

# Pump up the heart: calcium cycling defects and heart diseases

Yokohama City University School of Medicine

Susumu Minamisawa

(横浜市立大学 南沢 享)

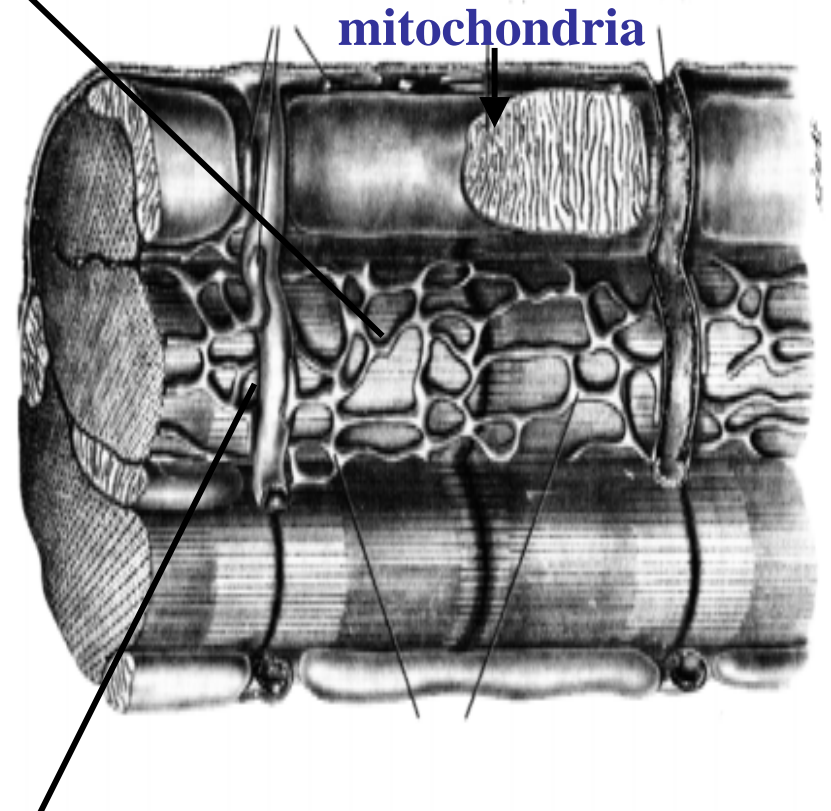
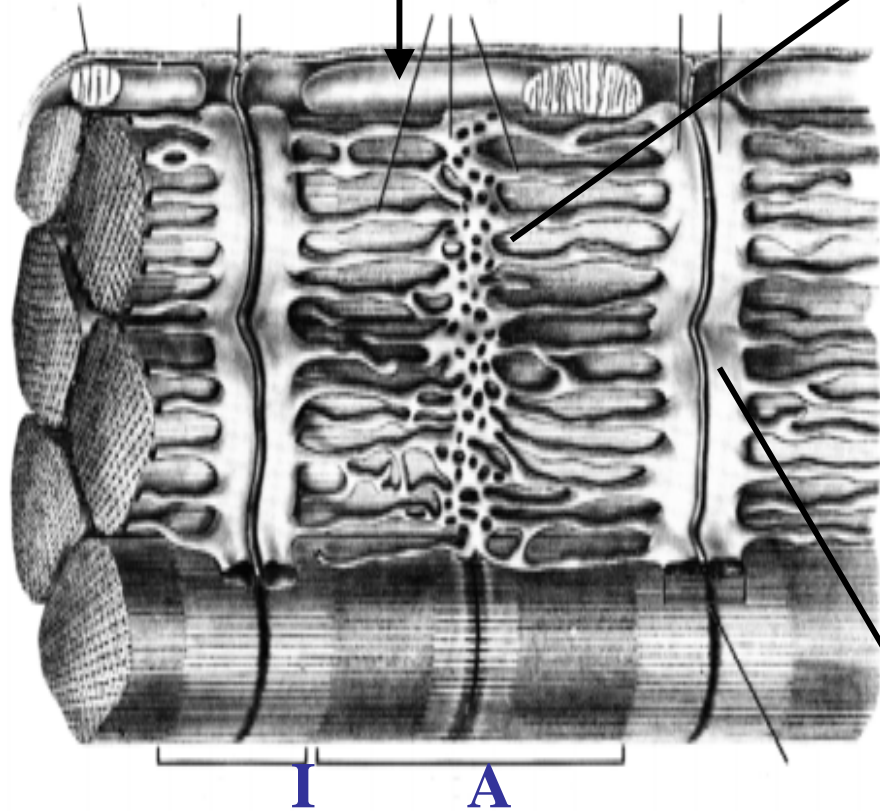
**skeletal muscle**

