

DBN1-mediated upregulation of GAB2 facilitates the migration and invasion of T-cell acute lymphoblastic leukemia cells

JIAXING SUN^{1-3*}, XIAOXING HUANG^{4*}, XINGRUO ZENG², YUFEI LEI², HENGJING HE²,
ZIMENG WEI², DI XIAO², QIUPING ZHANG^{2,5}, XINRAN LI^{6,7}, FULING ZHOU¹ and LIANG SHAO¹

¹Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, P.R. China;

²Department of Immunology, School of Basic Medical Science, Wuhan University, Wuhan, Hubei 430071, P.R. China;

³Luoyang Key Laboratory of Transplantation and Immunological Studies for Haematological Diseases, Department of Clinical Laboratory, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, Henan 471003, P.R. China; ⁴Department of Blood Transfusion, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, P.R. China; ⁵Hubei Provincial Key Laboratory of Developmentally Originated Disease, Wuhan University, Wuhan, Hubei 430071, P.R. China; ⁶Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, P.R. China; ⁷Hubei Provincial Clinical Research Center for Molecular Diagnostics, Wuhan, Hubei 430071, P.R. China

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Abstract. T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. The poor prognosis of T-ALL is closely associated with extensive leukemic infiltration into critical organs. Therefore, the mechanism underlying T-ALL infiltration is worth investigating. Databases and clinical samples were utilized to examine drebrin 1 (DBN1) expression in T-ALL. DBN1 knockdown cell lines were established by lentivirus transfection, and cell migration and invasion were examined using Transwell and Matrigel-Transwell assays. The molecular mechanism was investigated by RNA sequencing and further validated at the molecular level. Reverse transcription-quantitative PCR and

western blotting were employed to examine the expression of downstream molecules following DBN1 knockdown, with subsequent rescue experiments. DBN1-targeting microRNA (miR) predicted using bioinformatics websites was confirmed using dual-luciferase assays. In T-ALL cells, miRNA mimics transfection enabled functional validation, and investigations into the underlying molecular mechanisms encompassing rescue experiments. Clinical samples and publicly available databases revealed that DBN1 was upregulated in patients with T-ALL patients. DBN1 knockdown significantly decreased the migration and invasion of T-ALL cells *in vitro*. RNA sequencing revealed that downregulation of DBN1 could reduce Grb2-associated binding protein 2 (GAB2) expression. Western blotting revealed that GAB2 expression, and PI3K/AKT and MAPK/ERK signaling were decreased in DBN1-knockdown cells. GAB2 overexpression restored the phosphorylation of downstream effectors (AKT and ERK1/2). Bioinformatics and dual-luciferase reporter experiments identified miR-218-5p binding to the 3'-untranslated region of DBN1, which suppressed DBN1 expression. In addition, the experiments demonstrated that miR-218-5p acted as an upstream regulator of DBN1, and was involved in cell migration and invasion. Overall, DBN1 was upregulated in T-ALL, and its depletion inhibited cell migration and invasion through downregulation of GAB2 and consequent inhibition of AKT and ERK signaling cascades. The present data suggested that DBN1 could be a novel biomarker of T-ALL infiltration, which is a novel perspective in the field of leukemia research.

Correspondence to: Dr Liang Shao, Department of Hematology, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuhan, Hubei 430071, P.R. China
E-mail: liangsm@163.com

Dr Xinran Li, Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuhan, Hubei 430071, P.R. China
E-mail: xinran2827@whu.edu.cn

*Contributed equally

Abbreviations: T-ALL, T-cell acute lymphoblastic leukemia; GAB2, Grb2-associated binding protein 2; AML, acute myeloid leukemia; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; miRNA, microRNA; GEO, Gene Expression Omnibus; shRNA, short hairpin RNA; RNA-seq, RNA sequencing; FPKM, fragments per kilobase million; BM, bone marrow; DEG, differentially expressed gene

Key words: drebrin 1, T-ALL, migration, invasion, GAB2

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is characterized by diffuse infiltration of malignant hematopoietic cells expressing immature T-cell markers in the bone marrow (BM) (1,2). T-ALL accounts for 10-15 and 25% of the incidence of acute lymphoblastic leukemia in children and adults,

respectively (3,4). Invasiveness and recurrence contribute to T-ALL-related mortality (5). Dissemination of leukemia is associated with disease relapse, leading to the poor prognosis of patients with T-ALL (6). A lack of effective therapeutic targets has largely impeded the progress in T-ALL treatment. Therefore, further elucidation of the underlying molecular mechanisms of T-ALL infiltration is both theoretically and clinically significant.

Drebrin 1 (DBN1), which encodes a developmentally regulated brain protein, is an actin-binding protein originally identified in neurons (7,8). Nonetheless, its expression has been observed in various nonneuronal cells, including T lymphocytes and cancer cell lines (9,10). DBN1 contributes to cell motility and morphology (11,12). In particular, DBN1 drives local changes in plasticity and the formation of protrusions by assembling actin filaments, suggesting a role of DBN1 in cancer dissemination (13,14). Overexpression of DBN1 in glioma cells and prostate cancer cells results in alterations in cell morphology and augmented invasion *in vitro*, while knockdown of DBN1 decreases cell migration and invasion (15,16). Patients with luminal breast cancer exhibiting high DBN1 expression have higher recurrence rates and distant metastasis incidence compared with those with low DBN1 expression (17). However, to the best of our knowledge, the effect of DBN1 on leukemia cell infiltration in T-ALL has not yet been reported.

GAB2 is an oncogene and encodes Grb2-associated binding protein 2 (GAB2) (18,19). GAB2 is a scaffolding protein that contains various structural domains, and acts to transmit and amplify signals from both receptor and nonreceptor tyrosine kinases to downstream effectors (20). Previous studies have provided evidence that GAB2 potentiates the activation of the ERK and PI3K-Akt pathways (21,22), and GAB2 has been implicated in the tumor metastasis of hepatocellular carcinoma (23), gastric cancer (24) and leukemia (25). In leukemia, GAB2 has been reported to be associated with the development of acute myeloid leukemia (AML). For example, GAB2 can promote FMS-like tyrosine kinase-3 internal tandem duplication-mutant human AML aggressiveness via STAT5 signaling (26,27). High GAB2 expression has been shown to promote AML progression via the Src homology region 2-containing protein tyrosine phosphatase 2-ERK-cAMP-response element binding protein signaling pathway, as documented in a prior study (28).

The present study focused on investigating the roles of DBN1 and its downstream signaling molecules and pathways, including GAB2, in T-ALL infiltration. The present study also investigated the mechanism underlying the abnormal expression of DBN1 in T-ALL. The present study aimed to identify novel molecular markers to offer a novel perspective for the treatment of T-ALL.

Materials and methods

Collection of clinical samples and ethics statement. The present study was approved by the Institutional Ethics Committee of the Zhongnan Hospital of Wuhan University (Wuhan, China) and performed in accordance with the principles of The Declaration of Helsinki. Peripheral blood (PB) clinical samples from 10 patients with T-ALL and 6 healthy individuals

were collected at Zhongnan Hospital of Wuhan University (Wuhan, China) between November 2019 and November 2024. The patient cohort (n=10) comprised individuals aged 22-52 years (male-to-female ratio 3:2), while healthy controls (n=6) were aged 20-45 years (male-to-female ratio 1:1). The inclusion criteria for patients with T-ALL were as follows: Patients diagnosed with T-ALL for the first time and who had not received treatment. The exclusion criteria included the following: Patients with other hematological diseases; patients with malignant tumors; patients <18 years of age; and pregnant women. Each subject was sampled only once, and a 2-ml PB sample was obtained. Samples from patients with T-ALL were collected after diagnosis but before treatment. There was no clear restriction on the time of sample collection for healthy subjects. All PB samples were processed immediately upon collection for PB mononuclear cell (PBMC) isolation without intermediate storage steps. PBMCs were isolated from the PB of patients with T-ALL and healthy individuals.

PBMC isolation. The PBMC fractions were isolated using the Ficoll method. Fresh whole-blood samples were pre-diluted 1:1 with PBS and 2 ml diluted blood was then layered carefully on an equal volume of Ficoll Paque (TBD science). The PBMC fraction was isolated following centrifugation at 400 x g for 40 min at room temperature. Subsequently, the PBMC fraction was washed with PBS and centrifuged at 100 x g for 10 min at room temperature. Subsequently, PBMCs were subjected exclusively to reverse transcription-quantitative PCR (RT-qPCR) analysis.

Gene expression omnibus (GEO) and Oncomine databases. The Oncomine database [<http://www.oncomine.org>; Haferlach Leukemia dataset and Haferlach Leukemia 2 dataset (29)] was accessed to analyze DBN1 and GAB2 mRNA expression in T-ALL. In the present study, a P-value of 0.0001, fold change of 2 and top 10% gene rank were set as the thresholds. For GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) screening, the following standardized screening process was followed: i) Preliminary search: With 'T-cell acute lymphoblastic leukemia' (T-ALL) and 'Homo sapiens' as key words, 'array-based expression profiling' type data were screened in GEO DataSets; ii) inclusion criteria: T-ALL clinical samples or cell lines must be included; the case group (T-ALL) and healthy control group should be clearly distinguished; and iii) exclusion criteria: Sample sequencing datasets involving experimental intervention; datasets without matched control groups; and datasets with small sample sizes. Through the aforementioned process, the GSE48558 (30), GSE46170 (31), GSE26713 (32) and GSE13159 (33) datasets were initially obtained. Further analysis of the aforementioned datasets revealed that the GSE48558 dataset contained RNA sequencing (RNA-seq) data from T-ALL cell lines that could be included in the present study. The remaining three datasets all involved T-ALL clinical BM samples: GSE46710, which included 31 BM samples from patients with T-ALL and 7 healthy thymus tissues; GSE26713, which included 110 T-ALL BM samples and 7 healthy BM samples; and GSE13159, which included 170 BM samples from patients with T-ALL and 73 healthy BM samples. To ensure data quality, GSE13159 was selected

due to its large sample size for both case and control groups. GSE13159 and GSE48558 were selected to compare DBN1 and GAB2 expression in patients with T-ALL and healthy individuals.

