Washington University in St.Louis
School of Medicine

Experimental Molecular Therapeutic Strategies for Treating Barth Syndrome:

Elucidation of the Functional Role of the Mitochondrial Lipidome

Barth Syndrome Conference 2012

Michael Kiebish

Disclaimer

-All experiments and analysis were performed while at Washington University School of Medicine. The data and conclusions presented do not necessarily reflect the opinions of my current employer.

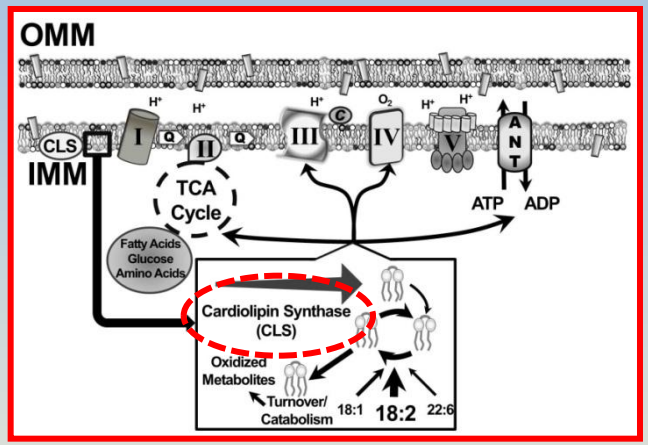
Overview

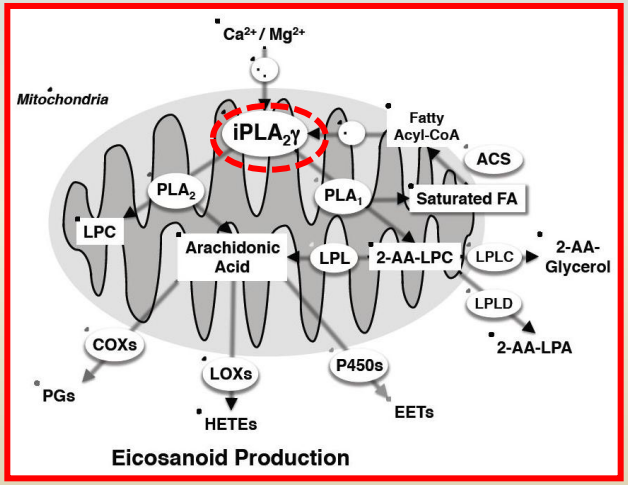
Barth Syndrome Mouse Model

Cardiac lipidomic, metabolic, and adaptive mechanisms

Effects of cardiac specific upregulation of cardiolipin synthase and the subsequent lipidomic and bioenergetic effects

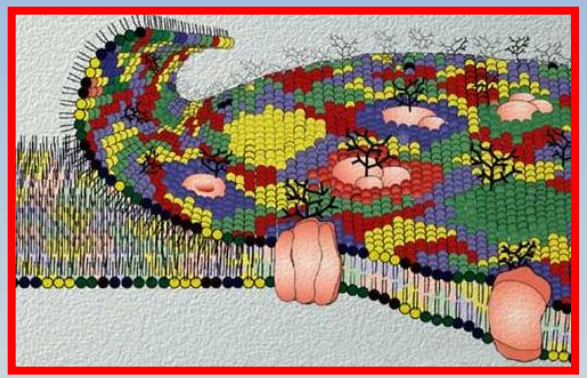
Targeting phospholipases by transgenically expressing/ablating iPLA$_2\gamma$ and its subsequent lipidomic effects



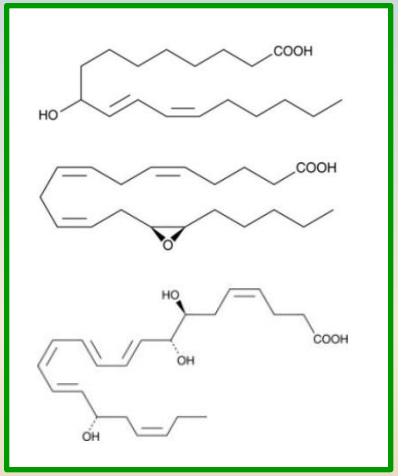


Novel interpretation of the role of the mitochondrial lipidome in health and disease as it relates to Barth syndrome

Multi-Omic Integrated Strategy to Elucidate Functional Changes in Pathophysiology

