The Linking of Cardiolipin Remodeling to Mitochondrial β-oxidation and Cardiolipin to T-cell function

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UNIVERSITY OF MANITOBA

the Manitoba Institute of Child Health

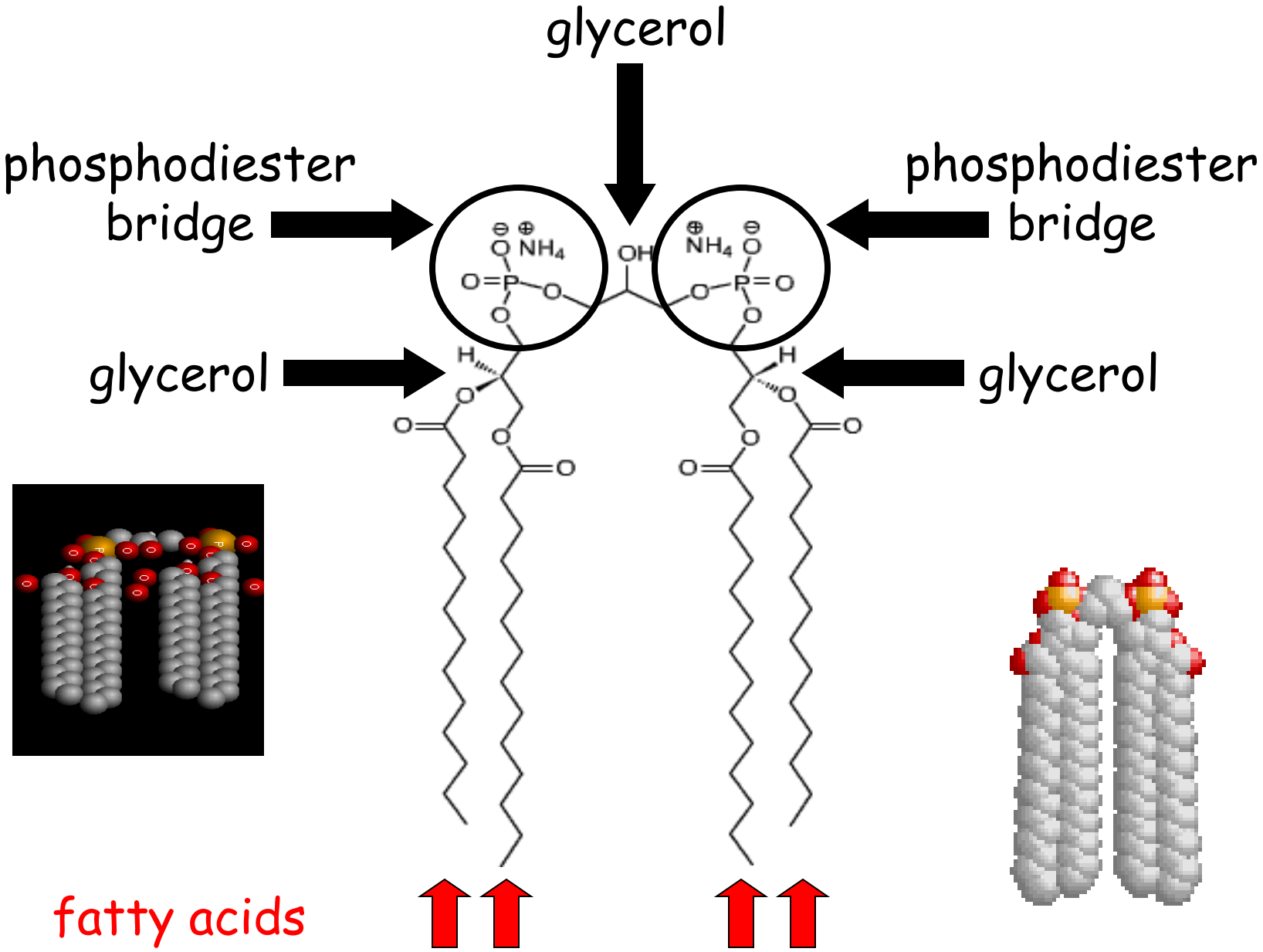
Research - for a healthy future.

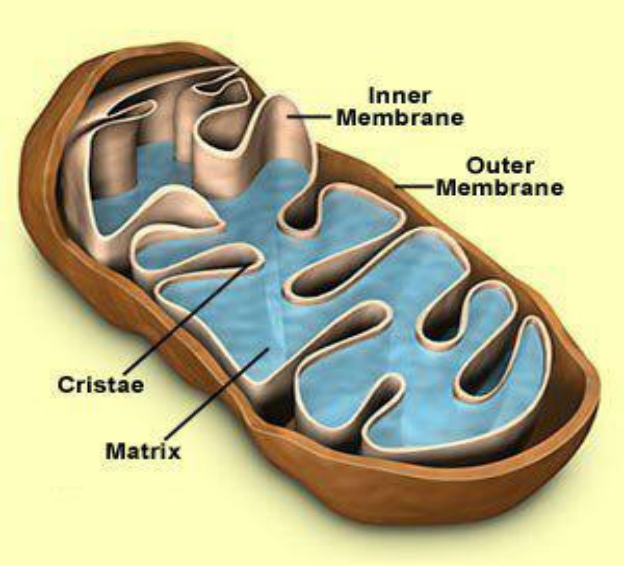

Winnipeg

Synopsis

- Brief Introduction to cardiolipin
- Role of Stomatin-like protein 2 in altering cardiolipin and mitochondrial function in T-cells
- Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling

Cardiolipin – *a unique phospholipid!*





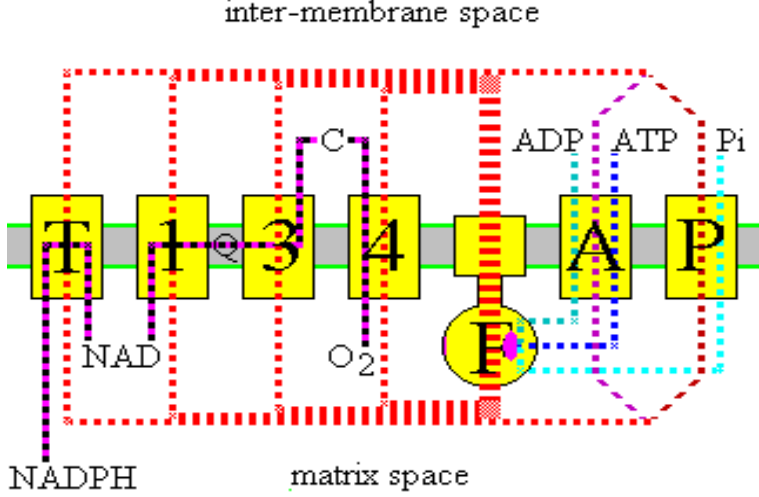
Cardiolipin

1. Major mitochondrial membrane phospholipid

- comprises 7-16% of the entire phospholipid mass of the cell depending upon the tissue

- 21% of phospholipid mass of the inner mitochondrial membrane

- synthesized on mitochondrial inner membrane



Cardiolipin

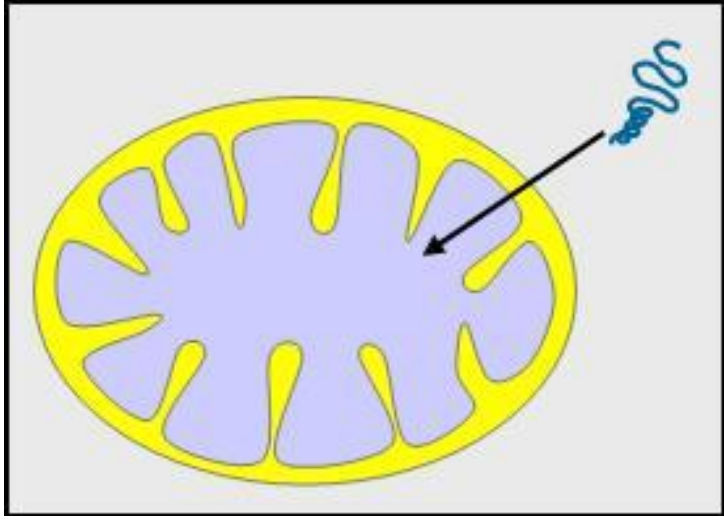
1. Major mitochondrial membrane phospholipid

2. Required for activation of enzymes of electron transport chain/respiratory supercomplex assembly

- both content and fatty acid composition are important

- e.g. delipidated cytochrome oxidase is reconstituted by addition of cardiolipin

- cardiolipin is the “glue” that holds the respiratory chain supercomplexes together



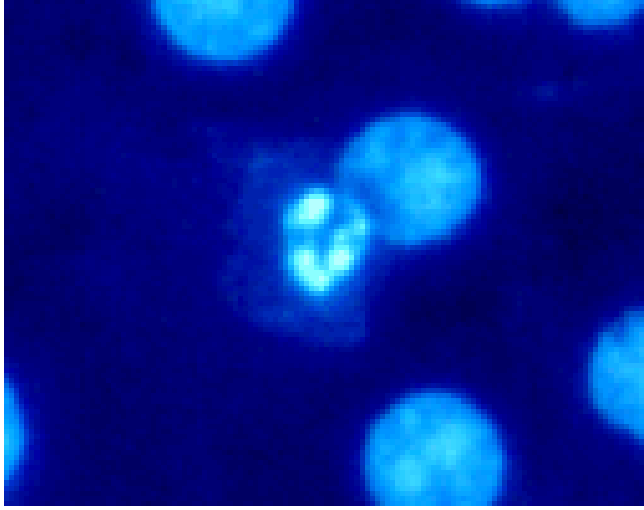
Cardiolipin

1. Major mitochondrial membrane phospholipid

2. Required for activation of enzymes of electron transport chain

3. Role in protein and lipid import into mitochondria

- CDP-DG, phosphatidylserine, malate dehydrogenase, ornithine carbamyltransferase precursor proteins



