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Original Article

Interfering with ITGB1-DT expression delays cancer progression and promotes cell sensitivity of NSCLC to cisplatin by inhibiting the MAPK/ERK pathway

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Abstract: Long non-coding RNA ITGB1-DT is involved in the regulation of cancer growth and metastasis. However, the roles of ITGB1-DT in non-small cell lung cancer (NSCLC) progression and sensitivity to cisplatin has not been elucidated. ITGB1-DT expression in NSCLC tissues, and the relationship between ITGB1-DT expression with NSCLC diagnosis, prognosis, clinicopathological features, and immune cell infiltration were investigated in The Cancer Gene Atlas (TCGA) database. The roles and mechanisms of ITGB1-DT in cell growth, migration, and drug sensitivity of NSCLC cells were explored in the cell model. The prognostic nomograms of ITGB1-DT-related genes were evaluated using bioinformatics. ITGB1-DT was overexpressed in NSCLC. Elevated ITGB1-DT expression was related to the late T stage, N stage, M stage, short overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) of NSCLC patients. ITGB1-DT was the independent risk factors for poor prognosis, and had diagnostic value for NSCLC patients. Interfering with the ITGB1-DT expression can inhibit the proliferation, migration, and invasion of A549, H1299, and drug-resistant A549/DDP, possibly due to the inhibition of p38 MAPK and ERK phosphorylation levels. ITGB1-DT expression was correlated with the levels of NSCLC immune infiltration cells, such as the TReg, Th, and NK cells. ITGB1-DT-related gene nomograms were associated with the prognosis, and were expected to evaluate the prognosis of NSCLC patients. In conclusion, inhibition of ITGB1-DT expression delayed the growth and metastasis of NSCLC using the MAPK/ERK signaling mechanism and enhanced the sensitivity of NSCLC to cisplatin drugs. These results indicate that ITGB1-DT might be a biomarker for evaluating the diagnosis and prognosis of NSCLC patients.

Keywords: ITGB1-DT, MAPK, NSCLC, overall survival, disease-specific survival, progression-free interval

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common cancers in the world [1]. Many studies have reported that the expression levels of long non-coding RNAs (lncRNAs) are related to the progression of NSCLC and the prognosis of cancer patients [2-7]. For instance, linc8087 expression is low in NSCLC tissues and cells, and is associated with short overall survival (OS). Moreover, linc8087 overexpression can inhibit the migration and invasion of NSCLC A549 and PC9 cells. In contrast, interfering with linc8087 expression promotes migration and invasion of NSCLC cells [2]. LncRNA SOX2-OT and PDK1 gene are overexpressed in NSCLC tissues, while miR-30d-5p is under-expressed. Inhibition of SOX2-OT expression can inhibit the proliferation, migration, and invasion of NSCLC cells and promote cell apoptosis. However, PDK1 overexpression or miR-30d-5p inhibition can reverse the inhibitory effect of SOX2-OT on NSCLC cells. SOX2-OT can also reduce CD8 T cell apoptosis through miR-30d-5p/PDK1 signaling axis and promote tumor growth *in vivo* [7]. Additionally, lncRNAs can regulate NSCLC drug sensitivity [8-12]. For instance, lncRNA SNHG1 is significantly overexpressed in cisplatin-resistant NSCLC tissues and cells. SNHG1 can promote drug resistance and malignant behavior of NSCLC cells to cisplatin. SNHG1 also increases DCLK1 levels

through sponge miR-330-5p, increasing the resistance and malignant behavior of NSCLC cells to cisplatin [9]. Compared with NSCLC A549 and H1299 cells, lncRNA FOXD3-AS1 is overexpressed in cisplatin-resistant A549/DDP and H1299/DDP cells. Overexpression of FOXD3-AS1 promotes the resistance of A549 and H1299 cells to cisplatin. Interfering with FOXD3-AS1 expression reduces the sensitivity of A549/DDP and H1299/DDP cells to cisplatin. FOXD3-AS1 promotes chemoresistance of NSCLC cells through the miR-127-3p/MDM2 signaling axis [12].

A few studies have assessed the roles of ITGB1-DT in the cancer [13-15]. Chang et al. recently showed that lncRNA ITGB1-DT is upregulated in lung adenocarcinoma (LUAD) tissues. The overexpression of ITGB1-DT is related to the OS and disease-free survival (DFS) in LUAD patients. Moreover, ITGB1-DT overexpression can promote the proliferation, migration and invasion of LUAD cells. Knockout of ITGB1-DT expression inhibits proliferation, migration and invasion of LUAD cells [14]. ITGB1-DT can regulate ARNTL2 expression through competitive binding to miR-30b-3p, thus promoting proliferation, migration, and invasion of LUAD cells [15]. However, the roles of ITGB1-DT in lung squamous cell carcinoma (LUSC) and the mechanism underlying NSCLC drug sensitivity are unknown. This study aimed to assess the expression level of ITGB1-DT in NSCLC tissues using the TCGA database and the relationship between ITGB1-DT expression with NSCLC diagnosis and patient prognosis using bioinformatics. The roles and mechanisms of ITGB1-DT in the growth and migration of NSCLC cells and cisplatin sensitivity were explored based on the cellular level model. The prognosis of NSCLC patients was evaluated using ITGB1-DT-related nomograms. This study may provide new candidate markers and therapeutic targets for the clinical diagnosis and treatment of NSCLC.

Materials and methods

NSCLC data source and identification of ITGB1-DT expression

Gene expression data of TPM and FPKM types and clinical data of NSCLC patients were downloaded from the Cancer Gene Atlas (TCGA) database. Data from the TCGA database were screened, extracted and identified, then used to investigate ITGB1-DT expression in pan-cancer tissues. The expression data of ITGB1-DT in cancer tissues of TCGA NSCLC patients and their paired para-cancerous tissues were then identified, and the expression of ITGB1-DT in paired tissues was analyzed.

The relationship between ITGB1-DT and clinicopathological features, diagnosis, and prognosis of NSCLC patients

The ITGB1-DT expression data in cancer tissues were matched with the clinicopathological characteristics and prognostic data of NSCLC patients. The relationship between ITGB1-DT expression and clinicopathological features, including age, gender, pathological stage, T stage, N stage, M stage, OS, DSS, and PFI of NSCLC patients, were then investigated. The relationship between ITGB1-DT and the diagnosis and prognosis of NSCLC was investigated through the Receiver Operating Curve (ROC) and Kaplan-Meier (K-M) survival analyses. The relationship between the ITGB1-DT expression with pathological stage, T stage, OS, DSS and PFI in NSCLC patients, and the construction of a prognostic nomogram were explored based on the univariate and multivariate COX regression analysis.

Cell culture

Lung bronchial epithelial cells (HBE) were cultured in DMEM medium containing 10% fetal Bovine Serum (FBS). NSCLC A549, H1299, and A549/DDP cells were cultured in RPMI-1640 medium containing 10% FBS. Penicillin-streptomycin (1%) was added to the whole-cell culture medium, and an appropriate amount of cisplatin was added to the A549/DDP cell culture medium.

NSCLC model construction

NSCLC cell models were constructed via cell transfection following the standard protocols. The interference cell models were detected using qRT-PCR after 24 h of transfection. ITGB1-DT interference sequences were: 5'-GGUCUAGCUGAGUUGACAATT-3', 3'-UUGUCAACUCAGCUAGACCTT-5'.

qRT-PCR

Total RNA was extracted from the NSCLC cells based on the standard methods. The cDNA of normal lung and cancer cells were reverse-transcribed using the reverse transcription kit following the kit instructions, then amplified by PCR to detect the expression levels of ITGB1-DT mRNA in HBE, A549, H1299, and A549/DDP cells. The primer sequences used were:

β-actin: forward: 5'-GTGGCCGAGGACTTTGATTG-3', reverse: 5'-CCTGTAACAACGCATCTCATATT-3'; ITGB1-DT: forward: 5'-TTCCCTGGATGTAGCCTCTCA-3', reverse: 5'-TCCGAAATCCATCCACATCT-3'.

Cell proliferation

The NSCLC model cells were digested and centrifuged, subcultured, and plated. CCK-8 reagent (10 μl) was then added after the NSCLC cells had adhered, and it was recorded as 0 h. Further, 10 μl of CCK-8 reagent was added at 24, 48, 72, and 96 h to detect the cell viability of the control and the experimental groups of ITGB1-DT expression. The experiment was repeated thrice.

Wound healing

NSCLC model cells were plated in 6-well plates. A 100 μl sterile pipette tip was used to scratch the cell surface when NSCLC cells reached 90-100% confluence. Serum-free medium was added after PBS exchange, then incubated. Images were obtained at 0 h, and 24 h after scratching to assess cell migration, and the migration distance was calculated. The experiment was repeated thrice.

Cell migration and invasion

The concentration of NSCLC model cell suspension was adjusted. Different cell suspensions were added to the upper chamber of the Transwell chamber, while complete medium (600 μl) was added to the lower chamber, then incubated at 37°C and 5% CO_2 for 24 h. The Transwell chamber was then washed twice using the phosphate buffered saline (PBS). The non-migrated and invaded cells on the membrane were wiped off using a cotton swab, and the cells were counted and developed. The experiment was also repeated thrice.

Immune-related analysis

Immune scoring of the TCGA NSCLC samples was conducted using an estimate and ssGSEA analysis. The expression of ITGB1-DT and the immune scoring samples were then merged in one-to-one correspondence. The relationship between ITGB1-DT expression and the level of immune cells was then investigated via correlation analysis.

The signaling mechanism of ITGB1-DT

The ITGB1-DT expression in TCGA NSCLC samples were determined, then the median value of ITGB1-DT expression was divided into ITGB1-DT overexpression and interference ITGB1-DT expression groups. The effect of ITGB1-DT expression on TCGA gene set was investigated based on Gene Set enrichment analysis (GSEA) [16, 17]. Nominal (NOM) $P < 0.05$ was the screening criterion for signaling mechanism.

Western blotting

Total protein was extracted from NSCLC cells using RIPA lysate. BCA method was used for protein quantification. The protein was separated using 10% SDS-PAGE, then transferred to PVDF membrane. The membrane was blocked, then incubated with primary antibodies (p38 MAPK, p-p38 MAPK, ERK, and p-ERK (1:1000)) at 4°C overnight. The membranes were then incubated with secondary antibody (1:10000) at room temperature for 1 h, after which it was washed. The relative expression levels of the proteins were calculated via semi-directional analysis. In detail, the gray values of p38 MAPK, p-p38 MAPK, ERK and p-ERK proteins were compared with the gray values of the internal reference. The relative ratio between the interference ITGB1-DT and control groups were compared to analyze the relative expression levels of p38 MAPK, p-p38 MAPK, ERK, and p-ERK proteins in the two groups.

Construction of ITGB1-DT correlation nomogram

The prognostic factors of NSCLC patients were analyzed using COX analysis. The ITGB1-DT-related genes and key prognostic genes were screened using VENN diagram. K-M survival analysis was then used to assess the relationship between the expression levels of ITGB1-DT-related genes and the prognosis of NSCLC patients. The expression of ITGB1-DT-related genes in NSCLC patients was assessed using univariate and multivariate COX regression analyses. The nomogram was used to evaluate the relationship between ITGB1-DT-related genes and the prognosis of NSCLC patients.

Statistical analysis

Wilcoxon rank-sum test analysis was used to explore the expression level of ITGB1-DT in TCGA pan-cancer tissues. COX regression and survival analyses were used to investigate the relationship between ITGB1-DT expression and prognosis of NSCLC patients. A t-test was used to evaluate statistical differences between the two groups in functional studies. $P < 0.05$ was

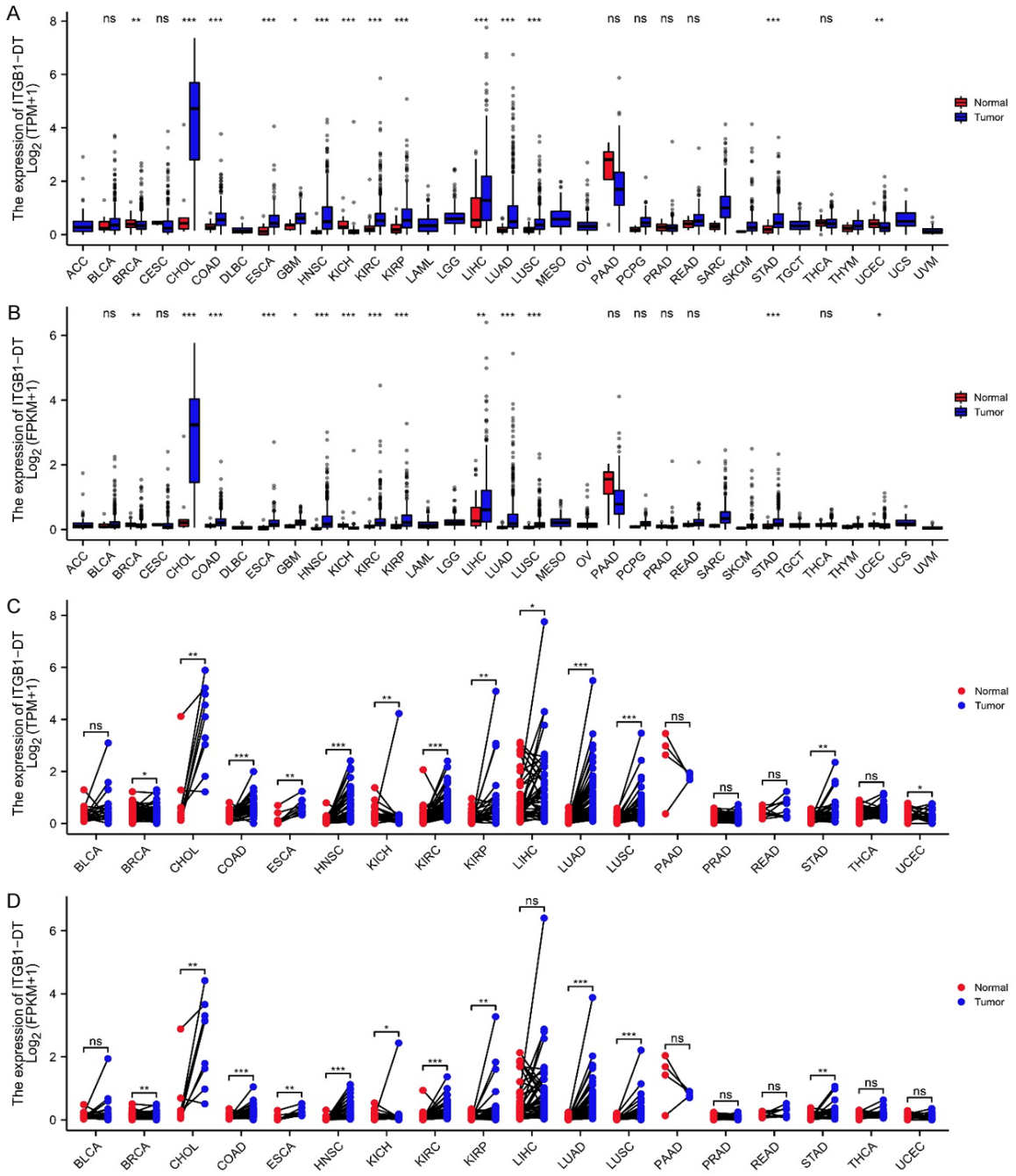


Figure 1. ITGB1-DT showed unusual expression in pan-cancer tissues in TCGA database. A. Unpaired data of TPM type; B. Unpaired data of FKPM type; C. Paired data of TPM type; D. Paired data of FKPM type. Note: TCGA, the Cancer Gene Atlas.

considered the statistically significant difference.

