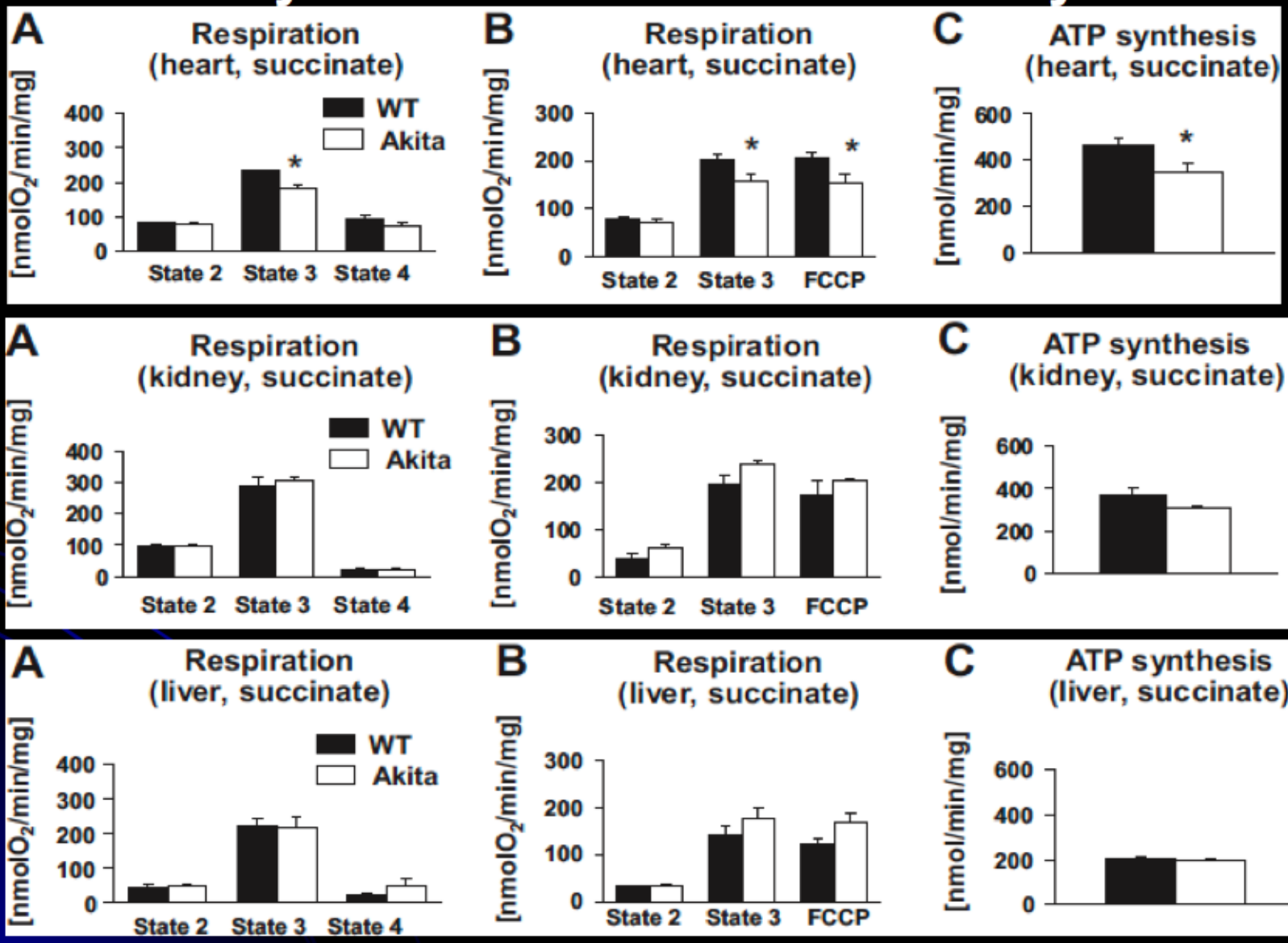
Analysis of Mitochondria Across Multiple Tissues in the Akita Mouse

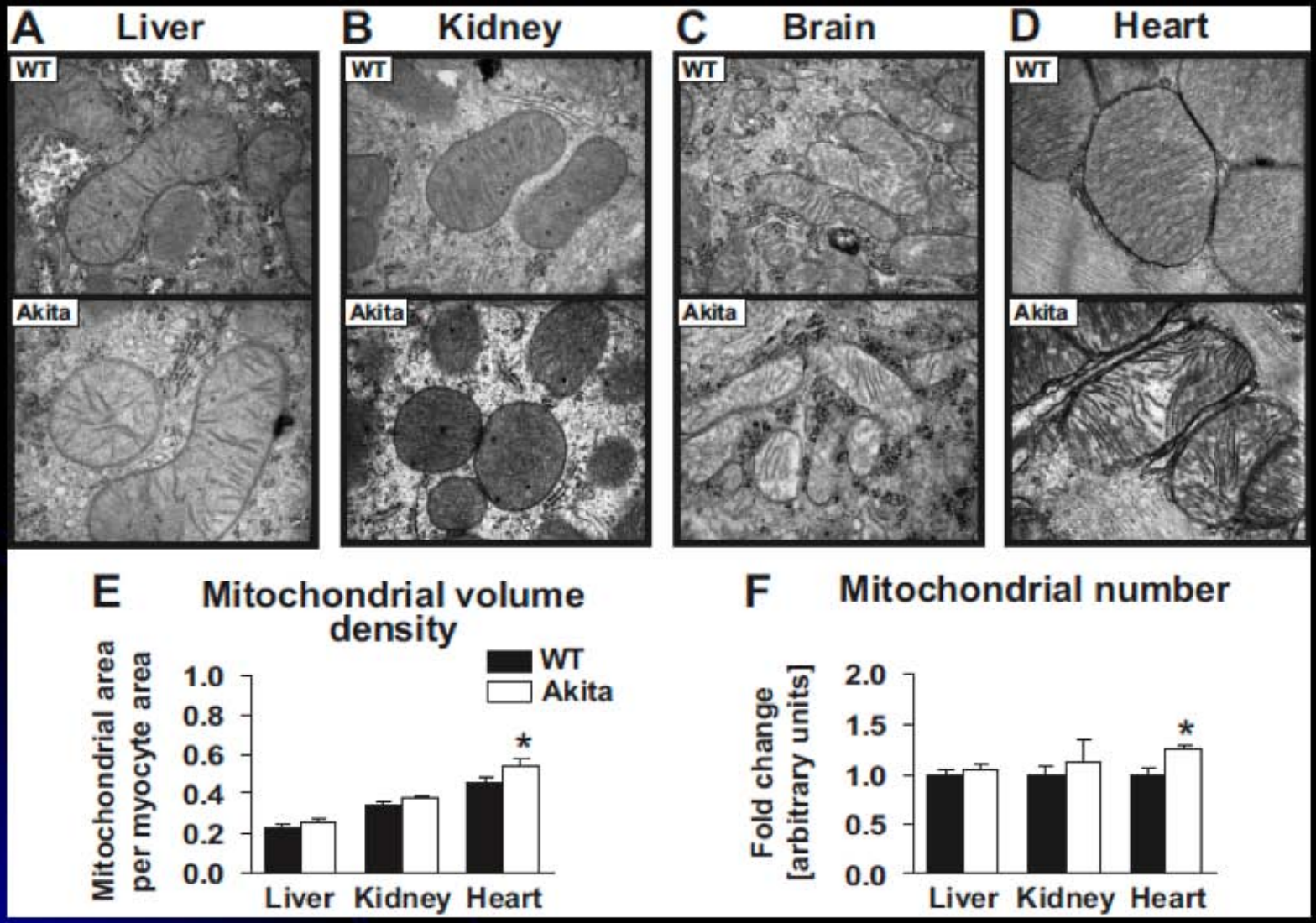
- Function
- Morphology
- Proteomics

Relative to Liver, Brain and Kidney the Diabetic Myocardium is Particularly Vulnerable to Mitochondrial Dysfunction



