Mouse model of human Barth syndrome

Zaza Khuchua, PhD
Cincinnati Children's Research Foundation
Cincinnati, OH, USA

Barry J. Byrne, MD, PhD
University of Florida
Department of Pediatrics
Gainesville, FL, USA

Background

- Barth (BTHS) syndrome is X-linked genetic disorder.
- Common symptoms include:
  - Cardiomyopathy
  - Skeletal myopathy
  - Neutropenia
  - 3-methylglutaconic aciduria
- BTHS caused by mutations in tafazzin (taz) gene.
- Taz gene is located on Xq28.
- Taz encodes mitochondrial acyltransferase.
- Mutations in taz gene cause cardiolipin deficiency.
- Cardiolipin is mitochondrial phospholipid and constitutes about 20% of inner mitochondrial membrane.

MODELS OF TAFAZZIN DEFICIENCY

- Yeast;
- Drosophila;
- Zebrafish;
- Various cell cultures.

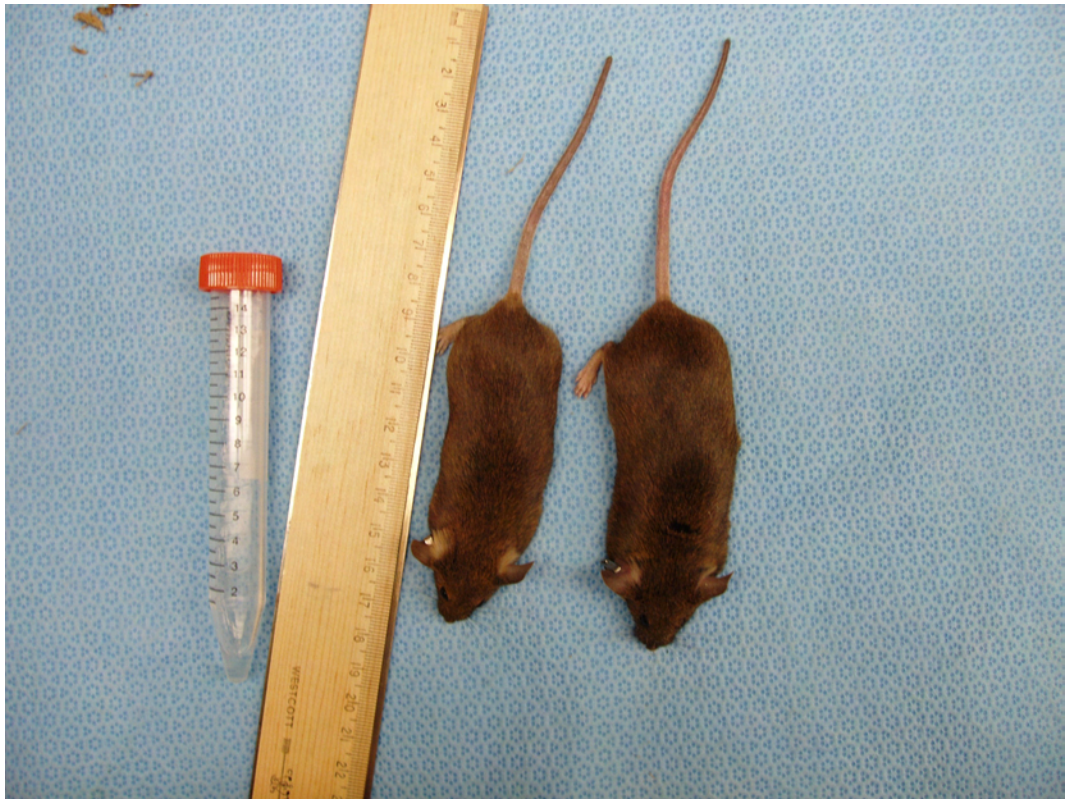
Urgent need for mouse model of Barth syndrome

- Several “floxed” alleles of taz have been generated in mouse ES cells;
- Problems lay with germline transmission of “floxed” taz allele.

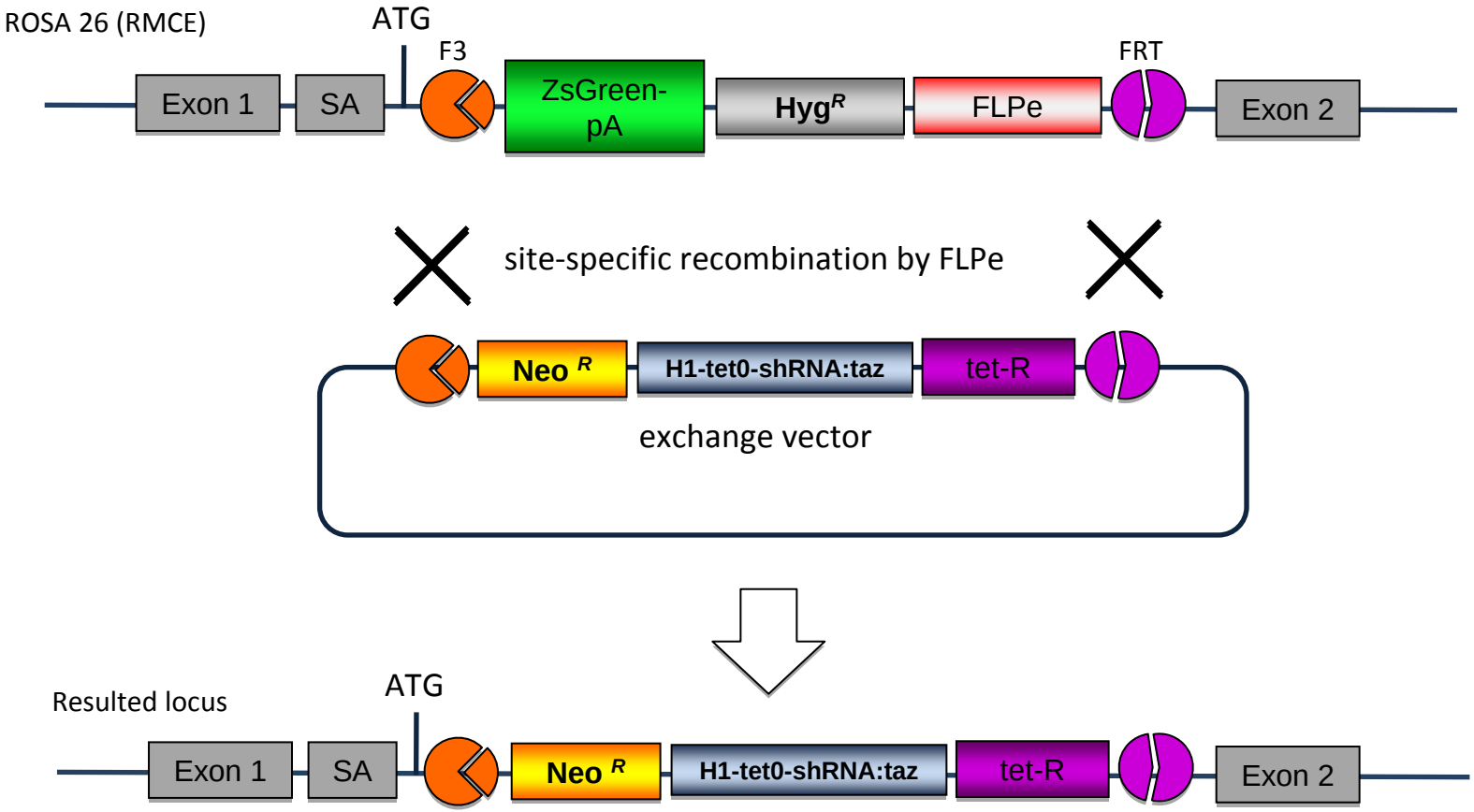
Tafazzin tet-on shRNA transgenic mice were generated by TaconicArtemis in 2009.

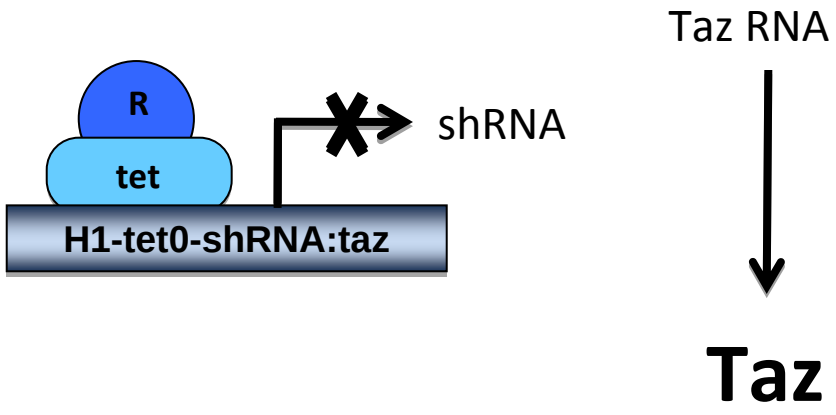
Work was initiated and funded by Barth Syndrome Foundation (www.barthsyndrome.org).

