

# Modeling Barth Syndrome using Patient-Specific, iPSC-derived Cardiomyocytes

William T. Pu

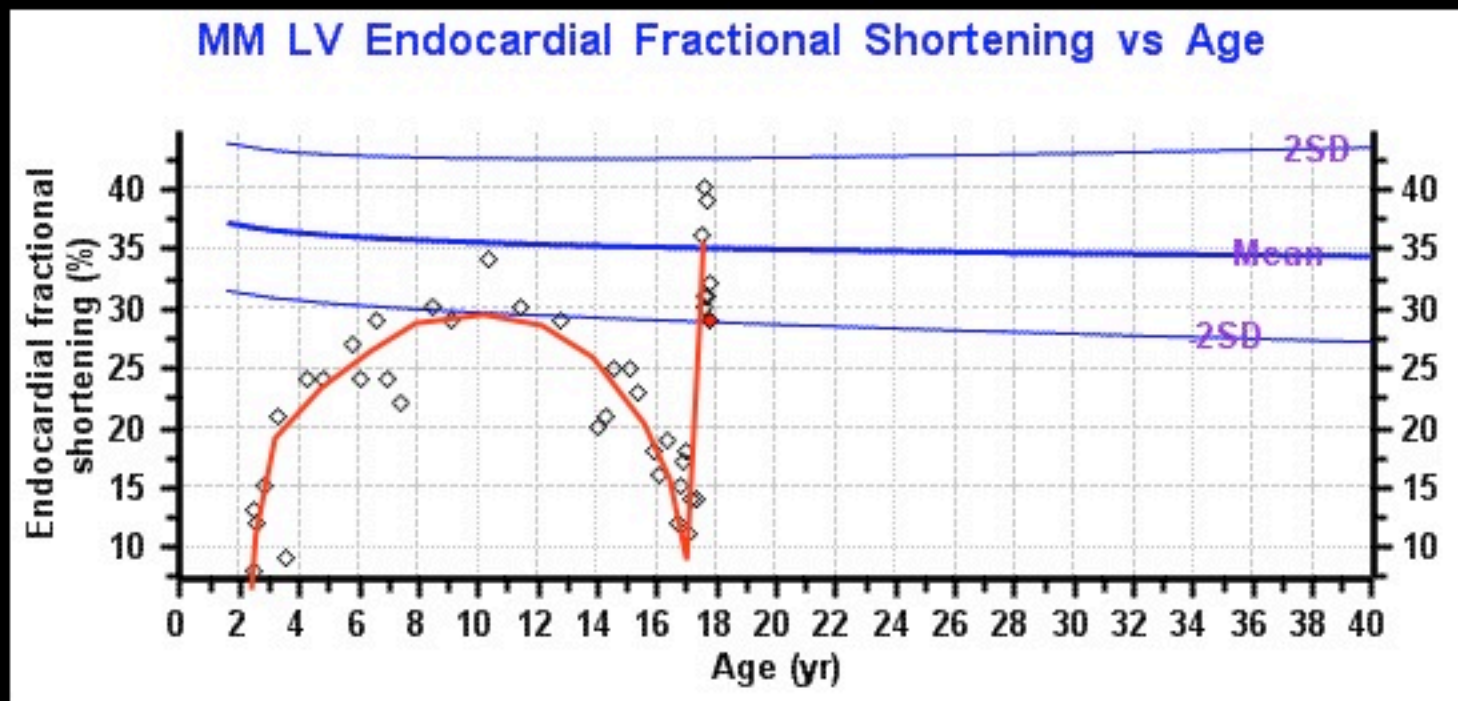
Department of Cardiology, Children's Hospital Boston  
Harvard Stem Cell Institute

June 2012

BSF Foundation Meeting



# Barth Syndrome -- a reversible cardiomyopathy?



- known single gene defect involving a metabolic pathway
- waxing and waning disease course rather than irreversibly progressive disease
- progress slowed until recently by lack of mammalian model systems

# Induced Pluripotent Stem Cells

## Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

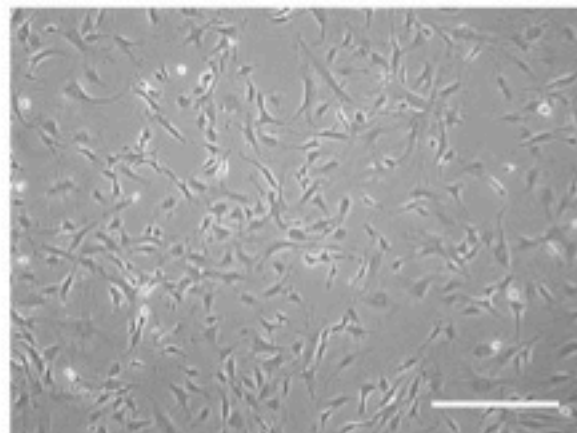
Kazutoshi Takahashi<sup>1</sup> and Shinya Yamanaka<sup>1,2,\*</sup>

<sup>1</sup>Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

<sup>2</sup>CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

\*Contact: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2006.07.024



## Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,<sup>1</sup> Koji Tanabe,<sup>1</sup> Mari Ohnuki,<sup>1</sup> Megumi Narita,<sup>1,2</sup> Tomoko Ichisaka,<sup>1,2</sup> Kiichiro Tomoda,<sup>3</sup> and Shinya Yamanaka<sup>1,2,3,4,\*</sup>

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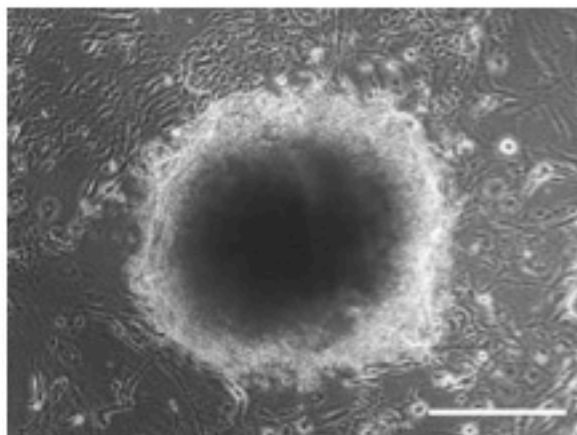
<sup>3</sup>Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA

<sup>4</sup>Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan

\*Correspondence: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2007.11.019

↓  
Reprogramming  
Factors





Normal iPSC: human embryogenesis

iPSC



Renewable, patient-specific disease model,  
e.g. LQTS, HCM, 22q11 del

- phenotype-genotype
- drug screening
- mutation discovery?



Patient  
fibroblast



Replacement  
Therapy

# Why an iPS model of BTHS?

- Human loss of function model, useful for studies of disease mechanism and interindividual variation.
- Renewable, patient-specific model of TAZ mutation for preclinical testing of BTHS treatment strategies.
- Potential for high throughput drug screening to discover novel approaches to treatment.

# iPSC Model of Barth Syndrome

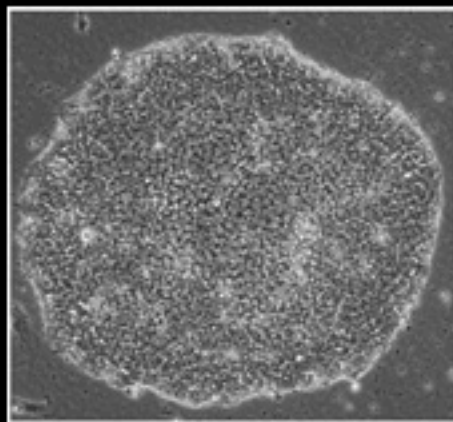
1. Generation and characterization of 2 iPSC lines, one from each of 2 patients with BTHS.
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4. Analysis of mitochondrial abnormalities in BTHS iCMs.
5. Treatments to reverse BTHS iCM mitochondrial abnormalities

# iPSC Models of Barth Syndrome

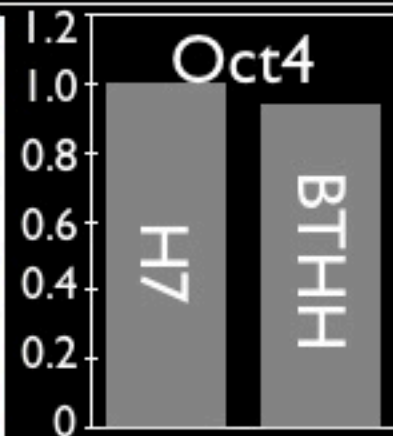
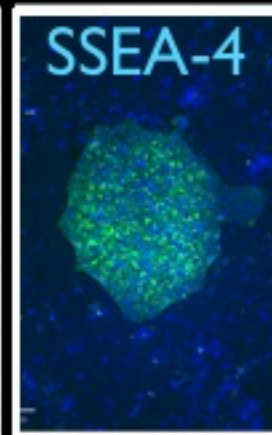
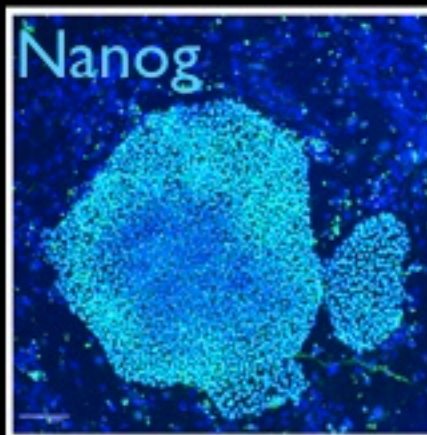
	BTH-H	BTH-C
Mutation	<p>ATGGGGACTG      ATGGGACTG</p>	<p>ACACTCCCAC      ACACCCCAC</p>
Protein	Asp173Thr.fsX11	Ser110Pro
Reprog.	OSKM retroviral	OSKML modRNA

# BTHH iPSC Quality Control

ESC-like morphology in feeder free culture



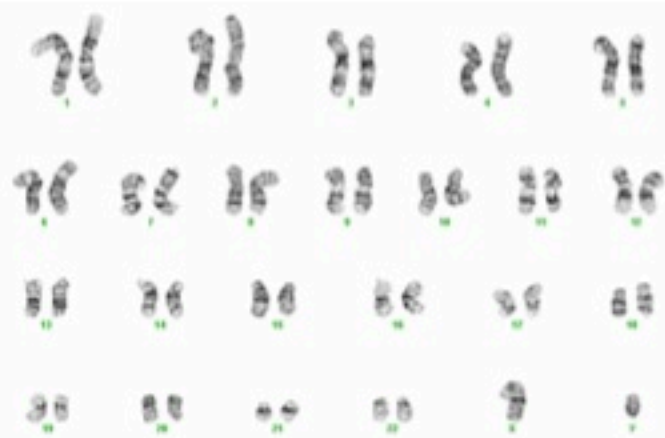
Expression of pluripotency markers





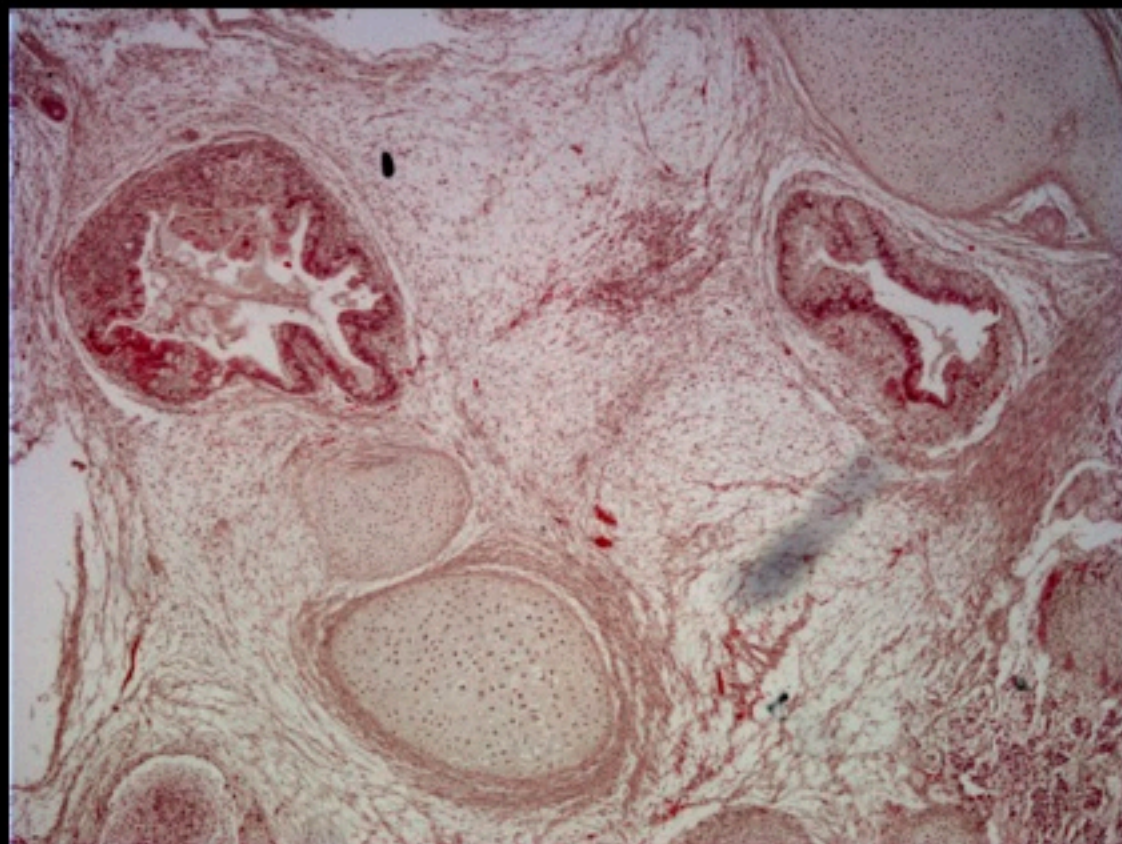
# BTHH iPSC Quality Control

Karyotype



46 XY

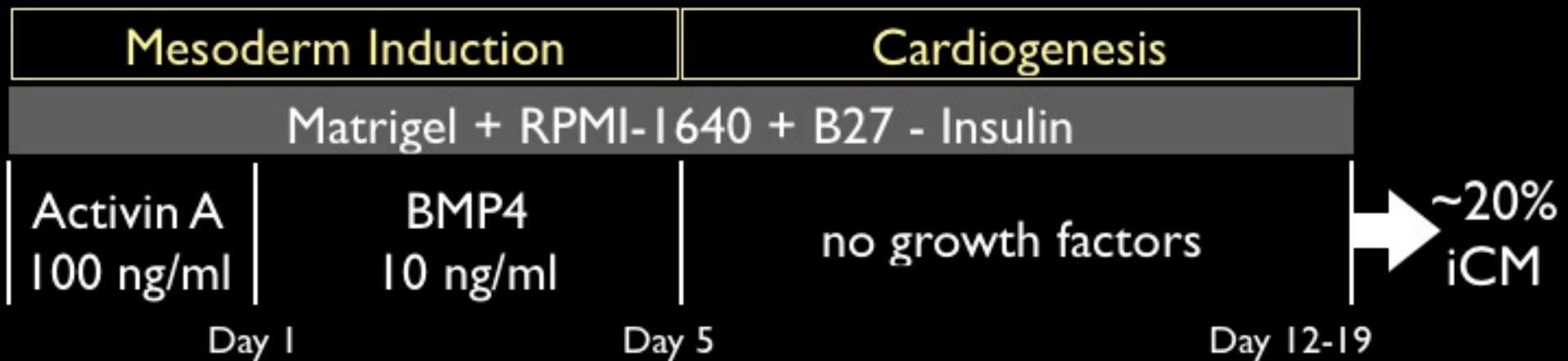
Teratoma Assay



# iPSC Model of Barth Syndrome

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# MACS CM Purification



# MACS CM Purification

Mesoderm Induction

Cardiogenesis

Matrigel + RPMI-1640 + B27 - Insulin

Activin A  
100 ng/ml

BMP4  
10 ng/ml

no growth factors

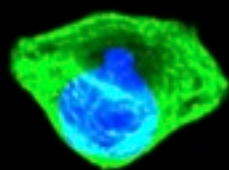
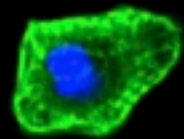
~20%  
iCM

Day 1

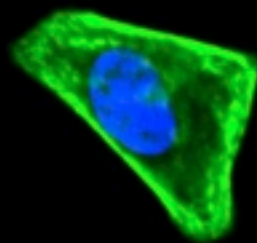
Day 5

Day 12-19

DAPI TNNI3



Control



BT HH



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Mesoderm Induction

Cardiogenesis

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Activin A  
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10 ng/ml

no growth factors

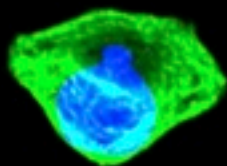
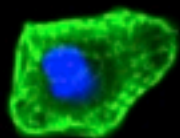
~20%  
iCM

