제 **111** 회 박사학위논문

지도교수 박 언 섭

Capmatinib suppresses lipogenesis and increases lipolysis in 3T3-L1 adipocytes through an irisin / adenine monophosphate-activated protein kinase-dependent pathway

캡마티닙의 **irisin/AMPK** 의존 경로를 통한 지방 전구세포에서의 지방합성 억제와 지방분해의 증가

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이 논문을 박사학위논문으로 제출함

2022 年 **8** 月

중앙대학교 대학원

의학과 병리학 전공

안 성 호

안 성 호의 박사학위논문으로 인정함

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Contents

I. Introduction

Increased prevalence of obesity has become an emerging public health issue in modern society due to the physical inactivity and high-calorie dietary habits (Kopelman et al., 2000, Visscher et al., 2001). Obesity is known to be associated with several metabolic disorders including type 2 diabetes, cardiovascular diseases, and elevated risk of premature death (Van Gaal et al., 2006, Apovian et al., 2016). Thus, not only proper behavioral interventions, but there are also further demands for developing safe and novel pharmacological treatment as an effective strategy for controlling obesity (Wadden et al., 2002, Srivastava et al., 2018). Obesity is defined by an excessive or abnormal accumulation of fat in the body, caused due to enlargement of adipose tissue to reserve excessive energy (Ogden et al., 2007). Imbalance between energy intake and expenditure affect the adipocytes by increasing the cell number and cell size. Accordingly, excessive energy consumption results in adipose tissue expansion and increase of adiposity (Lee et al., 2009, Heymsfield et al., 2017). Therefore, modulating adipocyte hyperplasia and hypertrophy can be a potential mechanism for further therapeutic strategy of controlling obesity.

Capmatinib (CAP) is a highly selective and potent adenosine triphosphate (ATP)-binding blocker of Mesenchymal-epithelial transition factor (c-Met) with an acceptable safety profile, which is evaluated to be a promising treatment option for patient with c-Met dysregulated non-small cell lung cancer (Bouattour et al., 2018, Vansteenkiste et al., 2019). c-Met is a member of the tyrosine kinase receptor family engaged in signaling cascade of cell proliferation, motility, and migration (Zhang et al., 2018). The abnormal stimulation of the hepatocyte growth factor/ c-Met axis is reported to be related with progression and spread of several human malignancies (Zhang et al., 2019, Eder et al., 2009). Thus, c-Met inhibitors are suggested to be potential therapeutic agents for various malignancies (Mo and Liu., 2017).

In recent study, c-Met inactivation also shows inhibition effect in differentiation of 3T3-L1 adipocytes, which may be promising targets for lowering excessive fat (Tang et al., 2017). Furthermore, CAP is suggested to attenuate acetaminophen-induced hepatic inflammation and oxidative stress (Saad et al., 2020), which are reported to intensify adipocyte differentiation (Lee et al., 2009) and closely related with development of obesity (Fernandez et al., 2011). Therefore, I hypothesized that CAP may present anti-obesity effects by regulating adipocyte differentiation and lipid

accumulation.

By using 3T3-L1 adipocytes, I examined the effects of CAP on adipocyte differentiation and lipolysis and explicated the mechanisms underlying these effects. The results of this study suggest that CAP can reduce lipid accumulation within adipocyte differentiation and increase lipolysis through the irisin/adenine monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway.

II. Materials and Methods

Cell culture and treatments

The 3T3-L1 cells (pre-adipocytes) (American Type Cell Culture, Manassas, VA, USA) were incubated in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplied with 10% fetal bovine serum (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C. For differentiation, the cells were grown to confluence (obtained on day 2 of culture) and cultured in a medium supplemented with MDI adipogenic cocktail (1 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, St Louis, MO, USA), and 0.5 μg/mL dexamethasone for two days), followed by cultivating in DMEM supplemented with 1 μM insulin for two days. The culture medium was replaced once every two or three days during the full differentiation period (seven days). The 3T3-L1 cells were treated with 0–1 nM CAP (Selleckchem, Houston, TX, USA) for four or seven days during experiments.

Western blot analysis and antibodies

Proteins of differentiated 3T3-L1 cells were prepared with lysis buffer (PRO-PREP ; Intron Biotechnology, Seoul, Republic of Korea) for 60 min at 4°C. Equal amounts of proteins (35 μg) were loaded to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 12% gel. The resolved proteins were transferred to a nitrocellulose membrane (Amersham Bioscience, Westborough, MA, USA). The membrane was probed with the indicated primary antibodies, followed by incubation with the secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology). The Immunoreactive signals were detected with using an enhanced chemiluminescence kit (Amersham Bioscience). The following antibodies were used for western blotting analysis: anti-SREBP1 (1:1000), anti-C/EBPα (1:1000), anti-FAS (1:1000), anti-SCD (1:1000), anti-phospho AMPK (Thr172; 1:1000), anti-AMPK (1:2500), anti-phospho mTOR (1:1500), anti-mTOR (1:1500), anti-phospho p38 (1:1500), anti-p38 (1:2500), anti-PGC1α (1:2500), anti-β-actin (1:2500) (all from Santa Cruz Biotechnology), and anti-FNDC5 (1:1,000) antibodies (Abcam, Cambridge, MA, USA).

Cell viability assay

Working solution of 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT; 2 mg/mL) was prepared in phosphate-buffered saline (Invitrogen). The cells were cultivated with MTT (100 μL/well) at 37°C under 5% CO_2, 95% air, and 100% humidity conditions for 3 h. Next, the formazan crystals were dissolved in 100 μL of dimethyl sulfoxide. The mixture plated was incubated at room temperature for 10 min. The optical density (OD) was measured using a multi-plate reader at wavelength of 570nm, with a reference wavelength of 630 nm.

Cell transfection

The short-interfering RNA (siRNA) oligonucleotides (20 nM) specific for AMPKα1 (si-AMPKα1) and FNDC5 (si-FNDC5) were purchased from Santa Cruz Biotechnology and transfected with si-AMPKα1 or si-FNDC5 to inhibit gene expression. Transfection was performed with Lipofectamine 2000 (Invitrogen), in compliance with the manufacturer's instructions. In short, the 3T3-L1 pre-adipocytes were differentiated and cultured until 75%

confluence and serum-starved for 12 h. The cells were transfected with siRNAs at a final concentration of 20 nM, and transfection was performed twice during the full differentiation period (seven days). The transfected 3T3-L1 adipocytes were harvested for protein extraction and stained with oil red O (day 7).

Oil red O staining and lipolysis assay

The 3T3-L1 adipocytes were fixed with 10% formalin for 30 min and stained with oil red O solution (Sigma) for 30 min at 37°C for measuring lipid accumulation. The samples were incubated with isopropanol at 25°C for 10 min with gentle agitation to extract the oil red O-stained lipids. Finally, by using a spectrometer, the OD of 100 μL of the isopropanol-extracted samples at 510 nm was analyzed.

The colorimetric lipolysis assay was achieved using the glycerol release assay kit (Abcam), in compliance with the manufacturer's guidelines.

Statistical analysis

GraphPad Prism version 7 for Windows (La Jolla, CA, USA) were used for performing all statistical analyses. Results are presented as the fold of the highest values (mean ± standard error of the mean). All experiments were performed in triplicates. Student's *t*-test or one-way analysis of variance were used in analyzing data.

III. Results

CAP reduces lipid accumulation and induces lipolysis in 3T3-L1 adipocytes

For optimizing the cell treatment condition and evaluating cytotoxic effects of CAP against 3T3-L1 adipocytes, I treated the adipocytes with CAP concentrations of 0–2 nM. Cell viability was measured using MTT assay. Treatment with CAP for seven days at concentrations of 0–1 nM did not show toxicity to the 3T3-L1 adipocytes (Fig. 1A). The lipid accumulation of 3T3-L1 adipocytes treated with CAP during differentiation was analyzed in accordance with following treatment schedule (Fig. 1B). The 3T3-L1 pre-adipocytes treated with CAP for four or seven days in the differentiation medium shows attenuation of lipid accumulation respectively (Fig. 2B, 2C). Moreover, seven days of treatment with CAP dose-dependently enhanced lipolysis in the 3T3-L1 adipocytes (Fig. 3).

CAP downregulates lipogenic gene expression in the fully differentiated adipocytes

To examine the effects of CAP against expression of lipogenic markers, I examined the 3T3-L1 adipocytes treated with CAP at day 7 post induction with western blotting analysis. Treatment with CAP dose-dependently attenuated the expression levels of adipogenic transcription factors, such as C/EBPα and processed SREBP1 (Fig. 4). Furthermore, the effect of CAP on the MDI-induced expression of late adipogenic markers, such as FAS and SCD1 was analyzed. CAP dose-dependently reduced the expression levels of FAS and SCD1 (Fig. 5).

CAP attenuates lipogenesis in 3T3-L1 adipocytes through the AMPK/mTORC1 dependent pathway

Activation of AMPK is suggested to downregulate lipogenesis in the adipocyte (Dagon et al., 2006). Furthermore, the AMPK/mTORC1 signaling pathway attenuates lipid accumulation in the adipocytes during adipogenesis (Lee et al., 2020). To demonstrate the underlying molecular mechanisms in CAP mediated suppression of 3T3-L1 adipocyte differentiation, I examined the phosphorylation of AMPK and mTORC1 with CAP treatment. Treatment with CAP dose-dependently facilitated AMPK phosphorylation in the 3T3-L1 pre-adipocytes. While CAP did not affect the basal mTORC1 phosphorylation levels (Fig. 6A, 6B, 6C). Treatment with CAP dose-dependently enhanced AMPK phosphorylation and downregulated mTORC1 phosphorylation in the differentiated 3T3-L1 adipocytes (Fig. 6D, 6E, 6F, 6G). Next, AMPK was knocked down by using siRNA. CAP-induced suppression of lipid accumulation was reduced in si-AMPKα1 transfected 3T-L1 adipocytes (Fig. 7). Moreover, CAP-induced upregulation of lipogenic marker expression was also mitigated in si-AMPKα1 transfected 3T-L1 adipocytes (Fig. 8).

CAP attenuates 3T3-L1 adipocyte differentiation and increases lipolysis through the induction of irisin secretion and the activation of AMPK-mediated signaling

Irisin is an adipomyokines which derived from its precursor FNDC5, studied to suppresses adipocyte differentiation (Ma et al., 2019) and induces lipolysis (Arhire et al., 2019). Additionally, p38 MAP kinase/PGC1α is suggested to promote irisin secretion in 3T3-L1 adipocytes (Gao et al., 2016). Thus, I examined the effect of CAP on the secretion of irisin in the 3T3-L1 cells. Treatment with CAP facilitated p38 phosphorylation, enhanced the expression of PGC1α and FNDC5 (Fig. 9A, 9B, 9C, 9D), consequently promoted irisin secretion (Fig. 9E) in the 3T3-L1 adipocytes. Furthermore, transfection with si-FNDC5 attenuated the CAP-induced decrease of lipid accumulation (Fig. 10B), enhancement of lipolysis (Fig. 10C), and upregulation of AMPK phosphorylation (Fig. 11A, 11B) in the 3T3-L1 adipocytes. Meanwhile, transfection with si-AMPKα1 did not affect CAP-induced FNDC5 expression (Fig. 11C, 11D). Thus, possible pathway of CAP affecting 3T3-L1 adipocytes through irisin, which is cleavage of FNDC5, and AMPK dependent pathway can be summarized as following schematic diagram (Fig. 12).

