

Loss of microRNA-155 Protects the Heart from Pathological Cardiac Hypertrophy

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1. Background - Hypertrophy, miR155
2. Results:
 - #1 – preliminary
 - #2 – miR155 knockout vs ...
 - #3 – miR155 knockout vs ... - ISOLATED CELL
 - #4 – miR155 and JARID2
3. Summary
4. Discussion

Background

Cardiac Hypertrophy

- Physiological: adaptive response to growth signals
- Pathological hypertrophy: response to stress signals
- Risk factor for arrhythmia and sudden cardiac death
- Molecular pathways not fully understood

miRNAs

- Short non-coding RNAs
- Regulation at post-transcriptional level
- Regulate 30-60% of protein-coding genes²
- Binding to mRNA, multiple targets
 - Inhibiting translation
 - Inducing degradation

miRNA155

- Big player among miRs
 - multiple already known targets
- Critical role shown in: ³
 - Cardiovascular disease
 - Hematopoietic lineage differentiation
 - Immunity
 - Inflammation
 - Viral infections
 - Cancer
 - Down Syndrome

miRNA155

Cardiovascular System:

- expressed in atherosclerotic plaques and proinflammatory macrophage -
KO → reduced plaque size ⁴
- Overexpression in human cardiomyocyte progenitor cells linked to protection from necrotic cell death in vitro ⁵

4. Nazari-Jahantigh M. Et al, J Clin Invest. (2012); 122:4190–4202.

5. Liu J. Et al, J Cell Mol Med. 2011; 15:1474–1482.

miRNA155

Cardiovascular System:

- Endogenous inhibition attenuated cardiac infiltration by monocyte-macrophages ⁶
- In vivo function in cardiomyocyte hypertrophy was reported ⁷

6. Corsten MF. Et al, Circ Res.2012; 111:415–425.

7. Heymans S. Et al, Circulation. 2013; 128:1420–1432

RESULTS #1

preliminary

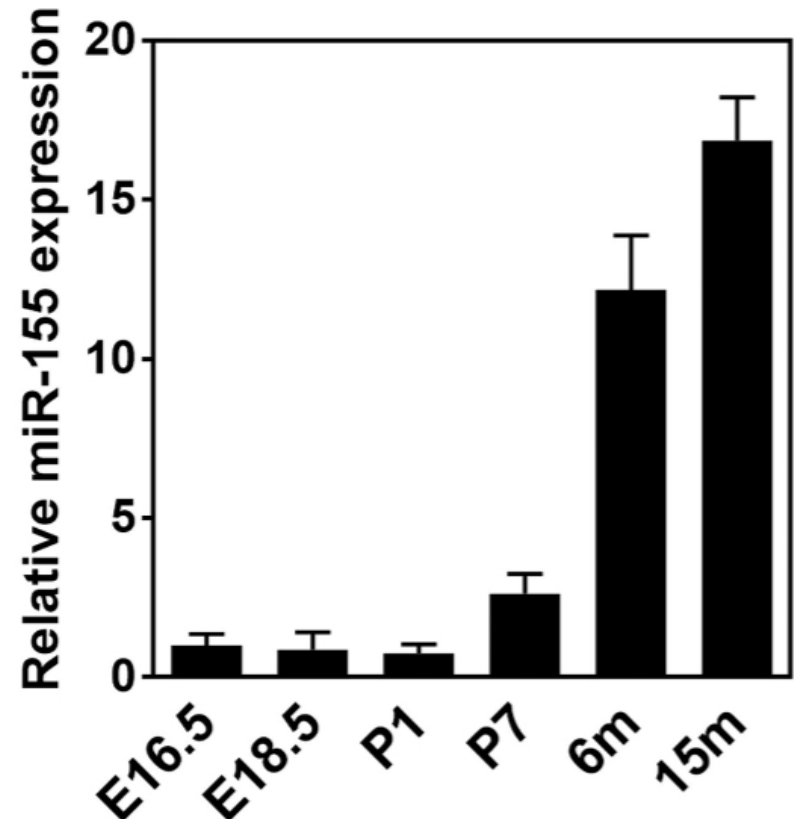
Expression in mice heart

Method: qPCR analyses

-increased in postnatal
day 7

-highest in 15 month old
mice

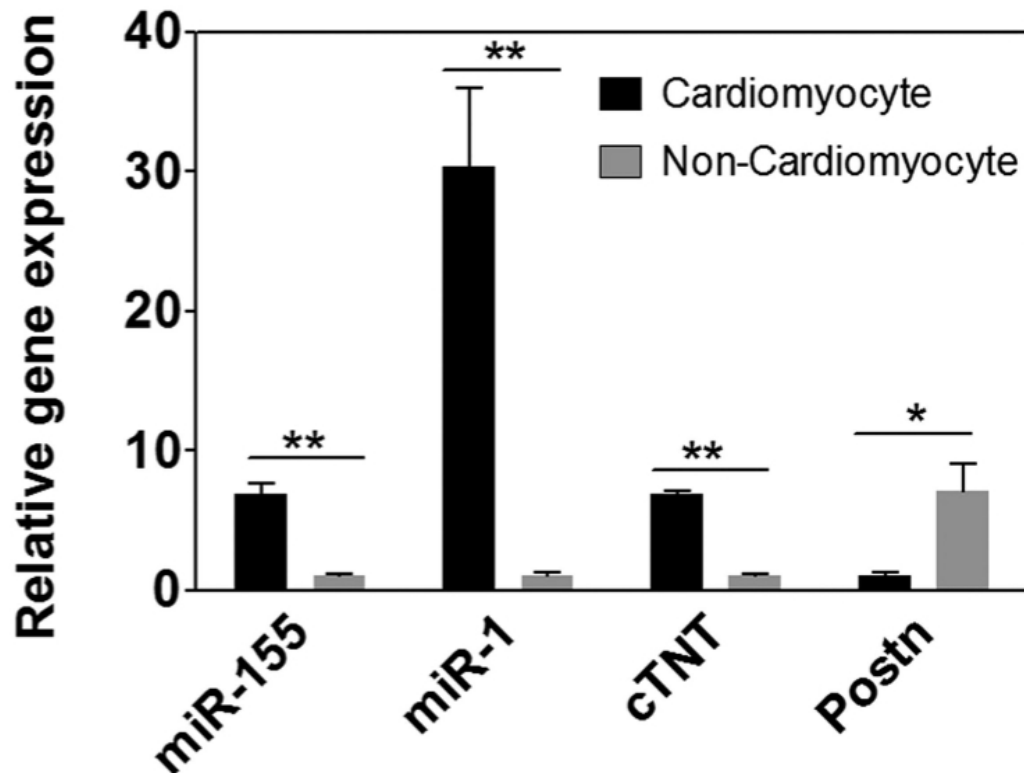
-important for
adult heart /remodeling?



Distribution

Method: qPCR analyses, Adult mouse hearts

→ miR155 mainly expressed in cardiomyocytes



Approach

Stimulation for hypertrophy was done in two ways:

- Transverse Aortic Constriction
- Calcineurin Transgenetic Mice

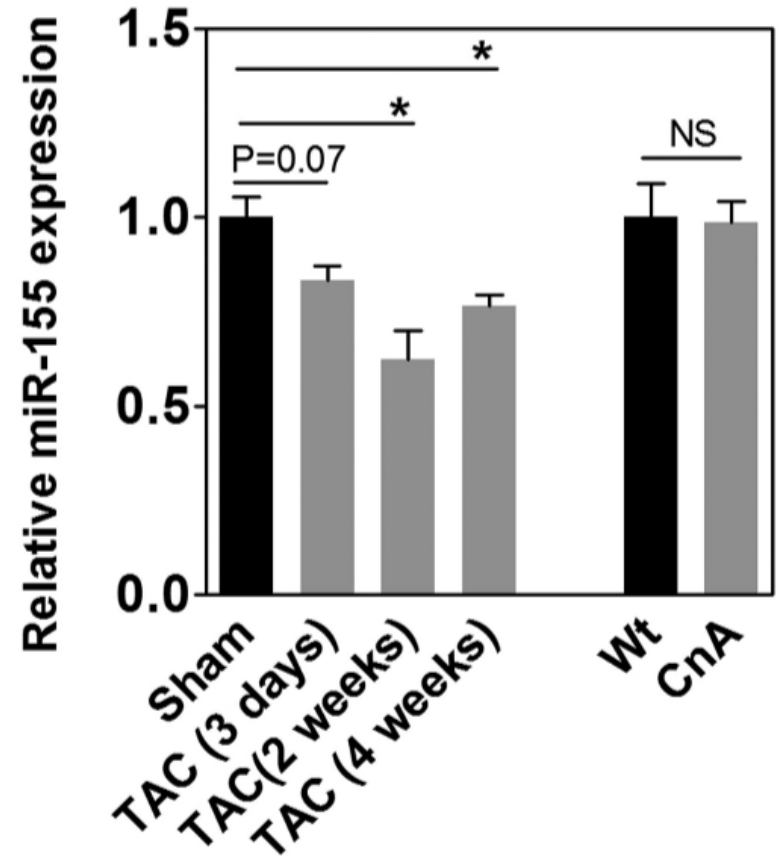
generated by Drs. Molkenin and Olson, were obtained from the Jackson lab(Tg(Myh6-Ppp3ca)37Eno)