Cardiolipin

1. Major mitochondrial membrane phospholipid

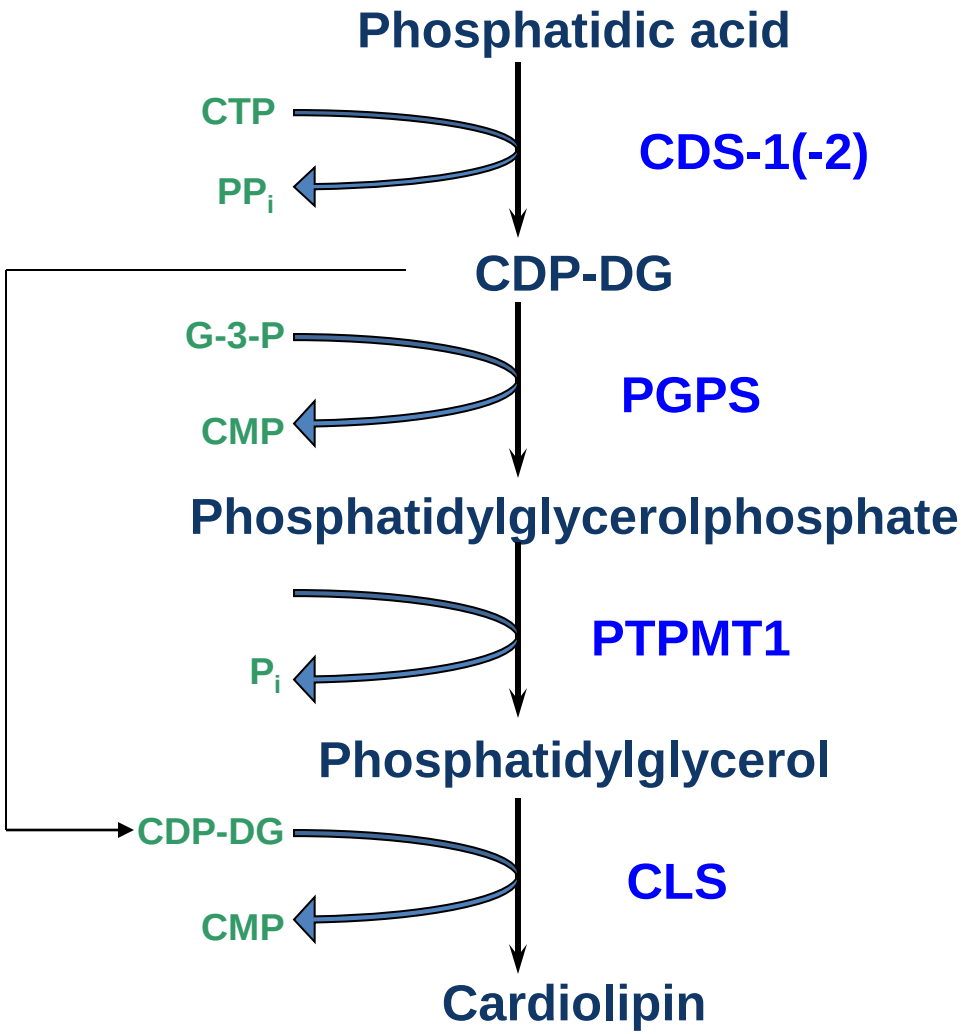
2. Required for activation of enzymes of electron transport chain

3. Role in protein and lipid import into mitochondria

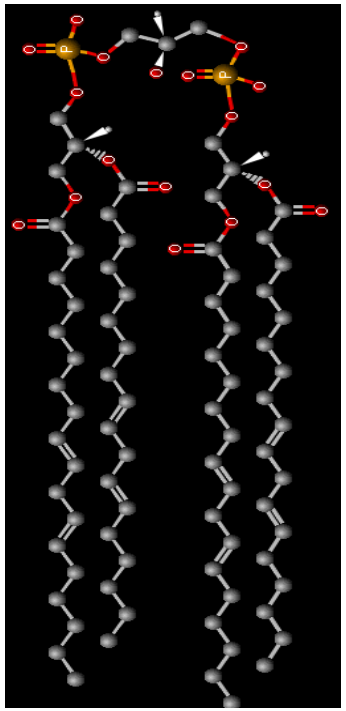
4. Regulator of apoptosis

- required for caspase-8 activation
- CL/MLCL binds to Bid/t-Bid, regulated by PLS3
> cytochrome c release from mitochondria

Mammalian Cardiolipin *de novo* Biosynthesis: "The CDP-DG Pathway"

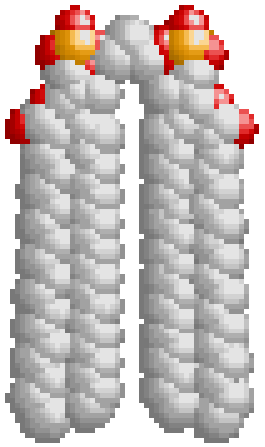


Human and Mammalian Cardiac Cardiolipin is Highly Enriched with 18:2 (Tetralinoleoyl-CL or L_4-CL)



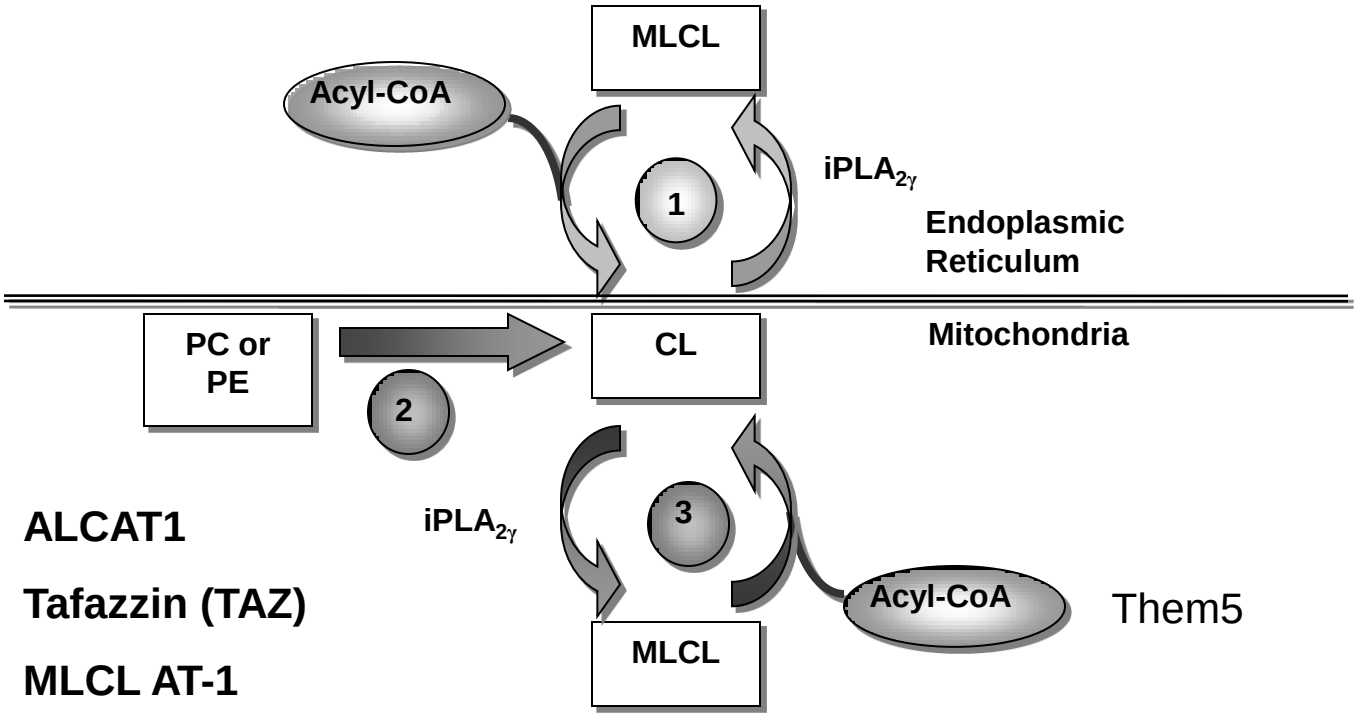
Major Cardiolipin Species

sn-1-*sn*-2	*sn*-2-*sn*-1	%
18:2-18:2	18:2-18:2	80
18:2-18:1	18:2-18:2	12
18:1-18:2	18:2-18:2	



Adapted from: Schlame et al. 2005 Chem. Phys. Lipids 138, 38-49

Cardiolipin Remodeling Pathways



1. **ALCAT1**
2. **Tafazzin (TAZ)**
3. **MLCL AT-1**
4. **Them5?**

Them5

Adapted from: Hauff and Hatch (2006) Prog. Lipid Res. 45, 91-101.

