Full Title:

AICAR induces apoptosis and inhibits migration of prostate cancer cells through an AMPK/mTOR-dependent pathway

Running Title:

AICAR inhibits the growth of prostate cancer cells through an AMPK/mTOR-dependent pathway

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Abstract

AICAR (5-aminoimidazole-4-carbox-amide-1-β-D-ribofuranoside), an AMP-activated protein kinase (AMPK) agonist, has demonstrated antitumor activities for several types of cancers. However, the activity of AICAR on the cell growth and metastasis of prostate cancer has not been extensively studied. Herein we examine the effects of AICAR on the cell growth and metastasis of prostate cancer cells, 22RV1 cells. Cell growth was performed by MTT assay and soft agar assay. Cell apoptosis was examined by Annexin V/PI staining and PARP cleavage Western blot. Cell migration was evaluated by wound-healing assay. The expression of EMT-related protein and the activity of the AMPK/ mTOR-dependent pathway were analyzed by Western blot. In addition, we also tested the effect of AICAR on the chemosensitivity to docetaxel using MTT assay. Our results indicated that AICAR inhibits cell growth, induces apoptosis, attenuates TGF-β-induced cell migration and EMT-related protein expression, and enhances the chemosensitivity to docetaxel through regulating the AMPK/mTOR-dependent pathway. Collectively, these findings support AICAR as a potential therapeutic agent for the treatment of prostate cancer.

Keywords: AICAR, AMPK, Prostate cancer, Metastasis, Chemosensitivity

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78 ## Introduction

79 Prostate cancer is the most common cancer and the second leading cause of
80 cancer-related death among men in the United States [1]. Current treatment options
81 for prostate cancer include surgery, hormonal therapy, chemotherapy, radiation
82 therapy, radiofrequency ablation, high-intensity focused ultrasound, cryotherapy, and
83 cancer vaccine [2, 3]. Androgen deprivation therapy (ADT) by surgical or chemical
84 castration has been the mainstay of treatment for advanced prostate cancer in the past
85 few decades. However, the majority of androgen-sensitive prostate cancer patients
86 will eventually develop resistance to ADT within 1 to 3 years and the disease will
87 become androgen-independent [4]. Currently, there is no effective therapy for
88 recurrent prostate cancer. Hence, a novel therapeutic method for prostate cancer is
89 needed.

90 The 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is
91 activated by increases in cellular ATP/AMP ratio and plays an important role in
92 regulating glycolytic activity and maintaining energy balance at both cellular and
93 whole body levels [5]. Previous studies indicated that AMPK exhibits intricate
94 relations with other energy/metabolite sensor pathways (e.g. SIRT1, Akt, mTOR,
95 PARPs, etc.) and acts in a coordinated fashion with these [6-10]. AMPK induction

96 leads to enhanced mitochondrial oxidation and mitochondrial biogenesis that have
97 been shown to exert anti-Warburg and anti-proliferative effects in several types of
98 cancers, such as leukemia [11], breast cancer [12], pancreatic cancer [13],
99 hepatocellular carcinoma [14], and prostate cancer [15]. These findings suggest that
100 AMPK activation may be used beneficially for cancer treatment.

101 The adenoside analog compound,
102 5-aminoimidazole-4-carbox-amide-1-β-D-ribofuranoside (AICAR), is intracellularly
103 converted by adenosine kinase to the non-phosphorylated derivative
104 amino-imidazolecarboxamide ribonucleotide (ZMP), an analogue of AMP, which
105 activates AMPK [16]. Therefore, AICAR is often used as an activator of AMPK [17]
106 to modulate cellular energy homeostasis, although AMPK-independent effects have
107 also been proposed [18, 19]. A previous study indicated that AICAR inhibited the
108 growth of androgen-independent (DU145, PC3) and androgen-sensitive (LNCaP)
109 cells after four days of treatment [20]. In addition, AICAR inhibited two key enzymes
110 involved in protein synthesis, mTOR and p70S6K, and blocked the ability of the
111 androgen R1881 to increase cell growth and the expression of two enzymes for *de*
112 *novo* fatty acid synthesis, acetyl CoA carboxylase and fatty acid synthase, in the
113 LNCaP cells [20]. A current study showed that AICAR induced AMPK-independent
114 programmed necrosis in prostate cancer cells [21]. Collectively, these studies indicate

115 that AICAR has potential in inhibiting the growth of prostate cancer. However, the
116 effect of AICAR on the apoptosis and migration of prostate cancer remains unclear. In
117 the present study, we evaluated the effects of AICAR on apoptotic activity, migration
118 and chemosensitivity through AMPK phosphorylation in human prostate cancer cells.
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Materials and Methods

Reagents

RPMI 1640 medium, penicillin, streptomycin and fetal bovine serum (FBS) were purchased from Gibco-BRL (Life Technologies, Grand Island, NY, USA). AICAR, bovine serum albumin (BSA), phosphate-buffered saline (PBS), RIPA buffer, protease inhibitor cocktail, phosphatase inhibitor cocktail, stripping buffer, thioglycollate medium, and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA). AICAR was purchased from Cayman Chemical (Ann Arbor, MI, USA). Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit was purchased from Thermo Fisher Scientific (Eugene, Oregon, OR, USA). BCA protein assay reagent was purchased from Thermo Scientific (Waltham, MA, USA). For western blotting, rabbit antibodies against human phospho-AMPK, AMPK, MYC, mTOR, PARP, phospho-p70S6K, p70S6K, TSC-1, TSC-2, β-actin and secondary antibodies were purchased from Cell Signaling (Farmingdale, NY, USA). Caspase-Glo 3/7 assay kit was purchased from Promega (Madison, WI, USA). Transforming growth factor-beta 1 was purchased from PeproTech (Rocky Hill, NJ, USA). Docetaxel (Taxothere, 20 mg/mL) was obtained from Sanofi Aventis (Germany).

