Butyrophilin-like 9 expression is associated with outcome in lung adenocarcinoma

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Abstract

Background: Lung adenocarcinoma (LUAD) is the most prevalent non-small cell lung cancer (NSCLC). Patients with LUAD have a poor 5-year survival rate. The use of immune checkpoint inhibitors (ICIs) for the treatment of LUAD has been on the rise in the past decade. This study explored the prognostic role of butyrophilin-like 9 (BTNL9) in LUAD.

Methods: Gene expression profile of butyrophilins (BTNs) was determined using the GEPIA database. The effect of BTNL9 on the survival of LUAD patients was assessed using Kaplan-Meier plotter and OncoLnc. Correlation between BTNL9 expression and tumor-infiltrating immune cells (TILs) was explored using TIMER and GEPIA databases. Further, the relationship between BTNL9 expression and drug response was evaluated using CARE. Besides, construction and evaluation of nomogram based on BTNL9 expression and TNM stage.

Results: BTNL9 expression was downregulated in LUAD and was associated with a poor probability of 1, 3, 5-years overall survival (OS). In addition, BTNL9 expression was regulated at epigenetic and post-transcriptional modification levels. Moreover, BTNL9 expression was significantly positively correlated with ImmuneScore and ESTIMATEScore. Furthermore, BTNL9 expression was positively associated with infiltration levels of B cells, CD4+ T cells, and macrophages. Kaplan-Meier analysis showed that BTNL9 expression in B cells and dendritic cells (DCs) was significantly associated with OS. BTNL9 expression was significantly positively correlated with CARE scores.

Conclusions: These findings show that BTNL9 is a potential prognostic biomarker for LUAD. Low BTNL9 expression levels associated with low infiltration levels of naïve B cells, and DCs in the tumor microenvironment are unfavorable for OS in LUAD patients.

Keywords: Butyrophilin-like 9, B cells, Dendritic cells, Prognosis, Lung adenocarcinoma

Background

Lung cancer is the most common cancer and the leading cause of cancer-related deaths globally and in China [1, 2]. Although the 5-year survival rate has increased over the past four decades, the OS is poor (5.6–20.6%) [3]. Immunotherapies have significantly improved cancer treatment during the past decade. For example, pembrolizumab used to treat naive advanced non–small-cell lung cancer (NSCLC) shows a 5-year survival rate of 23.2 and 29.6% in patients with a PD-L1 tumor proportion score ≥ of 50% [4]. Immune checkpoint inhibitors (ICIs) block immune checkpoint signaling, thus alleviating antitumor immunity, and significantly improving five year-OS in NSCLC.

PD-1/PD-L1 is the most widely used ICIs, whereas other immune checkpoints, such as LAG-3, TIGIT, TIM-3, and CTLA-4, are currently under development.
However, the biological role of immune checkpoint butyrophilins (BTNs) [6] in the regulation of NSCLC physiology and its underlying molecular mechanism remains to be fully elucidated. BTNs, including butyrophilin (BTN) and butyrophilin-like (BTLN), are related to the B7 family of co-stimulatory molecules. This family plays a significant role in T cell suppression, regulating epithelial cell and T cell interplays [7]. Human BTN genes are located in the MHC class I domain of the short arm of chromosome 6 (6p22.1). Human BTN genes are grouped into three subfamilies, which form phylogenetically related groups, including BTN1, BTN2, and BTN3. BTN1A1 belongs to the BTN1 subfamily, BTN2A1, BTN2A2, and BTN2A3 (BTN2A3P) belong to the BTN2 subfamily, whereas BTN3A1, BTN3A2, and BTN3A3 belong to the BTN3 subfamily. Moreover, butyrophilin-like proteins (BTNL: BTNL2, BTNL3, BTNL8, BTNL9, and BTNL10) and SKINT-like (SKINTL) are classified in the family of BTNs [7-9].

Lung adenocarcinoma (LUAD) has been the most prevalent histopathological subtype of NSCLC in China since 2014 [10]. In this study, we explored the relationship between the expression level of BTNs and LUAD prognosis. Significant survival-related BTNs were screened using GEPIA [11]. Datasets used for analysis in this study were retrieved from Gene Expression Omnibus [12], TIMER [13], KM plotter [14], UALCAN [15], OncoLnc [16], Oncomine [17], TissGDB [18] databases.

The findings from this study provide information on the relationship between immune checkpoint BTNL9 and tumor immune response. These findings show that BTNL9 can be used for the prognosis and development of immunotherapy for LUAD. A flow chart of the study design is shown in Fig. 1.

**Methods**

**Determination of expression profiles of BTNs genes**

Expression profiles of BTNs in LUAD were determined using the GEPIA database [19]. Default settings were used with \(| \log_{2} (FC) | > 1 \) and \( p \)-value Cutoff < 0.01 used as the cutoff criteria to determine differentially expressed genes. Log2( TPM + 1) was transformed for gene expression profile; jitter size was set at 0.4 for the plot. Notably, TCGA and GTex datasets were included in the analysis. mRNA expression profile of BTNL9 in different tumor types was determined using the Oncomine database [17] using default settings with a \( p \)-value of 0.01, a fold change of 1.5, and a top 10% gene ranking.

**Survival analysis**

Median gene expression was used as the cutoff point for survival analysis. Survival analysis of BTNL9 was performed using three online databases, including KM plotter [14], UALCAN [15], and OncoLnc databases [16]. TissGDB is a tissue-specific gene annotation database in

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![Fig. 1 A flow chart of the study design](image-url)
cancer [18]. Forest plot were generated using Cox pro-
portional hazard ratio (HR), and overall survival (OS)
and relapse-free survival (RFS) of 28 cancer types were
performed using 95% CI.

Estimation of infiltration level of immune cell type and
correlation with BTNL9
TIMER is a comprehensive resource for systematically
analyzing immune infiltration in diverse cancer types
based on the TCGA dataset [13, 20]. The Gene DE
module from TIMER was used to calculate BTNL9
mRNA expression in cross-carcinoma (*: P-value < 0.05;
**: P-value < 0.01; ***: P-value < 0.001). The expression
profile of BTNL9 in LUAD and its correlation with six
immune infiltration cells, including B cells, CD4+ T
cells, CD8+ T cells, macrophages, neutrophils, and DCs,
were analyzed using Gene and Survival module in
TIMER. Gene Corr module was used to determine the
correlation between BTNL9 expression and B and DC
cells [21]. The immune score and stromal score of each
TCGA tumor sample were estimated using Sangerbox
(http://sangerbox.com/Index).