Bugger et al., Diabetes, 2009

Altered Mitochondrial Morphology in Heart but not Liver, Kidney or Brain of Akita Mice



Bugger et al., Diabetes, 2009

Mitochondrial Proteome -Akita Mice

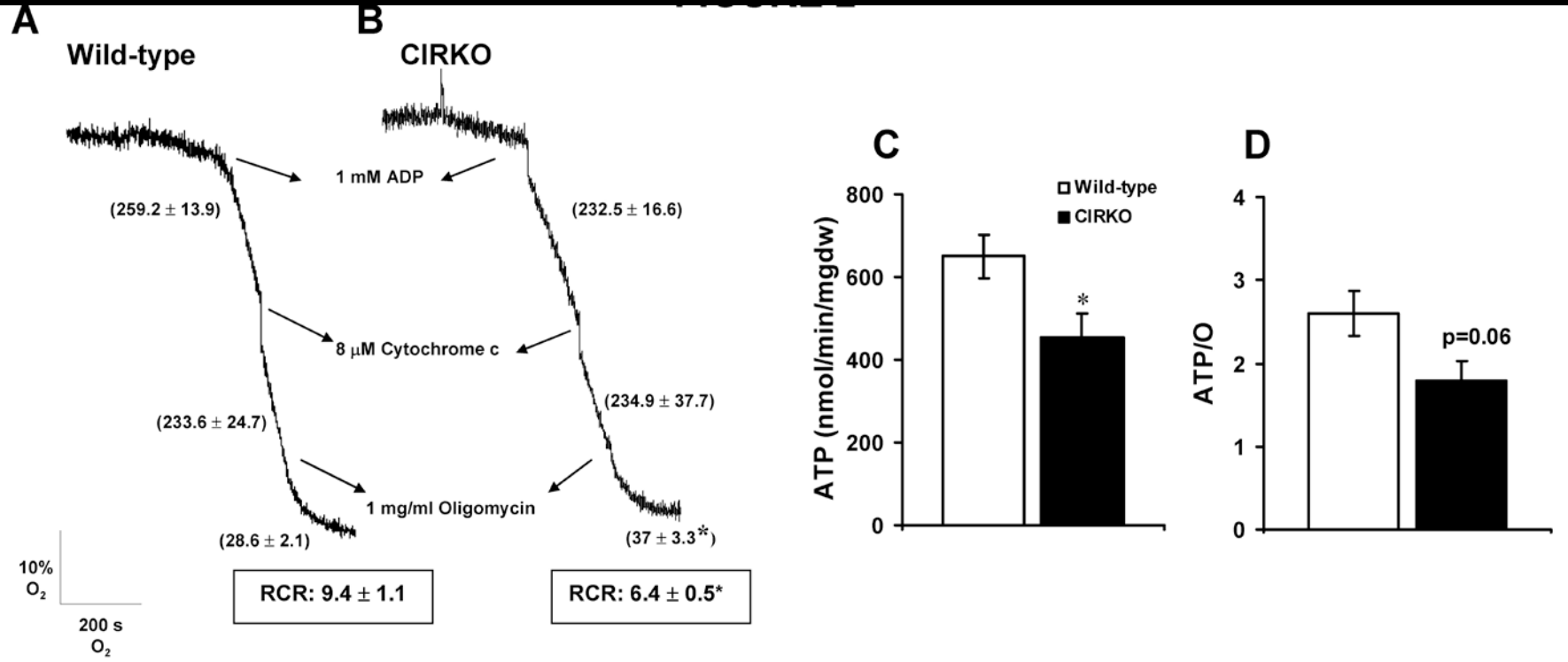
Table 1. Abundance of FAO proteins, TCA cycle enzymes, and OXPHOS subunits in mitochondria of liver, brain, heart and kidney of Akita, presented as fold change compared to WT. Highlighted cells indicate a significant difference ($p<0.05$) compared to WT (red = increased, yellow = decreased). n.d. = protein was not detected

Protein	Liver	Brain	Heart	Kidney
Fatty acid oxidation				
acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	0.75	1.27	1.41	1.69
acetyl-Coenzyme A dehydrogenase, long-chain	0.89	1.25	1.14	1.18
acetyl-Coenzyme A dehydrogenase, medium chain	0.96	1.12	1.03	0.94
acyl-Coenzyme A dehydrogenase, short chain	0.76	1.45	1.11	1.03
carnitine O-octanoyltransferase	0.70	n.d.	n.d.	0.68
dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenyme A isomerase)	0.83	1.14	1.11	1.32
enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	1.05	1.00	0.94	1.01
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	1.16	1.33	1.20	1.52
TCA cycle				
aconitase 2, mitochondrial	1.07	1.00	0.96	1.32
citrate synthase	0.93	0.97	0.86	1.25
fumarate hydratase 1	1.02	1.04	0.90	1.08
isocitrate dehydrogenase 3 (NAD+) alpha	n.d.	0.87	0.88	1.52
isocitrate dehydrogenase 3, beta subunit	n.d.	1.02	1.20	1.15
malate dehydrogenase 2, NAD (mitochondrial)	0.91	0.97	0.76	1.30
succinate-CoA ligase, GDP-forming, alpha subunit	1.03	0.89	1.19	1.18
succinate-Coenzyme A ligase, ADP-forming, beta subunit	0.93	0.89	1.00	1.22
succinate dehydrogenase Fp subunit	1.15	1.05	0.90	0.98
succinate dehydrogenase Ip subunit	1.19	0.98	0.96	1.01
Oxidative phosphorylation				
ATPase, H+/K+ transporting, nongastric, alpha polypeptide	n.d.	0.98	n.d.	n.d.
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	1.22	1.11	0.99	1.00
ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	1.23	1.03	1.09	1.01
ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit precursor	0.70	0.88	n.d.	0.94
ATP synthase, H+ transporting, mitochondrial F1 complex, gamma subunit	1.02	1.08	1.11	1.00
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	1.27	0.97	0.98	1.05
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	1.11	0.99	1.00	0.98
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	1.49	0.95	1.03	1.04
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F	1.41	1.28	1.04	1.03
cytochrome c oxidase subunit II	1.18	1.00	0.84	1.11
cytochrome c oxidase subunit IV isoform 1	1.32	1.01	1.11	1.09
cytochrome c oxidase, subunit Va	1.32	0.82	0.89	1.08
cytochrome c oxidase, subunit VIb polypeptide 1	1.15	1.11	0.95	1.01
cytochrome c oxidase, subunit VIIa 1	n.d.	n.d.	0.95	n.d.
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	1.33	1.03	0.76	1.22
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	1.19	1.06	1.03	1.04
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8	n.d.	0.99	0.99	0.97
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9	1.56	0.90	0.93	1.01
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	2.22	0.88	0.82	1.19
NADH dehydrogenase (ubiquinone) Fe-S protein 1	1.18	1.01	0.84	1.11
NADH dehydrogenase (ubiquinone) Fe-S protein 2	1.54	0.93	0.92	1.05
NADH dehydrogenase (ubiquinone) flavoprotein 1	1.37	0.99	0.87	1.09
PREDICTED: similar to ATP synthase coupling factor 6, mitochondrial precursor (ATPase subunit F6)	1.25	1.16	1.04	1.03
PREDICTED: similar to NADH dehydrogenase (ubiquinone) Fe-S protein 6	n.d.	1.11	0.95	1.28
ubiquinol-cytochrome c reductase core protein 1	1.10	0.99	0.88	1.05
ubiquinol cytochrome c reductase core protein 2	1.12	0.96	0.92	1.01
ubiquinol-cytochrome c reductase binding protein	0.84	0.92	1.09	0.98
ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	1.15	1.01	0.93	1.10
succinate dehydrogenase Fp subunit	1.15	1.05	0.90	0.98
succinate dehydrogenase Ip subunit	1.19	0.98	0.96	1.01

