

The Damaging Nature of Extracellular RNA in Atherosclerosis and Cardiovascular Disease

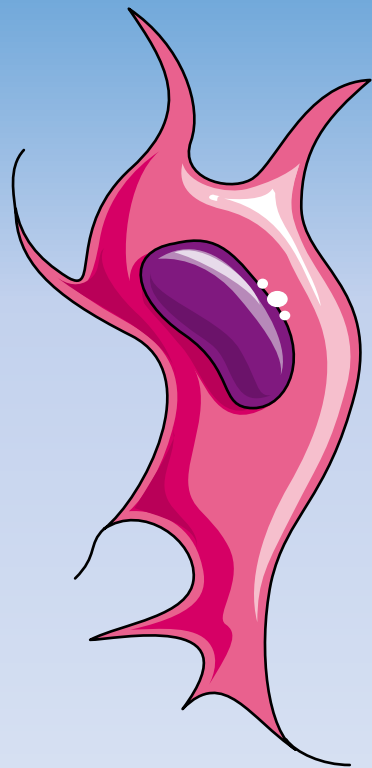


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Mechanisms Protecting Extracellular RNA (eRNA) From Degradation



Release of eRNA

Passive release
(cell death, necrosis)



Apoptotic bodies



Microparticles
Exosomes



Protein complexes



Lipoproteins (HDL)

Targeting eRNA

Type of eRNA

rRNA, mRNA, miRNA

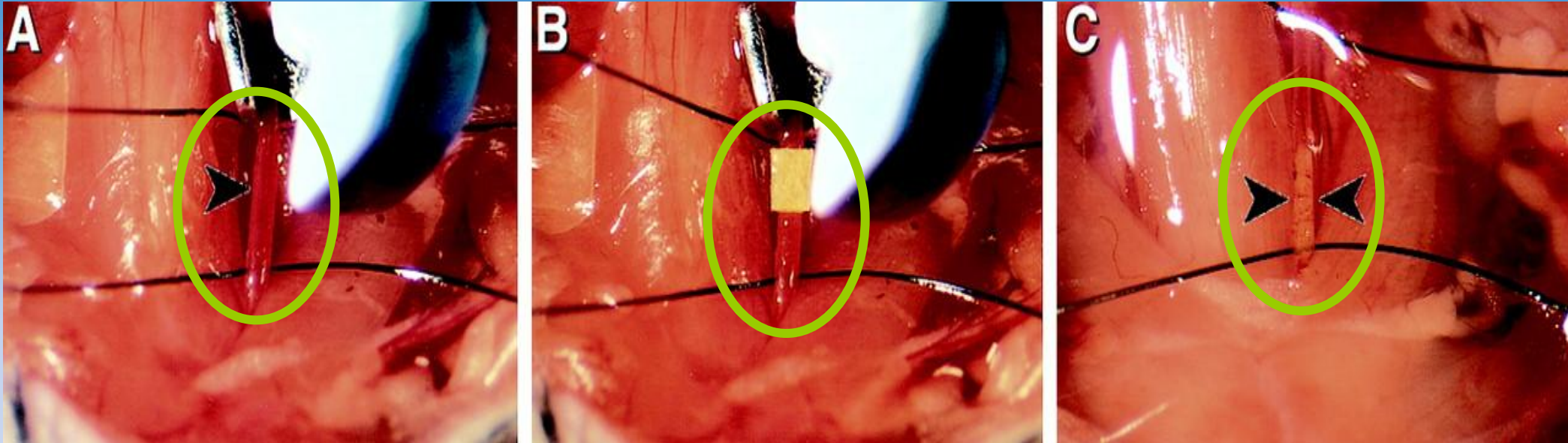
miRNA

rRNA, mRNA, miRNA

rRNA, miRNA

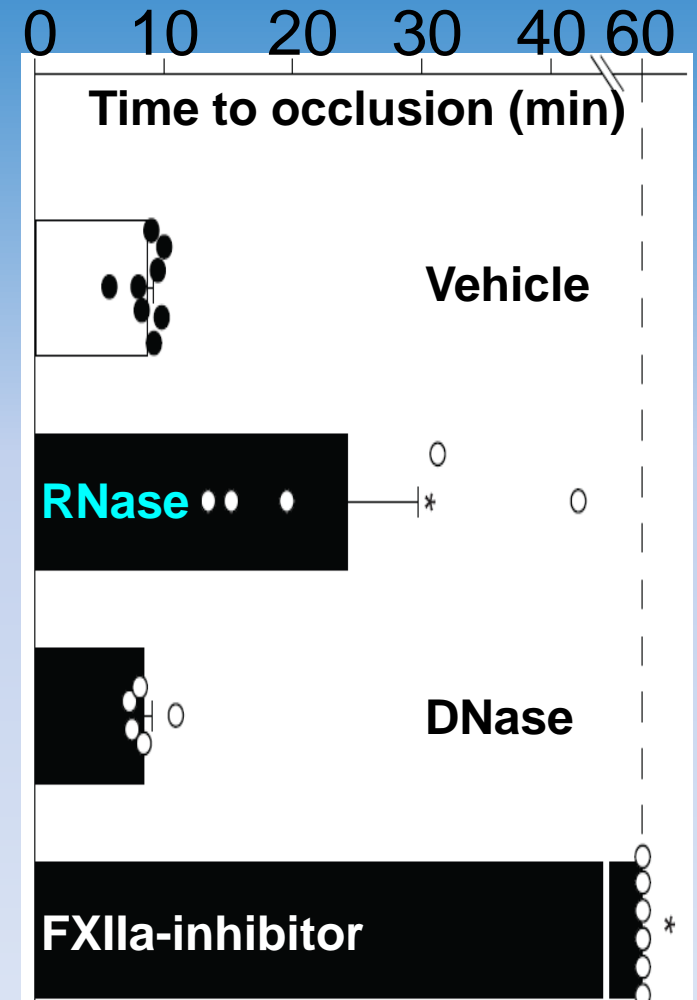
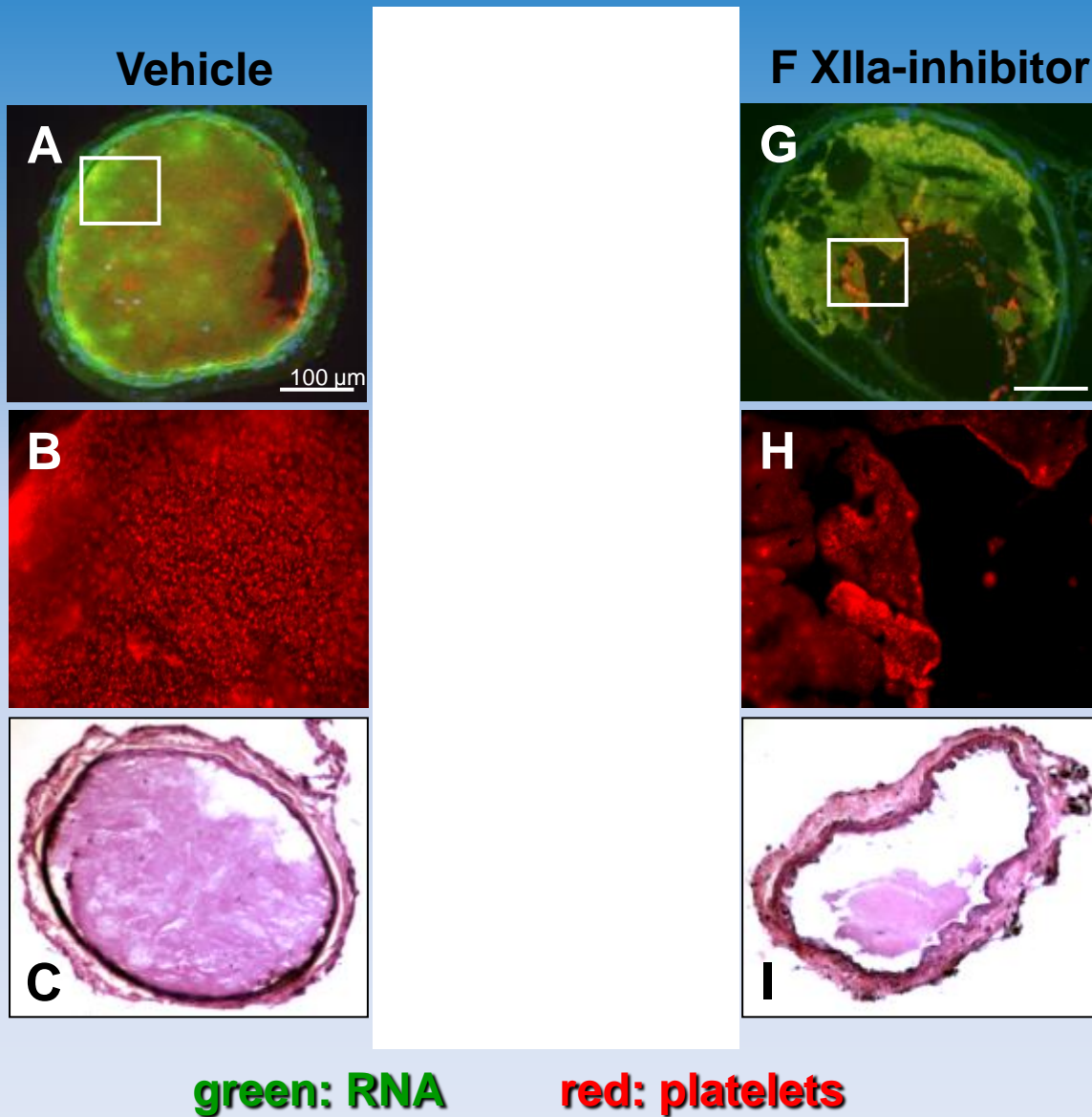
rRNA, miRNA

Carotid Artery Thrombosis Mouse Model Induced by FeCl₃



- A:** Preparation of left carotid artery, installation of ultrasound flow probe.
- B:** Application of filter paper soaked in 10% FeCl₃ for 3 min.
- C:** After about 8-9 min: formation of a platelet-rich thrombus

Procoagulant Activity of Extracellular RNA *in vivo*



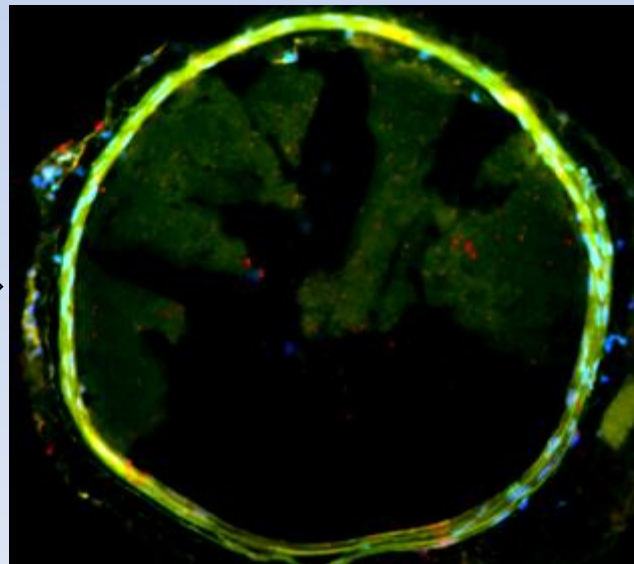
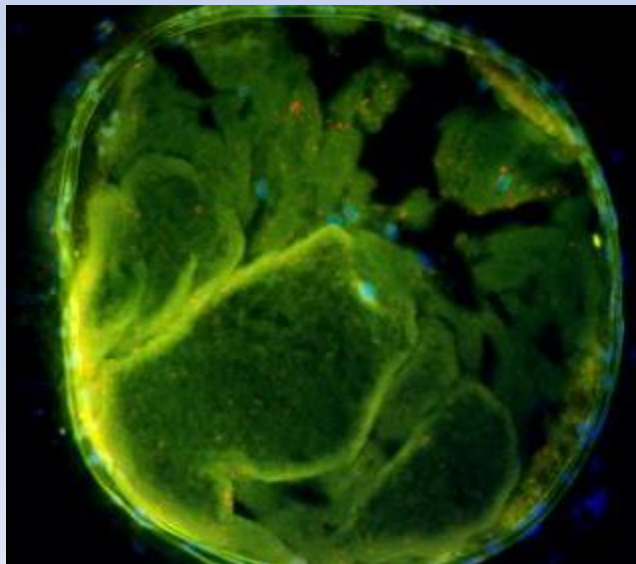
Kannemeier et al., PNAS 2007

