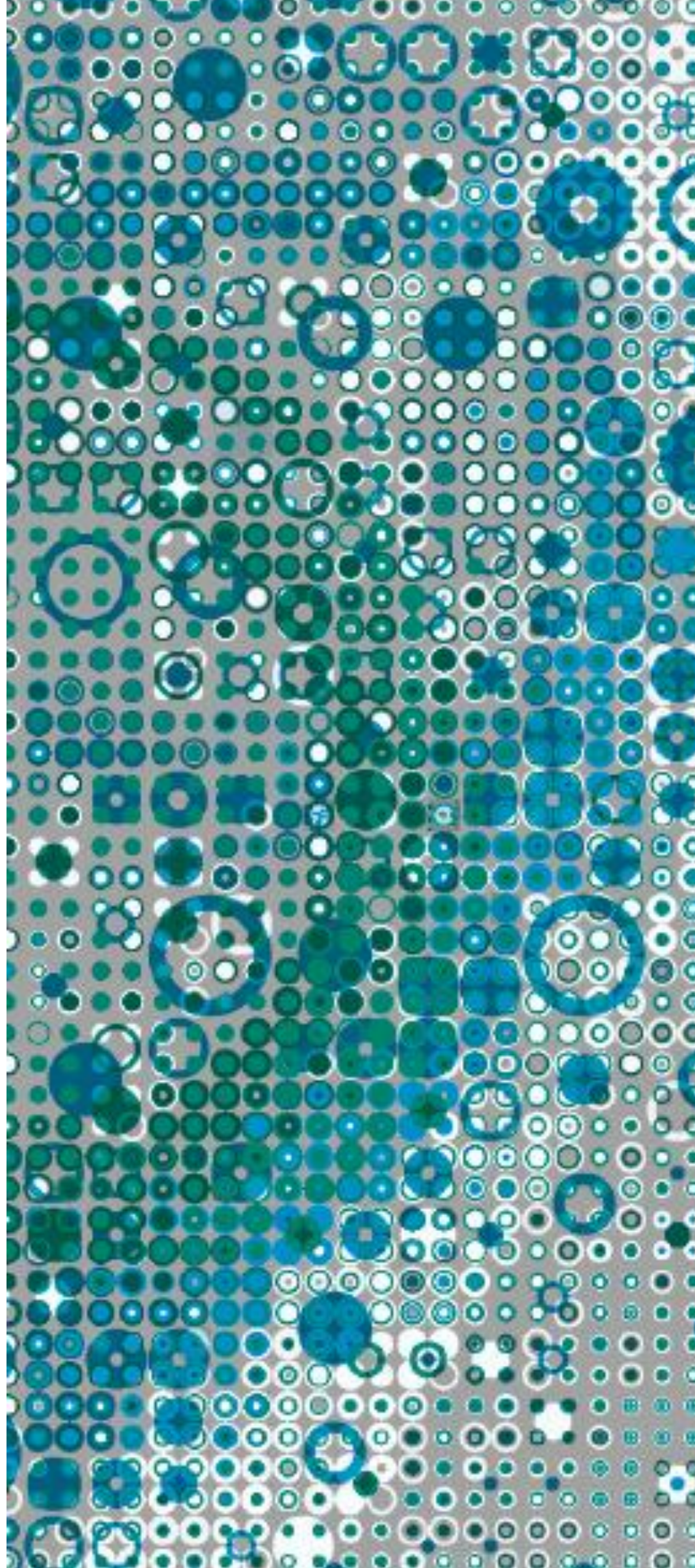
Hubrecht Institute

Developmental Biology and Stem Cell Research

MicroRNAs as therapeutic targets in Cardiac disease

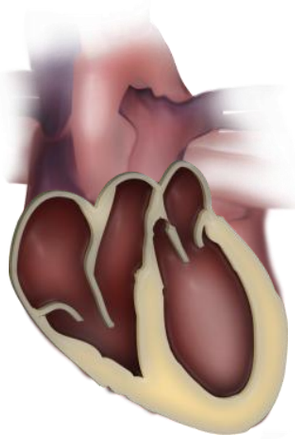
ESC Summer school Nice, June 2015

Eva van Rooij



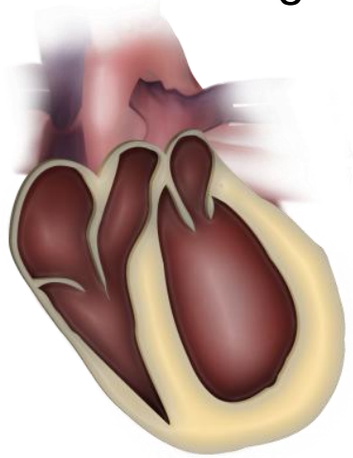
Remodeling of the heart

Normal Heart



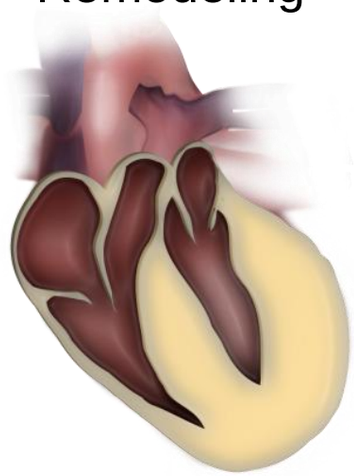
Exercise, Pregnancy

Physiologic Remodeling



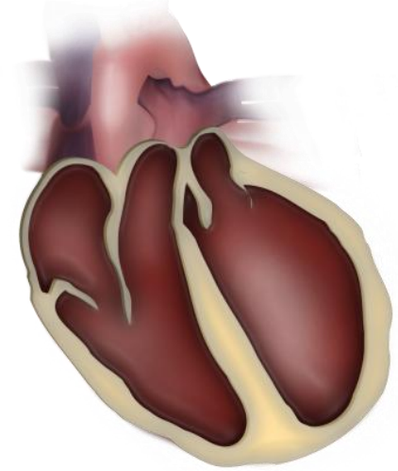
Hypertension, MI and Neurohumoral Activation

Pathologic Remodeling



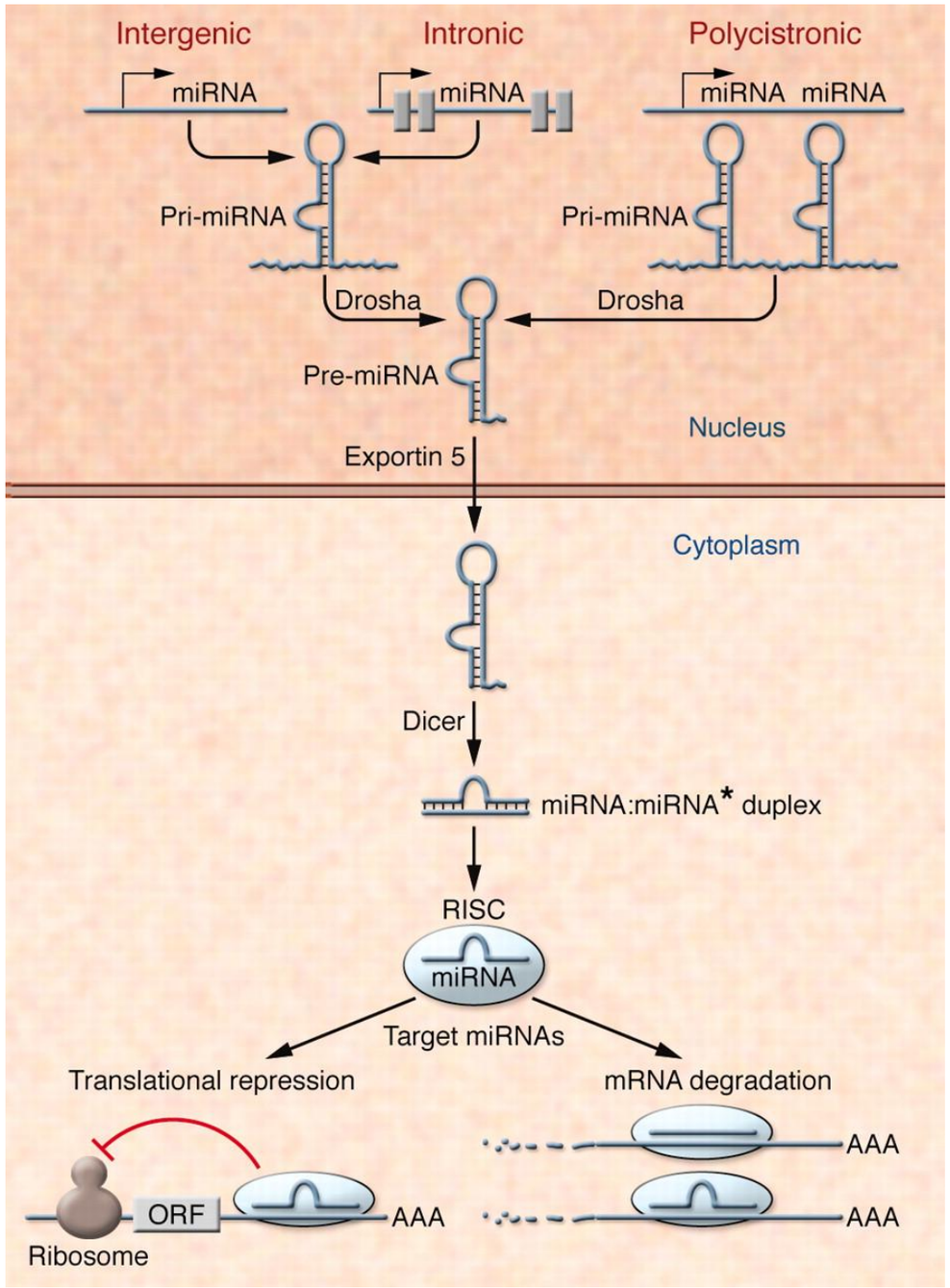
Stress

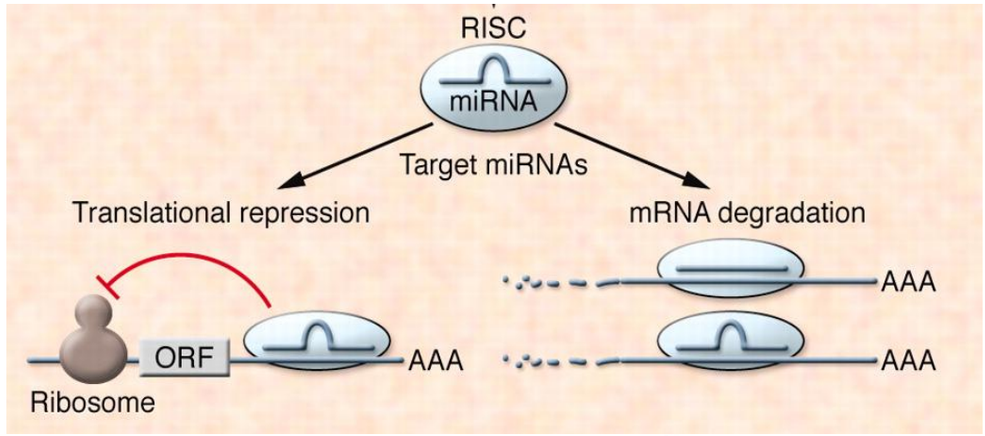
Heart Failure



- *Myocyte hypertrophy*
- *Apoptosis*
- *Fibrosis*
- *Metabolic shift*
- *Reduced cAMP generation*
- *Fetal gene activation (α → β - myosin switch)*

MicroRNA biogenesis & conservation





Seed

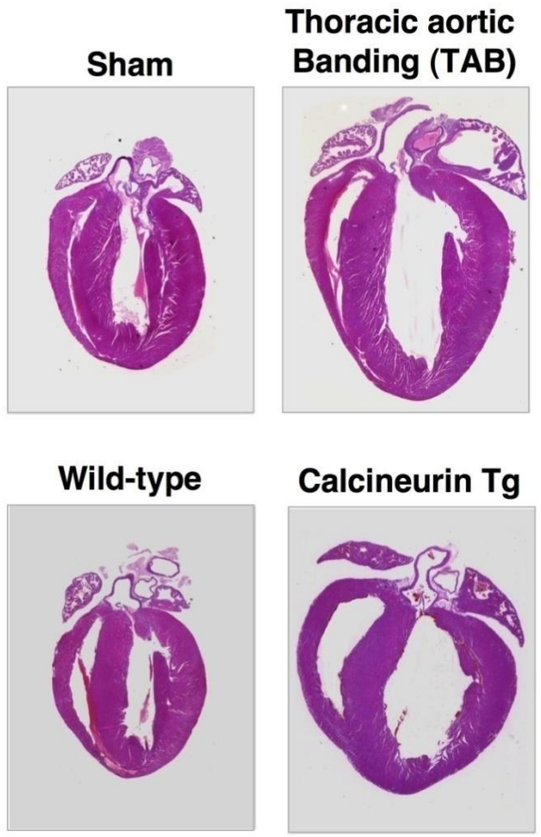
AAGUUUUGUACUUAACGACGAC **5' miRNA**

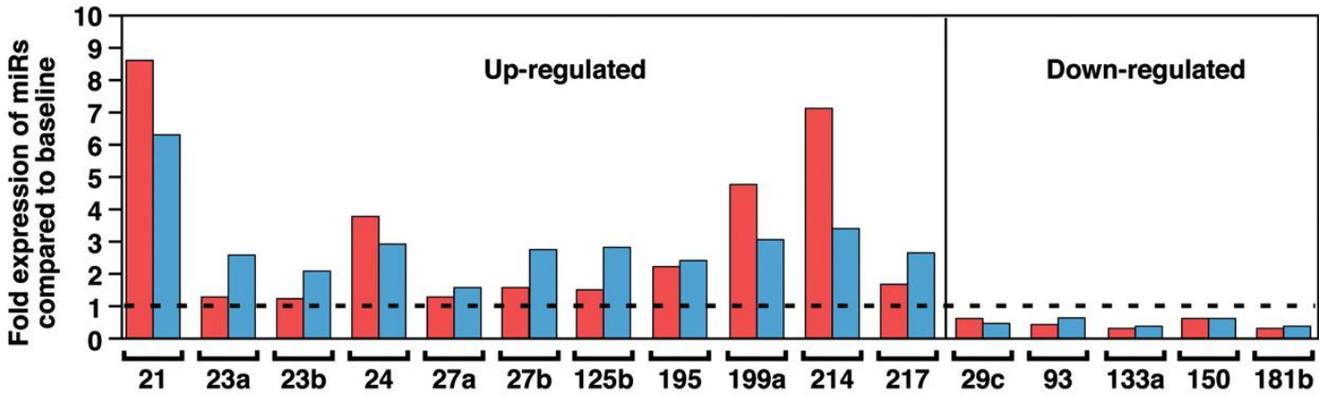
3' UTR target gene . . . GAAGUUACACA-GAAAUGCUGCUG . . .

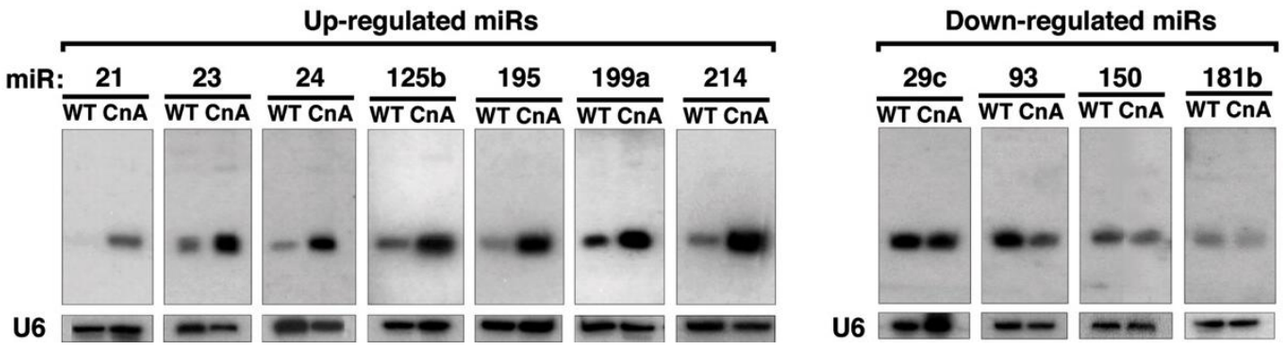
Fish miR-214	ACAGCAGGCACAGACAGGCAG.....
Chimpanzee miR-214	ACAGCAGGCACAGACAGGCAG.....
Rhesus Macaque miR-214	ACAGCAGGCACAGACAGGCAG.....
Rat miR-214	ACAGCAGGCACAGACAGGCAG.....
Opossum miR-214	ACAGCAGGCACAGACAGGCAG.....
Wild boar miR-214	ACAGCAGGCACAGACAGGCAG.....
Zebrafish miR-214	ACAGCAGGCACAGACAGGCAG.....
Xenopus miR-214	ACAGCAGGCACAGACAGGCAG.....
Cow miR-214	ACAGCAGGCACAGACAGGCAGU.....
Human miR-214	ACAGCAGGCACAGACAGGCAGU.....
Mouse miR-214	ACAGCAGGCACAGACAGGCAGU.....
Lizard miR-214	ACAGCAGGCACAGACAGGCAGU.....
Carp miR-214	ACAGCAGGCACAGACAGGCAGU.....

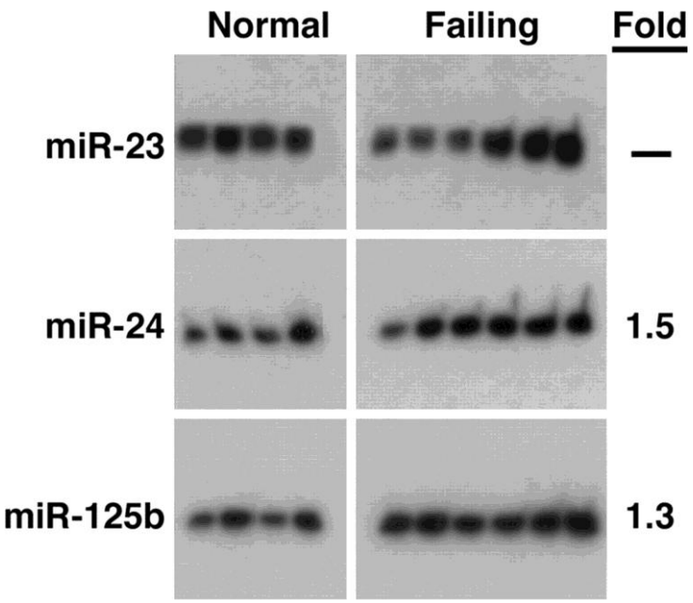
van Rooij & Olson JCI 2007

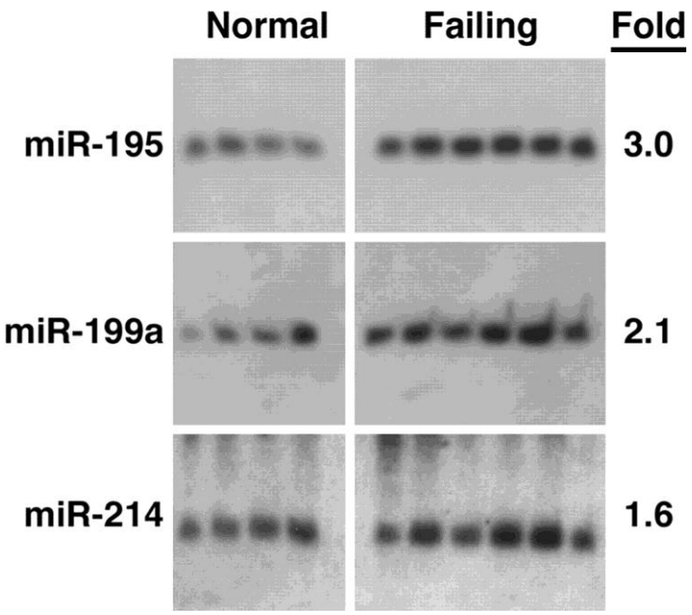
A microRNA signature of heart disease

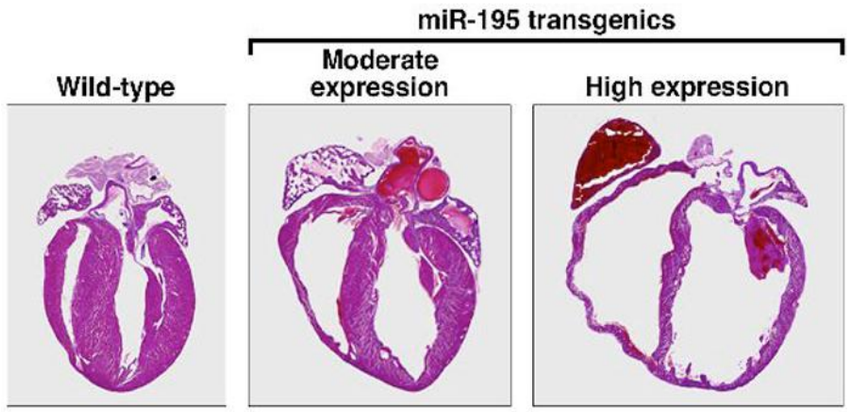






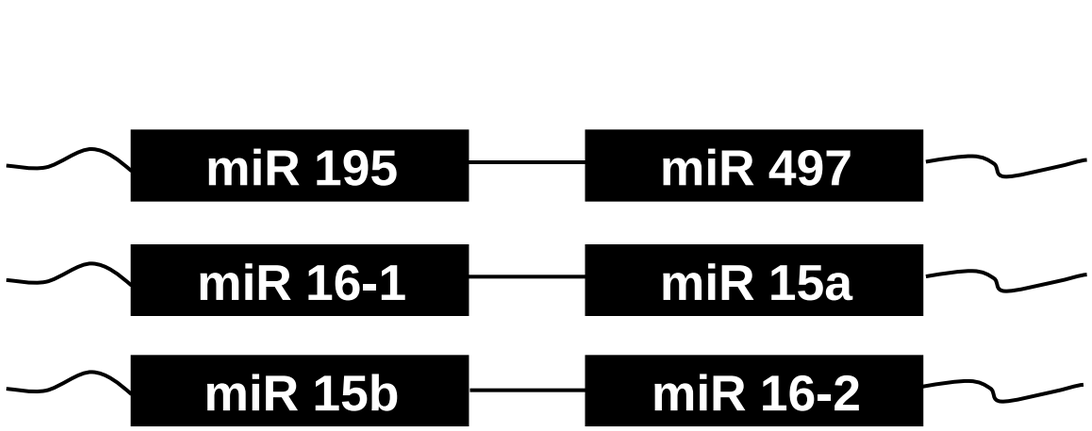






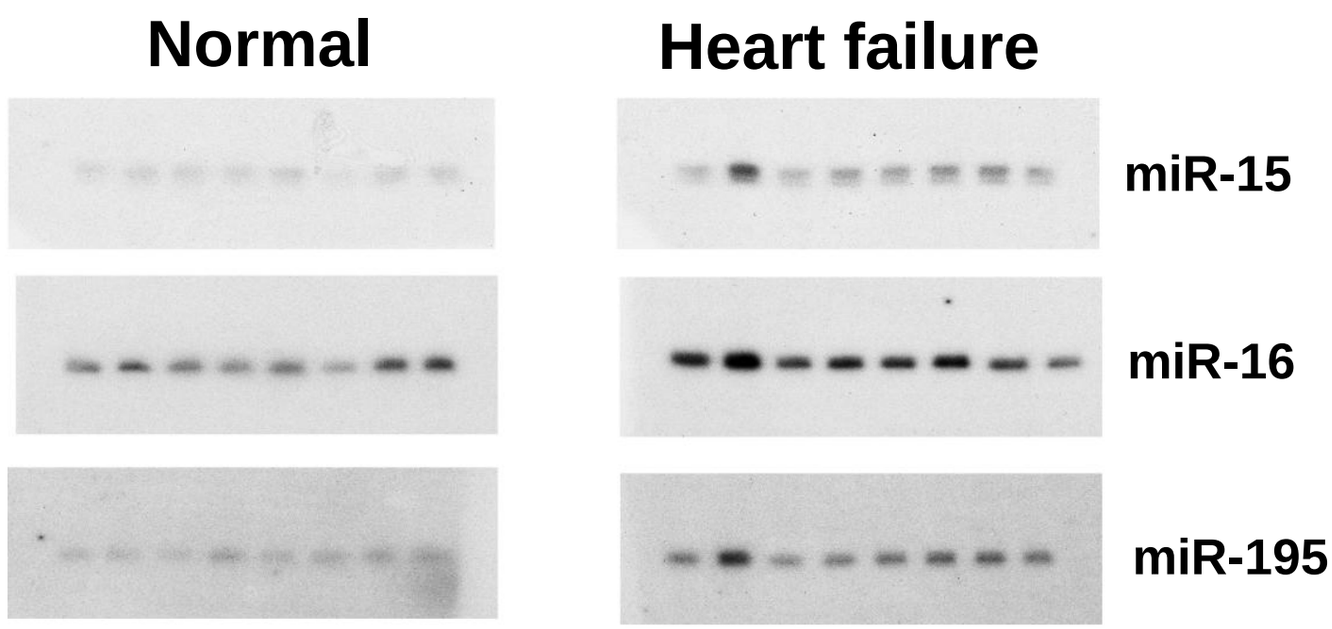
van Rooij et al. PNAS 2006

miR-15 family is upregulated in human heart failure



Seed

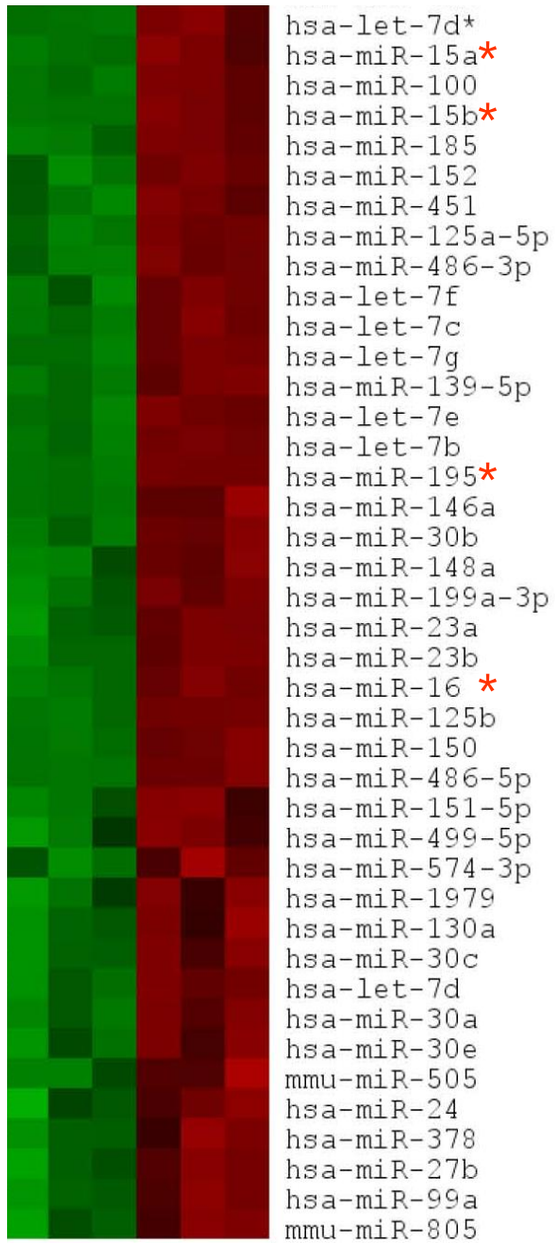
U**AGCAGCA**CAGAAAUAUUGGC-
C**AGCAGCA**CACUGUGGUUUGU
U**AGCAGCA**CGUAAAUAUUGGCG
U**AGCAGCA**CAUAAUGGUUUGUG
U**AGCAGCA**CAUCAUGGUUUACA
U**AGCAGCA**CGUAAAUAUUGGCG



