Mechanisms of heart failure: Involvement of Apoptosis

Molecular Mechanisms of Disease (CBNP5068Q)

Dominic Del Re, PhD – April 6, 2015

Overview

1. Background – basics of apoptosis

2. Cardiovascular disease, experimental model

3. Hippo signaling pathway

4. New mechanistic findings of heart injury


UCSD, La Jolla, CA

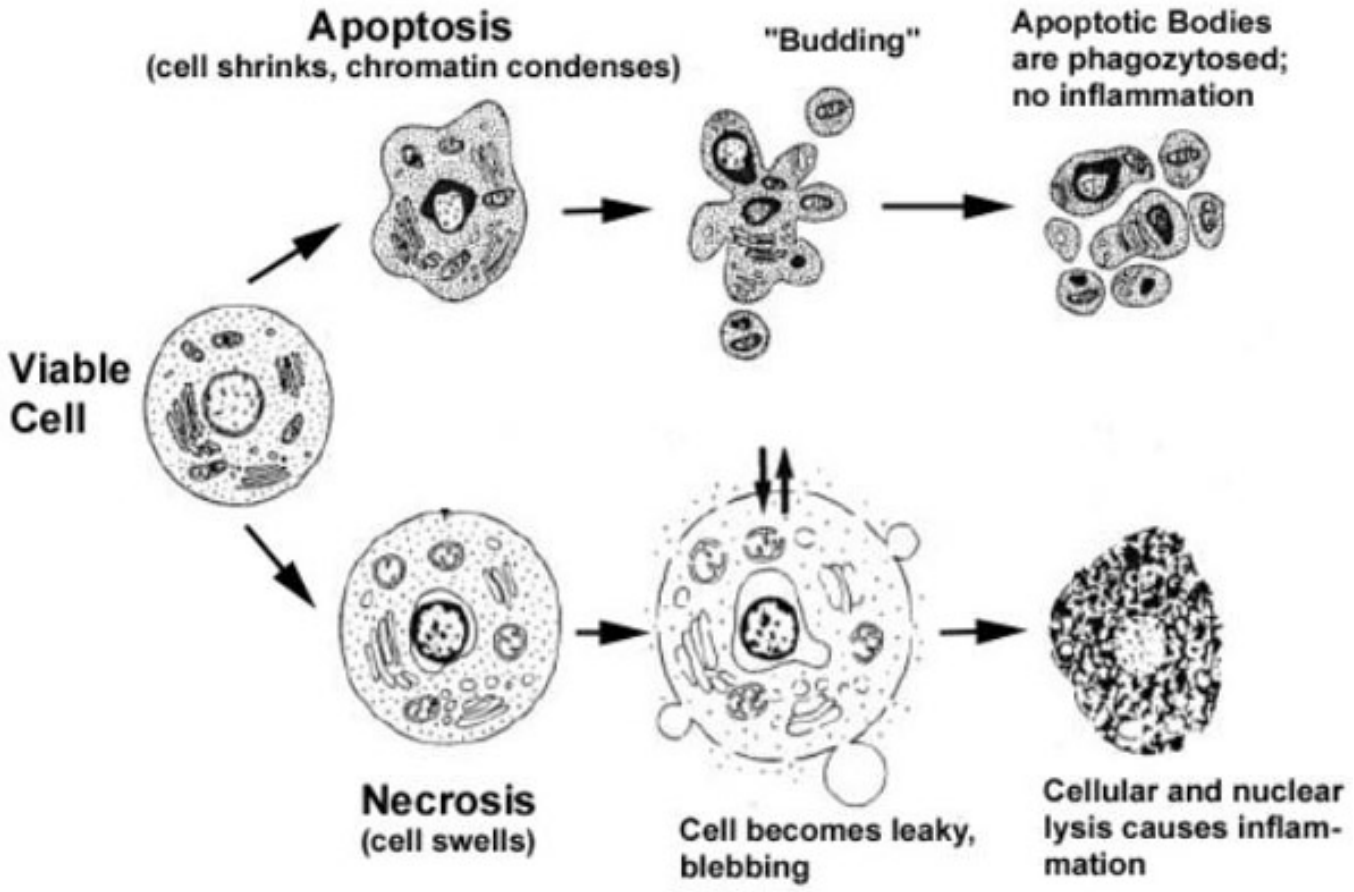
Accidental versus programmed cell death

A fly hits the windshield of a car on the highway…

Accidental cell death (ACD) – death caused by severe insult such as extreme physical, chemical or mechanical stimuli, is virtually immediate and does not involve specific molecular machinery

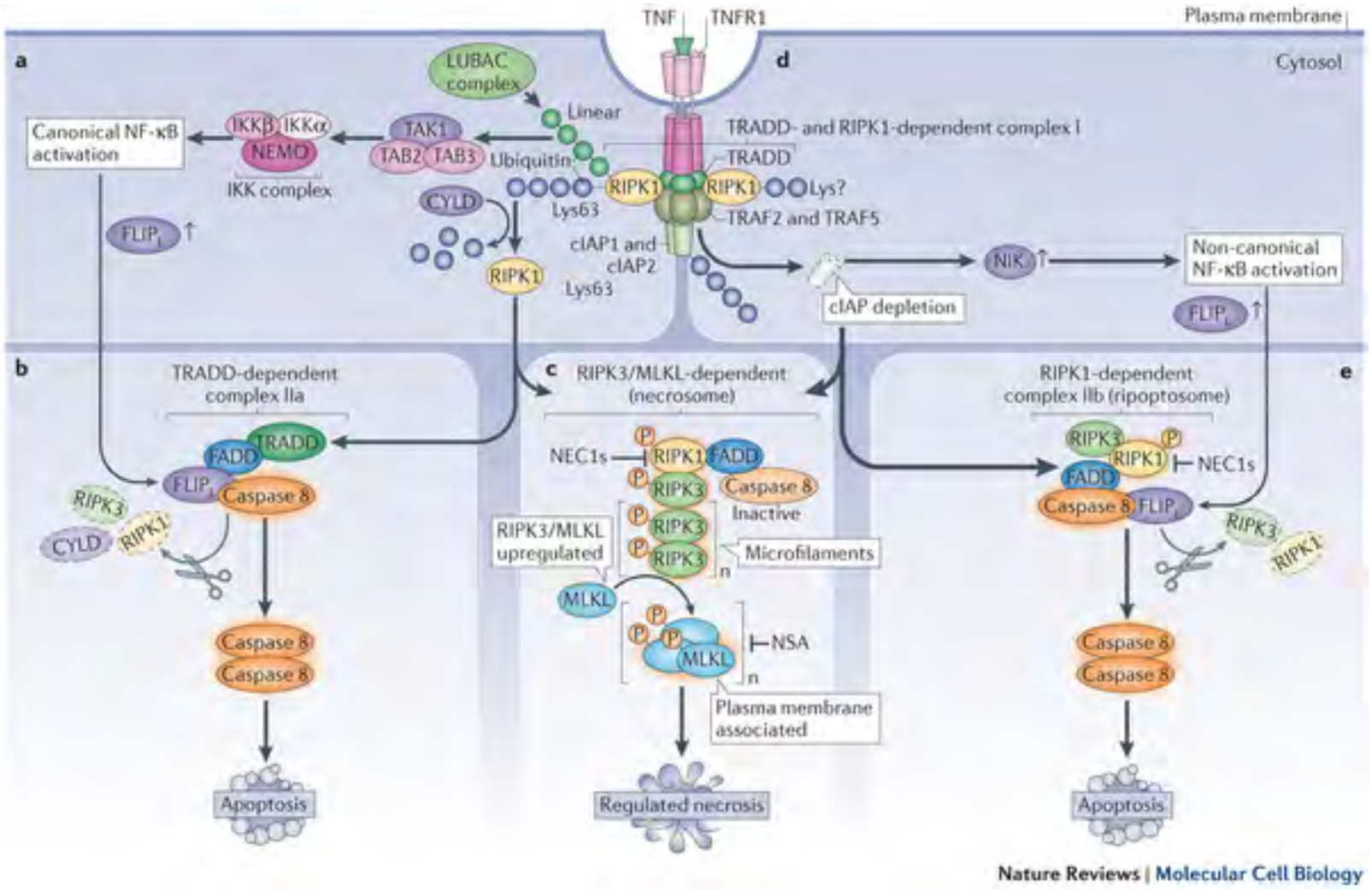
Programmed cell death (PCD) – death that requires genetically encoded molecular machinery, can be altered genetically or pharmacologically, and occurs relatively slower than ACD

Two main forms of programmed cell death



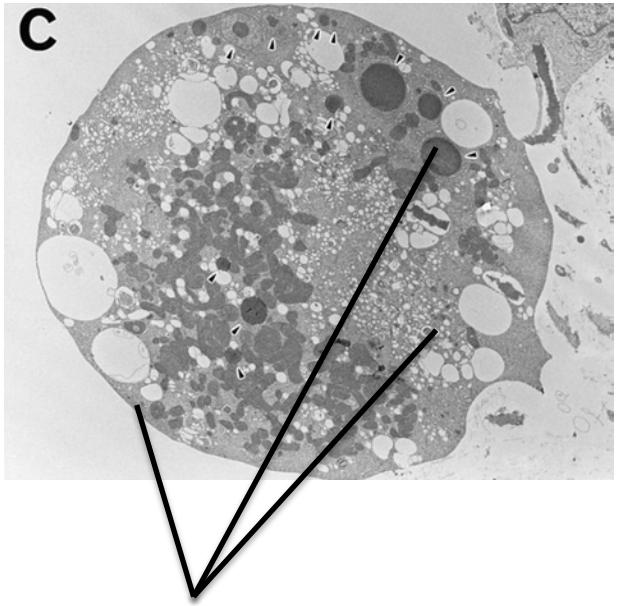
	Apoptosis	Necrosis
Morphological Features	1. Cytoplasmic shrinkage 2. Chromatin condensation 3. Nuclear fragmentation 4. Blebbing, PM intact 5. Apoptotic bodies	1. Cytoplasmic swelling 2. Organelle swelling 3. Irregular chromatin structure 4. Dilatation of nuclear membrane
Biochemical Features	1. Caspase-dependent 2. ATP-dependent 3. Phosphatidylserine PM exposure	1. Caspase-independent 2. RIPK1,3 and MLKL-dependent

Regulated necrosis – “Necroptosis”

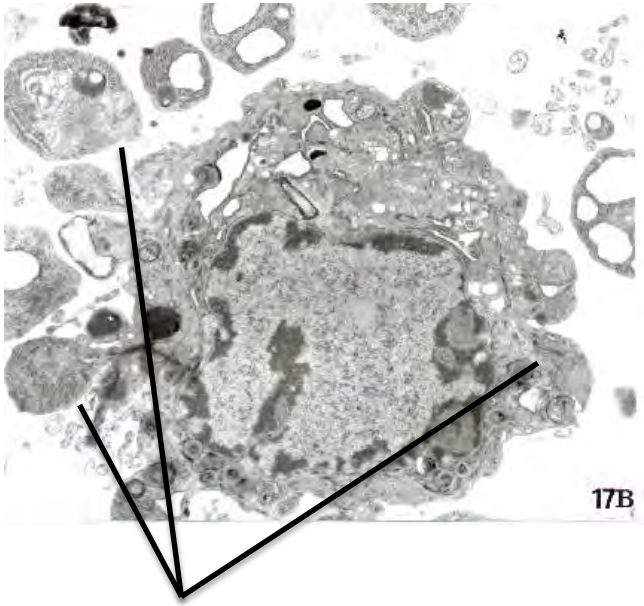


Apoptosis

The word "apoptosis" (ἀπόπτωσις) was used in Greek to describe the "dropping off" or "falling off" of petals from flowers, or leaves from trees.



vacuoles



blebbing

Apoptosis – Nobel Prize

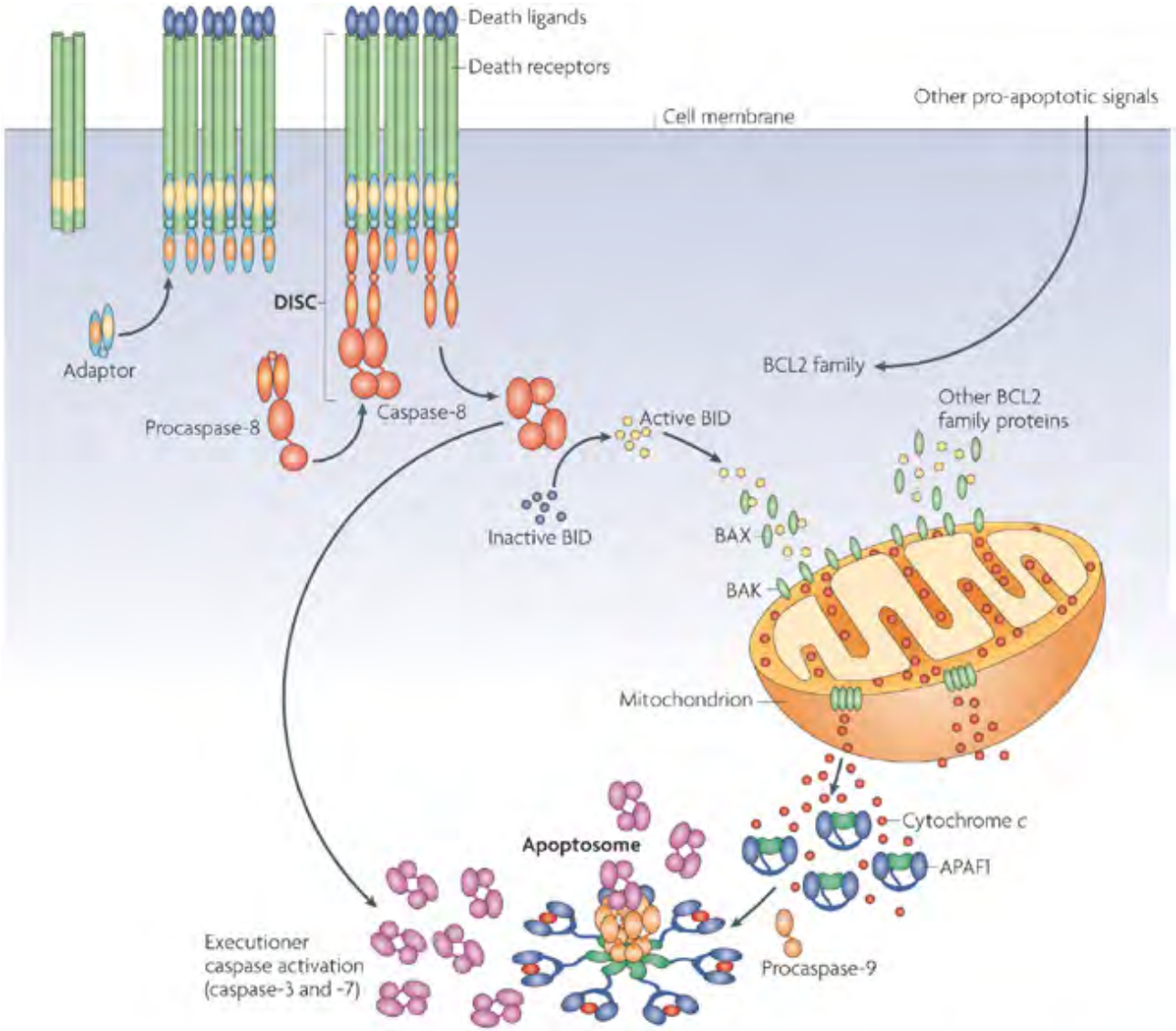
The 2002 Nobel Prize in Medicine was awarded to Sydney Brenner, H. Robert Horvitz and John E. Sulston for their work identifying genes that control apoptosis using the model organism *C. elegans.*