Turning Insight-Out: Extracellular RNA Between Defense and Disease

- **Extracellular RNA:**

- Blood coagulation, thrombosis
- Vascular permeability, oedema formation
- Inflammation, leukocyte trafficking
- Tumor progression and metastasis

(Kannemeier et al., PNAS 2007; Fischer et al., Blood 2007; FASEB J 2009; Thromb Haemost 2012; Cancer Res 2013; Jaax et al., Blood 2013)



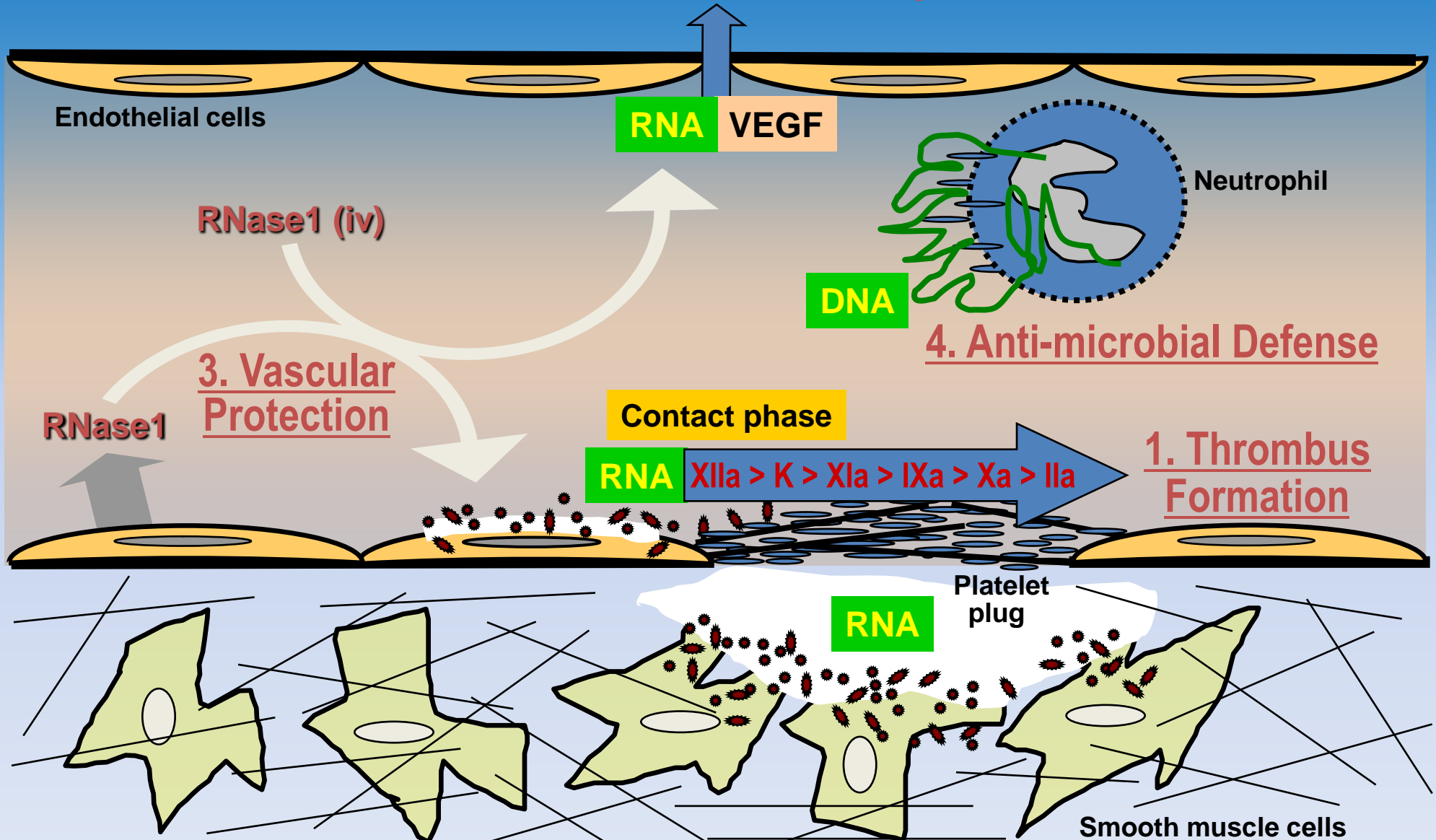
Kannemeier et al., PNAS 2007

Proposed mechanism

exRNA is a natural **auto-activation factor** for proteases of the **CONTACTPHASE** of blood coagulation.

Multiple Functions of Extracellular Nucleic Acids

2. Vascular Permeability

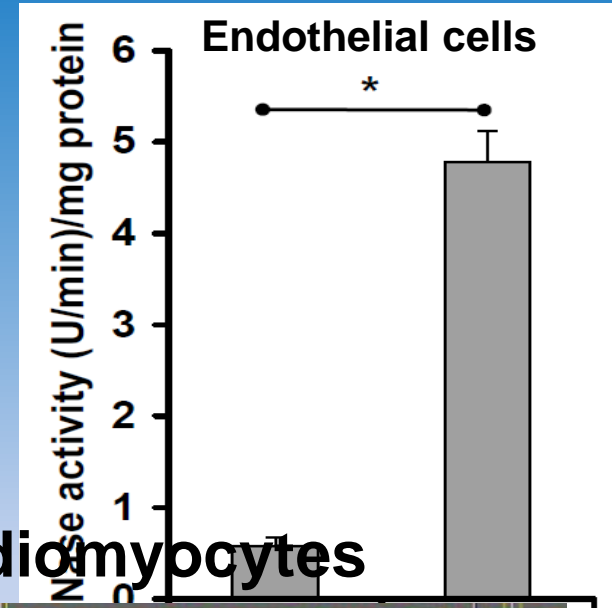
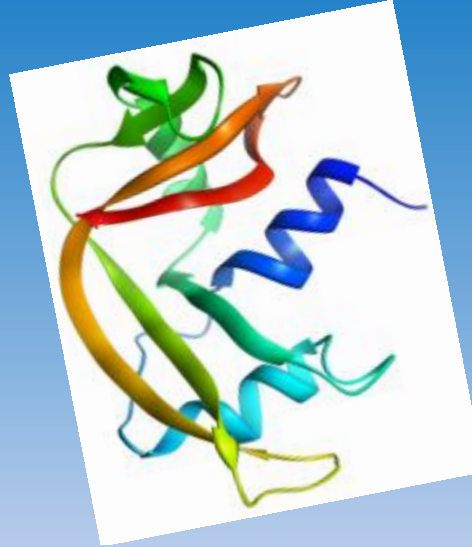


Fischer et al., Blood 2007; Kannemeier et al., PNAS 2007; Shibamiya et al., Blood 2009;
Fischer et al., Thromb. Haemost. 2011 & 2012; Can Res 2013; Jaax et al., Blood 2013

