




# CIRCULATING MicroRNAs IN RESPONSE TO EXERCISE AND MUSCLE INJURY

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*UMR-MD-1197*



# Circulating miRNAs as early markers of skeletal muscle injury

## Issues:

- Diagnostic of myofiber injury
- Prognosis
- Traumatology, toxicology and/or multi organ failure (MOF)

## Current problems:

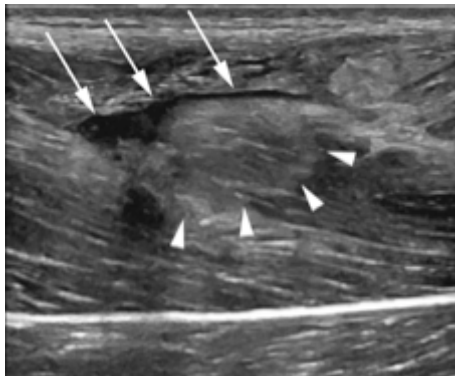
- High inter-individual variability of the routinely used markers (Creatine Kinase activity)
- Limited specificity

# Skeletal muscle injuries

- Intense exercise induces a large number of muscle injuries
- Exercise in the heat is a risk factor for heat stroke, with MOF including rhabdomyolysis

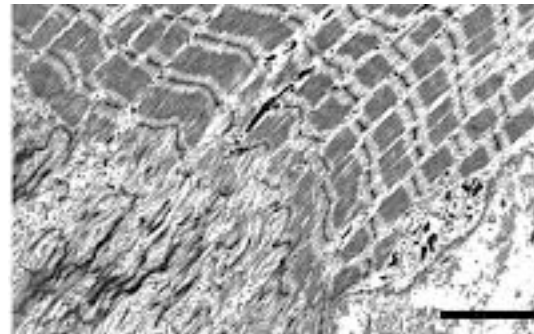


Macroscopic, focal injury



Crema, 2017

Microscopic injuries  
(ranging from pain to rhabdomyolysis)

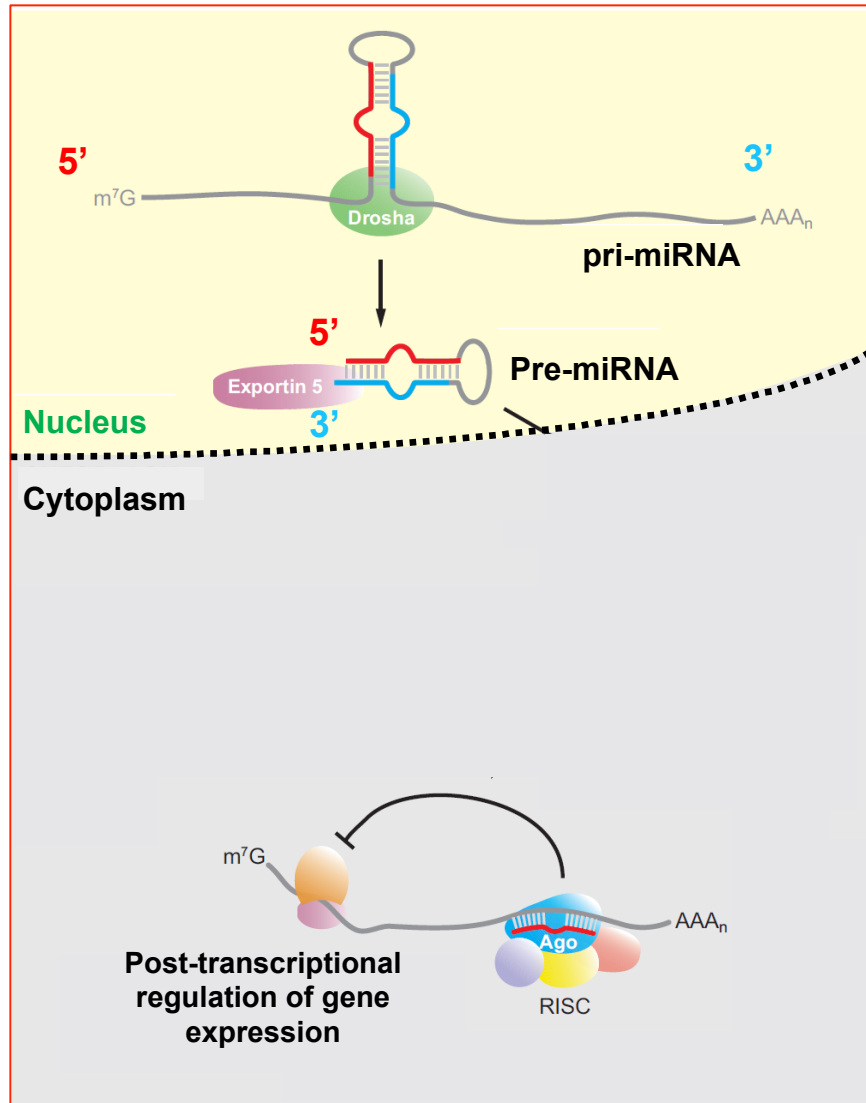


Tidball, 2011



Can circulating miRNA be biomarkers of muscle injury?

# Introduction to miRNAs



Adapted from Chang & Mendell, 2007

microRNA = miRNA = miR

- Small non-coding RNAs  
≈ 19 – 24 nucleotides
- Involved in the post-transcriptional regulation of gene expression

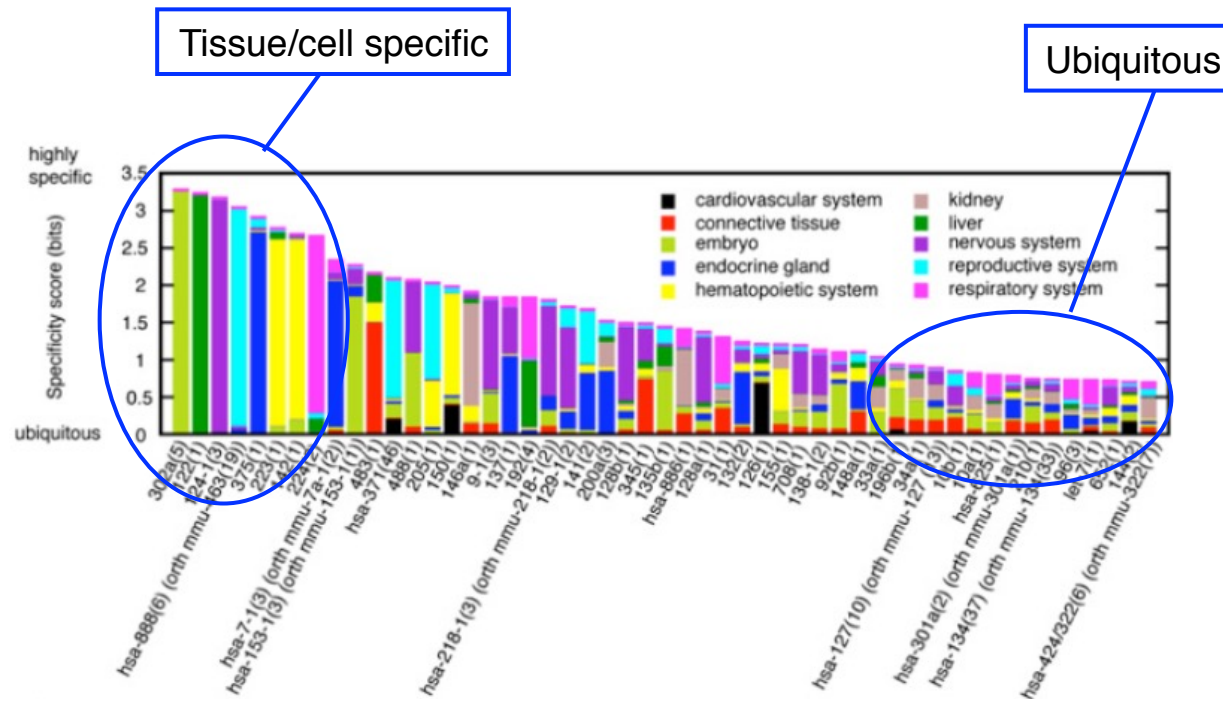
Nomenclature :

