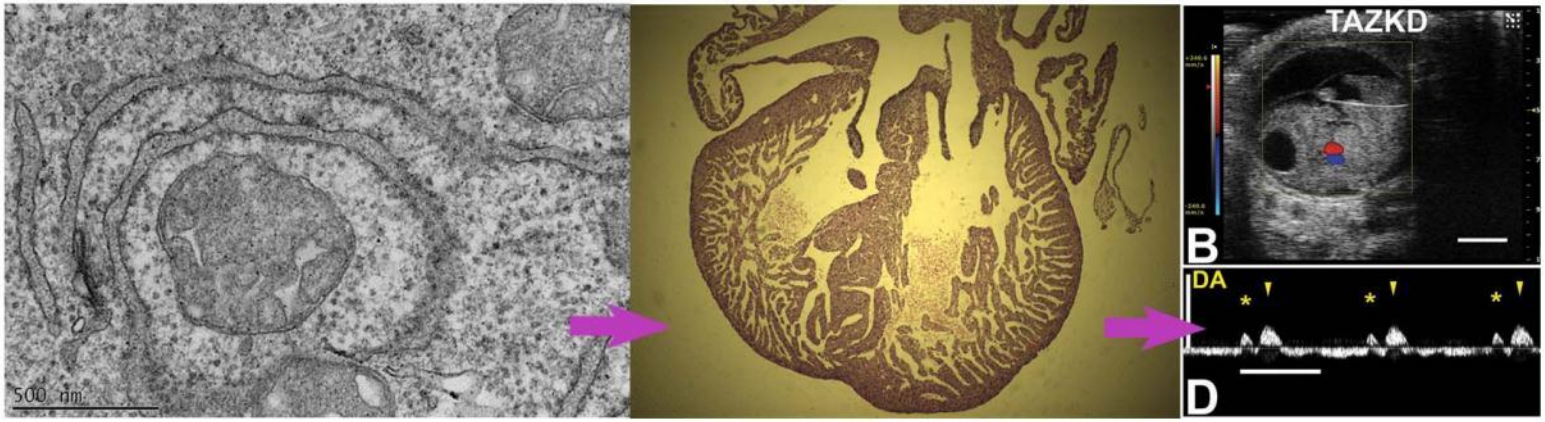
Barth Syndrome

6th International Scientific, Medical, and Family Conference

June 29, 2012



Developmental Noncompaction Cardiomyopathy in a Mouse Model of Barth Syndrome

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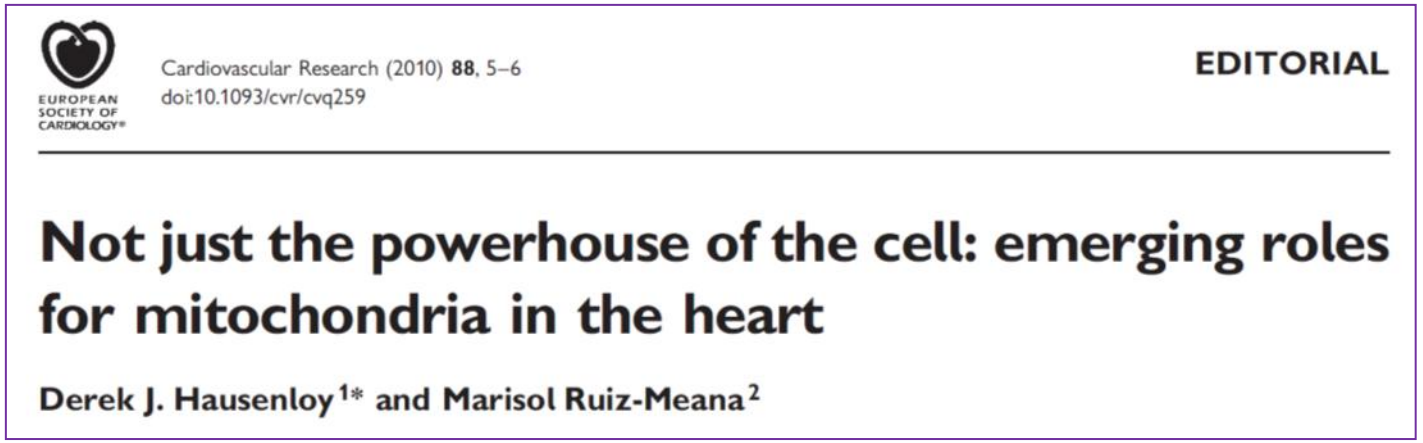
NYU LANGONE MEDICAL CENTER

nyu children's hospital

Barth Syndrome Foundation

Mitochondria & heart development

- Mitochondrial disorders
- Mitochondrial disorders as a category suggest a role of mitochondrial functioning in myocardial & heart development.
- Barth syndrome



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Cardiovascular Research (2010) **88**, 5–6
doi:10.1093/cvr/cvq259

EDITORIAL

Not just the powerhouse of the cell: emerging roles for mitochondria in the heart

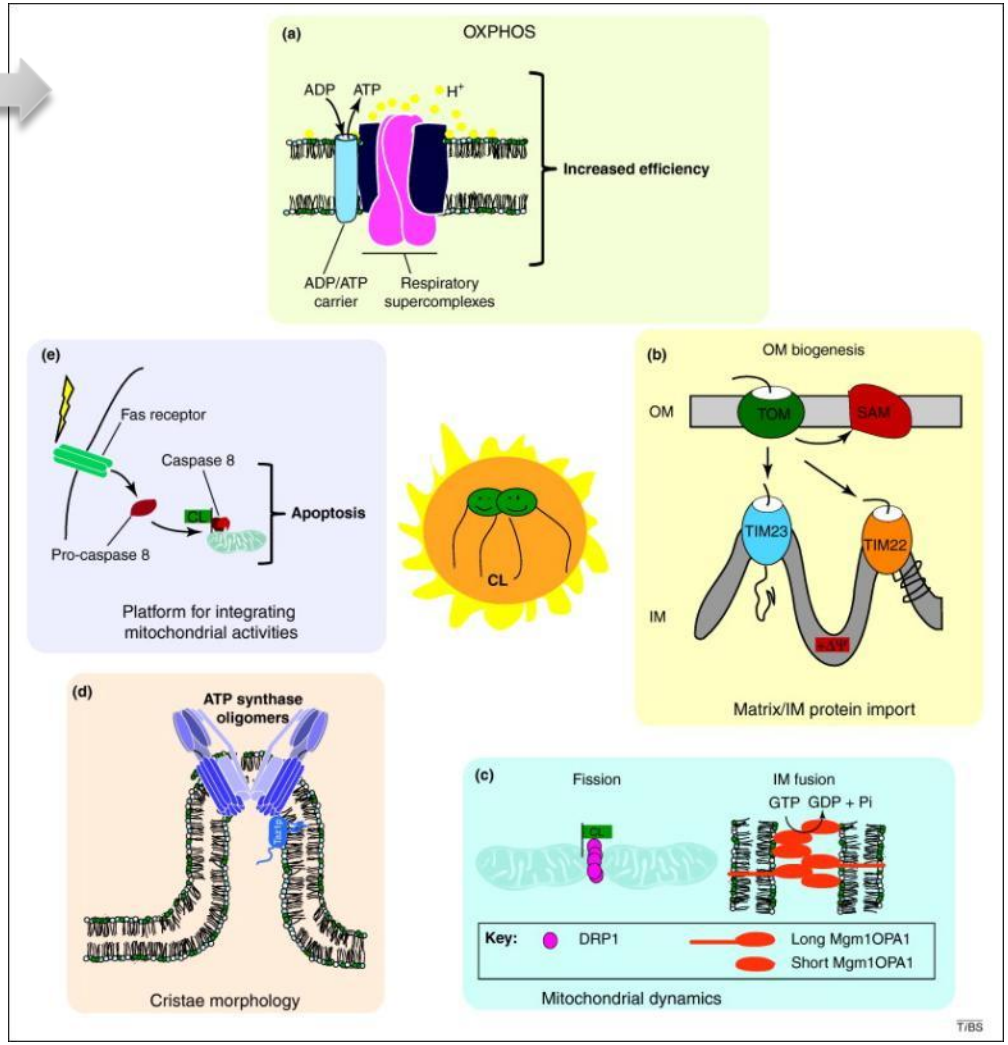
Derek J. Hausenloy[1]* and Marisol Ruiz-Meana[2]

Cardiolipin, the center of mitochondrial physiology

Tafazzin (taz) encodes for an acyltransferase involved in the maturation of the phospholipid cardiolipin

Mitochondrial functions:

- Bioenergetics
- Apoptosis
- Calcium homeostasis
- Cellular redox balance
- Biosynthetic pathways
- Transcriptional control, cellular proliferation pathways
- Heme synthesis reactions
- Immune responses



Claypool & Koehler, *TiBS* 2011

Barth syndrome: cardiolipin deficiency

- X-linked (Xq28): mutations in the *taz* gene

Journal of the Neurological Sciences, 1983, 62: 327–355
Elsevier

AN X-LINKED MITOCHONDRIAL DISEASE AFFECTING CARDIAC MUSCLE, SKELETAL MUSCLE AND NEUTROPHIL LEUCOCYTES

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(Received 1 November, 1982)
(Revised, received 11 August, 1983)
(Accepted 17 August, 1983)

SUMMARY

An X-linked recessive disease is reported in a large pedigree. The disease is characterised by a triad of dilated cardiomyopathy, neutropenia and skeletal

