



**Hematology Journal Club**

**The c-Myc-regulated miR-17-92 cluster mediates  
ATRA-induced APL cell differentiation**

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ORIGINAL ARTICLE

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# The c-Myc-regulated miR-17-92 cluster mediates ATRA-induced APL cell differentiation

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## Abstract

**Background:** Despite advances in the treatment of acute promyelocytic leukemia (APL) with all-trans-retinoic acid (ATRA), its underlying mechanism has not been fully elucidated. The oncogenic microRNA cluster miR-17-92 modulates multiple cellular processes, including survival, proliferation, and apoptosis. However, the role of miR-17-92 and its regulation has not yet been documented for APL.

**Methods:** We analyzed miR-17-92 expression in APL samples and cell lines by qRT-PCR. The expression of c-Myc was measured by western blot. Cell differentiation was assessed by measuring the surface CD11b antigen expression by flow cytometry analysis.

**Results:** We observed that miR-17-92 was upregulated in APL compared with healthy donors. Furthermore, we demonstrated that expressions of c-Myc and miR-17-92 are markedly suppressed during ATRA-induced NB4 cell differentiation. Importantly, we also demonstrated that miR-17-92 is directly regulated by c-Myc during the granulocytic differentiation of APL cells. Finally, the overexpression of miR-17-5p blocks ATRA-induced differentiation.

**Conclusions:** We report abnormal expression of the miR-17-92 cluster in APL cells, which is responsible for the differentiation block in blast cells in APL. In addition, we identified miR-17-92

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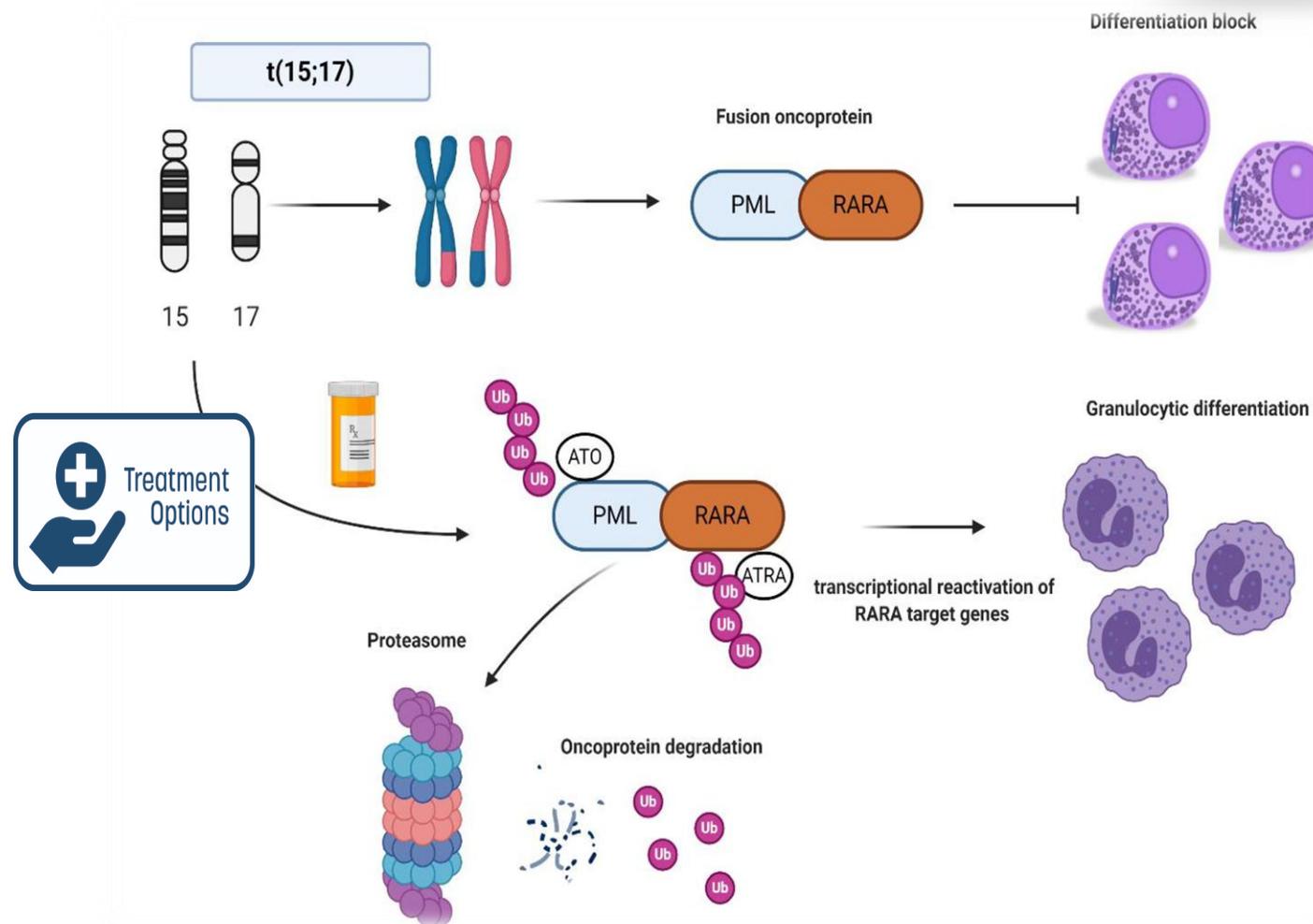
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- 3) Results
- 4) Discussion