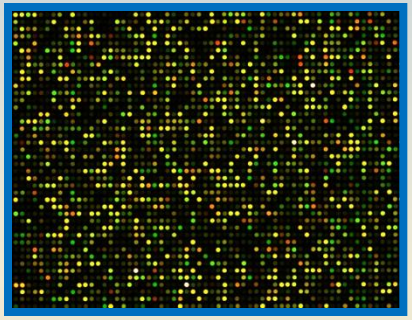


Lipidomic Analysis

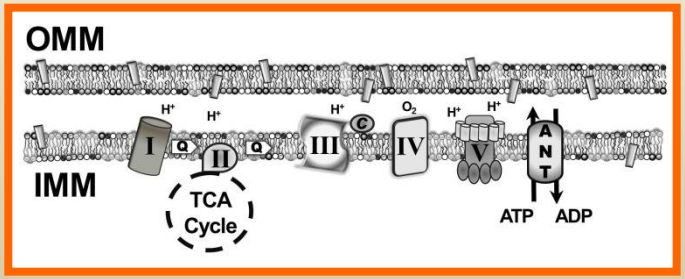


Signaling Lipidomic Analysis

Integrated Function



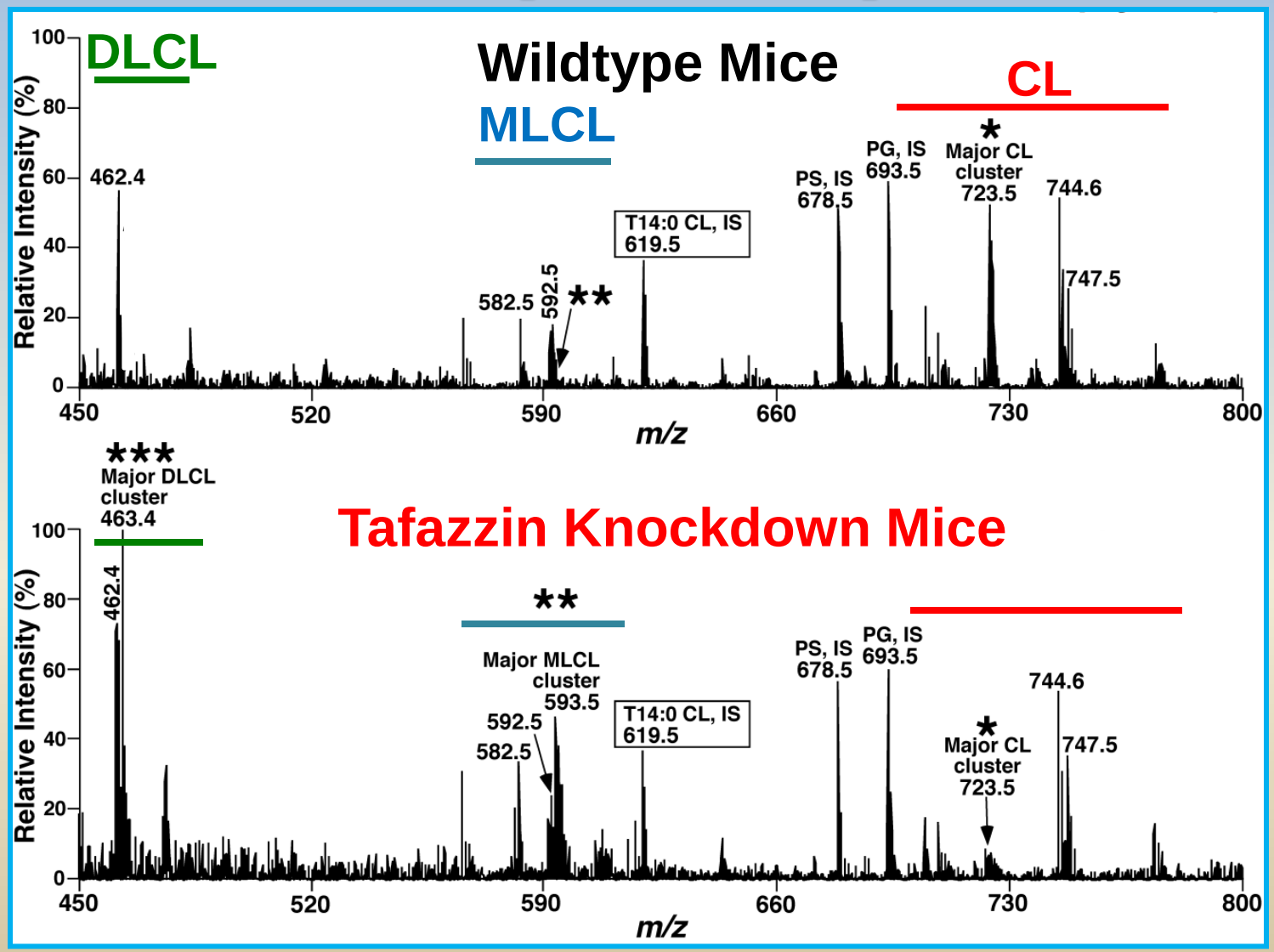
Transcriptomic Analysis



Bioenergetic Analysis

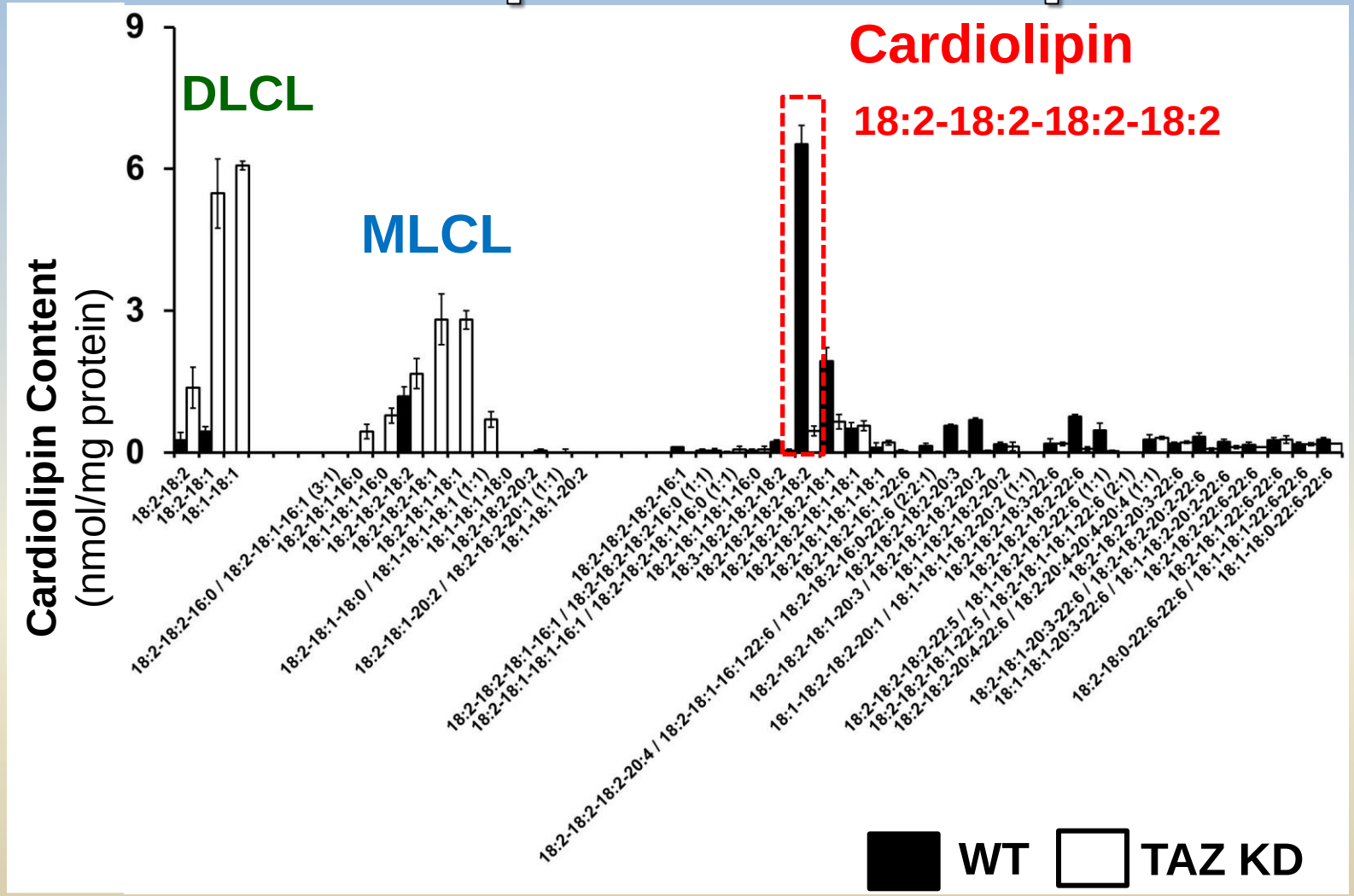
Barth Syndrome Mouse Model

Cardiac Anionic Lipidomic Spectrum



Barth Syndrome Mouse Model

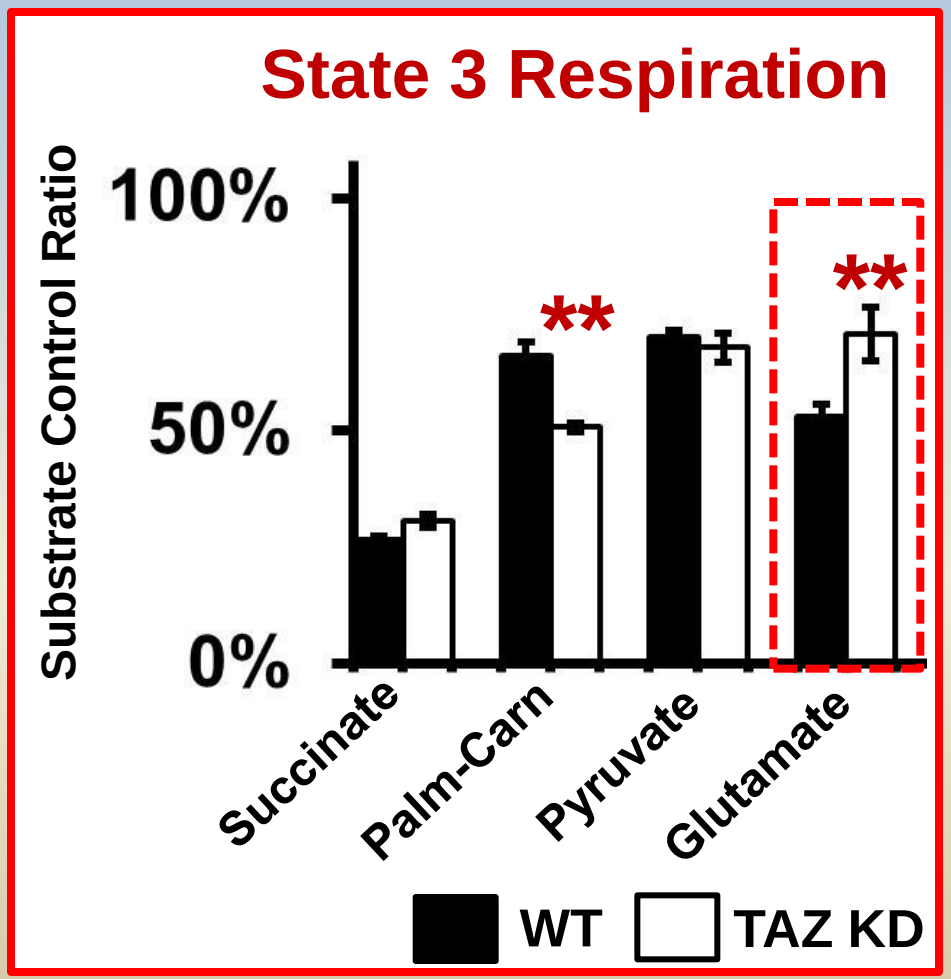
Cardiac Cardiolipin Molecular Species

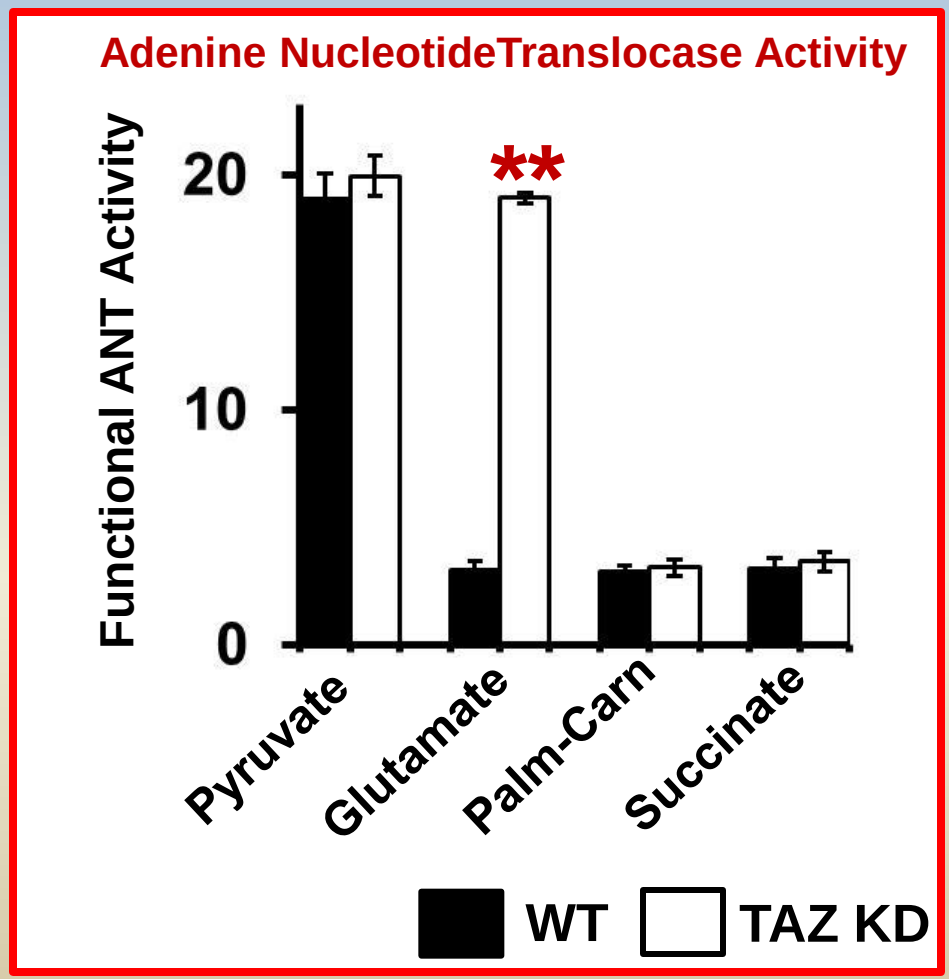


Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in 2 month old WT and Tafazzin knockdown mice (N = 4)

Barth Syndrome Mouse Model

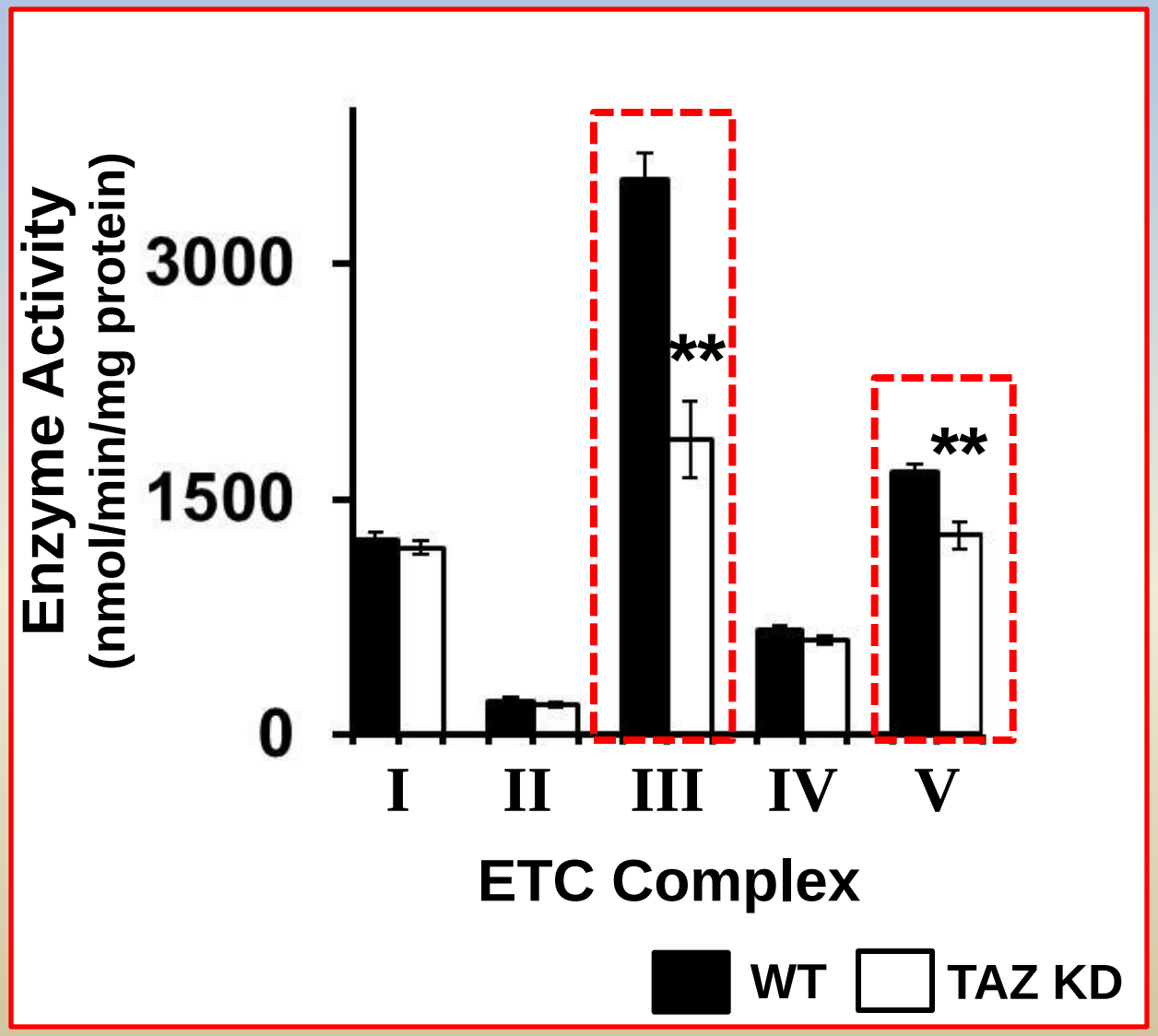
Respiratory and Enzymatic Characterization





