

# Markers of Mechanical Stress: Discovery of IL-33/ST2 in the Heart

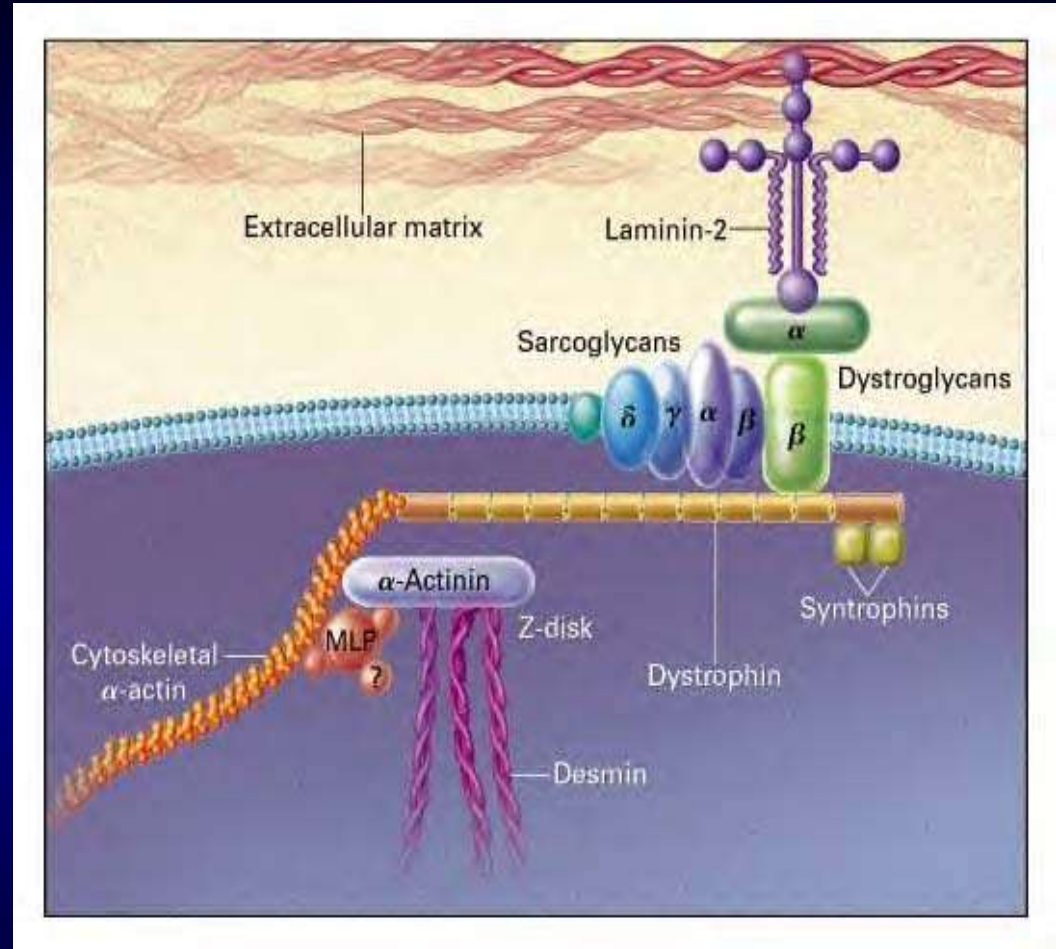
Richard T. Lee, M.D.



*The Unknown, 1912*

*John Charles Dollman (British, 1851-1934)*

Most of the molecular causes of heart failure disrupt the biomechanical balance between the cytoskeleton, the membrane, and the extracellular matrix.



*Hunter and Chien, NEJM, 1999*

# Transcriptional Profiling of Mechanically-Stimulated Cardiomyocytes

*Stimulus*



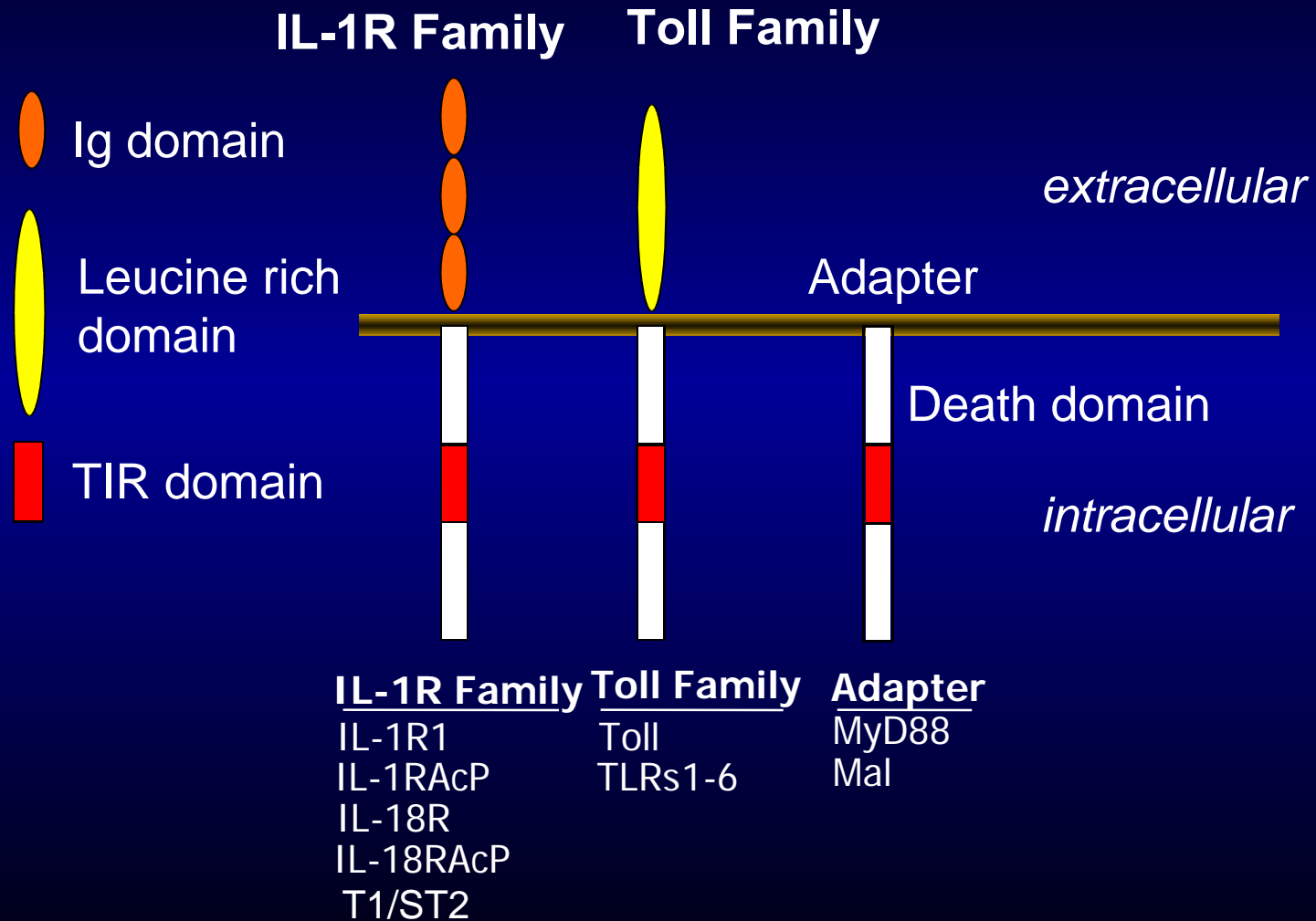
*Increased protein synthesis*  
*Decreased apoptosis*



