Metabolomics

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Knowledge that will change your world

Glucose and Mitochondrial Function

Wednesday, March 11, 2015

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Division of Molecular and Cellular Pathology

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NIH National Heart, Lung, and Blood Institute

ARW

Outline

- Define the question and model to determine the connection between metabolism and diabetic heart disease.
- Identify the molecular mechanisms by which glucose directly alters molecular function using systems biology.
    - Transcriptomics
    - Proteomics
    - Metabolomics
    - Methylomics and epigenetics

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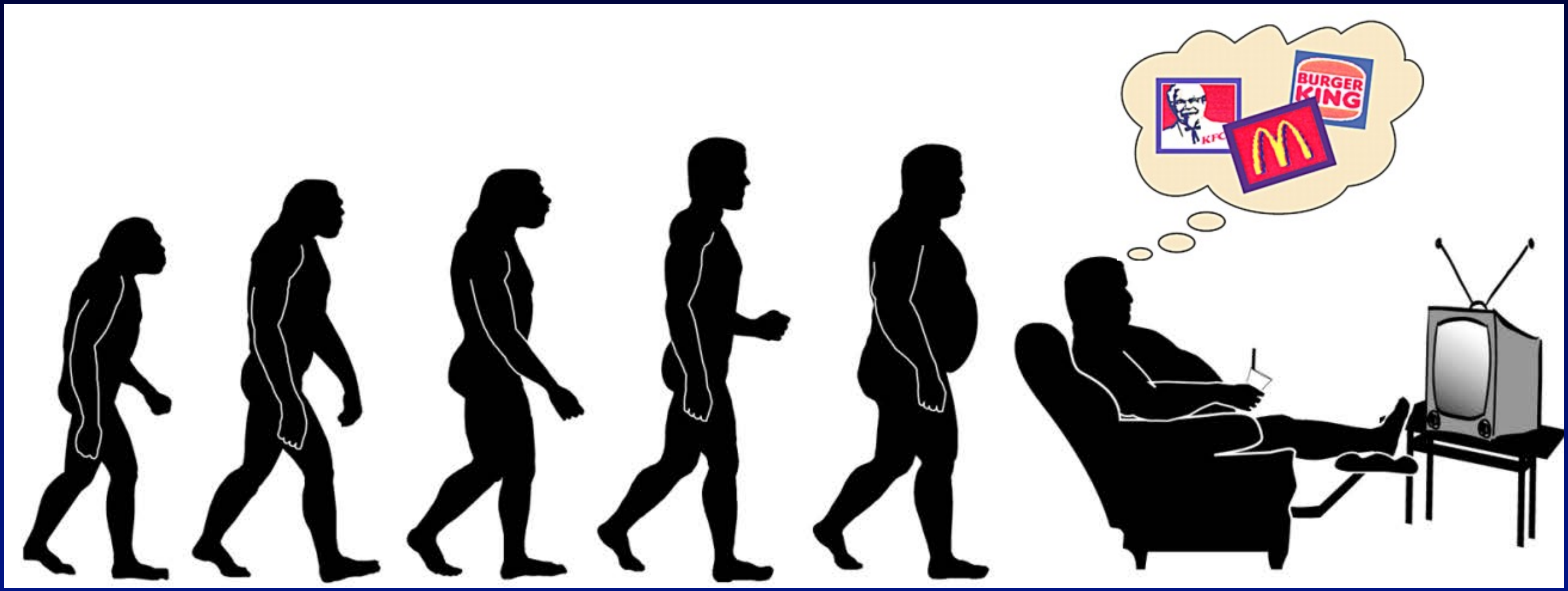
Obesity, Metabolic Syndrome, Diabetes, and Heart Failure



← 2.5 million years → ← 50 years →

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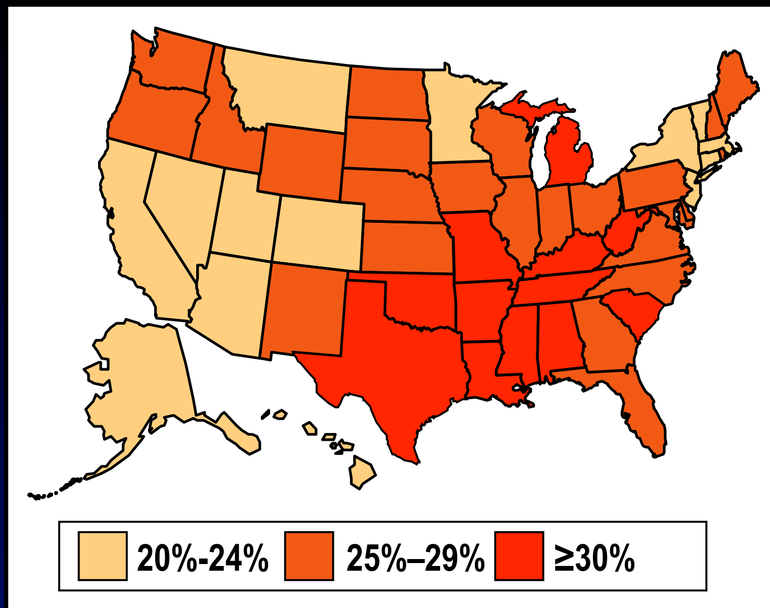
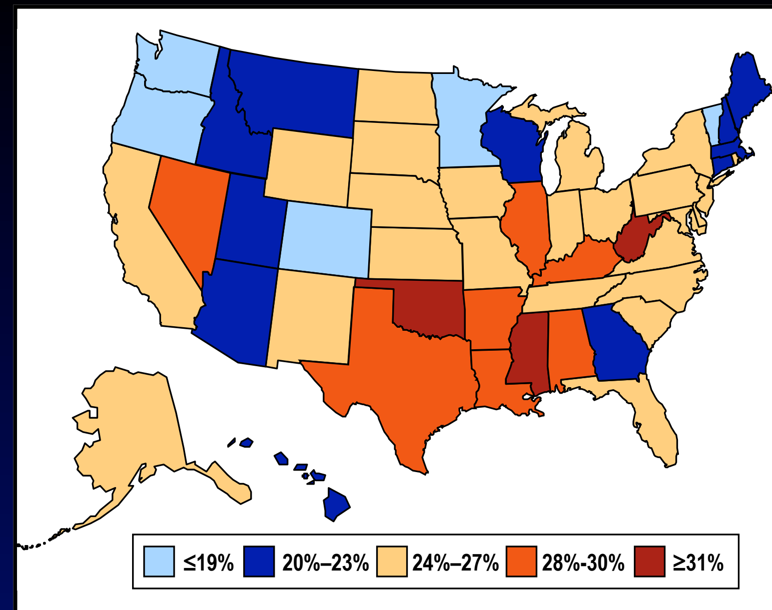
From: Roger Unger - UTSW

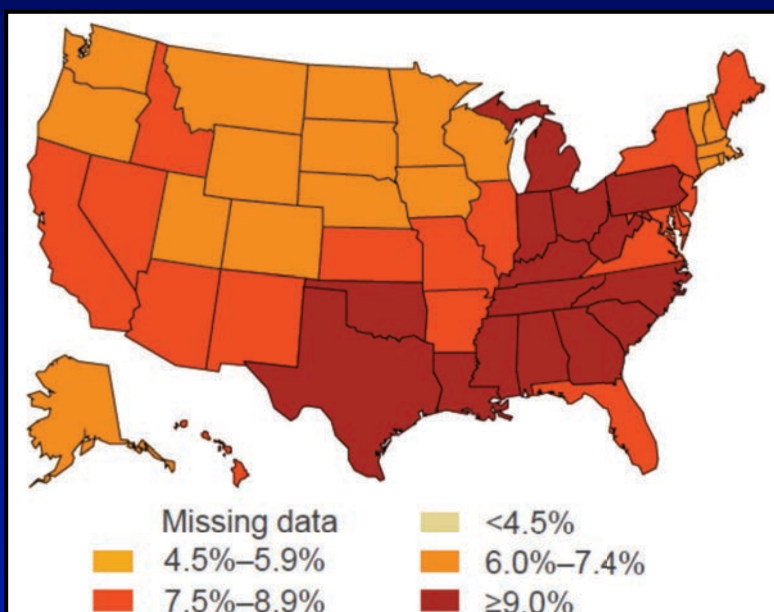
2010 – Obesity



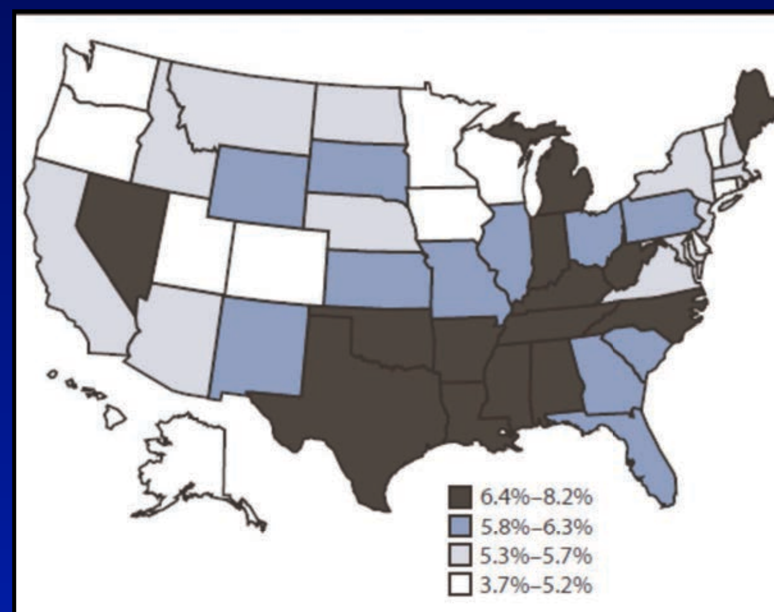
2010 – Physical Inactivity



2010 – Diabetes

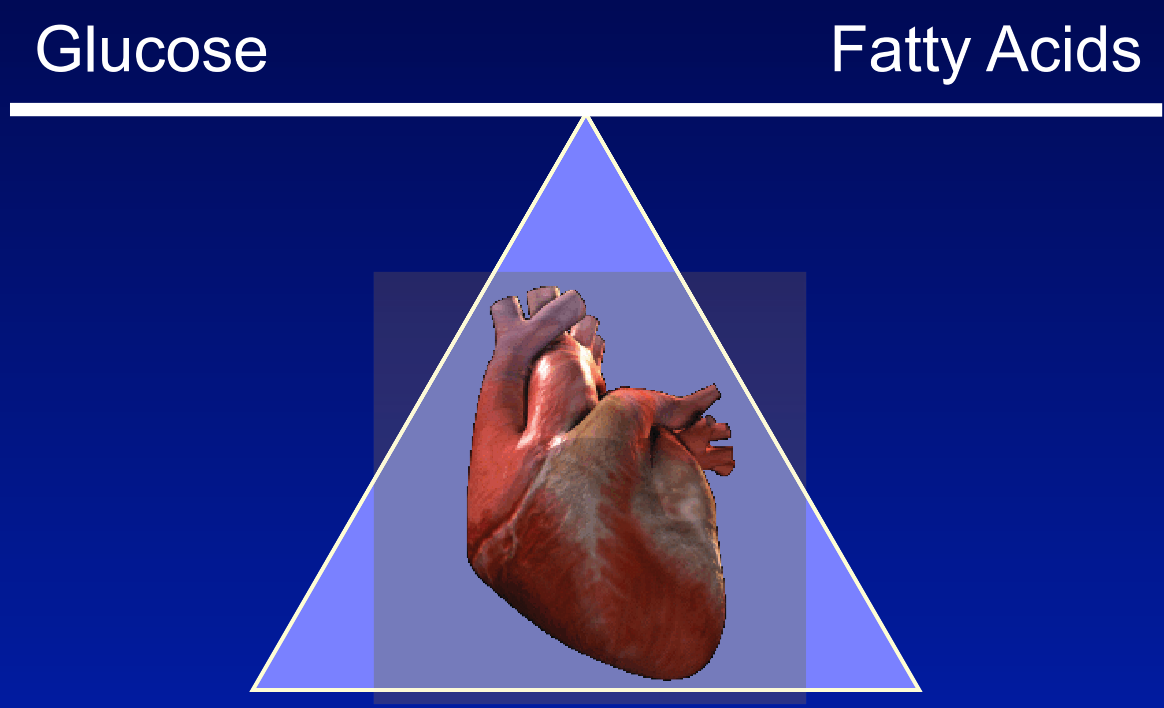


2010 – Heart Disease



www.cdc.gov/diabetes/statistics and www.cdc.gov/mmwr

Maintaining Cardiac Function Through Metabolic Substrate Balance



giphy.com

Studies on Myocardial Metabolism*

IV. Myocardial Metabolism in Diabetes

I. UNGAR, M.D., M. GILBERT, M.D., A. SIEGEL, M.S., J. M. BLAIN, M.D. *and* R. J. BING, M.D.

lactate usage and a slight decline in that of pyruvate. There is no change in utilization of amino acids by the heart in both species. Myocardial glucose consumption is reduced in dog and man relative to the elevation in blood glucose concentration. The myocardial usage of ketones is slightly increased in diabetic hearts of patients and significantly elevated in the dog. The main difference concerns the utilization of fatty acids; this is significantly increased in the human heart but is unchanged in the dog. Whether this is due to a species difference or to differences in type and severity of diabetes is not clear. Anesthesia, which was used in the dogs, may have played some part.

ARW

Ungar … Bing **1955** *Am J Med* 18(3):385

Metabolic Substrate Utilization in the Heart

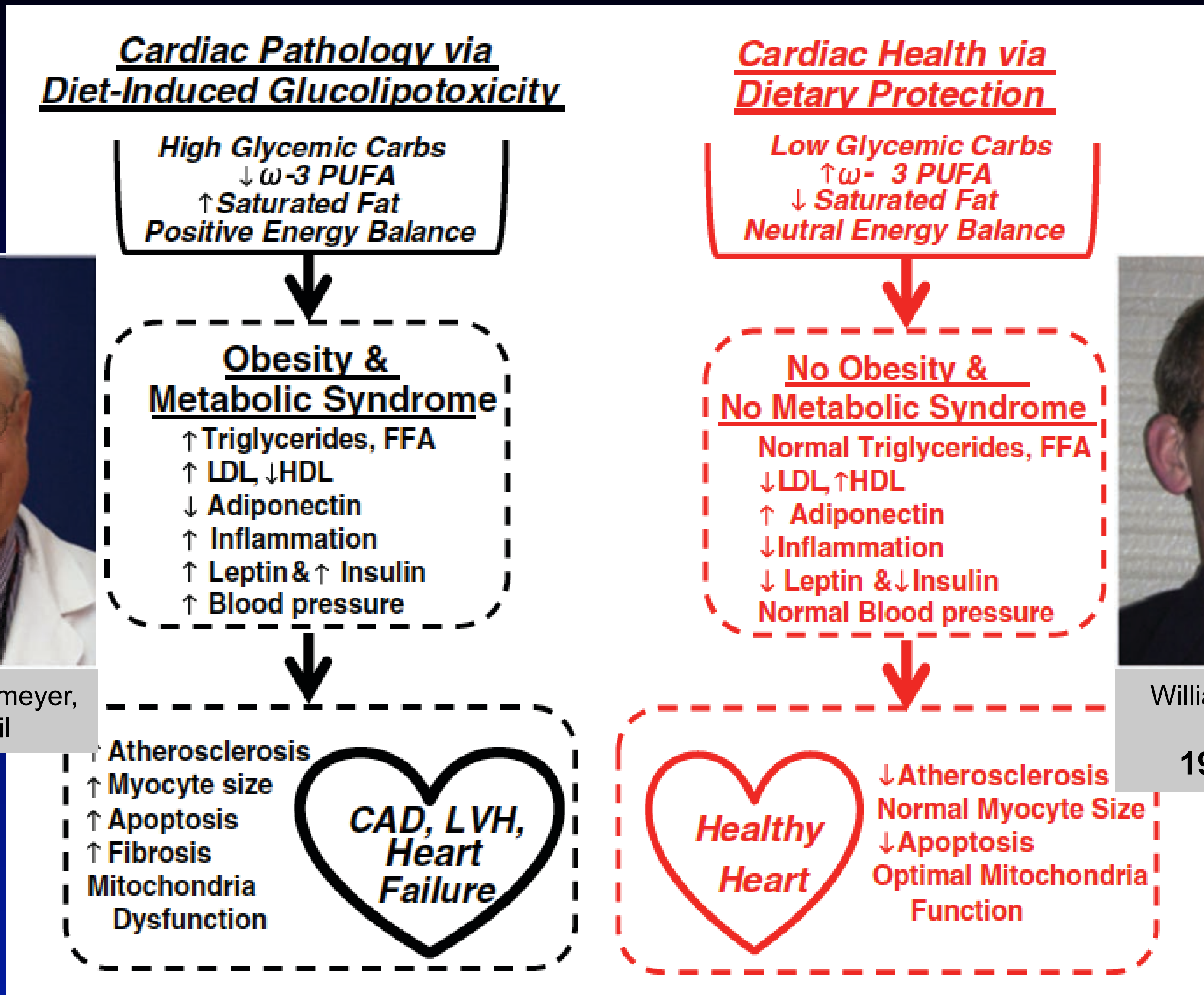
Table 2. Brief Overview of Myocardial Metabolism in Physiological and Pathophysiological Conditions

	MVo_2	Glucose Metabolism	Fatty Acid Metabolism
Aging	↑	↑	↓
Female sex	↑	↓	↑
Obesity	↑	—	↑
Diabetes, types 1 and 2	—↑	↓	↑
Hypertension: LV hypertrophy	—	↑	↓
Dilated cardiomyopathy	—	↑	↓
Ischemia	↓	↑	↓

Peterson and Gropler **2010** *Circ Cardiovasc Imaging* 3:211

Point/Counterpoint - The Right Balance?

Heinrich Taegtmeyer, MD, DPhil



William C. Stanley, PhD
1957 - 2013

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Taegtmeyer and Stanley **2011** *J Mol Cell Cardiol* 50(1):2

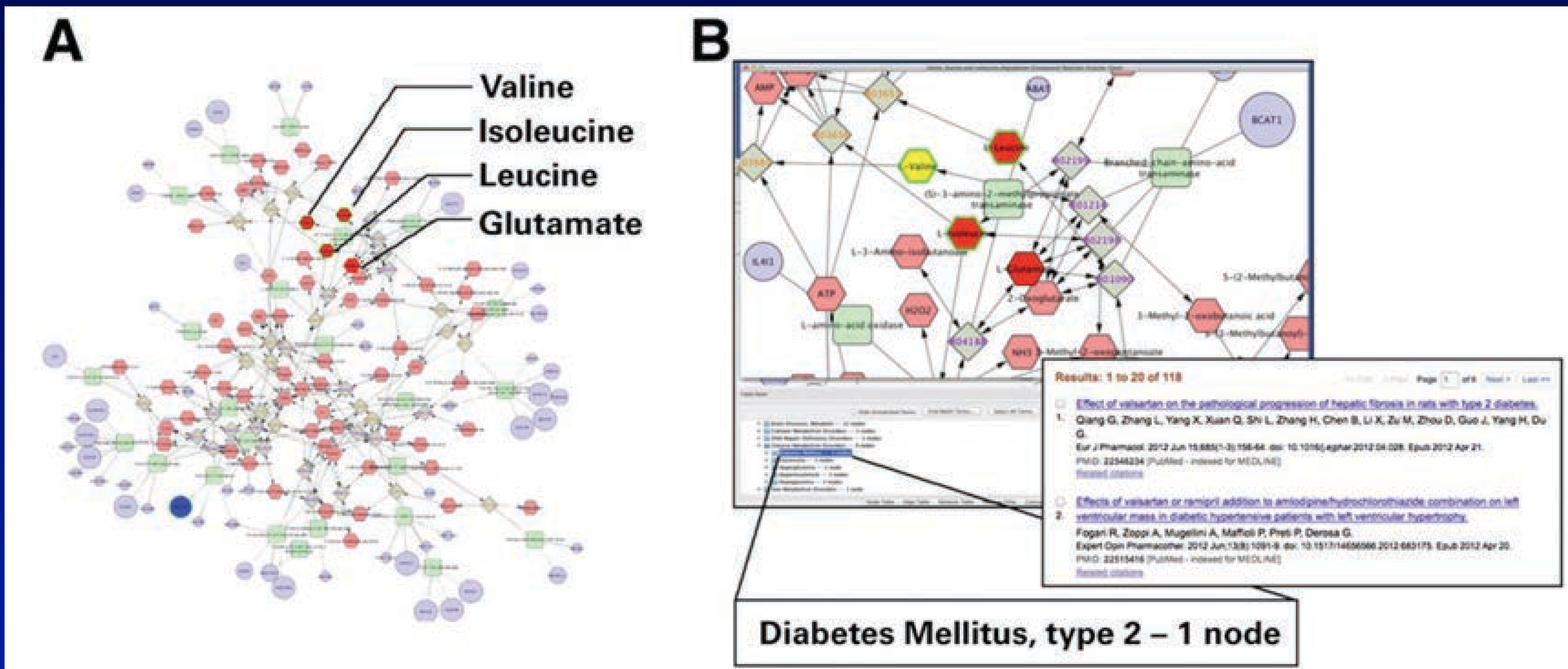
Diabetes and Metabolomics

Diabetes. 2015 Mar;64(3):718-732.

Metabolomics and Diabetes: Analytical and Computational Approaches.

Sas KM[1], Karnovsky A[2], Michailidis G[3], Pennathur S[4].

Metabolomics is an integral part for understanding disease processes … information garnered in the biomarker investigations, future research should shed more light on disease pathogenesis and explore new treatment options.



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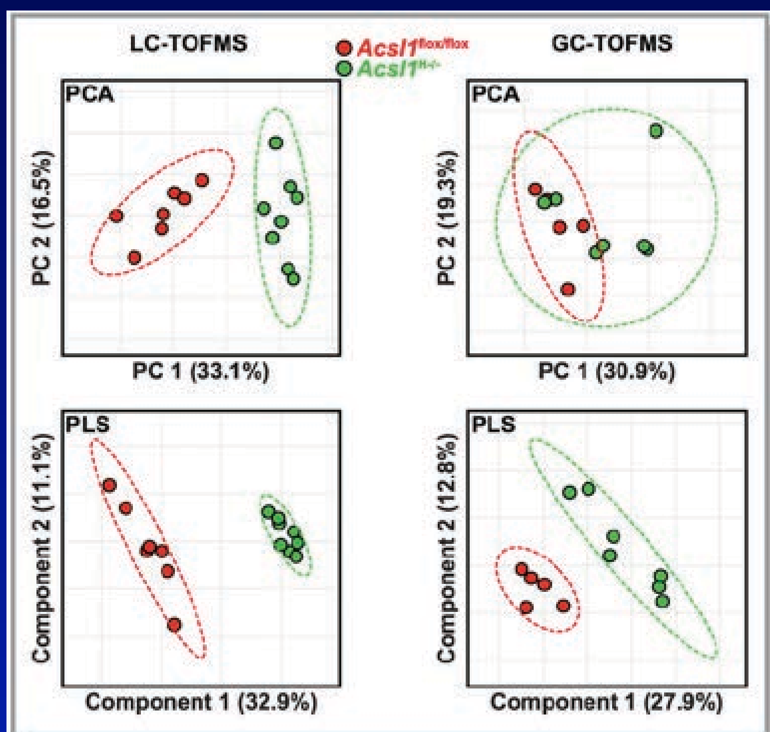
Heart failure and substrate switching

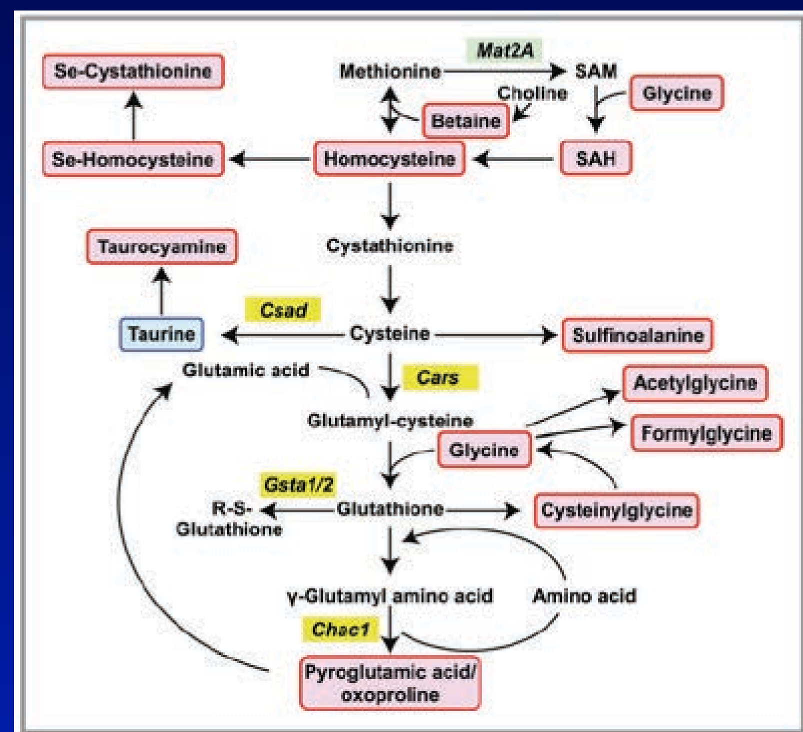
J Am Heart Assoc. 2015 Feb 24;4(2). pii: e001136. doi: 10.1161/JAHA.114.001136.

Cardiac energy dependence on glucose increases metabolites related to glutathione and activates metabolic genes controlled by mechanistic target of rapamycin.

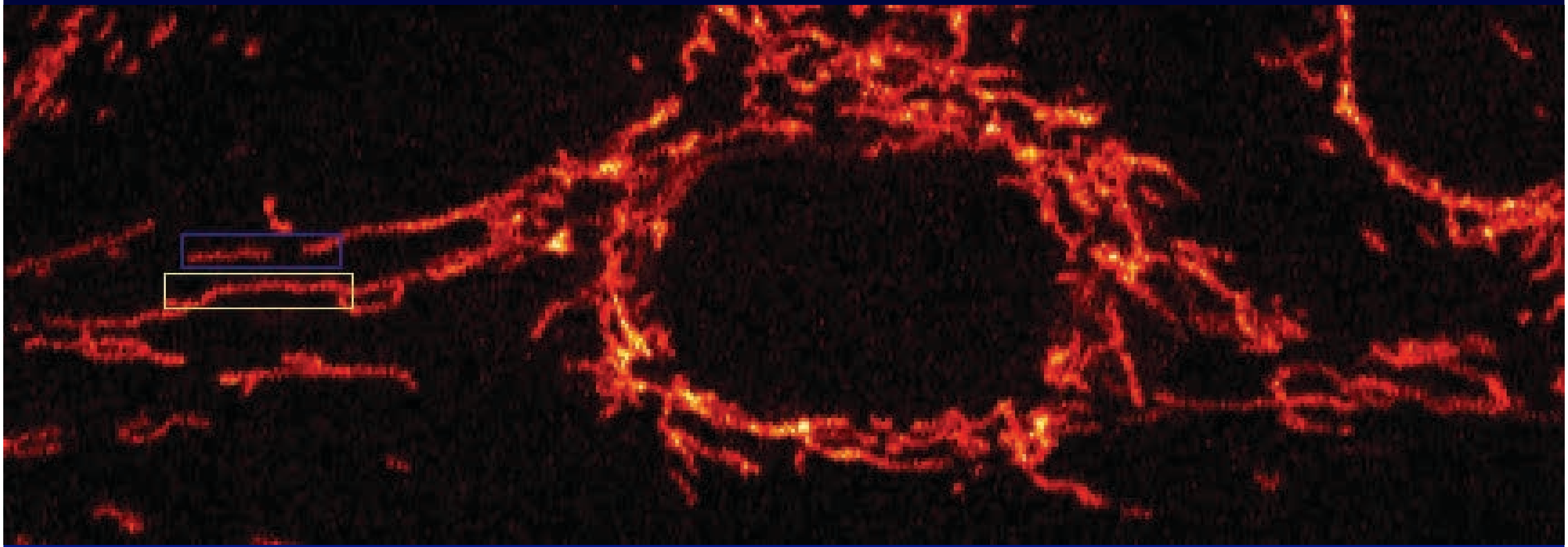
Schisler JC[1], Grevengoed TJ[2], Pascual F[2], Cooper DE[2], Ellis JM[2], Paul DS[2], Willis MS[3], Patterson C[1], Jia W[4], Coleman RA[2].

The hypertrophy, oxidative stress, and metabolic changes that occur within the heart when glucose supplants FA as a major energy source suggest that substrate switching to glucose is not entirely benign.





Mitochondria – a Dynamic Network



Fan … Brooks **2010** *Free Radic Biol Med* 49(11):1646

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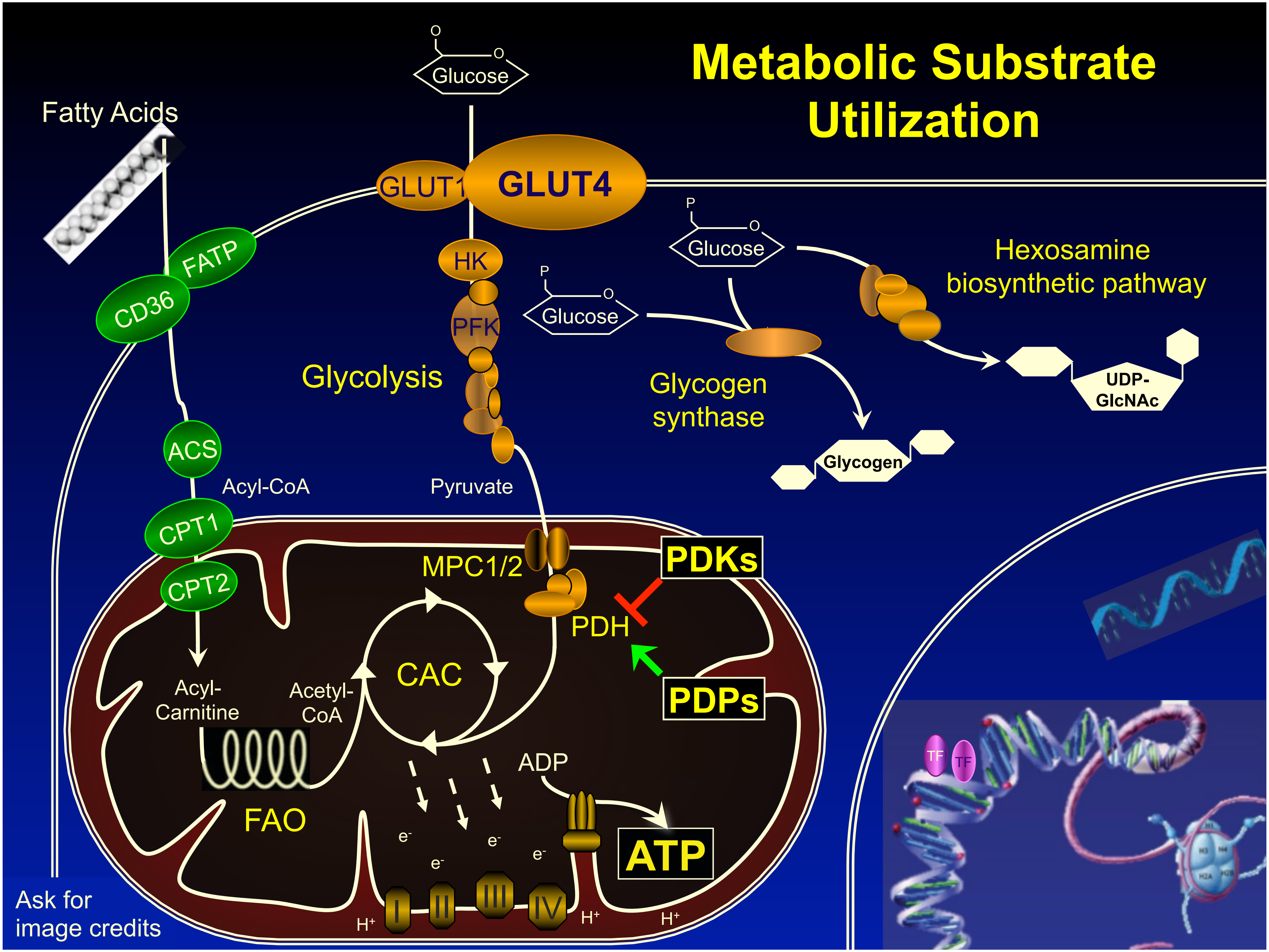
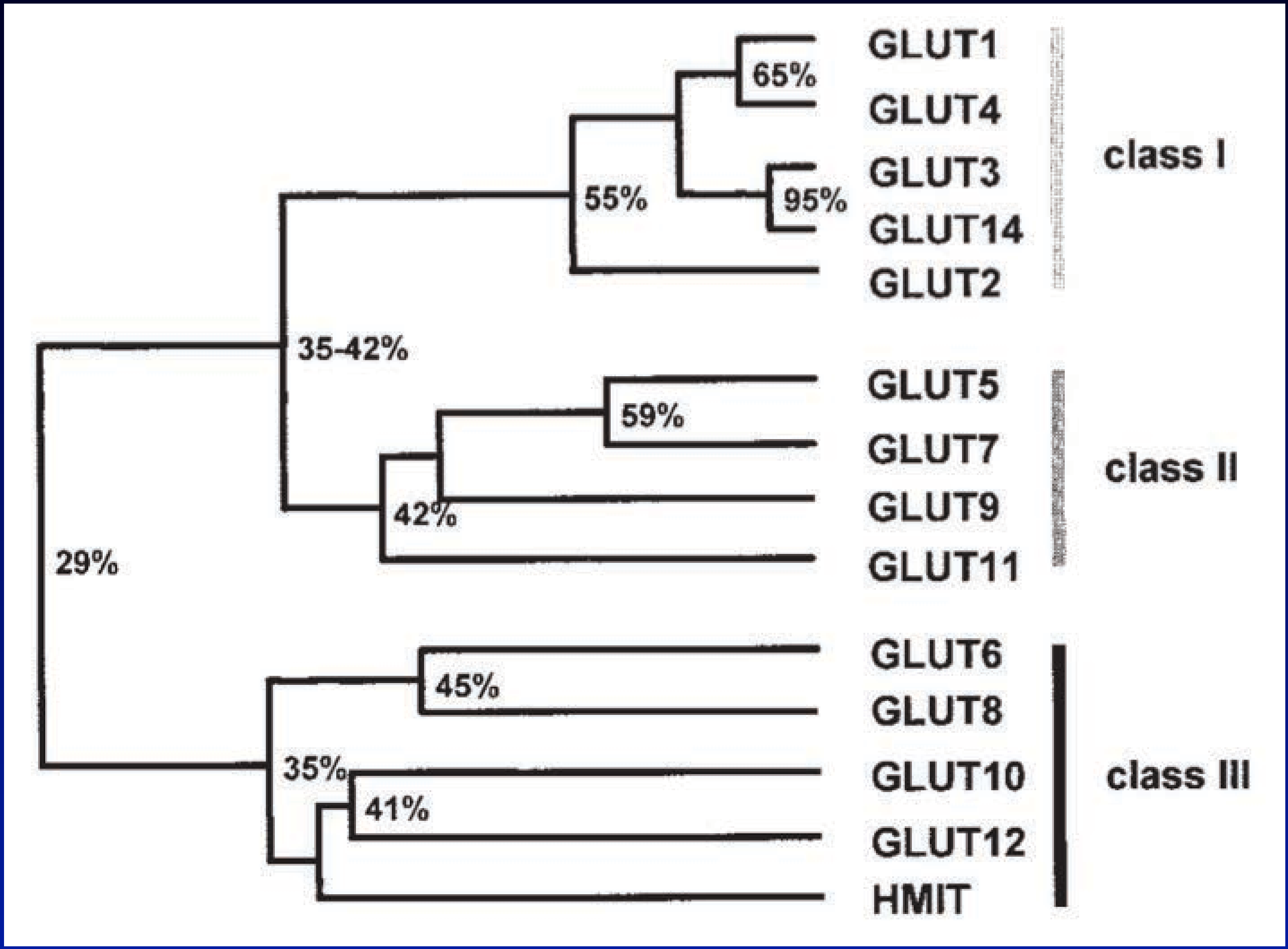
Metabolic Substrate Utilization



