# Arsenic disulfide promoted the demethylation of *PTPL1* in diffuse large Bcell lymphoma cells (#84081)



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# Arsenic disulfide promoted the demethylation of *PTPL1* in diffuse large Bcell lymphoma cells

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**Background.** Promoter methylation of tumor suppressor gene is crucial process in the pathogenesis ofcancer. Drugs with the capacity of demethylation may be candidates for the anti-cancer therapy. This study was designed to investigate the roles of *PTPL1* gene in diffuse large B cell lymphoma (DLBCL). Additionally, we investigated the effects of arsenic disulfide on *PTPL1* demethylation.

**Methods.** Based on two DLBCL cell lines (i.e. DB and SU-DHL-4 cells), we knocked down the expression of *PTPL1* using siRNA. Then we determined the DLBCL proliferation in the presence of *PTPL1* silencing. The methylation of *PTPL1* in DLBCL cells was analyzed by methylation specific PCR (MSPCR). The function of arsenic disulfide in the demethylation of *PTPL1* was determined in DLBCL cell lines using 5 μM, 10 μM and 20 μM arsenic disulfide, respectively. To investigate the potential mechanism on the arsenic disulfide mediated demethylation, we measured the mRNA expression of DNMT1, DNMT3b and MBD2,respectively.

**Results.** *PTPL1* served as a tumor suppressor gene in DLBCL cells, which was featured by the fact that *PTPL1* knockdown promoted the proliferation of DLBCL cells. *PTPL1* was hypermethylated in DLBCL cells. Arsenic disulfide promoted the *PTPL1* demethylation in a dose-dependent manner, which may be related to the inhibition of DNA methyltransferases (DNMTs) and the increase of methyl-CpG-binding domain 2 (MBD2).

**Conclusion.** *PTPL1* may be a tumor suppressor gene in DLBCL progression. *PTPL1* methylation could bereversed by arsenic disulfide in a dose-dependent manner. Our study may provide a theoretical basis for the clinical application of arsenic disulfide in DNA methylation-related diseases.

# Arsenic disulfide promoted the demethylation of *PTPL1* in diffuse large B cell lymphoma cells

**Short Title: *PTPL1* demethylation by $As_2S_2$ in DLBCL**

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## Abstract

**Background.** Promoter methylation of tumor suppressor gene is crucial process in the pathogenesis of cancer. Drugs with the capacity of demethylation may be candidates for the anti-cancer therapy. This study was designed to investigate the roles of *PTPL1* gene in diffuse large B cell lymphoma (DLBCL). Additionally, we investigated the effects of arsenic disulfide on *PTPL1* demethylation.

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**Conclusion.** *PTPL1* may be a tumor suppressor gene in DLBCL progression. *PTPL1* methylation could be reversed by arsenic disulfide in a dose-dependent manner. Our study may provide a theoretical basis for the clinical application of arsenic disulfide in DNA methylation-related

diseases.

## Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma in adults accounting for about 30-40% (*Goldfinger and Cooper, 2022*). It is an aggressive malignant lymphoma with untreated median survival of less than 12 months. After treatment with the combination-chemotherapy regimen CHOP consisting of doxorubicin, prednisone, vincristine, and cyclophosphamide, patients can achieve a progression-free survival rate of approximately 40% and a long-term survival rate of 50% (*Fisher et al., 1993*). Besides, CD20 monoclonal antibody (rituximab) further improves the treatment outcome and prognosis of DLBCL patients. Unfortunately, about 20-40% of patients still show no response or even rapid progression and relapse after treatment (*Chapuy et al., 2018*).

Most DLBCL patients have epigenetic heterogeneity, and varied degrees of heterogeneity were related to different prognosis (*Cerhan et al., 2014; Y. Jiang and A. Melnick, 2015*). DNA methylation is one form of epigenetic modification. Studies have shown that DNA hypermethylation in tumor suppressor genes could lead to transcriptional silencing, thereby leading to the loss or attenuation of functions (*Lopez et al., 2022*). PTPL1 is a protein tyrosine phosphate (PTP) encoded by the human *PTPN13* gene, which can exert tumor suppressor role by antagonizing protein tyrosine kinase (PTK) (*Freiss and Chalbos, 2011*). Promoter methylation of *PTPN13/PTPL1* has been confirmed in a variety of malignancies including non-small cell lung cancer, ovarian cancer, prostate cancer, and breast cancer (*Bompard et al., 2002; Castilla et al., 2012; J. Wang et al., 2022; Wang et al., 2018*). In DLBCL and follicular lymphoma, hypermethylation could also be detected in the majority of the *PTPL1* gene promoter, along with

attenuation or silencing of *PTPL1* (*Wang et al., 2016*). Because this epigenetic inheritance of methylation is reversible, activation of tumor suppressor genes induced by pharmacologic demethylation is considered an attractive therapeutic strategy to block tumor growth and progression.

Arsenic trioxide (ATO, $As_2O_3$), which induces DNA demethylation, has been utilized as an anti-cancer agent by suppressing cancers of the liver, prostate, and breast apparently through demethylation and apoptosis (*Thomas et al., 2010; Xia et al., 2012*). Arsenic disulfide ($As_2S_2$), the main component of traditional Chinese medicine (TCM) realgar, has been reported to exhibit similar antitumor effects to ATO with lower toxicity (*Skoczynska and Skoczynska, 2022*). Zhao et al (*Zhao et al., 2018*) showed that $A_{S2}S_2$ could exert anticancer efficacies via apoptosis induction, cell cycle arrest, and pro-survival signal inhibition in human breast cancer cells. Besides, $As_2S_2$ induced apoptosis and autophagy through the activation of ROS/JNK and suppression of Akt/mTOR signaling pathways in osteosarcoma (*Wang et al., 2017*). Furthermore, $A_{S2}S_2$ had obvious antitumor effect on mouse model of human lymphoma transplanted tumor in a dose dependent manner (*Wang, Li, & Li, 2021*).

