SICOM & AOCO 2024
SOMS International Conference on Obesity & Metabolism
in conjunction with Asia-Oceania Conference on Obesity
Hosted by SOMS Society for Korean Obesity and Metabolism Studies
Co-Hosted by Asia Oceania Association for the Study of Obesity

Biology of brown/beige adipocytes and therapeutic potential for the treatment of obesity

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Iwate Medical University

Iwate Medical University

Morioka City / Iwate

Contents of Presentation

- Therapeutic potential of brown/beige adipocytes for the treatment of obesity

- Suppression of adipose tissue fibrosis by PRDM16-GTF2IRD1 complex impact systemic glucose metabolism

- Development of a novel treatment for obesity by activating brown/beige adipocytes

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Pathology and Factors of Obesity

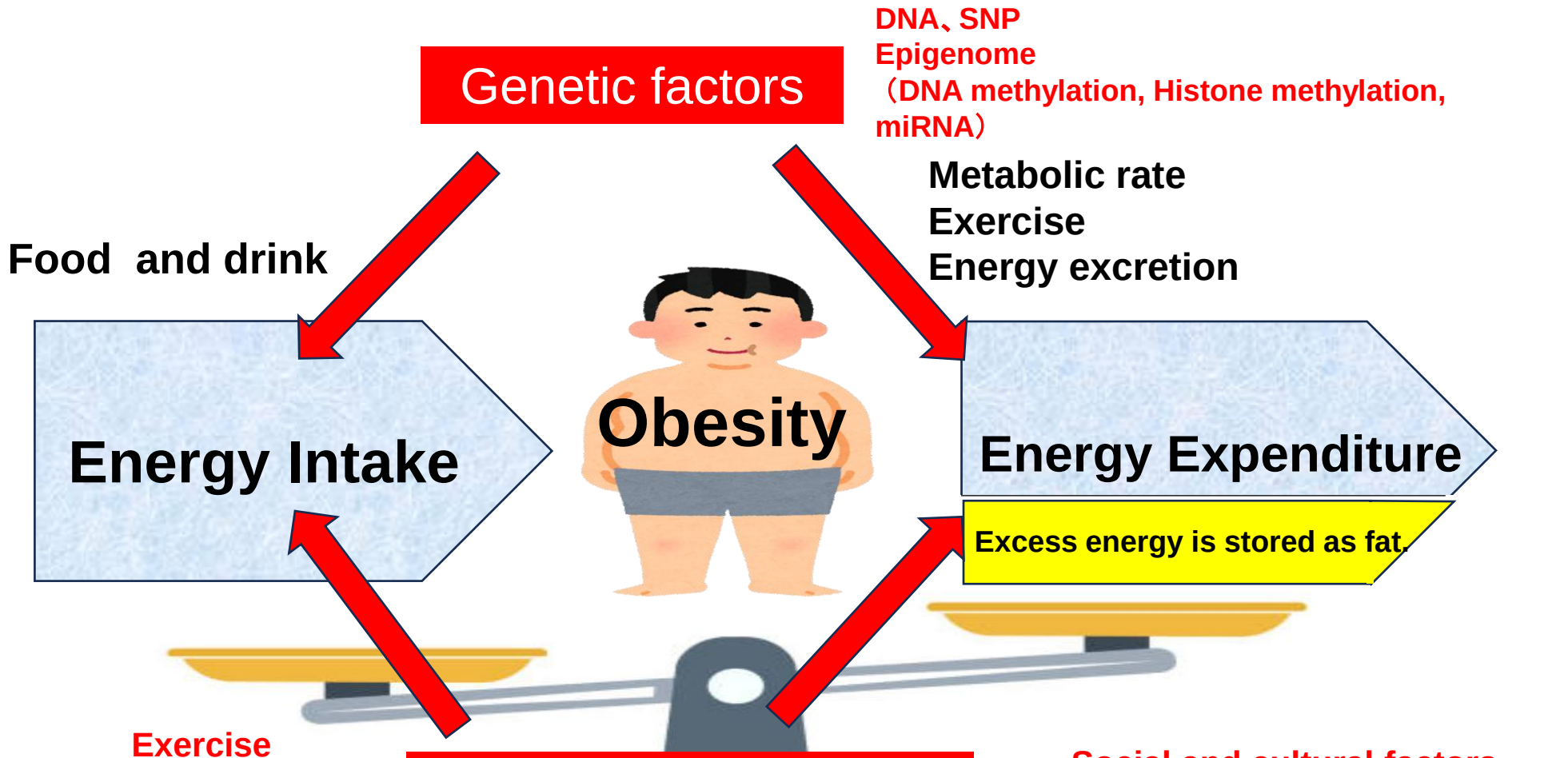
Food and drink

Energy Intake

Metabolic rate
Exercise
Energy excretion

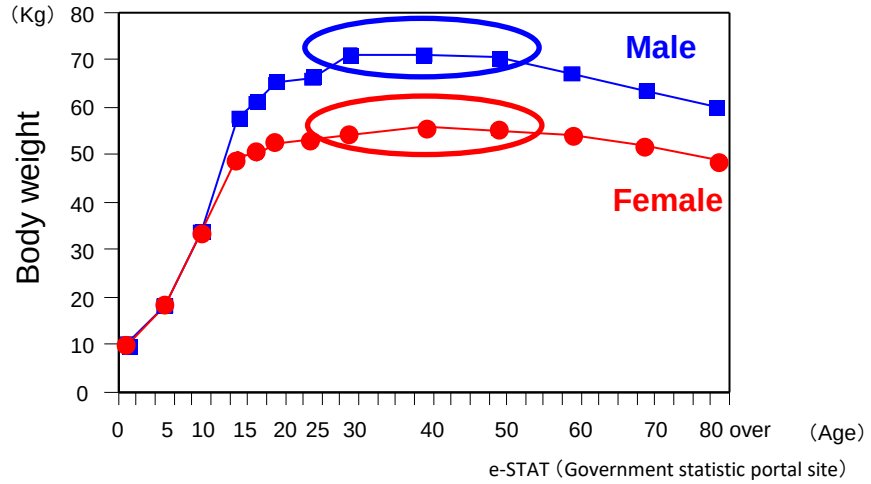
Energy Expenditure

Pathology and Factors of Obesity



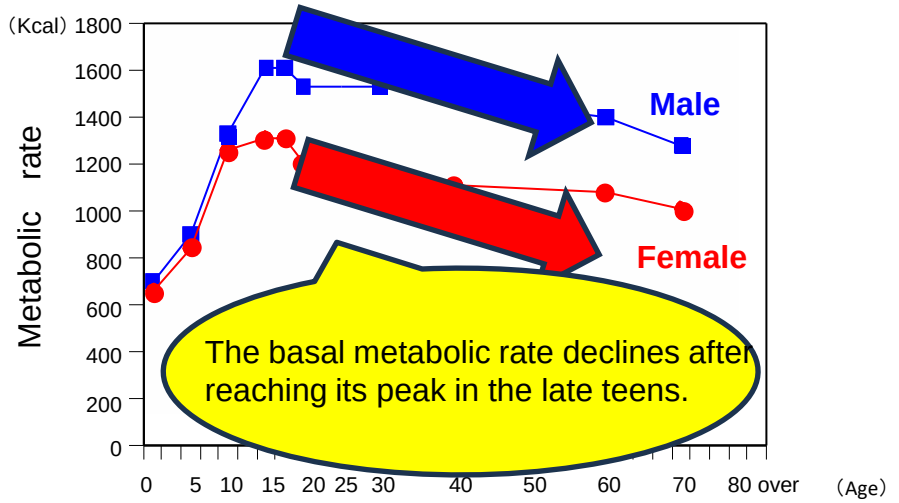
Changes in average weight and basal metabolic rate by age

Average body weight



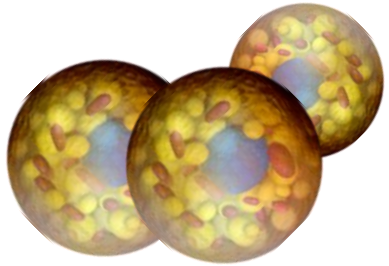
e-STAT (Government statistic portal site)

Average metabolic rate



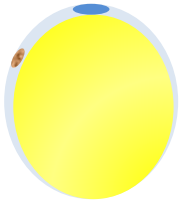
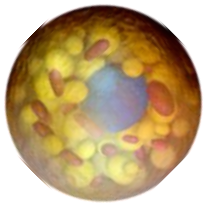
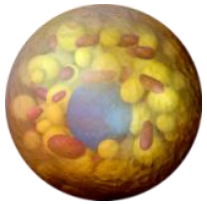
日本人の食事基準量2020から

One of the reasons for this decreased metabolism is a decrease in the activity of brown/beige adipocytes.



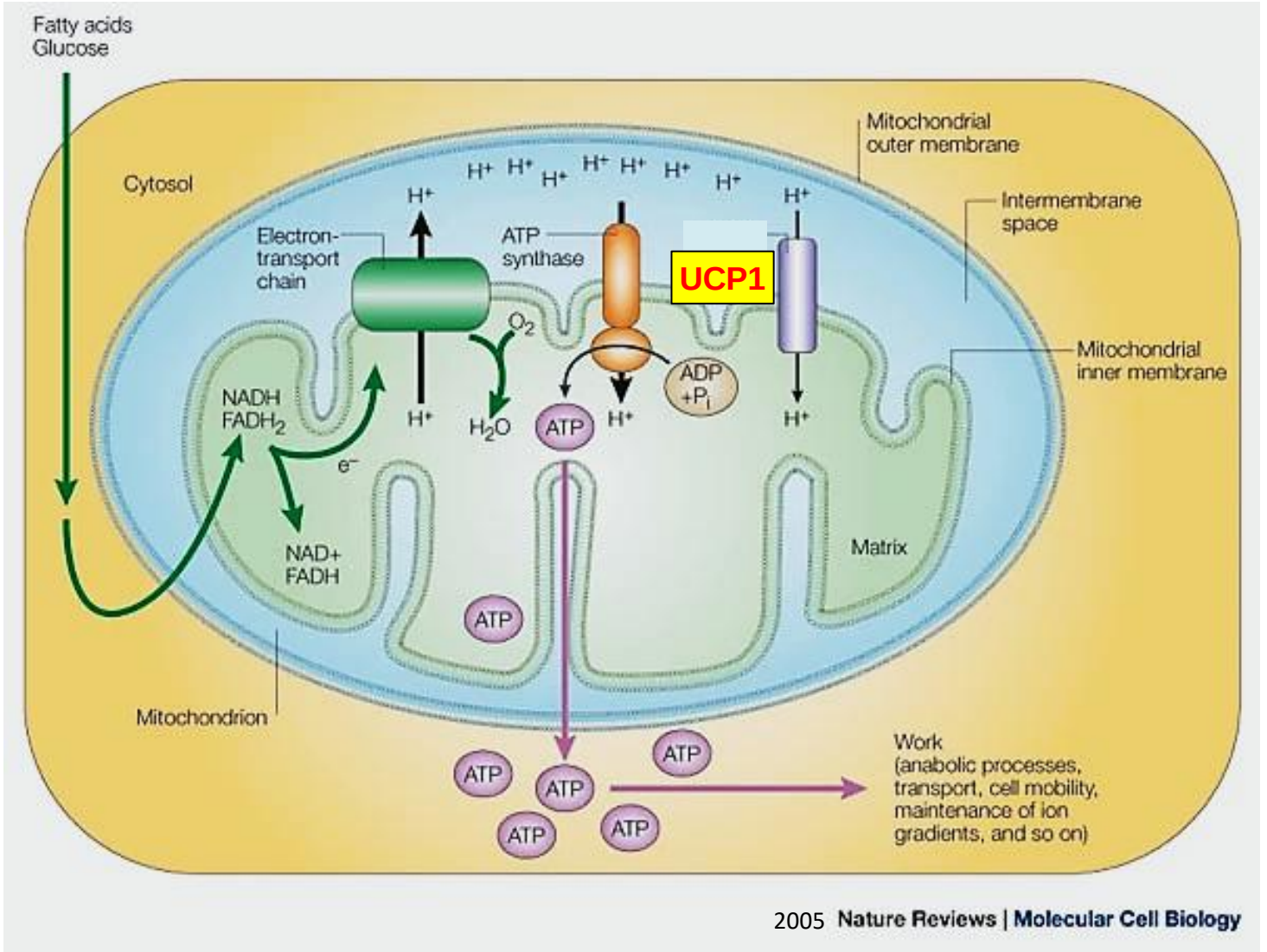
White adipocyte v.s. Brown/beige adipocyte