Synopsis

- Brief Introduction to cardiolipin

Role of Stomatin-like protein 2 in altering cardiolipin and mitochondrial function in T-cells

Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling

Stomatin-like protein 2 (SLP-2)

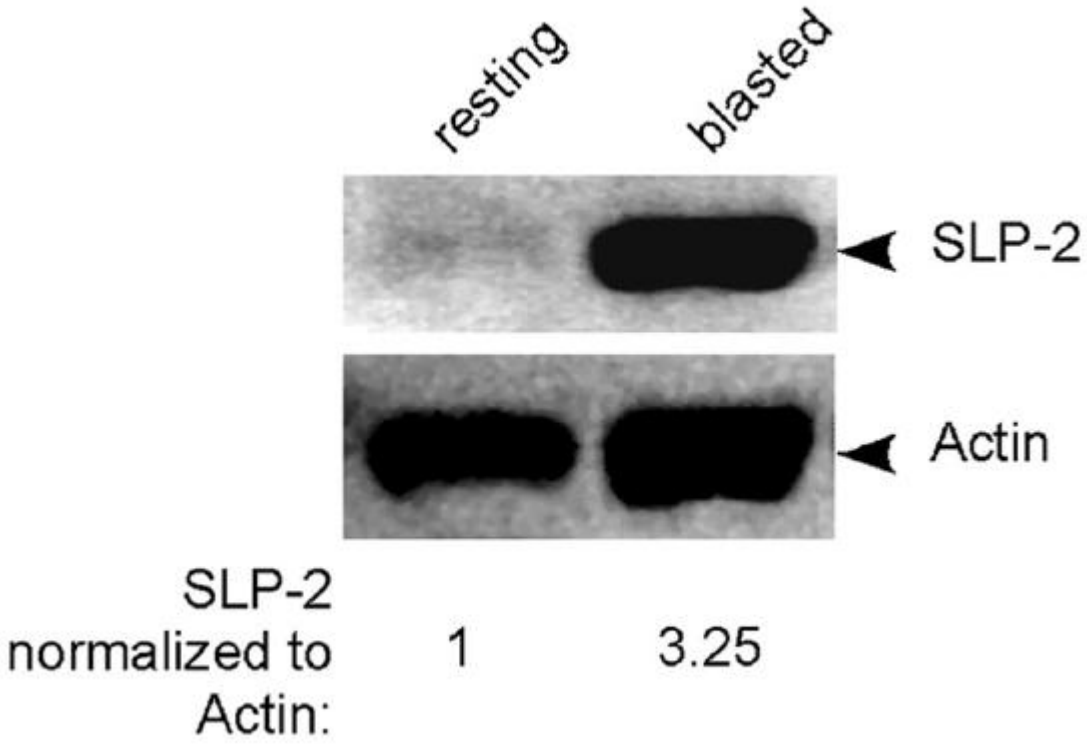
- stomatin – prohibitin – flotillin – HflC/K (SPFH) superfamily

- highly conserved family of proteins that mediate interactions with cell membranes

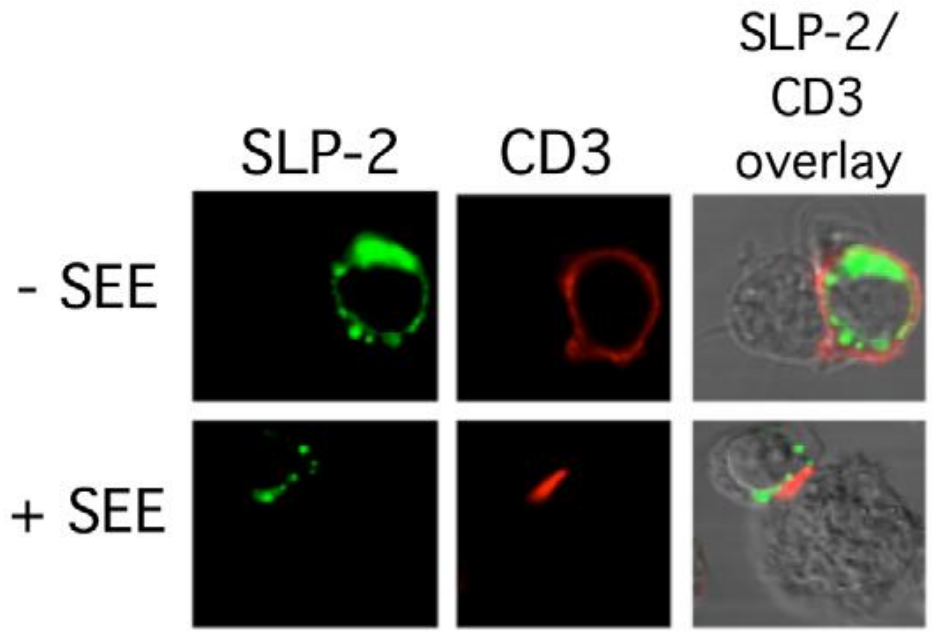
- upregulated in many cancers

- modulates MMP and ATP production

SLP-2 is Upregulated Upon Peripheral Blood Mononuclear Cell Activation



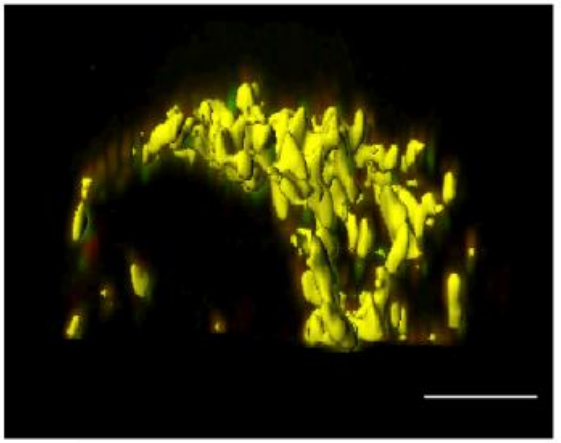
SLP-2 Localizes to the Immunological Synapse Upon Jurkat T-Cell Activation

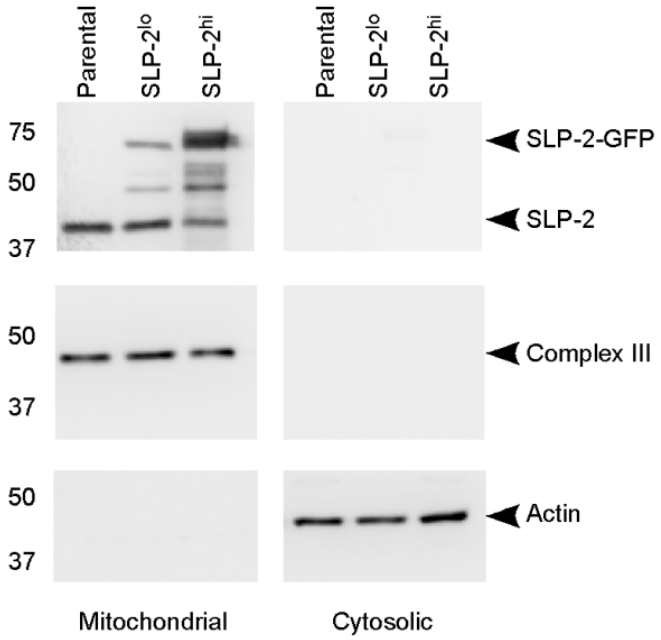


SLP-2 polarizes to the immunological synapse during T cell activation (*Christie et al 2012 PloS One*)

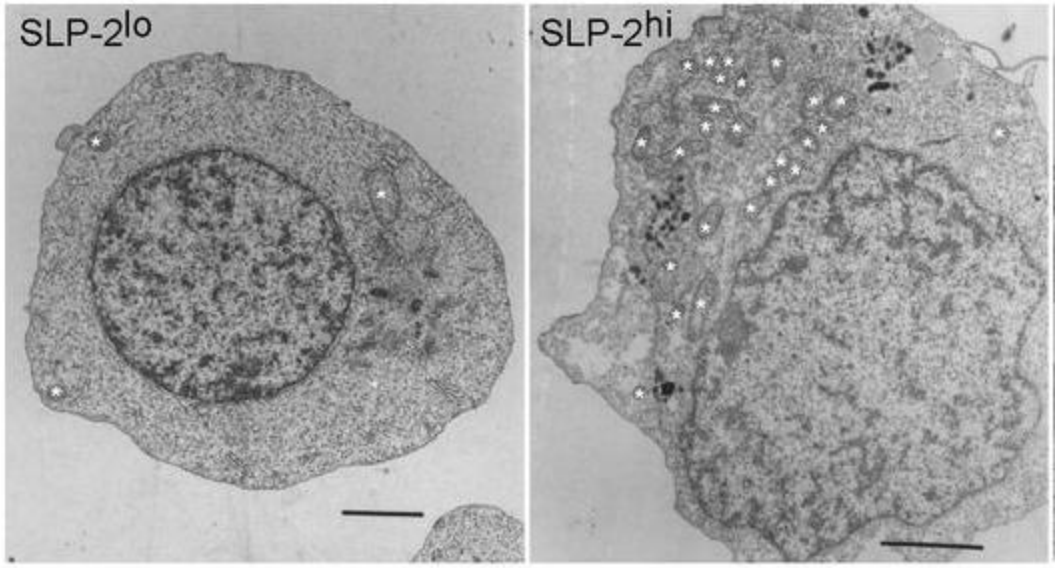
SLP-2 expression increases effector responses whereas down-regulation of SLP-2 correlates with loss of TCR signaling and activation (*Kirchhof et al. 2008 J. Immunol*.)

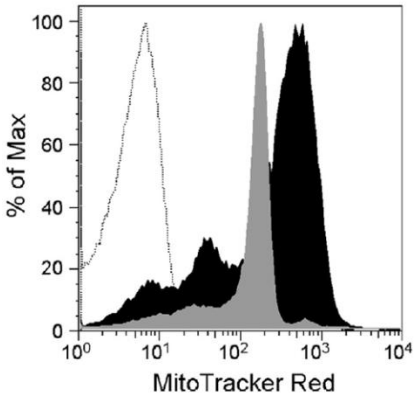
SLP-2 is Localized Primarily to Mitochondria in Jurkat T-Cells

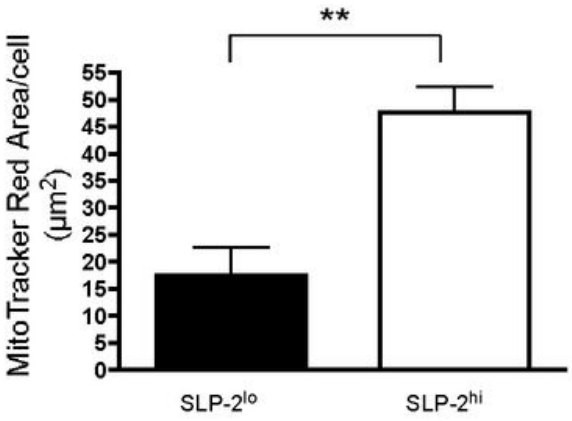


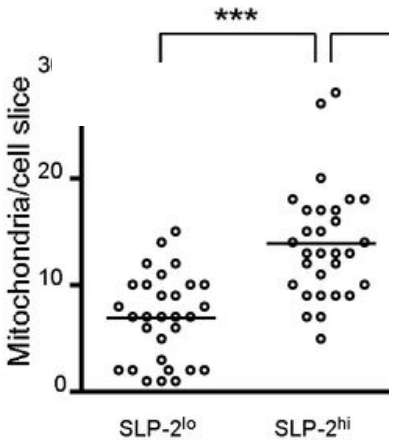


Expression of SLP-2 in T-cells Increases Number of Metabolically Active Mitochondria

