

Mouse model of human Barth syndrome

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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.

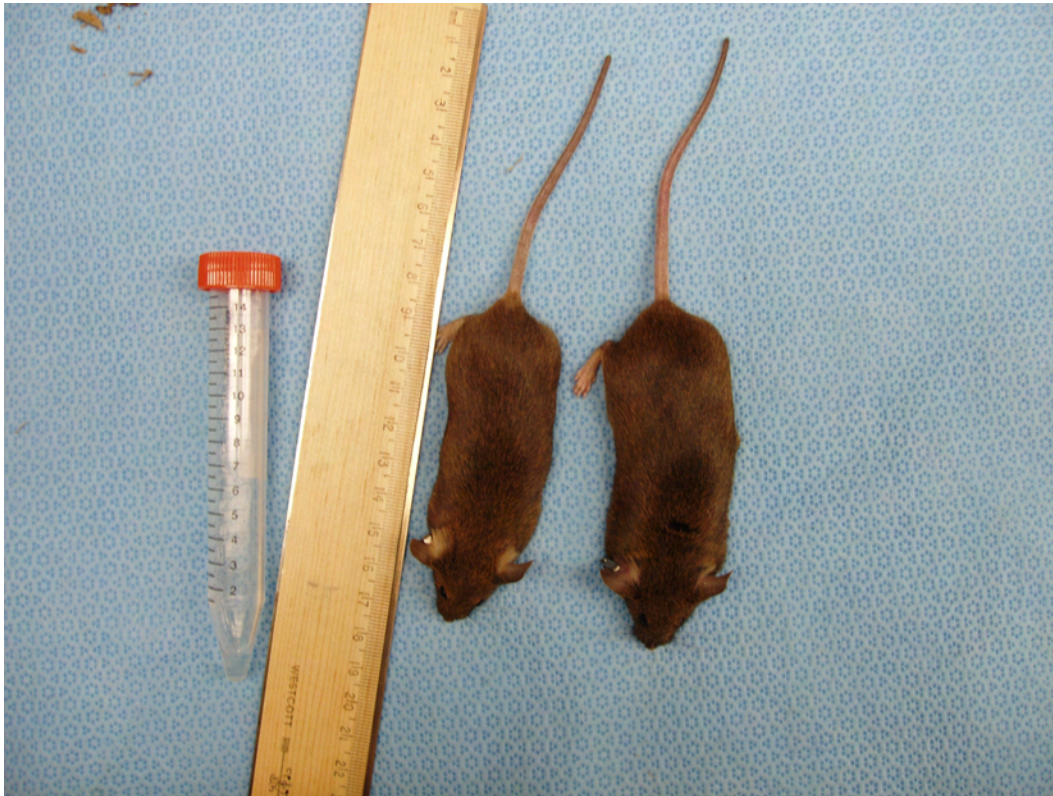
Urgent need for mouse model of Barth syndrome

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

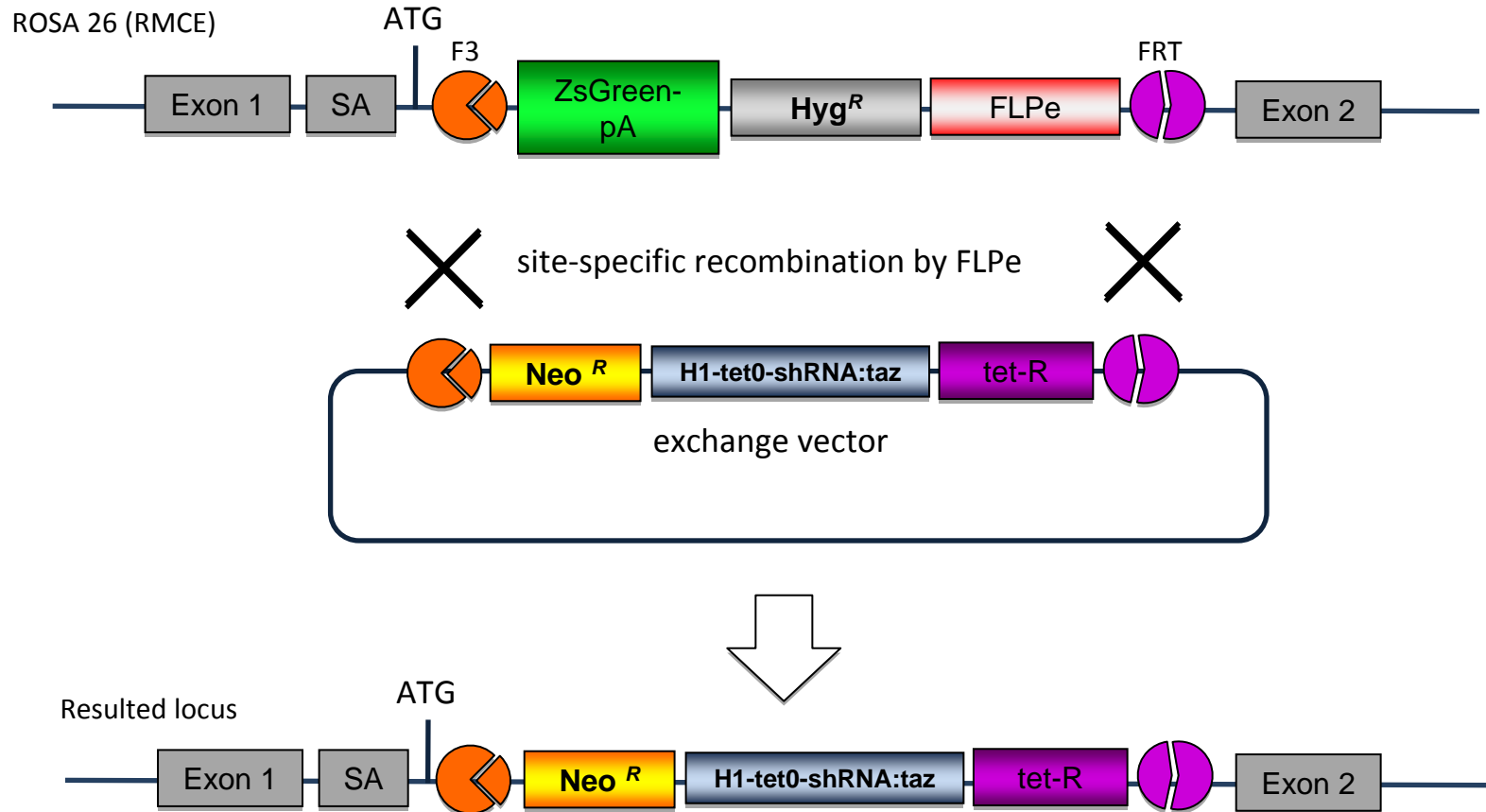
Tafazzin tet-on shRNA transgenic mice were generated by TaconicArtemis in 2009.

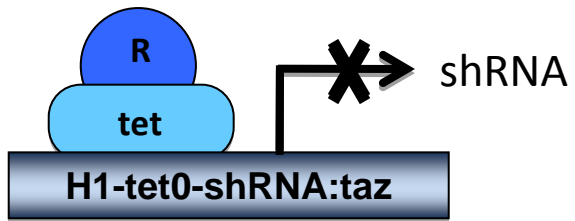
Work was initiated and funded by Barth Syndrome Foundation (www.barthsyndrome.org).