Results

ITGB1-DT is overexpressed in NSCLC tissues

In this study, ITGB1-DT was significantly overexpressed in the CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, and STAD tissues, while it was lowly expressed in BRCA, KICH, and UCEC tissues based on the unpaired data of TCGA TPM type (**Figure 1A**). Moreover, ITGB1-DT was significantly overexpressed in the CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, and STAD tissues, while it was lowly expressed in the BRCA,

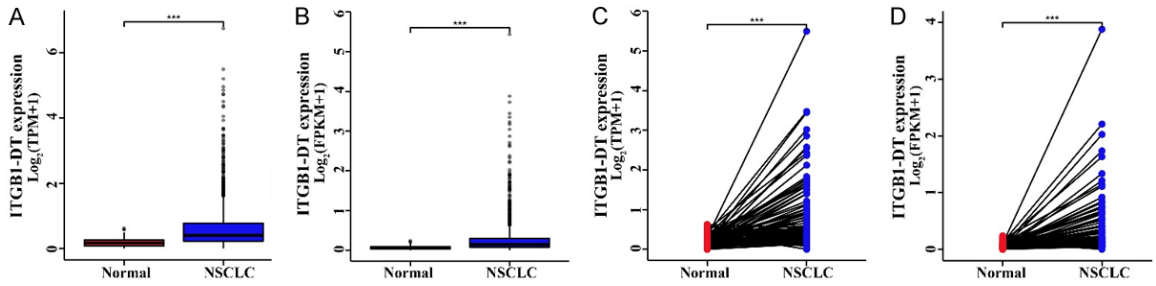


Figure 2. ITGB1-DT was overexpressed in NSCLC tissues in TCGA database. A. Unpaired data of TPM type; B. Unpaired data of FKPM type; C. Paired data of TPM type; D. Paired data of FKPM type. Note: TCGA, the Cancer Gene Atlas; NSCLC, non-small cell lung cancer.

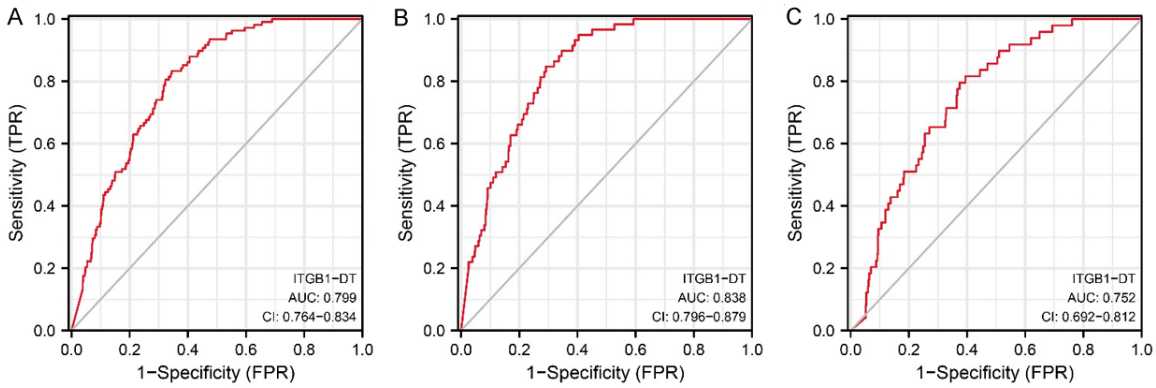
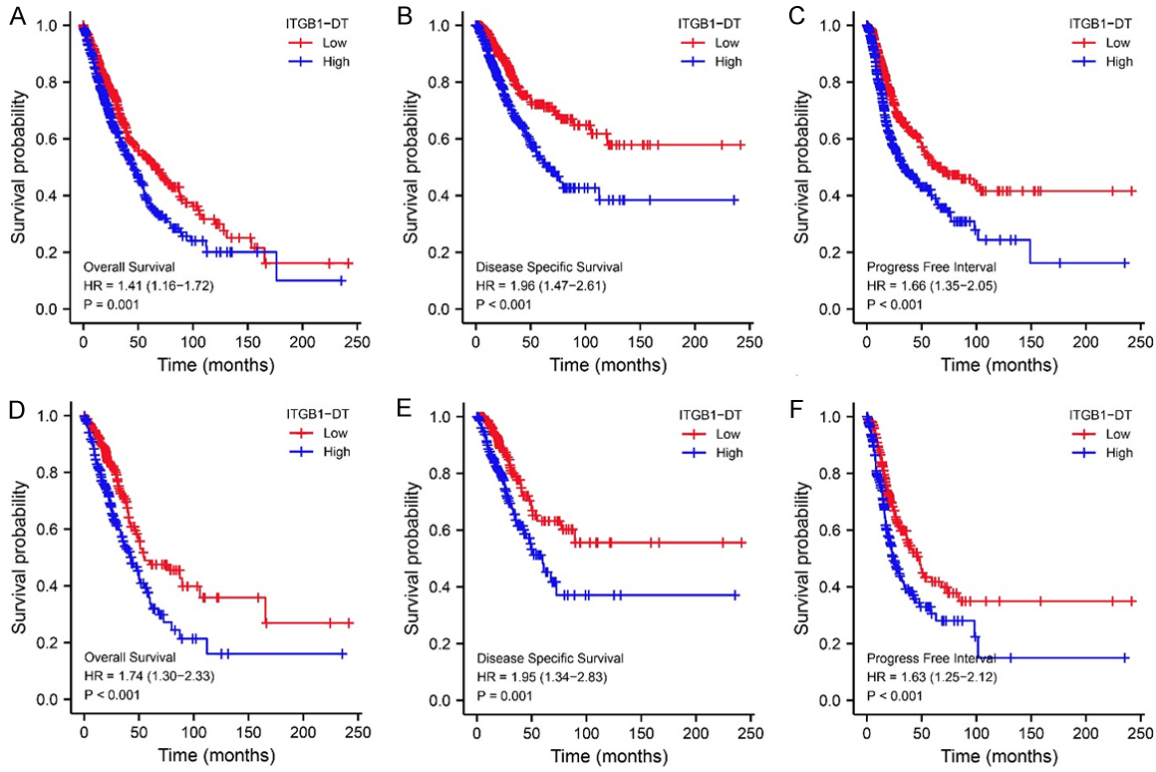


Figure 3. Diagnostic values of ITGB1-DT expression level in NSCLC as determined using ROC analysis. A. NSCLC; B. LUAD; C. LUSC. Note: NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; ROC, Receiver Operating Curve.



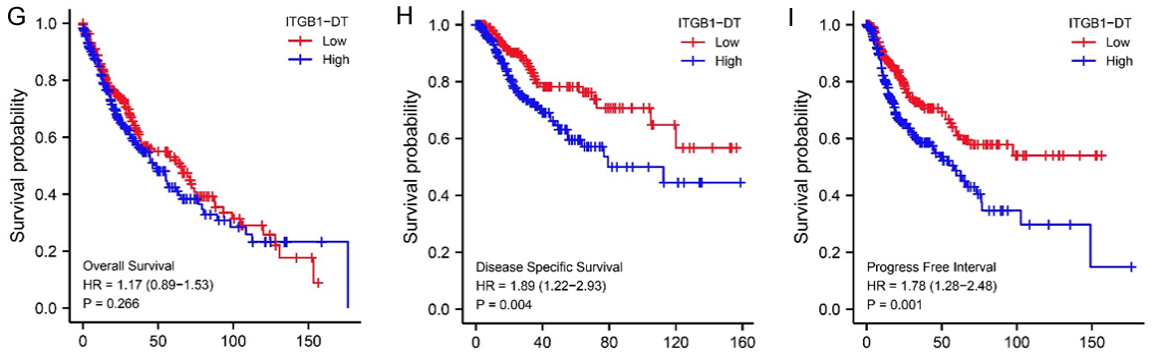


Figure 4. ITGB1-DT overexpression was associated with dismal prognosis in NSCLC as determined using the K-M survival analysis. A-C. OS, DSS, and PFI for NSCLC patients; D-F. OS, DSS, and PFI for LUAD patients; G-I. OS, DSS, and PFI for LUSC patients. Note: NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; K-M, Kaplan-Meier; OS, overall survival; DSS, disease specific survival; PFI, Progress free survival.

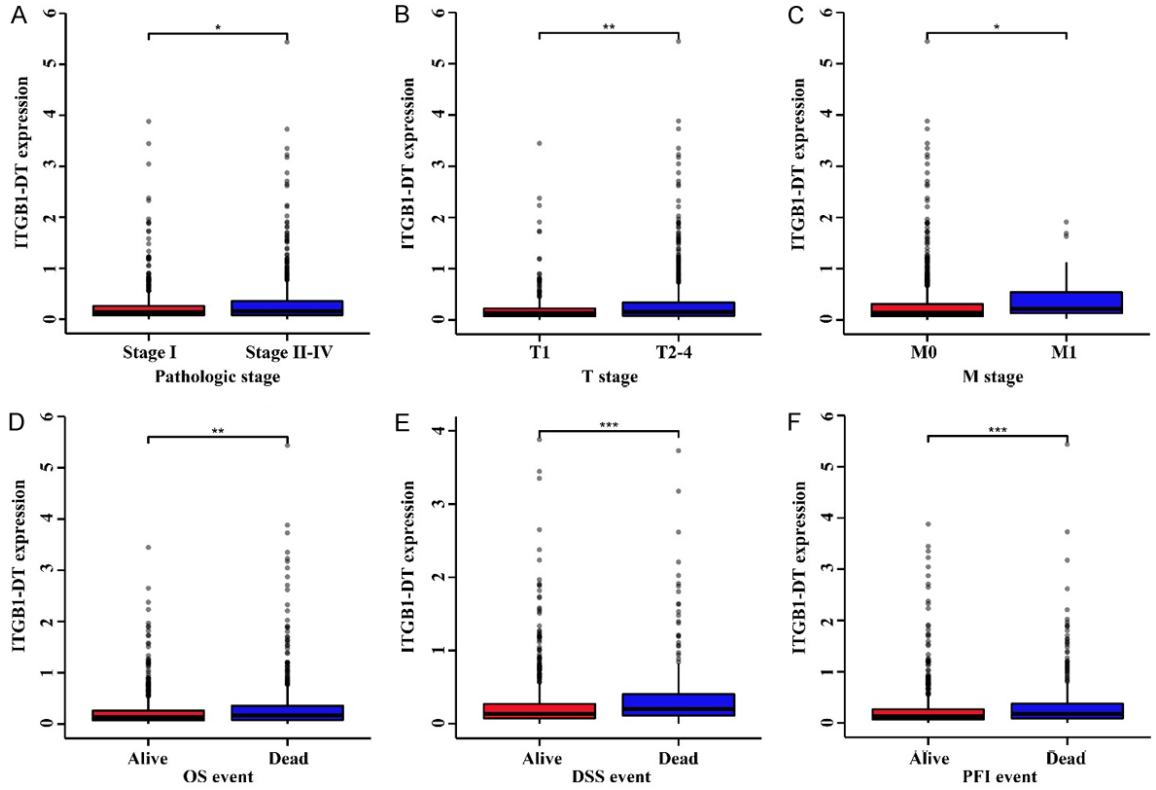


Figure 5. The expression of ITGB1-DT in NSCLC tissues based on the clinicopathologic features. A. Pathologic stage; B. T stage; C. M stage; D. OS status; E. DSS status; F. PFI status. Note: NSCLC, non-small cell lung cancer; OS, overall survival; DSS, disease specific survival; PFI, Progress free survival.

KICH, and UCEC tissues based on the unpaired data of TCGA FKPM type (**Figure 1B**).