Day 1

Day 5

Day 12-19

DAPI TNNI3



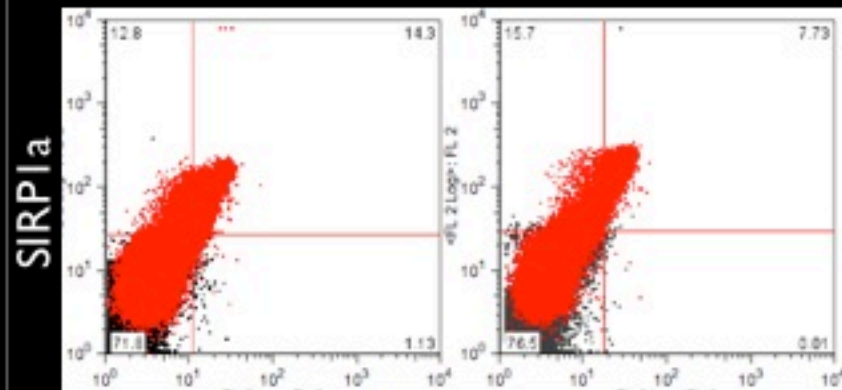
Control



BTHH

Control

BTHH



VCAMI

# iCM Beating

Ctrl

BT HH

BT HC

# iCM Beating

Ctrl

BT HH

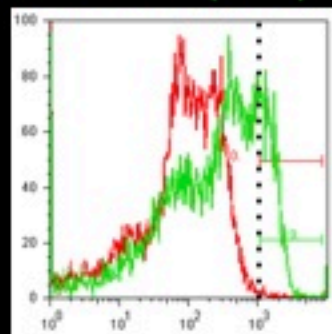
BT HC

# MACS iCM Purification

Matrigel  
Diff to iCMs

isotype (0%)

VCAMI (19%)



VCAMI



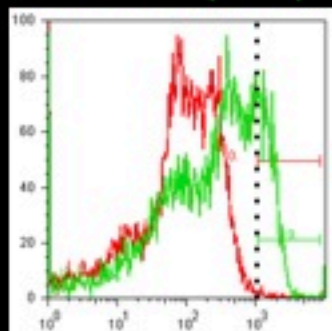
# MACS iCM Purification

Matrigel  
Diff to iCMs

Collagenase  
VCAMI MACS

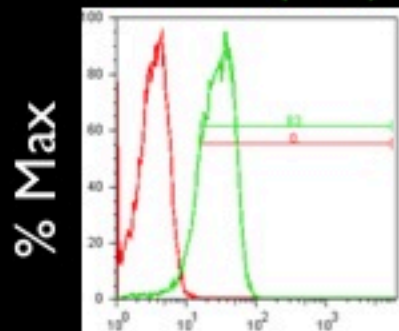
Enriched  
iCMs

isotype (0%)  
VCAMI (19%)

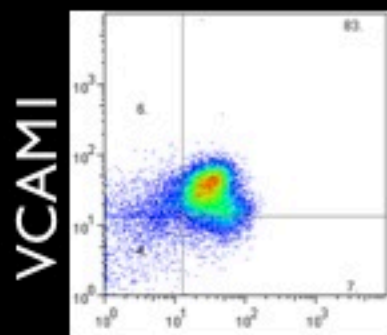


VCAMI

isotype (0%)  
VCAMI (83%)



VCAMI



TNNT2

# MACS iCM Purification

Matrigel  
Diff to iCMs

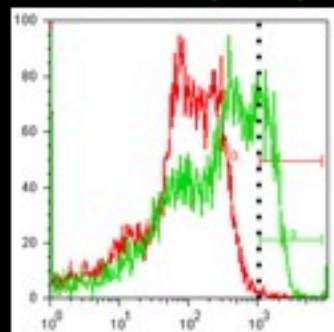
Collagenase  
VCAMI MACS

Enriched  
iCMs

Gelatin/Fibronectin  
Plating

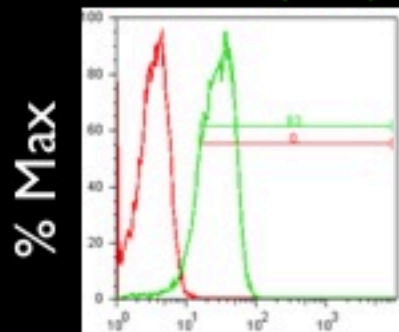
Assay

isotype (0%)  
VCAMI (19%)

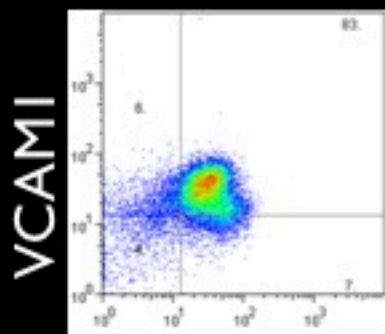


VCAMI

isotype (0%)  
VCAMI (83%)

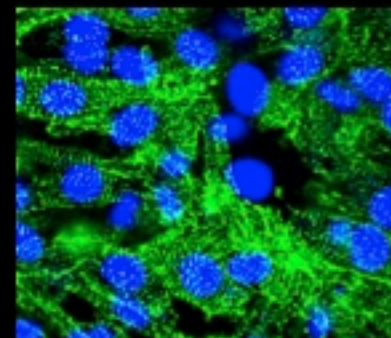


VCAMI



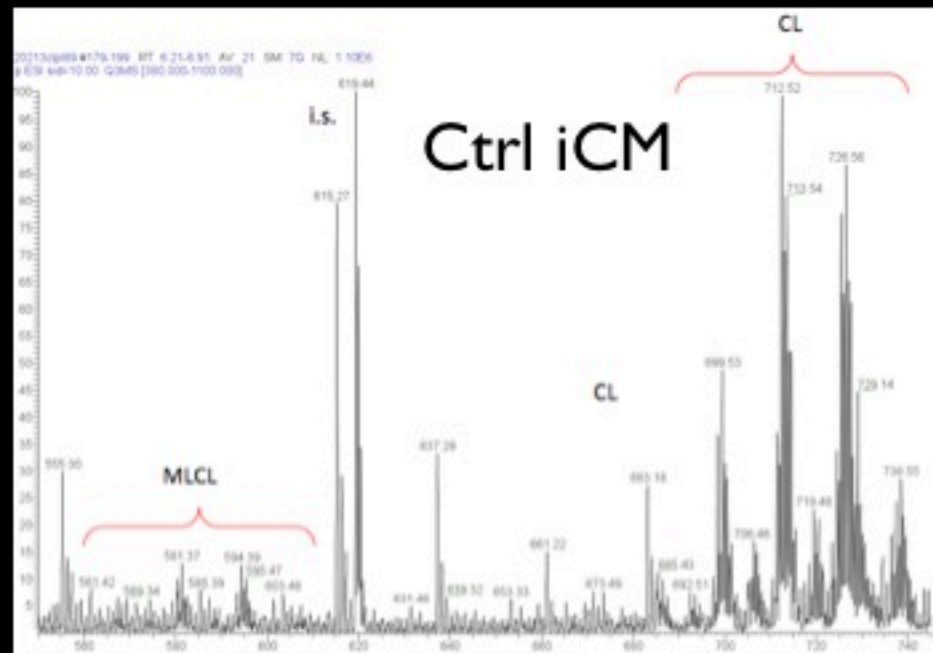
TNNT2

Actinin DAPI

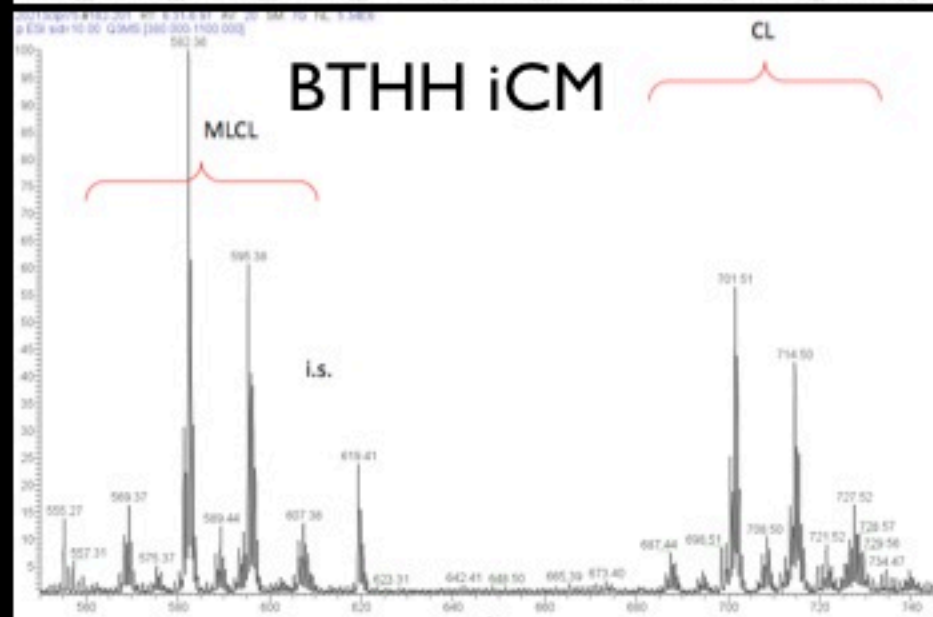


# CL deficiency in BTHS iCMs

Abundance



Abundance



Mass/charge

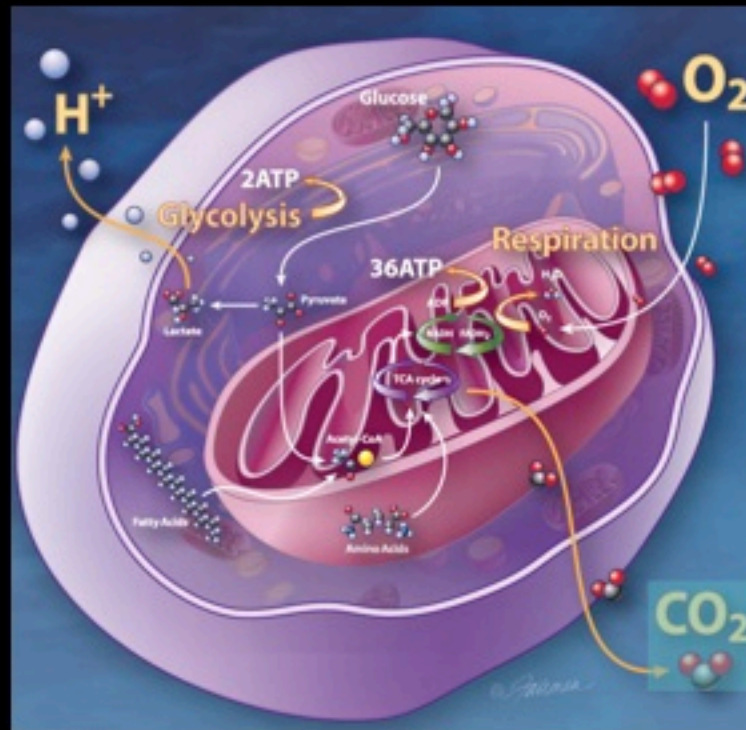
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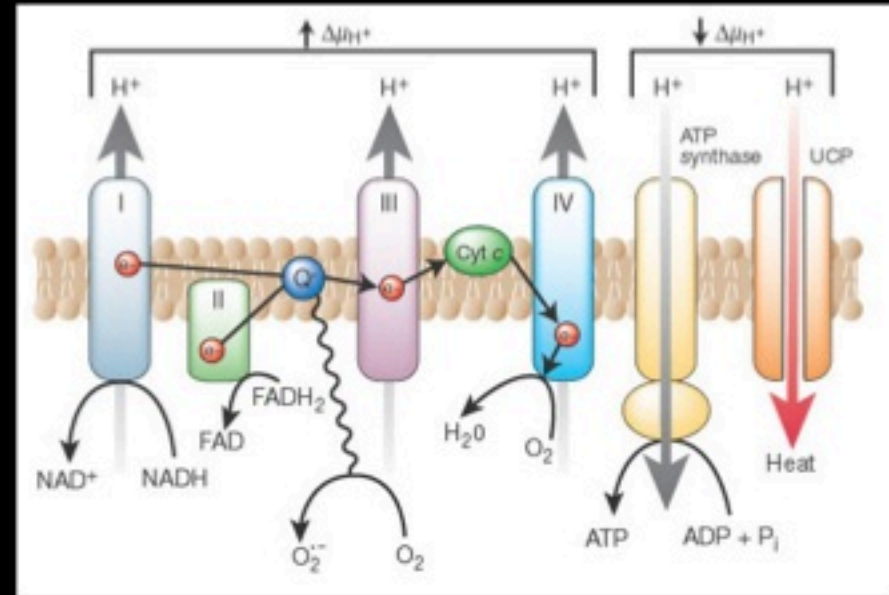
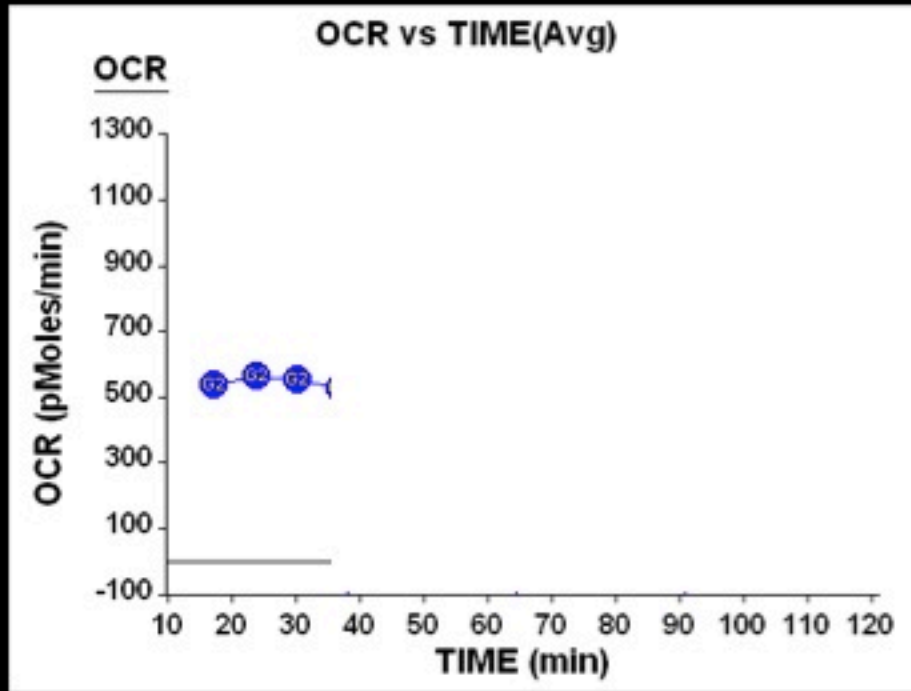
# Seahorses Biosciences extracellular flux analyzer

