수의학 석사 학위논문

# The role of IRBIT in white and brown adipocyte

백색지방과 갈색지방에서 IRBIT 역할 연구

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**이 혜 림**

# ABSTRACT

# The role of IRBIT in white and brown adipocyte

## 백색지방과 갈색지방에서 IRBIT 역할 연구

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The IP3 pseudo-protein, a novel IP3 R binding protein released with inositol 1,4,5-trisphosphate (IRBIT) has been known to regulate calcium release from Endoplasmic Reticulum (ER) to cytosol. IRBIT is highly expressed in Brown Adipose Tissue (BAT), however, the role of IRBIT has not been reported in BAT. Here we show that IRBIT acts as a critical regulator of brown adipocyte activity and white adipocyte "browning". IRBIT expression level was increased after CL 316,243 ($\beta$3-adrenergic receptor agonist) treatment in adult adipose tissues. Thus, we used brown and white adipocyte to figure out

the potential role of IRBIT. So we found out the functional loss of IRBIT enhanced the brown adipocyte activity and white adipocyte browning in a CaMK2 dependent manner. These findings suggest an important role of IRBIT in the regulation of energy expenditure via β3-adrenergic signaling pathway.

**Keywords** : IRBIT, brown adipose tissue, β3-adrenergic receptor agonist, CaMK2, browning

**Student Number** : 2016-22298

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# 1. Introduction

The prevalence of obesity and its related metabolic diseases is increasing worldwide. (Cannon et al., 2004). Obesity is caused by an imbalance between energy intake and energy expenditure. The excess energy is stored as triglycerides especially in white adipocytes. White adipose tissue (WAT) stores excess energy as fat. Brown adipose tissue (BAT) burns fat into energy. The recent discovery of brown adipose tissue (BAT) in adults has brought about a new interest in alternative therapies that activate BAT to treat obesity and associated metabolic diseases (Broeders et al., 2015; Kajimura et al., 2015; Rosen et al., 2014). Hence, promoting BAT recruitment is an important aspect to be considered for those who do not possess sufficient amounts of active BAT, such as the obese and elderly population (Kajimura et al., 2015). BAT activation by endogenous pathways that regulate metabolic activity causes healthy weight loss (Bowers et al., 2007). Therefore, finding novel gene which regulates energy metabolism or taking pharmacological approach to enhance BAT activity is essential.

IRBIT, which is the abbreviation for IP3R binding protein released with inositol 1,4,5-trisphosphate. This protein was originally identified as a molecule that interacts with the intracellular calcium channel, IP3R. IRBIT is pseudo-protein of IP3 so it binds to IP3R instead of IP3 and blocks IP3R activity. (Ando et al., 2006; Ando et al, 2014). Katsuhiko Mikoshiba and other groups have reported that IRBIT contributes to electrolyte transport, mRNA processing, and the maintenance of genomic integrity (Ando et al., 2014; Arnaoutov et al., 2014; He et al, 2010; Kiefer et al, 2009; Park et al. 2013; Shirakabe et al, 2006; Yang et al, 2009). However, there is no work about the function of IRBIT in adipose tissue. There is compelling evidence that suggests IRBIT suppresses CAMK2$\alpha$ kinase activity and contributes to catecholamine homeostasis through TH phosphorylation (Kawaai et al, 2015).

Therefore, we hypothesized that IRBIT would suppress CAMK2 activation, and this would consequently regulate energy and lipid metabolism.

In present study, we show that targeted knockdown of IRBIT expression was associated with increased phosphorylation of CaMK2. It increased Sirt1 phosphorylation and PGC1$\alpha$ expression which are CaMK2 downstream signaling pathway and resulted in "browning" of white adipocyte and increased brown adipocyte activity. Results reported here suggest that IRBIT expression might be an important regulator of energy metabolism in adipocyte, and potential therapeutic targets for treating obesity, diabetes and their associated metabolic disorders.

# 2. Materials and Methods

## Animals

Mice were housed in a 12:12 light : dark cycle at 25 ° C, and chow and water were provided ad libitum. To stimulate brown adipose tissue activity, 11weeks-old mice were treated with daily intraperitoneal injections of 1mg/kg bodyweight CL-316,243 (Tocris) dissolved in PBS. For same reason, 11weeks-old mice were placed in a 6 ° C cold chamber and 30 ° C warm condition for 3 days. The experiments were performed according to the "Guide for Animal experiments" (Edited by Korean Academy of Medical Sciences) and approved by the Institutional Animal Care and Use Committee (IACUC SNU-161222-4-2) of the Seoul National University.

## Cell Culture

Primary mouse SVFs from brown adipose tissues were isolated and differentiated as described. Derivation of clonal cell lines via limiting dilution was performed as described (Harms M et al., 2013). For adipocyte differentiation assay, confluent cultures of clonal lines were exposed to induction DMEM/F-12 GlutaMAX (Invitrogen, CA, USA) containing dexamethasone (5 mM), Indomethacin (125uM), insulin (20 nM), isobutylmethylxanthine (0.5 mM), T3 (1 nM), and 10% fetal bovine serum (FBS) (Invitrogen, CA, USA). Day 4 after induction, cells were maintained in media containing insulin (20 nM), T3 (1 nM), and 10% FBS until they were ready for collection.

The mouse white pre-adipocyte cell line 3T3-L1 (purchased from the Korean Cell Line Bank). Dulbecco's Modified Eagle's Medium (DMEM), (4.5 g/L glucose, 4mM glutamine) (Welgene, Korea), supplemented with 10% Bovine Calf Serum (BCS) (Gibco, Waltham, MA, USA) at 37 ° C in 5% CO2. 3T3-L1 cell line was cultured to confluence and then induced differentiation

after 2 days (Day 0). 3T3-L1 cell line was differentiated in DMEM with 10% Fetal Bovine Serum (FBS) (Welgene, Korea) supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Merck, Germany), 1 μM dexamethasone (Merck, Germany), T3 (1 nM) and 5 μg/ml insulin (Merck, Germany). After 48 h, cells were refed in DMEM with 10% FBS containing only T3 and insulin at the above concentrations. The media were refreshed every day for additional period of 6 days. For knockdown study, small interfering RNA (siRNA) directed against IRBIT mRNA (NM_145542.3) was from bioneer. siRNA transfections were performed with Lipofectamine RNAiMAX (Invitrogen, CA, USA), according to the manufacturer's protocol.

