

Mechanistic basic science: molecules or man - Cells and animals are what counts.

Volker Adams, PhD

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Cardiac regeneration and stem cell therapy

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Current Opinion in Organ Transplantation 2008, 13:536–542

Purpose of review

Significant recent developments have occurred in the field of cardiac regeneration and stem-cell therapy. Understanding the new technological advances in cell therapy will ultimately allow us to achieve a goal of cell-based cardiac repair.

Recent findings

We discuss the latest cell-based therapies (including skeletal myoblasts, bone-marrow-derived cells, stem cells), the recent clinical trials in humans and different delivery techniques.

Summary

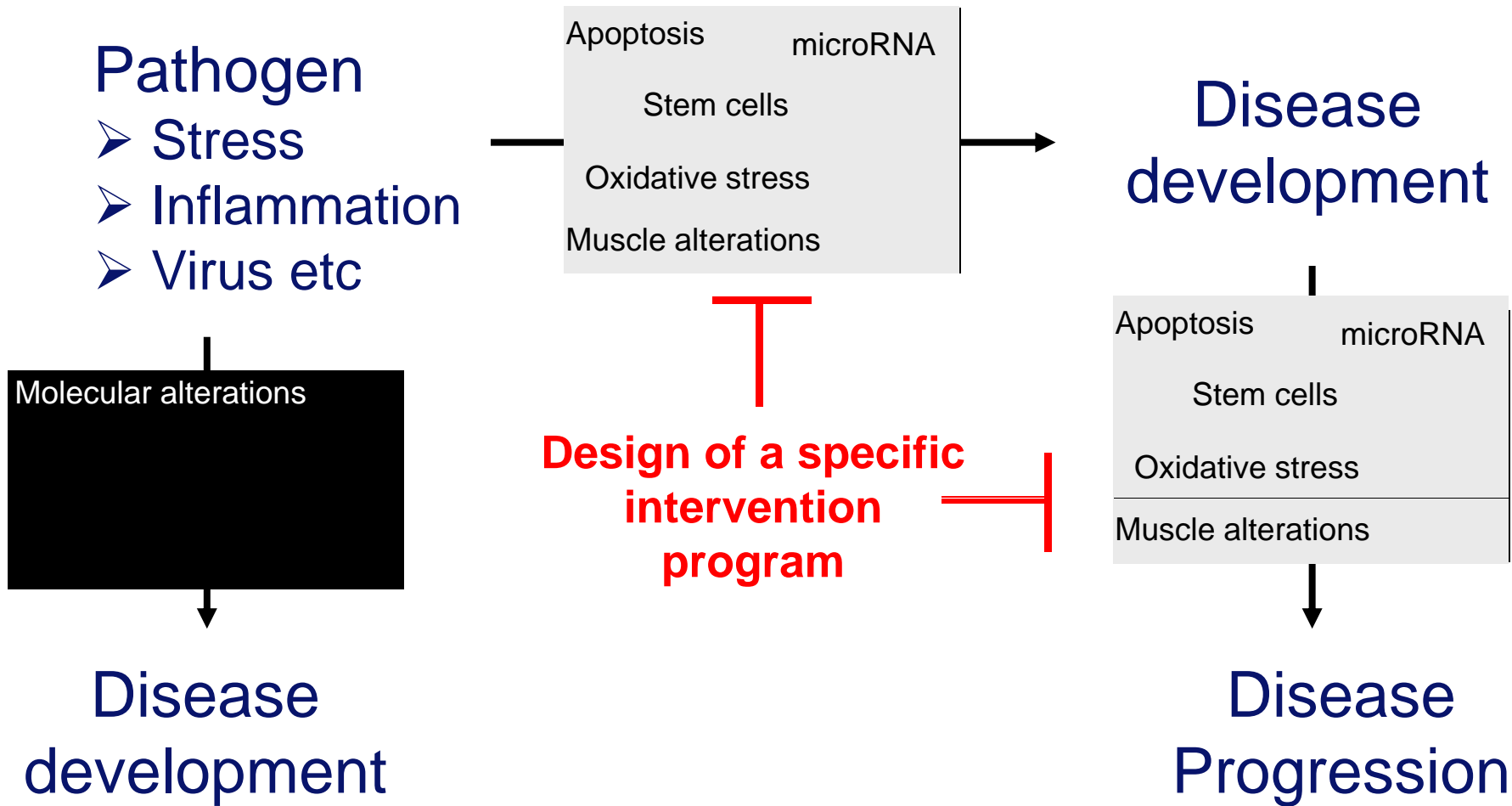
Ongoing unraveling of the mechanistic and clinical complexities underlying cardiac regeneration will advance the field of regenerative medicine. Substantial progress is being made by the parallel conduct of basic science studies and clinical trials that are incorporating mechanistic evaluations.

Keywords

cardiac regeneration, cell-based therapies, stem cells

- The mechanistic understanding of the disease development will help to design strategies for its prevention -

Why Mechanistic Basic Science



Examples of Mechanistic Basic Science Leading to the Design of an Intervention

- **Development of AS and exercise**
- **Loss of muscle mass**
- **MicroRNA and**

Examples of Mechanistic Basic Science Leading to the Design of an Intervention

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Development of Aortic Valve Stenosis

Oxidative stress
Inflammation
Hypercholesteremia
Smoking
Diabetes

Aortic valve sclerosis

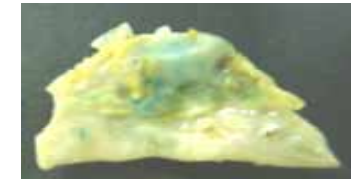
Aortic valve stenosis
with calcification

Mouse model



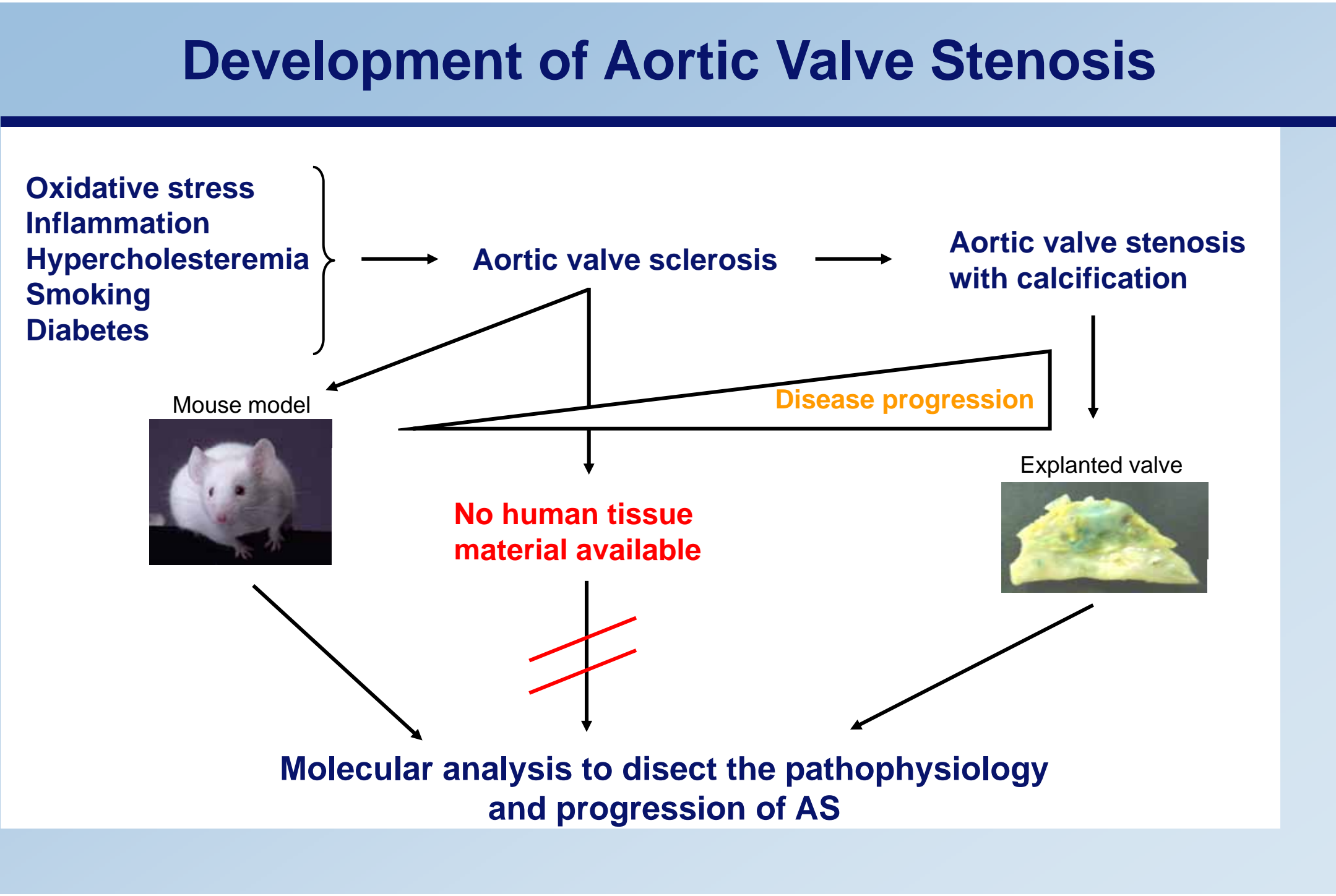
Disease progression

Explanted valve

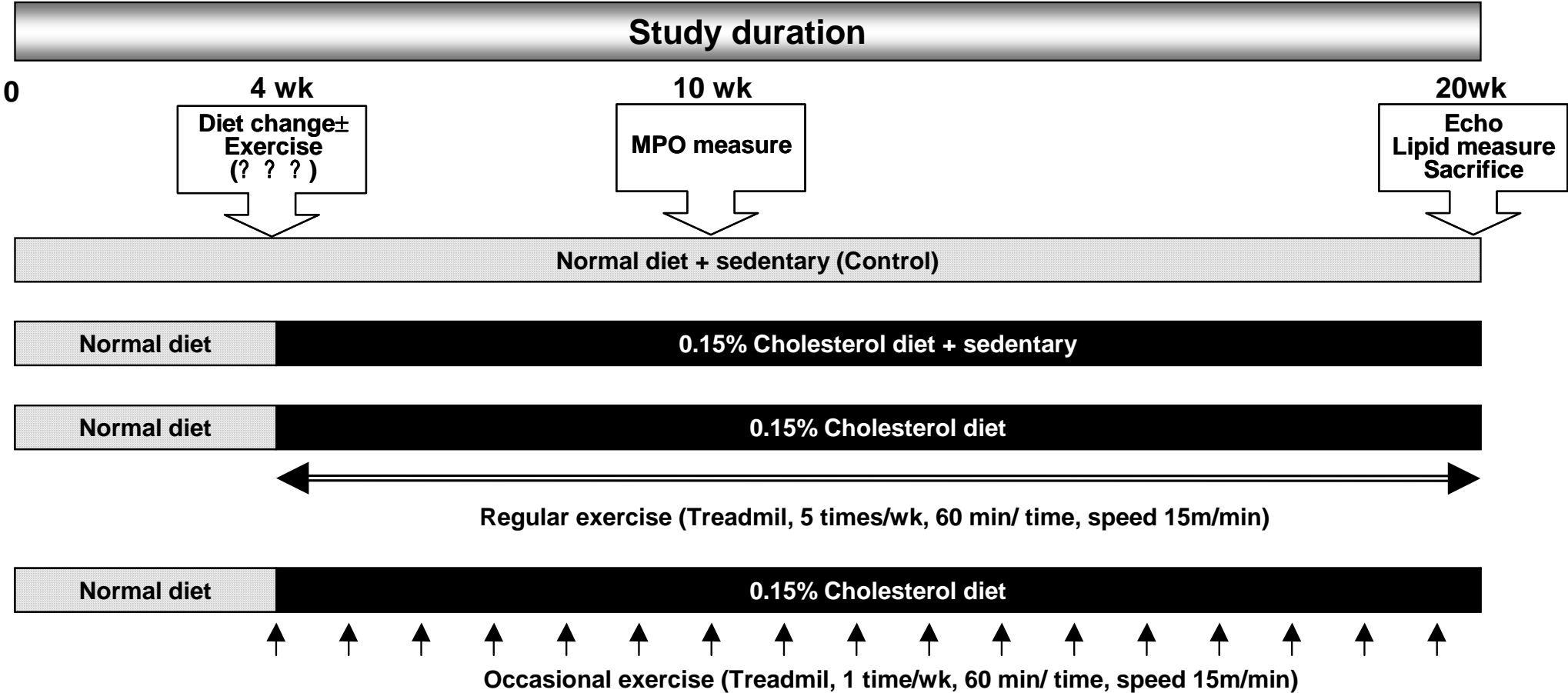


No human tissue
material available

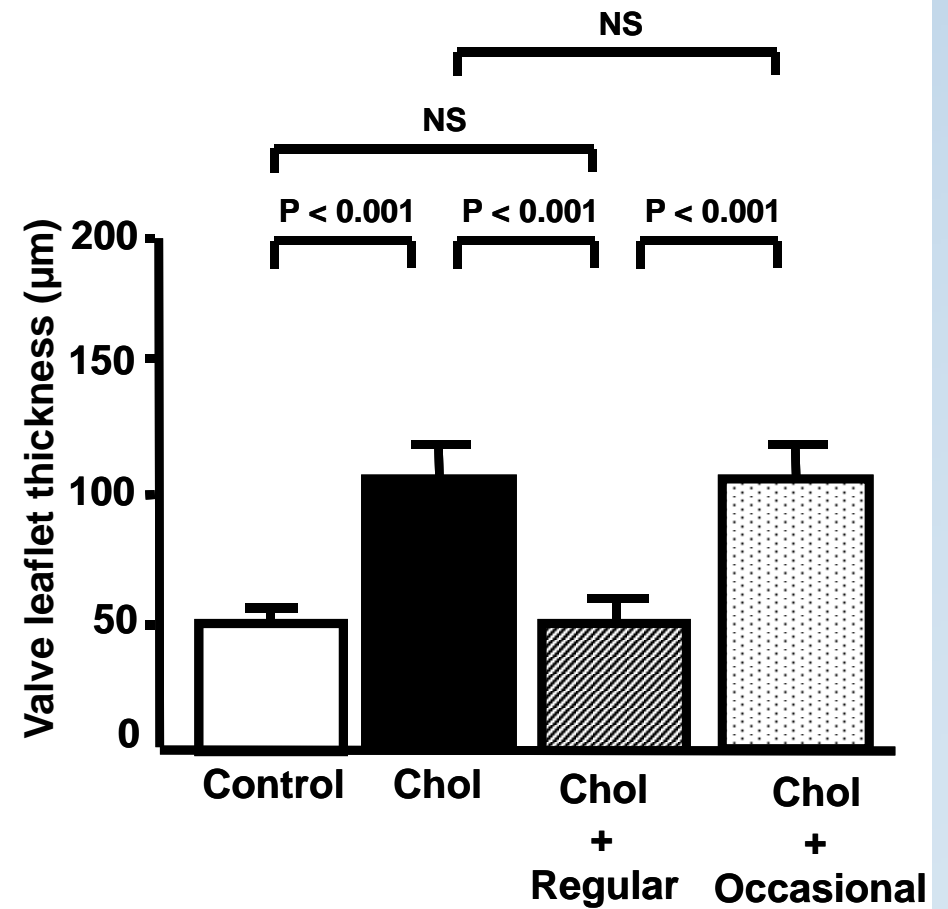
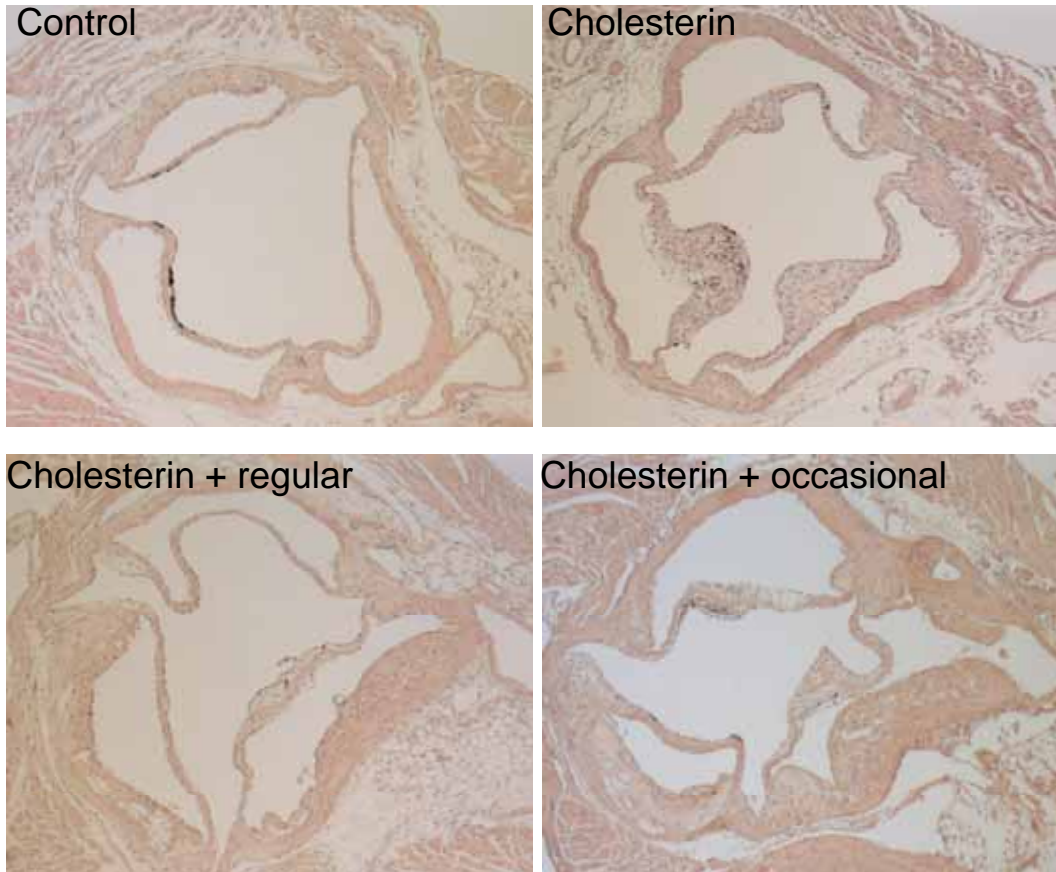
Molecular analysis to dissect the pathophysiology
and progression of AS