Facilitative Glucose Transporters: GLUTs
"Solute Carrier Family, SLC2A"



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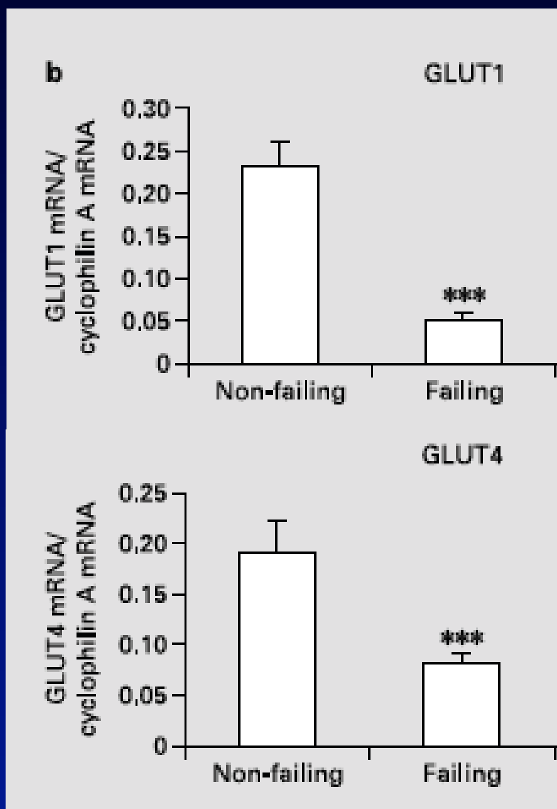
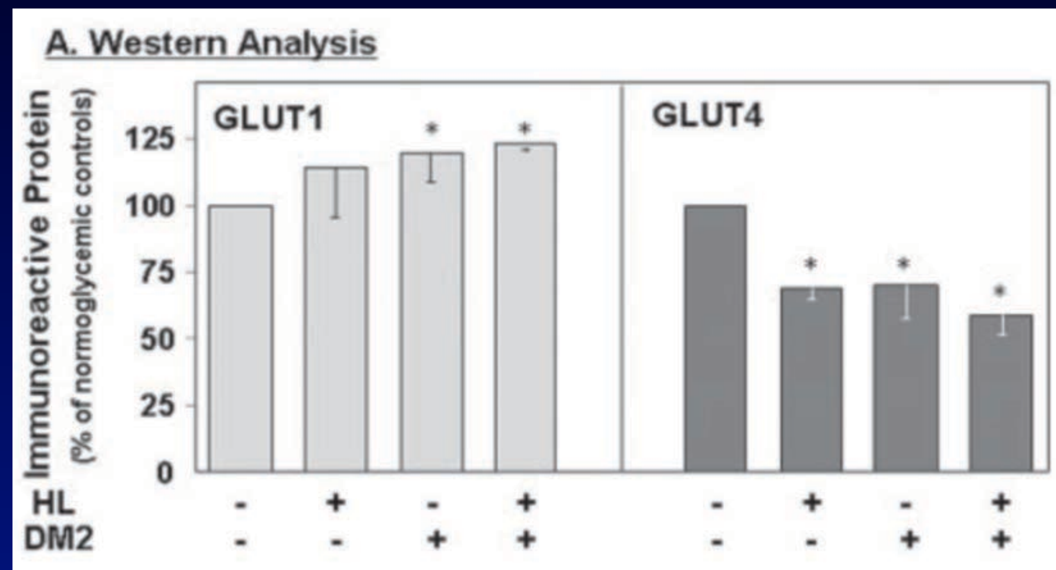
Scheepers … Schurmann **2004** *J Parenter Enteral Nutr* 28:364

Changes in Human Heart GLUT Levels

RNA
Human heart failure



Protein
Human heart diabetes



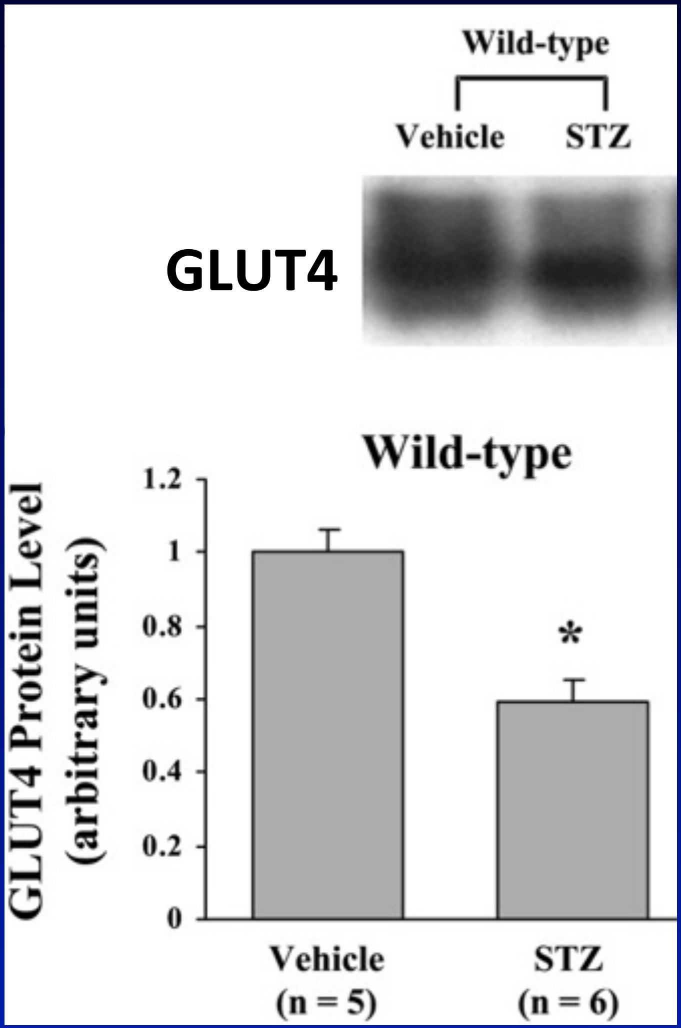
Biopsies obtained during coronary bypass surgery
HL = hyperlipidemia
DM2 = diabetes mellitus type 2

Razeghi … Taegtmeyer **2002** *Cardiology* 280(41):34786

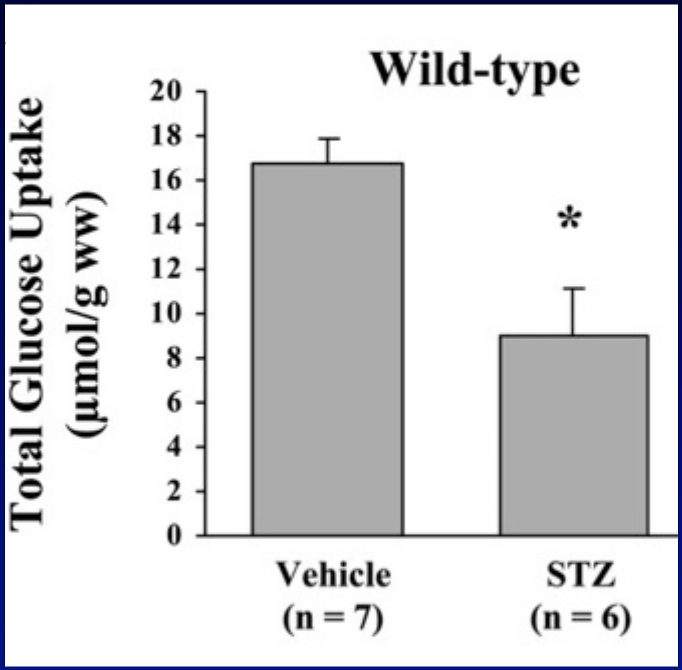
Armoni … Karnieli **2005** *J Biol Chem* 280(41):34786

Glucose Utilization and Rodent Models of Type 1 Diabetes

Protein
Diabetic Mouse Heart



Glucose Uptake
Diabetic Mouse Heart



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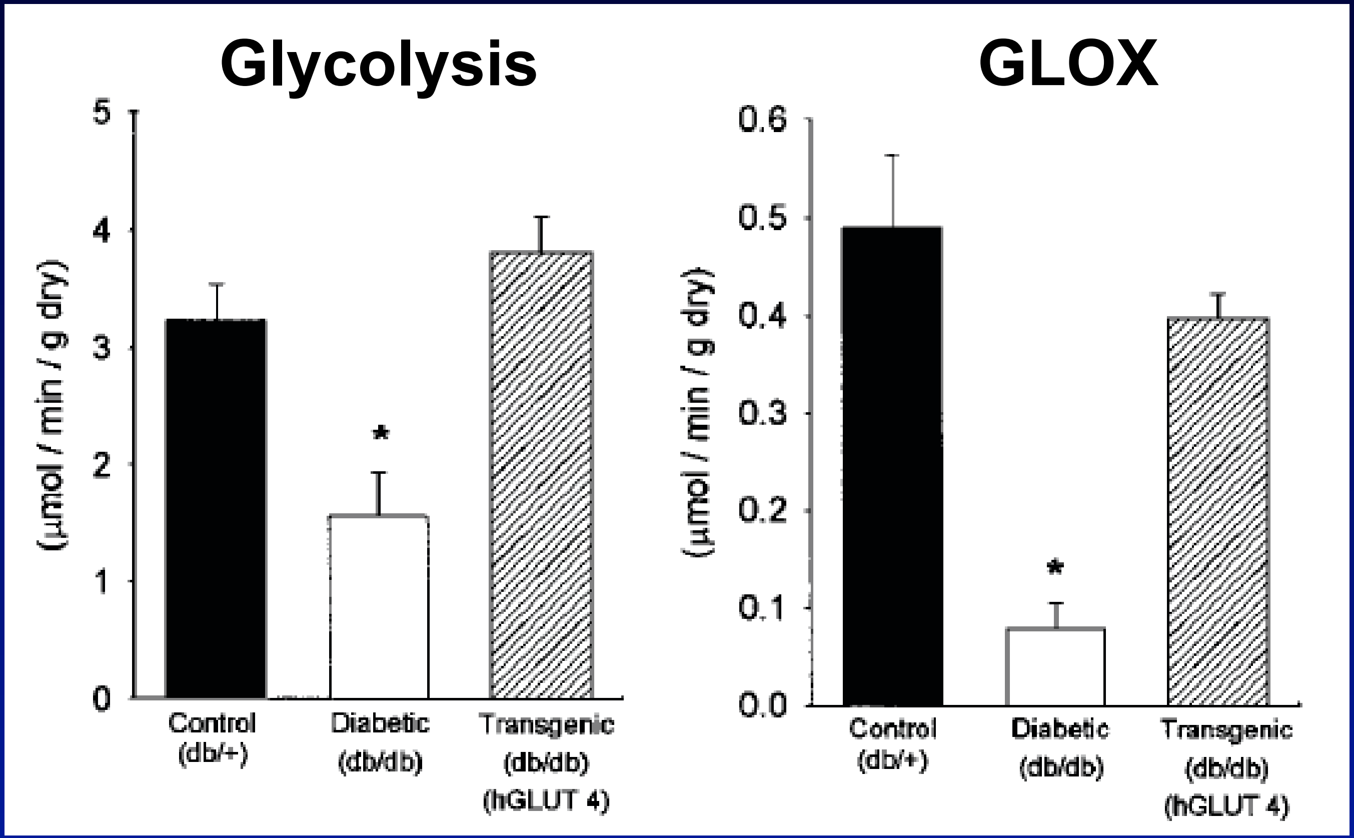
Panagia … Clarke **2005** *Am J Physiol 288:H2677*

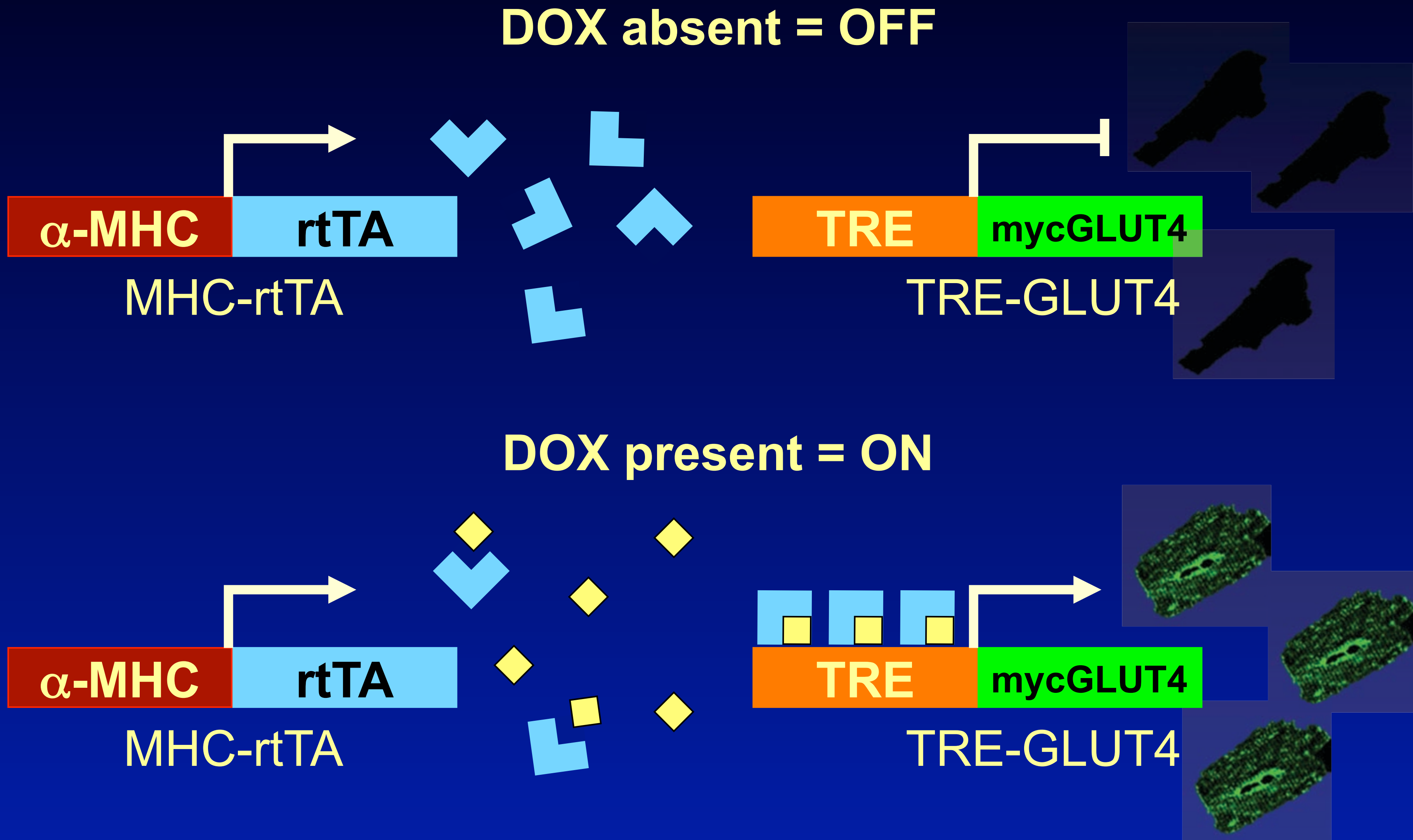
Constitutive GLUT4 Expression Prevents Development of Glucose Utilization Defects



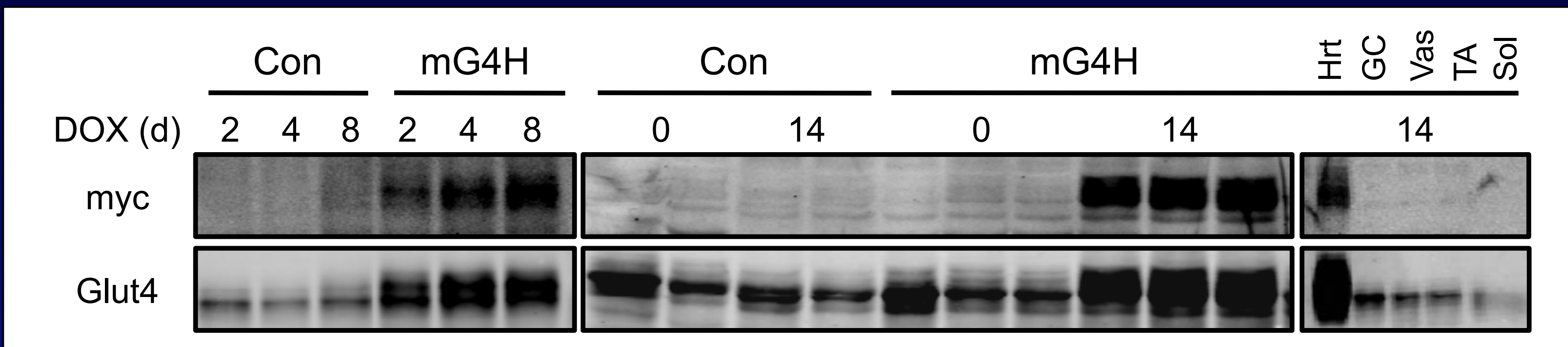
Belke … Severson **2000** *Am J Physiol* 279:E1104

Question: Is the change in cardiac metabolic substrate flexibility adaptive or maladaptive?

Inducible Cardiomyocyte-Specific GLUT4 Expression (mG4H)

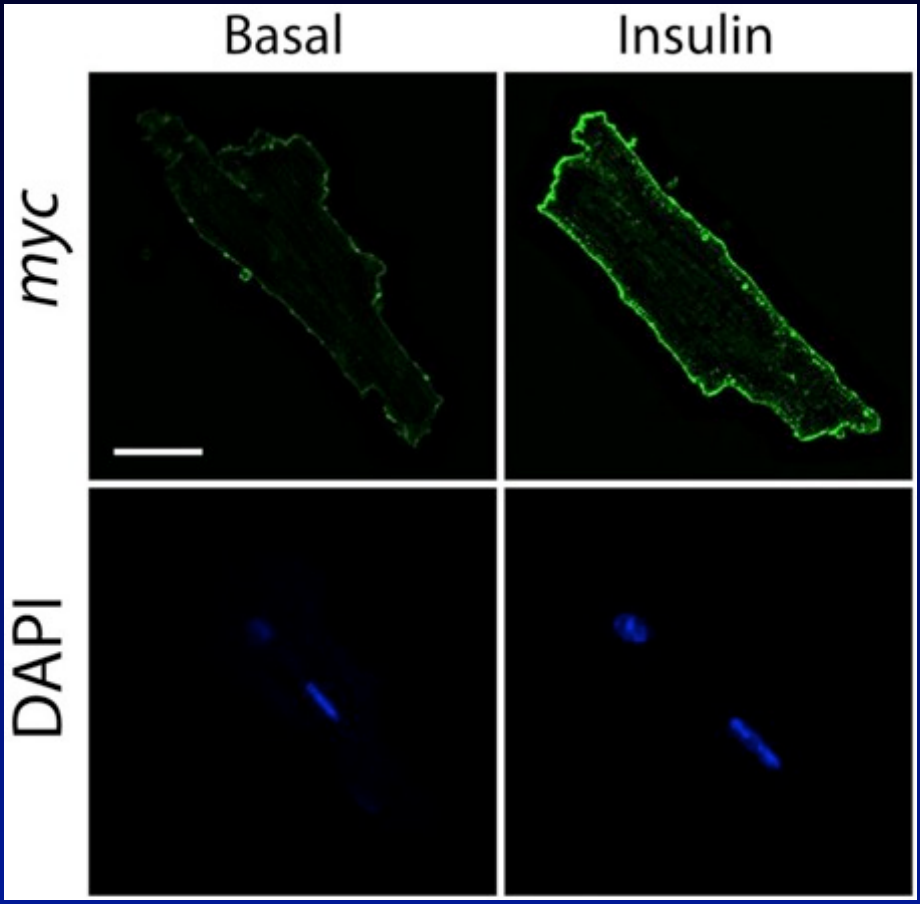


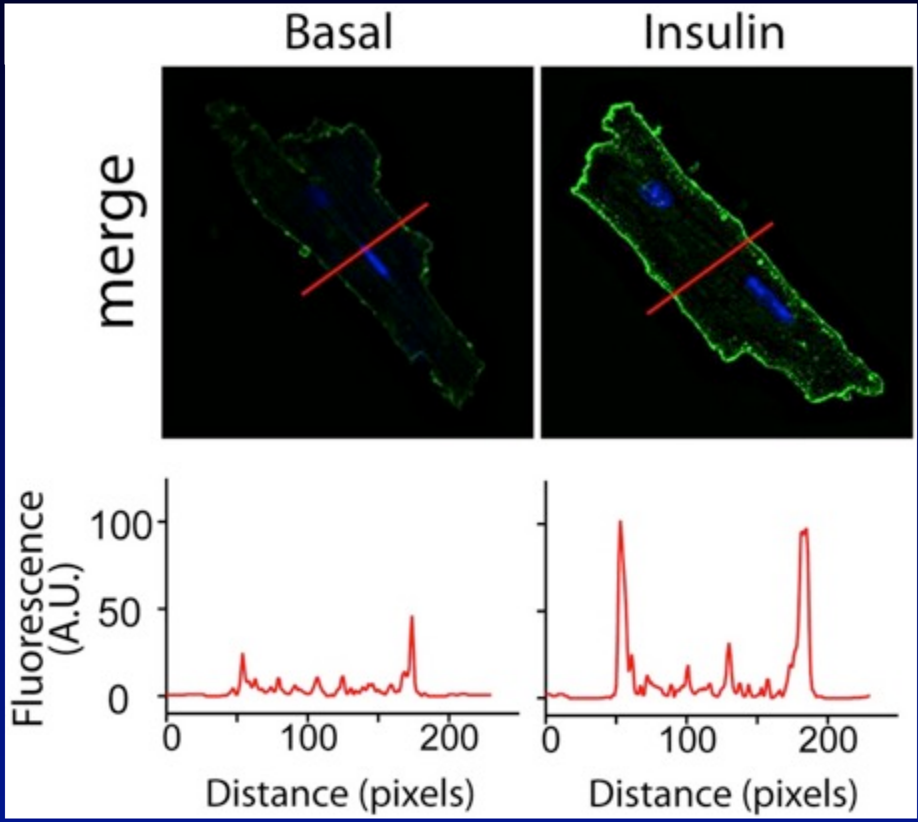
mG4H Mice Exhibit Inducible Cardiac-Specific Expression of GLUT4



Hrt = Heart
GC = Gastrocnemius
Vas = Vastus lateralis
TA = Tibialis anterior
Sol = Soleus

Insulin-induced GLUT4 Vesicle Fusion and Exofacial Myc-Epitope Exposure

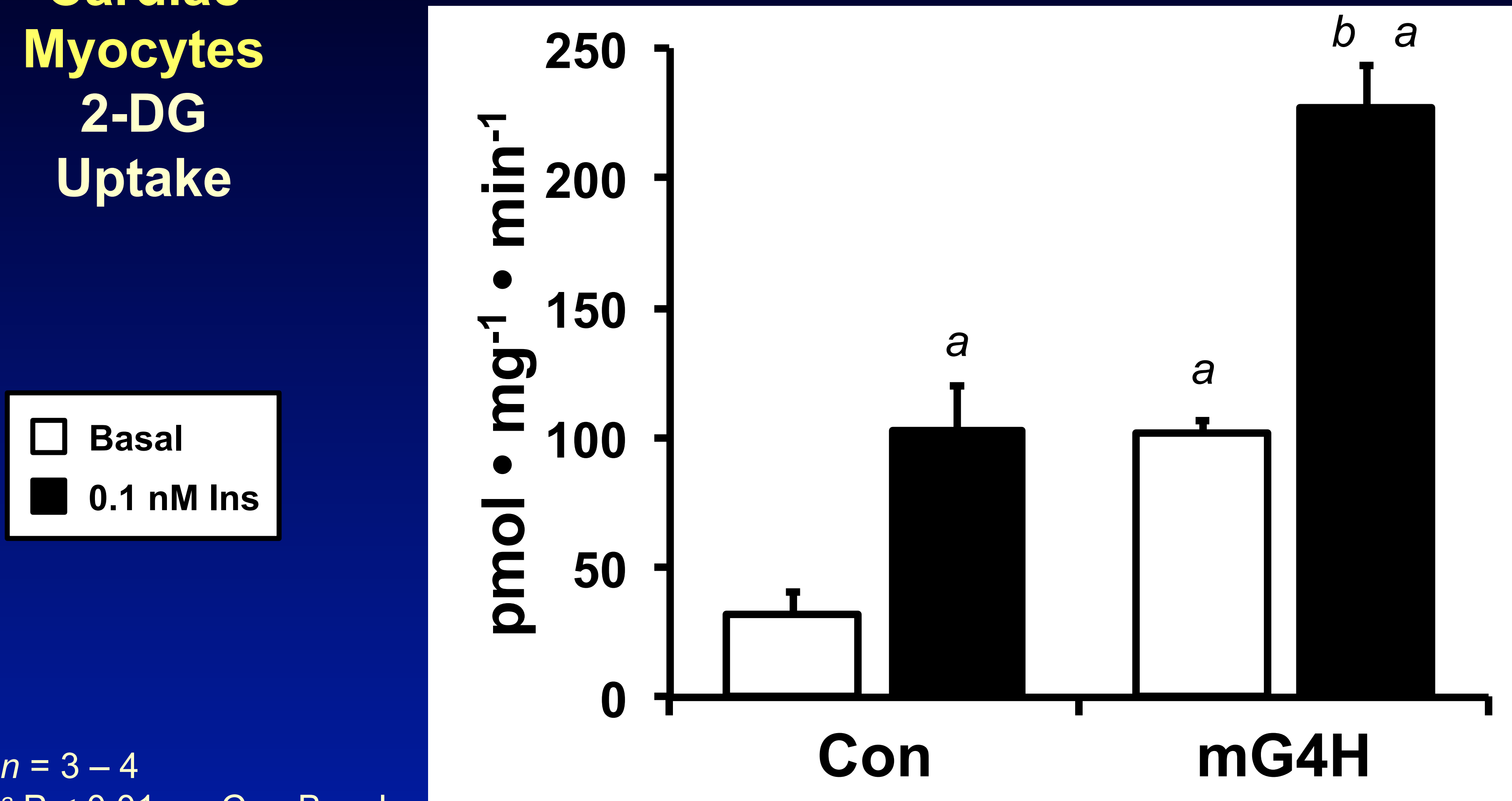




Ariel Contreras-Ferrat

GLUT4 Induction Increases Basal and Insulin-Stimulated Glucose Uptake

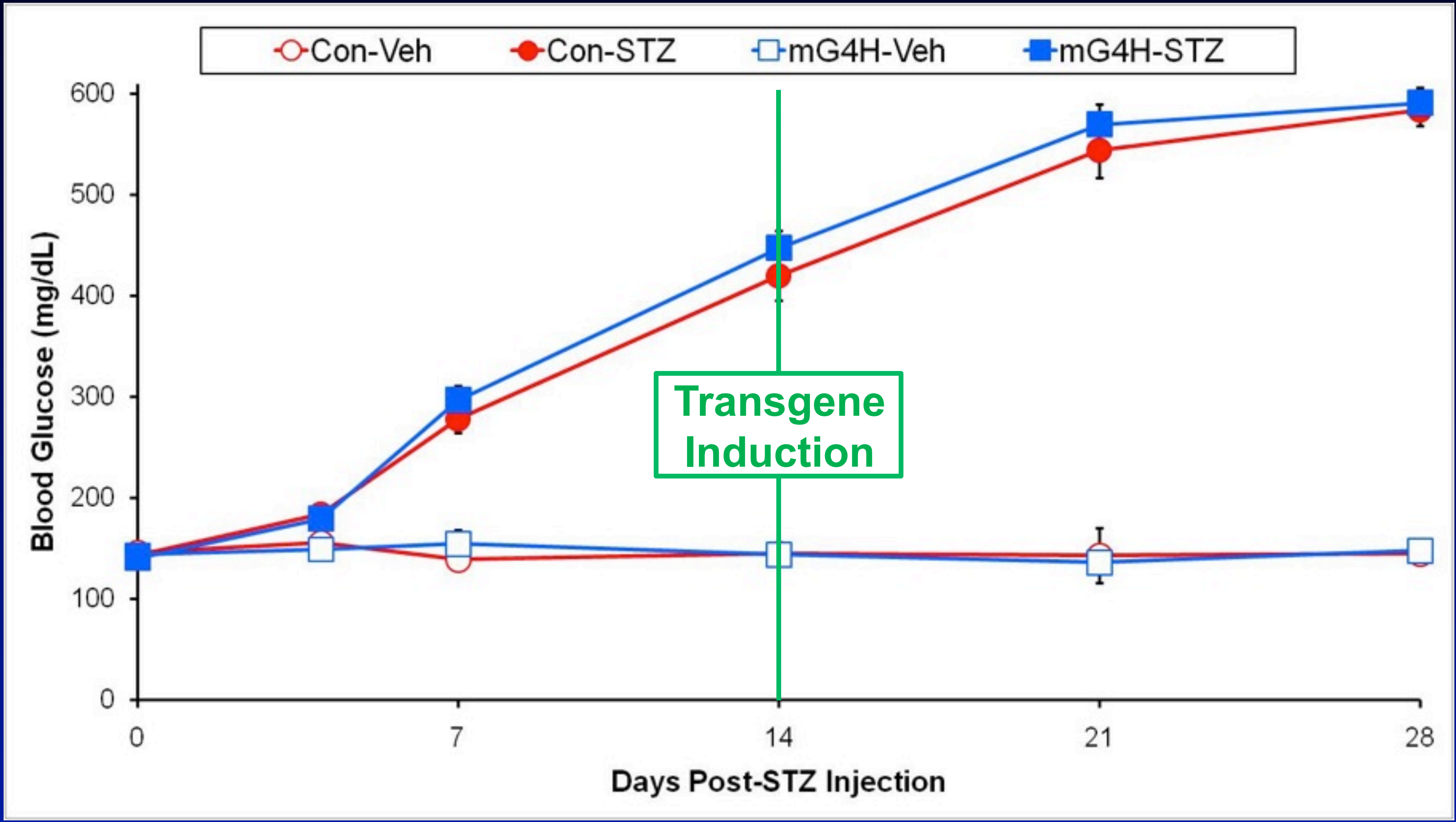
Cardiac Myocytes 2-DG Uptake



n = 3 – 4
[a] P < 0.01 vs. Con-Basal
[b] P < 0.001 vs. All

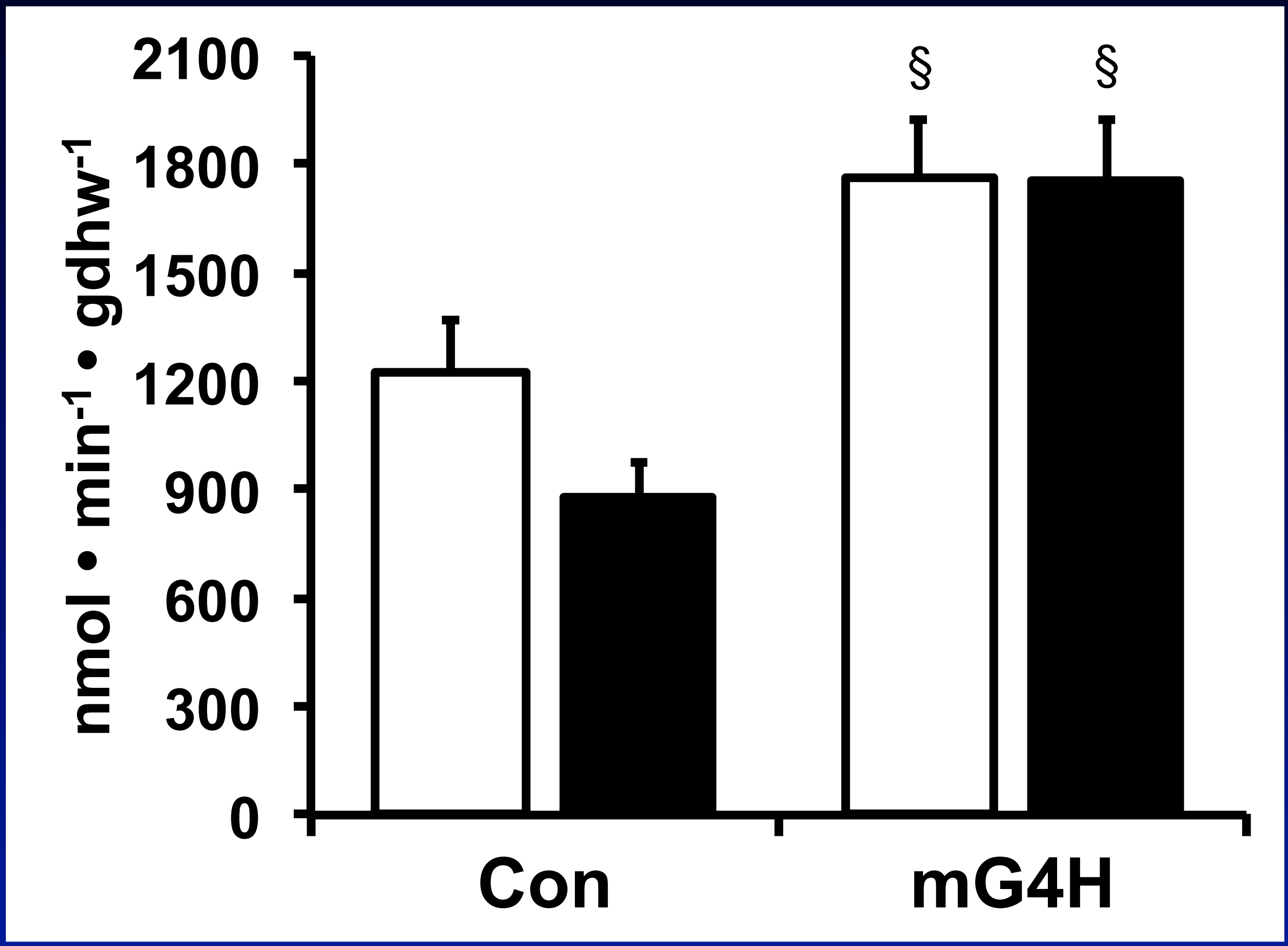
Renata O. Pereira

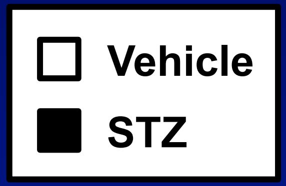
Streptozotocin (STZ)-Induced Hyperglycemia is Not Altered by Transgene Induction



