A microscopic image of tissue, likely a blood vessel, showing various cellular structures and colors (red, green, blue, yellow). A central white box contains the title text.

Bring out your dead: Cell Death and its Consequences in Inflammatory Vascular Disease

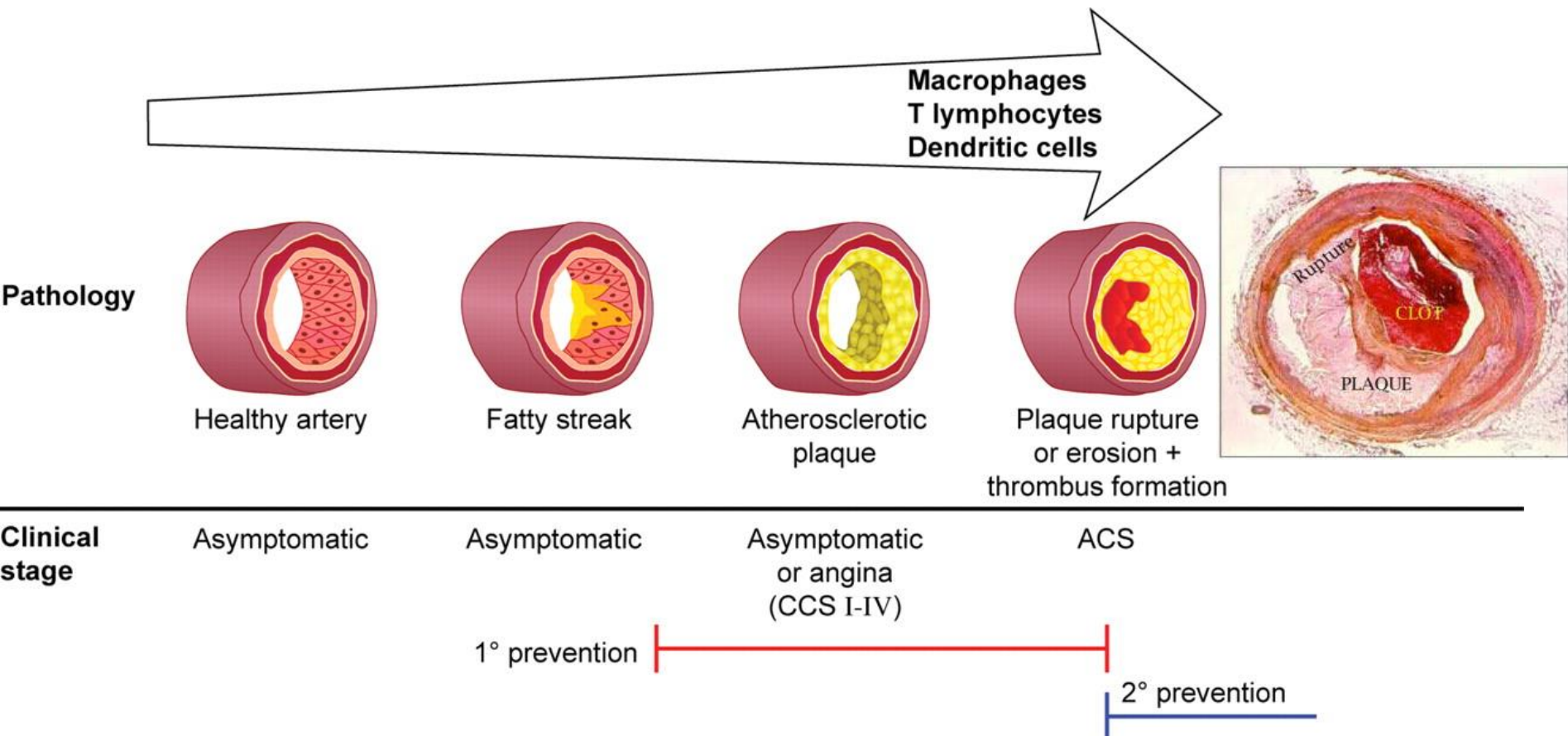
Katey Rayner

Assistant Professor,
Faculty of Medicine
uOttawa



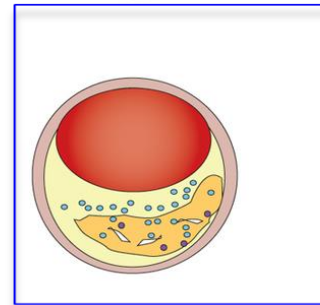
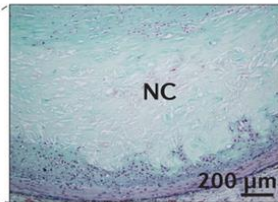
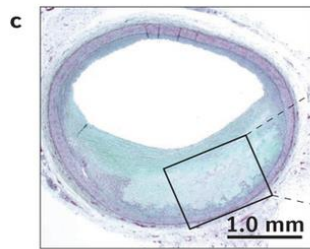
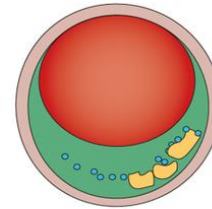
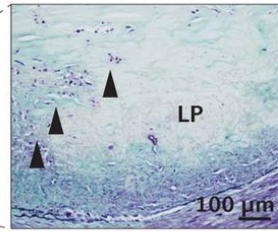
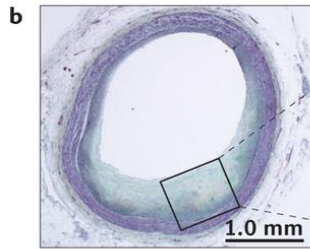
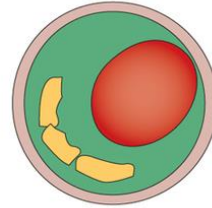
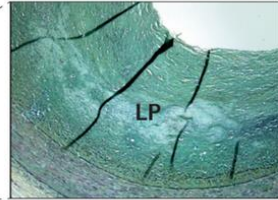
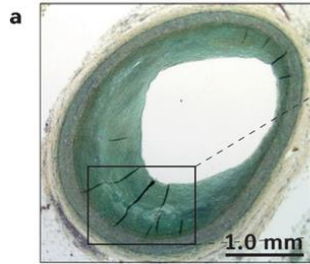
UNIVERSITY OF OTTAWA
HEART INSTITUTE
INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

The vulnerable plaque

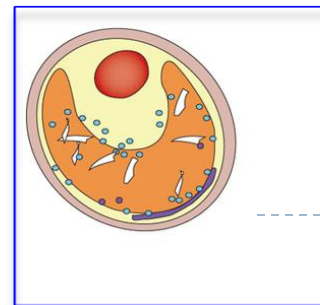
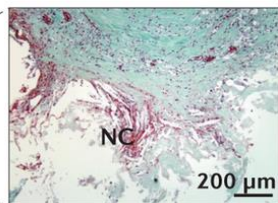
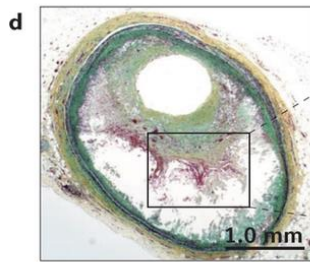


Not all plaques are created equal

- Artery wall
- Lumen
- Smooth muscle cells
- Macrophage foam cells
- Extracellular lipid
- Collagen
- Necrotic core
- Cholesterol clefts
- Calcified plaque

