

# MiR-545-3p Upregulated in Osteoporosis and Regulates The Apoptosis of Osteoblasts by Targeting SIRT6

**Qi-Ming Ma**

the Third Affiliated Hospital of Anhui Medical University

**Xiao-Jing Li**

Longhua Branch of Shenzhen People's Hospital

**Shao-Bao Pei**

the Third Affiliated Hospital of Anhui Medical University

**Bo-Wen Li**

the Fourth Affiliated Hospital of China Medical University

**Guo-Song Han**

the Third Affiliated Hospital of Anhui Medical University

**Shuang Cao**

the Third Affiliated Hospital of Anhui Medical University

**Ting Jiang** (✉ [jiangting70@163.com](mailto:jiangting70@163.com))

The First Affiliated Hospital to Anhui University of Chinese Medicine <https://orcid.org/0000-0002-6847-0020>

---

## Research article

**Keywords:** Osteoporosis, Apoptosis, MiR-545-3p, SIRT6

**Posted Date:** September 30th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-81465/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** The imbalance of proliferation and apoptosis plays an important role in the pathogenesis of osteoporosis. MicroRNAs play an important role in the apoptosis of osteoblasts cells. However, the role and potential mechanism of miR-545-3p in regulating osteoblast apoptosis remain unclear. The purpose of this study was to investigate the effect of miR-545-3p on osteoblast cells apoptosis and explore the mechanism of osteoporosis.

**Methods:** Osteogenic medium was used to induce the differentiation of osteoblasts MC3T3-E1 to construct the osteoporosis model, and the expression of ALP, Runx2, OCN were detected by western blot; miR-545-3p mRNA was detected by RT-PCR. Transfected with miR-545-3p mimics into MC3T3-E1, then flow cytometry was used to detected the changes of apoptosis status. The expression of apoptosis related proteins Bcl-2 and Bax were detected by western blot. Bioinformatics was used to analyze the binding protein of miR-545-3p, and luciferase reporter gene experiment was used to verify whether miR-545-3p directly targets SIRT6; RT-qPCR and western blot were used to detect the expression level of SIRT6 after transfection of miR-545-3p mimics or miR-545-3p inhibitor. After co-transfection of miR-545-3p mimics and pcDNA3.1-SIRT6, the apoptosis status of osteoblasts was analyzed by flow cytometry, and the expression of apoptosis related proteins Bcl-2 and Bax were detected by western blot.

**Results:** The expression of miR-545-3p in patients with osteoporosis was significantly higher than that of normal in GEO database ( $P<0.05$ ). After osteoblasts were cultured in osteogenic medium, the expression of ALP, Runx2 and OCN was increased, and the expression of miR-545-3p was decreased. Flow cytometry analysis showed that overexpression of miR-545-3p promoted the apoptosis of osteoblasts. Western blot results showed that overexpression of miR-545-3p promoted the expression of Bax and decreased the expression of Bcl-2. Bioinformatics analysis showed that miR-545-3p could target SIRT6. The results of real-time PCR and western blot showed that SIRT6 expression was significantly inhibited by miR-545-3p mimics ( $P<0.05$ ). Luciferase reporter gene assay showed that miR-545-3p significantly inhibited luciferase activity of wild-type SIRT6-3'UTR plasmid transfected cells ( $P<0.05$ ), but had no effect on luciferase activity of mutant SIRT6-3'UTR plasmid transfected cells ( $P<0.05$ ). However, co-transfection of miR-545-3p mimics and pcDNA3.1-SIRT6 could reduce the apoptosis, and western blot results showed that co-transfection promoted the expression of Bcl-2 and decreased the expression of Bax.

**Conclusions:** miR-545-3p can promote the apoptosis of osteoblasts by inhibiting the expression of SIRT6, which provides a certain idea for the treatment of osteoporosis.

## Introduction

Osteoporosis is a kind of bone degenerative disease characterized by the destruction of the body bone unit structure and accelerated bone loss, which seriously affects people's quality of life and even endangers their lives [1]. It is predicted that by 2050, the number of osteoporotic fracture patients in China

will reach to 5.99 million [2]. Bone fragility and fracture susceptibility in patients with osteoporosis are associated with mortality and poor quality of life [3].

Although various drugs have been developed to inhibit bone resorption and increase bone formation, their side effects limit their long-term use [4]. The cost of treatment of senile osteoporotic fracture will become a heavy burden of social medical treatment, and the prevention and treatment of senile osteoporosis has important medical and economic significance. Therefore, it is necessary to further study the role of molecular and cellular factors.

According to the characteristics of bone metabolism in senile osteoporosis, it is important to improve the differentiation and activity of osteoblasts to enhance the synthesis of bone matrix [5]. In recent years, miRNAs have been reported as a key posttranscriptional regulator involved in osteoblast induced bone formation and osteoclast mediated bone remodeling [6]. They regulate the expression of multiple genes involved in many biological processes, such as osteoblast cell growth, differentiation, apoptosis and tumorigenesis by binding specifically to the 3' untranslated region of target mRNAs [7]. And reported shows that functional characterization of genetic factors involved in the pathogenesis of osteoporosis may provide new ideas for the development of targeted therapy [8]. The increased miR-194-5p in the mesenchymal stem cells (MSCs) of ovariectomized (OVX) mice significantly suppressed bone formation by target Wnt family member 5a (Wnt5a) [9]. Mir300 negatively regulates the differentiation of osteoblasts by targeting the crosstalk between Smad3,  $\beta$ -Catenin and Runx2 [10]. Similarly, Li et al. indicated that miR-545-3p downregulated in osteogenesis and played an inhibitory role in osteogenic differentiation [11].

Above all, osteoblasts apoptosis inhibited the process of differentiation, which revealing its great ability as a therapeutic target for bone related diseases. However, there are few reports on the mechanism of miR-545-3p regulating osteoblast apoptosis. In this study, we aim to establish the osteogenic differentiation model of osteoblast MC3T3-E1 cells in vitro and to explore the exact role of miR-545-3p on apoptosis in the process of osteogenesis.