GLUT4 Induction Increases Glycolysis and Rescues Diabetic Cardiac Glycolytic Defects

Isolated Working Hearts Glycolysis





n = 6 – 10
§ $P < 0.01$ vs. Con
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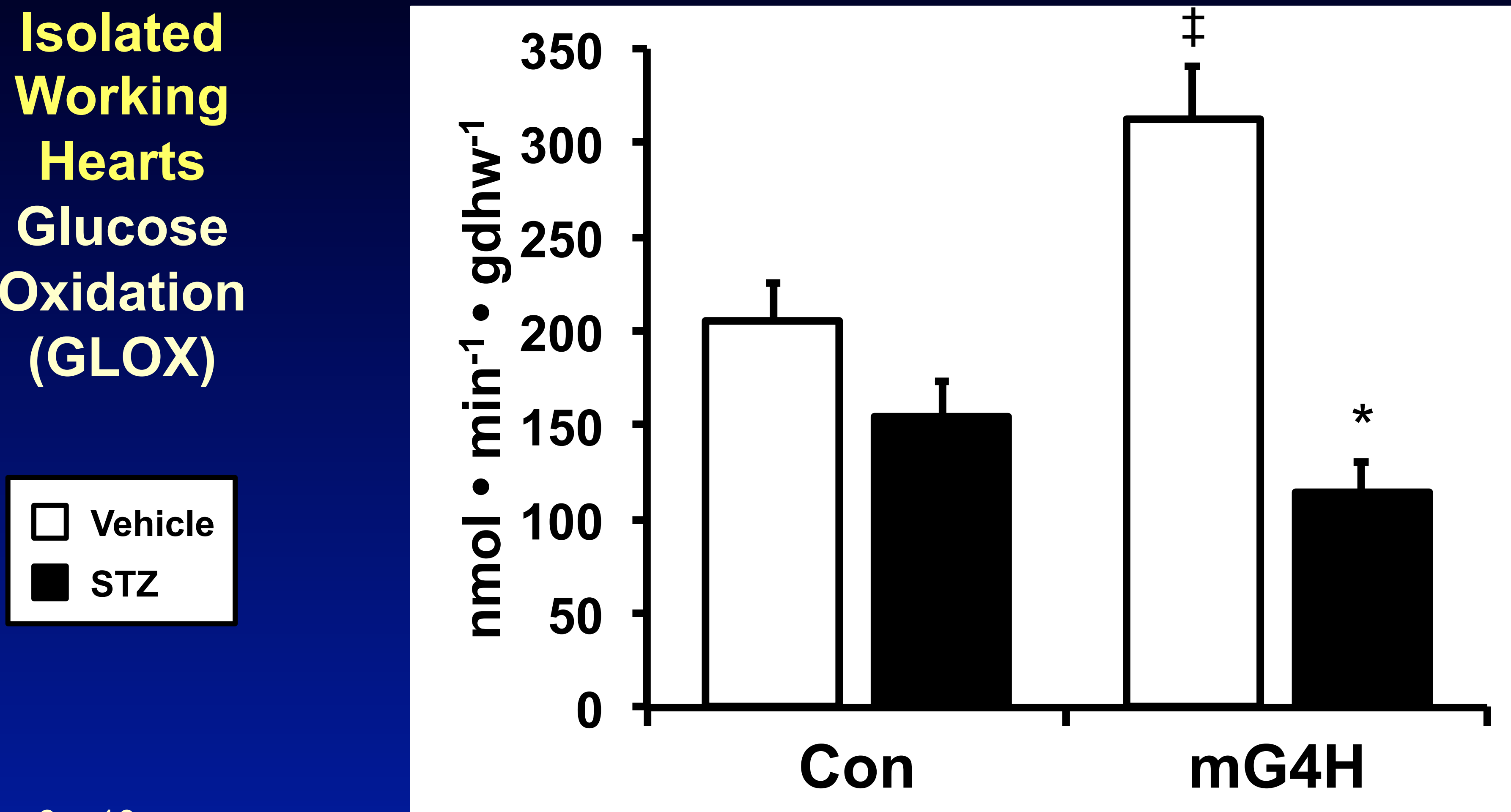
Joseph Tuinei
Wende … Abel *in prep*

GLUT4 Induction Increases GLOX but Accelerates Diabetic Cardiac GLOX Defects

Isolated Working Hearts Glucose Oxidation (GLOX)

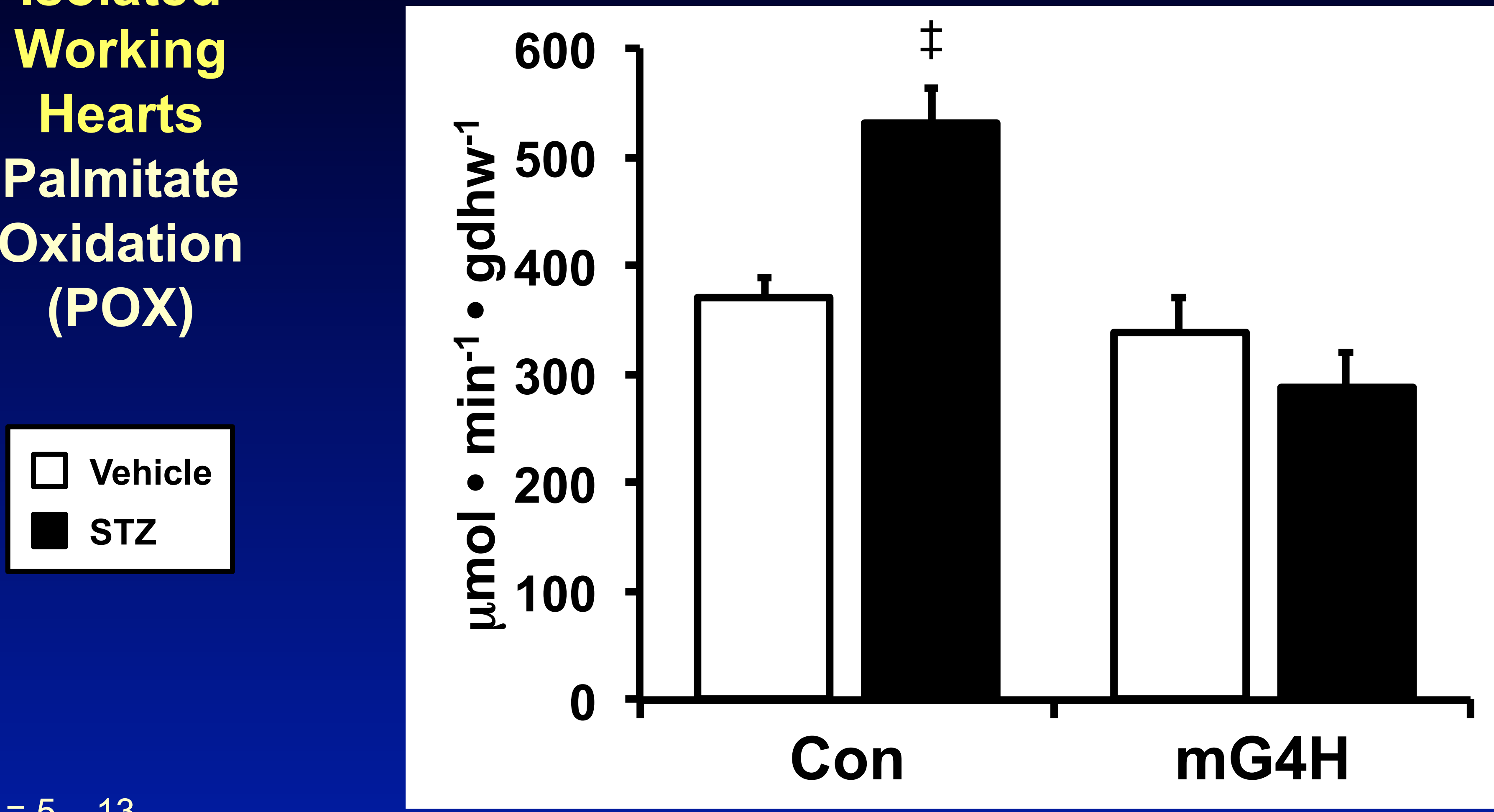


n = 6 – 10
‡ P < 0.001 vs. All
* P < 0.01 vs. Veh
ARW

Joseph Tuinei
Wende … Abel *in prep*

GLUT4 Induction Prevents Increased Cardiac POX in Diabetes

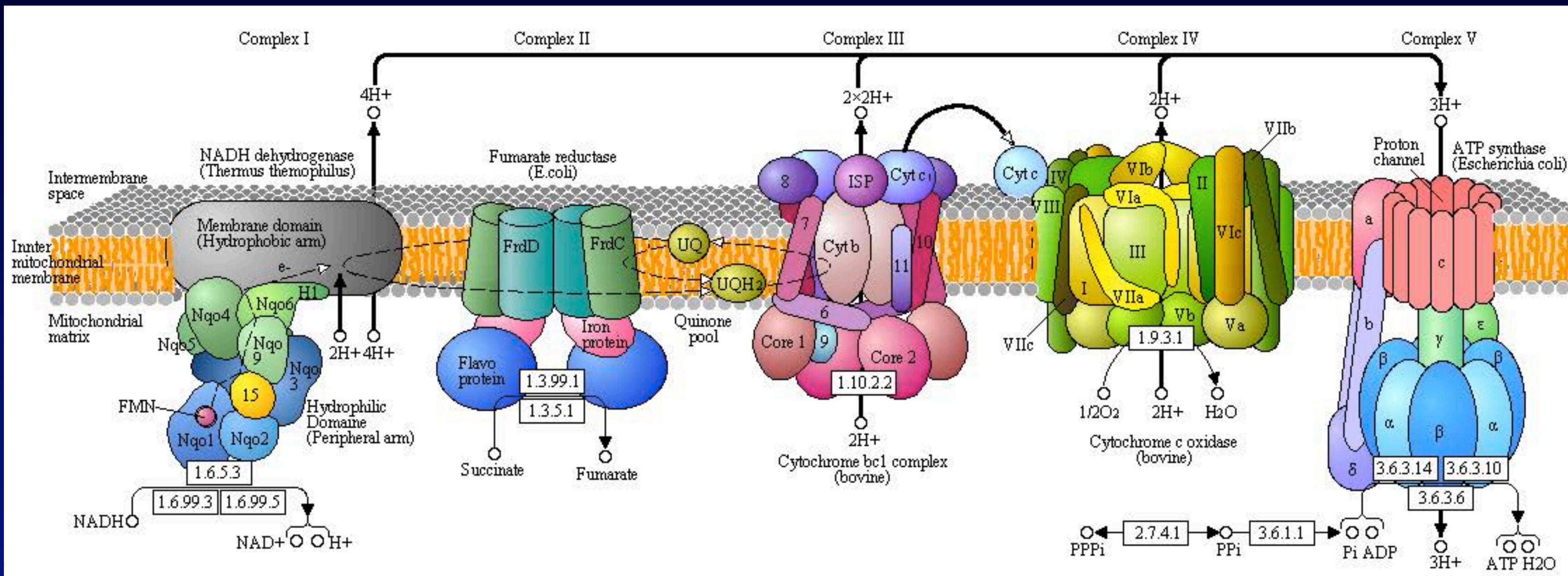
Isolated Working Hearts Palmitate Oxidation (POX)



n = 5 – 13
‡ P < 0.001 vs. All
ARW

Joseph Tuinei

Oxidative Phosphorylation



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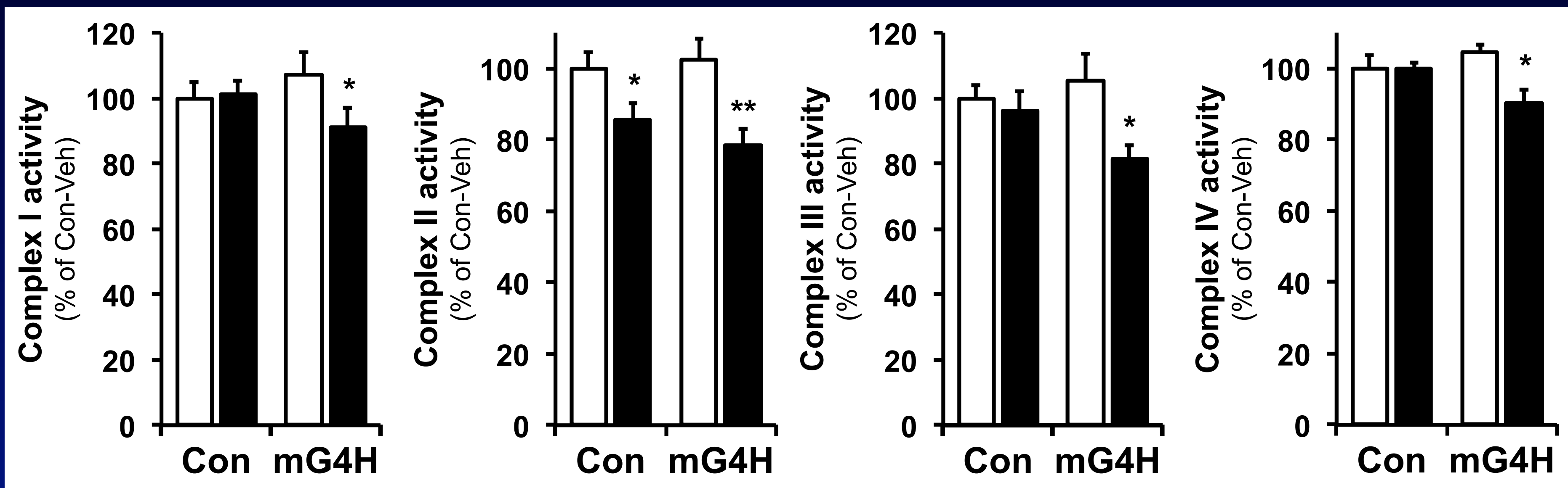
www.genome.jp/kegg/pathway.html

GLUT4 Induction Accelerates Development of Mitochondrial Dysfunction



n = 3 – 4
* P < 0.05
ARW

Oleh Khalimonchuk
Wende … Abel *in prep*

Conclusion – Part 1

In the context of diabetes, enhancing glucose delivery by expression of GLUT4 accelerates the progression of mitochondrial dysfunction.

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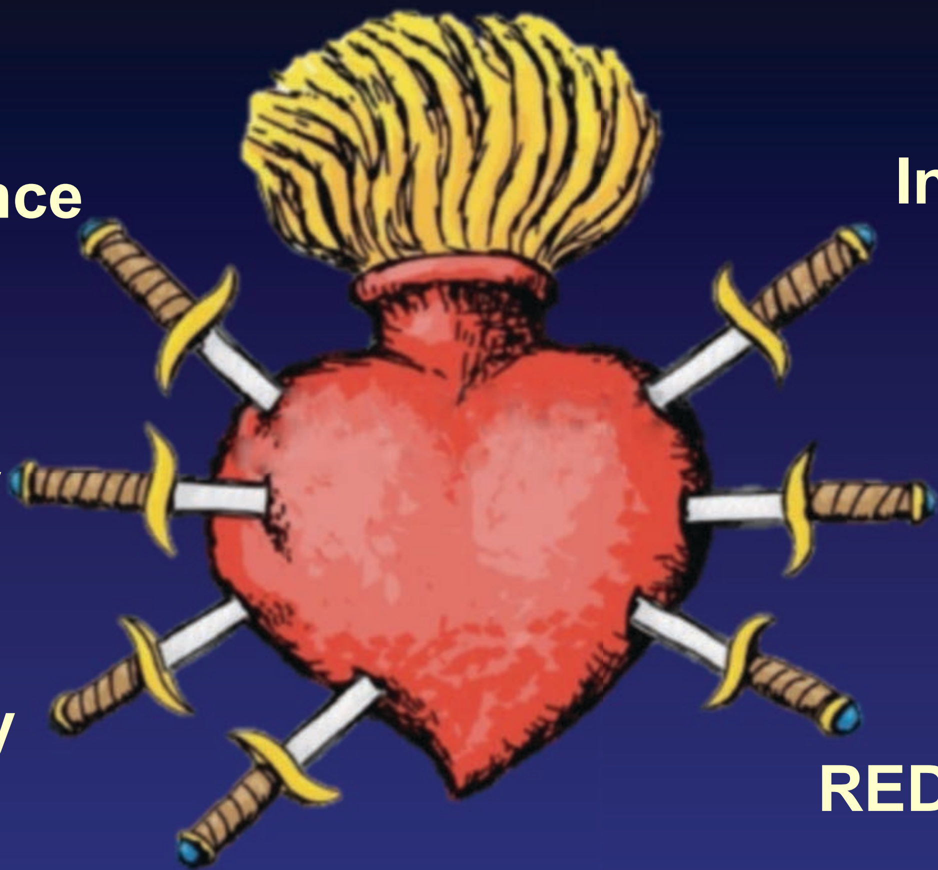
Diabetic Cardiomyopathy
"Death by a Thousand Cuts…"



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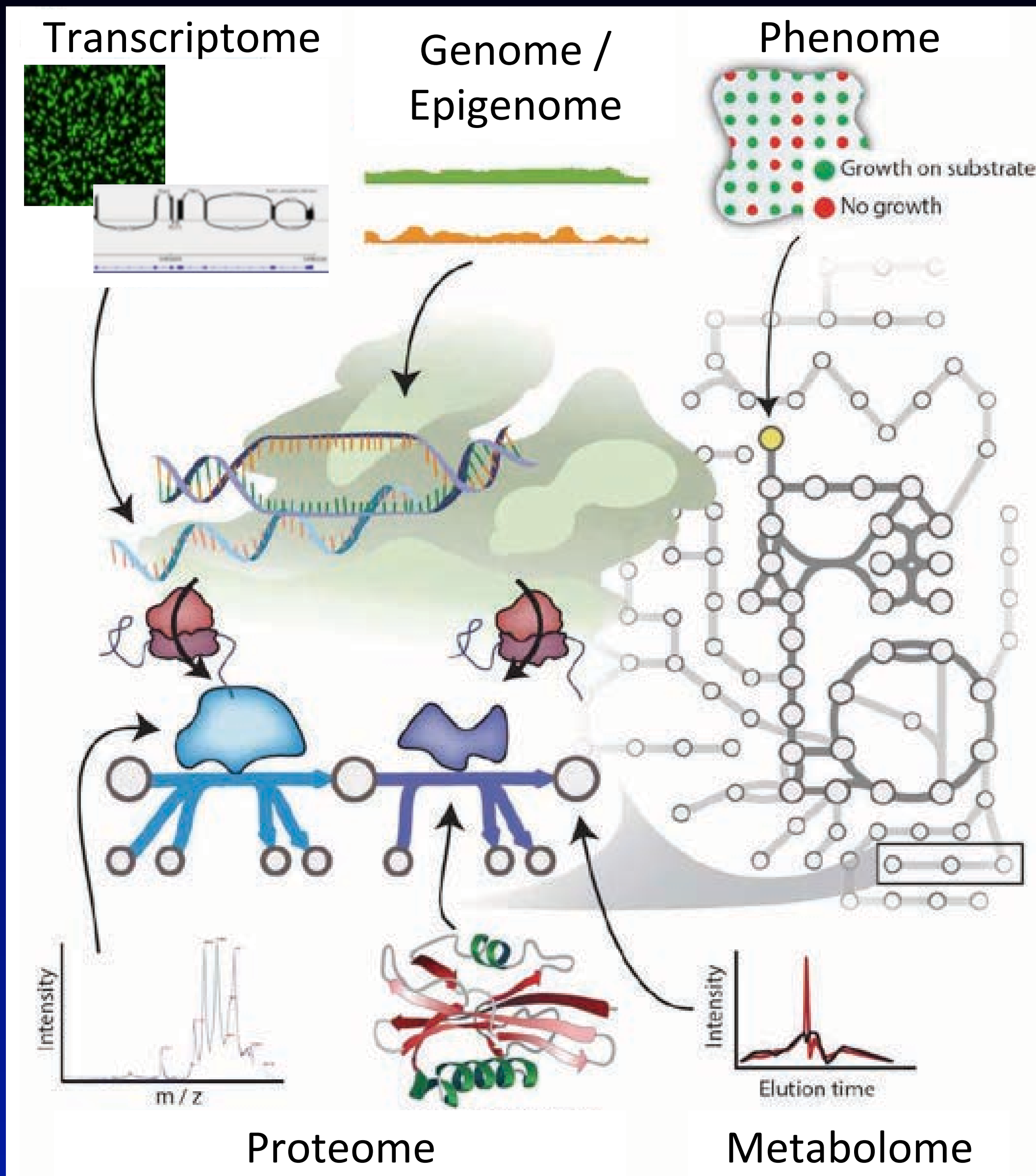
Adapted from Wende, Symons, and Abel **2012** *Curr Hypertens Rep* 14(6):517

Systems Biology



Phenome

Obesity, diabetes, heart failure, BHI, etc.

Transcriptome

Northerns, qPCR, microarray RNA-seq, miR, lncRNA, etc.

Proteome

Mass spec, western blot, Co-IP, IHC, PTMs, etc.

Metabolome

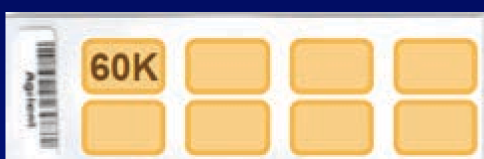
Glucometer, ELISA, GC-MS, HPLC, NMR, fluxomics, etc.

Genome / Epigenome

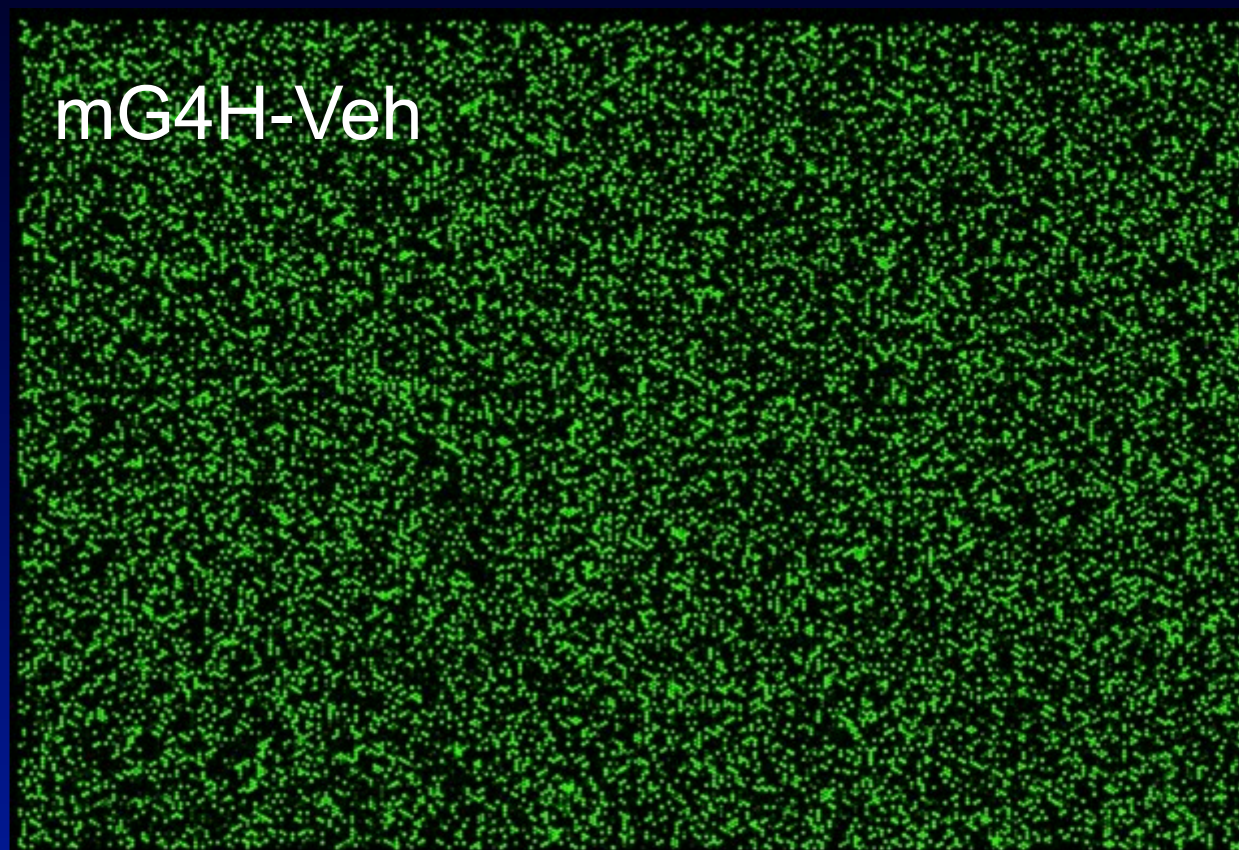
Southerns, sequencing, GenBank, ENCODE, ChIP-seq, bsDNA-seq, etc.

Adapted from Lewis and Abdel-Haleem **2013** *Front Physiol* 4:237

Transcriptomic Analysis Using the Agilent SurePrint G3 60K Microarray



181.9 MB



Microarray and Bioinformatic cores – Brian Dalley and Brett Milash
Wende … Abel *in prep*

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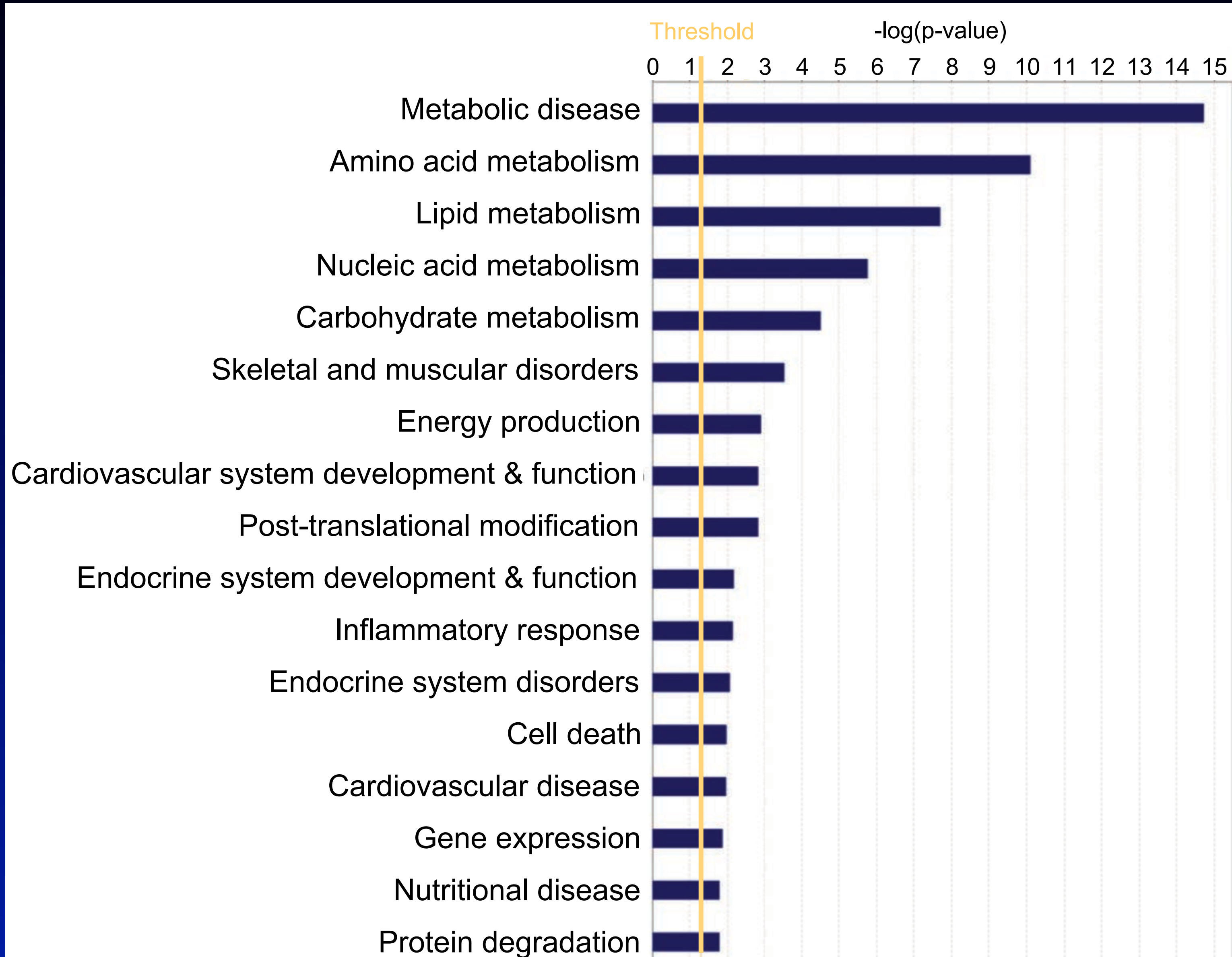
Pathway Analysis of Microarray

