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ORIGINAL ARTICLE

Regulatory effects of ΔFosB on proliferation and apoptosis of MCF-7 breast cancer cells

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Abstract Matrix metalloproteinase-9 (MMP-9) plays a vital role in tumor angiogenesis, cell migration, and invasiveness because it can degrade almost all basement membrane and extracellular matrix components. MMP-9 has been reported in many cancers including breast cancer, lung cancer, and colon cancer. ΔFosB in mammary epithelial cells has been shown to regulate cell proliferation, differentiation, and death. We found that ΔFosB increased the expression of MMP-9 in MCF-7 breast cancer cells. ΔFosB overexpression in MCF-7 cells increased cellular viability and decreased cell apoptosis. SB-3CT, an inhibitor of MMP-9, promoted apoptosis, inhibited cell proliferation, induced cell cycle arrest, and downregulated the expression of antiapoptotic genes Bcl-2 and Bcl-xl in MCF-7 cells. ΔFosB increased the number of MCF-7 cells in G2/M and S phases, upregulated the expression of Bcl-2 and Bcl-xl, and protected MCF-7 cells from apoptosis induced by MMP-9 inhibition. We also found that ΔFosB overexpression in MCF-7 cells inhibited Ca^{2+}-induced apoptosis and promoted cell proliferation. Therefore, ΔFosB may be a potential target in breast cancer cell apoptosis by regulating the expression of MMP-9.

Hui Li and Lihui Li contributed equally to this work.

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Introduction

The activator protein 1 (AP-1) family of transcription factors regulates various biological processes [1] and plays important roles in many types of cancer including breast, liver, ovarian, and colorectal tumors [2, 3]. As an important member of the AP-1 family [4], ΔFosB has been caught in cell proliferation, differentiation, and death [5]. ΔFosB regulates the transcription activating ability of Jun [6], and c-Jun overexpression can produce highly invasive tumors in MCF-7 breast cancer cells [7]. ΔFosB could promote primary neuronal precursor cells' proliferation and enhance neurogenesis after transient forebrain ischemia [8]. Furthermore, ΔFosB promotes rat embryo cells survival dependent on the mitochondrial pathway of caspase-9 and caspase-3 [5]. In addition, the overexpression of ΔFosB in transgenic mice induces an increase in bone mass and a decreased mass of adipose tissue by elevating bone formation [9].

Matrix metalloproteinase-9 (MMP-9), a representative member of the MMP family [10], plays important roles in cell fate decisions because of its ability to degrade type IV collagen [11, 12]. MMP-9 overexpression in MCF-7 human mammary carcinoma cells induces migration and invasion [13, 14]. MMP-9 activity has also been shown to be important for the angiogenic and metastatic phenotypes of human tumors. MMP-9 upregulation in three tumor models, the K14 HPV16 skin cancer, the RIP1 TAg2 insulinoma, and DU145 prostate tumor, results in an increase in tumor growth and angiogenesis by activating the gene expression of vascular endothelial growth factor (VEGF) and intercellular adhesion molecule 1 (ICAM-1) [15–17].

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Table 1 Primers designed for quantitative real-time PCR (RT-qPCR)

Gene	Primer sequences
MMP-9	Forward: AGCCCACATAGTCCACCTGA
	Reverse: CCCATTAGCACGCACGAC
ΔFosB	Forward: ACCCACCCTCATCTCTTCCAT
	Reverse: GCCACTGCTGTAGCCACTCA
Bcl-2	Forward: ATGTGTGTGGAGAGCGTCAACC
	Reverse: TGAGCAGAGTCTTCAGAGACAGCC
Bcl-xl	Forward: CGGGCATTCAGTGACCTGAC
	Reverse: TCAGGAACCAGCGGTTGAAG
Bax	Forward: CCCTTTTGCTTCAGGGTTTC
	Reverse: GCCACTCGGAAAAAGACCTC
Bad	Forward: GAGCCGAGTGAGCAGGAAGAC
	Reverse: CACTCATCCTCCGGAGCTCGC
CyclinD1	Forward: TCTAAGATGAAGGAGACCATC
	Reverse: GCGGTAGTAGGACAGGAAGTTGTT
P53	Forward: CCCCTCCTCAGCATCTTATCC
	Reverse: CACCTCAAAGCTGTTCCGTCC
caspase-3	Forward: AGAACTGGACTGTGGCATTGAG
	Reverse: GCTTGTCGGCATACTGTTTCAG
caspase-9	Forward: ATGGACGAAGCGGATCGGCGGCTCC
	Reverse: GCACCACTGGGGGTAAGGTTTTCTAG
GAPDH	Forward: GCAAGTTCCACGGCACAG
	Reverse: GGTTCACGCCCATCACAA
UXT	Forward: TGTGGCCCTGGGATATGGTT
	Reverse: GGCTGTTGCTGAGCTCTGTG
MRPL39	Forward: AAGTTCTCTTTTGTTGGCATCC
	Reverse: TTGGTCAGAGCTCCAGAAGT

The transcription of MMP-9 gene depends on several transcription factors, including AP-1, NF-κB, and Sp-1 [18, 19]. Blocking AP-1 activity in brain tumor inhibits MMP-9 expression and activity, as well as MMP-9-dependent cell invasiveness [20]. The inhibition of AP-1 activation represses MMP-9 transcriptional expression via MAPK signaling pathways that is implicated in inhibiting MCF-7 cells invasion [21]. Inhibiting the ability of AP-1 binding to MMP-9 exhibits a cell invasion suppressive effect in MDA-MB-231 human breast carcinoma cell lines [22]. However, the interactions between ΔFosB and MMP-9 and their roles in cell proliferation and apoptosis in human breast cancer cells are poorly understood.

We hypothesized that ΔFosB could protect MCF-7 cells from apoptosis by regulating the expression of MMP-9. Indeed, we demonstrate here that ΔFosB promotes MMP-9 expression and protects MCF-7 cells from SB-3CT- and calcium-induced apoptosis. We further demonstrate that MMP-9 can mediate ΔFosB induction of antiapoptotic protein Bcl-2 in MCF-7 cells. Thus, these results establish a role for ΔFosB in promotion of breast cancer cells survival.

