Our previous studies have shown that PLAGL2 suppresses cell migration and proliferation in Hirschsprung's disease[13], and it also increases colon cancer growth via binding to the MYH9 promoter[14]. PLAGL2 could promote the development stomach cancer by promoting the deubiquitination of Snail1 protein[15]. In addition, PLAGL2 could also promote the development of CRC through the Wnt signaling pathway[29]. However, the relationship between PLAGL2 and immunotherapy has not been reported. Here, we explored the expression and prognosis of PLAGL2 in various cancers, and further investigated the relationship between PLAGL2 and immune infiltration for the first time. These studies indicate that PLAGL2 might act as a prognostic biomarker and target for anti-tumor immunotherapy in human cancers.

Firstly, we explored the expression of PLAGL2 through Oncomine, TIMER and SangerBox websites. Consistent with previous studies, the results showed that PLAGL2 was overexpressed in most types of cancer. These results indicate that PLAGL2 does promote the occurrence and development of human cancer.

Then, we investigated the relationship between PLAGL2 expression and prognosis in various cancers. We can find that overexpression of PLAGL2 might predicted a poor prognosis in STAD, which proves that

PLAGL2 may serve as a potential prognostic biomarker.

Following that, we investigated PLAGL2 expression in several immunological and molecular subtypes of human cancer to evaluate its probable biochemical pathway. The findings revealed that PLAGL2 expression differs considerably across immunological subtypes and molecular subtypes of most cancer types, suggesting that PLAGL2 is a viable diagnostic pan-cancer biomarker that plays a role in immune regulation. Furthermore, we demonstrated that the expression of PLAGL2 varies significantly across clinical subgroups. PLAGL2 is differently expressed in most malignancies with various clinical features, indicating that PLAGL2 may have a role in tumor development and progression.

Previous researches have shown that tumor infiltrating lymphocytes (TIL) in TME might act as an independent predictor of the prognosis of cancer patients and the effect of immunotherapy[30, 31]. Our research shows that the expression of PLAGL2 is correlated with various immunostimulatory and immunosuppressive cytokines, which prove evidence for the potential immune function of PLAGL2.

We also analyzed the PLAGL2 co-expression network, and evaluated the prognostic potential of genes positive co-expression with PLAGL2 in STAD through Kaplan-Meier plotter. The results show that most of co-expression genes have a significant effect on the prognosis, which further indicates that PLAGL2 could applied as a prognostic biomarker for STAD.

Finally, we further designed experiments to explore the function of PLAGL2 in STAD. Consistent with previous studies, PLAGL2 was significantly overexpressed in STAD tissues compared with that of adjacent normal tissues. And PLAGL2 can promote the proliferation of STAD cells both in vivo and in vitro.

However, this work has certain limitations, despite the fact that we did a thorough and systematic examination of PLAGL2 and used many databases for cross-validation. First, there are discrepancies between microarray and sequencing data from different databases, as well as a lack of granularity and specificity, which might contribute to system bias. Secondly, although in vivo/in vitro experiments have been carried out and proved the role of PLAGL2 in STAD, its regulatory mechanism needs to be further explored. Third, while we found that PLAGL2 expression is related to immune cell infiltration and prognosis in STAD, we don't have direct evidence that PLAGL2 affects prognosis through immune infiltration. As a result, the mechanism through which PLAGL2 contributes to immune modulation remains unknown. Further study is required to determine the specific procedure. In the future, prospective investigations on the expression of PLAGL2 and its involvement in human cancer immune infiltration will be required, as well as the effective development and testing of novel anti-tumor immunotherapy medicines for PLAGL2.

Conclusions

Our findings showed that data mining successfully identifies PLAGL2 expression and putative regulatory networks in STAD, laying the groundwork for additional research into the function of PLAGL2 in

carcinogenesis.

Abbreviations

ACC	Adrenocortical carcinoma
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
ESCA	Esophageal carcinoma
FPPP	FFPE Pilot Phase II
GBM	Glioblastoma multiforme
GBMLGG	Glioma
HNSC	Head and Neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIPAN	Pan-kidney cohort (KICH+KIRC+KIRP)
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute Myeloid Leukemia
LGG	Brain Lower Grade Glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma

STAD	Stomach adenocarcinoma
SKCM	Skin Cutaneous Melanoma
STES	Stomach and Esophageal carcinoma
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma
OS	Osteosarcoma
ALL	Acute Lymphoblastic Leukemia
NB	Neuroblastoma
WT	High-Risk Wilms Tumor

Declarations

Ethics approval and consent to participate

Our research was approved by the Human Research Ethics Committee of Huazhong University of Science and Technology.

Patient consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Author's contributions

Zili Zhou and Li Yan collected the data, analyzed and interpreted the data, and wrote the manuscript. Lin Wang prepared draft figures and tables. All authors read and approved the final manuscript for publication

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Tables

Table 1. Correlation of PLAGL2 mRNA expression and prognosis in STAD with different clinicopathological factors by Kaplan-Meier plotter.

Clinicopathological factors	Overall survival			Post progression survival		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
SEX						
Femal	236	1.74(1.22-2.48)	0.0018	149	1.82(1.18-2.81)	0.006
Male	544	1.77(1.43-2.2)	1.50E-07	149	2.16(1.65-2.83)	9.60E-09
Stage						
1	67	2.56(0.89-7.38)	0.071	31	8.33(0.99-70.06)	0.02
2	140	2.1(1.13-3.92)	0.016	105	2.09(1.02-4.28)	0.038
3	305	1.57(1.18-2.1)	0.002	142	1.53(1-2.35)	0.051
4	148	1.12(0.76-1.64)	0.57	104	1.14(0.72-1.79)	0.58
Stage T						
1	14			3		
2	241	1.49(0.97-2.29)	0.065	196	1.54(0.98-2.42)	0.061
3	204	1.49(1.06-2.11)	0.022	150	1.35(0.92-1.99)	0.12
4	38	1.27(0.56-2.9)	0.57	29	0.85(0.33-2.15)	0.73
Stage N						
0	74	1.9□0.8-4.49	0.14	41	1.57(0.47-5.27)	0.46
1+2+3	422	1.5□1.16-1.96□	0.0022	337	1.41(1.06-1.87)	0.018
1	225	2.06□1.36-3.14□	0.00056	169	2.37(1.47-3.82)	0.00025
2	121	1.31□0.83-2.05□	0.24	105	1.24(0.77-2)	0.37
3	76	1.17□0.69-1.99□	0.56	63	1.19(0.67-2.12)	0.54
Stage M						
0	444	1.45□1.1-1.91□	0.009	342	1.5(1.11-2.03)	0.0077
1	56	1.1□0.62-1.96□	0.74	36	1.09(0.53-2.25)	0.82
Differentiation						