IV. Discussion

Prevention and control of obesity is important strategy for public health promotion because obesity is related with numerous medical conditions and comorbidities (Lumeng et al., 2011, Wyatt et al., 2006). This study, for the first time, proved that CAP reduces lipid accumulation and induces lipolysis through the irisin/AMPK-dependent pathway in 3T3-L1 adipocytes and its further potential usefulness as anti- obesity drug. Treatment with CAP attenuated lipogenesis, increased lipolysis, and facilitated irisin expression and AMPK phosphorylation in the 3T3-L1 adipocytes. Moreover, Transfection with si-AMPKα1 or si-FNDC5 reduced the diminution of lipid accumulation and enhancement of lipolysis in the 3T3-L1 adipocytes induced by CAP. Interestingly, transfection with si-FNDC5 decreased upregulation of AMPK phosphorylation induced by CAP.

In development of obesity, Adipocyte hypertrophy and hyperplasia are crucial components (Lee et al., 2009). Thus, regulating the excessive accumulation of lipids and stimulating lipolysis of intracellular lipids deposit can be an effective therapeutic strategy for controlling obesity in addition to proper restriction of calorie intake and behavioral change. In this study, treatment with CAP attenuated lipid accumulation and downregulated the expression of early and late lipogenic markers in the 3T3-L1 cells at days 4

or 7 post-induction. These results suggest that CAP reduces lipids accumulation by suppressing the expression of adipogenic genes. Nonetheless, this study has a limitation that the results does not quantitatively reflect the change of numbers of adipocytes, which plays a central role in hyperplasia. Thus, further studies are needed to investigate the mitotic clonal expansion phase, and effect of CAP in increase of adipocyte number.

AMPK is a master regulator for maintaining cellular energy homeostasis. Under energy-depletion conditions, AMPK upregulates ATP generation by promoting the expression of catabolism-related genes (Garcia and Shaw, 2017). Moreover, AMPK controls the energy balance at the cellular and physiological levels (Hardie et al., 2012). Thus, therapeutic potential of AMPK has been widely reported and studied as a treatment of metabolic syndrome, which is mainly caused due to the chronic imbalance of energy metabolism (Fryer and Carling, 2005). Previous studies have explained that activation of AMPK reduces insulin resistance induced by hyperlipidemia in various cell types, such as myocytes (Jung et al., 2020), hepatocytes (Kwon et al., 2020), and adipocytes (Jung et al., 2020), as well as in the animal models (Jung et al., 2020, Jung et al., 2018b). AMPK activation also mitigates obesity-mediated non-alcoholic fatty acid liver disease (Jung et al., 2018a) and atherosclerosis (Zhao et al., 2020). Furthermore, AMPK is

suggested to be related with differentiation of adipocyte. Previous study reported that resveratrol inhibits adipocyte differentiation through the AMPK-mediated signaling pathway (Chen et al., 2011). The AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR) is known to impair adipogenesis in the 3T3-L1 adipocytes through the canonical Wnt pathway (Lee et al., 2011). Besides, several studies also demonstrated that activation of AMPK suppresses mTORC1 phosphorylation, which consequently results in the attenuation of lipogenesis(Lee et al., 2020, González et al., 2020). In this study, CAP treatment increased AMPK phosphorylation and diminished mTORC1 phosphorylation. Furthermore, transfection with si-AMPKα1 mitigated the effects of CAP on MDI-induced mTORC1 phosphorylation, lipid accumulation, and upregulation of lipogenic gene expression. These results suggest that AMPK/mTORC1 signaling pathway mediates CAP-induced suppression of lipogenesis in the adipocytes.

Irisin was first identified as myokine, which is secreted by skeletal muscles in response to exercise through a PGC1α-dependent mechanism (Boström et al., 2012, Gouveia et al., 2016). Irisin is reported to show capacity of browning of adipocyte and thermogenesis mediated by stimulation of uncoupling protein 1 (UCP1) expression (Boström et al., 2012), and leads to consequent increase in energy expenditure. Further studies investigated that irisin is also an adipokine and it is related with muscle- adipose tissue

intervention through regulatory feedback (Roca-Rivada et al., 2013, Moreno-Navarrete et al., 2013, Rodríguez et al., 2017). Treatment with recombinant irisin shows result of inhibition of lipid accumulation in human adipocytes through the upregulation of adipose triglyceride lipase and attenuation of fatty acid synthase (Huh et al., 2014). Additionally, irisin is reported to suppress adipogenesis through the inhibition of Wnt signaling (Ma et al., 2019). Nonetheless, biological function and effect of irisin is still remains controversial as some study suggested negative effect of irisin (Maak et al., 2021, Buscemi et al., 2018). So, I tried to figure out the function and molecular mechanisms of irisin in adipocytes leastly, based on the findings of previous studies. I examined the effect of CAP on FNDC5 expression and secretion of irisin in the 3T3-L1 adipocytes. As a result, CAP dose-dependently facilitated p38 phosphorylation, increased PGC1α and FNDC5 expression, and promoted subsequent secretion of irisin in the 3T3-L1 cells. Furthermore, transfection with si-FNDC5 decreased the effects of CAP on lipogenesis, lipolysis, and AMPK phosphorylation. In contrast, transfection with si-AMPKα1 did not affect FNDC5 expression. These findings implied that the CAP-induced secretion of irisin stimulates AMPK phosphorylation, which results in the suppression of adipogenesis and induction of lipolysis in the adipocytes. Still, further studies are needed to investigate the mechanisms underlying how CAP induces activation of p38/PGC1α signaling and following increase of FNDC5 expression.

On the other hand, CAP has variety of side effects such as nausea, vomiting, fatigue, dyspnea, edema, and loss of appetite (Mathieu et al., 2022) since it has been firstly approved for treating aggressive lung cancer. So, further in vivo trial is needed to investigate whether the side effects are related with or affect potential anti-obesity effect of CAP.

In conclusion, this study proved and emphasized that treatment with CAP attenuated lipid accumulation and enhanced the expression of lipogenic markers during 3T3-L1 adipocyte differentiation through an AMPK-dependent pathway. Mechanistically, CAP activates the AMPK/mTORC1 signaling pathway, which results in the inhibition of lipogenesis and induction of lipolysis in the adipocytes through the upregulated secretion of irisin by autocrine mediated mechanism. These results are expected to be able to break new ground in developing further potential anti-obesity drugs

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VI. Appendix

Fig. 1. A

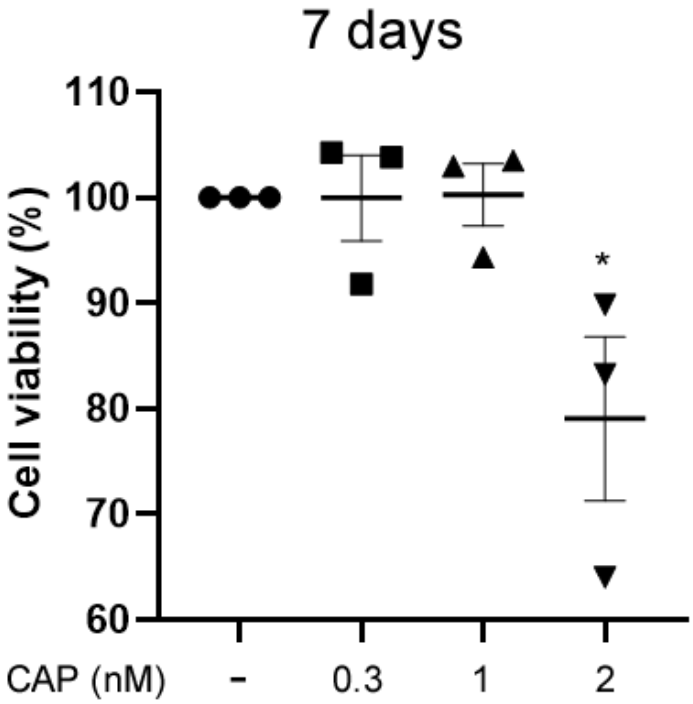


Fig. 1. B

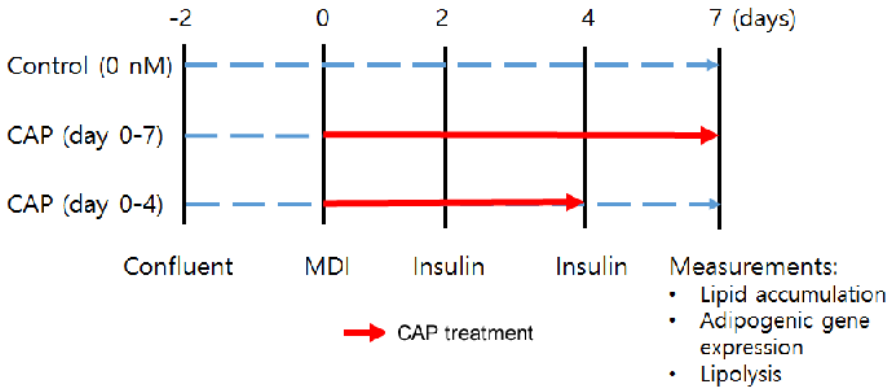
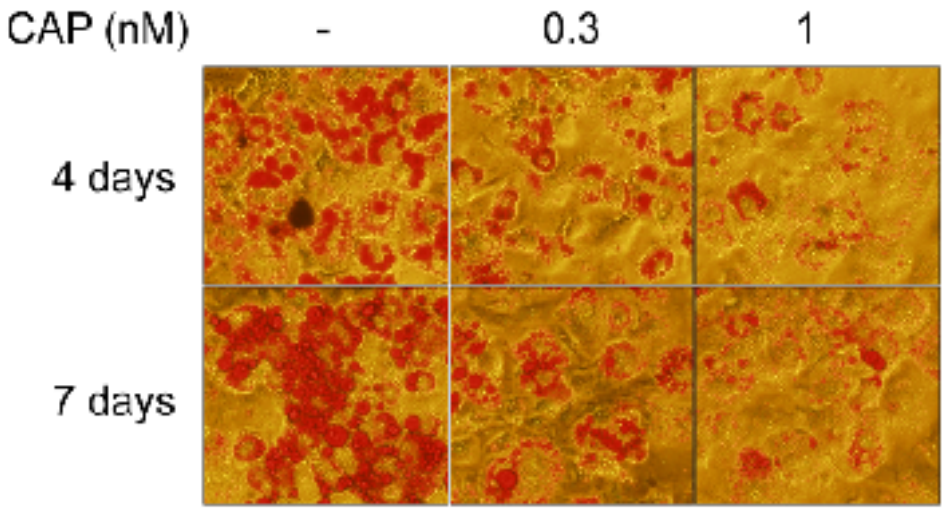