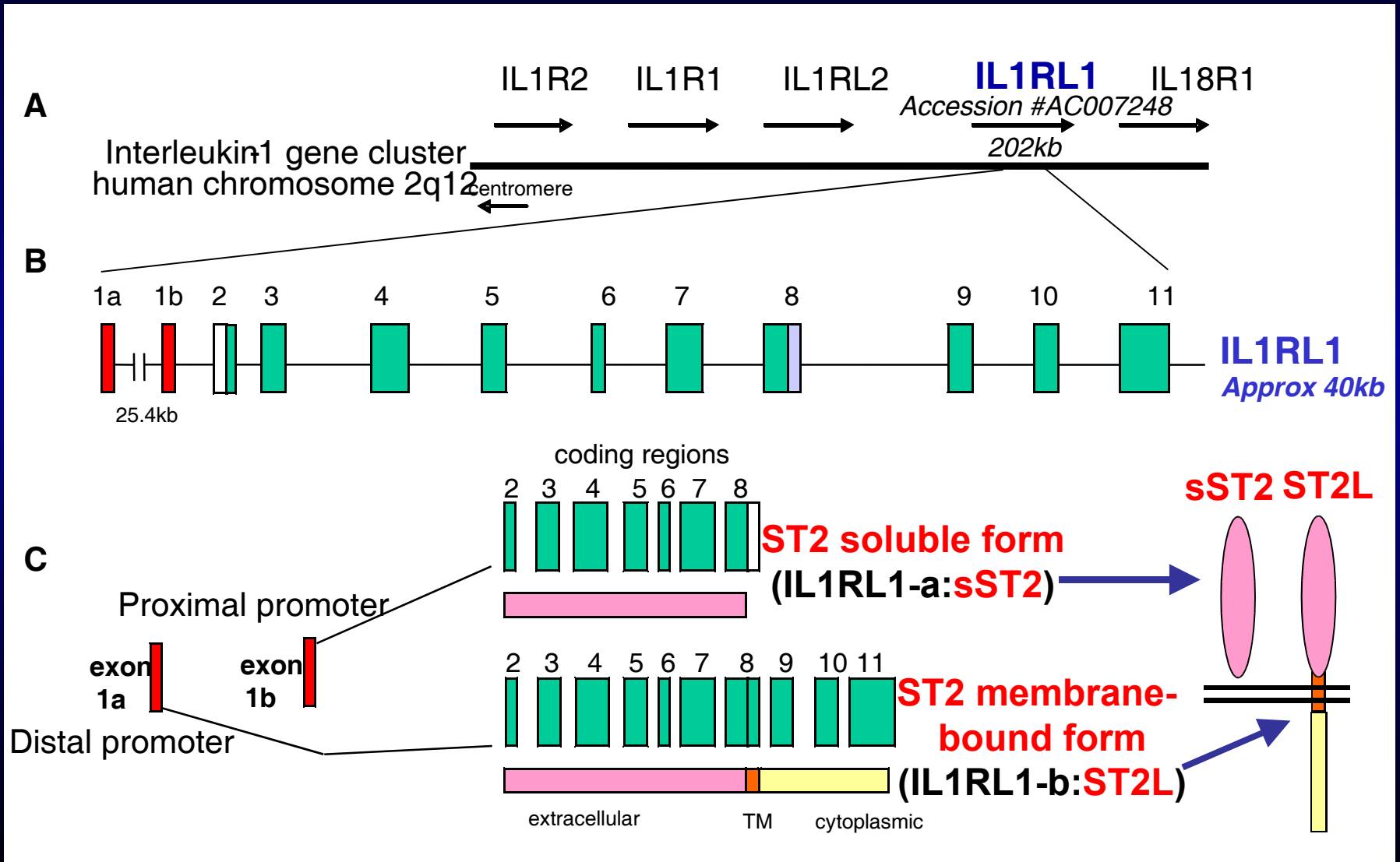
6 hr  
35/10,000 genes

48 hr  
No Differences

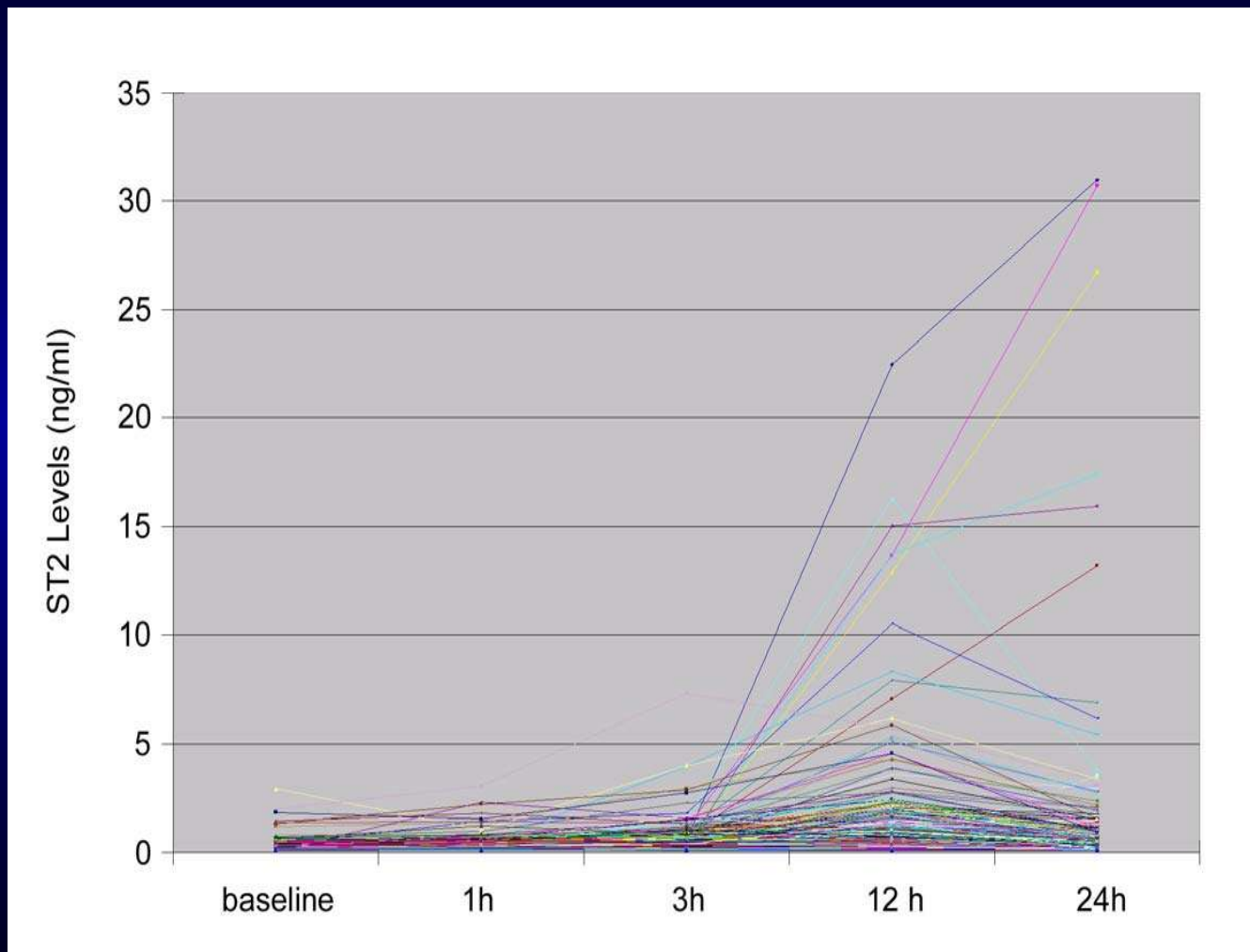
# ST2 is a Member of IL-1 Receptor Family



