

# ITGBL1 promotes EMT, invasion and migration by activating NF- $\kappa$ B signaling pathway in prostate cancer

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**Background:** Integrin beta-like 1 (ITGBL1) was extensively demonstrated to contribute the metastasis and progression in a variety of cancers. However, its role of ITGBL1 in prostate cancer (PCa) is still not reported.

**Methods:** Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot were performed to detect ITGBL1 expression in PCa tissues and cell lines. Immunohistochemical (IHC) staining of ITGBL1 in 174 PCa tissues was performed. The influence of ITGL1 expression in PCa cells epithelial-mesenchymal transition (EMT), migration and invasion was investigated. Notably, the possible mechanisms underlying the action of ITGBL1 in vivo and vitro assays were explored.

**Results:** We analyzed PCa dataset from The Cancer Genome Atlas (TCGA) and found that ITGBL1 was upregulated in PCa tissues. Overexpression of ITGBL1 is positively associated with the progression and lymph node metastasis in PCa patients. Furthermore, upregulating ITGBL1 enhanced the invasion, migration abilities and EMT in PCa cells. Conversely, downregulating ITGBL1 exhibited an opposite effect. Our findings further demonstrated that ITGBL1 promoted invasion and migration via activating NF- $\kappa$ B signaling in PCa cells.

**Conclusion:** Therefore, our results identify a novel metastasis-related gene in PCa, which will help to develop a novel therapeutic strategy in metastatic PCa.

**Keywords:** ITGBL1, EMT, NF- $\kappa$ B signaling, lymph node metastasis and prostate cancer

## Introduction

Prostate cancer (PCa) is one of the prevalent malignancies in male worldwide and occupies approximately 20% of newly diagnosed cancers in males.<sup>1</sup> In China, although substantial progress in the treatment of localized PCa, such as radical prostatectomy and androgen ablation, metastatic PCa remains incurable due to limited therapeutic methods. The prime causes for the PCa patients deaths are mostly attributed to invasion and metastasis.<sup>2,3</sup> Thus, exploring the underlying mechanism in metastasis of PCa is crucial to develop therapeutic avenues to treat or prevent PCa metastasis.

Integrin beta-like 1 (ITGBL1) encodes a class of  $\kappa$ -integrin-associated protein with ten tandem integrin EGF-like repeats since discovered from an osteoblast cDNA library.<sup>4</sup> Unlike the most family members of EGF-like protein, neither an RGD (Arg-Gly-Asp)-binding domain nor a transmembrane domain was found in ITGBL1, implying that ITGBL1 exerts its function differing from those of integrin.<sup>5</sup> Recently, the functional roles of ITGBL1 in the development and

metastasis in numerous cancers have been extensively studied. For example, transcriptional upregulation of ITGBL1 by Runx2 promoted the bone metastasis via TGF- $\beta$  signaling pathway in breast cancer;<sup>6</sup> in addition, ITGBL1 has been reported to promote the adhesion and migration of ovarian cancer cell via FAK/SRC and Wnt/PCP signaling;<sup>7</sup> in gastrointestinal cancers, including colorectal cancer and gastric cancer, high level of ITGBL1 was closely associated with the invasive phenotypes of cancer cells, and predicted poor prognosis.<sup>8,9</sup> Therefore, the existing literature supports the idea that ITGBL1 functions as an important pro-metastatic oncogene in multiple human cancer types. Currently, however, any information regarding the clinical significance and functional role of ITGBL1 remain scanty in the metastasis of PCa.

In the present study, our results showed that ITGBL1 was elevated in PCa tissues, which was positively associated with poor clinicopathological characteristics and lymph node metastasis, as well as predicted poor progression-free survival. Furthermore, upregulating ITGBL1 promoted, while silencing ITGBL1 inhibited the invasion, migration abilities and EMT in PCa cells. Our findings further revealed that ITGBL1 promoted the migration and invasion abilities of PCa cells by activating NF- $\kappa$ B signaling. Therefore, our results unravel a new mechanism responsible for ITGBL1-induced invasion and migration abilities in PCa cells, suggesting that ITGBL1 may be used as a potential therapeutic target in metastatic PCa.

## Materials and methods

### Cell lines and cell culture

Normal prostate epithelial cells RWPE-1, primary PCa cell lines 22RV1, bone metastatic PCa cells PC-3 and VCaP, brain metastatic PCa DU145, lymph node metastatic PCa LNCaP were obtained from Shanghai Chinese Academy of Sciences cell bank (China). All were cultured according to the previous study.<sup>10</sup>

### Patients and tumor tissues

A total of 174 paraffin-embedded PCa tissues and 8 fresh PCa tissues were collected at The First hospital of Xiangtan city (Xiangtan, Hunan, China). The clinical and pathological evidence were required for diagnosis of PCa patients. Prior patients' consents and approval from the Institutional Research Ethics Committee were obtained from The First hospital of Xiangtan city. Furthermore, the patient consent was written informed consent, and in accordance with the

**Table 1** The relationship between ITGBL1 and clinicopathological characteristic in 174 patients with prostate cancer

Age (years)	Number of cases	ITGBL1 expression		P values
		Low	High	
Age (years)				
≤71	88	45	43	0.439
>71	86	49	37	
Differentiation				
Well/moderate	48	28	20	0.610
Poor	126	67	59	
Serum PSA				
<81.3	87	53	34	<0.001*
>81.2	87	23	65	
Gleason grade				
≤7	102	73	29	<0.001*
>7	72	22	50	
Operation				
TURP	42	22	20	0.485
Needle biopsy	143	63	80	
PP	83	39	44	
TURP+BO	15	10	5	
BO	12	5	7	
LNM-status				
N0	106	40	66	<0.001*
NI	68	74	21	

**Abbreviation:** PSA, prostate-specific antigen; TURP, Trans Urethral Resection Prostate; PP, Prior Prostatectomy; BO, Bilateral Orchiectomies; SD, Standard deviation; IHC, Immunological Histological Chemistry; LNM, lymph node metastasis.

Declaration of Helsinki. Table 1 summarized the clinicopathological characteristics of PCa patients.