Cell culture. 293T cells (CRL-11268), and MOLT4 (CRL-1582) and Jurkat (TIB-152) human T-ALL cell lines were purchased from American Type Culture Collection. 293T cells were cultured in DMEM (Biological Industries) supplemented with 10% FBS (Biological Industries) and 1% penicillin/streptomycin (Beyotime Institute of Biotechnology) with 5% CO₂ at 37°C. T-ALL cell lines were cultured in RPMI-1640 medium (Biological Industries) supplemented with 10% FBS (Biological Industries), 1% L-glutamine (HyClone™; Cytiva) and 1% penicillin/streptomycin (Beyotime Institute of Biotechnology) with 5% CO₂ at 37°C.

RNA isolation and RT-qPCR. Total RNA was isolated from cells using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA was synthesized from 1 µg total RNA using HiScript III RT SuperMix for qPCR (Vazyme Biotech Co., Ltd.) according to the manufacturer's instructions (thermocycling conditions: 25°C for 5 min, followed by 37°C for 15 min and 85°C for 5 sec) or the Bulge-Loop miRNA qRT-PCR Starter Kit (Guangzhou RiboBio Co., Ltd.) according to the manufacturer's instructions (thermocycling conditions: 42°C for 60 min and 70°C for 10 min). After reverse transcription, qPCR amplification was performed with the ChamQ SYBR qPCR Master Mix Q311-02/03 version 7.1 kit (Vazyme Biotech Co., Ltd.) using the QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific, Inc.). The thermocycling conditions were: 95°C for 30 sec (initial denaturation); 40 cycles of 95°C for 10 sec (denaturation) and 60°C for 30 sec (annealing/extension); followed by melt curve analysis at 95°C for 30 sec, 65°C for 60 sec and 95°C for 15 sec. MicroRNA (miRNA/miR)-218-5p expression was normalized to U6 expression, while DBN1 and GAB2 expression was normalized to GAPDH expression. The expression fold-changes were analyzed using the 2^{-ΔΔC_q} relative quantitative method (34). The RT-qPCR primers (Bulge-loop™ miRNA RT-qPCR Prime Sets) specific for miR-218-5p (MQPS0000843-1-100) and U6 (MQPS0000002-1-100) were purchased from Guangzhou RiboBio Co., Ltd. The other primers used are listed in Table SI.

Protein isolation and western blotting. The cells were lysed in RIPA buffer including 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, EDTA, 1% NP-40 and 0.5% sodium deoxycholate (Beyotime Institute of Biotechnology) supplemented with protease inhibitor PMSF (Beyotime Institute of Biotechnology) on ice. The protein concentration was determined using a BCA protein assay kit (Jiangsu CoWin Biotech Co., Ltd.), and whole lysates were mixed with SDS loading buffer (Beyotime Institute of Biotechnology). Samples were heated at 100°C for 5 min and 20 µg total protein per sample was separated on 10% SDS-polyacrylamide gels. The separated proteins were subsequently transferred to PVDF membranes. The membranes were blocked with TBS with 0.1% Tween-20 containing 5%

BSA (Beyotime Institute of Biotechnology) for 2 h at room temperature, and incubated with primary antibodies overnight at 4°C. After incubation with the primary antibodies, the membranes were incubated with secondary antibodies for 1 h at room temperature. Bands were detected using ECL western blotting detection reagents (Epizyme Biotechnology Co., Ltd.) according to the manufacturer's protocol. GAPDH and actin were used as loading controls. The resulting bands were semi-quantified using ImageJ (v.1.52; National Institutes of Health): Relative abundance=(target band intensity)/(GAPDH or actin intensity). All antibodies used are listed in Table SII.

Lentivirus infection. Lentivirus-based vectors were used for DBN1 knockdown and GAB2 overexpression. For knockdown of DBN1, two independent short hairpin RNAs (shRNAs/shs) and scrambled controls (sequences in Table SIII) were cloned into Plko.1-plasmids (Beijing Zoman Biotechnology Co., Ltd.). 293T cells (1x10⁵ cells per well) were seeded and cultured overnight. Using the second-generation lentiviral packaging system, 293T cells were co-transfected with the following plasmids to produce lentiviruses: 1 µg Plko.1-plasmid containing scramble control (sh-NC) or shRNA DBN1 (sh-DBN1-1/2), 0.75 µg psPAX2 packaging plasmid and 0.5 µg pMD2.G envelope plasmid (Beijing Zoman Biotechnology Co., Ltd.), at a mass ratio of 4:3:2. GAB2 was cloned into GV341 plasmids (Shanghai GeneChem Co., Ltd.) for overexpression. The lentivirus production protocol followed the same methodology as aforementioned. After 48 h of incubation with 5% CO₂ at 37°C, the lentiviral supernatant was filtered through a 0.45-µm low-protein-binding filter to remove cellular debris. Briefly, 5x10⁵ MOLT4 or Jurkat cells were incubated with 500 µl lentiviral particles (MOI, 10) in the presence of 8 µg/ml Polybrene (Santa Cruz Biotechnology, Inc.). Cells were subsequently spinoculated (800 x g for 90 min at room temperature) for transduction and then incubated for 24 h with 5% CO₂ at 37°C. The medium was refreshed at 24 h post-transduction, and the transfected cells were then screened with 0.5 µg/ml puromycin for 2 weeks. During the screening period, the proportion of fluorescent cells was observed and the efficiency of silencing and overexpression was verified by RT-qPCR or western blotting. After screening, stable transgenic cell lines were obtained for culturing (0.2 µg/ml puromycin was used for maintenance) and subsequent experiments.

Transwell and Matrigel-Transwell assays. Cell migration and invasion were examined in a 24-well plate using 8-µm-pore-size polycarbonate inserts (Costar; Corning, Inc.). A total of 1x10⁵ cells suspended in 200 µl RPMI-1640 culture medium with 1% FBS were added to the upper chambers, while 600 µl RPMI-1640 culture medium with 10% FBS was added to the lower chamber. After 12 h of incubation with 5% CO₂ at 37°C, the cells in the lower well were recovered and counted. For the invasion assay, the chamber was coated with 100 µl of 1:10 diluted Matrigel (BD Biosciences), and then polymerized for 4 h at 37°C. A total of 1x10⁵ cells suspended in 200 µl RPMI-1640 culture medium with 1% FBS were added to the upper chambers, while 600 µl RPMI-1640 culture medium with 10% FBS was added to the lower chamber. The cells were allowed to migrate for 24 h with 5% CO₂ at 37°C. Quantification was performed by counting the mean number

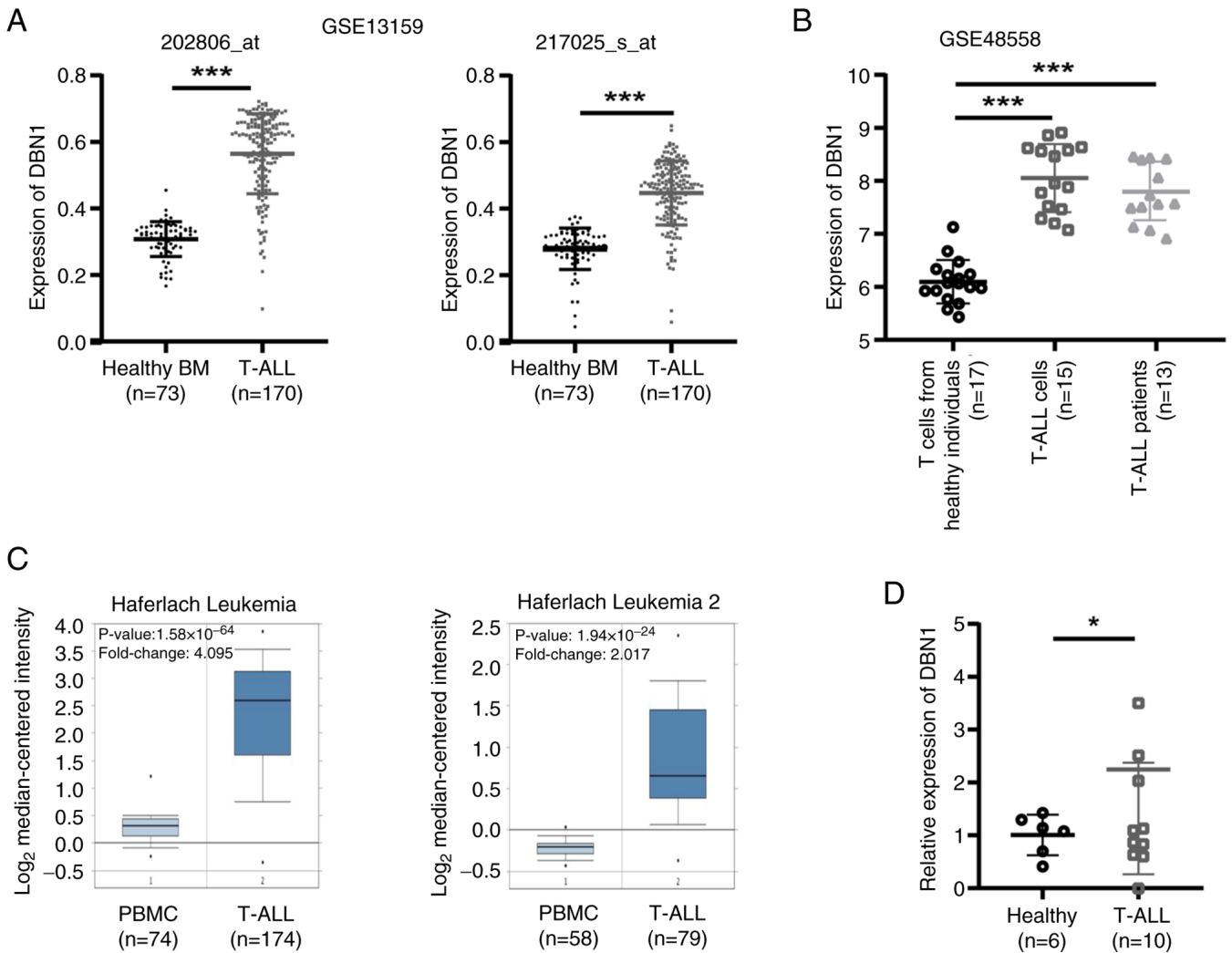


Figure 1. DBN1 is highly expressed in T-ALL. (A) DBN1 expression in BM cells from patients with T-ALL (n=170) and healthy individuals (n=73) (GSE13159). 202806_at and 217025_s_at are the probe names of DBN1. Statistical significance was determined using the Wilcoxon rank sum test. (B) DBN1 expression in T cells from healthy individuals (n=17), T-ALL cells (n=15) and samples from patients with T-ALL (n=13) (GSE48558). Statistical significance was determined using the Kruskal-Wallis test followed by the Bonferroni post hoc test. (C) DBN1 expression in samples from patients with T-ALL and healthy PBMCs in Oncomine datasets (Haferlach leukemia and Haferlach leukemia 2). Statistical significance was determined using the Wilcoxon rank sum test. (D) DBN1 expression in the PBMCs from 10 patients with T-ALL and 6 healthy individuals. Statistical significance was determined using an unpaired t-test. * $P < 0.05$, *** $P < 0.001$. BM, bone marrow; DBN1, drebrin 1; PBMC, peripheral blood mononuclear cell; T-ALL, T-cell acute lymphoblastic leukemia.

of cells in three microscopy fields per chamber under a light microscope (35-37).