However, the demethylation role of $As_2S_2$ in DLBCL remains unclear. In the present study, we knocked down *PTPL1* in two DLBCL cell lines (i.e. DB and SU-DHL-4 cells) using siRNA to investigate the role of *PTPL1* in DLBCL proliferation. Promoter methylation of *PTPL1* in DLBCL cell lines was detected using methylation specific polymerase chain reaction (MSPCR). We then analyzed the effects of arsenic disulfide on *PTPL1* methylation. The results obtained from this study may contribute to a better understanding of the role of *PTPL1* in DLBCL and highlight that

arsenic disulfide may be a potential new therapeutic approach to improve the poor outcomes associated with DLBCL.

## Materials & Methods

### *Cell culture*

Two human DLBCL cell lines (i.e. DB and SU-DHL-4 cell lines, generously donated by Qilu Hospital of Shandong University) and one normal human GM12878 cell line were cultured in Iscove's modified Dulbecco's medium (IMDM, Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) at 37°C in an incubator with 5% $CO_2$. Cells in the logarithmic growth phase were collected for subsequent experiments.

### *Screening of small interfering RNA (siRNA)*

To screen siRNA with better knockdown efficiency, DB and SU-DHL-4 cells of logarithmic phase were plated into 6-well plates and transfected respectively using three siRNAs against *PTPL1* for 48 h with riboFect™ CP (RIB Bio, Guangzhou, China) according to the manufacturer's instructions. Three siRNAs were siRNA1 (sequence, GGATGATGTTAGTCTAATA), siRNA2 (sequence, CCACCATGCTGCAATTGAA), and siRNA3 (GCATGAGACTACAAAGACA). Cells transfected with randomized control siRNA were used as a negative control (NC) group, Knockdown efficiencies of three siRNAs was verified using reverse transcription PCR (RT-PCR) and Western blotting.

### *RT-PCR*

Total RNA was extracted from cells using TRIzol reagent (ThermoFisher, CA, USA) according to

the manufacturer's instructions. After the synthesis of cDNA by RT-PCR, reverse transcription amplification of *PTPL1* was conducted with the specific primers (5'-CAACAATGGTCAGCAACAG-3'; 5'-CACCACAAAGCCCTTCA-3'). The specific primers were designed based on the sequence of the transcript CDS regions of the *PTPL1* gene searched in NCBI. The amplification conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. GAPDH was used as an internal reference. A fluorescence quantitative PCR instrument (CFX Connect, Bio-Rad) was used to analyze the data.

***Western blotting***

Total protein was extracted from cells in each group using RIPA lysis buffer (P0013D, Beyotime, China). The protein content was evaluated using Pierce™ Rapid Gold BCA Protein Assay Kit (A53225, Thermo Fisher). Next, the protein was separated on 10% SDS-PAGE gel, transferred onto PVDF membranes (IPVH00010, Millipore, Bedford, USA), and blocked with 5% skimmed milk under room temperature for 1 h. Subsequently, the membranes were incubated overnight at 4°C with anti-PTPL1 goat polyclonal primary antibody (1:1000; AF3577, R&D) and anti-GAPDH mouse monoclonal primary antibody (1:10000; ab8245, abcam). Then the membranes were washed in Tris-Buffered Saline and Tween (TBST) and incubated with HRP-conjugated donkey anti goat secondary antibody (1:3000; E-AB-1050, Elabscience) or HRP-conjugated goat anti-mouse secondary antibody (1:3000; E-AB-1001, Elabscience) at room temperature for 1 h. After washing three times in TBST, images were acquired with an Electro-Chemi-Luminescence (ECL) chemiluminescence kit (P0018M, Biyuntian Bio, China). Signal intensities of bands were quantified using Image J software.

***Silencing of PTPL1***

To investigate the roles of *PTPL1* in DLBCL, DB and SU-DHL-4 cells were divided into the following groups: i) control group, with no transfection; ii) negative control (NC) group, transfected with randomized control siRNA; iii) siRNA group, transfected with the best siRNA from the three siRNAs. Transfection was performed using riboFect™ CP (RIB Bio, Guangzhou, China), according to the manufacturer's instructions. Briefly, 2 mL of cell suspension was seeded into a 6-well plate, and the cell density was adjusted to $1\times10^5$-$5\times10^5$ cells/well to achieve a cell density of 30-50% at transfection. Cells were then cultured at 37°C overnight in an incubator with 5% $CO_2$. Next, 10 μL of siRNA solution (20 μM, diluted with 120 μL of 1X riboFect™ CP buffer) and 12 μL riboFect™ CP reagent were added for transfection. The mixture was incubated at room temperature for 15 min and was transferred to a serum-containing medium. After mixing gently, the cells were cultured at 37°C for 48 h in an incubator with 5% $CO_2$. Cell proliferation was observed using microscopy.

***CCK-8 analysis for cellular proliferation***

DB and SU-DHL-4 cells ($1\times10^5$/mL, 100μL) in each group were added into the wells of 96-well plate and incubated in an incubator with 5% $CO_2$ at 37℃ for 48h. Afterwards, cells were incubated with 10 μL/well CCK-8 reagents for about 1 h. Absorbance at 450 nm was measured with a microplate reader. The cell proliferation rate was calculated as the ratio of the OD value in treatment group and the control group.

***DNA extraction and methylation***

DB and SU-DHL-4 cells in the logarithmic growth phase without any treatment were washed twice

143 with PBS, followed by genomic DNA extraction using a commercial kit (Omega, USA)
144 accordance to the manufacturer's instructions. Afterwards, DNA methylation was performed using
145 200 ng genomic DNA with a commercial kit (Epigentek, USA).