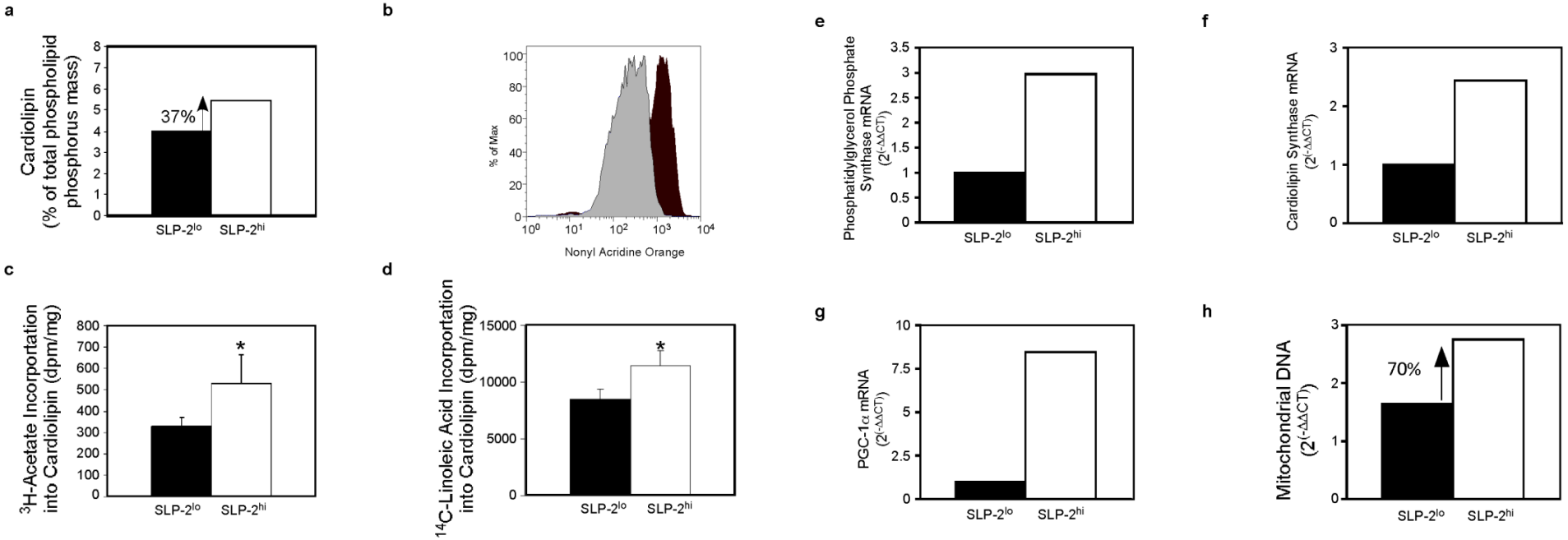




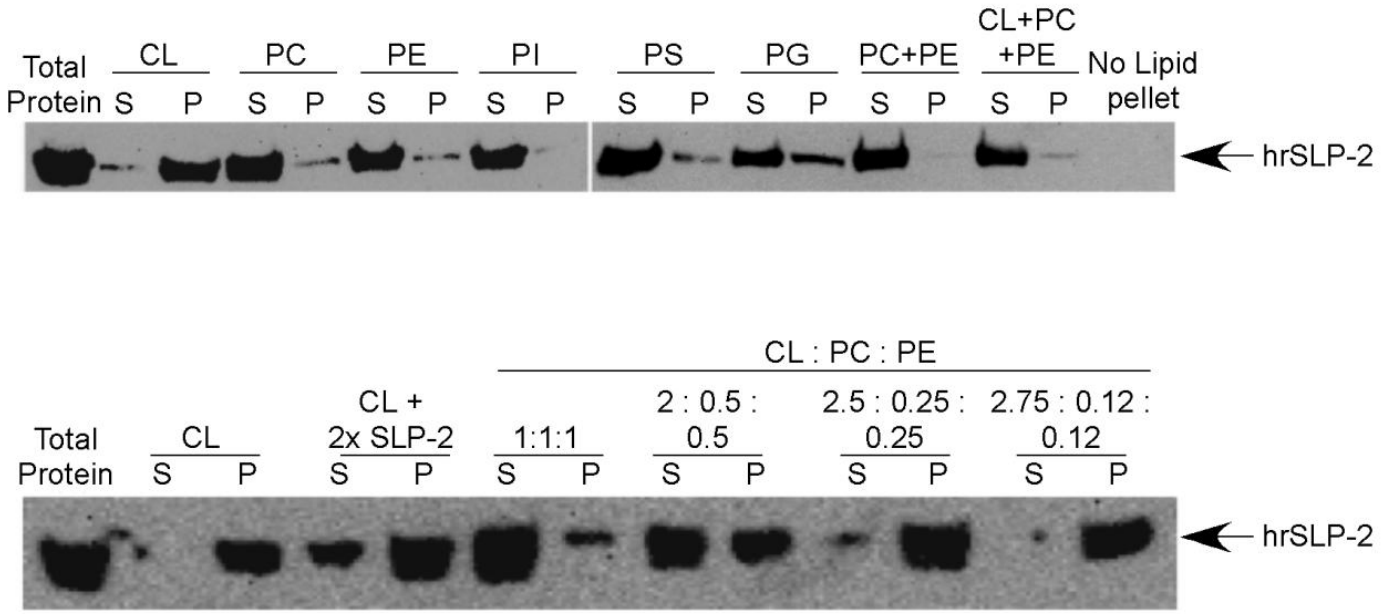




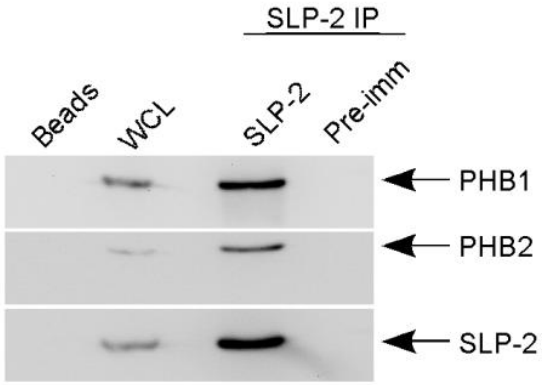
SLP-2 Expression in T-Cells Stimulates Cardiolipin and Mitochondrial Biogenesis