poorly differentiated	165	1.1□0.74-1.64□	0.64	49	1.49(0.78-2.85)	0.23
moderately differentiated	67	1.26□0.66-2.41□	0.49	24	0.81(0.32-2.02)	0.65
well differentiated	32	0.83□0.35-1.96□	0.67	0		
Treatment						
surgery alone	380	1.4(1.05-1.87)	0.021	277	1.35(0.98-1.85	0.061
5 FU based adjuvant	152	1.26(0.89-1.79)	0.19	135	1.49(1.04-2.14)	0.03
other adjuvant	76	0.79(0.33-1.91)	0.6	74	0.82(0.34-1.98)	0.66
HER2 status						
HER2 negative	532	1.69(1.35-2.12)	4.60E-06	334	1.7(1.27-2.27)	0.00029
HER2 positive	343	1.39(1.07-1.81)	0.012	164	2.24(1.55-3.23)	1.10E-05

Table 2. Correlation analysis between PLAGL2 and relate gene markers of immune cells in STAD.

Description	Gene markers	None Core	P	Purity Core	P
CD8+ T cell	CD8A	-0.011	0.831	-0.22	***
	CD8B	0.157	***	-0.121	**
T cell(general)	CD3D	-0.029	0.58	-0.315	***
	CD3E	0.018	0.722	-0.335	***
	CD2	0.029	0.573	-0.303	***
B cell	CD19	0.051	0.32	-0.218	***
	CD79A	-0.051	0.318	-0.268	***
Monocyte	CD86	-0.005	0.927	-0.286	***
	CSF1R	0.054	0.299	-0.208	***
TAM	CCL2	-0.13	**	-0.205	***
	CD68	0.151	***	-0.159	***
	IL10	0.032	0.534	-0.254	***
M1 Macrophage	NOS2	0.284	***	-0.094	0.0663
	IRF5	0.201	***	-0.111	**
	PTGS2	-0.085	0.0986	-0.126	**
M2 Macrophage	CD163	0.086	0.0938	-0.19	***
	VSIG4	-0.04	0.44	-0.166	***
	MS4A4A	-0.043	0.403	-0.191	***
Natural killer cell	KIR2DL1	0.026	0.615	-0.077	0.137
	KIR2DL3	0.002	0.974	-0.132	**
	KIR2DL4	-0.026	0.614	-0.165	***
	KIR3DL1	-0.019	0.707	-0.124	**
	KIR3DL2	-0.002	0.972	-0.161	***
	KIR3DL3	0.072	0.16	-0.02	0.703
Dendritic cell	HLA-DPB1	-0.095	0.0635	-0.293	***
	HLA-DQB1	-0.075	0.142	-0.282	***
	HLA-DRA	-0.066	0.197	-0.276	***

	HLA-DPA1	-0.061	0.235	-0.276	***
	BDCA-1	-0.041	0.425	-0.285	***
	BDCA-4	0.027	0.594	-0.173	***
	CD11c	0.096	0.0617	-0.224	***
TH1	TBX21	0.063	0.221	-0.254	***
	STAT4	0.117	0.0232	-0.245	***
	STAT1	0.302	***	-0.104	0.042
	TNF	0.117	0.023	-0.281	***
	INF-α	0.115	**	-0.033	0.519
TH2	GATA3	-0.087	0.0925	-0.174	***
	STAT6	0.316	***	0.011	0.836
	STAT5A	0.126	0.0144	-0.132	0.0101
	IL13	-0.042	0.418	-0.002	0.971
Tfh	BCL6	0.033	0.525	-0.135	***
TH17	STAT3	0.236	***	-0.071	0.165
	IL17A	0.237	***	-0.122	0.0173
Treg	FOXP3	0.214	***	-0.241	***
	CCR8	0.19	***	-0.168	***
	STAT5B	0.305	***	-0.023	0.664
	TGFB1	0.031	0.548	-0.169	***
T cell exhaustion	PD-1	0.105	**	-0.175	***
	CTLA4	0.177	***	-0.197	***
	LAG3	0.02	0.699	-0.227	***
	TIM-3	0.022	0.665	-0.245	***
	GZMB	-0.001	0.988	-0.254	***

(*P < 0.05, **P < 0.01, ***P < 0.001)

Table 3. Clinicopathological analysis of PLAGL2 expression in STAD.

Parameters	N	PLAGL2		P-value
		High	Low	
Age(years)				
60	36	28	8	0.1
≥60	21	12	9	
Gender				
Male	33	25	8	0.28
Femal	24	15	9	
Size of tumor				
3cm	24	14	10	0.096
≥3cm	33	26	7	
Differentiation				
Well-moderate	26	17	9	0.469
Poor	31	23	8	
T Stages				
T1-T2	22	12	10	0.041
T3-T4	35	28	7	
N Stages				
N0	16	8	8	0.038
N1-2	41	32	9	
M Stages				
M0	39	25	14	0.14
M1	18	15	3	