**cardiac muscle**

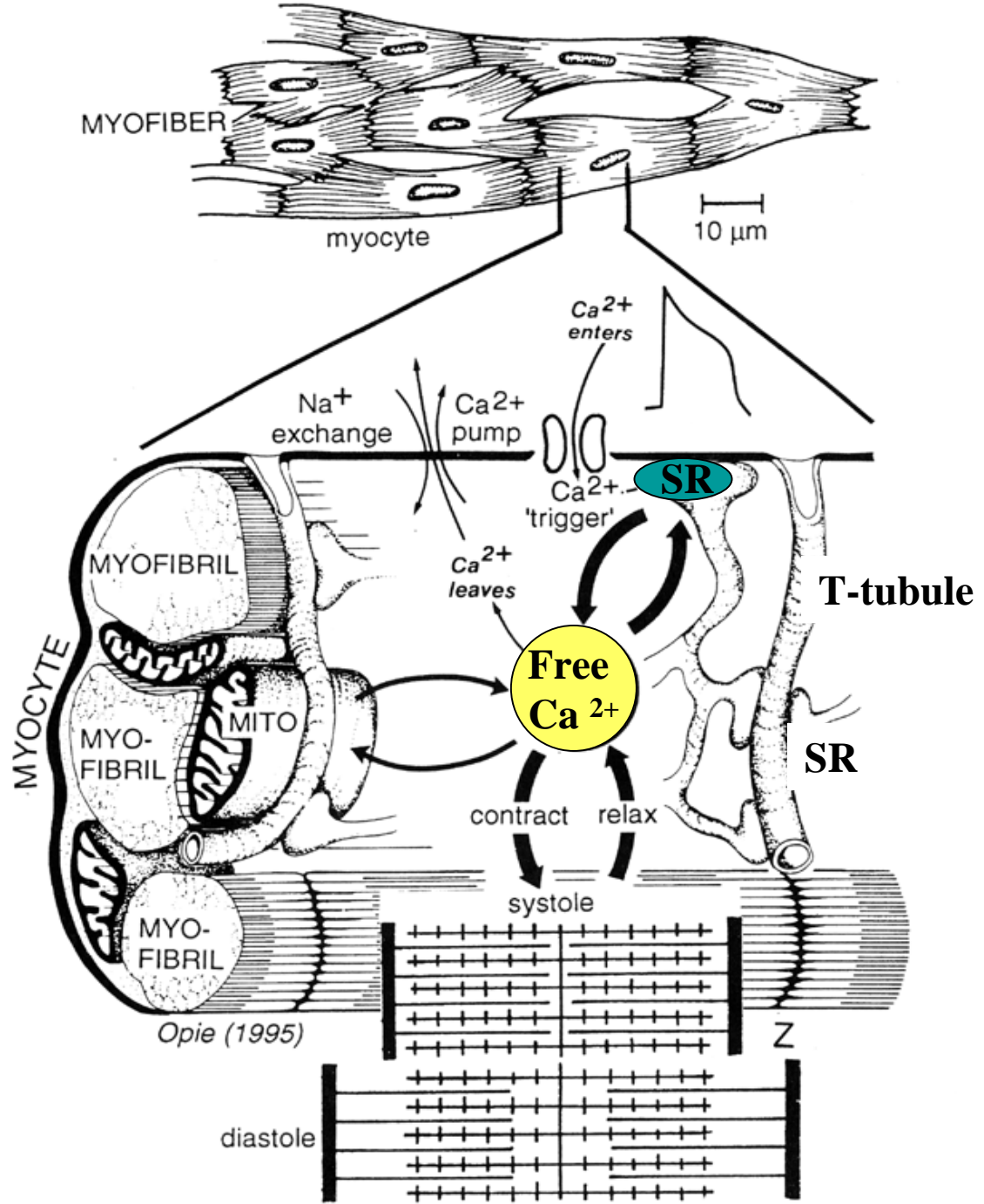
**mitochondria**

**Sarcoplasmic reticulum**

**mitochondria**

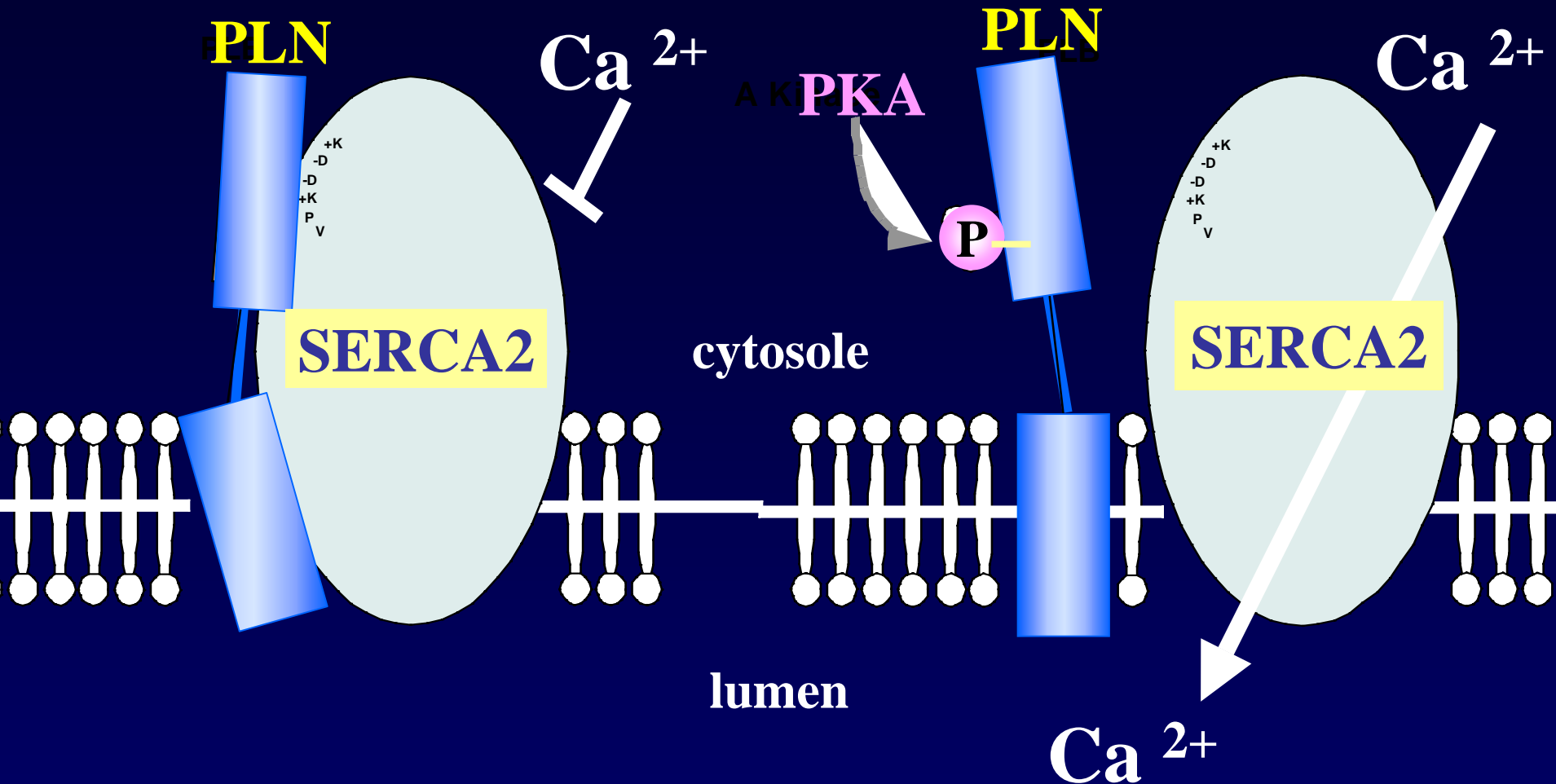


**T-tubule**



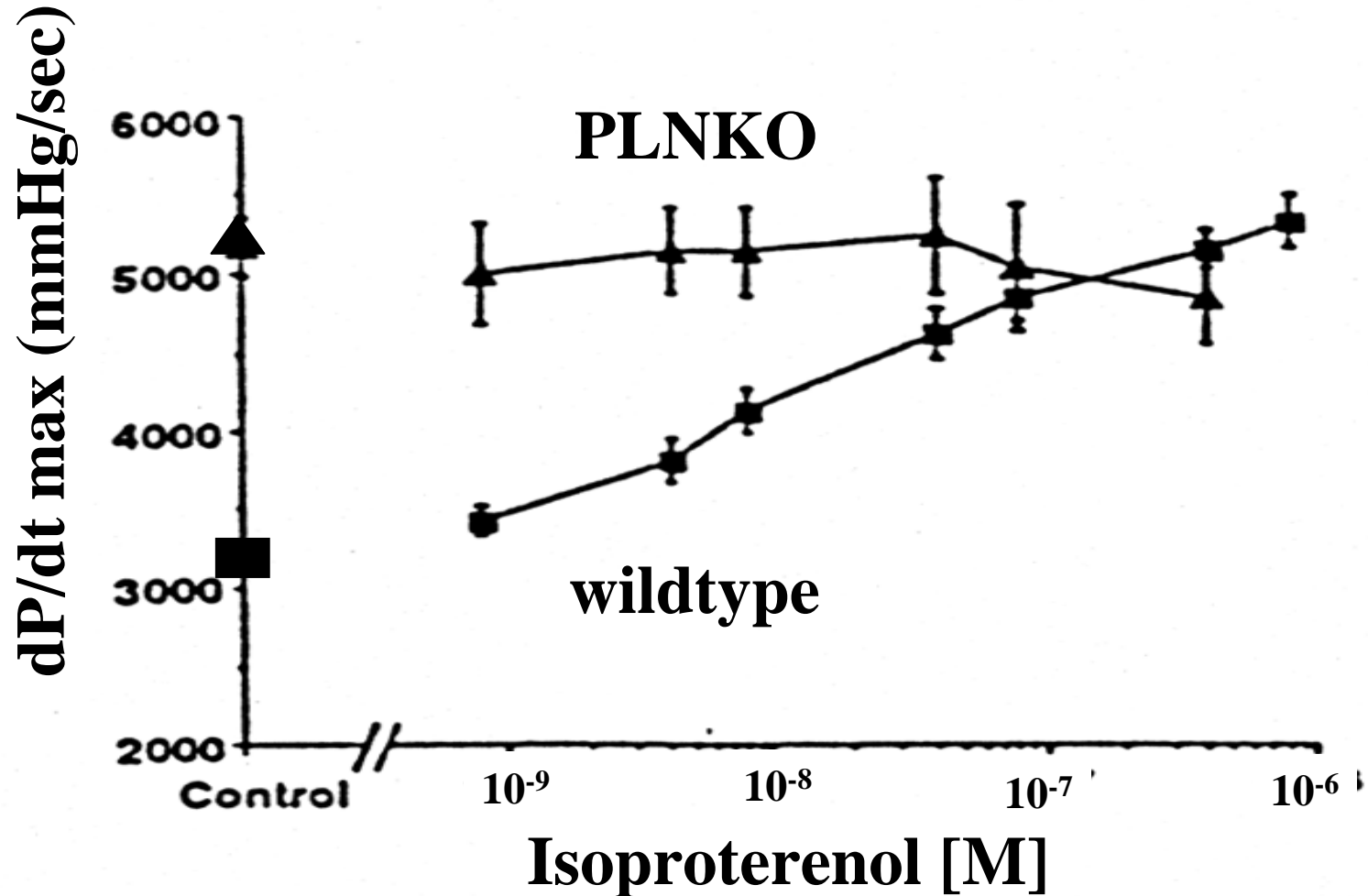
Opi. The Heart,  
3rd edition

# Phospholamban is an endogenous inhibitor of SERCA activity

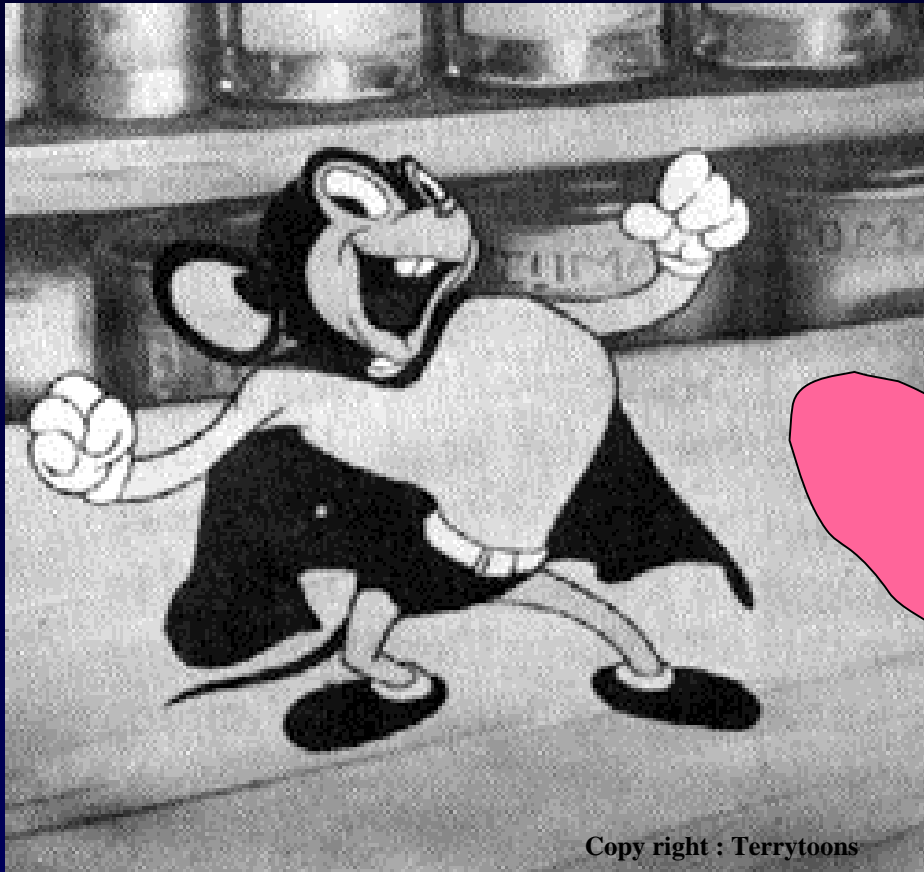


**SERCA2a and Phospholamban are  
critical regulators of calcium  
cycling in the heart**

# PLNKO Mice Exhibit Enhanced Myocardial Contractility and Loss of $\beta$ -Agonist Stimulation



# Gene Complementary Strategy



PLNKO mouse

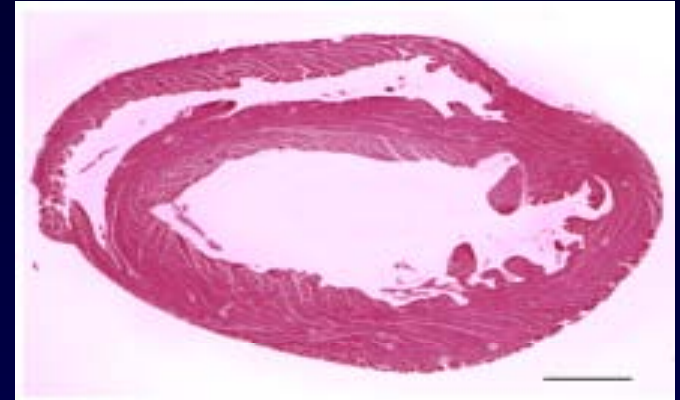
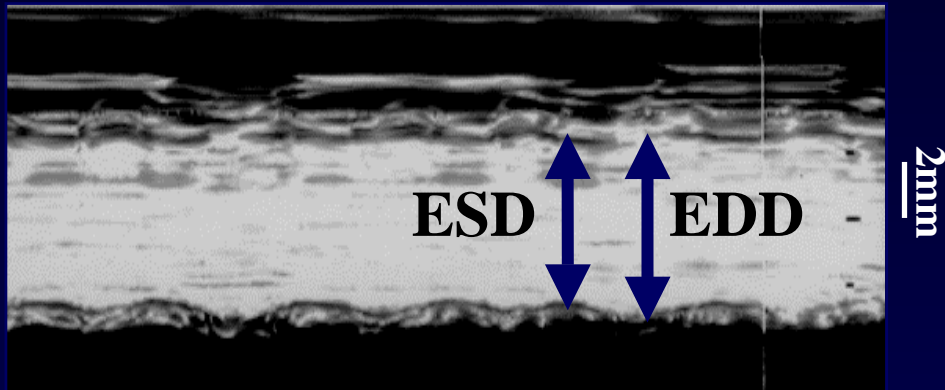


MLPKO mouse

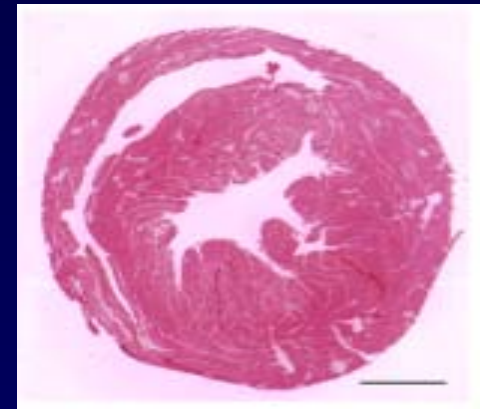
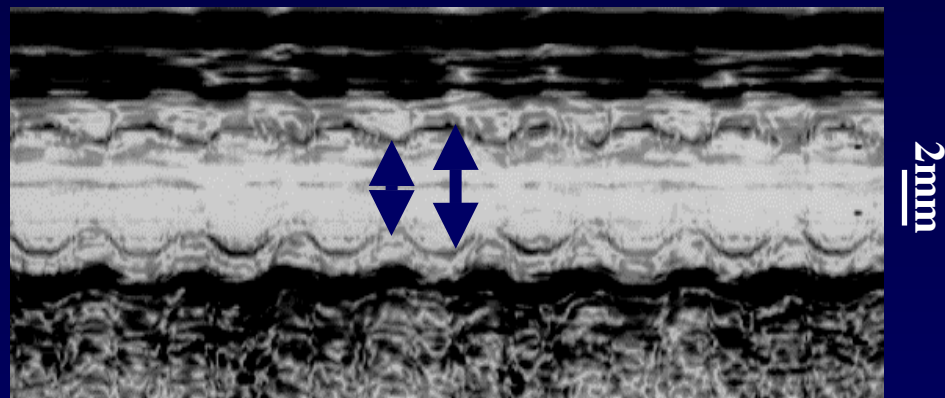
# Phospholamban gene ablation prevents the progression of dilated cardiomyopathy

**MLP KO**

**FS 9%**



**MLP KO/ PLN KO** **FS 32%**

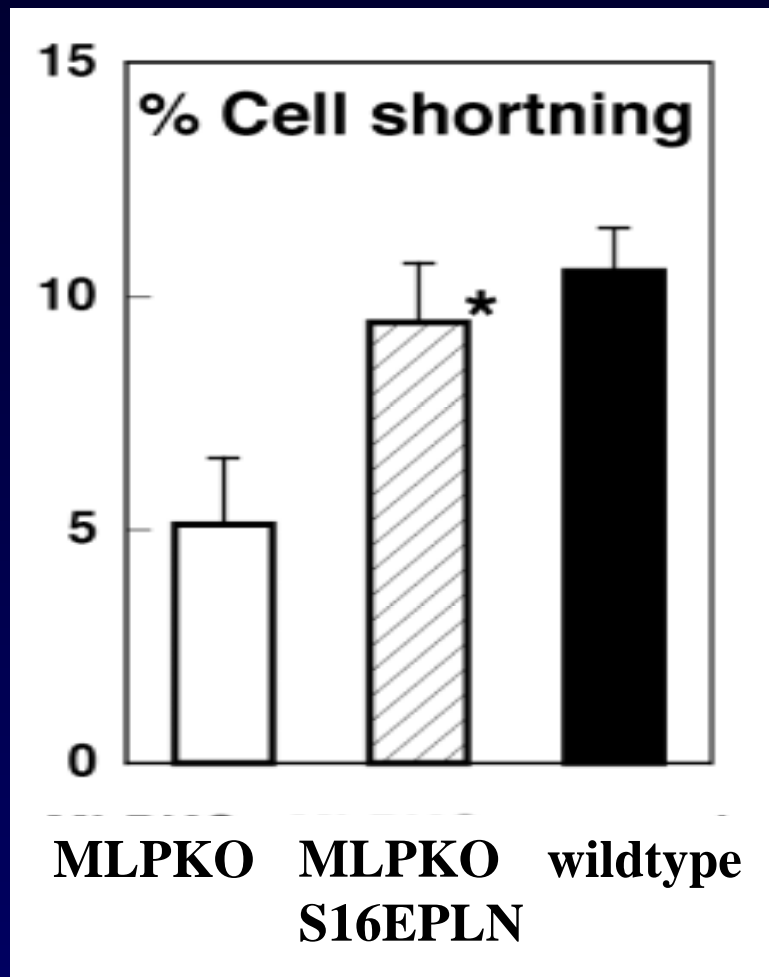


200 msec

Minamisawa et al. Cell 1999



# S16E PLN mutant gene transfer by adenoviral vector improved myocyte contractility in DCM model mice



# **Enhancement of calcium uptake *via* the sarcoplasmic reticulum is a potent therapeutic strategy for heart failure**

- **Strategies to increase SERCA2a protein**
- **Strategies to modulate SERCA2a to increase calcium transport**
- **Strategies to decrease PLB protein**
- **Strategies to disrupt the interaction between SERCA2a and PLN**

## **The advantages of phospholamban as a prime target to increase SERCA2a activity (1)**

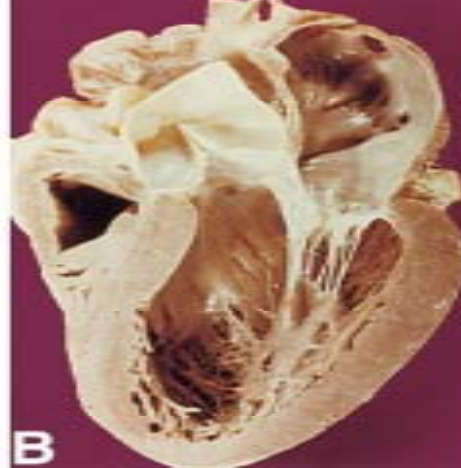
- **PLN is the strongest (probably only) endogenous inhibitor of SERCA2a in the ventricles.**
- **PLN is a terminal effector of  $\beta$ -adrenergic signalling pathways.**
- **PLN expression is highly cardiac specific and is much higher in ventricles than atria.**
- **PLN inhibition does not affect chronotropic responses.**

## **The advantages of phospholamban as a prime target to increase SERCA2a activity (2)**

- **PLN is a small protein. The genetic modification can be easily manipulated.**
- **PLN is remarkably conserved between species.**
- **PLN-deficient mice have not displayed any adverse events so far.**



