



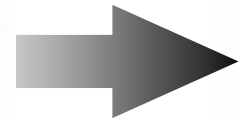
# **Cardiomyocyte Autophagy in the Pathogenesis of Heart Failure**

**Joseph A. Hill, MD, PhD**

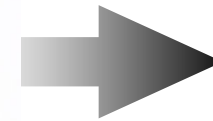
**October 12, 2007**

# Heart Failure Progression

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**cell  
growth**



**cell  
death**

~~necrosis~~

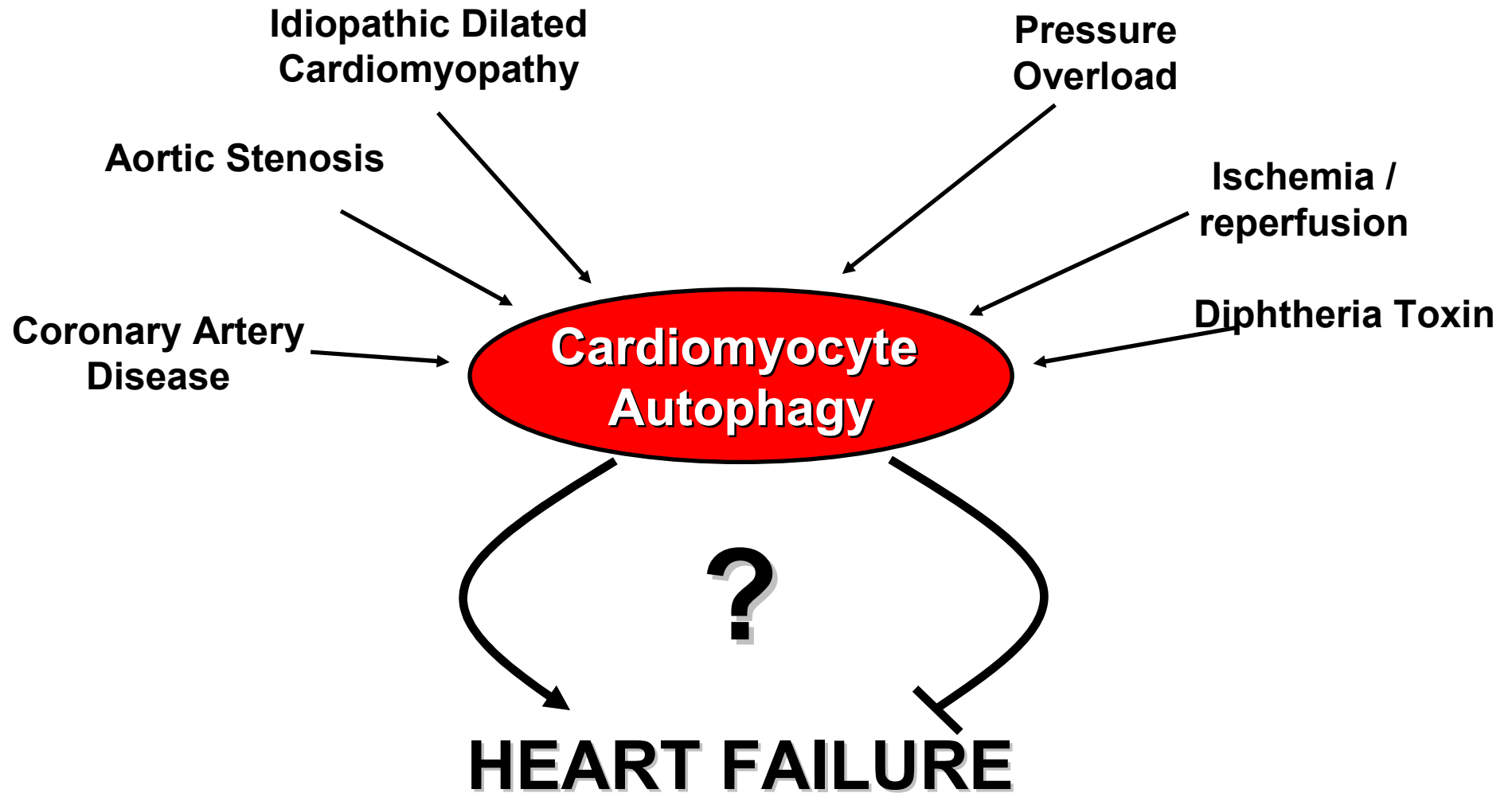
~~apoptosis~~

autophagy



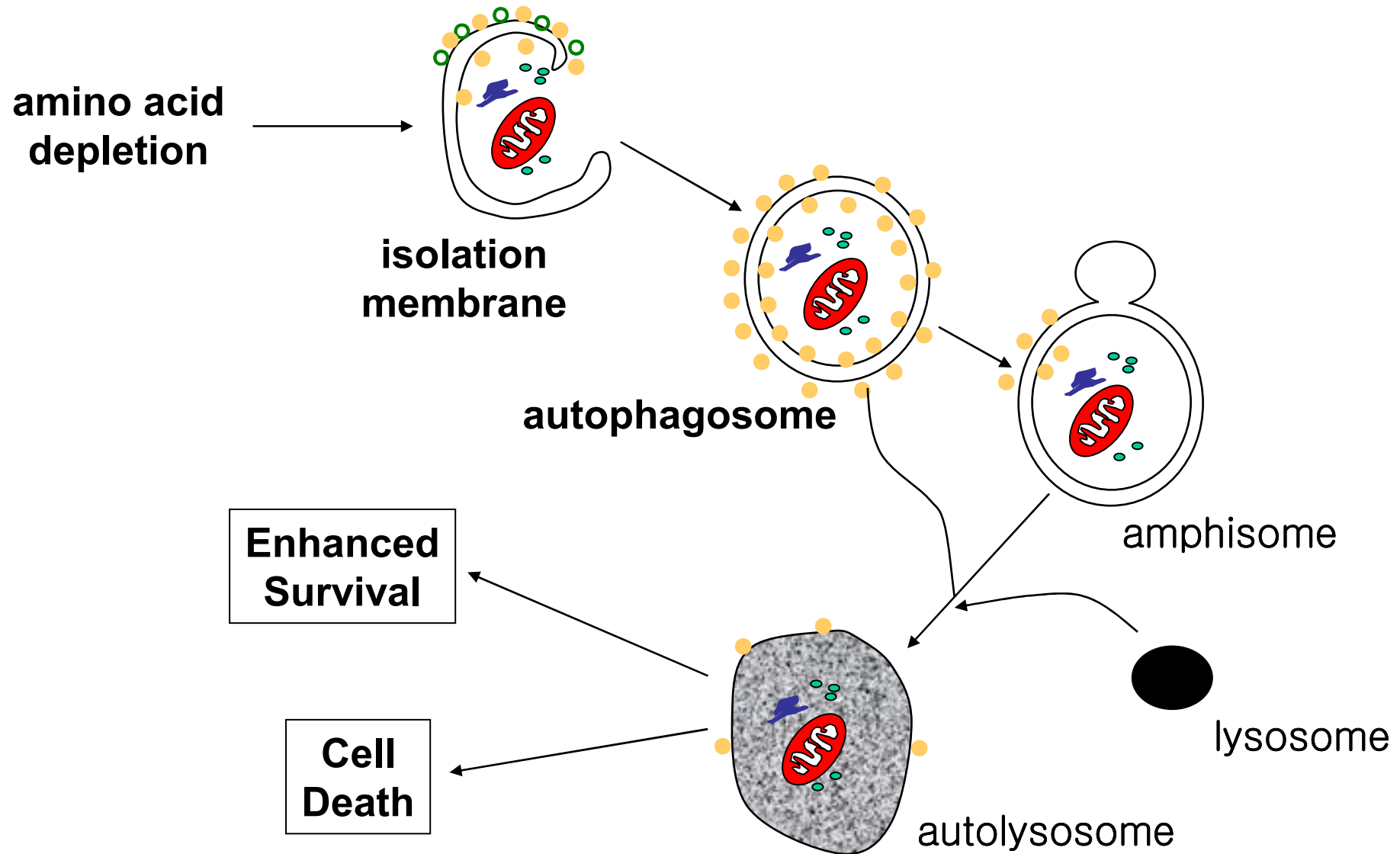
# Autophagy in Heart Disease

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# Autophagy Pathway

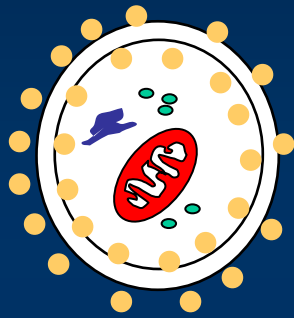
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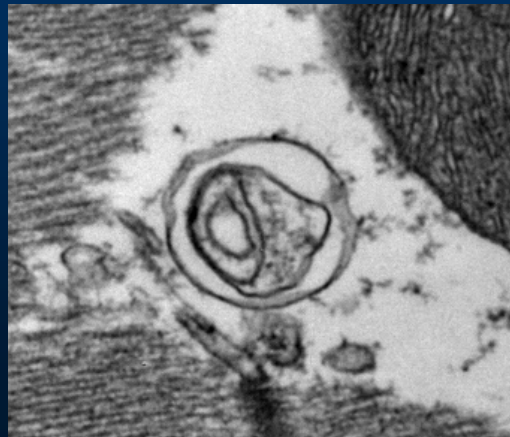


# Experimental Markers of Autophagy

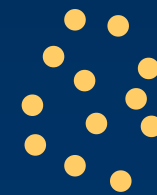
## Morphological Markers



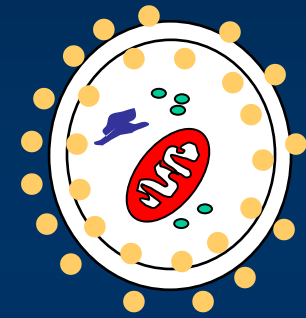
Autophagosomes



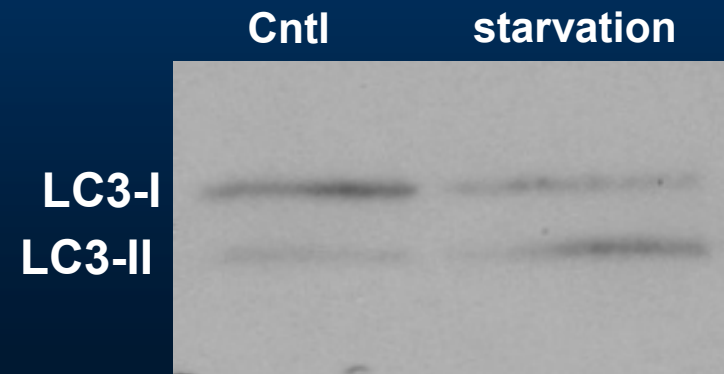
## LC3 Processing



LC3-I  
(18kD)



LC3-II  
(16kD)

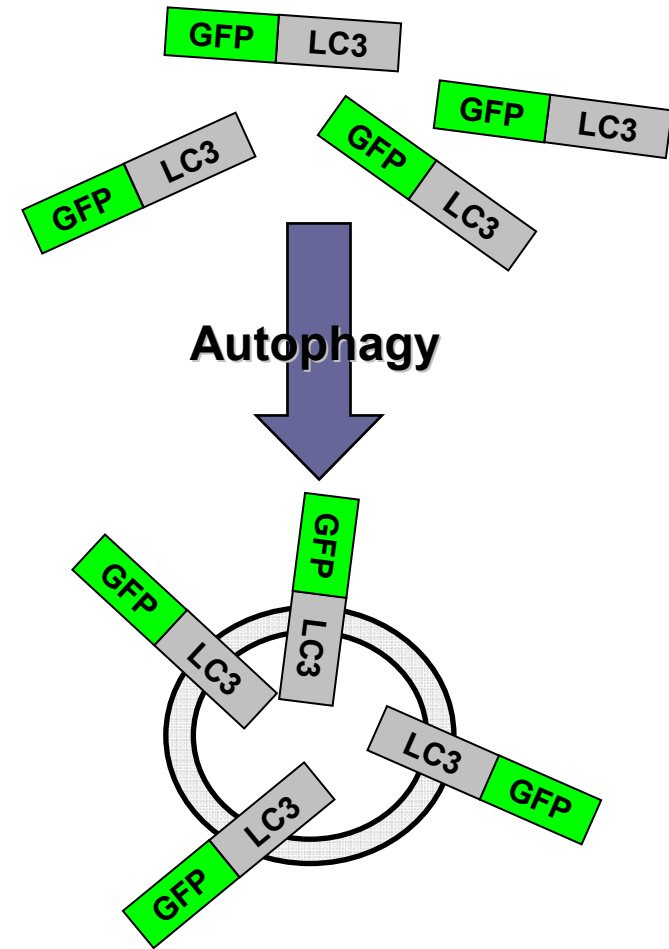


# Monitoring Autophagy *In Vivo*

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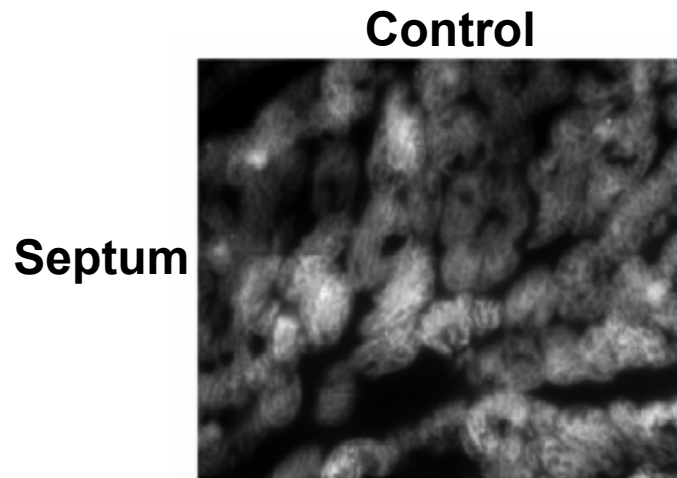


