

# **University of Utah -AMDCC Steering Committee Update March 2005**

**E. Dale Abel M.D., Ph.D. on behalf of  
Donald McClain M.D., Ph.D. (PI)  
Sheldon Litwin M.D.**



# Glucose

# FFA

*reguly* \*

\* Regulated Transport

\*

Cytosol

Glucose  
↓  
Glycolysis  
↓  
Pyruvate

## F-Acyl-CoA

Nucleus

Acetyl-CoA  
MCD  
ACC

PPAR-alpha Target Genes

1: PPAR- $\alpha$   
2: RXR

‡: PPAR- $\alpha$  Targets

Malonyl-CoA

CPT1&2

Citrate

Pyruvate

Acetyl-CoA

F-Acyl-CoA

Oxaloacetate

Citrate

TCA Cycle

NAD, FAD

ADP

ATP

ADP

ATP

ADP

Cr

PCr

ATP

ADP

PDH

PDH

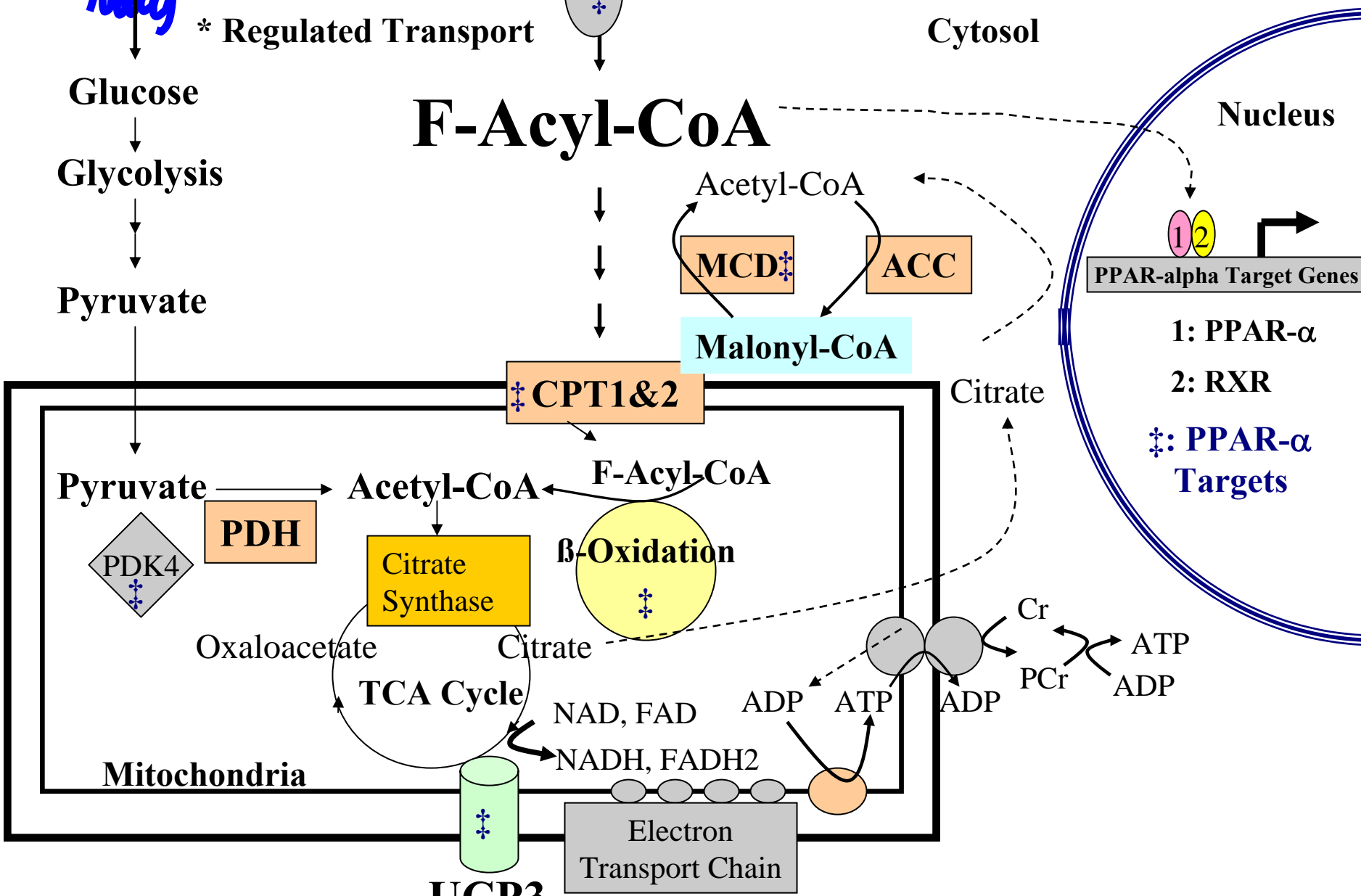
Citrate Synthase

$\beta$ -Oxidation

Mitochondria

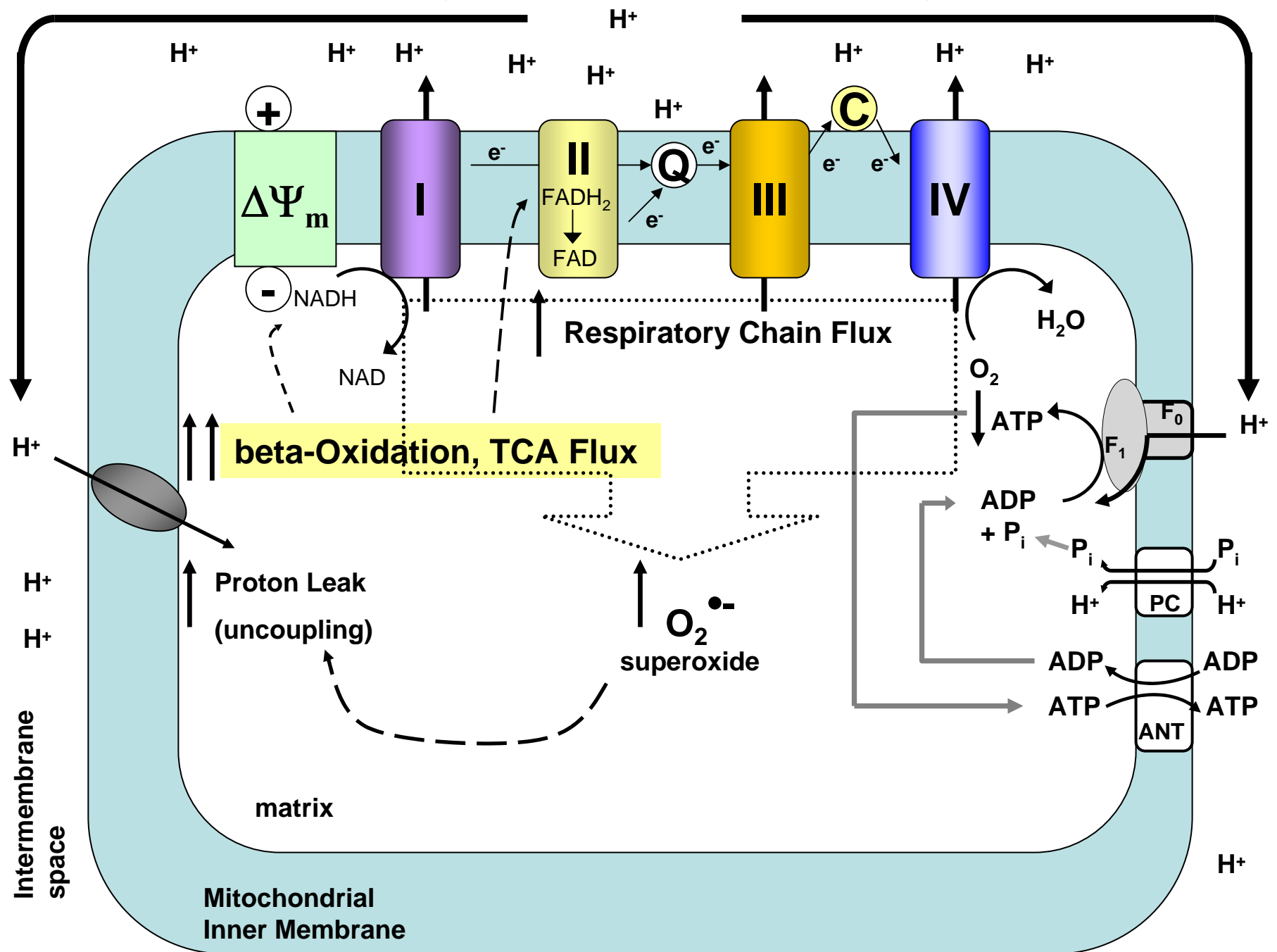
Electron Transport Chain

UCP3



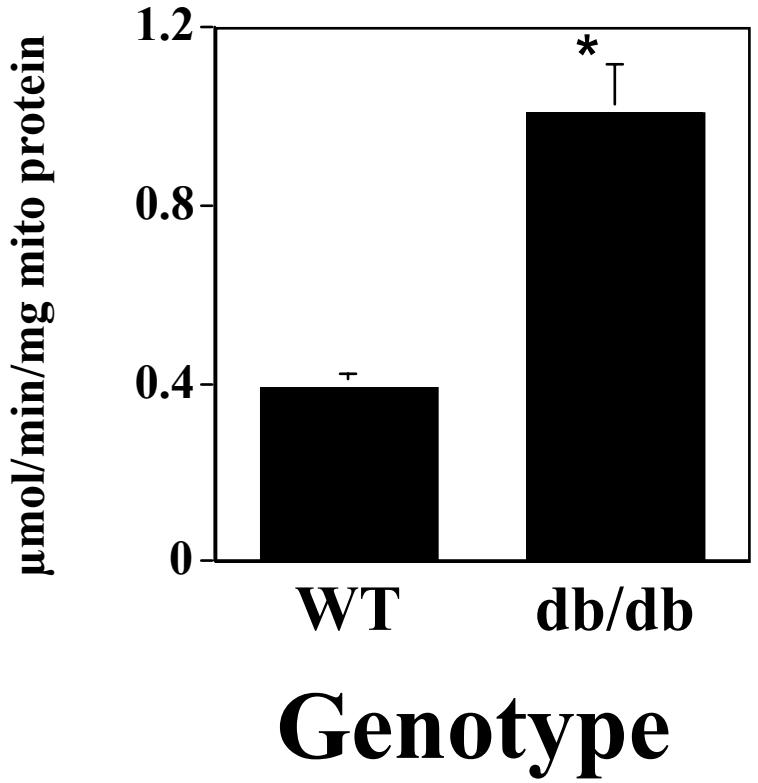
futile/ uncoupled proton cycling

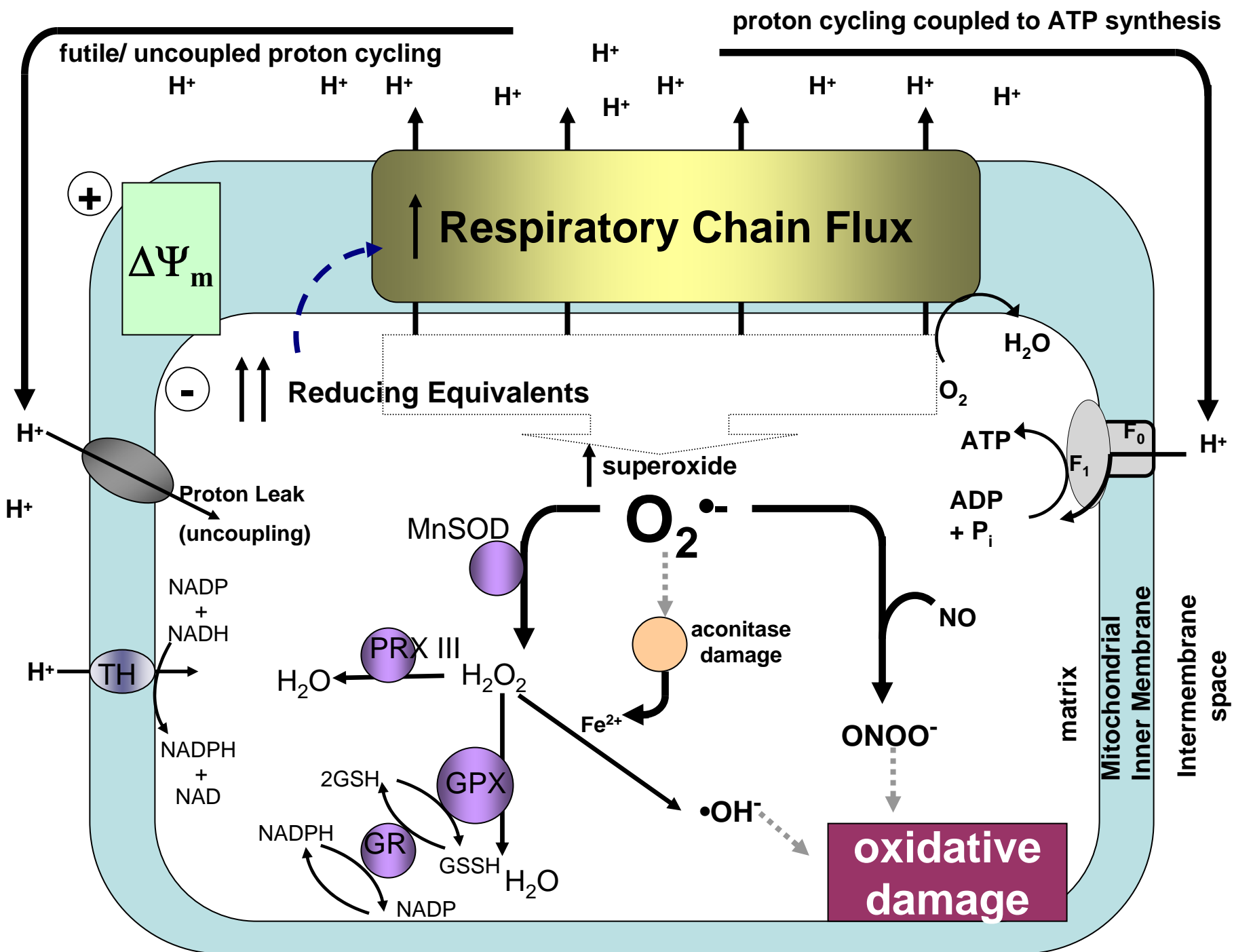
proton cycling coupled to ATP synthesis

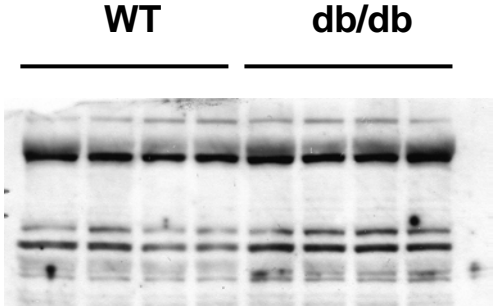


# Increased ROS Production in db/db Mitochondria

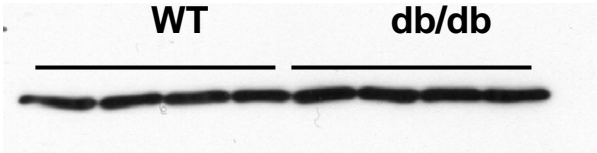
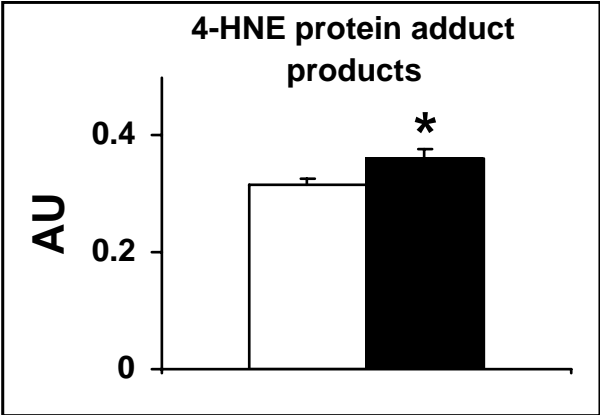
## Mitochondrial H<sub>2</sub>O<sub>2</sub> Generation



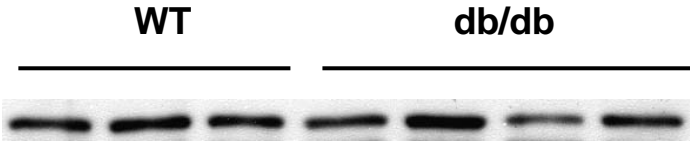