hsa-miR-133a-3p

specie  
number  
variant  
Position

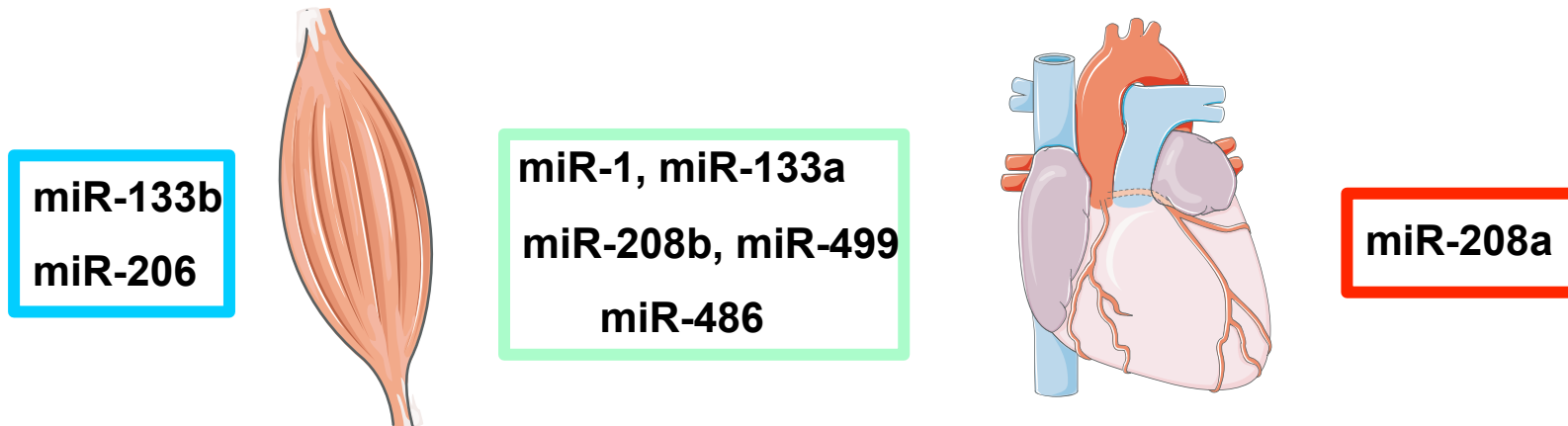
Exception : let-7 family

# Tissue specificity of miRNAs



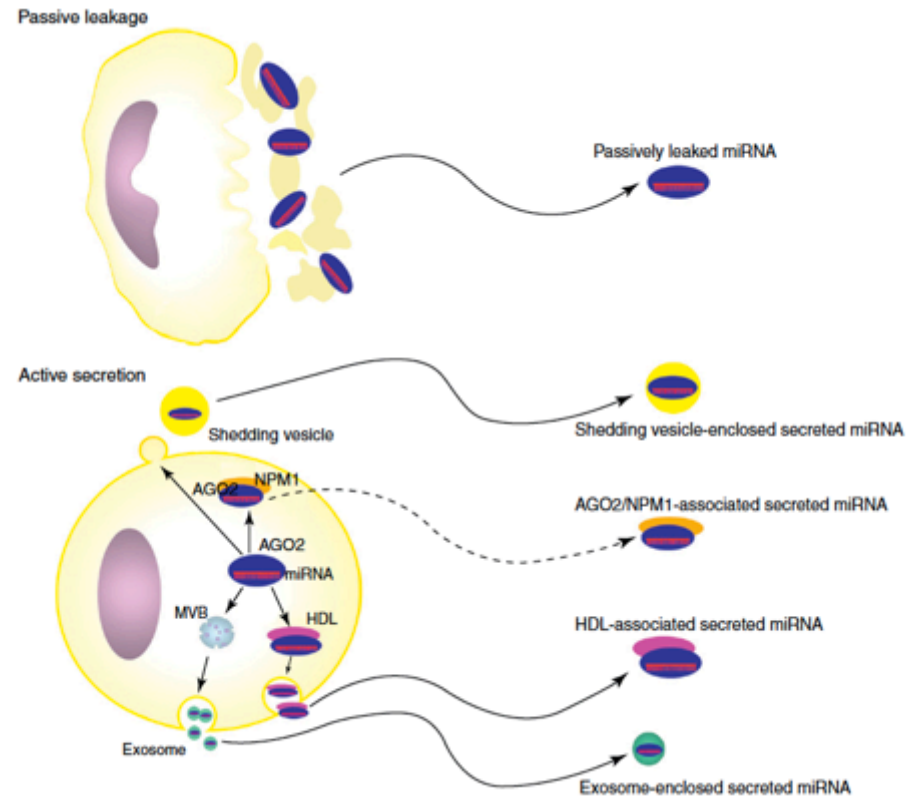
# Muscle miRNAs: myomiRs

Some miRNAs are muscle-specific or muscle-enriched



Involved in various biological processes including muscle cell proliferation or differentiation

# Extracellular miRNAs



Chen, 2012

miRNAs are found in most biofluids, including plasma, saliva and urines.  
miRNAs are biomarkers of some cancers



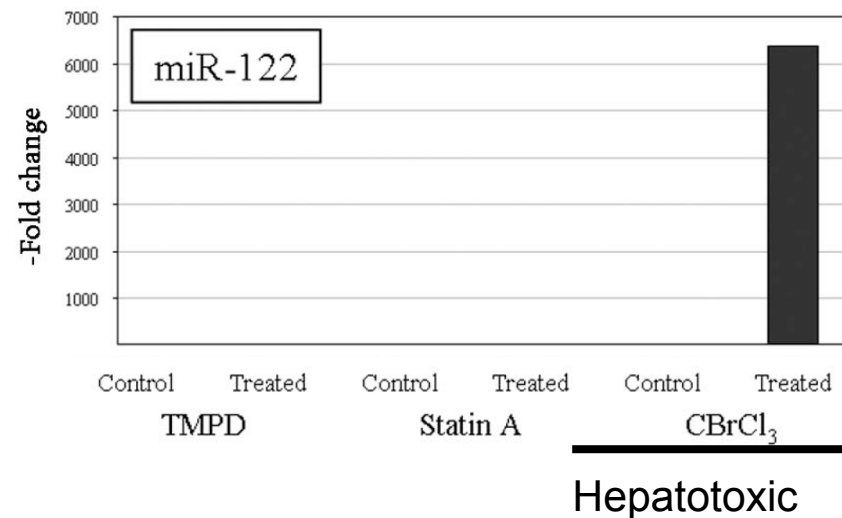
# miRNAs as biomarkers of tissue injury

Clinical Chemistry 55:11  
1977–1983 (2009)

Molecular Diagnostics and Genetics


## Plasma MicroRNAs as Sensitive and Specific Biomarkers of Tissue Injury

Omar F. Laterza,<sup>1</sup> Lee Lim,<sup>2</sup> Philip W. Garrett-Engle,<sup>3</sup> Katerina Vlasakova,<sup>4</sup> Nagaraja Muniappa,<sup>4</sup>  
Wesley K. Tanaka,<sup>1</sup> Jason M. Johnson,<sup>3</sup> Joseph F. Sina,<sup>4</sup> Thomas L. Fare,<sup>5</sup> Frank D. Sistare,<sup>4</sup> and  
Warren E. Glaab<sup>4\*</sup>



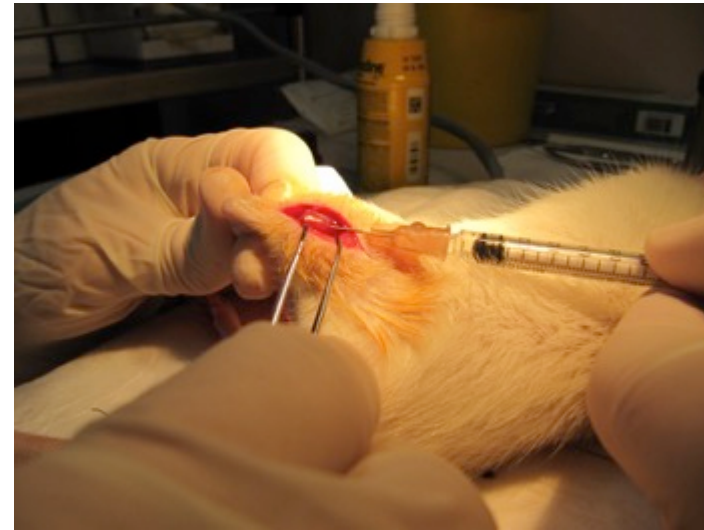
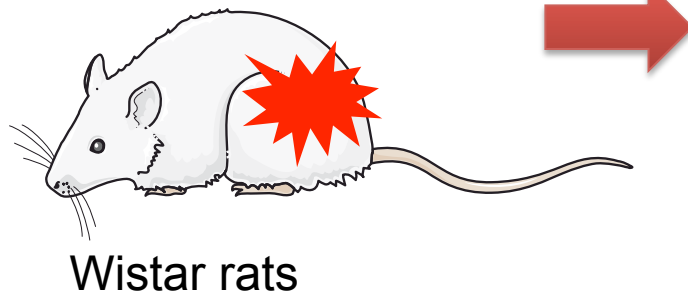
Laterza et al., 2009



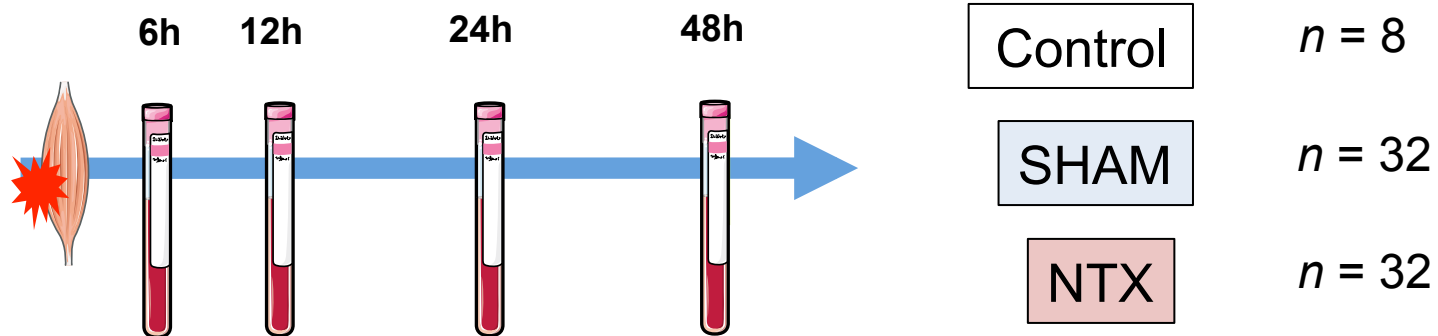


Can circulating miRNA be used as biomarkers of acute muscle injury ?

