# *Circ_0002232* Acts as a Potential Biomarker for AML and Reveals a Potential ceRNA Network of *Circ_0002232/miR-92a-3p/PTEN*

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# Abstract

**Background:** *PTEN*, known as a classical tumor suppressor, has been reported to be down-expressed in acute myeloid leukemia (AML) and affected the progression of AML patients. CircRNAs, an emerging type of non-coding RNAs, could act as competing endogenous RNAs (ceRNAs) and has been reported to regulate the expression of *PTEN* through sponging miRNA in many solid tumors. But there are rarely studies focused on the role of *circ-PTEN* in AML. Our research was aimed to investigate the expression level of *circ_0002232*, one of circular RNAs of *PTEN*, reveal the clinical significance and potential ceRNA interaction network in AML of it.

**Methods:** *Circ_0002232* expression in 117 AML patients and 48 controls was detected by using Real-time quantitative PCR. The diagnostic value of *circ_0002232* expression was evaluated by receiver operating characteristic curve. Kaplan-Meier curves were used to analyse the impact of *circ_0002232* for overall survival. CeRNA network of *circ_0002232* was predicted by using interaction prediction websites.

**Results:** Compared with controls, *circ_0002232* was notably low-expressed in AML ($P$<0.001). According to the result of receiver operating characteristic curve, *circ_0002232* expression could distinguish AML patients from controls ($P$<0.001). There were significant differences in patients' age ($P$=0.002), FAB classifications ($P$=0.025), white blood cell count ($P$=0.034) and platelet count ($P$=0.047) between low-expressed *circ_0002232* group and high-expressed *circ_00022332* group. Moreover, there was a positive correlation between *circ_0002232* expression and patients' age (Pearson r=0.256, $P$=0.0053). Interestingly, we found that patients in low-expressed *circ_0002232* group had better overall survival both in whole AML ($P$=0.019) and non-APL AML ($P$=0.044). Remarkably, the expression of *circ_0002232* was positively correlated with *PTEN* (Pearson r=0.769, $P$<0.001). Furthermore, there was a negative correlation in AML between *circ_0002232* and *miR-92a-3p* (Pearson r=-0.262, $P$=0.032), *miR-92a-3p* and *PTEN* (Pearson r=-0.358, $P$=0.019). Interaction prediction websites revealed that *circ_0002232* might regulate the expression of *PTEN* through sponging *miR-92a-3p* and affect the process of AML.

**Conclusions:** *Circ_0002232*, one of circRNAs of *PTEN*, was remarkably down-regulated in AML and could act as a promising biomarker for the diagnosis of AML. In addition, there might be a potential ceRNA interaction network of *circ_0002232/miR-92a-3p/PTEN* in AML.


# Background

Acute myeloid leukemia (AML), the most common malignant myeloid disease in adults, is characterized by loss of differentiation of blasts (myeloid progenitor cell) and clonal amplification in the peripheral blood and bone marrow[1,2]. It had poor prognosis in the past[2]. Cytogenetics analyses play a crucial role to identify subgroups of AML with different outcomes[3]. Meanwhile, identifying molecular genetic markers also help to divide AML patients into different groups and refine their prognosis[3].

In recent years, non-coding RNAs have increasingly caught researchers' attention. A wide variety of studies have showed that non-coding RNAs participate the process of controlling cell differentiation

through regulating expression of the gene[4].

Circular RNAs (circRNAs) are an emerging class of non-coding RNAs and are characterized by having covalent binding between the 3' and 5' ends which are generated by the mechanism of reverse splicing[5]. Due to the conserved characteristic across species and tissue, circRNAs have been found to be ideal diagnostic and prognostic biomarkers for disease, especially cancer[6]. For example, according to Xia et al., their study indicated that high-expressed of *circ_0067934* in esophageal cancer was related with poor proliferation. Up-regulated expression of *circ_0067934* was an unfavorable factor for esophageal squamous cell carcinoma[7]. Shao et al. revealed that *circ_0014717* expression significantly decreased in gastric carcinoma. The level of its expression was related to tumor staging and distal metastasis. Due to the stable expression of *circ_0014717*, it had been regarded as ideal biomarker for clinical detection of gastric cancer[8].

Moreover, circular RNAs, which have also been named as competing endogenous RNAs (ceRNAs), could participate the process of regulating gene expression by acting as miRNA sponges[5]. Actually, circRNAs play an essential regulatory role in diseases through interacting with disease-related miRNAs[9]. For example, Weng W et al. illustrated that over-expressed *ciRs-7* acted as miRNA sponge to abolish the tumor suppressive effect of *miR-7* and promoted tumorgenesis in colorectal cancer[10]. *Circ_FBLIM1* had been found to function as ceRNA and regulate *FBLIM1* expression through binding with *miR-346*. This process promoted the progression of hepatocellular cancer[11]. But there are few studies focused on the diagnostic and prognostic value of circular RNAs or their function acting as ceRNA in malignant hematonosis.

Phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) serves as a classic tumor suppressor[12]. It mainly participates the homeostasis of the phosphatidylinositol 3 kinase *(PI3K)/AKT* pathway[12]. And losing the suppressive function of *PTEN* plays an essential role in the occurrence of cancer. *PTEN* have been found to be down-expressed in several solid cancers, like prostate cancer and breast cancer[13,14]. Furthermore, some researches illustrated that the expression of *PTEN* transcript was remarkably lower in AML than controls and inactivation of *PTEN* promoted AML progression[15,16].

To our knowledge, circular RNAs of *PTEN* have seldom been studied in cancer, let alone AML. *Circ_0002232* is one of circRNAs of *PTEN*. The purpose of this research was to analyse *circ_0002232* expression in AML and to invastigate its clinical relevance. We wanted to find whether it could serve as a biomarker for diagnosis and prognosis of AML and reveal the potential ceRNA network behind it.