## Methods

### Chemicals and materials

MC3T3-E1 obtained from Chinese Academy of Sciences (Shanghai, China). The miR-545-3p mimics and miR-545-3p inhibitor were purchased from Genepharma (Shanghai, China), pcDNA3.1-SIRT6 was constructed by Genepharma (Shanghai, China), Lipofectamine 3000 transfection reagent was obtained from Genepharma (Shanghai, China). Apoptosis assay kit was obtained from Thermo Fisher (Shanghai, China). Alkaline phosphatase (ALP), Run family transcription factor 2 (Runx2) and osteocalcin (OCN),  $\beta$ -actin and SIRT6 antibodies were purchased from Abcam (Cambridge, UK).

### GEO analysis and R-based open-source software

We obtained clinical data from GSE103473 with 192 miRNAs in 12 mutation-positive (median age 39 years, range 11-76 years) and 12 mutation-negative (35 years, range 9-59 years) of osteoporosis patient or normal. Gene differential expression was performed by the limma package which is a core component of Bioconductor, an R-based open-source software. 'ggplot2' package was used to achieve the visualization of above analysis.

## Cell culture

MC3T3-E1 cultured with growth medium (GM) prepared by modified Eagle medium (MEM) (Gibco® Invitrogen, Grand Island, NY), 10% fetal bovine serum (FBS) (Hyclone; USA) and 1% Penicillin-Streptomycin Solution (Gibco) (ratio 9:1:1). The growth medium was cultured in incubator with constant temperature of 37°C and 5% CO<sub>2</sub> saturation humidity. The medium was changed every 2 or 3 days.

## Osteogenic induction

In order to induce osteoblasts, 10 mmol/L sodium β-glycerophosphate, 50 mg/ L vitamin C and 10 nmol/L dexamethasone were mixed as osteogenic medium (OM) and added into the culture medium for osteogenic differentiation.

## Cell transfection

The miR-545-3p mimics and miR-545-3p inhibitor were purchased from Genepharma (Shanghai, China). pcDNA3.1-SIRT6 was constructed by Genepharma (Shanghai, China), pcDNA3.1 blank vector was used as negative control (NC). MC3T3-E1 were seeded in 6-well plates at the rate of  $5 \times 10^5$ /well. When the cells reached 70% fusion, the cells were transfected by Lipofectamine 3000 (Invitrogen company, USA) with miR-545-3p mimics or miR-545-3p inhibitor or the corresponding negative control (transfected with empty plasmid). The culture medium was changed after 6 hours, and the cells were harvested for real-time qPCR detection after 48 hours, and western blotting was used after 72 hours.

## Quantitative real-time PCR

All of miRNA expression was detected by qRT-PCR analysis. Trizol reagent (Invitrogen, USA) was used to lyse cells for the extraction of total RNA. 2 μg total RNA was performed by MicroRNA kit (Invitrogen, Shanghai) for reversing transcribed. 20 μl final reaction mixture was used for PCR amplifications by using the SYBR Green PCR Master mix (Thermo Fisher, Shanghai). The expression of results was standardized by β-actin. Factors of expression level of RNA was calculated by the  $2^{-\Delta\Delta Ct}$  value. Primer sequence are follows:

miR-545-3p: forward 5'- TGCGCTCAGCAAACATTTATTG-3',

reverse 5'- CCAGTGCAGGGTCCGAGGTATT-3';

β-actin: forward 5'-AGCGAGCATCCCCAAAGTT-3',

reverse 5'-GGGCACGAAGGCTCATCATT-3'

### **Apoptosis assay**

Performing 48 hours transfection, the cells were obtained, and then, these cells were washed for three times by PBS. Then, 100  $\mu$ L buffer was used to suspend cells, and 5  $\mu$ L 1X Annexin-V-FITC and 5  $\mu$ L 1X PI were added in the suspension about 15 min and keep darkness. And then flow cytometer was performed to evaluate the cell apoptosis of each group and the assays was performed for more than three times.

### **Western blot assay**

The cells were prepared by RIPA (Beytime, Shanghai) after collected at 4°C for 2 hours. 10% SDS-PAGE gels were used for protein extracts and then proteins were moved onto PVDF membrane (Thermo Fisher, Beijing). And then blocking in 5% BSA for 2 hours at 37°C, all the membranes were incubated with the ALP, Runx2, OCN, SIRT6 (1:1000, Abcam) or  $\beta$ -actin (1:1000, Abcam) primary antibodies overnight at 4°C. Then after the primary antibody incubation, it next incubates with horseradish peroxidase (HRP). The grey value of these proteins was measured by using Image J Software.

### **Luciferase reporter assay.**

The 3'UTR specific targeting site of SIRT6 for miR-545-3p and by polymerase chain reaction (PCR), we amplified mutant sequences of a specific site and then the sequences were inserted into the pre-miR-RB-REPORT (Thermo Fisher, Beijing). After 24h transfection, we used the Luciferase assay system (Thermo Fisher Scientific, Beijing) performing the assay and these results with the Renilla luciferase were normalized.

### **Statistically analysis**

All data analyzed by using the SPSS 22.0 (SPSS, Chicago, IL), and are presented as the mean  $\pm$  standard deviation (SD). \* $P$ <0.05 considered statistical significance.

## **Results**

### **Comparative analysis of differences of GEO database**

To analyze the differentially expressed miRNAs in patients with osteoporosis, we performed a bioinformatics analysis of osteoporosis patient and normal in the GSE103473 data sets. The results were evaluated using Differential expression analysis, Among the differentially expressed miRNAs in GSE103473, 100 were upregulated and 87 were downregulated in osteoporosis patient compared with normal. Among them, 10 genes were significantly increased (Fig. 1A). The expression of miR-545-3p was significantly different between osteoporosis patients and normal controls (Fig. 1B)

### **Expression level of miR-545-3p in MC3T3-E1 cells by osteogenic induction**

In order to confirm the expression of miR-545-3p in the process of osteogenic differentiation, we used osteogenic medium (OM) to detect the osteogenic differentiation marker proteins alkaline phosphatase (ALP), Run family transcription factor 2 (Runx2) and osteocalcin (OCN) levels (Fig.1A). And it was found that the expression of miR-545-3p decreased with the increase of ALP, Runx2 and OCN expression in OM group (Fig.1B).

