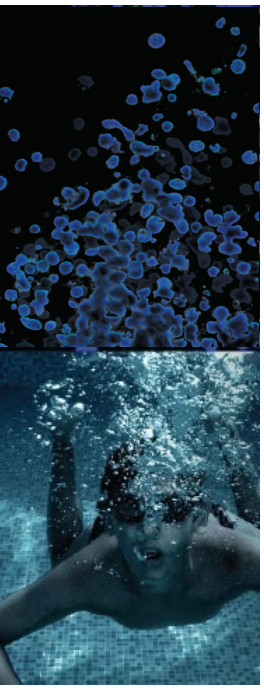
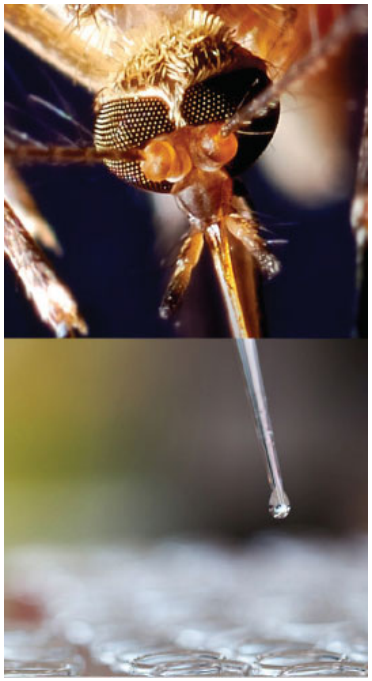
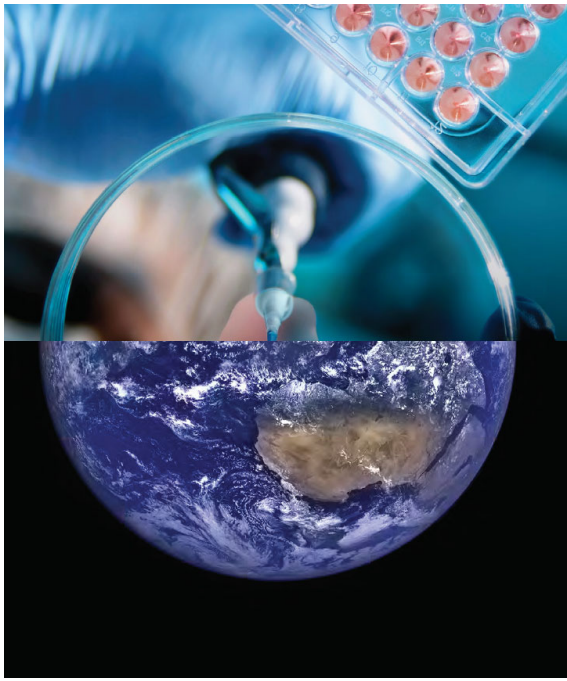
The Development of Standard In Vitro Models for Studying Metabolic Diseases

Aaron M. Cypess, MD, PhD, MMSc
Senior Investigator and Chief
Translational Physiology Section, DEOB,
NIDDK, NIH

Credible Leads to Incredible™







ATCC Webinar Series

Development of Standard *in vitro* Models of Human White (ATCC CRL-4063™) and Brown Adipose Tissue (ATCC CRL-4062™)

Aaron M. Cypess, MD, PhD, MMSc

Senior Investigator and Chief, Translational Physiology Section, Diabetes, Endocrinology, and Obesity Branch, NIDDK, NIH

NIH National Institute of Diabetes and Digestive and Kidney Diseases

Objectives

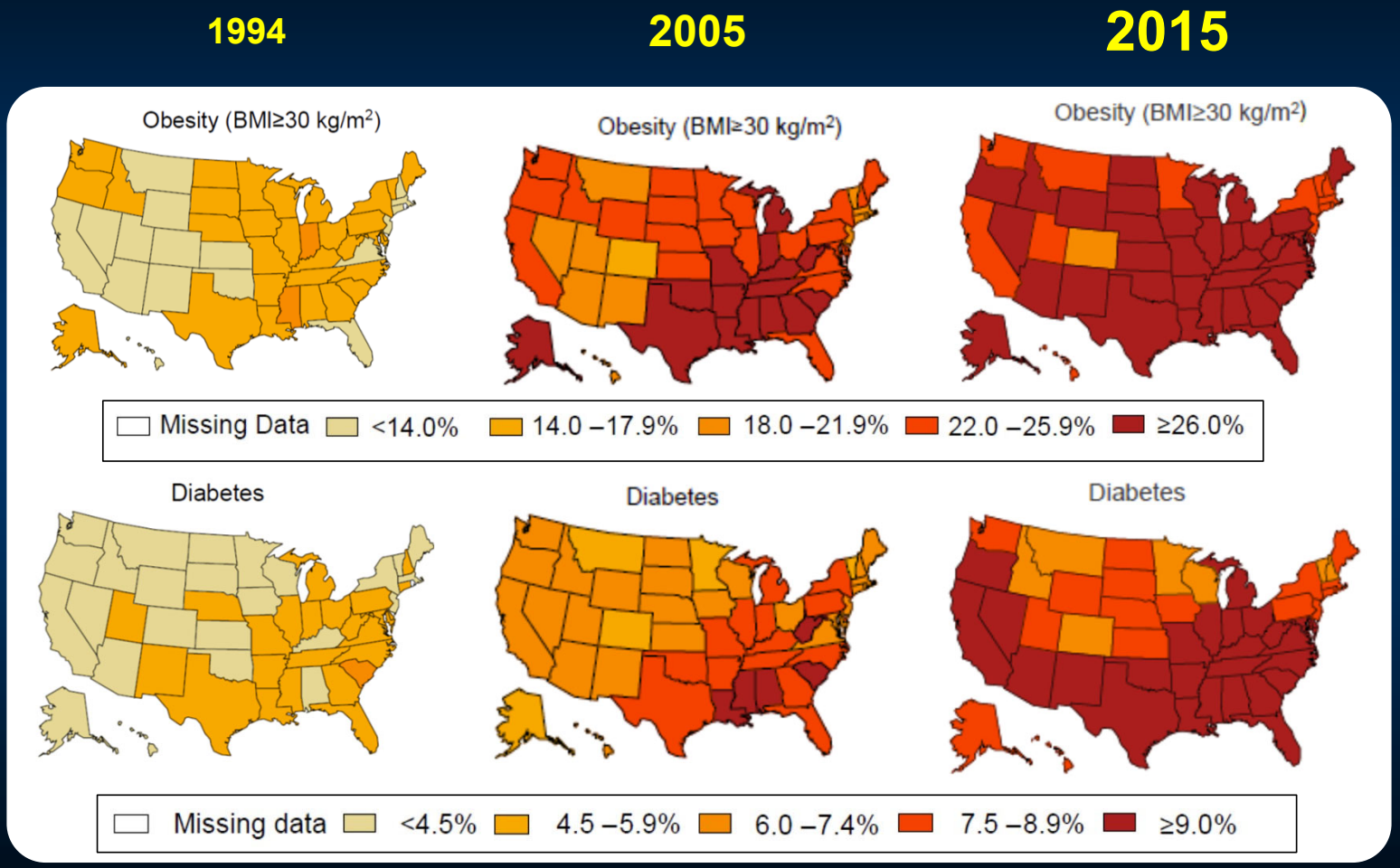
1. Describe the functional and physiological roles of human white and brown adipose tissue (WAT and BAT).

2. Appreciate the physiological responses to chronic treatment with the β3-adrenergic receptor (AR) agonist mirabegron.

3. Identify the types of *in vitro* model systems that can be used to study human adipocyte physiology.

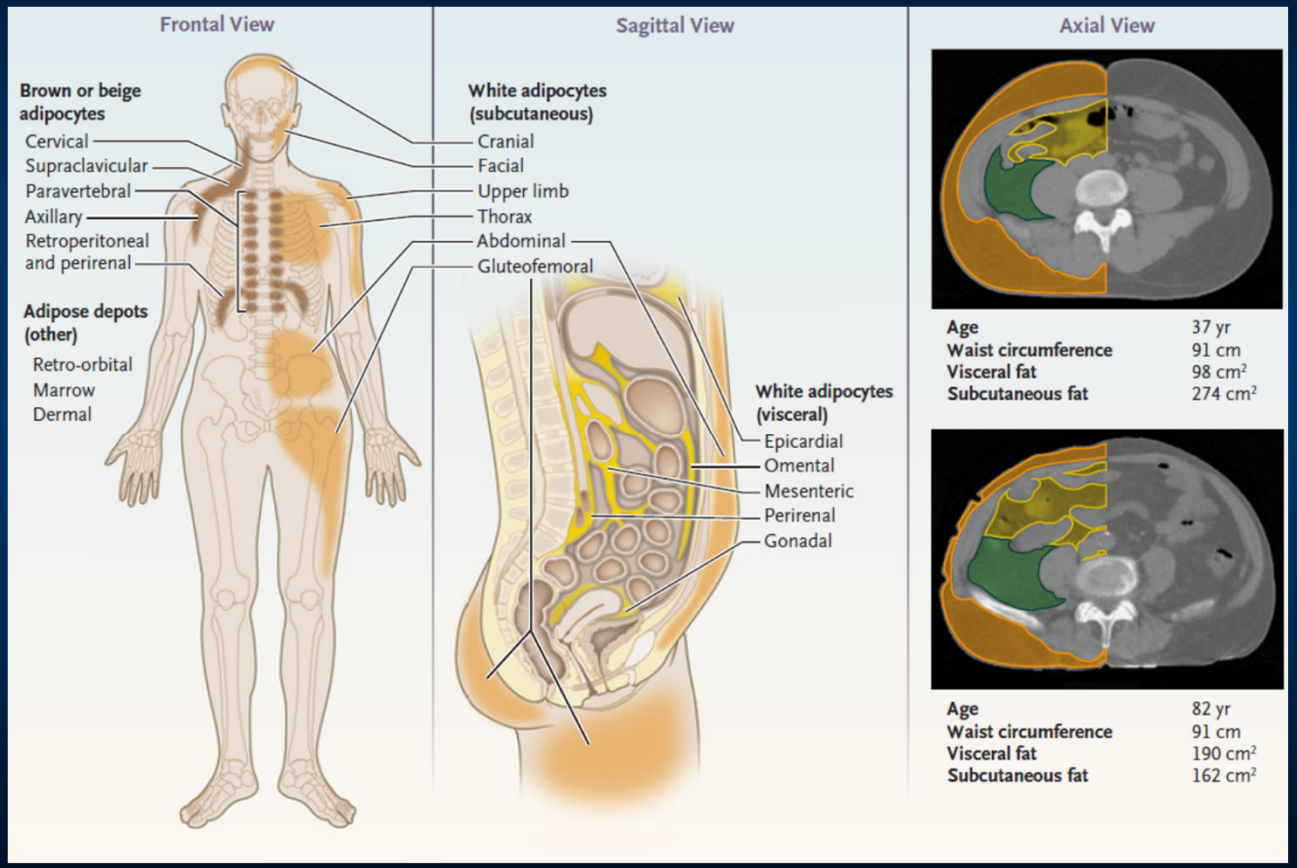
4. Understand the derivation and then the genetic, molecular, and functional characterization of the new immortalized, clonal human white (ATCC CRL-4063™) and brown (ATCC CRL-4062™) preadipocytes.

Is Adipose Tissue Simply Something that in Excess Causes Diabetes?



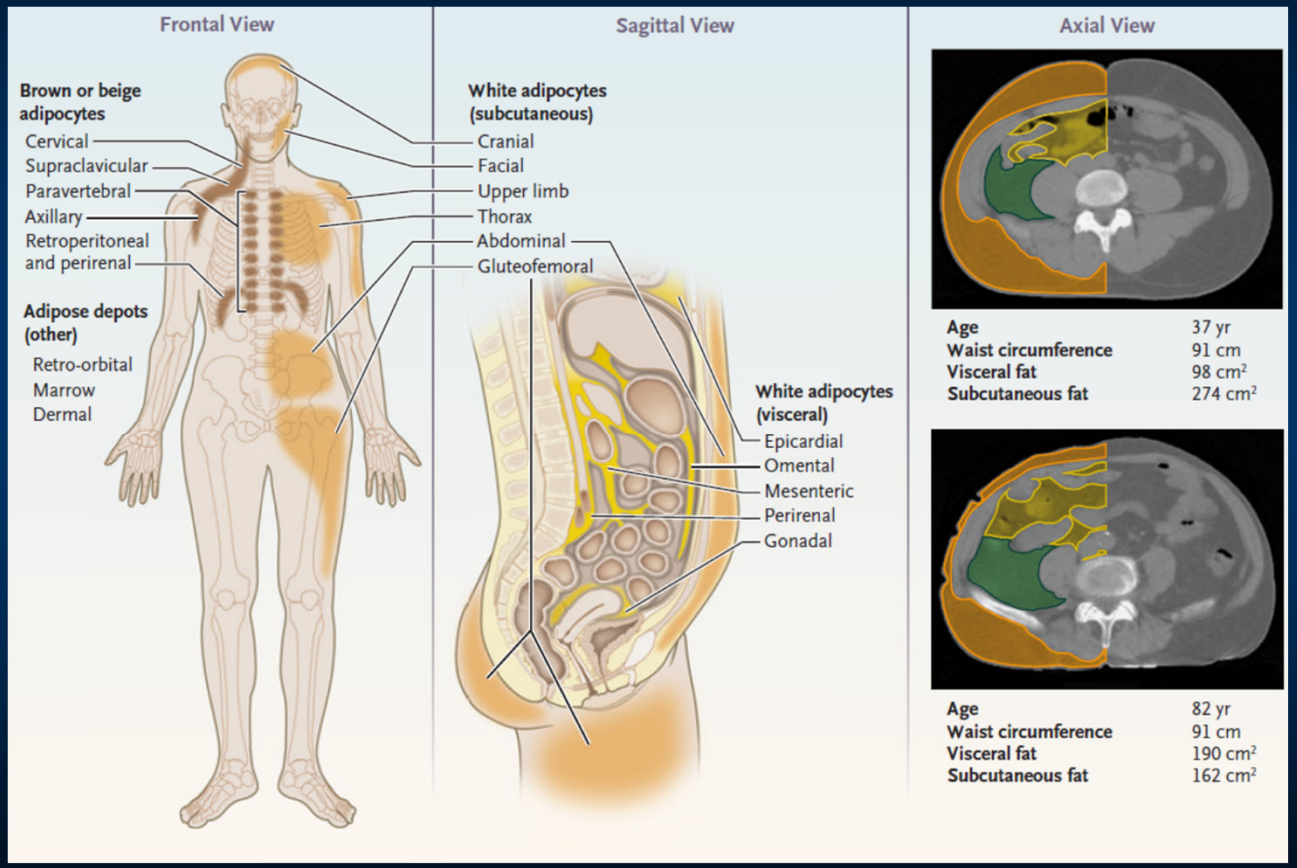
https://www.cdc.gov/diabetes/statistics/slides/maps_diabetesobesity_trends.pdf; https://mapsontheweb.zoom-maps.com/post/625814139904917504/obesity-rate-by-state-in-brazil; https://pophealthmetrics.biomedcentral.com/articles/10.1186/s12963-020-00209-0

Reassessing: Human Adipose Tissue is Polychromatic and Found Throughout the Body with Functional Diversity



Cypess AM N Engl J Med 2022;386:768-779

Reassessing: Human Adipose Tissue is Polychromatic and Found Throughout the Body with Functional Diversity



- Human WAT begins to develop in the second trimester of pregnancy, and by birth, both visceral and subcutaneous depots are well established.

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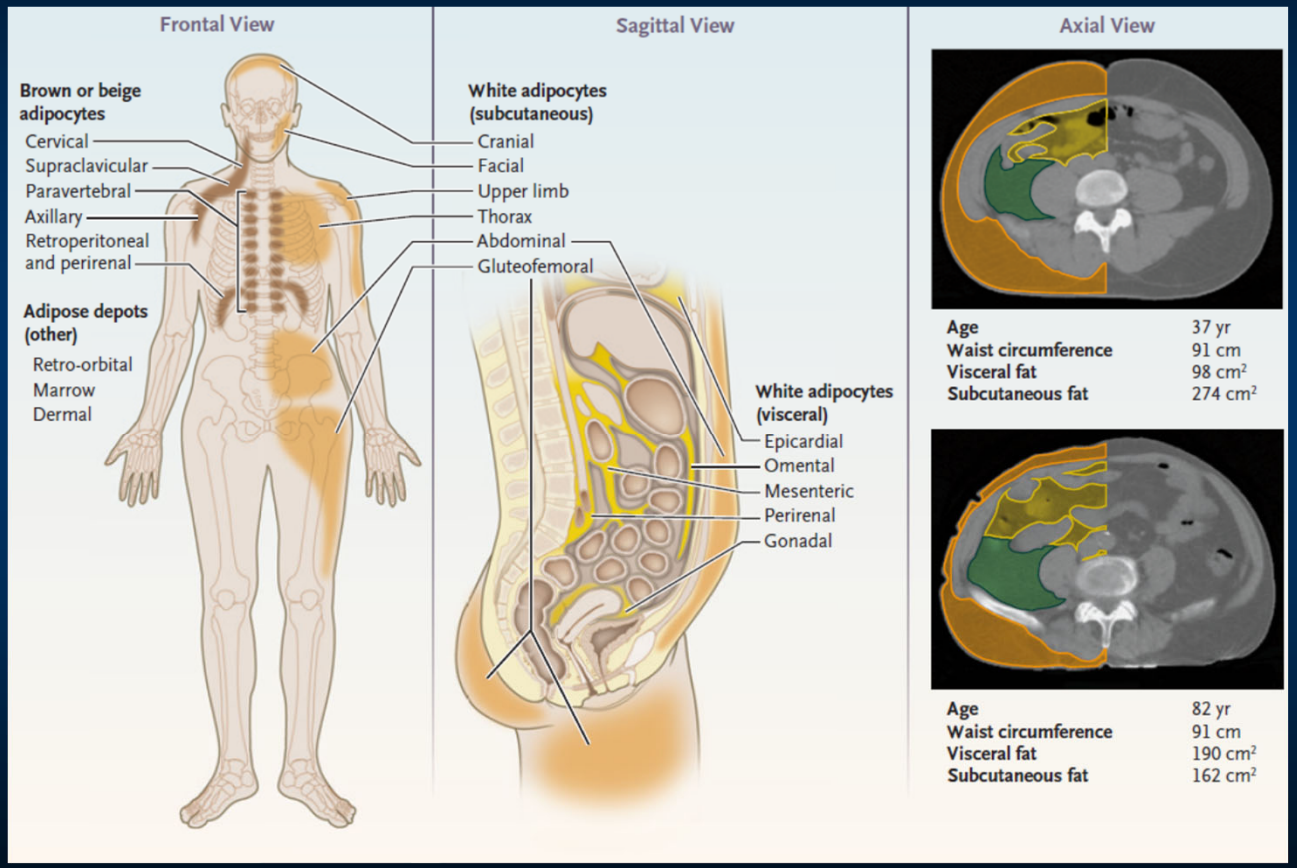

- Human WAT begins to develop in the second trimester of pregnancy, and by birth, both visceral and subcutaneous depots are well established.
- BAT first arises during the late 2nd trimester and protects newborns from cold while they develop the ability to shiver.