Alteration in Hypertrophy

Method: qPCR analyses

-Decreased Expression
stimulated by pressure
overload

-No Alteration in
Transgene mice



Knockout mice vs. Wild type

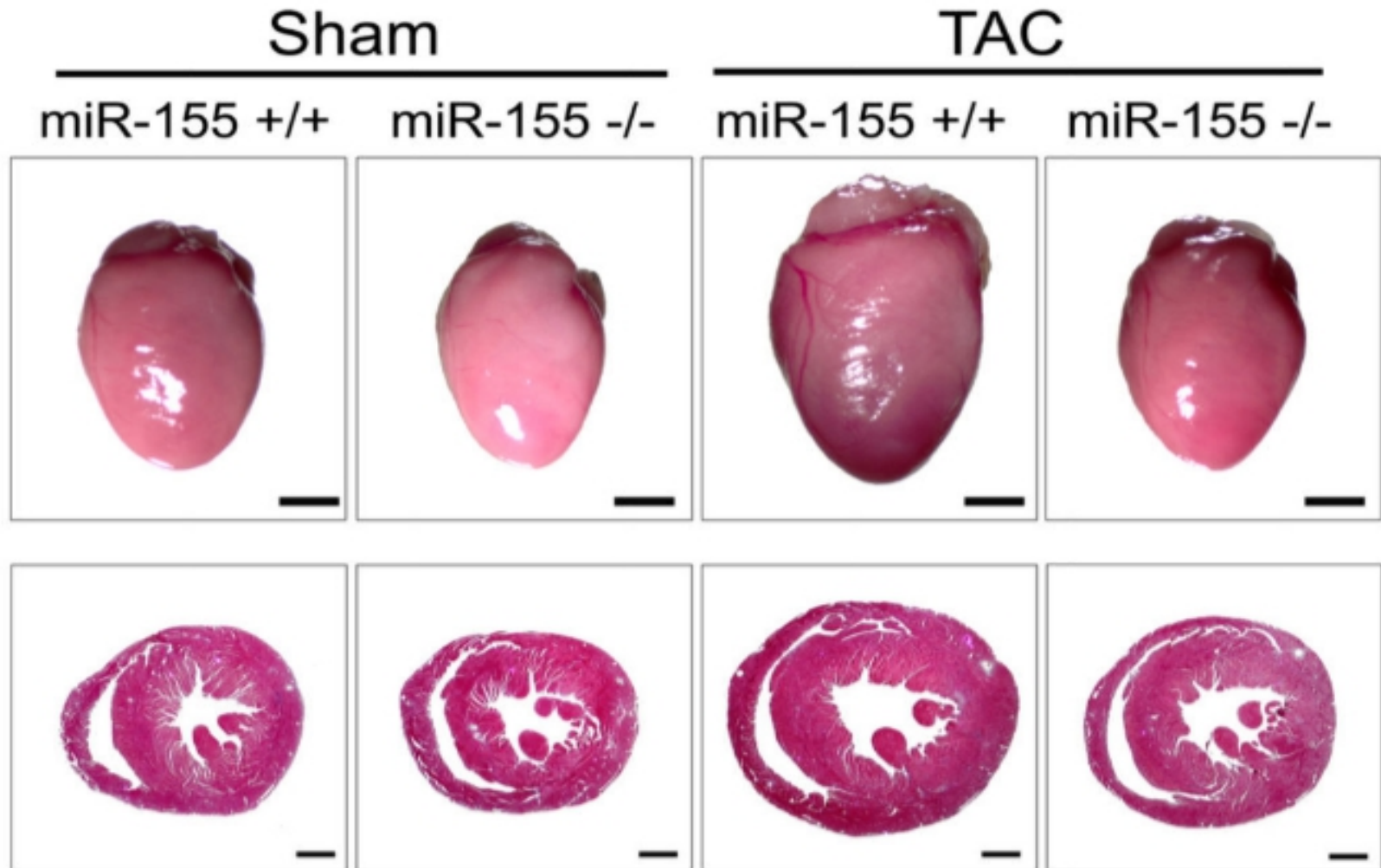
MiR155 is putatively involved in the remodeling mechanism → further investigation: miR155 knockout mice.

Normal mouse development and heart function under physiological conditions within miR155 null mice compared to their wildtype littermates.

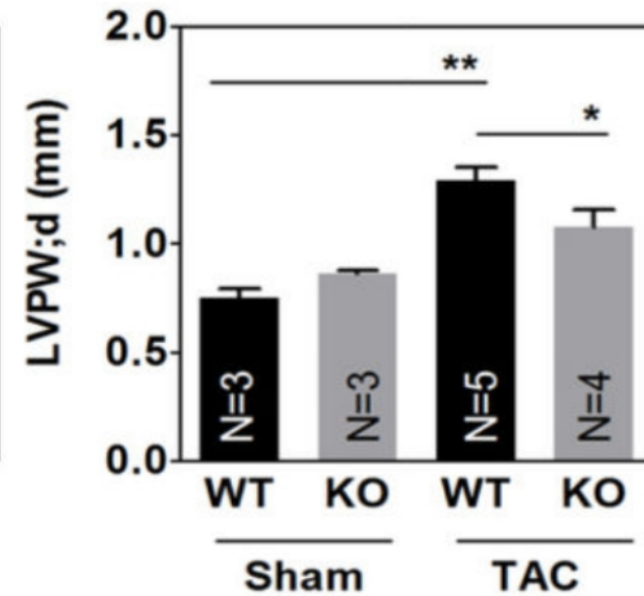
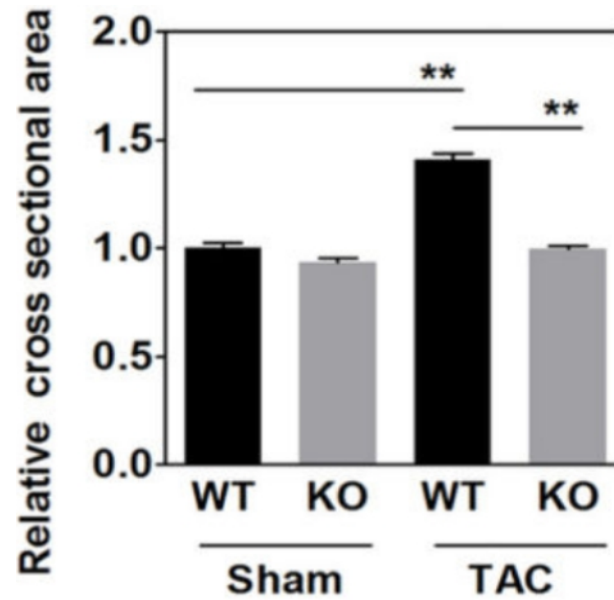
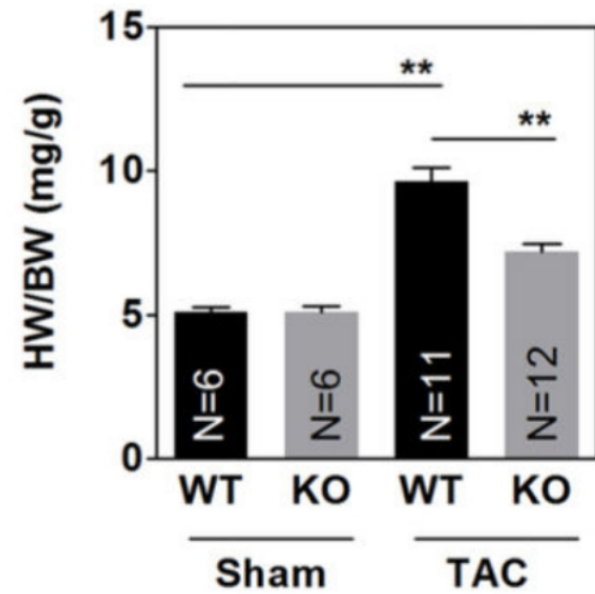
Knockout mice vs. Wild type

RESULTS #2 KO vs ...

Macroscopic Alteration



Biometric Alteration



Histologic Alteration

Sham

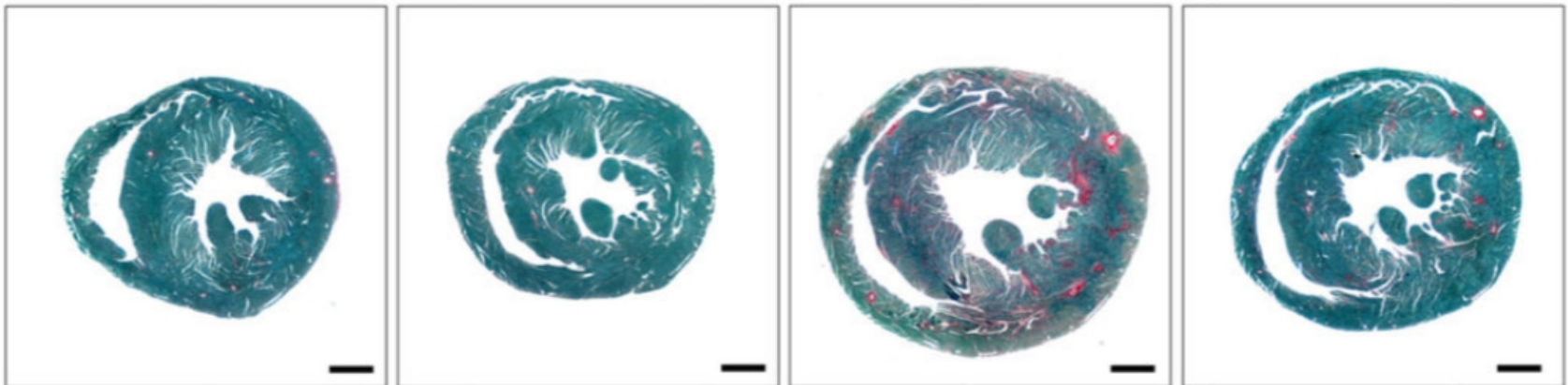
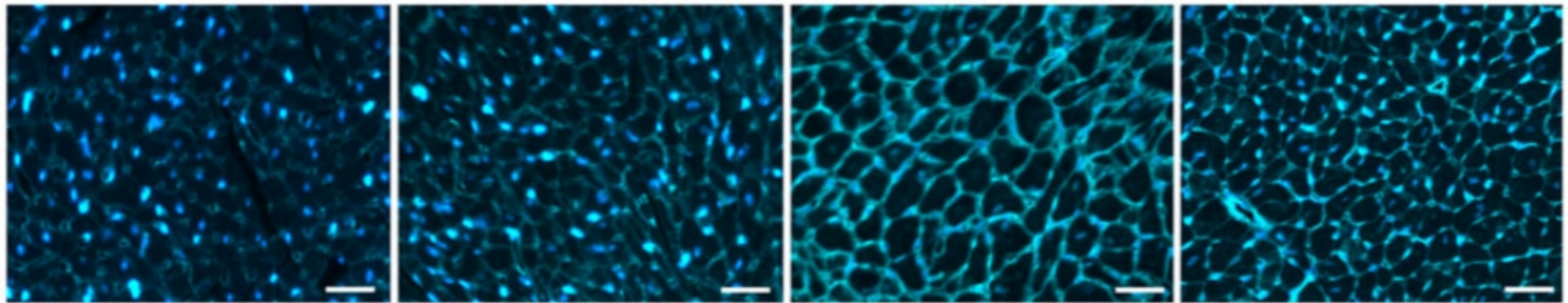
TAC