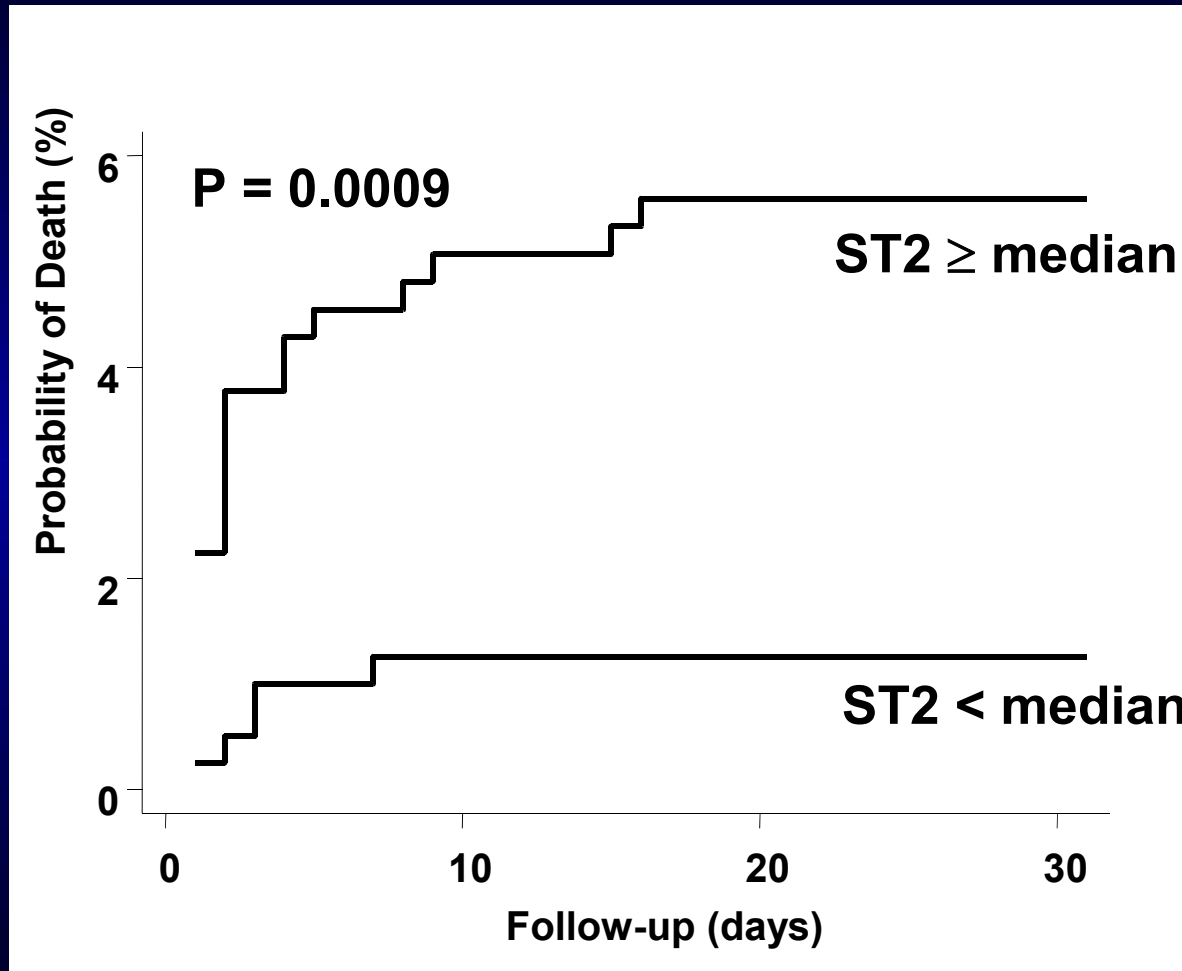
# ST2 Membrane and Soluble Isoforms



# ST2 Serum Levels During MI



# ST2 Serum Levels AT PRESENTATION Predict Outcome After MI



Shimpo, *Circulation* 2004

# Is ST2 Just a Marker of MI?

- We wanted to test the hypothesis that serum ST2 may be a biomarker in the absence of infarction.
- 162 patients with severe non-ischemic heart failure
- ST2 measured at baseline and at 2 weeks