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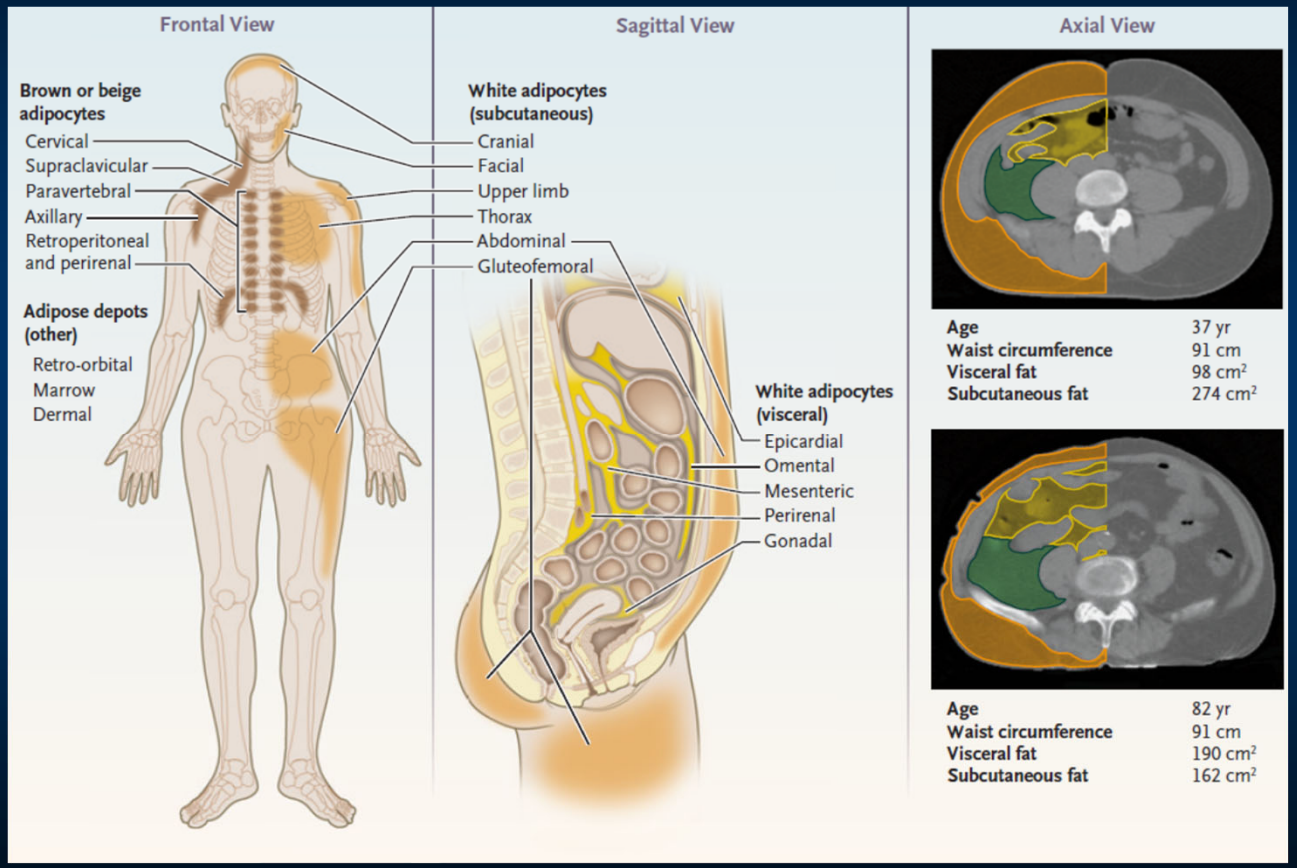

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- Human WAT begins to develop in the second trimester of pregnancy, and by birth, both visceral and subcutaneous depots are well established.
- BAT first arises during the late 2nd trimester and protects newborns from cold while they develop the ability to shiver.
- In lean women, the entire WAT depot ranges from 20 to 30 kg (30 to 40% of total body mass) and in lean men it is 10 to 20 kg in men (15 to 25% of total body mass).

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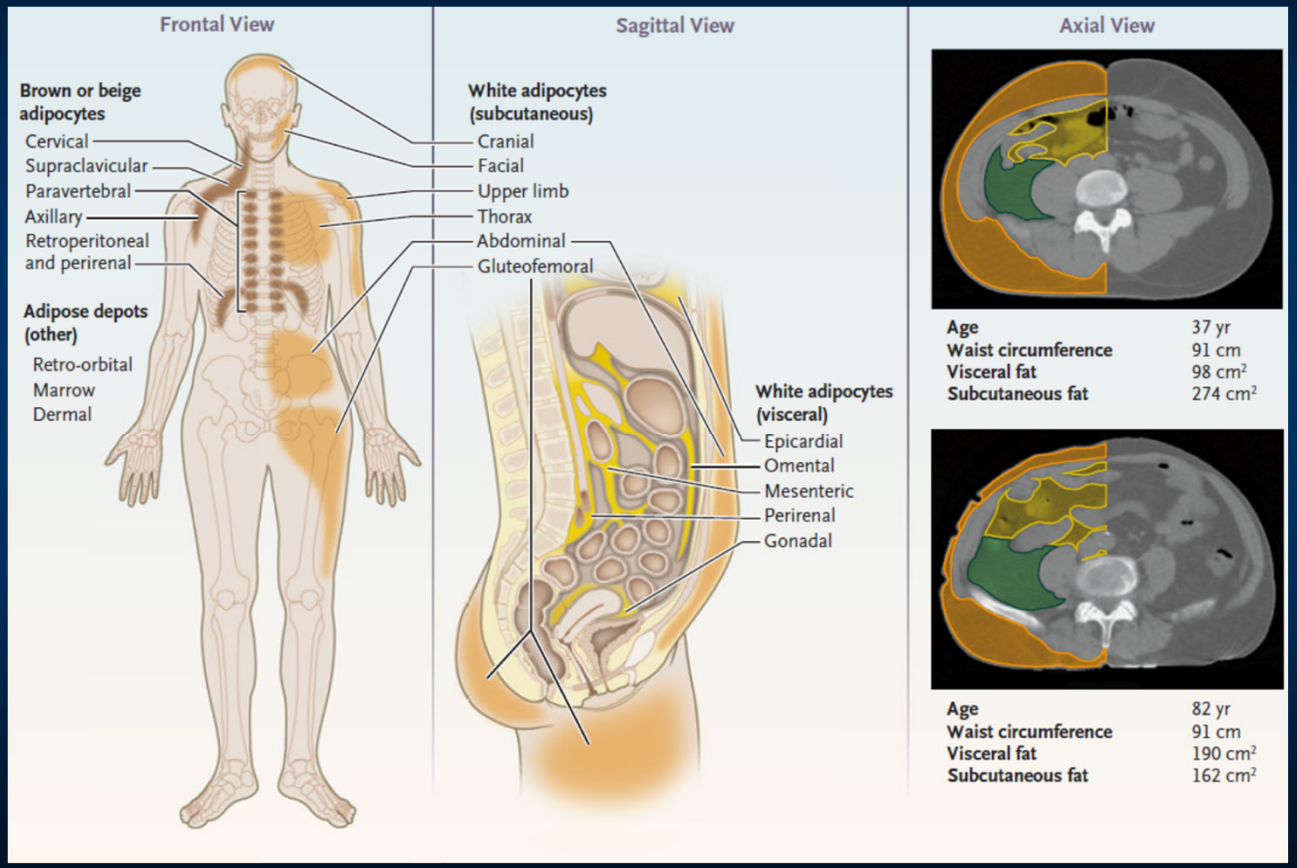

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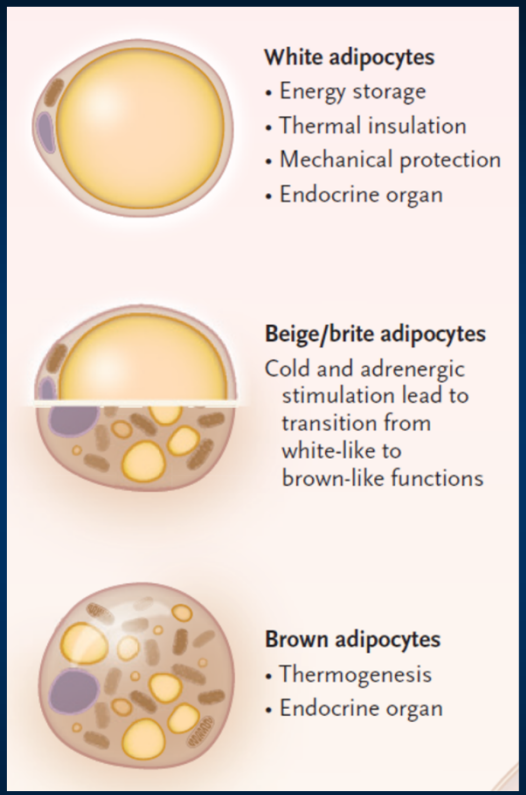
- BAT first arises during the late 2nd trimester and protects newborns from cold while they develop the ability to shiver.
- In lean women, the entire WAT depot ranges from 20 to 30 kg (30 to 40% of total body mass) and in lean men it is 10 to 20 kg in men (15 to 25% of total body mass).

For Adipocytes, Form Reflects Function

- White adipocytes store energy = droplets
- Brown adipocytes expend energy = mitochondria
- Beige adipocyte = in between
- Both have the ability to generate heat



Cypess AM N Engl J Med 2022;386:768-779

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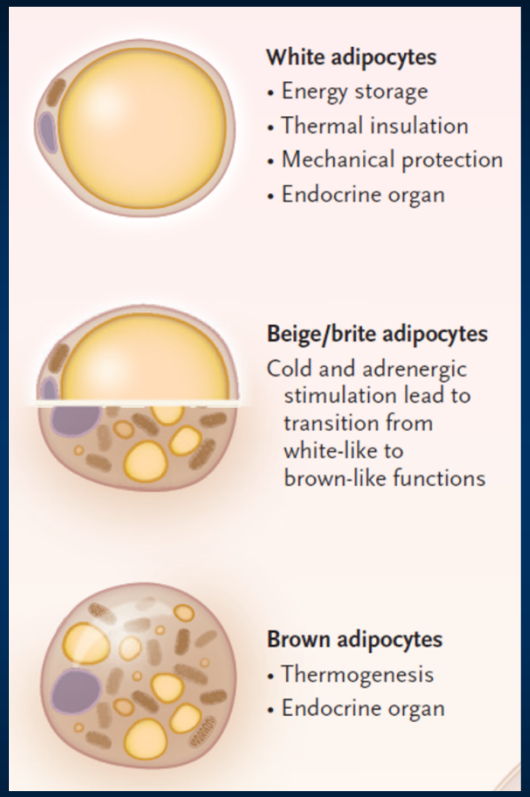
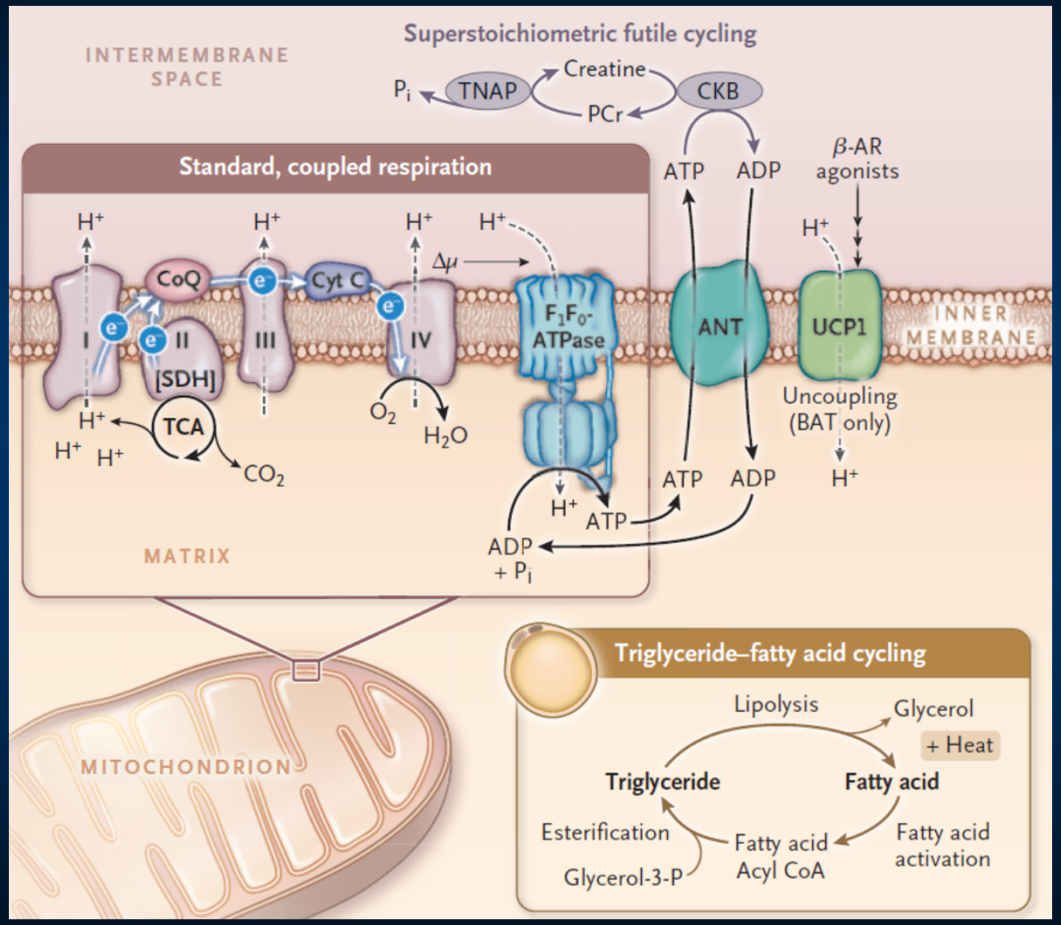
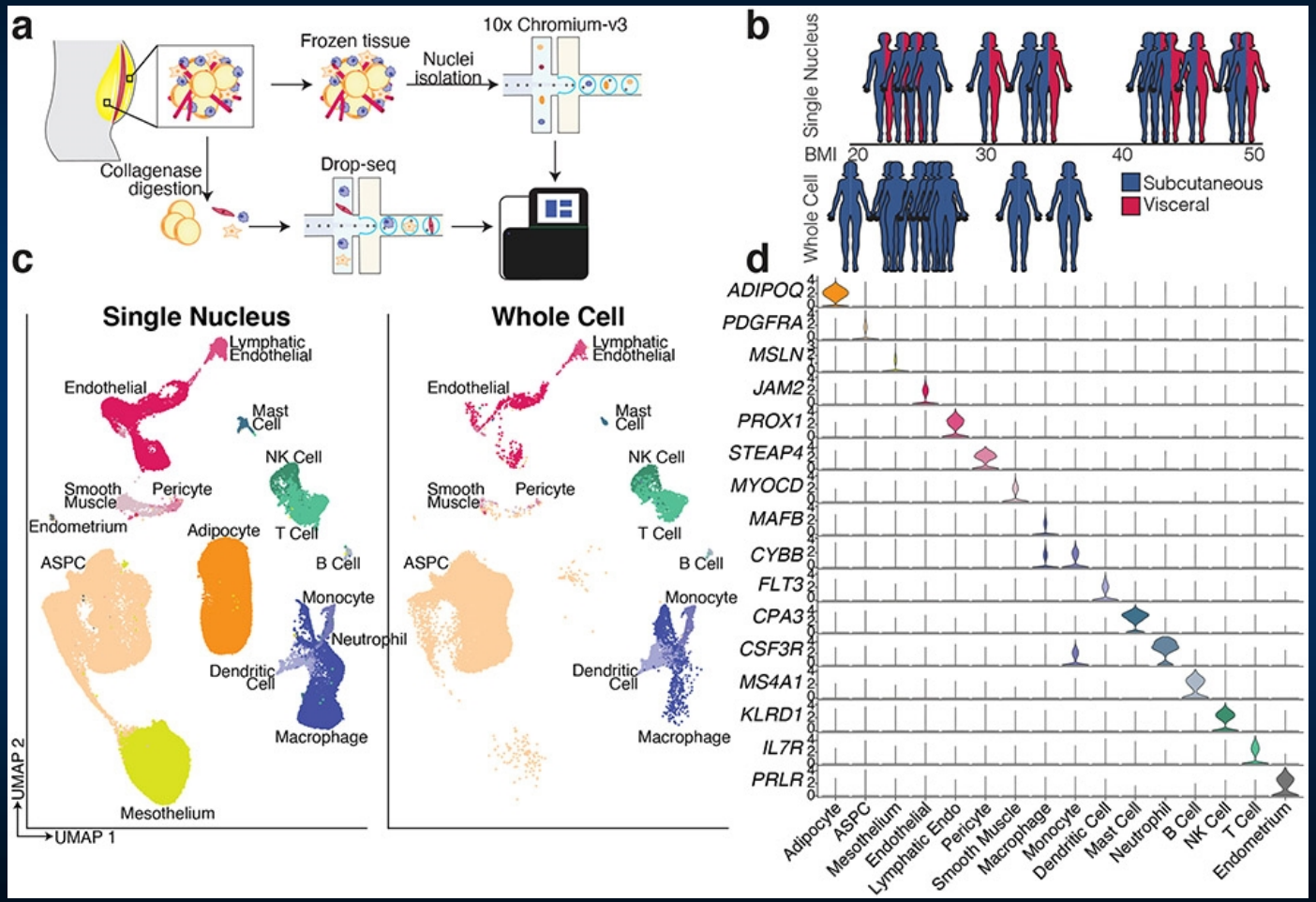




Cypess AM N Engl J Med 2022;386:768-779

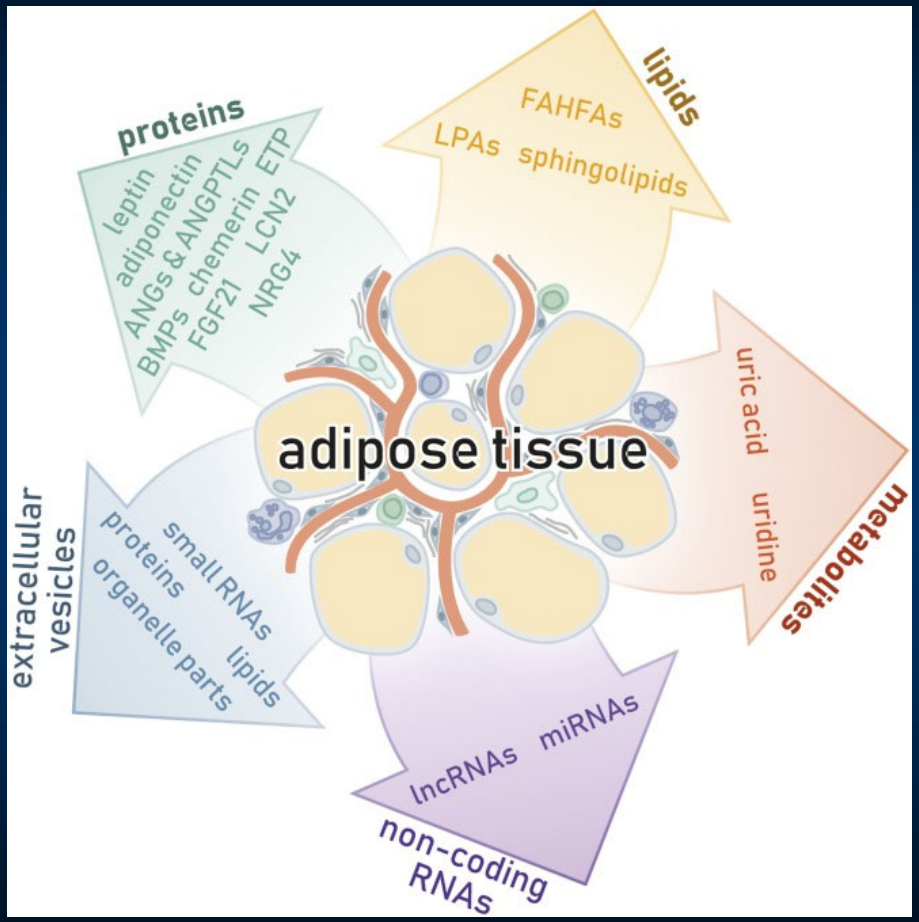
Remember that Tissues are Made up of a Diversity of Cell Types