Fig. 2. A



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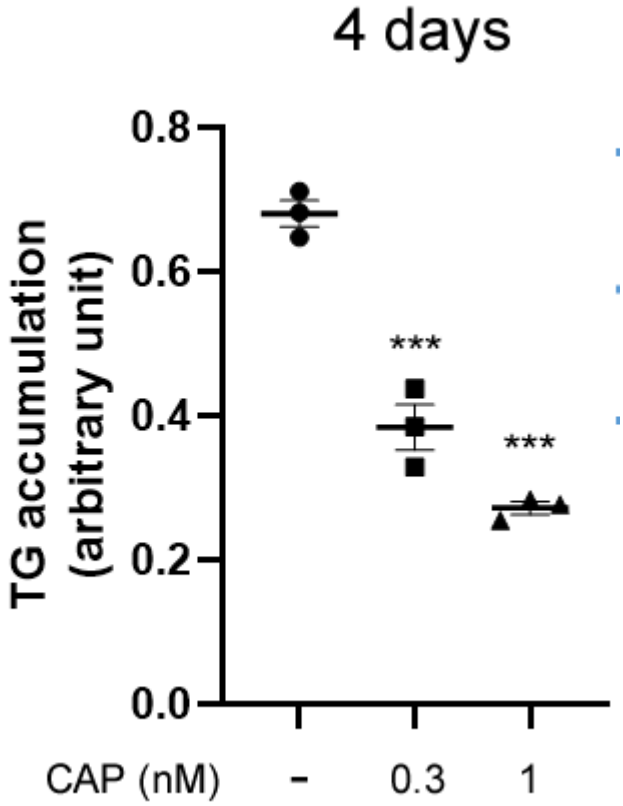
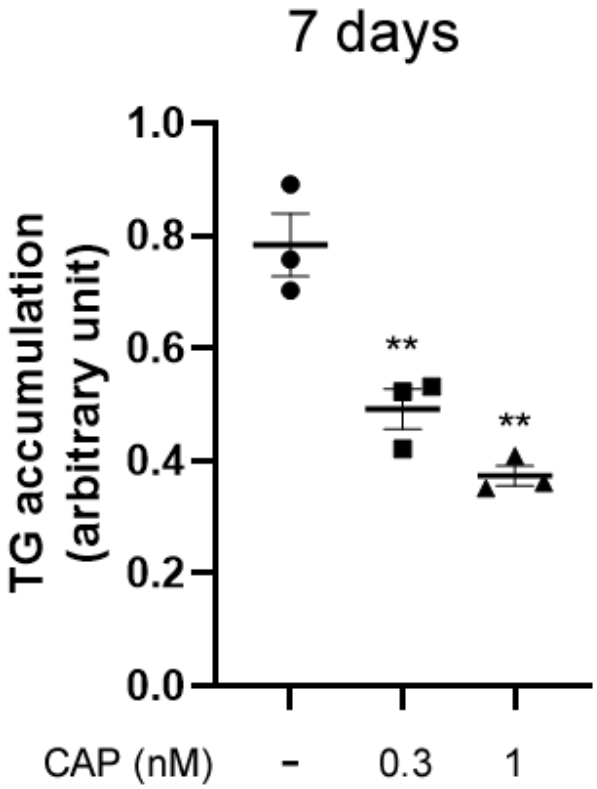


Fig. 2. C



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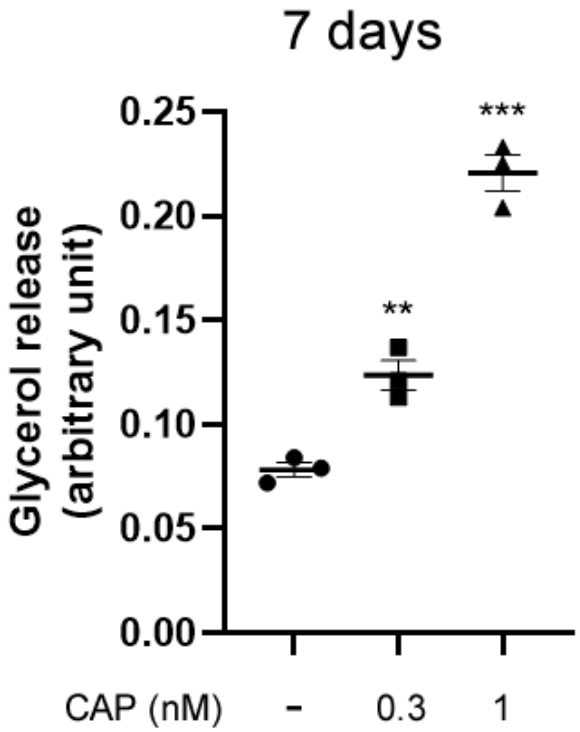

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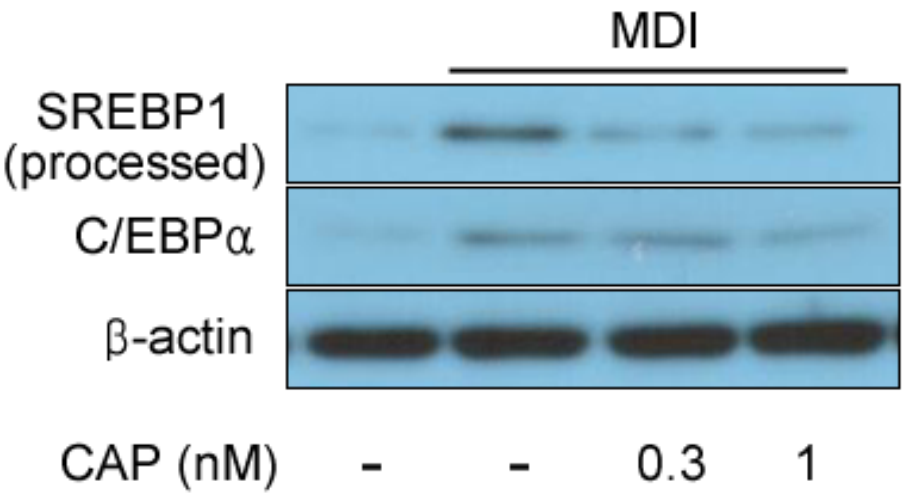

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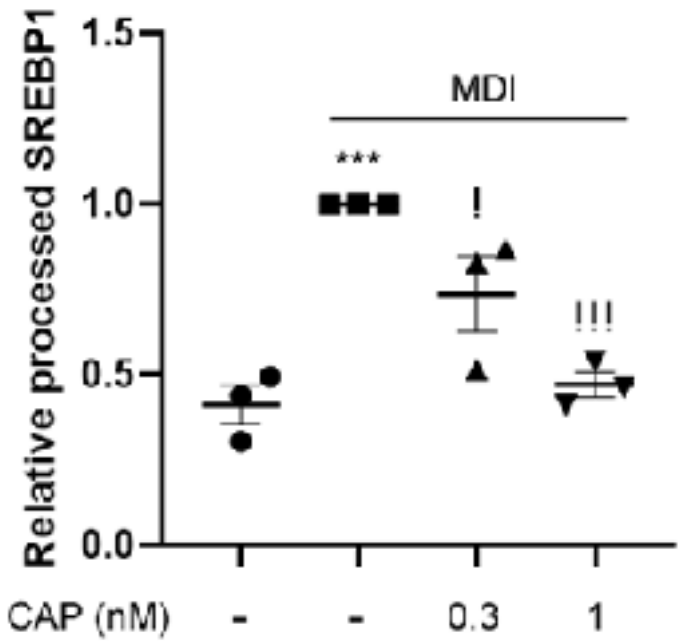

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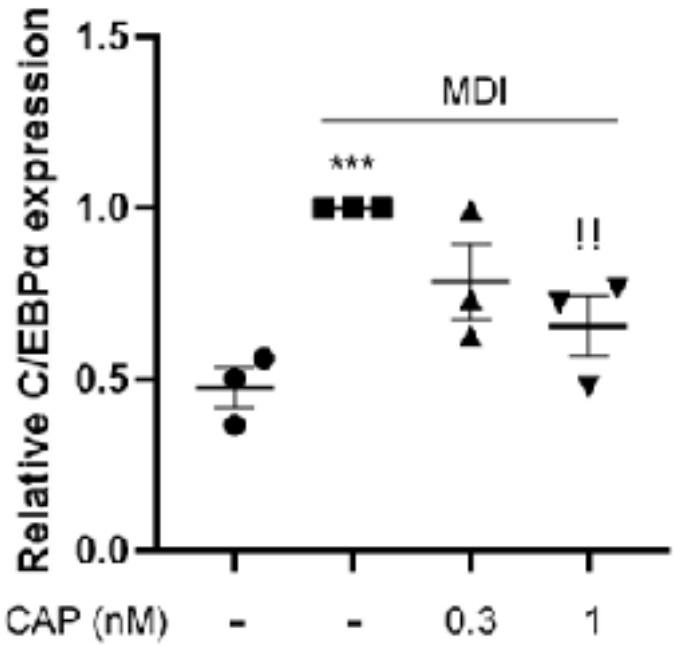

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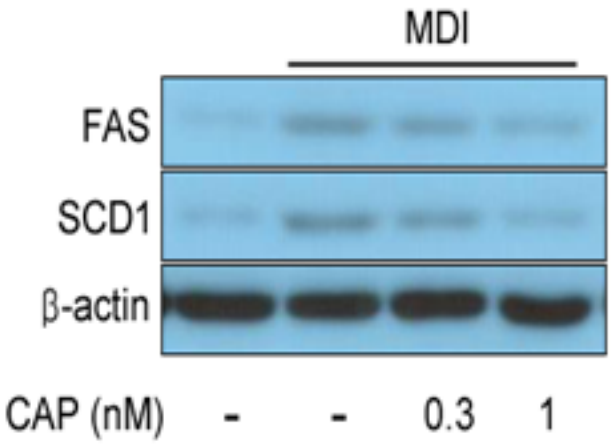
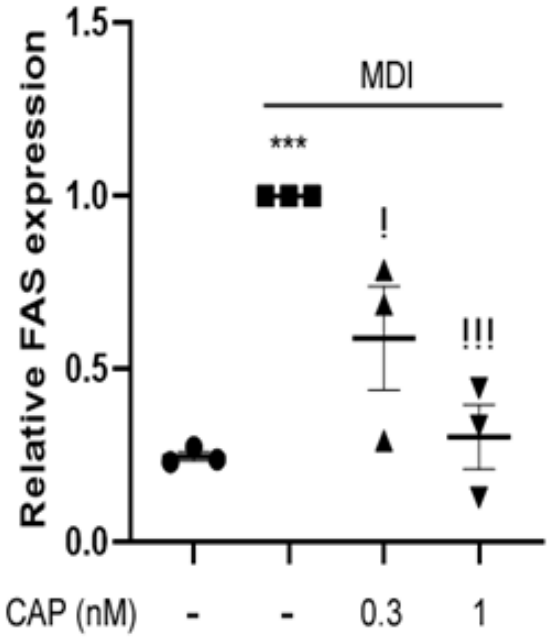