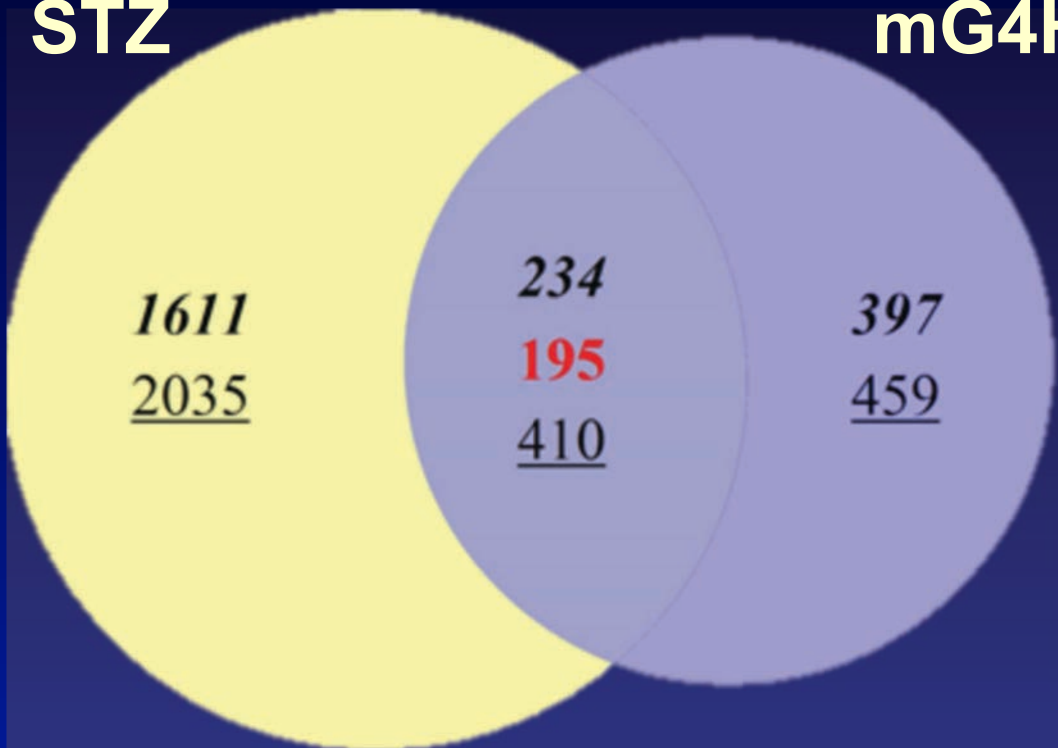


Wende … Abel *in prep*

Glucose Regulated Gene Expression

Mouse STZ

Mouse mG4H



0 *= up-regulated*

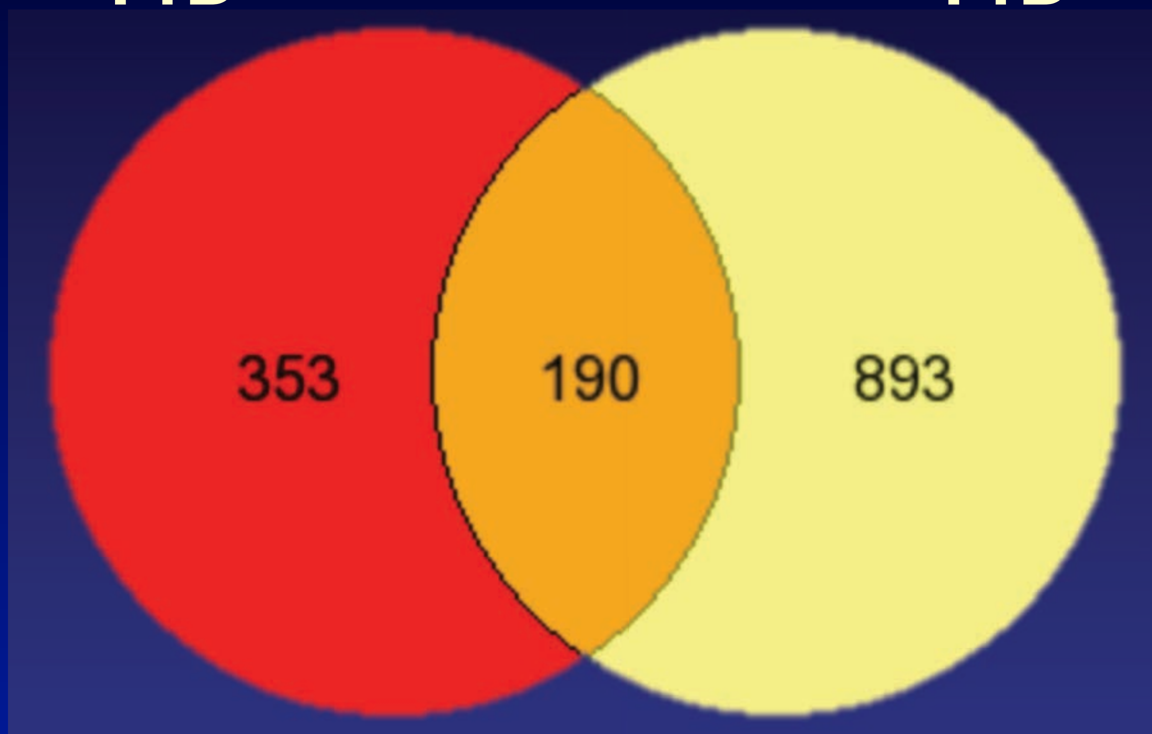
0 = contra-regulated

0 = down-regulated

Wende, unpublished

Species Conservation of Gene Expression Changes in Diabetes

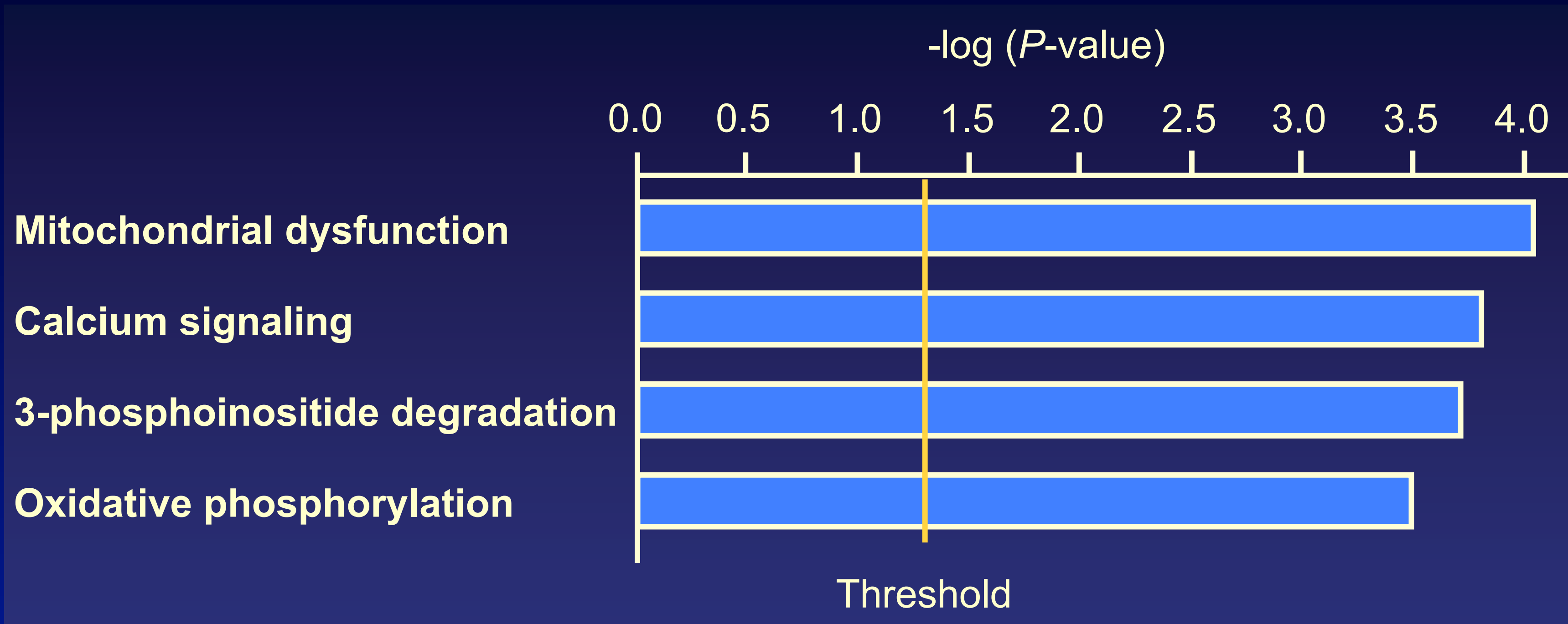
Human T1D **Mouse T1D**



ARW

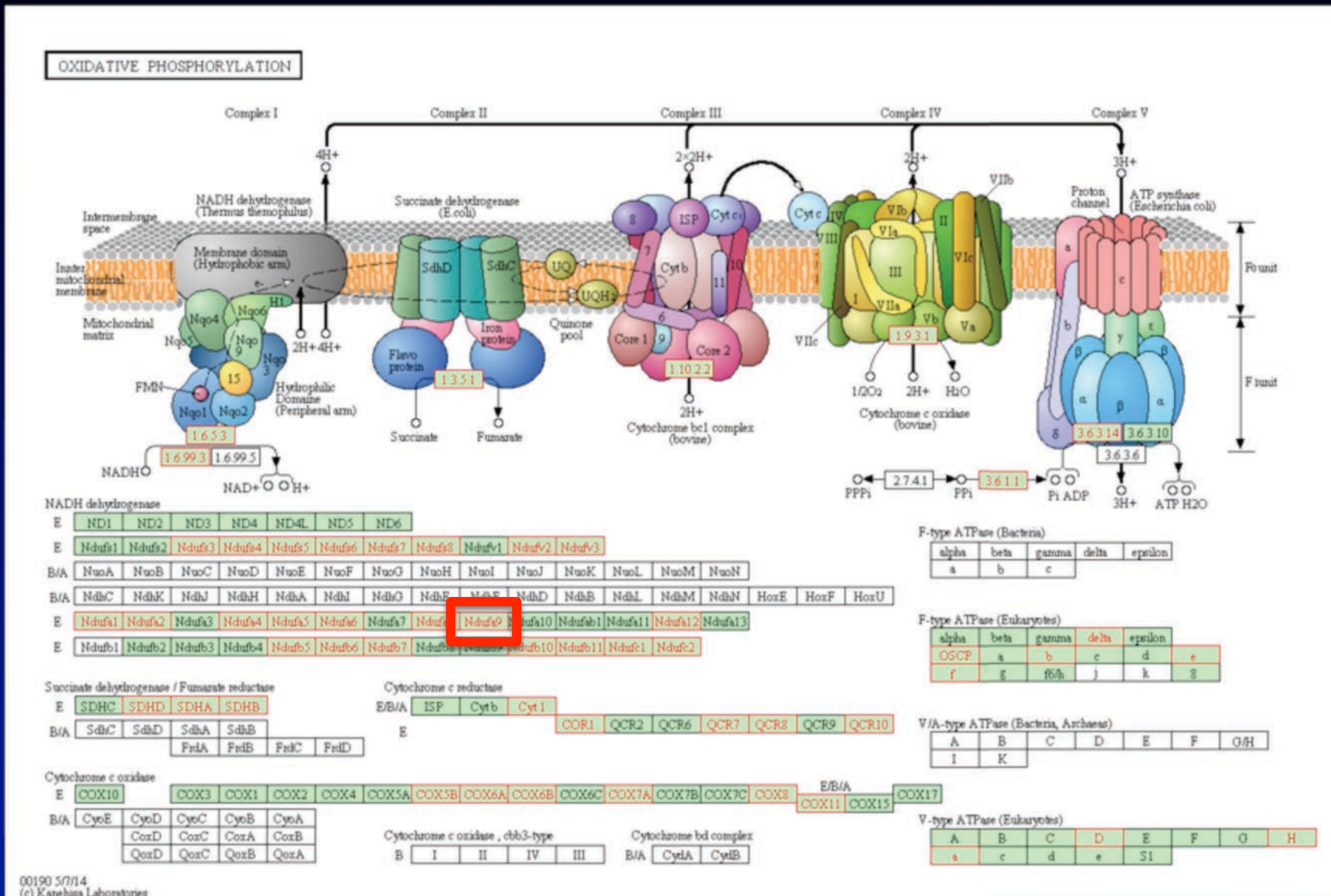
Drakos … Wende, unpublished

Species Conservation of Gene Expression Changes in Diabetes



Drakos … Wende, unpublished

Oxidative Phosphorylation



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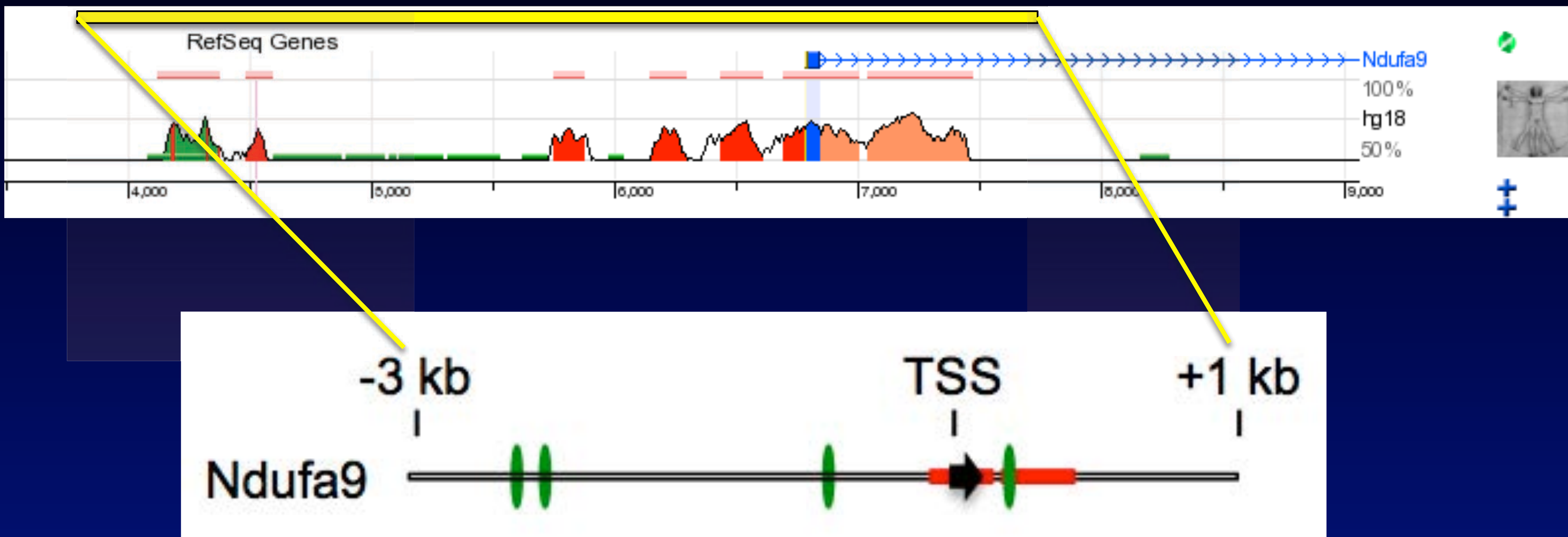
GeneSifter using KEGG

Ndufa9 Gene Promoter Structure



KEY

TSS = Transcription start site


= CpG island


= Sp1 RE

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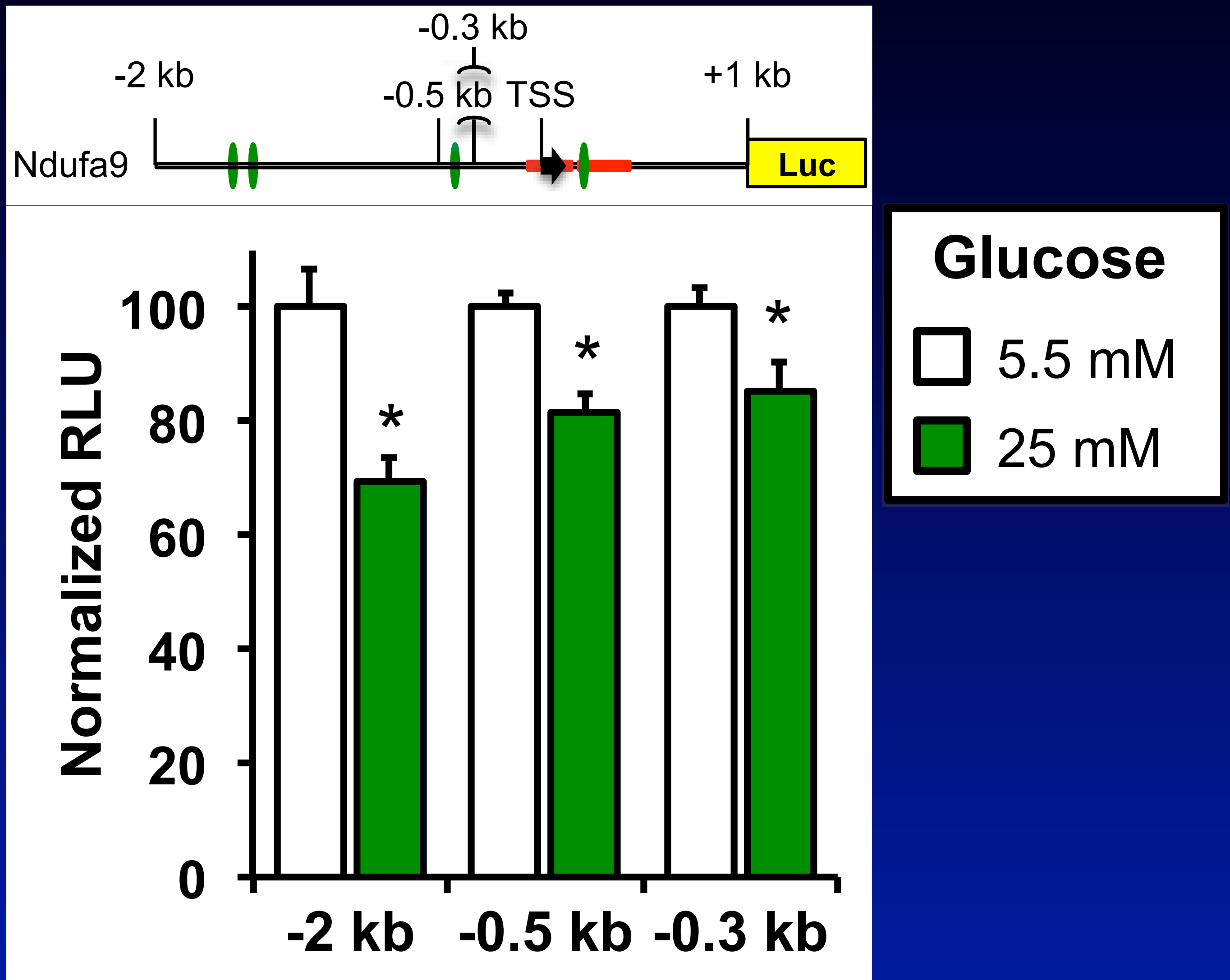
http://ecrbrowser.dcode.org

Ndufa9 Gene Promoter Mapping

Transient Transfection Promoter Activity



C_2C_{12} Myotubes
n = 9
* P < 0.05

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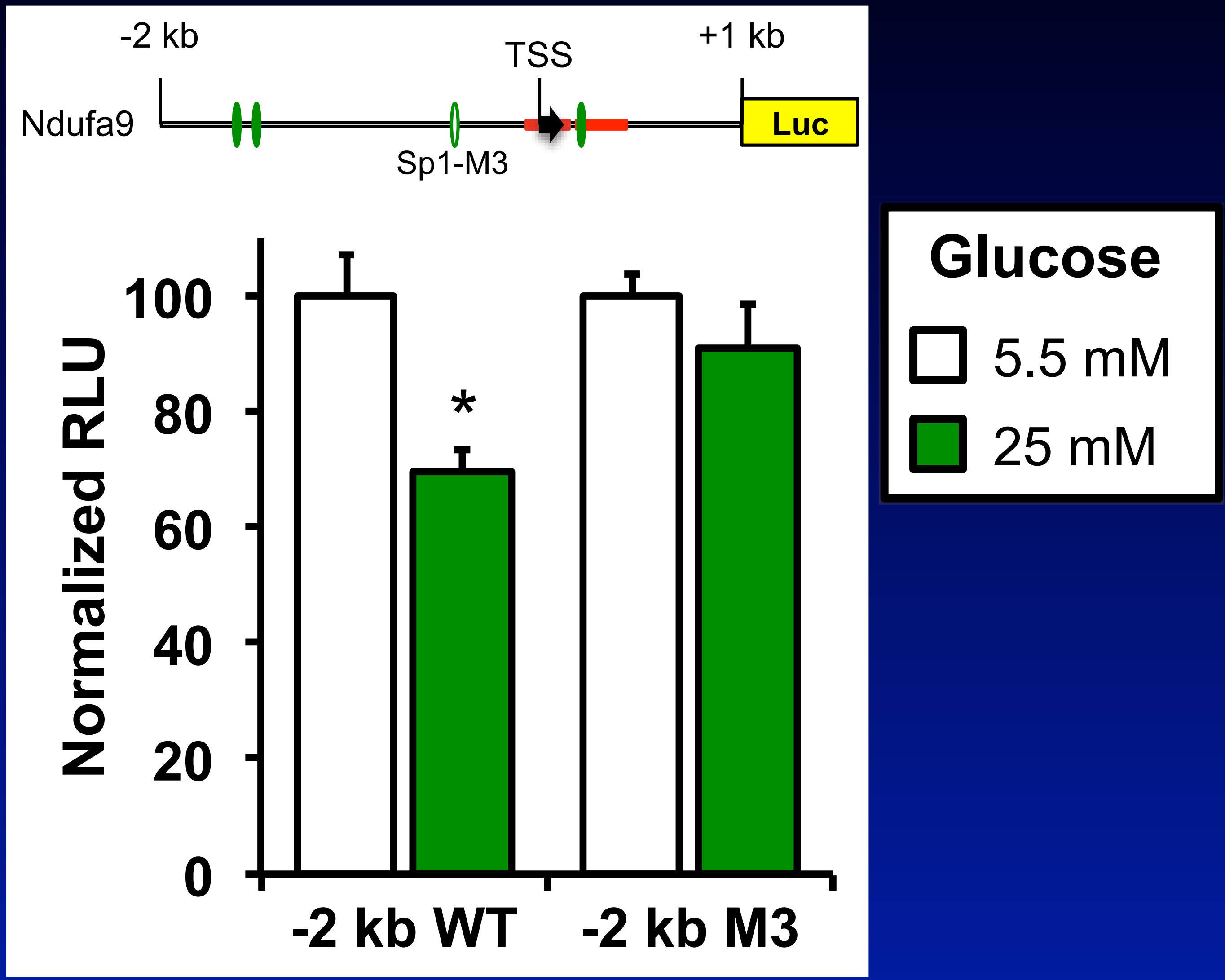
Wende … Abel *in prep*

Ndufa9 Gene Promoter Mapping

Transient Transfection Promoter Activity



C_2C_{12} Myotubes
n = 9
* P < 0.05

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Wende … Abel *in prep*

Ndufa9 Gene Promoter Mapping

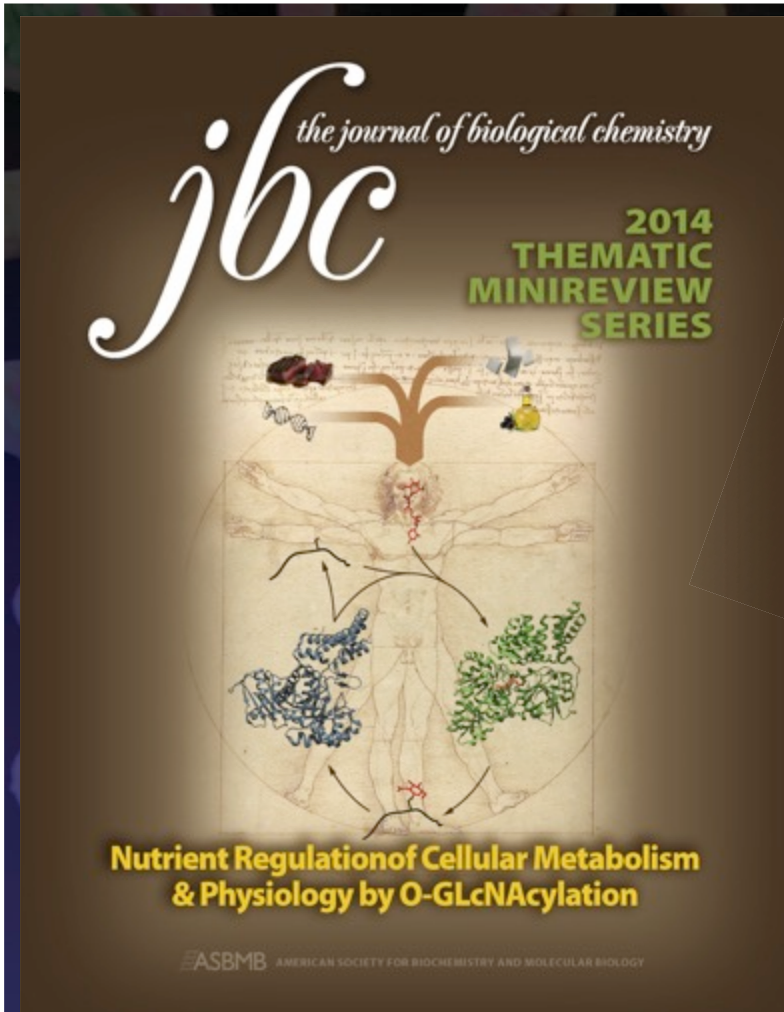
Transient Transfection Promoter Activity

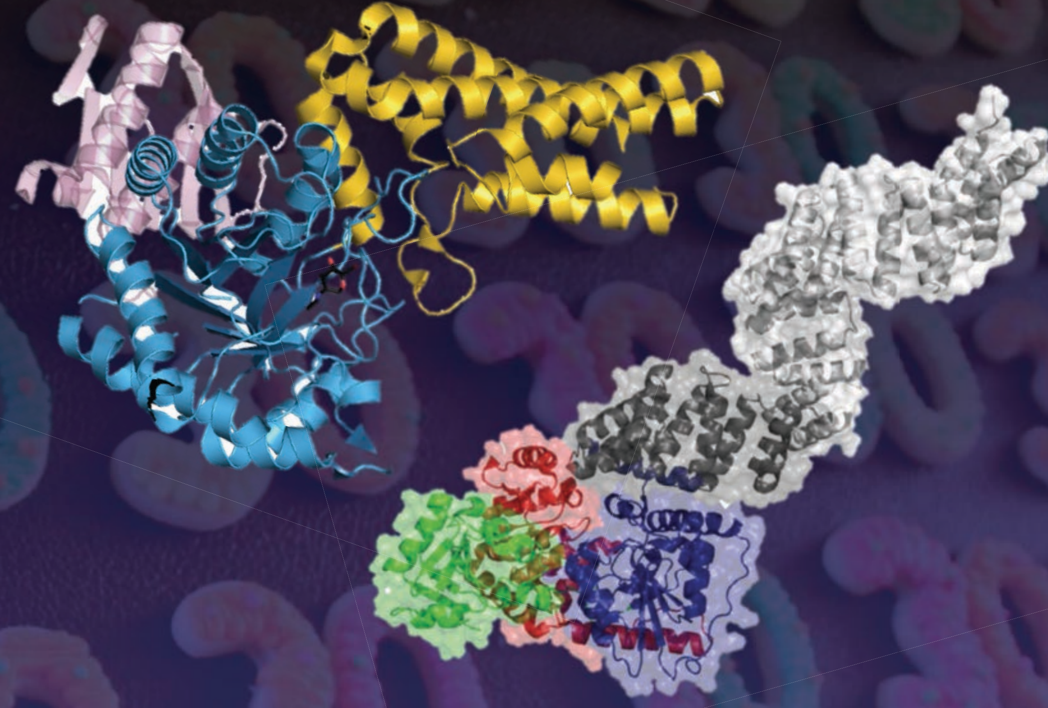
C_2C_{12} Myotubes
n = 9
* P < 0.05

ARW

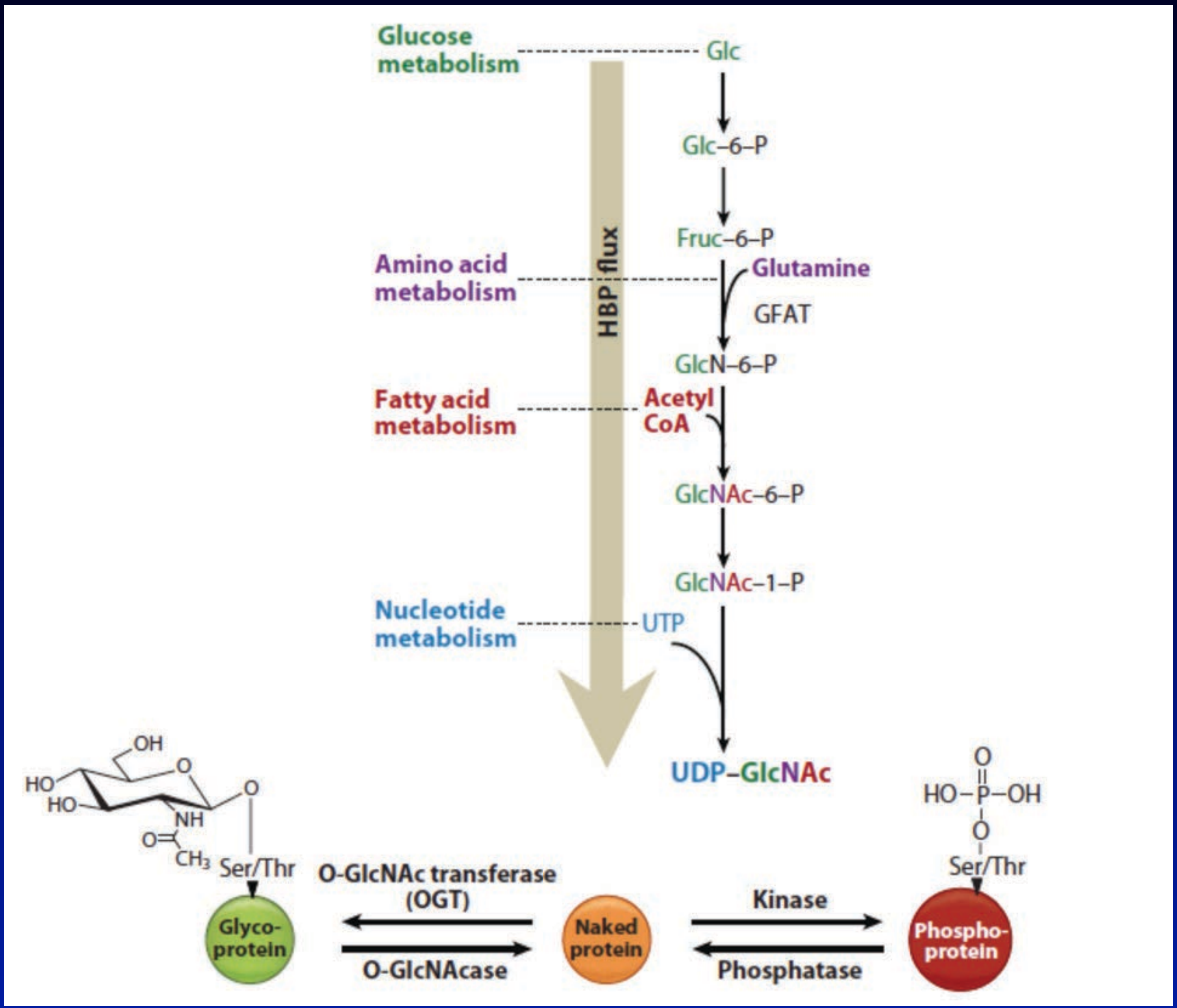
Wende … Abel *in prep*

O-GlcNAcylation





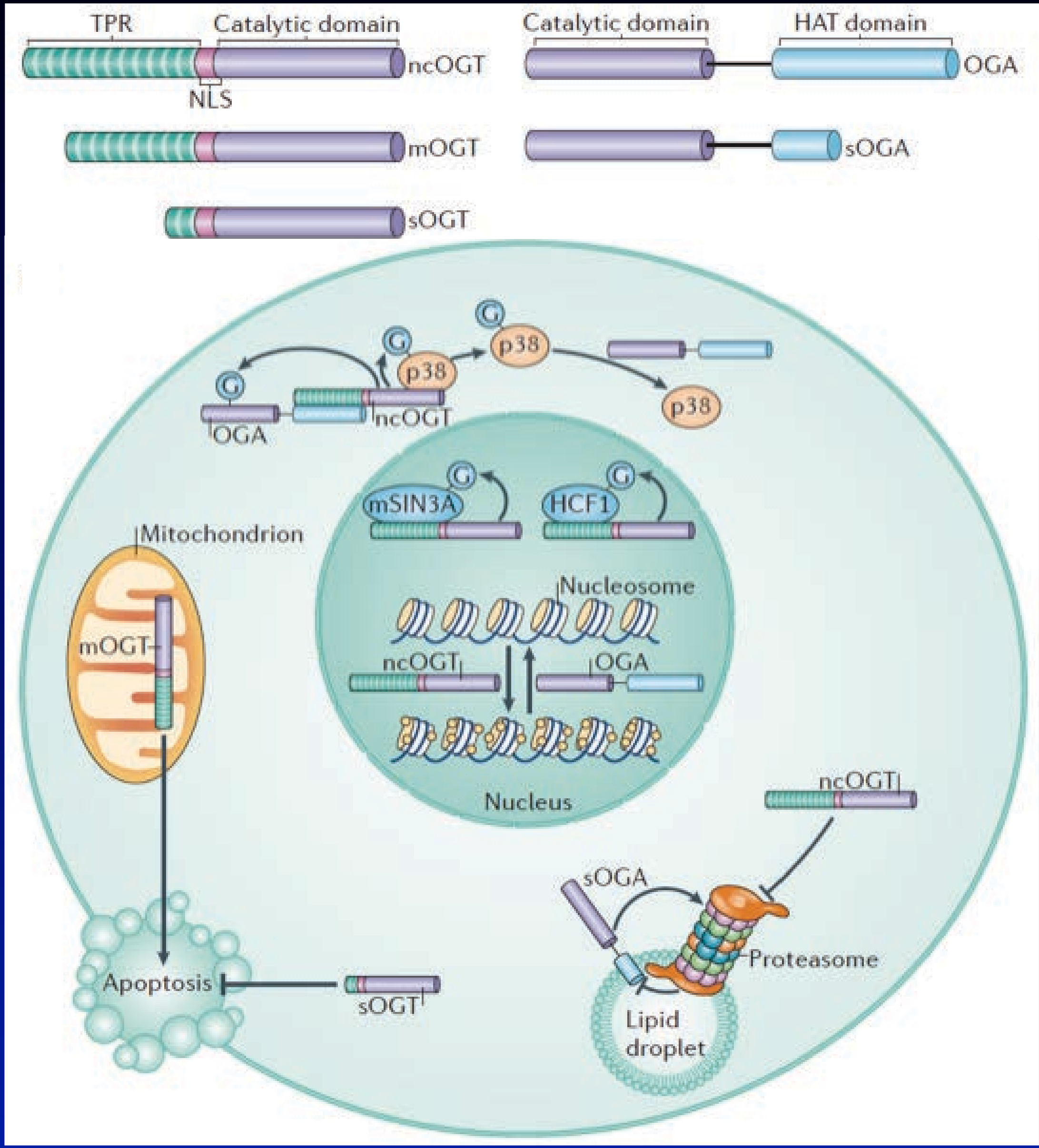
Metabolic Integration: Protein *O*-GlcNAcylation