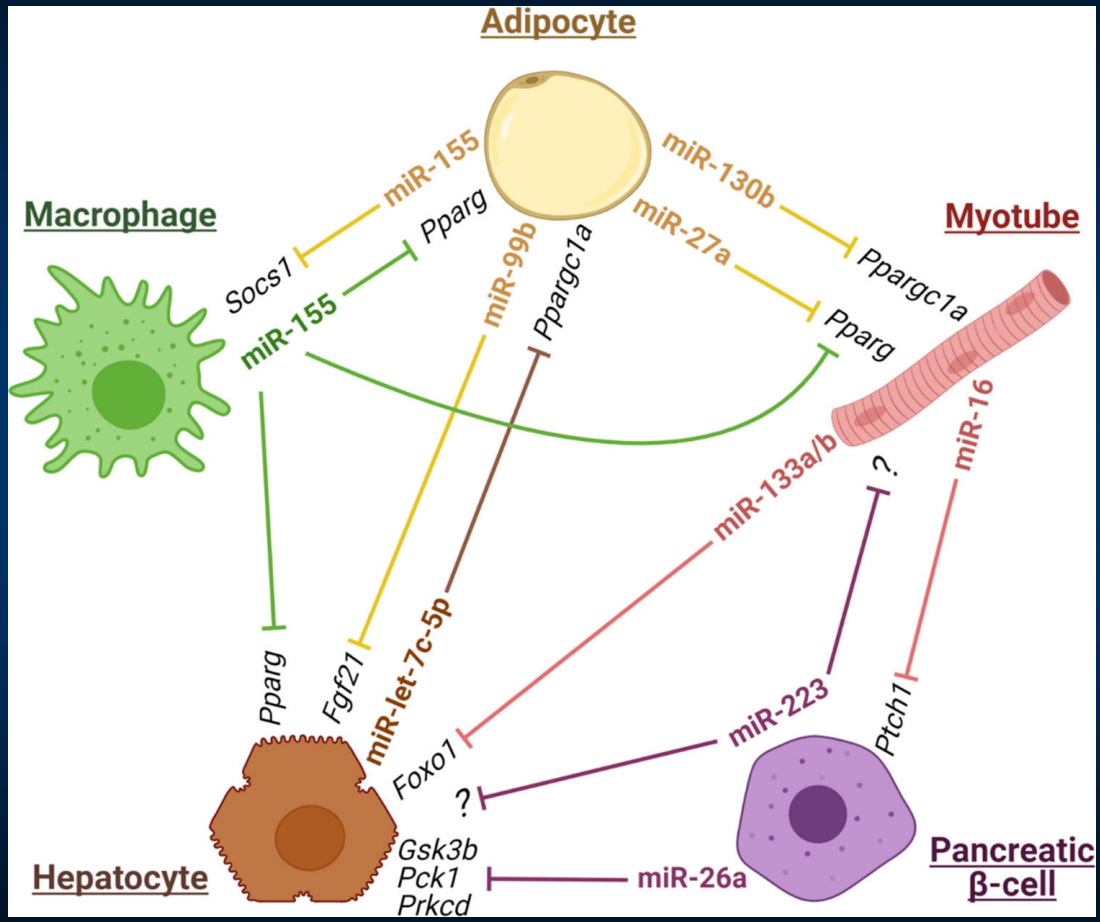
- Single cell atlas of human WAT shows many cell types.
- Besides adipocytes, there are stem cells, several types of immune cells, endothelial cells, and others, with distinct marker genes.
- Each type can act locally but also impact organs throughout the body.



Emont MP…Rosen ED Nature 2022:603,926–933

The Roles of Adipose Tissues are only Beginning to be Discovered

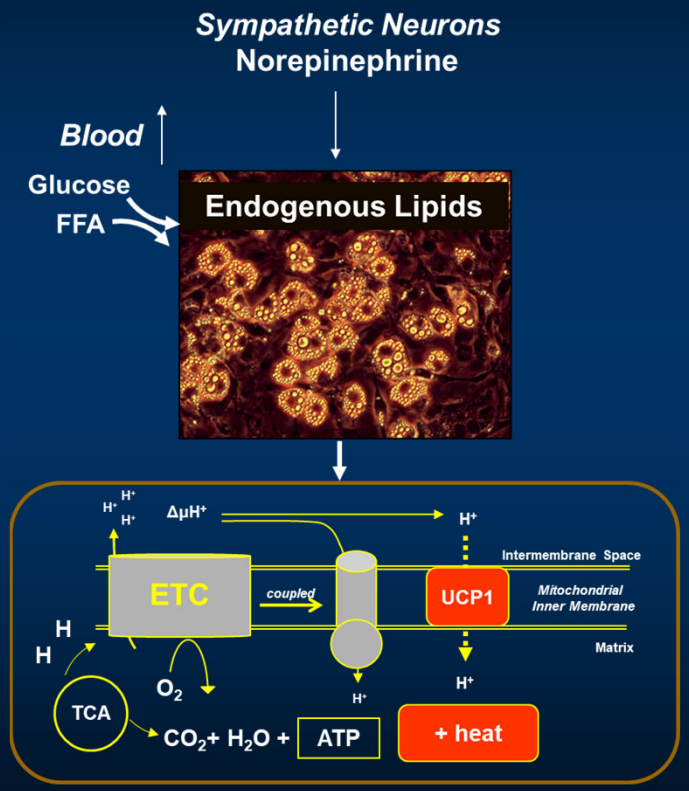




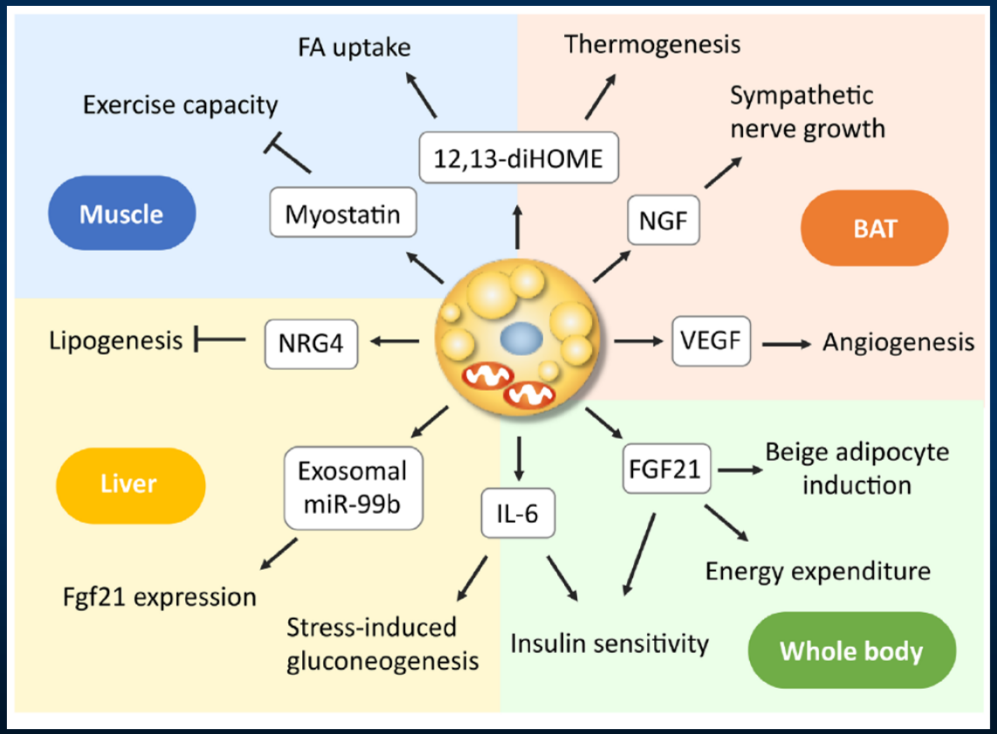
Funcke J-B Scherer PE J Lipid Res 2019;60:1648; Brandao BB Lino M Kahn CR J Physiol 2022;600:1155;

What Could Human BAT Do? Consider Both Functional and Endocrine

Thermogenic Organ

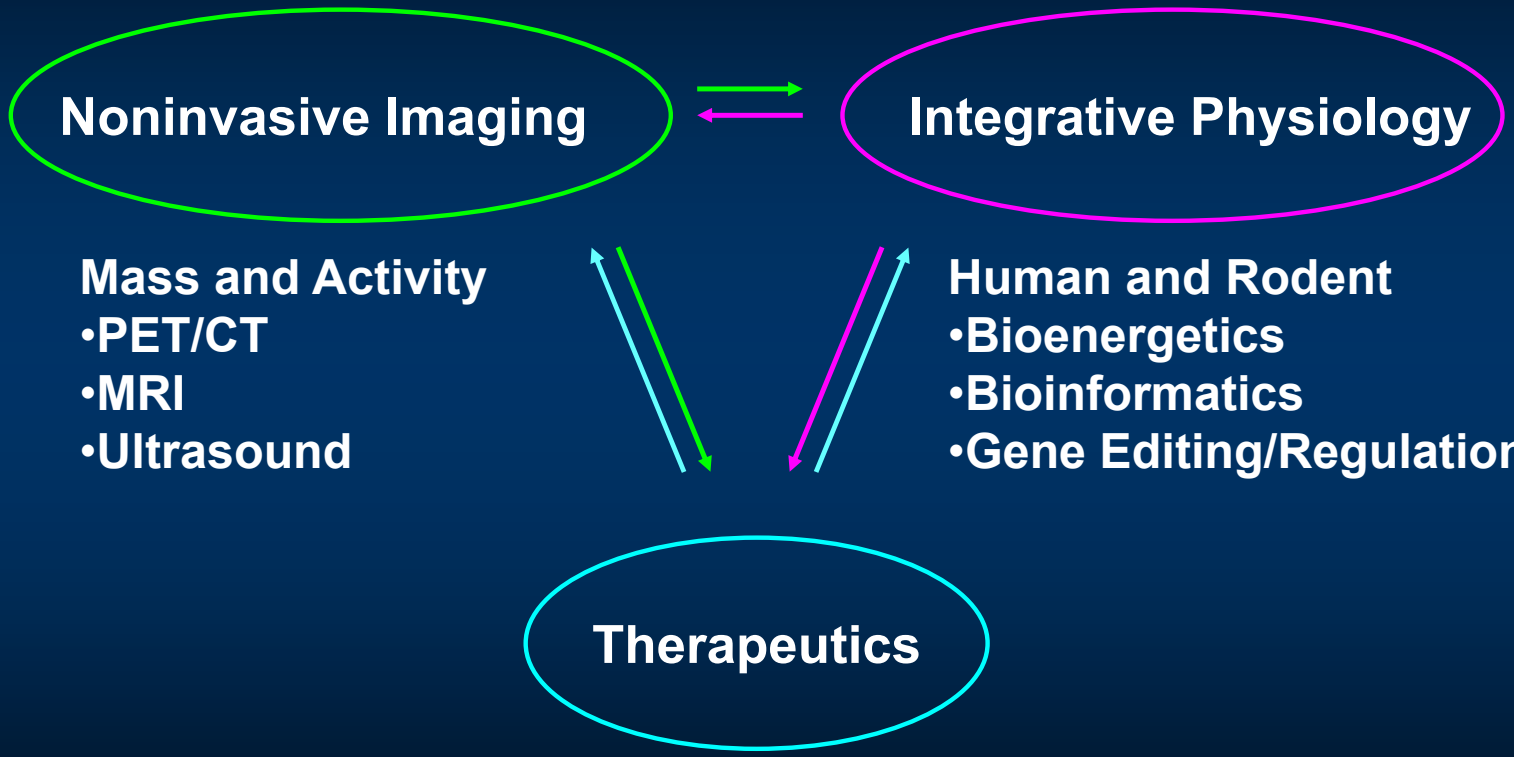


Endocrine Organ



Nedergaard J & Cannon B. Cell Metab. 2011;13:238-40. Yuko O-O and Saito M Diabetes Metab J. 2021;45:840-852

How We Approach the Study of the Cellular Physiology and Endocrine Roles of Human BAT and WAT



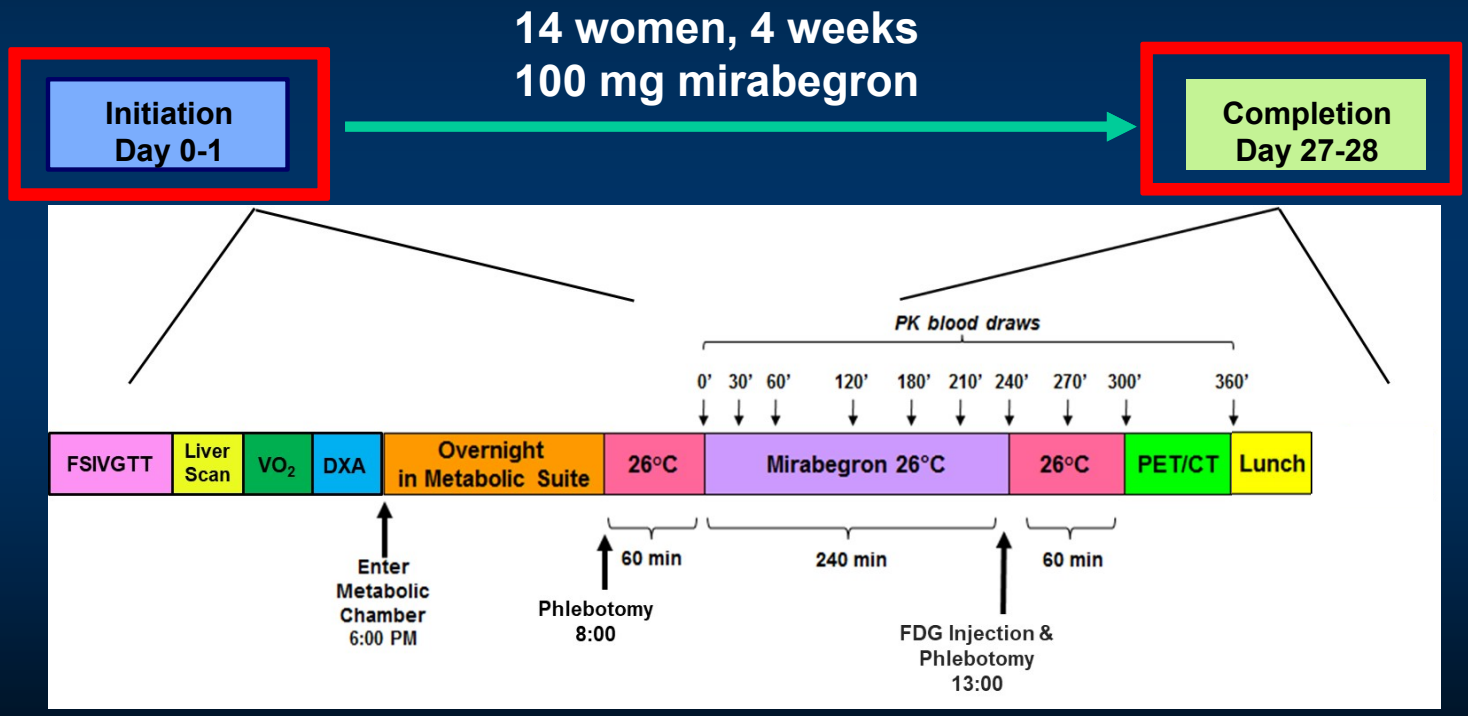
•Mild Cold Exposure (15-18 °C)

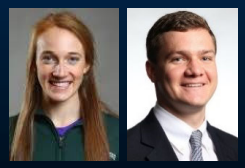
•β3-adrenergic receptor agonists - mirabegron

β3-Adrenergic Receptor Agonists

Chronic Mirabegron Treatment "CMT" - 17-DK-0054

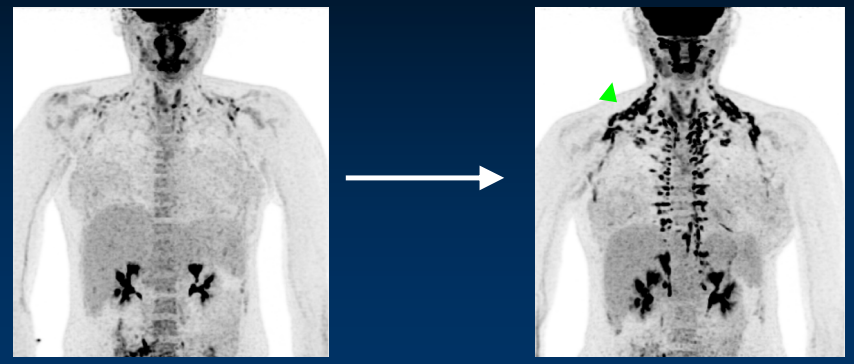
Cohort 1 - The primary endpoint is the change in detectable BAT metabolic activity associated with daily dosing of mirabegron over a 4-week period.