A

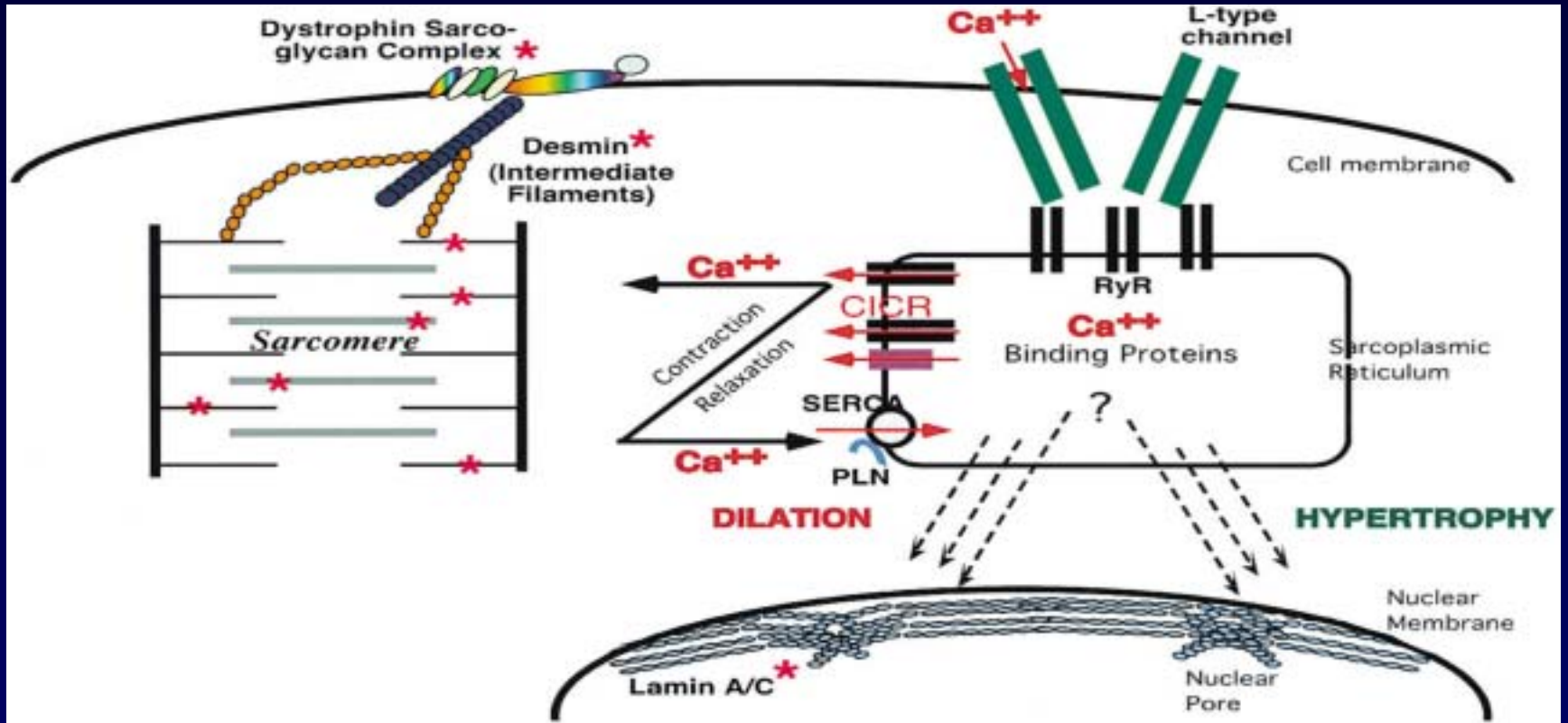


B

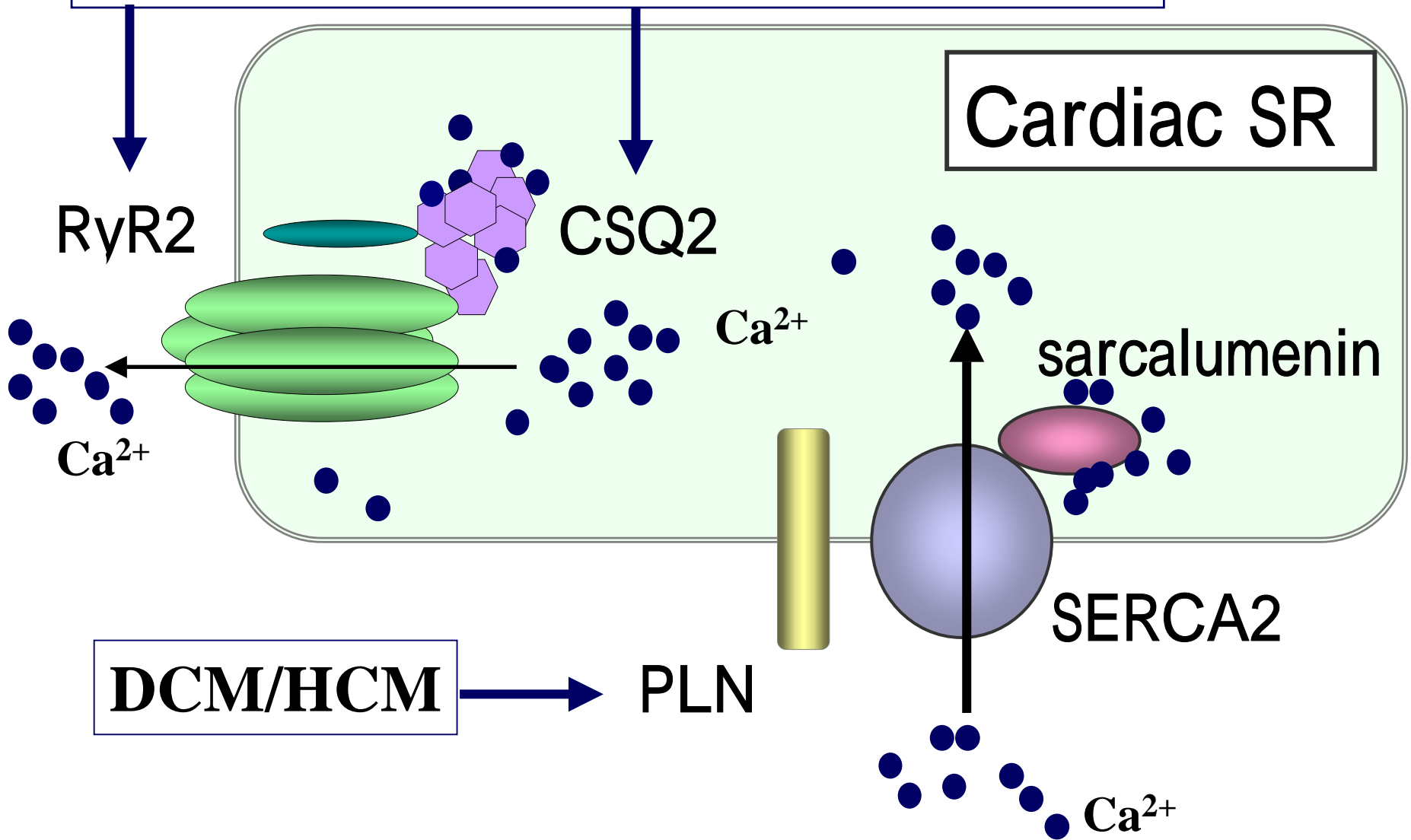


C

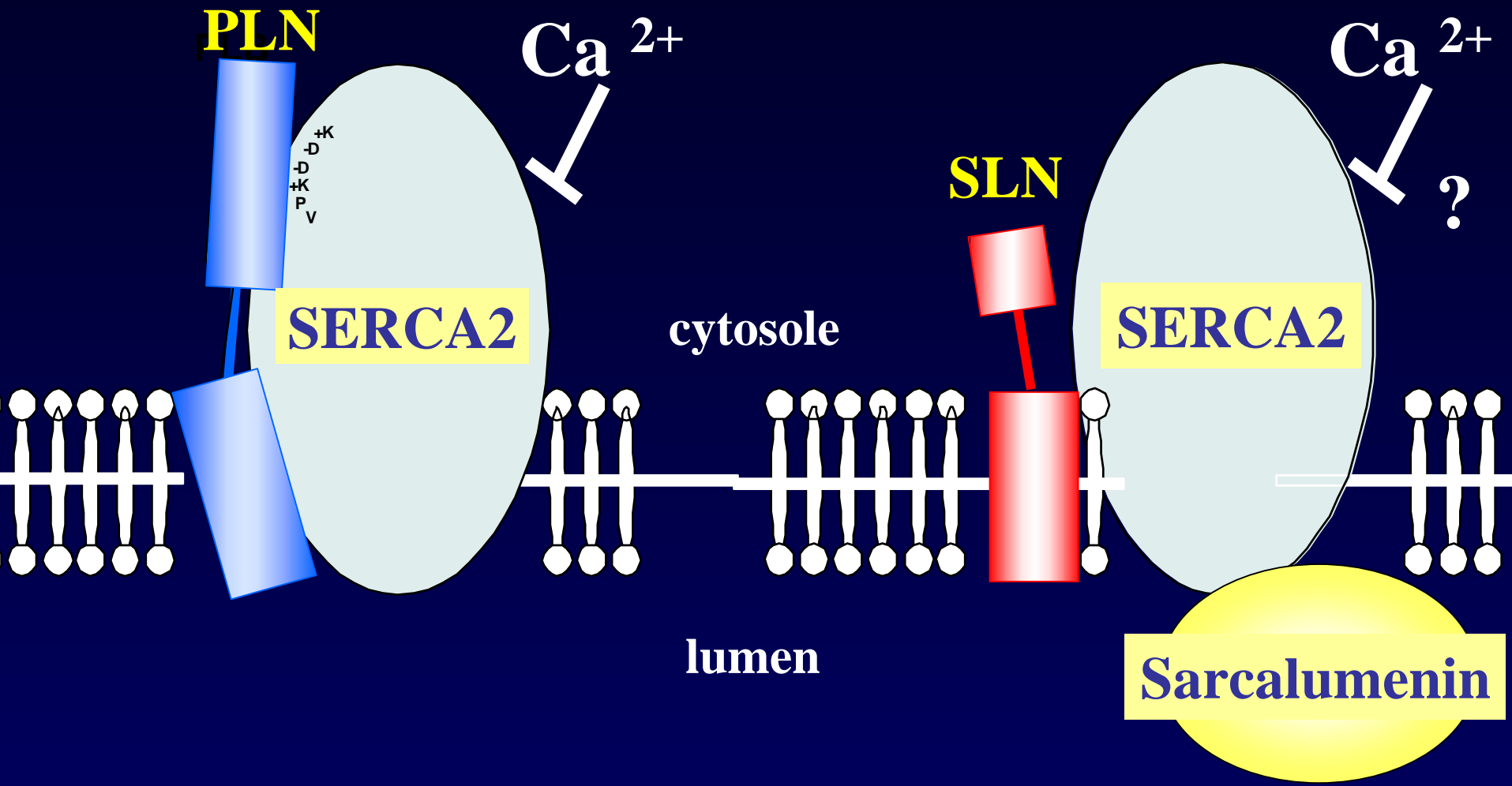
**HCM** ← **Gene Mutations** → **DCM**



# Arrhythmogenic Right Ventricular Dysplasia Catecholamine-sensitive Ventricular Tachycardia



**Calcium cycling defects due to a  
gene mutation of the SR proteins  
cause cardiomyopathy**



**PLN**

$Ca^{2+}$

**SERCA2**

cytosole

**SLN**

$Ca^{2+}$

**SERCA2**

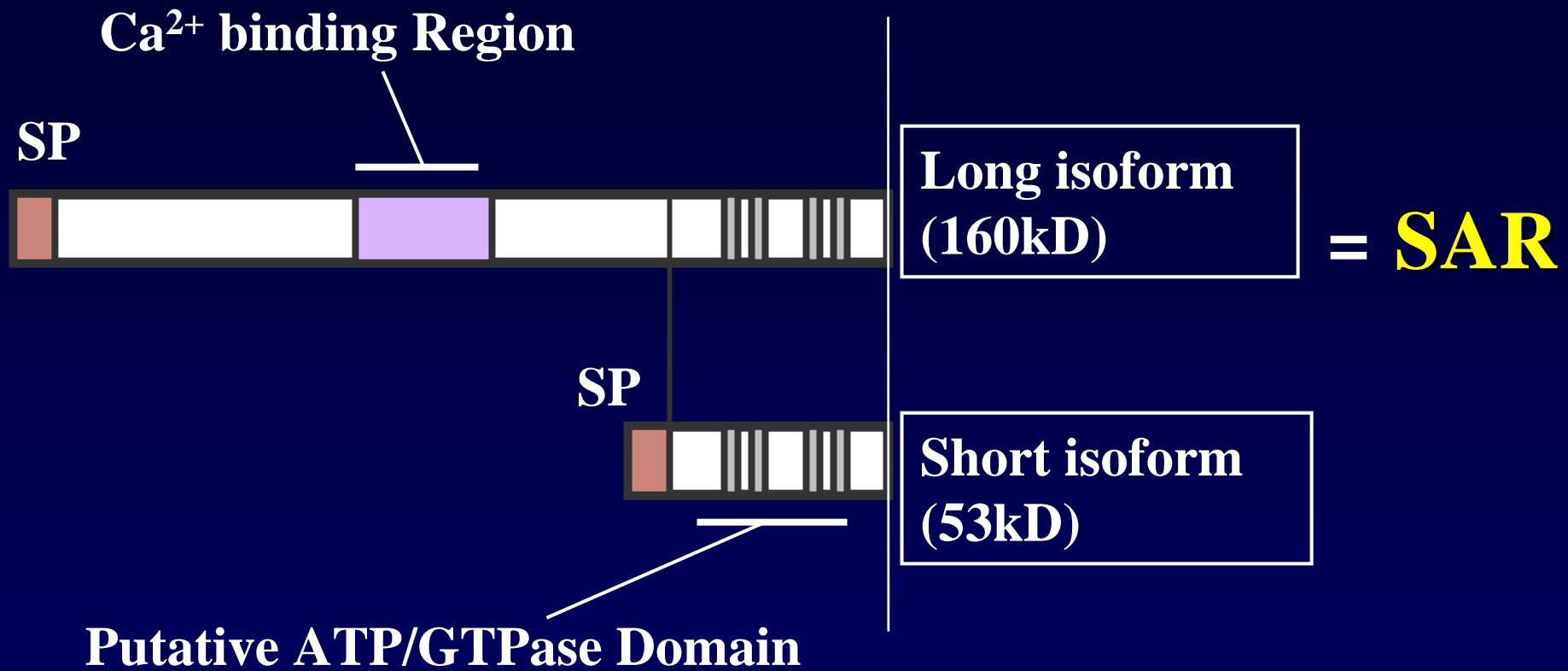
lumen

**Sarcalumenin**

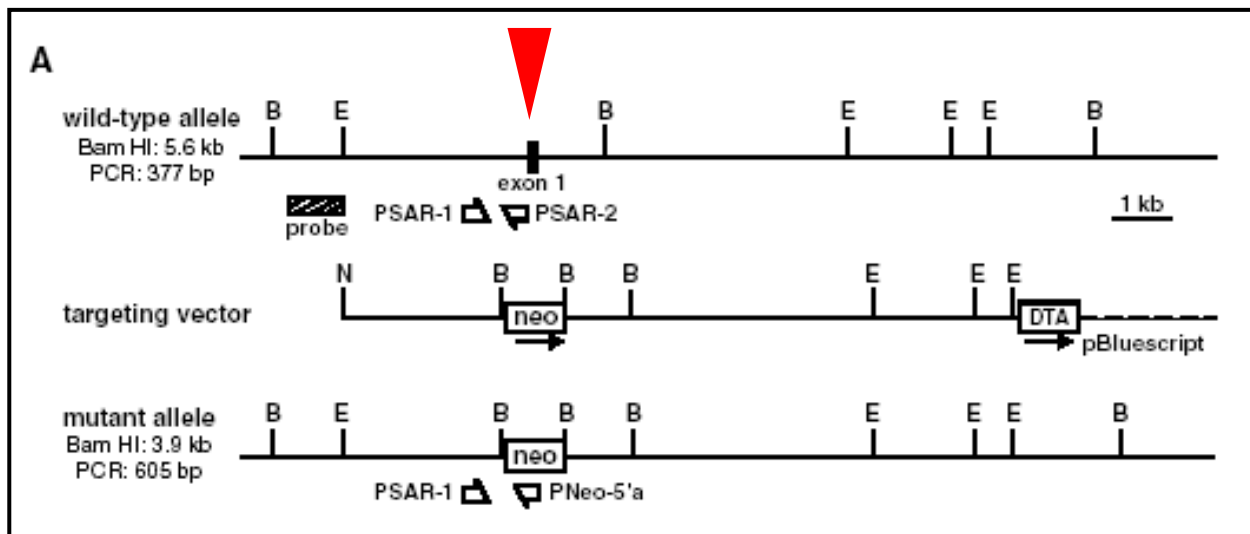


# Sarcalumenin(SAR)

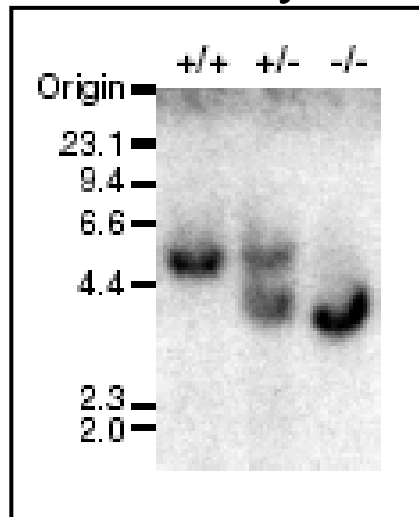
is a luminal  $\text{Ca}^{2+}$  binding glycoprotein in the longitudinal SR of striated muscles.