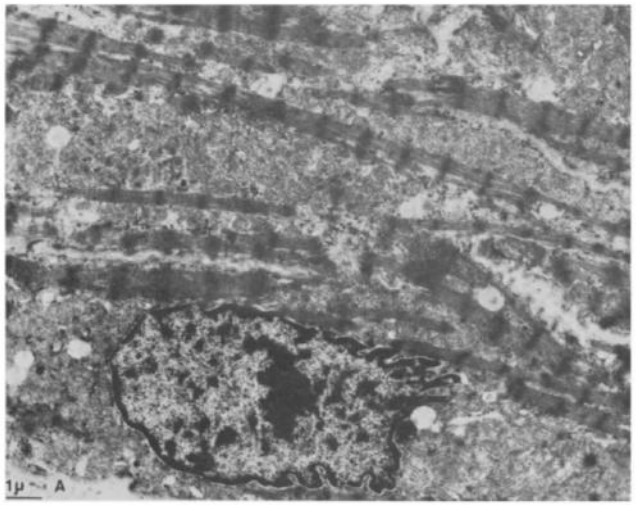
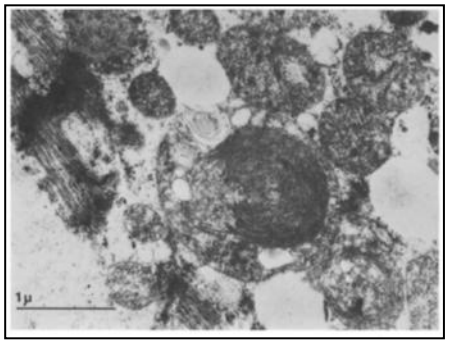
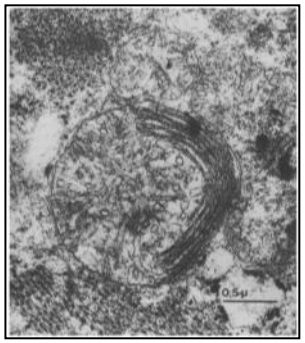
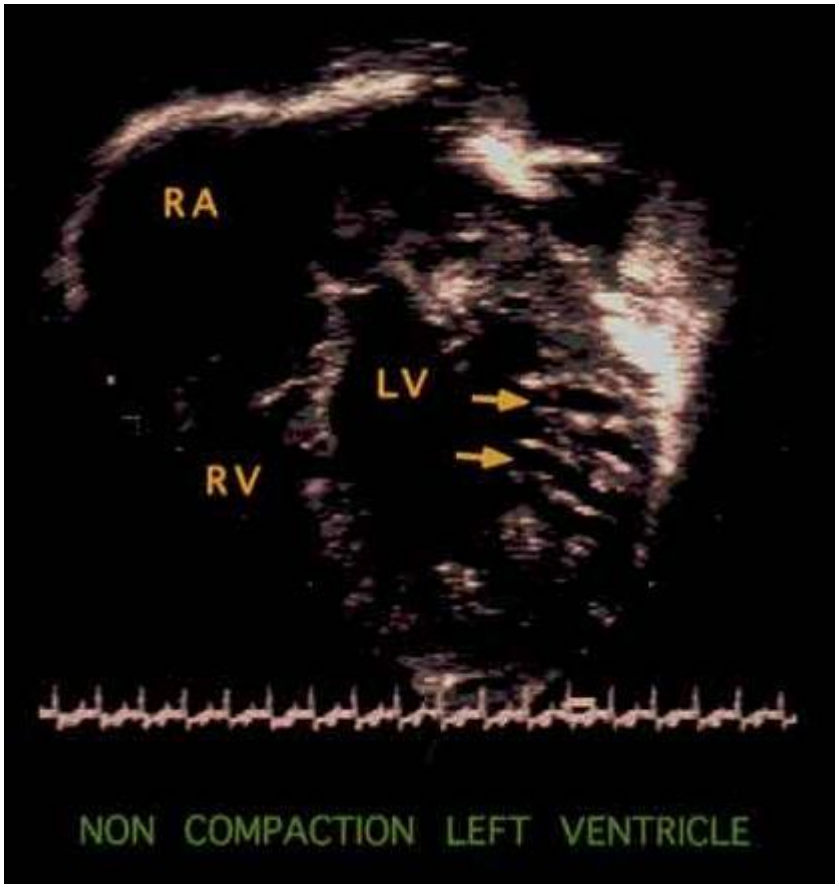


Fig. 4.4. Case V-12. Cardiac myofibres from left ventricle. Longitudinal section prepared for electron microscopy. The fibres have a relative lack of myofibrils, the expanded sarcoplasm is studded with mitochondria.

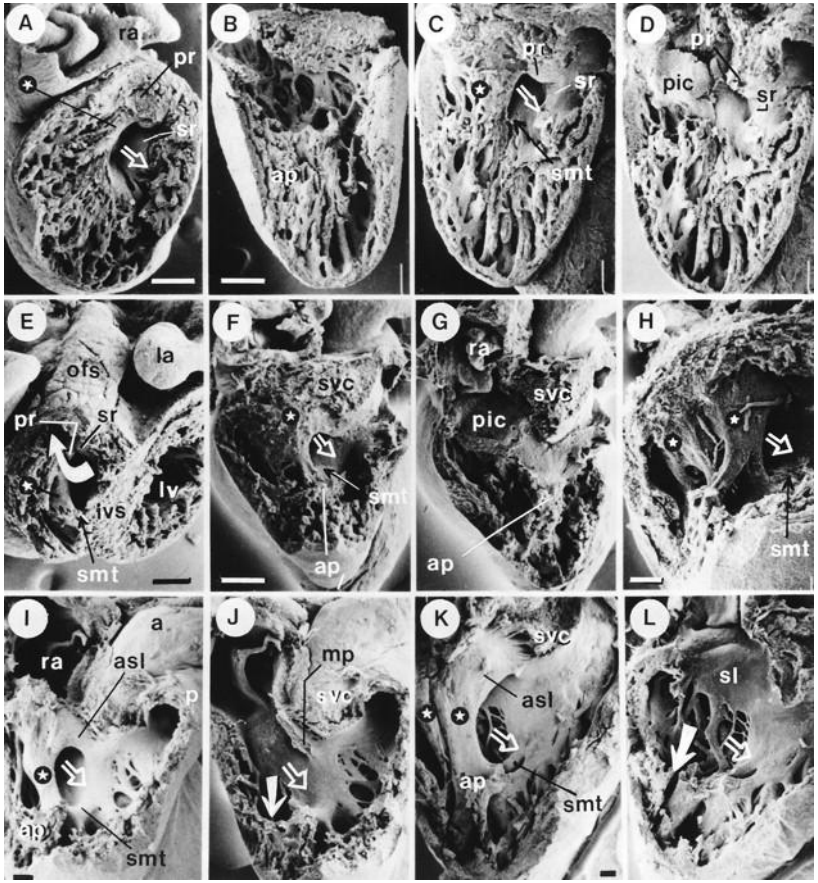




Myocardial trabeculation & compaction



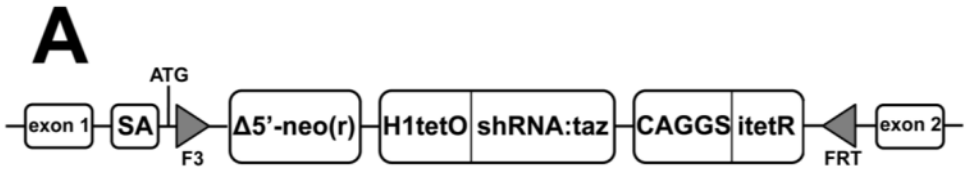
LV noncompaction in Barth syndrome
Towbin & Bowles, 2001

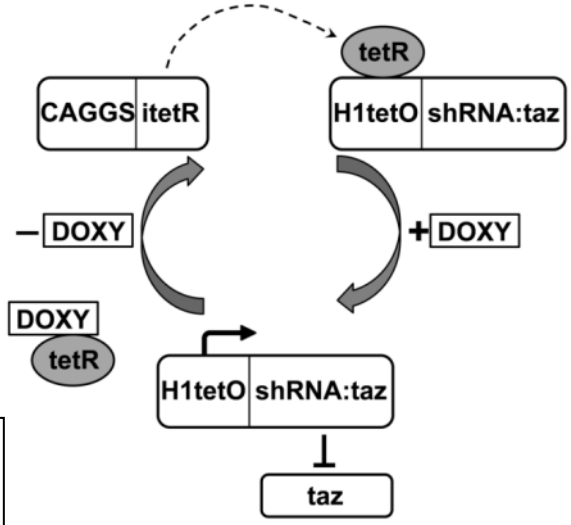


Trabeculation & compaction in human embryonic hearts
Lamers et al., 1995

Model for Barth syndrome?

- Model organisms: yeast, Drosophila, zebrafish
- Traditional mouse knockout genetics: unsuccessful
- Proprietary shRNA knockdown strategy





(TaconicArtemis)

Tafazzin Knockdown in Mice Leads to a Developmental Cardiomyopathy With Early Diastolic Dysfunction Preceding Myocardial Noncompaction

Colin K. L. Phoon, MPhil, MD; Devrim Acehan, PhD; Michael Schlame, MD; David L. Stokes, PhD; Irit Edelman-Novemsky, PhD; Dawen Yu, PhD; Yang Xu, PhD; Nitya Viswanathan, MD; Mindong Ren, PhD

Background—Barth syndrome is a rare, multisystem disorder caused by mutations in *tafazzin* that lead to cardiolipin deficiency and mitochondrial abnormalities. Patients most commonly develop an early-onset cardiomyopathy in infancy or fetal life.

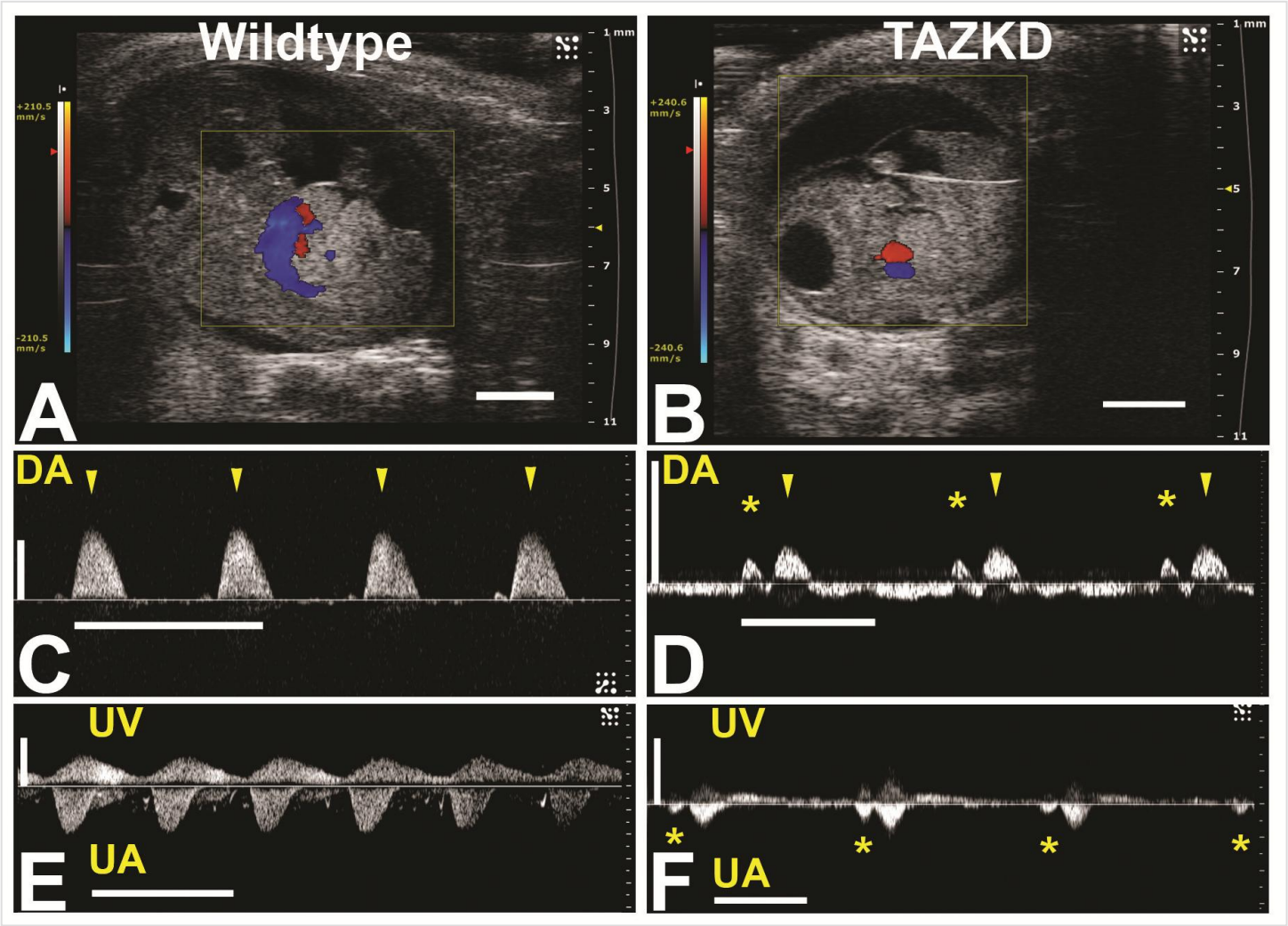
Methods and Results—Knockdown of *tafazzin* (TAZKD) in a mouse model was induced from the start of gestation via a doxycycline-inducible shRNA transgenic approach. All liveborn TAZKD mice died within the neonatal period, and in vivo echocardiography revealed prenatal loss of TAZKD embryos at E12.5-14.5. TAZKD E13.5 embryos and newborn mice demonstrated significant *tafazzin* knockdown, and mass spectrometry analysis of hearts revealed abnormal cardiolipin profiles typical of Barth syndrome. Electron microscopy of TAZKD hearts demonstrated ultrastructural abnormalities in mitochondria at both E13.5 and newborn stages. Newborn TAZKD mice exhibited a significant reduction in total mitochondrial area, smaller size of individual mitochondria, reduced cristae density, and disruption of the normal parallel orientation between mitochondria and sarcomeres. Echocardiography of E13.5 and newborn TAZKD mice showed good systolic function, but early diastolic dysfunction was evident from an abnormal flow pattern in the dorsal aorta. Strikingly, histology of E13.5 and newborn TAZKD hearts showed myocardial thinning, hypertrabeculation and noncompaction, and defective ventricular septation. Altered cellular proliferation occurring within a narrow developmental window accompanied the myocardial hypertrabeculation-noncompaction.