ARW

Hart … Lagerlof **2011** *Annu Rev Biochem* 80:825

O-GlcNAc Cycling



Hanover … Love **2012** *Nat Rev Mol Cell Biol* 13(5):312

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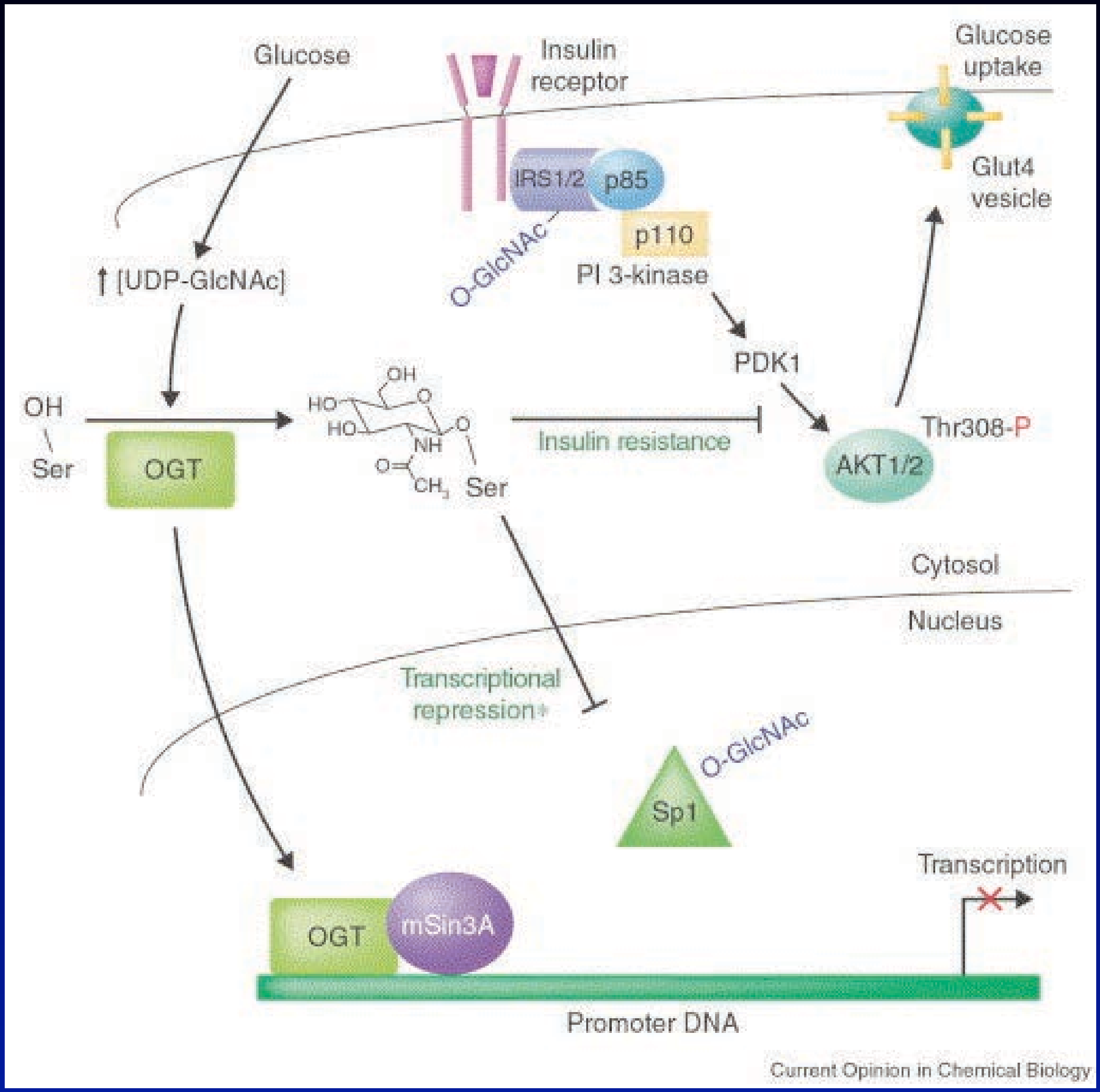
GlcNAc Regulation of Sp1



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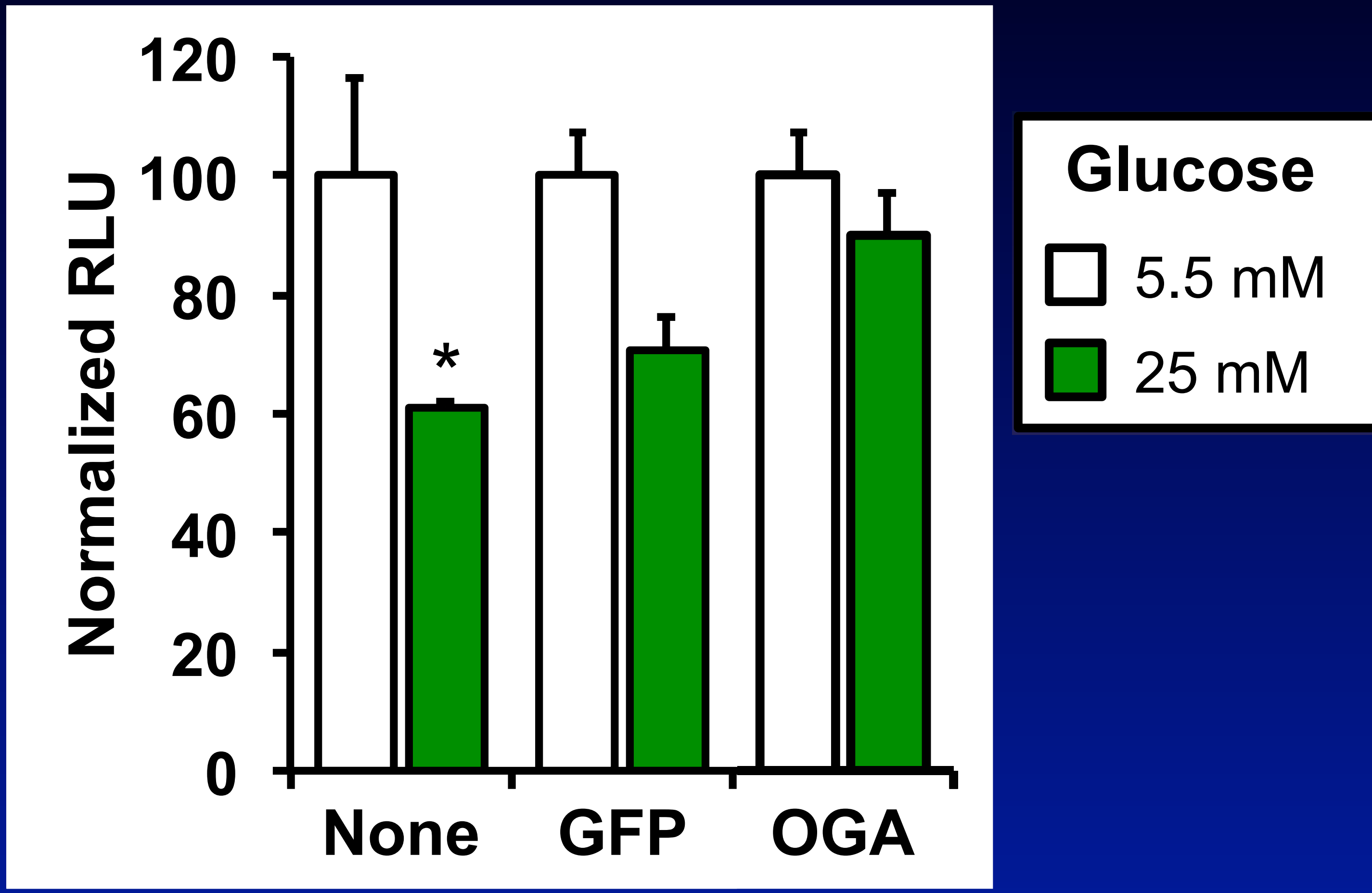
Vosseller … Hart **2002** *Curr Opin Chem Biol* 6(6):851

GlcNAcylation Regulates *Ndufa9* Gene Expression

Transient Transfection Promoter Activity



C_2C_{12} Myotubes
n = 3
* P < 0.05
ARW

Li Wang
Wende … Abel *in prep*

Conclusion – Part 2

Enhanced glucose delivery regulates oxidative capacity via transcriptional mechanisms including GlcNAcylation of transcription factors.

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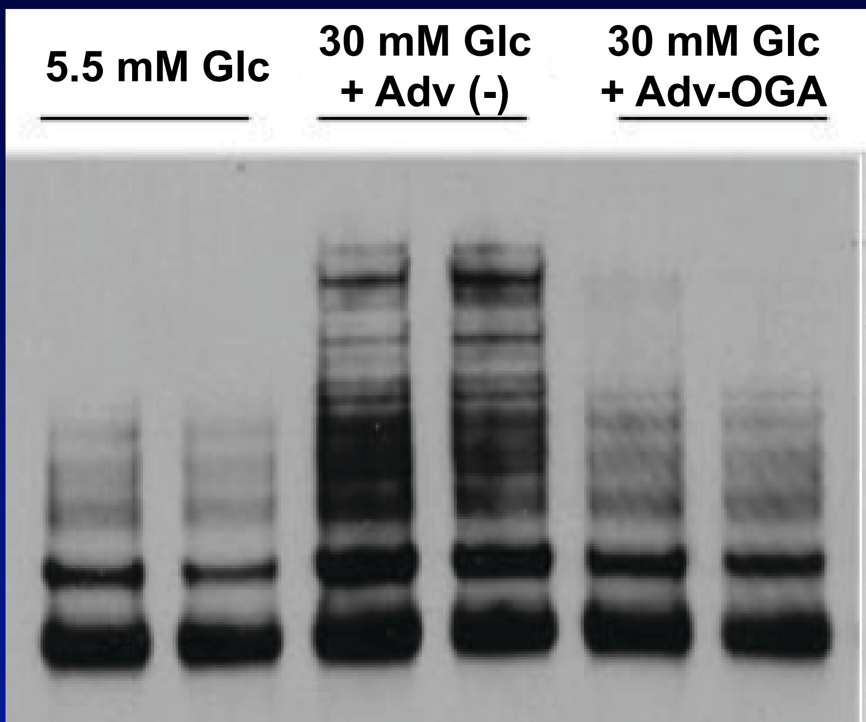
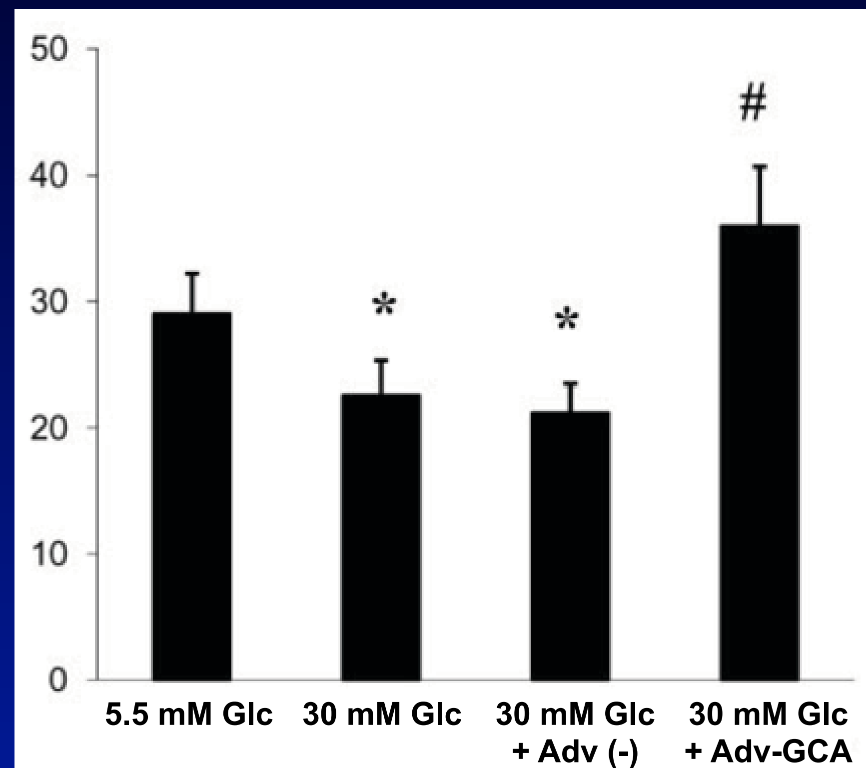
Mitochondrial Protein *O*-GlcNAcylation and Neonatal Cardiomyocyte Metabolic Function

Mitochondrial Protein *O*-GlcNAcylation



Complex I Activity



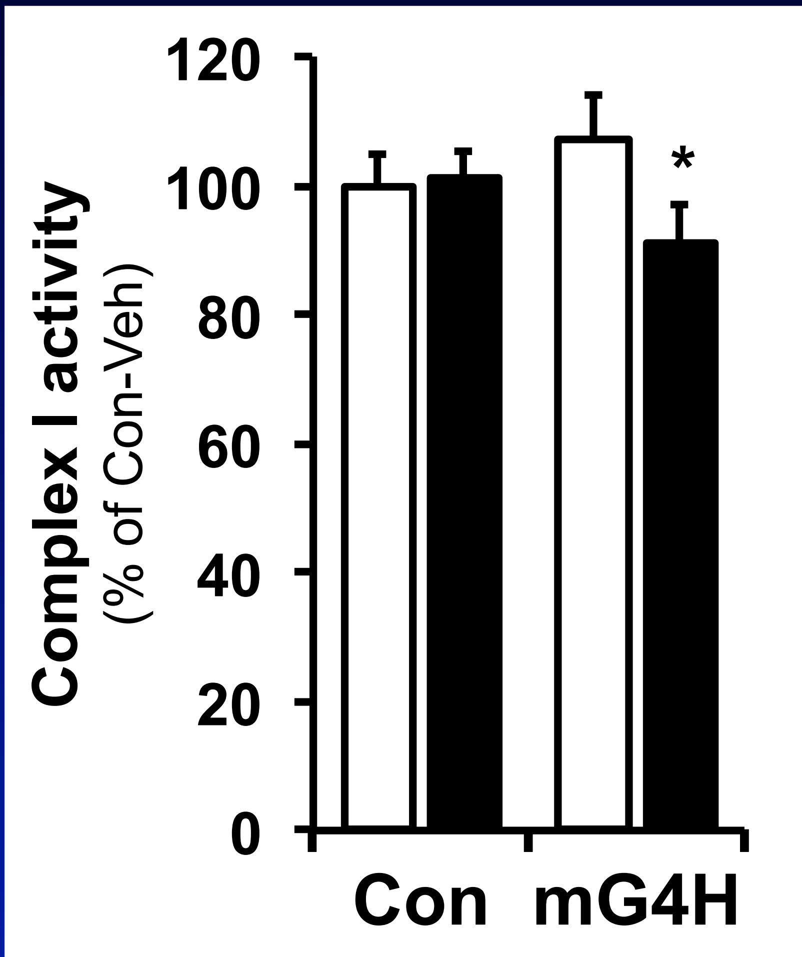
O-GlcNAcylation of NDUFA9

ARW

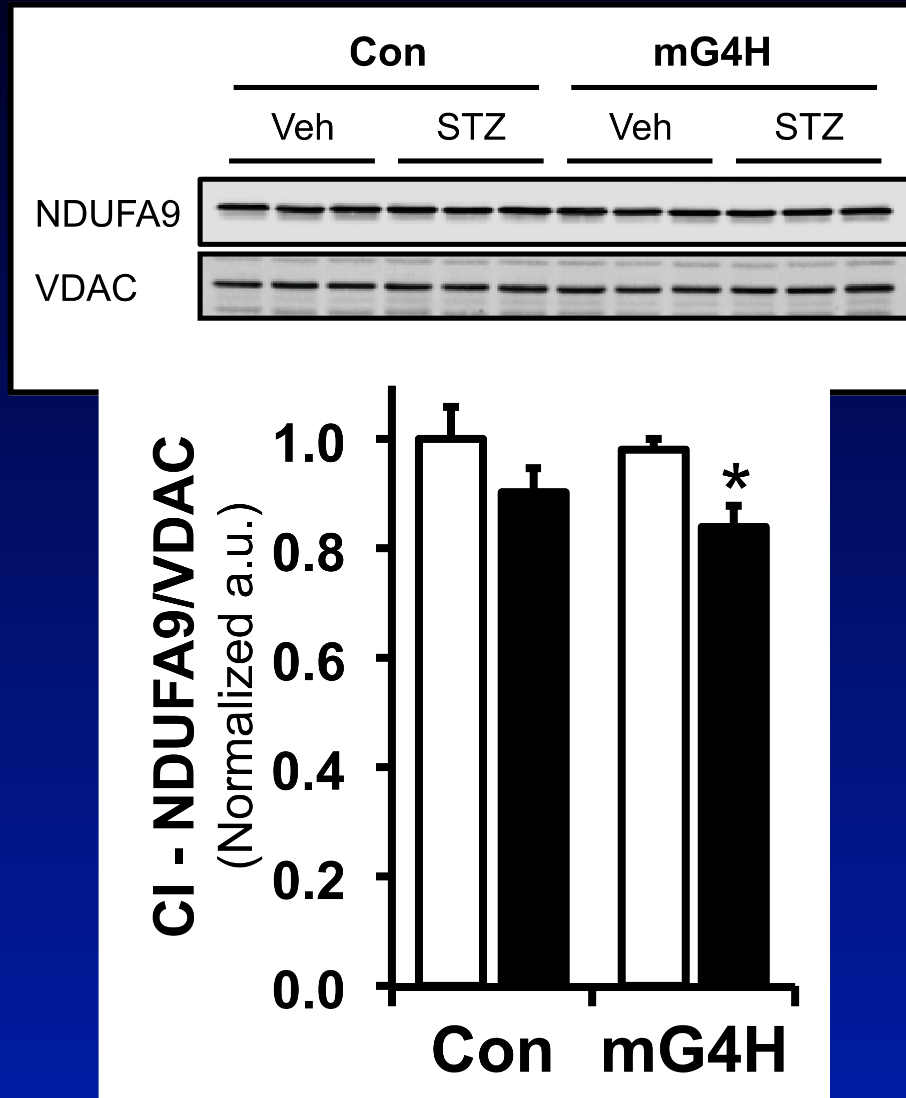
Hu … Dillmann **2009** *J Biol Chem* 284(1):547

NDUFA9 – Complex I

OXPHOS Activity



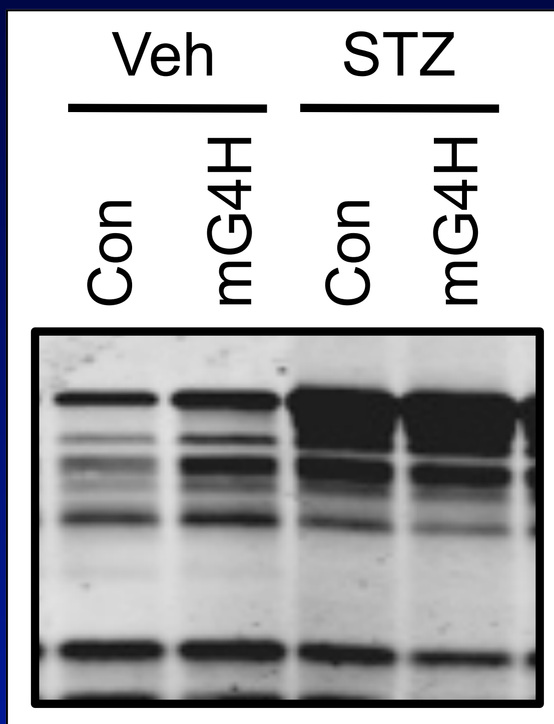
Western Blot



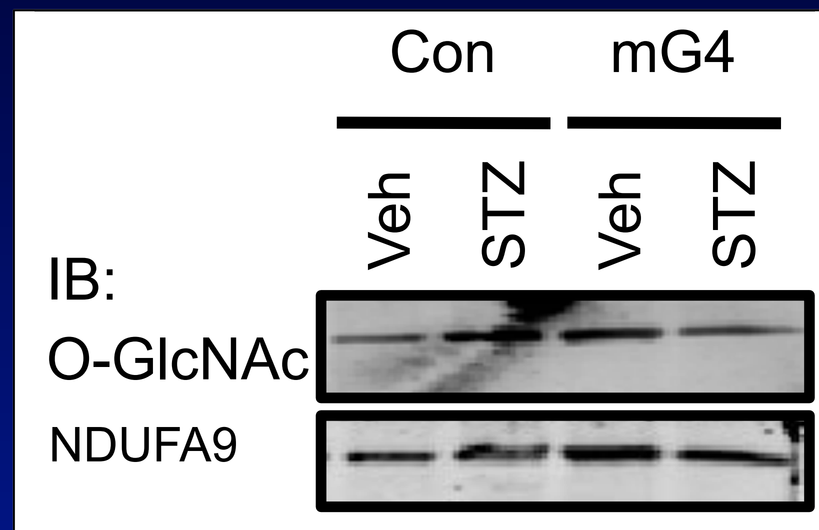
Chase A. Andrizzi

GLUT4 Induction Alters Mitochondrial Protein *O*-GlcNAcylation

Mitochondrial Protein
O-GlcNAcylation



NDUFA9
Immunoprecipitation

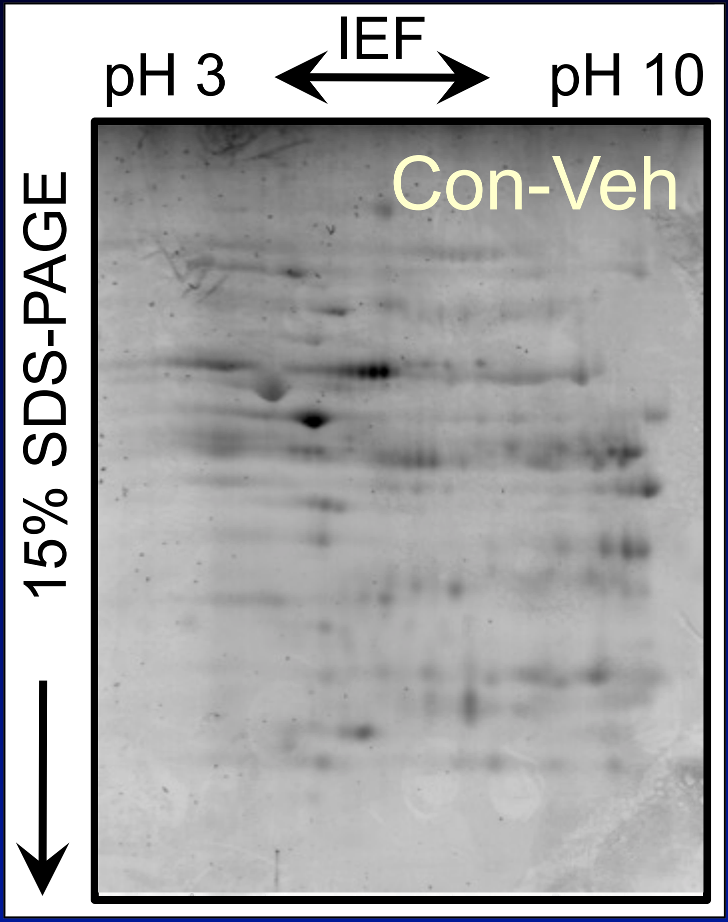


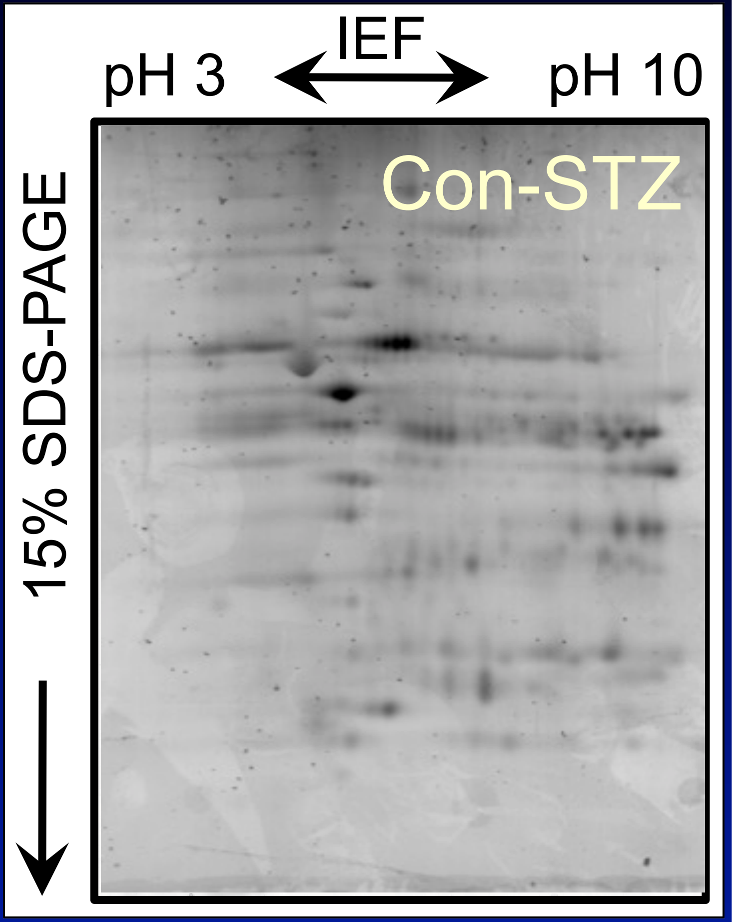
ARW

Wende … Abel *in prep*

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Isolated Mitochondria
2D-PAGE
Pro-Q
Emerald





Hansjörg Schwertz
Wende, unpublished

ARW

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome

Symbol	Description	Gene ID	*O*-GlcNAc Target	LC-MS/MS PTM	Pathway
ATP5A1	ATP synthase F1 complex, α subunit 1	11946	known[1,2]	Ac-	OXPHOS
ATP5H	ATP synthase F0 complex, subunit d	71679	novel	Ac-	OXPHOS
ATP5O	ATP synthase F1 complex, O subunit	28080	novel		OXPHOS
ETFB	Electron transferring flavoprotein, β polypeptide	110826	novel	Ac-	OXPHOS
UQCRC1	Ubiquinol-cytochrome c reductase core protein 1	22273	known[1]		OXPHOS
UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	66694	novel		OXPHOS
MDH2	Malate dehydrogenase 2, NAD (mitochondrial)	17448	novel	Ac-	TCA
OGDH	Oxoglutarate dehydrogenase (lipoamide)	18293	known[3]	P-	TCA
SUCLA2	Succinate-Coenzyme A ligase, ADP-forming, β subunit	20916	novel	Ac-, P-	TCA
DLAT	Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)	235339	known[1]		Glycolysis/ TCA
ACADL	Acyl-Coenzyme A dehydrogenase, long-chain	11363	novel	P-	FAO
HADHB	Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), β subunit	231086	novel	P-	FAO

PTM = post-translational modification; Ac- = acetylation; P- = phosphorylation; OXPHOS = Oxidative phosphorylation; TCA = Tricarboxylic acid cycle; FAO = Fatty acid β-oxidation

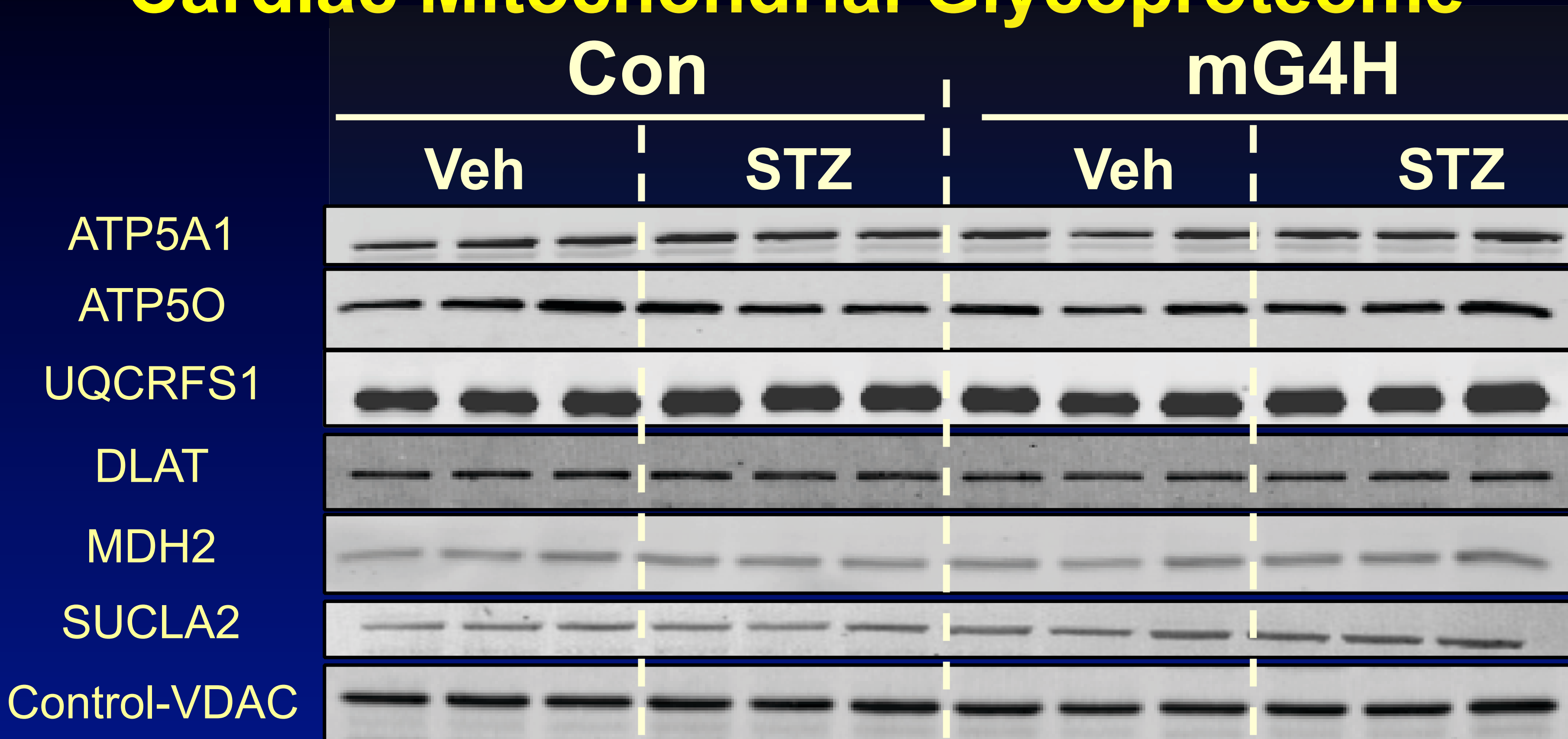
[1]Clark et al 2008 J Am Chem Soc 130(35): 11576; Previously identified modification by *O*-GlcNAc in rat brain and HeLa cells.
[2]Teo et al 2010 Nat Chem Biol 6(5):338; Previously identified modification by *O*-GlcNAc in rat liver.
[3]Nandi et al 2006 Anal Chem 78(2):452; Previously identified modification by *O*-GlcNAc in HeLa cells.