Study Design

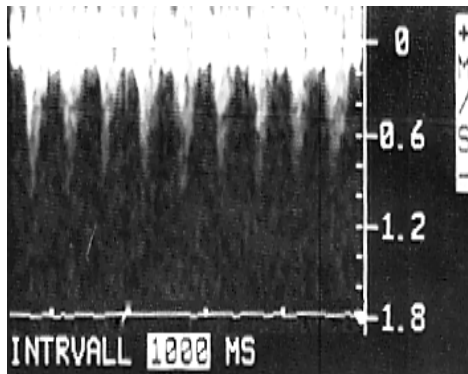


Results – Valve Thickness

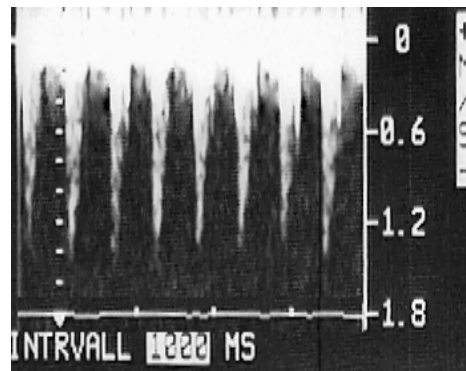


Results – Blood Flow Velocity

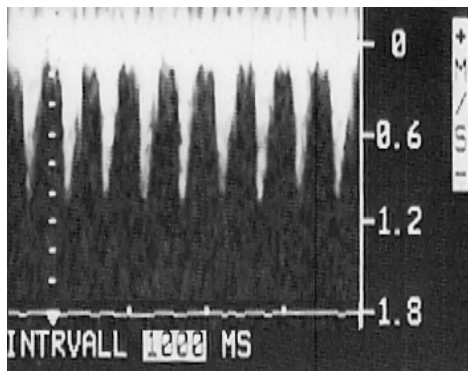
Control



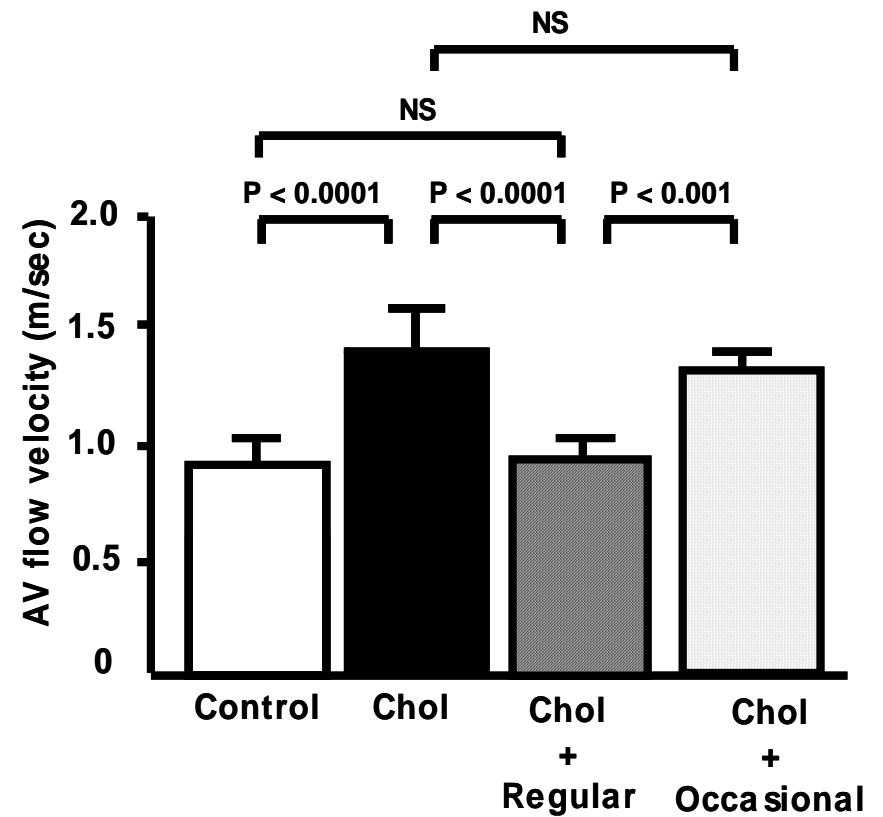
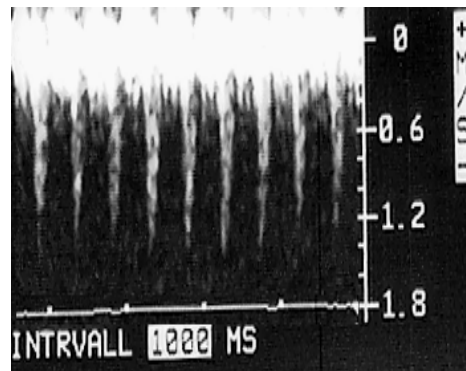
Cholesterin



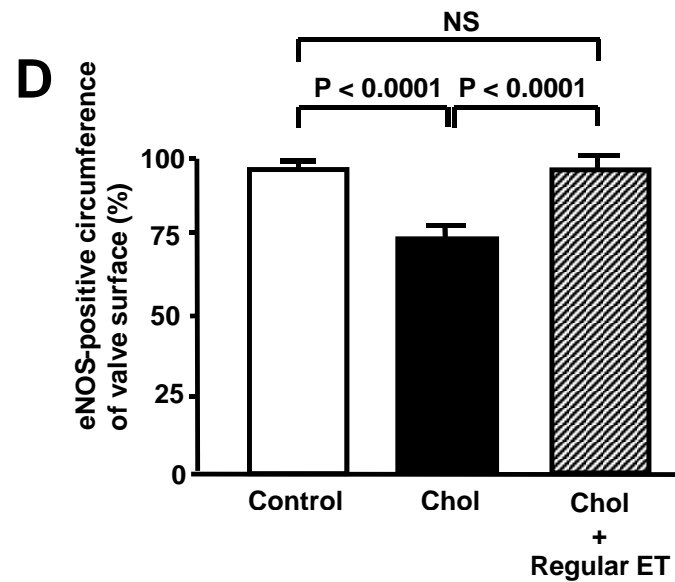
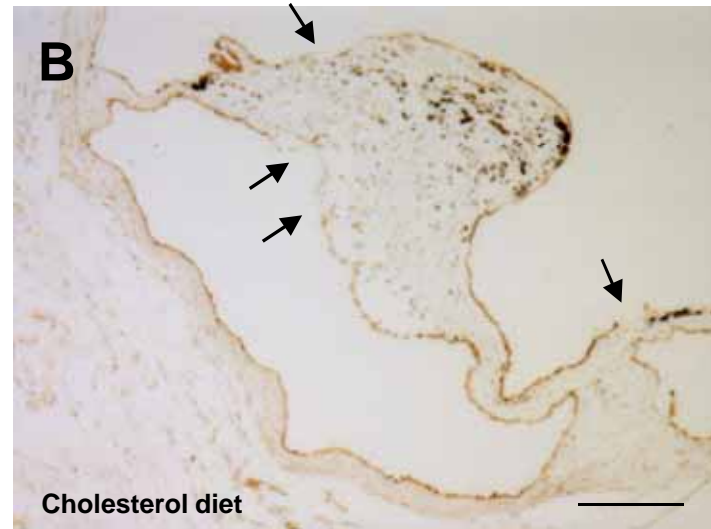
Chol+Regular



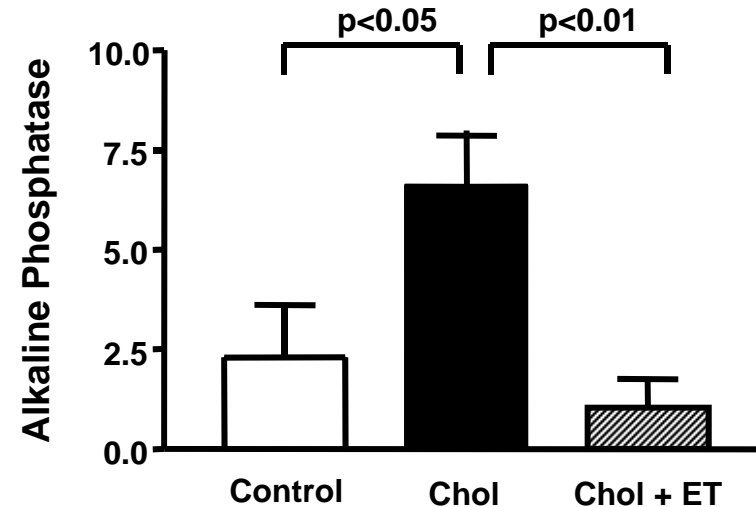
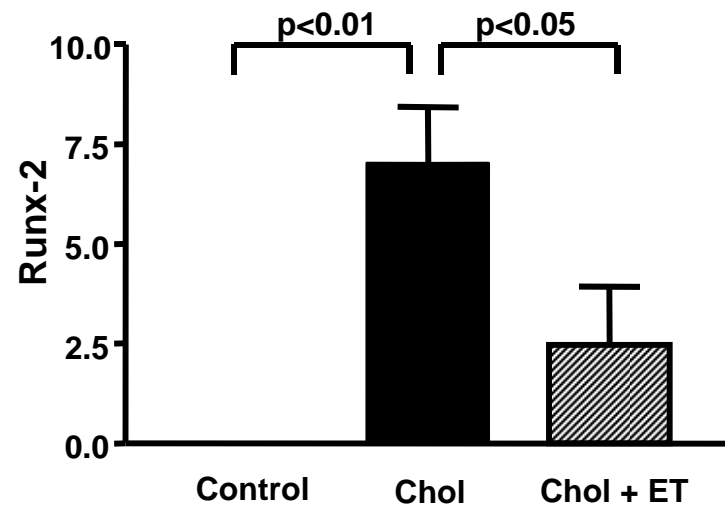
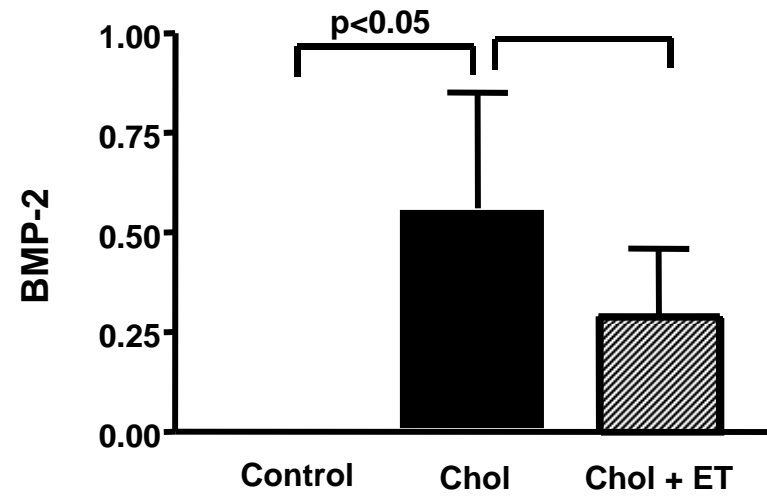
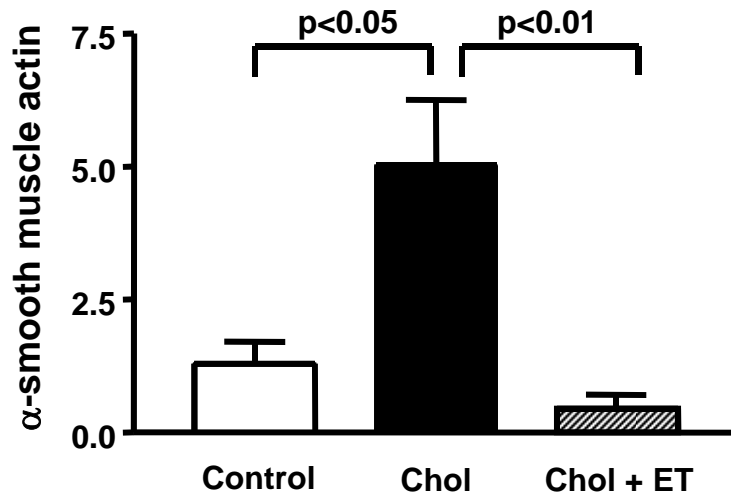
Chol+Occasional