Conclusions—In this murine model, tafazzin deficiency leads to a unique developmental cardiomyopathy characterized by ventricular myocardial hypertrabeculation-noncompaction and early lethality. A central role of cardiolipin and mitochondrial functioning is strongly implicated in cardiomyocyte differentiation and myocardial patterning required for heart development. (***J Am Heart Assoc.* 2012;1:e000455 doi: 10.1161/JAHA.111.000455.**)

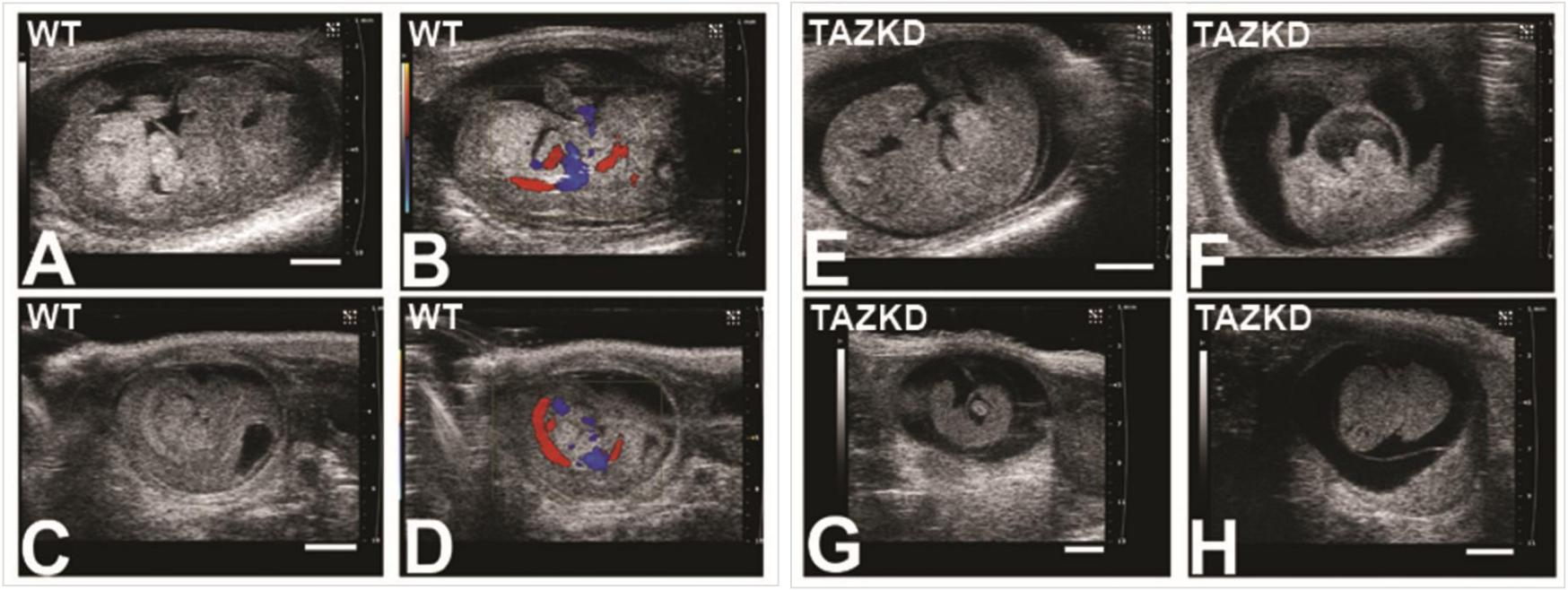
Key Words: Barth syndrome • cardiolipin • mitochondrial disease • noncompaction cardiomyopathy • tafazzin

Phoon et al. *J Am Heart Assoc* 2012

Cardiac dysfunction in TAZKD embryos



Taz knockdown leads to prenatal lethality

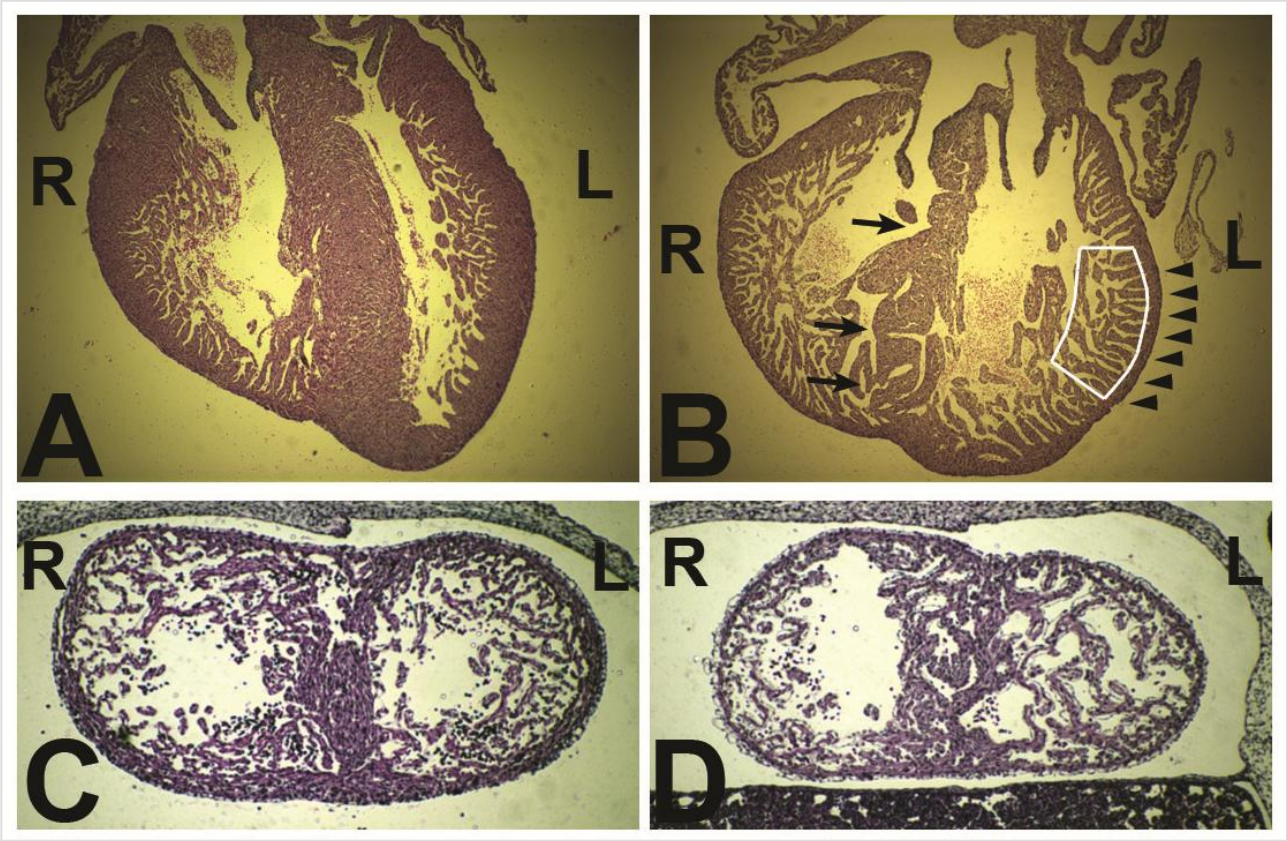


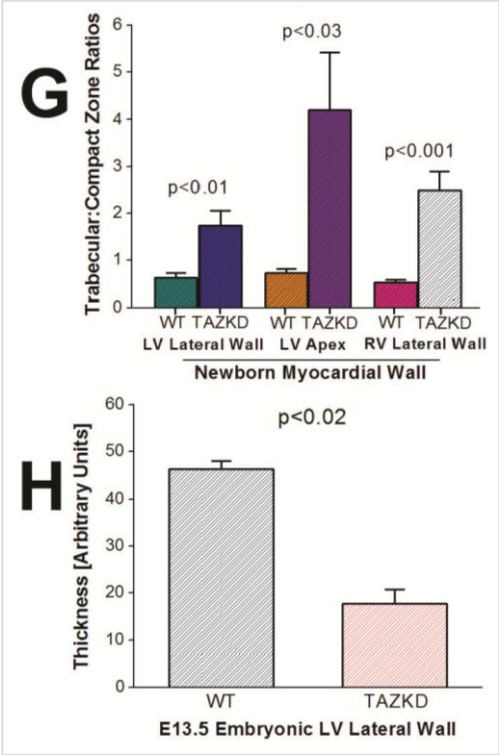
Evidence for pre-/perinatal lethality

- Uninduced litters: expected Mendelian ratios at birth
- One litter imaged at E14.5:
  - 8 live+2 resorbed embryos at E14.5
  - 6 live pups born, all WT

STAGE	TOTAL	WT Alive	WT Dead	TAZKD Alive	TAZKD Dead
E12.5	14	7	1	3	3
E13.5	67	31	2	29	5
E14.5	28	18	1	3	6
Newborn	60	35	0	13	12