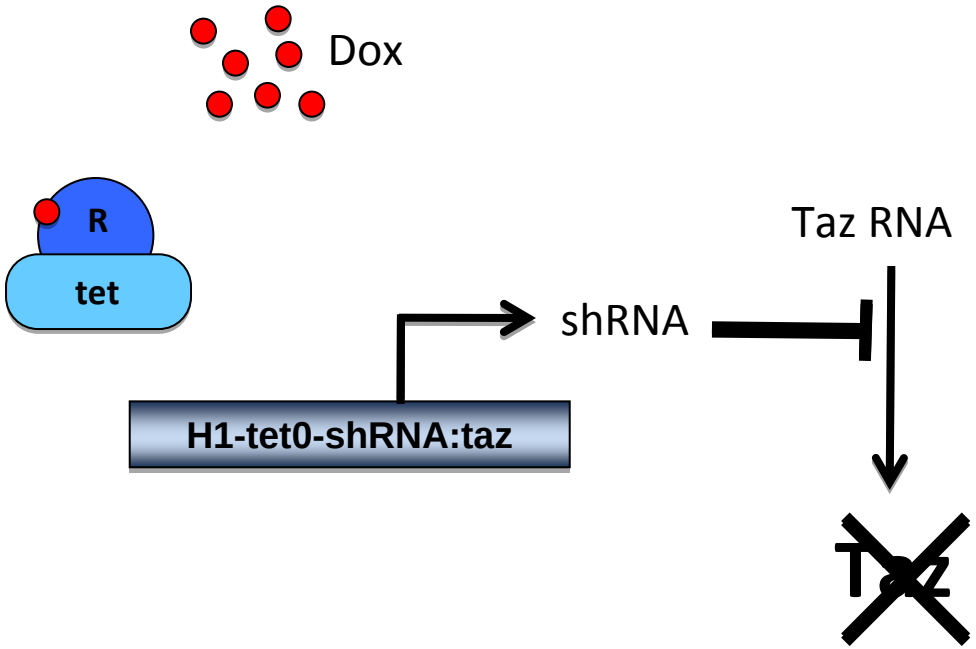
Mice available from Jackson Laboratory:
Gt(ROSA)26Sor<tm37(H1/tetO-RNAi:Taz)Arte



RECOMBINASE MEDIATED CASSETE EXCHANGE




R
tet
H1-tet0-shRNA:taz
shRNA
Taz RNA
Taz

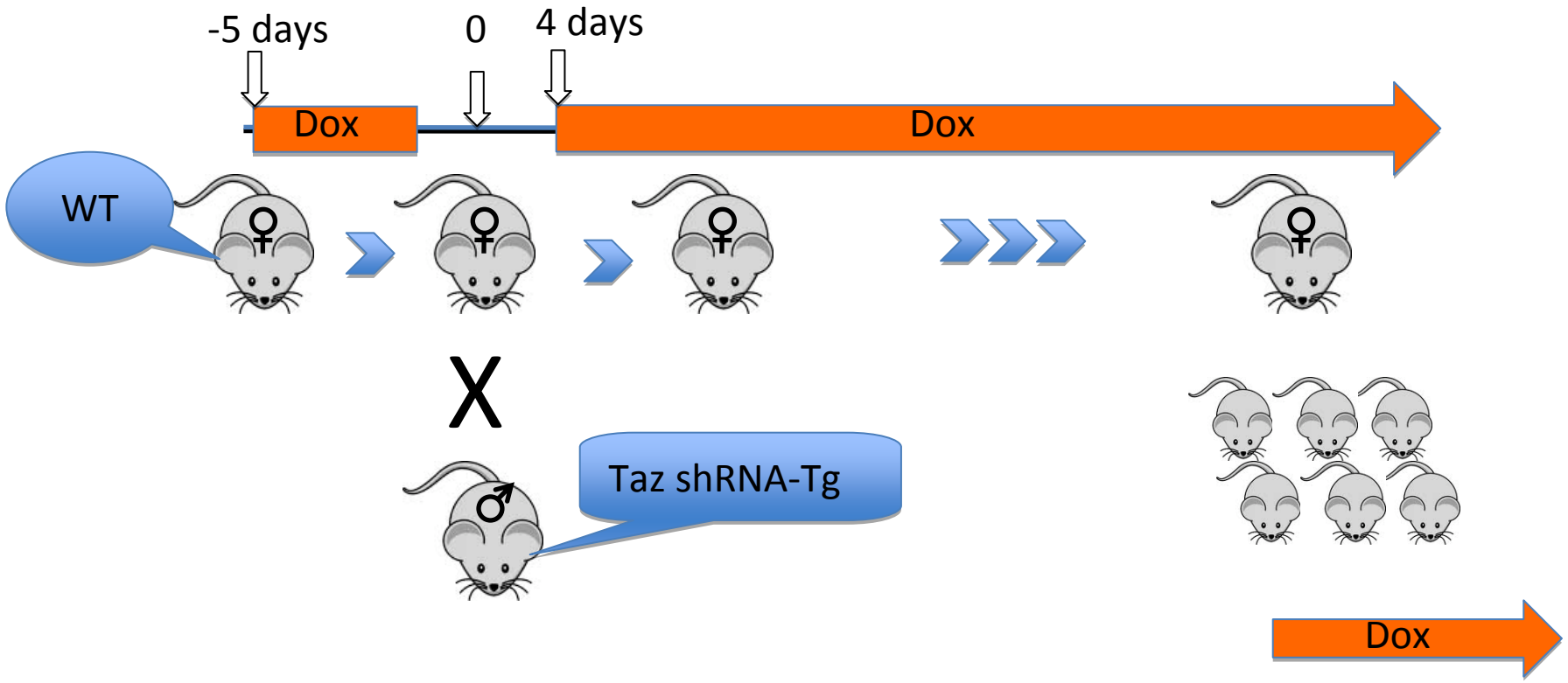

Dox
R
tet
Taz RNA
shRNA
H1-tet0-shRNA:taz

Important Questions:

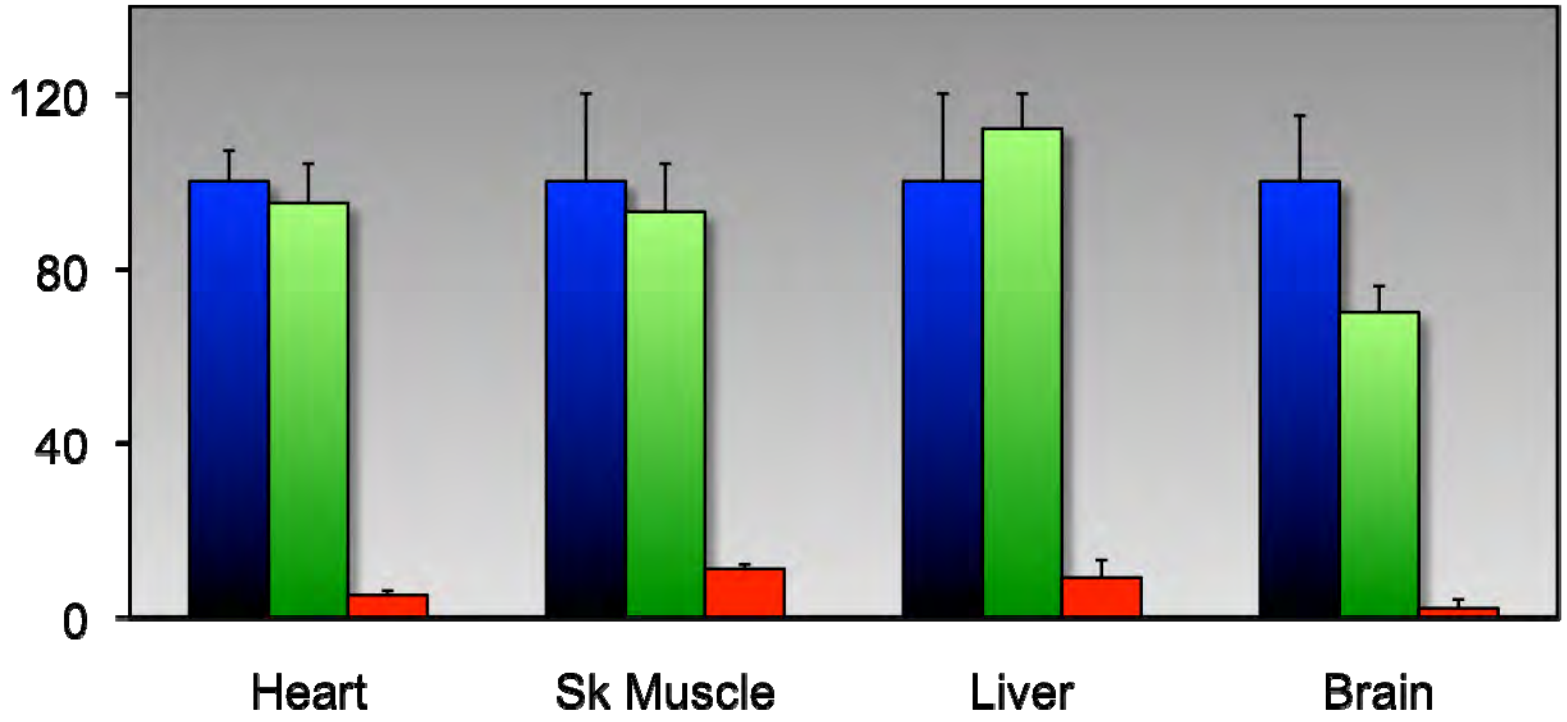
1. How efficient is shRNA mediated silencing in different tissues?
2. How tight is regulation by Dox?
3. What are side-effects of chronic Dox administration?
4. Is it reversible?

