Supplementary

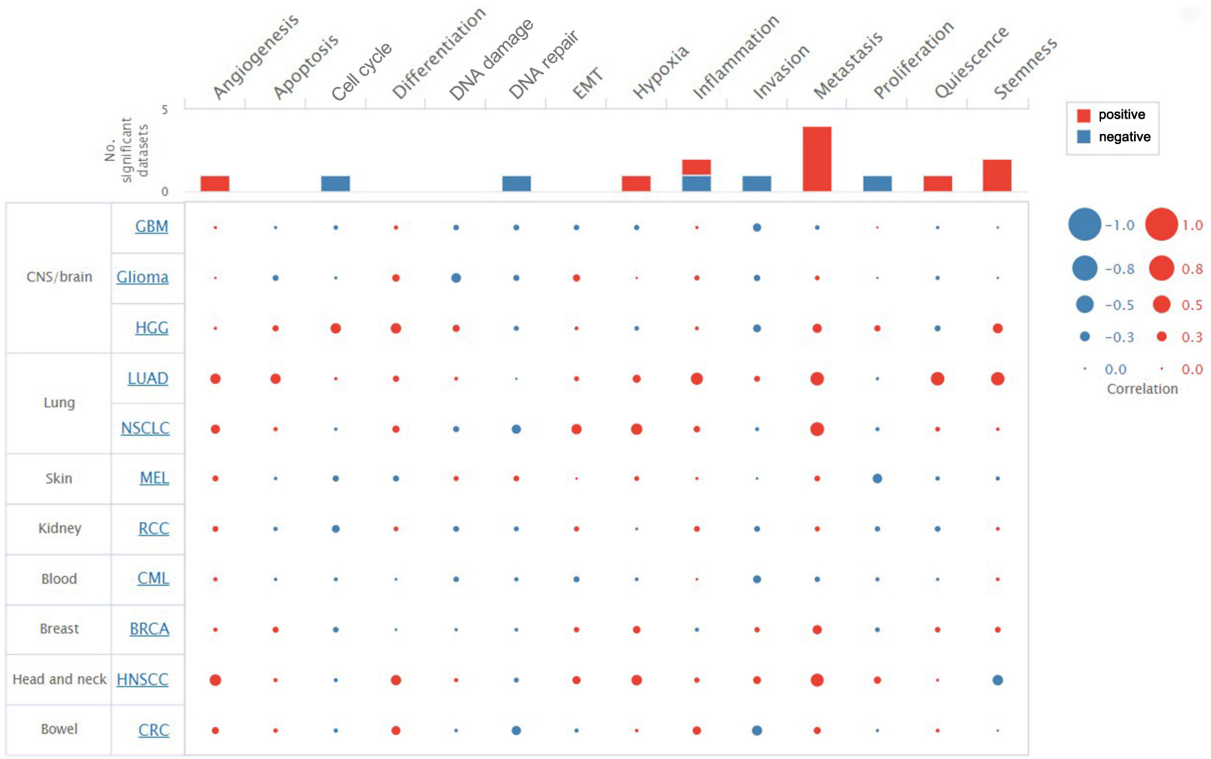

Figure S1 Functional states analysis of *ITGB4* in CancerSEA database. *ITGB4* was positively associated with most of the functional states of lung adenocarcinoma cells, especially with inflammation and metastasis in CancerSEA database. EMT, Epithelial-Mesenchymal Transition; No., Number; CNS, central nervous system; GBM, glioblastoma; HGG, high-grade glioma; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; MEL, melanoma; RCC, renal cell carcinoma; CML, chronic myelogenous leukemia; BRCA, breast cancer; HNSCC, head and neck squamous cell carcinoma; CRC, colorectal cancer.