miR-155 +/+

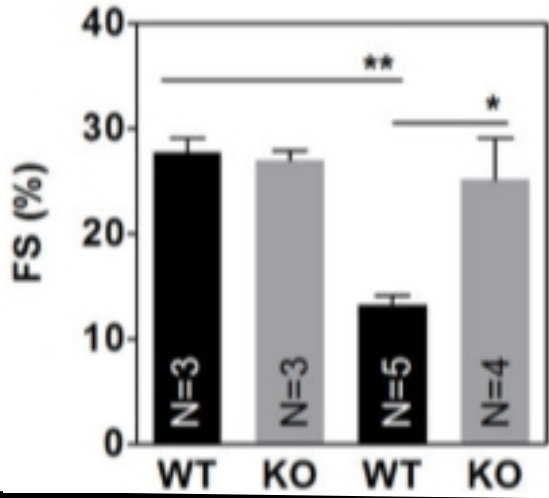
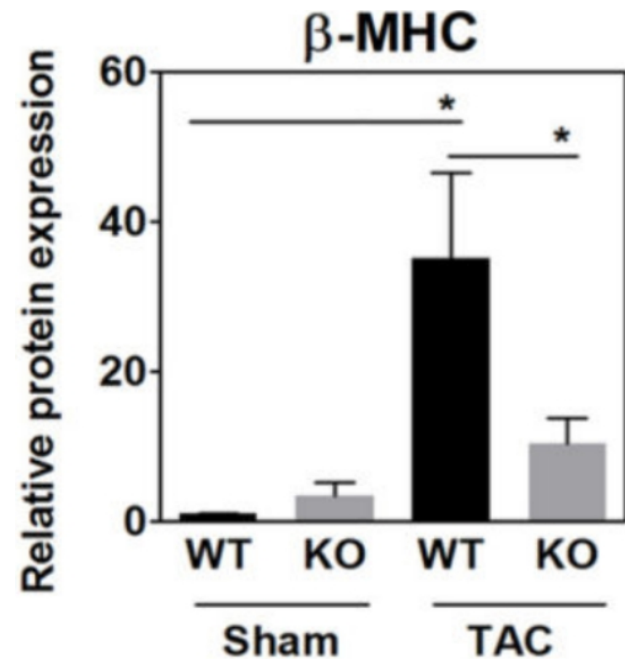
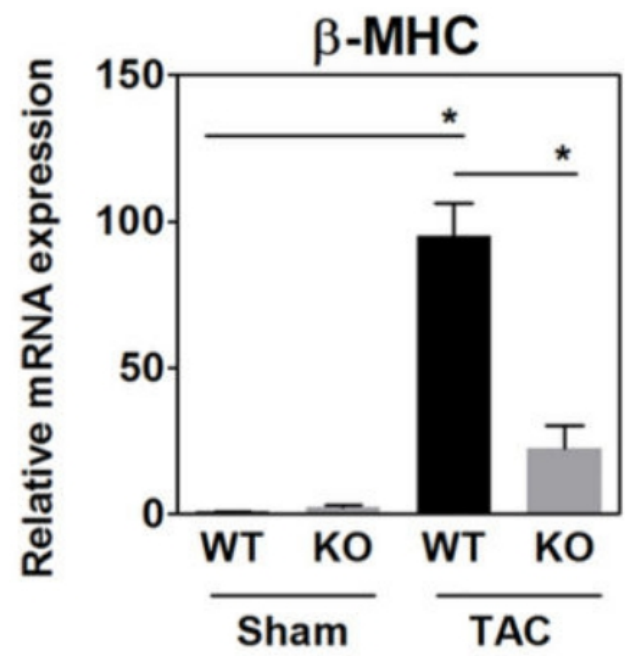
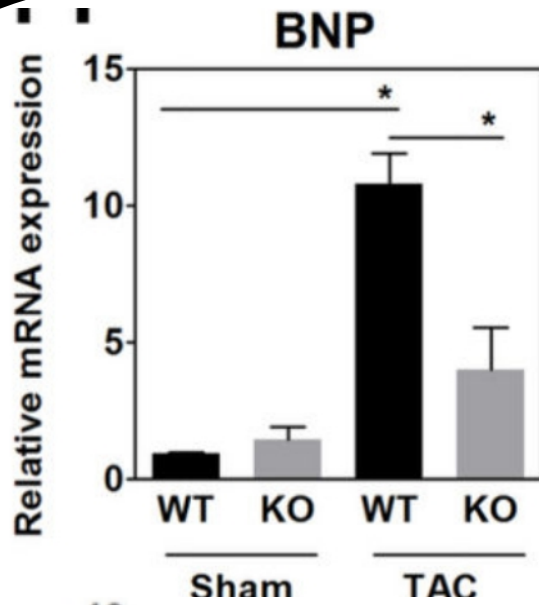
miR-155 -/-

miR-155 +/+

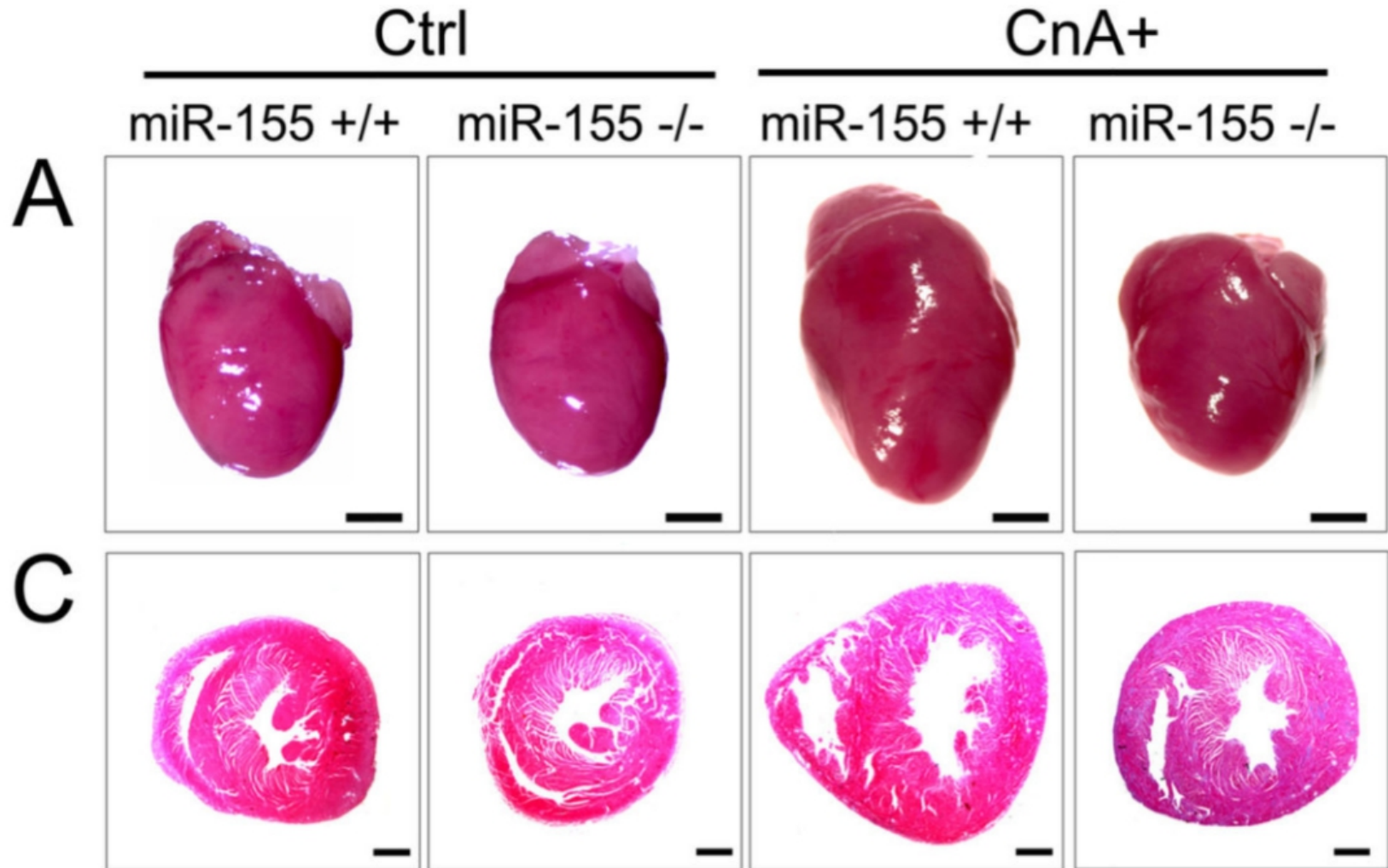
miR-155 -/-



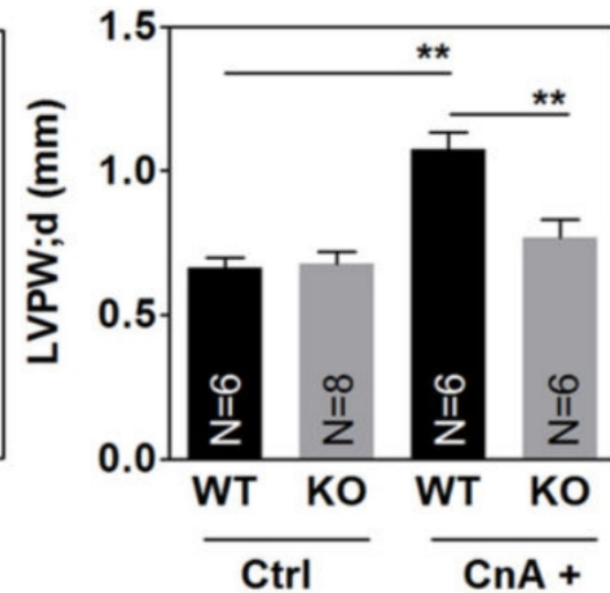
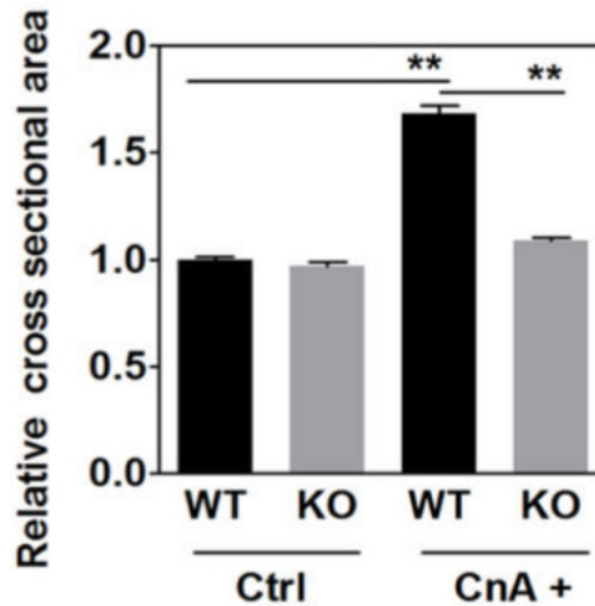
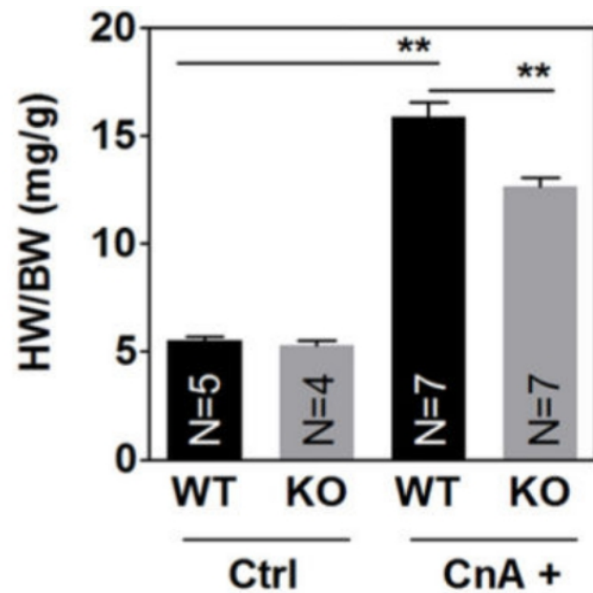
Hypertrophic/ functional Markers