Adipocyte

	White adipocyte	Brown/beige adipocyte	
		Uncoupling protein 1 (UCP1)	Thermogenic function
			
Location in mice	Subcutaneous, aroundl organs (epididymus, mesentery)	Interscapular, axillary, perirenal area	Scattered in the subcutaneous adipose tissue
Location in human	Abdominal, subcutaneous, and intraperitoneal	Accumulated between the shoulder blades (infants only)	Scattered in subcutaneous adipose tissue such as the neck, supraclavicular, and axillary region
Existence form	Pre-existing	Pre-existing	Inducible
Structure	Univesicular (single/large) lipid droplet Few Mitochondria	Multivesicular (multiple/small) lipid droplets Rich Mitochondria	

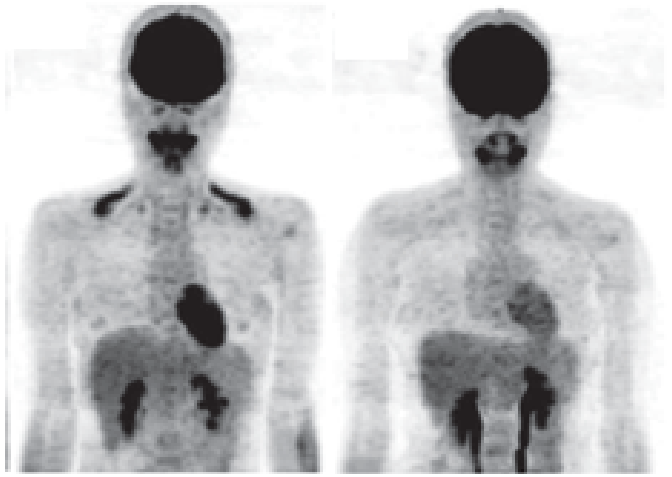
Function of UCP1

Brown/beige adipocyte

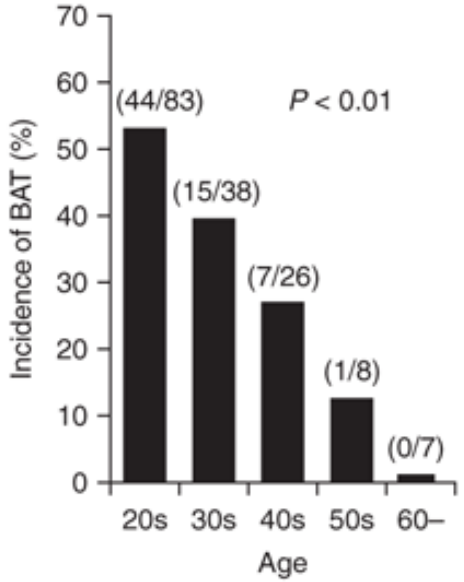


2005 Nature Reviews | Molecular Cell Biology

Evaluation of brown fat cells by PET/CT scan



There are differences in BAT activity even within the same age.



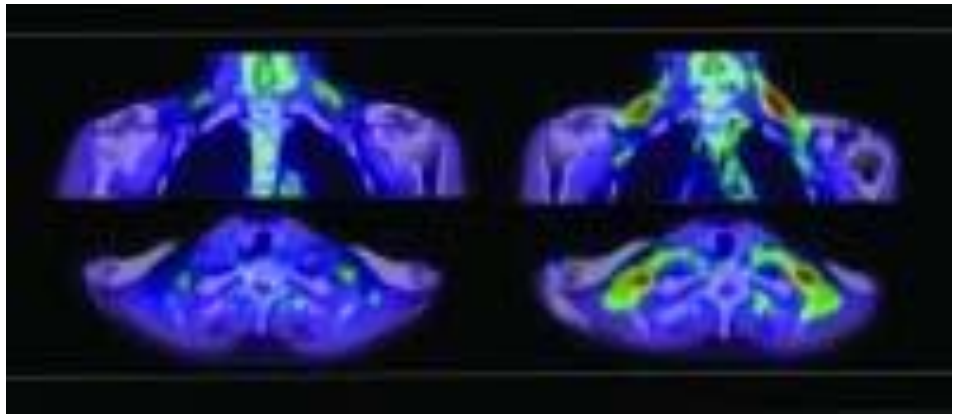
As age increases, BAT activity tends to decrease.

Yoneshiro et al, Obesity. 2011

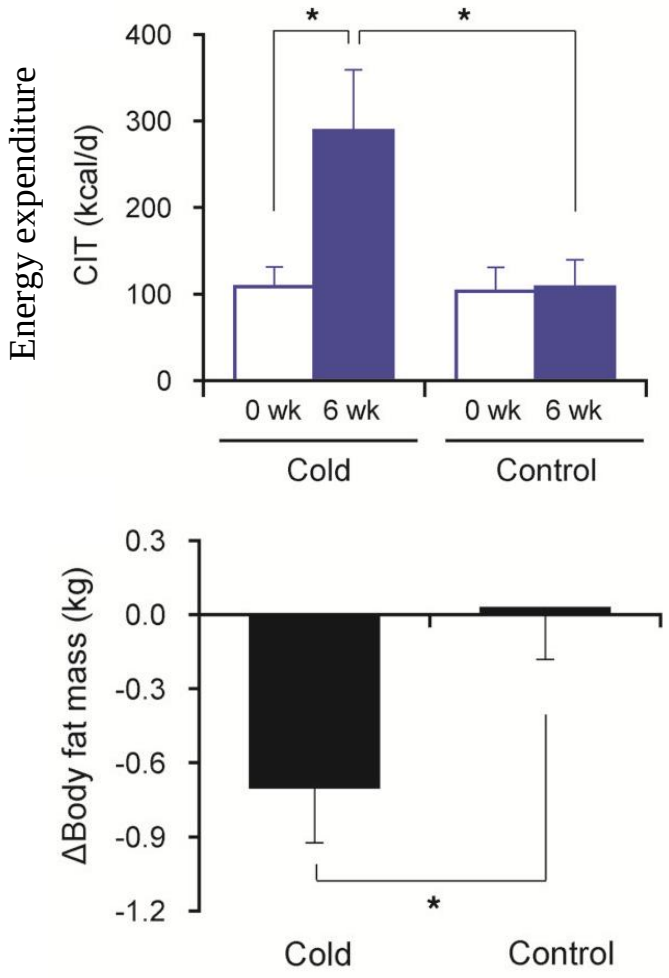
Recruitment of beige adipocytes and basal metabolism by chronic cold exposure

Cold exposure（2hour 19℃　6 weeks）

Before **After**



Browning



Chronic cold exposure recruites beige adipocytes and increases metabolic rates.

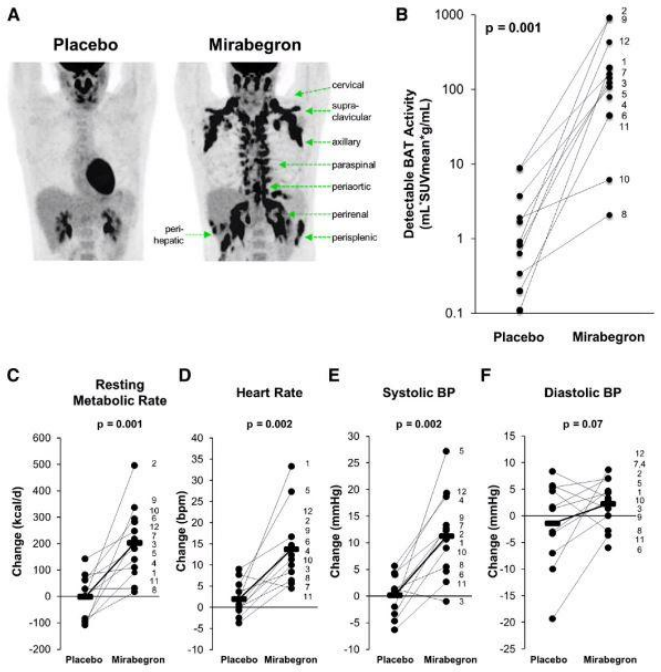
Yoneshiro et al, JCI. 2013: 123 3404-8

Effectiveness of β3 adrenergic receptor agonists to activate brown/beige fat cells

Mirabecron（Betanis®） β3 Adrenergic Receptor Agonist

Activation of Human Brown Adipose Tissue by a β3-Adrenergic Receptor Agonist

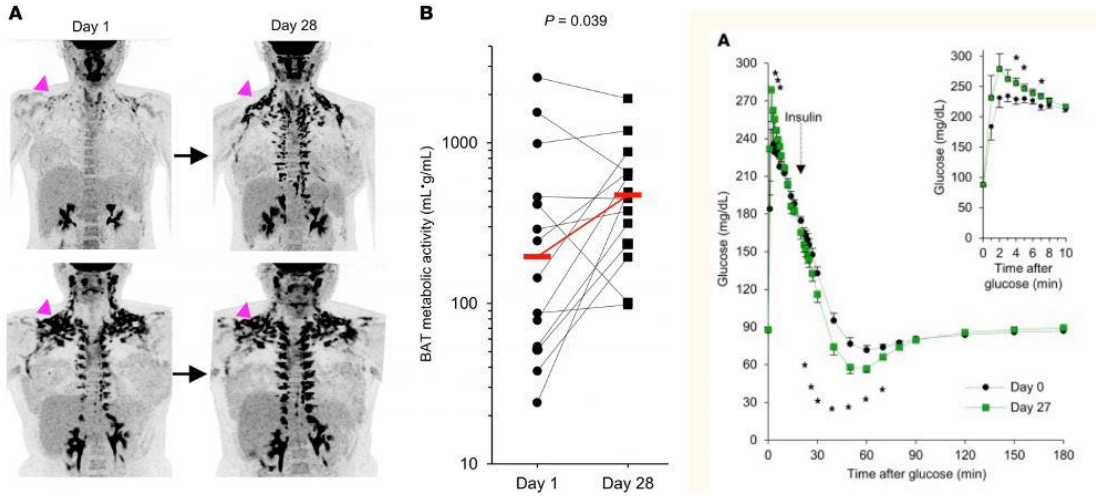
(200mg 12w)



Cell Metabolism 21 33-38, 2015

Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity

(100mg 4w)



O'mara AE. et al. J Clin Invest. 130(5): 2209–2219. 2020

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＜Our Research Concept＞

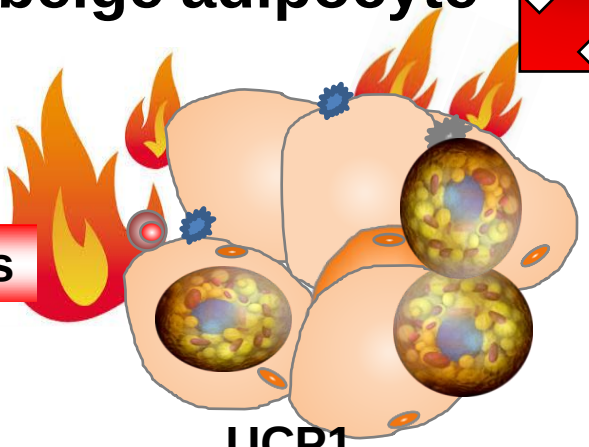
Food

Excessive calorie intake

Exercise

Cold exposure

Brown/beige adipocyte



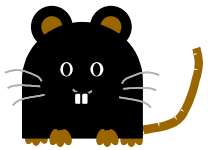
Thermogenesis

UCP1

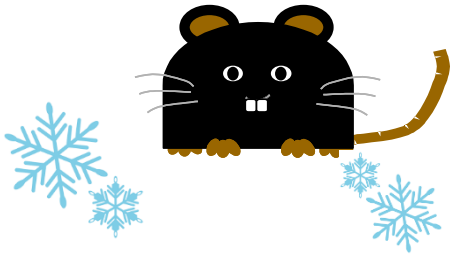
<Discover theTherapy>
Increase the number
or Brown/beige adipocytes
Activate

<Biology>

Wild type mice



UCP1 deficient mice

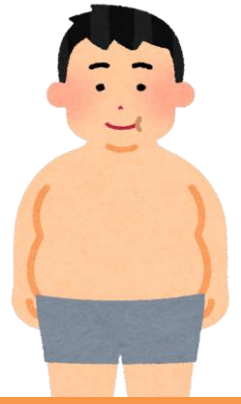


UCP1 dependent

UCP1 independent

Creatine futile cycle
Calcium (Serca) futile cycle
N-acyl amino acids (Pm20d1)