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



RECOMBINASE MEDIATED CASSETTE EXCHANGE

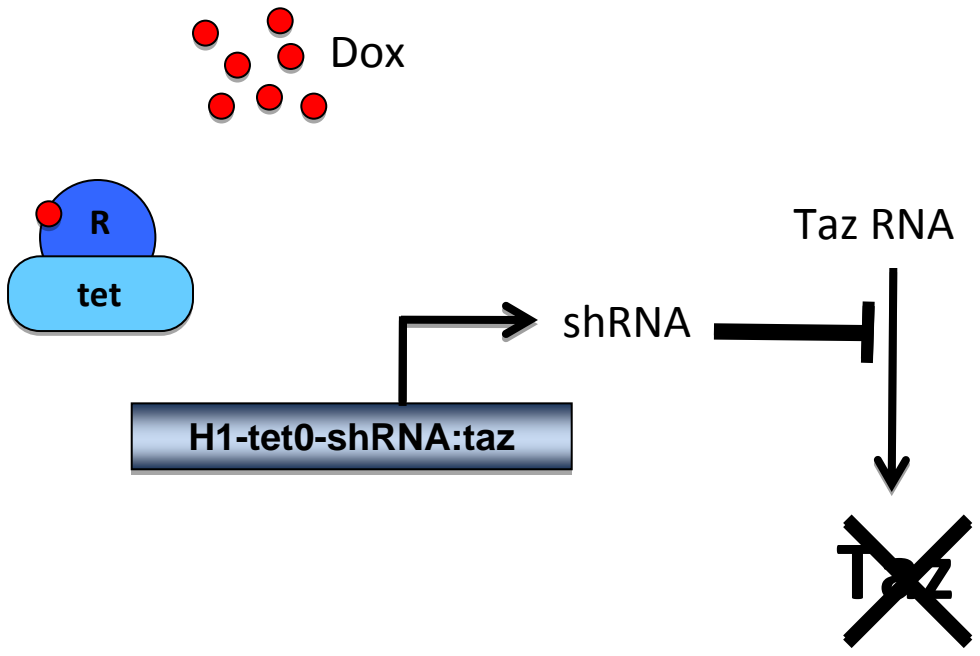




Taz RNA



Taz

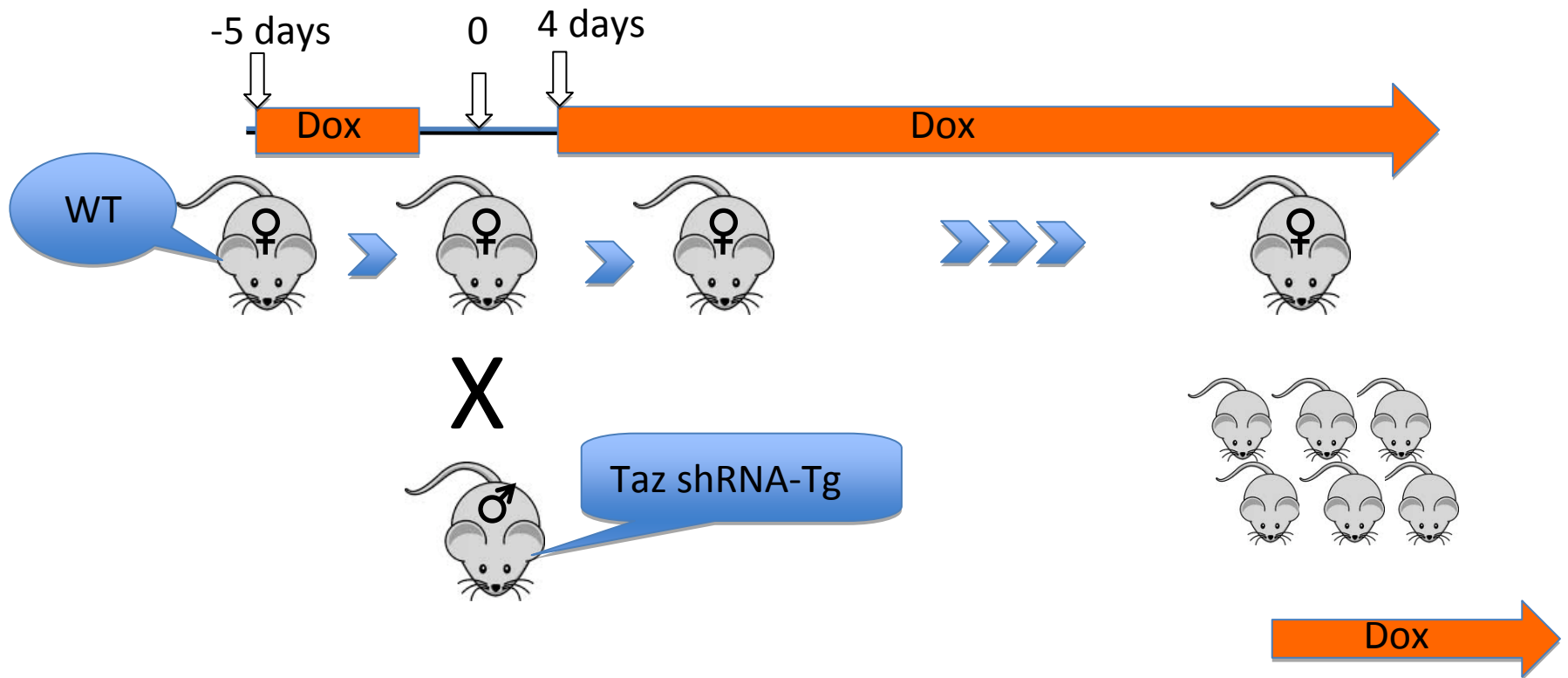


Important Questions:

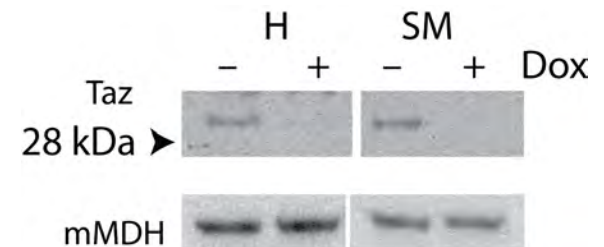
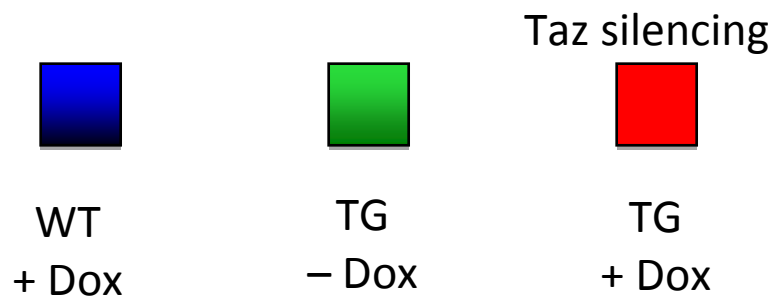
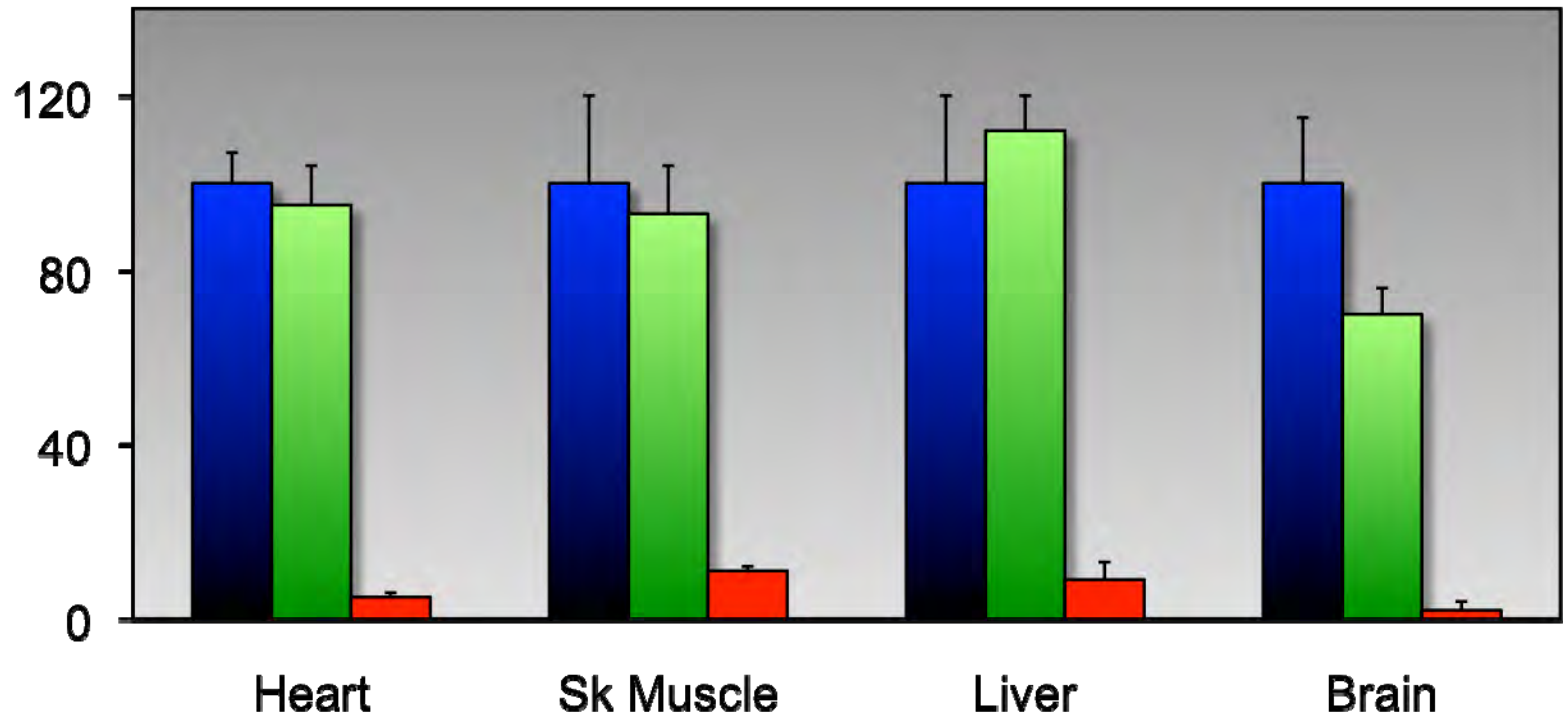
1. How efficient is shRNA mediated silencing in different tissues?
2. How tight is regulation by Dox?
3. What are side-effects of chronic Dox administration?
4. Is it reversible?