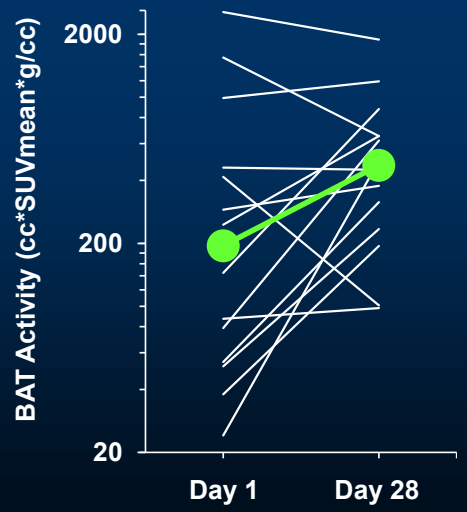
O'Mara AE…Cypess AM JCI 2020;130:2209-2219

Mirabegron Increased BAT Metabolic Activity & Volume

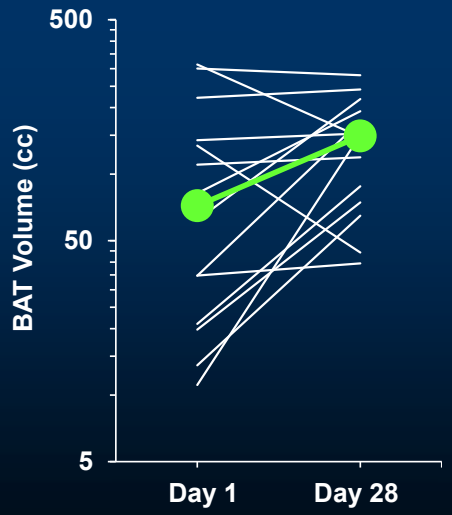


O'Mara AE…Cypess AM
JCI 2020;130:2209-2219

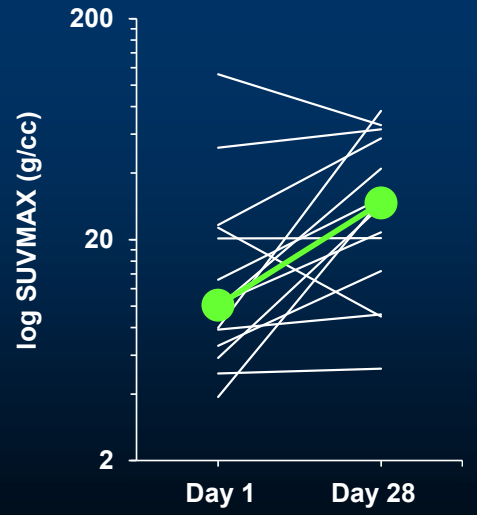
BAT Metabolic Activity = +278
P = 0.039



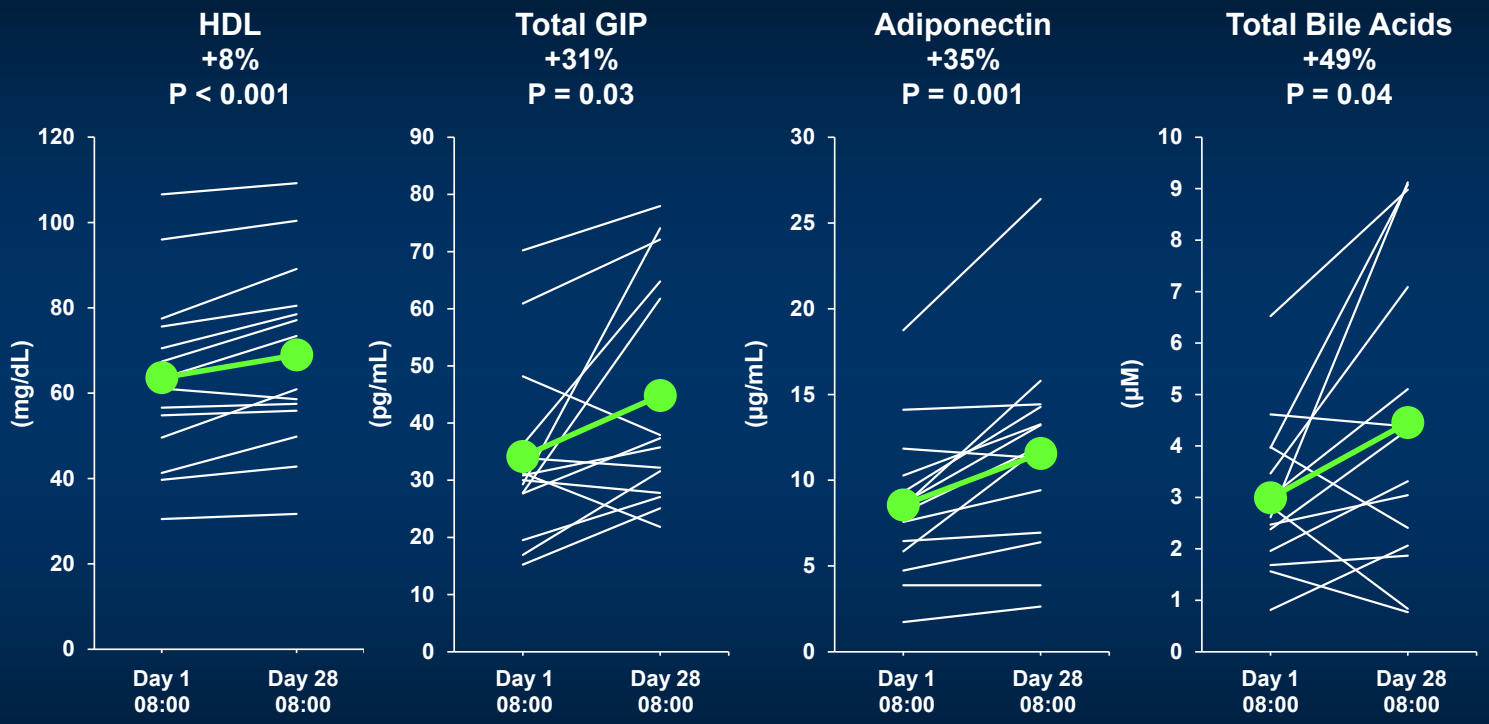
BAT Volume = +77 mL
P = 0.036



Max BAT Activity = +19 mg/mL
P = 0.017



Chronic Mirabegron Raised HDL, tGIP, Adiponectin, and Bile Acids



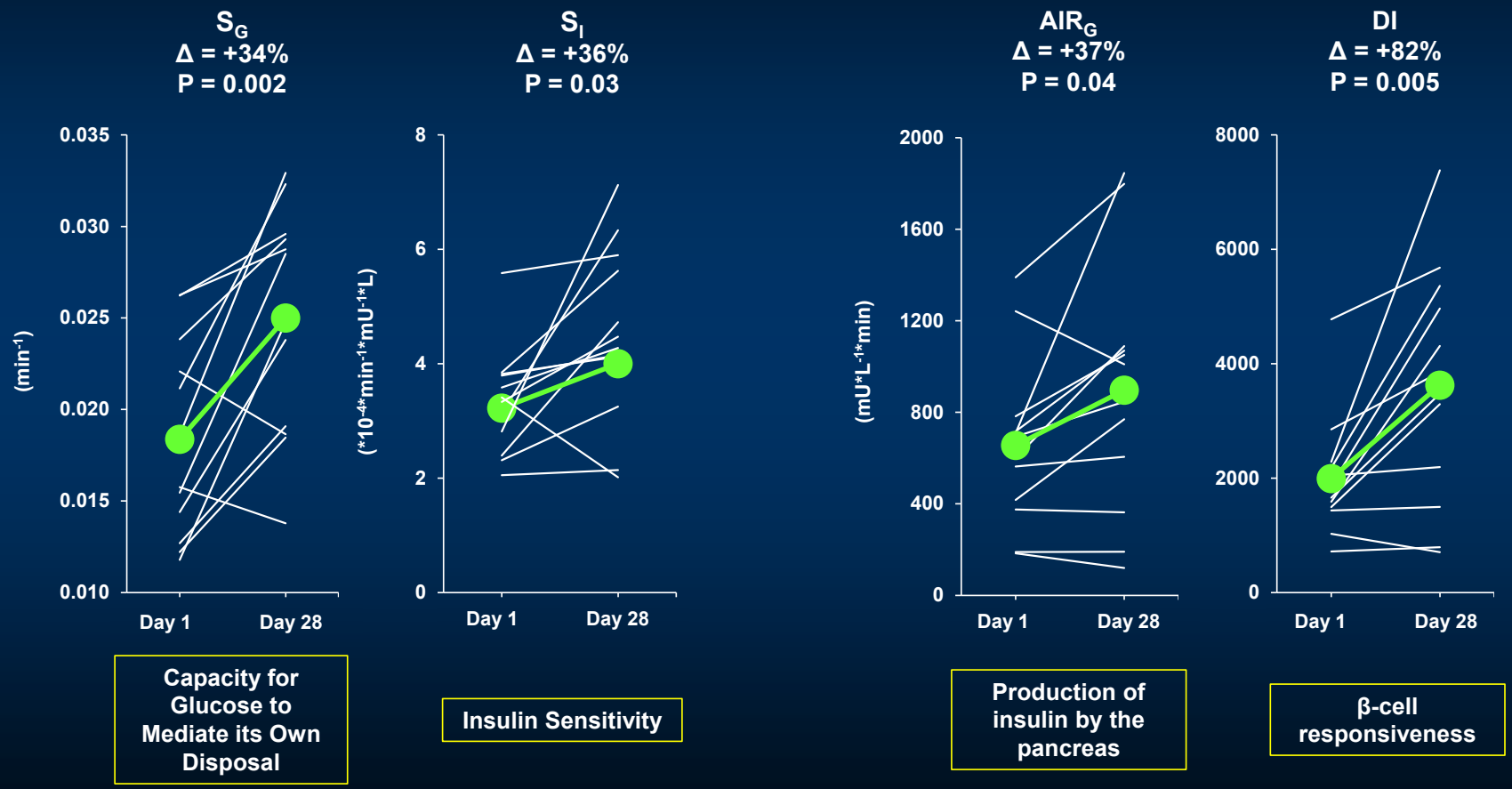
Bile Acids

- **Surfactants that help absorb dietary lipids, cholesterol, and Rx**
- **Bind to FXR, LXR, PXR, TGR5**
- **Liver – homeostasis of glucose, lipid, lipoprotein, & BA metabolism**
- **CV – cardioprotective**
- **GI – modify microbiome**
- **Brain – neuroprotective; appetite suppression**
- **SkM – insulin sensitivity**
- **BAT – thermogenesis**
- **Immune – modulating**

Shapiro H…Elinav E J Exp Med 2018;215:383-396

O'Mara AE…Cypess AM JCI 2020;130:2209-2219

Mirabegron Caused Improvements in Both Glucose Uptake and Pancreatic β-Cell Function

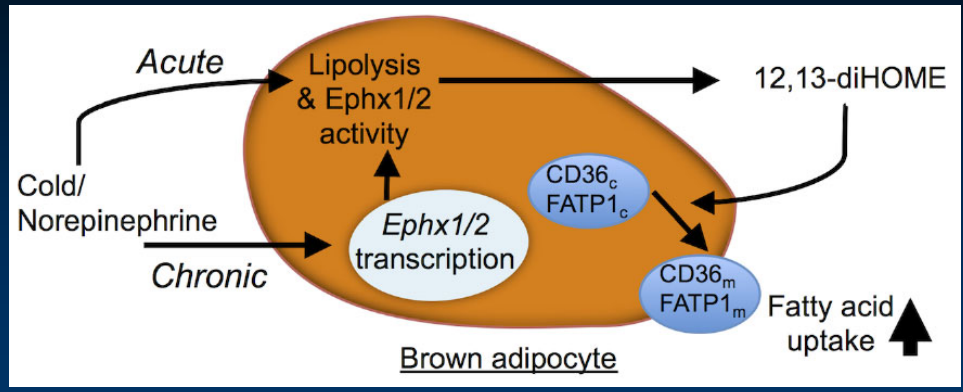


O'Mara AE…Cypess AM JCI 2020;130:2209-2219

Leveraging Banked Plasma and Tissue for Discovery

The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue and skeletal muscle

- Lynes et al., Nat Med 2017;23:631-637
- Cypess AM…Kahn CR PNAS USA 2012;109:10001-10005

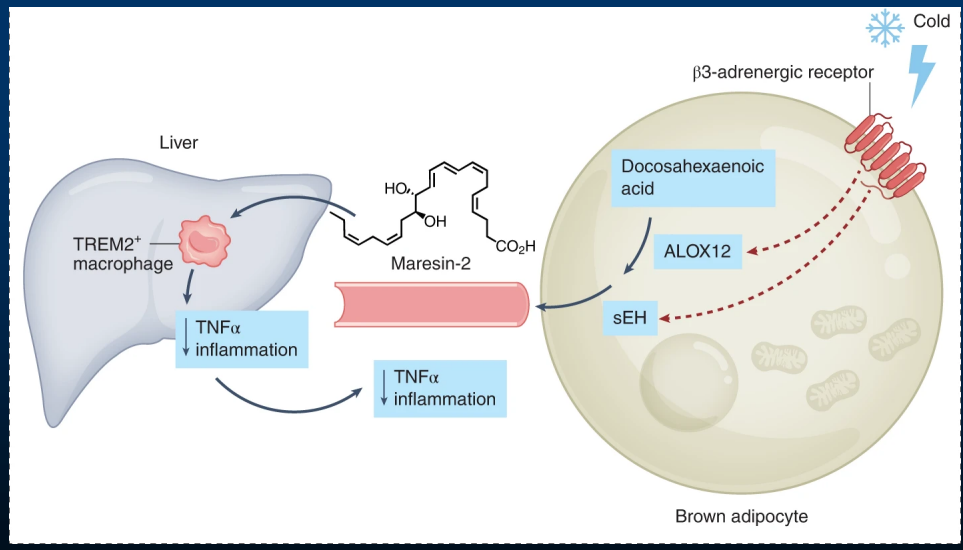


12-Lipoxygenase Regulates Cold Adaptation and Glucose Metabolism by Producing the Omega-3 Lipid 12-HEPE from Brown Fat

- Leiria et al., Cell Metab 2019;30:768–783
- Cypess AM…Kahn CR PNAS USA 2012;109:10001-10005

Brown adipose tissue-derived MaR2 contributes to cold-induced resolution of inflammation

- Sugimoto et al. Nat Metab 2022;4:775-790
- Villarroya et al., Nat Metab 2022;4:649-650

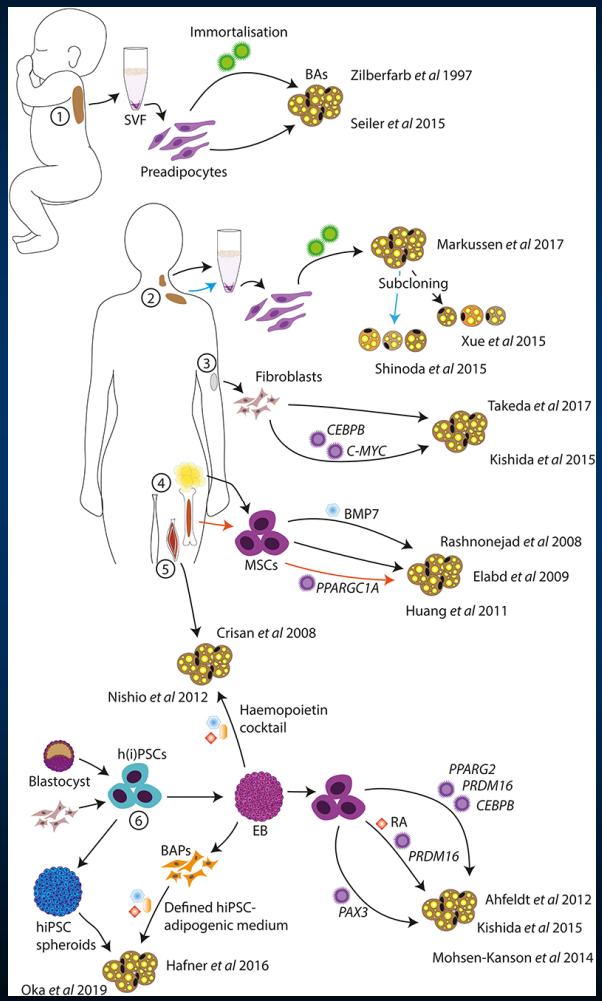


Summary of Chronic Mirabegron Treatment Studies

- Chronic treatment with mirabegron for 4 weeks leads to the following:
  - Increases in BAT metabolic activity
  - Increases in REE, 6% above baseline
  - Increases in HDL, total bile acids, and adiponectin
  - Improvements in both glucose uptake and β-cell function
- Targeting the β3-AR may be a new approach to treating obesity-related complications such as T2DM as well as components of the metabolic syndrome.

How can the underlying mechanisms remain be determined given that BAT and WAT likely have important roles?

Determining Physiological Mechanisms using *in vitro* Models



- The difficulty in accessing and obtaining human BAT and the lack of suitable human brown adipocyte models have hampered understanding the physiological role of human brown adipocytes.

- A few models to study human BAT have been developed including:
  - Human multipotent adipose-derived stem cells (hMADS)
  - Multipotent mesenchymal precursor cells (MPCs)
  - Pluripotent stem cells (hPSC)
  - Primary culture derived from adult BAT
  - Immortalized human BAT cell lines

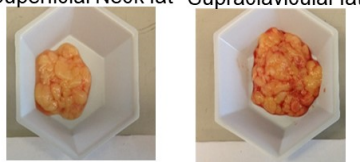
Samuelson and Vidal-Puig et al., 2020. Studying Brown Adipocytes Tissue in a Human in vitro context.

New Models, Part 1
Primary Human Preadipocytes

Isolation of Primary Human White and Brown Preadipocytes from SVF

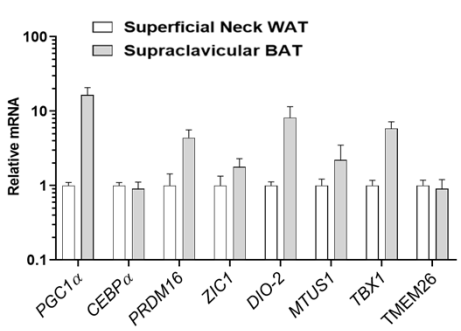
16 year-old female human (advanced malignancy)

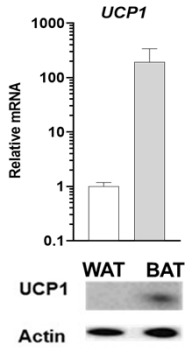
Superficial Neck fat Supraclavicular fat

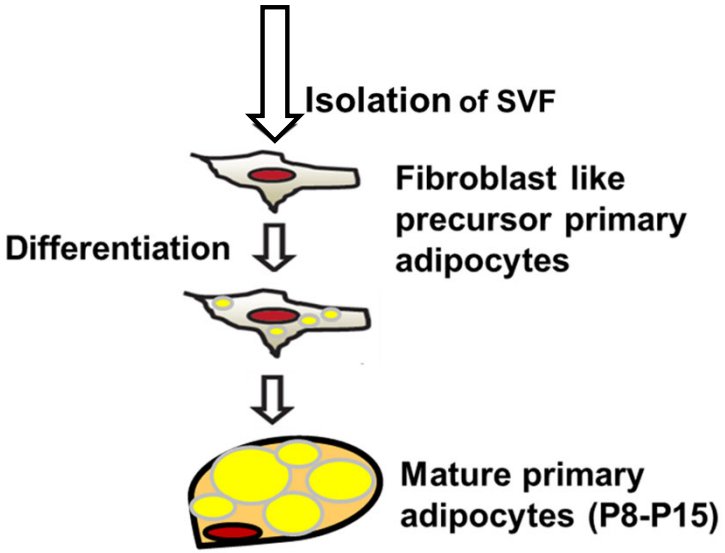


In collaboration with NIH, Pathology core (NCI)

