

***Integrins and Associated  
Proteins: Implications for  
Heart Failure***

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**VA Healthcare – San Diego**



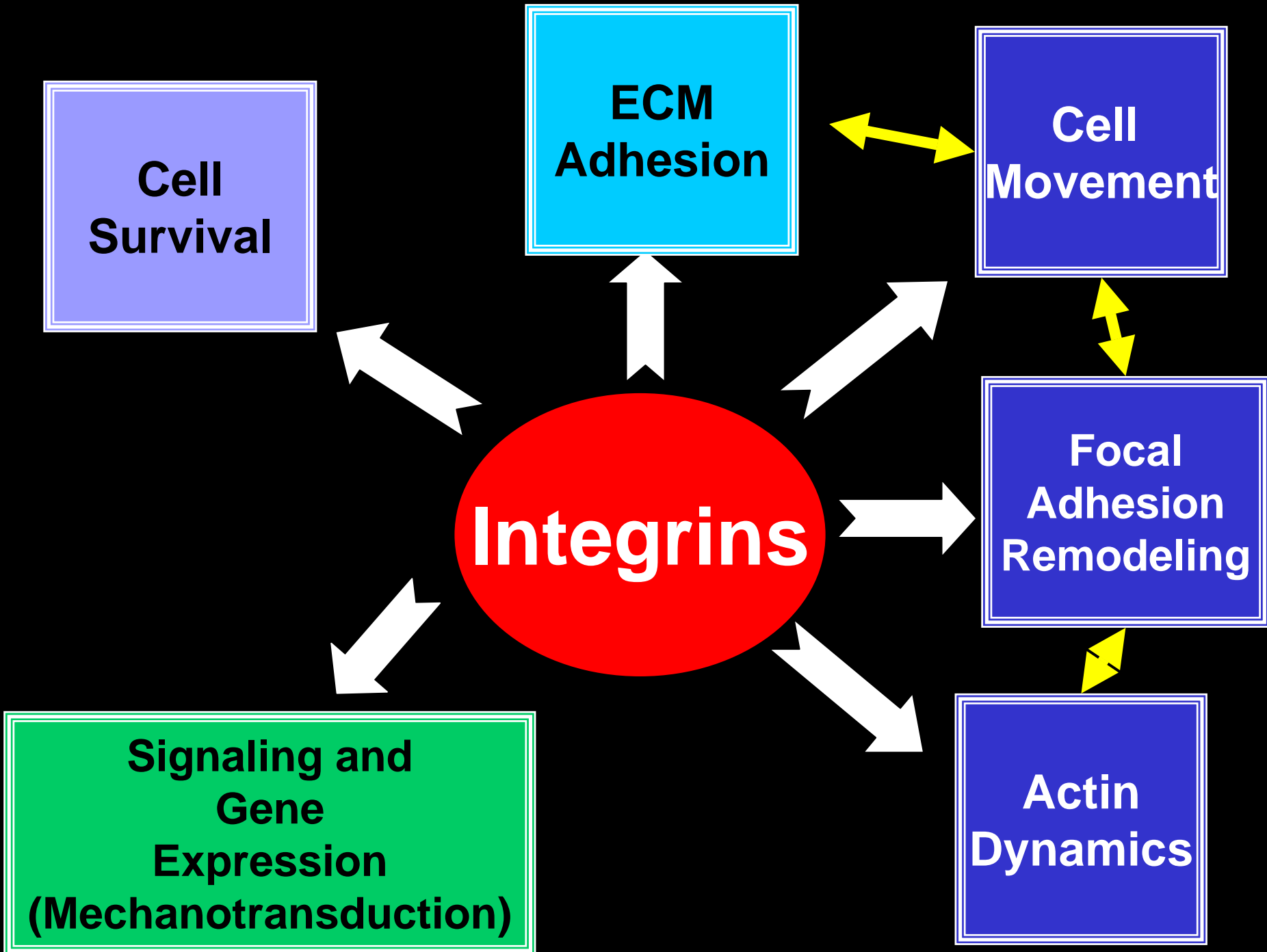
- **General concepts**
- **Normal integrin expression on the cardiac myocyte is required for:**
  - 1) **preservation of basal heart function,**
  - 2) **ability of the ventricle to tolerate hemodynamic stress, and**
  - 3) **mechanical and adrenergic signaling**
- **Discuss Potential links of integrins and related proteins to heart disease**

# General Properties of Integrins

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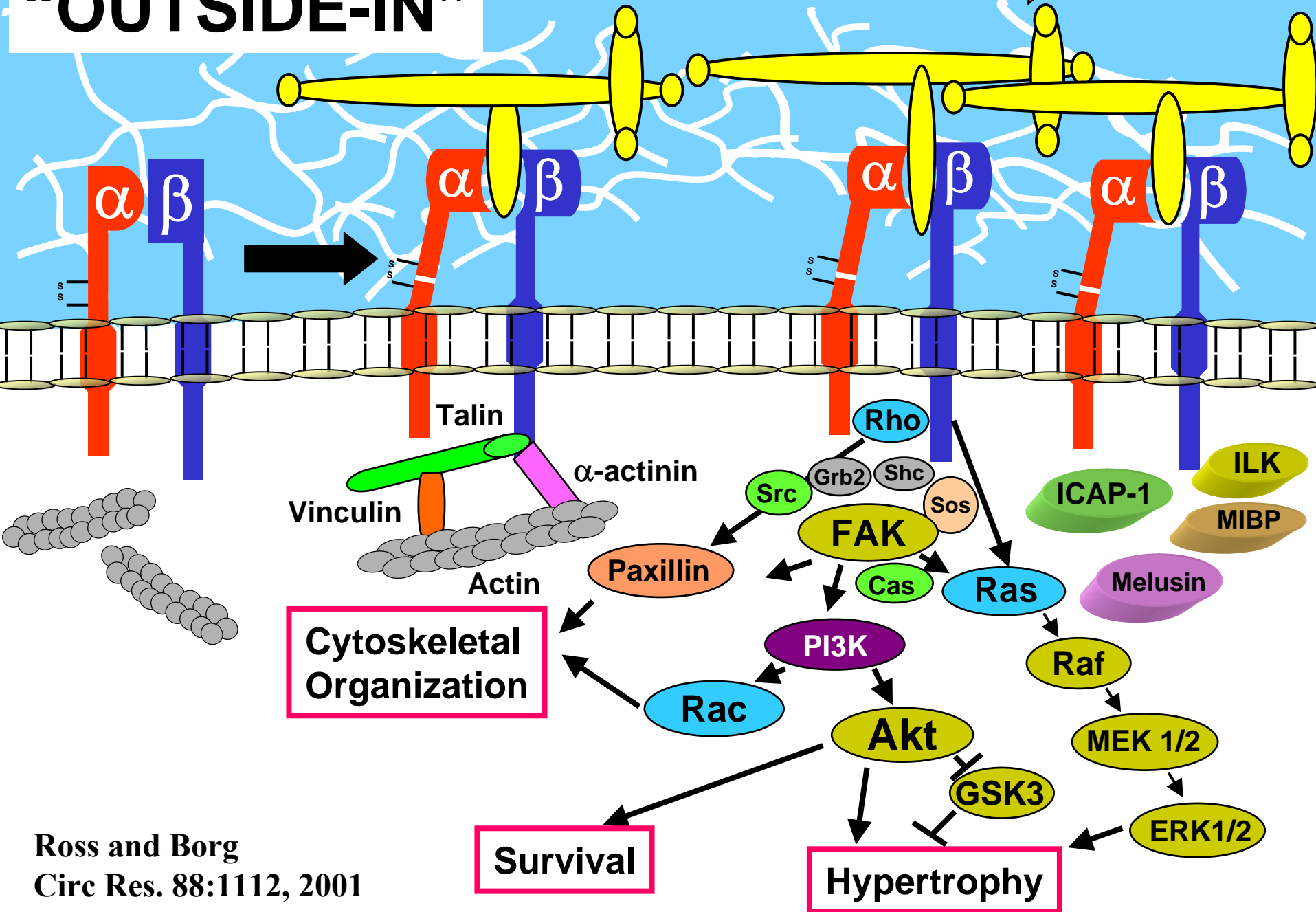
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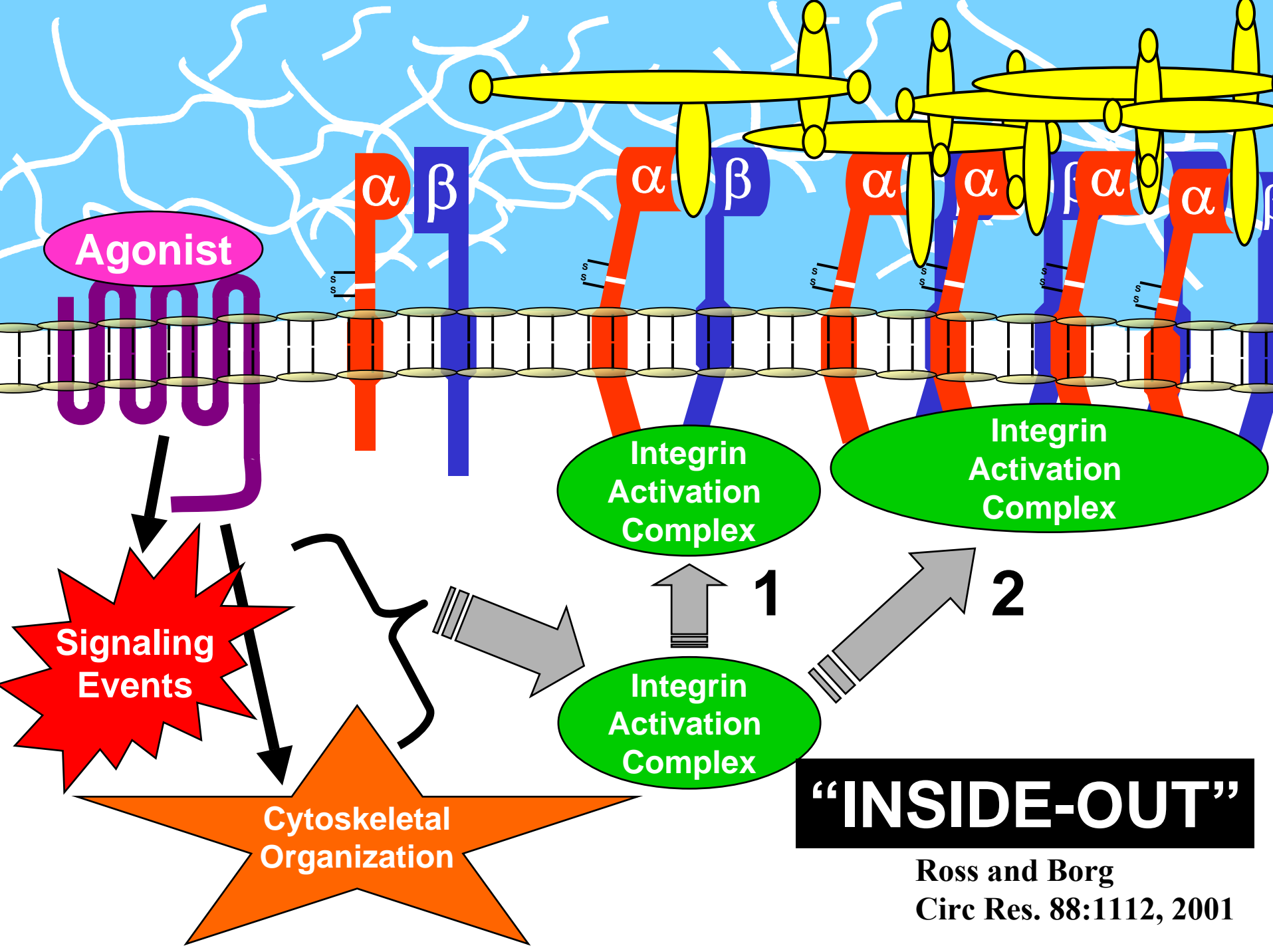
- Large family with over 24 receptors composed of 18  $\alpha$  and 8  $\beta$  integrin subunits
- Different heterodimeric receptors can bind similar ligands: e.g. collagen, laminin and fibronectin
- Many integrins can be expressed on one cell
- Integrin expression can change developmentally and with disease state (e.g. embryonic vs. adult, normal vs. hypertrophic myocardium)