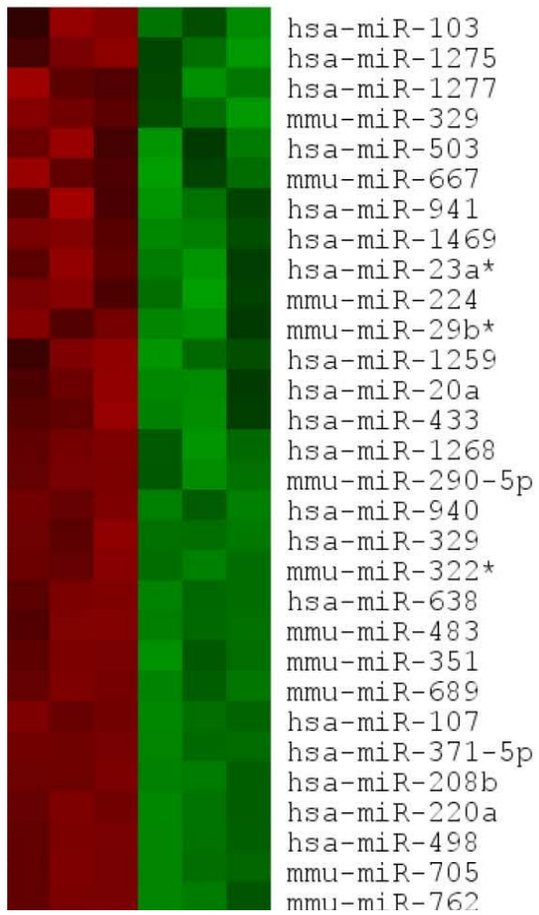
“Ongoing loss of cardiac myocytes is responsible for the development and worsening of heart failure .”

Cardiac microRNA regulation during neonatal cell cycle arrest

Up-regulated

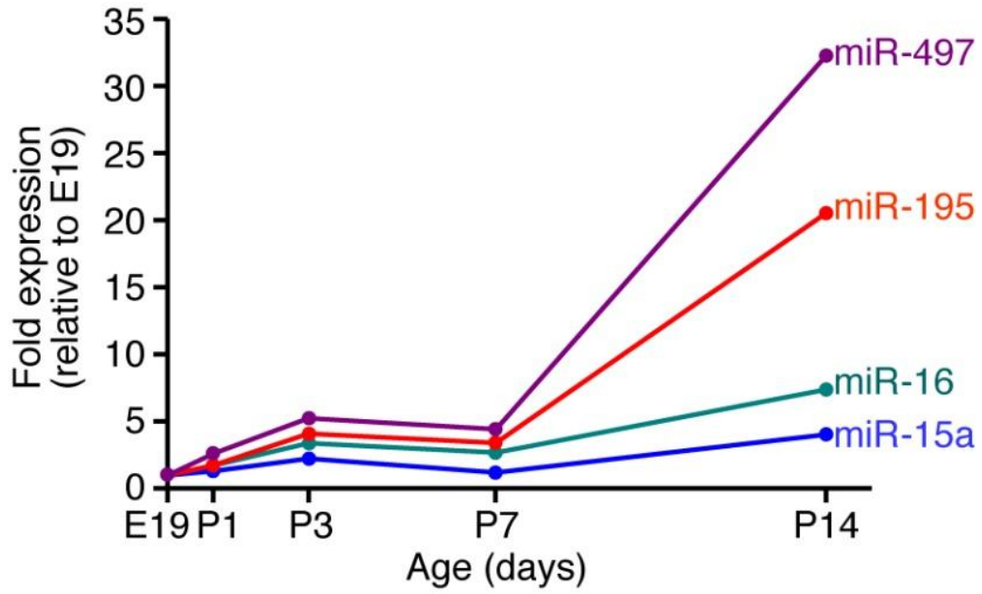


Down-regulated



p1 versus p10 hearts

Up-regulation of miR-15 family



Porrello et al. Circ Res 2011

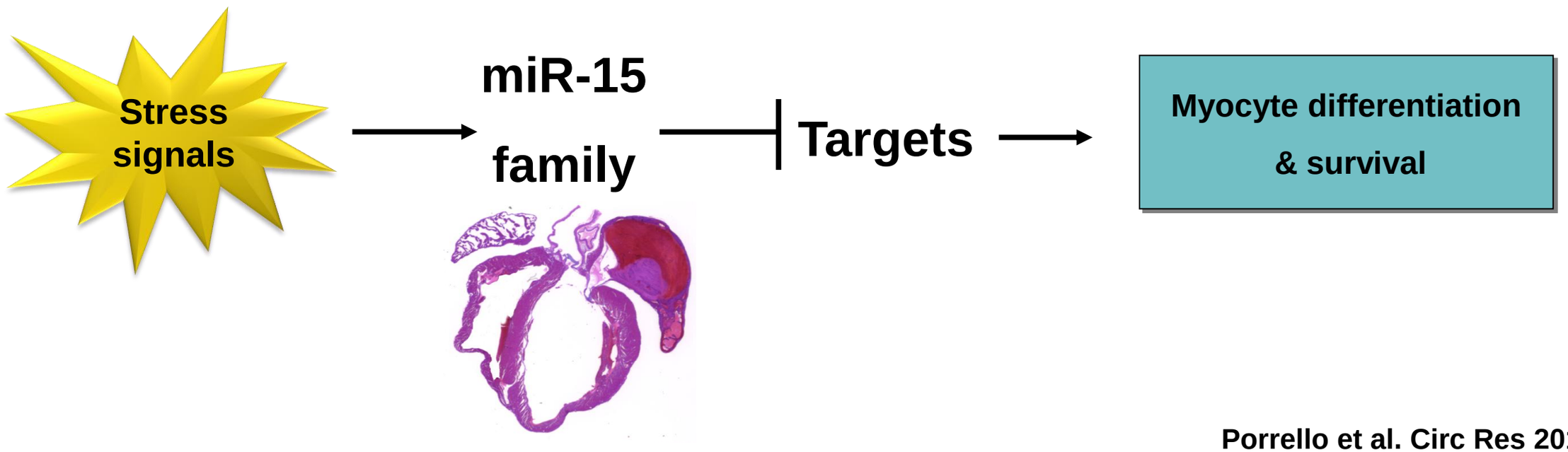
miR-15 controls cell cycle and survival

Cyclins:

-Cyclin E1
-Cyclin D2
-Cyclin T2
-Cyclin M2
-Cyclin D1
-Cyclin D3

Cell cycle / survival:

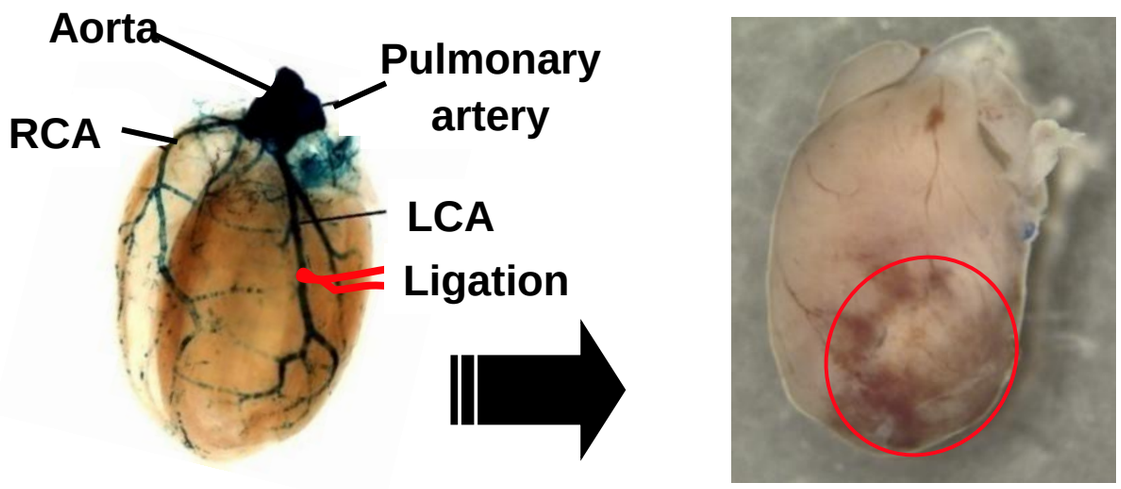
-CDCA4
-CDC42
-BCL2L2
-CDC27
-Smad7
-E2F7
-Smad5
-PDCD4
-PBBP6
-CDC37L1
-CHEK1
-CDC25A
-CDC14B
-CDK5R1
-Smad3
-CAPRIN1
-CDC14A
-E2F3
-Tcl-1
-PDCD6IP
-CDC23
-G0S2
-CCNJL
-CDC25B
-CDCA7L
-CRKRS
-PAK7
-CDK6
-BCL2

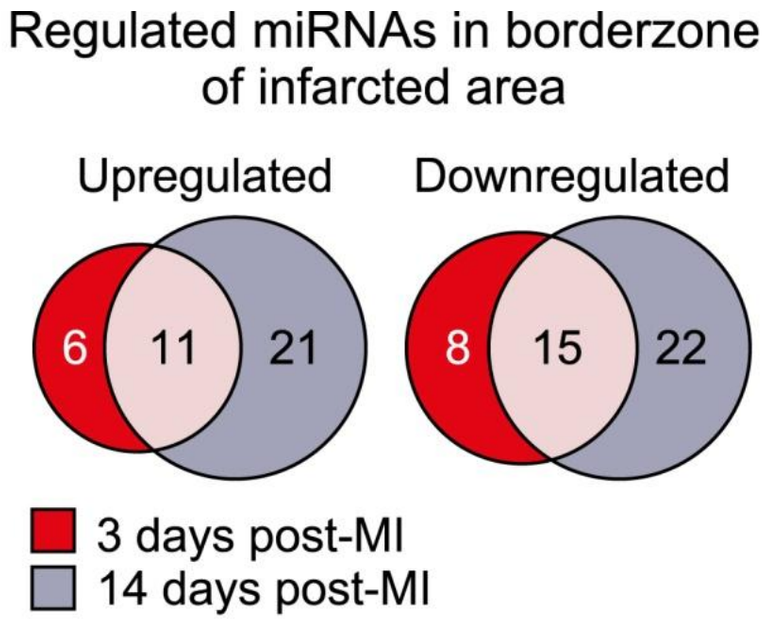


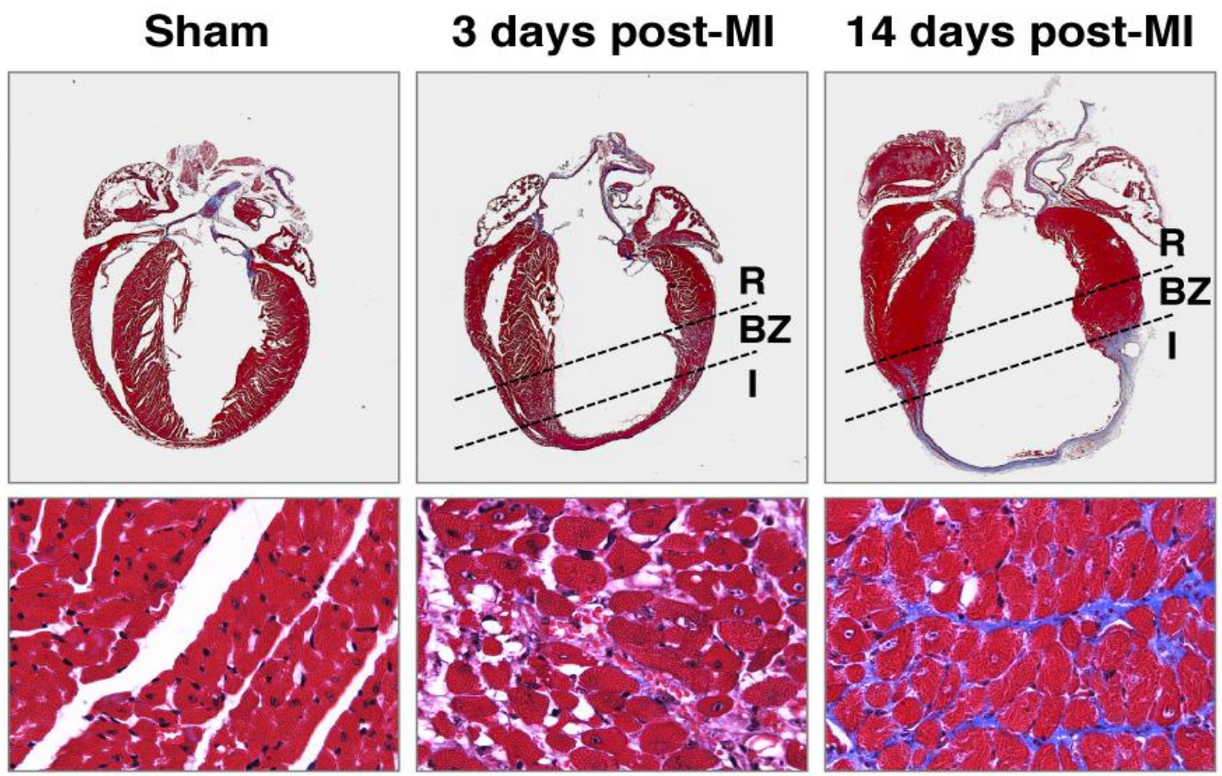
Porrello et al. Circ Res 2011

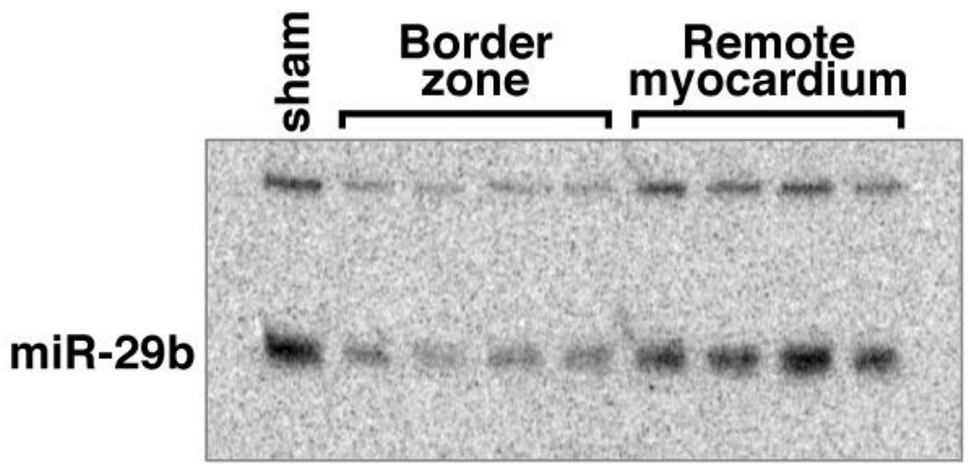
Hullinger et al. Circ Res 2012

MicroRNA expression in response to ischemic injury



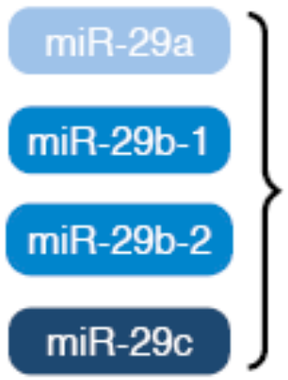




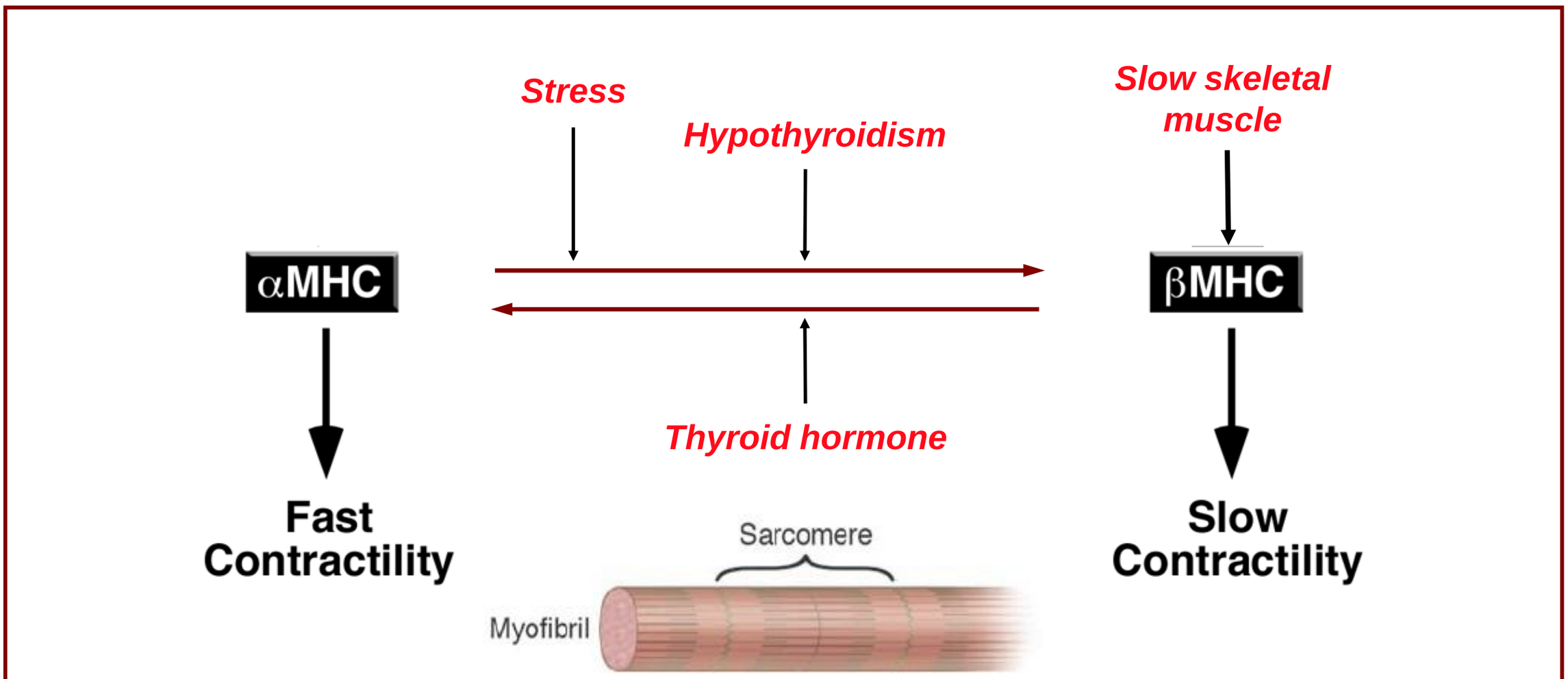


van Rooij et al. PNAS 2008

miR-29 regulates ECM



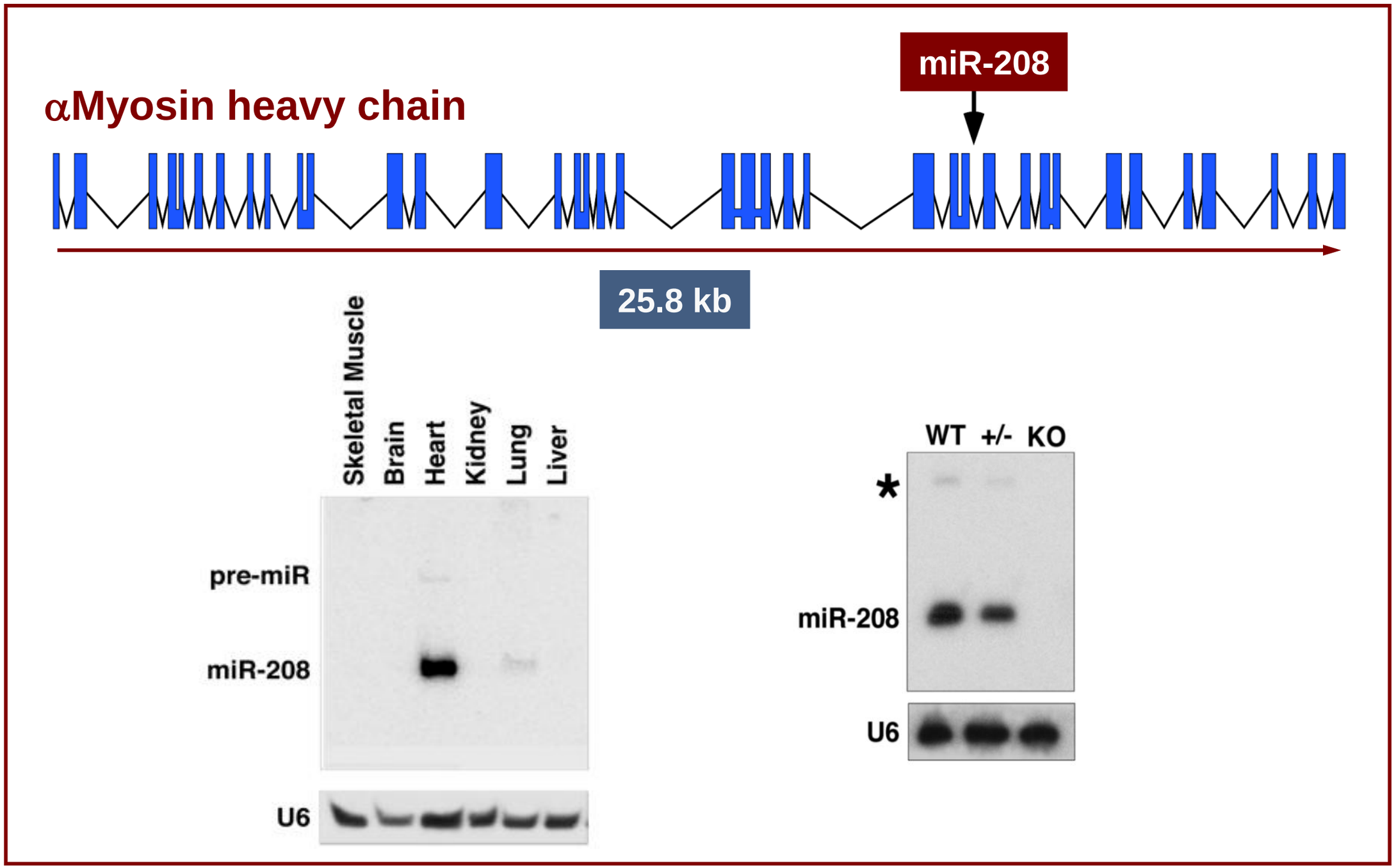
Cardiac myosin switching



αMHC - high ATPase activity
- downregulated during heart disease
- upregulated by thyroid hormone