Extracellular  
acidification rate  
(ECAR):  
glycolysis

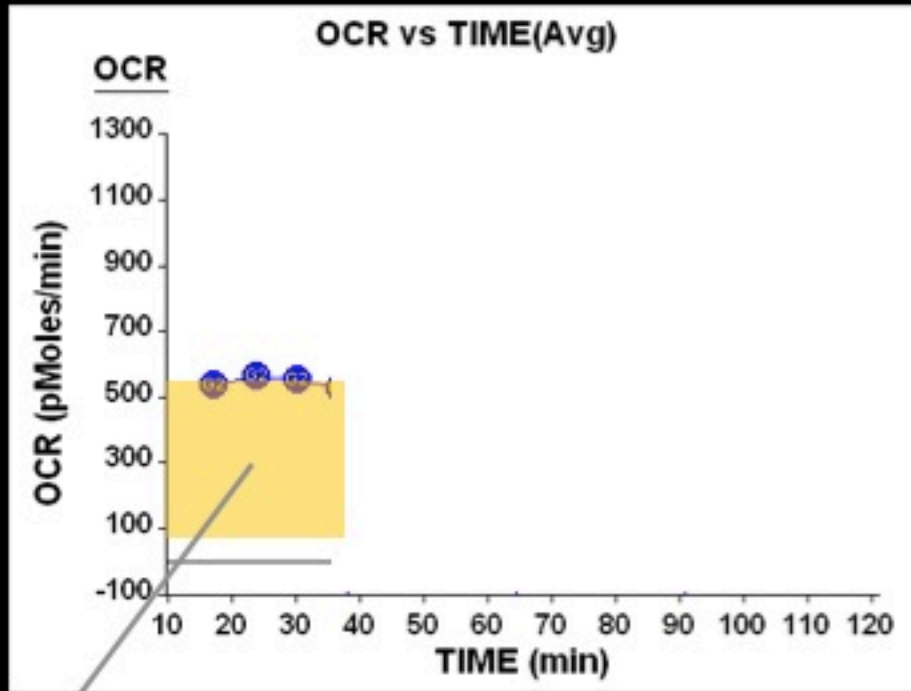


Oxygen  
consumption  
rate (OCR):  
oxidative  
respiration

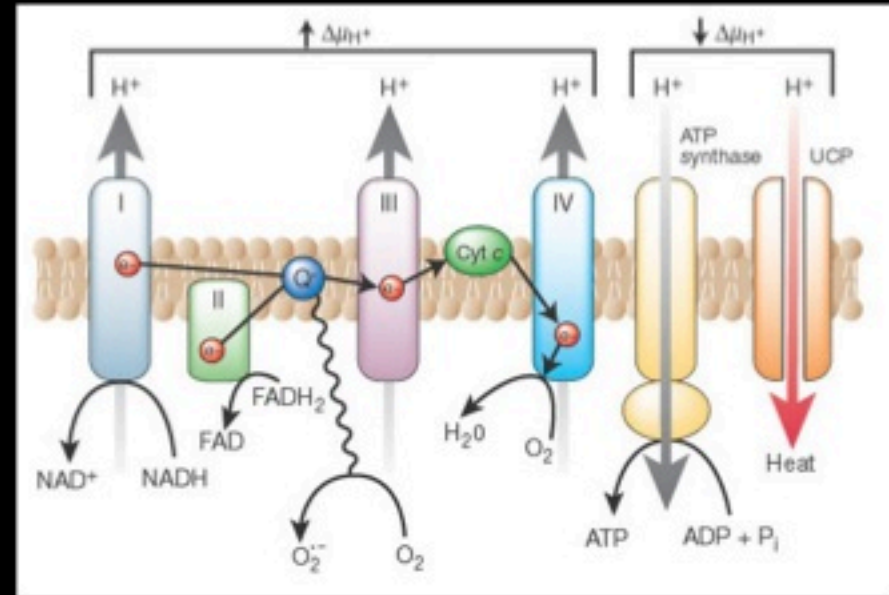
# Probing mitochondrial function



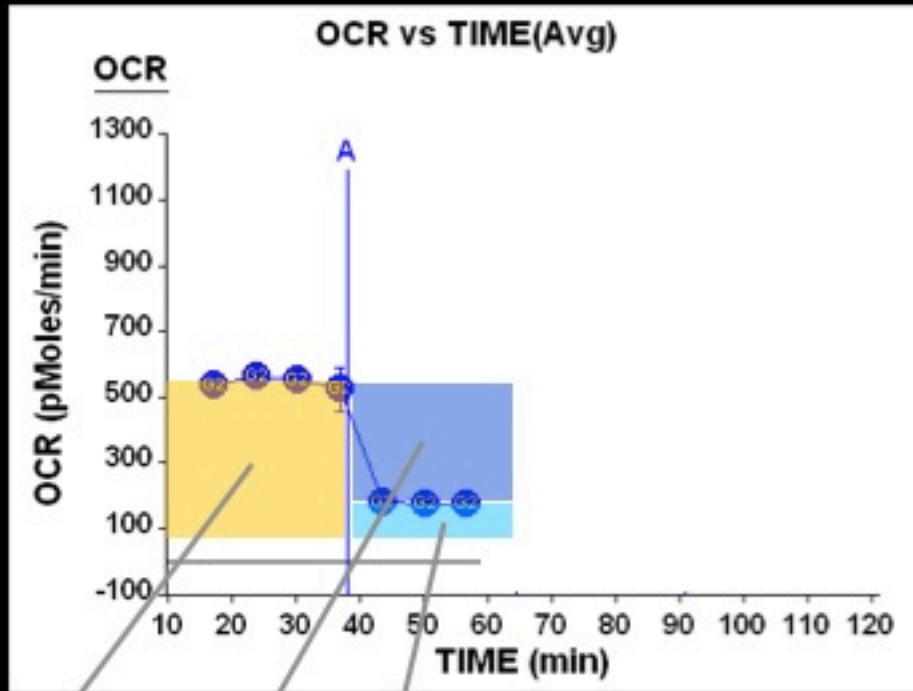
# Probing mitochondrial function



Basal  
Resp



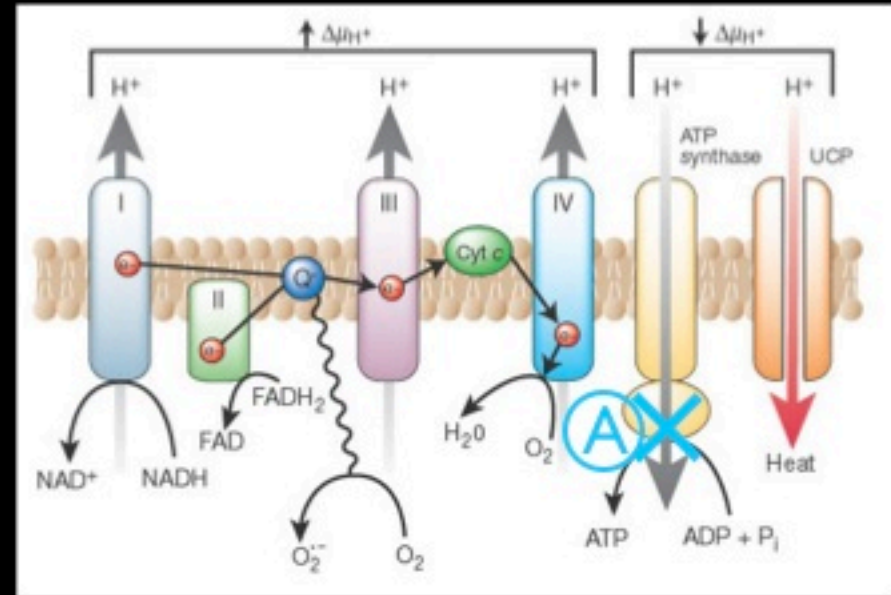
# Probing mitochondrial function



Basal  
Resp

ATP  
Gen

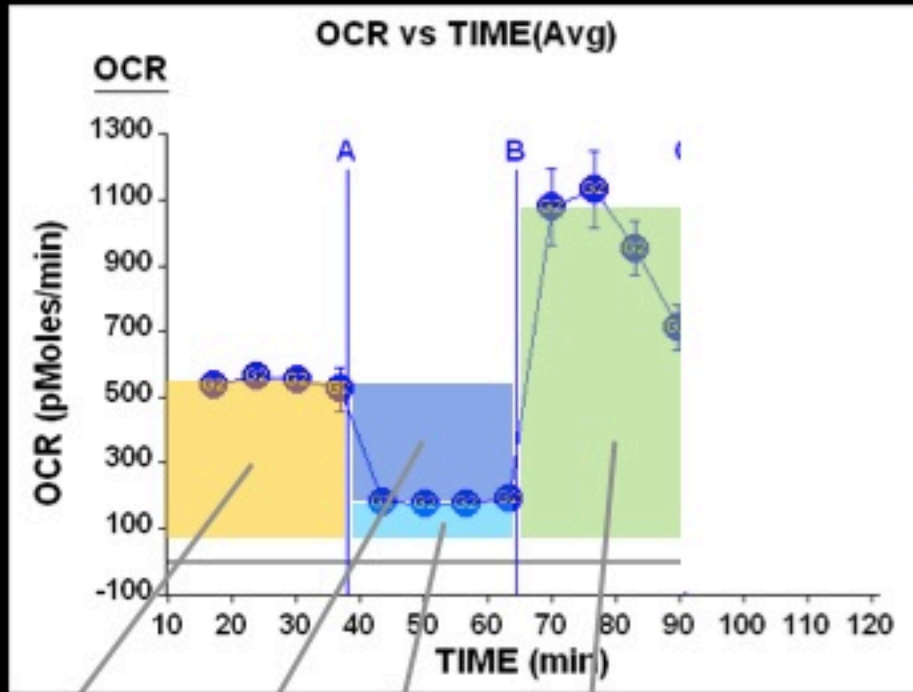
H<sup>+</sup>  
Leak



A. Oligomycin



# Probing mitochondrial function

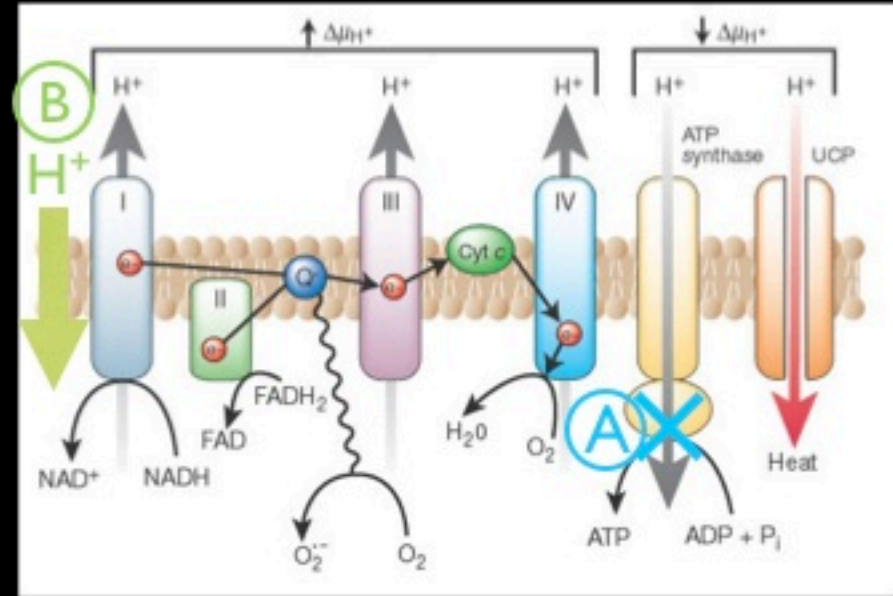


Basal  
Resp

ATP  
Gen

H<sup>+</sup>  
Leak

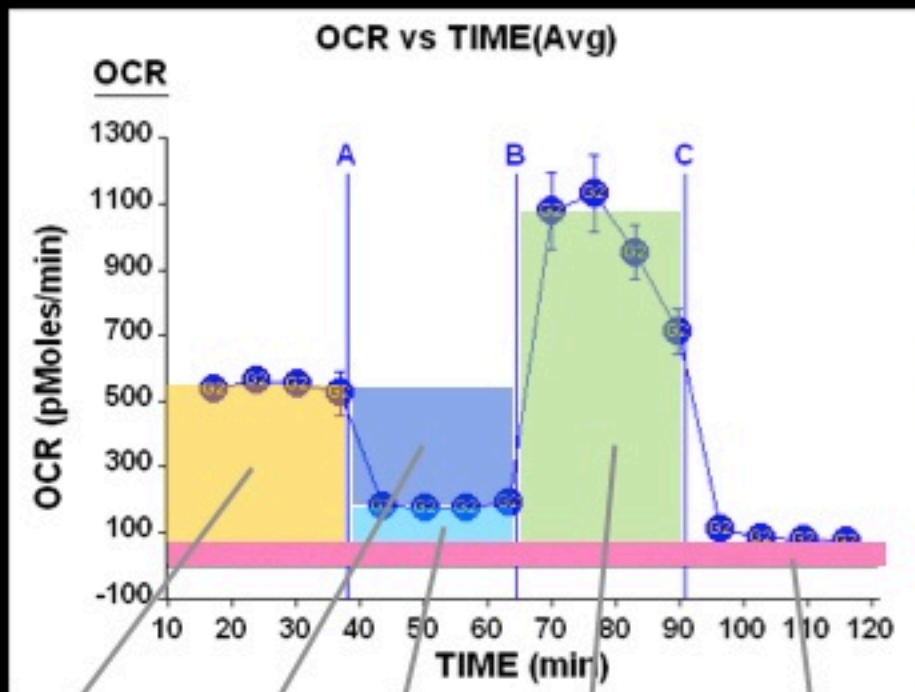
Resp  
Capacity



A. Oligomycin

B. FCCP

# Probing mitochondrial function



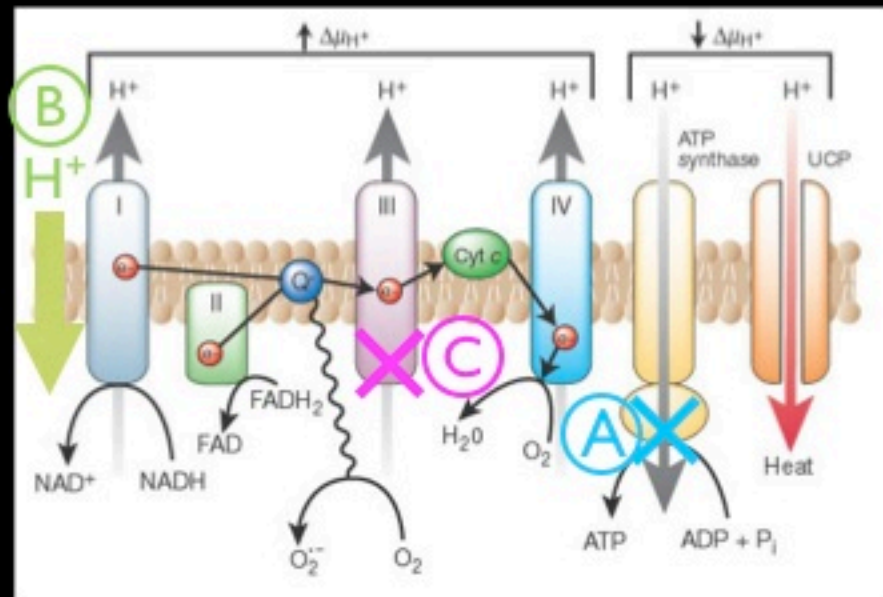
Basal  
Resp

ATP  
Gen

H<sup>+</sup>  
Leak

Resp  
Capacity

Non-  
mitochondrial

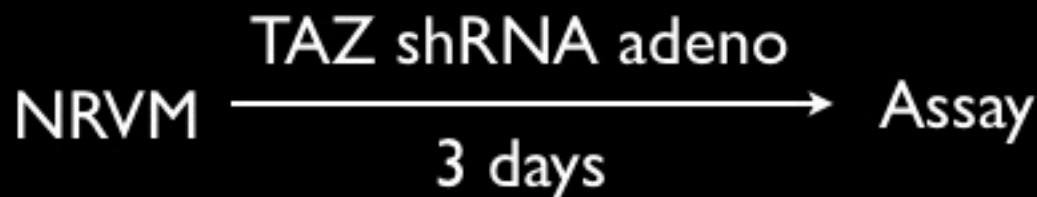


A. Oligomycin

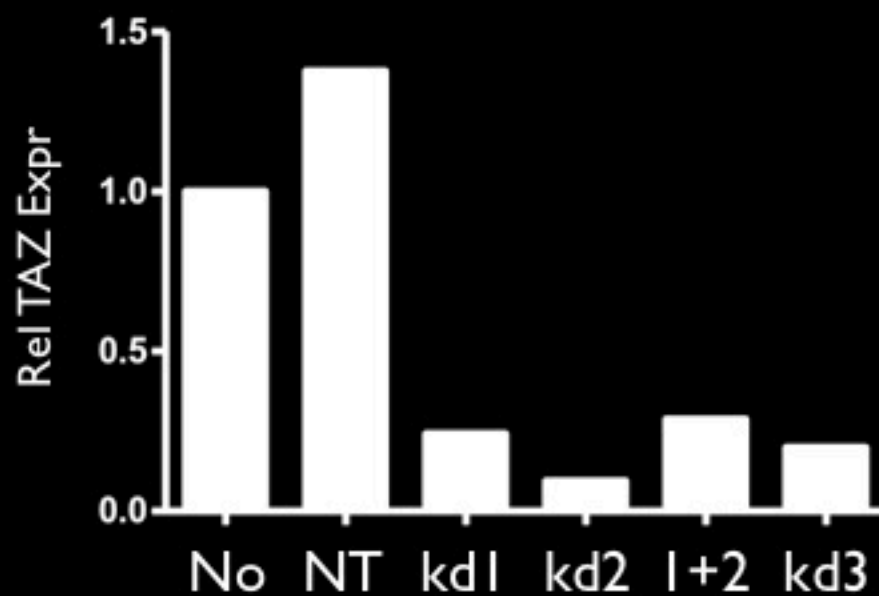
B. FCCP

C. Antimycin

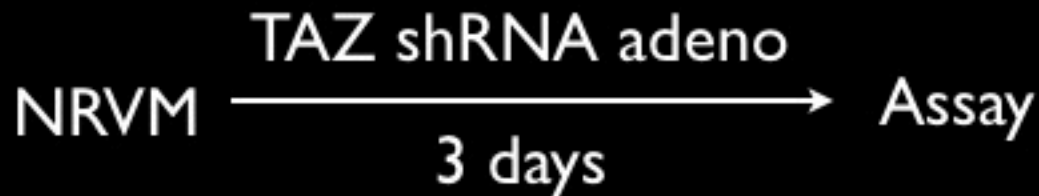
# NRVM TAZ Knockdown Model



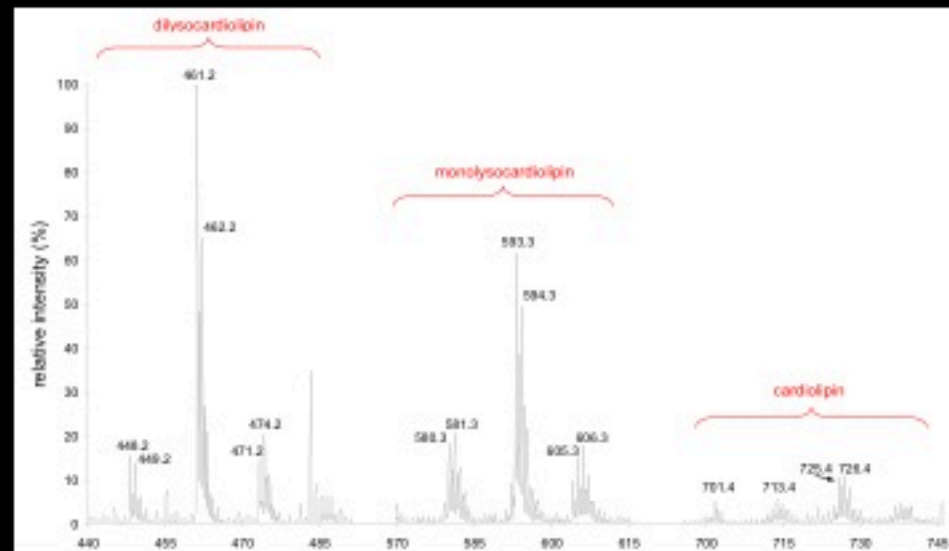
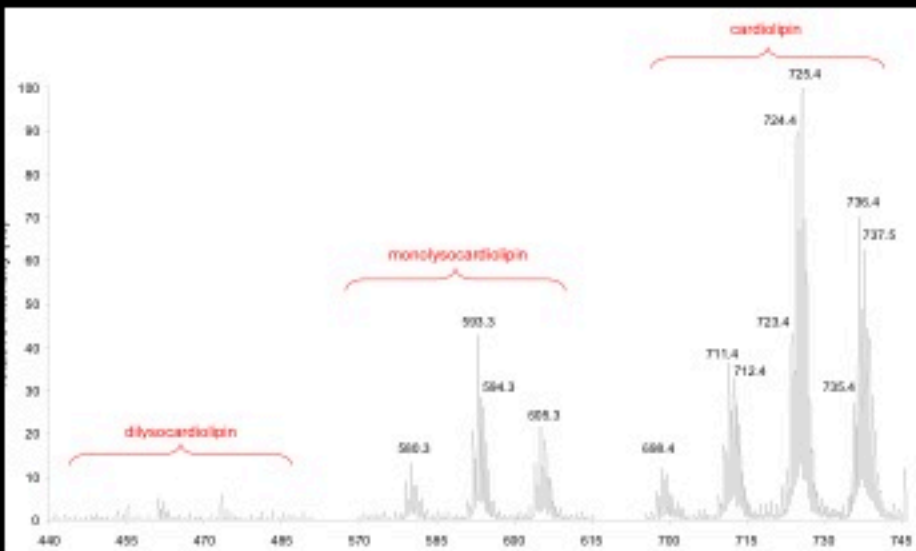
## Validation of Knockdown