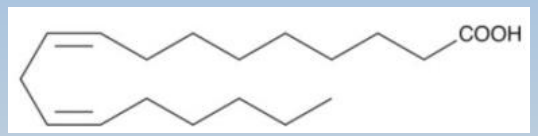
Barth Syndrome Mouse Model

Electron Transport Chain Activities

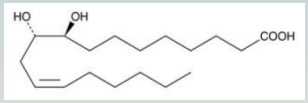


Oxidized Lipidomics

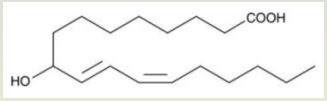
Linoleic



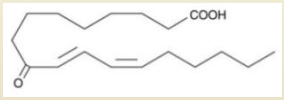
DiHOMEs



HODEs



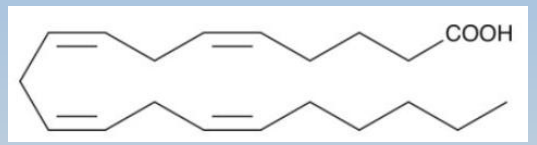
oxoODE



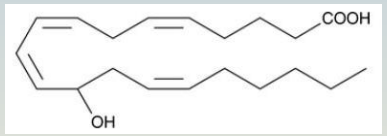
EpOME

Calcium Influx
Immune Response

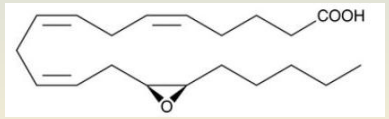
Arachidonic



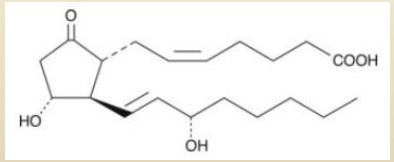
HETEs



EETs

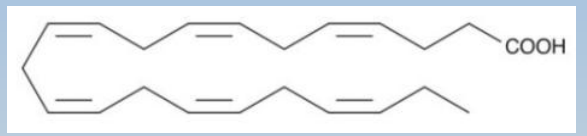


Prostanoids

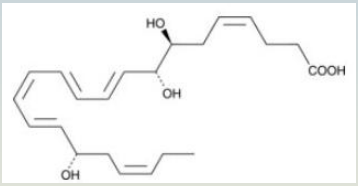


Vasodilation
Vasoconstriction
Inflammation

DHA

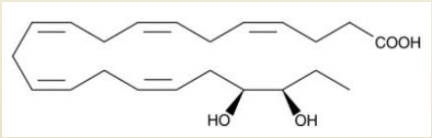


RVD



DiHDoHE

DiHDPA

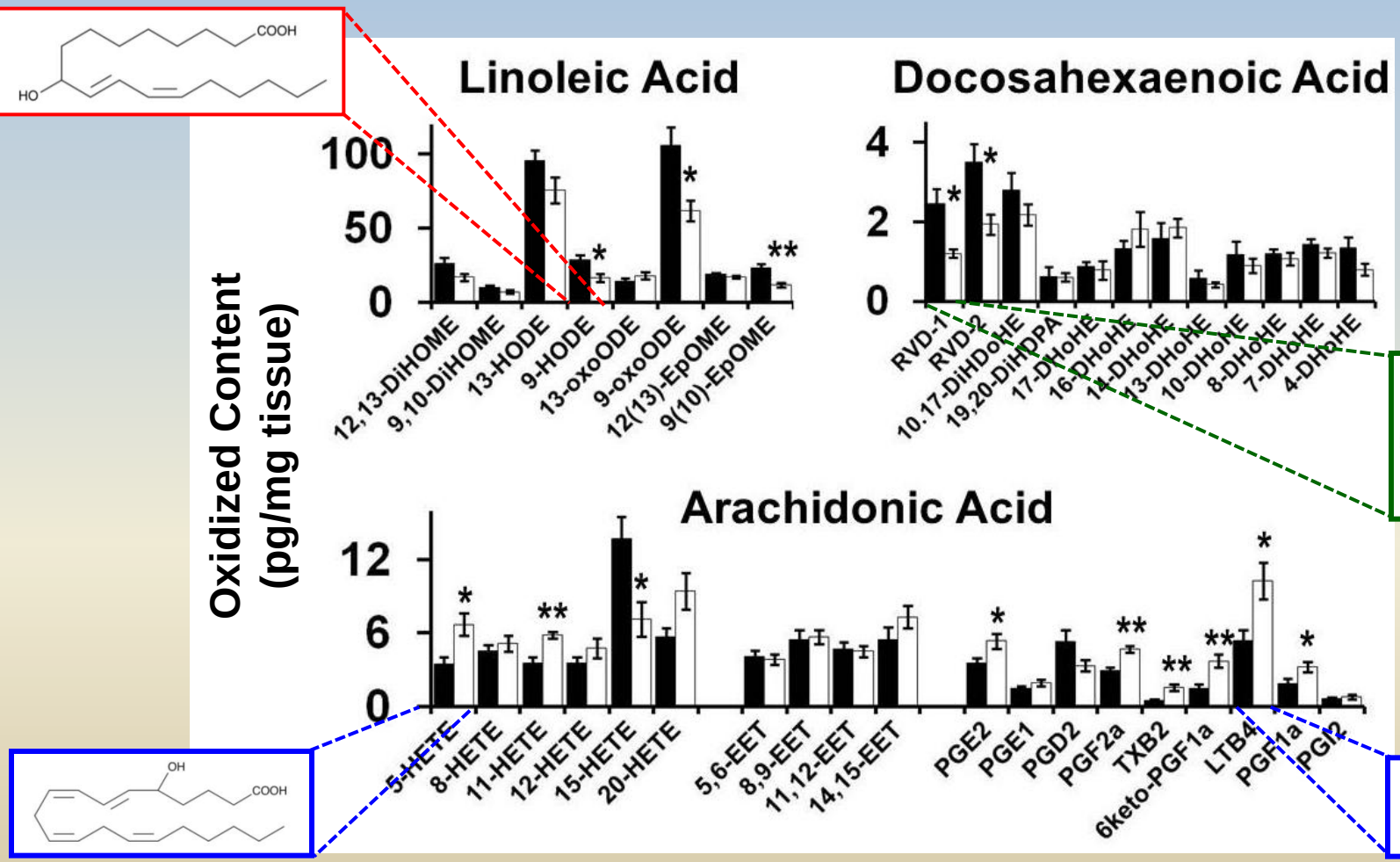


DHoHE

Anti-Inflammatory

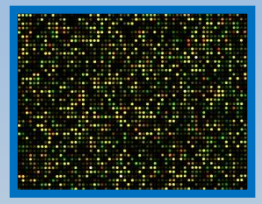
Barth Syndrome Mouse Model

Cardiac Oxidized Lipid Analysis



Barth Syndrome Mouse Model

Transcriptomics/GSEA Analysis



Protein Translation

Aminoacyl tRNA Biosynthesis

Peptide Chain elongation

Nucleotide Metabolism

Purine Metabolism

Gene Expression

Amino Acid Synthesis and Interconversion

Valine, Leucine, and Isoleucine Biosynthesis

Hypoxia and p53 in Cardiovascular System

GTP Hydrolysis

Metabolism of Proteins

One Carbon pool by Folate

Branched chain amino acid catabolism

IL12 and STAT4 Dependent Signaling Pathway in Th1 Development

Bioactive Peptide Induced Signaling Pathway

Valine, Leucine, and Isoleucine Degradation

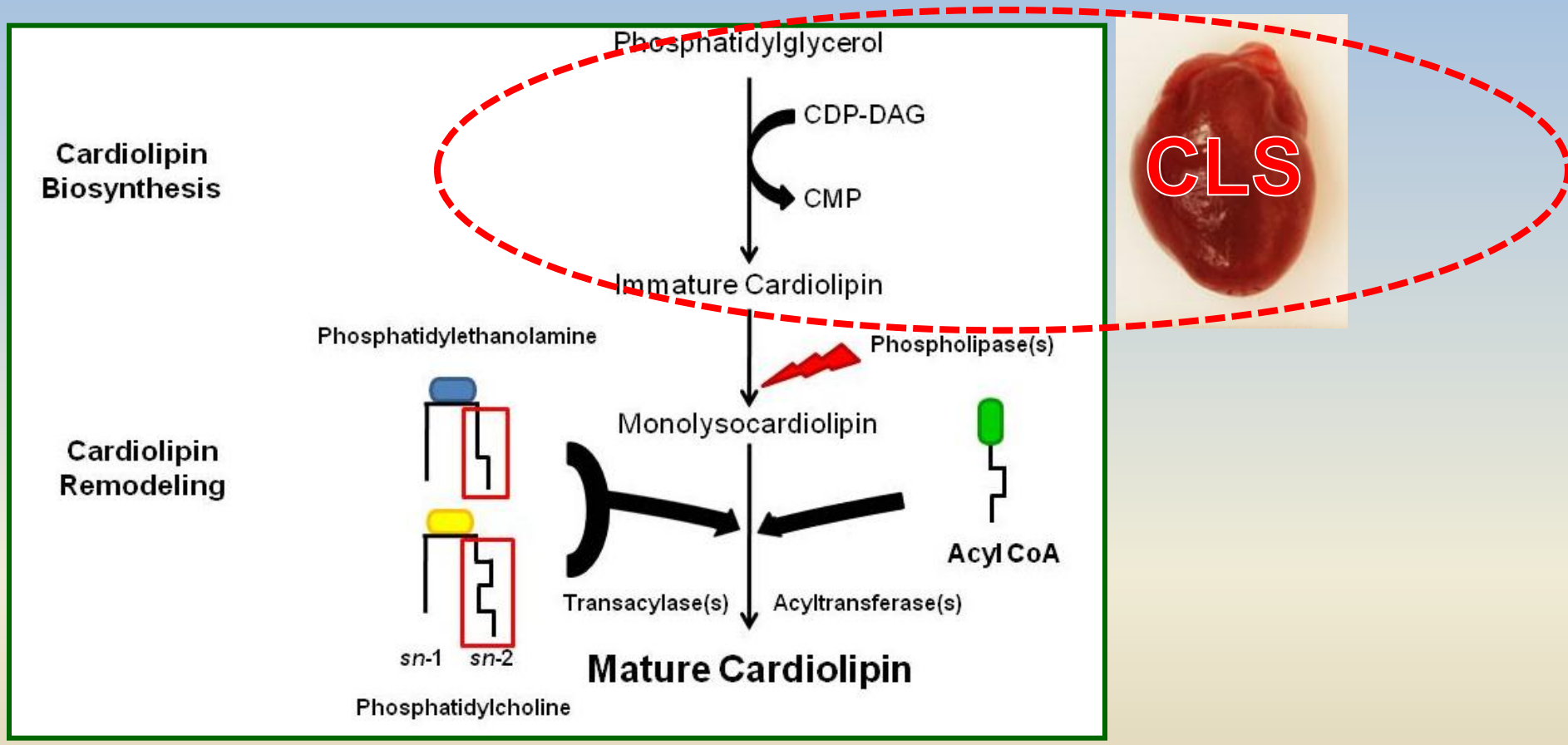
Transcription factor CREB and its extracellular signals

TAK1/p38 MAPK Activation

Barth Syndrome Mouse Model

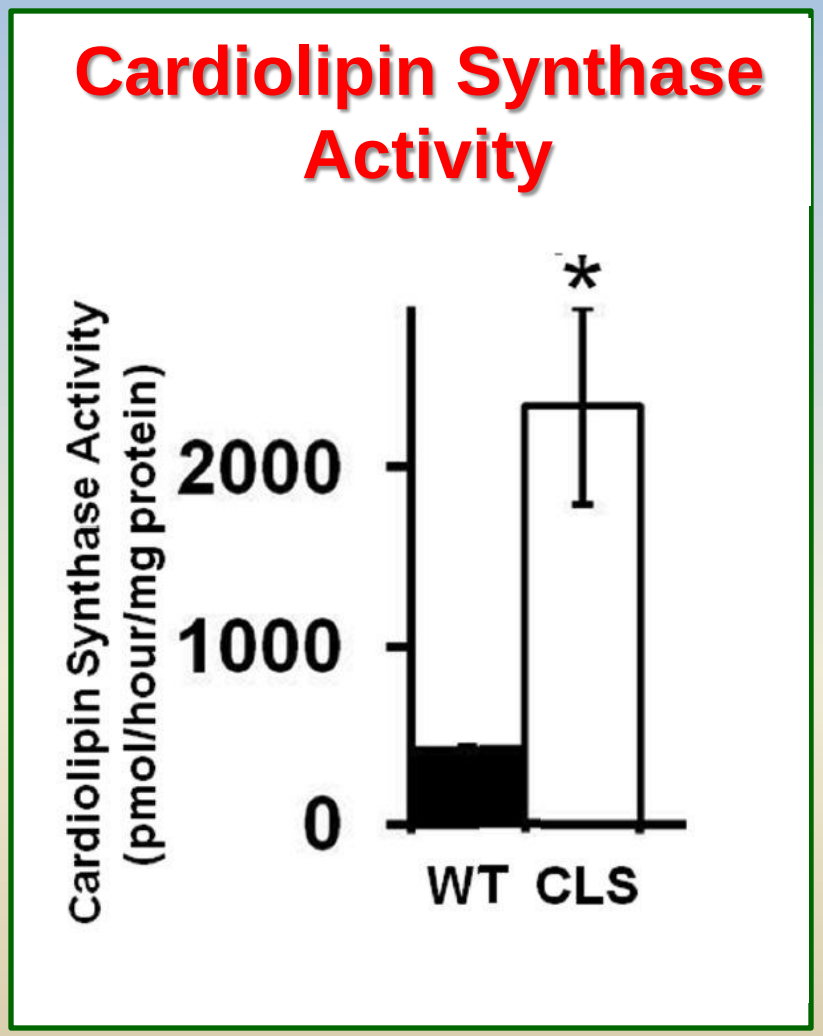
- **Demonstrates the lipidomic abnormalities discovered in boys with Barth syndrome**
- Displays altered substrate utilization (decreased fatty acid and increased glutamate stimulated respiration)
- **Demonstrates altered mediator lipidomic signature**
- **Compensatory enzyme kinetics (Adenine nucleotide translocase and Electron transport chain) and gene expression (microarray analysis)**