# 2. Materials And Methods

### 2.1 Patients and samples

A total of 165 samples, including 48 controls and 117 de novo AML patients, were provided by the Affiliated People's Hospital of Jiangsu University. This study was approved by Human Research Ethics

Committee of the Affiliated People's Hospital of Jiangsu University. Patients involved in this study were clearly diagnosed and classified according to guidelines of World Health Organization (WHO) and French-American-British (FAB) criteria[17,18]. To refine the group, total 117 AML samples we used included 91 non-acute promyelocytic leukemia AML (non-APL AML) samples and 53 normal karyotype AML (CN-AML) samples. Bone marrow (BM) specimen was collected after every participator signed informed consent. Extraction of bone marrow mononuclear cells (BMNCs) were conducted by using Lymphocyte Separation Medium (TBD sciences corporation, Tianjin, China). The vital clinical and laboratory features of these patients were listed in Table 1.

**Table 1. Comparison of clinical and laboratory characteristics between AML patients with low and high *circ_0002232* expression.**

| Patient's parameters | Low (n=88) | High (n=29) | *P* value |
|---|---|---|---|
| Sex, male/female | 59/29 | 15/14 | 0.183 |
| Median age, years (range) | 54(21-81) | 64(20-88) | 0.002* |
| Median WBC, $\times 10^9$/L (range) | 14.25(0.3-528.0) | 35.35(1.1-207.5) | 0.034* |
| Median hemoglobin, g/L (range) | 78(34-144) | 82(42-119) | 0.578 |
| Median platelets, $\times 10^9$/L (range) | 35.5(3-415) | 51.5(9-382) | 0.047* |
| BM blasts, % (range) | 47.75(1.00-109.00) | 30.50(6.50-92.00) | 0.776 |
| CR (+/-) | 39/37 | 16/8 | 0.241 |
| FAB | | | 0.025* |
| M0 | 0(0%) | 1(4.2%) | |
| M1 | 4(4.9%) | 0(0%) | |
| M2 | 39(47.6%) | 6(25%) | |
| M3 | 14(17.1%) | 2(8.3%) | |
| M4 | 17(20.7%) | 9(37.5%) | |
| M5 | 7(8.5%) | 6(25%) | |
| M6 | 1(1.2%) | 0(0%) | |
| Karyotype classification | | | 0.286 |
| Favorable | 24(27.3%) | 4(13.8%) | |
| Intermediate | 53(60.2%) | 20(69.0%) | |
| Poor | 9(10.2%) | 3(10.3%) | |
| No data | 2(2.3%) | 2(6.9%) | |
| Karyotype | | | 0.289 |
| Normal | 40(40.5%) | 12(41.4%) | |
| t(8;21) | 9(10.2%) | 1(3.4%) | |
| t(15;17) | 14(15.9%) | 2(6.9%) | |
| +8 | 2(2.3%) | 3(10.3%) | |
| complex | 8(9.1%) | 3(10.3%) | |
| others | 13(14.7%) | 6(20.6%) | |
| No data | 2(2.3%) | 2(6.9%) | |
| Gene mutation | | | |
| *CEBPA* (+/-) | 10/65 | 0/18 | 0.200 |
| *NPM1* (+/-) | 8/67 | 0/18 | 0.347 |
| *FLT3*-ITD (+/-) | 11/64 | 1/17 | 0.450 |
| *C-KIT*(+/-) | 4/71 | 1/17 | 0.533 |
| *N/K-RAS* (+/-) | 3/61 | 2/11 | 0.196 |
| *IDH1/2* (+/-) | 0/75 | 1/17 | 0.194 |
| *DNMT3A* (+/-) | 5/70 | 1/17 | 1.000 |
| *U2AF1* (+/-) | 1/74 | 1/17 | 0.351 |
| *SRSF2(+/-)* | 1/63 | 0/13 | 1.000 |

WBC, white blood cell; BM blast, bone marrow blast; FAB, French-American-British criteria.
*indicated statistical significance (P<0.05).

## 2.2 RNA isolation and reverse transcription

The process of isolating total RNA from BMNCs was conducted by using Trizol reagent (Invitrongen, Carlsbad, USA). Reverse transcription mixture contains 2μg of total RNA from each sample, 10mM of dNTPs, 10μM of random hexamers, 80U of RNase inhibitor, and 200U of reverse transcriptase (MBI Fermentas corporation, Hanover, USA). The reverse transcript system was incubated at 25℃ for 10 min, at 42℃ for 60 min and store at -20℃.

## 2.3 Real-time quantitative PCR

The expression of *circ_0002232*, *miR-92a-3p* and *PTEN* was detected by real-time quantitative PCR (RQ-PCR) with specific primers listed in Additional file 1. The PCR reaction systems of detecting *circ_0002232* and *PTEN* were SYBR Premix Ex Taq II (TaKaRa, Japan) and the reaction system of detecting the expression of *miR-92a-3p* was miScript SYBR green PCR kit (Qiagen, Duesseldorf, Germany). 7500 Thermocycler (Applied Biosustems, CA, USA) was used to perform reaction system. A housekeeping gene (*ABL*) was used to calculate the quantity of *circ_0002232* and *PTEN*. And the quantity of *miR-92a-3p* was valued by *U6*. Relative expression level of *circ_0002232*, *miR-92a -3p* and *PTEN* were calculated by using $2^{-\Delta\Delta CT}$ formula.

## 2.4 Gene mutation detection

Mutations of gene *NPM1*, *N/K-RAS*, *DNMT3A*, *c-KIT*, *U2AF1*, *IDH1/2* and *SRSF2* were detected by High Resolution Melting analysis[19-22]. Direct DNA sequencing were used to detect mutation of gene *CEBPA* and *FLT3-ITD*.

## 2.5 Bioinformatics and statistical analysis

Micro RNAs which might bind with *circ_0002232* were predicted by circRNA-miRNA interation prediction websites, miRanda (http://miranda.org.uk) and RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/submission.html). Target genes of *miR-92a-3p* were predicted by miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/index.php), miRDB (http://www.mirdb.org) and TargetScan (http://www.targetscan.org/vert_72/).