Induction of Taz knockdown

Doxycycline was administered with rodent chow, formulated by Purina Mills (625 mg / kg)



TAFAZZIN KNOCKDOWN

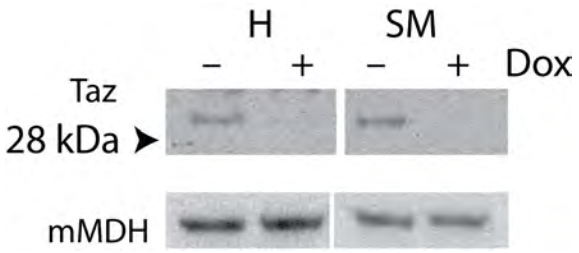


Taz silencing

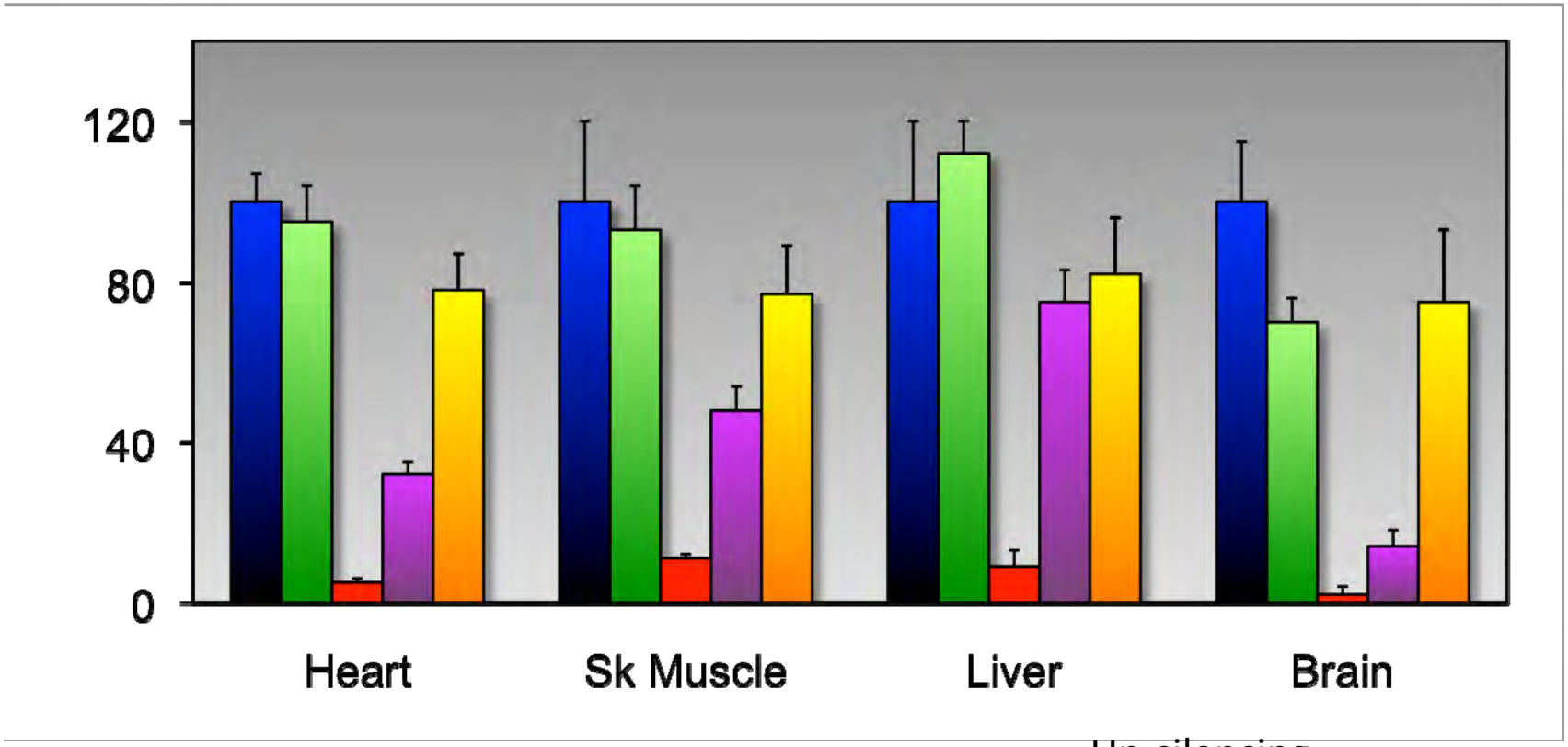
WT
+ Dox

TG
– Dox

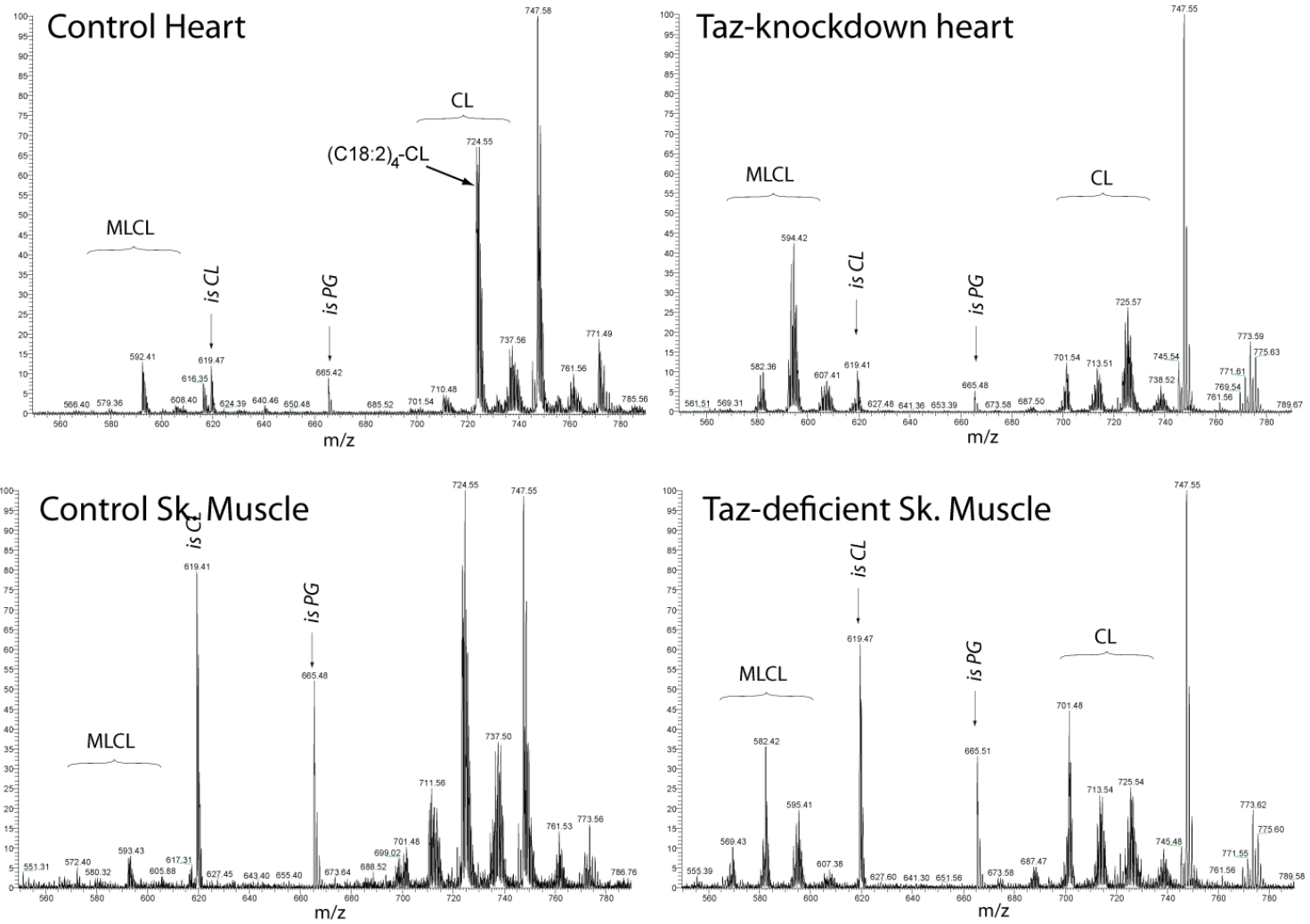
TG
+ Dox