### RNA extraction, transfection, and real-time PCR

The total RNA from tissues or cells was extracted using TRIzol (Life Technologies) according to the manufacturer's instructions. mRNA was reverse transcribed using the Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). cDNA was quantified and amplified on ABI 7500HT system (Applied Biosystems, Foster City, CA, USA) using SYBR Green I (Applied Biosystems). Real-time PCR was carried out as described previously.<sup>11</sup> The primer sequence of ITGBL1 was forward, 5'-GACTGCAAAGCAGGCTGGTATG-3'; reverse, 5'-GGAGGATAGCAGGTGCATTTGC-3'. The endogenous control for mRNA was GAPDH. Relative fold expressions were calculated with the comparative threshold cycle ( $2^{-\Delta\Delta C_t}$ ) method as previously described.<sup>12</sup>

## Plasmid, small interfering RNA and transfection

The cDNA of ITGBL1 was purchased from (Vigene Biosciences, Shandong, China) and cloned into the pSin-EF2 plasmid (Cambridge, MA, USA). Silencing ITGBL1 was carried out by cloning two short hairpin RNA (shRNA) oligonucleotides into the pSUPER-puro-retro vector (OligoEngine, Seattle, WA, USA). The control and pNF $\kappa$ B-luc plasmids were purchased from Promega Corporation. shRNAs or plasmids were transfected using Lipofectamine 3000 (Life Technologies) as previously described.<sup>13</sup>

## Western blotting

Western blotting was performed as described previously.<sup>14</sup> The E-cadherin (Cat#. 3195), Vimentin (Cat#. 5741) and Fibronectin (Cat#. 4706) antibodies were purchased from Cell Signaling Technology, ITGBL1 (Cat#: Ag11521) and p65 (Cat#. 10745-1-AP) from Proteintech, p84 (Cat#. PA5-27816) from Invitrogen. After stripped, the membranes were re probed with an  $\alpha$ -tubulin antibody (Sigma-Aldrich, USA) as the loading control.

## Invasion and migration assay

The migration and invasion assays were carried out using Transwell chamber consisting of 8-mm membrane filter inserts (Corning) without or with coated Matrigel (BD Biosciences) respectively as described previously.<sup>15</sup> Briefly, cells were trypsinized and resuspended in serum-free medium after serum starvation for 24 h. Thereafter,  $1.5 \times 10^5$  cells were added to the upper chamber, while the lower chamber was filled with medium containing 10% FBS. After incubation for 48 h, cells that had invaded through the coated membrane to the lower surface were fixed and stained. The cell count was performed under a microscope (100 $\times$ ), and the random 10 fields were captured. The total cell number in these 10 fields were used to compare the effect of ITGBL1 on the invasion/migration ability of different groups of PCa cells.

## Luciferase assay

Cells ( $4 \times 10^4$ ) were plated into the triplicate in 24-well plates and carried out as previously described.<sup>16</sup> Briefly, the indicated number of cells were transfected with 100 ng the control or pNF $\kappa$ B reporter luciferase plasmid, plus the pRL-TK Renilla plasmid with concentration of 5 ng (Promega) as manufacturer's protocol. A Dual-Luciferase Reporter Assay

Kit (Promega) was used to measure Luciferase and Renilla signals 36 h after transfection. Briefly, 5 copies of an NF- $\kappa$ B response element (NF- $\kappa$ B-RE) were transferred into the pGL3-basic by restriction endonuclease, which is followed by the luciferase reporter gene. Cells were plated in 24-well plates, proliferating to 60–80% confluence after 24 h of culture, and the pGL3(NF- $\kappa$ B/luc) or pGL3, plus pRL-TK Renilla plasmid (Promega) were transfected into cells using Lipofectamine 3000 (Life Technologies). Forty-eight hours after transfection, the transfection medium was replaced with fresh RPMI-1640 medium; cells were harvested and washed with PBS, and lysed with passive lysis buffer (Promega). The cell lysates were analyzed immediately using Synergy™ 2 microplate system (BioTek, Winooski, VT, US). Luciferase and Renilla luciferase were measured using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The luciferase activity of each lysate was normalized to Renilla luciferase activity.

## Immunohistochemistry

The immunohistochemistry (IHC) was carried out as previously described.<sup>17</sup> The IHC scores were obtained from two independent investigators to further comparatively evaluating ITGBL1 expression in PCa tissues. The IHC score was calculated as the product of the staining intensity score and the proportion of positive tumor cells, which were evaluated as previously described.<sup>18</sup>

## Tumor xenografts

The twenty BALB/c-nu mice (6-week-old) were randomly divided into four groups.  $1 \times 10^6$  22Rv1 tumor cells were injected subcutaneously into the right flanks of each mouse in 100  $\mu$ l of RPMI mixed with Matrigel. In overexpression of ITGBL1 with JSH-23 treatment group, the ITGBL1 overexpression 22RV1 cells were treated with JSH-23 (20  $\mu$ mol/L)<sup>19</sup> for 24h and then implanted subcutaneously in the right flanks of each mouse. In overexpression of ITGBL1 with Y2409881 treatment group, the Y2409881 (50 mg/kg)<sup>20</sup> was intraperitoneally given on every three days for 4 weeks. An external caliper and the equation  $(4/3) \pi r^3$  were used to calculate the tumor volume, where  $r = (\text{length} + \text{width})/4$ . On day 28, the tumors tissues were collected and weighed. In addition to the approval from the Institutional Animal Care and Use Committee of Sun Yat-sen University, laboratory animal-guideline for ethical review of animal welfare based on the State Standard of the People's Republic of China was followed for the welfare of the animals.