Wende, unpublished

ARW

dbOGAP http://cbsb.lombardi.georgetown.edu and YinOYang www.cbs.dtu.dk

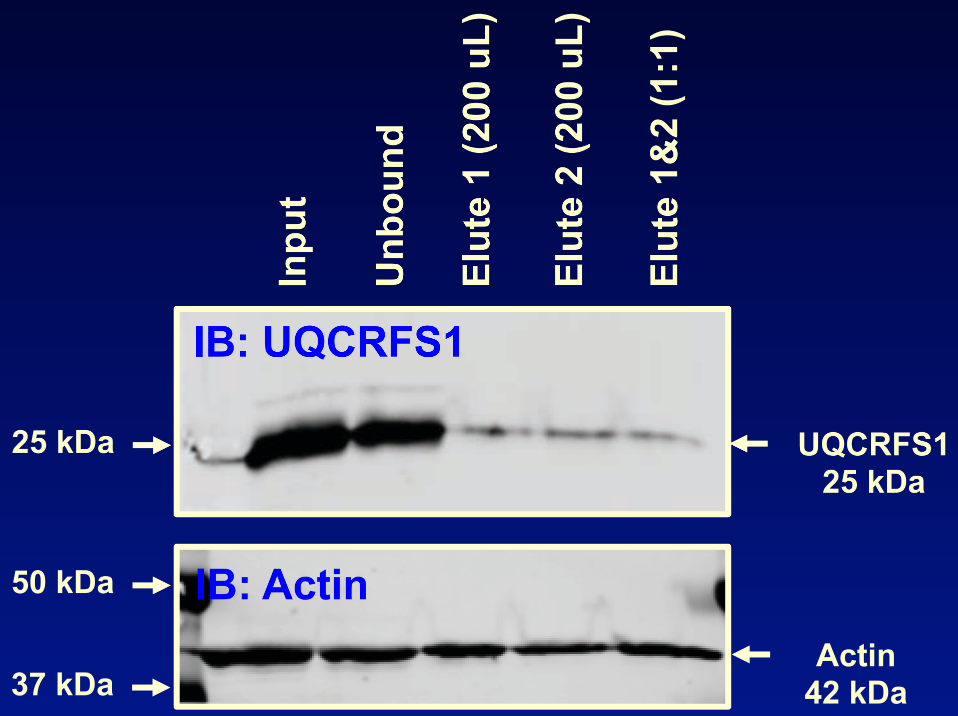
GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome



ARW

Chase Andrizzi

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome



Lamario Williams, Manoja Brahma, and Chase Andrizzi

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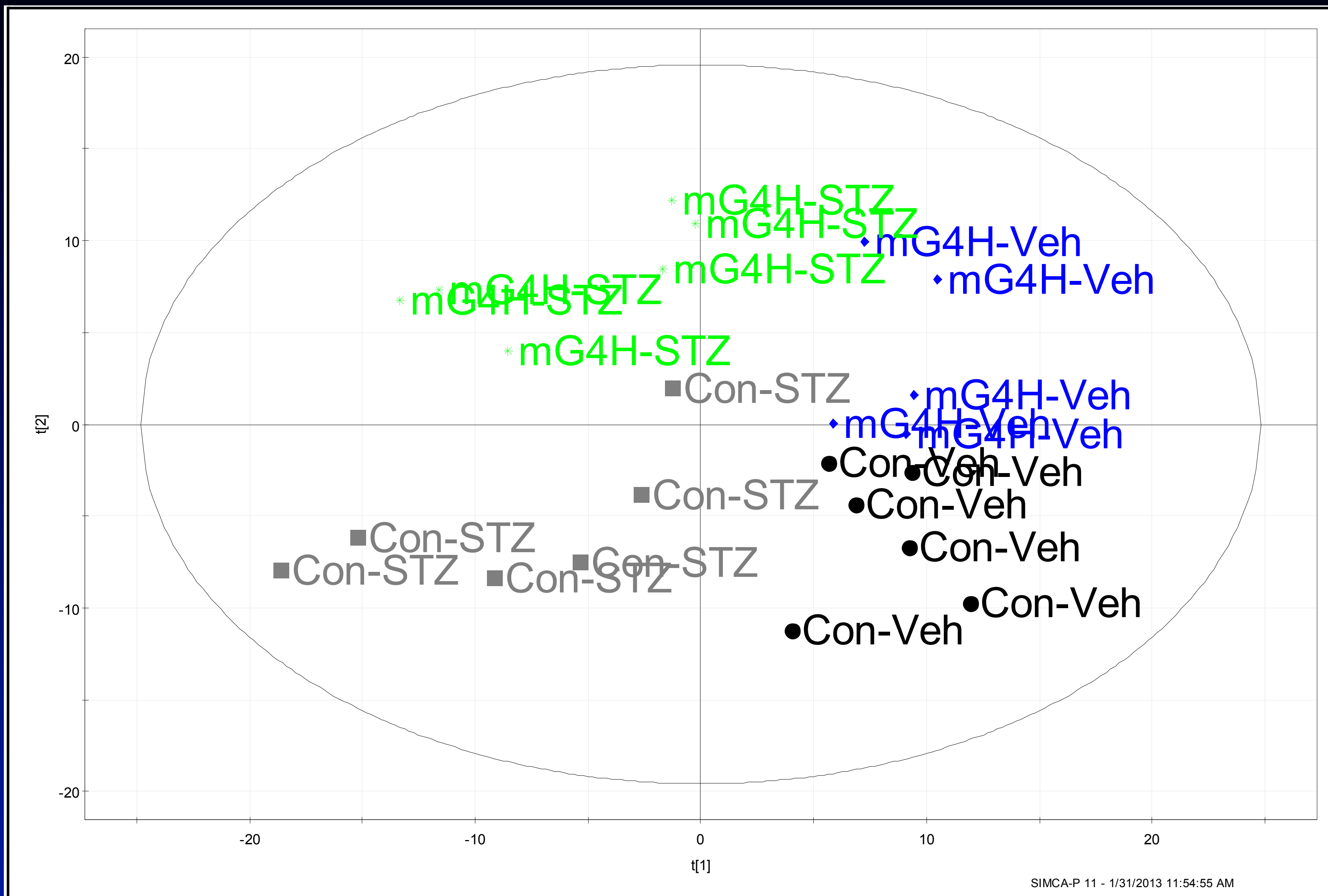
Metabolomics



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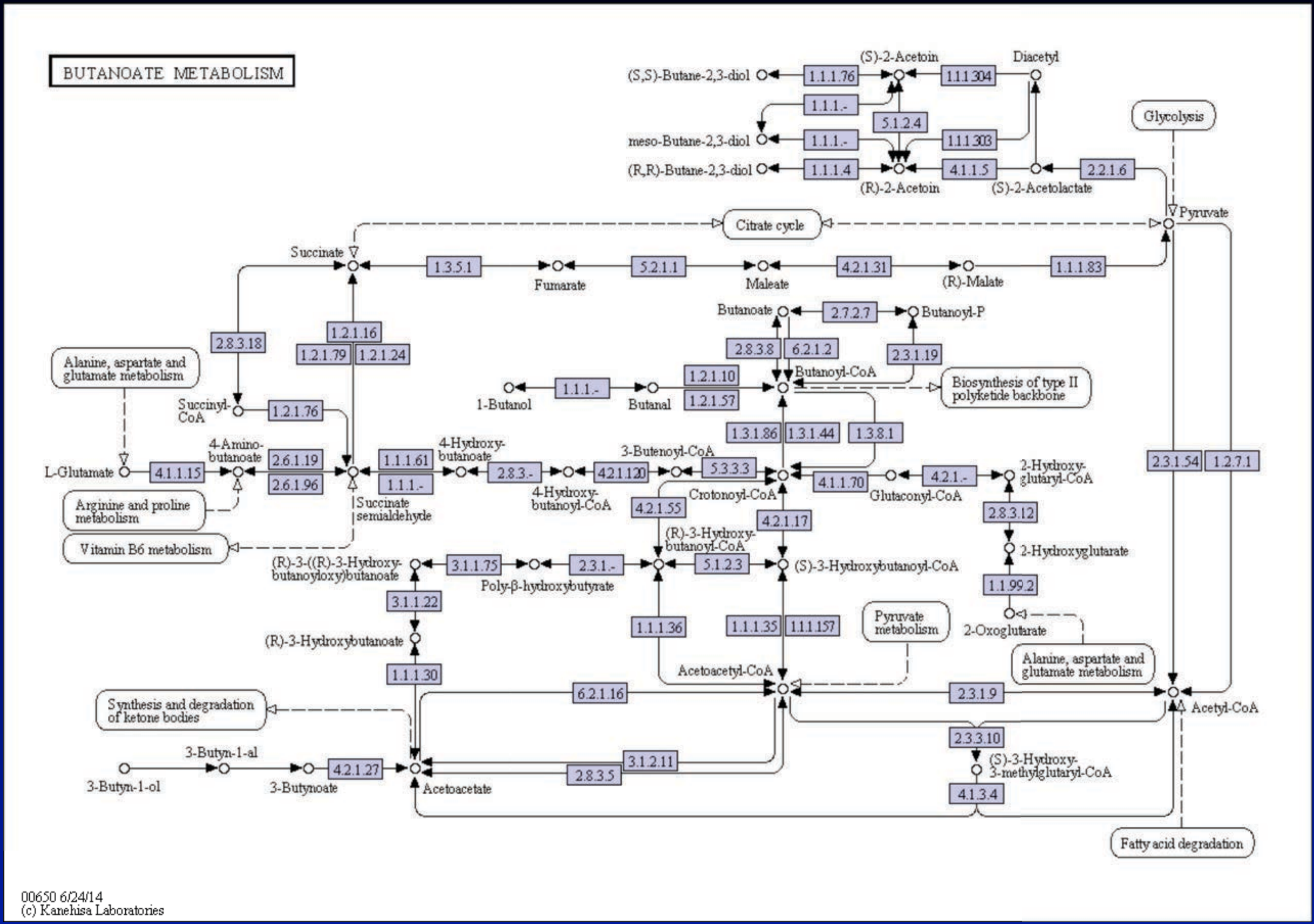
Wende, unpublished

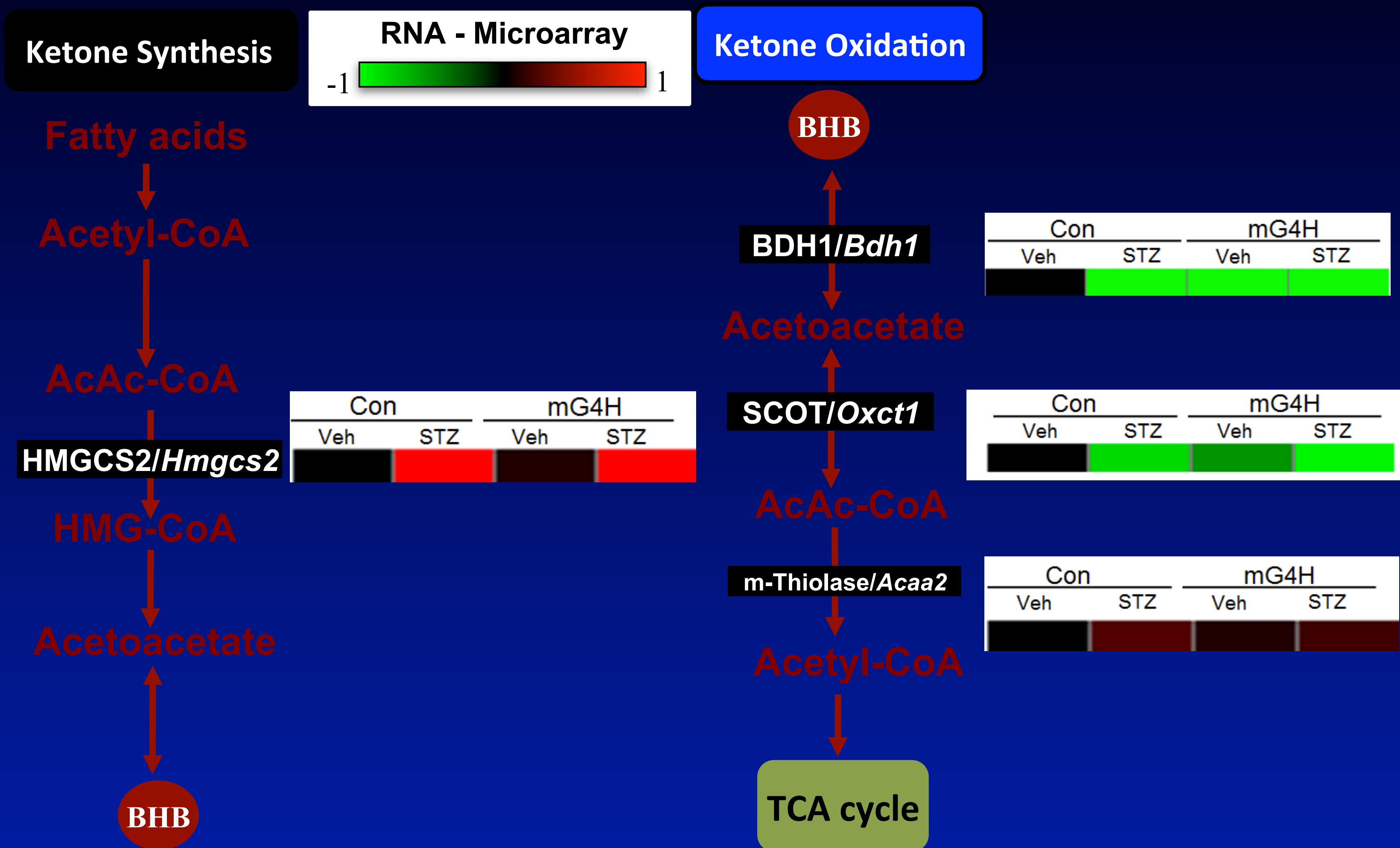
Metabolomics



ARW

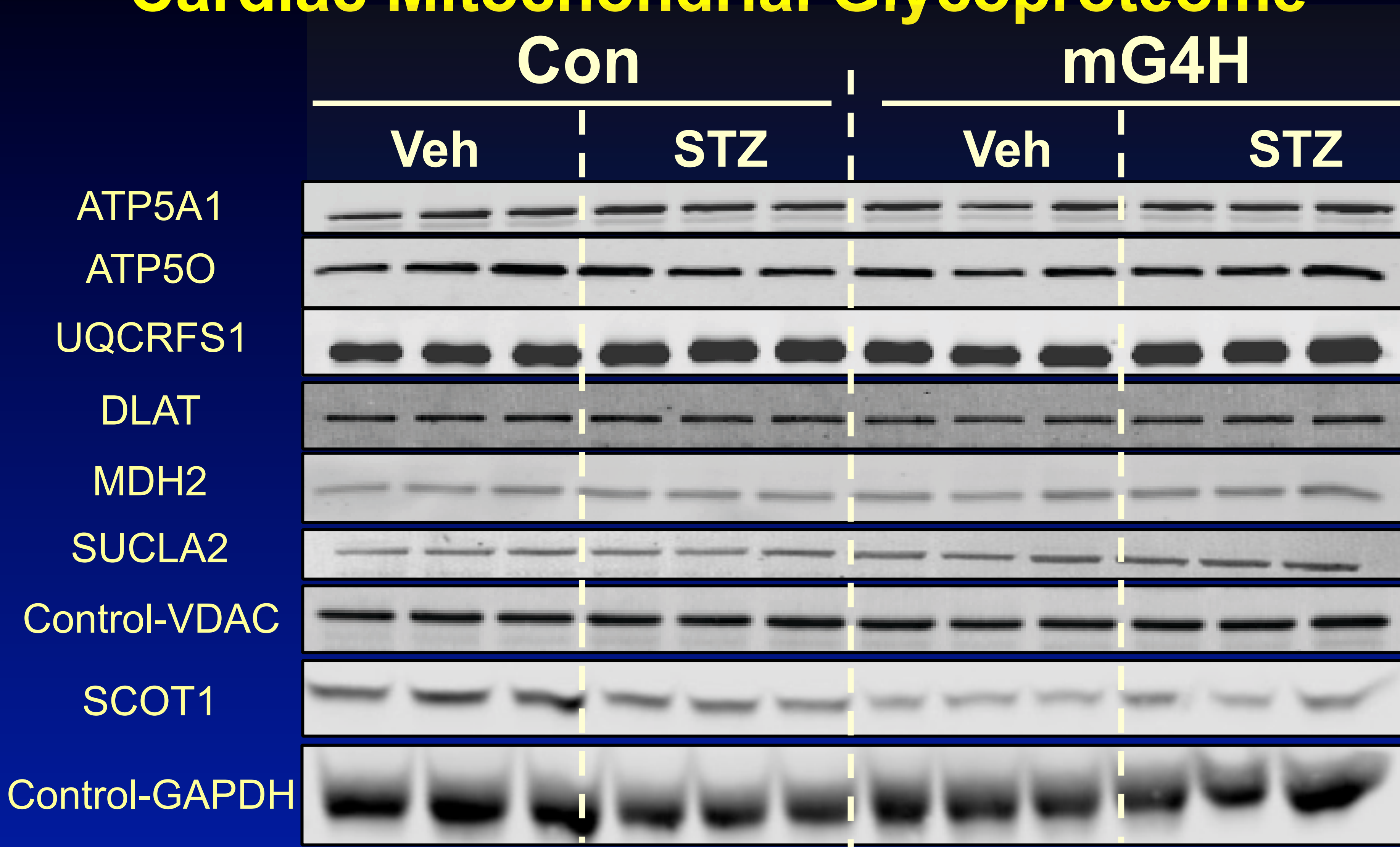
Wende, unpublished

GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome



Manoja Brahma

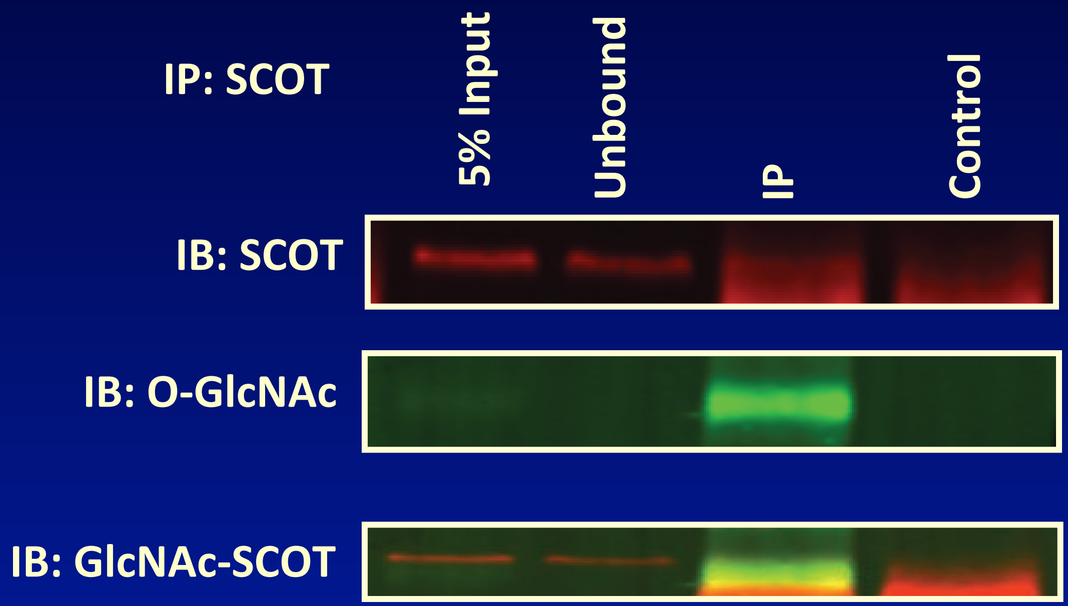
GLUT4 Induction Alters the Cardiac Mitochondrial Glycoproteome



Lamario Williams, Manoja Brahma, and Chase Andrizzi

GLUT4 Induction Alters Mitochondrial Protein *O*-GlcNAcylation

SCOT immunoprecipitation
from LV of 24h fasted mice



Manoja Brahma

Conclusion – Part 3

Enhanced cardiac glucose delivery alters metabolic flux through other pathways and regulates the mitochondrial proteome via *O*-GlcNAcylation.

ARW

From Human to Mouse and Back Again

Broad Institute Communications

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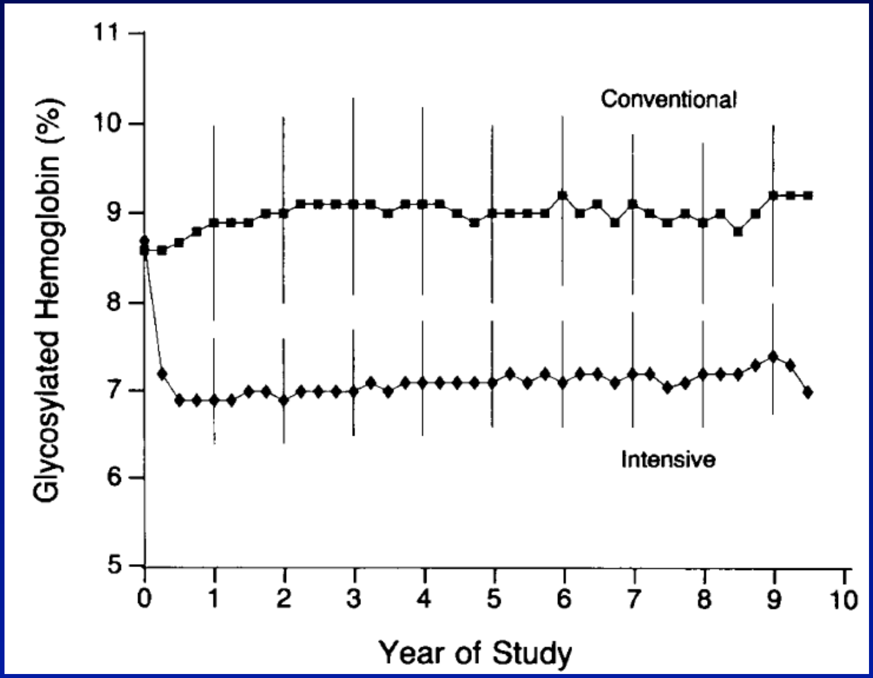
Epigenetics - Programming
DCCT: Diabetes Control and Complications Trial

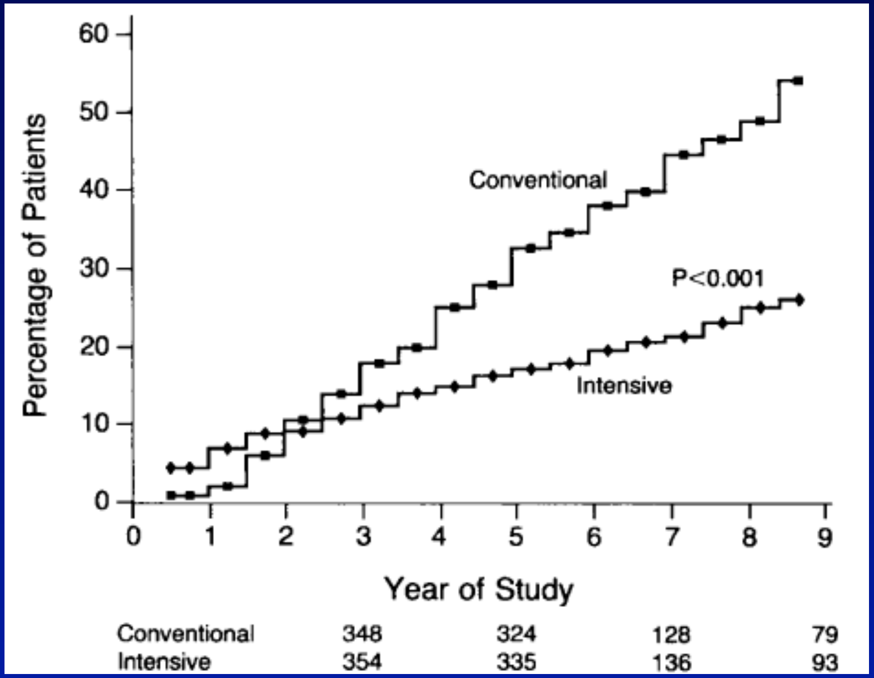
The New England
Journal of Medicine

Volume 329 SEPTEMBER 30, 1993 Number 14

THE EFFECT OF INTENSIVE TREATMENT OF DIABETES ON THE DEVELOPMENT AND PROGRESSION OF LONG-TERM COMPLICATIONS IN INSULIN-DEPENDENT DIABETES MELLITUS

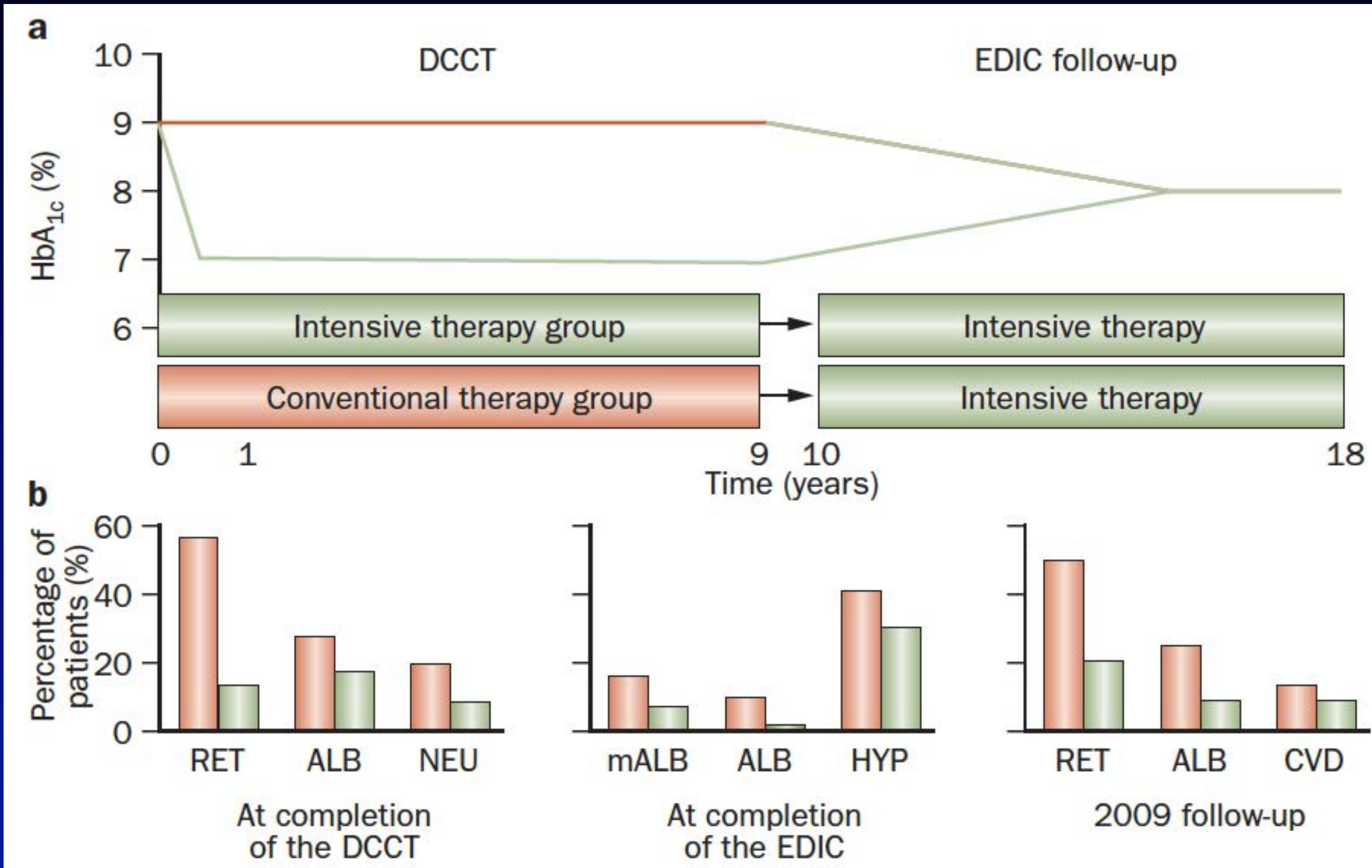
The Diabetes Control and Complications Trial Research Group*





Epigenetics - Memory

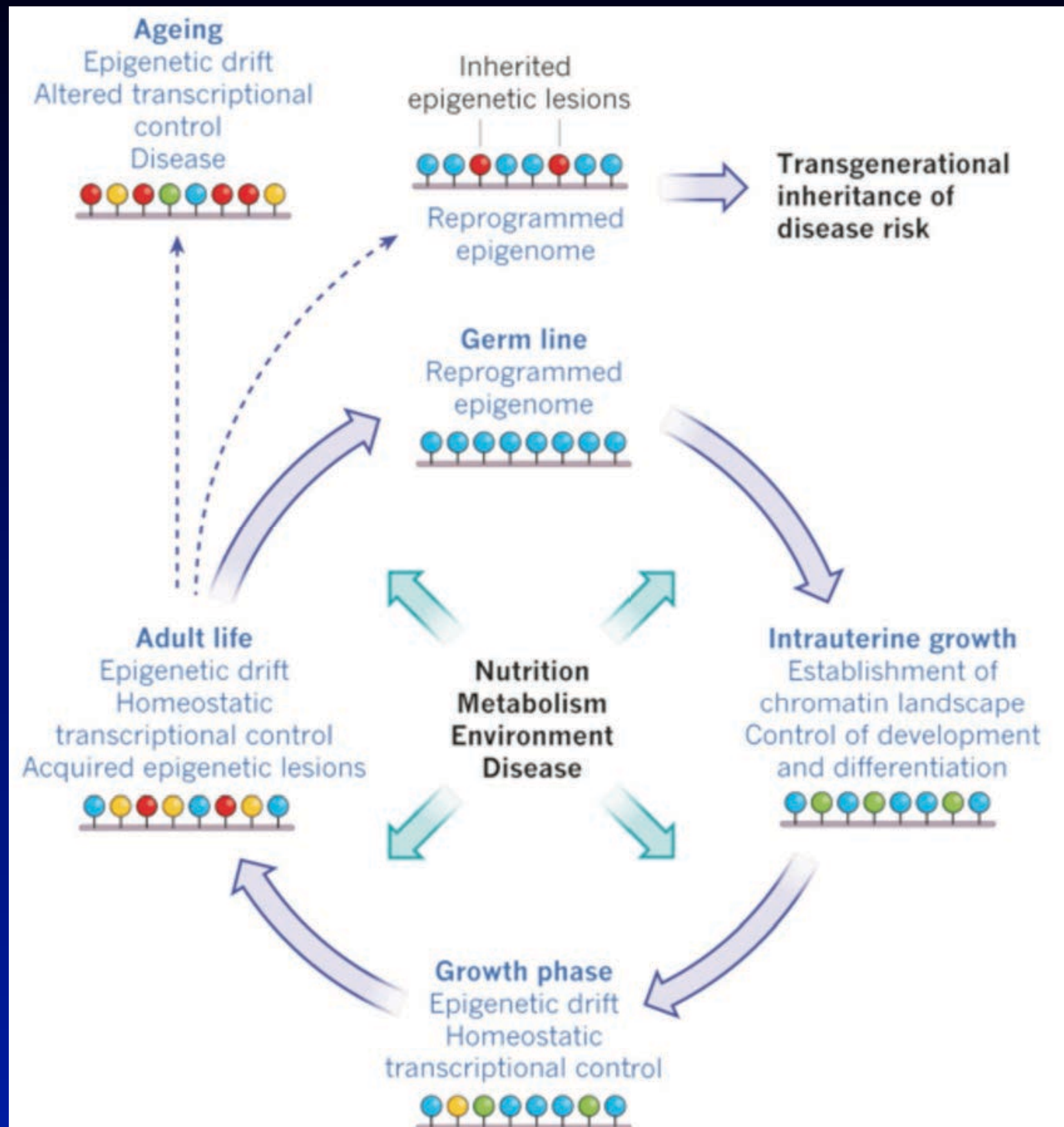
EDIC: Epidemiology of Diabetes Interventions Trial



ARW

Pirola … El-Osta **2010** *Nat Rev Endocrinol* 6(12):665

Epigenetics: Transgenerational and Drift



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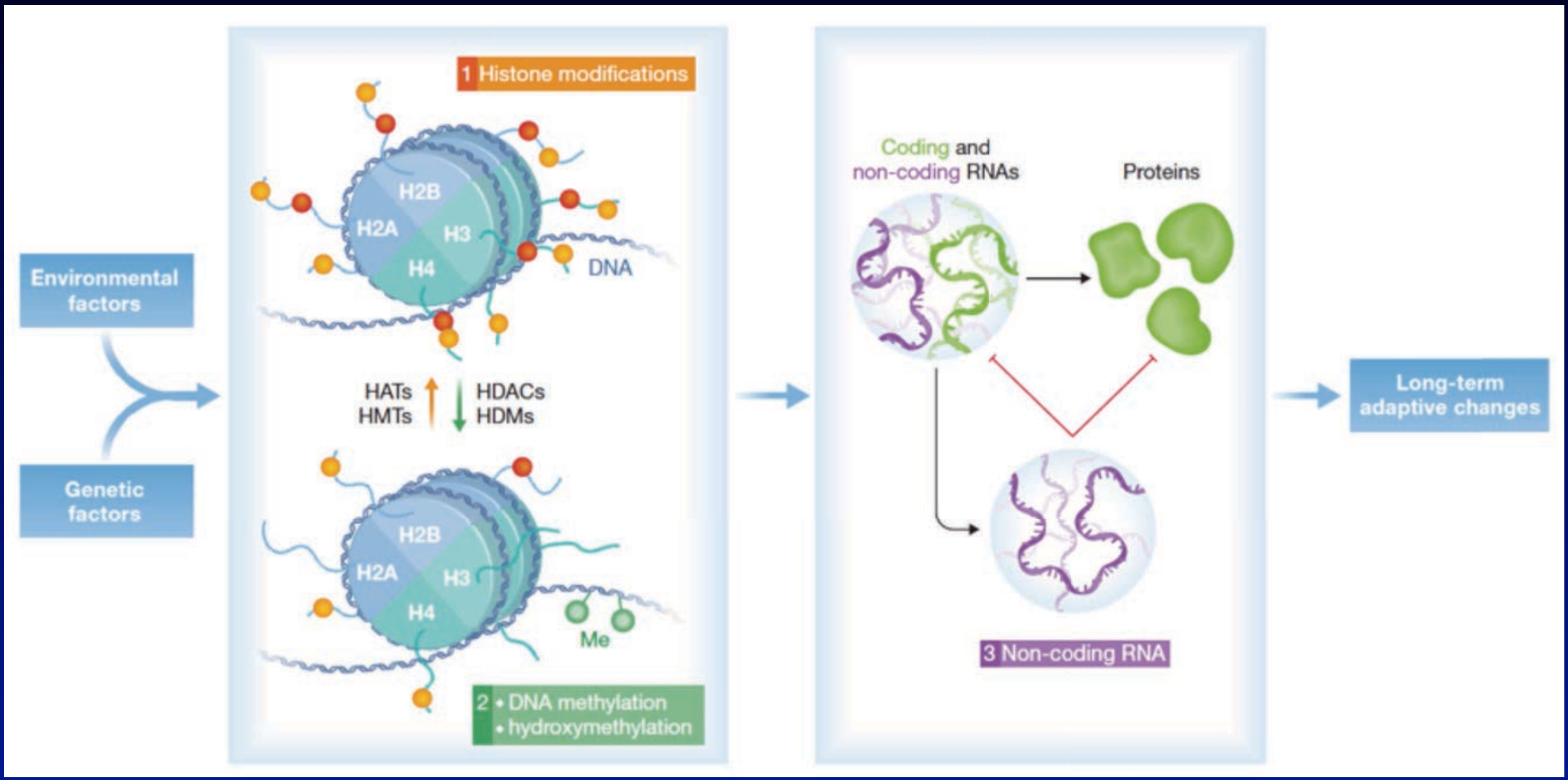
Gut and Verdin **2013** *Nature* 502:489

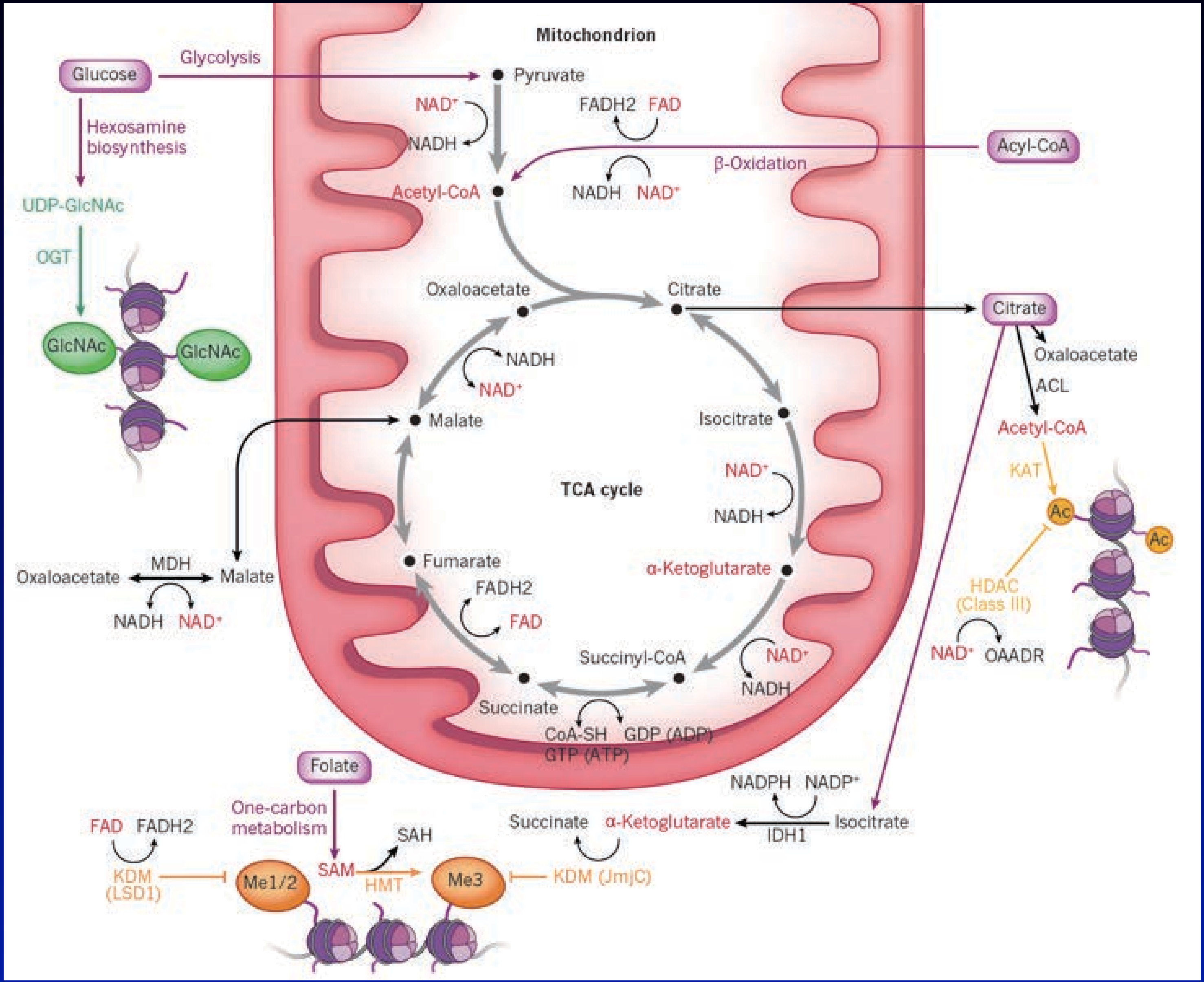
Epigenetic Code



ARW

Fischer **2014** *EMBO J* 33(9):945:489

Metabolite Signaling to Chromatin



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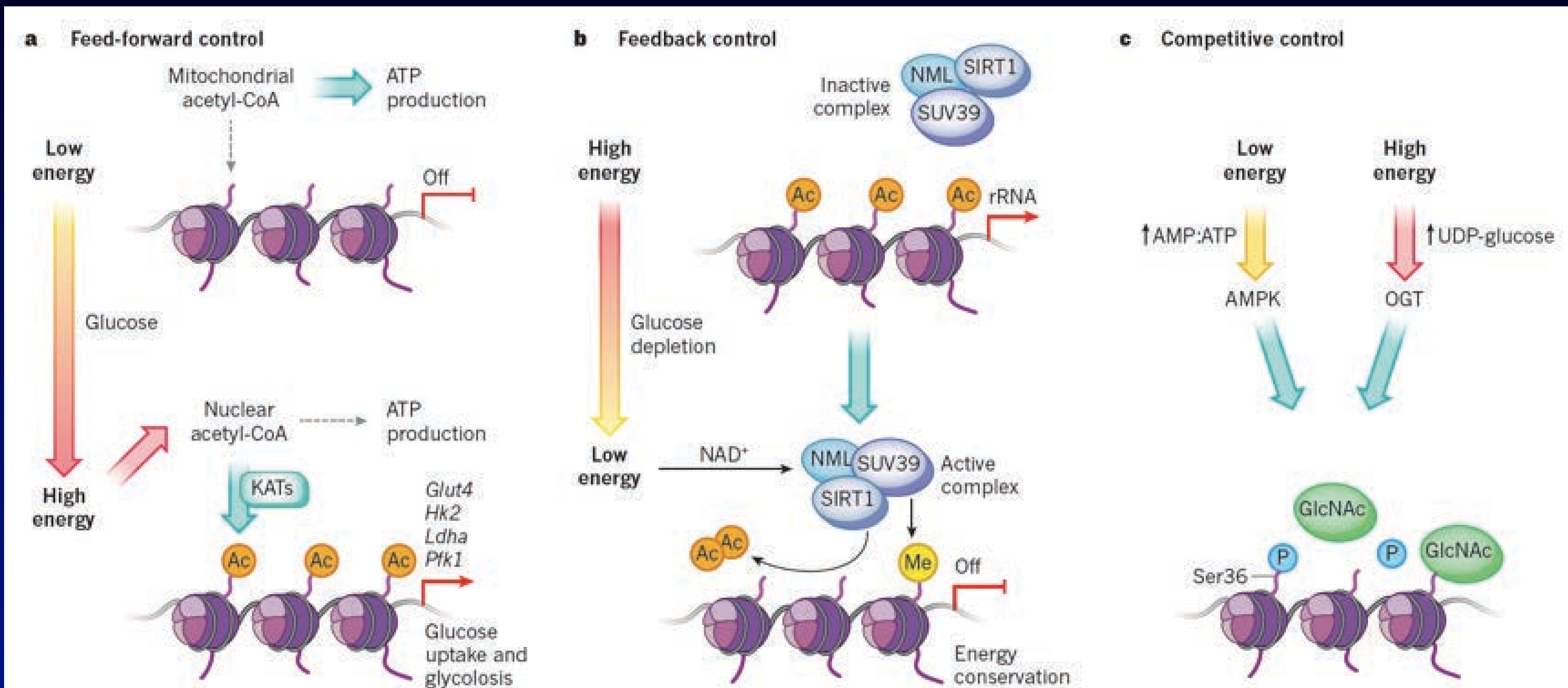
Gut and Verdin **2013** *Nature* 502:489

How does GlcNAc fit in?