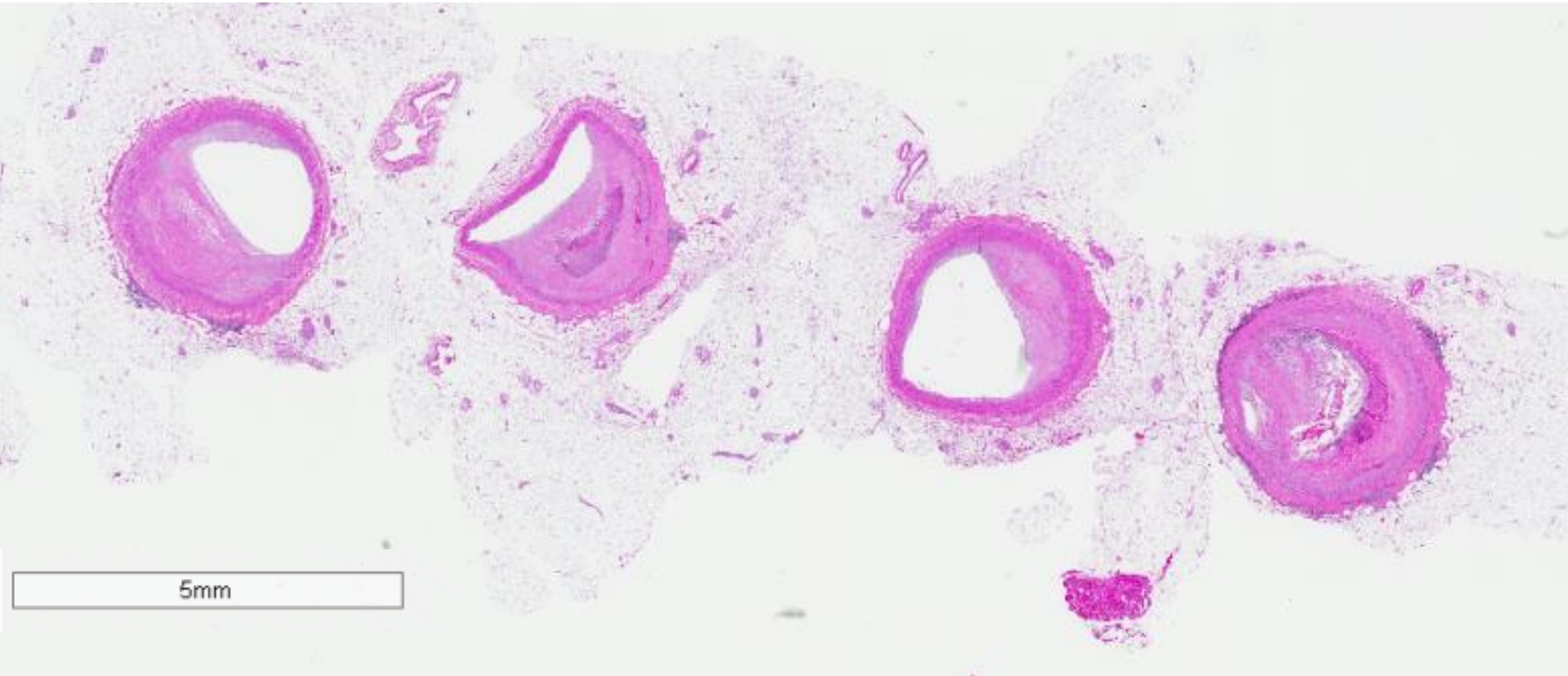


Foam cells & lipid-rich

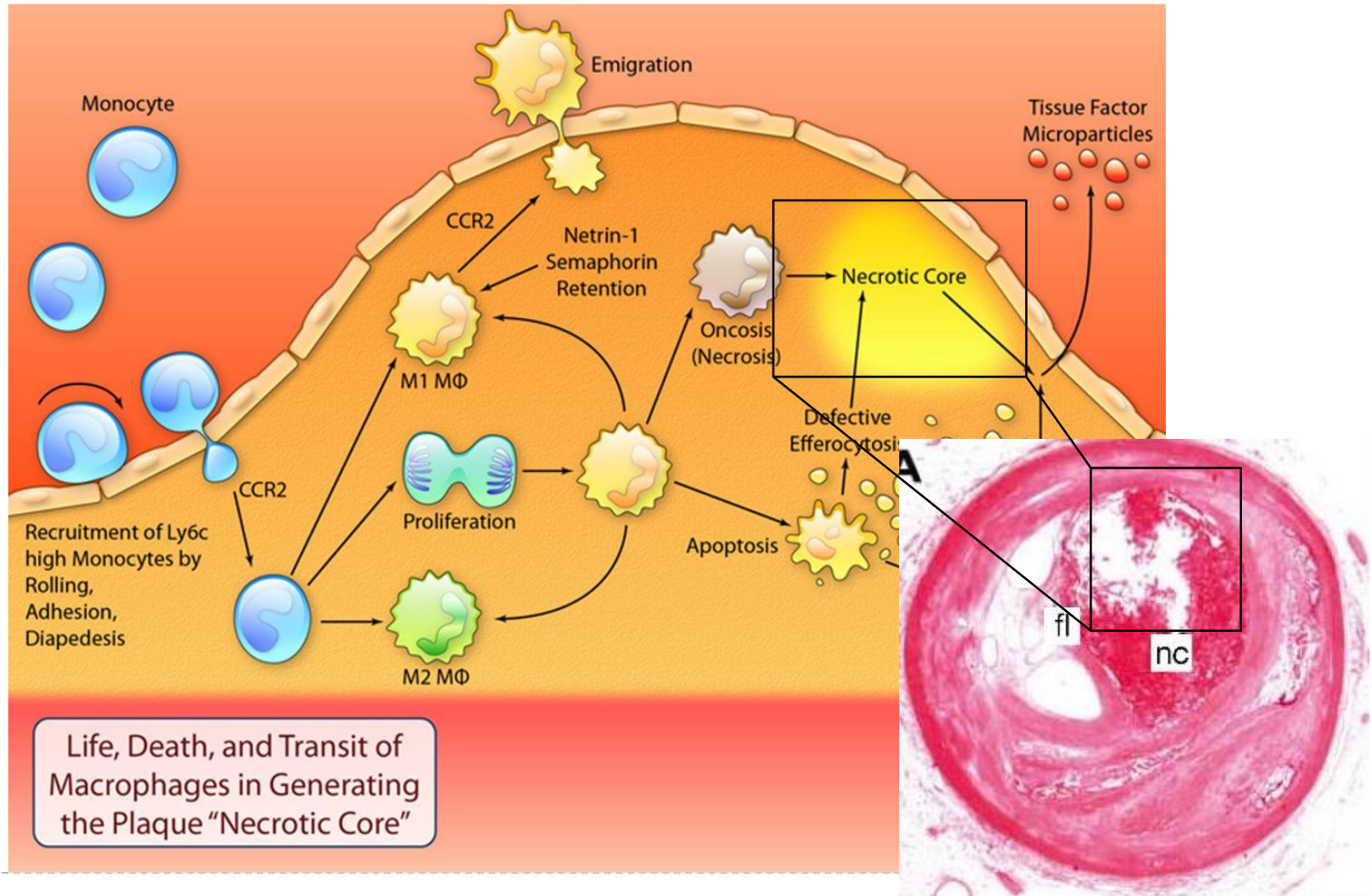


Necrotic core

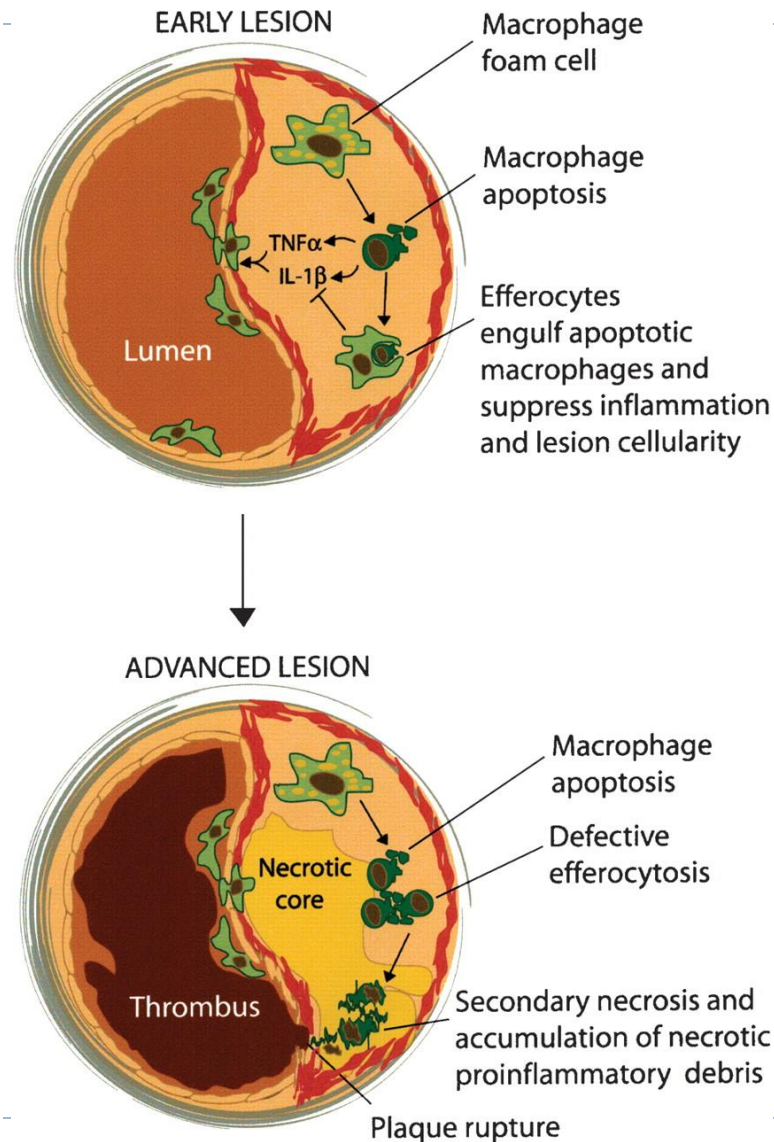
Not all plaques are created equal



Inflammation and the necrotic core

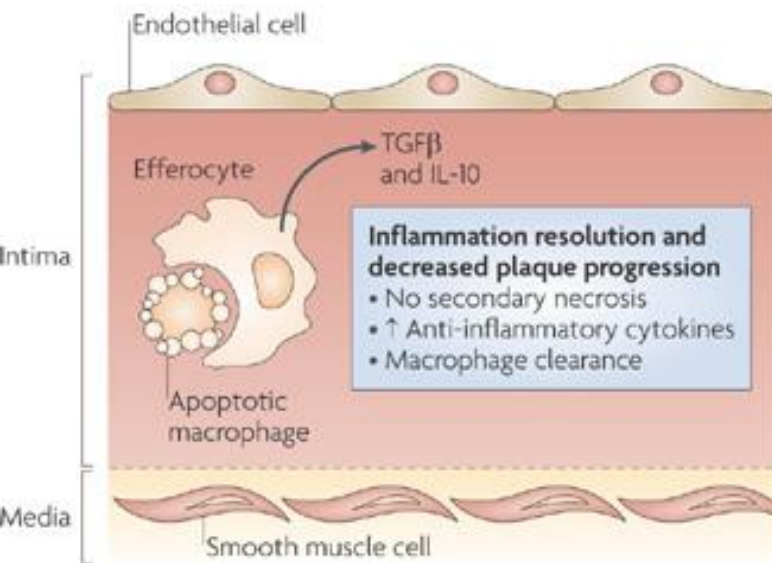


Cell death in atherosclerosis