Fig. 5. A

Fig. 5. B



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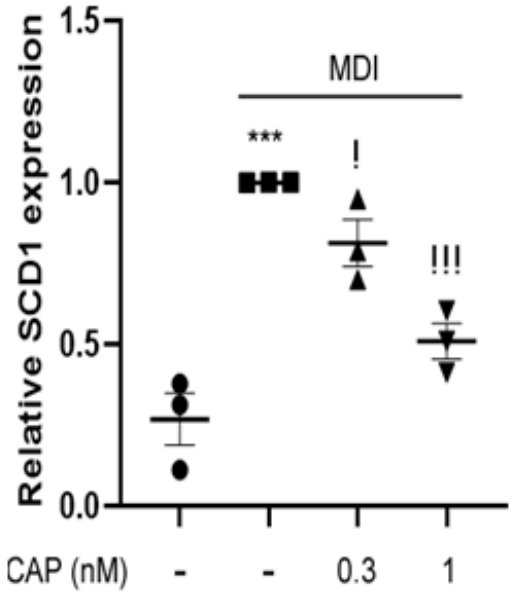

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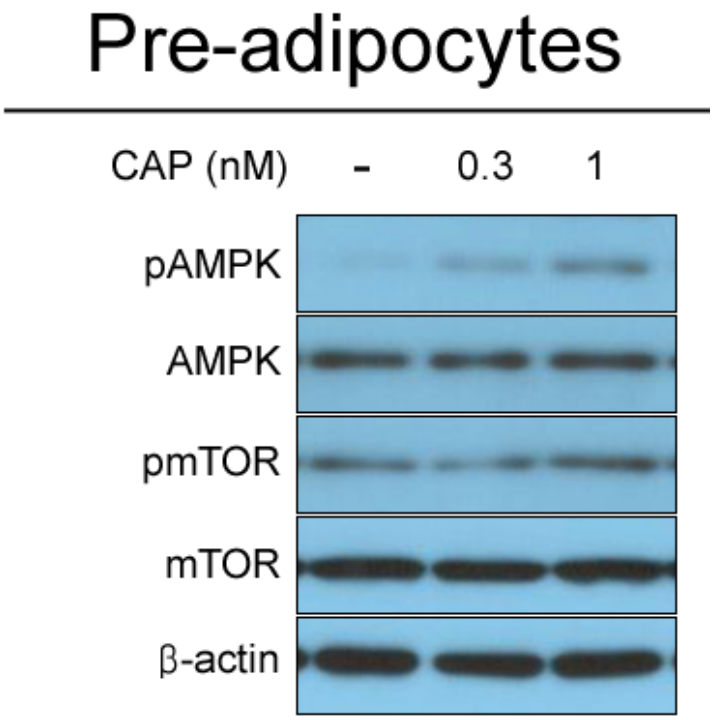

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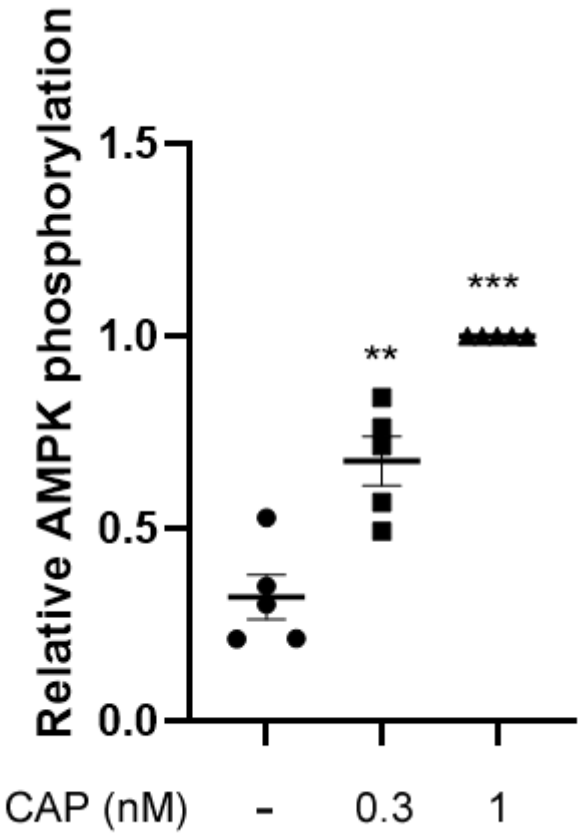

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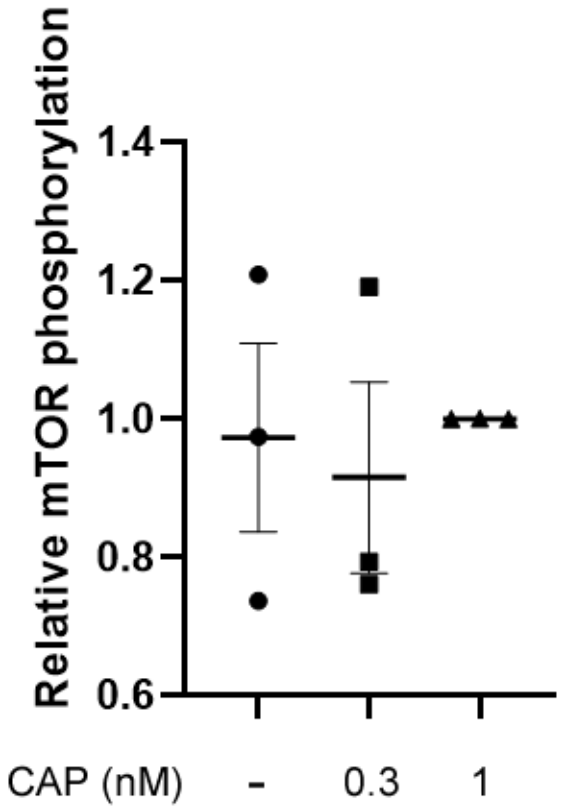

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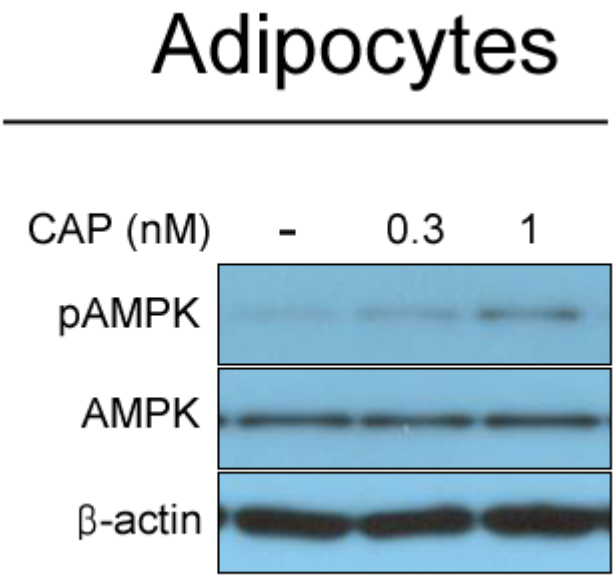

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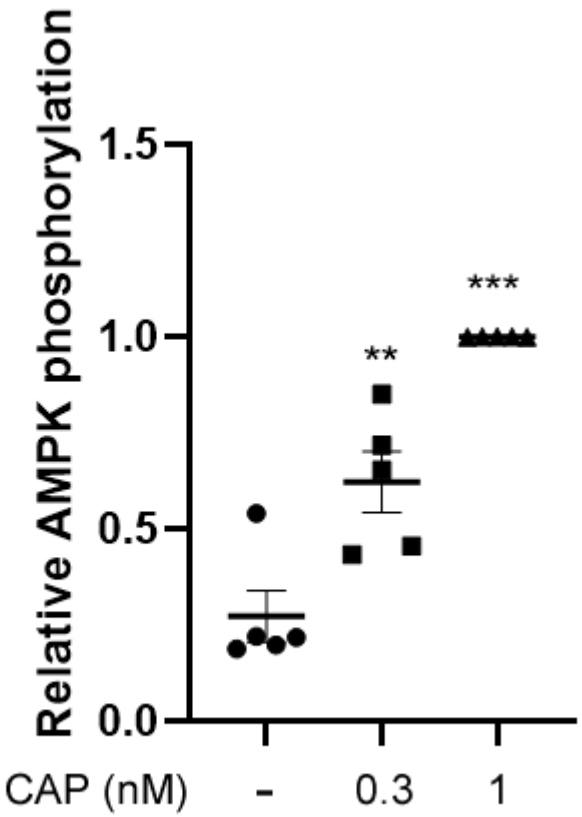

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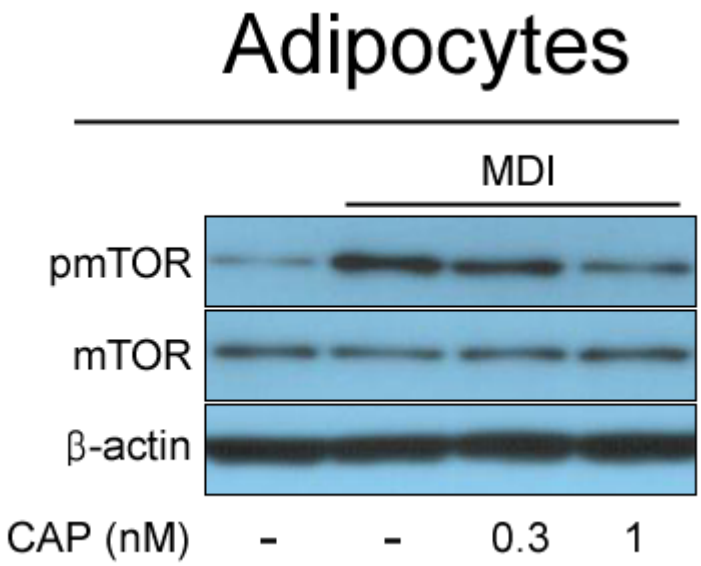