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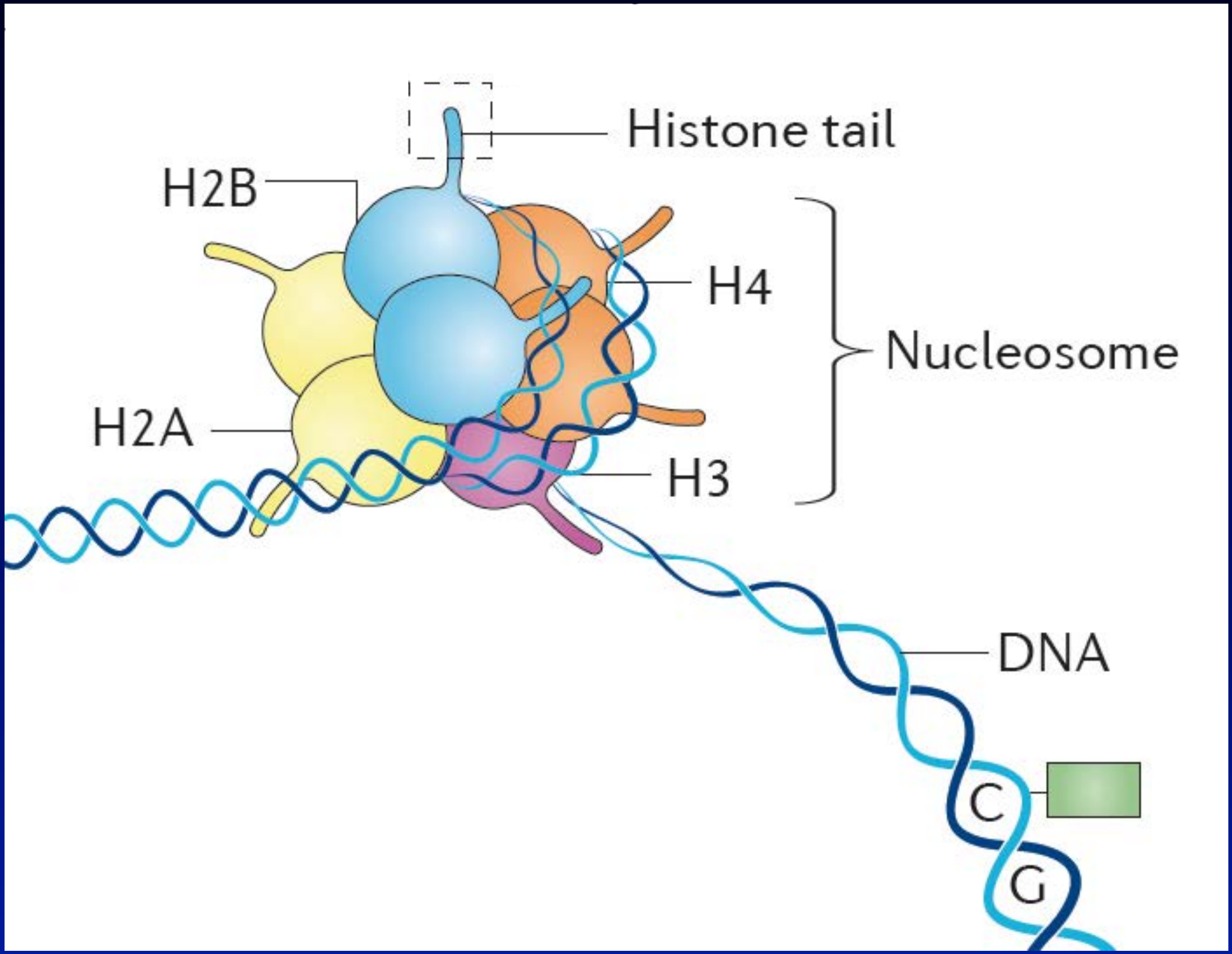
Gut and Verdin **2013** *Nature* 502(7472):489

Chromatin Regulation



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Gräff and Tsai **2013** *Nat Rev Neurosci* 14(2):97

How does GlcNAc fit in?

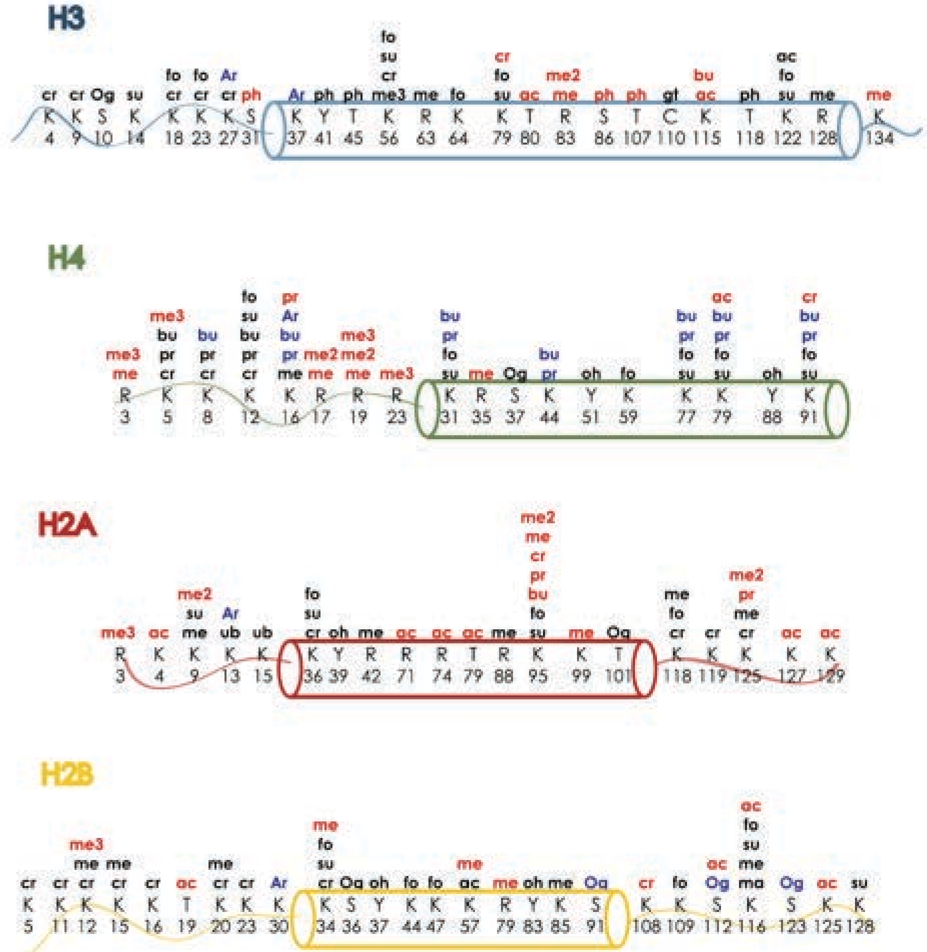
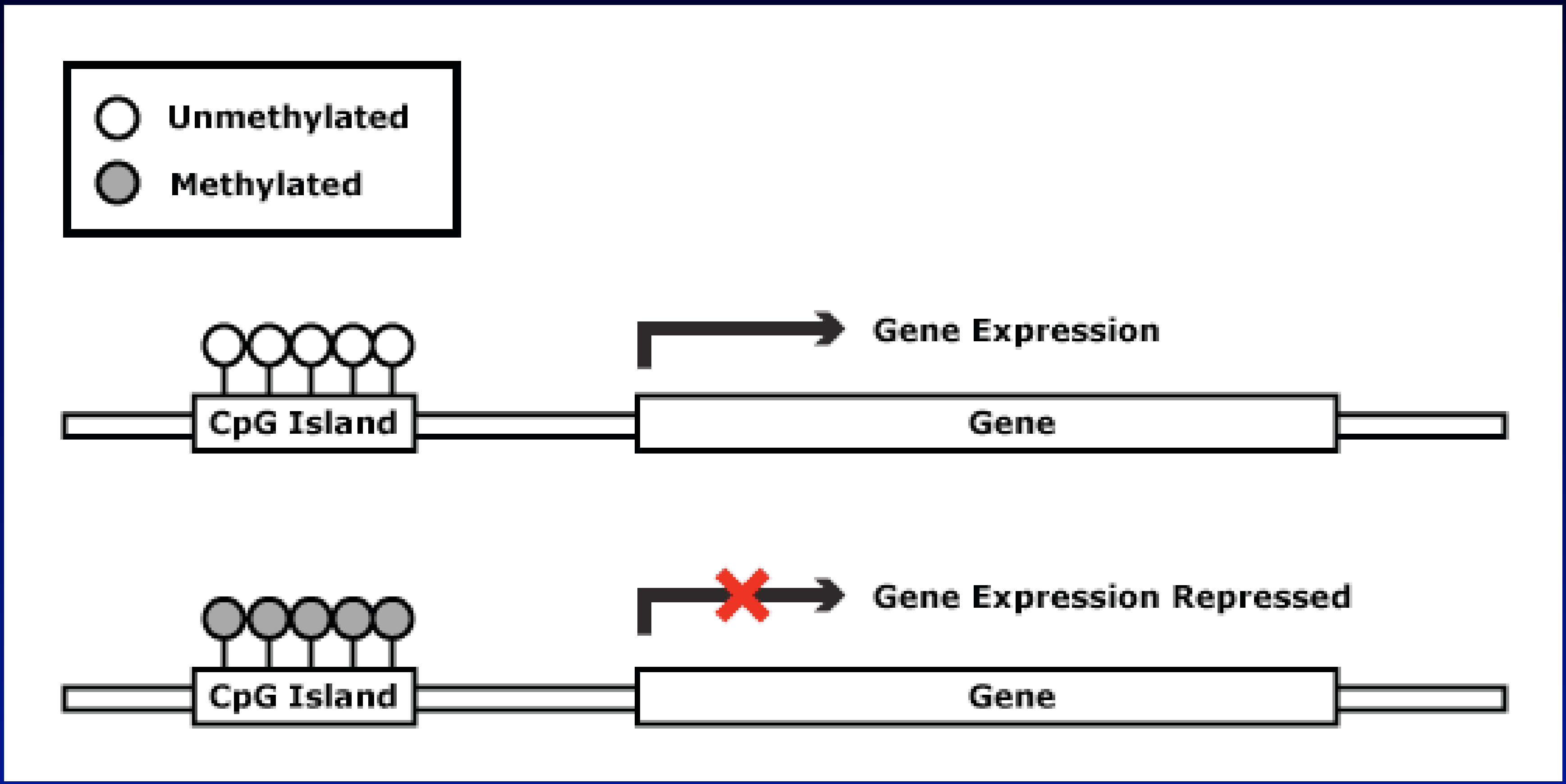


Figure 1 Recently identified modifications on the core histones. Black, modifications found *in vivo* in human; red, modifications found in mouse brain; blue, modifications found *in vitro*. ac, acetylation; Ar, ADP-ribosylation; bu, butyrylation; cr, crotonylation; fo, formylation; gt, glutathionylation; ma, malonylation; me, methylation; Og, O-glcNAcylation; oh, hydroxylation; pr, propionylation; su, succinylation; ph, phosphonylation; ub, ubiquitination.

ARW

Arnaudo … Garcia **2013** *Epigenetics Chromatin* 6(1):24

DNA Methylation 101



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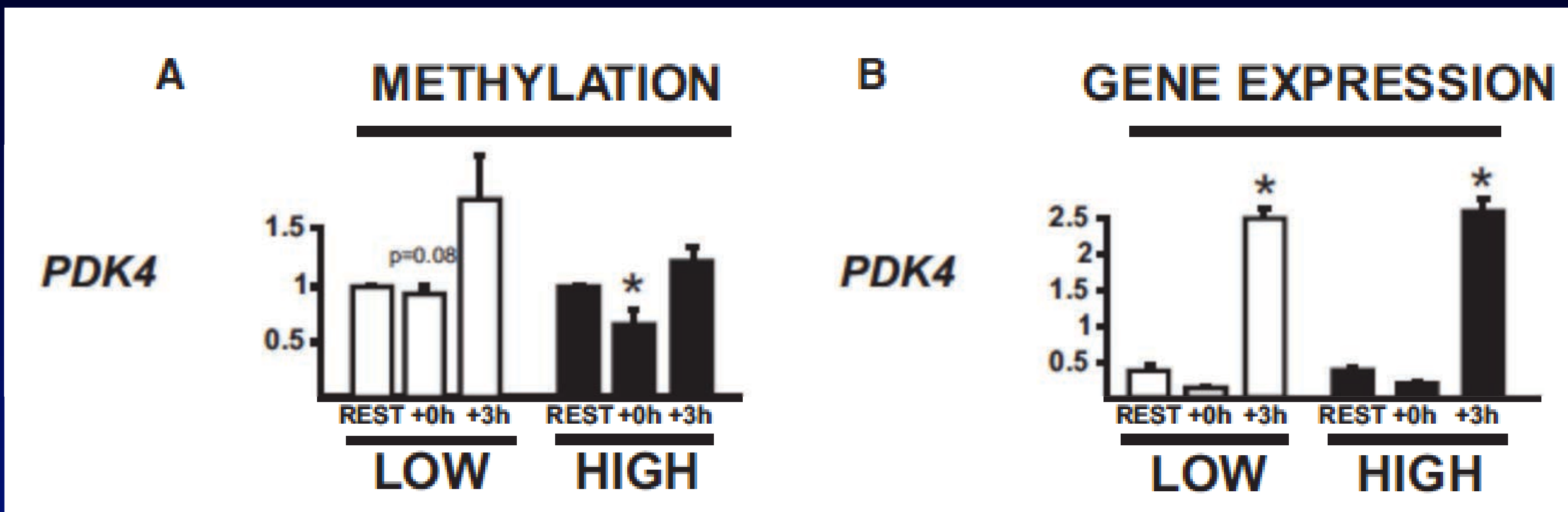
ucsf.edu

Exercise Alters DNA Methylation of Key Metabolic Genes



Low = 40% VO_{2peak} High = 80% VO_{2peak}

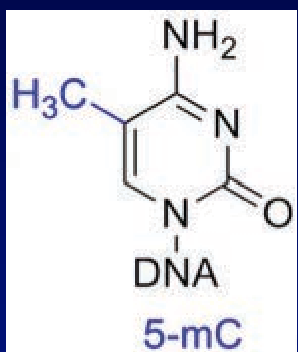
Subjects fasted overnight and then consumed a high carbohydrate diet 4 hr prior to exercise.

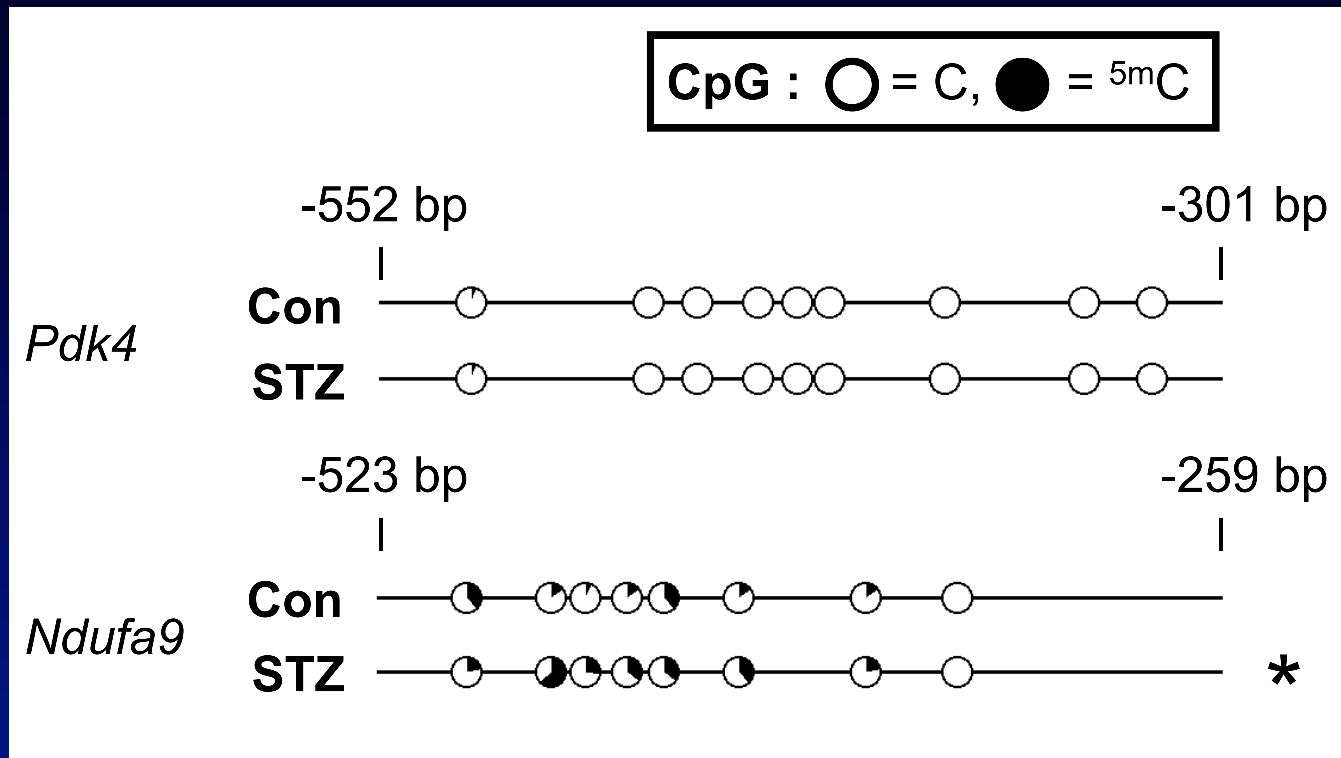
ARW

Barres and Zierath **2012** *Cell Metab* 15:405

Diabetes Regulated Cardiac DNA Methylation

Targeted bsDNA-seq 5-mCpG





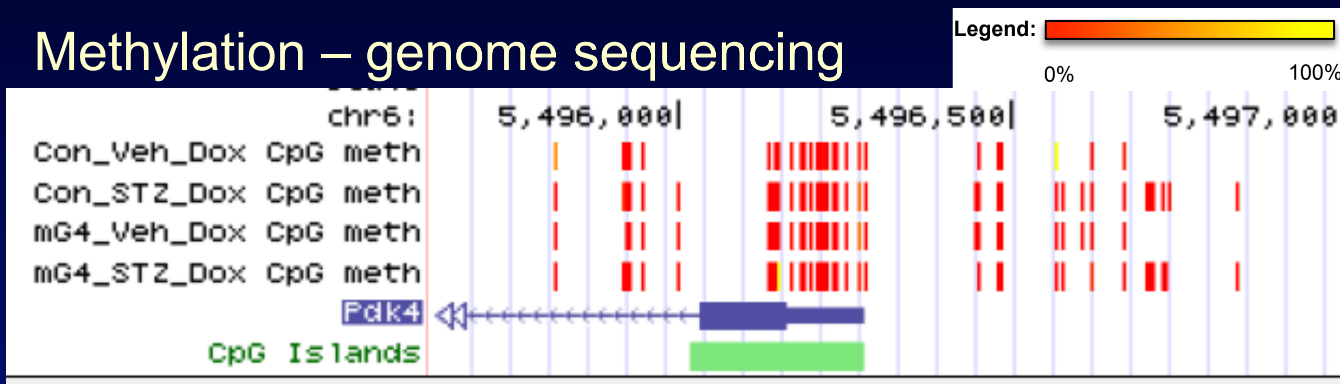
Heart, LV
n = 10
* P < 0.05

Wende, unpublished

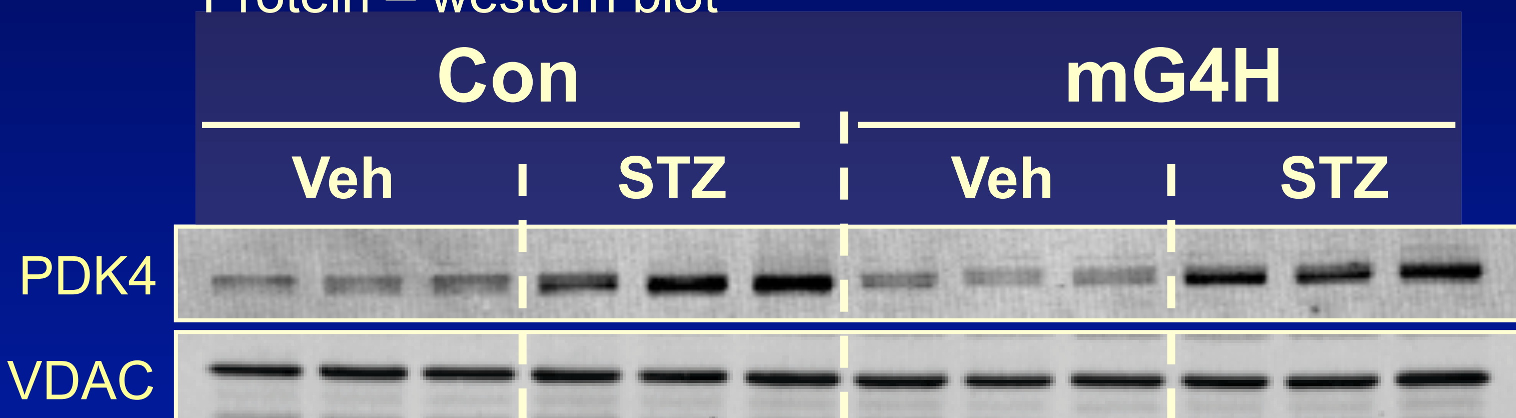
Methylation and Expression

RNA – microarray

Methylation – genome sequencing



Protein – western blot



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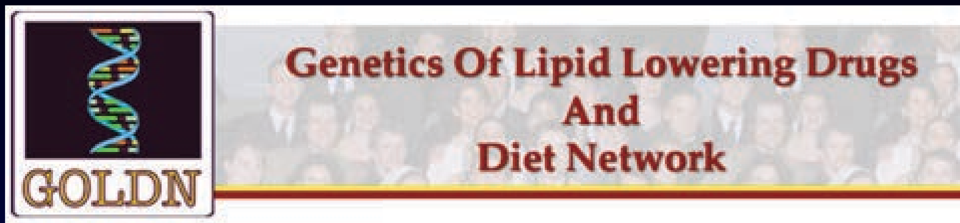
GeneSifter and Zymo/UCSC Genome Browser

Other Human/Mouse Comparisons



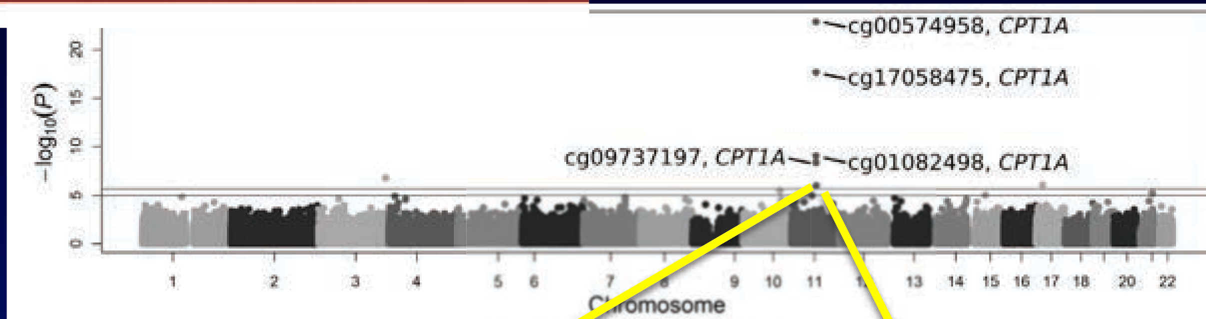


Figure 2. Epigenome-wide association Manhattan plot for VLDL-C in the discovery dataset (n=991). VLDL-C indicates very-low-density lipoprotein cholesterol.

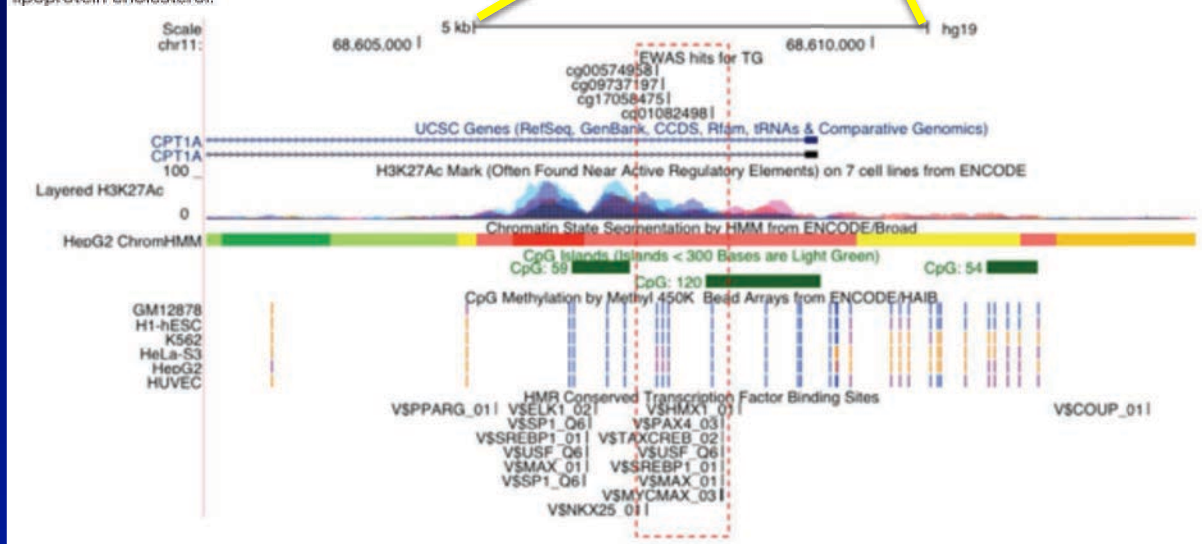


Figure 3. ENCODE annotation of the promoter region and intron 1 of *CPT1A*. Top CpGs for TG are positioned within the gene along with CpG islands, cell line chromatin state (ChromHMM), cell line methylation at CpG sites on the Methyl450 Beadchip according to Hudson Alpha Institute for Biotechnology (HAIB; note blue, purple, and orange highlights correspond to low, medium and high methylation state, respectively), and HMR conserved transcription factor binding sites. CpG indicates cytosine-(phosphate)-guanine; and TG, triglyceride.