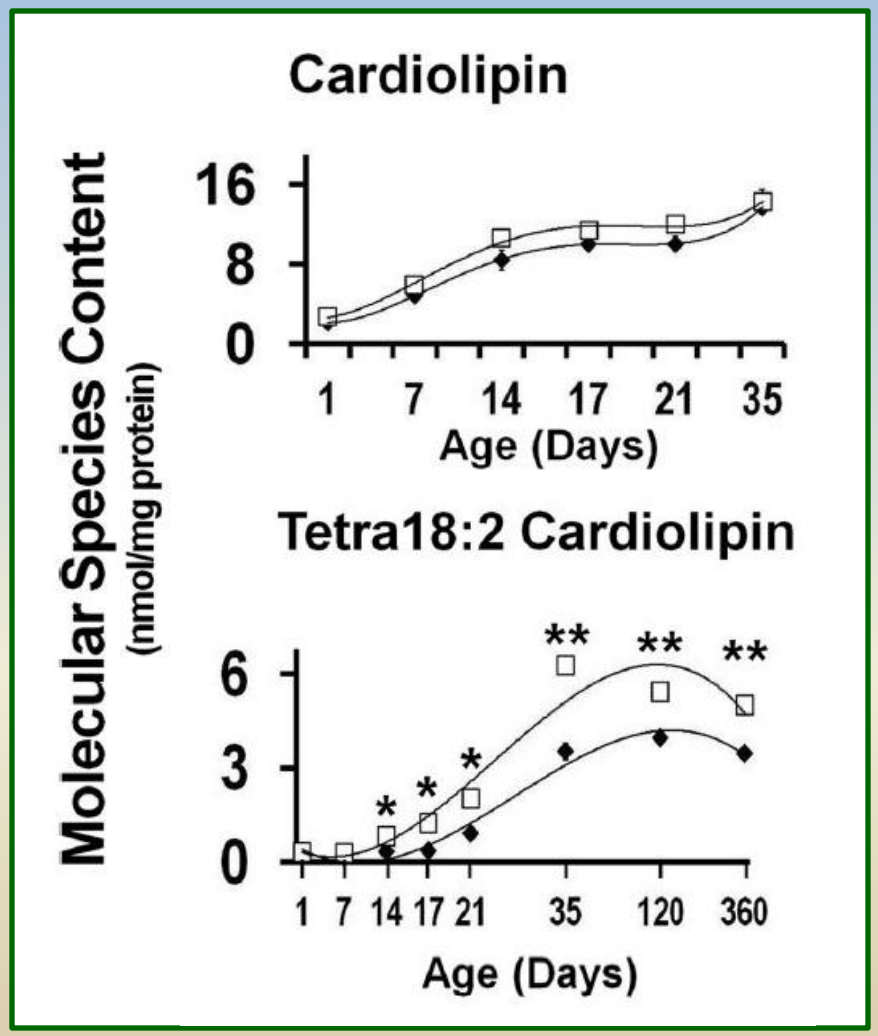
Cardiolipin Synthase (CLS) Mouse Model



The CLS Model Provides a Molecular Therapeutic Tool to Investigate the Role of Cardiolipin in Attenuating Mitochondrial Dysfunction in Disease

Cardiolipin Synthase Mouse Model

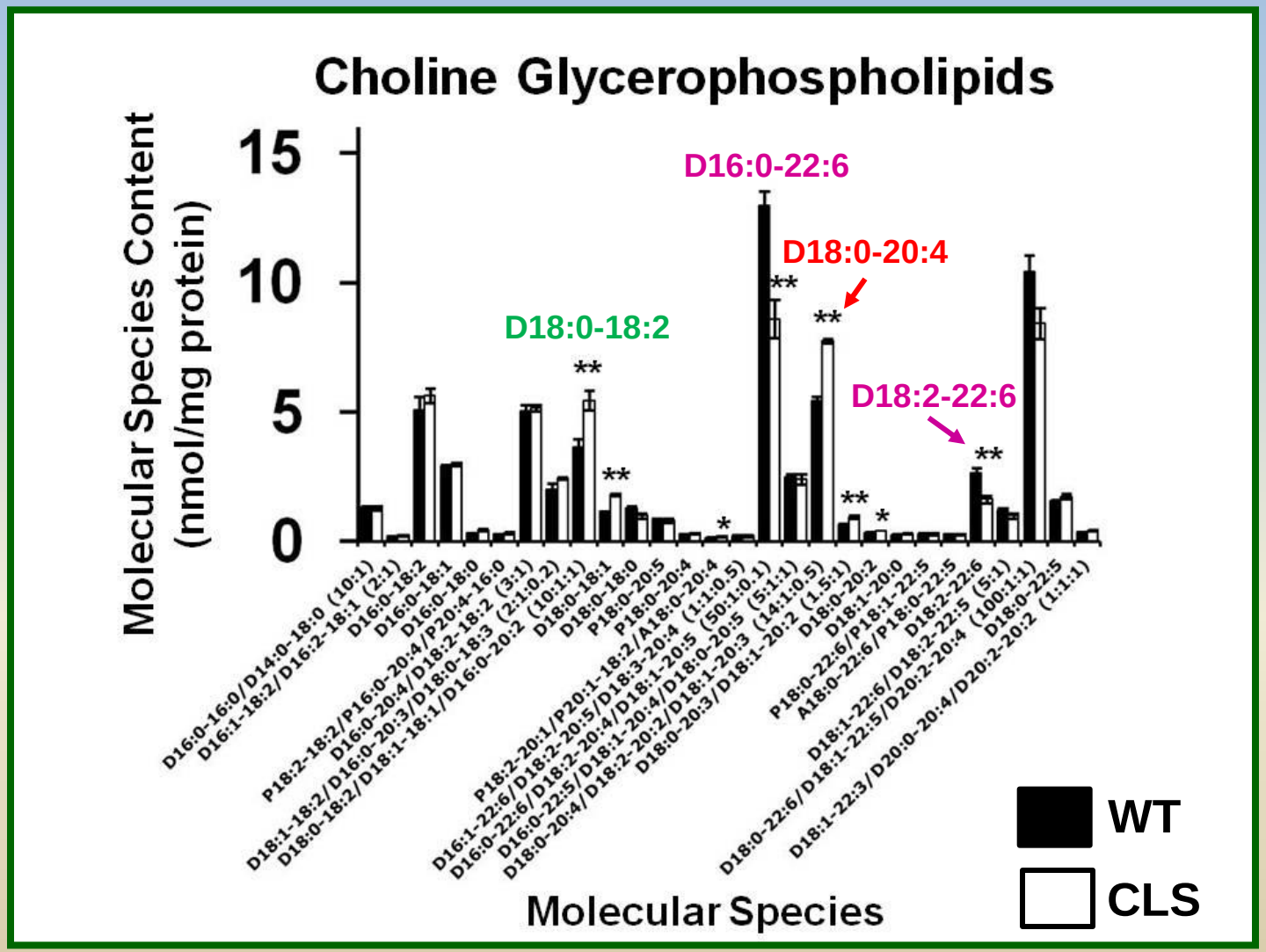




Kiebish *et al* 2012 (in press JBC)

Black Diamonds (Wildtype) and White Squares (CLS)

Cardiolipin Synthase Mouse Model

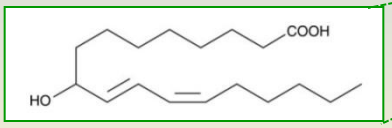


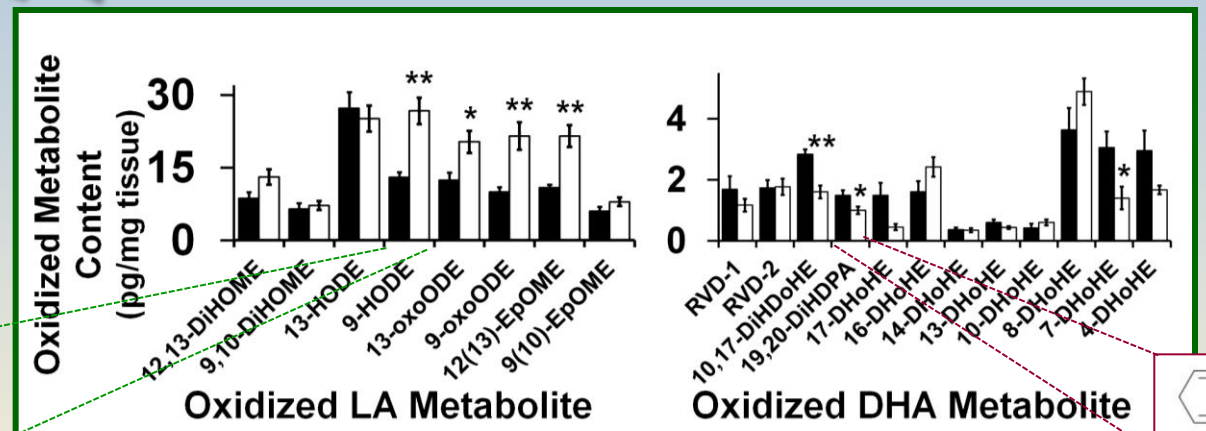
Kiebish *et al* 2012 (in press JBC)