Predicting binding of miRNA and IncRNA to BTNL9
miRMap [22], TargetScan [23], and miRWalk [24] were
used to predict miRNAs that can bind to BTNL9. Pre-
dicted miRNAs obtained from the three databases were
further verified using the starBase database [25].

Gene set enrichment analysis (GSEA) of BTNL9 high and
low expression groups
Sangerbox is a tool developed by Hangzhou Mugu Tech-
nology Co., Ltd. GSEA was used to perform KEGG and
HALLMARK pathway analysis for the BTNL9 high and
low expression groups based on the TCGA database.

Estimating drug response for LUAD
Computational Analysis of Resistance (CARE) is a soft-
ware that uses compound screening data to identify
genome-scale biomarkers for targeted therapeutic strat-
egies. Pearson correlation analysis between the gene expres-
sion profile of the cancer sample and the CARE
scoring vector was used to group the patient as a re-
sponder or a non-responder [26].

Construction and evaluation of nomogram
We acquired TCGA LUAD RNA-seq data from the Uni-
versity of California, Santa Cruz (UCSC) Xenabrowser
(https://xenabrowser.net/). After screening, samples with
missing clinical data and 0 days overall survival time
were excluded, and a total of 501 samples were included.
Next, we randomly divided the TCGA-LUAD cohort
(n = 501) in a 7 to 3 ratio into a training (n = 352) and
testing dataset (n = 149). We performed the R package
“rms” to construct a nomogram based on the TNM
stage and expression profile of BTNL9 using the training
dataset. To evaluate the usefulness of the nomogram,
the R package “ROCsurvival” was used to construct
ROC for the prediction of the 1-, 3- and 5- year OS. The
R package “ggDCA” was executed to create a decision
analysis curve to evaluate the clinical utility of the
nomogram. Finally, R package “rms” was applied to per-
form a calibration curve to evaluate the precision for
predicting 1-, 3- and 5-year OS prediction of the LUAD
cohort.

Statistical analysis
The relationship between BTNL9 expression and single
cancer cell biological behavior of LUAD was determined
using Pearson correlation analysis and Spearman’s cor-
relation analysis of the correlation between BTNL9 and
tumor mutation burden (TMB). In all the studies, P <
0.05 was considered statistically significant.

Results
The high expression level of BTNL9 was associated with
favorable survival of LUAD
Gene expression profile of BTN5s, including BTN1A1,
BTN2A1, BTN2A2, BTN2A3P, BTN3A1, BTN3A2,
BTN3A3, BTN1A1, BTN1B3, BTN1L2, BTN1L3, BTN1L8, BTN1L9,
and SKINTL was evaluated in normal and tumor lung
tissues (Fig. 2A). Analysis showed that expression levels
of BTN1L8 and BTN1L9 were significantly lower in tumor
tissues compared with that of normal tissues (Fig. 2A).
Furthermore, the expression level of BTN1L9 was signifi-
cantly negatively correlated with the clinical stage, lymph
node metastasis stage, and p53 mutation. Concurrently,
the expression level of BTN1L8 was significantly nega-
tively correlated with the clinical stage and N stage (Fig.
2B). However, survival analysis showed that BTN1L8
was not significantly correlated with OS, whereas BTN1L9
was significantly correlated with OS in LUAD (Fig. 2C).
Validation of the prognosis value of BTN1L9 in LUAD
cohorts using PrognoScan [27] showed that BTN1L9 ex-
pression was significantly correlated with RFS and OS in
the GSE31210 dataset (n = 204). In addition, the expres-
sion level of BTN1L9 was significantly associated with OS
in GSE3141 cohort (n = 111) (Supplementary Table 1). An-
alysis using OncoLnc, UALCAN, and KM plotter
showed that high expression of BTN1L9 is significantly
associated with better OS in LUAD (Fig. 2D). Although
the two survival curves crossover occurred after 150
months (Fig. 2C), it is well beyond 5-years (60 months),
and survival curves in the verification databases didn’t
show crossover. Thus, we considered the results in this
study reliable and stable. These findings imply that
BTN1L9 is a critical immune checkpoint of BTNs in
LUAD.
Fig. 2 (See legend on next page.)
**Fig. 2** BTNL9 Expression and prognosis in LUAD. (A) BTNs expression panel in LUAD tissues compared with adjacent tissues in GEPIA database. (B) Correlation between BTNL9 and BTNL9 expression and LUAD clinical stages, N stage, and p53 mutation in the UALCAN database (BTNL9 expression compared with LUAD clinical stages (Normal-vs-Stage1, \( P = 1.01E-04 \); Normal-vs-Stage2, \( P = 7.08E-03 \); Normal-vs-Stage3, \( P = 1.36E-04 \); Normal-vs-Stage4, \( P = 1.78E-08 \)), N stage (Normal-vs-N0, \( P = 6.56E-04 \); Normal-vs-N1, \( P = 8.27E-10 \); Normal-vs-N2, \( P = 5.06E-04 \); Normal-vs-N3, \( P = 1.97E-10 \); N0-vs-N1, \( P = 3.27E-03 \); N0-vs-N3, \( P = 6.79E-04 \), and p53 status (Normal-vs-T P53-Mutant, \( P = 1.27E-06 \); Normal-vs-T P53-Non-Mutant, \( P = 1.80E-03 \)). (C) BTNL9 expression comparison with LUAD clinical stages (Normal-vs-Stage1, \( P = 1.21E-12 \); Normal-vs-Stage2, \( P = 3.60E-12 \); Normal-vs-Stage3, \( P = 2.40E-12 \); Normal-vs-Stage4, \( P = 2.25E-12 \)), N stage (Normal-vs-N0, \( P = 1.08E-12 \); Normal-vs-N1, \( P = 1.85E-12 \); Normal-vs-N2, \( P = 1.02E-12 \); Normal-vs-N3, \( P = 1.67E-12 \); N0-vs-N1, \( P = 1.02E-03 \); N2-vs-N3, \( P = 3.01E-02 \)), and p53 status (Normal-vs-T P53-Mutant, \( P = 1.85E-12 \); Normal-vs-T P53-Non-Mutant, \( P = 4.10E-12 \); T P53-Mutant-vs-T P53-Non-Mutant, \( P = 1.77E-04 \)). (D) Correlation between BTNL9 and BTNL9 expression with overall survival of LUAD using GEPIA database. (E) Correlation between BTNL9 expression and LUAD overall survival using OncoLnc, UALCAN, and KM plotter databases. *: \( P \)-value < 0.05; **: \( P \)-value < 0.01; ***: \( P \)-value < 0.001