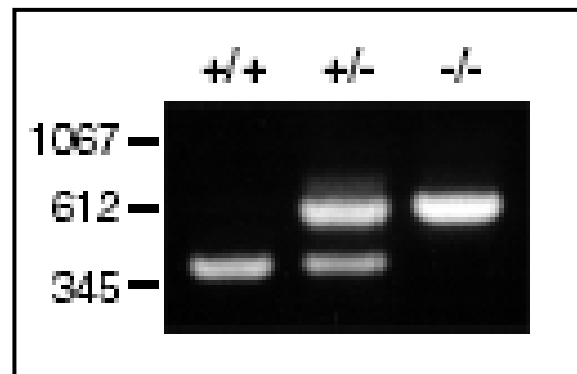
# Generation of SAR-KO mice



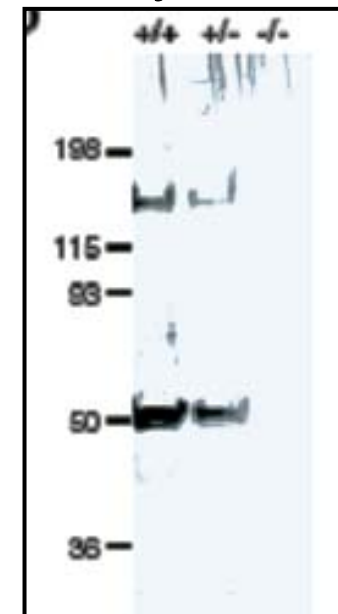
## Southern blot analysis



## PCR analysis

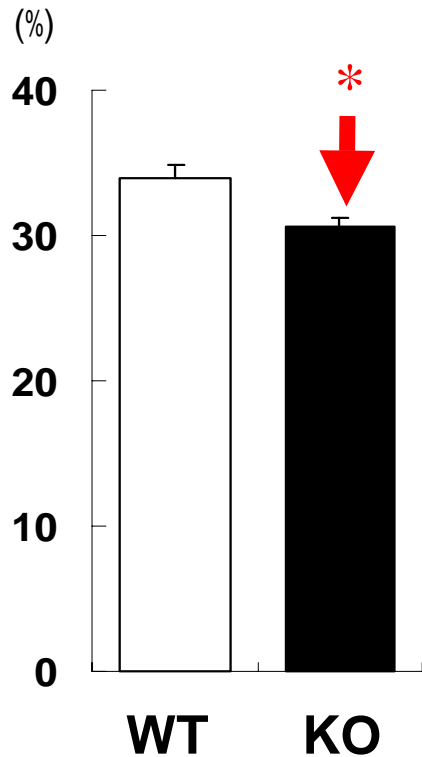


## Immunoblot analysis

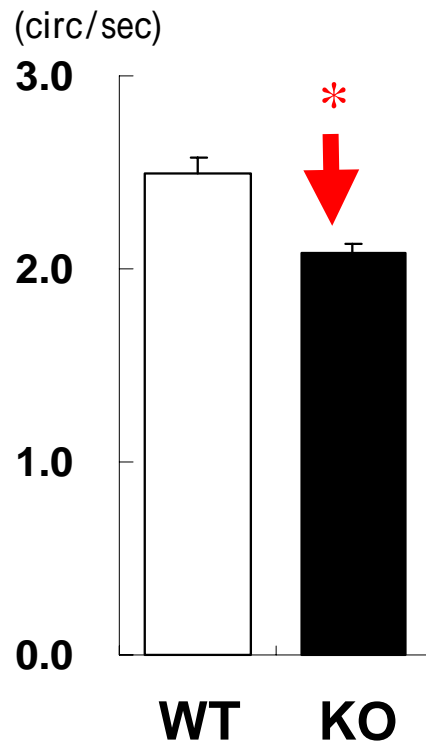


# Mild systolic dysfunction was detected in 6-week SAR-KO mice

## A. LV%FS



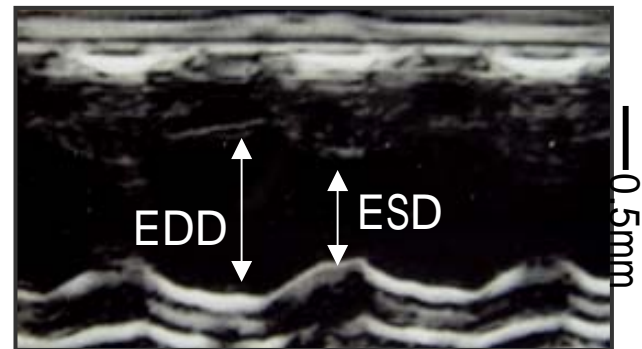
## B. Vcfc



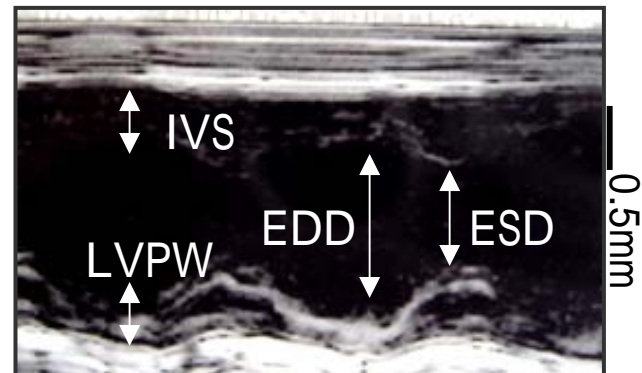
## C.