# NRVM TAZ Knockdown Model

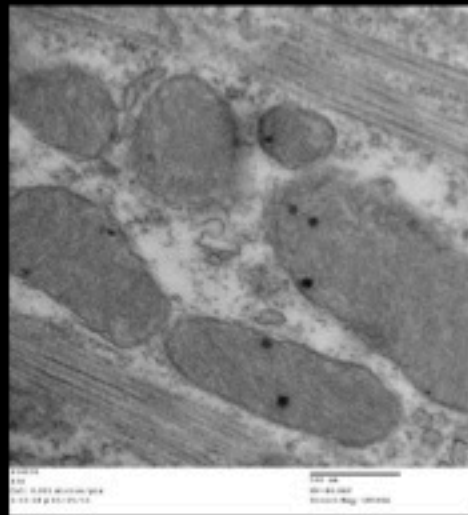


## Validation of CL Depletion

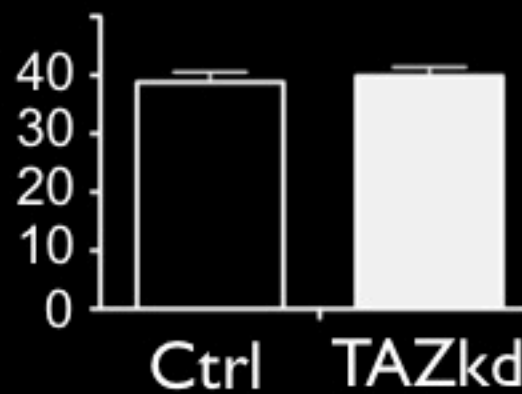


# Mitochondrial morphology in NRVM TAZ Knockdown Model

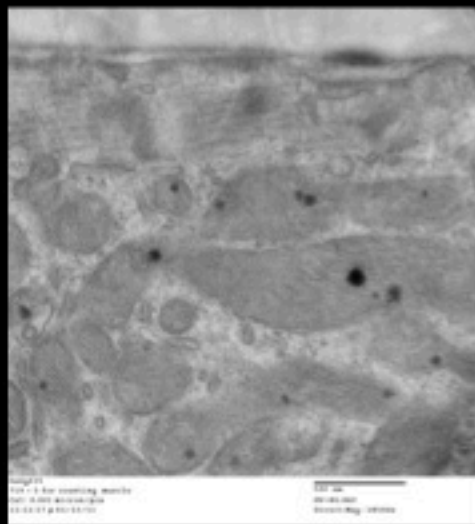
CTRL



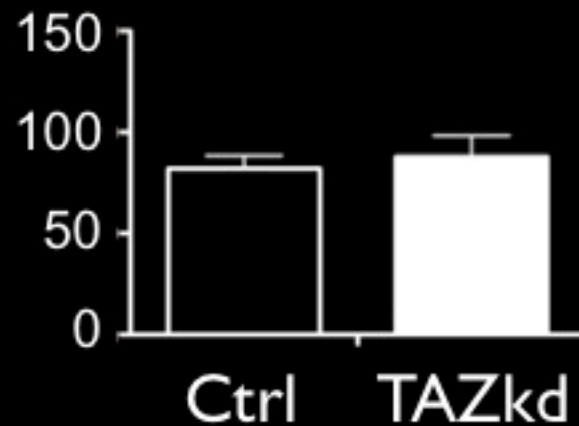
Mito Area/Cell  
Area (%)<sup>50</sup>



TAZ shRNA

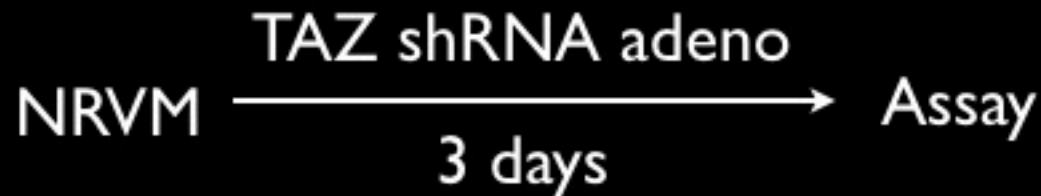


Mito Num/  
CM section

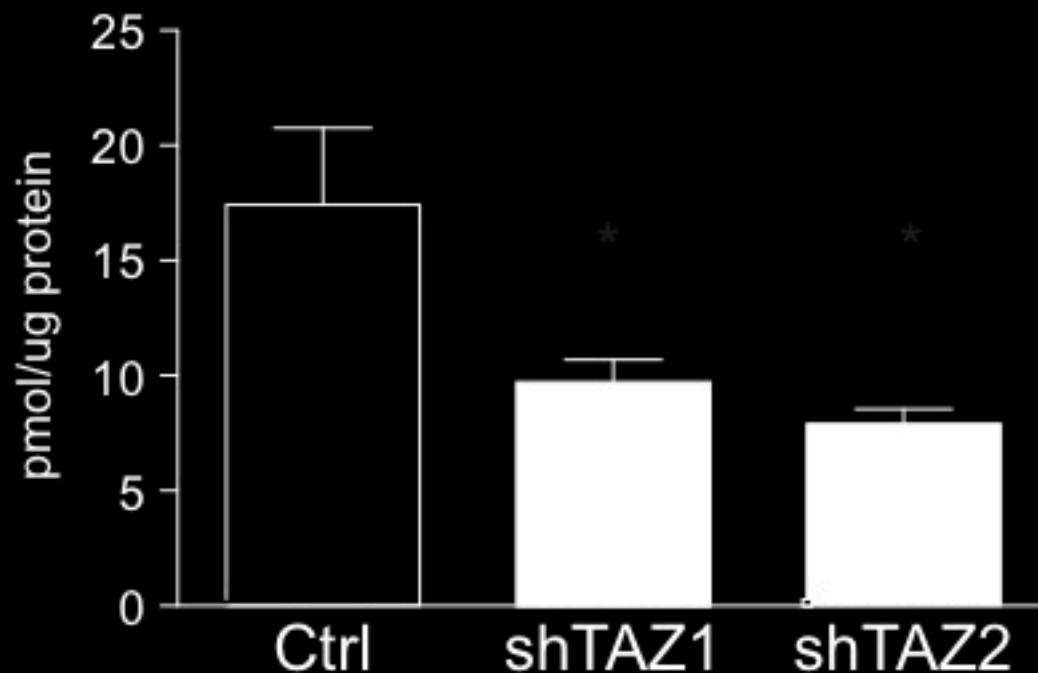




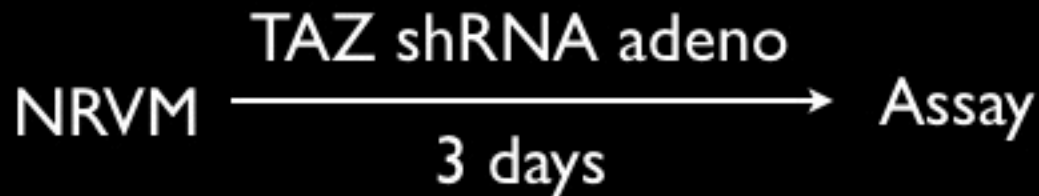
# NRVM TAZ Knockdown Model



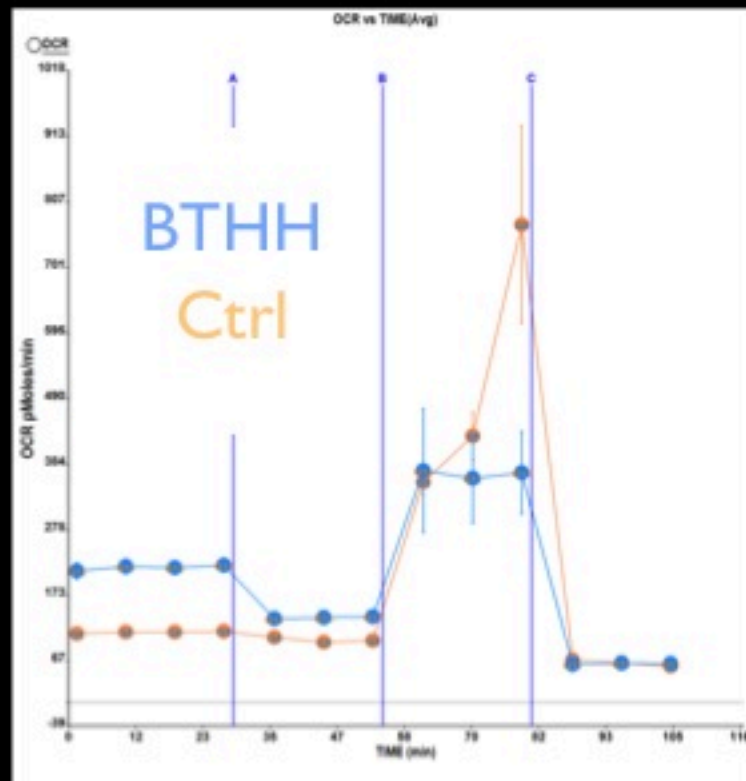
Intracellular ATP content



# NRVM TAZ Knockdown Model

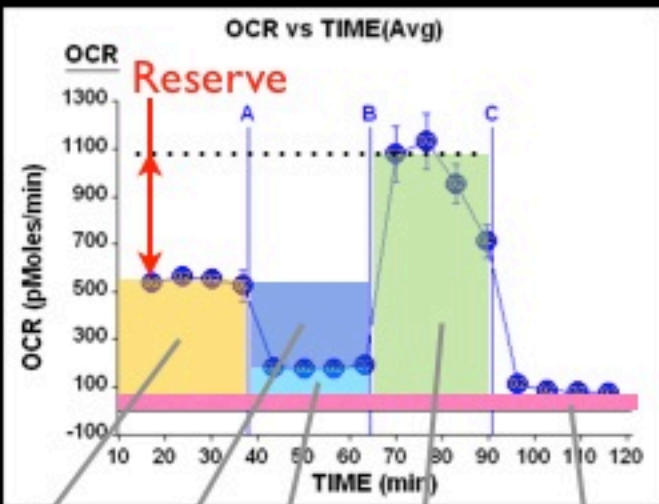


## Mitochondrial Phenotype

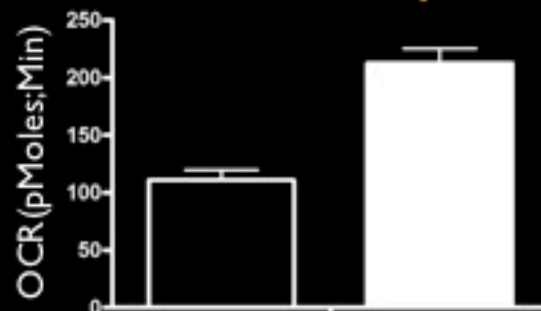


# Mitochondrial function after TAZ depletion in NRVM

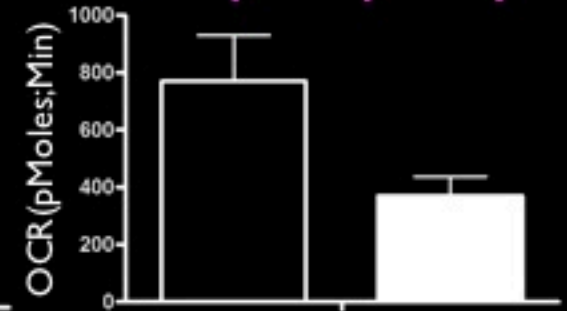
Ctrl shRNA  
 TAZ shRNA



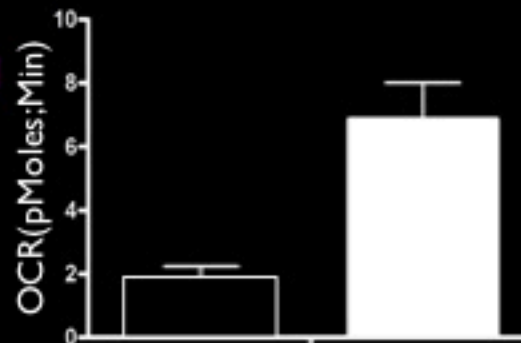
**Basal Resp**



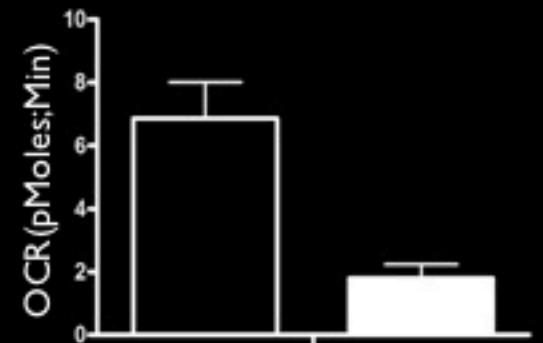
**Resp Capacity**



**ATP Gen**



**Resp Reserve**



Basal  
Resp

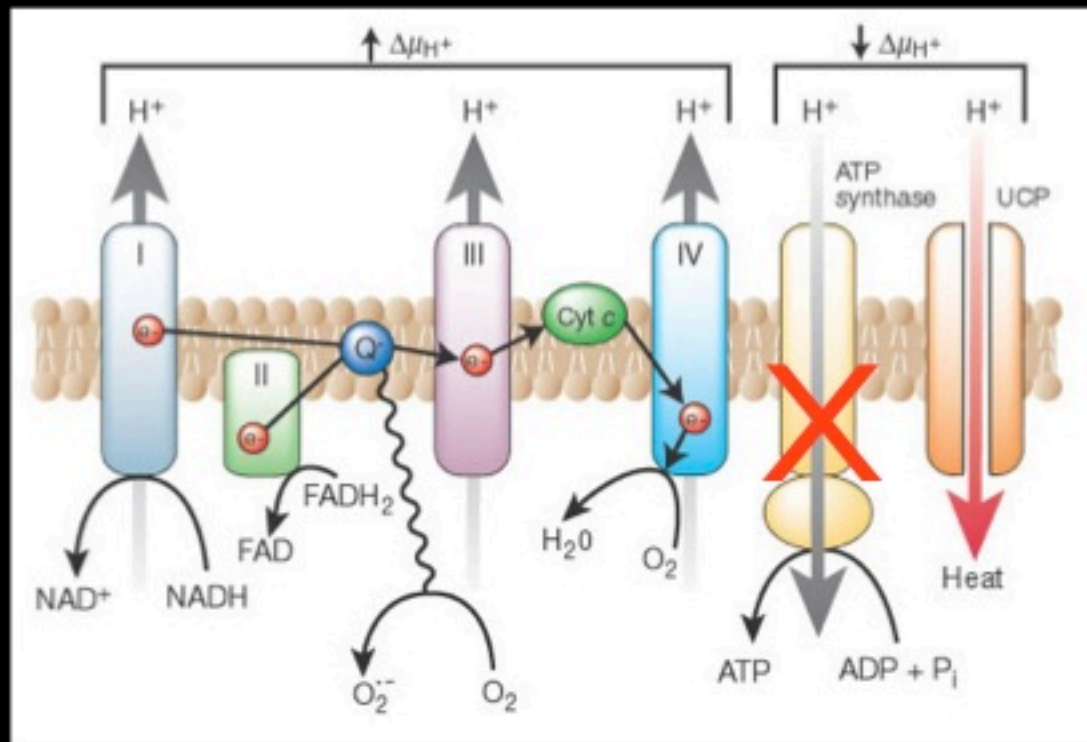
ATP  
Gen

H<sup>+</sup>  
Leak

Resp  
Capacity

Non-  
mitochondrial





### Baseline

CL deficiency makes F<sub>0</sub>F<sub>1</sub>  
ATP synthase activity limiting

Incr transmemb  
gradient

Decr ATP