Figures

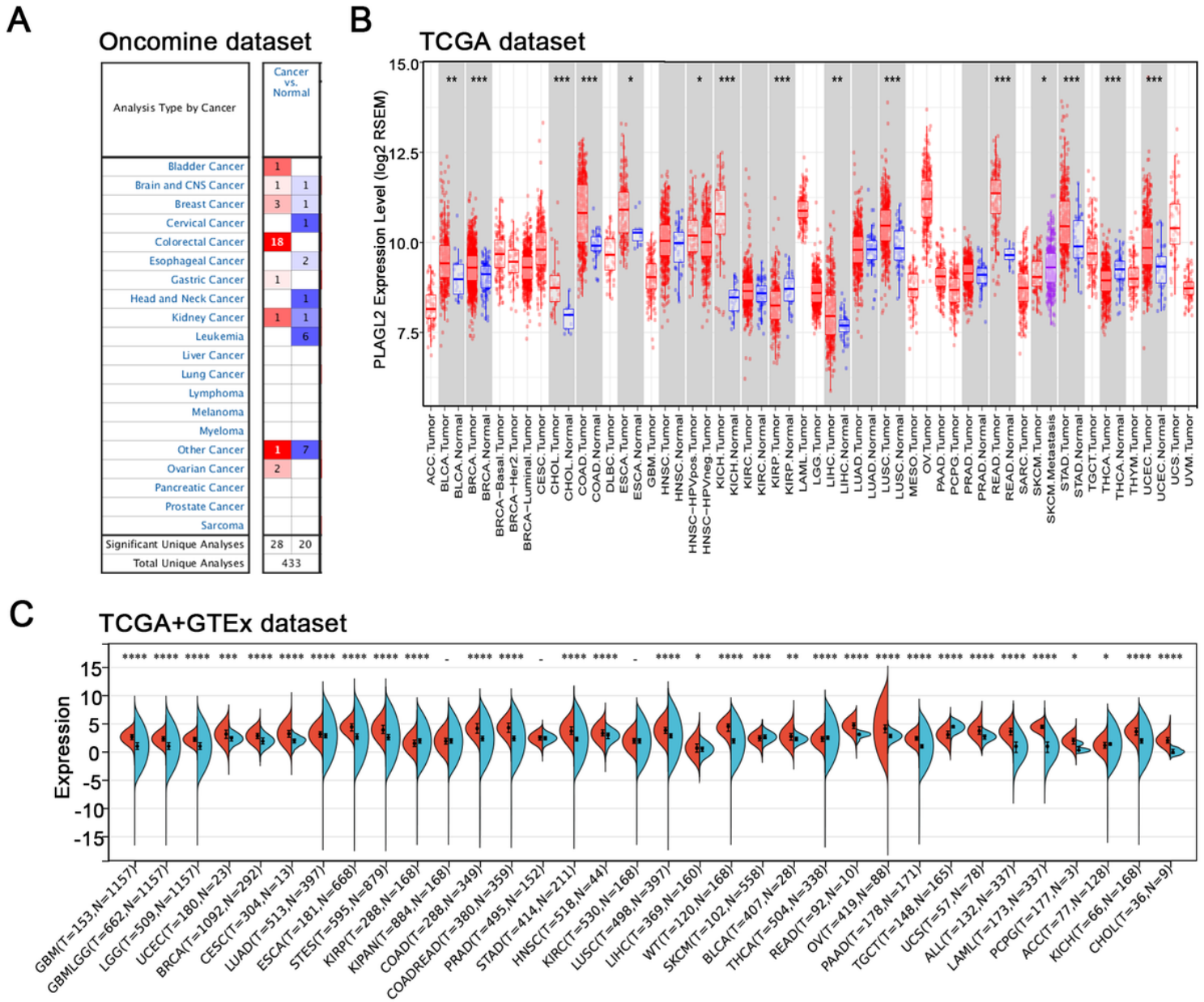

Figure 1

The expression of PLAGL2 in human cancers

(A) The expression level of PLAGL2 in different cancers and paired normal tissue in the Oncomine database. (B) The expression level of PLAGL2 in different cancer types from the TCGA database analyzed by the TIMER database (*P < 0.05, **P < 0.01, ***P < 0.001). (C) The expression level of PLAGL2 in different cancer types from the TCGA and GTEx by SangerBox website (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001)