# introduction

## APL

- distinctive subtype of AML
- chromosomal translocation t(15;17)
- PML/RAR $\alpha$  fusion
- ATRA



# introduction

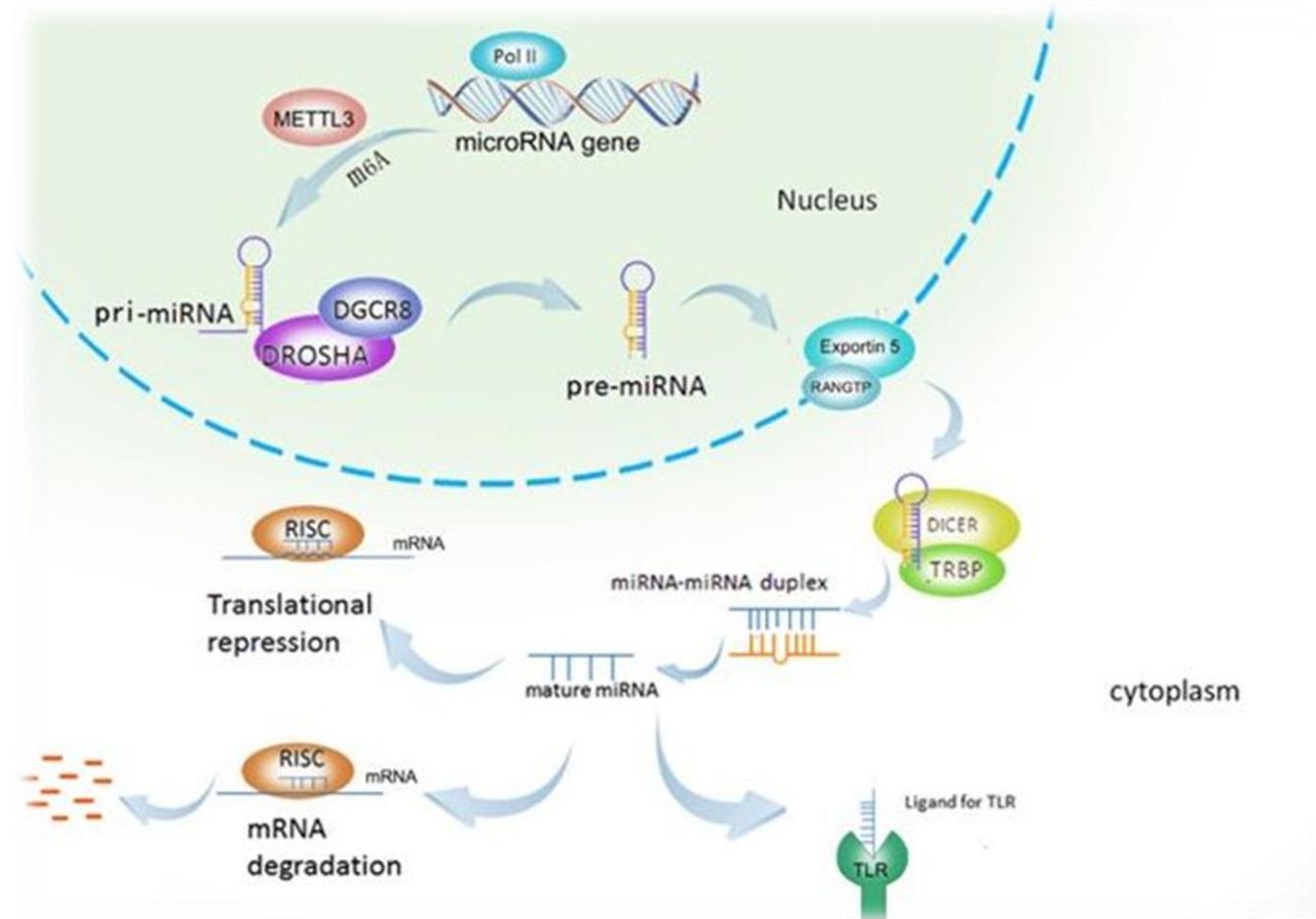
## miRNA

- ✓ differentiation
- ✓ development
- ✓ Apoptosis

1) Tumor suppressor miRNA

2) Oncogene miRNA ⇒ miR-17-92

- miR-17
- miR-18a
- miR-19a
- miR-19b-1
- miR-20a
- miR-92a





# Materials and methods

- I. Patients and samples
- II. Cell lines and cell culture
- III. Quantitative real-time PCR analysis  $\Rightarrow$  SYBR Green
- IV. Flow cytometry analysis  $\Rightarrow$  ITGAM/CD11b antigen
- V. Transfection
- VI. Western blotting
- VII. Chromatin immunoprecipitation assay