# Methodology



Notexin injection in *soleus* muscle

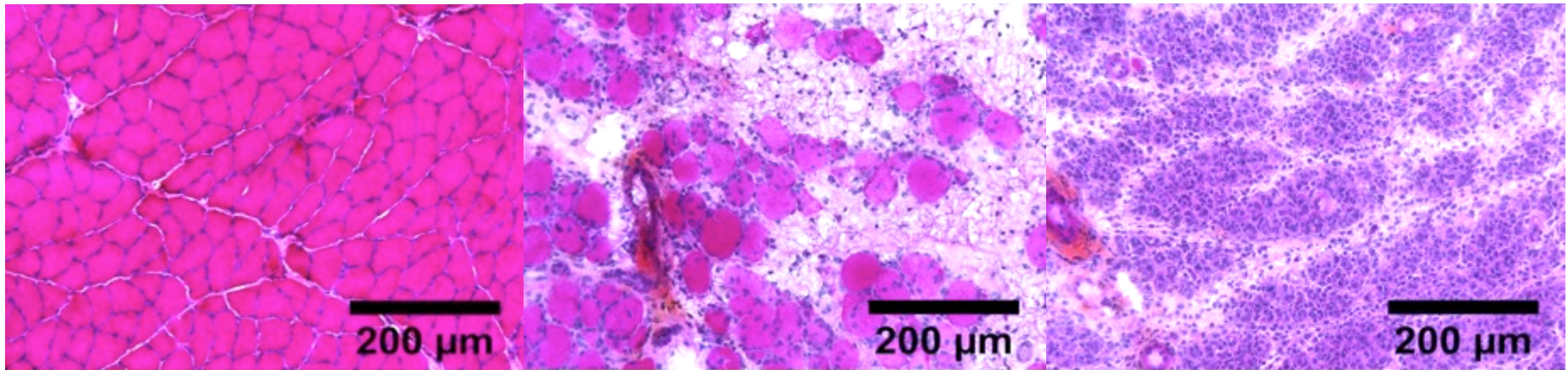


# ○ — Characterization of muscle injury

Control

NTX 24h

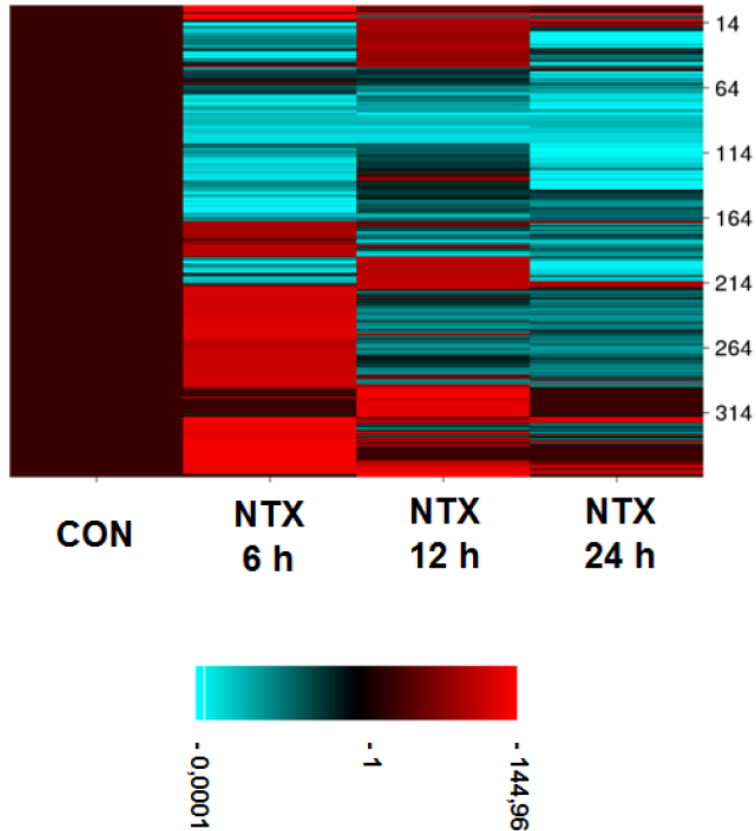
NTX 48h



HES staining

- Extensive necrosis
- Oedema
- Mononuclear cells infiltration

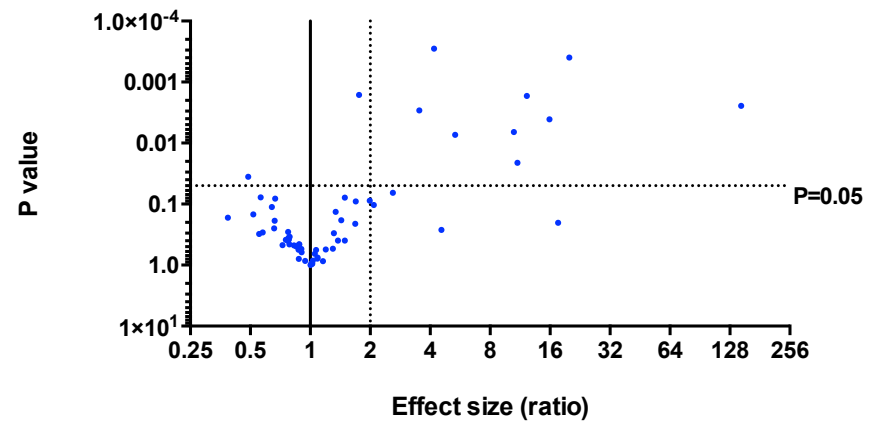
# PCR-array profiling and miRNA selection



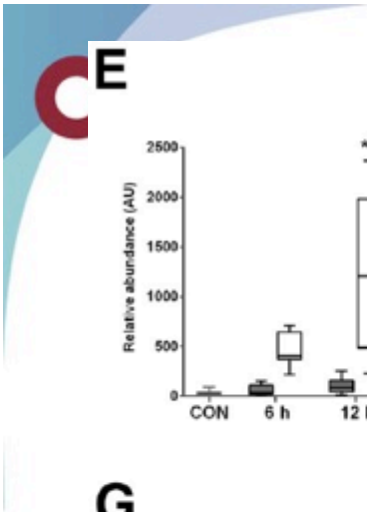
780 miRNA on pools of plasma

## Selection of 74 miRNA

- *Fold-change*  $\geq 1.5$  as compared to controls
- Stable miRNA (references)
- Cardiac myomiR miR-208a

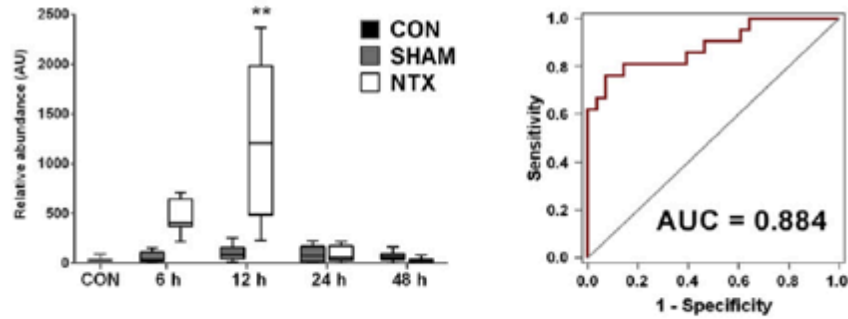


Candidates,  $n = 8$



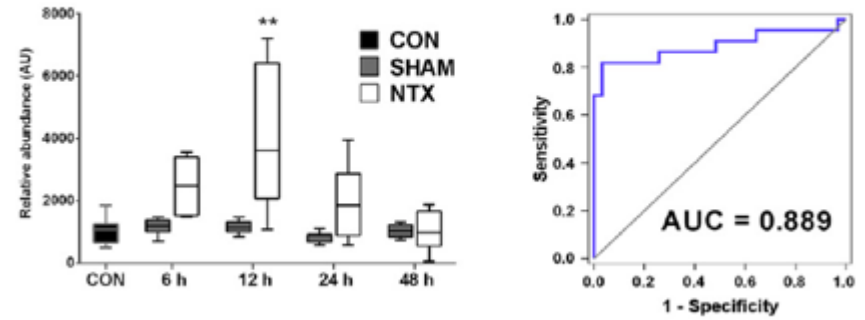
**E**

**rno-miR-1-3p**



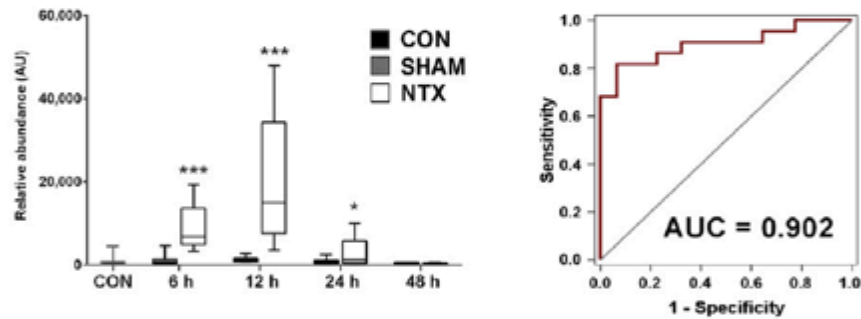
**K**

**rno-miR-378a-3p**



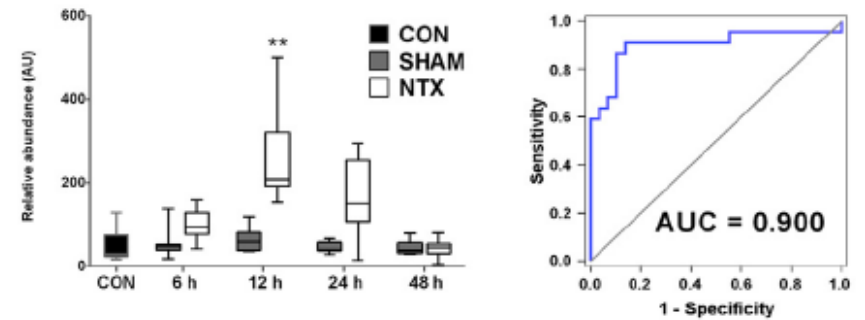
**G**

**rno-miR-133b-3p**



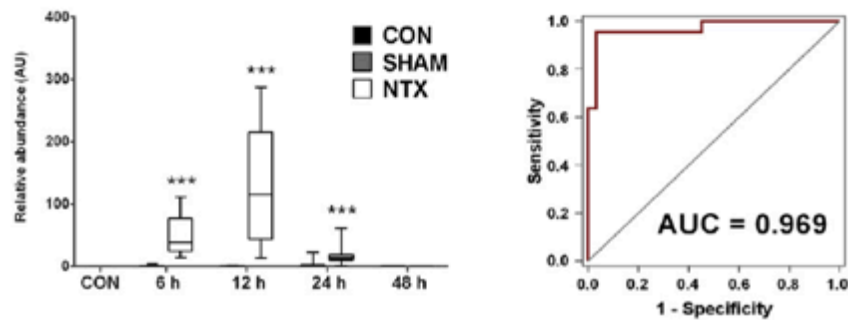
**L**

**rno-miR-434-3p**



**I**

**mmu-miR-208b-3p**



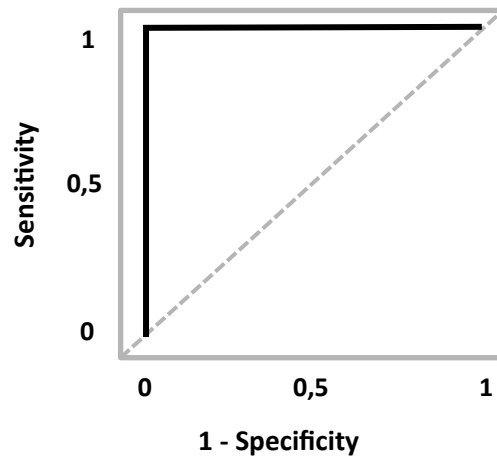
Non muscle-specific miRNA

MyomiRs

Siracusa, 2016

# Evaluating diagnostic performances

Receiver-operating characteristic (ROC) curves



Area under the curve (AUC)

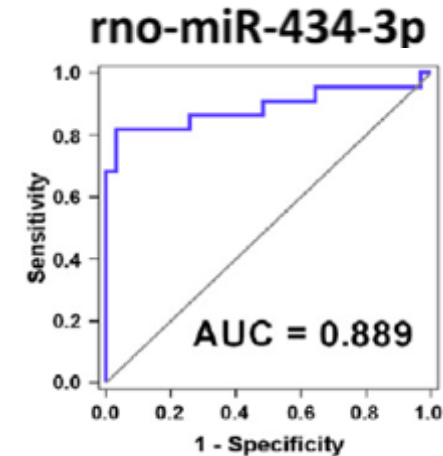
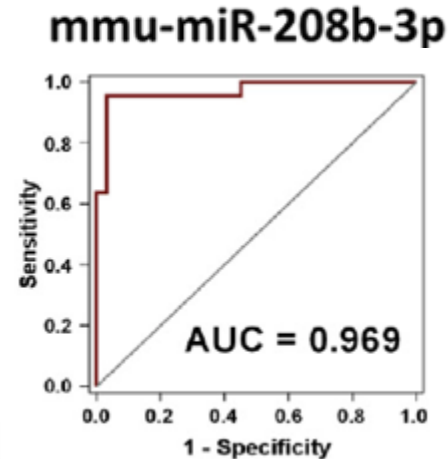
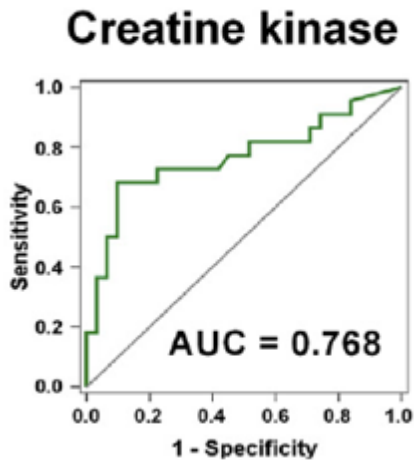
AUC = 0.5