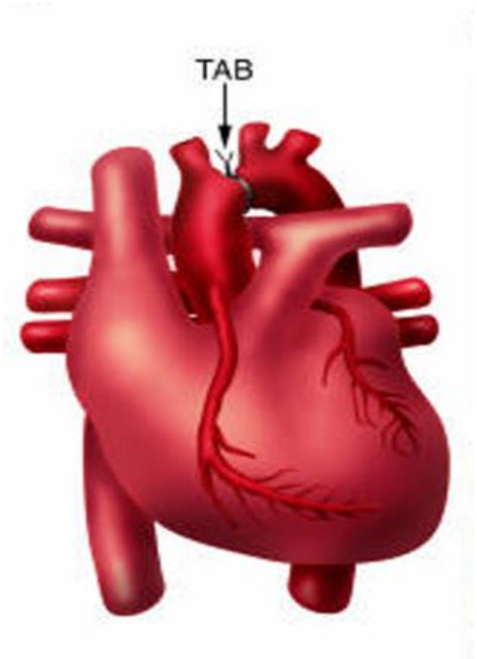
βMHC - low ATPase activity
- upregulated during heart disease
- upregulated by hypothyroidism

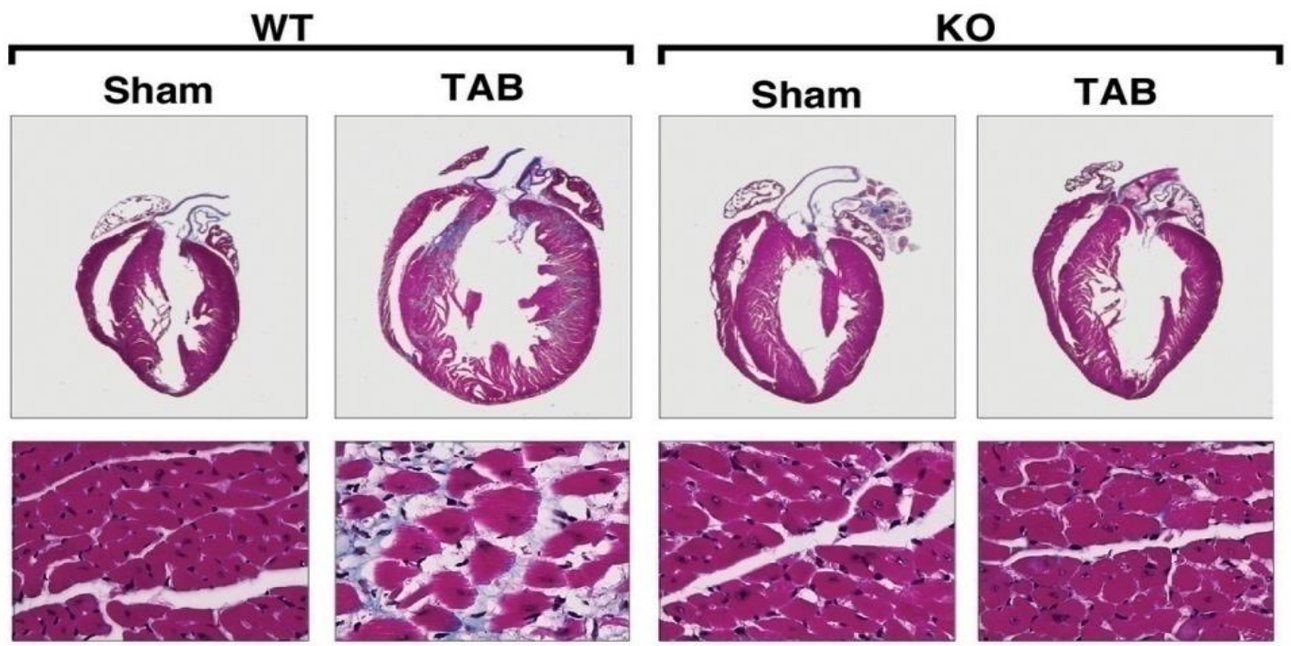
miR-208 is cardiac specific and co-expressed with αMHC

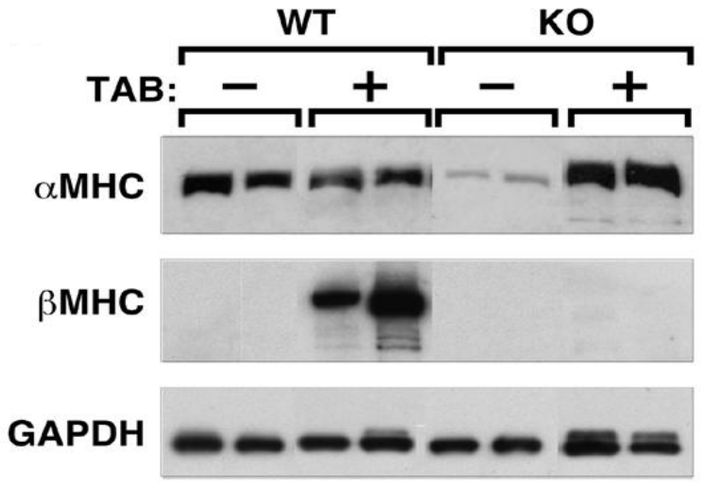


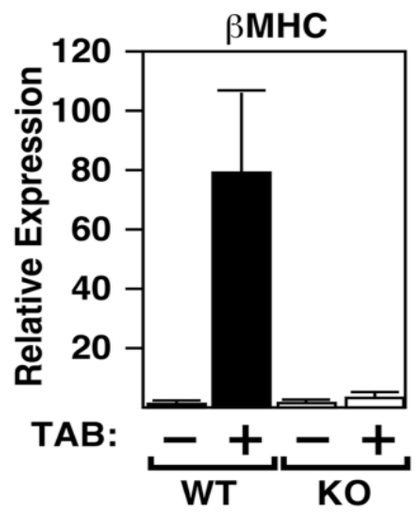
van Rooij et al. Science 2007

Genetic deletion of miR-208 blocks stress-induced remodeling and βMHC expression

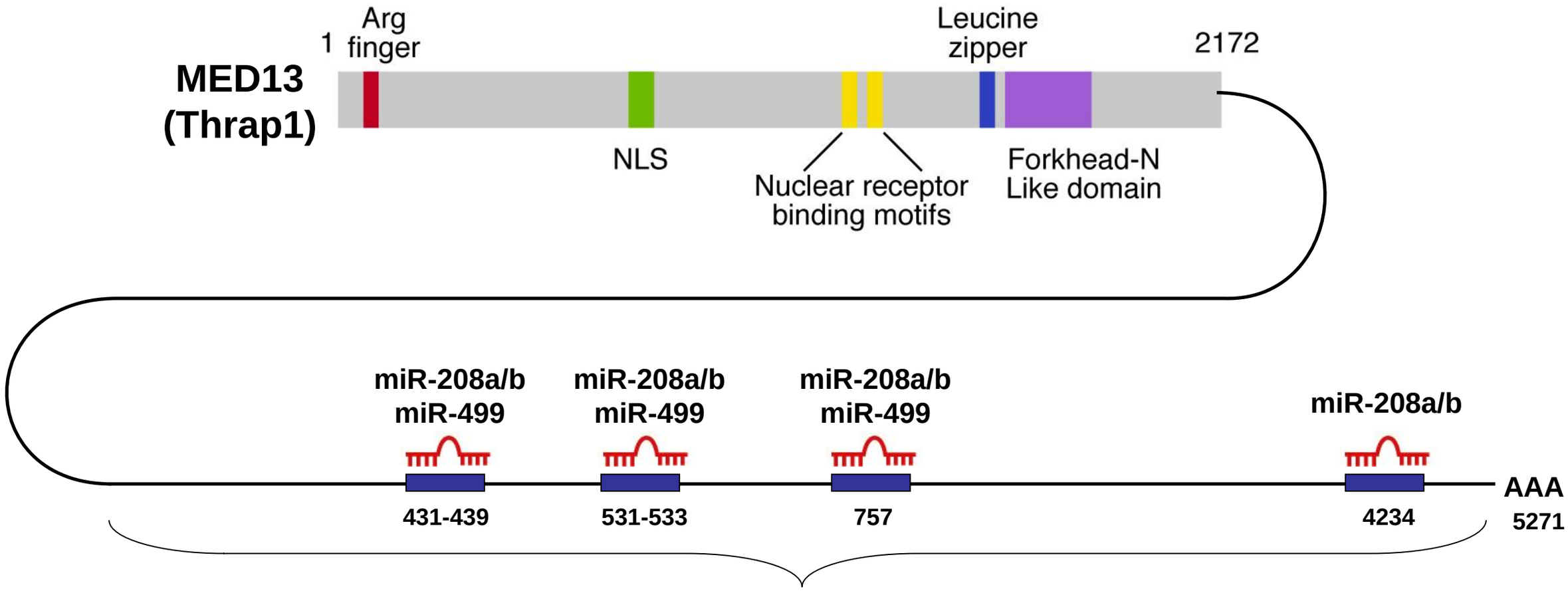


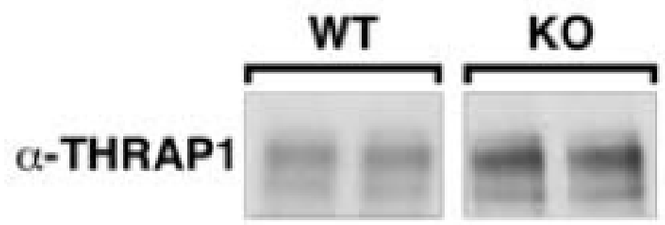




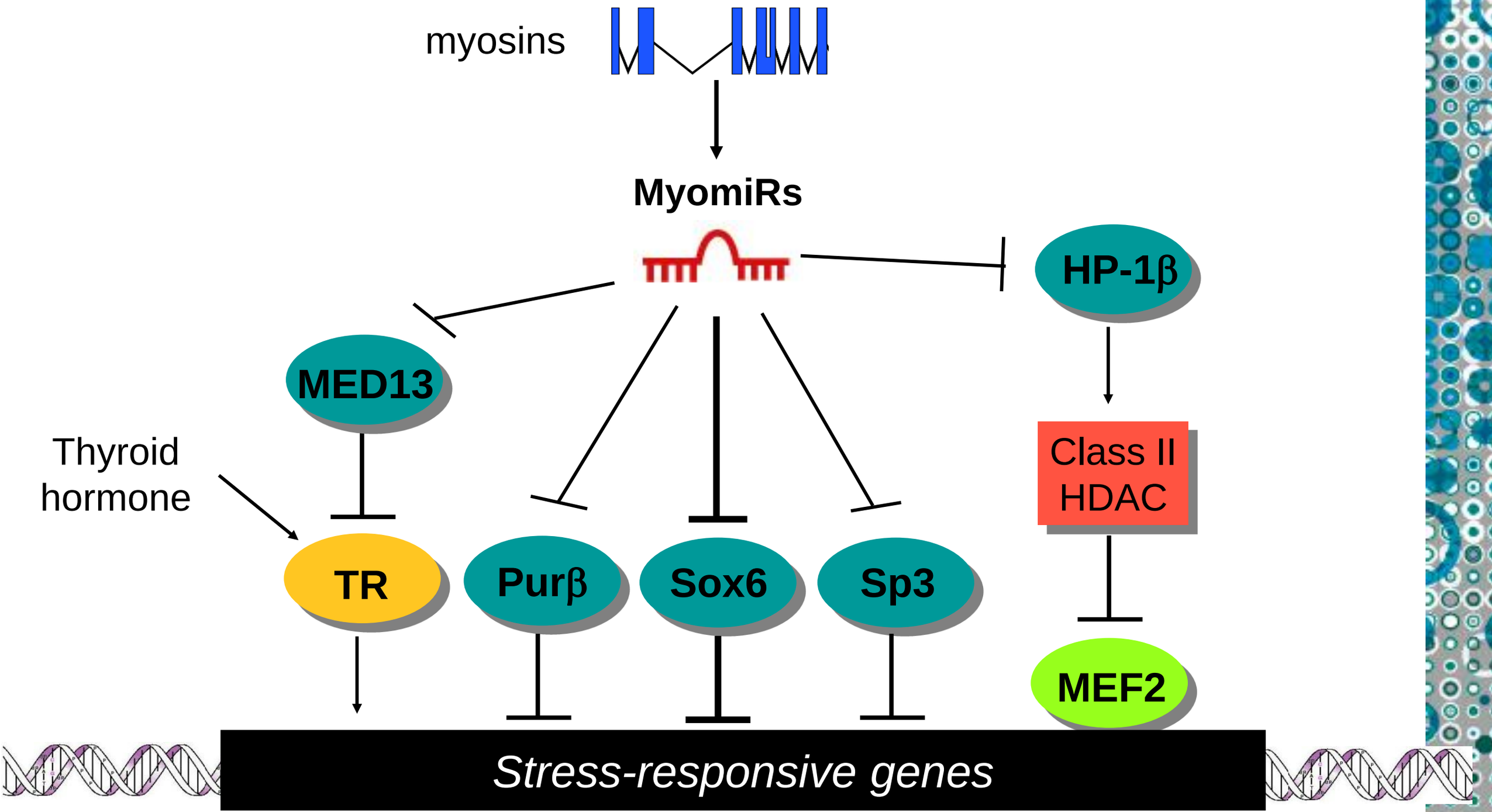


miR-208 targets Thrap1 / MED13





miR-208 controls a network of transcriptional repressors

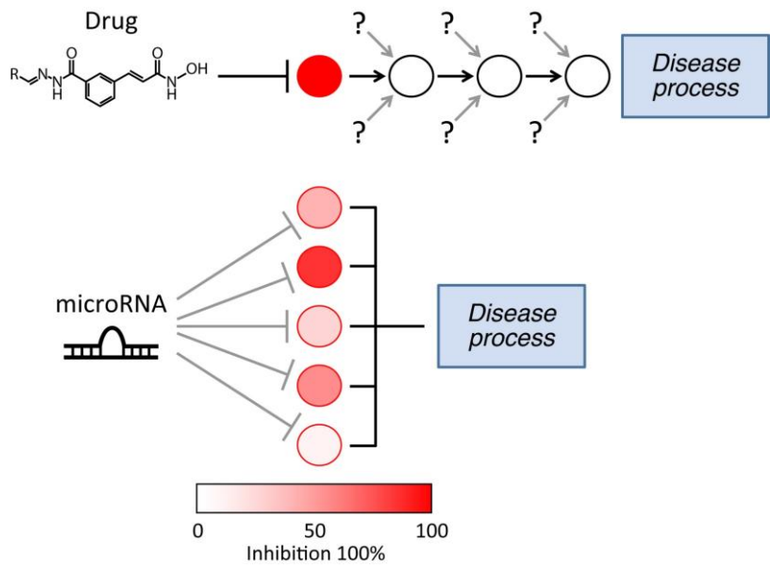


cardiac fibrosis and hypertrophy

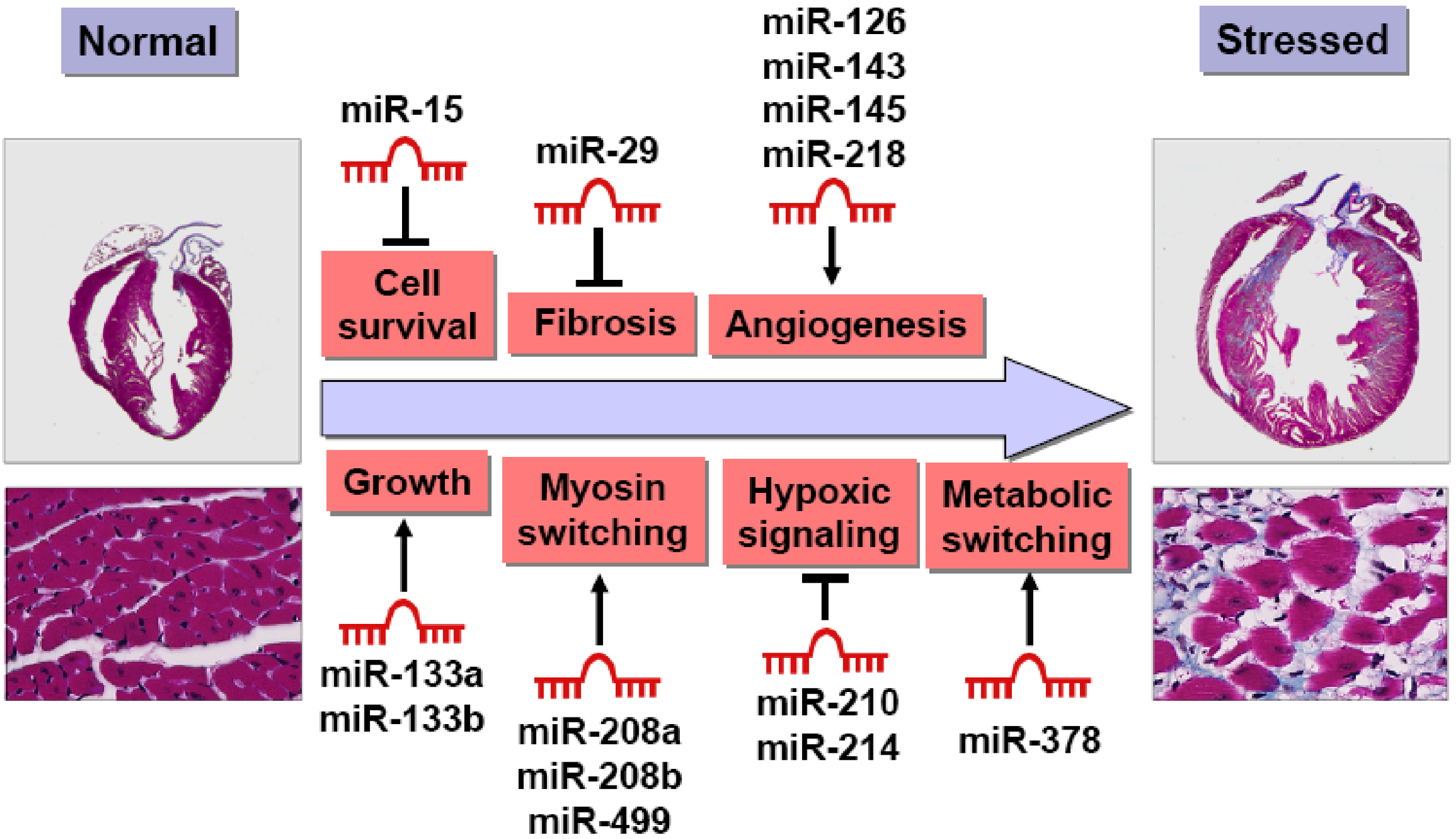
van Rooij et al. Dev Cell 2009

Basics of microRNA biology

- MiRNAs are conserved across species
- MiRNAs are regulated during disease
- MiRNAs are more important under stress conditions, i.e. during disease
- The genome can contain multiple copies of a microRNA and they often target multiple genes involved in a similar cellular process or signaling pathway
- Intronic miRNAs often influence the function of the hostgene
- Presence of multiple binding sites within an UTR can correlate to the relevance of the miRNA/mRNA interaction
- The level of gene regulation is moderate, but the combined influence of these relatively small changes can have a profound downstream effects

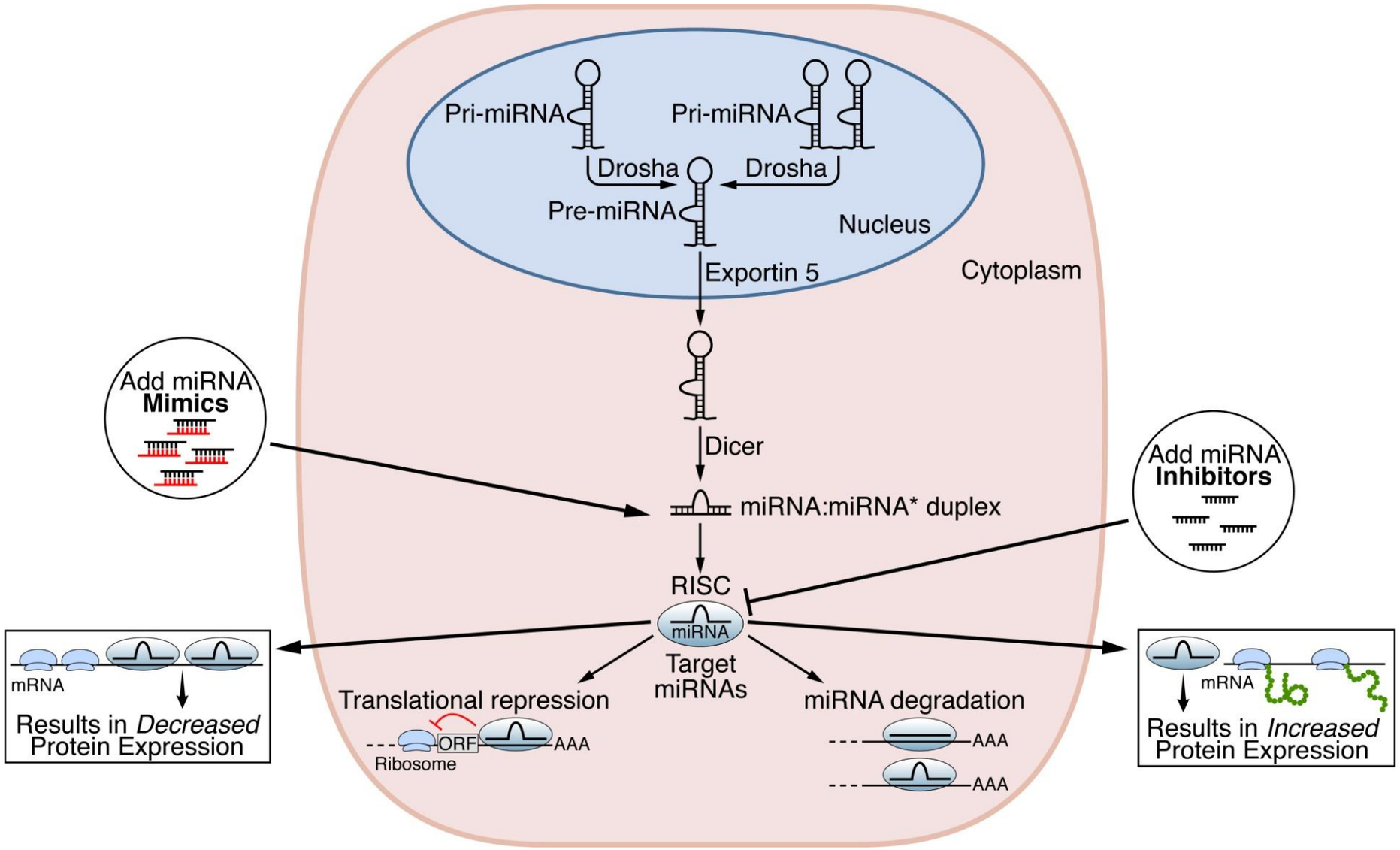


MicroRNA function in cardiac disease

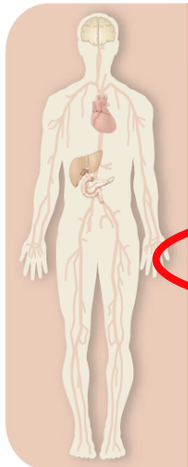


van Rooij et al., PNAS, 2006, 2008; Science, 2007; JCI, 2007; Dev Cell, 2009; Wang et al., Dev Cell, 2008; Liu et al., Genes & Dev, 2008; Xin et al., Genes & Dev, 2009; Williams et al., Science, 2009