ITGB1-DT was significantly overexpressed in the CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, and STAD tissues, while it was lowly expressed in the BRCA, KICH and UCEC tissues expression based on the paired data of TCGA TPM type (**Figure 1C**). ITGB1-DT was significantly overexpressed in the CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LUAD, LUSC, and STAD tissues, while it was under-expressed in the BRCA and KICH tissues based on the paired data of TCGA FK-

Table 1. Risk factors predicting poor overall survival of NSCLC patients

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
T stage	1019				
T1	289	Reference			
T2	571	1.404 (1.096-1.799)	0.007	1.262 (0.938-1.698)	0.125
T3	117	2.258 (1.614-3.161)	< 0.001	1.811 (1.104-2.972)	0.019
T4	42	2.750 (1.760-4.296)	< 0.001	1.819 (1.003-3.298)	0.049
N stage	1000				
N0	660	Reference			
N1	222	1.539 (1.223-1.936)	< 0.001	1.131 (0.750-1.704)	0.557
N2	111	2.059 (1.549-2.737)	< 0.001	1.992 (1.102-3.599)	0.022
N3	7	1.511 (0.375-6.086)	0.562	1.506 (0.340-6.679)	0.590
M stage	792				
M0	760	Reference			
M1	32	2.269 (1.439-3.577)	< 0.001	1.963 (1.074-3.588)	0.028
Gender	1022				
Female	410	Reference			
Male	612	1.164 (0.949-1.428)	0.145		
Pathologic stage	1010				
Stage I	533	Reference			
Stage II	280	1.611 (1.271-2.042)	< 0.001	1.193 (0.772-1.845)	0.427
Stage III	164	2.262 (1.750-2.924)	< 0.001	1.029 (0.536-1.976)	0.931
Stage IV	33	3.108 (1.969-4.906)	< 0.001		
Age	1006				
≤ 65	445	Reference			
> 65	561	1.265 (1.034-1.548)	0.022	1.348 (1.070-1.697)	0.011
Smoker	996				
No	90	Reference			
Yes	906	0.883 (0.617-1.263)	0.496		
Race	855				
Asian	16	Reference			
Black or African American	84	0.754 (0.295-1.929)	0.556		
White	755	0.812 (0.335-1.968)	0.645		
ITGB1-DT	1022				
Low	511	Reference			
High	511	1.403 (1.151-1.709)	< 0.001	1.308 (1.046-1.634)	0.018

Note: NSCLC, non-small cell lung cancer.

PM type (**Figure 1D**). ITGB1-DT was significantly overexpressed in NSCLC tissues based on the TCGA database (**Figure 2**).

ITGB1-DT overexpression has diagnostic values and can predict poor prognosis in NSCLC patients

ROC analysis showed that the area under the curve (AUC) of ITGB1-DT for NSCLC diagnosis was 0.799 (95% CI; 0.764-0.834) (**Figure 3A**). The AUC of ITGB1-DT for LUAD and LUSC diagnoses were 0.838 (95% CI; 0.796-0.879) (**Figure 3B**) and 0.752 (95% CI; 0.692-0.812) (**Figure 3C**), respectively. K-M survival analysis indicated that ITGB1-DT overexpression was associated with poor prognosis in NSCLC patients (**Figure 4**). Moreover, TGB1-DT overexpression was associated with short OS, DSS and PFI in NSCLC patients (**Figure 4A-C**). TGB1-DT overexpression was also associated with

Table 2. Risk factors predicting poor disease-specific survival of NSCLC patients

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
T stage	932				
T1	274	Reference			
T2	517	1.534 (1.078-2.184)	0.017	1.272 (0.827-1.954)	0.273
T3	108	2.884 (1.832-4.540)	< 0.001	2.087 (1.034-4.212)	0.040
T4	33	3.006 (1.508-5.993)	0.002	1.818 (0.737-4.487)	0.195
N stage	915				
N0	618	Reference			
N1	196	1.996 (1.457-2.735)	< 0.001	1.258 (0.717-2.209)	0.424
N2	95	2.712 (1.855-3.964)	< 0.001	2.585 (1.075-6.217)	0.034
N3	6	0.000 (0.000-Inf)	0.991	0.000 (0.000-Inf)	0.995
M stage	709				
M0	681	Reference			
M1	28	2.819 (1.560-5.093)	< 0.001	2.986 (1.407-6.340)	0.004
Gender	935				
Female	375	Reference			
Male	560	1.050 (0.793-1.390)	0.732		
Pathologic stage	923				
Stage I	498	Reference			
Stage II	255	2.204 (1.574-3.086)	< 0.001	1.470 (0.796-2.711)	0.218
Stage III	141	3.193 (2.229-4.573)	< 0.001	1.134 (0.429-2.995)	0.800
Stage IV	29	4.526 (2.500-8.195)	< 0.001		
Age	919				
≤ 65	418	Reference			
> 65	501	0.993 (0.752-1.310)	0.958		
Smoker	909				
No	85	Reference			
Yes	824	0.835 (0.513-1.359)	0.469		
Race	800				
Asian	15	Reference			
Black or African American	80	2.686 (0.363-19.903)	0.333		
White	705	2.448 (0.342-17.50)	0.372		
ITGB1-DT	935				
Low	468	Reference			
High	467	1.997 (1.501-2.657)	< 0.001	1.605 (1.160-2.221)	0.004

Note: NSCLC, non-small cell lung cancer.

short OS, DSS and PFI in LUAD patients (**Figure 4D-F**). TGB1-DT overexpression was correlated with the DSS and PFI of LUSC patients, and it was not significantly related to the OS of LUSC patients (**Figure 4G-I**).

ITGB1-DT overexpression was associated with the pathological stage, T stage, M stage, and poor prognosis in NSCLC patients

ITGB1-DT overexpression was associated with advanced pathological stage, advanced T stage, and M stage in NSCLC patients based on grouping according to clinicopathological characteristics (**Figure 5A-C**). ITGB1-DT overexpression was associated with poor prognosis in NSCLC patients (**Figure 5D-F**). ITGB1-DT overexpression was associated with OS, DSS, and PFI of deceased patients.

ITGB1-DT overexpression is a risk factor for poor prognosis of NSCLC patients

Univariate COX regression analysis showed that the T stage, N stage, M stage, pathological

Table 3. Risk factors predicting poor progression-free interval of NSCLC patients

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
T stage	1020				
T1	289	Reference			
T2	572	1.461 (1.131-1.887)	0.004	1.424 (1.041-1.950)	0.027
T3	117	2.761 (1.978-3.853)	< 0.001	2.019 (1.196-3.408)	0.009
T4	42	1.431 (0.780-2.625)	0.247	0.954 (0.436-2.085)	0.905
N stage	1001				
N0	660	Reference			
N1	223	1.462 (1.154-1.853)	0.002	0.991 (0.648-1.517)	0.969
N2	111	1.561 (1.141-2.135)	0.005	1.151 (0.578-2.292)	0.688
N3	7	0.622 (0.087-4.435)	0.635	0.000 (0.000-Inf)	0.992
M stage	793				
M0	761	Reference			
M1	32	1.829 (1.084-3.085)	0.024	2.082 (1.084-4.001)	0.028
Gender	1023				
Female	410	Reference			
Male	613	1.005 (0.816-1.237)	0.966		
Pathologic stage	1011				
Stage I	533	Reference			
Stage II	281	1.664 (1.311-2.112)	< 0.001	1.365 (0.877-2.126)	0.168
Stage III	164	1.919 (1.452-2.536)	< 0.001	1.513 (0.715-3.204)	0.279
Stage IV	33	2.342 (1.398-3.926)	0.001		
Age	1007				
≤ 65	446	Reference			
> 65	561	1.014 (0.824-1.247)	0.897		
Smoker	997				
No	90	Reference			
Yes	907	0.721 (0.510-1.019)	0.064		
Race	855				
Asian	16	Reference			
Black or african american	84	0.872 (0.367-2.071)	0.756		
White	755	0.816 (0.364-1.833)	0.623		
ITGB1-DT	1023				
Low	512	Reference			
High	511	1.663 (1.352-2.045)	< 0.001	1.477 (1.164-1.875)	0.001

Note: NSCLC, non-small cell lung cancer.

stage, age, and ITGB1-DT expression were the risk factors for the OS, DSS and PFI of NSCLC patients. Moreover, multivariate COX regression analysis found that T stage, N stage, M stage, age, and ITGB1-DT expression were independent risk factors for the OS of NSCLC patients (**Table 1**). Multivariate COX regression analysis found that the T stage, N stage, M stage, and ITGB1-DT expression were independent risk factors for the DSS and PFI of NSCLC patients (**Tables 2** and **3**). The prognostic nomograms were constructed based on COX analysis (**Figures 6** and S1).

Interfering with ITGB1-DT expression attenuates NSCLC cell growth, migration, and cisplatin resistance

Compared with HBE cells, ITGB1-DT was significantly overexpressed in A549 and H1299 cells (**Figure 7A**). Compared with A549 cells, ITGB1-DT was significantly overexpressed in A549/

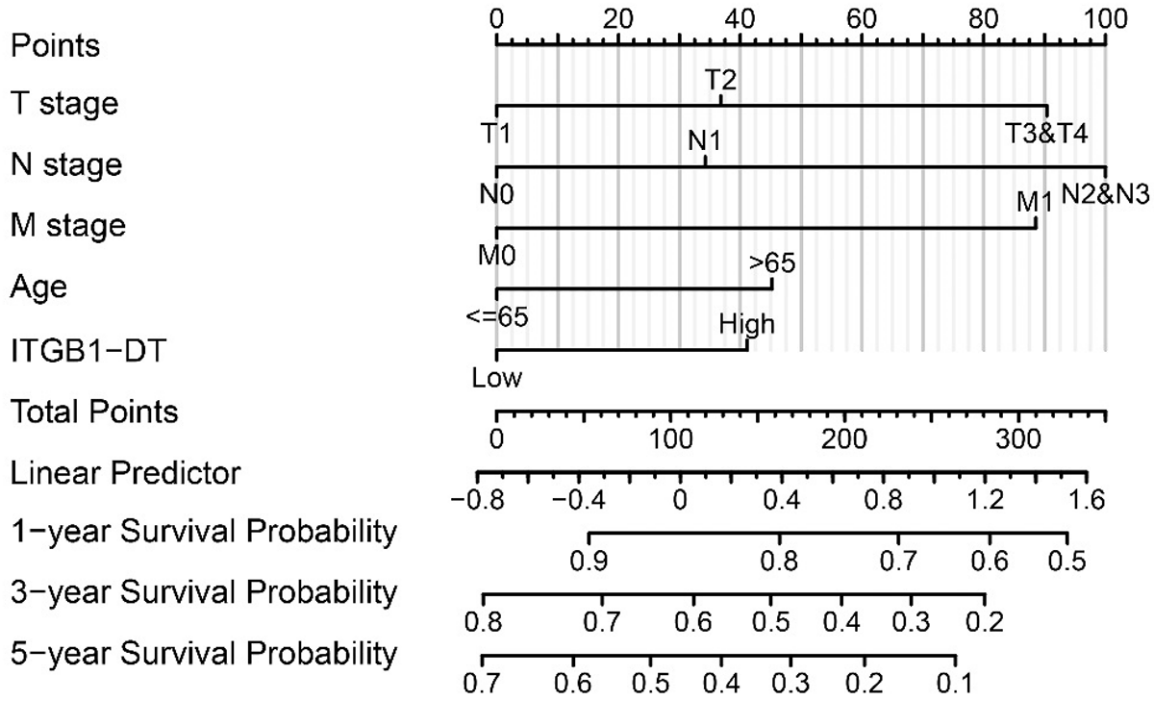


Figure 6. A nomogram based on ITGB1-DT and clinical characteristic factors for predicting the OS of NSCLC. Note: NSCLC, non-small cell lung cancer; OS, overall survival.

DDP cells (**Figure 7A**). CCK-8, wound healing, and Transwell experiments showed that interfering with ITGB1-DT expression inhibited cancer cell proliferation, migration, and invasion in A549, H1299, and A549/DDP cells (**Figures 7** and **8A-C**).

Interfering with ITGB1-DT expression inhibits NSCLC cell growth, migration, and cisplatin resistance via the MAPK/ERK signaling mechanism

Herein, ITGB1-DT expression was low in multiple cancer signaling pathways, including the insulin signaling pathway, GNRH signaling pathway, ABC transporters, adipocytokine signaling pathway, calcium signaling pathway, PPAR signaling pathway, FC epsilon RI signaling pathway, MAPK signaling pathway, WNT signaling pathway (Figure S2). Western-blotting showed that interfering with ITGB1-DT expression inhibited p-p38 MAPK and p-ERK protein expression in NSCLC cells while it did not significantly affect p38 MAPK and ERK protein expression levels (**Figure 8D-F**).

ITGB1-DT expression is associated with immune cell infiltration in NSCLC

Correlation analysis showed that ITGB1-DT expression was associated with immune cell infiltration in NSCLC (**Figure 9**). ITGB1-DT expression was also correlated with the NSCLC immune score, stromal score, and estimate score (**Figure 9A-C**). Moreover, ITGB1-DT expression was significantly correlated with the level of NSCLC aDC, CD8 T cell, macrophages, and other immune cells (**Figure 9D-T**).

Functions and mechanisms of ITGB1-DT-related genes

ITGB1-DT-related genes were involved in various functions, such as keratinocyte differentiation, epidermal cell differentiation, negative regulation of megakaryocyte differentiation, DNA replication-dependent nucleosome assembly, chemical carcinogenesis, drug metabolism, pentose and glucuronate interconversions, steroid hormone biosynthesis (**Figure 10**).