### **Upregulation of miR-545-3p inducing osteoblast cell apoptosis**

To explore the role of miR-545-3p in osteoblasts, we transfected miR-545-3p mimics or miR-545-3p inhibitor into osteoblasts to up-regulate or down-regulate the expression of miR-545-3p. RT-qPCR was used to detect the transfection efficiency, the result shows the expression of miR-545-3p in control group and overexpressed group was 1:19.37 (Fig. 2A), and the expression of control group and miR-545-3p inhibitor group was 1:0.26 (Fig. 2B). Next, we detected the expression of osteoblast differentiation markers after transfected miR-545-3p mimics or miR-545-3p inhibitor. After transfection of miR-545-3p mimics, the expression of ALP, Runx2 and OCN decreased significantly, while the expression of ALP, Runx2 and OCN increased after transfection with miR-545-3p inhibitor, which indicated that miR-545-3p had a certain inhibitory effect on osteoblast differentiation (Fig. 2C). We know that apoptosis is one of the important markers to prevent the differentiation of osteoblasts. Therefore, we examined the effect of miR-545-3p on osteoblast apoptosis. Apoptosis was detected by flow cytometry. The results showed that the apoptotic index of normal osteoblasts was (2.35±1.52%), while miR-545-3p inhibitor group was (0.75±1.26%), miR-545-3p mimics group was (12.64±1.33%), which indicated that overexpression of miR-545-3p could promote the apoptosis of osteoblasts (Fig. 2D). To further confirm this result, we then detected two groups of apoptosis related proteins Bcl-2 and Bax by western blot. The results show that the trend is consistent with the flow cytometry results (Fig. 2E).

### **Upregulation of miR-545-3p limit SIRT6 expression**

In order to explore how miR-545-3p regulates osteoblast apoptosis, we used bioinformatics database and found that miR-545-3p has multiple binding sites with SIRT6 (Fig. 3A). To further verify the regulation of miR-545-3p on SIRT6 expression, we detected SIRT6 expression after overexpression or knockdown of miR-545-3p. The results showed that SIRT6 mRNA expression was decreased or increased by overexpression or knockdown compared with control group (Fig. 3B). Then, Western blot was used to detect the expression of SIRT6 protein after overexpression of miR-545-3p, and the results were consistent (Fig. 3C). After that, luciferase reporter gene experiment was used to verify whether miR-545-3p directly targets SIRT6. The results showed that miR-545-3p significantly inhibited the luciferase activity of wild-type SIRT6-3'UTR plasmid transfected cells ( $P<0.05$ ), but had no effect on the luciferase activity of mutant SIRT6-3'UTR plasmid transfected cells ( $P<0.05$ ). Therefore, SIRT6 significantly inhibited the luciferase activity of wild-type SIRT6-3'UTR plasmid transfected cells, but had no effect on the luciferase activity of mutant SIRT6-3'UTR plasmid transfected cells (Fig. 3D). Therefore, combined with informatics and experimental science, we speculate that SIRT6 is a direct target of miR-545-3p.

### **MiR-545-3p regulates osteoblast apoptosis by targeting SIRT6**

To further explore whether miR-545-3p regulates osteoblast apoptosis by inhibiting SIRT6. We used osteoblast cells to transfected miR-545-3p mimics and miR-545-3p mimics+pcDNA3.1-SIRT6 respectively. RT-qPCR was used to detected the transfection efficiency, the result shows the expression of pcDNA3.1 and pcDNA3.1-SIRT6 was 1:6.14 (Fig. 4A). And western blot used to detect the expression of SIRT6 (Fig. 4B). Then, we measured the apoptosis index by flow cytometry, the results showed that the apoptotic index of normal osteoblasts was ( $2.13\pm 1.18\%$ ), while miR-545-3p mimics group was ( $7.16\pm 1.34\%$ ), miR-545-3p mimics+pcDNA3.1-SIRT6 group was ( $2.66\pm 1.57\%$ ), which indicated that SIRT6 overexpression could reduce the apoptosis induced by miR-545-3p (Fig. 4C). To further confirm this result, we detected the expression of Bcl-2 and Bax by Western blot, which was consistent with the results of flow cytometry (Fig. 4D).

## Discussion

In this study, we first revealed that miR-545-3p regulates osteoblast apoptosis by regulating silent mating type information regulation 2 homolog 6 (SIRT6). Sirtuins family play an important role in the process of osteoblasts differentiation. Such as peroxisome proliferator-activated receptor (PPAR)  $\gamma$  promotes osteogenic differentiation in a SIRT1-dependent manner [12]. SIRT6 is a member of Sirtuins family in mammalian, which located in the nucleus and play an important role in many aspects of cell activity such as transcriptional silencing, apoptosis regulation, fat mobilization and life span regulation [13].

SIRT6 regulated osteoclast mainly through osteoblast paracrine manner and its inhibition may enhances osteoclast activation regulated by the hyperactive NF- $\kappa$ B signaling [14]. And the loss of SIRT6 in osteoblasts activates osteoclasts, which leads to osteopenia [15].

Zhao et al. reported that miR-128 inhibits osteoblast differentiation in osteoporosis by down regulating SIRT6 expression, thus accelerating the development of osteoporosis [16]. Osteogenic differentiation of rat bone mesenchymal stem cells (BMSCs) can be interfered by miR-186 target SIRT6. Toshifumi et al. indicated that SIRT6 interacts with Runx2 and osterix (Osx), which are the two key transcriptional regulators of osteoblastogenesis, and SIRT6 deficiency leads to hyperacetylation of dickkopf-related protein 1 (DKK1) promoter H3K9, which is a powerful negative regulator of osteoblast formation [17]. The apoptosis of osteoblasts induced by hypoxia is dependent on glycolytic activity. And SIRT6 is a negative regulator of inflammation can reduce periapical lesions by inhibiting glycolysis and apoptosis of osteoblasts [18]. Similarly, SIRT6 has a therapeutic effect on periapical lesions through suppression of chemokine (C-C motif) ligand 2 (CCL2) synthesis [19]. However, Xiao et al. indicated that SIRT6 have an inhibitory effect on osteogenic differentiation of BMSCs [20]. In our study, we found overexpression of SIRT6 attenuates the inhibitory effect of miR-545-3p on osteoblasts. This suggests that more comprehensive and in-depth studies are needed.