https://dx.doi.org/10.21037/tlcr-24-50

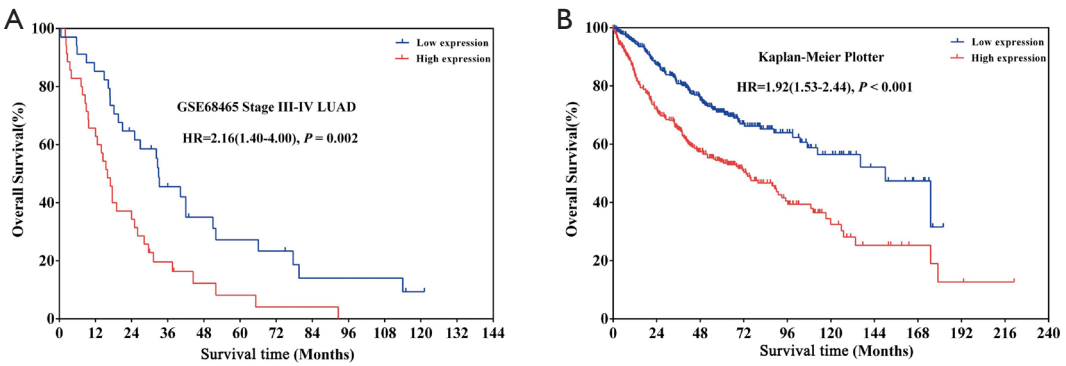

Figure S2 *ITGB4* expression is significantly associated with a worse prognosis of patients with LUAD. (A) 69 advanced LUAD patients (stage III–IV) from the GSE68465 dataset. (B) 719 LUAD patients from the Kaplan-Meier plotter (http://kmplot.com). HR, hazard ratio; LUAD, lung adenocarcinoma.

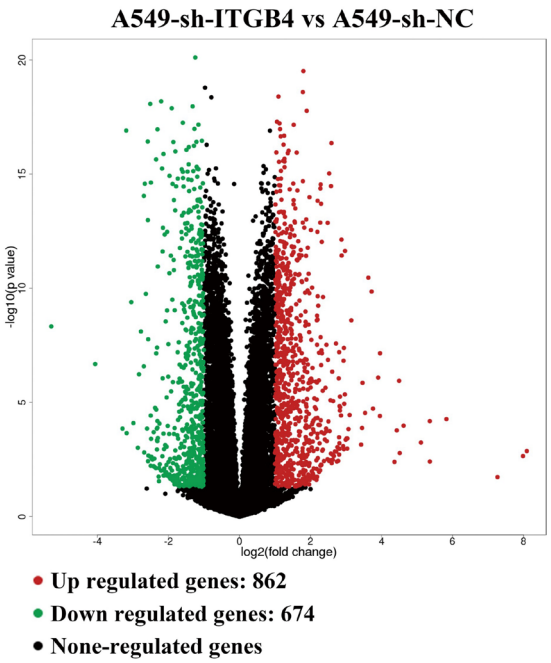

Figure S3 Volcano plot of the differentially expressed genes in RNA transcriptome sequencing data. RNA transcriptome sequencing analysis was performed to analyze the gene expression profile in A549 cells with *ITGB4* silencing. A volcano plot showed the differentially expressed genes. sh-ITGB4, short hairpin RNAs against ITGB4; sh-NC, short hairpin RNAs negative control.

https://dx.doi.org/10.21037/tlcr-24-50

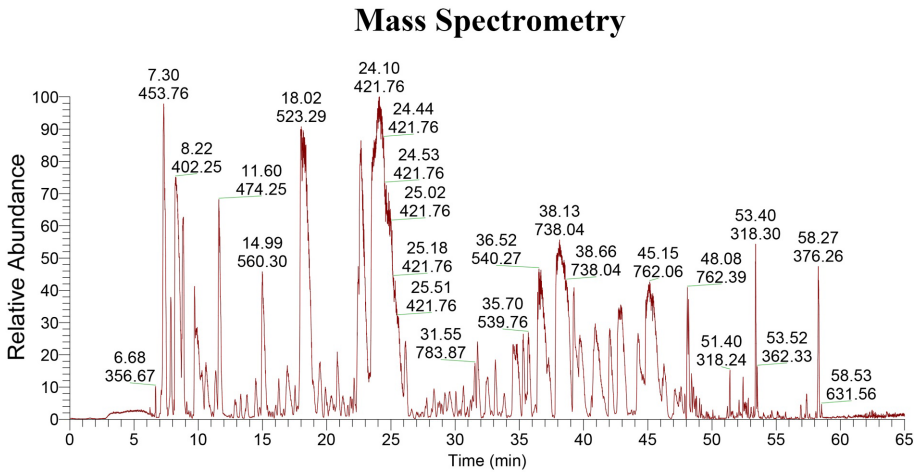

Figure S4 Mass spectrometry analysis of the proteins in the immunoprecipitation assay of *ITGB4* in A549 cells.

