Lenti-shRNA-mediated FAM54A knockdown suppresses the proliferation and induces apoptosis of human Burkitt lymphoma cells

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Research article

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Abstract

Background Burkitt lymphoma is a kind of non-Hodgkin B-cell-derived malignancy, derived from germinal center B cells. FAM54A has been proved to be involved in various physiological and pathological processes of cancers, but the biological function of FAM54A in Burkitt lymphoma remains unclear. Thus, the aim of our research was to elucidate the roles of FAM54A in proliferation, apoptosis and cell cycle of Burkitt lymphoma.

Methods Burkitt lymphoma cell line (Namalwa) was chosen to perform the following experiments. FAM54A-shRNA and negative control-shRNA lentivirus that were synthesized, respectively by Qiagen were used to transfect targeted cells in order to knockdown FAM54A or as negative control. Then, cell proliferation, cell cycle and cell apoptosis were detected by using MTS assay, propidium iodide staining and Annexin V-APC staining, respectively.

Results Our results showed that high expression of FAM54A protein was found in Namalwa cell line. Furthermore, MTS analysis exhibited that knockdown of FAM54A obviously inhibited cell proliferation in Namalwa cells. What's more, cell cycle analysis showed that knockdown of FAM54A induced Namalwa cell apoptosis and arrested cell cycle in G2/M phase.

Conclusions These findings suggest that FAM54A is essential for Namalwa cell proliferation and may be a potential therapeutic target for the treatment of Burkitt lymphoma.

Background

Burkitt lymphoma (BL) is a kind of non-Hodgkin B-cell-derived malignancy from germinal center (GC) B cells [1–3] with highly invasive and proliferative ability. Clinically, it is divided into three subtypes: endemic, sporadic and immune deficiency related [4]. Many literatures have reported that the occurrence of the disease is related to epstein-barr (EB) virus infection, human immunodeficiency virus (HIV) infection and chromosomal translocation. Among the factors that cause BL, the formation of MYC translocation caused by the fusion of MYC gene and immunoglobulin gene is a key event, resulting in abnormal expression of the gene, so it can further promote the proliferation of BL cells [5, 6].

FAM54A is a highly conserved gene family [7] that controls embryonic development and cell differentiation. Aberrant regulation of the gene is associated with the occurrence of malignant tumors [8]. However, the effects of FAM54A on BL is still undetermined, so the aim of this research was to explore the biological function and potential mechanism of FAM54A in human BL. To our knowledge, it was the first time to deeply study the role of FAM54A in BL pathogenesis and confirm obviously high expression level of FAM54A in BL tissues by western blotting (WB). Knockdown of FAM54A with the Lenti-shRNA system [9] significantly inhibited cell proliferation. Furthermore, we found that knockdown of FAM54A could induce cell apoptosis and render cell cycle arrest in G2/M phases. These outcomes indicate that FAM54A may play a essential role in cell proliferation and progression of BL.

Methods

Cell lines and cell culture

Human BL cell line (Namalwa) and human renal epithelial cells (293T) were purchased from Biological Science (Shanghai, China) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with penicillin (100 U/ml)-streptomycin (100 μg/ml) and 10% fetal bovine serum (FBS) in 5% CO_2 at 37°C.

Western blot analysis

The cells were harvested and lysed using ice-cold lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% SDS, 1 mM EDTA, 1% NP-40) including 1 mM protein inhibitor and 1 mM Phenylmethylsulfonyl Fluoride (PMSF) for 30 minutes on ice. The pyrolysis products were centrifuged at 10,000×g at 4˚C for 10 minutes and the supernatant was collected. The protein concentration was detected by Bicinchoninic acid (BCA) detection kit (HyClone-Pierce, USA). Total proteins were separated using 12.5% sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) according to the previous reported method [10] and then transferred to Polyvinylidene Fluoride (PVDF) membrane. Mouse anti-GAPDH and FAM54A antibodies were used to detect targeted proteins (Santa Cruz Biotechnology, USA). Finally, the proteins were visualized by use of enhanced chemiluminescence (ECL) Regent (Piscataway, NJ, USA)

Recombinant lentiviral vector production

According to the principle of RNA interference design, multiple RNA interference target sequences were designed based on *FAM54A* sequence (Accession no. NM_001099286). After evaluation and determination by a design software, the target sequence (5'-AATTGTGGAAATGCAGGAA-3') from the full-length sequence of *FAM54A* was selected as the most suitable interference target by Genechem company (Shanghai, China). After detecting of knockdown efficiency, the stem-loop oligonucleotides were synthesized and inserted into lentivirus-based GV115-GFP vectors with agel/EcoRI sites (Genechem, shanghai, China). Lentivirus particles were prepared as a previous report [11].

Cells transfection

For lentivirus transfection, the target cells were cultured in a 6-well microplate, and then *FAM54A*-shRNA lentivirus and negative control (NC)-shRNA lentivirus was added according to a multiplicity of infection (MOI). After 72 hours of transfection, the cells were observed by a fluorescence microscope (NIB900, Olympus, Japan). After 120 hours of transfection, the cells were collected to detect the knockdown efficiency by WB.

Cell proliferation assay

Cell growth ability was measured by the MTS (3-(4,5-dimethylthiazol-2-yl)-5- (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (CellTiter 96® AQueous one Solution Cell proliferation Assay,

Promega, USA) [12]. In brief, cells transfected with *FAM54A*-shRNA or NC-shRNA lentivirus were placed into 96-well plates at a cell density of 2×10^3 cells/well, respectively. 1, 2, 3, 4 and 5 day after incubation, the medium was substituted with 100 μl of fresh serum-free RPMI-1640 medium with 20 μl CellTiter 96® AQueous one Solution reagent in each well. After incubation for 4 h at 37 ℃, the 96 well plate was placed on the oscillator and vibrated for 2-5 minutes, then the OD (Optical density) value in each well was read by detecting the absorbance at 490 nm using a Microplate Spectrophotometer.