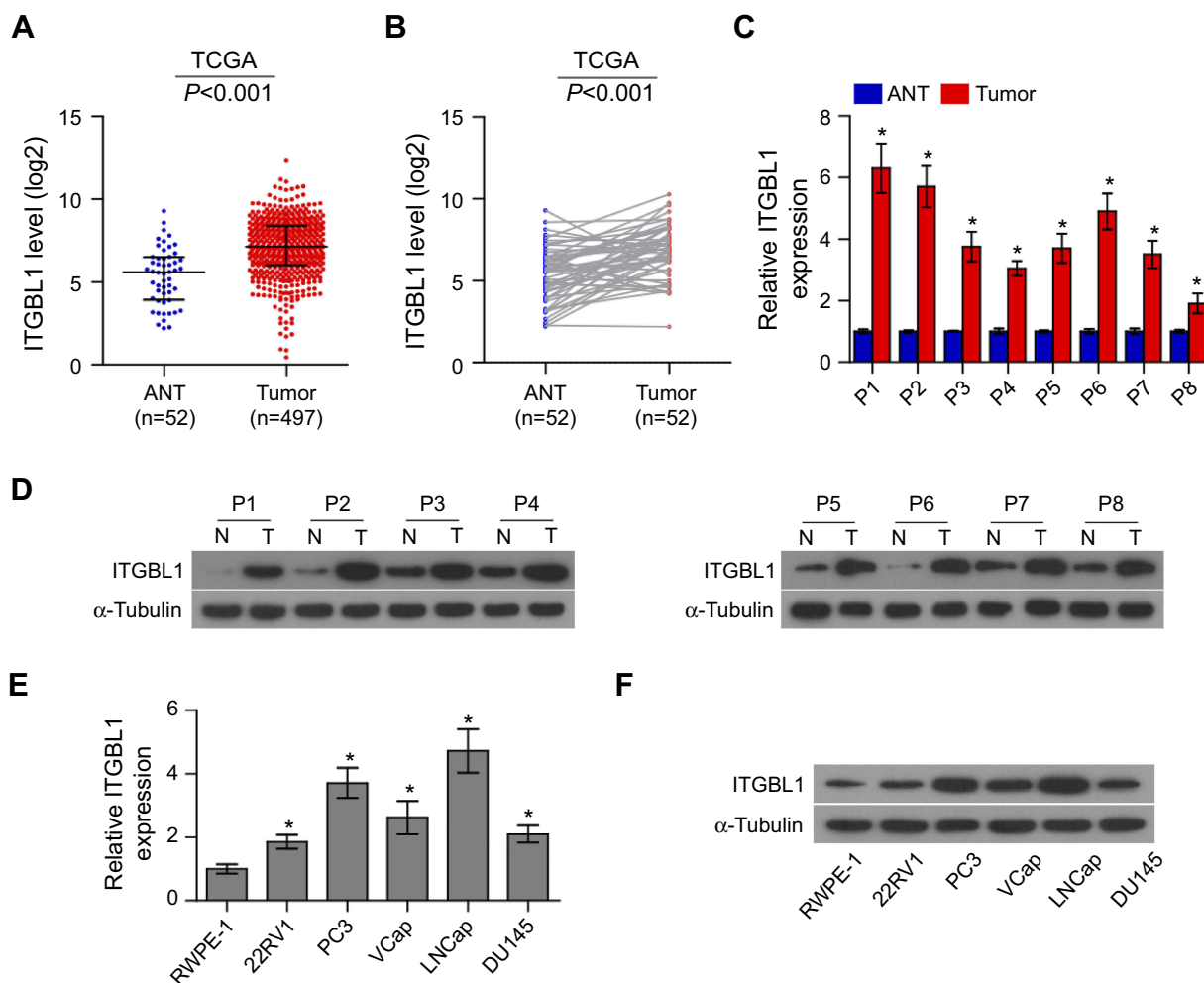
## Statistical analysis

Statistical significance was determined by GraphPad 5.0 software (USA), and  $P$  values  $\leq 0.05$  considered significant. Statistical significance between two groups or multiple groups was determined by student's *t* test (unpaired two sample) or One-way ANOVA respectively. The association between ITGBL1 expression and clinicopathological characteristics of PCa patients was analyzed via the chi-square test. The Kaplan Meier method was used to plot survival curves and compared by the log-rank test. repetition of at least 3 times was applied to achieve reproducibility.

## Results

### ITGBL1 is upregulated in PCa tissues

To determine the expression levels of ITGBL1 in PCa, the PCa dataset from The Cancer Genome Atlas (TCGA) was first analyzed. As shown in Figure 1A, ITGBL1 was found to be upregulated in 497 PCa tissues compared to the 52 adjacent normal tissues (ANT) (Figure 1A). Consistently, ITGBL1 expression level was upregulated in PCa tissues compared to those in the matched ANT (Figure 1B). The expression of ITGBL1 in our PCa tissues was further examined, and the results showed that ITGBL1 expression was