# “OUTSIDE-IN”

Mechanical Tension 





Agonist

$\alpha$

$\beta$

$\alpha$

$\beta$

$\alpha$

$\alpha$

$\beta$

$\alpha$

Integrin Activation Complex

Integrin Activation Complex

Integrin Activation Complex

1

2

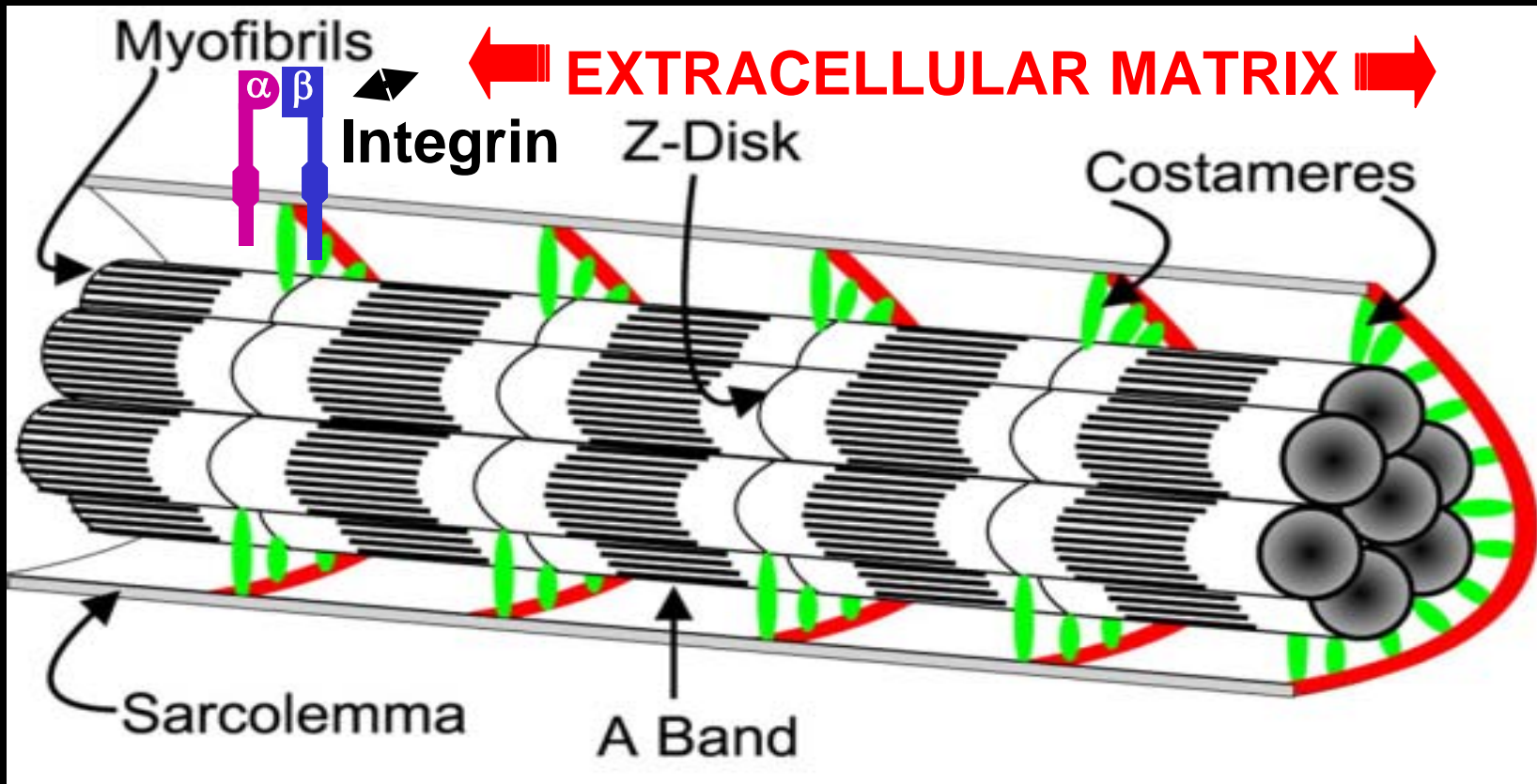
Signaling Events

Cytoskeletal Organization

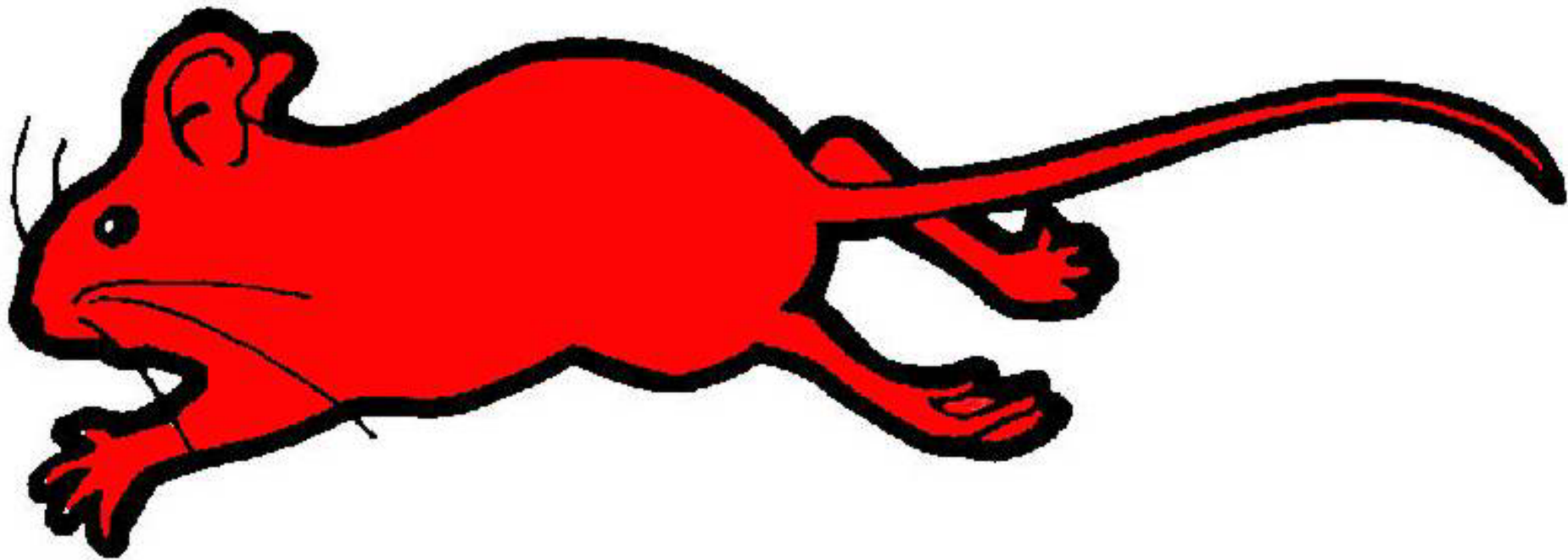
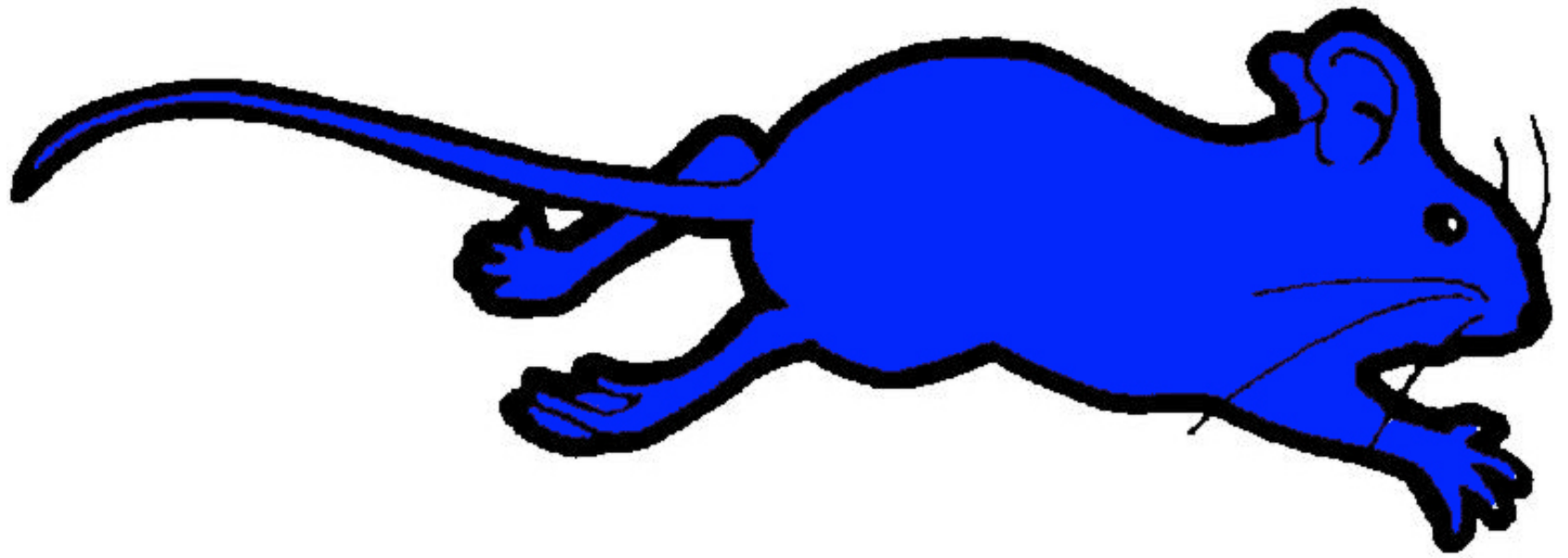
**"INSIDE-OUT"**

Ross and Borg  
Circ Res. 88:1112, 2001

**Integrins are located at Costameres:**  
structures that registers with the Z-disk of myofibrils and therefore couple the sarcomeres with the sarcolemma.



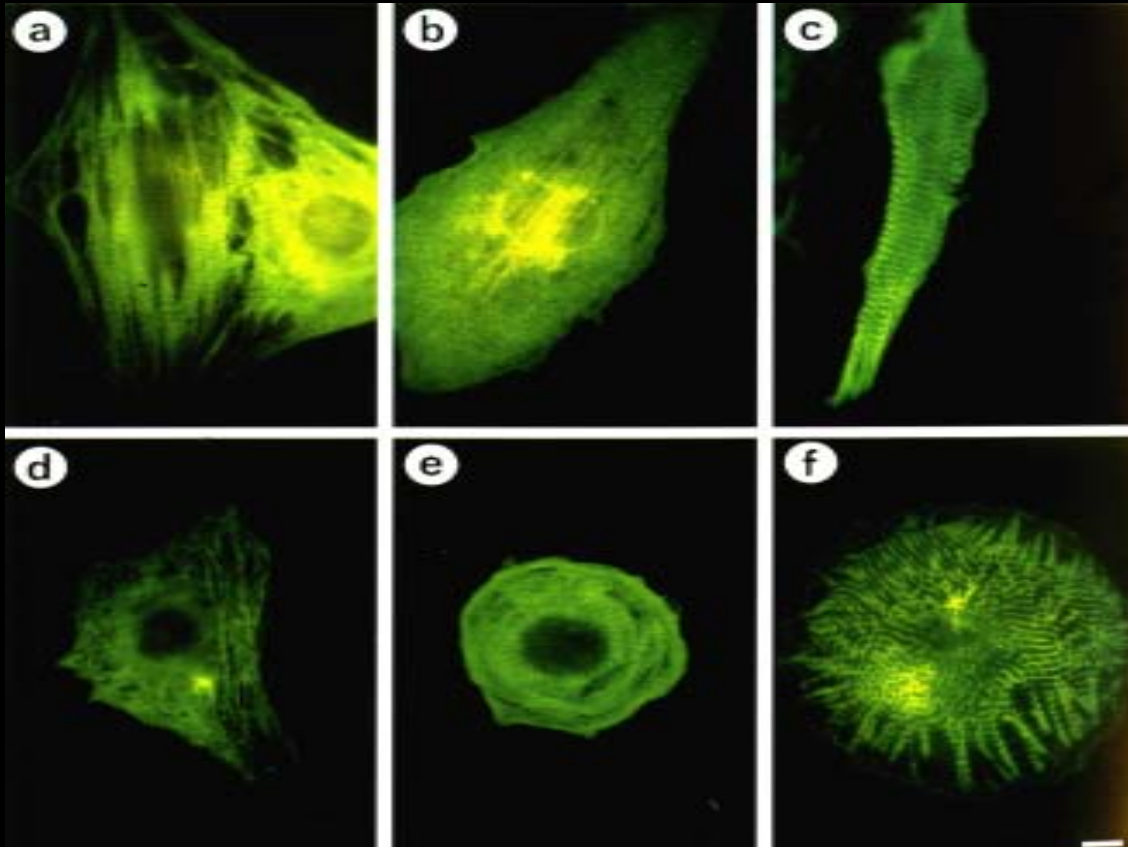
Adapted from: Ervasti JM. J Biol Chem. 18;278:13591-4, 2003.



# $\beta 1$ Integrin Knockout in all Mouse Cells

- Mice die early in gestation before the heart has formed.
- Abnormal myofibrils are found in cardiac-like cells derived from integrin null embryoid bodies

WT



KO

Fassler and Meyer,  
Genes and  
Development, 9:1896,  
1995

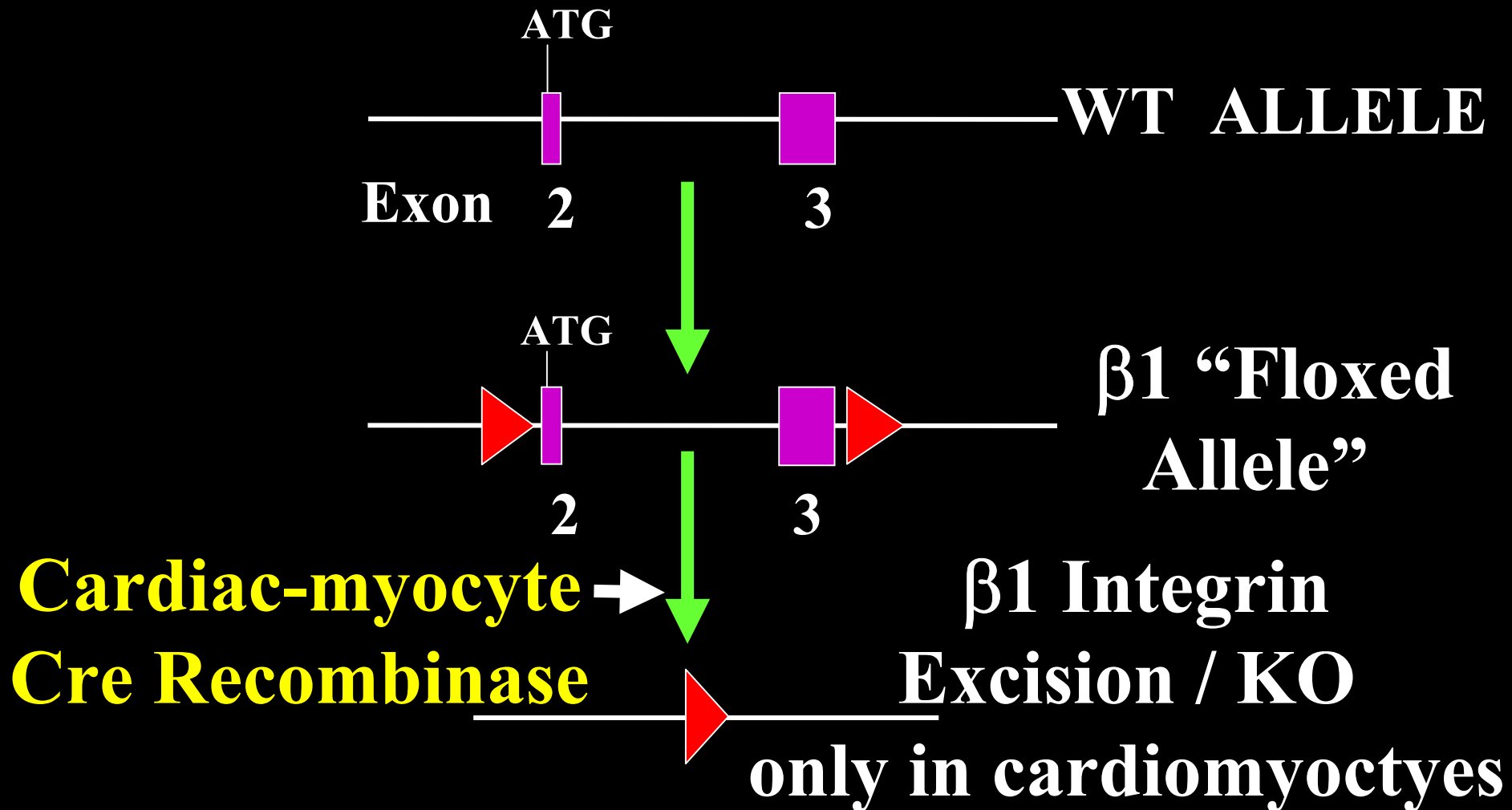
Fassler, et.al., J. Cell  
Science, 109:2989,  
1996.