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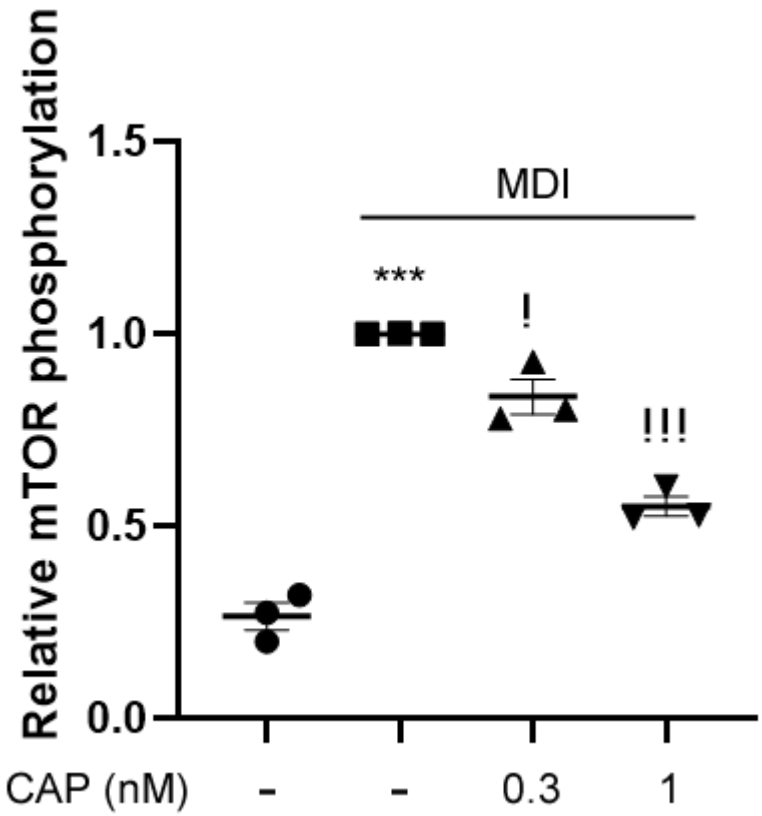

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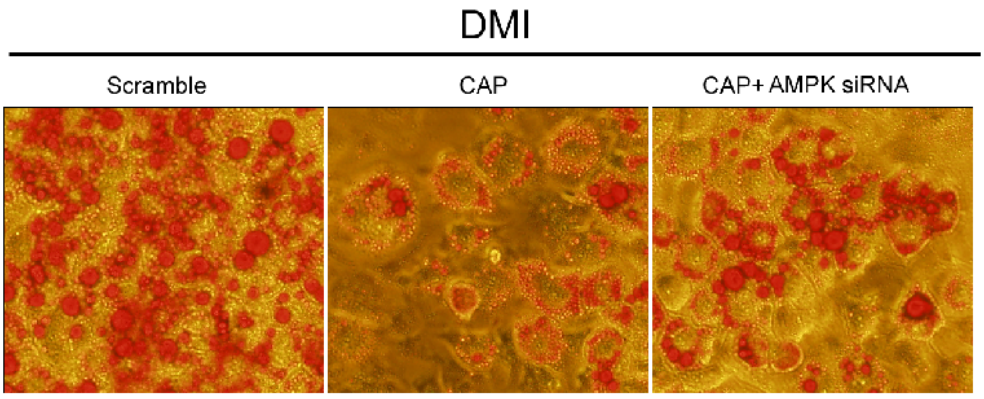

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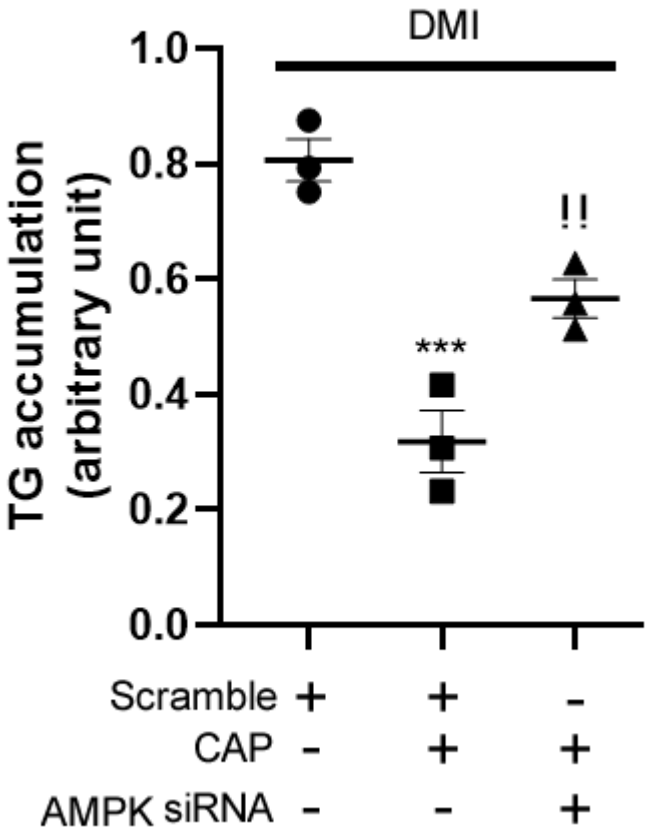

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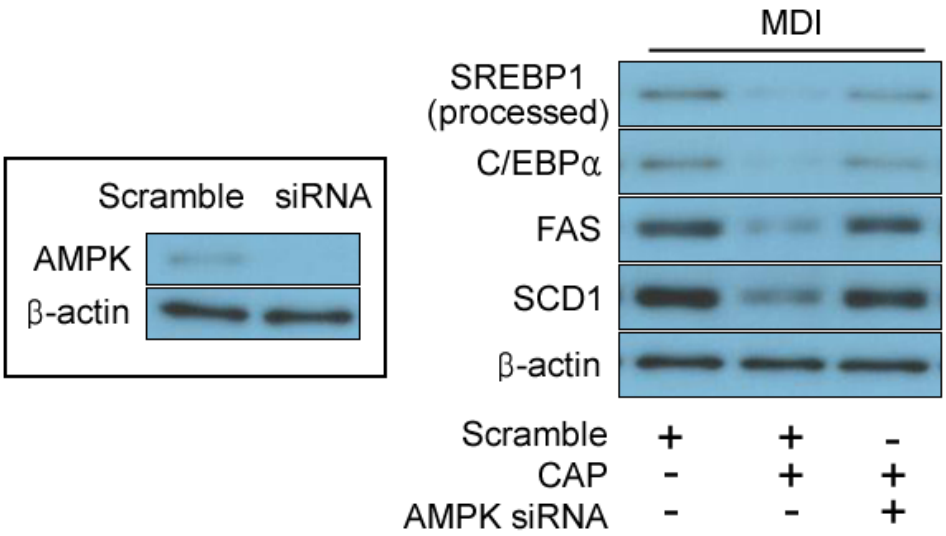

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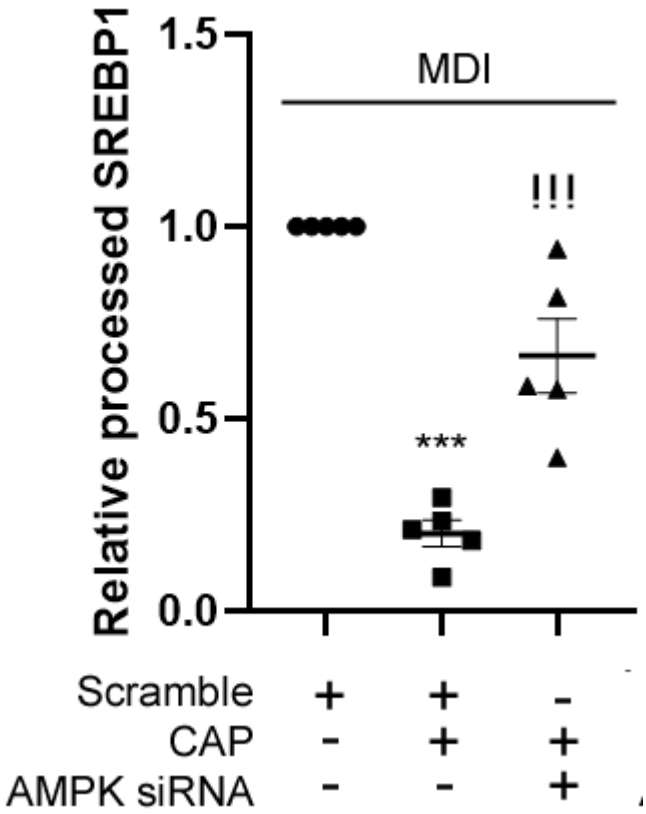

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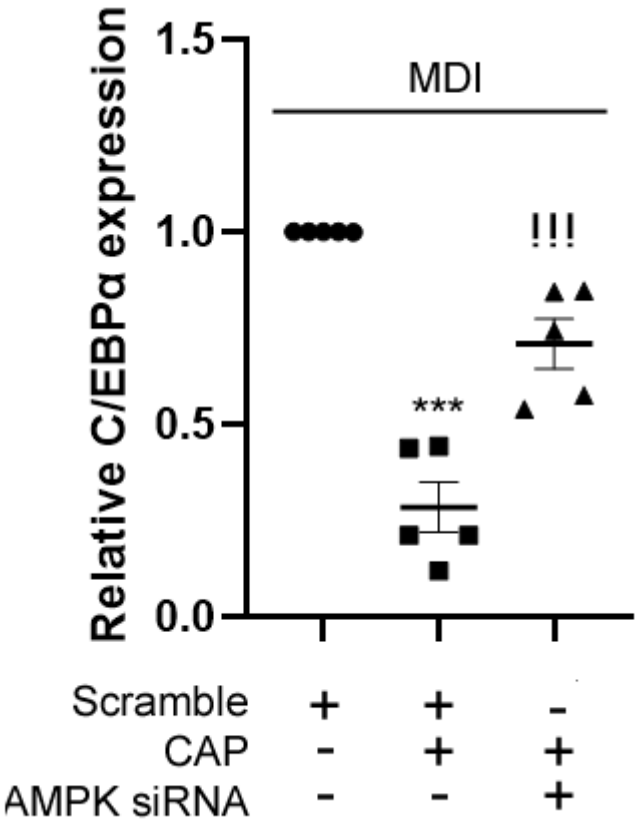

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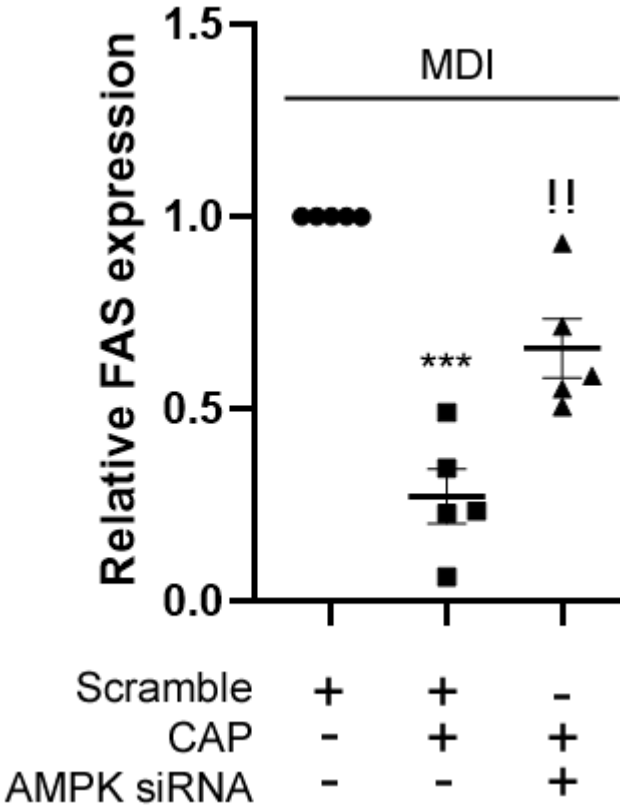