MicroRNA modulation



Feb 2007, n=2



Bringing MicroRNA Discoveries to the Clinic

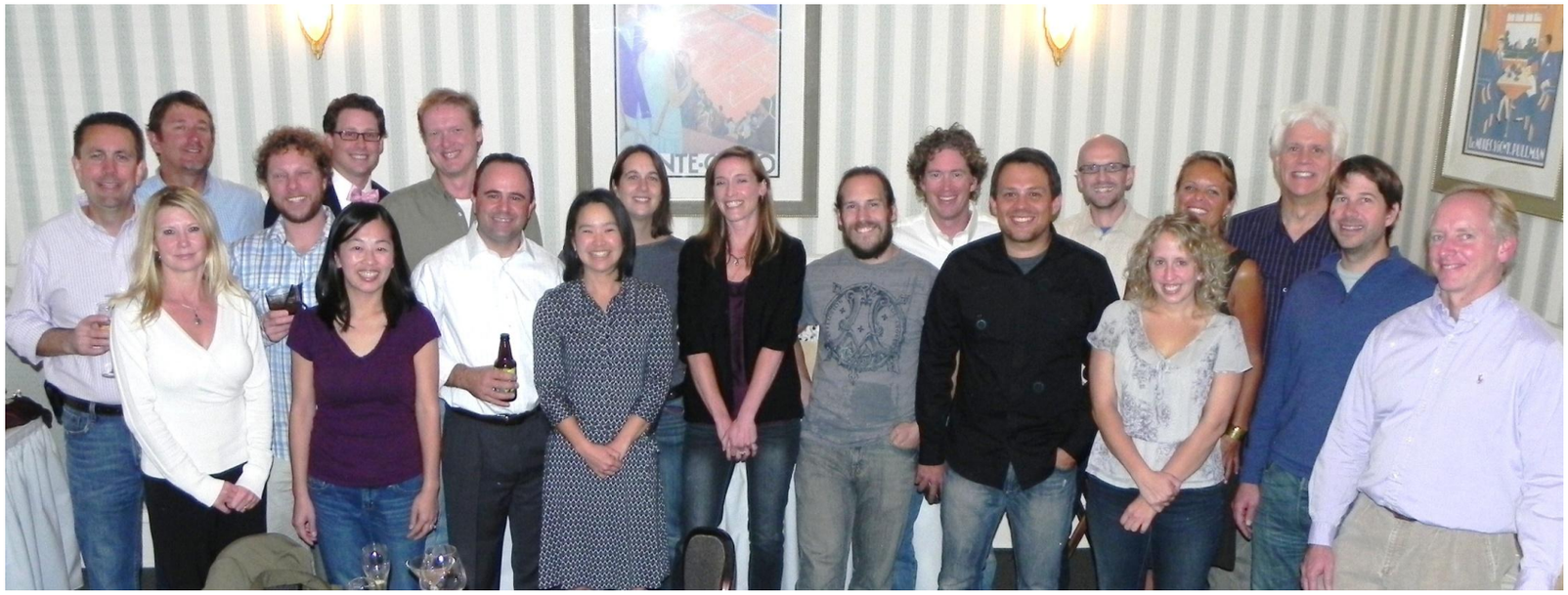
Company	Location	Disease Focus	Founded
Asuragen	Austin, Texas	Cancer diagnostics	2006
Crogen Pharmaceuticals	Philadelphia, Pennsylvania	Cancer	2004
Miragen Therapeutics	Boulder, Colorado	Cardiovascular and muscle diseases	2007
Regulus Therapeutics	Carlsbad, California	Viral diseases, cancer	2007
Rosetta Genomics	Rehovot, Israel, and Jersey City, New Jersey	Cancer	2000

Science, 28 March 2008

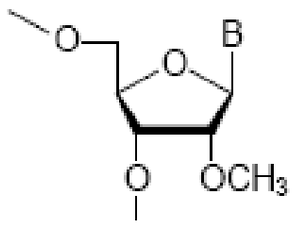
October 2011

SERVIER

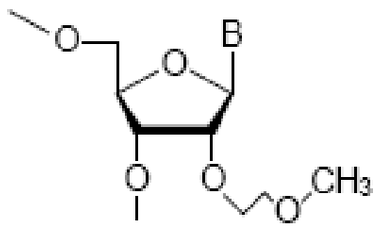
Servier and Miragen Sign $352 Million Partnership Agreement for the Research, Development and Commercialization of MicroRNA-targeting Drugs for Cardiovascular Disease



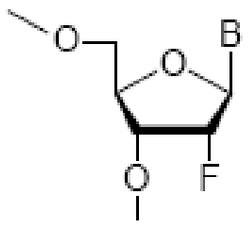
AntimiR chemistries



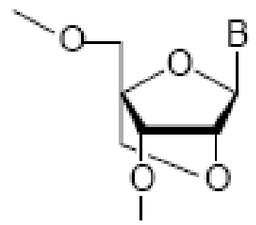
'-O-Methyl
2′-O-Me)



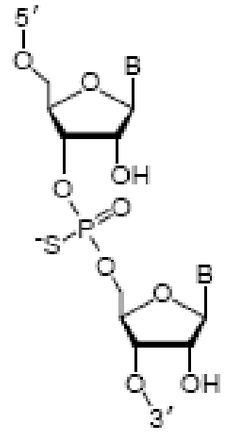
2′-O-Methoxyethyl
(2′-O-Moe)



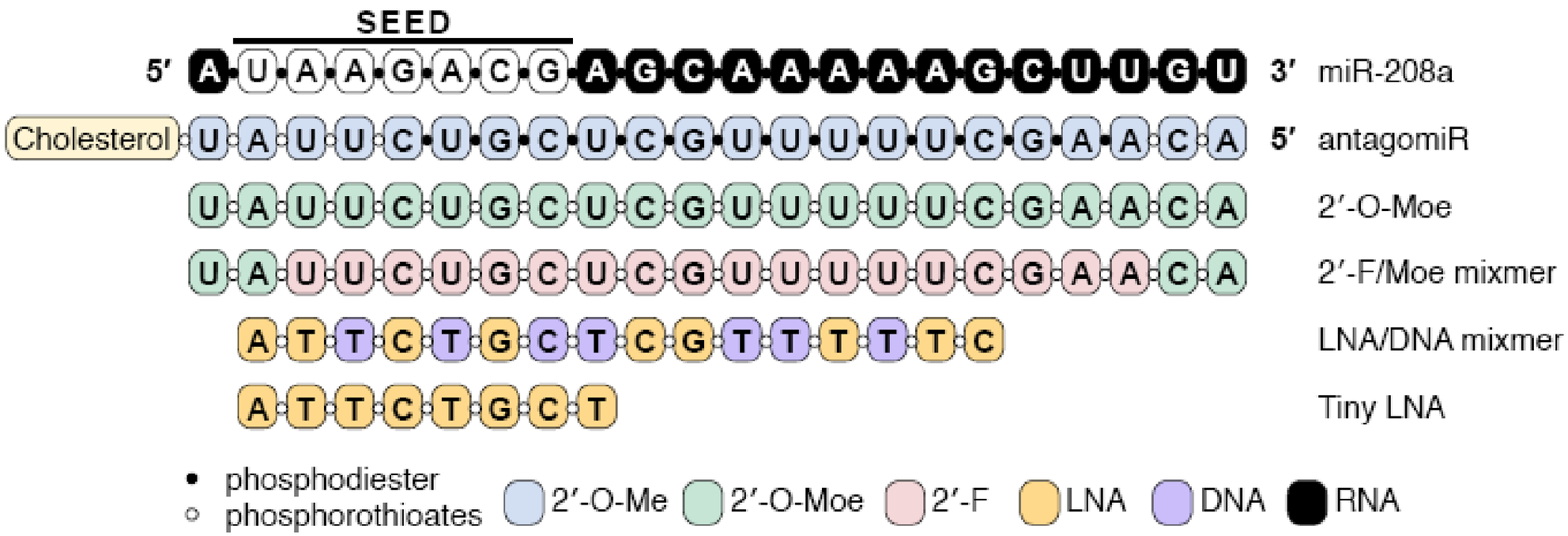
2′-Fluoro
(2′-F)



Locked Nucleic Acid
(LNA)



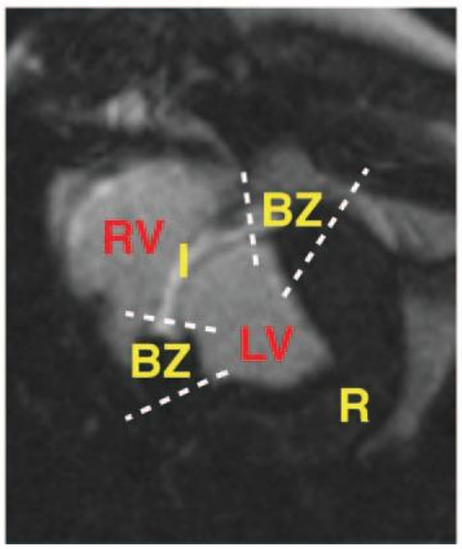
RNA phosphorothioate
(PS)

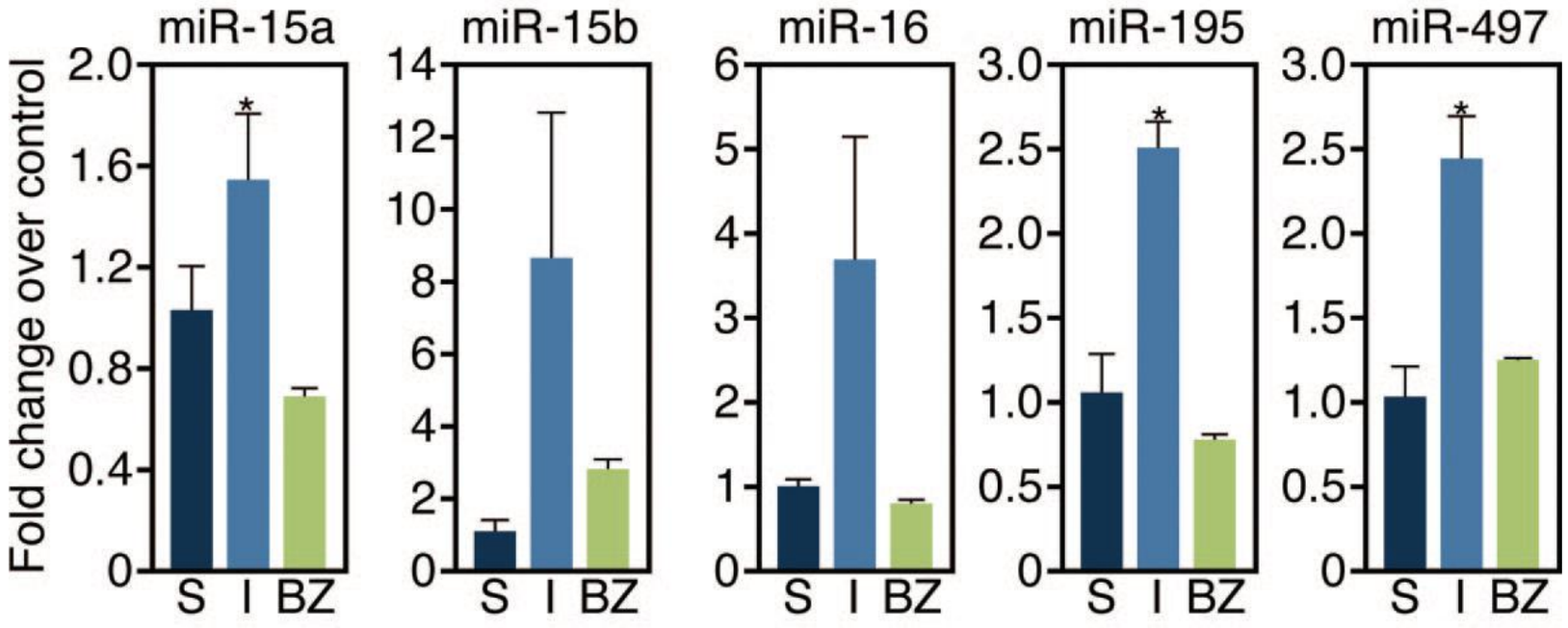


santaris pharma a/s
RNA Medicines for the 21st Century

van Rooij and Olson. Nature Rev Drug Discovery

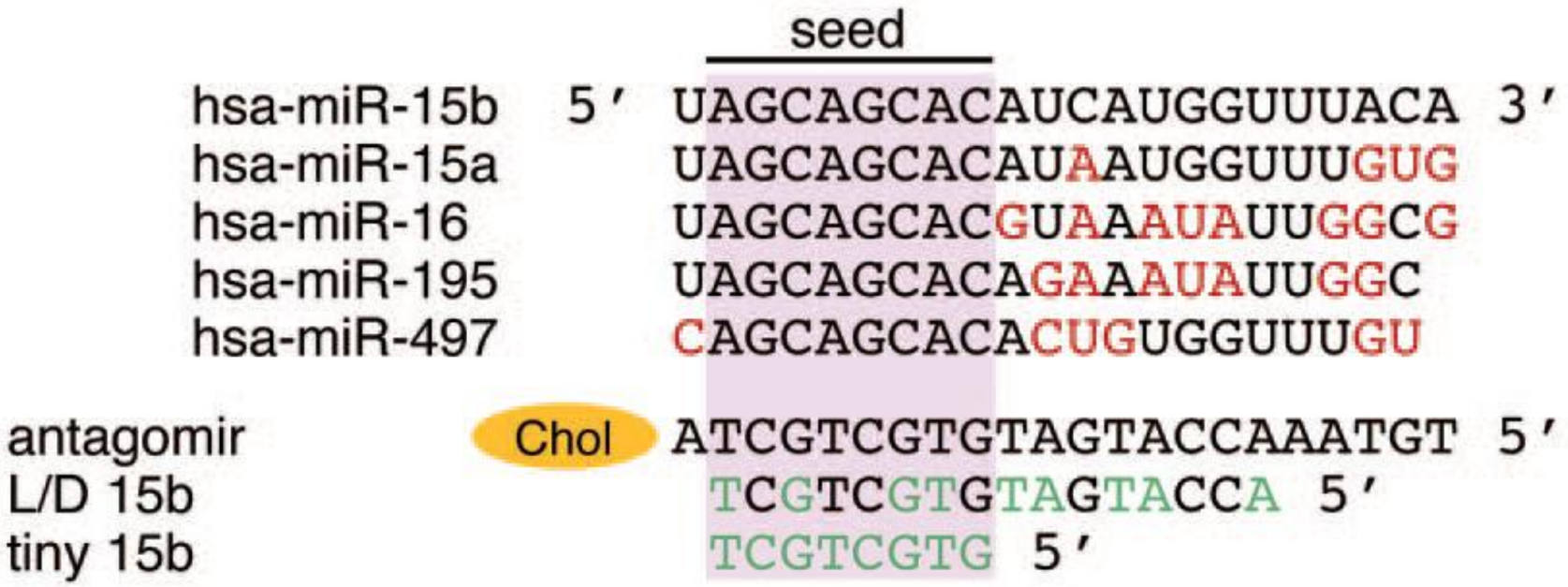
miR-15 family upregulated in response to ischemic injury in pigs





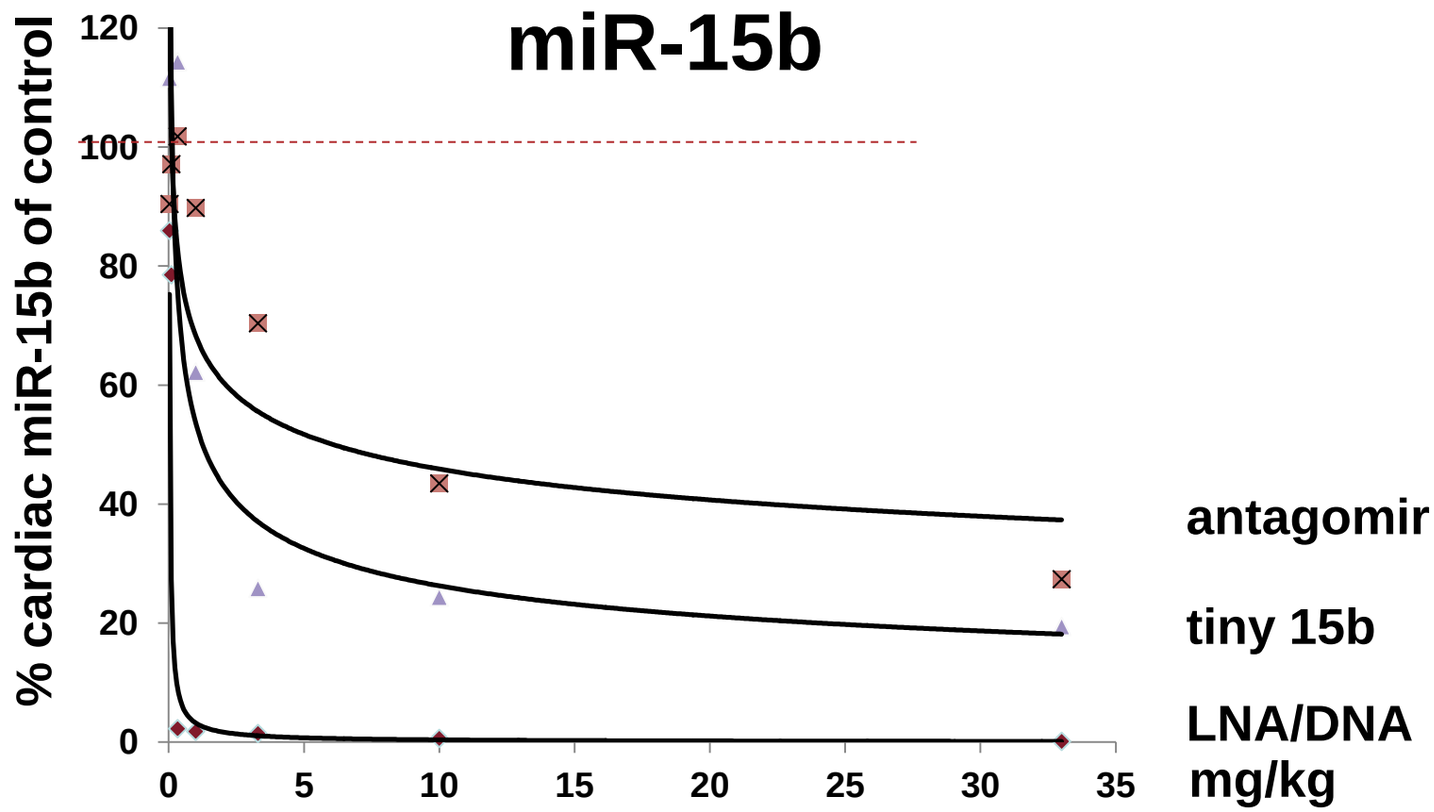
Hullinger et al. Circ Res 2011

miR-15 family inhibition using antimiRs

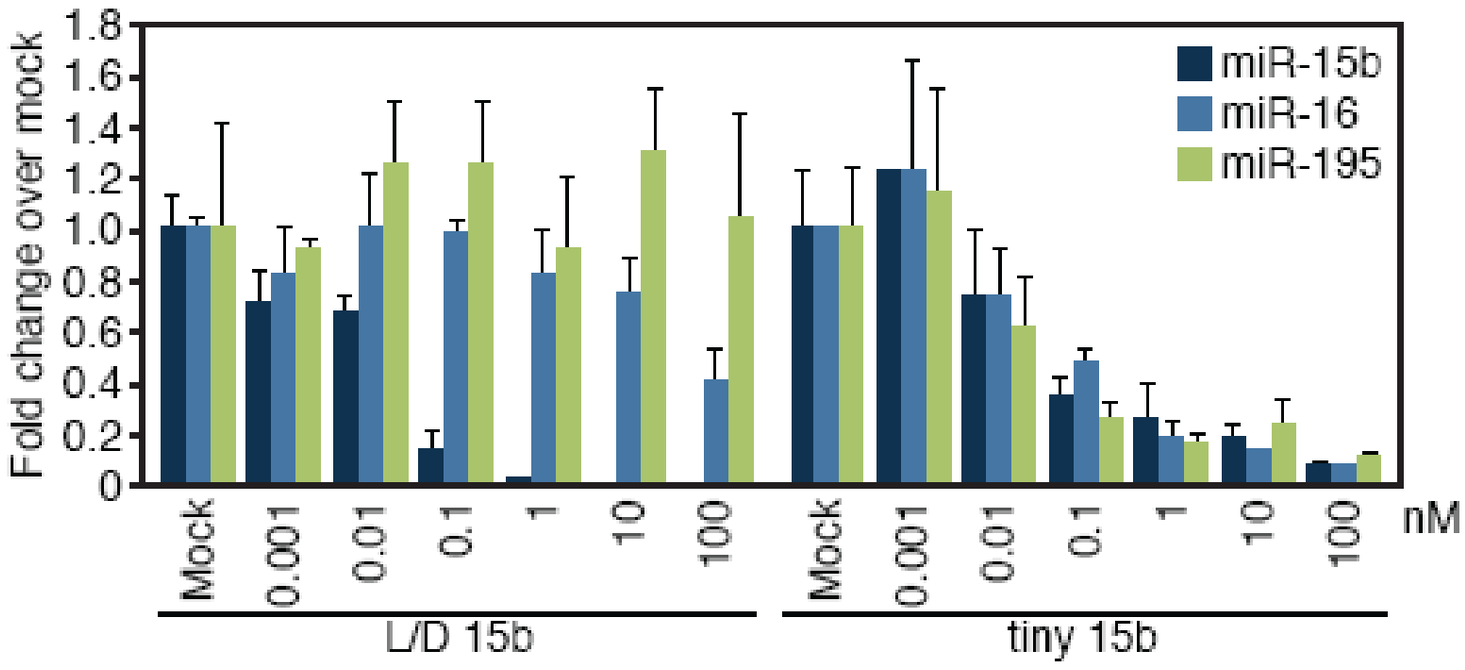


Experimental set-up:

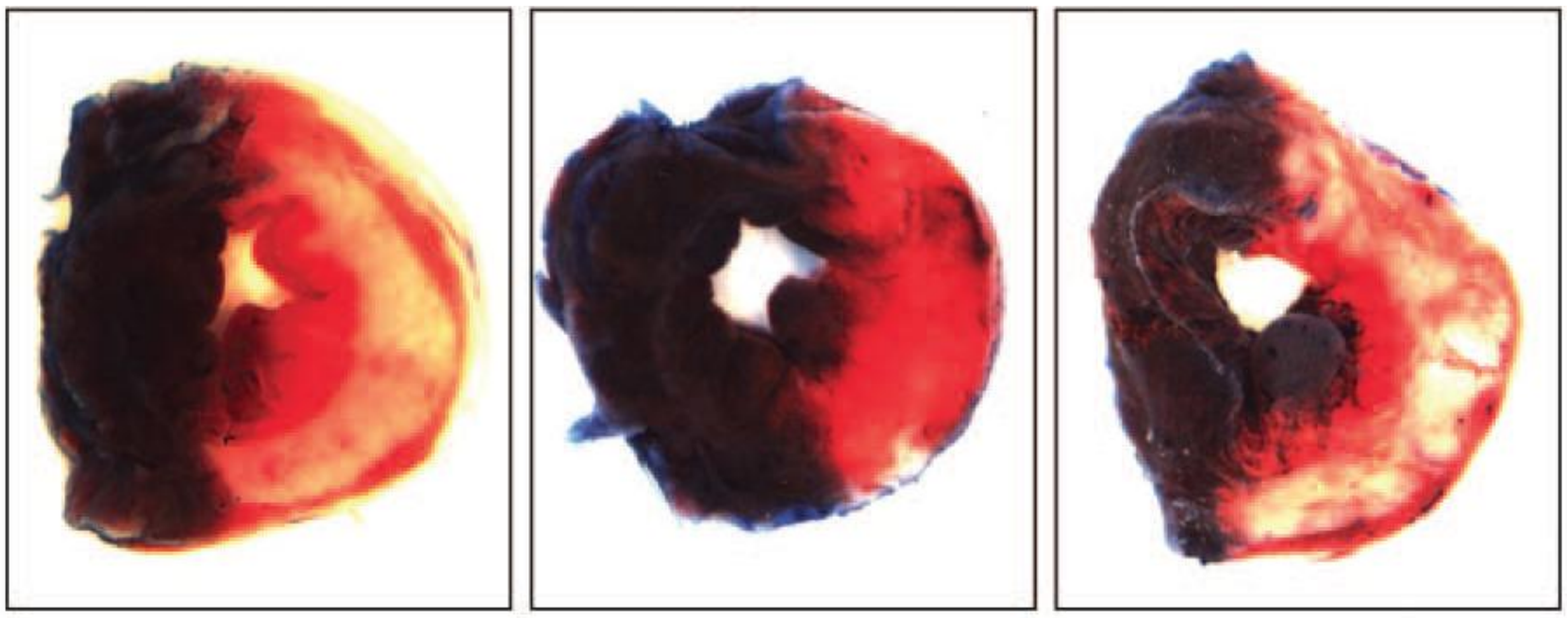
-IV injection C57Bl6
-Doses ranging from 0.033 mg/kg to 33 mg/kg
-Collected tissues 7 days after injection
-Determined knockdown by realtime PCR analysis