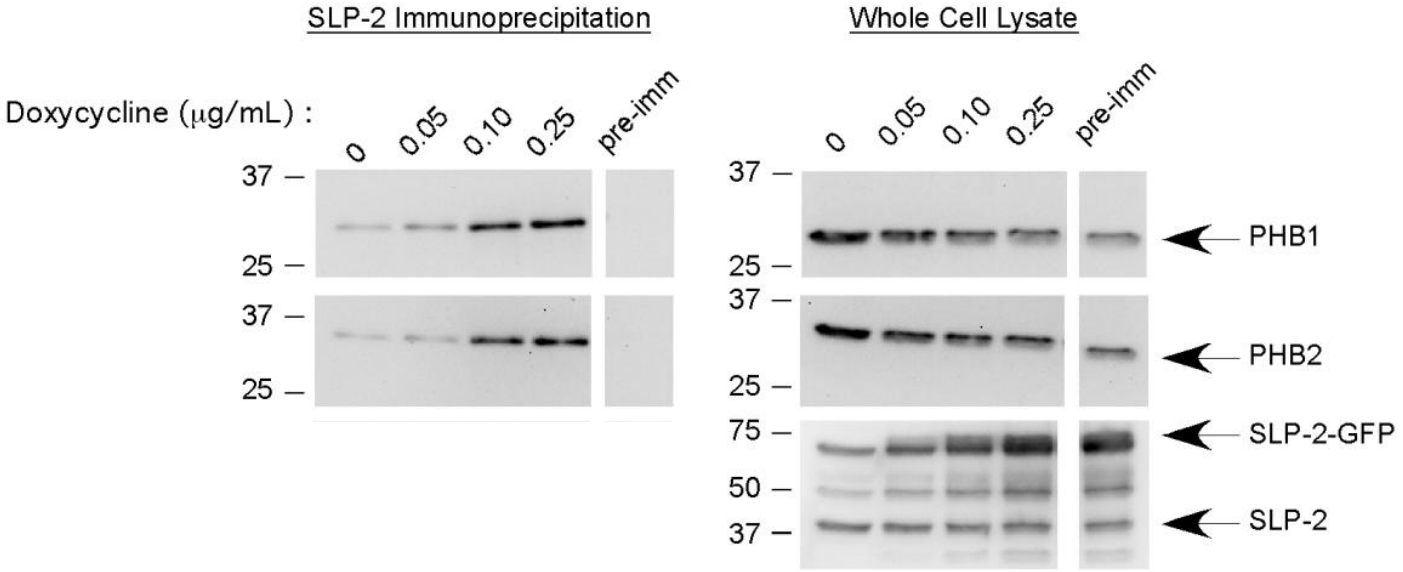


SLP-2 Binds to Cardiolipin

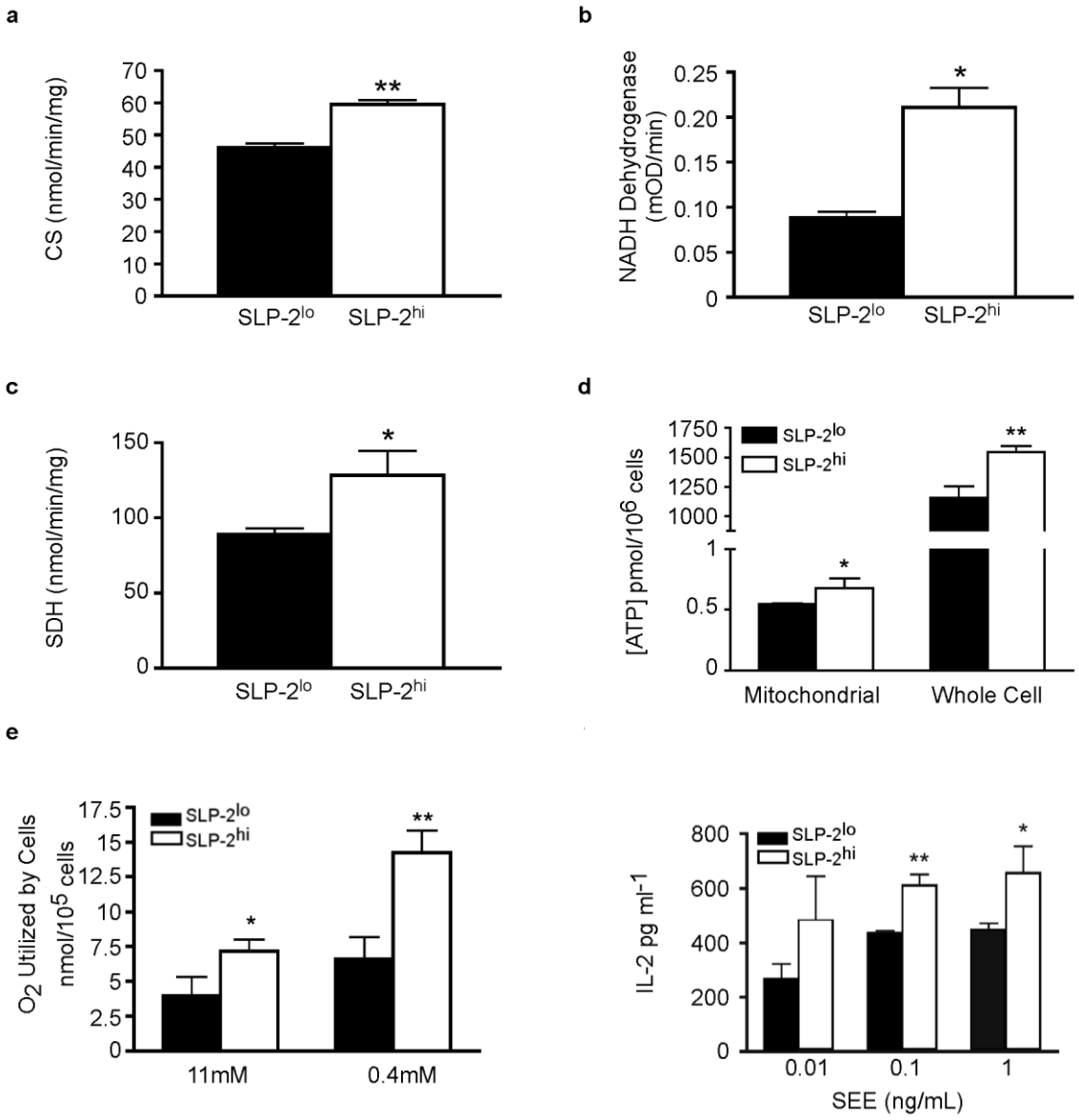


SLP-2 Interacts with Prohibitins

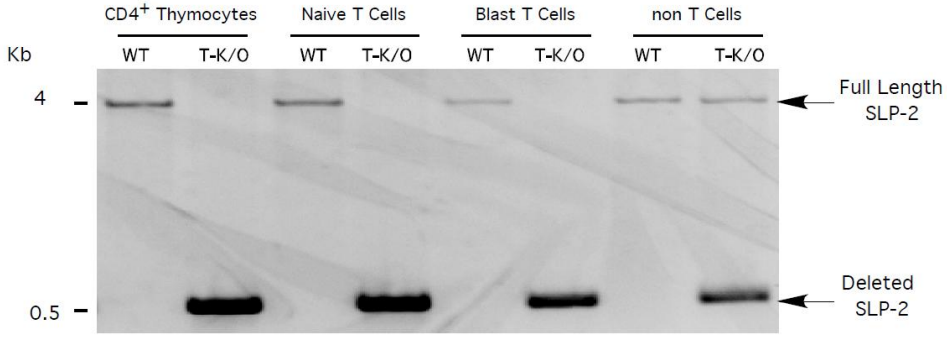


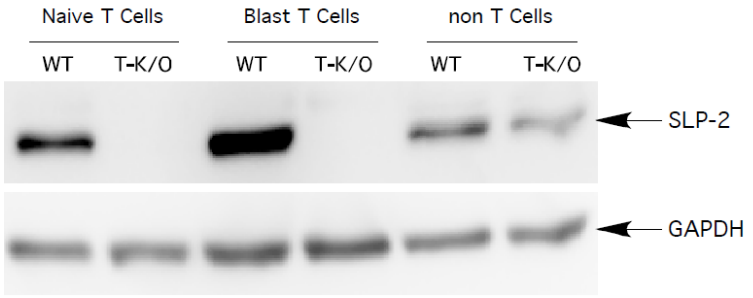


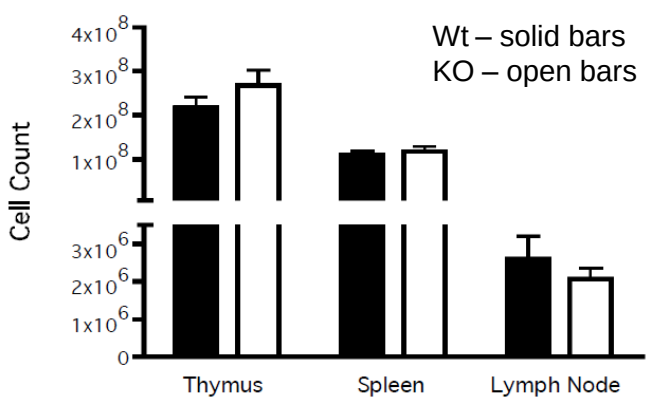
SLP-2 Expression in T-Cells Stimulates Mitochondrial Activity and Interleukin-2 Secretion in Response to T-Cell Stimulation

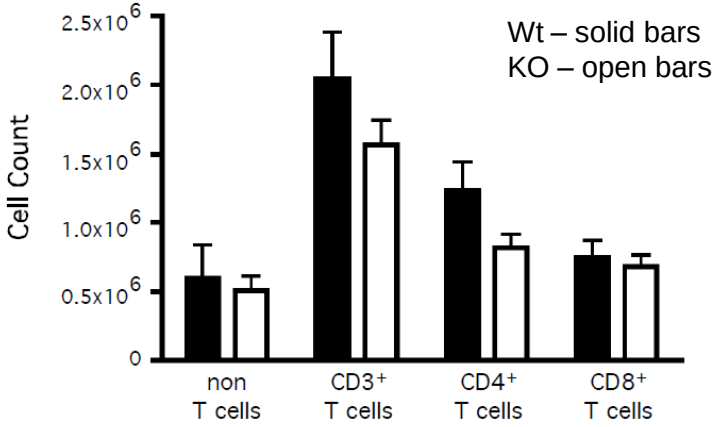


T-Cell Specific SLP-2 Knock Out Mice Exhibit Near Normal T-cell Number

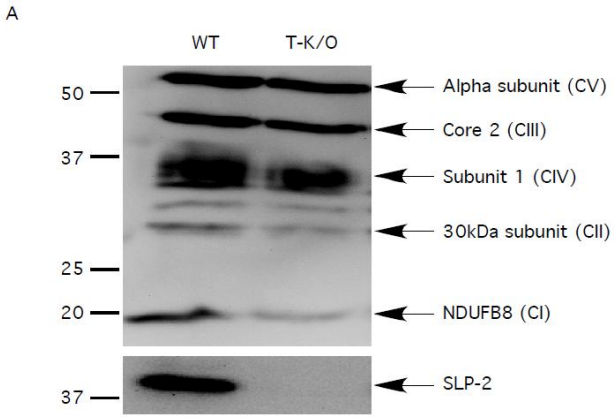


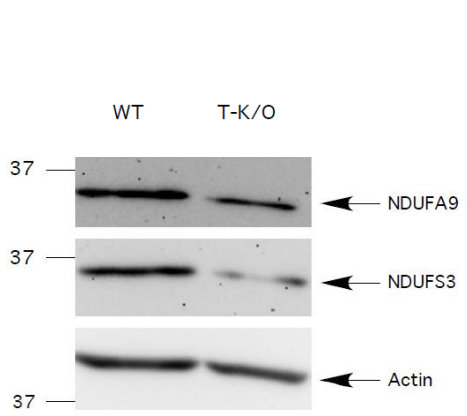


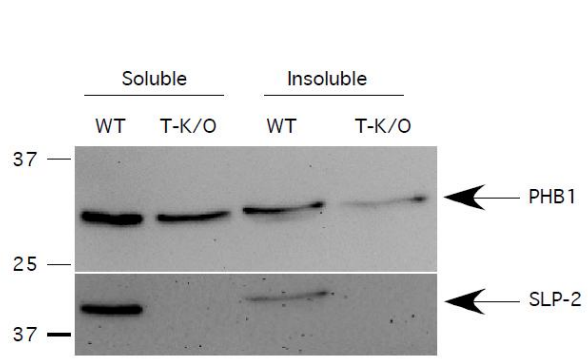


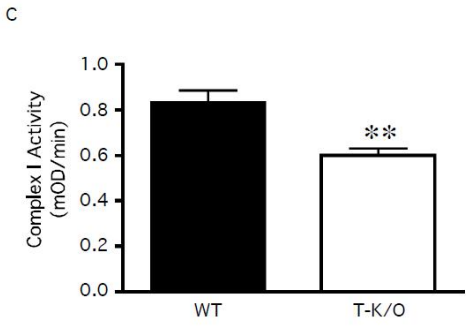


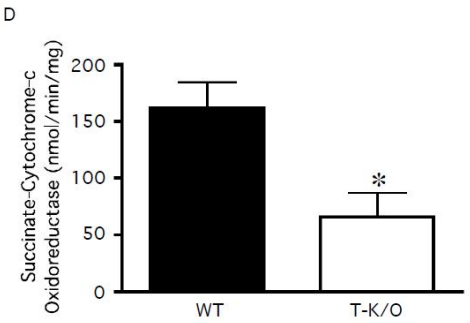
T-Cells Deficient in SLP-2 Exhibit Reduced Mitochondrial Complex Protein/Activities, insoluble Prohibitin-1 and Cardiolipin