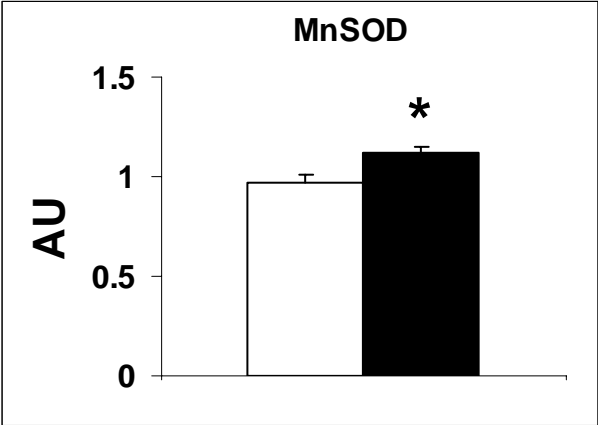




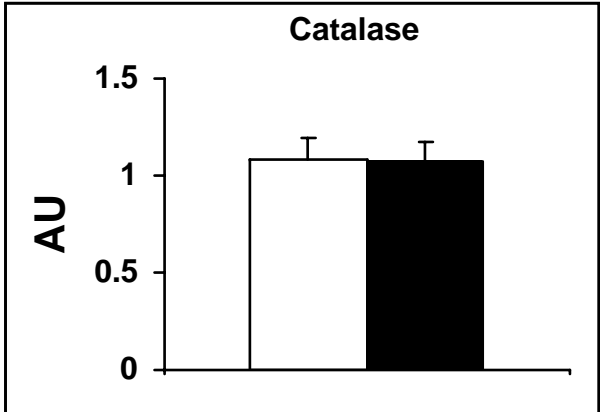
4 HNE Adducts



MnSOD



Catalase



# Metabolic Basis for Cardiac Dysfunction in Diabetes and Obesity

## Increased Fatty Acid Delivery

### MITOCHONDRIA

Increased FFA Flux

Decreased Glucose Utilization

Increased Mitochondrial ROS

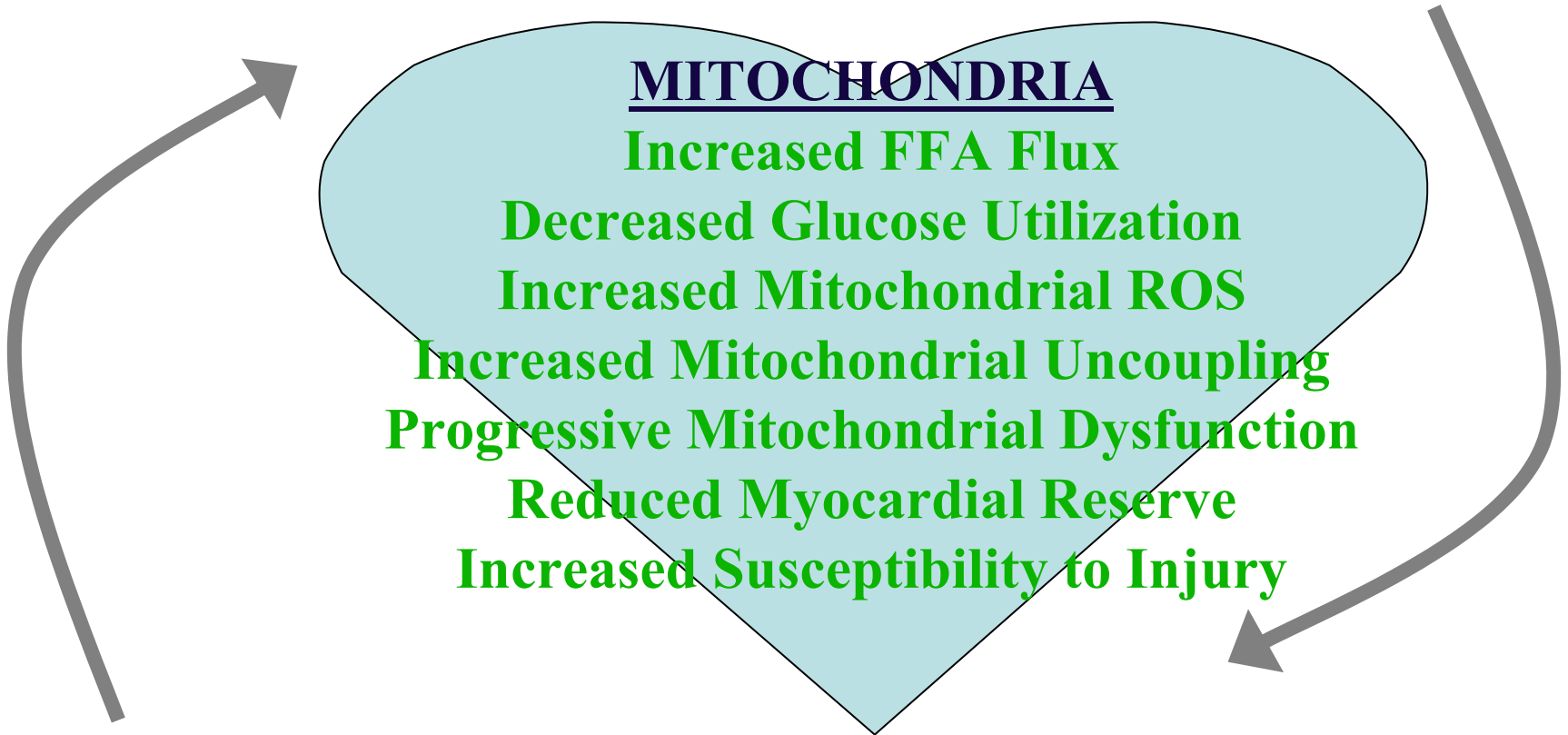
Increased Mitochondrial Uncoupling

Progressive Mitochondrial Dysfunction

Reduced Myocardial Reserve

Increased Susceptibility to Injury

## Insulin Resistance





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## University of Utah -Overview Summary of Current Models

Animal model	Background Strain	Current Status
Ob/ob ✓	c57Bl/6J	Final/advanced phenotyping
Db/db ✓	c57BlKS	Final/advanced phenotyping
Akita ✓	C57Bl/6J	Early Phenotyping
CIRKO ✓	Mixed	Final/advanced phenotyping
UCP-DTA ✓	FVB	Ongoing phenotyping
Dominant Negative PI3Kinase ✓	FVB	Phenotyping in Progress
Inducible CIRKO ✓	Mixed	Early Phenotyping
ACS-CIRKO ✓	Mixed	Colony Established
STZ ✓	Various	CIRKO-STZ in progress
GLUT4 Heart Specific	Mixed	Ongoing phenotyping



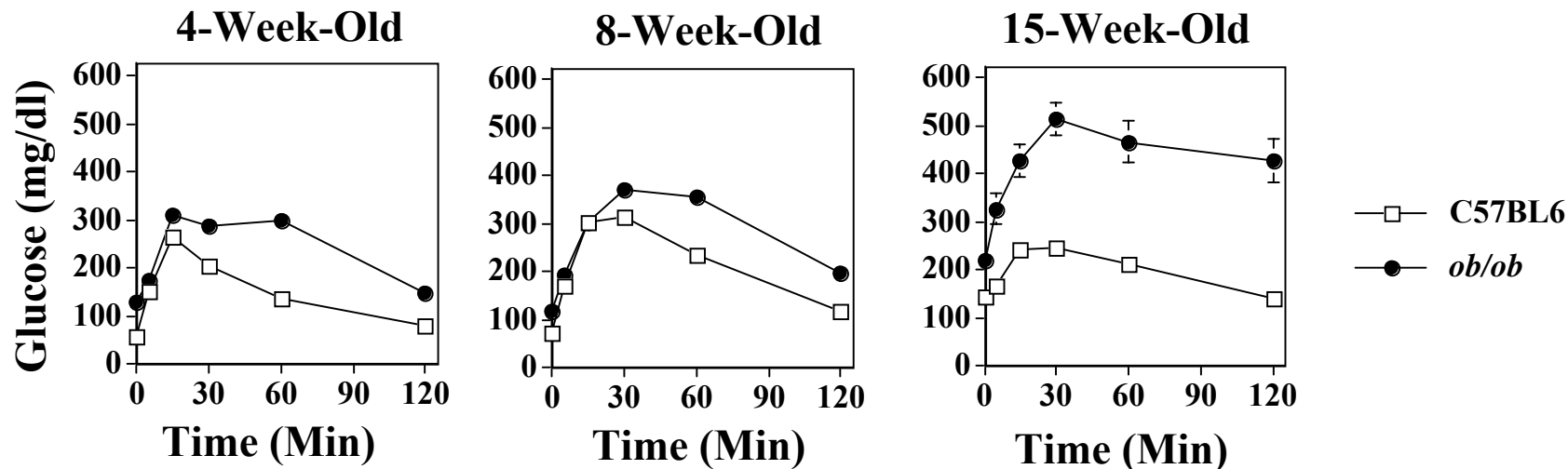
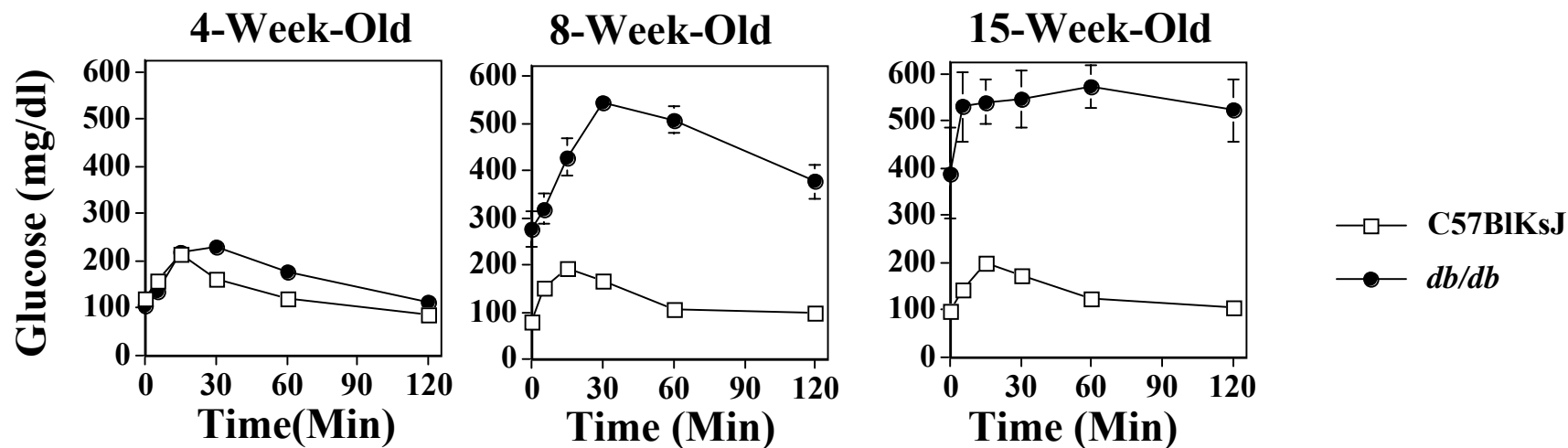
# EAC Comments

