

# Identification of TLN1 as a prognostic biomarker to effect cell proliferation and differentiation in Acute Myeloid Leukemia

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## Research Article

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# Abstract

The protein Talin1 encoded by the *TLN1* gene is a focal adhesion-related protein that binds to various cytoskeletal proteins and plays an important role in cell adhesion and movement. Recent studies have shown that it is overexpressed in prostate cancer, liver cancer, and oral squamous cell carcinoma, and is closely related to tumor progression and metastasis. This study integrated bioinformatics and functional analysis to reveal the prognosis and potential functions of *TLN1* in AML. The results showed that the expression level of *TLN1* was abnormally increased in AML and localized in the cell membrane and cytoplasm, which is a significant prognostic indicator of overall survival(OS). Enrichment analysis of related genes showed that *TLN1* is related to neutrophil mediated immunity, neutrophil activation and may regulate important signal pathways in hematological tumors including tyrosine kinase receptor, FLT3 and PIK3/AKT. The PPI network shows that *TLN1* and *MYH9* may be involved in the process of AML tumors together with *PIP5K1C*, *ROCK1*, *S100A4*, *MYO1A* and *WAC*. Immune infiltration analysis explains that *TLN1* is associated with multiple immune cells and may be an important immune marker in AML. Furthermore, molecular biology experiments confirmed that *TLN1* is related to the proliferation, differentiation and cycle of AML cells. Silencing *TLN1* can inhibit the proliferation of AML cells and promote differentiation through the Talin1/P-AKT/CREB signaling pathway.

## Introduction

Acute myeloid leukemia (AML) is an aggressive hematological tumor characterized by the abnormal proliferation and differentiation of immature myeloid cells a poor prognosis and is prone to drug resistance and recurrence<sup>1</sup>. According to cancer statistics 2020, 19940 new cases and 11180 deaths are expected to arise in America in 2020<sup>2</sup>. Although the development and application of new drugs have greatly extended the median survival time of patients, most patients still experience recurrence, refractory or even death, and the overall survival rate is not satisfactory. A total of 57.4% of patients experience recurrence with genetic alterations that are difficult to treat<sup>3</sup>. The prognosis is even worse for these patients, only approximately 10% of patients surviving long term, with a median survival of 5 to 10 months<sup>4</sup>. Therefore, there is an urgent need to find new treatment targets and treatment plans in clinical practice to improve the remission rate and survival rate of patients and reduce adverse reactions after chemotherapy.

Integrin family adhesion molecules are the most important molecules that mediate the interaction between cells and the extracellular matrix. They recognize and bind the corresponding ligands in the extracellular matrix to provide attachment points for cell attachment. At the same time, it transmits signals from the extracellular environment to the cell, and regulates gene expression by activating multiple signal pathways, thereby regulating cell movement, proliferation and apoptosis<sup>5</sup>. Talin1 is a cytoskeletal protein with a weight of about 270 kDa encoded by *TLN1*, which is the first adaptor protein to be confirmed to bind to integrin. It's binding to the intracellular segment of the integrin subunit is the key link for integrin activation<sup>6</sup>. Talin1 contains 18 domains that bind at least 11 different fatty acid

components, including vinculin and actin<sup>7</sup>. Recent studies have shown that the overexpression of *TLN1* in oral squamous cell carcinoma, liver cancer, and prostate cancer is related to tumor invasion, metastasis, or poor differentiation. Molecular biology experiments have confirmed that it does promote the growth and invasion of tumor cells. Furthermore, the overexpression of *TLN1* can significantly enhance the drug resistance of triple-negative breast cancer<sup>8</sup>. However, the role and mechanism of *TLN1* in leukemia is still unclear. This study uses bioinformatics methods and molecular biology experiments to explore the functional role of *TLN1* in the occurrence and development of AML.

## Materials And Methods

### Data Collection

The data of RNA expression data, DNA methylation and transcript were downloaded from UCSC XENA (<https://xenabrowser.net/datapages/>), the RNAseq data in TPM format TCGA and GTEX processed uniformly by the Toil process<sup>9</sup>, including AML patients of TCGA and normal controls of GTEX.

### Molecular correlation analysis

To explore the related genes of *TLN1* in AML, we used the LinkedOmics database(<http://www.linkedomics.org/>) to draw a volcano map, heat map, Pearson correlation coefficient or scatter plot.