# Cardiac Myocyte–Specific Excision of the $\beta 1$ Integrin Gene Results in Myocardial Fibrosis and Cardiac Failure

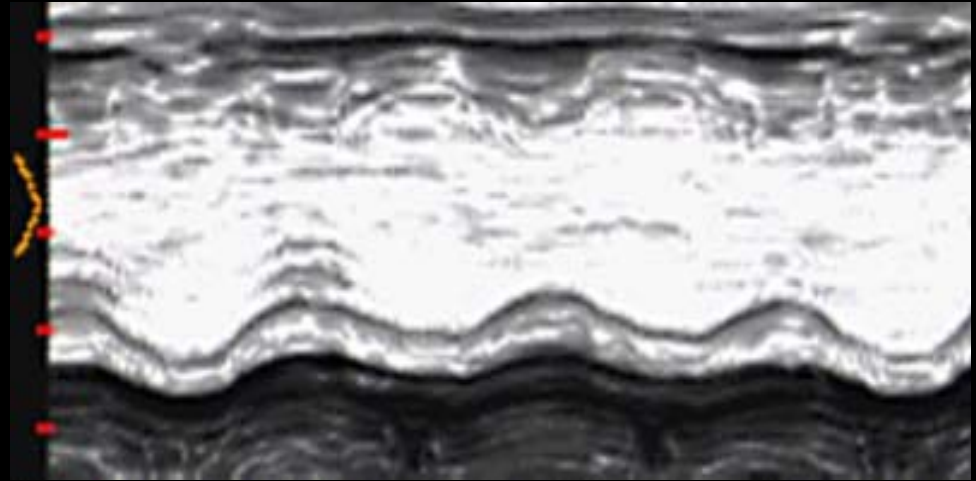
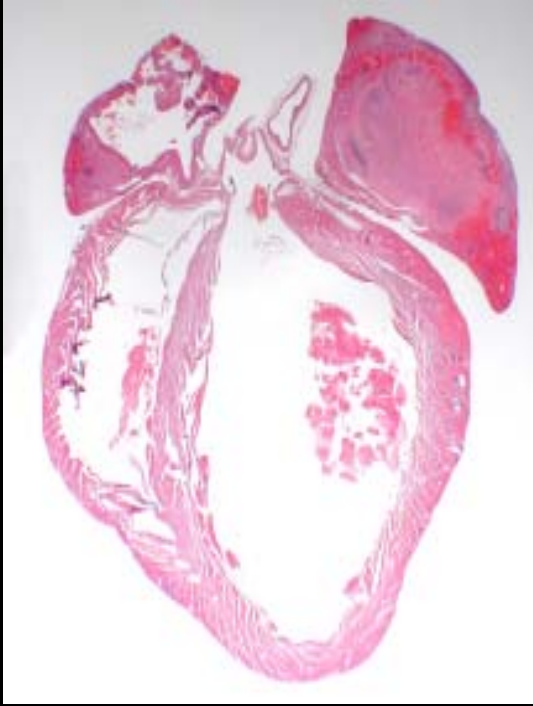
Shaw-Yung Shai, Alice E. Harpf, Christopher J. Babbitt, Maria C. Jordan, Michael C. Fishbein, Ju Chen, Michelle Omura, Tarek A. Leil, K. David Becker, Meisheng Jiang, Desmond J. Smith, Simon R. Cherry, Joseph C. Loftus, Robert S. Ross

**Abstract**—Integrins link the extracellular matrix to the cellular cytoskeleton and serve important roles in cell growth, differentiation, migration, and survival. Ablation of  $\beta 1$  integrin in all murine tissues results in peri-implantation embryonic lethality. To investigate the role of  $\beta 1$  integrin in the myocardium, we used Cre-LoxP technology to inactivate the  $\beta 1$  integrin gene exclusively in ventricular cardiac myocytes. Animals with homozygous ventricular myocyte  $\beta 1$  integrin gene excision were born in appropriate numbers and grew into adulthood. These animals had 18% of control levels of  $\beta 1$  integrin protein in the heart and displayed myocardial fibrosis. High-fidelity micromanometer-tipped catheterization of the intact 5-week-old  $\beta 1$  integrin knockout mice showed depressed left ventricular basal and dobutamine-stimulated contractility and relaxation (LV  $dP/dt_{max}$  and LV  $dP/dt_{min}$ ) as compared with control groups ( $n=8$  to 10 of each,  $P<0.01$ ). Hemodynamic loading imposed by 7 days of transverse aortic constriction showed that the  $\beta 1$  integrin knockout mice were intolerant of this stress as they had 53% survival versus 88% in controls ( $n=15$  each). By 6 months of age, mice with depressed ventricular expression of  $\beta 1$  integrin developed a dilated cardiomyopathy that was not evident in any control animals and had patchy decrease in glucose metabolism as determined by positron emission tomography. Myocyte membrane integrity as determined via Evan's blue dye staining was disrupted in the  $\beta 1$  integrin knockout mice. This model provides strong evidence for the importance of  $\beta 1$  integrin in cardiac form and function and indicates that integrins can be linked to development of cardiomyopathies.

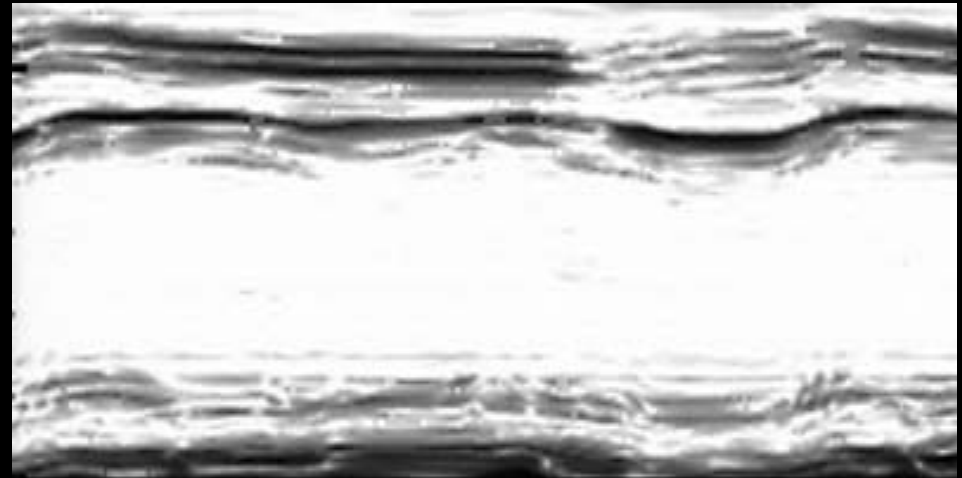
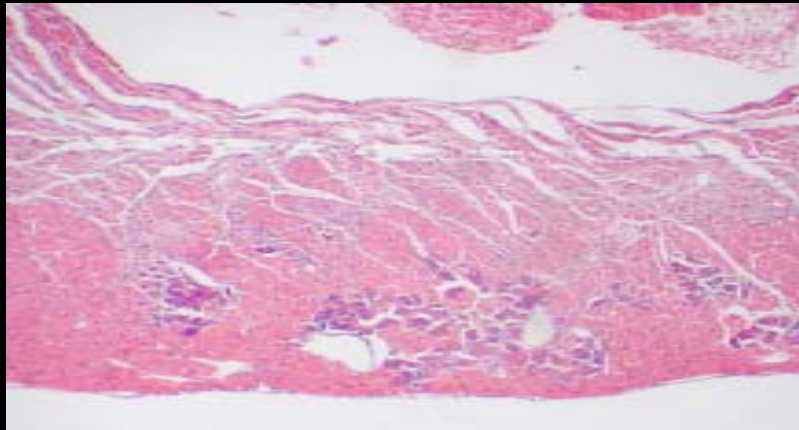
# Use of Cre-Lox to Knockout the $\beta 1$ Integrin “Floxed” Gene only in Cardiac Myocytes



# $\beta 1$ Cre-lox KO Mice: Fibrosis and Development of a Dilated Cardiomyopathy



Wild-type



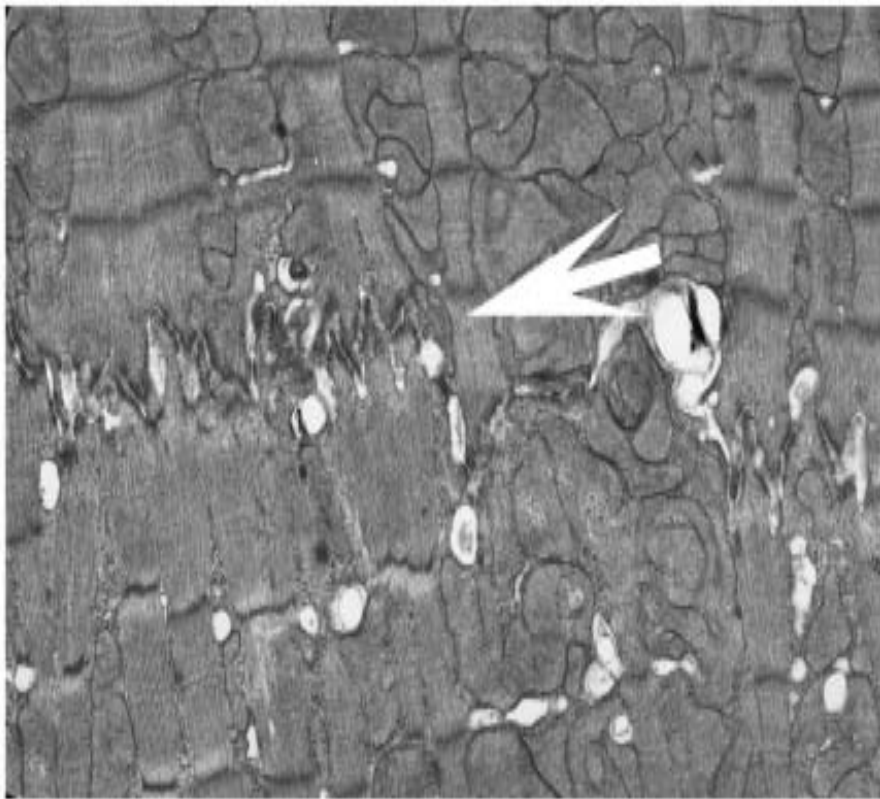
$\beta 1$  Cre-lox KO

# Cardiac Myocyte $\beta 1$ Integrin Gene Excision leads to Abnormal Intercalated Disks

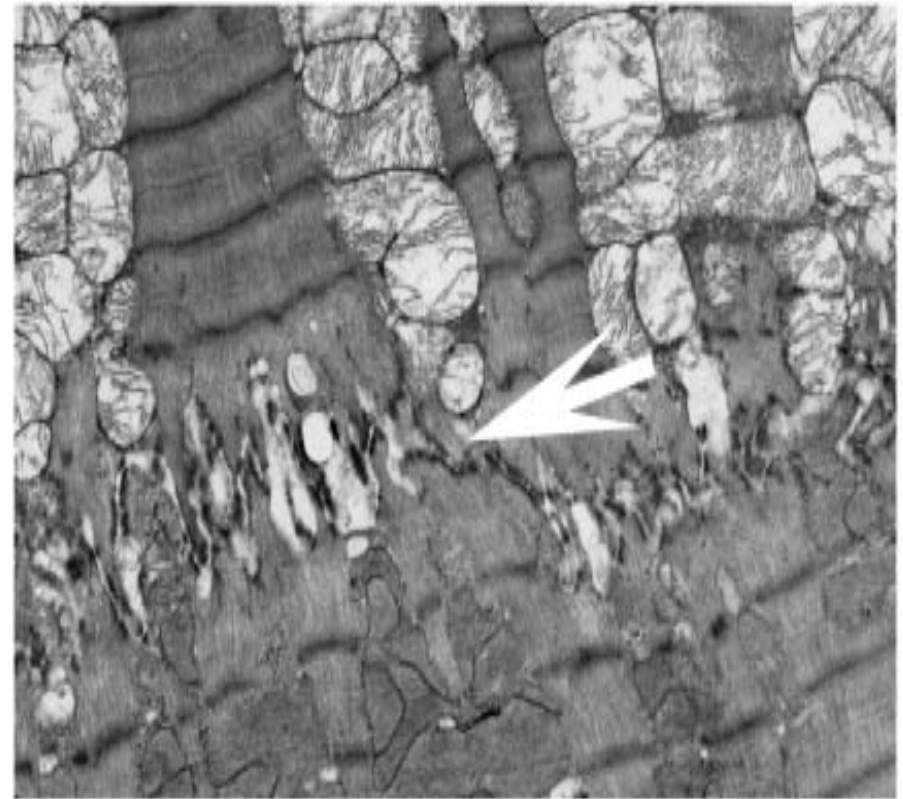
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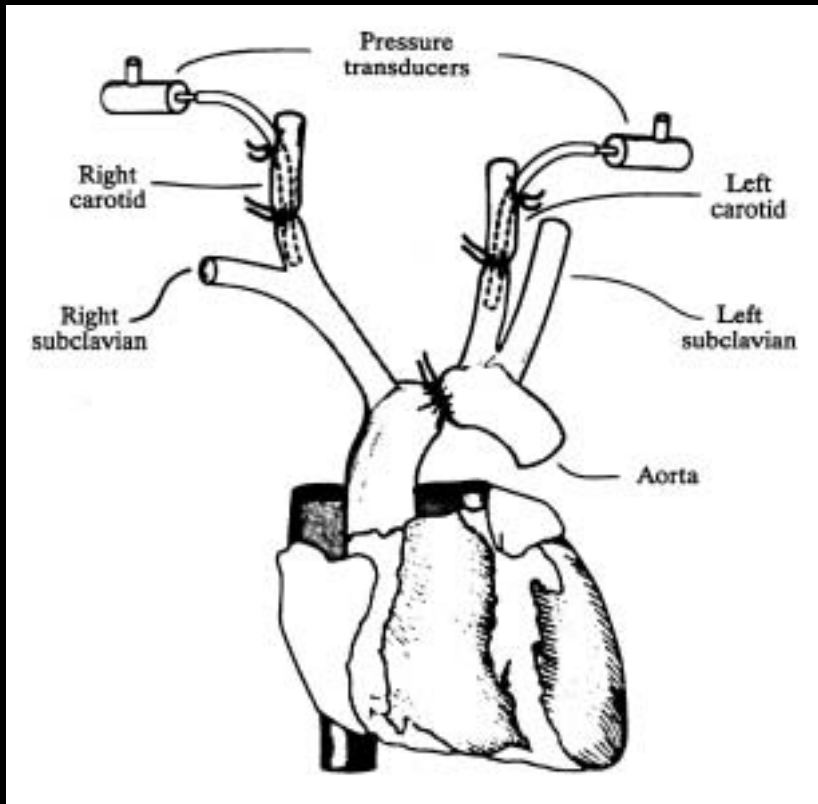
$\beta 1$  Lox - No Cre