- Utah Center could offer a core service to perform these studies since it is not duplicated within the consortium.
- **Whole Animal Metabolic Phenotyping: Glucose Clamps, Serum Analysis- Insulin, FFA, Leptin, Adiponectin**
- **Intact Animal Cardiovascular Phenotyping: Invasive LV Catheterization, Mouse Echocardiography, Aortic Banding and Coronary Artery Ligation Surgery, EKG telemetry.**
- **Isolated Hearts: Isovolumic and Working Heart Preparations for Determination of Cardiac Function, Substrate Metabolism and Oxygen Consumption**
- **Mitochondrial Phenotyping: Bioenergetics, Respiration, ATP, Mitochondrial Enzyme Assays**
- **Histological Assessment: Light Microscopy, Immunofluorescence, Electron Microscopy**

# EAC Comments

- Other models of diabetes should be phenotyped - **Akita Mice, High-Fat Feeding**
- Measure cardiac function in older mice and correlate insulin sensitivity with cardiac function - **ob/ob and db/db mice now studied out to 40 weeks of age (hyperglycemia persists in db/db mice on the c57BLKS background in our hands)**
- ob/ob and db/db mice are extreme models of obesity and insulin resistance - **Studies (including metabolism and mitochondrial evaluations) are underway in high-fat fed c57Bl6, studies in UCP-DTA mice are on going**
- Capillary growth is impaired in the heart (in response to hypertrophic stimuli. Is this true in the retina?)-**These observations were made in CIRKO mice. Retinal studies are suited to generalized models of diabetes**

# Glucose Tolerance Tests *ob/ob* and *db/db* mice

**A****B**

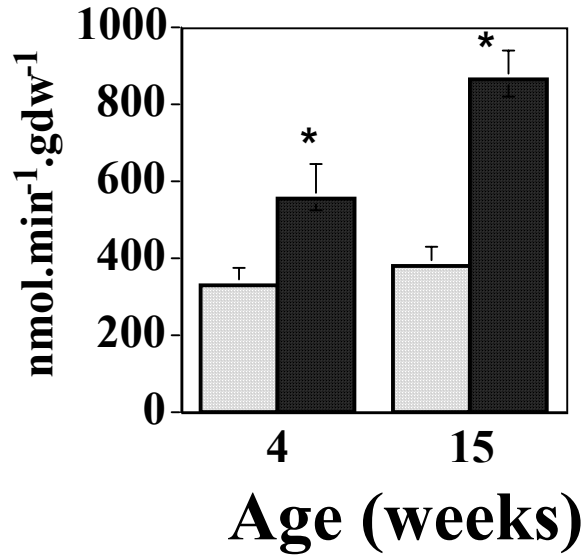
# Blood Glucose in db/db on the c57BlKS background at 40-wks of Age

	BW	Glucose		Insulin	
		0 Min	30Min	0 Min	30Min
Db/db	59±3	564±7	>600*±0	1.6±0.2	2.1±0.3
Control	27±0.5	99±3	152±3	0.15±0.04	0.30±0.03

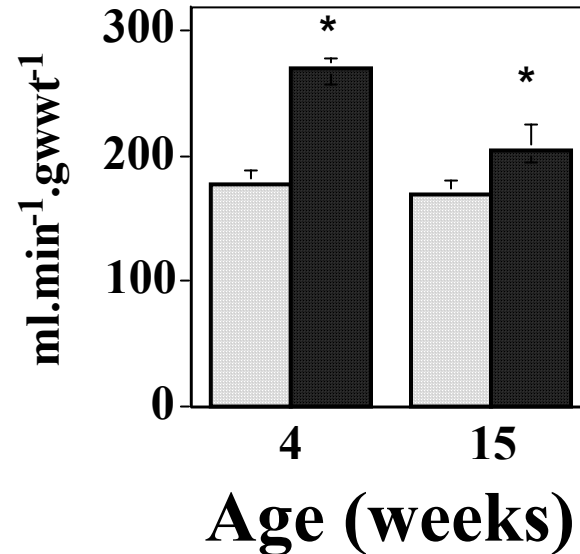
\* 600 mg/dl is the maximum value on the glucometer