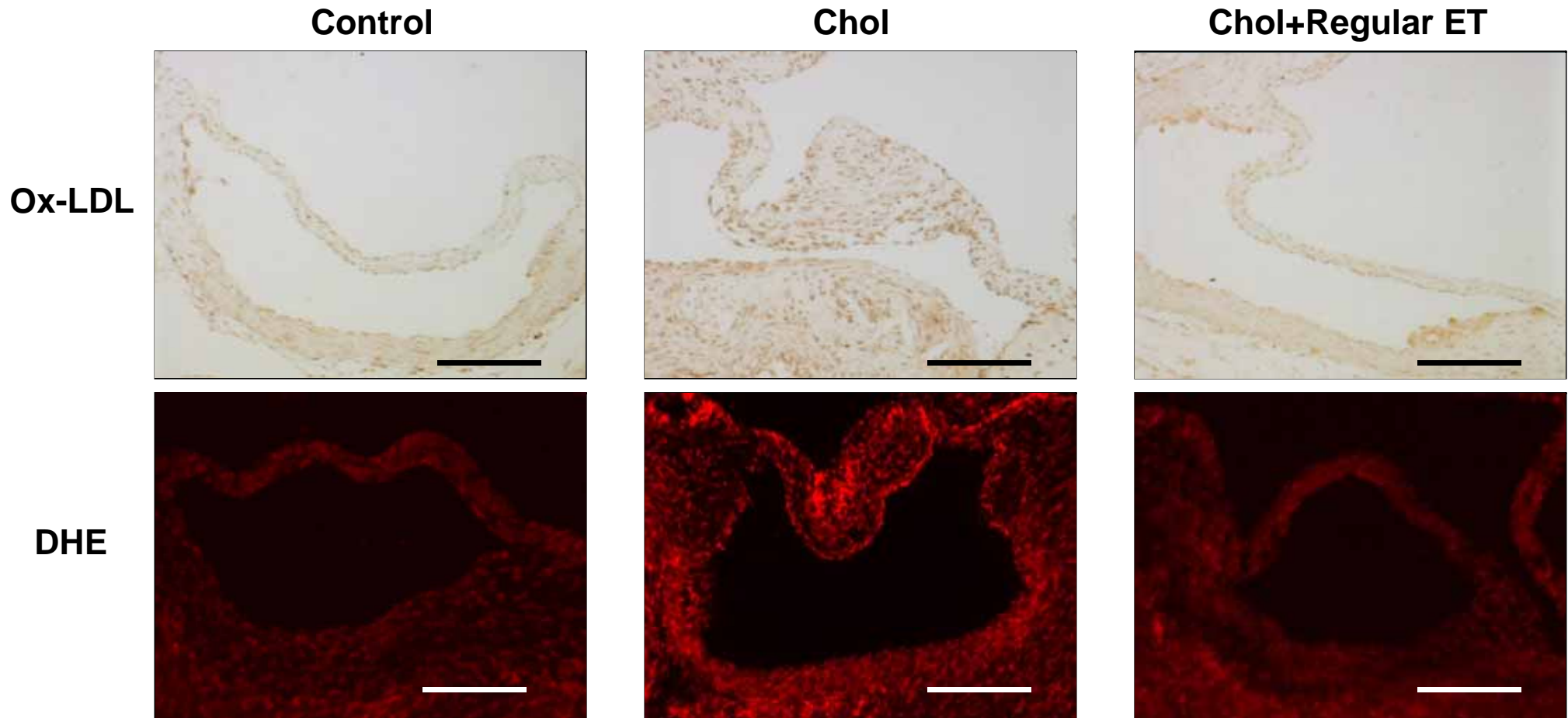
Results – Staining of Endothelial Cells with ecNOS antibody

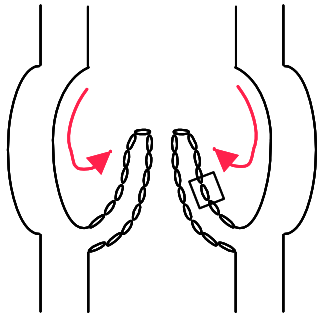


Results –mRNA Expression of Osteoblastic Genes

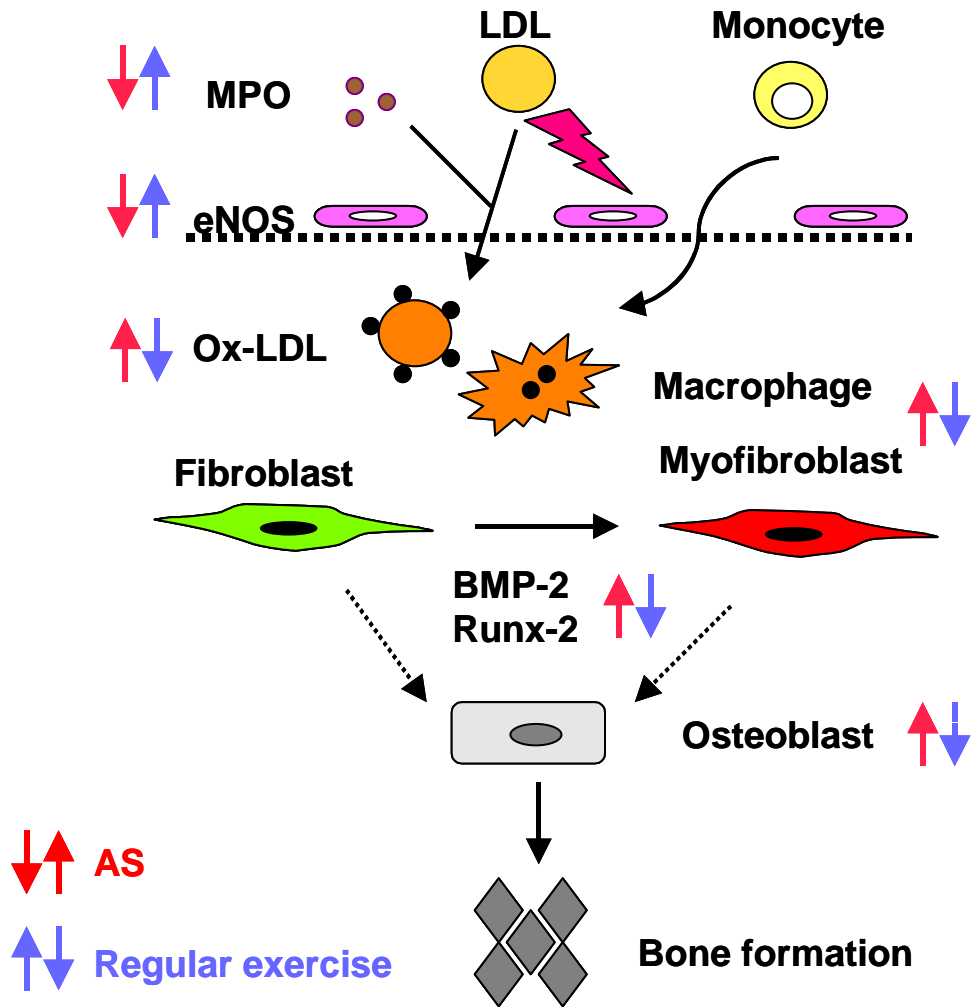


Results – Oxidative Stress

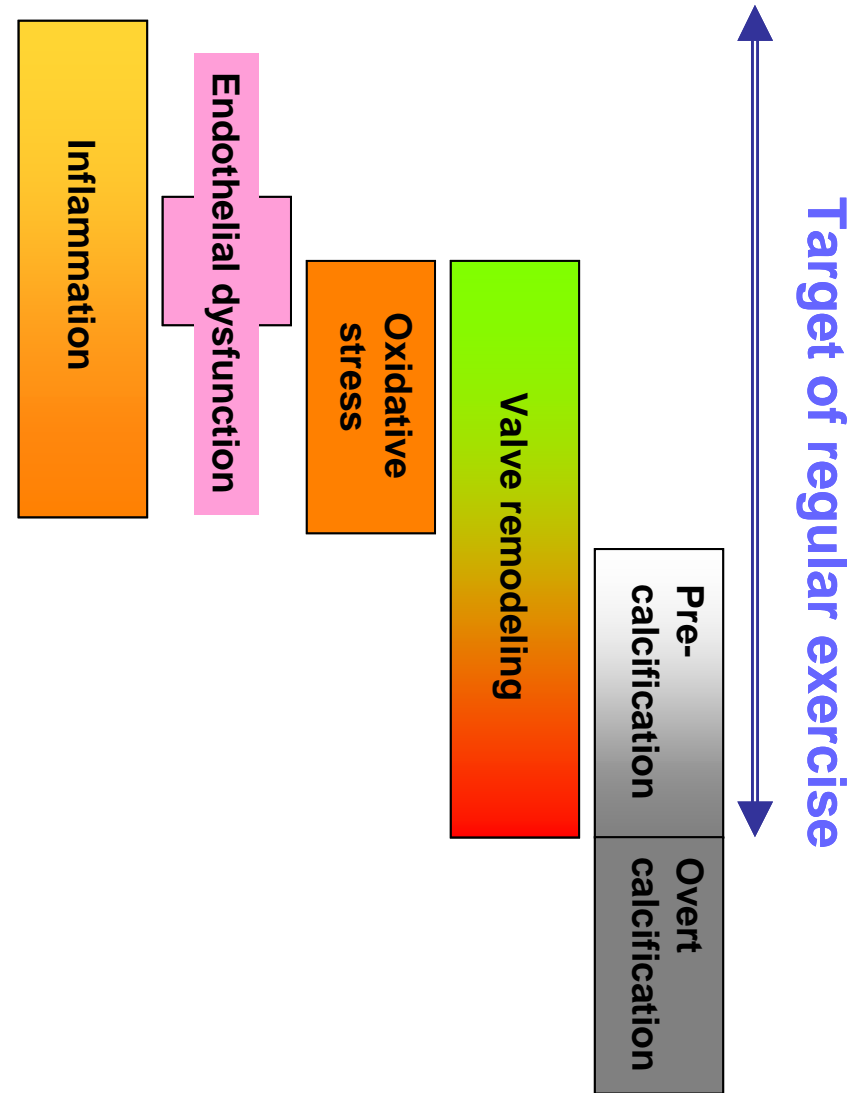




A



B



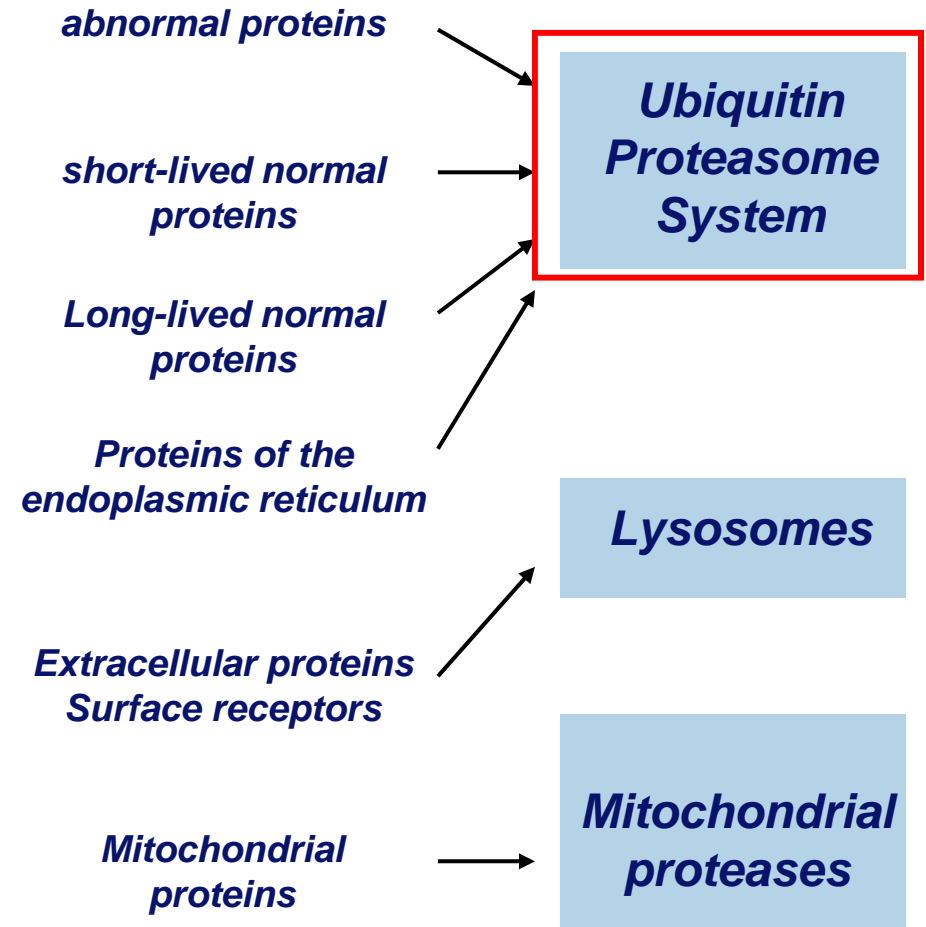
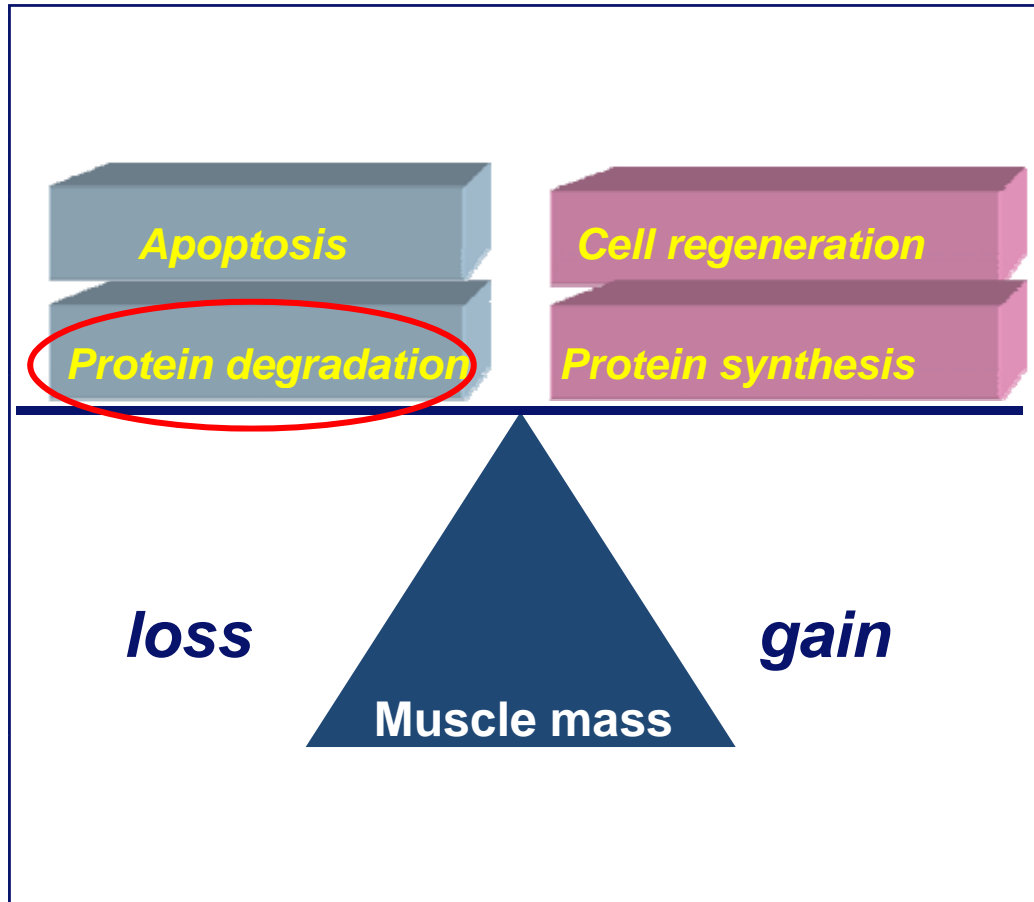
Examples of Mechanistic Basic Science Leading to the Design of an Intervention

- **Development of AS and exercise**
- **Loss of muscle mass**
- **MicroRNA and**