### Stress

Reduced ETC  
activity

Decr Resp  
Reserve



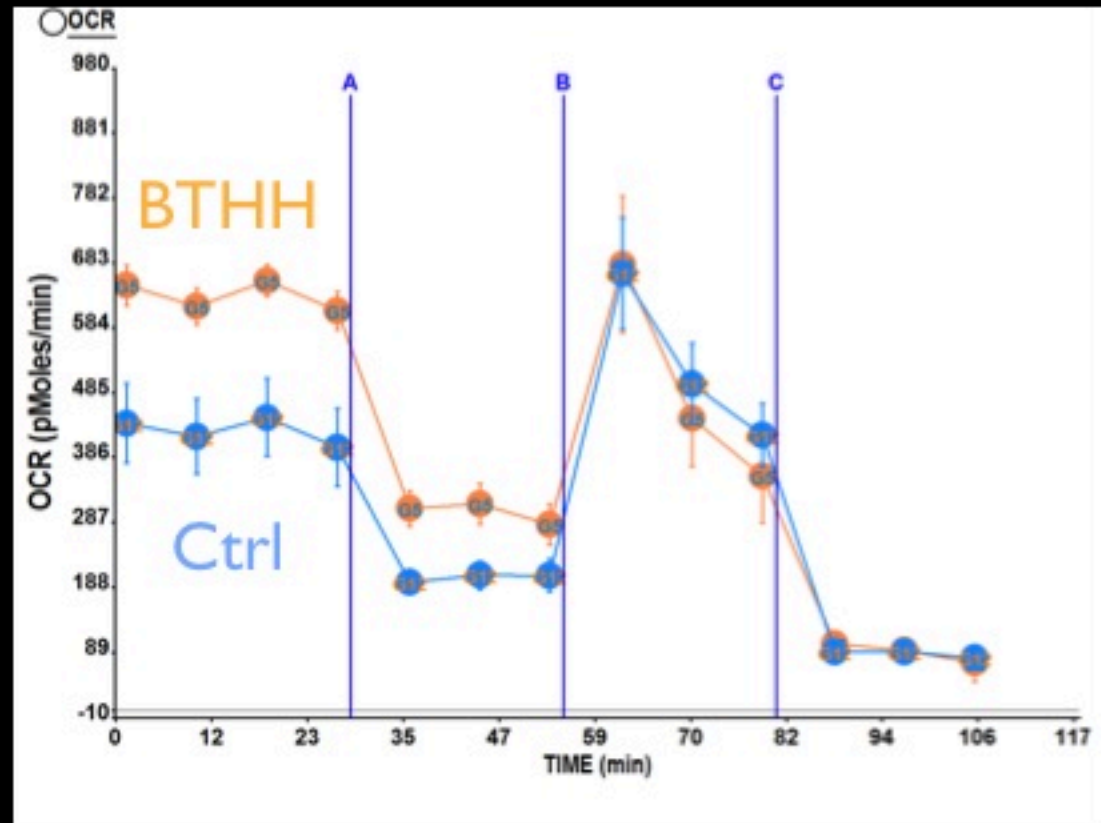
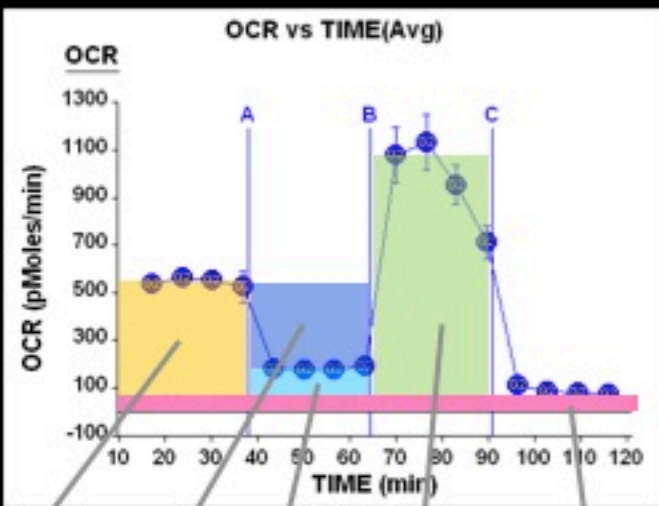
# Summary: NRVM TAZ kd model of BTHS

1. Rapid TAZ and CL depletion indicate short half-life of both.
2. Mitochondrial functional abnormalities occur prior to detectable morphological abnormalities.
3. Primary effect of CL deficiency at baseline is limitation of F<sub>0</sub>/F<sub>1</sub> ATP synthase activity.

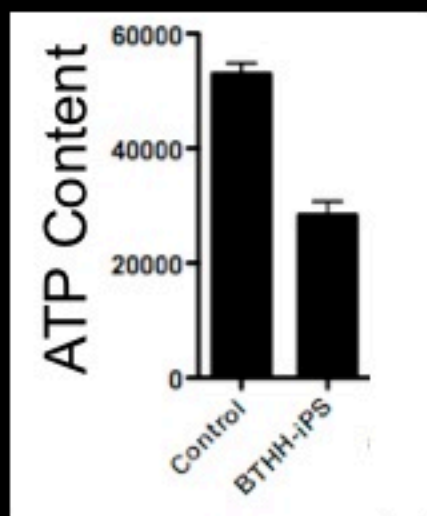
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# Analysis of BTHS iCM Mitochondrial Activity



# Analysis of BTHS iCM Mitochondrial Activity



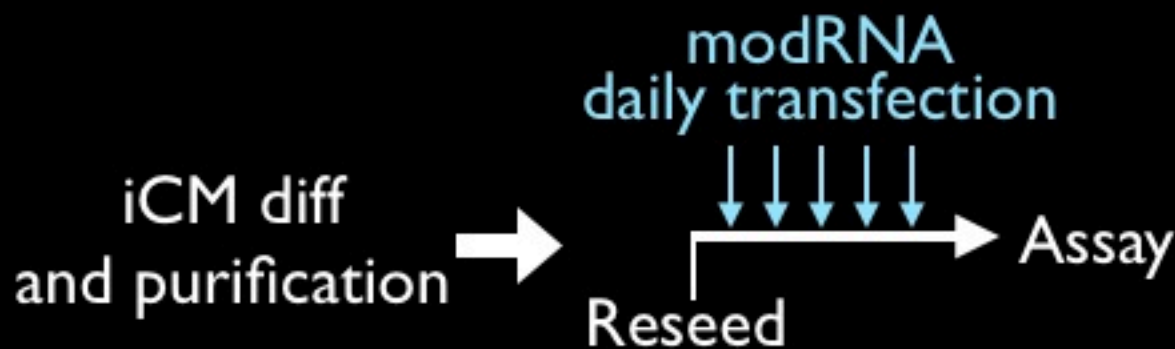
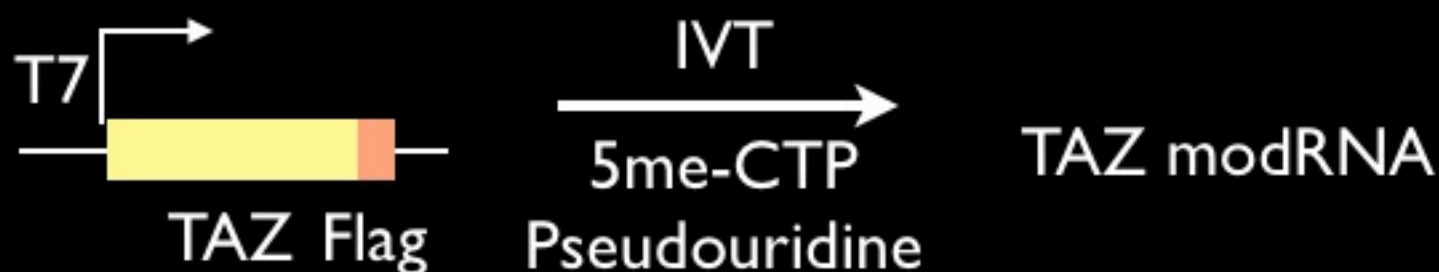
Higher basal OCR and decreased ATP content suggest decreased mitochondrial efficiency.

# Is the phenotype due to TAZ mutation or to other genetic difference?

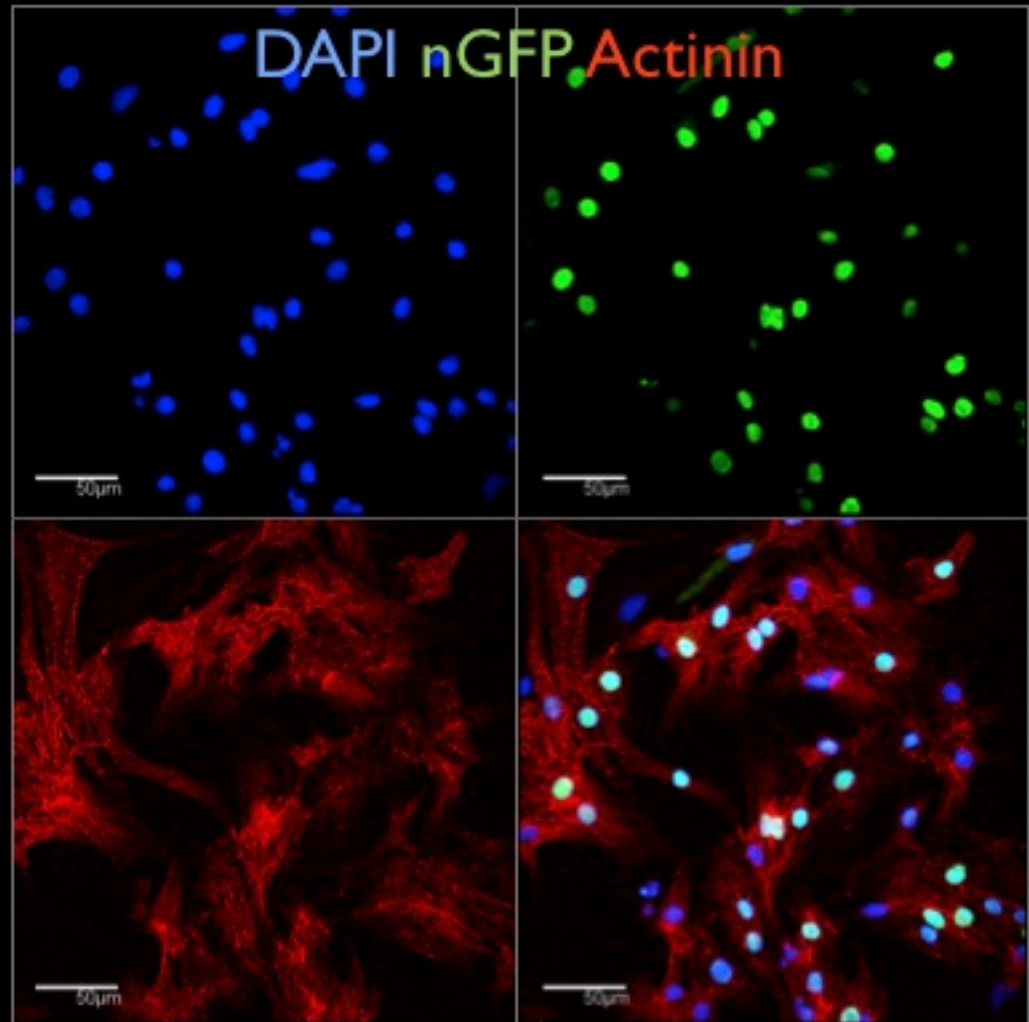
1. Analysis of a neonatal rat ventricular CM TAZ knockdown model.
2. TAZ modRNA rescue of the BTBS iPSC phenotype



# TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype



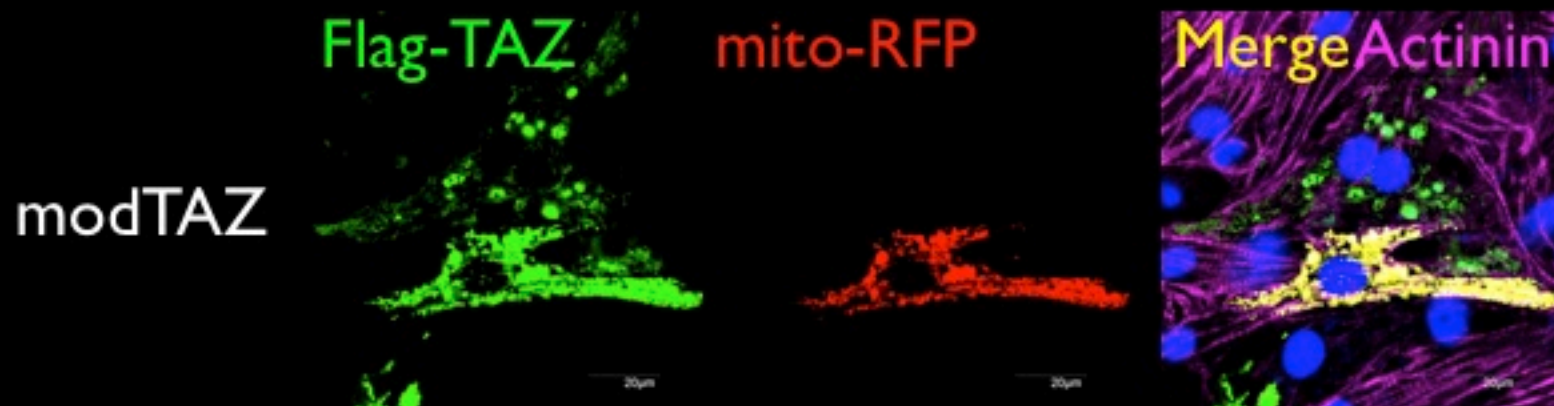
# Efficient modRNA transfection into NRVMs



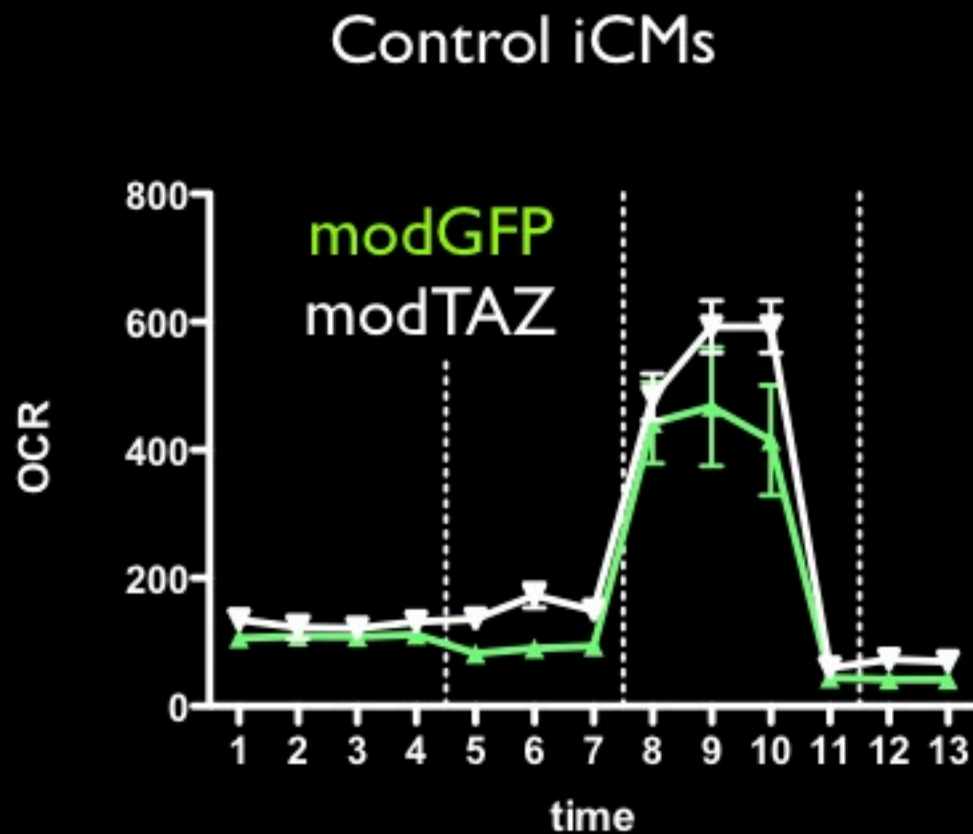
# TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype



# TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

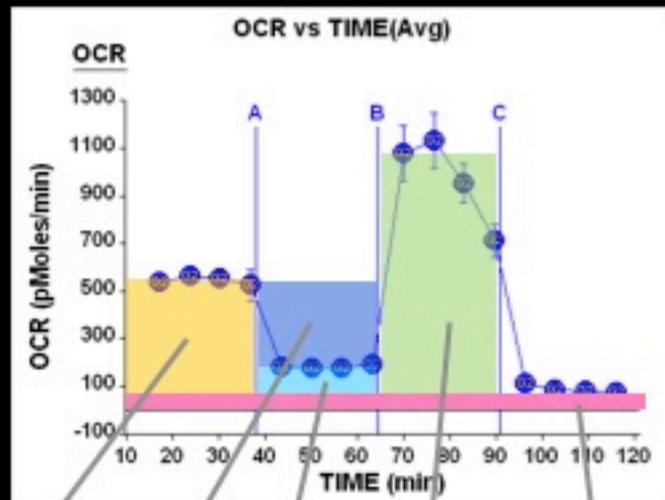


# TAZ overexpression did not cause substantial mitochondrial phenotype





# TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype



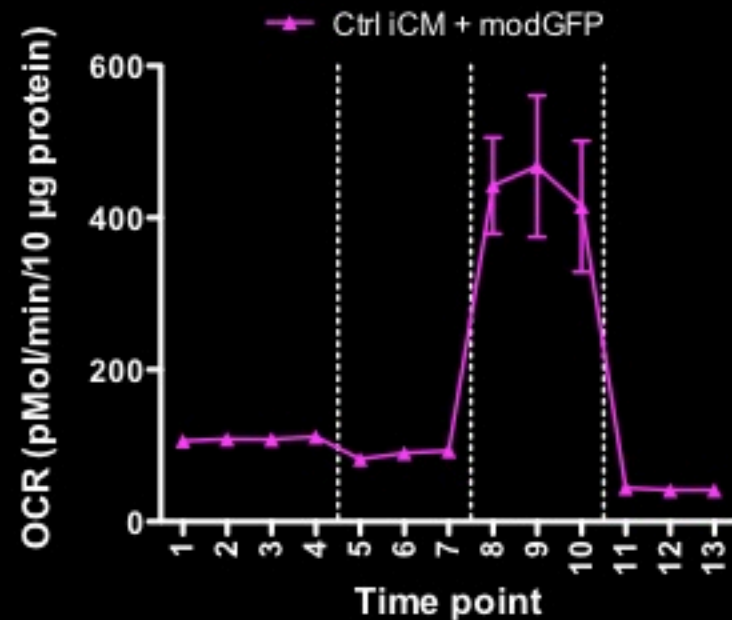
Basal  
Resp

Non-  
mitochondrial

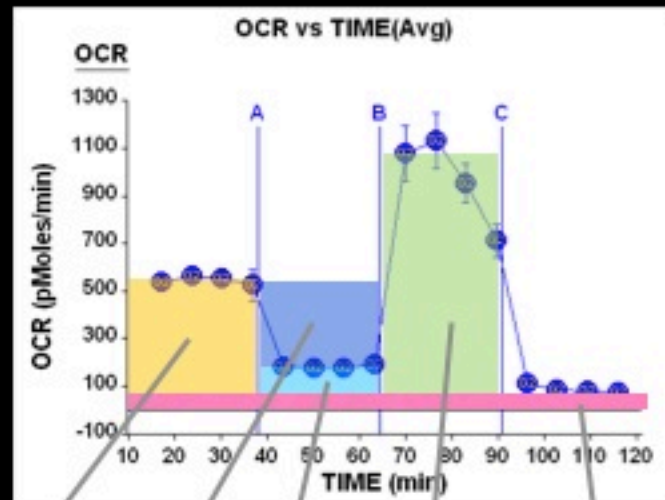
ATP  
Gen

H<sup>+</sup>  
Leak

Resp  
Capacity



# TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype



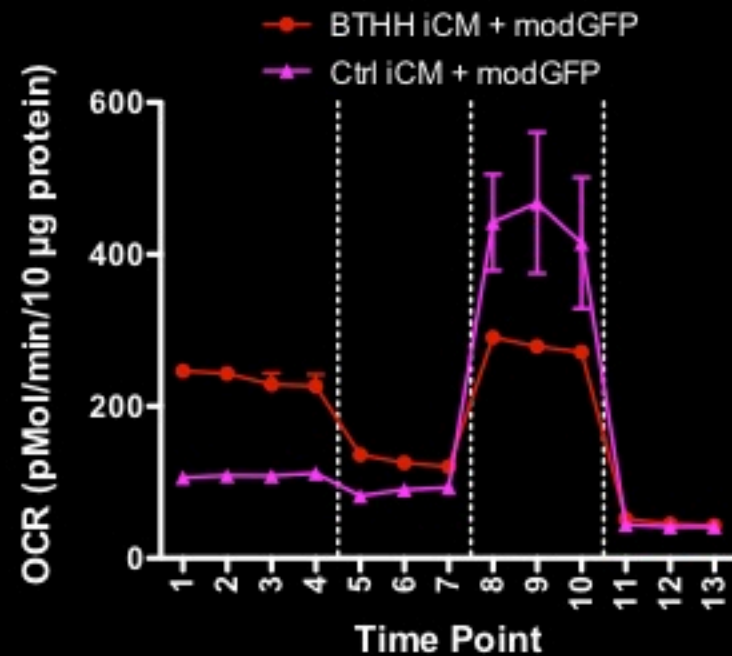
Basal  
Resp

ATP  
Gen

H<sup>+</sup>  
Leak

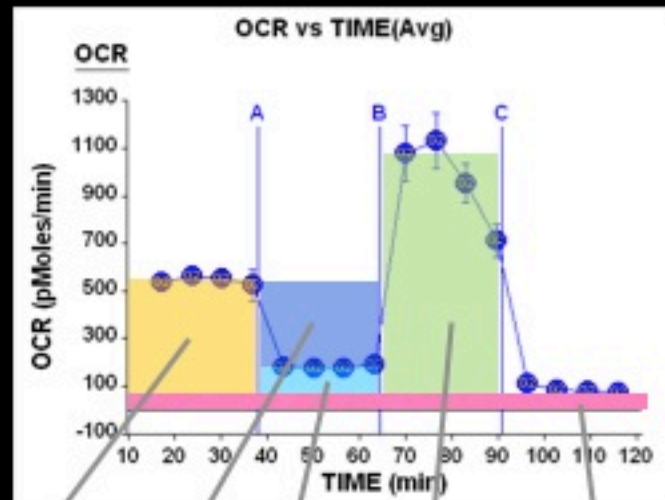
Resp  
Capacity

Non-  
mitochondrial

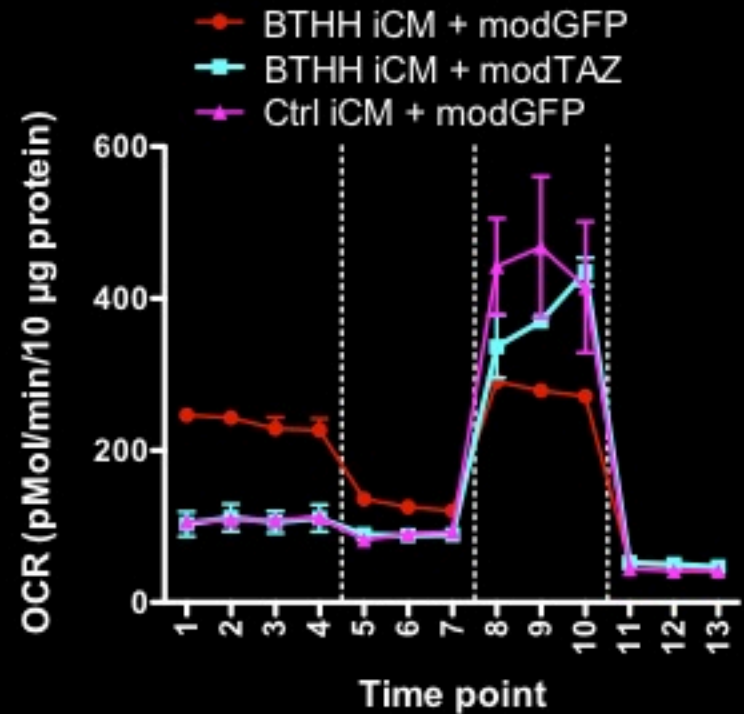


Time Point

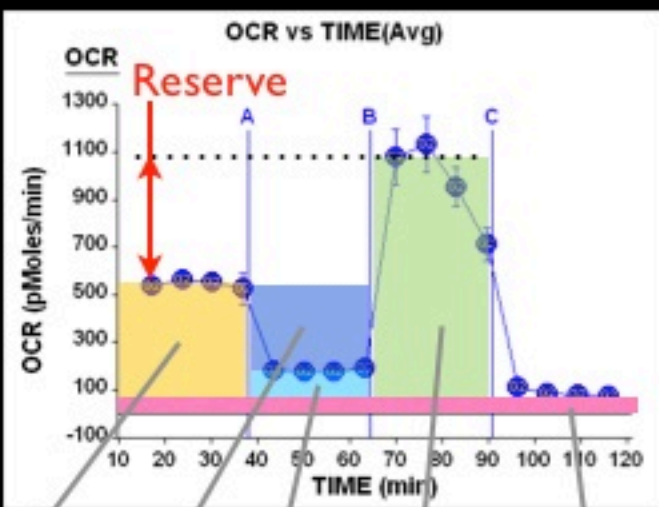
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Basal Resp  
ATP Gen  
H<sup>+</sup> Leak  
Resp Capacity  
Non-mitochondrial

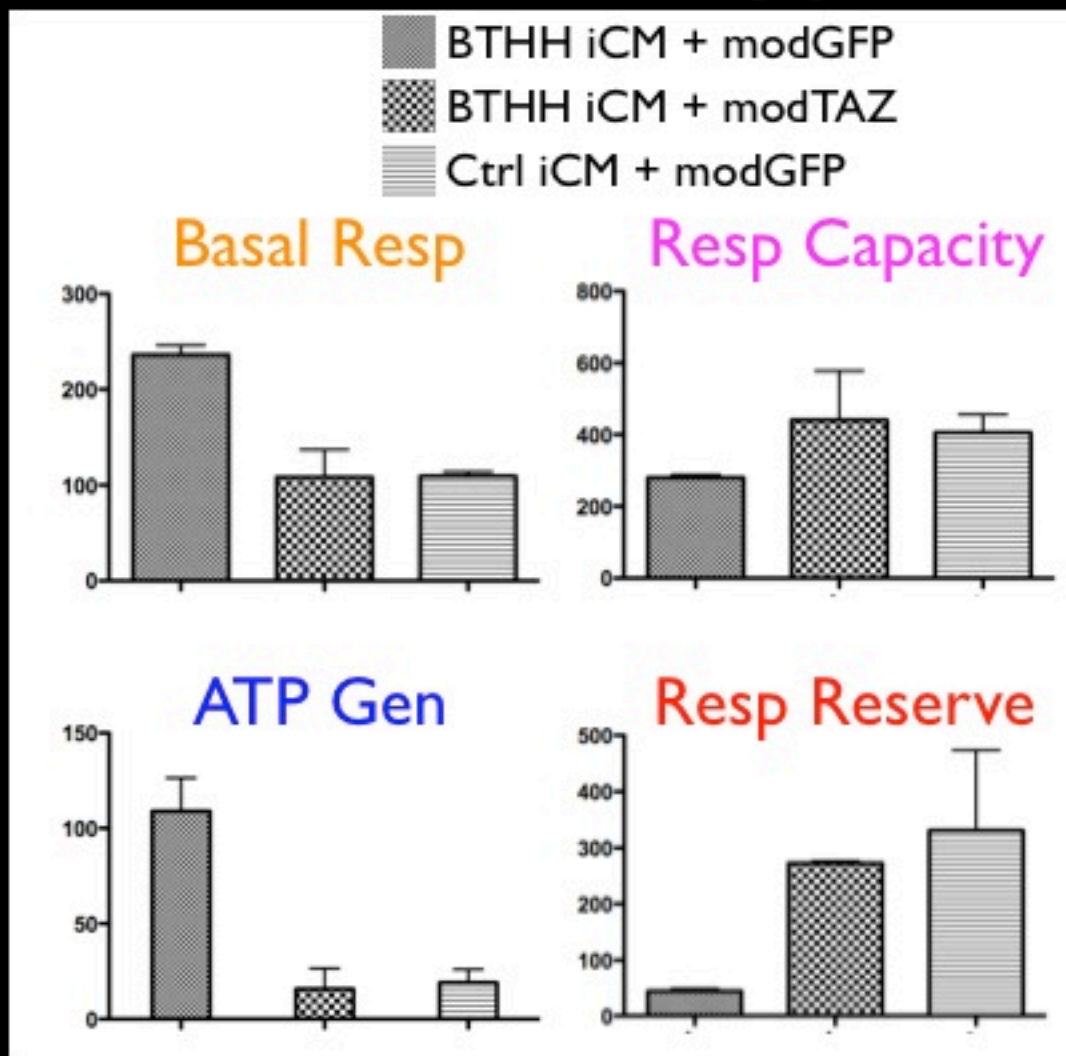


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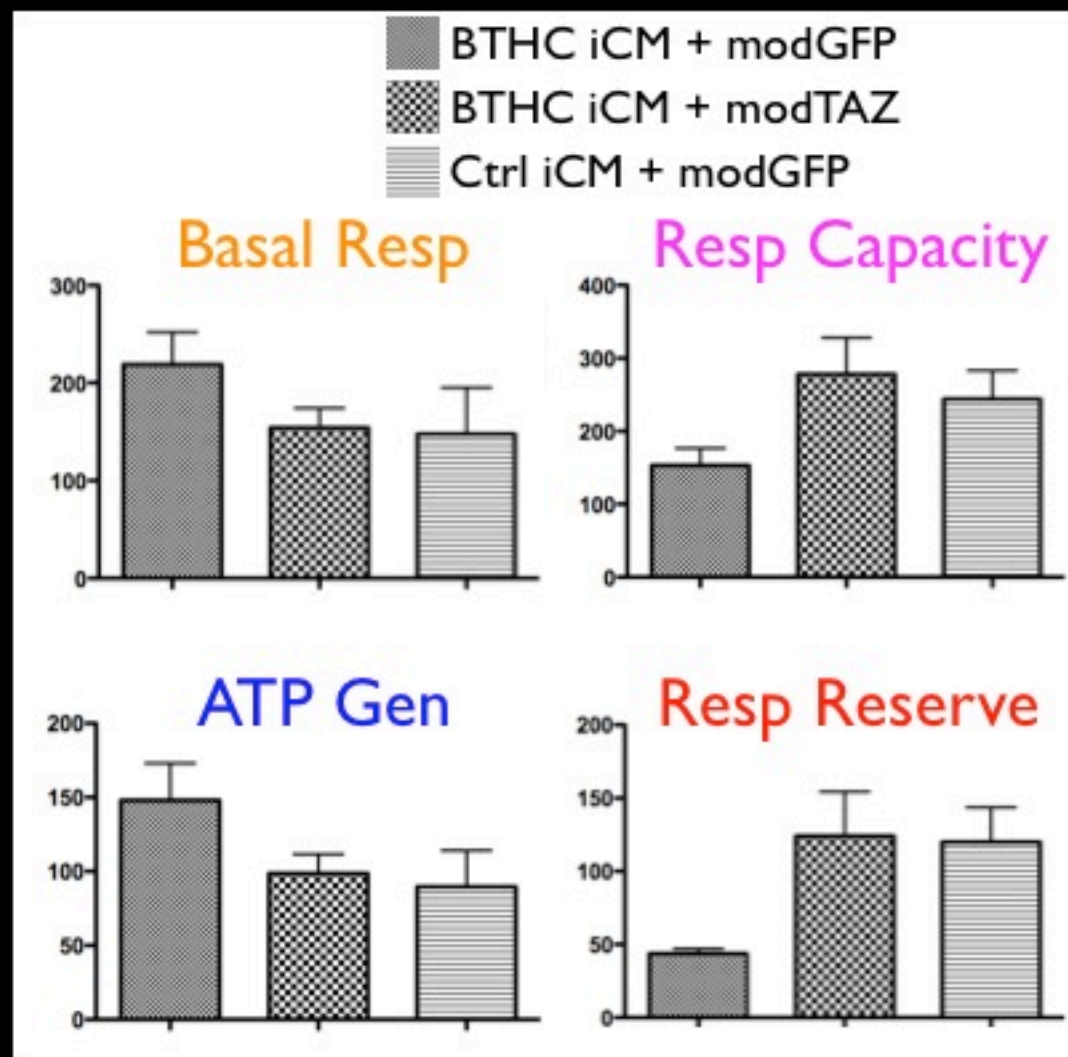
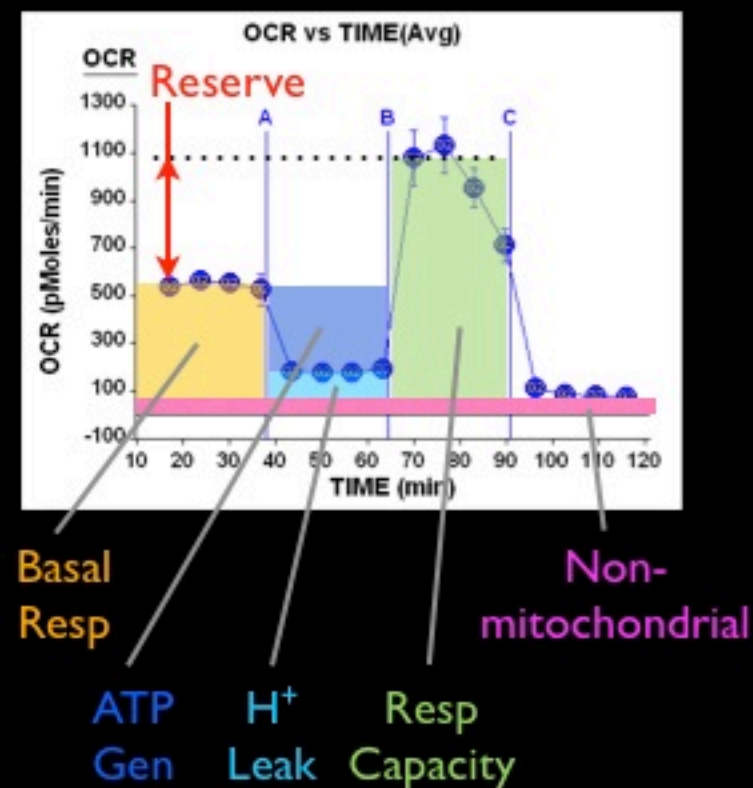