# Abnormal Substrate Metabolism and Increased $MVO_2$ Precede the Onset of Hyperglycemia in db/db mice

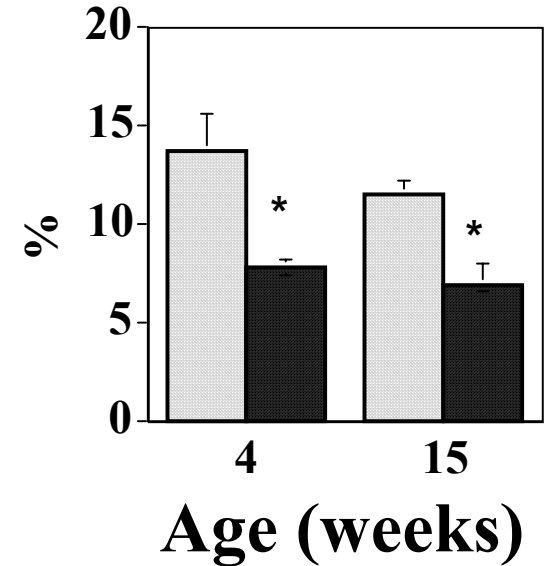
### FA Oxidation Rates



### $MVO_2$

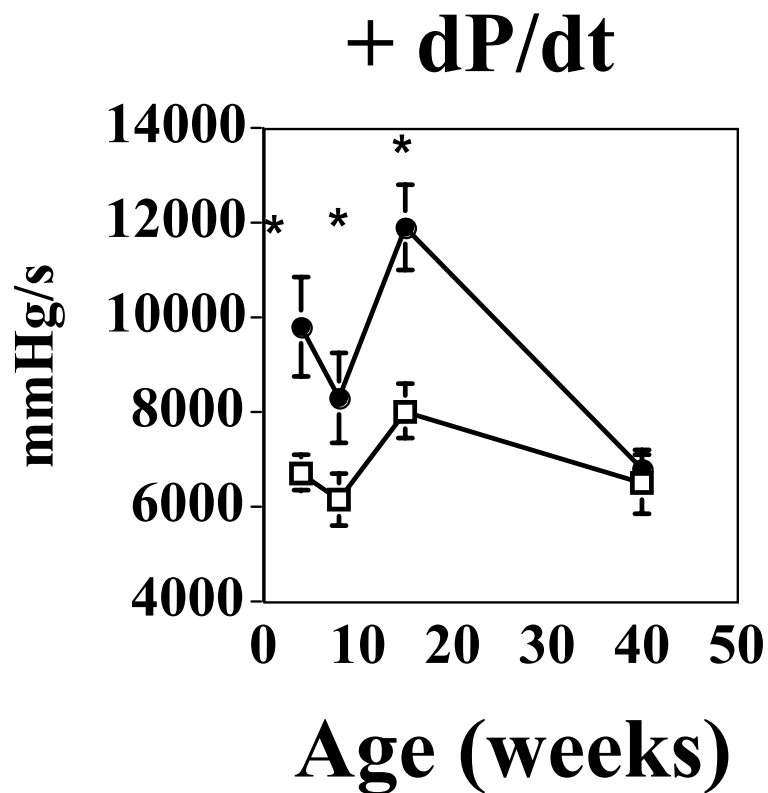


### Cardiac Efficiency

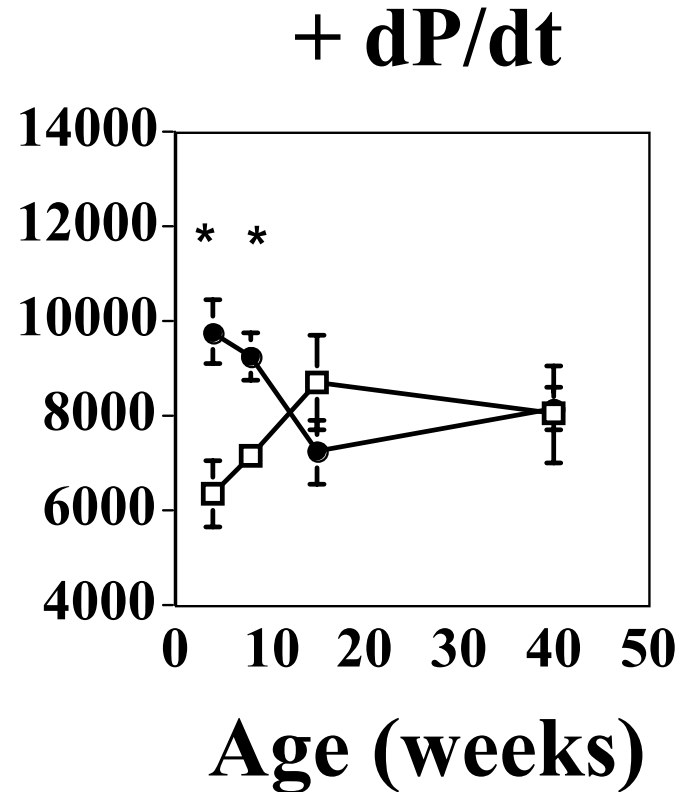


□ db+  
■ db/db

# Hemodynamics ob/ob and db/db mice



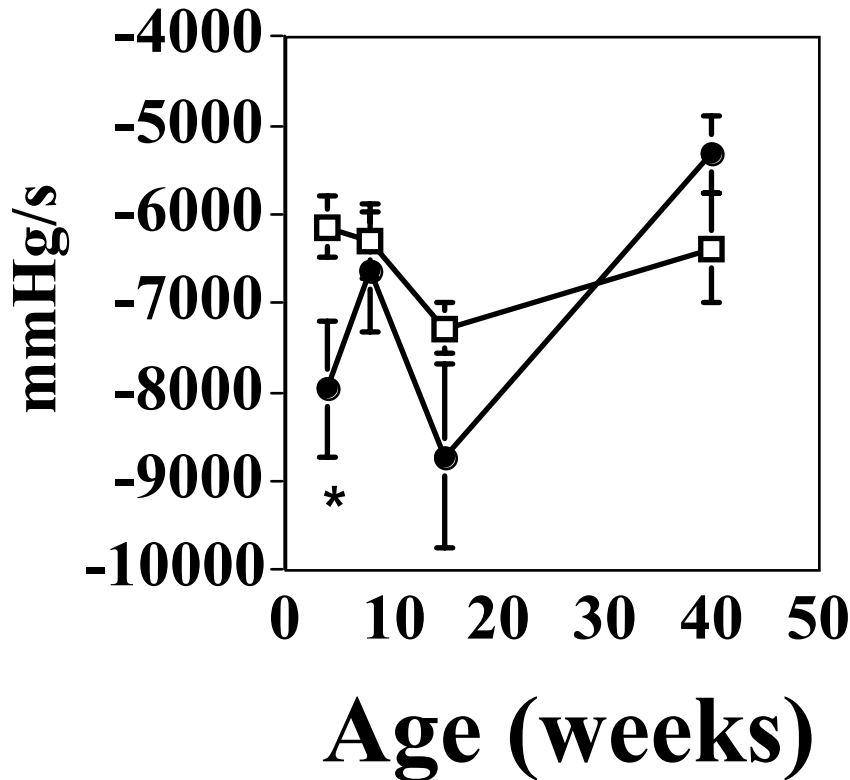
—□— ob+  
—●— obob



—□— db+  
—●— dbdb

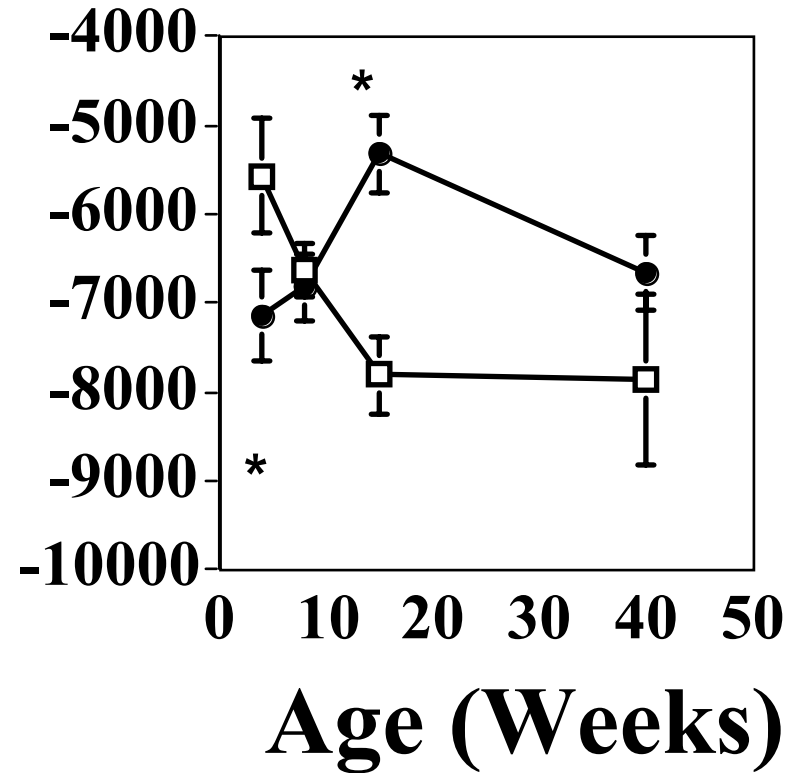
# Hemodynamics ob/ob and db/db mice

## - dP/dt



—□— ob+  
—●— obob

## -dP/dt



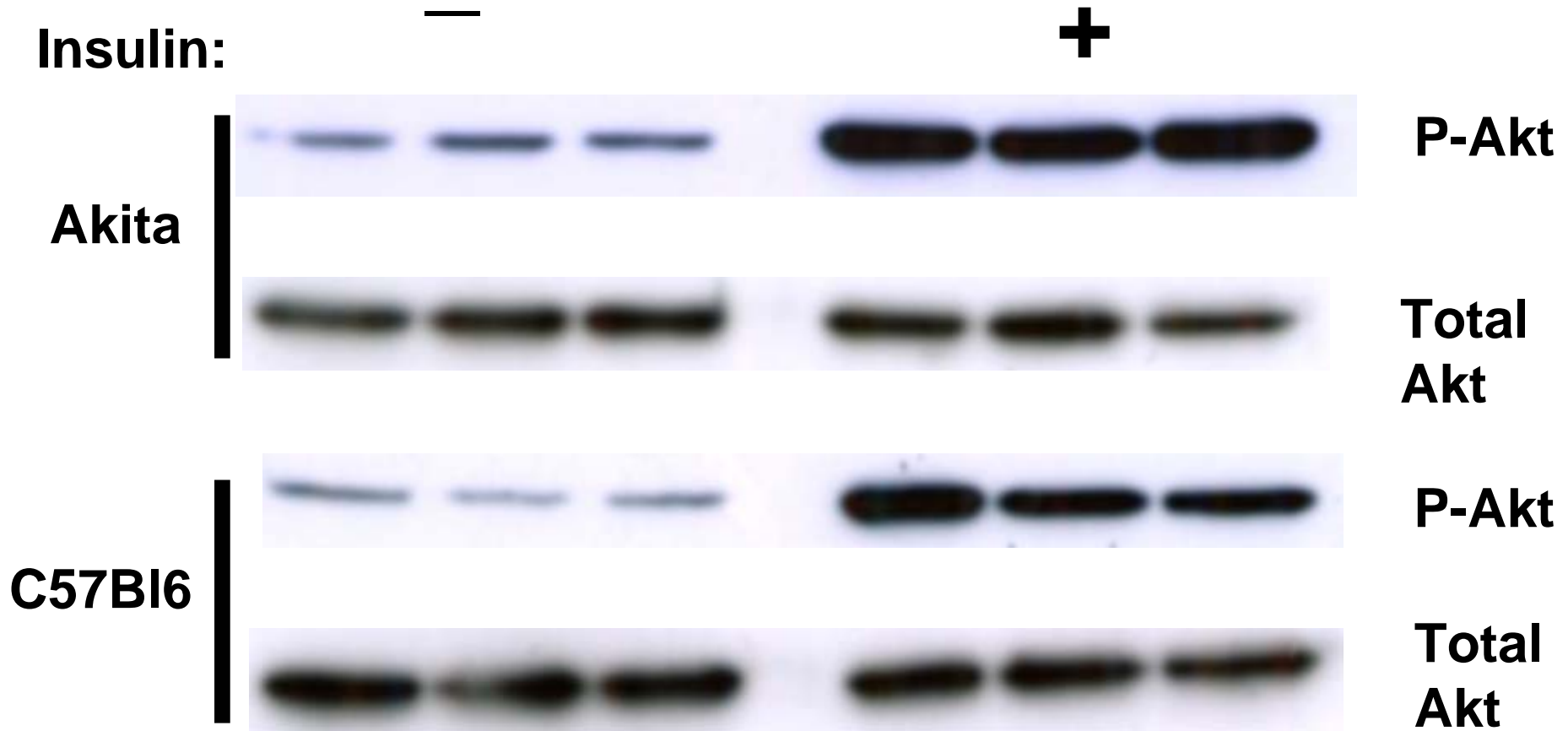
—□— db+  
—●— dbdb

# Akita versus Ob/ob Mice

<b>Parameter</b>	<b>Akita</b>	<b>Ob/ob</b>
Hyperglycemia	Severe from Weaning	Progressive
Hyperinsulinemia	No	Yes
Obesity	No	Yes
Myocardial Insulin Resistance	No	Yes
Cardiac Dysfunction	Yes	Yes but different basis



# Hearts from 8-Wk Old Akita Mice are Insulin Sensitive

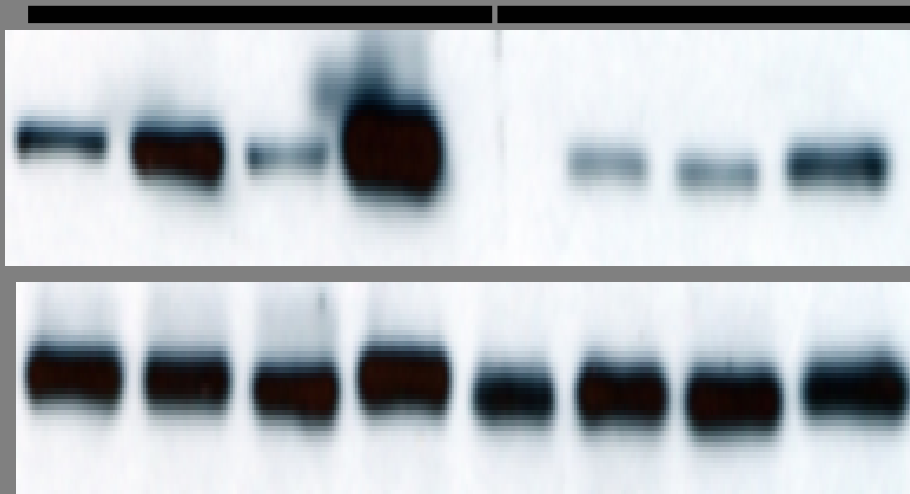


# Insulin-Stimulated Activation of Akt is Impaired in ob/ob mouse hearts

Wildtype                      ob/ob

p-Akt

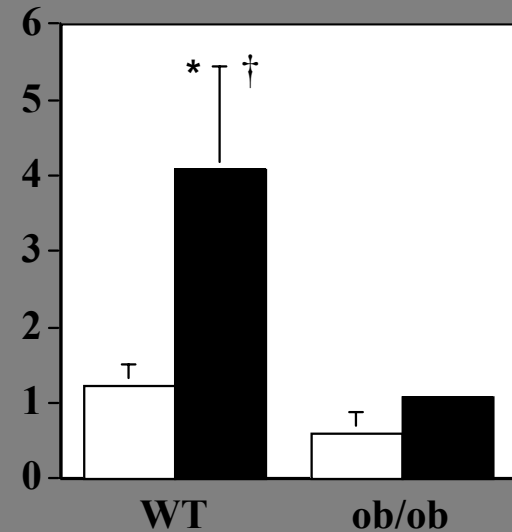
t-Akt



Insulin: - + - + - + - +

Densitometry (Arbitrary Units)

pAkt/Akt



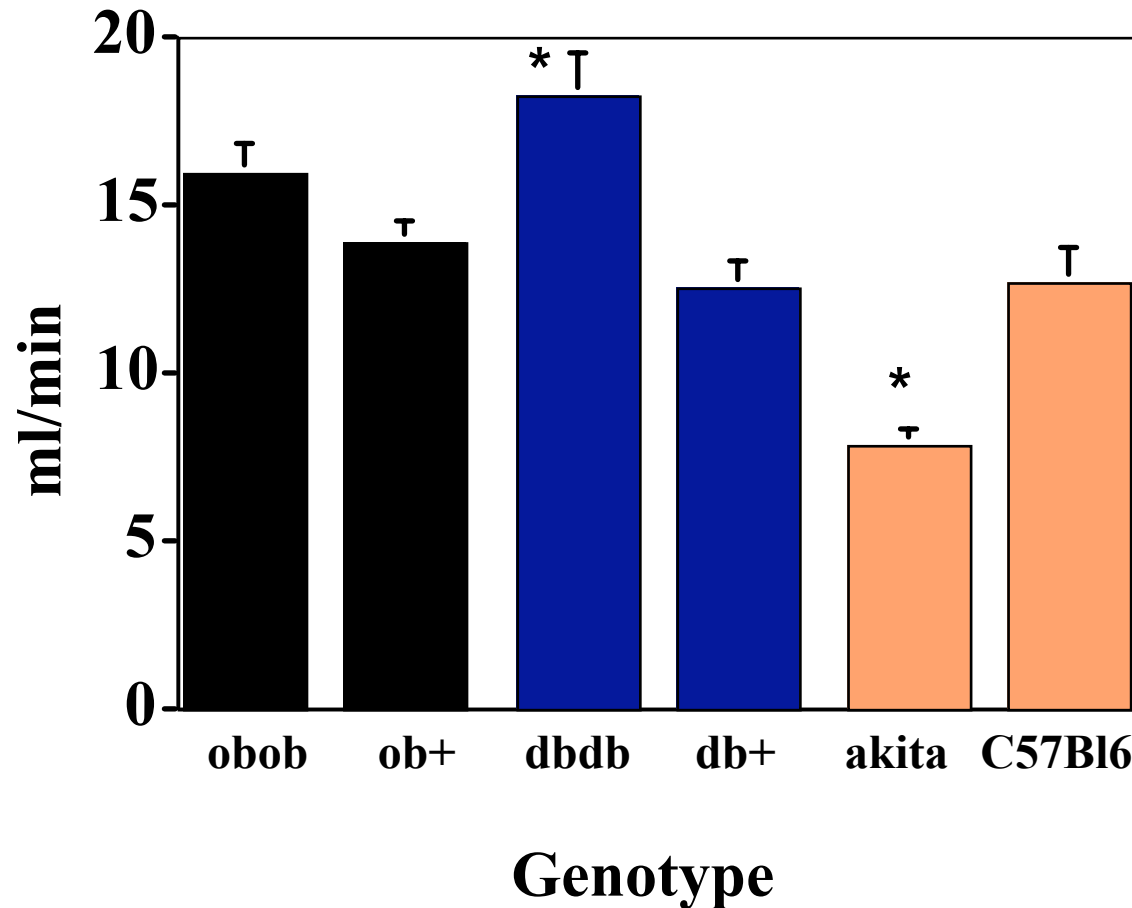
Genotype

□ Insulin 0

■ Insulin 1nM

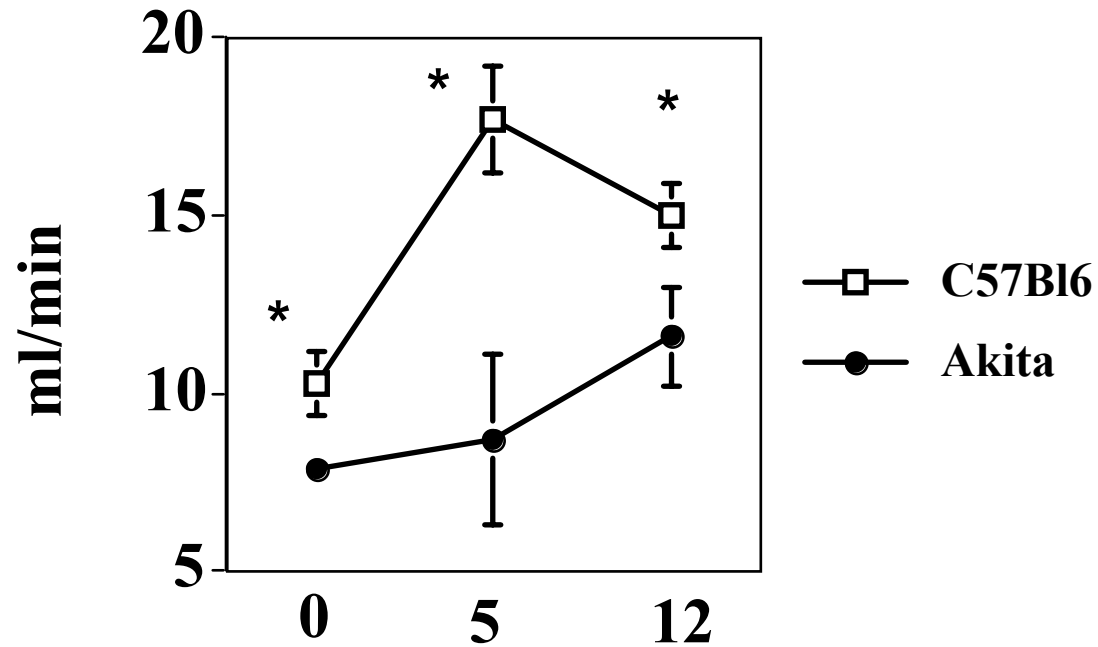
# Cardiac Output Across 3-Models of Diabetes