Obesity



Accumulation of visceral fat

Inactive

Increased basal metabolism

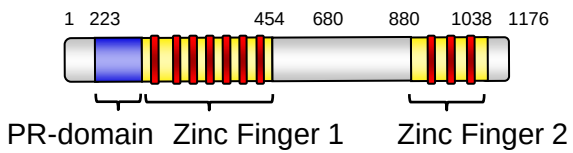
Anti-obesity

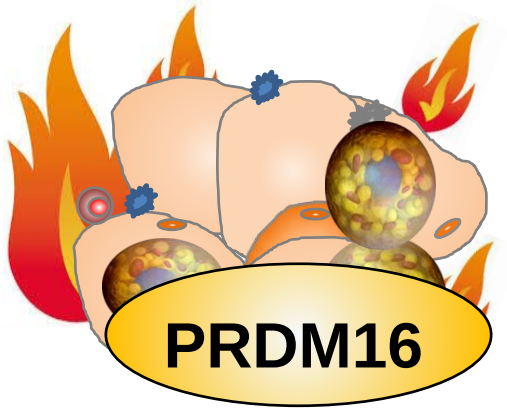
Improved metabolism

Healthy Longevity

Active

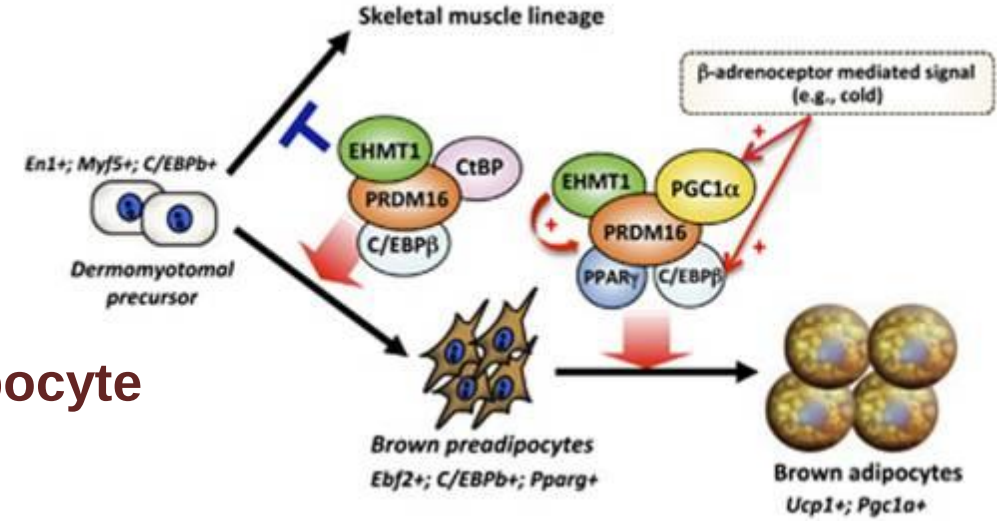
Transcription factor PRDM16



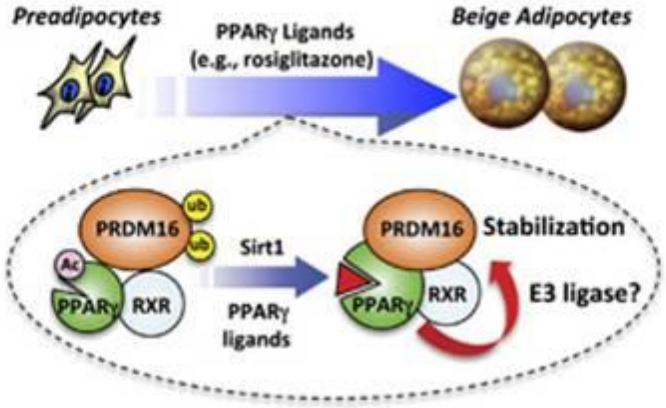


Transcription factor

Brown adipocyte



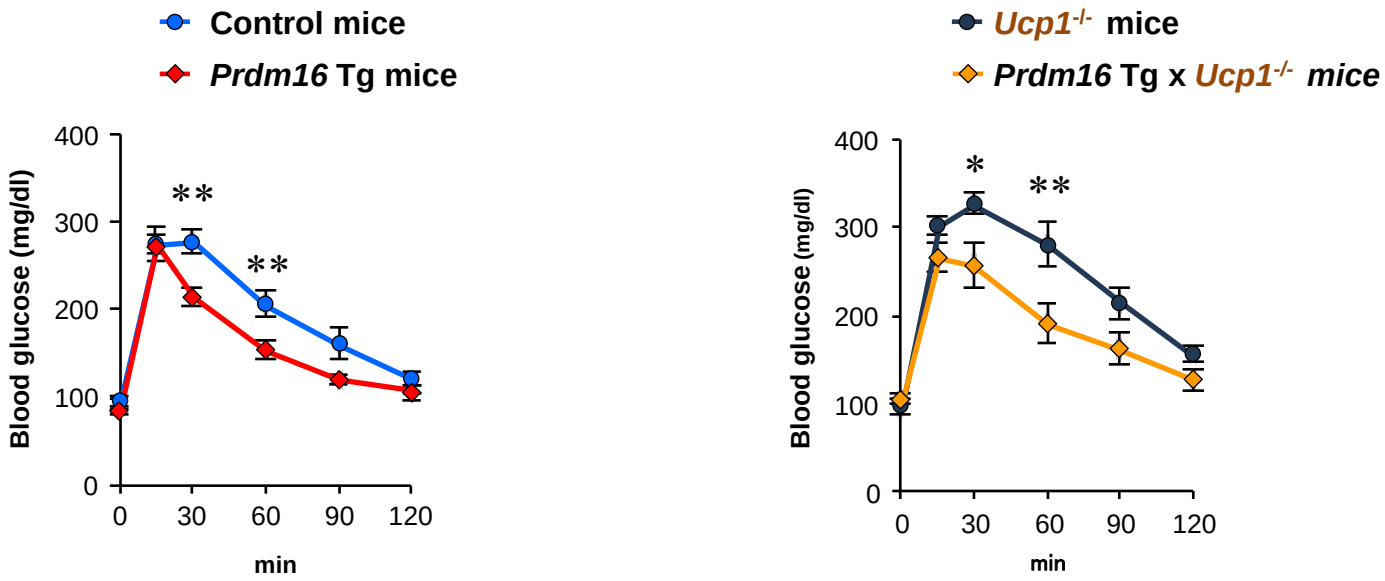
Beige adipocyte



PRDM16 is a transcription factor that directly binds to PPARγ and C/EBPβ and plays an important role in the brown and beige adipogenesis.

Seale P et al. Nature 2008 and JCI 2011.

PRDM16 improved systemic glucose tolerance



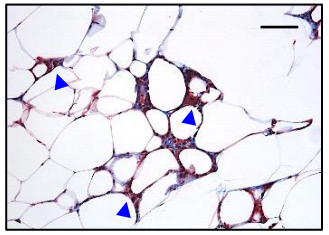
Overexpression of PRDM16 in adipose tissue improved glucose tolerance.

Crossed onto an UCP1-deficient background,
overexpression of PRDM16 improved glucose tolerance.

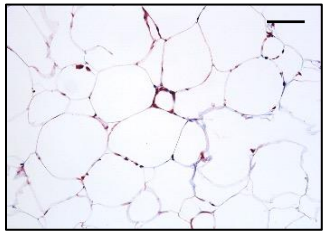
→ PRDM16 has the function to enhance the systemic glucose metabolism independent of UCP1.

PRDM16 suppressed adipose fibrosis

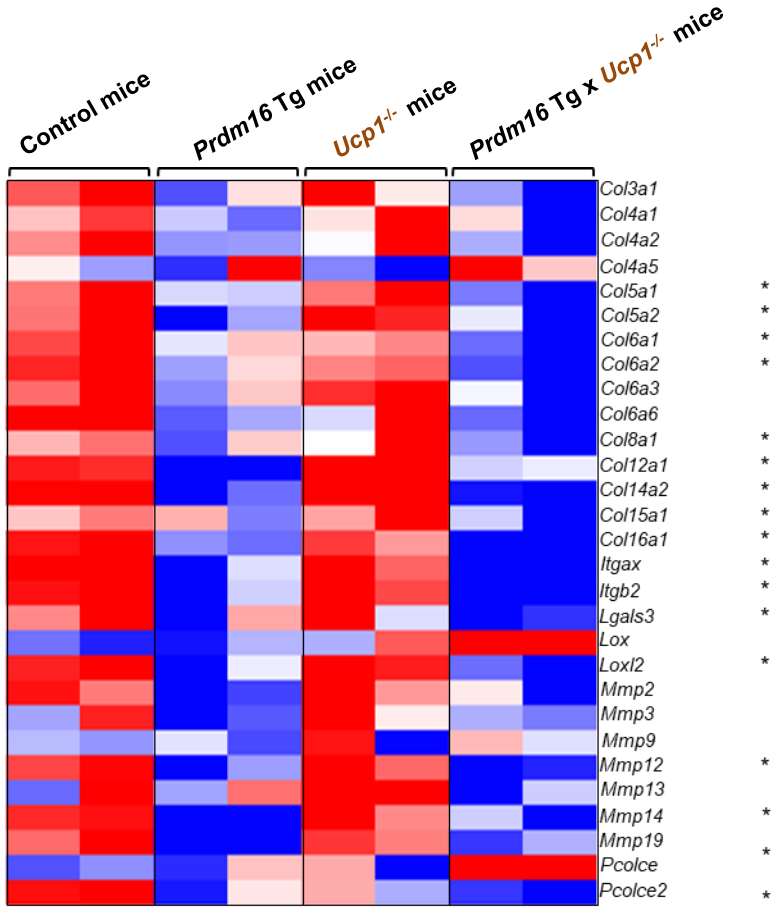
Control mice



***Prdm16* Tg mice**



Masson's Trichrome staining



Overexpression of PRDM16 also suppressed adipose fibrosis in UCP1-deficient mice.

Suppression of adipose fibrosis by PRDM16 is independent of UCP1.

Question

PRDM16 improved systemic glucose homeostasis
independent of UCP1.

PRDM16 suppressed adipose fibrosis
independent of UCP1.

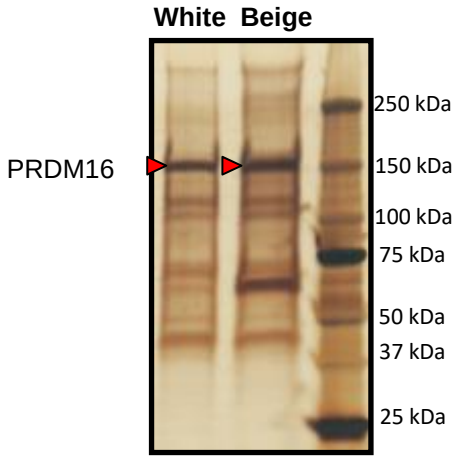
What is the mechanism of PRDM16?

How to transcript the target genes?

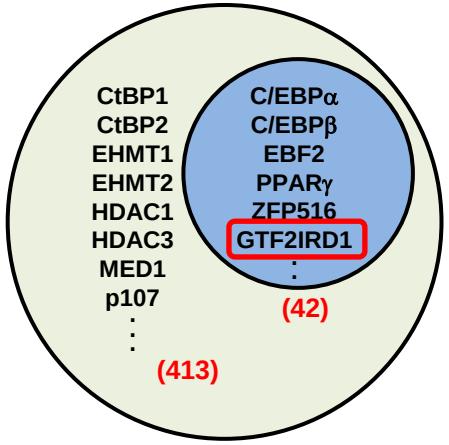
Methods < IP-MS and RNA-seq>

IP-Mass spectometry

Interaction with PRDM16

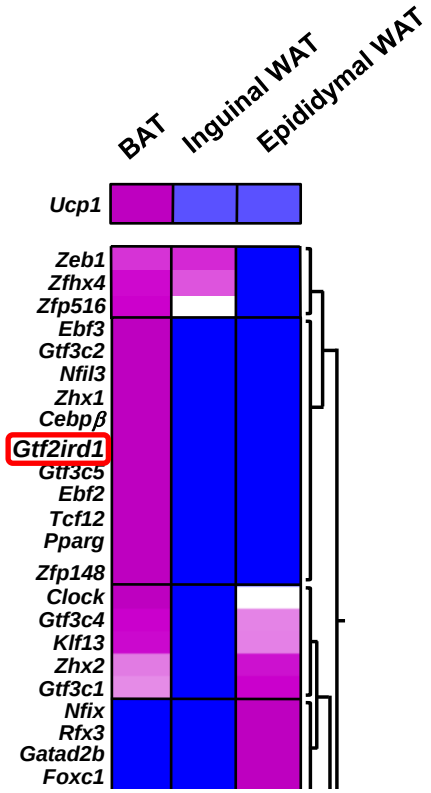


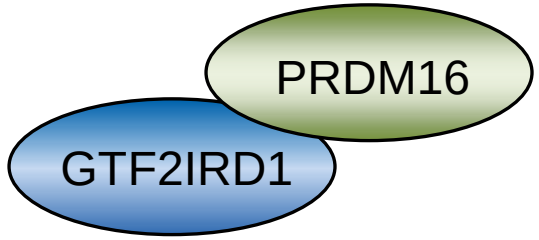
Transcriptional Complex with PRDM16



DNA-binding transcription factors

RNA-sequence analysis

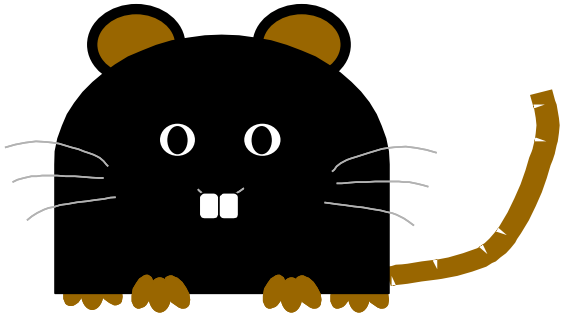




GTF2IRD1 was identified by IP-MS and RNA-seq.