Our study established a new mechanism of osteoblast differentiation in miR-545-3p. Circulating miR-545-3p inhibits the expression of SIRT6, thus promoting osteoblast apoptosis and inducing osteoporosis.

Therefore, the therapeutic intervention targeting miR-545-3p has a great prospect in the treatment of osteoporosis.

## **Abbreviations**

MSCs

Mesenchymal stem cells; Wnt5a:Wnt family member 5a; GM:Growth medium; OM:Osteogenic medium; ALP:Alkaline phosphatase; Runx2:Run family transcription factor 2; OCN:osteocalcin; SIRT6:Silent mating type information regulation 2 homolog 6.

## **Declarations**

### **Acknowledgements**

Not applicable.

### **Authors' contributions**

Qi-Ming Ma and Xiao-Jing Li design the study and make a manuscript; Shao-Bao Pei and Bo-Wen Li performs the experiment study; Guo-Song Han is responsible for bioinformatics data; Shuang Cao is responsible for data collation; Ting Jiang supervise the experiment. All authors prove the final study.

### **Funding**

No funding was received for the implementation of this study.

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

We declare that the materials described in the manuscript will be freely available to all scientists for non-commercial purposes

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

Not applicable.

### **Consent for publication**

Not applicable.

### **Competing interests**

All authors declare that they have no conflict of interest.

### **Author details**

<sup>1</sup> Department of Orthopedics and Traumatology, the First Affiliated Hospital to Anhui University of Chinese Medicine, 117 Meishan Road, Hefei 230031, Anhui Province, China.

<sup>2</sup> Department of Spinal Surgery, the Third Affiliated Hospital of Anhui Medical University (the First People's Hospital of Hefei City), 390 Huaihe Road, Hefei 230000, Anhui Province, China.

<sup>3</sup> Department of Orthopedics, Longhua Branch of Shenzhen People's Hospital, 101 Longguan East Road, Longhua District, Shenzhen 518109, Guangdong Province, China.

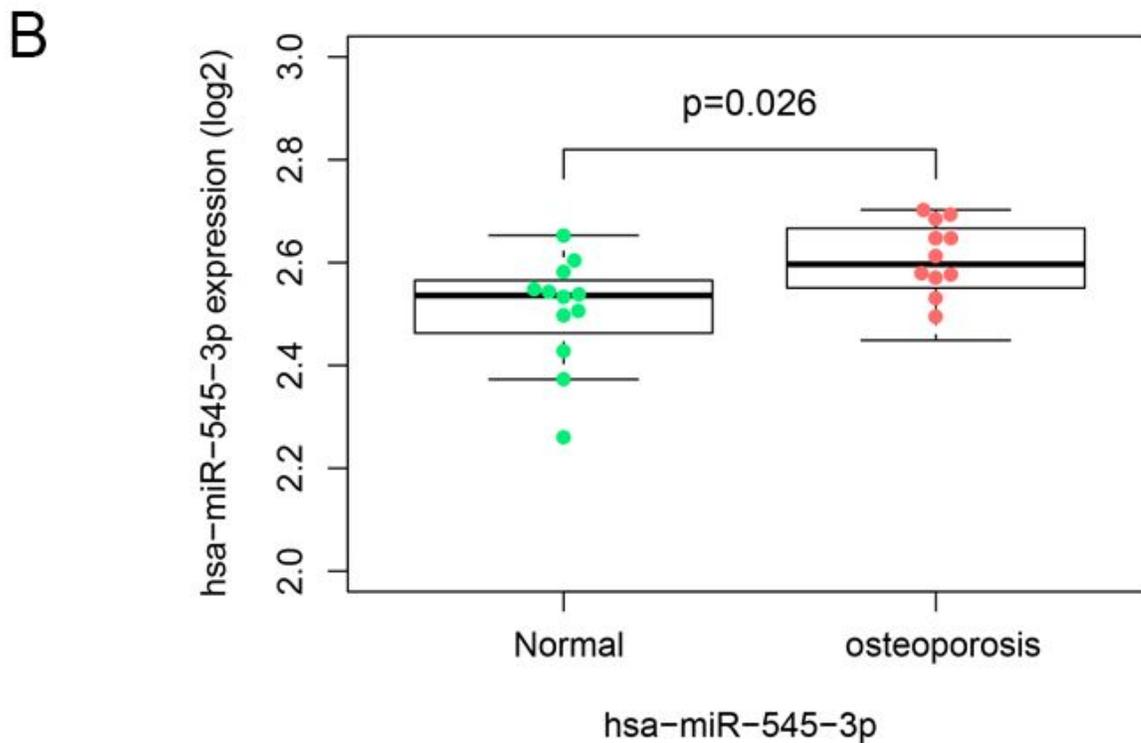
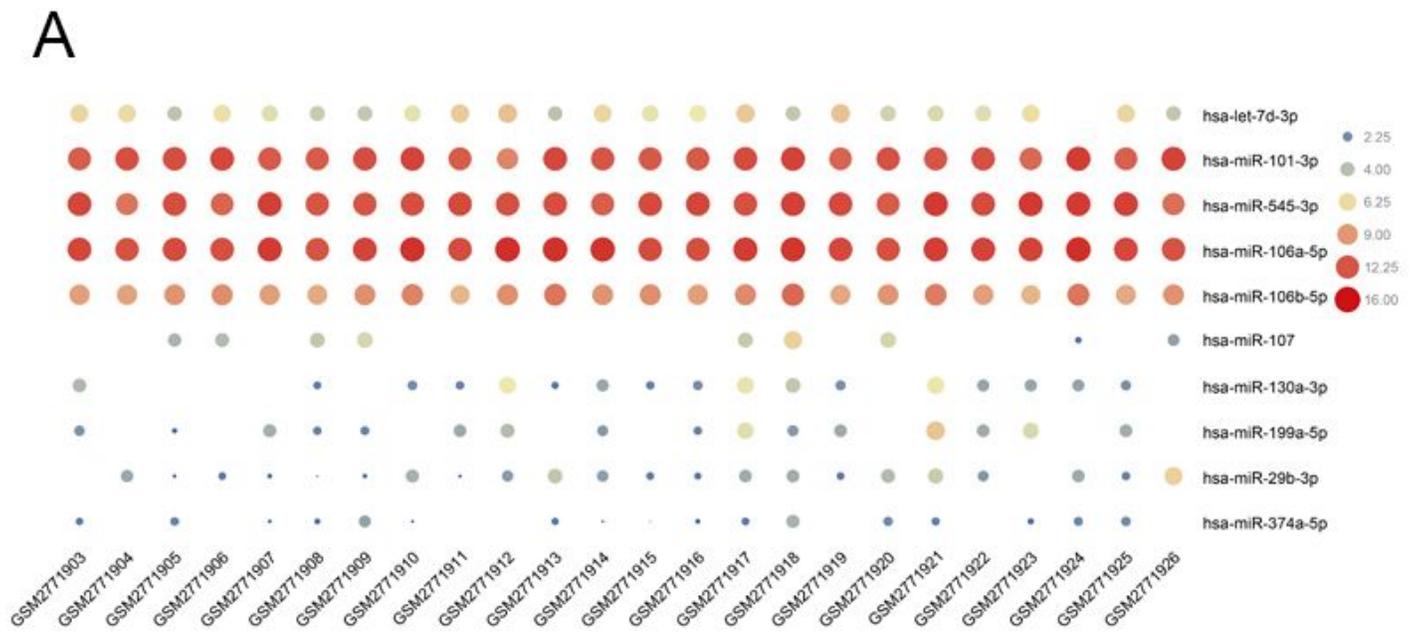
<sup>4</sup> Department of General Surgery, the Fourth affiliated Hospital of China Medical University, 4 Chongshan East Road, Huanggu District, Shenyang 110032, Liaoning Province, China.