RNA-seq. MOLT4 cells transfected with shRNA-DBN1 and the scramble control were used for RNA-seq. RNA sequencing services were provided by Novogene Co., Ltd. The data can be accessed via National Center for Biotechnology Information BioProject (ID: PRJNA726293). All RNA-seq experiments were performed in biological replicates (three biological replicates per group). Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The integrity of RNA was assessed by 1% agarose gel electrophoresis and further validated using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Inc.). A total of 1 μ g RNA per sample was used to generate an RNA-seq library using the NEBNext® Ultra™ RNA Library Prep Kit (cat. no. E7775L; New England BioLabs, Inc.) according to the manufacturer's

protocol. Briefly, mRNA with poly A was enriched using the NEBNext® Ultra™ RNA Library Prep Kit (Illumina, Inc.), and was then enzymatically fragmented into small pieces. Subsequently, the cleaved RNA was reverse-transcribed into first strand cDNA using the NEBNext® Ultra™ II RNA First Strand Synthesis Module (cat. no. E7775L; New England BioLabs, Inc.). Second strand cDNA synthesis was subsequently performed using the NEBNext Ultra™ RNA Second Strand Synthesis Module (cat. no. E7775L; New England BioLabs, Inc.). Thermal cycling was performed according to the manufacturer's protocol. PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer according to the NEBNext® Ultra™ RNA Library Prep Kit protocol. The thermocycling conditions were: 98°C for 30 sec (initial denaturation); 15 cycles of 98°C for 10 sec (denaturation) and 60°C for 75 sec (annealing/extension); 65°C for 5 min (final extension). The PCR products were purified using an AMPure XP system

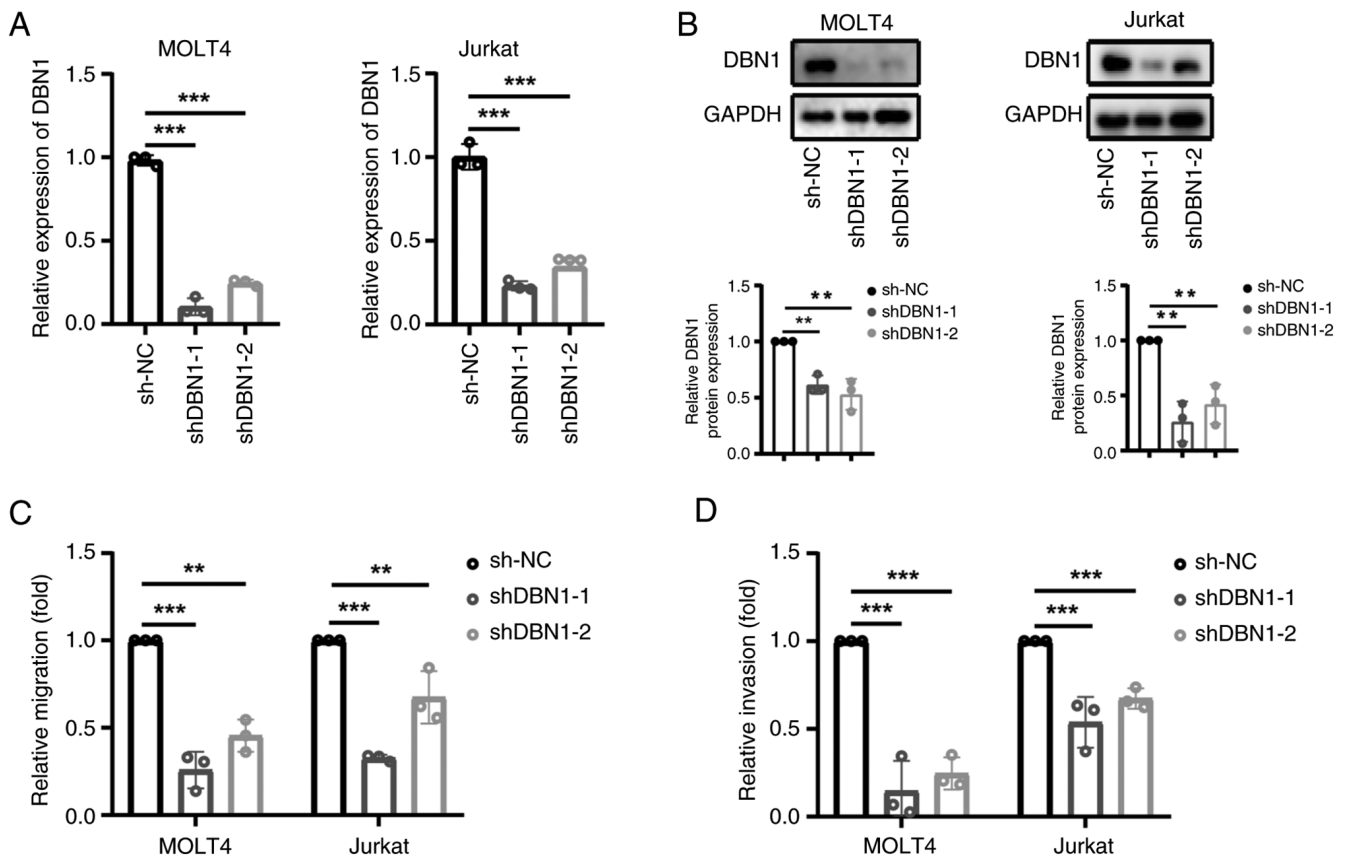


Figure 2. Knockdown of DBN1 inhibits cell migration and invasion *in vitro*. (A) Reverse transcription-quantitative PCR analysis of DBN1 mRNA expression in DBN1-knockdown MOLT4 and Jurkat cells (sh-1 and sh-2). (B) Western blot analysis of DBN1 protein expression in DBN1-knockdown MOLT4 and Jurkat cells (sh-1 and sh-2). The intensities of the protein bands were determined using ImageJ. (C) Transwell assays showing the migration tendency of DBN1-knockdown cells. (D) Matrigel-Transwell assays showing the invasion tendency of leukemia cells after DBN1 knockdown. The data were normalized and compared with those in the sh-NC control group. Statistical significance was determined using one-way ANOVA followed by the Bonferroni test. ** $P < 0.01$, *** $P < 0.001$. DBN1, drebrin 1; NC, negative control; sh, short hairpin RNA.

(Beckman Coulter, Inc.), and the quality was tested using an Agilent Bioanalyzer 2100 system (Agilent Technologies, Inc.) to create the final cDNA library. Post-amplification libraries were initially quantified with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Inc.). Based on concentration measurements, libraries were normalized to 2 nM prior to downstream applications. Hybridization and cluster generation were performed on a cBot Cluster Generation System (Illumina, Inc.) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina NovaSeq 6000 platform (Illumina, Inc.), and 150-bp paired-end reads were generated. After pretreatment (filtering and quality control) of the raw reads, clean reads were aligned to the human reference genome using HISAT2 (v2.0.5; <https://github.com/open-estuary/hisat2>). Afterwards, the fragments per kilobase million (FPKM; currently the most commonly used method for estimating gene expression levels) value of each gene was calculated based on the length of the gene and the read count mapped to this gene. The DESeq2 R package (v1.16.1; <https://bioconductor.org/packages/release/bioc/html/DESeq2.html>) in R (v4.0.3; <https://www.r-project.org/>) was applied to compare different groups of samples based on the FPKM values. Genes with an adjusted P-value < 0.05 according to DESeq2 were considered to be differentially expressed.

miRNA prediction. The putative miRNAs of DBN1 were predicted using the miRanda database (<http://www.microrna.org/>), TargetScan (<http://www.targetscan.org/>) and miRDB databases (<http://www.mirdb.org/>).

miRNA mimic transfection. The miR-218-5p mimic (miR10000275-1-5) and negative control (miR1N0000001-1-5) were obtained from Guangzhou RiboBio Co., Ltd. The oligonucleotides were transfected into MOLT4 and Jurkat cells at a final concentration of 100 nM using Lipofectamine RNAiMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. After 48 h of incubation with 5% CO₂ at 37°C, RNA was extracted for RT-qPCR, while protein was extracted for western blotting at 72 h post-transfection.