Hasegawa Y and Kajimura S., Cell Metabolism 2018

Generation of *Gtf2ird1* Tg mice

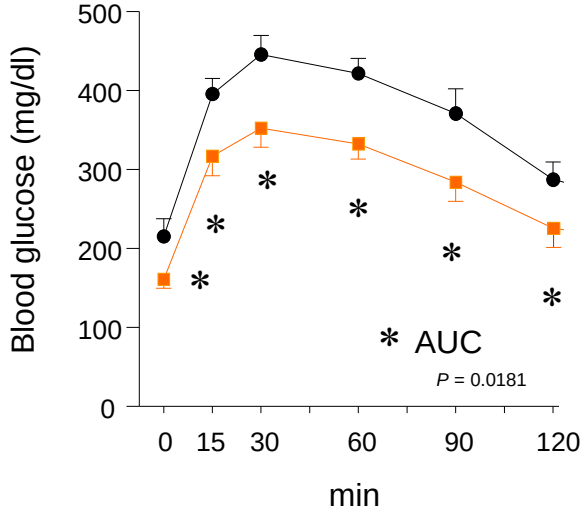


Control mice

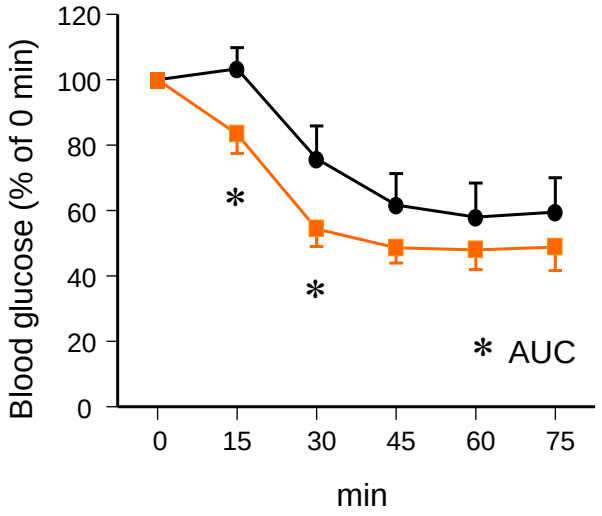
Gtf2ird1 Tg mice

10 weeks of HFD

Glucose Tolerance Test

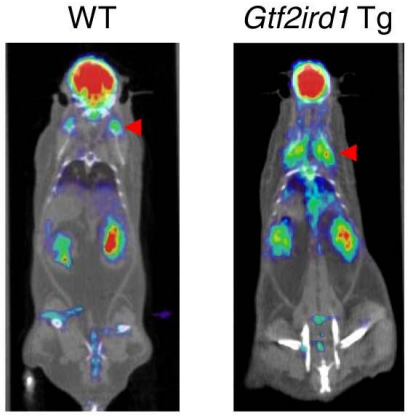


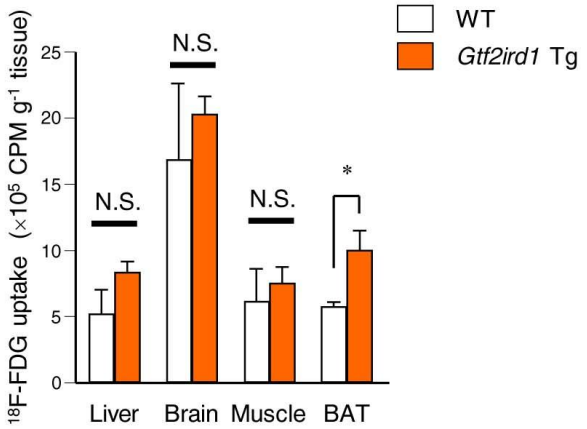
Insulin Tolerance Test



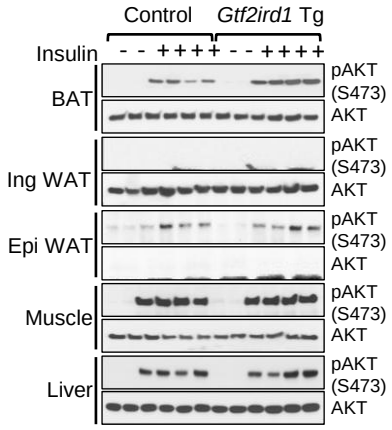
Metabolic phenotype of *Gtf2ird1* Tg mice

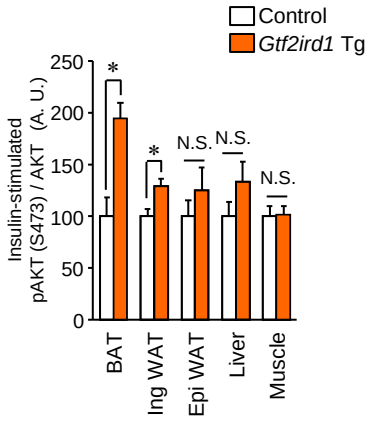
PET/CT scan





Insulin signaling

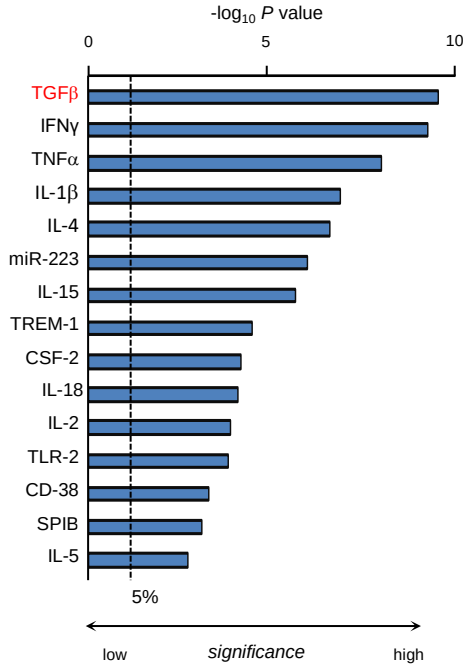




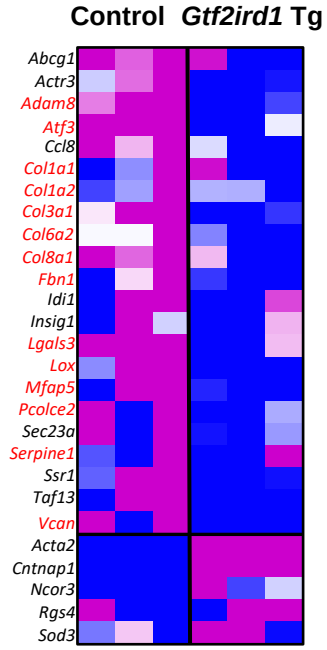
GFT2IRD1 increased FDG uptake in BAT through enhanced insulin signaling.

TGFβ signaling pathway was repressed by overexpression of *Gtf2ird1*

Repressed signaling

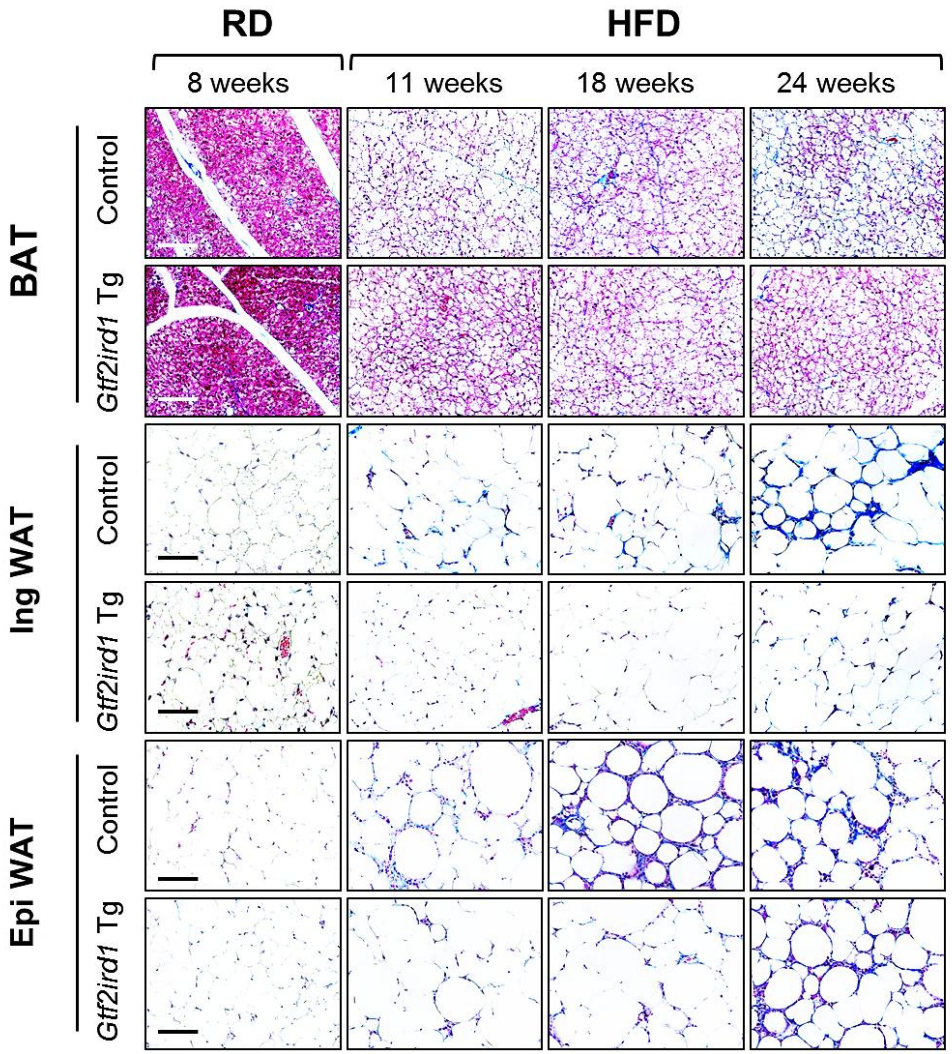


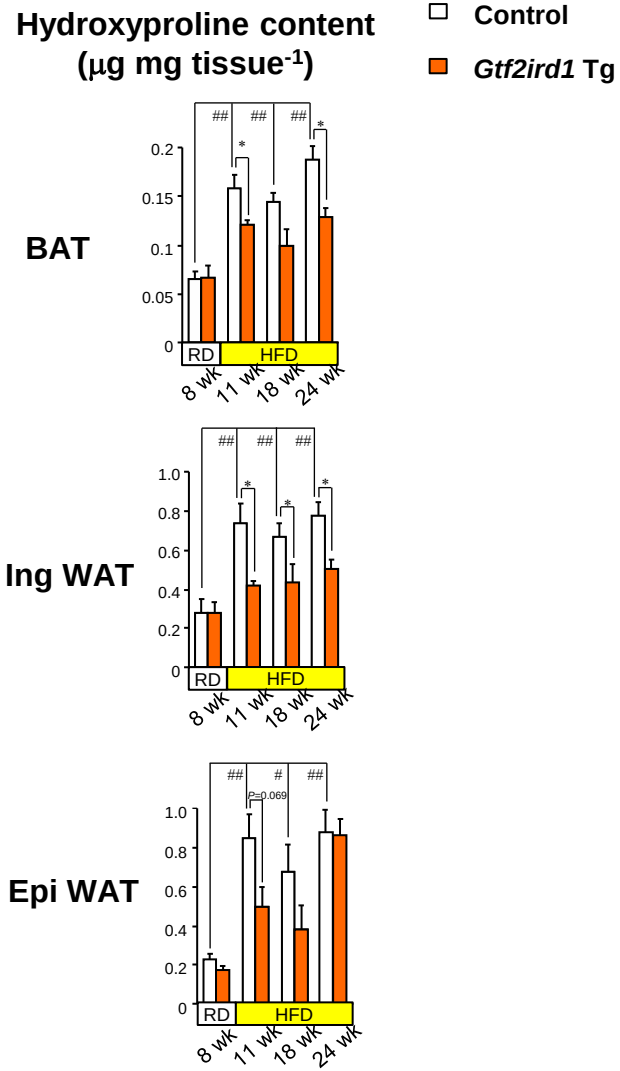
TGFβ target genes



TGFβ signaling pathway was repressed by Metascape analysis.