The prognosis roles of ITGB1-DT-related genes

Cox regression analysis was used to screen the prognostic factors in NSCLC patients. Venn diagram showed that the overlapping genes were *CLMP, FEZF1, NTSR1, FIBCD1, ARL14EPL, GPR-78, PPBP, SNORA54, FETUB, MUC2, HS3ST5, AREG, SERPINA4, SNORA71D, HSD17B13, ACSM6, BTF3P12, SLC5A7, KLK7, BTBD18, FS-*

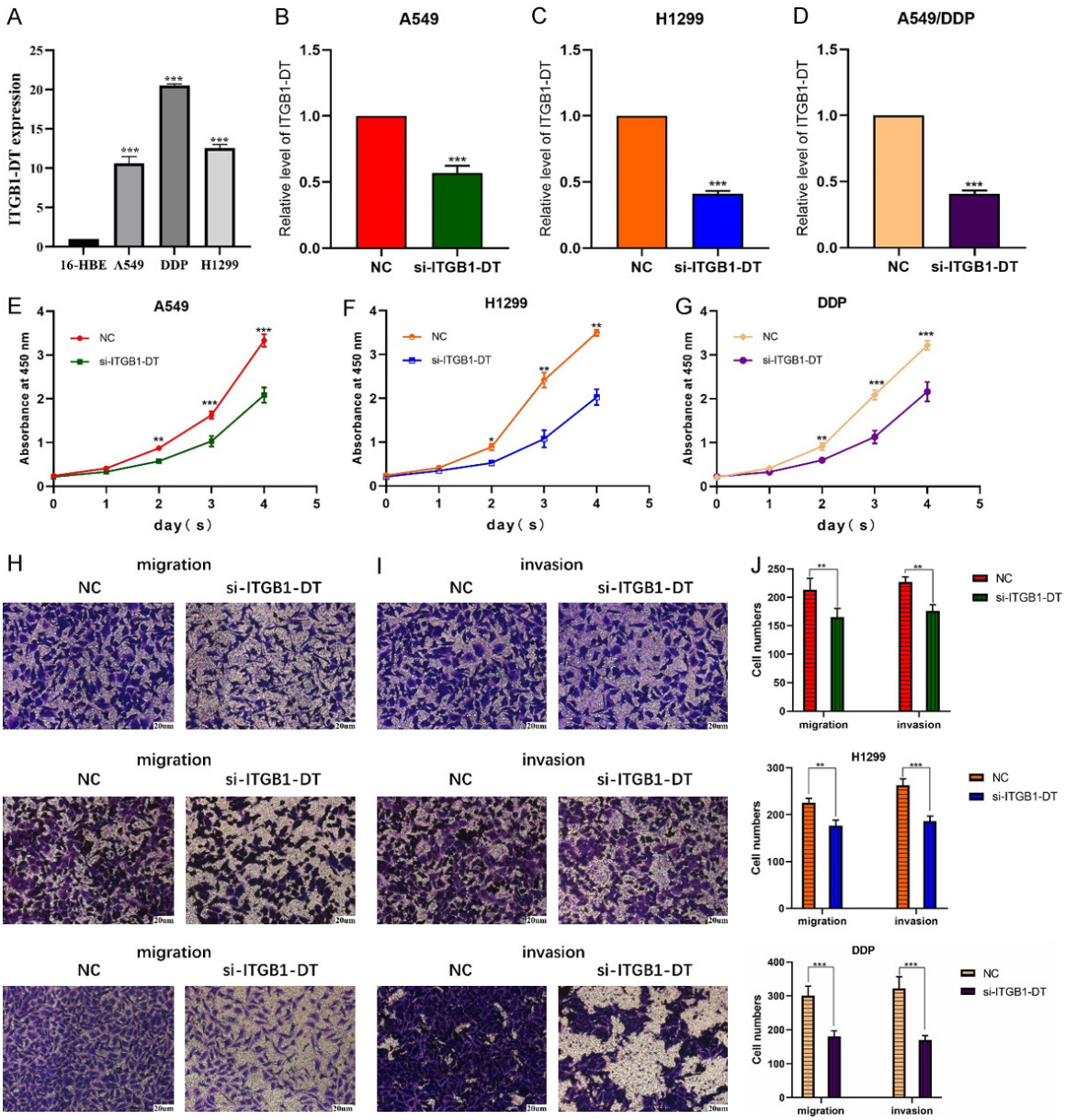


Figure 7. Interfering with ITGB1-DT expression inhibited NSCLC cell growth, migration and invasion of NSCLC cells. Note: NSCLC, non-small cell lung cancer.

IP2, GGTLC1, LIPK, SERPINA3, SERPINA5, BNIP3P42, KLK6, RHOF, ARL14, CREB3L3, APCDD1L, ANGPTL3, EPHA5, GPR22, OLFM1, CRHR2, CYP2A6, TH, SLC13A2, VEGFC, TMA-16P2, CDK5R2, EREG, HHIPL2, G0S2, CRIS-P3, AC007496.3, L1CAM, EIF5P1, SNORA22, TCN1, REG4, FGA, PNPLA5, CPHL1P, FAM-133A, AL096712.1, Y_RNA, SNORA12, TR-IM15, AC025031.5, TAS2R43, AL136310.1, FAHD2P1, TUBB8P11, LCN1, ADH1, COL19A1, among other genes. K-M survival analysis showed that *AC025031.5, AL162578.1, APCD-D1L, AREG, ARL14, CCK, CDK5R2, CIDEC, CR391992.1, CREB3L3, CRHR2, DKK1, EREG, FEZF1, FIBCD1, FLNC, FSIP2, GPR78, HSD-17B13, INSYN2B, KCNF1, LAMC2, LCN1, LIPK, NTSR1, OLFM1, TCN1,* and *VEGFC* were significantly associated with the OS of NSCLC patients (**Figures 11** and S3).

Furthermore, *AREG*, *CIDEC*, *CR391992.1*, *CRHR2*, *DKK1*, *RETREG2*, *FIBCD1*, *KCNF1*, *LAMC2*, *LCN1*, *LIPK*, *NTSR1*, *OLFM1*, *TCN1*, and *VEGFC* expressions were significantly associated with

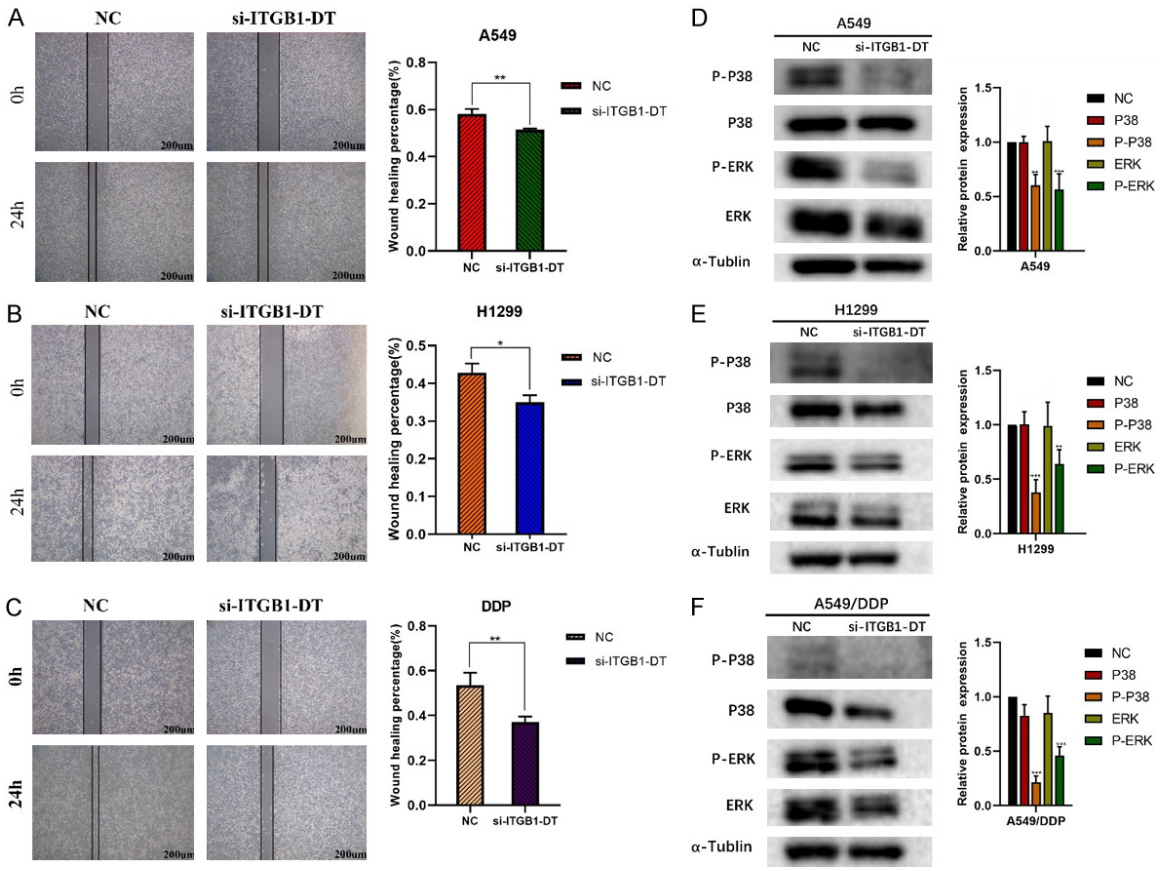


Figure 8. Inhibition of ITGB1-DT expression inhibited NSCLC cell migration via the MAPK/ERK pathway. Note: NSCLC, non-small cell lung cancer.

the OS of LUAD patients (**Figure 12**). The expression levels of *AL1625781*, *FEZF1*, *FLNC*, *INSYN2B*, and *LIPK* were significantly associated with the OS of LUSC patients (Figure S4).

Construction of nomogram based on ITGB1-DT-related prognostic genes

Univariate Cox regression analysis found that the expression levels of *AL162578.1, AREG, CIDEC, CR391992.1, CRHR2, DKK1, EREG, FEZF1, FIBCD1, FLNC, INSYN2B, KCNF1, LAMC2, LCN1, LIPK, NTSR1, OLFM1, TCN1*, and *VEGFC* were the risk factors of OS in NSCLC patients (**Table 4**). Multivariate Cox regression analysis found that the expression levels of AL162578.1, CIDEC, DKK1, and OLFM1 were the independent risk factors of the OS in NSCLC patients (**Table 4**). A nomogram of NSCLC patients was then constructed based on the ITGB1-DT prognosis genes (**Figure 13A**).

Furthermore, univariate Cox regression analysis found that the expression levels of *AREG, CIDEC, CR391992.1, CRHR2, DKK1, FIBCD1, KCNF1, LAMC2, LCN1, LIPK, NTSR1, OLFM1, TCN1,* and *VEGFC* were the risk factors of OS in LUAD patients (**Table 5**). Multivariate Cox regression analysis found that the expression levels of *CR391992.1, DKK1, NTSR1, OLFM1,* and *TCN1* were the independent risk factors of OS in LUAD patients (**Table 5**). A nomogram of LUAD patients was then constructed based on the ITGB1-DT prognosis genes (**Figure 13B**). Univariate Cox regression analysis found that the expression levels of *AL162578.1, FEZF1, FLNC, INSYN2B*, and *LIPK* were the risk factors of OS in LUSC patients (**Table 6**). Multivariate Cox regression analysis found that the expression levels of *CR391992.1, DKK1,* and *TCN1* were the independent risk factors of OS in LUSC patients (**Table 6**). A nomogram of LUAD patients was also constructed based on the ITGB1-DT related prognosis genes (**Figure 13C**).

Herein, T stage, N stage, and M stage were the risk factors for dismal prognosis in cancer

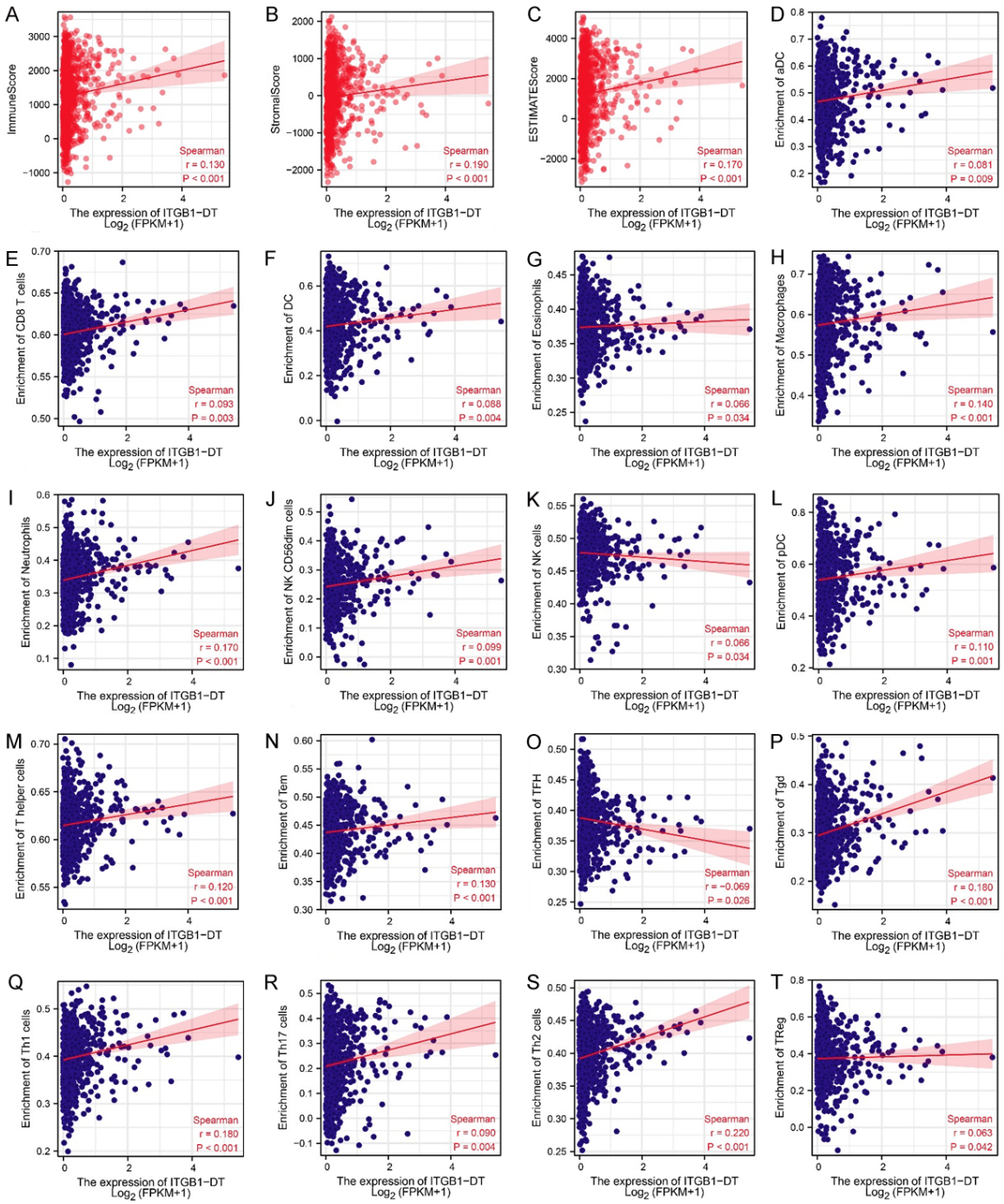


Figure 9. ITGB1-DT expression level was associated with immune infiltration in NSCLC. Note: NSCLC, non-small cell lung cancer.

patients, and Cox regression analyses showed that ITGB1-DT overexpression was the risk factors for poor prognosis in cancer patients. Therefore, the nomograms of NSCLC, LUAD, and LUSC patients were constructed (**Figures 14**, S5 and S6).