$\alpha$ MHC-GFP-LC3  
"Reporter Mouse"



# Starvation induces autophagy in cardiac myocytes *in vivo*

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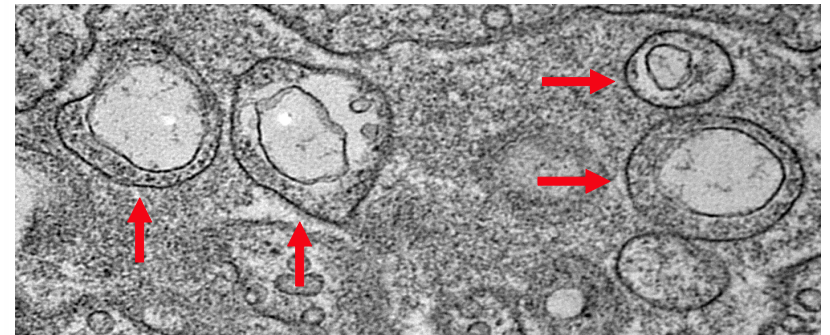
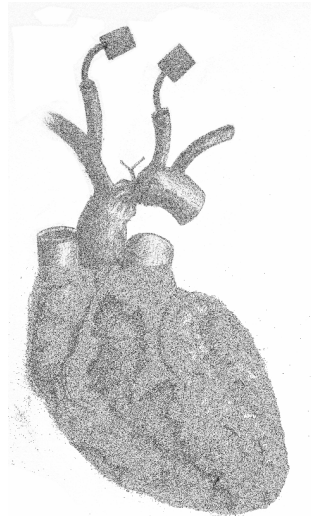
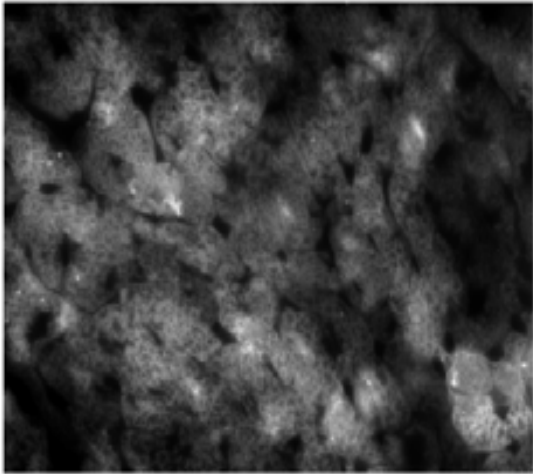


# Pressure overload triggers cardiac autophagy

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Sham

Septum

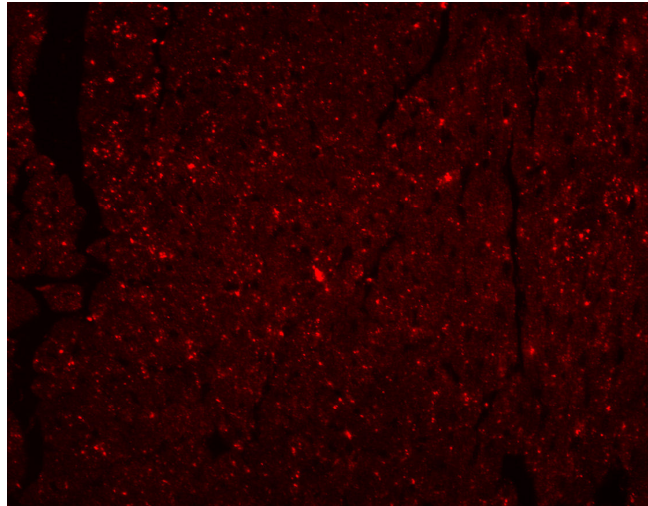


# Autolysosomes are increased in pressure-overload heart failure

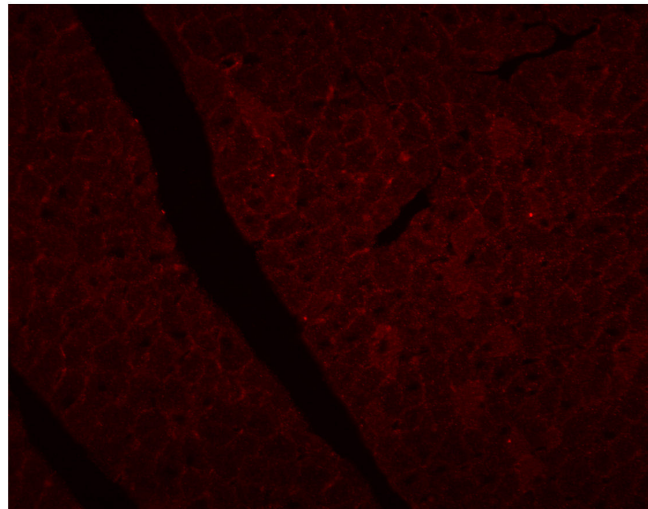
---

Sham

LAMP-1

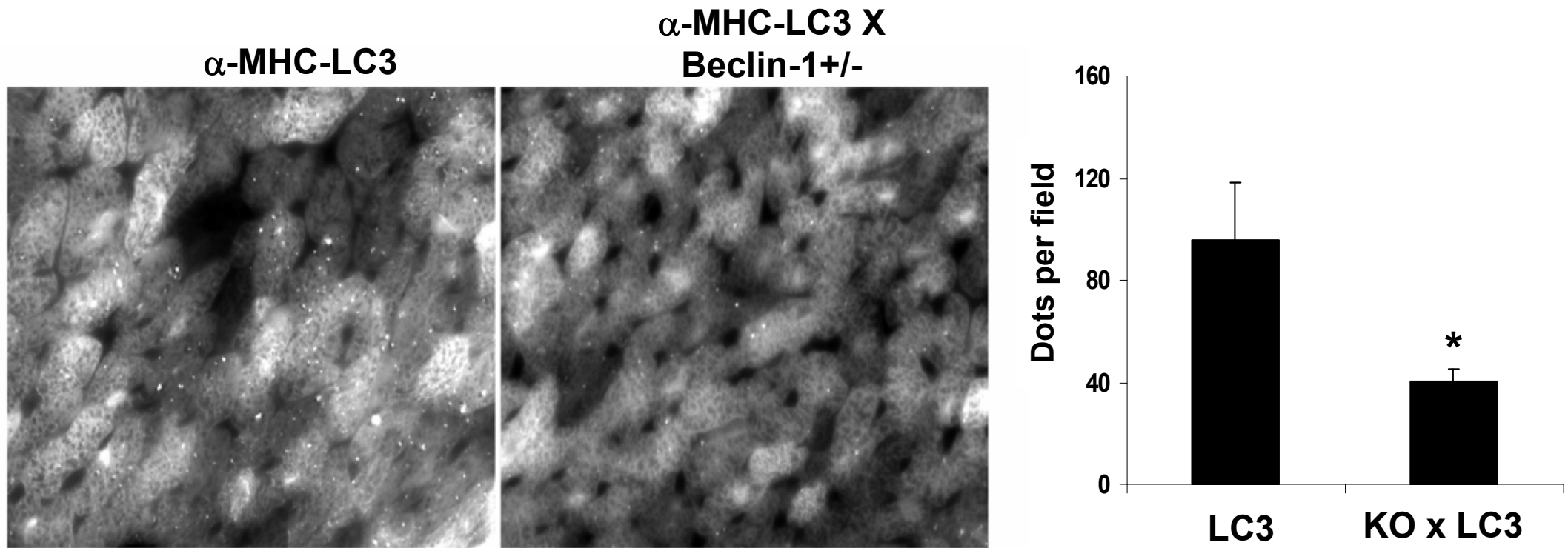


Cathepsin D



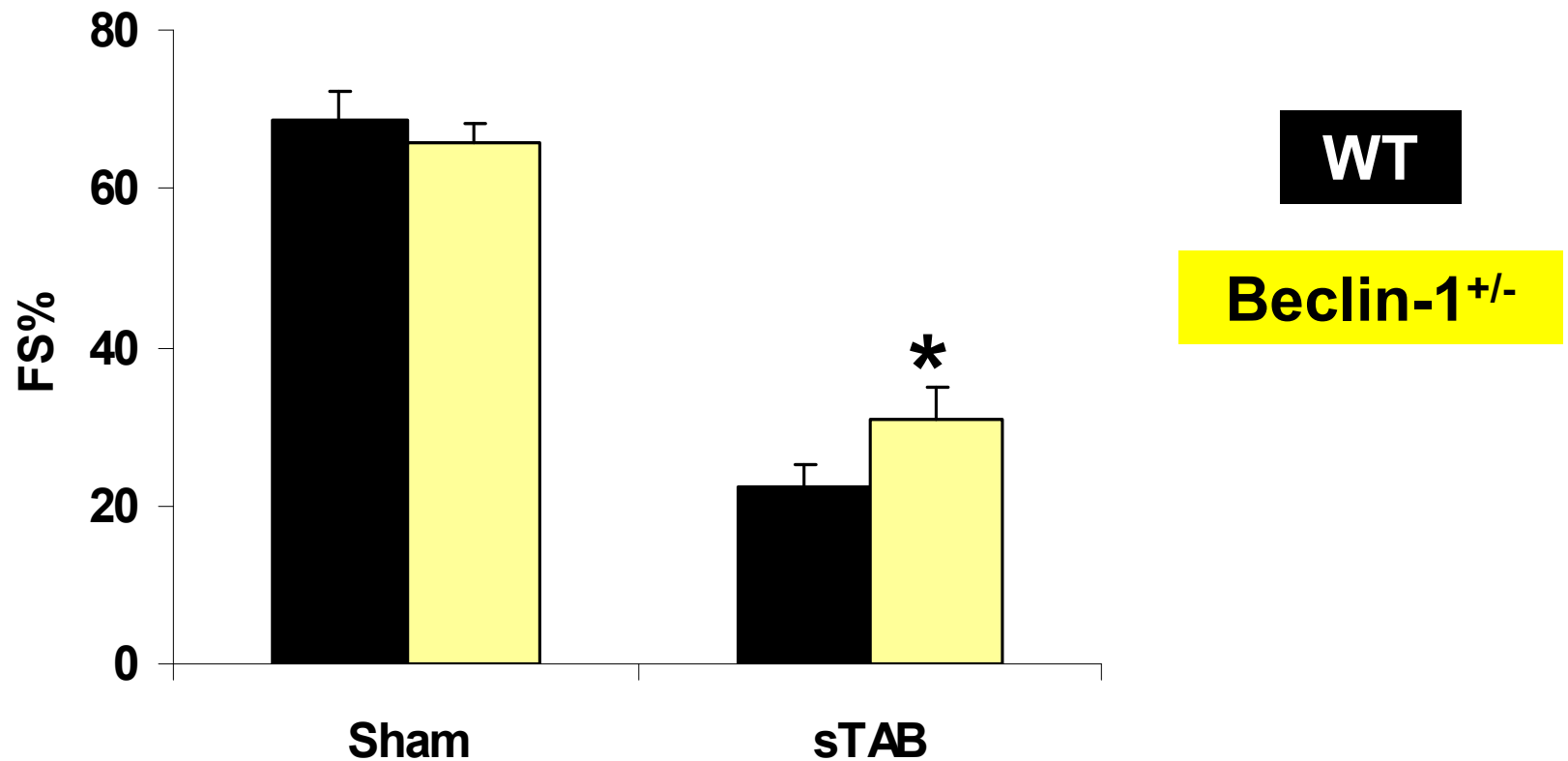
# Beclin-1 inactivation diminishes load-induced cardiomyocyte autophagy