Dual luciferase assay. 293T cells (1 × 10⁵ cells per well) were seeded and cultured overnight. The pmiR-RB-REPORT DBN1-wild-type (WT) and pmiR-RB-REPORT DBN1-mutant (MUT) plasmids (Guangzhou RiboBio Co., Ltd.) were transfected into cells together with miR-218-5p mimic or negative control. Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used. The luciferase activity was measured after 48 h of transfection using the dual-luciferase reporter gene assay system (Promega Corporation). Relative

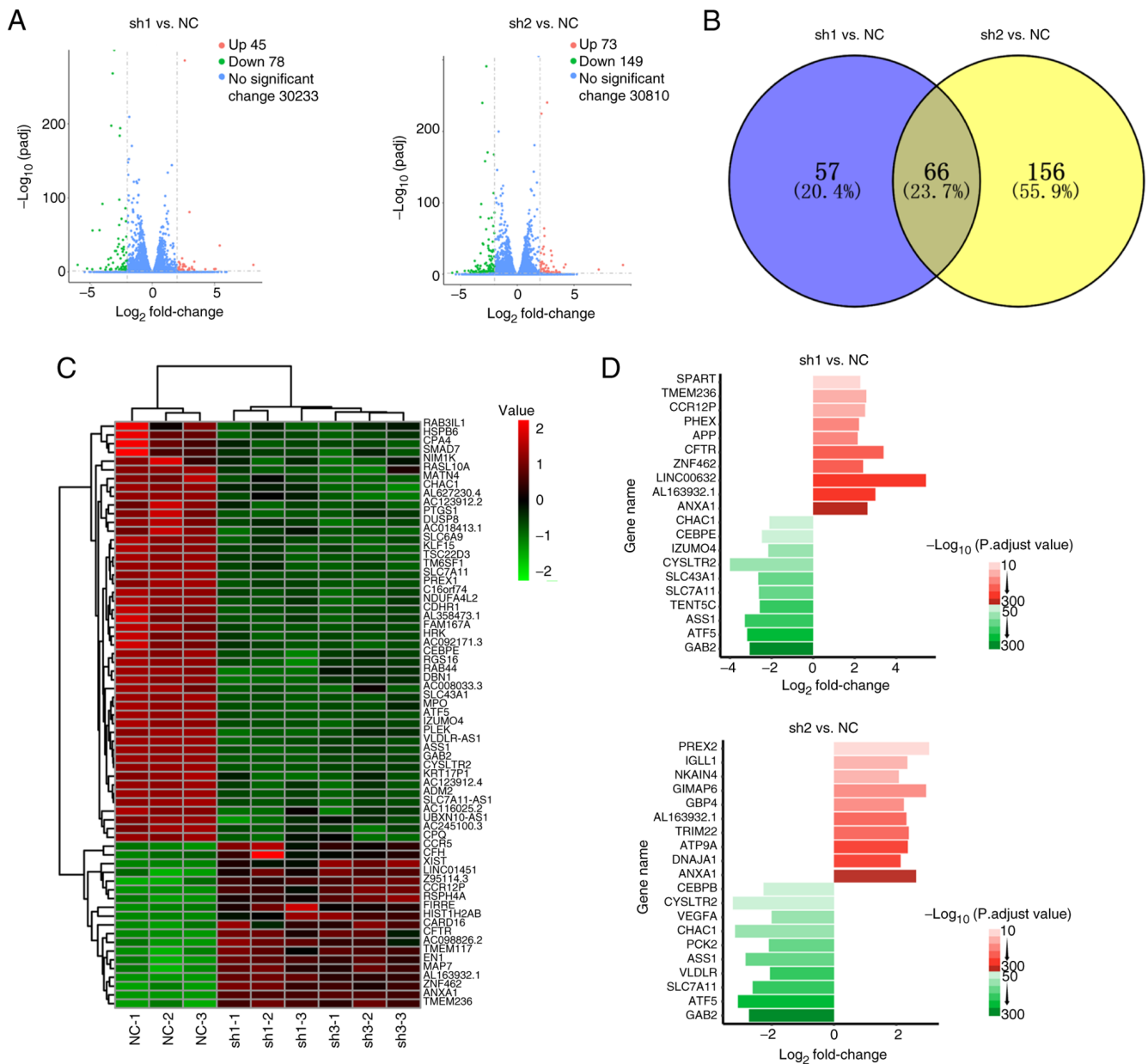


Figure 3. RNA-seq analysis in DBN1-knockdown MOLT4 cells. (A) Volcano plot of transcriptome profiling in MOLT4 shDBN1-1 cells vs. NC cells (left). Genes significantly upregulated (red) or downregulated (green) following DBN1 silencing are highlighted. Volcano plot for the MOLT4 shDBN1-2 group knockdown and control samples based on RNA-seq data. The 'value' represents the relative expression value (Z-score) obtained after normalization. (D) Top 10 genes with the most significant upregulation or downregulation in the shDBN1-1 group (left) and shDBN1-2 group (right). Red and green bars represent upregulated and downregulated DEGs, respectively. The color gradient reflects significance levels, with darker shades denoting lower P-values. DBN1, drebrin 1; DEG, differentially expressed gene; NC, negative control; padj, adjusted P-value; RNA-seq, RNA sequencing; sh, short hairpin RNA.

luciferase activity was calculated as the ratio of *Renilla*/firefly luminescence and normalized to the negative control (NC) group. The sequences of DBN1-WT and DBN1-MUT are shown in Table SIV.

Statistical analysis. GraphPad Prism (v.8.0; Dotmatics) and SPSS (v.21.0; IBM Corp.) were used for statistical analysis and figure generation. Data are presented as the mean $\pm$ SEM of three technical replicates per group and were verified in three independent experiments. The normal distribution of data was assessed with the Shapiro-Wilk test. If data passed, an unpaired t-test and one-way ANOVA followed by Bonferroni

test were used to compare the data in different groups. Otherwise, nonparametric tests (Wilcoxon rank sum test or the Kruskal-Wallis test followed by the Bonferroni post hoc test) were used. The correlation between miRNA and mRNA expression was analyzed using Spearman's correlation analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

DBN1 expression is upregulated in T-ALL. The Oncomine and GEO databases were utilized to determine the expression

Table I. Top 10 genes that are significantly upregulated or downregulated in the negative control group vs. the short hairpin RNA-1 group.

Gene name	Gene biotype	Fold change	P-value	P. adjust
GAB2	Protein coding	-3.048506011	$<1 \times 10^{-300}$	$<1 \times 10^{-300}$
ATF5	Protein coding	-3.170630317	1.16×10^{-272}	6.49×10^{-269}
ASS1	Protein coding	-3.284095496	9.15×10^{-202}	3.08×10^{-198}
TENT5C	Protein coding	-2.563717302	2.49×10^{-198}	6.99×10^{-195}
SLC7A11	Protein coding	-2.603990354	4.77×10^{-188}	1.15×10^{-184}
SLC43A1	Protein coding	-2.640875881	4.16×10^{-101}	3.04×10^{-98}
CYSLTR2	Protein coding	-3.995678746	2.37×10^{-95}	1.48×10^{-92}
IZUMO4	Protein coding	-2.150518857	1.54×10^{-89}	8.92×10^{-87}
CEBPE	Protein coding	-2.461485244	5.43×10^{-75}	2.18×10^{-72}
CHAC1	Protein coding	-2.103684776	6.53×10^{-72}	2.50×10^{-69}
ANXA1	Protein coding	2.613074631	2.66×10^{-290}	2.24×10^{-286}
AL163932.1	lincRNA	2.991092183	3.45×10^{-84}	1.76×10^{-81}
LINC00632	Antisense	5.426264996	1.56×10^{-38}	1.82×10^{-36}
ZNF462	Protein coding	2.402268334	8.73×10^{-21}	3.97×10^{-19}
CFTR	Protein coding	3.384719604	4.39×10^{-16}	1.31×10^{-14}
APP	Protein coding	2.139437504	9.25×10^{-16}	2.66×10^{-14}
PHEX	Protein coding	2.201167771	2.01×10^{-14}	5.04×10^{-13}
CCR12P	Transcribed unprocessed pseudogene	2.500934200	2.40×10^{-12}	4.70×10^{-11}
TMEM236	Protein coding	2.555311280	2.92×10^{-12}	5.66×10^{-11}
SPART	Protein coding	2.261114841	4.77×10^{-12}	9.03×10^{-11}

lincRNA, long intergenic noncoding RNA; P. adjust, adjusted P-value.

levels of DBN1. Upregulated DBN1 expression was observed in BM samples from patients with T-ALL compared with in those from healthy individuals in the GSE13159 dataset (Fig. 1A). Analysis of the GSE48558 dataset showed that DBN1 levels in both 15 T-ALL cells (from four T-ALL cell lines) and 13 samples from patients with T-ALL were significantly higher than those in T cells from healthy individuals (Fig. 1B). Furthermore, DBN1 expression was also upregulated in PBMCs from patients with T-ALL compared with in those from their healthy counterparts in the Oncomine database Haferlach leukemia dataset ($P=1.58 \times 10^{-64}$; fold change, 4.095) and Haferlach leukemia 2 dataset ($P=1.94 \times 10^{-24}$; fold change, 2.017) (Fig. 1C). To further confirm these results, PB was collected from 6 healthy donors and 10 patients with T-ALL to determine the expression levels of DBN1. Consistently, the expression levels of DBN1 in patients with T-ALL were significantly higher than those in healthy individuals (Fig. 1D). Overall, these data suggested that the expression levels of DBN1 were significantly upregulated in T-ALL.

Knockdown of DBN1 inhibits T-ALL cell migration and invasion. A previous study has reported the involvement of DBN1 in cancer cell migration and invasion (38). To determine the functional roles of DBN1 in T-ALL, shRNA DBN1 was transfected into MOLT4 and Jurkat cells to knock down DBN1 expression. RT-qPCR and western blotting demonstrated that

DBN1 expression was downregulated at both the mRNA and protein levels, indicating the successful construction of DBN1-knockdown cells (Fig. 2A and B). Notably, Transwell experiments demonstrated that migration was significantly reduced in DBN1-knockdown cells (Figs. 2C and S1A). Furthermore, the invasion of T-ALL cells was also negatively impacted by DBN1 knockdown (Figs. 2D and S1B).

DBN1 upregulates GAB2 expression in T-ALL. To further investigate the underlying molecular mechanism by which DBN1 increased the infiltration of T-ALL cells, RNA-seq was performed to determine the differentially expressed genes (DEGs) in DBN1-knockdown cells (Fig. 3A). A total of 66 genes with significant changes in expression ($|\text{Log}_2\text{FoldChange}| \geq 2$; adjusted P-value < 0.05) were identified in both the shDBN1-1 and shDBN1-2 groups compared with the NC group (Fig. 3B and C). Based on the adjusted P-value, the top 10 most significantly upregulated and downregulated DEGs were identified (Tables I and II). Notably, the results showed that annexin A1 (ANXA1) and GAB2 were the most significantly upregulated and downregulated DEGs, respectively (Fig. 3D).