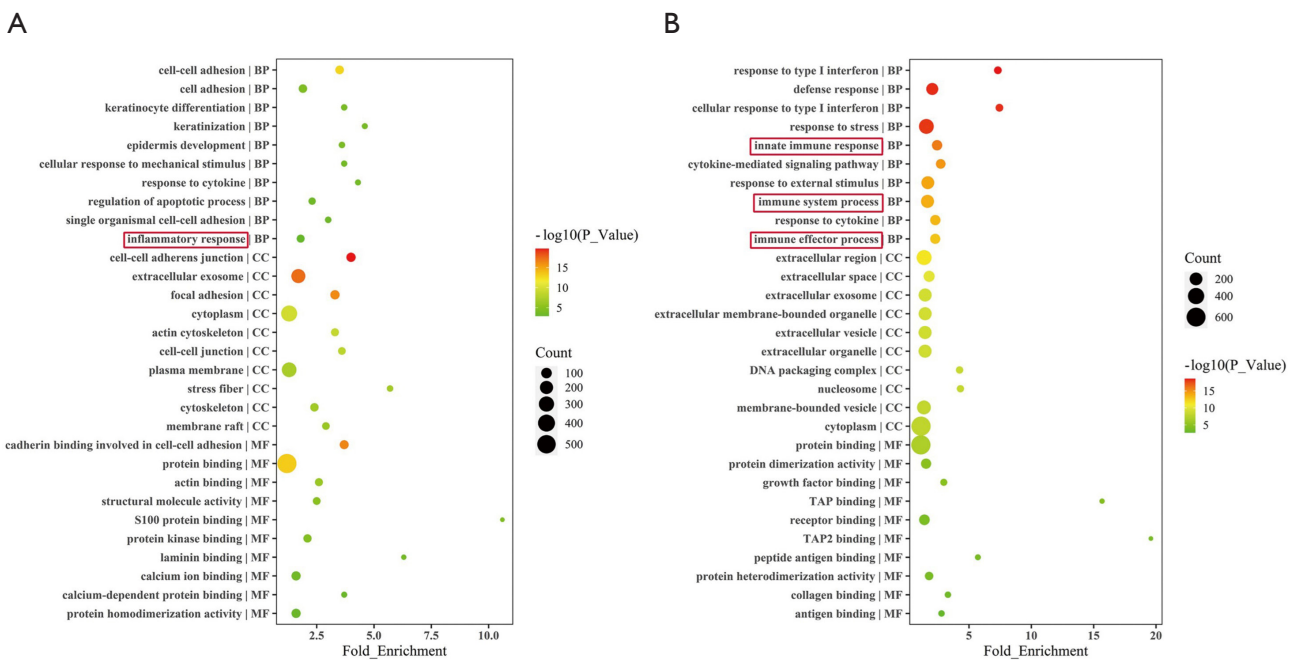

Figure S5 Gene Ontology analysis based on RNA transcriptome sequencing data and TCGA dataset. (A) Highly enriched BP term of *ITGB4* in TCGA dataset was inflammatory response. (B) Highly enriched BP terms of *ITGB4* in RNA transcriptome sequencing dataset were innate immune response, immune system process and immune effector process. TCGA, The Cancer Genome Atlas; CC, Cellular Component; MF, molecular function; BP, biological process.