## Immunoblotting

For immunoblot analysis, cells were lysed in RIPA buffer containing 50mM Tris-HCl(ph7.4), 1% NP-40, 1% Triton X-100, 0.25% Na-deoxycholate, 150Mm NaCl, 1mM EDTA, 10% Glycerol, Proteinase inhibitor cocktail and Phosphatase-inhibitor cocktail (GenDEPOT, Barker, TX, USA). Total Protein lysates were boiled with 5Xsample buffer that contained 1M Tris buffer (Ph6.8), 50% glycerol, 10% SDS, 2-Mercaptoethanol, 1% BPB, DW. Subsequently, Protein lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a PVDF membrane (Millipore, Billerica, Massachusetts, USA). The PVDF membrane blots were blocked in 5% BSA in Tris-buffered saline with Tween 20 (TBS-T) and incubated 1-3hours in Room Temperature with rabbit anti-IRBIT (CST94248S, Cell Signalling), rabbit anti-PGC1α (ab54481, abcam), rabbit anti-CaMK2 (CST4436S, Cell Signalling), rabbit anti-phospho CaMK2 (CST3356S, Cell Signalling), rabbit anti-UCP1 (ab10983, abcam), rabbit anti-Sirt1 (CST9475 Cell Signalling), rabbit anti-phospho SIRT1 (CST2314S, Cell Signalling) and mouse anti β-Actin(S1734, Sigma Aldrich), α-tublin (SC 8035, Santa Cruze Biotechnology). Peroxidase labled anti-rabbit IgG(PI-1000 VECTOR laboratories INC), Peroxidase labled anti-mouse IgG(PI-2000 VECTOR laboratories INC) were used as a secondary antibody. Proteins were visualized by ECL chemiluminescence (Abclon, Seoul, Korea). Immunoreactive

signals were detected through their enhanced chemiluminescence and recorded using the MicroChemi 4.2 system (DNR Bio-Imaging Systems, Jerusalem, Israel).

## Oxygen Consumption Assays

At day 6 of differentiation, oxygen consumption was measured at 37° C in cultured fat cells by using seahorse XF24 system (Seahorse Bioscience). For CL316,243-induced respiration assays, fully differentiated fat cells were incubated with 1 uM CL316,243 for 12 hr before measuring oxygen consumption. Cells were maintained in a 5% CO2 incubator at 37° C, and 1 h before the experiment, the cells were washed and incubated in 600㎕ of DMEM containing 10 mM glucose (DMEM-high glucose) pH 7.4 at 37° C in a non-CO2 incubator. 1 mM oligomycin (Merck, Germany) was added to block ATP production, rendering uncoupled respiration. After this, the mitochondrial uncoupler FCCP (Merck, Germany) was added into the media. Finally, ETC activity was blocked by the addition of rotenone or antimycin A (Merck, Germany).

## Quantitative PCR(qPCR) analysis

Total RNA was extracted using a total RNA purification system(Invitrogen) following to the manufacturer's protocol. Extracted mRNA was reverse-transcribed using PrimeScript 1st strand cDNA Synthesis kit (Takara). Quantitative PCR was performed with SYBR Green dye using the ABI Step One Real Time PCR instrument (Applied Biosystems, Cheshire, U.K.). For relative quantification of gene expression, we used the comparative Ct method ( $\Delta\Delta$Ct ). The specific primers sequences used for PCR are listed in Supplementary Table1.

## Statistics

Statistical analysis was performed using the Student's t-test between two groups. $P < 0.05$ was considered significantly.

Table 1. Primer sequences for quantitative RT-PCR

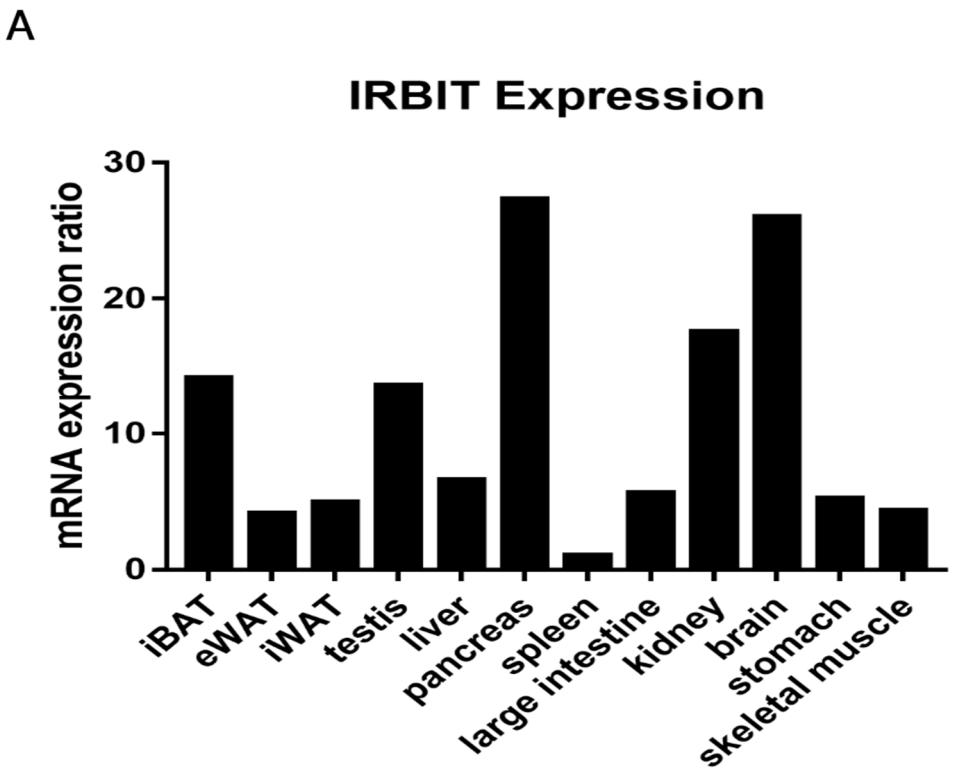
| Gene | Forward (5′ -> 3′) | Reverse (5′ -> 3′) |
|---|---|---|
| IRBIT | TACTTTGCTTGGTCATCTG | CAGATGACCAAGCAAAGTA |
| PGC1α | CCCTGCCATTGTTAAGACC | TGCTGCTGTTCCTGTTTTC |
| UCP1 | ACTGCCACACCTCCAGTCATT | CTTTGGCTCACTCAGGATTGG |
| 36B4 | GAGGAATCAGATGAGGATATGGGA | AAGCAGGCTGACTTGGTTGC |
| β-Actin | GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT |
| aP2 | ACACCGAGATTTCCTTCAAACTG | CCATCTAGGGTTATGATGCTCTTC |
| C/EBPα | GACATCAGCGCCTACATCGA | TCGGCTGTGCTGGAAGAG |
| Cox4 | GTACCGCATCCAGTTTAACGA | CCATACACATAGCTCTTCTCCCA |
| Cox5a | TTGATGCCTGGGAATTGCGTAAAG | AACAACCTCCAAGATGCGAACAG |
| Cox5b | ATCAGCAACAAGAGAATAGT | GTAATGGGTTCCACAGTTGGG |
| COX7a1 | CAGCGTCATGGTCAGTCTGT | AGAAAACCGTGTGGCAGAGA |
| PPARγ | CTGGCCTCCCTGATGAATAAAG | AGGCTCCATAAAGTCACCAAAG |
| Cidea | TGCTCTTCTGTATCGCCCAGT | GCCGTGTTAAGGAATCTGCTG |

| | | |
|---|---|---|
| Dio2 | CAGTGTGGTGCACGTCTCCAA<br>T | TGAACCAAAGTTGACCACCAG |

# 3. Results

## IRBIT expression in mouse tissues.

To study the role of IRBIT, We first performed quantitative RT-PCR analysis to measure IRBIT mRNA abundance in each tissue. As previously reported, IRBIT mRNA was ubiquitously expressed. However, we found out IRBIT was expressed especially in brain, pancreas, kidney, testis and iBAT (interscapular Brown adipose tissue) (Fig 1A). To examine whether IRBIT has a role in adipose tissue function, we performed IRBIT transcript and protein expression level in iBAT, epididymal white adipose tissue (eWAT) and inguinal white adipose tissue (ingWAT). IRBIT was highly expressed in iBAT among three adipose tissues (Fig 1B, C). These results indicate that IRBIT may have a role in brown adipose tissue which increases energy expenditure (Cannon and Nedergaard, 2004).