**Fig. 3** mRNA expression level of BTNL9 in pan-cancer and hazard ratio of relapse-free survival and overall survival in LUAD patients. (A) Increased or decreased expression of BTNL9 in various tumors compared with adjacent tissues using the Oncomine database. (B) BTNL9 expression in pan-cancer analysis using TCGA dataset analyzed by TIMER (* \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \)). (C) BTNL9 expression and hazard ratio of relapse-free survival, and (D) overall survival using the TissGDB database.
Furthermore, the clinical distribution of BTNL9 was explored. TCGA-LUAD dataset was divided into the high and low groups based on the median gene expression level of BTNL9. The clinical characteristics, including age, gender, race, TNM staging, ECOG score, EGFR mutations, KRAS mutations, and radiotherapy information, were compared between the two groups. Analysis showed no significant difference in these clinical characteristics between the two groups (data not shown).

Pan-cancer gene expression and prognostic value of BTNL9
To further understand BTNL9 expression in pan-cancer, analysis of the dataset was performed using the Onco-mine database. The findings showed that BTNL9 expression level was significantly lower in breast cancer, one colon cancer cohort, lung cancer, kidney cancer, and crabtree uterus cancer than normal tissues. However, BTNL9 expression was significantly higher in the brain and CNS cancer, colorectal cancer, esophageal cancer, leukemia, and lymphoma, than in normal tissue (Fig. 3A and Supplementary Table 2). Further, the BTNL9 expression profile was explored using TCGA RNA sequencing data (TIMER). The BTNL9 expression level was significantly downregulated in Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cholangiocarcinoma (CHOL), Esophageal carcinoma (ESCA), Glioblastoma multiforme (GBM), Head and Neck squamous cell carcinoma (HNSC), Kidney renal papillary cell carcinoma (KIRP), LUAD, and LUSC compared with normal tissues. In contrast, BTNL9 was significantly increased in Colon adenocarcinoma (COAD), Kidney Chromophobe (KICH), and Kidney renal clear cell carcinoma (KIRC) compared with normal tissues (Fig. 3B). Analysis using the TissGDB database gave a correlation coefficient of BTNL9 expression with LUAD’s RFS HR of 0.87 [95% CI (0.79, 0.96)], and that
for the correlation between $BTNL9$ expression and OS HR was 0.87 [95% CI (0.8,0.96)] (Fig. 3C, D).

**Upstream and downstream regulatory network of $BTNL9$**

A previous study reports that the expression level of $BTNL9$ in LUAD is significantly lower than that in normal tissues. DNA methylation is a biological process through which methyl groups are added to DNA molecules by Methyltransferases (DNMTs). DNA methylation of a gene promoter region functions by inhibiting gene transcription. Correlation analysis between $BTNL9$ expression and DNA methylation marker DNMTs (including DNMT1, DNMT2, DNMT3A, DNMT3B) was conducted using the GEPIA tool. Analysis showed that expression of $BTNL9$ in normal lung tissue was positively correlated with DNMTs ($r = 0.35$, $P = 0.0059$); however, there was no correlation with DNMTs in LUAD ($r = -0.019$, $P = 0.67$) (Fig. 4A, B). These findings show that DNA methylation may be involved in the pathogenesis of LUAD. To further explore the upstream regulation mechanisms of the $BTNL9$ expression, miRNAs that bind to $BTNL9$ were predicted by using miRMap [22], TargetScan [23], and miRWalk [24] databases. A total of 248 miRNAs common predicted miRNAs from the three databases were obtained (Fig. 4C) and used starBase [25] to validate the predicted binding miRNAs. Analysis showed that, has-miR-30b-3p, has-miR-4709-3p and has-miR-6514-3p were significantly positively correlated with $BTNL9$ expression ($r = 0.312$, $P = 5.25E-13$, $r = 0.277$, $P = 1.74E-10$, and $r = 0.103$, $P = 0.02$.

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**Fig. 5** Low expression of $BTNL9$ significantly enriches proteasome and promotes tumor malignancy in LUAD. (A, B, C, D) High and low $BTNL9$ expression groups were presented using the Sangerbox tool and GSEA for KEGG and HALLMARK pathways. (E) the t-SNE plot shows scRNA analysis of $BTNL9$ expression, and (F) molecular function of $BTNL9$ in LUAD using CancerSEA database shows that $BTNL9$ is significantly negatively correlated with tumor invasion, metastasis, EMT, proliferation, hypoxia, and DNA damage in LUAD.
respectively, Fig. 4D). In addition, the three miRNAs were highly expressed and significantly correlated with higher OS of LUAD patients (HR = 0.66, P = 0.0058, HR = 0.63, P = 0.0023, and HR = 0.73, P = 0.036, respectively) (Fig. 4D). Although the two survival curves of hsa-miR-4709-3p and hsa-miR-6514-3p crossover occurred after 100 months, it is well beyond 5-years (60 months). Thus, we considered the results in this study reliable. Furthermore, 18 lncRNAs were predicted to bind to BTNL9 using LncMap [28] database (Supplementary Table 3). These findings were verified using LncACTdb2.0 [29] database. LncRNA AP001462.6 was predicted to bind to BTNL9, and the high expression level of AP001462.6 was significantly correlated with a high OS of LUAD patients (P = 0.049) (Fig. 4E).