Basal Resp  
Non-mitochondrial

ATP Gen  
H<sup>+</sup> Leak  
Resp Capacity



# Results replicated in BTHC iCMs





# Summary of BTHS iCM mitochondrial abnormalities



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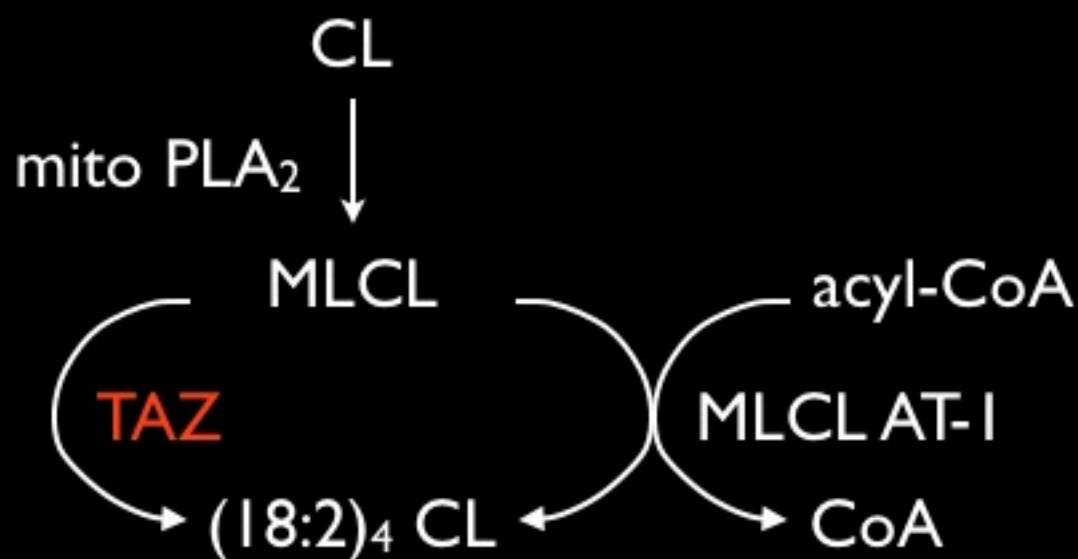
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- Provides positive control for development of assays to screen for therapeutic compounds
- TAZ modRNA overexpression in control iCMs did not cause measurable abnormalities >>> amenable to gene therapy.

# iPSC Model of Barth Syndrome

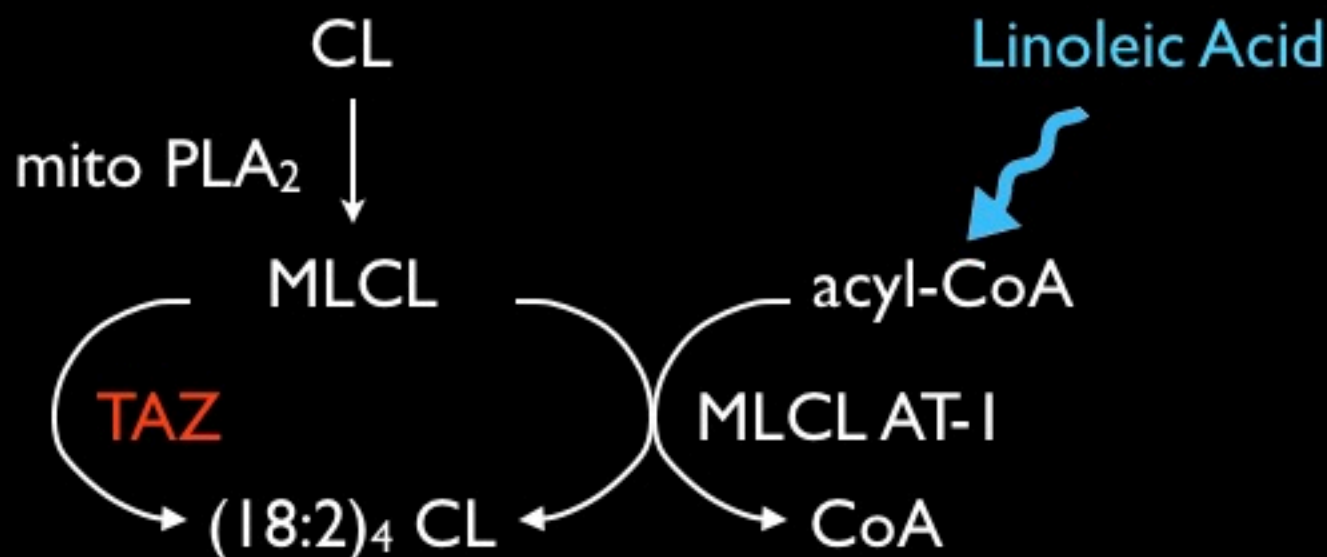
1. Generation and characterization of 2 iPSC lines, one from each of 2 patients with BTHS.
2. Differentiation and purification of BTHS iPSC-derived cardiomyocytes (iCMs).
3. Analysis of mitochondrial abnormalities in a neonatal rat ventricular cardiomyocyte model
4. Analysis of mitochondrial abnormalities in BTHS iCMs.
5. Treatments to reverse BTHS iCM mitochondrial abnormalities



# Targeted Barth Syndrome Treatment

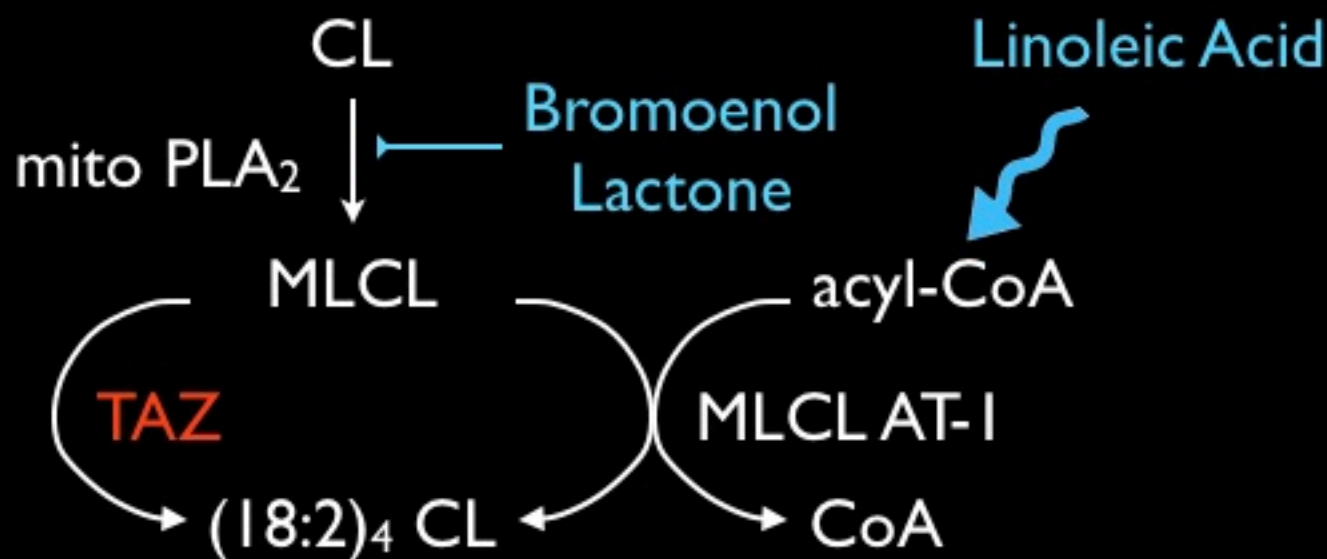


# Targeted Barth Syndrome Treatment



Valianpour, J. Lipid Res., 2003: LA increased CL in BTHS fibroblasts

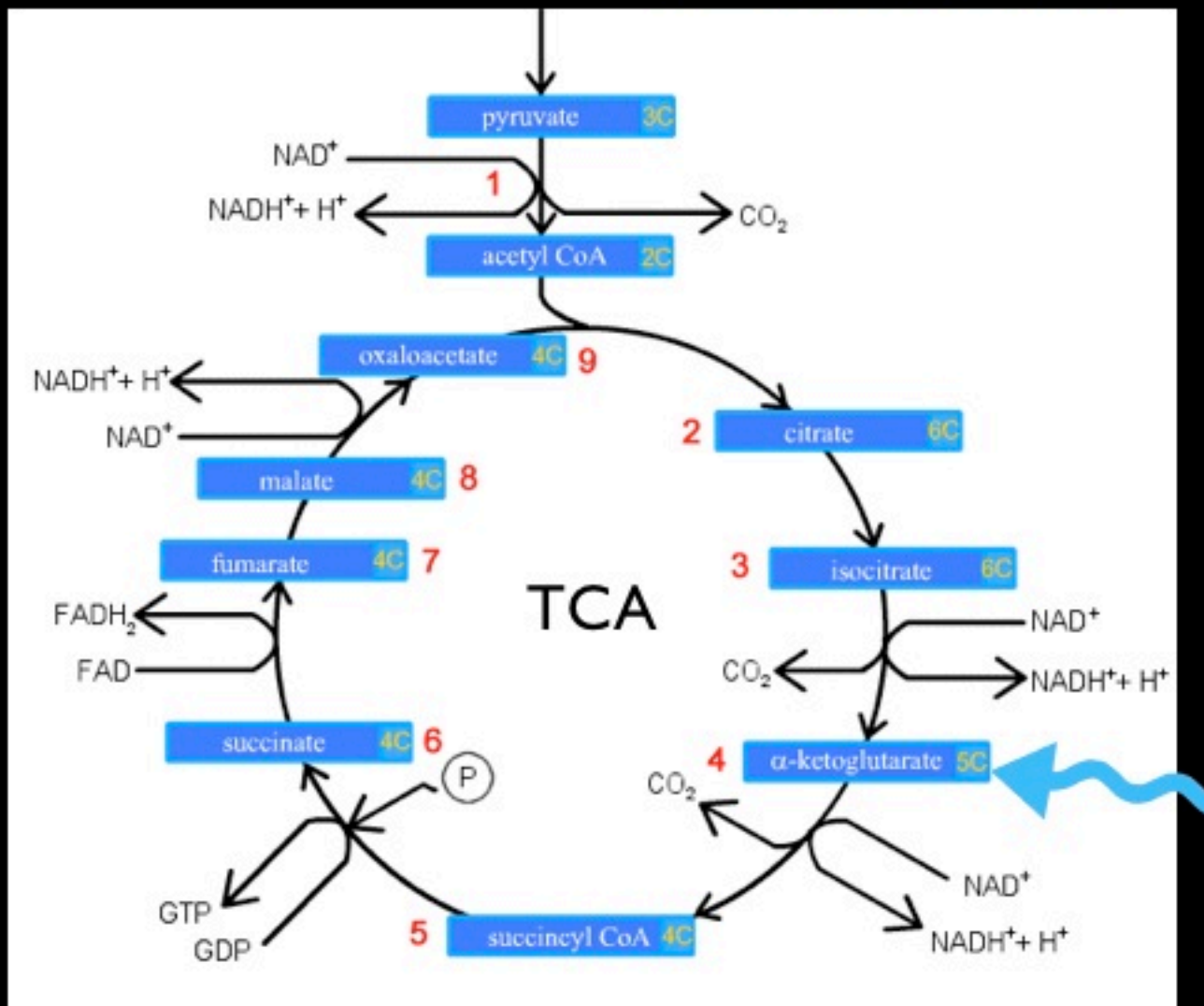
# Targeted Barth Syndrome Treatment



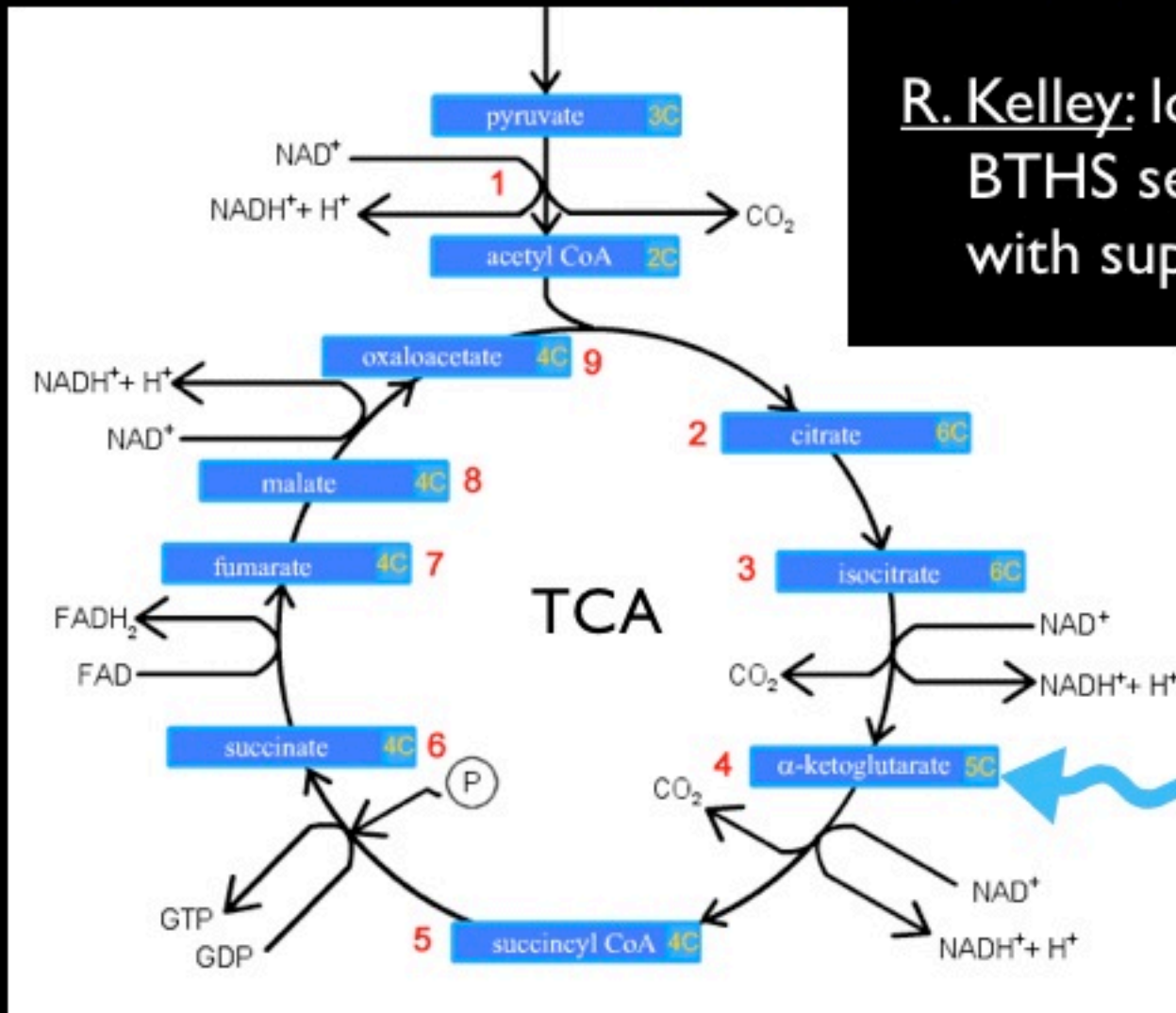
Valianpour, J. Lipid Res., 2003: LA increased CL in BTHS fibroblasts