### GO|KEGG and Gene Set Enrichment Analysis(GSEA) enrichment analysis

Genes associated with *TLN1* with the threshold for  $|\text{pearson correlation coefficient}| > 0.5$  and  $p < 0.01$  were applied for GO|KEGG enrichment analysis and GSEA enrichment analysis<sup>10</sup>. Use `org.Hs.eg.db` package version 3.10.0 for gene ID conversion and `clusterProfiler` package<sup>11</sup> version 3.14.3 for enrichment analysis in R. The GSEA enrichment analysis data set uses `C2.all.v7.0.symbols.gmt`.

### Pathway activity and drug sensitivity

Use the Gene Set Cancer Analysis (GSCA) database to predict cancer pathway activity and drug sensitivity related to *TLN1*.

### Protein-protein interaction(PPI) network and immune infiltration analysis

Used GeneMANIA to construct a PPI network and predict the part function of *TLN1*<sup>12</sup>. Used version 1.34.0 of GSVA package to perform immune infiltration of *TLN1* in AML. The correlation analysis method uses Spearman.

## Cell culture

The human AML cell lines KG-1, MOLM13, Kasumin-1, K562, THP-1 and HL60 were provided by the Department of Hematology, The First Affiliated Hospital of Zhejiang University, Hangzhou, China. AML cells were cultured with RPMI-1640 containing 10% fetal bovine serum at 37 °C and 5%CO<sub>2</sub> saturated humidity.

## Patient sample source

Human primary bone marrow cells were obtained from the First Affiliated Hospital of the University of Science and Technology of China. After obtaining the consent of the admitted AML patient, 3-5 ml of bone marrow or peripheral blood was collected. After mononuclear cells were extracted by density gradient centrifugation, the protein was extracted for subsequent experiments. Human primary cells were obtained after obtaining informed consent from AML patients according to the Declaration of Helsinki. This study was approved by the Ethics Committee of the First Affiliated Hospital of the University of Science and Technology of China.

## Immunofluorescence staining and Wright–Giemsa staining

Collect the cells growing in the logarithmic phase, wash with PBS and smear. After fixation with 4% paraformaldehyde for 15 minutes, use goat serum to block overnight at 4°C. After the primary antibody was incubated for six hours, washed with PBST three times at 4°C, and incubated with the secondary antibody(Dy light594) for 1 hour at room temperature. The slides were sealed with DAPI-containing anti-fluorescence quenching sealing tablets and observed under Zeiss LSM780 confocal microscope to collect images. Cell-smear was performed as previously described, Wright's Giemsa dye solution for 30 seconds, add distilled water dropwise to dye for 15 minutes. Wash the slides last, and microscopy was performed on Axio microscope using 100X/1.25 NA oil objective.

## qRT-PCR and and small interfering RNA transfection

Trizol reagent(Invitrogen, China) was used to separate total ribonucleic acid from cultured cells and used PrimeScript RT Master Mix(Takara) to obtain cDNA. At last, use TB Green Premix Ex Taq(Takara) to make realtime PCR. The *TLN1* primer used for the amplification was as follows: 5'-AGTGACGGACAGCATCAACCAG-3'and 5'- GGATTCTCCAGGAGTTCTCGGA-3(Tongyong Biotech, China). Cells were transfected with siRNA using Lipofectamine 8000(Beyotime Biotechnology, China). The

targeting sequence of small interfering RNAs were siRNA1 5'-GGUACAGAAUCUAGAGAAATT-3' and 5'-UUUCUCUAGAUUCUGUACCTT-3', siRNA2 5'-UGUUAUUUCCUCCUUUUUCUC-3' and 5'-GAAAAAGGAGGAAUAACAGG-3' (Tongyong Biotech, China).

## Western Blotting

After using siRNA transfection 48 h, the cells in each group were lysed with RIPA lysate and centrifuged for half an hour (4 °C, 13 000 r/min). Other western blotting steps are the same as previous studies[16]. Caspase-3 antibody was purchased from Cell Signalling Technology (Beverly, MA, USA). Phosphorylated-AKT, CREB, Talin1 and PARP were purchased from Proteintech Technology (Wuhan, China). Total AKT was purchased from Zen-bioscience (Chengdu, China). This procedure was repeated 3 times for each protein sample.

## Cell cycle analysis

The cells were treated with siRNA transfection for 48 hours and fixed overnight with 70% ethanol at -4°C. After two times of PBS cleaning, PI staining was used for 30 minutes on the flow cytometer for testing and the Cytexpert software was used to analyze data.