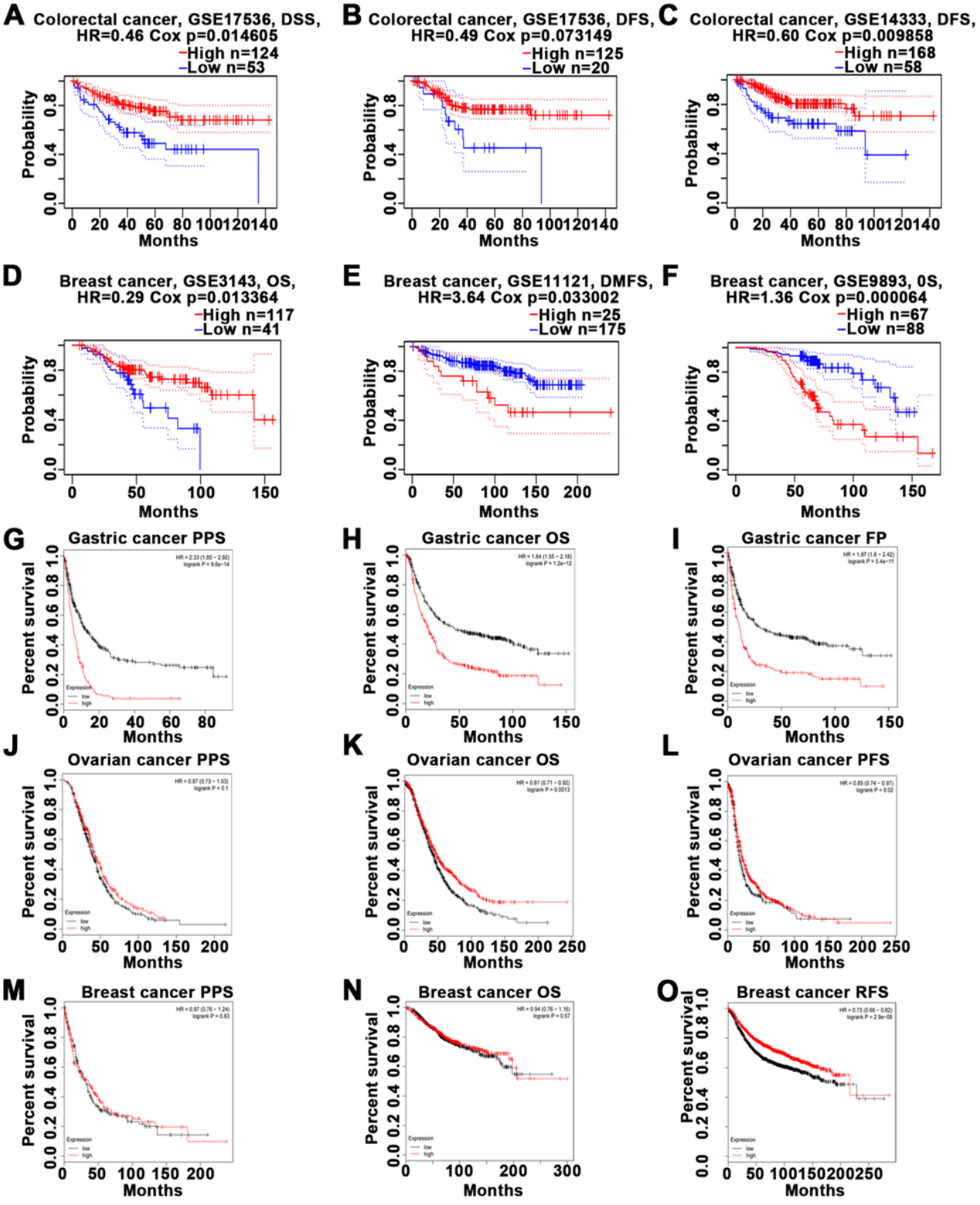

Figure 2

Prognostic potential of PLAGL2 in cancers

(A-F) Prognostic potential of PLAGL2 in colorectal cancer(A-C) and breast cancer(D-F) analyzed by PrognoScan. (G-O) Prognostic potential of PLAGL2 in gastric cancer(G-I), ovarian cancer(J-L) and breast cancer(M-O) analyzed by Kaplan-Meier plotter.

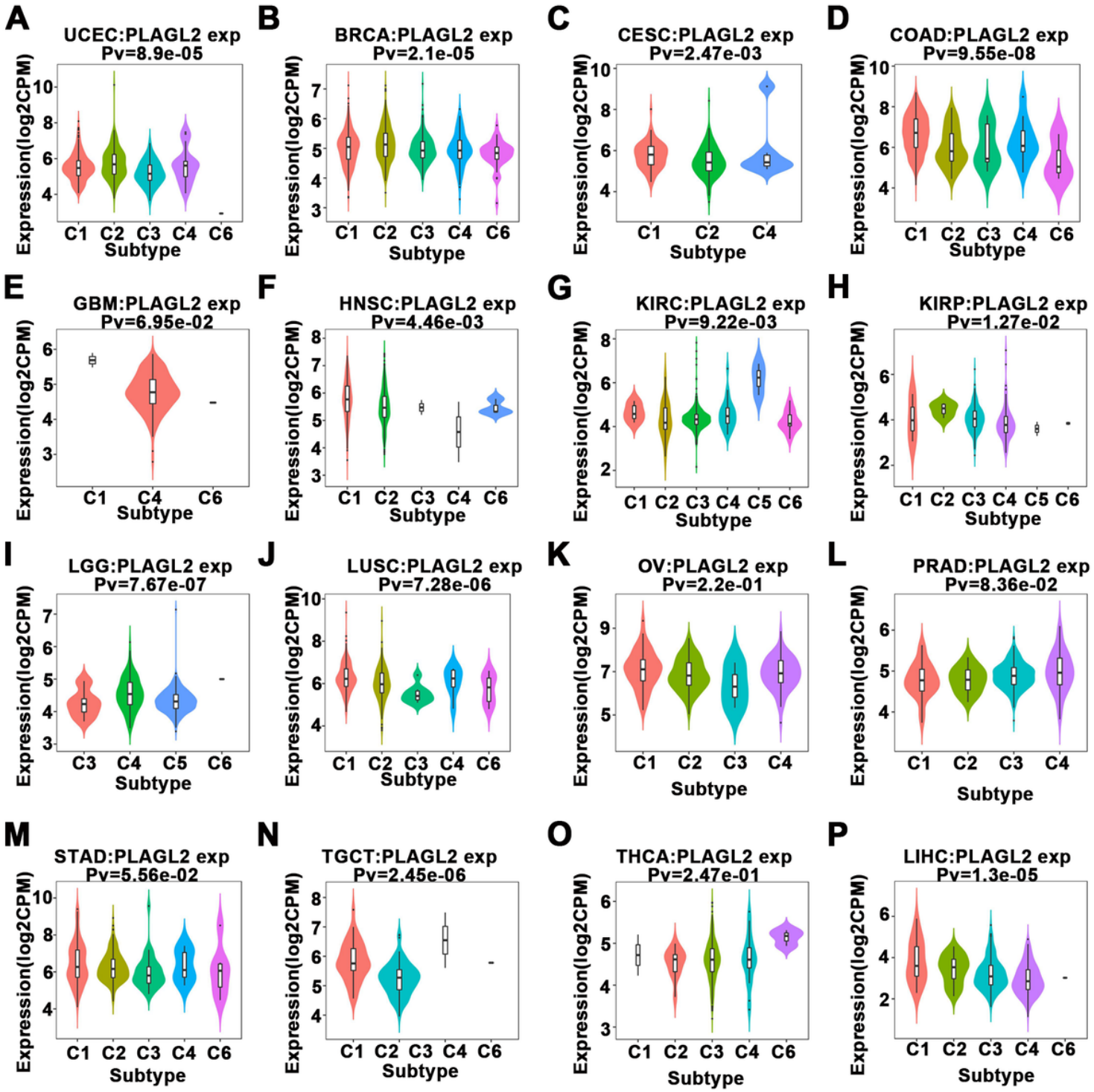

Figure 3

Relationship between PLAGL2 expression and immune subtypes in human cancers

The relationship between PLAGL2 expression and pan-cancer immune subtypes. (A) in UCEC, (B) in BRCA, (C) in CESE, (D) in COAD, (E) in GBM, (F) in HNSC, (G) in KIRC, (H) in KIRP, (I) in LGG, (J) in LUSC, (K) in OV, (L) in PRAD, (M) in STAD, (N) in TCGT, (K) in THCA, (L) in LIHC. C1 (wound healing), C2 (IFN-γ dominant), C3 (inflammation), C4 (lymphocyte depletion), C5 (immune quietness) and C6 (TGF-b dominant)