Brown and Beige adipocyte markers





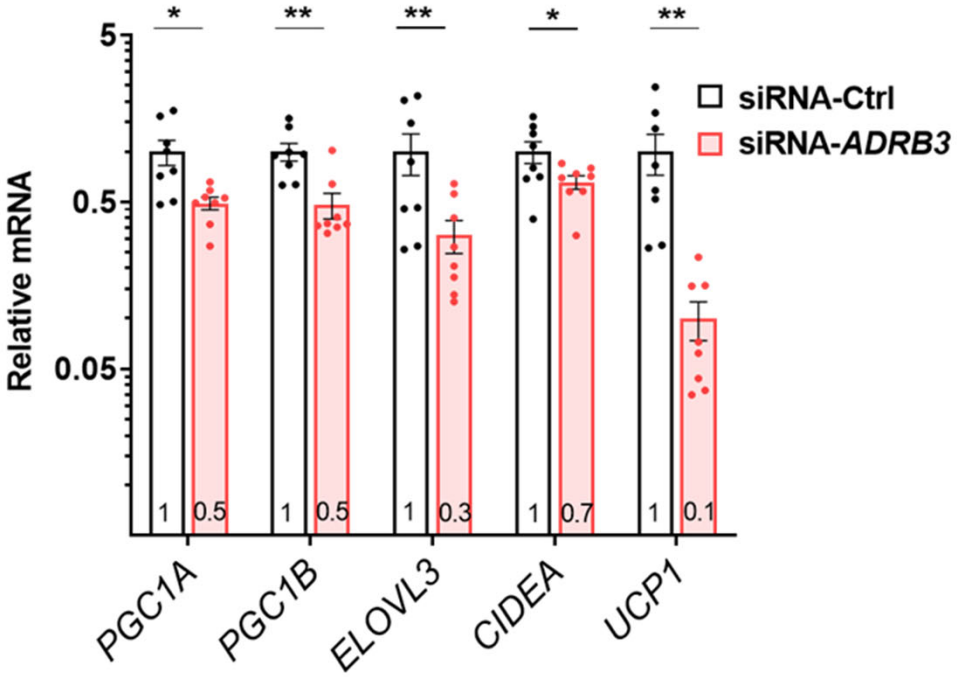


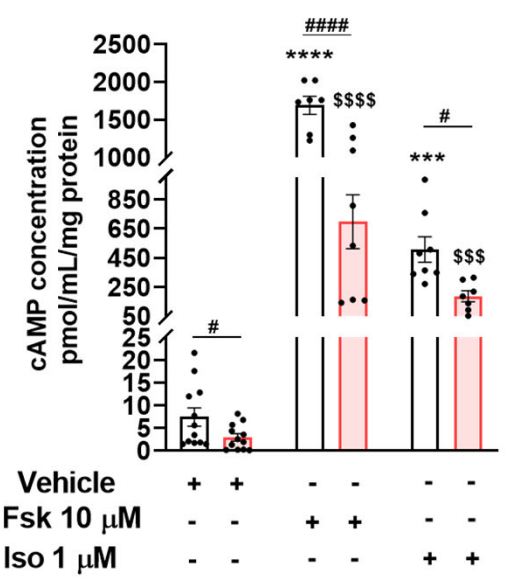
Primary non-transformed cells (vs immortalized):

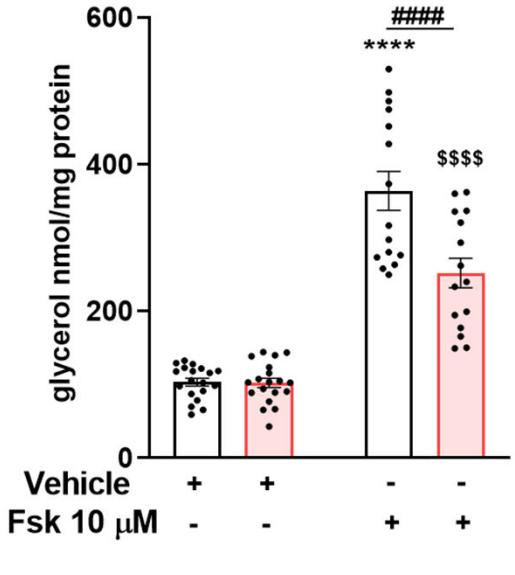
- **resemble more their original tissue characteristics and physiology**
- **entopic gene expression and protein function**

Cero C…Cypess AM JCI Insight 2021;6(11):e139160

Knockdown of *ADRB3* in Human Thermogenic Adipocytes Lowers Expression of Thermogenic Genes and Reduces Lipolysis

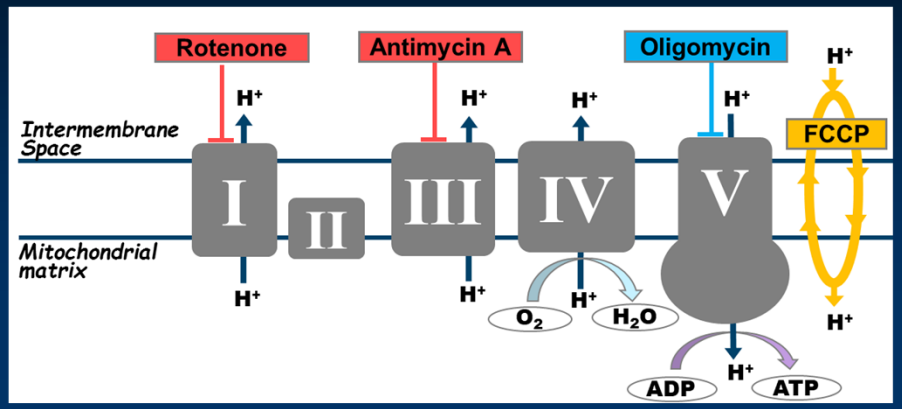




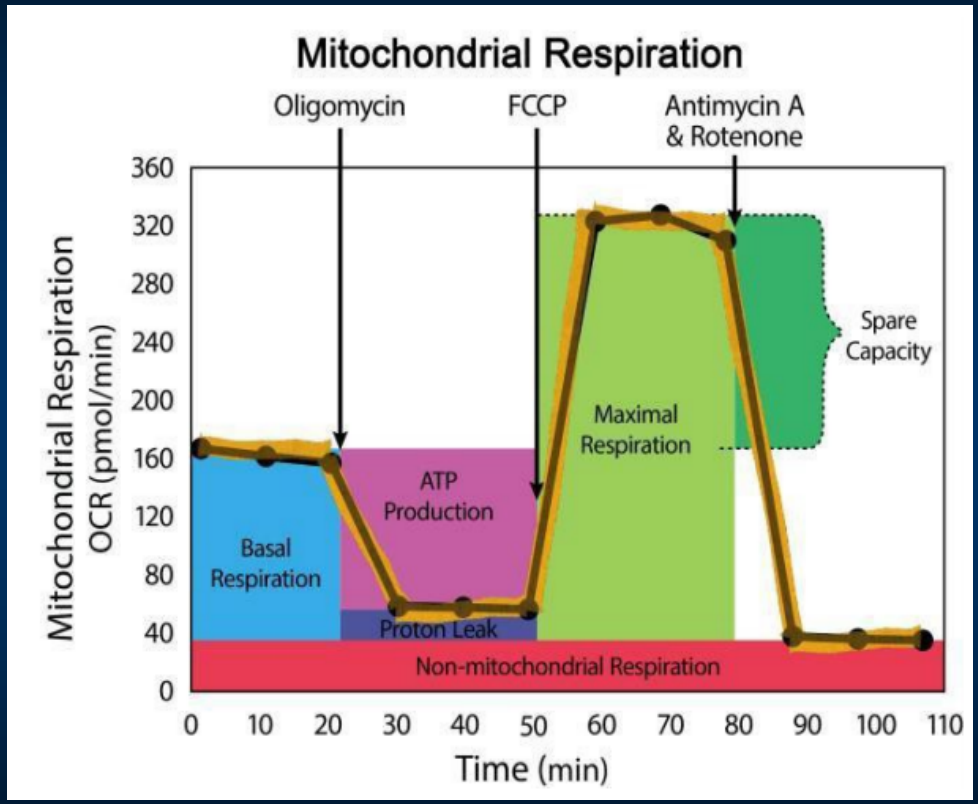


Cero C… Cypess AM JCI Insight 2021;6(11):e139160

Measuring Mitochondrial Bioenergetics Using the Agilent Seahorse XFe96 Analyzer

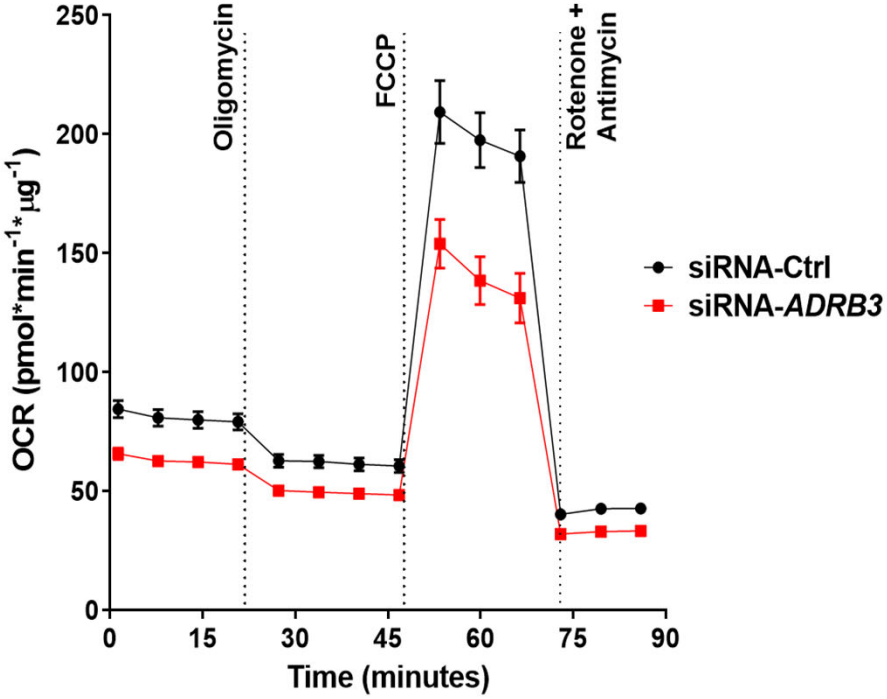


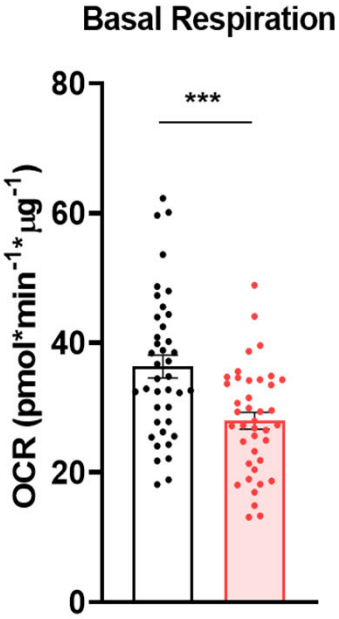
- Basal respiration = (last rate measurement before first injection) – (non-mitochondrial respiration rate);
- ATP Production – shows ATP produced by the mitochondria that contributes to meeting the energetic needs of the cell
- H+ leak = (minimum rate after oligo injection) – (non-mitochondrial respiration rate)
- Maximal respiration = (maximum rate after FCCP injection) – (non-mitochondrial respiration rate);
- Non-mitochondrial respiration = minimum rate measurement after Rotenone/Antimycin A injection

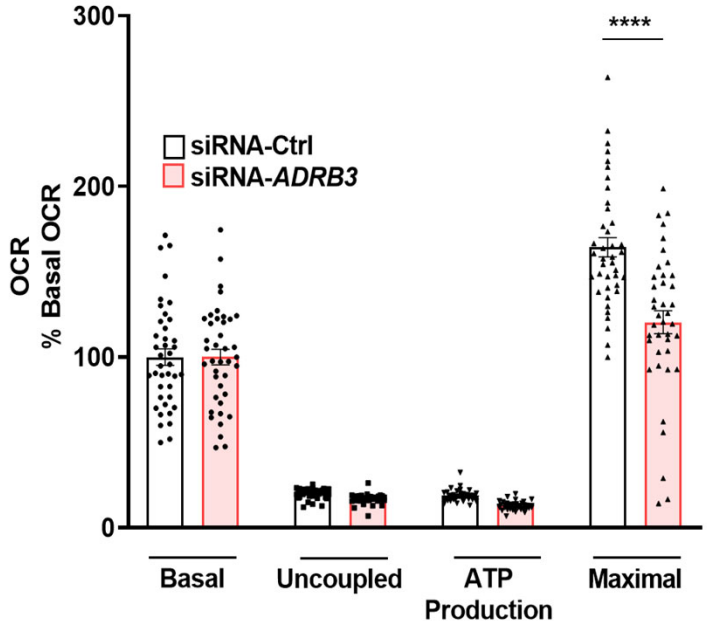


https://www.agilent.com/cs/library/usermanuals/public/XF_Cell_Mito_Stress_Test_Kit_User_Guide.pdf

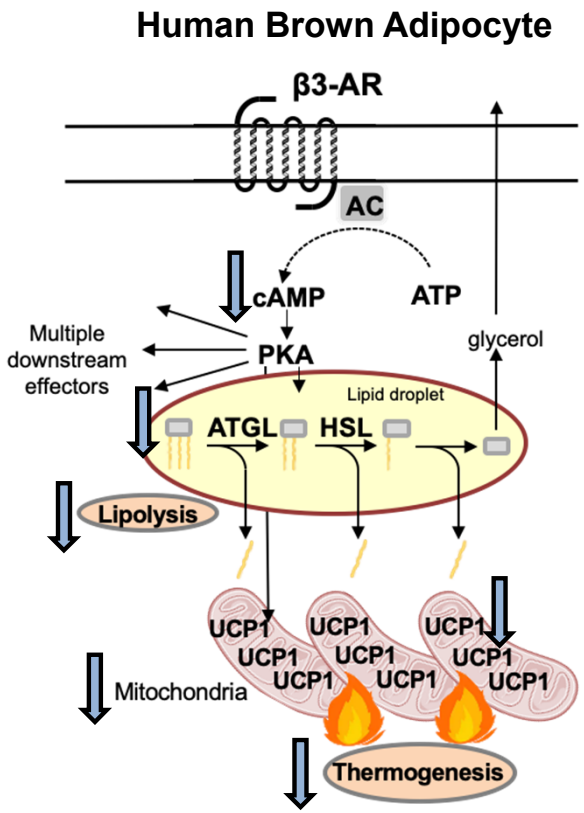
The β3-AR in Human Thermogenic Adipocytes Contributes to both Basal and Maximal Oxygen Consumption Rates







The β3-AR Has Functional Roles in the Cellular Physiology of Human Brown Adipocytes



- Upon differentiation, primary human brown adipocytes from the supraclavicular fat depot increase expression of adipogenic, β-AR, and thermogenic genes
- Human brown adipocytes express functional β3-ARs
- Knocking down the β3-ARs in human brown adipocytes lowers:
  - basal cAMP and reduces cAMP-mediated downstream signaling and lipolysis
  - mitochondrial content & cellular respiration
  - UCP1 expression and UCP1-mediated thermogenesis

Cero C…Cypess AM JCI Insight 2021;6(11):e139160

New Models, Part 2

Immortalized Clonal Human Preadipocytes

white (ATCC CRL-4063™)

brown (ATCC CRL-4062™)

Endocrinology, 2023, **164**, 1–22
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Research Article

ENDOCRINE SOCIETY

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Standardized In Vitro Models of Human Adipose Tissue Reveal Metabolic Flexibility in Brown Adipocyte Thermogenesis

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Development of Standard *in vitro* Models of Human White and Brown Adipose Tissue

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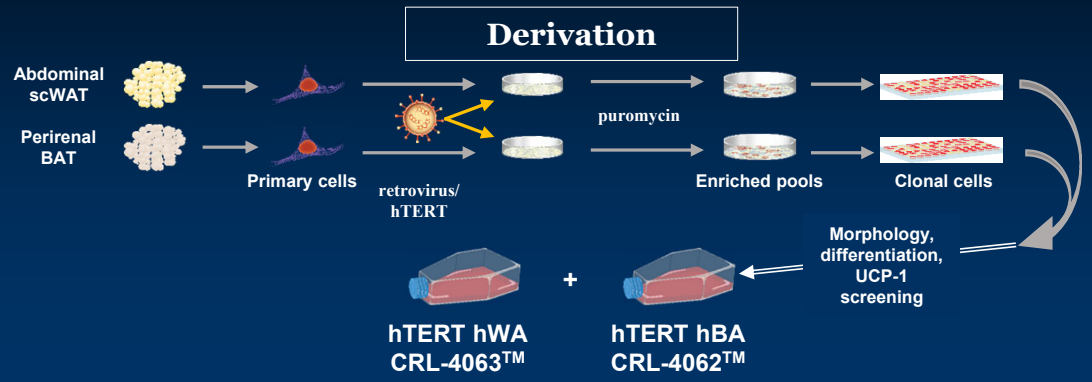
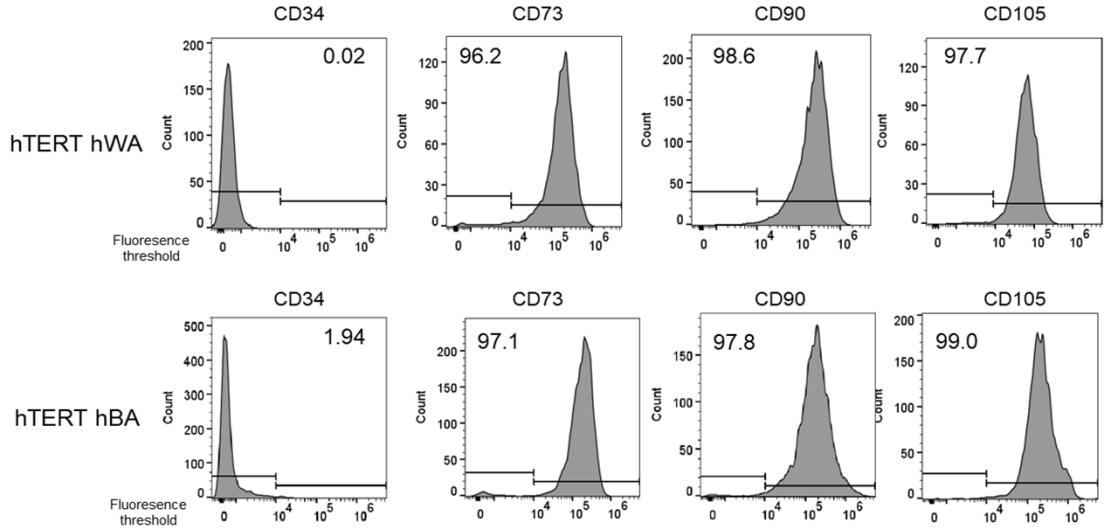
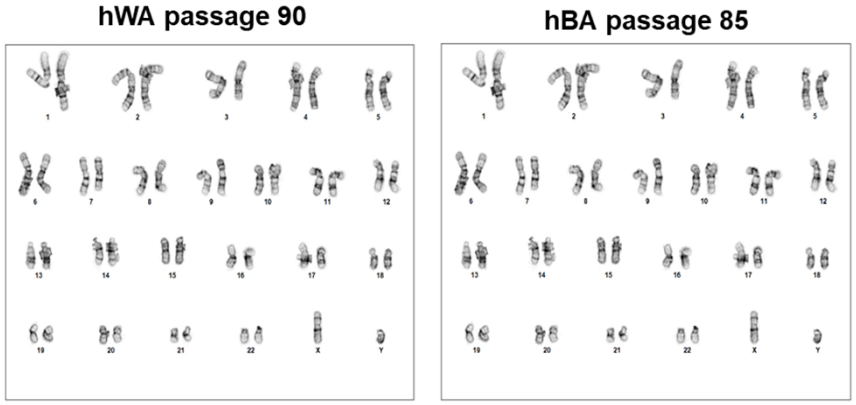
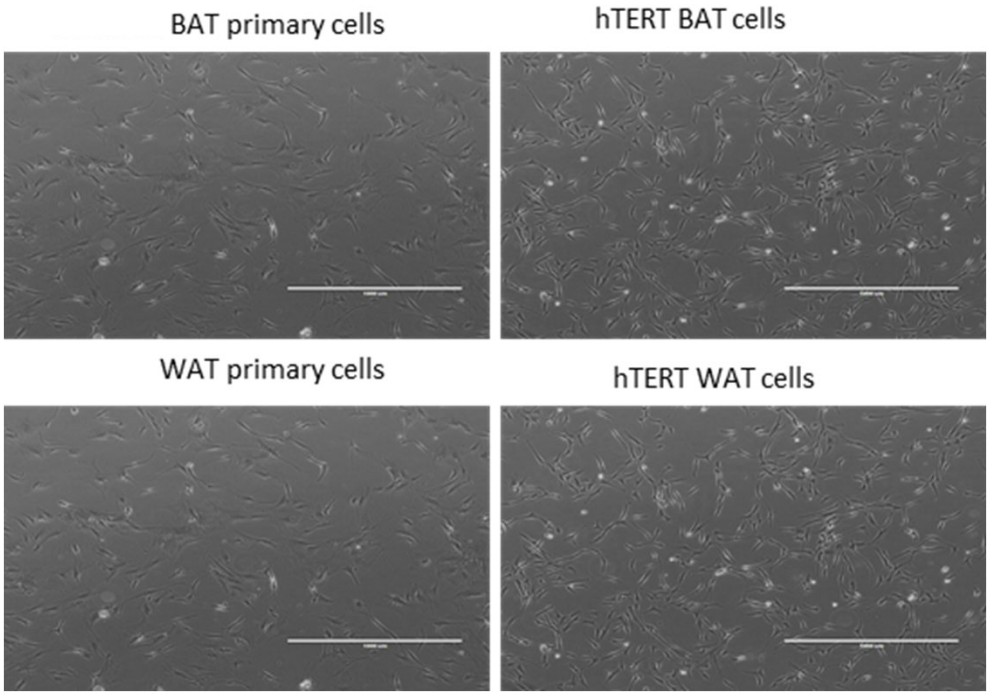
Endocrinology 2023
Nov 2;164(12):bqad161