TAFAZZIN KNOCKDOWN

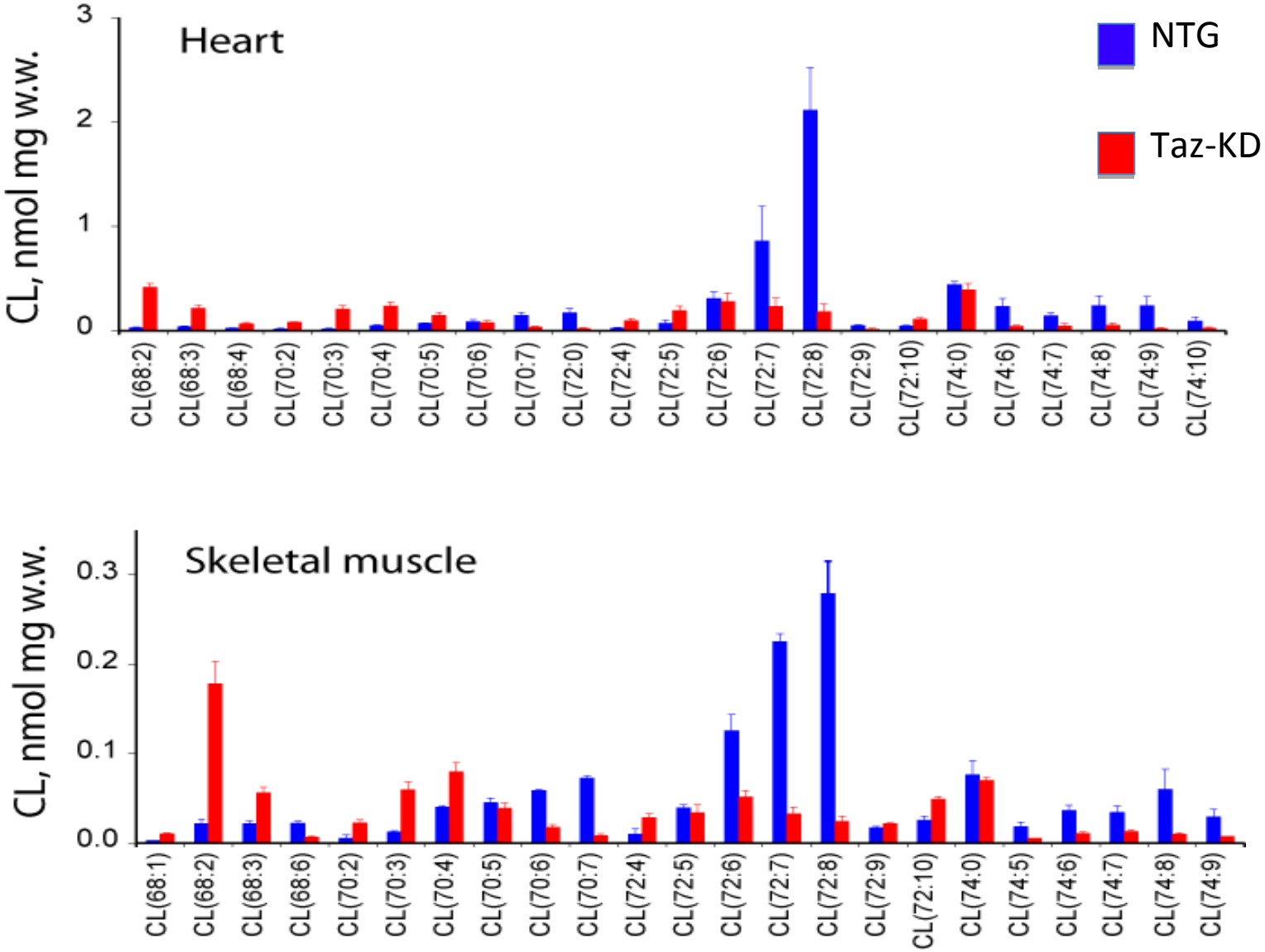


Cardiolipin analysis



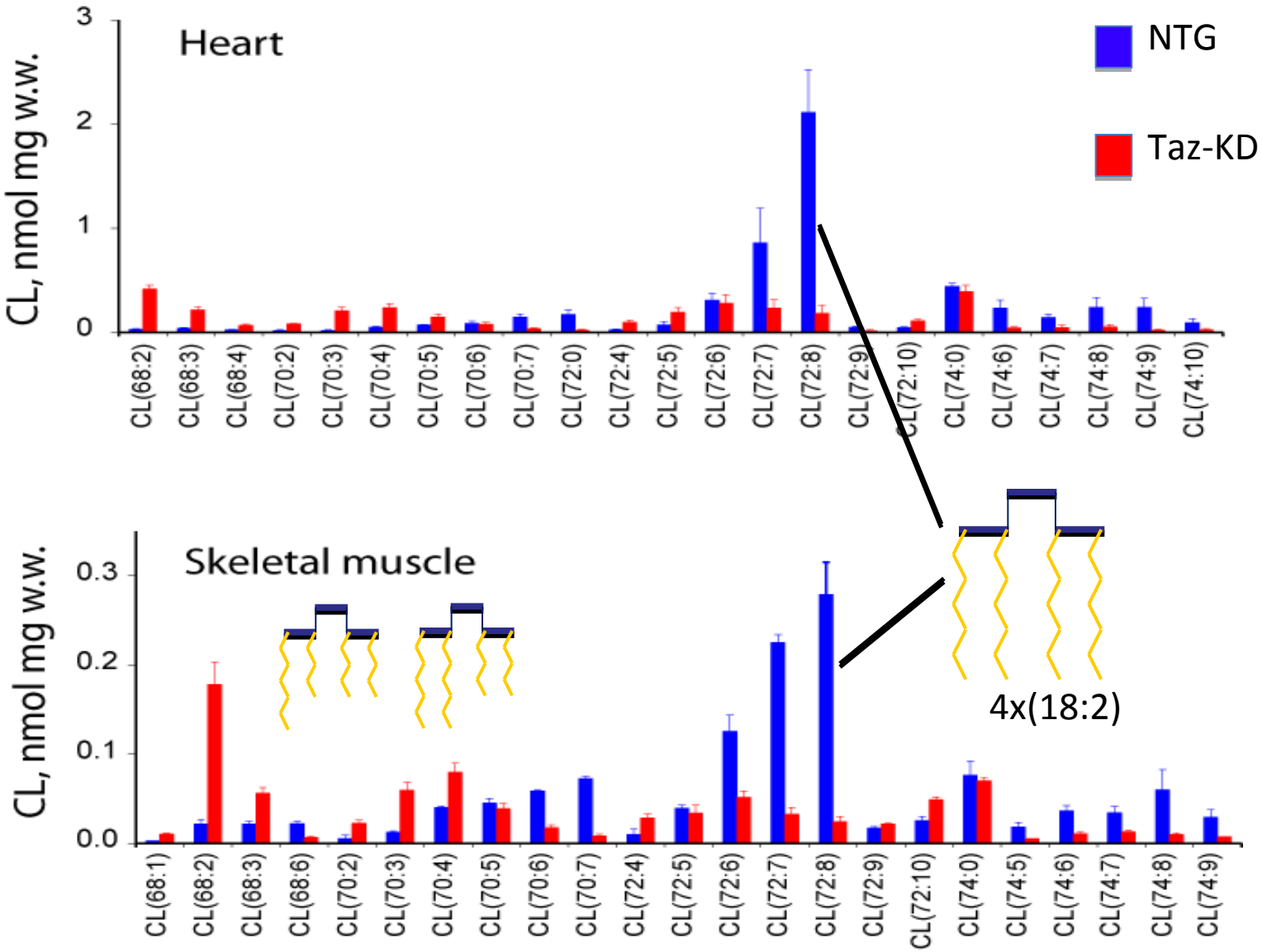
Acehan et al. JBC 2010 (in press)

CARDIOLIPIN IN HEART AND MUSCLE

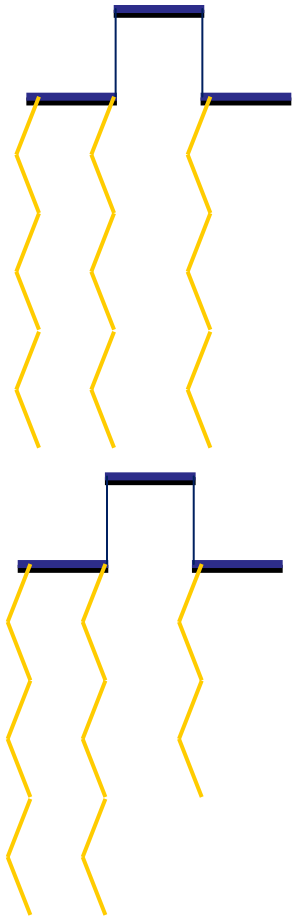


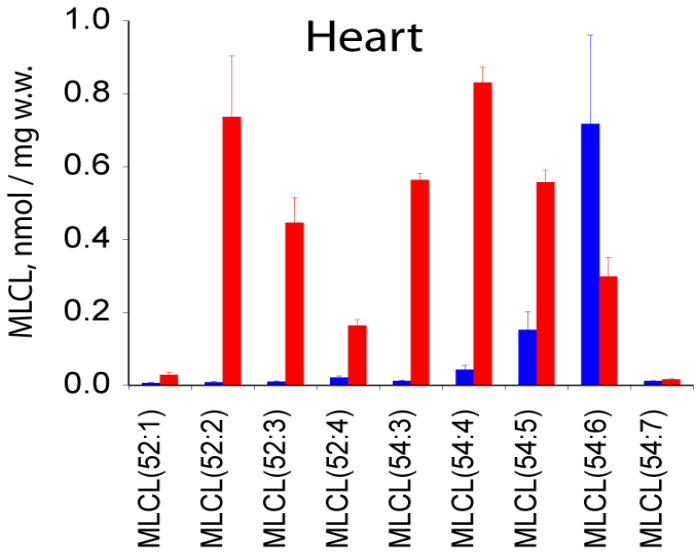
Acehan et al. JBC 2010 (in press)