The effect of DBN1 on downstream genes was further evaluated through experiments. RNA-seq experiments revealed that the expression levels of GAB2 and ANXA1 were affected by DBN1. RNA-seq results demonstrated that

Table II. Top 10 genes that are significantly upregulated or downregulated in the negative control group vs. short hairpin RNA-2 group.

Gene name	Gene biotype	Fold change	P-value	P. adjust
GAB2	Protein coding	-2.725381687	9.58x10 ⁻²⁹⁶	8.81x10 ⁻²⁹²
ATF5	Protein coding	-3.081651034	3.35x10 ⁻²⁴⁴	1.54x10 ⁻²⁴⁰
SLC7A11	Protein coding	-2.611266971	1.39x10 ⁻¹⁷⁴	3.19x10 ⁻¹⁷¹
VLDLR	Protein coding	-2.053021828	9.51x10 ⁻¹⁷²	1.94x10 ⁻¹⁶⁸
ASS1	Protein coding	-2.834883932	3.42x10 ⁻¹⁶²	6.30x10 ⁻¹⁵⁹
PCK2	Protein coding	-2.087801383	2.06x10 ⁻¹¹⁷	2.11x10 ⁻¹¹⁴
CHAC1	Protein coding	-3.174572015	8.43x10 ⁻¹⁰²	6.20x10 ⁻⁹⁹
VEGFA	Protein coding	-2.002472857	9.28x10 ⁻⁹⁰	5.50x10 ⁻⁸⁷
CYSLTR2	Protein coding	-3.245798544	2.02x10 ⁻⁸³	1.06x10 ⁻⁸⁰
CEBPB	Protein coding	-2.257193054	1.55x10 ⁻⁸²	7.91x10 ⁻⁸⁰
ANXA1	Protein coding	2.635796148	7.03x10 ⁻²⁴⁵	4.31x10 ⁻²⁴¹
DNAJA1	Protein coding	2.137149770	4.69x10 ⁻²²⁹	1.73x10 ⁻²²⁵
ATP9A	Protein coding	2.372149849	1.97x10 ⁻⁶⁷	6.73x10 ⁻⁶⁵
TRIM22	Protein coding	2.398735825	8.43x10 ⁻⁵⁷	2.13x10 ⁻⁵⁴
AL163932.1	lincRNA	2.324620236	1.78x10 ⁻⁴¹	2.14x10 ⁻³⁹
GBP4	Protein coding	2.243162862	2.71x10 ⁻³⁸	2.91x10 ⁻³⁶
GIMAP6	Protein coding	2.958049617	1.08x10 ⁻³⁵	1.01x10 ⁻³³
NKAIN4	Protein coding	2.079971882	2.52x10 ⁻³²	2.00x10 ⁻³⁰
IGLL1	Protein coding	2.355764004	1.81x10 ⁻³⁰	1.26x10 ⁻²⁸
PREX2	Protein coding	3.051253803	5.41x10 ⁻²⁹	3.47x10 ⁻²⁷

lincRNA, long intergenic noncoding RNA; P. adjust, adjusted P-value.

knockdown of DBN1 significantly diminished the levels of GAB2 mRNA (Fig. 4A), which was subsequently verified via RT-qPCR in DBN1-knockdown MOLT4 and Jurkat cells (Fig. 4B). Furthermore, knockdown of DBN1 also decreased GAB2 expression at the protein level in T-ALL cells, as validated by western blotting (Fig. 4C). RNA-seq results revealed that ANXA1 mRNA levels were elevated in DBN1-knockdown MOLT4 cells (Fig. 4D). RT-qPCR further validated the results in DBN1-knockdown MOLT4 and Jurkat cells, although this was not significant for the shDBN1-1 group in Jurkat cells (Fig. 4E). Notably, ANXA1 protein upregulation aligned with transcriptional changes exclusively in MOLT4 cells, achieving statistical significance specifically in the shDBN1-1 group. Conversely, Jurkat cells exhibited an opposite trend in the shDBN1-1/2 groups, although these changes were not statistically significant (Fig. 4F). Collectively, unlike ANXA1, GAB2 was downregulated at both the RNA and protein levels after DBN1 knockdown. Therefore, we hypothesized that DBN1 may mediate the infiltration of T-ALL through GAB2. Additionally, the present results demonstrated that GAB2 was highly expressed in patients with T-ALL compared with healthy controls based on the GEO database (GSE13159 and GSE48558 datasets), Oncomine database (Haferlach leukemia dataset) and patient samples (Fig. 5A-D). As shown in Fig. 5E, a positive correlation was observed between DBN1 and GAB2 in clinical samples collected in the present study, including samples from healthy patients and patients with T-ALL.

DBN1 promotes T-ALL cell migration and invasion mediated by GAB2 and downstream PI3K/AKT and ERK pathways. Overexpression of GAB2 has been linked to aberrant activation of ERK and PI3K/AKT in various malignant tumors, such as colorectal and endometrial cancer (22,39). Knockdown of DBN1 in MOLT4 cells not only decreased GAB2 expression but also inhibited the phosphorylation of AKT at position 473 and the phosphorylation of ERK1/2 at position 202/204 (Fig. 6A). Consistently, knockdown of DBN1 in Jurkat cells had similar effects (Fig. 6B). Furthermore, overexpression of GAB2 in DBN1-knockdown cells led to upregulated phosphorylation of AKT and ERK1/2 (Fig. 6C). The effectiveness of the GAB2 overexpression plasmid is shown in Fig. S2. These results indicated that DBN1 promoted T-ALL infiltration via the PI3K/AKT and ERK axes.

DBN1 is targeted by miR-218-5p. The present study subsequently explored the mechanism responsible for the upregulation of DBN1 expression in T-ALL. miRNAs are known to function by regulating target genes in various cancer types. Based on the analysis using three bioinformatics databases (TargetScan, miRanda and miRDB), miR-218-5p and miR-876-5p were predicted to be potential upstream regulators of DBN1 (Fig. 7A). Among the two candidates, miR-218-5p was of particular interest, as its higher Context++ score suggested a higher possibility (Table III). Therefore, miR-218-5p was selected for further analysis in the present study. Dual-luciferase gene reporter assays were conducted to determine whether DBN1 harbors miR-218-5p binding

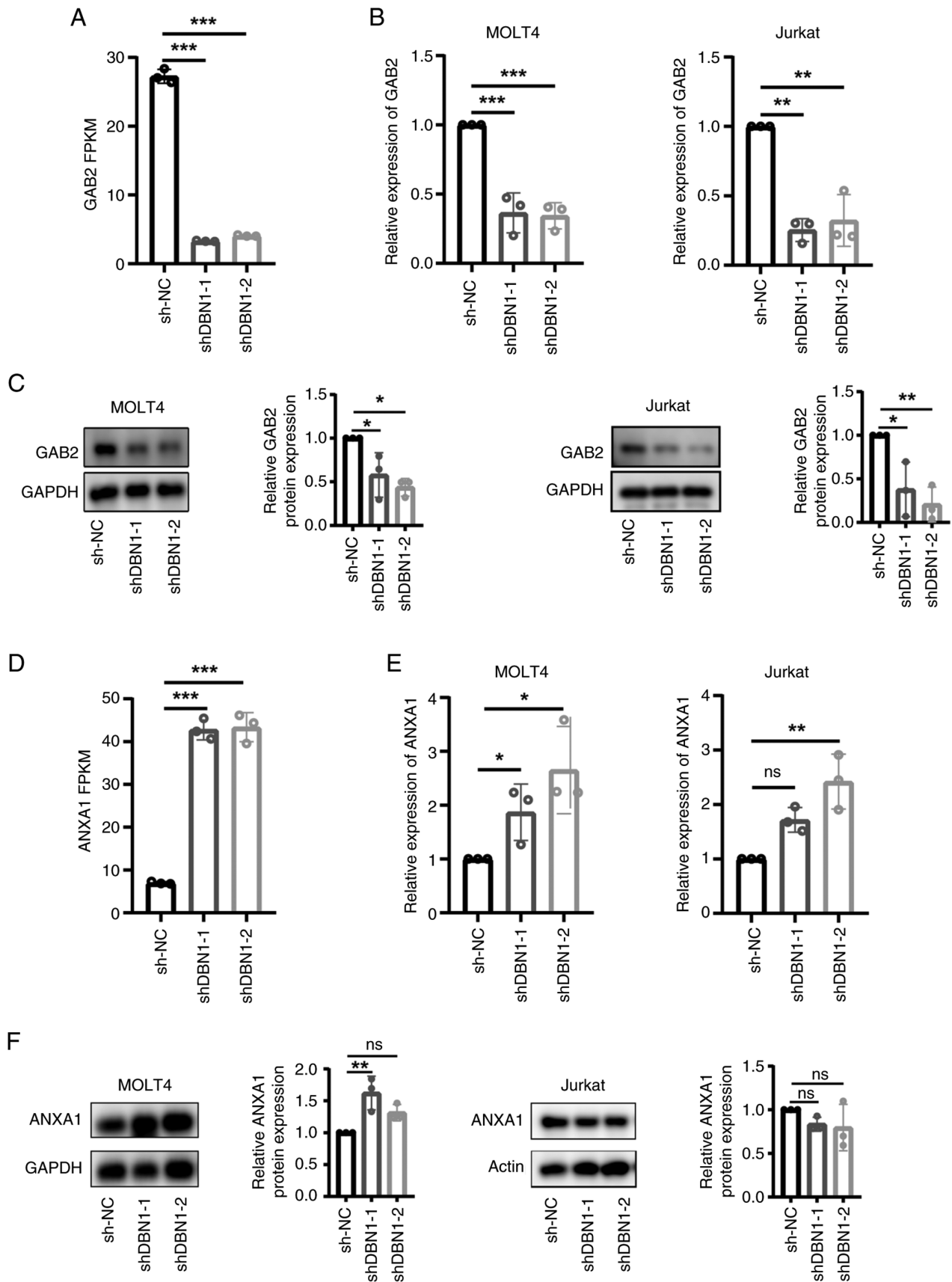


Figure 4. DBN1 regulates GAB2 expression. (A) GAB2 FPKM in the NC and DBN1 knockdown groups. (B) RT-qPCR was performed to assess the levels of GAB2 transcripts after DBN1 silencing. (C) Western blot analysis of GAB2 in NC and DBN1 knockdown cells. The intensities of the protein bands were determined using ImageJ. (D) ANXA1 FPKM in the NC and DBN1 knockdown groups. (E) RT-qPCR was performed to determine the levels of ANXA1 transcripts after DBN1 silencing. (F) Western blot analysis of ANXA1 in NC and DBN1-knockdown cells. The intensities of the protein bands were determined using ImageJ. Statistical significance was determined using one-way ANOVA followed by the Bonferroni test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ANXA1, annexin A1; DBN1, drebrin 1; FPKM, fragments per kilobase million; GAB2, Grb2-associated binding protein 2; NC, negative control; ns, not significant; RT-qPCR, reverse transcription-quantitative PCR; sh, short hairpin RNA.