https://dx.doi.org/10.21037/tlcr-24-50

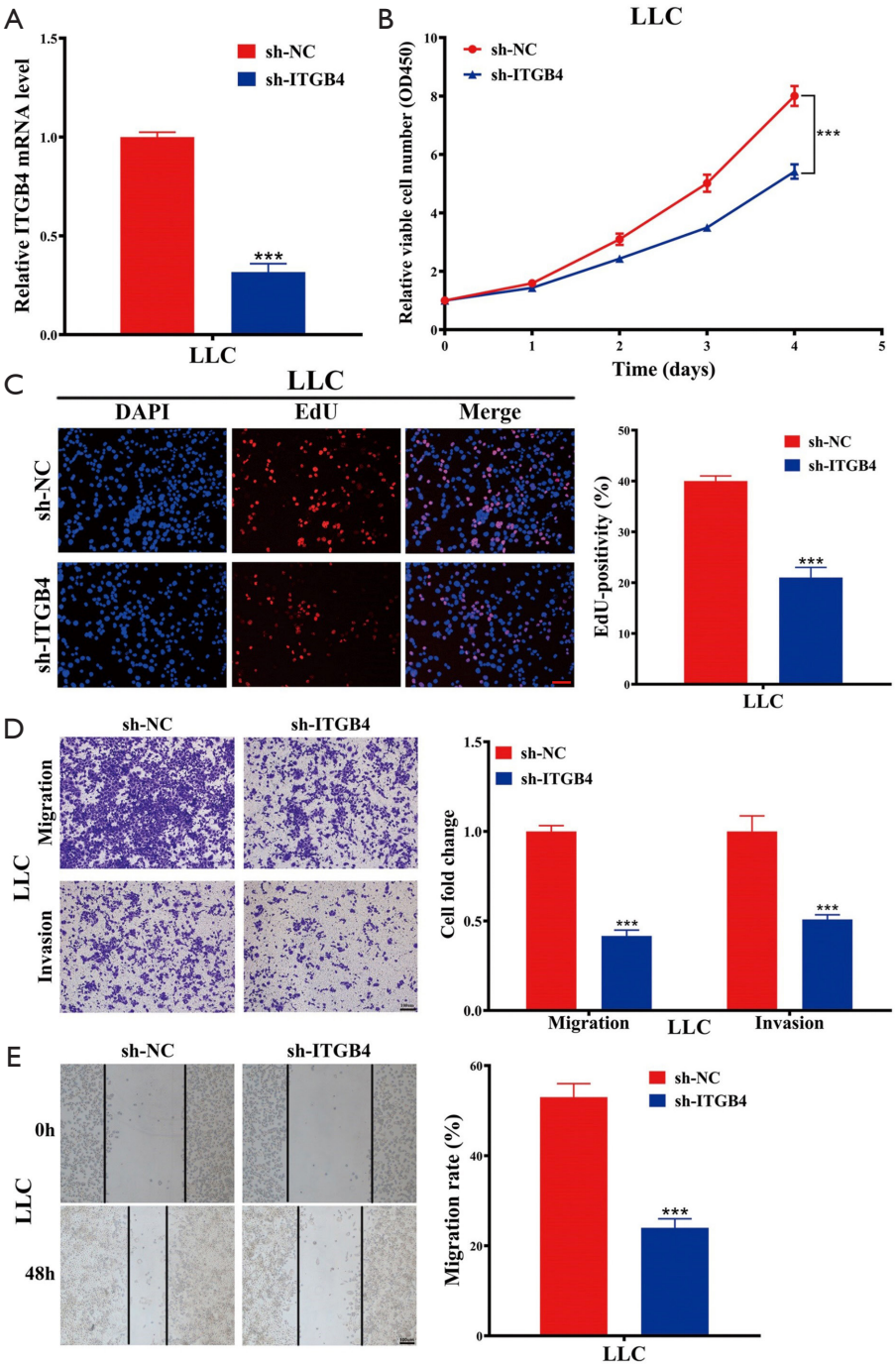

Figure S6 *ITGB4* promotes LLC cell proliferation, migration and invasion *in vitro*. (A) The effectiveness of *ITGB4* knockdown in LLC cells was confirmed by qRT-PCR analysis. (B) Cell proliferation curves were determined by CCK-8 assay after knocking down *ITGB4* in LLC cells. (C) EdU assay was conducted to evaluate cell proliferation following *ITGB4* knockdown in LLC cells. Scale bar, 50 μm. (D) Transwell assays were performed to assess the migration and invasion abilities of LLC cells upon *ITGB4* silencing. Scale bar, 100 μm. (E) Wound healing assay was carried out to evaluate the migration of LLC cells. Scale bar, 100 μm. Values are expressed as the mean ± standard deviation. *, P<0.05; **, P<0.01; ***, P<0.001. sh-ITGB4, short hairpin RNAs against ITGB4; sh-NC, short hairpin RNAs negative control; OD450, optical density at 450 nanometer; DAPI, 4',6-diamidino-2-phenylindole; EdU, 5-ethynyl-2-deoxyuridine; qRT-PCR, quantitative reverse transcription polymerase chain reaction; LLC, Lewis lung carcinoma.

https://dx.doi.org/10.21037/tlcr-24-50

Table S1 Characteristics of twenty paired lung adenocarcinoma samples used in this study