Contribution of Myocardial Insulin Resistance to Oxidative Stress, Mitochondrial Uncoupling and Reduced Cardiac Efficiency

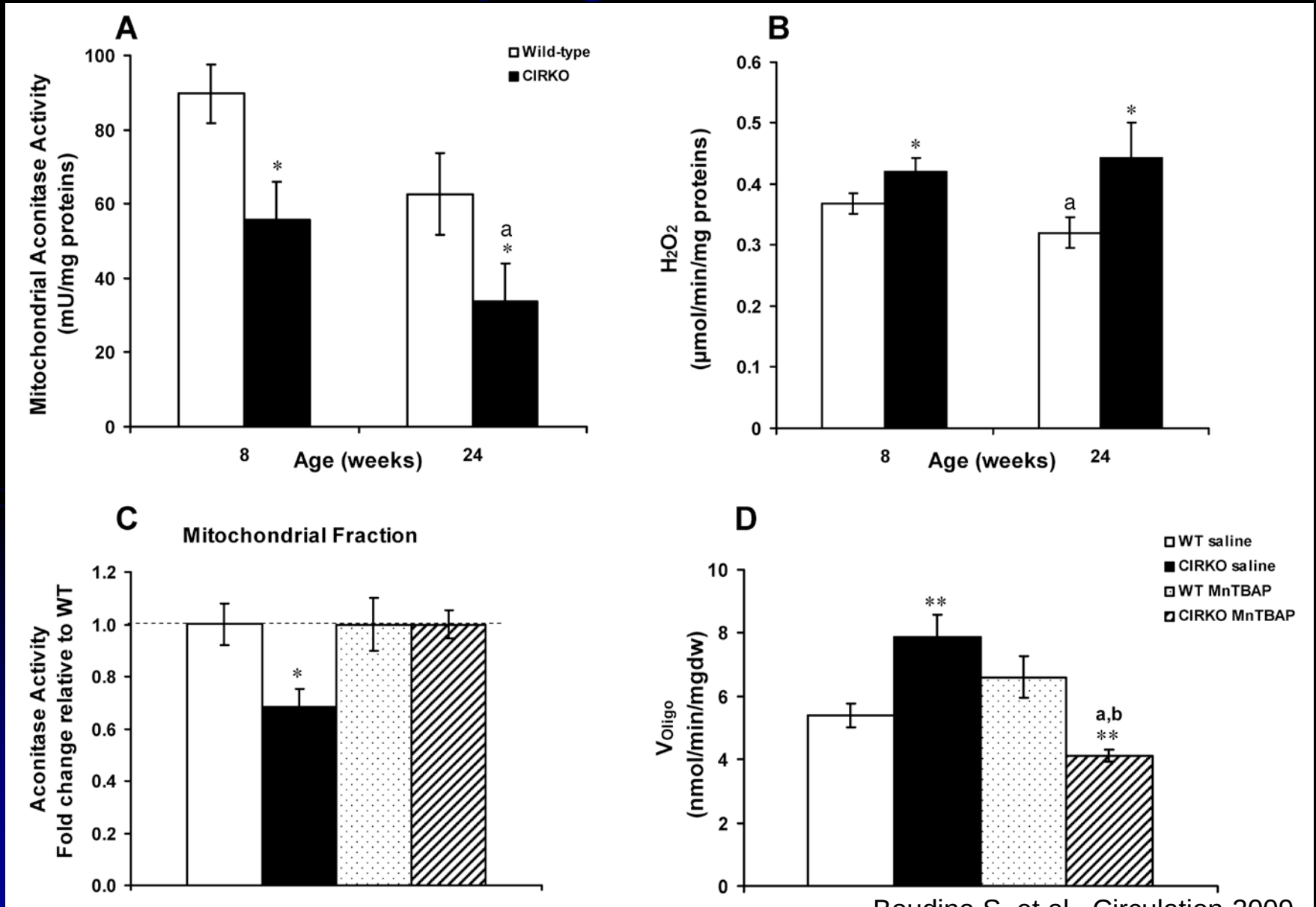
- Mitochondrial uncoupling is present in ob/ob and db/db hearts but not in Akita or STZ diabetes
- Ob/ob and db/db hearts are insulin resistant, whereas Akita hearts are insulin sensitive
- Ob/ob and db/db mitochondria overproduce ROS, but Akita hearts do not.
- Can insulin resistance alone induce oxidative stress?