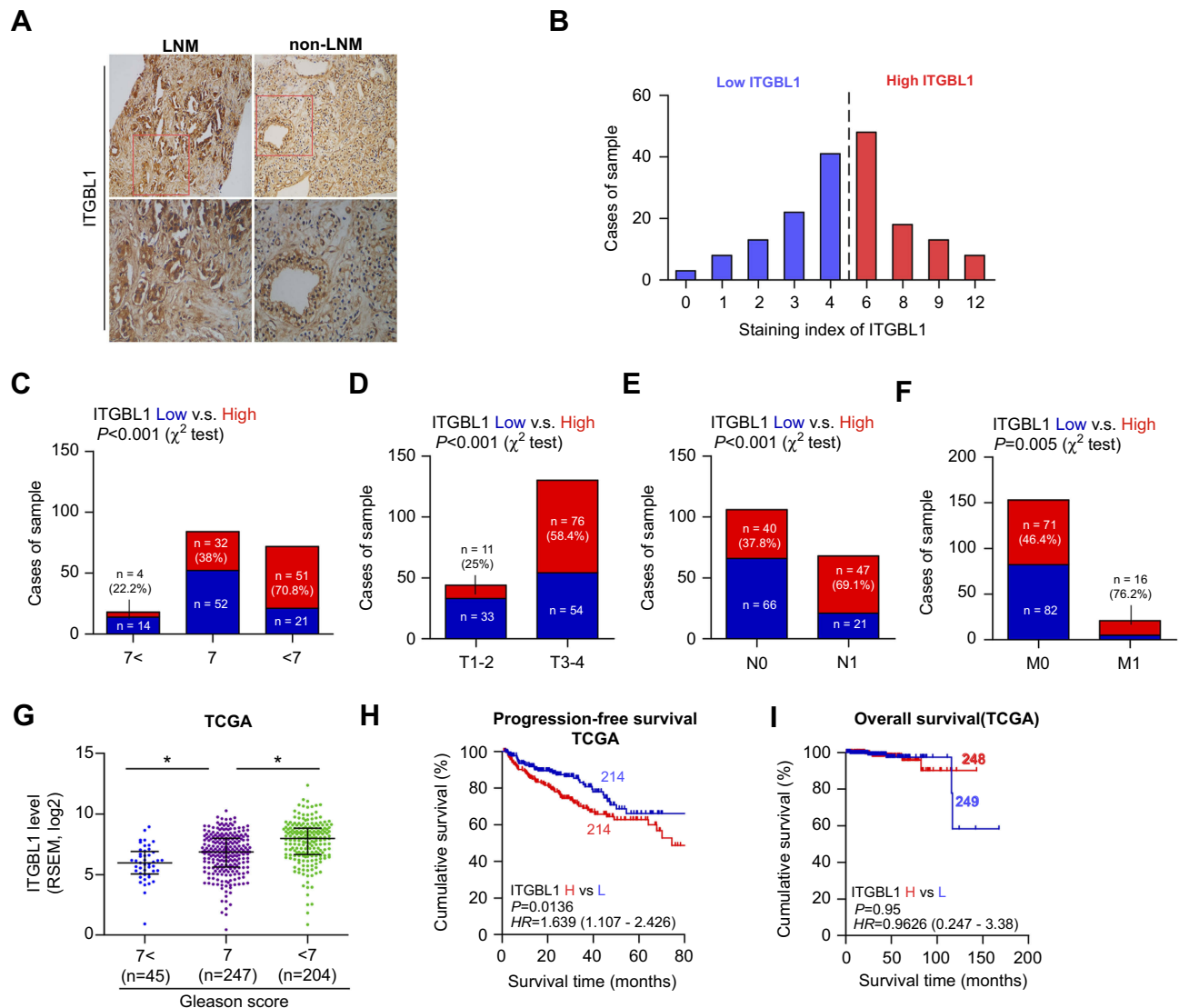
**Figure 1** ITGBL1 is upregulated in PCa tissues. **(A)** In the mRNA sequencing of PCa dataset from TCGA, ITGBL1 expression levels were upregulated in PCa tissues compared with the normal adjacent tissues (ANT). PCa: n=497; ANT=52. **(B)** ITGBL1 expression levels were upregulated in 52 paired PCa tissues compared with the matched normal adjacent tissues as assessed by the mRNA sequencing of PCa dataset from TCGA. **(C)** The real-time PCR analysis of ITGBL1 expression in 8 paired PCa samples. Transcript levels were normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. Error bars represent the mean  $\pm$  S.D. of three independent experiments.  $*P < 0.05$ . **(D)** Western blot analysis of ITGBL1 expression in 8 paired PCa samples.  $\alpha$ -Tubulin served as the loading control. **(E)** The real-time PCR analysis of ITGBL1 expression levels in the normal prostate epithelial cell (RWPE-1), primary PCa cell 22RV1, bone metastatic PCa cell lines (PC-3 and VCaP), brain metastatic cell line DU145 and lymph node metastatic cell line LNCaP. Transcript levels were normalized to GAPDH expression. Error bars represent the mean  $\pm$  S.D. of three independent experiments.  $*P < 0.05$ . **(F)** Western blot analysis of ITGBL1 expression in a normal prostate epithelial cell (RWPE-1), primary PCa cell 22RV1, bone metastatic PCa cell lines (PC-3 and VCaP), brain metastatic cell line DU145 and lymph node metastatic cell line LNCaP.  $\alpha$ -Tubulin served as the loading control.

**Abbreviations:** TCGA, the cancer genome atlas; ANT, the normal adjacent tissues; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ITGBL1, integrin beta-like 1.

elevated in PCa tissues compared to those in the matched ANT (Figure 1C and D). We further evaluated ITGBL1 expression in PCa cells and found that the expression levels of ITGBL1 were differentially upregulated in PCa cells compared with those in one normal prostate epithelial cells RWPE-1, particularly in metastatic PCa cells, including LNCaP and PC-3 (Figure 1E and F). Taken together, these results suggest that overexpression ITGBL1 may contribute to the development and metastasis of PCa.

## Overexpression of ITGBL1 correlates with lymph node metastasis of PCa

We further analyzed ITGBL1 expression via immunohistochemical (IHC) in 174 PCa tissues, and found that ITGBL1 expression in PCa tissues with lymph node metastasis was elevated compared to that in PCa tissues without lymph node metastasis (Figure 2A and B). Furthermore, ITGBL1 expression levels were positively associated with Gleason grade, serum PSA levels, tumor size, distant metastasis and lymph



**Figure 2** Overexpression of ITGBL1 is associated with lymph node metastasis of PCa. (A) Representative images of ITGBL1 expression in PCa tissues with lymph node metastasis and PCa tissues without lymph node metastasis via immunohistochemistry (IHC). (B) The IHC staining index of ITGBL1 expression in PCa tissues. (C) Percentages and number of samples showed high or low ITGBL1 expression in our PCa patients with different Gleason scores. (D) Percentages and number of samples showed high or low ITGBL1 expression in our PCa patients with different tumor size. (E) Percentages and number of samples showed high or low ITGBL1 expression in our PCa patients with different distant metastasis. (F) Percentages and number of samples showed high or low ITGBL1 expression in our PCa patients with different distant metastasis. (G) Percentages and number of samples showed high or low ITGBL1 expression in PCa tissues with different Gleason scores from TCGA. \* $P < 0.05$ . (H) Kaplan-Meier progression-free survival curves for PCa patients stratified by high and low expression of ITGBL1 as assessed by analyzing the RNA sequencing dataset of PCa from TCGA. (I) Kaplan-Meier overall survival curves for PCa patients stratified by high and low expression of ITGBL1 as assessed by analyzing the RNA sequencing dataset of PCa from TCGA.