Loss of Muscle Weight is Associated With Increased Protein Degradation

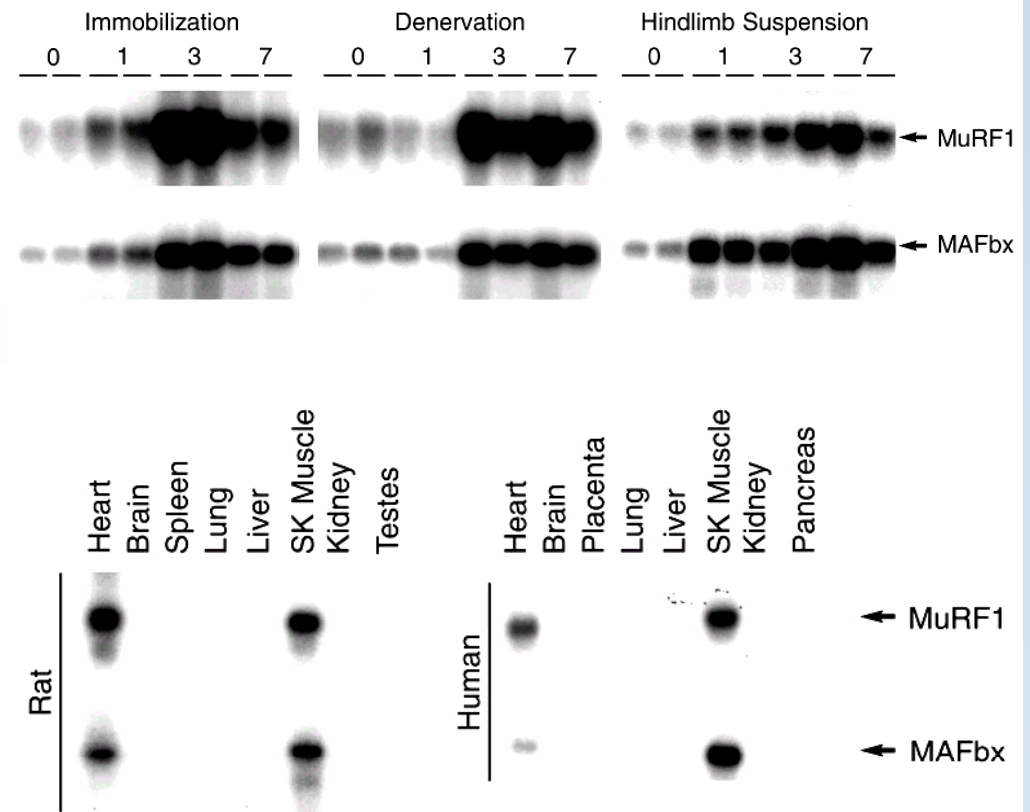
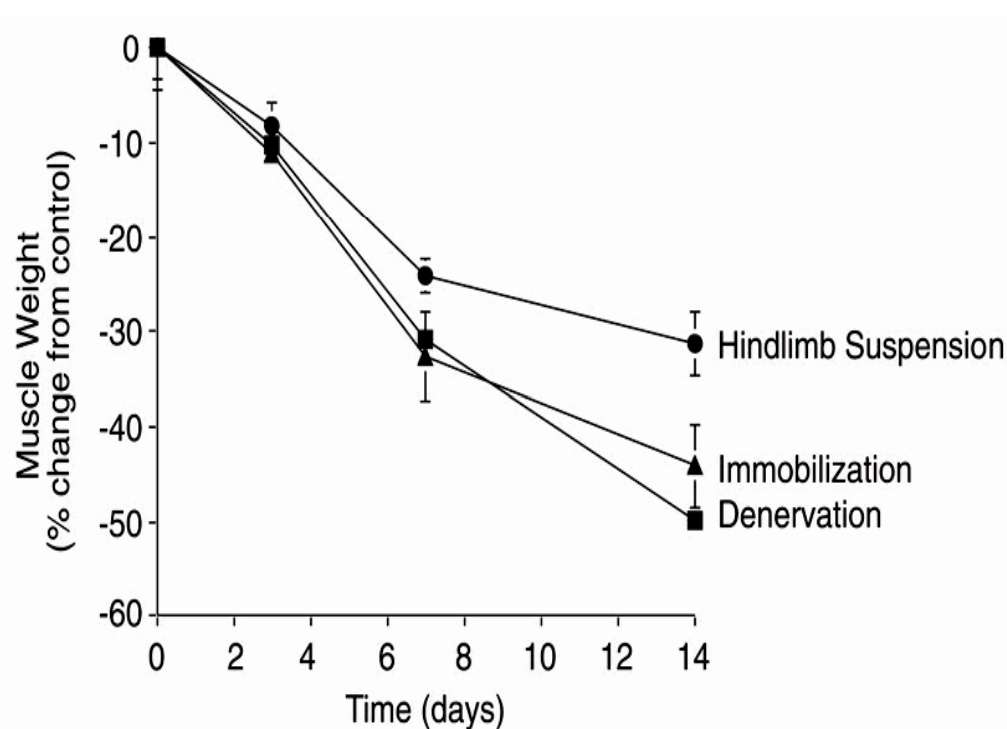
| Condition | Sample | Muscle weight | | Protein degradation | |
|---|----------|---------------|--------|---|------------|
| | | (mg) | % loss | (pmol Tyr mg ⁻¹ 2h ⁻¹) | % increase |
| Fasting ^a | Control | 137.3 ± 1.8 | | 230 ± 11 | |
| | Fasting | 118.4 ± 1.7 | 14 | 342 ± 13 | 49 |
| Tumor implantation ^b (Yoshida hepatoma) | Control | 45.0 ± 0.6 | | 251 ± 13 | |
| | Tumor | 38.6 ± 0.7 | 14 | 410 ± 17 | 63 |
| Chronic renal failure ^c (7/8 nephrectomy) | Control | 28.6 ± 1.4 | | 150 ± 13 | |
| | CRF | 20.3 ± 1.5 | 29 | 236 ± 32 | 57 |
| Diabetes ^c (streptozotocin) | Control | 39.4 ± 1.0 | | 149 ± 5 | |
| | Diabetes | 31.6 ± 1.1 | 20 | 208 ± 8 | 40 |

Possible Pathways for Muscle Protein Degradation

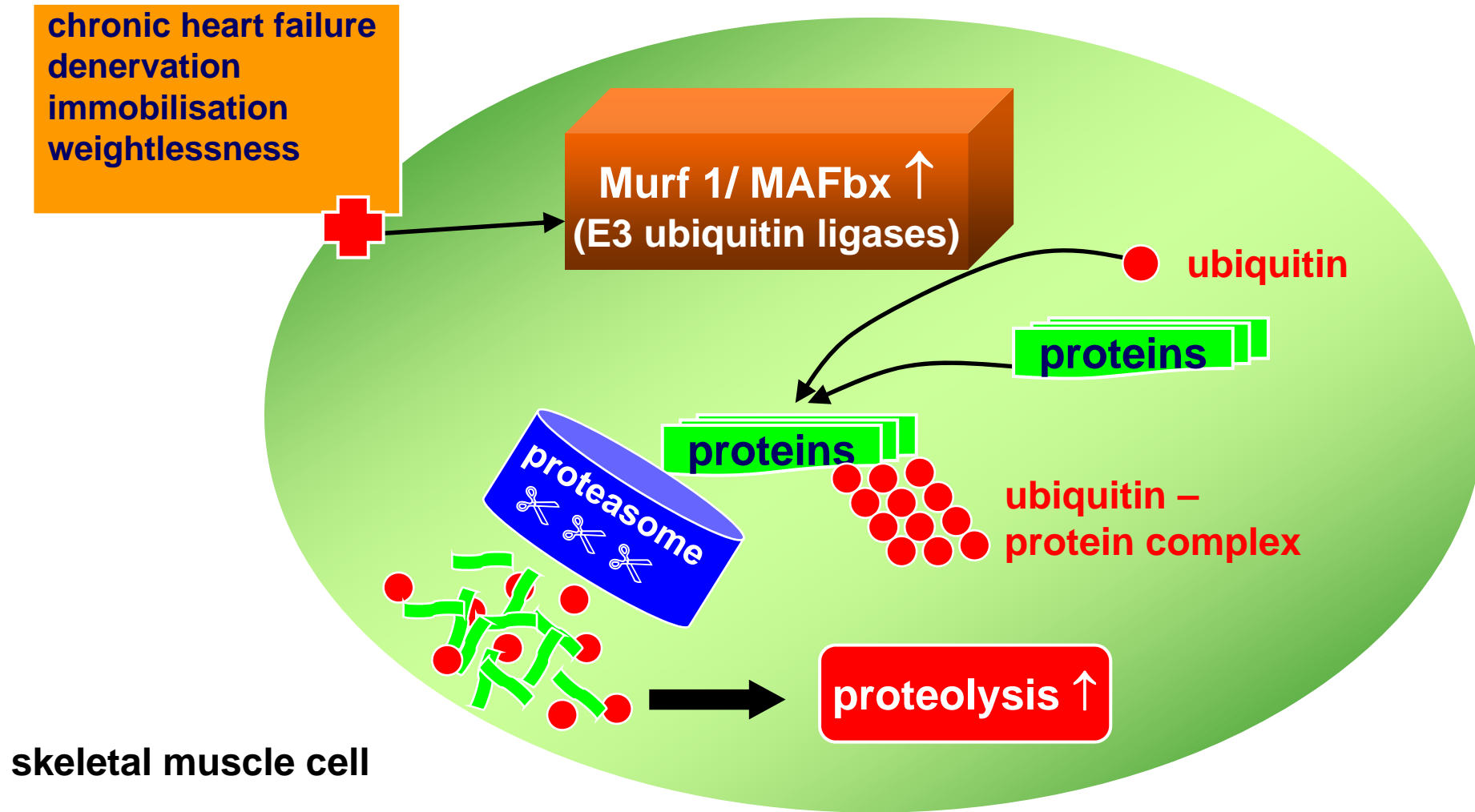


Ubiquitin Dependent Ligases in Skeletal Muscle

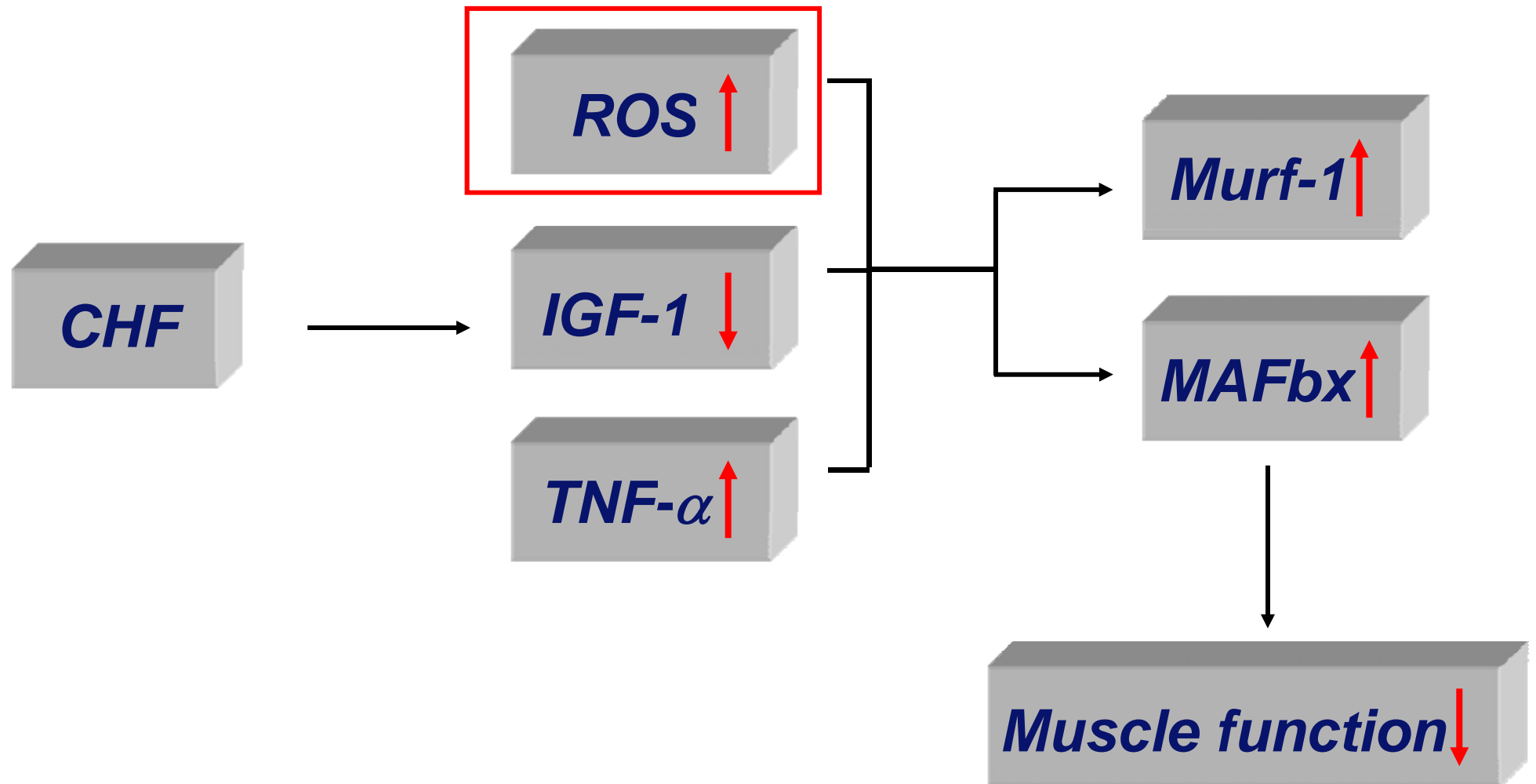
Comparison of mRNA from rat medial gastrocnemius muscle that had been immobilized for 3 days to mRNA from control muscle via differential display approach. The identified genes were furthermore studied in other models of muscle atrophy like denervation and hindlimb suspension.