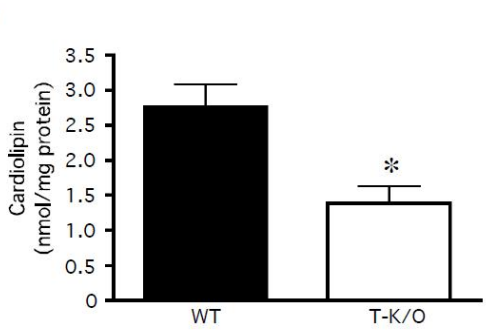




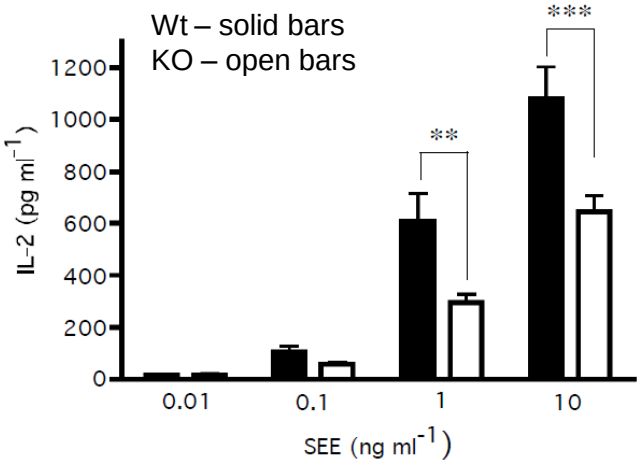


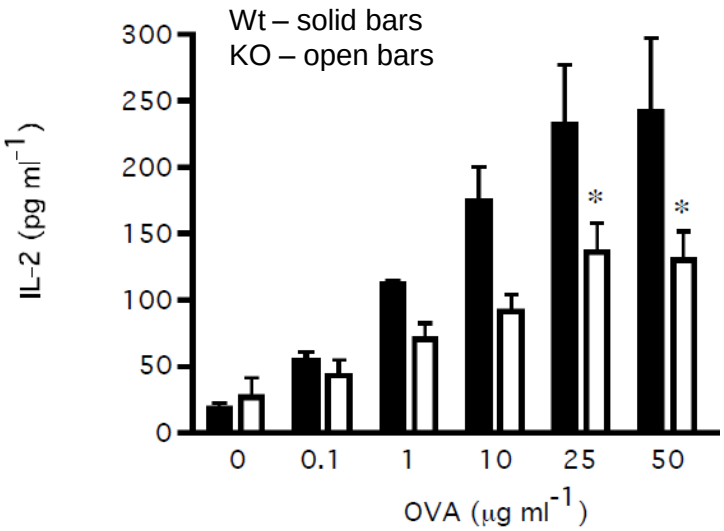


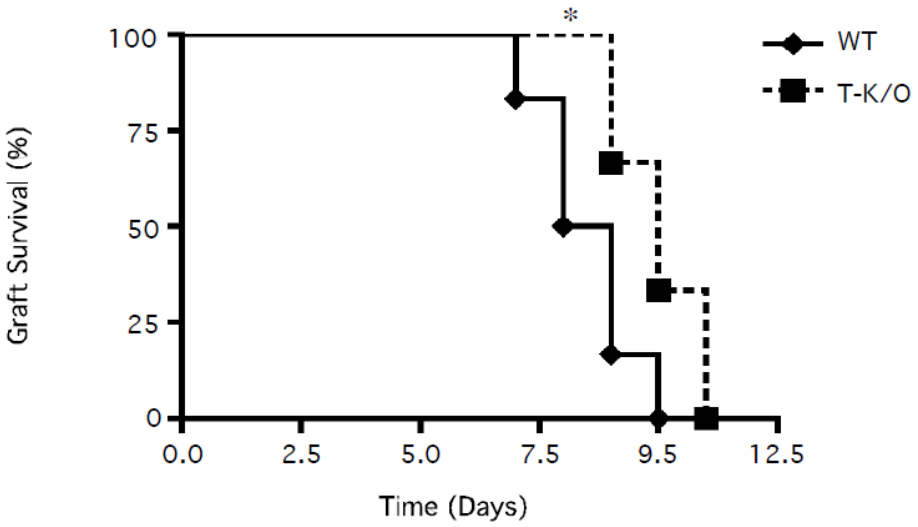




T-Cell Specific SLP-2 Knock Out Reduces *in vivo* Interleukin-2 Secretion in Response to T-Cell Stimulation and Delays Cardiac Allograft rejection







June 2012 Vol 32, Issue 11

Summary I:

SLP-2 expression in T-cells stimulates cardiolipin and mitochondria biogenesis/activity and T-cell ability to respond to stimuli

Christie et al., Mol. Cell. Biol. 2011, 31:3845-3856.

SLP-2 KO in T-cells reduces cardiolipin in PHB complex fractions, mitochondrial metabolism and T-cell ability to respond to stimuli linking cardiolipin to T-cell function

Christie et al., J. Immunol. 2012 (in revision)

SLP-2 functions to recruit prohibitins to cardiolipin-enriched microdomains in which ETC complexes are optimally assembled

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- Brief Introduction to cardiolipin
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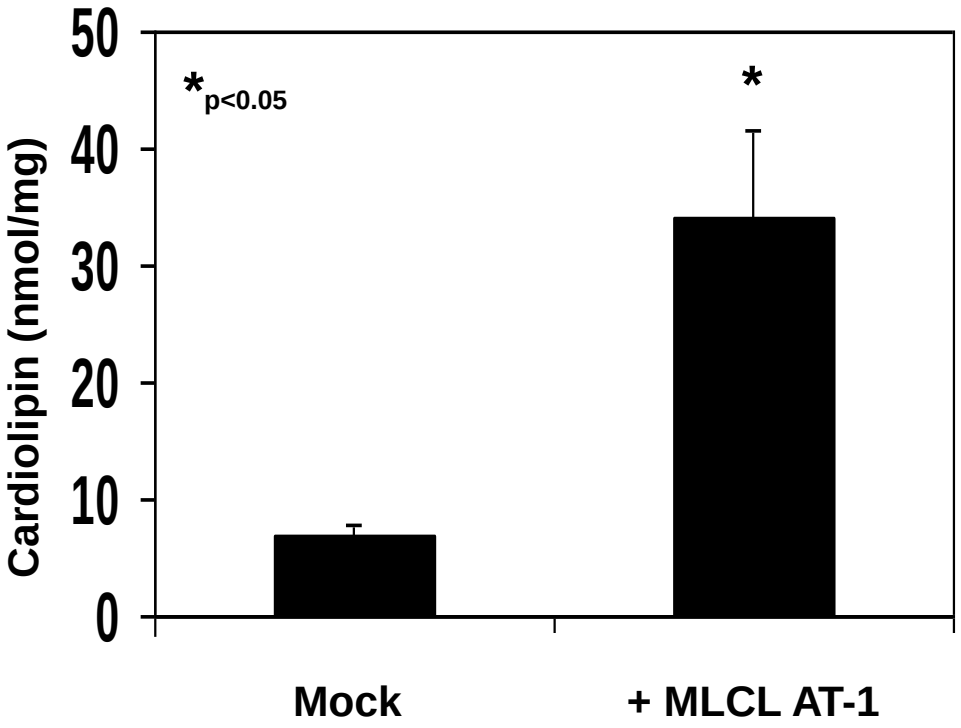
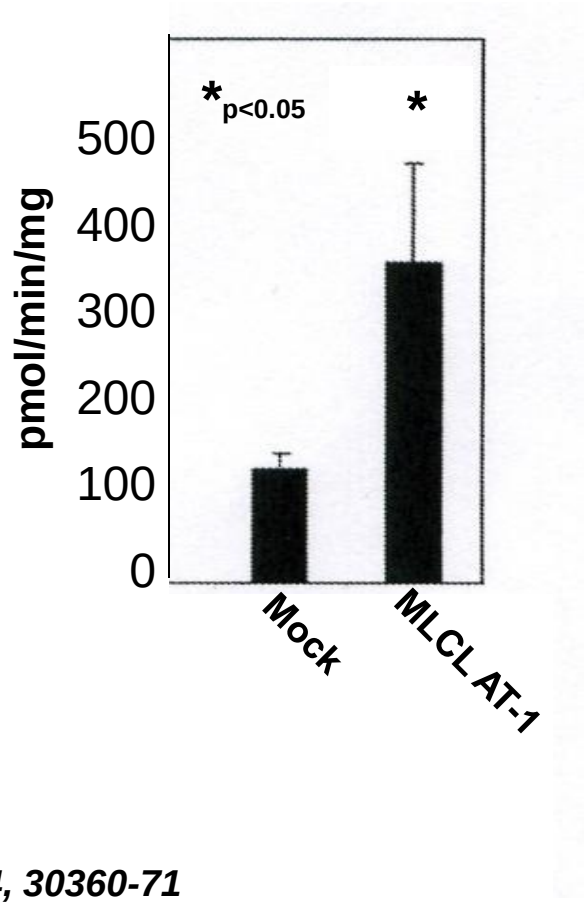
- Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling