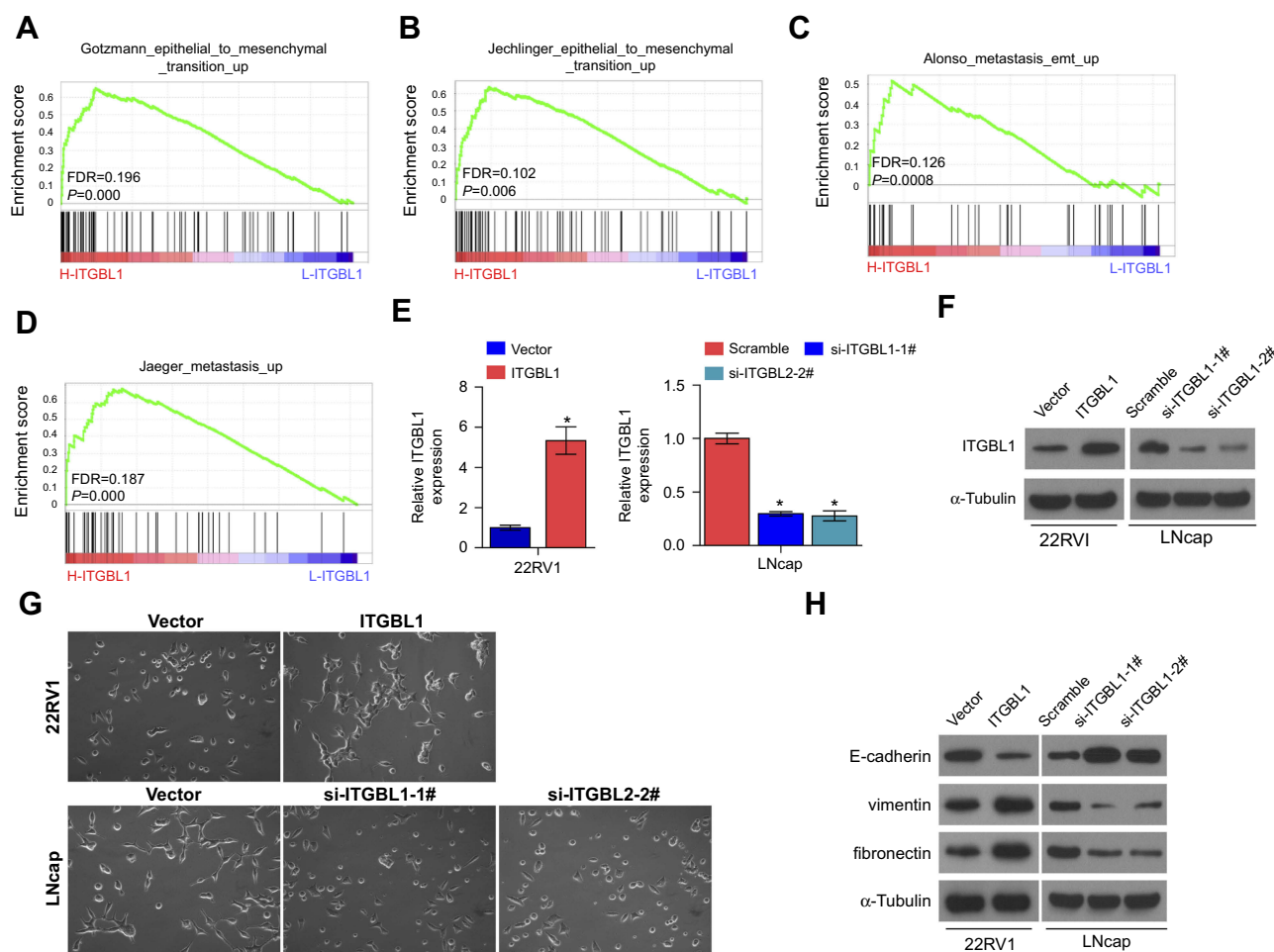
**Abbreviations:** IHC, immunohistochemistry; ITGBL1, integrin beta-like 1; TCGA, the cancer genome atlas.

node metastasis in PCa patients (Figure 2C–F and Table 1). The association of ITGBL1 expression with clinicopathological features in PCa dataset from TCGA was further analyzed, and the results showed that high expression of ITGBL1 correlated with Gleason grades (Figure 2G) and poor progression-free survival (Figure 2H), but not with overall survival in PCa patients (Figure 2I). The results above indicate that overexpression of ITGBL1 correlated with progression and lymph node metastasis in PCa patients.

## Upregulation of ITGBL1 enhances EMT, invasion and migration

Gene Set Enrichment Analysis (GSEA) was performed to investigate the biological role of ITGBL1 in PCa. As

shown in Figure 3A–D, the expression level of ITGBL1 was positively and strongly related with epithelial-mesenchymal transition (EMT)-associated gene signatures and metastatic phenotypes of cancer cells. Next, we constructed ITGBL1-stably expressing PCa cells by ectopically overexpressing ITGBL1 in 22RV1 cells, as well as endogenously knocking down ITGBL1 in LNCaP cells, respectively (Figure 3E and F). Upregulating ITGBL1 converted the epithelial profile to a mesenchymal phenotype in 22RV1 cells (Figure 3G). Conversely, silencing ITGBL1 converted the mesenchymal phenotype to the epithelial profile in LNCaP cells (Figure 3G). Western blot analysis revealed that upregulating ITGBL1 increased the expression of mesenchymal marker vimentin and fibronectin in PCa cells and decreased the expression of



**Figure 3** ITGBL1 promotes EMT in PCa cells. **(A–D)** Gene set enrichment analysis (GSEA) revealed that ITGBL1 expression significantly and positively correlated with the EMT signatures and metastatic phenotypes. **(E)** The real-time PCR analysis of ITGBL1 expression in the indicated PCa cells compared to the corresponding controls. Transcript levels were normalized by GAPDH expression. \* $P < 0.05$ . **(F)** Western blot analysis of ITGBL1 expression in the indicated PCa cells compared to the corresponding controls.  $\alpha$ -Tubulin served as the loading control. **(G)** Upregulation of ITGBL1 converted a cobblestone-like or a short spindle-shaped epithelial morphology to a stick-like or long spindle-shaped mesenchymal profile in 22RV1 cells. Conversely, silencing ITGBL1 converted a stick-like or long spindle-shaped mesenchymal profile to a cobblestone-like or a short spindle-shaped epithelial morphology in LNCaP cells. **(H)** Upregulation of ITGBL1 decreased E-cadherin expression and increased Vimentin and Fibronectin expression in 22RV1 cells while silencing ITGBL1 increased E-cadherin expression and decreased Vimentin and Fibronectin expression in LNCaP cells.  $\alpha$ -Tubulin served as the loading control.

epithelial marker E-cadherin, and silencing ITGBL1 displayed the opposite effect in these PC cells (Figure 3H). Furthermore, upregulating ITGBL1 promoted, while downregulating ITGBL1 repressed the invasion and migration ability of PCa cells (Figure 4A and B). These findings demonstrated that ITGBL1 promotes the migration, invasion, and EMT in PCa cells.