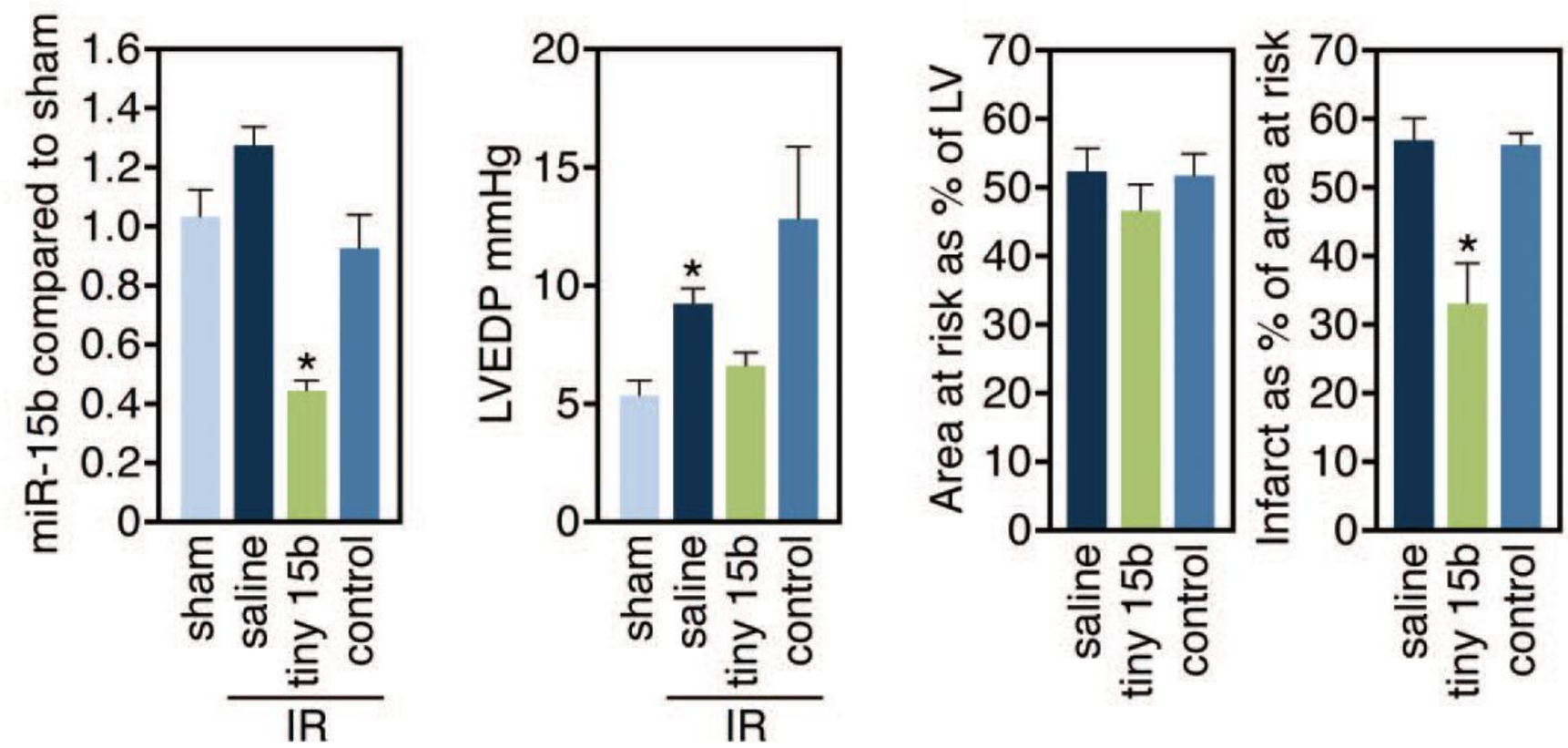


miR-15 family inhibition induces target derepression in cardiomyocytes



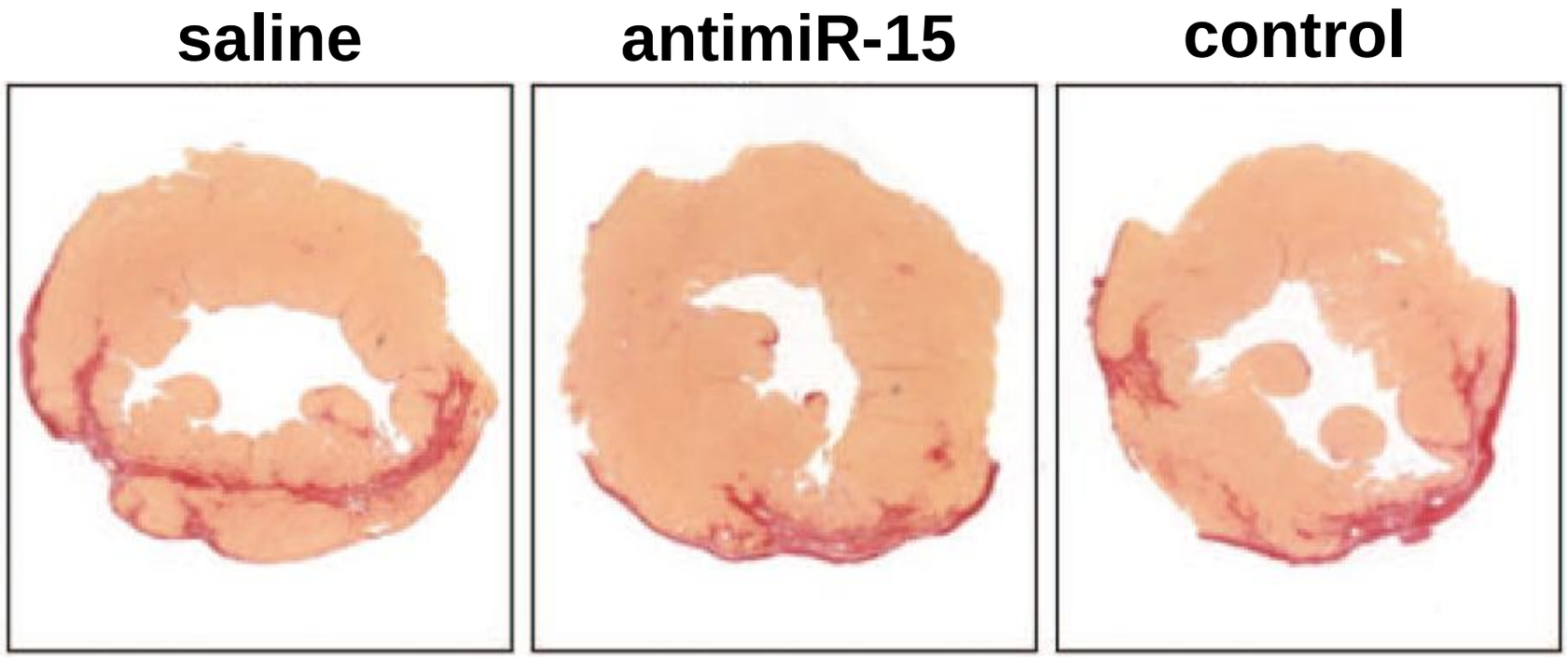
AntimiR-15 reduces infarct size in response to ischemic injury

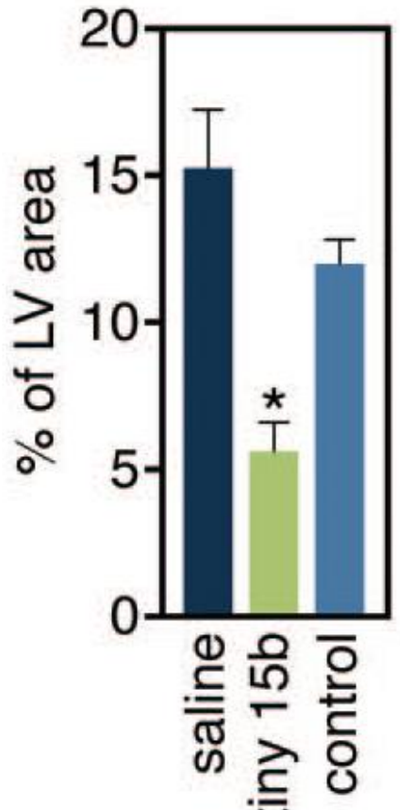


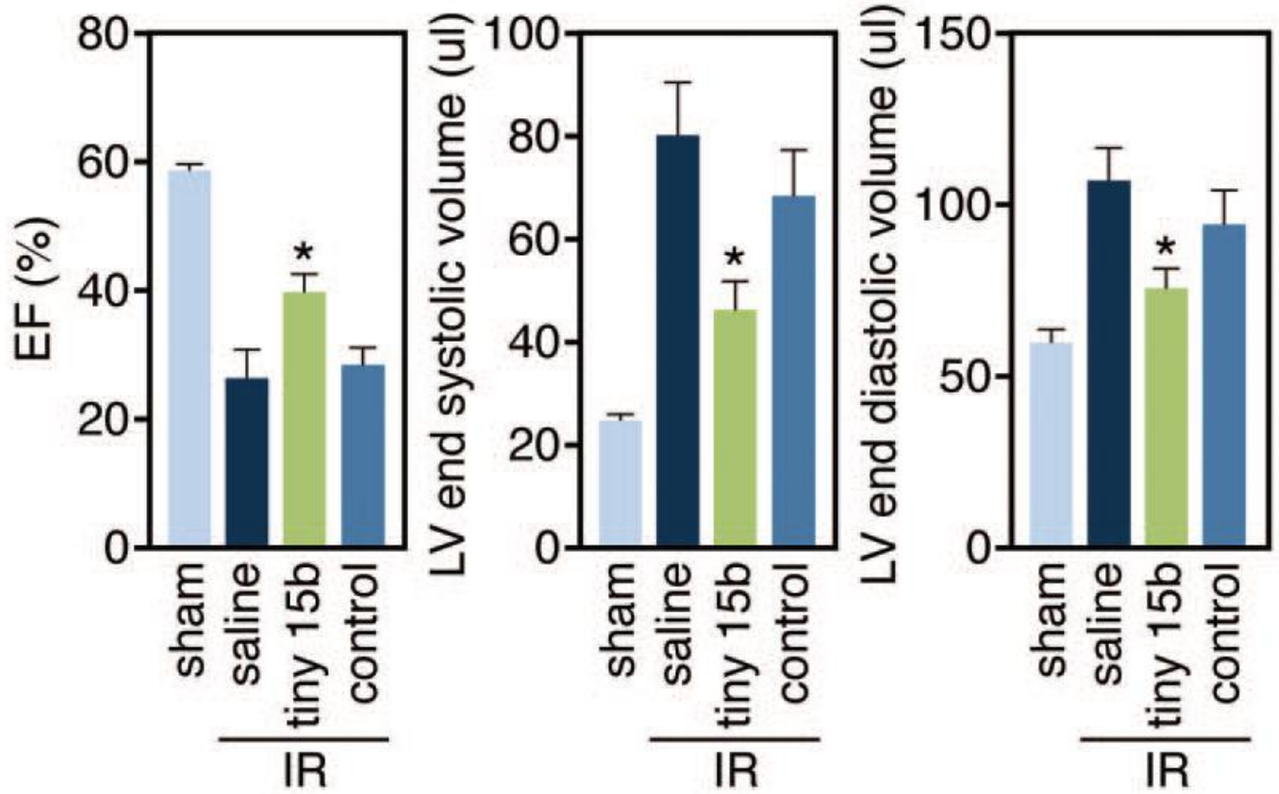


Hullinger et al. Circ Res 2011

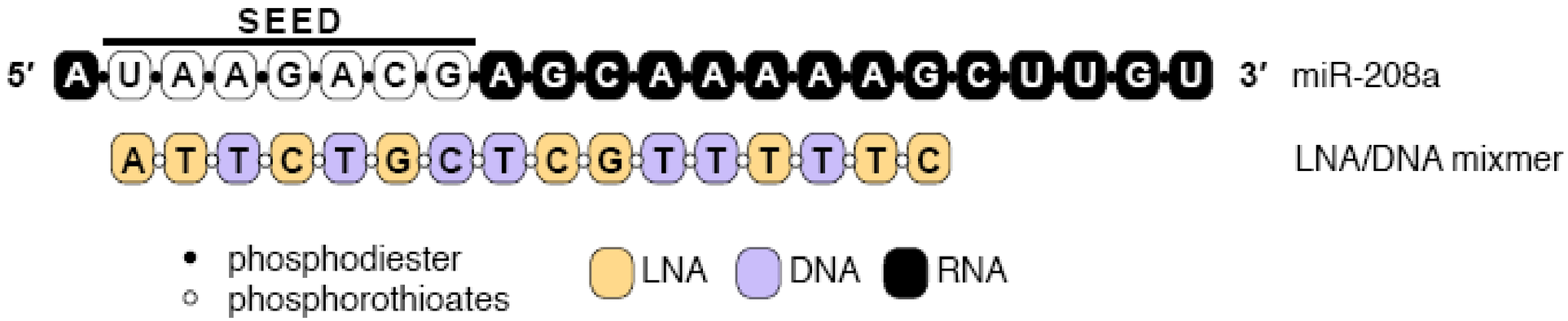
miR-15 inhibition reduces remodeling and improves cardiac function in response to ischemic damage



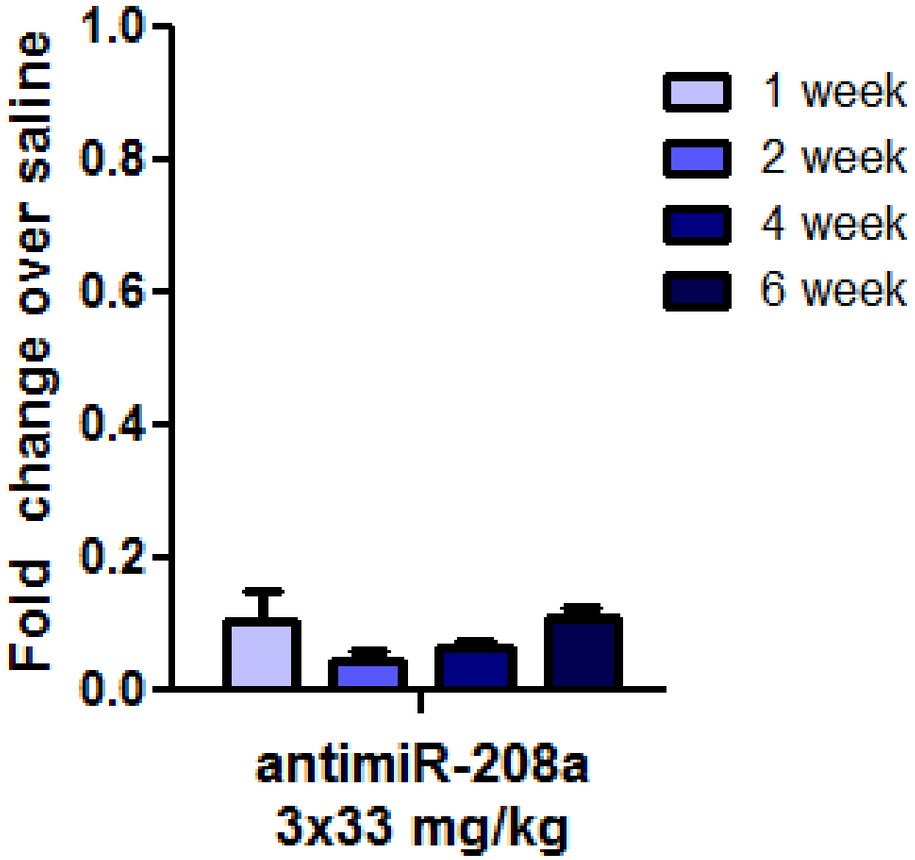


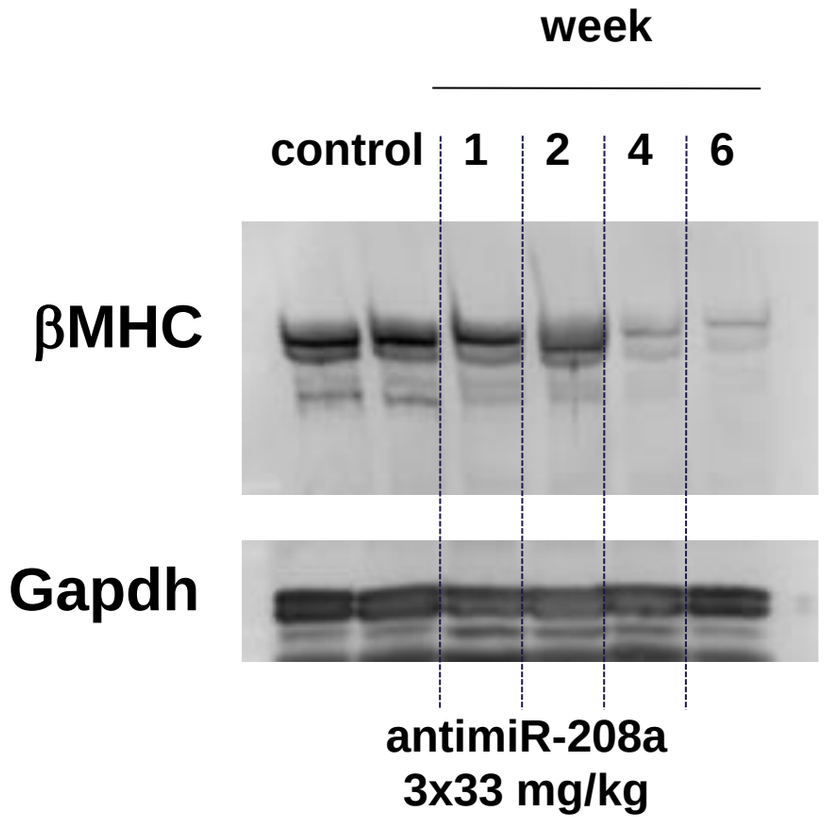


AntimiR-208a



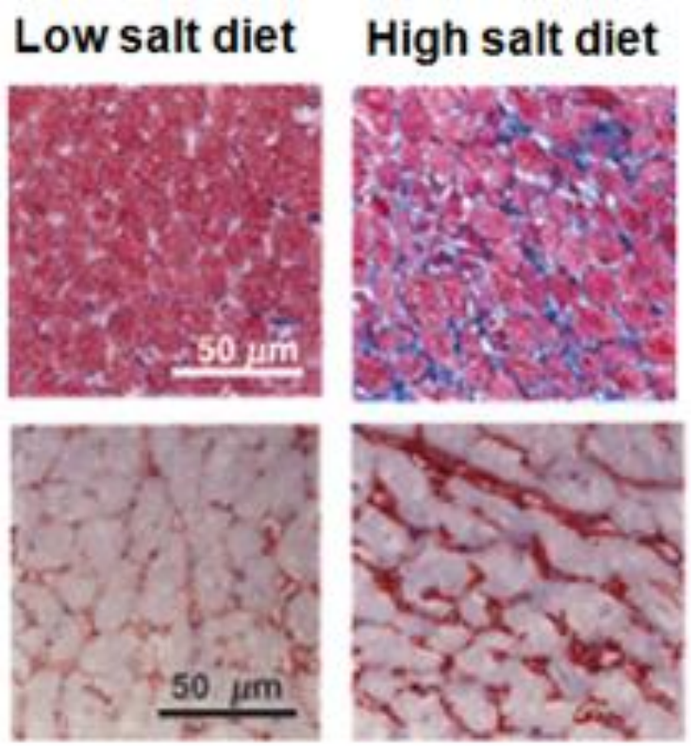
miR-208





Montgomery et al. Circ 2012

Dahl Salt-Sensitive rat model

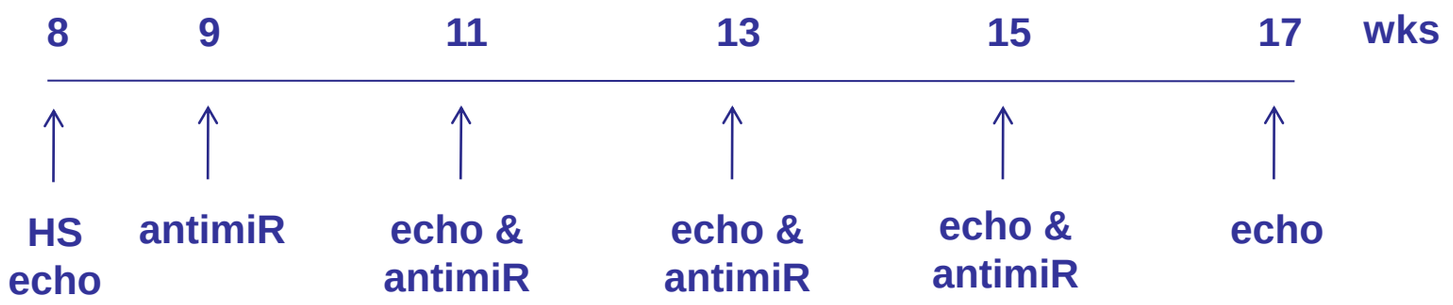


Dahl Salt-Sensitive Model is a high-quality rodent model for diastolic heart failure

High salt diet causes continuous increase in blood pressure which leads to remodeling of the heart

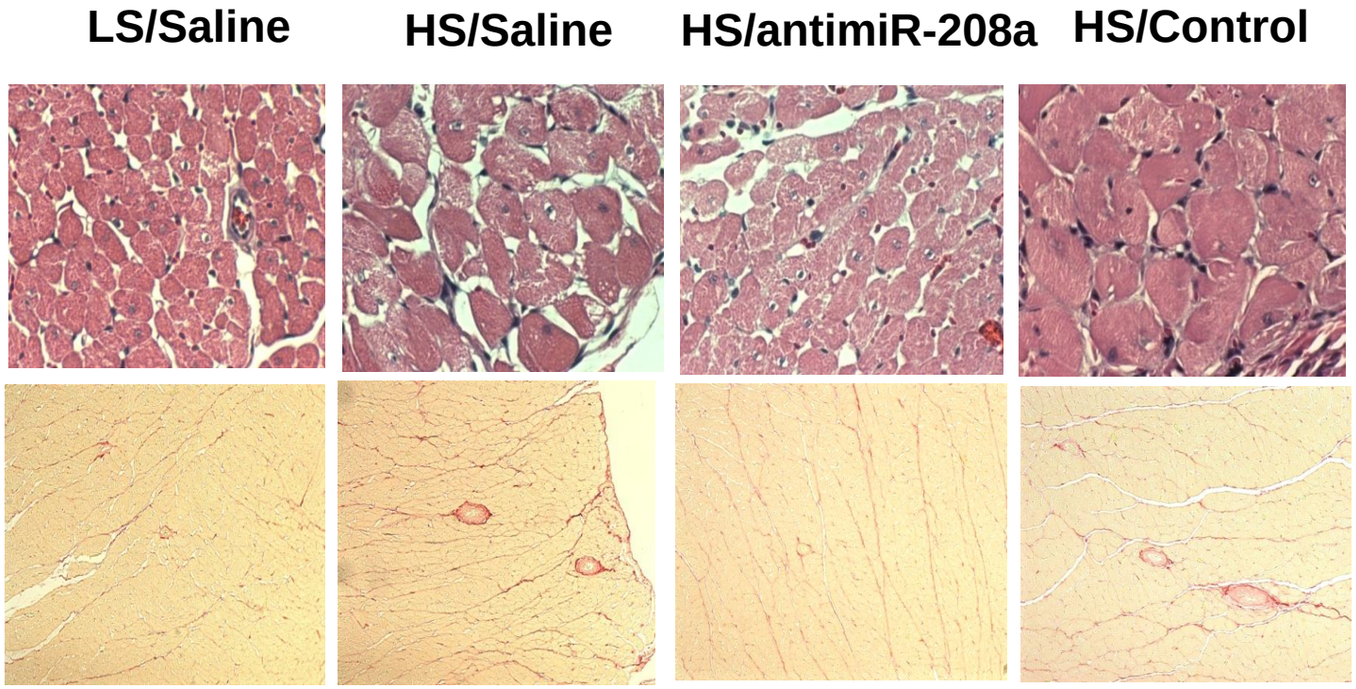
- hypertrophy
- fibrosis
- increase hypertrophic markers
- switch from αMHC to βMHC

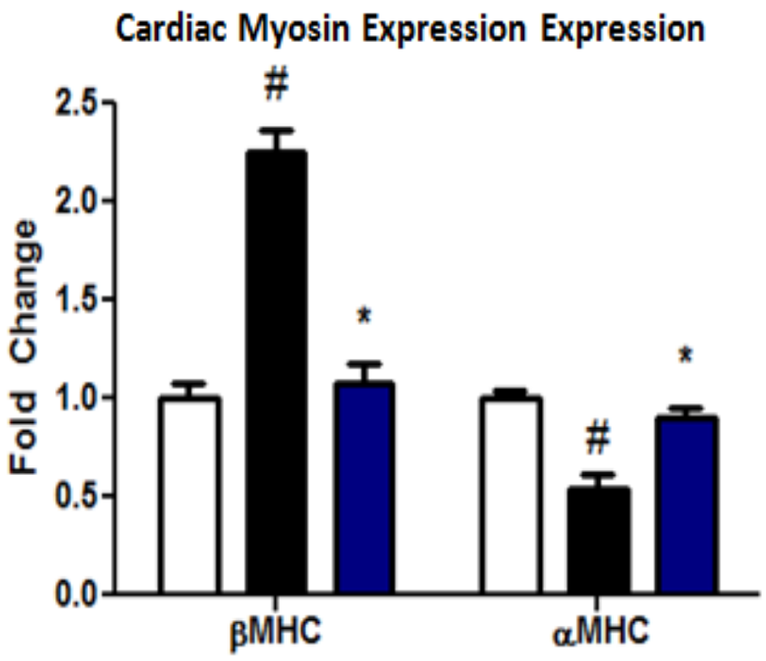
Stiffening of the ventricle diminishes elasticity, relaxation of the LV