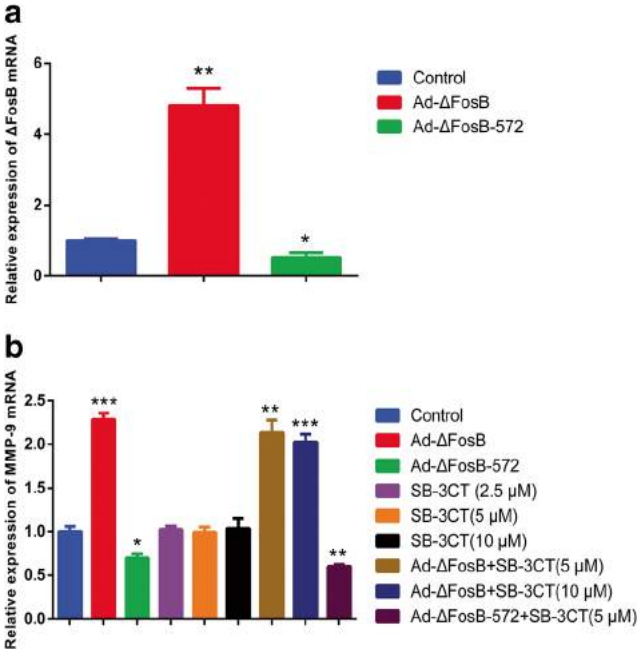

Fig. 1 ΔFosB increases MMP-9 expression in cultured MCF-7 breast cancer cells. **a** Relative expression levels of ΔFosB mRNA in MCF-7 cells transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h. **b** Cells were transduced for 48 h with Ad-ΔFosB or Ad-ΔFosB-572, followed by exposure to different concentrations of SB-3CT for 24 h. MMP-9 mRNA levels were analyzed by quantitative real-time PCR (RT-qPCR). Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB, *SB-3CT* an inhibitor of MMP-9

Materials and methods

Cell culture and reagents

MCF-7 cells, which were originally purchased (ATCC number HTB-22), were cultured in DMEM/F-12 with 10 % fetal bovine serum (FBS), penicillin, and streptomycin (10 KU/L; Harbin Pharmaceutical Group, China) in 5 % CO_2 at 37 °C. ΔFosB overexpression recombinant adenoviruses Ad-ΔFosB and interference recombinant adenoviruses Ad-ΔFosB-572 were constructed in our laboratory [23]. SB-3CT, an inhibitor of MMP-9, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell treatment and transduction

Before the experiments, the MCF-7 cells were cultured in 60-mm culture dishes (NUNC, Denmark) with complete DMEM/F-12 medium which contains 10 % FBS and penicillin/streptomycin (10 KU/L; Harbin Pharmaceutical Group, China). The MCF-7 cells at nearly 80~90 % confluence were plated at $5\times10^5\sim1\times10^6$ cells/well in 6-well plates or 1×10^4 cells/well in 96-well plates in

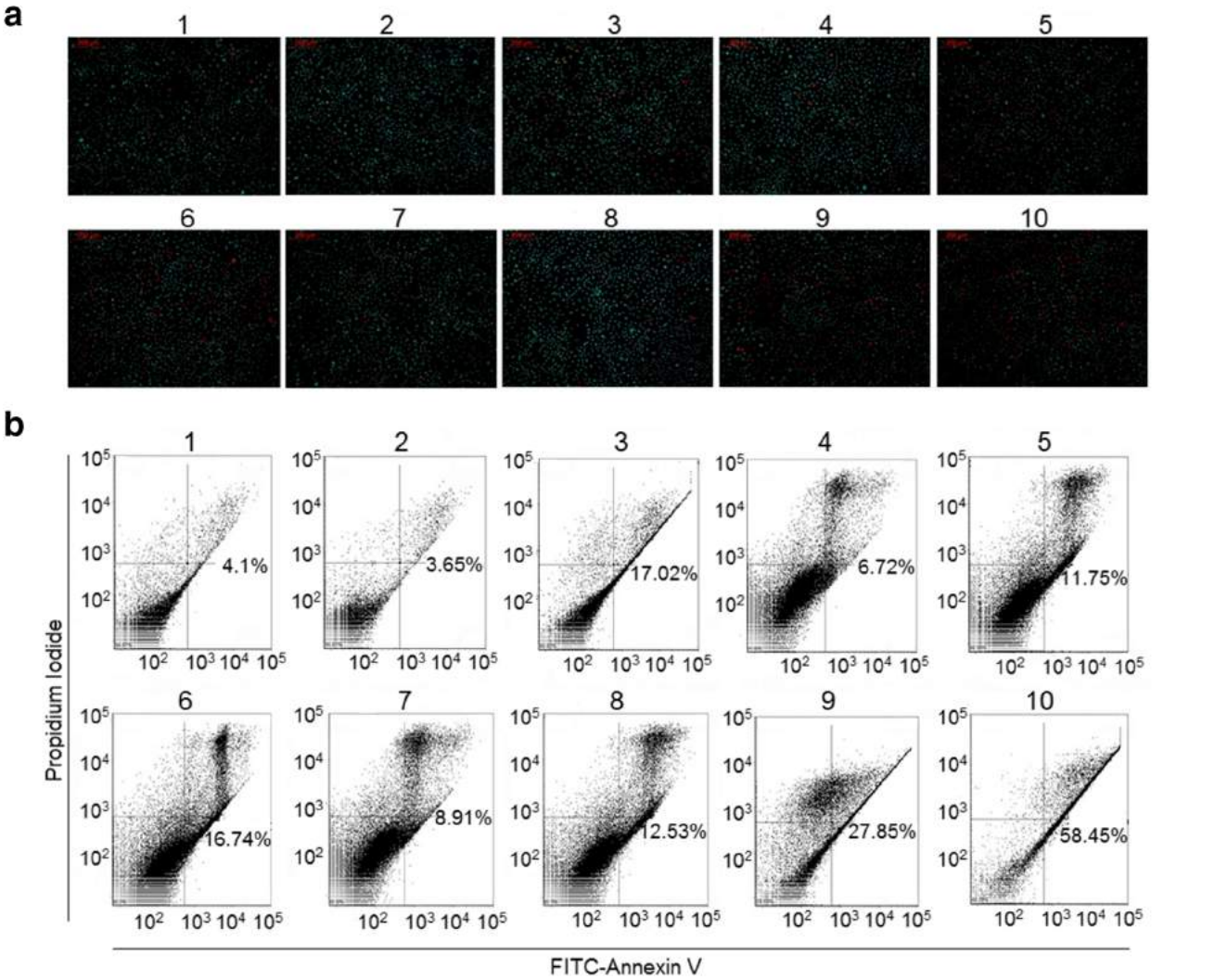

Fig. 2 ΔFosB-dependent protection from SB-3CT-induced cell apoptosis in MCF-7 breast cancer cells. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of SB-3CT for 24 h. Cell apoptosis was determined by Hoechst33342/PI dual staining assays (**a**) and annexin V-FITC/PI binding followed by flow cytometry (**b**). For Hoechst33342/PI dual staining assays, Hoechst33342^{-}/PI^{-} cells (live cells) were in *light blue* and *lighter red*; Hoechst33342^{+}/PI^{+} cells (late apoptotic cells) were in *dark blue* and *bright red*. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB, *SB-3CT* an inhibitor of MMP-9

complete DMEM/F-12 medium and incubated overnight at 37 °C with 5 % CO_2 and air. When MCF-7 cells grow to approximately 80–90 % confluence, they were treated with (a) 0, 2.5, 5, or 10 μM SB-3CT, respectively; (b) 0, 1, 2, 4, or 8 mM $CaCl_2$, respectively; (c) Ad-ΔFosB or Ad-ΔFosB-572, respectively; (d) Ad-ΔFosB+SB-3CT, Ad-ΔFosB-572+SB-3CT, Ad-ΔFosB+$CaCl_2$, or Ad-ΔFosB-572+$CaCl_2$. After 24 or 48 h incubation, the MCF-7 cells with different treatments were used for Hoechst33342/PI staining analysis, cell proliferation assay, cell apoptosis/cycle assay, and RNA extraction.