## Statistical analysis

Statistical obtained from TCGA and GTEX were analyzed by R-3.6.1. Other datas were processed using GraphPad Prism 8, Flowjo and Cytexpert software.  $P < 0.05$  was considered statistically significant.

## Results

### TCGA samples information and *TLN1* expression analysis in pan-cancers and AML

The RNAseq data of the AML and the normal control samples are uniformly processed by the Toil process downloaded from UCSC XENA, and the RNAseq data in TPM (transcripts per million reads) format are analyzed and compared after log<sub>2</sub> conversion. We found that *TLN1* is abnormally highly expressed in 12 tumors including AML, compared with normal tissues (Figure1A and B). Then we used The Human Protein Atlas(<https://www.proteinatlas.org/>) to analyze the expression level of *TLN1* in human blood cells, found that the RNA expression of *TLN1* showed low cell-type specificity (Figure1C). Finally, we used UCSC XENA to analyze the RNA expression, exon expression and DNA methylation of *TLN1* in the TCGA AML cohort(Figure1D). We found that DNA methylation level was significantly increased at the probe of cg14025149 and cg14095761(Figure1E).

# The relationship between the expression of *TLN1* and the prognosis and clinical characteristics of patients

We used survival package(3.2-10 version) for statistical analysis of survival data and survminer package [0.4.9 version] for visualization and drawing Kaplan-Meier survival curves. The high expression of *TLN1* is related to poor overall survival(OS) in 7 tumors including AML(Figure2A-G). ROC analysis for AML patients shows that *TLN1* was a significant prognostic marker for OS(Figure2H). Next, we downloaded the clinical characteristics of TCGA patients and divided them into high expression groups and low expression groups according to the median of TPM(Table1). We used univariate Cox regression analysis to evaluate prognostic variables related to OS. The univariate Cox model showed that high *TLN1* expression is closely related to poor OS(HR=1.728 (95% Confidence interval:1.128–2.647)). In addition, age (>60 years), Cytogenetic risk(intermediate and poor) were also poor prognostic factors for AML patients(Figure2M). Therefore, we used the nomogram to show the correlation between *TLN1* expression and prognosis. The Conformity Index (C-index) was 0.721(95% Confidence interval:0.693-0.748) (Figure2N). The results of the multiple hypothesis test (Dunn's test) show that the groups of intermediate and poor are higher than the average of Favorable, the difference is statistically significant ( $P<0.05$ )(Figure2I). Mann-Whitney U test (Wilcoxon rank-sum test) results show that the expression level of *TLN1* increased significantly when the age>60, the median of the difference between the two groups is 0.327(0.08-0.545), the difference is statistically significant ( $P=0.009$ )(Figure2K). Similarly, the expression of *TLN1* was also related to the later FAB classifications and dead of OS events(Figure2J and L).

## *TLN1* related gene enrichment analysis

To investigate genes related to *TLN1*, we used LinkedOmics to analyze co-expressed genes of *TLN1* in AML<sup>13</sup>. After screening with  $P<0.05$  and |correlation coefficient|>0.5, we got 285 genes revealed a significant positive association with *TLN1*, while 165 genes indicated significantly negative associations(Figure3A-C). Then we used 450 genes related to *TLN1* for GO|KEGG and GSEA enrichment analysis. GO enrichment analysis results showed that *TLN1* may be involved in neutrophil mediated immunity, neutrophil activation and neutrophil degranulation etc(Figure3D and E). We also analyzed the bubble chart and chord chart for several main enrichment functions(Figure3F-H), the enrichment function notes are in supplementary 1. In addition, GSEA enrichment analysis results show that *TLN1* may be related to multiple signaling pathways in hematological tumors, including tyrosine kinase receptor, PI3K/AKT/mTOR and FLT3 signaling pathway(Figure4A-K). Next, the analysis of cancer pathway activity showed that *TLN1* may participate in the activation of EMT, PI3K/AKT, RAS/MAPK and RTK pathways(Figure4L). Complementary, drug sensitivity analysis showed *TLN1* may be related to the resistance of PD-153035, austocystin-D and Afatinib. (5Z)-7-Oxozeaenol, AZD6482 and BX-795 may provide a therapeutic strategy for targeting *TLN1*(Figure4M and N).