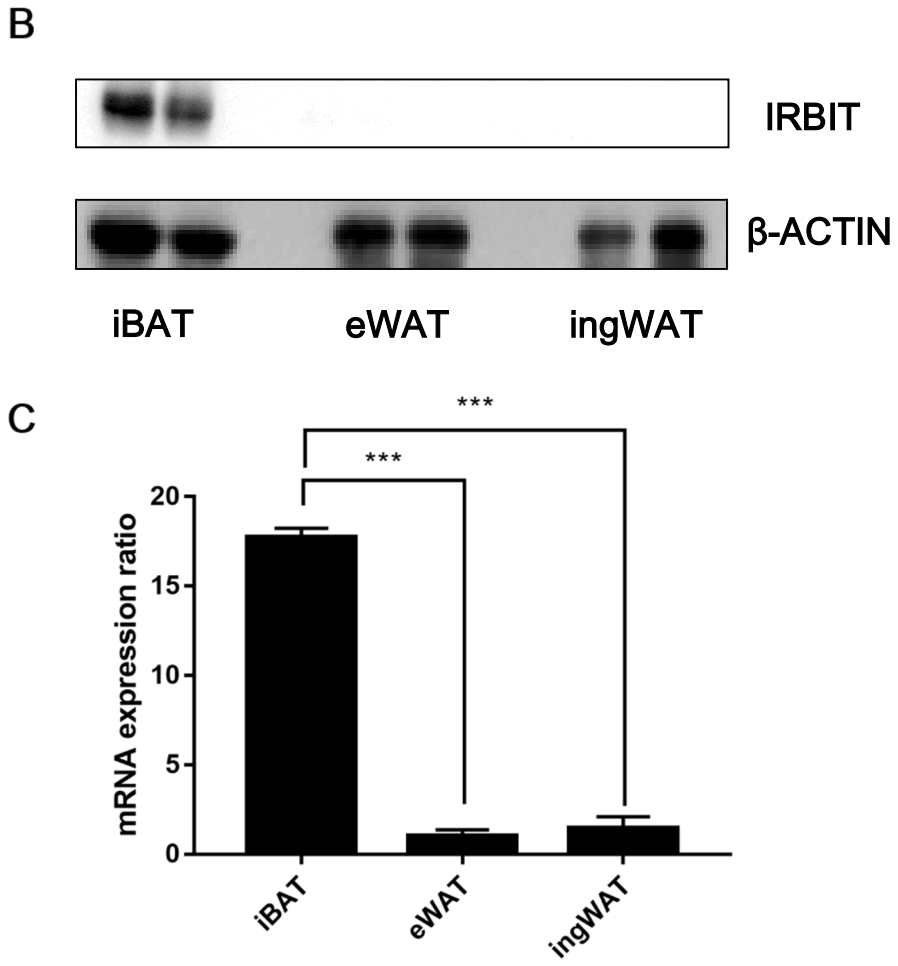
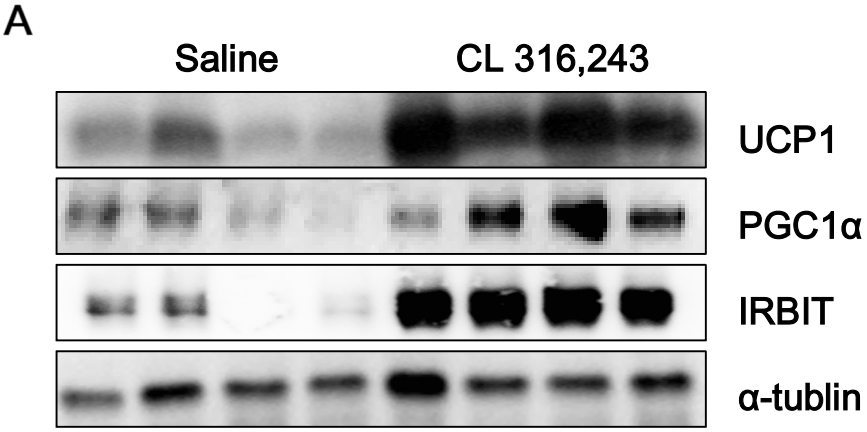



Figure 1. IRBIT expression in adipose tissues.

(A) Relative mRNA expression levels of IRBIT in adult mouse tissues. Relative mRNA expression levels were measured by qRT-PCR. qRT-PCR analysis of RNA isolated from tissues of B6/N mouse. (B) IRBIT protein expression in adipose tissues. (C) This was confirmed by qRT-PCR. Values were normalized to $\beta$-Actin. (*$P$ < 0.05, ** $P$ < 0.01, *** $P$ < 0.001 )

To examine the expression level of IRBIT in activated brown adipose tissue, we treated CL 316,243 which was $\beta$3-adrenergic receptor agonist in B6/N mice. This action was carried out to induce iBAT activation. IRBIT expression was significantly increased when BAT was activated after CL treatment which was demonstrated by the UCP1 and PGC1$\alpha$ pretein levels (Fig 2A). This was confirmed by IRBIT gene expression level which was accompanied by the rise of brown adipocyte marker gene UCP1 and PGC1$\alpha$ (Fig 2B). Also, IRBIT gene expression level was increased during cold-induced thermogenesis and CL316,243 treatment in ingWAT. Browning effect of white adipose tissue was confirmed by UCP1 gene level which was increased more than ~150 fold. These data shows the possibility that IRBIT is involved in brown adipocyte activation pathway and browning effect in white adipocyte.