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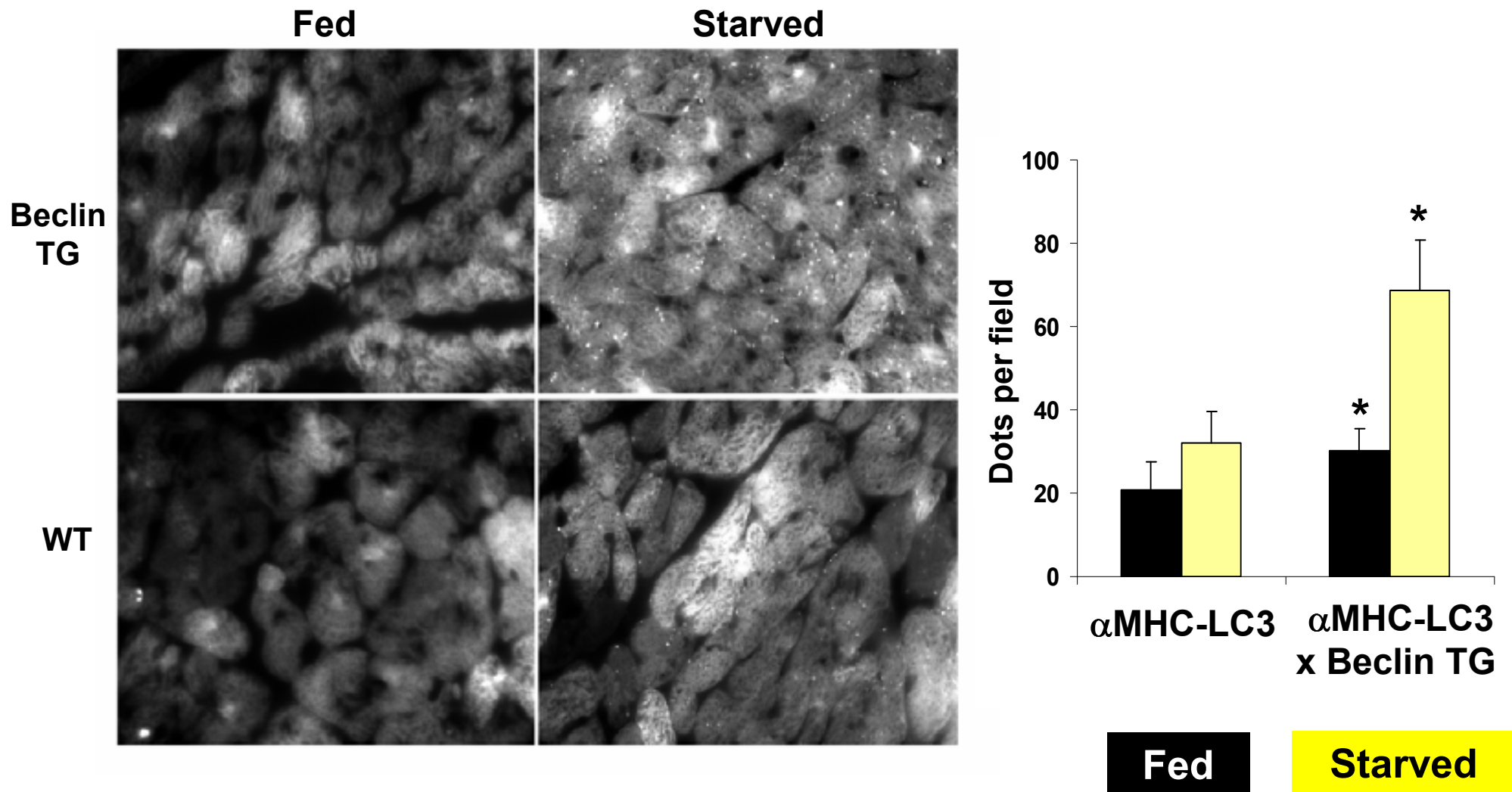
# Pathological ventricular remodeling is modestly blunted by Beclin-1 inactivation and consequent diminished autophagy

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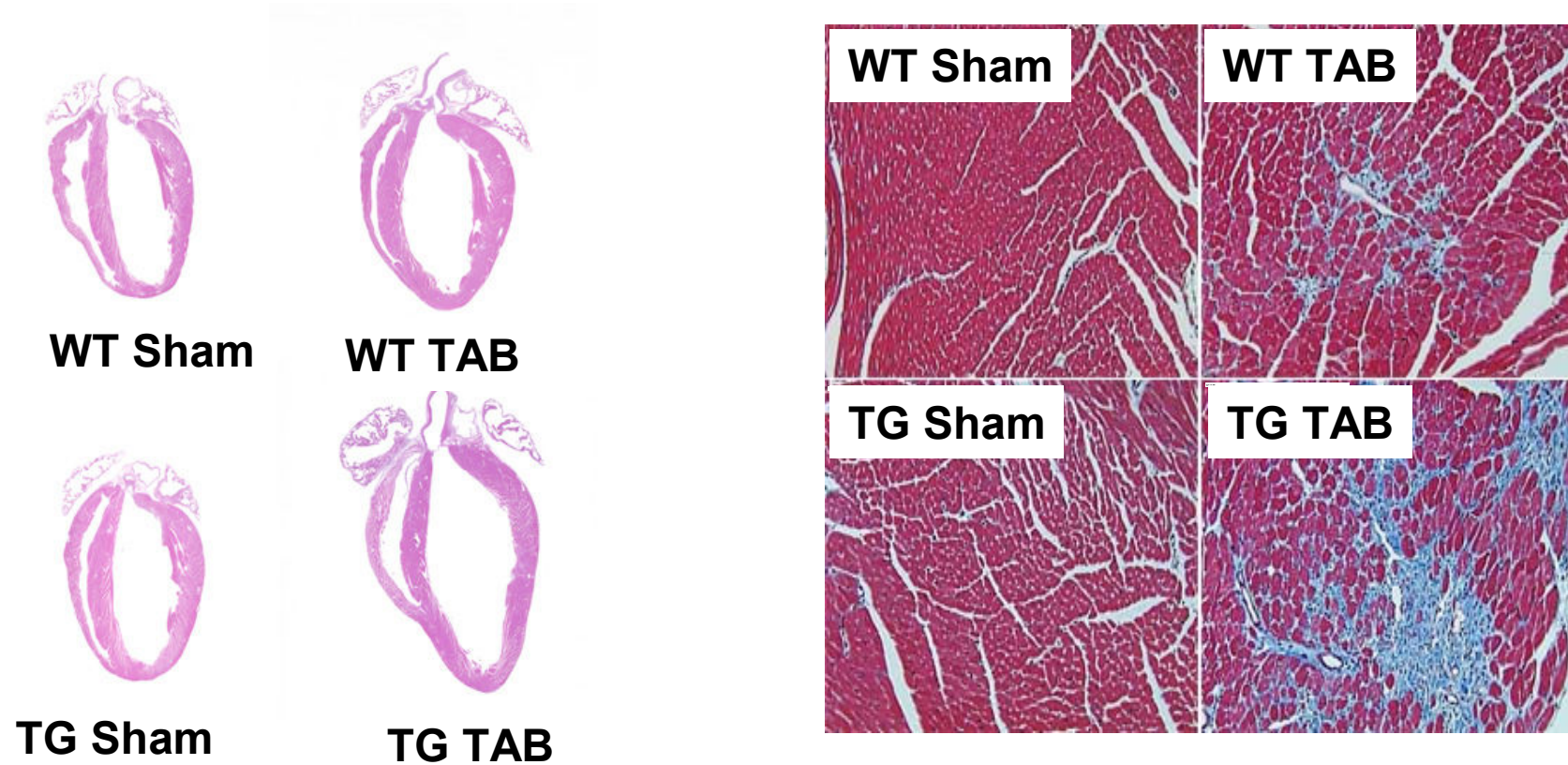
# Beclin-1 over-expression promotes cardiomyocyte autophagy



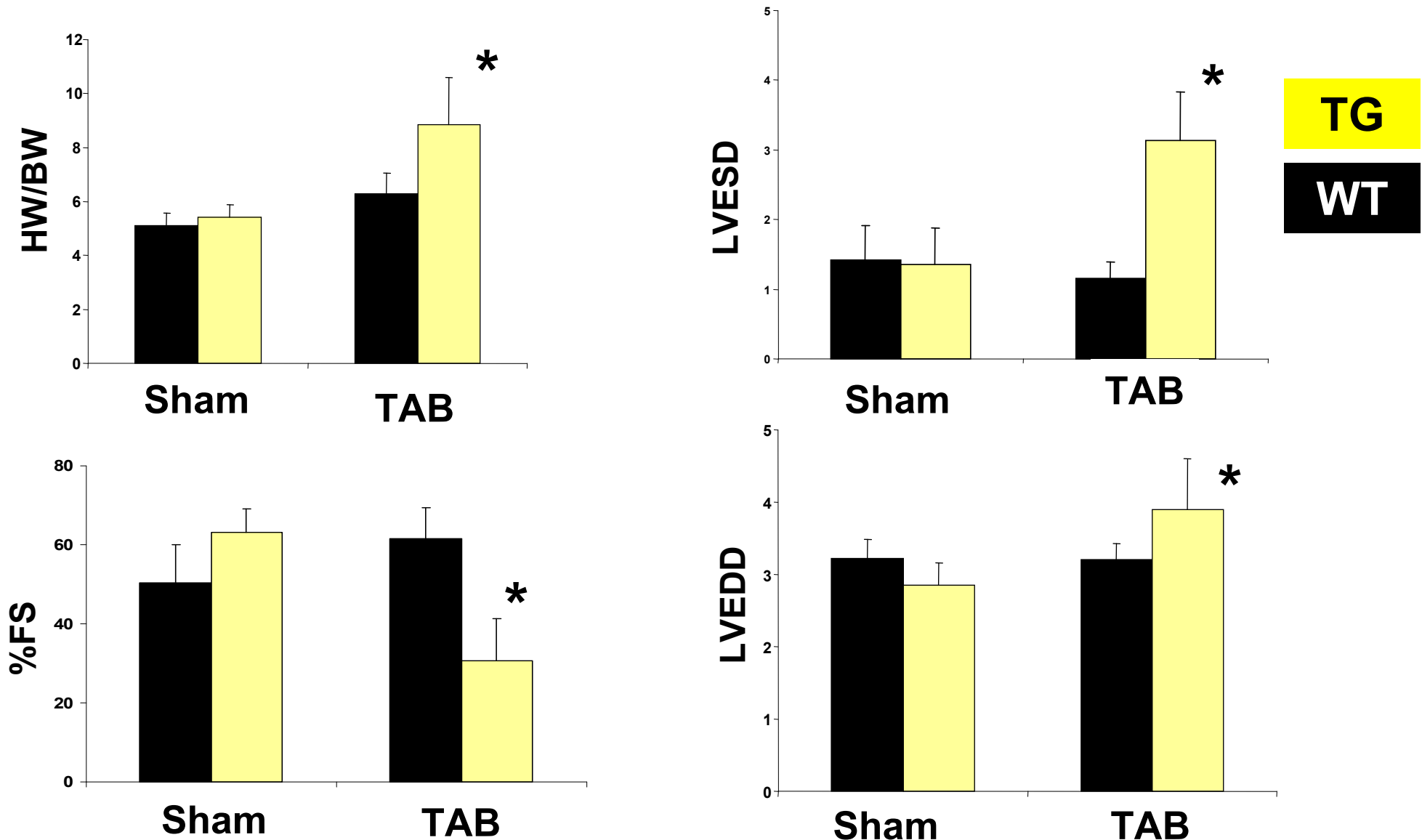


# Beclin-1 over-expression accentuates TAB-induced pathological remodeling

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# Beclin-1 over-expression accentuates TAB-induced pathological remodeling

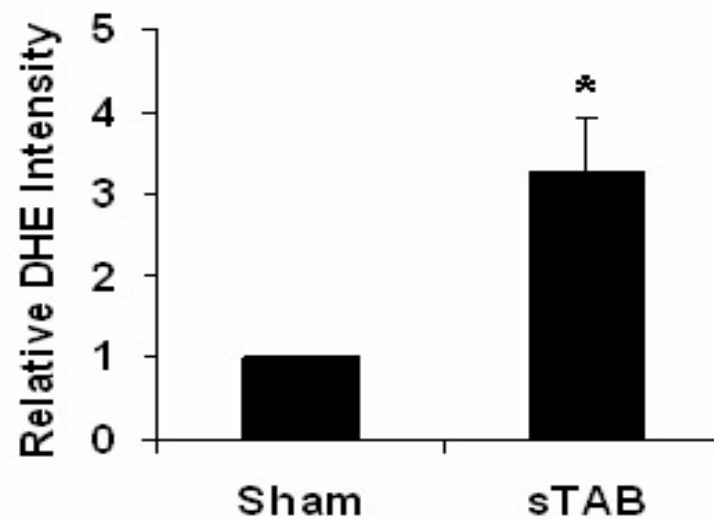
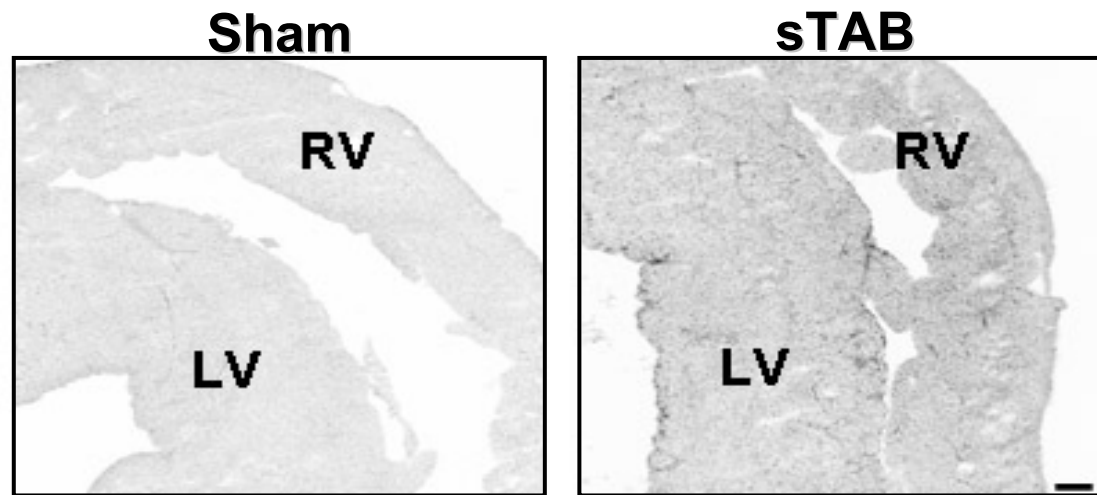