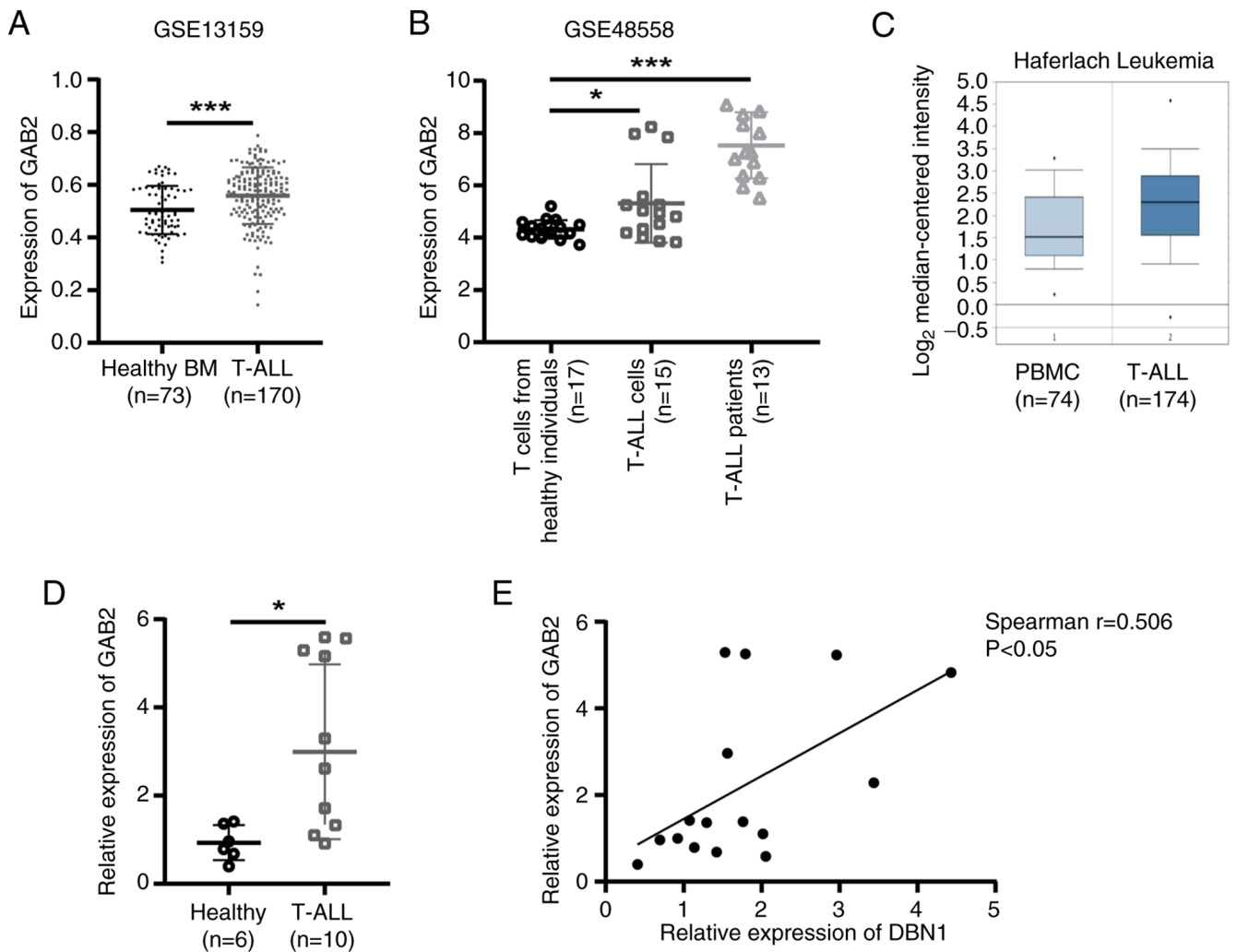


Figure 5. GAB2 is highly expressed in T-ALL. (A) GAB2 expression in the GSE13159 dataset. BM from patients with T-ALL (n=170) and BM from healthy individuals (n=73). Statistical significance was determined using the Wilcoxon rank sum test. (B) GSE48558. T cells from healthy individuals (n=17), T-ALL cells (n=15) and samples from patients with T-ALL (n=13). Statistical significance was determined using the Kruskal-Wallis test followed by the Bonferroni post hoc test. (C) Oncomine dataset (Haferlach leukemia). Statistical significance was determined using the Wilcoxon rank sum test. (D) GAB2 expression in 10 T-ALL clinical PB samples and 6 healthy donor PB samples. Statistical significance was determined using an unpaired t-test. (E) Spearman's correlation between DBN1 and GAB2 expression based on healthy and T-ALL samples. * $P<0.05$, *** $P<0.001$. BM, bone marrow; DBN1, drebrin 1; GAB2, Grb2-associated binding protein 2; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; T-ALL, T-cell acute lymphoblastic leukemia.

sites. The luciferase activity in the DBN1-WT⁺ miR-218-5p mimic group was reduced compared with that in the NC group, whereas the luciferase activity in the DBN1-MUT group was not affected (Fig. 7B). Furthermore, MOLT4 and Jurkat cells were transfected with miR-218-5p mimics to confirm the effect of miR-218-5p on DBN1. RT-qPCR analysis demonstrated that miR-218-5p expression levels in MOLT4 and Jurkat cells transfected with miR-218-5p mimics were significantly elevated compared with those in the NC group (Fig. S3). miR-218-5p mimics significantly inhibited DBN1 expression at both the mRNA and protein levels (Fig. 7C and D). Furthermore, RT-qPCR results showed that miR-218-5p was downregulated in PB in T-ALL samples (Fig. 7E), and a negative correlation between DBN1 and miR-218-5p expression was observed in clinical samples collected in the present study, including samples from healthy patients and patients with T-ALL (Fig. 7F). Furthermore, a negative correlation was observed between GAB2 and miR-218-5p expression in clinical samples collected in the

present study, including samples from healthy patients and patients with T-ALL; however, this was not statistically significant (Fig. 7G). A possible reason for this is the small number of clinical samples, which needs further exploration. These results indicated that DBN1 was a direct target of miR-218-5p in T-ALL cells. Furthermore, functional examination of miR-218-5p was conducted. Transwell assays revealed that the migration and invasion of T-ALL cells in the miR-218-5p mimic group were decreased compared with those of cells in the NC group (Figs. 8A and S4). These results indicated that high miR-218-5p expression inhibited cell migration and invasion. Given the observation of the targeting relationship between miR-218-5p and DBN1, and the regulation of downstream signaling pathways by DBN1 through GAB2, the role of miR-218-5p in these pathways was evaluated. Compared with those of cells transfected with mimic-NC, transfection of miR-218-5p mimics resulted in reduced expression levels of DBN1 and GAB2, as well as decreased levels of AKT and ERK1/2 phosphorylation (Fig. 8B). In addition,