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154 **Cell culture**

155 Human prostate cancer cell line, 22Rv1, purchased from Bioresource Collection and
156 Research Center (Food Industry Research and Development Institute, Hsinchu,
157 Taiwan), and was cultured in RPMI 1640 supplemented with antibiotics (100 U/mL
158 penicillin and 100 U/mL streptomycin) and 10% (v/v) FBS in a humidified
159 atmosphere of 5% CO_2 at 37˚C and passaged every 2-3 days to maintain growth.

160

161 **MTT assay**

162 22Rv1 cells were seeded in a 96-well plate at a concentration of 1 x 10^5 cells, and
163 were allowed to acclimatize overnight. Cells were treated with various concentrations
164 of the AICAR (0, 0.5, 1 and 3 mM) for 24 hr. Cell viability was measured by the
165 ability of viable cells to reduce MTT to formazan based on the ability of living cells
166 utilized thiazolyl blue and converted it into purple formazan. The concentration of
167 formazan is measured by determining the OD at 570 nm using a microplate reader
168 (BioTek Instruments, Inc., Winooski, VT, USA). The results are given as relative
169 percentage to the untreated control. To detect the synergistic effects of AICAR, cells
170 were treated with different doses of the AICAR (0, 0.5, and 1 mM) combined with
171 different doses of docetaxel for 24 and 48 hr.

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173 **Soft agar colony formation assay**

174 Noble agar (BD Biosciences, Franklin Lakes, NJ, USA) was dissolved in complete
175 medium and coated with 0.5% agar solution on the bottom of 6-well plates. After
176 solidifying, top agar medium mixture (0.3%) containing 5 x 10^3 cells was added, and
177 incubated at 37 ˚C in a humidified atmosphere of 5% CO_2 for 3 weeks. Colonies were
178 stained with 0.05% crystal violet-10% ethanol in PBS. Photographs of the
179 stained colonies were captured using Bio-Rad ChemiDoc XRS$^+$ system (Bio-Rad
180 Laboratories, Inc., Hercules, CA, USA) and quantified using ImageJ software
181 (National Institutes of Health, Bethesda, MD, USA).

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183 **Apoptosis assay**

184 22Rv1 cells were treated with different doses of AICAR (0, 0.5, 1, and 3 mM) for 24
185 hr. Cells were trypsinized, washed twice by cold PBS, and stained with Alexa Fluor®
186 488 Annexin V and propidium iodide (PI) according to manufacturer's protocol
187 (Thermo Fisher Scientific, Rockford, IL, USA). Apoptotic cells were determined
188 using FC500 flow cytometer (Beckman-Coulter, Fullerton, CA, USA). Ten thousand
189 events were collected per sample. Data were analyzed by CXP analysis software
190 (Beckman-Coulter, Fullerton, CA, USA).

191

192 **Western blot**

193 Cells were lysed by RIPA buffer with protease inhibitors and phosphatase inhibitors
194 according to the manufacturer's protocol (Sigma Aldrich, St. Louis, MO, USA). The
195 protein concentration was determined using the BCA protein assay reagent according
196 to the manufacturer's instructions (Thermo Scientific, Waltham, MA, USA). Cellular
197 protein extracts were separated by electrophoresis using 8% SDS polyacrylamide gel
198 and were electroblotted onto PVDF membranes. The membranes were incubated with
199 blocking solution for 1 hr at room temperature, followed by incubation overnight with
200 primary antibodies at 4 ˚C (1:1000). Blots were washed three times with Tris-buffered
201 saline/Tween 20 (TBST) and incubated with a 1:5000 dilution of horseradish
202 peroxidase conjugated secondary antibody for 1 hr at room temperature. Blots were
203 again washed three times with TBST and developed using an ECL
204 chemiluminescence substrate (Thermo Scientific, Waltham, MA, USA). The signals
205 were captured and the band intensities were quantified using Bio-Rad ChemiDoc
206 XRS$^+$ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

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208 **Caspase 3/7 activity assay**

209 22Rv1 cells were seeded in a 96-well white plate at a concentration of 2.5 x 10^4 cells,

and were allowed to acclimatize overnight. Cells were treated with various concentrations of the AICAR (0, 0.5, 1, and 3 mM) for 24 hr. A total of 100 μL Caspase-Glo® 3/7 reagent was added to each well, gently mixed contents of wells using a plate shaker at 300~500 rpm for 30 seconds, and then samples were incubated for 30 min at RT. Enzyme activity is directly proportional to luminescence, which was measured using a luminescence microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Data were normalized relative to the caspase 3/7 activity of cells treated with DMSO alone.

Migration assay

SPLScar™ Block (SPL life sciences, Korea) was placed in a 24-well plate, seeded 5 x 10^4 cells in each side of block, and was allowed to acclimatize overnight. The block was removed, replaced flash medium, and treated cells with 5 ng/ml TGF-β1 and different concentrations of AICAR. Cell migration was monitored under a phase-contrast microscope and the migratory distance was calculated.