Induction of Taz knockdown

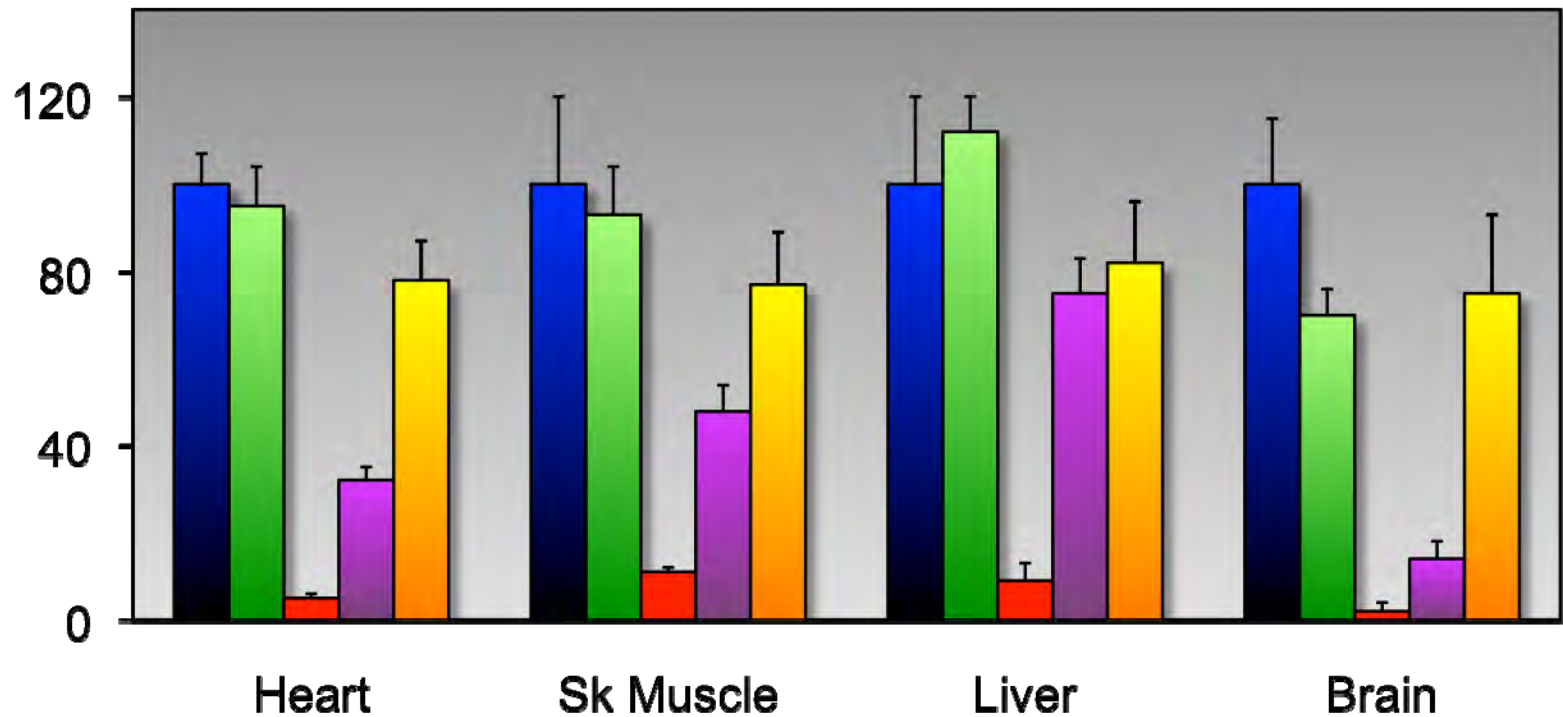
Doxycycline was administered with rodent chow, formulated by Purina Mills (625 mg / kg)



TAFAZZIN KNOCKDOWN



TAFAZZIN KNOCKDOWN



Un-silencing



WT
+ Dox



TG
- Dox



TG
+ Dox

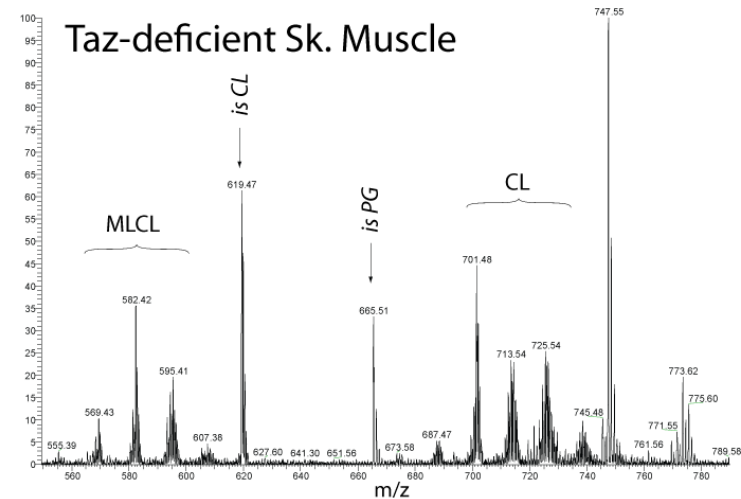
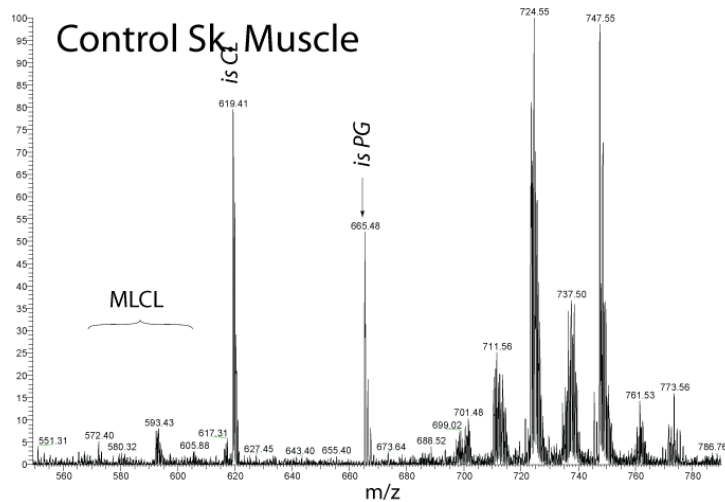
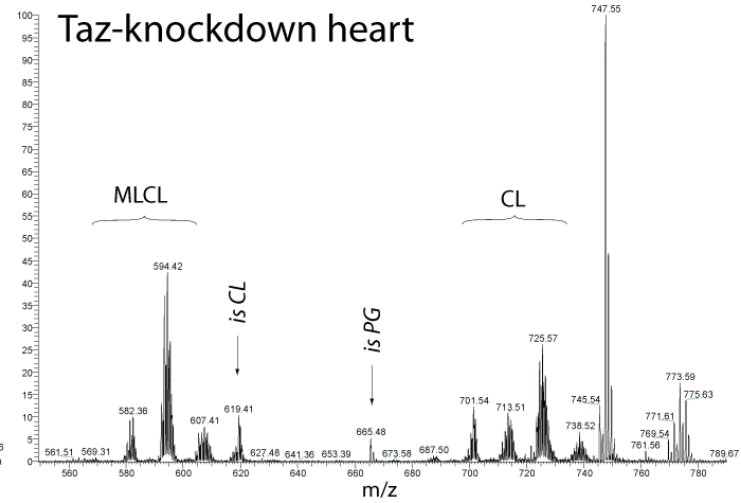
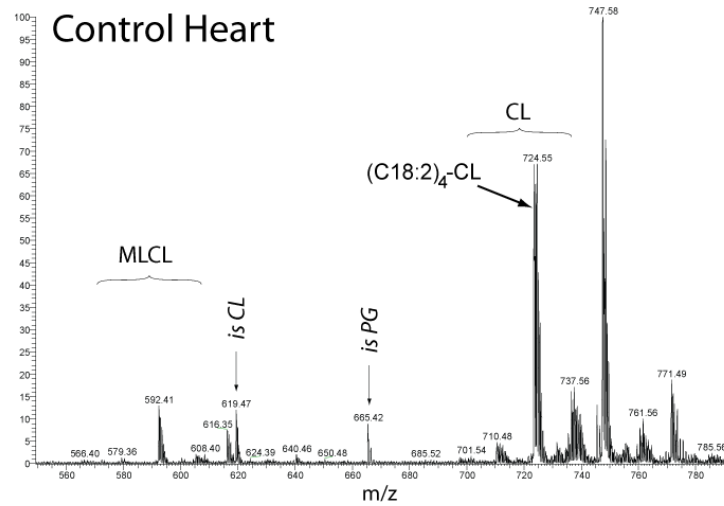