## PPI and immune infiltration analysis of *TLN1*

In the previous correlation analysis, *MYH9* and *FLNA* showed a very significant correlation, and the correlation coefficients were 0.76 ( $p < 0.001$ ) and 0.71 ( $p < 0.001$ ). Therefore, we analyzed the expression and prognosis of *MYH9* and *FLNA* in the TCGA AML cohort. The results showed that *MYH9* was abnormally highly expressed in AML patients and both of *MYH9* and *FLNA* were associated with poor prognosis (Figure 5A). Using GeneMANIA to predict *TLN1* and *MYH9*-related PPI network results showed that the functions were concentrated in blood coagulation, cell-cell junction organization, coagulation, hemostasis, homotypic cell-cell adhesion, platelet activation (Figure 5B). We co-expression analysis of the top fifteen genes with the highest scores in the PPI network (Figure 5C), and the expression of *PIP5K1C*, *ROCK1*, *S100A4*, *MYO1A* and *WAC* is abnormally increased in AML (Figure 5D-H). This showed that *TLN1* may work together with *PIP5K1C*, *ROCK1*, *S100A4*, *MYO1A* and *WAC* in AML. The results of immune infiltration analysis of *TLN1* using R showed that *TLN1* expression was significant associations with multiple immune cells including macrophages ( $p < 0.001$ ,  $r = 0.428$ ), Tem ( $p < 0.001$ ,  $r = 0.426$ ), iDC ( $p < 0.001$ ,  $r = 0.421$ ) etc (Figure 5I). This means that *TLN1* may be an important marker of immune infiltration in AML.

## Expression of *TLN1* in AML samples and cell lines

To further investigate the expression of *TLN1* in AML, we verified the expression level of *TLN1* in AML cell lines using qRT-PCR and Western blot (Figure 6A and B). The result showed that the mRNA and protein expression levels of Talin1 were the highest in the acute monocytic leukemia cell line (THP-1). Next, we verified the protein expression level of Talin1 in the mononuclear cells of normal people and AML patients. The result showed that Talin1 expression was low or undetectable in normal people, and the expression level is abnormally increased in most AML patients (Figure 6C and D). In addition, we used immunofluorescence staining to observe the localization of Talin1 in AML cell lines and the results showed that Talin1 is mainly located in the cell membrane and cytoplasm (Figure 6E).

## Silenced *TLN1* can inhibit the proliferation of AML cells and promote differentiation

After transfecting THP-1 and HL60 cell lines with small interfering RNA (siRNA), the expression level of *TLN1* was detected by qRT-PCR. As shown in Figure 7A, both siRNA1 and siRNA2 could significantly reduce the expression of *TLN1*. Growth curve analysis showed that silenced *TLN1* significantly reduced the proliferation ability of AML cells (Figure 7E). Wright Giemsa compound staining results showed that silenced *TLN1* leads to the transformation of AML cell nuclei from round to rod-shaped nuclei and pyknotic nuclei and the nucleus of THP-1 appears pyknotic and deep staining (Figure 7B). This means that silencing *TLN1* could inhibit proliferation and promote the differentiation of AML cells. Flow cytometry was used to analyze the effect of *TLN1* on the cell cycle, as shown in Figure 7C and D, the proportion of apoptotic cells of HL60 and THP-1 after *TLN1* silence increased significantly. At the same time, the proportion of THP-1 cells in G0/G1 phase increased significantly, but this phenomenon was not found in

HL60. We also tested the expression level of apoptosis protein silenced by *TLN1*, as shown in Figure7F, the expression of Caspase3 in *TLN1* silenced group cells was significantly increased, but PARP did not change significantly. This means that the silencing of *TLN1* may lead to apoptosis of AML cells. In summary, these results indicate that *TLN1* participates in the progression of AML by regulating the proliferation, apoptosis, differentiation and cell cycle of AML cells.

## ***TLN1* regulates the proliferation and differentiation of AML cells by Talin1/p-AKT/CREB signaling pathway**

According to the enrichment analysis result of GSEA, *TLN1* may be related to the PI3K/AKT signaling pathway. Therefore, we tested the expression levels of AKT and P-AKT after *TLN1* silence. Our results showed that interfering with the expression of Talin1 reduced the expression level of P-AKT. Similarly, the expression level of CREB was also downregulated which was a signal molecule downstream of AKT and related to cell proliferation and differentiation(Figure7G). These results indicated that *TLN1* may regulate the proliferation and differentiation of AML cells by regulating the expression of Talin1/p-AKT/CREB.