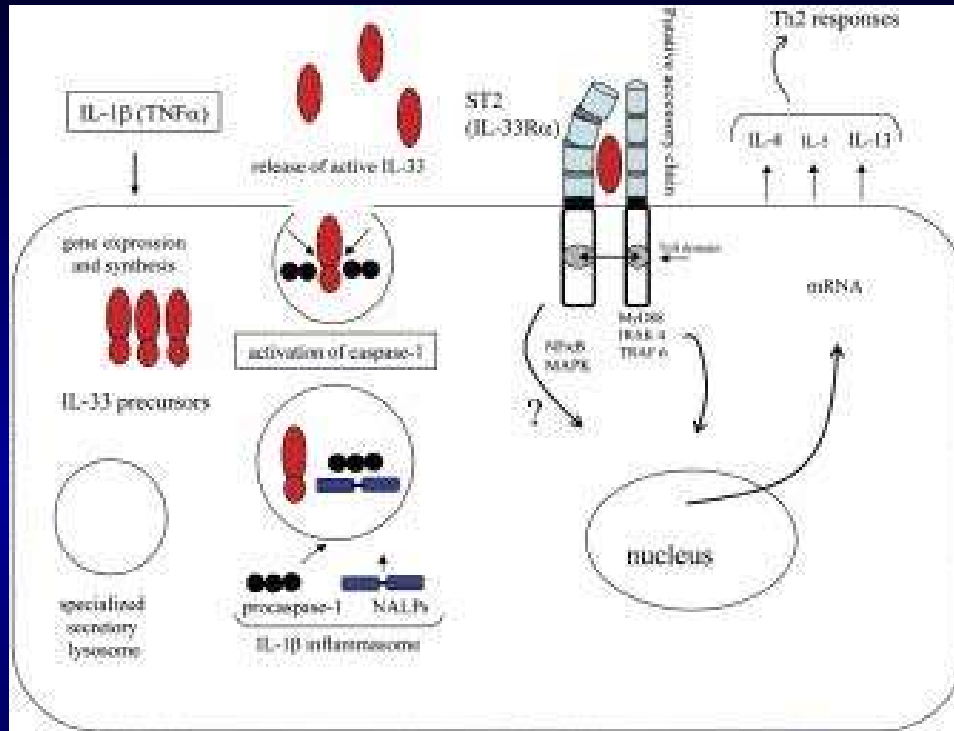


# ST2 and Death in CHF Patients

- Higher ST2 in patients who died.
- Increase in ST2 from baseline to 2 weeks predicted mortality ( $p=0.04$ ) and this remained independently predictive in multivariate analysis that included BNP, ANP, clinical parameters.

*Circulation 2003*

# IL-33 is a ligand for ST2

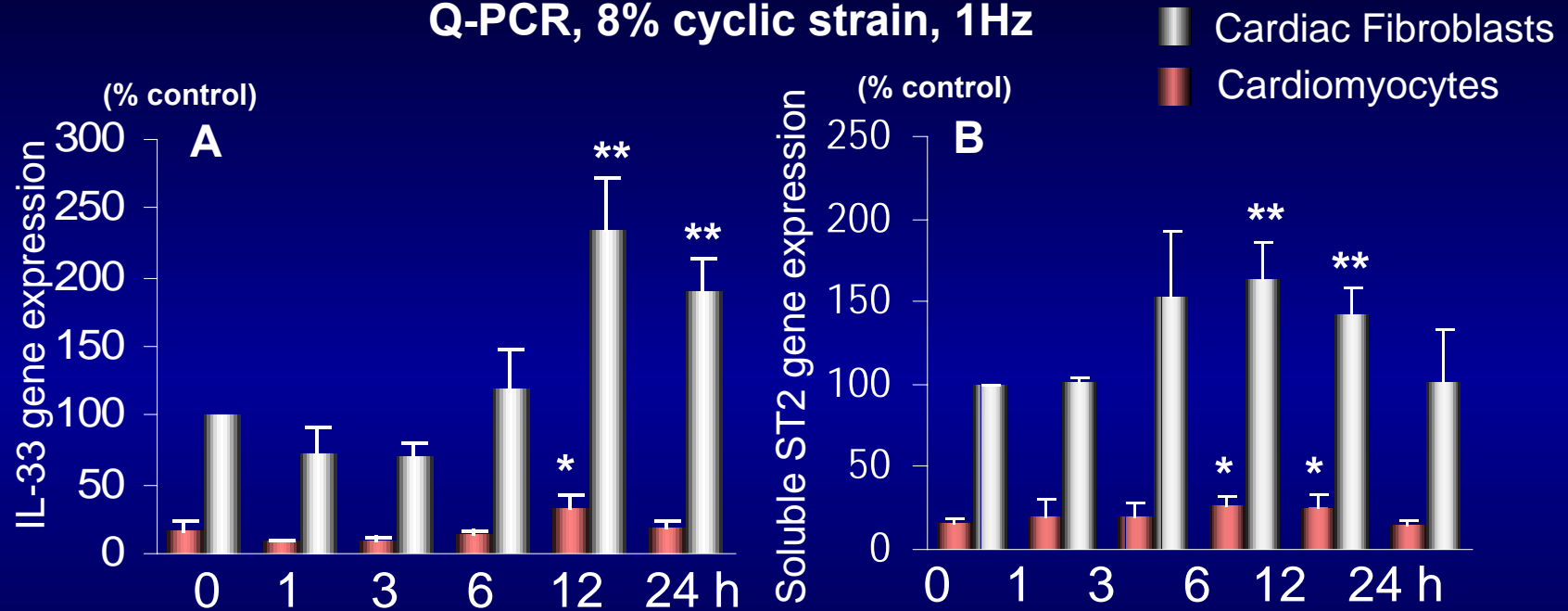


Dinarello, Immunity 2005

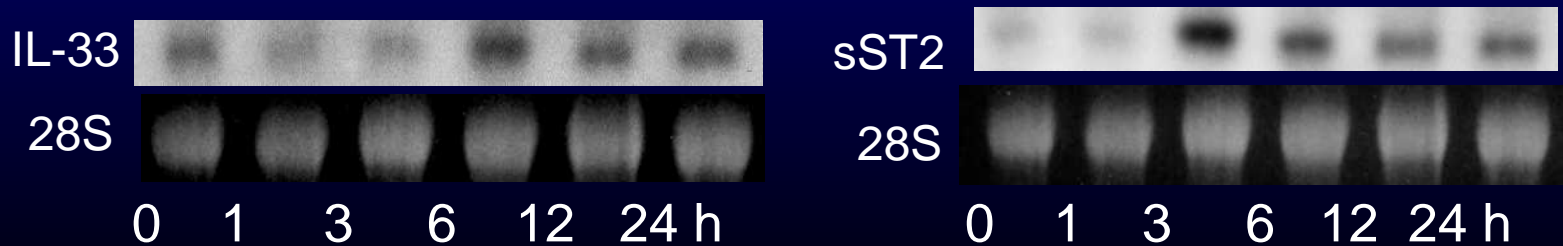
Assuming that IL-33 is similar to the other two members of the IL-1 ligand family, IL-1 $\beta$  and IL-18, the IL-33 precursor enters the specialized secretory lysosome. Following activation of caspase-1, the IL-33 precursor is cleaved, the membrane of the secretory lysosome fuses with the cell membrane, and IL-33 is released as an active cytokine. Mature IL-33 binds to ligand binding ST2. Similar to IL-1 and IL-18 receptors, a heterodimeric signalling complex is formed with a second accessory chain, SIGIRR.