146 ***Methylation specific PCR (MSPCR)***

147 MSPCR was conducted to measure the *PTPL1* methylation as previously described (*Wang et al.,*
148 *2016*). Briefly, two pairs of primers (primer M: 5'-TATAGAAATAAGGTTGAGAGGTAGC-3',
149 5'-CGAACGACAAAATTCCTAACG-3'; primer U: 5'-
150 AATATAGAAATAAGGTTGAGAGGTAGT-3'; 5'-ACCAAACAACAAAATTCCTAACAC-
151 3') were used to amplify methylated DNA and non-methylated DNA, respectively. The
152 amplification conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 30 s,
153 58°C for 30 s (for methylated DNA) or 60°C for 30 s (for non-methylated DNA), and 72°C for 30
154 s, and72°C for 10 min. Finally, the amplified PCR products were subjected to DNA agarose gel
155 electrophoresis (1.5%), followed by observation using a gel imaging system.

156 ***PTPL1 gene methylation after $As_2S_2$ treatment***

157 To investigate the effects of $As_2S_2$ on PTPL1 gene methylation, GM12878, DB and SU-DHL-4
158 cell lines were treated with 5μM, 10μM, and 20μM of $As_2S_2$ (Sigma-Aldrich, Missouri, America;
159 dissolved in 1 M NaOH and adjusted pH value to 7.35-7.45 using HCL) for 72h, respectively. The
160 cells without $A_{S2}S_2$ treatment were used as control. The mRNA levels of DNMTs (i.e. DNMTl and
161 DNMT3) and methyl-CpG-binding domain 2 (MBD2) were detected. DNMTl primers were 5'-
162 CAACGGGCAGATGTTTCA-3' and 5'-TCCTCACATTCATCCACCA-3'. DNMT3B primers
163 were 5'-GAGAAAGCTAGGGTGCGA-3' and 5'-CACTGGTTGCGTGTTGTT-3'. MBD2

primers were 5'-AGTAAGCCCCAGTTGACACG-3' and 5'-AACTGACACAGGCTGCTTGA-3'. GAPDH (5'-ACAACTTTGGTATCGTGGAAGG-3' and 5'-GCCATCACGCCACAGTTTC-3') was used as an internal reference.

***Statistical analysis***

SPSS 21.0 was used to statistical analysis. One-way analysis of variance (ANOVA) was used for significance test. The significance level was set at $P < 0.05$.

## Results

***Selection of optimal siRNAs for the knockdown of PTPL1 gene***

Among three siRNAs, siRNA2 (sequence: CCACCATGCTGCAATTGAA) had the best knockdown efficiency (**Fig. 1**). Therefore, siRNA2 was used to transfect DB and SU-DHL-4 cells to silence *PTPL1* in subsequent experiments.

***PTPL1 knockdown promoted DLBCL cell proliferation***

Micrographs and CCK-8 result together showed that compared with control and NC groups, siRNA group showed significant increase in cellular proliferation rate ($P < 0.01$, **Fig. 2**). This indicated that *PTPL1* knockdown promoted the proliferation of DB and SU-DHL-4 cells and PTPL1 exhibited the role of suppressing DLBCL. Therefore, *PTPL1* gene served as a tumor suppressor gene in the DLBCL.

***Increased PTPL1 promoter methylation in DLBCL cell lines***

Methylation was characterized by the appearance of amplification products of primer M. Unmethylation was characterized by the appearance of amplification products of primer U. The amplification products of both primer M and primer U indicated partial methylation. The

amplification of primer M was observed in DB and SU-DHL-4 cell lines (**Fig. 3**), indicating the promoter methylation of *PTPL1*.

***Arsenic disulfide inhibited the methylation of PTPL1 in a dose-dependent manner***

$As_2S_2$ treatment showed no effects on *PTPL1* methylation levels in GM12878 cell line, which demonstrated that *PTPL1* methylation was not present in normal cell lines (**Fig. 4**). In contrast, $As_2S_2$ treatment significantly reduced *PTPL1* methylation levels in both DB and SU-DHL-4 cell lines ($P < 0.01$). Besides, compared with NC group, the *PTPL1* methylation was attenuated in three $As_2S_2$ treatment groups (**Fig. 5**), especially 20 μM $AS_2S_2$ group. These indicated that $As_2S_2$ exhibited demethylation role, showing a dose-dependent manner.

***Arsenic disulfide regulated DNMTl, DNMT3b and MBD2 mRNA expression***

To investigate the potential mechanisms on arsenic disulfide mediated PTPL1 methylation inhibition, we performed RT-PCR to analyze the mRNA expression of three crucial enzymes involved in the methylation including DNMT1, DNMT3b and MBD2. RT-PCR results showed that arsenic disulfide significantly decreased the mRNA expression of DNMT1 and DNMT3b and significantly increased the mRNA expression of MBD2 (**Fig. 6**). Such phenomenon showed a dose-dependent manner.

## Discussion

DLBCL is the most common lymphoid neoplasm with dismal outcomes (*Campo et al., 2011*). DLBCL inherits the cytosine methylation patterns instability that exist in the germinal center B cells from which DLBCL arises and displays variable degrees of epigenetic heterogeneity. Greater epigenetic heterogeneity is linked with poor clinical outcome (*Yanwen Jiang and Ari Melnick,*

206 *2015*). Fortunately, the epigenetic alterations provide a number of additional targets that can be
207 pharmacologically modified and hold the promise for improved patient outcomes (*Shaknovich et*
208 *al., 2010*). *PTPL1/PTPN13* maps to the human chromosomal locus 4q21 (*S.-H. Yeh et al., 2006*)
209 and encodes a high-molecular-weight (270 kDa) non-receptor type phosphatase. Studies have
210 shown that the *PTPN13/PTPL1* gene had genetic polymorphisms, and some mutations could lead
211 to the deletion of the entire catalytic phosphatase domain or the inhibition of the phosphatase
212 activity (*Zhu et al., 2008*). In addition to genetic polymorphisms, epigenetic regulation of
213 *PTPN13/PTPL1* expression has been demonstrated in cancers. Therefore, we hypothesized that
214 the regulation of *PTPL1* could modulate DLBCL progression. Consistent with the hypothesis, the
215 findings in the present study indicated that *PTPL1* served as a tumor suppressor gene (TSG) and
216 showed hypermethylation in DLBCL cells, and arsenic disulfide promoted the demethylation of
217 *PTPL1* gene.