# Cardiomyocyte Autophagy

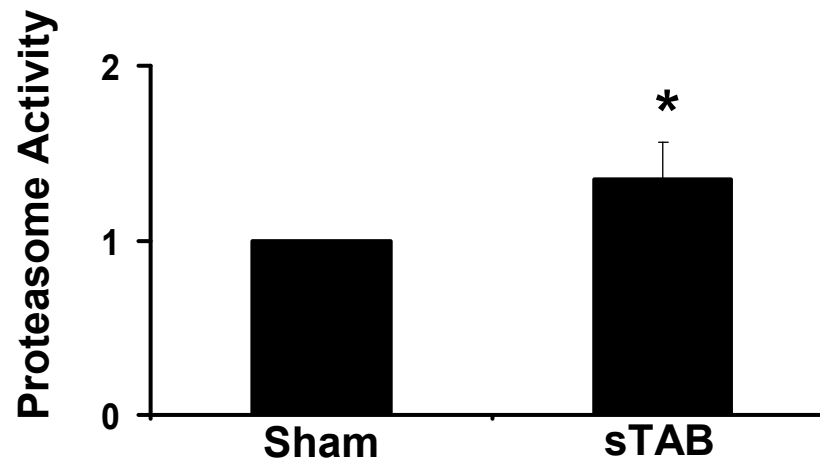
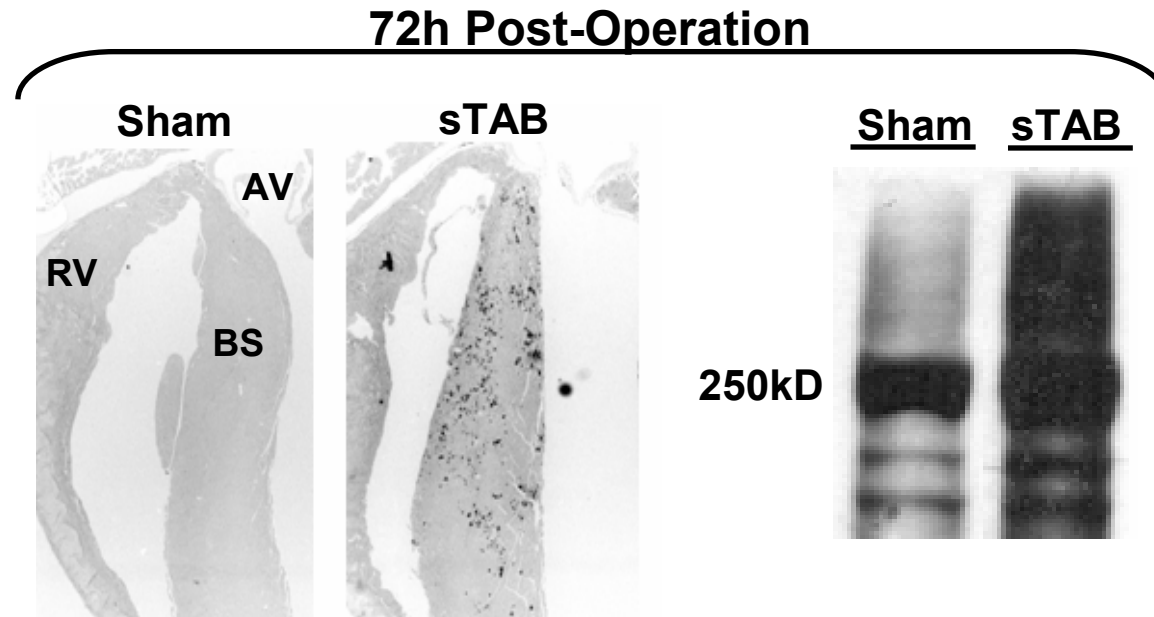
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What are the proximal activating triggers?

# ROS generation is an early response to pressure overload

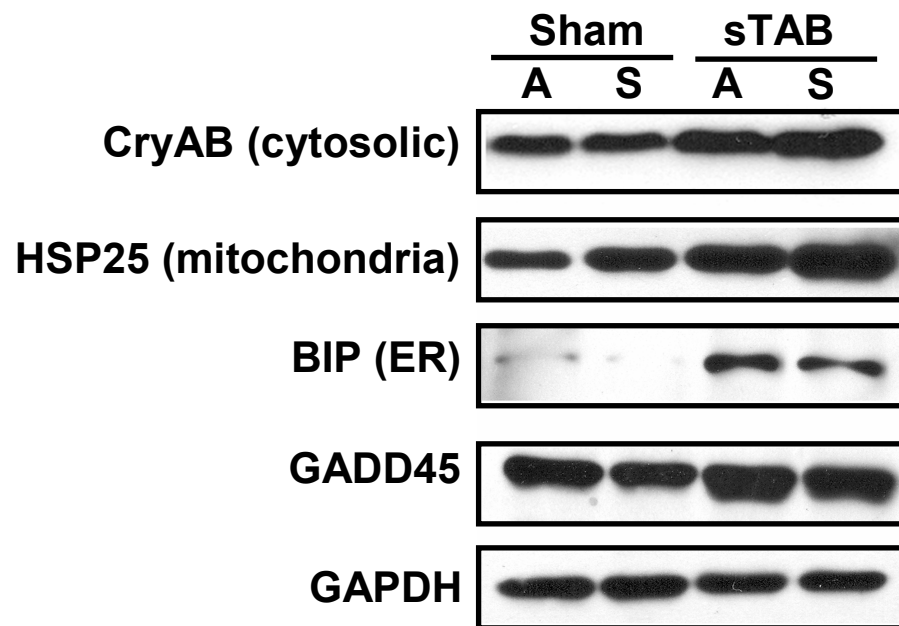


# Polyubiquitinated proteins accumulate in stressed myocardium



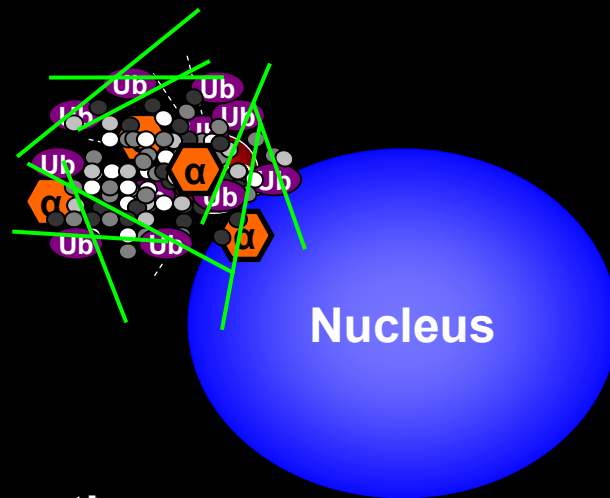
# Increased levels of chaperone proteins

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# Pressure-Overload Induced Aggresome Formation

**Aggresome:** A perinuclear, cytoplasmic inclusion containing damaged proteins ensheathed in a cage of IFs, formed specifically at the MTOC.



— = Vimentin

α = CryAB

Ub = Ubiquitin

# **Desmin-Related Cardiomyopathy**

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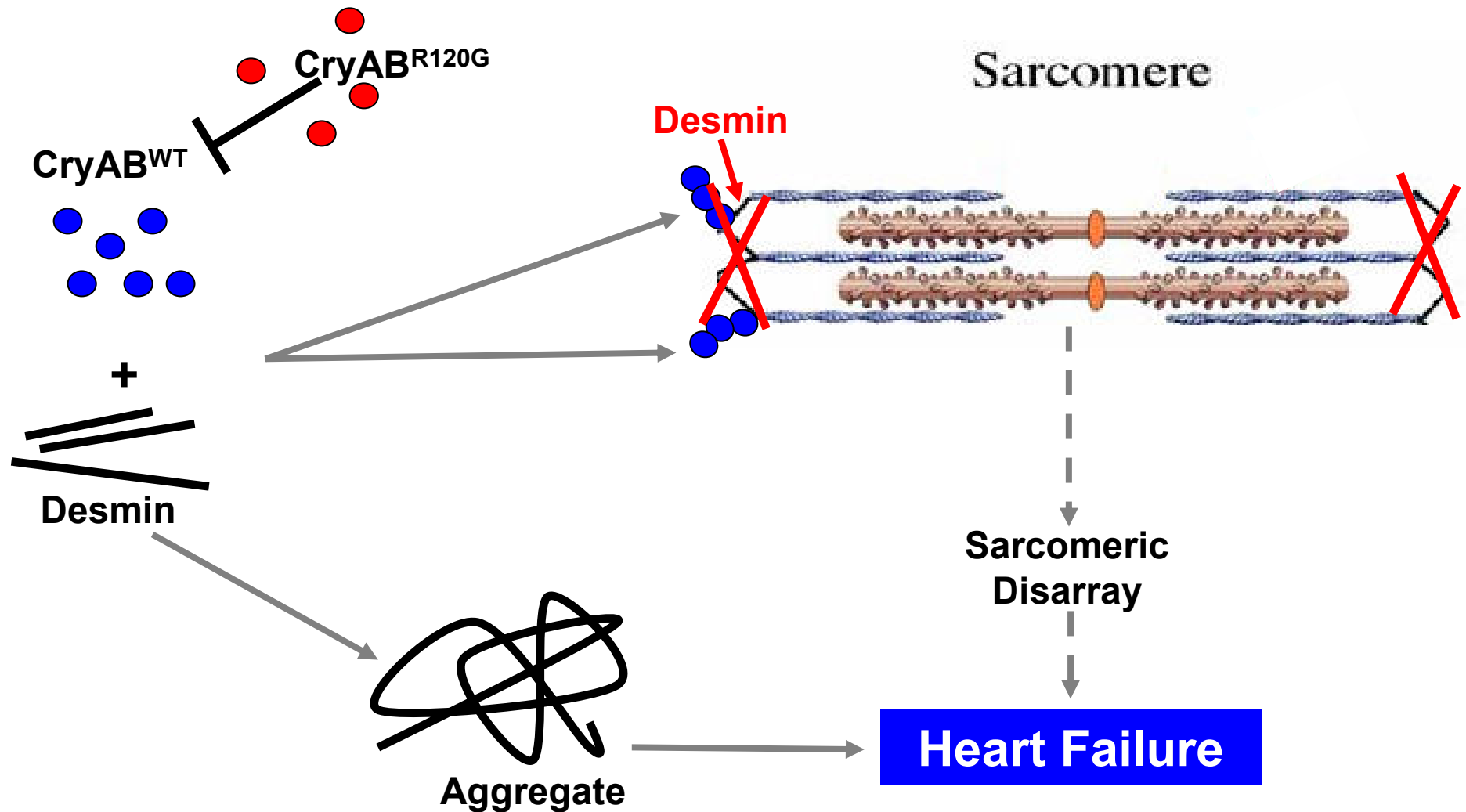
**Due to dysfunctional chaperone proteins, misfolded peptides precipitate in the myocyte cytoplasm leading to intracellular aggregates.**

**This condition is analogous to neurodegenerative proteinopathies, such as Parkinsonism, Huntingtonism, and Alzheimer's disease.**



# CryAB<sup>R120G</sup>-associated DRCM

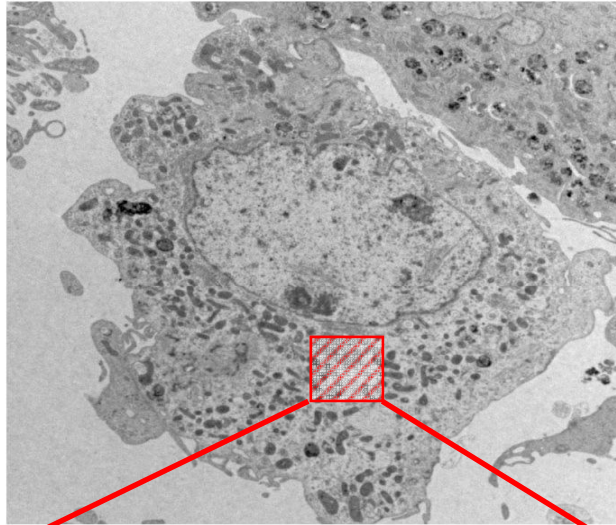
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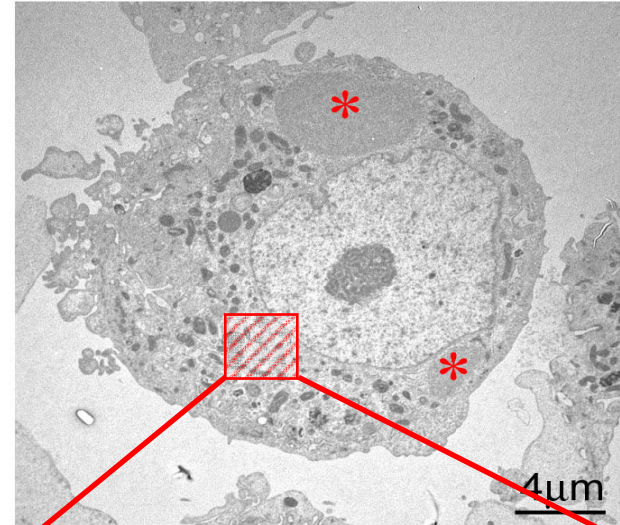
# CryAB<sup>R120G</sup> is a potent activator of cardiomyocyte autophagy

