

Utility of Circulating microRNAs in Cardiovascular Disease

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- **Introduction**
- **Biology of microRNAs**
- **Circulating microRNAs**
 - **Stability and packaging**
 - **c-miR as potential biomarkers**
 - **c-miR as intercellular messengers**
 - **Future therapeutic perspectives**
- **Conclusions**

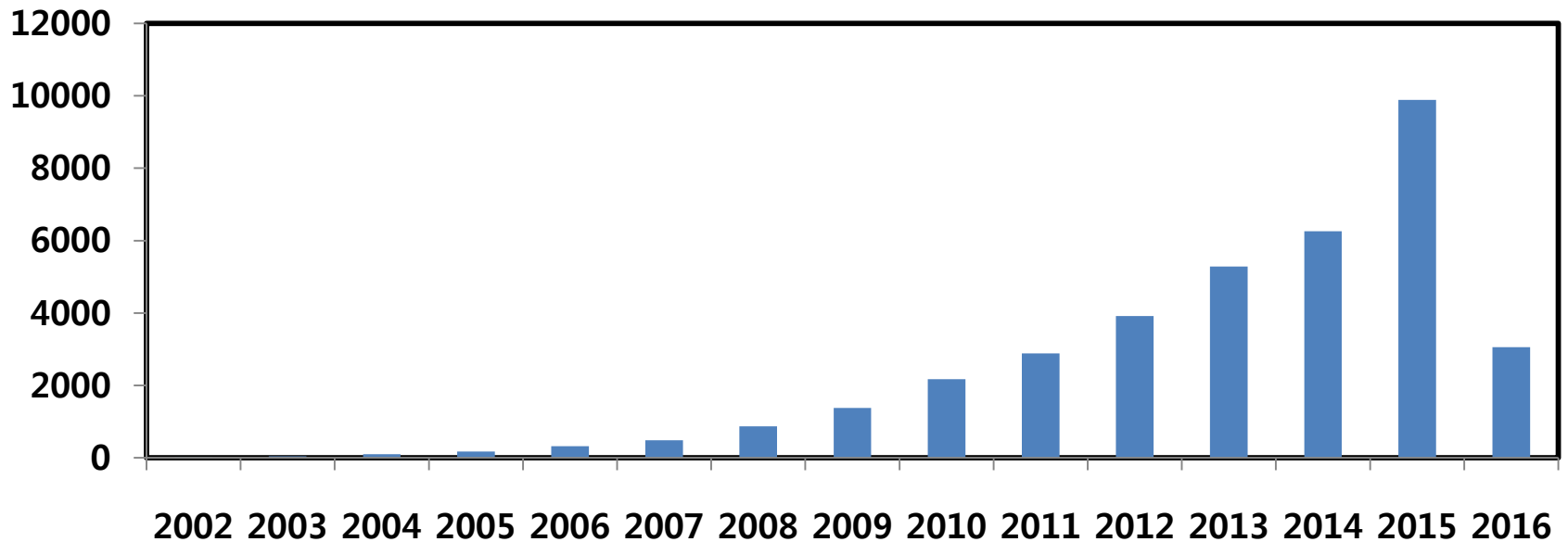
Introduction

What is microRNAs ?

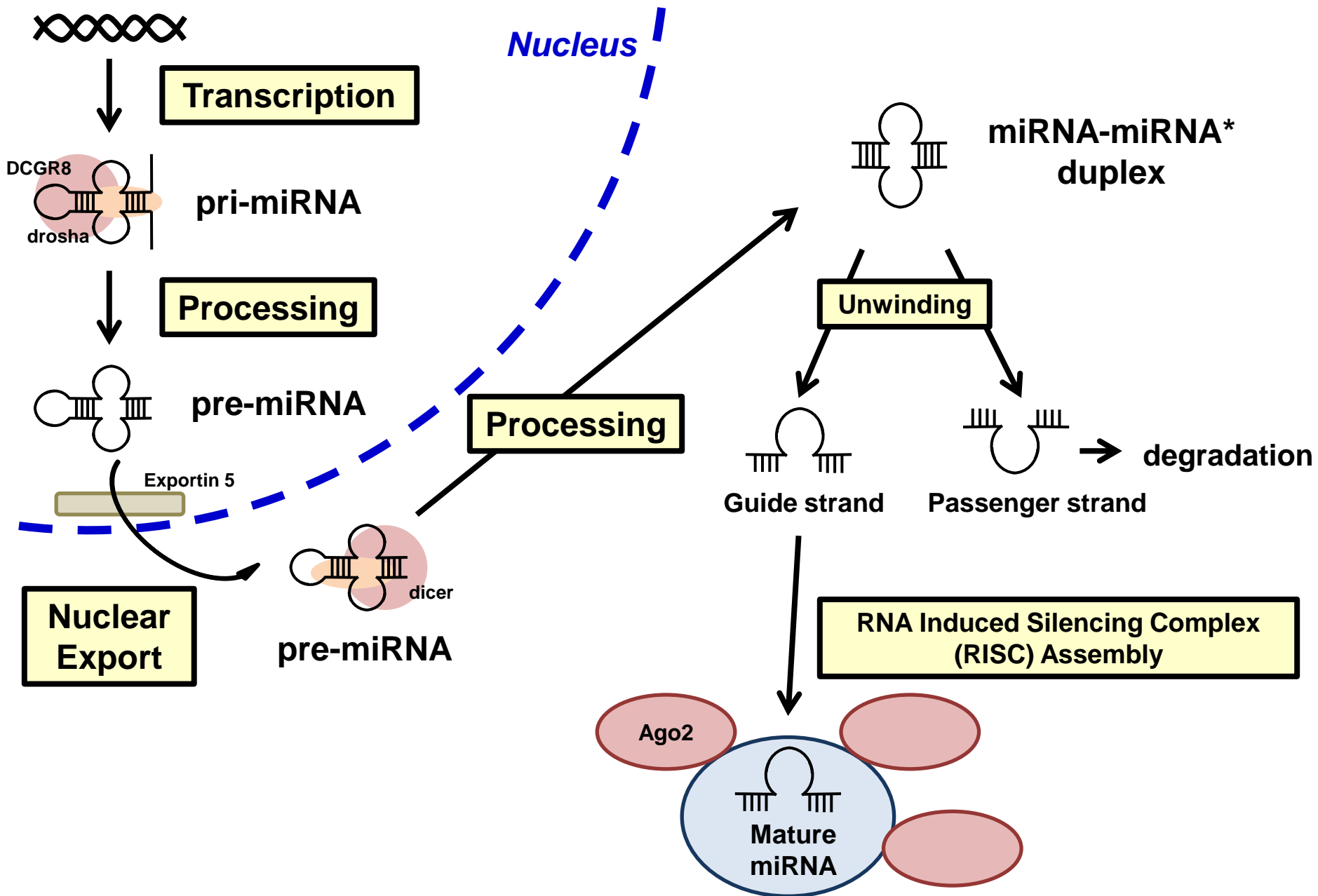
- **Small, evolutionarily conserved, non-protein coding RNA molecules**
- **Mediate post-transcriptional gene regulation by binding the 3' UTR of mRNAs**
- **First description in the nematode worm, 1993**
- **First description of biological activity in mammalian cells, 2001**
- **> 2,500 miRNAs identified in human**
- **> 1,500 miRNAs defined gene regulatory functions**

Publication regarding microRNAs

No. of Articles



Biology of microRNAs

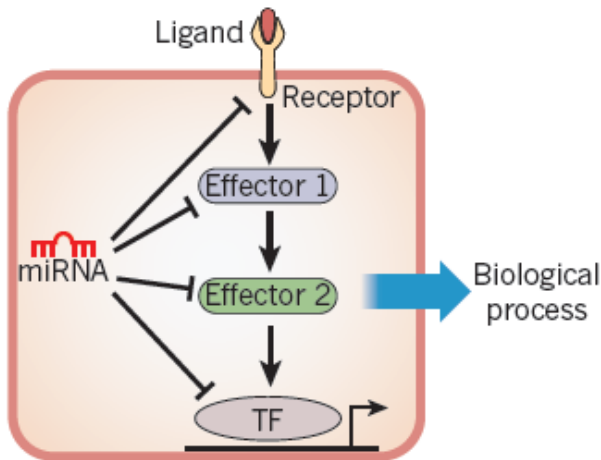


Mechanisms of Action

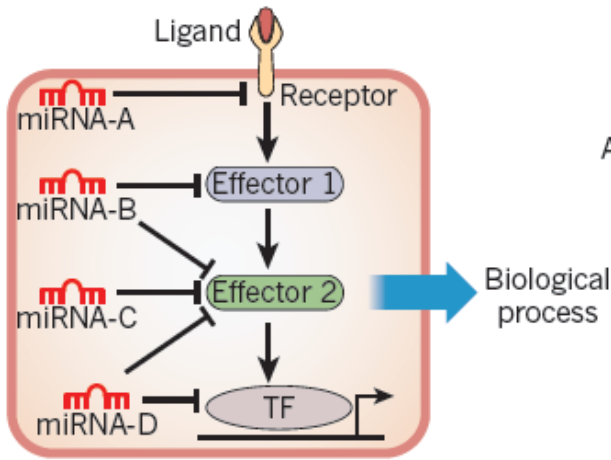
- miR-induced silencing complex (miRISC)
- 3' untranslated region (UTR) of mRNAs
- Seed sequence (nucleotides 2 ~ 7/8) at 5' end of microRNA
- The rest of the miR bind imperfectly – bulges and mismatches
- mRNA degradation or translational repression