Statistical analysis

All data are expressed as means ± SD. Each value is the mean of three independent experiments. Statistical analysis was assessed via Student's *t*-test using IBM SPSS

229 Statistics v.19 (IBM Corp., Armonk, NY, USA), and the significant difference was set

230 at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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Results

AICAR inhibits the growth of prostate cancer cells

The 22Rv1 cell line is an androgen-independent prostate cancer epithelial cell line that is representative of clinical recurrent prostate cancer [22]. Firstly, we examined the effect of AICAR on the growth of 22Rv1 prostate cancer cells. Cells were treated with various concentrations (0, 0.5, 1 and 3 mM) of AICAR for 24 hr. Cell viability was analyzed by MTT assay. The experimental results showed that AICAR inhibits the growth of 22Rv1 cells in a dose-dependent manner (Fig. 1A). In addition, we further examined whether AICAR affects the colony growth under anchorage-independent condition. Cells were treated with different concentrations (0, 0.25, 0.5 and 1 mM) of AICAR and then conducted a soft agar assay. The experimental results showed that AICAR suppressed growth of 22Rv1 cells in soft agar in a dose-dependent manner (Fig. 1B).

AICAR induces apoptosis in prostate cancer cells

To further test whether AICAR induces apoptosis in prostate cancer cells, 22Rv1 cells were treated with various concentrations (0, 0.5, 1 and 3 mM) of AICAR for 24 hr. Apoptotic cells were detected with Annexin V/PI staining using flow cytometry. Our

267 results demonstrated that AICAR induced apoptosis (Fig. 2A). Poly(ADP-ribose)
268 polymerase (PARP) is a nuclear enzyme involved in DNA repair which is cleaved by
269 caspase-3 during apoptosis [23]. We further examined whether AICAR affects the
270 expression of PARP in 22Rv1 cells. As shown in Figure 2B, AICAR increased the
271 expression of cleaved PARP, an apoptosis marker, in 22Rv1 cells. In addition, we
272 also examined the activity of caspase 3/7 using a luminescent substrate based assay.
273 Our results indicated that AICAR increased the activity of caspase 3/7 in 22Rv1 cells
274 (Fig. 2C).
275
276 **AICAR inhibits transforming growth factor-beta (TGF-β)-induced migration**
277 **and epithelial to mesenchymal transition (EMT)**
278 TGF-β signaling is well known as a key regulator of many biological processes in
279 prostate cancer including inducing EMT, migration and metastasis [24]. To examine
280 whether AICAR affects TGF-β-induced migration and EMT in prostate cancer cells,
281 22Rv1 cells were treated with 5 ng/mL TGF-β and various concentrations (0, 0.25 and
282 0.5 mM) of AICAR. Cell migration was performed by counting the average wound
283 space. Our results showed that AICAR significantly inhibited TGF-β-induced
284 migration (Fig. 3A and 3B). In addition, we also analyzed the expression of
285 EMT-related proteins using Western blot. As shown in Figure 3C, AICAR inhibited

286 TGF-β-induced EMT through inhibiting the expression of mesenchymal marker,
287 N-cadherin, and enhancing the expression of epithelial marker, E-cadherin.
288
289 **AICAR exhibits synergistic effect with docetaxel treatment**
290 Docetaxel-based therapy has been applied as the first-line chemotherapy for
291 metastatic castration-resistant prostate cancer (mCRPC) for a decade due to its ability
292 to improve the median overall survival (OS) by around 3 months [25, 26]. To examine
293 whether AICAR affects the therapeutic efficiency of docetaxel-based therapy in
294 prostate cancer, 22Rv1 cells were treated with various doses (0, 0.5 and 1 mM) of
295 AICAR in the presence or absence of docetaxel for 24 hr. Cell survival was analyzed
296 by MTT assay. As shown in Fig. 4, AICAR exhibited synergistic effect with
297 docetaxel treatment in prostate cancer cells.
298
299 **AICAR inhibits the growth of prostate cancer cells through activating an**
300 **AMPK/mTOR-dependent pathway**
301 AMPK is an upstream regulator in modulating the activation of TSC1 and TSC2
302 which consequently inhibits cell survival and proliferation via repressed
303 mTOR-p70S6K-MYC signaling pathway [27, 28]. To investigate the mechanism of
304 action of AICAR in regulating the activity of AMPK-dependent pathway, 22Rv1 cells

were treated with different concentrations (0, 0.5, 1 and 3 mM) of AICAR. The expression of phospho-AMPK, AMPK, TSC-1, TSC-2, mTOR, phospho-p70S6K, p70S6K and MYC was analyzed by Western blot. As shown in Fig. 5A, AICAR promoted the phosphorylation of AMPK in 22Rv1 cells. In addition, our experimental results showed that AICAR enhanced the expression of TSC1 and TSC2 (Fig. 5B), whereas AICAR reduced the expression of mTOR and MYC as well as decreased the phosphorylation of p70S6K (Fig. 5C). These results suggest that AICAR inhibits the growth of prostate cancer cells through an AMPK/mTOR-dependent pathway (Fig. 5D).