Moreover, proteins implicated in binding BTNL9 were analyzed using the STRING [30] database. Analysis showed a total of 7 proteins that bind to BTNL9 (Fig. 4F). The top 2 binding proteins, including HTRA4 and TM4SF19, were predicted using the cytoHubba module of Cytoscape [31] (Fig. 4G). HTRA4 gene encodes a member of the Htra protease family. HTRA4 plays a role as a secreted oligomeric chaperone protease to degrade misfolded secretory proteins [19]. We hypothesized that low expression of BTNL9 in LUAD might be related to degradation through ubiquitination. Analysis using Ubibrowser [32] database showed that E3 (MARCH8) ligases could bind the substrate BTNL9 (Supplementary Table 4). In addition, BTNL9 has a potential E3 recognizing domain and two potential E3 identifying motifs (Fig. 4H, I).

**Low expression of BTNL9 significantly enriches proteasome and increases cancer malignancy**

Gene Set Enrichment Analysis (GSEA) analysis for KEGG and HALLMARK was performed using the Sangerbox tool to explore the two groups’ biological pathways. The findings showed that the top 3 significantly enriched KEGG pathways in the high BTNL9 expression group were vascular smooth muscle contraction, phosphatidylinositol signaling system, and abc transporters (Fig. 5A). On the other hand, the top 4 significantly enriched KEGG pathways in the low BTNL9 expression group were pathways implicated in Parkinson’s disease, oxidative phosphorylation, DNA replication, and proteasome pathways (Fig. 5B). GSEA for the HALLMARK pathway showed that the top 3 pathways associated with high BTNL9 expression were bile acid metabolism, heme metabolism, and Wnt/beta-catenin signaling pathways. Further, the top 4 pathways associated with low BTNL9 expression were E2F targets, glycolysis, myc targets v1, and mTORC1 signaling (Fig. 5C, D). These findings imply that BTNL9 is involved in LUAD metabolic reprogramming.

Metabolic reprogramming is a hallmark of cancer, and intrinsic and extrinsic factors contribute to various metabolic phenotypes in tumors. As cancer develops from pre-tumor lesions to local, clinically obvious malignant tumors to metastatic cancer, metabolism changes the phenotype and dependence [33]. Single-cell RNA (scRNA) analysis of LUAD using CancerSEA [34] database (Fig. 5E) showed that BTNL9 expression is significantly negatively correlated with tumor malignant features including invasion (r = −0.53, P < 0.0001), metastasis (r = −0.35, P = 0.011), EMT (r = −0.47, P = 0.0006), proliferation (r = −0.37, P = 0.0086), Hypoxia (r = −0.36, P = 0.011), and DNA damage (r = −0.34, P = 0.017) (Fig. 5F). This finding implies that low expression of BTNL9 is significantly associated with the malignant features of LUAD.

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**Fig. 6 Correlation between BTNL9 and tumor-infiltrating immune cells and OS prognosis.** (A) Correlation analysis between BTNL9 and TMB using Sangerbox tool, (B) somatic mutation pattern of BTNL9 in LUAD, and (C, D, E) ESTIMATE scores. (F) Correlation between BTNL9 and TME infiltrating immune cells analyzed using TIMER database, and (G) overall survival
| Immune cell | Marker | TIMER | GEPIA |
|-------------|--------|-------|-------|
| | | Spearman’s $\rho$ | $P$ Value | Spearman’s $\rho$ | $P$ Value | Spearman correlation coefficient | $P$ Value | Spearman correlation coefficient | $P$ Value |
| DC | CD1C | 0.36 | 1.65E-17 | 0.34 | 1.03E-14 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | HLA-DPA1 | 0.23 | 1.64E-07 | 0.20 | 8.47E-06 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | HLA-DPB1 | 0.29 | 8.39E-12 | 0.27 | 8.03E-10 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | HLA-DQB1 | 0.21 | 1.31E-06 | 0.18 | 8.16E-05 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | HLA-DRA | 0.18 | 4.54E-05 | 0.14 | 0.0020 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | ITGAX | 0.24 | 1.63E-08 | 0.22 | 1.10E-06 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | NCR1 | 0.04 | 0.35980601 | 0.01 | 0.8450 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| | NRP1 | 0.03 | 0.41981037 | 0.03 | 0.4973 | —0.022 | 0.87 | 0.33 | 1.5E-13 |
| cDC1s | CD8A | 0.03 | 0.55316497 | —0.03 | 0.5613 | —0.028 | 0.83 | 0.22 | 1.20E-06 |
| | CLEC9A | 0.40 | 1.00E-21 | 0.38 | 1.16E-18 | —0.028 | 0.83 | 0.22 | 1.20E-06 |
| | XCR1 | 0.41 | 2.70E-22 | 0.39 | 7.37E-20 | —0.028 | 0.83 | 0.22 | 1.20E-06 |
| cDC2s | CLEC12A | 0.22 | 3.50E-07 | 0.18 | 3.61E-05 | —0.028 | 0.83 | 0.22 | 1.20E-06 |
| | ESAM | 0.51 | 1.01E-35 | 0.50 | 2.89E-33 | —0.028 | 0.83 | 0.22 | 1.20E-06 |
| B cell | CD19 | 0.23 | 1.93E-07 | 0.20 | 5.30E-06 | 0.017 | 9.00E-01 | 0.37 | 8.30E-17 |
| | FCER2 | 0.40 | 7.97E-21 | 0.38 | 2.18E-18 | 0.017 | 9.00E-01 | 0.37 | 8.30E-17 |
| | MS4A1 | 0.34 | 6.45E-16 | 0.33 | 2.59E-14 | 0.017 | 9.00E-01 | 0.37 | 8.30E-17 |
| | SDC1 | 0.07 | 0.0917 | 0.09 | 0.0523 | 0.017 | 9.00E-01 | 0.37 | 8.30E-17 |
| Naïve B cell | CD19 | 0.23 | 1.93E-07 | 0.20 | 5.30E-06 | 0.082 | 0.54 | 0.43 | 8.60E-23 |
| | CD22 | 0.50 | 2.44E-34 | 0.51 | 1.10E-34 | 0.082 | 0.54 | 0.43 | 8.60E-23 |
| | CD83 | 0.33 | 6.56E-15 | 0.31 | 2.09E-12 | 0.082 | 0.54 | 0.43 | 8.60E-23 |
| | MS4A1 | 0.34 | 6.45E-16 | 0.33 | 2.59E-14 | 0.082 | 0.54 | 0.43 | 8.60E-23 |
| | TCL1A | 0.25 | 1.57E-08 | 0.21 | 1.31E-06 | —0.19 | 0.15 | 0.059 | 0.19 |
| Plasma B cell | CD38 | 0.04 | 0.3699 | 0.08 | 0.0746 | —0.19 | 0.15 | 0.059 | 0.19 |
| | TNFR SF17 | 0.04 | 0.4053 | 0.08 | 0.0814 | —0.19 | 0.15 | 0.059 | 0.19 |
Correlation between \textit{BTNL9} and infiltrating immune cell markers