Cell cycle analysis

Cell cycle distribution was analyzed using propidium iodide (PI) following the manufacturer's instructions as previously described [13]. Briefly, Namalwa cells that were transfected with *FAM54A*-shRNA or NC-shRNA lentivirus were collected, washed twice with ice-cold phosphate-buffered saline (PBS), fixed with ice-cold 70% ethanol at 4˚C for 1 h, and stained with PI (Genechem, Shanghai, China). Finally, cell cycle analysis was performed by flow cytometry. Each experiment was performed in triplicates.

Cell apoptosis detection

The apoptosis rate of cells was determined by Annexin V-APC stainning according to the kit protocols (Santacruz, USA). The Namalwa cells (6×10^5cells/well) were plated into 6-well plate after infection with *FAM54A*-shRNA or NC-shRNA lentivirus. After 5 days of cultivation. They were washed, collected, and resuspended in PBS buffer and the cell concentration was adjusted to 1×10^6 /ml. Then, the 5 μl Annexin V (0.1 μg/μl) was added into 100 μl cell suspension and incubated at room temperature in dark for 30 min on ice. The cells given different treatment were subjected to Fluorescent-Activated Cell Sorting (FACS) analysis. All experiments were performed in triplicates.

Statistical analysis

The statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Student's t-test was used for raw data analysis. For all statistical tests, p-values less than or equal to 0.05 were considered to be statistical significance.

Results

FAM54A protein was highly expressed in human BL cell line

The expression level of FAM54A protein in human BL cell line (Namalwa) and human renal epithelial cells (293T) were examined by WB. As depicted in Fig. 1, the expression of FAM54A at the protein level was significantly high in the Namalwa cells.

Figure 1. The expression level of FAM54A protein in 293T cells and Namalwa cells. WB was used to analyze FAM54A protein expression level in both cell lines. GAPDH was used as a loading control.

Determination of knockdown efficiency by WB

Knockdown efficiency was determined by detecting FAM54A protein expression level in infected 293T cells with *FAM54A*-shRNA or NC-shRNA lentivirus by WB. Compared to NC-shRNA lentivirus, the expression level of the protein in 293T cells transfected with *FAM54A*-shRNA lentivirus was greatly decreased, which indicated a efficient knockdown of the target gene (Fig. **2**).

Figure 2. Knockdown of FAM54A protein expression in 293T cells. WB was used to detect the expression of FAM54A protein in *FAM54A*-shRNA or NC-shRNA lentivirus-transfected 293T cells. GAPDH was used as a loading control.

Lentivirus-mediated knockdown of *FAM54A* in the human BL cell line Namalwa

To explore the function of *FAM54A*, we knocked down *FAM54A* in namalwa cell line. After 3 days of transfection, fluorescence imaging technology was applied to reflect the transfection results of cells. Results showed that the percentage of infected cells was >50% for both the *FAM54A*-shRNA or NC-shRNA lentivirus (Fig. 3). FAM54A protein expression levels were assessed by WB at day 5 post infection with either the *FAM54A*-shRNA or NC-shRNA lentivirus. The results in Namalwa cell line were similar to those in 293T cells. FAM54A-shRNA cultures had obviously lower protein expression compared to NC-shRNA lentivirus (Fig.4).

Figure 3. Evaluating infection efficiency with *FAM54A*-shRNA or NC-shRNA lentivirus in Namalwa cells. Namalwa cell line were transfected with *FAM54A*-shRNA or NC-shRNA lentivirus and observed with fluorescent microscopy on the third day after infection. About 50% of the cells expressed green fluorescent protein (GFP). Magnification, 100×. Fluorescent and white light images of the cells are shown.

Figure 4. Knockdown of FAM54A protein expression in cell line Namalwa. FAM54A protein expression was analyzed by WB in *FAM54A*-shRNA and NC-shRNA lentivirus-transfected Namalwa cells. GAPDH was used as a loading control.

Knockdown of *FAM54A* inhibits human BL cell proliferation in vitro

In order to study the effect of *FAM54A* on cell growth, *FAM54A*-shRNA lentivirus was transfected into Namalwa cells to silence the gene. Then, cells expressing *FAM54A*-shRNA or NC-shRNA lentivirus were implanted into 96-well plates and analyzed by MTS for 5 days. The effect of *FAM54A* downregulation on the proliferation of Namalwa cells was detected by MTS assay. Compared to NC, the absorbance of *FAM54A*-shRNA-transfected Namalwa cells at 490 nm was significantly lower (Fig. 5), which meant that the speed of proliferation of *FAM54A*-shRNA lentivirus-transfected cells was slower than that of NC ($p<0.05$). Thus, the experimental data showed that the downregulation of *FAM54A* noticeably suppressed cell proliferation.

Figure 5. Effect of *FAM54A* knockdown on Namalwa cell growth. Cells were implanted into 96-well plates and infected with *FAM54A*-shRNA or NC-shRNA lentivirus and cell growth was evaluated every day for 5 days (NC vs. *FAM54A*-shRNA, $p<0.05$).