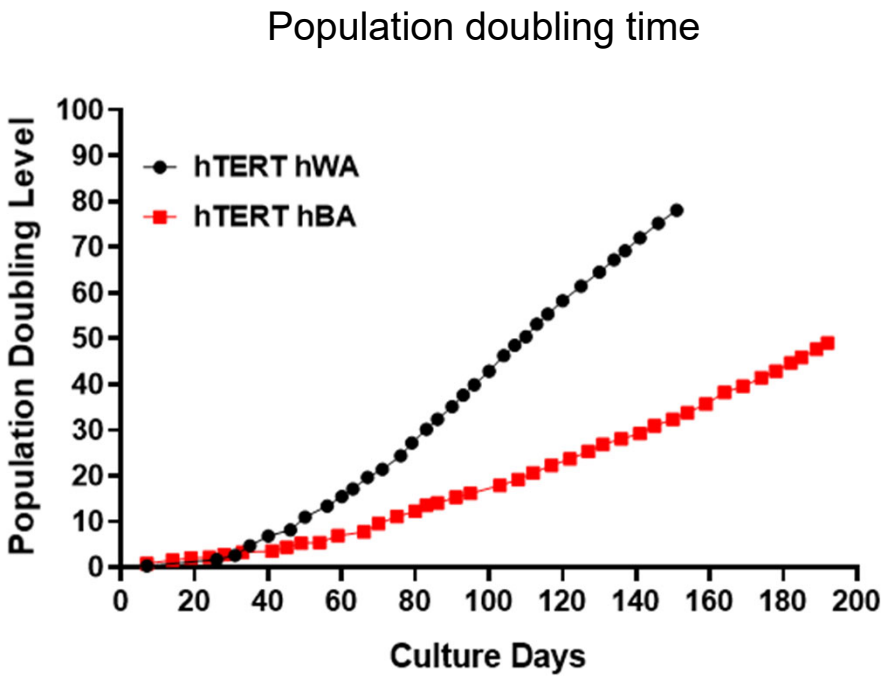






The Immortalized Preadipocytes were Successfully Isolated and Grow with Distinct Doubling Times





Adipogenic Differentiation Protocols – Depend on Cell Types

Growing cells in DMEM + 10% FBS + 1% Pen/Strep (and hFGF 4.0 ng/ml)

hWA

Induction Media 1
x 7 days

- Advanced DMEM/F12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 µM Human Insulin
- 0.1 µM Dexamethasone
- 0.5 mM IBMX
- 33 µM Biotin
- 2 nM T3
- 30 µM Indomethacin
- 17 µM Pantothenate
- 2 µM Rosiglitazone

Induction Media 2
x 18-23 days

- Advanced DMEM/F12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 µM Human Insulin
- 0.1 µM Dexamethasone
- 0.5 mM IBMX
- 33 µM Biotin
- 2 nM T3
- 30 µM Indomethacin
- 17 µM Pantothenate

hBA

Pre-Induction Media
x 6 days

- Advanced DMEM/F12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 µM Human Insulin
- 2 nM T3
- 3.3 nM BMP7

Induction Media #1
x 7 days

- Advanced DMEM/F12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 µM Human Insulin
- 0.1 µM Dexamethasone
- 0.5 mM IBMX
- 33 µM Biotin
- 2 nM T3
- 30 µM Indomethacin
- 17 µM Pantothenate
- 2 µM Rosiglitazone

Induction Media #2
x 12-17 days

- Advanced DMEM/F12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 µM Human Insulin
- 0.1 µM Dexamethasone
- 0.5 mM IBMX
- 33 µM Biotin
- 2 nM T3
- 30 µM Indomethacin
- 17 µM Pantothenate

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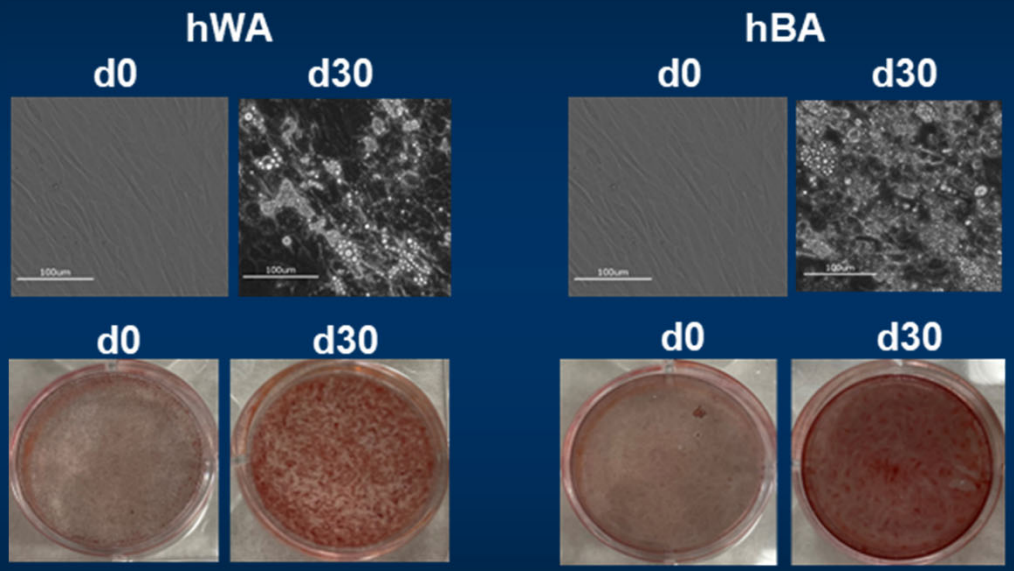
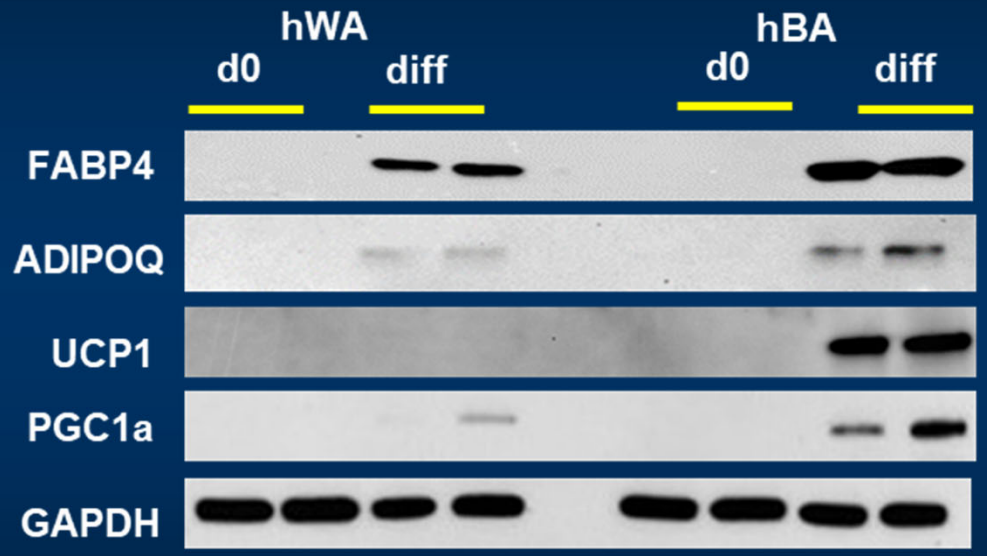
Immortalized Clonal hWA and hBA Showed Expected Lipid Accumulation and Protein Expression





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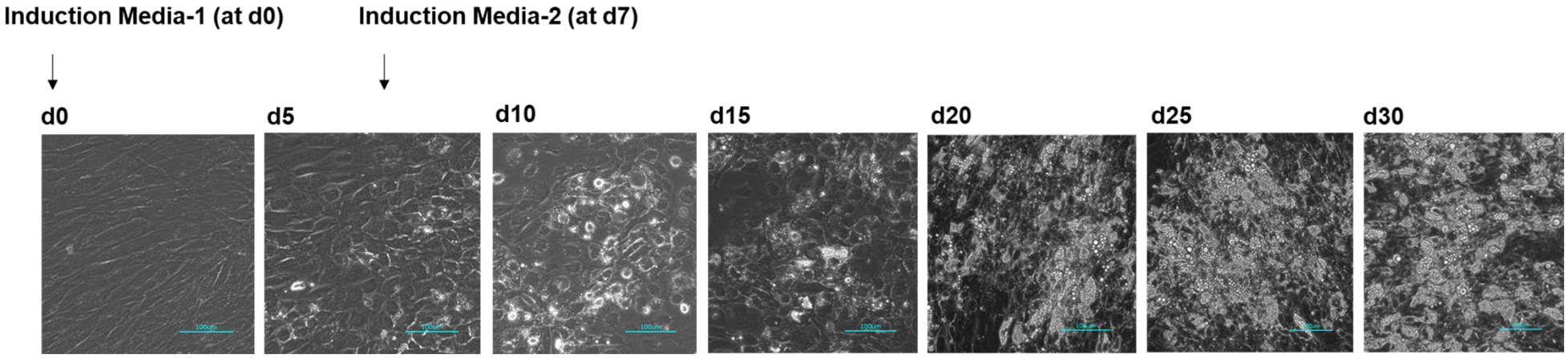
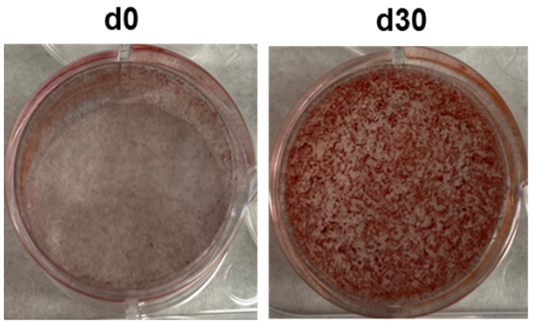
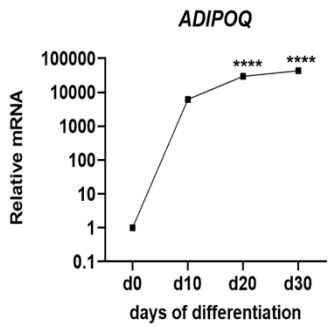
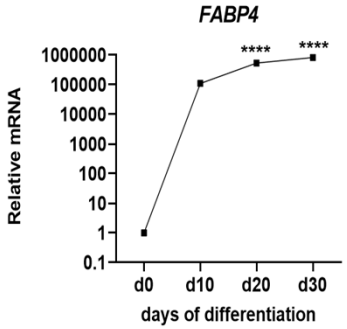
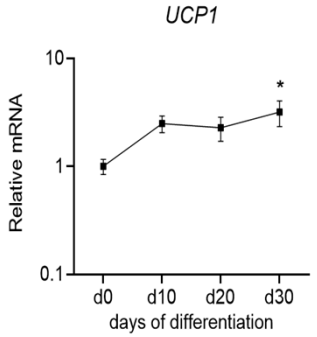
Differentiation and molecular characteristics of CRL-4063-MCB (hWA) at passage 7



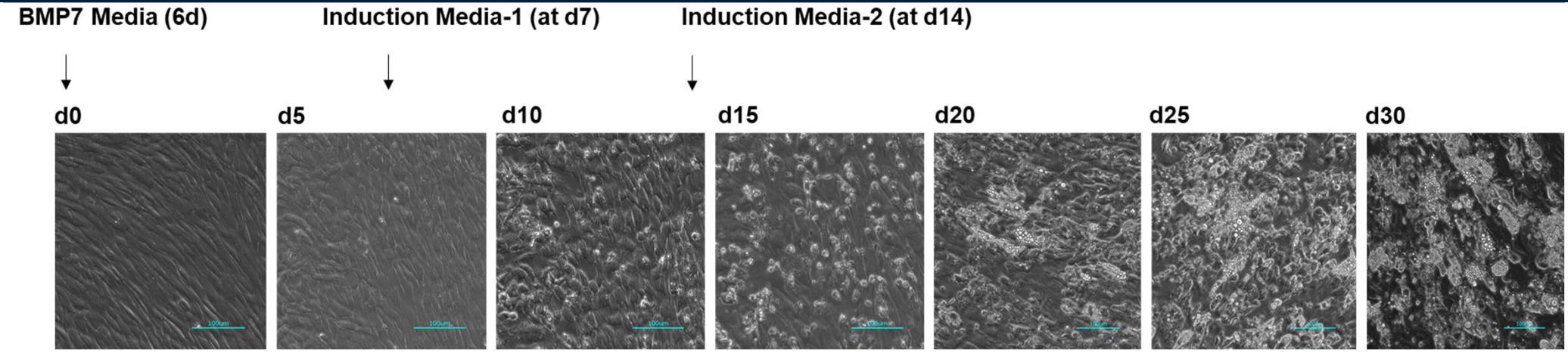


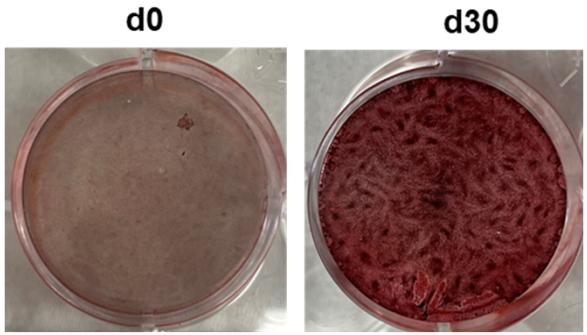


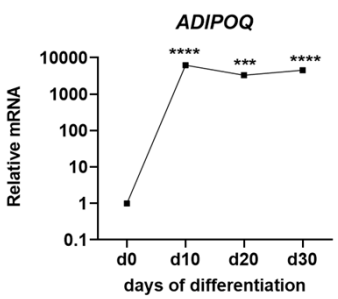


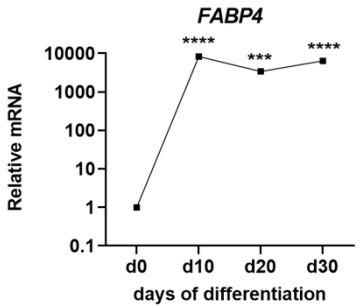


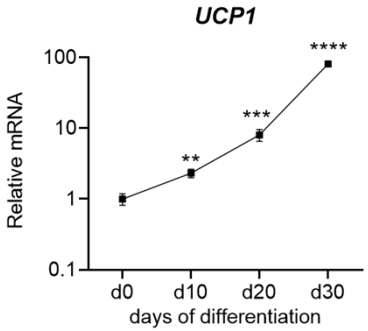
Differentiation and molecular characteristics of CRL-4062-MCB (hBA) at passage 7