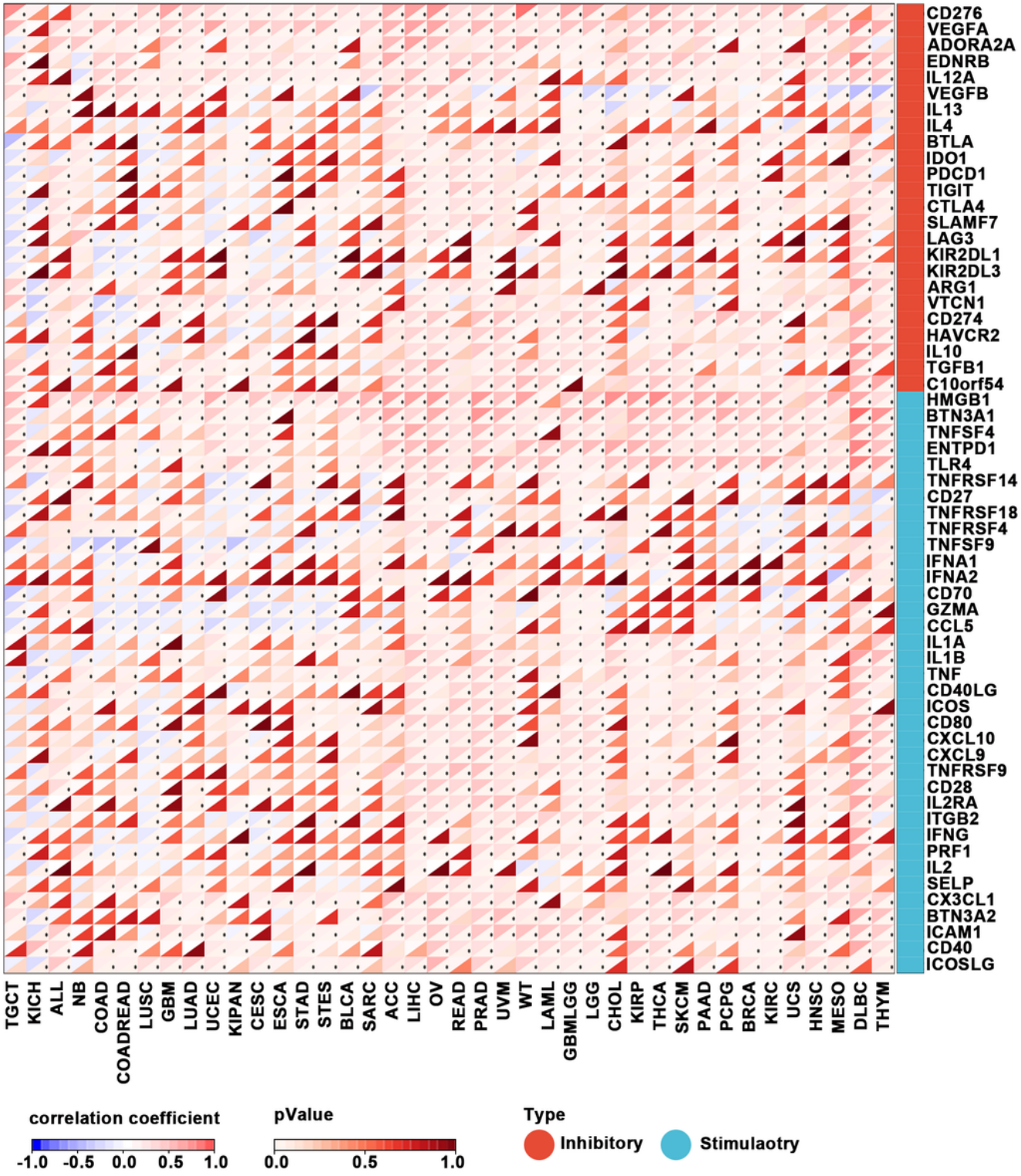

Figure 4

Relationship between PLAGL2 expression and immune checkpoint (ICP) genes in human cancers

The relationship between PLAGL2 expression and pan-cancer immune checkpoint genes. *P < 0.05, **P < 0.01, ***P < 0.001.