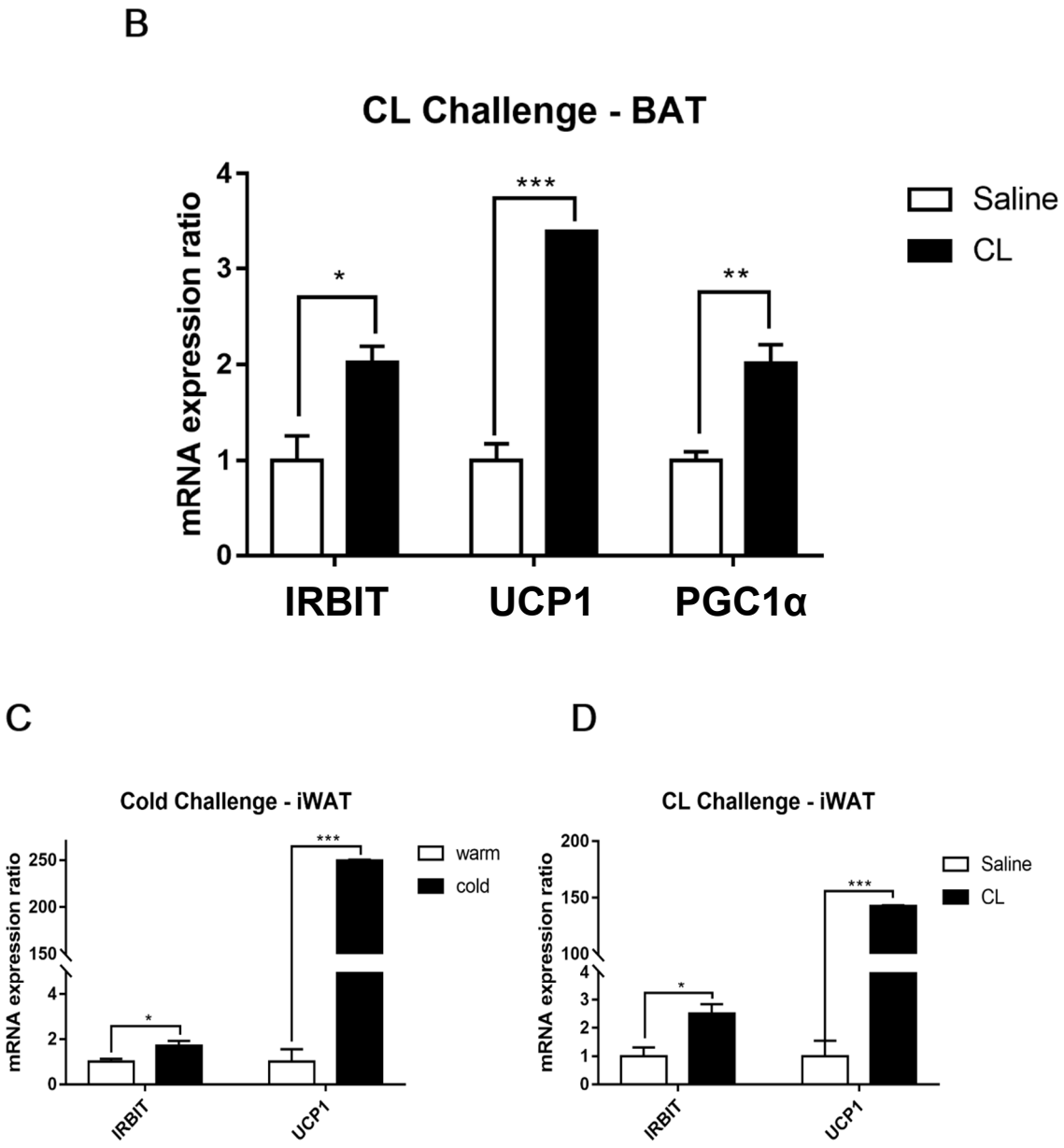


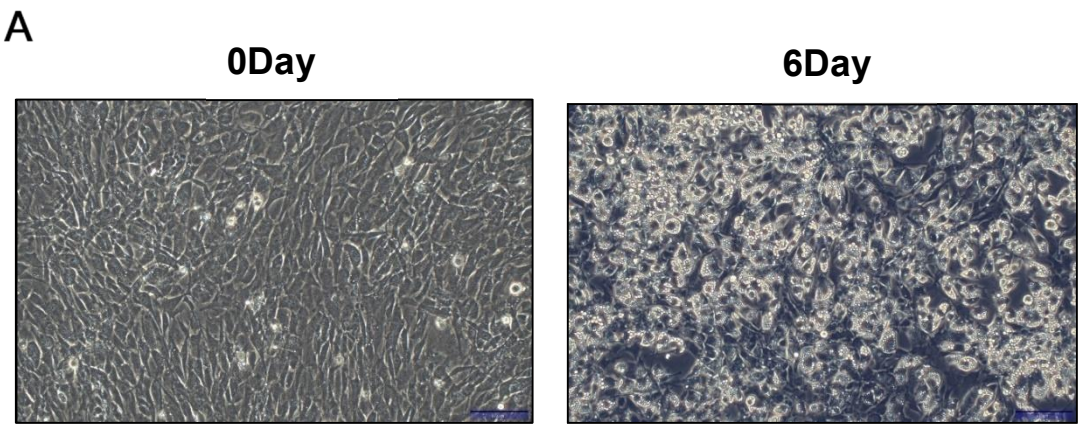

Figure 2. IRBIT expression was increased in response to cold-induced thermogenesis and CL 316,243 treatment.

(A) IRBIT protein expression pattern in brown adipose tissue after CL 316,243 ($\beta$-adrenergic receptor 3 agonist) treatment. Brown adipose tissue activation was confirmed by increased UCP1 and PGC1$\alpha$ expression. (B) IRBIT mRNA expression pattern in brown adipose tissue after CL 316,243 treatment. Brown adipose tissue activation was confirmed by increased UCP1

and PGC1$\alpha$ expression. (C) IRBIT mRNA expression pattern in inguinal white adipose tissue after cold induced thermogenesis. Browning of white adipose tissue was confirmed by increased UCP1 expression. (D) IRBIT mRNA expression pattern in inguinal white adipose tissue after CL 316,243 treatment. Relative mRNA expression levels were measured by qRT-PCR. Values were normalized to $\beta$-Actin. qRT-PCR analysis of RNA isolated from iBAT and ingWAT of B6/N mice (N=4). (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ )

## The effect of IRBIT on brown adipocyte function

We next examined whether IRBIT functions in brown adipocytes by using immortalized primary brown adipocyte. We differentiated brown preadipocyte to investigate the pattern of IRBIT during brown adipogenesis. We started to differentiate when the confluency was 80%, which named 0day. Then we differentiated cell as mentioned in materials and methods until day6 (Fig 3A). To evaluate expression pattern of IRBIT and brown adipocyte-selective genes, cell lysates were prepared during the differentiation process. We also treated CL 316,243 on day 6 to see the IRBIT expression level after brown adipocyte activation. IRBIT gene expression was significantly increased as well as mitochondrial markers (Cox4, Cox5a, Cox5b, Cox7a1) and brown adipocyte markers (Dio2, Cidea, UCP1, PGC1$\alpha$) (Fig 3B). We therefore believed that IRBIT was involved in brown adipocyte activation.