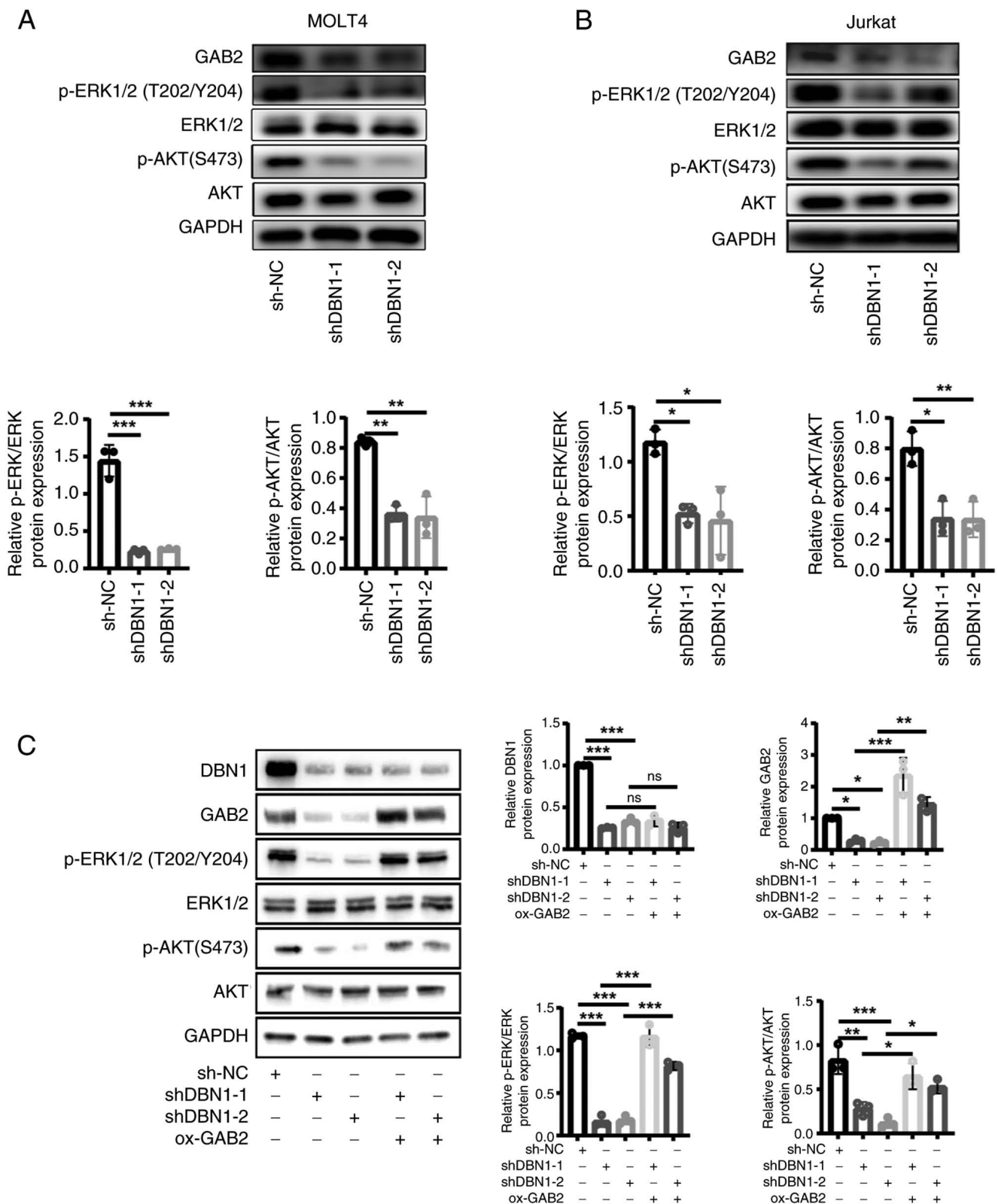


Figure 6. Downregulation of DBN1 inhibits both GAB2 expression and the activation of the PI3K/AKT/ERK pathways. (A) Inhibitory effects on the expression levels of GAB2, and phosphorylated AKT and ERK1/2 in the DBN1-knockdown MOLT4 cells. (B) Jurkat cells. (C) Overexpression of GAB2 rescued the expression levels of phosphorylated AKT and ERK1/2 in DBN1-knockdown MOLT4 cells. The intensities of the protein bands were determined using ImageJ. Statistical significance was determined using one-way ANOVA followed by the Bonferroni test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DBN1, drebrin 1; GAB2, Grb2-associated binding protein 2; NC, negative control; ns, not significant; ox-, overexpression; p-, phosphorylated; sh, short hairpin RNA.

overexpression of GAB2 in cells transfected with miR-218-5p mimic revealed that, compared with those in the miR-218-5p mimic group, the expression levels of DBN1 in the miR-218-5p

mimic + GAB2-overexpression group did not change, but the phosphorylation levels of AKT and ERK1/2 were increased (Fig. 8C). These data suggested that miR-218-5p acts as an

Table III. Predicted miRNAs and drebrin 1 interaction data based on TargetScan.

miRNA	Site type	Context ⁺⁺ score	Context ⁺⁺ score percentile	Weighted context ⁺⁺ score	Conserved branch length	PCT
miR-218-5p	8mer	-0.50	99	-0.02	5.292	0.91
miR-876-5p	8mer	-0.34	98	-0.01	3.503	N/A

miR/miRNA, microRNA; N/A, not applicable; PCT, preferentially conserved targeting.

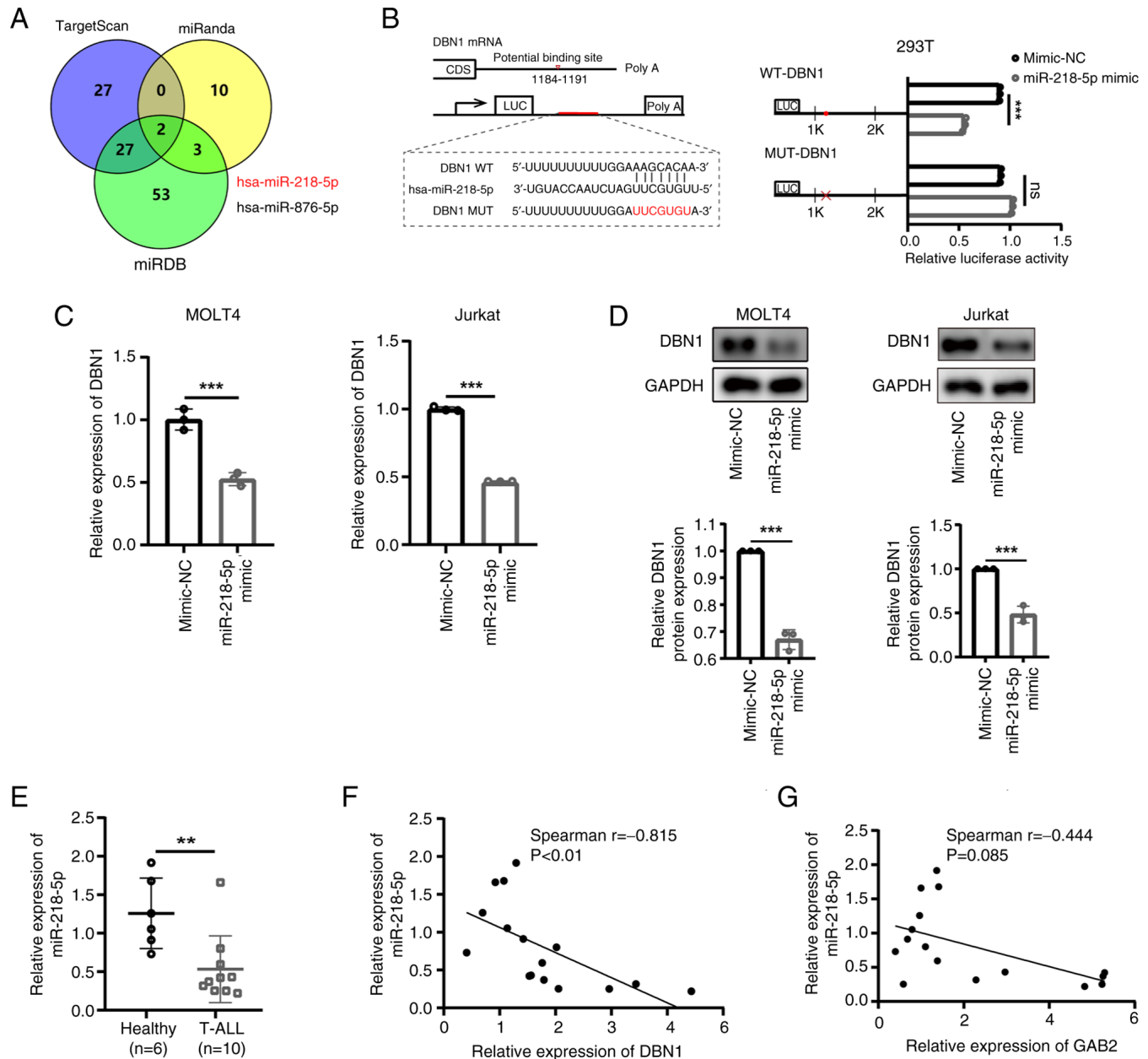


Figure 7. miR-218-5p regulates DBN1 expression through direct binding to the 3'-untranslated region of DBN1. (A) Bioinformatics analysis was performed using the TargetScan, miRanda and miRDB databases. (B) Predicted miR-218-5p binding sites in DBN1 (WT) and the designed mutant sequence (MUT). Relative luciferase activity was determined after cells were transfected with WT or MUT. Statistical significance was determined using an unpaired t-test. (C) Reverse transcription-quantitative PCR demonstrated miR-218-5p-mediated suppression of DBN1 expression in T-ALL cells. Statistical significance was determined using an unpaired t-test. (D) Western blotting demonstrated miR-218-5p-mediated suppression of DBN1 expression in T-ALL cells. The intensities of the protein bands were determined using ImageJ. Statistical significance was determined using an unpaired t-test. (E) Expression levels of miR-218-5p in samples from healthy individuals and T-ALL samples. Statistical significance was determined using an unpaired t-test. (F) Spearman's correlation between miR-218-5p and DBN1 expression in healthy individuals and patients with T-ALL. (G) Spearman's correlation between miR-218-5p and GAB2 expression in healthy individuals and patients with T-ALL. **P<0.01, ***P<0.001. CDS, coding sequence; DBN1, drebrin 1; GAB2, Grb2-associated binding protein 2; LUC, luciferase; miR, microRNA; MUT, mutant; NC, negative control; ns, not significant; T-ALL, T-cell acute lymphoblastic leukemia; WT, wild-type.