Statistical analysis was conducted by using spss software version 22.0. The diagnostic value of *circ_0002232* expression was evaluated by receiver operating characteristic (ROC) curve and area under the ROC curve (AUC). The differences of categorical variables between the two groups were analysed by using Pearson Chi-square analysis or Fisher exact test and the differences of continuous variables were evaluated by using Mann-Whitney U test. To explore prognostic potential of *circ_0002232*, Kaplan-Meier curves were used to analyse the impact of *circ_0002232* for overall survival (OS) and Cox regression analysis were used to assess its independent prognostic value. Pearson correlation analysis was used

respectively to examine the correlationship among the expression of *circ_0002232, miR-92a-3p*, platelet count and the patients' age. *P* value less than or equal to 0.05 (two-sided) was considered statistically significant in all analyses.

# 3. Results

### 3.1 *Circ_0002232* expression in AML and controls

In our experiment, the expression level of *circ_0002232* in de novo AML (median 0.0492, range 0.000215-1.066) was notablely decreased compared with that in controls (median 0.468, range 0.00693-40.518) (*P*<0.001, Figure 1). In addition, *circ_0002232* expression level was remarkably down-regulated in non-APL AML patients (*P*=0.0021, Figure 1) and in CN-AML patients (*P*=0.027, Figure 1).

### 3.2 Differentiating ability of *circ_0002232* expression

The capacity of *circ_0002232* expression to distinguish AML patients from controls was analysed by ROC curve (AUC:0.846, 95% CI:0.782-0.910, *P*<0.001, Figure 2A). It indicated that *cric_00022232* could act as a significant marker in differentiating between AML patients and controls. In addition, the remarkable significance was found in non-APL AML patients (AUC:0.841, 95% CI:0.774-0.909, *P*<0.001, Figure 2B).

### 3.3 Clinical and laboratory characteristics of AML patients

For the purpose of exploring the relationship between clinical parameters and *circ_0002232* expression, we obtained the cut-off value, which had the maximum sum of sensitivity and specificity according to ROC curve analysis, and divided 117 AML patients into low-expressed group (*circ_0002232*$^{low}$) and high-expressed group (*circ_0002232*$^{high}$). Hence, we used 0.165 as the cut-off value, whose sensitivity was 0.813 and specificity was 0.759. There were no significant discrepancies between the two groups in sex, hemoglobin, BM blasts, complete remission (CR), karyotypes and nine gene mutations (*P*>0.05, Table 1).

However, remarkable differences were observed in FAB classifications (*P*=0.025), white blood cell (WBC) count (*P*=0.034) and platelet count (*P*=0.047) between *circ_0002232*$^{low}$ and *circ_0002232*$^{high}$ groups. Age of the patients in *circ_0002232*$^{low}$ group were notably younger than those in *circ_0002232*$^{high}$ group (*P*=0.002). Moreover, we found that there was a positive correlationship between *circ_0002232* expression and patients' age (Pearson r=0.256, $r^2$=0.0656, *P*=0.0053, Figure 3A). There was a tend of the correlation between the expression of *circ_0002232* and platelet count, but it was not statistically significant (Pearson r=0.176, $r^2$=0.0310, *P*=0.059, Figure 3B).

### 3.4 Correlation between *circ_0002232* expression and patients' clinical outcome

Survival analysis included 90 AML patients and excluded 27 patients who were failed to follow up. Median follow-up time of included patients was 8 months, which range from 1 months to 90 months. According to Kaplan-Meier analysis, *circ_0002232*$^{low}$ group had significantly longer OS (*P*=0.019)

compared with *circ_0002232*$^{high}$ group in whole AML (Figure 4A). In non-APL AML, patients in low-expressed *circ_0002232* group tended to have better prognosis (*P*=0.044, Figure 4B). However, in low age group (age<40y), we found that patients with high *circ_0002232* expression tended to have better OS, but it was not statistically significant(*P*=0.287, Figure 4C).

Univariate analysis, including age (≤60y or >60y), WBC count (≥30×$10^9$/L or <30×$10^9$/L), karyotype classification, *circ_0002232* expression with *P*<0.20, showed that expression of *circ_0002232* could be used as a valuable factor for AML patients' prognosis. However, according to multivariate analysis, expression of *circ_0002232* could not act as an independent factor for OS (*P*=0.609) among AML patients (Table 2).

**Table 2. Univariate and multivariate analyses of prognostic variables for overall survival in whole AML patients**

| Variables | Overall survival | | | |
|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | |
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Age | 2.544 (1.553-4.168) | <0.001 | 1.242 (0.690-2.235) | 0.470 |
| WBC | 3.016 (1.840-4.943) | <0.001 | 2.217 (1.274-3.858) | 0.005* |
| Karyotype classifications | 2.026 (1.468-2.796) | <0.001 | 1.975 (1.318-2.959) | 0.001* |
| *Circ_0002232* expression | 1.875 (1.071-3.280) | 0.028 | 0.815 (0.373-1.783) | 0.609 |
| *FLT3*-ITD mutation | 0.876 (0.395-1.941) | 0.745 | - | - |
| *NPM1* mutation | 1.693 (0.720-3.979) | 0.228 | - | - |
| *CEBPA* mutation | 0.885 (0.377-2.075) | 0.778 | - | - |
| *c-KIT* mutation | 0.581 (0.141-2.391) | 0.452 | - | - |
| *N/K-RAS* mutation | 2.752 (1.072-7.067) | 0.035 | 2.981 (1.150-7.730) | 0.025* |
| *IDH1/2* mutation | 5.405 (0.707-41.327) | 0.104 | 4.023 (0.512-31.599) | 0.186 |
| *DNMT3A* mutation | 1.635 (0.649-4.122) | 0.297 | - | - |
| *U2AF1* mutation | 4.679 (1.089-20.102) | 0.038 | 1.774 (0.221-14.206) | 0.589 |
| *SRSF-2* mutation | 2.652 (0.359-19.616) | 0.339 | - | - |

HR, hazard ratio; CI, confidence interval; WBC, white blood cell. Prognositic variables included WBC (≥30×$10^9$ vs. <30×$10^9$ /L), patients' age (≤60 vs. >60 years), Karyotype classifications (favorable vs. intermediate vs. poor), *circ_0002232* expression level (Low vs. High), and gene mutations (mutant vs. wild-type). Variables with *P*<0.200 in univariate analysis were included into multivariate analysis.
*indicated statistical significance (P<0.05).