Mitochondria from CIRKO Mice Exhibit FA-Induced Mitochondrial Uncoupling



Boudina S, et al., Circulation 2009

Oxidative Stress Contributes to Mitochondrial Uncoupling in CIRKO Hearts

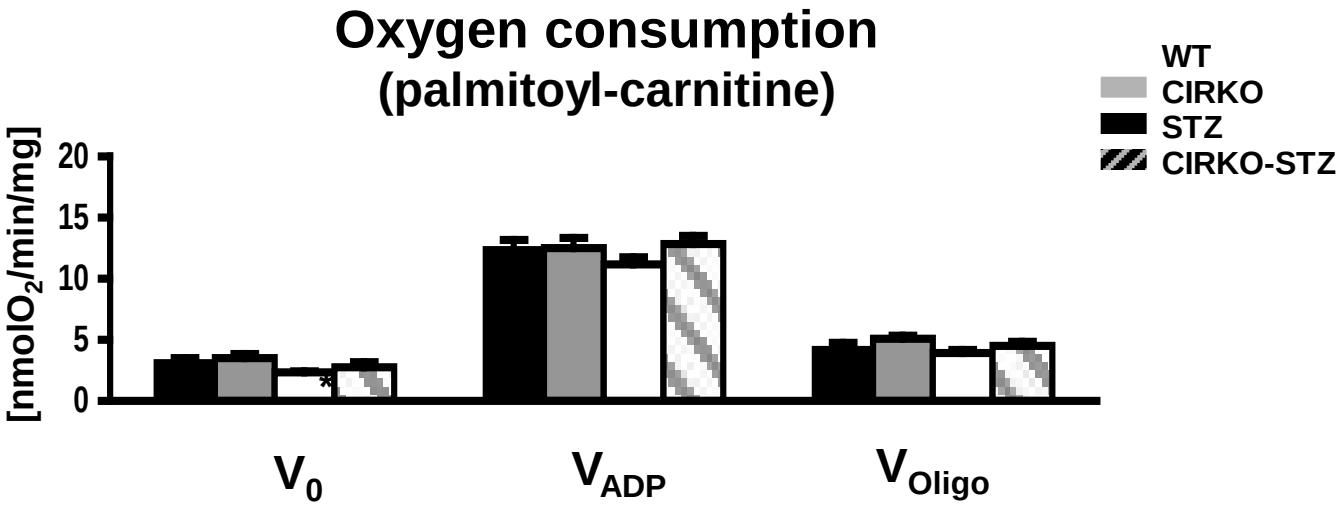


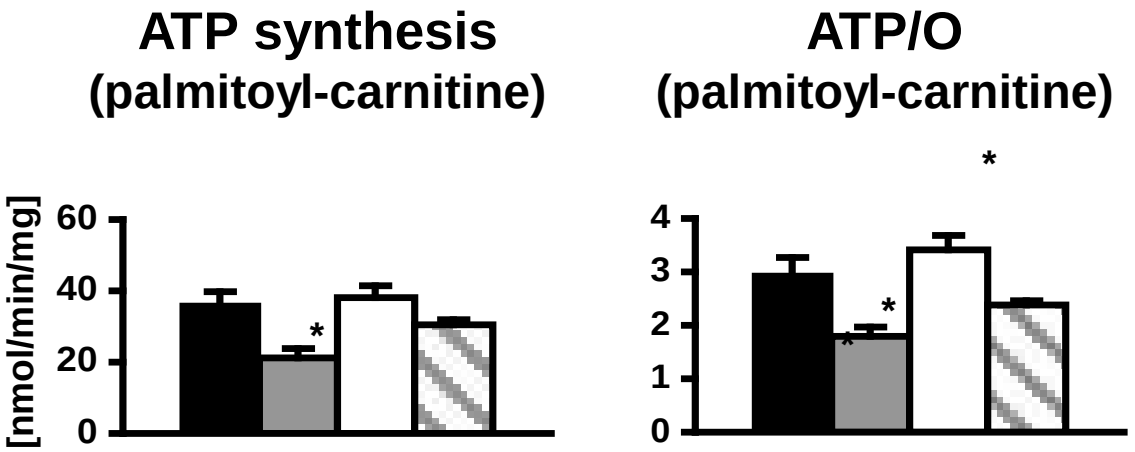
Boudina S, et al., Circulation 2009

Myocardial Insulin Resistance Promotes Oxidative Stress

Will Insulin Resistance Increase Mitochondrial Uncoupling and Decrease Cardiac Eficiency in STZ Hearts?

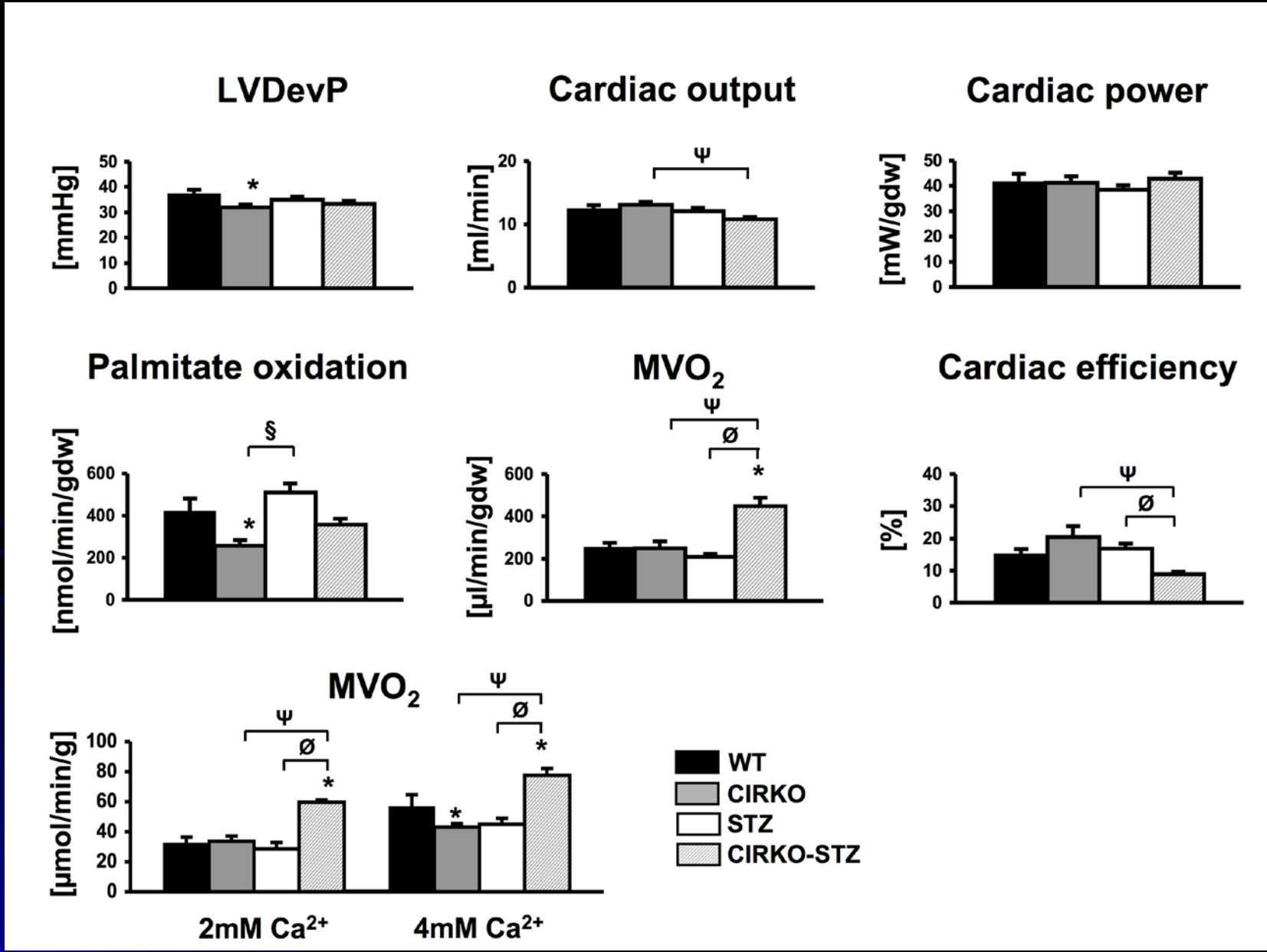
Mitochondrial Uncoupling in CIRKO and CIRKO-STZ Hearts but not in STZ Hearts





n=6-7

MVO_2 and Cardiac Efficiency are Synergistically Impaired in STZ-CIRKO mice



Mitochondrial FAO Protein Abundance is Reduced in CIRKO, but Increased in STZ and CIRKO-STZ Hearts