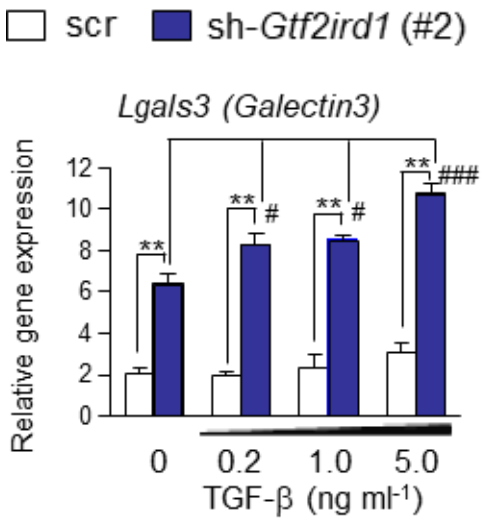
Histological Analysis in Adipose Tissue Overexpressed *Gtf2ird1*

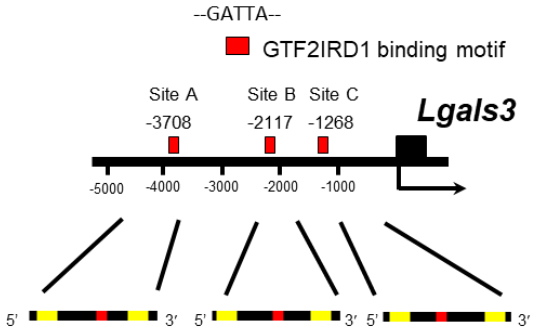




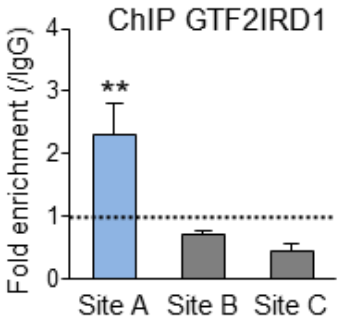
Search for the genes directly regulated by GTF2IRD1

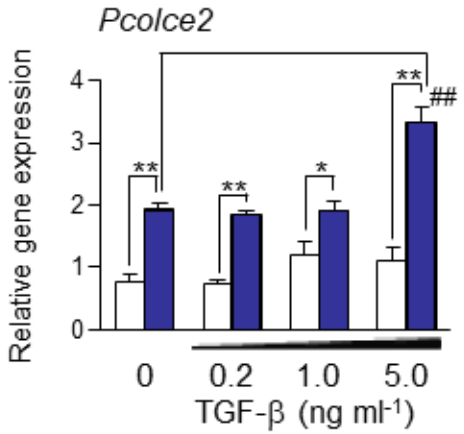
Knockdown of *Gtf2ird1*

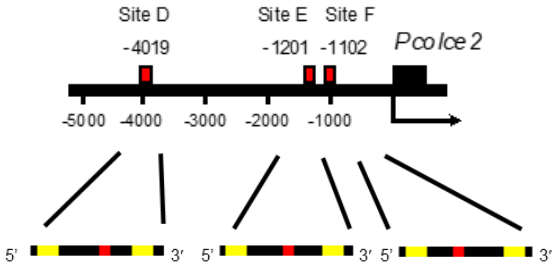


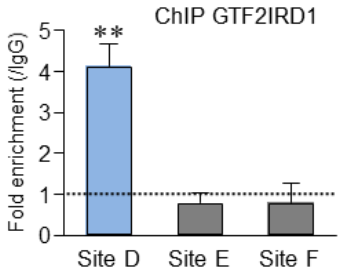


ChIP-qPCR



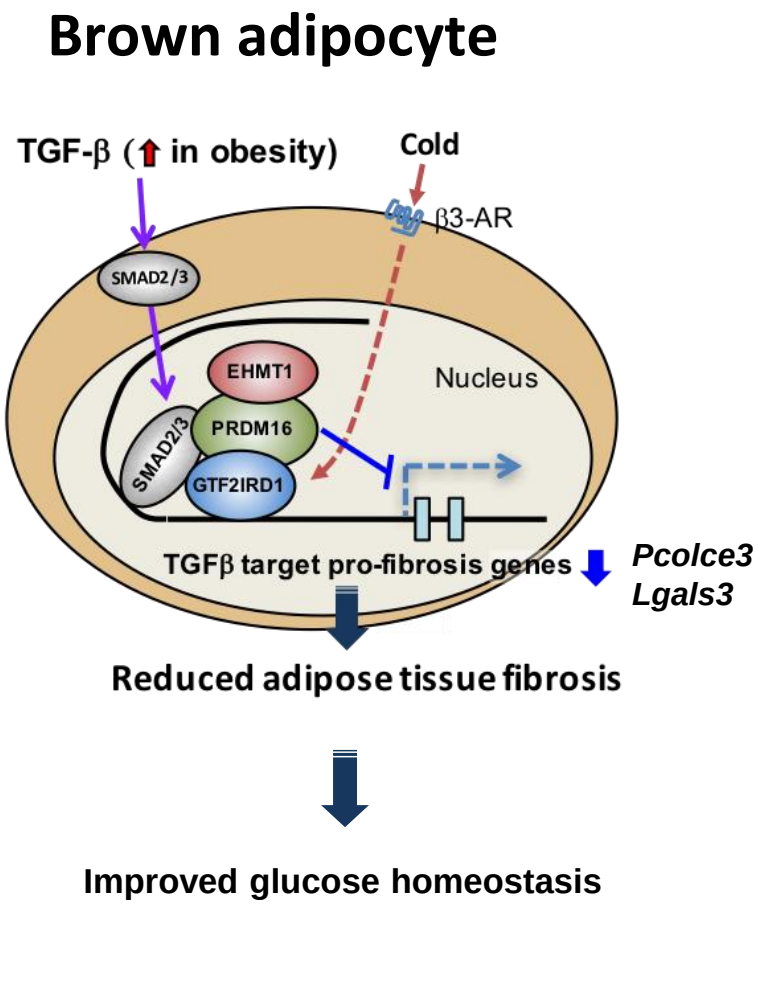


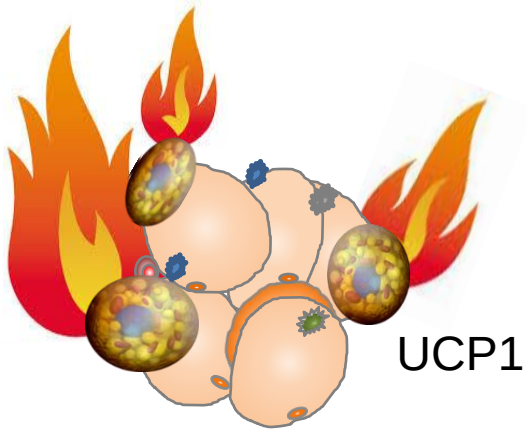


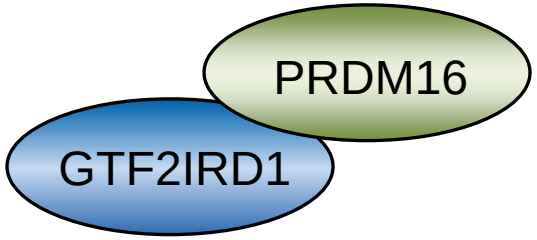


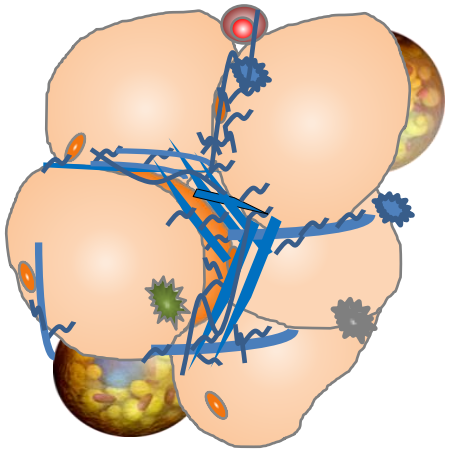
Cultured brown adipocytes

Proposed Scheme









Hasegawa Y and Kajimura S., *Cell Metabolism* 2018

Presentation Content

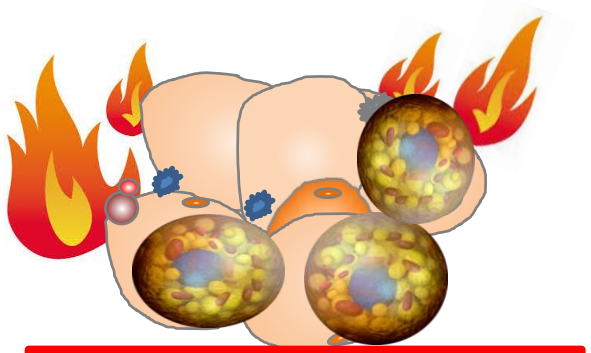
- Therapeutic potential of brown/beige adipocytes for the treatment of obesity

- Suppression of adipose tissue fibrosis by PRDM16-GTF2IRD1 complex impact systemic glucose metabolism

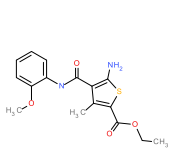
- Development of a novel treatment for obesity by activating brown/beige adipocytes