Sydney Brenner


H. Robert Horvitz

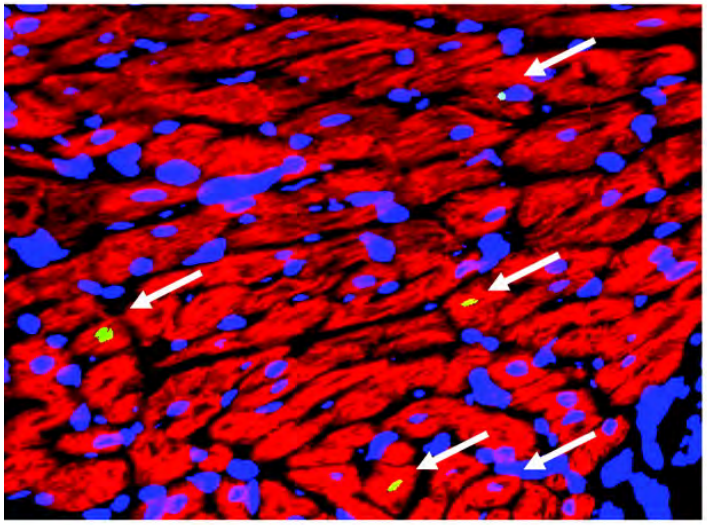

John E. Sulston

Overview of apoptotic signaling

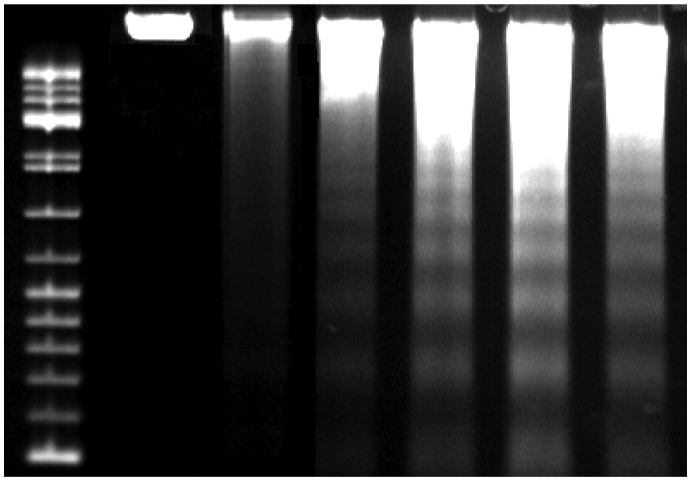


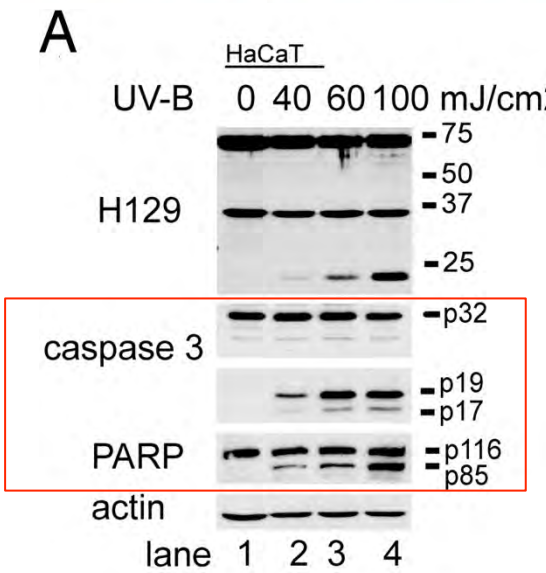
Common assays for Apoptosis

TUNEL

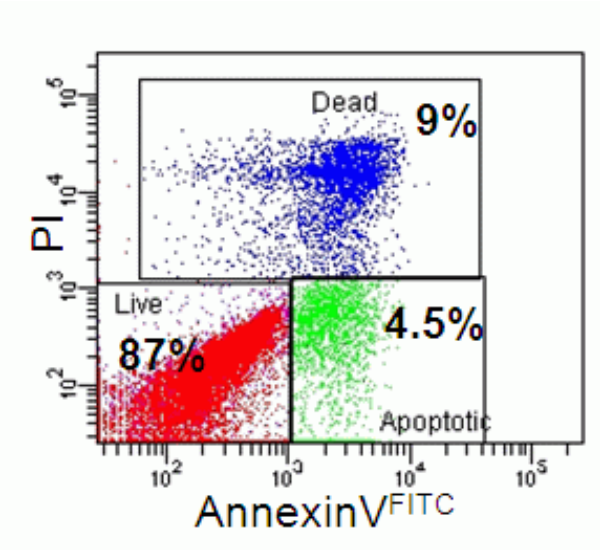


DNA laddering





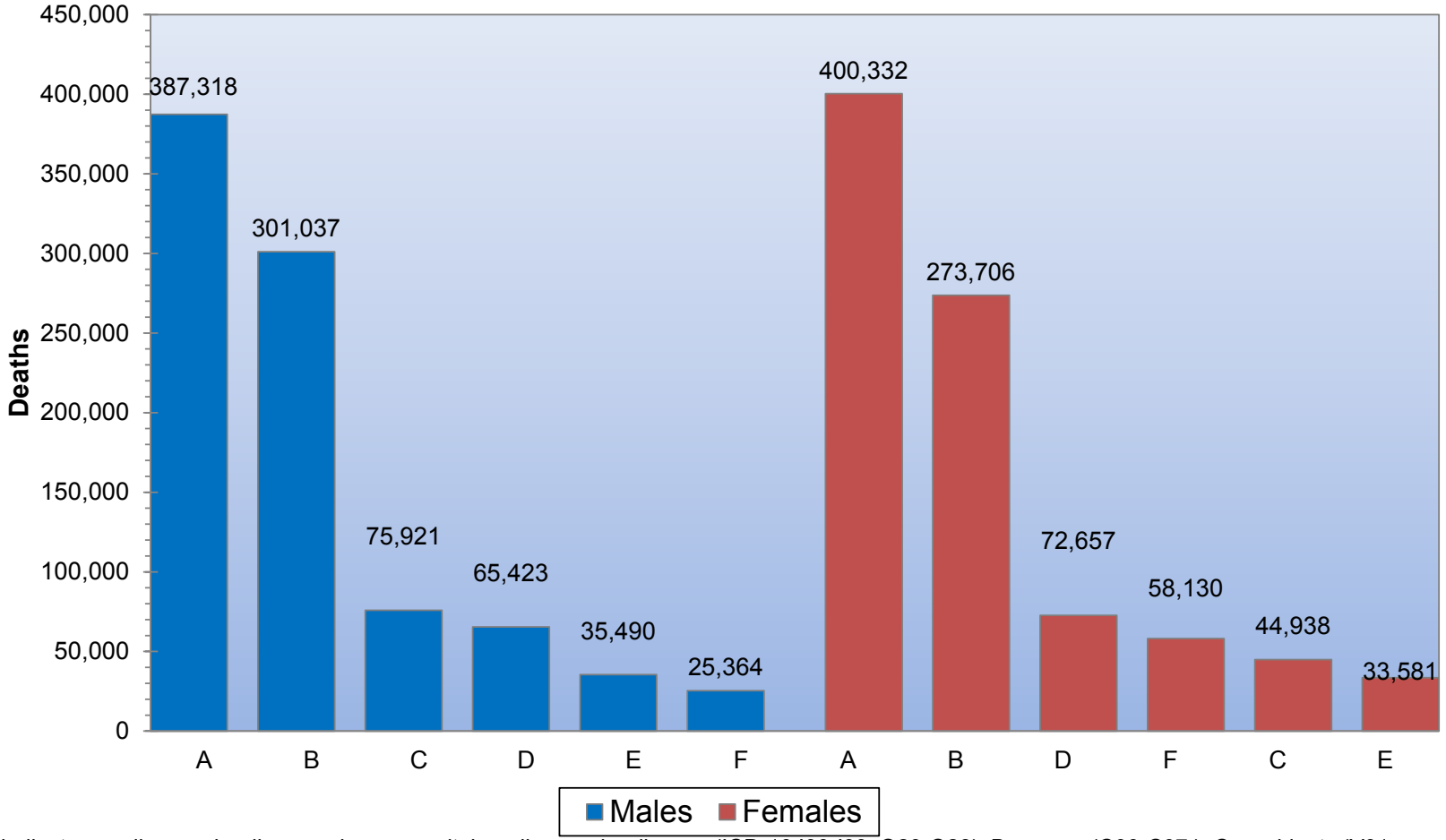
Caspase-3 and PARP cleavage



Flow Cytometry

Why study heart disease?

Cardiovascular disease and other major causes of death for all males and females (United States: 2010)



A indicates cardiovascular disease plus congenital cardiovascular disease (ICD-10 I00-I99, Q20-Q28); B, cancer (C00-C97); C, accidents (V01-X59,Y85-Y86); D, chronic lower respiratory disease (J40-J47); E, diabetes mellitus (E10-E14); F, Alzheimer disease (G30). Source: National Center for Health Statistics and National Heart, Lung, and Blood Institute.

Go AS et al. Published online in *Circulation* Dec. 18, 2013

Why study heart disease?

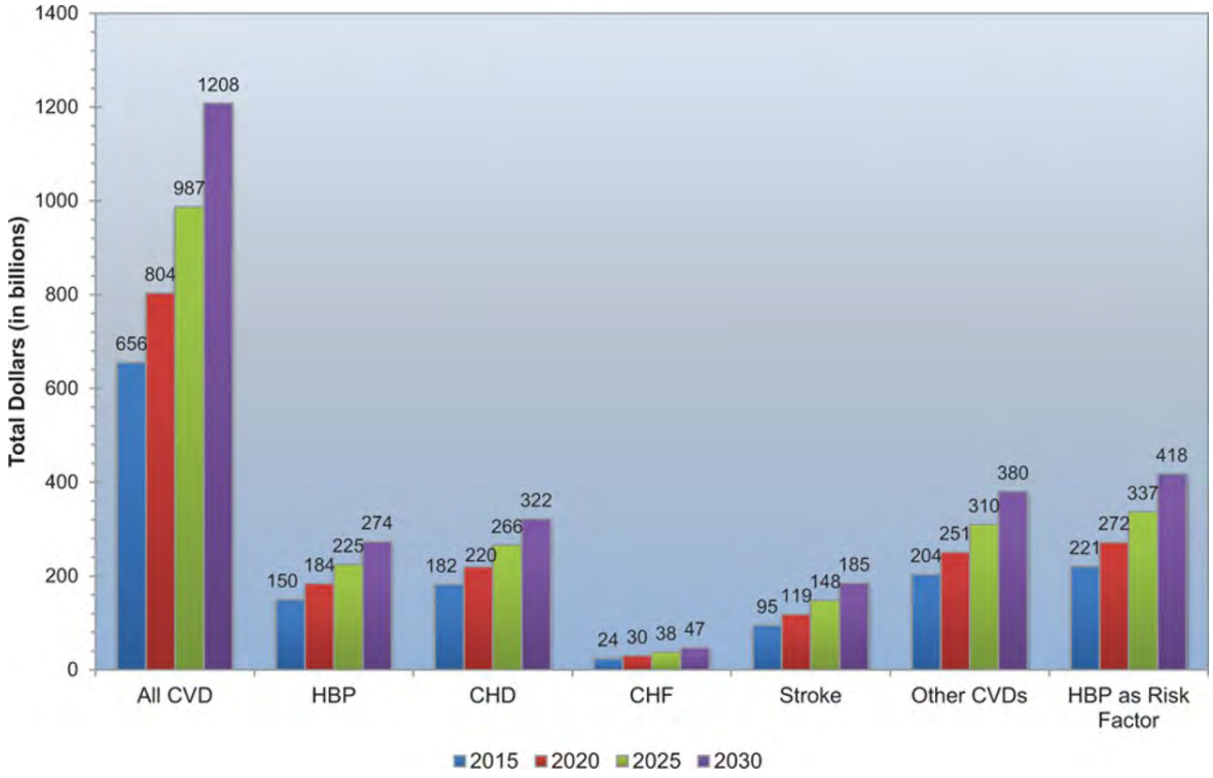
<u>In the United States (2010)</u>

83,600,000 with CVD

787,650 deaths

5,802,000 hospital discharges

Cost: $315.2 billion



Go et al. 2014. Heart Disease and Stroke Statistics—2014 Update - A Report From the American Heart Association. *Circulation*.

Relevance of apoptosis to heart disease

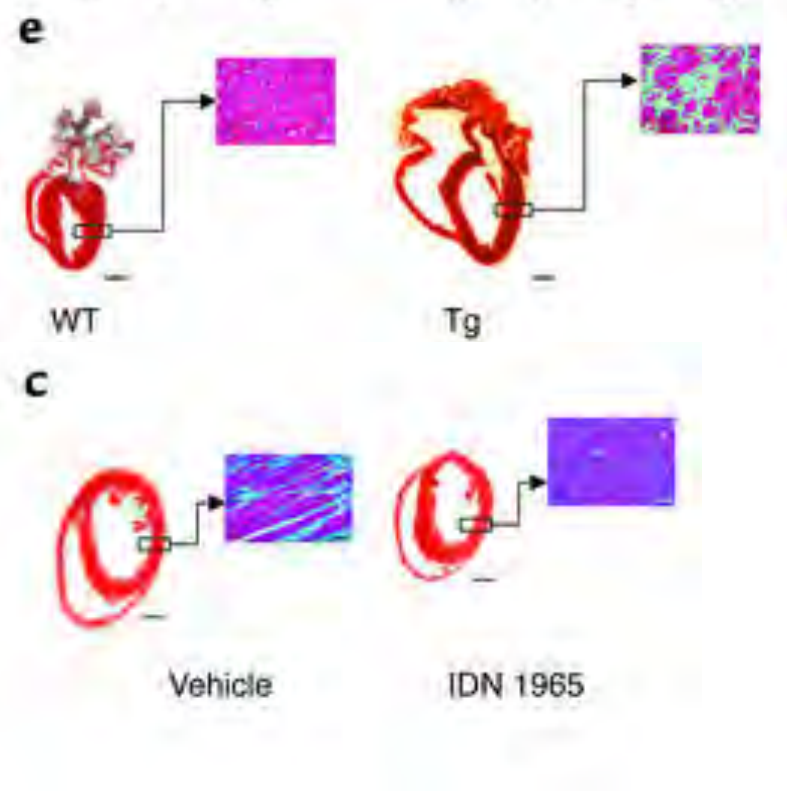
- Olivetti, G., et al. 1997. Apoptosis in the failing human heart. N. Engl. J. Med. 336:1131–1141.
- Saraste, A., et al. 1997. Apoptosis in human acute myocardial infarction. Circulation. 95(2):320-3.
- Guerra, S., et al. 1999. Myocyte death in the failing human heart is gender dependent. Circ. Res. 85(9):856-66.

Wencker, D. et al. 2003. A mechanistic role for cardiac myocyte apoptosis in heart failure. J. Clin. Invest. 111(10):1497-504.

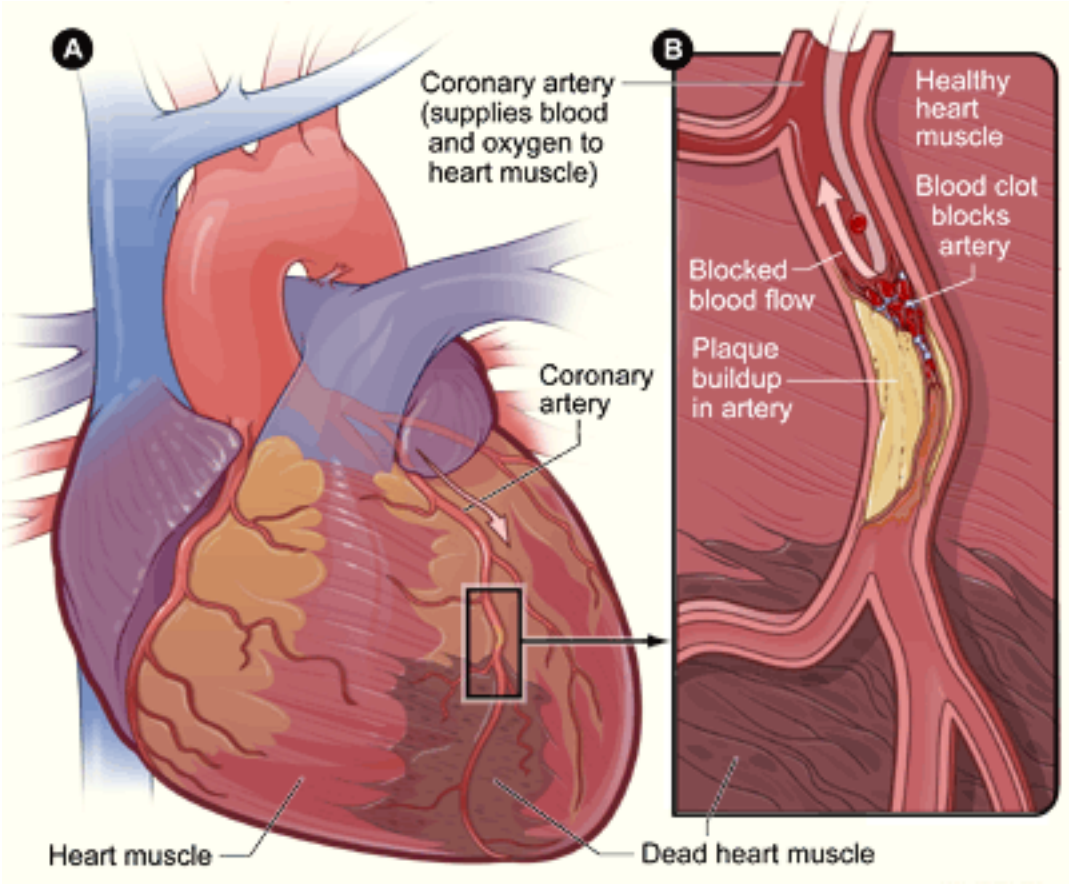
Table 1
Frequency of cardiac myocyte apoptosis in human heart failure vs. FKBP–caspase-8 mice

Species	Apoptotic frequency (myocytes per 10^5 nuclei)	
	Controls	Dilated cardiomyopathy
Human heart failure		
Olivetti et al. (8)	1	237
Saraste et al. (9)	11	119
Guerra et al. (10)	2	80–180
FKBP–caspase-8 mice	1.6	23

Apoptosis was assessed using the TUNEL assay.