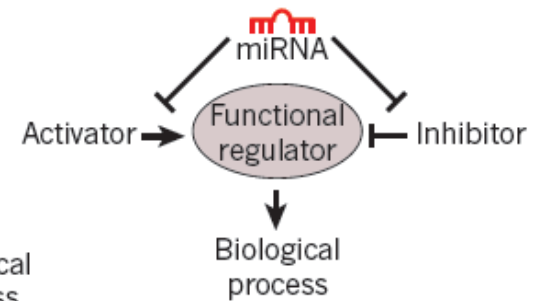
Concepts of miRNA function



**Multiplicity of
miRNA targets**

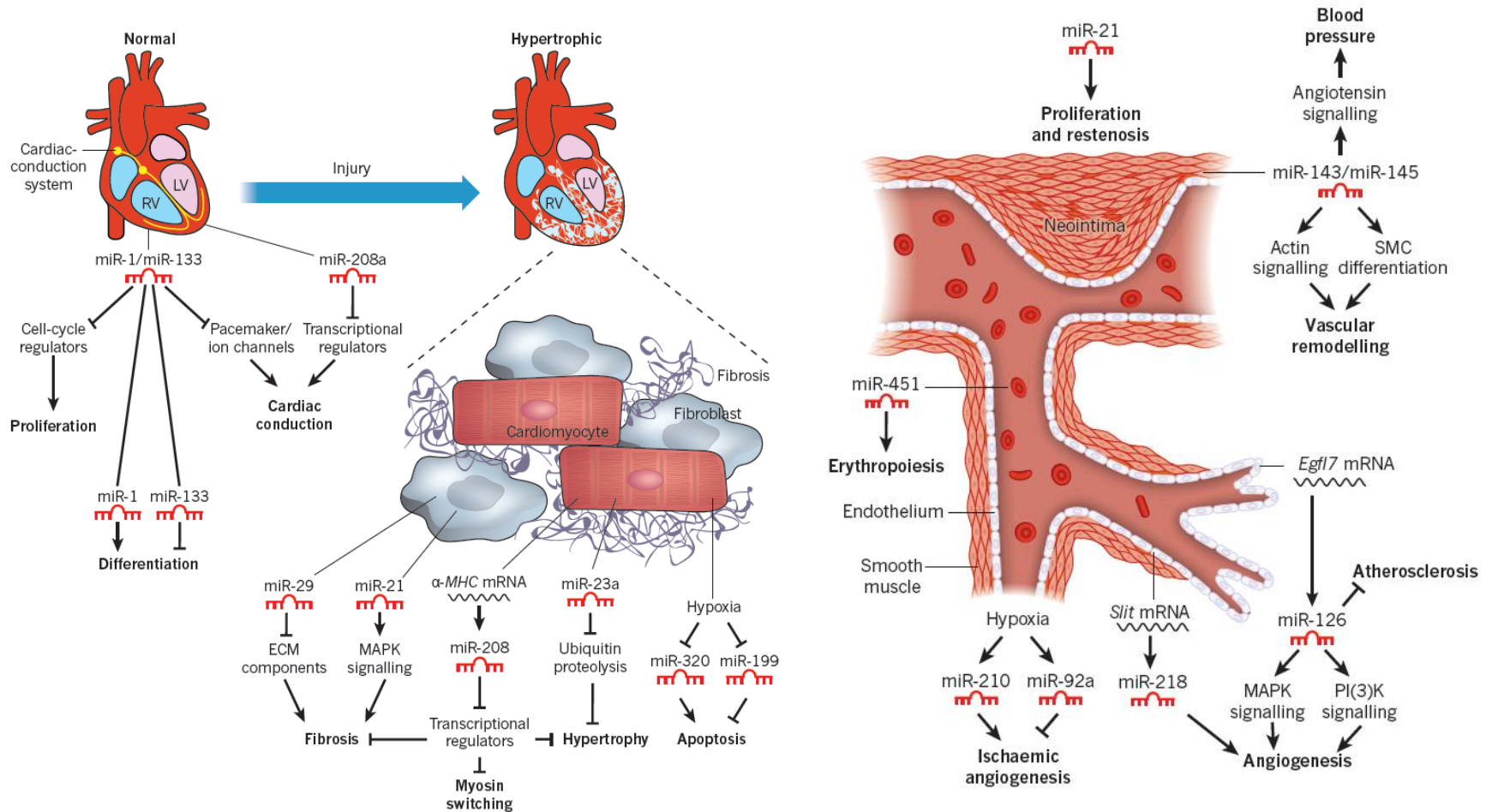


**miRNA cooperativity
and redundancy**



**Physiological
buffer**

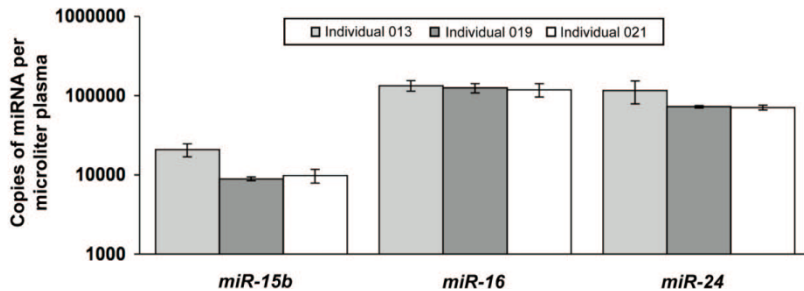
Role of miRNAs in CV system



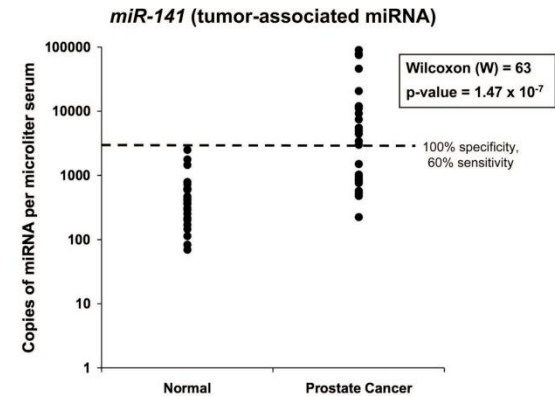
Circulating microRNAs

First description of extracellular miRNAs

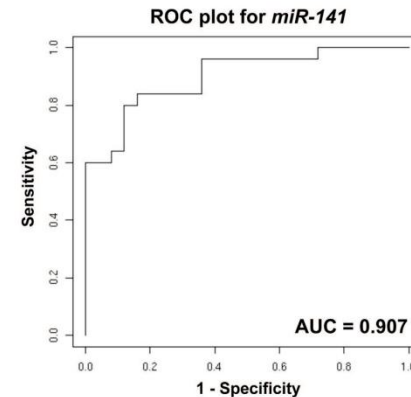
- Mitchell PS, et al. Circulating microRNAs as stable blood-based markers for cancer detection.



A



B



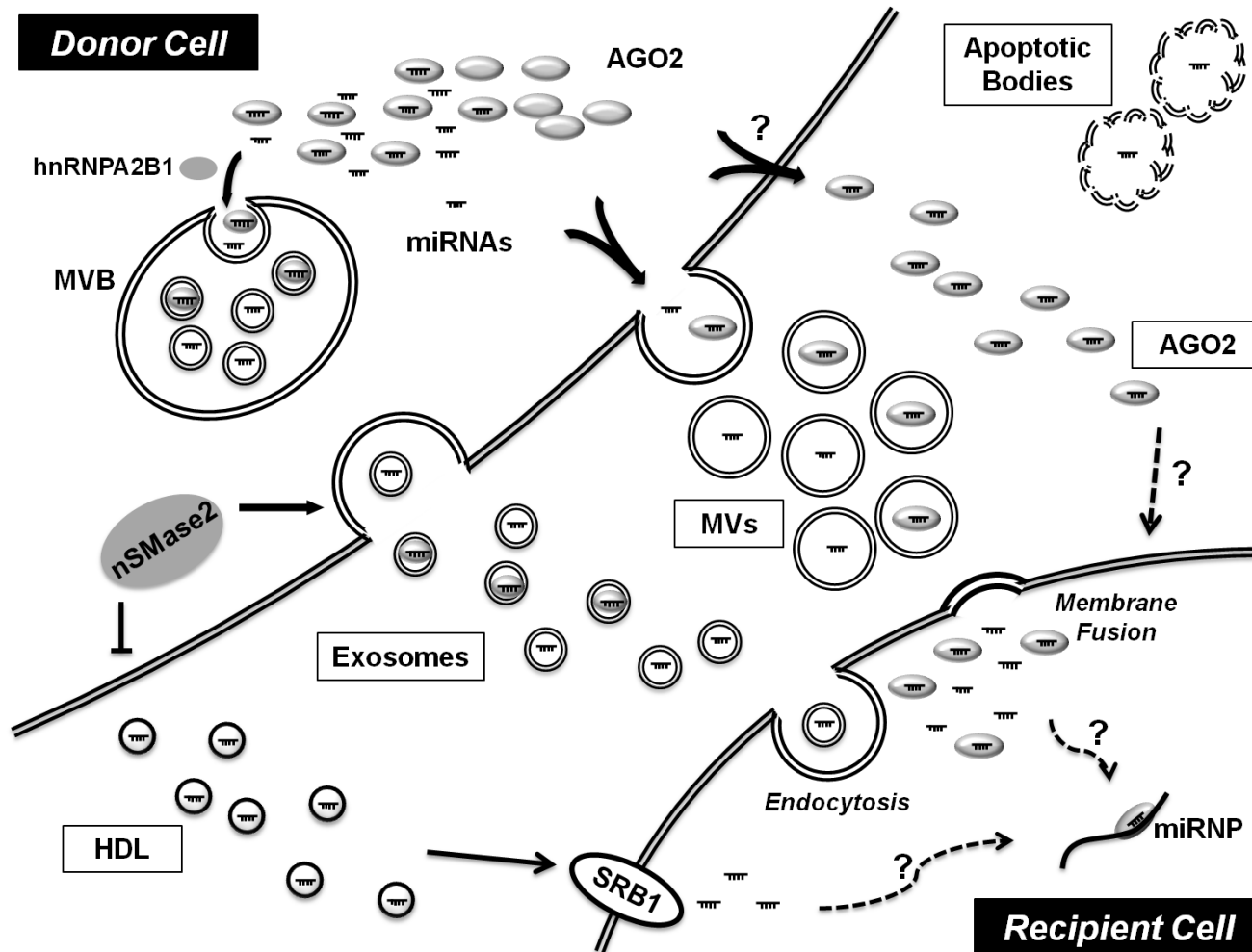
Stability of Extracellular miRNAs

- **Stable even under harsh conditions such as boiling, low/high pH, extended storage at RT, and multiple freeze-thaw cycles**
- **Synthetic miRNAs added to plasma → rapidly degraded by RNase activity**
- **Detergents or proteinase K facilitate extracellular miRNAs degradation**

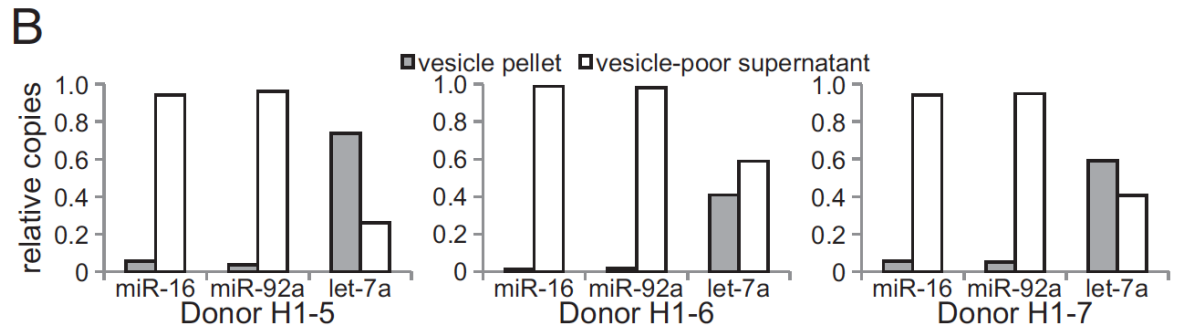
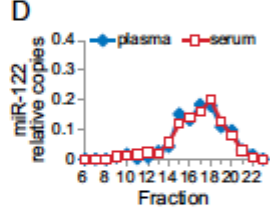
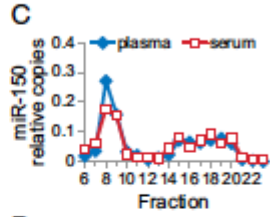
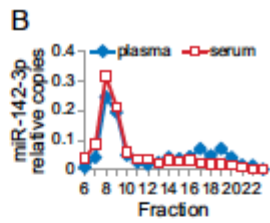
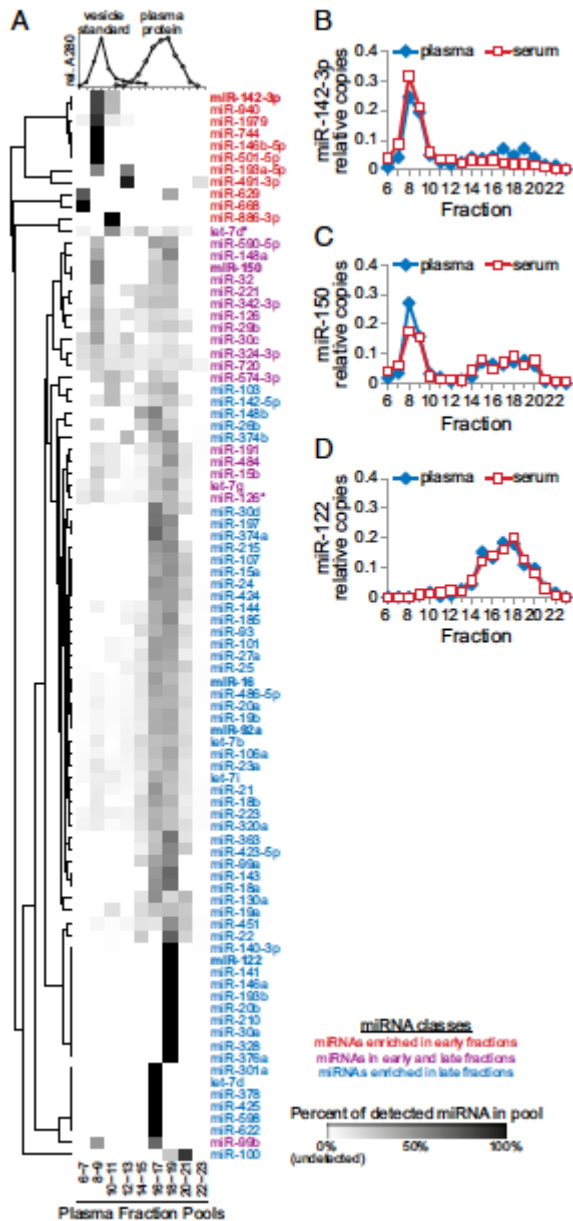
Mechanisms for Protection