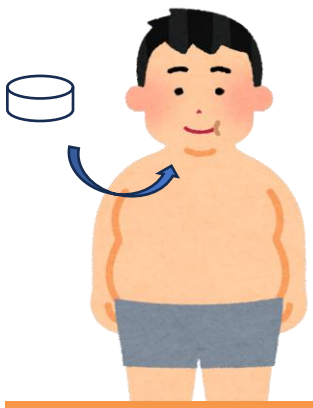
Research concept

Brown/beige adipocytes



<Therapy>
Increase the number
or
Activate



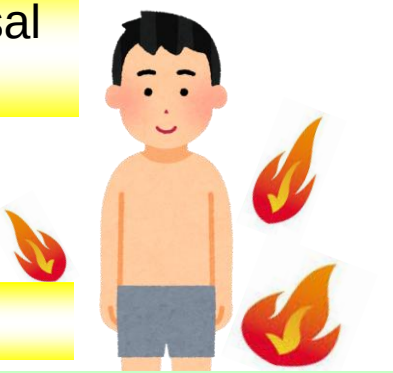


Accumulation of visceral fat

Inactive

Increased basal metabolism

Anti-obesity



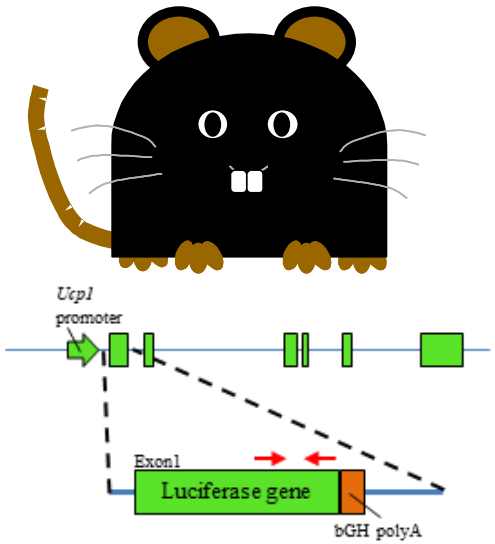
Improved metabolism

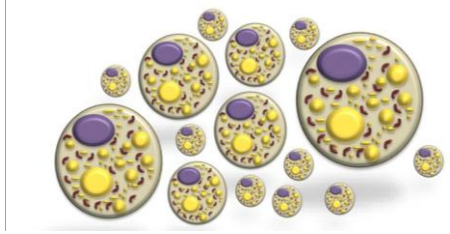
Healthy Longevity

Active

Method

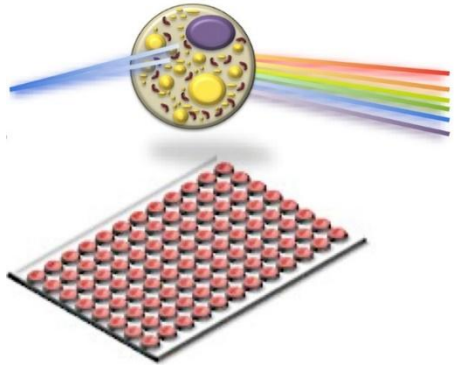
Generation of Thermo mice





Immortal inguinal adipocytes

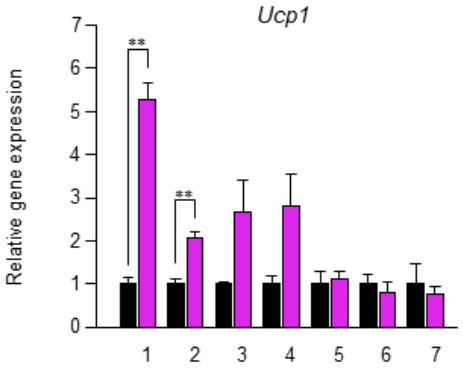
Luciferase assay



High throughput screening

4800 chemical compounds

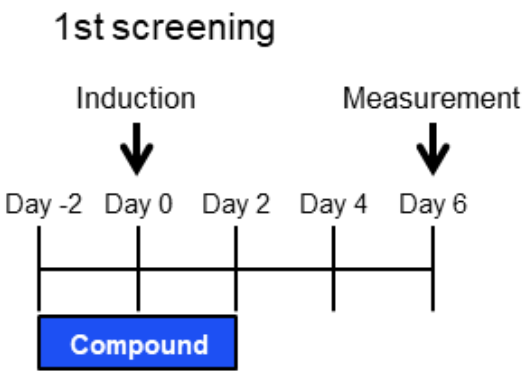
Gene expression analysis

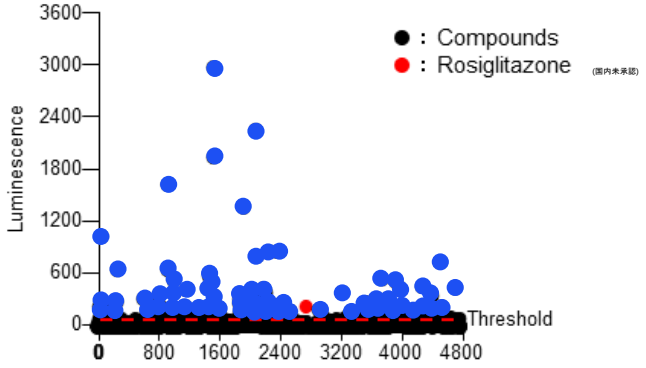


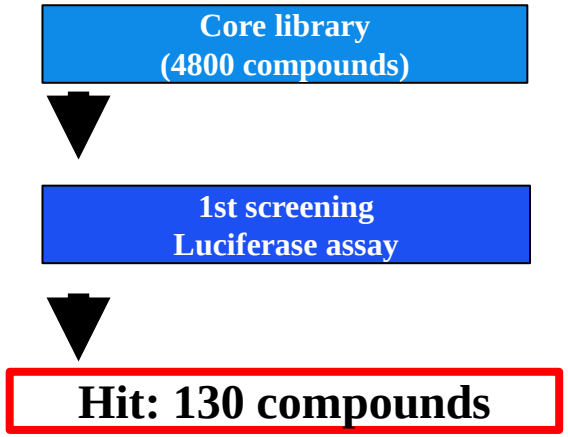
Galmozzi A, Hasegawa Y. *Cell Reports*. 2014 11;9(5):1584-93.

1st screening

We screened 4800 compounds in the 1st screening.



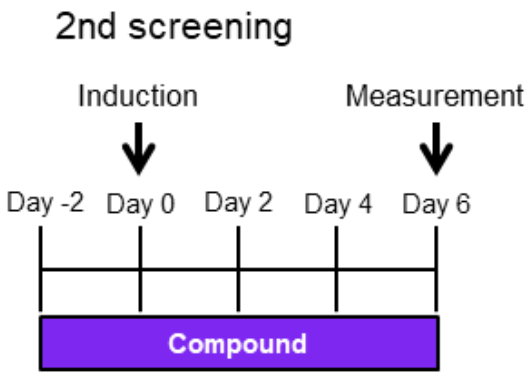


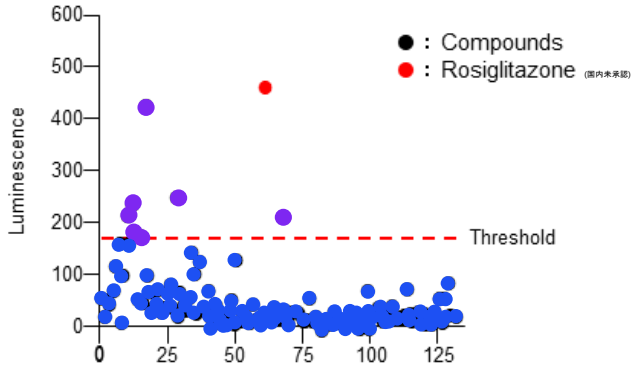


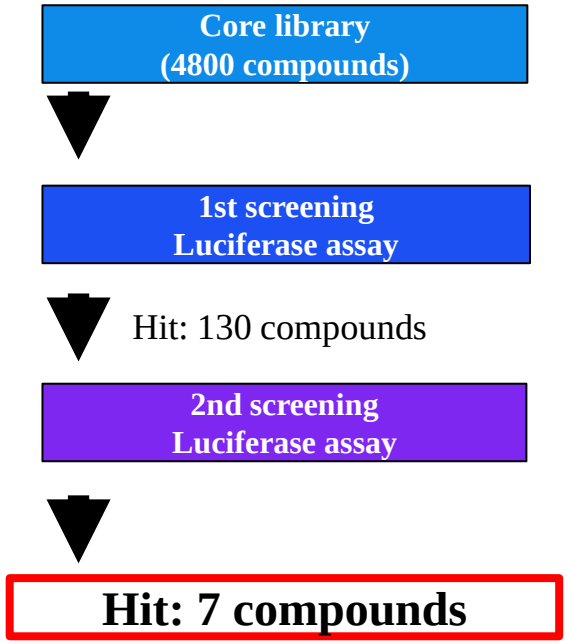
Onodera K, Hasegawa Y, et al. ***Obesity***. 2024 Feb;32(2):324-338.

2nd screening

We screened 130 compounds in the 2nd screening.

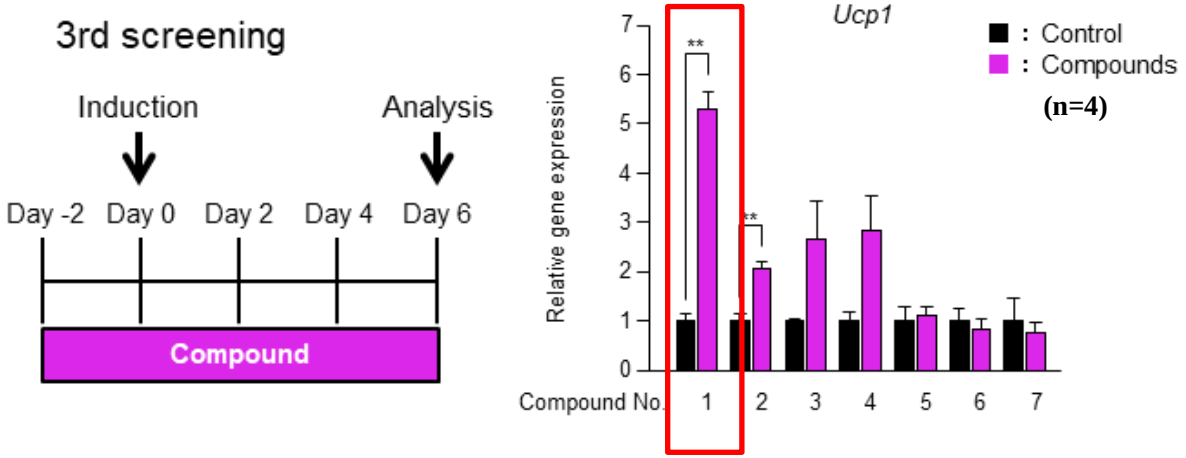


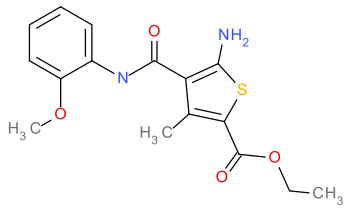




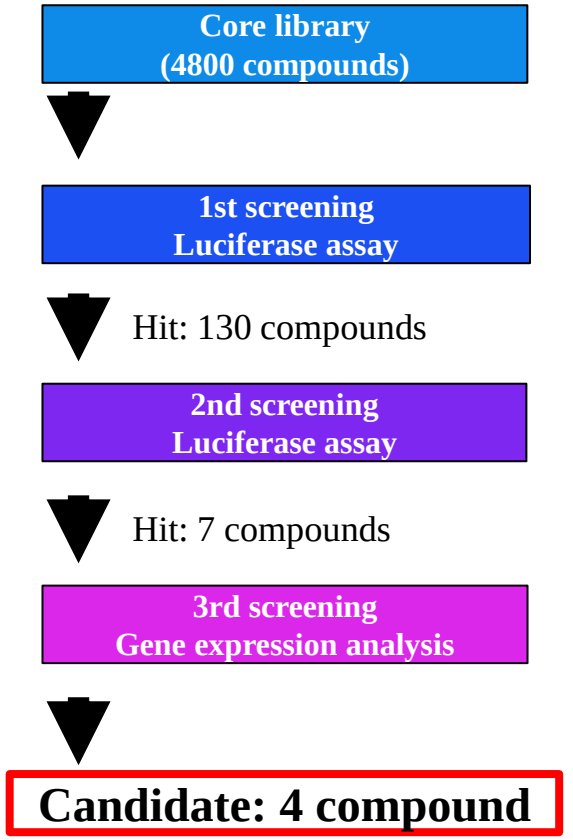
3rd screening

We analyzed the *Ucp1* gene expression in the 3rd screening.