TG
+/- Dox
1 M

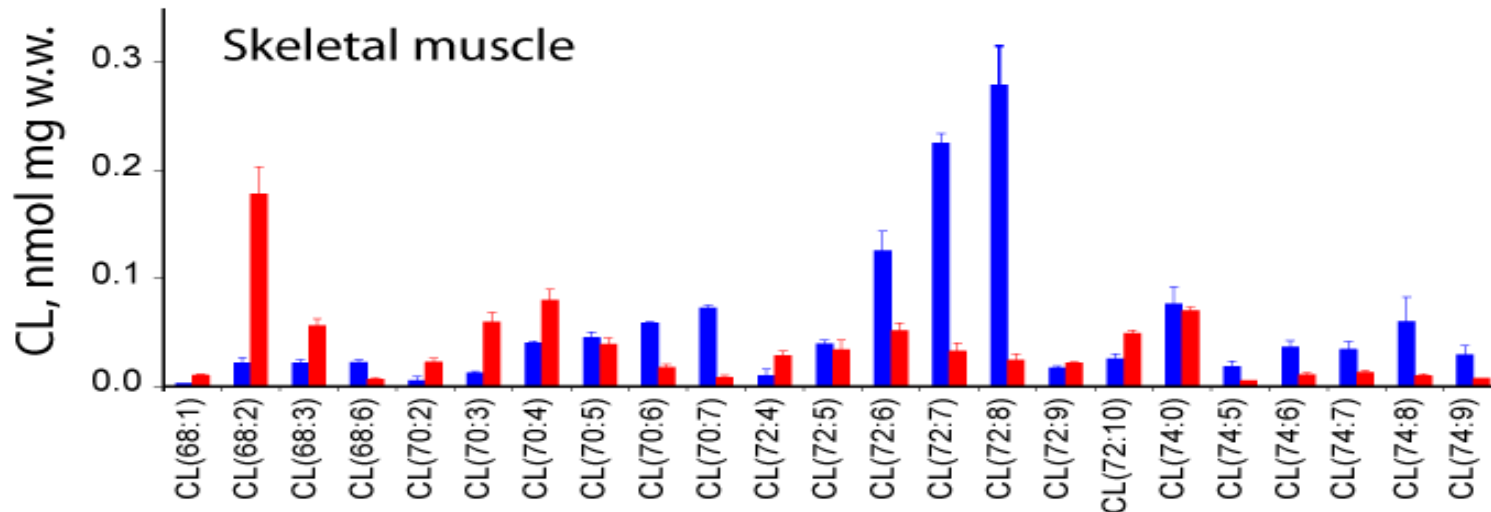
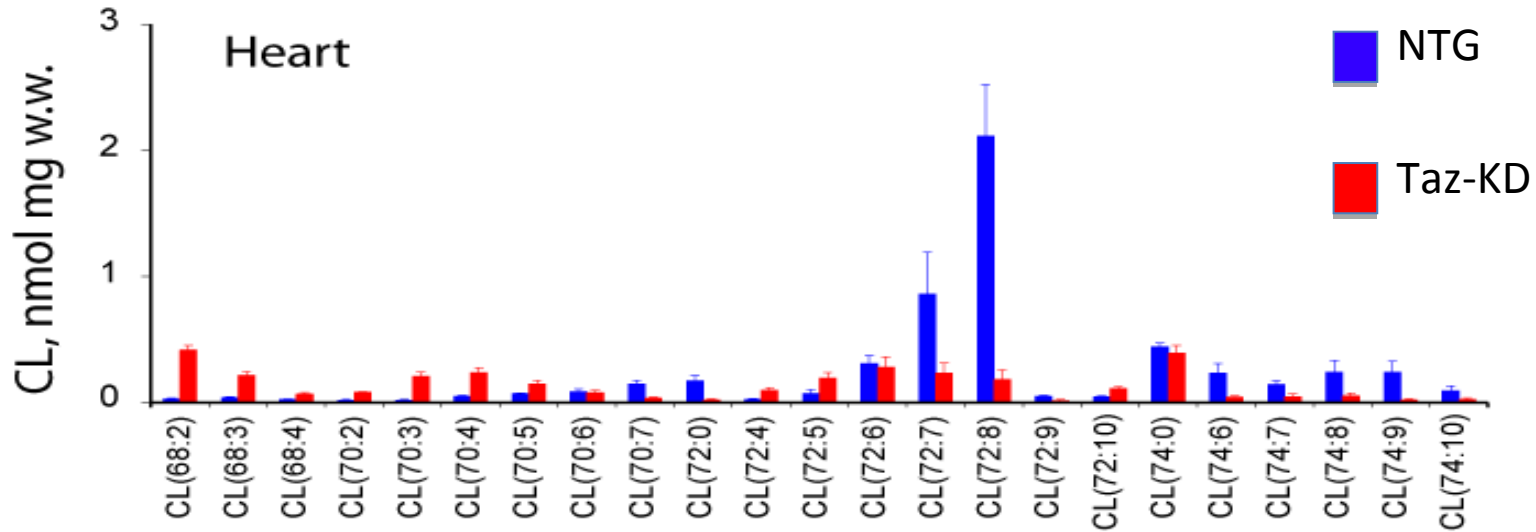


TG
+/- Dox
2 1/2 M

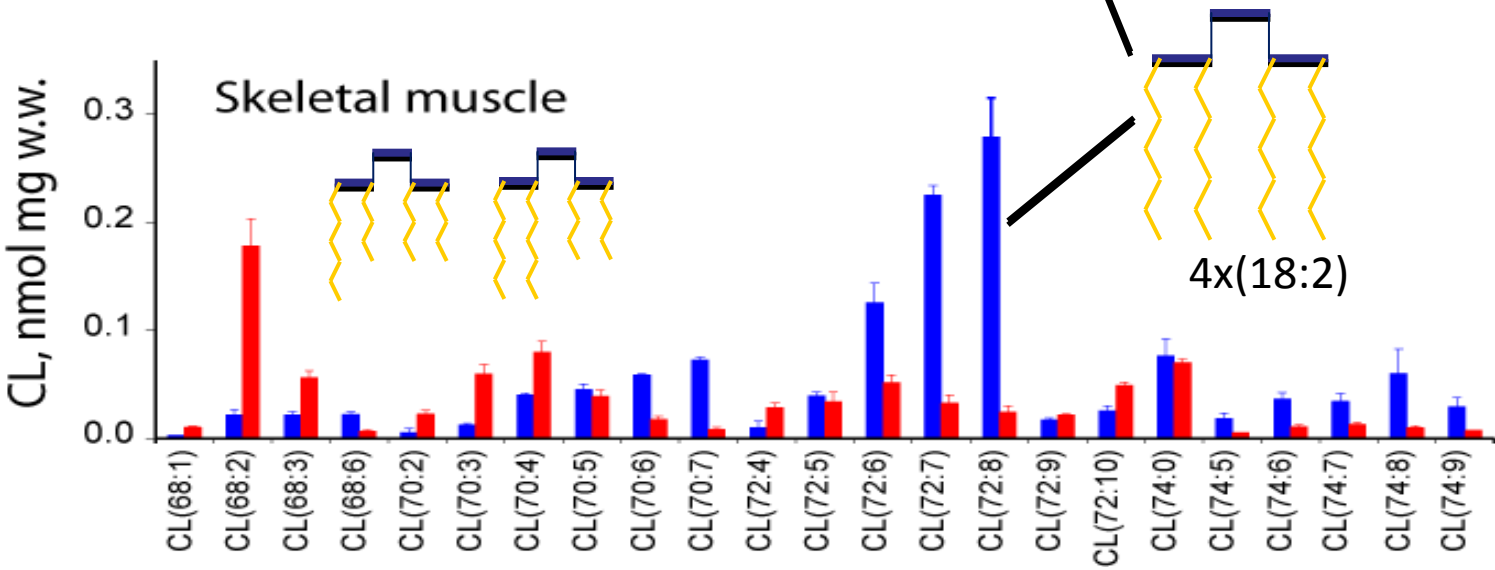
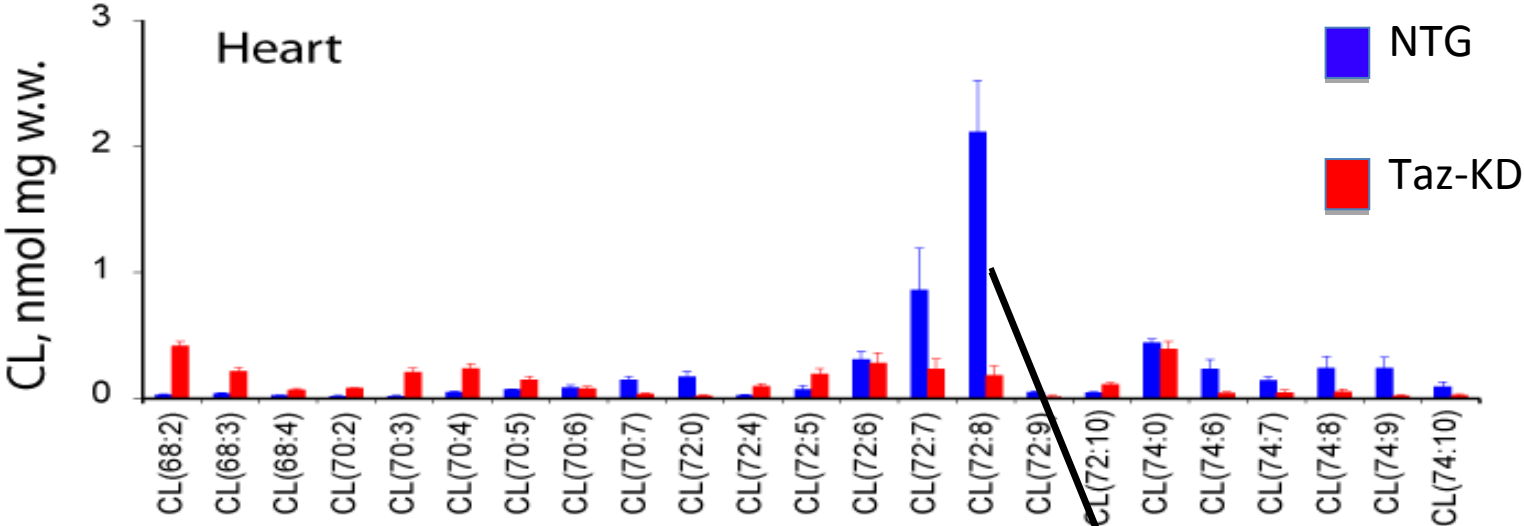
Cardiolipin analysis