Total RNA extraction and quantitative real-time PCR

Total RNA extraction was done using Trizol reagent (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions. The cDNA was synthesized using the PrimeScript RT kit (Takara Bio Inc., Otsu, Japan). Quantitative real-time PCR (RT-qPCR) primers were designed using the Primer 5.0 software (Table 1). GAPDH, ubiquitously expressed transcript (UXT), and mitochondrial ribosomal protein L39 (MRPL39) were used for normalization of the RT-qPCR data [24]. RT-qPCR was performed with the SYBR Premix ExTaqTM

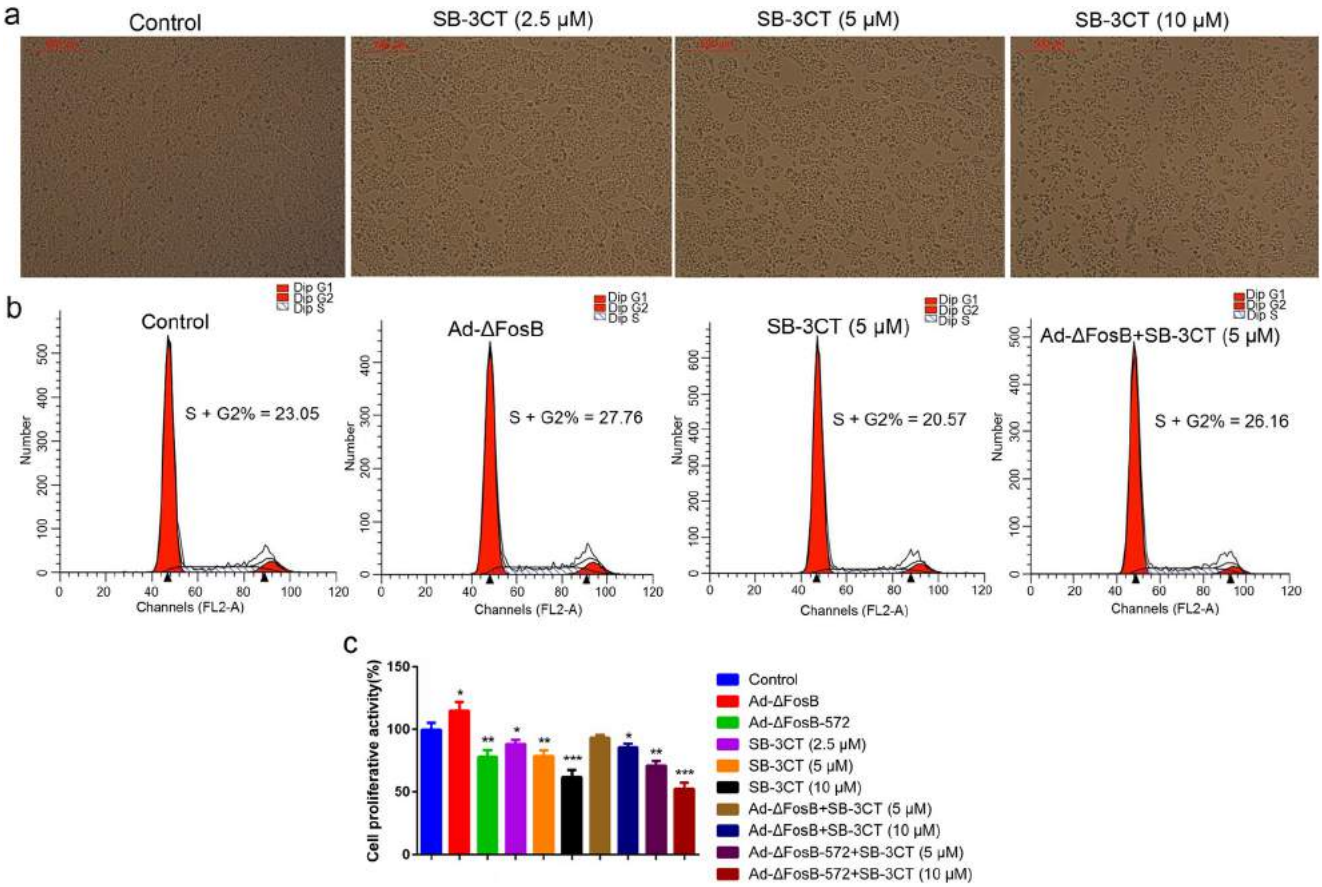

Fig. 3 Effects of ΔFosB on the cell proliferation and cycle in MCF-7 breast cancer cells are mediated by MMP-9. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of SB-3CT for 24 h (**a**) and were subsequently subjected to cell cycle assay (**b**) and cell proliferation analysis by Cell Counting Kit-8 (CKK-8) assays (**c**). *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB, *SB-3CT* an inhibitor of MMP-9. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

kit (Takara Bio Inc., Otsu, Japan) on a Bio-Rad CFX-96 sequence detector (Bio-Rad Laboratories Inc., Hercules, CA). The RT-qPCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method. Every sample was tested in triplicate.

Cell proliferation assay

The MCF-7 cells were plated in 96-well plates in a 100 μL volume and treated with SB-3CT, $CaCl_2$, Ad-ΔFosB, Ad-ΔFosB-572, respectively. For the Cell Counting Kit-8 (CCK-8) assay after the treatment, each well was incubated with 10 μL of CCK-8 reagent (Vazyme, China) and 90 μL refreshed DMEM/F-12 medium at 37 °C for 1∼4 h. Then the absorbance was detected at 450 nm using a microplate reader (Molecular Devices, USA).

Cell cycle assay

Cell cycle analysis was performed using Cell Cycle Testing Kit (Multisciences, China). The MCF-7 cells were grown in 6-well plates (1×10^6 cells/well). After treating with SB-3CT, $CaCl_2$, Ad-ΔFosB, Ad-ΔFosB-572, respectively, the cells were harvested and centrifuged at 800 g/min for 5 min. the supernatant was discarded, and the cells were washed once by cold phosphate buffered saline (PBS). MCF-7 cells were re-suspended using 1 mL of reagent A and 10 μL of reagent B, subsequently blended by vortexing for 5∼10 s and incubated for 30 min, and then analysis was done by flow cytometry.