Ubiquitin proteasome pathway

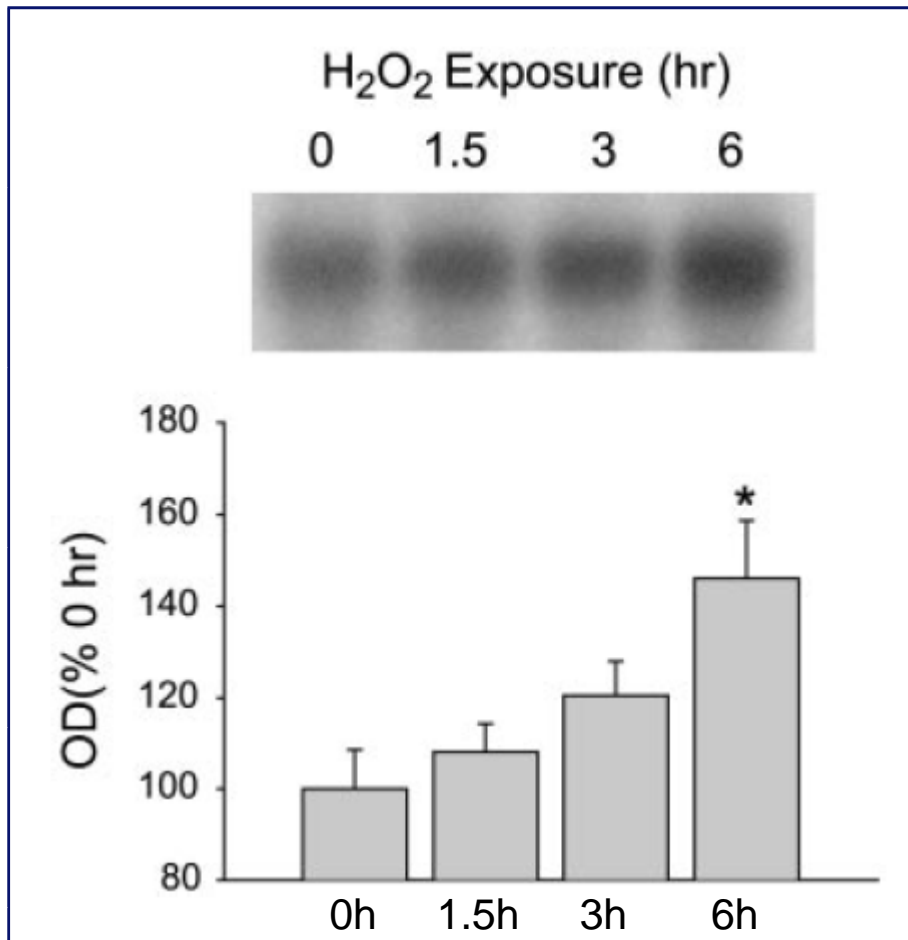


Possible Mediators for Regulating Murf-1/MAFbx

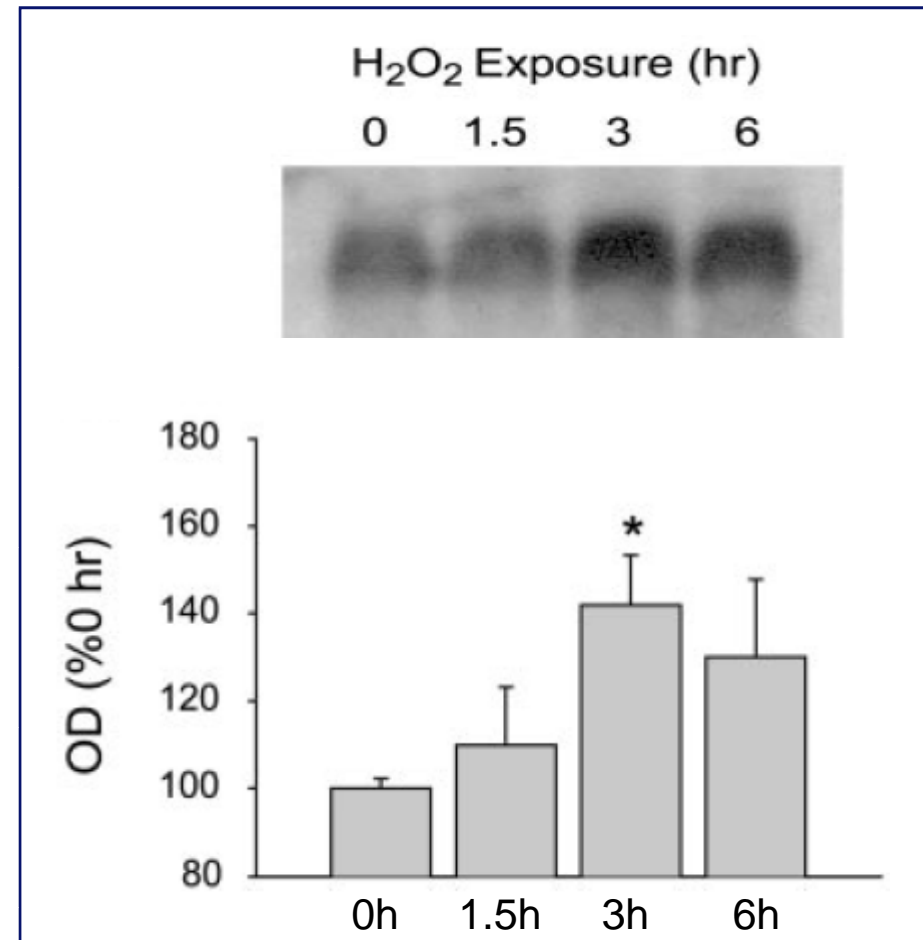


Activation of MAFbx/Murf-1 in C2C12 Cells by Hydrogen Peroxides

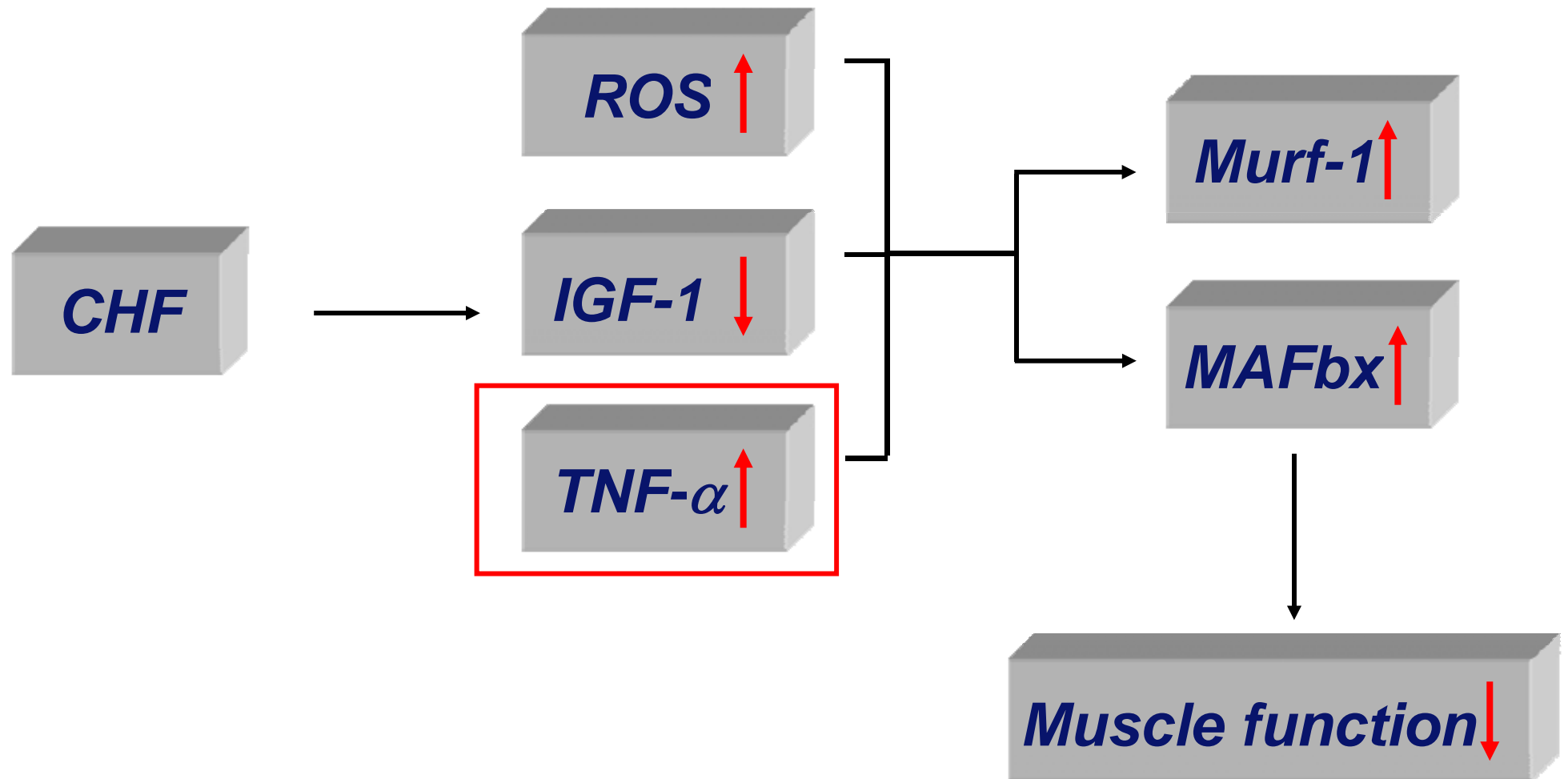
Murf-1



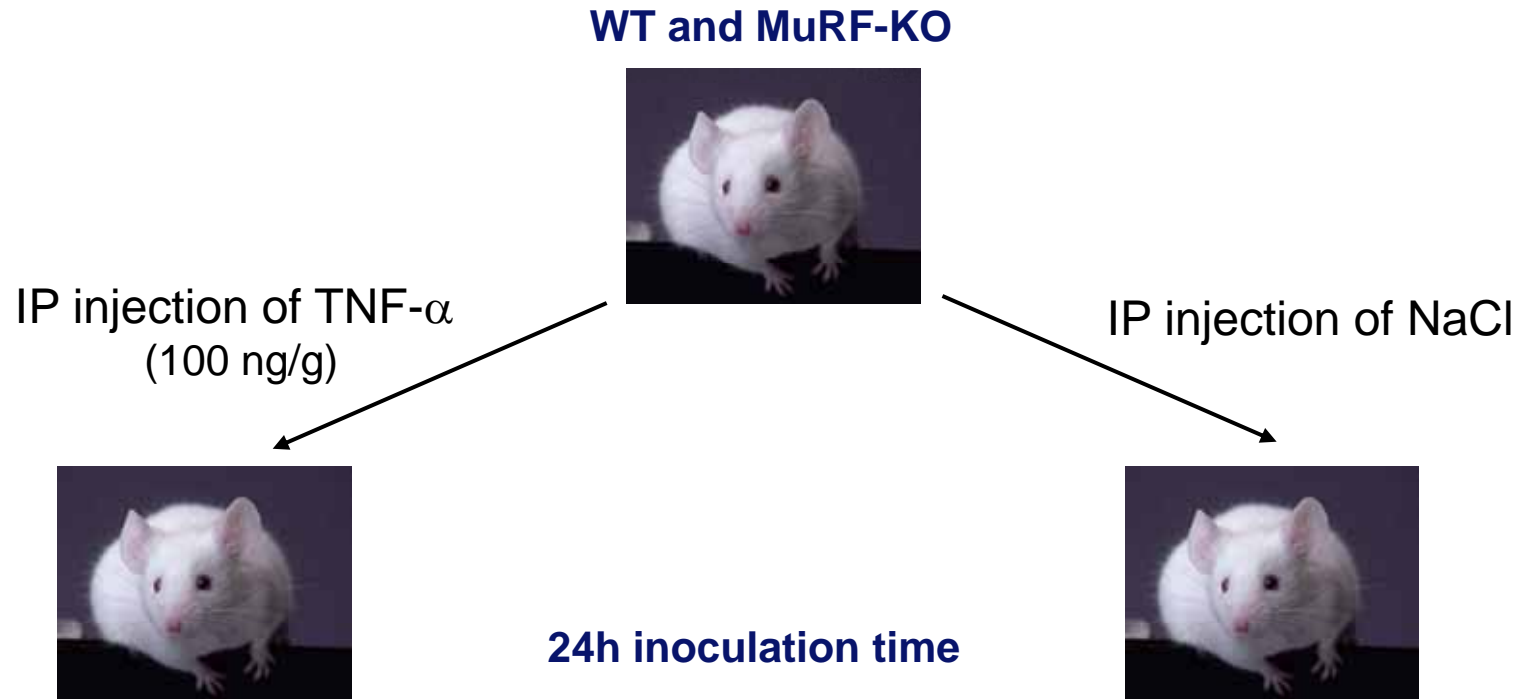
MAFbx



Possible Mediators for Regulating Murf-1/MAFbx

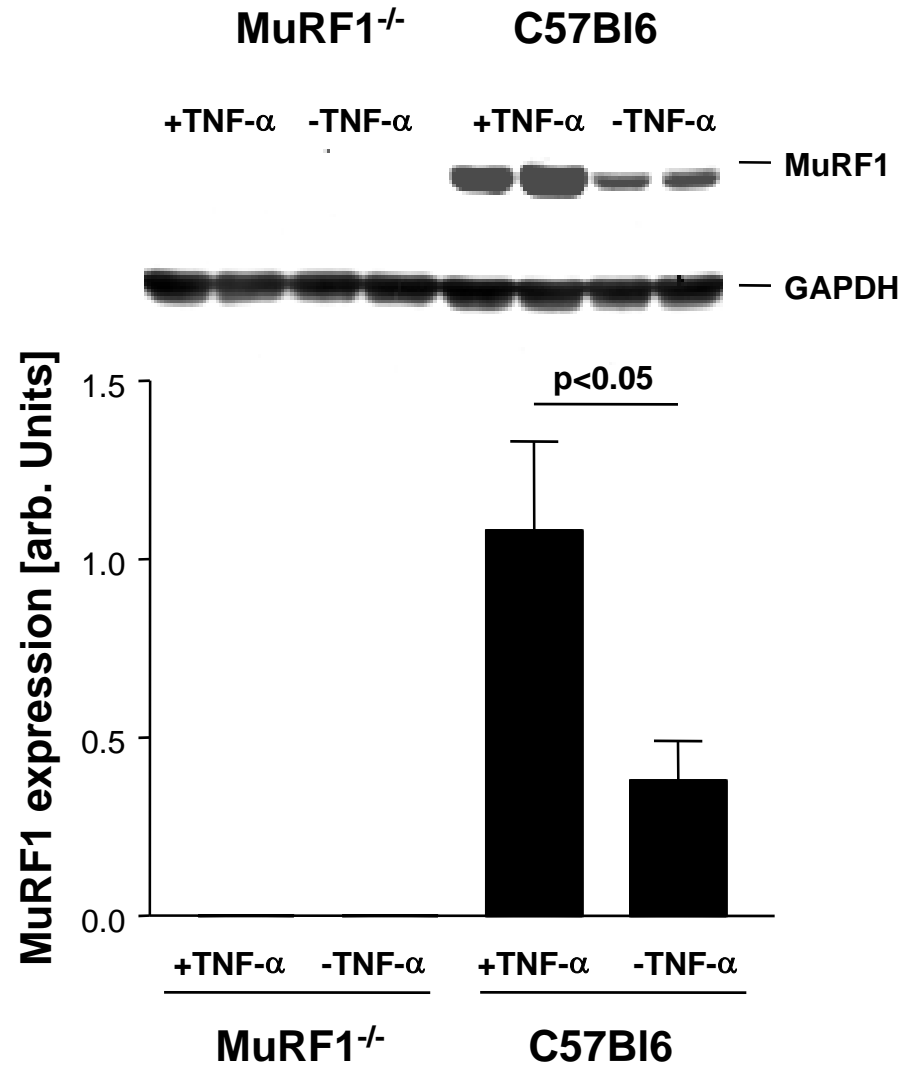
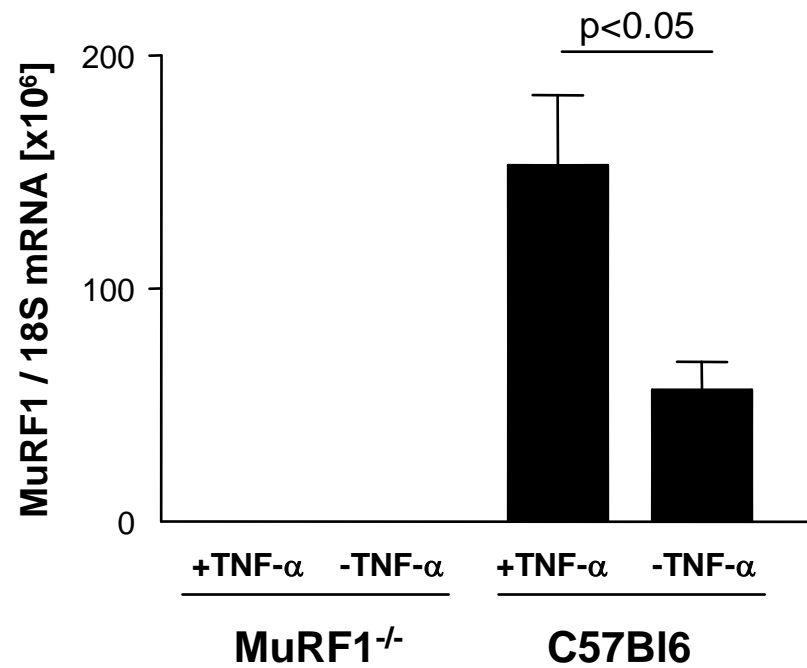