\*  $P < 0.05$ , vs. WT

WT

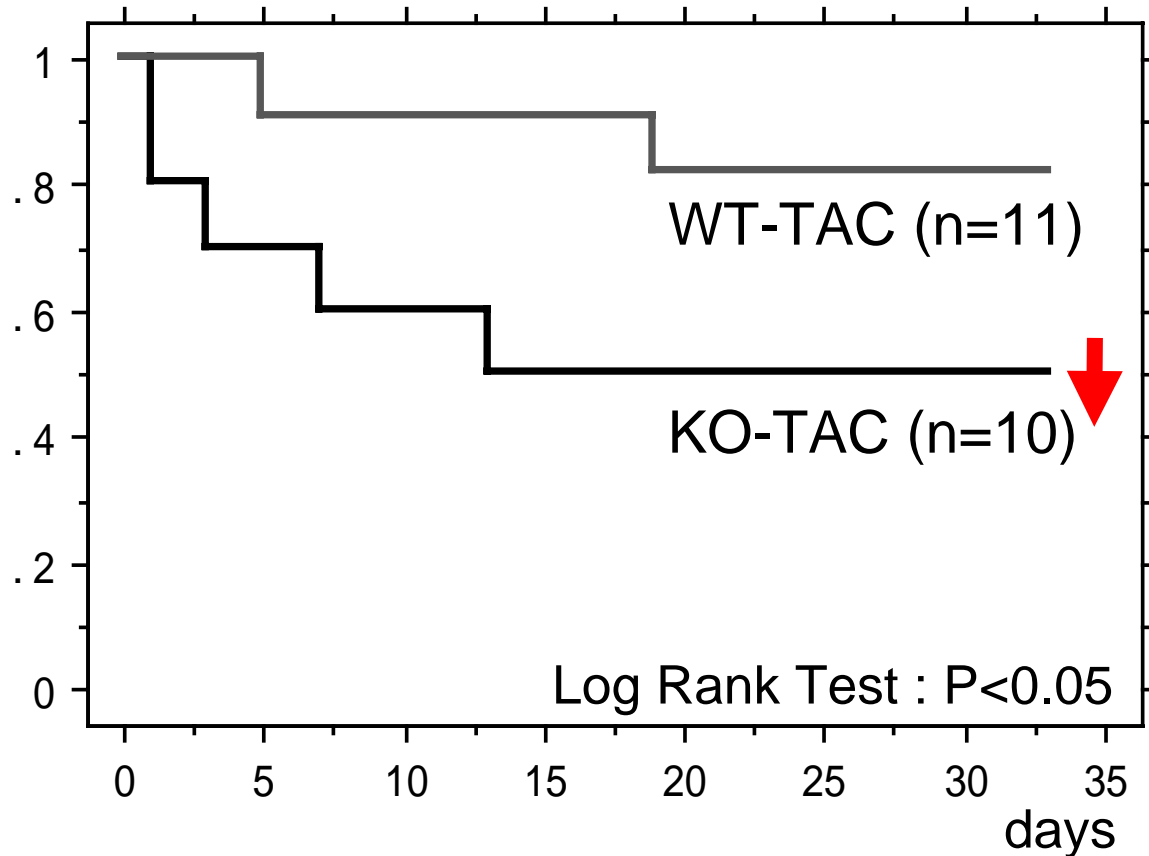


KO

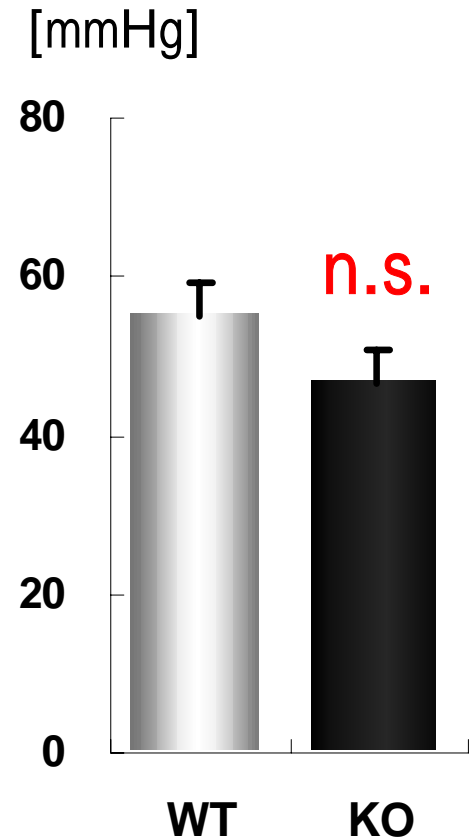


# Survival Rates after TAC were Lower in SAR-KO Mice than in WT Mice

A. Survival Rates

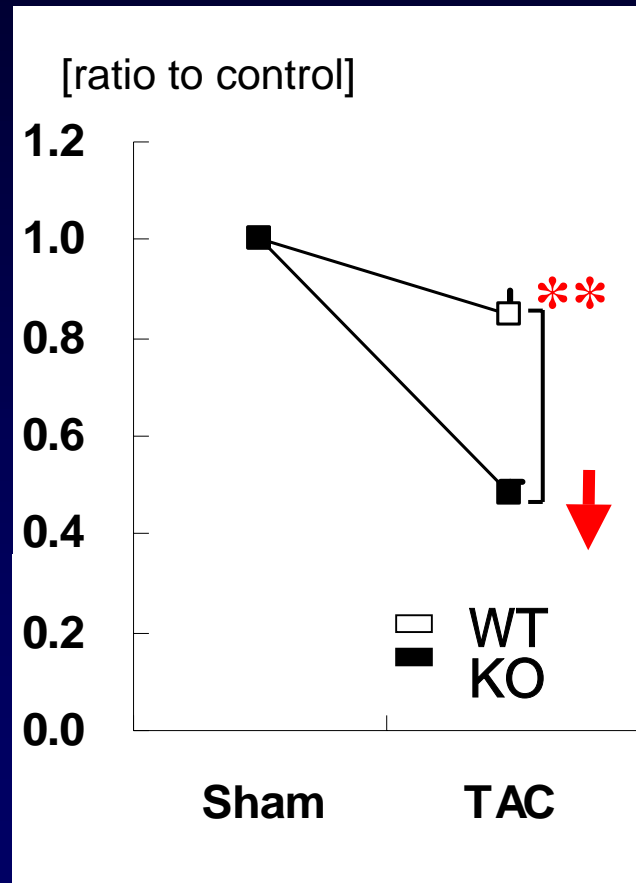


B. Pressure Gradients

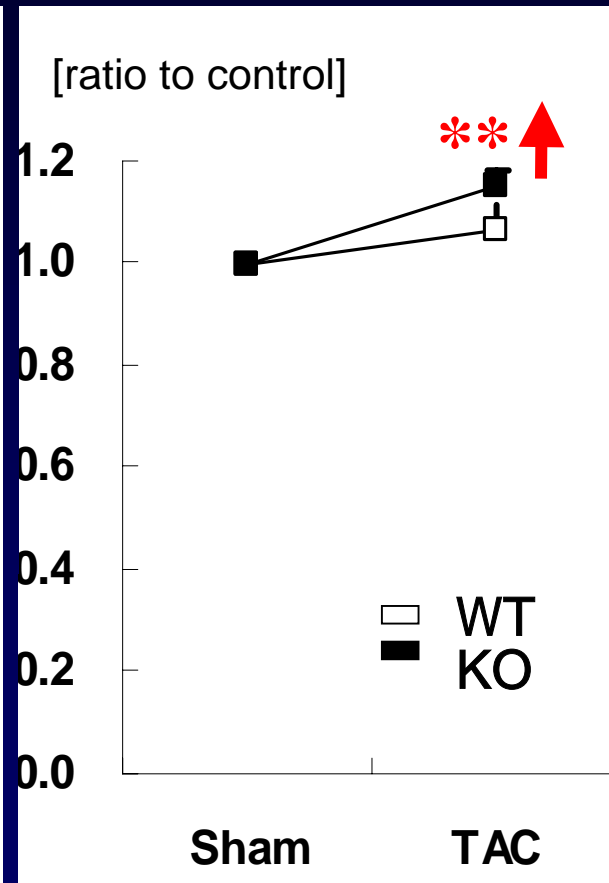


# SAR Deficiency Caused the Progressive Dysfunction in Response to Pressure Overload

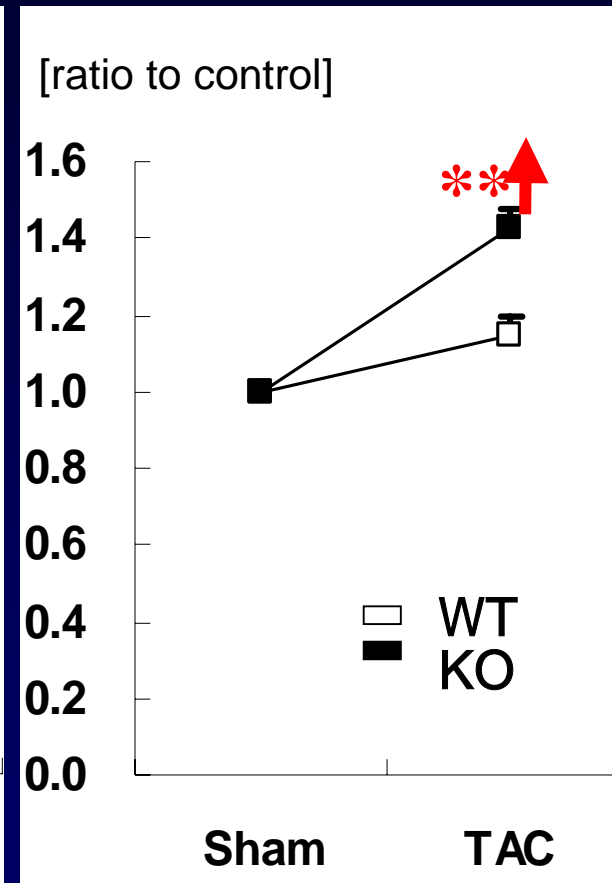
## A. dP/dt



## B. tau

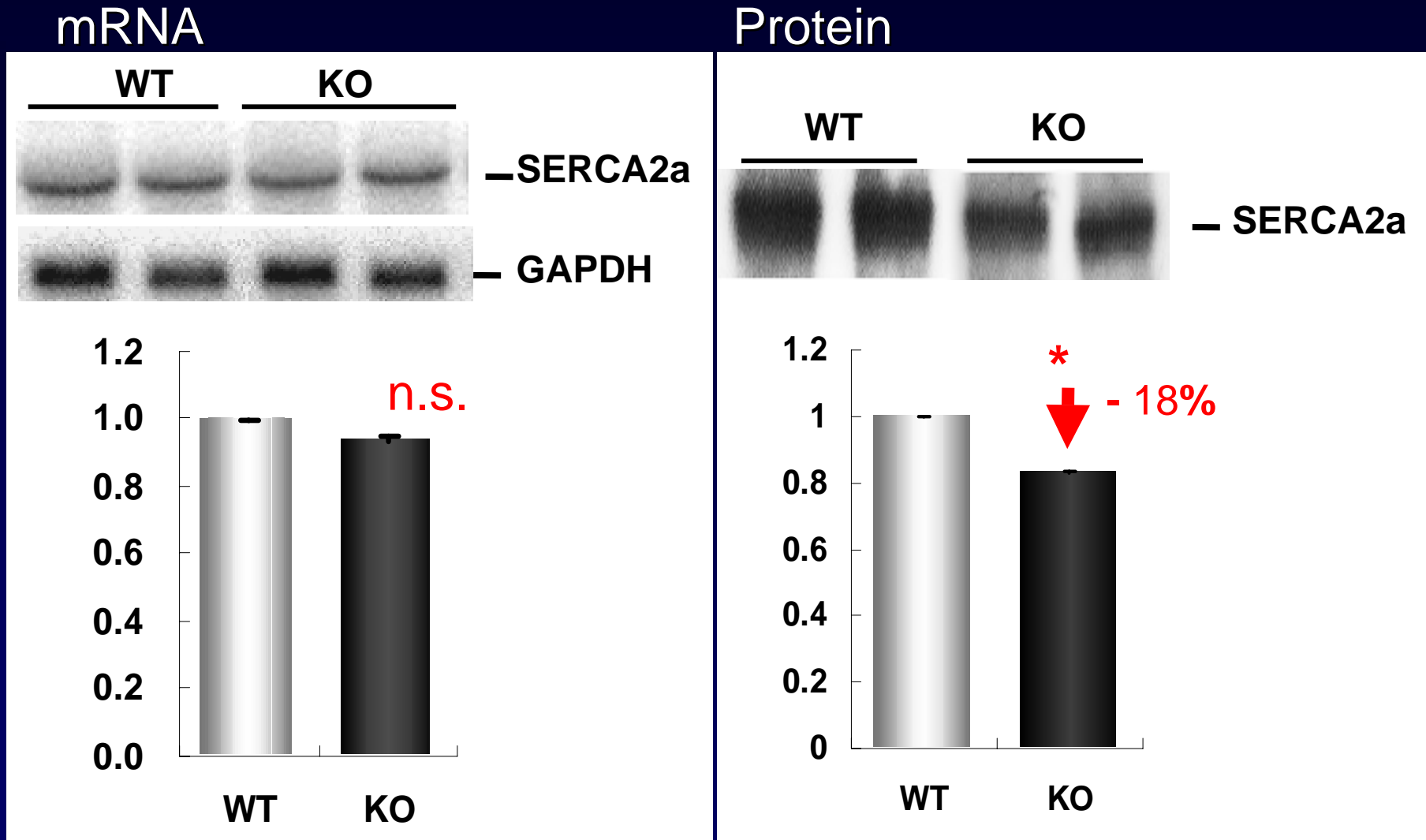


## C. LVEDP



\*  $P < 0.05$ , vs. WT

# SERCA2a Protein, but not mRNA was Decreased in SAR-KO Mice



\*  $P < 0.05$ , vs. WT

# Co-localization of SAR and SERCA2a Co-expressed in HEK-293T Cells

**mSERCA2 (FITC)**

**SAR (TRITC)**

**Overlay**

DIC/FL  
L

DIC/FL

DIC/FL

FL

FL

FL

**Excitation:488nm**

**Filter:BP505-530**

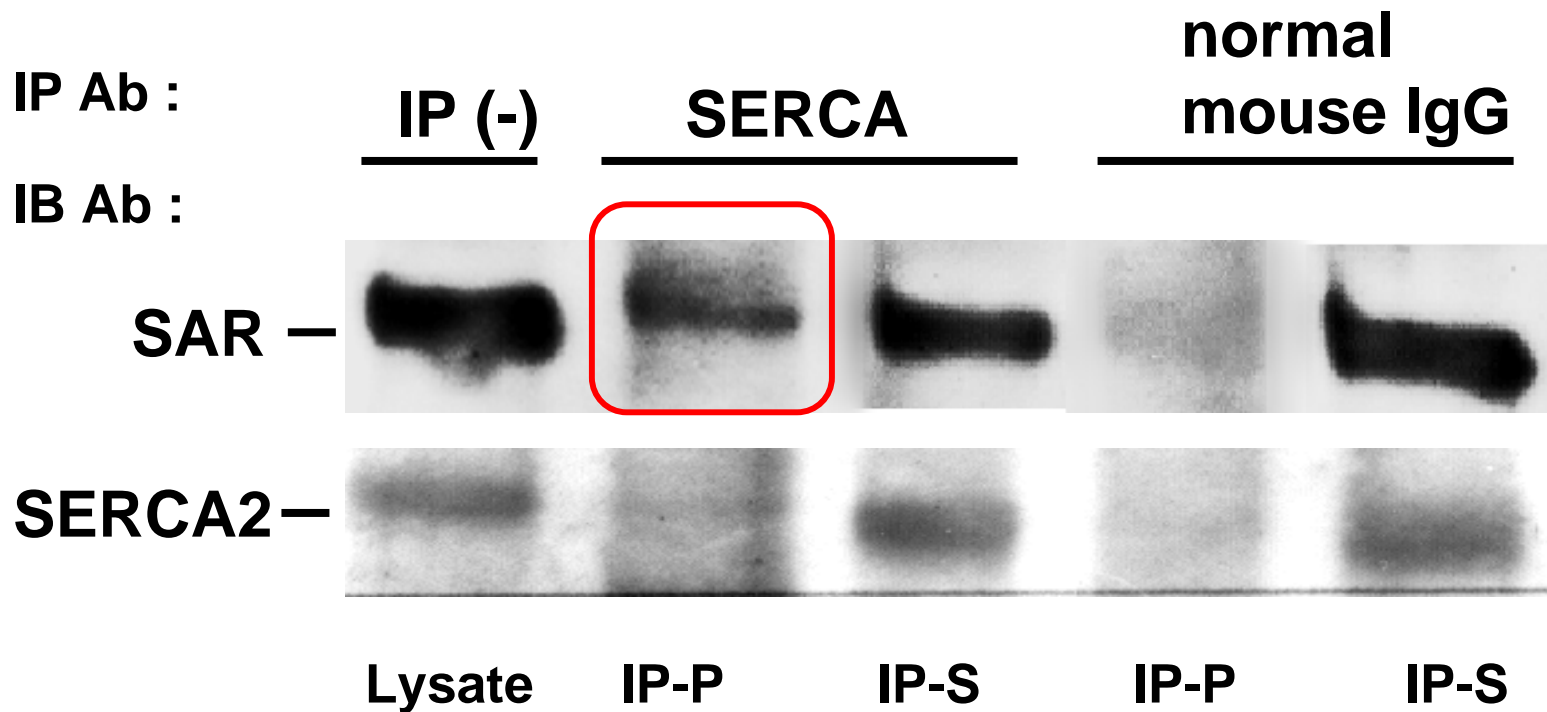
**Excitation:543nm**

**Filter:BP560-615**

\* LSM510 (Carl Zeiss)

\* Scale bar : 10uM

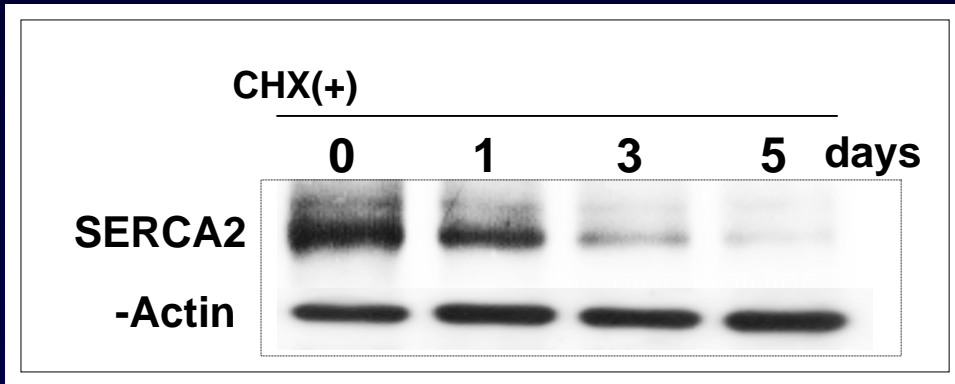
# Interactions between SAR and SR proteins in cardiac muscles



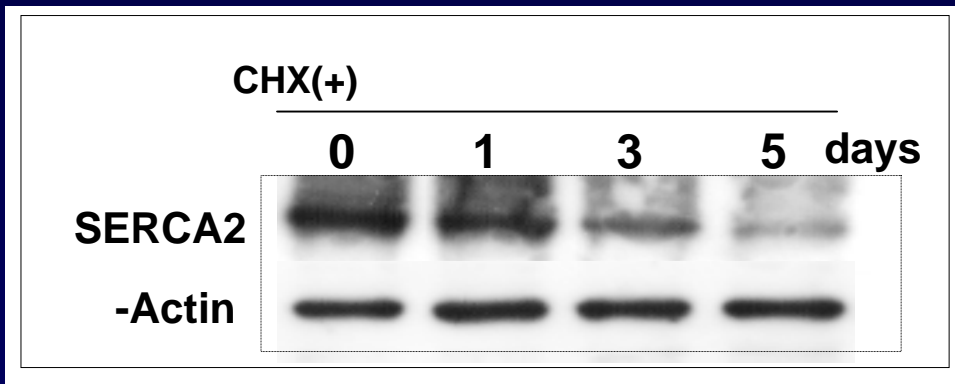


# Rapid Protein Degradation of SERCA2a without SAR

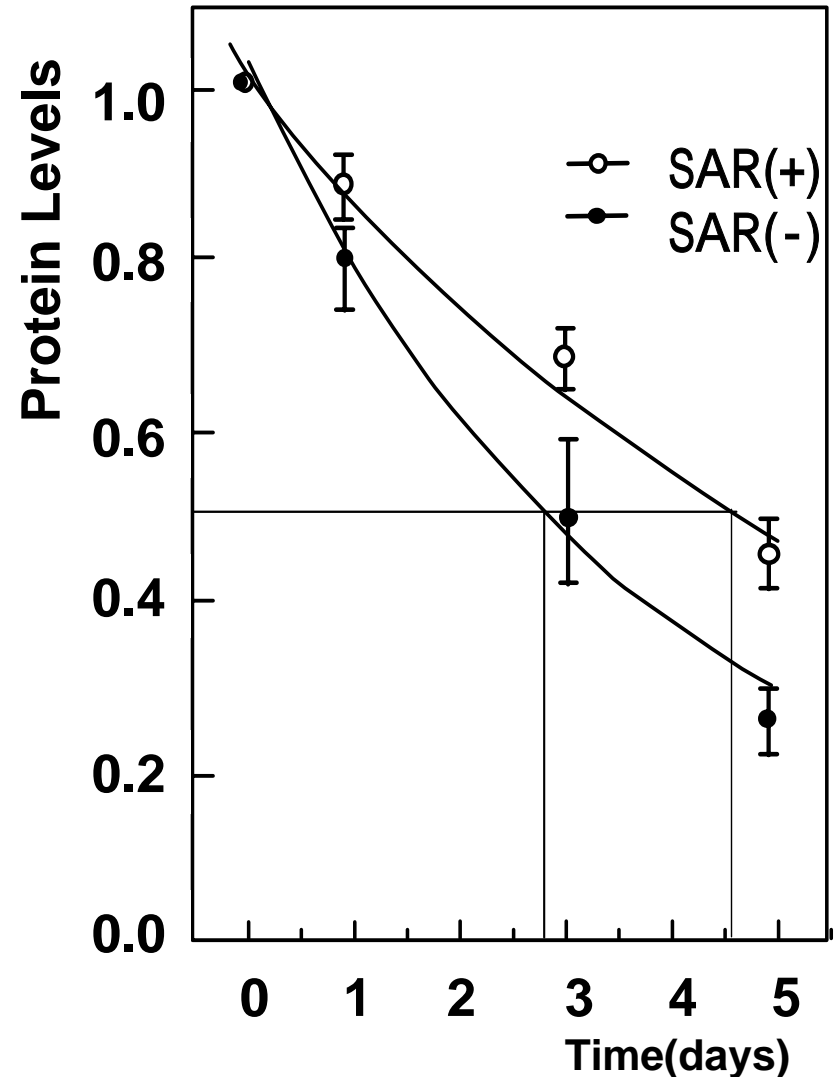
## 1) SAR(-)



## 2) SAR(+)



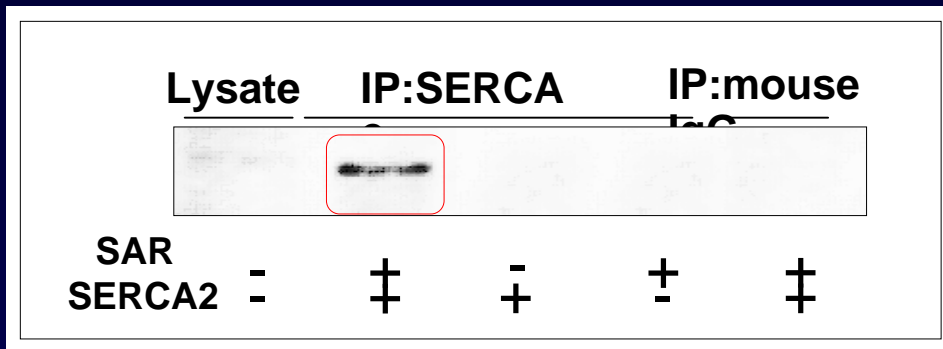
## 3) Protein Half-life



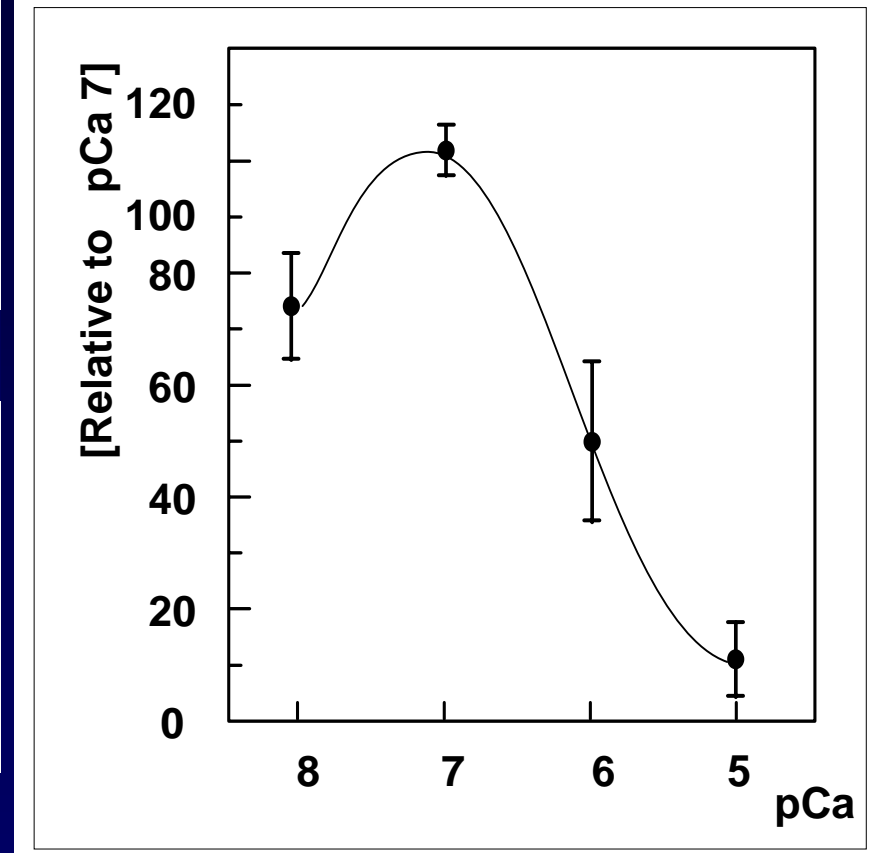
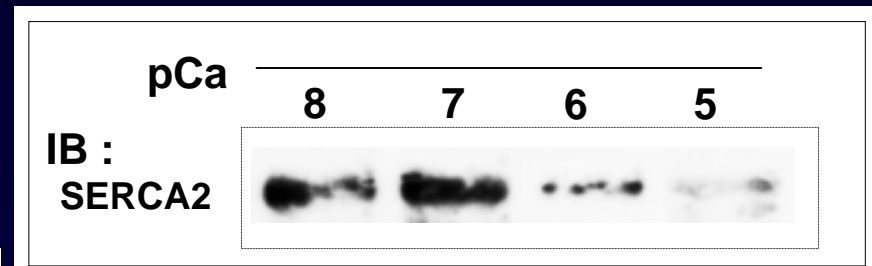
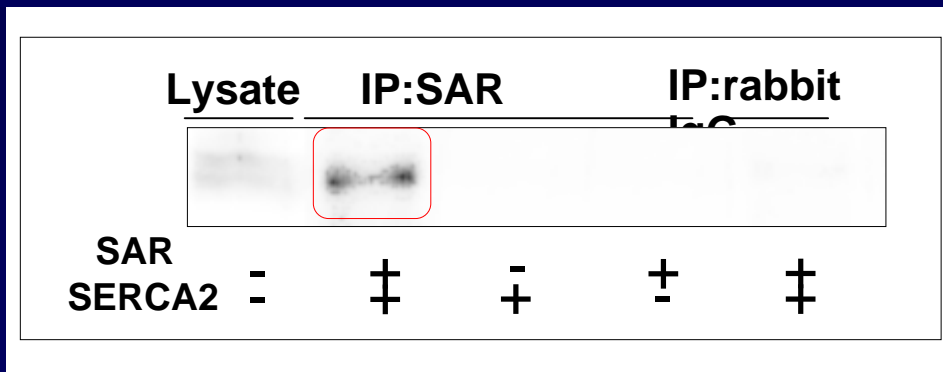
# Interactions between SAR and SERCA2a were Dissociated by Elevated $\text{Ca}^{2+}$