Hoechst33342/PI dual staining assays

Apoptosis and necrosis were distinguished via PI and Hoechst 33342 double staining using the Hoechst 33342 and PI dyes

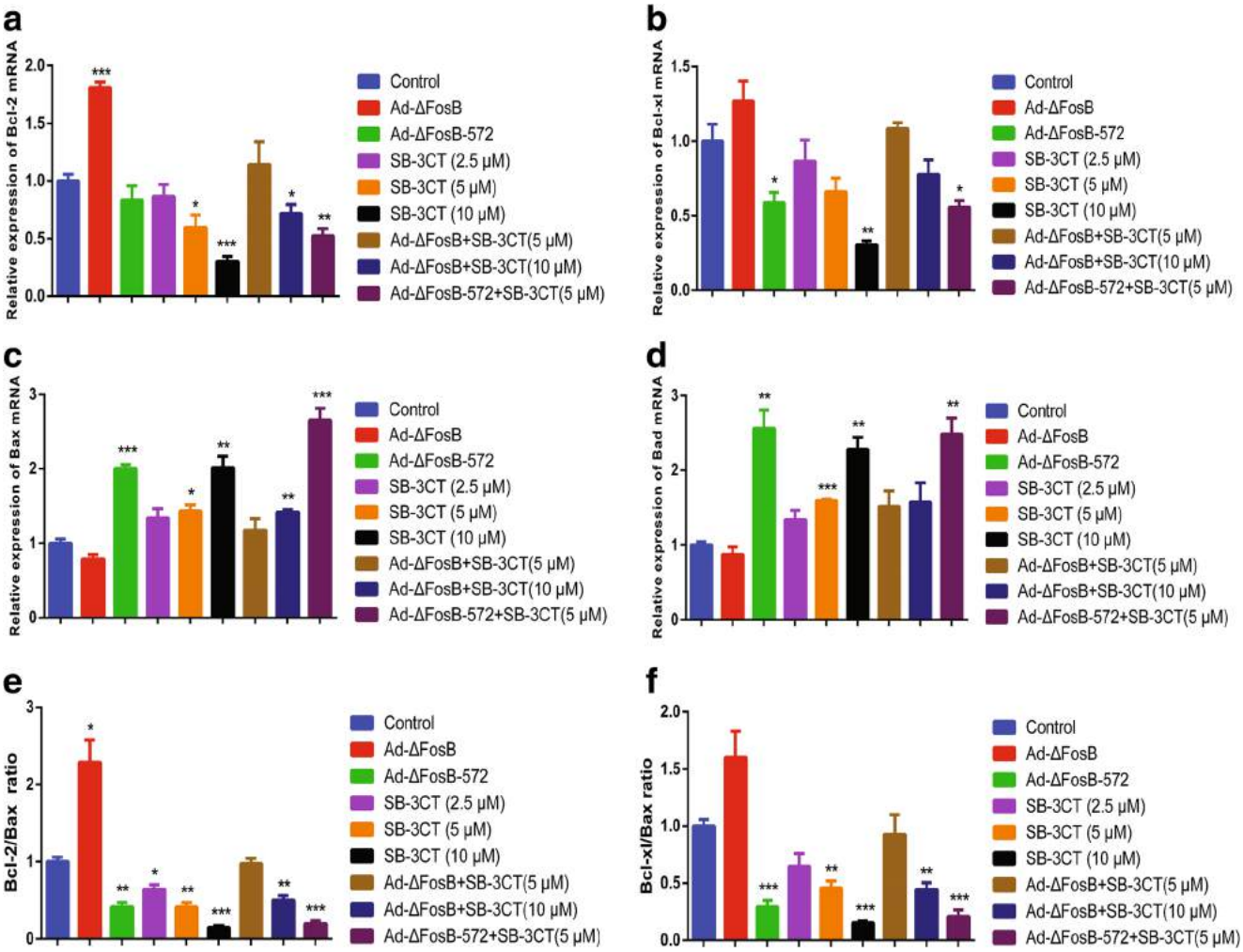

Fig. 4 MMP-9 mediates ΔFosB-induced increase of Bcl-2 expression in MCF-7 breast cancer cells. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of SB-3CT for 24 h. mRNA levels of cell survival-related genes Bcl-2 (**a**), Bcl-xl (**b**), Bax (**c**), Bad (**d**) were analyzed by RT-qPCR. Bcl-2/Bax (**e**) and Bcl-xL/Bax (**f**) ratios were also shown. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB, *SB-3CT* an inhibitor of MMP-9. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

(Solarbio, China). The MCF-7 cells were grown in 6-well plates (1×10^6 cells/well) and treated with SB-3CT, $CaCl_2$, Ad-ΔFosB, Ad-ΔFosB-572, respectively. After treatment, the MCF-7 cells were stained with Hoechst33342 (10 μg/mL) for 5 min and washed with PBS twice. Then the MCF-7 cells were stained with PI (10 μg/mL) washed with PBS again. Lastly, the cell staining results were observed under the fluorescence microscope.

Cell apoptosis measurement by Annexin V-FITC/PI staining assay

The MCF-7 cells were grown in 6-well plates (1×10^6 cells/well). After different treatments, the cells were harvested and centrifuged at 800 r/min for 5 min. Then the pellet was diluted at a density of 1×10^6 cells/mL with 1×binding buffer. Each 100 μL of the sample solution was incubated with 5 μL of Annexin V-FITC (Vazyme, China) and 5 μL of PI (Vazyme, China) for 15 min in the dark. Then each sample solution was mixed with 400 μL PBS. Finally, each sample was analyzed using a flow cytometry instrument and the data were analyzed by FCS Express 4 Plus research software.

Statistical analyses

All treatments were replicated three times, and the data were analyzed by GraphPad Prism 6 software (GraphPad software Inc., La, Jolla, USA) using one-way analysis of variance