Knockdown of *FAM54A* in Namalwa cells increases cell apoptosis

In order to detect whether the expression of FAM54A protein affects the apoptosis of BL cells, we knock out *FAM54A* in Namalwa cells. Annexin V staining and flow cytometry were applied to check apoptosis rate (Fig. 6A). As shown in Figure 6B, detailed data showed that apoptosis rate of *FAM54A*-shRNA lentivirus-transfected cells was significantly higher than that of NC (NC 2.97±0.02% vs. FAM54A-shRNA 4.93 ±0.07%, $p < 0.001$). These results implied that the lack of the FAM54A protein expression is a critical factor of cell apoptosis in Namalwa cells.

Figure 6. *FAM54A* knockdown increases apoptosis of Namalwa cells. (A) cell apoptosis was determined by annexin V staining and flow cytometry. Experiment was repeated in triplicate. (B) The quantitative data was shown. Note that compared with NC, apoptosis in *FAM54A*-shRNA lentivirus-transfected group was significantly increased (*** $p <0.001$).

Knockdown of *FAM54A* renders cell cycle arrest in human BL cell in vitro

In order to determine the detailed mechanism that *FAM54A* knocdown led to cell proliferation inhibition, cell cycle distribution of *FAM54A*-shRNA or NC-shRNA lentivirus-transfected cells was analyzed by flow cytometry (Fig. 7A). The cell cycle distribution of NC-shRNA lentivirus-transfected cells was shown as follows: (G0/G1 phase, 39.91±0.75 %; S phase, 36.19 ±0.99 %; and G2/M phase, 23.91 ±0.28 %) and cell cycle distribution of *FAM54A*-shRNA lentivirus-transfected group was also shown as follows: (G0/G1 phase, 36.46 ±0.36 %; S phase, 33.16 ±0.69 %; and G2/M phase, 30.38 ±0.59 %). As shown in Figure 7B, compared with NC group, the cell proportion of *FAM54A*-shRNA lentivirus-transfected group showed a significant decrease in G0/G1 phase ($p<0.05$), but showed a significant increase in G2/M phase ($p<0.05$). To sum up, the data indicates that *FAM54A* regulates cell proliferation and downregulation of *FAM54A* can render cell cycle arrest in G2/M phase.

Figure 7. Knockdown of *FAM54A* results in cell cycle arrest. (A) Cell cycle distribution was analyzed by flow cytometry. Each experiment was repeated in triplicates. (B) compared with NC group, the cell proportion of *FAM54A*-shRNA lentivirus-transfected group showed a significant decrease in G0/G1 phase (* $p<0.05$); however, it showed a significant increase in G2/M phase (* $p<0.05$).

Discussion

In the world, BL is one of the most common causes of cancer-related death, because it is difficult to be perceived in early stage and the disease has developed to late stage when it is diagnosed accurately, so the best treatment time may not be utilized [14]. Therefore, early diagnosis and targeted therapy are key to better prognosis of patients with BL. Additionally, FAM54A, aslo named as Mitochondrial fission regulator 2 (MTFR2) or DUF729 domain-containing protein 1 (DUFD1) plays an important role in regulating the structure and function of mitochondria. FAM54A protein is also related to membrane-enriched subcellular fractions, including mitochondria. It has been proven that inhibition of Dufd1 expression in testicular germ cell lines can seriously impair oxygen consumption, suggesting that the

gene is necessary for mitochondrial respiration [15]. Therefore, the downregulation of FAM54A expression in tumor cells will be beneficial to inhibit the consumption and utilization of oxygen and synthesis of Adenosine triphosphate (ATP) by tumor cells, and further leads to the death of tumor cells due to hypoxia. This mechanism may provide a novel therapeutic strategy against tumors. At the same time, this protein is also an important nuclear protein and is considered as a key molecular target in cancers, infectious diseases and some other diseases. In 2009, Persson et al reported that FAM54A has a positive relevance with inflammation and tumor progression [8]. In order to determine this critical molecular marker that may provide a novel strategy and idea for the early diagnosis and targeted treatment of BL, we explored the role of FAM54A in tumorigenesis and progression of BL.

First of all, we measured the expression level of FAM54A protein in Human BL cell line (Namalwa) and the results exhibited that expression level of FAM54A protein was very high in cancer cell line. So, we speculated that it probably exerted a role to promote cell growth in BL and acted as an oncogene.

Next, we transfected the Namalwa cells using FAM54A-shRNA lentivirus, which significantly downregulated the endogenous expression level of FAM54A protein. Then, the cell proliferation viability was detected by MTS assay. Our results showed that knockdown of FAM54A obviously leaded to a inhibition in cell proliferation in BL. Furthermore, results of cell cycle and apoptosis analysis disclosed that downregulation of FAM54A negatively regulated BL progression by inducing apoptosis and blocking cell cycle in Namalwa cells.

Conclusions

Although, detailed reference literatures on FAM54A are very limited because few studies are carried out to explore it carefully, our present study states that FAM54A protein is expressed highly in human BL cell line. Knockdown of FAM54A suppresses cell proliferation by increasing cell cycle arrest and inducing cell apoptosis. These findings not only elucidate the effects of FAM54A on BL, but also can provide a novel strategy and idea used for improvement of prognosis of BL and promote the development of early diagnosis and targeted therapy of human BL. However, the detailed molecular mechanism involved in effects of FAM54A on BL still needs further to be further investigated.