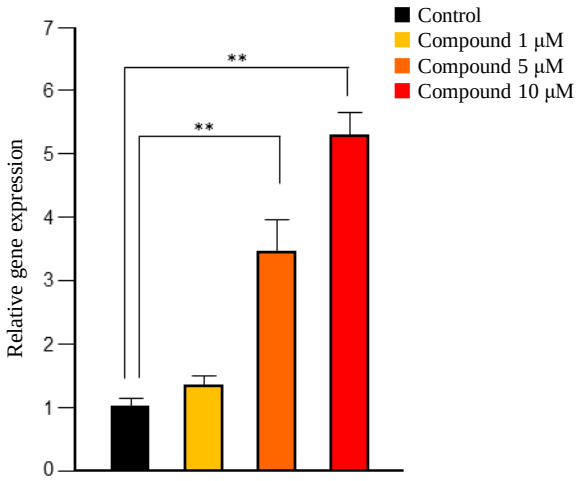


Ethyl 5-amino-4-((2-methoxyphenyl)carbamoyl)-3-methylthiophene-2-carboxylate

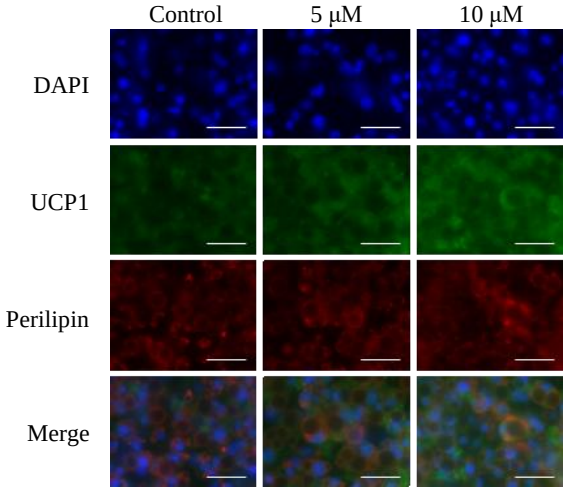


Expression of UCP1

Ucp1

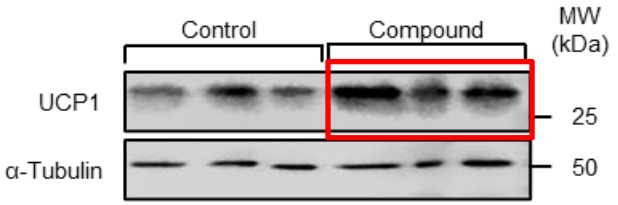


Immunostaining



Scale bar 40 μm

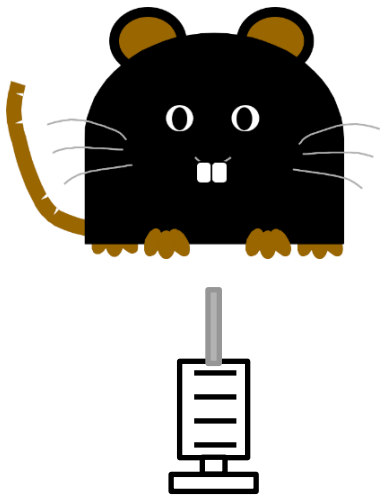
Expression of UCP1 protein



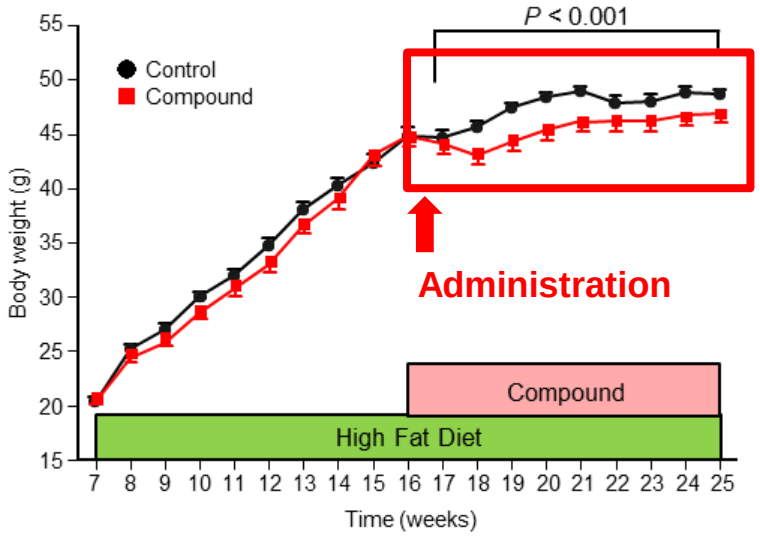
Expression of UCP1 is increased by this compound.

Effect of weight loss by this compound

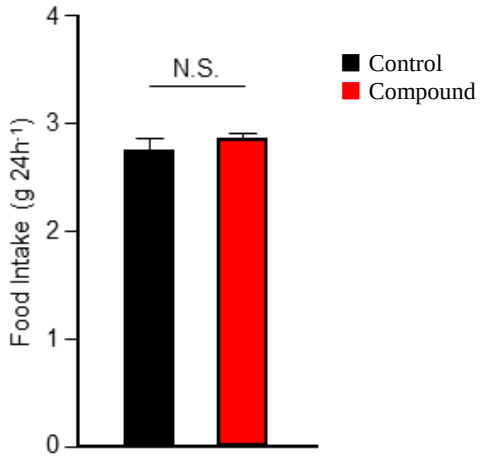
Oral administration



Body weight



Food intake



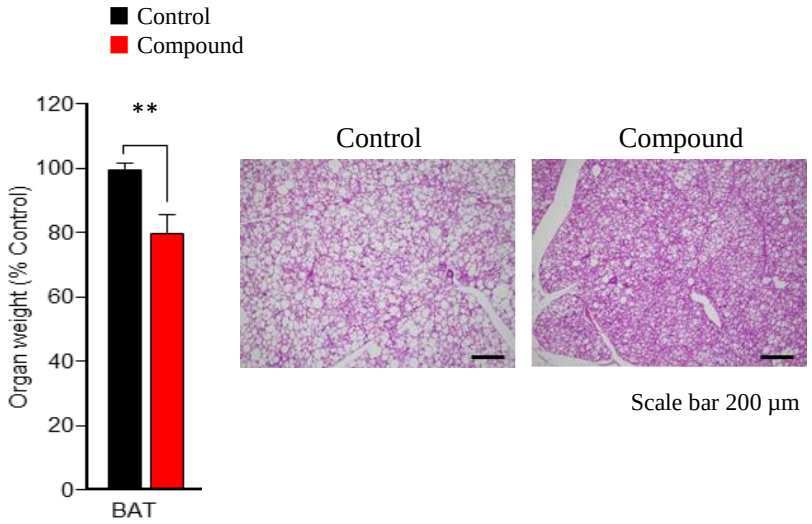
The identified compounds were orally administered daily to obese model mice, which were wild-type mice fed a high-fat diet for 9 weeks.

After starting administration of this compound, weight gain was suppressed.

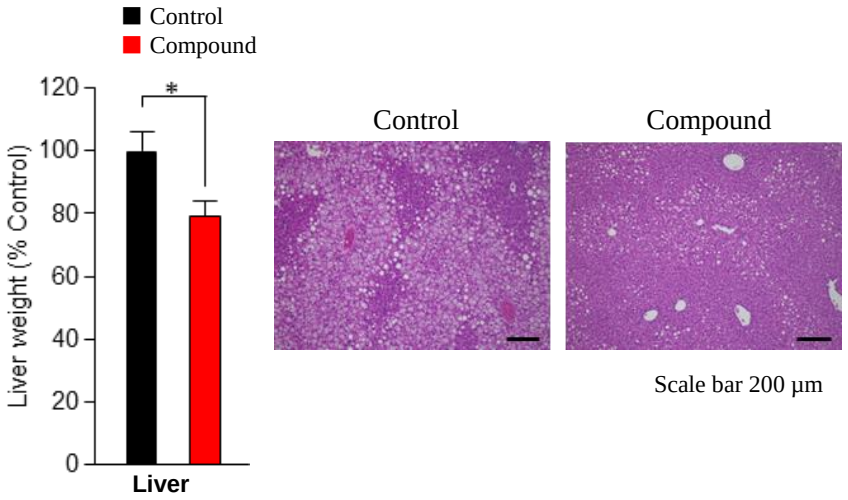
There was no difference in food intake.

Effects on adipose tissue and liver by this compound

BAT



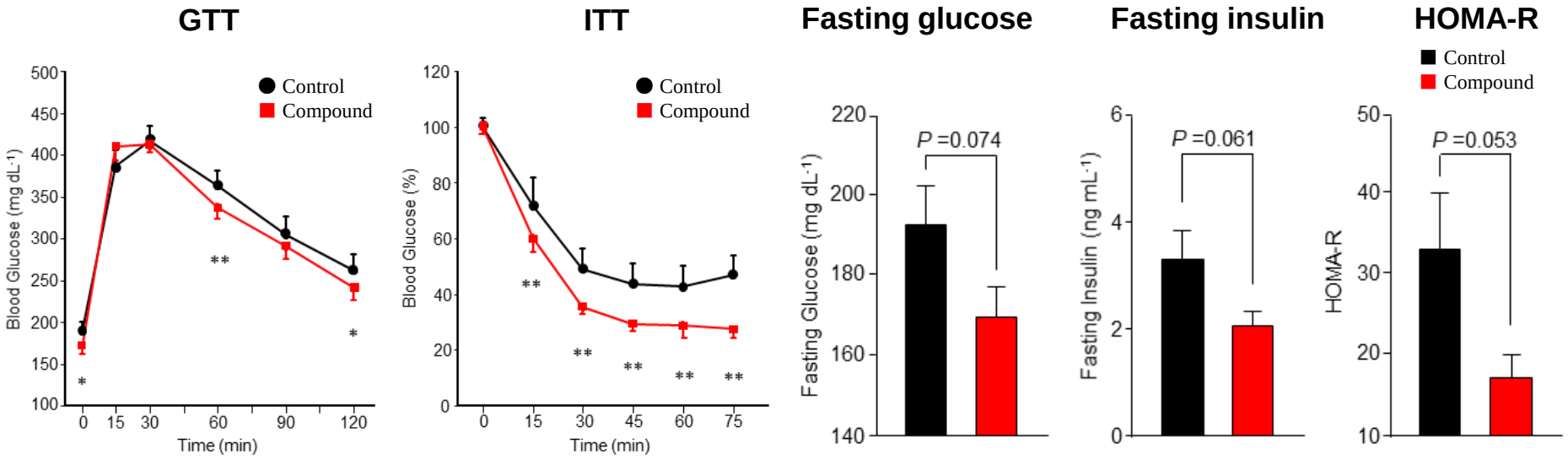
Liver



The weight of brown adipose tissue and liver was decreased by this compound.

Accumulation of fat was decreased in both BAT and liver.

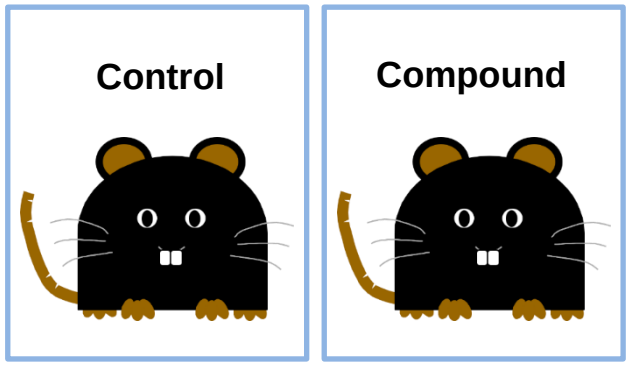
Effects on glucose tolerance and insulin sensitivity



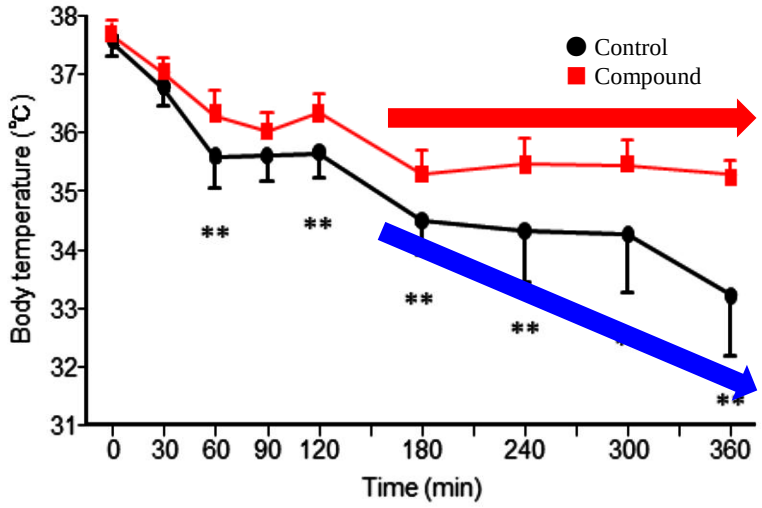
Glucose tolerance and insulin sensitivity were improved.
Levels of HOMA-R were apparently lower.