Sample ID	Gender	Age (years)	Tumor size (cm)	Subtypes	Differentiation	EGFR	Ki-67 (%)
JSPH01	Female	66	2.2	Acinar	I–II	E21 p. L858R	5
JSPH02	Male	72	5.0	Acinar	II	NA	10
JSPH03	Female	70	1.3	Acinar	I–II	E21 p. L858R	NA
JSPH04	Male	64	2.5	Micropapillary	II–III	Wild type	50
JSPH05	Male	67	2.5	Micropapillary	II–III	NA	30
JSPH06	Male	66	3.5	Solid	III	NA	60
JSPH07	Male	52	2.0	Micropapillary	II–III	NA	NA
JSPH08	Female	52	1.5	Acinar	I–II	NA	NA
JSPH09	Male	75	2.7	Papillary	II–III	Wild type	15
JSPH10	Male	73	1.6	Acinar	I–II	E21 p. L858R	5
JSPH11	Male	57	2.0	Acinar	I–II	E19	10
JSPH12	Male	50	1.5	Micropapillary	II–III	NA	NA
JSPH13	Female	55	2.0	Acinar	II	E19	NA
JSPH14	Female	56	3.0	Micropapillary	II–III	E20	25
JSPH15	Male	64	2.6	Acinar	II–III	E21 p. L858R	30
JSPH16	Male	59	2.0	Acinar	II	NA	5
JSPH17	Female	66	1.3	Solid	II–III	Wild type	20
JSPH18	Female	66	2.0	Acinar	I–II	E21 p. L858R	8
JSPH19	Female	56	1.5	Papillary	II	E21 p. L858R	NA
JSPH20	Female	58	1.3	Papillary	II	E21 p. L858R	NA

NA, not available; EGFR, estimated glomerular filtration rate.

Table S2 Sequences of siRNAs and shRNAs

Name	siRNA sequence (5' to 3')
sh-NC	TTCTCCGAACGTGTCACGT
sh-ITGB4#1 (human)	GCCTACTGCACAGACGAGATGTTCA
sh-ITGB4#2 (human)	CCTATAGCTACTACGAGAAGCTTCA
sh-ITGB4 (mouse)	CCGGCATCATGAACCGCAATGATGA
si-TFAP2A	Sense: GGGUAUUAACAUCCCAGAU
	Antisense: AUCUGGGAUGUUAAUACCCGG

siRNAs, small interfering RNAs; shRNAs, short hairpin RNAs; NC, negative control.

https://dx.doi.org/10.21037/tlcr-24-50

Table S3 Primers for quantitative reverse transcription polymerase chain reaction

Name	Primer sequence (5' to 3')
ITGB4	
Forward	CTCCACCGAGTCAGCCTTC
Reverse	CGGGTAGTCCTGTGTCCTGTA
TFAP2A	
Forward	GCTGGGCACTGTAGGTCAATC
Reverse	TGGGAGTAAGGATCTTGCGACT
β-actin	
Forward	CATGTACGTTGCTATCCAGGC
Reverse	CTCCTTAATGTCACGCACGAT

Table S4 Antibodies used in the study

Name	Company	Catalog number
ITGB4	Abcam	ab182120
p65	Abcam	ab32536
p-p65	Abcam	ab183559
IκBα	Abcam	ab32518
p-IκBα	Abcam	ab133462
IKKα/β	Abcam	ab178870
p-IKKα/β	Abcam	ab194528
TFAP2A	Abcam	ab52222
Anti-rabbit IgG, HRP-linked antibody	Abcam	ab6721
GAPDH	Abcam	ab181602
Recombinant human laminin-5 protein	Abcam	ab190413
Lamin B1	Abcam	ab133741
Anti-human CD4 antibody (IHC)	Abcam	ab133616
Anti-human CD8 antibody (IHC)	Abcam	ab245118
Anti-mouse CD4 antibody (IHC)	Abcam	ab183685
Anti-mouse CD8 antibody (IHC)	Abcam	ab217344
Ki-67	Abcam	ab279653

IgG, immunoglobulin G; IHC, immunohistochemistry.

https://dx.doi.org/10.21037/tlcr-24-50

Table S5 The top 20 proteins identified through mass spectrometry in the immunoprecipitation assay of ITGB4 in A549 cells

No.	Gene names	Unique peptides	Abundances (IP: ITGB4)	Abundances (IP: IgG)
1	*LIMA1*	15	658002991	0
2	*AIFM1*	16	456653323	0
3	*TPM4*	19	423985832	0
4	*ITGB4*	17	385194144	0
5	*DBN1*	13	285183992	0
6	*HIST1H1E*	21	227387554	0
7	*CAPZA2*	12	196453716	0
8	*CSNK1A1*	17	182016528	0
9	*UBAP2L*	11	179244107	0
10	*RPS2*	18	174538733	0
11	*KRT31*	14	153244804	0
12	*ZNF185*	12	133074577	0
13	*TNKS1BP1*	16	115573483	0
14	*NFKBIA*	13	109079161	0
15	*HNRPC*	10	90935096	0
16	*RPL18*	9	76517107	0
17	*PABPC4*	10	68809882	0
18	*DDX5*	11	55688484	0
19	*ERC1*	14	47490237	0
20	*MYO1D*	12	29366955	0

https://dx.doi.org/10.21037/tlcr-24-50