List Of Abbreviations

BL: Burkitt lymphoma; GC: Germinal center; EB: Epstein barr; HIV: Human immunodeficiency virus; WB: Western blotting; RPMI: Roswell park memorial institute; FBS: Fetal bovine serum; PMSM: Phenylmethylsulfonyl fluoride; BCA: Bicinchoninic acid; SDS-PAGE: Sodium dodecyl sulfate-poly acrylamide gel electrophoresis; PVDF: Polyvinylidene fluoride; ECL: Enhanced chemiluminescence; GFP: Green fluorescent protein; PI: Propidium iodide; MOI: Multiplicity of infection; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium; OD: Optical density; FACS: Fluorescent-activated cell sorting; PBS: Phosphate-buffered saline; NC: Control-transfected; MTFR2:

Mitochondrial fission regulator 2; DUFD1: DUF729 domain-containing protein 1; ATP: Adenosine triphosphate; GFP: Green fluorescent protein.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed 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

YG, WXH, YB and XCY analyzed and interpreted the all experimental data. ZRY, CMX, LRF and ZHD performed the relevant experiments, respectively. YG and JZS were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Not applicable

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Figures

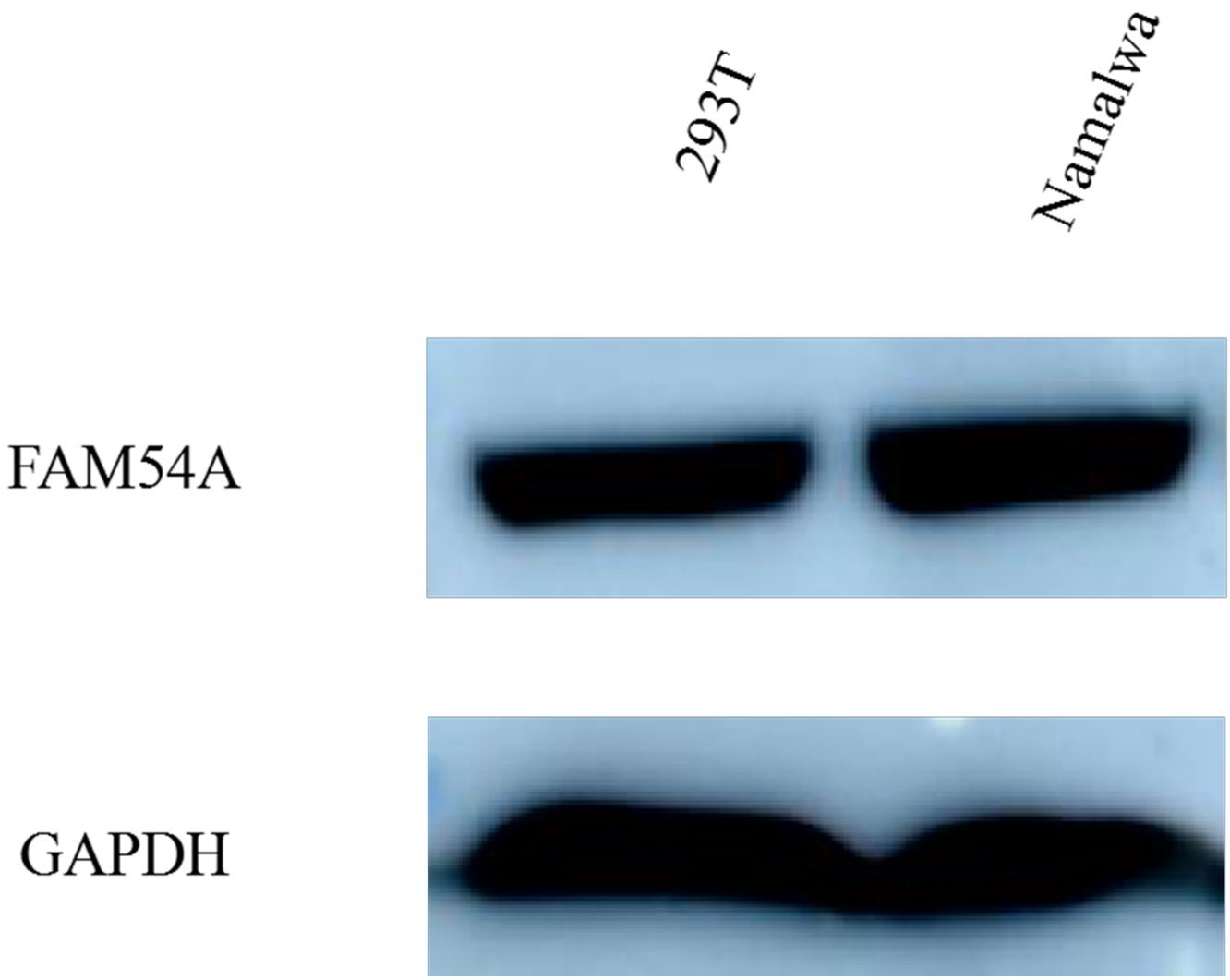

Figure 1

The expression level of FAM54A protein in 293T cells and Namalwa cells. WB was used to analyze FAM54A protein expression level in both cell lines. GAPDH was used as a loading control.