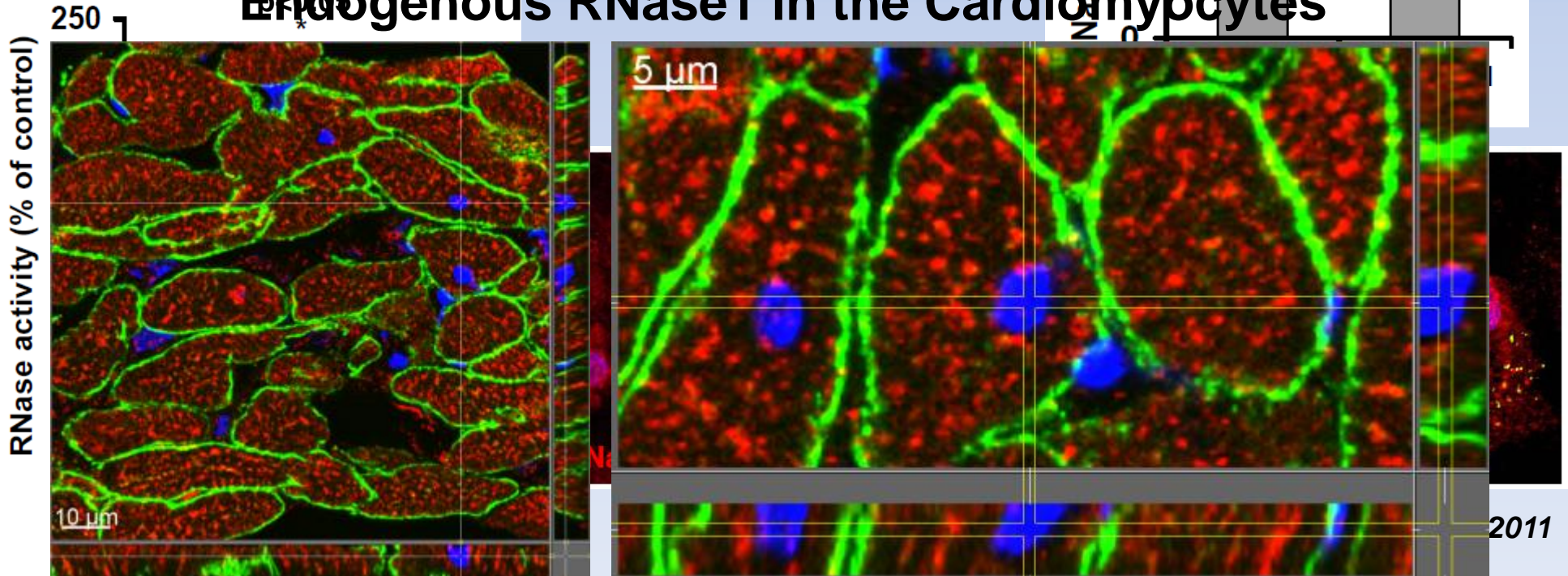
Expression and Distribution of RNase1

Extracellular RNase1

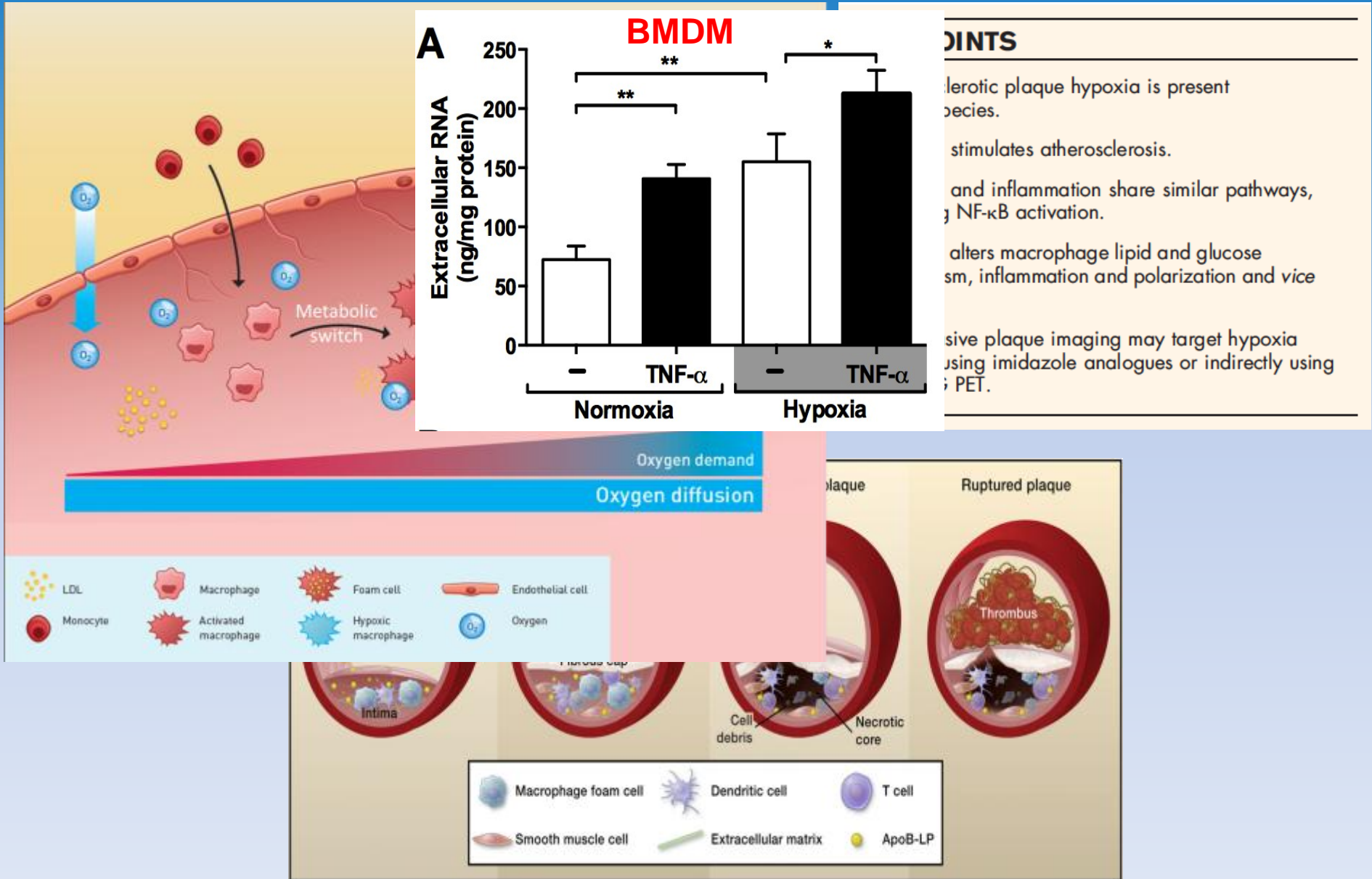
- Member of the RNaseA-family
- Thermostable enzyme (17 kDa)
- Major pancreatic ribonuclease
- Non-toxic factor for host cells
- Major vascular RNase



Endogenous RNase1 in the Cardiomyocytes



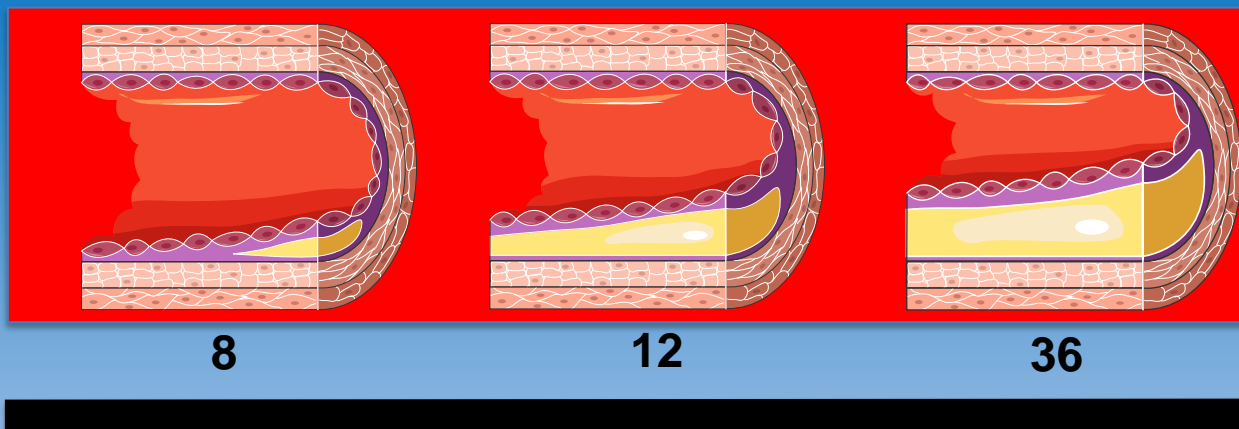
Systemic Hypoxia Promotes Atherosclerosis



POINTS

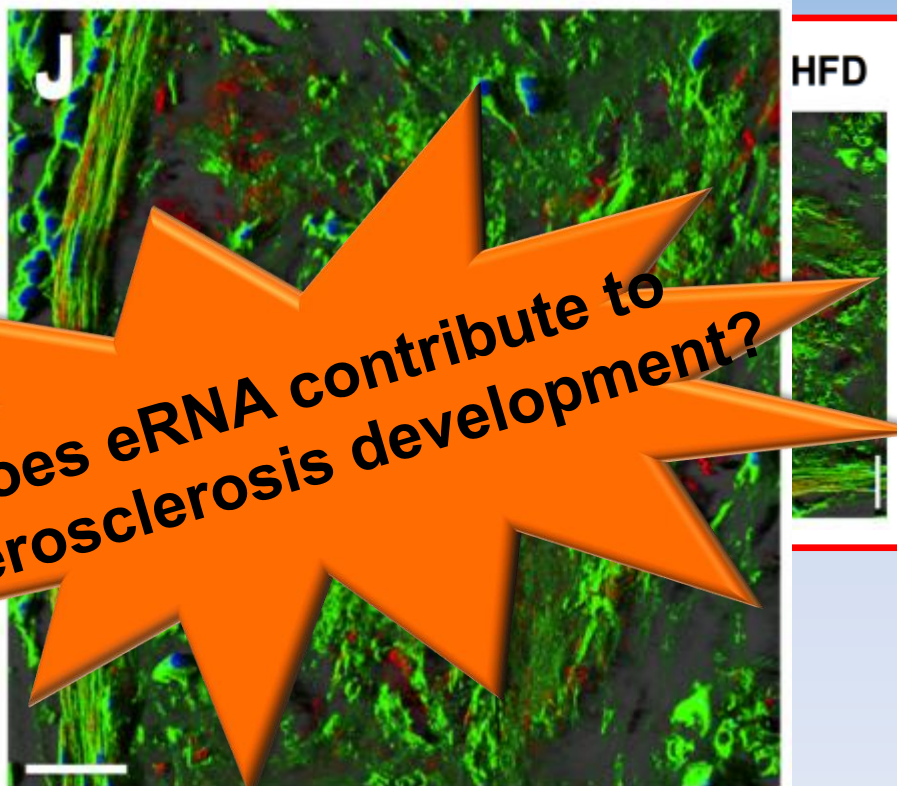
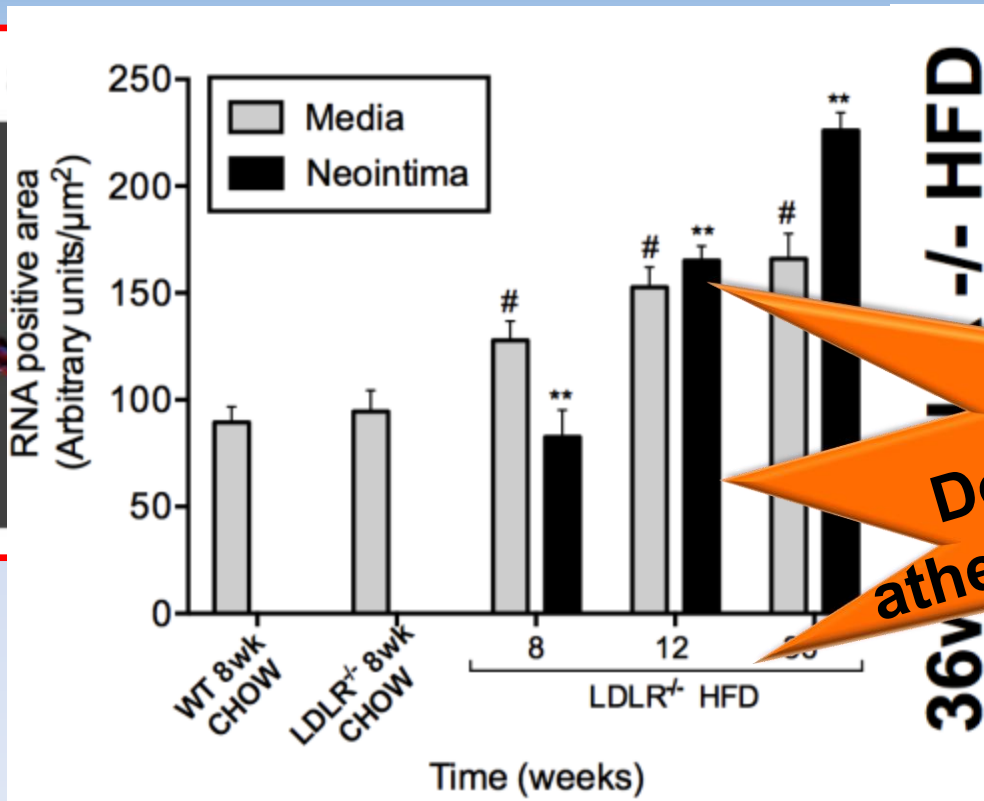
- Atherosclerotic plaque hypoxia is present in all species.
- Hypoxia stimulates atherosclerosis.
- Hypoxia and inflammation share similar pathways, including NF- κ B activation.
- Hypoxia alters macrophage lipid and glucose metabolism, inflammation and polarization and vice versa.
- Advanced plaque imaging may target hypoxia using imidazole analogues or indirectly using PET.