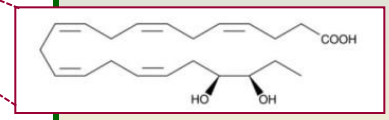
Cardiolipin Synthase Mouse Model

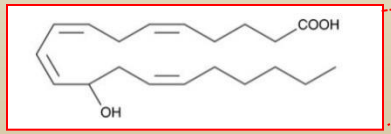
Oxidized Lipid Species

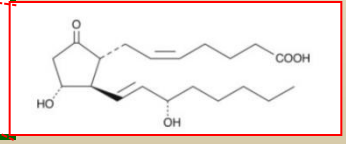
18:2 → 20:4 → 22:6







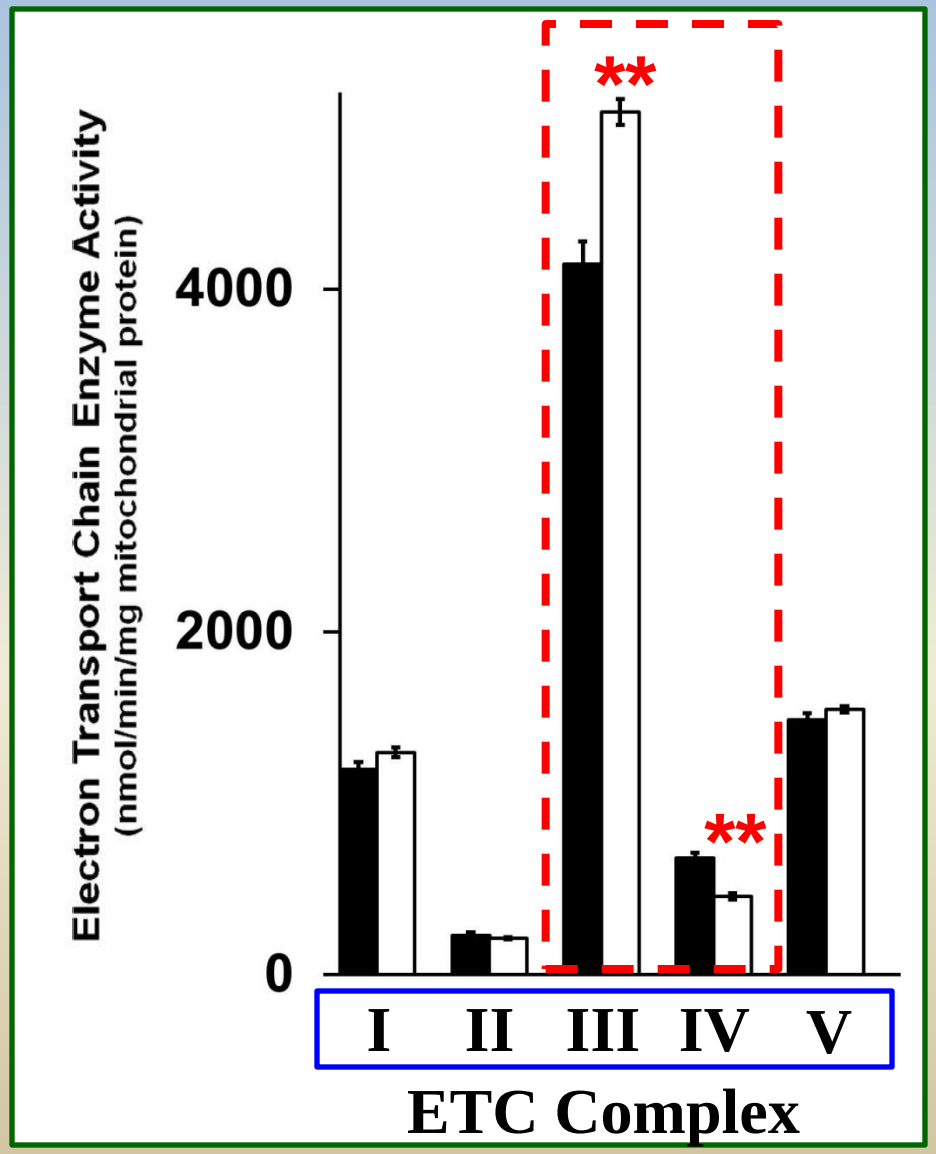


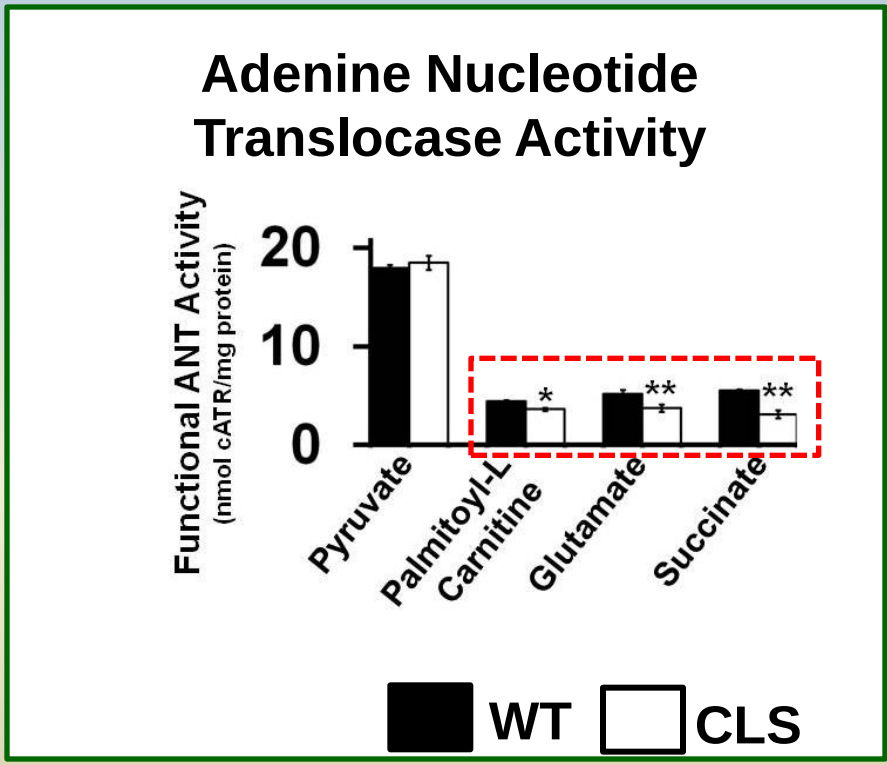


WT CLS

Cardiolipin Synthase Mouse Model

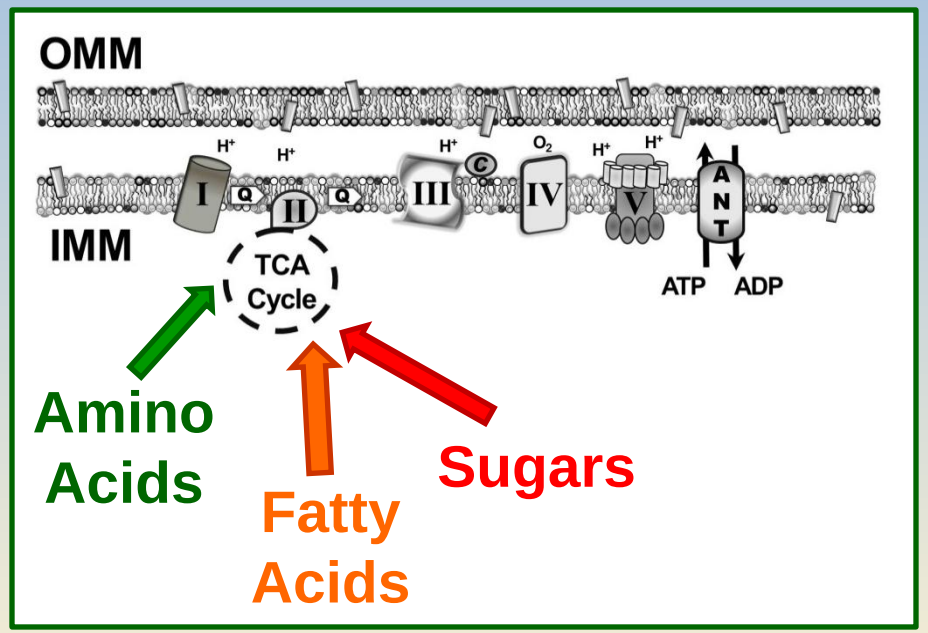
Mitochondrial Enzyme Activities





Cardiolipin Synthase Mouse Model

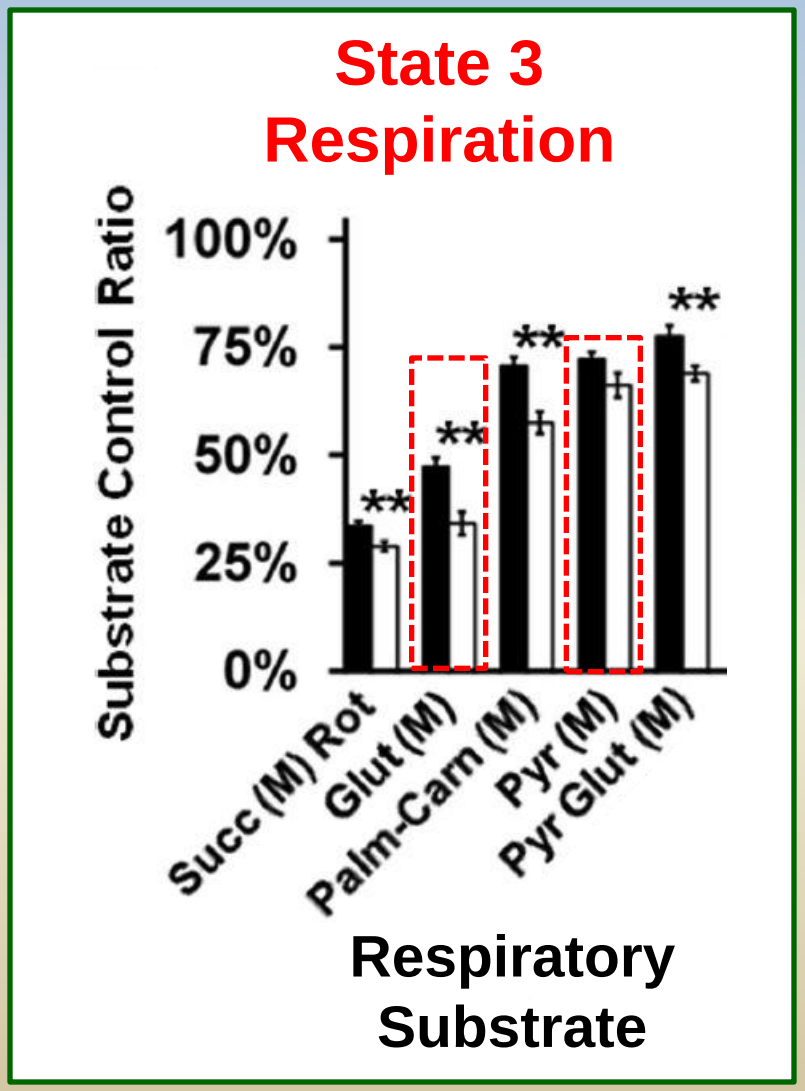
Mitochondrial Respiration



Pyruvate = Normal

Glutamate = ⬇

Palm-Carnitine = ⬇



Cardiolipin Synthase Mouse Model

Transcriptomics/GSEA Analysis

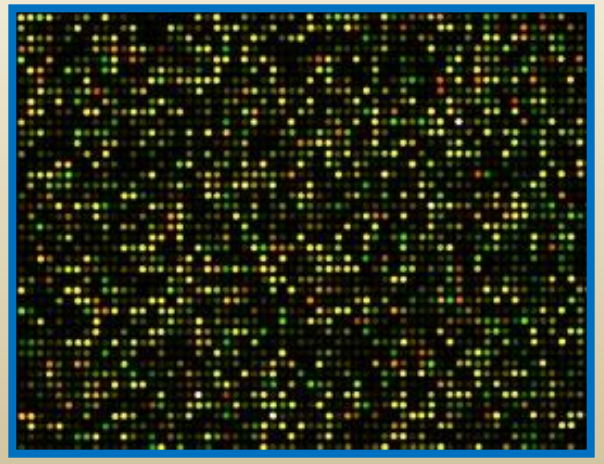
Aminoacyl tRNA Biosynthesis

P53 Signaling Pathway

Glycine, Serine, Threonine Metabolism

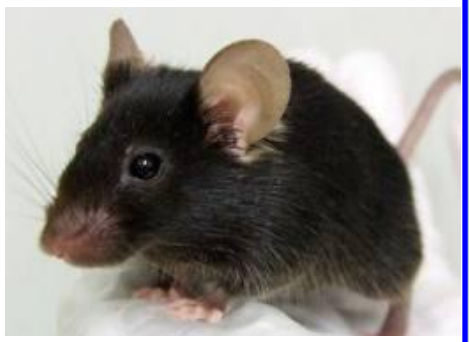
Amino Acid Synthesis and Interconversion