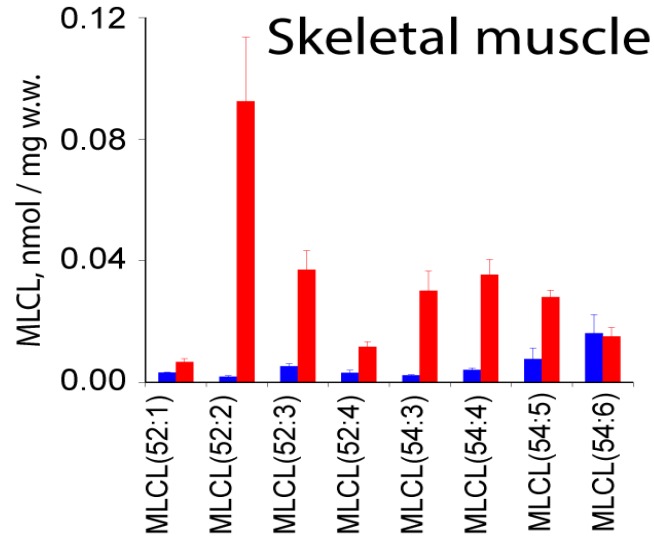
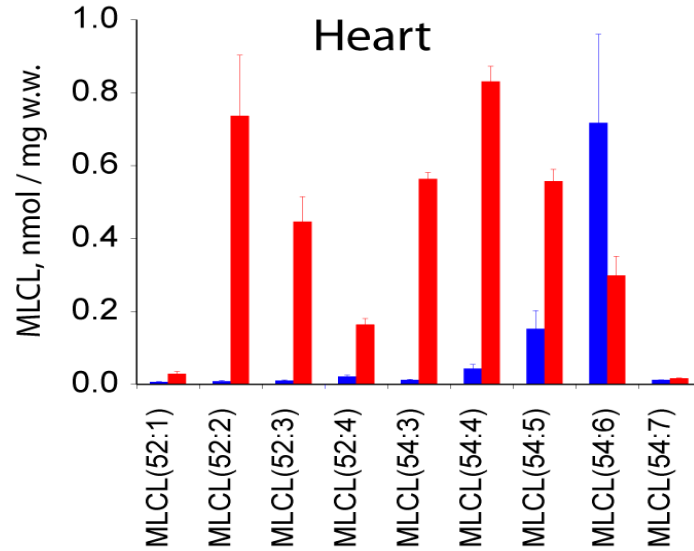
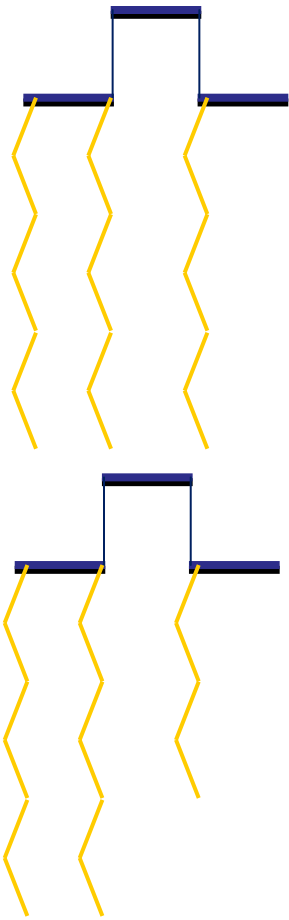
CARDIOLIPIN IN HEART AND MUSCLE



CARDIOLIPIN IN HEART AND MUSCLE



MLCLs IN HEART AND MUSCLE



CARDIAC MRI

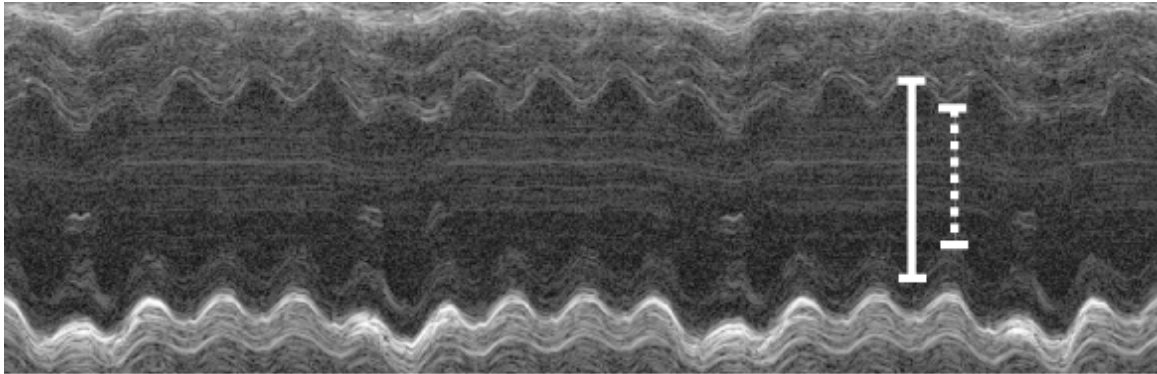


Control

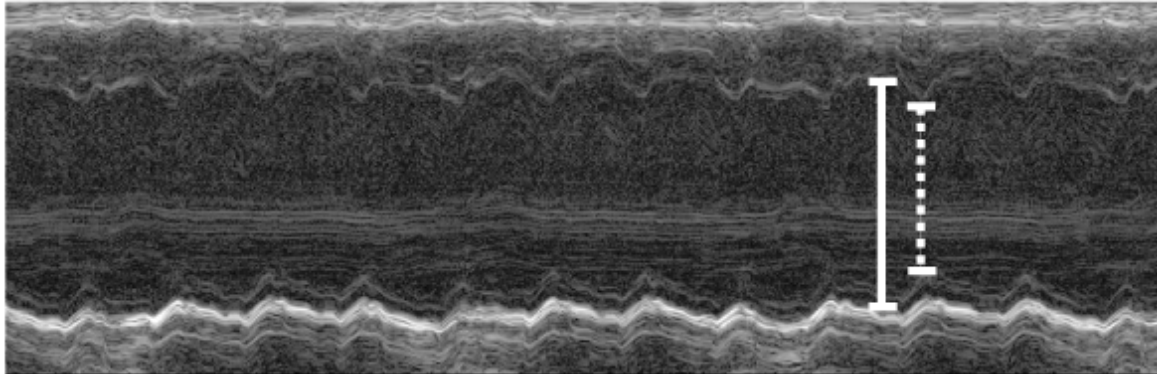


Taz Knockdown

ECHOCARDIOGRAPHY (8M)



NTG

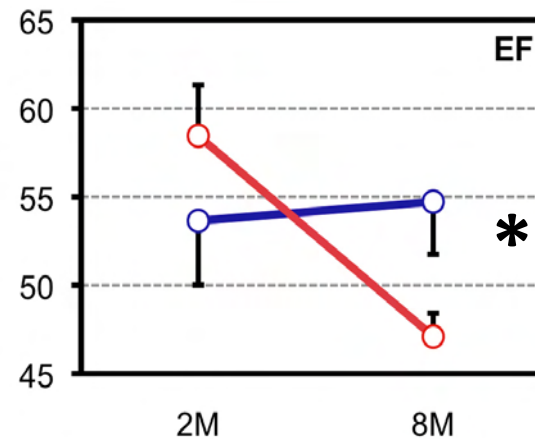
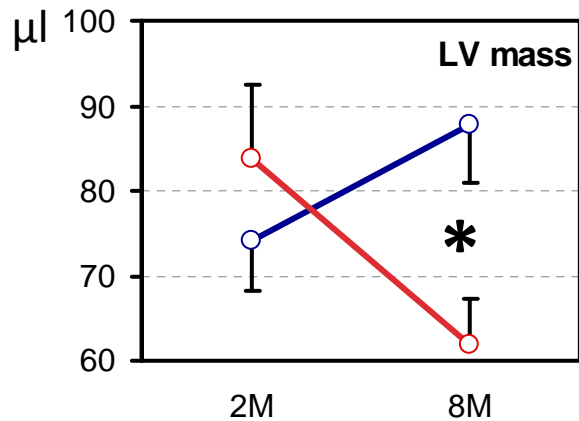
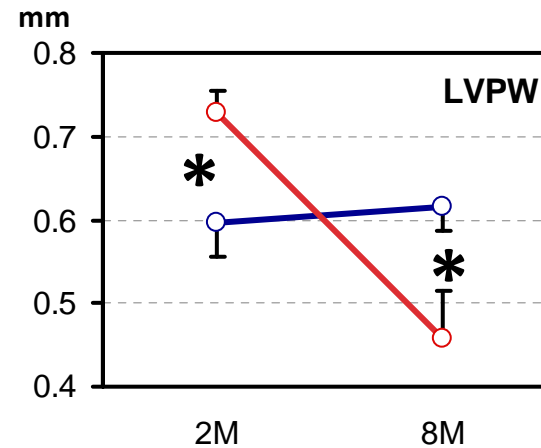
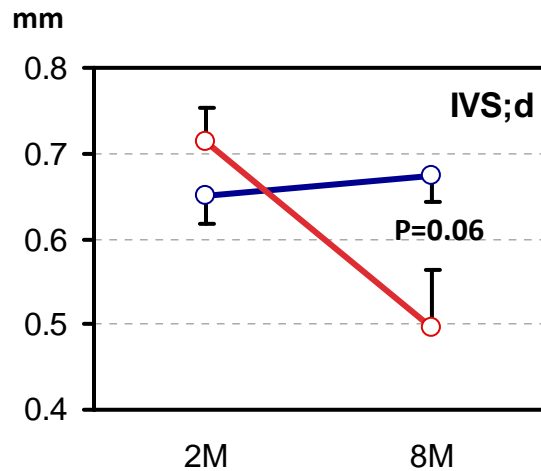