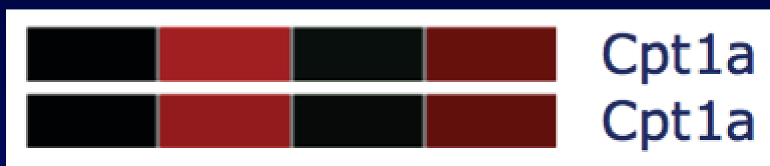
ARW

Irvin … Arnett **2014** *Circulation* 130:565

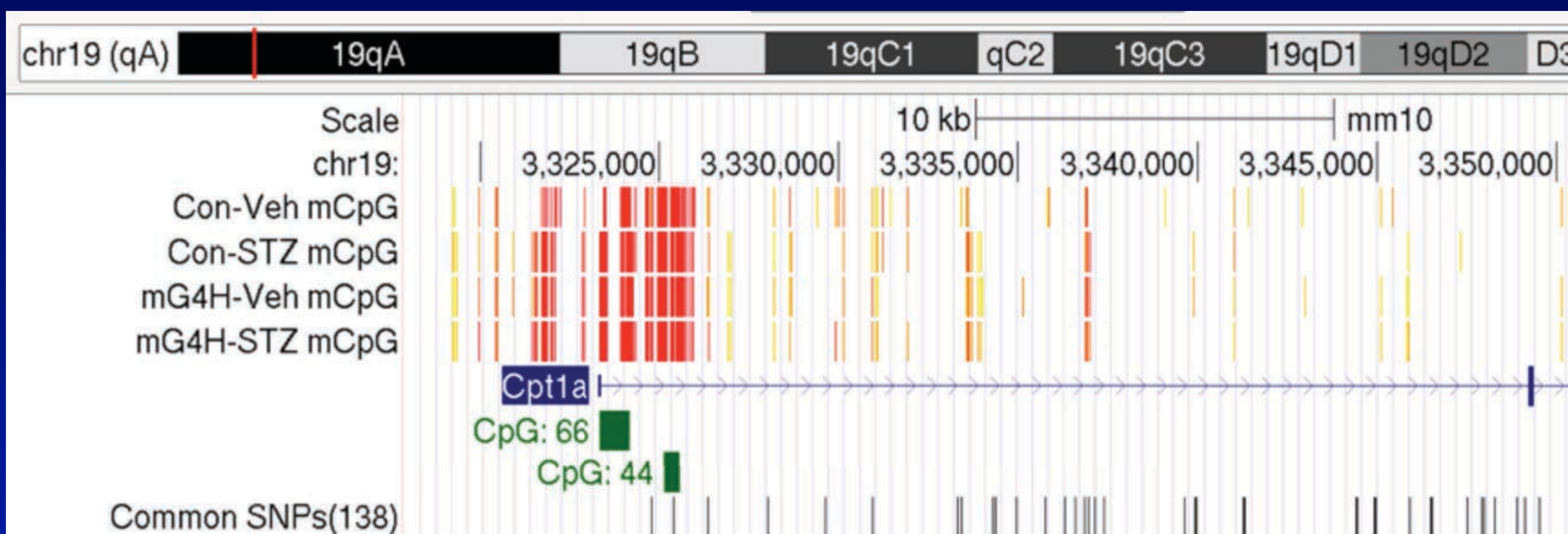
Other Human/Mouse Comparisons

Mouse
Gene Expression

Con Veh | Con STZ | mG4H Veh | mG4H STZ | **GENE**

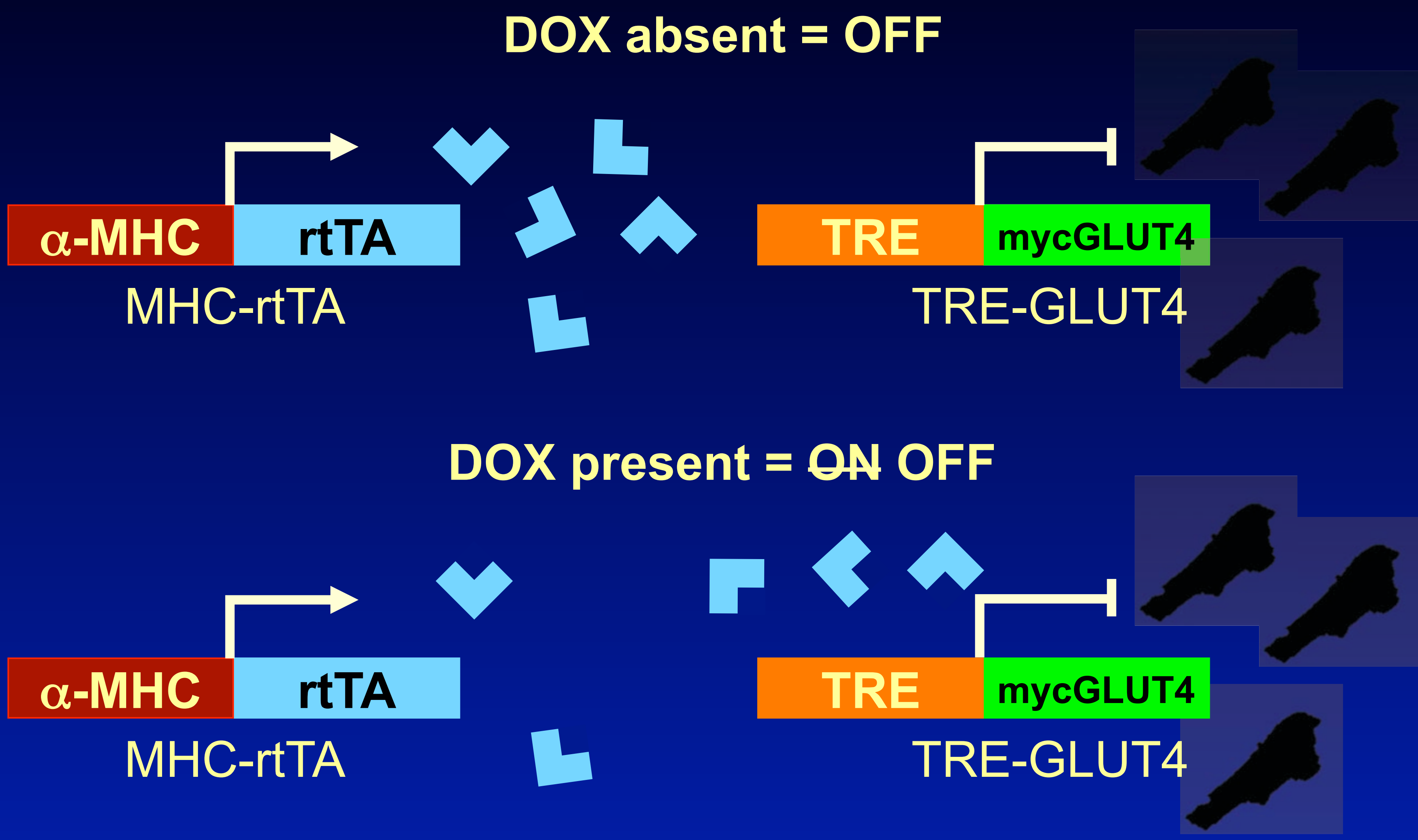


Mouse
DNA Methylation



Wende, unpublished

Where Does Glycemic Memory Fit In?



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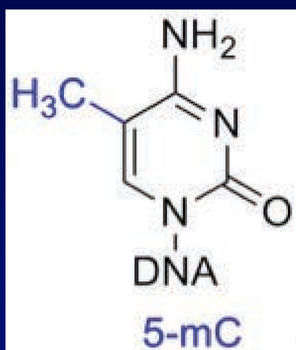
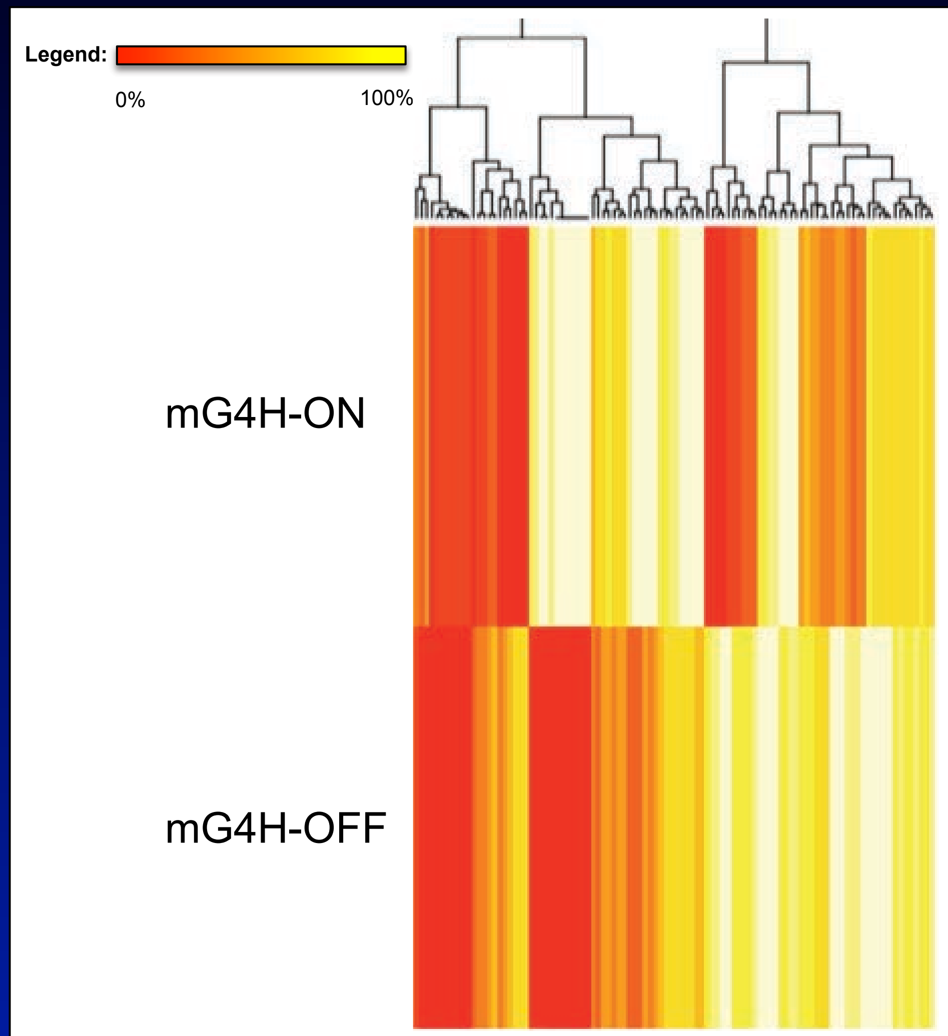
Glucose Cycling Alters Epigenetic Programming

Genomewide bsDNA-seq 5-mCpG

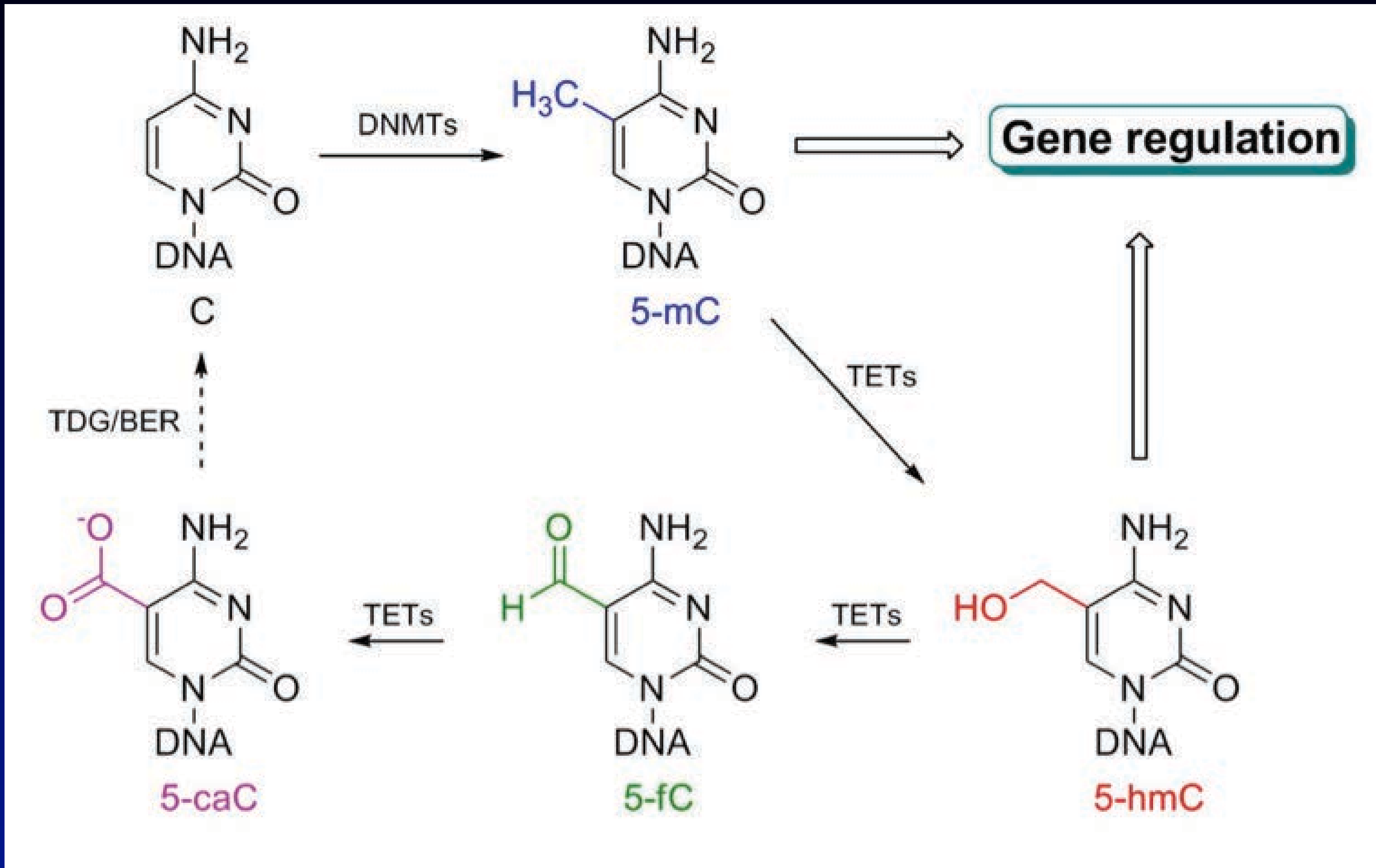




Heart, LV

Zymo Research
Wende, unpublished

Background

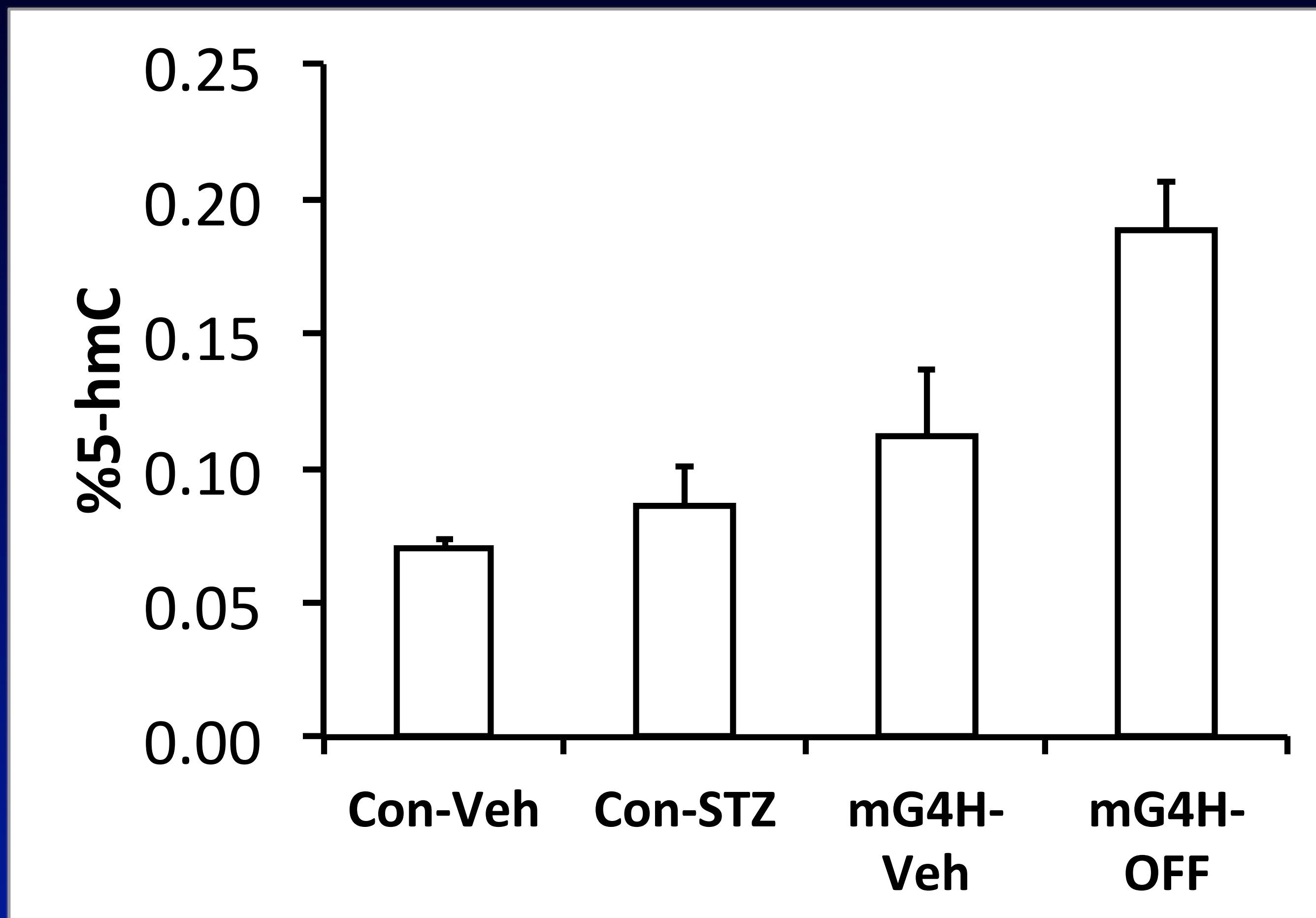


5-hmC
Wyatt and Cohen **1952** *Nature* 170(4338):1072
Kriaucioni and Heintz **2009** *Science* 324(5929):929
Tahiliani … Rao **2009** *Science* 324(5929):930

ARW http://chemistry.uchicago.edu/faculty/faculty/person/member/chuan-he.html

Glucose Cycling Alters Epigenetic Programming

5-hmCpG ELISA

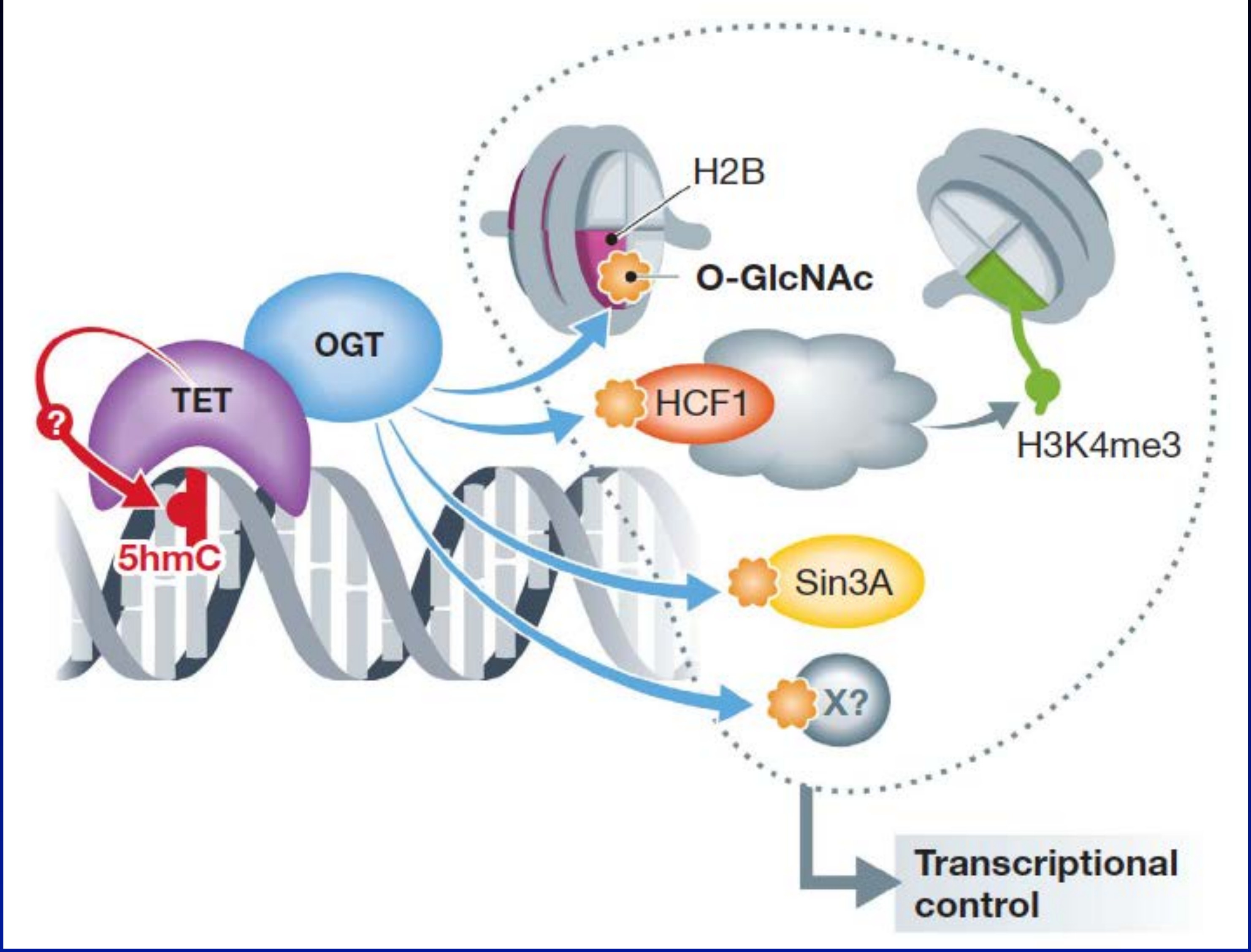


Heart, LV

ARW

Zymo Research
Wende, unpublished

How does GlcNAc fit in?



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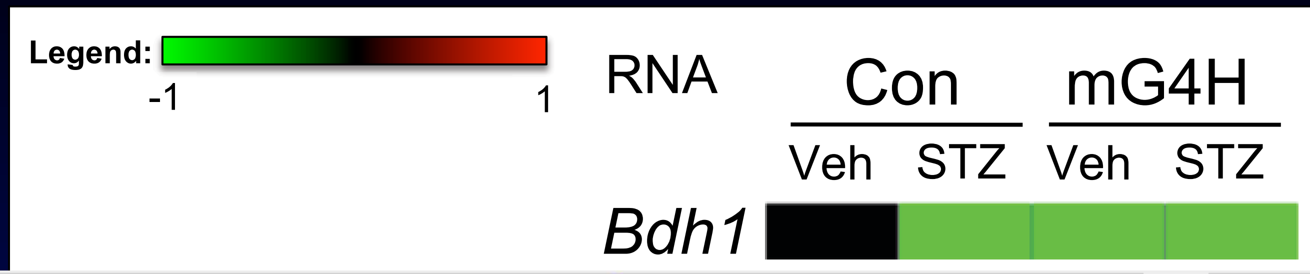
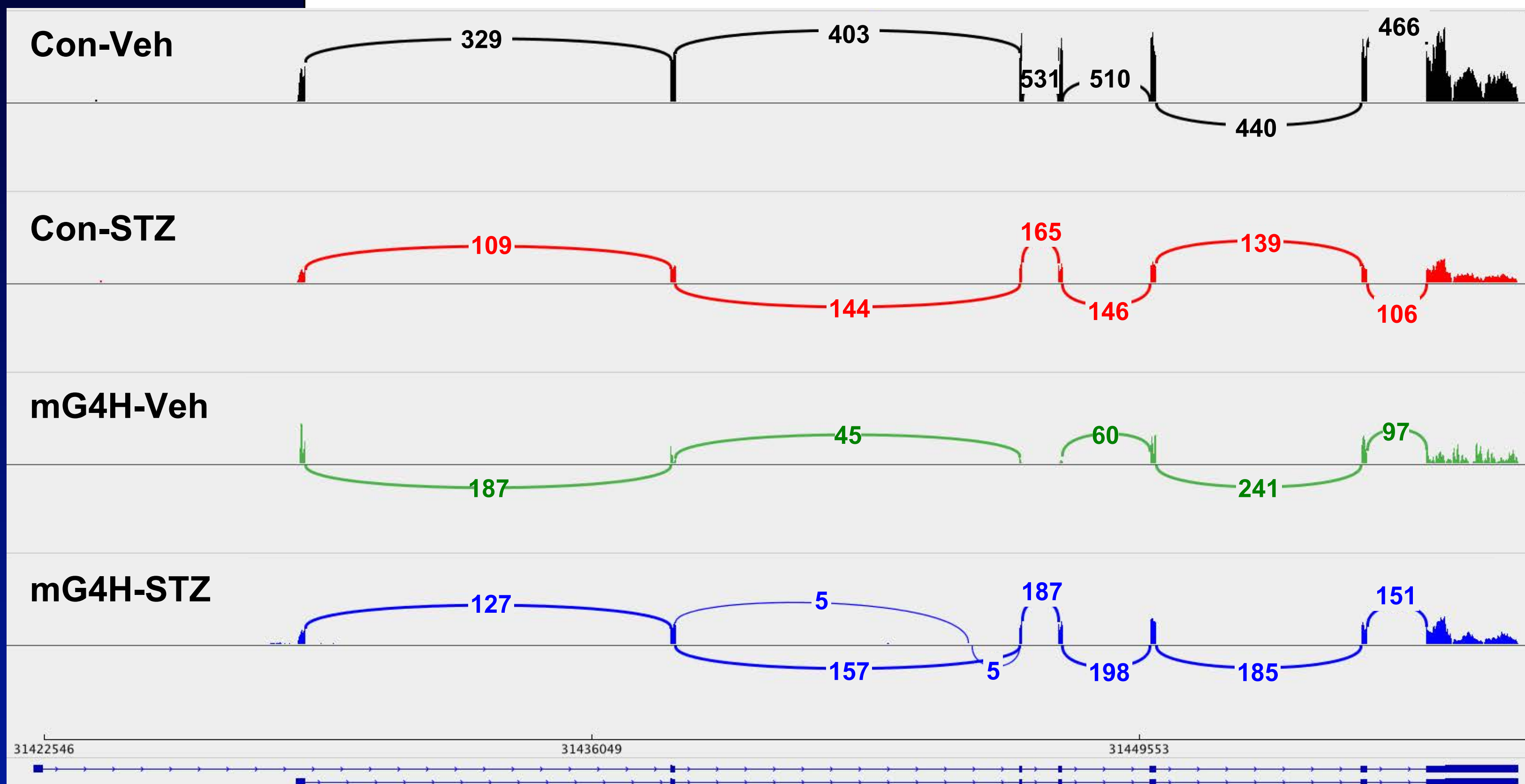
Mariappa … Aalten **2013** *EMBO J* 32:612

Tissue Specific Promoter Utilization

RNA-seq Transcripts



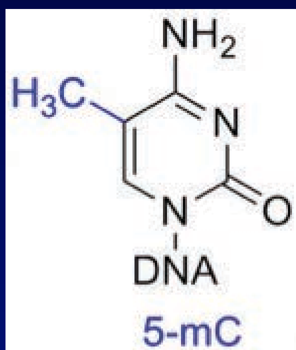


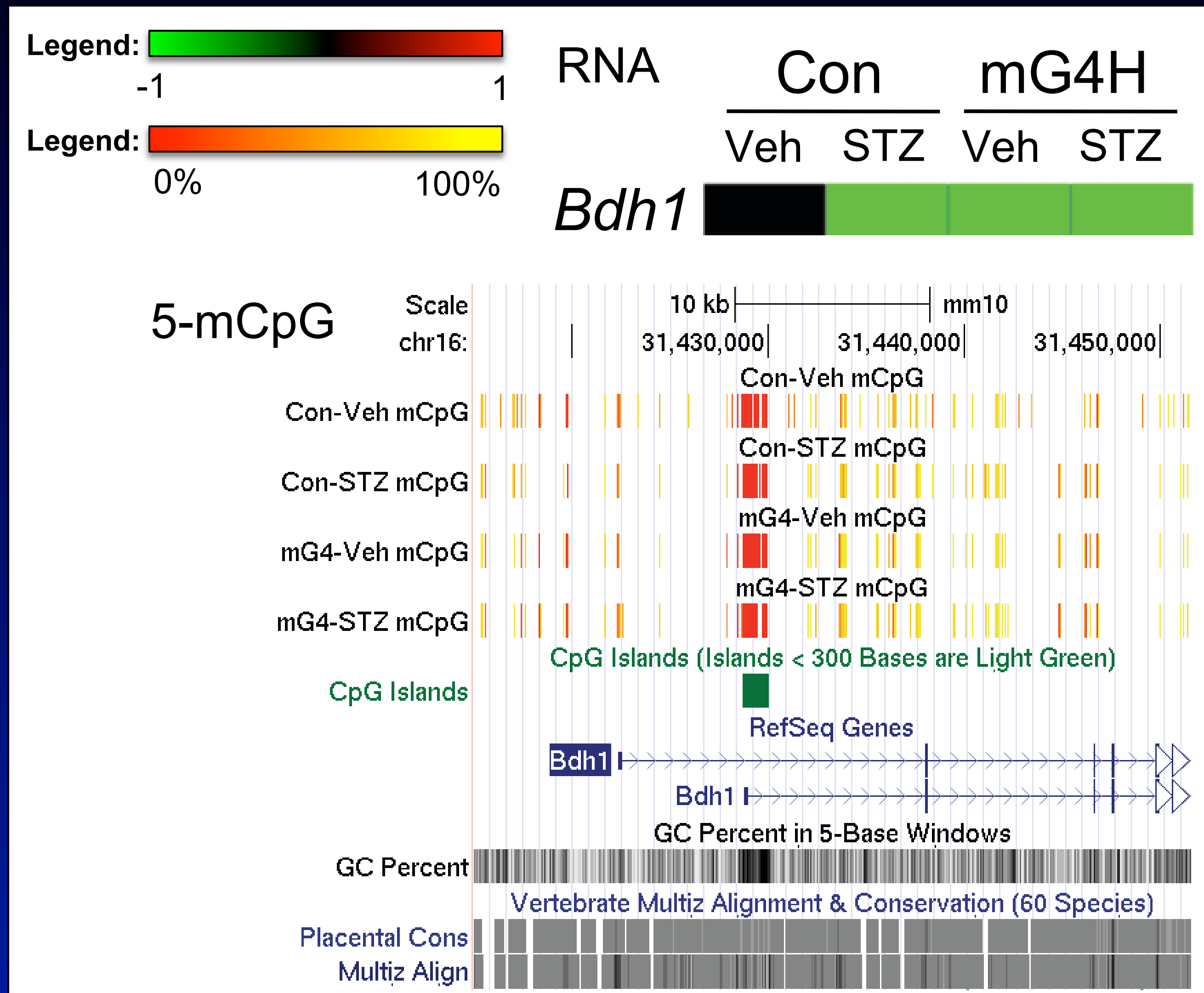
Heart, LV
$n = 3$

Heflin Center for Genomic Sciences, UAB
Nye … Wende, unpublished

Combined Transcriptome/Methylome

Genomewide
bsDNA-seq
5-mCpG



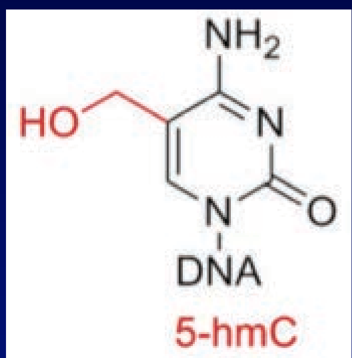


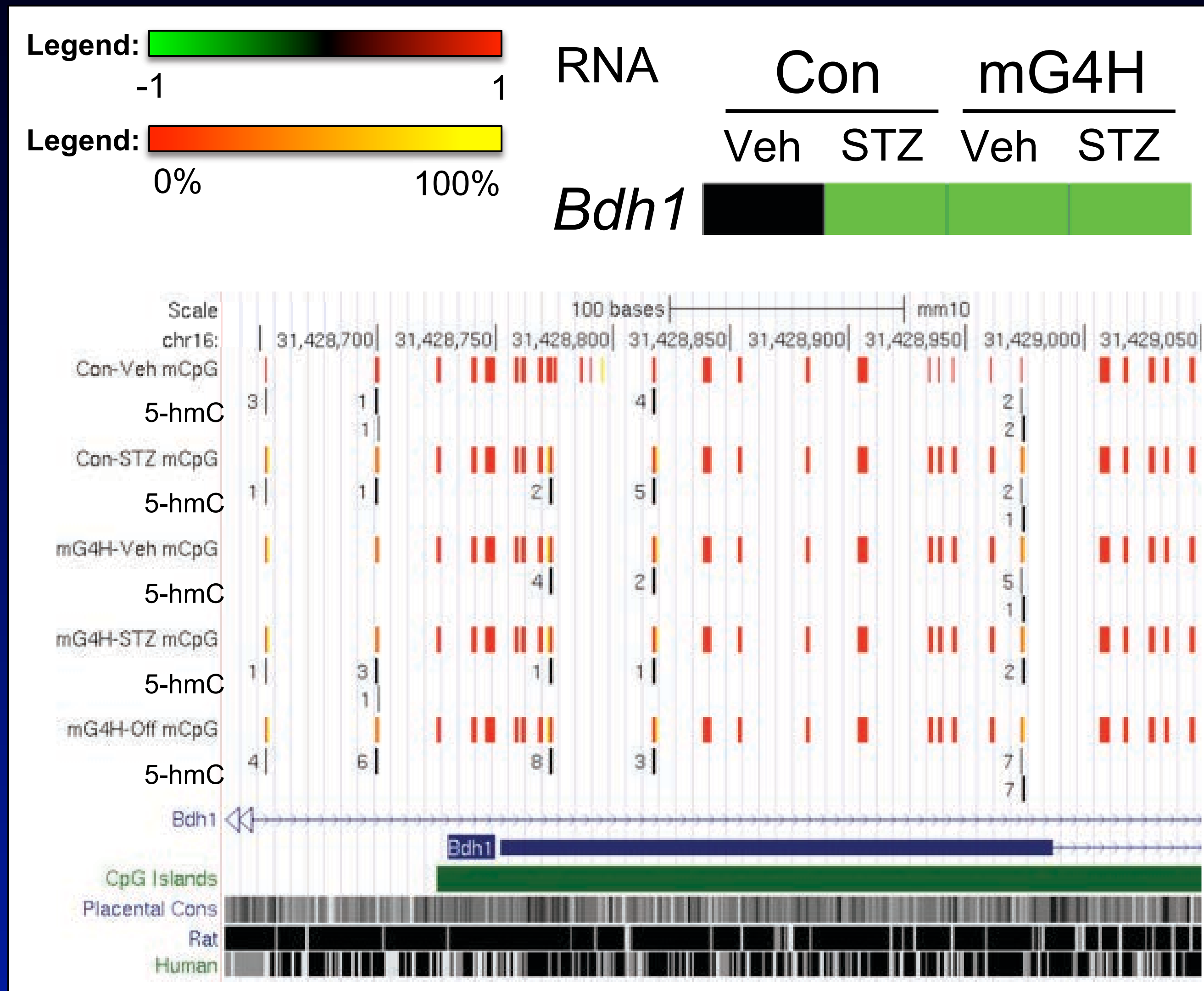
Heart, LV

Zymo Research and UCSC Genome Browser
Wende, unpublished

Combined Transcriptome/Methylome

Genomewide
RRHP
5-hmCpG





Heart, LV

Zymo Research and UCSC Genome Browser
Nye … Wende, unpublished

Conclusion – Part 4

Cellular glucose fluctuations regulates the epigenome via histone modifications and controlling the machinery for DNA methylation.

ARW

Sugar Gumming Up the Works

ARW

giphy.com

Overall Summary

Using combined methylomics, transcriptomics, proteomics, and metabolomics we have begun to define the mechanism of glucotoxicity.

ARW

Acknowledgements

Thomas J Bailey – Undergrad; *Cox6a2*
Manoja K. Brahma – Postdoc; *Bdh1/Oxct1*
Mark C. McCrory – Lab Manager; *Hmgcs2*
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Mark Pepin – MSTP; *Abat*
Lamario J Williams – Undergrad; UQCRFS1/ROS

UAB Collaborators

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John C. Chatham
David K. Crossman
Steve M. Pogwizd
Martin E. Young

E. Dale Abel

John C. Schell
Joseph Tuinei
many others…

Stavros G. Drakos

Nikos A. Diakos

Hansjörg Schwertz

Oleh Khalimonchuk – UNL

Other Colleagues & Mentors past and present

UAB – Knowledge that will change your world
Division of Molecular and Cellular Pathology

NIH National Heart, Lung, and Blood Institute
K99R00 HL111322
R00 HL111322-S1

NIH National Institute of Diabetes and Digestive and Kidney Diseases
U24 DK076169

American Heart Association
AHA 0725064Y

JDRF – Improving Lives. Curing Type 1 Diabetes.
JDRF 51002608