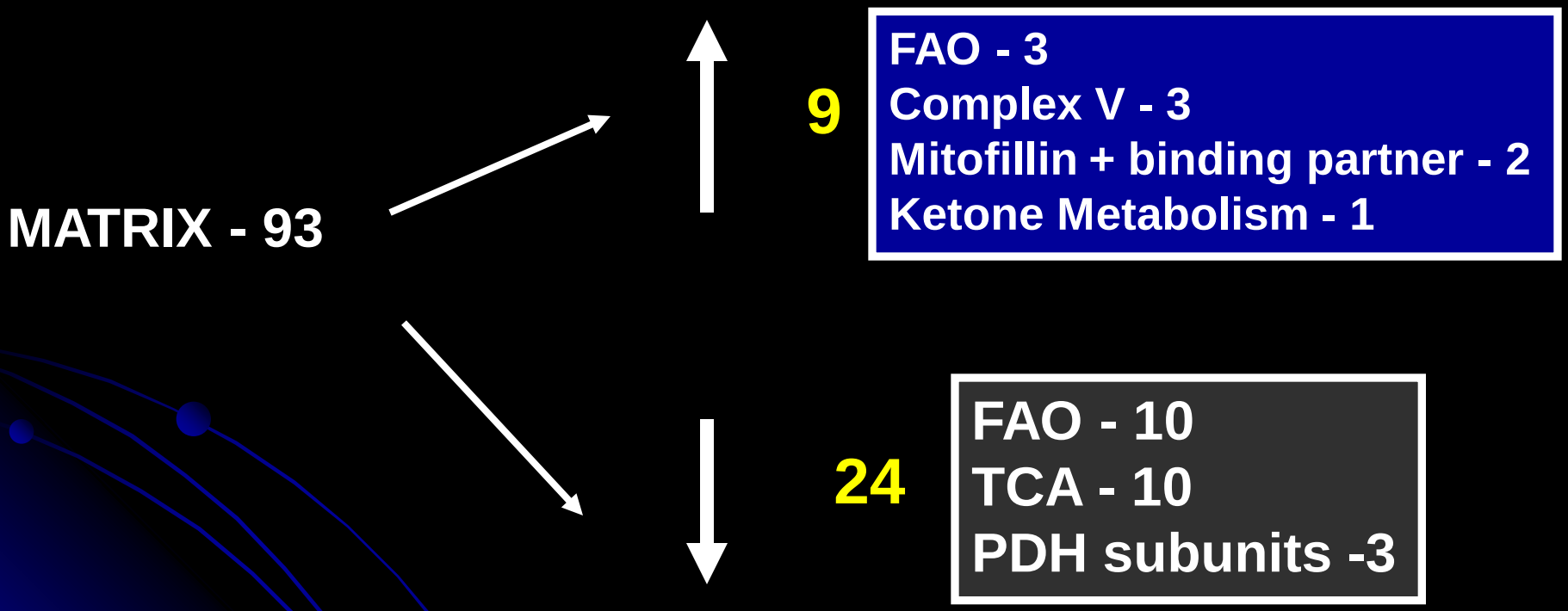
decreased
increased

Mitochondrial proteomics

Fatty acid oxidation protein	CIRKO	STZ	CIRKO-STZ
acetyl-Coenzyme A acyltransferase 2	0.86	1.49	1.10
acetyl-Coenzyme A dehydrogenase, long-chain	0.99	1.28	1.15
acetyl-Coenzyme A dehydrogenase, medium chain	1.06	1.33	1.11
acyl-Coenzyme A dehydrogenase, short chain	n.d.	n.d.	n.d.
acyl-Coenzyme A dehydrogenase, very long chain	0.83	1.02	1.12
carnitine palmitoyltransferase 1b, muscle	0.85	0.88	1.02
carnitine palmitoyltransferase 2	0.68	1.04	0.94
dodecenoyl-Coenzyme A delta isomerase	0.86	1.43	1.25
electron transferring flavoprotein, alpha polypeptide	0.99	1.32	1.11
electron transferring flavoprotein, beta polypeptide	1.03	1.28	1.12
electron transferring flavoprotein, dehydrogenase	0.82	1.10	0.96
enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	1.10	1.02	0.87
hydroxyacyl-Coenzyme A dehydrogenase, alpha subunit	0.88	1.27	1.11
hydroxyacyl-Coenzyme A dehydrogenase, beta subunit	0.90	1.19	1.03

Proteomic Survey of CIRKO Mitochondria

244 proteins identified.



Boudina et al, Circulation 2009

Proteomic Survey of CIRKO Mitochondria

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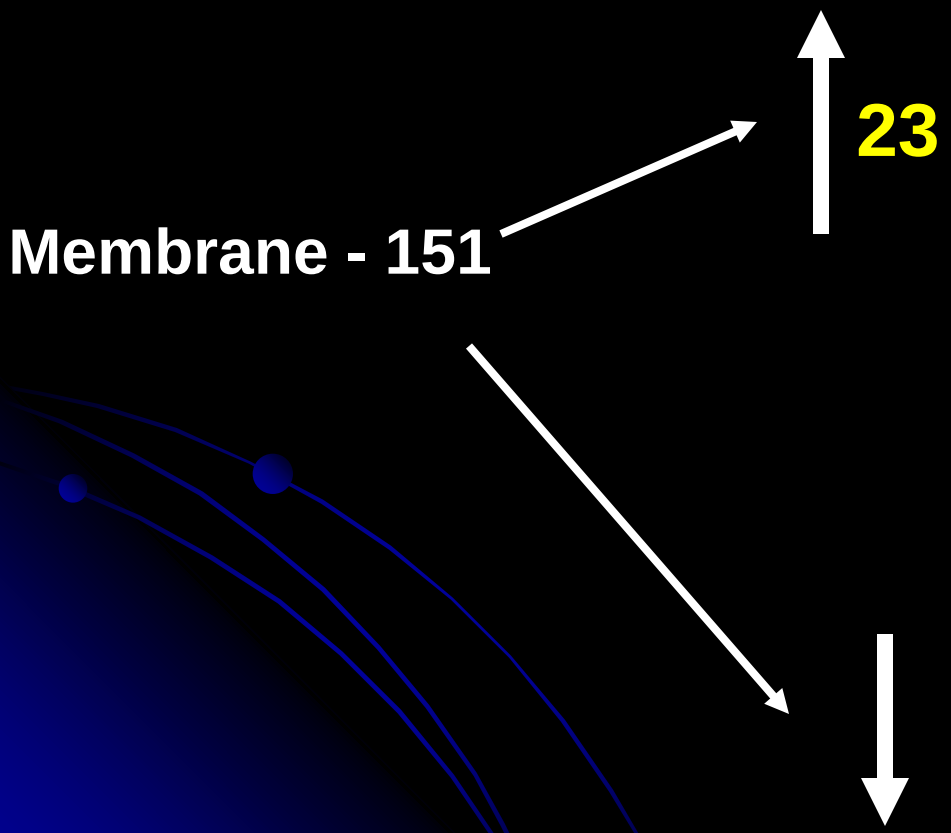
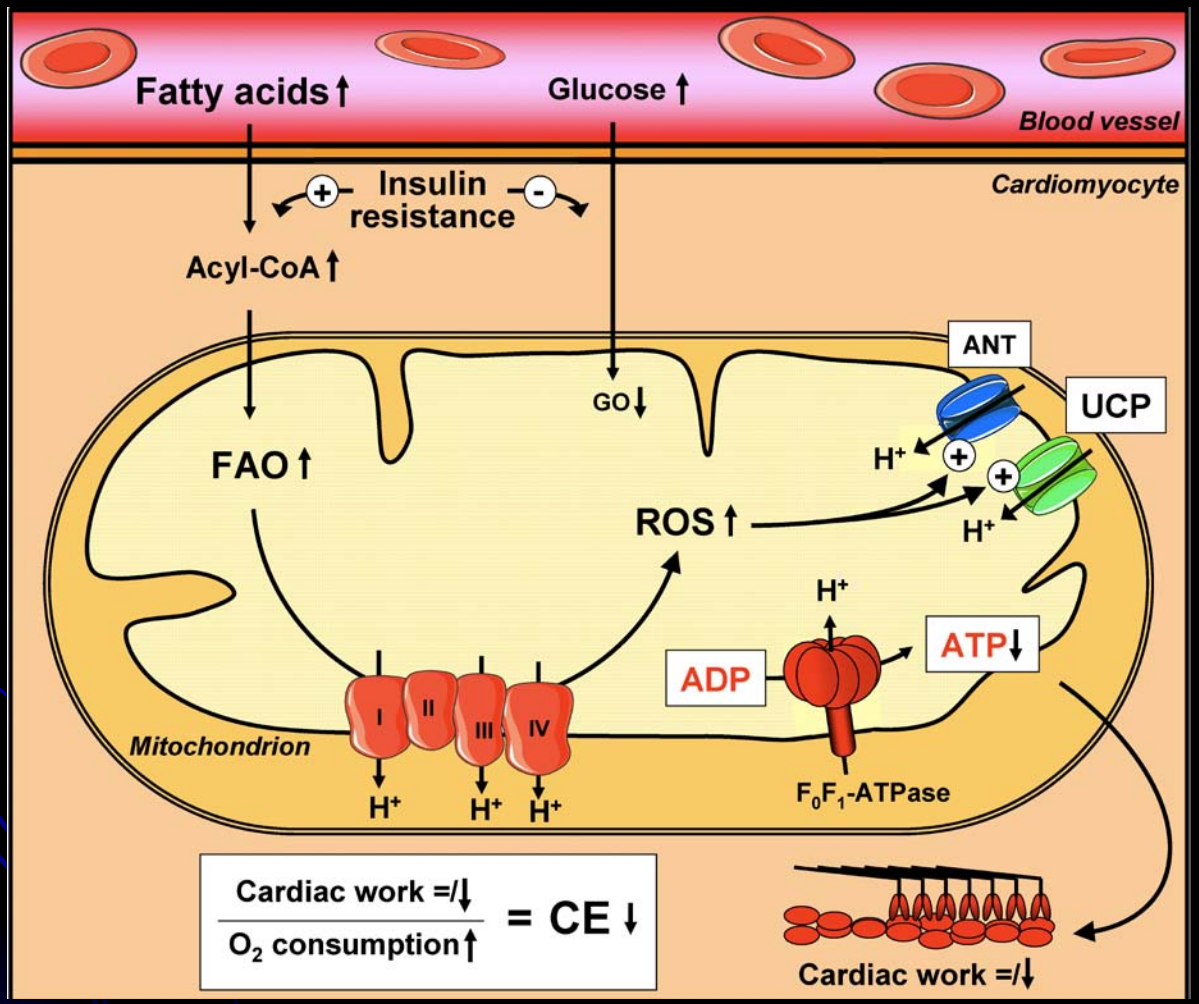