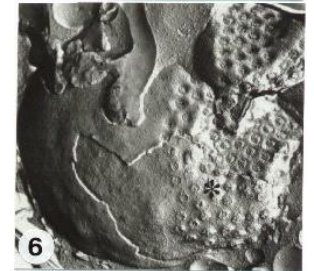
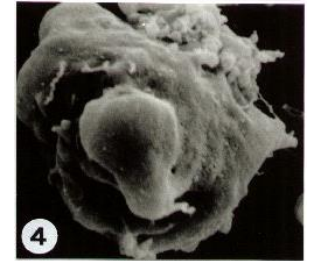
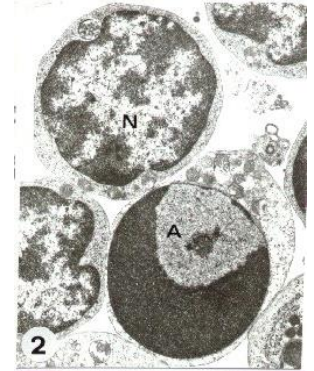
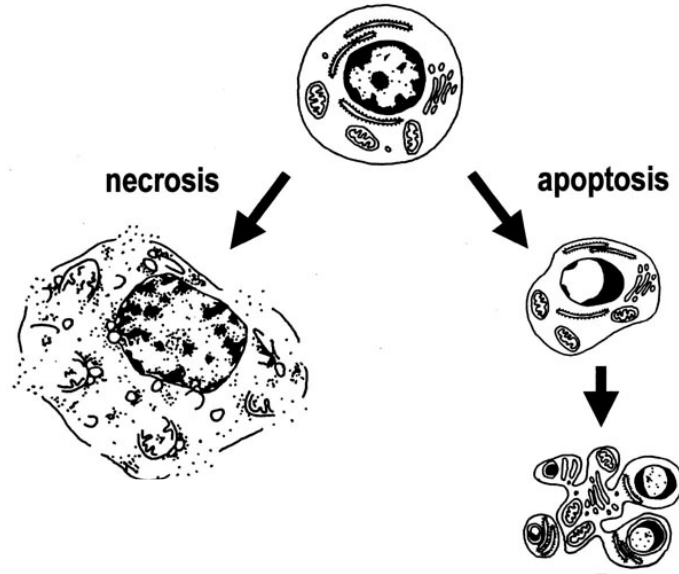
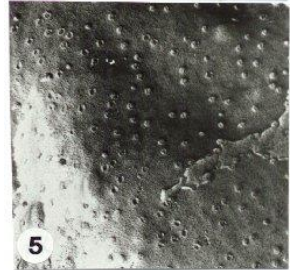
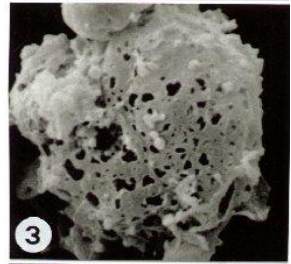
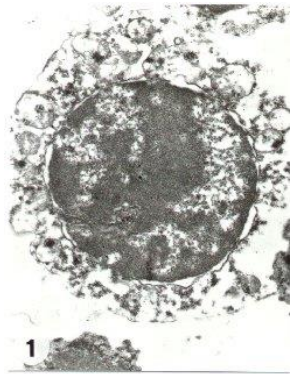
Cell death in atherosclerosis