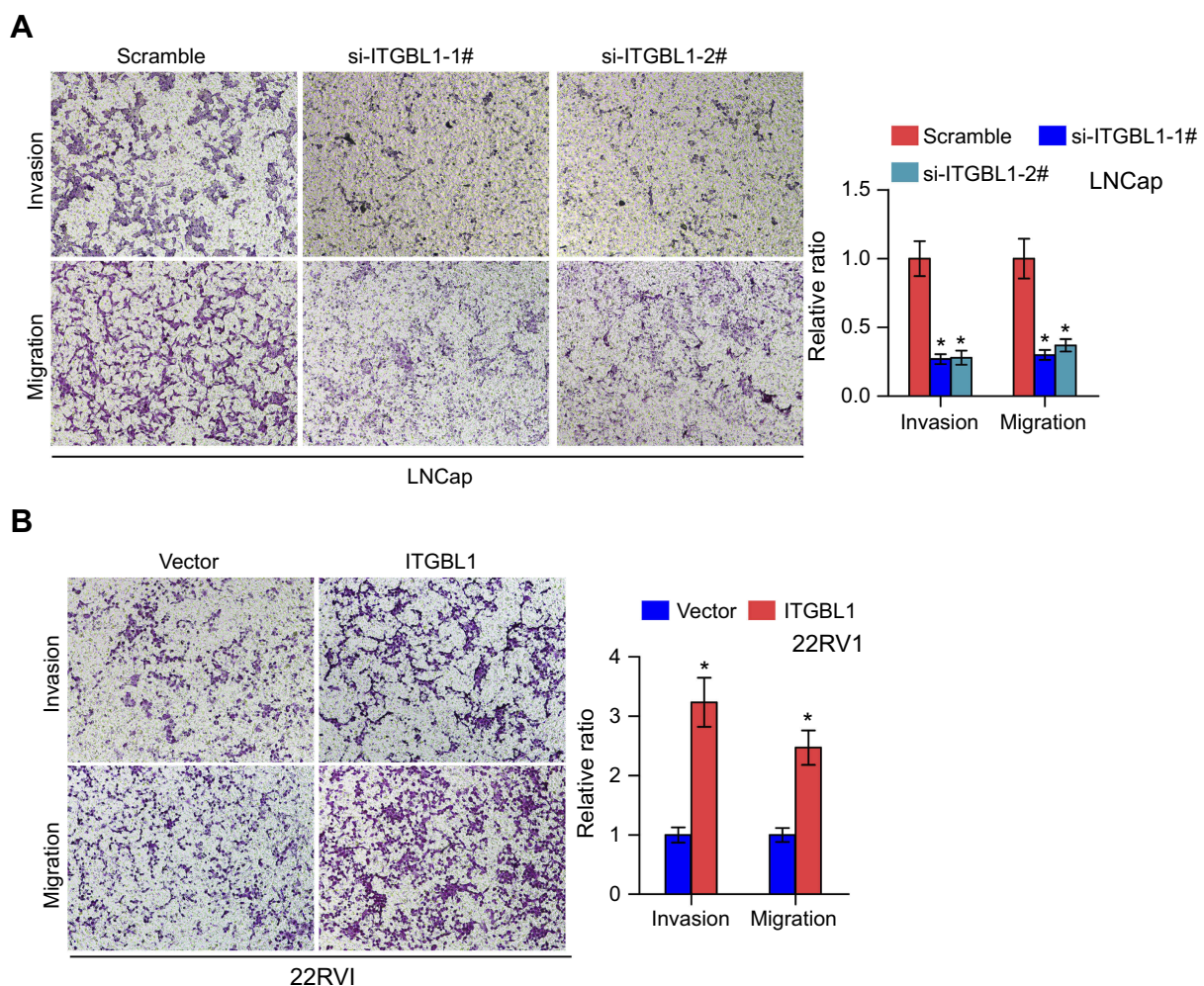
### ITGBL1 activates NF- $\kappa$ b signaling pathway

To determine the mechanism underlying the metastasis-stimulating role of ITGBL1 in PCa cells, GSEA based on ITGBL1 expression was performed, and our results revealed that overexpression of ITGBL1 was positively associated with hyperactivity of NF- $\kappa$ B signaling (Figure 5A and B) that has been identified to promote the tumorigenesis and metastasis in several human cancers.<sup>21–23</sup> Our results showed that upregulating ITGBL1 increased, while downregulating

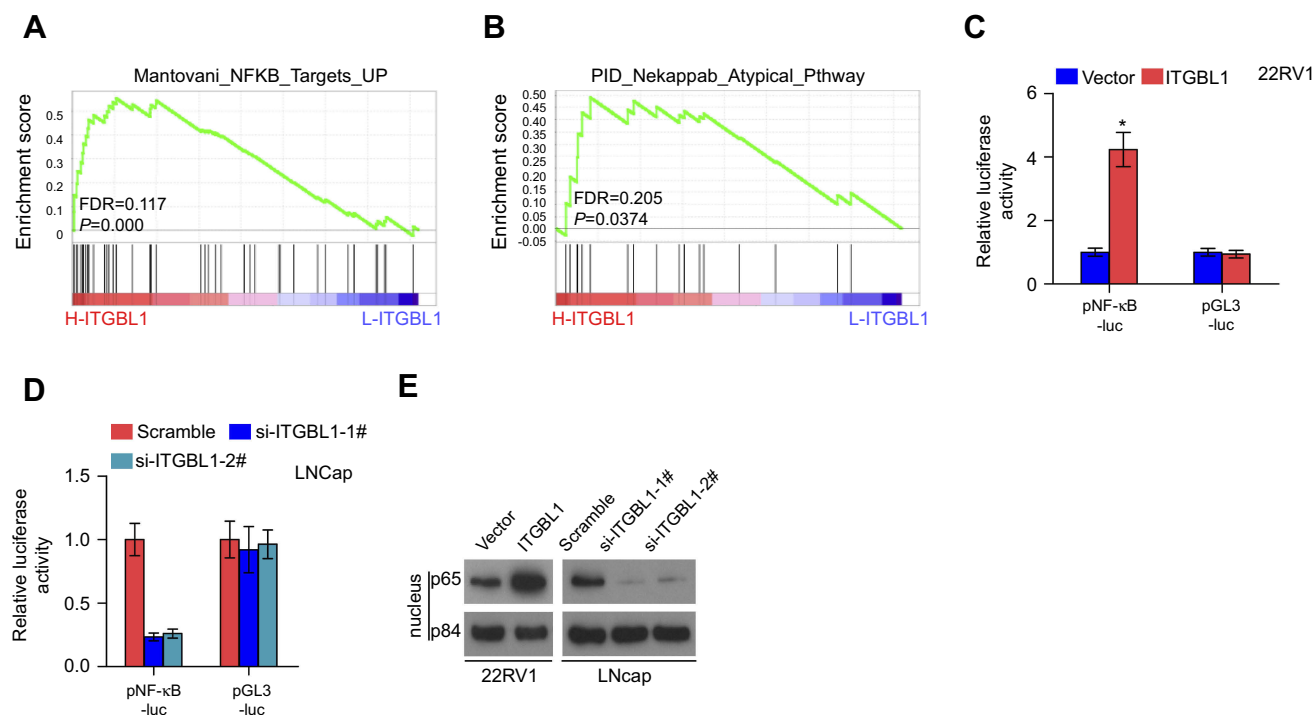
ITGBL1 decreased NF- $\kappa$ B-dependent luciferase activity in PCa cells via luciferase assay (Figure 5C and D). Moreover, upregulating ITGBL1 increased, while silencing ITGBL1 decreased nuclear translocation of NF- $\kappa$ B/p65 via cellular fractionation and western blotting analysis (Figure 5E). These results indicate that ITGBL1 activates NF- $\kappa$ B signaling in PCa cells.