- **Encapsulation of miRNAs in membrane-derived vesicles**
 - **Exosomes, microvesicles, or apoptotic bodies**
- **RNA-binding proteins**
 - **Nucleophosmin I, or Argonaute proteins**
- **Lipoprotein complexes such as HDL**

Several mechanisms for packaging of extracellular miRNAs for transport

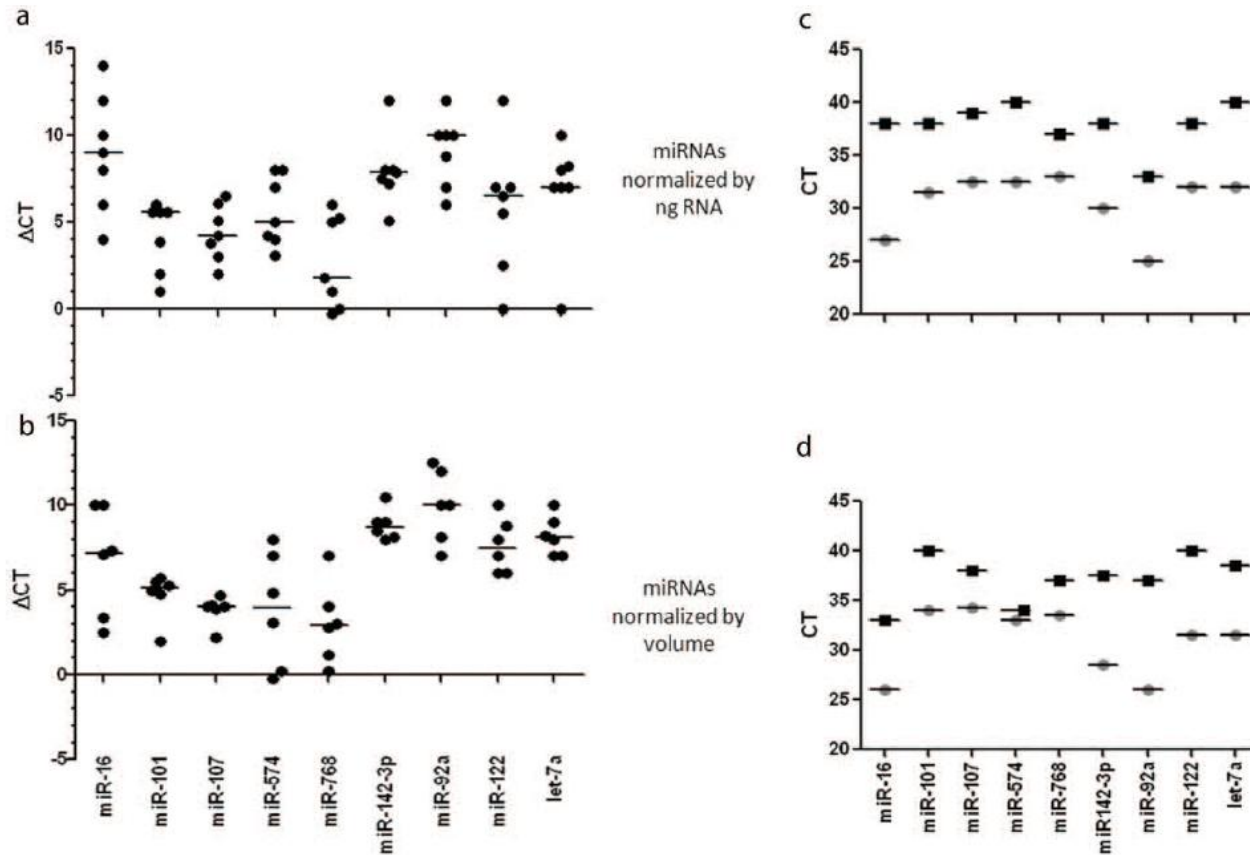


Main fraction of extracellular microRNAs (1)



- A majority of miRNAs was found in nonvesicle-associated complexes bound to AGO2

Main fraction of extracellular microRNAs (2)



- The Majority of MicroRNAs Detectable in Serum and Saliva Is Concentrated in Exosomes

Main fraction of extracellular microRNAs (3)

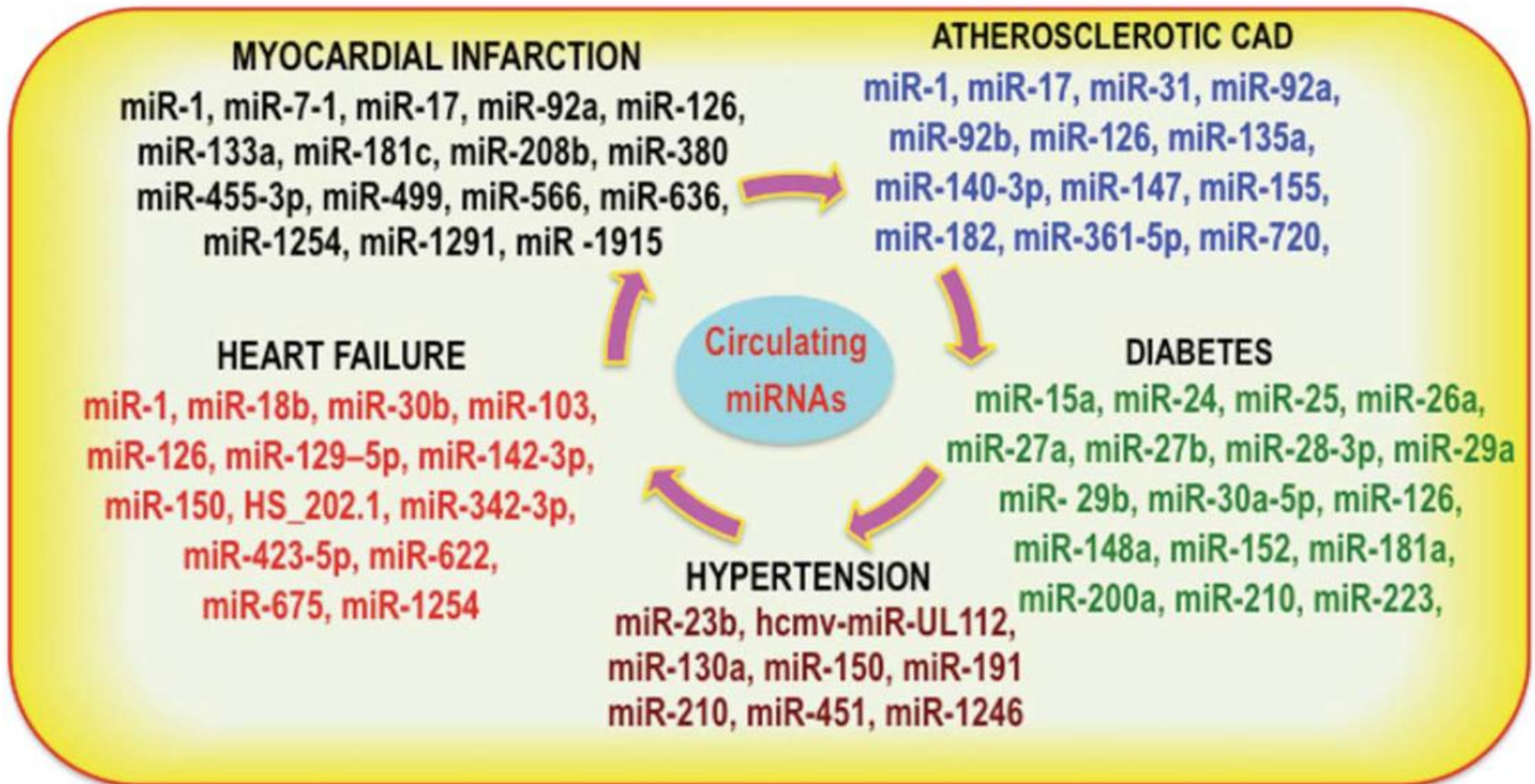
- **This discrepancy may arise from differences in technique for microvesicle isolation and RNA extraction**
- **Association with AGO2 with microRNAs – critical role in stabilizing in extracellular space in both vesicle and RNA-protein complexes**