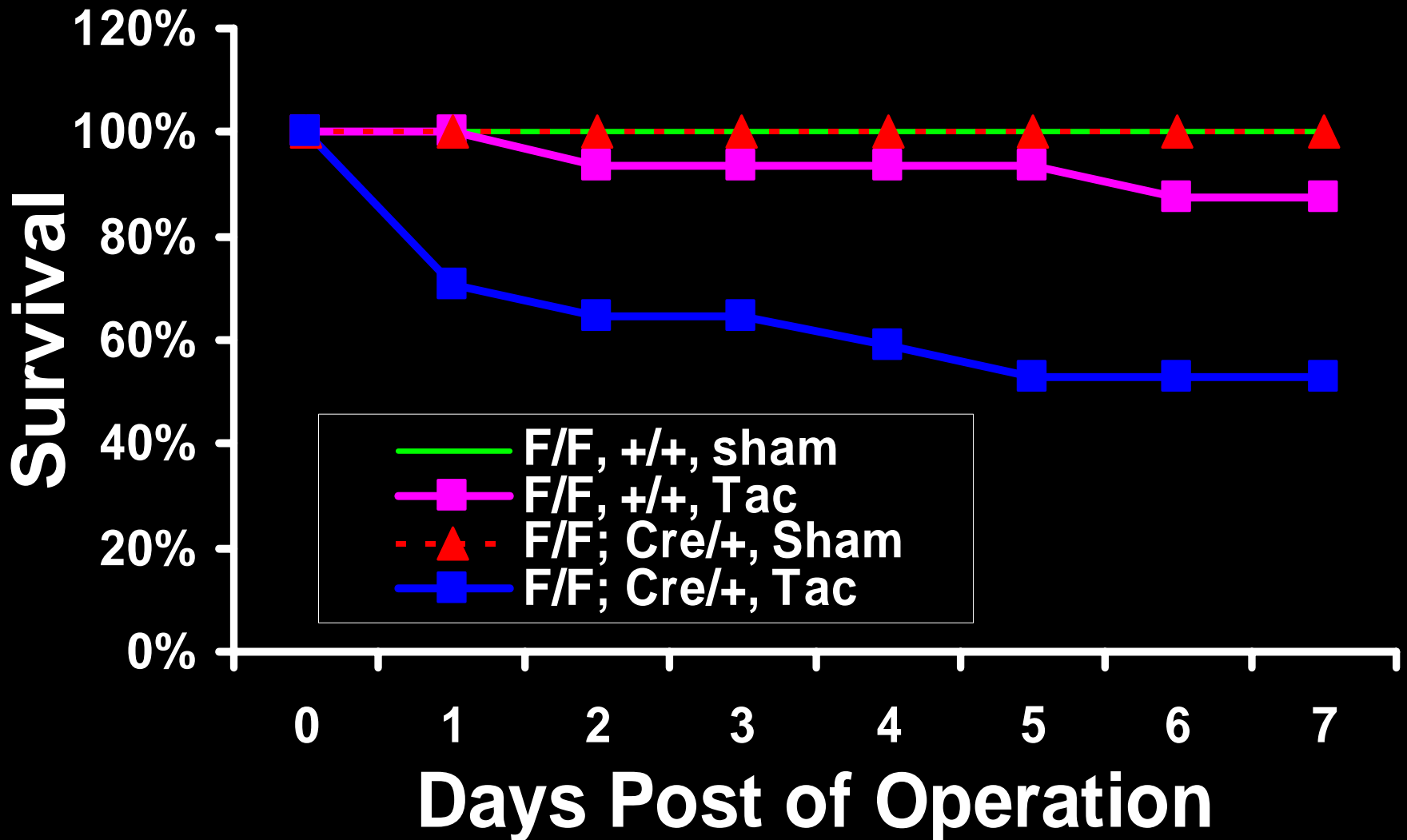
$\beta 1$  Lox / Myocyte Cre

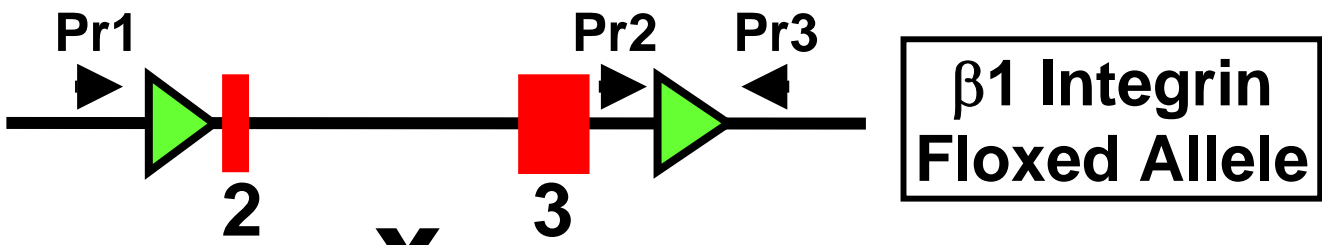
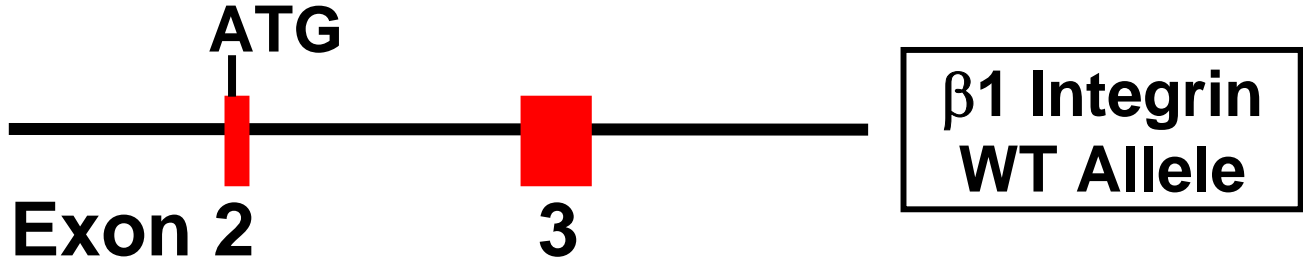


# Transverse Aortic Constriction as a model of Mechanical Stress on the Left Ventricle

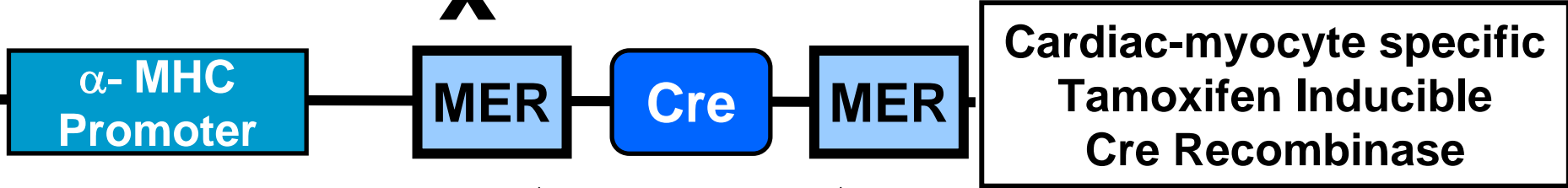


# Homozygous $\beta 1$ KO Mice are Intolerant of Mechanical Stress (Aortic Banding)

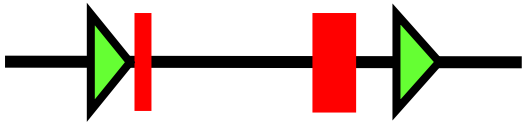
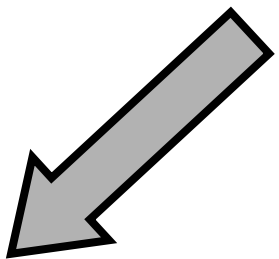




X

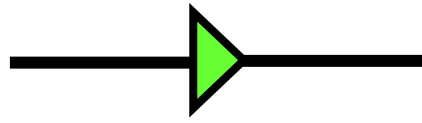
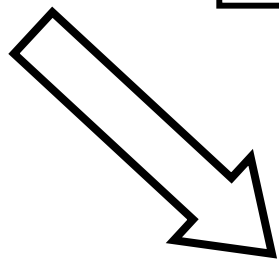


Peanut Oil Injection



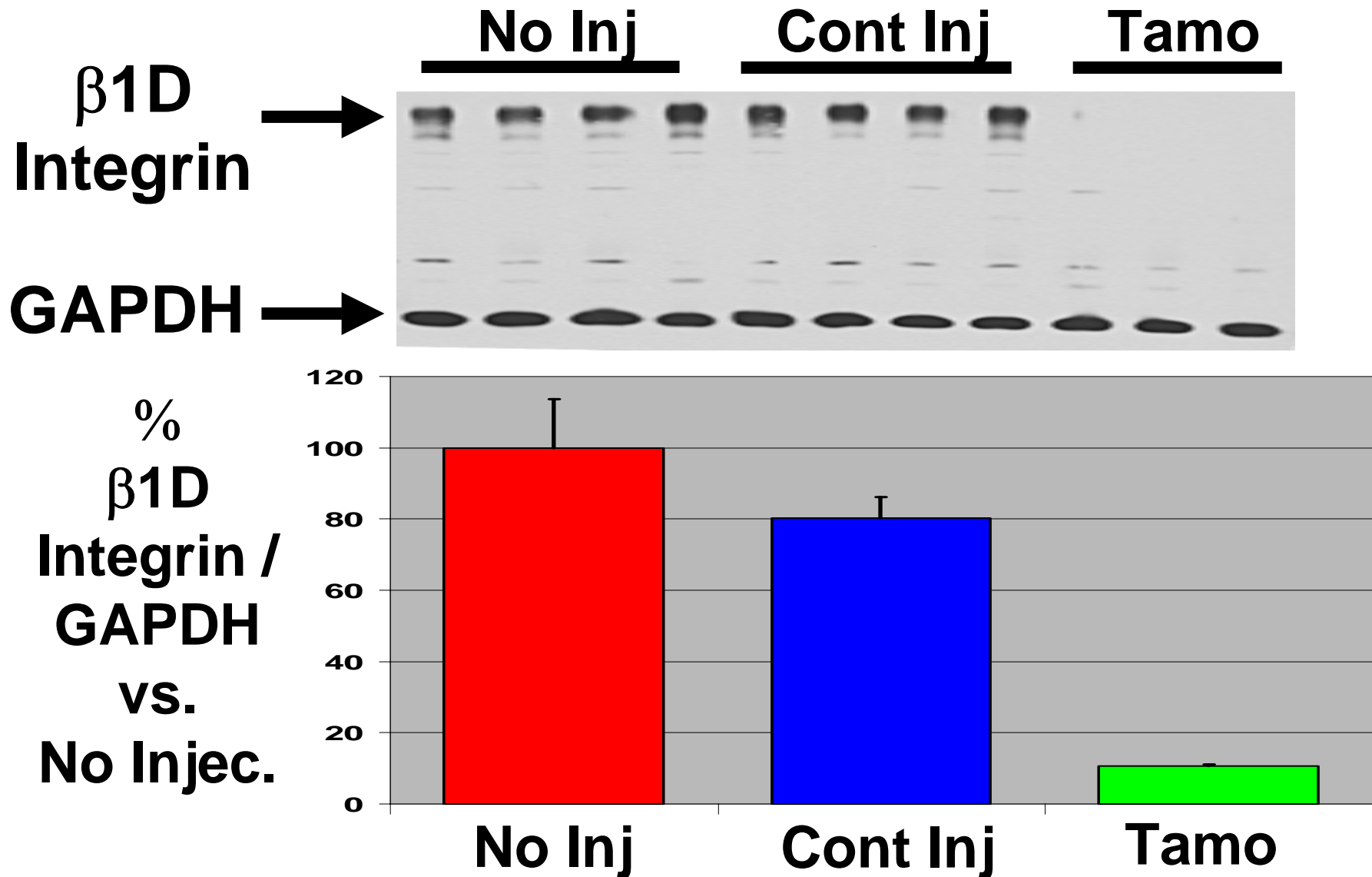
Intact Allele (Control)

Tamoxifen Injection

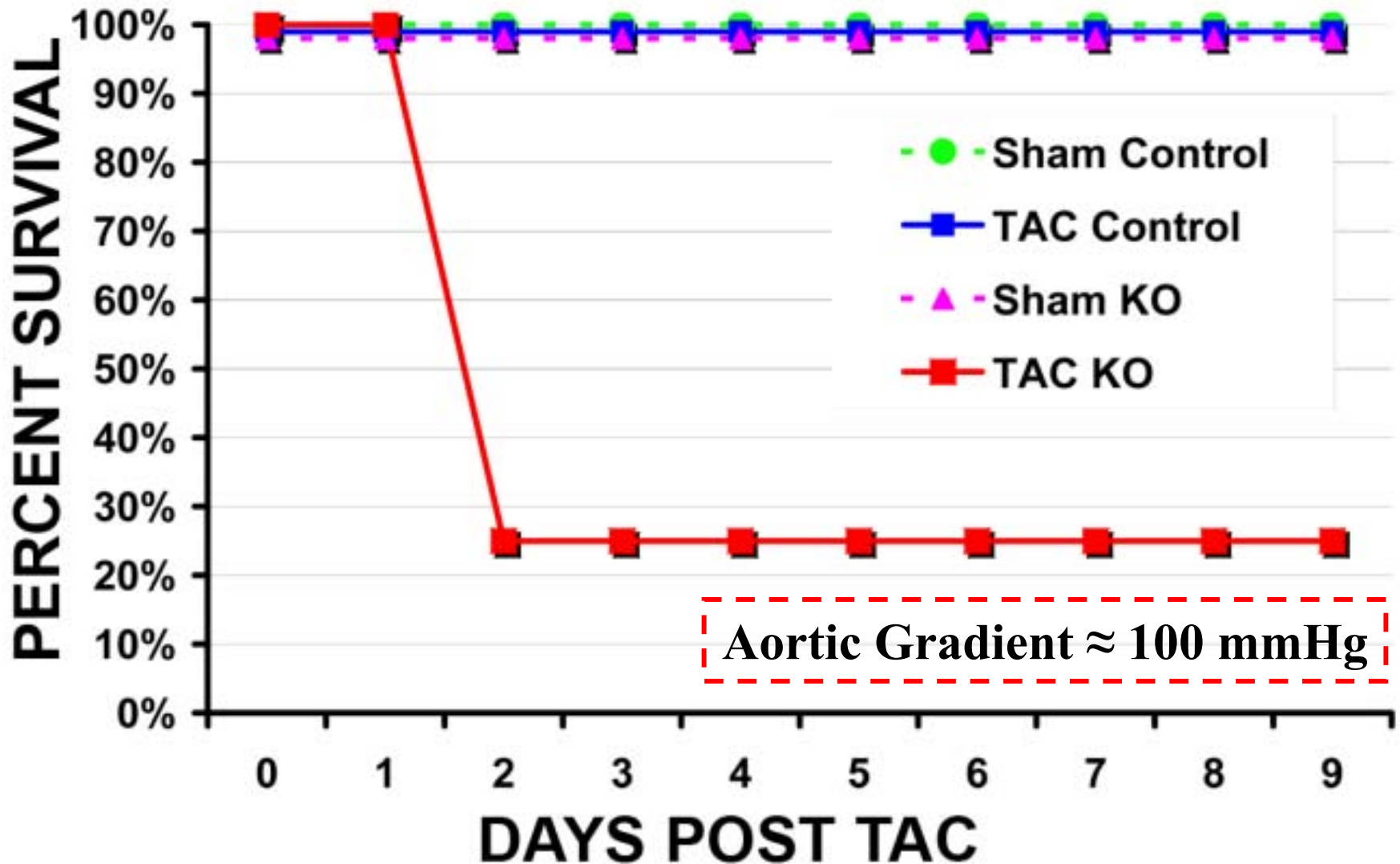


Excised Allele (KO)