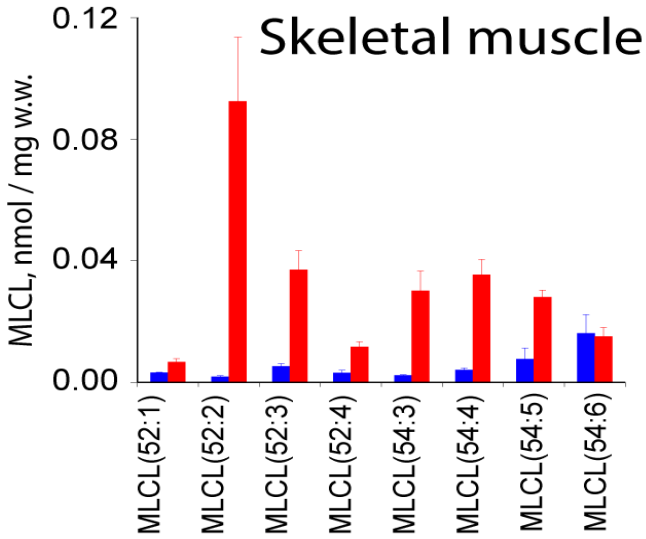
CARDIOLIPIN IN HEART AND MUSCLE



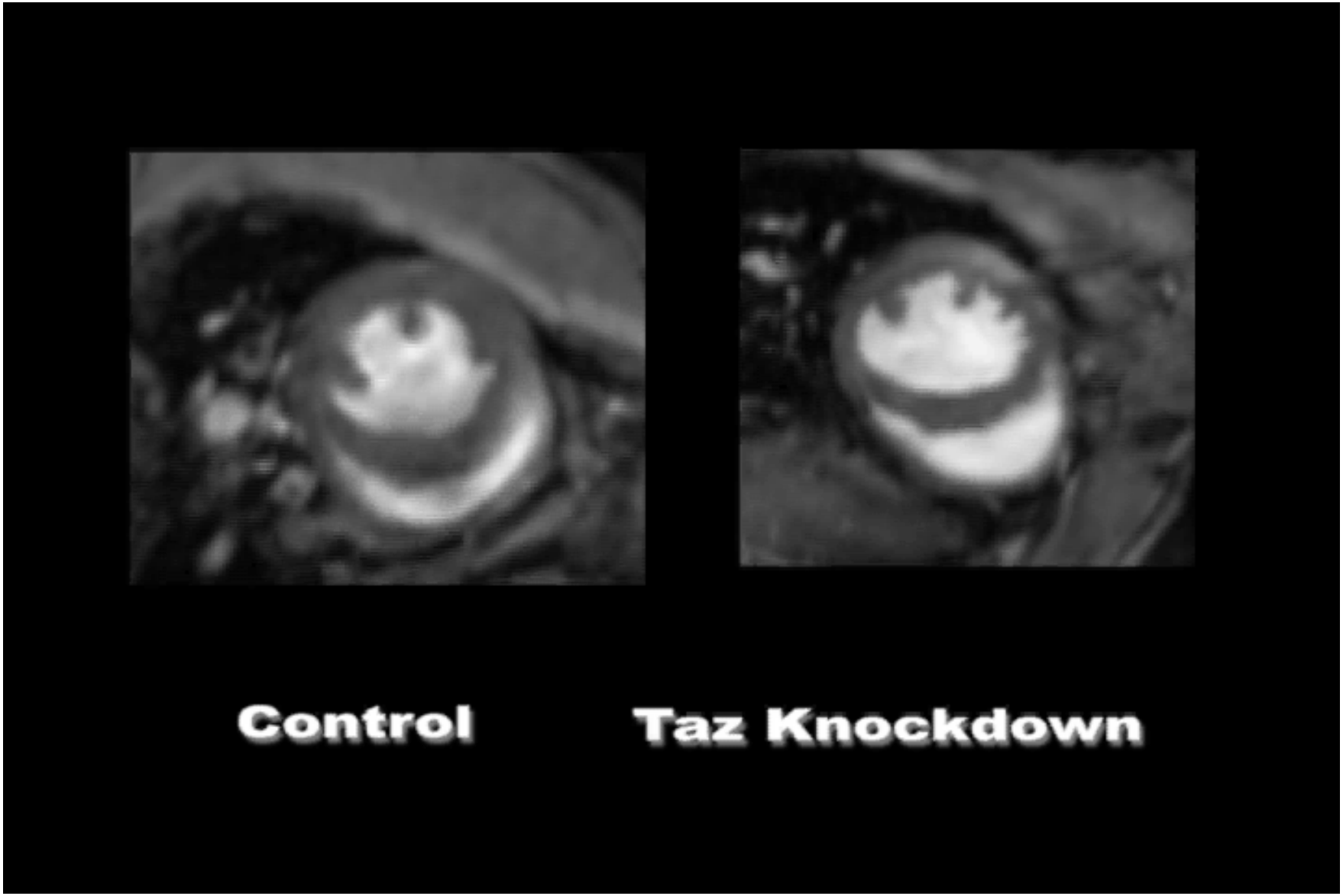
MLCLs IN HEART AND MUSCLE





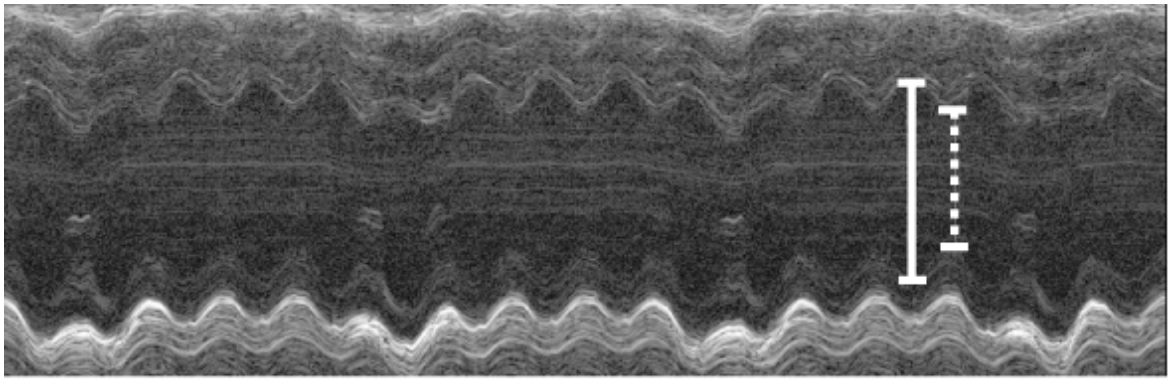


CARDIAC MRI

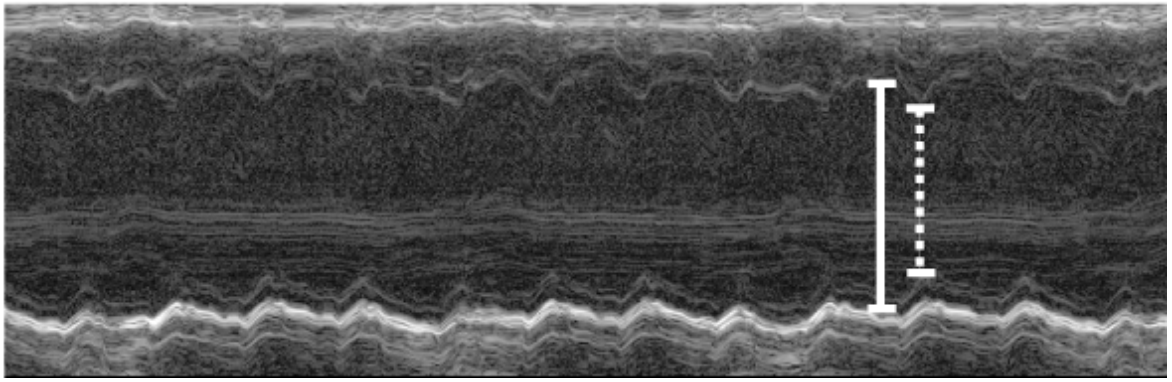


Acehan et al. JBC 2010 (in press)

ECHOCARDIOGRAPHY (8M)



NTG



Taz-KD

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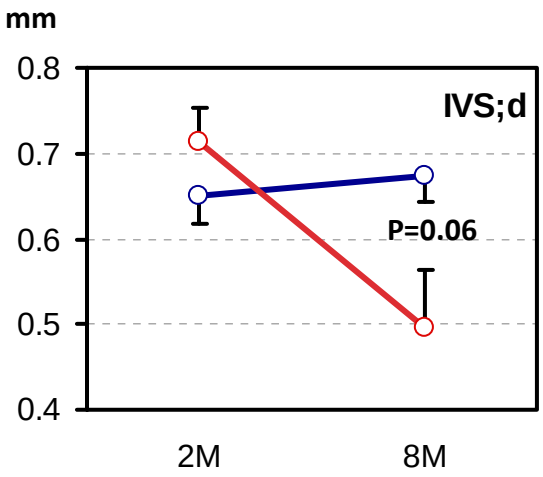
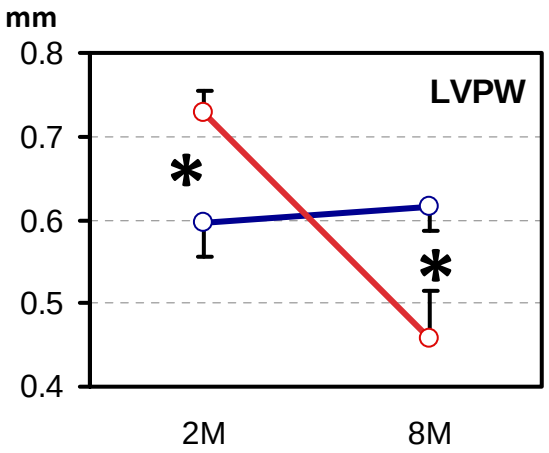
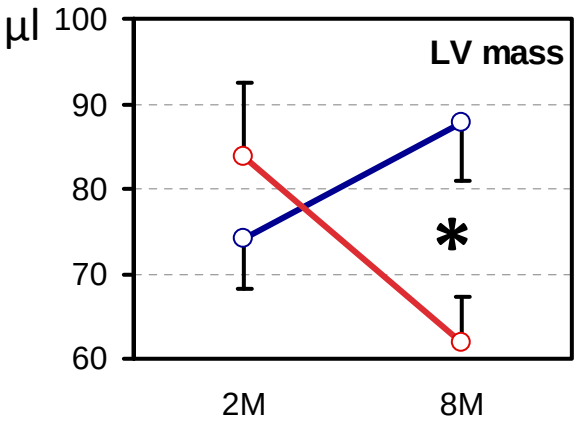
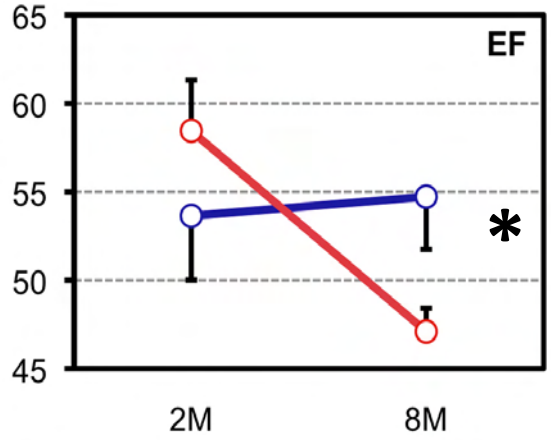
LOSS OF TAZ ALTERS CARDIAC PARAMETERS
Wall thickness and LV wall mass