Several mechanisms to control the packaging and sorting of miRNA release

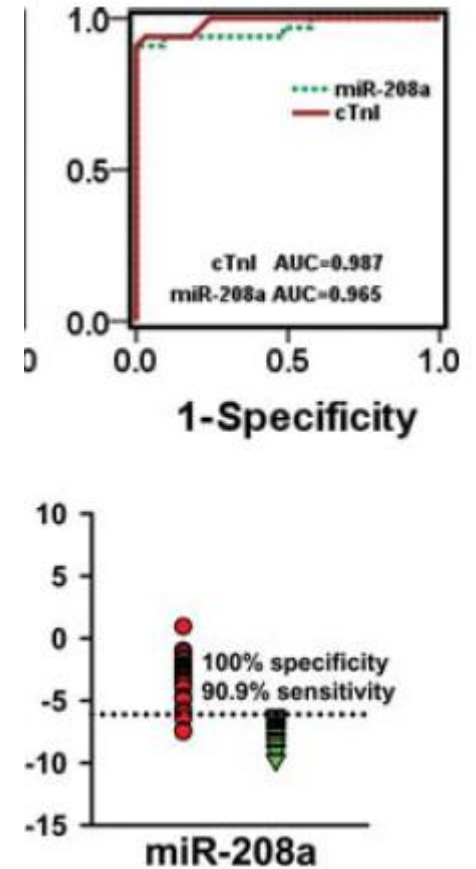
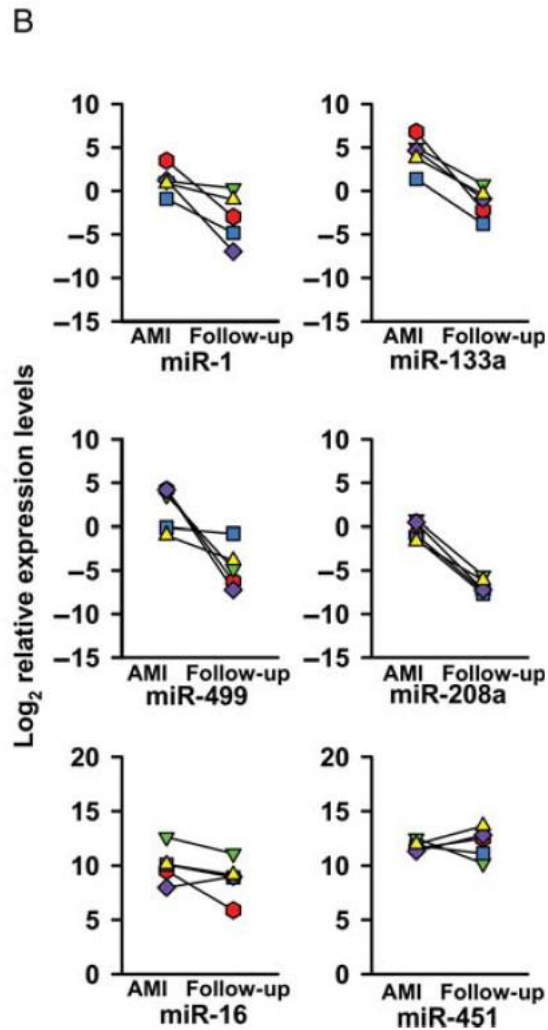
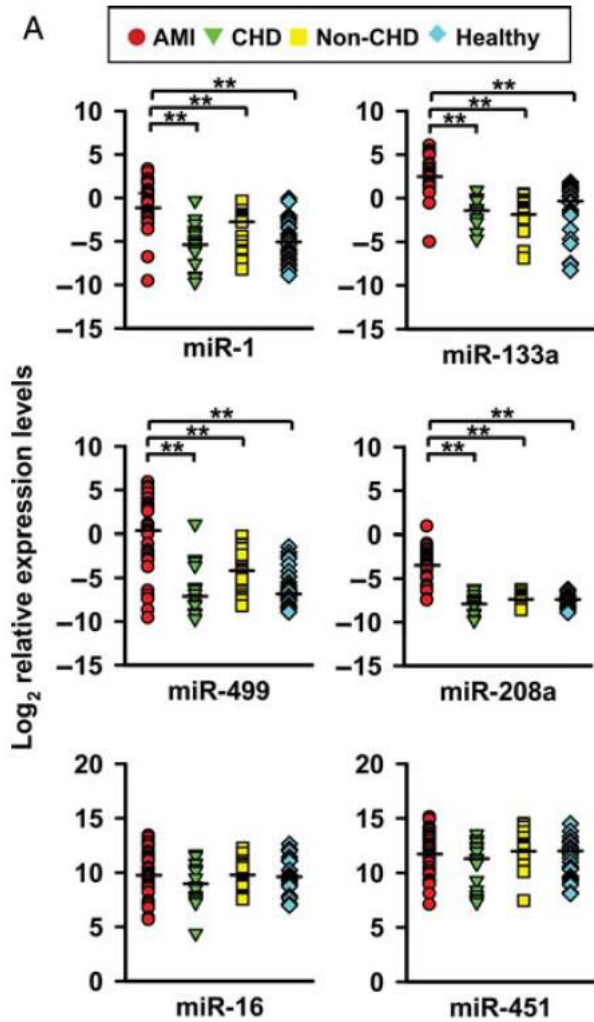
- **Level of miRNA expression**
- **Cellular energetics (Intracellular ATP levels)**
- **Protein components of RISC – AGO2, GW182**
- **Sumoylated heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1)**

c-miRNAs as Potential Biomarkers

C-miRs as Diagnostic Biomarkers for CVD



Cardiac specific miR-208 in AMI



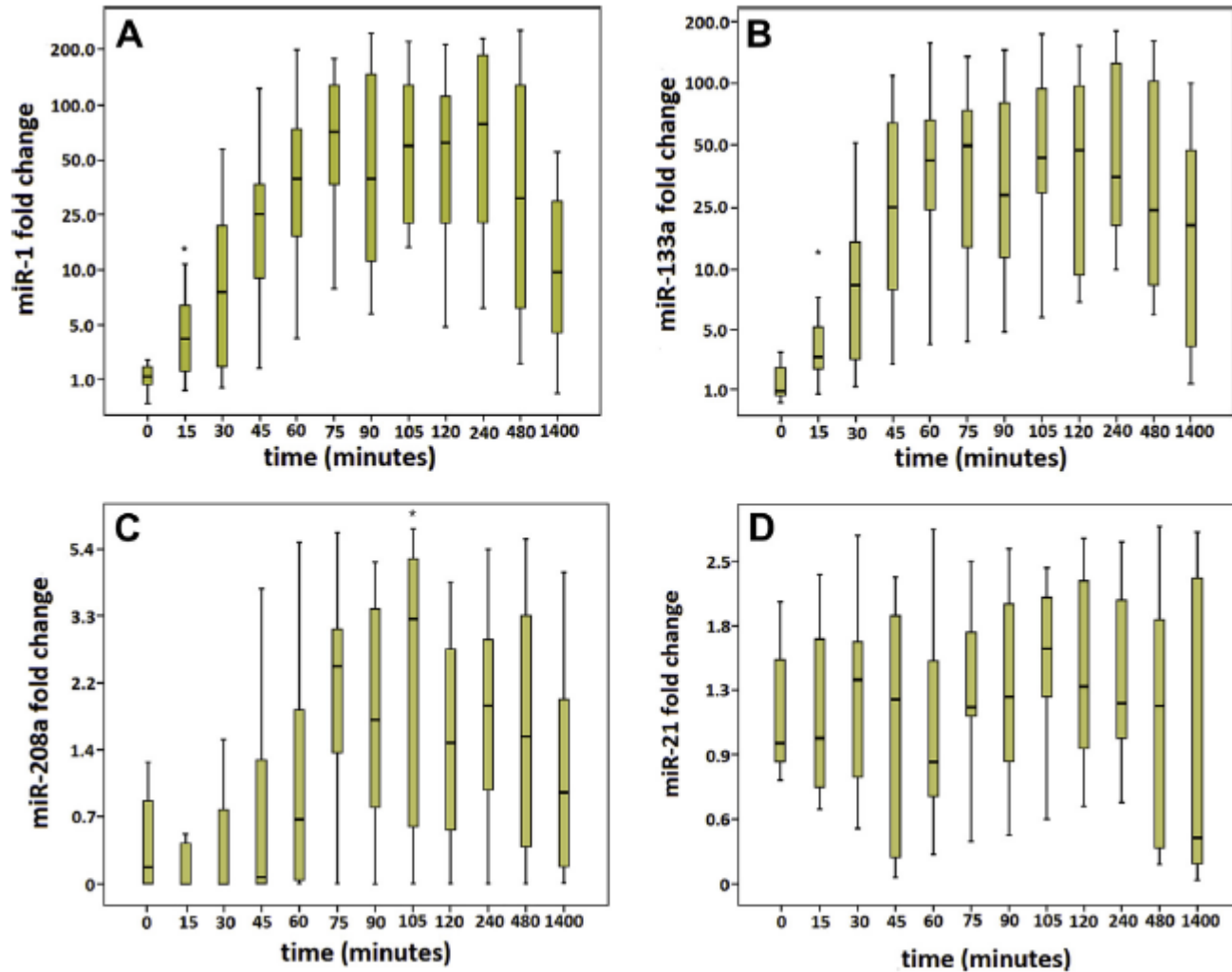
Early assessment of ACS in ED

- the potential diagnostic value of c-miRs

Table 4. AUCs and Odds ratios of miRNAs in suspected ACS patients in a clinical model ($n = 332$)

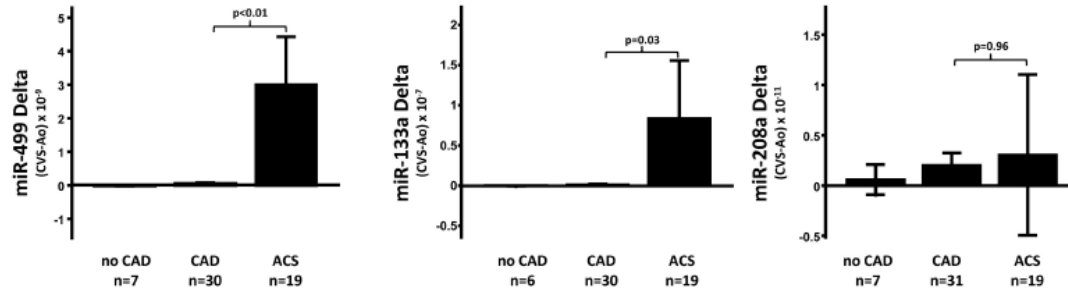
Marker	AUC	95% CI
Clinical model (CM)	0.72	0.66–0.78
CM + cardiac troponin	0.88	0.85–0.92
CM + cardiac hs-troponin T	0.89	0.85–0.92
CM + cardiac hs-troponin T with		
miR-1	0.92 ^a	0.90–0.95
miR-208a	0.89	0.85–0.93
miR-499	0.92 ^a	0.89–0.95
miR-21	0.92 ^a	0.89–0.95
miR-146a	0.90	0.87–0.94
miR-1 + miR-499 + miR-21	0.94 ^a	0.92–0.97