Interactions between SAR and SERCA2a Co-expressed in HEK-293 T-Cells (IP)

a. IB : SAR



b. IB : SERCA2a



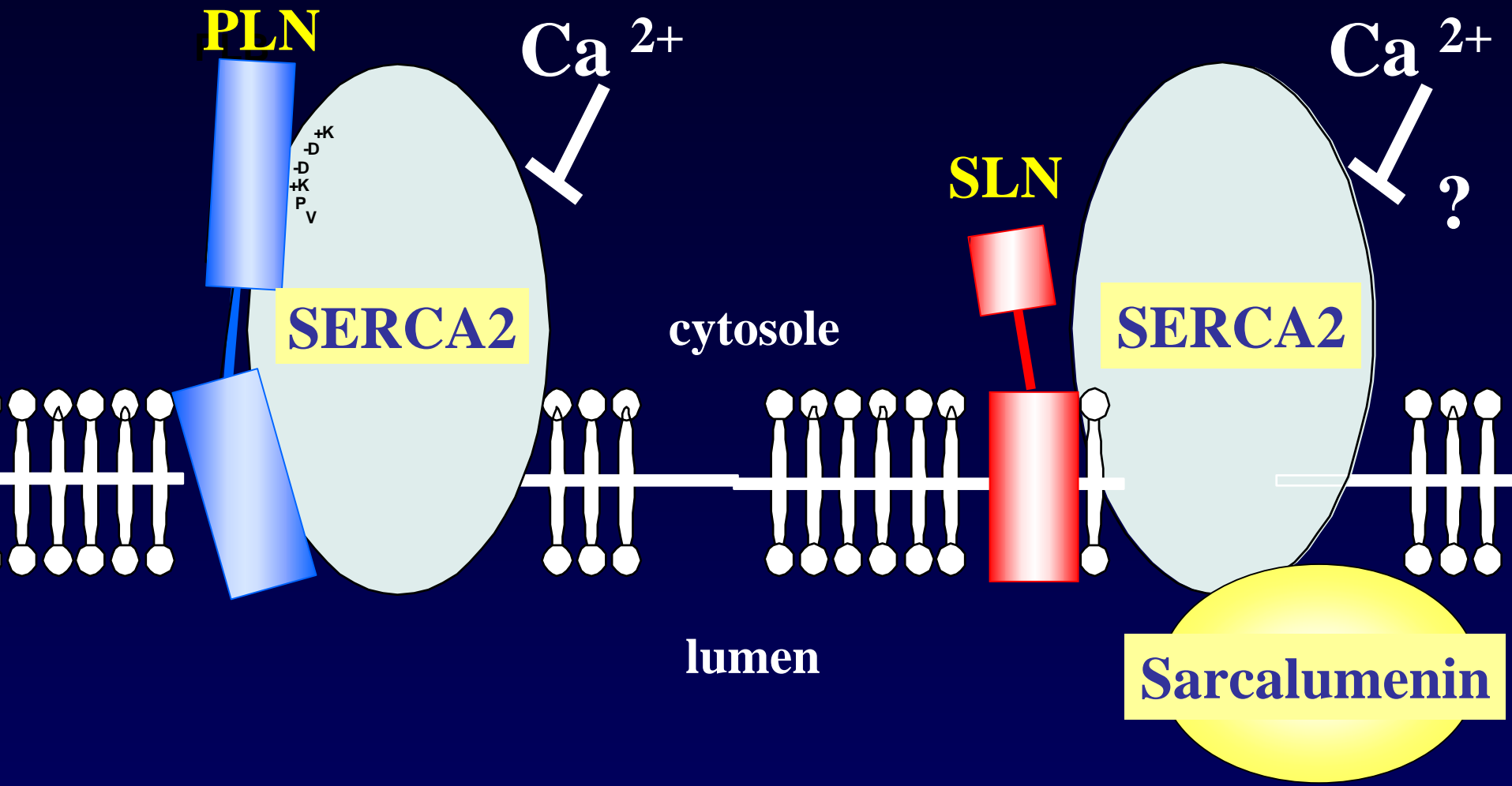
# Summary

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**-SAR deficiency induced the progressive impairment of cardiac function, especially in response to pressure overload.**

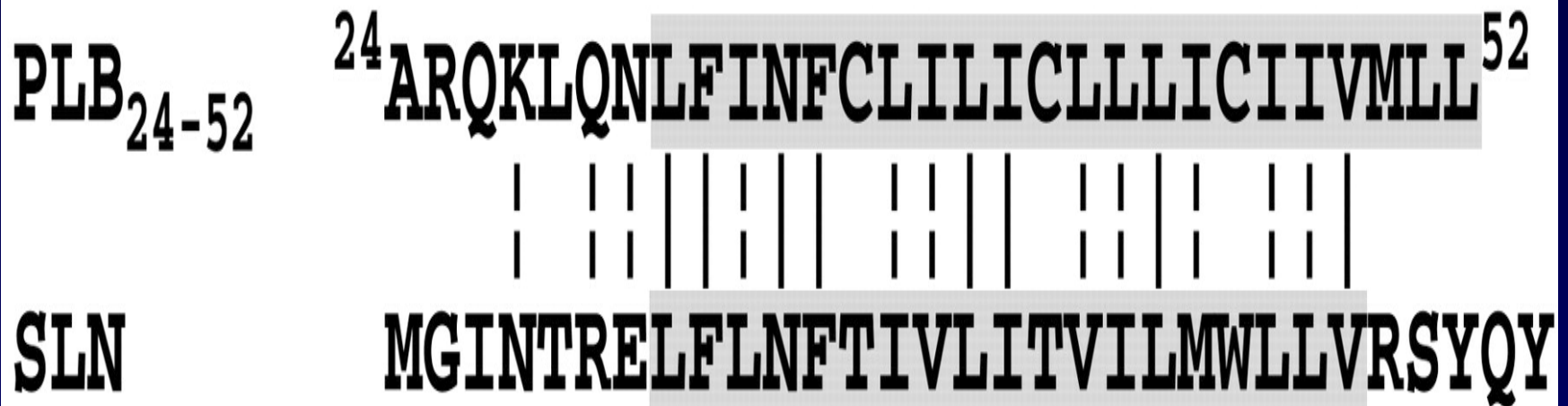
**-SAR interacts with SERCA2a in a  $\text{Ca}^{2+}$ -dependent manner.**

**-The physical interaction of SAR with SERCA2a increases the protein stability of SERCA2a by its presumable chaperone-like activity.**

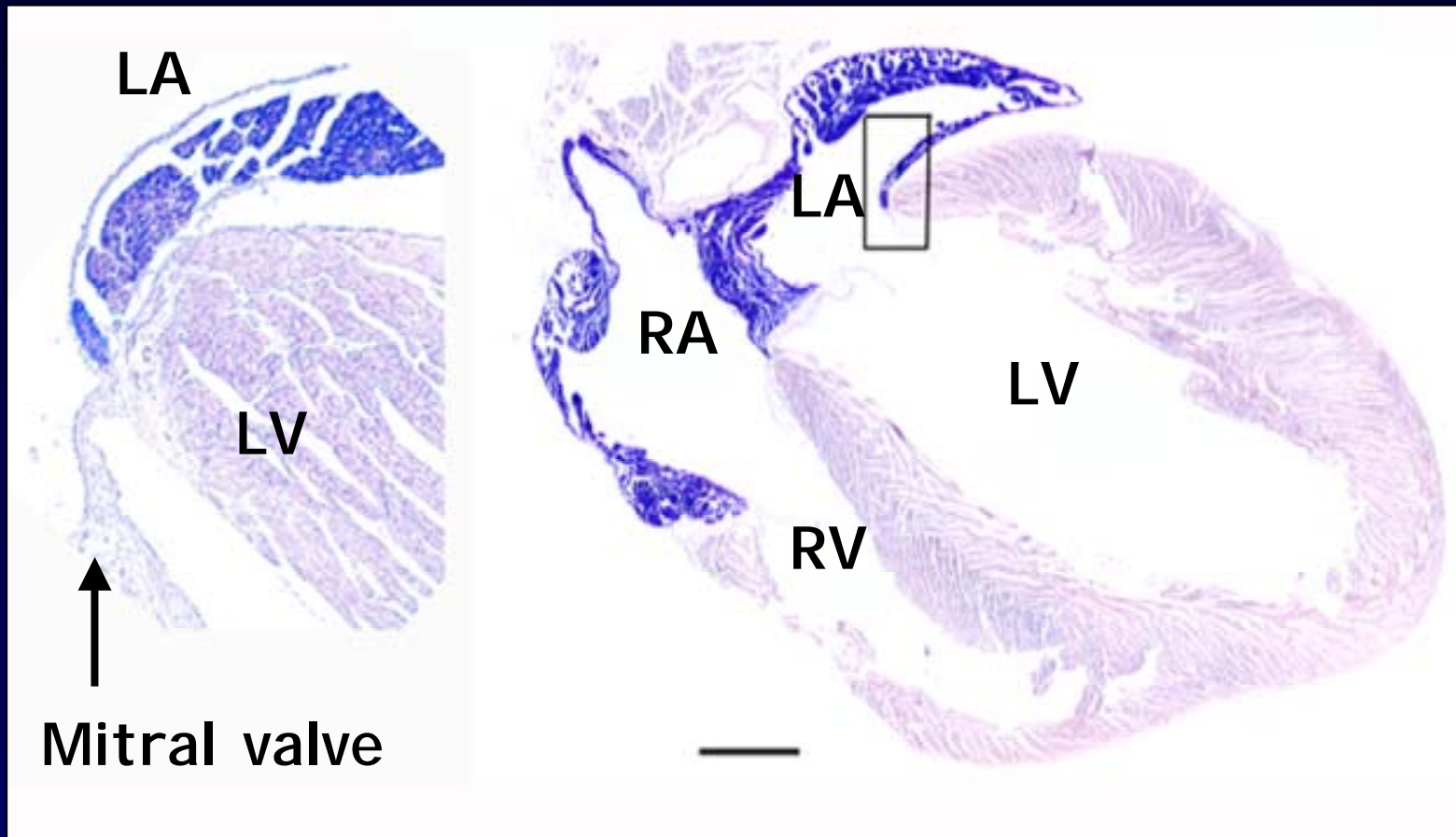


Sarcophilin (SLN) is a 31-amino acid proteolipid in the SR.

SLN is a shorter homologue of PLN.

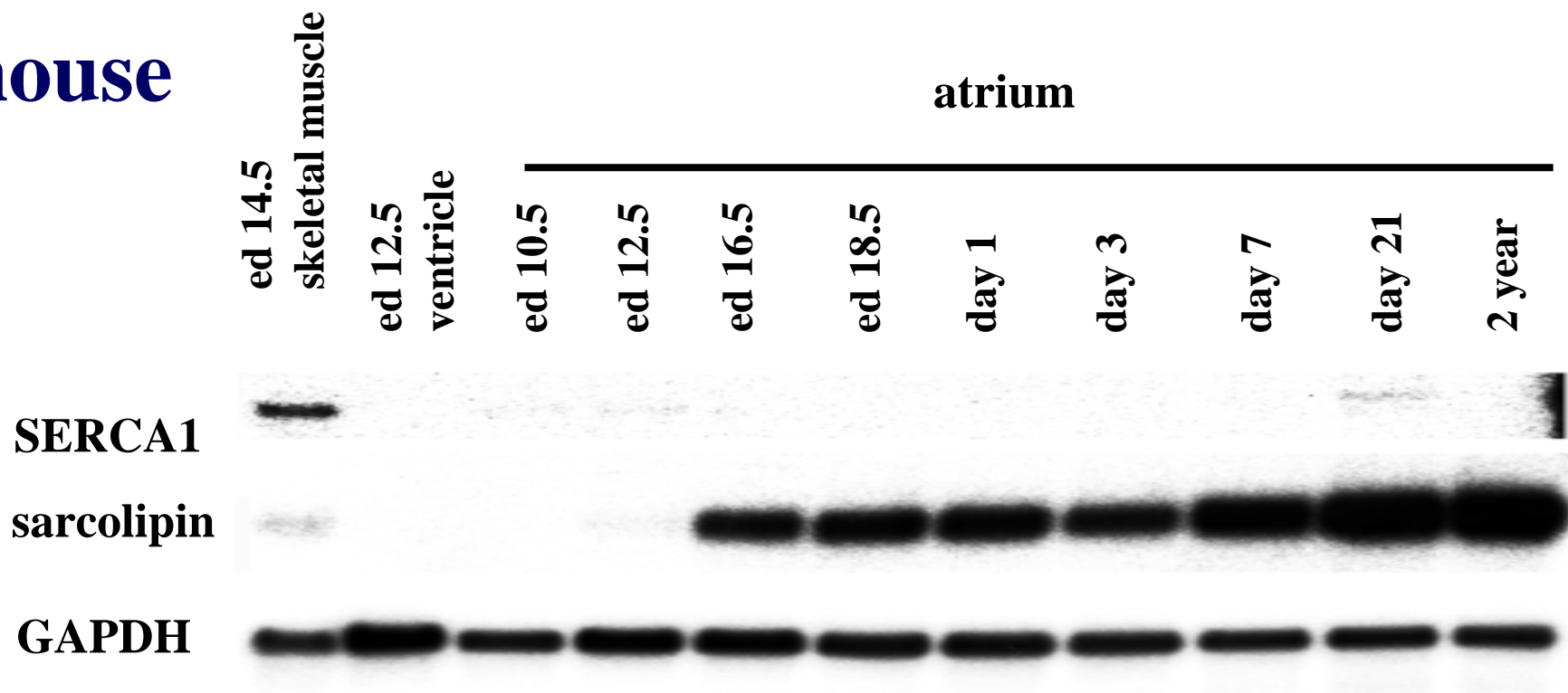


The expression of mouse sarcolipin mRNA was most abundant in the atria and was not detected in the ventricles.