218 *PTPL1*, a huge tyrosine phosphatase with multiple domains, prevents the conclusive
219 determination of a positive or negative effect of *PTPL1* on tumorigenesis. To date, the roles of
220 *PTPL1* in the pathogenesis and progression of tumors remains controversial. Li et al demonstrated
221 that *PTPL1* over-expression increased resistance to Fas-induced apoptosis by the anti-Fas antibody
222 CH-11 in Jurkat and TMK-1 cells (*Li et al., 2000*). Ungefroren et al found the functional role of
223 *PTPL1* as a potential inhibitor of Fas-mediated apoptosis in pancreatic cancer cells (*Ungefroren*
224 *et al., 2001*). In astrocytoma cells, the knockdown of *PTPL1* by RNA interference led to increased
225 apoptosis and increased sensitivity to Fas-induced cell death (*Foehr et al., 2005*). Some studies
226 have confirmed the negative action of *PTPL1* on Fas-mediated apoptosis in colon cancer,

melanoma and myeloid cells using SLV inhibitory peptide (*Huang et al., 2008; Yao et al., 2004*), or RNA interference (*Schickel et al., 2010; Xiao et al., 2010*). Conversely, *PTPL1* expression was sufficient to block the IRS-1/PI3K/Akt signaling pathway to inhibit the insulin-like growth factor-I effect on cell survival and to induce apoptosis (*Dromard et al., 2007*). In addition, Wang et al found that *PTPL1* played a crucial suppressive role in the pathogenesis of lung cancer through counteracting the Src/ERK/YAP1 pathway (*Jing Wang et al., 2022*). The findings of Zhu et al indicated that the knockdown of *PTPL1* enhanced the migration and invasion capabilities of A549 cells through enhancing TGF-β1-induced EMT (*Zhu et al., 2021*). In the present study, we used siRNA to induce the silencing of *PTPL1* in DB and SU-DHL-4 cell lines to clarify the positive or negative effects of *PTPL1* in DLBCL progression. The results showed that *PTPL1* knockdown promoted DLBCL cellular proliferation, suggesting that *PTPL1* served as a TSG and played an anti-tumor role in DLBCL. The contradictory roles of *PTPL1* in different cancers may be due to the unique characteristics of each cancer type and different functional domains in *PTPL1*.

The loss of TSG function can result in a set of functional capabilities for malignant growth, usually including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, and tissue invasion and metastasis (*Wang and Wang, 2013*). TSG may become epigenetically silenced by hypermethylation of CpG islands located in their promoter regions (*Mehta et al., 2015*). *PTPL1* was downregulated or silenced in multiple cell lines such as non-Hodgkin lymphoma. Interestingly, methylation of *PTPL1* was detected by MSPCR in almost all cell lines with reduced or silenced *PTPL1* expression (*S. H. Yeh et al., 2006; Ying et al., 2006*). In this study, we detected

248 the *PTPL1* promoter methylation in two DLBCL cell lines (i.e. DB and SUDHL4 cells) using
249 MSPCR. Consistently, our data suggested that *PTPL1* was methylated in both cell lines, which
250 inhibited *PTPL1* expression and promoted DLBCL proliferation. Interestingly, unlike genetic
251 alterations, DNA methylation, an epigenetic modification, is reversible (*Kedhari Sundaram et al.,*
252 *2019*). Therefore, demethylation may restore the expression of tumor suppressor genes, and then
253 inhibit tumor progression, which provides a new idea for the treatment of tumors. Recently,
254 decitabine and azacitidine have been approved for the treatment of hematological malignancies as
255 epigenetic targeting drugs (*Blecua, Martinez-Verbo, & Esteller, 2020*). Nevertheless, numerous
256 patients do not respond to these drugs and eventually relapse (*Bazinet and Bravo, 2022*). Therefore,
257 there is still a need to develop new drugs targeting DNA methylation.

258 Our data showed that arsenic disulfide promoted the demethylation of *PTPL1* gene, which bring
259 new information for the development of anti-cancer agents. As important TCM components,
260 arsenic drugs, including arsenic disulfide ($As_2S_2$), arsenic tetrasulfide ($As_4S_4$), ATO ($As_2O_3$),
261 exhibit favorable anti-tumor effects in various tumors especially blood-related malignancies
262 (*Wang et al., 2013*). The FDA approved ATO for the treatment of acute promyelocytic leukemia
263 in 2000 (*Jing et al., 1999*). Compared with ATO, arsenic disulfide showed comparable anti-tumor
264 effects and more advantages, including lower toxicity of oral administration (*Zhao et al., 2019*).
265 To date, the mechanism of arsenic disulfide against tumors is still unclear. In a previous study,
266 arsenic disulfide exerted anti-tumor role by induction of autophagy and apoptosis, as well as cell
267 cycle arrest (*Zhao et al., 2018*). In this study, there was decreased *PTPL1* gene methylation in cell
268 lines treated with $As_2S_2$, in a dose dependent manner. Overexpression of DNA methyltransferases

(DNMTs), such as DNMT1, DNMT3A and DNMT3B, could promote DNA hypermethylation, was closely related to the prognosis in cancer patients (*Weisenberger, Lakshminarasimhan, & Liang, 2022*). Besides, MBD2 is a component of the MeCP1 complex and functions as a demethylase (*Feng and Zhang, 2001*). For the expression of DNMTs, the RNA expression of DNMT1 and DNMT3b showed significant decrease after $As_2S_2$ treatment, while the mRNA expression of MBD2 showed significant increase in these cells. These suggested that the inhibition of DNMTs and the increase of MBD2 were potential mechanisms of $As_2S_2$-induced PTPL1 demethylation.