# IL-33 is a Mechanically-Induced Cardiac Fibroblast Gene

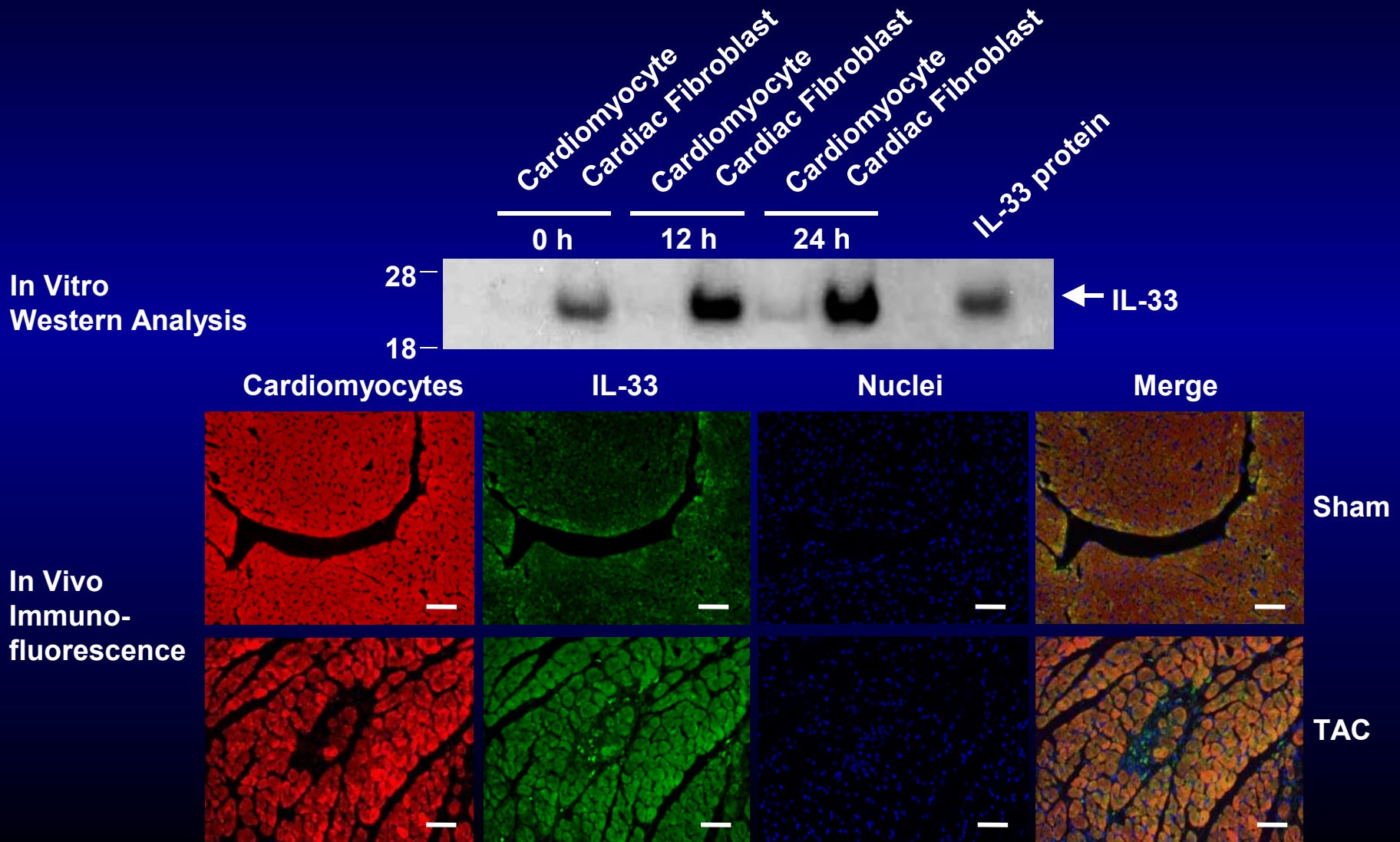
Q-PCR, 8% cyclic strain, 1Hz



Northern Analysis in Cardiac Fibroblasts



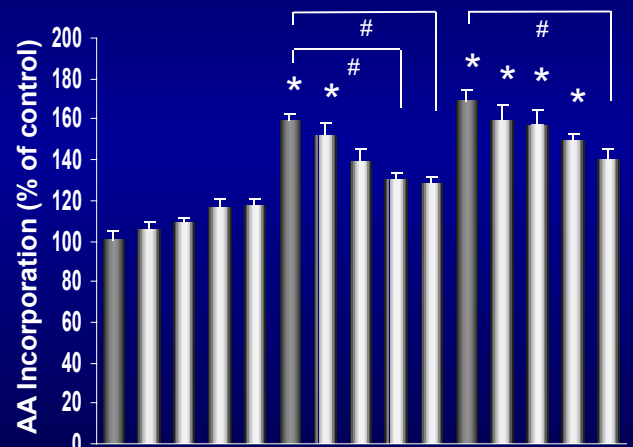
# Gene Expression of IL-33/ST2 is Induced By Stress



# IL-33 Blocks Hypertrophic Stimuli Through ST2 in Cardiomyocytes

A

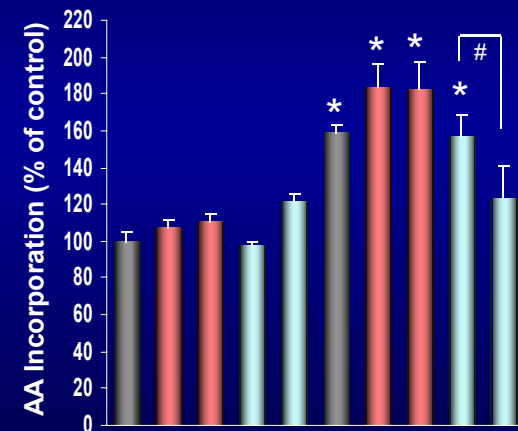
IL33 blocks hypertrophic stimuli



Angiotensin-II (0.1 $\mu$ M)	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
Phenylephrine (50 $\mu$ M)	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
IL-33 (ng/ml)	-	0.11	10	100	-	0.11	10	100	-	0.11	10	100	-	-	