TAZKD mice exhibit noncompaction





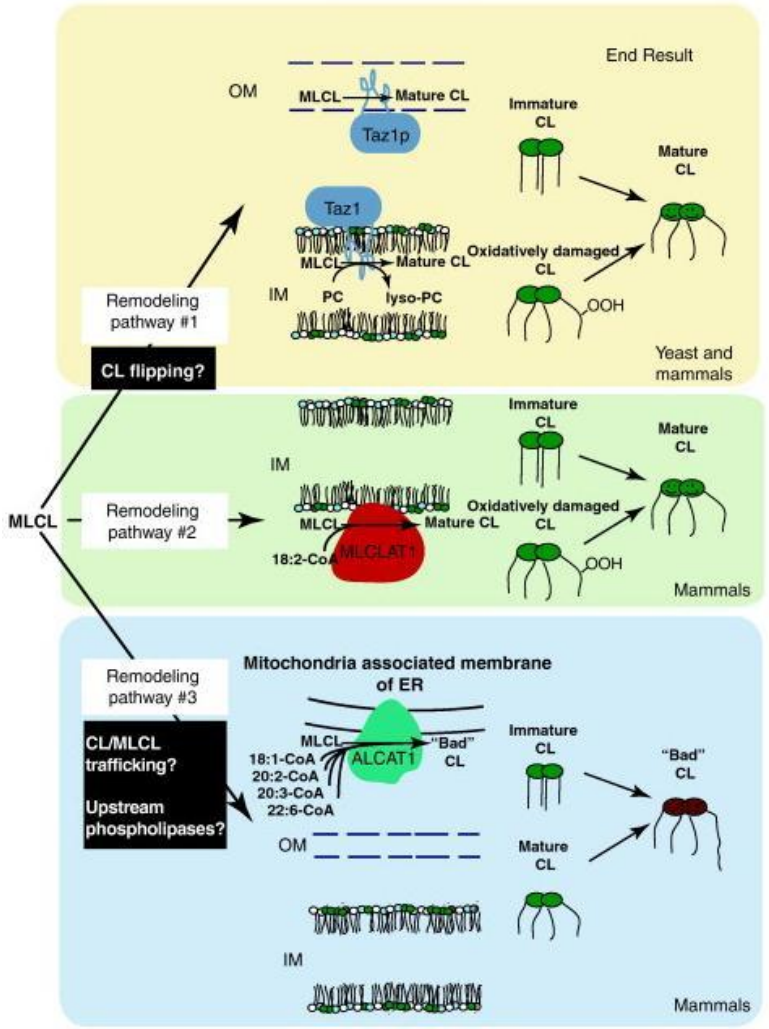
E13.5 Embryos In Vivo	End-diastolic Area (biV) (mm^2)	Fractional Area Shortening	Dorsal Ao peak velocity (mm/s)	Isovolumic Relaxation Time (msec)
WT	1.969 ±0.057	42.1% ±1.7	103 ±8	53 ±8
TAZKD	1.832 ±0.072	45.5% ±1.3	78* ±8	42 ±9

***p < 0.05**

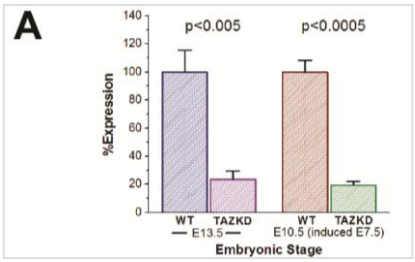
Newborn Mice (few hours old)	End-diastolic Area (LV only) (mm^2)	Fractional Area Shortening	LV diastolic wall thickness (mm)
WT	1.413 ±0.070	50.8% ±1.4	0.26 ±0.01
TAZKD	1.375 ±0.058	49.4% ±1.1	0.26 ±0.01

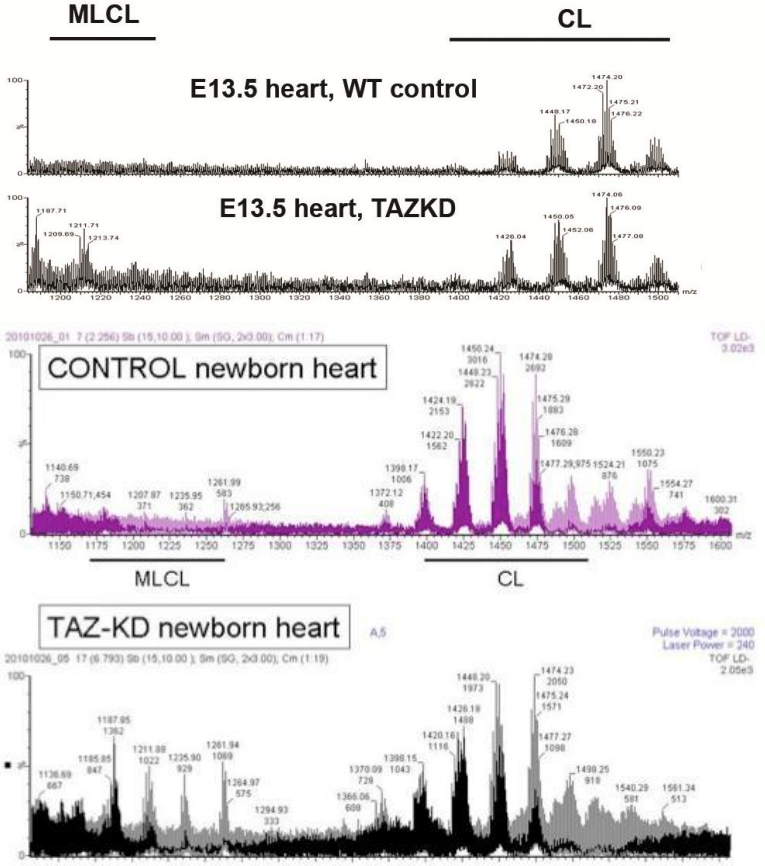
***No significant differences in any indices of heart size or function**