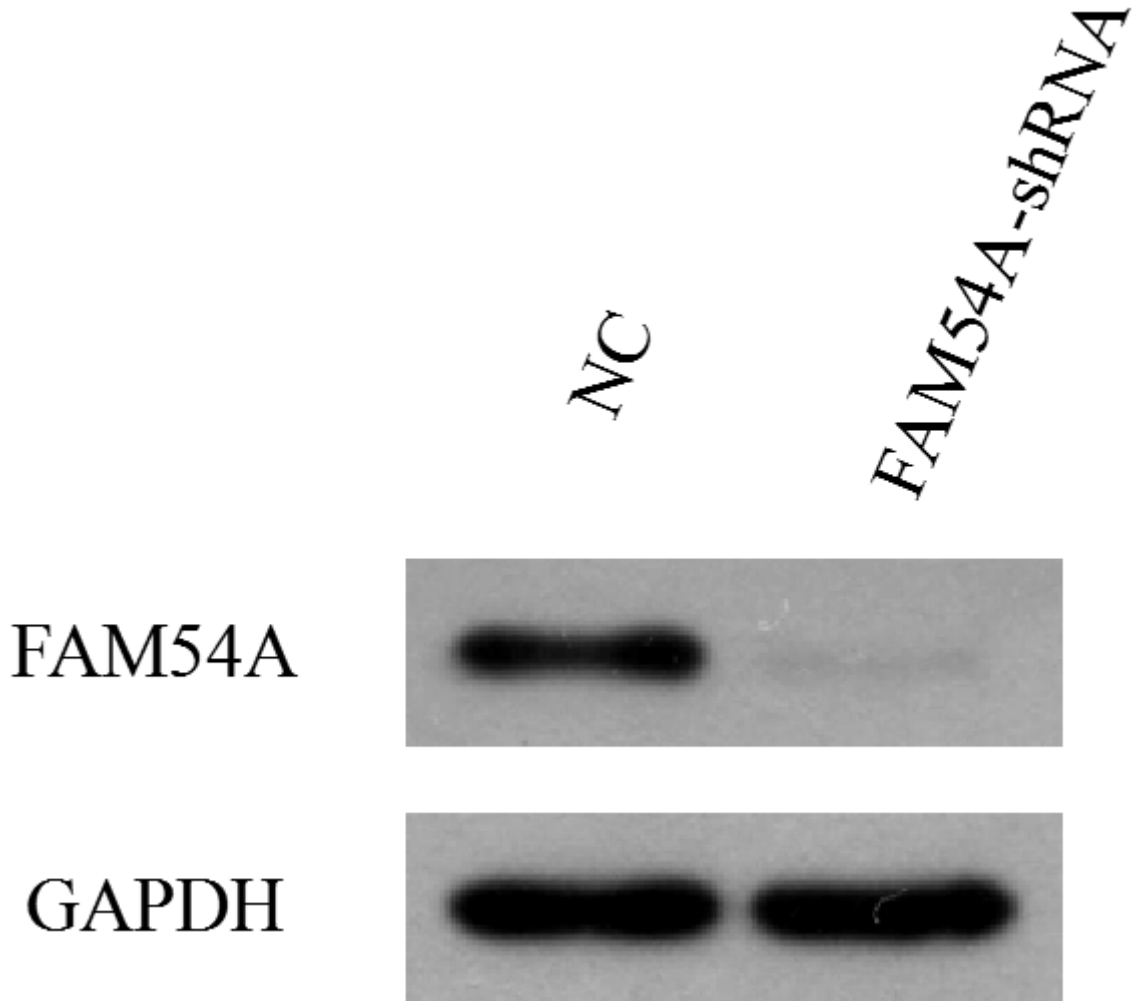

Figure 2

Knockdown of FAM54A protein expression in 293T cells. WB was used to detect the expression of FAM54A protein in FAM54A-shRNA or NC-shRNA lentivirus-transfected 293T cells. GAPDH was used as a loading control.