Release Kinetics of c-miRs in HCMP undergoing Septal Ablation

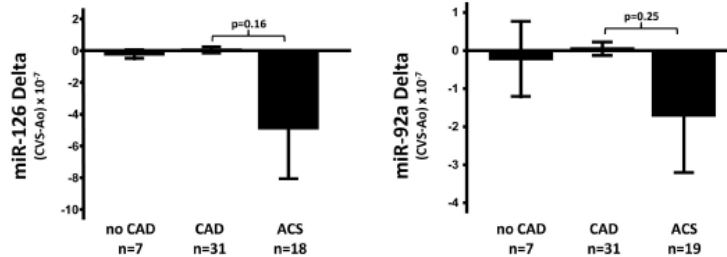


Transcoronary Concentration Gradients of c-miRNAs

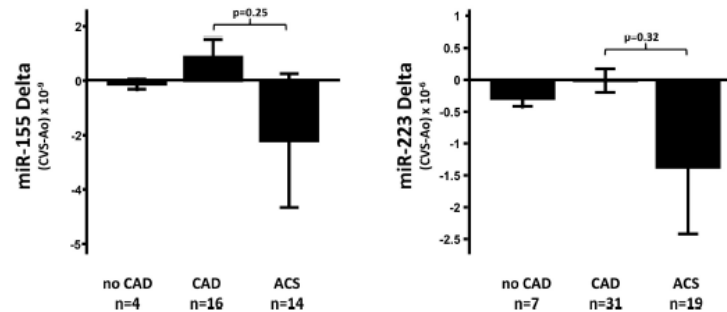
A muscle-enriched miRs



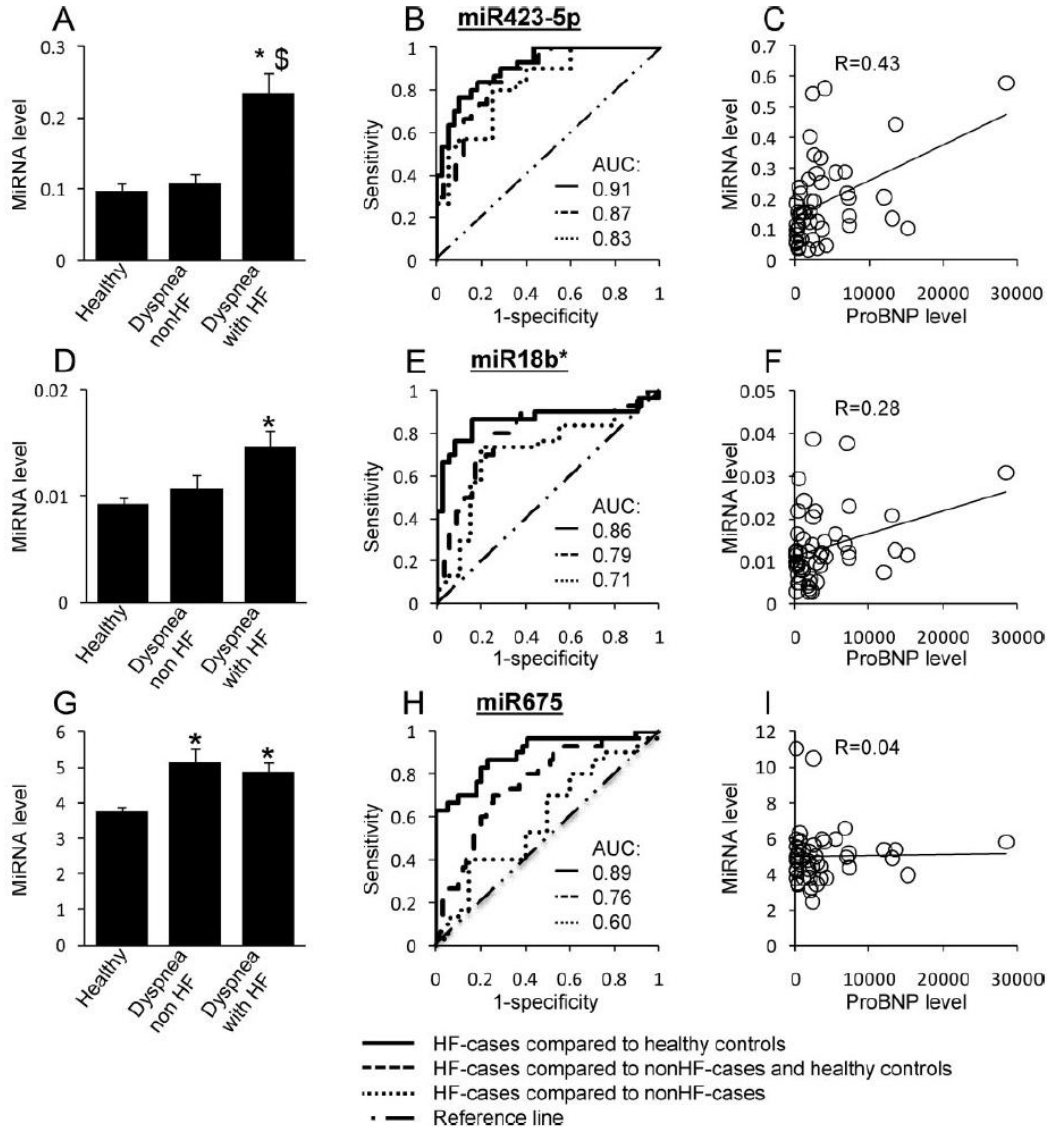
B vascular-related miRs



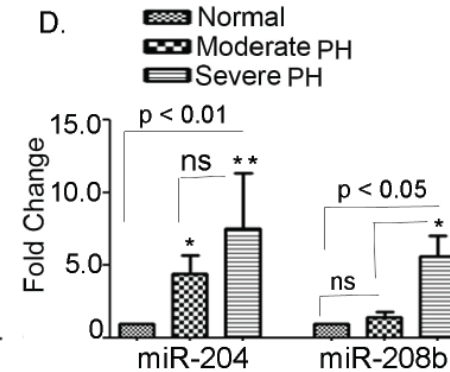
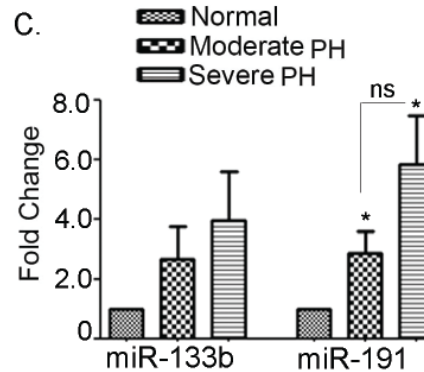
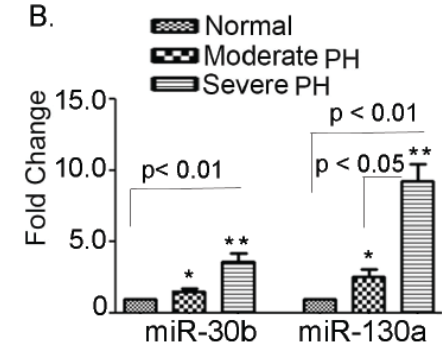
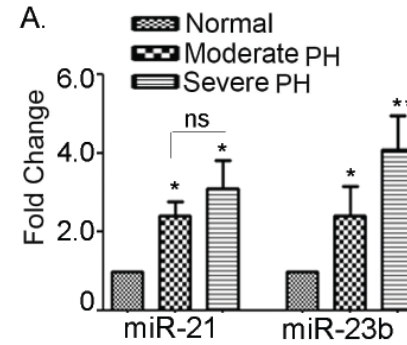
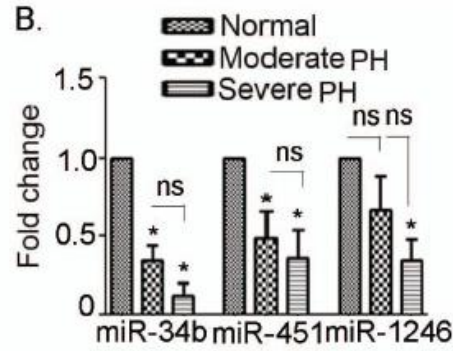
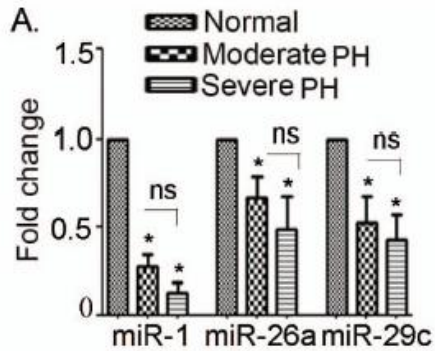
C leukocyte- and platelet-related miRs



miR-423-5p as a Biomarker for HF



c-miRNAs as Biomarkers for PH



Mild PH: mean PAP > 25 mmHg

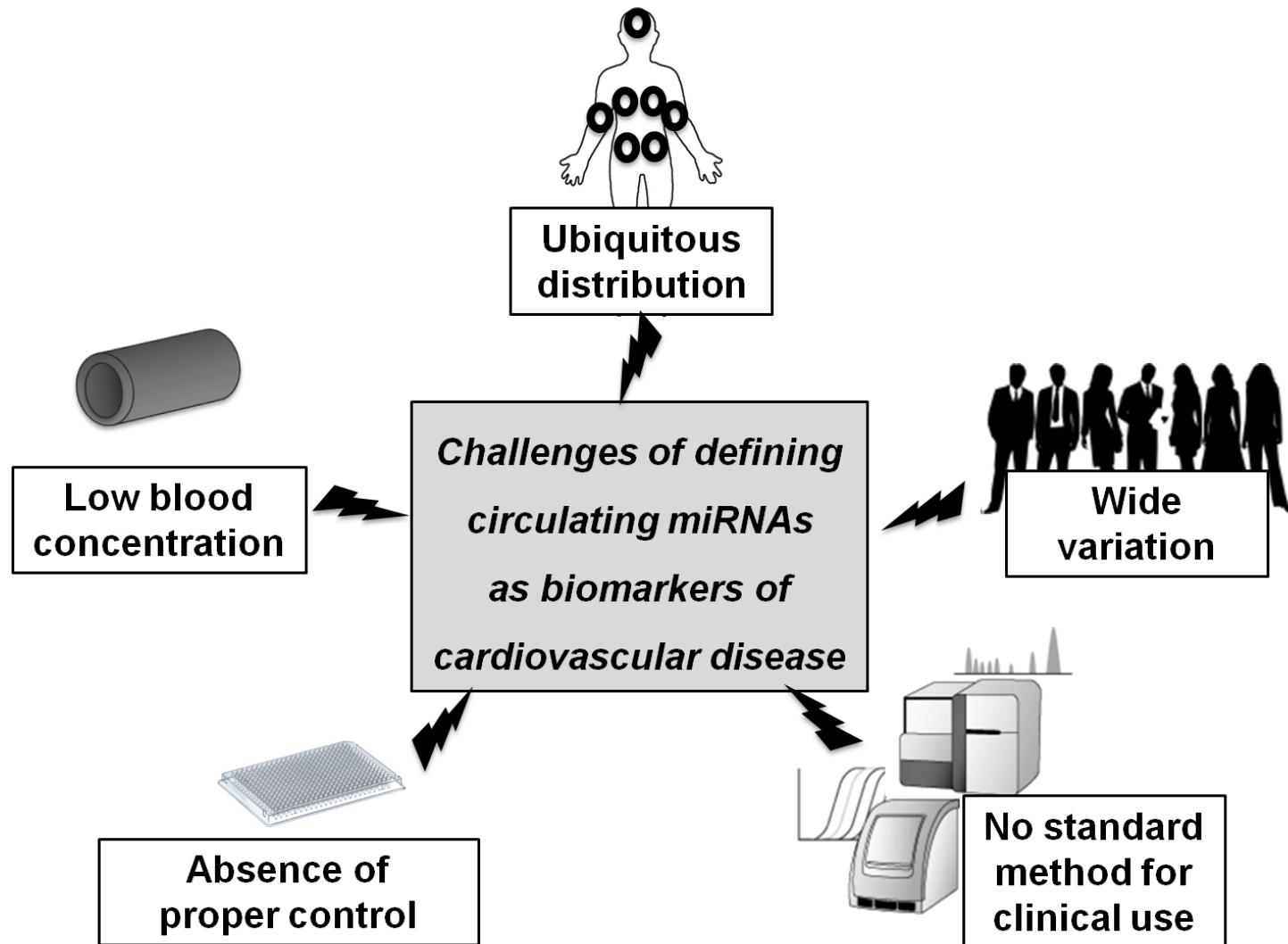
Moderate PH: mean PAP > 35 mmHg

Severe PH: mean PAP > 40 mmHg

Challenges in interpretation of released miRNAs

- **By-product from damaged cells or additional roles as intercellular messengers**
- **Same c-miRs altered in a variety of clinical situations**