## Cardiac Output



# Impaired Response of Akita Mice to Isoproterenol

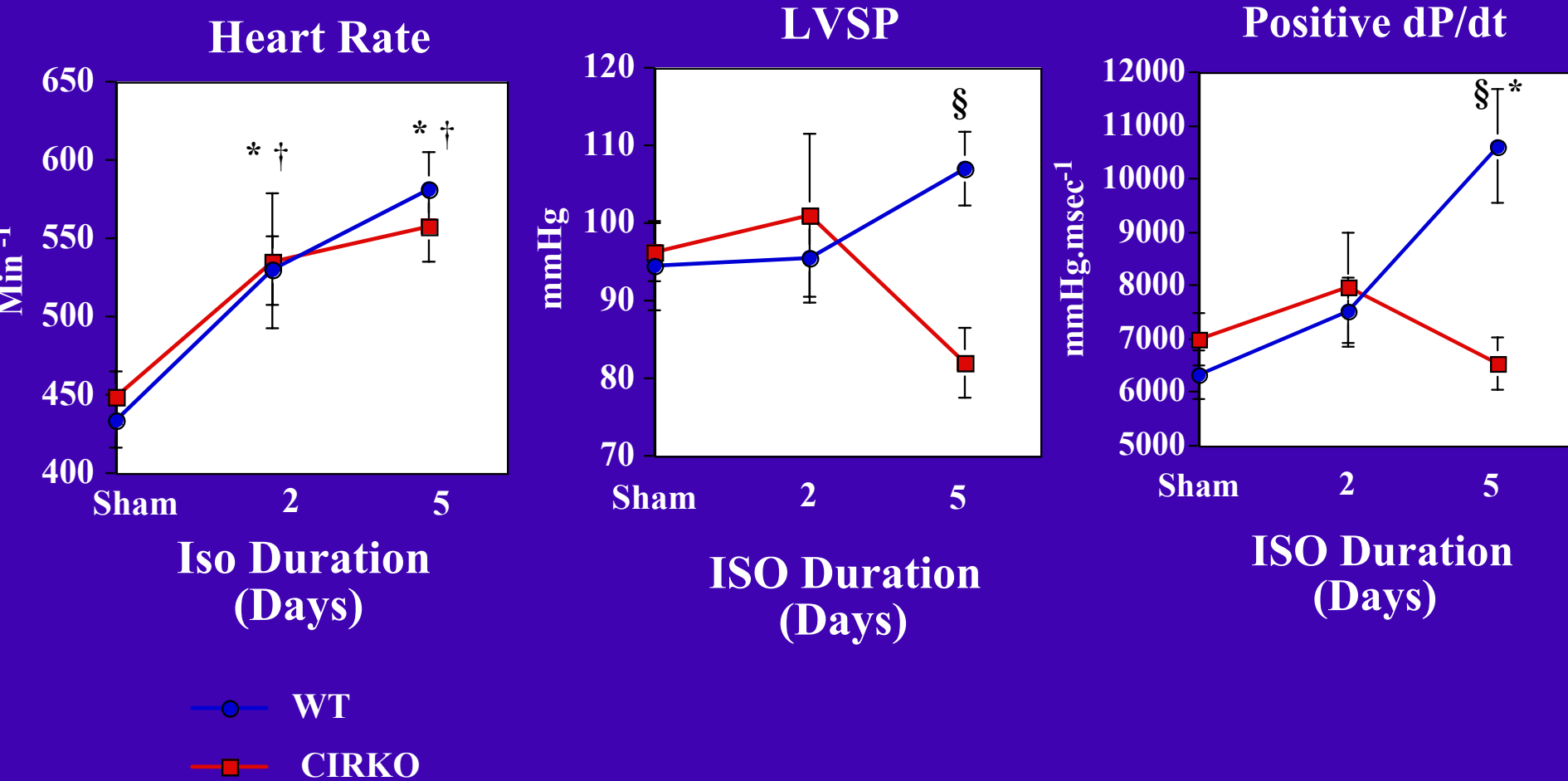
## Cardiac Output



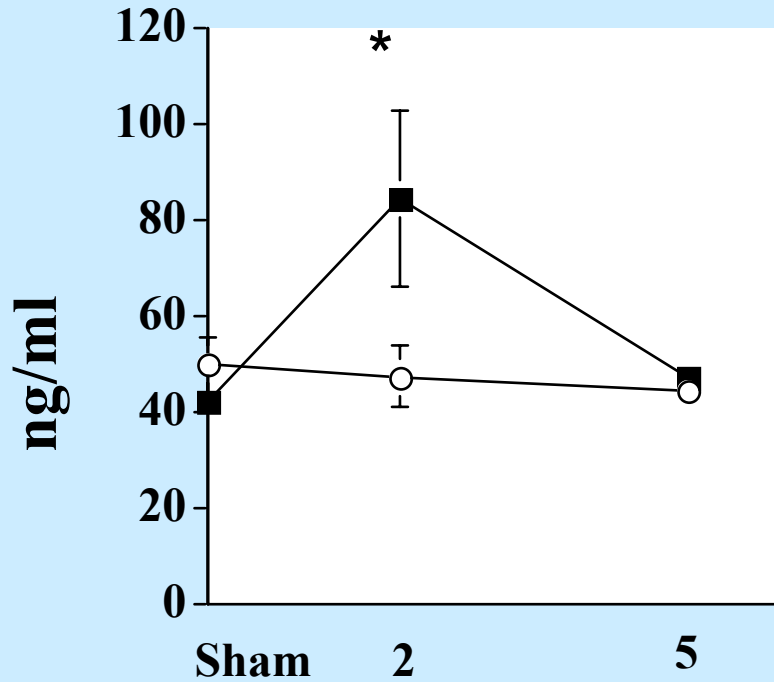
ISO Duration (Days)

**Insulin Signaling Modulates  
The Metabolic Response to  
Pressure Induced Hypertrophy**

# Blunted Hemodynamic Response of CIRKO Hearts to 5 Days of Isoproterenol



# Serum Troponin



**Duration of Isoproterenol  
(Days)**

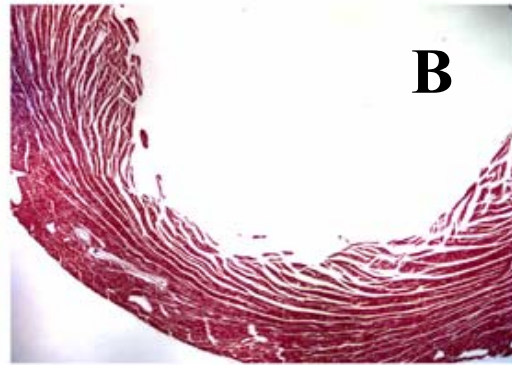
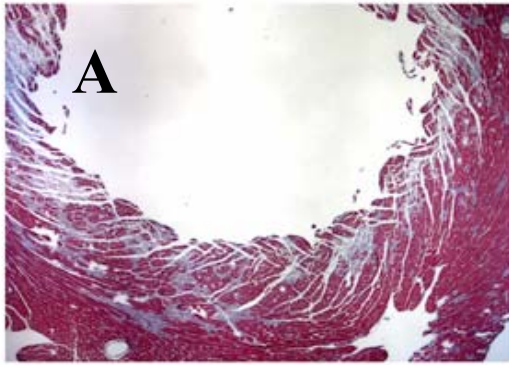
—○— WT

—■— CIRKO

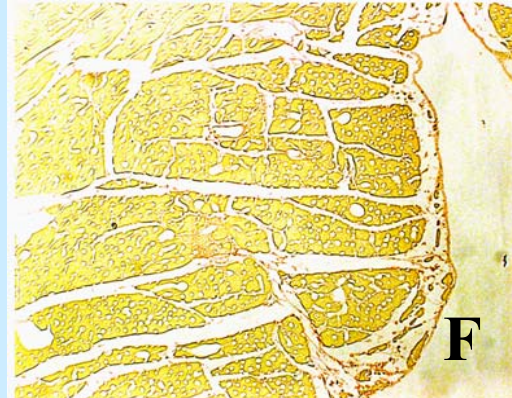
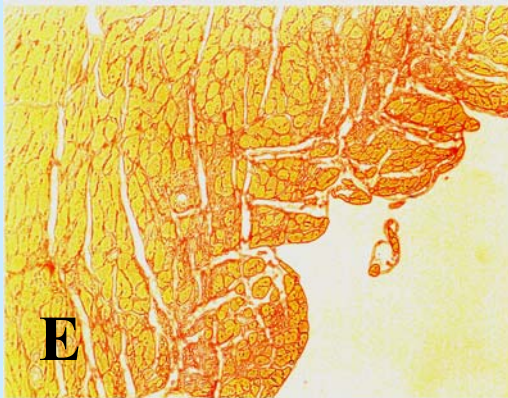
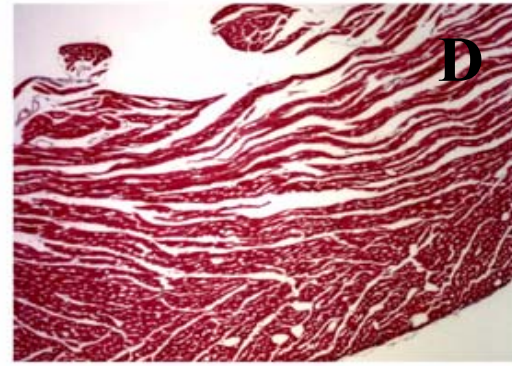
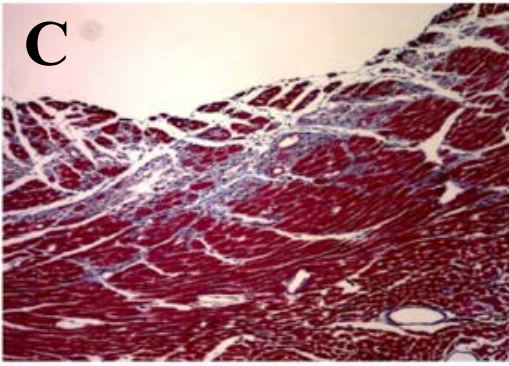
CIRKO ISO

WT ISO

4X

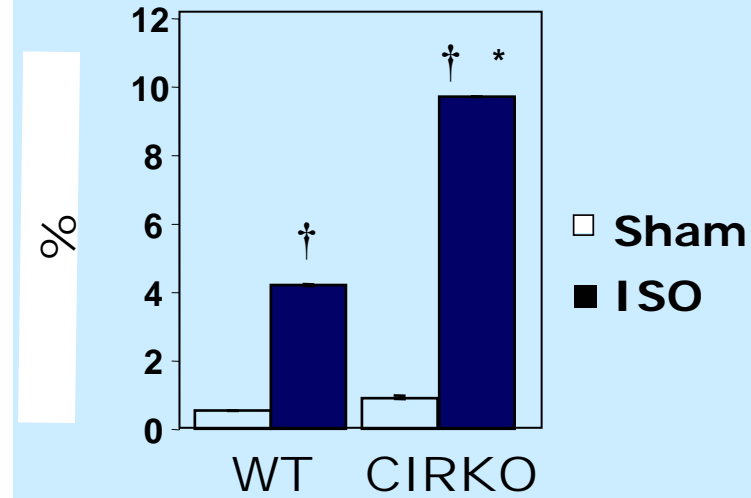


20X



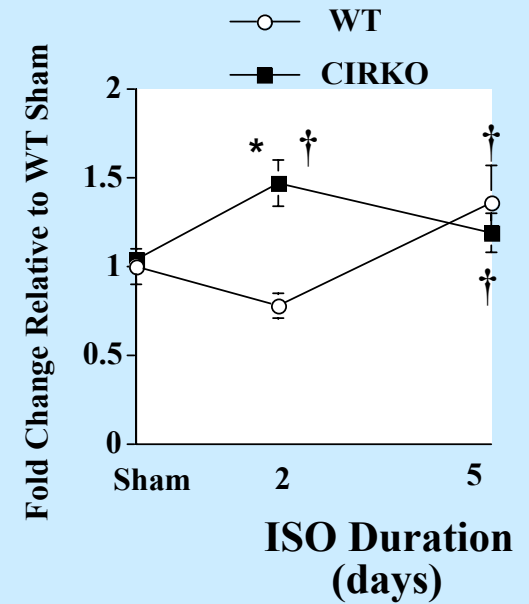
G

## Collagen Volume Fraction



## CTGF mRNA Abundance

H



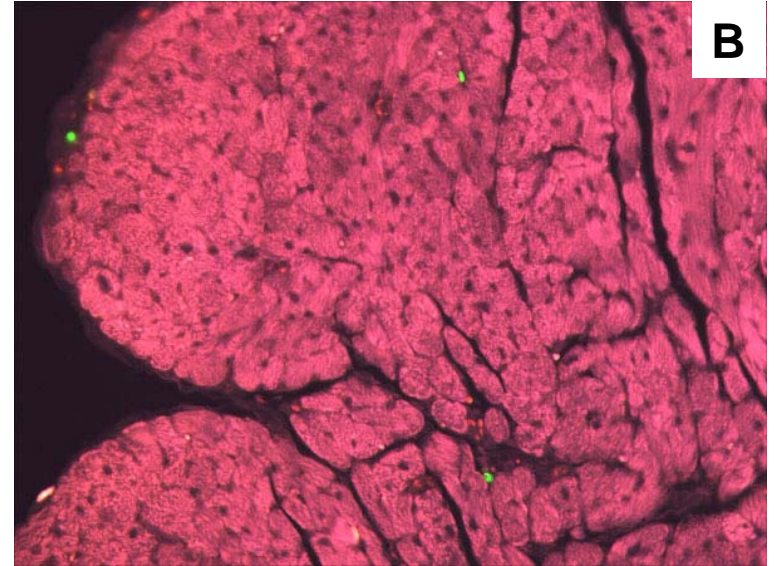
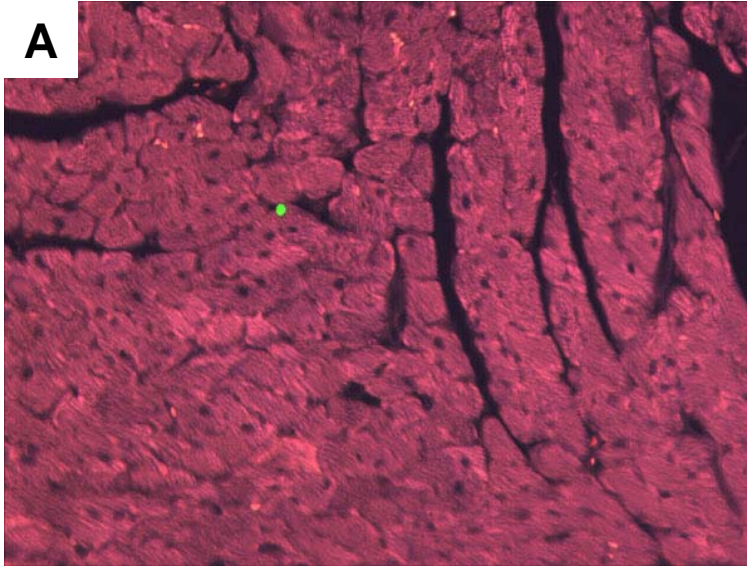