Non informative biomarker

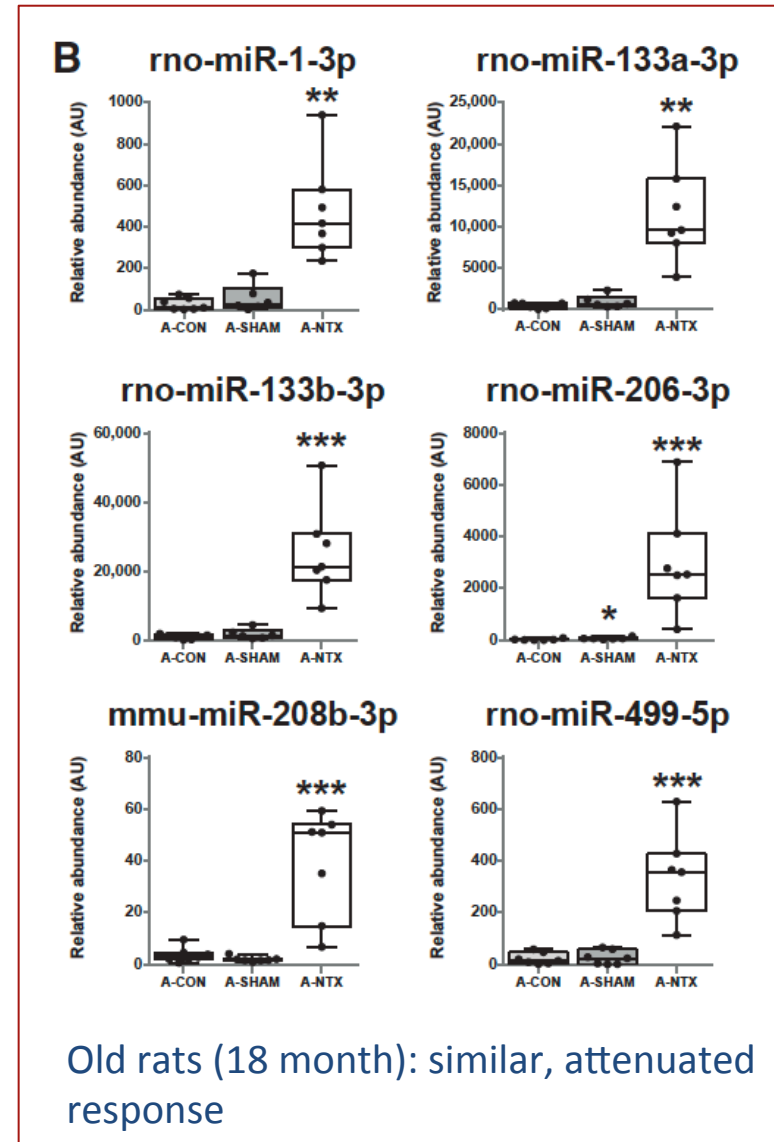
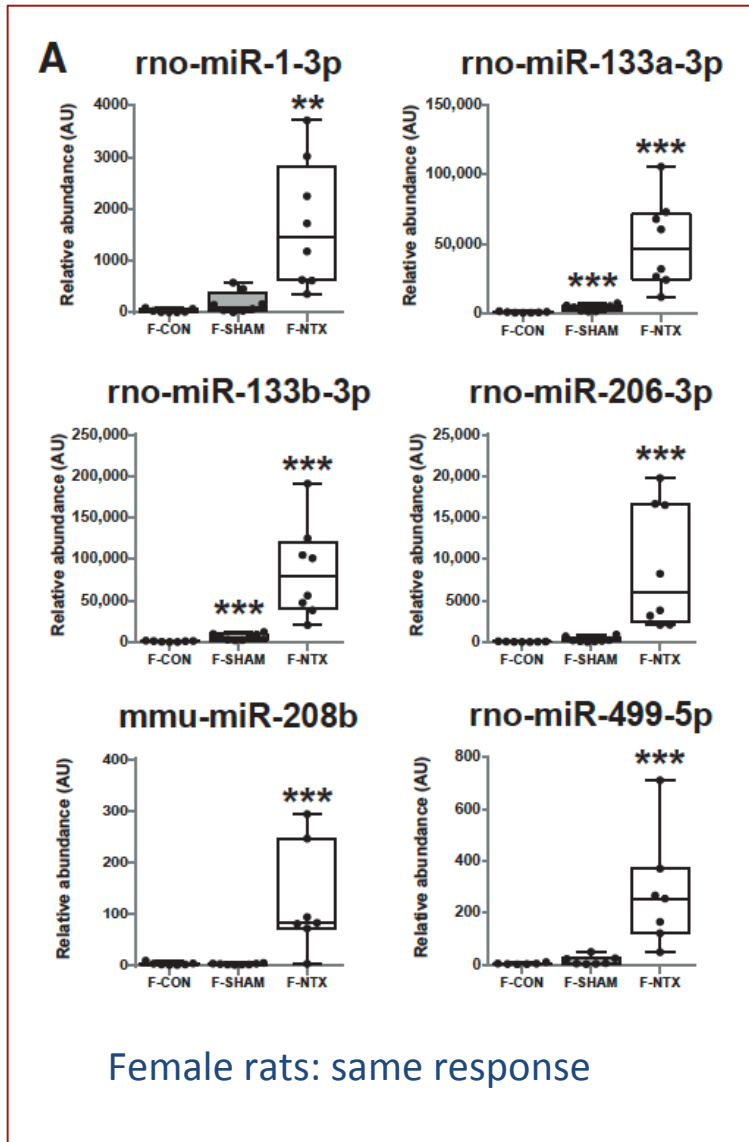
AUC = 1

Perfect biomarker

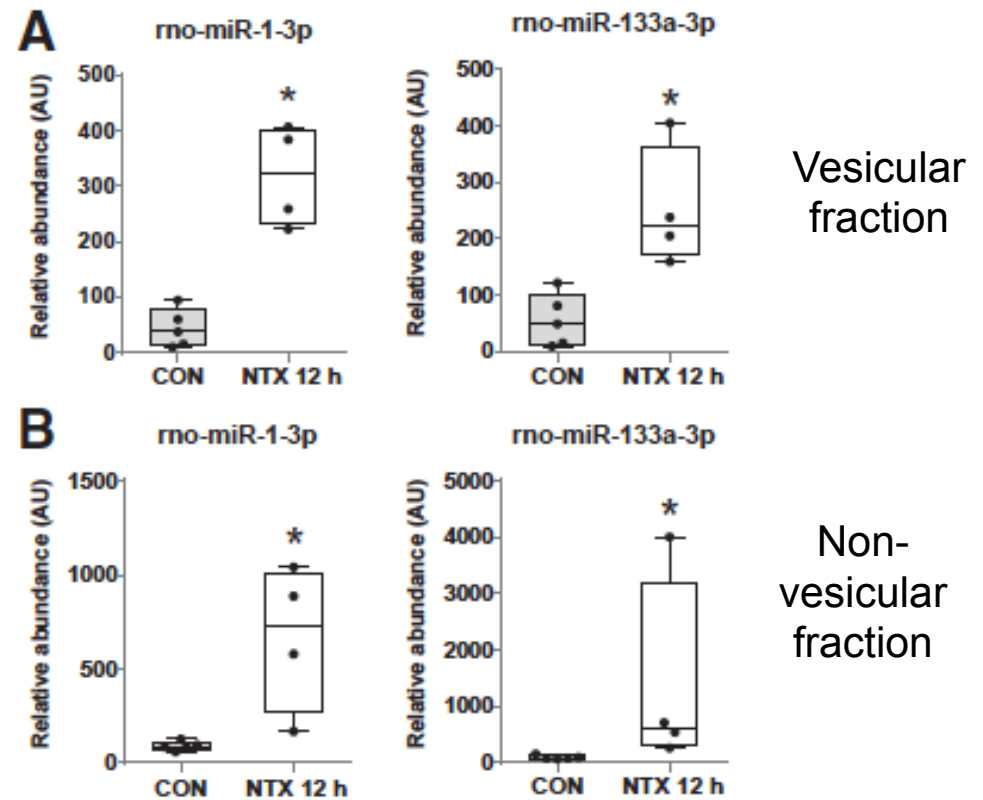
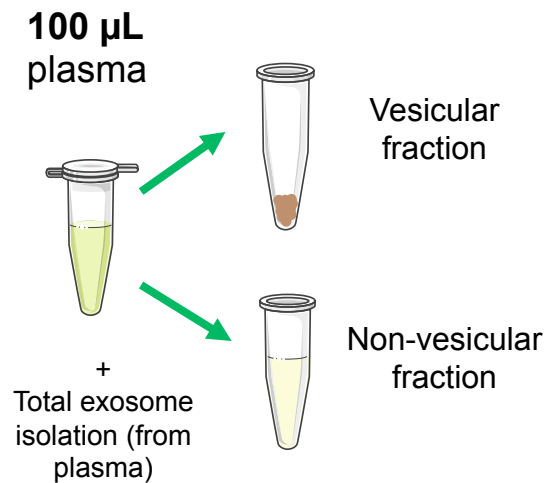
ROC curves analysis in the first 24 hours



# Influence of gender and age

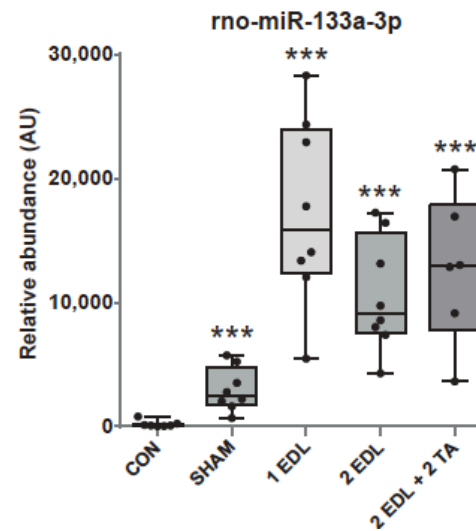
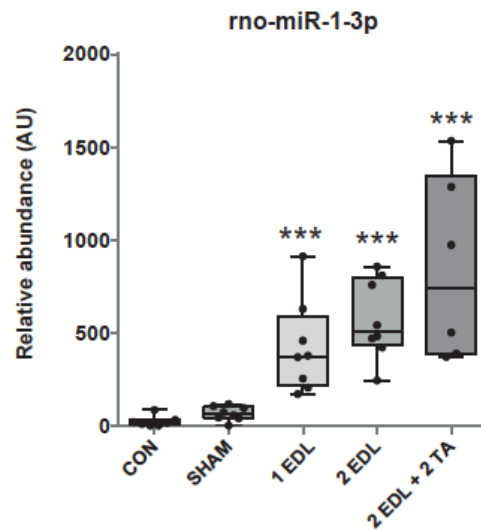