Discussion

LncRNA is closely related to the occurrence and development of cancers and the progression of NSCLC [18-22]. For instance, the significantly upregulated lncRNA AFAP1-AS1 in

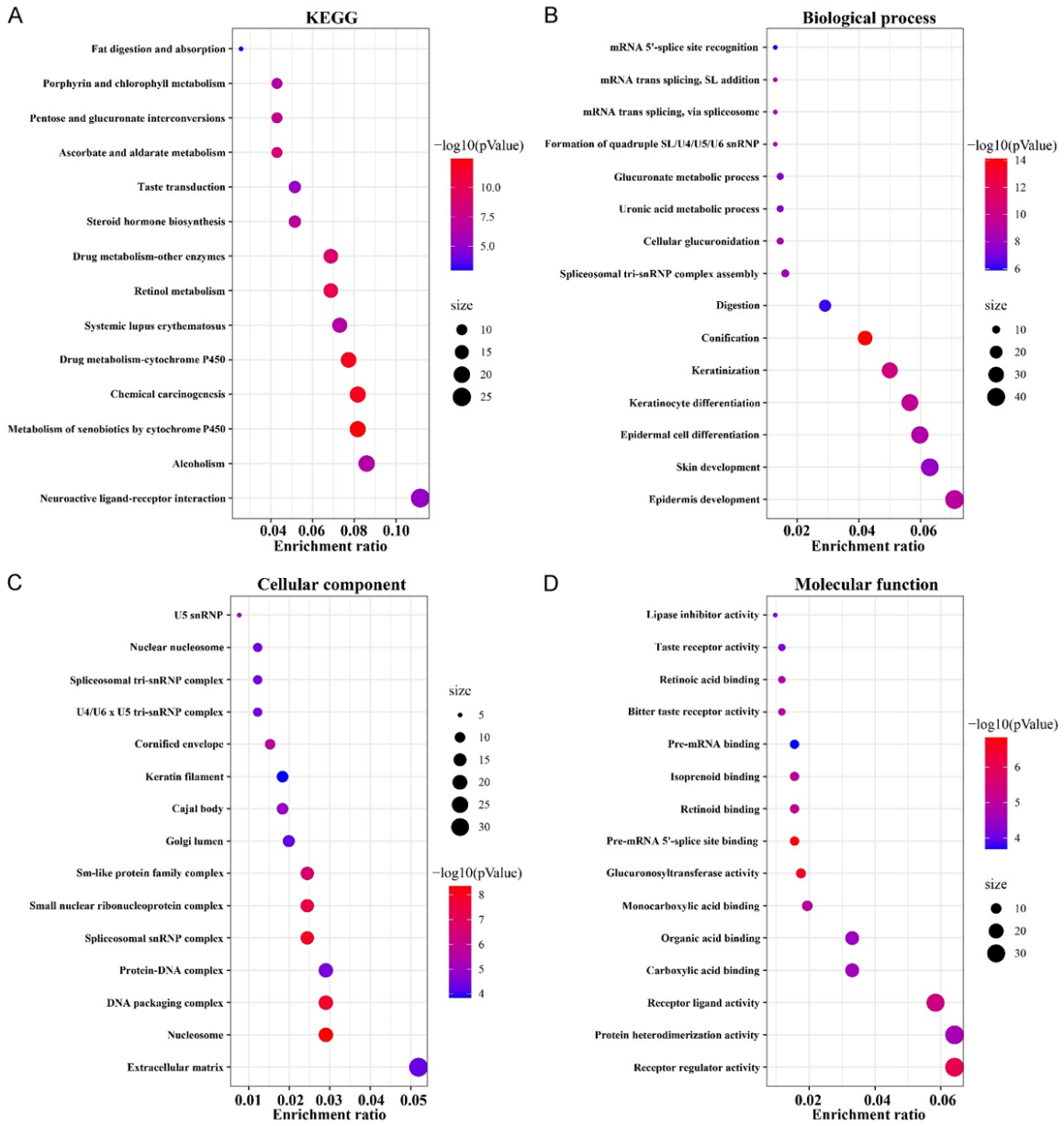


Figure 10. Functions and mechanisms of ITGB1-DT-related genes. A. Signaling pathways using KEGG analysis; B. Biological process; C. Cellular component; D. Molecular function.

NSCLC tissues is correlated with clinical outcomes of NSCLC patients. Inhibition of AFAP1-AS1 expression in NSCLC cells decreases cell growth ability *in vitro* and *in vivo*. AFAP1-AS1 epigenetically represses p21 expression by interacting with EZH2 and recruiting EZH2 to the p21 promoter region [19]. The significantly upregulated lncRNA ANRIL in NSCLC tissues is correlated with lymph node metastasis, tumor size and poor prognosis in NSCLC patients. Downregulation of ANRIL expression inhibits cell proliferation and induces apoptosis *in vitro* and *in vivo* [22]. Chang et al. reported that ITGB1-DT expression is significantly upregulated in LUAD tissues. Moreover, ITGB1-DT overexpression is associated with late clinical stage and poor prognosis of LUAD patients. ITGB1-DT overexpression also promotes LUAD cell proliferation, migration, invasion, and metastasis. ITGB1-DT knockdown inhibits LUAD cell proliferation, migration, and invasion. ITGB1-DT can exert oncogenic effects through the ITGB1/WNT/β-catenin/MYC signaling mechanism, which constitutes a positive feedback mecha-

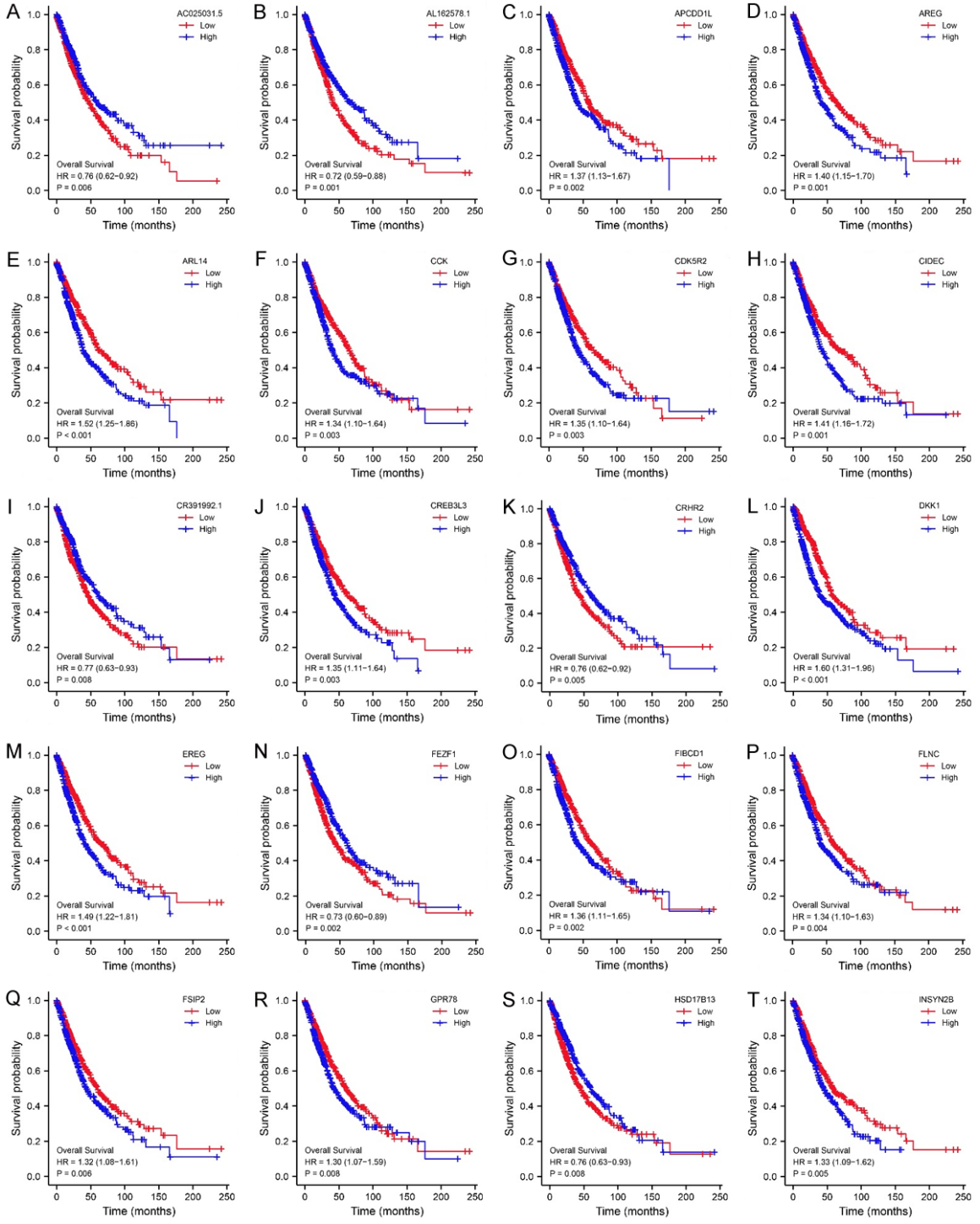


Figure 11. The prognostic roles of ITGB1-DT-related genes in NSCLC patients. A. AC025031.5; B. AL162578.1; C. APCDD1L; D. AREG; E. ARL14; F. CCK; G. CDK5R2; H. CIDEC; I. CR391992.1; J. CREB3L3; K. CRHR2; L. DKK1; M. EREG; N. FEZF1; O. FIBCD1; P. FLNC; Q. FSIP2; R. GPR78; S. HSD17B13; T. INSYN2B. Note: NSCLC, non-small cell lung cancer.

nism [14, 15]. However, the mechanism of ITGB1-DT in NSCLC progression, especially LUSC, is unclear. In this study, ITGB1-DT was highly expressed in NSCLC, LUAD, and LUSC tissues based on the TCGA database data. Moreover, ITGB1-DT could predict the diagnosis

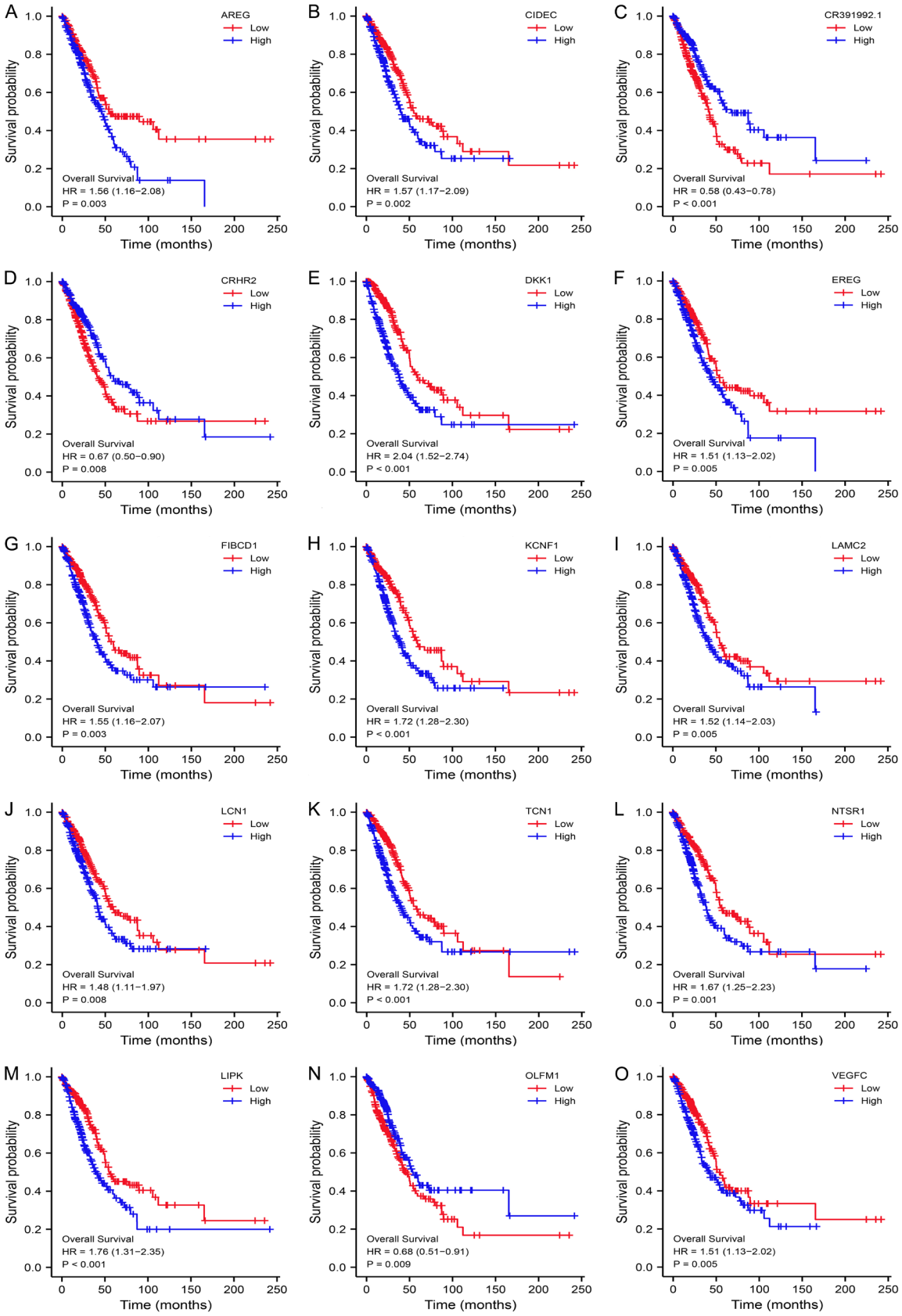


Figure 12. The prognostic roles of ITGB1-DT-related genes in LUAD patients. A. AREG; B. CIDEC; C. CR391992.1; D. CRHR2; E. DKK1; F. EREG; G. FIBCD1; H. KCNF1; I. LAMC2; J. LCN1; K. LIPK; L. NTSR1; M. OLFM1; N. TCN1; O. VEGFC. Note: LUAD, lung adenocarcinoma.