AHSP Pathway

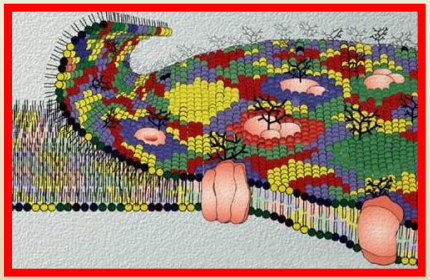


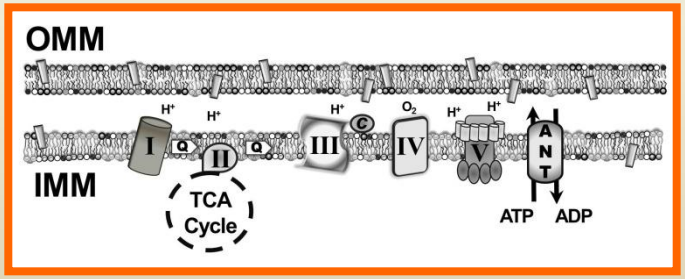
CLS-TG/Tafazzin KD Mouse Model

Cardiac specific Tg-Cardiolipin Synthase Mouse Model



Tafazzin inducible shRNA Knockdown Mouse Model

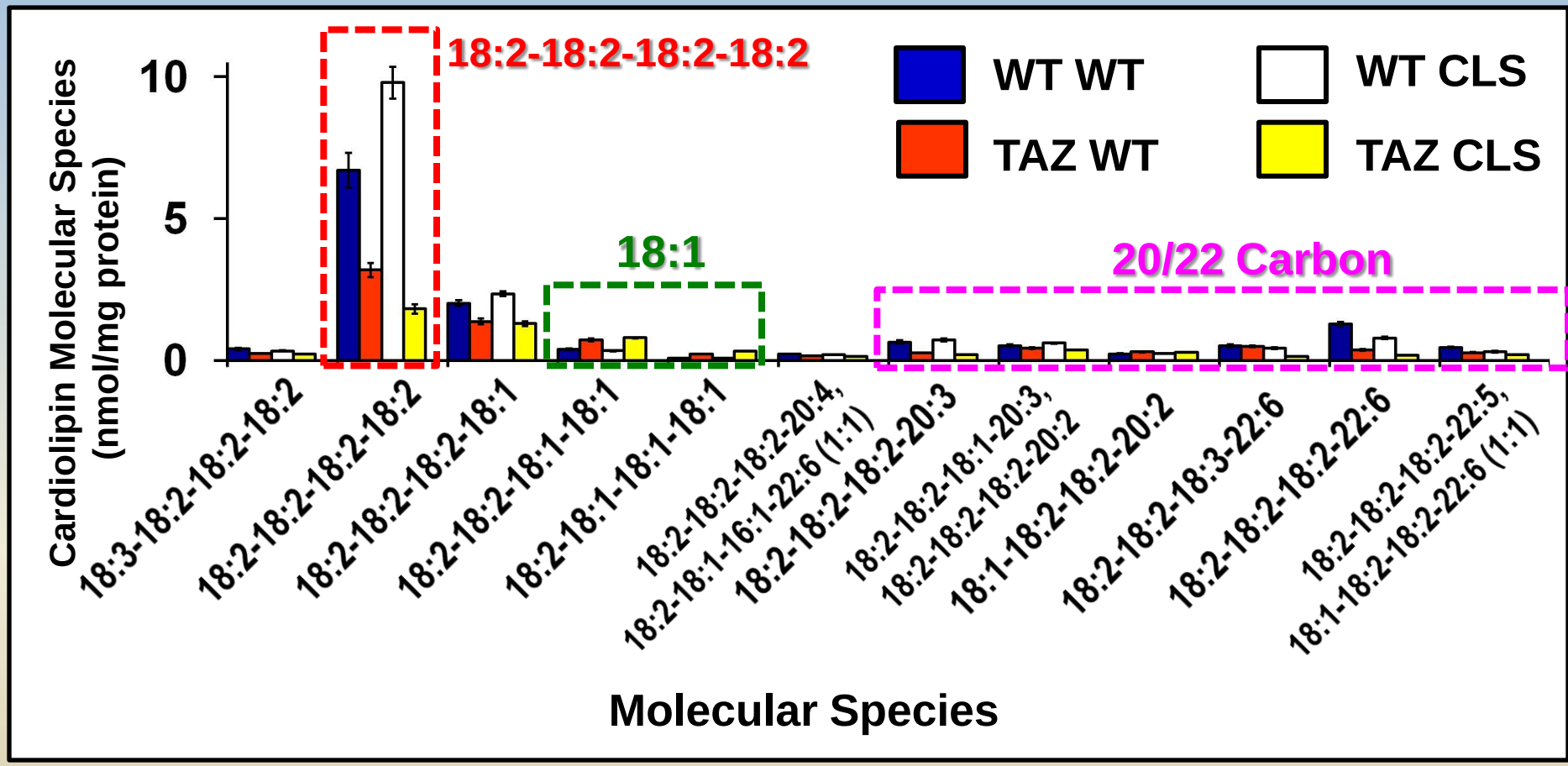




Goal was to use cardiolipin synthase over expression in the heart to attenuate altered cardiolipin molecular species in Barth Syndrome, thus restoring the homeostatic balance of mitochondrial substrate utilization

CLS-TG/Tafazzin KD Mouse Model

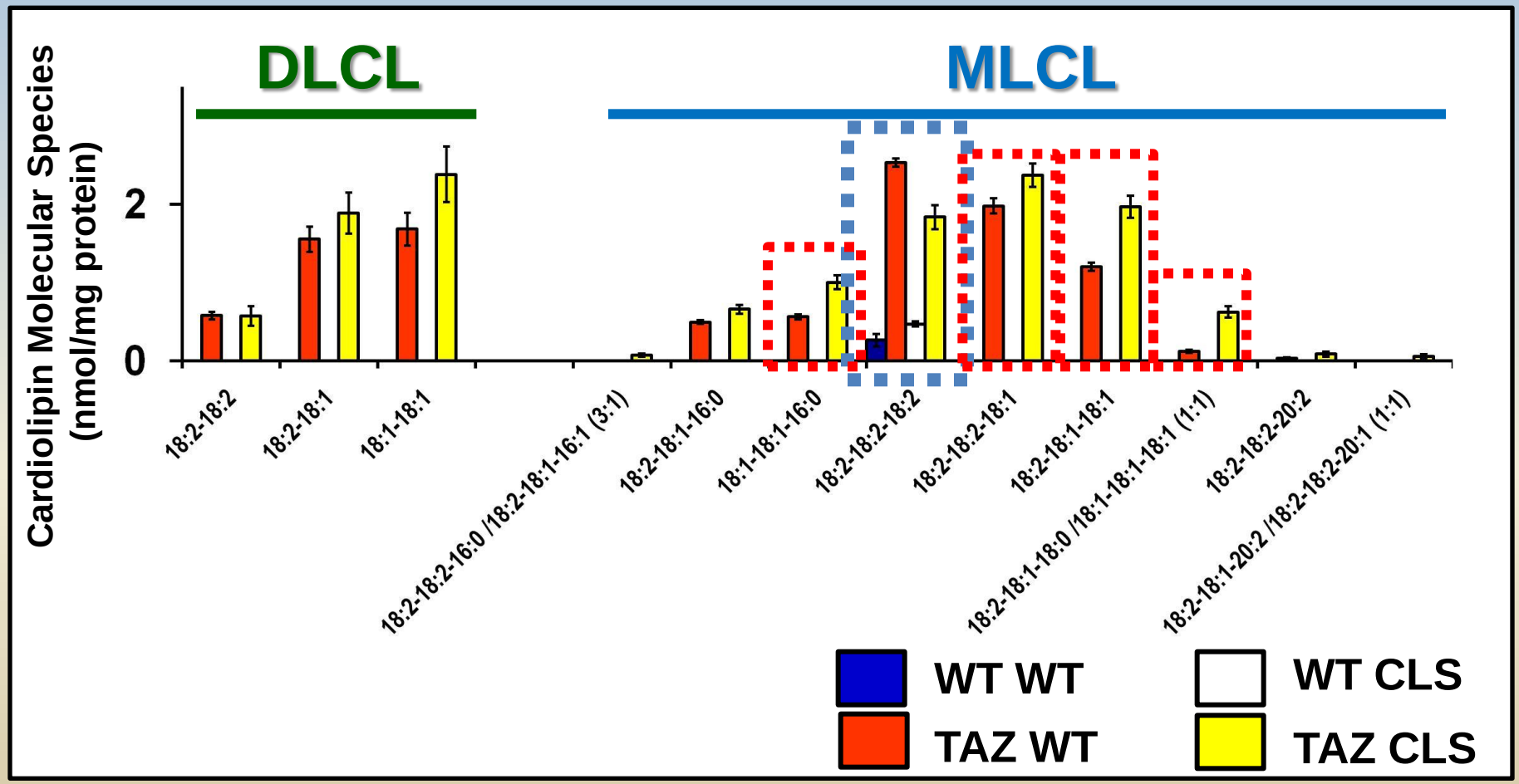
Cardiolipin Molecular Species



Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated with doxycyline for 2 months (N = 4)

CLS-TG/Tafazzin KD Mouse Model

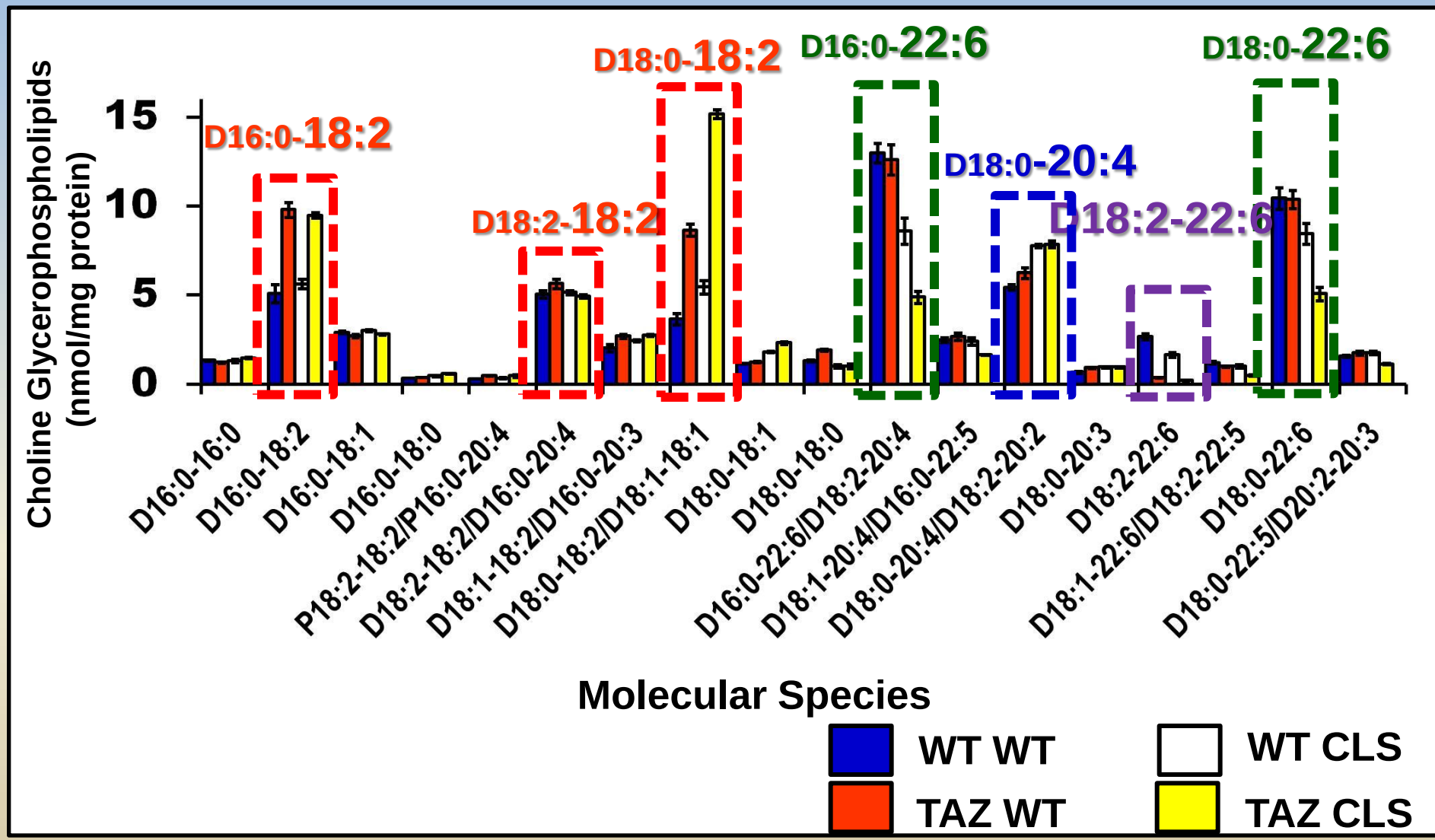
Di/Monolyso - Cardiolipin Molecular Species



Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated with doxycyline for 2 months (N = 4)