Macroscopic Alteration



Biometric Alteration





Histologic Alteration

Ctrl

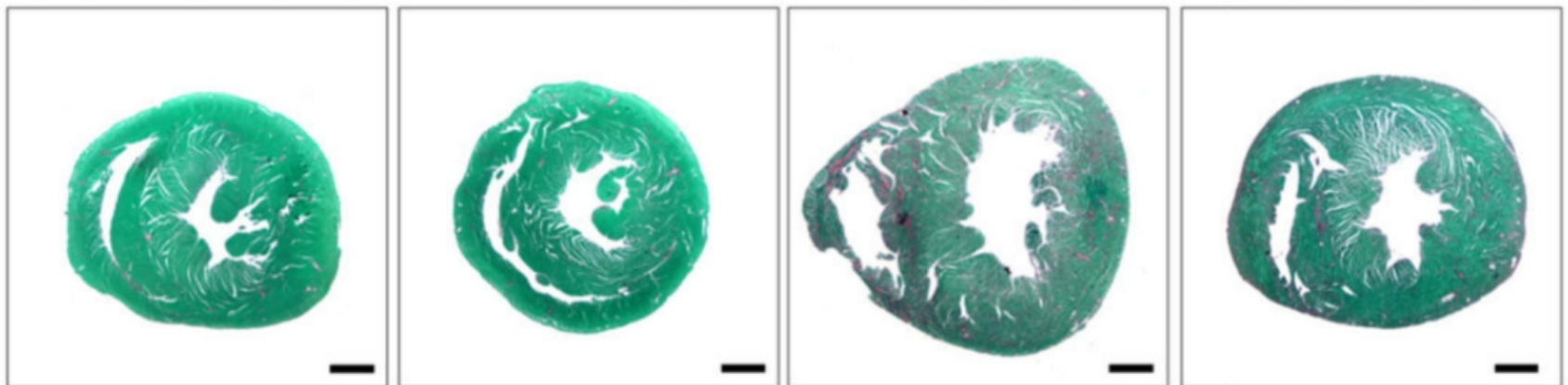
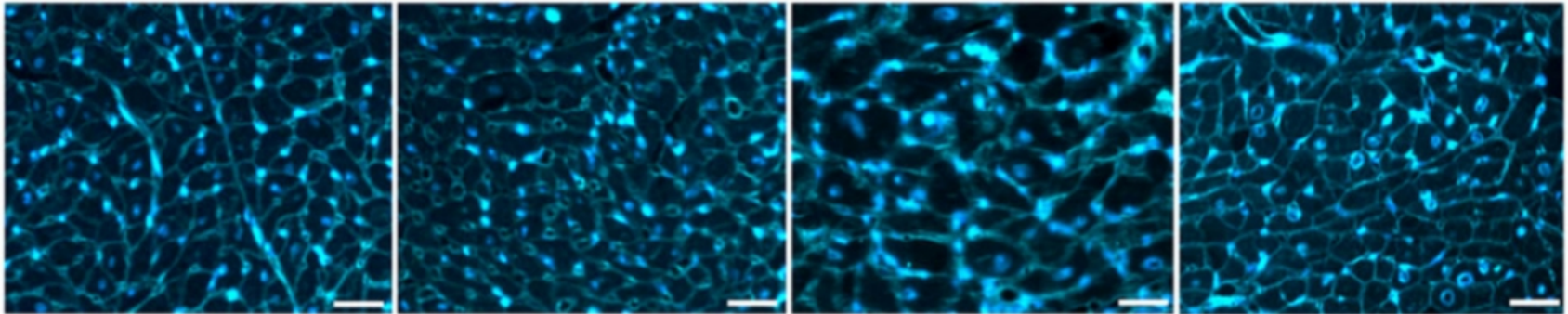
CnA+

miR-155 +/+

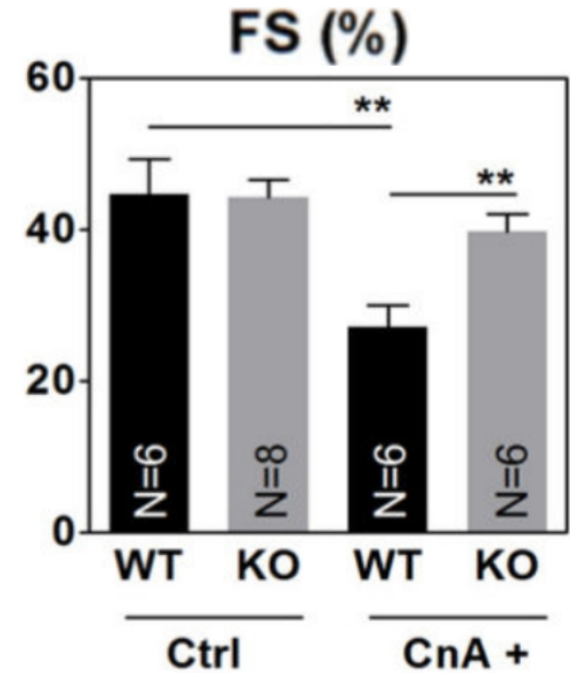
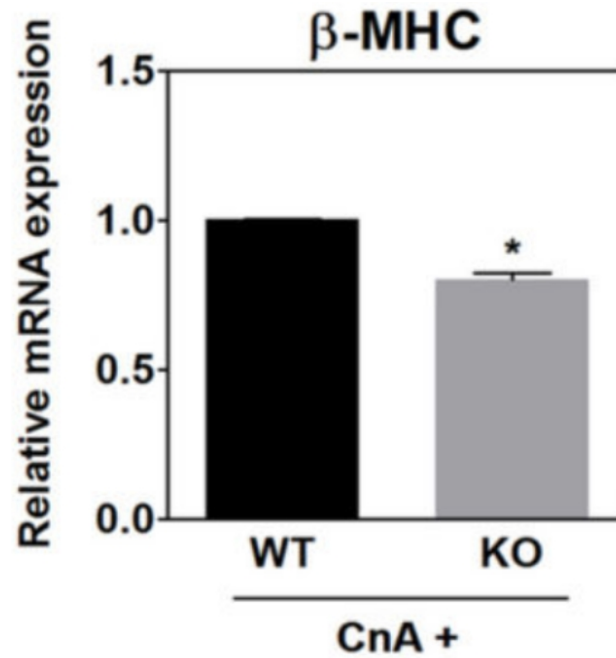
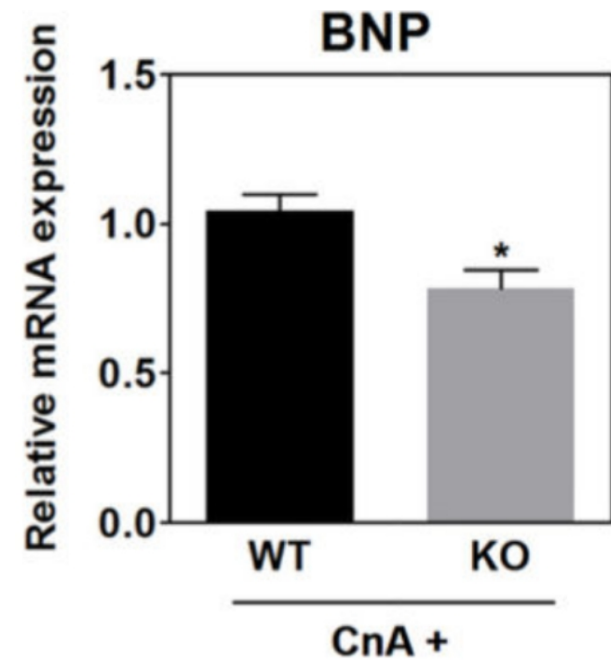
miR-155 -/-

miR-155 +/+

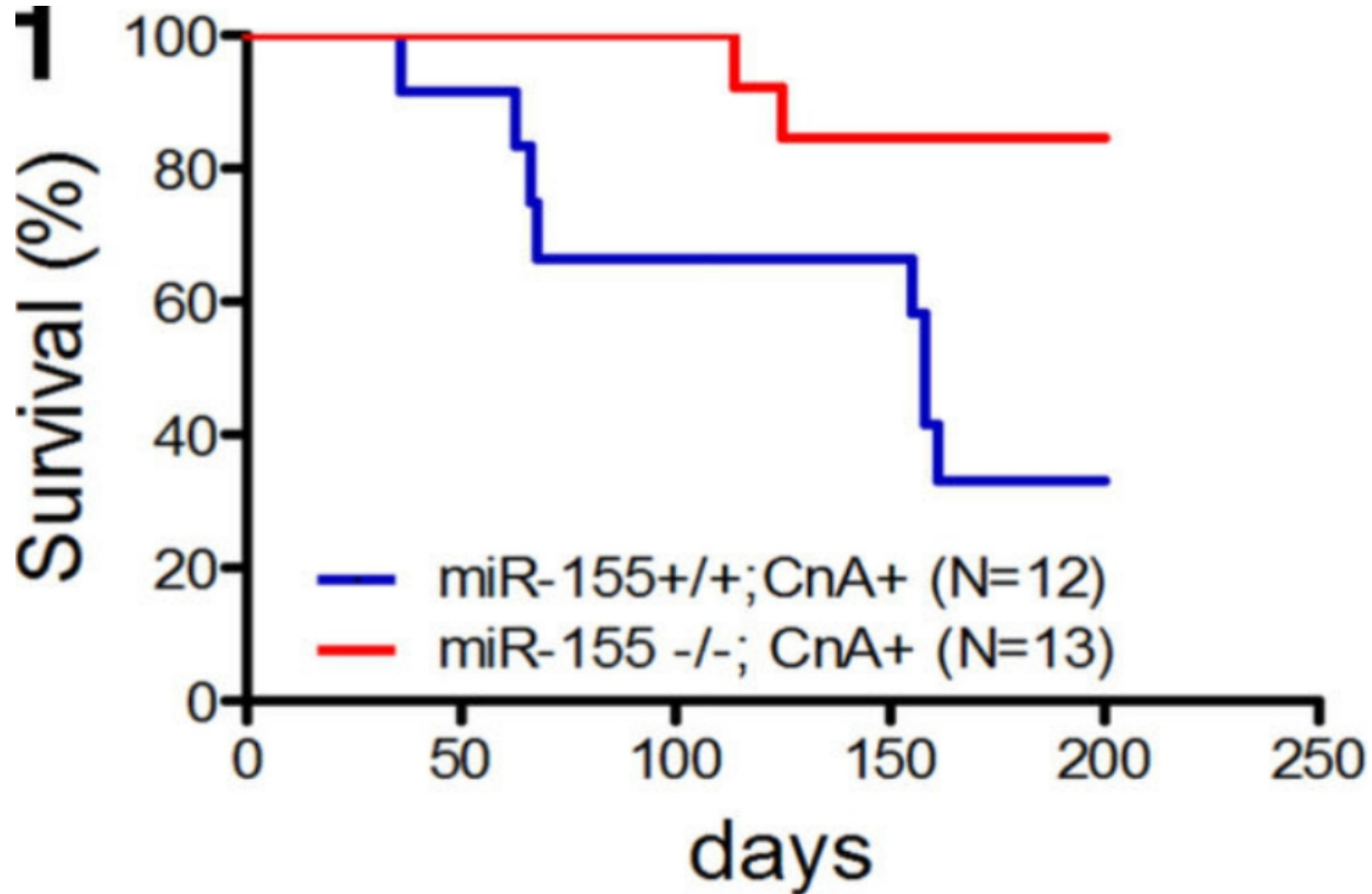
miR-155 -/-



Hypertrophic/ functional Markers



Survival



Overcoming the limitation

miR155 apparently does affect the formation of a hypertrophic heart in mice, since :

- KO-mice show with both stressors less signs of hypertrophy in every way that was examined

But: miR155 is involved in many regulatory mechanisms, it still could be an effect of other points of its activity but in the cardiomyocytes.