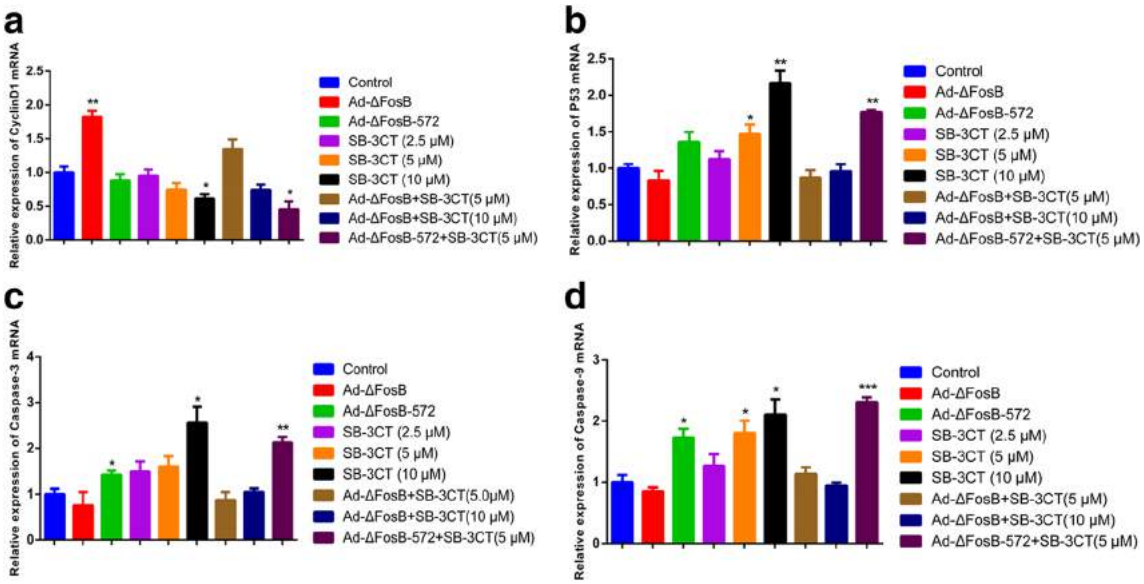

Fig. 5 Effects of ΔFosB on the expression of cell survival-related genes in MCF-7 breast cancer cells mediated by MMP-9. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of SB-3CT for 24 h. mRNA levels of cell survival-related genes CyclinD1 (**a**), p53 (**b**), caspase-3 (**c**), caspase-9 (**d**) were analyzed by RT-qPCR. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB, *SB-3CT* an inhibitor of MMP-9. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

(ANOVA) and Tukey's test. Values are means±SEM for three individuals, using the following significant values: *P<0.05, **P<0.01, and ***P<0.001.

Results

ΔFosB increases MMP-9 expression in MCF-7 cells

Given that AP-1 induces MMP-9 overexpression in mammary epithelial cells and breast cancer cells [25], we hypothesized that ΔFosB/AP-1 in MCF-7 cells could similarly upregulate MMP-9 expression. The cells transduced with ΔFosB overexpression recombinant adenoviruses Ad-ΔFosB increased messenger RNA (mRNA) levels of MMP-9. MMP-9 mRNA levels decreased in MCF-7 cells transduced with ΔFosB interference recombinant adenoviruses Ad-ΔFosB-572, and ΔFosB overexpression increased the mRNA expression of MMP-9 (Fig. 1). We also found the inhibition effect of Ad-ΔFosB-572 on the mRNA expression of MMP-9 could be abrogated by ΔFosB overexpression (Fig. S1).

MMP-9 mediates the antiapoptotic effects of ΔFosB in MCF-7 cells

To assess whether the protective effect of ΔFosB is dependent on MMP-9, we pretreated MCF-7 cells with SB-3CT to analyze the effects of ΔFosB on cell survival. FITC-Annexin V/PI and Hoechst33342/PI staining assays showed an increased number of apoptotic MCF-7 cells after being treated with SB-3CT (Fig. 2 and Fig. S2). To further assay the supposed role of MMP-9 in ΔFosB-dependent cells protection actions, MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572, respectively. ΔFosB overexpression inhibited SB-3CT-induced apoptosis, and ΔFosB knockdown aggravated MCF-7 cell death (Fig. 2 and Fig. S2).

In order to confirm that MMP-9 is a key factor implicated in ΔFosB antiapoptotic actions in MCF-7 cells, we performed cell cycle and CCK-8 cell viability assays to evaluate the effects of SB-3CT on cell survival. We found that SB-3CT inhibited cell viability, and this effect was improved by ΔFosB overexpression in MCF-7 cells (Fig. 3). Cell cycle analysis revealed that SB-3CT significantly increased the number of MCF-7 cells in G0/G1 phase and subsequently decreased the proportion of cells in S and G2/M phases and thus confirmed the growth inhibitory effect of SB-3CT (Fig. 3). However, ΔFosB exhibited an increase in G2/M and S phases and improved the effect of SB-3CT on cell cycle (Fig. 3).

Thus, these observations suggest that the pro-survival role of ΔFosB in MCF-7 can be partly dependent on MMP-9.

MMP-9 mediates ΔFosB-induced increase of Bcl-2 expression in MCF-7 cells

Previous studies have described that antiapoptotic protein Bcl-2 overexpression inhibits apoptosis in mammary epithelial cells [26]. To characterize whether Bcl-2 could be regulated by ΔFosB in mammary carcinoma cells through a MMP-9-dependent pathway, we pretreated MCF-7 cells with SB-3CT