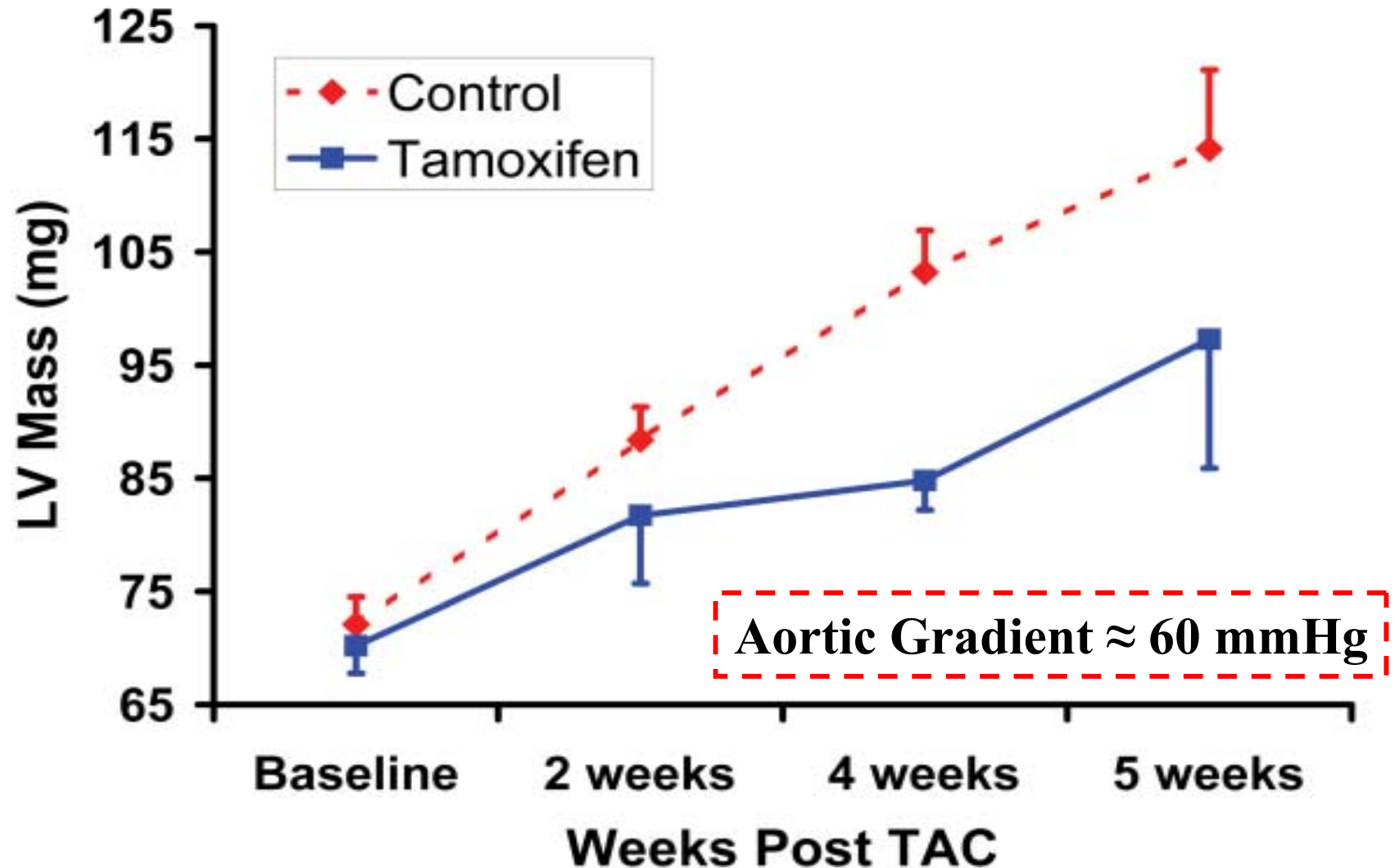
# Tamoxifen injection causes significant reduction of $\beta$ -1 Integrin Expression



# Aortic constriction (TAC) causes high mortality when $\beta 1$ integrin is reduced in the adult heart



# LV Mass Increase is Blunted following Modest Aortic Constriction (TAC) in $\beta$ -1 Integrin KO Mice

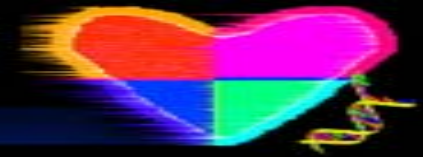


# What about signaling events in the cardiac-myocyte integrin KO mice?

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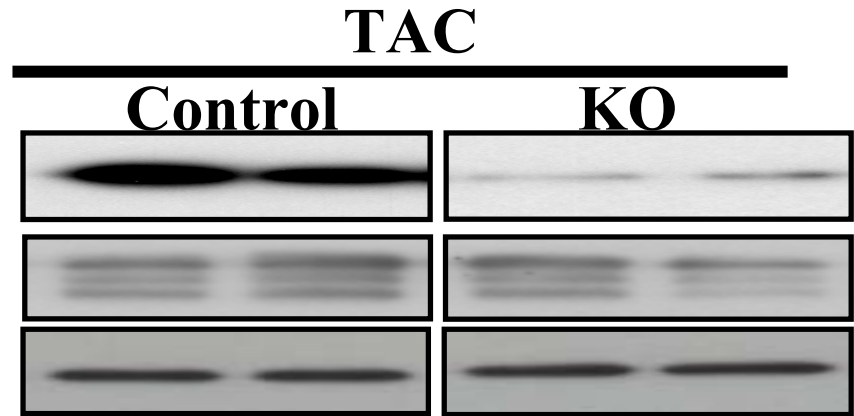
1. Would signaling be abnormal in the resting heart or in the heart following mechanical loading induced by aortic constriction?
2. Would signaling from non-integrin receptors be disturbed?  
( $\beta$  adrenergic receptors)



# Hypertrophic and Survival Signaling is abnormal in $\beta 1$ KO Hearts after Aortic Banding

**p38**

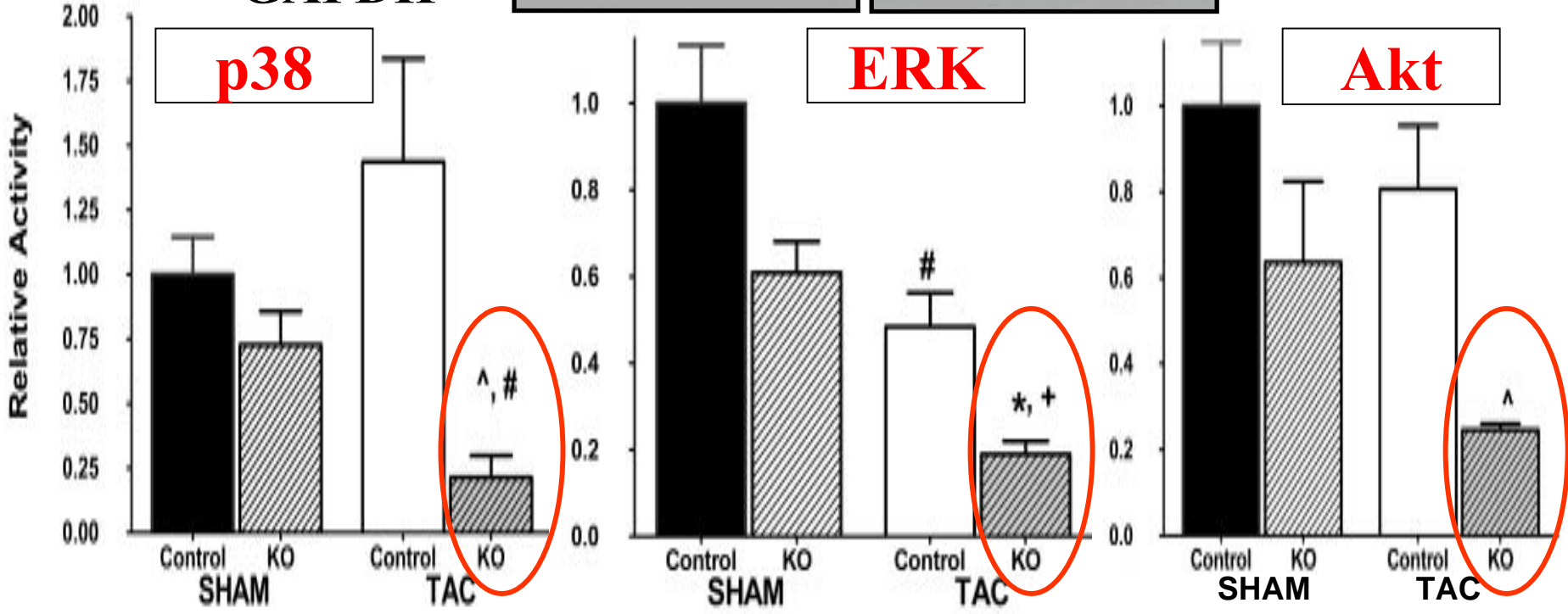
P-ATF2  
T-ATF2  
GAPDH

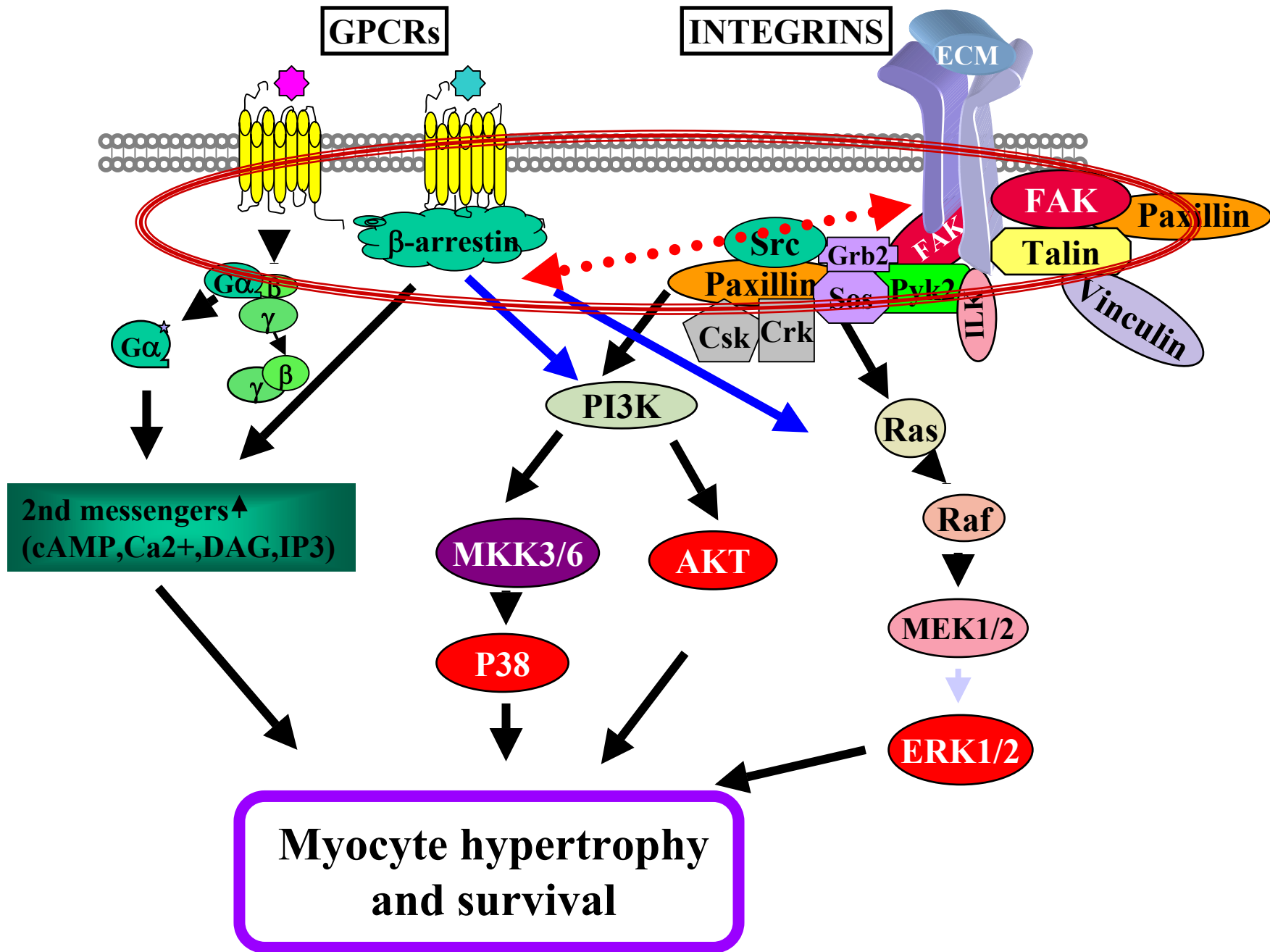


**p38**

**ERK**

**Akt**





# Adrenergic Signaling is abnormal in $\beta 1$ Knockout Myocytes

**Akt**

**Control**

**KO**

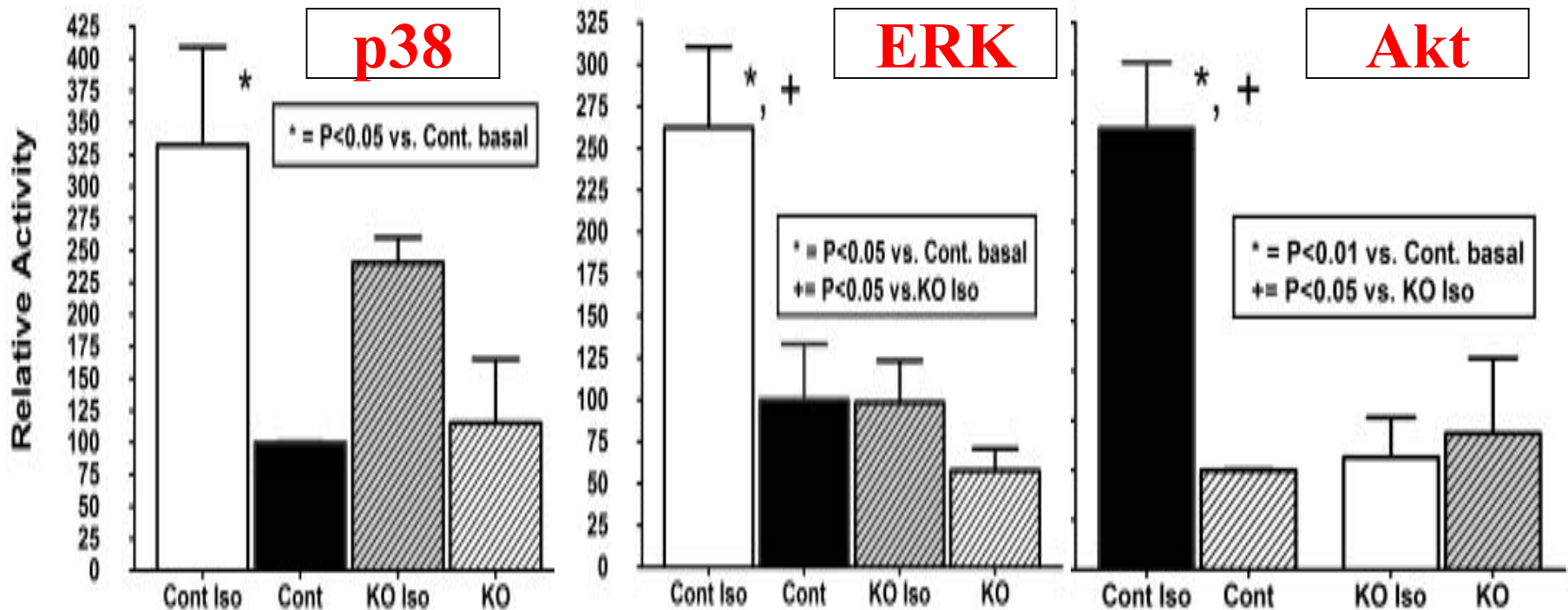
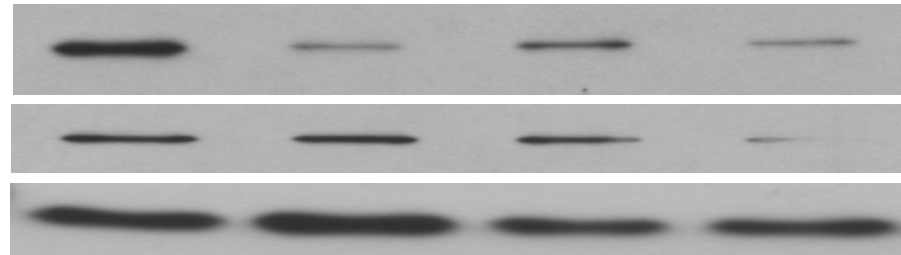
*ISO*