Discussion

AMPK is an important regulator of cellular energy homeostasis [29]. Several agents have been demonstrated to activate AMPK, including metformin and phenformin, which increase the AMP:ATP ratio, the nucleoside AICAR which is metabolized to an AMP mimetic, and A769662, which is a direct activator of AMPK [30]. Although a number of studies indicated an anti-cancer role for AMPK [9, 11, 12, 14, 15, 31], the effect of an AMPK activator, AICAR, in regulating the pathological processes of prostate cancer remains enigmatic. Previous studies have shown that AICAR could exert cytotoxic effect on cancer cells via AMPK-dependent and/or AMPK-independent mechanisms [16, 31-33]. However, the cytotoxic effect on prostate cancer remains controversial. Guo et al. indicated that AICAR induces programmed necrosis, but not apoptosis, in prostate cancer cells, LNCaP, PC-3 and PC-82 cells through AMPK-independent pathways [21]. In contrast, Sauer et al. demonstrated that AICAR induces apoptosis of DU-145 prostate cancer cells through the AMPK/mTOR-dependent signaling pathway [16]. Our experimental results indicated that AICAR induces apoptosis, but not necrosis, in 22Rv1 prostate cancer cells. These results point out that AICAR inhibits cell growth and induces cell death in different prostate cancer cell lines probably through different mechanisms. In

addition, AMPK is an upstream regulator in modulating the activation of TSC1 and TSC2, which consequently inhibits cell survival and proliferation via repressed mTOR-p70S6K-MYC signaling pathway [27, 28]. Our experimental results showed that AICAR enhances the expression of TSC1 and TSC2, whereas it reduces the expression of mTOR and MYC as well as decreases the phosphorylation of p70S6K in 22Rv1 prostate cancer cells. We suggest that AICAR induces apoptosis and inhibits migration of prostate cancer cells through an AMPK/mTOR-dependent pathway.

Cancer metastasis is the major cause of morbidity and mortality; approximately 80% of the men who have expired from prostate cancer possessed bone metastases [34]. Our experimental results indicated that AICAR suppresses TGF-β-induced cell migration and inhibits TGF-β-induced EMT by decreasing the expression of a mesenchymal-related marker, N-cadherin and enhancing the expression of an epithelial-related marker, E-cadherin in 22Rv1 prostate cancer cells. A recent study also indicated that treatment of AICAR suppresses migration and invasion in PC3 and PC3M prostate cancer cells [15]. These results implied that AICAR might have potential in inhibiting the metastatic activity in prostate cancer.

Docetaxel is a first-line treatment for CRPC, which confers survival advantages of approximately 2 months for patients with low overall survival benefit [35]. However, treatment with docetaxel usually causes adverse side effects including hair

loss, myelosuppression, neurotoxicity, diarrhea etc. Our experimental results showed that AICAR has synergistic effect with docetaxel treatment. These results suggest that AICAR increases the sensitization of prostate cancer cells to docetaxel-induced apoptosis, which might have benefit for reducing toxicity of chemotherapy in prostate cancer patients.

In summary, our experimental results indicated that AICAR inhibits cell growth, induces apoptosis, attenuates cell migration, enhances chemosensitivity to docetaxel, and suppresses the activation of the AMPK/mTOR-dependent signaling pathway. These results suggest that AICAR appears as a new potential anticancer agent for treating prostate cancer.

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Competing interests:

The authors declare that they have no conflict of interest.

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571

572

573 **Figure legends**

574 Figure 1. The effect of AICAR on the growth of prostate cancer cells. (A) 22Rv1 cells
575 were treated with different concentration of AICAR for 24 hr. The cell viability was
576 measured by the MTT assay. (B) 22Rv1 cells were treated with different
577 concentration of AICAR, and then grown in soft agar, an anchorage-independent
578 condition, for 3 weeks. Colonies were stained with crystal violet and captured using
579 Bio-Rad ChemiDoc XRS$^+$ system. Data are represented as means ± SD of triplicate
580 values and statistical significance was determined using the Student's *t*-test (***p*<
581 0.01; ****p*< 0.001).

582

583 Figure 2. Effect of AICAR on the apoptosis in 22Rv1 prostate cancer cells. Cells were
584 incubated with different concentrations of AICAR for 24 hr. (A) Cells were collected,
585 stained with Annexin V and PI, and analyzed by flow cytometry. Data are
586 representative of at least three independent experiments with similar results. (B) The
587 expression of PARP was determined by Western blot. Actin was used as a loading
588 control in Western blot. (C) Cellular caspase 3/7 activities were analyzed with
589 caspase-glo assay kit. Data are represented as means ± SD of triplicate values and

590 statistical significance was determined using the Student's *t*-test (***$p< 0.001$).

591

592 Figure 3. Effect of AICAR on the cell migration activity in 22Rv1 prostate cancer
593 cells. Cells were seeded in SPLScar^{TM} Block overnight, the block was then removed
594 and cells were treated with 5 ng/ml TGF-β1 and different concentrations of AICAR.
595 (A) Cell migration was monitored under a phase-contrast microscope. Data are
596 representative of at least three independent experiments with similar results. (B) The
597 migratory distance was calculated. Data are represented as means ± SD of triplicate
598 values and statistical significance was determined using the Student's *t*-test (*$p< 0.05$;
599 **$p< 0.01$; ***$p< 0.001$). (C) The expression of N-cadherin and E-cadherin was
600 analyzed by Western blot. Actin was used as a loading control in Western blot. The
601 Western blotting results are representative of results obtained in three separate
602 experiments.

603

604 Figure 4. Effect of AICAR on the sensitivity of 22Rv1 prostate cancer cells to
605 docetaxel treatment. Cells were treated with different concentrations of AICAR in the
606 presence or absence of docetaxel for 24 hr. The cell viability was measured by the
607 MTT assay. Data are represented as means ± SD of triplicate values and statistical
608 significance was determined using the Student's t -test (*$p< 0.05$; **$p< 0.01$).