Cardiolipin biochemistry is altered

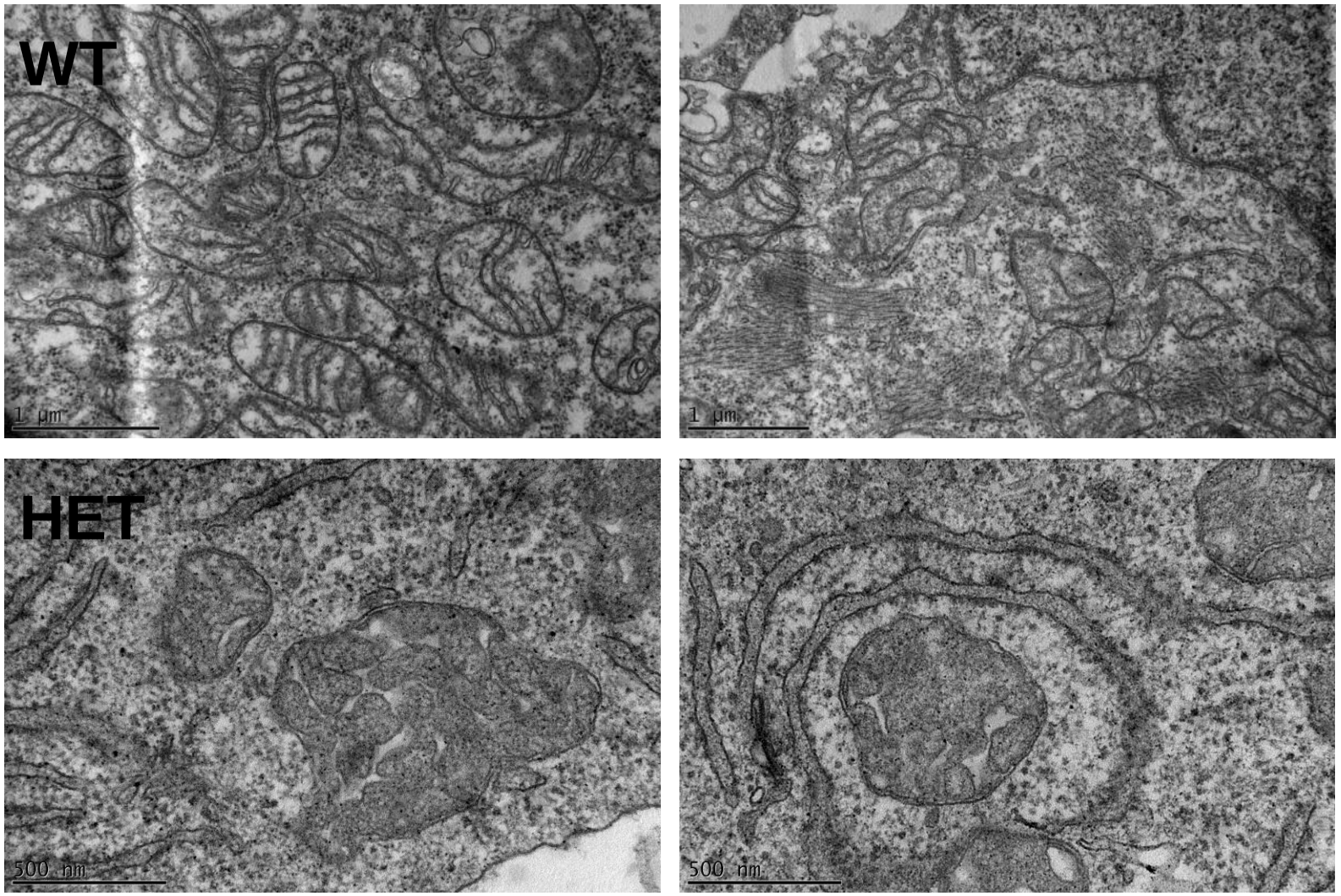


Claypool & Koehler, *TiBS* 2011

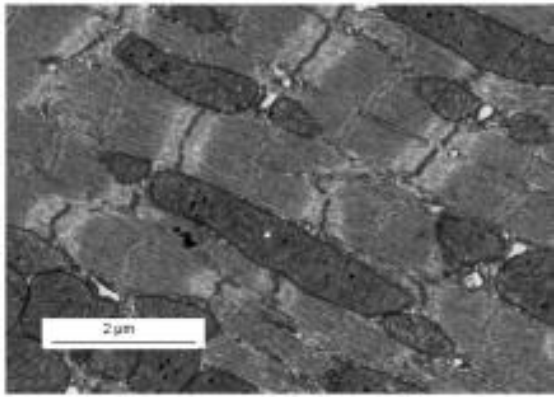




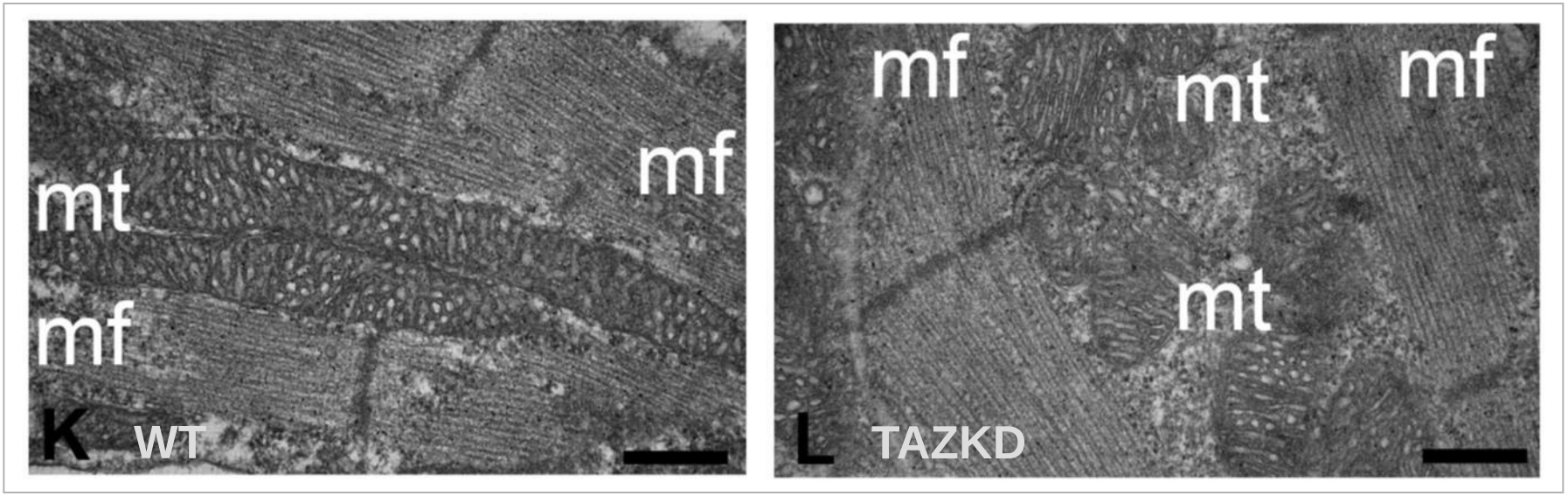
Mitochondria are abnormal: E13.5



Mitochondria-myofibril alignment

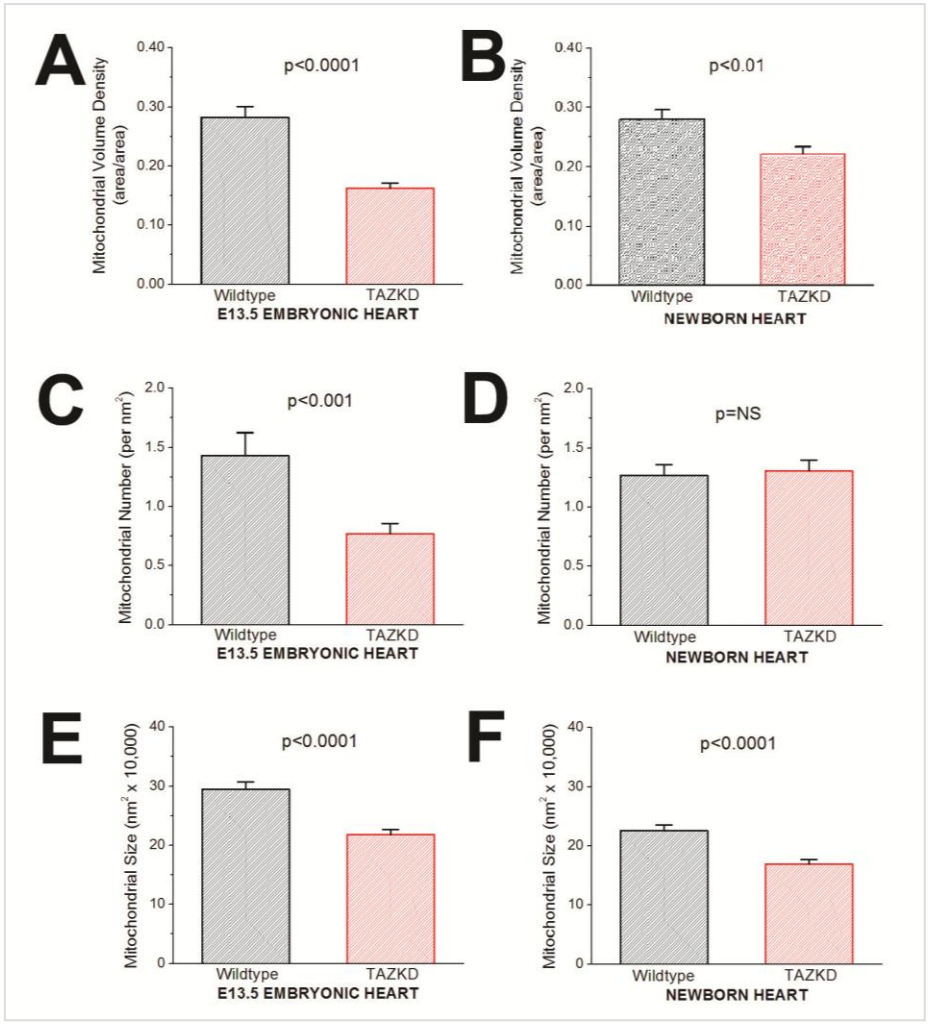


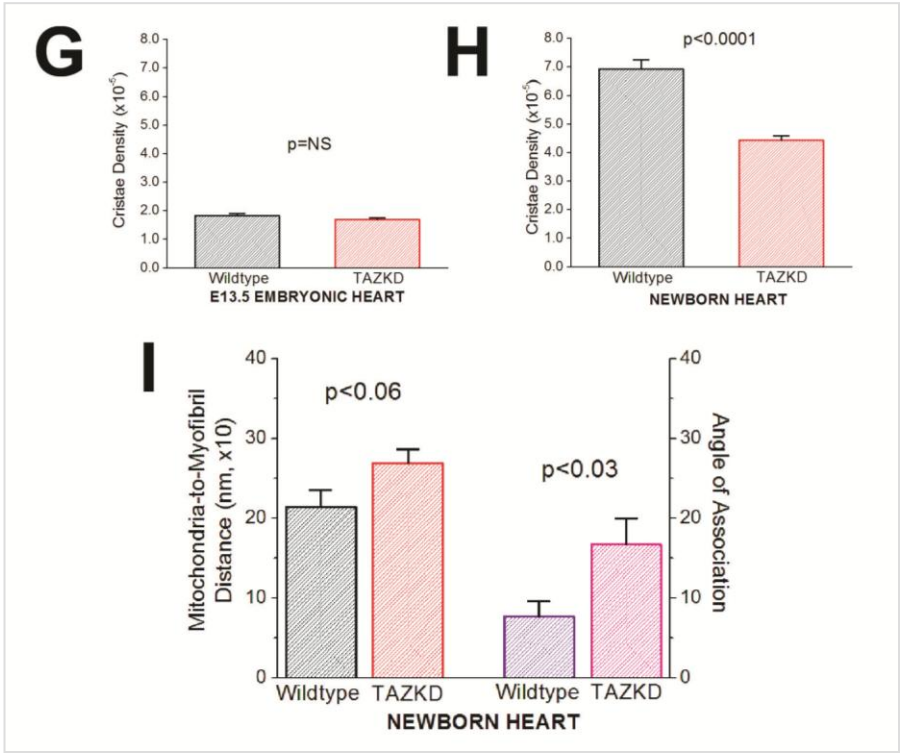
Ong, *Cardiovasc Res* 2010



Newborn myocardium

Abnormal mitochondrial morphometrics





Cardiomyocytes: Less differentiated?

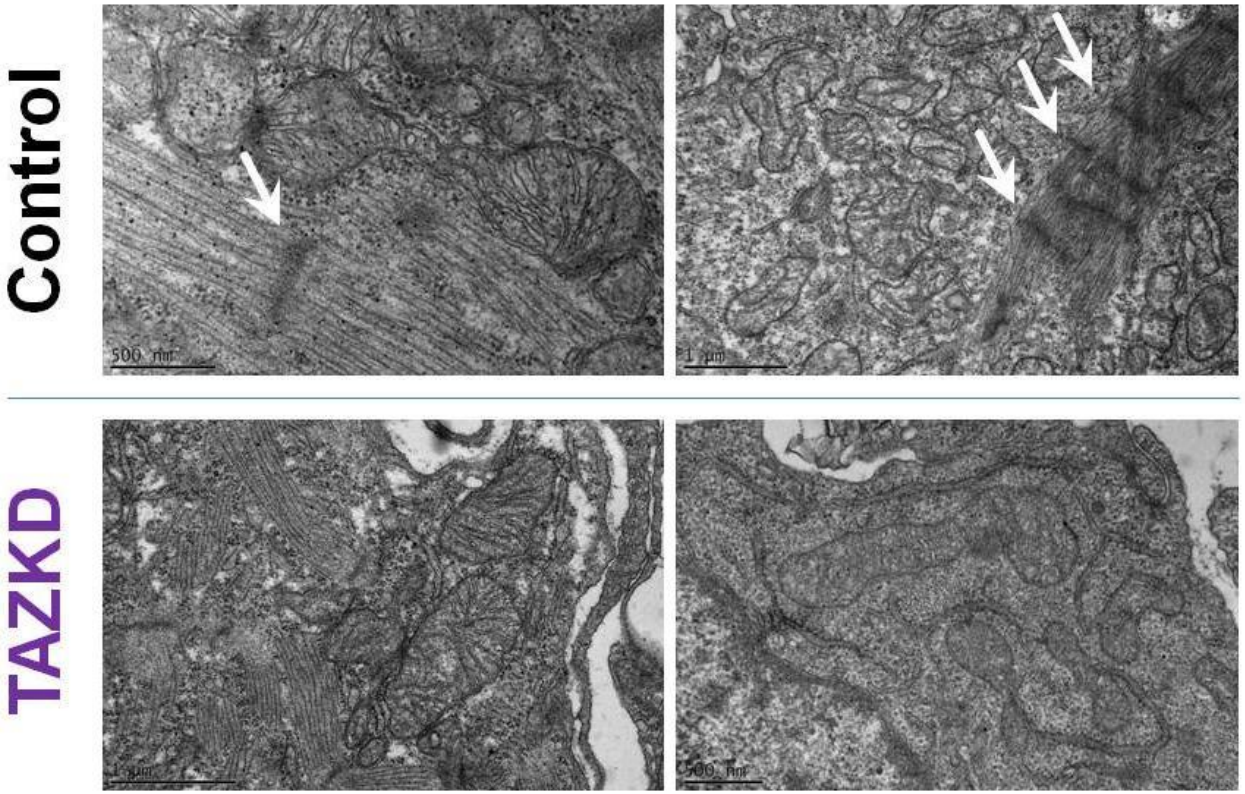
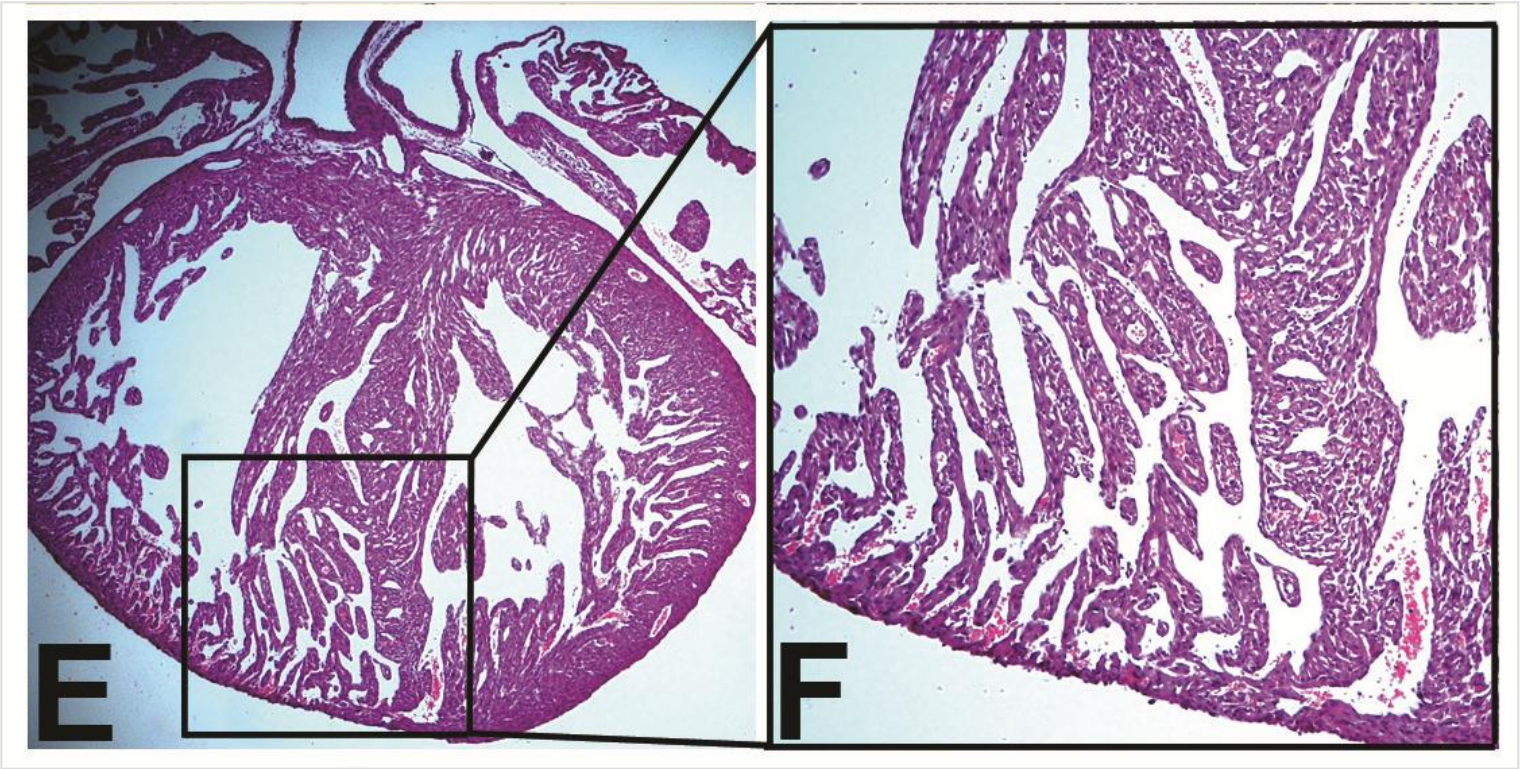