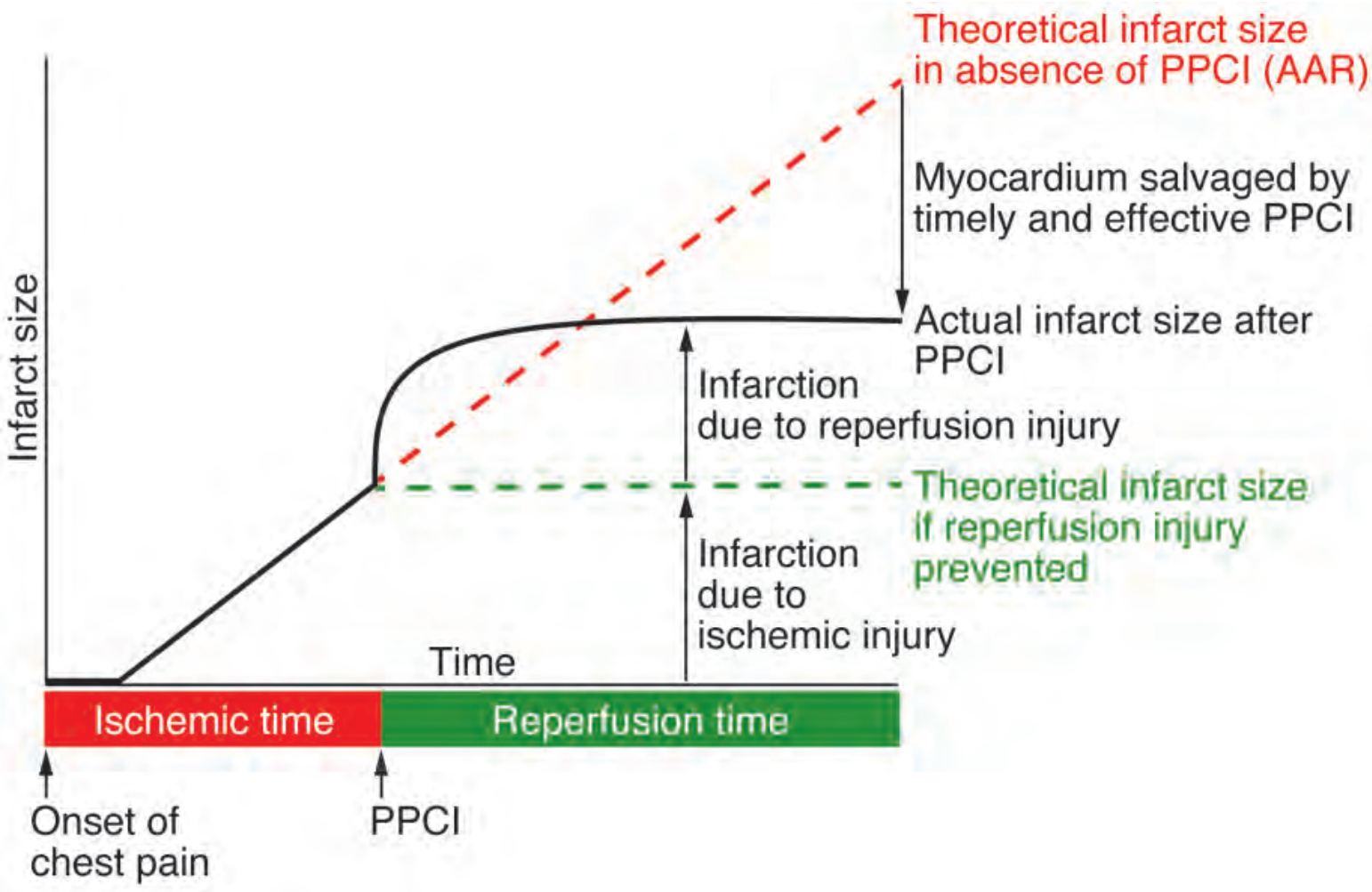


Acute myocardial infarction – major contributor to heart failure

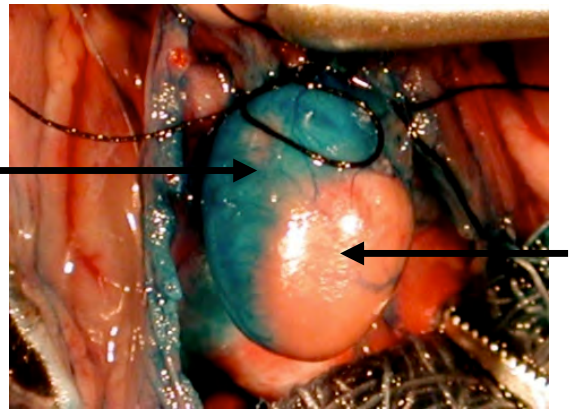


- Stop in blood flow causes heart muscle injury
- Reperfusion therapy still gold standard of treatment
- Resulting scar contributes to arrhythmia (electrical) and reduced contractile function (mechanical)

Acute myocardial infarction – major contributor to heart failure



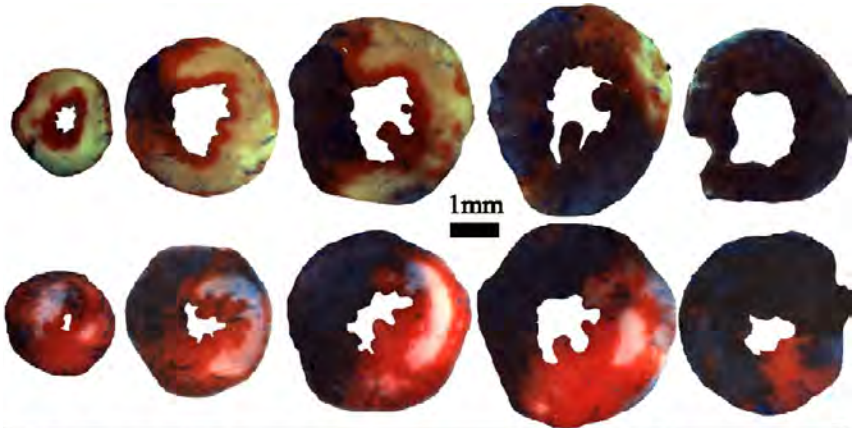
Ischemia-Reperfusion model



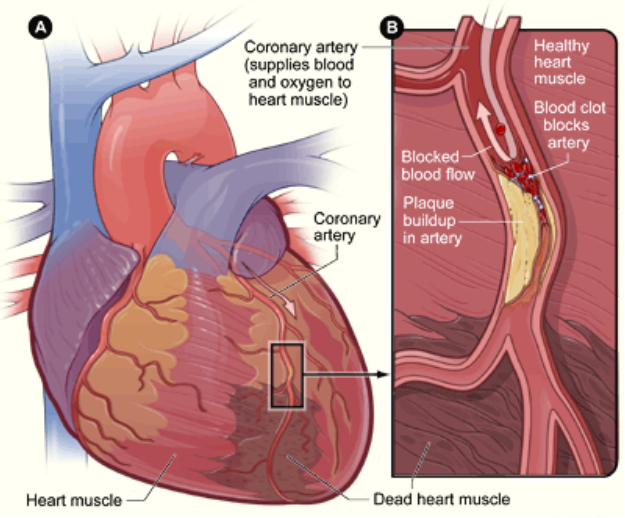
Coronary artery occlusion
(20-45 min)

Reperfusion period
(24 hrs)

Measure
Infarct
(TTC)



Why study heart disease?



"Although improvements in myocardial reperfusion continue to take place in terms of new antiplatelet and antithrombotic agents, there is still no effective therapeutic strategy for preventing myocardial reperfusion injury."

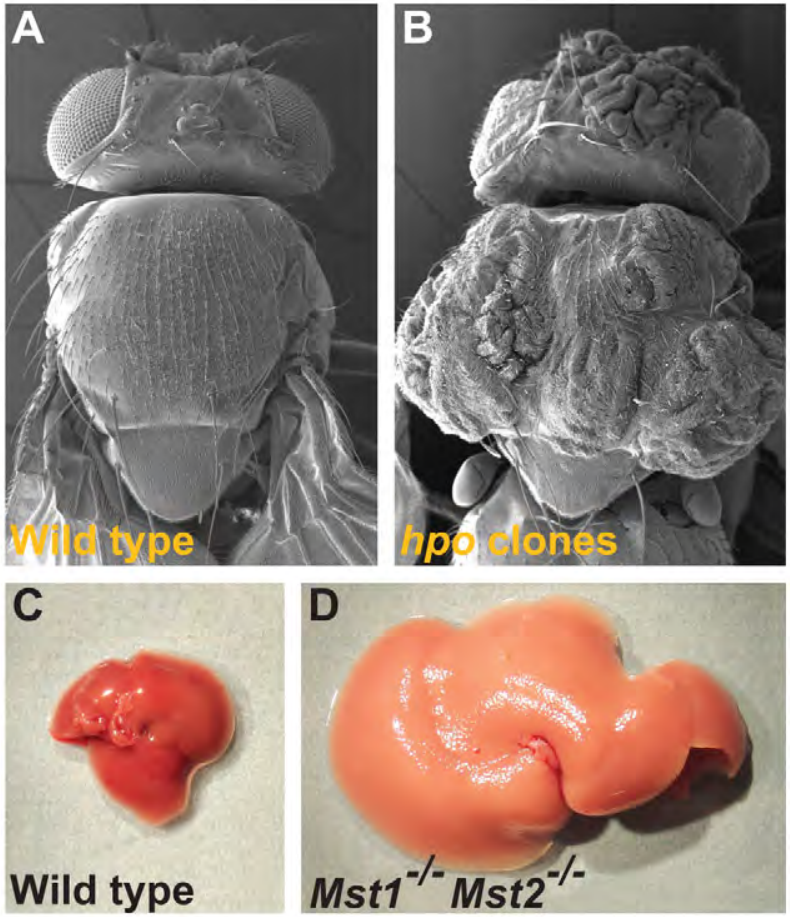
- Derek J. Hausenloy and Derek M. Yellon
University College London

Myocardial ischemia-reperfusion injury: a neglected therapeutic target.
Hausenloy DJ, Yellon DM.
J Clin Invest. 2013 Jan 2;123(1):92-100.

Why study heart disease?

New and improved therapies for treating patients with acute MI are needed

The HIPPO signaling pathway

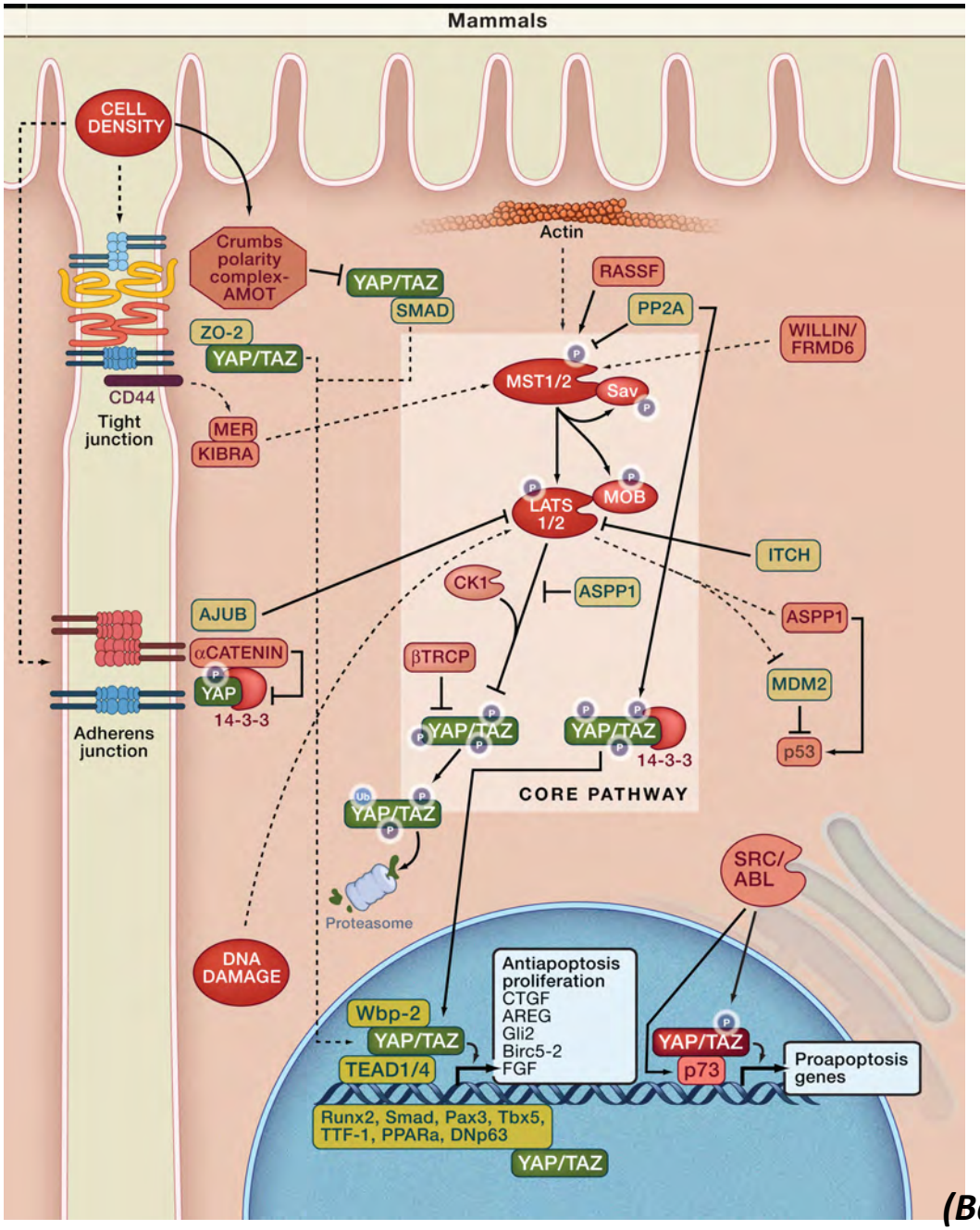


- Discovered in Drosophila - mutant screen for overgrowth phenotype
- Highly conserved from flies to mammals
- Regulates cell proliferation and survival
- Important modulator of organ size
- Involved in human disease - cancer

hpo = Mst1/Mst2

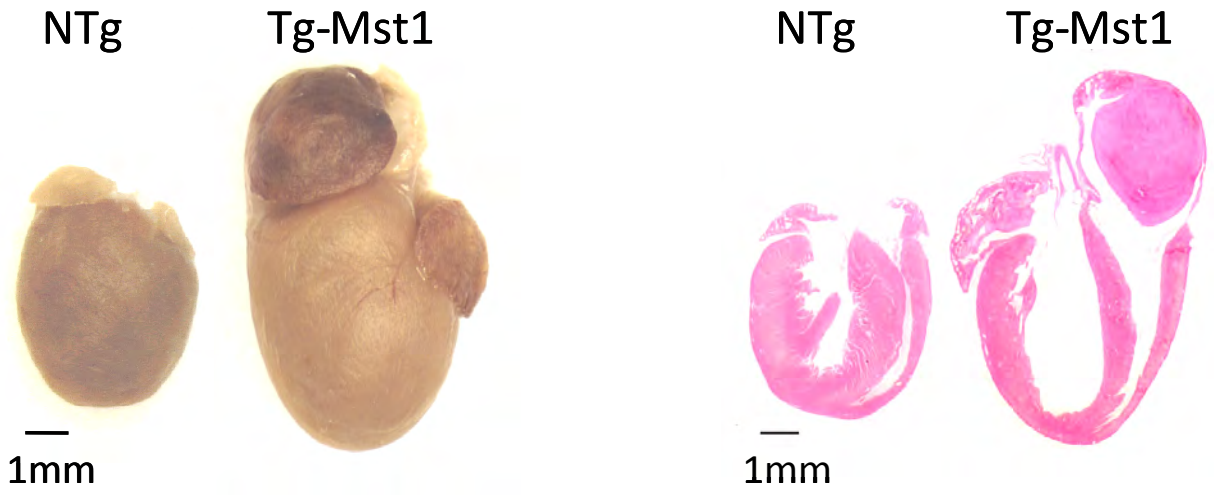
(fly) (mammals)

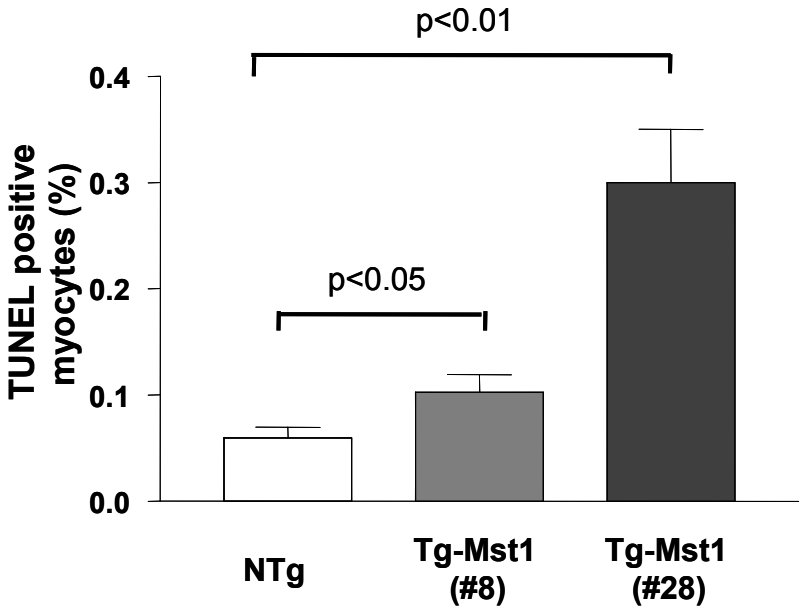
Overview of the HIPPO signaling pathway in mammalian cells



(Badouel and McNeill 2011)

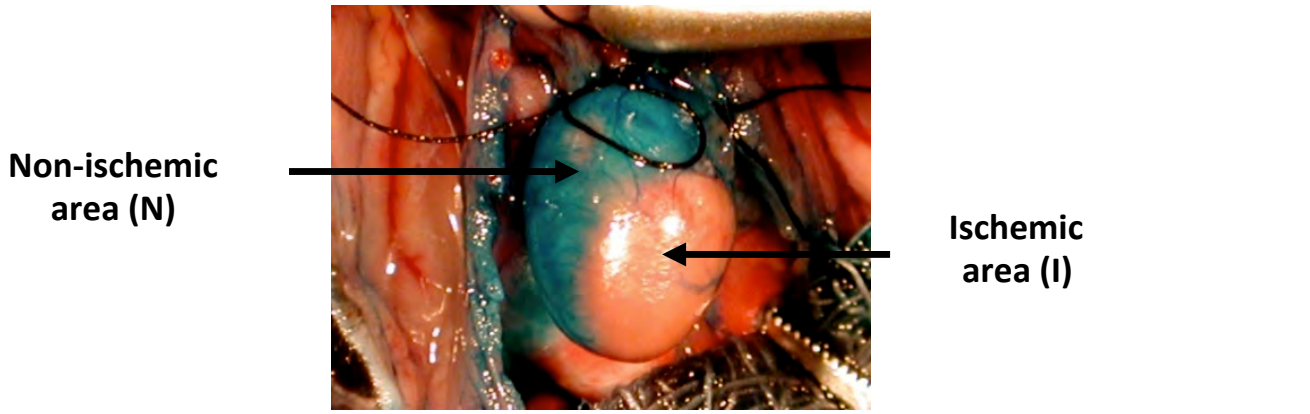
Overexpression of Mst1 causes dilated cardiomyopathy with increases in apoptosis