609

610 Figure 5. Effect of AICAR on the AMPK/mTOR-dependent pathway in 22Rv1
611 prostate cancer cells. Cells were treated with different concentrations of AICAR for 2
612 hr. The expression of (A) phospho-AMPK, AMPK, (B) TSC1 and TSC2 was
613 examined by Western blot. Cells were treated with different concentrations of AICAR
614 for 6 hr. (C) The expression of mTOR, cMYC, phosphor-p70S6K and p70S6K was
615 determined by Western blot. Actin was used as a loading control in Western blot. The
616 Western blotting results are representative of results obtained in three separate
617 experiments. (D) Proposed mechanism of the anticancer effects induced by AICAR in
618 22Rv1 prostate cancer cells.

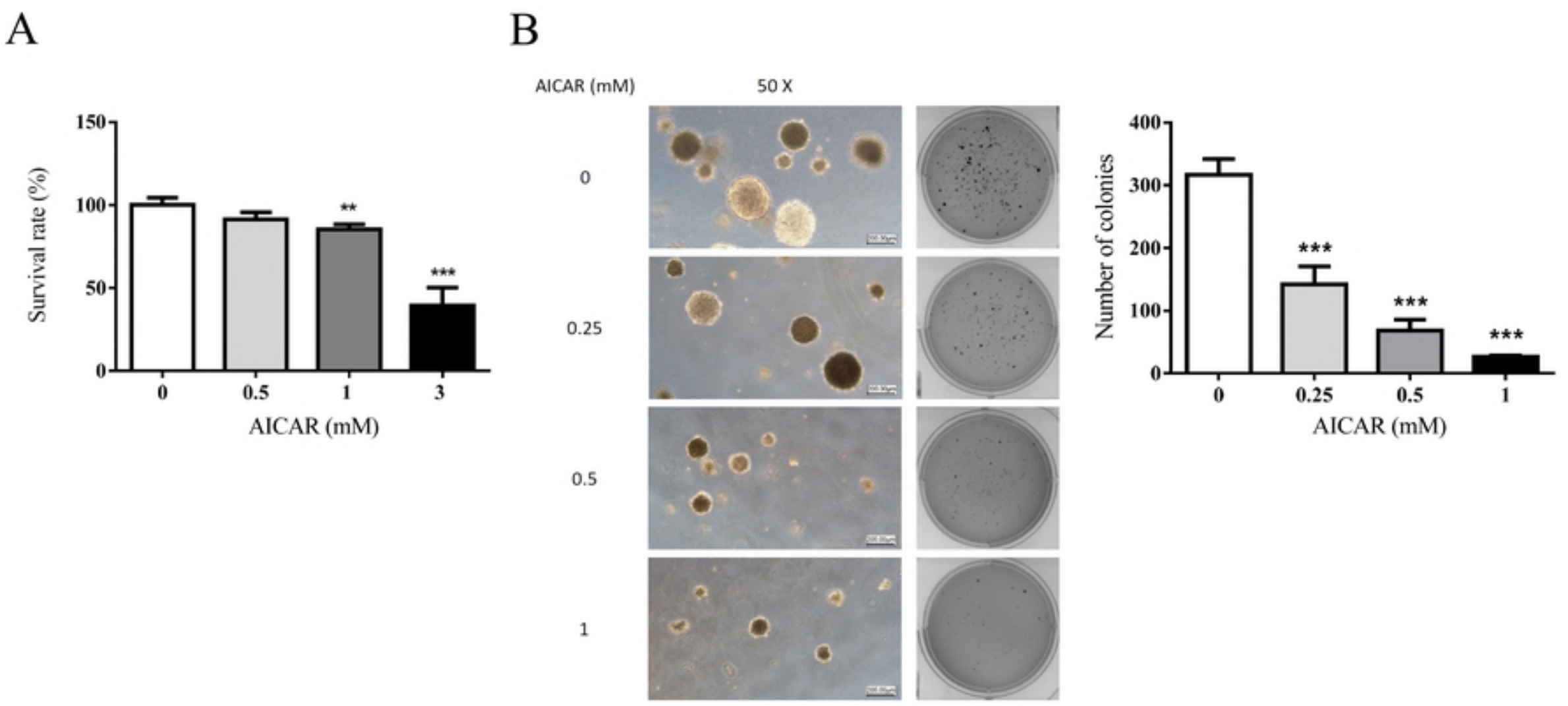
A
Survival rate (%)
150
100
50
0
**
***
0
0.5
1
3
AICAR (mM)
B
AICAR (mM)
50 X
0
0.25
0.5
1
Number of colonies
400
300
200
100
0
***
***
***
0
0.25
0.5
1
AICAR (mM)