# Materials and methods

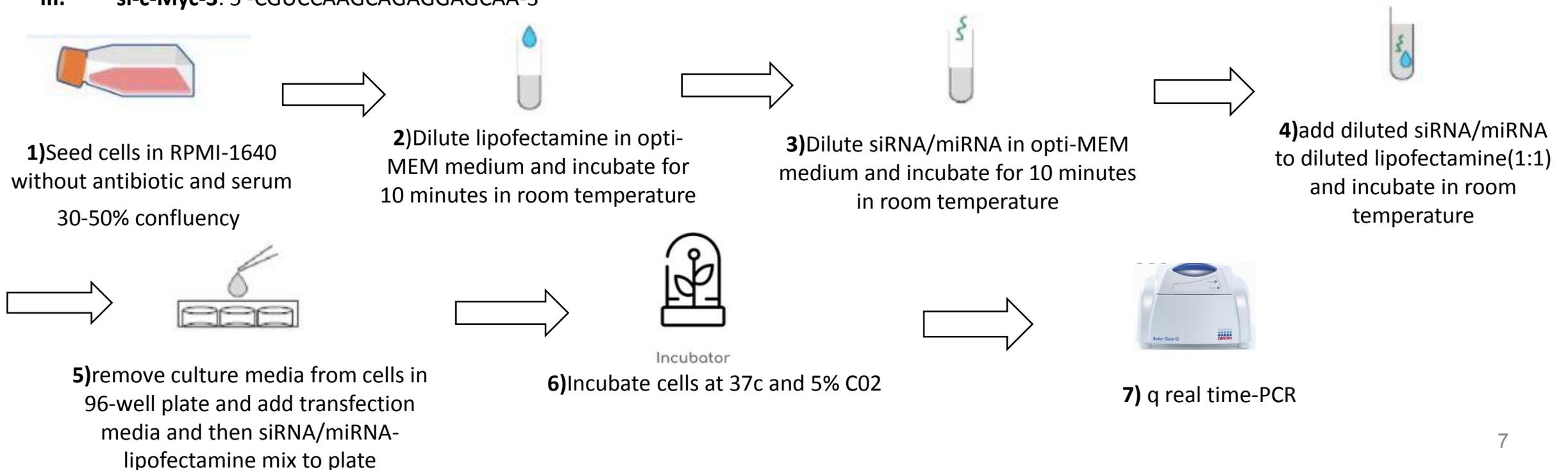
## Transfection

### The siRNA sequences used to target c-Myc

- I. si-c-Myc-1: 5'-CGAUGUUGUUUCUGUGGAA-3'
- II. si-c-Myc-2: 5'-GCUUGUACCUUGCAGGAUCU-3'
- III. si-c-Myc-3: 5'-CGUCCAAGCAGAGGAGCAA-3'

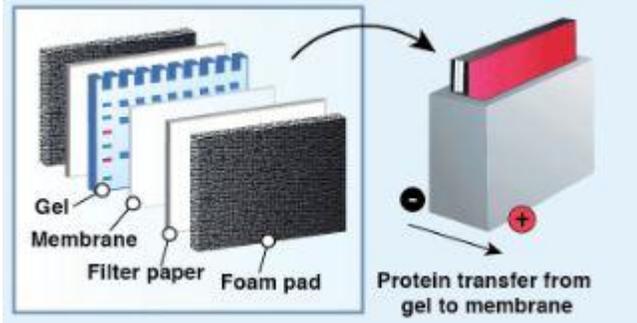
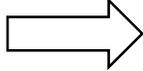
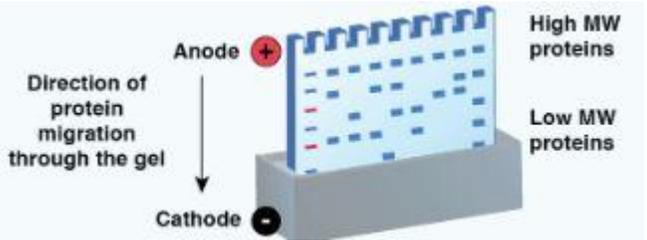
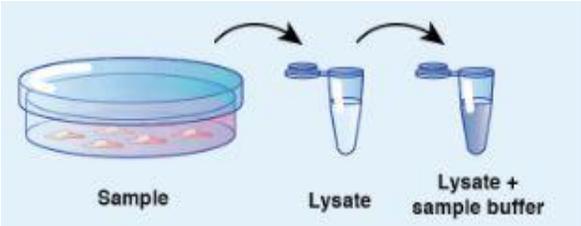
### miRNA

- miR-17-5p agomir
- miRNA agomir controls



# Materials and methods

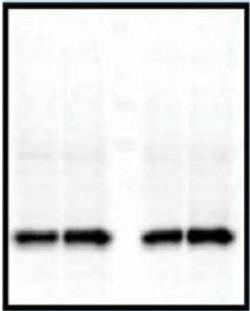
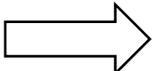
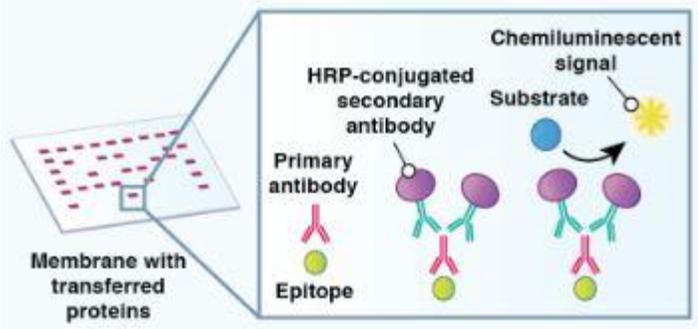
## Western blotting