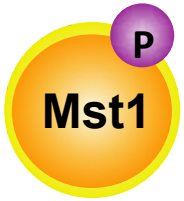




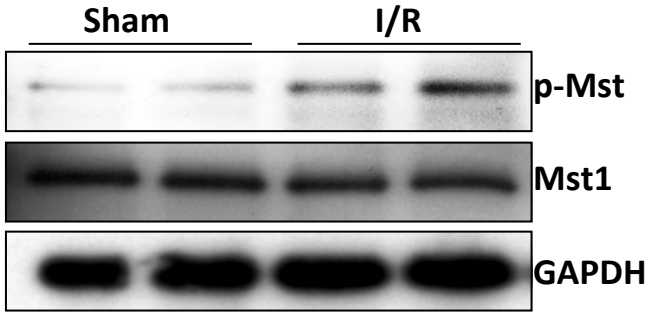
(Yamamoto et al JCI 2003)

Mst1 is activated by ischemia-reperfusion (I/R)

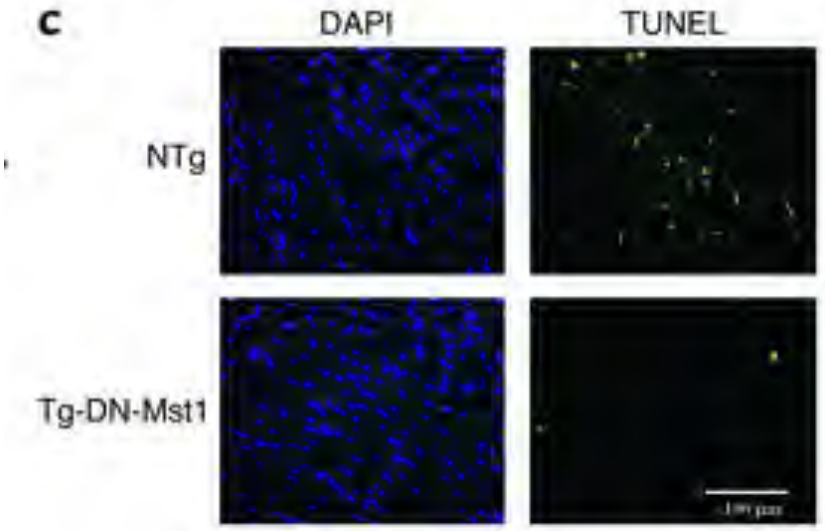


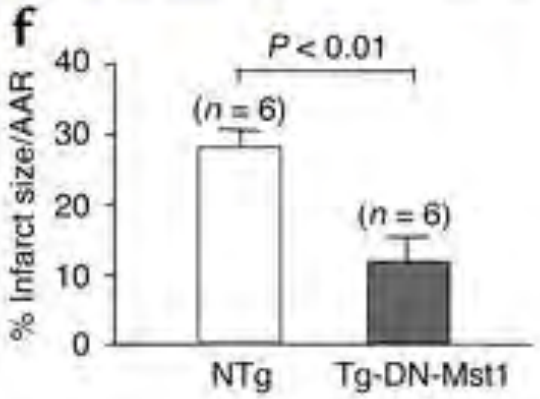


= Activated



Inhibition of Mst1 attenuates apoptosis and decreases injury caused by ischemia/reperfusion





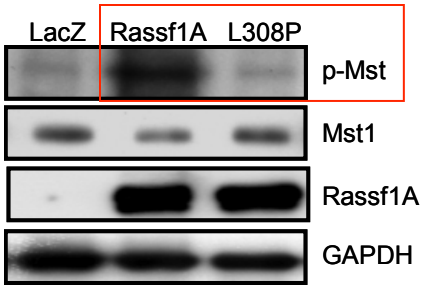
Mst1 is a critical mediator of ischemia/reperfusion injury and a potential therapeutic target

How is Mst1 regulated in the heart during stress?
What are the downstream targets of Mst1?

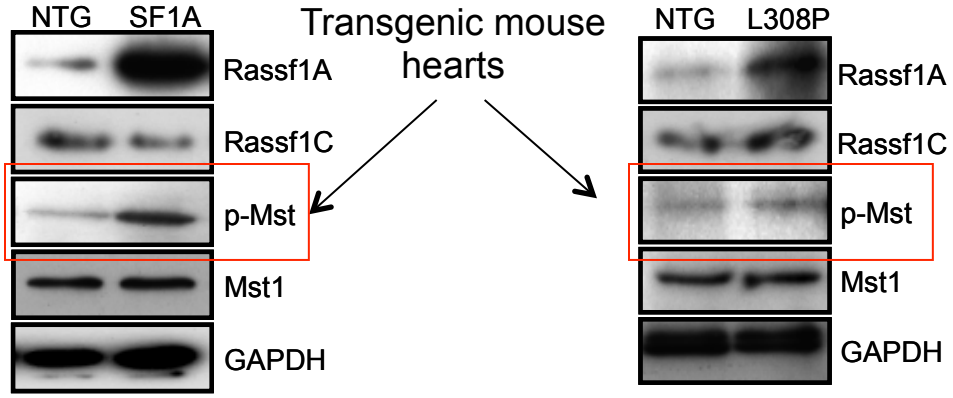
(Yamamoto et al JCI 2003)

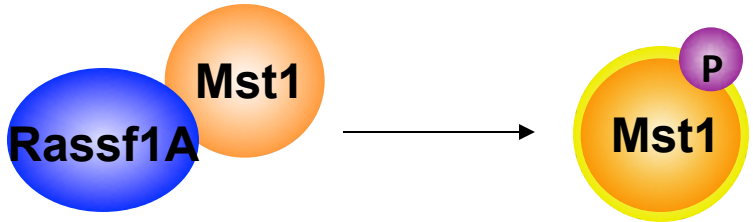
RASSF1A promotes activation of Mst1 in cardiomyocytes and mouse heart

Mst activation

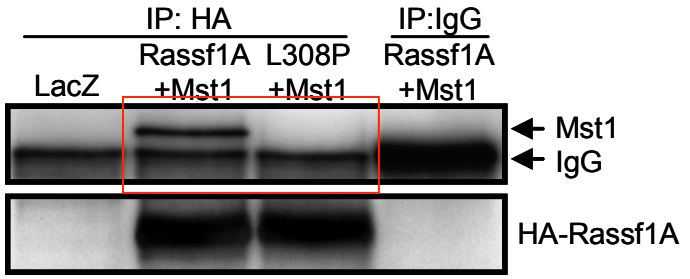


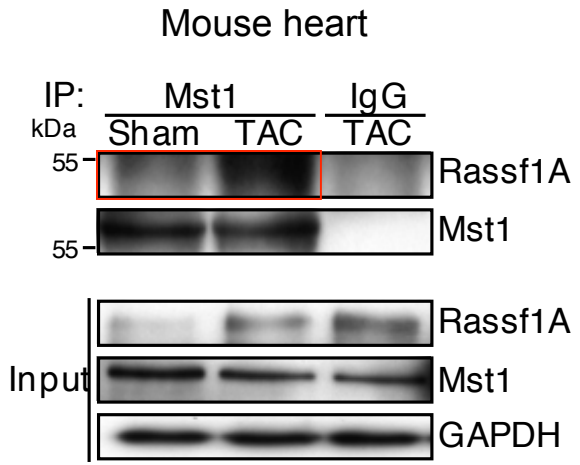
cardiomyocytes

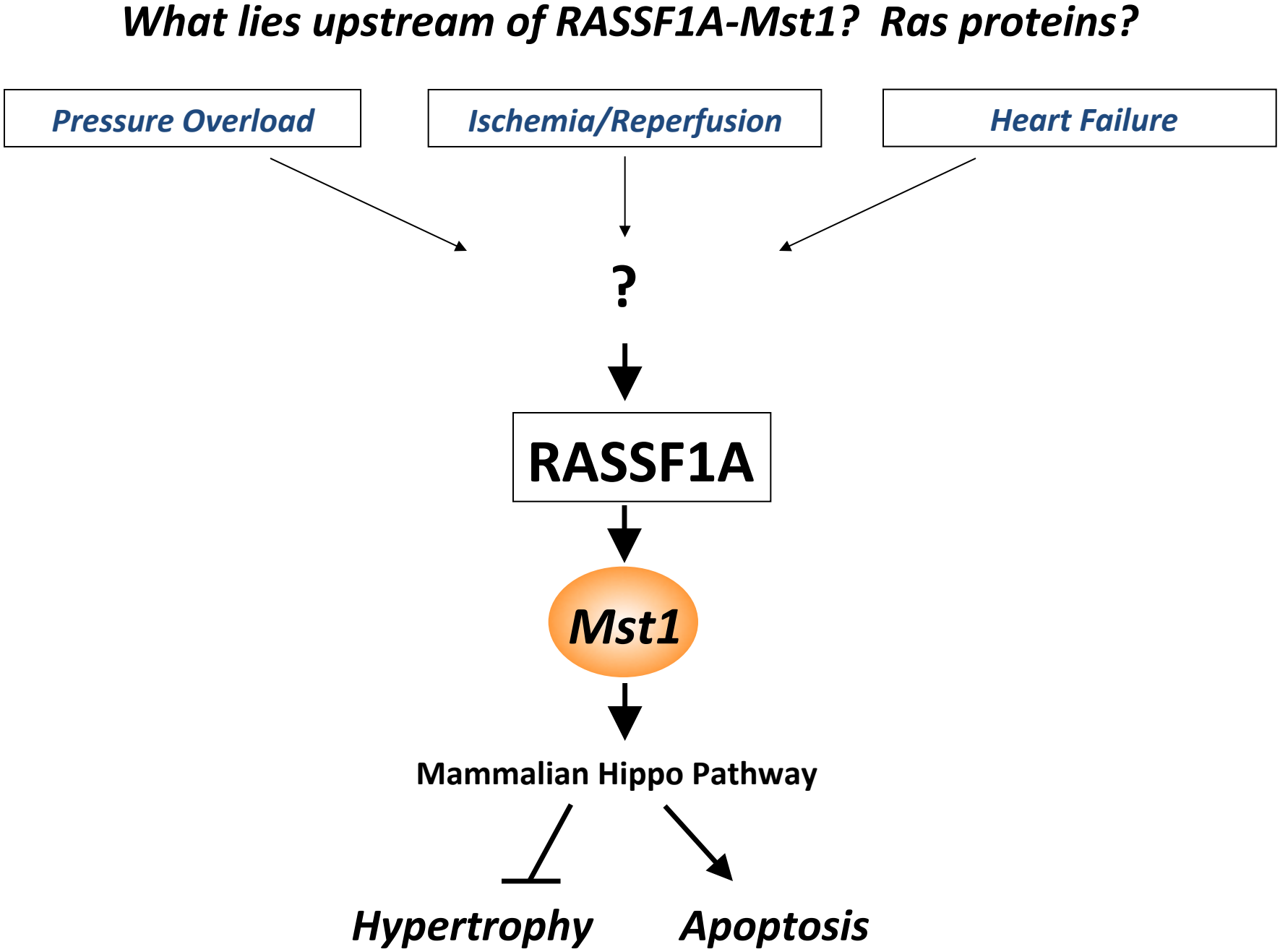




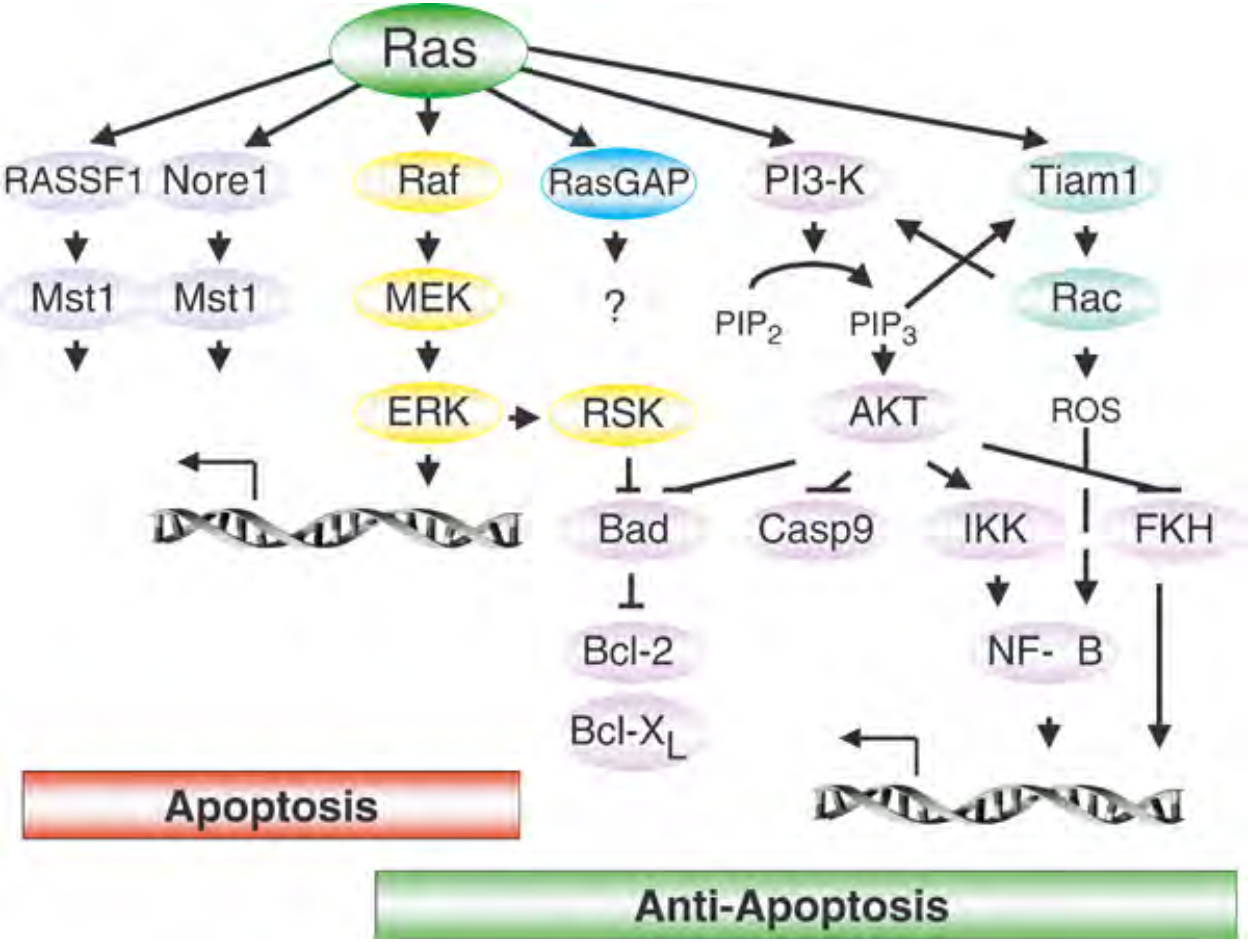
RASSF1A-Mst1 interaction





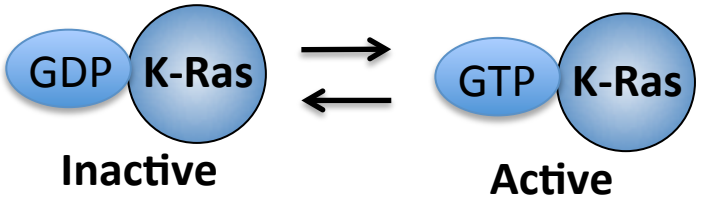

What lies upstream of RASSF1A-Mst1? Ras proteins?
Pressure Overload
Ischemia/Reperfusion
Heart Failure
?
RASSF1A
Mst1
Mammalian Hippo Pathway
Hypertrophy
Apoptosis

Ras proteins can stimulate both survival and apoptotic signaling

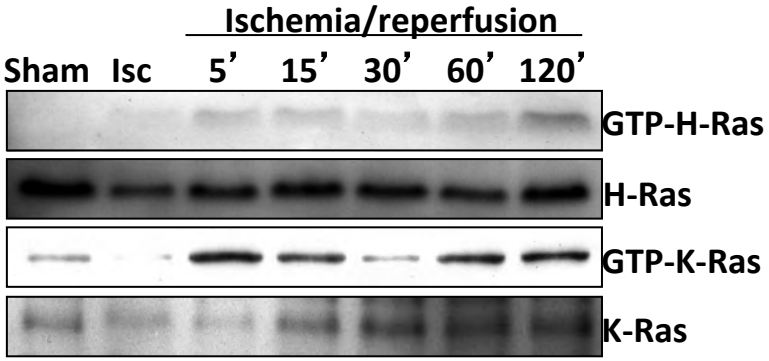


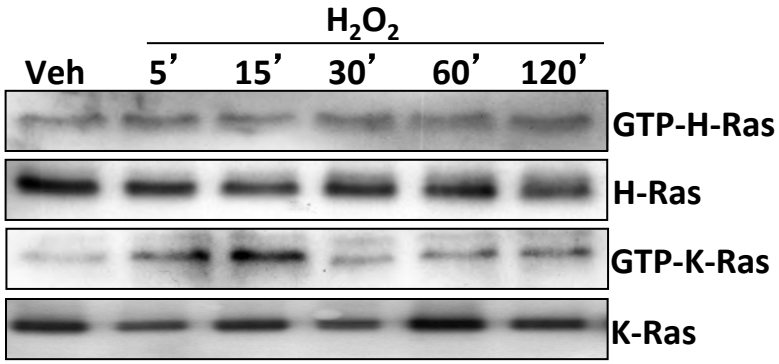
(Cox and Der Oncogene 2003)

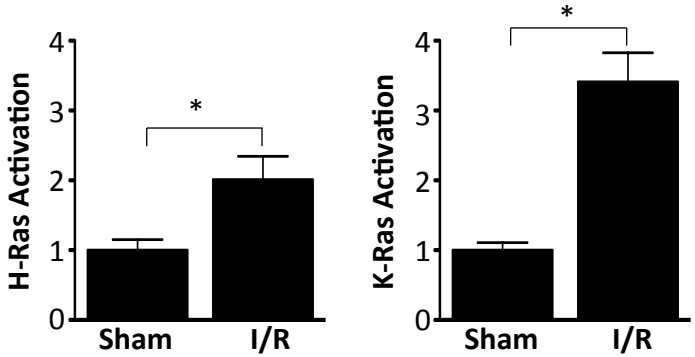
Are Ras isoforms activated by oxidative stress in the heart?