### 3.5 Correlation between expression of *circ_0002232* and *PTEN* in AML

The expression of *PTEN* in AML (median 1.984, range 0.00701-88.0896) was remarkably down-regulated compared with controls (median 3.330, range 0.842-103.788) (*P*=0.0057, Figure 5A). Furthermore, the expression of *circ_0002232* was positively correlated with its parental gene *PTEN* (Pearson r=0.769, $r^2$=0.592, *P*<0.001, Figure 5B).

### 3.6 Potential interaction network of *circ_0002232/ miR-92a-3p/PTEN*

CircRNA-miRNA interaction prediction websites were used to predict miRNAs which might bind with *circ_0002232.* Through searching literature, we finally choose *miR-92a-3p* (Figure 6A, 6B). The expression of *miR-92a-3p* were detected in controls and AML patients. *MiR-92a-3p* was notably up-expressed in AML (median 6.215, range 0.0610-218.199) compared with controls (median 0.472, range 0.00815-2.964)

(*P*=0.0087, Figure 6E). Pearson correlation analysis revealed that *circ_0002232* expression was negatively correlated with *miR-92a-3p* expression in AML (Pearson r=-0.262, $r^2$=0.0688, *P*=0.032, Figure 6F). Moreover, prediction websites showed the potential binding sites between *miR-92a-3p* and *PTEN* (Figure 6C, 6D). According to result of Pearson analysis, *miR-92a-3p* had negative correlation with *PTEN* (Pearson r=-0.358, $r^2$=0.129, P=0.019, Figure 6G).

# 4. Discussion

CircRNAs known as a novel category of non-coding RNAs exist widely in mammalian cells[23]. They have been considered as ideal biomarkers for disease because of their conservative feature across species. There are a few studies concentrated on the role of circRNAs in hematological malignancies. For instance, *circ_0004277* expression had been reported to be down-regulated in AML. And the expression of *circ_0004277* tended to up-regulated when the patients got complete remission and down-regulated again when they got relapsed. *Circ_0004277* expression changed dynamically with process of AML, which proved that it could be used as AML biological marker[24].

According to what we know, this is the first report foused on the expression of circular RNA of *PTEN* in AML. In this study, *circ_0002232* expression in AML was notably down-regulated compared with that in controls. The same results were found in groups of non-APL AML and CN-AML. Accordng to ROC curve analysis, *circ_0002232* could act as a valuable marker to identify AML patients and control groups.

Identifying the relation between the expression of *circ_0002232* and clinical characteristic, we found that the expression level of *circ_0002232* was positively correlated with platelet count. *Circ_0002232*$^{low}$ group tended to have lower platelet count. There already have several reports focused on the abnormal platelet count and dysfunction in AML[25]. Low platelet count was associated with poor prognosis and recovery of platelet was concerned with relapse-free survival rate after chemotherapy in AML[26,27]. Moreover, *circ_0002232*$^{low}$ group also tended to have lower hemoglobin, and higher percentage of blast compared with *circ_0002232* high expression group. This means *circ_0002232*$^{low}$ group have more severe myelosuppression and more serious infiltration in BM. Hence, low expression of *circ_0002232* is an adverse factor of AML.

Unexpectedly, results of Kaplan-Meier analysis revealed that OS of patients with low-expressed *circ_00002232* were longer than that of patients with high-expressed *circ_00002232* in whole AML. *PTEN*, parental gene of *circ_0002232*, plays a role of tumor suppressor in many diseases. At the beginning of our experiment, we proposed that patients with low-expressed *circ_0002232* might have shorter overall survival time, which was obviously contract with current results.

However, our study indicated that patients in *circ_0002232*$^{low}$ group were significantly younger than those in *circ_0002232*$^{high}$ group. In other words, old patients were liable to have high expression of *circ_0002232*. Pearson analysis was used to confirm this result, which revealed that the *circ_0002232* expression was positively correlated with patients' age. Age is an important risk factor for AML. Survival

time of AML patients tends to decrease with increased age[28,29]. We suppose that it may help us to understand this conflicting result. The correlation between age and *circ_0002232* expression led to this reverse result.

Then according to the expression level of *circ_0002232*, we divided the patients (age<40y) into two groups and compared the differences in survival time. The result showed that *circ_0002232*$^{high}$ group tended to have better OS compared with *circ_0002232*$^{low}$ group. This result confirmed our conjecture. But due to the limitation of our experiment size, this result wasn't statistically significant. In the future, additional experiments are needed to enlarge sample size and identify the relationship between *circ_0002232* expression and OS in different age subgroups.

Moreover, the phenomenon of circRNAs acting as miRNA sponges in regulating proliferation, metastasis and relapse of gastrointestinal cancer have been reported in some studies[5]. In this research, prediction websites revealed that there were potential binding sites among *circ_0002232*, *miR-92a-3p*, and *PTEN*. *MiR-92a-3p* expression has been revealed to be up-regulated in several solid cancers including breast cancer and brain glioma[30,31]. According to our experiment, *miR-92a-3p* expression of AML patients was obviously up-regulated compared with controls and was negatively correlated with the expression of *circ_0002232*.

Furthermore, high-expressed *miR-92a* have been found to regulate colorectal cell migration and invasion by reducing the expression level of *PTEN*[32]. Alteration of *miR-92a* also promoted its effect on metastatic behavior of nasopharyngeal carcinoma cell by targeting *PTEN*[33]. Notably, we found that the expression level of *miR-92a-3p* was also negatively correlated with *PTEN* in AML. Hence, we proposed that *circ_0002232* might regulate *PTEN* expression through sponging *miR-92a-3p* and affect the process of AML. In the future, we plan to design more experiments like knock-out and over-expressed experiments to explore the mechanism of this pathway in AML.