Table 4. ITGB1-DT-related prognostic genes in NSCLC patients determined using the Cox regression analysis

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
AL162578.1	1022				
Low	511	Reference			
High	511	0.698 (0.572-0.850)	< 0.001	0.728 (0.594-0.893)	0.002
AREG	1022				
Low	511	Reference			
High	511	1.398 (1.147-1.704)	< 0.001	1.120 (0.884-1.418)	0.347
CIDEC	1022				
Low	511	Reference			
High	511	1.402 (1.150-1.708)	< 0.001	1.295 (1.055-1.589)	0.013
CR391992.1	1022				
Low	511	Reference			
High	511	0.758 (0.622-0.924)	0.006	0.852 (0.692-1.048)	0.129
CRHR2	1022				
Low	511	Reference			
High	511	0.765 (0.628-0.932)	0.008	0.949 (0.768-1.172)	0.625
DKK1	1022				
Low	511	Reference			
High	511	1.614 (1.321-1.972)	< 0.001	1.540 (1.242-1.911)	< 0.001
EREG	1022				
Low	511	Reference			
High	511	1.488 (1.220-1.814)	< 0.001	1.133 (0.879-1.461)	0.335
FEZF1	1022				
Low	511	Reference			
High	511	0.734 (0.602-0.894)	0.002	0.842 (0.683-1.039)	0.109
FIBCD1	1022				
Low	511	Reference			
High	511	1.355 (1.113-1.650)	0.002	1.039 (0.826-1.308)	0.743
FLNC	1022				
Low	511	Reference			
High	511	1.339 (1.099-1.632)	0.004	1.077 (0.857-1.353)	0.527
INSYN2B	1022				
Low	511	Reference			
High	511	1.324 (1.086-1.613)	0.005	1.114 (0.889-1.398)	0.348
KCNF1	1022				
Low	511	Reference			
High	511	1.372 (1.126-1.671)	0.002	1.114 (0.893-1.391)	0.338
LAMC2	1022				
Low	511	Reference			
High	511	1.487 (1.220-1.812)	< 0.001	1.004 (0.796-1.267)	0.973
LCN1	1022				
Low	511	Reference			
High	511	1.320 (1.084-1.607)	0.006	1.024 (0.825-1.270)	0.831
LIPK	1022				
Low	511	Reference			
High	511	1.437 (1.180-1.751)	< 0.001	1.141 (0.916-1.422)	0.238

NTSR1	1022				
Low	511	Reference			
High	511	1.292 (1.061-1.573)	0.011	1.037 (0.834-1.291)	0.742
OLFM1	1022				
Low	511	Reference			
High	511	0.768 (0.631-0.935)	0.009	0.807 (0.658-0.989)	0.038
TCN1	1022				
Low	511	Reference			
High	511	1.309 (1.075-1.595)	0.007	1.135 (0.922-1.398)	0.231
VEGFC	1022				
Low	511	Reference			
High	511	1.336 (1.097-1.628)	0.004	1.036 (0.828-1.295)	0.758

Note: NSCLC, non-small cell lung cancer.

of NSCLC, LUAD, and LUSC. ITGB1-DT overexpression was associated with short OS, DSS, and PFI of NSCLC and LUAD patients. It was also associated with short DSS and PFI in LUSC patients. ITGB1-DT overexpression was an independent risk factor in NSCLC patients, similar to Chang et al. study. These results demonstrate that ITGB1-DT is a suitable prognostic marker in NSCLC patients.

Chang et al. found that ITGB1-DT overexpression promotes LUAD cell proliferation, migration, and invasion [14, 15]. Herein, interfering with ITGB1-DT expression promoted NSCLC sensitivity to cisplatin. Moreover, interfering with the expression of ITGB1-DT inhibited proliferation, invasion, and migration in A549/DDP cells. GSEA analysis indicated that interference of ITGB1-DT expression affected insulin signaling pathway, GNRH signaling pathway, ABC transporters, Adipocytokine signaling pathway, Calcium signaling pathway, PPAR signaling pathway, FC epsilon RI signaling pathway, MAPK signaling pathway, WNT signaling pathway, etc. The MAPK signaling pathway, WNT signaling pathway, and ABC transporters are associated with NSCLC growth metastasis and cisplatin resistance [23-30]. For instance, lncRNA SDPR-AS is lowly expressed in NSCLC tissues and cells. Downregulation of SDPR-AS enhances NSCLC cell proliferation, migration, and invasion and inhibits apoptosis. However, SDPR-AS overexpression inhibits cell proliferation, migration, and invasion and enhances apoptosis. SDPR-AS regulates the malignant behavior of NSCLC cells by regulating SDPR expression and participating in the p38 MAPK/ERK signaling pathway [23]. LncRNA NNT-AS1 is highly expressed in drug-resistant cells. Interfering with the expression of NNT-AS1 increases the IC50 value of drug-resistant cells, inhibits the proliferation of drug-resistant cells and promotes apoptosis and cell cycle arrest. NNT-AS1 promotes the resistance of NSCLC cells to DDP through the MAPK/Slug signaling pathway [24]. Herein, western-blotting showed that the interference with ITGB1-DT expression affected the MAPK signaling mechanism. Moreover, interfering with ITGB1-DT expression in NSCLC cells inhibited p-p38 MAPK and p-ERK protein expression, while it did not significantly affect p38 MAPK and ERK protein expression. However, the effect of interfering with ITGB1-DT expression on other signaling mechanisms requires further studies.

Presently, immunotherapy is the key treatment method for cancer, which can improve the prognosis of cancer patients [31-33]. Zhang et al. observed higher objective response rates in PD-L1-overexpressing NSCLC patients than in PD-L1-underexpression group based on meta-analysis [33]. Epidermal growth factor receptor (EGFR) phosphorylation can activate JANUS kinase 2 (JAK2), signal transducer and activator of transcription 3 (STAT3), thus regulating PD-L1 expression. Nobiletin can inhibit PD-L1 expression via EGFR/JAK2/STAT3 signaling pathway. miR-197 enhances antitumor immunity by regulating STAT3 and PD-L1 expression levels. Nobiletin can induce PD-1/PD-L1 blockade, a key factor in immune escape mechanisms [34]. Herein, ITGB1-DT expression was significantly correlated with NSCLC immune score, stromal score, and estimate score, as well as NSCLC aDC, CD8 T, macrophages, and other

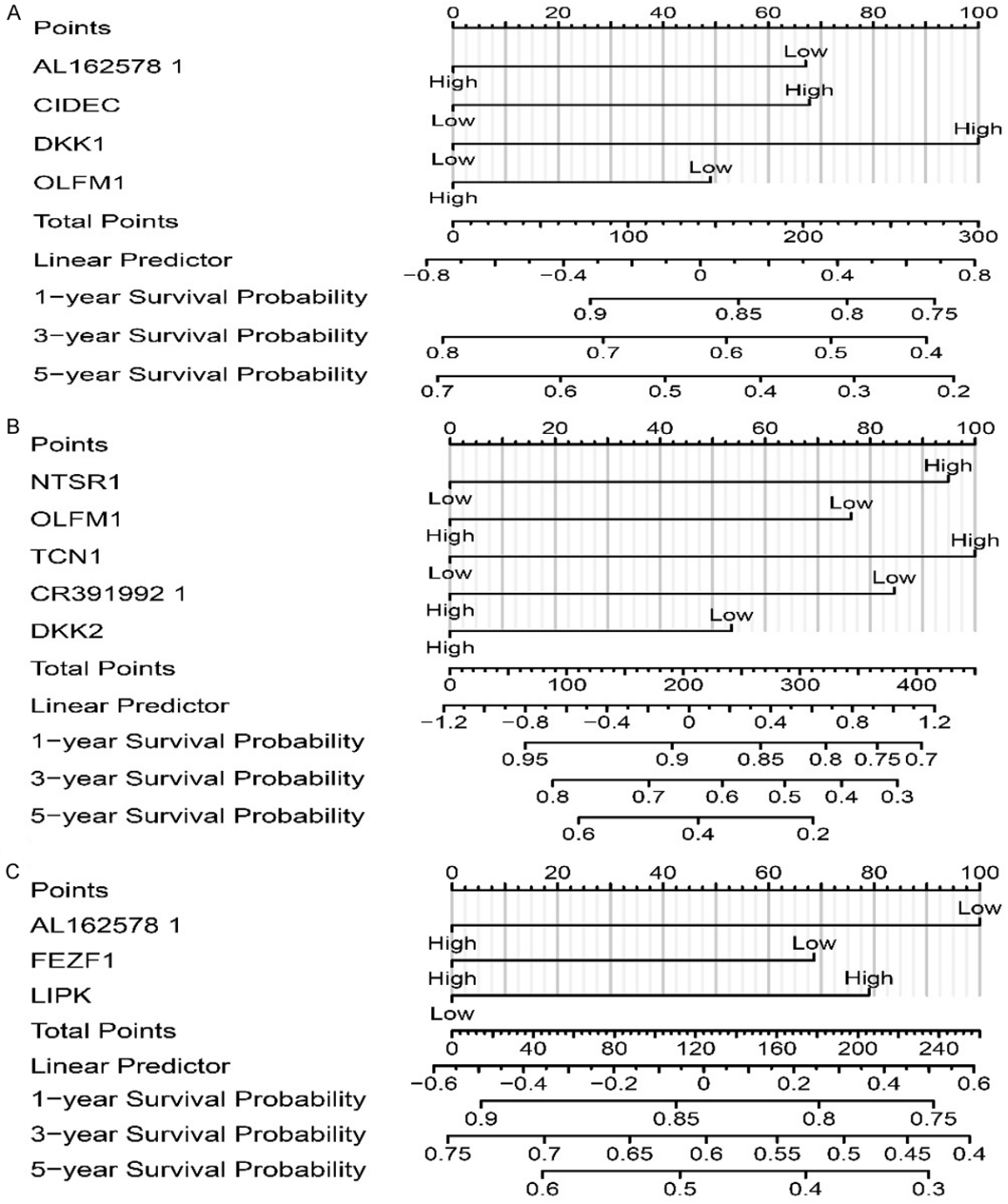


Figure 13. Construction of a nomogram based on ITGB1-DT-related prognostic genes. A. NSCLC; B. LUAD; C. LUSC. Note: NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

immune levels. However, the roles of ITGB1-DT in immune infiltration in NSCLC should be validated based on cell models. Overall, this study explored the roles of ITGB1-DT in NSCLC using bioinformatics analysis and basic research. However, future basic research is needed to confirm the results.

Conclusions

In this study, the overexpressed ITGB1-DT in NSCLC tissues was correlated with high T stage, N stage, M stage, short OS, DSS, and PFI of NSCLC patients. NSCLC patients with elevated ITGB1-DT expression have poor OS, DSS, and

Table 5. ITGB1-DT-related prognostic genes in LUAD patients determined using the Cox regression analysis

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
AREG	526				
Low	263	Reference			
High	263	1.553 (1.162-2.077)	0.003	1.156 (0.836-1.598)	0.381
CIDEC	526				
Low	263	Reference			
High	263	1.572 (1.177-2.099)	0.002	1.119 (0.815-1.536)	0.486
CR391992.1	526				
Low	263	Reference			
High	263	0.583 (0.435-0.782)	< 0.001	0.713 (0.524-0.971)	0.032
CRHR2	526				
Low	263	Reference			
High	263	0.686 (0.514-0.917)	0.011	0.838 (0.619-1.134)	0.252
DKK1	526				
Low	263	Reference			
High	263	2.038 (1.516-2.738)	< 0.001	1.486 (1.067-2.068)	0.019
RETREG2	526				
Low	263	Reference			
High	263	0.874 (0.657-1.164)	0.358		
FIBCD1	526				
Low	263	Reference			
High	263	1.537 (1.150-2.052)	0.004	1.126 (0.812-1.562)	0.478
KCNF1	526				
Low	263	Reference			
High	263	1.744 (1.299-2.340)	< 0.001	1.341 (0.960-1.873)	0.085
LAMC2	526				
Low	263	Reference			
High	263	1.548 (1.159-2.068)	0.003	0.805 (0.558-1.160)	0.244
LCN1	526				
Low	263	Reference			
High	263	1.489 (1.115-1.988)	0.007	1.078 (0.787-1.476)	0.642
LIPK	526				
Low	263	Reference			
High	263	1.755 (1.310-2.351)	< 0.001	1.179 (0.824-1.687)	0.368
NTSR1	526				
Low	263	Reference			
High	263	1.666 (1.246-2.229)	< 0.001	1.401 (1.021-1.921)	0.037
OLFM1	526				
Low	263	Reference			
High	263	0.679 (0.507-0.909)	0.009	0.636 (0.467-0.867)	0.004
TCN1	526				
Low	263	Reference			
High	263	1.718 (1.285-2.297)	< 0.001	1.419 (1.030-1.954)	0.032
VEGFC	526				
Low	263	Reference			
High	263	1.483 (1.110-1.982)	0.008	1.190 (0.868-1.631)	0.280

Note: LUAD, lung adenocarcinoma.

Table 6. ITGB1-DT-related prognostic genes in LUSC patients determined using the Cox regression analysis

Characteristics	N	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P	HR (95% CI)	P
AL162578.1	496				
Low	248	Reference			
High	248	0.632 (0.480-0.831)	0.001	0.648 (0.491-0.855)	0.002
FEZF1	496				
Low	248	Reference			
High	248	0.665 (0.507-0.874)	0.003	0.739 (0.559-0.977)	0.034
FLNC	496				
Low	248	Reference			
High	248	1.414 (1.078-1.855)	0.012	1.323 (0.993-1.761)	0.056
INSYN2B	496				
Low	248	Reference			
High	248	1.492 (1.137-1.958)	0.004	1.208 (0.903-1.618)	0.203
LIPK	496				
Low	248	Reference			
High	248	1.441 (1.098-1.891)	0.008	1.326 (1.005-1.750)	0.046

Note: LUSC, lung squamous cell carcinoma.