## References

1. Imerb N, Thonusin C, Chattipakorn N, Chattipakorn SC: **Ageing, obese-insulin resistance, and bone remodeling.** *Mech Ageing Dev* 2020, **191**:111335.
2. Si L, Winzenberg TM, Jiang Q, Chen M, Palmer AJ: **Projection of osteoporosis-related fractures and costs in China: 2010-2050.** *Osteoporos Int* 2015, **26**(7):1929-1937.
3. Fuggle NR, Curtis EM, Ward KA, Harvey NC, Dennison EM, Cooper C: **Fracture prediction, imaging and screening in osteoporosis.** *Nat Rev Endocrinol* 2019, **15**(9):535-547.
4. Ishtiaq S, Fogelman I, Hampson G: **Treatment of post-menopausal osteoporosis: beyond bisphosphonates.** *J Endocrinol Invest* 2015, **38**(1):13-29.
5. Wang S, Deng Z, Ma Y, Jin J, Qi F, Li S, Liu C, Lyu FJ, Zheng Q: **The Role of Autophagy and Mitophagy in Bone Metabolic Disorders.** *Int J Biol Sci* 2020, **16**(14):2675-2691.
6. Hao L, Li J, Tian Y, Wu J: **Changes in the MicroRNA Profile of the Mandible of Ovariectomized Mice.** *Cell Physiol Biochem* 2016, **38**(4):1267-1287.
7. Chen Z, Wu Y, Kong J, Li Y, Wang H, Zhao H, Wu Z: **Insertion/deletion polymorphism in the 3' untranslated region of COL1A2 disrupts its interaction with microRNA-382 and leads to decreased susceptibility to osteoporotic fracture.** *J Cell Biochem* 2019, **120**(8):12402-12411.
8. Yang Y, Yujiao W, Fang W, Linhui Y, Ziqi G, Zhichen W, Zirui W, Shengwang W: **The roles of miRNA, lncRNA and circRNA in the development of osteoporosis.** *Biol Res* 2020, **53**(1):40.
9. Mi B, Yan C, Xue H, Chen L, Panayi AC, Hu L, Hu Y, Cao F, Sun Y, Zhou W *et al*: **Inhibition of Circulating miR-194-5p Reverses Osteoporosis through Wnt5a/beta-Catenin-Dependent Induction of Osteogenic Differentiation.** *Mol Ther Nucleic Acids* 2020, **21**:814-823.
10. Kaur T, John AA, Sharma C, Vashisht NK, Singh D, Kapila R, Kapila S: **miR300 intervenes Smad3/beta-catenin/RunX2 crosstalk for therapy with an alternate function as indicative biomarker in osteoporosis.** *Bone* 2020:115603.
11. Li L, Qiu X, Sun Y, Zhang N, Wang L: **SP1-stimulated miR-545-3p inhibits osteogenesis via targeting LRP5-activated Wnt/beta-catenin signaling.** *Biochem Biophys Res Commun* 2019, **517**(1):103-110.