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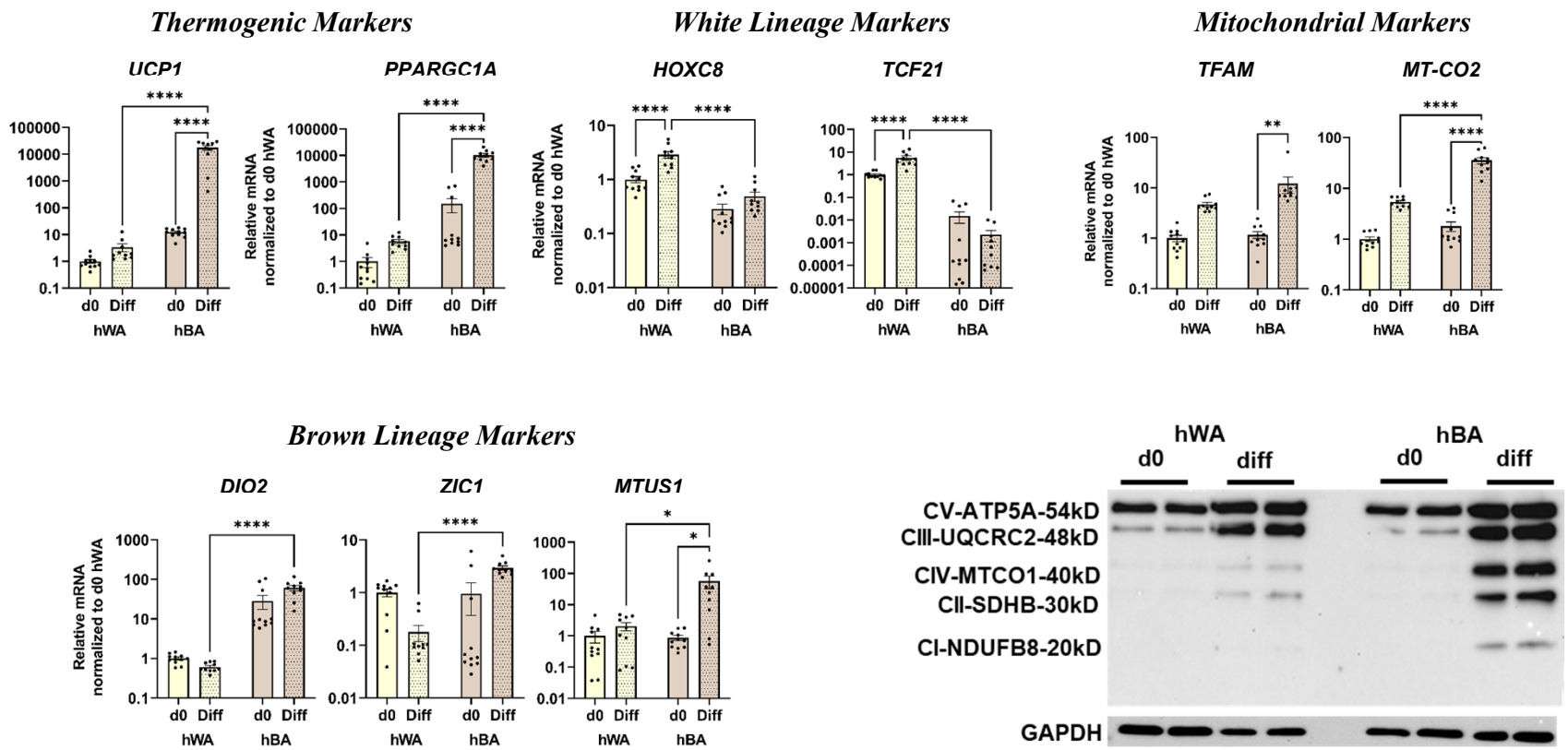
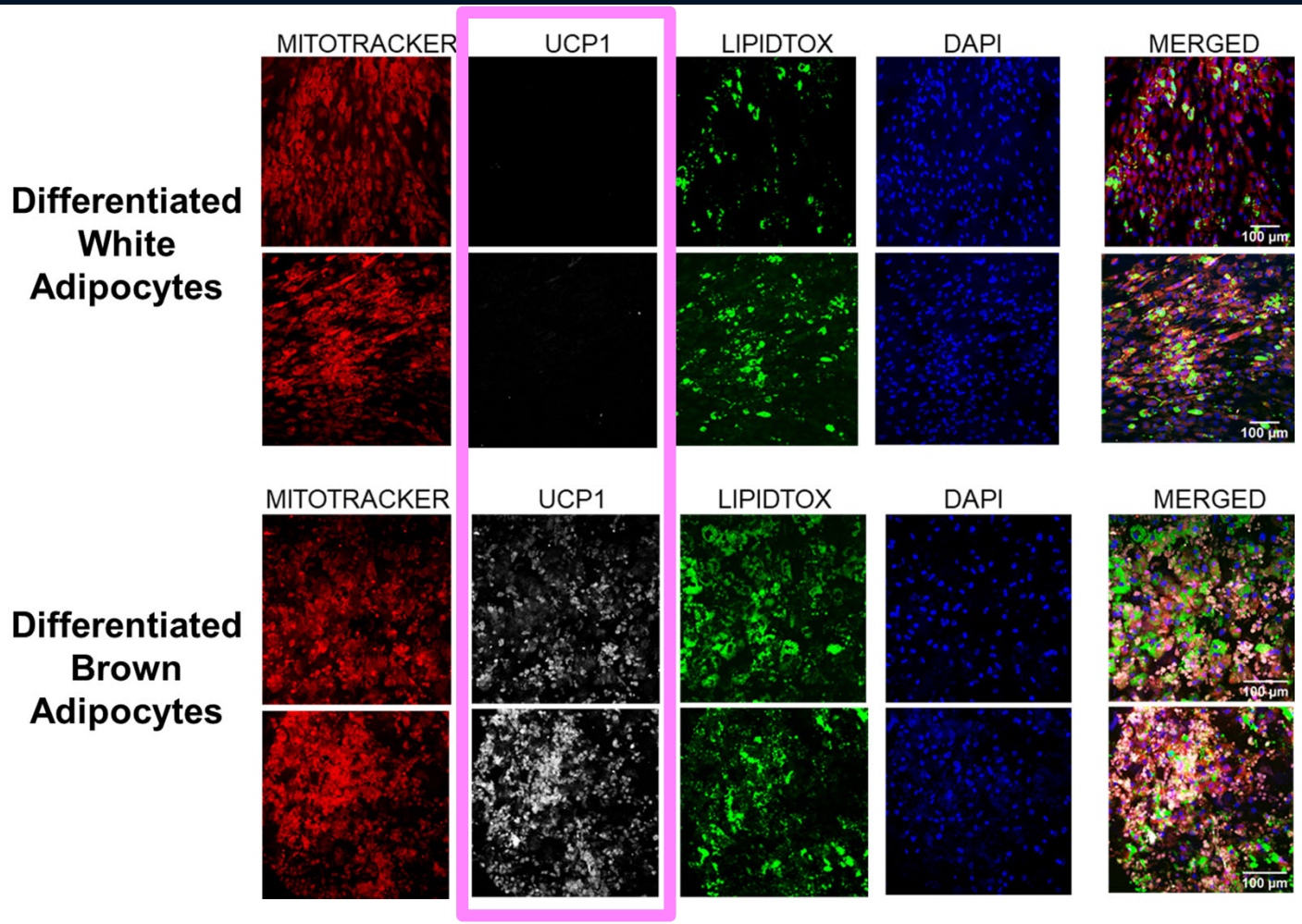
hWA/hBA Expressed Expected White and Brown Adipocyte Functional and Lineage Markers



Cero C…Cypess AM Endocrinology 2023 Nov 2;164(12):bqad161; Waldén TB…Nedergaard J. AJPEM 2012;302:E19-31

Mitochondria of hBA-CRL-4062™ express UCP1



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Interim Summary 1

- We have generated immortalized, clonal white and brown human preadipocytes from abdominal subcutaneous and perirenal depots, respectively.
- The differentiated cells expressed the genes and proteins standardly associated with each cell type.
- In doing so, we have established renewable and scalable model systems that have the molecular and structural machinery to investigate their cellular physiology.
- In the next set of experiments, we will demonstrate the functionality of these cell types.

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How do the new cells compare to other offerings from ATCC?

- Adipose-Derived Mesenchymal Stem Cells; Normal, Human PCS-500-011™.
- These are fibroblast-like cells that are best grown in Mesenchymal Stem Cell Basal Media supplemented with Mesenchymal Stem Cell Growth Kit Low serum components with 2% FBS.
- Multipotent, capable of differentiating down the adipogenic, osteogenic, and chondrogenic lineages.
- The cells are cryopreserved at the second passage to ensure the highest viability and plating efficiency.

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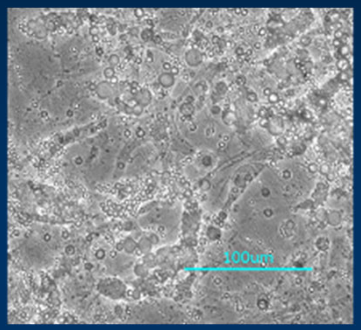
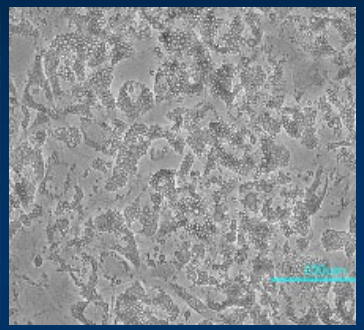
Three Different Differentiation Media Cause White Adipogenesis

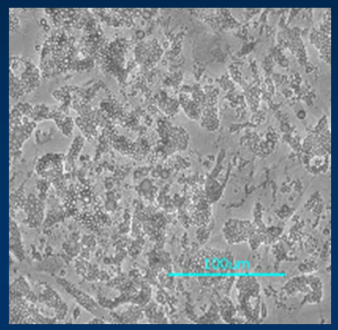
PCS-500-011 Standard

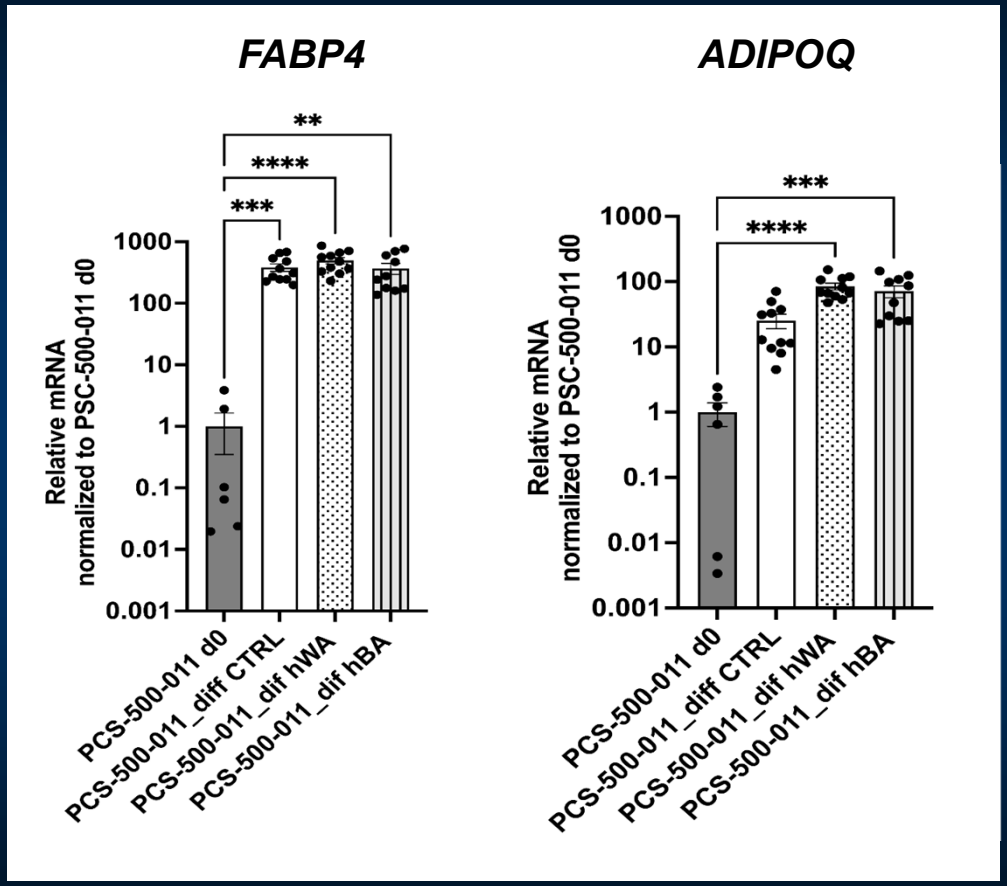


PCS-500-011 hWA

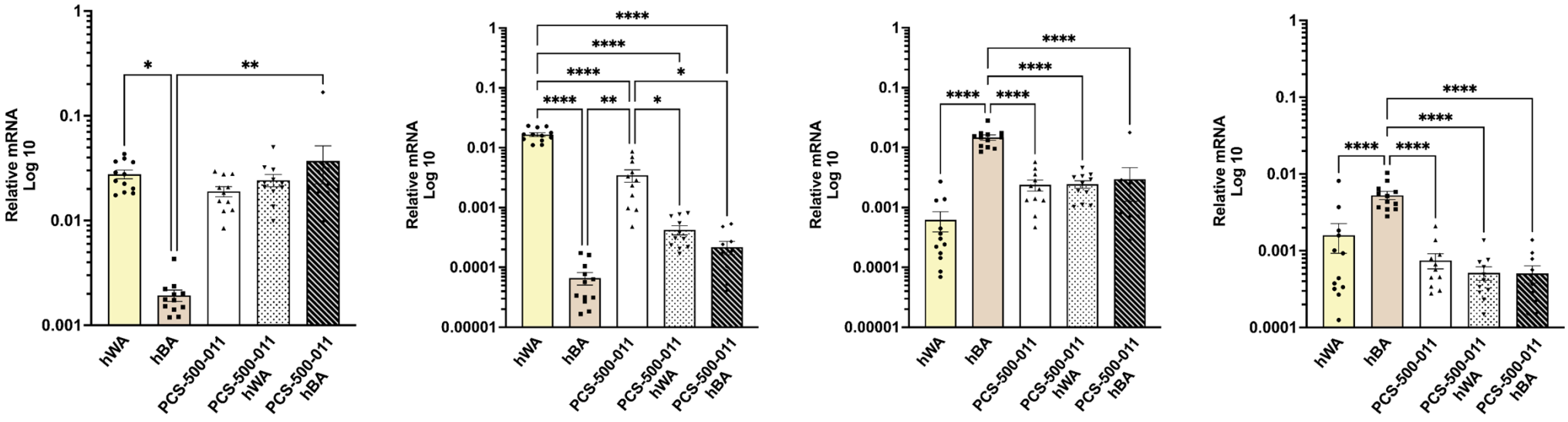


PCS-500-011 hBA





The Mesenchymal Stem Cells *Cannot* be Induced to Become Brown



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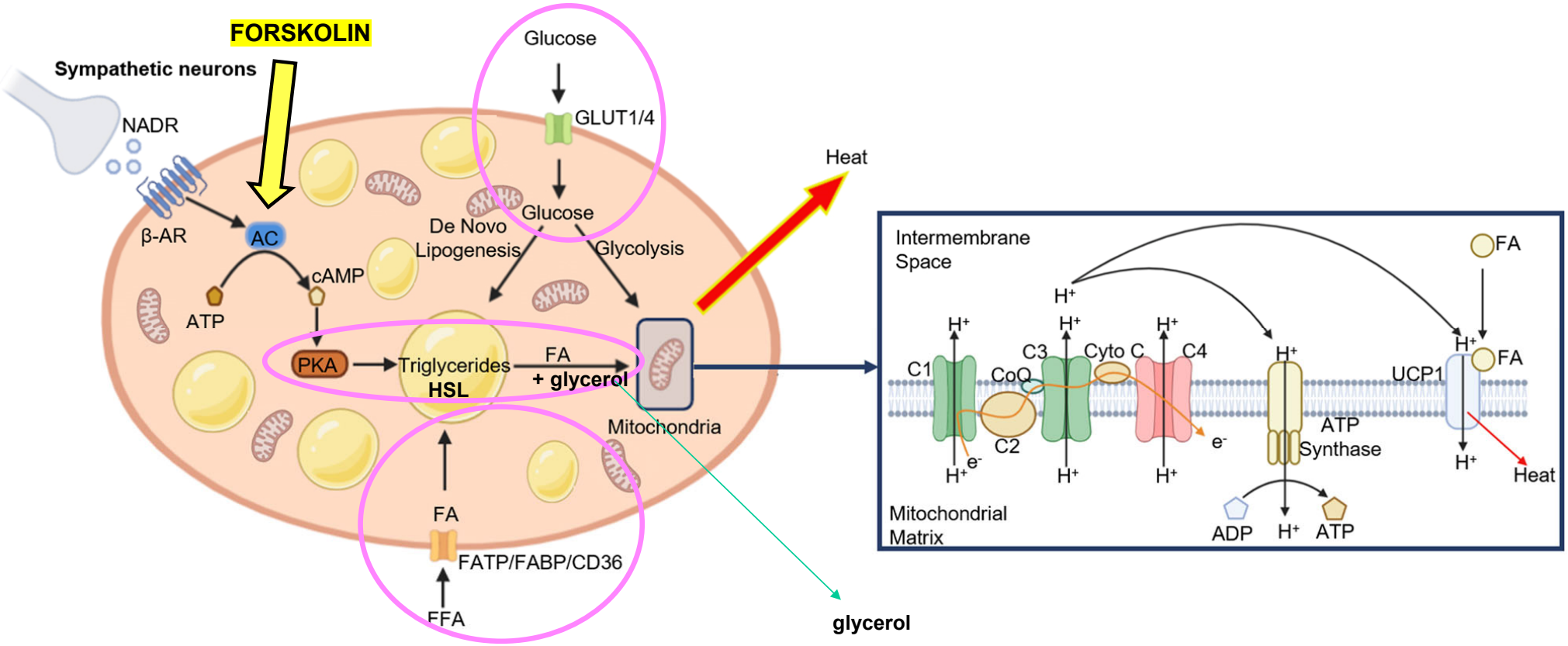
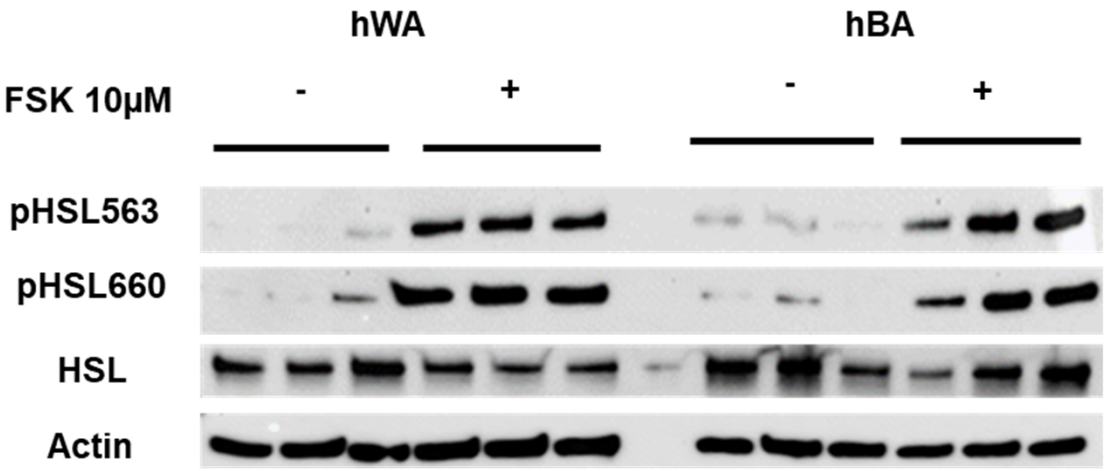
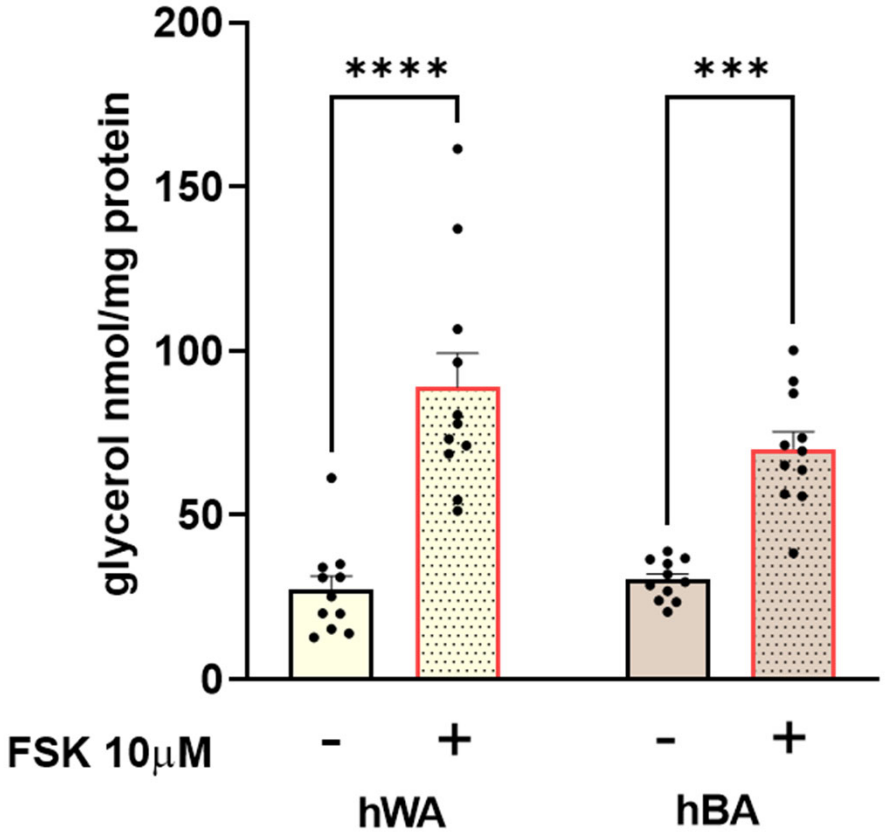
Activation of Brown Adipocytes Starts with Adrenergic Activation, Causing Lipolysis and then Thermogenesis



Modified from McNeill et al., 2021. MECHANISMS IN ENDOCRINOLOGY: Human brown adipose tissue as a therapeutic target: warming up or cooling down?