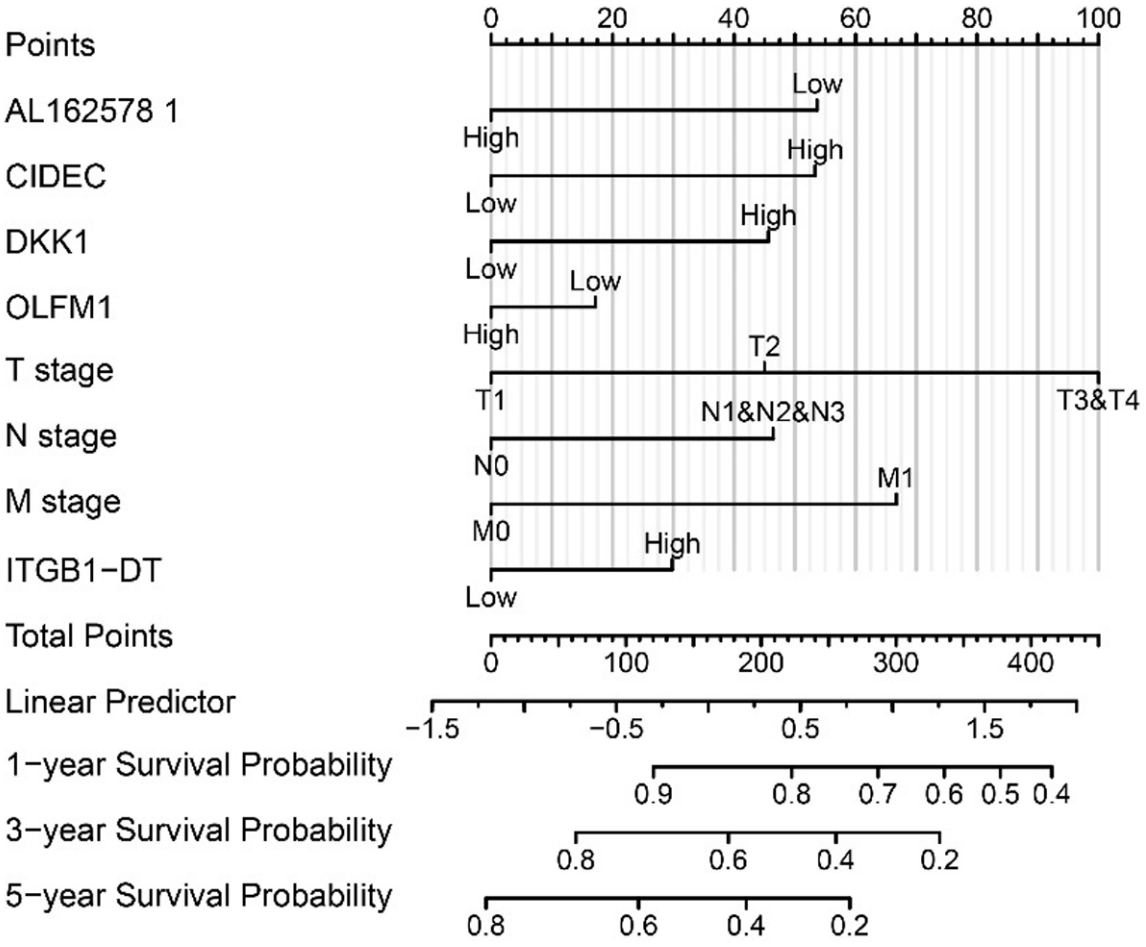


Figure 14. Construction of nomogram based on ITGB1-DT-related prognostic genes and TNM stage in NSCLC. Note: NSCLC, non-small cell lung cancer.

PFI. Therefore, ITGB1-DT expression is an independent risk factor for poor prognosis in cancer patients and has diagnostic value for NSCLC. Interfering with the expression of ITGB1-DT inhibited the proliferation, migration, and invasion of NSCLC A549, H1299, and drug-resistant A549/DDP cells. Moreover, interfering with ITGB1-DT expression delayed the growth and metastasis of NSCLC through the MAPK/ERK signaling mechanism, and enhanced the sensitivity of NSCLC to cisplatin. Therefore, ITGB1-DT may be a biomarker for the diagnosis and prognosis of NSCLC patients.

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Disclosure of conflict of interest

None.

Abbreviations

NSCLC, Non-small cell lung cancer; lncRNA, Long non-coding RNA; OS, Overall survival; DSS, Disease-specific survival; PFI, Progression-free interval; LUAD, Lung adenocarcinoma; DFS, Disease-free survival; LUSC, Lung squamous cell carcinoma; TCGA, The Cancer Gene Atlas.

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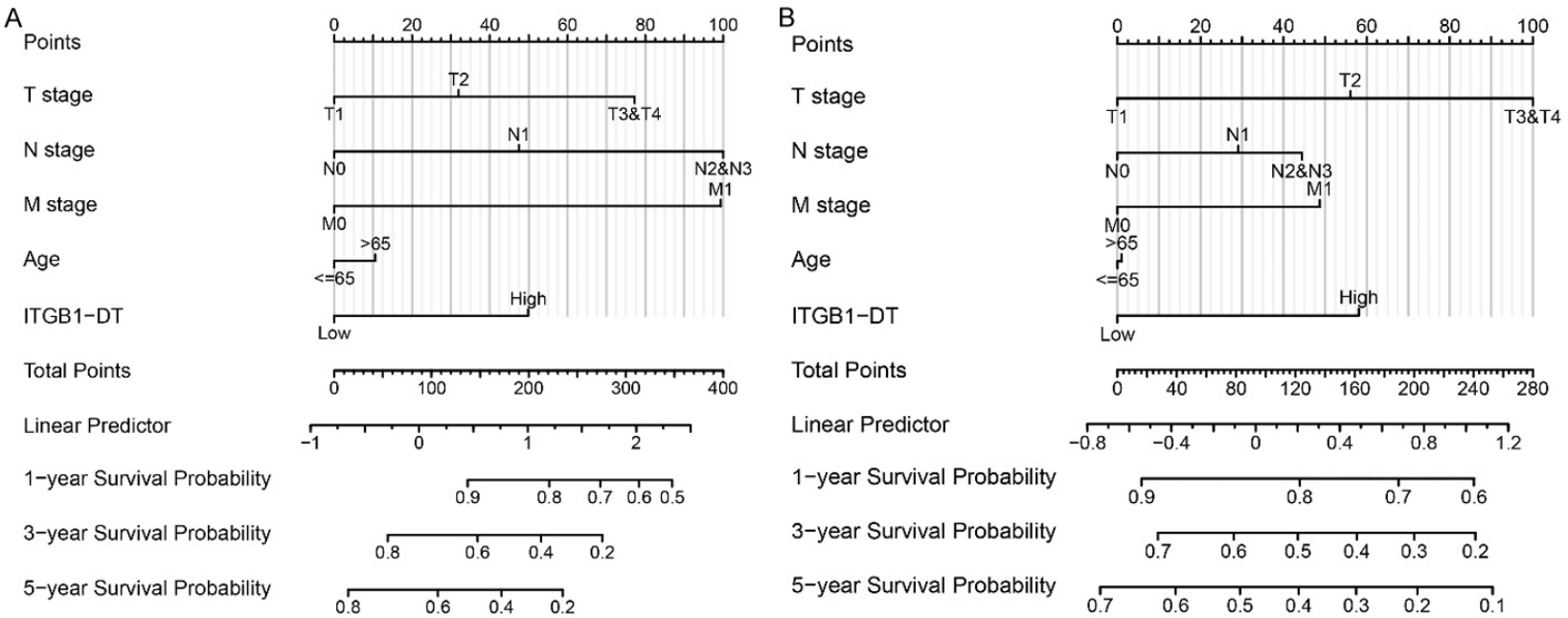
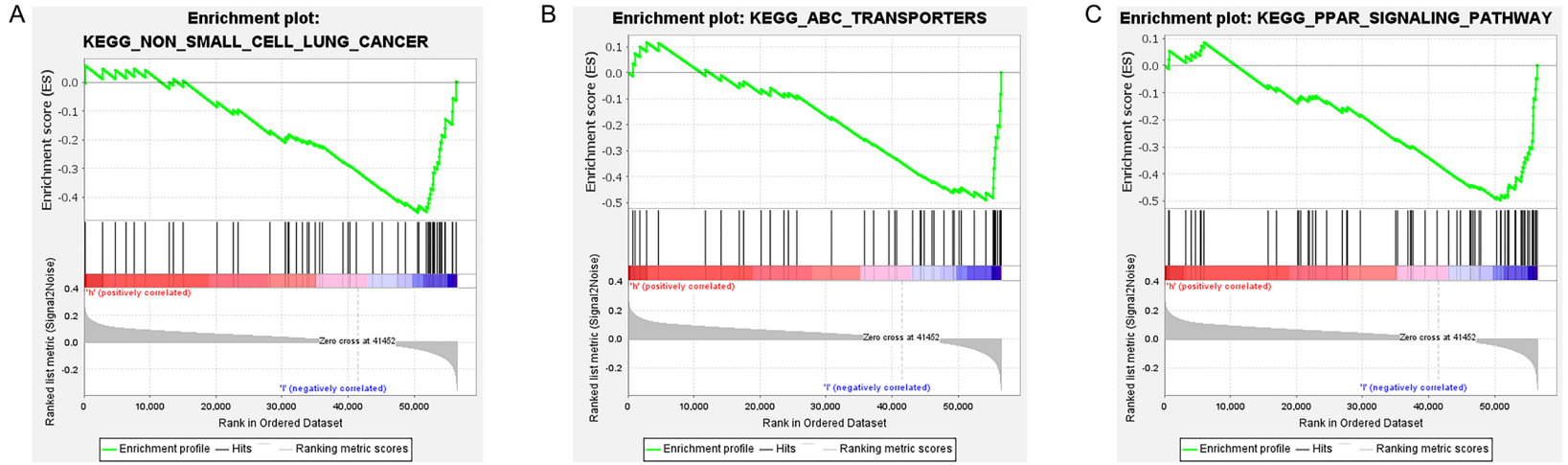


Figure S1. A nomogram based on ITGB1-DT and clinical characteristic factors for predicting DSS and PFI NSCLC. Note: NSCLC, non-small cell lung cancer; DSS, disease-specific survival; PFI, progression-free interval.



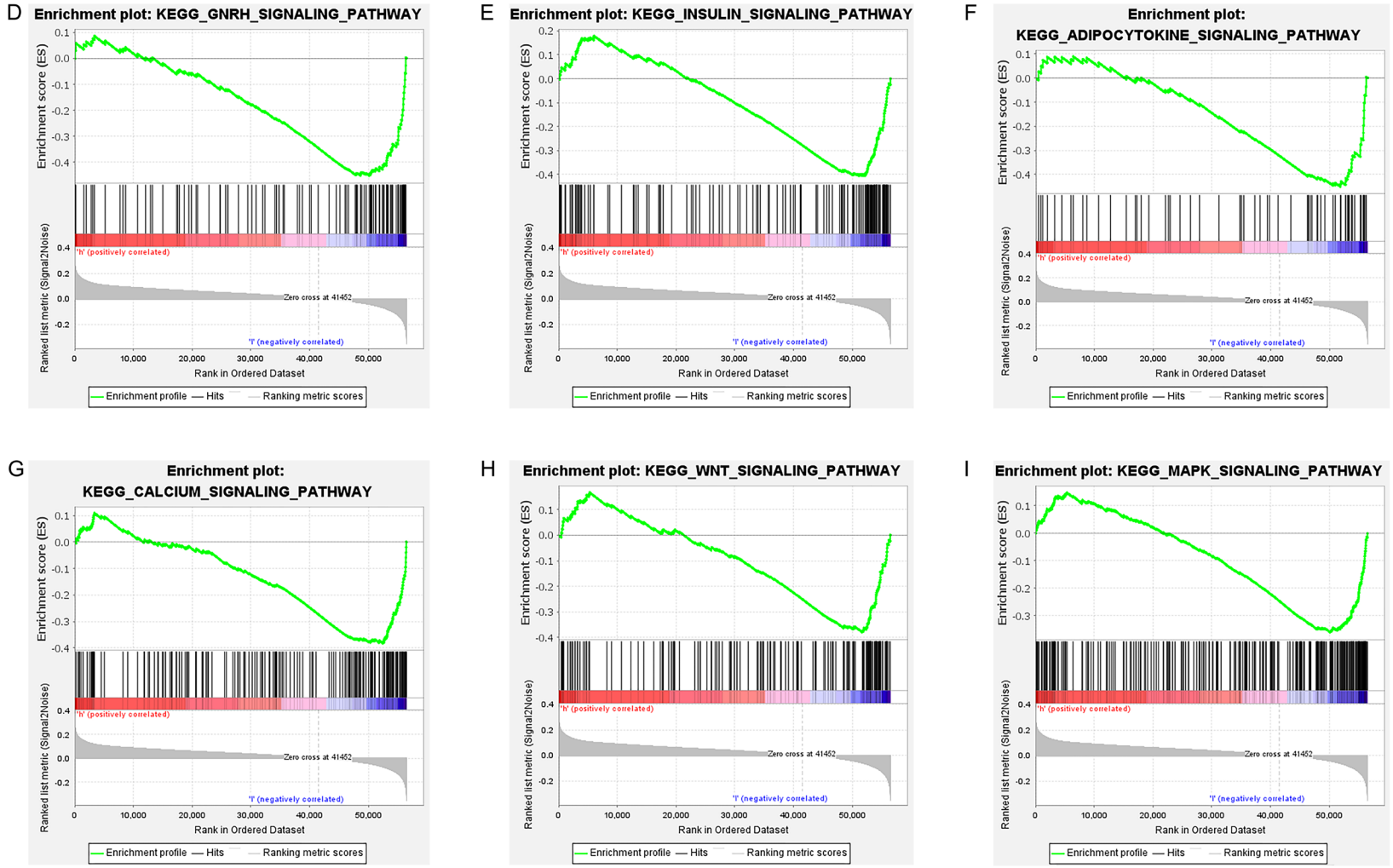


Figure S2. The signaling pathway of ITGB1-DT in NSCLC using GSEA analysis. Note: GSEA, Gene Set enrichment analysis; NSCLC, non-small cell lung cancer.