# TUNEL STAINING OF CIRKO HEARTS

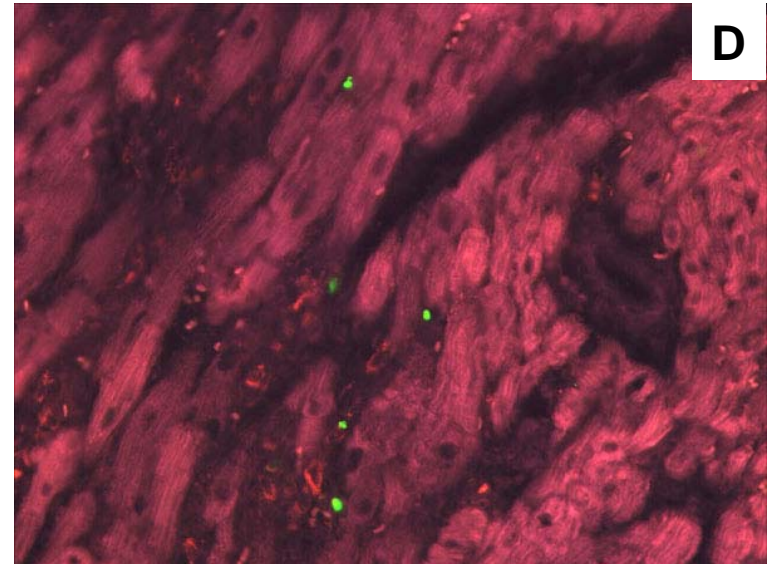
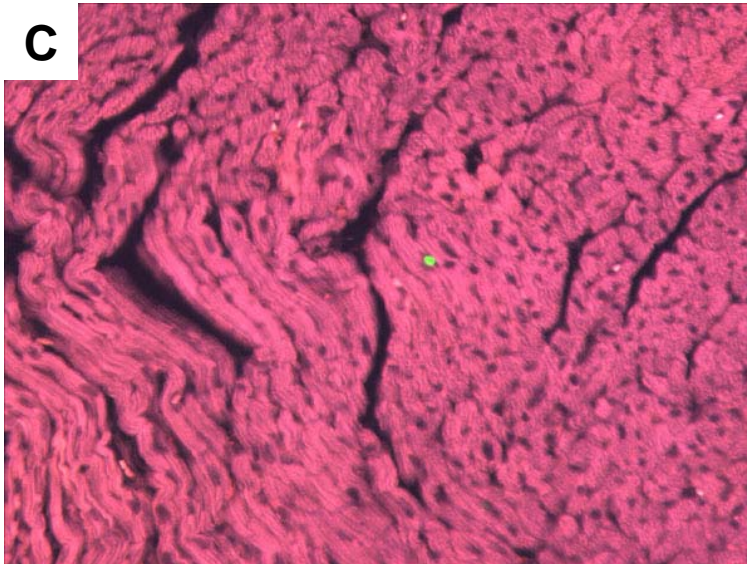
SHAM

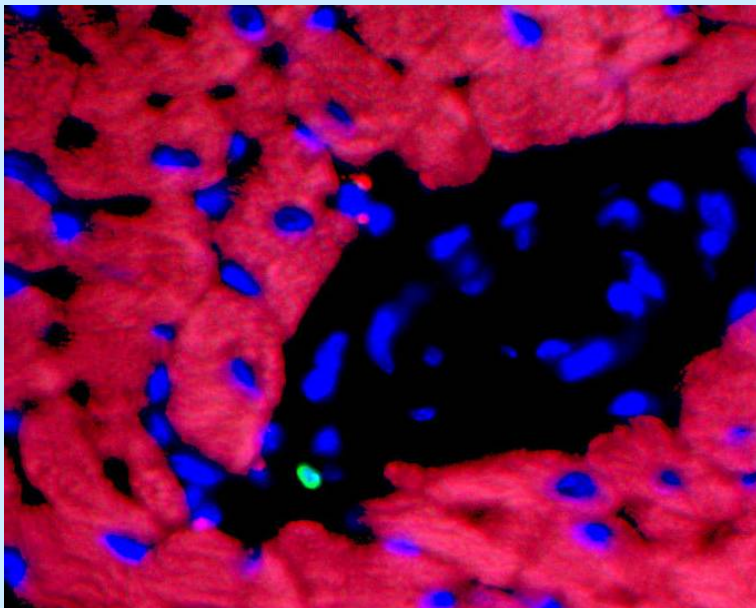
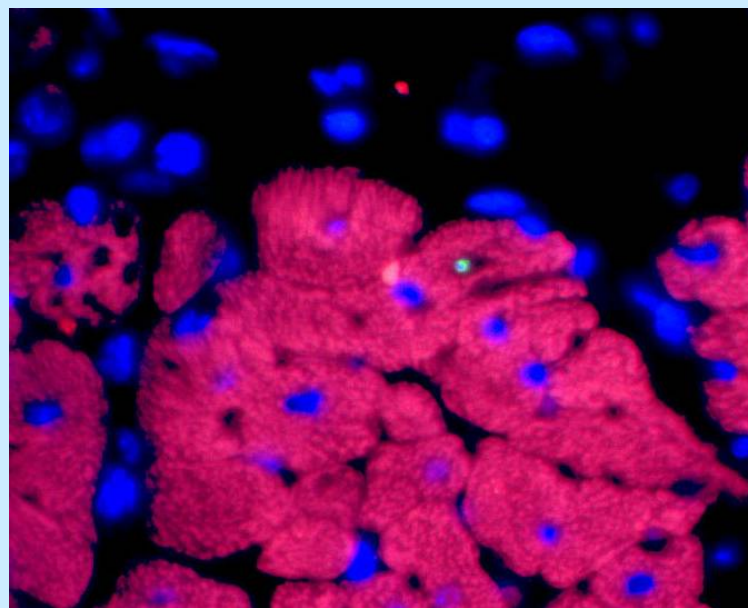
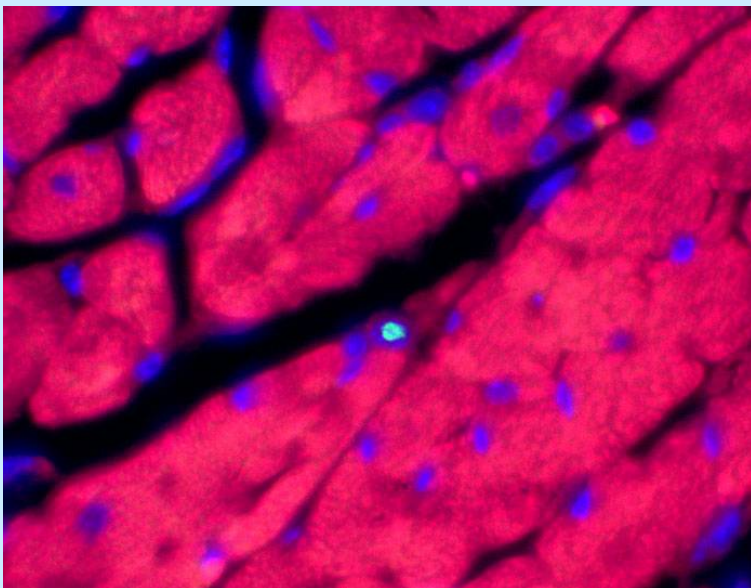
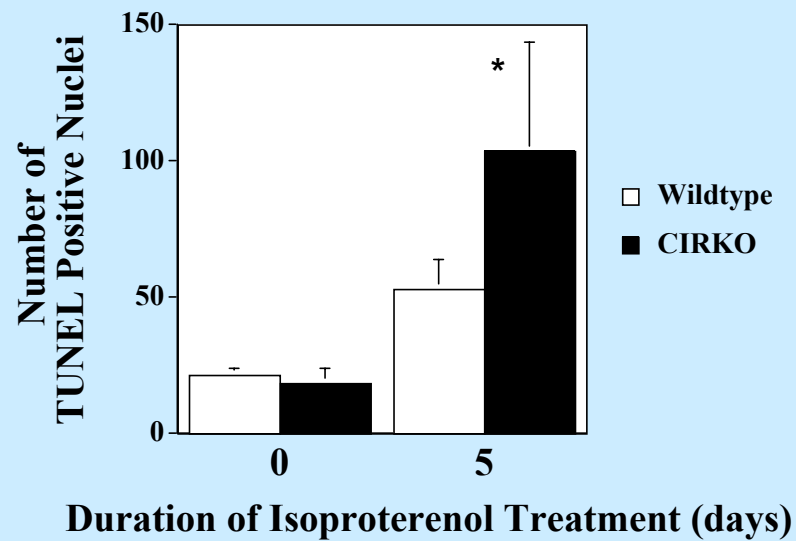
ISO

Wild  
Type



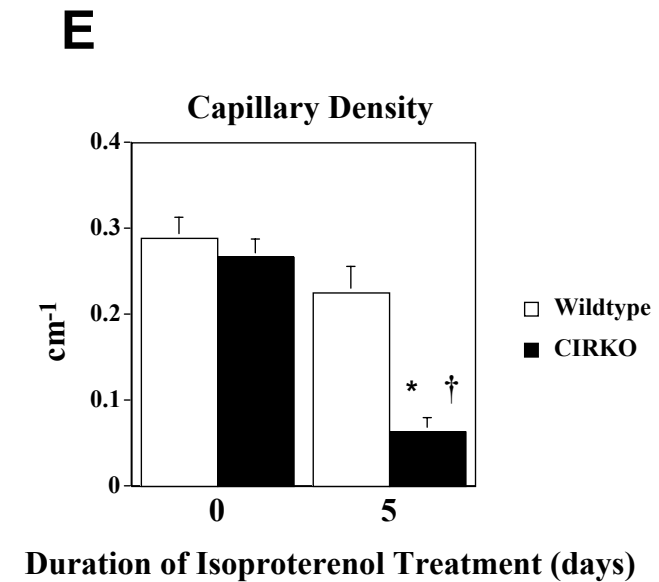
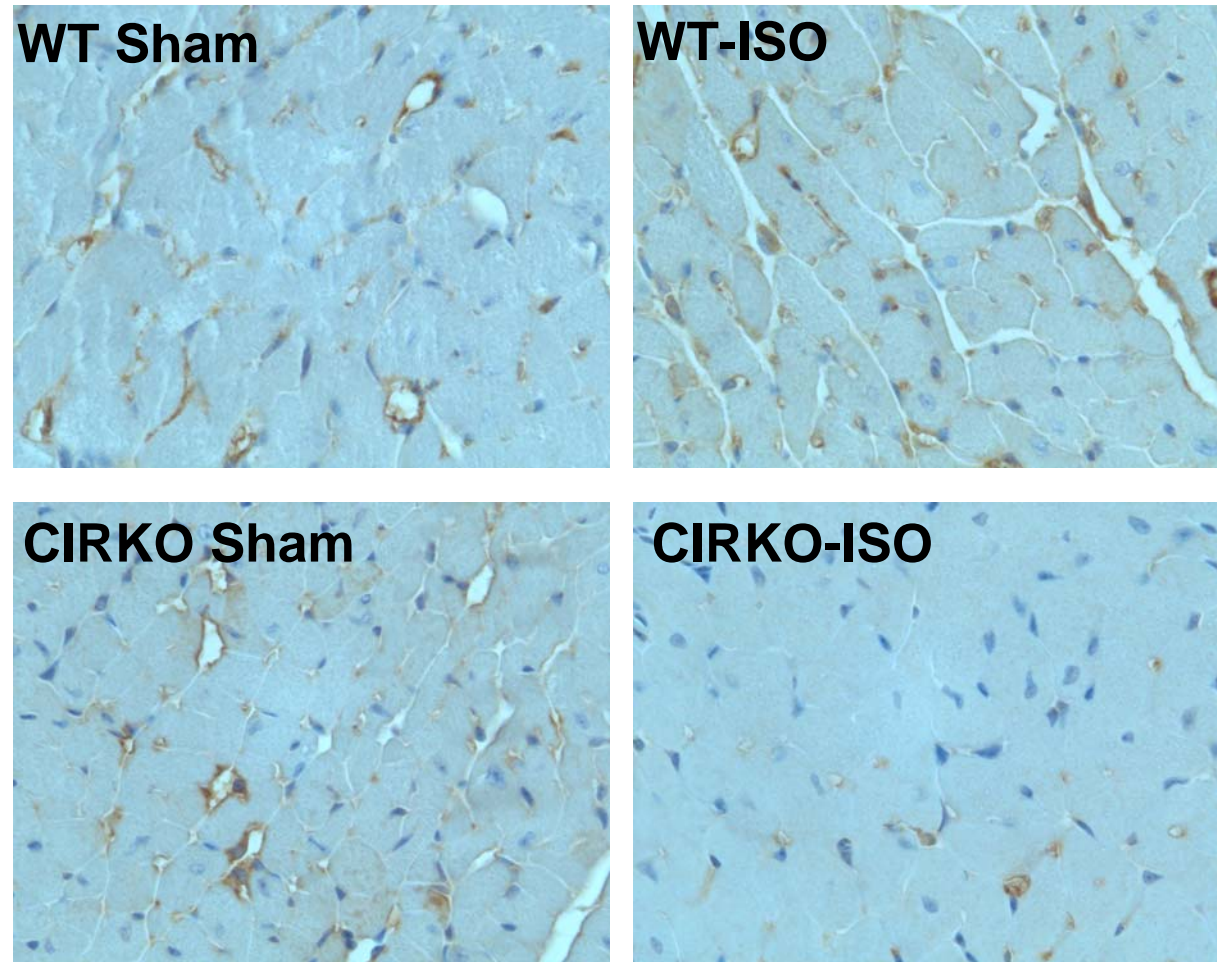
CIRKO