Study Design and Methods

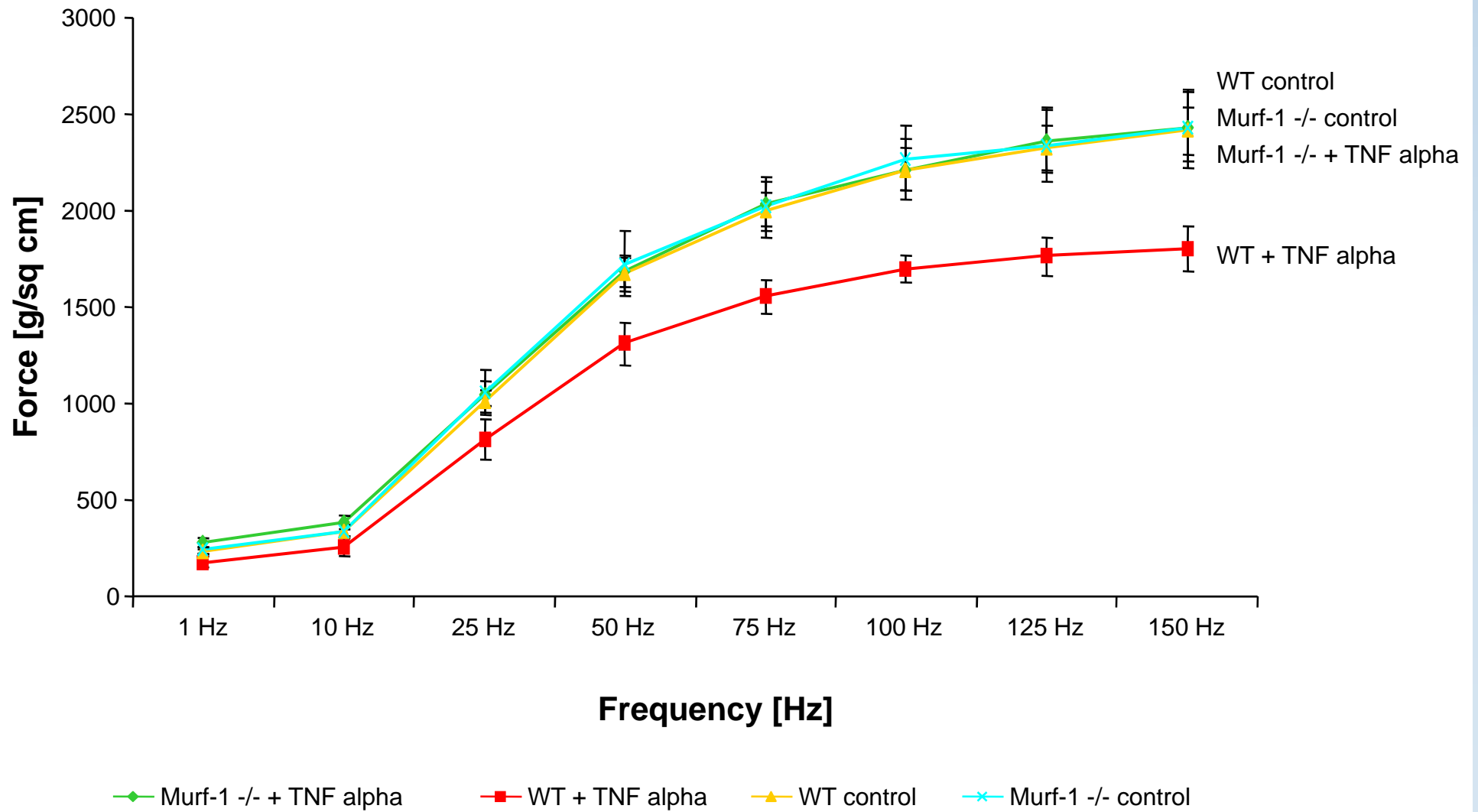


- Measurement of muscle contractility (soleus, force-frequency relation)
- Expression analysis of MuRF1 and contractile proteins

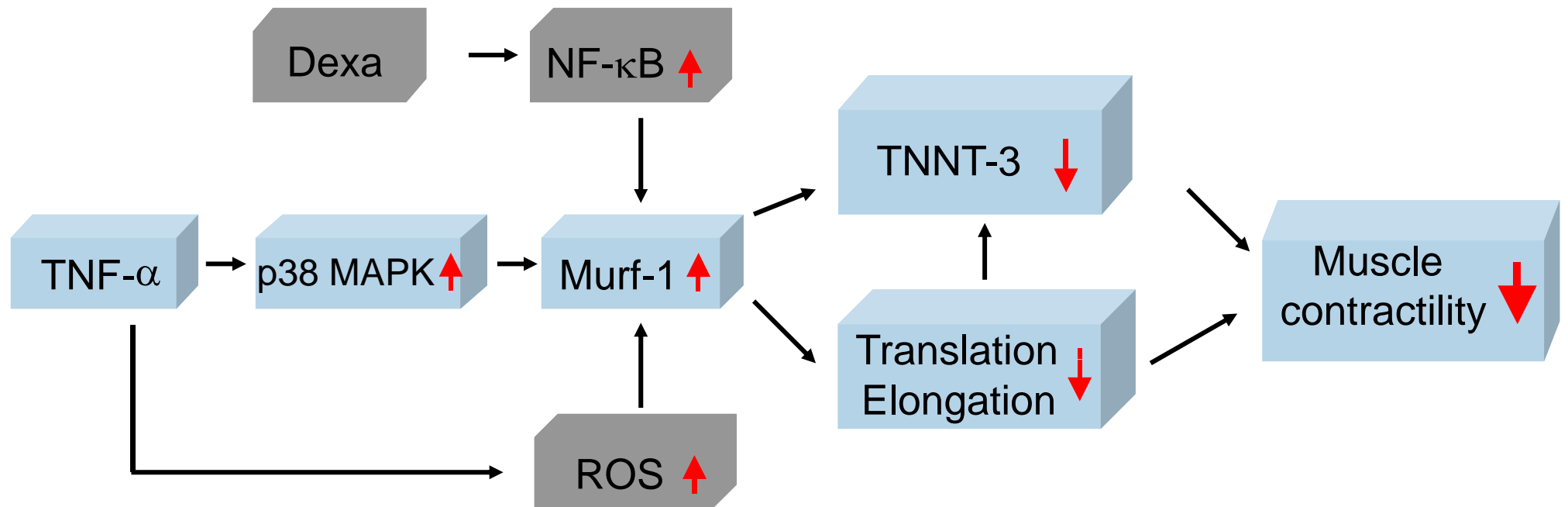
Murf-1 Protein Expression after TNF- α IP injection



Force Frequency Relation in Soleus Muscle

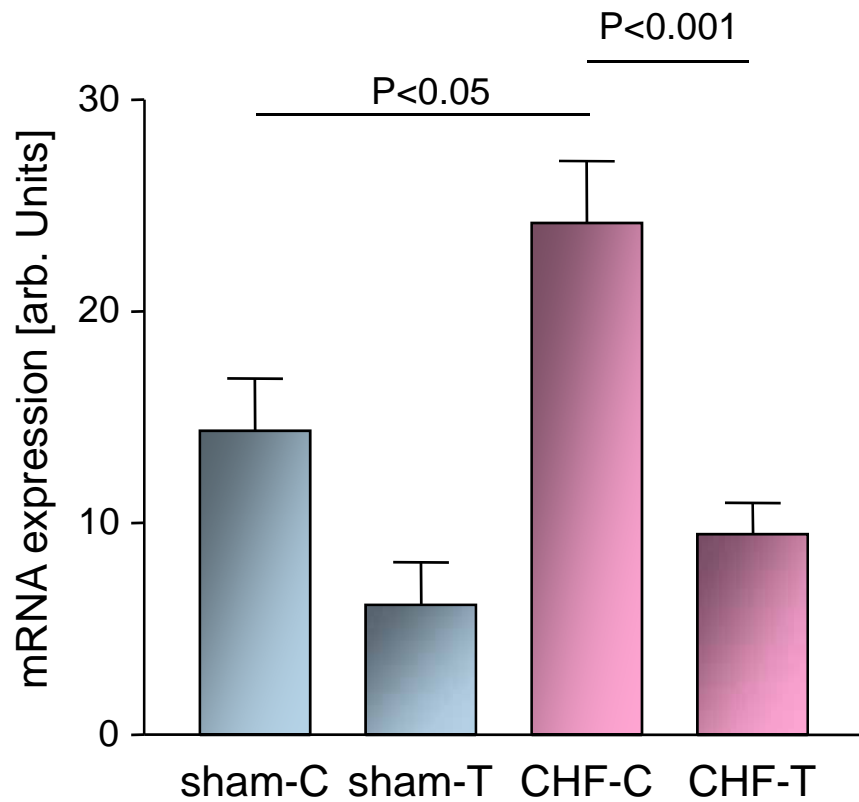


Pathophysiological Model for the TNF- α Induced Reduction in Muscle Contractility

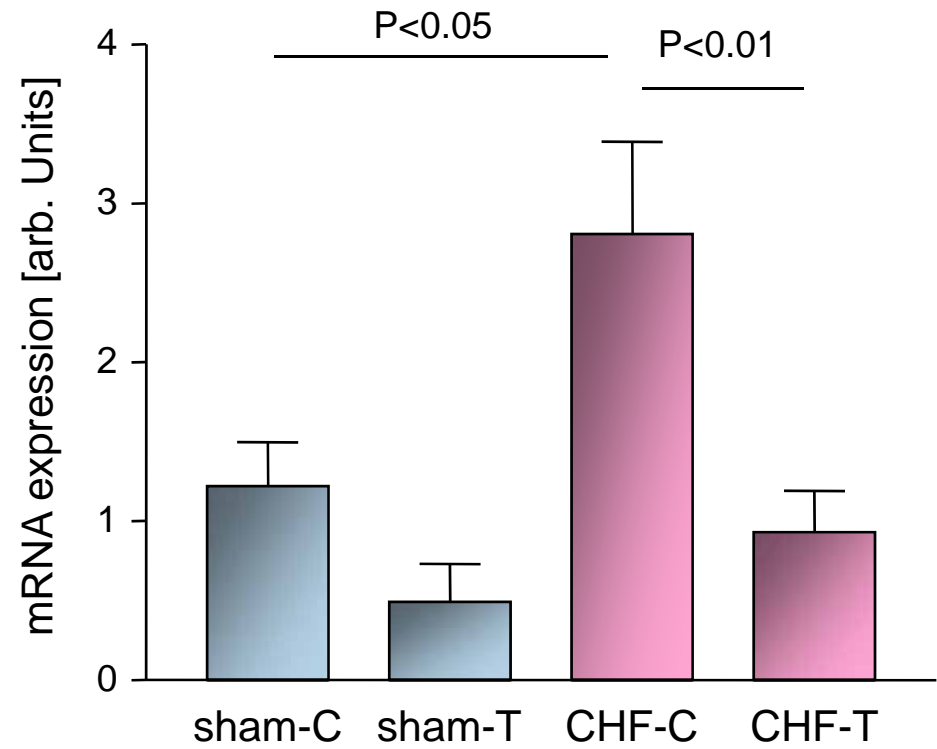


Exercise Training and MAFbx / Murf-1 Expression - animal study -

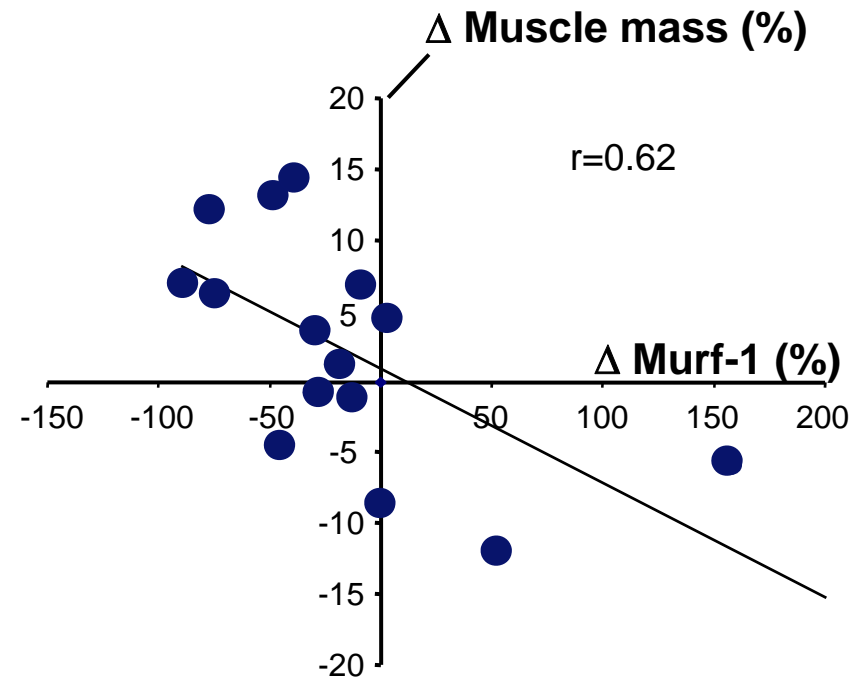
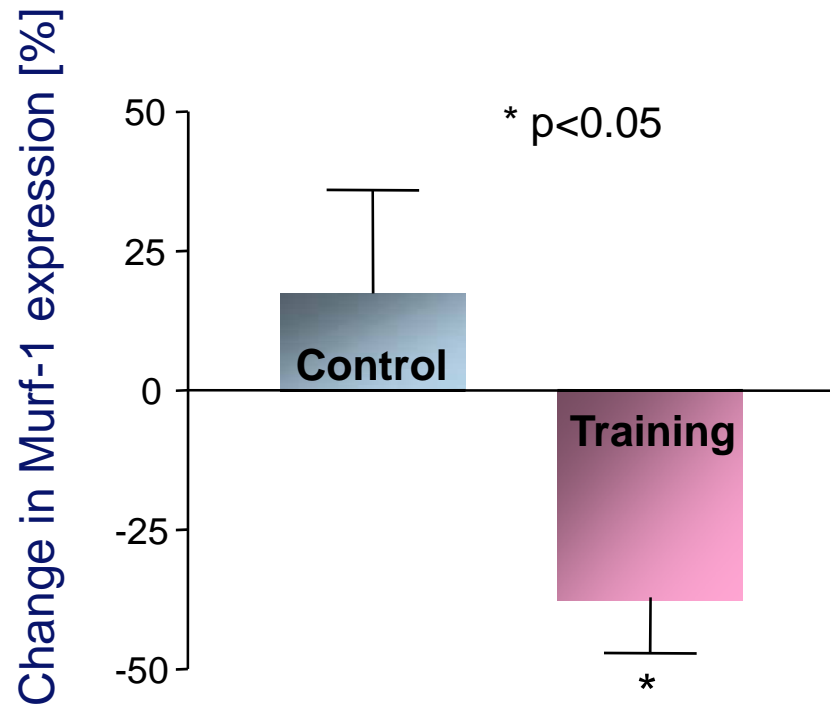
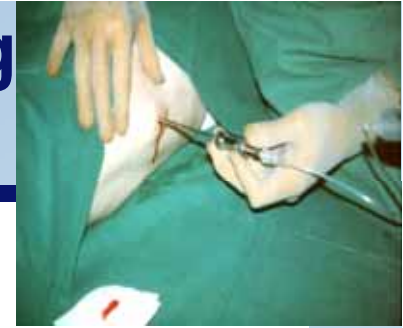
MAFbx