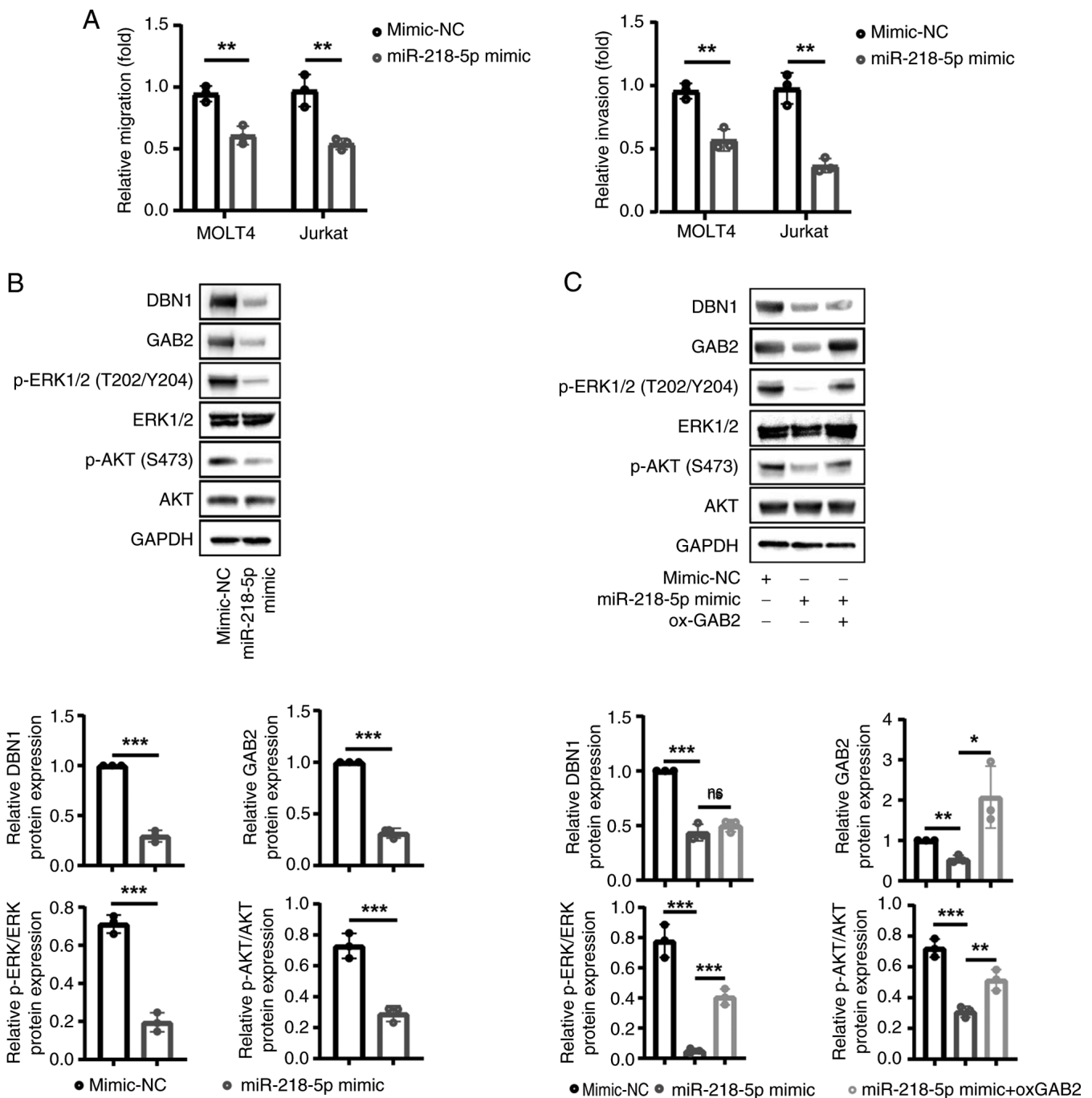


Figure 8. miR-218-5p is involved in T-ALL cell migration and invasion. (A) Effects of miR-218-5p mimics transfection on T-ALL cell migration and invasion. Statistical significance was determined by one-way ANOVA followed by the Bonferroni test. (B) Western blot analysis of GAB2, and phosphorylated and total AKT and ERK1/2 in the MOLT4 cells after miR-218-5p mimic transfection. Statistical significance was determined using an unpaired t-test. (C) Western blot analysis of the effects of GAB2 overexpression on the levels of phosphorylated AKT and ERK1/2 in MOLT4 cells transfected with miR-218-5p mimic. The intensities of the protein bands were determined using ImageJ. Statistical significance was determined using one-way ANOVA followed by the Bonferroni test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DBN1, drebrin 1; GAB2, Grb2-associated binding protein 2; miR, microRNA; NC, negative control; ns, not significant; ox-, overexpression; p-, phosphorylated; T-ALL, T-cell acute lymphoblastic leukemia.

upstream regulator of DBN1, and is involved in cell migration and invasion.

Discussion

The development of effective chemotherapeutic drugs and hematopoietic stem cell transplantation technology have improved the cure rate of T-ALL to 60-85% (40,41). However,

leukemia infiltration remains a crucial factor affecting disease progression and recurrence (42). Transendothelial migration is an essential step to allow leukemic cells to exit the bloodstream into target tissues or organs (6,43). A previous study has found that actin cytoskeleton remodeling is associated with the mechanisms of leukemic T-cell extravasation (44). Therefore, cytoskeleton-related molecules appear to be possible targets for the treatment of leukemic infiltration.

DBN1 has been reported to be involved in the reorganization of the actin cytoskeleton (45,46). However, its role in T-ALL remains elusive. The present study explored the role of DBN1 in T-ALL infiltration and the corresponding molecular mechanism, and offered a potential therapeutic target for limiting or even reversing T-ALL infiltration.

Several studies have demonstrated that DBN1 expression is upregulated in several tumors, such as skin tumors and lung cancer (47,48), especially in metastatic cancer cells (49). The present results revealed that DBN1 was highly expressed in T-ALL based on analysis of clinical T-ALL samples and databases. Considering that actin cytoskeleton remodeling is an important process in cancer metastasis and invasion, DBN1, as a key actin-binding protein, may promote tumor metastasis and infiltration (13,15). High DBN1 expression promotes glioma cell migration and invasion (16). Decreased migration and invasion of MOLT4 and Jurkat cells following DBN1 silencing were observed in the present study. Collectively, these findings highlight the role of DBN1 in T-ALL cell metastasis and invasion, which, to the best of our knowledge, has not been previously reported.

To investigate the downstream molecular mechanisms involved, RNA-seq was employed, and GAB2 was revealed to be the most significantly downregulated DEG in DBN1-knockdown cells. GAB2 is a known activator of the PI3K/AKT and ERK signaling pathways (21), and their roles in tumor metastasis and progression have been reported previously (18,50). The present study revealed that GAB2 was highly expressed in T-ALL. High levels of GAB2 have been shown to promote cancer metastasis by activating the PI3K signaling pathway in ovarian cancer (51). In AML, high levels of GAB2 have been observed, and these promoted AML migration via the ERK signaling pathway (28). Previous studies have also reported the involvement of the PI3K/AKT and ERK signaling pathways in the metastasis and invasion of leukemia (49,52,53). Activation of the MEK/ERK and AKT pathways has also been shown to promote the proliferation and migration of T-ALL cells (53). In the present study, knockdown of DBN1 led to reduced expression levels of GAB2 and suppressed ERK1/2 phosphorylation (MEK/ERK pathway) and AKT phosphorylation (AKT pathway). Notably, the rescue experiment demonstrated that restoring GAB2 expression in DBN1-knockdown cells restored phosphorylation of key downstream effectors (AKT and ERK1/2). These aforementioned results support the current findings that GAB2 and PI3K/AKT and ERK signaling could be downstream mediators of DBN1 in T-ALL. However, further studies are required to confirm these results.

miRNAs belong to a class of small noncoding RNAs that function in the posttranscriptional regulation of gene expression (54). miRNAs inhibit the expression of target genes via sequence-specific interactions with the 3'-untranslated regions, which drives mRNA degradation and translation inhibition (55,56). For instance, upregulation of CD47 in leukemia cells aids the evasion of macrophage phagocytosis, and CD47 expression can be suppressed by miR-708, thus promoting macrophage phagocytosis for T-ALL eradication (57). Therefore, we hypothesized that certain miRNAs could be crucial for the regulation of DBN1 in T-ALL cells. miR-218-5p was identified using the TargetScan, miRanda and miRDB databases, and could be a modulator of DBN1 expression. The

subsequent experiments demonstrated that DBN1 was a downstream target of miR-218-5p, and DBN1 expression could be inhibited by miR-218-5p binding to the 3'-untranslated region of DBN1 mRNA. miR-218-5p is a highly conserved miRNA, which serves the role of a tumor suppressor in bladder cancer and other tumors (58,59). For example, miR-218-5p downregulation is associated with enhanced proliferation, migration and invasion of tumor cells in cervical cancer (60). The present findings indicated that miR-218-5p was downregulated in T-ALL, which led to the upregulation of DBN1. Additionally, the present study revealed that transfection with miR-218-5p mimics could inhibit the migration and invasion of MOLT4 and Jurkat cells, as well as reduce the levels of DBN1, downstream GAB2, phosphorylated AKT and phosphorylated ERK1/2. In addition, overexpression of GAB2 could reverse the decrease in phosphorylated AKT and phosphorylated ERK1/2 levels caused by miR-218-5p mimics transfection. These findings suggest that miR-218-5p may exert its effects by downregulating DBN1 expression, thereby inhibiting the GAB2 pathway and its downstream PI3K/AKT and ERK pathways.

The present study has limitations that are worth noting. Firstly, the expression differences of ANXA1 in the two DBN1-knockdown T-ALL cell lines (ANXA1 protein expression was upregulated in DBN1-knockdown MOLT4 cells but downregulated in DBN1-knockdown Jurkat cells) are a phenomenon worth exploring. These differences may stem from the distinct biological backgrounds of the two cell lines, despite both being T-ALL cell lines. Jamal *et al* (36) conducted RNA-seq on MOLT4 and Jurkat cells, and found that numerous genes differed between the two cell lines. However, the present study did not conduct corresponding experiments to explore the relevant mechanisms, which should be included in our future research scope. In the future, single-cell sequencing or other technologies could effectively address these limitations, and reveal findings regarding the differences in protein translation or post-translational modifications between MOLT4 and Jurkat cells. Secondly, due to the scarcity of T-ALL clinical specimens and the prioritization of samples for nucleic acid extraction, validation of DBN1 and GAB2 expression was limited to the RNA level, with direct protein level evidence lacking. Future studies remain necessary to perform verification at the protein level.

In conclusion, the present study demonstrated that increased DBN1 expression could promote migration and invasion in T-ALL cells, and the underlying mechanism was revealed. Significant upregulation of DBN1 was observed in both clinical samples and the T-ALL databases (GEO and Oncomine). Further investigation using DBN1-knockdown cell lines suggested the role of DBN1 in cell migration and invasion, with GAB2 and PI3K/AKT and ERK pathways appearing to be the downstream mediators. In addition, the present study reported that miR-218-5p was an upstream regulator of DBN1. Overall, the present study may expand the understanding of T-ALL infiltration, and might suggest DBN1 as a potential novel biomarker for T-ALL. However, further studies should be performed to validate this finding.

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Availability of data and materials

The RNA sequencing data generated in the present study may be found in the National Center for Biotechnology Information repository under accession number PRJNA726293 or at the following URL: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA726293/>. The other data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

JS and XH performed experiments, conducted data analysis and wrote the original draft. XZ, YL and HH performed the bioinformatics analysis (including OncoPrint and GEO database analysis and microRNA prediction), assisted with experiments and revised the manuscript. ZW and DX contributed to the design and standardization of all figures, including statistical analysis and data visualization, and systematically revised the manuscript to ensure accurate presentation of the research findings. QZ and FZ were involved in conception and design, revised the manuscript, and acquired funding. LS and XL were involved in conception and design and project administration, and acquired funding. JS and XH confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of the Zhongnan Hospital of Wuhan University (Wuhan, China; scientific ethical approval no. 2019094) and performed in accordance with the principles of The Declaration of Helsinki. All the participants agreed to the use of their samples in this scientific research, and all peripheral blood samples collected were obtained with the written informed consent of the participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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