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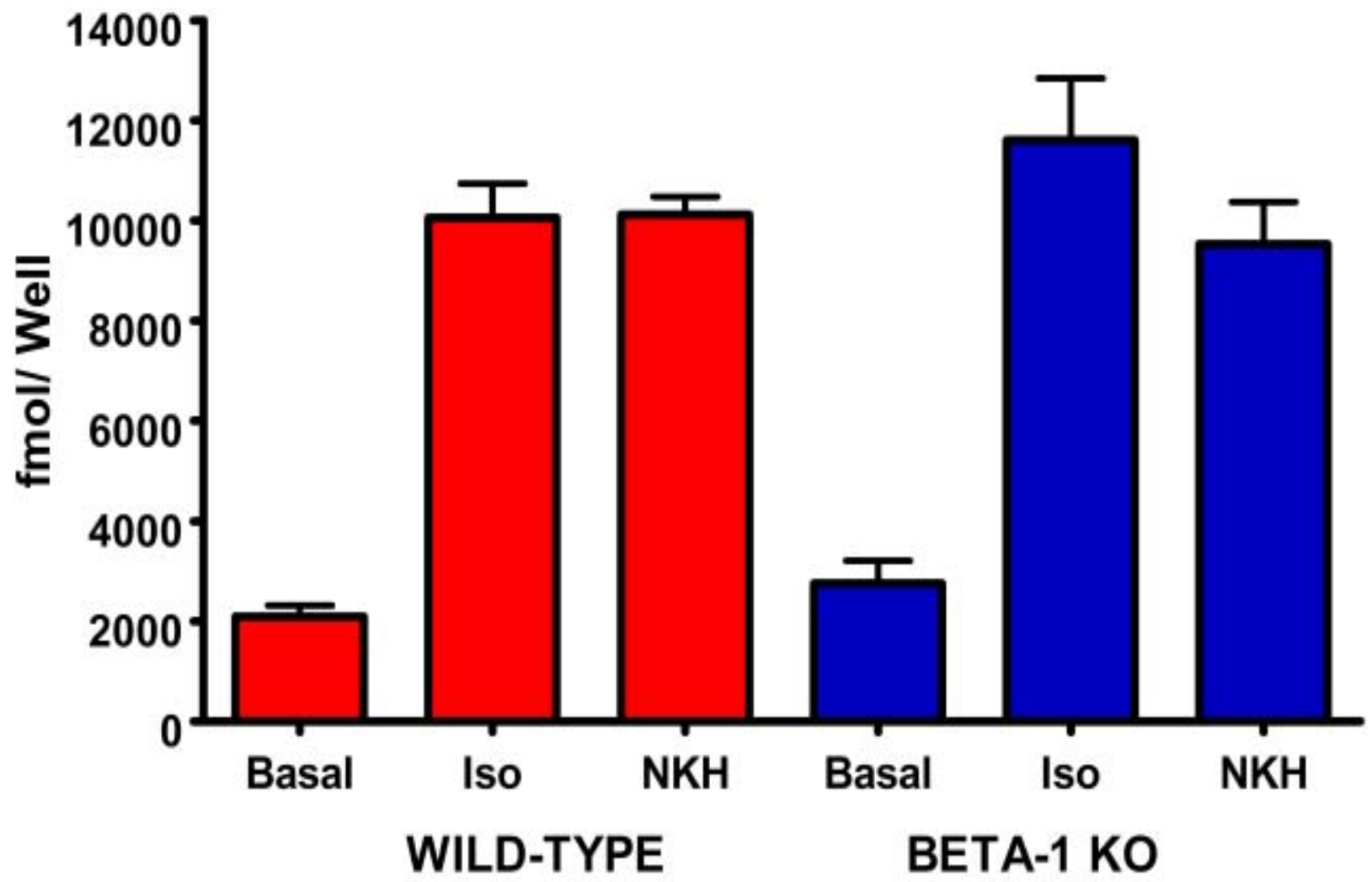
**Phospho-GSK3 $\beta$**

**Total GSK3 $\beta$**

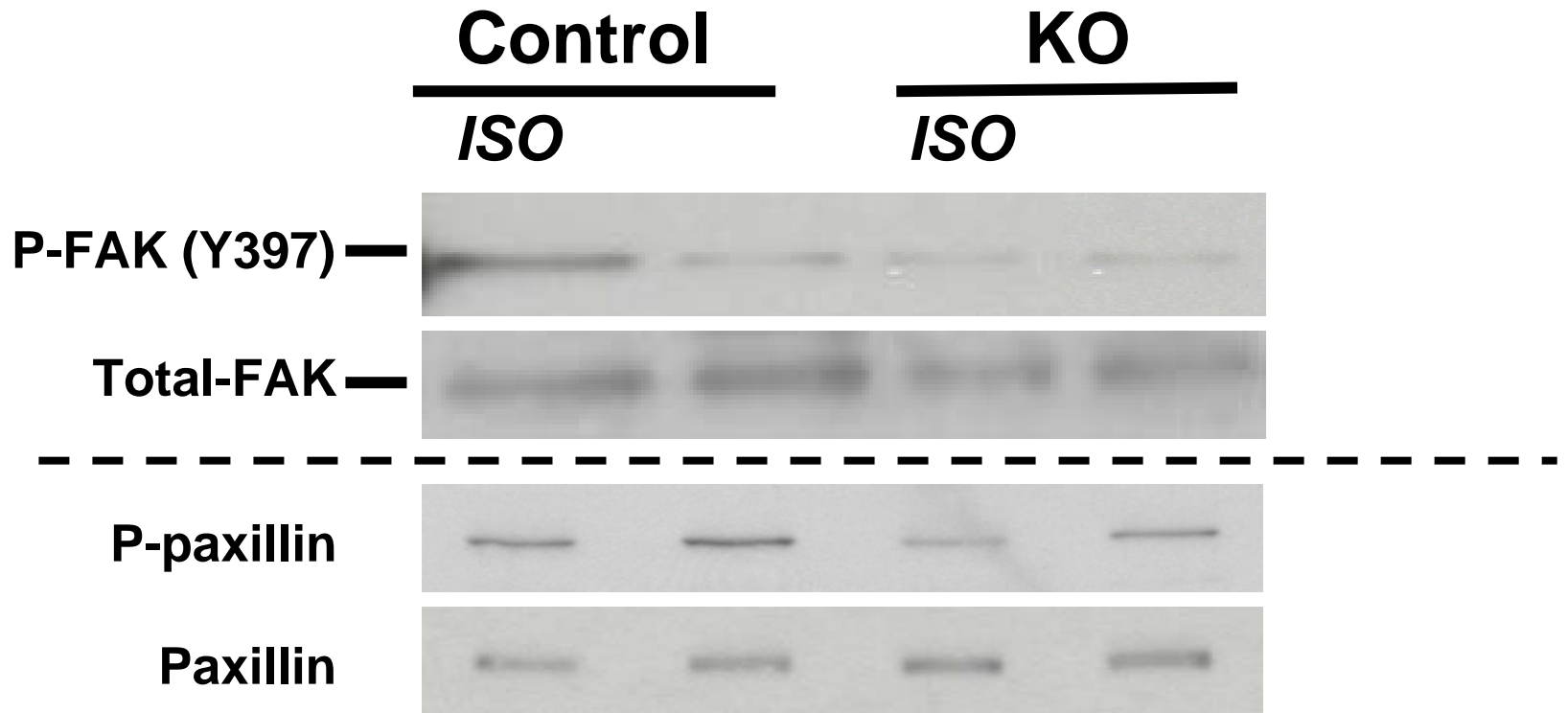
**GAPDH**



# cAMP Activity is unchanged in $\beta$ -1 Integrin Cardiac Myocytes



# FAK and Paxillin Activity in $\beta$ -1 Integrin Knockout Cardiac Myocytes

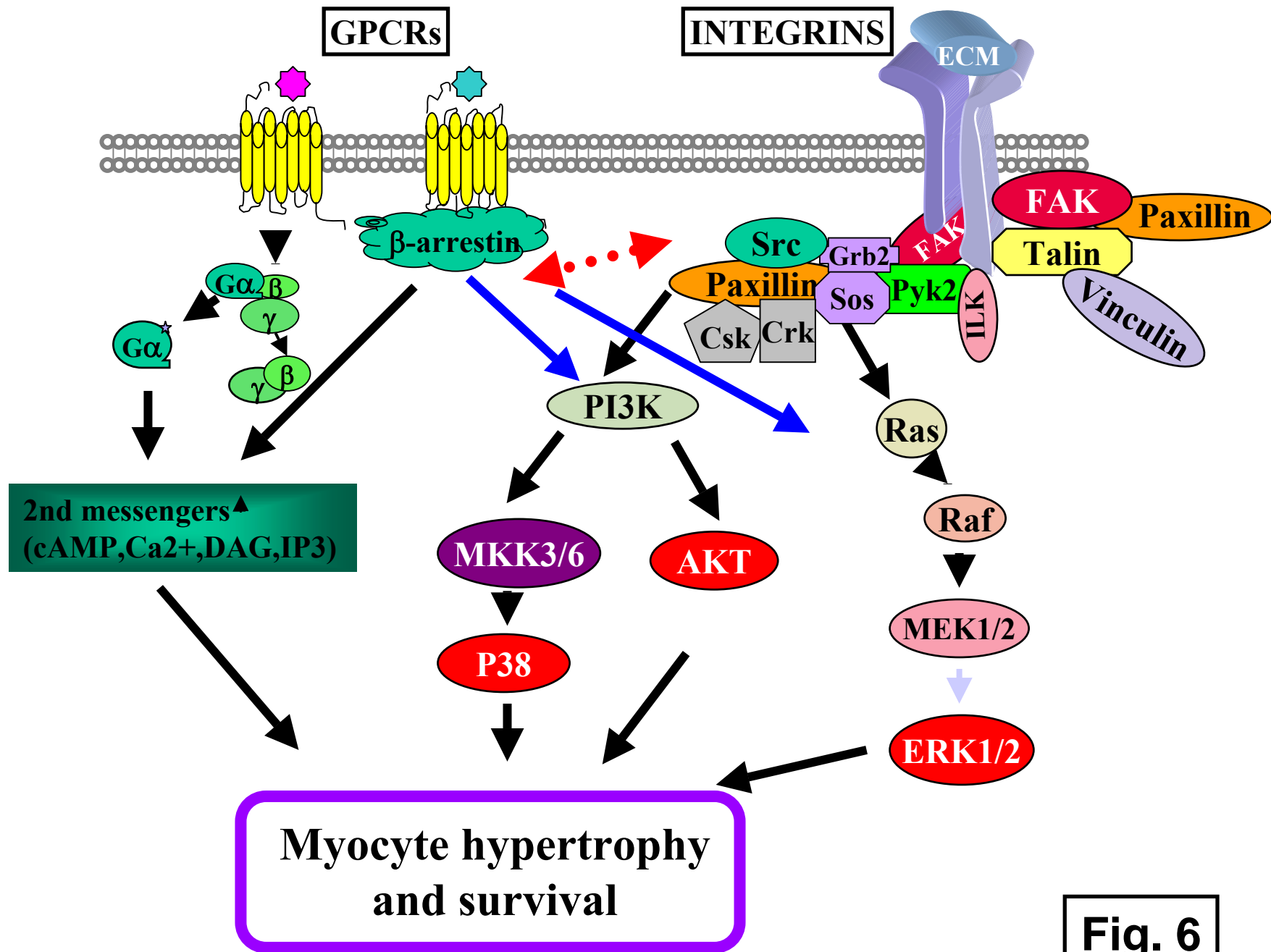


# **Summary: $\beta$ 1 Integrin Cardiac-specific KO**

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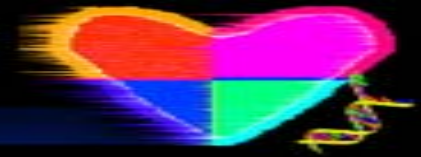
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- **Defective mechanotransduction**
- **Blunted hypertrophic response**
- **Altered hypertrophic and survival signaling after aortic constriction**
- **Abnormal Isoproterenol activation of p38, ERK1/2, Akt and FAK in KO myocytes.**
- **cAMP generation was normal.**