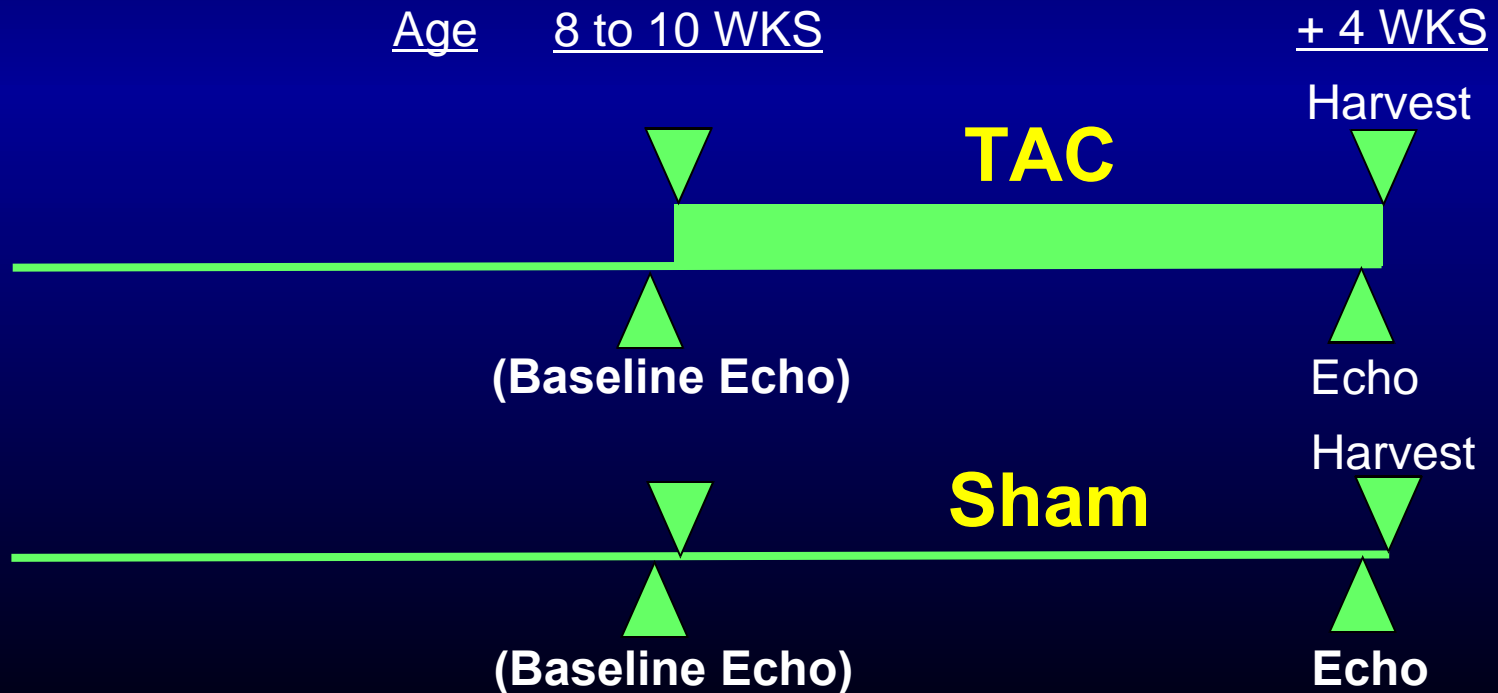
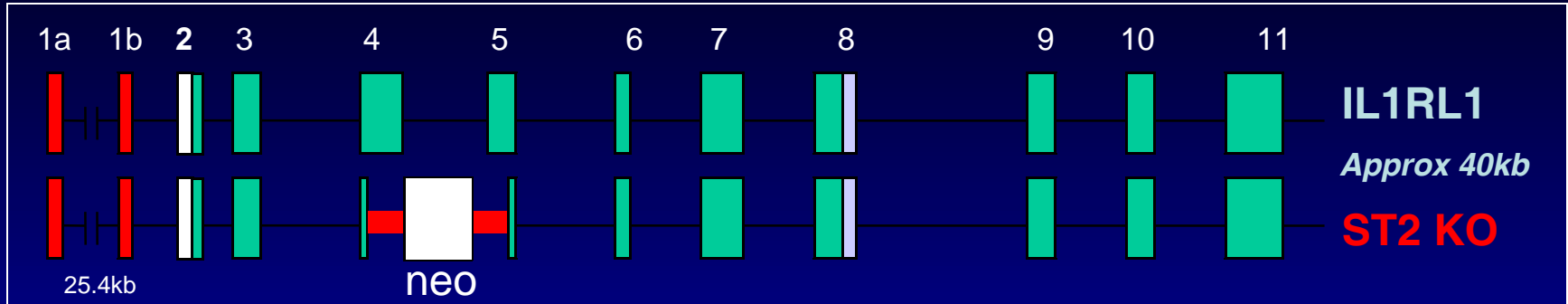
B

Blocking the ST2L receptor eliminates the effect of IL-33

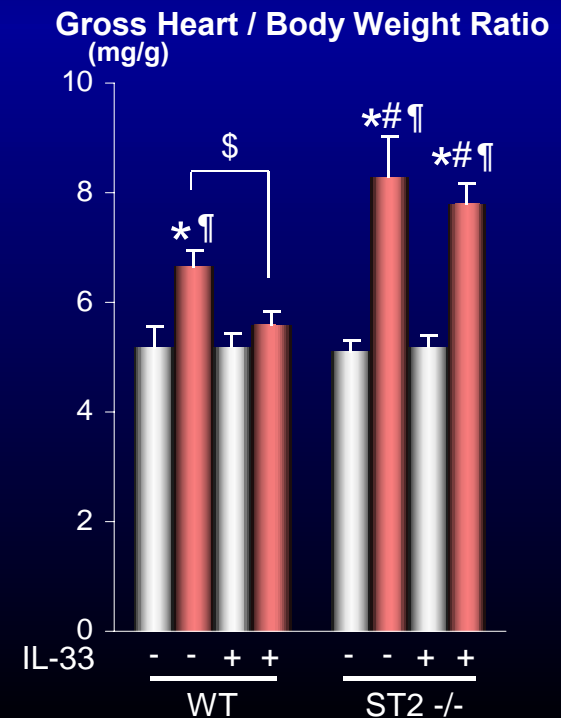
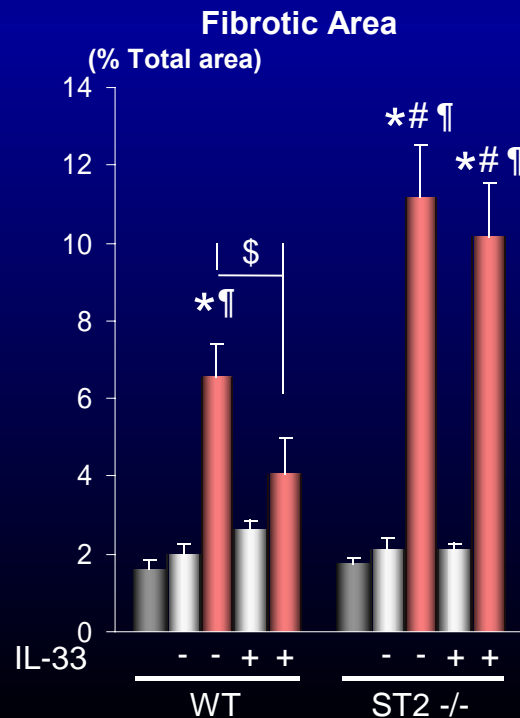
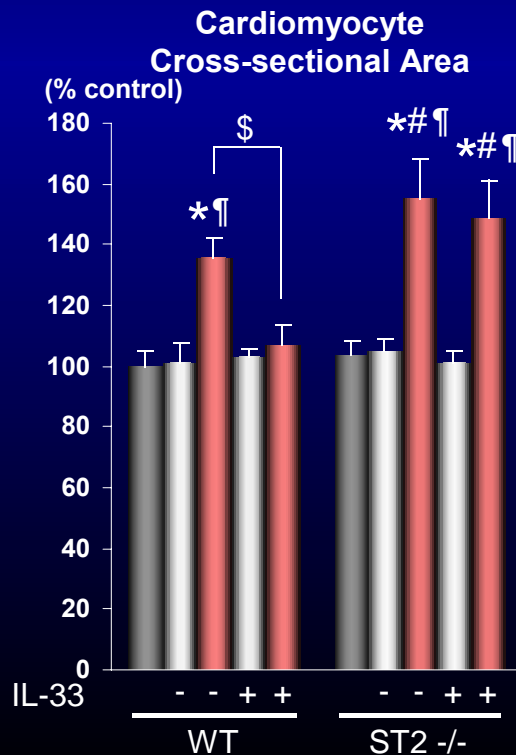
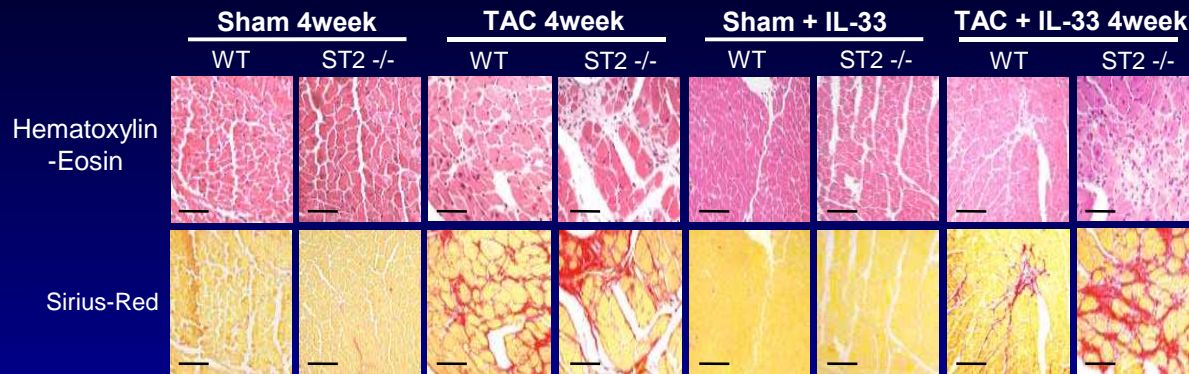