Taz-KD

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LOSS OF TAZ ALTERS CARDIAC PARAMETERS

Wall thickness and LV wall mass

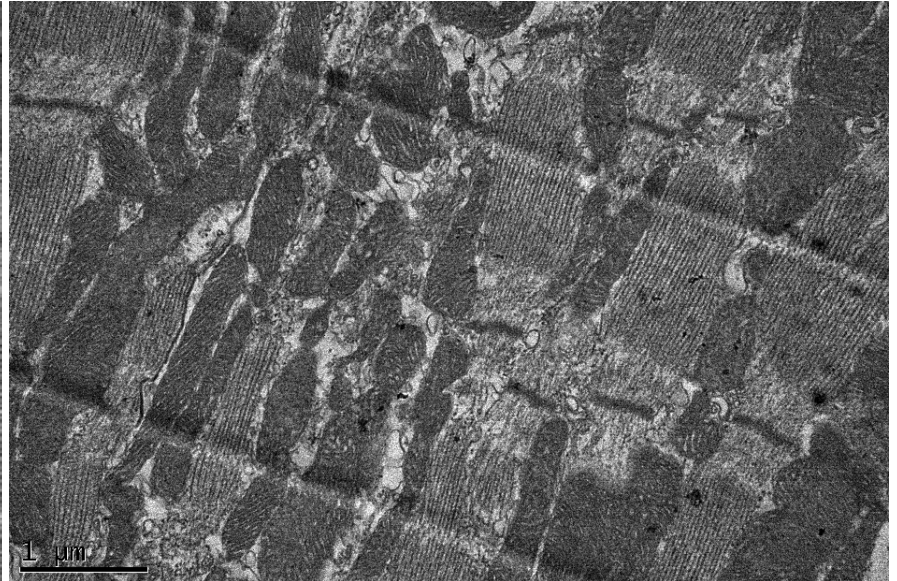
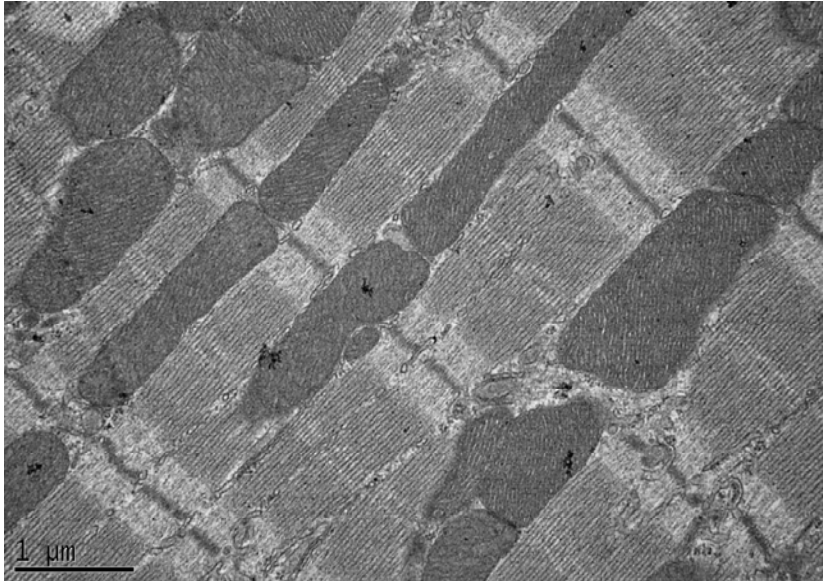


■ NTG

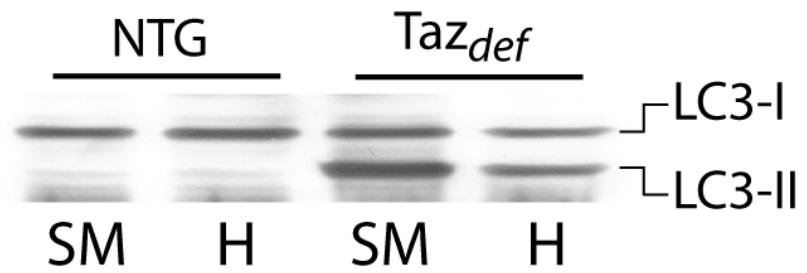
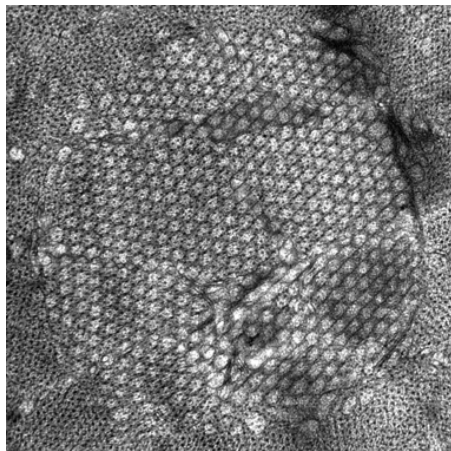
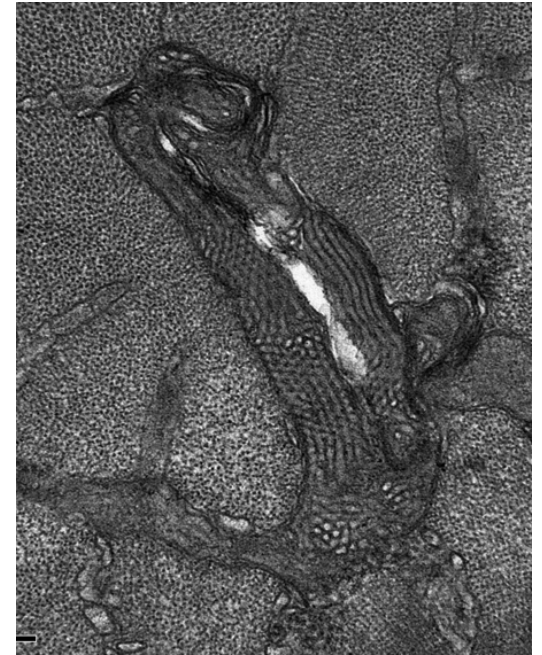
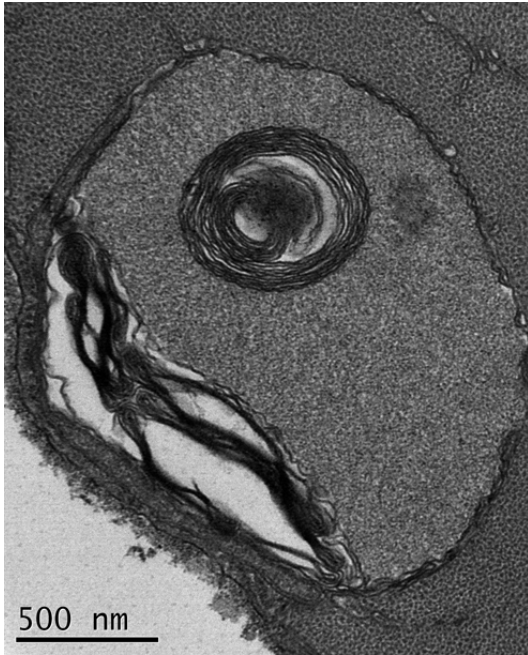
■ Taz knockdown