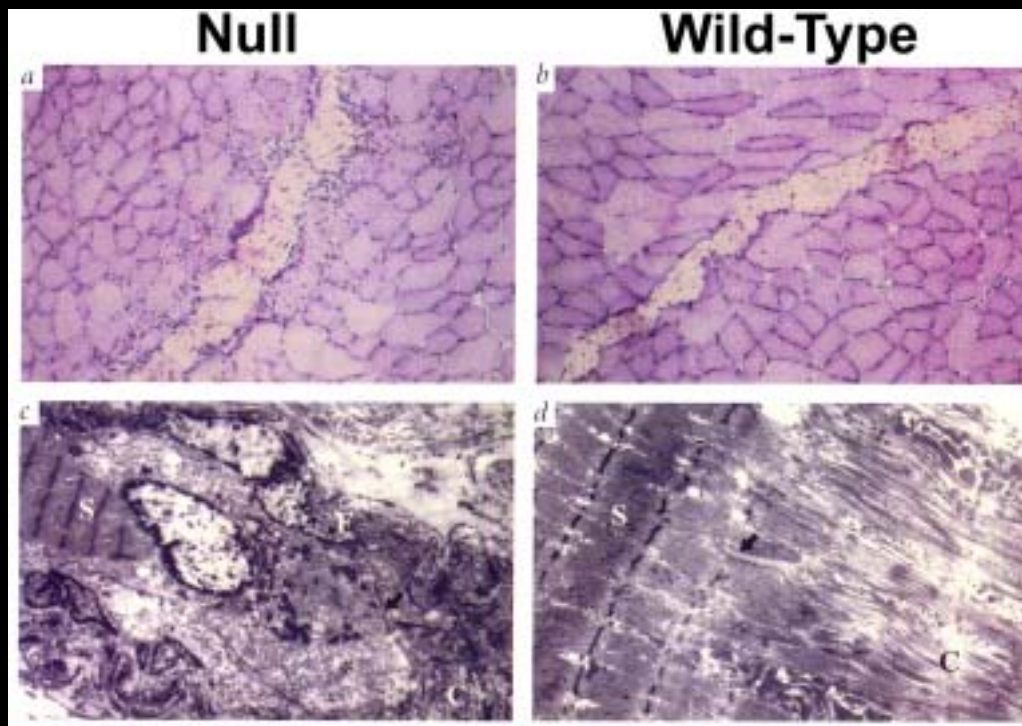
**Fig. 6**

**Are there cardiac diseases  
caused by abnormalities in  
Integrins or related  
proteins??**

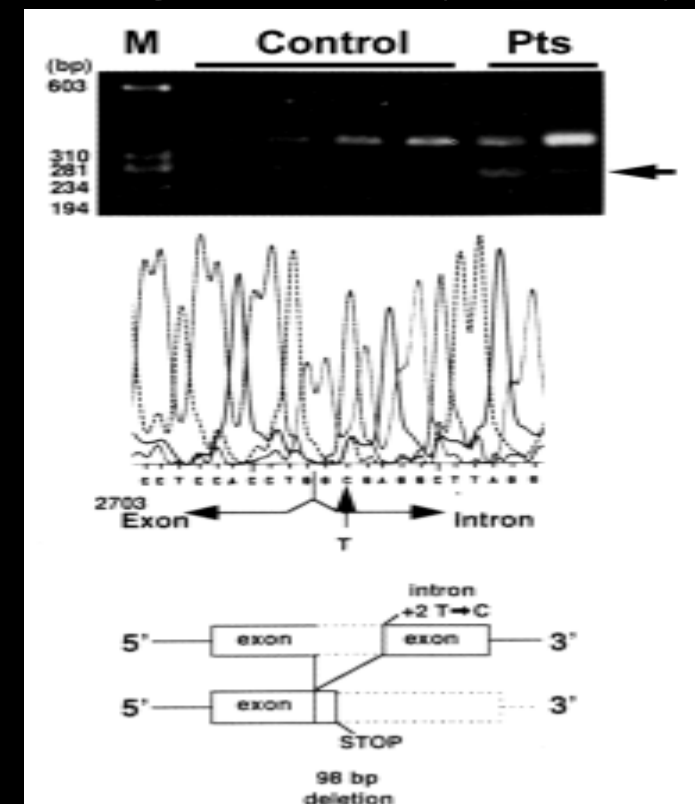


# $\alpha 7$ Integrin Mutations / Deficiency Cause Muscular Dystrophy in Mouse and Man

## $\alpha 7$ KO Mouse

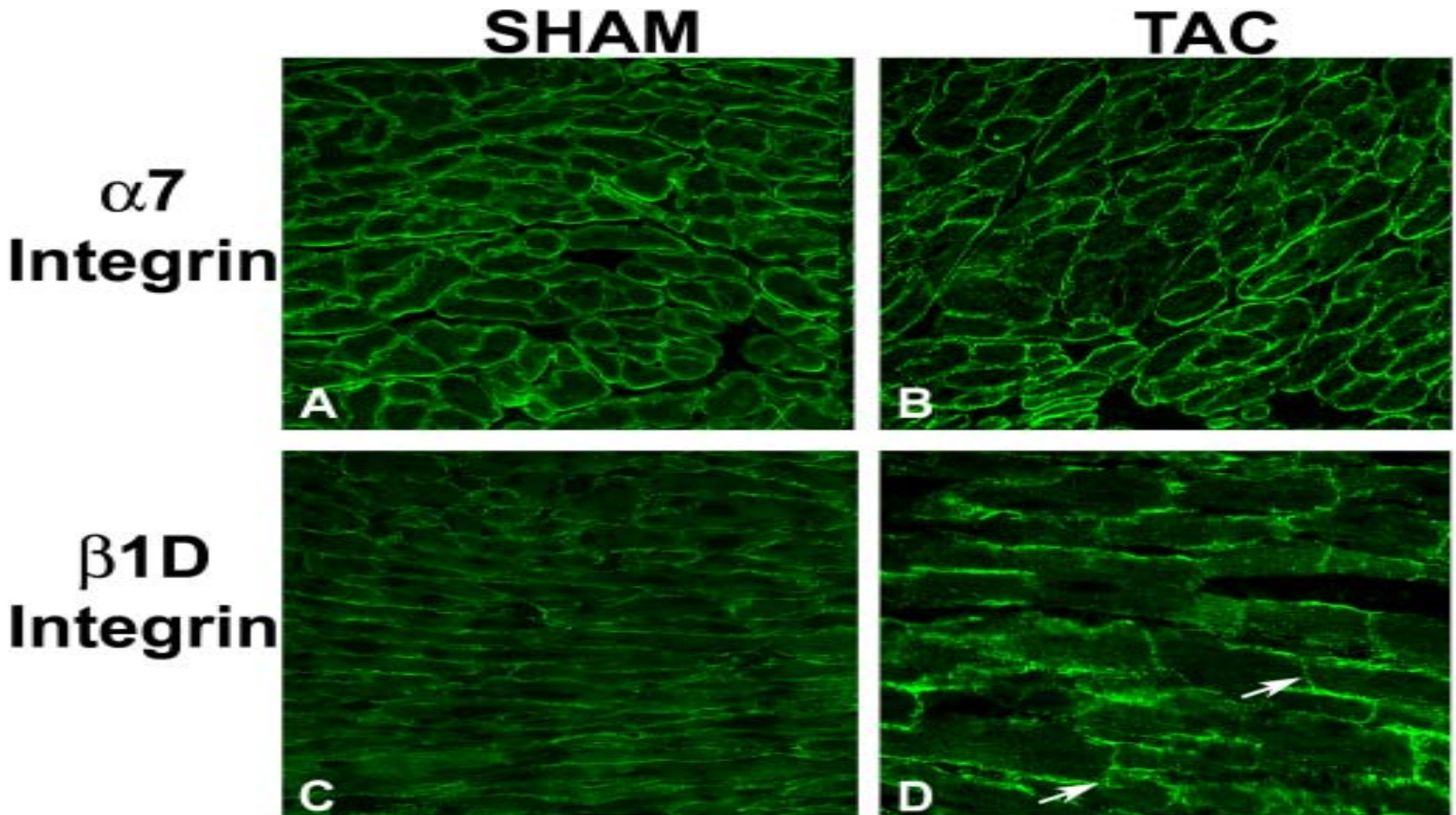


## $\alpha 7$ Mutation in congenital myopathy



Mayer, et. al., Nat. Gen. 17:318, 1997  
Hayashi, et. al., Nat. Gen. 19:94, 1998

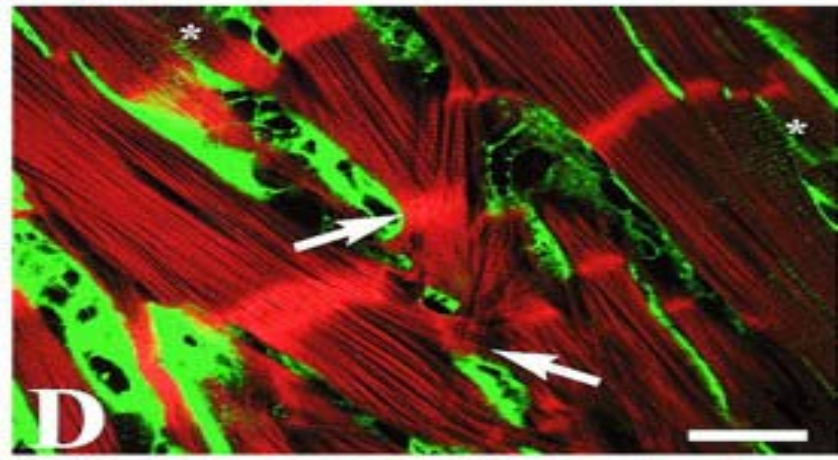
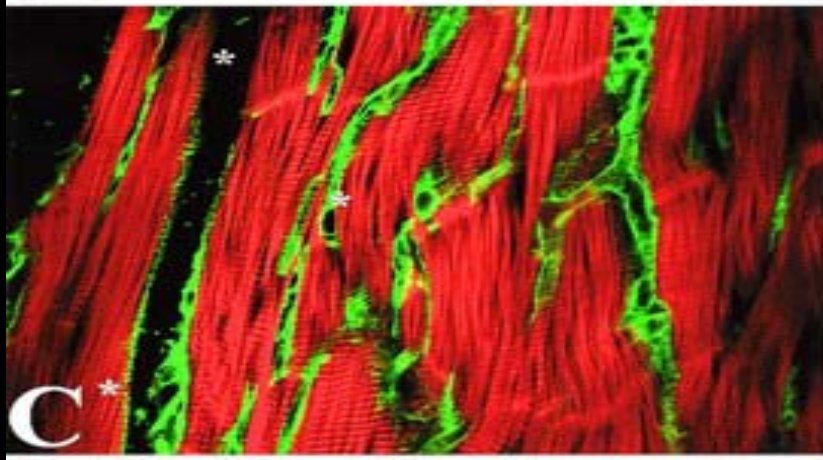
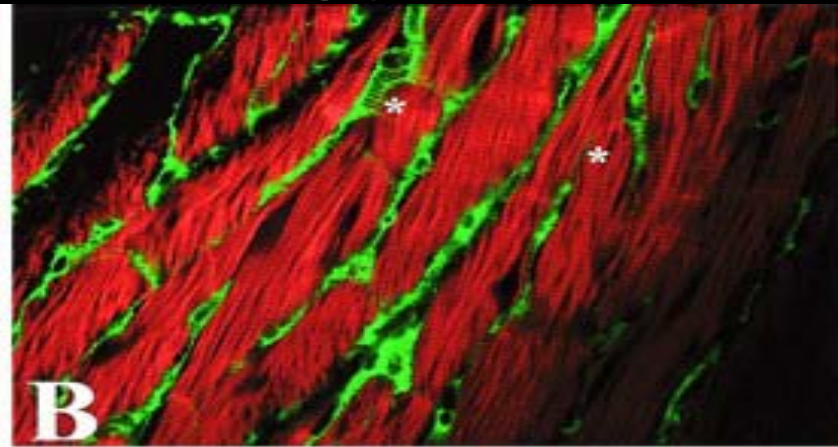
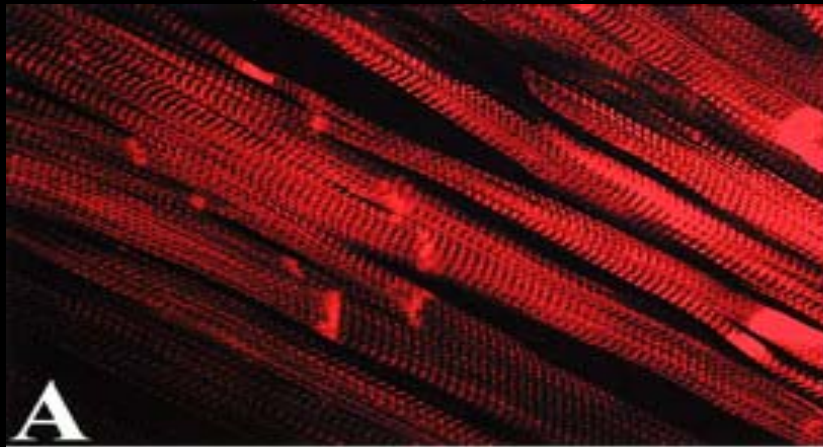
# $\alpha 7$ and $\beta 1$ Integrins are increased in Hypertrophied Left Ventricle of the Mouse