a Early atherosclerosis

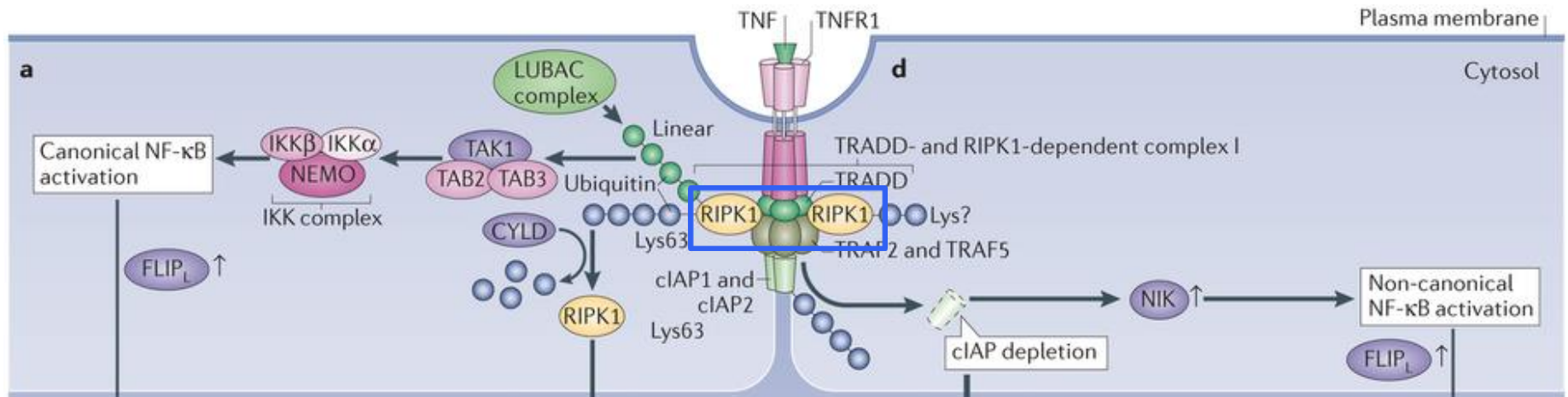


Nature Reviews | Immunology

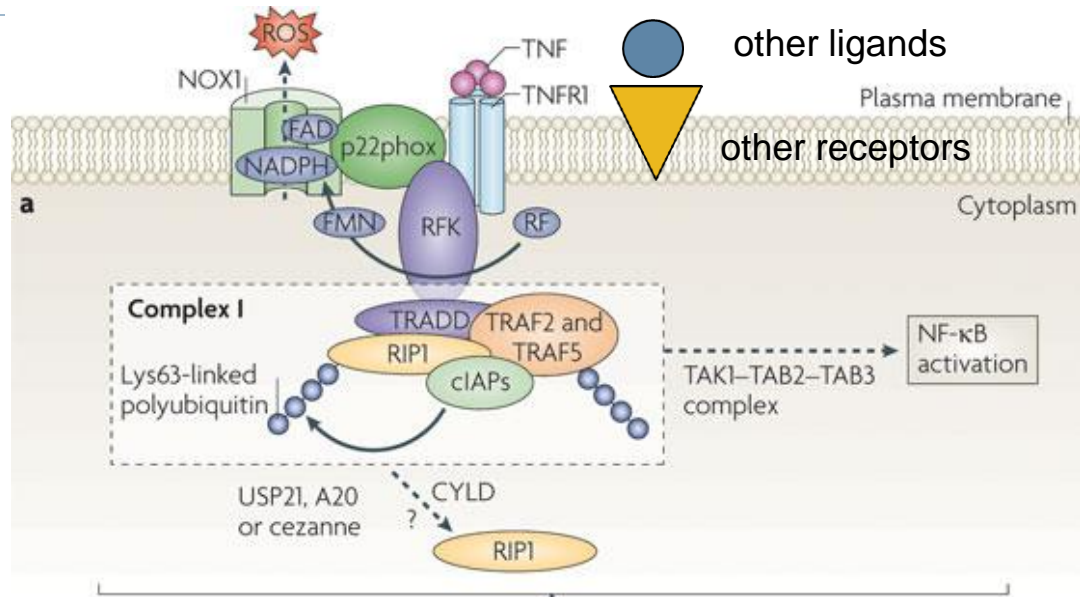
Cell death...How to die?



Necroptosis = programmed cell necrosis

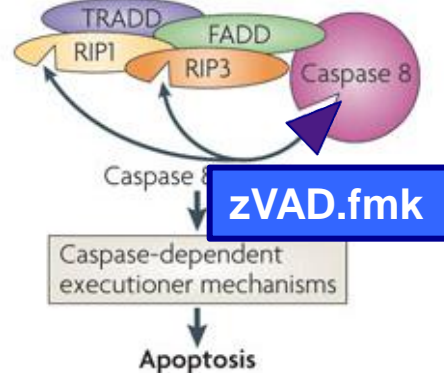


Necroptosis vs apoptosis: a balance



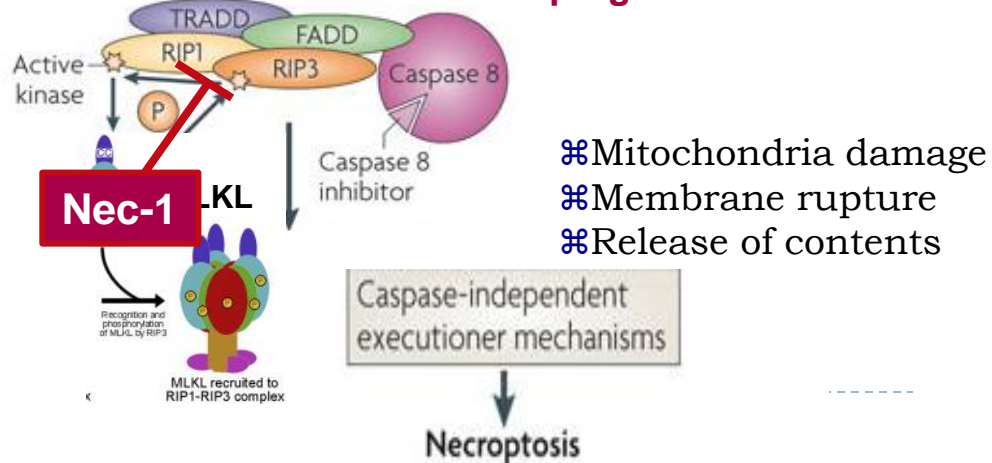
Caspase-8 active

d



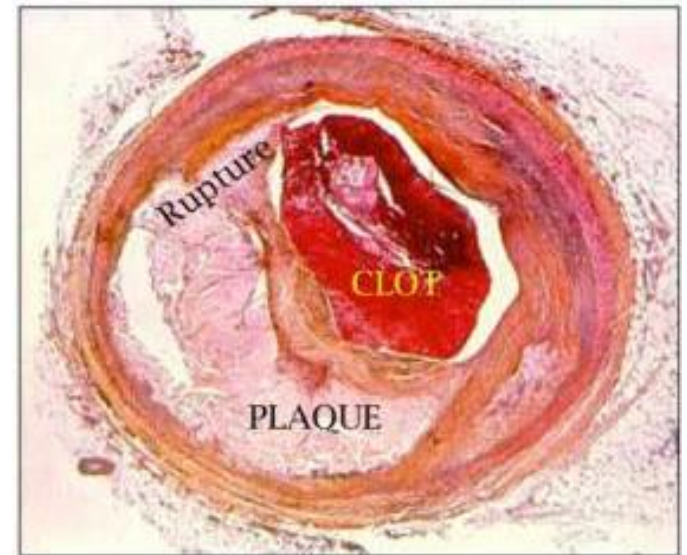
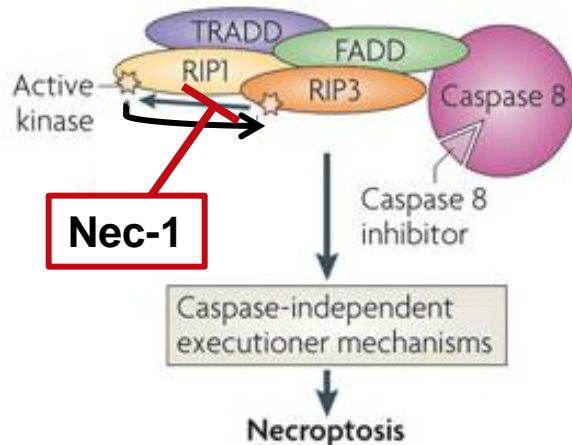
Caspase-8 inactive
Upregulation of RIP1/RIP3

c



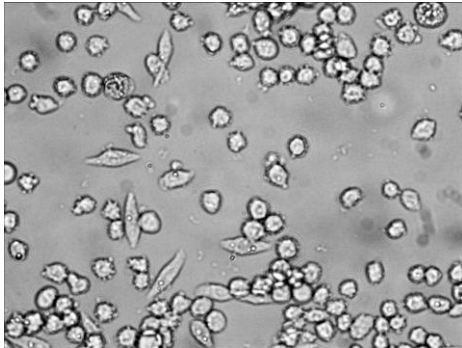
HYPOTHESIS:

Necroptotic cell death in the plaque releases inflammatory mediators and underlies necrotic core formation



Model of *in vitro* necroptosis

Macrophages in culture

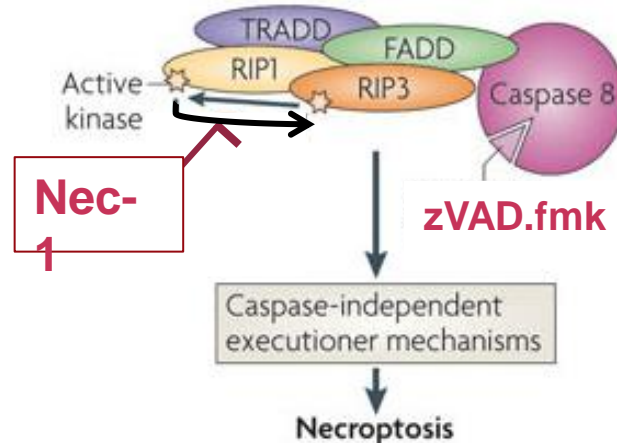


+ oxidized LDL \longrightarrow
24h

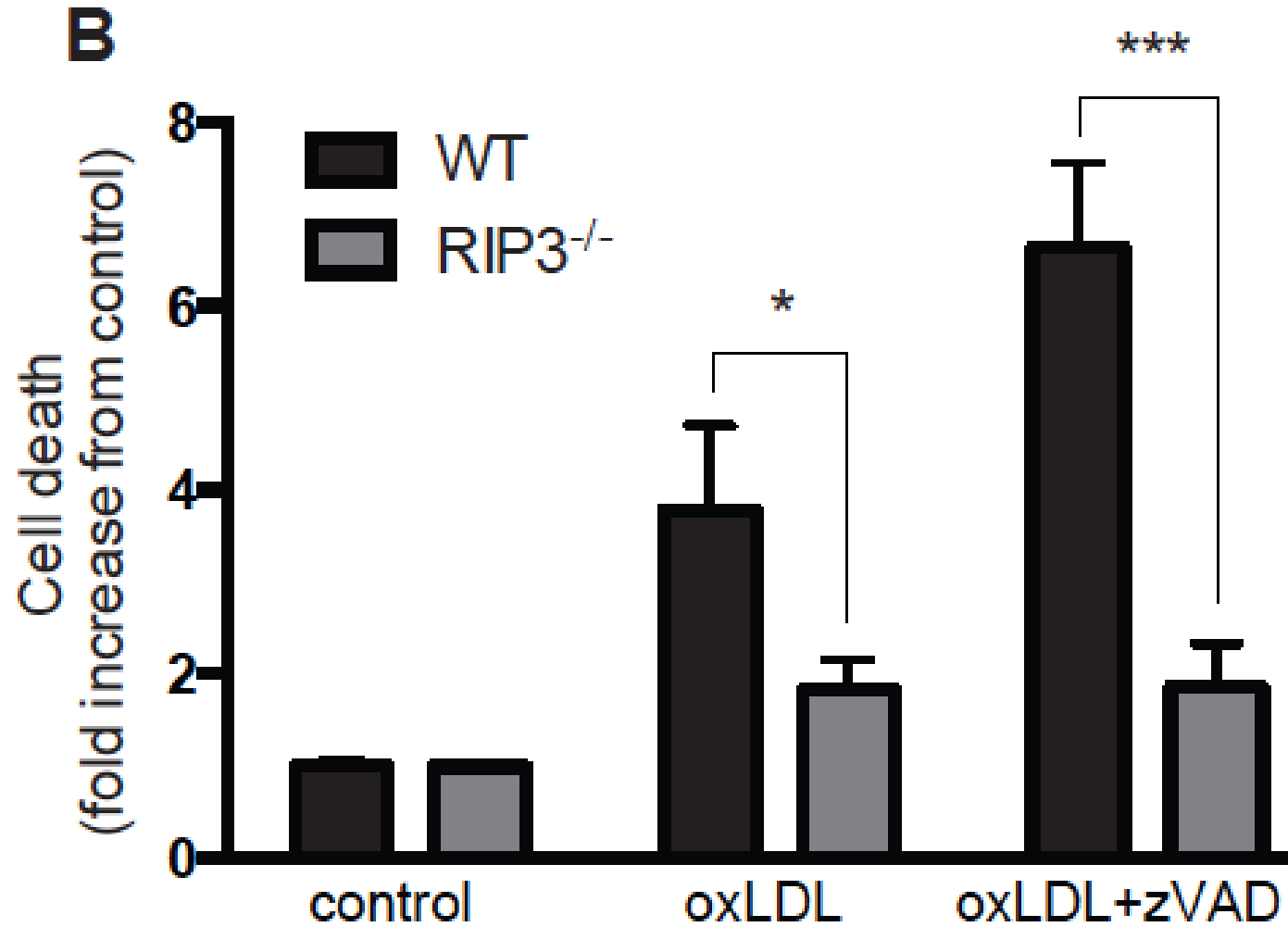
% Cell death

+ **Nec-1** (inhibitor of NECROPTOSIS)