1) NB4 cells were lysed in RIPA buffer with protease inhibitor

2) Protein extracts were separated by SDS-PAGE

3) transferred to a PVDF membrane

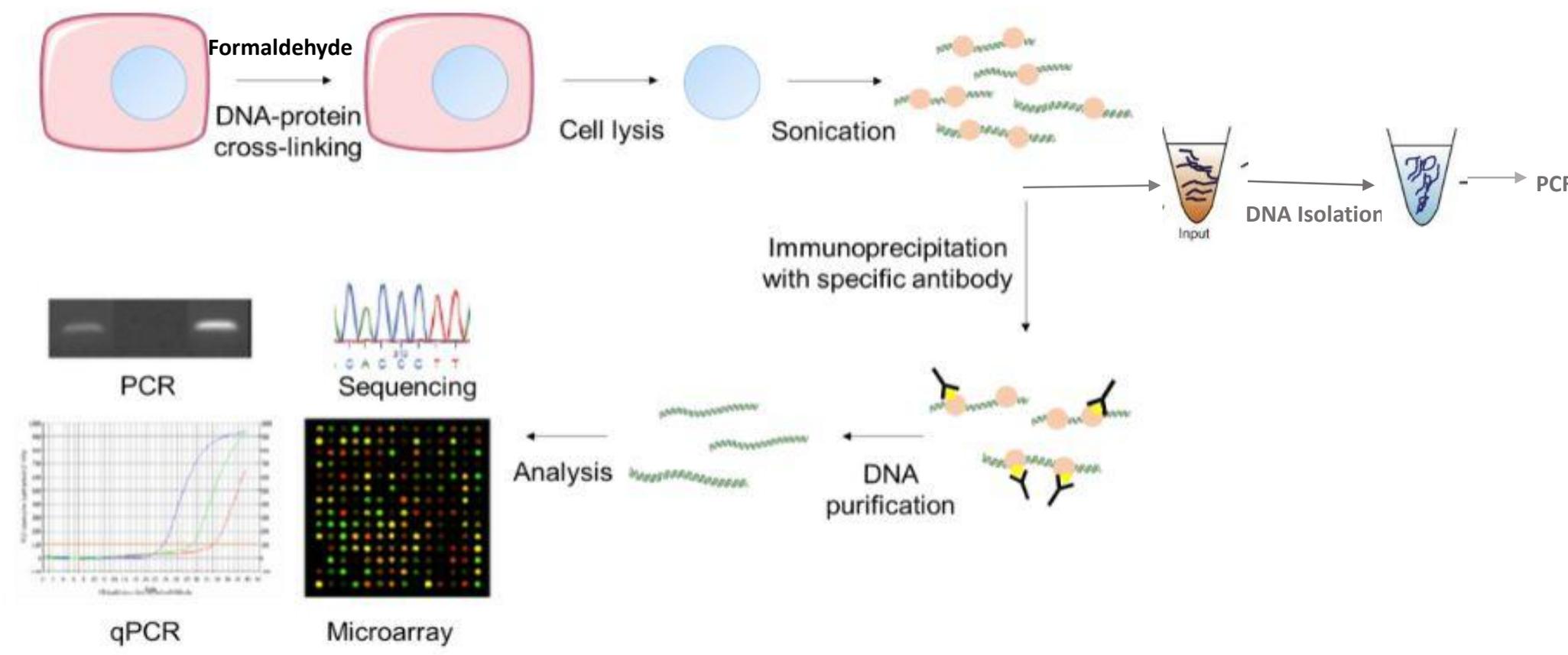


4) The blots were incubated with anti-c-Myc and anti-β-actin antibodies