Therapeutic Inhibition of miR-208a Improves Cardiac Function and Survival During Heart Failure

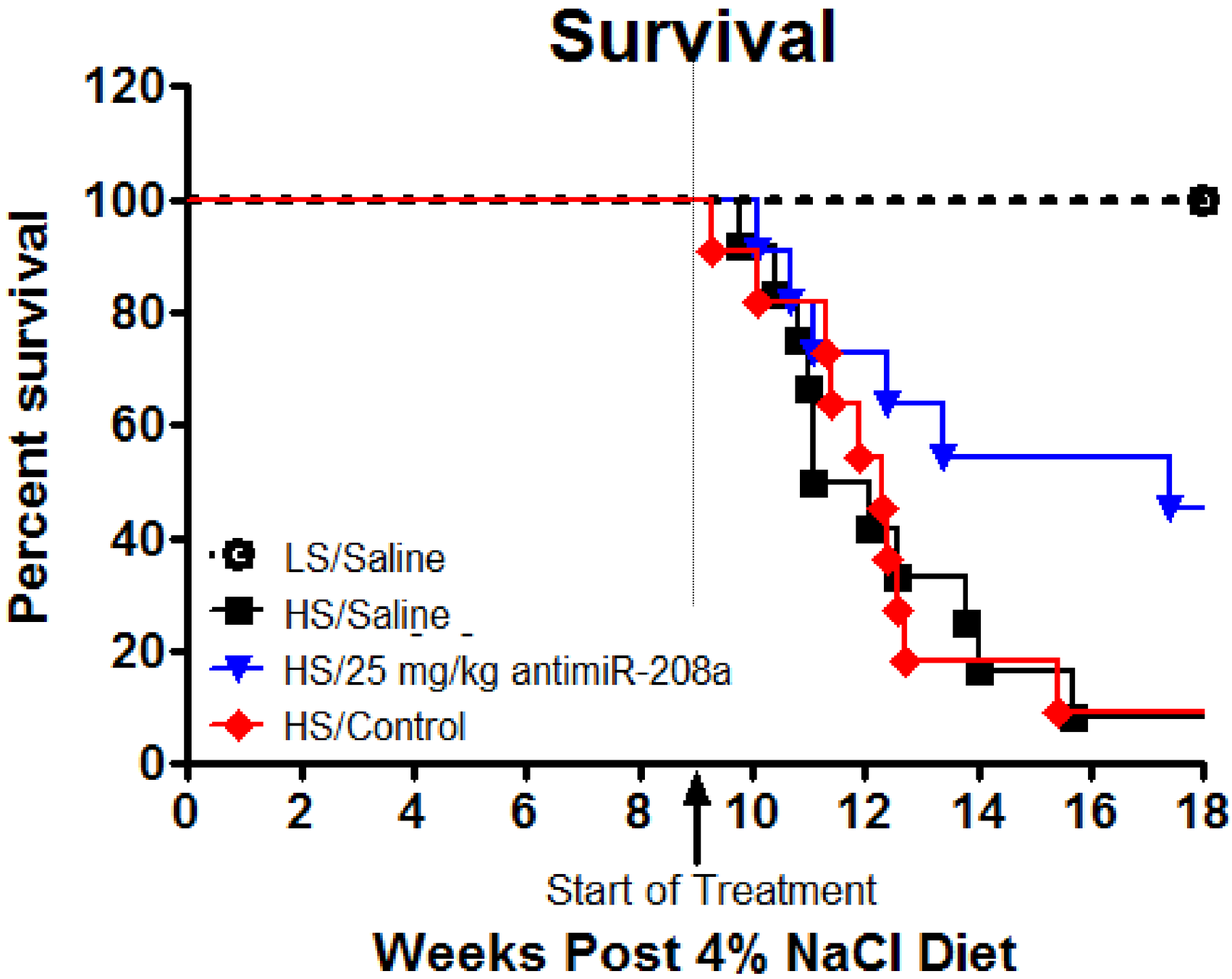
Rusty L. Montgomery, PhD; Thomas G. Hullinger, PhD; Hillary M. Semus, MS; Brent A. Dickinson, BS; Anita G. Seto, PhD; Joshua M. Lynch, BS; Christianna Stack, MS; Paul A. Latimer, BS; Eric N. Olson, PhD; Eva van Rooij, PhD



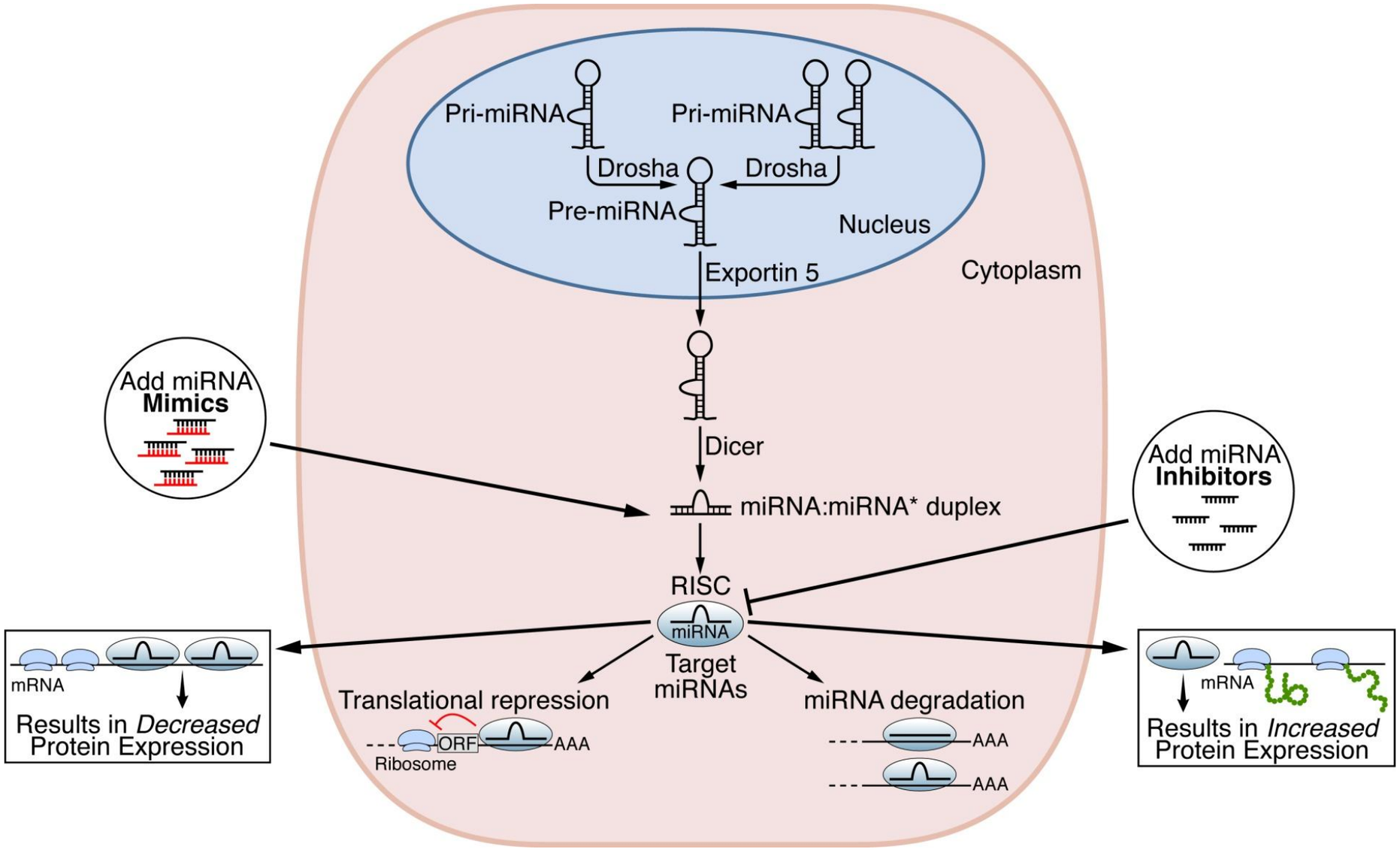


Montgomery et al. Circ 2012

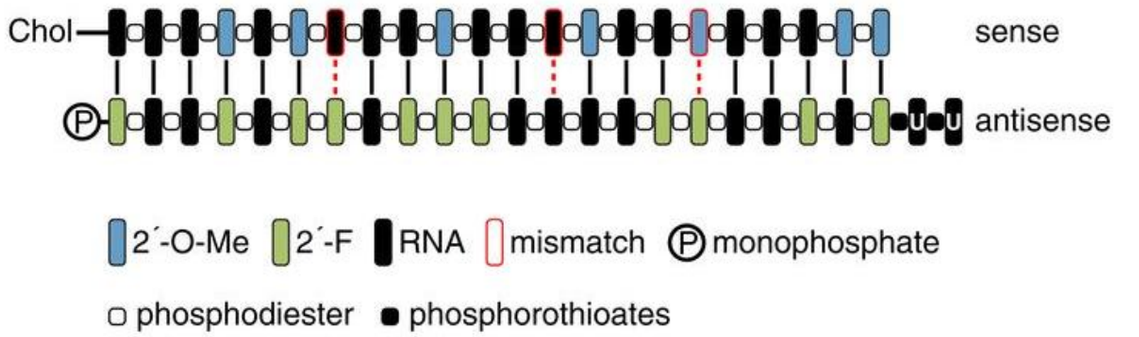
AntimiR-208 improves survival during heart failure

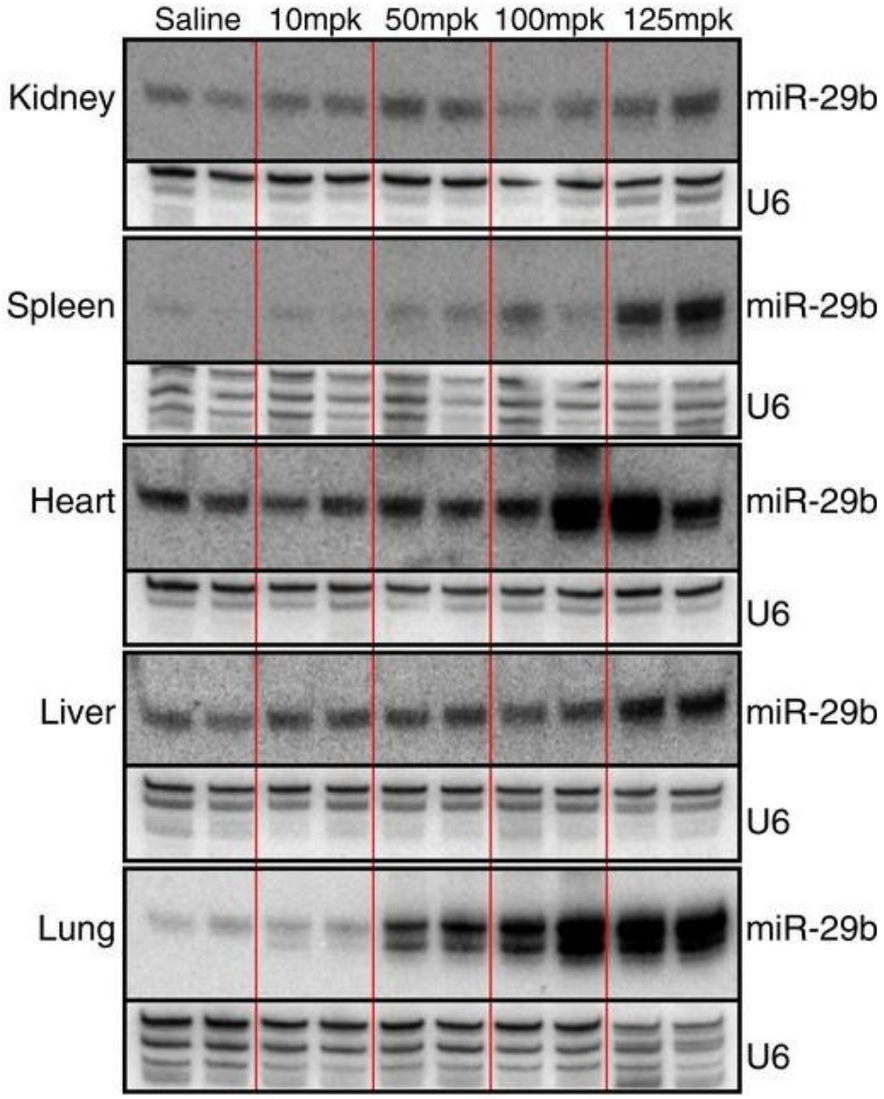


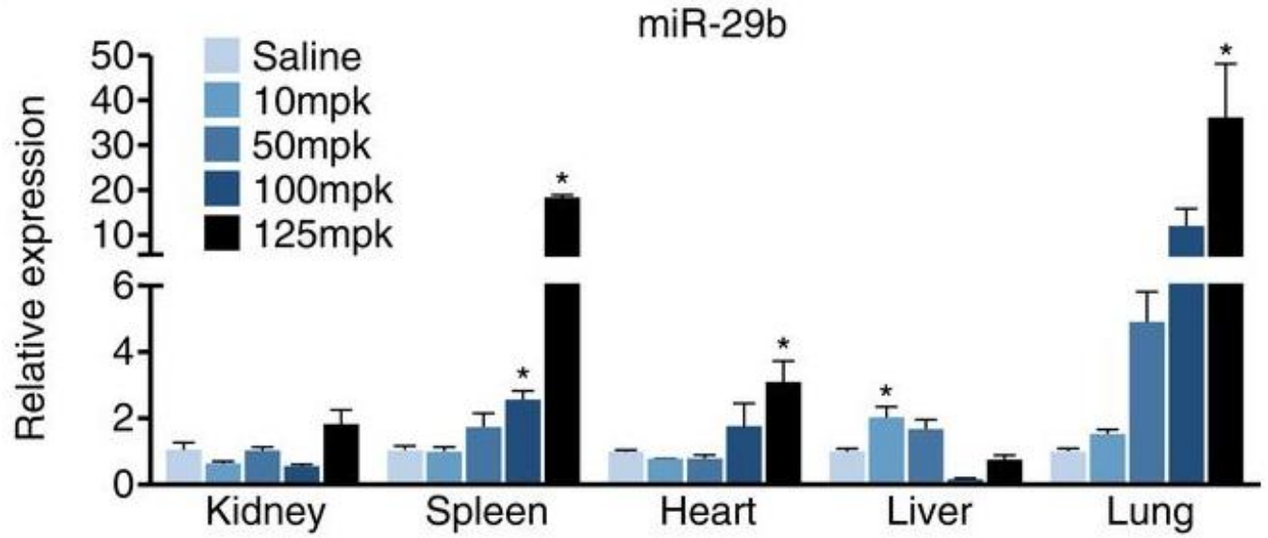
MicroRNA modulation



MicroRNA mimicry in multiple tissues

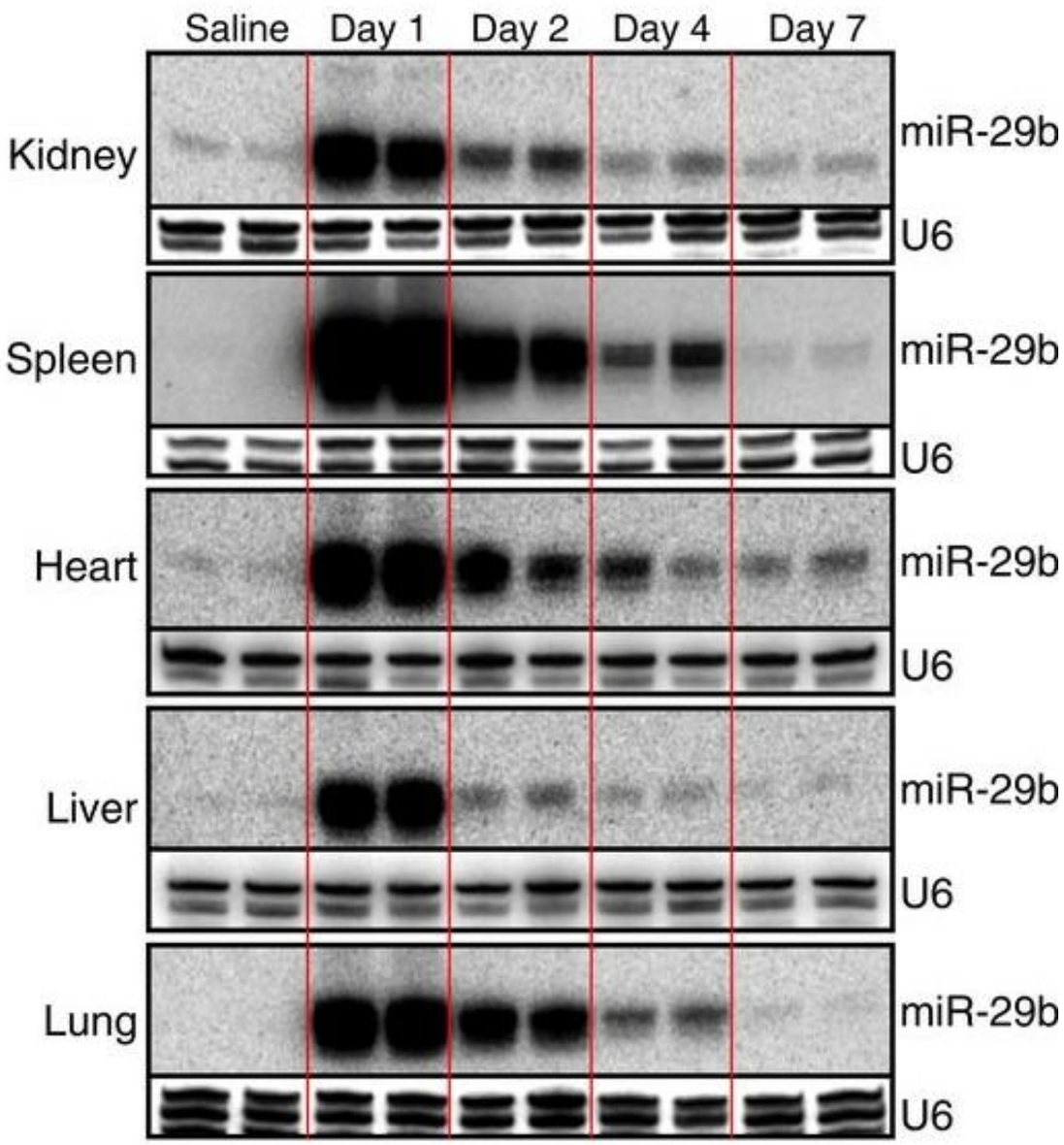


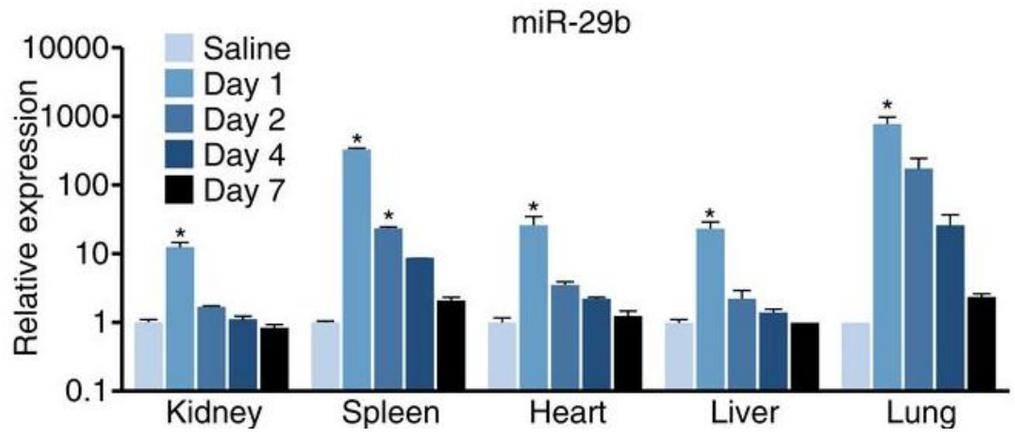




Montgomery et al. EMBO Mol Med 2014

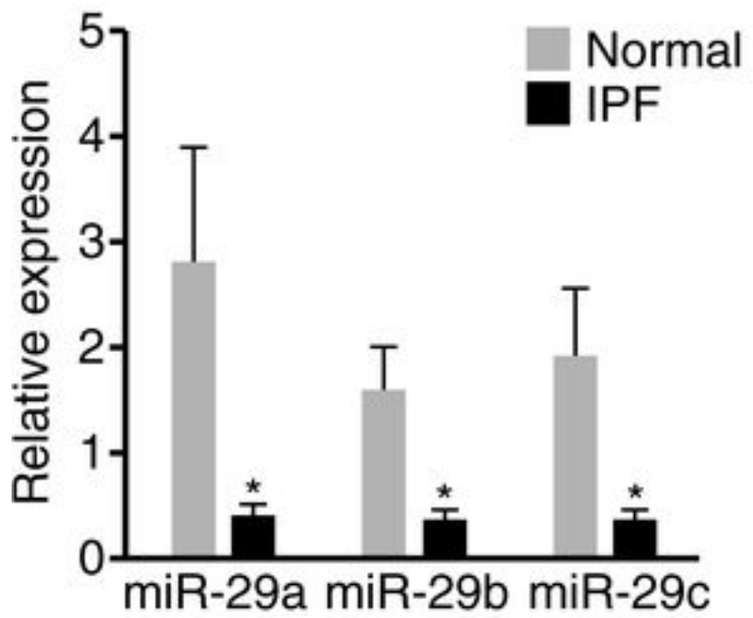
The stability of a microRNA mimic is tissue dependent