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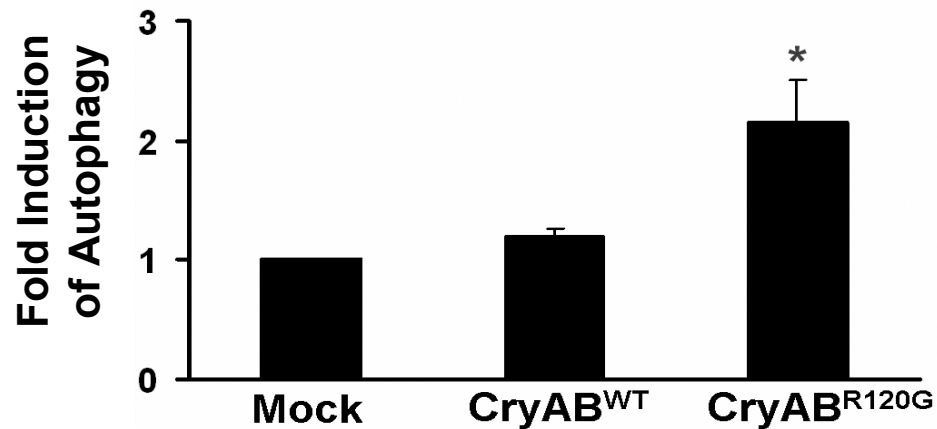
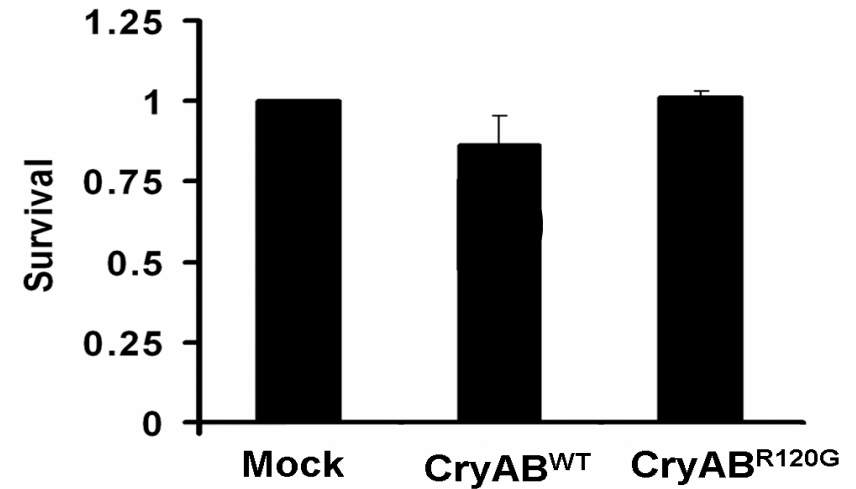
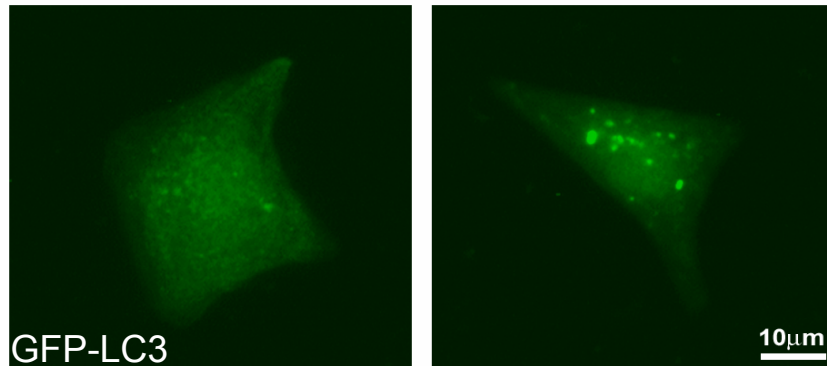
CryAB<sup>WT</sup>



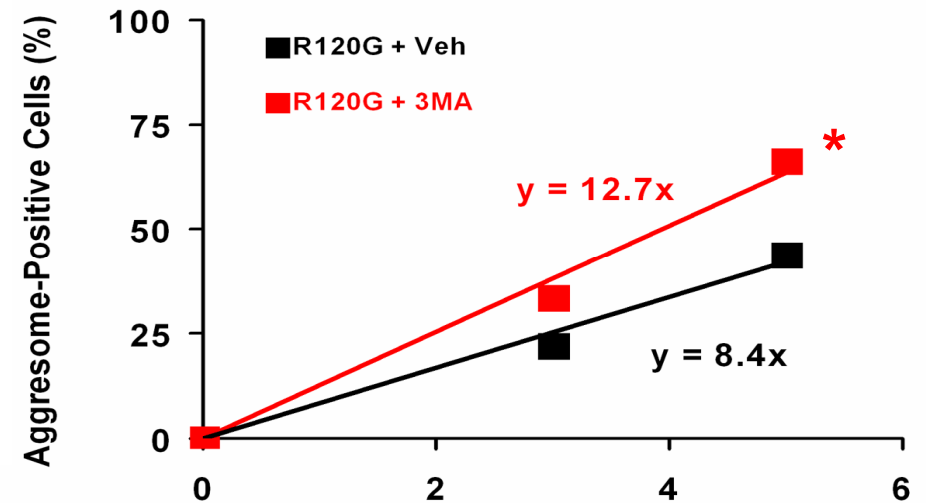
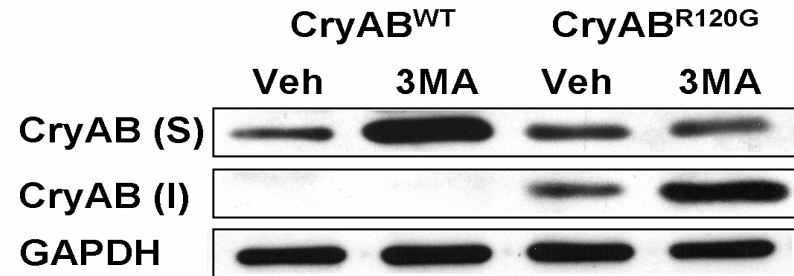
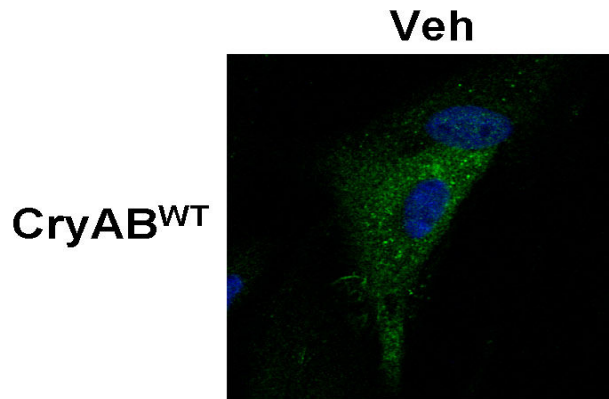
CryAB<sup>R120G</sup>



# CryAB<sup>R120G</sup> is a potent activator of cardiomyocyte autophagy

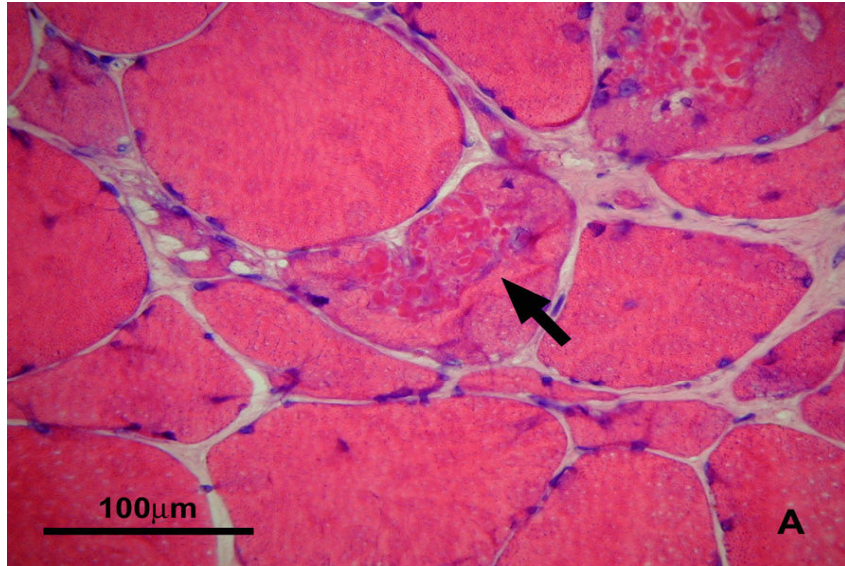


# Autophagic activity clears CryAB<sup>R120G</sup>-induced aggregates



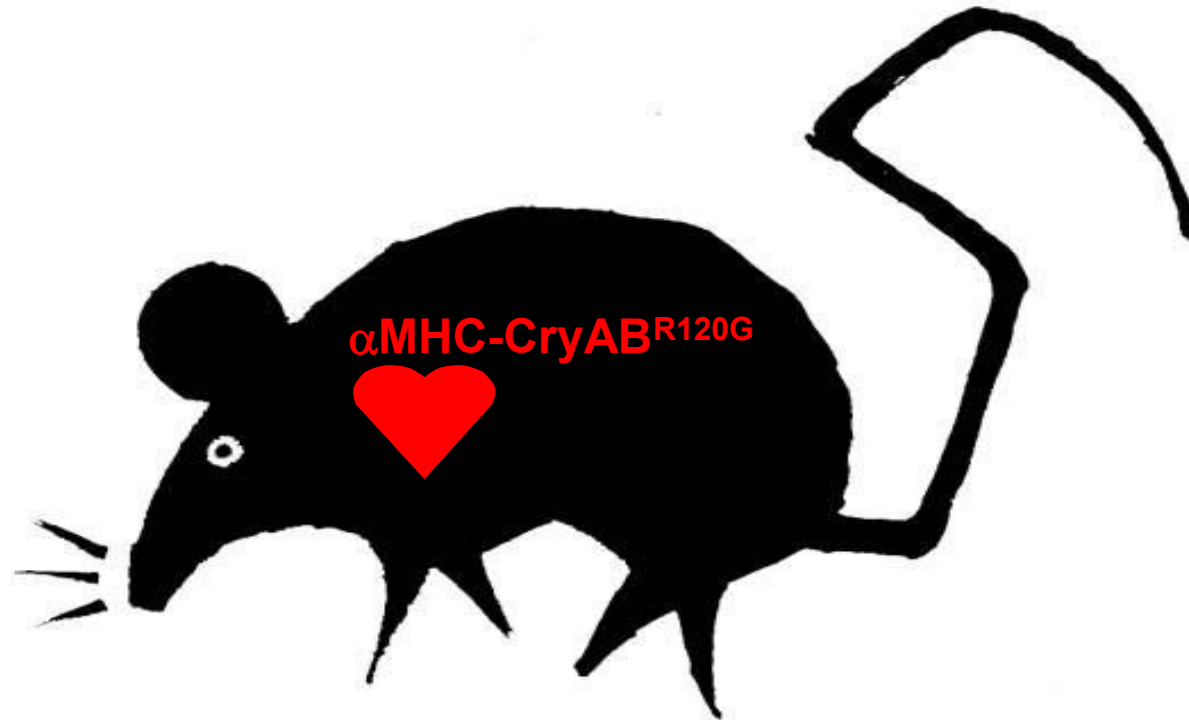
# Autophagy in patients with MFM

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# Model of DRCM

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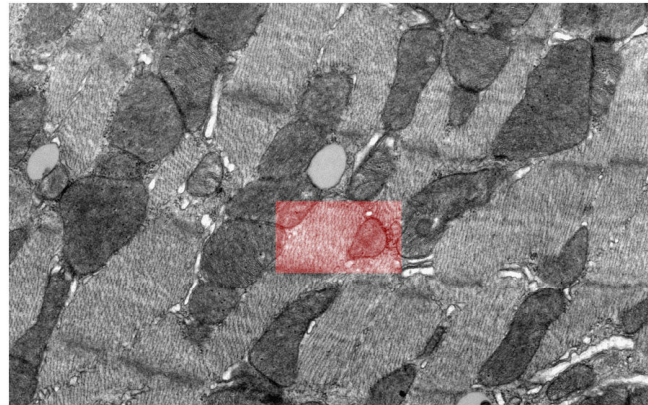
**First signs of cardiac disease at 9m**  
**Congestive heart failure and death at ~1yr**  
**Extensive intracellular aggregates**  
**Cardiomyocyte-restricted expression**