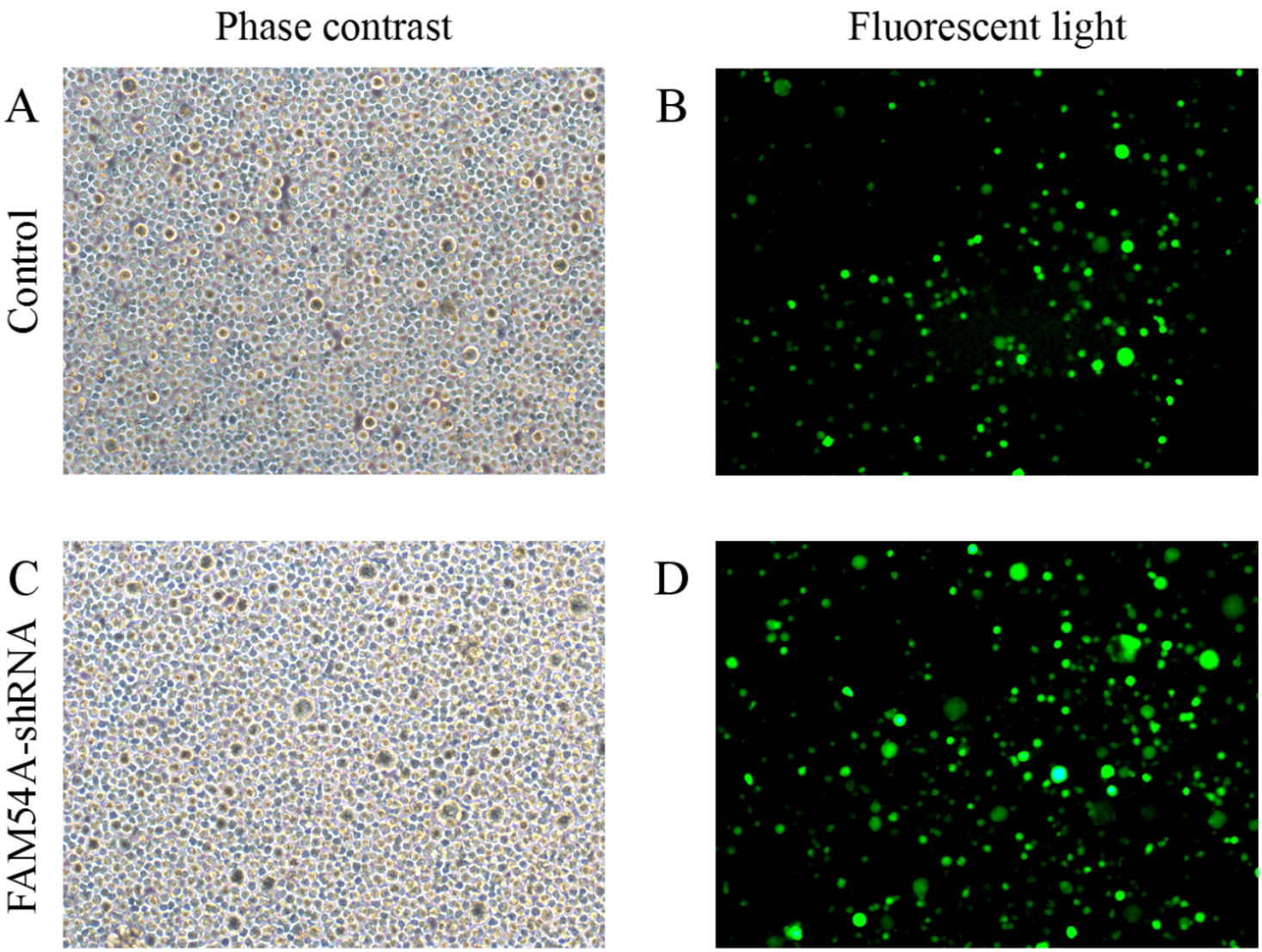

Figure 3

Evaluating infection efficiency with FAM54A-shRNA or NC-shRNA lentivirus in Namalwa cells. Namalwa cell line were transfected with FAM54A-shRNA or NC-shRNA lentivirus and observed with fluorescent microscopy on the third day after infection. About 50% of the cells expressed green fluorescent protein (GFP). Magnification, 100×. Fluorescent and white light images of the cells are shown.