Angiotensin-II (0.1 $\mu$ M)	-	-	-	-	-	+	+	+	+	+
IL-33 (ng/ml)	-	-	10	-	10	-	-	10	-	10
anti-ST2 Ab ( $\mu$ g/ml)	-	100	100	-	-	-	100	100	-	-
IgG2a ( $\mu$ g/ml)	-	-	-	100	100	-	-	-	100	100

# *In vivo* TAC Model of ST2 Null Mice



# IL-33 Eliminates Cardiomyocyte Hypertrophy and Fibrosis *in Vivo*



# IL-33 Blocks Gene Expression of Hypertrophic Markers *in vivo*

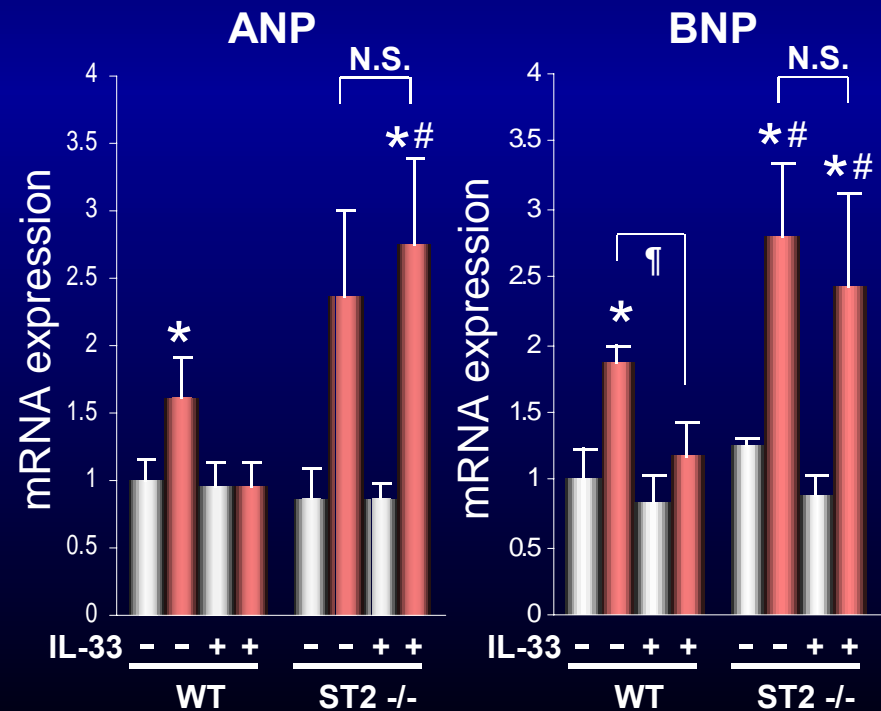
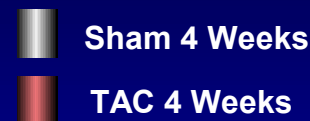
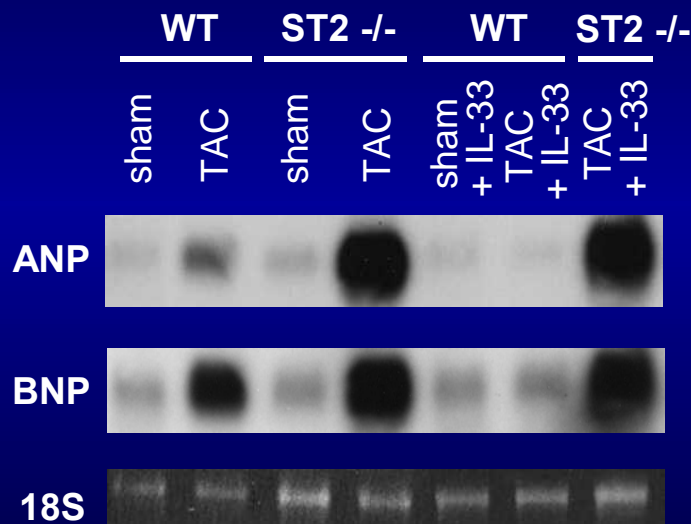
Northern Analysis in the *in Vivo* Heart