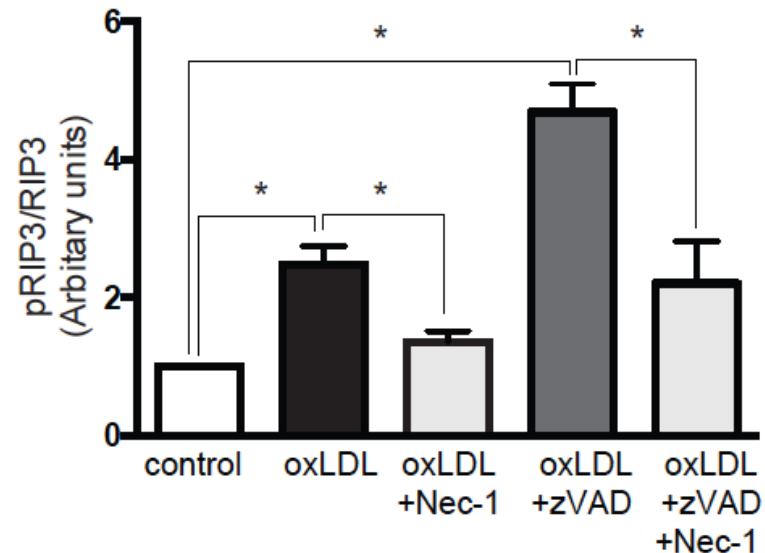
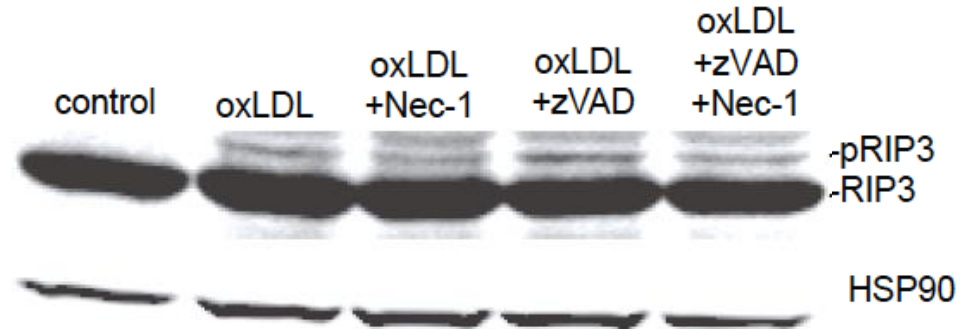
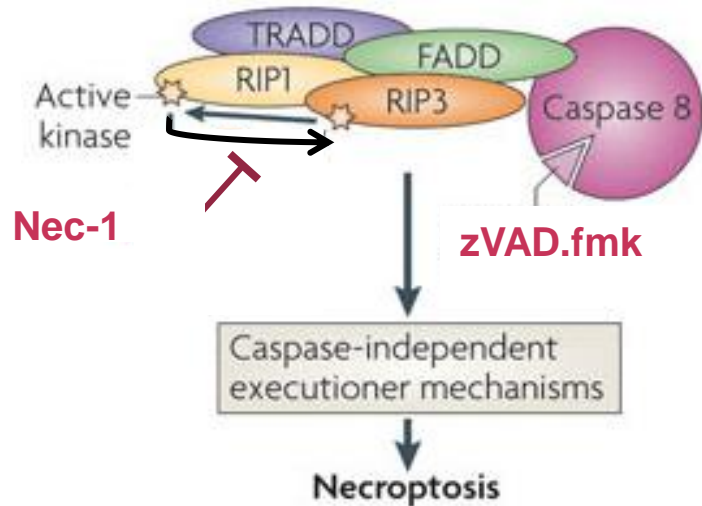
+ **zvad** (inhibitor of apoptosis, \rightarrow NECROPTOSIS)



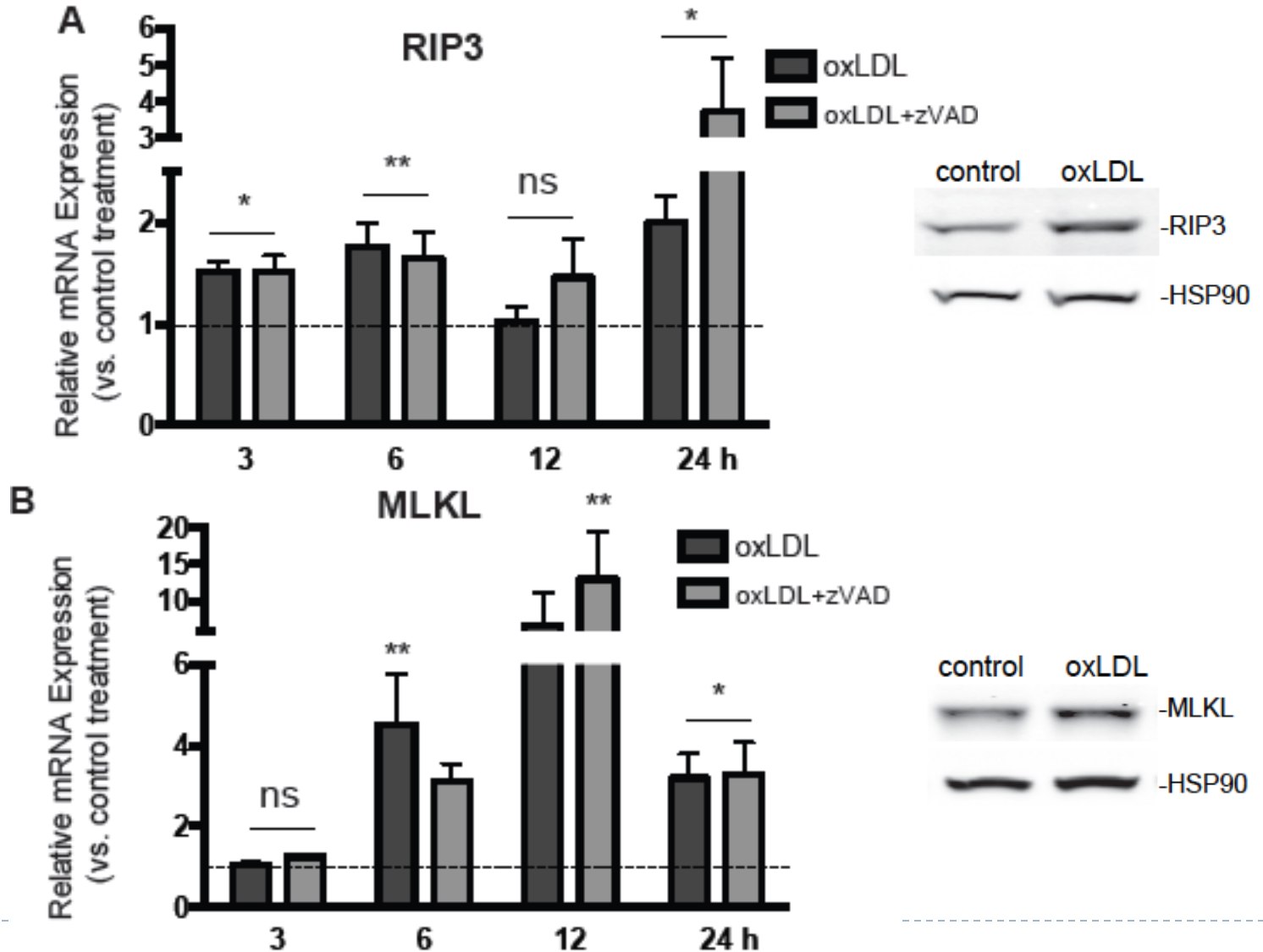
RIP3^{-/-} BMDMs do not undergo oxLDL-induced necroptosis



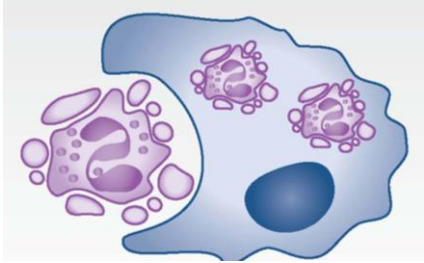
Phosphorylation of RIP3 – key determinant in driving macrophage necroptosis