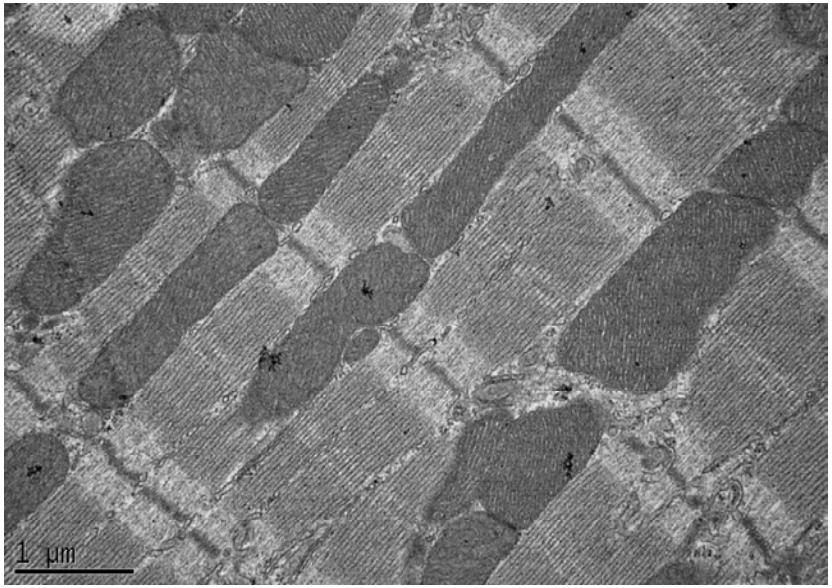




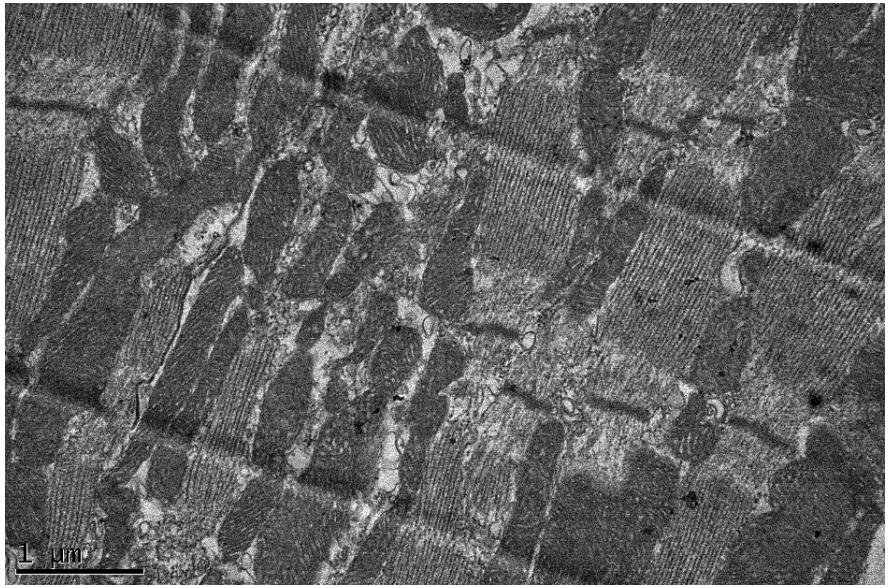


NTG Taz knockdown

CONTROL HEART



TAFAZZIN KNOCKDOWN HEART



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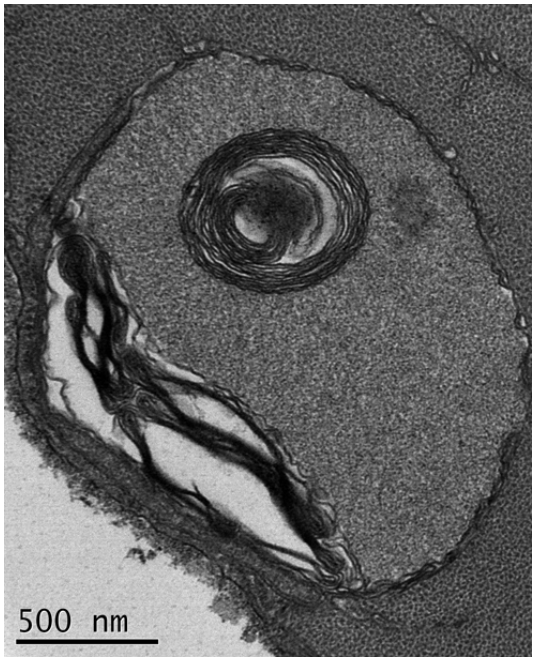
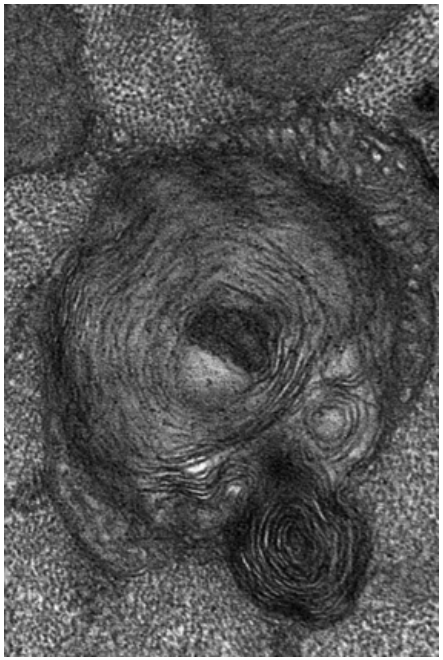
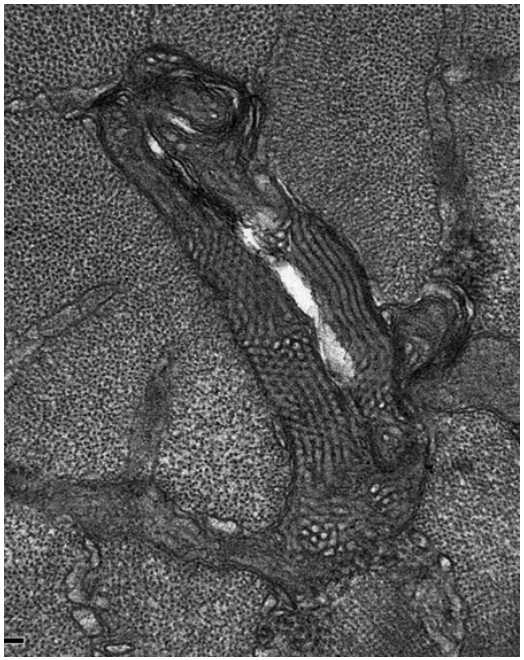
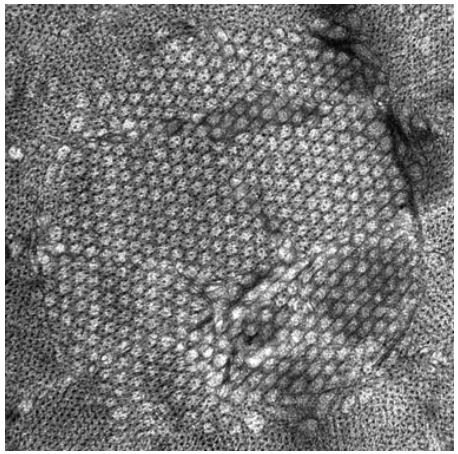
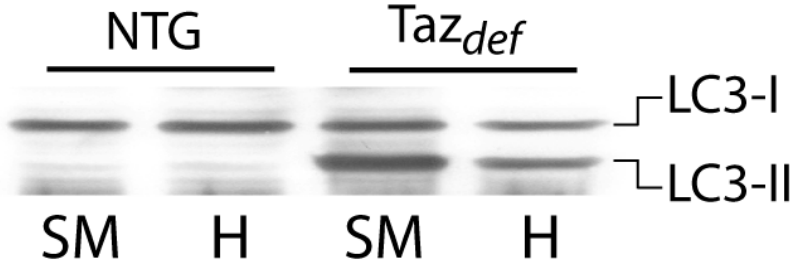