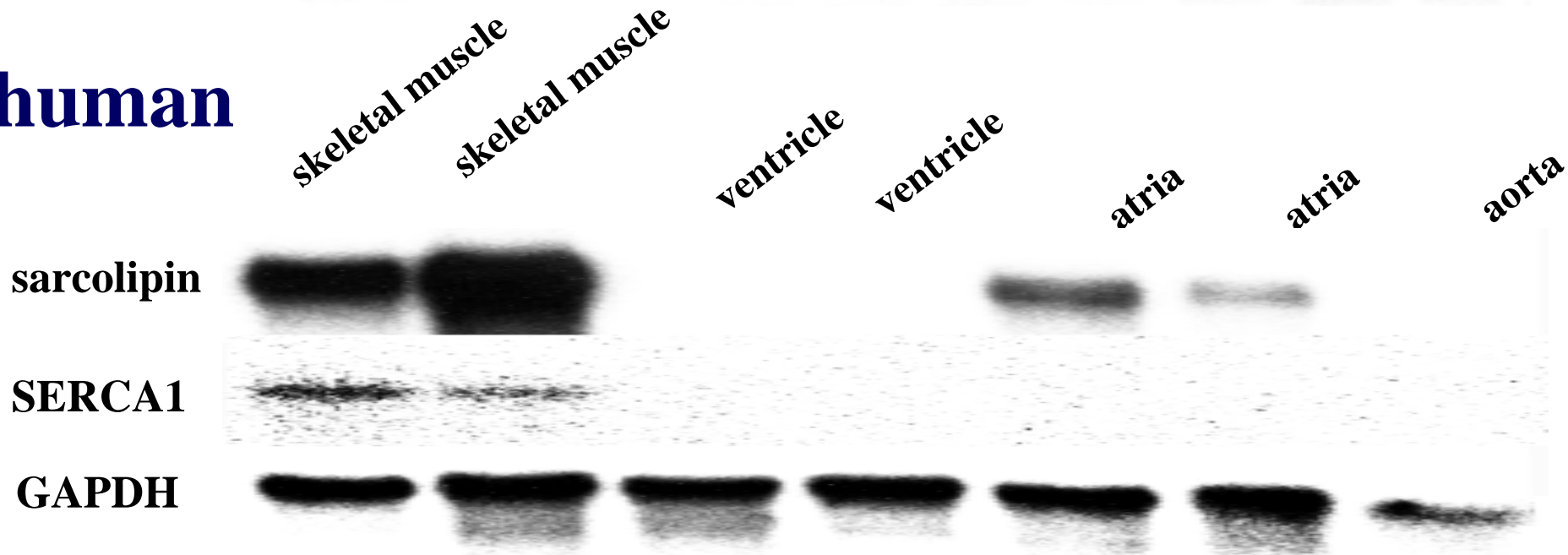


# mouse

## atrium

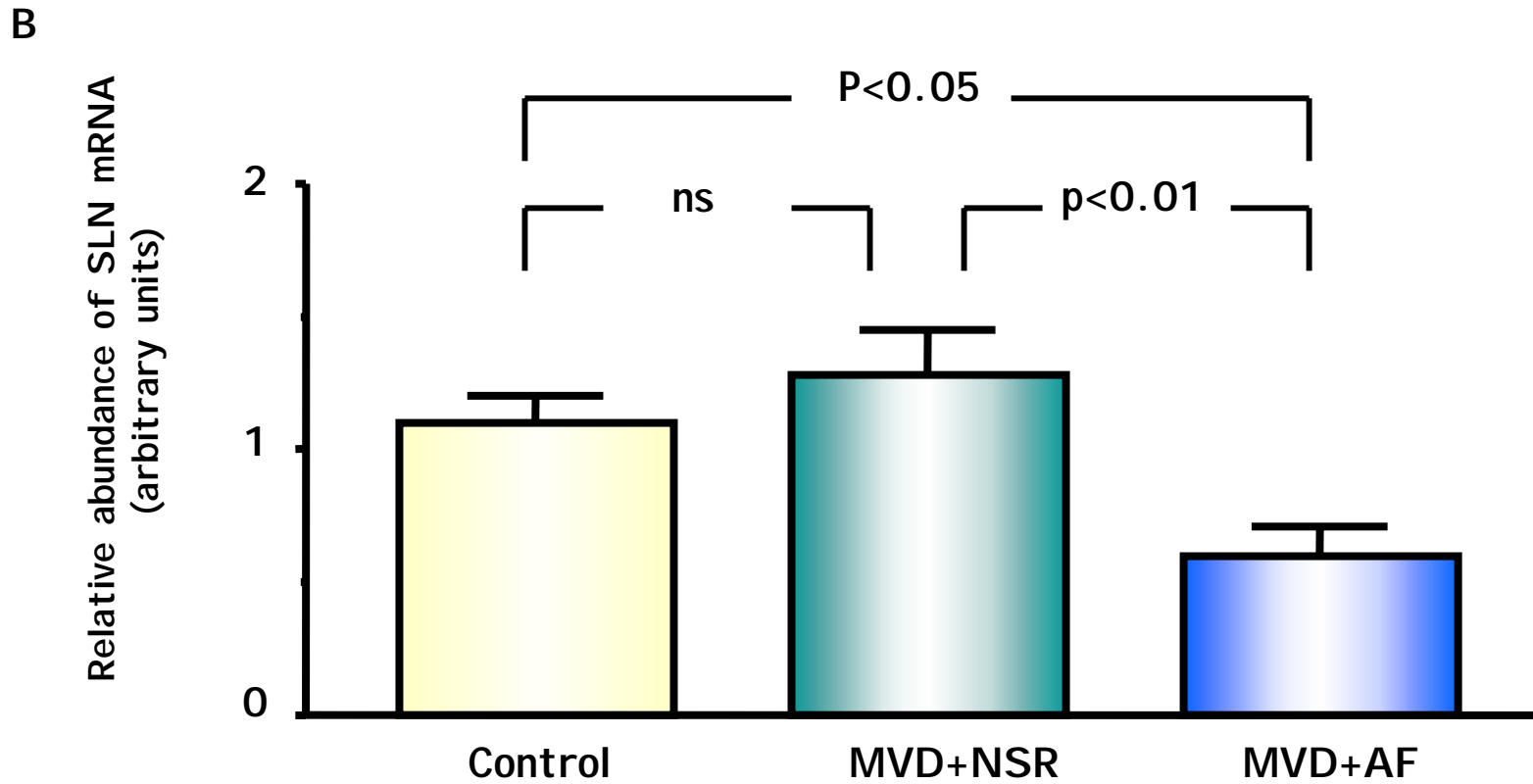
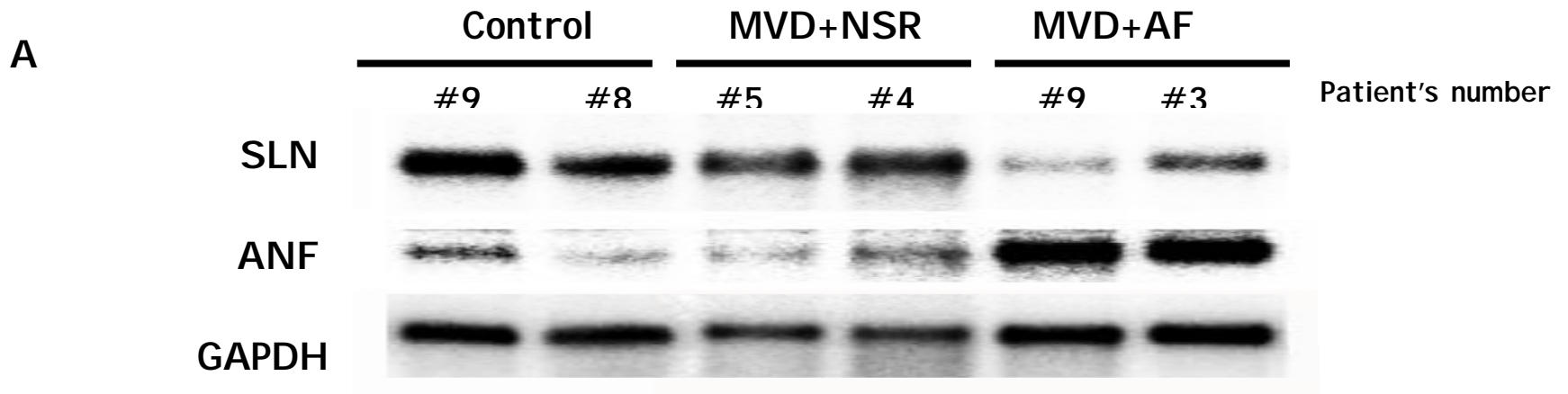


# human



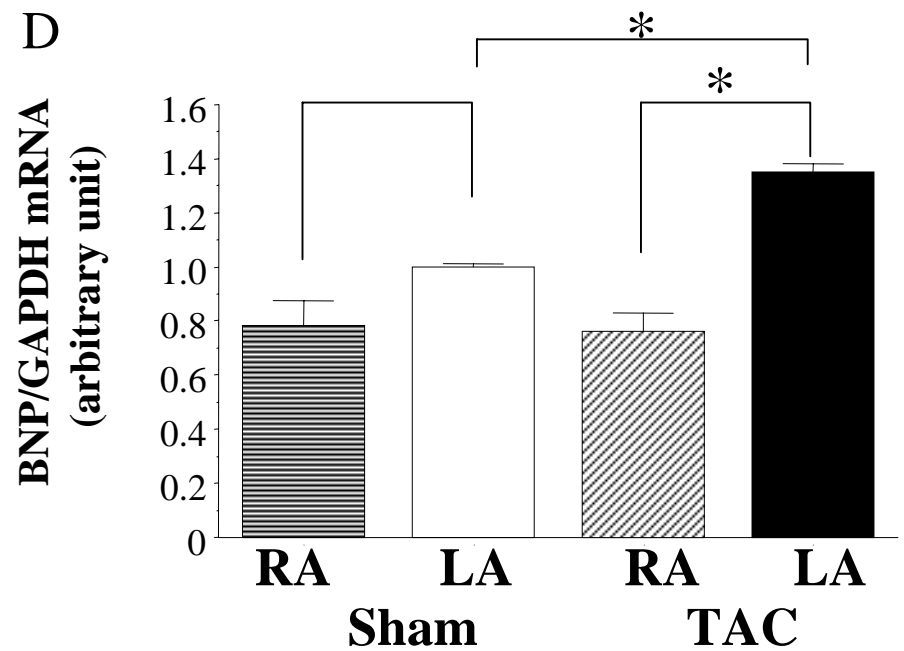
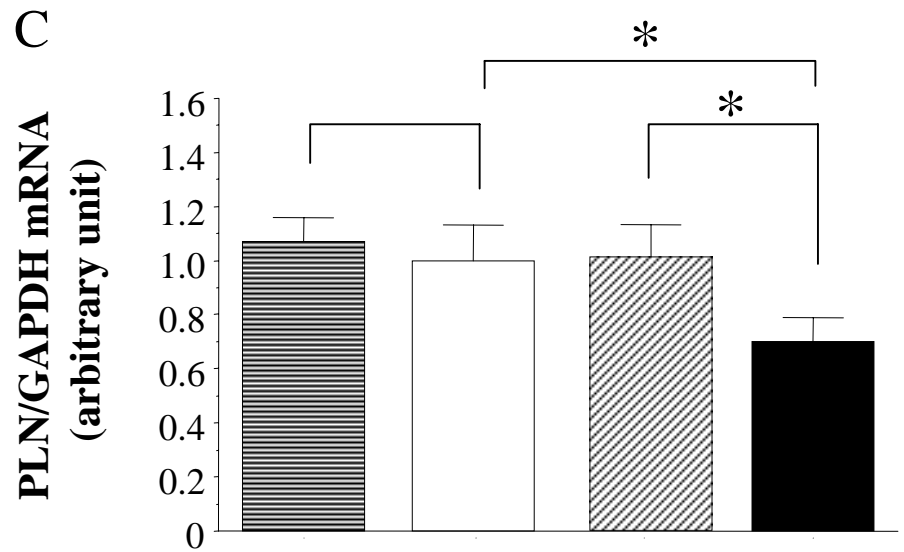
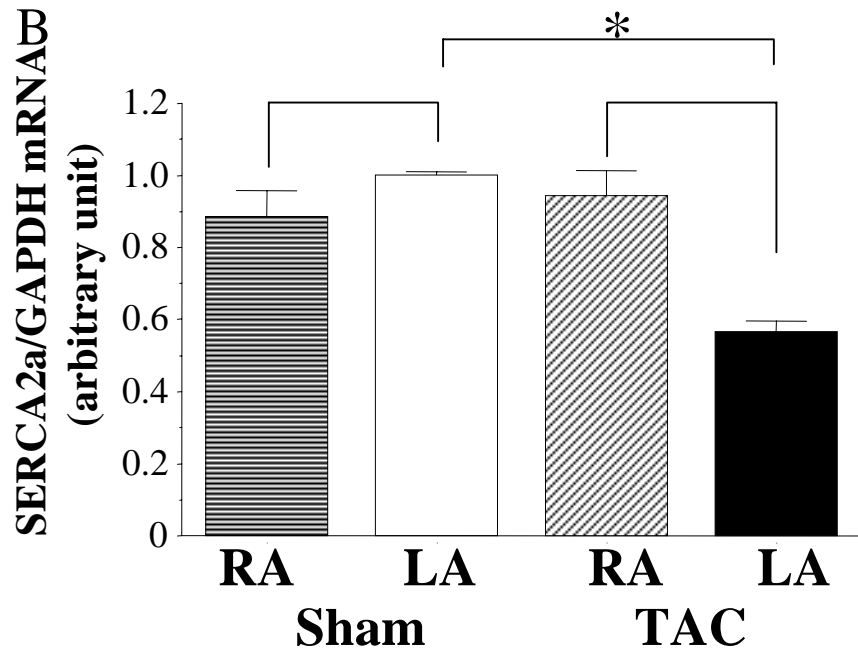
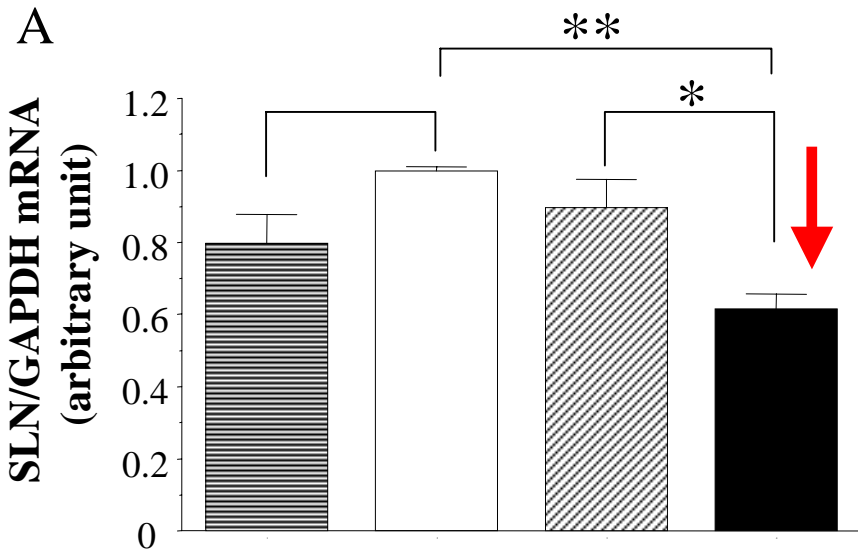
**Abnormal intracellular  $\text{Ca}^{2+}$   
homeostasis may be an  
important modulator of  
sustained atrial  
fibrillation (AF).**