CLS-TG/Tafazzin KD Mouse Model

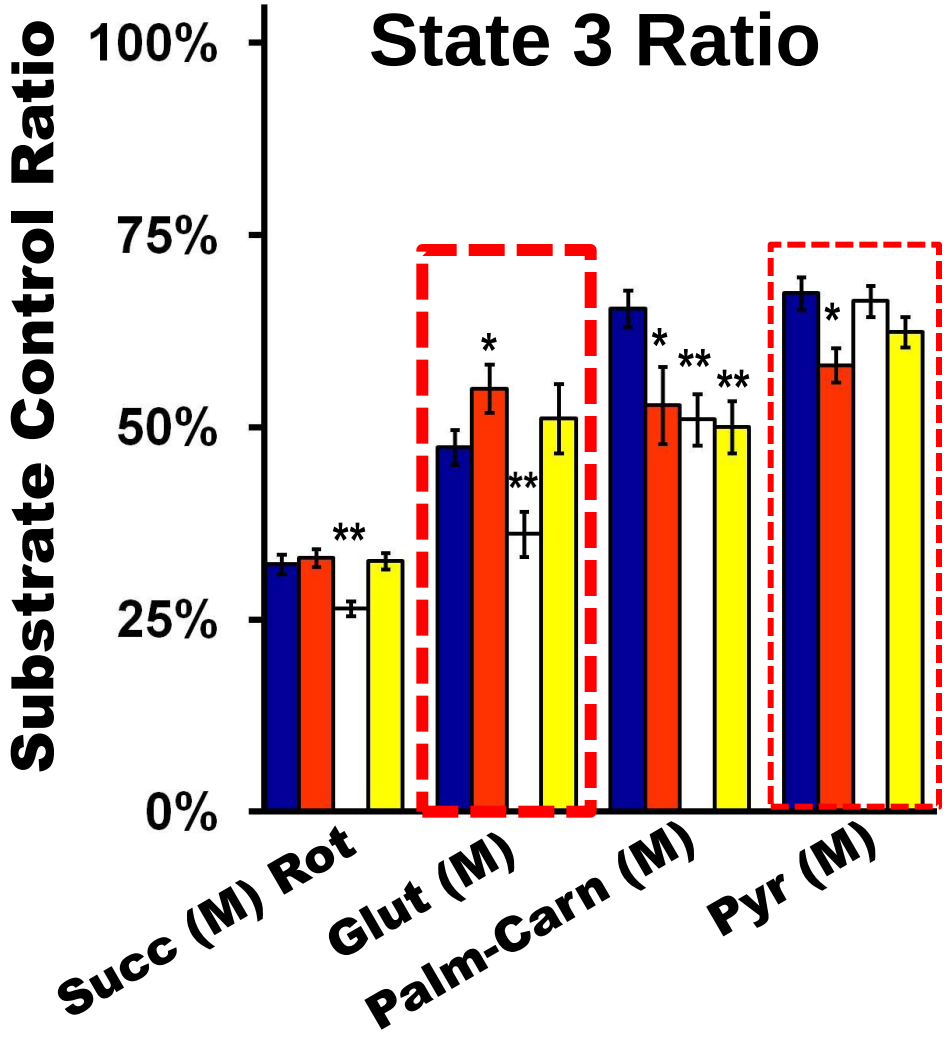
Choline Glycerophospholipid Molecular Species

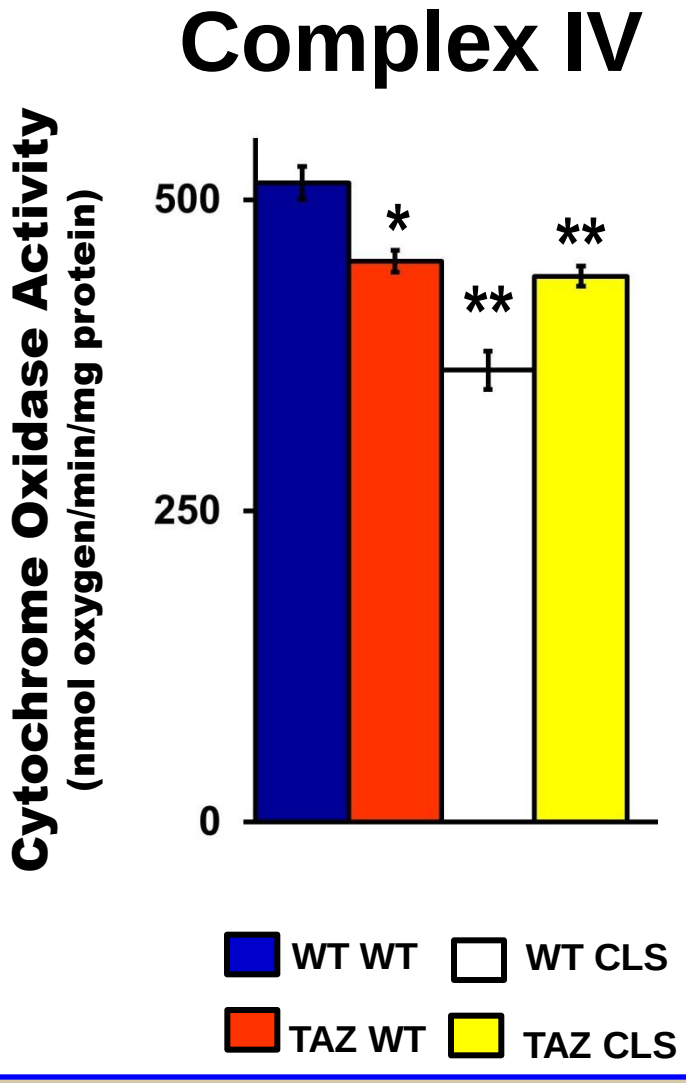


Values represent the mean ± S.E. choline glycerophospholipid molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated on doxycyline for 2 months (N = 4)

CLS-TG/Tafazzin KD Mouse Model

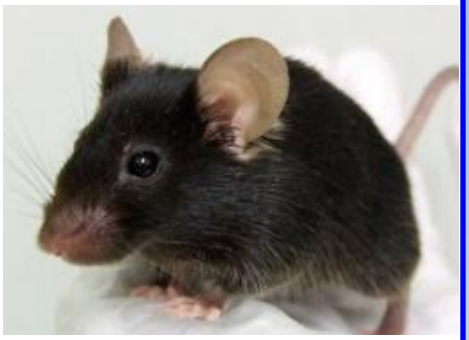
Bioenergetic Analysis





CLS-TG/Tafazzin KD Mouse Model

Cardiac specific Tg-Cardiolipin Synthase Mouse Model



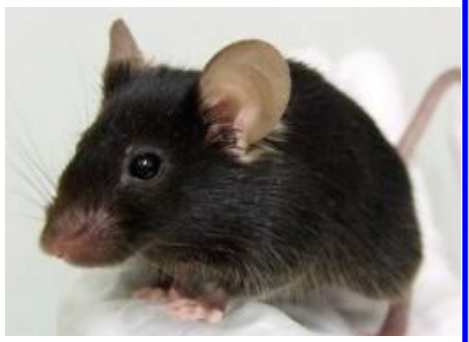
Tafazzin inducible shRNA Knockdown Mouse Model

- **Although cardiolipin molecular species were less remodeled in the Taz X CLS mouse model, the overall bioenergetic phenotype was attenuated, possibly suggesting that alternative factors may be influencing bioenergetics**

iPLAγ-TG and KO Mouse Model

iPLA$_2$γ KO

iPLA$_2$γ-TG

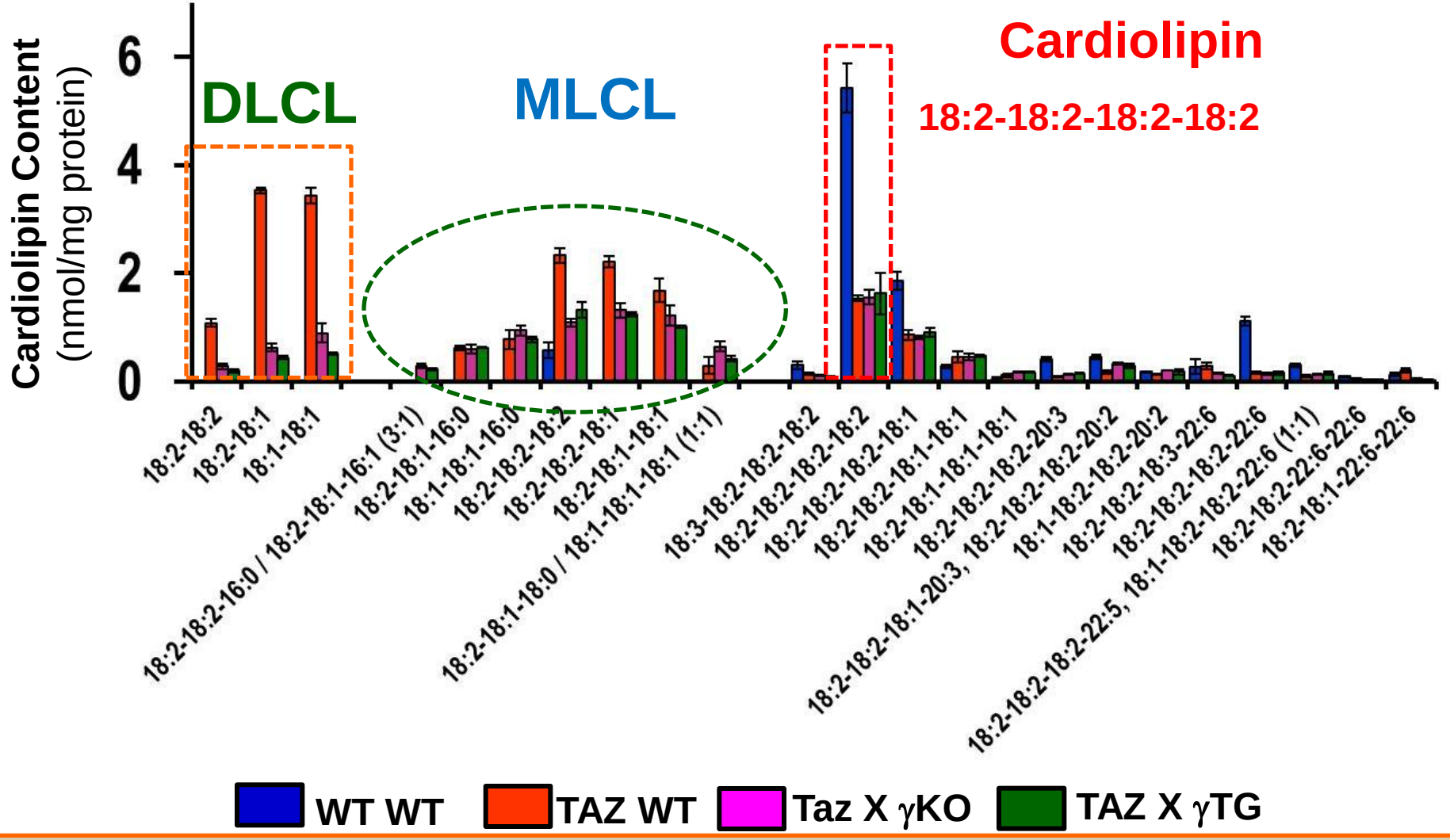


Tafazzin inducible shRNA Knockdown Mouse Model

Does iPLA$_2$γ expression attenuate dysfunctional cardiolipin remodeling as well as effect survival of the cross?