Challenges in Defining c-miRs as Biomarkers



c-miRNAs as Intercellular Messengers

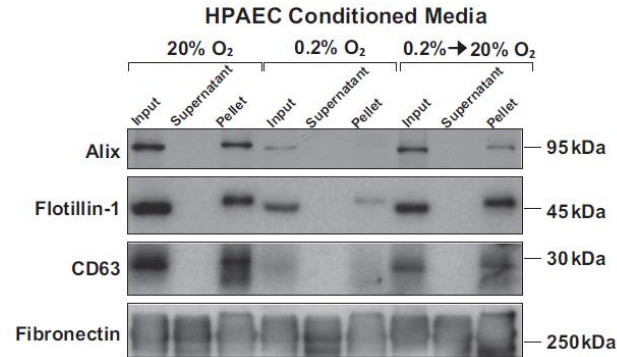
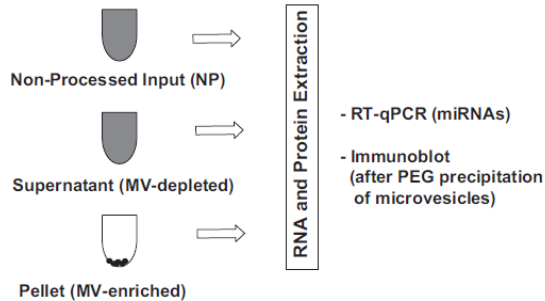
Several mechanisms for delivery of extracellular miRNAs

- **Intercellular transfer of miRNAs within microvesicles → endocytosis, membrane fusion, or phagocytosis**
- **HDL-associated miRNAs → scavenger receptor class B type 1 (SRB1)**
- **Hypoxia induced miR-210 can be delivered to recipient EC via AGO2-RNA complex**

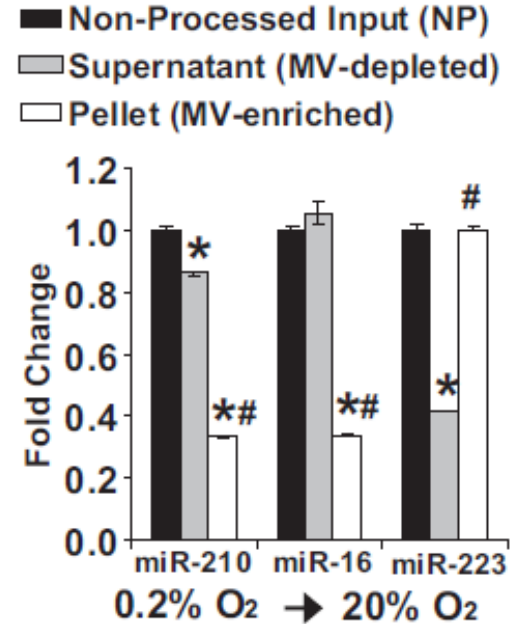
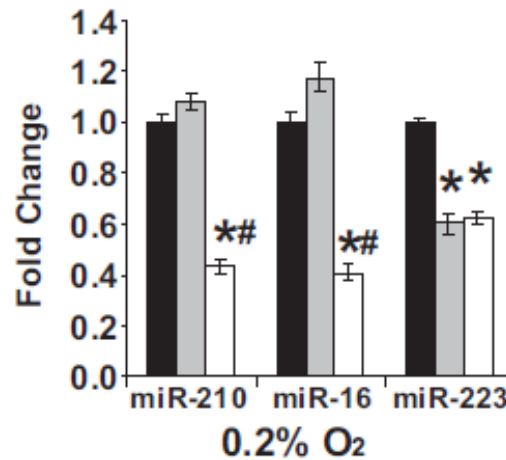
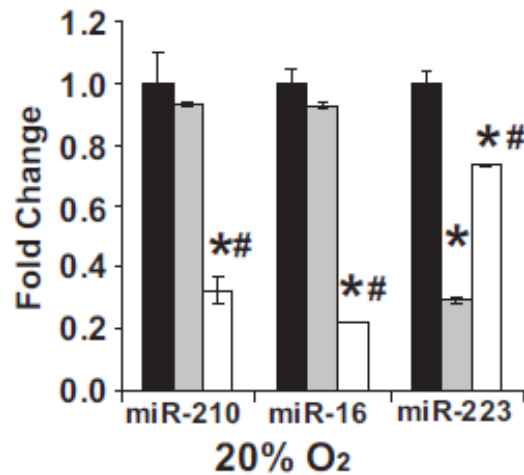
miRNA delivery in CV system

***In vitro* studies**

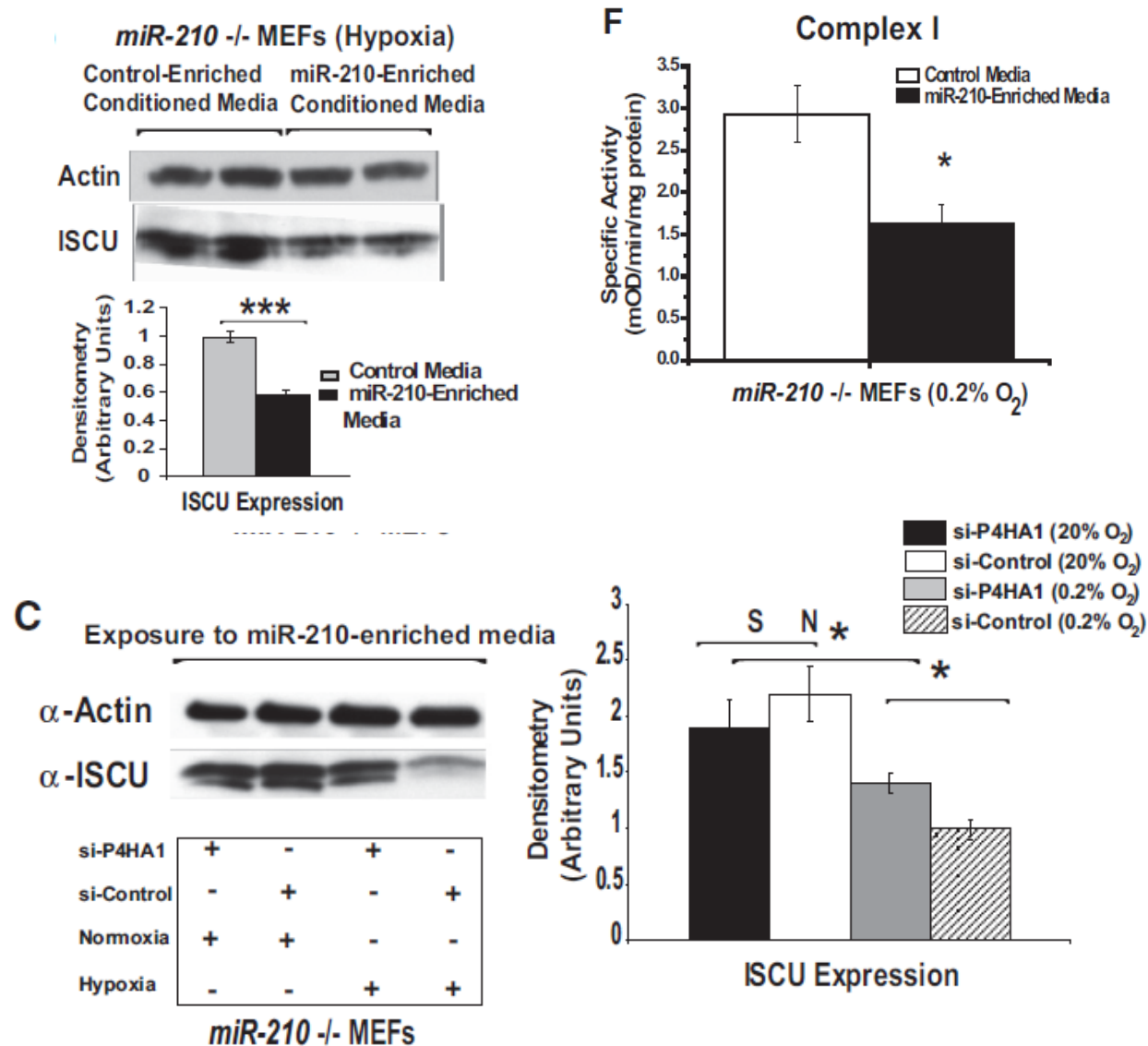
Distinct pattern of packaging miRNAs for release



B HPAEC Conditioned Media



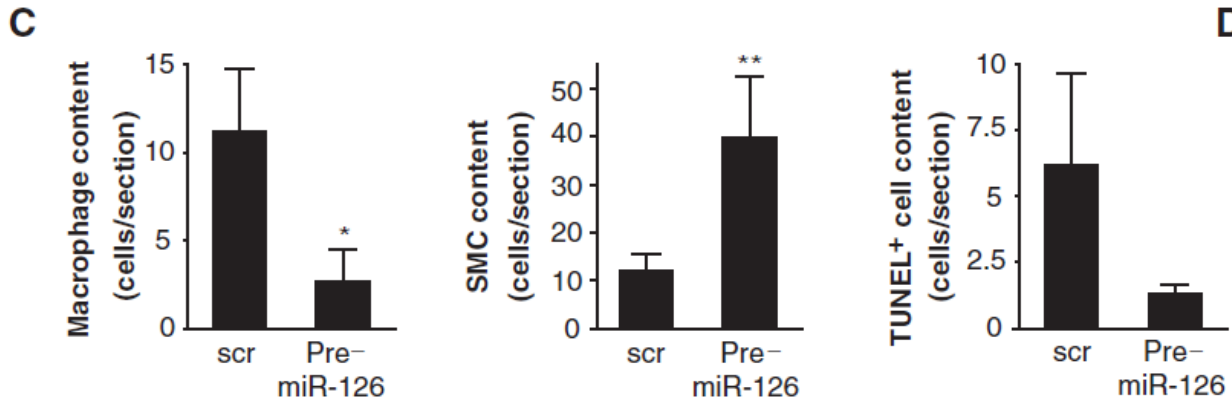
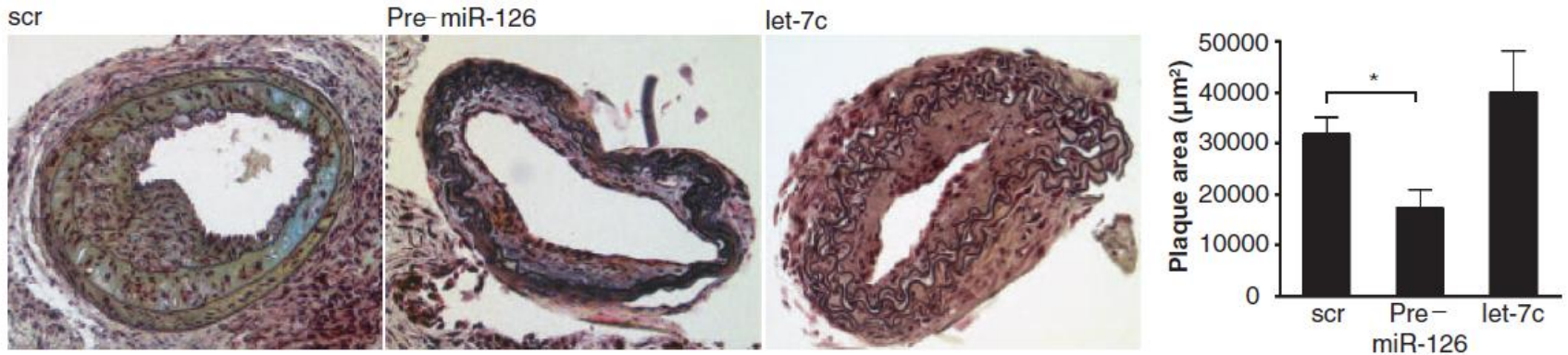
Released miR-210 delivered into recipient cells