## **Discussion**

The protein Talin1 encoded by *TLN1* is a focal adhesion cytoskeleton protein that binds and activates integrins, and integrins recruit other focal adhesion kinases (FAKs). Studies have shown that Talin is an indispensable factor for endothelial cell-mediated angiogenesis<sup>14</sup>. In patients with multiple myeloma and chronic lymphocytic leukemia, Talin1 can also promote the abnormal transport of malignant B cells to the bone marrow and lymph nodes by regulating  $\alpha 4\beta 1$  integrin activity. The abnormal homing of these malignant cells to the bone marrow microenvironment can induce cell adhesion-mediated drug resistance<sup>15</sup>. In addition, Arundhati Halder et al. analyzed one publicly available chronic myeloid leukemia(CML) RNA-sequencing dataset (SRA073794) found that Talin1(*TLN1*)was upregulated by 6 times in CML-blast crisis(BC) compared with the CML-chronic phase(CP) stage<sup>16</sup>. These results imply that *TLN1* may play an important role in hematopoietic malignancies.

In this study, we used a variety of bioinformatics analysis and functional analysis methods to explore the expression, prognostic role and biological function of *TLN1* in AML. Our results show that the RNA and protein of Talin1(*TLN1*) are abnormally highly expressed in AML samples, which may be a prognostic marker of AML patients. Enrichment analysis results show that *TLN1* is related to multiple functions of neutrophils and may be involved in multiple important signal pathways in hematopoietic malignancies.

Non-muscle myosin heavy chain9(*MYH9*) is widely expressed in various tissues and cells. *MYH9* is abnormally highly expressed in esophageal squamous cell carcinoma<sup>17</sup>, breast cancer<sup>18</sup>, epithelial ovarian cancer<sup>19</sup> and acute myeloid leukemia<sup>20</sup> and is associated with poor prognosis. Researchers report that *MYH9* degrades GSK3 $\beta$  protein through TRAF6-induced ubiquitination, enhancing the stem cell characteristics, migration, invasion, growth and sorafenib resistance of HCC cells<sup>21</sup>. This means that

*MYH9* may be a promising target gene for cancer treatment. Our analysis found that *TLN1* strongly correlates with *MYH9*, and *TLN1* may work with *MYH9*, *PIP5K1C*, *ROCK1*, *S100A4*, *MYO1A* and *WAC* to promote the growth and invasion of AML cells. However, the mechanism of action between *TLN1* and *MYH9* needs more experiments to verify.

The transcription factor CREB (cAMP response element binding protein) is a nuclear protein that selectively binds to the cyclic adenylate response element to regulate cell proliferation, differentiation and apoptosis<sup>22</sup>. Research has shown that the expression level of CREB in acute leukemia cells is significantly higher than that of normal bone marrow cells, and the high expression of CREB is associated with a poor prognosis.<sup>23</sup> In addition, CREB can be used as a proto-oncogene to be activated through the AKT signaling pathway, which can regulate cell production in the blood and affect the phenotype of leukaemia<sup>24</sup>. Our study found that interference with the expression of *TLN1* can reduce the level of CREB by down-regulating the expression of P-AKT. Therefore, the Talin1/P-AKT/CREB signal pathway regulated by *TLN1* may become a new therapeutic target for AML.

The 13 rod-shaped of Talin1(R1-R13) domains are entangled into a compact globular structure<sup>25, 26</sup>, and the R4 domain is buried in the core to protect R4 from being combined with other proteins to function<sup>27</sup>. The conformational changes of Talin1 can be regarded as a collection of open and closed states, which can be reversibly changed by changes in the microenvironment in the body. This autoinhibition mode can appropriately turn off the adhesion components to control cell attachment and migration<sup>28</sup>. We speculate that this autoinhibition state may change in AML patients, resulting in abnormally abnormal activation of Talin1, thereby promoting the proliferation of AML cells. In conclusion, *TLN1* is a potential prognostic and therapeutic biomarker of AML, and it can affect the immune microenvironment to promote the proliferation of AML cells and inhibit differentiation and regulate multiple signal pathways in hematologic malignancies. However, the regulatory effect of *TLN1* on the immune microenvironment and the interaction between *TLN1* and *MYH9* need to be further confirmed by experimental data.

## Abbreviations

AML: Acute myeloid leukemia

TPM: Transcripts per million reads

CREB: cAMP response element binding protein

PPI: Protein-protein interaction

GSEA: Gene Set Enrichment Analysis

GSCA: Gene Set Cancer Analysis

TCGA: The cancer genome atlas

GTEX: Genotype-Tissue Expression

OS: Overall survival

MYH9: Non-muscle myosin heavy chain9

## **Declarations**

### **Ethics approval and consent to participate**

This study was conducted under the approval of the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China. All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethics Committee. All patients and/or legal guardians were informed prior to experiments and signed written informed consents.