# Conclusion

Our experiment revealed *circ_0002232,* one of circRNAs of *PTEN*, was remarkably down-regulated in AML and could act as a promising biomarker for the diagnosis of AML. In addition, there might be a potential ceRNA interaction network of *circ_0002232/miR-92a-3p/PTEN* in AML.

# Abbreviations

PTEN: phosphatase and tensin homolog; circRNAs: circular RNAs; ceRNA: competing endogenous RNAs; AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; CN-AML: normal karyotype AML; RQ-PCR: real-time quantitative PCR; BM: bone marrow; BMNCs: BM mononuclear cells; FAB classification: French-American-British classification; WHO criteria: World Health Organization criteria; ROC: receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval; OS: overall survival; CR: complete remission; WBC: white blood cell.

# Declarations

### Ethics approval and consent to participate

This study was approved by Human Research Ethics Committee of the Affiliated People's Hospital of Jiangsu University. All patients signed informed consents to participate in our research.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

XYS leaded whole process of experiments, analysed data and written manuscript. XZ, QZ, JMK, DLW, YYY, and JY were contributed to collect patients' data and revised the manuscript. JL and JQ were involed in acquiring data and providing useful suggestion during the experiments. ZQD mainly took charge for experiments design and the manuscript revision. All authors read and approved the final manuscript.


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# Figures

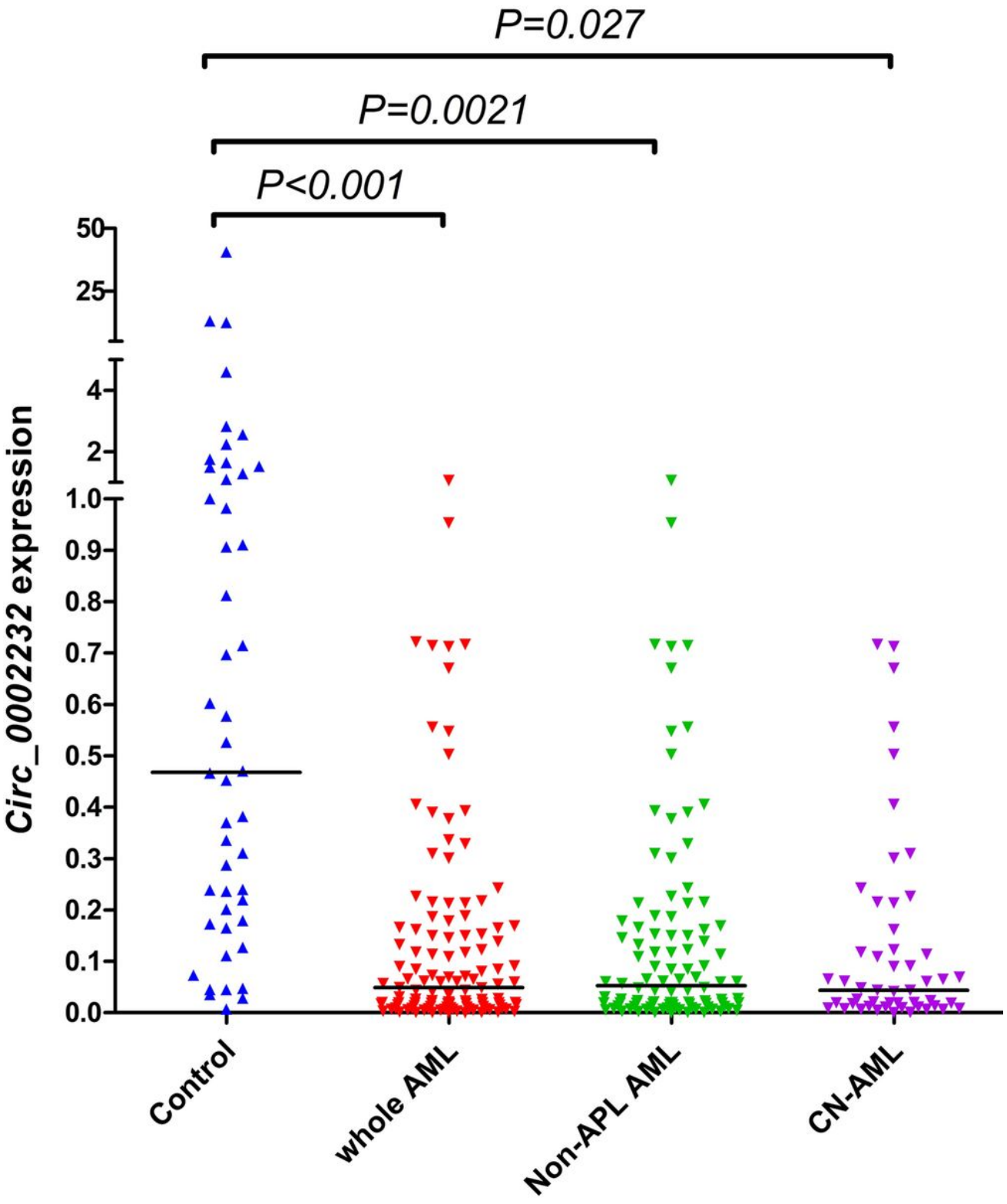



**Figure 1**

Relative expression level of circ_0002232 in controls and AML. The expression level of circ_0002232 in controls, whole AML, non-APL AML and CN-AML patients were measured by using RQ-PCR. Each dot represents a single sample and horizontal line represents the median level of expression.