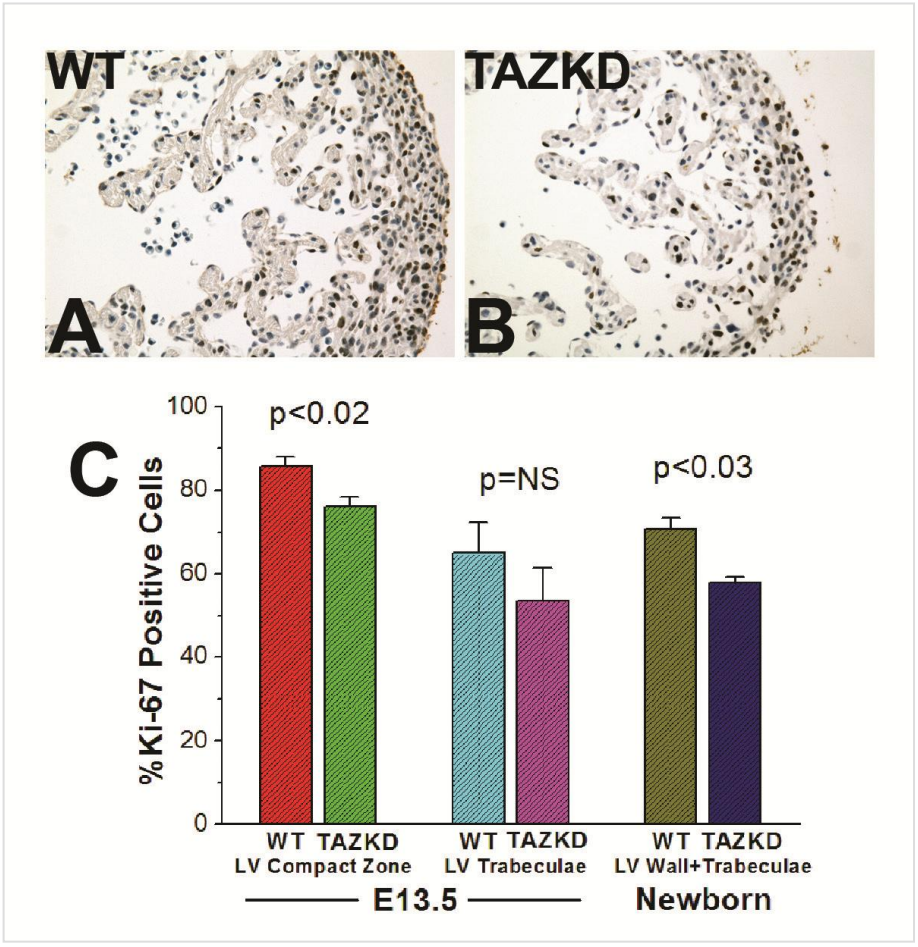
Figure 8. Representative EM's from 2 control and 2 E13.5 TAZKD embryos suggest TAZKD cardiomyocytes are less well-differentiated: myofibrils are lacking in Z-bands (arrows in control mice) and appear less well-organized.

Developmental window of noncompaction



Induced at E10.5

Abnormal cellular proliferation



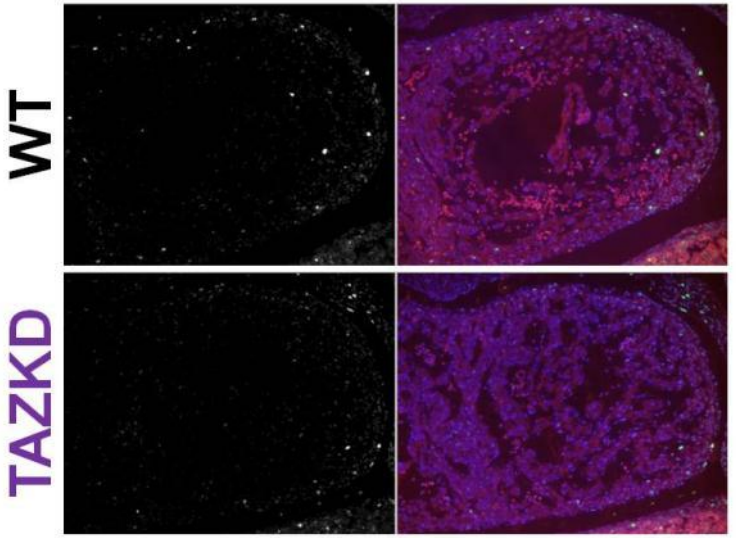


Figure 7. Phosphohistone-H3 (PHH3) immunofluorescence staining of representative E13.5 WT and TAZKD left ventricular sections. Left panels: PHH3, single-channel; right panels: PHH3 (green) merged with DAPI (blue) and troponin (red). Differential cardiomyocyte proliferation in trabecular and compact layers is evident.

Microarray data: E12.5 myocardium

DAVID GO Terms (Functional annotation clusters)	Enrichment Score	Up/Down
Metal ion binding, zinc finger	1.6-5.6	Up/Down
Steroid hormone, nuclear hormone receptor	3.5	Down
Synaptic transmission, neurotransmitter, neuron	2.7-3.5	Down
Protein dimerization, protein binding	2.7	Up
Apoptosis, programmed cell death	2.6	Up
DNA binding, transcription, regulation of RNA metabolic process	2.5	Down
Membrane glycoprotein	2.0	Down
Cell adhesion, cell-cell adhesion	2.0	Down
Cell morphogenesis, neuron morphogenesis	1.8	Down

Role of reactive oxygen species (ROS)?

STEM CELLS
EMBRYONIC STEM CELLS/INDUCED PLURIPOTENT STEM CELLS

Mitochondrial Function Controls Proliferation and Early Differentiation Potential of Embryonic Stem Cells

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• Differentiation

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Review

Mitochondria and calcium signaling in embryonic development

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Developmental Cell
Article

Cell PRESS

The Permeability Transition Pore Controls Cardiac Mitochondrial Maturation and Myocyte Differentiation

Jennifer R. Hom,[1,8] Rodrigo A. Quintanilla,[2,8] David L. Hoffman,[1] Karen L. de Mesy Bentley,[5] Jeffery D. Molkentin,[6] Shey-Shing Sheu,[3,7] and George A. Porter, Jr.[1,3,4,*]

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Developmental Cell
Article

The Permeability Transition Pore Controls Cardiac Mitochondrial Maturation and Myocyte Differentiation

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*Correspondence: george_porter@urmc.rochester.edu
DOI 10.1016/j.devcel.2011.08.008

SUMMARY

Although mature myocytes rely on mitochondria as the primary source of energy, the role of mitochondria in the developing heart is not well known. Here, we find that closure of the mitochondrial permeability transition pore (mPTP) drives maturation of mitochondrial structure and function and myocyte differ-

that mitochondria are important to the development of the heart, as dysfunction of the mitochondrial electron transport chain (ETC) can cause heart malformation and embryonic death between E8.5 and E10.5, suggesting that mitochondrial function is essential to cardiac function and survival of the embryo (Ingraham et al., 2009; Larsson et al., 1998).

Mitochondria in the adult heart are well characterized and occupy over 30% of the cell volume. It is thought that complex

Increased ROS: BTHS, TAZKO cells

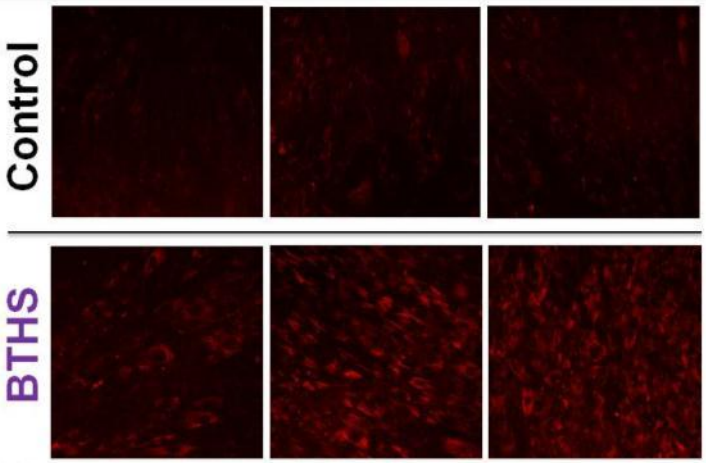


Figure 2. MitoSOX™ Red shows increased mitochondrial ROS in fibroblasts from Barth syndrome patients (BTHS, bottom) vs. controls (top). Cells were plated at comparable density.