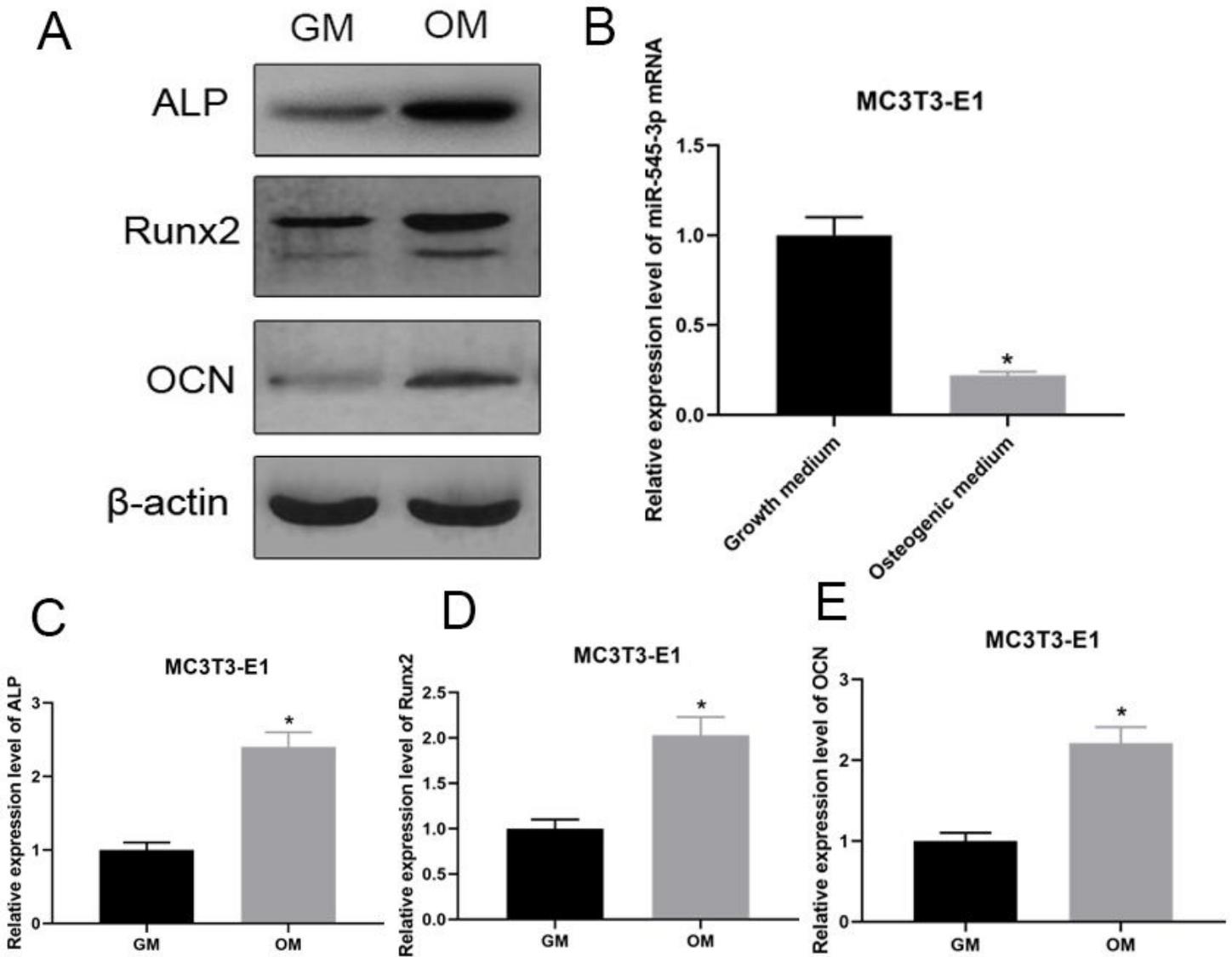
12. Gong K, Qu B, Wang C, Zhou J, Liao D, Zheng W, Pan X: **Peroxisome Proliferator-Activated Receptor alpha Facilitates Osteogenic Differentiation in MC3T3-E1 Cells via the Sirtuin 1-Dependent Signaling Pathway.** *Mol Cells* 2017, **40**(6):393-400.
13. Song L, Chen X, Mi L, Liu C, Zhu S, Yang T, Luo X, Zhang Q, Lu H, Liang X: **Icariin-induced inhibition of SIRT6/NF-kappaB triggers redox mediated apoptosis and enhances anti-tumor immunity in TNBC.** *Cancer Sci* 2020.
14. Zhang D, Jing J, Lou F, Li R, Ping Y, Yu F, Wu F, Yang X, Xu R, Li F *et al.*: **Evidence for excessive osteoclast activation in SIRT6 null mice.** *Sci Rep* 2018, **8**(1):10992.
15. Kim SJ, Piao Y, Lee MG, Han AR, Kim K, Hwang CJ, Seo JT, Moon SJ: **Loss of Sirtuin 6 in osteoblast lineage cells activates osteoclasts, resulting in osteopenia.** *Bone* 2020, **138**:115497.
16. Zhao J, Liu S, Zhang W, Ni L, Hu Z, Sheng Z, Yin B: **MiR-128 inhibits the osteogenic differentiation in osteoporosis by down-regulating SIRT6 expression.** *Biosci Rep* 2019, **39**(9).
17. Sugatani T, Agapova O, Malluche HH, Hruska KA: **SIRT6 deficiency culminates in low-turnover osteopenia.** *Bone* 2015, **81**:168-177.
18. Kok SH, Hou KL, Hong CY, Chao LH, Hsiang-Hua Lai E, Wang HW, Yang H, Shun CT, Wang JS, Lin SK: **Sirtuin 6 Modulates Hypoxia-induced Apoptosis in Osteoblasts via Inhibition of Glycolysis: Implication for Pathogenesis of Periapical Lesions.** *J Endod* 2015, **41**(10):1631-1637.
19. Lee YL, Lin SK, Hou KL, Kok SH, Lai EH, Wang HW, Chang JZ, Yang H, Hong CY: **Sirtuin 6 attenuates periapical lesion propagation by modulating hypoxia-induced chemokine (C-C motif) ligand 2 production in osteoblasts.** *Int Endod J* 2018, **51** Suppl 2:e74-e86.
20. Xiao J, Qin S, Li W, Yao L, Huang P, Liao J, Liu J, Li S: **Osteogenic differentiation of rat bone mesenchymal stem cells modulated by MiR-186 via SIRT6.** *Life Sci* 2020, **253**:117660.