Murf-1



Change in Murf-1 expression by exercise training - NYHA III Study -

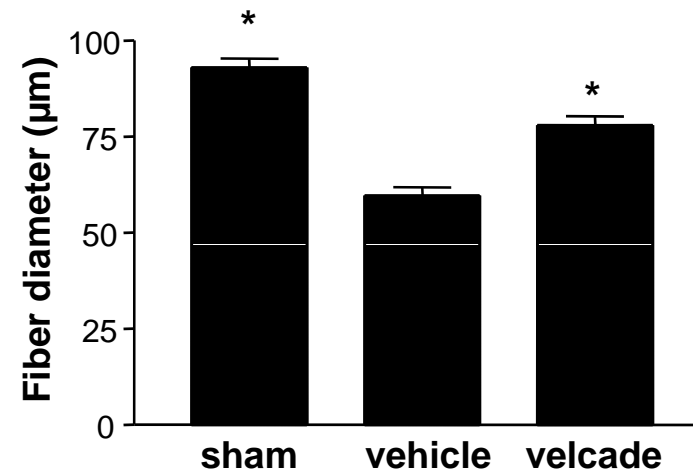
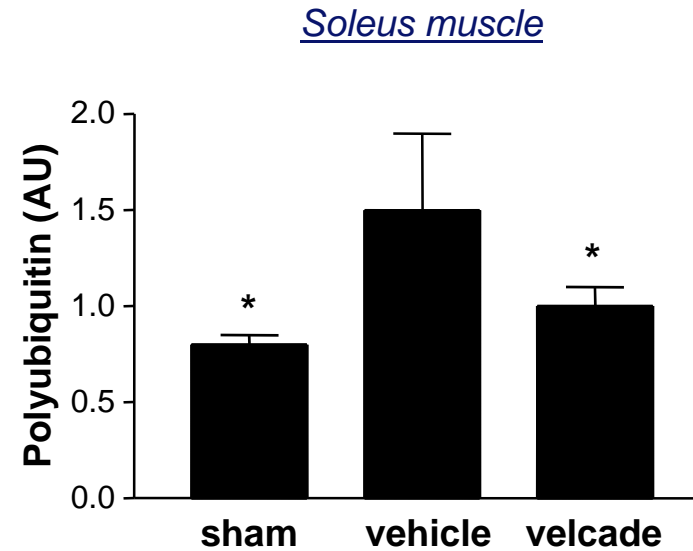
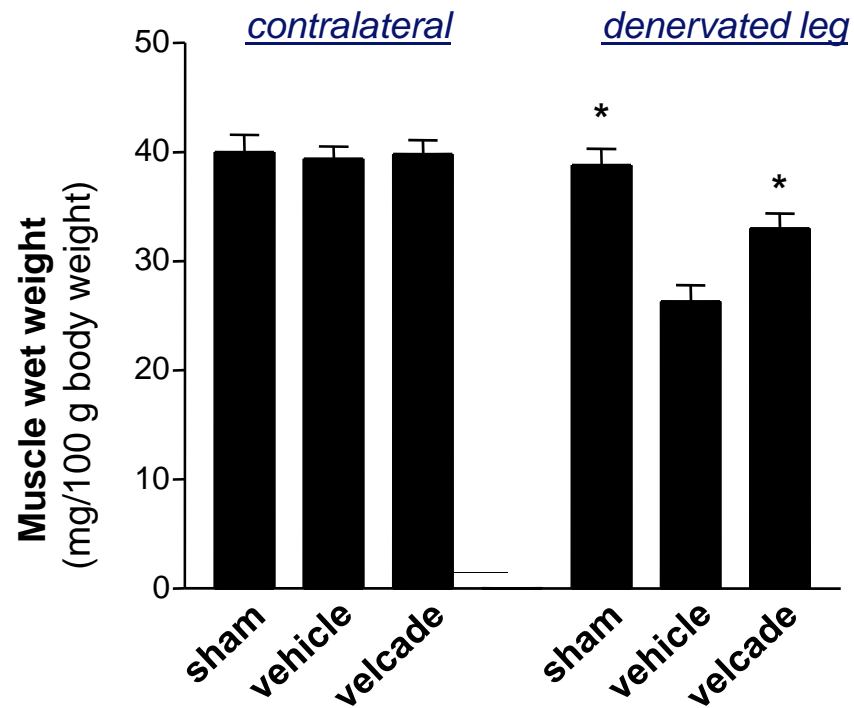


Reduction in Atrophy by Proteasome Inhibition

Rats with denervated hindlimb,
Contralateral leg served as control

Administration of vehicle
(7 days)

Administration of Velcade
(7 days, each day)



Examples of Mechanistic Basic Science Leading to the Design of an Intervention

- **Development of AS and exercise**
- **Loss of muscle mass**
- **MicroRNA**



Science in medicine

MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets

Eva van Rooij and Eric N. Olson

Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA.



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CLINICAL IMPLICATIONS
OF BASIC RESEARCH

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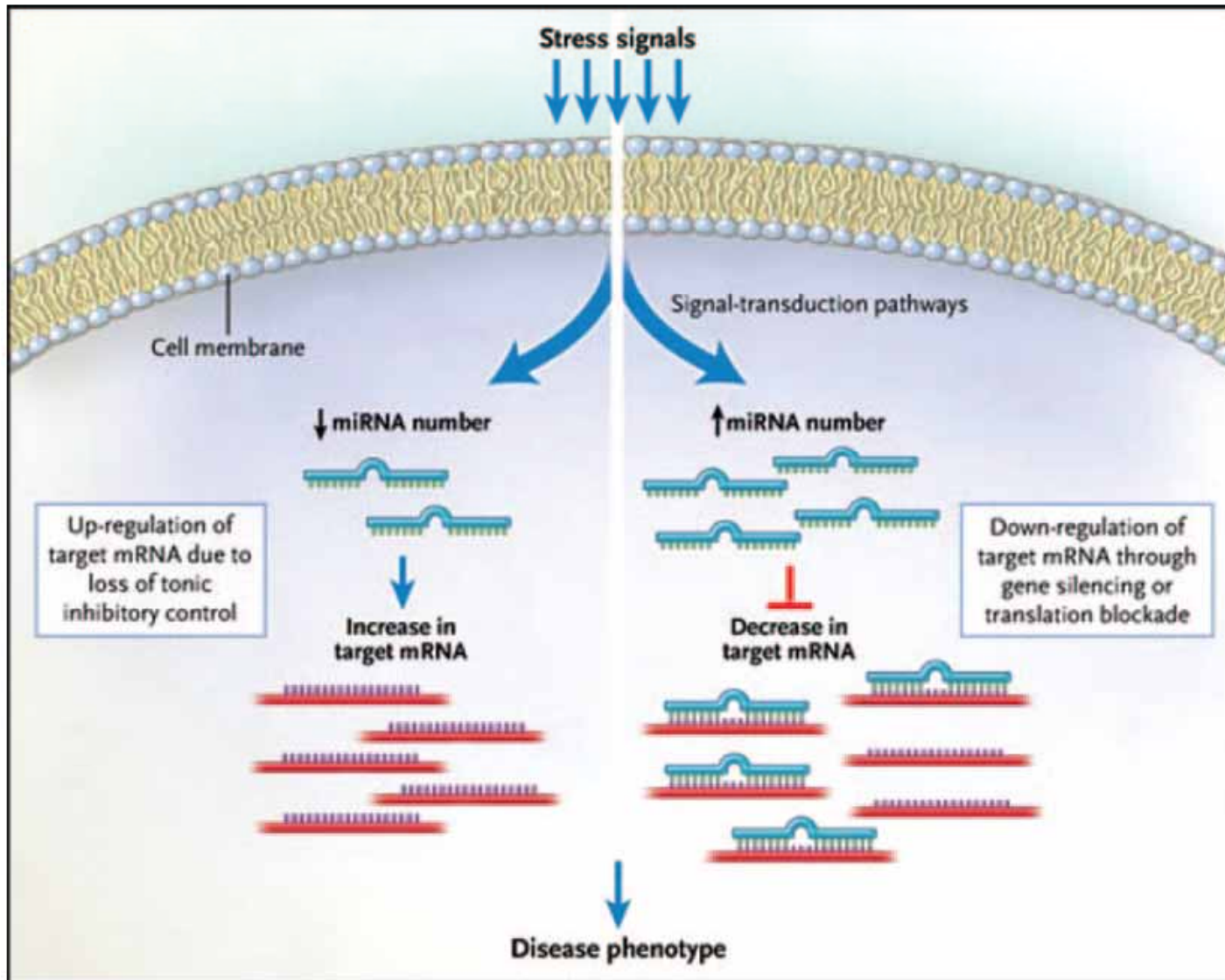
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MicroRNAs and the Failing Heart

Douglas L. Mann, M.D.

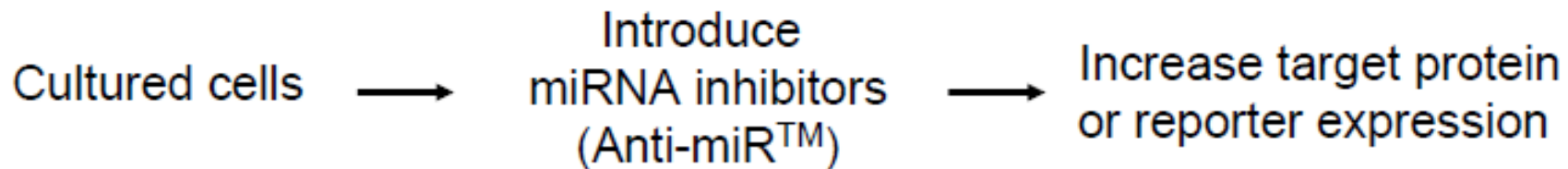
MicroRNAs (miRNA) are single-stranded RNA molecules of 21-23 nucleotides in length, which regulate gene expression. miRNAs are encoded by genes from whose DNA they are transcribed but miRNAs are not translated into protein (non-coding RNA); instead each primary transcript (a pri-miRNA) is processed into a short stem-loop structure called a pre-miRNA and finally into a functional miRNA. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down-regulate gene expression

MicroRNA (miRNA)



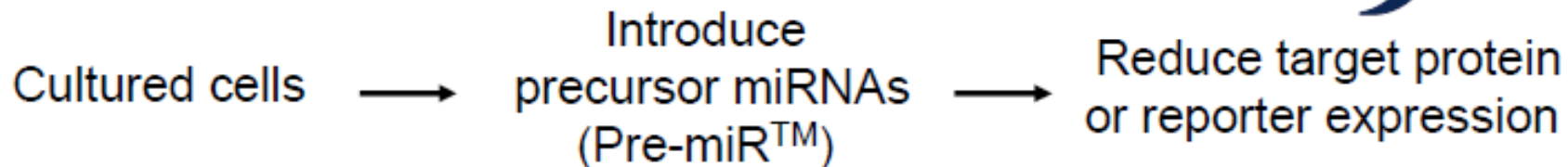
MicroRNA

Loss-of-function experiment

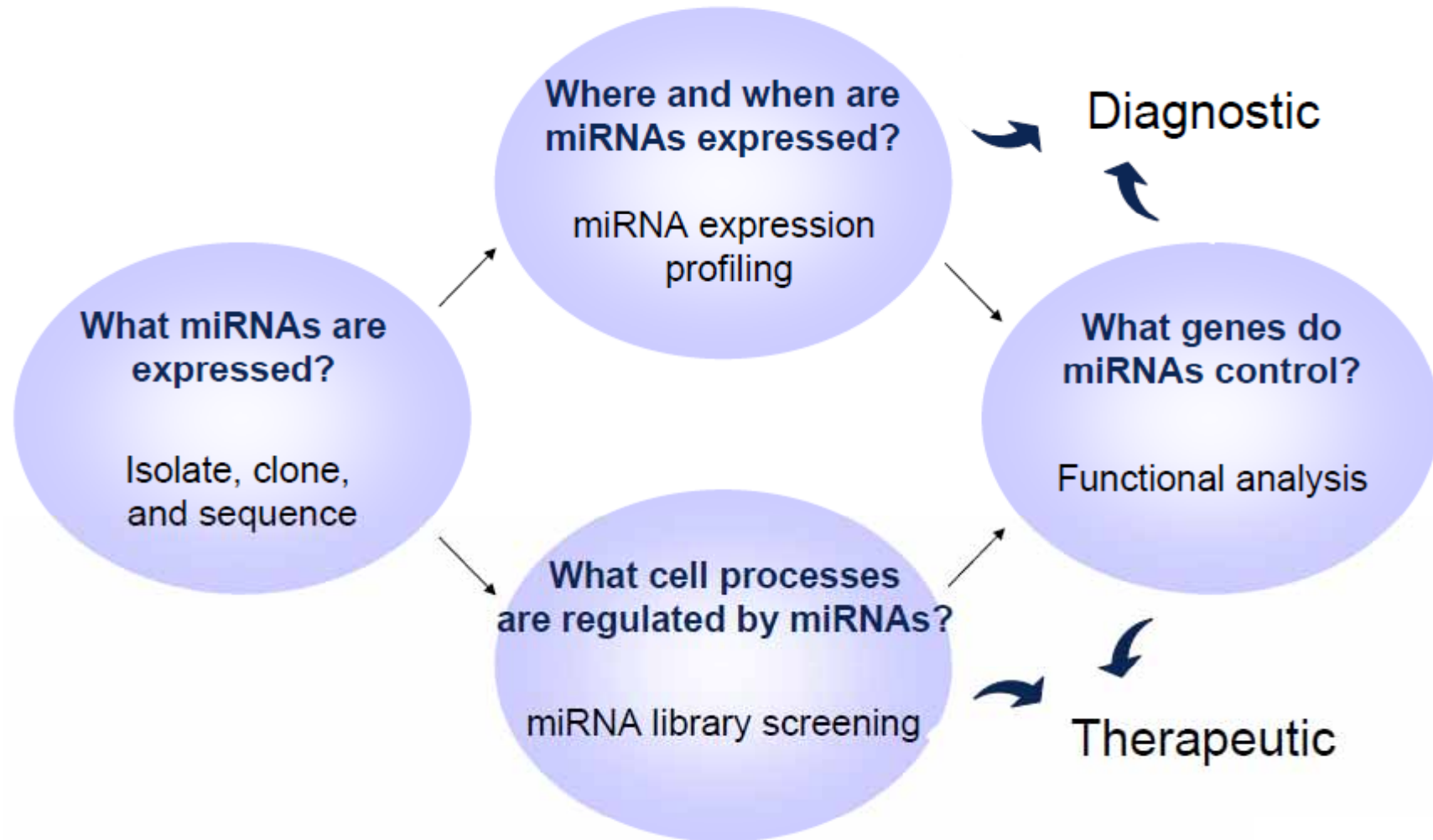


↓
Target gene ID
Gene function
Cellular process
Phenotype

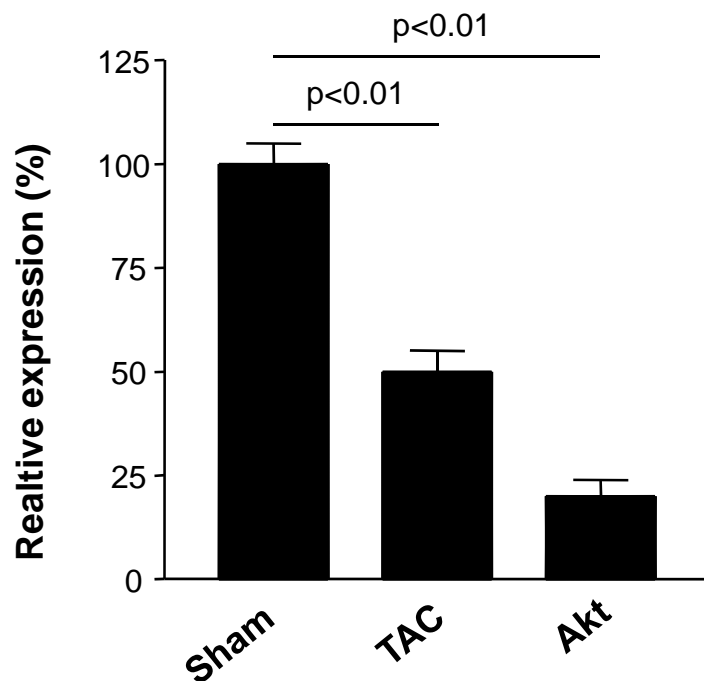
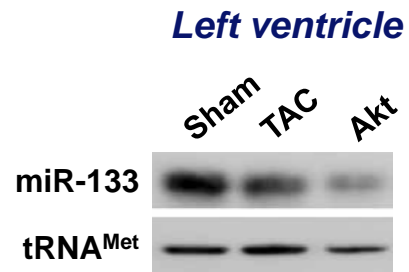
Gain-of-function experiment