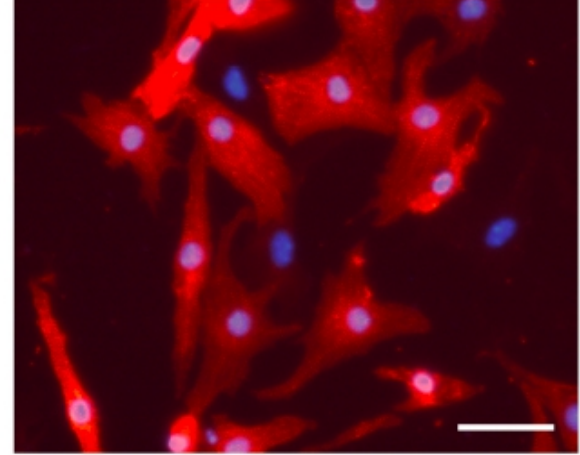
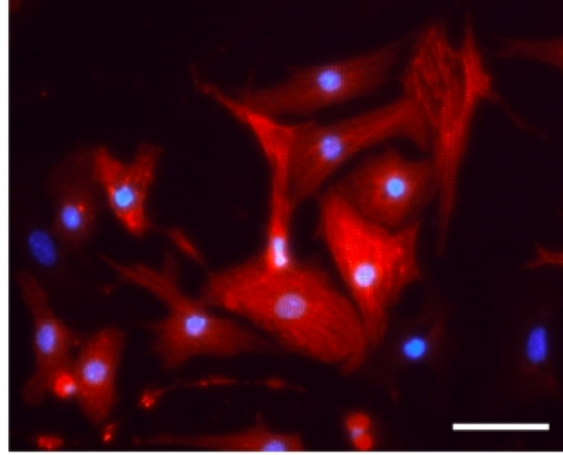
Knockout mice
vs. Wild type

RESULTS #3
KO vs ...
Isolated cells

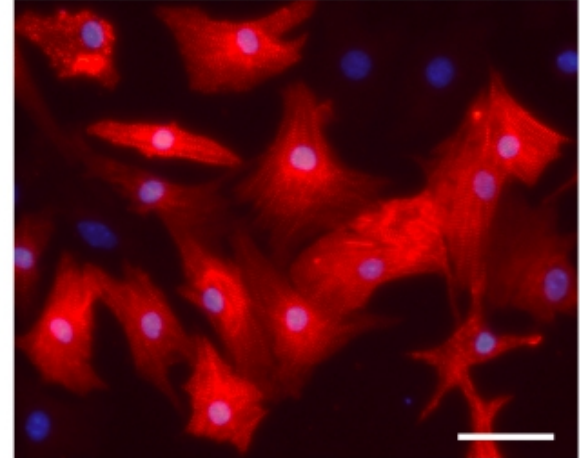
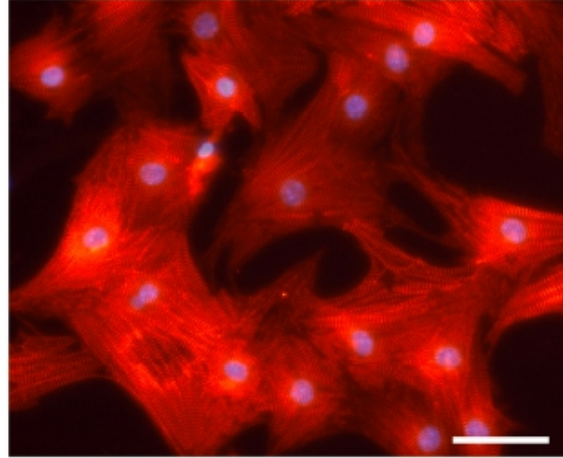
Individual Cells WT

KO

Ctrl

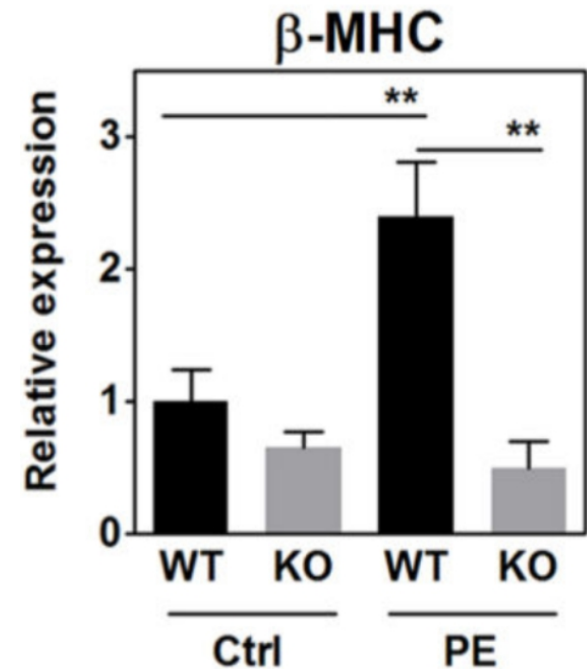
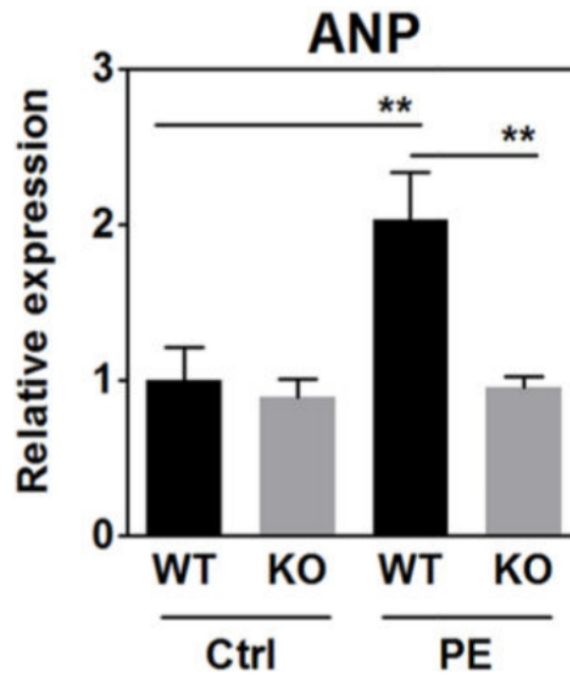
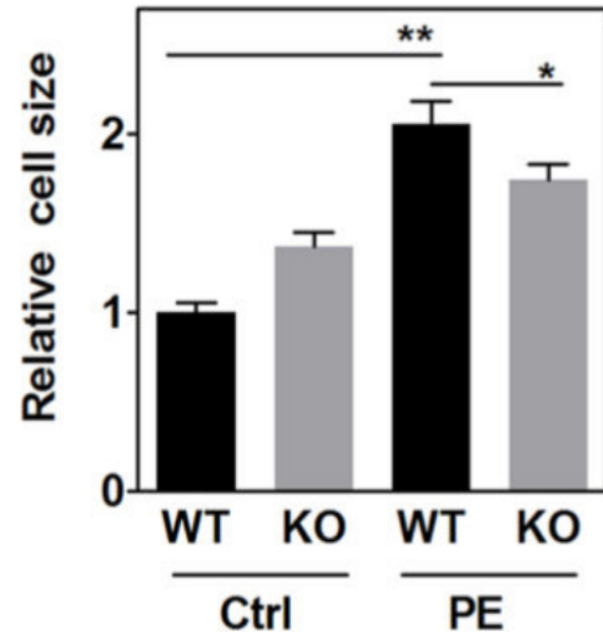


PE



α -Actinin/DAPI

Individual Cells

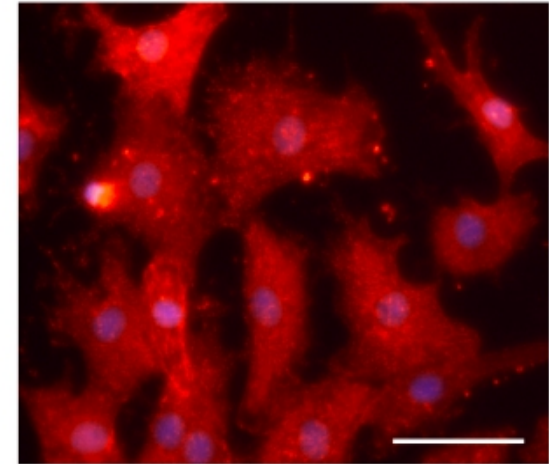
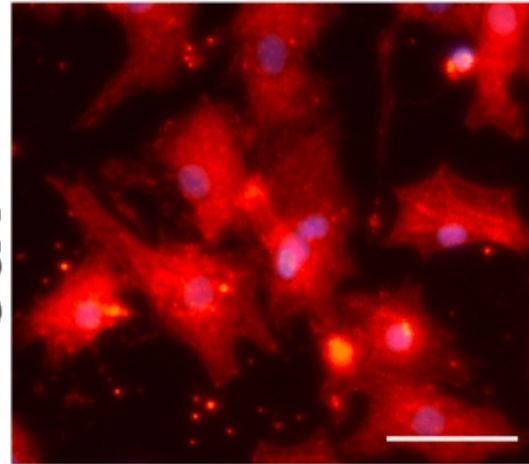