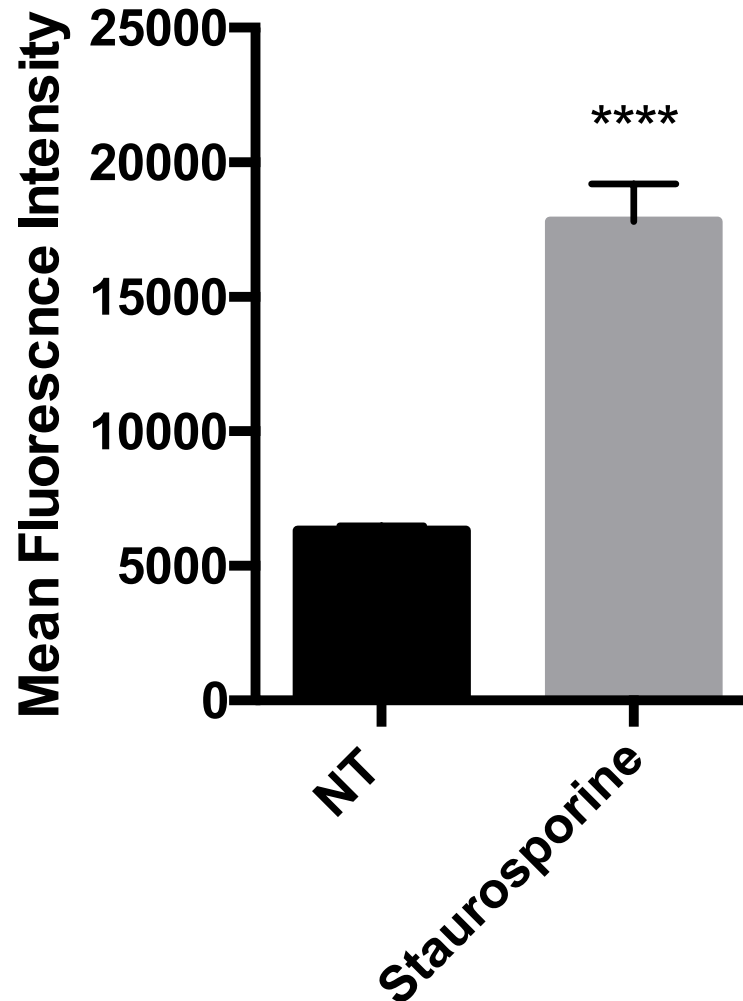
Activation of gene expression by oxLDL



Necroptotic cells are not efficiently efferocytosed

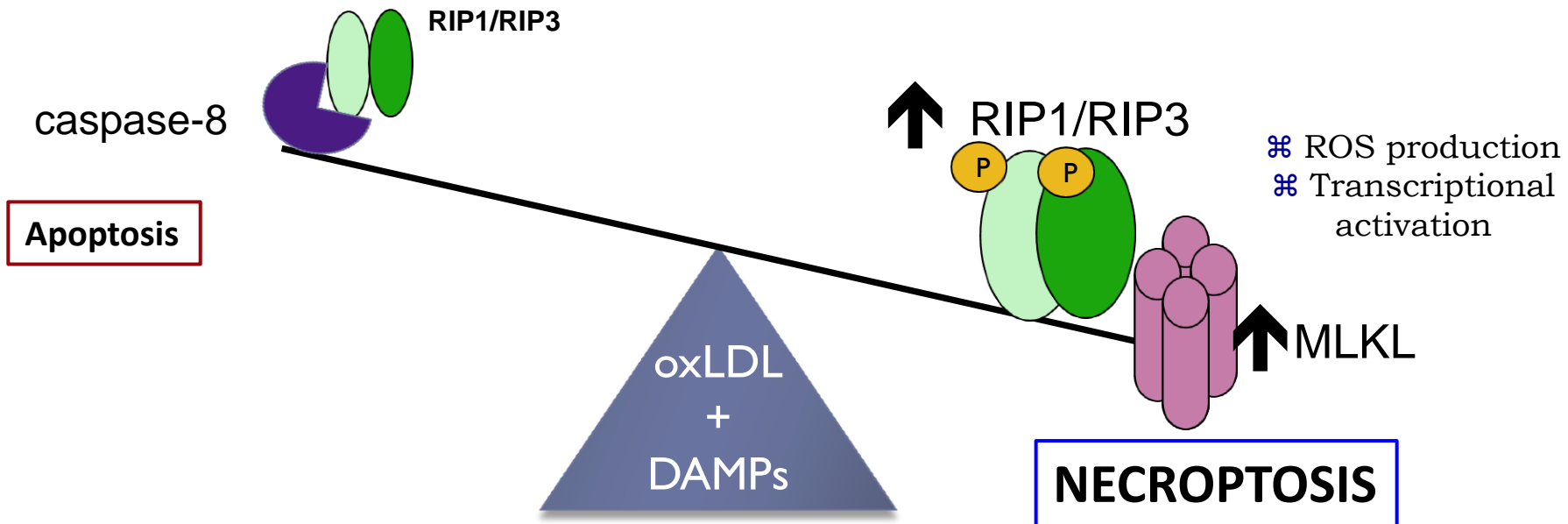


In vitro efferocytosis

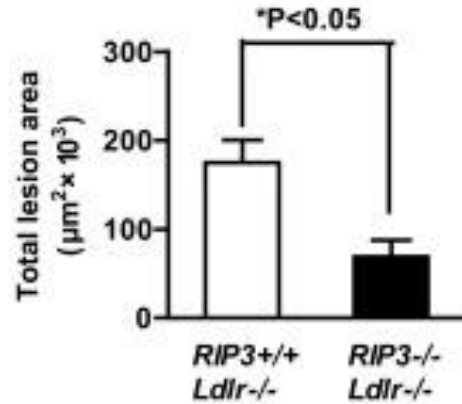
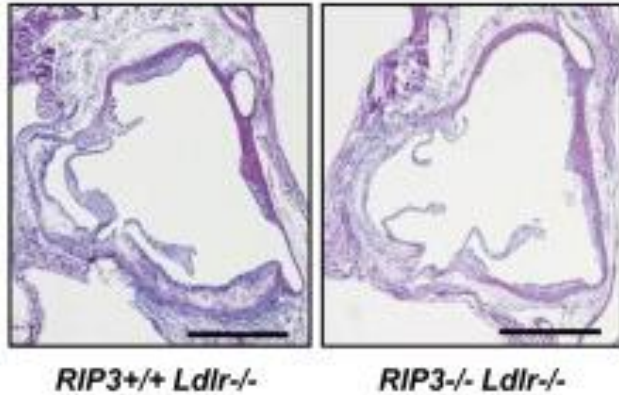


Summary: In vitro findings

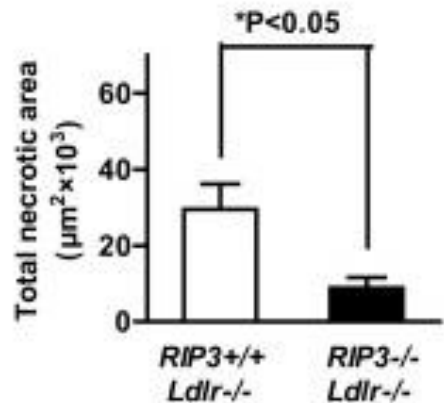
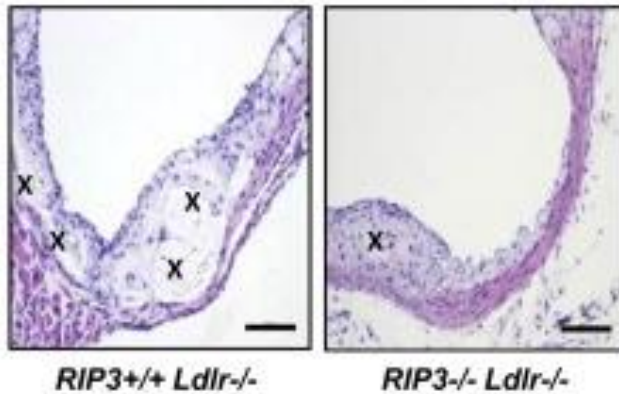
- ▶ Atherogenic ligands oxLDL + DAMPs activate necroptosis in the absence of chemical inhibitors of apoptosis
- ▶ Via upregulation of RIP3 and MLKL
- ▶ Reduced efferocytosis of necroptotic cells



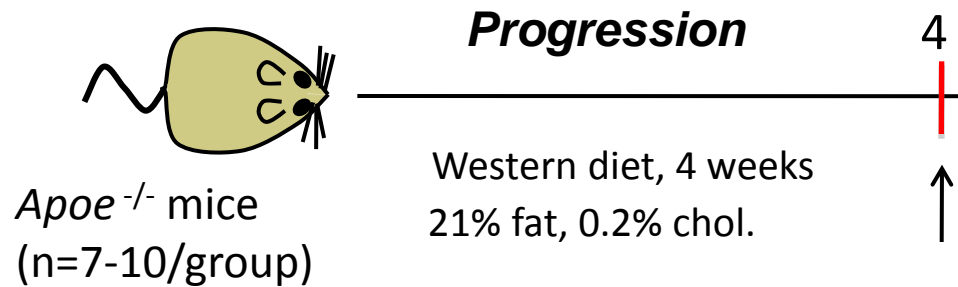
RIP3^{-/-} mice have decreased atherosclerosis



When RIP3 is knocked-out, atherosclerosis is reduced.