# Vesicular versus non vesicular fraction of the plasma?





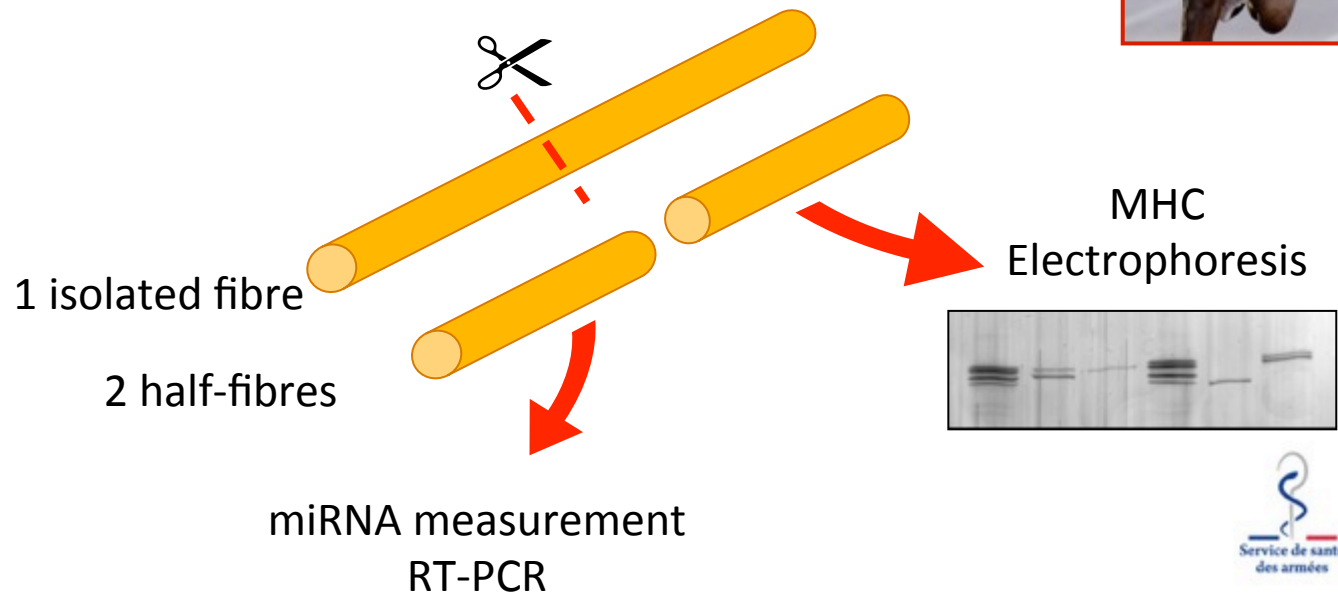
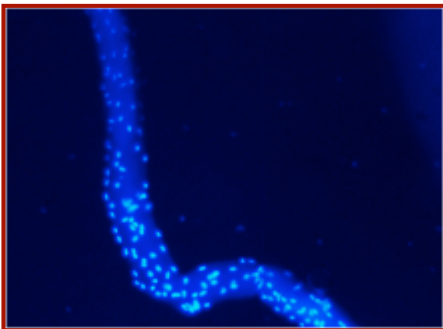
# Is the miRNA response proportional to the injury?



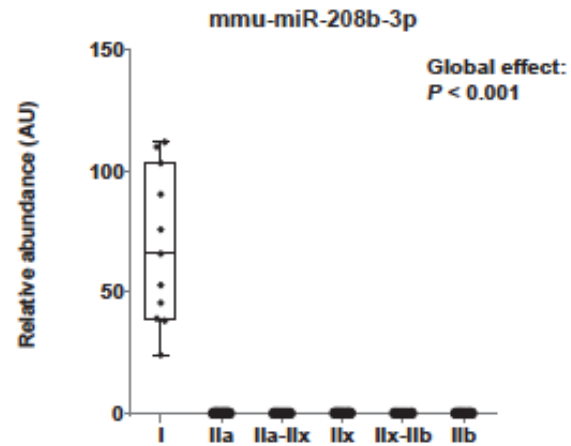
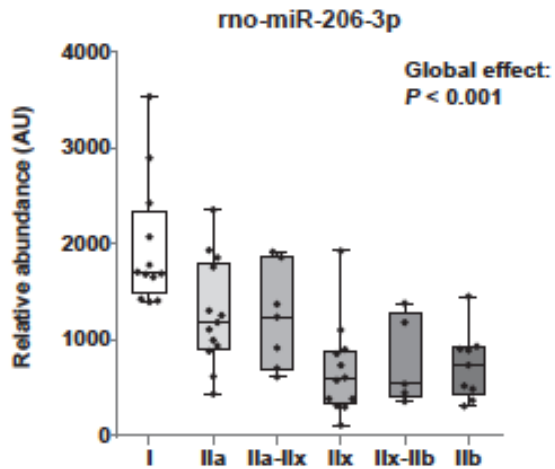
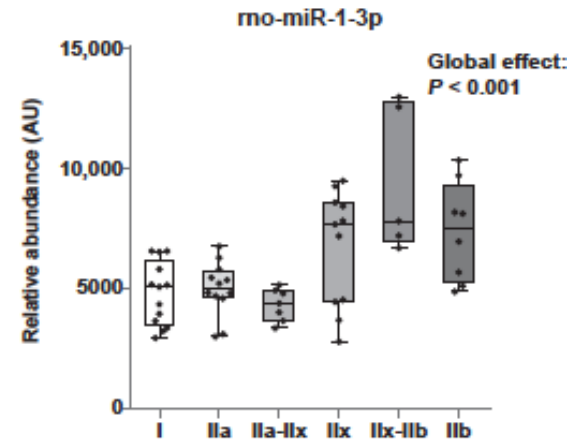
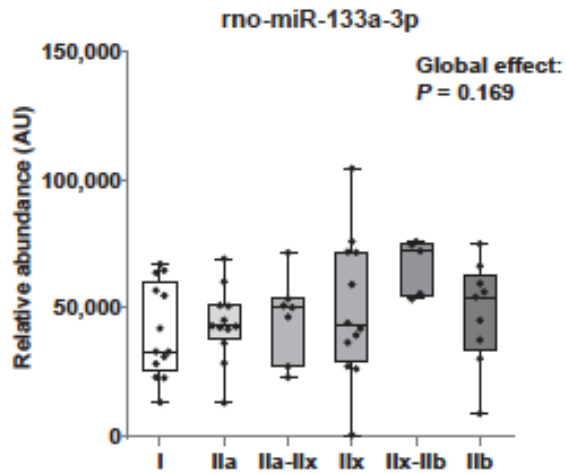


# miRNAs are differentially expressed in slow and fast muscle fibres

I      IIa      IIx      IIb



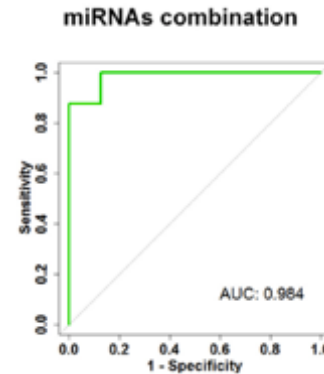
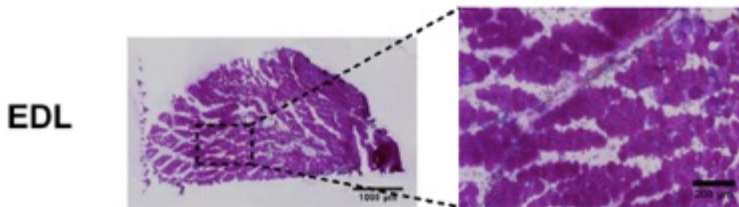
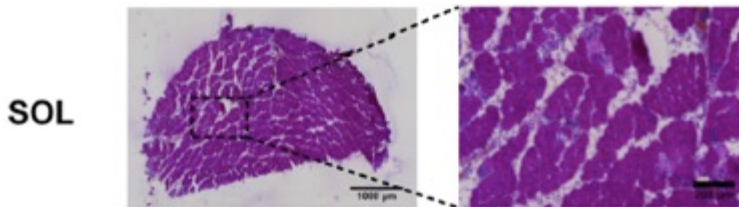
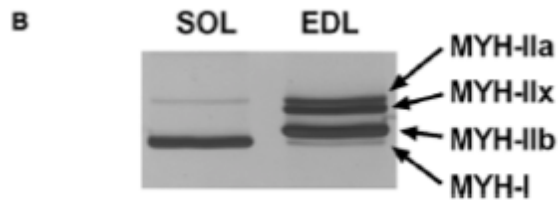
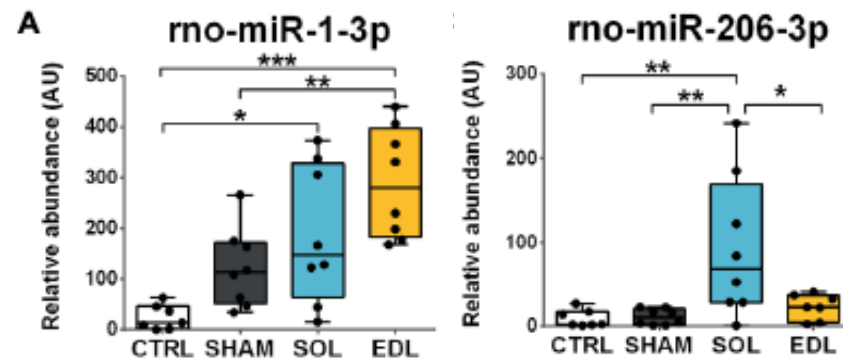
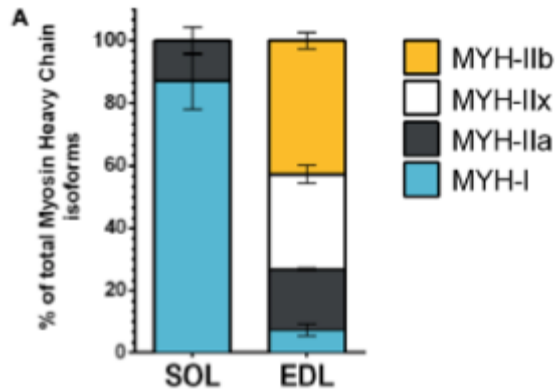
# miRNAs are differentially expressed in slow and fast muscle fibres



Slow

Fast

# Can circulating miRNA profile give information on muscle phenotype?



Diagnostic performance of a combination of 3 circulating miRNA (SOL/EDL)



# In Human?

## Concentric

Treadmill  
Speed : 3,6 km.h<sup>-1</sup>  
Slope : 25%  
Duration: 30 min  
Load 12% of total body weight  
**Forwards**