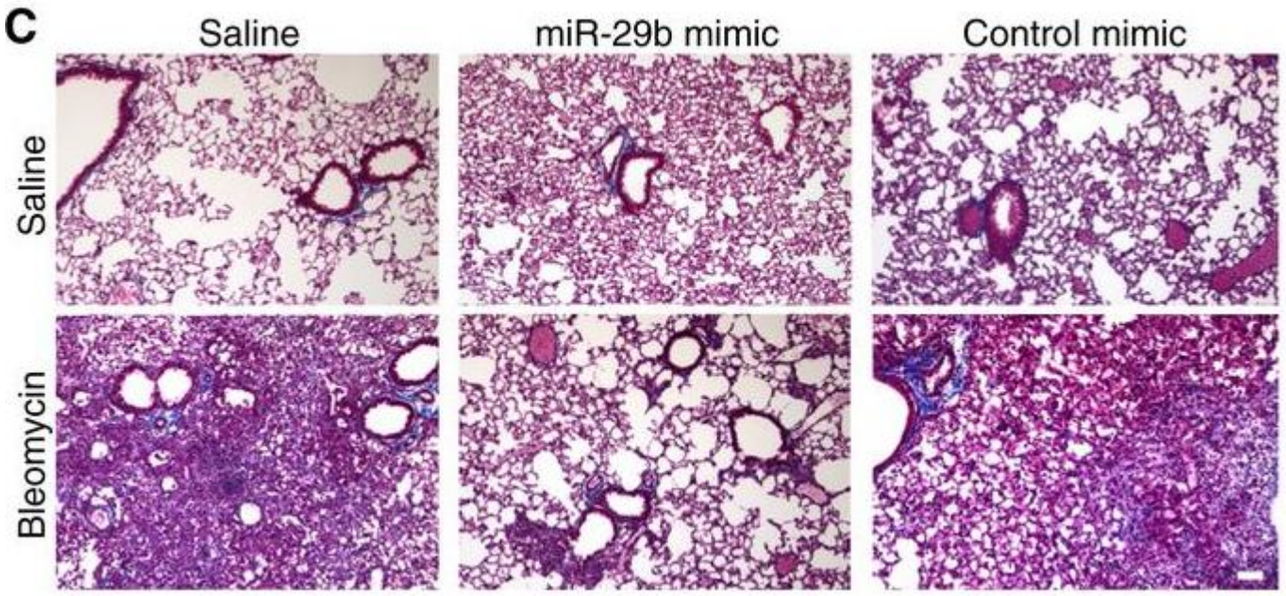


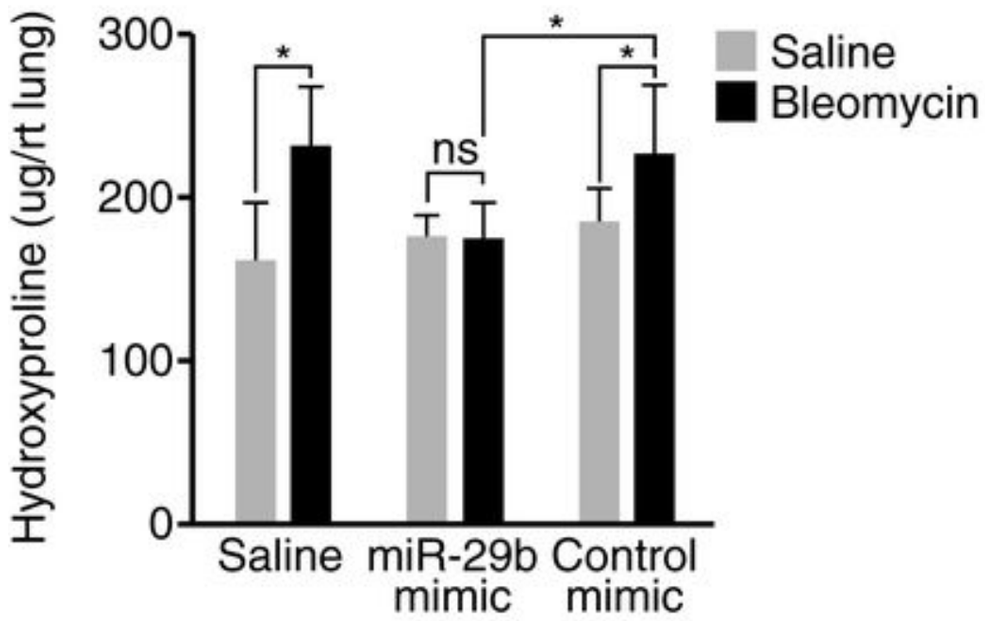


100 mg/kg iv

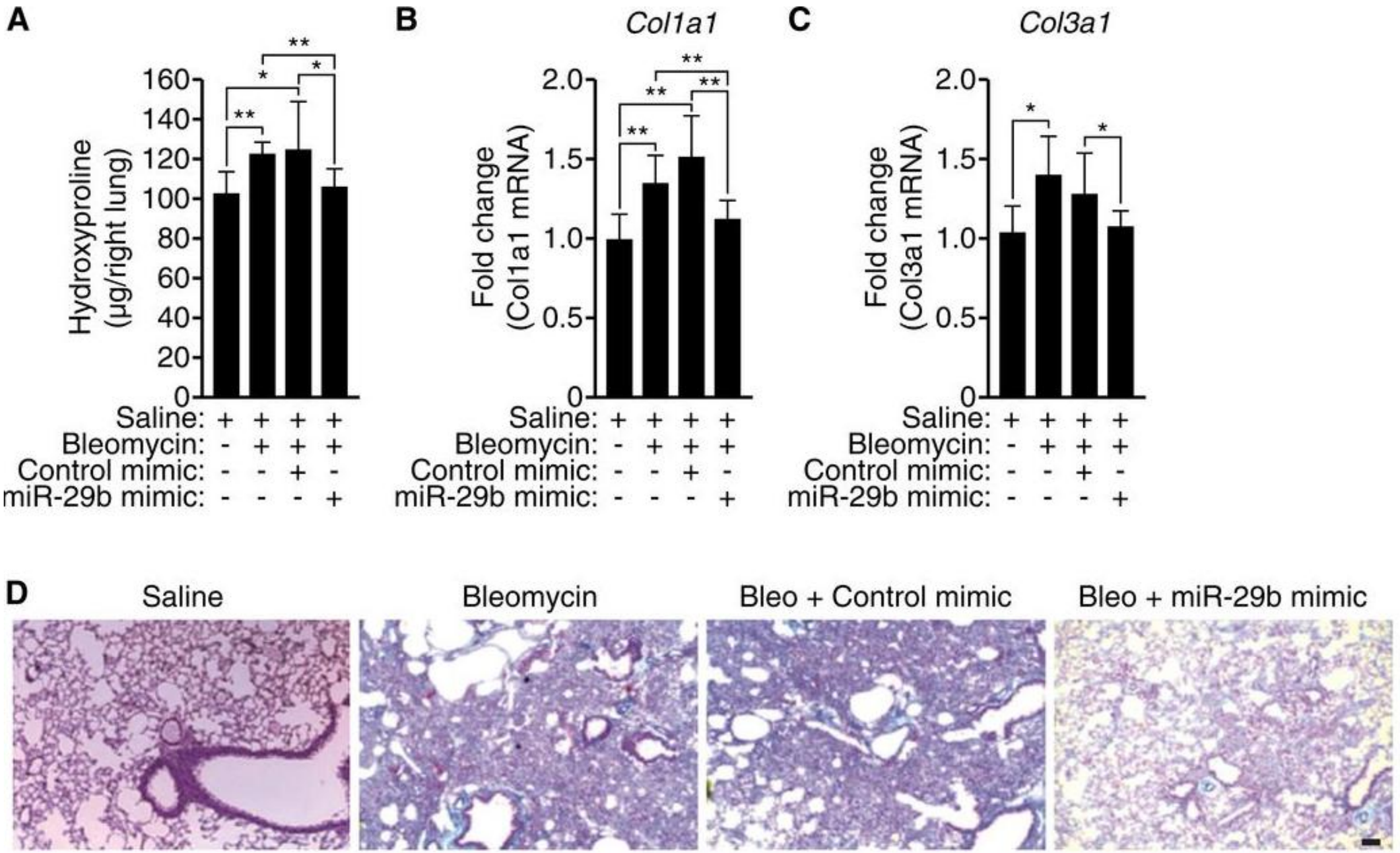
miR-29 mimicry blocks pulmonary fibrosis



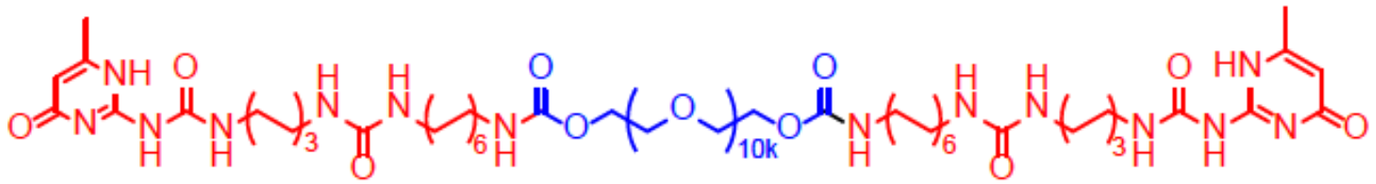


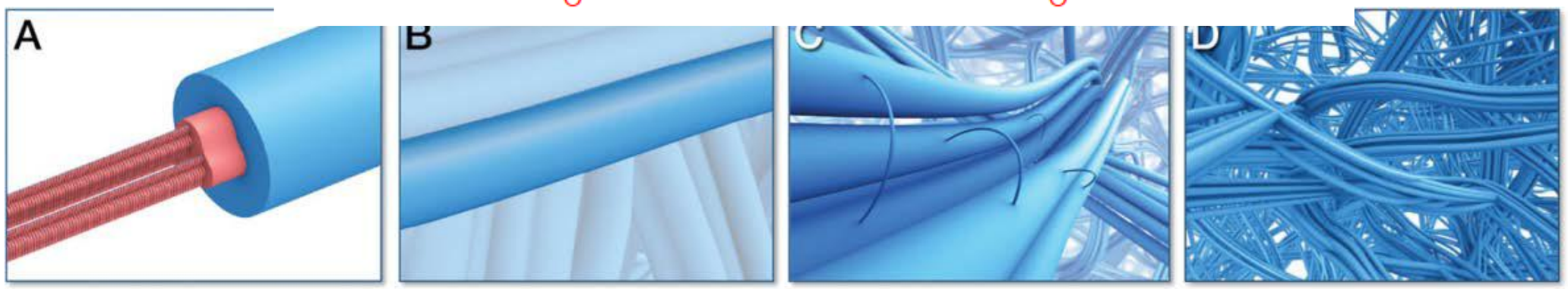


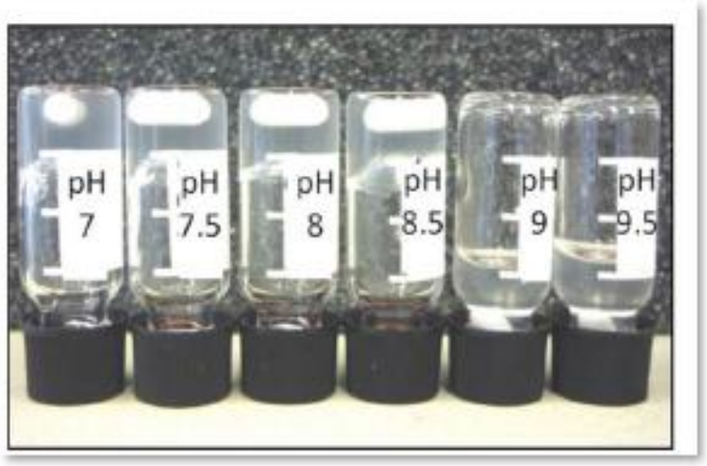
miR-29 mimicry reverses signs of pulmonary fibrosis

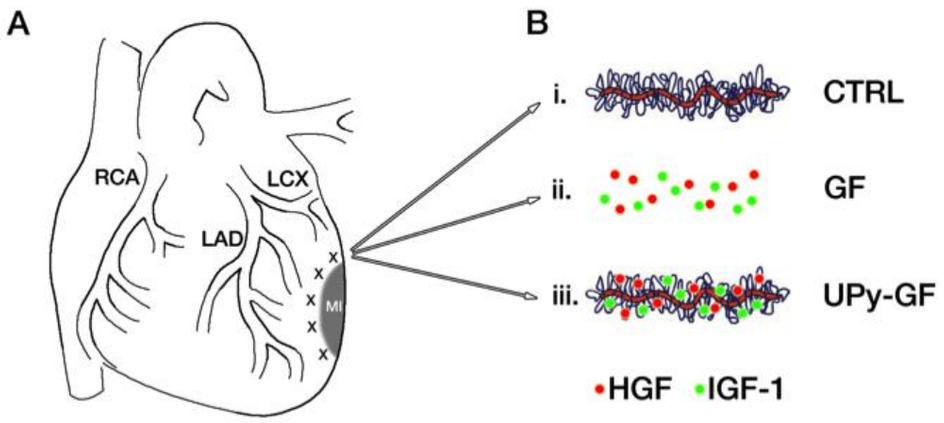


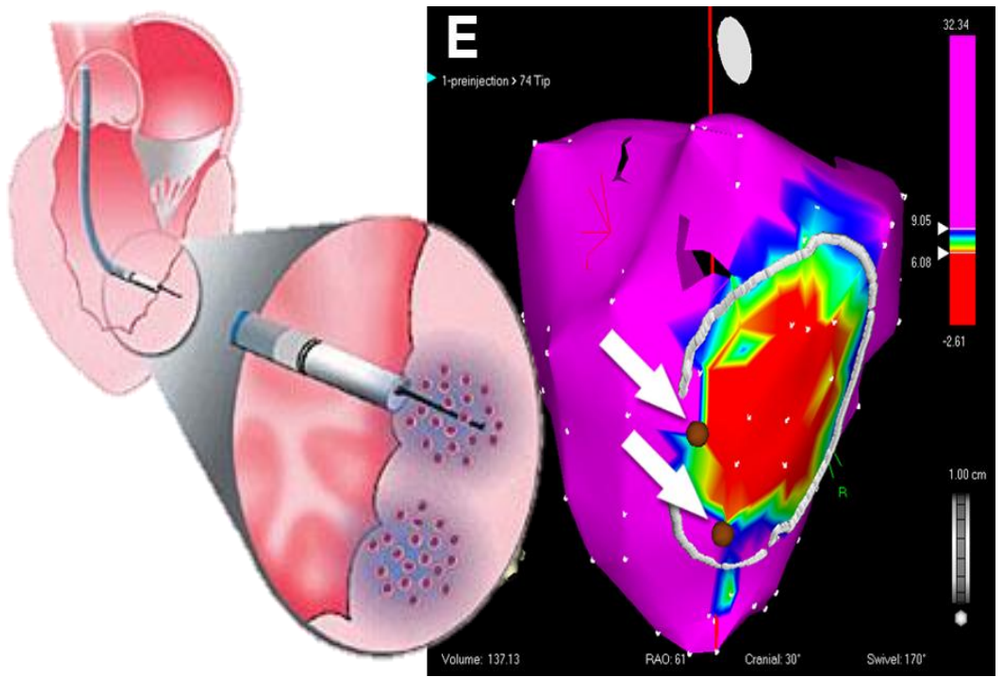
pH sensitive Upy hydrogel for cardiac delivery of novel therapeutics





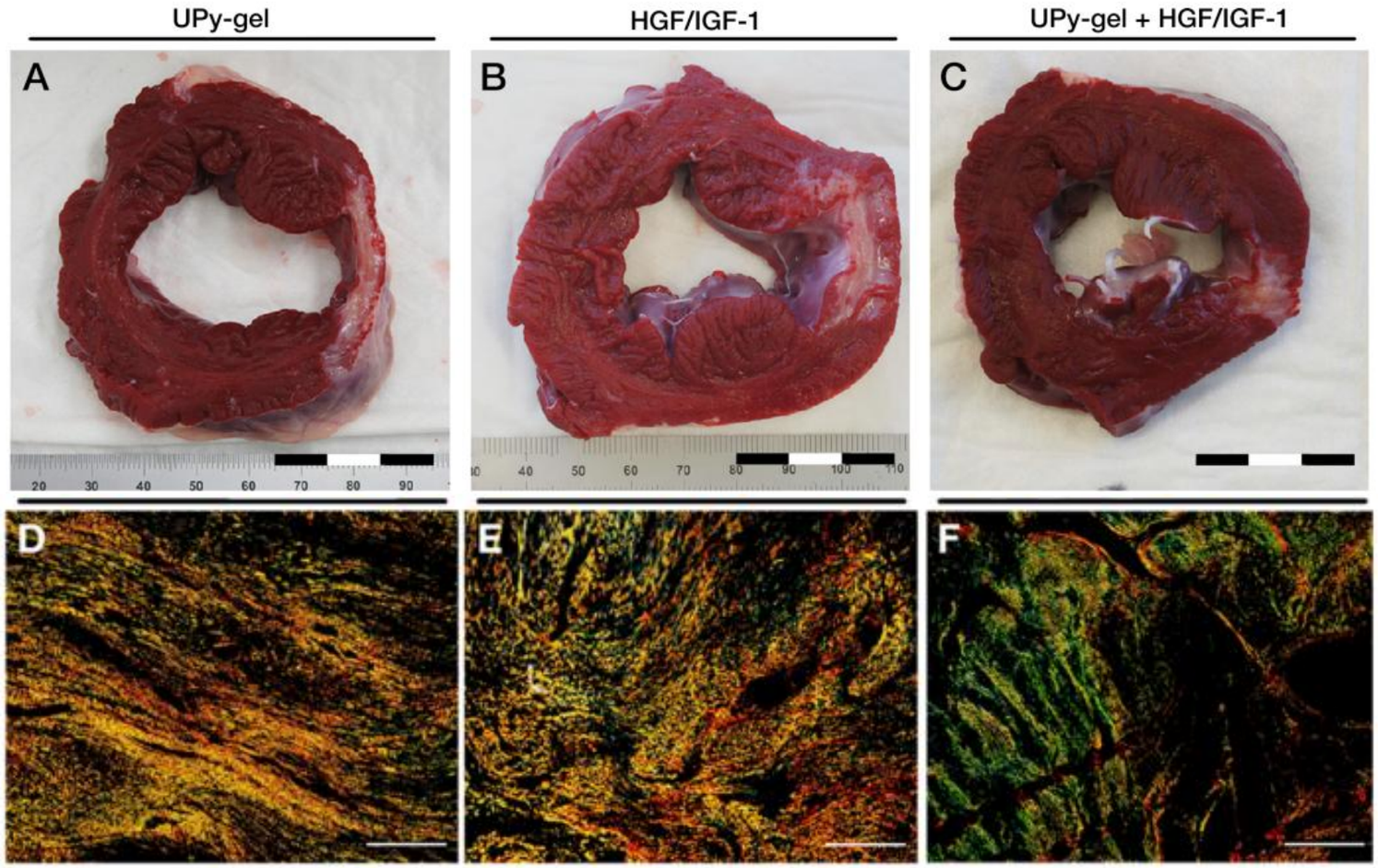


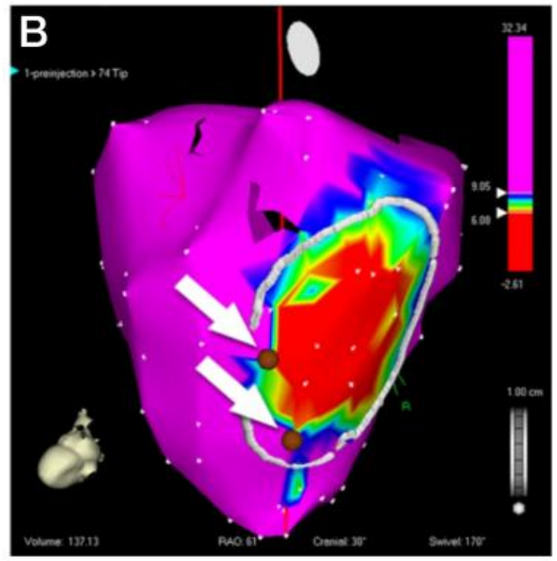


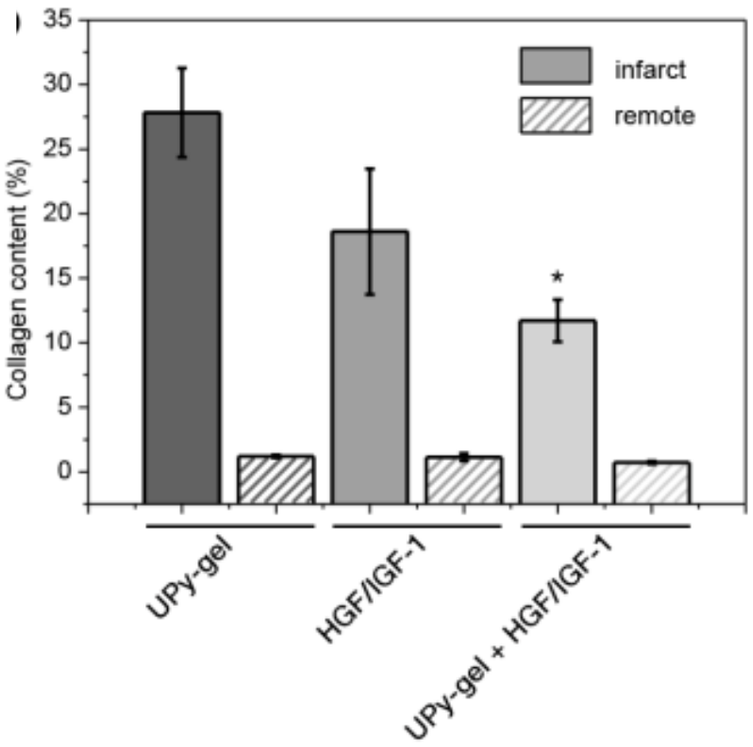


Dankers, TU/e

In vivo delivery of Upy hydrogel in porcine myocardial infarction model





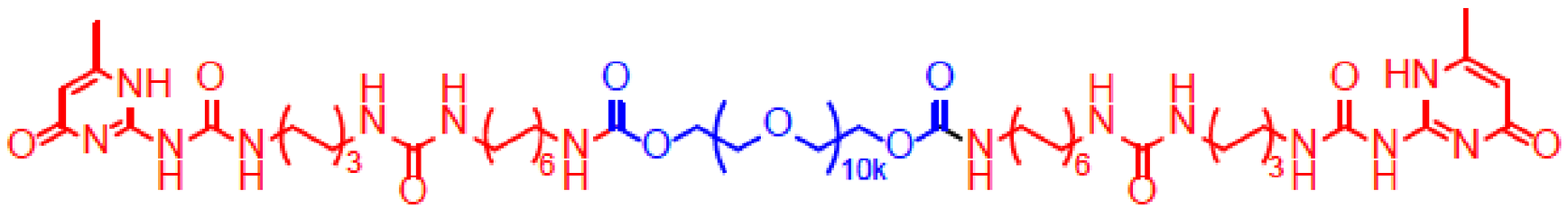


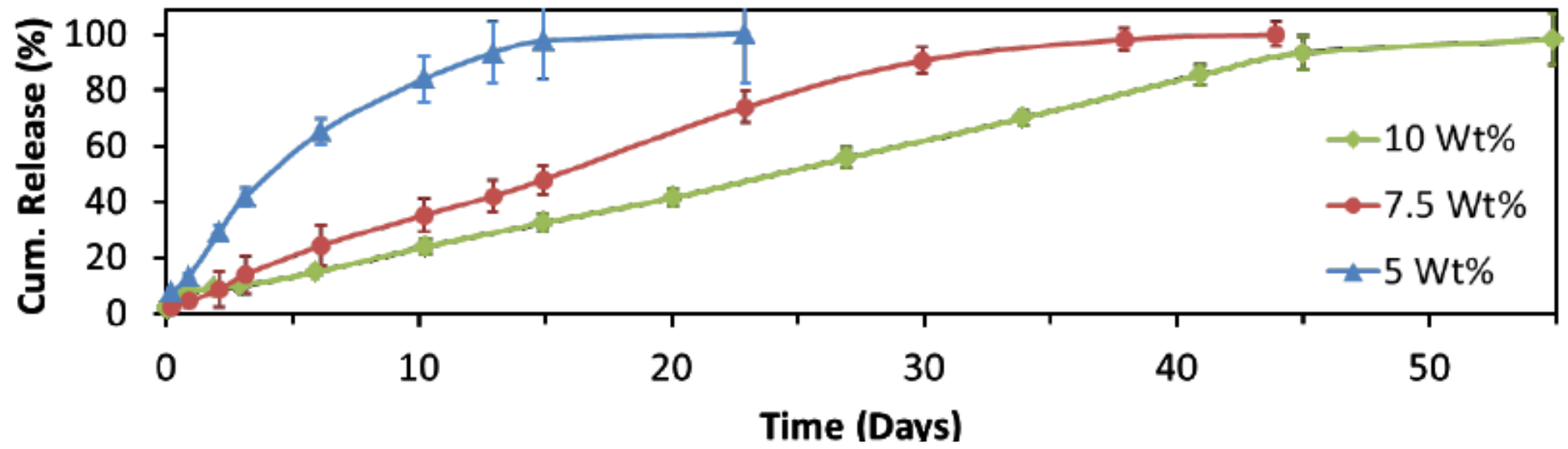
Towards **other drugs**
and possibly other formulations