CONTROL HEART

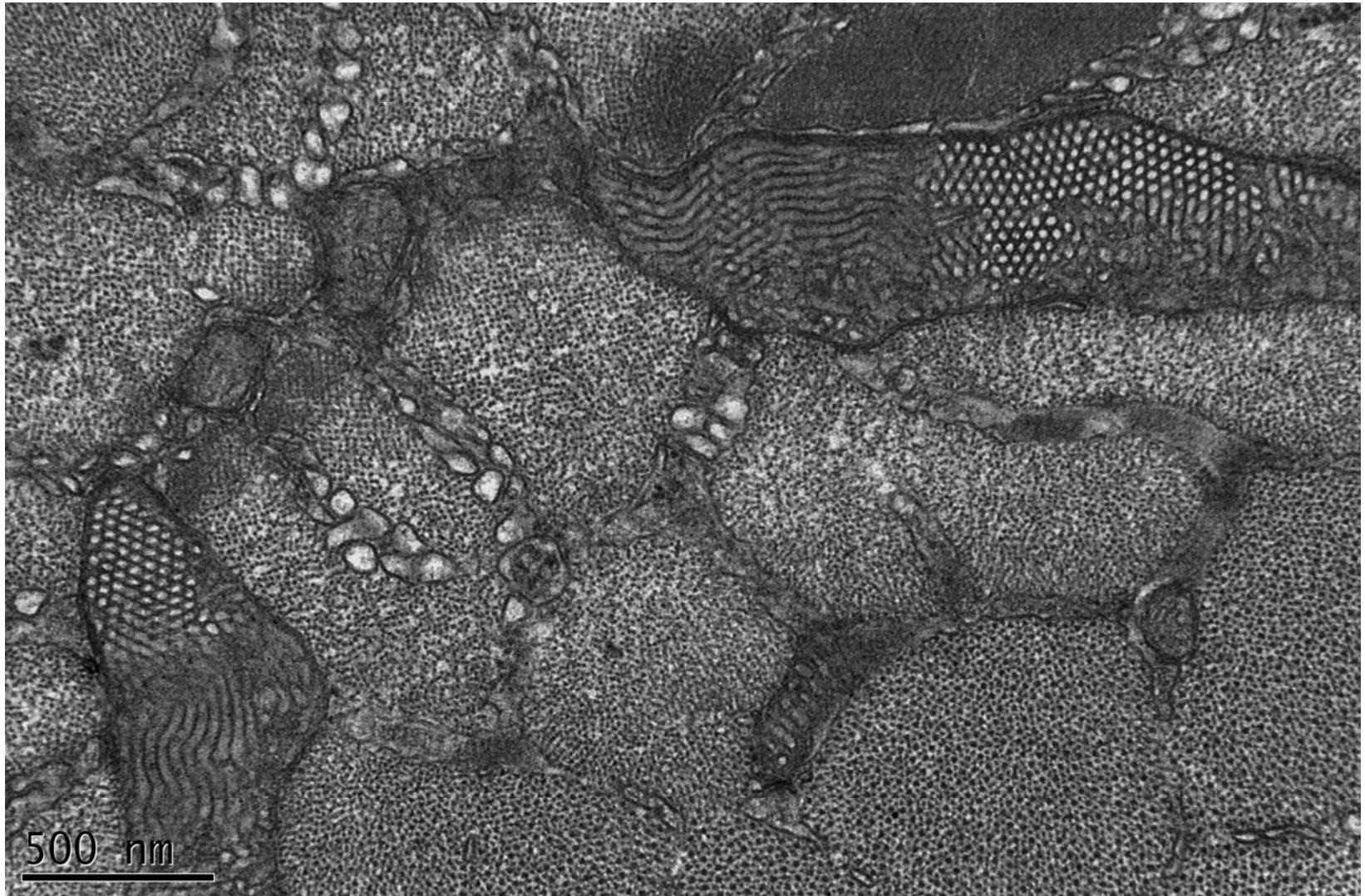
TAFAZZIN KNOCKDOWN HEART



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TFAZZIN KNOCKDOWN SKELETAL MUSCLE



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.

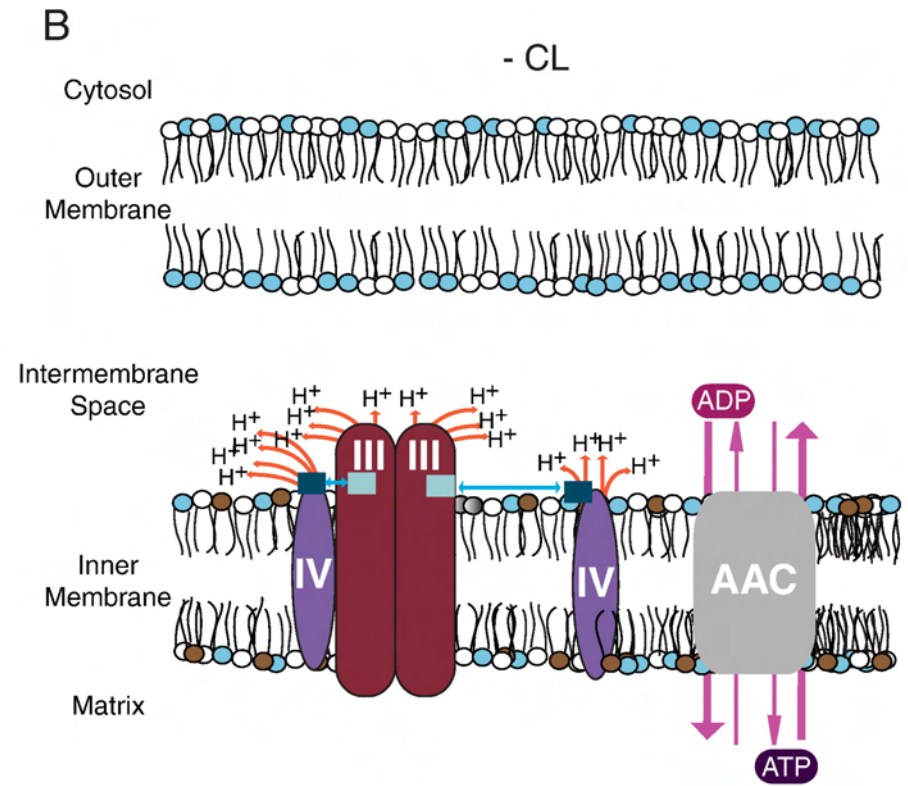
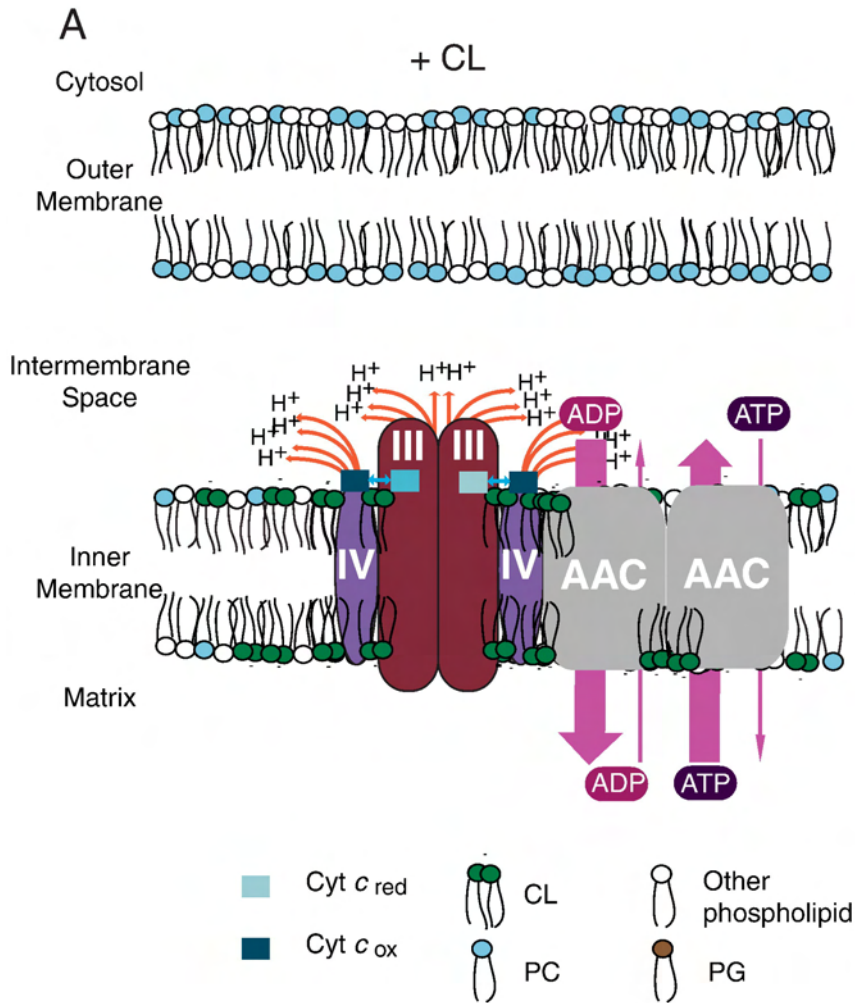
3. What are side-effects of chronic Dox administration?