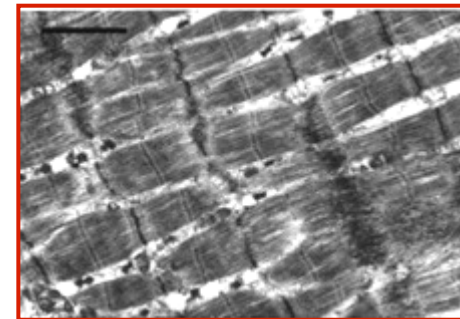
High intensity  
**No injury**



10 healthy subjects  
2 sessions, 6 to 8  
weeks washout

## Eccentric

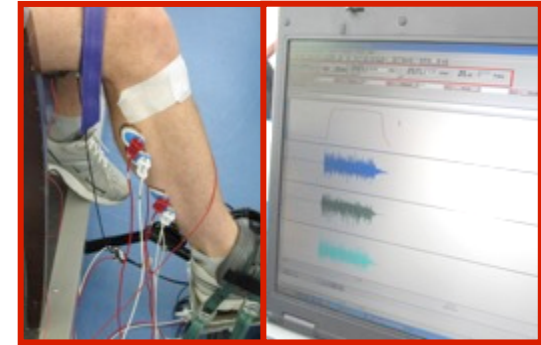
Treadmill  
Speed : 3,6 km.h<sup>-1</sup>  
Slope : 25%  
Duration: 30 min  
Load 12% of total body weight  
**Backwards**



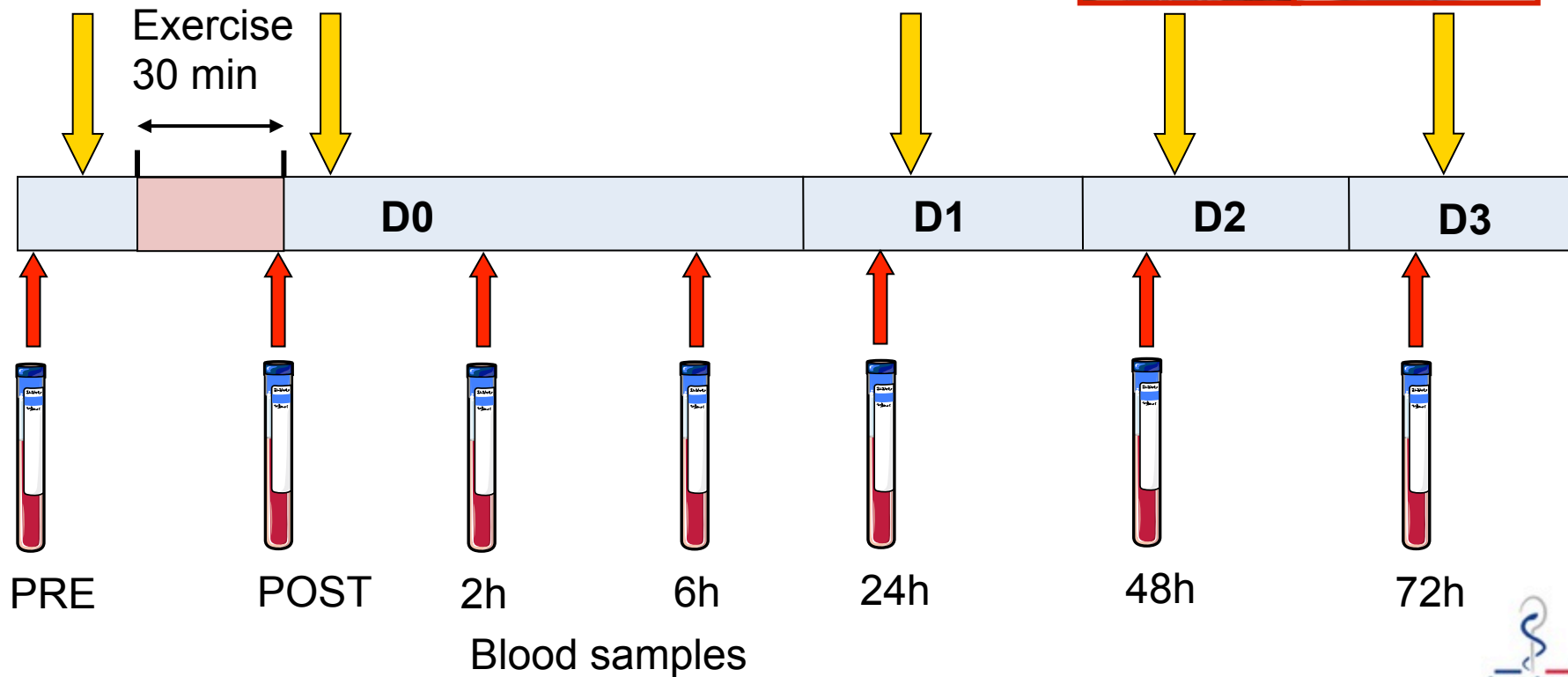
Feasson, 2002

Moderate intensity  
**DOMS**

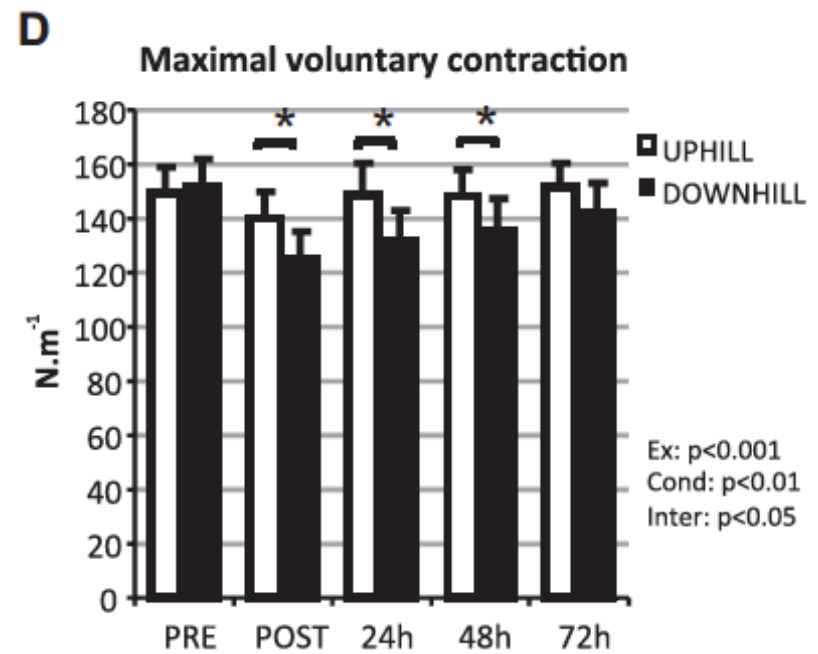
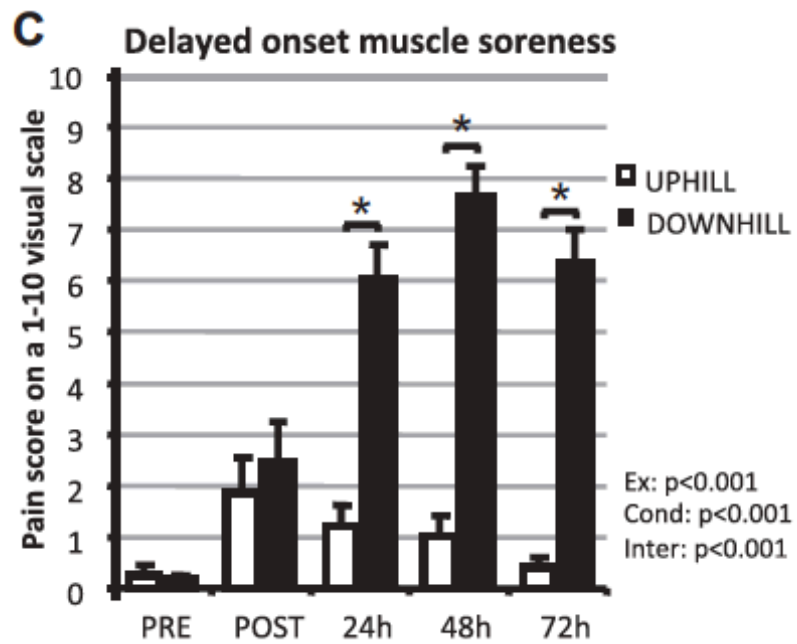
# Experimental protocol

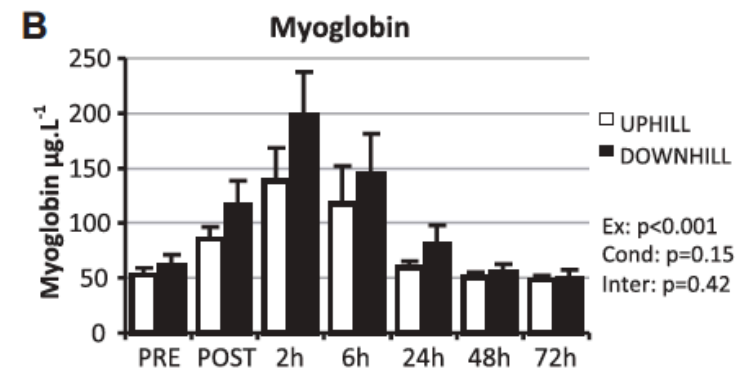
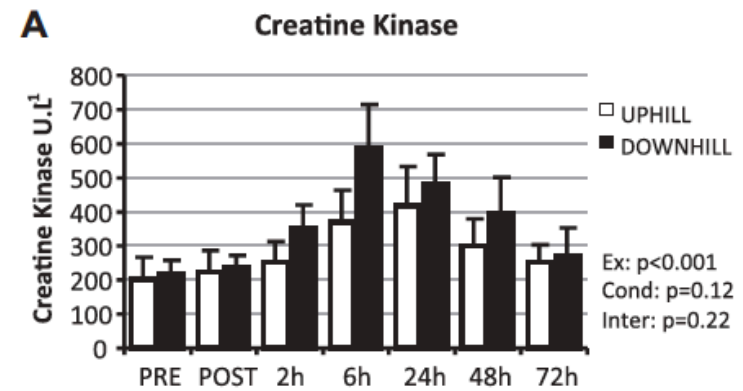
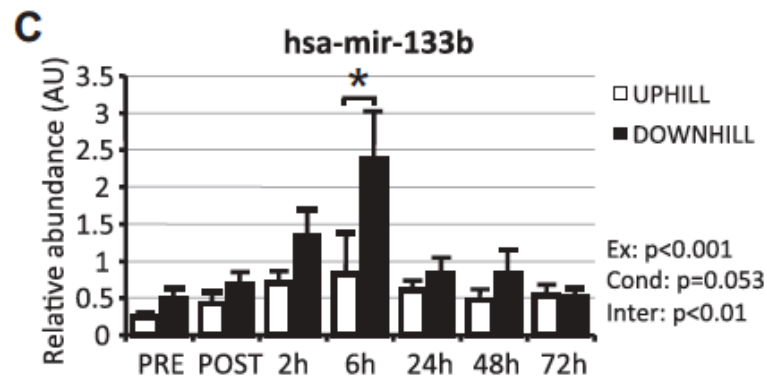
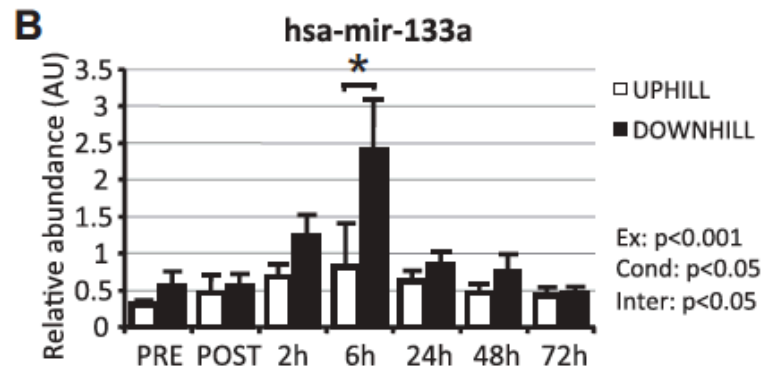
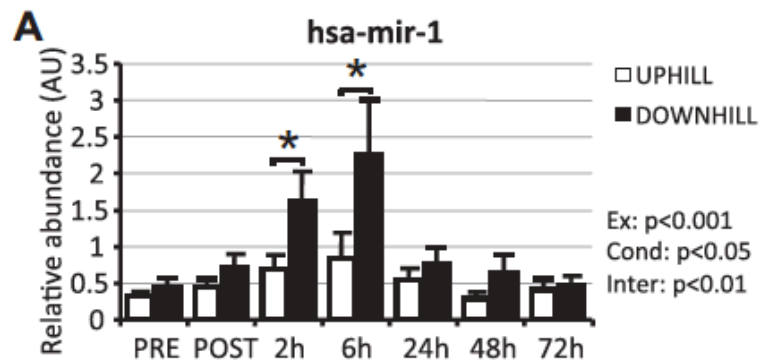