# $\beta 1$ Integrin Shedding in the Transition to Heart Failure: Reducing cell adhesion?

Pre-immune

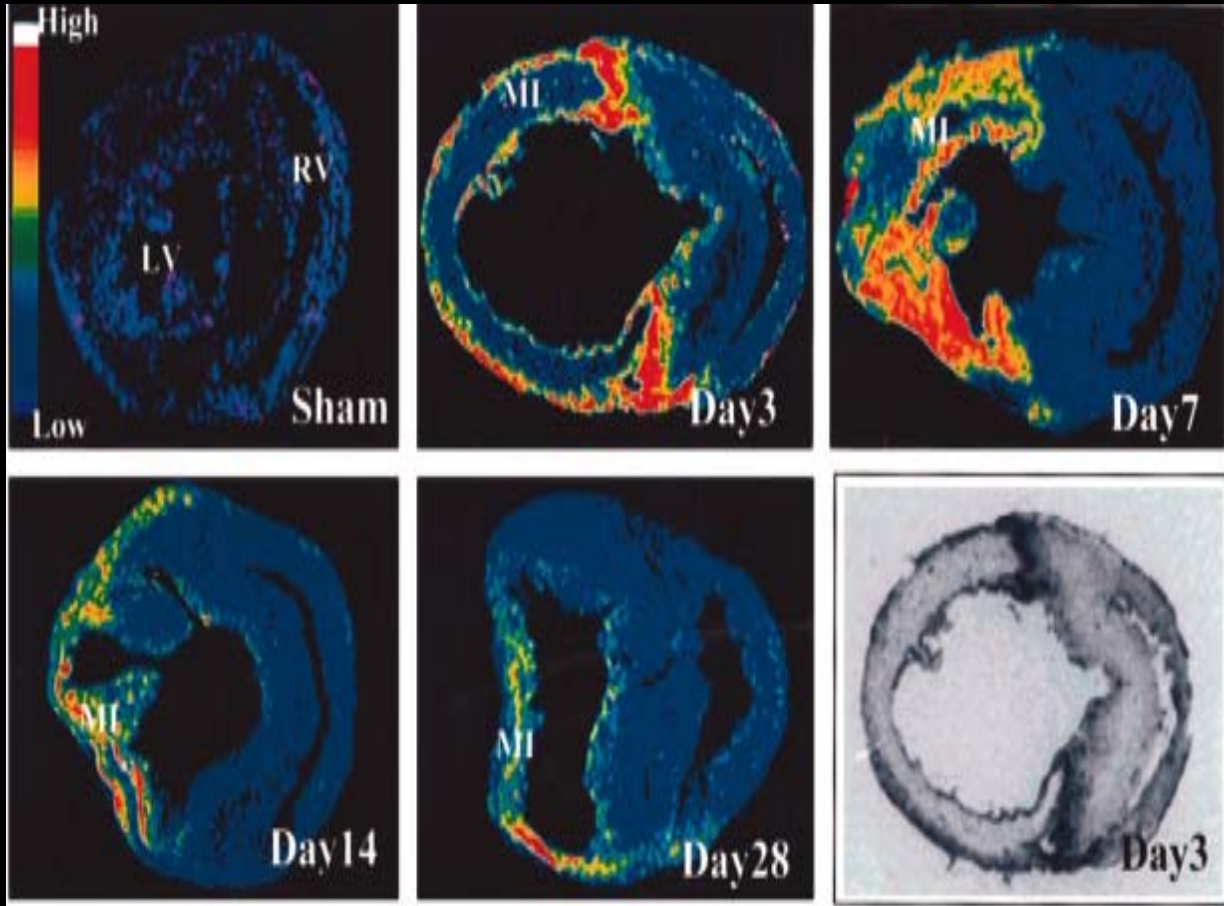
Control



4-Week AS

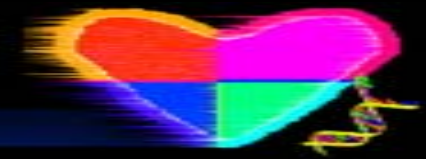
7-Week AS

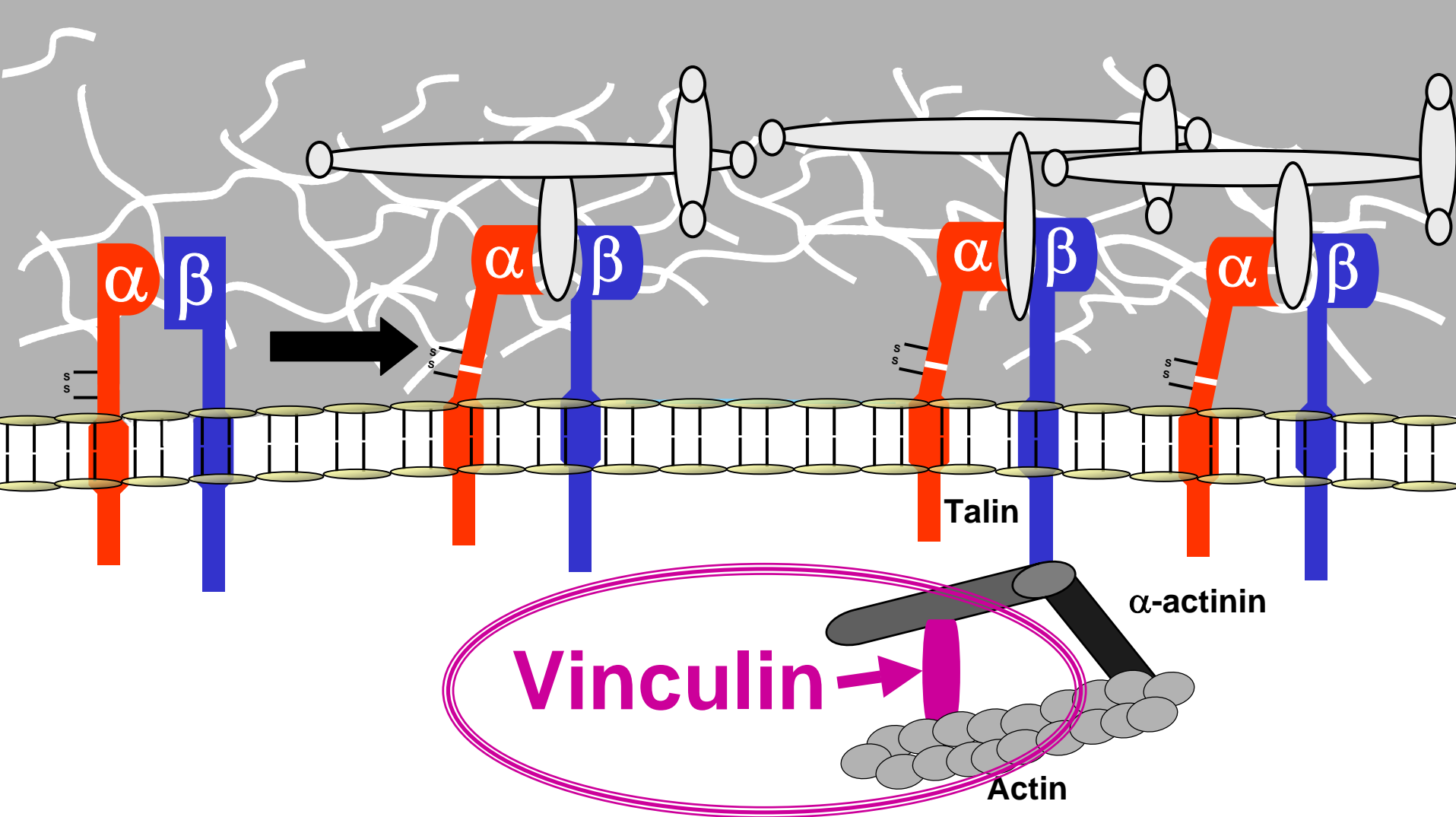
# Integrins and Myocardial Infarction



$\beta 1$  integrin transcript was increased in the peri-infarct zone by day 3 post-MI and decreases by day 14-28.

Sun M, Opavsky MA, Stewart DJ, Rabinovitch M, Dawood F, Wen WH, Liu PP. 25;:1046-52 Circulation, 2003

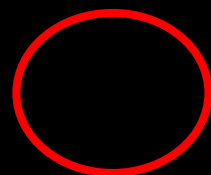




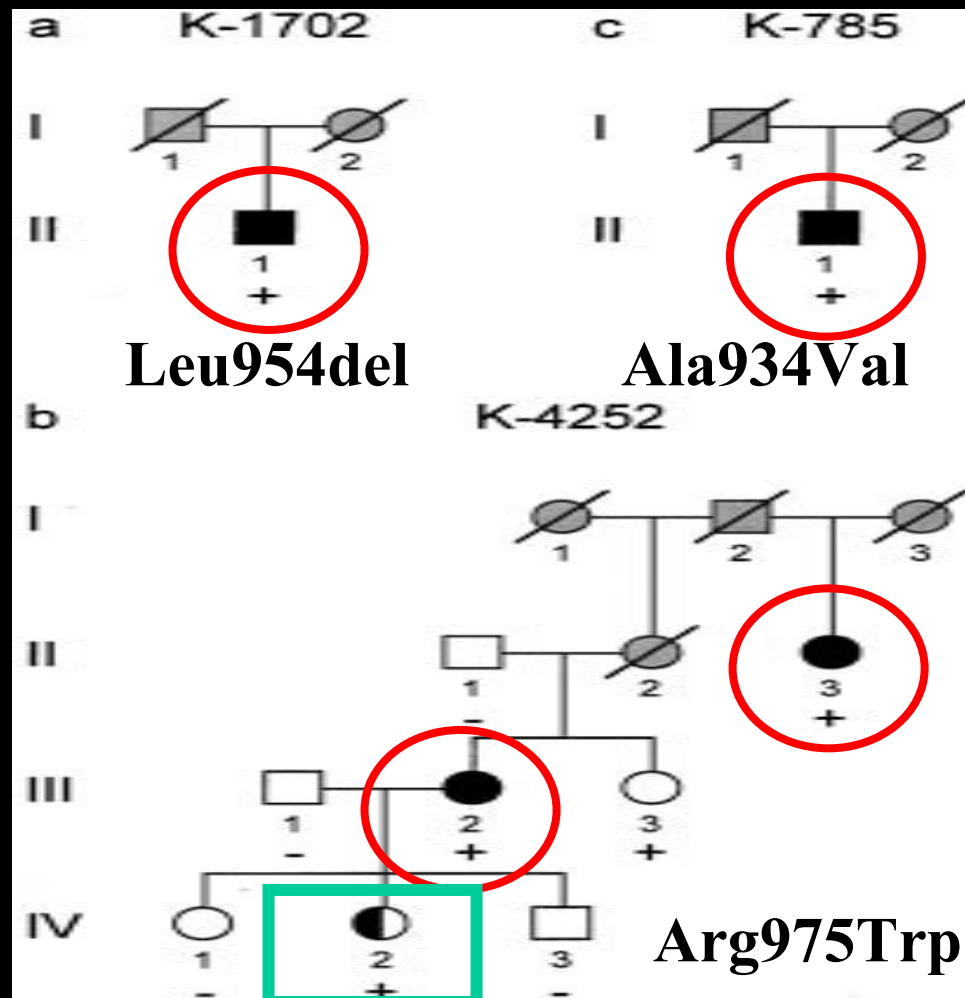
Ross and Borg  
Circ Res. 88:1112, 2001

# Metavinculin a Vinculin splice-variant:

## 3 heterozygous genotypes cases IDCM

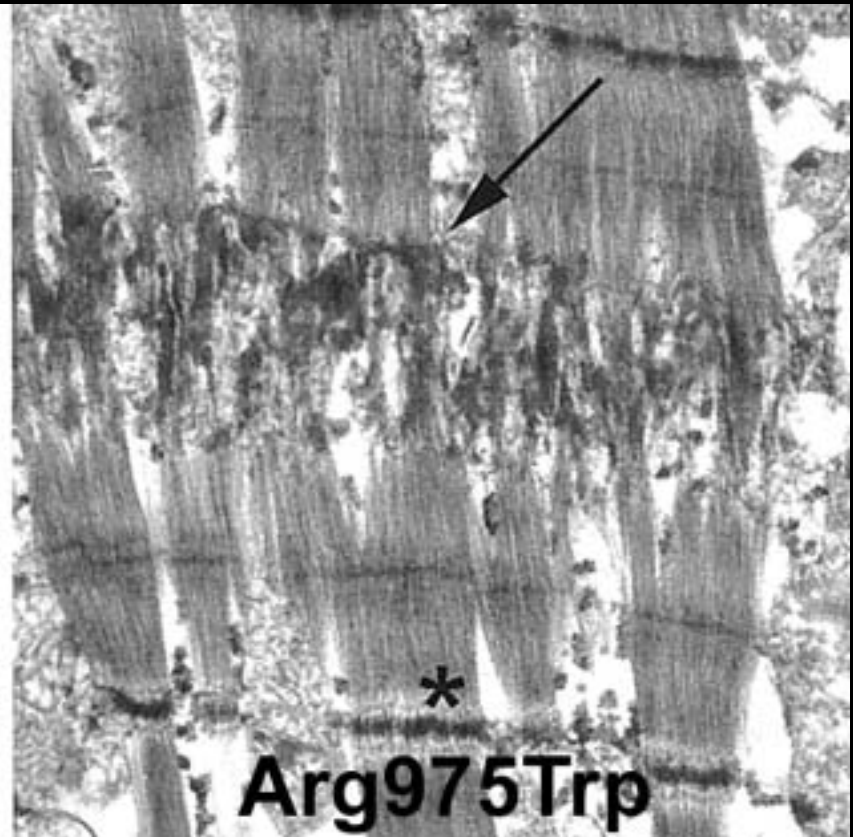
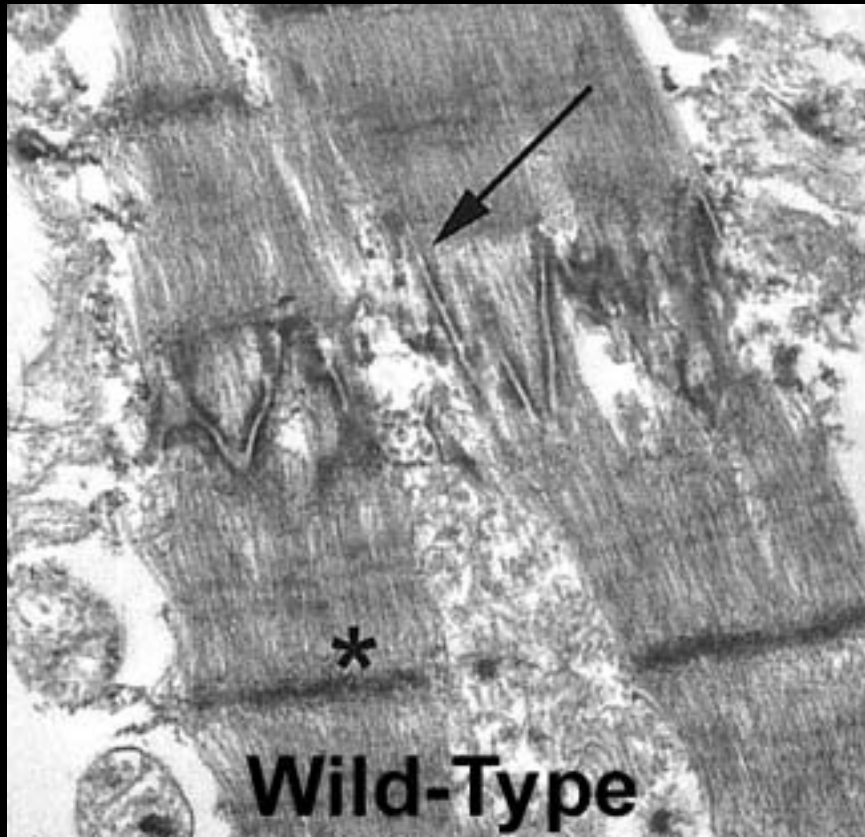
 = IDCM

 = Dilated Ventricle



Olson, et.al.  
Circ. 105:431, 2002

# Endomyocardial Biopsy from a Metavinculin Mutant Heart: Abnormal Intercalated Disks



Olson, et.al.  
Circ. 105:431, 2002

# **Integrins and the Pathological Myocardium**

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- **No current integrin myocardial disease in man**
- **Skeletal muscle myopathies linked to integrin mutations**
- **Integrin “shedding” from the myocyte surface may play a role in the transition from compensated function to heart failure.**

# Integrins and the Pathological Myocardium - II

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- Myocyte integrin expression may increase to allow for “firmer” adhesion of the stressed myocyte following pressure loading or MI
- **Meta-Vinculin** which indirectly binds integrins, has been identified in patients with DCM

**Ying Wu**

**Maria Manso**

**Alice Zemljic-Harpf**

**Laila Elsherif**

**Seok-Min Kang (Yonsei)**

**Steve Padilla**

**Michael S. Huang**

**Uyen Nguyen**

**Joel Miller**

**Afrina Qutubuddin**

**Shaw-Yung Shai (Tulane)**

**Univ. Cincinnati**

**Jeff Molkentin**

**UCLA**

**Scott Henderson**

**Burnham Institute**

**Eileen Adamson**

**UCSD**

**Yusu Gu**

**Nancy Dalton**

**Mei Hua Gao**

**Toshitaka Yajima**

**Andrea Thor**

**Guy Perkins**

**Patricia Reid**