## Figures



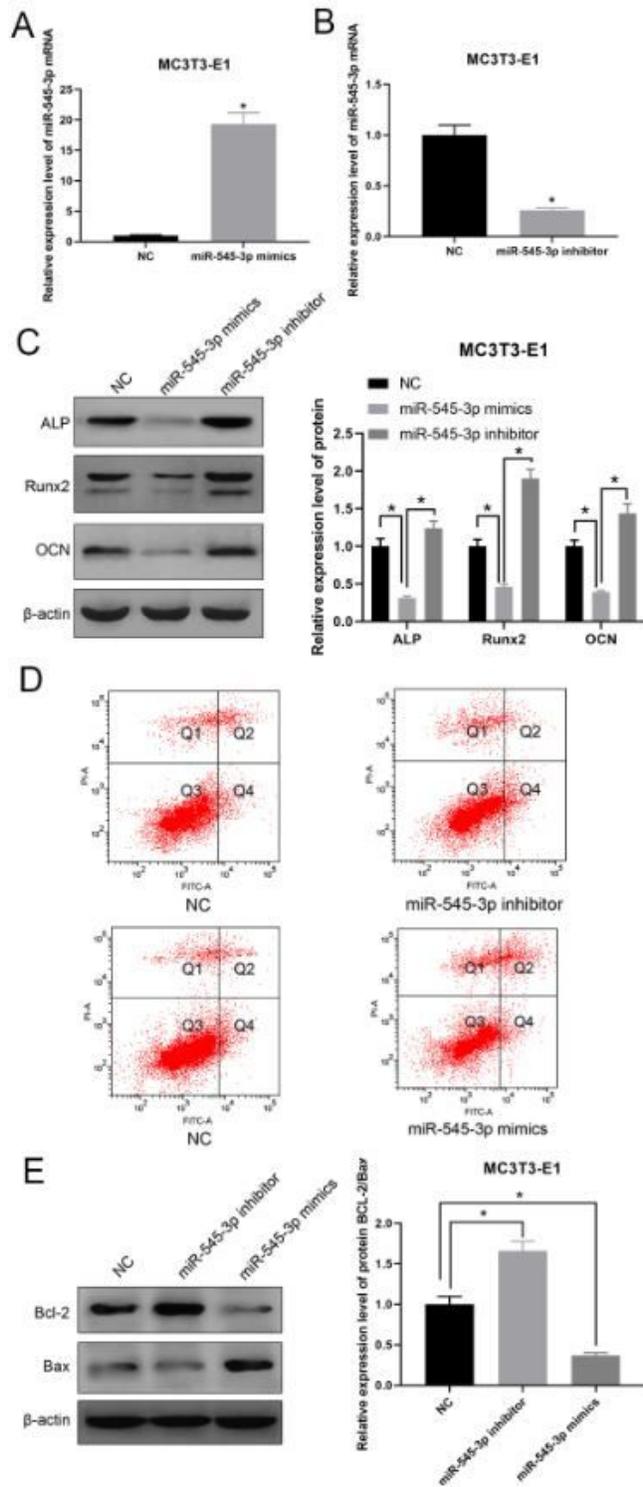
**Figure 1**

a Differential expression analysis of GSE103473 data sets. b The expression of miR-545-3p was significantly different between osteoporosis patients and normal controls.



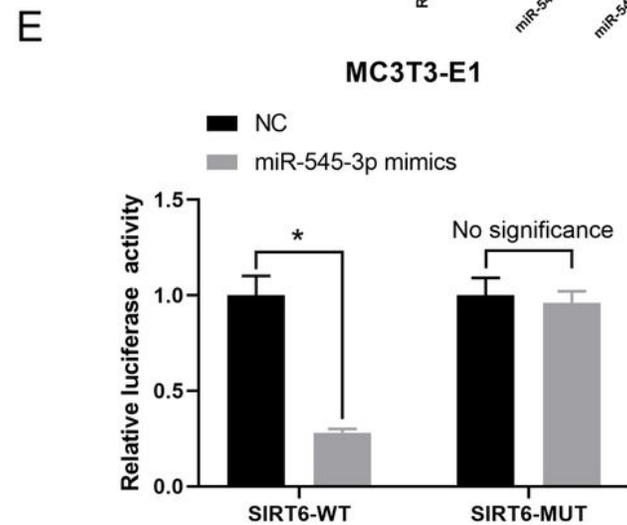
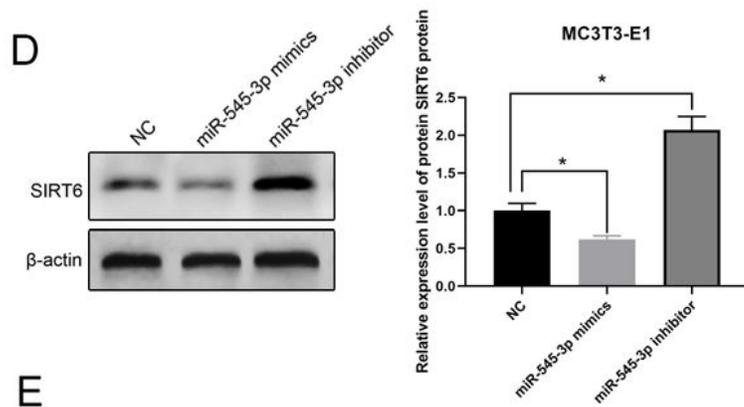
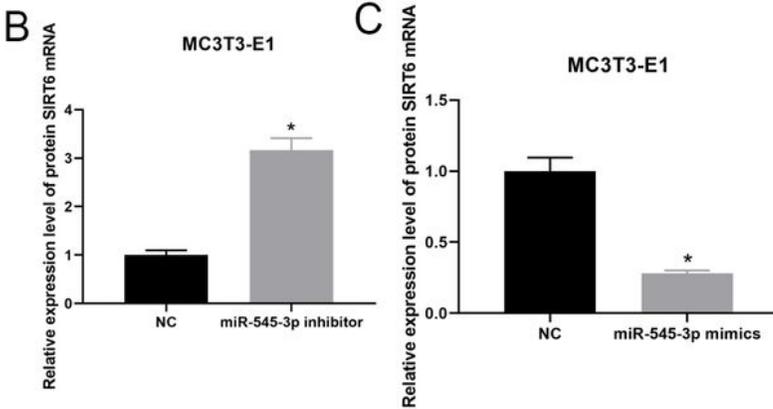
**Figure 2**

a The expression level of proteins ALP, Runx2 and OCN under osteogenic medium (OM) culture or growth medium (GM). b The expression level of miR-545-3p mRNA under osteogenic medium (OM) culture or growth medium (GM).