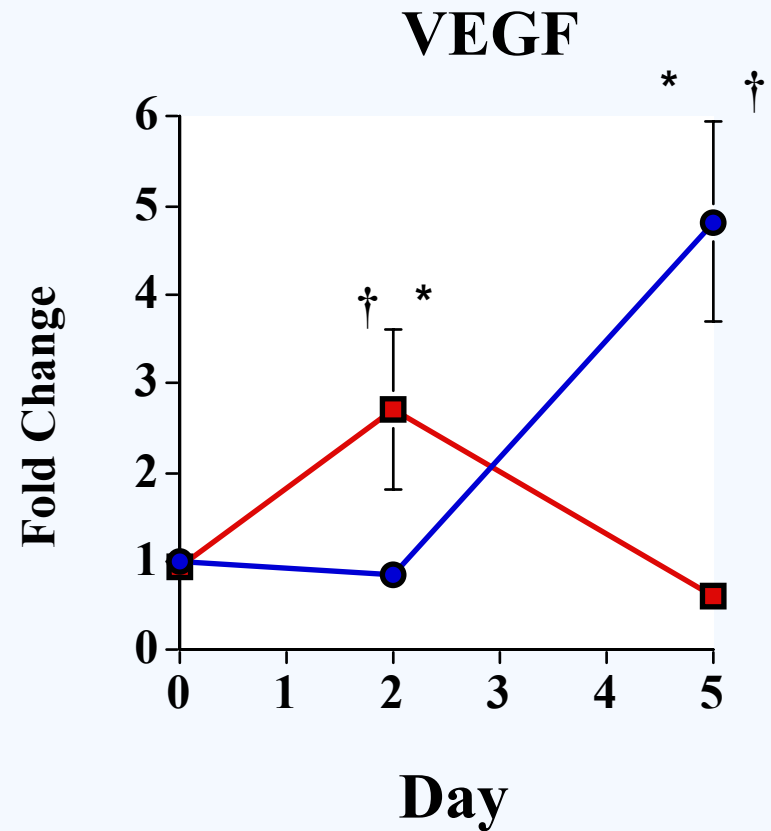
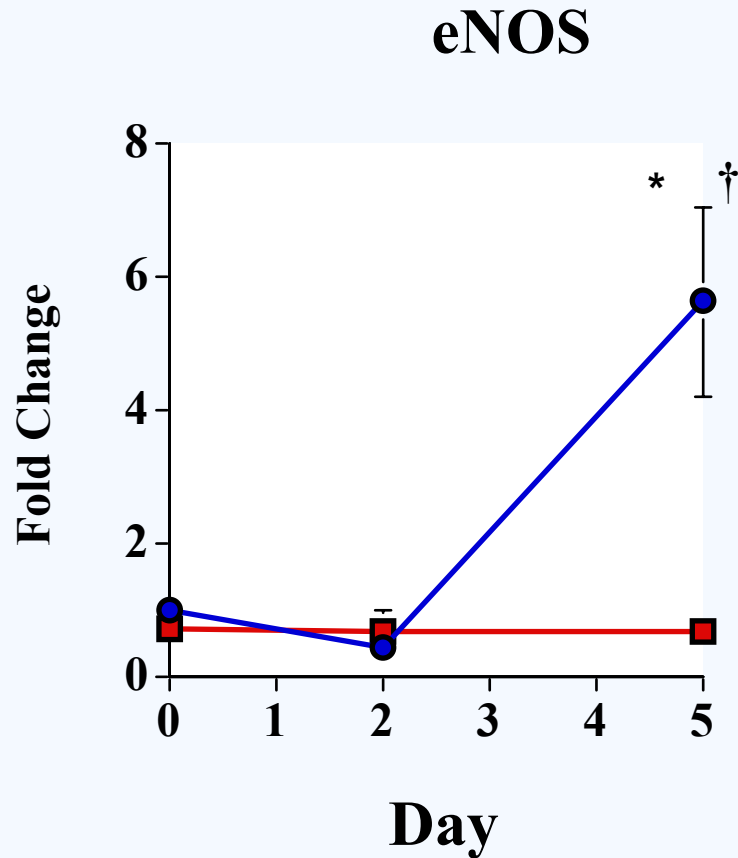
**E****F****G****H**



# Reduced Capillary Density in ISO Treated CIRKO Hearts



# Impaired Angiogenic Gene Expression



- WT
- CIRKO

# Expression Levels of Hypoxia Inducible Factor -1 (HIF-1 $\alpha$ ) are Reduced in CIRKO Hearts

HIF1

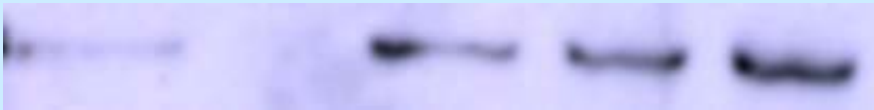


WT

Tubulin



HIF1

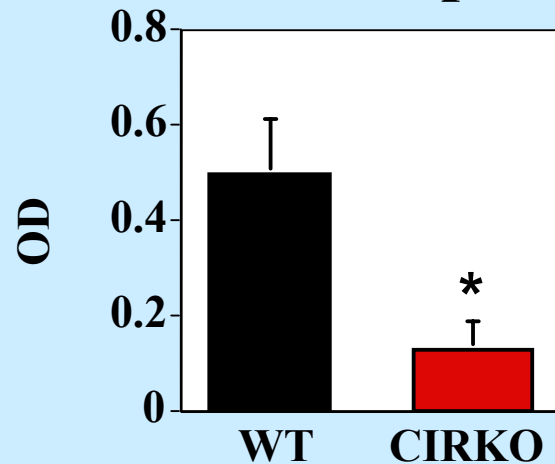


CIRKO

Tubulin



HIF-1 Alpha



**Extrinsic Cues**



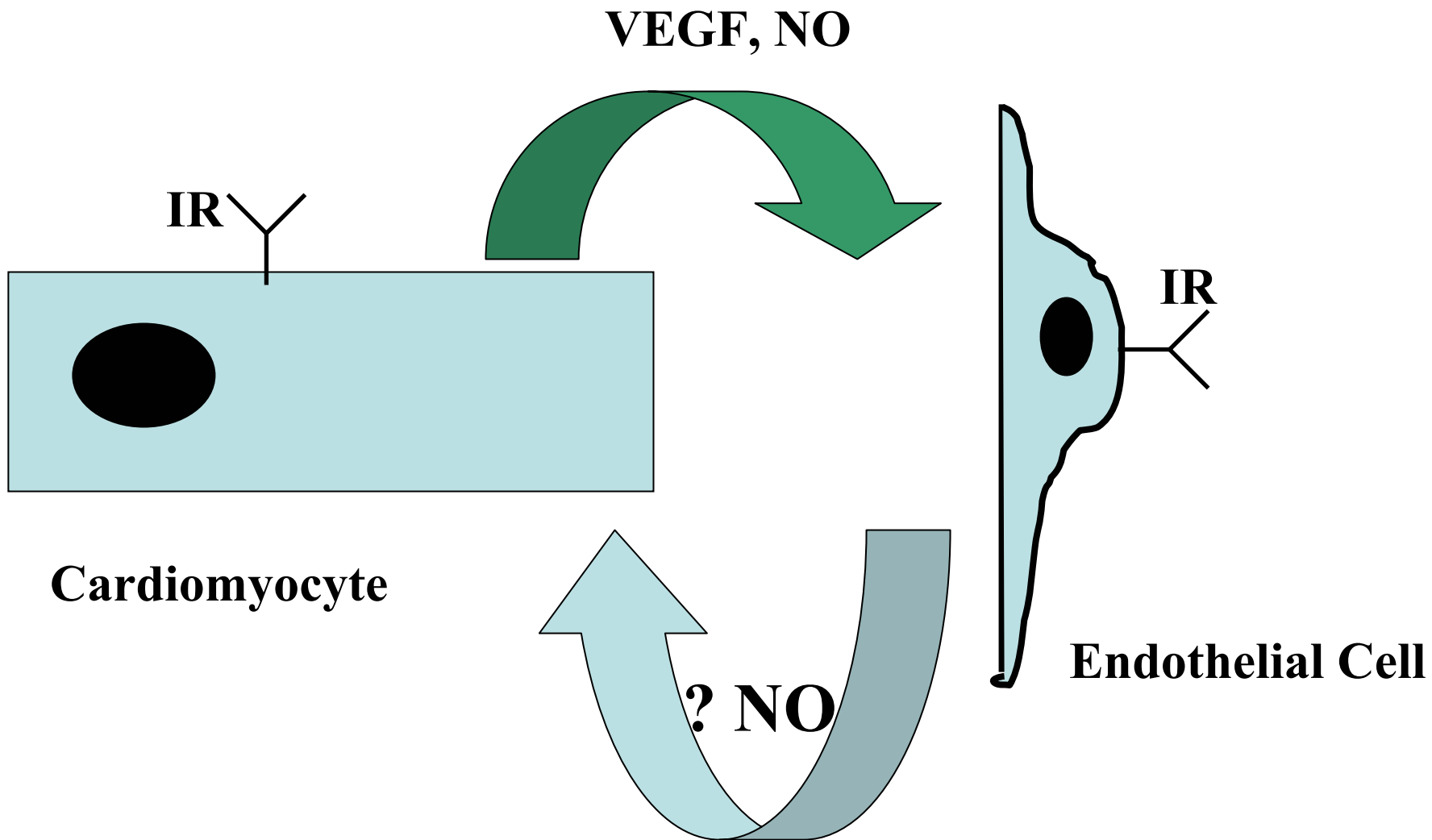
**Insulin Signaling**



**HIF1-Alpha**



**Regulation of GLUT1 and VEGF**



**Mouse Models (3) Which Are  
Extensively Phenotyped  
(Near Complete)**



# Phenotyping to Date



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<b>Criteria/Validation</b>	<b>Db/db mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Trichrome pending. Increased myocardial lipid.
Cardiac Hypertrophy	No
Invasive Assessment of LV Function	↑LVSP, ↑LVDP as early as 4-weeks of age, ↑dP/dt (4 wks), contractility declines after 15 weeks of age.
Isolated Hearts	Decreased LV Function much worse than ob/ob.
Cardiac Metabolism/Mitochondrial Function	↓Glucose Ox, ↑FA OX & MVO <sub>2</sub> Mitochondrial Dysfunction is present. Precedes hyperglycemia and worsens thereafter
Gene Expression	MHC isoforms switched.
Response to Stress	Pending
Electrophysiology	Prolonged action potential.

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>Ob/ob Mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Trichrome pending, Increased myocardial lipid.
Cardiac Hypertrophy	Yes
Invasive Assessment of LV Function	↑LVSP, ↑LVEDP as mice age, dp/dt↑ at 4 weeks, ↓ at 8-weeks.
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	↓Glucose Ox, ↑FA- OX & MVO <sub>2</sub> Mitochondrial dysfunction is present. Insulin signaling in cardiomyocytes is impaired. Precedes Hyperglycemia
Gene Expression	MHC isoforms switched.
Response to Stress	Aortic Banding in Progress
Electrophysiology	NA

# Phenotyping to Date



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<b>Criteria/Validation</b>	<b>CIRKO Mice</b>
Ejection Fraction	Reduced
Replacement/Interstitial Fibrosis	Increased. Accelerated by Hypertrophy. May Reflect Impaired Angiogenesis
Cardiac Hypertrophy	Age dependent
Invasive Assessment of LV Function	LV Function reduced, $\pm$ Increased Diastolic Pressures.
Isolated Hearts	Decreased LV Function.
Cardiac Metabolism/Mitochondrial Function	$\downarrow$ Glucose Ox, $\uparrow$ FA OX & $MVO_2$ (young) $\downarrow$ FA OX (Old). Mitochondrial dysfunction is present. $\uparrow$ ROS
Gene Expression	MHC isoforms switched.
Response to Stress	Impaired response to ischemia and pressure overload.
Electrophysiology	Prolonged action potential

# **Mouse Models (3) Which We Are Now Focusing On**

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>dNPI3K</b>
Ejection Fraction	Normal
Replacement/Interstitial Fibrosis	Increased following aortic banding
Cardiac Hypertrophy	Not at baseline
Invasive Assessment of LV Function	Pending
Isolated Hearts	Mild reduction in performance
Cardiac Metabolism/Mitochondrial Function	↓ Glucose Ox, ↓ FA- OX Mitochondrial dysfunction is present. Insulin signaling in cardiomyocytes is impaired.
Gene Expression	MHC isoforms not switched, ANF increased.
Response to Stress	Decreased Function After Aortic Banding
Electrophysiology	NA

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>CIRKO+ ACS Mice</b>
Ejection Fraction	Pending
Replacement/Interstitial Fibrosis	Pending
Cardiac Hypertrophy	Pending
Invasive Assessment of LV Function	Pending
Isolated Hearts	Pending
Cardiac Metabolism/Mitochondrial Function	Pending.
Gene Expression	Pending
Response to Stress	Pending
Electrophysiology	Pending

**The Colony is now Established and is Being Expanded for Phenotyping**

# Phenotyping to Date



<b>Criteria/Validation</b>	<b>Akita Mice</b>
Ejection Fraction	In Progress
Replacement/Interstitial Fibrosis	Pending
Cardiac Hypertrophy	Pending
Invasive Assessment of LV Function	Pending.
Isolated Hearts	Pending
Cardiac Metabolism/Mitochondrial Function	Pending
Gene Expression	Pending
Response to Stress	In Progress
Electrophysiology	Pending