HEARTS



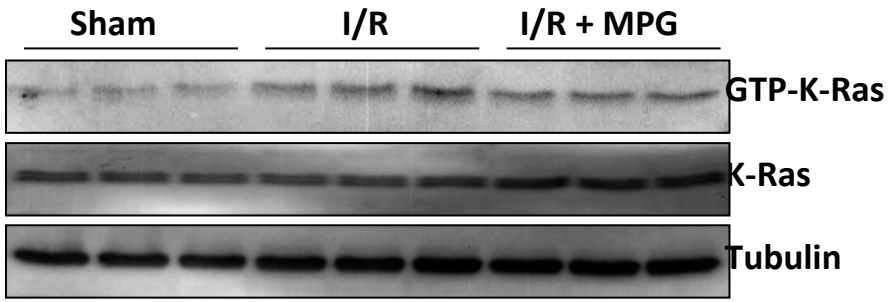


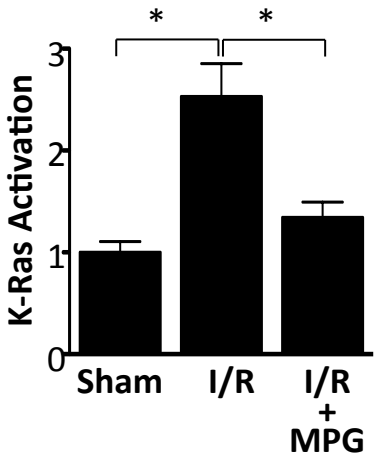


CARDIOMYOCYTES

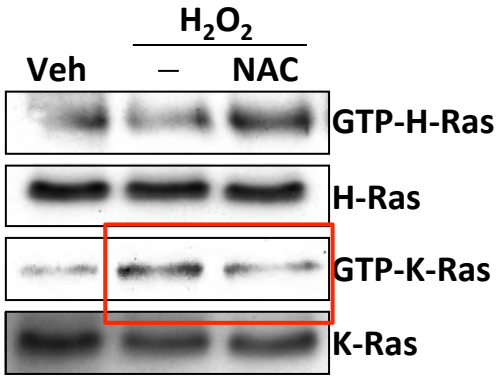
Is Ras activation mediated by oxidation?

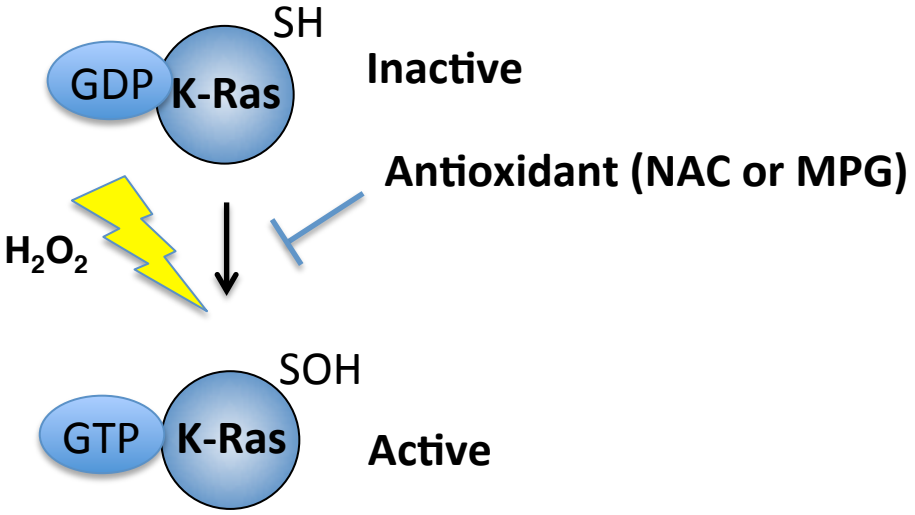
HEART



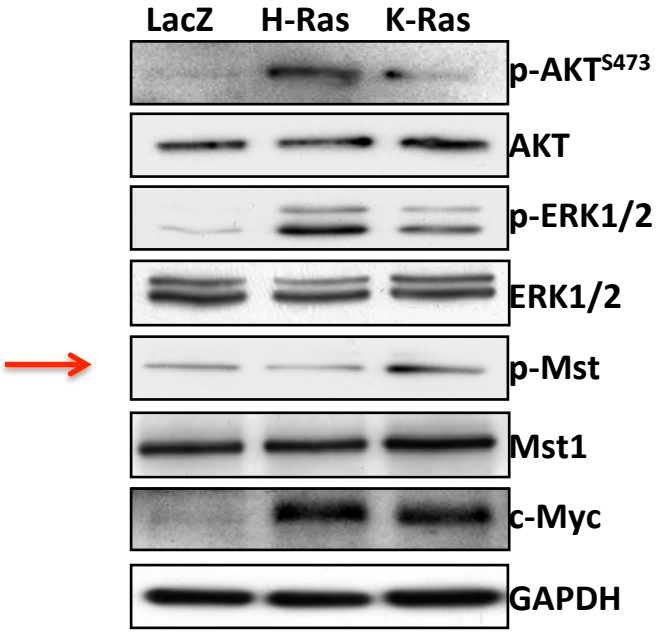


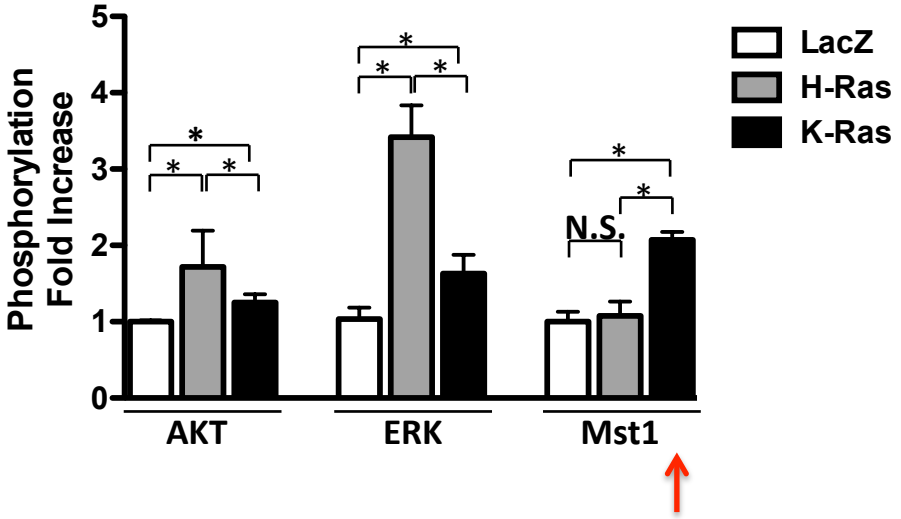
CARDIOMYOCYTES

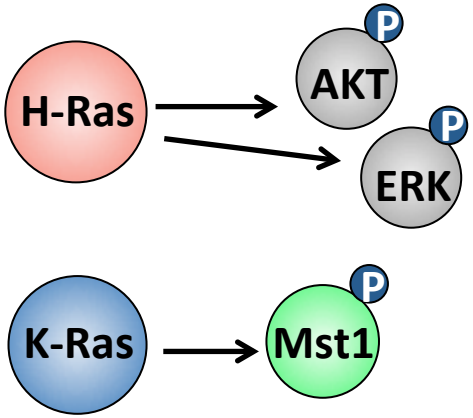




H-Ras and K-Ras initiate divergent signaling

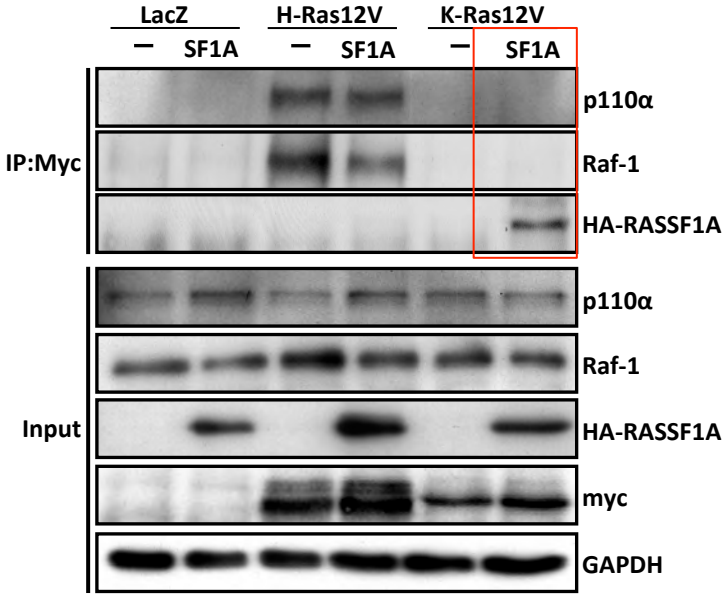


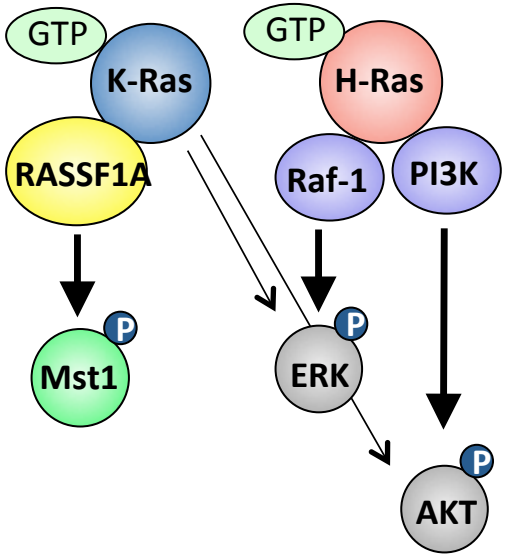




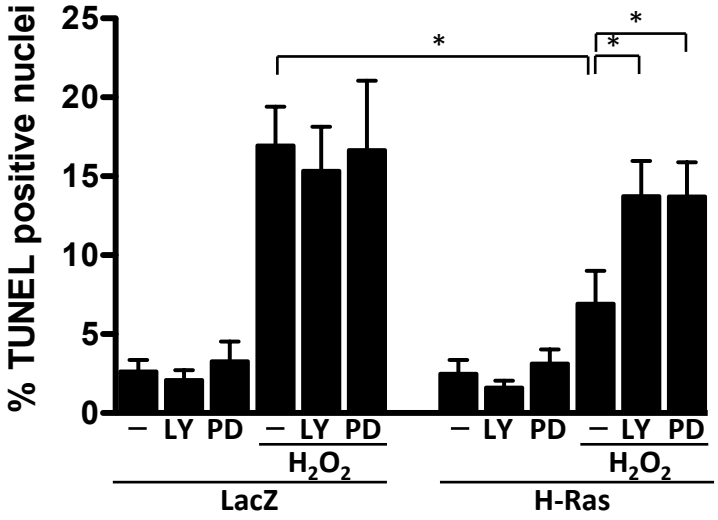
H-Ras and K-Ras initiate divergent signaling

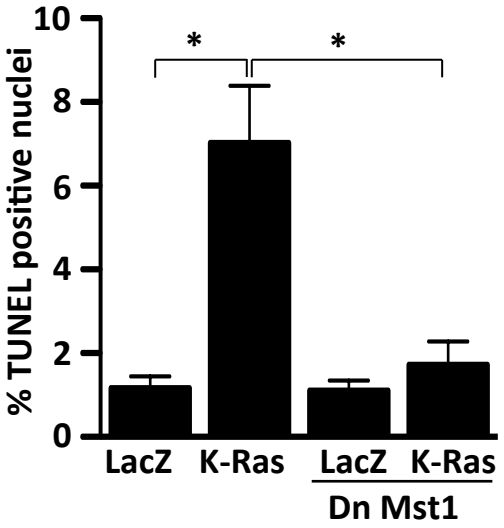
Cardiomyocytes





H-Ras is protective while K-Ras promotes apoptosis through Mst1





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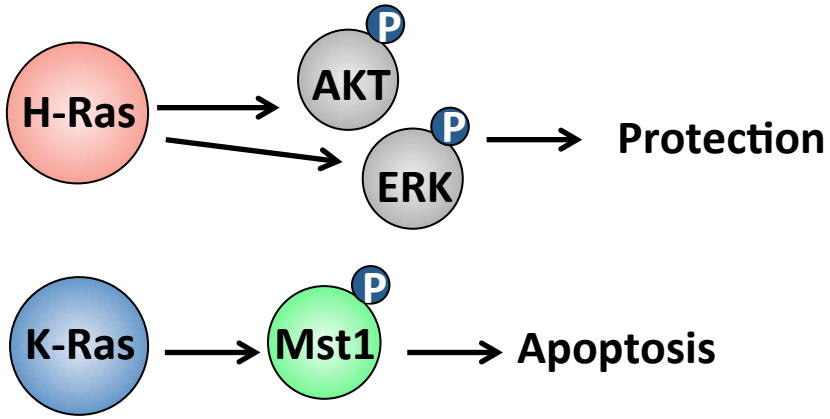
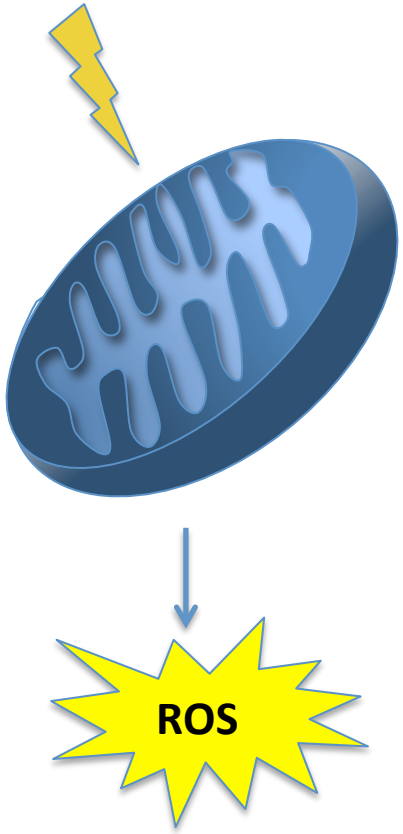


Mitochondria as a source of reactive oxygen species (ROS)