B

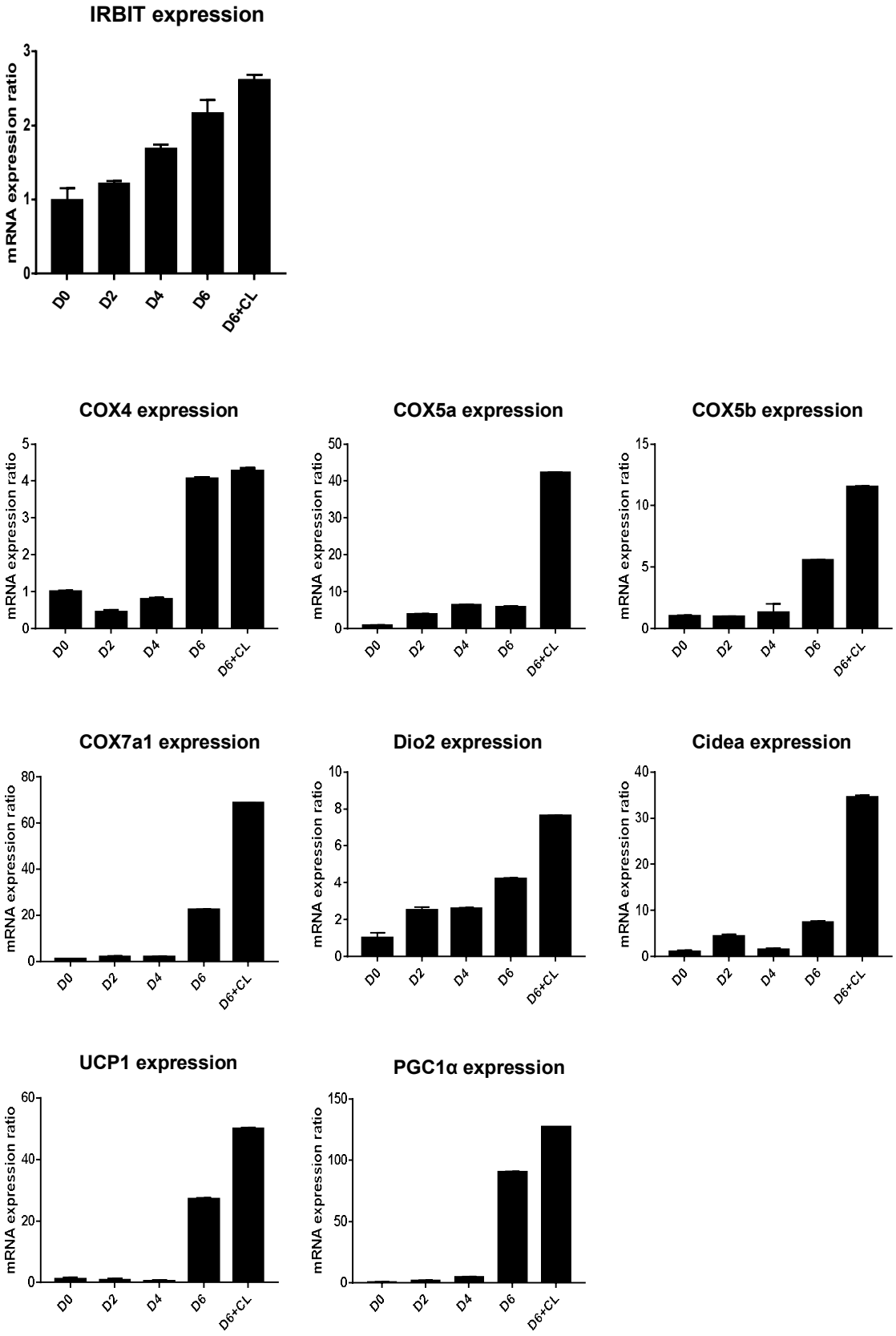
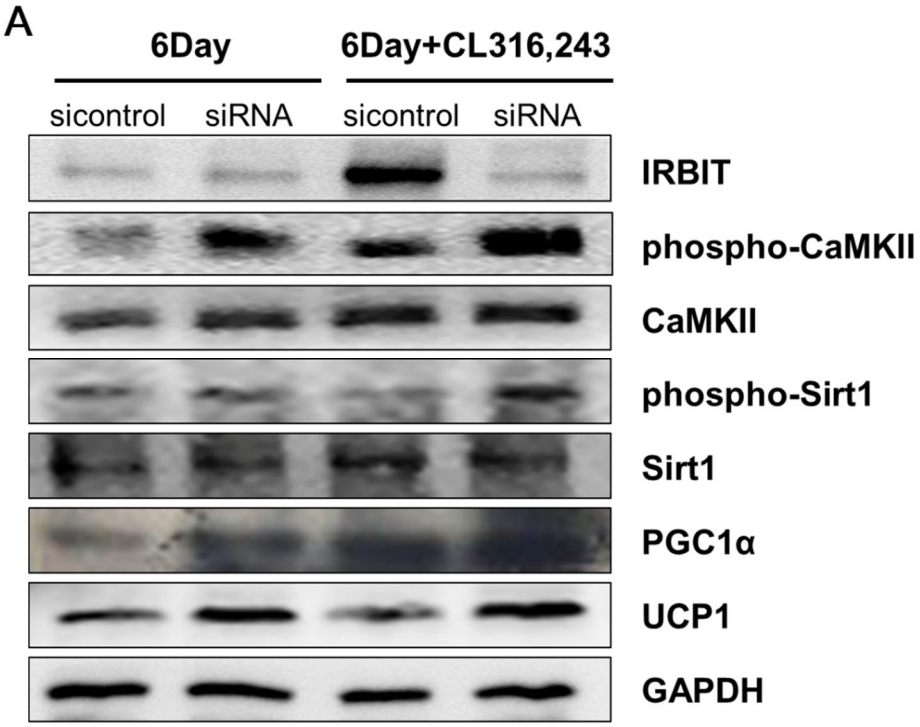

IRBIT expression
mRNA expression ratio
D0
D2
D4
D6
D6+CL
COX4 expression
COX5a expression
COX5b expression
COX7a1 expression
Dio2 expression
Cidea expression
UCP1 expression
PGC1α expression

**Figure 3. IRBIT expression during brown adipogenic differentiation.** (A) Primary brown adipocyte was differentiated for 6days (X 400). (B) Relative mRNA expression patterns of IRBIT, mitochondrial markers (Cox4, Cox5a, Cox5b, Cox7a1) and brown adipocyte markers (Dio2, Cidea, UCP1, PGC1α) were listed. Relative mRNA expression levels were measured by qRT-PCR. Values were normalized to 36B4.

## The functional loss of IRBIT induces brown adipocyte activation mechanism

Previously, it has been reported that IRBIT suppressed CaMK2 phosphorylation by attaching to CAMK2 kinase domain (Kawaai et al, 2015). And also, previous studies have shown that Sirt1, PGC1α are downstream signals of CAMK2 in brown adipocyte (Baskaran P et al, 2017). Based on these facts, when IRBIT was deficient, CAMK2 and Sirt1 phosphorylation and PGC1α would be increased. So we transfected the IRBIT specific small interference RNA into differentiated brown adipocyte. And we treated CL316,243 to activate brown adipocyte. First of all, Brown adipocyte marker genes PGC1α and UCP1 were highly expressed in CL treated siRNA compared to CL treated sicontrol. Secondly, phosphorylation of CAMK2 and Sirt1 were increased in CL treated siRNA compared to CL treated sicontrol. Consequently, these findings suggest that IRBIT deficiency brown adipocyte activation in a CaMK2 dependent manner (Fig 4A). Also, we examined the effect of IRBIT deficiency on mitochondrial activity. We measured the OCR (Oxygen Consumption Rate) in differentiated brown adipocyte after CL 316,243 treatment. We found that IRBIT deficiency significantly enhanced overall oxygen consumption rate in brown adipocyte (Fig. 6B, C). Therefore, we concluded that the loss of IRBIT increased brown adipocyte activity in a CaMK2 dependent manner.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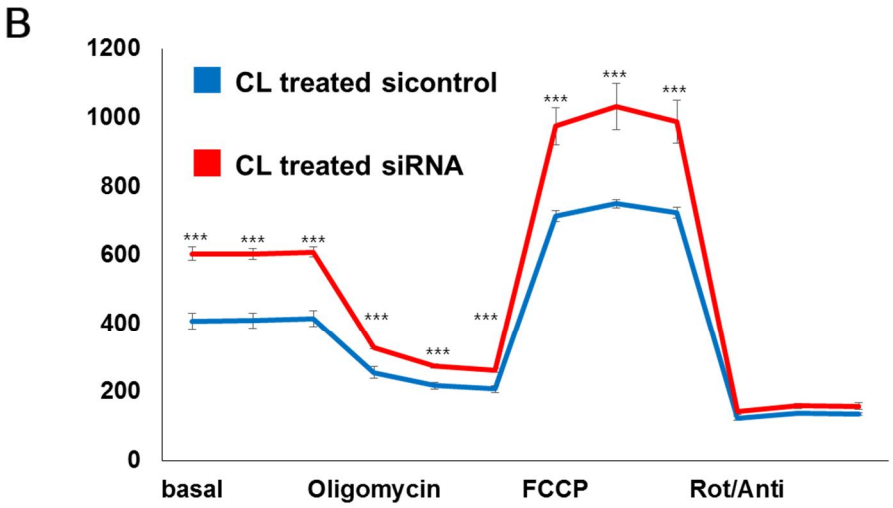
UCP1
GAPDH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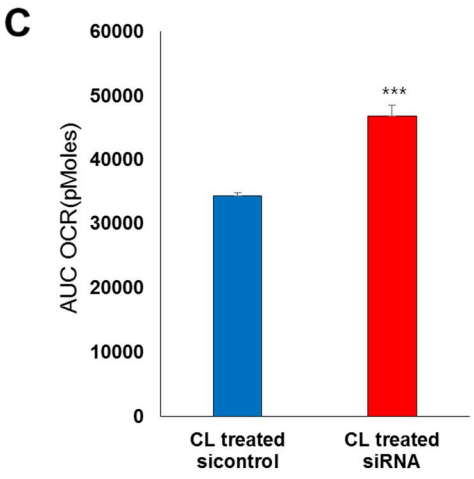
C
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CL treated sicontrol
CL treated siRNA