5) detection step

# Materials and methods

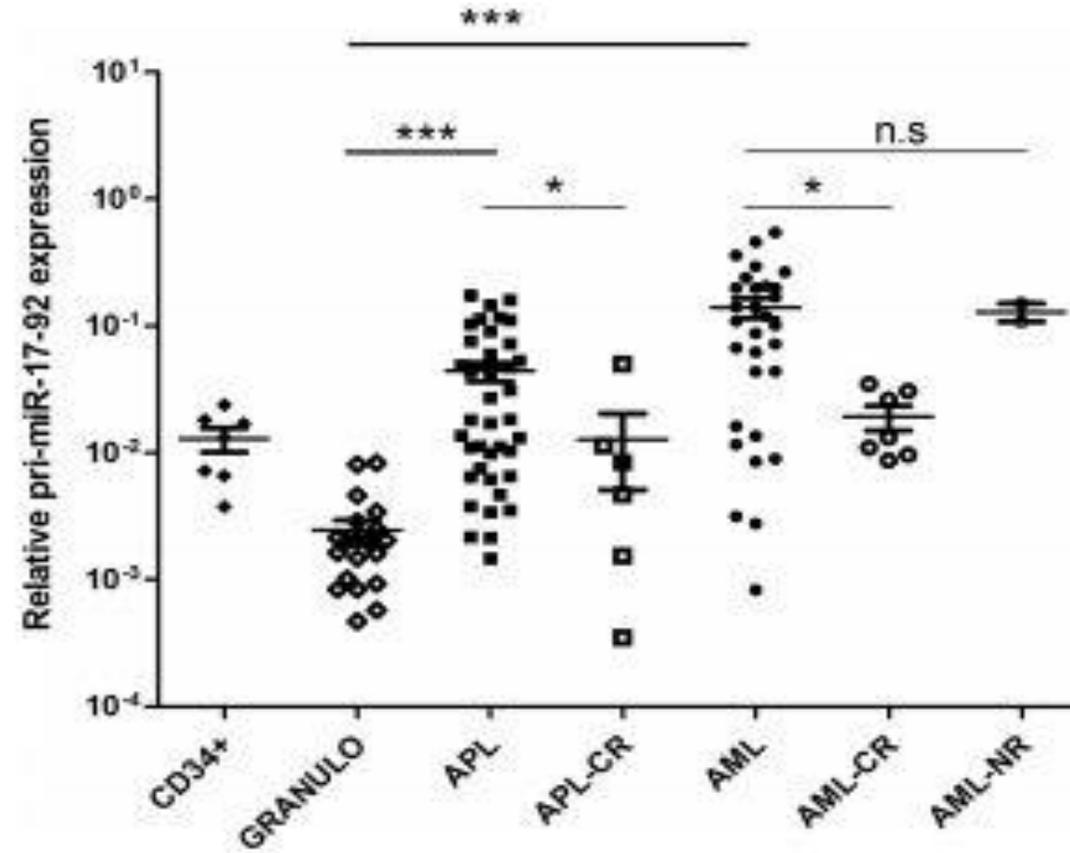
## Chromatin immunoprecipitation assay



# Results

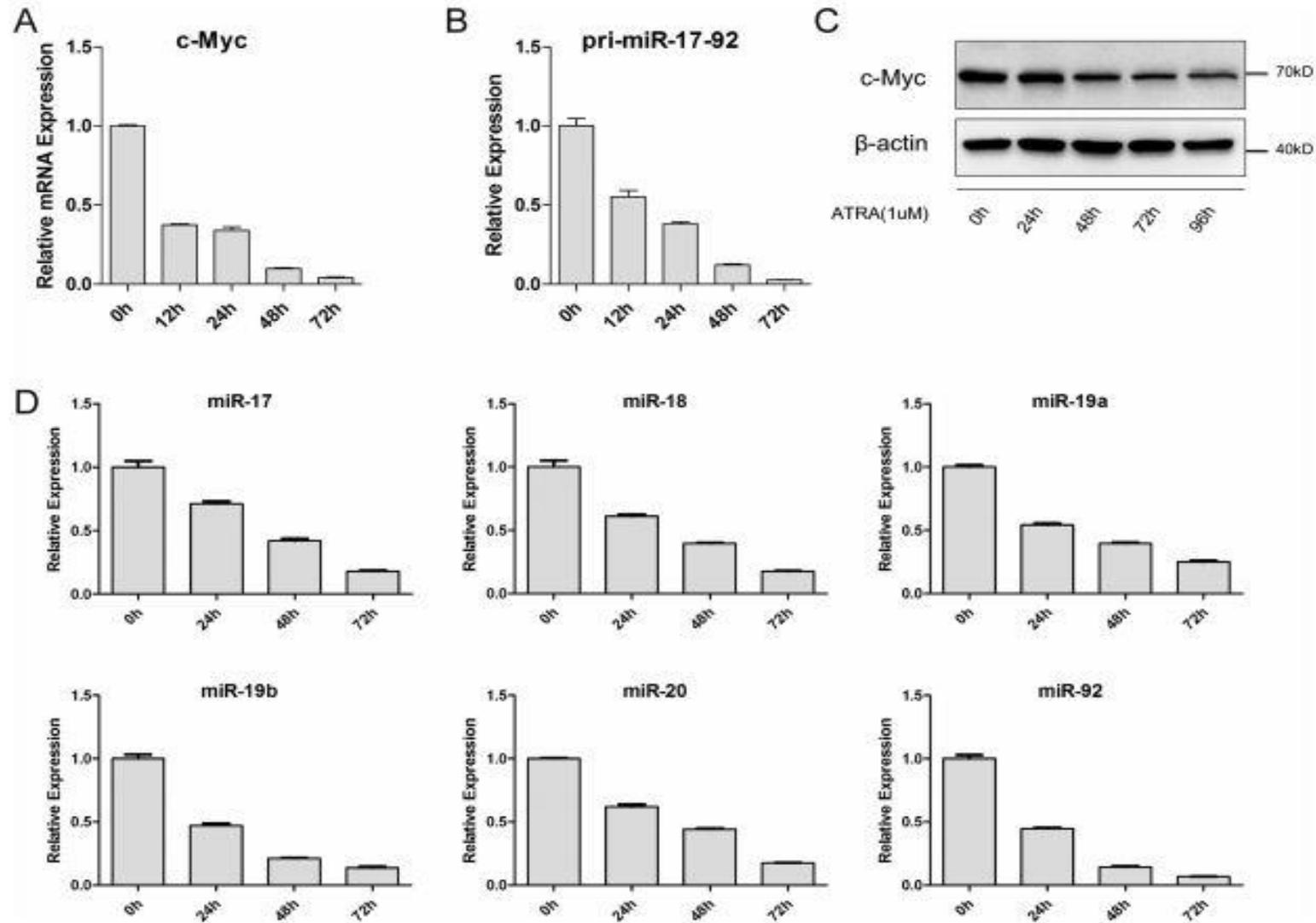


□ pri-miR-17-92 is upregulated in APL