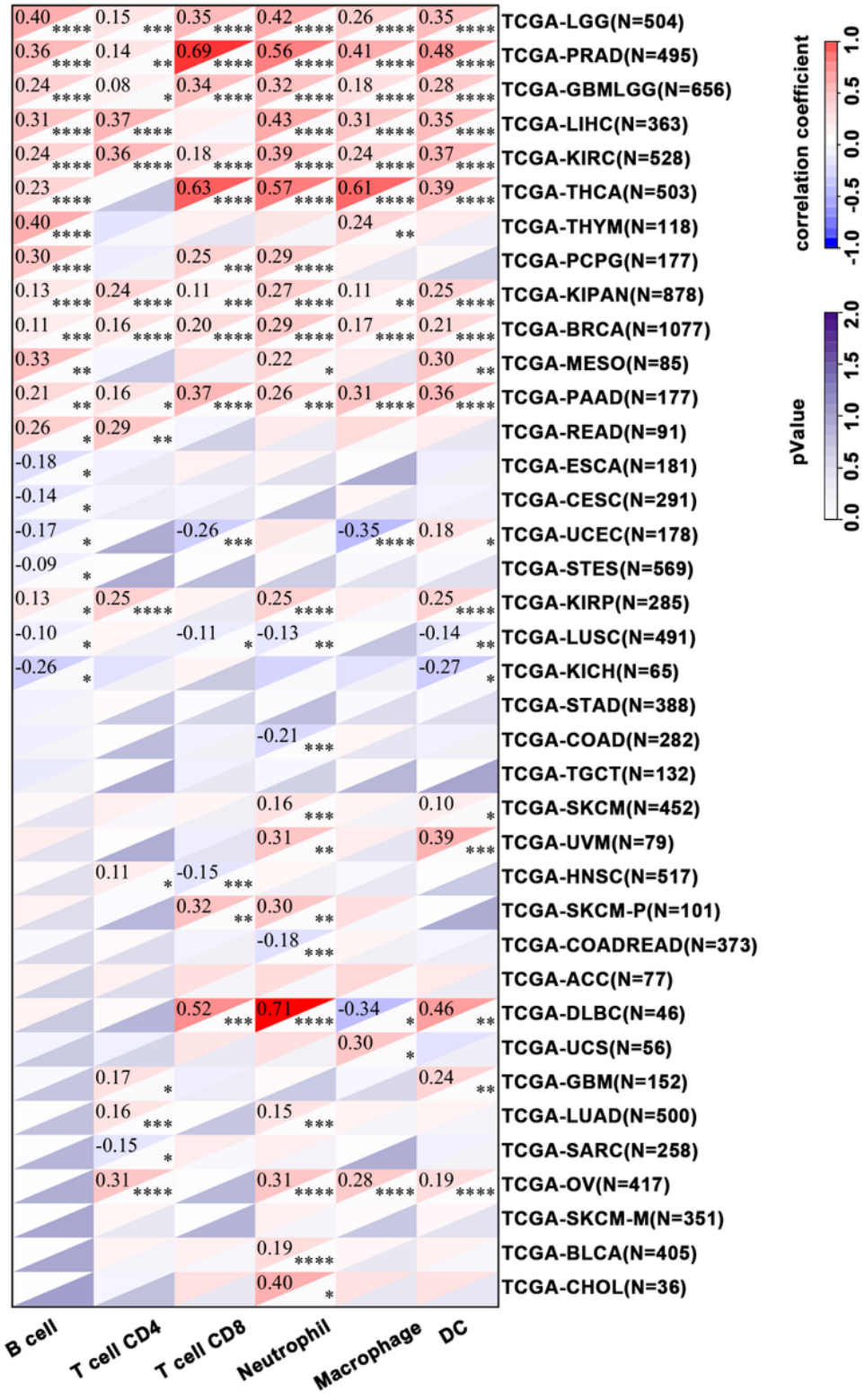

Figure 5

Relationship between PLAGL2 and immune cell infiltration in human cancers

The relationship between PLAGL2 expression and immune cell infiltration in the TME. *P < 0.05, **P < 0.01, ***P < 0.001.

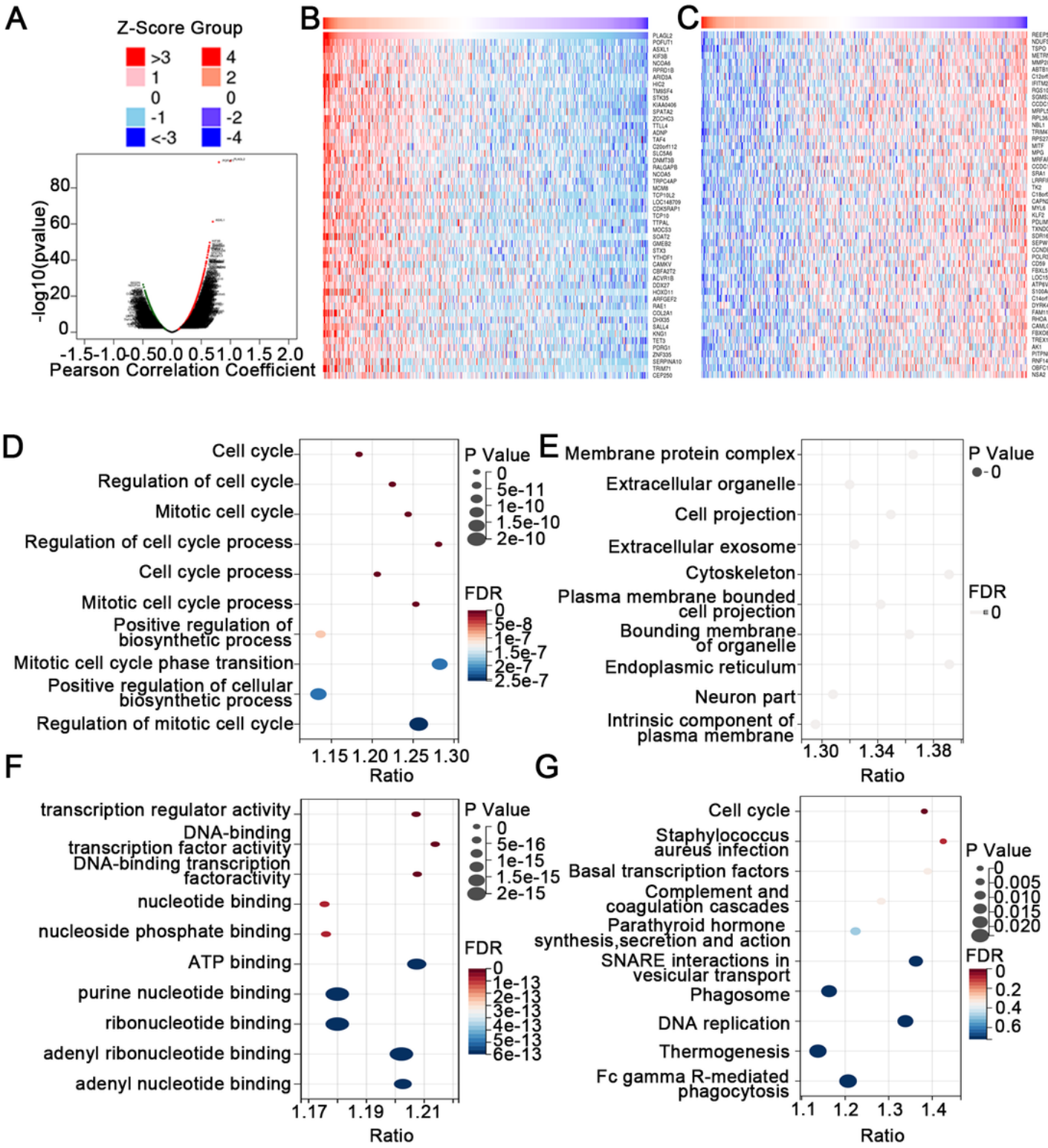

Figure 6

The regulation network of PLAGL2 in stomach cancer

(A)Highly correlated genes of PLAGL2 tested by Pearson test in STAD cohort. (B) Top 50 positive co-expression genes and (C) negative co-expression genes of PLAGL2 in heat map in STAD, (D-G) GO and

KEGG pathway analyses of co-expression genes correlated with PLAGL2 in gastric cancers, BP: biological processes(D), CC: cellular components(E), MF: molecular functions(F), KEGG pathway(G).