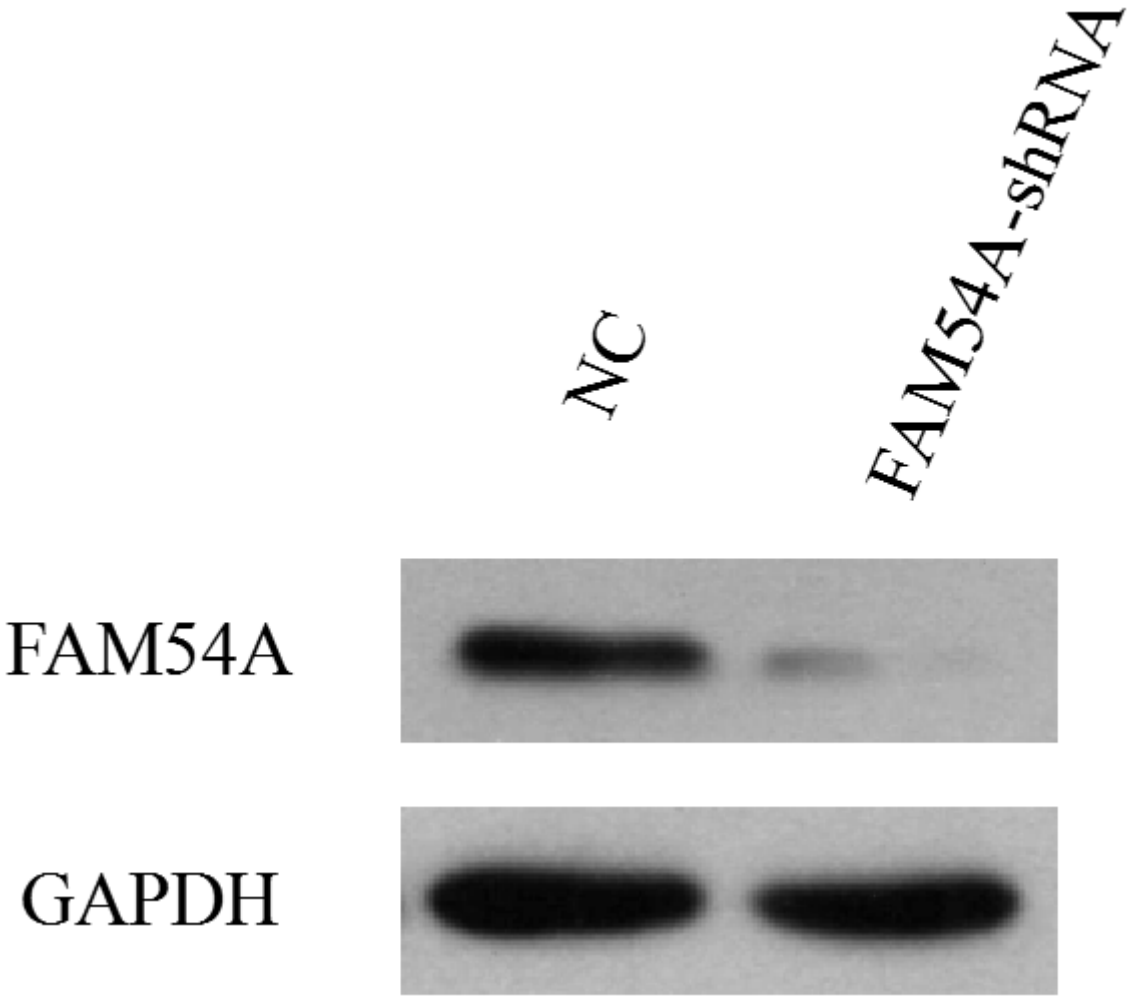

Figure 4

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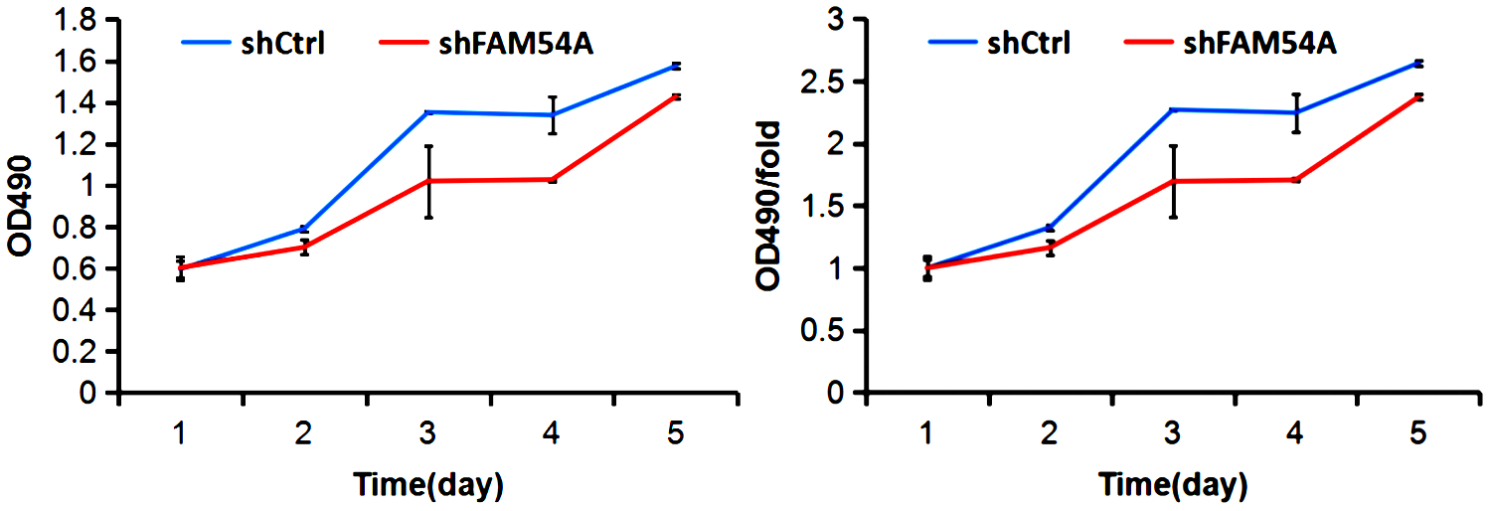

Figure 5

Effect of FAM54A knockdown on Namalwa cell growth. Cells were implanted into 96-well plates and infected with FAM54A-shRNA or NC-shRNA lentivirus and cell growth was evaluated every day for 5 days (NC vs. FAM54A-shRNA, p<0.05).