Figure 1

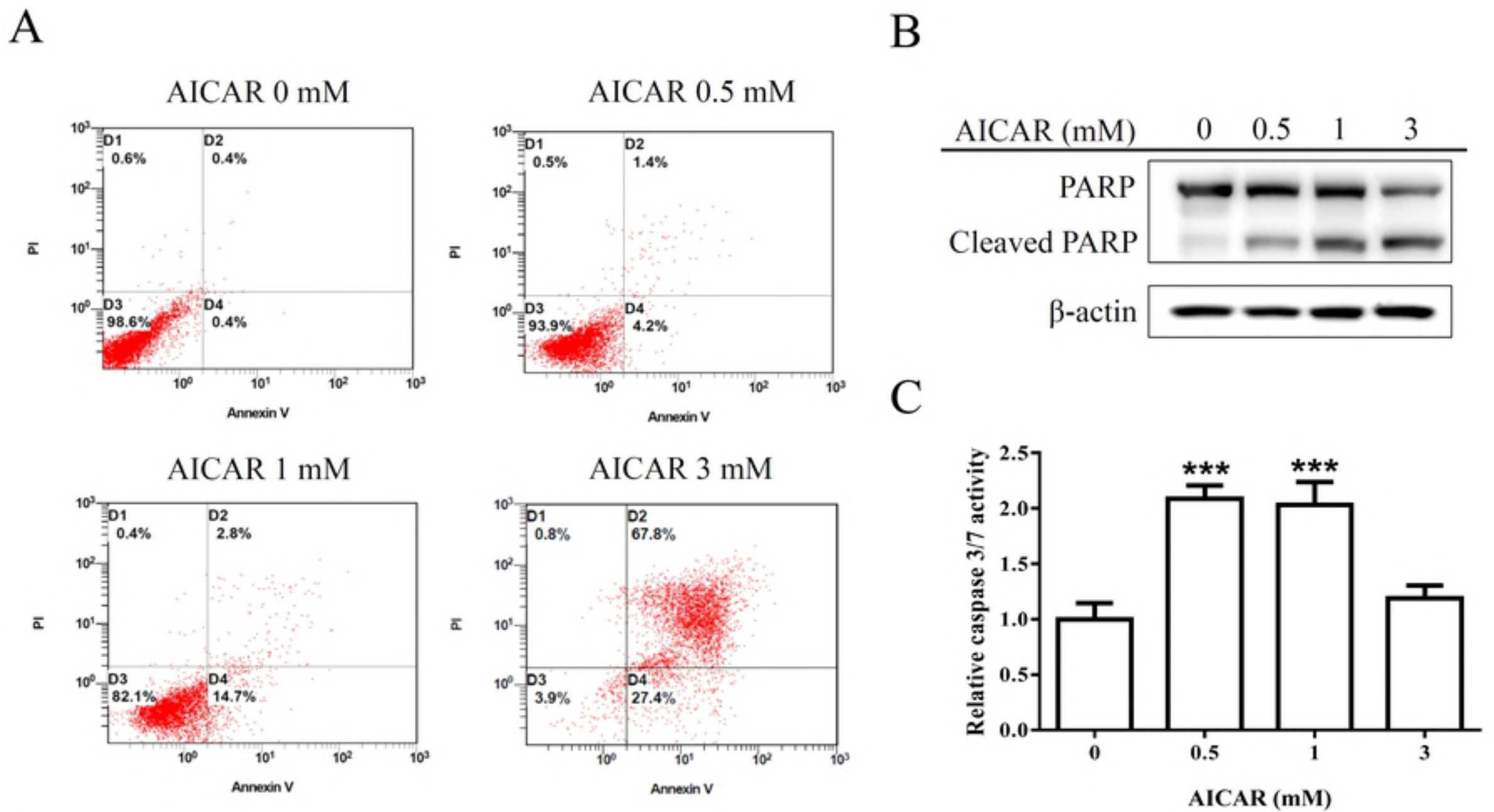
A
AICAR 0 mM
D1
0.6%
D2
0.4%
D3
98.6%
D4
0.4%
PI
Annexin V
AICAR 0.5 mM
D1
0.5%
D2
1.4%
D3
93.9%
D4
4.2%
PI
Annexin V
AICAR 1 mM
D1
0.4%
D2
2.8%
D3
82.1%
D4
14.7%
PI
Annexin V
AICAR 3 mM
D1
0.8%
D2
67.8%
D3
3.9%
D4
27.4%
PI
Annexin V
B
AICAR (mM)
0
0.5
1
3
PARP
Cleaved PARP
β-actin
C
Relative caspase 3/7 activity
***
***
AICAR (mM)