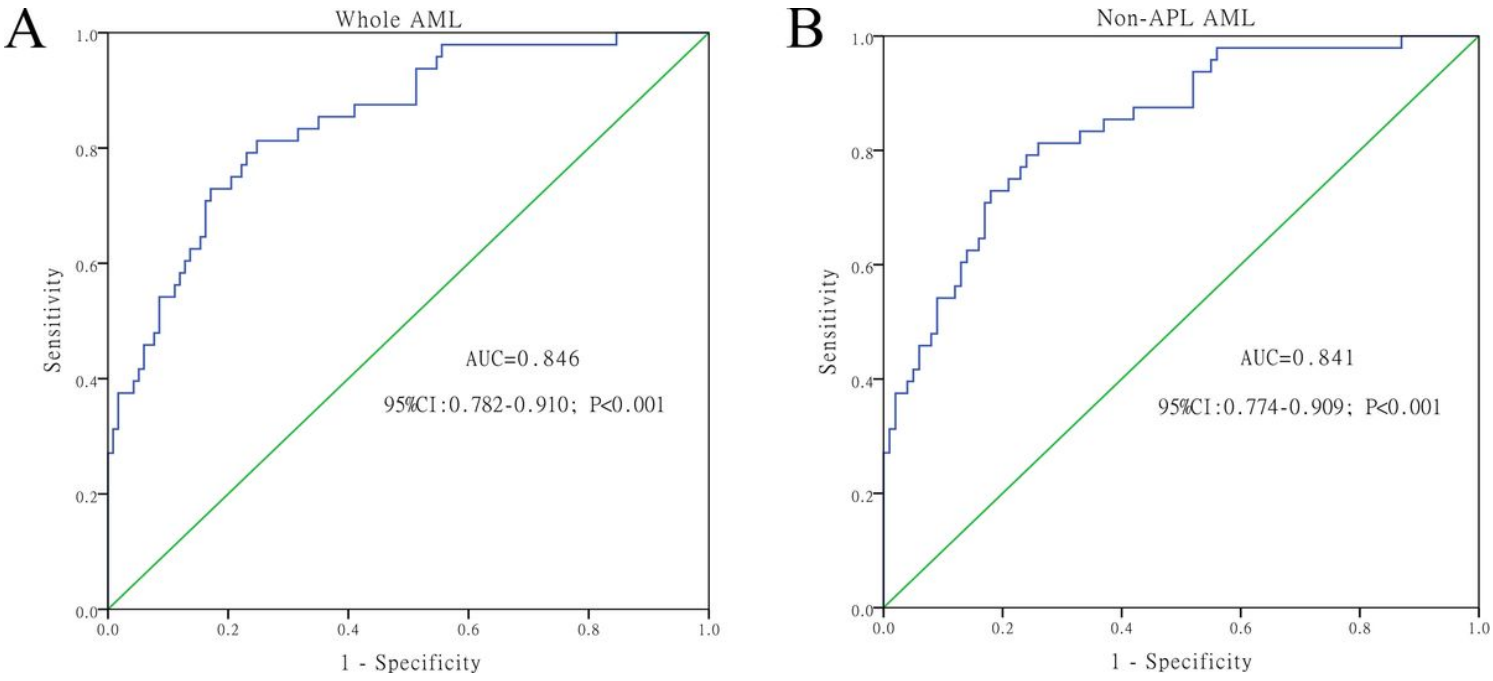



**Figure 2**

ROC curve analysis of circ_0002232 for distinguishing AML patients from controls: A: Whole AML; B: non-APL AML.