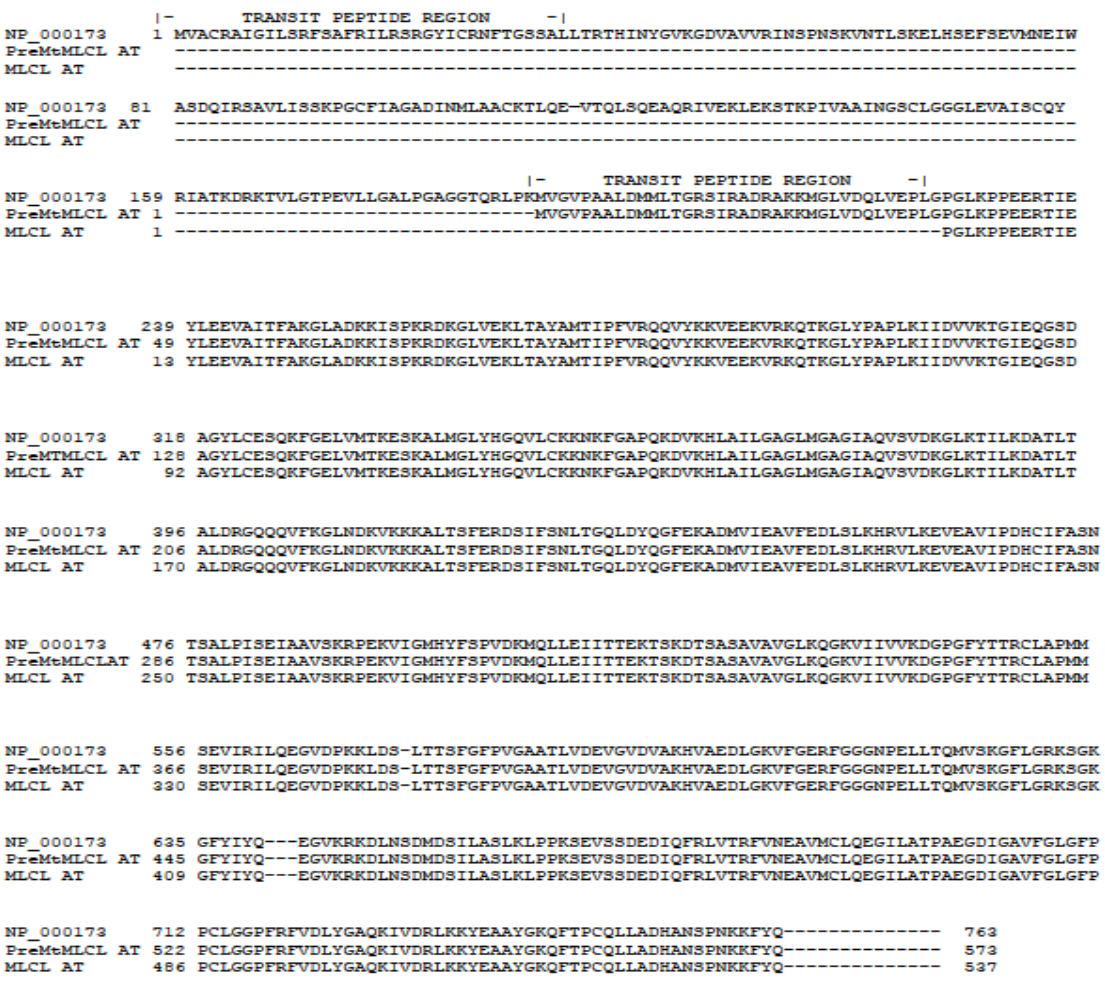
Expression of human MLCL AT-1 in Barth Syndrome lymphoblasts (patient ΔTAZ1) increases cardiolipin and mitochondrial Complex II + III activity





Taylor and Hatch (2009) J. Biol. Chem. 284, 30360-71

Human MLCL AT-1 is a shortened version (59 kDa) of the α subunit of Human Trifunctional Protein (74 kDa)



Human MLCL AT-1 is a shortened version (59 kDa) of the α subunit of Human Trifunctional Protein (74 kDa)

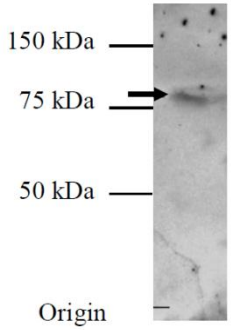
Trifunctional protein

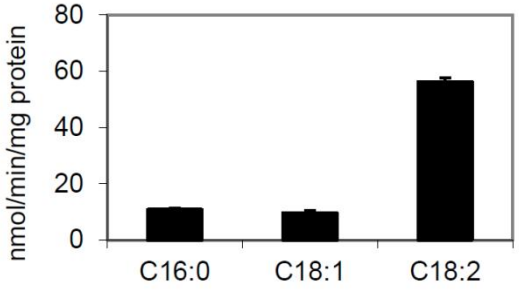
- multifunctional, membrane-bound beta-oxidation enzyme protein catalyzing three enzyme activities:
  - long-chain enoyl-Coenzyme A hydratase
  - long-chain 3-hydroxyacyl-Coenzyme A-dehydrogenase
  - long-chain 3-oxoacyl-Coenzyme A thiolase

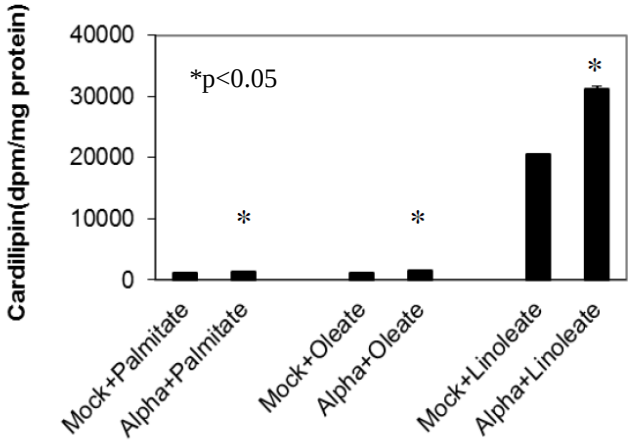
- heterocomplex of two subunits, 4 alpha and 4 beta

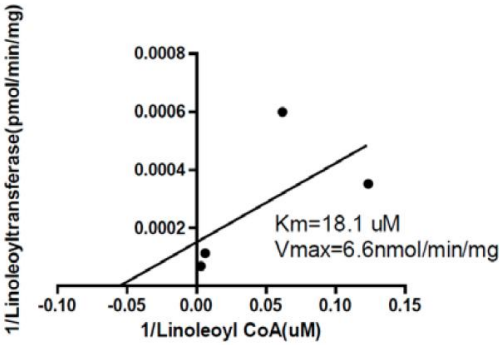
```
                |-      TRANSIT PEPTIDE REGION     -|
NP_000173     1 MVACRAIGILSRFSAFRILRSRGYICRNFTGSSALLTRTHINYGVKGDVAVVRINSPNSKVNTLSKELHSEFSEVMNEIW
PreMtMLCL AT    ------------------------------------------------------------------------------
MLCL AT         ------------------------------------------------------------------------------

NP_000173    81 ASDQIRSAVLISSKPGCFIAGADINMLAACKTLQE-VTQLSQEAQRIVEKLEKSTKPIVAAINGSCLGGGLEVAISCQY
PreMtMLCL AT    ------------------------------------------------------------------------------
MLCL AT         ------------------------------------------------------------------------------

                                                |-     TRANSIT PEPTIDE REGION     -|
NP_000173   159 RIATKDRKTVLGTPEVLLGALPGAGGTQRLPKMVGVPAALDMMLTGRSIRADRAKKMGLVDQLVEPLGPGLKPPEERTIE
PreMtMLCL AT  1 -------------------------------MVGVPAALDMMLTGRSIRADRAKKMGLVDQLVEPLGPGLKPPEERTIE
MLCL AT       1 ------------------------------------------------------------PGLKPPEERTIE
```

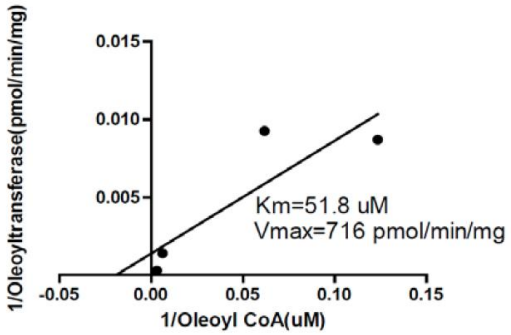
Human recombinent α subunit of Trifunctional Protein exhibits MLCL AT *in vitro* activity and stimulates [1-^{14}C]fatty acid incorporation into cardiolipin in Hela cells

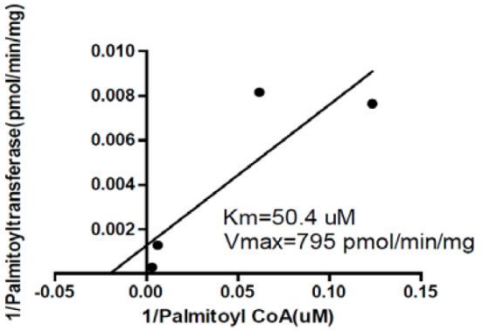












MLCL AT-1 is likely a splice variant of the α subunit of Trifunctional Protein

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                |-      TRANSIT PEPTIDE REGION     -|
NP_000173     1 MVACRAIGILSRFSAFRILRSRGYICRNFTGSSALLTRTHINYGVKGDVAVVRINSPNSKVNTLSKELHSEFSEVMNEIW
PreMtMLCL AT    ----------------------------------------------------------------------------------
MLCL AT         ----------------------------------------------------------------------------------

NP_000173    81 ASDQIRSAVLISSKPGCFIAGADINMLAACKTLQE-VTQLSQEAQRIVEKLEKSTKPIVAAINGSCLGGGLEVAISCQY
PreMtMLCL AT    ----------------------------------------------------------------------------------
MLCL AT         ----------------------------------------------------------------------------------