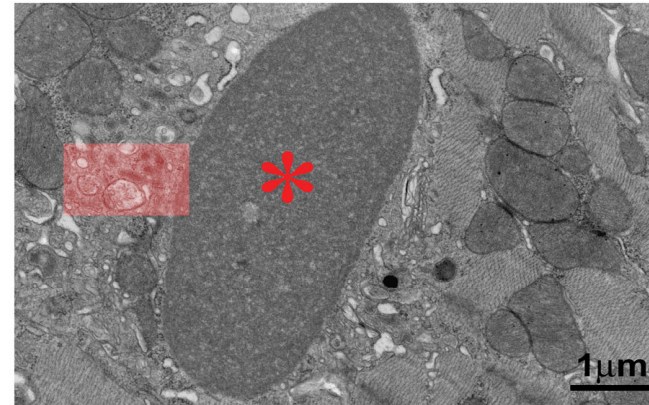
# Mutant CryAB expression induces autophagy *in vivo*

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Wildtype



$\alpha$ MHC-CryAB<sup>R120G</sup>



Heart  
6,000X

# Is CryAB-induced autophagy adaptive or maladaptive?

## Genotype

*Wildtype*

*Beclin 1<sup>+/-</sup>*

*αMHC-CryAB<sup>R120G</sup>*

*Beclin 1<sup>+/-</sup> X αMHC-CryAB<sup>R120G</sup>*

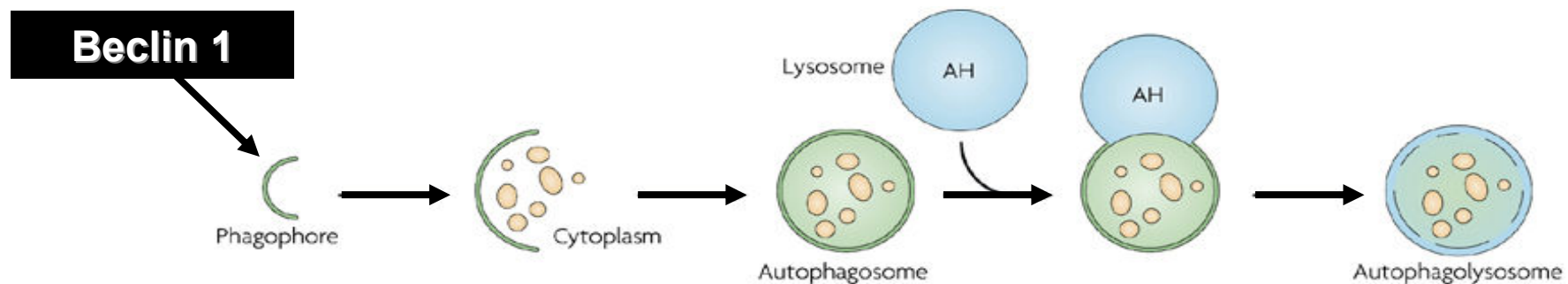
## Phenotype

Baseline

Blunted autophagy, no stress

Over-expression of mutant protein

Mutant protein with blunted autophagy

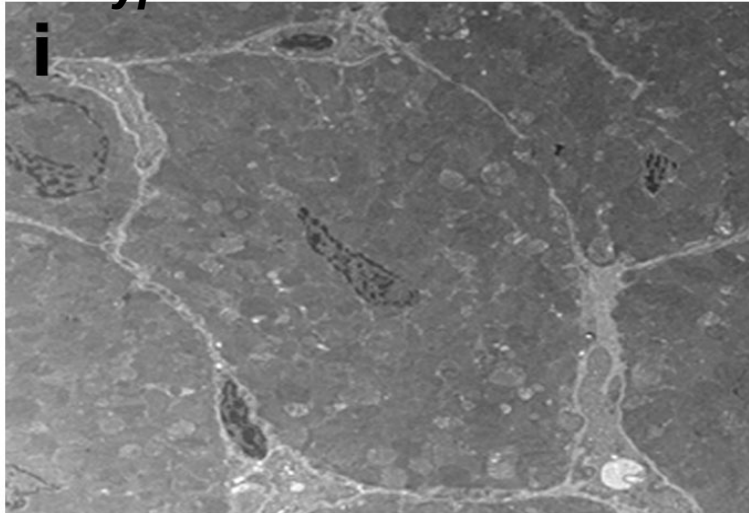




# Blunting autophagy increases pathological remodeling

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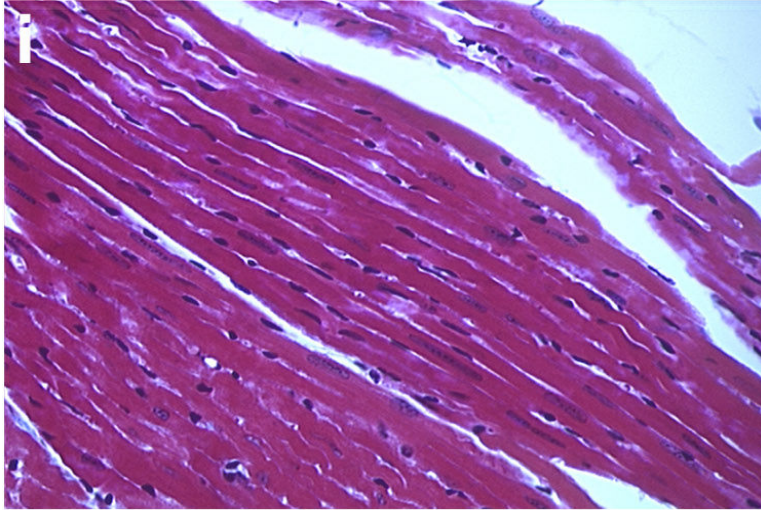
*Wildtype*



# Blunting autophagy increases pathological remodeling

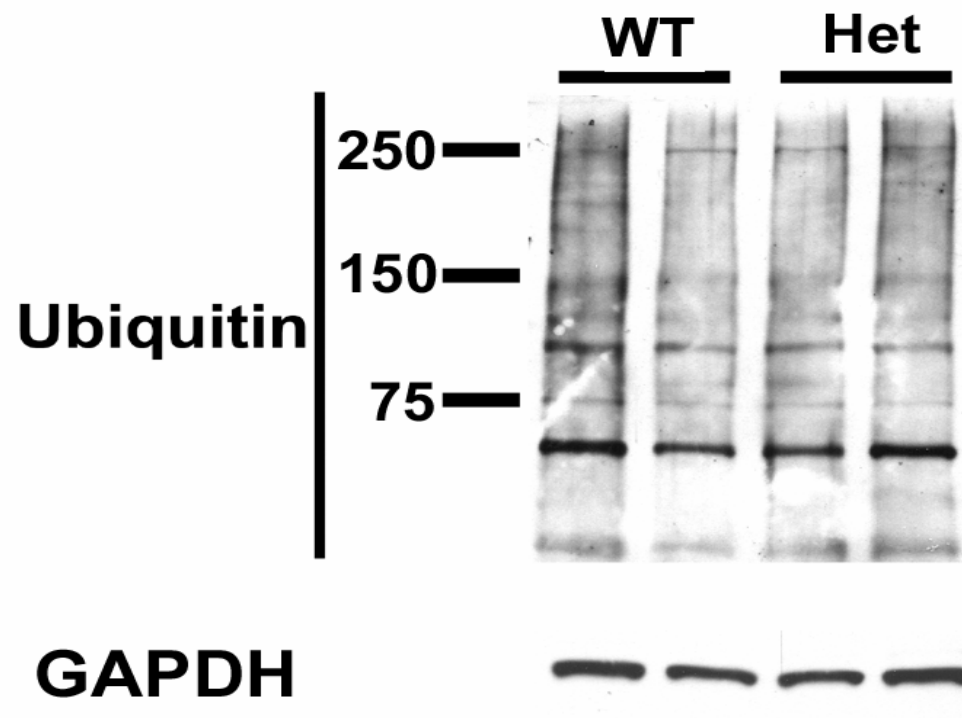
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*Wildtype*