miRNA delivery in CV system

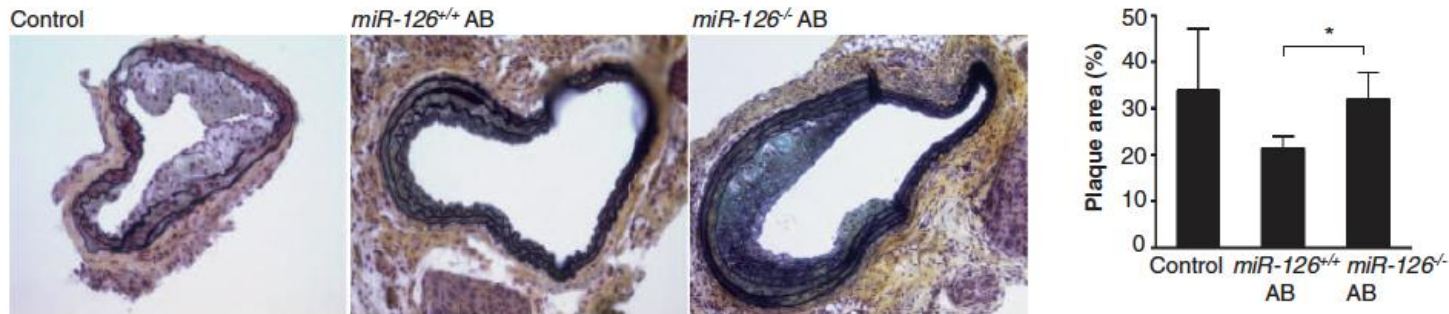
In vivo studies

Delivery of MicroRNA-126 by Apoptotic Bodies Induces CXCL12-Dependent Vascular Protection

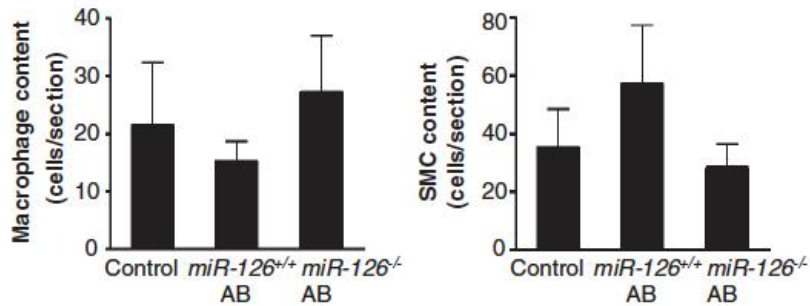


Delivery of MicroRNA-126 by Apoptotic Bodies Induces CXCL12-Dependent Vascular Protection

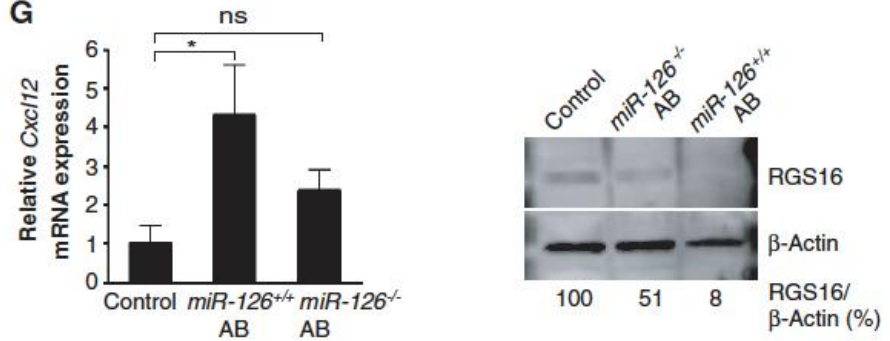
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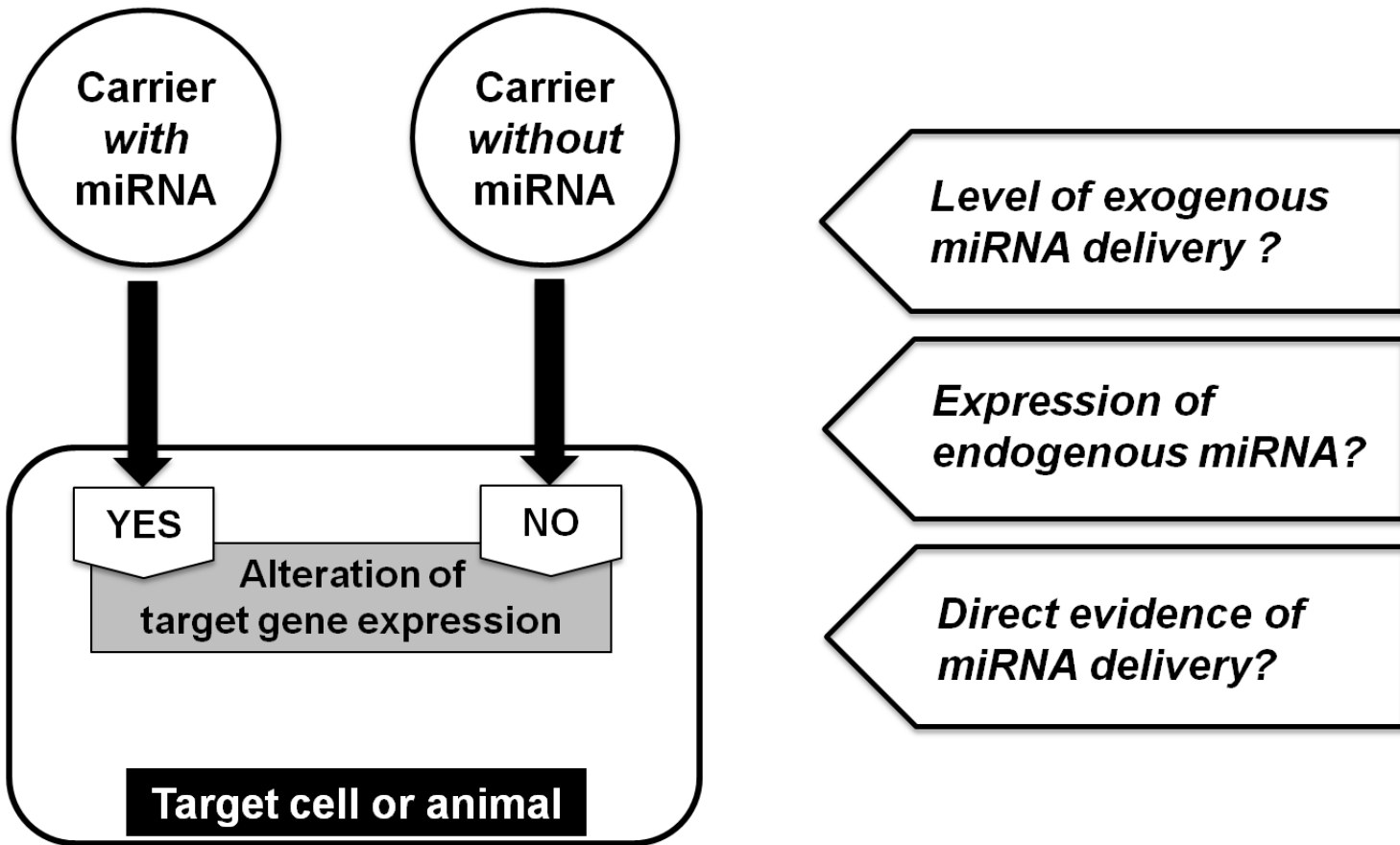
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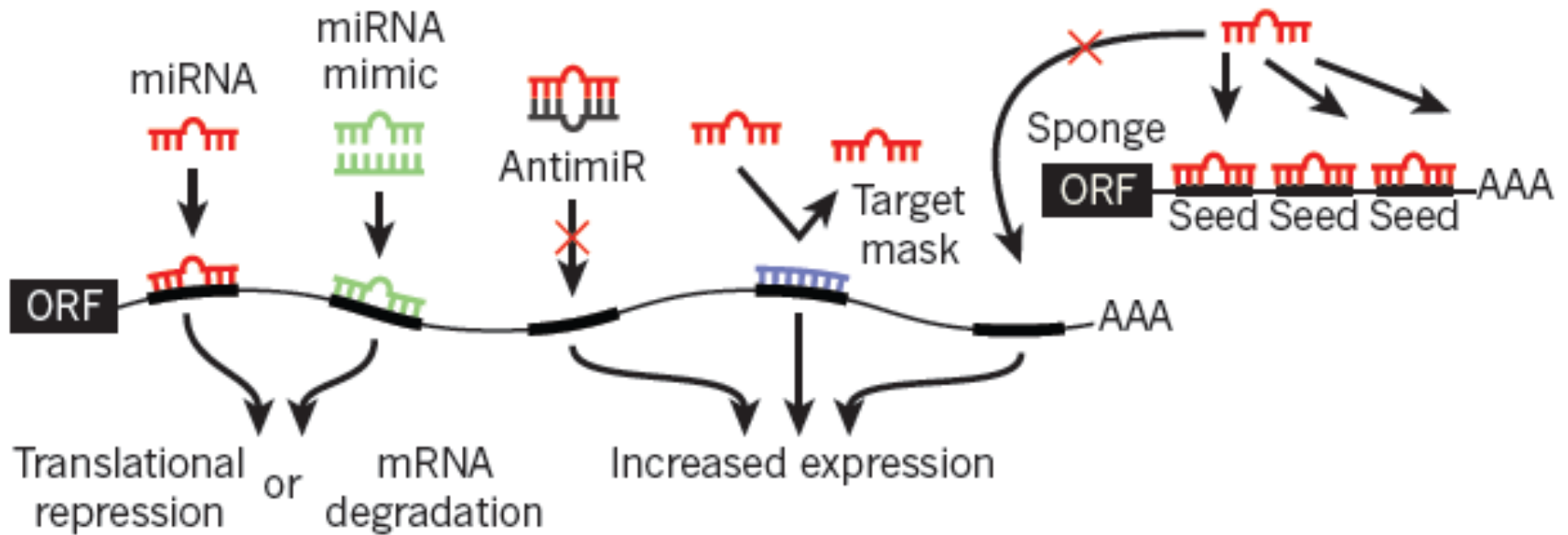