Appearance of Extracellular RNA in Atherosclerotic Lesions



High Fat Atherogenic Diet

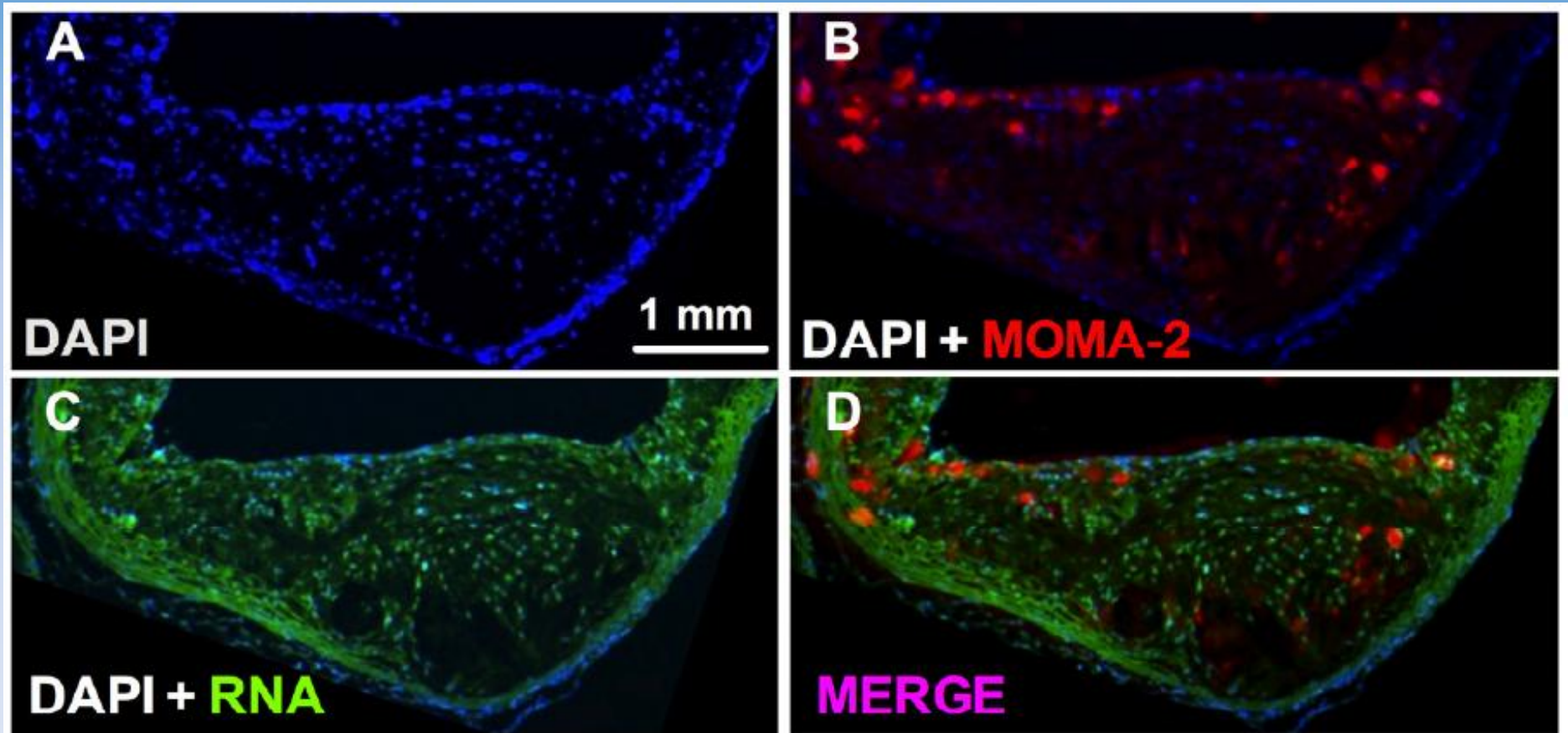
Weeks on diet



Does eRNA contribute to atherosclerosis development?

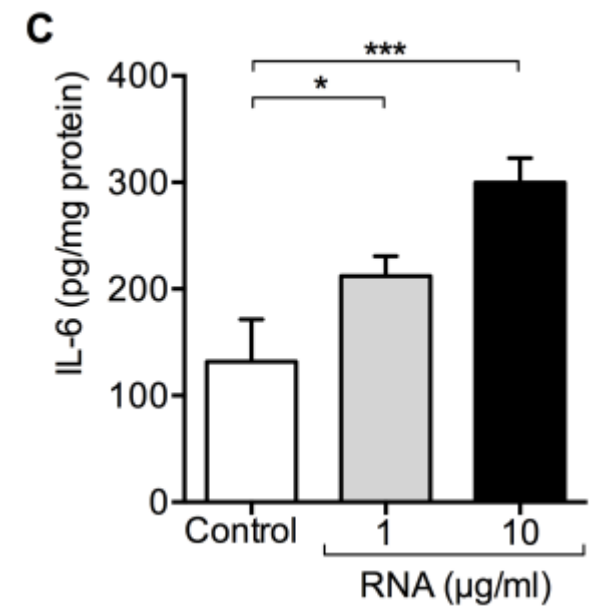
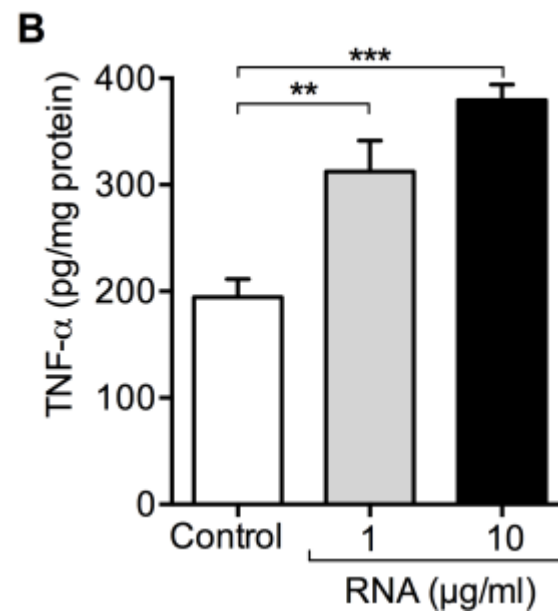
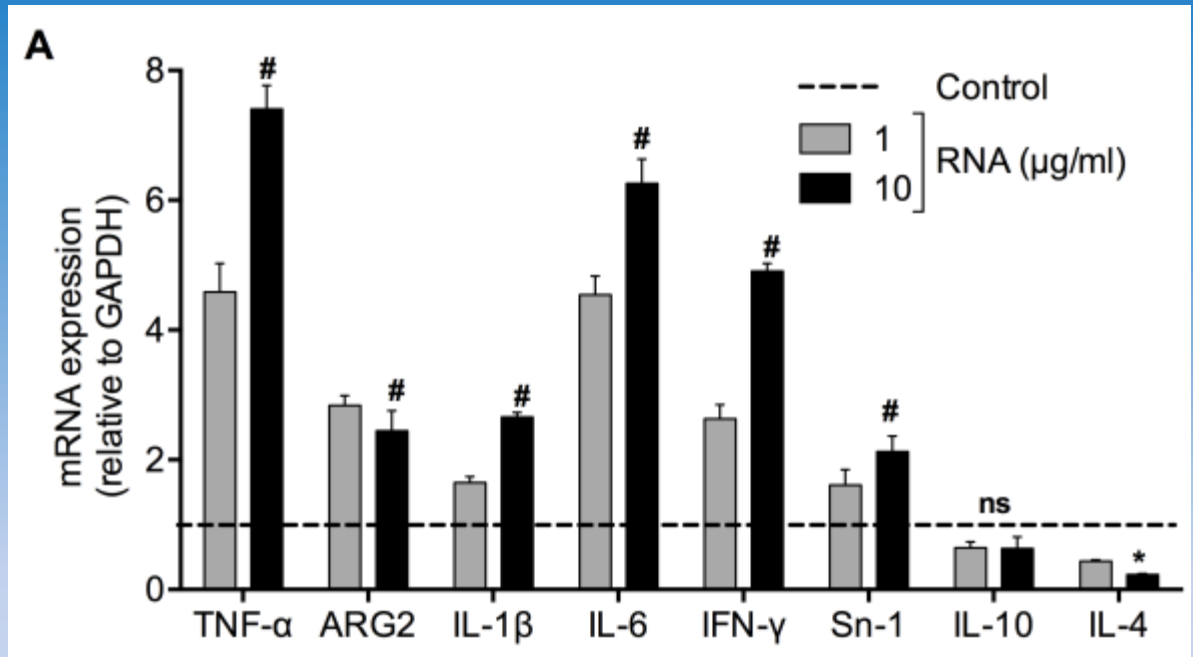
Colocalization of Extracellular RNA and Macrophages in Atherosclerotic Lesions of LDL-R^{-/-} Mice (36 Weeks HFD)

Atherosclerosis-prone LDLR^{-/-} mice: High-fat-diet (William Boisvert, Hawaii)



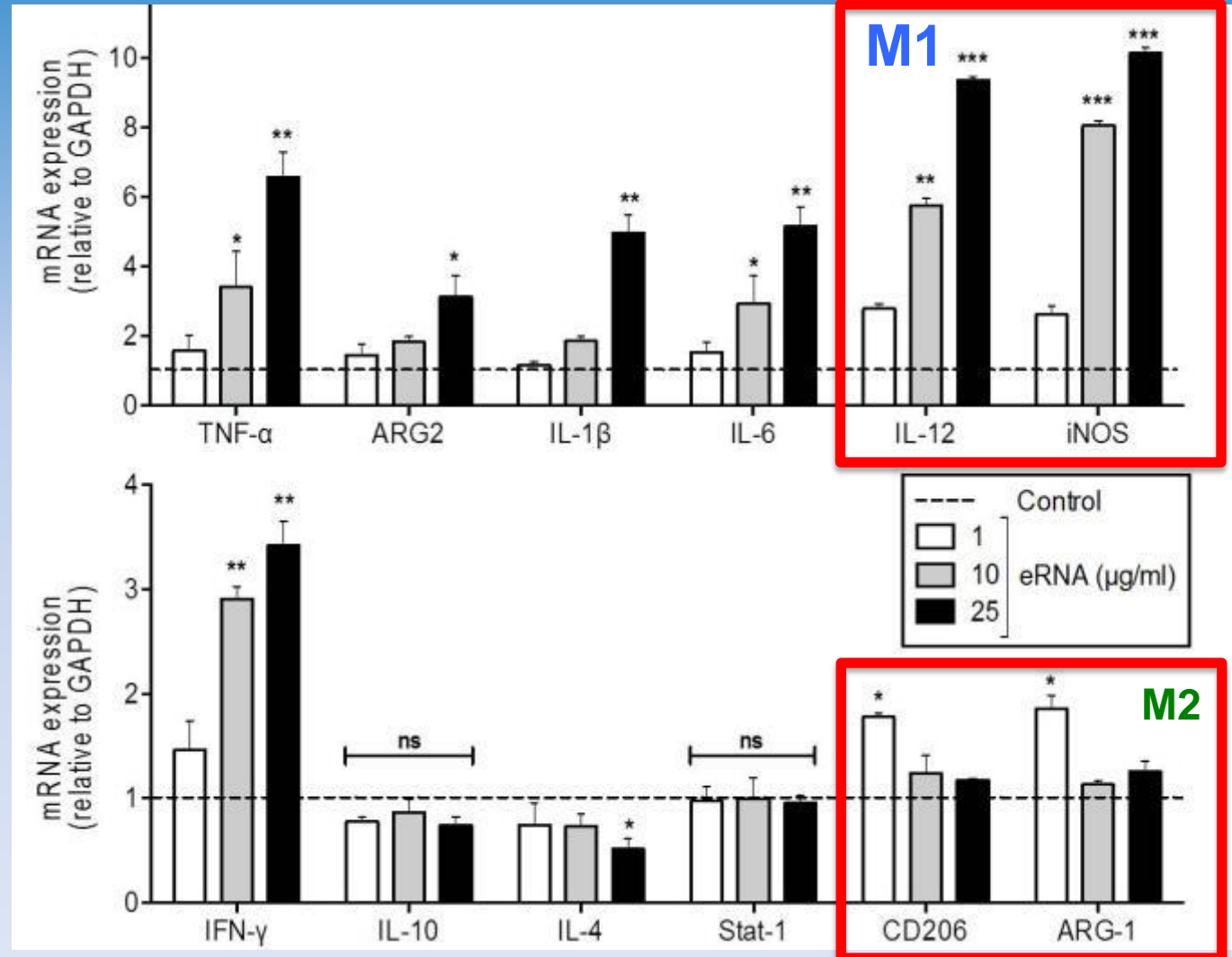
Extracellular RNA-mediated Cytokine Production in BMDM