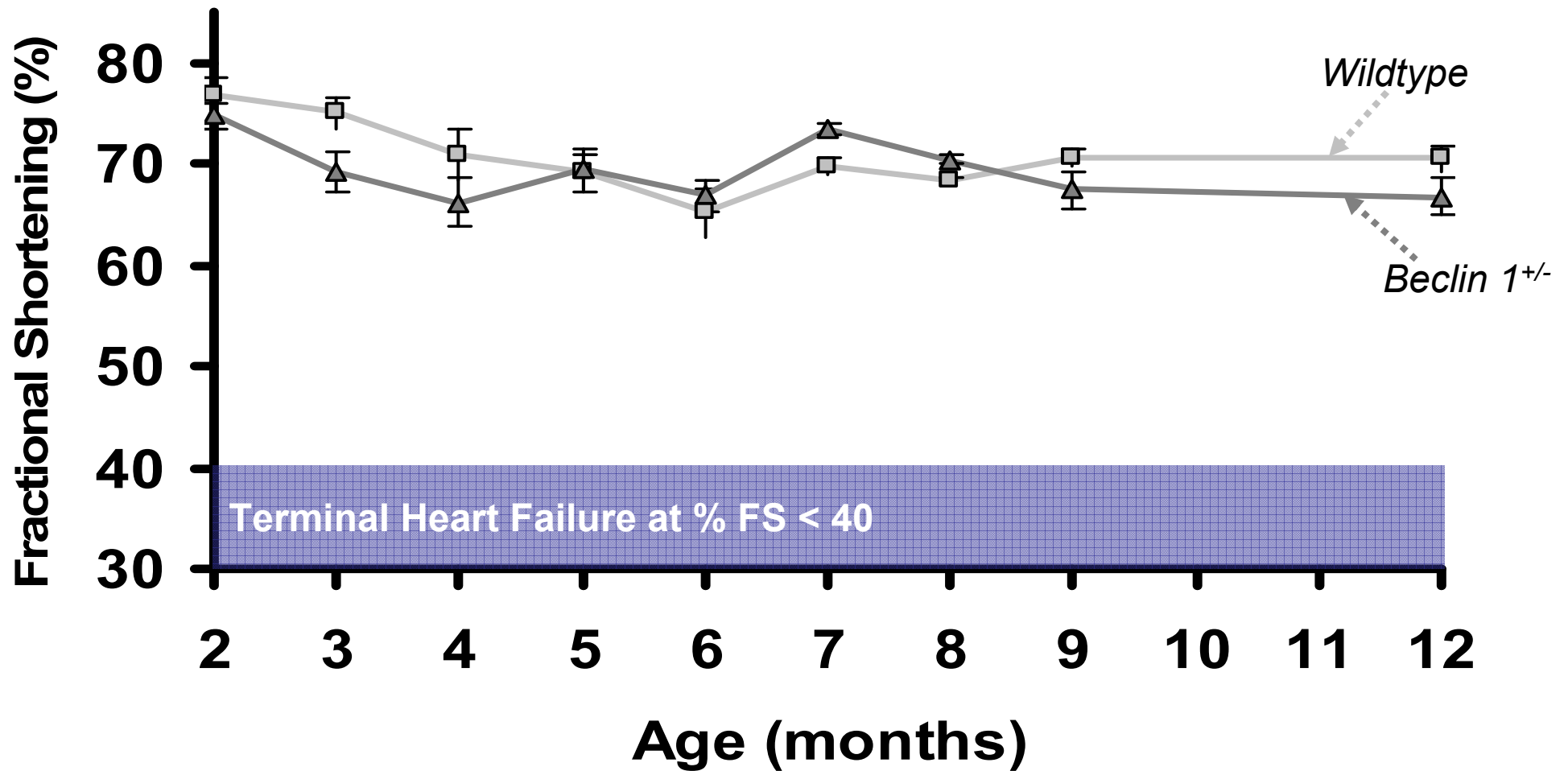


# Blunting autophagy increases pathological remodeling

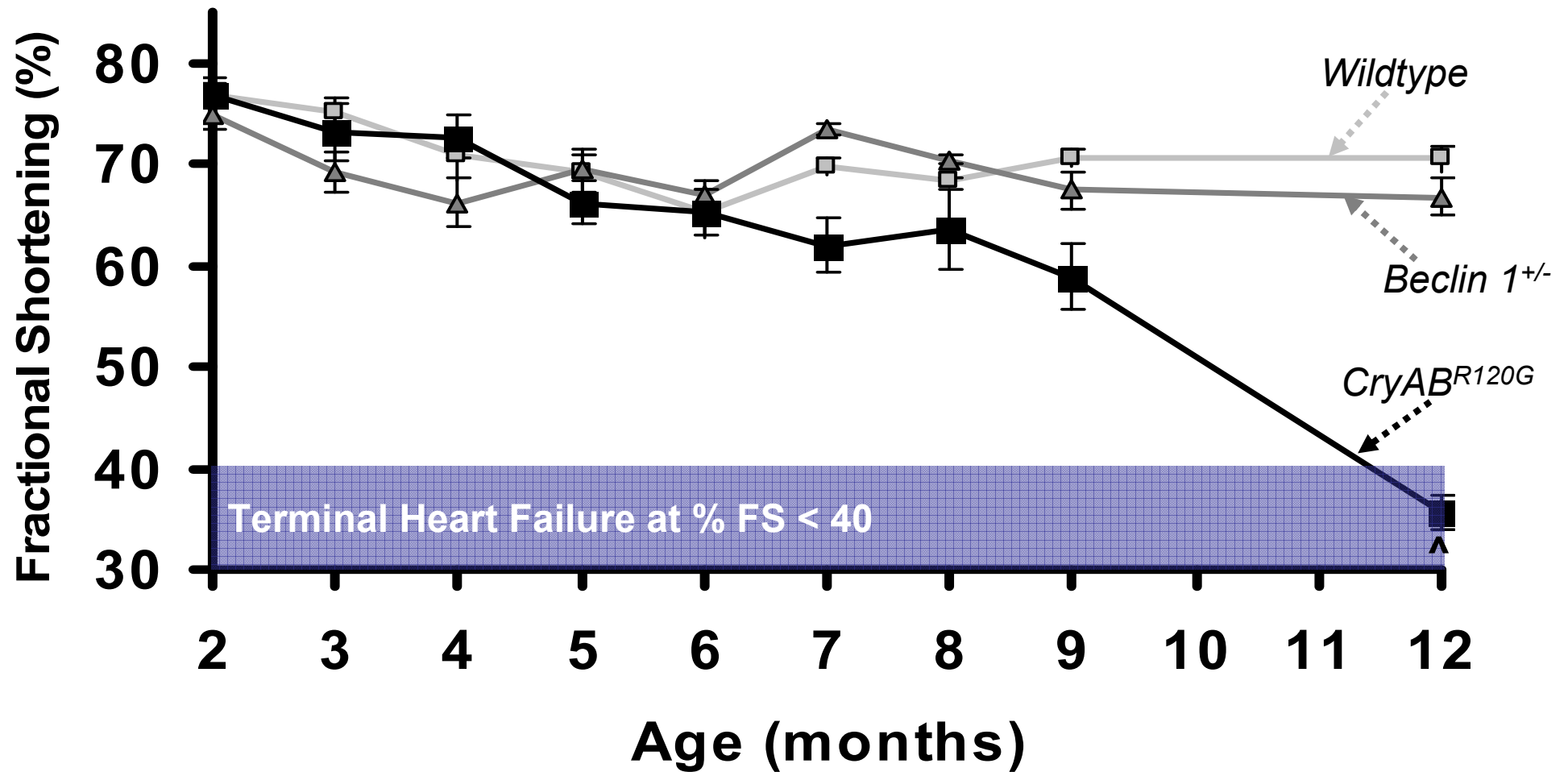
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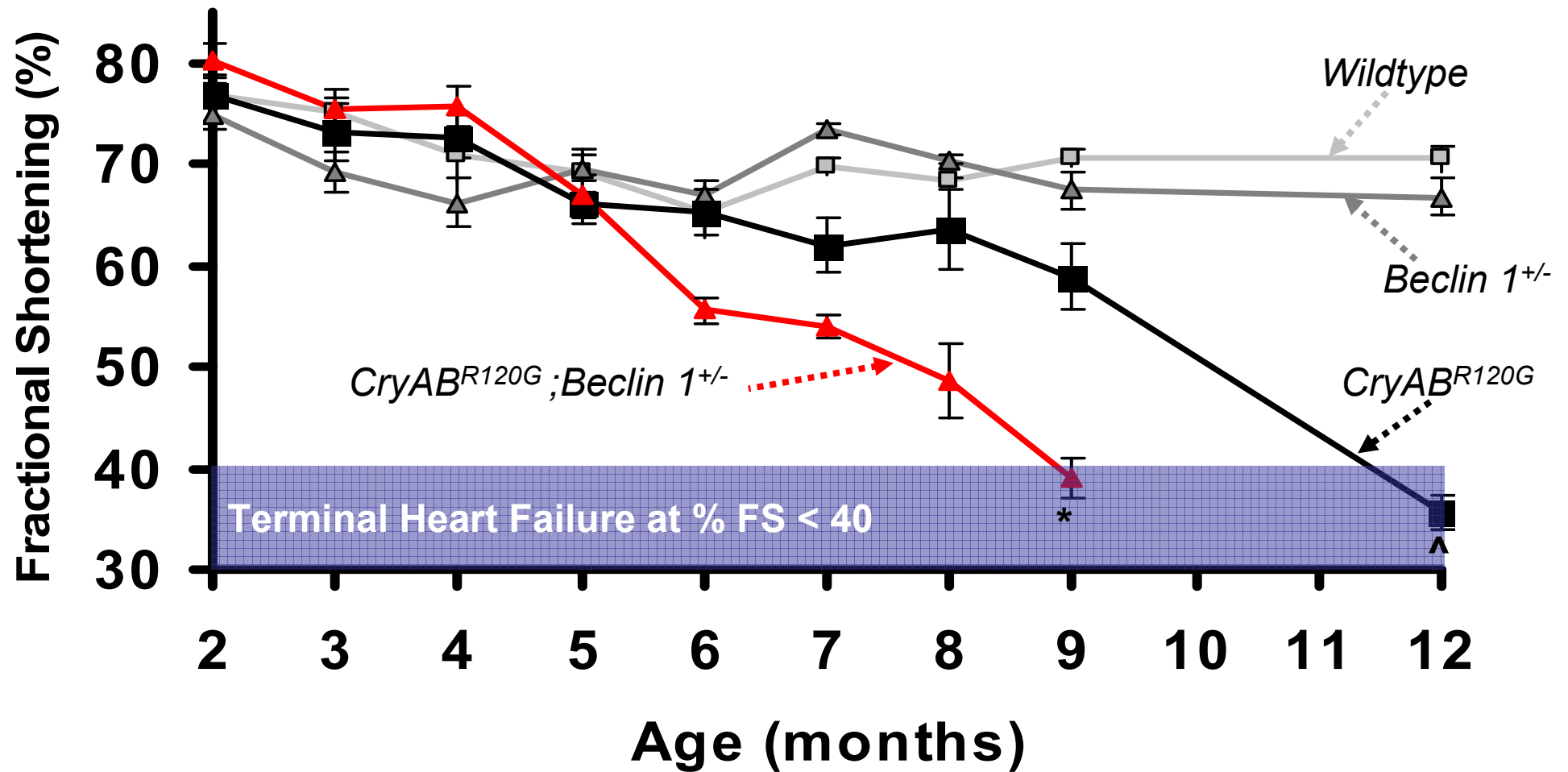
# Autophagy attenuates CryAB<sup>R120G</sup>-induced heart failure



# Autophagy attenuates **CryAB<sup>R120G</sup>-induced heart failure**



# Autophagy attenuates $CryAB^{R120G}$ -induced heart failure



# Autophagy attenuates $CryAB^{R120G}$ -induced heart failure

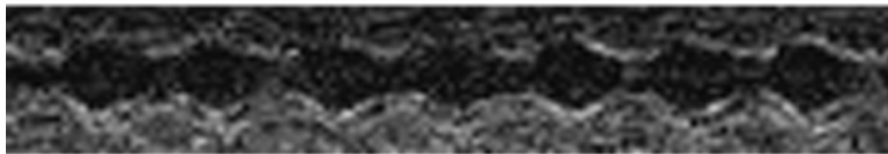
*Wildtype*



*beclin 1<sup>+/-</sup>*



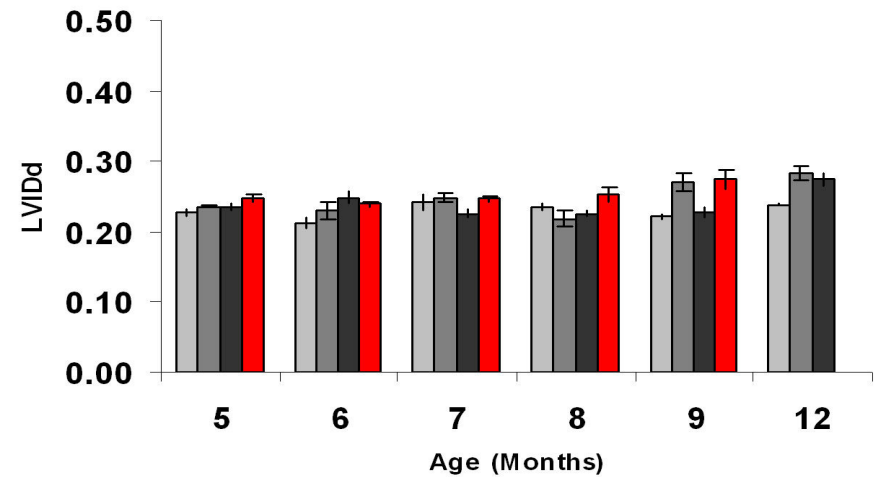
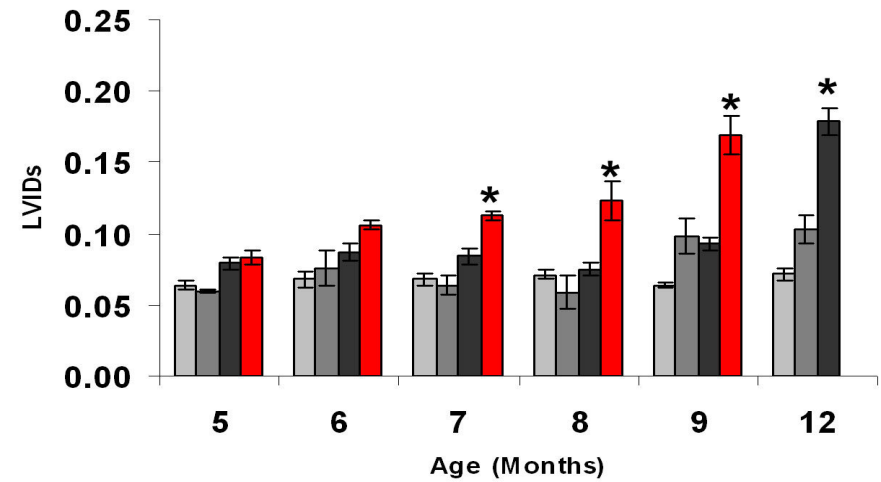
*CryAB<sup>R120G</sup>*



*CryAB<sup>R120G</sup>;beclin 1<sup>+/-</sup>*



30ms



# Conclusion

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- **Cardiomyocyte autophagy is a robust remodeling response to diverse forms of cardiac stress.**
- **Depending on the context, autophagic activity is either adaptive or maladaptive.**



# Acknowledgments

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## Hill Lab

Andy Blag  
Jeff Berry  
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Vien Le  
Xiang Luo  
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Oktay Rifki  
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Paul Tannous  
Hongxin Zhu

## ...gone but not forgotten

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Jun Cheng  
Minjie Jiang  
Bill Kutschke  
Greg Lu  
Yan Ni  
Bridgid Nolan  
Ken Richardson  
Yanggan Wang  
Zhengyi Wang

## Collaborators

Beverly Rothermel, PhD  
Eric Olson, PhD  
Rhonda Bassel-Duby, PhD  
Beth Levine, MD  
James Richardson, DVM, PhD