Spearman’s correlation analysis of the correlation between expression of \textit{BTNL9} and tumor mutation burden (TMB) in the TCGA LUAD cohort showed that \textit{BTNL9} is significantly negatively correlated with TMB ($P = 1.4\text{E}-9$) (Fig. 6A). Analysis of somatic mutation pattern of \textit{BTNL9} in LUAD using the SangerBox tool showed that the mutation frequency of \textit{BTNL9} in LUAD was 1.41% (Fig. 6B). Genetic mutations are implicated in the tumor microenvironment (TME) [35]; therefore, the relationship between the expression of \textit{BTNL9} and the immune score was determined using the ESTIMATE algorithm in the SangerBox tool. Analysis showed that \textit{BTNL9} was significantly positively correlated with ImmuneScore ($r = 0.129$, $P = 0.003$) and ESTIMATEScore ($r = 0.106$, $P = 0.016$). However, the expression of \textit{BTNL9} was not significantly correlated with StromalScore (Fig. 6C-E).

Analysis of the correlation between gene expression of \textit{BTNL9} and infiltrating level of immune cells using TIMER [13] database showed that \textit{BTNL9} was negatively correlated with tumor purity ($r = -0.124$, $P = 5.6\text{E}-03$). On the other hand, gene expression of \textit{BTNL9} was significantly positively correlated with B cells ($r = 0.24$, $P = 8.88\text{E}-8$), CD4$^+$ T cells ($r = 0.283$, $P = 2.24\text{E}-10$) and macrophages ($r = 0.209$, $P = 3.35\text{E}-6$) (Fig. 6F). Moreover, survival analysis showed that high expression of \textit{BTNL9} in B cells ($P = 0.000$) and DC cells ($P = 0.048$) was correlated with better OS for LUAD (Fig. 6G).

A detailed analysis of TME infiltrated DC and B cells using the TIMER database showed that DC and its subtypes cDCs1 and cDCs2 [36] are associated with \textit{BTNL9} expression before and after purity adjustment. GEPIA database analysis showed that normal lung tissue was not correlated with DC and its subtypes cDCs1 and cDCs2. However, DC and its subtypes were significantly positively correlated with \textit{BTNL9} expression (Table 1), implying that DCs regulated by \textit{BTNL9} may participate in LUAD immune response. B cells are heterogeneous and include two subtypes: naïve B cells and plasma B cells [37]. TIMER analysis showed that total B cells and naïve B cells were significantly correlated with \textit{BTNL9} expression before and after purity adjustment. However, plasma B cells were not associated with \textit{BTNL9} expression before and after purity adjustment. GEPIA analysis showed that total B cells and naïve B cells were not correlated with \textit{BTNL9} expression in normal lung tissues; however, they were significantly positively correlated with \textit{BTNL9} expression in LUAD tissues. Plasma B cells showed no correlation with \textit{BTNL9} in both normal tissues and LUAD tissues (Table 1), indicating that \textit{BTNL9} may play a role in promoting naïve B cell antitumor immune response.

High expression of \textit{BTNL9} is associated with tyrosine kinase inhibitors response

\textit{BTNL9} expression was significantly positively correlated with CARE scores for several compounds retrieved from Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC, previously named CGP), and Cancer Therapeutics Response Portal (CTRP) cohorts, mainly including antiangiogenic tyrosine kinase inhibitors Axitinib, Nilotinib, Sorafenib, Pazopanib, Masitinib, and Ki8751 (Fig. 7, and Table 2). These findings show that immune checkpoint inhibitors based on \textit{BTNL9} plus antiangiogenic tyrosine kinase inhibitors could be developed as a potential chemotherapy-free combination treatment strategy for LUAD.

Independent predictive power of \textit{BTNL9} based on multivariate analysis

We used the R package “survival V3.2–10” to construct a Cox model, including known important clinical variables for OS, such as TNM stage, primary therapy outcome, and \textit{BTNL9} expression. We also used multivariate analysis to explore whether \textit{BTNL9} expression was an independent OS factor for TCGA-LUAD patients. The results demonstrated that higher \textit{BTNL9} expression significantly ($p = 0.049$) and independently increased OS (HR $= 0.67$, 95% CI 0.45–0.99) (Table 3).
| Drug           | Target      | t-value  | p-value  |
|---------------|-------------|----------|----------|
| IOX2          | EGLN1       | 5.91996  | 4.56E-09 |
| PHA-793887    | CDK9        | 4.92172  | 1.02E-06 |
| OSI-027       | MTOR        | 4.55428  | 5.99E-06 |
| Ispinesib     | KIF11       | 4.42265  | 1.09E-05 |
| Nilotinib     | ABL1        | 4.3886   | 1.30E-05 |
| Axitinib      | PDGFRA      | 4.33456  | 1.64E-05 |
| NG25          | MAP 4 K2    | 4.29746  | 1.92E-05 |
| Nilotinib     | KIT         | 4.26379  | 2.26E-05 |
| BMS345541     | IKBKB       | 4.12048  | 4.13E-05 |
| GSK525762A    | BRD2        | 3.92075  | 2.95E-05 |
| CAY10603      | HDAC6       | 3.91591  | 9.69E-05 |
| TubastatinA   | HDAC6       | 3.89552  | 0.000105 |
| GSK525762A    | BRD4        | 3.83187  | 0.000136 |
| CGP dataset   |             |          |          |
| PHA-793887    | CDK1        | 3.4184   | 0.000658 |
| Fluorouracil  | TYMS        | 3.09815  | 0.002008 |
| TPCA-1        | IKBKB       | 3.09042  | 0.002061 |
| 1,256,580–46-7| ALK         | 3.01459  | 0.002646 |
| CAL-101       | PIK3CD      | 2.99145  | 0.002852 |
| Belinostat    | HDAC6       | 2.82403  | 0.004852 |
| AT7519        | CDK9        | 2.81016  | 0.00506  |
| CP-466722     | ATM         | 2.78047  | 0.000542 |
| Enzastaurin   | PRKCB       | 2.76951  | 0.000573 |
| SB590885      | BRAF_V600E.Mutation | 2.6446 | 0.008337 |
| Vorinostat    | HDAC6       | 2.54112  | 0.011233 |
| Nutlin-3      | MDM2        | 2.94731  | 0.003295 |
| Dasatinib     | EPHA2       | 3.23912  | 0.001305 |
| Quizartinib   | FLT3        | 3.33865  | 0.000877 |
| 1,173,900–33-8| PIK3CB      | 3.55986  | 0.00039  |
| Enzastaurin   | PRKCB       | 2.76951  | 0.000573 |
| CCLE dataset  |             |          |          |
| Panobinostat  | HDAC1       | 3.29253  | 0.001066 |
| abraxane      | TUBB        | 2.83794  | 0.00473  |
| Palbociclib   | RB1         | 2.64989  | 0.008358 |
| Topotecan     | TOP1        | 2.45344  | 0.014498 |
| Sorafenib     | FLT3        | 4.25026  | 2.56E-05 |
| CTRP dataset  |             |          |          |
| 9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione | NR3C1 | 5.30047 | 1.51E-07 |
| abraxane      | TUBB        | 4.50938  | 7.51E-06 |
| Alisertib     | AURKB       | 4.50069  | 7.83E-06 |
| PAC-1         | CASP3       | 4.27611  | 2.16E-05 |
| 660,868–91-7  | PLK1        | 4.21764  | 2.79E-05 |
| Gossypol      | BCL2        | 4.19093  | 3.12E-05 |
| Decitabine    | DNMT1       | 4.18042  | 3.24E-05 |
| 722,544–51-6  | AURKB       | 4.14645  | 3.74E-05 |
Table 2 Loss of BTN19 expression may promote drug resistance toward many targeted therapies in CGP, CCLE, and CTRP cohorts (Continued)