Max voluntary contraction  
Pain questionnaires



## Evaluation of muscle injury

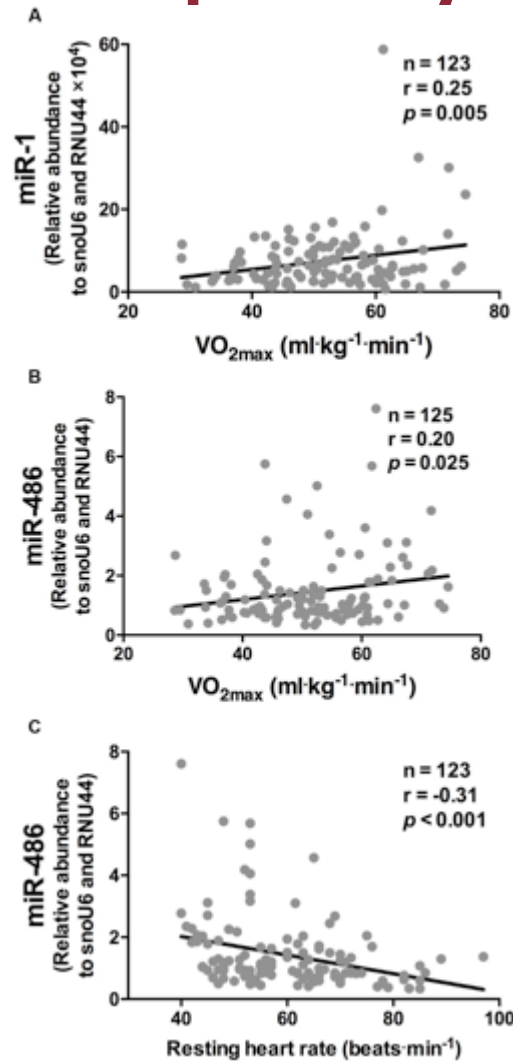




Banzet, 2013

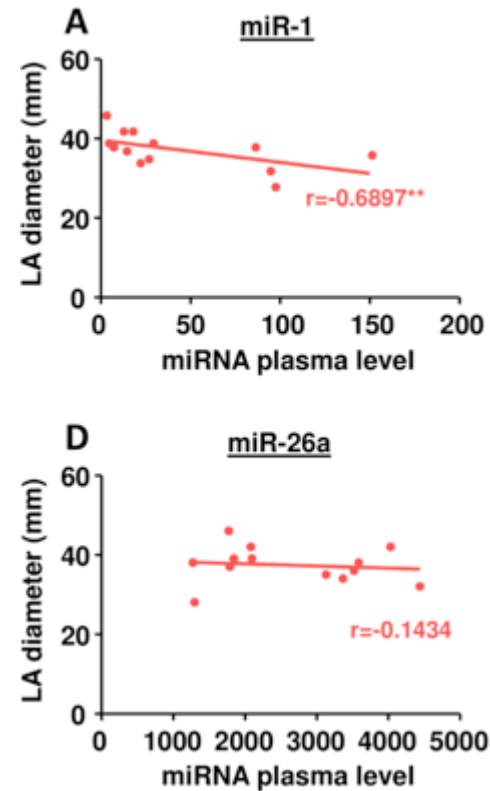


# Could miRNA be biomarkers of cardio-respiratory fitness ?



Denham, 2016

61 endurance athletes, 67 healthy controls

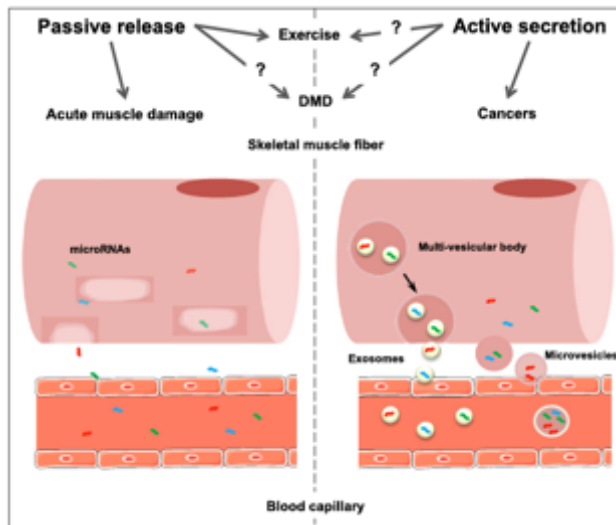


Clauss, 2016

Elite runners

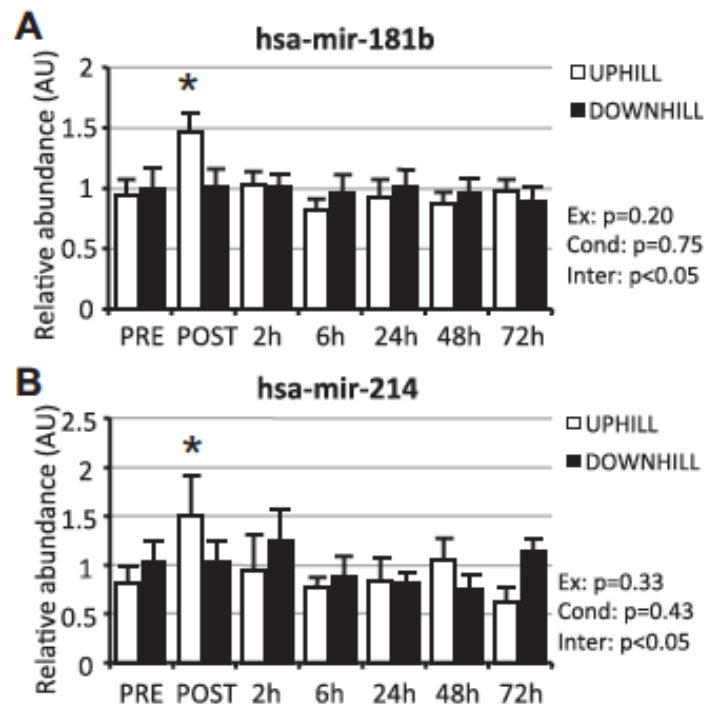
Lack of experimental evidence to support this hypothesis

- Circulating miRNAs are efficient biomarkers of both acute and chronic muscle injury.
- Their Performances are complementary to commonly used markers.
- Limitation for their clinical use: technical



Siracusa, 2018b

# Circulating miRNA in response to non-damaging exercise



Concentric, non damaging exercise also induces changes in plasma miRNA

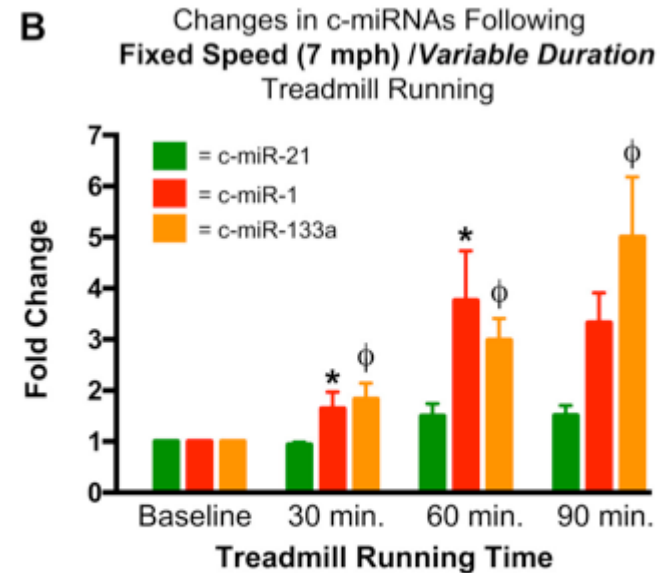
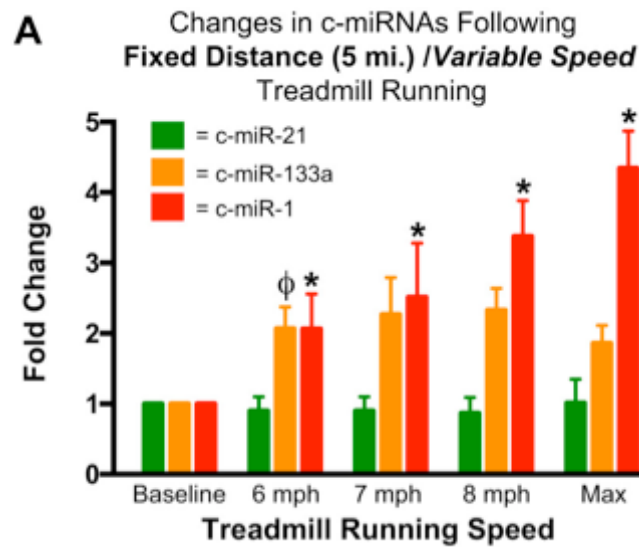
- miRNA involved in the immune response
- miRNA involved in angiogenesis

Could these miRNAs mediate biological information ?