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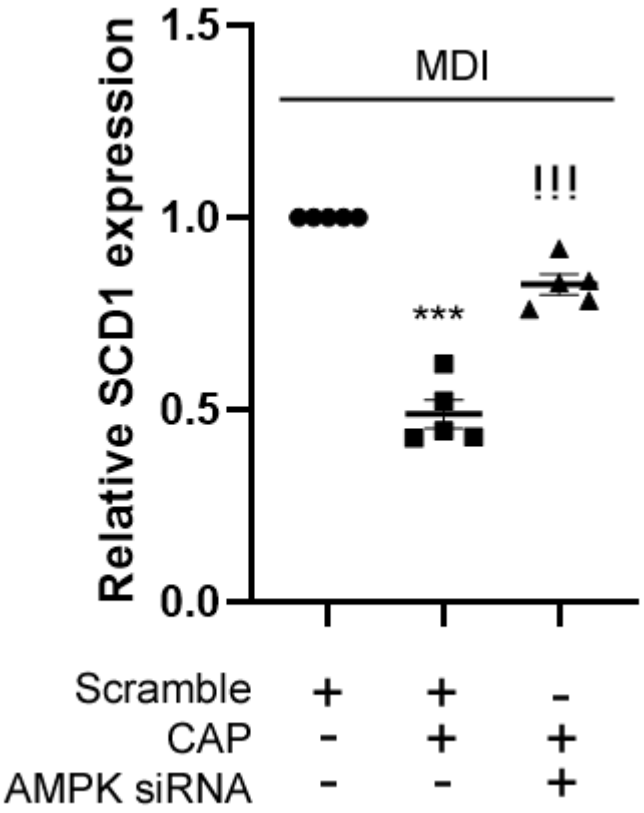

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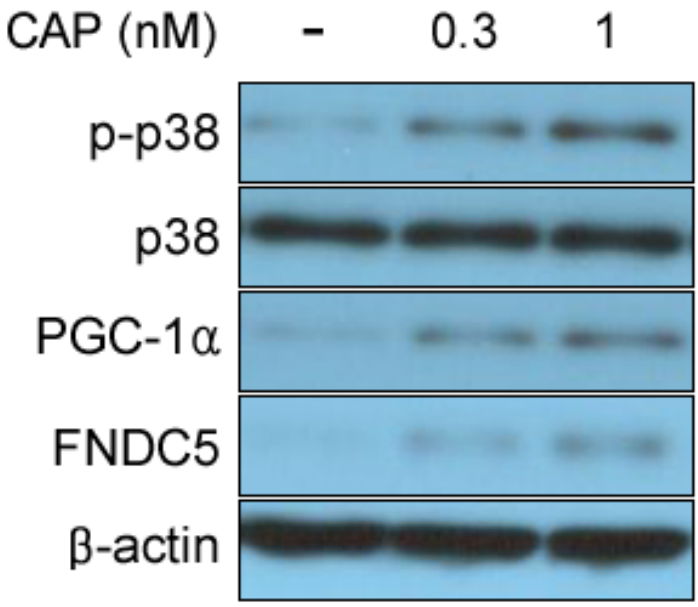

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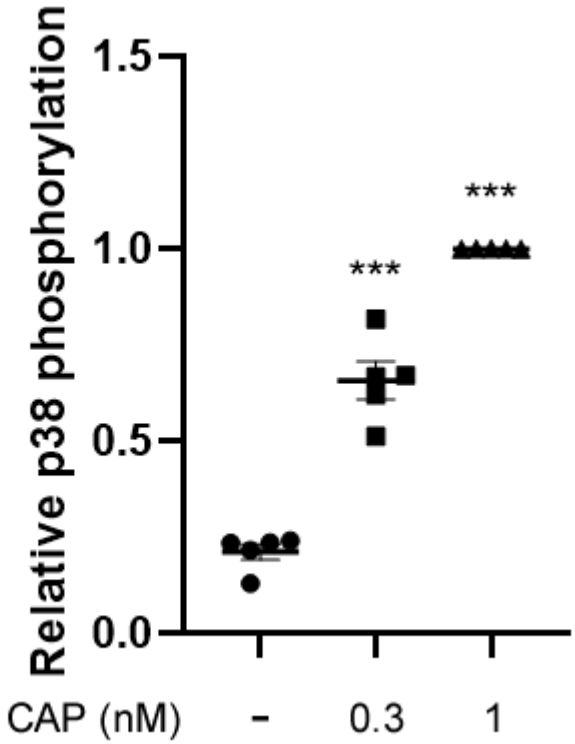

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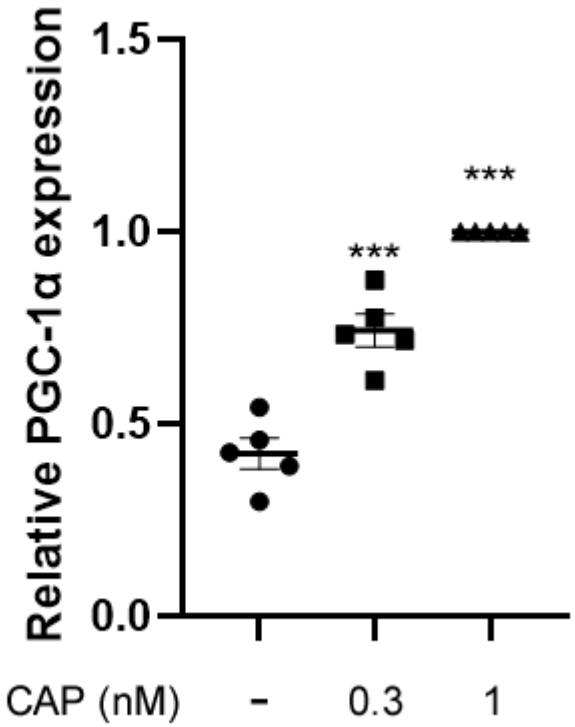

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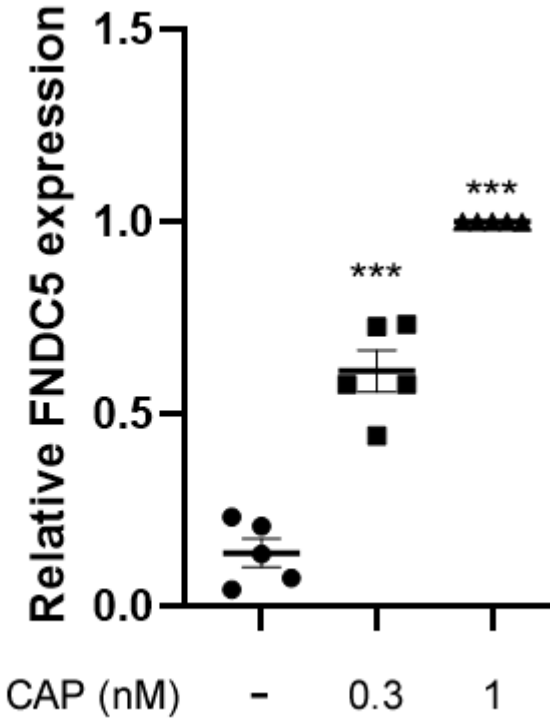

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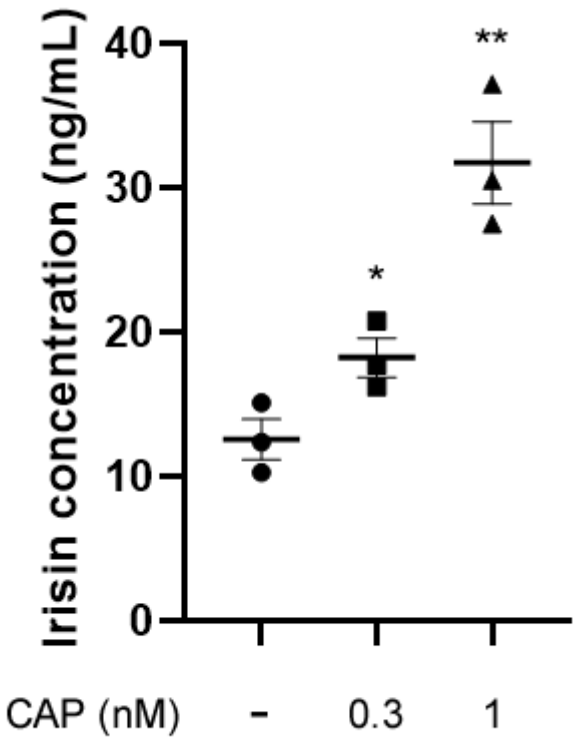

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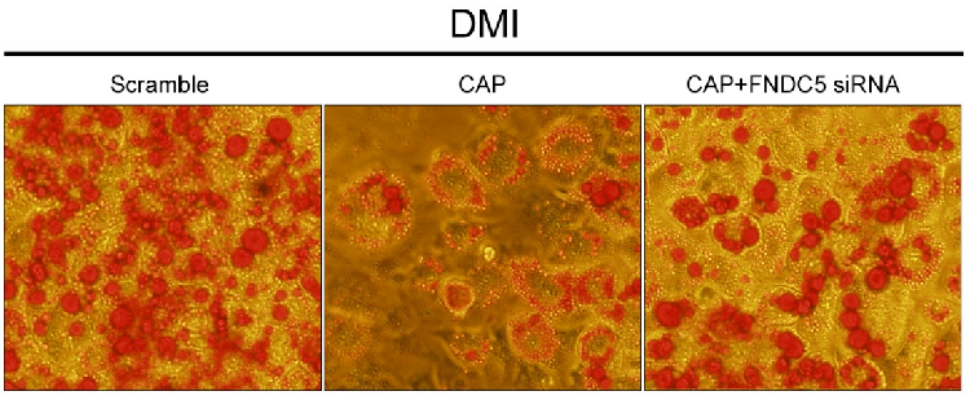