Figure 2

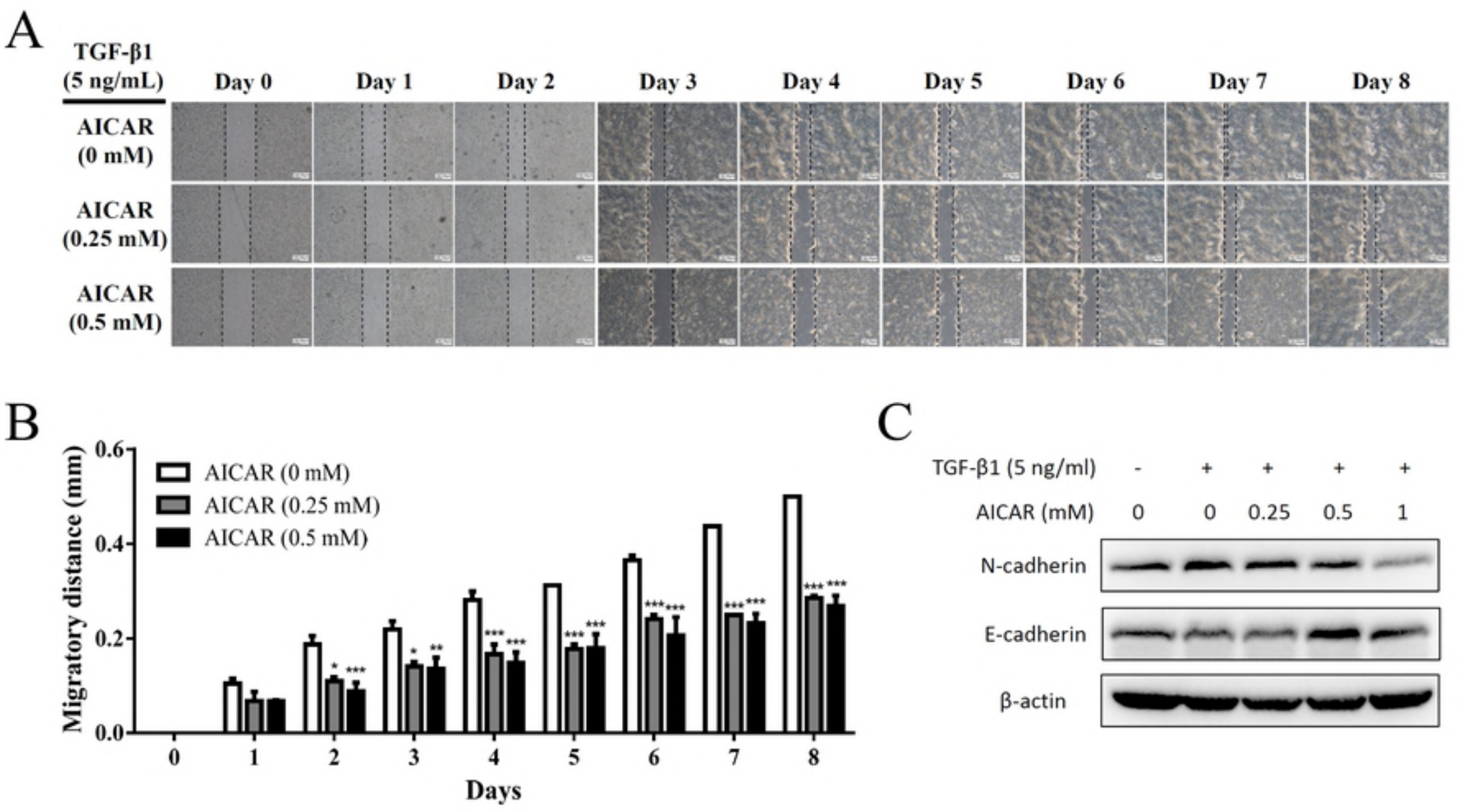
A
TGF-β1
(5 ng/mL)
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
AICAR
(0 mM)
AICAR
(0.25 mM)
AICAR
(0.5 mM)
B
Migratory distance (mm)
0.6
0.4
0.2
0.0
AICAR (0 mM)
AICAR (0.25 mM)
AICAR (0.5 mM)
0
1
2
3
4
5
6
7
8
Days
C
TGF-β1 (5 ng/ml)
-
+
+
+
+
AICAR (mM)
0
0
0.25
0.5
1
N-cadherin
E-cadherin
β-actin

Figure 3

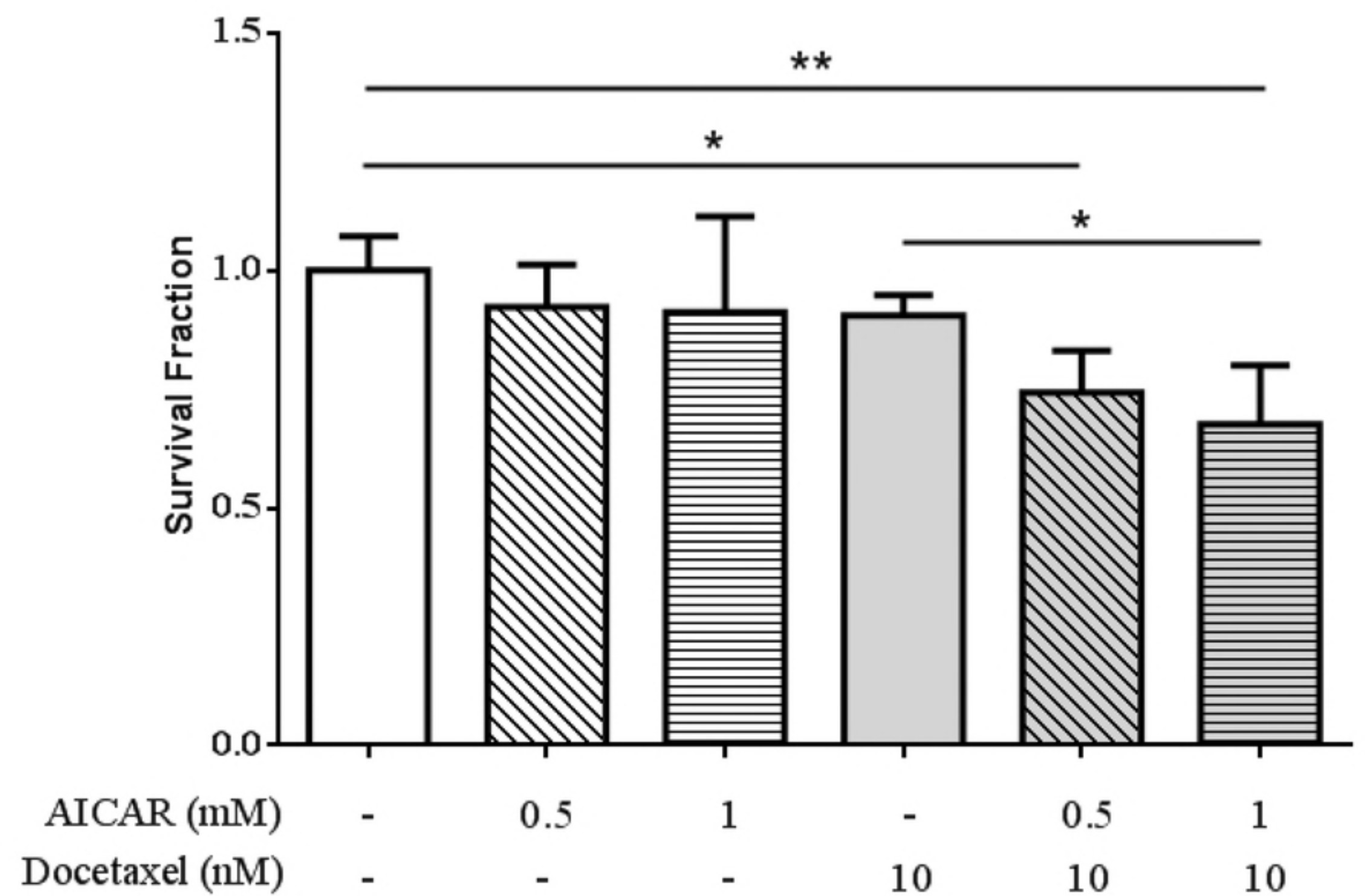
1.5
1.0
0.5
0.0
Survival Fraction
**
*
*
AICAR (mM) - 0.5 1 - 0.5 1
Docetaxel (nM) - - - 10 10 10