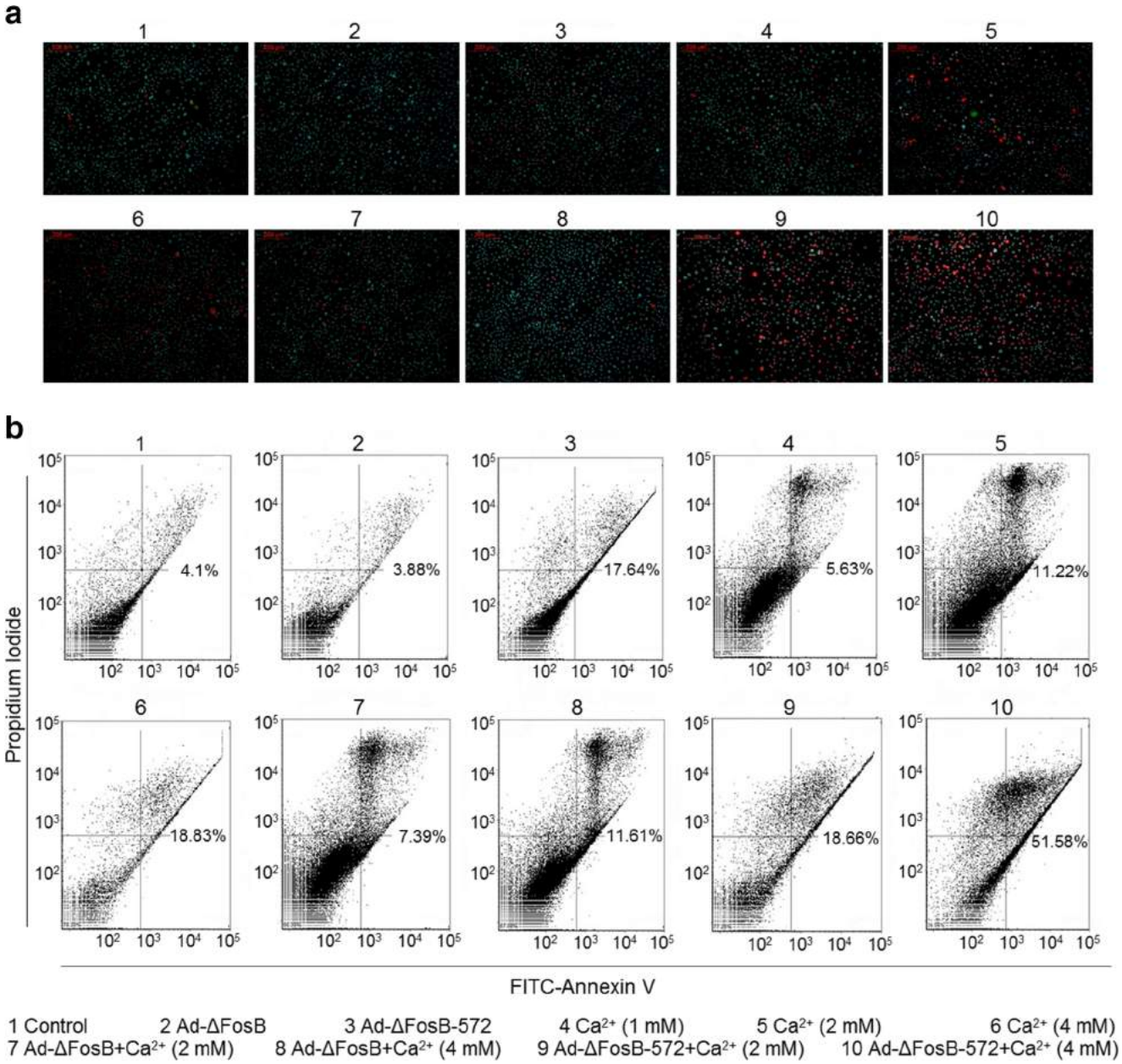

Fig. 6 ΔFosB-dependent protection from Ca^{2+}-induced cell apoptosis in MCF-7 breast cancer cells. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h, and subsequently exposed to different concentrations of Ca^{2+} for 24 h. Cell apoptosis was determined by Hoechst33342/PI dual staining assays (**a**) and Annexin V-FITC/PI binding followed by flow cytometry (**b**). For Hoechst33342/PI dual staining assays, Hoechst33342^{-}/PI^{-} cells (live cells) were in *light blue* and *lighter red*; Hoechst33342^{+}/PI^{+} cells (late apoptotic cells) were in *dark blue* and *bright red*. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB

and analyzed Bcl-2 expression in the presence or absence of Ad-ΔFosB or Ad-ΔFosB-572. We found that ΔFosB promoted the expression of Bcl-2, and SB-3CT inhibited this upregulation in MCF-7 cells (Fig. 4a). Then we wanted to know whether the expression of other Bcl-2 family members was regulated by ΔFosB: pro-survival gene Bcl-xl and proapoptotic genes Bax and Bad. We found that ΔFosB markedly increased the mRNA levels of Bcl-xl and decreased the expression of Bax and Bad in MCF-7 cells (Fig. 4b–d). In response to external stimuli, cellular Bcl-2/Bax or Bcl-xl/Bax ratio is a rheo-stat of regulating cell apoptosis. We found that ΔFosB increased both Bcl-2/Bax and Bcl-xl/Bax ratios in MCF-7 cells (Fig. 4e, f) and then SB-3CT specifically abolished the increased ratios. Moreover, we also detected the expression of tumor growth-related genes p53, CyclinD1, caspase-3, and caspase-9. We found that ΔFosB increased CyclinD1 expression and this upregulation was inhibited by SB-3CT in MCF-7 cells (Fig. 5a). By contrast, SB-3CT increased the mRNA levels of p53, caspase-3, and caspase-9, and

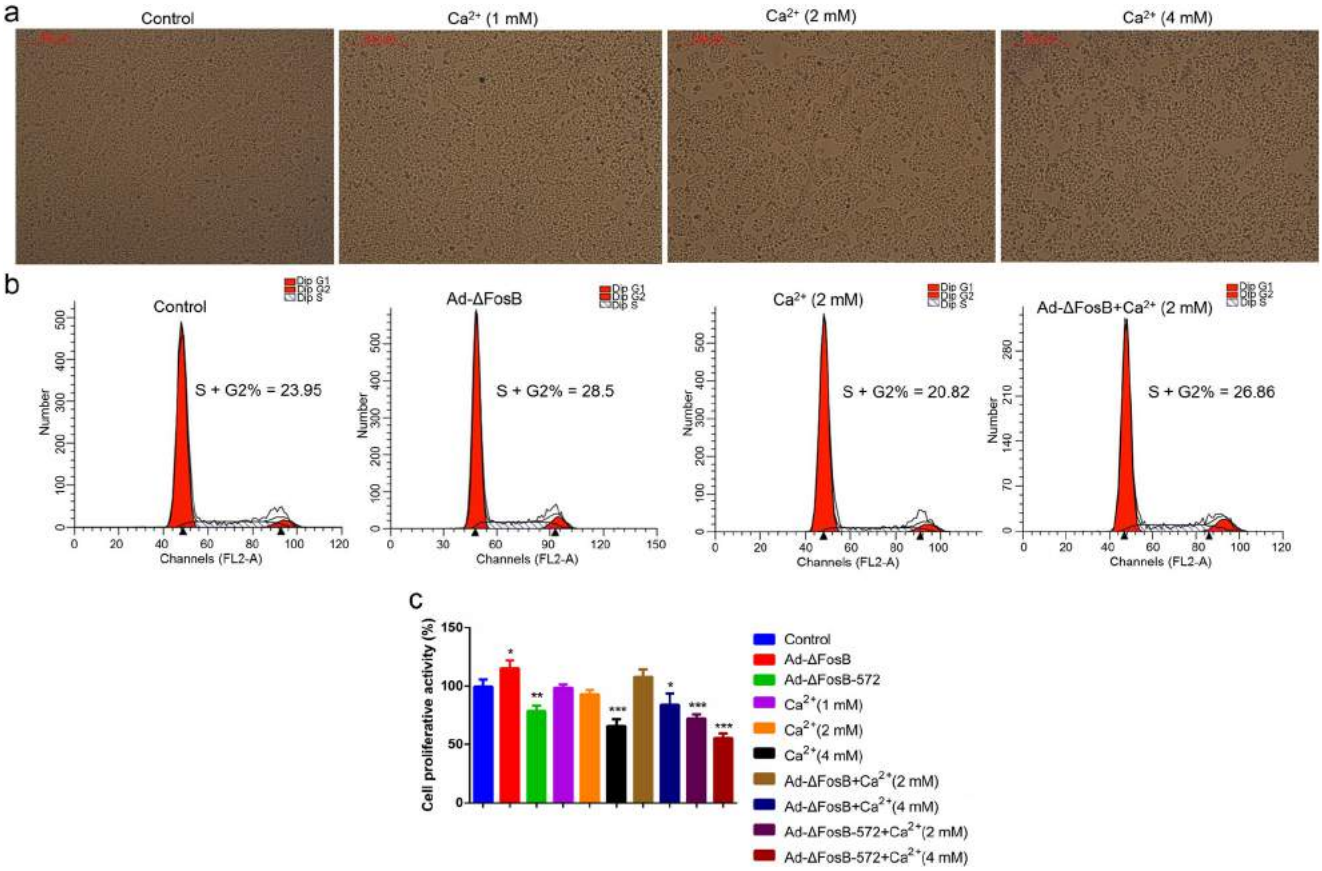

Fig. 7 Effects of ΔFosB on the cell cycle and proliferation in MCF-7 breast cancer cells. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h (**a**) and subsequently exposed to different concentrations of Ca^{2+} for 24 h and were subjected to cell cycle assay (**b**) and cell proliferation analysis by Cell Counting Kit-8 (CKK-8) assays (**c**). *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

this effect was specifically abrogated by ΔFosB overexpression (Fig. 5b–d).