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

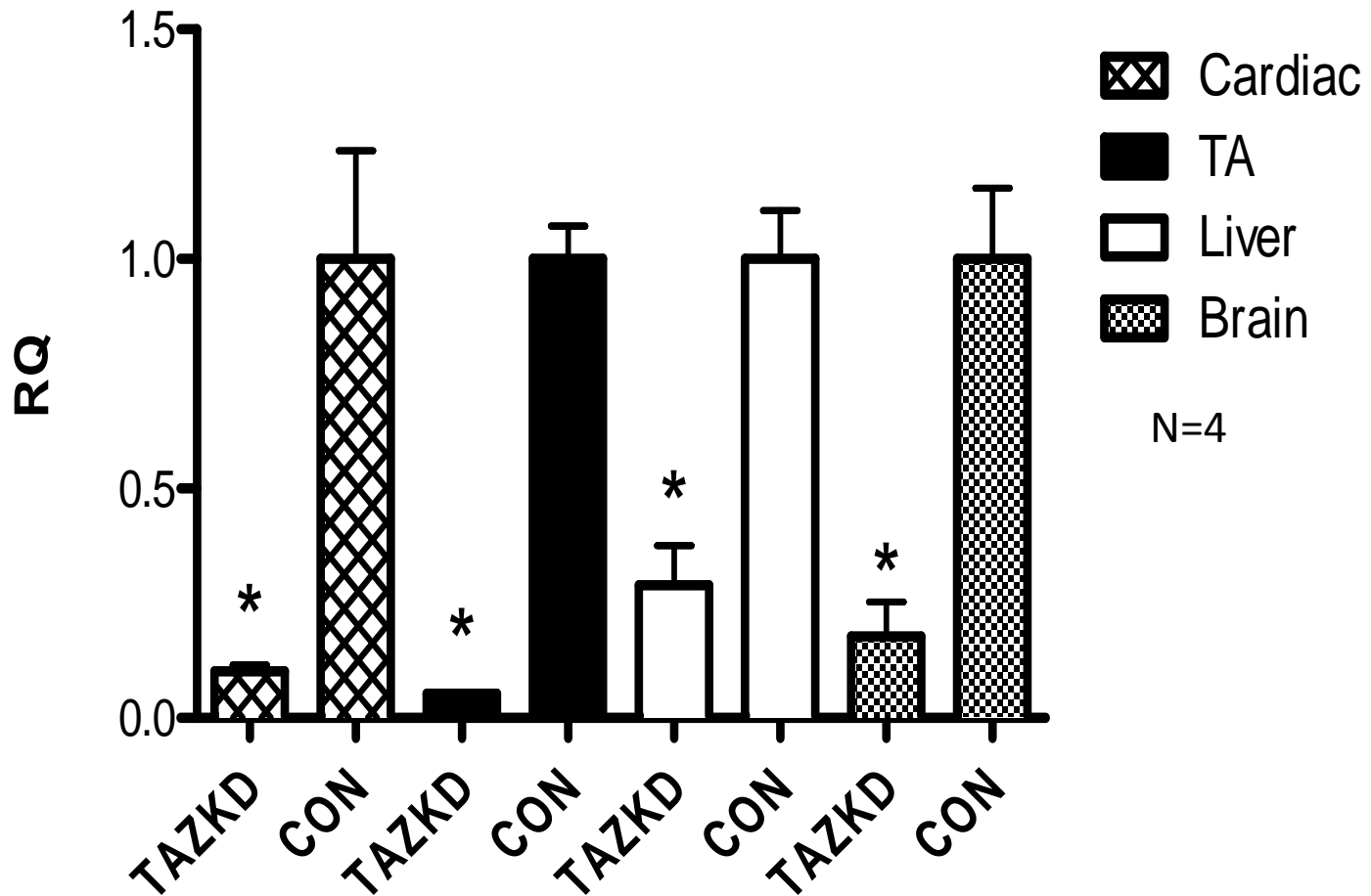
4. Is it reversible?

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

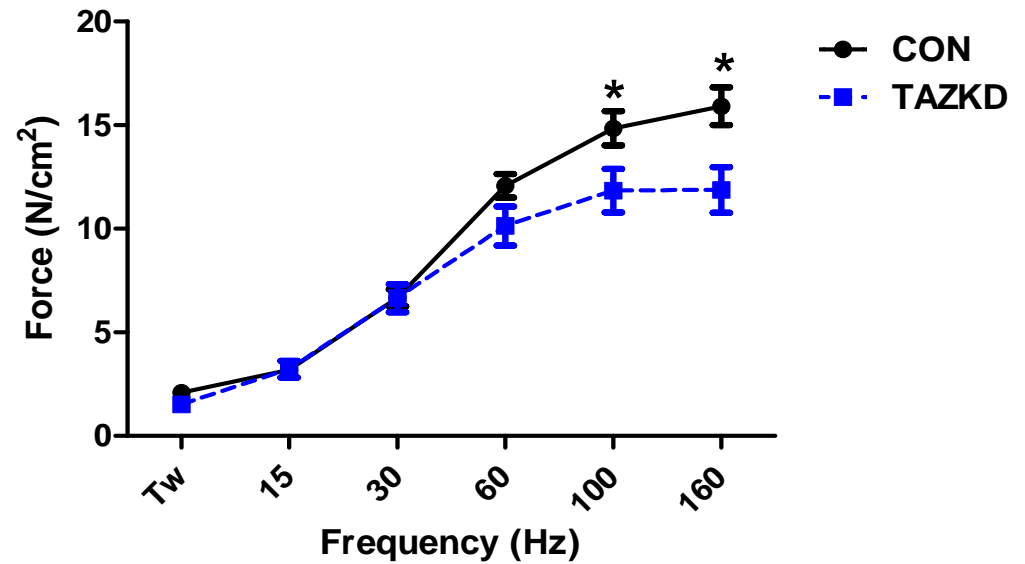
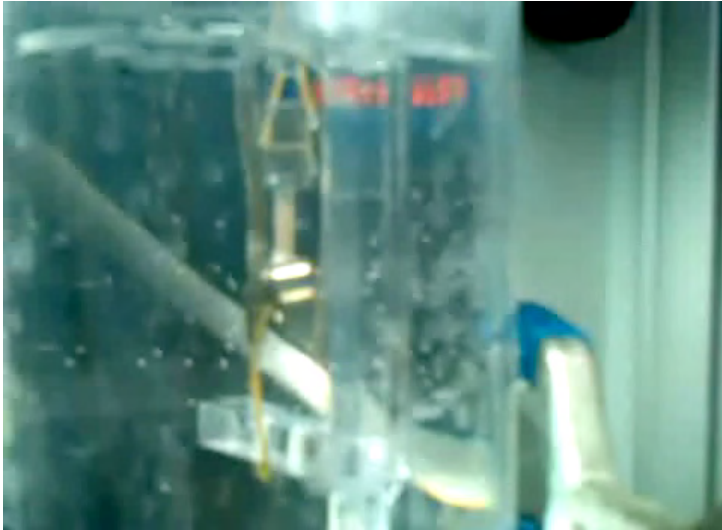
Impact of Cardiolipin on IMM



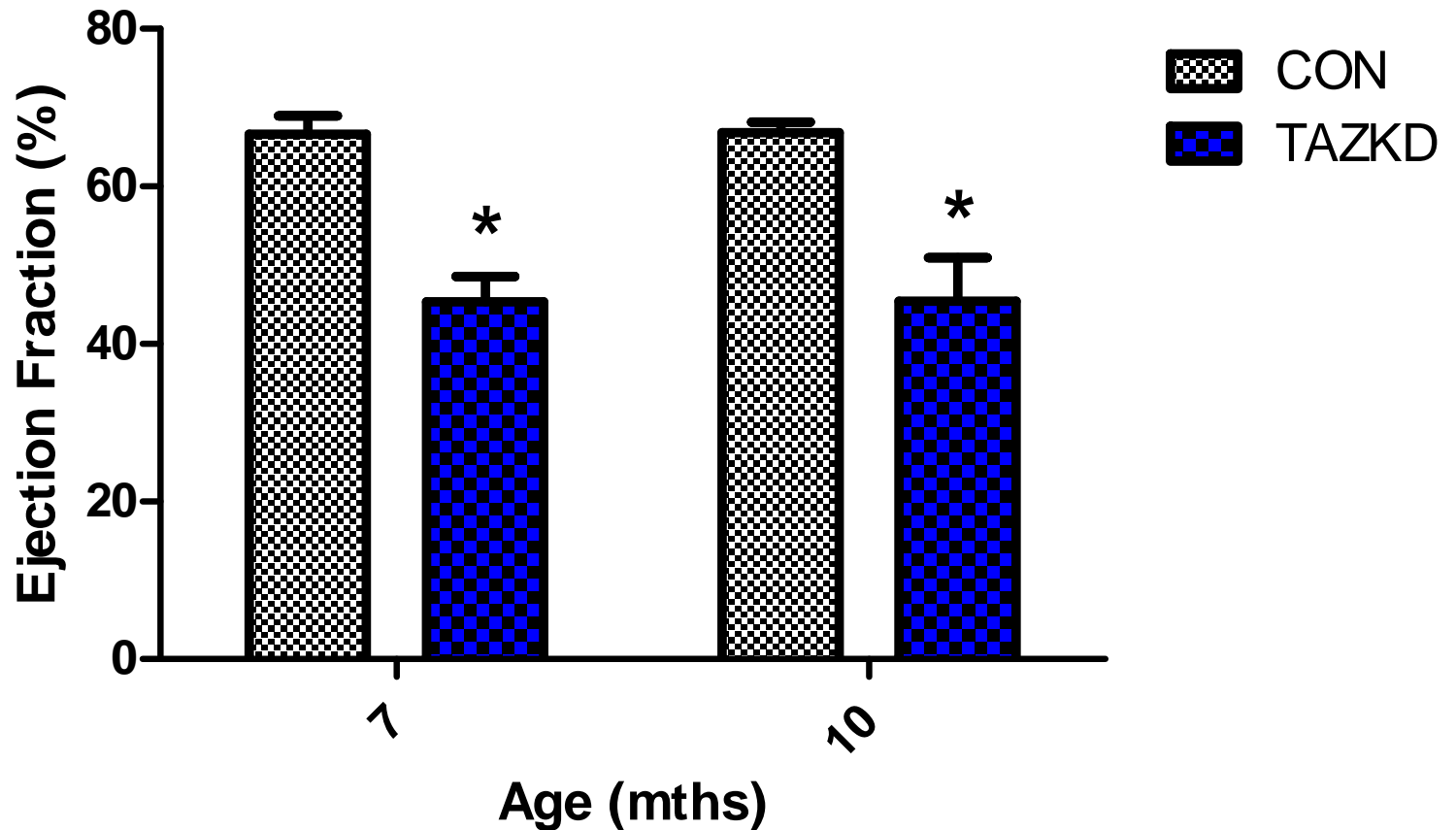
Dox shRNA-induction causes significant TAZ knockdown



TAZKD Results in Impaired Contractility in the Soleus



TAZKD Results in Reduced Cardiac Function



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



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

Acknowledgements

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