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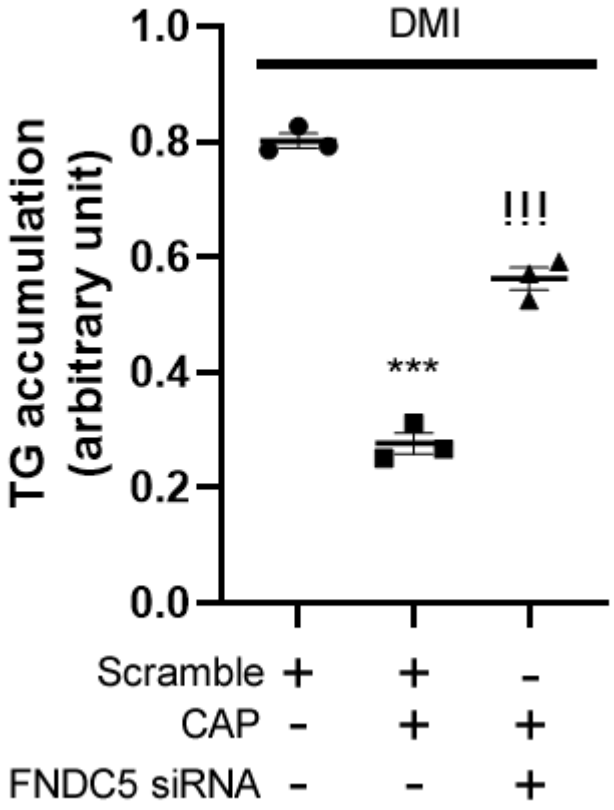

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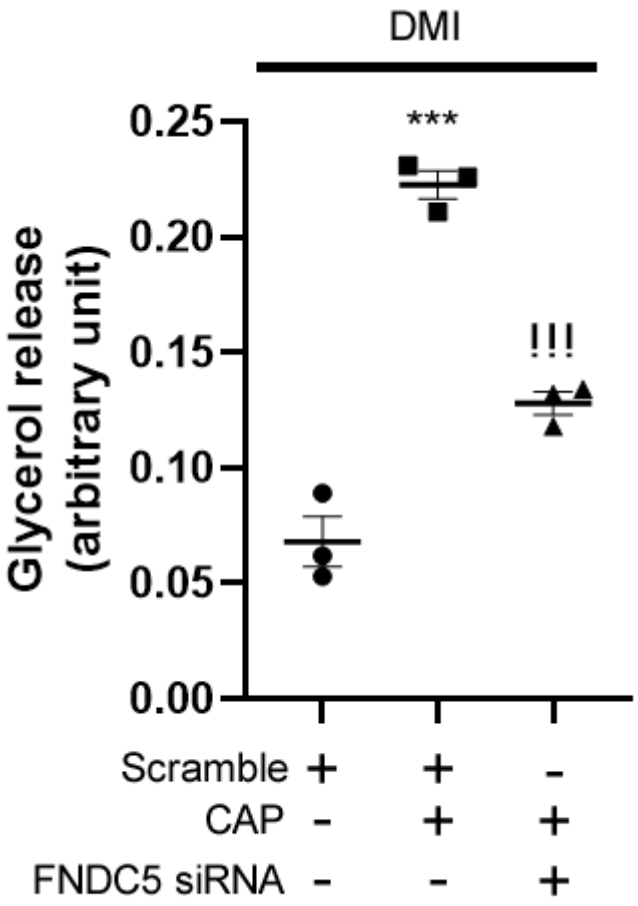

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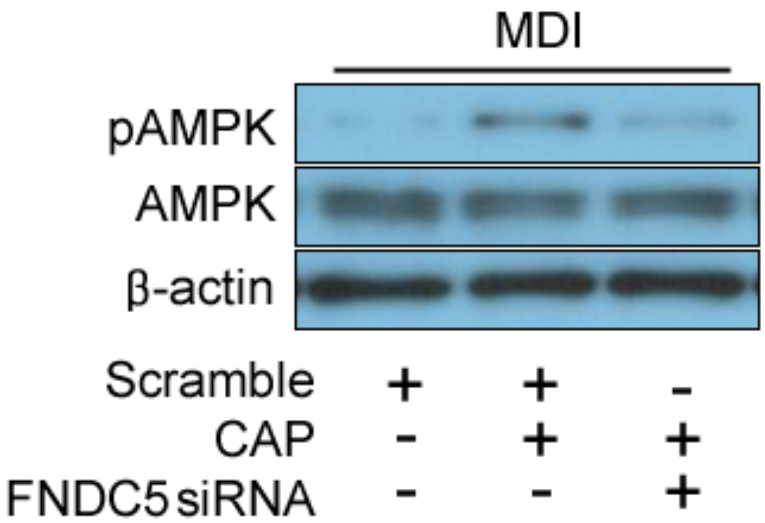

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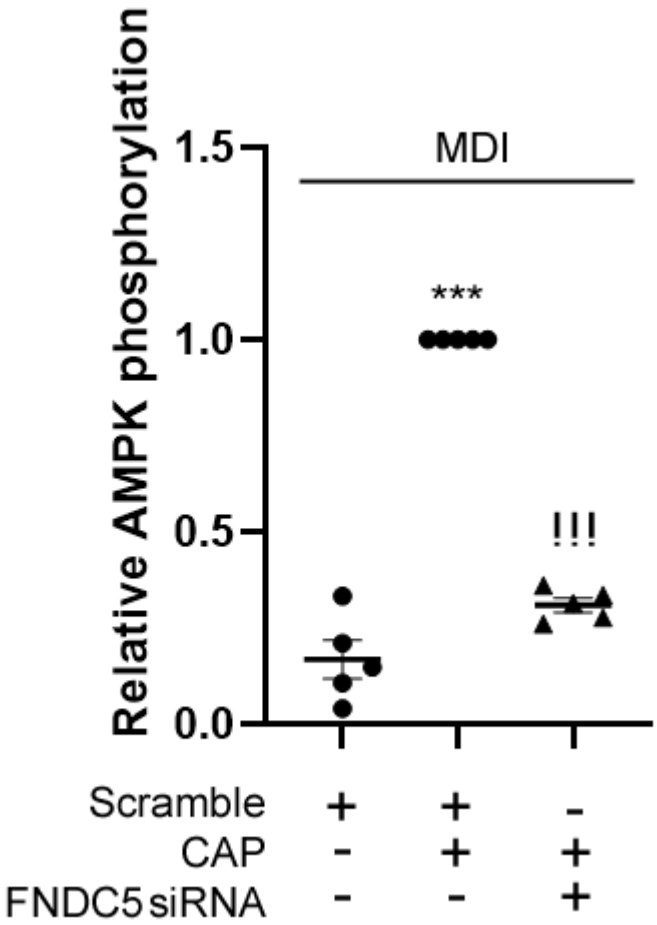

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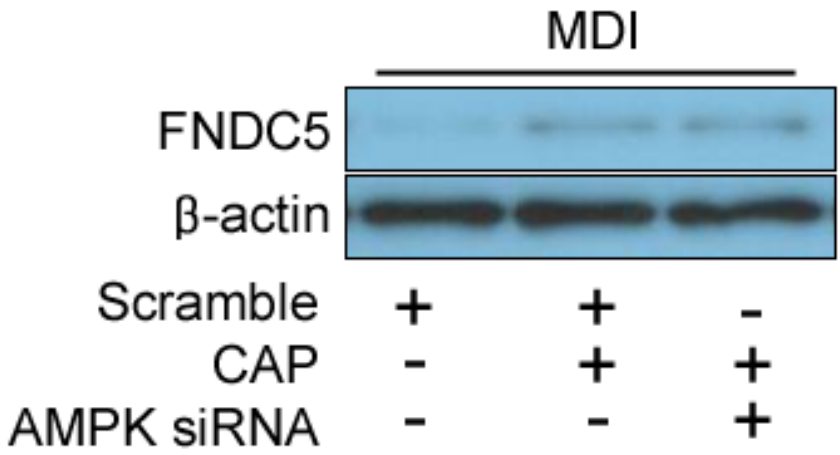

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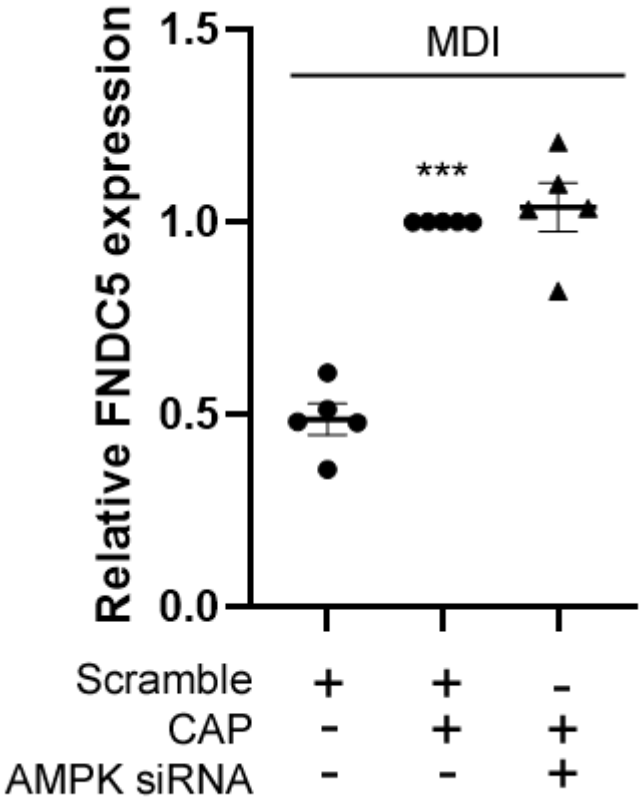

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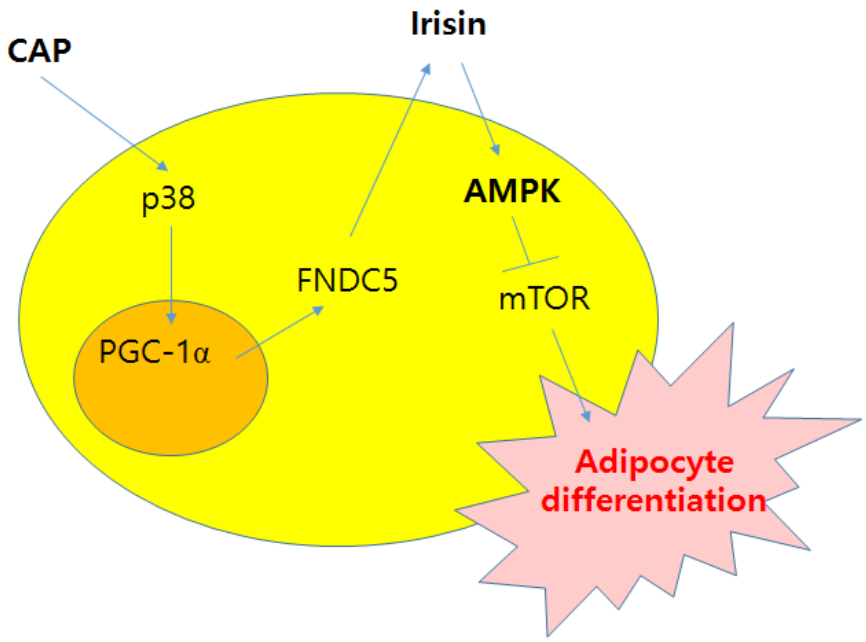

Figure legends.