Figure 4

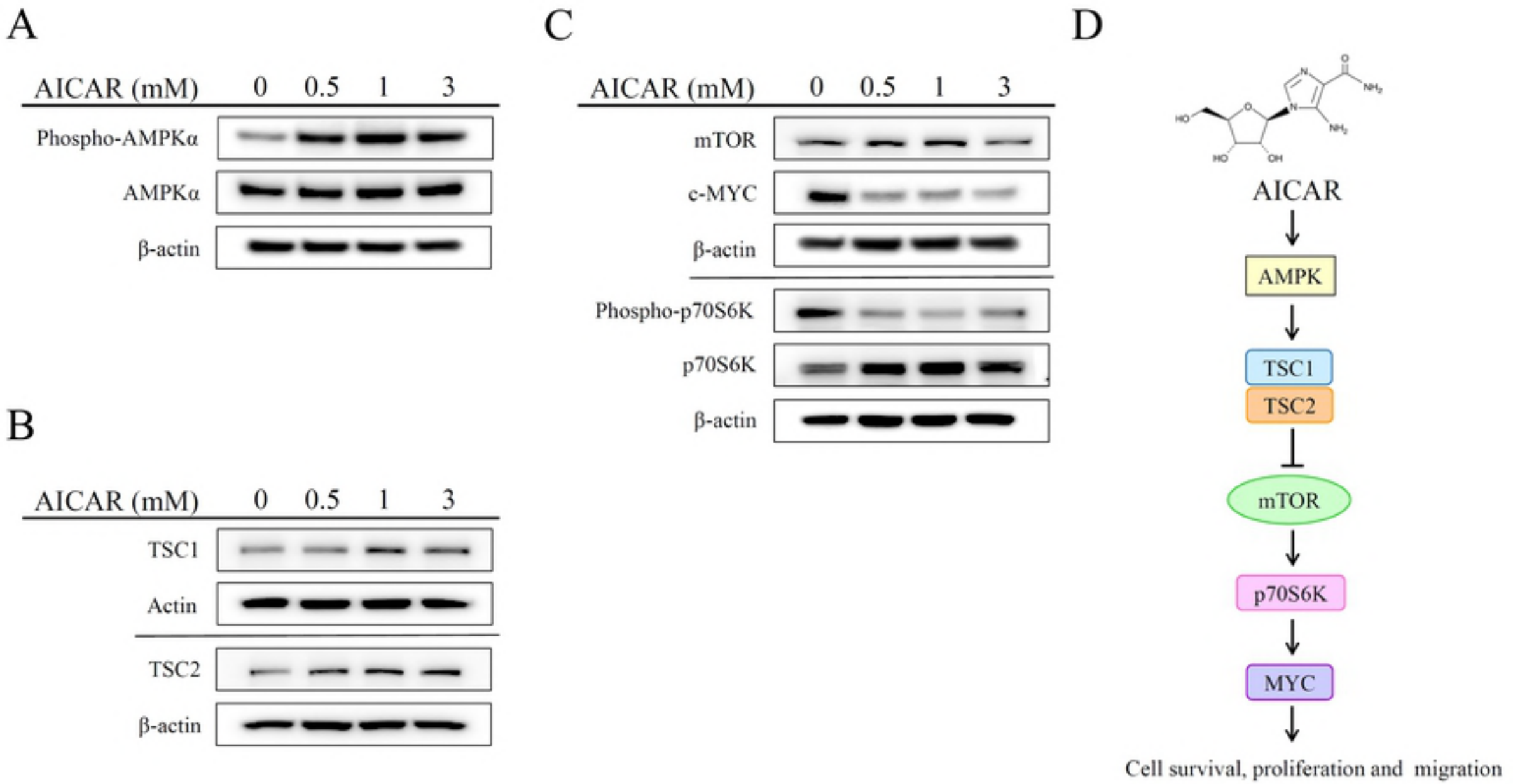
A
AICAR (mM) 0 0.5 1 3
Phospho-AMPKα
AMPKα
β-actin
B
AICAR (mM) 0 0.5 1 3
TSC1
Actin
TSC2
β-actin
C
AICAR (mM) 0 0.5 1 3
mTOR
c-MYC
β-actin
Phospho-p70S6K
p70S6K
β-actin
D
AICAR
AMPK
TSC1
TSC2
mTOR
p70S6K
MYC
Cell survival, proliferation and migration

Figure 5