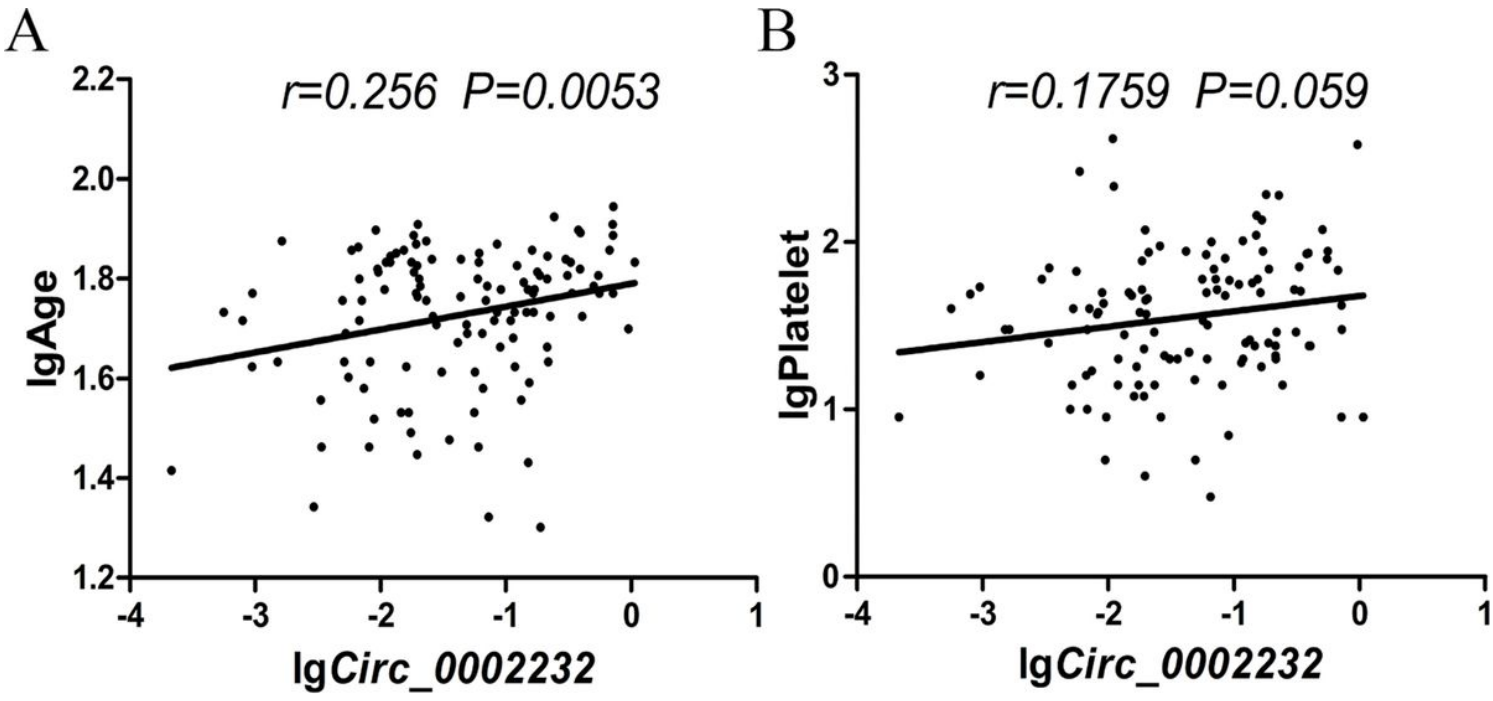
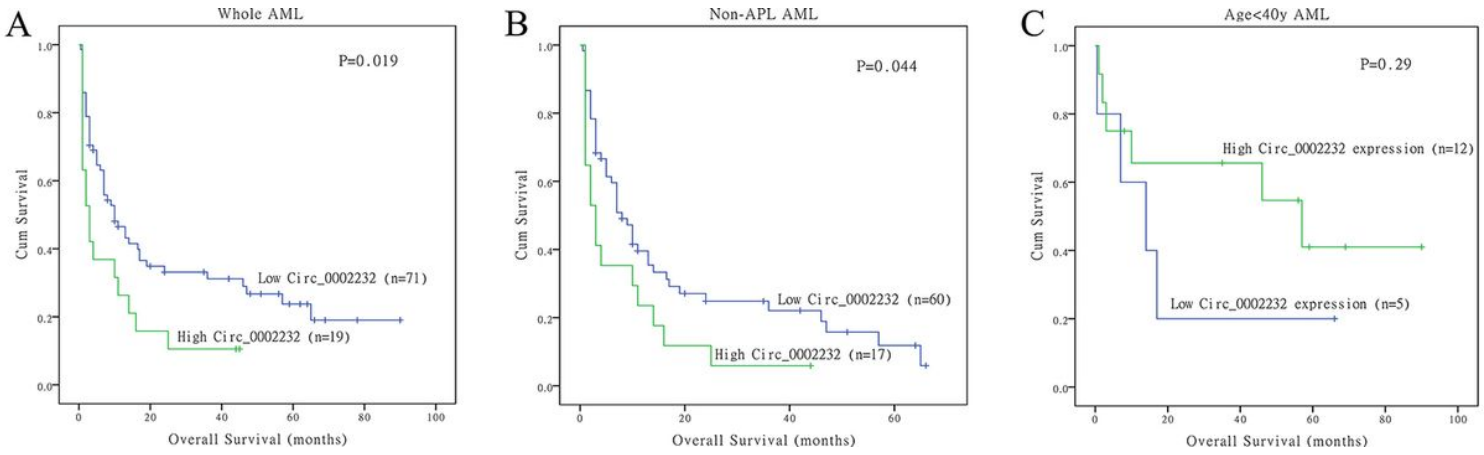