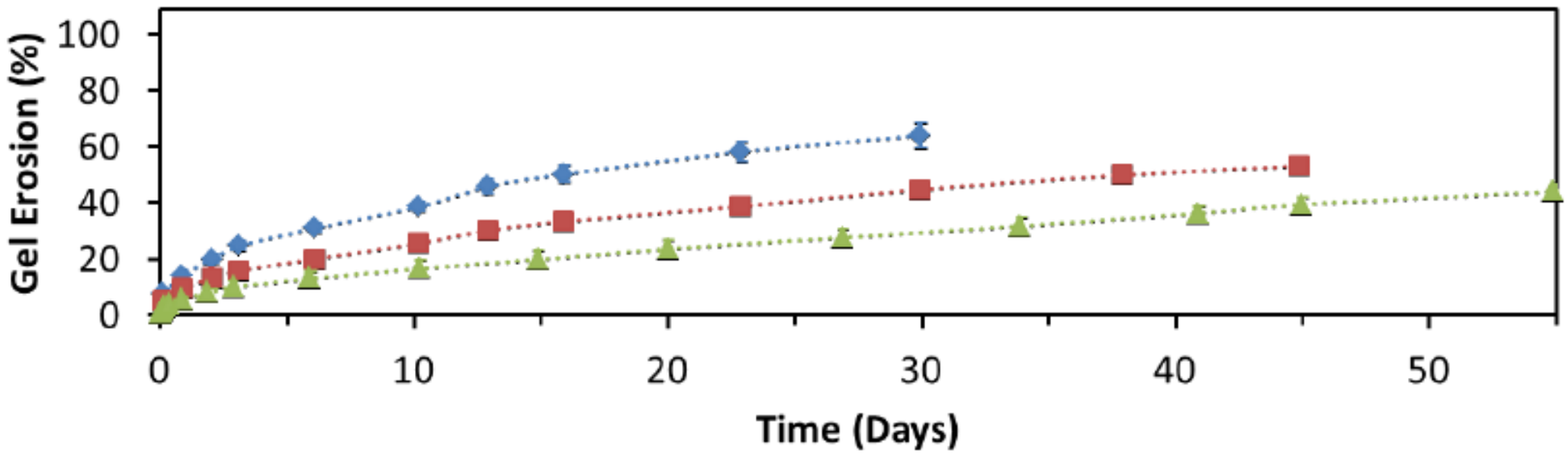
Dankers, TU/e

TU/e and UMC Utrecht

pH sensitive Upy hydrogel for cardiac delivery of microRNA therapeutics



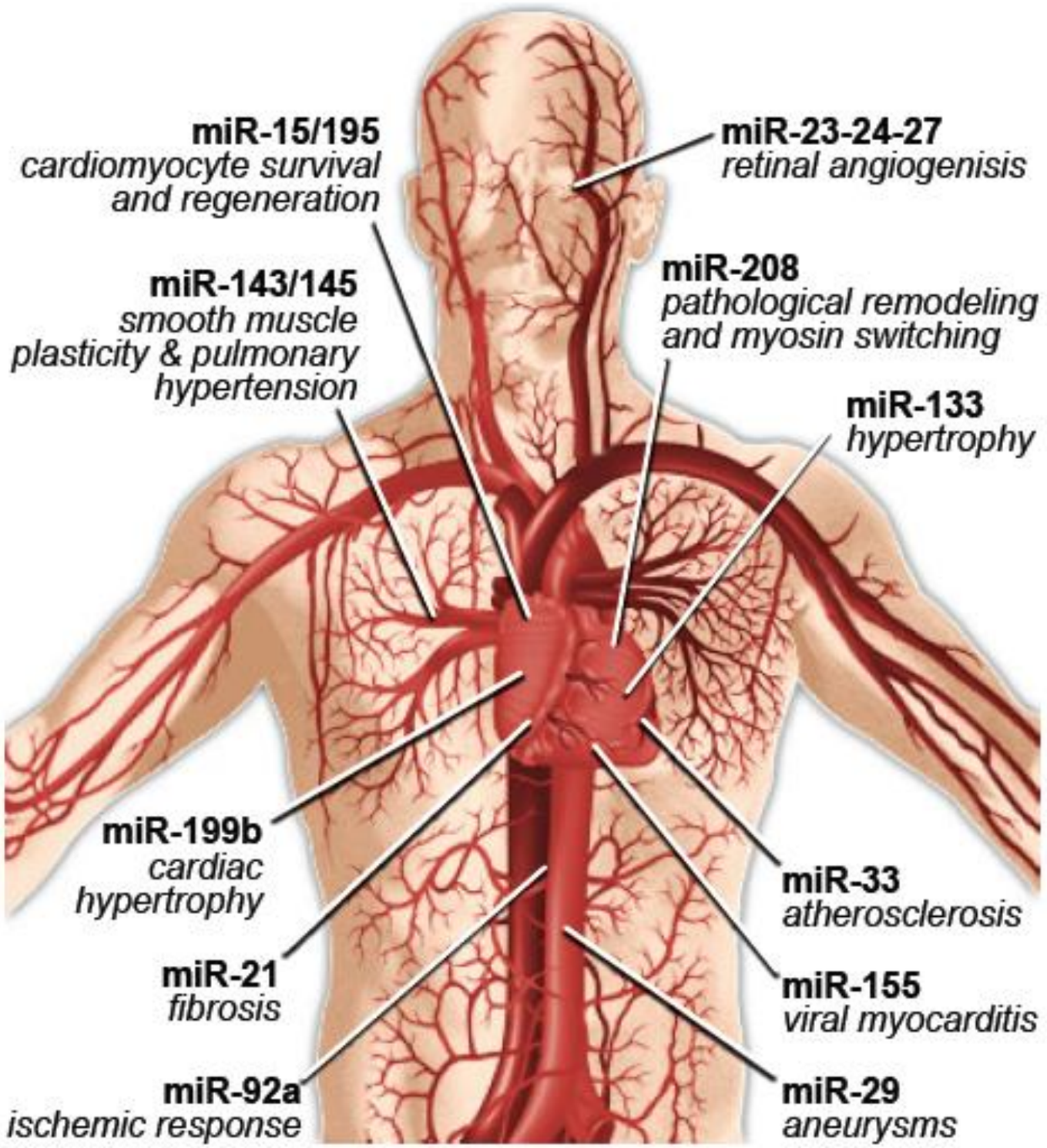




MicroRNA therapeutics

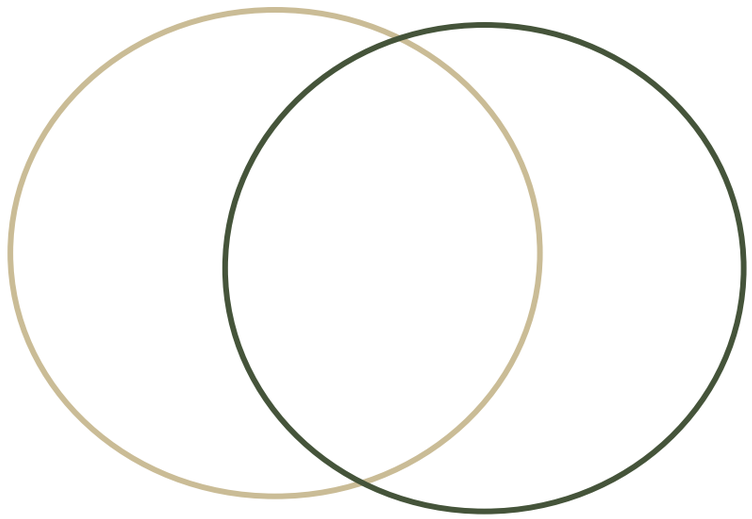
- AntimiRs can induce longlasting, potent and specific inhibition of a microRNA
- AntimiRs can be used to target cardiac microRNAs, but preferentially deliver to the kidney and liver – *what are the implications for chronic indications?*
- AntimiRs can be delivered subcutaneously (but do not cross the GI tract) – *will an injectable be attractive enough?*
- MicroRNA mimic can increase a microRNA in vivo

Efficacy studies using antimiRs in rodents

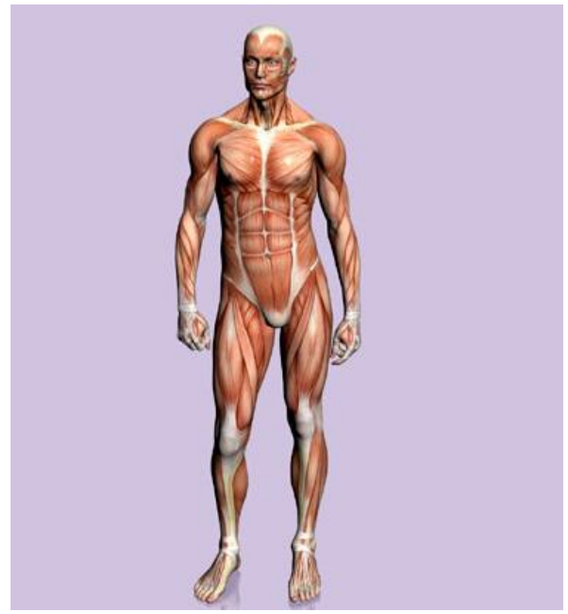


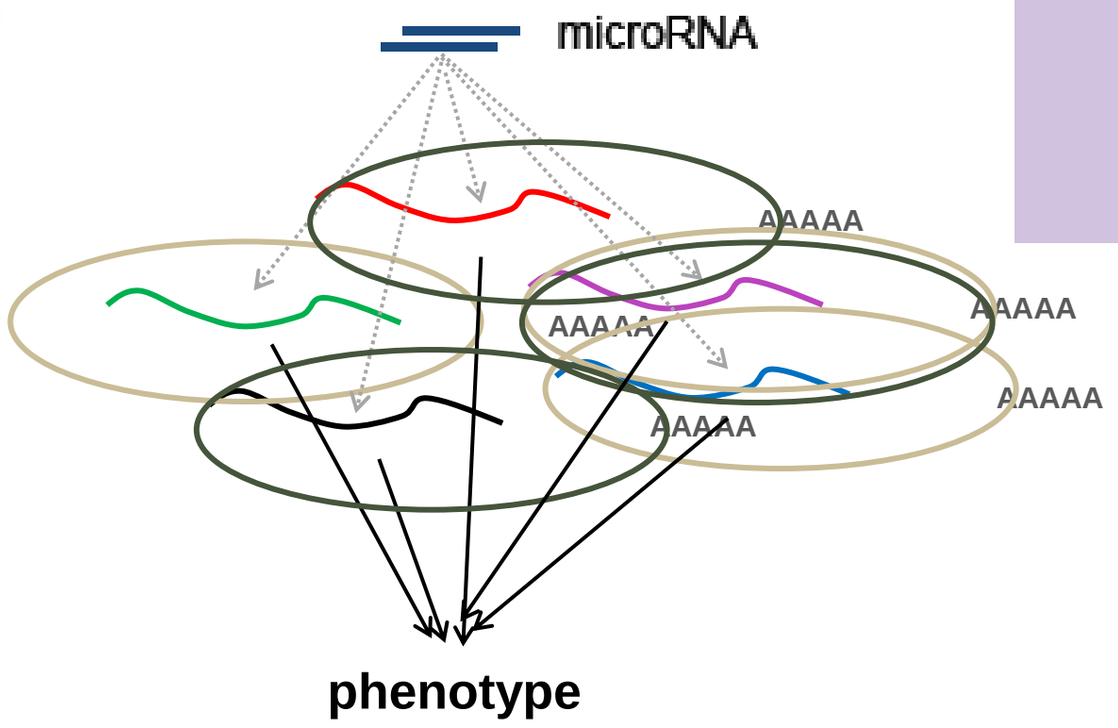
Species dependent target regulation

Target regulation in mice



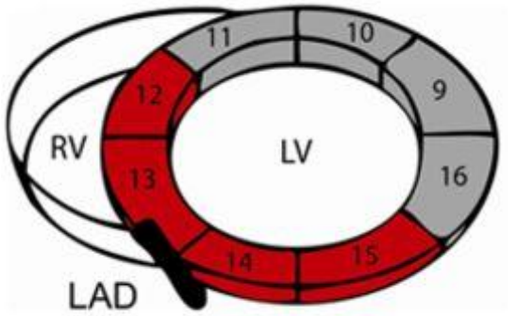
Target regulation in humans



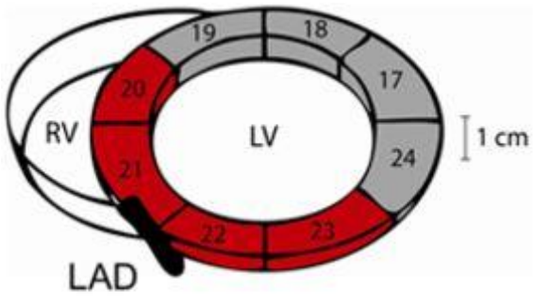


MicroRNA-92a Controls Angiogenesis and Functional Recovery of Ischemic Tissues in Mice

Angelika Bonauer,[1] Guillaume Carmona,[1] Masayoshi Iwasaki,[1] Marina Mione,[2] Masamichi Koyanagi,[1] Ariane Fischer,[1] Jana Burchfield,[1] Henrik Fox,[1,3] Carmen Doebele,[1] Kisho Ohtani,[1] Emmanouil Chavakis,[1,3] Michael Potente,[1,3] Marc Tjwa,[4] Carmen Urbich,[1] Andreas M. Zeiher,[3] Stefanie Dimmeler[1]*

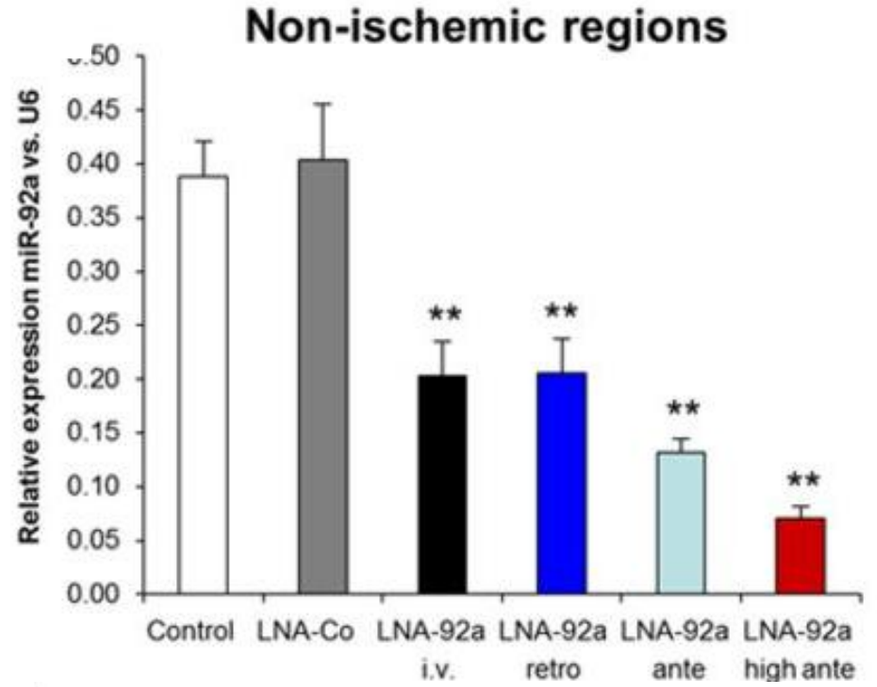


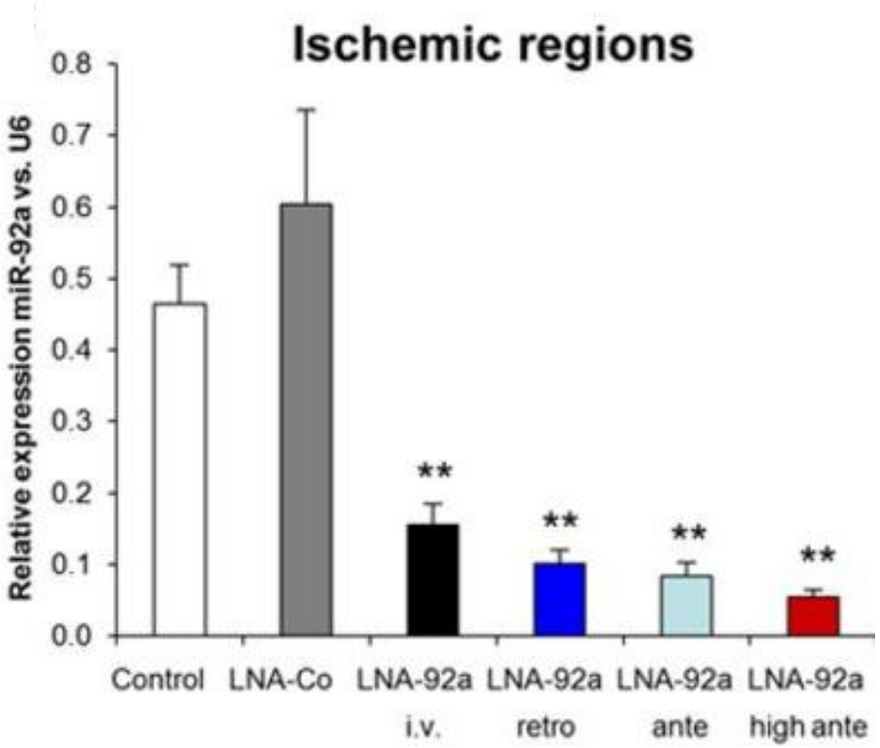
Slice 2



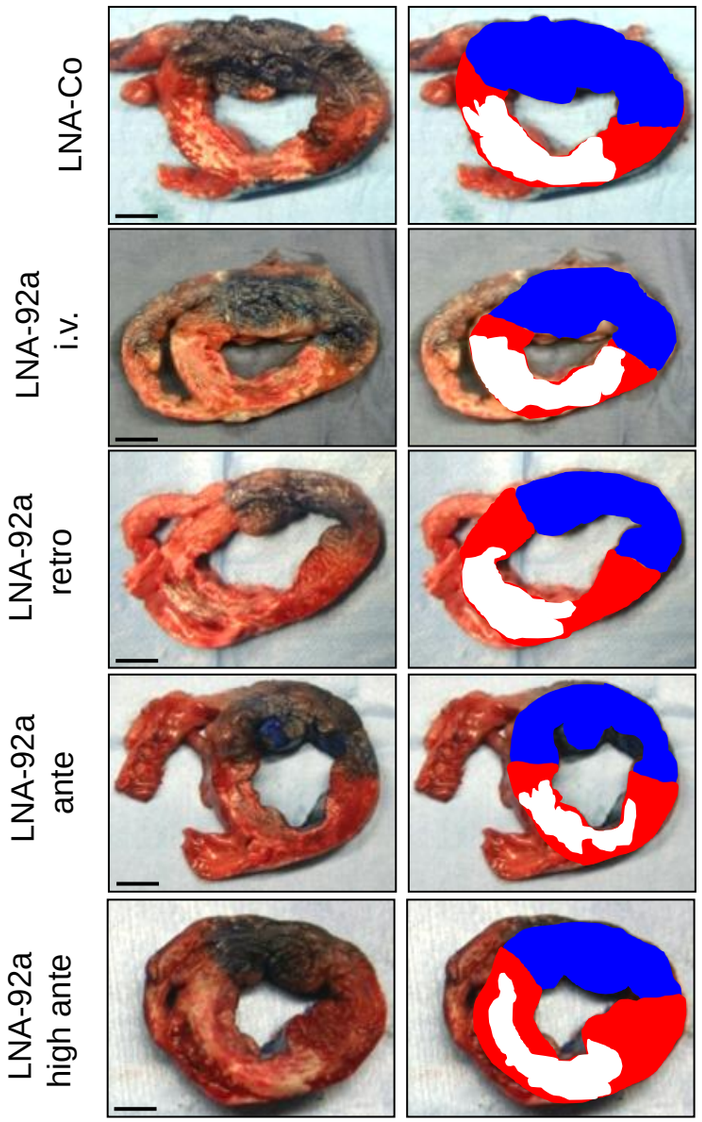
Slice 3

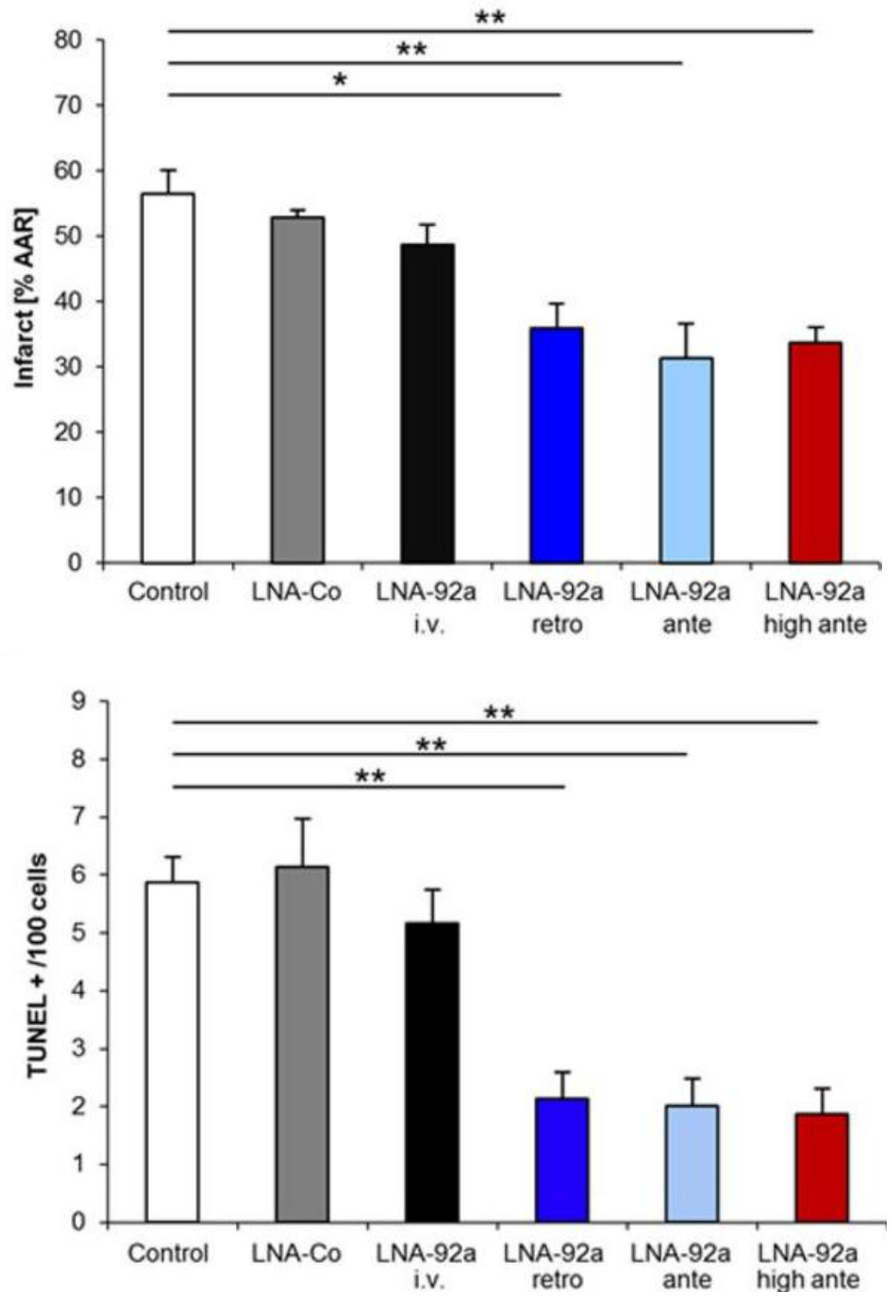
Non-ischemic regions
Ischemic regions





miR-92a inhibition reduces infarct size and enhances cardiac function in a porcine IR model





Hinkel et al. Circ 2013

ORIGINAL ARTICLE

Treatment of HCV Infection by Targeting MicroRNA

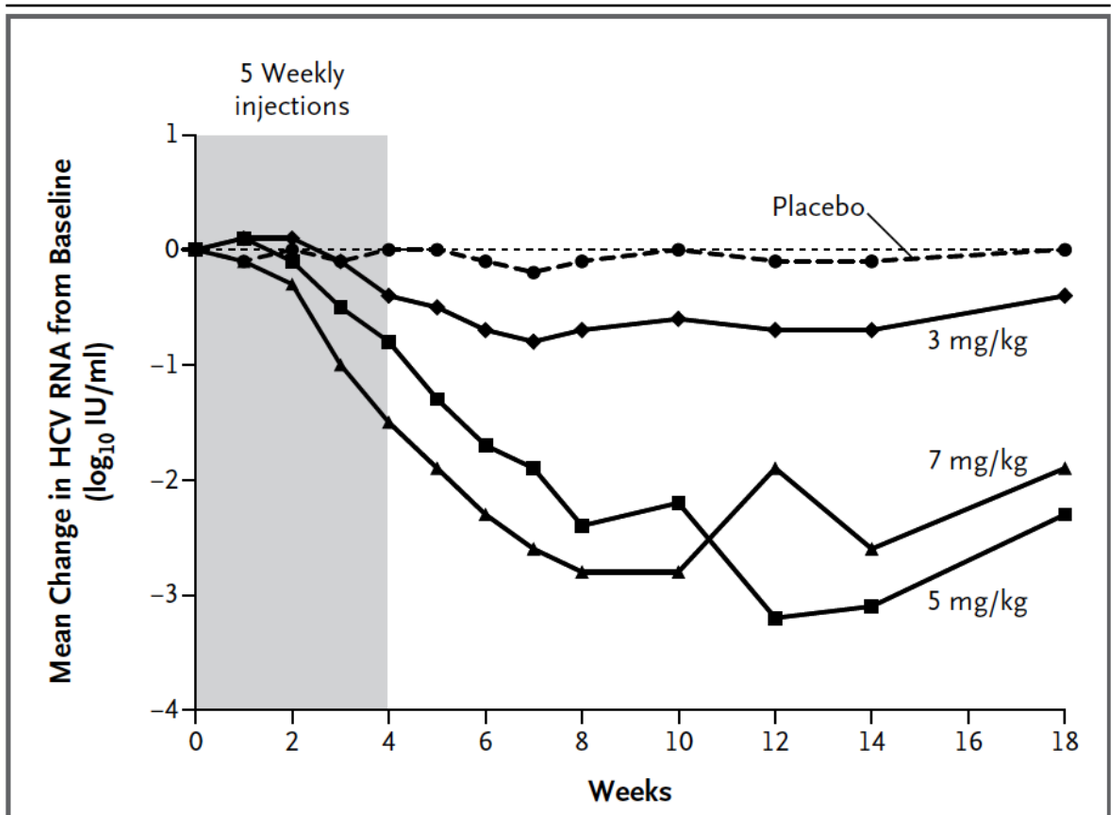


Figure 2. Change from Baseline in HCV RNA Levels.

Shown are the mean changes in HCV RNA levels from baseline for patients receiving 3 mg, 5 mg, or 7 mg of miravirsen per kilogram of body weight, as compared with placebo. Miravirsen was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed line indicates no change from baseline. The HCV RNA levels during the use of pegylated interferon and ribavirin in some patients were not included in this analysis.

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RNA Medicines for the 21st Century

May 2013

Hubrecht Institute

Developmental Biology and Stem Cell Research

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Prof. Olson, UTSW

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