500 nm

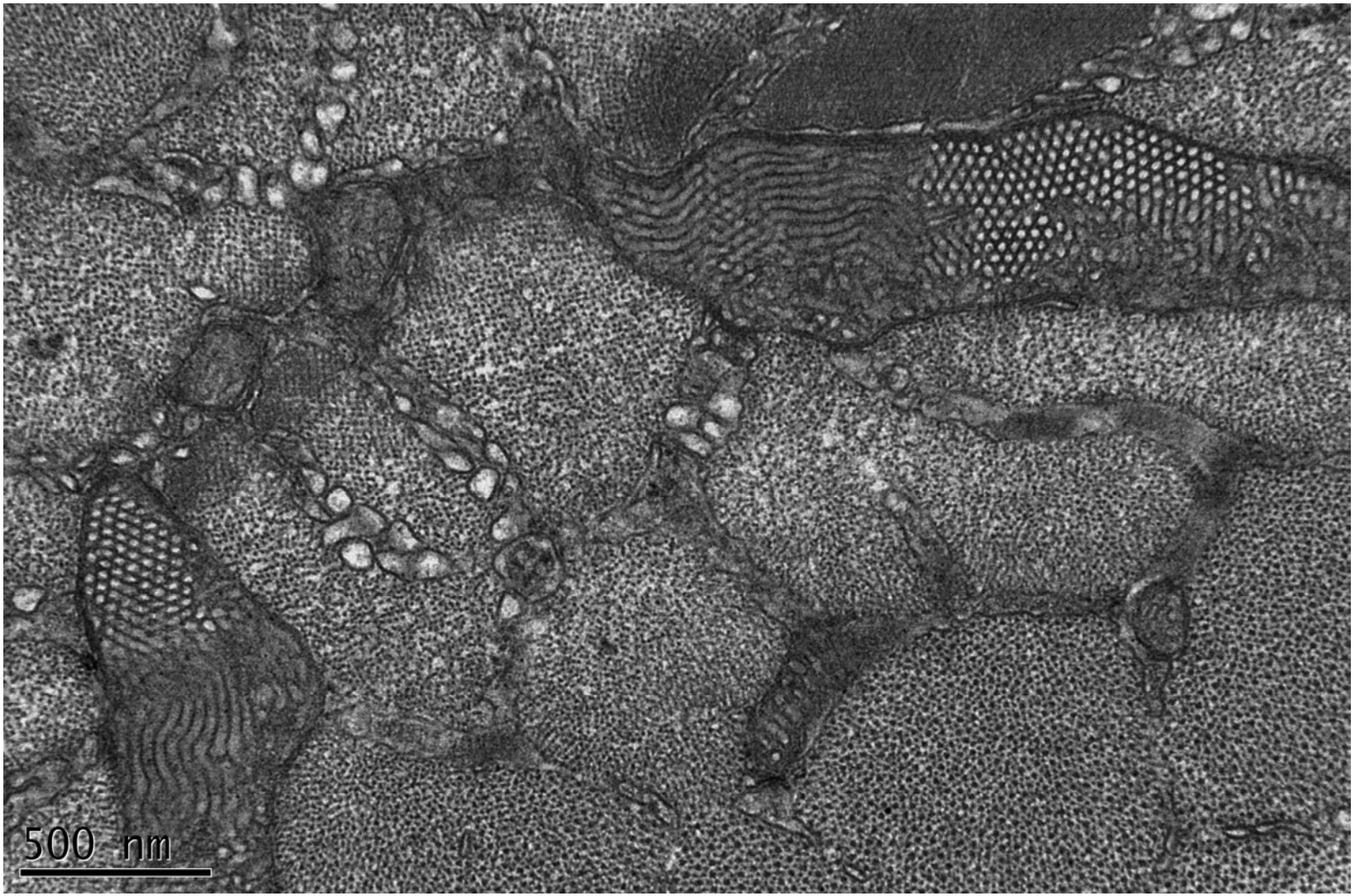







NTG
Taz*def*
LC3-I
LC3-II
SM
H
SM
H

TAFAZZIN KNOCKDOWN SKELETAL MUSCLE



Acehan et al. JBC 2011

Questions Answered:

1. How efficient is shRNA mediated silencing?

   An shRNA mediated silencing of taz is very efficient in heart, muscle, liver and brain

2. How tight is regulation by Dox?

   Dox very efficiently controls shRNA expression in heart, skeletal muscle and liver. In brain control is less tight and we observed ~35% reduction of taz mRNA level.

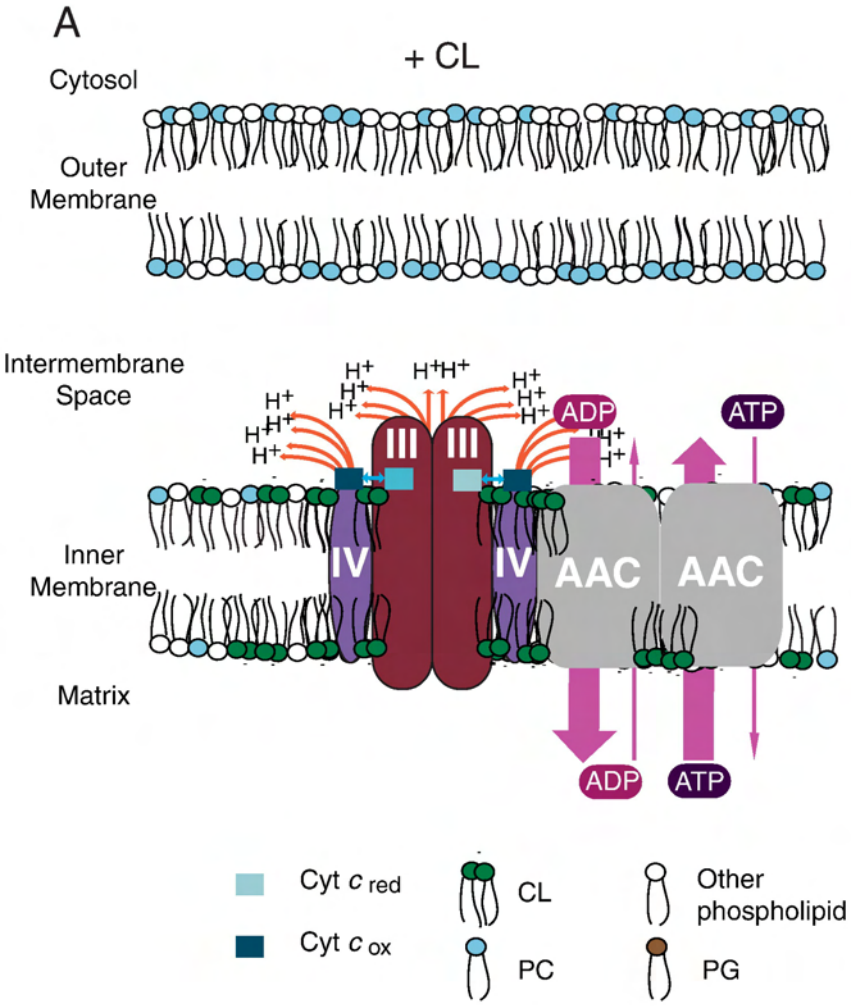
3. What are side-effects of chronic Dox administration?

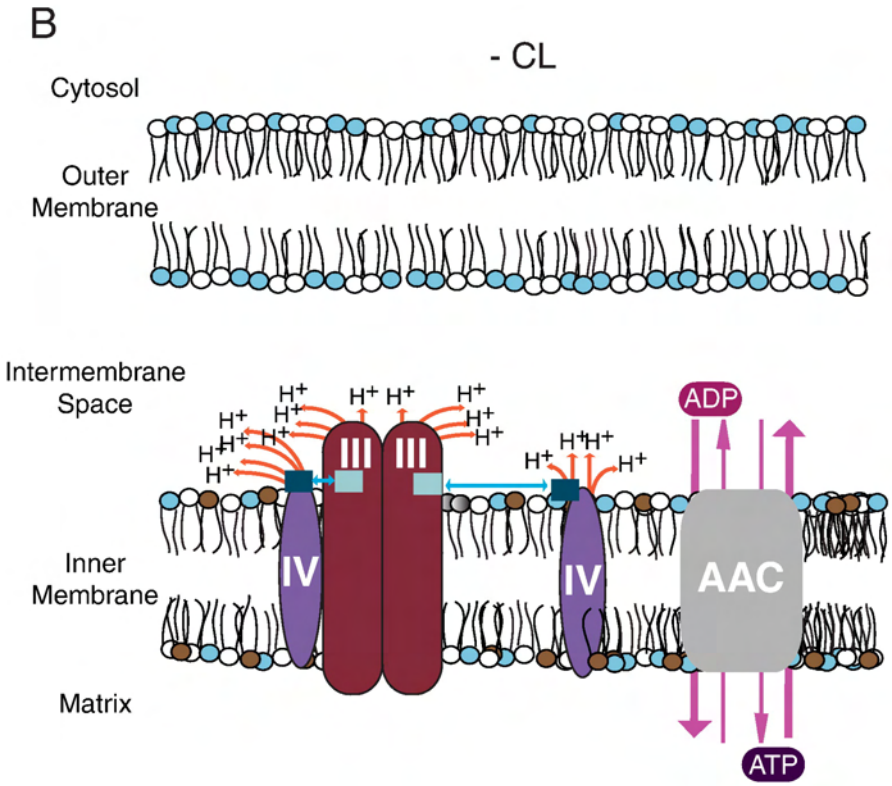
   We didn't find any adverse effects of prolonged dox administration in control animals.

4. Is it reversible?

   We found that withdrawal from dox restores taz level to 75-90% of normal in 2.5 months.