This study has some limitations. First, the optimal dose for arsenic disulfide demethylation remains unclear. Secondly, although we confirmed the demethylation role of arsenic disulfide on methylated *PTPL1*, the exact mechanisms are still not well defined. Furthermore, biological system does not work in isolation. Although $As_2S_2$ treatment induced the demethylation of *PTPL1* by reducing the expressions of DNMT1 and DNMT3B, whether $As_2S_2$ could cause the disrupted normal methylation pattern remains unknown. More studies in the future are required to focus on the delivery of $As_2S_2$ to the *PTPL1* DNA promoter using tools such as CRISPR, thereby inducing specific demethylation of *PTPL1*.

## Conclusion

In summary, *PTPL1* was a tumor suppressor gene in DLBCL progression. *PTPL1* methylation could be reversed by arsenic disulfide in a dose-dependent manner. Our data may provide a reference for the clinical application of arsenic disulfide in DNA methylation-related diseases and provide ideas for DLBCL treatment.

## Acknowledgement

None.

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## Figure Legends

**Figure 1.** Knockdown efficiencies of three siRNAs verified using RT-PCR and Western blotting. (**A**) PTPL1 mRNA expression in control, NC, siRNA1, siRNA2, and siRNA3 groups detected by RT-PCR; (**B**) Western blotting bands and PTPL1 protein expression in control, NC, siRNA1, siRNA2, and siRNA3 groups. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

**Figure 2.** Cell proliferation and proliferation rate of control group, NC group and siRNA group in DB and SU-DHL-4 cell lines. **$P < 0.01$.

**Figure 3.** *PTPL1* methylation in DB and SU-DHL-4 cell lines detected by MSPCR.

**Figure 4.** *PTPL1* methylation levels before and after arsenic disulfide (20μM) treatment in GM12878, DB and SU-DHL-4 cell lines. **$P < 0.01$ and ***$P < 0.001$.

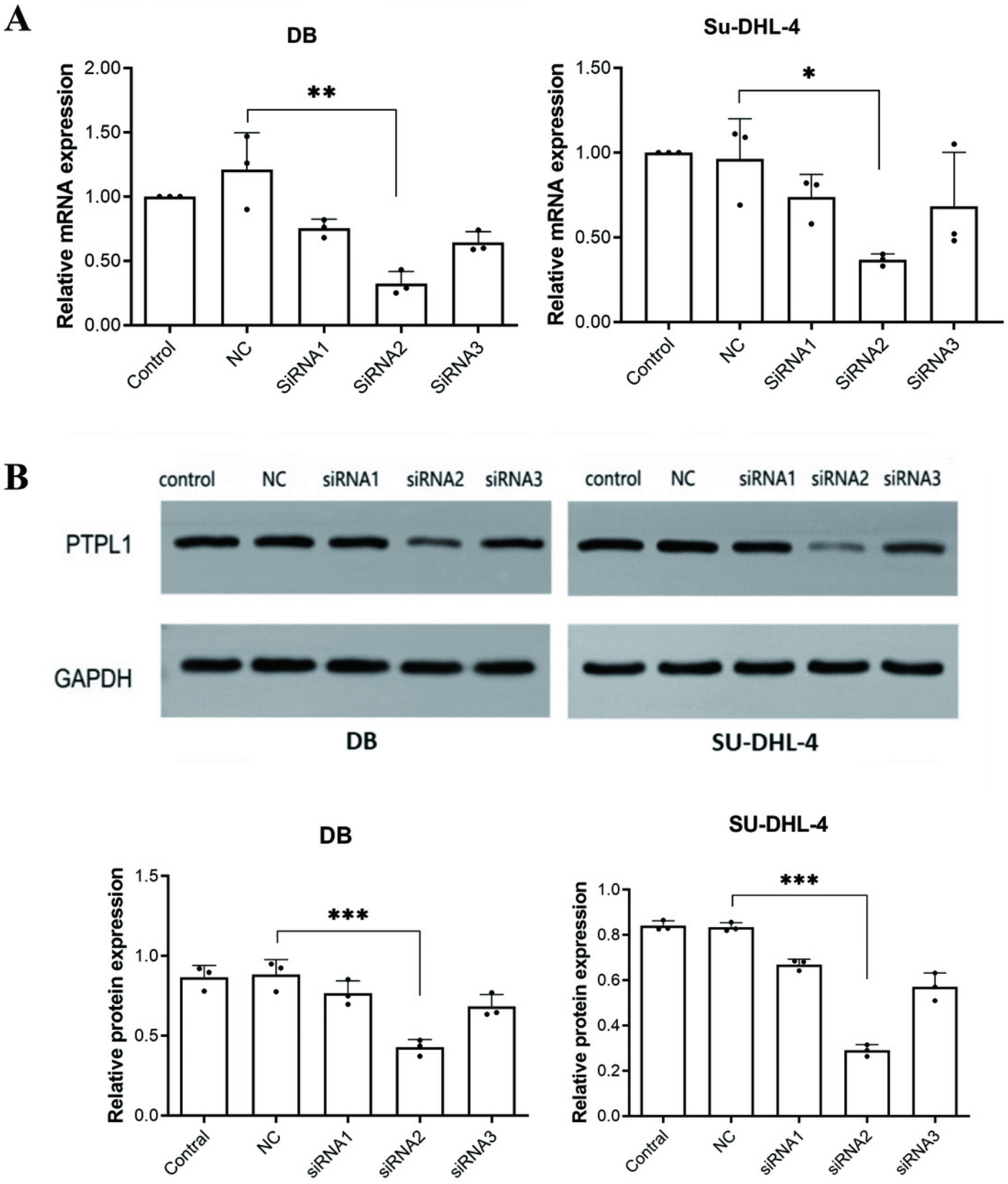
**Figure 5.** *PTPL1* methylation in DB and SU-DHL-4 cells treated with different doses of arsenic disulfide detected by MSPCR.

**Figure 6.** DNMTl, DNMT3b, and MBD2 mRNA expression in DB and SU-DHL-4 cells treated with different doses of arsenic disulfide detected by RT-PCR. *$P < 0.05$ and **$P < 0.01$.