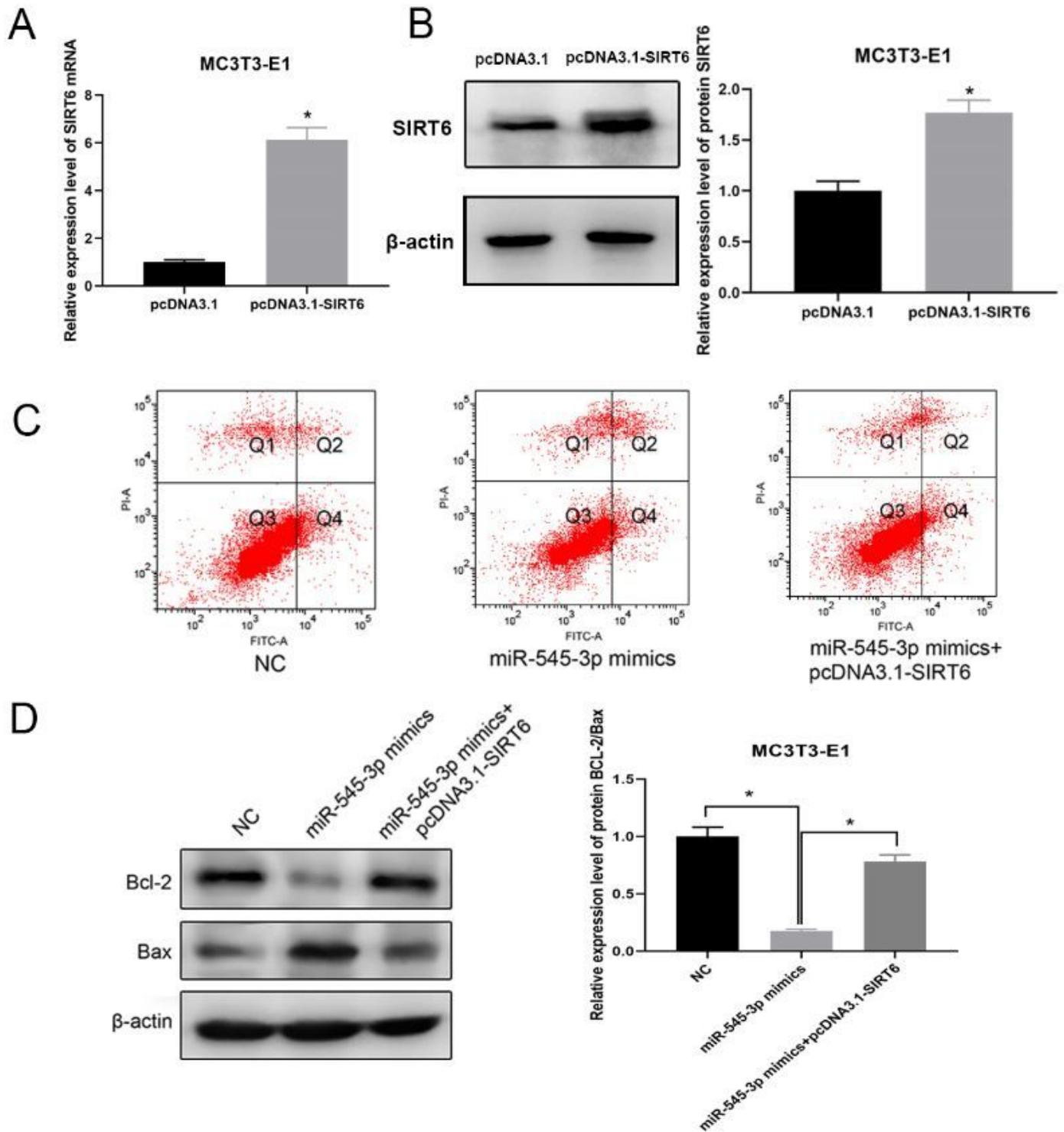
**Figure 3**

a The expression level of miR-545-3p mRNA under transfected miR-545-3p mimics. b The expression level of miR-545-3p mRNA under transfected miR-545-3p inhibitor. c The expression level of proteins ALP, Runx2 and OCN under transfected miR-545-3p mimics or miR-545-3p inhibitor. d The apoptotic index of osteoblasts under transfected miR-545-3p mimics or miR-545-3p inhibitor. e The expression level of proteins Bcl-2 and Bax under transfected miR-545-3p mimics or miR-545-3p inhibitor.



**Figure 4**

a Target gene prediction of miR-545-3p by Targetscan. b The expression level of SIRT6 mRNA under transfected miR-545-3p inhibitor. c The expression level of SIRT6 mRNA under transfected miR-545-3p mimics. d The expression level of protein SIRT6 under transfected miR-545-3p mimics or miR-545-3p inhibitor. e Detection of SIRT1 binding to miR-545-3p by luciferase reporter gene assay.



**Figure 5**

a The expression level of SIRT6 mRNA under transfected pcDNA3.1-SIRT6. b The expression level of protein SIRT6 under transfected pcDNA3.1-SIRT6. c The apoptotic index of osteoblasts under transfected miR-545-3p mimics or miR-545-3p mimics+pcDNA3.1-SIRT6. d The expression level of proteins Bcl-2 and Bax under transfected miR-545-3p mimics or miR-545-3p mimics+pcDNA3.1-SIRT6.