### The activity of NF- $\kappa$ b signaling is essential for ITGBL1-induced invasion and migration, and tumorigenesis of PCa cells

The function of NF- $\kappa$ B signaling in the metastasis-stimulating effect of ITGBL1 on PCa cells was further investigated using NF- $\kappa$ B signaling inhibitors JSH-23 and LY2409881. As shown in Figure 6A, JSH-23 or LY2409881 abolished the effect of ITGBL1 on the activity of NF- $\kappa$ B signaling. Furthermore, JSH-23 and LY2409881 dramatically reduced the migration



**Figure 4** ITGBL1 promotes invasion and migration abilities in PCa cells. **(A and B)** Invasion assay indicated upregulating ITGBL1 enhanced, while silencing ITGBL1 reduced the invasion and migration ability of PCa cells. Histograms show the relative invasion ability in the indicated cells.\* $P < 0.05$ .



**Figure 5** ITGBL1 promotes invasion and migration ability via activating NF- $\kappa$ B signaling pathway in PCa cells. **(A and B)** GSEA revealed that ITGBL1 expression was positively associated with NF- $\kappa$ B signaling. **(C and D)** NF- $\kappa$ B transcriptional activity was assessed by luciferase reporter constructs in the indicated PCa cells. Error bars represent the mean  $\pm$  S.D. of three independent experiments. \* $P < 0.05$ . **(E)** Western blotting of nuclear NF- $\kappa$ B/p65 expression in the indicated PCa cells. The nuclear protein p84 was used as the nuclear protein marker, respectively.

and invasion abilities in ITGBL1-overexpressing PCa cells (Figure 6B). Importantly, the pro-tumorigenic role of ITGBL1-overexpressing PCa cells was partially blocked by LY2409881 and JSH-23 respectively (Figure 6C–E). Therefore, these results demonstrate that ITGBL1 activates NF- $\kappa$ B signaling pathway, which further promotes invasion, migration and tumorigenesis of PCa cells.