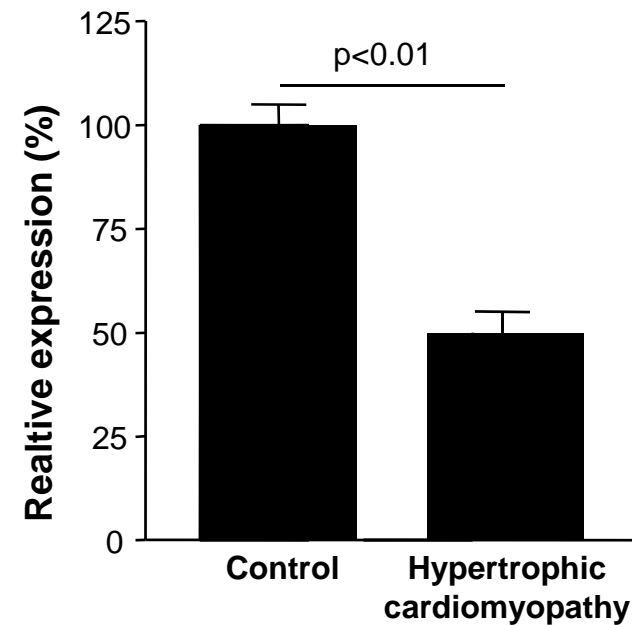
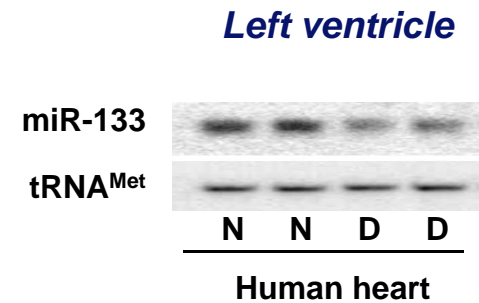
MicroRNA – Fundamental Questions



MicroRNA-133 and Cardiac Hypertrophy



TAC = transaortic constriction,
Akt = Akt transgenic mice



miR-133 is downregulated in cardiac hypertrophy

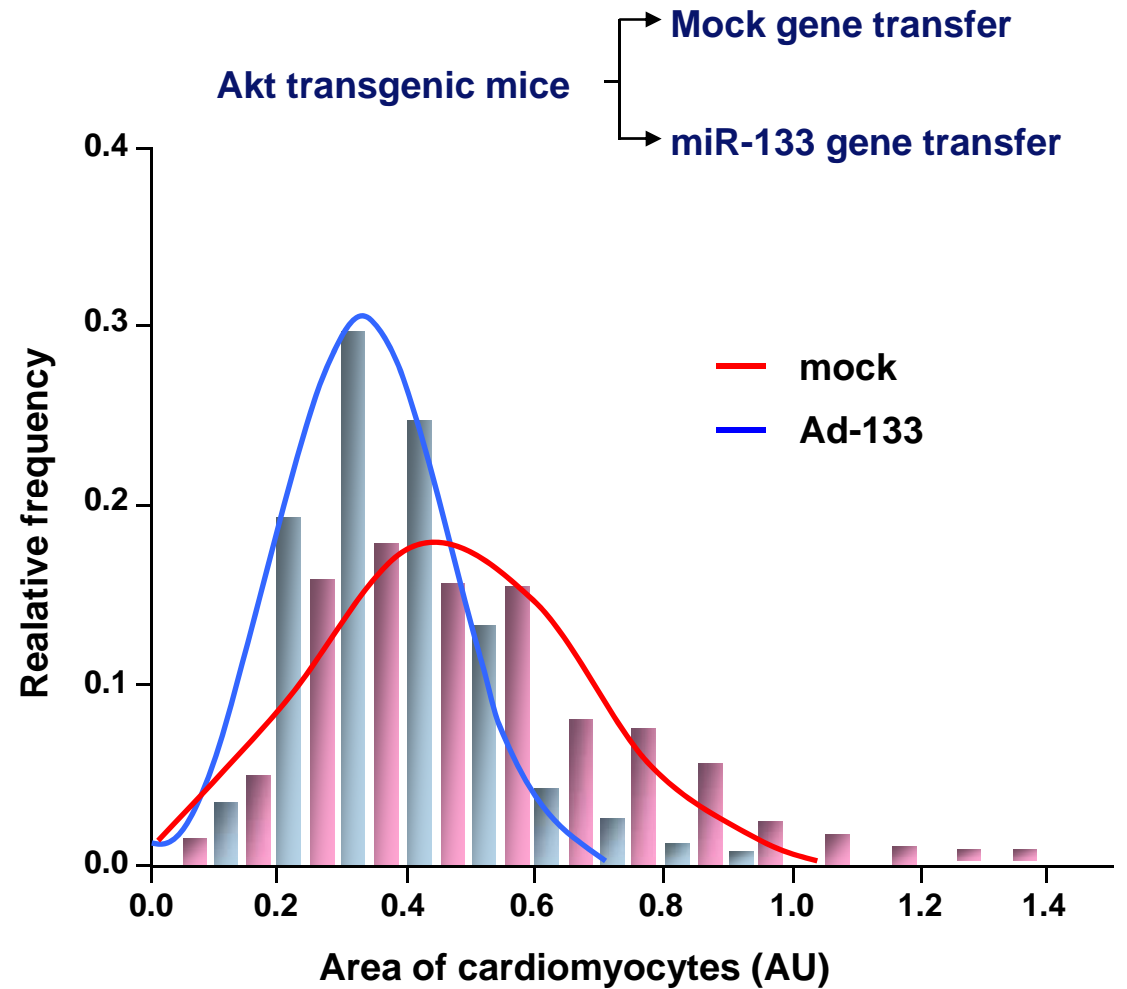
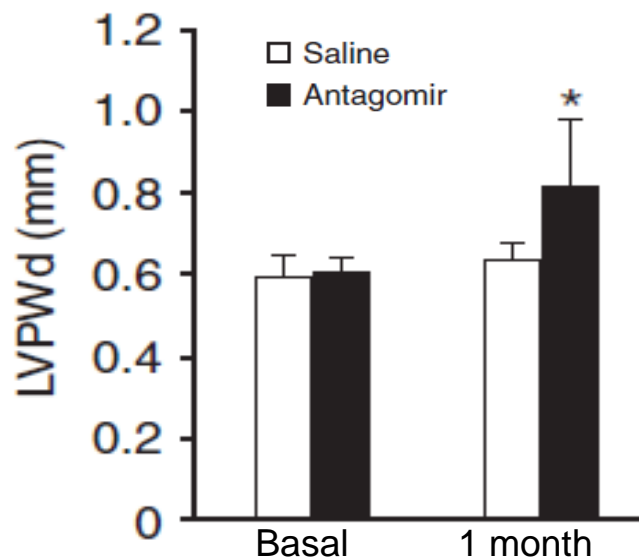
MicroRNA-133 and Cardiac Hypertrophy

- loss of function and gain of function -

1 month of antagomir 133 infusion



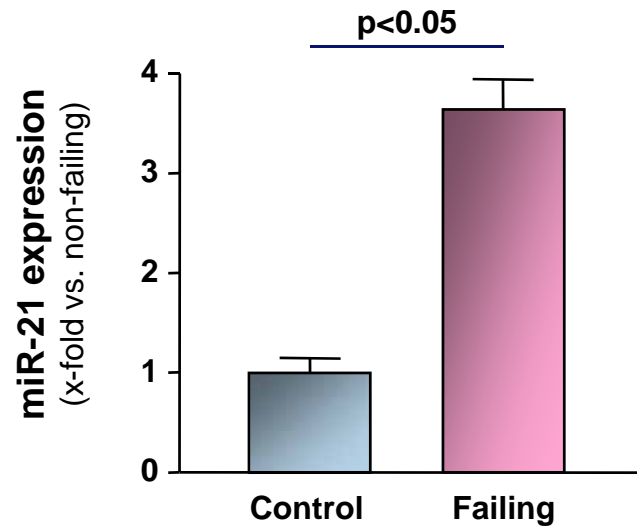
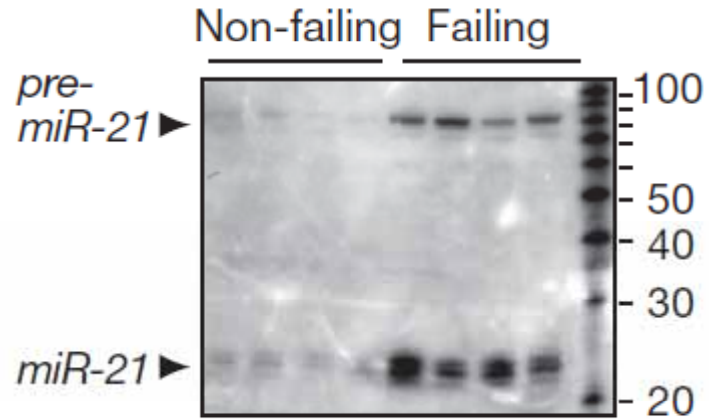
Diastolic left ventricular wall thickness



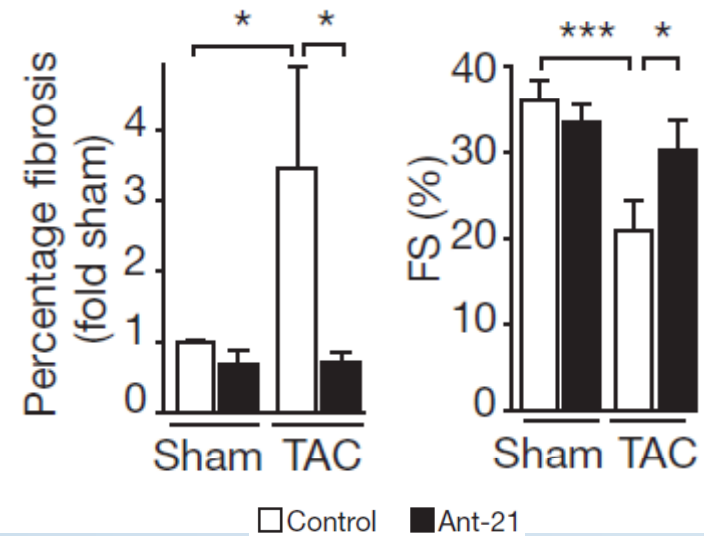
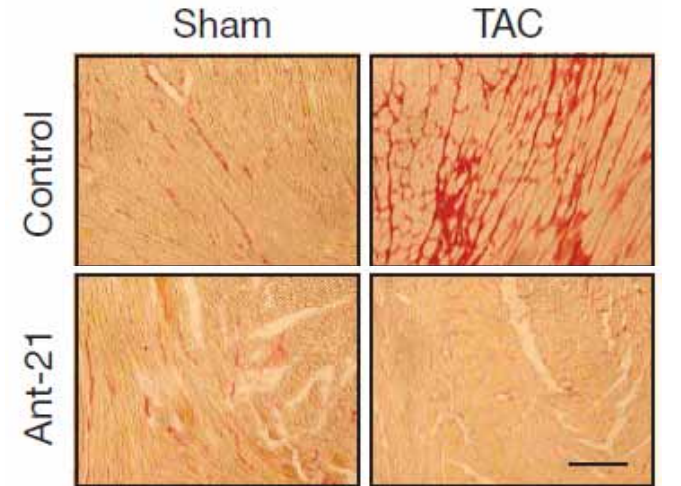
Presence of miR-133 attenuates cardiac hypertrophy

MicroRNA-21 and Failing Heart

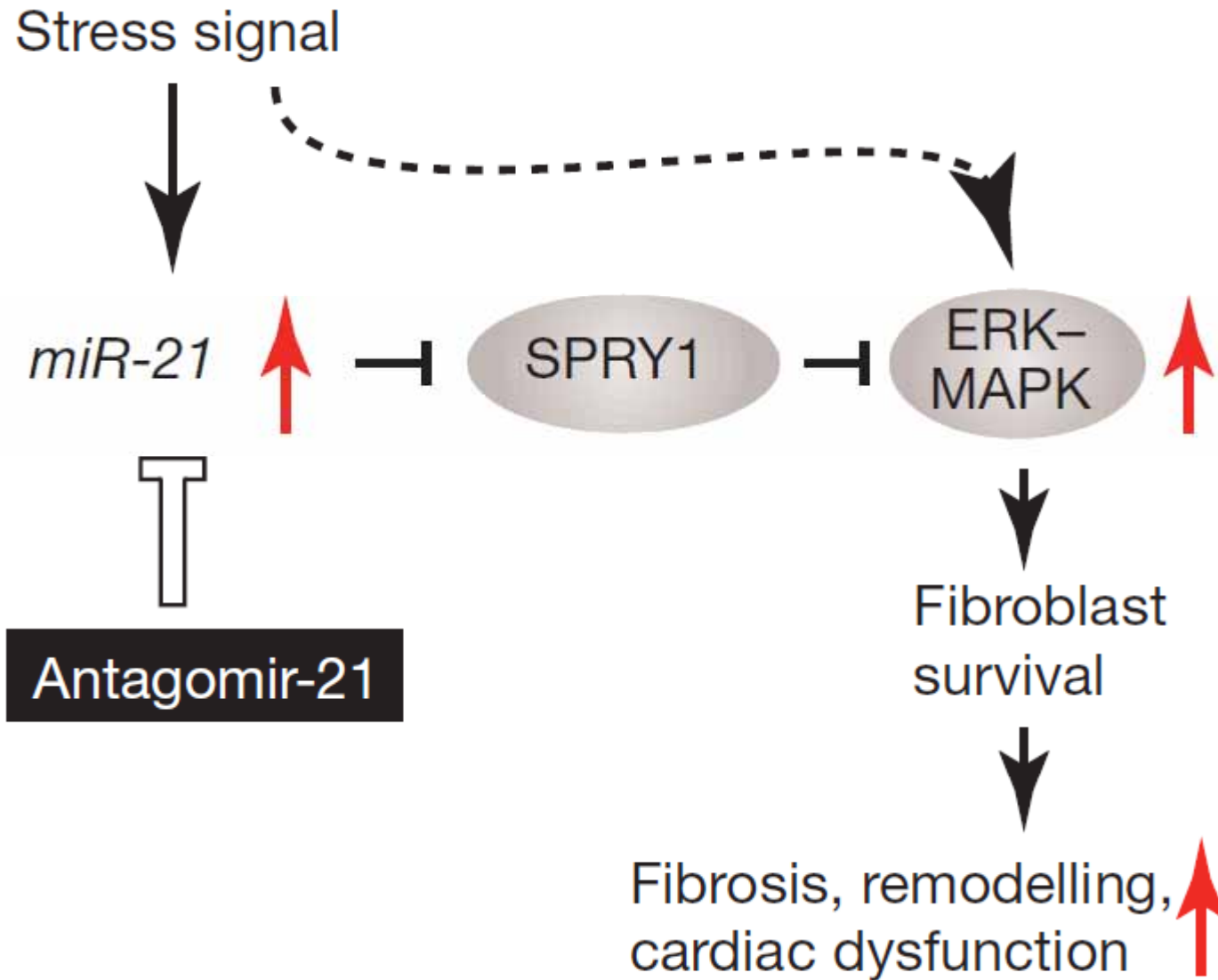
Human left ventricular myocardium



Sirius red staining for fibrosis



Working Model of miR-21 in Failing Heart



Why Mechanistic Basic Science