Complex I - 5
ADP/ATP Translocases -3
TCA - 4
Complex V -2
PDH Subunits -2
1 each from - Ketone Metabolism, FAO, Complex IV, NNT1, Tu translation elongation factor 1

FAO - 12
Cytochrome C - 5
Complex III - 2
TCA - 2
Mito CK- 2
Complex V -1

Boudina et al, Circulation 2009

ROS-Mediated Mitochondrial Uncoupling that Limits Mitochondrial ATP Generation and Cardiac Energetic Reserves is a Characteristic of Type 2 Diabetes but not Type 1 Diabetes and Might be Related to Insulin Resistance



Bugger H, Abel ED, Clinical Science-114(3):195-210, 2008

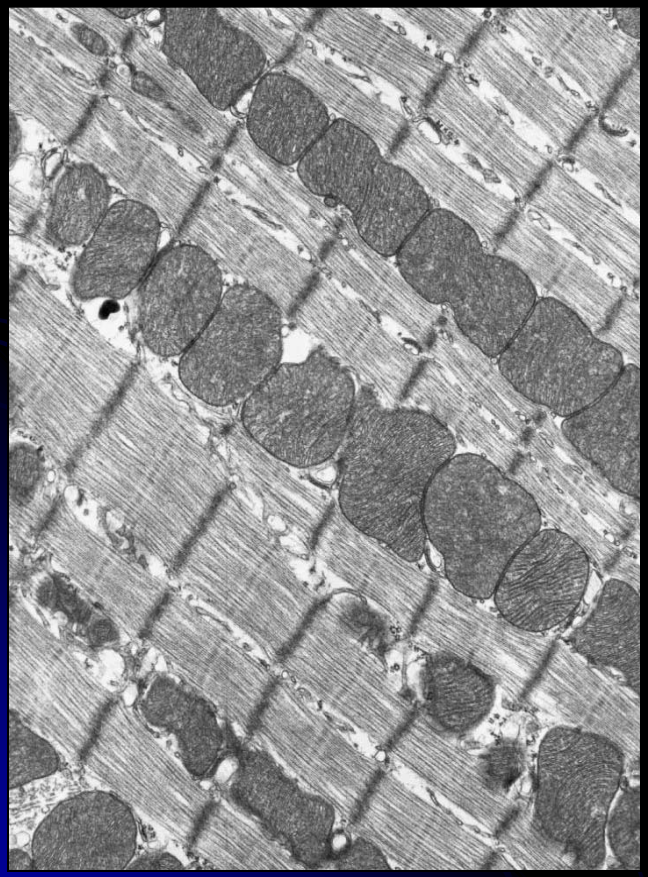
Abnormal Mitochondrial Dynamics in Lipotoxic Cardiomyopathy

Increased mitochondrial volume density and number but reduced mitochondrial size in ACS mouse hearts