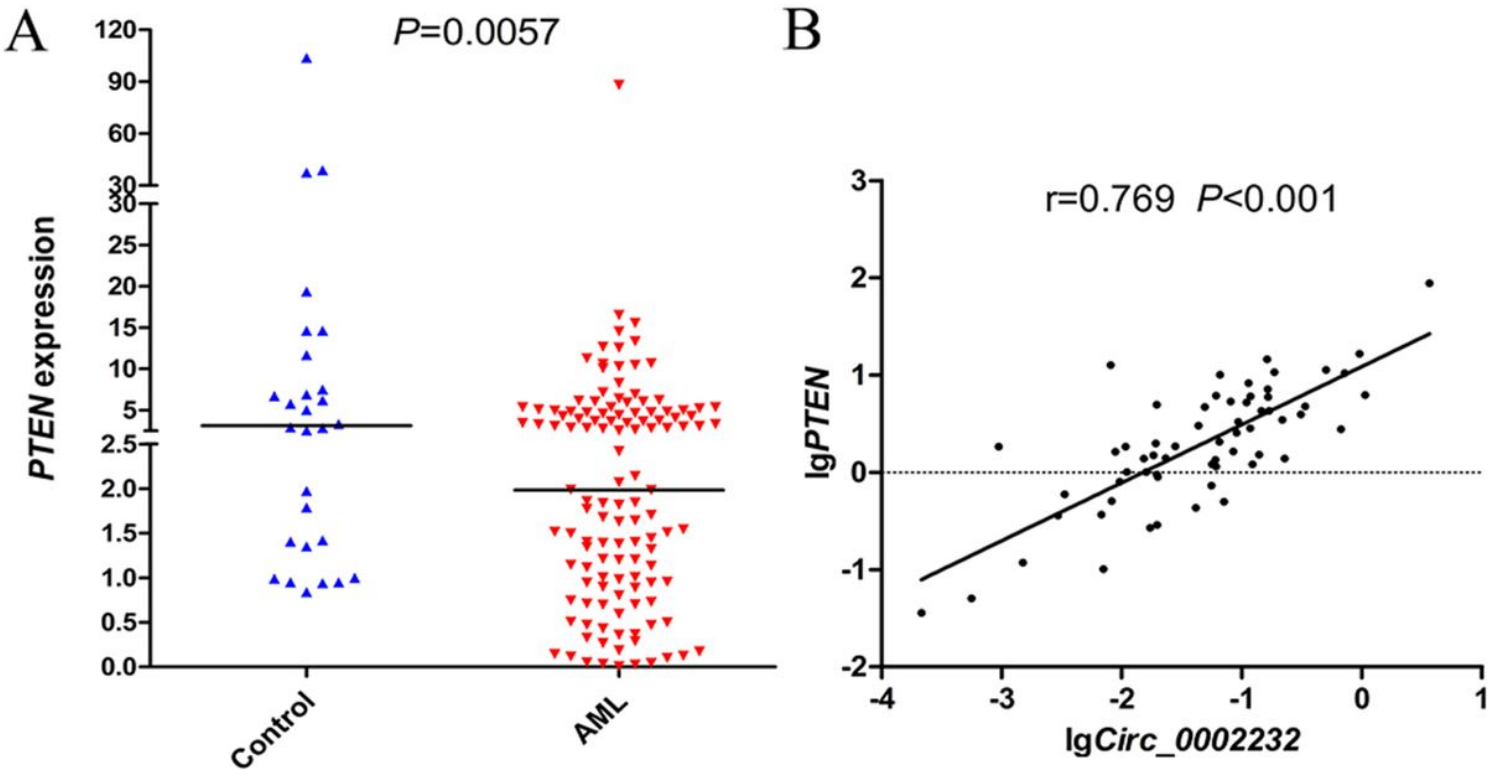

**Figure 3**

Pearson correlation analysis: A: correlationship between patients' age and circ_0002232 expression in AML; B: correlationship between platelet count and circ_0002232 expression in AML.



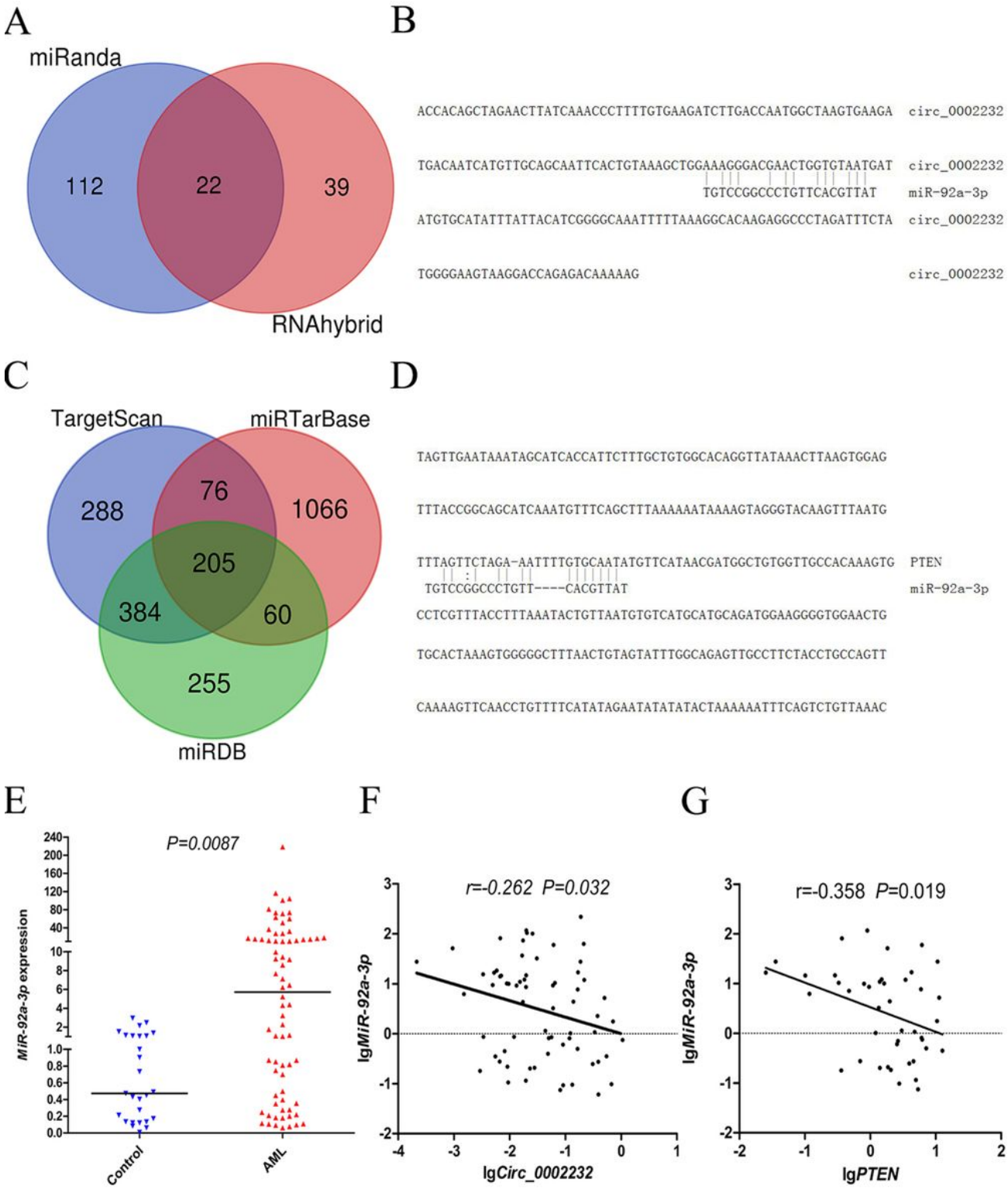

## Figure 4

Kaplan-Meier analysis showed the differences in overall survival between circ_0002232low and circ_0002232high group: A: overall survival among whole AML; B: overall survival among non-APL AML; C: overall survival among AML (age<40y).




## Figure 5

A: Relative expression level of PTEN in controls and whole AML. B: Pearson correlation analysis between the expression of PTEN and circ_0002232 in AML.




## Figure 6

A: Venn results of microRNAs which could bind with circ_0002232 predicted by miRanda and RNAhybrid. B: Binding sites between circ_0002232 and miR-92a-3p predicted by miRanda. C: Venn results of genes which could bind with miR-92a-3p predicted by TargetScan, miRTarBase and miRDB. D: Binding sites between miR-92a-3p and PTEN predicted by miRTarBase. E: Relative expression level of miR-92a-3p in

controls and AML. F: Pearson correlation analysis between the expression of circ_0002232 and miR-92a-3p in AML. G: Pearson correlation analysis between the expression of miR-92a-3p and PTEN in AML.

## Supplementary Files

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