                                                |-     TRANSIT PEPTIDE REGION     -|
NP_000173   159 RIATKDRKTVLGTPEVLLGALPGAGGTQRLPKMVGVPAALDMMLTGRSIRADRAKKMGLVDQLVEPLGPGLKPPEERTIE
PreMtMLCL AT 1 --------------------------------MVGVPAALDMMLTGRSIRADRAKKMGLVDQLVEPLGPGLKPPEERTIE
MLCL AT      1 ------------------------------------------------------------PGLKPPEERTIE
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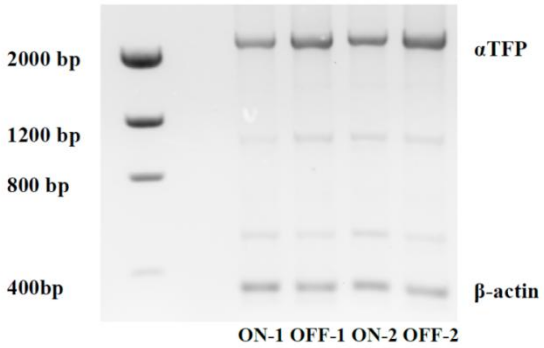
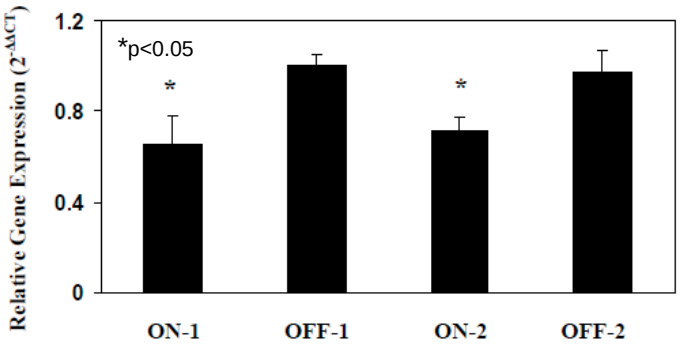
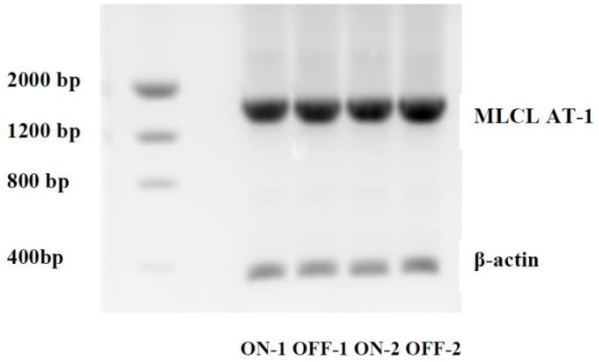
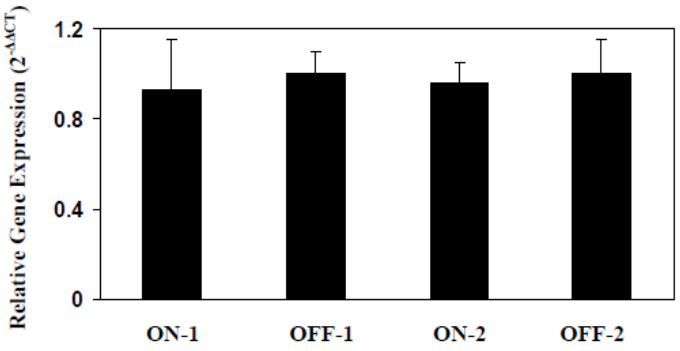
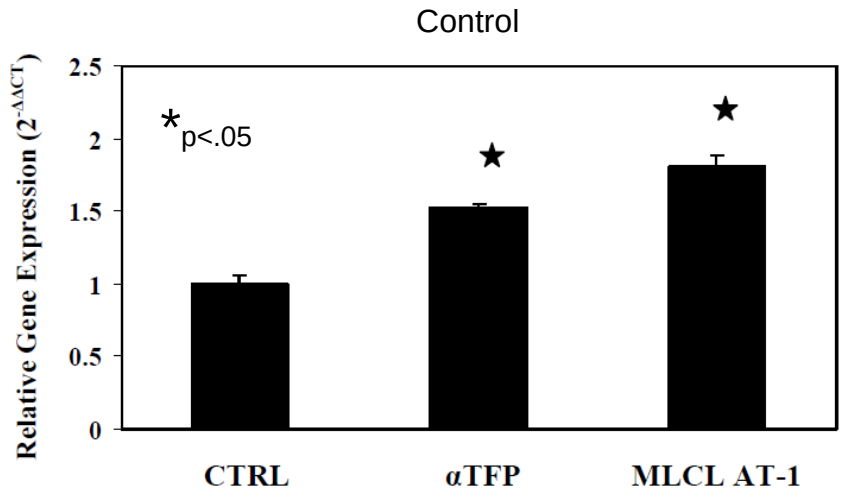




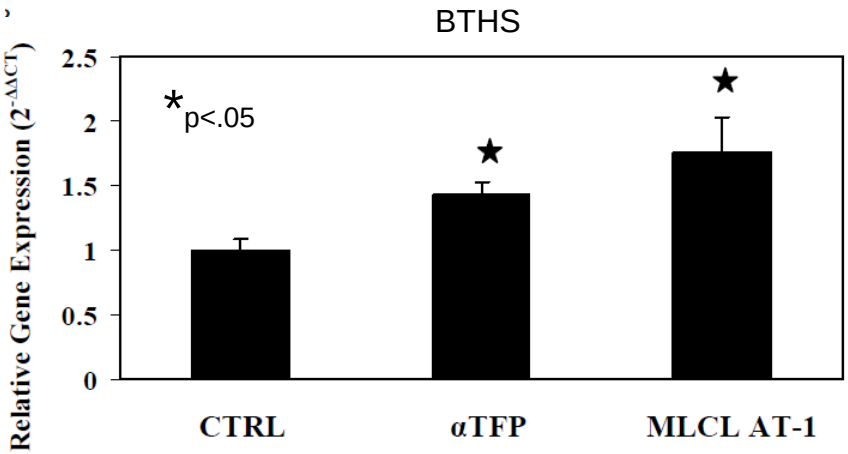


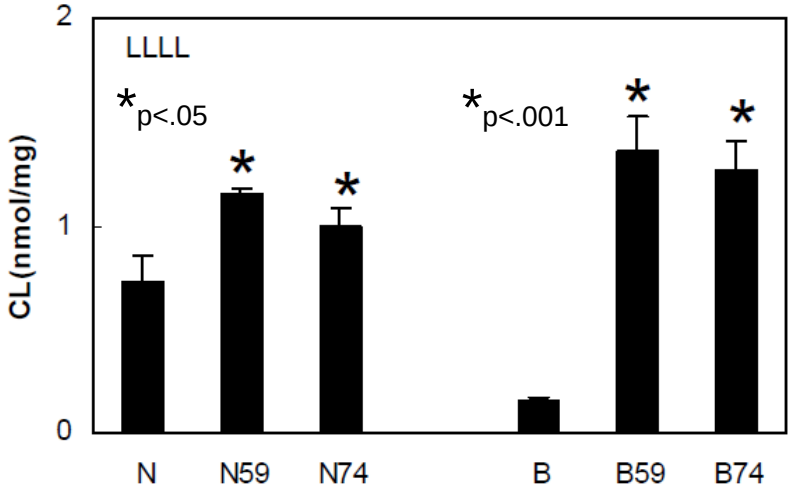


Expression of MLCL AT-1 or α subunit of Trifunctional Protein in Normal or BTHS Lymphoblasts increases L_4-cardiolipin

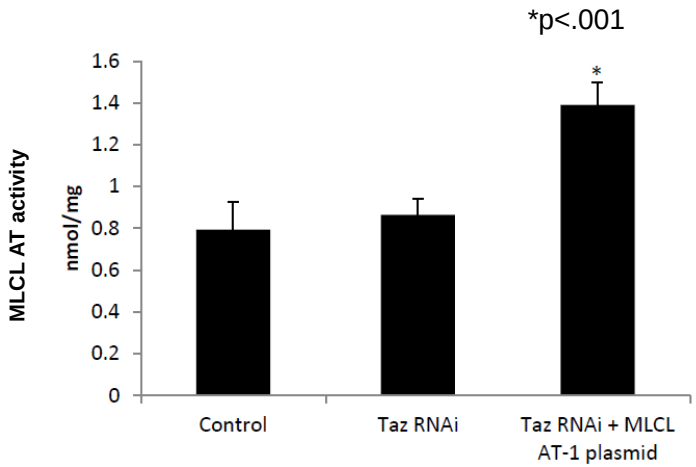


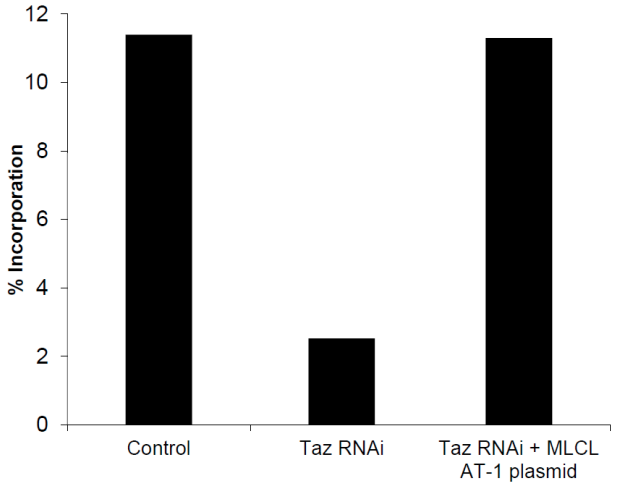
Lysophospholipid	Enzyme activity (pmol/min/mg protein)
Monolysocardiolipin	56.3 ± 1.3
Lysophosphatidic acid	ND
Lysophosphatidylglycerol	ND
Lysophosphatidylcholine	ND
Lysophosphatidylethanolamine	ND





MLCL AT enzyme activity is not increased by knock down of TAZ in normal human lymphoblasts but MLCL AT-1 expression restores [1-^{14}C]linoleate into cardiolipin after TAZ knock down





Summary II:

αTFP exhibits MLCL AT activity and expression of αTFP stimulates cardiolipin remodeling with linoleate and increases L_4-cardiolipin levels in normal and BTHS lymphoblasts linking an enzyme of β-oxidation to cardiolipin remodeling

Taylor et al. Biochem. J. 2012 (submitted)

MLCL AT-1 activity is not increased by *TAZ* knock down indicating that *TAZ* and MLCL AT-1 may not complement each other in cardiolipin remodeling but MLCL AT-1 expression may compensate for loss of *TAZ*

Current Studies:

1. Will expression of MLCL AT-1 or αTFP in Taz knock down mice attenuate development of the cardiac defects?

2. Role of SLP-2 in mitochondrial dysfunction in BTHS

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