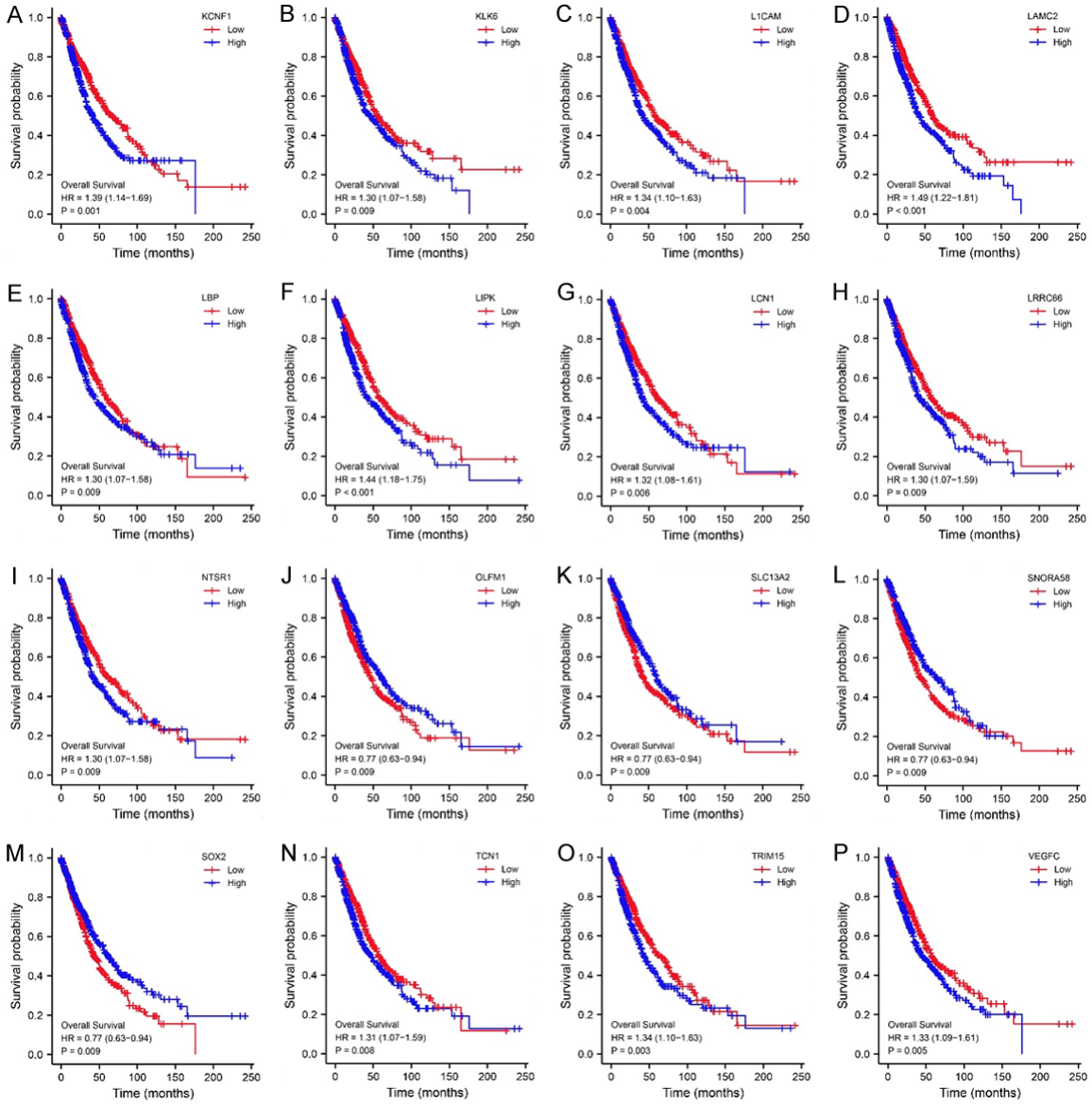


Figure S3. The prognosis roles of ITGB1-DT-related genes in NSCLC patients. Note: NSCLC, non-small cell lung cancer.

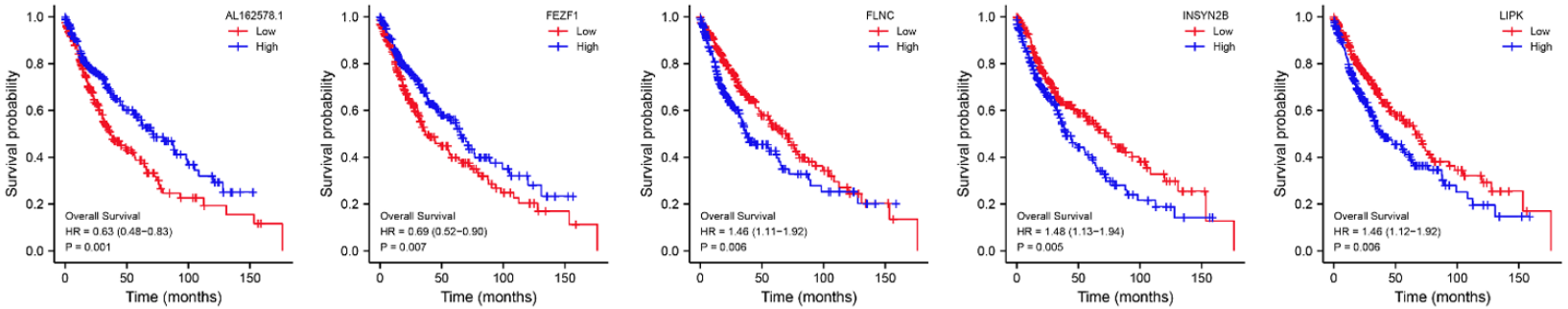


Figure S4. The prognosis roles of ITGB1-DT-related genes in LUSC patients. Note: LUSC, lung squamous cell carcinoma.

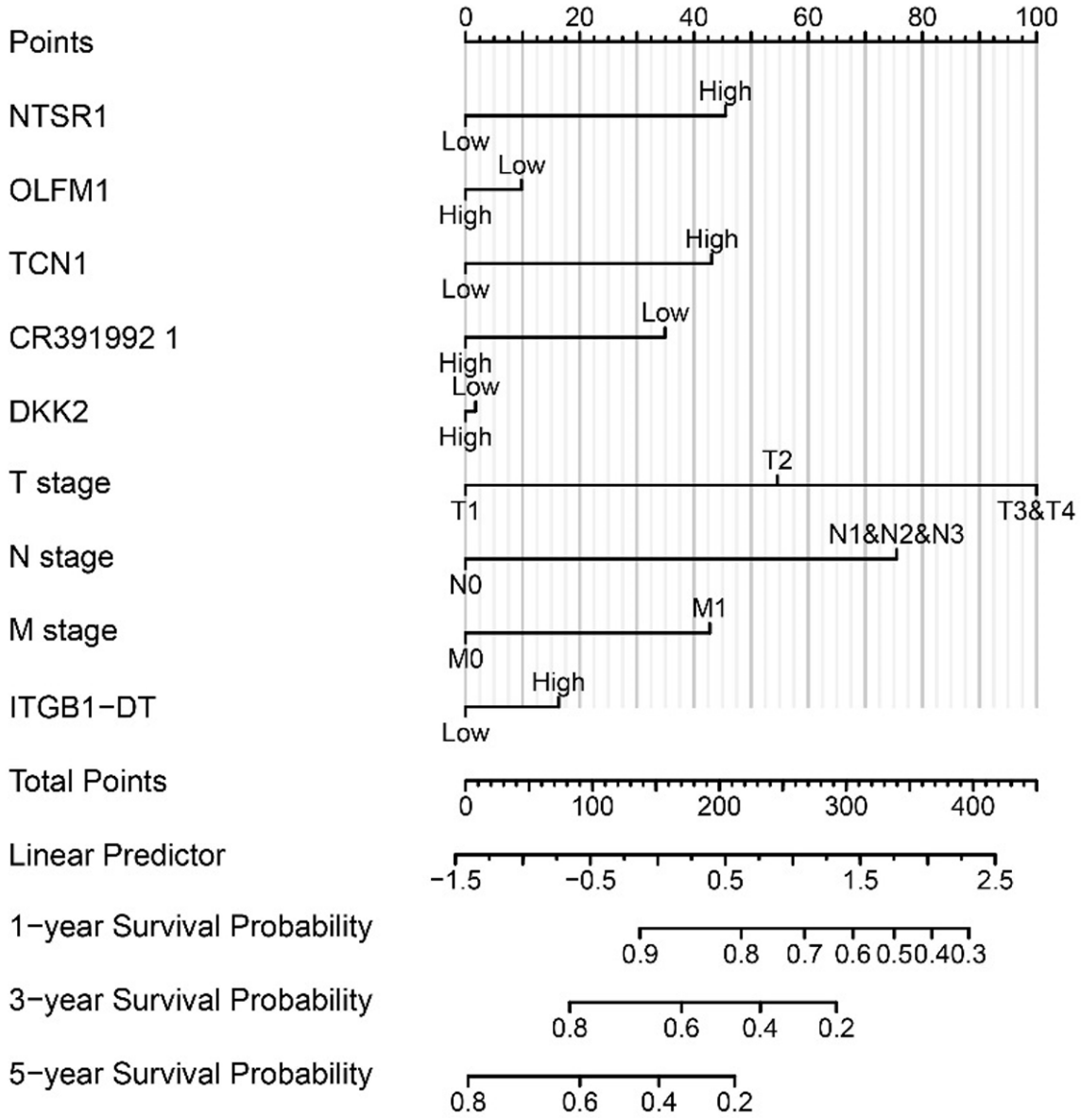


Figure S5. Construction of nomogram in ITGB1-DT-related prognostic genes and TNM stage for predicting outcomes of LUAD patients. Note: LUAD, lung adenocarcinoma.

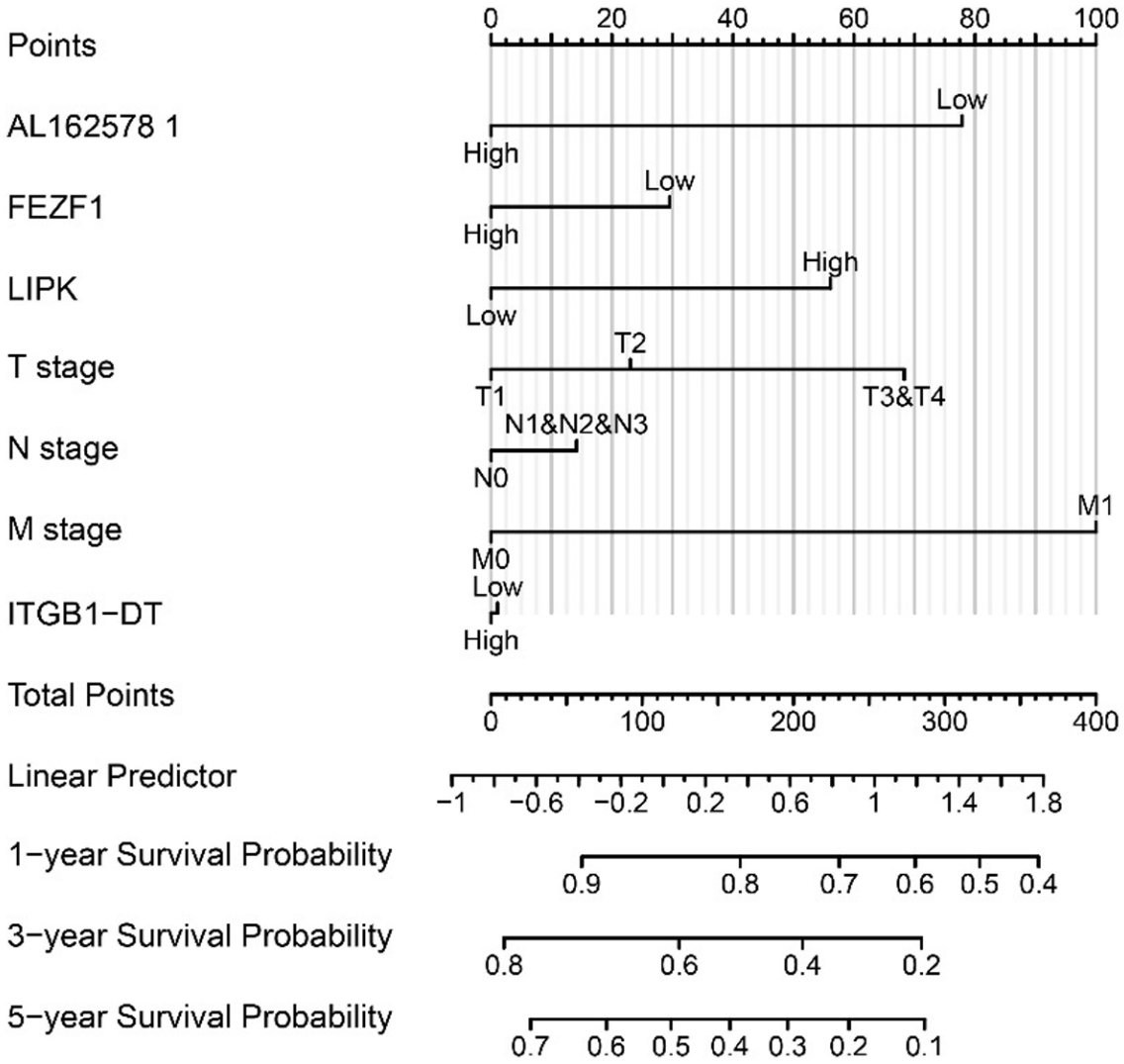


Figure S6. Construction of nomogram based on ITGB1-DT-related prognostic genes and TNM stage for predicting outcomes of LUSC patients. Note: LUSC, lung squamous cell carcinoma.