Individual Rat-Cells

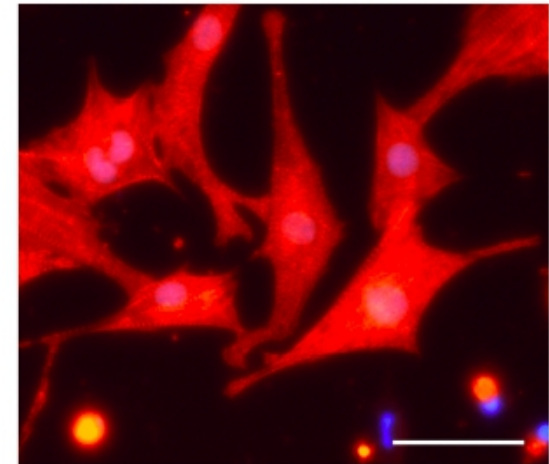
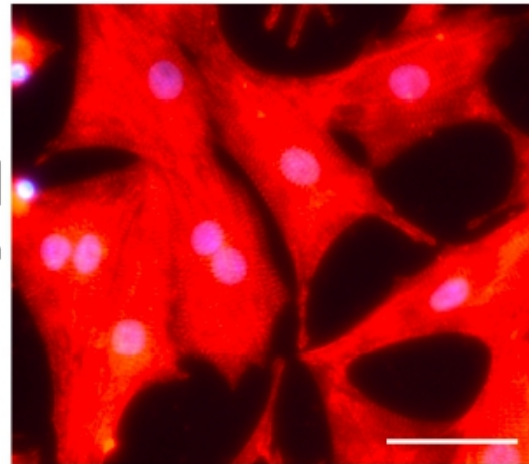
Ctrl Inhibitor

miR-155 Inhibitor

Ctrl

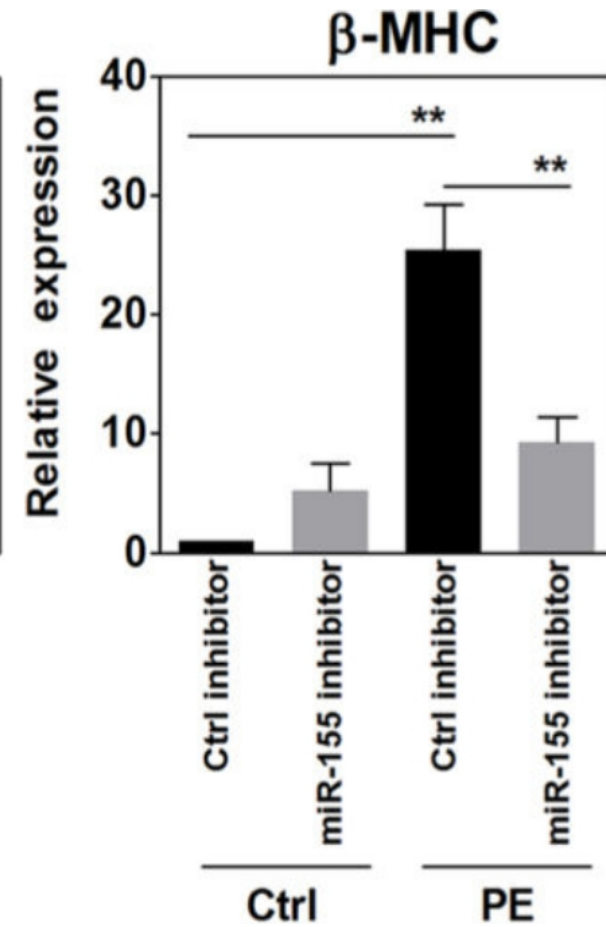
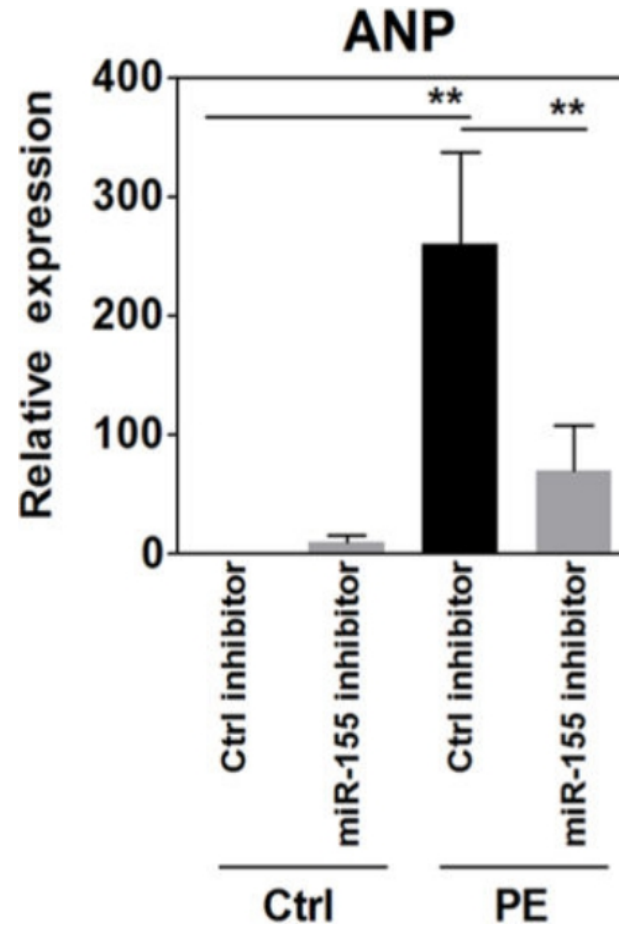
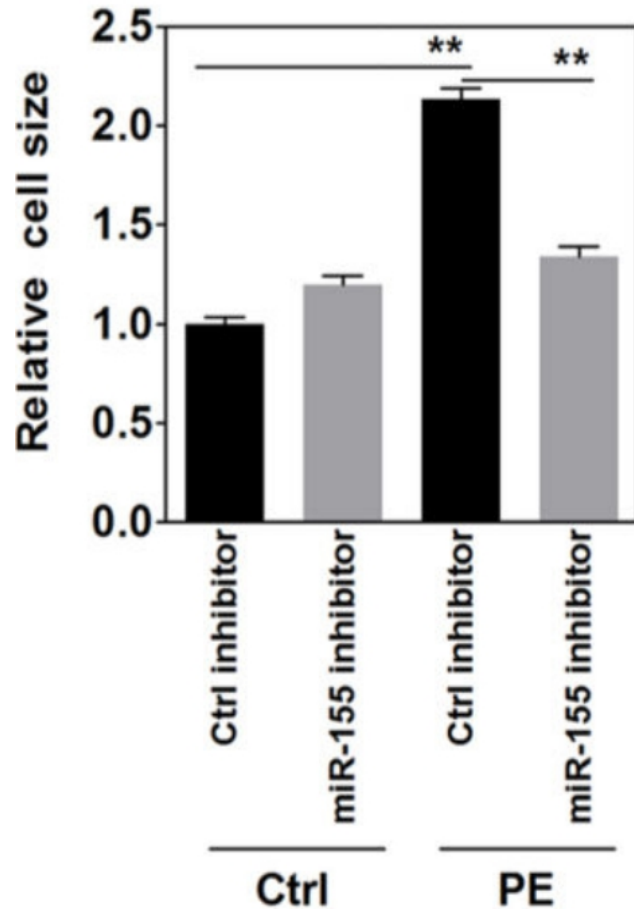