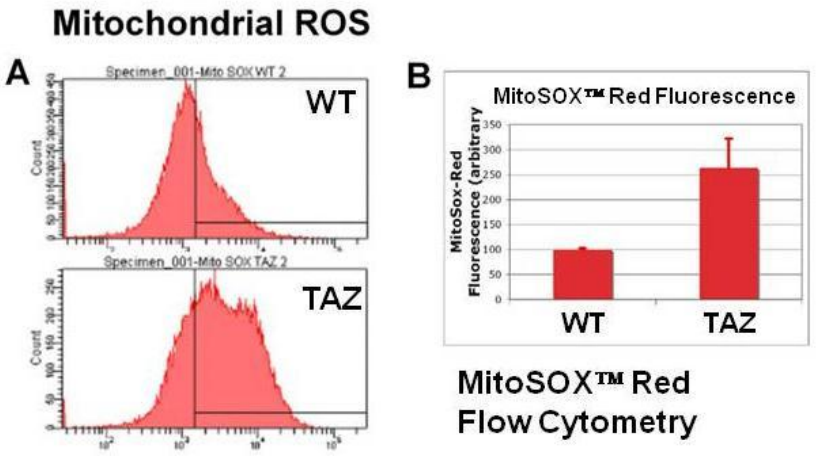


Figure 3. **A,B)** Mouse embryoid-derived fibroblast-like cells in which *tafazzin* was knocked out showed a 2 to 3-fold increase in mitochondrial superoxide in TAZ cells over wildtype (WT). (ESC's courtesy of Dr. Zaza Khuchua; Acehan 2009)

ROS: E12.5 ventricular myocardium

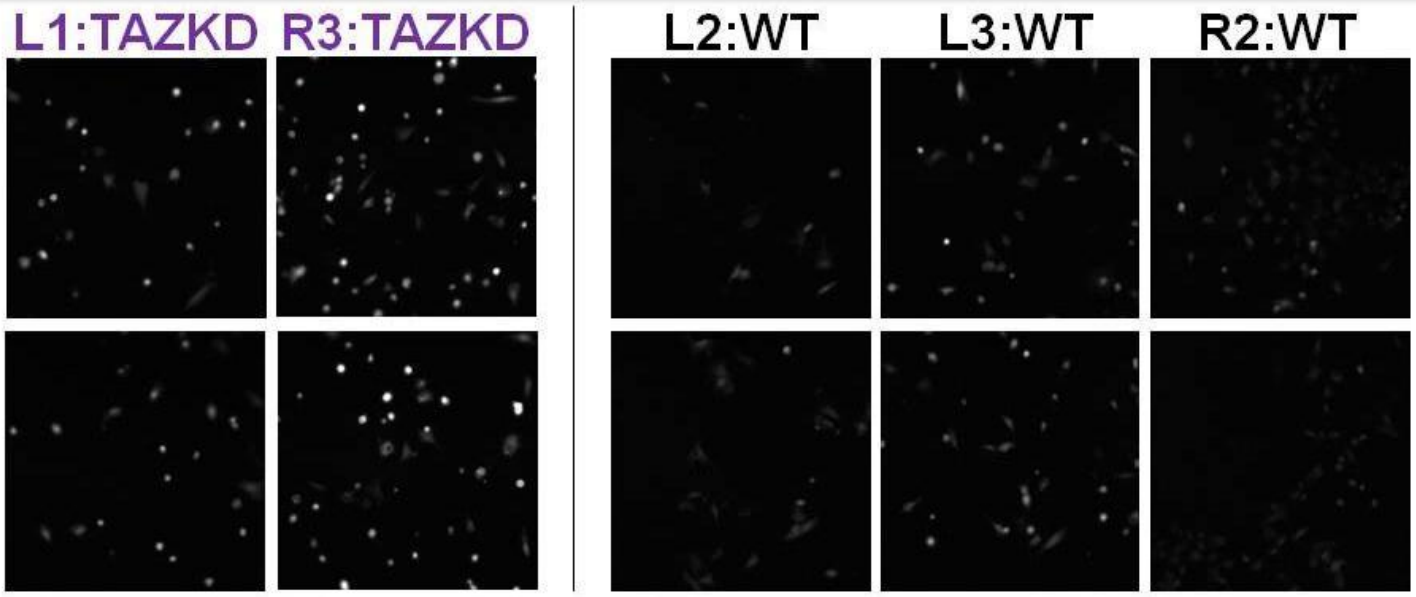


Figure 4. DCF staining shows increased ROS in E12.5 ventricular cardiomyocytes from tafazzin-knockdown (TAZKD, left) vs. wildtype (WT, right). Two representative regions of interest from each plate are shown. Cells were plated at comparable density.

ROS: E18.5 ventricular myocardium

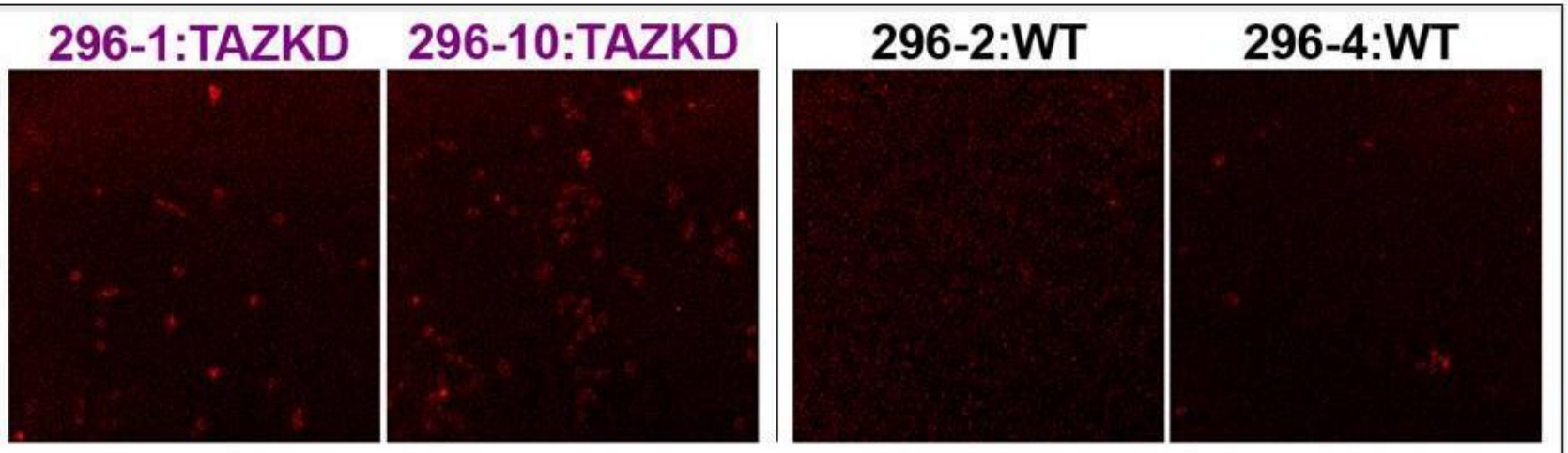
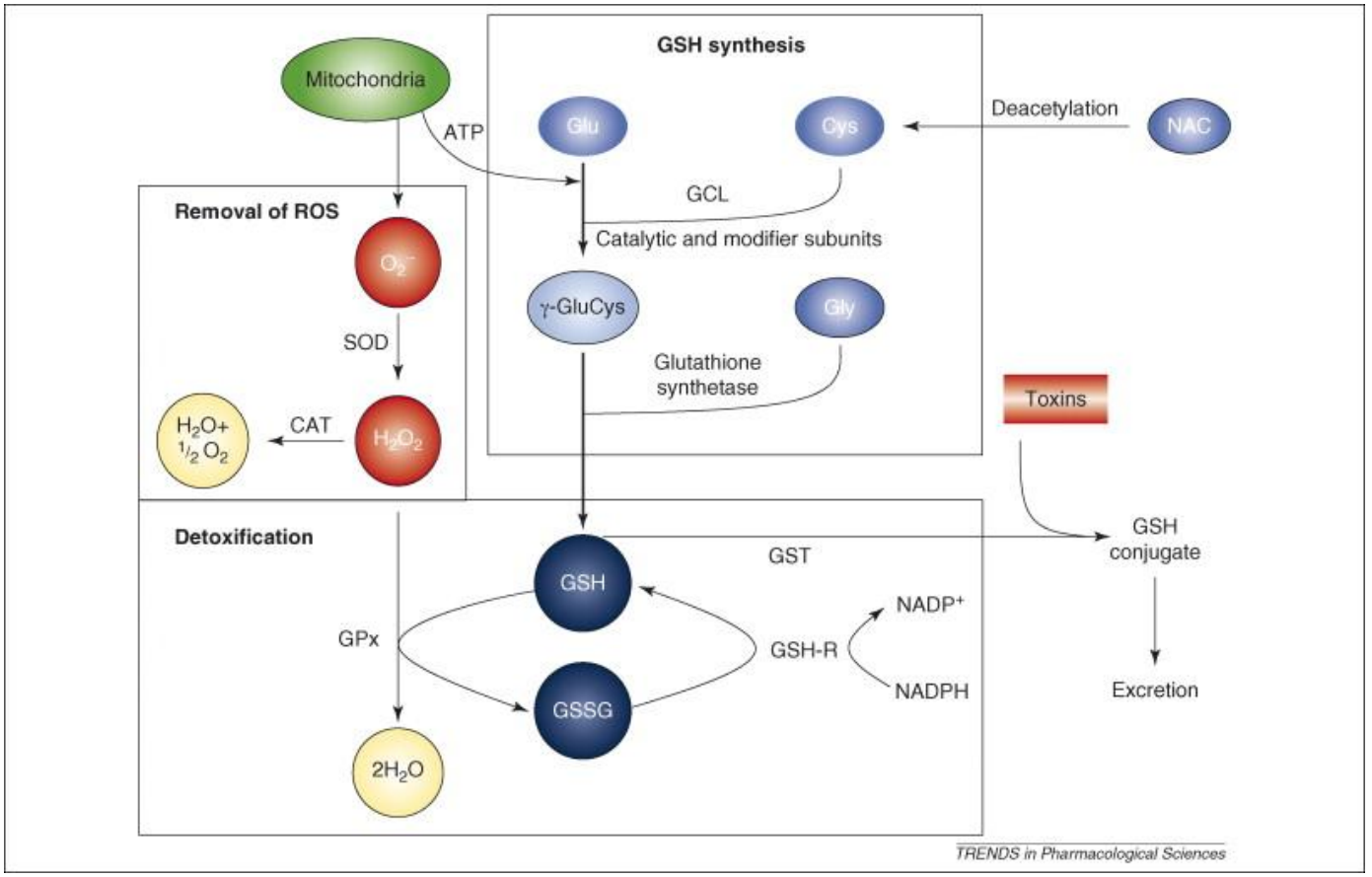


Figure 5. MitoSOX™ Red shows increased mitochondrial ROS in E18.5 *taz*-knockdown (TAZKD, left panels) ventricular cardiomyocytes vs. wildtype (WT, right panels). Cells were plated at comparable density.