Model of c-miRs as Intercellular Messengers



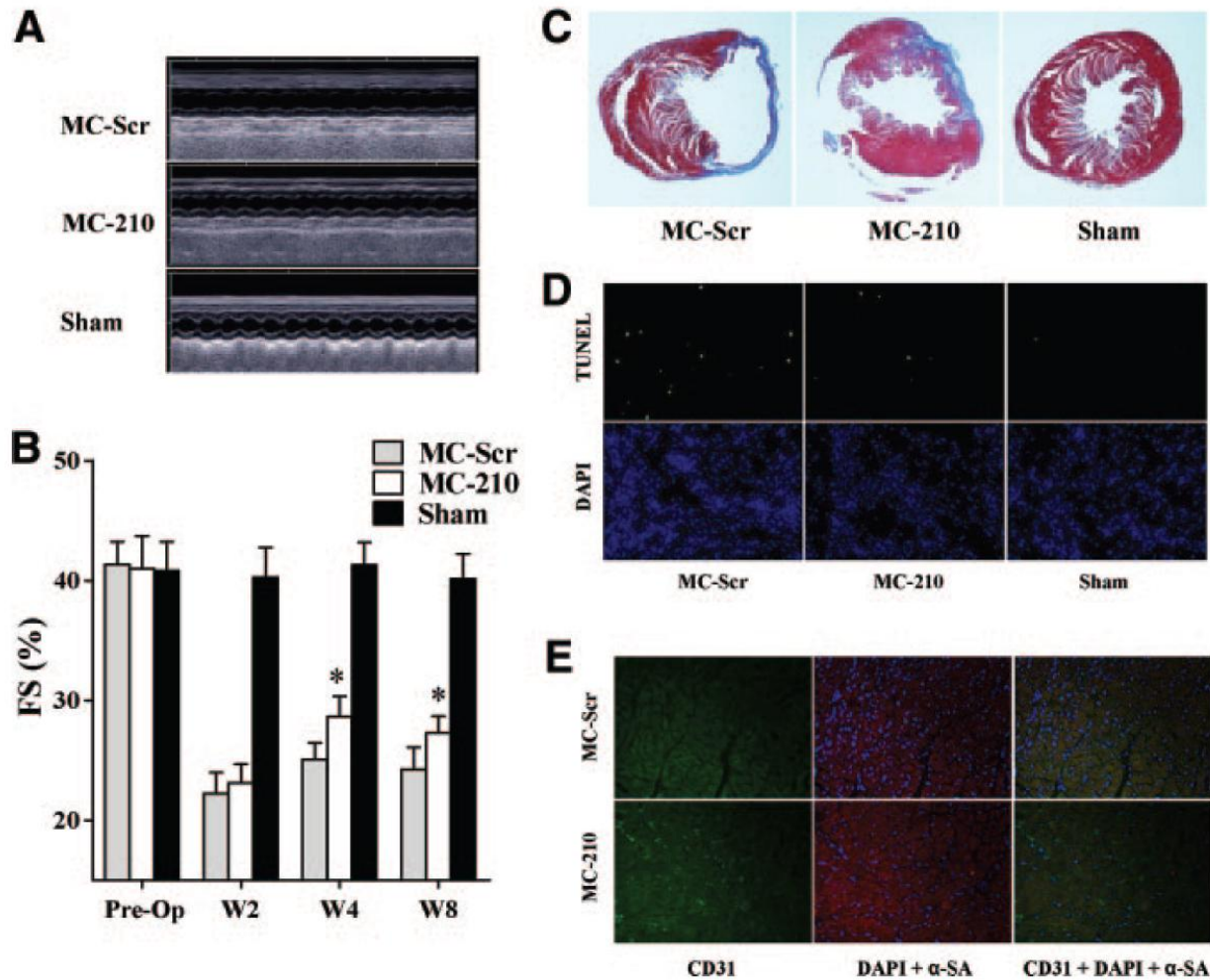
Future Therapeutic Perspectives

Oligonucleotide manipulation of miRNA function



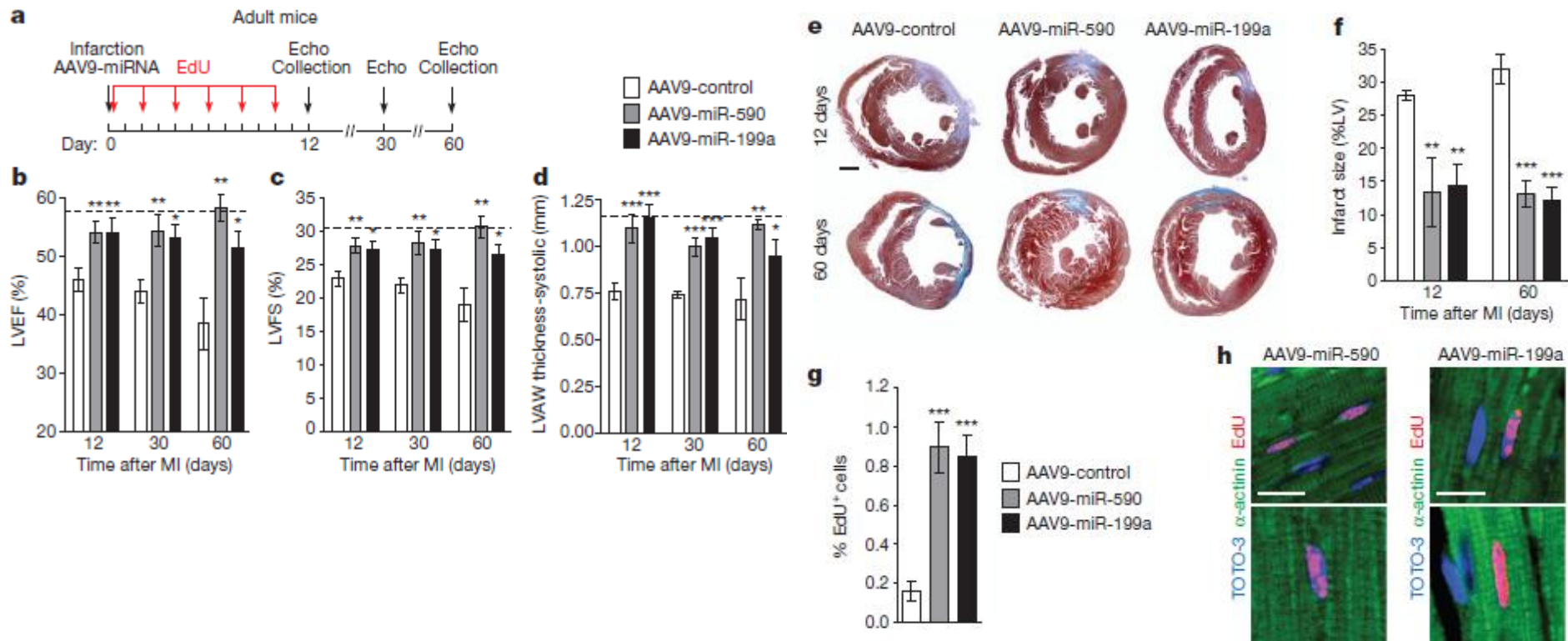
miR-210 as a novel Tx for IHD

Intramyocardial injection of minicircle DNA plasmid in murine MI model

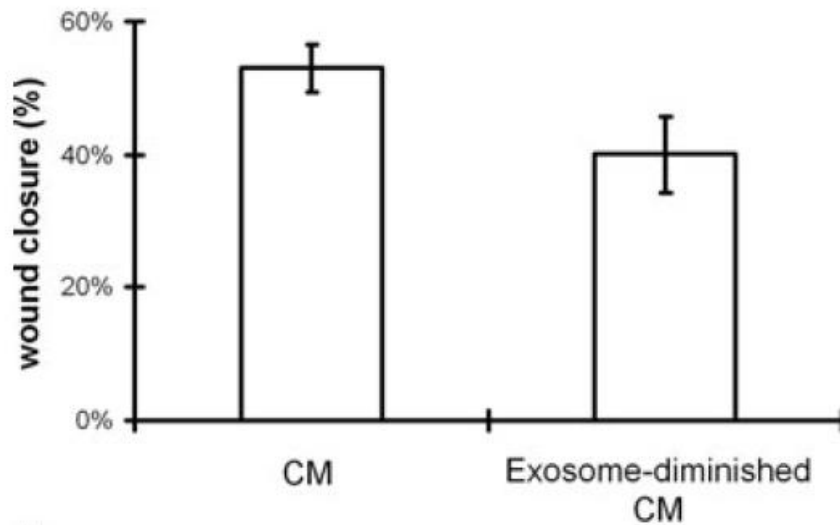


miR-210 as a novel Tx for IHD

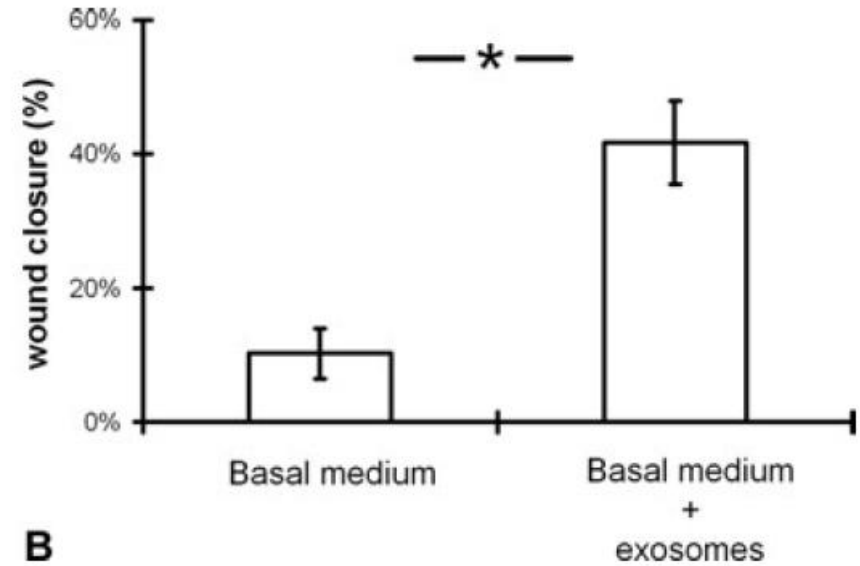
Intracardiac injection using adenoviral vectors in murine MI model



Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells



A



B

Challenges

- **Mode of delivery ?**
 - **Engineered exosomes; small size, stability, ability to cross membranes, modification of exosomal surface for effective targeting**
- **Purification method**
- **Determination of half-life and clearance**
- **Specificity of delivery**

Conclusions

- **Increasing attention to the role of c-miRNAs in CV disease**
- **C-miRNAs could play important roles in the prediction, diagnosis, and tailored treatment of CV disease in the near future**
- **It is hoped that a better understanding of c-miRNAs from packaging and release to uptake will be forthcoming**

A scenic sunset over a large body of water. The sky transitions from a deep blue at the top to a bright orange near the horizon. In the foreground, dark silhouettes of pine branches frame the top and left sides. A single bird is captured in flight against the orange sky. The water in the middle ground shows gentle ripples. The overall mood is peaceful and serene.

Thank you for your attention !