WT Bone Marrow Derived Macrophages (BMDM)
Isolation and
24h Stimulation



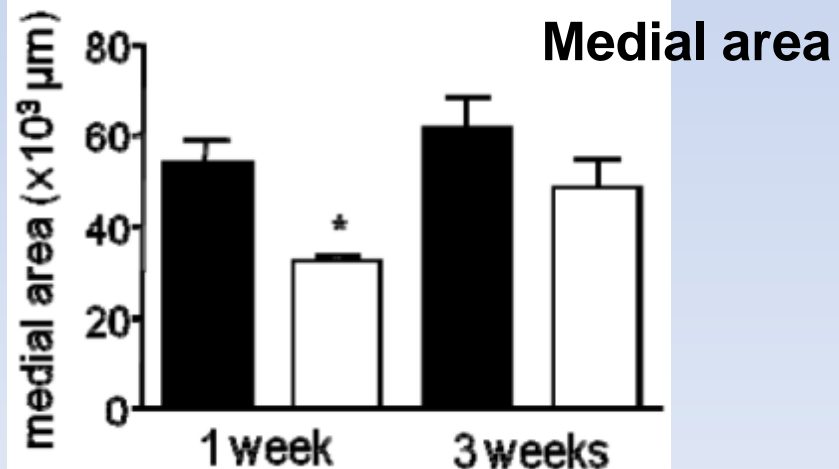
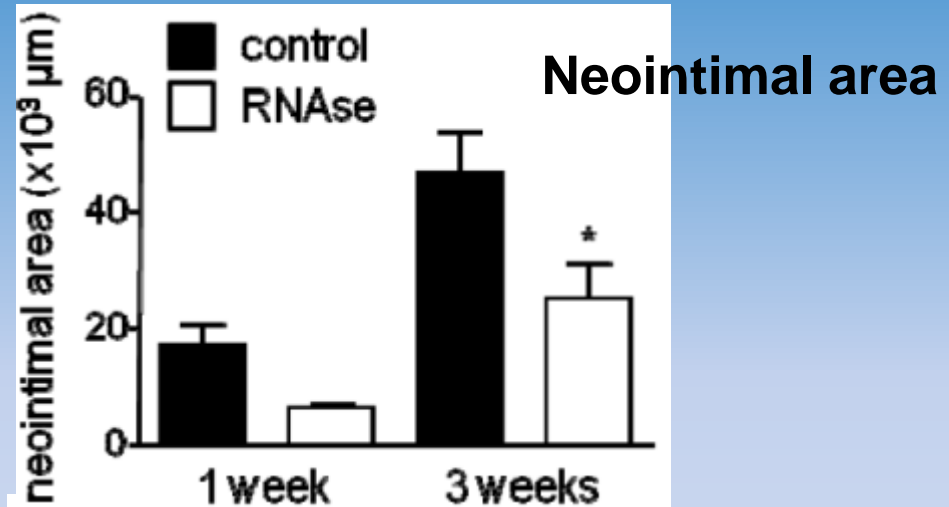
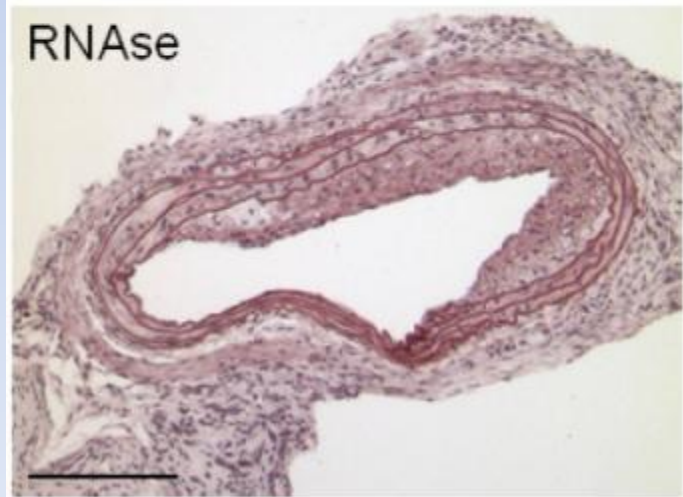
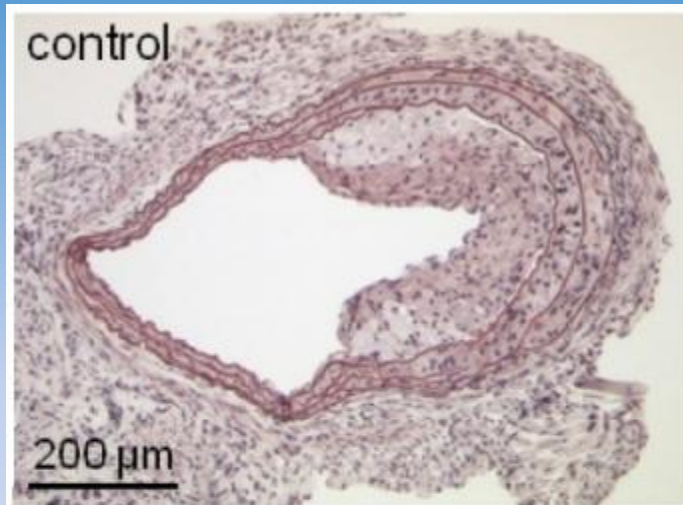
M-CSF - WT Bone Marrow
Derived Macrophages (BMDM)
Isolation and
24h Stimulation