**The Colony is now Established and is Being Expanded for Phenotyping**

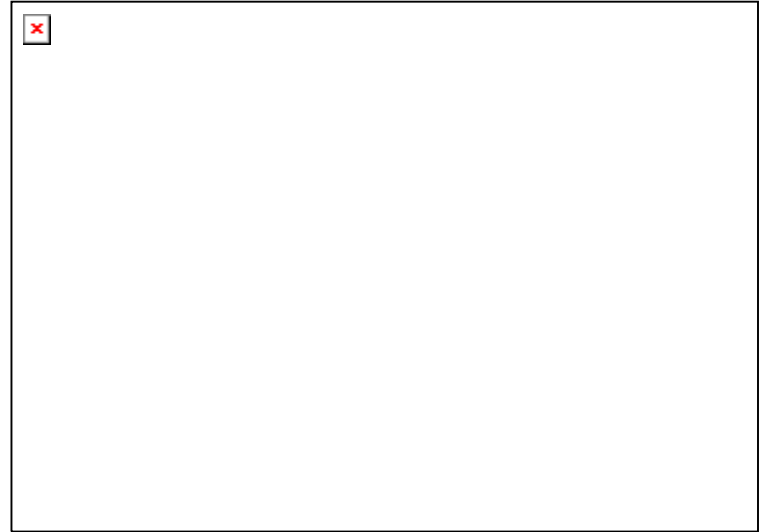
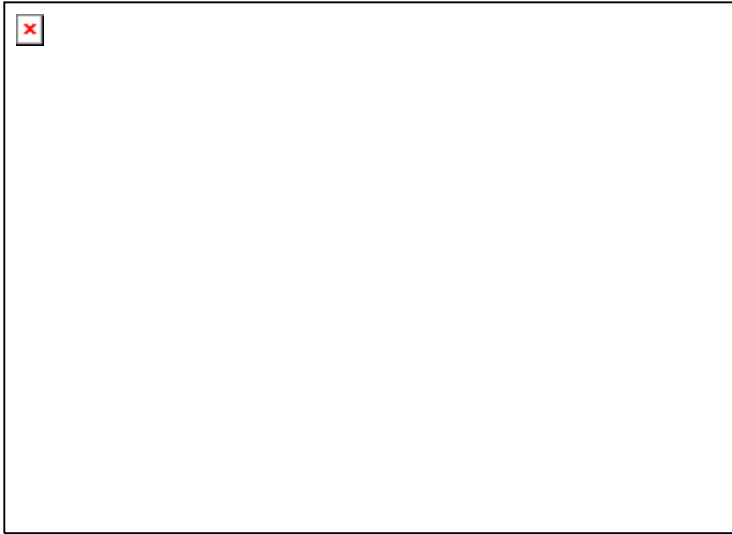
# Current Pertinent Collaborations

- Metabolic characterization of mouse models of lipotoxic cardiomyopathy from the Goldberg Lab (Columbia)
- Cardiac Specific PPAR $\gamma$  KO -Metabolism, Banding, Injury - UCLA
- Histology of Pig Hearts - UNC Chapel Hill
- Phenotyping of PGC-1 $\alpha$  KO mitochondria - Washington University
- IUGR Hearts - University of Utah



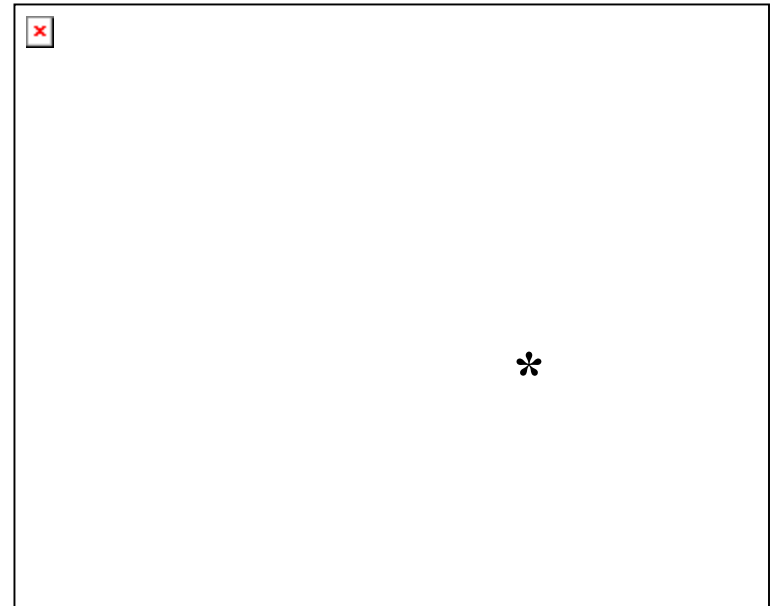
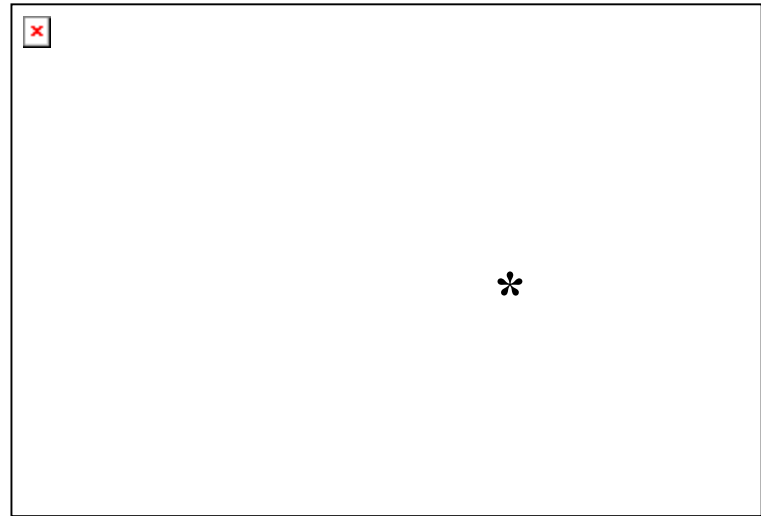
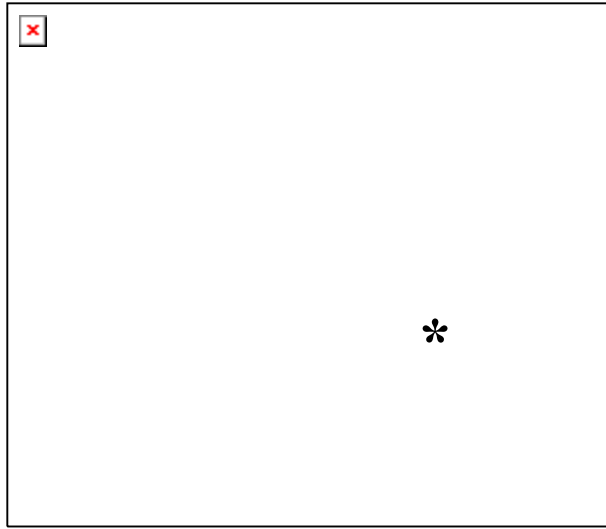


# Targeted Proteomic Analysis of ob/ob Mitochondria



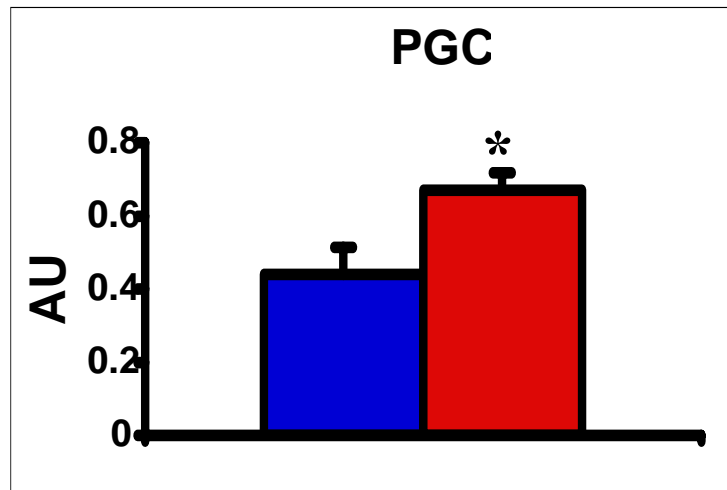
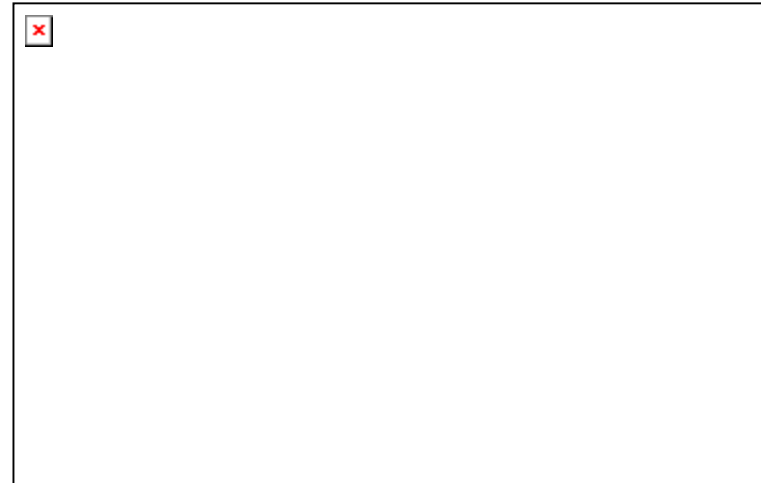
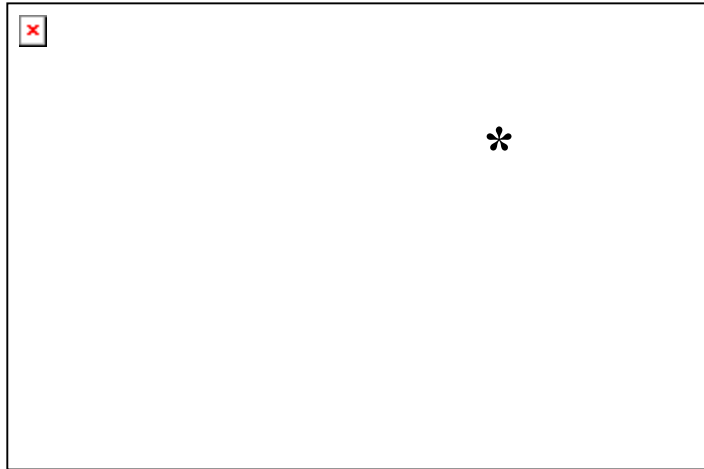
■ WT ■ ob/ob

# Targeted Proteomic Analysis of ob/ob Mitochondria



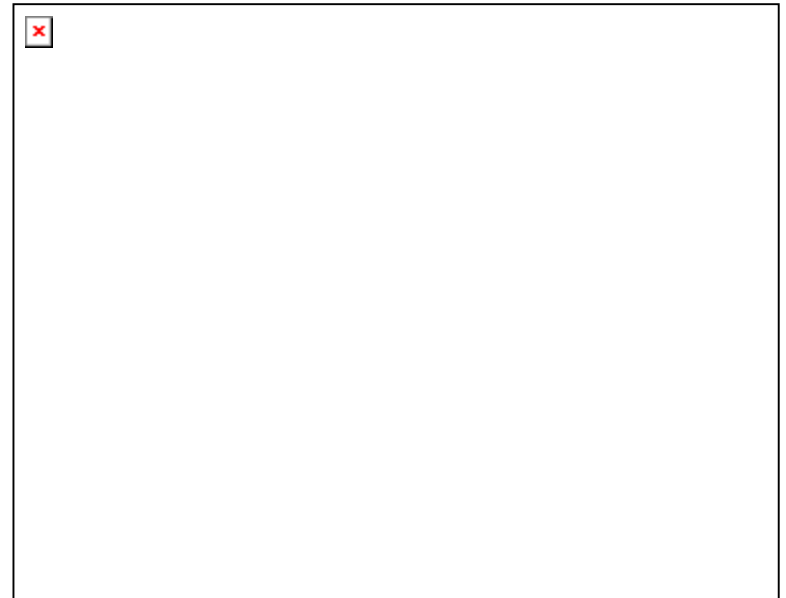
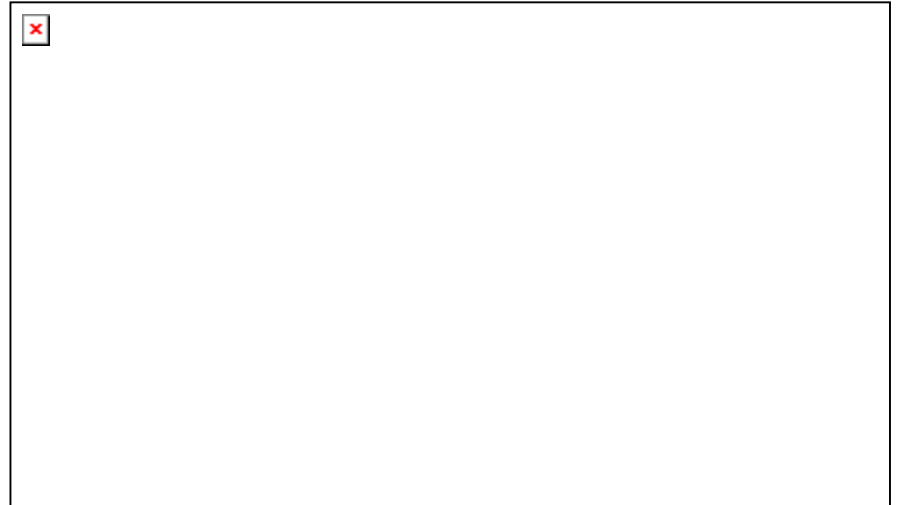
■ WT ■ ob/ob

# Targeted Proteomic Analysis of db/db Mitochondria



■ WT ■ db/db

# Targeted Proteomic Analysis of db/db Mitochondria



■ WT ■ db/db