Upon Differentiation, Both hWA and hBA Synthesize the Proteins Necessary for Lipolysis and Are Functional





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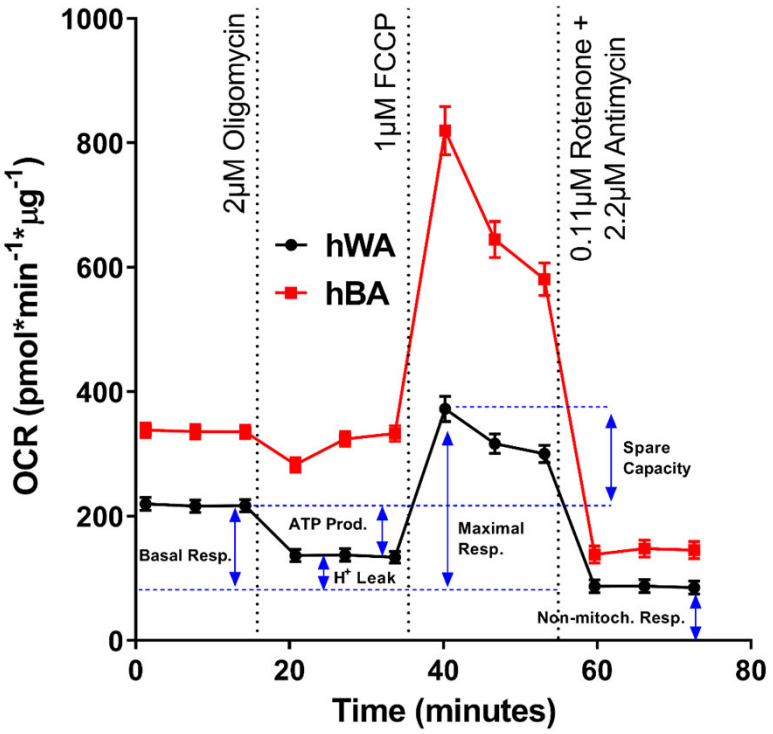
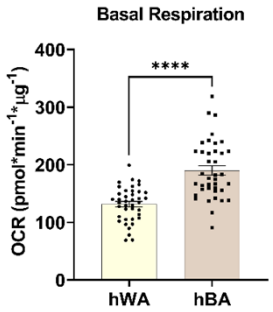
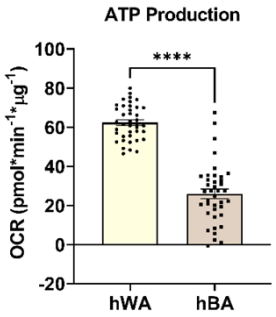
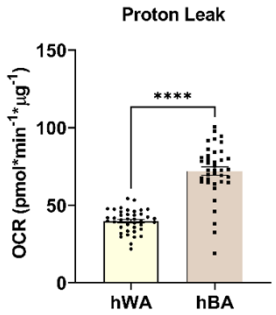
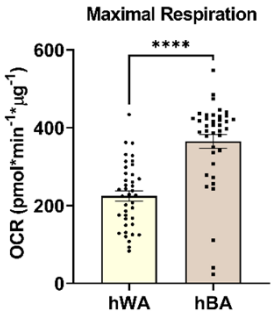
hBA Have a Higher Content of Thermogenic Mitochondria and Higher Basal and Maximal Metabolic Rate

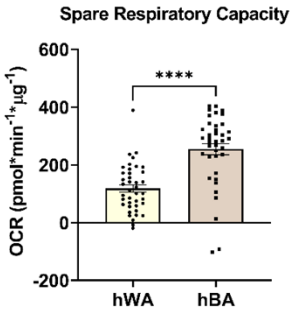


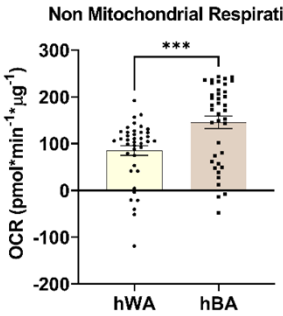












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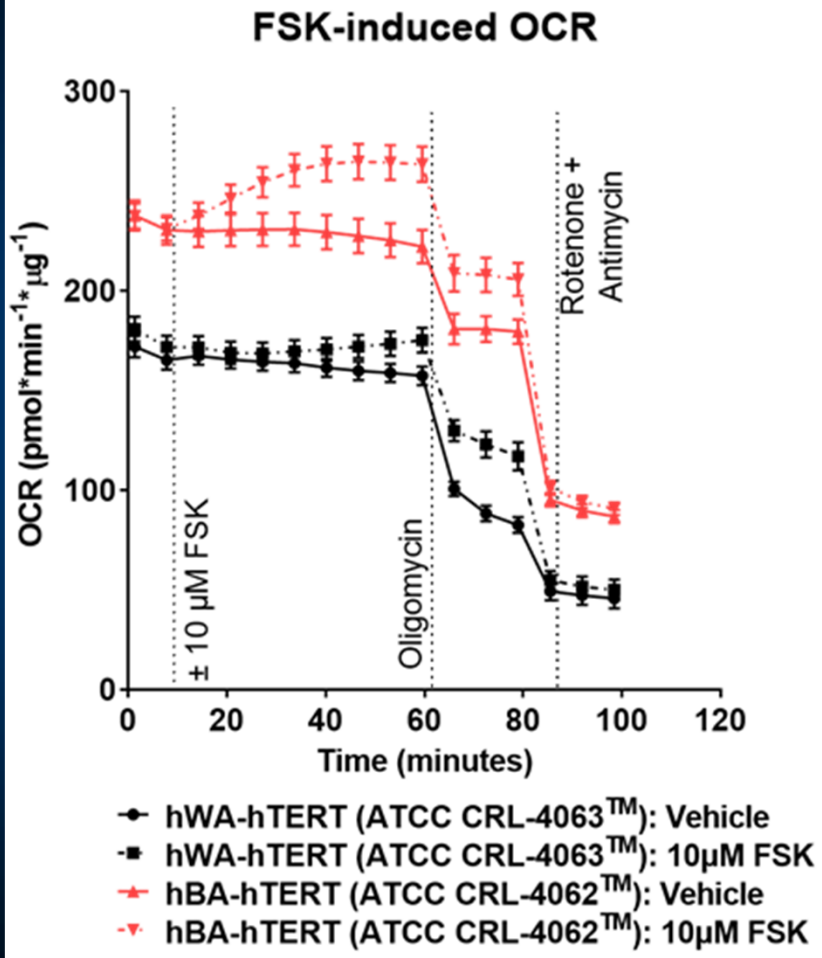
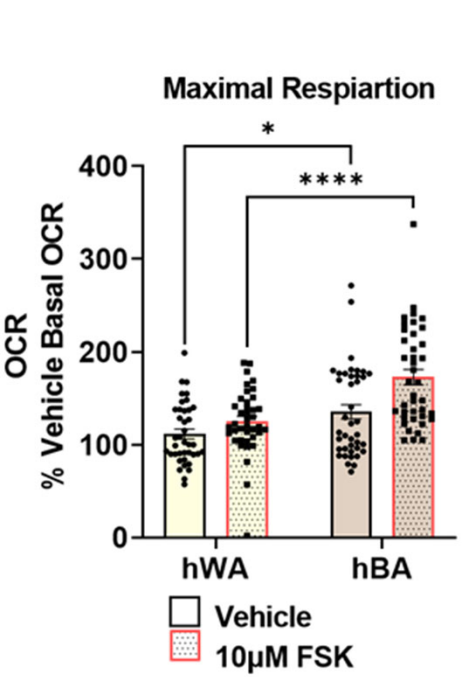
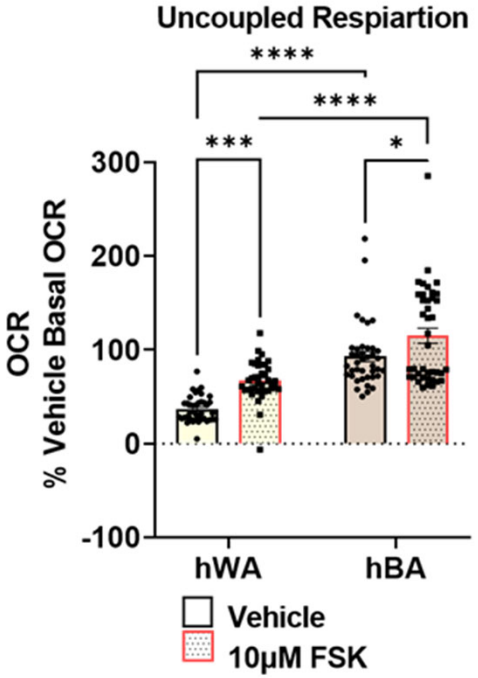
Forskolin Increases Cellular Respiration in Both hBA and hWA







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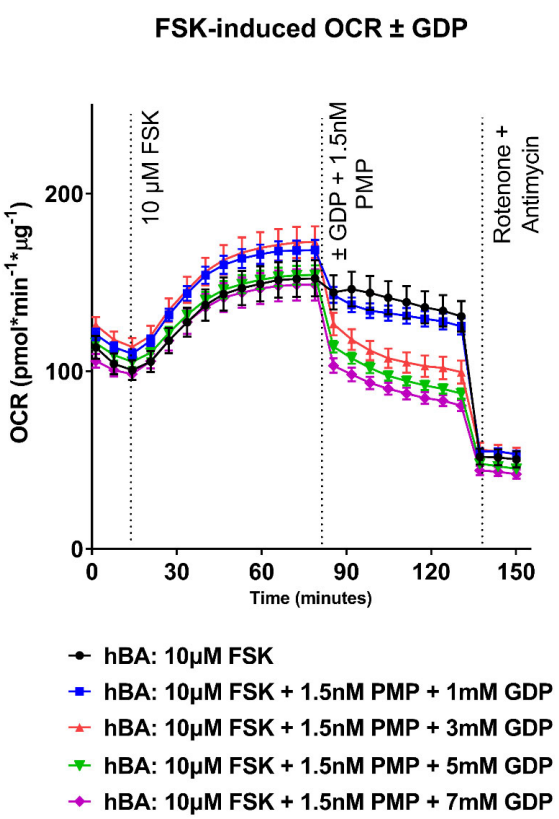
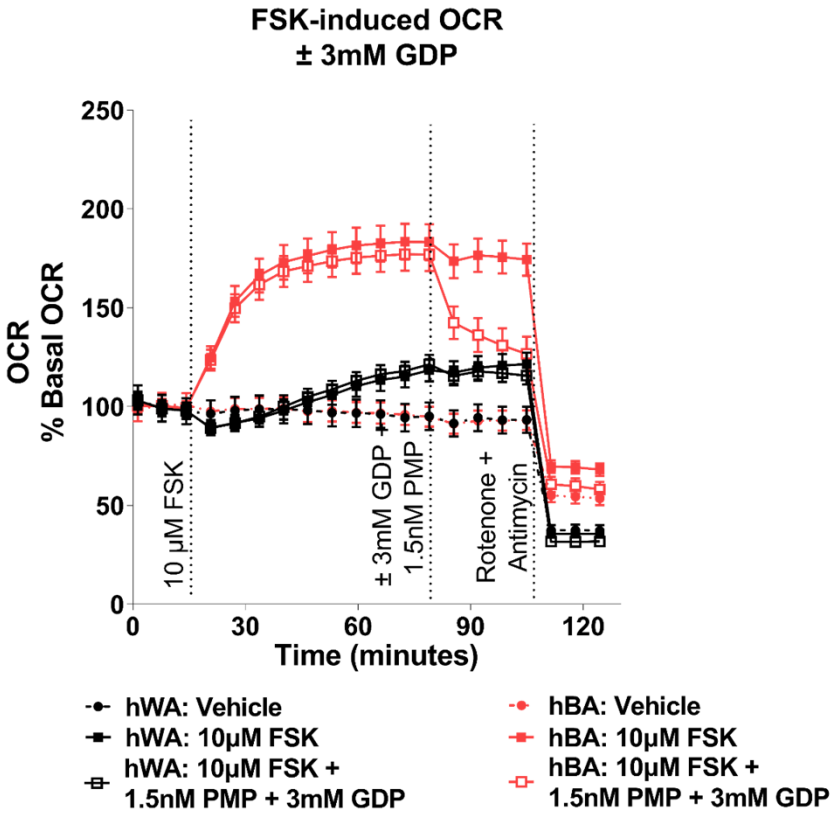
For Thermogenesis, hBA Used Both UCP1 *and* non-UCP1-Mediated Processes





- Forskolin increased hBA OCR
- GDP blocks UCP1-mediated futile cycling thermogenesis.
- There was a dose-response effect of GDP on reducing OCR, maximum at 35-45%
- GDP [3 mM] lowered OCR in hBA by 35% but had no effect on hWA

❖ The majority of adrenergic thermogenesis in hBA is NOT from UCP1

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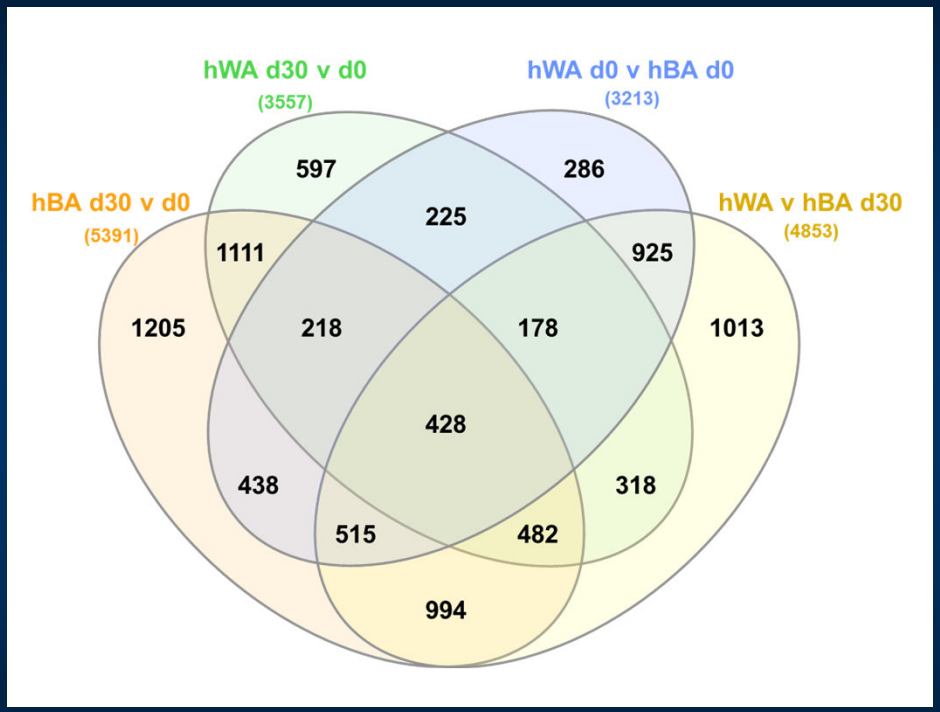
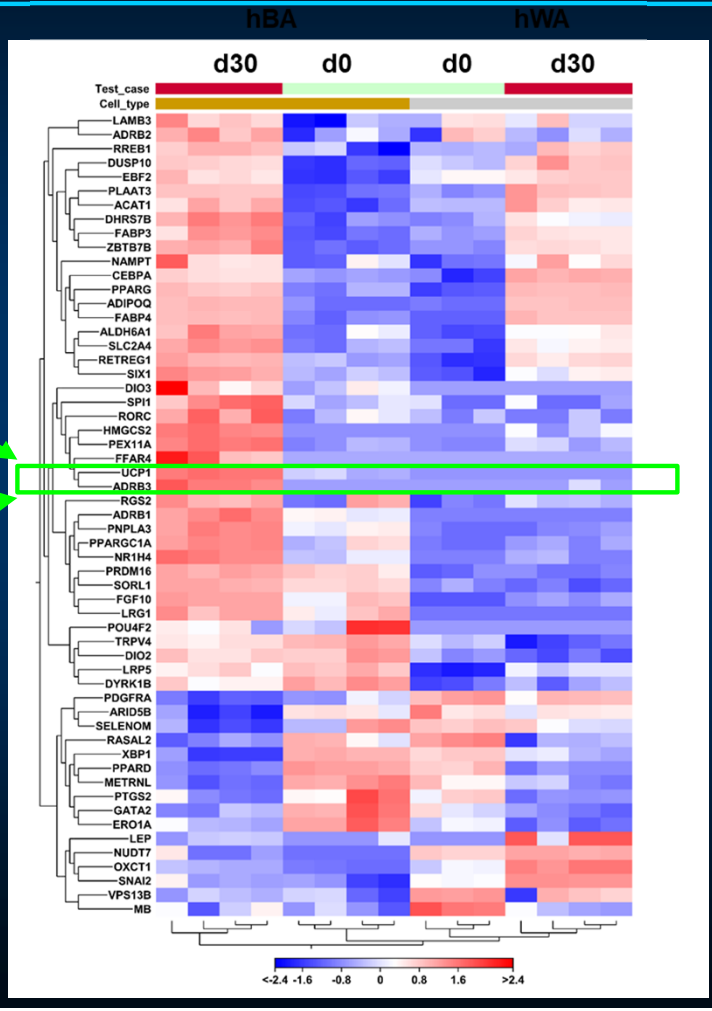
RNA-seq Transcriptomics Gives Genetic Signatures of the Different Adipocyte States





UCP1

β3-AR

Cero C…Cypess AM Endocrinology 2023 Nov 2;164(12):bqad161

Interim Summary 2

- hBA have an overall increased cellular respiration (higher UCP1 expression and mitochondrial content).
- Both cell types have a functional lipolytic machinery to undergo cAMP/PKA activated lipolysis (similar phosporylation of HSL and glycerol release).
- RNA sequencing data are consistent with the PCR data and can be utilized to corroborate and likely identify new physiological relationships.
- The data highlight new genes and pathways that are different in these two cell lines, opening many possibilities of new research.

Final Summary

- Adipose tissue is polychromatic. It can be white and brown (and more), and each tissue depot contains multiple cell types. Many of the endocrine roles and their mediators remain to be determined.
- To help study the mechanism underlying the different functions, we generated and characterized a pair of immortalized, clonal human brown (hBA) and white (hWA) preadipocytes.
- The differentiated hWA and hBA phenocopied primary adipocytes in terms of adrenergic signaling, lipolysis, and thermogenesis.
- Transcriptomics via RNA-seq were consistent with the functional studies and established a molecular signature for each cell type.
- These standardized cells are anticipated to become a common resource for future physiological, pharmacological, and genetic studies of human adipocytes.

Thank You

NIH

National Institute of Diabetes and Digestive and Kidney Diseases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Nhuquynh Nguyen

Paige Studlack
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Annie Pierce
Alex Jiang
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James Johnson
Alison Baskin

Cheryl Cero
Joyce Linderman
Zahraa Abdul-Sater

DEOB
Marc Reitman
Kong Chen
Sushil Rane
Rebecca Brown

Mass Spectrometry Core
Peter Walter
Hongyi Cai

Laboratory Core
Mary Walter

UOB
Marston Linehan

TOSB
Jeremy Davis

NICHD
Karel Pacak

MDB
William Simonds
Lee Weinstein

EOB
Naris Nilubol

BMIB
Ahmed Gharib
Ron Ouwerkerk

CRC PET Department
Peter Herscovitch
Corina Millo
William Dieckmann
Craig Barker

CRC Radiology and Imaging Sciences
Brad Wood
Elliot Levy
Sheng Xu

ATCC

Fang Tian
Weiguo Shu
Diana Douglas
Michael Maddox

Jonathan Jacobs
Amy Reese
Ajeet Singh

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For more information: www.atcc.org/hTERT

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