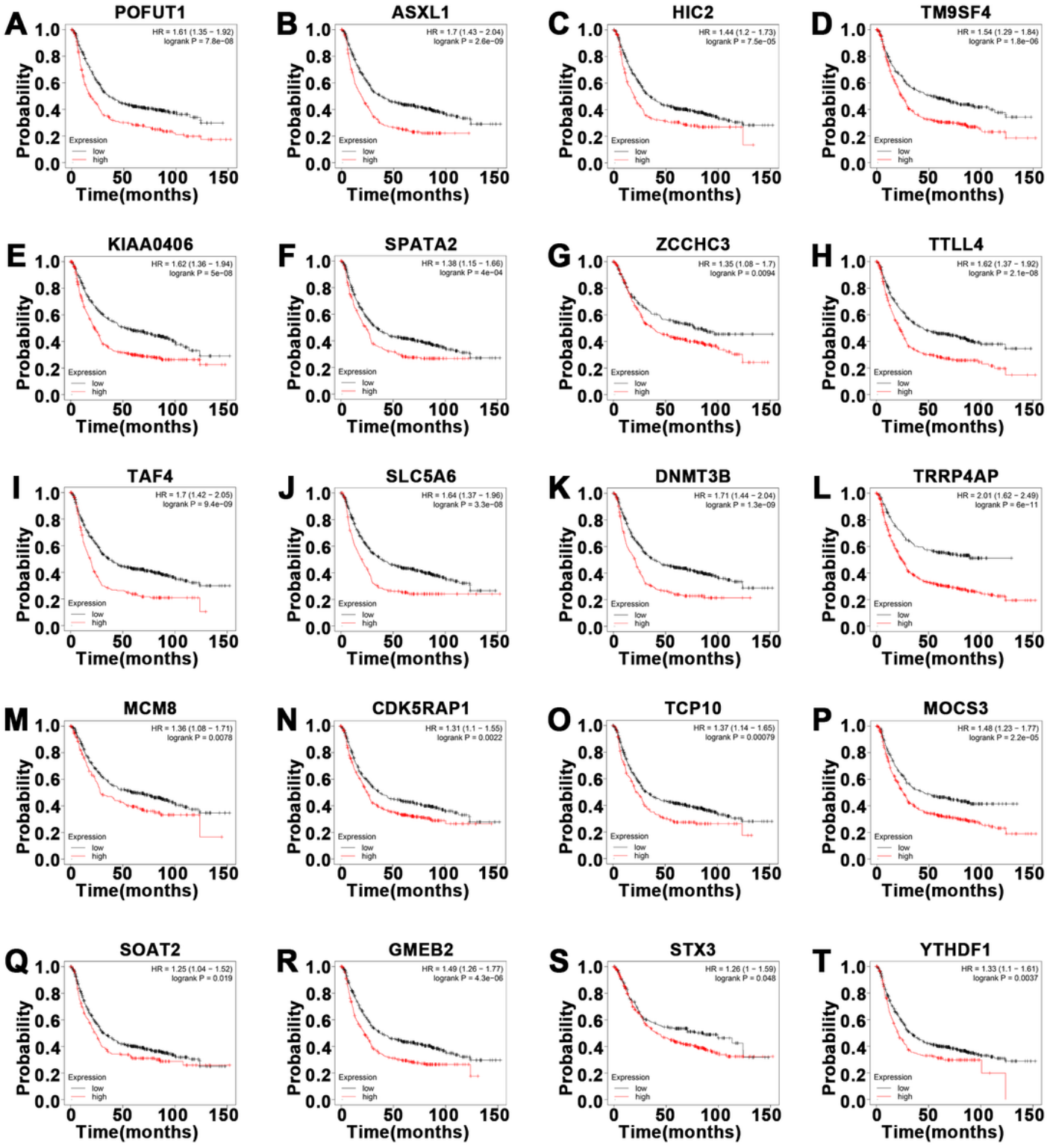

Figure 7

Prognostic potential of PLAGL2 co-expression genes in stomach cancers

Prognostic potential of PLAGL2 co-expression genes in gastric cancers. (A)POFUT1, (B)ASXL1,(C)HIC2, (D)TM9SF4,(E)KIAA0406,(F)SPATA2,(G)ZCCHC3,(H)TTLL4,(I)TAF4,(J)SLC5A6,(K)DNMT3B,(L)TRRP4AP, (M)MCM8,(N)CDK5RAP1,(O)TCP10,(P)MOCS3,(Q)SOAT2,(R)GEMB2,(S)STX3,(T)YTHDF1.