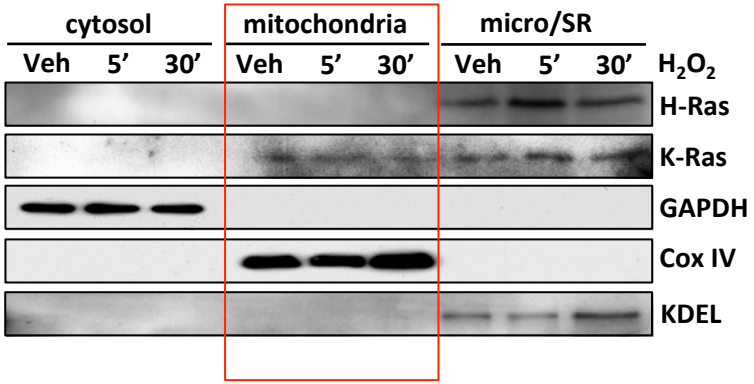
Ischemia/reperfusion
Oxidative Stress



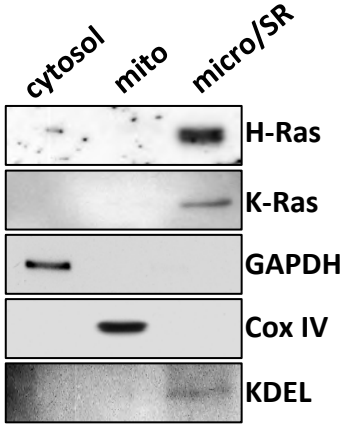
mitochondrion

Differences in H-Ras and K-Ras subcellular localization

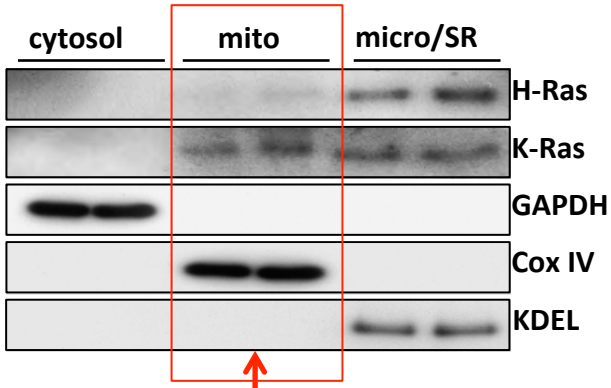
Cardiomyocytes



Cardiac Fibroblasts

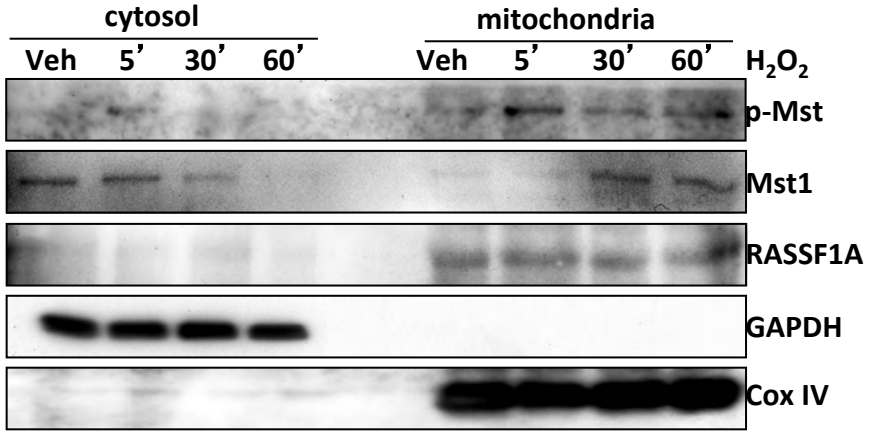


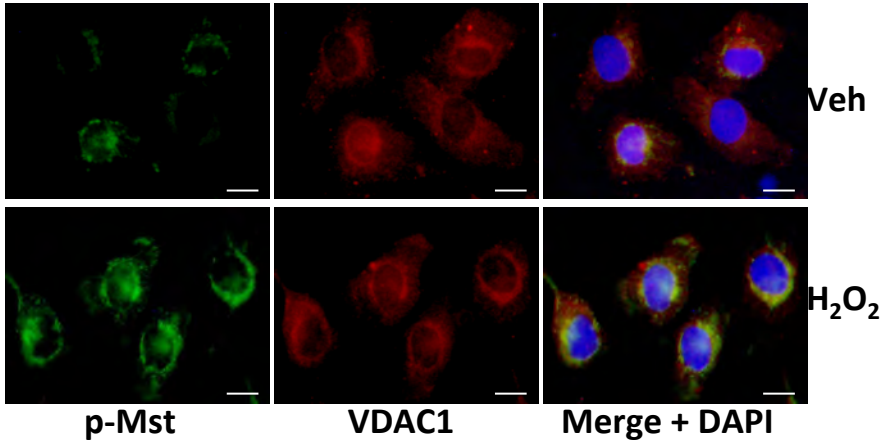
Heart

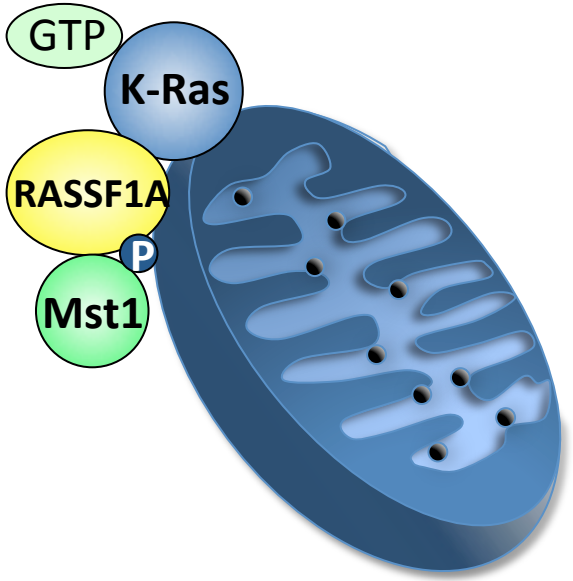


Only K-Ras in mitochondria-enriched fractions

Mst1 translocates to mitochondria following oxidative stress

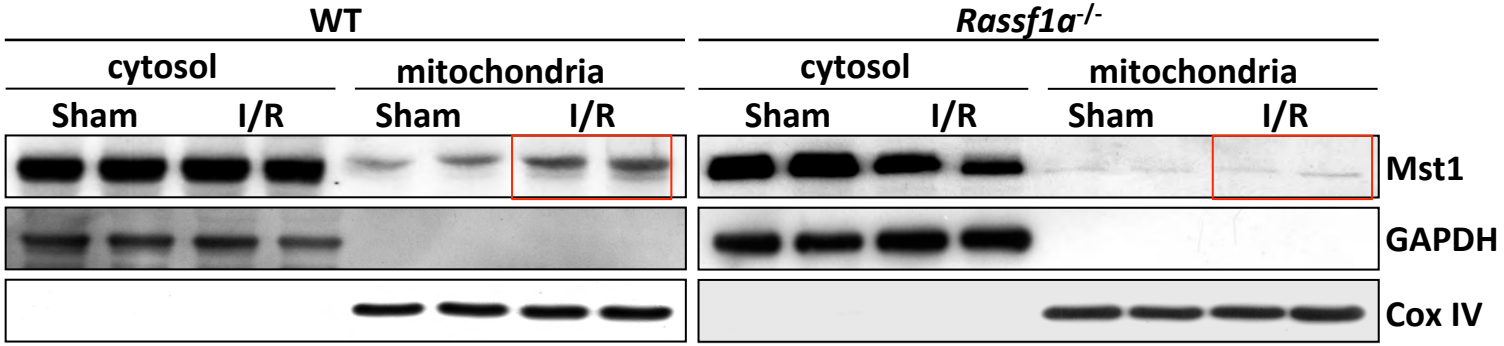


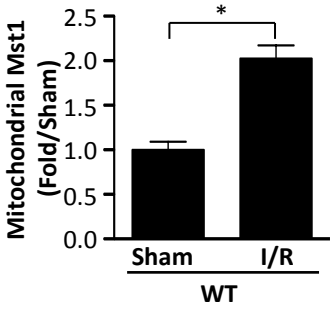


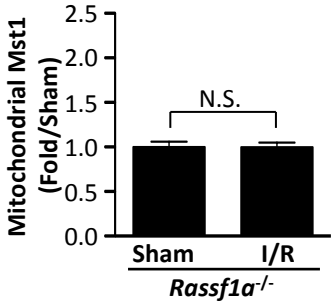


Mst1 translocation requires RASSF1A

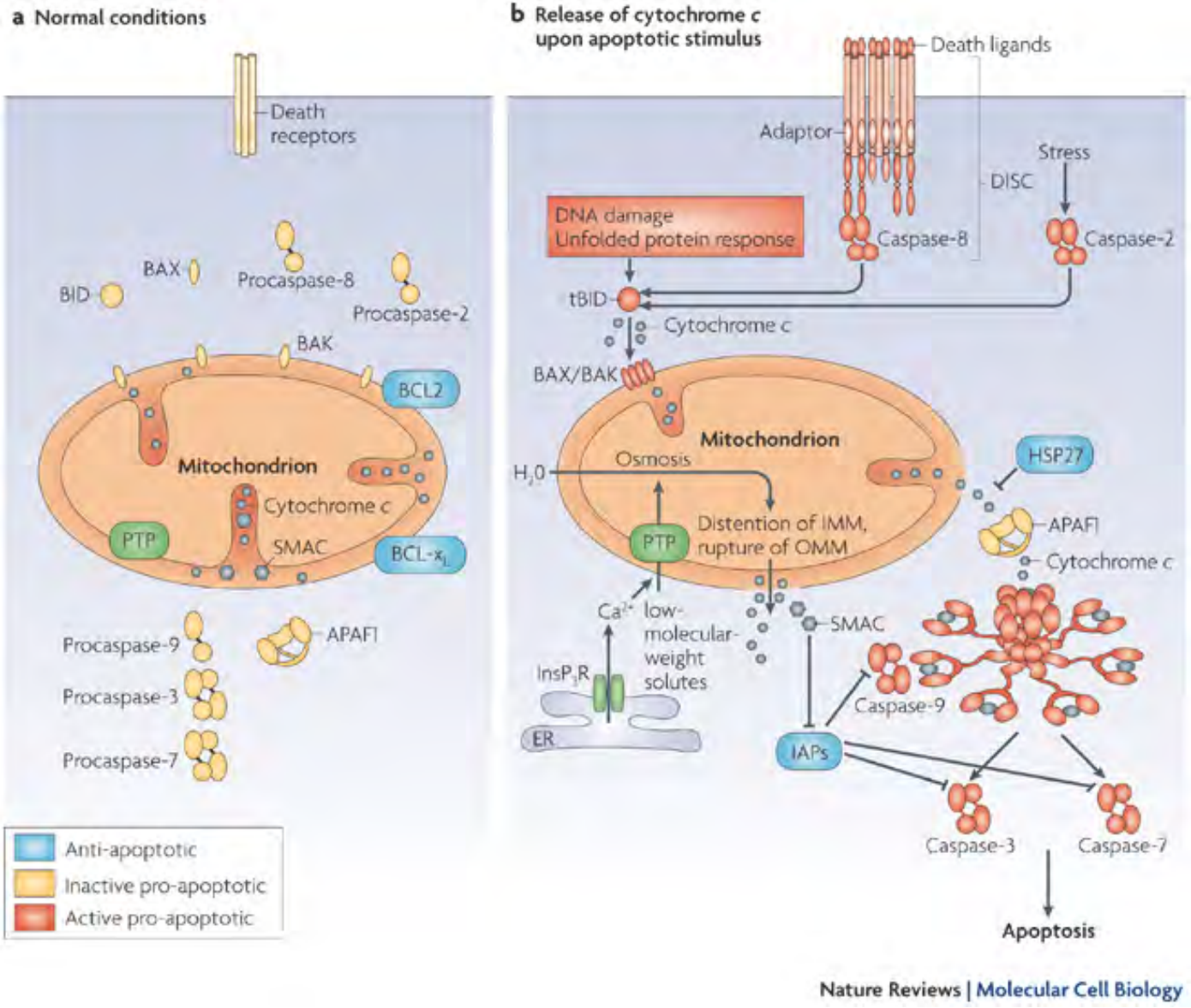
Heart



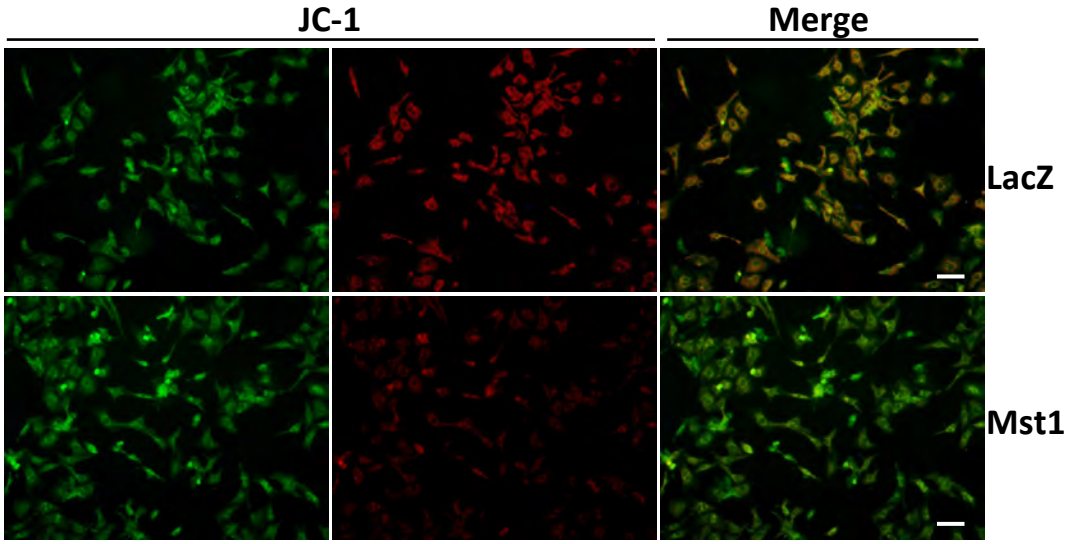


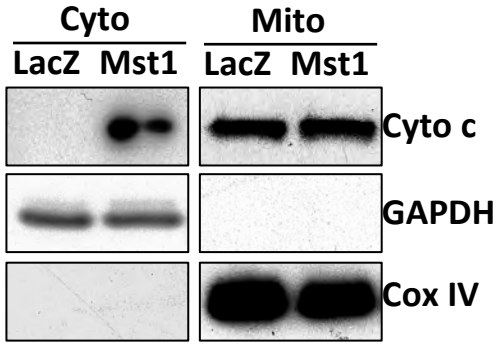


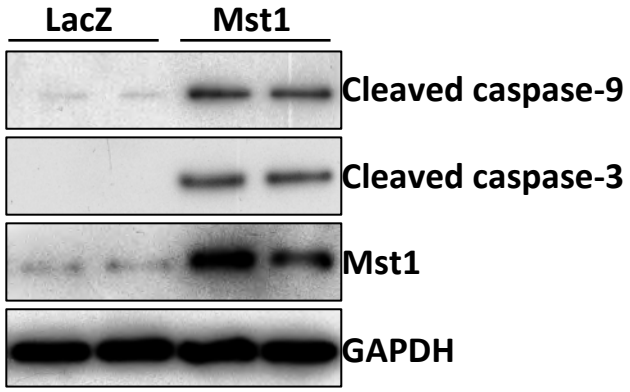
Intrinsic pathway of apoptosis



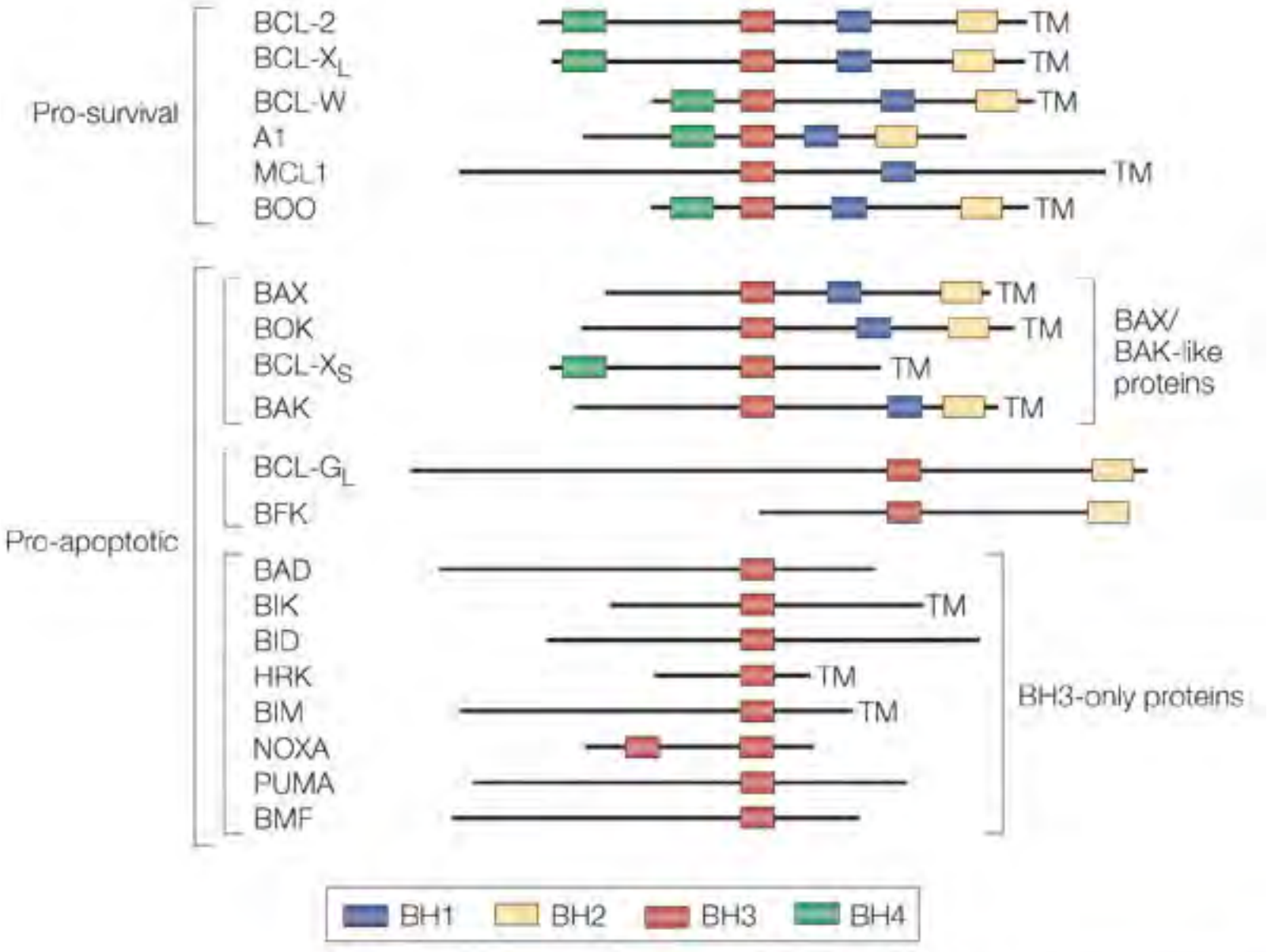
What is the function of Mst1 at mitochondria?