Banzet, 2013



# Circulating miRNA in response to non-damaging exercise



$\phi$  = denotes  $p < 0.05$  compared to previous experimental condition (c-miR-133a)

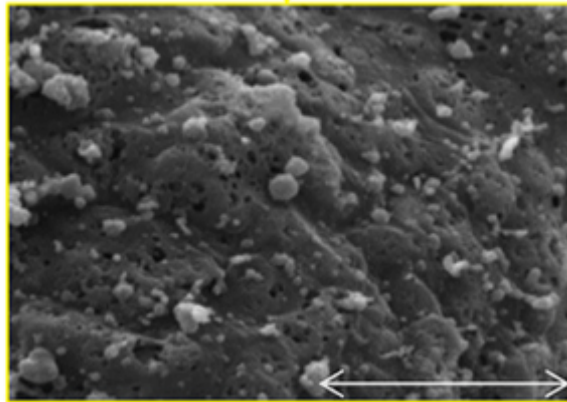
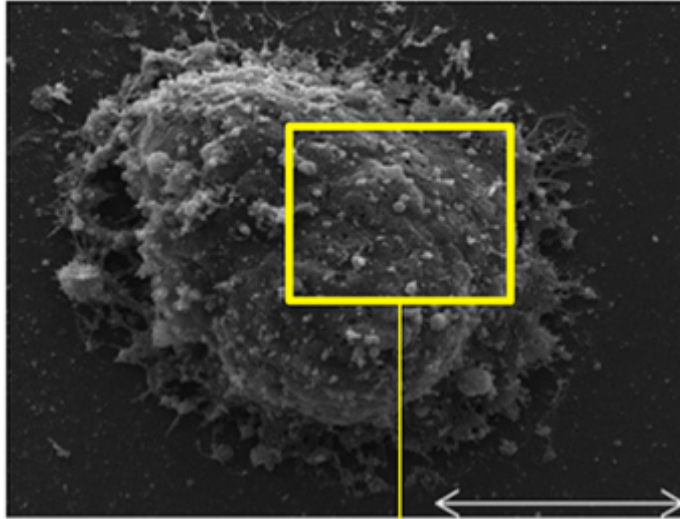
\* = denotes  $p < 0.05$  compared to previous experimental condition (c-miR-1)

Healthy young men

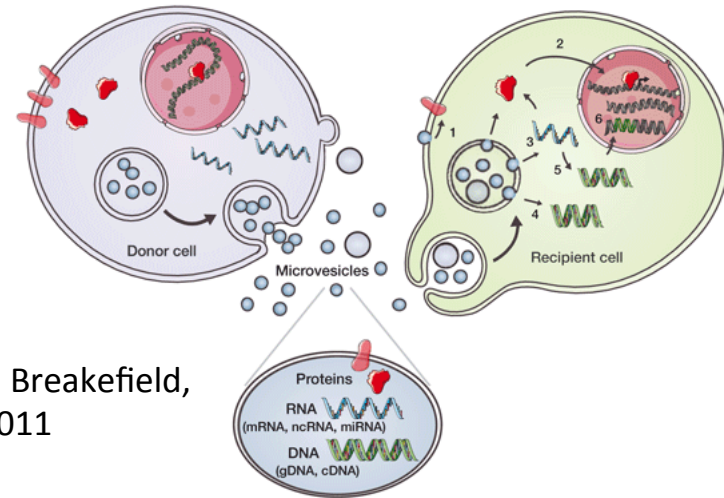
Ramos, 2018



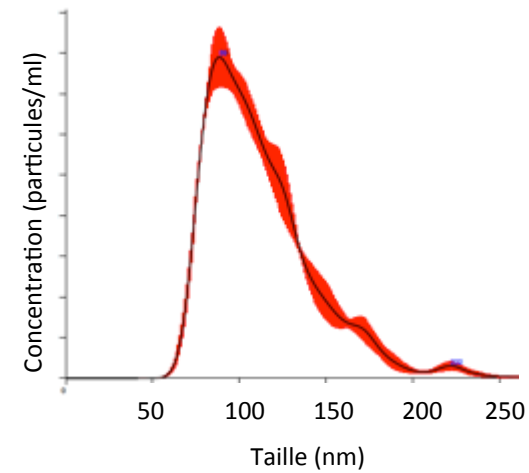
# Extracellular vesicles and exercise



Saha, 2016

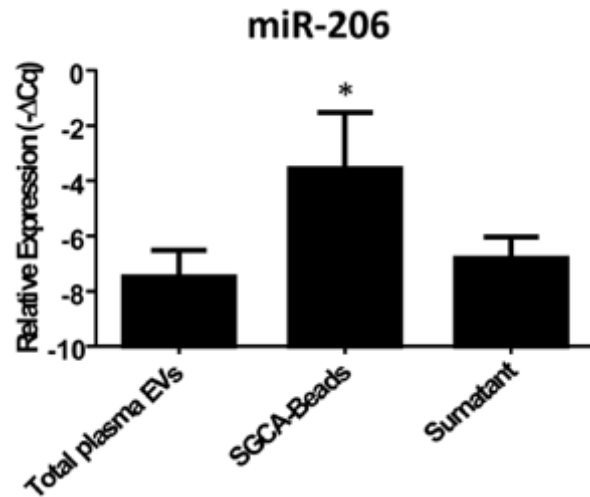


O Breakefield, 2011



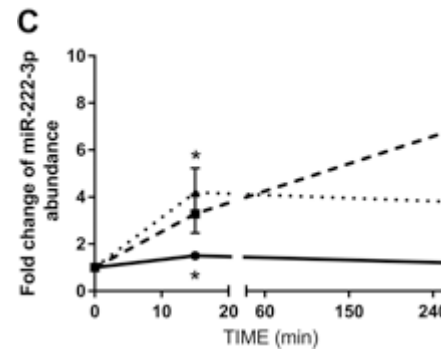
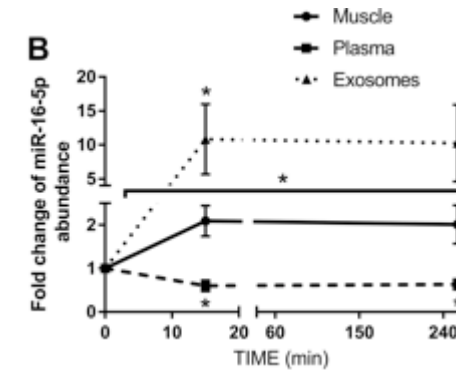
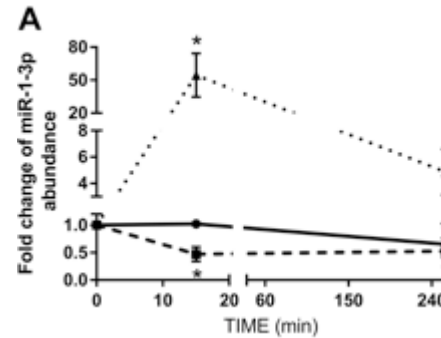


# Extracellular vesicles and exercise



Guescini, 2015

Skeletal muscle fibres release miRNA containing EV

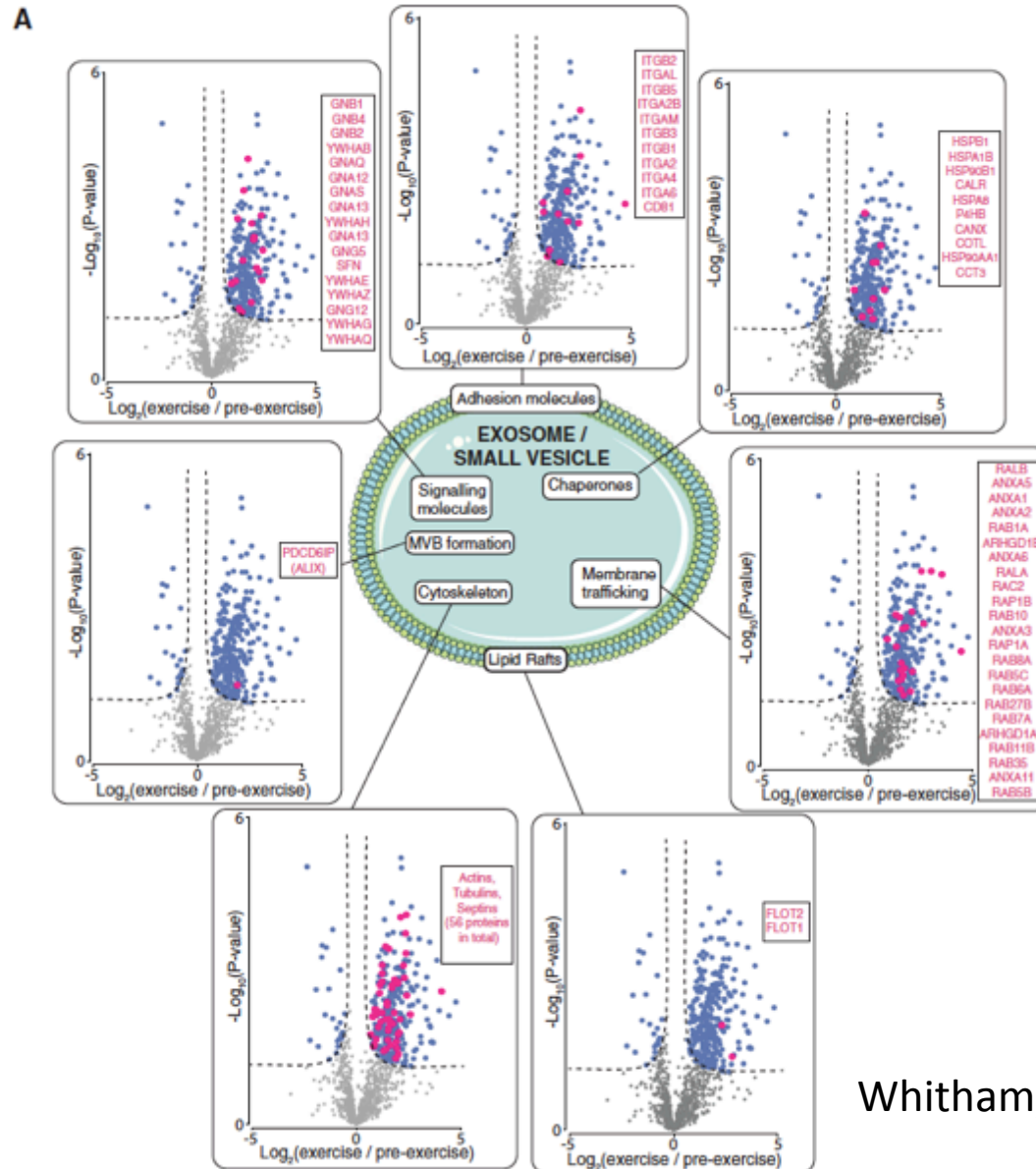


D'Souza, 2018

single bout of high-intensity interval cycling exercise



# Extracellular vesicles and exercise



- EV are released during exercise
- Exercise modifies the protein profile of circulating EV
- Exercise-liberated EV have a propensity to localize in the liver

Whitham, 2018



# CONCLUSIONS

- miRNAs are new biomarkers of acute and chronic muscle injury.
- There are numerous possible applications in the diagnosis of other tissue injury, with combined analysis giving new tools in MOF
- Non damaging exercise modifies circulating EV landscape
- Exercise-related miRNAs could be active molecules





# Acknowledgments

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