Analysis of cold tolerance

4℃ refrigerated chamber



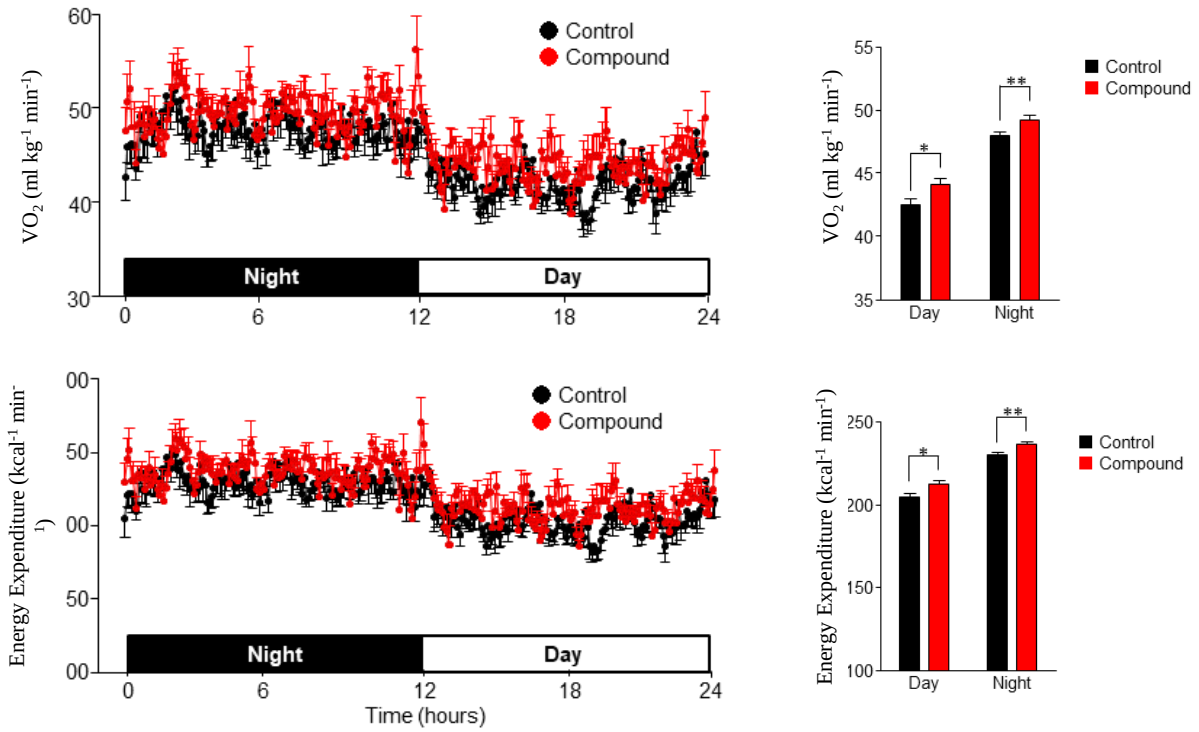
Changes in rectal temperature after 4℃ cold stimulation



In the compound-administered group, the decrease in rectal temperature was suppressed.

Cold tolerance was enhanced by this compound.

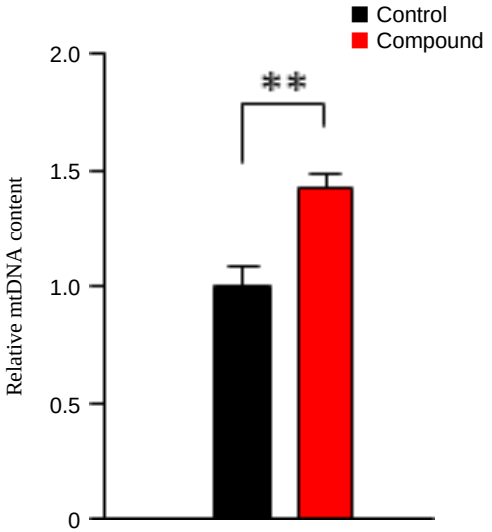
Measurement of Basal Metabolic Rate



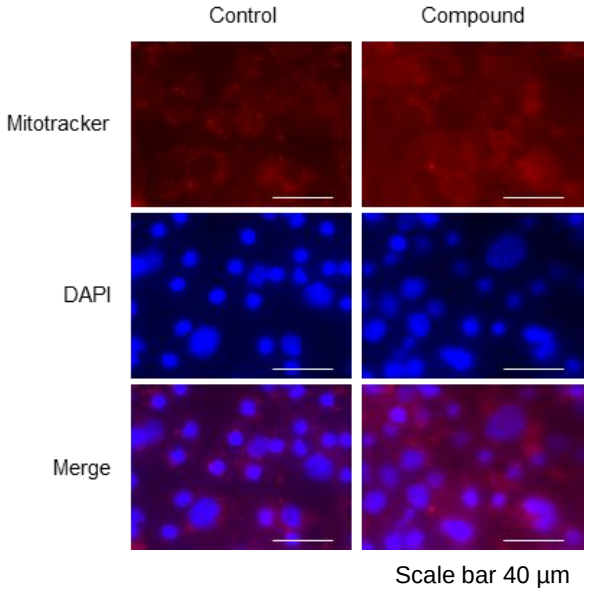
Basal metabolism rate was increased throughout the day.

Analysis of Mitochondrial function

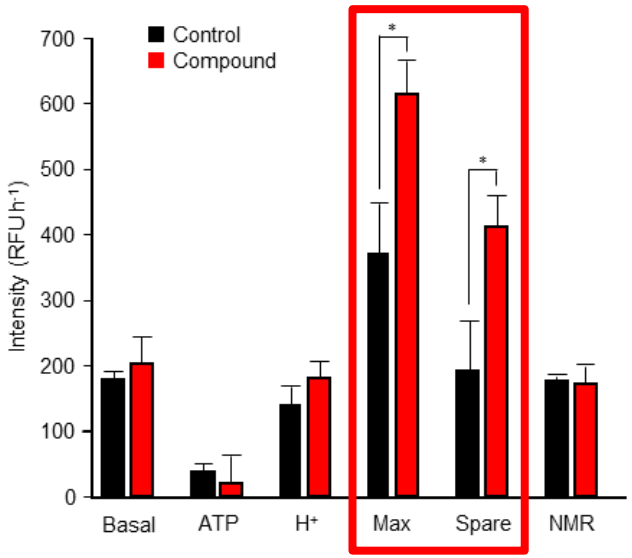
Mitochondrial DNA content



Mitotracker staining



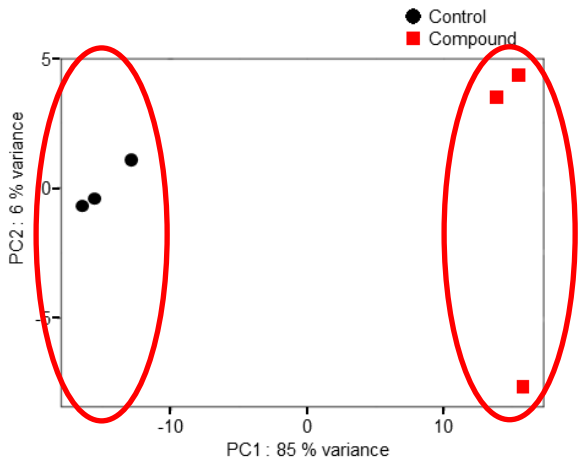
Oxygen consumption



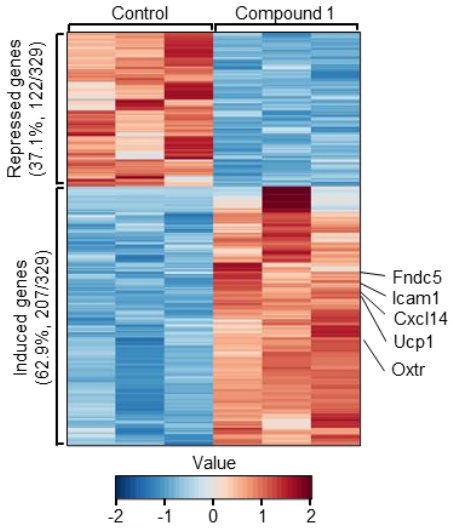
Increased mitochondrial expression induce the increase in basal metabolic rate.

Identification of activated signal pathways by RNA sequence analysis

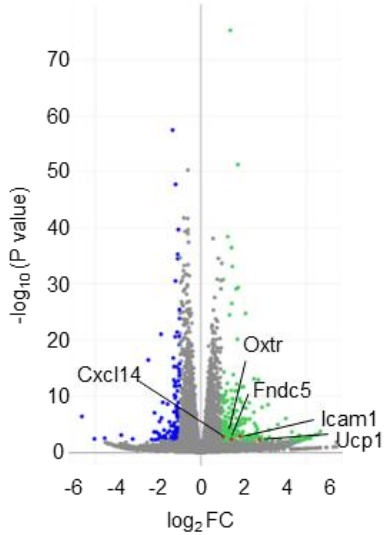
Principal component analysis



Heat map



Volcano plot



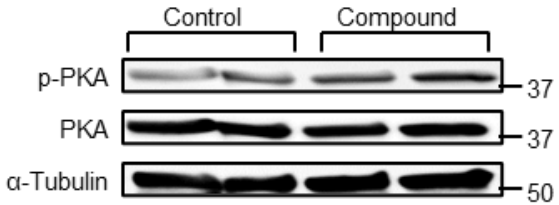
This compound significantly changes the genetic profiles of brown adipocytes.

The identified compound increases the expression of genes downstream of the PKA-p38 MAPK signal pathway, such as Fndc5 (Irisin), Icam1, Cxcl14, and Oxtr.

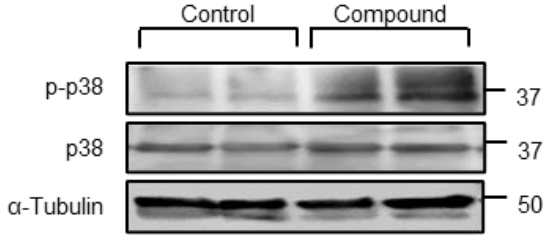
It was suggested that the compound enhances thermogenesis in adipocytes via the PKA-p38 MAPK signal pathway.

Analysis of PKA-p38 MAPK signal pathway

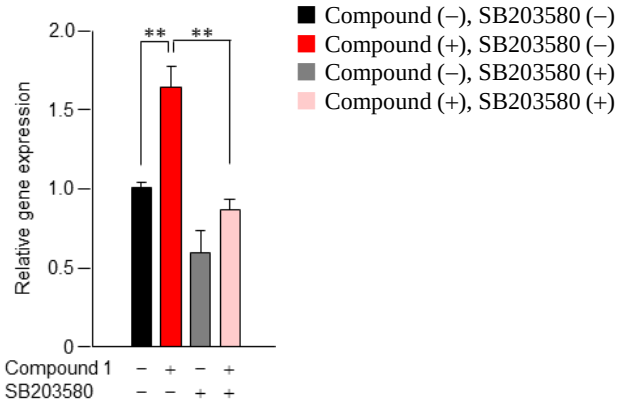
PKA



p38 MAPK



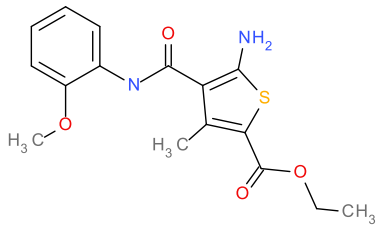
Ucp1



Administration of this compound increased the phosphorylation of PKA and p38 MAPK, indicated the activation of the PKA-p38 MAPK signal pathway.

Administration of a p38 MAPK inhibitor (SB203580) suppressed the *Ucp1* activation by this compound. It was revealed that the identified compound increases the expression of *Ucp1* through the PKA-p38 MAPK signal pathway.

Mechanistic action of identified compound



Identified compound

↓

PKA

↓

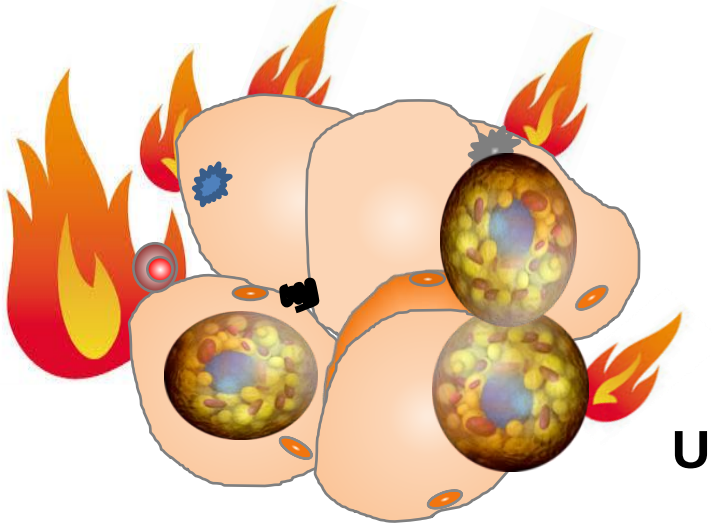
p38 MAPK

↘ ↗

KCNC2 ***Gm5627***

→

Brown/beige adipocytes



UCP1 ↑

Thermogenesis
Increase in basal metabolic rate

Anti-obesity effect

Onodera K, Hasegawa Y, et al**.** ***Obesity***. 2024 Feb;32(2):324-338.

Conclusions

- Brown/beige adipocytes have the therapeutic potential for the treatment of obesity

- Activation of PRDM16-GTF2IRD1 axis suppresses adipose tissue fibrosis and ameriolates systemic glucose metabolism.

- Identified compound has anti-obesity effects by activating brown/beige adipocytes via PKA-p38MAPK pathway.

Acknowledgement

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