## Figure 4. IRBIT deficiency enhanced brown adipocyte activity.

Brown primary cells were transfected with IRBIT specific siRNA and compared to siControl. CL 316,243 was treated to activate brown adipocyte. (A) Western blot analysis of the effect of IRBIT on brown adipocyte activation. (B) Oxygen consumption rates (OCR) measured in differentiated brown adipocyte. (C) AUC (Area Under the Curve) of OCR graph. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

## The effect of IRBIT on white adipocyte function

We also examined the role of IRBIT in white adipocyte using 3T3-L1 cell line. The 3T3-L1 cell line is generally used as a model for differentiation of committed white pre-adipocytes into white adipocytes (Foster and Bartness, 2006). The 3T3-L1 cell line was induced to differentiate into white adipocytes as described under "Materials and Methods". Then, we put IRBIT-specific siRNA (small interference RNA) into 3T3-L1 cells and compared with sicontrol cells. We analyzed mRNA expression of genes which involved in white adipogenic differentiation were measured by qRT-PCR. The mature adipocyte markers including a carrier protein for fatty acids (aP2), enhancer binding protein $\alpha$ (C/EBP $\alpha$), peroxisome proliferator-activated receptor $\gamma$ (PPAR $\gamma$), and IRBIT gene expressions were significantly increased during white adipocyte differentiation. However, when IRBIT mRNA expression was successfully down-regulated, there was no significant change on white adipocyte specific marker genes (PPAR $\gamma$, C/EBP $\alpha$, aP2) expression. Therefore, we concluded that loss of IRBIT had no significant effect on white adipocyte differentiation.

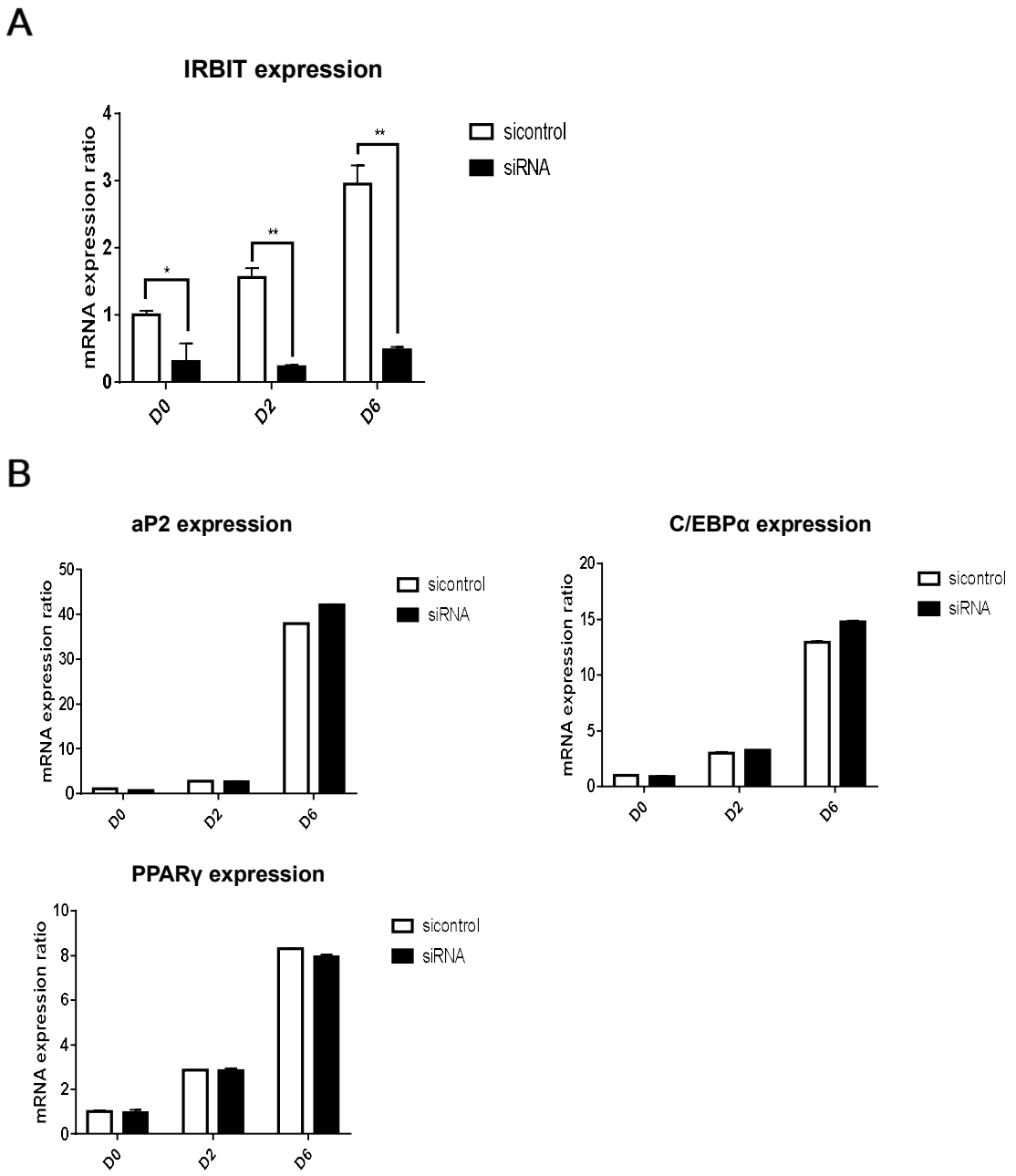
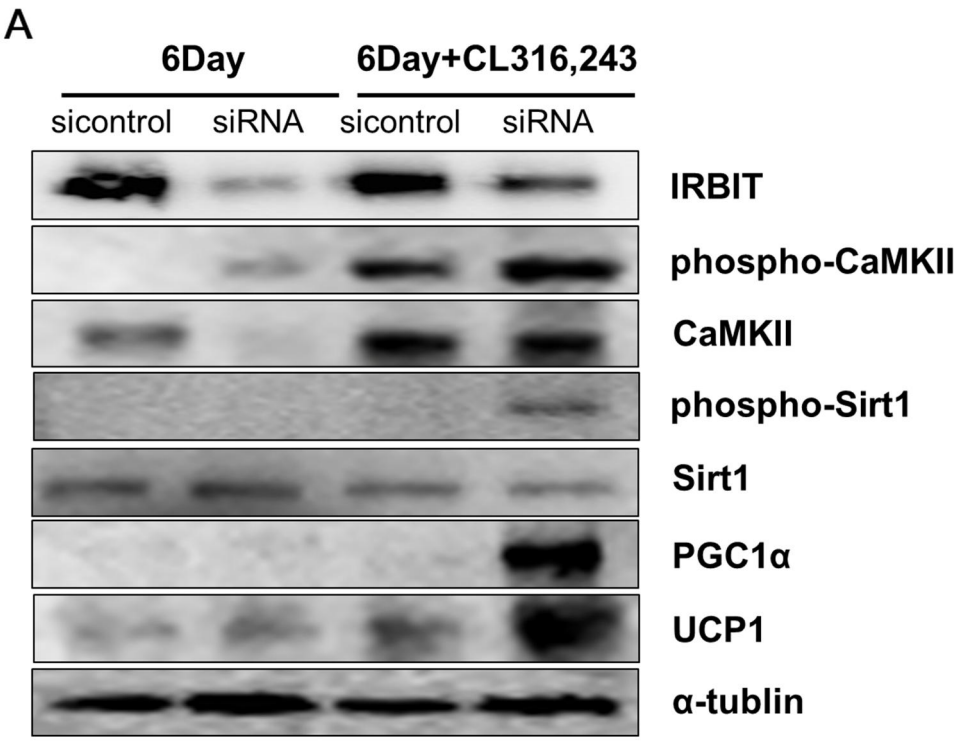