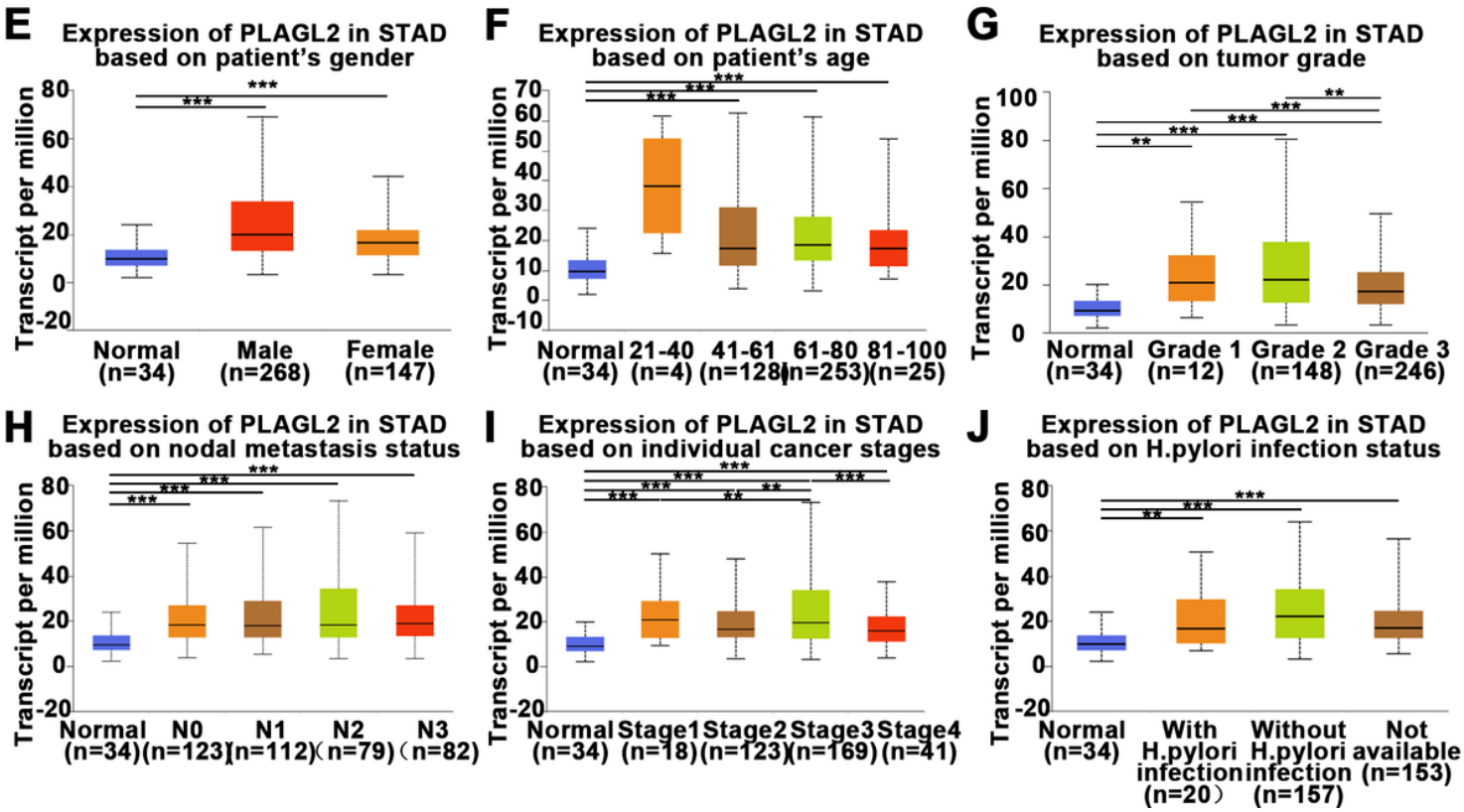

Figure 8

Relationship between PLAGL2 and different clinical subgroups in STAD

PLAGL2 differential expression in STAD with individual patient gender(A), age(B), tumor grade(C), lymph node metastasis status(D), cancer stages(E), and Helicobacter pylori infection status(F) (*P < 0.05, **P < 0.01, ***P < 0.001).

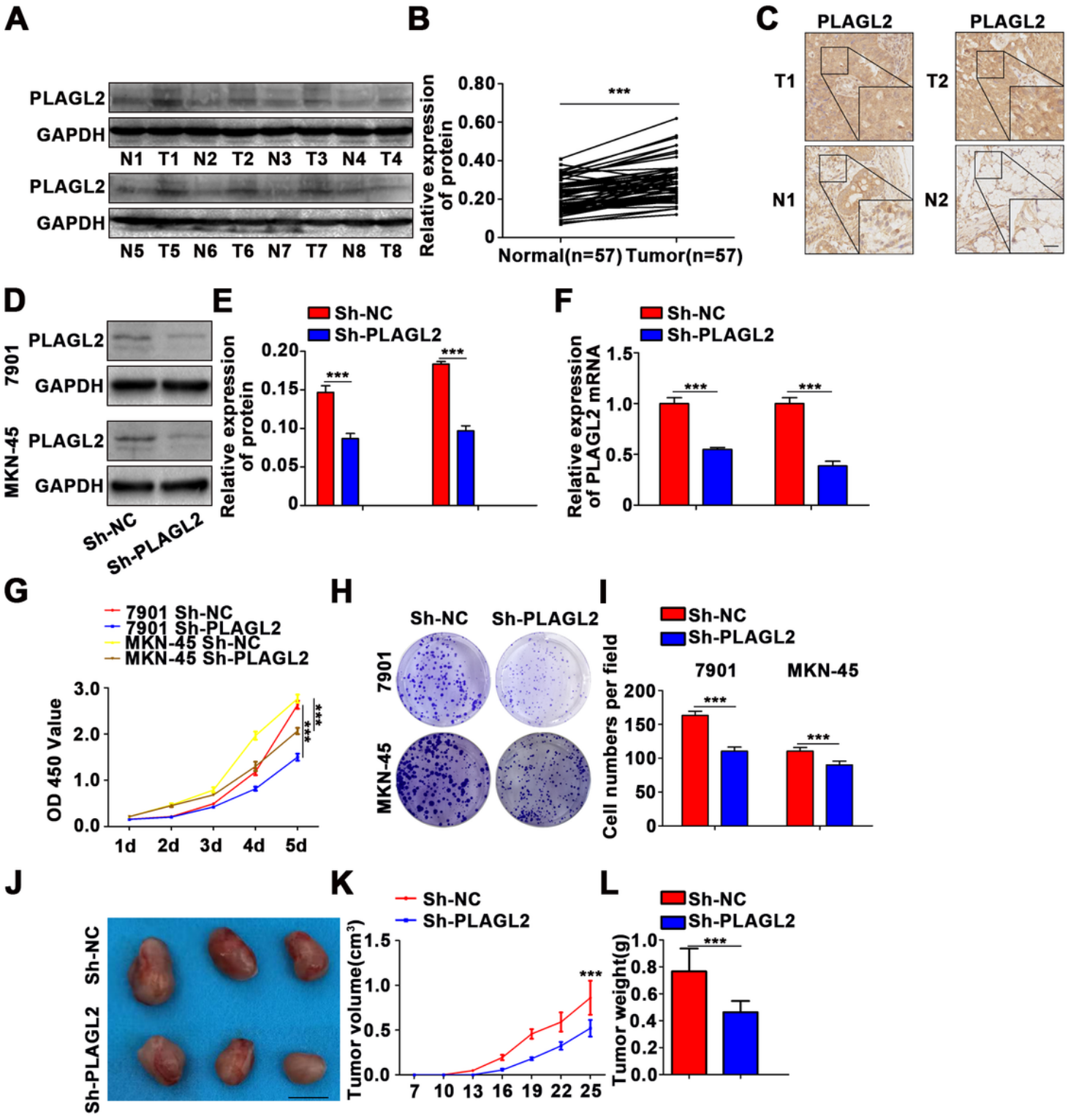

Figure 9

The function of PLAGL2 in STAD cells

(A-B) The expression of PLAGL2 in STAD tissues and adjacent normal tissues detected by WB. (C) The expression of PLAGL2 in STAD tissues and adjacent normal tissues detected by IHC (scale bar: 50μm). (D-F) The expression of PLAGL2 was knocked down by sh-PLAGL2 in 7901 and MKN-45. (G) CCK8

assays revealed that PLAGL2 promotes proliferation in STAD cells. (H-I) Colony formation assays revealed that PLAGL2 promotes proliferation in STAD cells. (J-L) Subcutaneous xenograft tumors grew slower in the sh-PLAGL2 group than in the sh-NC group. Tumor weights in the sh-PLAGL2 group were lower than those in the sh-NC group (scale bar: 1 cm). (*P < 0.05, **P < 0.01, ***P < 0.001)

Supplementary Files

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