\* :  $p < 0.05$  vs. WT Sham

# :  $p < 0.05$  vs. the same treatment in WT

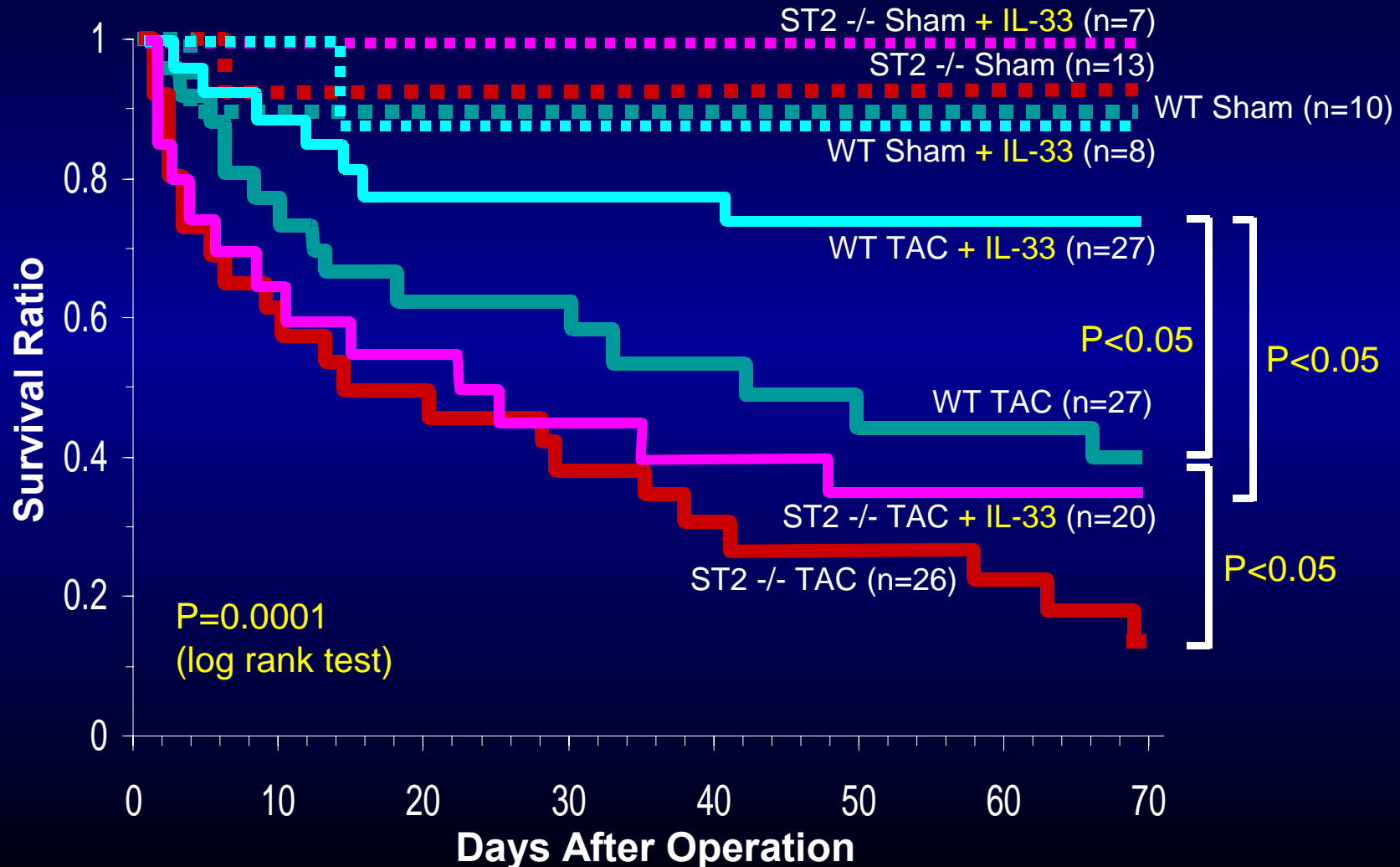
¶ :  $p < 0.05$  vs. Sham in the same group

\$ :  $p < 0.05$





# IL-33 Improves Survival Under Pressure Overload via ST2 *in Vivo*

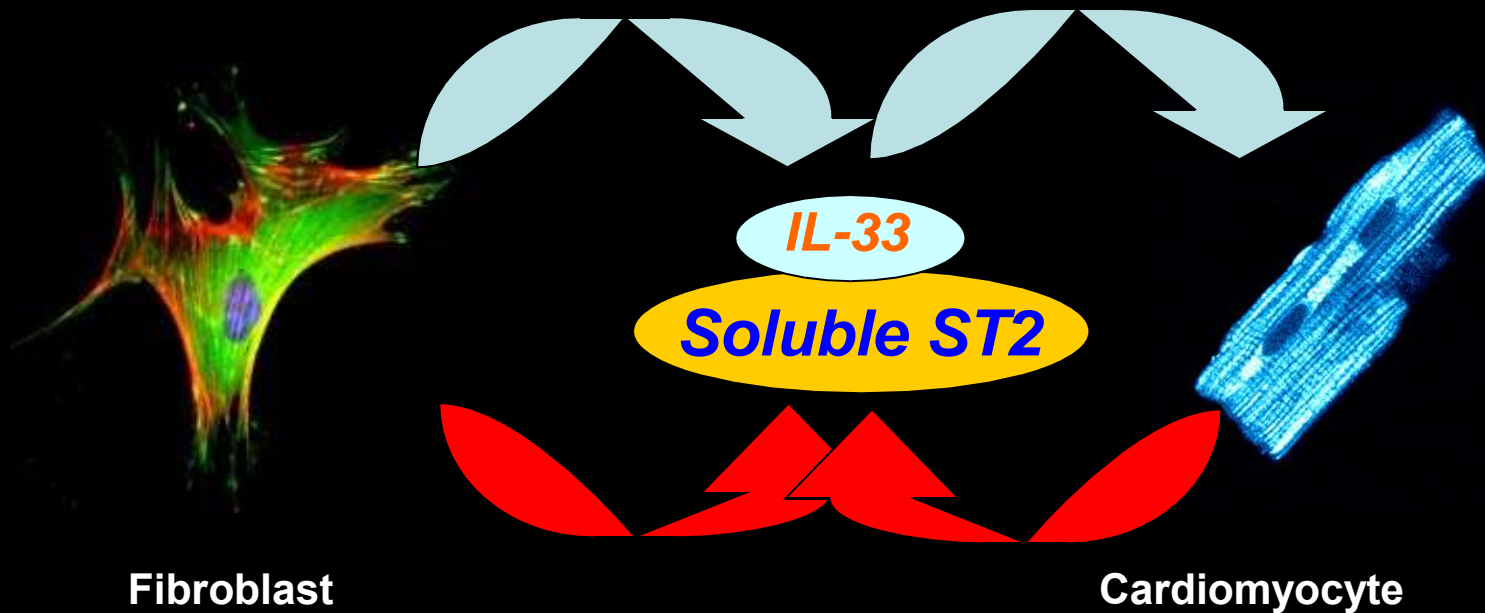


# IL-33/ST2 signaling is a new cardioprotective fibroblast-cardiomyocyte cytokine pathway



**IL-33: Cardioprotection, Reduced Fibrosis**

# IL-33/ST2 signaling is a new cardioprotective fibroblast-cardiomyocyte cytokine pathway



**IL-33: Cardioprotection, Reduced Fibrosis**  
**Soluble ST2: Decoy Receptor for IL-33**

# Conclusions

- Although there was no apparent biological link, discovery studies revealed that the IL-1 receptor family member soluble ST2 is a powerful biomarker for cardiovascular disease.
- IL-33 is the ligand for the membrane ST2L and soluble ST2
- Soluble ST2 can act as a decoy receptor, blocking IL-33
- In vivo, IL-33 is cardioprotective and reduces cardiac fibrosis after pressure overload

# Thanks to My Lab