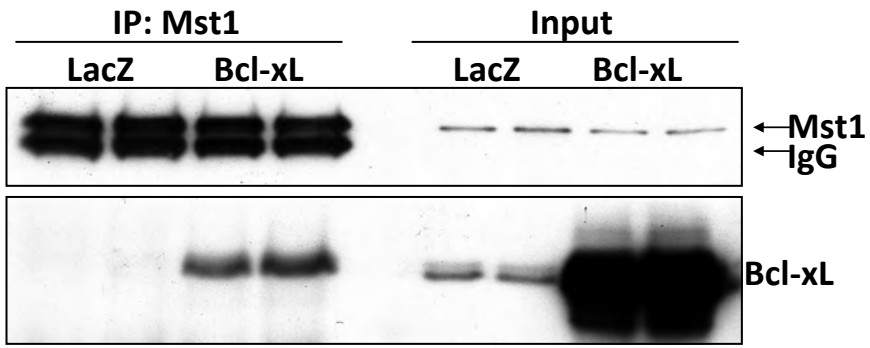


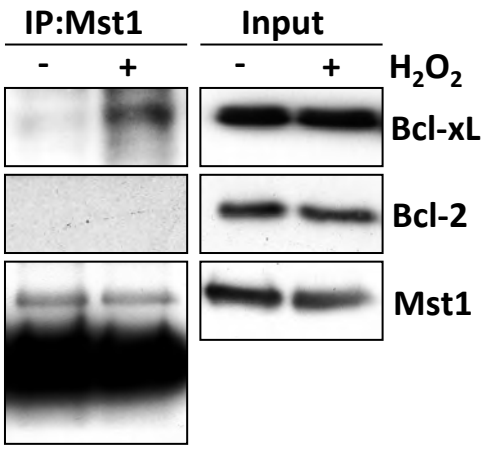


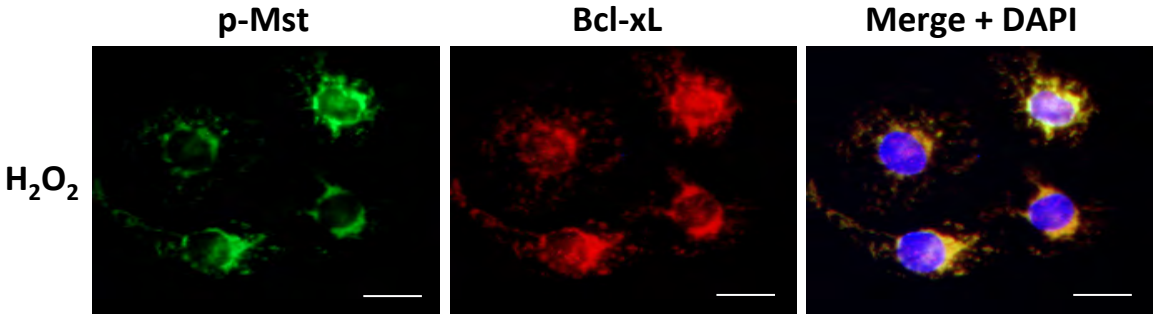
Bcl-2 family members



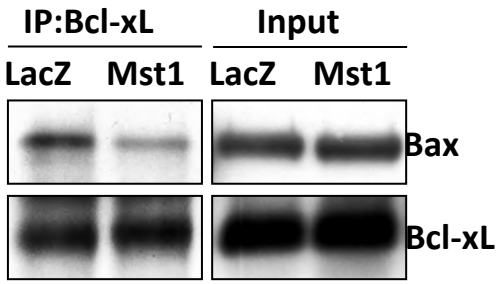
What is the mitochondrial target of Mst1?

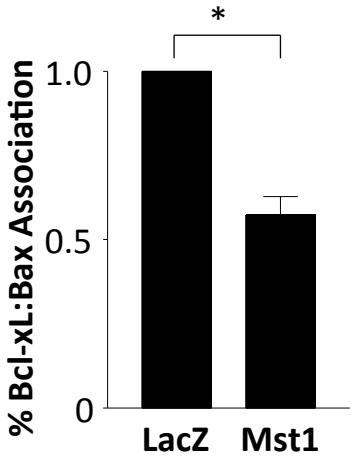


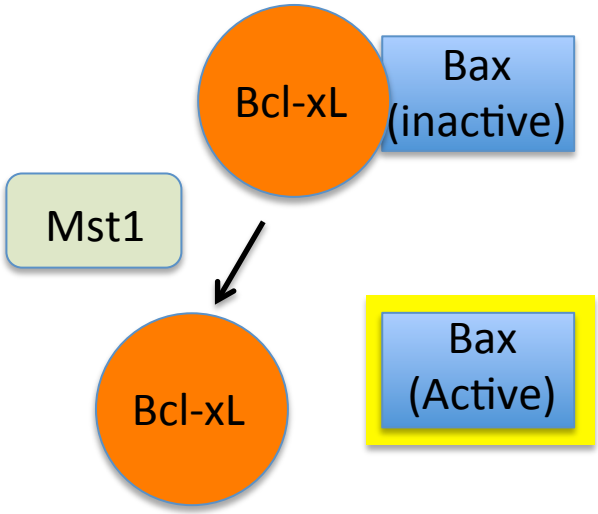


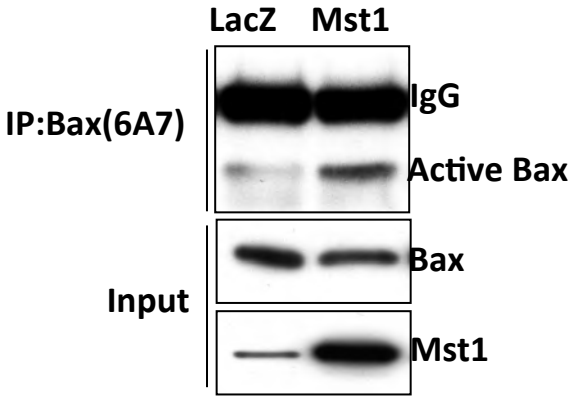


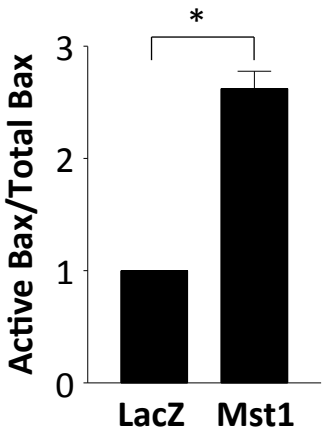
Mst1 disrupts Bcl-xL-Bax interaction and promotes Bax activation



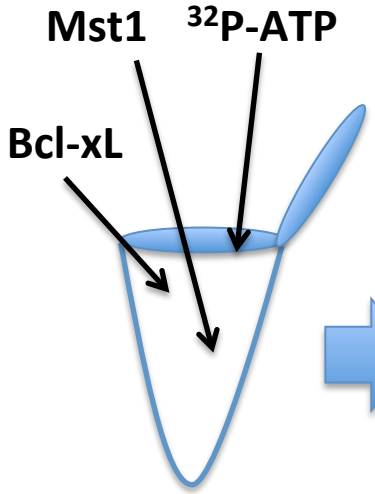




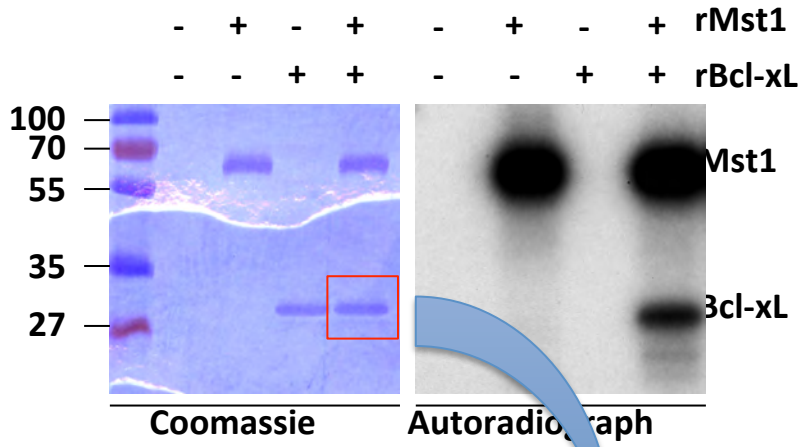


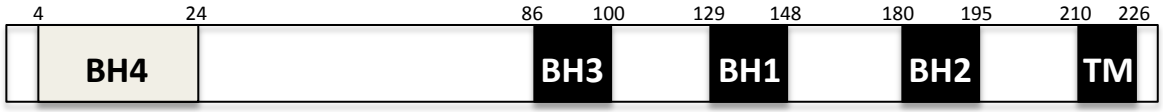


Mst1 phosphorylates the BH4 domain of Bcl-xL



SDS-PAGE





Proteomics analysis

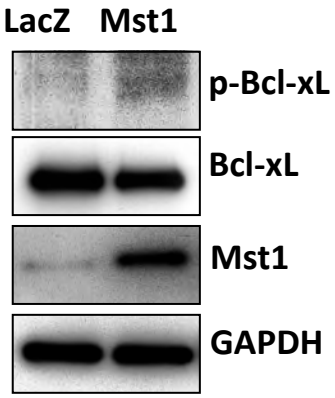
rat SNRELVVDFLSYKLSQKGYSW
mouse SNRELVVDFLSYKLSQKGYSW
human SNRELVVDFLSYKLSQKGYSW

α_1-helix

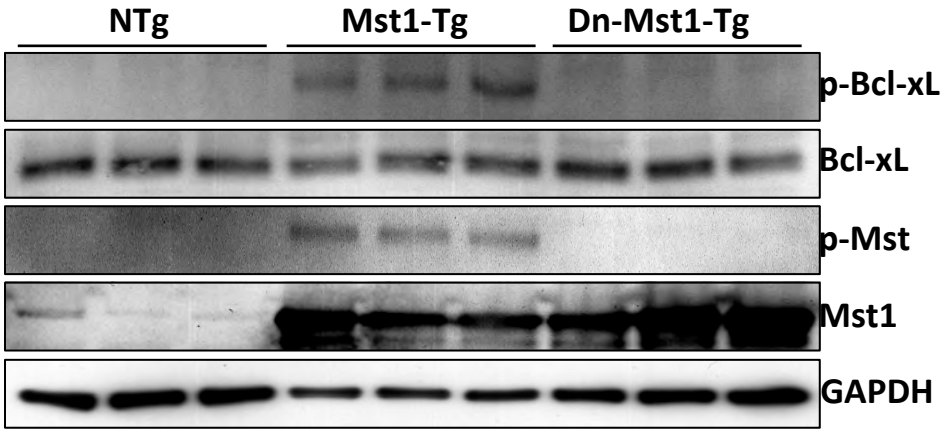
Dr Hong Li, Proteomics Core, NJMS

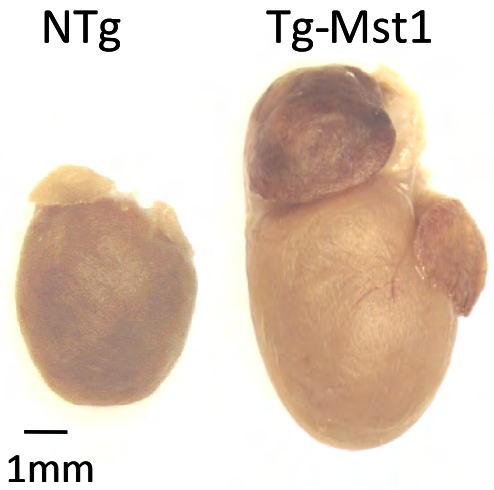
Mst1 expression elicits Bcl-xL phosphorylation