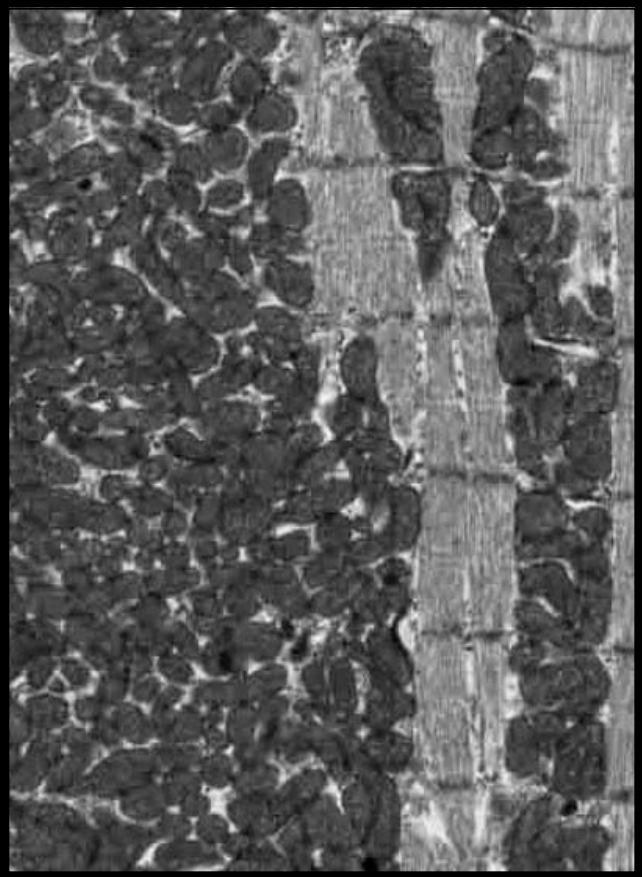
Electron microscopy

24 weeks

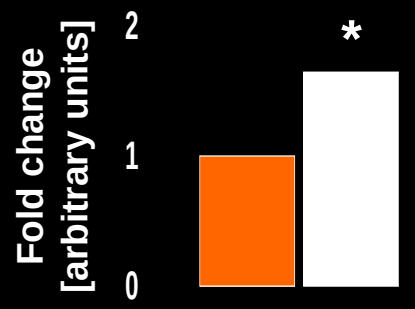
WT



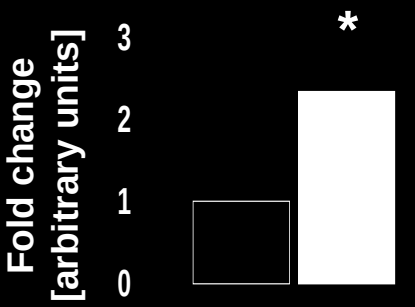
ACS



Mitochondrial volume density

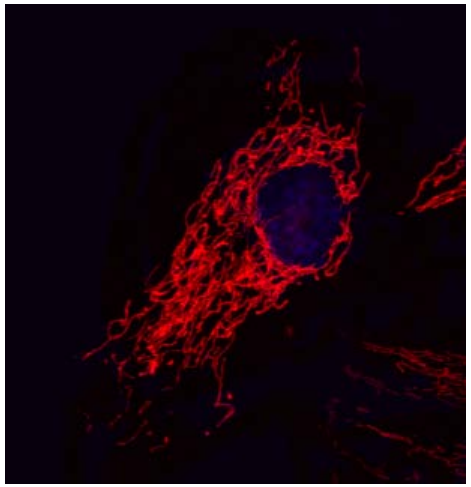


Mitochondrial number

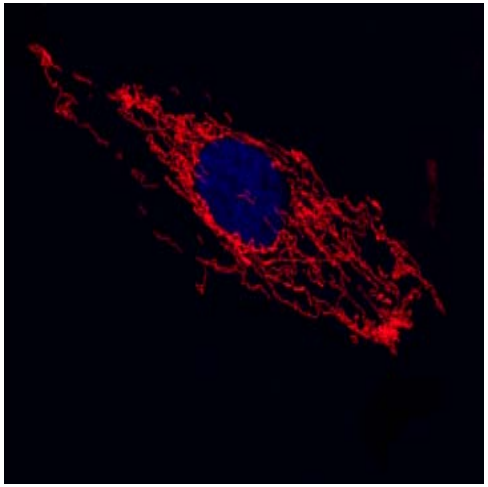


Palmitate Leads to Fragmentation of the Mitochondrial Network

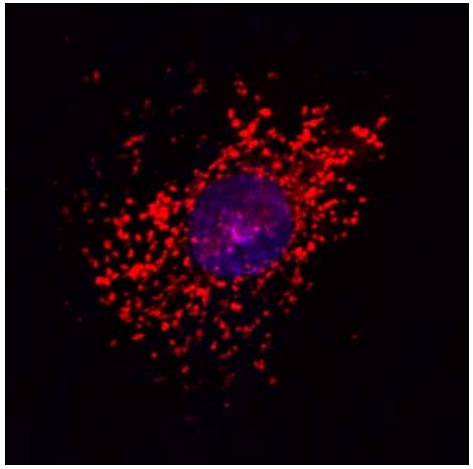
Vector no palmitate



ACS no palmitate



Vector 500uM



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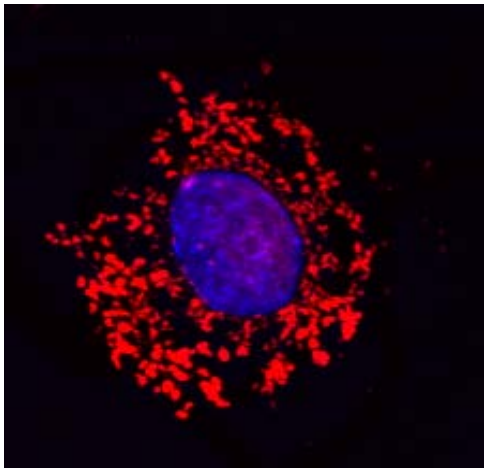
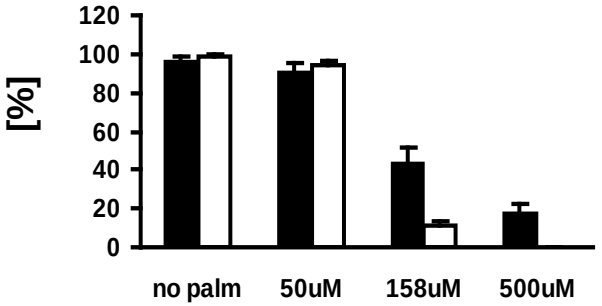
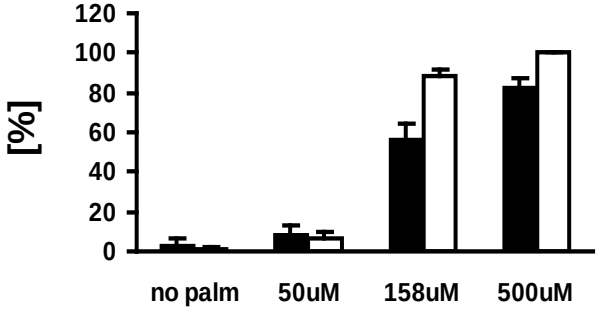