Figure 5. The loss of IRBIT showed no significant effect on white adipocyte differentiation. 3T3-L1 cells were transfected with IRBIT-specific siRNA. (A) The effect of IRBIT siRNA on IRBIT expression. (B) Relative mRNA expression patterns of genes including aP2, PPAR$\gamma$, C/EBP$\alpha$ which are main factors in white adipogenesis. Relative mRNA expression levels were measured by qRT-PCR and values were normalized to $\beta$-Actin. ($*P < 0.05$, $** P < 0.01$, $*** P < 0.001$)

## The loss of IRBIT enhanced browning effect on white adipocyte

Although IRBIT had no effect on white adipogenesis, we decided to examine the effect of IRBIT on white adipocyte "browning" since IRBIT was increased in CL316,243 treated inguinal white adipose tissue (Fig 2D). So we transfected the IRBIT specific small interference RNA into differentiated white adipocyte. And we treated CL316,243 to induce "browning" effect in 3T3-L1. Interestingly, IRBIT deficiency enhanced brown adipocyte activation pathway in white adipocyte. Brown adipocyte marker genes PGC1$\alpha$ and UCP1 were highly expressed in CL treated siRNA compared to CL treated sicontrol. Phosphorylation of CAMK2 and Sirt1 were also increased in CL treated siRNA compared to CL treated sicontrol. When IRBIT expression was successfully knock downed, phosphorylated form of CaMK2 and Sirt1 expressions were increased after CL316,243 treatment. Consequently, these findings suggest that IRBIT deficiency induce browning in a CaMK2 dependent manner in white adipocyte (Fig 6A). Also, we examined the effect of IRBIT deficiency on mitochondrial activity. We measured the OCR (Oxygen Consumption Rate) in differentiated 3T3-L1 6 days after CL 316,243 treatment. We found that IRBIT deficiency slightly enhanced overall oxygen consumption rate (Fig. 6B, C). Therefore, we concluded that loss of IRBIT increased browning effect on white adipocyte.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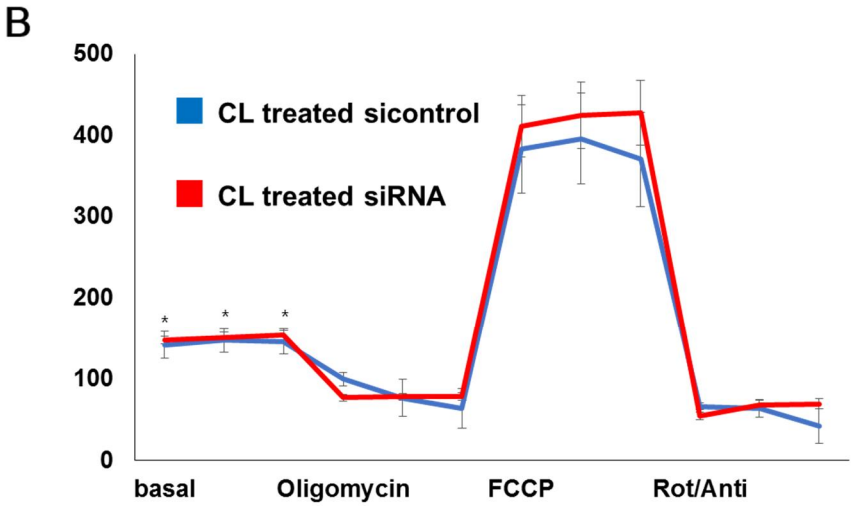
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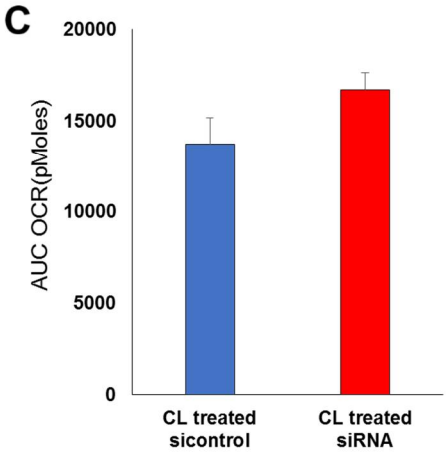
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AUC OCR(pMoles)
CL treated sicontrol
CL treated siRNA

**Figure 6. IRBIT deficiency enhanced browning effect on white adipocyte.** 3T3-L1 cells were transfected with IRBIT specific siRNA and compared to siControl. CL 316,243 was treated to induce browning effect on white adipocyte. (A) Western blot analysis of the effect of IRBIT on browning on white adipocyte. (B) Oxygen consumption rates (OCR) measured in differentiated 3T3-L1. (C) AUC (Area Under the Curve) of OCR graph. (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ )

# 4. Discussion

The functions of IRBIT in adipose tissues have been unclear, although IRBIT is highly expressed in iBAT. Here, we provide evidence that IRBIT acts as a critical regulator of energy metabolism in adipocyte. Previously, it has been revealed that IRBIT bound to calcium calmodulin-dependent kinase 2 alpha (CaMK2$\alpha$) and suppressed its phosphorylation. CaMK2$\alpha$ subsequently controlled tyrosine hydroxylase (TH) phosphorylation which increased the biosynthesis of catecholamines (Kawaai et al, 2015). TH is also a marker of sympathetic innervation (Rosen et al, 2014). Catecholamine induces lipolysis in adipose tissue (Jocken et al, 2008). These factors made us think IRBIT might have a function in fat metabolism.