Malhotra et al., PNAS, 2009: Bromoenol lactone increased CL in BTHS lymphocytes

# Targeted Barth Syndrome Treatment



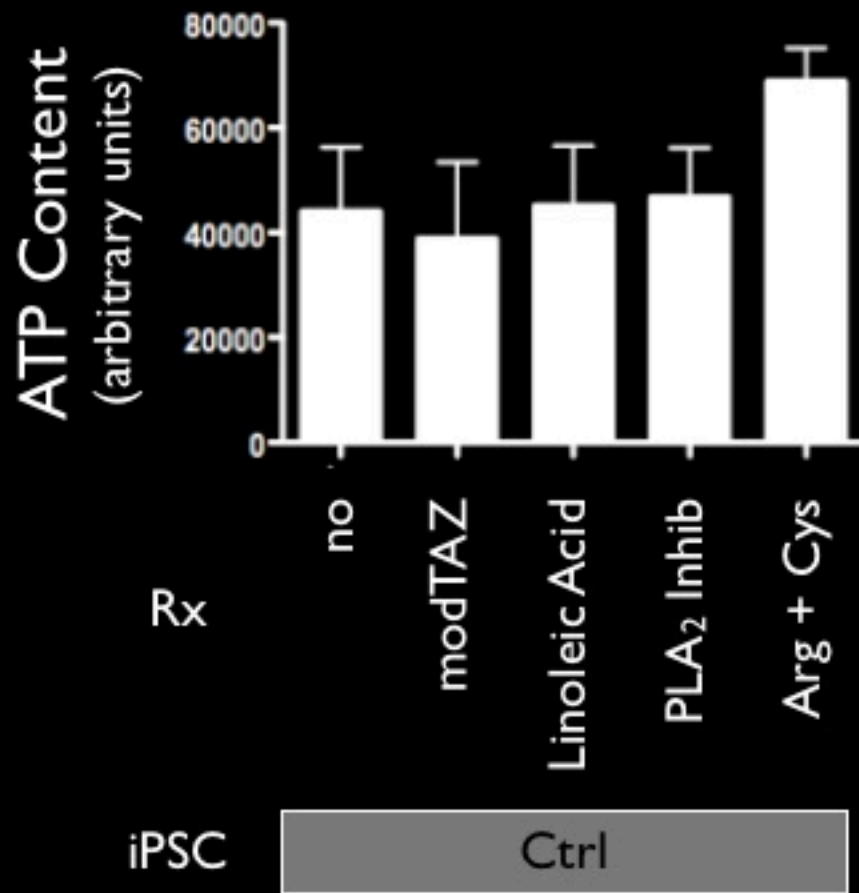
# Targeted Barth Syndrome Treatment



R. Kelley: low R and C in some BTHS serum. FS improved with supplementation.

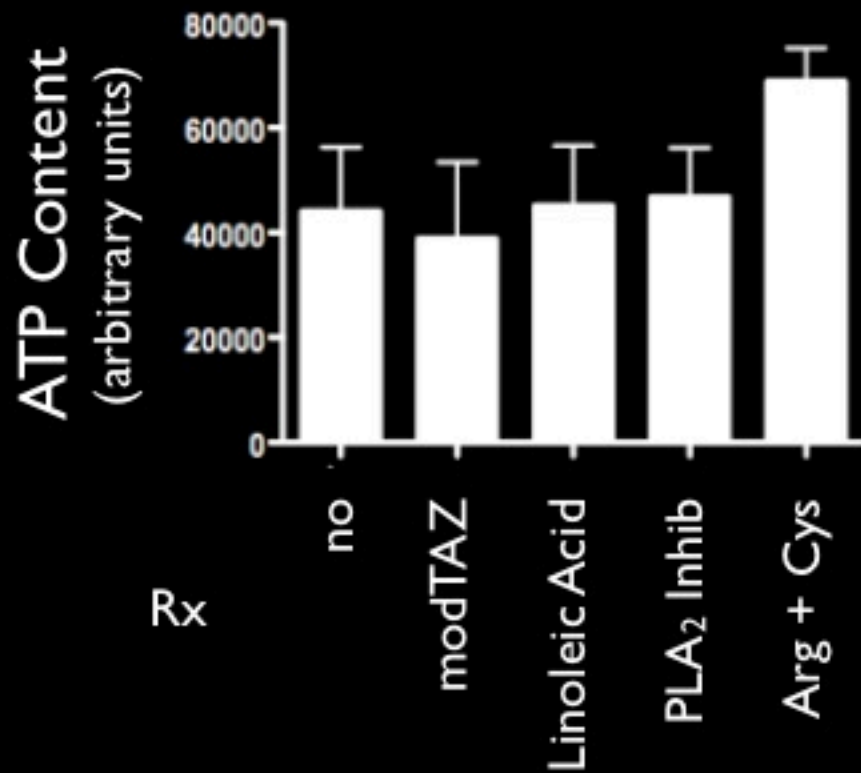
Arginine & Cysteine

# Targeted Barth Syndrome Treatment



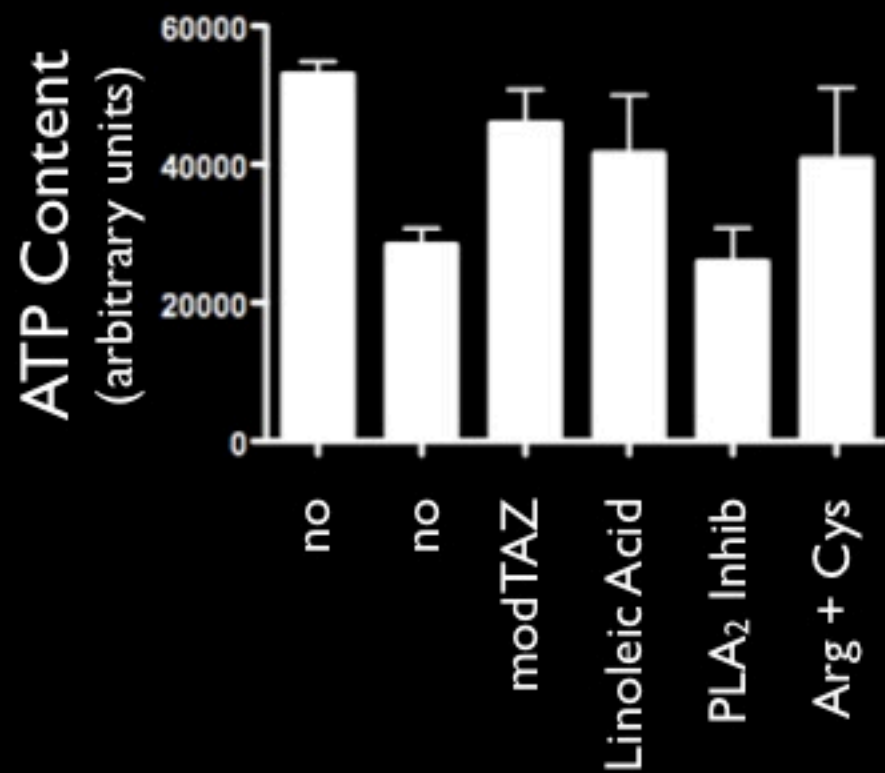


# Targeted Barth Syndrome Treatment



iPSC

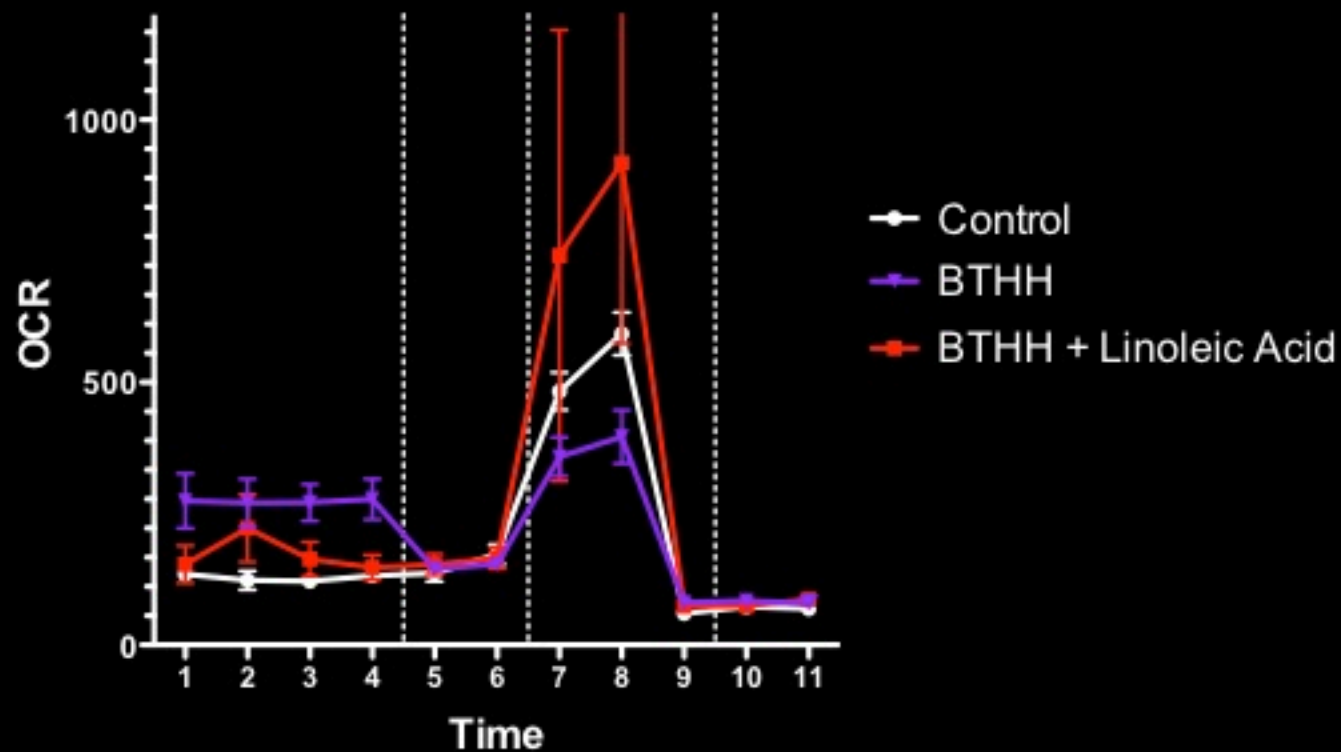
Ctrl



Ctrl

BTHH

# Rescue of BTSH mitochondrial abnormalities by linoleic acid



Summary: treatments to rescue BTHS  
iCM mitochondrial abnormalities

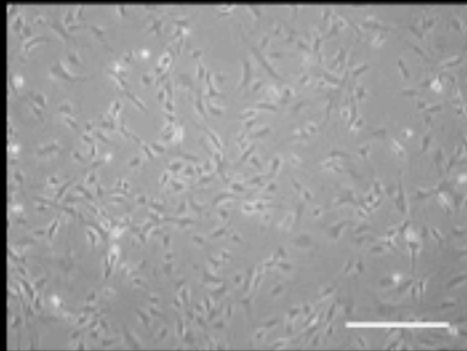
# Summary: treatments to rescue BTHS iCM mitochondrial abnormalities

- Established a renewable, human cardiomyocyte model for analyzing proposed treatments of BTHS cardiomyopathy.

# Summary: treatments to rescue BTHS iCM mitochondrial abnormalities

- Established a renewable, human cardiomyocyte model for analyzing proposed treatments of BTHS cardiomyopathy.
- Showed that linoleic acid and arginine + cysteine supplementation normalize mitochondrial function in BTHS iCMs.

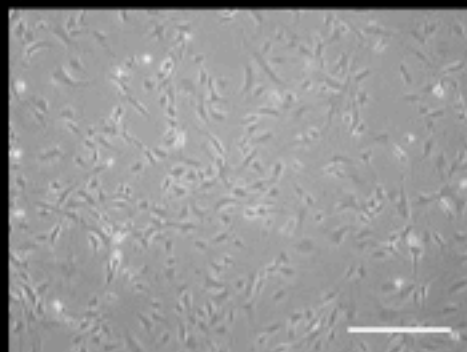
# Overall Summary



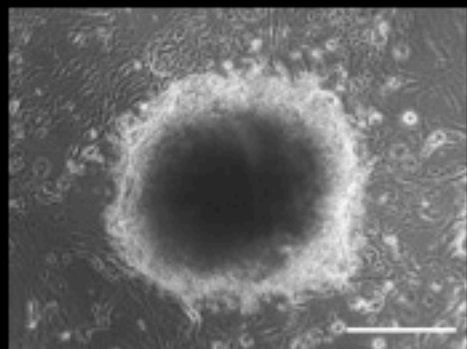
BTHS skin fibro



# Overall Summary

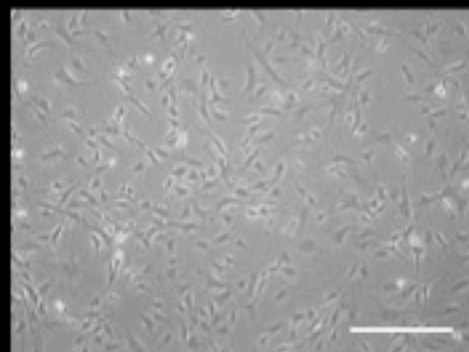


BTHS skin fibro



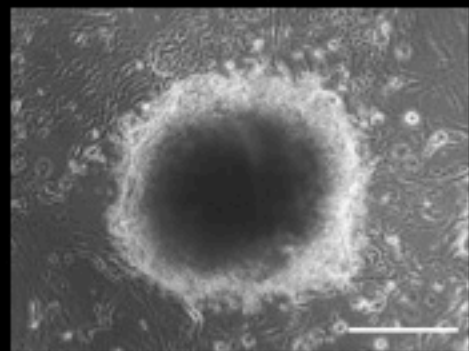
BTHS iPSC

# Overall Summary



BTHS skin fibro

OSKM  
↓



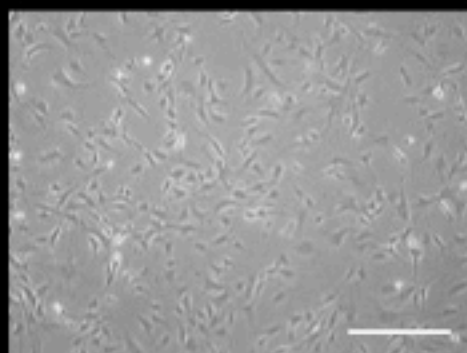
BTHS iPSC

CM  
diff  
↗



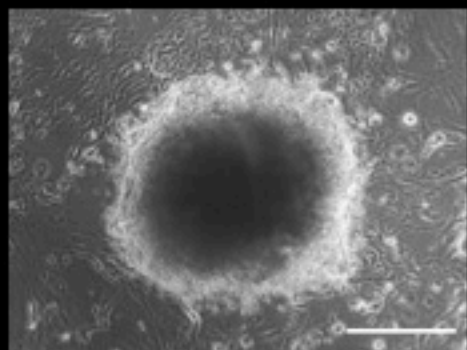
BTHS iCM

# Overall Summary



BTHS skin fibro

OSKM



BTHS iPSC

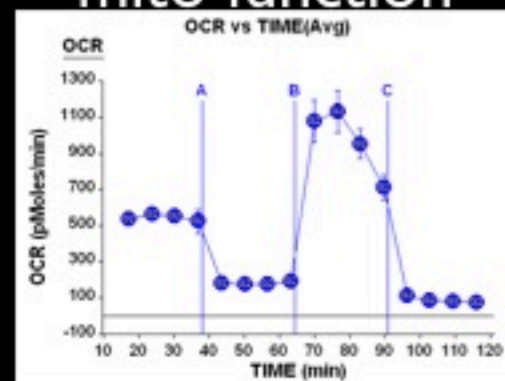
CM  
diff



BTHS iCM



mito function



inefficient mito fn

- low ATP, high OCR
  - reduced resp reserve
- modTAZ rescue shows rapid reversibility
- platform for screening of therapeutic strategies
- linoleic acid, R+C suppl

# Recruitment

Seeking additional patients with BTHS for generation of iPS cells.

- informed consent.
- ~2 mm skin punch biopsy, obtained with topical local anesthetic.

# Distribution

- Both BTHH and BTHC low passage fibroblasts available from BSF biorepository -- need to address expansion and further distribution.
- Will soon distribute BTHH and BTHC iPCs lines via a stem cell repository, e.g. Wicell.



# Acknowledgements



Barth Syndrome  
Foundation



## Pu Lab

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David Clapham (CHB)

Saumya Das (BIDMC)

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