PE

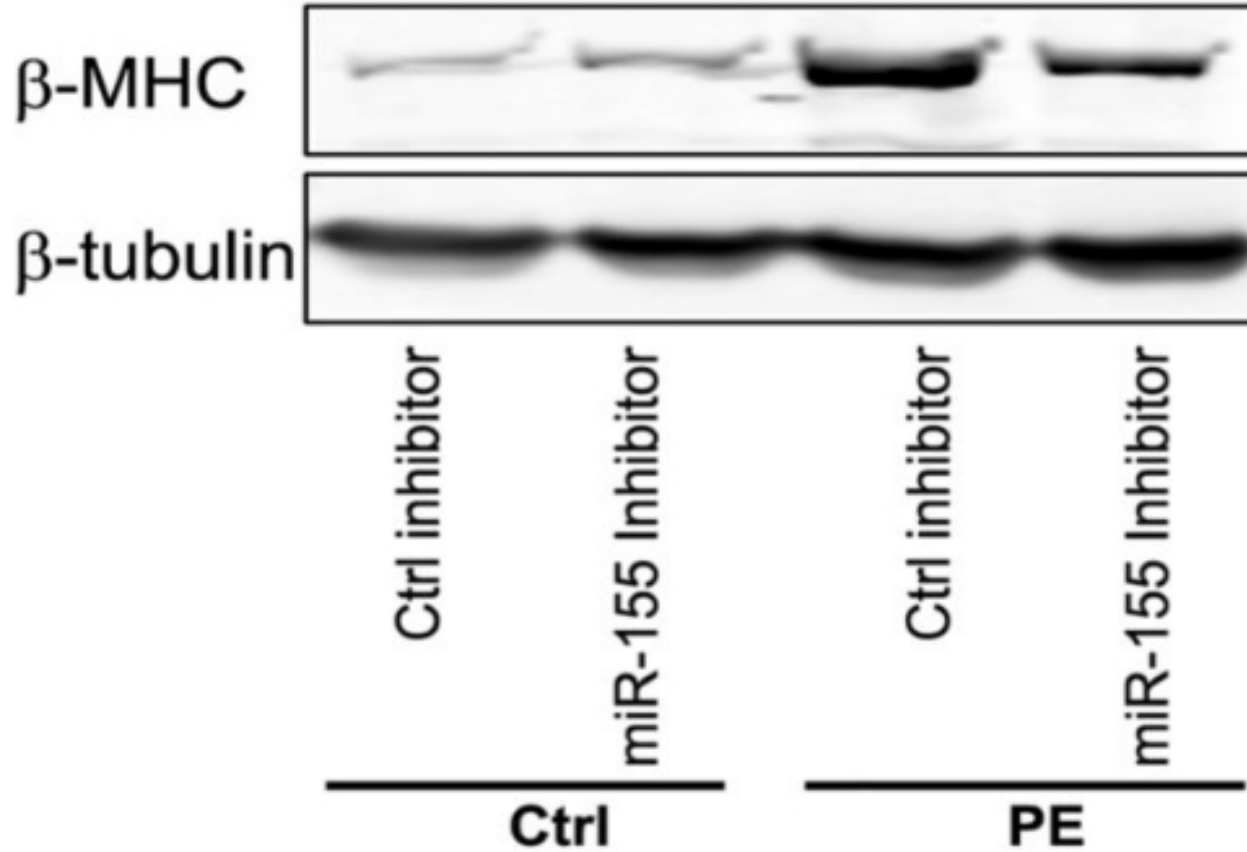


α -Actinin/DAPI

Individual Cells



Individual Cells



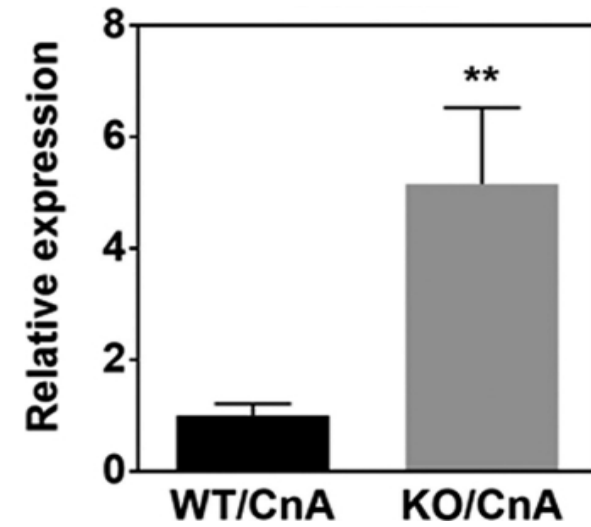
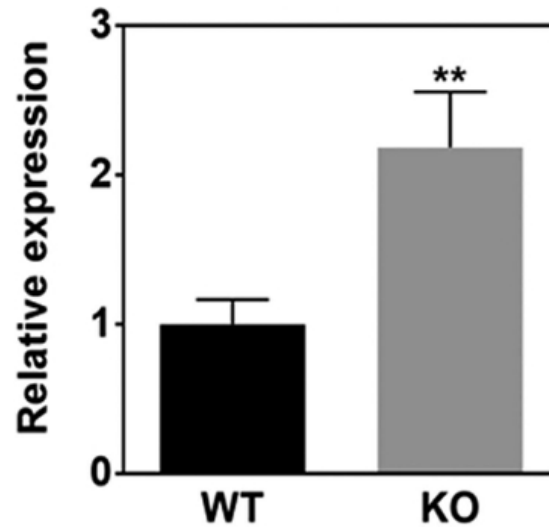
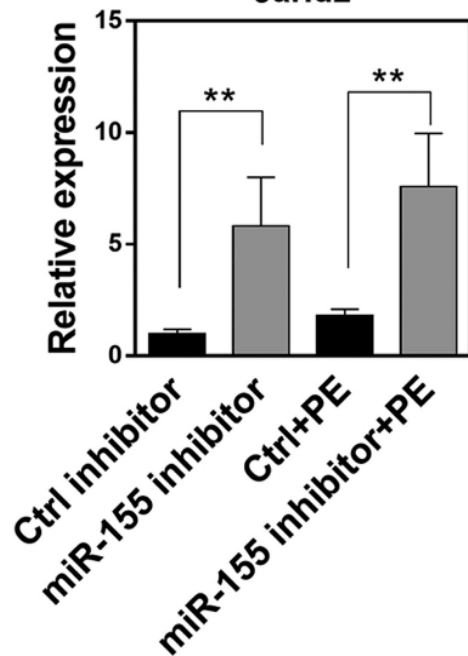
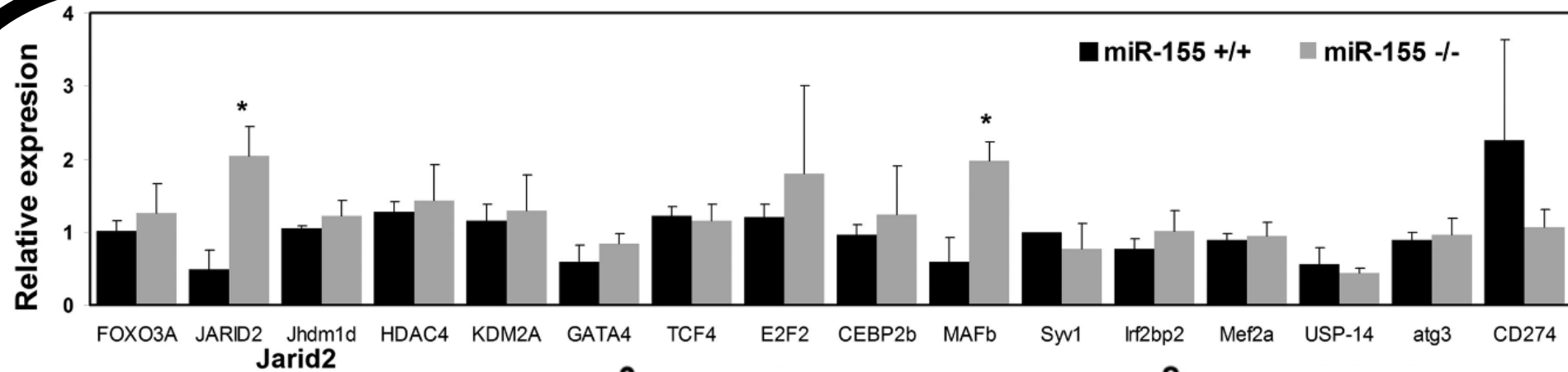
Knockout mice vs. Wild type

RESULTS #4 miR155 and JARID2

Role of Jarid2

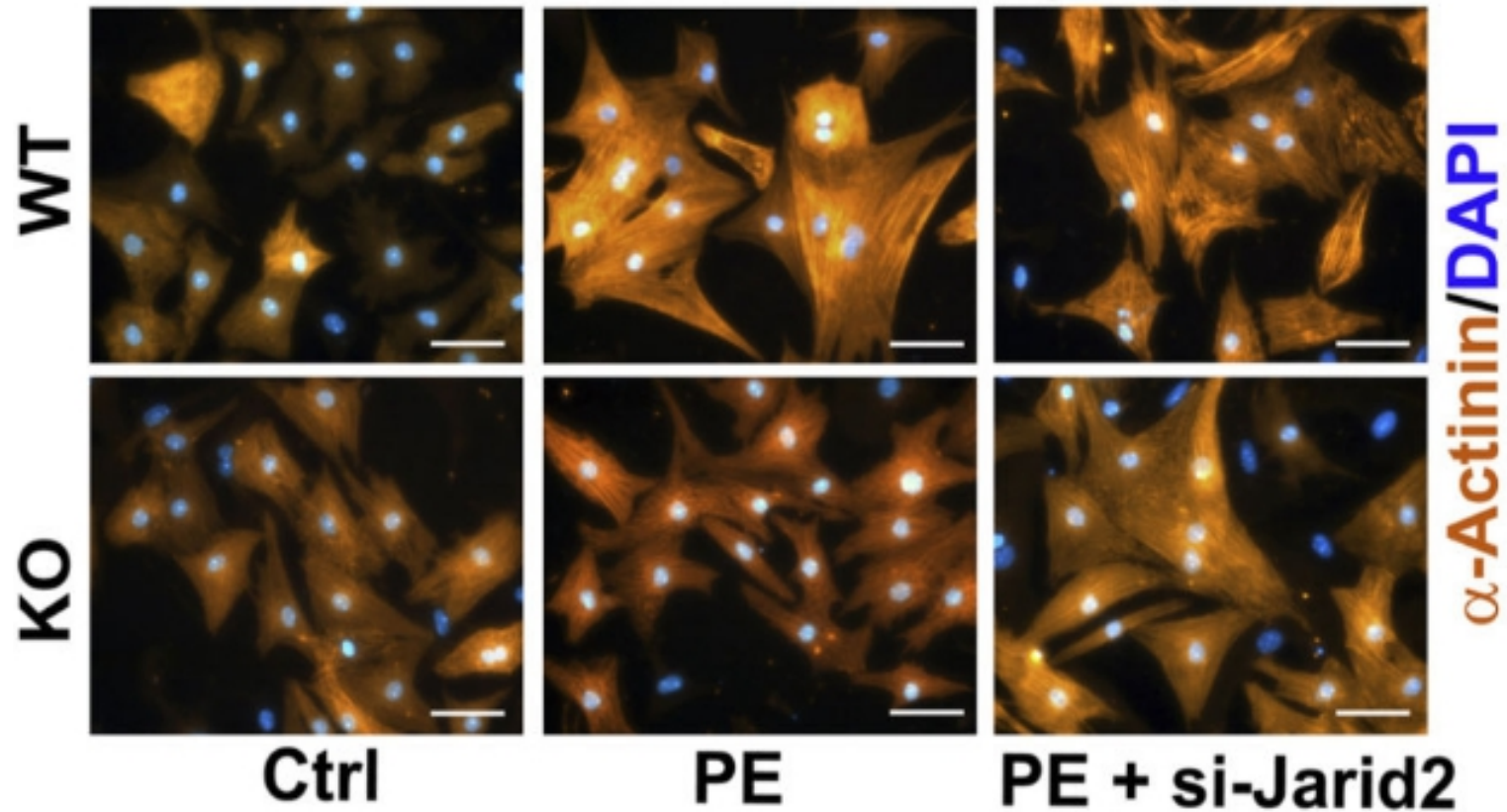
- Previously studies examined a role in cardiac hypertrophy
- Canonical target in T-lymphocytes
- Simulations expected it in cardiomyocytes as well

Relative Expression of Jarid2

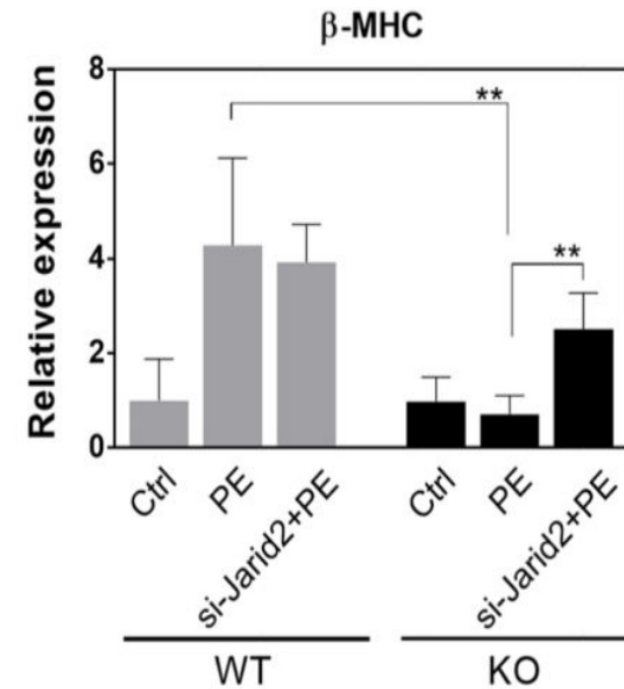
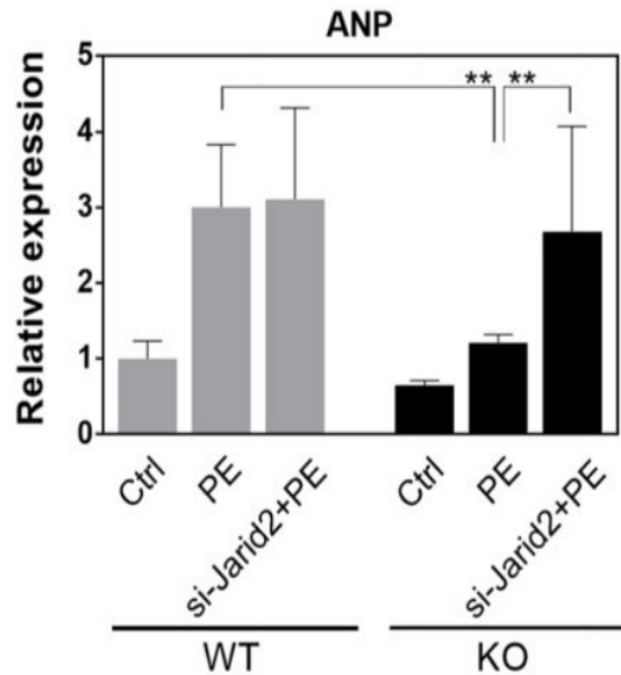
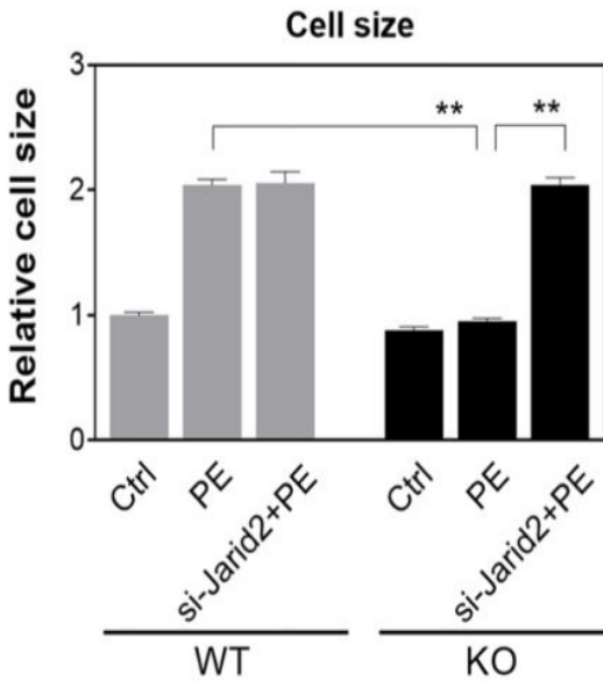