IRBIT was ubiquitously expressed in mice and human, however, the function of IRBIT was nearly unrevealed in fat tissues. Here we provide the evidence of the IRBIT function in fat metabolism. IRBIT was highly expressed not only in brain but in Brown Adipose Tissue (BAT). We supposed that IRBIT has a certain role in BAT to activate energy expenditure. So we treated CL316,243 ($\beta$3-Adrenergic receptor agonist) to B6/N wild type mouse, IRBIT expression was increased including brown adipocyte-selective marker genes UCP1 and PGC1$\alpha$ not only in BAT also in ingWAT (inguinal white adipose tissue).

Thus, we used brown primary cell to figure out the function of IRBIT in Brown adipocyte. IRBIT expression was significantly increased after differentiation and activation of brown adipocyte. Then we showed the effect of IRBIT on brown adipocyte by transfecting IRBIT specific siRNA on differentiated and activated brown adipocytes. IRBIT deficiency increased protein expressions of UCP1, PGC1$\alpha$ and CaMK2, Sirt1 phosphorylation were enhanced. This finding showed that IRBIT induce brown adipocyte activation in a CaMK2 dependent manner. Oxygen consumption rate was significantly increased when IRBIT was deficient. We also used 3T3-L1 cell line to see the effect of the loss of IRBIT on white adipocyte differentiation and browning.

We found out IRBIT expression was increased during white adipogenic differentiation but there was no significant effect on white adipogenesis. We showed the IRBIT deficiency enhanced browning effect by OCR data. This phenomenon could be explained by And this could be explained by increased protein expressions of beige adipocyte selective genes UCP1, PGC1$\alpha$ (Wu et al, 2012) and CaMK2, Sirt1 phosphorylation were enhanced in IRBIT deficient white adipocytes. In conclusion, IRBIT might be an important regulator of lipid metabolism.

We have delineated a novel mechanism by showing IRBIT downstream protein expressions which were affected by loss of IRBIT in brown and white adipocyte. However, this work presents specific role of IRBIT only in vitro. Further studies are, therefore, required to specifically show the phenotype of IRBIT knockout mouse metabolism. The strategy of increasing the number of brown or beige adipocytes to combat metabolic diseases has recently been studied intensively. We believe that our results offer novel insight into brown adipocyte function, which might be useful for developing therapeutic strategies aimed at enhancing brown and beige adipocyte thermogenic activity.

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# 국문초록

IRBIT 는 IP3 R binding protein released with inositol 1,4,5-trisphosphate 의 약자로, IP3 와 같은 구조를 가지고 있다고 알려져 있다. 세포질그물(Endoplasmic Reticulum)에 존재하는 IP3 수용체에 IP3 대신 붙어 IP3R 을 통해 세포질그물에서 세포질로 분출되는 칼슘의 농도를 조절하는 역할을 한다. IP3 농도가 낮은 상태에서는 세포질그물에 붙은 IP3 Receptor Protein 에 IRBIT 가 IP3 대신 붙어서 존재하고 있다가 IP3 농도가 높을 때에는 IRBIT 가 떨어져 나오면서 세포질액 (cytosol)에서 그 역할을 하는데, 현재까지 mRNA processing 에 관여하거나, 유전체 항상성을 유지하는 등의 역할이 알려져 있었다. 또한 이전 연구에서, IRBIT 가 CaMK2α (Ca2+/calmodulin-dependent protein kinase 2α)의 kinase domain 에 상호작용하여 그 활성을 억제한다는 것을 밝힌 바가 있다. CaMK2 는 TH(Tyrosine hydroxylase)의 활성을 증가시키는데 TH 는 카테콜아민계 호르몬을 합성하는 효소이며 카테콜아민은 지방조직에서 지방분해를 유도하는 호르몬이기에 IRBIT 가 지방조직에서 역할을 할 것이라 생각할 수 있다.

하지만 이 결과는 뇌와 HEK293 세포주에서 연구된 결과였고, 지방세포에서의 역할은 아직까지 연구된 바가 없다. 이에 이 논문에서는 IRBIT 가 CaMK2 의 작용을 억제하여 결과적으로 갈색지방세포의 활성화에 관여할 것이라는 가설을 세우고, IRBIT 의 발현과 그 역할을 규명하는 실험을 진행하였다.

마우스 전 장기 중 알려진 대로 뇌조직에 IRBIT 유전자 발현량이 많았지만, 갈색지방 조직에서도 그 발현량이 많다는 것을 확인하였다. 이를 토대로 B6/N 마우스에 CL 316,243 (베타 3-아드레날직 수용체 길항제)를 처리하여 갈색지방을 활성화 했을 때, IRBIT 의 단백질과 유전자 발현 모두 대조군에 비해 상당히 증가하였다. 또한, 추위로 열발생을 증가하거나, CL 316,243 의 처리로 샅부위 (inguinal region)의 백색지방 조직의 갈색지방화 유도 시에도, IRBIT 의 발현이 증가하였다. 결과를 토대로 IRBIT 가 지방조직에서 어떠한 기능을 할 것이라고

생각하였기에, 백색지방 세포주인 3T3-L1 와 갈색지방세포를 사용하여 분화과정과 활성화 과정에서 IRBIT 가 미치는 영향을 조사하였다.

그 결과, 백색지방세포와 갈색지방세포의 분화 과정에서 IRBIT 는 분화가 진행될수록 증가하는 양상을 보인다. 먼저, 갈색지방세포에서 IRBIT 의 유전자 발현을 감소시켰을 때, 갈색지방세포의 활성이 증가한다는 사실을 사립체 (mitochondria) 활성 실험에서 확인하였다. 이는 IRBIT 의 발현이 줄어들면 CaMK2 의 활성이 증가하고, 그의 하위 신호체계 중 Sirt1 의 인산화가 늘어나고, 갈색지방세포 특이적 유전자인 UCP1, PGC1$\alpha$의 발현이 증가하기 때문이라는 것을 western blot 실험을 통해 확인하였다.

또한, 백색지방 세포주인 3T3-L1 에서 분화 과정에 IRBIT 의 발현을 감소시켰을 때 백색지방 분화에 영향을 주는지 확인하였다. 그 결과, 분화 후에 백색지방 특이적 유전자들(aP2, C/EBP$\alpha$, PPAR$\gamma$)의 발현에 차이가 없었기에 IRBIT 가 백색지방세포의 분화에 미치는 영향은 적다는 결론을 내렸다. 하지만, 3T3-L1 의 분화 후 CL 316,243 처리로 갈색지방화 (browning)를 유도 했을 때, IRBIT 의 발현을 감소시킨 경우 사립체의 활성이 증가한다는 것을 관찰하였다. 이 현상 또한, 백색 지방세포에서 IRBIT 의 발현이 감소하면서 CaMK2 인산화와 그 하위 신호체계가 활성화되어 IRBIT 가 백색지방의 갈색지방화에 영향을 미친다는 사실을 western blot 실험을 통해 확인하였다.

결과적으로, 이 논문에서는 IRBIT 가 갈색지방세포의 활성화와 백색지방세포의 갈색지방화에 관여하며, IRBIT 의 발현이 감소할 경우 지방대사의 활성이 증가한다는 사실을 보여주고 있다.

**주요 단어** IRBIT, 갈색지방조직, 베타 3-아드레날직 수용체 길항제, CaMK2, 갈색지방화

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