Recombinant mouse **M-CSF**-driven BMDM-differentiation

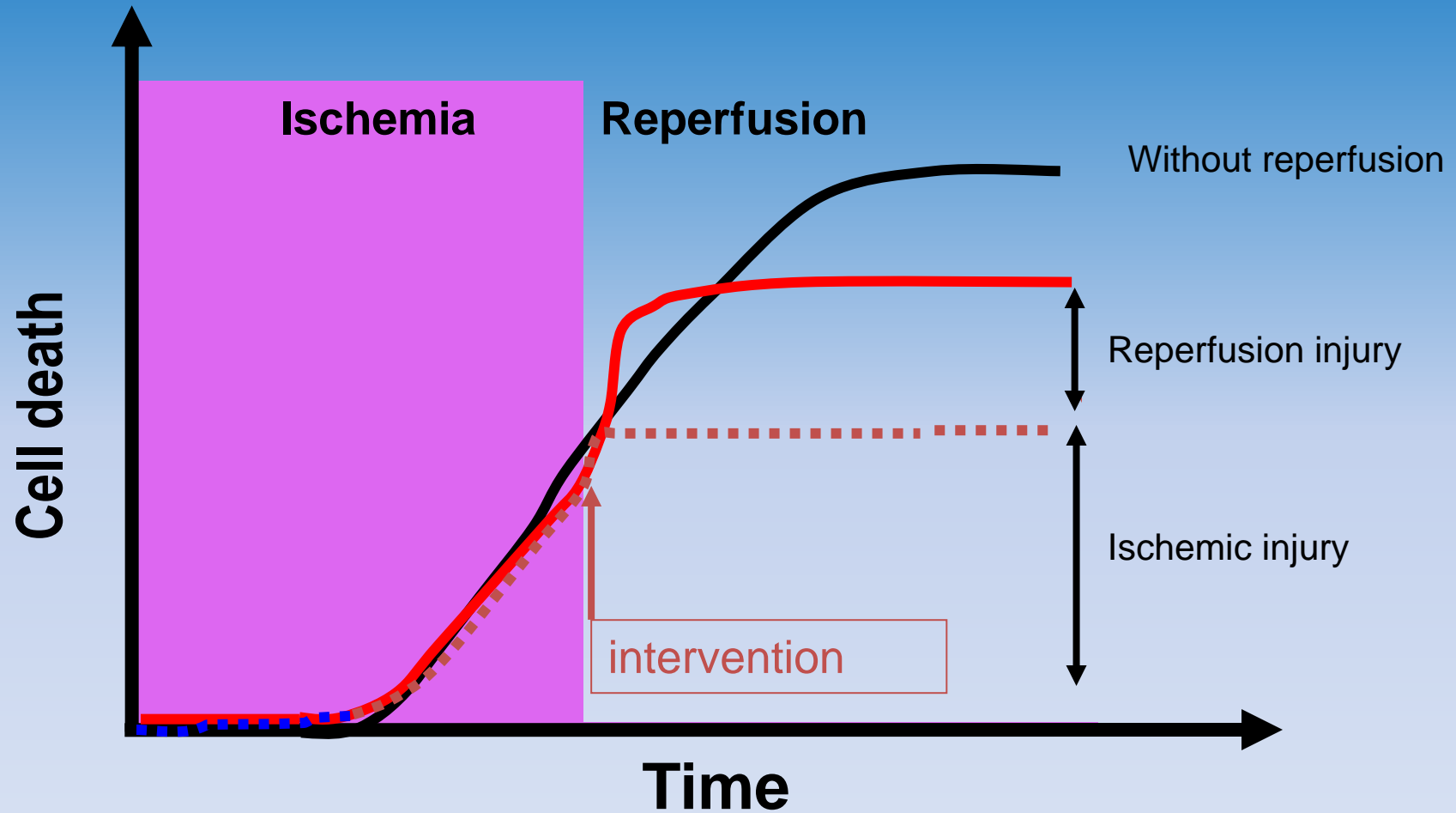


RNase1 Treatment of Atherosclerosis-Prone Apo-E^{-/-} Mice

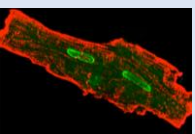
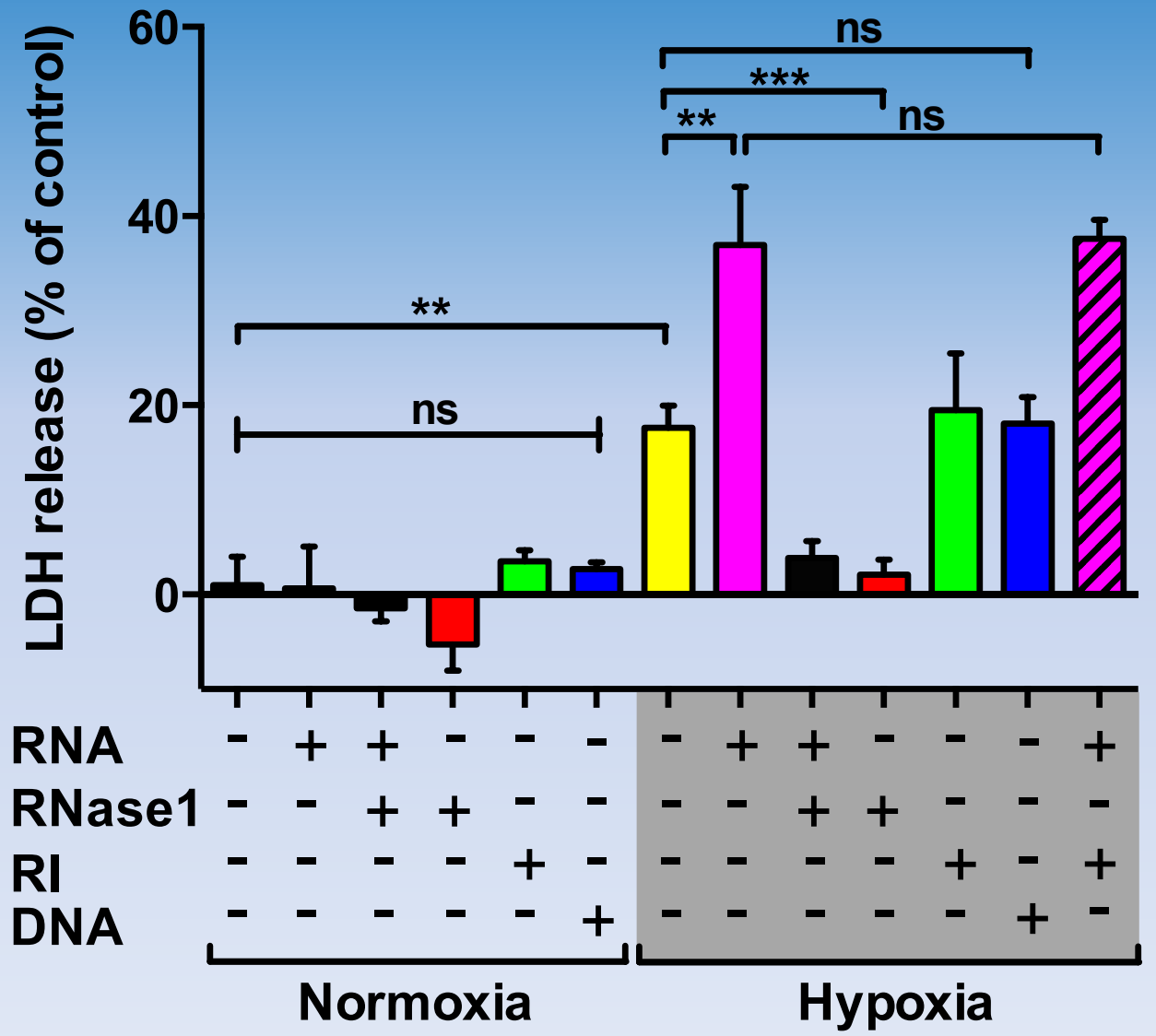
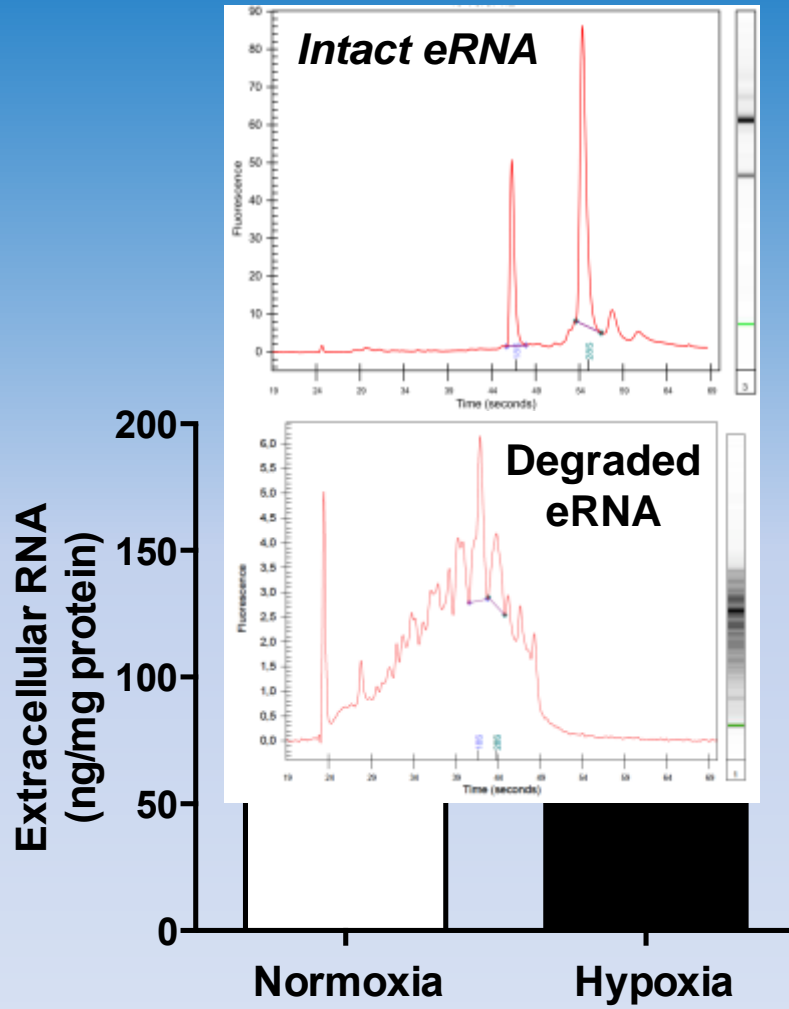
Atherosclerosis-prone apo-E^{-/-} mice: Wire-induced vessel injury (Alma Zerneck, Aachen/Würzburg)



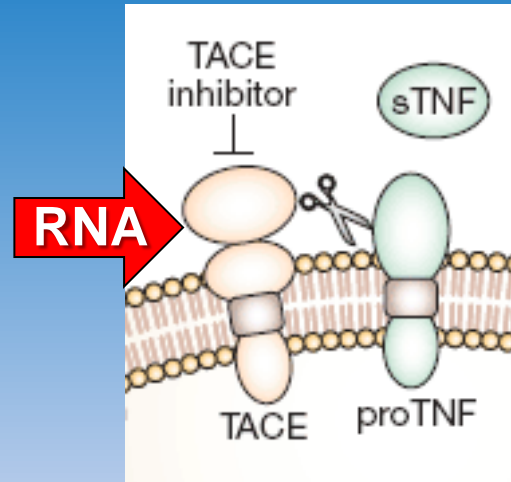
Ischemia/Reperfusion Injury



Extracellular RNA (but not DNA) Induces Cell Death Under Hypoxic Conditions

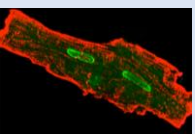
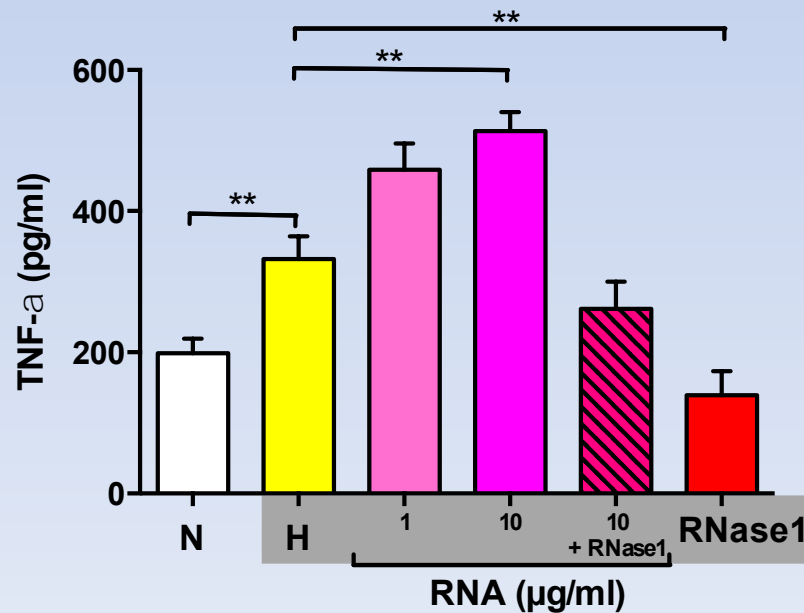


Extracellular RNA promotes TACE-induced cleavage of membrane-bound proTNF.

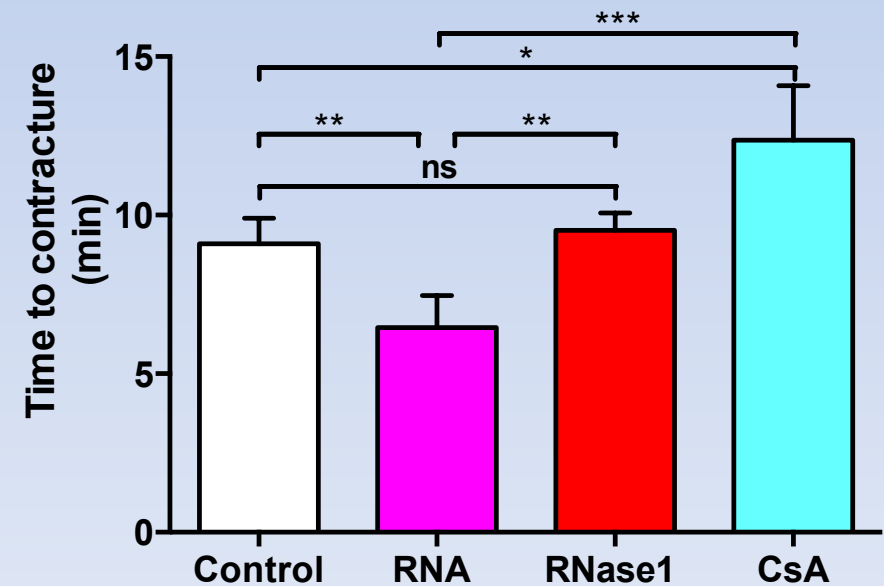
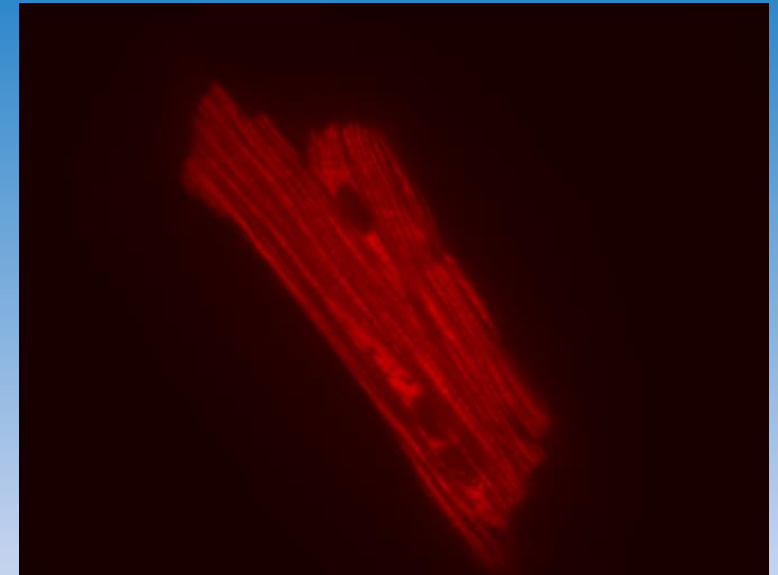
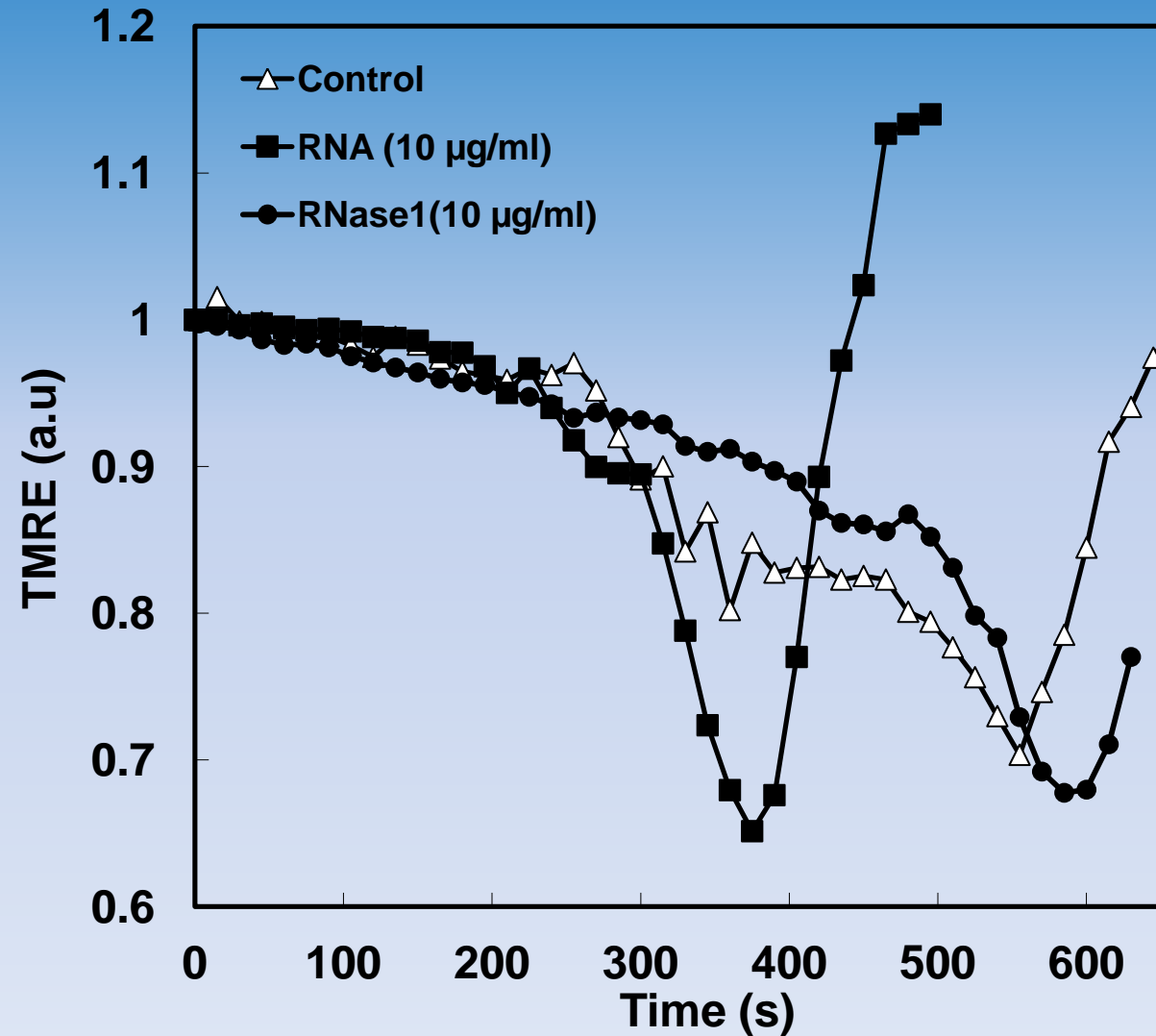