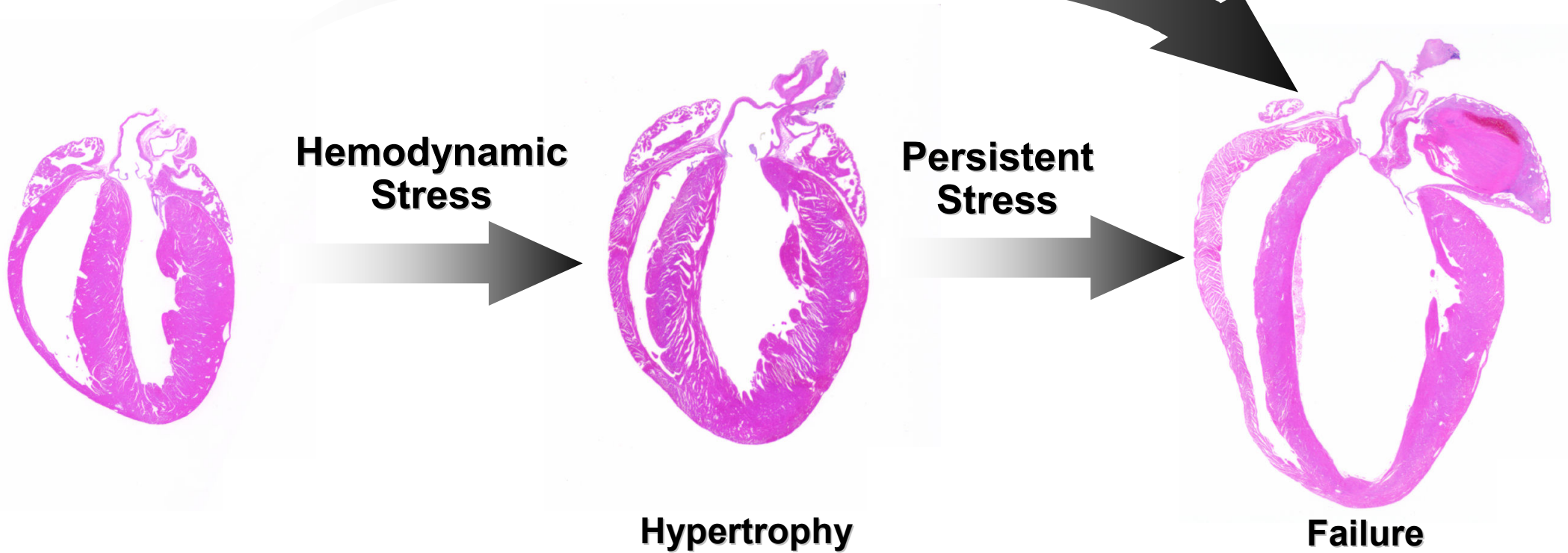




# The Biological Paradigm

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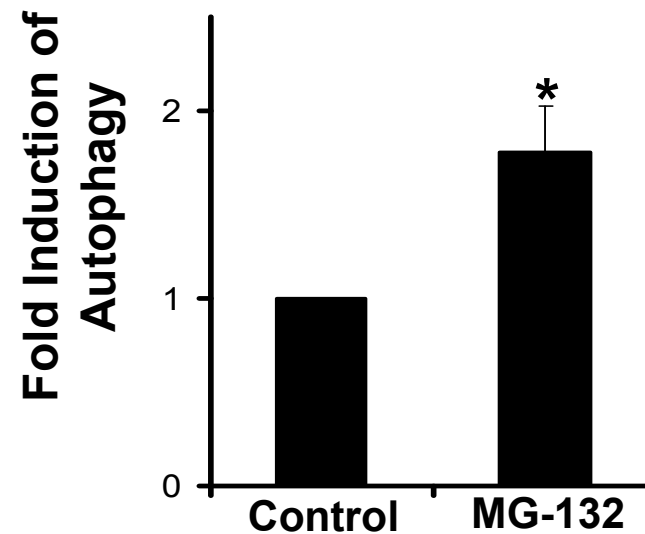
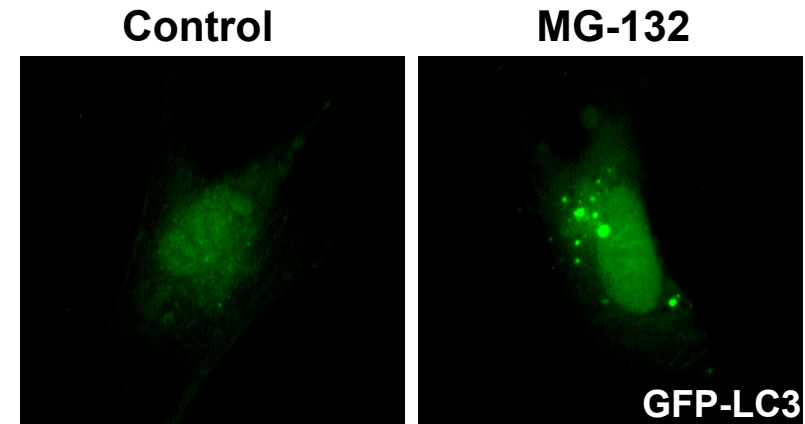
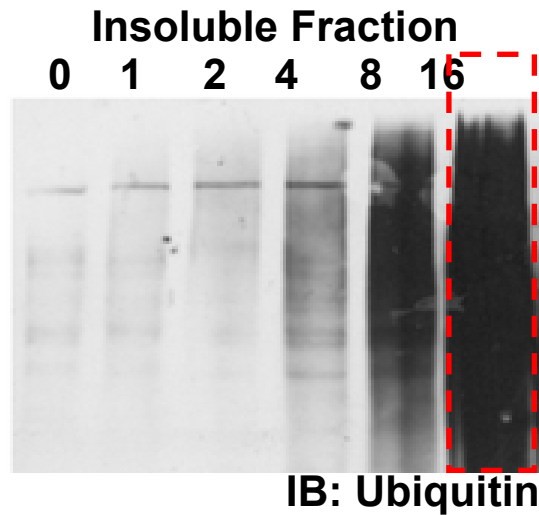
Severe Stress





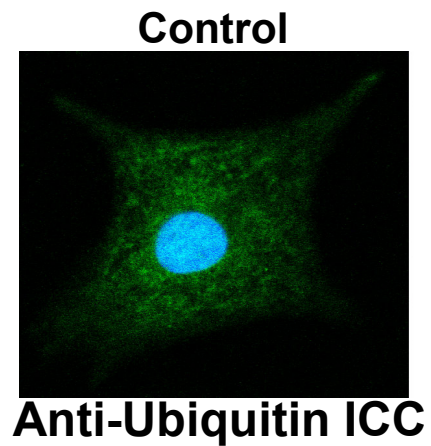
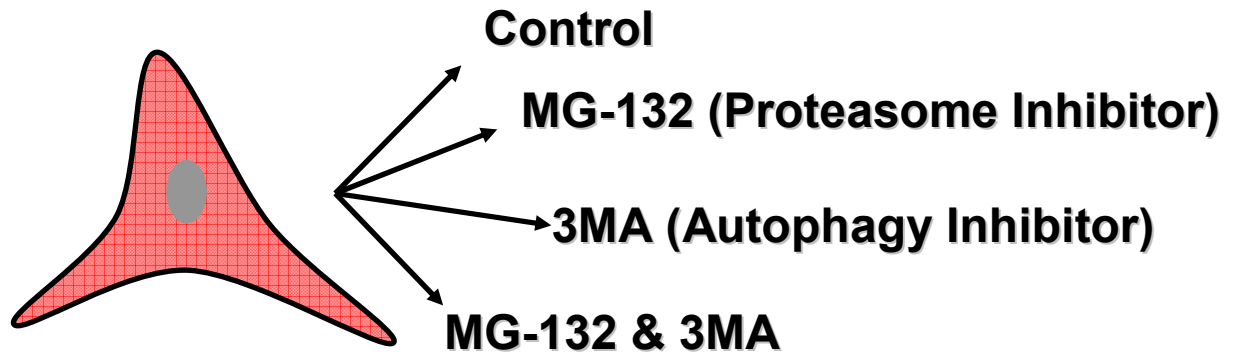
# Accumulation of Ubiquitinated Protein Induces a Dramatic Increase in Autophagy

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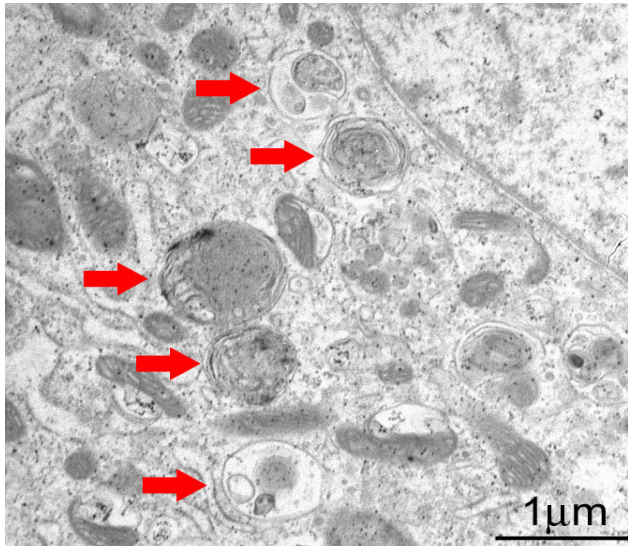
# Formation of Aggresome-Like Bodies

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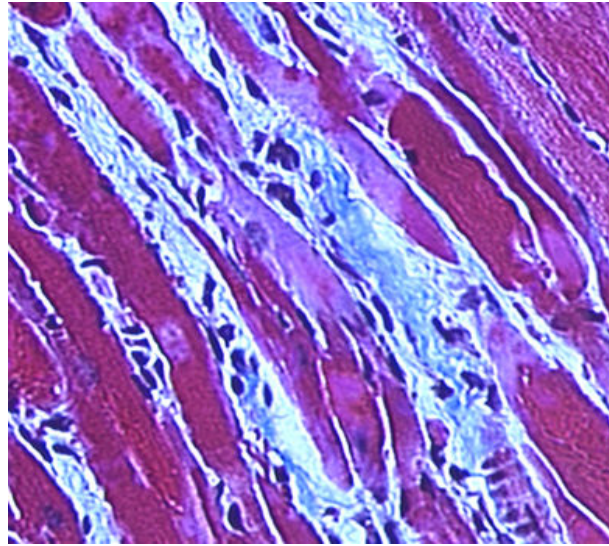


# DRCM Conclusions

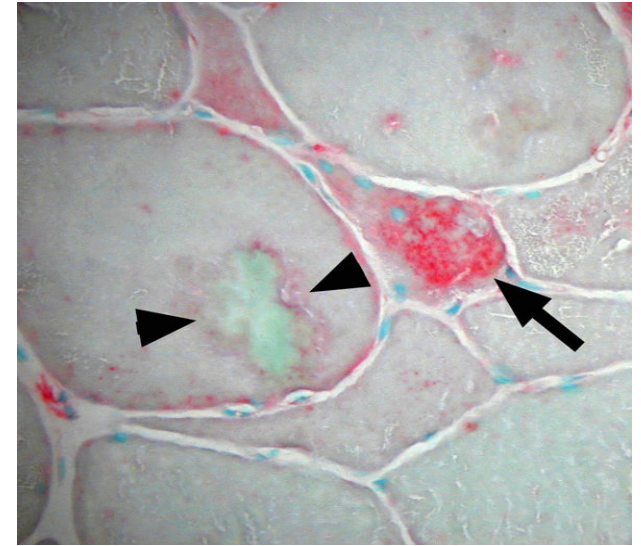
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***In Vitro***



***In Vivo***

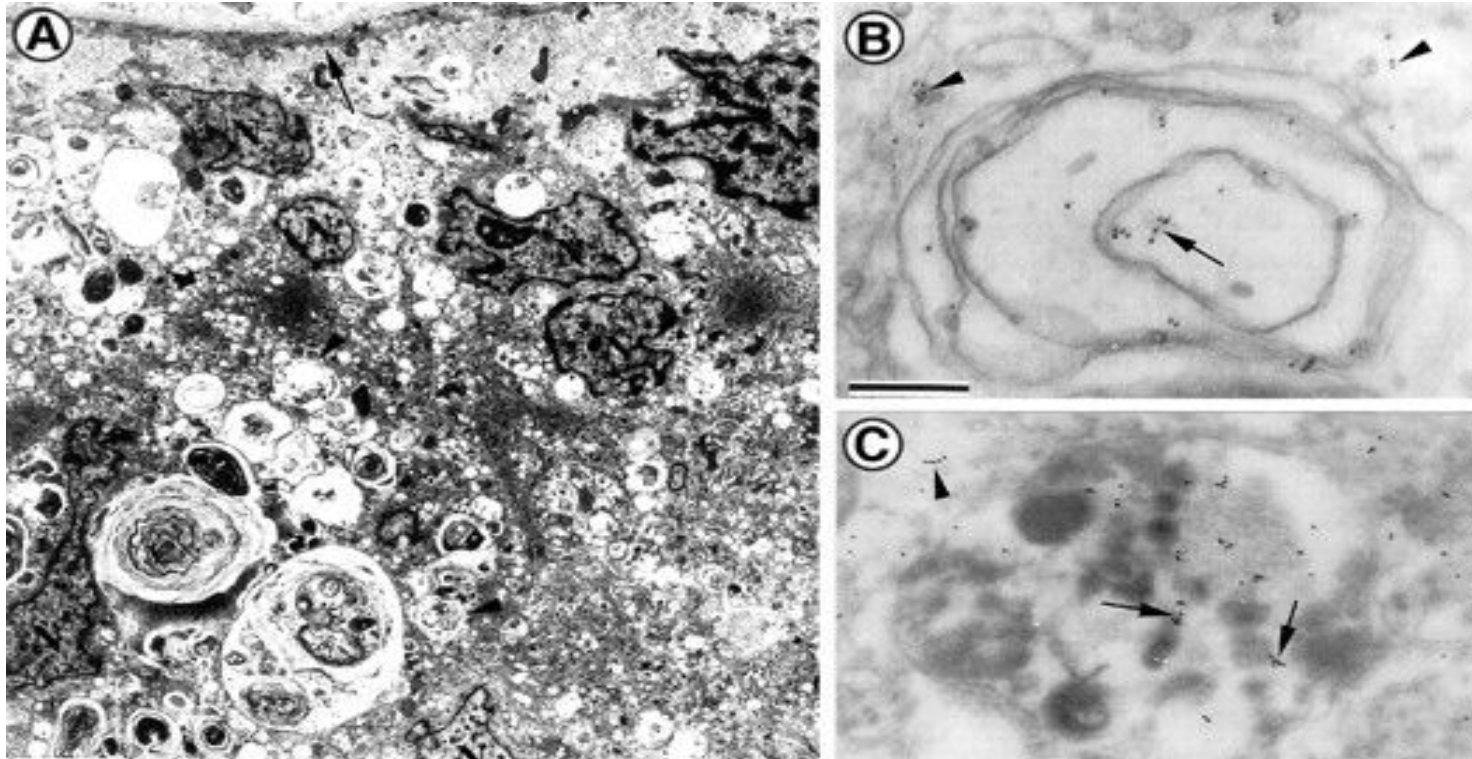


**In Human**



# Autophagy in heart disease

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# Starvation induces autophagy in cardiac myocytes *in vitro* and *in vivo*

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