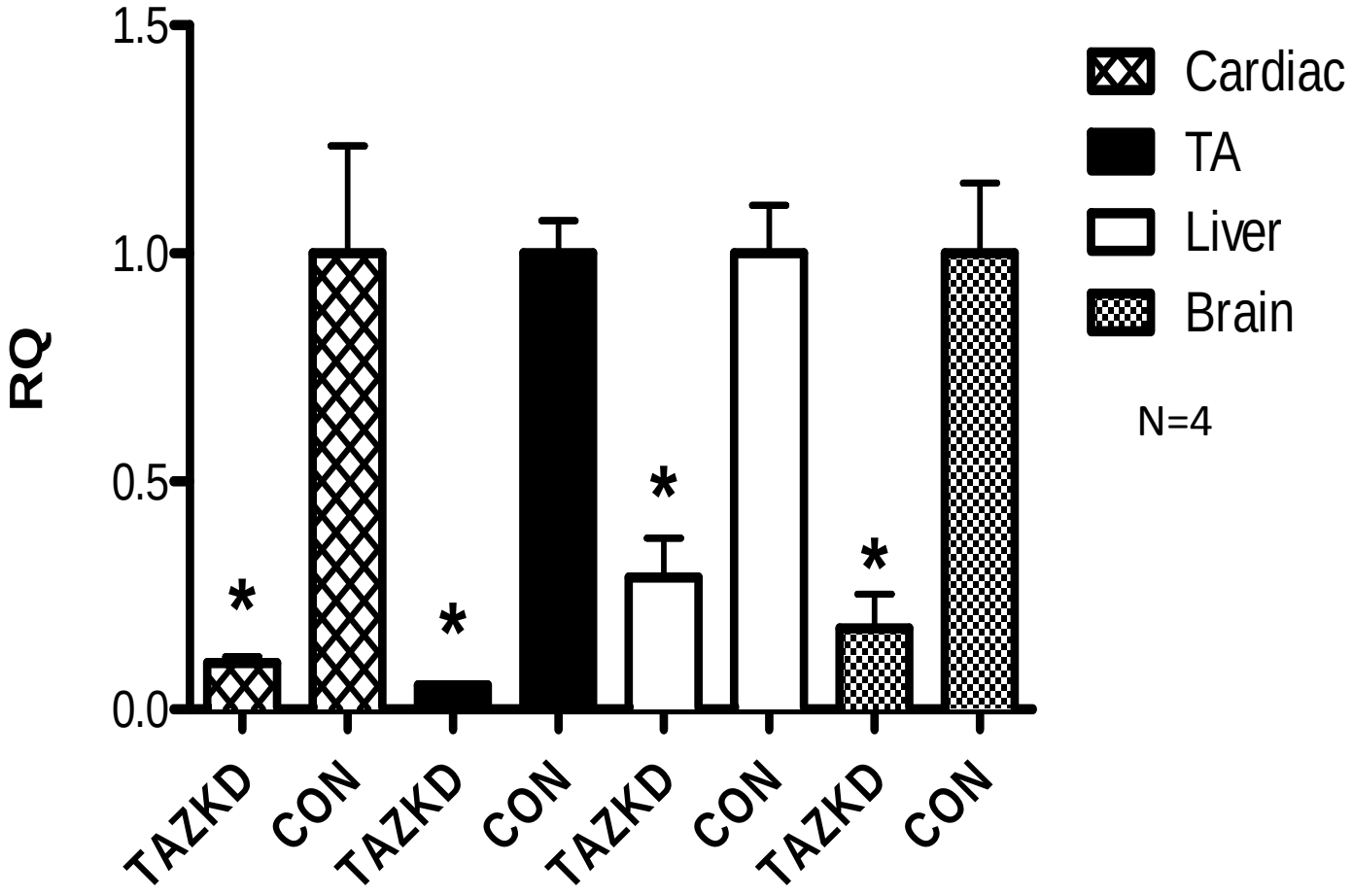
Impact of Cardiolipin on IMM





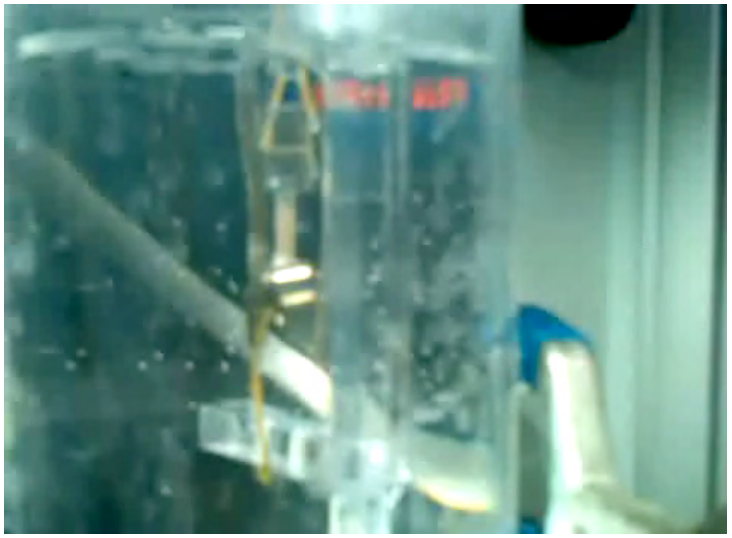
Claypool et al. 2008

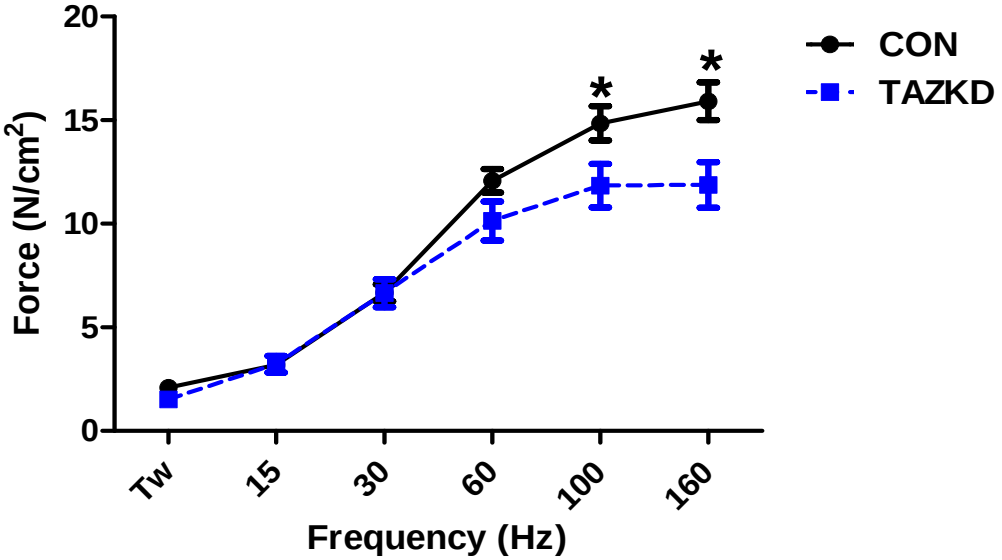
Dox shRNA-induction causes significant TAZ knockdown



Soustek *et al.*, HGT, 2011

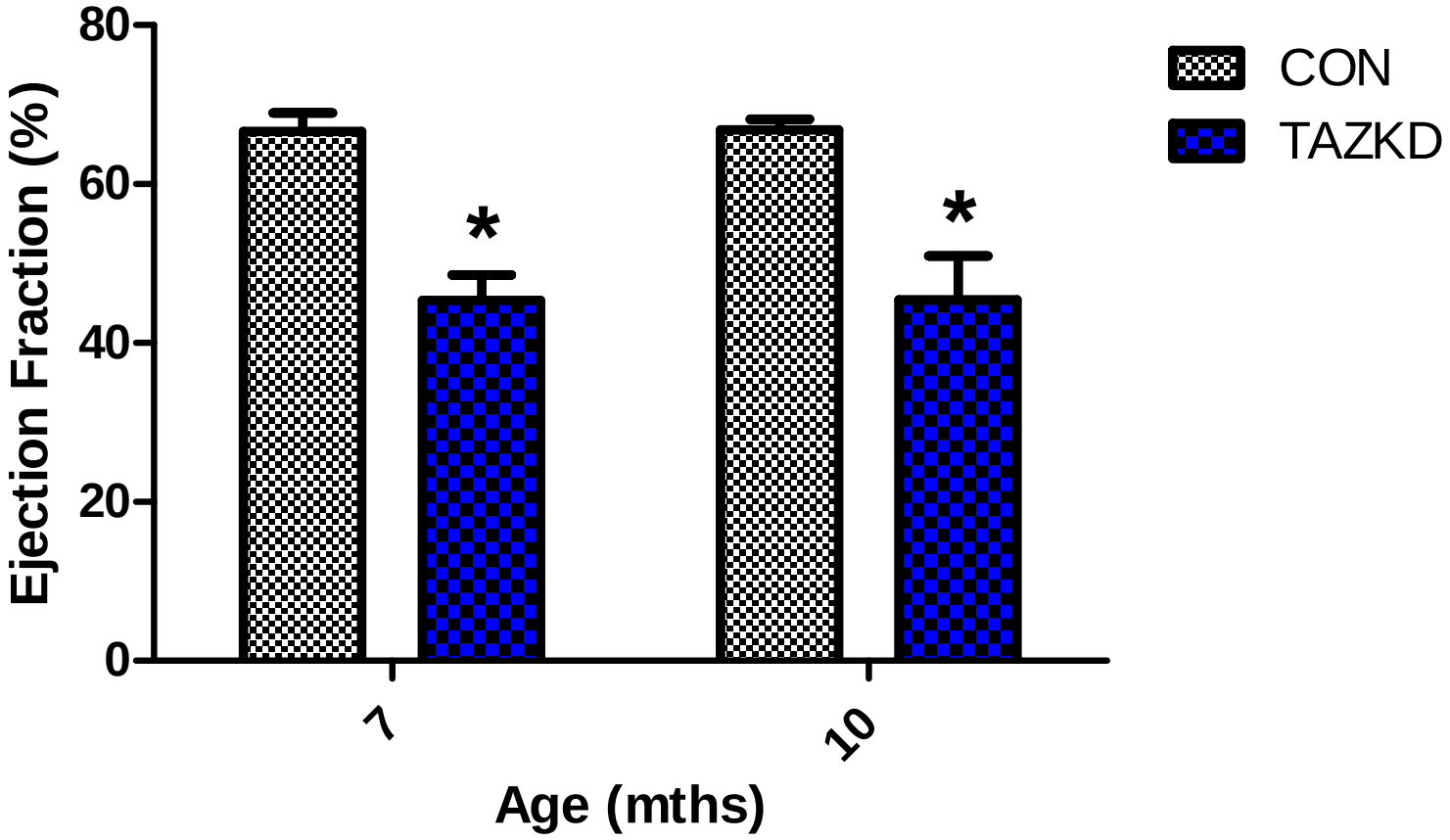
TAZKD Results in Impaired Contractility in the Soleus





Soustek *et al.*, HGT, 2011

TAZKD Results in Reduced Cardiac Function



Soustek *et al.*, HGT, 2011

Transduction of pTR-Myc-TAZ-FL in HEK293 Cells

Western Blot : C-Myc Antibody (1:200)

Acknowledgements

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- Meghan Soustek
- Al Lewin
- Cathryn Mah
- Denise Cloutier
- Darien Falk

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