Fig. 1. Cell viability assay and differentiation schedule

(A) The effect of CAP (0–2 nM) treatment for 24 h on the viability of fully differentiated 3T3-L1 cells at day 7 post-induction was analyzed using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. (B) Schematic diagram of the 3T3-L1 cell differentiation schedule. Three independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control).

Fig. 2. Capmatinib (CAP) attenuates lipogenesis in the 3T3-L1 adipocytes.

(A) Oil red O staining results of differentiated 3T3-L1 cells treated with CAP (0–1 nM) for four (B) or seven days (C). The intracellular lipid levels were quantified by extracting lipids with isopropanol. Three independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control).

Fig. 3. Capmatinib (CAP) induces lipolysis in the 3T3-L1 adipocytes.

The fully differentiated 3T3-L1 cells treated with CAP (0–1 nM) for seven days were subjected to glycerol release assay. Three independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control).

Fig. 4. Capmatinib (CAP) downregulates lipogenic gene expression in the 3T3-L1 adipocytes.

The western blotting analysis shows the expression levels of processed SREBP1, C/EBPα in the 3T3-L1 adipocytes treated with CAP (0–1 nM) for seven days. Three independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{***}P<0.05$ (compared with the protein levels in the pre-adipocytes). $^{!!!}P<0.001$, $^{!!}P<0.01$, and $^{!}P<0.05$ (compared with the protein levels in the MDI-stimulated cells). MDI; adipogenic cocktail containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin mixture.

Fig. 5. Capmatinib (CAP) downregulates lipogenic gene expression in the 3T3-L1 adipocytes.

The western blotting analysis shows the expression levels of FAS (B), and SCD1 (C) in the 3T3-L1 adipocytes treated with CAP (0–1 nM) for seven days. Three independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{***}P<0.05$ (compared with the protein levels in the pre-adipocytes). $^{!!!}P<0.001$, $^{!!}P<0.01$, and $^{!}P<0.05$ (compared with the protein levels in the MDI-stimulated cells). MDI; adipogenic cocktail containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin mixture.

Fig. 6. CAP suppresses lipid accumulation in the 3T3-L1 adipocytes through the adenine monophosphate-activated kinase (AMPK) /mammalian target of rapamycin (mTOR) signaling pathway

By using the western blotting analysis, the levels of phosphorylated AMPK and mTOR in the 3T3-L1 pre-adipocytes (A), (B), (C) and 3T3-L1 adipocytes (D), (E), (F), (G) treated with CAP (0–1 nM) for seven days were examined. Three or five independent experiments were performed. $^{***}P<0.001$ and $^{**}P<0.01$ (compared with the levels in the control cells or pre-adipocytes). $^{!!!}P<0.001$, $^{!!}P<0.01$, and $^{!}P<0.05$ (compared with the levels in the MDI-stimulated or CAP-treated cells).

Fig. 7. CAP suppresses lipid accumulation in the 3T3-L1 adipocytes through the adenine monophosphate-activated kinase (AMPK) /mammalian target of rapamycin (mTOR) signaling pathway

The si-AMPKα1-transfected 3T3-L1 adipocytes treated with CAP (1 nM) for seven days were stained with oil red O (A). The intracellular lipid levels were quantified by extracting lipids with isopropanol (B). Three or five independent experiments were performed. $^{***}P<0.001$ and $^{**}P<0.01$ (compared with the levels in the control cells or pre-adipocytes). $^{!!!}P<0.001$, $^{!!}P<0.01$, and $^{!}P<0.05$ (compared with the levels in the MDI-stimulated or CAP-treated cells).

Fig. 8. CAP suppresses lipid accumulation in the 3T3-L1 adipocytes through the adenine monophosphate-activated kinase (AMPK) /mammalian target of rapamycin (mTOR) signaling pathway

The expression levels of processed SREBP1 (B), C/EBPα (C), FAS (D), and SCD1 (E) in the siRNA-transfected 3T3-L1 adipocytes treated with CAP (1 nM) for seven days were analyzed using western blotting. Three or five independent experiments were performed. $^{***}P<0.001$ and $^{**}P<0.01$ (compared with the levels in the control cells or pre-adipocytes). $^{!!!}P<0.001$, $^{!!}P<0.01$, and $^{!}P<0.05$ (compared with the levels in the MDI-stimulated or CAP-treated cells).

Fig. 9. Irisin regulates the activity of adenine monophosphate-activated protein kinase (AMPK), which mitigates lipid accumulation and facilitates lipolysis in the 3T3-L1 adipocytes.

The 3T3-L1 adipocytes treated with CAP (0–1 nM) for seven days were subjected to western blotting analysis (A) to analyze the levels of phosphorylated p38 (B), PGC1α (C), and FNDC5 (D) and enzyme-linked immunosorbent assay to evaluate the irisin concentration (E). Three or five independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control). $^{!!!}P<0.001$ (compared with the levels in the CAP-treated cells). MDI; adipogenic cocktail containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin mixture.

Fig. 10. Irisin regulates the activity of adenine monophosphate-activated protein kinase (AMPK), which mitigates lipid accumulation and facilitates lipolysis in the 3T3-L1 adipocytes.

The si-FNDC5-transfected 3T3-L1 adipocytes treated with CAP (1 nM) for seven days were subjected to oil red O staining (A), The intracellular lipid levels (B) and glycerol release assay (C). The intracellular lipid levels were quantified by extracting lipids with isopropanol. Three or five independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control). $^{!!!}P<0.001$ (compared with the levels in the CAP-treated cells). MDI; adipogenic cocktail containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin mixture.

Fig. 11. Irisin regulates the activity of adenine monophosphate-activated protein kinase (AMPK), which mitigates lipid accumulation and facilitates lipolysis in the 3T3-L1 adipocytes.

The si-FNDC5-transfected 3T3-L1 adipocytes treated with CAP (1 nM) for seven days were subjected to western blotting analysis to examine the levels of phosphorylated AMPK (A), (B) and the si-AMPKα1 transfected 3T3-L1 adipocytes treated with CAP (1 nM) for seven days were subjected to western blotting analysis to examine the levels of FNDC5 (C), (D). Three or five independent experiments were performed. $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ (compared with the levels in the control). $^{!!!}P<0.001$ (compared with the levels in the CAP-treated cells). MDI; adipogenic cocktail containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin mixture.

Figure 12. Schematic diagram of the effect of Capmatinib on 3T3-L1 adipocytes through irisin/ AMPK - dependent pathway.

The schematic diagram which explains possible pathway of CAP mediating 3T3-L1 adipocytes through irisin/ AMPK dependent pathway.

국문 초록

캡마티닙의 **irisin/AMPK** 의존 경로를 통한 지방 전구세포에서의 지방합성 억제와 지방분해의 증가

안 성호

의학과 병리학 전공

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비만은 현대 사회에서 급증하는 건강 문제로서, 다양한 대사 질환과 질병에 관련이 있으며 생활 방식의 중재, 식습관의 변화와 더불어 새로운 약제의 개발을 필요로 한다. 캡마티닙은 mesenchymal-to-epithelial transition (c-Met) 억제제로서 비소세포폐암의 새로운 치료제로 사용이 시도되고 있는 약물이다. c-Met 억제제는 다양한 악성종양의 치료에 연구가 이루어지고 있으며 최근 연구에서 3T3-L1 지방 전구세포의 분화를 억제하는 효과가 있음이 알려져 있고 또한 캡마티닙은 다양한 염증 매개물질과 산화 스트레스를 감소시키는 것으로 연구된 바 있다.

따라서 본 연구에서는 캡마티닙의 새로운 비만치료제로서의 가능성을 연구하고자 지방 전구세포에서의 지방생성과 지방분해에 미치는 효과를 확인하고자 하였다. 캡마티닙의 처리는 용량 의존적으로 3T3-L1지방 전구세포의 분화과정에 있

어 지방의 축적과 지방생성 관련 유전자 및 전사인자의 발현을 감소시켰으며 지방의 분해는 촉진하는 결과를 보였다. 또한 캡마티닙의 처리는 adenosine monophosphate-activated protein kinase (AMPK)의 인산화를 촉진하였으며 mTORC1의 인산화는 억제, FNDC5의 발현과 irisin의 분비는 촉진하는 결과를 보였다.

si-AMPK 혹은 si- FNDC5 로 형질 전환된 지방 세포에서는 캡마티닙으로 인해 유도되는 지방축적 억제와 지방분해의 촉진 효과가 감소하는 결과를 보였다. 추가적으로, si- FNDC5 로 형질 전환된 세포는 캡마티닙으로 인해 유도되는 AMPK의 인산화가 감소하였으나 반대로 si-AMPK로 형질 전환된 경우 캡마티닙으로 유도되는FNDC5의 발현에 변화가 보이지 않아 경로의 선후관계를 유추할 수 있었다. 결론적으로 본 연구의 결과는 캡마티닙이 irisin/AMPK 관련 경로를 통해 지방 전구세포에 영향을 주는 것을 알 수 있었으며, 추후 항 비만 치료를 위한 약제로서 사용될 수 있는 가능성을 제시하였다.

핵심어: 캡마티닙, 지방 전구 세포, **irisin, AMPK** 의존 경로, 지방 합성, 지방 분해

Abstracts

Capmatinib suppresses lipogenesis and increases lipolysis in 3T3-L1 adipocytes through an irisin / adenine monophosphate-activated protein kinase-dependent pathway

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The obesity is rapidly emerging public health issue, which is closely associated with numerous metabolic disorders, and demands novel therapeutic agents. Capmatinib (CAP) is a novel mesenchymal-to-epithelial transition (c-Met) inhibitor prescribed for treatment of non-small cell lung cancer. The inactivation of c-Met also suggested to show inhibition effect in differentiation of 3T3-L1 adipocytes. Additionally, CAP is reported to reduce pro-inflammatory mediators and oxidative stress in several studies. Thus, I examined the effect of CAP on lipogenesis and lipolysis in the adipocytes in

this study. Treatment of CAP dose-dependently attenuated lipid accumulation, lipogenic gene expression and increased lipolysis in 3T3-L1 adipocytes differentiation. Additionally, CAP treatment enhanced adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, downregulated mTORC1 phosphorylation, upregulated FNDC5 expression and irisin secretion. Transfection with si-AMPK or si- FNDC5 reduced the CAP induced suppression of lipogenesis and enhancement of lipolysis, Additionally, transfection with si- FNDC5 mitigated the CAP- induced phosphorylation of AMPK while si-AMPK did not affect the CAP-induced FNDC5 expression. In conclusion, these results suggest that the CAP mediate adipocytes through irisin/AMPK dependent pathway and that CAP can be a further potential pharmacologic agent for controlling obesity.

Keywords: Capmatinib, 3T3-L1 adipocytes, irisin, AMPK, lipogenesis. lipolysis