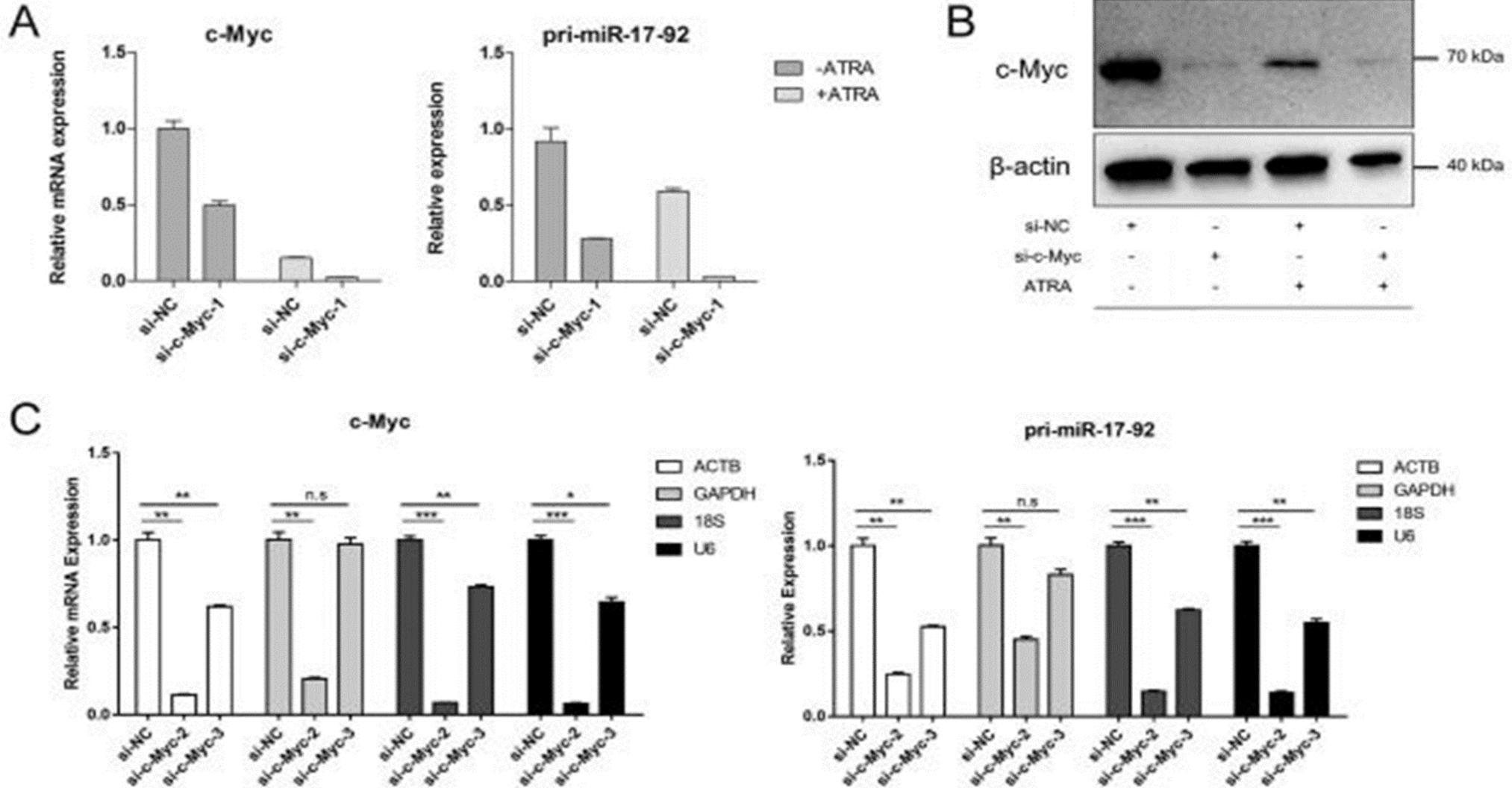
# Results

□ c-Myc and pri-miR-17-92 are suppressed during ATRA-induced cell differentiation



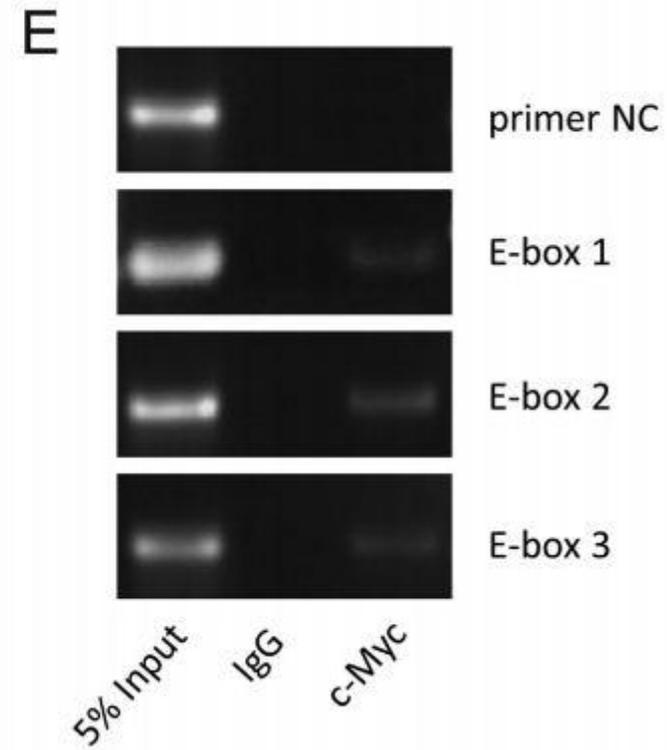
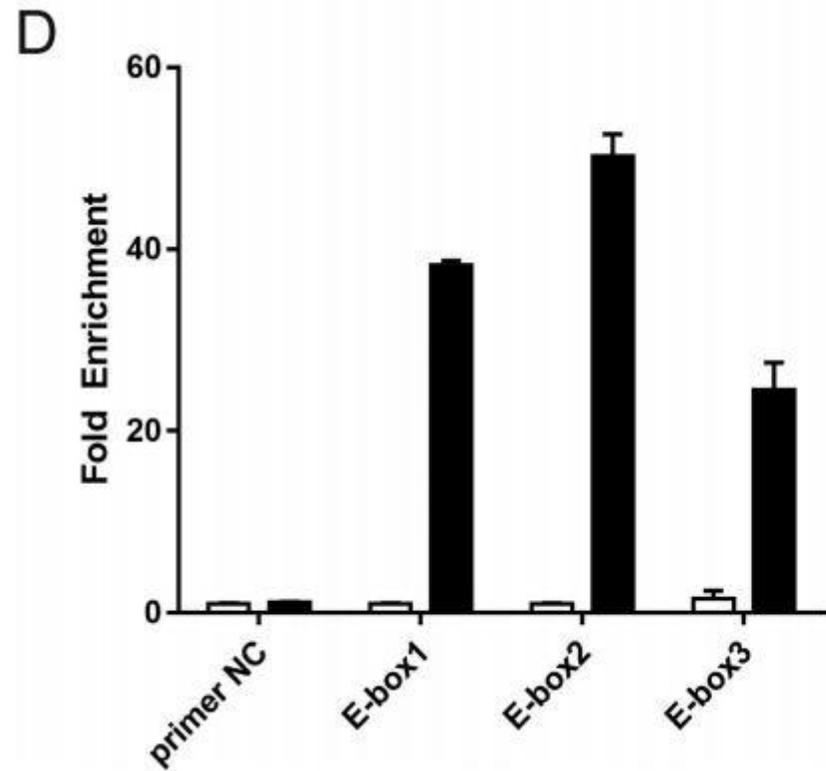
# Results