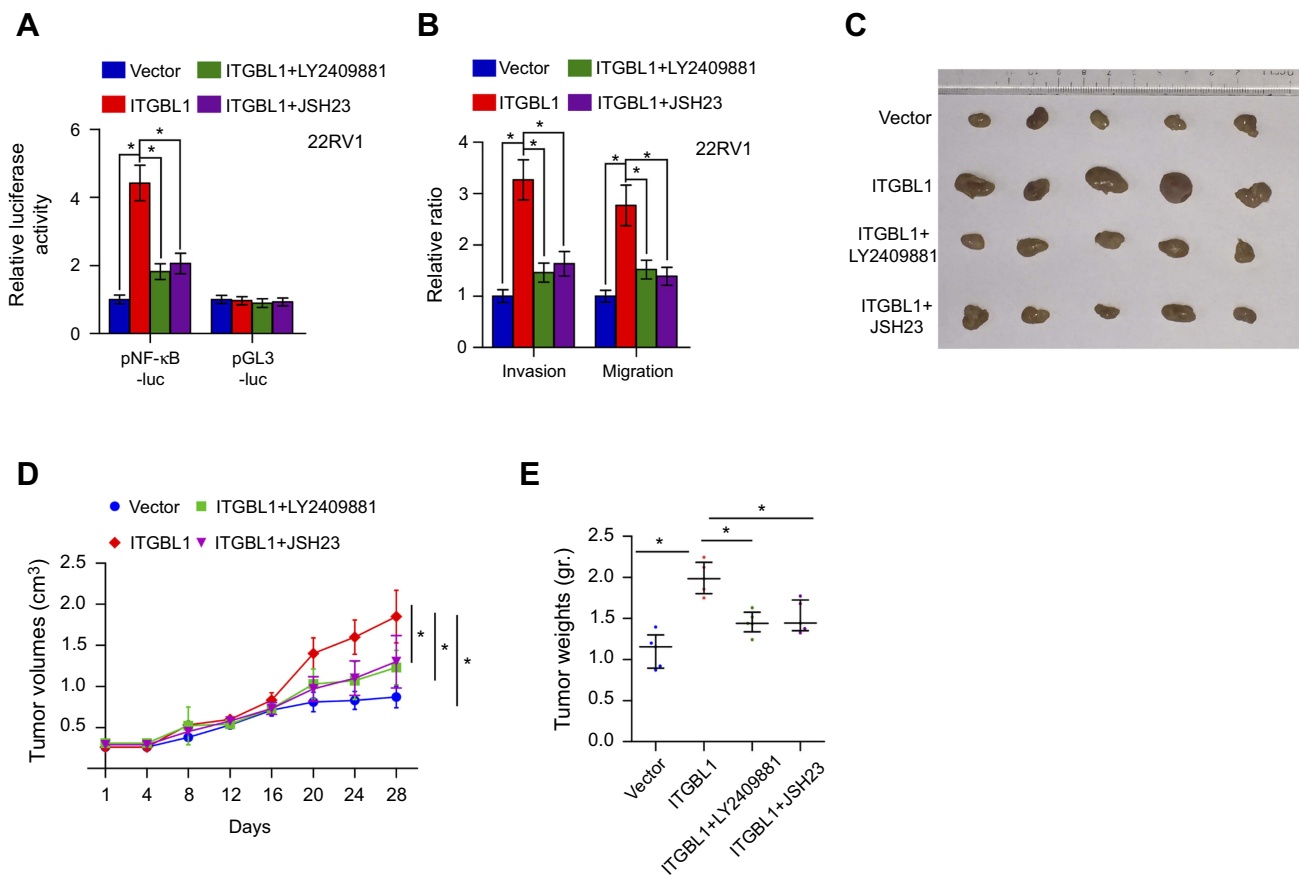
## Discussion

A number of studies have shown that ITGBL1 was upregulated in various types of cancers, including colorectal cancer,<sup>8</sup> breast cancer<sup>6</sup> and gastric cancer,<sup>9,24</sup> which contributed to the metastasis and progression of cancers via the varying mechanism. Strikingly, ITGBL1 expression was downregulated in non-small cell lung cancer (NSCLC), and upregulating ITGBL1 inhibited invasion and migration abilities of NSCLC cells,<sup>25</sup> indicating that ITGBL1 functions its role as a tumor suppressor in NSCLC. These findings imply that the anti- or pro-tumor roles of ITGBL1 vary based on tumor type. Notably, ITGBL1 was found to be overexpressed in PCa tissues by transcriptomic analysis.<sup>26</sup> However, the biological function and clinical significance of ITGBL1 in PCa is

still unclear. In the current study, our results revealed that ITGBL1 expression was upregulated in PCa tissues, which was positively associated with progression and metastasis in PCa patients. Functional experiments in vitro showed that upregulating ITGBL1 enhanced, while silencing ITGBL1 inhibited the EMT, invasion and migration in PCa cells. Therefore, our results indicate that ITGBL1 plays an oncogenic role in PCa cells.

Since been identified, the vast majority of studies have reported that ITGBL1 functions as a metastasis-related gene in several cancers, which promotes the migration, invasion, and EMT of cancer cells, resulting in the distant metastasis of cancer.<sup>6–9</sup> Consistent with these observations, our results showed that ITGBL1 in PCa tissues with lymph node metastasis was upregulated compared to that in PCa tissues without lymph node metastasis. Furthermore, upregulating ITGBL1 promoted the development of EMT, inducing the epithelial profile to a mesenchymal phenotype, enhancing Vimentin and Fibronectin expression and reduced E-cadherin expression, as well as aggravated the migration and invasion ability in PCa cells. Conversely, silencing ITGBL1 yielded an opposite effect on PCa cells. Collectively, our results determine a metastasis-stimulating role of ITGBL1 in PCa.





**Figure 6** The activity of NF- $\kappa$ B signaling is essential for ITGBL1-induced invasion and migration, and tumorigenesis of PCa cells. **(A)** NF- $\kappa$ B signaling inhibitors LY2409881 and JSH-23 decreased the stimulatory effect of ITGBL1 on NF- $\kappa$ B transcriptional activity in PCa cells respectively. Error bars represent the mean  $\pm$  s.d. of three independent experiments. \* $P$ <0.05. The NF $\kappa$ B luciferase activity results presented in **(A)** were independent experiments. **(B)** NF- $\kappa$ B signaling inhibitors LY2409881 and JSH-23 attenuated the stimulatory effects of ITGBL1 on invasion and migration abilities of PCa cells. Error bars represent the mean  $\pm$  s.d. of three independent experiments. \* $P$ <0.05. **(C)** Representative images of the tumors are shown in the xenograft model of nude mice. **(D)** Tumor volumes in the indicated groups were measured every 4 days. Data presented are the mean  $\pm$  s.d. \* $P$ <0.05. **(E)** Tumor weights in the tumor tissue from the indicated mice groups. \* $P$ <0.05.

As mentioned above, our study demonstrated that up-regulating ITGBL1 promoted, while silencing ITGBL1 repressed the EMT in PCa cells. In fact, accumulating evidence have reported that EMT is identified to be one of the critical steps in tumor metastasis, including PCa, by allowing cancer cells acquire mesenchymal features that permit escape from the primary tumor.<sup>27–30</sup> Given the crucial role of EMT in metastasis of PCa and our findings in this study, we infer that ITGBL1 promote lymph node metastasis of PCa via inducing EMT of PCa cells.

Accumulating evidence has shown that ITGBL1 can be used as a potential marker to predict prognosis in cancer patients. For example, overexpression of ITGBL1 was demonstrated to predict poor prognosis in gastric cancer or colorectal cancer patients;<sup>8,9,24</sup> moreover, ITGBL1 has been identified to be a prognostic gene to be able to predict progression-free survival in multiple myeloma patients.<sup>31</sup> Here, our results indicated that

overexpression of ITGBL1 was positively associated with Gleason grade, tumor size, distant metastasis and lymph node metastasis in PCa patients. Consistently, our results further revealed that high expression of ITGBL1 predicted poor progression-free survival in PCa patients through analyzing the ITGBL1 expression in PCa dataset from TCGA. These findings indicate that ITGBL1 harbors great promise as a candidate biomarker for the progression-free and metastasis-free survivals in PCa patients. However, a solid conclusion requires further investigation in the following work.

In summary, our findings reveal that ITGBL1 promotes the metastasis, invasion, and EMT by activating NF- $\kappa$ B signaling in PCa cells, providing the evidence that ITGBL1 plays a pro-metastatic role in PCa. Therefore, better identification of the biological function of ITGBL1 in PCa will facilitate the development of novel anti-metastasis therapeutic measures in PCa.

## Acknowledgments

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## Ethical approval

For the use of these clinical materials for research purposes, prior patients' consents and approval from the Institutional Research Ethics Committee were obtained. All mouse experiments were approved by The Institutional Animal Care and Use Committee of Sun Yat-sen University and the approval-No.was L102012018120P.

## Disclosure

The authors report no conflicts of interest in this work.

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