Histologic insight on effects of Jarid2

Neonatal mouse cardiomyocytes

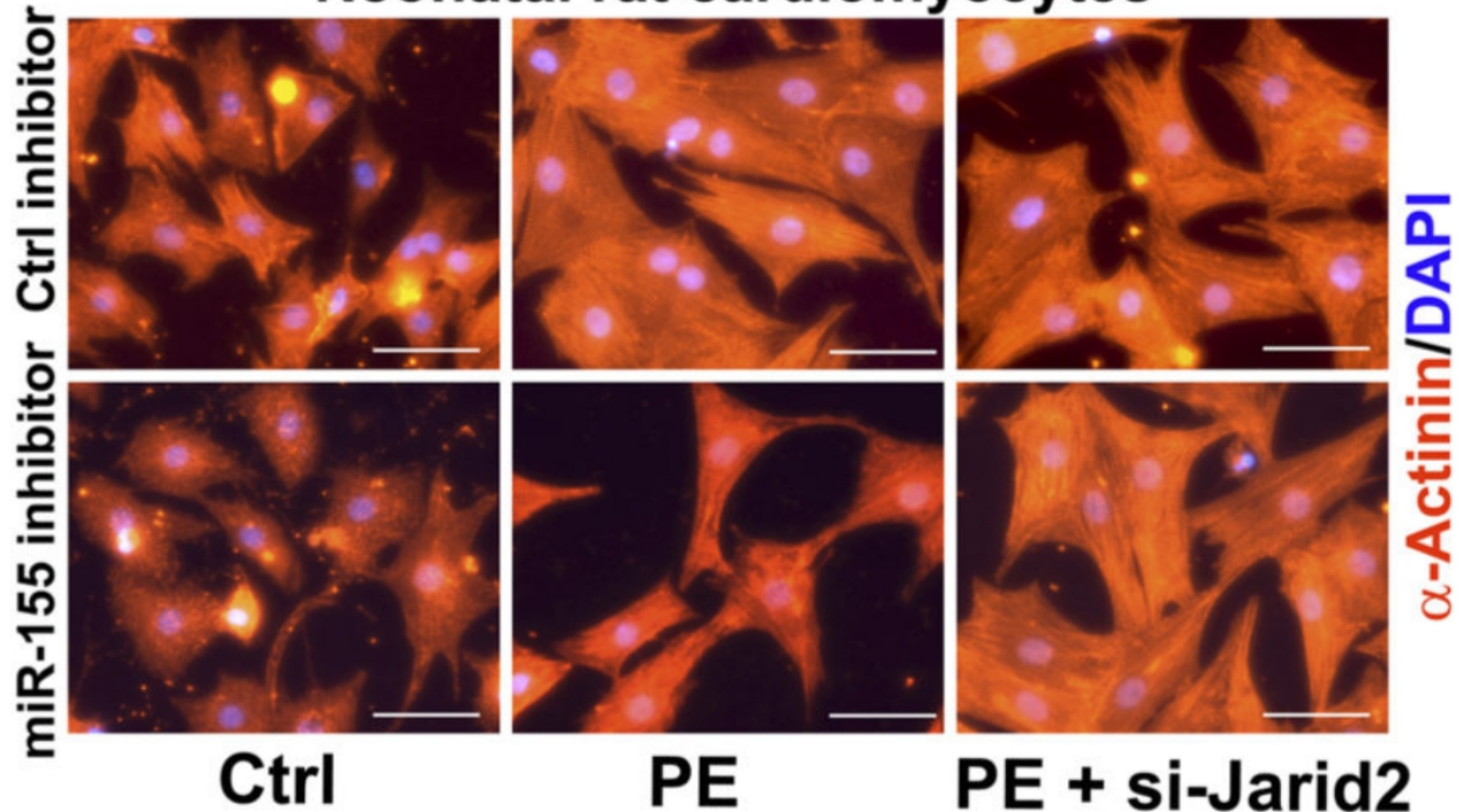


Effects of Jarid2



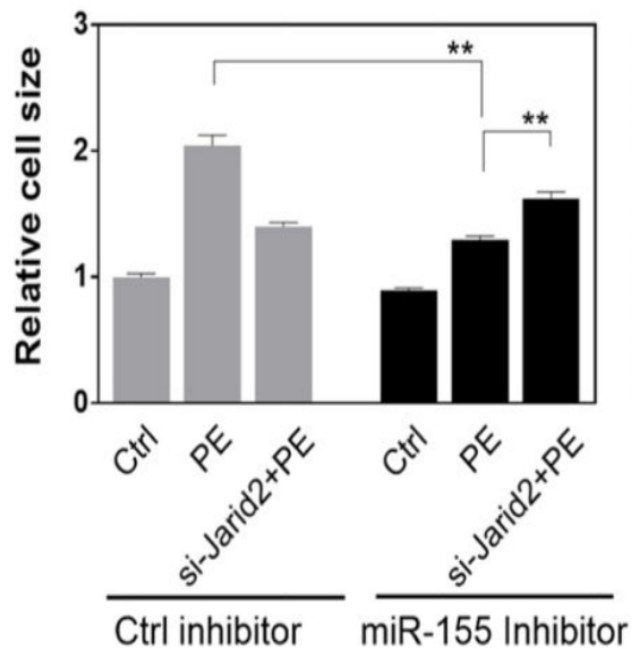
Histologic insight on effects of Jarid2

Neonatal rat cardiomyocytes

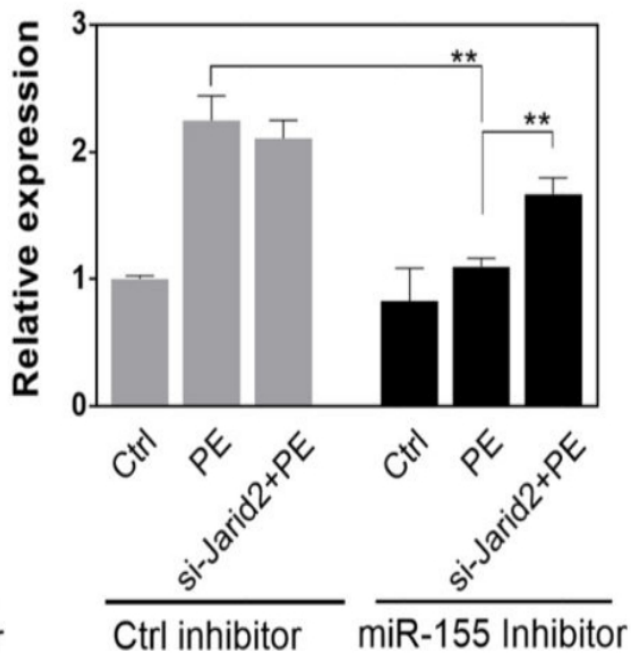


Effects of Jarid2

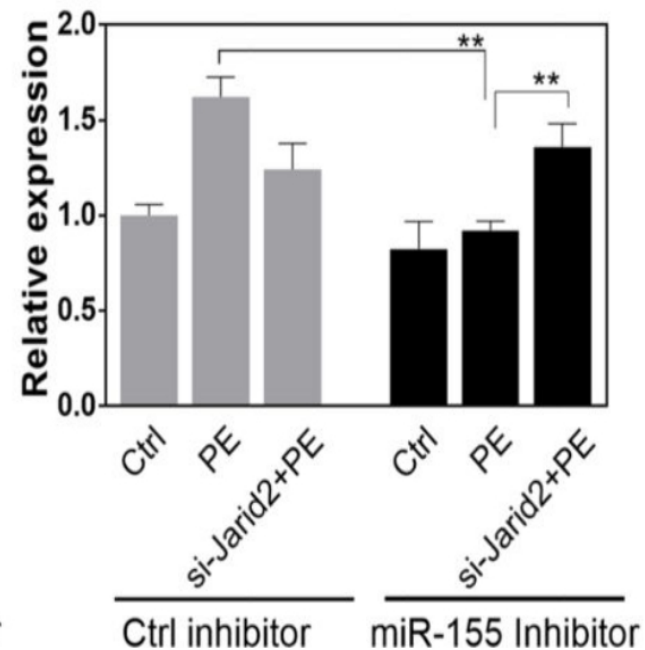
Cell size



ANP



β -MHC



Role of Jarid2

→ Jarid2 knockdown de-represses this loss-of miR-155 phenotype when treated with PE

- Also ANF and β -MHC expression was partly restored

Results were confirmed with rat-cardiomyocytes

Summary

- miR-155 is required for the development of cardiac hypertrophy in response to stress
- Inhibition of miR-155 protects cardiac function in a mouse model of cardiac hypertrophy.
- miR-155 could be a therapeutic target for the treatment of pathological cardiac hypertrophy.
- Jarid2 was identified as a direct miR-155 target that mediates its function in cardiomyocytes

Discussion

- β -MHC mutations involved in 40% of hypertrophic heart diseases – alterations in miR155 effects? ⁸
- Expression pattern (especially miR155) differs strongly from neonatal to adult → only neonatal cells were isolated
- Remodeling necessary to a certain point – adverse effects?

Discussion

- Future: more specific miR155 knockout
- Inhibition of Jarid2 could partly rescue the effect of mir155 loss
- But Jarid2-inhibition reduced PE-induced hypertrophy → distinct role of Jarid2
- Wide field of not yet known miR155 derived mechanisms in the heart
- Putative therapeutic target for cardiac defects