| Drug | Target | t-value | p-value |
|------|--------|---------|---------|
| Etoposide | TOP2B | 4.0066 | 6.76E-05 |
| 3,5-bis(4-methylbenzylidene)piperidin-4-one | USP13 | 3.92444 | 9.47E-05 |
| 180,002–83-9 | CNR2 | 3.91953 | 9.68E-05 |
| CICLOPIROX | RRM1 | 3.90668 | 0.000102 |
| Dabrafenib | BRAF_V600EMutation | 3.84863 | 0.00014 |
| BRD-K62801835–001–01-0 | EZH2 | 3.80929 | 0.000151 |
| BI2536 | PLK1 | 3.69725 | 0.000233 |
| Nilotinib | ABL1 | 3.67464 | 0.000255 |
| Cerulenin | HMGC51 | 3.66163 | 0.000268 |
| TW-37 | BCL2 | 3.585 | 0.000358 |
| zebularine | DNMT1 | 3.57912 | 0.000366 |
| Pevonedistat | NAE1 | 3.55057 | 0.000409 |
| BAS02002358 | GPER1 | 3.52883 | 0.000442 |
| KU-60019 | ATM | 3.52842 | 0.000443 |
| narciscasine | RHOA | 3.47702 | 0.000536 |
| Ki8751 | PDE4A | 3.43765 | 0.000619 |
| 4ly1 | HDAC1 | 3.39584 | 0.000719 |
| SCHEMBL12182311 | EIF4E | 3.35831 | 0.000822 |
| Belinostat | HDAC1 | 3.35122 | 0.000886 |
| Masitinib | PDGFRA | 3.32101 | 0.000941 |
| BIBR1532 | TERT | 3.31812 | 0.000949 |
| UNII-UZ77T1VFBM | BIRC5 | 3.30878 | 0.000985 |
| Nutlin-3 | MDM2 | 3.2982 | 0.001018 |
| CHEMBL2058177 | EIF4E | 3.29052 | 0.001045 |
| NSC373989 | MDM2 | 3.24218 | 0.001239 |
| Imatinib | ABL1 | 3.22213 | 0.001327 |
| Tacrolimus | PPP3CB | 3.13945 | 0.001761 |
| Olaparib | PARP1 | 3.07789 | 0.002159 |
| SMR0001317659 | PDE4A | 3.03729 | 0.002467 |
| Axitinib | PDGFRA | 3.02572 | 0.002563 |
| Pubchem_92131101 | KIF11 | 3.01909 | 0.002617 |
| abraxane | TUBB1 | 3.01218 | 0.002678 |
| GSK461364 | PLK1 | 2.97879 | 0.002987 |
| Sorafenib | PDGFRA | 2.93438 | 0.003443 |
| Nutlin-3 | PDE4A | 2.88952 | 0.003968 |
| SMR000068650 | S1PR2 | 2.88871 | 0.003992 |
| MK-1775 | WEE1 | 2.88827 | 0.003985 |
| BRD-K53855319–001–01-2 | SIRT1 | 2.87063 | 0.004217 |
| Apicidin | HDAC1 | 2.7849 | 0.005485 |
| TelomeraseInhibitorIX | TERT | 2.7825 | 0.005527 |
| Pluripotin | MAPK1 | 2.76988 | 0.005741 |
| PHA-793887 | CDK1 | 2.75543 | 0.005999 |
| Vorinostat | HDAC1 | 2.74595 | 0.006174 |
Development of a nomogram predicting OS

A nomogram predicting the 1-, 3- and 5-year OS for TCGA-LUAD was constructed based on BTNL9 expression and TNM stage (Fig. 8A). We built the ROC for the training dataset and the testing dataset and calculated the area under the ROC (AUC) to validate the accuracy of the nomogram. The AUCs for 1-, 3- and 5-year OS were 0.642, 0.645, and 0.607 in the training set (Fig. 8B); 0.727, 0.545, and 0.631 in the testing set (Fig. 8C). These results suggested that the nomogram showed a consistent accuracy in the training and testing dataset. We then conducted decision curve analysis (DCA) to evaluate the clinical usefulness, and the result showed that the nomogram provided an additional benefit compared to the “treat-all” and “treat-none” strategies in both the training and testing dataset (Fig. 8D-E). Finally, to compare the consistency of the model predictions with actual clinical outcomes, calibration curves for 1-year, 3-year, and 5-year OS were created for the training and testing dataset (supplementary Fig. 1A-F). The calibration curves showed consistent agreement between the predicted and observed values for 1-, 3- and 5-year OS.