N-acetylcysteine



Berk M. TiPS 2008

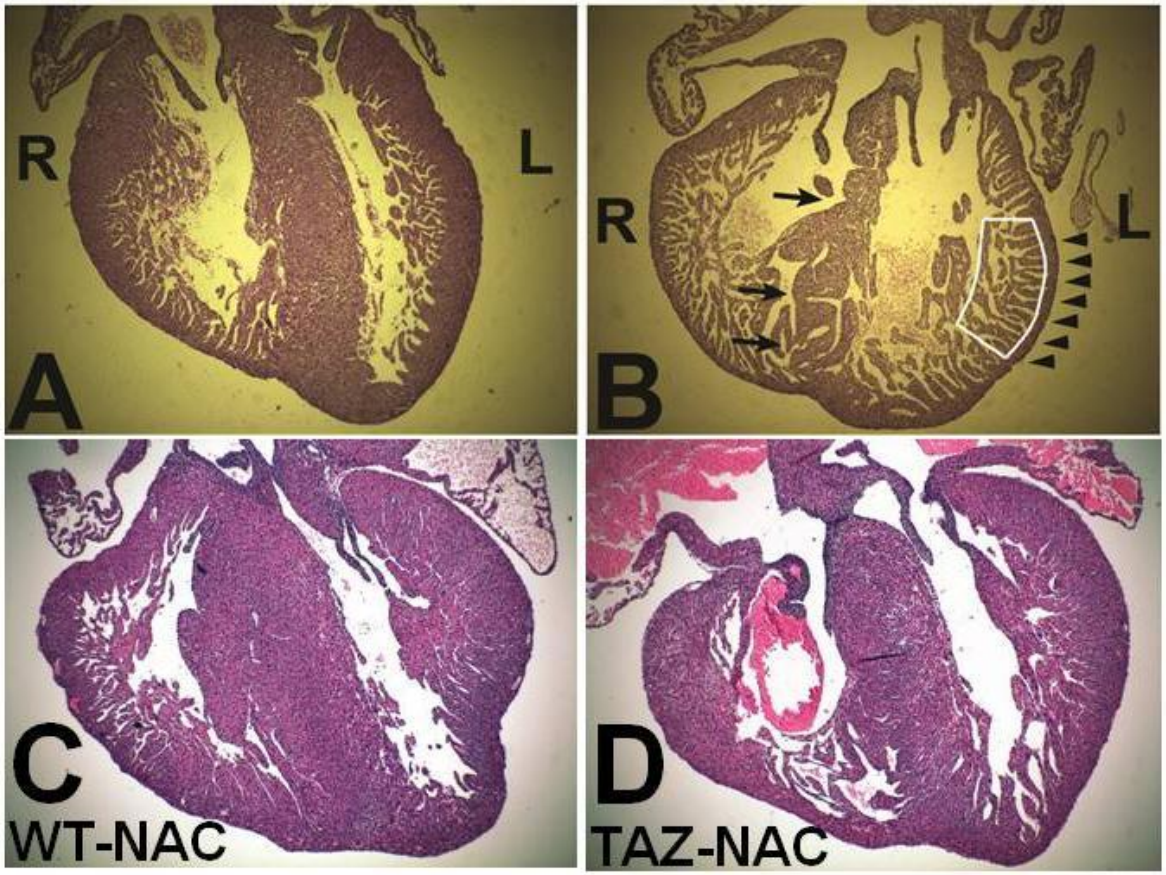


Figure 6. N-acetylcysteine (NAC) partially rescues the HT-NC phenotype in TAZKD newborn mice. **A)** Wildtype; **B)** TAZKD with HT-NC and ventricular septal defects. **C,D)** Newborns of pregnant mothers fed NAC: **(C)** Wildtype and **(D)** TAZKD. (**A** & **B** adapted from Phoon 2012)

Genes Associated with Human LV Noncompaction	Genes Related to Compact Zone & Trabecular Formation, & Cell Cycle Control, Animal Models	Transcriptional Regulators, Factors	PI3K/Akt Pathway-Related Genes
Lmx1b	Rxra (retinoid X receptor alpha)	Zinc finger proteins (many)	Akt-related: Akt2
Nr0b1	Jumonji-related: Jmjd6	GATA's: GATA6	Pleckstrin-related: Ph1db1
	BMP-related: BMPr1b	Klf14	Igf-related: Igfbp2, Igfals
	Neuregulin-related: Nrg2	Nkx family: Nkx2.2	NfκB-related: Nfκbie, Nfκb2
	MAPK-related: Map3k9, Map4k2	p53-related: Trp53rk, Trp53inp2; Ankrd11, MDM2, Timm50	Bcl2-related: Bad, Bnip2, Bmf, Hrk
	Notch-related: Dlk2	Sp2, Sp6	Eef1a2
	E2f2	Cdk9	Rgs2
	Snn		Egfr

Table 1

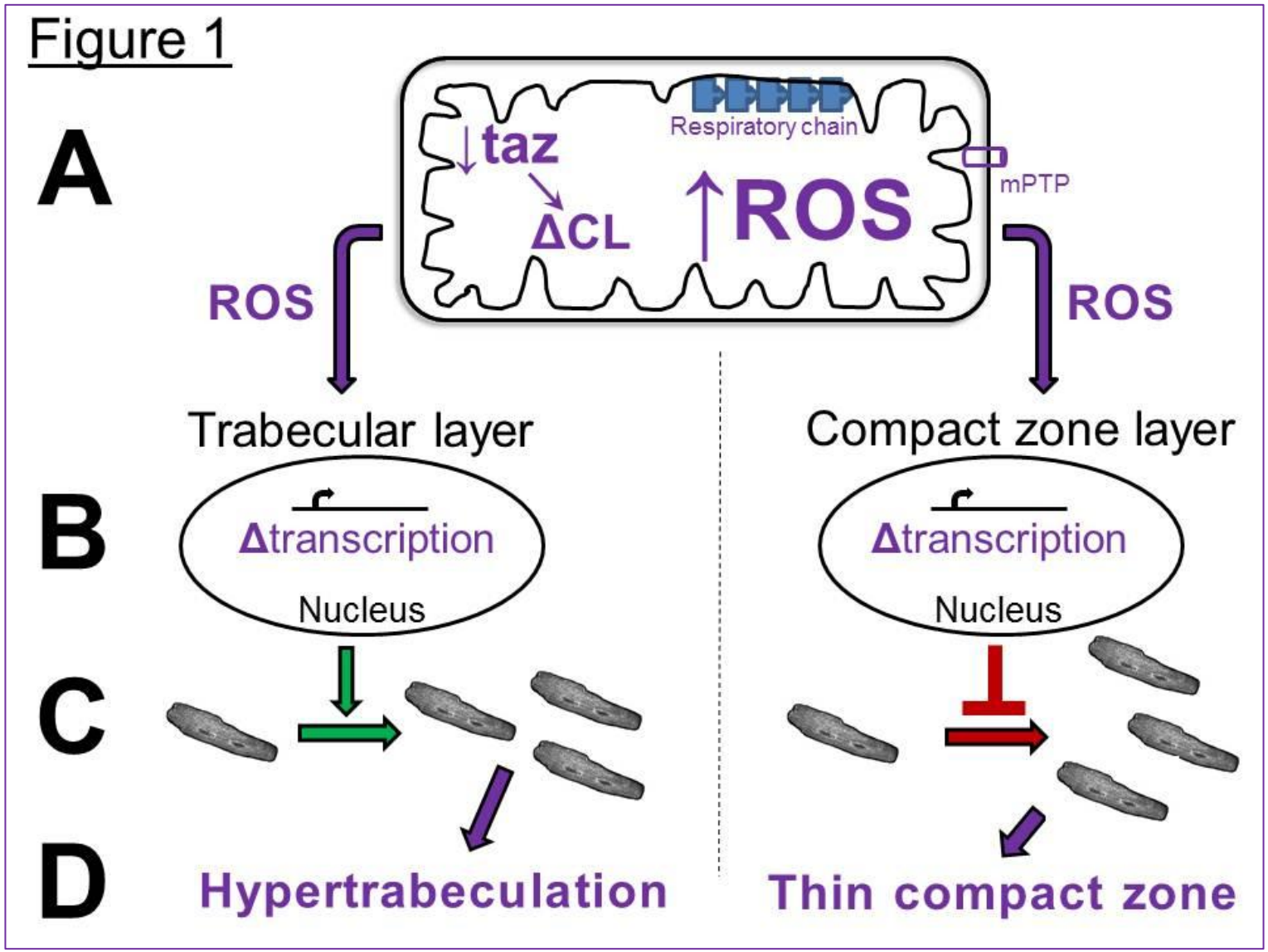


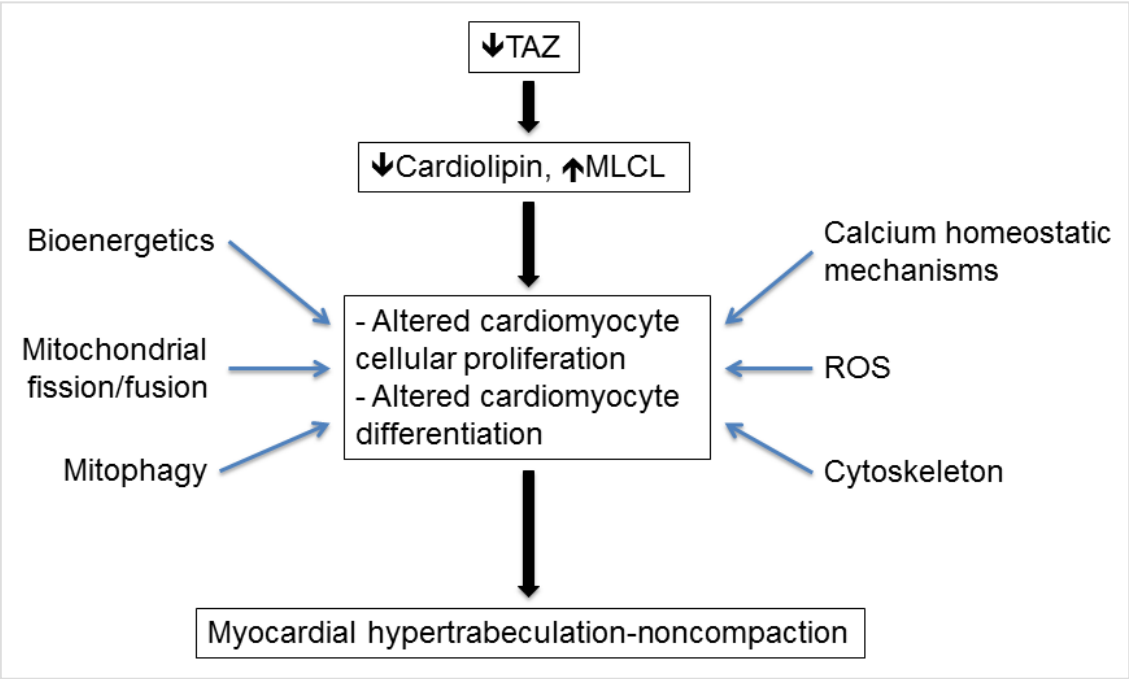
Figure 1
A
taz
ΔCL
Respiratory chain
ROS
mPTP
ROS
ROS
Trabecular layer
Compact zone layer
B
Δtranscription
Nucleus
Δtranscription
Nucleus
C
D
Hypertrabeculation
Thin compact zone

Conclusions

- The TAZKD mouse is a good model for human BTHS.
  - Ventricular hypertrabeculation-noncompaction
  - Myocardial wall thinning
  - Abnormal mitochondrial morphometrics
  - Abnormal mitochondrial functioning: ROS
- Tafazzin knockdown in embryonic vs. adult hearts indicates entirely different roles for mitochondria.
- Mitochondria & heart development: an emerging field
  - Myocardial patterning: possible role of mito-ROS
  - Not just bioenergetics!

Future directions

- How does cardiolipin contribute to mitochondrial development & normal cardiac myoarchitecture?
  - Cell cycling pathways
  - ROS, Ca^{2+} homeostasis, ECM, cell adhesion, cytoskeleton



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