# Figure 1

Figure 1.

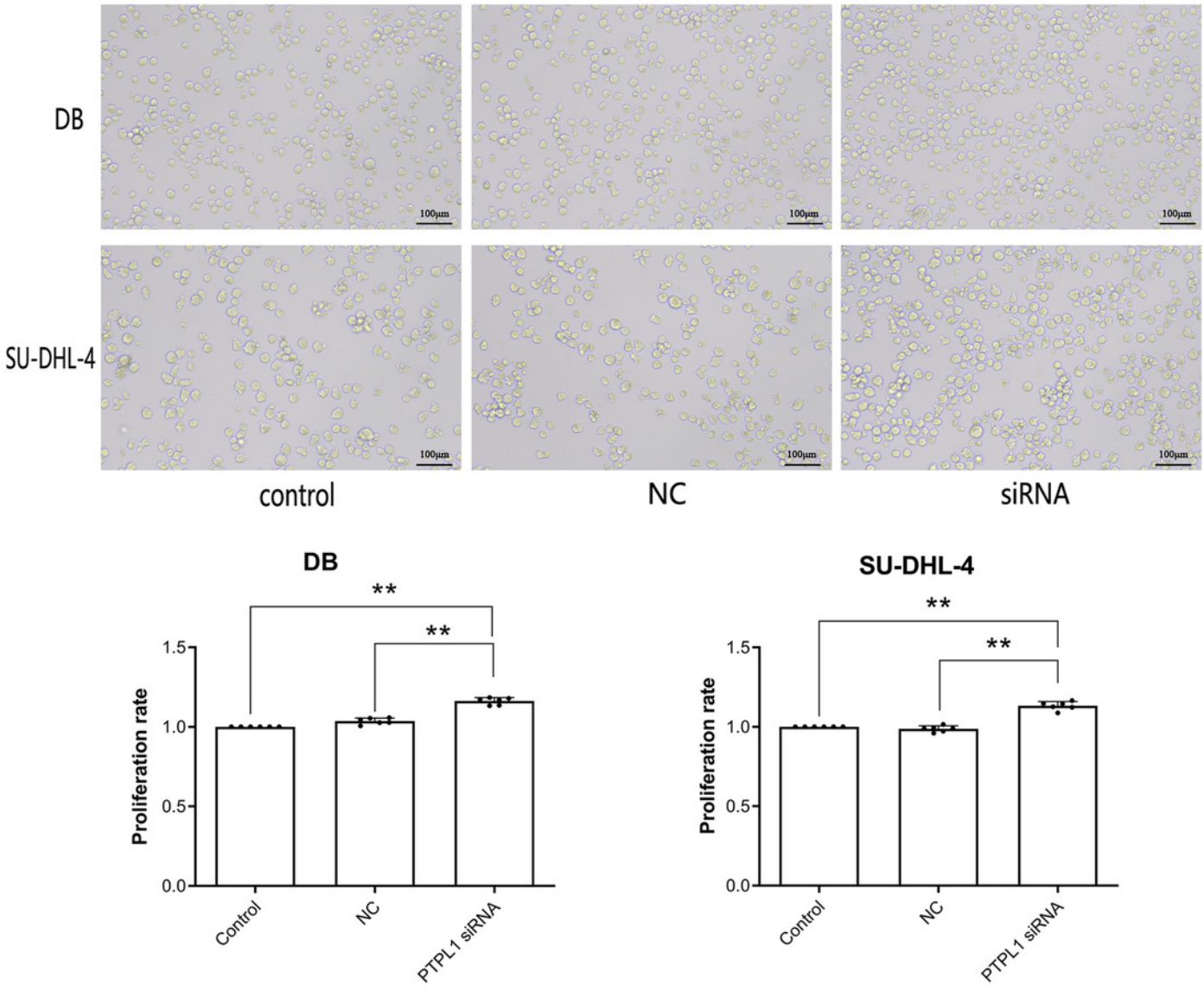
Knockdown efficiencies of three siRNAs verified using RT-PCR and Western blotting. (**A**) PTPL1 mRNA expression in control, NC, siRNA1, siRNA2, and siRNA3 groups detected by RT-PCR; (**B**) Western blotting bands and PTPL1 protein expression in control, NC, siRNA1, siRNA2, and siRNA3 groups. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.


A
DB
Relative mRNA expression
2.00
1.50
1.00
0.50
0.00
**
Control
NC
SiRNA1
SiRNA2
SiRNA3
Su-DHL-4
Relative mRNA expression
1.50
1.00
0.50
0.00
*
Control
NC
SiRNA1
SiRNA2
SiRNA3
B
control
NC
siRNA1
siRNA2
siRNA3
control
NC
siRNA1
siRNA2
siRNA3
PTPL1
GAPDH
DB
SU-DHL-4
DB
Relative protein expression
1.5
1.0
0.5
0.0
***
Contral
NC
siRNA1
siRNA2
siRNA3
SU-DHL-4
Relative protein expression
1.0
0.8
0.6
0.4
0.2
0.0
***
Contral
NC
siRNA1
siRNA2
siRNA3