Tubular mitochondria

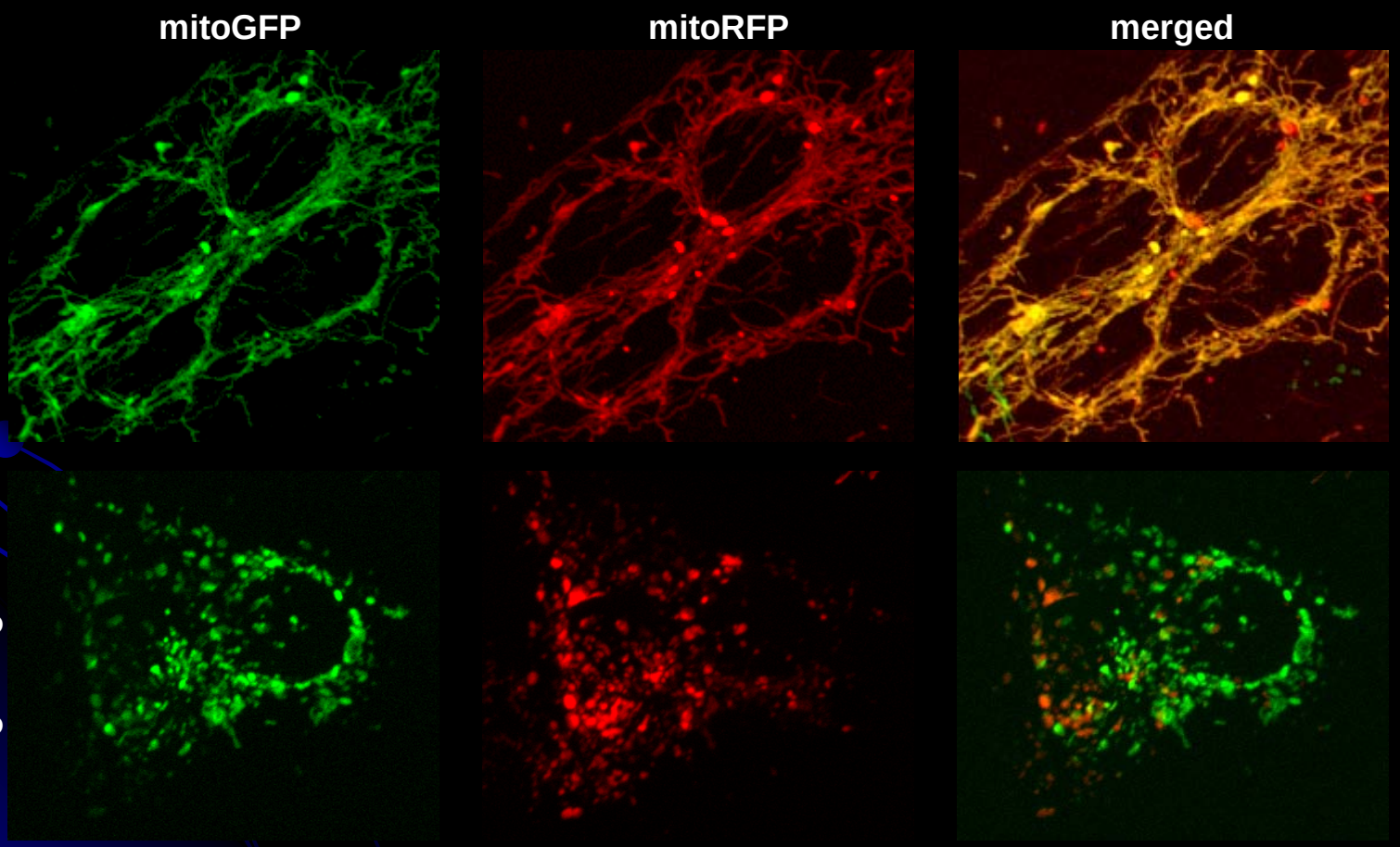


Fragmented mitochondria



Palmitate Impairs Mitochondrial Fusion

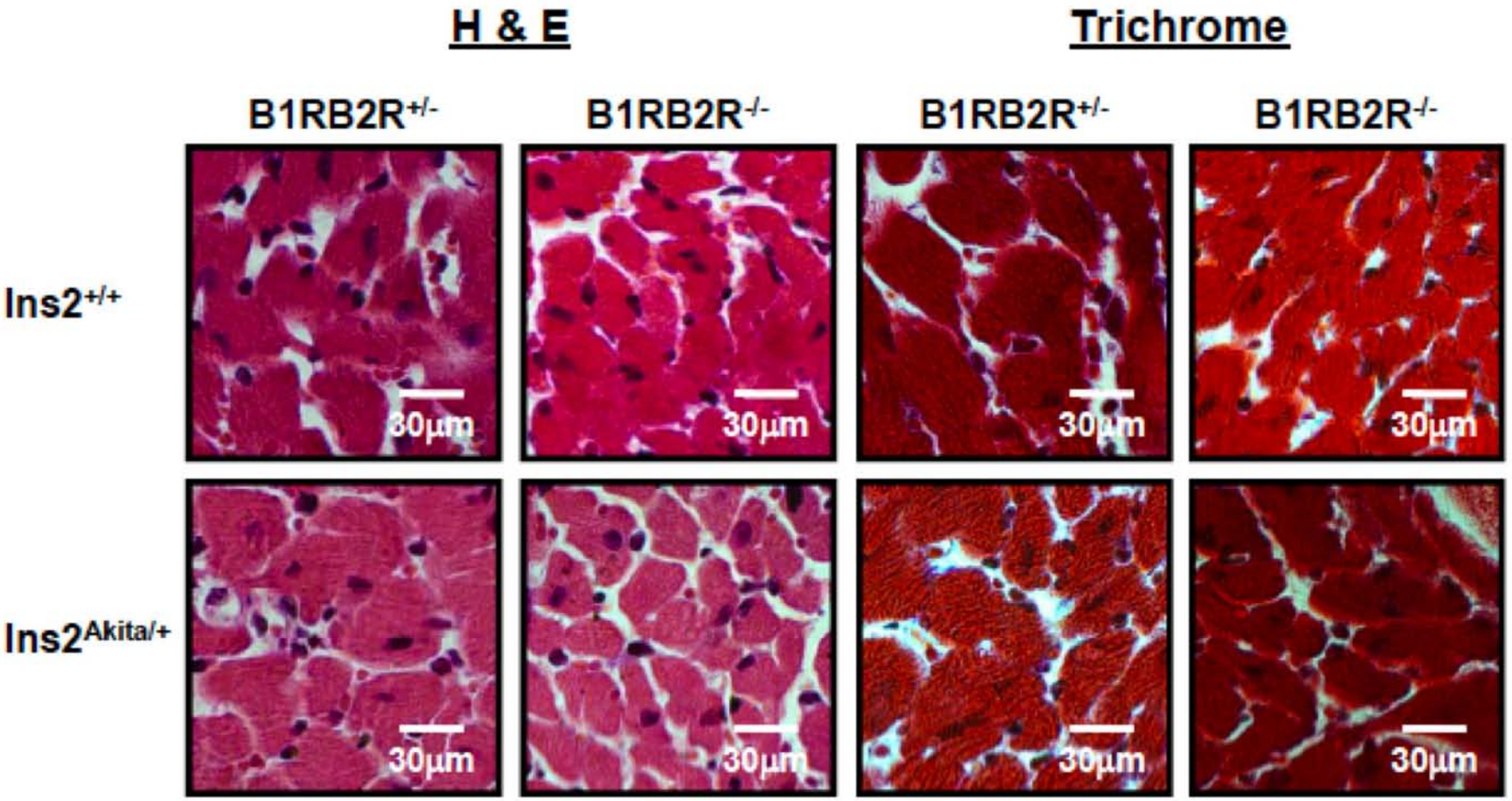
PEG Fusion Assay



Collaborative Study between UNC-Smithies/Maeda and University of Utah

Characterization of Cardiac Phenotype of B1RB2RKO mice on the Akita Background

No Synergistic Impact on Cardiac Structure



No Synergistic Impact on Cardiac Function

Parameter	$Ins2^{+/+}$ $B1RB2R^{+/-}$	$Ins2^{+/+}$ $B1RB2R^{-/-}$	$Ins2^{Akita/+}$ $B1RB2R^{+/-}$	$Ins2^{Akita/+}$ $B1RB2R^{-/-}$
No. of mice	7	8	9	9
Aortic systolic pressure (mmHg)	96 ± 3	95 ± 4	86 ± 2[a]	94 ± 3
Aortic diastolic pressure (mmHg)	63 ± 4	61 ± 2	61 ± 3	68 ± 3
LVSP (mmHg)	99 ± 3	99 ± 3	88 ± 1[a]	90 ± 2[a]
LVDP (mmHg)	0.8 ± 1.1	0.2 ± 0.7	2.7 ± 1.0	1.0 ± 0.9
LVEDP (mmHg)	13 ± 3	8 ± 2	11 ± 2	13 ± 3
LV Dev P (mmHg)	98 ± 3	98 ± 3	85 ± 1[a]	89 ± 2[a]
+dP/dt (mmHg • sec^{-1})	7550 ± 270	7921 ± 471	6342 ± 207[a]	6760 ± 363[a]
-dP/dt (mmHg • sec^{-1})	-7412 ± 604	-7975 ± 617	-5855 ± 152[a]	-6373 ± 431[a]
Heart rate (beat • min^{-1})	478 ± 14	490 ± 17	453 ± 10	484 ± 21

Data are mean ± SEM. -dP/dt, peak rate of LV pressure decline; LV Dev P, left ventricle developed pressure; LVSP, LV systolic pressure. [a] $P < 0.05$ vs. $Ins2^{+/+}$ same B1B2R genotype .