Discussion

Immune checkpoint inhibition or adoptive cell therapy has significantly changed the cancer treatment paradigm.

Table 2 Loss of BTNL9 expression may promote drug resistance toward many targeted therapies in CGP, CCLE, and CTRP cohorts (Continued)

| Drug                  | Target      | t-value | p-value |
|-----------------------|-------------|---------|---------|
| I-BET151              | BRD4        | 2.73756 | 0.006332|
| SCHEM12182311         | EIF4A2      | 2.68306 | 0.007448|
| 112,522–64-2          | HDAC2       | 2.66828 | 0.007855|
| RG108                 | DNMT1       | 2.65008 | 0.008212|
| PF184                 | KBKB        | 2.63052 | 0.008701|
| Pazopanib             | PDGFRA      | 2.63026 | 0.008704|
| JQ-1                  | BRD4        | 2.59578 | 0.009615|
| SCHEM13833318         | HDAC1       | 2.56094 | 0.010633|
| CAY10603              | HDAC6       | 2.55261 | 0.010887|
| GSK25762A             | BRD4        | 2.51599 | 0.012071|
| 4CA-620               | PLK1        | 2.43094 | 0.01529 |
| BCP9000801            | MDM2        | 2.41963 | 0.015783|
| Belinostat            | HDAC6       | 2.37335 | 0.018128|
| Tipifarnib            | FNTA        | 2.36404 | 0.018536|
| prima-1               | TP53        | 2.35318 | 0.018879|
| BMS345541             | KBKB        | 2.35317 | 0.018872|
| Gemcitabine           | RRM1        | 2.3315  | 0.019984|
| SMR001317659          | PDE4B       | 2.28061 | 0.022841|
| Doxorubicin           | TOP2B       | 2.26369 | 0.023866|
| Rigosertib            | PIK3CA      | 2.22431 | 0.026433|
| 3,5-di-tert-butylchalcone | RARA | −2.40858 | 0.016249|
| Dasatinib             | EPHA2       | −2.95126| 0.003261|

Table 3 Independent predictive power of BTNL9 based on multivariate analysis

| Characteristics | Total(N) | Univariate analysis | P value | Multivariate analysis | P value |
|-----------------|----------|---------------------|---------|-----------------------|---------|
| T stage (T3&T4 vs. T1&T2) | 501      | Hazard ratio (95% CI) | < 0.001 | 1.911 (1.136–3.217)   | 0.015 |
| N stage (N1&N2&N3 vs. N0) | 492      | 2.606 (1.939–3.503) | < 0.001 | 1.863 (1.260–2.755)   | 0.002 |
| M stage (M1 vs. M0) | 360      | 2.111 (1.232–3.616) | 0.007   | 1.662 (0.790–3.496)   | 0.181 |
| Primary therapy outcome (PD&SD vs. CR&PR) | 419 | 2.786 (1.978–3.924) | < 0.001 | 2.951 (1.949–4.468)   | < 0.001 |
| BTNL9 (High vs. Low) | 504      | 0.686 (0.511–0.921) | 0.012   | 0.669 (0.448–0.999)   | 0.049 |

# CR complete response, PR partial response, SD stable disease, PD progressive disease
and has resulted in an age of chemotherapy-free NSCLC [38]. Immune checkpoint inhibitors figure prominently in achieving chemotherapy-free cancer treatment. BTNs are immune checkpoints in several cancer types; however, the functions of BTNs have not been explored in LUAD. This study shows that BTNL9 is poorly expressed in LUAD tissues, and its low expression is correlated with a lower probability of 1, 3, 5-year OS based on a nomogram model. In addition, this team explored the mechanisms behind BTNL9 low expression. This study shows that mutated p53 results in a significant decrease in BTNL9 expression (Fig. 2B). Approximately 46% of LUAD patients possess p53 mutation [39]. Breast cancer exhibits a low expression level of
BTN1L9, which can be targeted to inhibit proliferation and metastasis through the p53/CDC25C and p53/GADD45 signaling pathway [40]. In addition, BTN1L9 plays a role as a transcriptional modulator through epigenetic regulation and post-transcriptional modification.

DNA methylation is the most common form of DNA modification. It plays a vital role in normal cell physiology, and increased DNA methylation, and loss of demethylation, are observed in different cancer types. DNMTs are implicated in abnormal DNA methylation. Gene body hypermethylation activates oncogenes, and promotion of hypermethylation causes suppression of tumors [41]. GEPIA analysis showed that BTN1L9 and DNMTs correlate significantly with normal lung tissues but not LUAD tumorigenesis (Fig. 4A, B). However, the molecular mechanism of regulation of BTN1L9 by DNMTs in LUAD has not been explored.

miRNA and IncRNA are non-coding RNAs involved in tumor promotion and suppression, depending on the tumor type [41]. A total of 3 miRNAs (hsa-miR-30b-3p, hsa-miR-4709-3p, and hsa-miR-6514-3p) were significantly positively correlated BTN1L9 in LUAD, and their high expression was significantly associated with longer OS. Previous studies reported that hsa-miR-30b-3p plays a role as an antitumor miRNA [42, 43]. Hsu Y-L et al. reported that BTN1L9 acts as a tumor suppressor in LUAD and is regulated by hsa-miR-183-5p; however, the specific regulatory network was not reported [44]. In addition, IncRNA AP001462.6 was shown to bind to BTN1L9, and the high expression level of this IncRNA was significantly correlated with longer OS in LUAD patients (Fig. 4E).

Moreover, analysis of the protein interaction network of BTN1L9 showed that the interacting proteins played a role in immune regulation, protease hydrolysis, and serine/threonine kinase regulation. Notably, protease hydrolysis is related to ubiquitination and degradation of proteins. Analysis showed that BTN1L9 has a potential E3 recognizing domain binding site (Fig. 4H, I). These findings indicate that BTN1L9 in LUAD may be regulated by DNA methylation and non-coding RNA. In addition, BTN1L9 protein may be held by ubiquitination and degradation after translation.