# Figure 2

Figure 2

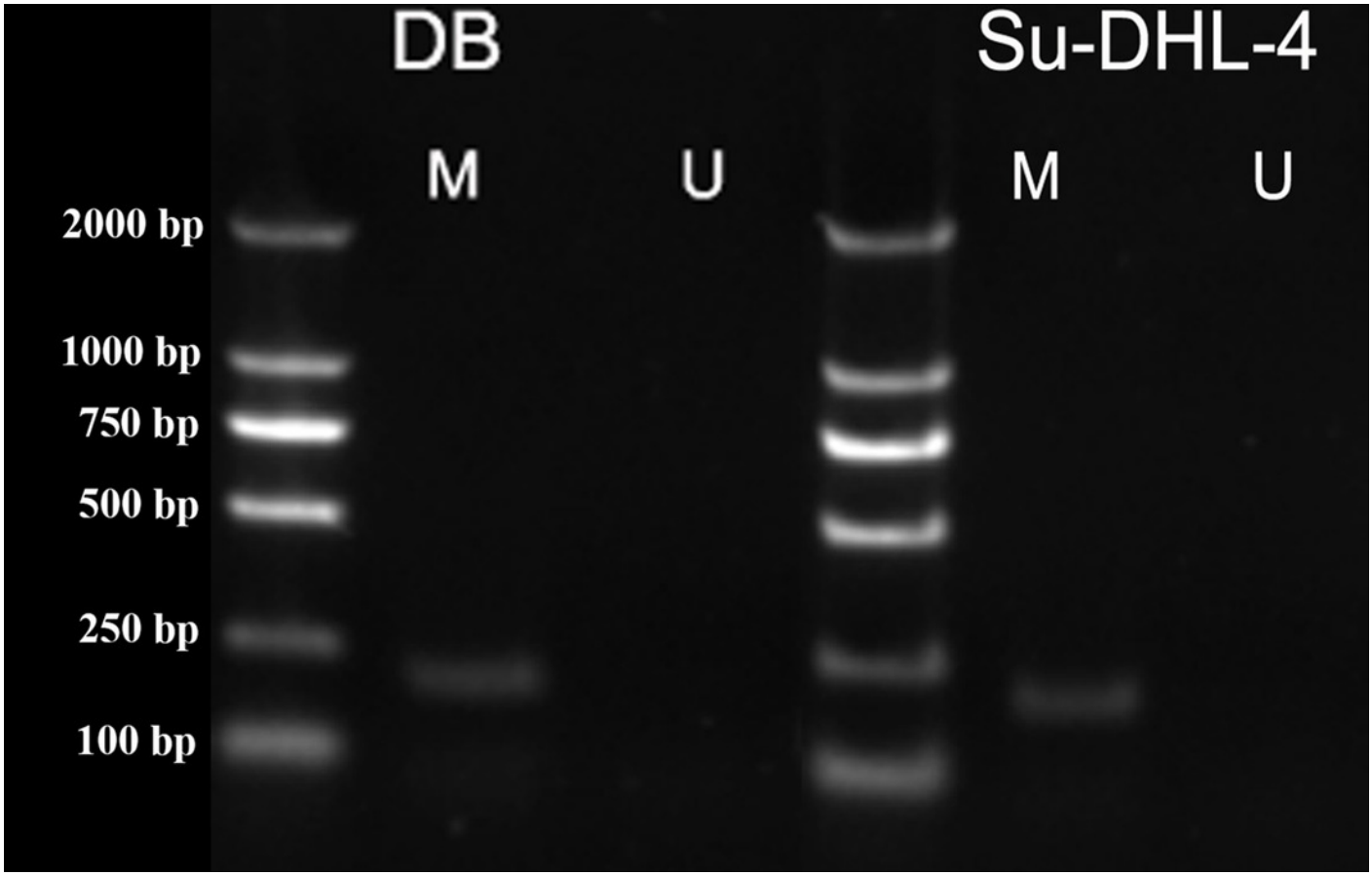
Cell proliferation and proliferation rate of control group, NC group and siRNA group in DB and SU-DHL-4 cell lines. **$P < 0.01$.



# Figure 3

Figure 3

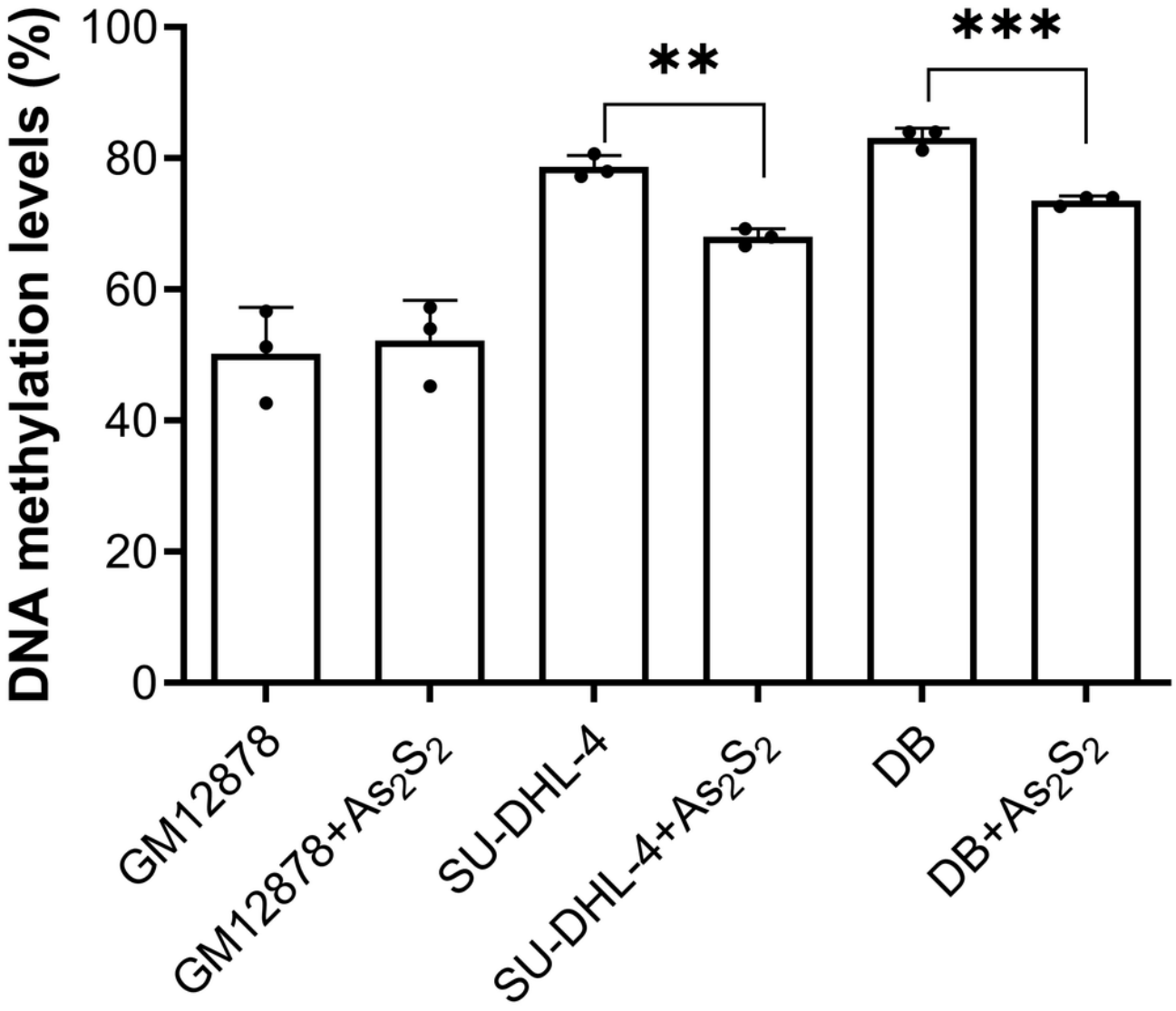
*PTPL1* methylation in DB and SU-DHL-4 cell lines detected by MSPCR.