□ c-Myc promotes pri-miR-17-92 expression by binding to the miR-17-92 promoter



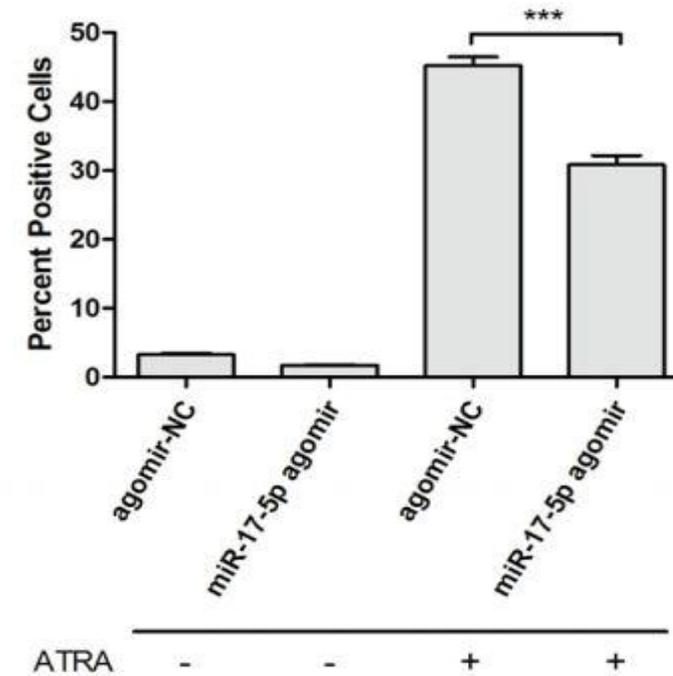
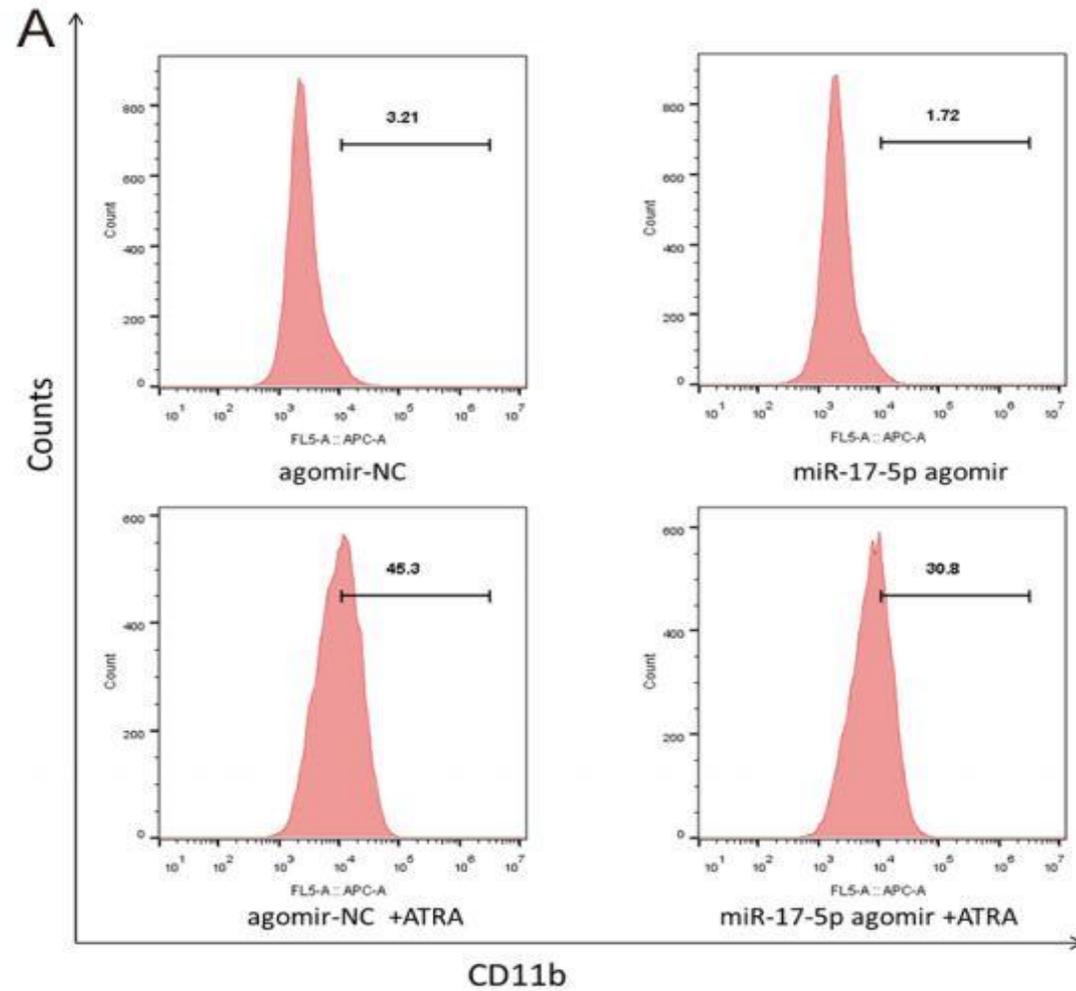
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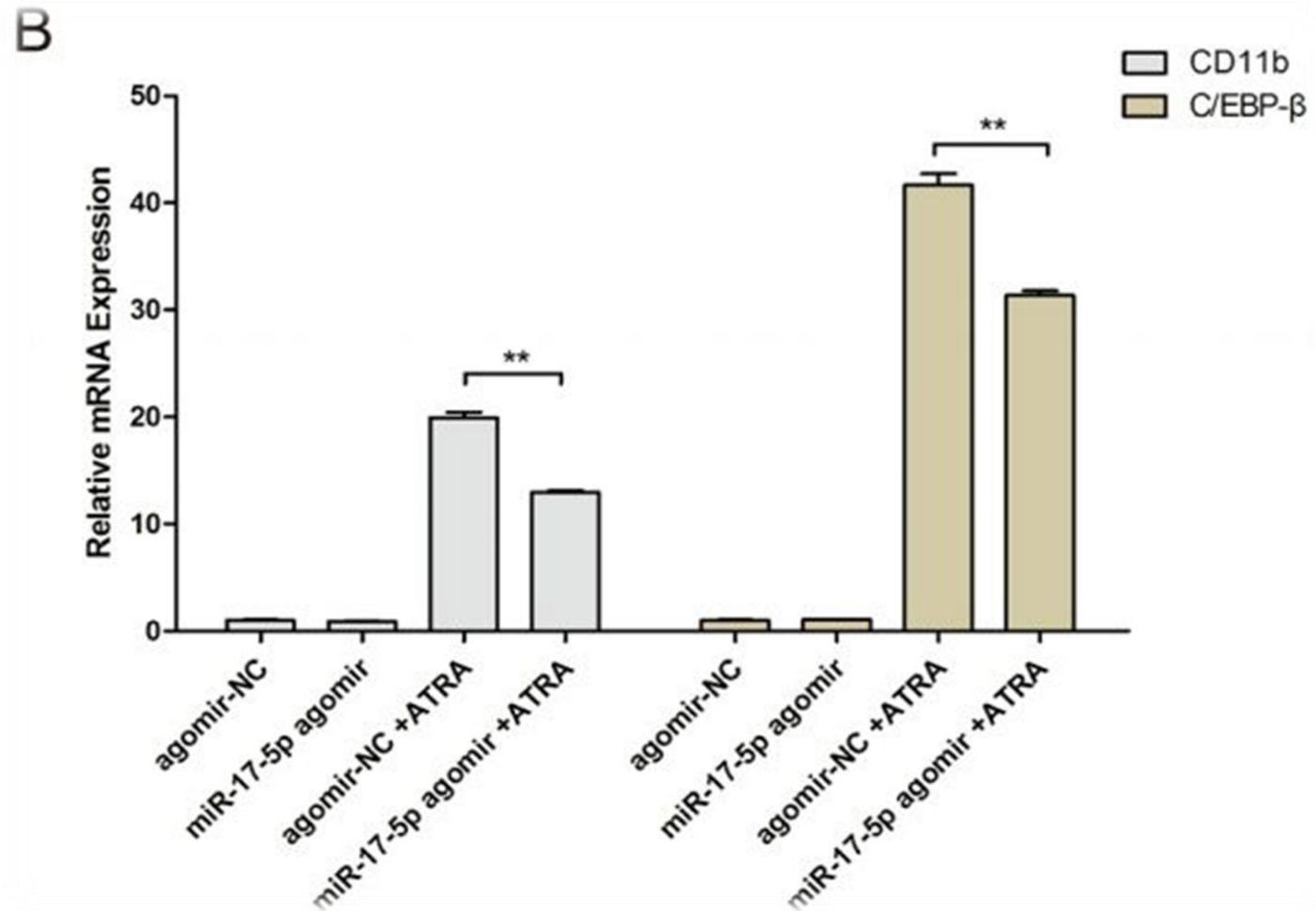
# Results

Overexpression of miR-17-5p blocks ATRA-induced APL cell differentiation



# Results

Overexpression of miR-17-5p blocks ATRA-induced APL cell differentiation



# *Discussion*



Thanks for your  
attention!!