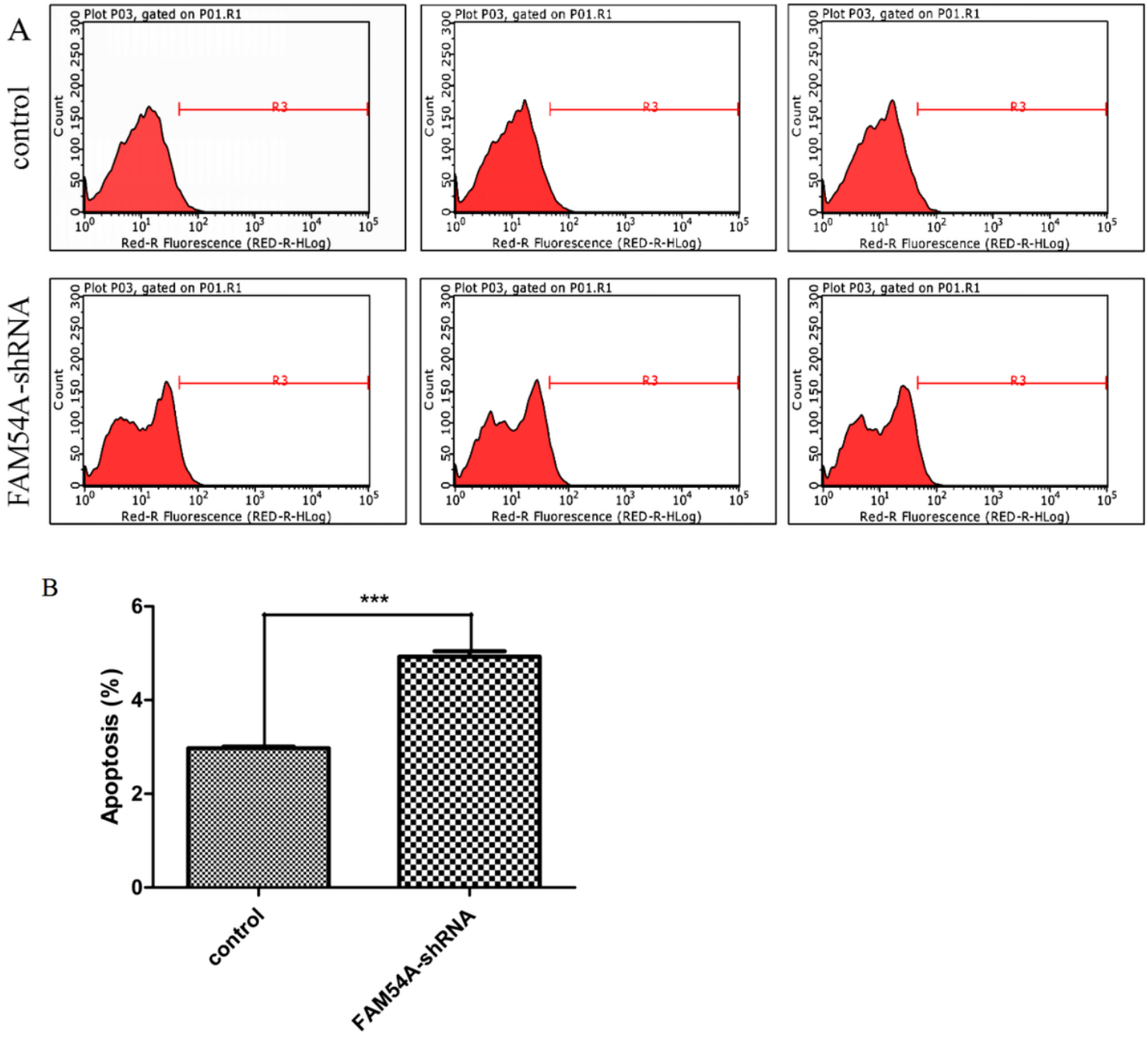

Figure 6

FAM54A knockdown increases apoptosis of Namalwa cells. (A) cell apoptosis was determined by annexin V staining and flow cytometry. Experiment was repeated in triplicate. (B) The quantitative data was shown. Note that compared with NC, apoptosis in FAM54A-shRNA lentivirus-transfected group was significantly increased (*** p <0.001).

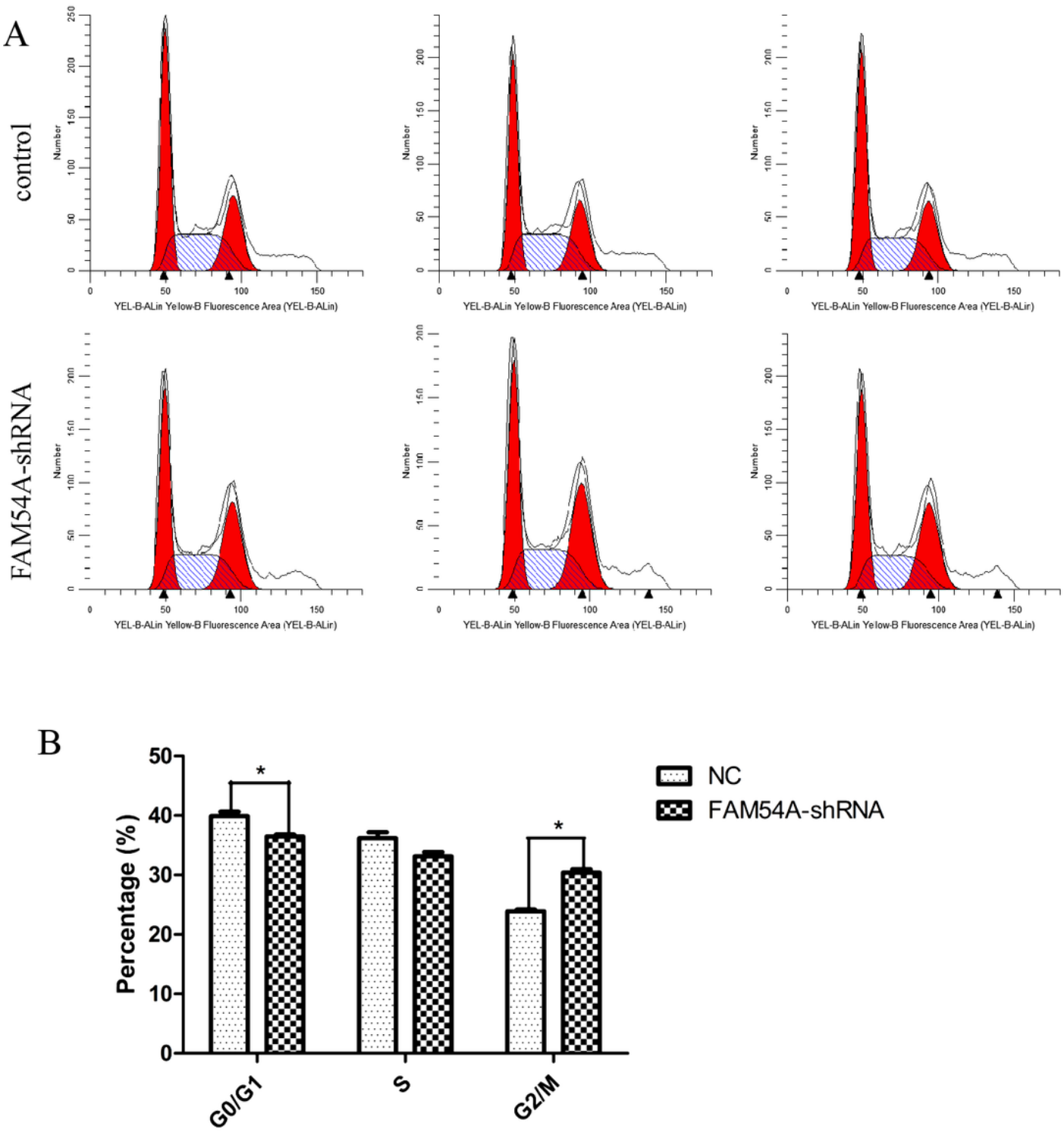

Figure 7

Knockdown of FAM54A results in cell cycle arrest. (A) Cell cycle distribution was analyzed by flow cytometry. Each experiment was repeated in triplicates. (B) compared with NC group, the cell proportion of FAM54A-shRNA lentivirus-transfected group showed a significant decrease in G0/G1 phase (* $p<0.05$); however, it showed a significant increase in G2/M phase (* $p<0.05$).

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