# Figure 4

Figure 4

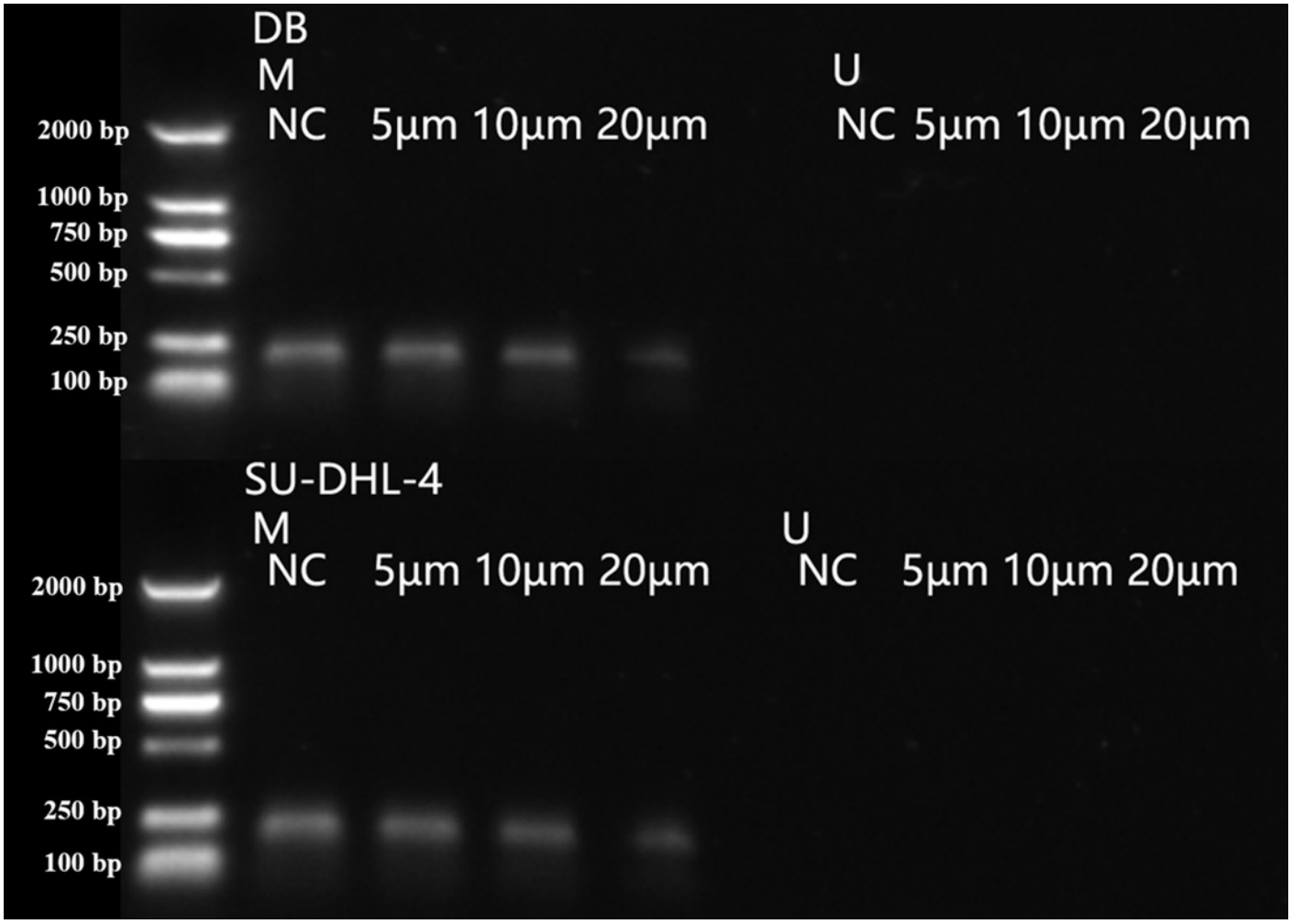
*PTPL1* methylation levels before and after arsenic disulfide (20μM) treatment in GM12878, DB and SU-DHL-4 cell lines. ***P* < 0.01 and ****P* < 0.001.



# Figure 5

Figure 5

*PTPL1* methylation in DB and SU-DHL-4 cells treated with different doses of arsenic disulfide detected by MSPCR.



# Figure 6

Figure 6

DNMTl, DNMT3b, and MBD2 mRNA expression in DB and SU-DHL-4 cells treated with different doses of arsenic disulfide detected by RT-PCR. *$P < 0.05$ and **$P < 0.01$.