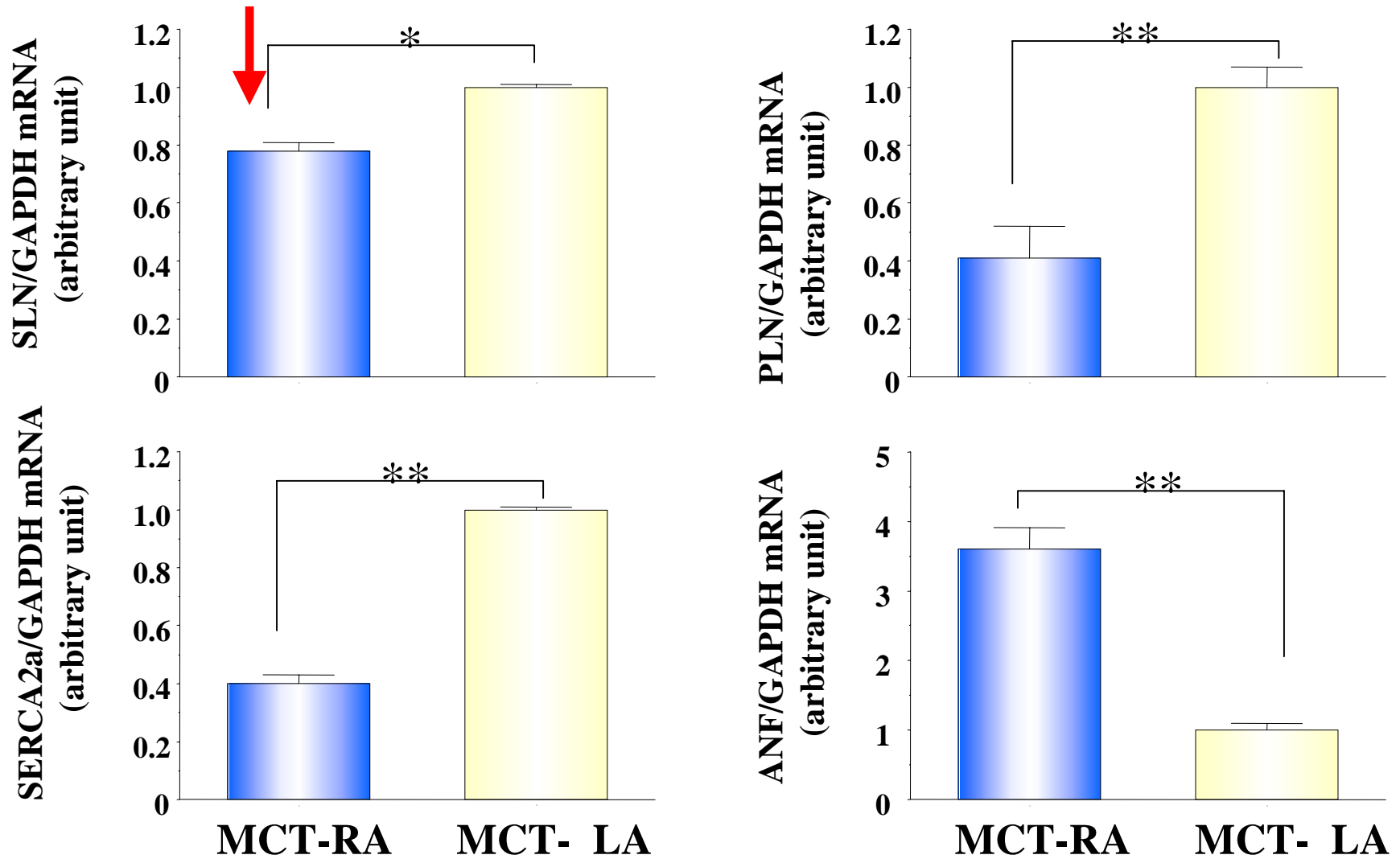


**Pressure overload  
down-regulates SLN mRNA  
in the loaded atria.**

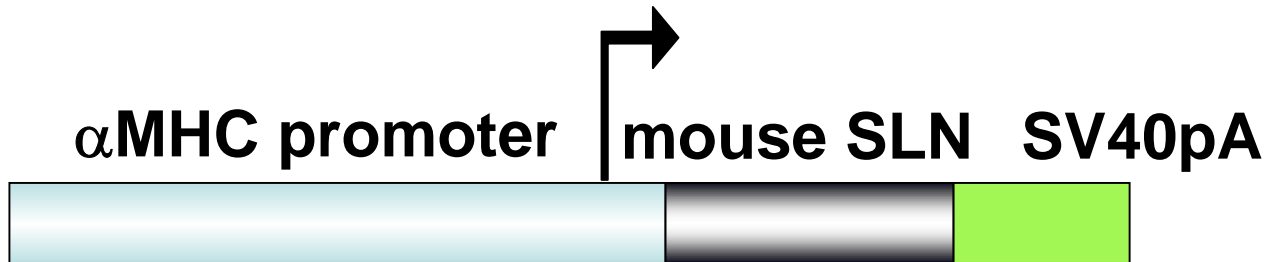
# Transverse aortic constriction down-regulated SLN mRNA in LA



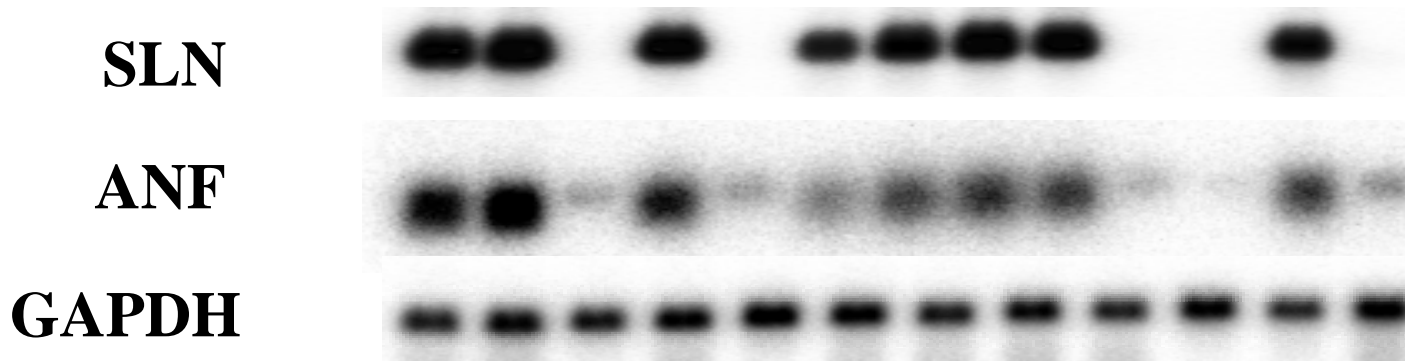
# Monocrotaline down-regulated SLN mRNA in RA



# Heart-specific SLN Transgenic Mice

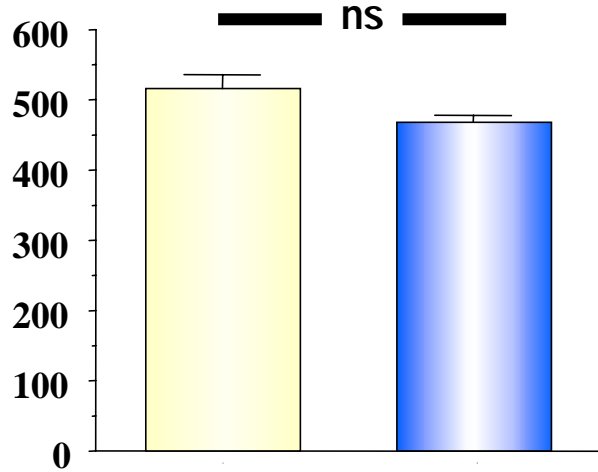


## Northern Blot Analysis of Ventricular Tissues

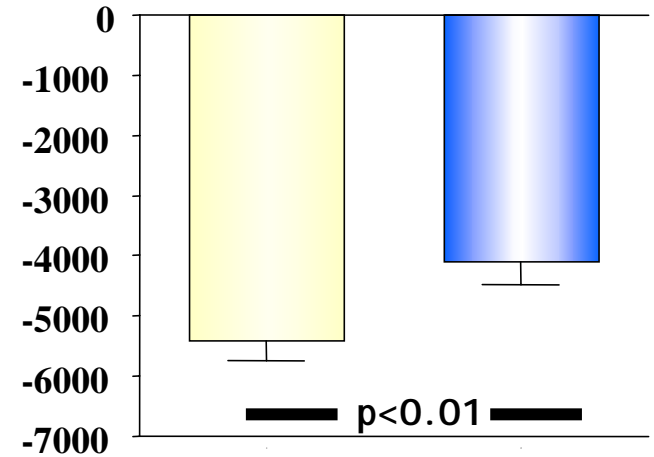


# *In vivo* hemodynamic assessment on SLN transgenic mice

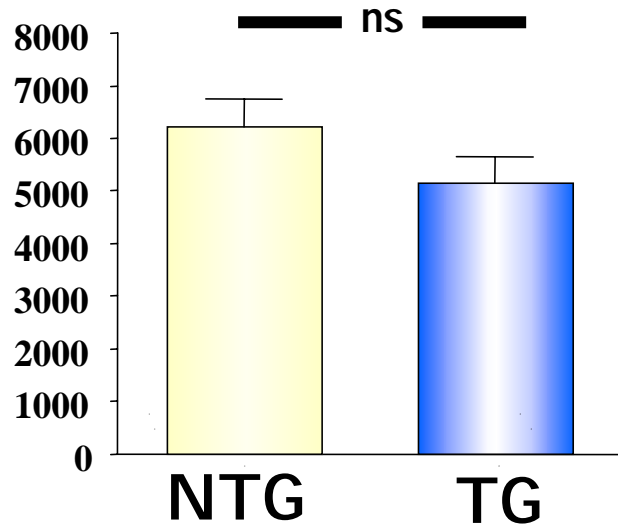
Heart Rate (bpm)



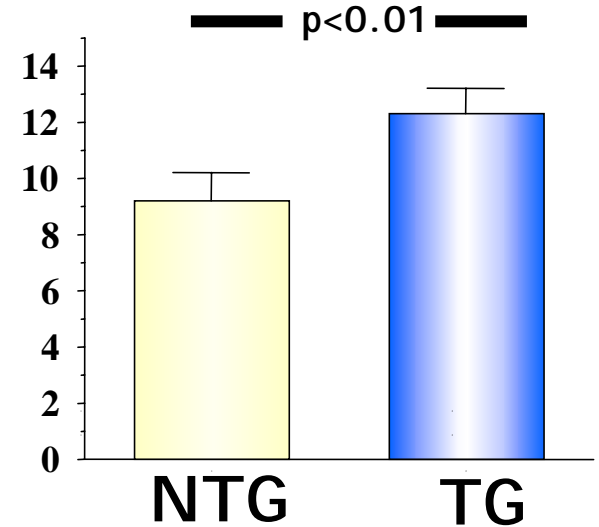
dP/dtmin (mmHg/sec)



dP/dtmax (mmHg/sec)

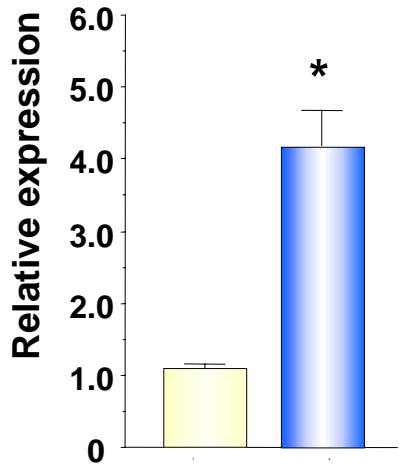


Tau (sec)

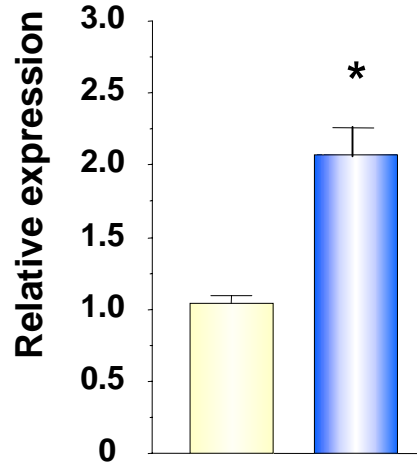


# ANF and BNP mRNAs were increased in SLN TG ventricles

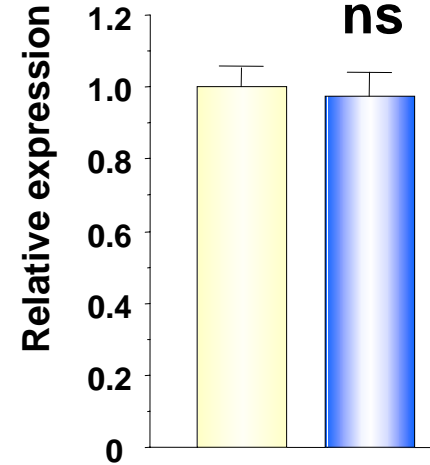
## ANF



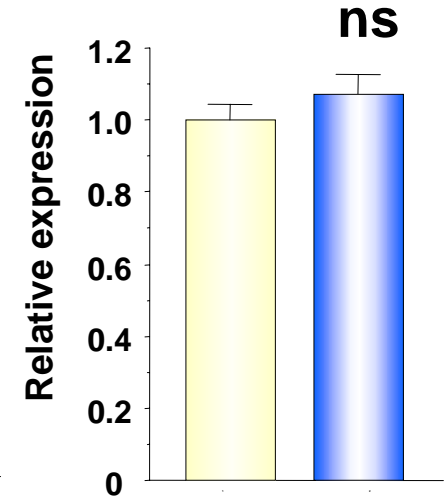
## BNP



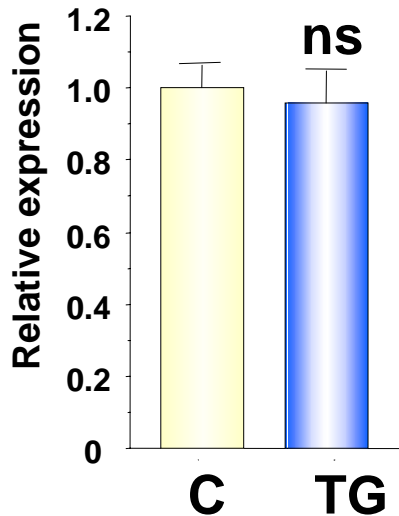
## SERCA2a



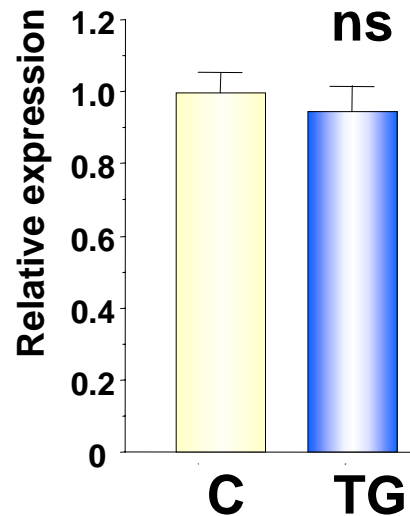
## PLN



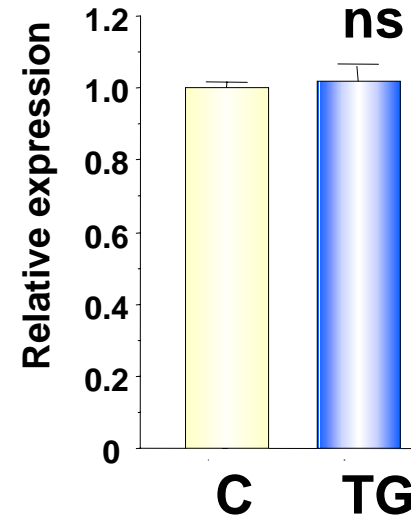
## RyR2



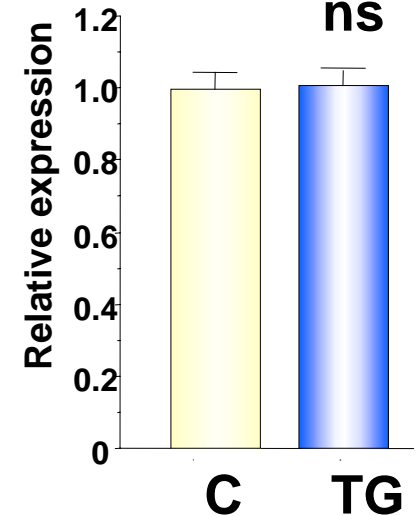
## CSQ2



## NCX1



## HRC



# Summary

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**-SLN is an atrial type of PLN homologue and inhibits diastolic cardiac function by inhibiting the SERCA2a activity.**

**-SLN is down-regulated in the atria with atrial fibrillation and with pressure-overload, which may affect the atrial function through the change in atrial  $\text{Ca}^{2+}$  cycling.**



*University of California,  
San Diego*

Masahiko Hoshijima  
John Ross, Jr.  
Kenneth R. Chien

*University of Maryland*

Yibin Wang

*University of Cincinnati*

Evangelia G. Kranias

*Tohoku University*

Hiroshi Takeshima

*Tokyo Women's Medical University*

Rumiko Matsuoka

*National Institute of Health Sciences*

Yoji Sato

*Yamaguchi University*

Yasuhiro Ikeda  
Tomoko Ohkusa

*Yokohama City University*

Miei Shimura  
Yoshihiro Ishikawa

# The clinical profile of the patient with mutation -77A G

**Sex: female**

**Age: 58 years old**

**Family history: father (cardiomyopathy)  
a brother (HCM)**

**Clinical symptom: palpitation at the age of 56 years**

**The 12-lead ECG : LVH and ST-T wave abnormality**

**Echocardiography:**

**The wall thickness of LV**

**septal wall : 30 mm**

**posterior wall: 13 mm**

**The dimension of LV**

**diastolic: 48 mm**

**systolic: 27mm**