We performed a GSEA analysis to explore the biological function of BTN1L9 in LUAD. Functional analysis showed low expression levels of BTN1L9 in energy metabolism (oxidative phosphorylation, glycolysis, myc targets v1 [45], and mTORC1 signaling [46]), DNA replication, and protease hydrolysis. Metabolic reprogramming triggers selective gene amplification and a large gene family, which drives cellular functions to promote cancer cell growth and proliferation [45]. The above functions were subsequently verified from a single cell perspective. Findings showed that BTN1L9 was significantly negatively correlated with cancer cell malignant behaviors such as proliferation, invasion, EMT, metastasis, and hypoxia. This result indicates that BTN1L9 may play a role in LUAD tumor suppression.

TME is a potential predictor of response to an immune checkpoint inhibitor. Analysis of the relationship between BTN1L9 and TME showed that the mutation frequency of BTN1L9 in LUAD was about 1.14%, and BTN1L9 was significantly negatively correlated with TMB. Furthermore, BTN1L9 was significantly positively correlated with ImmuneScore and ESTIMATEScore. Previous studies report that high ImmuneScore and ESTIMATEScore are positively associated with a good prognosis of LUAD [47]. This finding shows that BTN1L9 plays an important role in TME immune regulation. Moreover, the correlation between BTN1L9 and TILs showed that BTN1L9 was significantly negatively correlated with tumor purity, and previous studies report that low tumor purity is associated with poor prognosis [48]. Although BTN1L9 was significantly correlated with B, CD4+ T, and macrophages, survival analysis showed that BTN1L9 was only significantly correlated with B cells and DC cells.

DCs act pivotally in shaping innate and adaptive immune responses because they have a unique ability to initiate T-cell responses and promote their differentiation into effector lineages [36]. B cells play antigen presentation, cytotoxicity, and antibody production functions, which are essential in adaptive immunity [49]. TIMER and GEPIA database analysis showed that the expression level of BTN1L9 was not correlated with levels of DCs (cDC1s and cDC2s) in normal adjacent tissues; however, BTN1L9 was significantly associated with levels of DCs (cDC1s and cDC2s) in LUAD tissues (Table 1). cDC1s can migrate to tumor-draining lymph nodes, activate and attract T cells, secrete cytokines, and present antigens in TME, promoting local cytotoxic T cells [50]. cDC2s present antigens to MHC II, activate CD4+ T cells, and effectively polarize TILs into anti-tumor T helper cell 1 (Th1) or Th17 phenotype [51]. The BTN1L9 expression level was not correlated with B cells (naïve B cells) in normal adjacent tissues; however, it was significantly associated with levels of B cells (naïve B cells) in LUAD tissues, except for plasma B cells. This finding implies that BTN1L9 regulates the function of naïve B cells in TME. Previous studies report that naïve B cells are down-regulated in advanced NSCLC and are correlated with poor prognosis [37]. Furthermore, CARE database analysis showed that BTN1L9 expression is associated with effective antiangiogenic tyrosine kinase inhibitors response (Fig. 7, and Table 2). More data are being awaited to confirm this preliminary observation.

Notably, this study had a few limitations. Firstly, our findings are entirely based on public databases using
bioinformatics analysis, and therefore further molecular biology experiments should be performed to verify these results. In addition, all findings presented here were developed using Database algorithms. Secondly, BTNL9 may not be detected by histopathology due to a lack of LUAD tissue samples. Further, OS analyses can’t be performed based on histopathology results. Finally, the study did not verify the role of BTNL9 in predicting immune responses in LUAD patients due to the lack of clinical cohorts of immunotherapy-treated LUAD patients. Taken together, results and conclusions based entirely on bioinformatics are informative and can lay down the foundations for more robust studies, but they do not replace experimentation.

Conclusion
In summary, the findings of this study show an association between immune checkpoint BTNL9 and OS in LUAD patients. Transcriptional regulation and post-transcriptional regulation are potential mechanisms for down-regulating BTNL9 expression, resulting in more malignant biological characteristics in LUAD. BTNL9 may modify the TME by enrichment of naïve B cells and DCs and promoting immune response and antiangiogenic tyrosine kinase inhibitors response.

Abbreviations
LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; NSCLC: Non-small cell lung cancer; BTLN: Butyrophilin; BTLN1: Butyrophilin-like; GEPIA: Gene expression profiling interactive analysis; TIMER: Tumor immune estimation resource; CARE: Computational Analysis of Resistance; GEO: Gene expression omnibus; ECOG: Eastern Cooperative Oncology Group; DNM5: Methyltransferases; GSEA: Gene set enrichment analysis; scRNA: single-cell RNA; SNV: Single nucleotide mutations; TMB: Tumor mutation burden; CCLE: Cancer Cell Line Encyclopedia; GDSC: Genomics of Drug Sensitivity in Cancer; CTRP: Cancer Therapeutics Response Portal; TILs: Tumor-infiltrating immune cells; TME: Tumor microenvironment.

Supplementary Information
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Additional file 5. Supplementary Fig. 1. The calibration curves for predicting 1-year, 3-year, and 5-year OS in LUAD. (A, B, and C) Calibration curves for 1, 3, 5-year OS in training dataset; (D, E, and F) Calibration curves for 1, 3, 5-year OS in testing dataset.

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Authors’ contributions
ZC, TD conceptualized and designed the study. WM, JL, ZC, JM, NH, and SZ contributed to data collection. ZC, WM, and TD analyzed and interpreted data. ZC drafted the manuscript. All authors approved the final version of the manuscript.

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Availability of data and materials
All data in this study were retrieved from public and open-source databases. All databases and their web address were listed below: GEPIA (http://gepia2.cancer-pku.cn/index), Oncomine (https://www.oncomine.org/), KM plotter (http://www.kmplot.com), UALCAN (http://ualcan.path.uab.edu/index.html), OncoLnc (http://www.oncolnc.org/), TissGDB (https://bioinfouth.uu.edu/TissGDB/index.html?csrt=1756789353851237153), TIMER (http://timer.comp genomics.org/), Sangerbox (http://sangerbox.com/index), miRWalk (https://mirmap.expanded.org/), TargetScan (http://www.targetscan.org/vert_72/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/), starBase (http://starbase.sysu.edu.cn/panCancer.php), CancerSEA (biocc.hrbmu.edu.cn/CancerSEA/goSearch).

Declarations
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Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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