**TACE / ADAM 17
Tumor Necrosis Factor-
a Converting Enzyme**

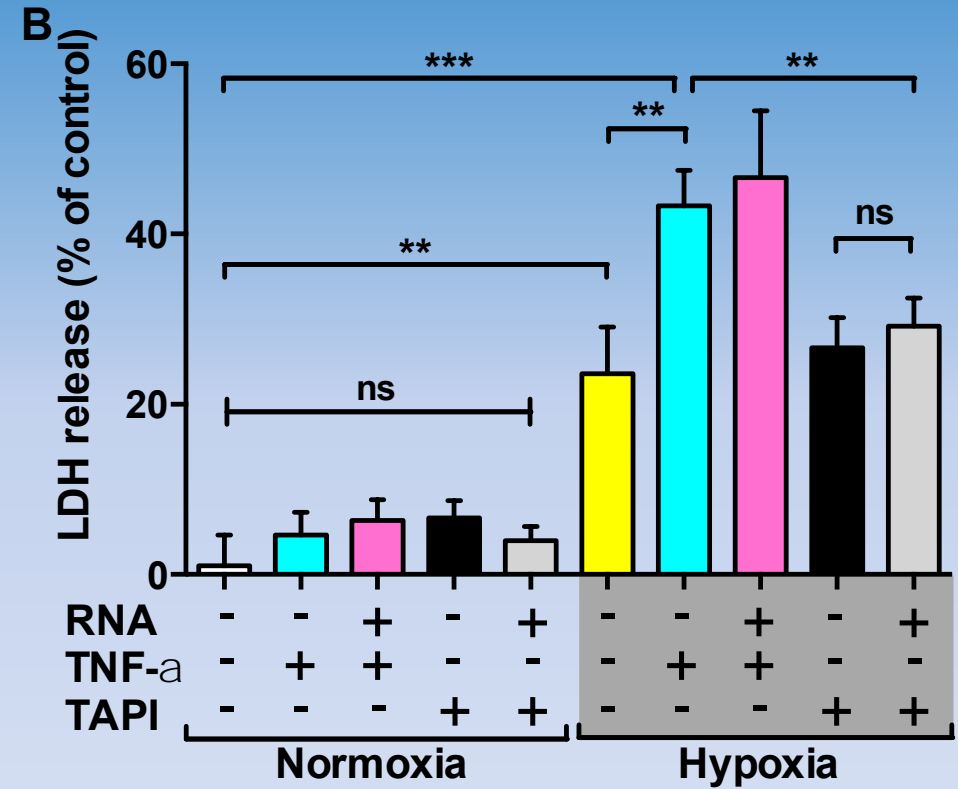
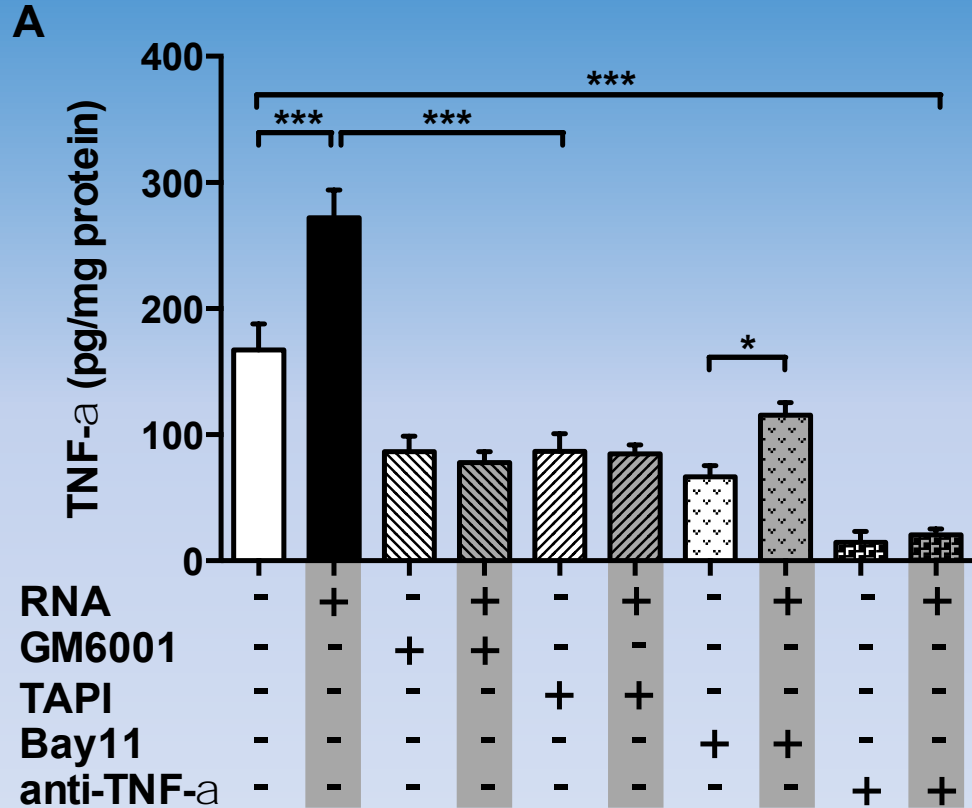
Fischer et al., Thromb Haemost 2012



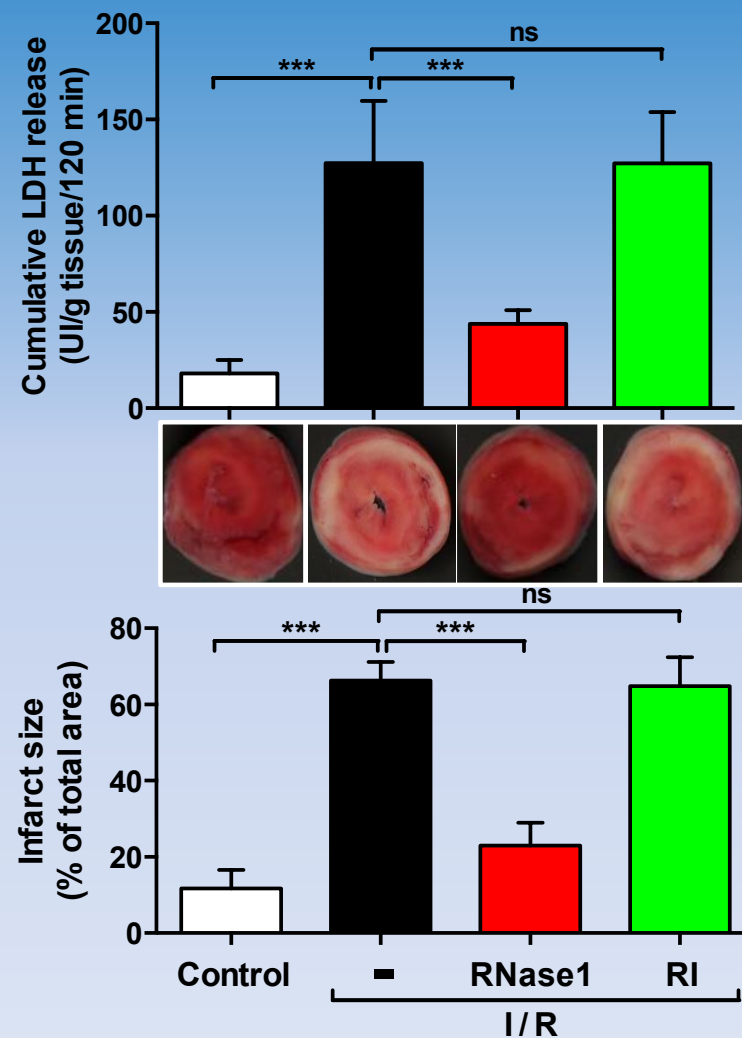
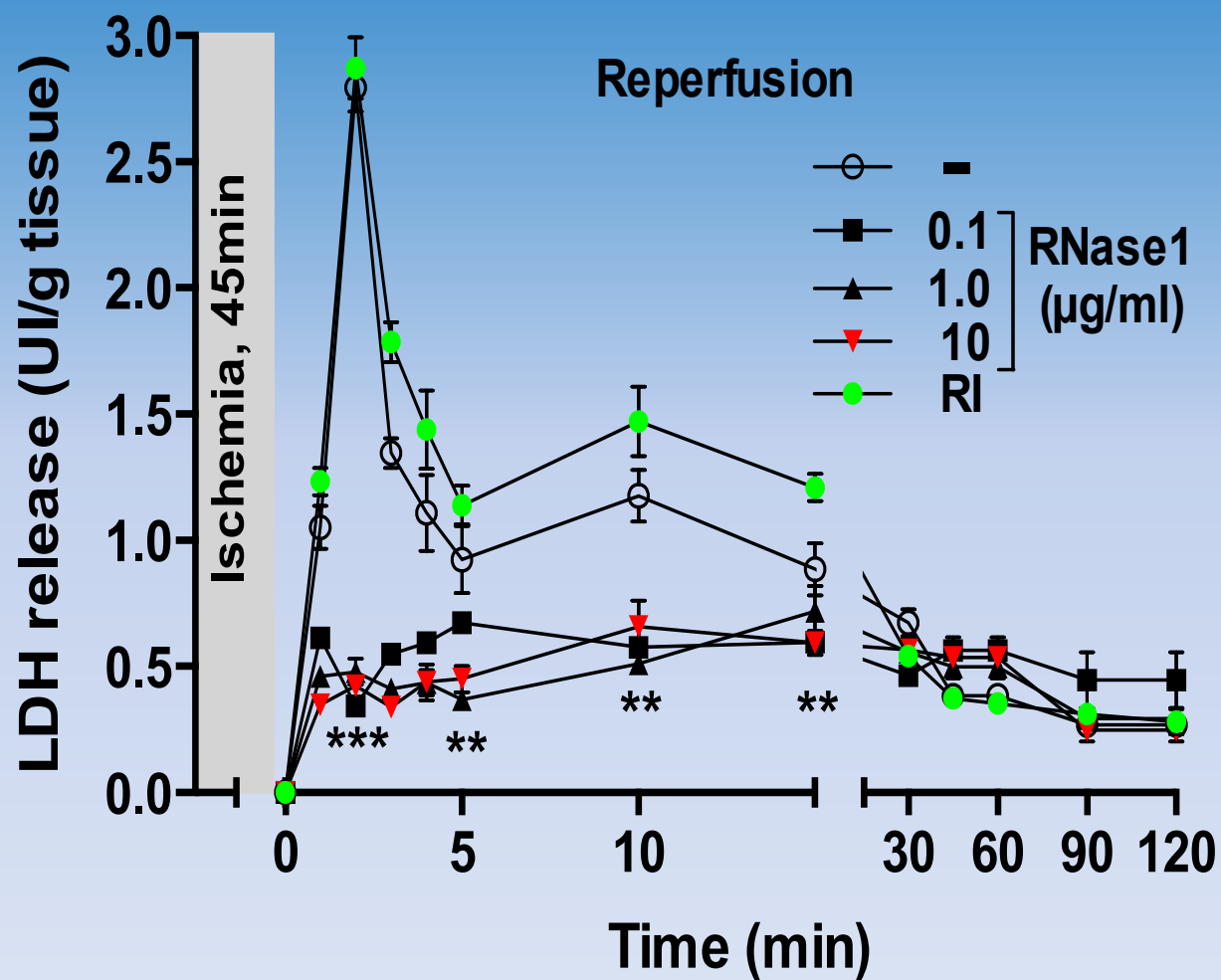
RNA Enhanced the Time to Cell Shortening as a Consequence of mPTP Opening