Collectively, these results support the notion that MMP-9 can mediate ΔFosB induction of antiapoptotic proteins in breast cancer cells.

ΔFosB overexpression inhibits calcium-induced apoptosis in the MCF-7 cells

Calcium modulates many aspects of cell life, and intracellular calcium levels should be kept a dynamic equilibrium to avoid causing cellular dysfunction and death [27]. Our previous findings showed that ΔFosB overexpression in the goat mammary epithelial cell was associated with decreased intracellular calcium levels [23]. To explore the role of ΔFosB in calcium-induced apoptosis, we pretreated MCF-7 cells with different doses of $CaCl_2$ to analyze the effects of ΔFosB on cell survival. We found that calcium induced a dose-dependent increase of the mRNA expression of ΔFosB and MMP-9, and ΔFosB overexpression inhibited calcium-induced apoptosis (Fig. 6, Figs. S3 and S4). CCK-8 cell viability assays revealed that ΔFosB overexpression protected MCF-7 cells from calcium-induced cells' proliferation inhibition and ΔFosB interfering suppressed mammary epithelial cells' proliferation (Fig. 7c). We found that Ca^{2+} increased the number of MCF-7 cells in G0/G1 phase accompanied by decreasing the percentage of S and G2-M cells (Fig. 7b). ΔFosB overexpression in Ca^{2+}-treated MCF-7 cells increased the number of cells in G2/M and S phases and decreased the proportion of cells in G0/G1 phases (Fig. 7b). Consistent with this, calcium decreased the Bcl-2/Bax and Bcl-xl/Bax ratios, and ΔFosB specifically abrogated this effect (Fig. 8). Furthermore, calcium increased the mRNA levels of p53, caspase-3, and caspase-9 and inhibited CyclinD1 expression. This effect was specifically abrogated by ΔFosB overexpression (Fig. 9). Taken together, ΔFosB protects MCF-7 cells from calcium-induced apoptosis.

Discussion

We propose that ΔFosB, a member of AP-1 family, promotes MCF-7 cell proliferation and inhibits apoptosis by regulating

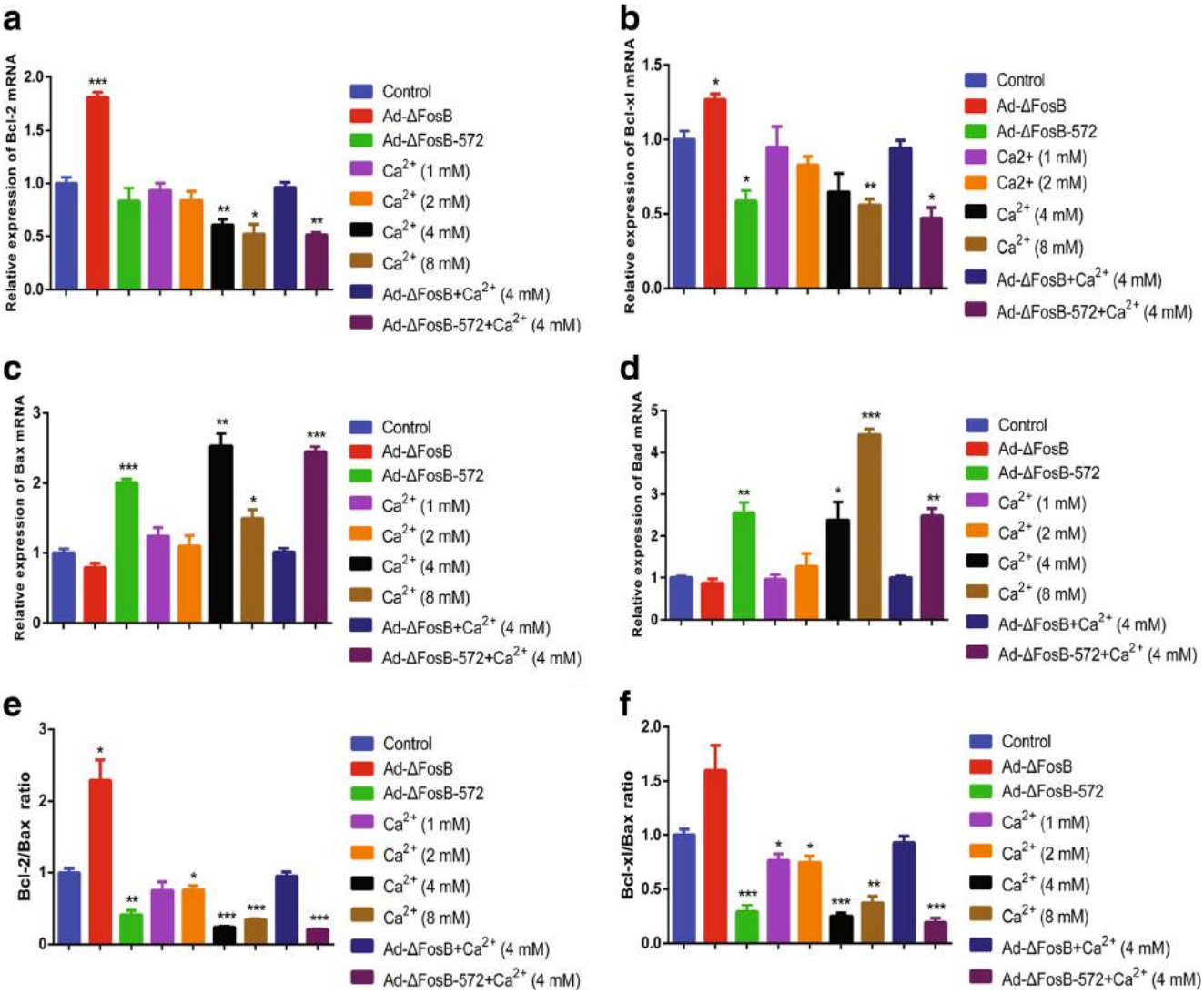

Fig. 8 Ca^{2+} mediates ΔFosB-induced increase of Bcl-2 expression in MCF-7 breast cancer cells. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of Ca^{2+} for 24 h. mRNA levels of cell survival-related genes Bcl-2 (**a**), Bcl-xl (**b**), Bax (**c**), Bad (**d**) were analyzed by RT-qPCR. Bcl-2/Bax (**e**) and Bcl-xL/Bax (**f**) ratios were also shown. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