CARDIOMYOCYTES

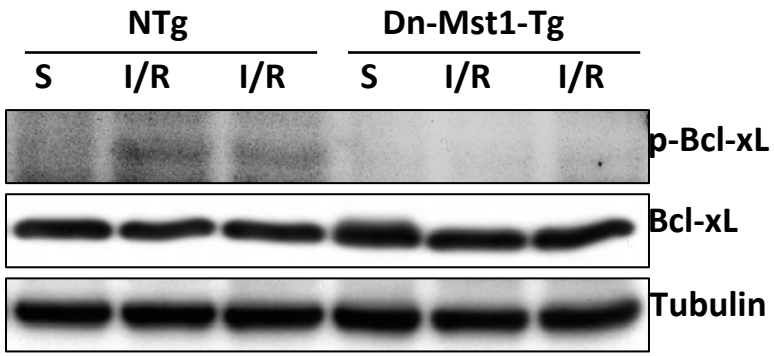


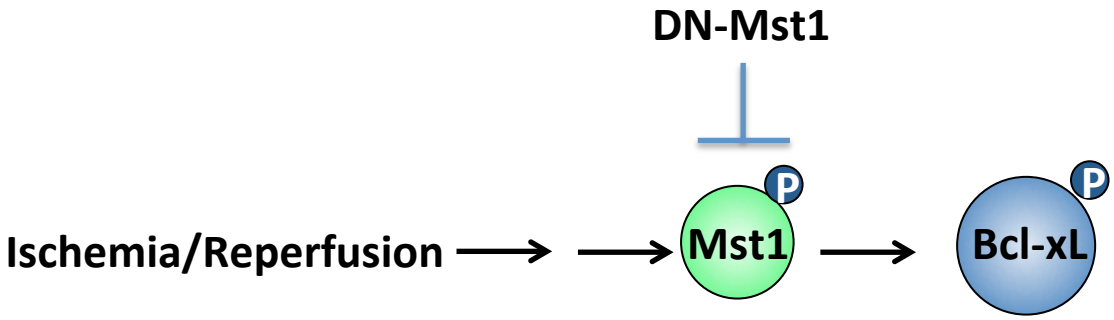
HEART



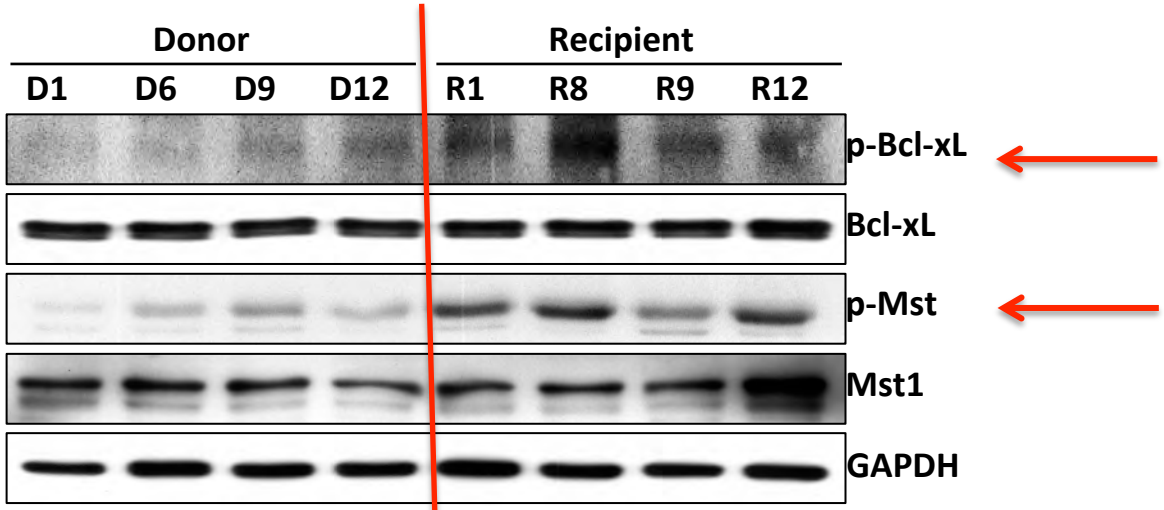


Endogenous Mst1 mediates Bcl-xL phosphorylation

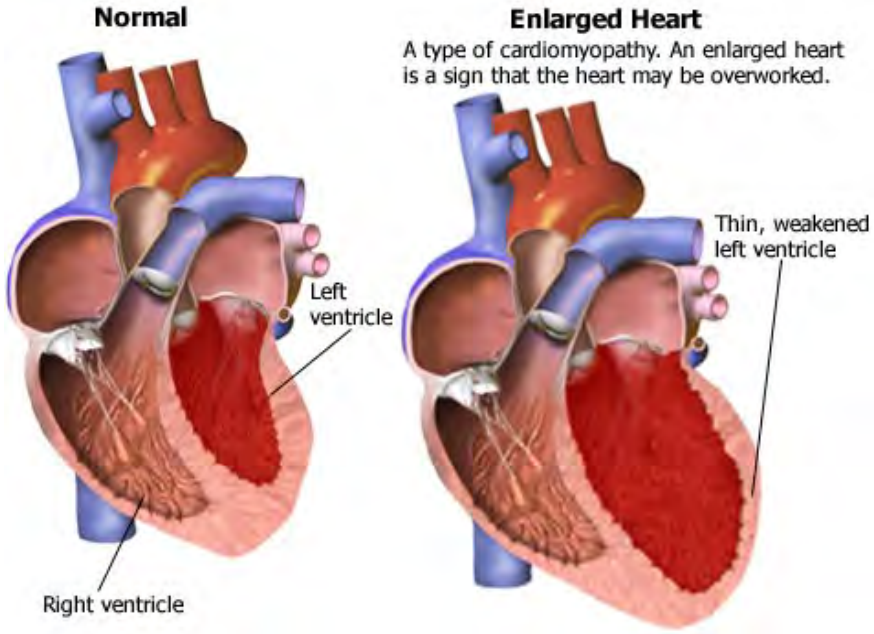




Bcl-xL phosphorylation is increased in human failing hearts

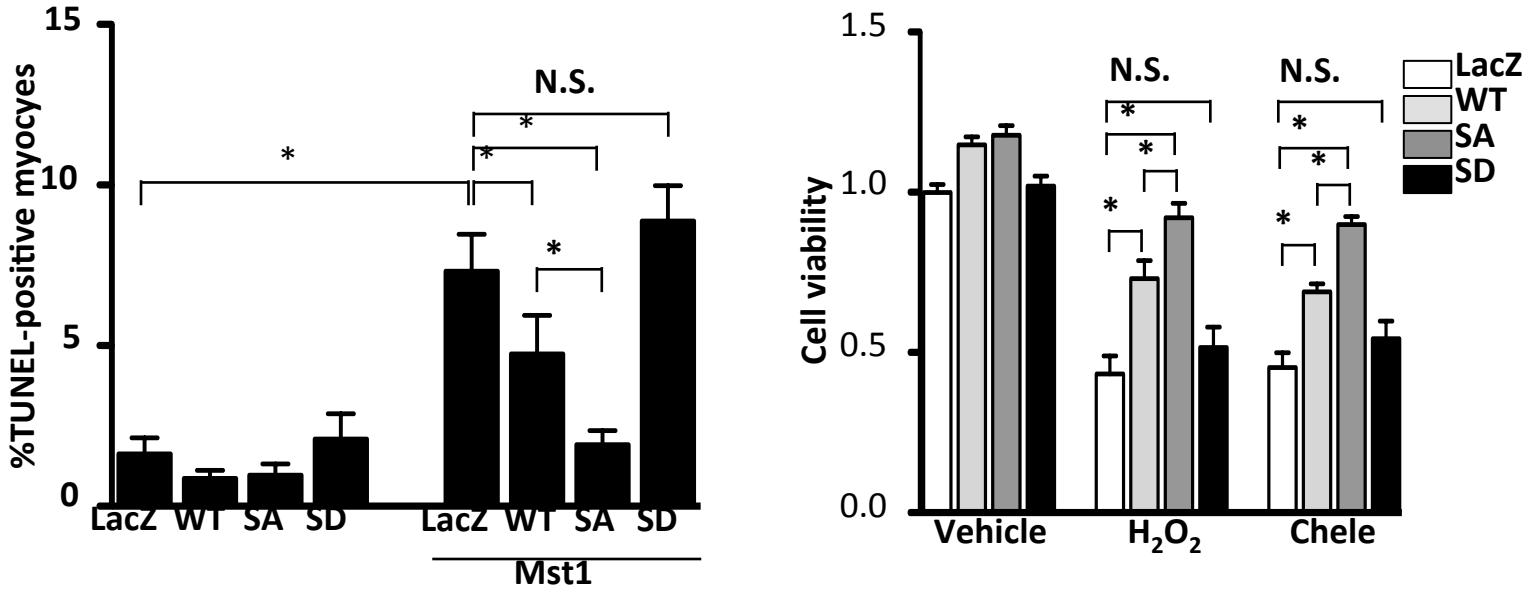


HEALTHY **FAILING**



Phosphorylated Bcl-xL has compromised protective capacity

Cardiomyocytes

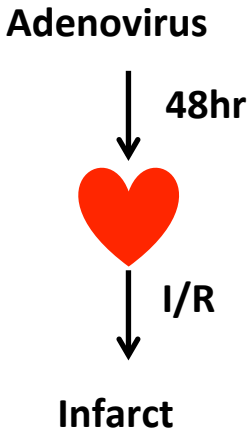


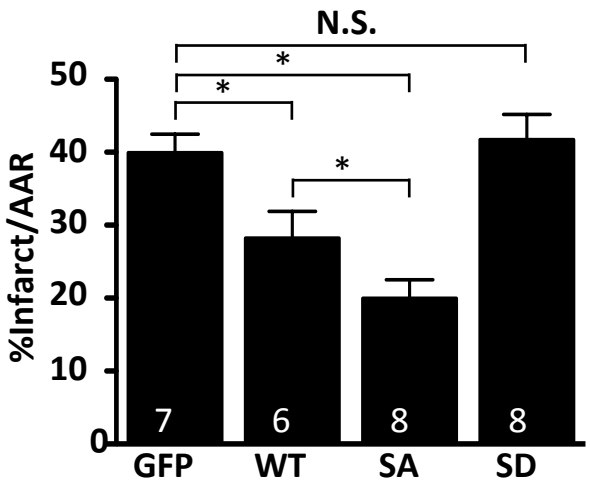
Bcl-xL mutants:

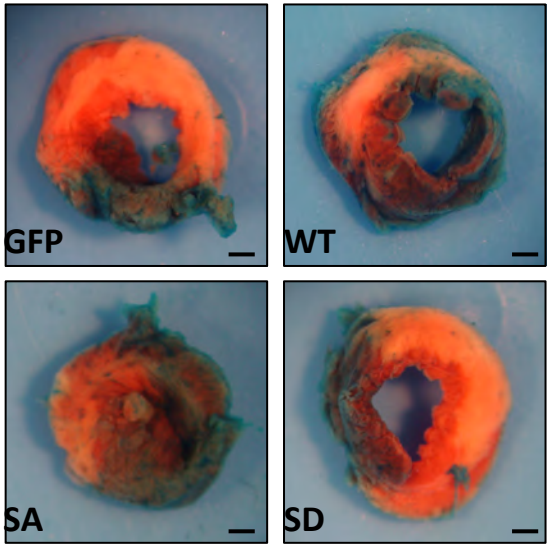
SA=Ser to Ala=non-phosphorylatable - - - extra protection

SD=Ser to Asp=phosphomimetic - - - no protection

Phosphorylated Bcl-xL has compromised protective capacity





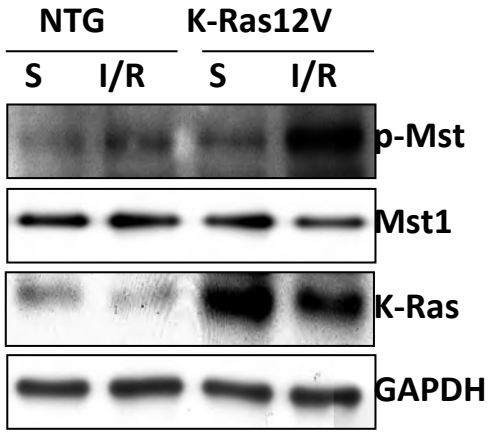


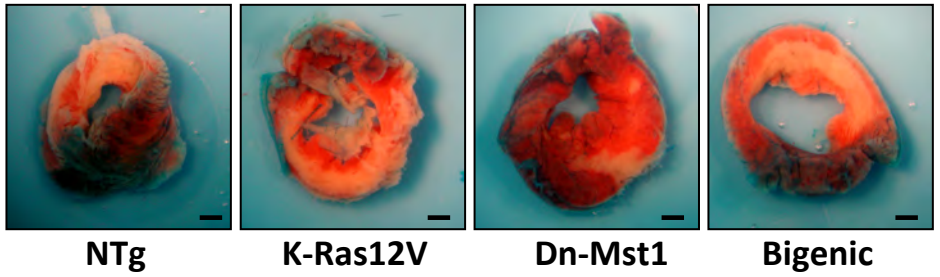
<u>Bcl-xL mutants:</u>

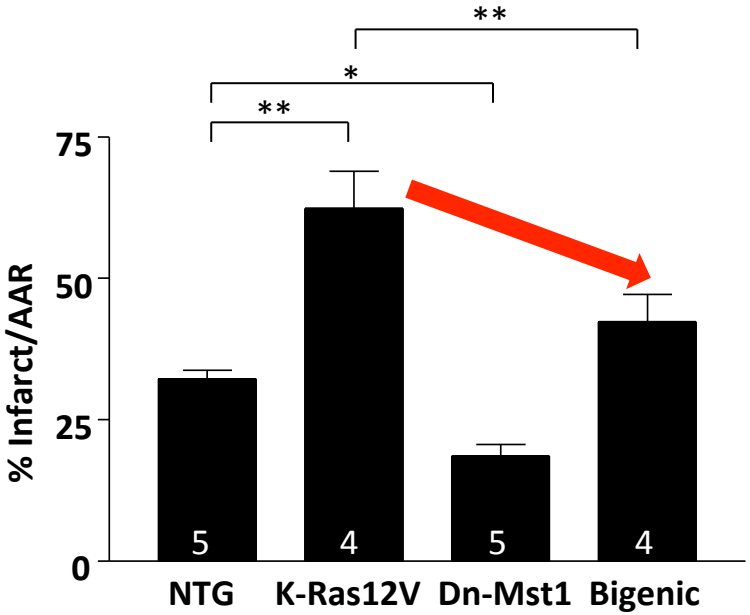
SA=Ser to Ala=non-phosphorylatable - - - extra protection

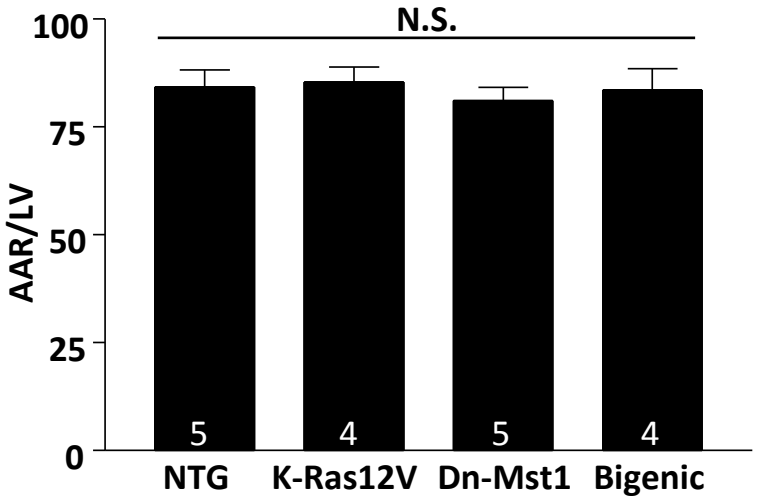
SD=Ser to Asp=phosphomimetic - - - no protection

K-Ras12V expression promotes myocardial injury in vivo

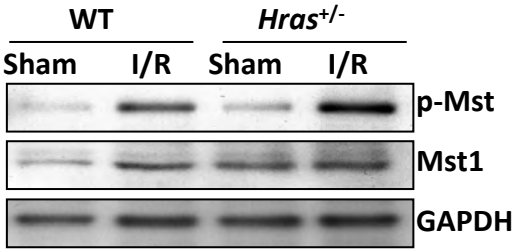


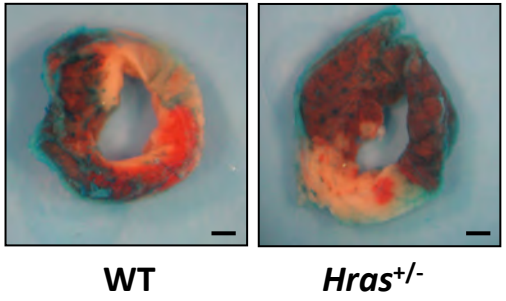


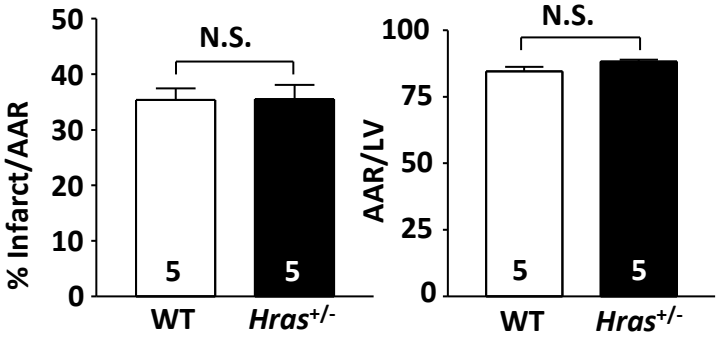


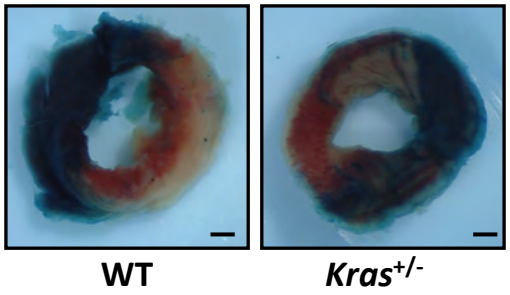


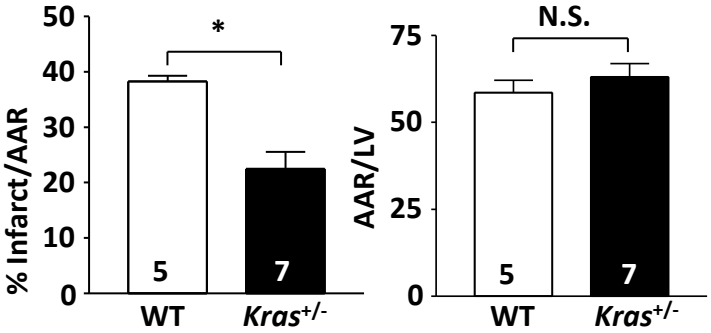
K-Ras deletion protects against I/R injury in vivo

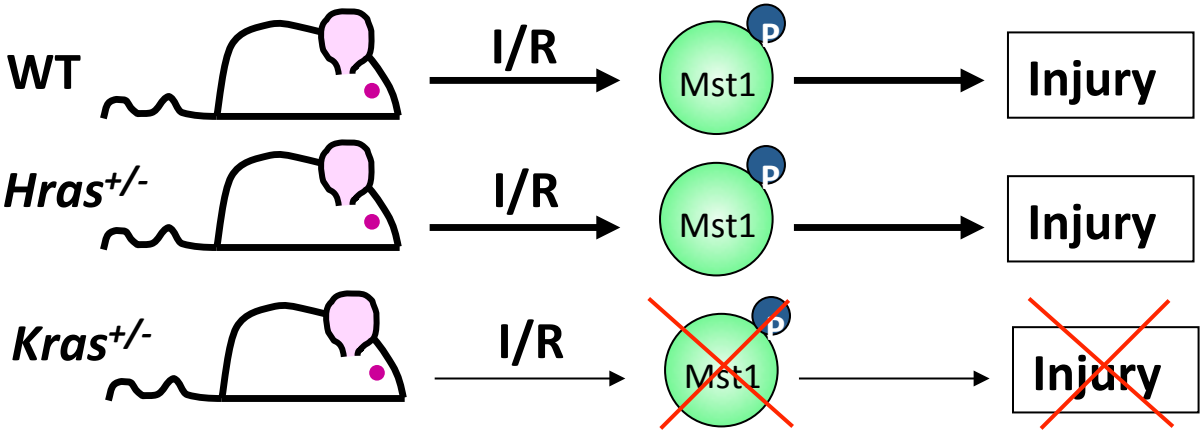






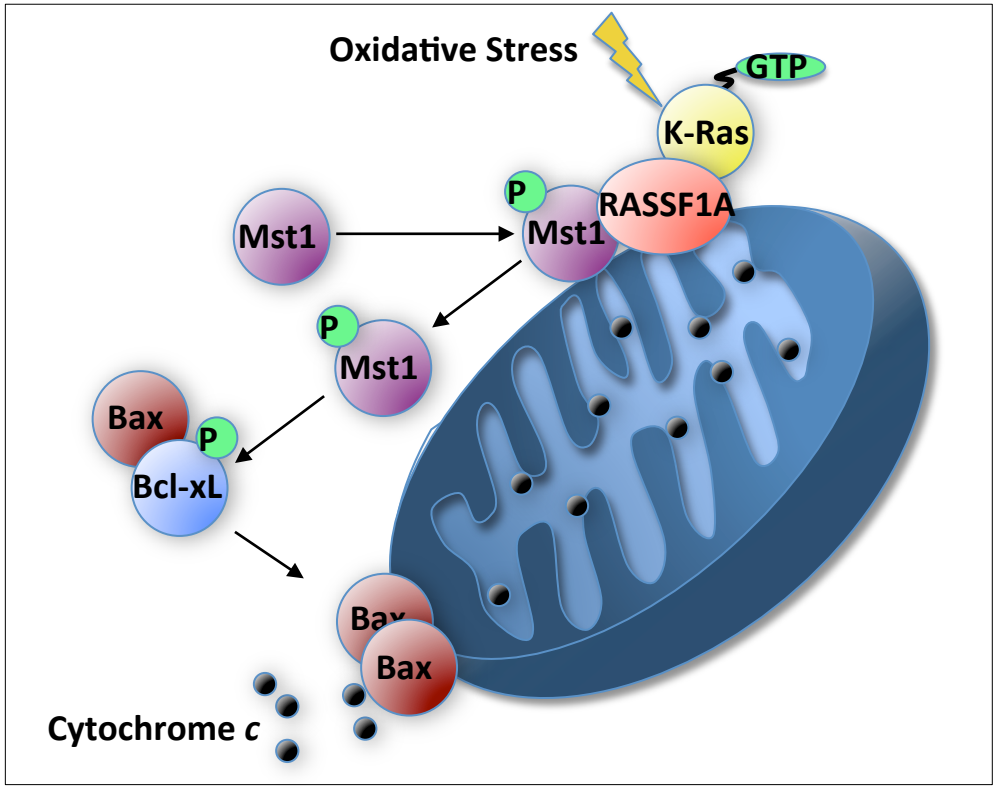






Summary

- Hippo signaling is an important regulator of heart injury and failure
- K-Ras is activated during I/R through an oxidative dependent mechanism
- K-Ras and H-Ras show selective signal transduction in cardiomyocytes
- K-Ras/RASSF1A/Mst1 form a complex at mitochondria leading to Mst1 activation
- Mst1 phosphorylates the BH4 domain of Bcl-xL leading to activation of Bax and cardiomyocyte apoptosis
- Inhibition of endogenous K-Ras *in vivo* attenuates Mst1 activation and confers cardioprotection



More Questions – Future Directions

- Why is K-Ras at mitochondria of cardiomyocytes and not other cell types?
- How does phosphorylation of Serine 14 of Bcl-xL prevent Bax association?
- Are there additional subcellular localizations of Hippo signaling?

Potential Therapeutic Implications

- Is it possible to target/inhibit this pathway?
- Can Mst1 activation be prevented? RASSF1A binding? Kinase activity?
- Optimal timing of treatment for MI patients? Reperfusion?