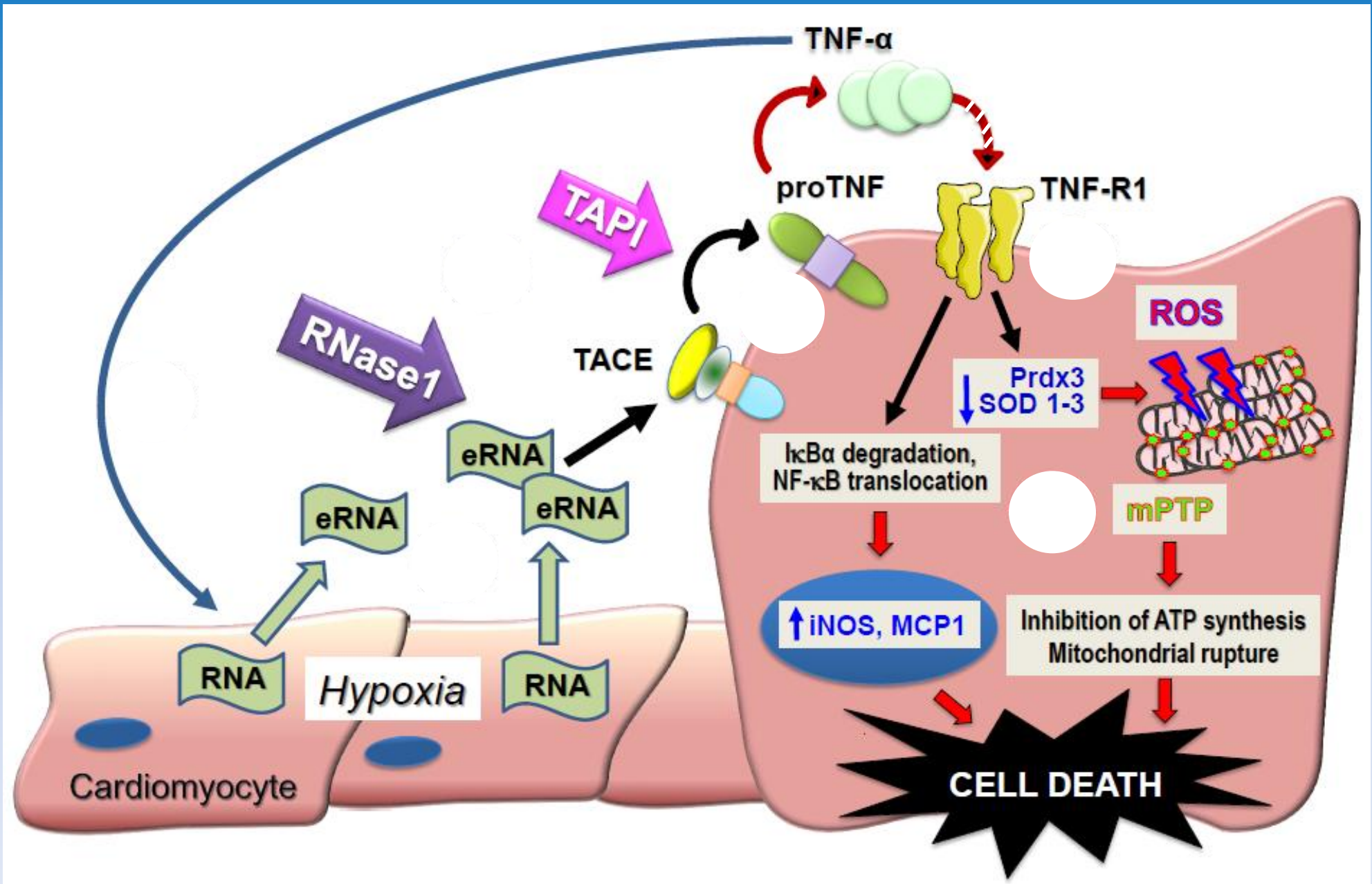
TNF- α Induces Cell Death Under Hypoxic Conditions that is Prevented by TAPI



RNase1 Improves Ventricular Recovery by Reducing Infarct Size

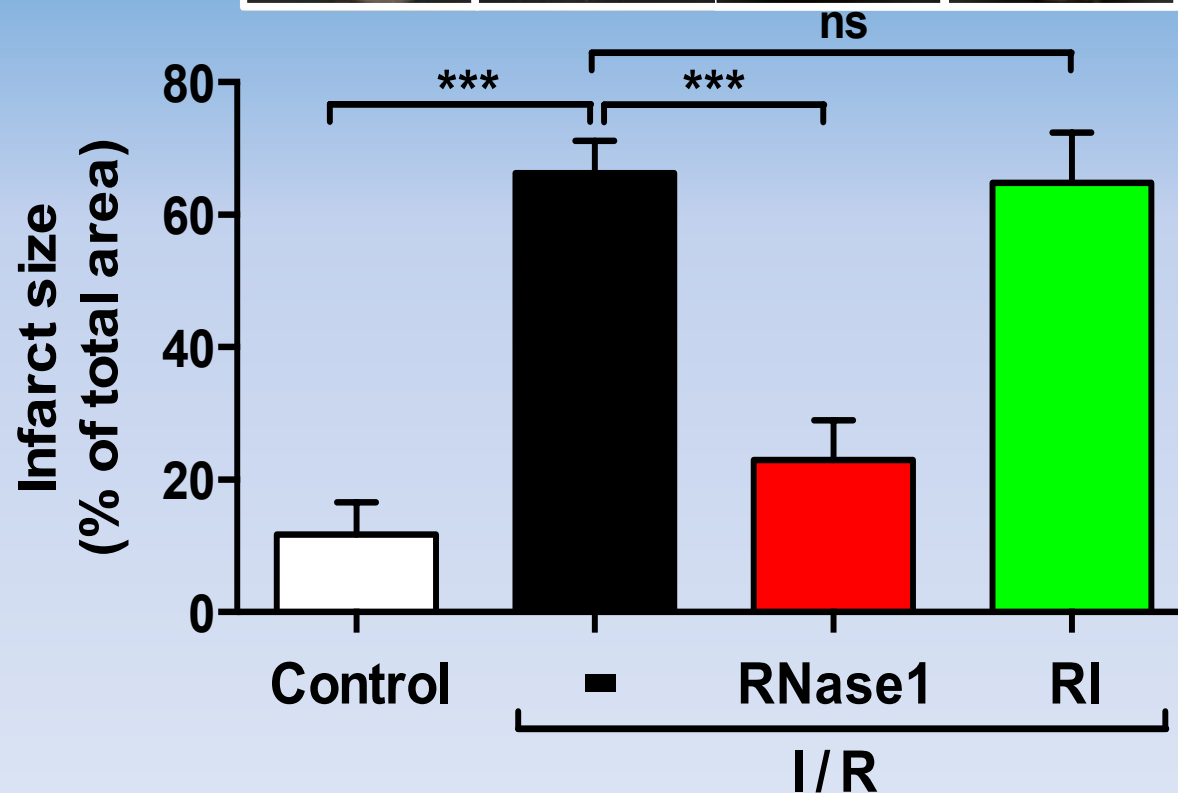
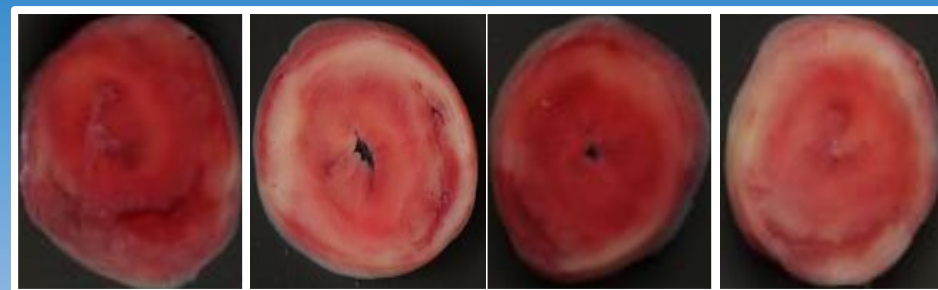
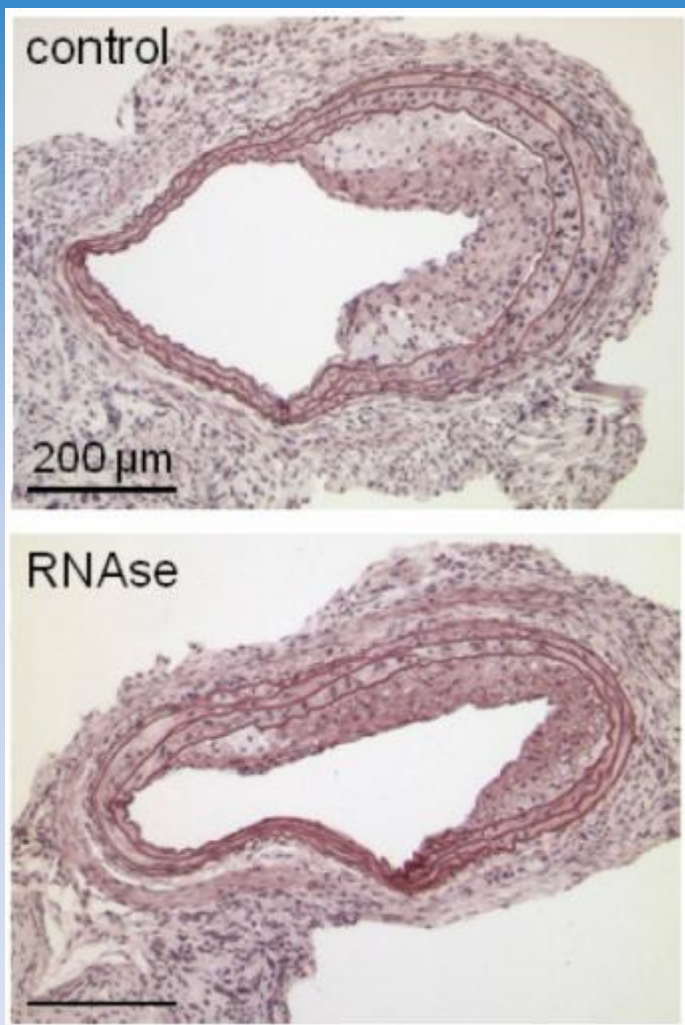


Extracellular RNA and Cardiovascular Disease





RNase1 *in vivo* Protects Against Neointimal Hyperplasia and Myocardial Infarction



RNase1 Treatment of Atherosclerosis-Prone *Apo-E*^{-/-} Mice (Wire Injury)

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