### **Consent for publication**

Not applicable.

### **Conflicts of interest**

The authors declare no competing financial interests.

### **Availability of data and materials**

All analyzed data are included in this published article and its supplementary information file. The data underlying this study are freely available from UCSC XENA (<https://xenabrowser.net/datapages/>). The authors did not have special access privileges. All data included in this study are available upon request by contact with the corresponding author.

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### **Author contributions**

C.Z.M. and W.W.Z. designed the experiments; D.C .and X.L.C. carried out experiments and wrote the manuscript; Y.Y and X.Y.G analyzed the results; W.W.Z. submitted and revised the paper.

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Not applicable.

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## Table

Table 1 is available in the Supplementary Files section.

## Figures

## Figure 1

*TLN1* expression in tumor and normal tissues in pan-cancer data of The Cancer Genome Atlas(TCGA) and GTEx(A). *TLN1* expression in tumor and normal tissues in AML(B) . RNA expression of *TLN1* in blood cells(C). Heatmap showing the correlations between *TLN1* methylation , exon expression and mRNA expression in AML via UCSC Xena(D). Analysis of methylation probe of *TLN1* in AML(E). Data were shown as mean  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

## Figure 2

Correlation between *TLN1* expression and prognosis. High expression of *TLN1* is associated with poor prognosis of Brain Lower Grade Glioma(A), Adrenocortical carcinoma(B), Oral squamous Cell Carcinoma(C), Colon adenocarcinoma(D), Glioma(E), Cervical squamous cell carcinoma and endocervical adenocarcinoma(F), AML(G). ROC analysis of *TLN1* in the prognosis of AML(H). Correlation between *TLN1* and clinical characteristics of AML(I-L). Univariate cox analysis of *TLN1* in AML(M). The nomogram of age (>60 years), Cytogenetic risk, *TLN1* in the prognosis of AML(N).

## Figure 3

A Pearson test was used to analyze the association of *TLN1* with genes expressed in AML cohort(A). Heat maps showing genes positively (B) and negatively (C) related to *TLN1* in AML(TOP 50). Bubble chart and visual network of GO|KEGG enrichment analysis(D-E). Analysis of the joint correlation coefficient of GO|KEGG gene enrichment(F-H).

## Figure 4

GSEA analysis of *TLN1* related genes. GSEA analysis of the top ten enrichment pathways of Enrichment Mountain plots(A). Gen enrichment score plots of the top ten enrichment pathways(B-K). ES, enrichment score; NES, normalized ES; ADJ $P$ -val, adjusted  $P$ -value. *TLN1*-related Cancer pathway activity(L). *TLN1*-related drug sensitivity using GDSC (M) and CTRP (N) database.

## Figure 5

The expression and prognosis of *MYH9* and *FLNA* in AML(A). PPI network of *TLN1* and *MYH9* (GeneMANIA) (B). Heat map of co-expressed genes of *TLN1* and *MYH9*(C). *PIP5K1C*, *ROCK1*, *S100A4*, *MYO1A* and *WAC* expression in tumor and normal tissues in AML(D-H). Correlation between expression of *TLN1* and immune infiltration(I).

## Figure 6

*TLN1* expression in AML cell lines(A-B). The expression of *TLN1* in normal people and AML patients(C-D). Subcellular localization of *TLN1* in THP-1 and HL60(E).

## Figure 7

HL60 and THP-1 cells were transfected with siRNA, the level of *TLN1* was evaluated by qRT-PCR(A). Wright–Giemsa staining of control and *TLN1*-silenced cells(B). The flow cytometric analysis was carried out to analysis the cell cycle distribution(C-D). The proliferation of THP-1 and HL60 was examined by cell counts(E). The expression levels of apoptosis-related proteins were evaluated by Western blot in control and *TLN1*-silenced cells(F). The expression levels of Talin1/p-AKT/CREB proteins were evaluated by Western blot in control and *TLN1*-silenced cells (G). Data were shown as mean  $\pm$  SD. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [AMLNORMALTLN1expression.xlsx](#)
- [GSEA.xlsx](#)
- [supplementary1.xlsx](#)
- [tln1pancerexpression.xlsx](#)