MMP-9 expression. Transcription factor AP-1 could bind in MMP-9 promoter region and leads to the promotion of MMP-9 gene transcription [22, 28]. We showed that ΔFosB was found to increase MMP-9 mRNA levels and protect MCF-7 mammary carcinoma cells from death by increasing Bcl-xl and Bcl-2 expression and inactivating the proapoptotic proteins Bad and Bax. ΔFosB triggers cell proliferation in mammary epithelial cells with an increased expression of Runx2 [23], and high levels of Runx2 could induce a metastatic phenotype of breast cancer cells [29]. MMP-9 induces tumor growth and angiogenesis and necessary for tubular network formation in mammary carcinoma cells [13]. In vivo, primary breast tumors developing from MMP-9 knockdown cells present favorable prognosis, and MMP-9 overexpression tightly correlates with the most aggressive subtypes of breast cancer [30]. Our results suggested that MMP-9 has a marked consequence on cell viability and apoptosis in MCF-7 cells. Therefore, MMP-9 could be a mediator of the antiapoptotic effects of ΔFosB in the MCF-7 cells.

Intracellular calcium levels maintain at low levels, and many apoptotic pathways are triggered by intracellular calcium toxicity [27, 31]. Bcl-2 family proteins play vital roles in regulating Ca^{2+} signals because of their ability to either directly or indirectly affect the endoplasmic reticulum (ER) store content by interacting with inositol 1,4,5-trisphosphate receptor [32, 33]. As pro-survival genes, Bcl-2 and Bcl-xl inhibit ER Ca^{2+} release, whereas the proapoptotic Bcl-2 family members can promote ER Ca^{2+} release and mitochondrial Ca^{2+} uptake [33, 34]. ΔFosB increases the expression of Runx2 and Smad4 in

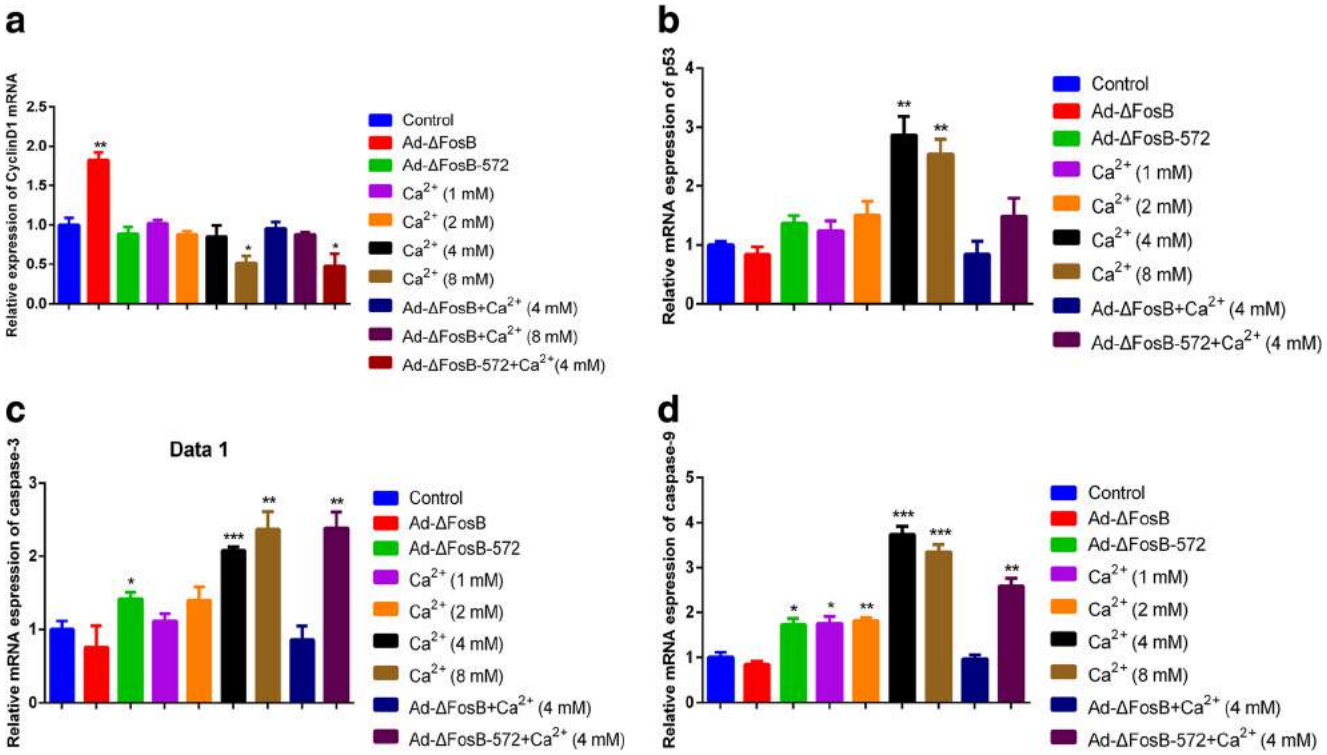

Fig. 9 Effects of Ca^{2+} on the expression of cell survival-related genes in MCF-7 breast cancer cells mediated by ΔFosB. MCF-7 cells were transduced with Ad-ΔFosB or Ad-ΔFosB-572 for 48 h and subsequently exposed to different concentrations of Ca^{2+} for 24 h. mRNA levels of MMP-9 and cell survival-related genes CyclinD1 (**a**), p53 (**b**), caspase-3 (**c**), caspase-9 (**d**) were analyzed by RT-qPCR. *Ad-ΔFosB* the overexpression recombinant adenoviruses of ΔFosB, *Ad-ΔFosB-572* the interference recombinant adenoviruses of ΔFosB. Values are means±SEM (n=3). *P<0.05; **P<0.01; ***P<0.001

goat mammary epithelial cells along with decreasing intracellular Ca^{2+} levels [23]. ΔFosB in mammals also increases bone formation and bone mass, especially promoting osteoblast differentiation [4, 9]. In this study, we found that ΔFosB overexpression protected MCF-7 cells from high intracellular Ca^{2+} levels and induced cell death with suppressing the mRNA levels of Bcl-2 and Bcl-xl.

In summary, our findings show that ΔFosB increases the expression of MMP-9 and exhibits a significantly high survival and proliferation in MCF-7 cells. We also report that ΔFosB can protect MCF-7 cells from calcium-induced apoptosis and promote cell cycle and proliferation. Thus, ΔFosB may be a potential target in breast cancer cell apoptosis by regulating the expression of MMP-9.

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Compliance with ethical standards

Conflicts of interest None.

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