(iPLAγ-TG or KO) X Tafazzin KD Mouse Model

Cardiolipin Molecular Species



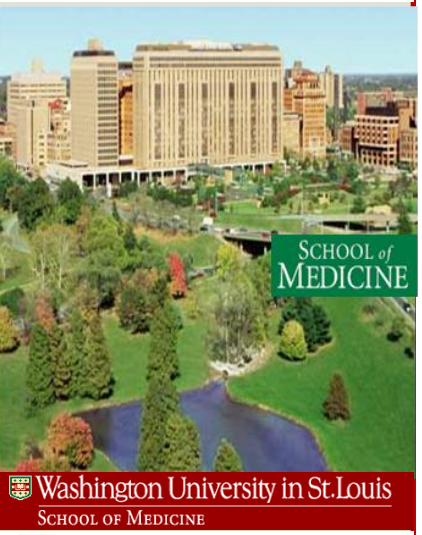
Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in WT, Taz, Taz x iPLAγKO, and Taz x iPLA2γ-TG treated on doxycyline for 2 months (N = 4)

Conclusion

- Through the utilization of various transgenic models as well as investigating dynamic flux of the lipidome, we can elucidate the lipidomic/bioenergetic connection that initiates pathological sequalae or that can be identified for therapeutic efficacy

- Regulation of the mitochondrial lipidome may hold unknown mechanism that go well beyond just structure and enzyme kinetics

- The inducible Tafazzin shRNA knockdown mouse model of Barth Syndrome is an invaluable tools to discover unknown mechanism that embody the Barth Syndrome phenotype which can be therapeutically targeted



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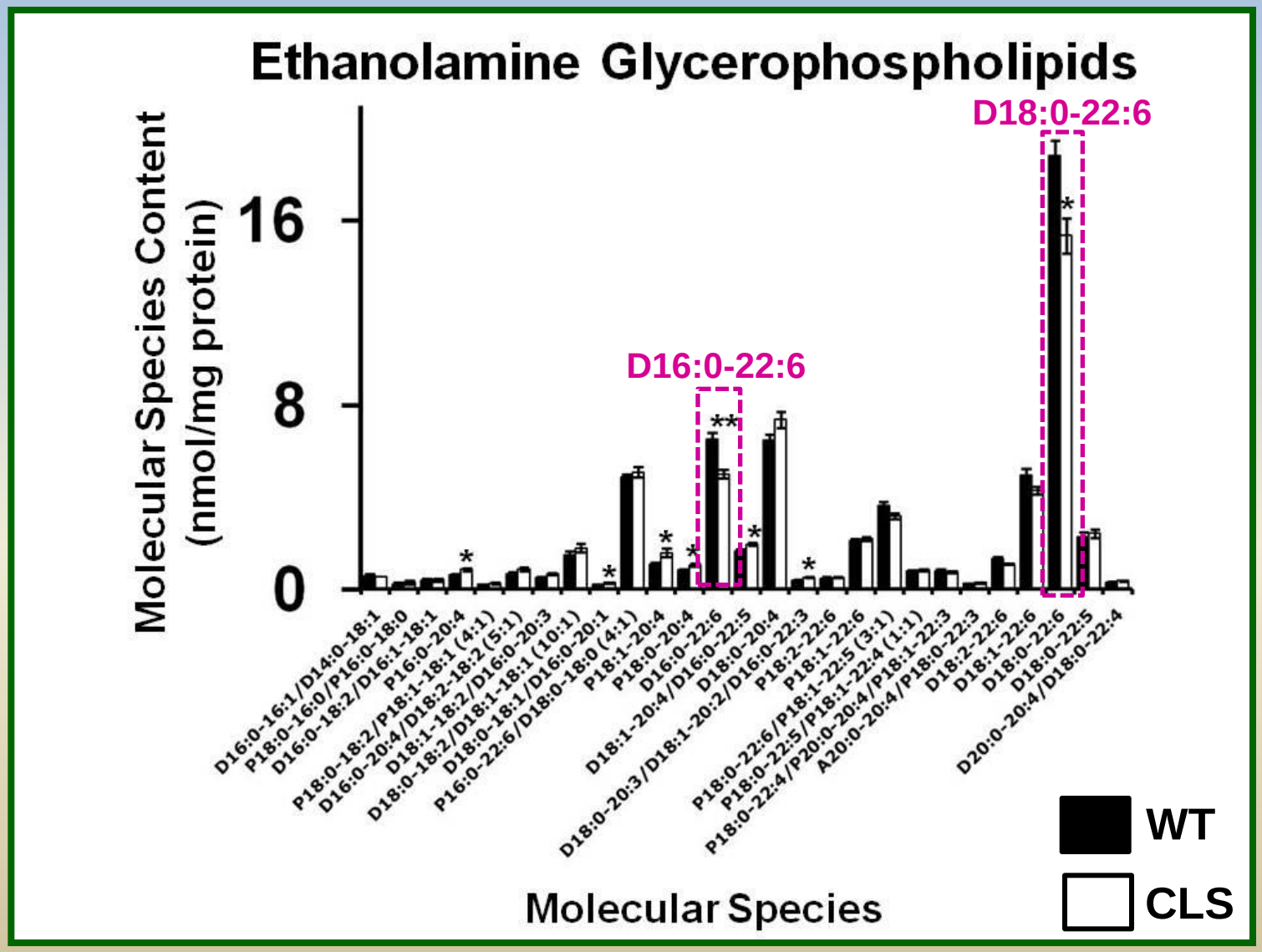
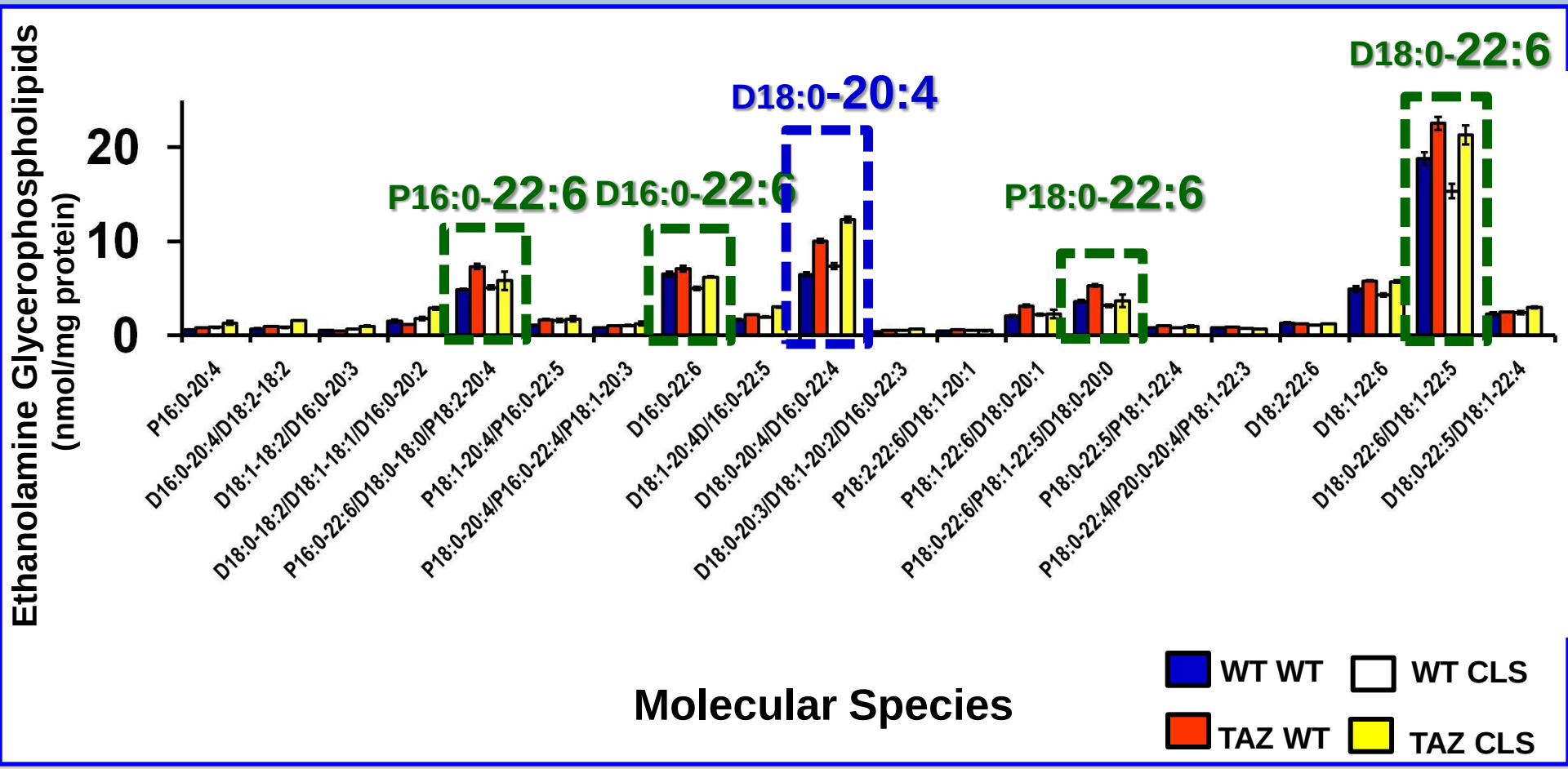
Cardiolipin Synthase Mouse Model



CLS-TG/Tafazzin KD Mouse Model

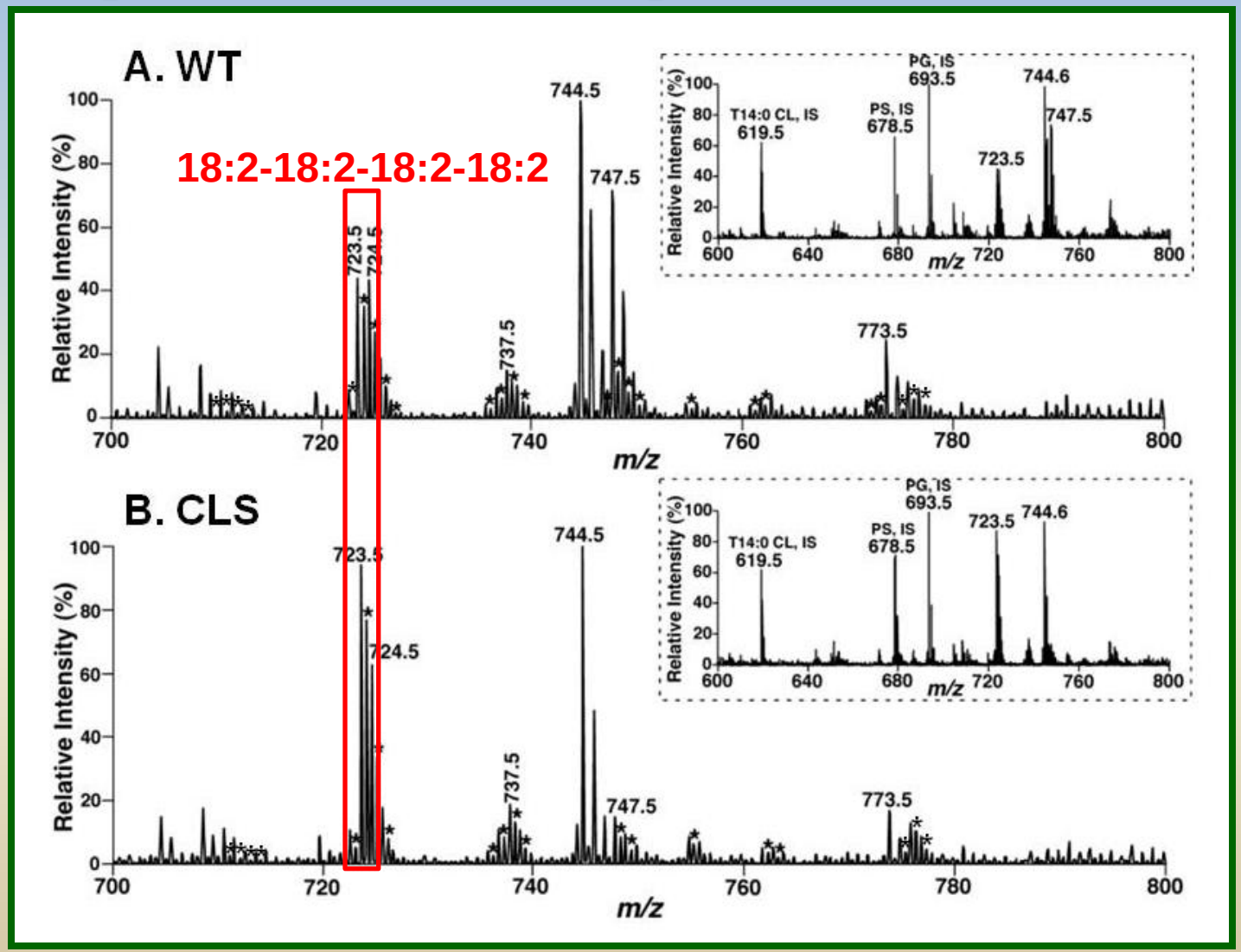
Ethanolamine Glycerophospholipid Molecular Species



Values represent the mean ± S.E. ethanolamine glycerophospholipid molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated on doxycyline for 2 months (N = 4)

Cardiolipin Synthase Mouse Model

Cardiolipin Molecular Species



Kiebish *et al* 2012 (in press JBC)