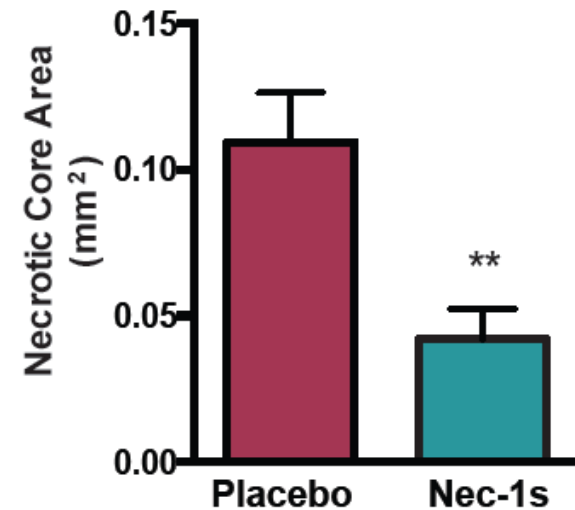
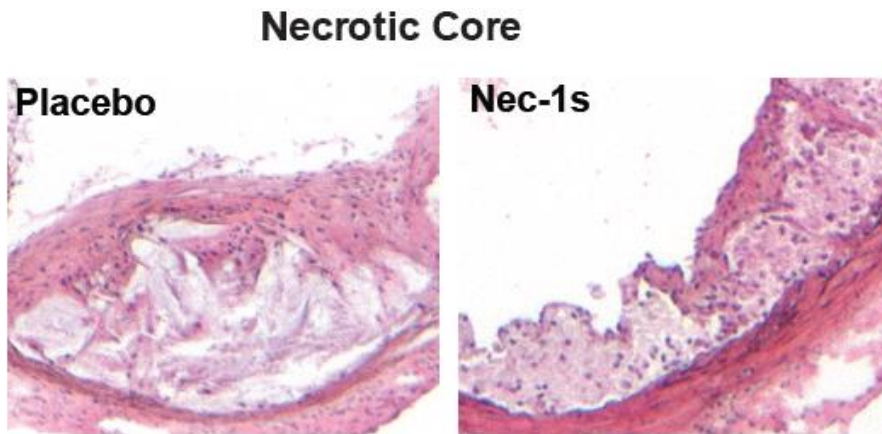
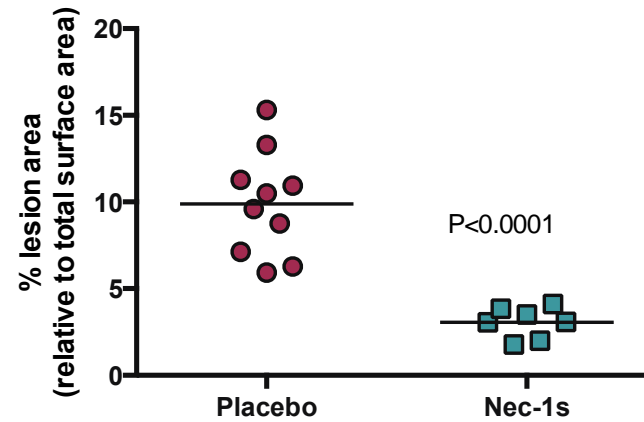
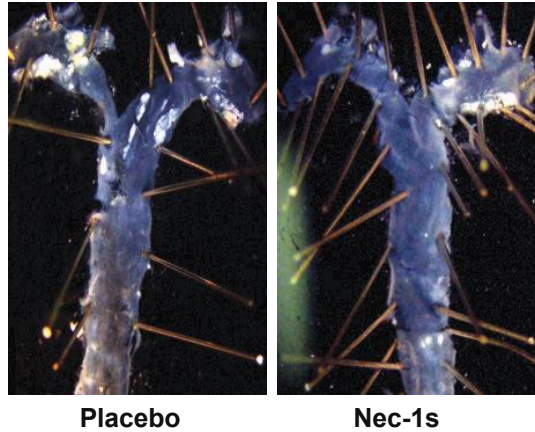
Can we use necroptosis as a target for therapeutic intervention?



Atherosclerosis Intervention Model with Nec-1s

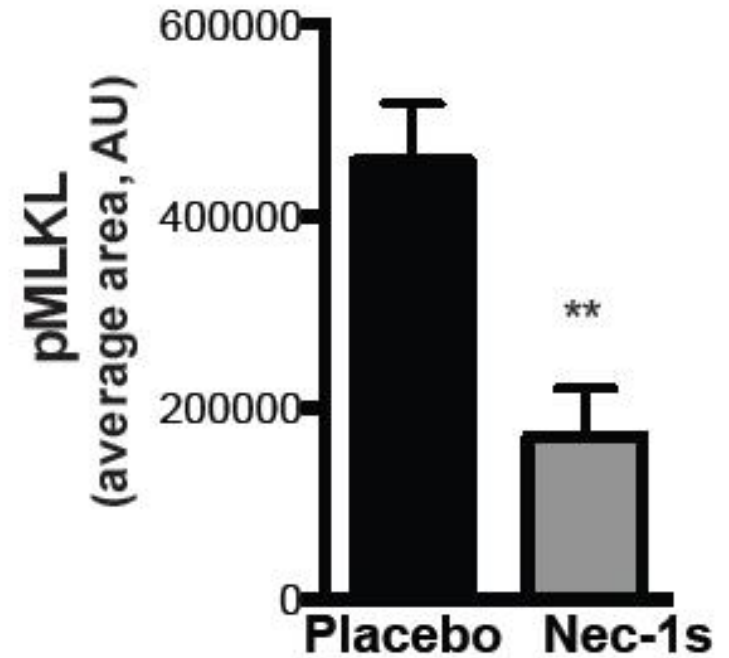
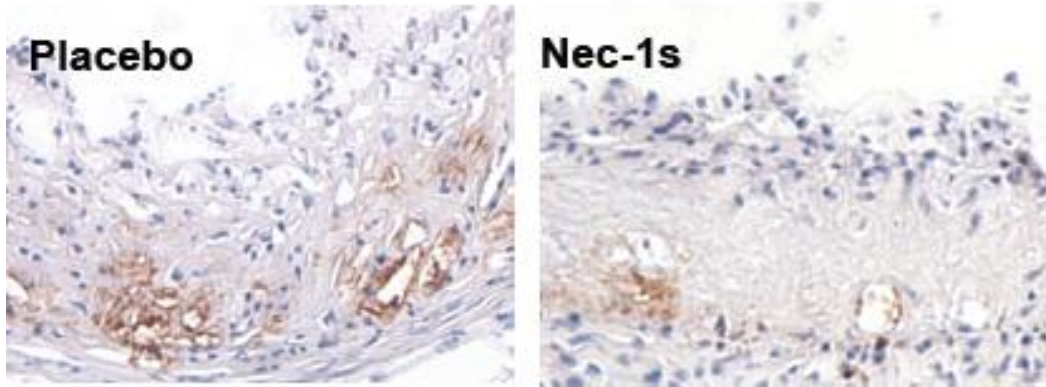


Inhibiting necroptosis reduces lesion size & necrotic core

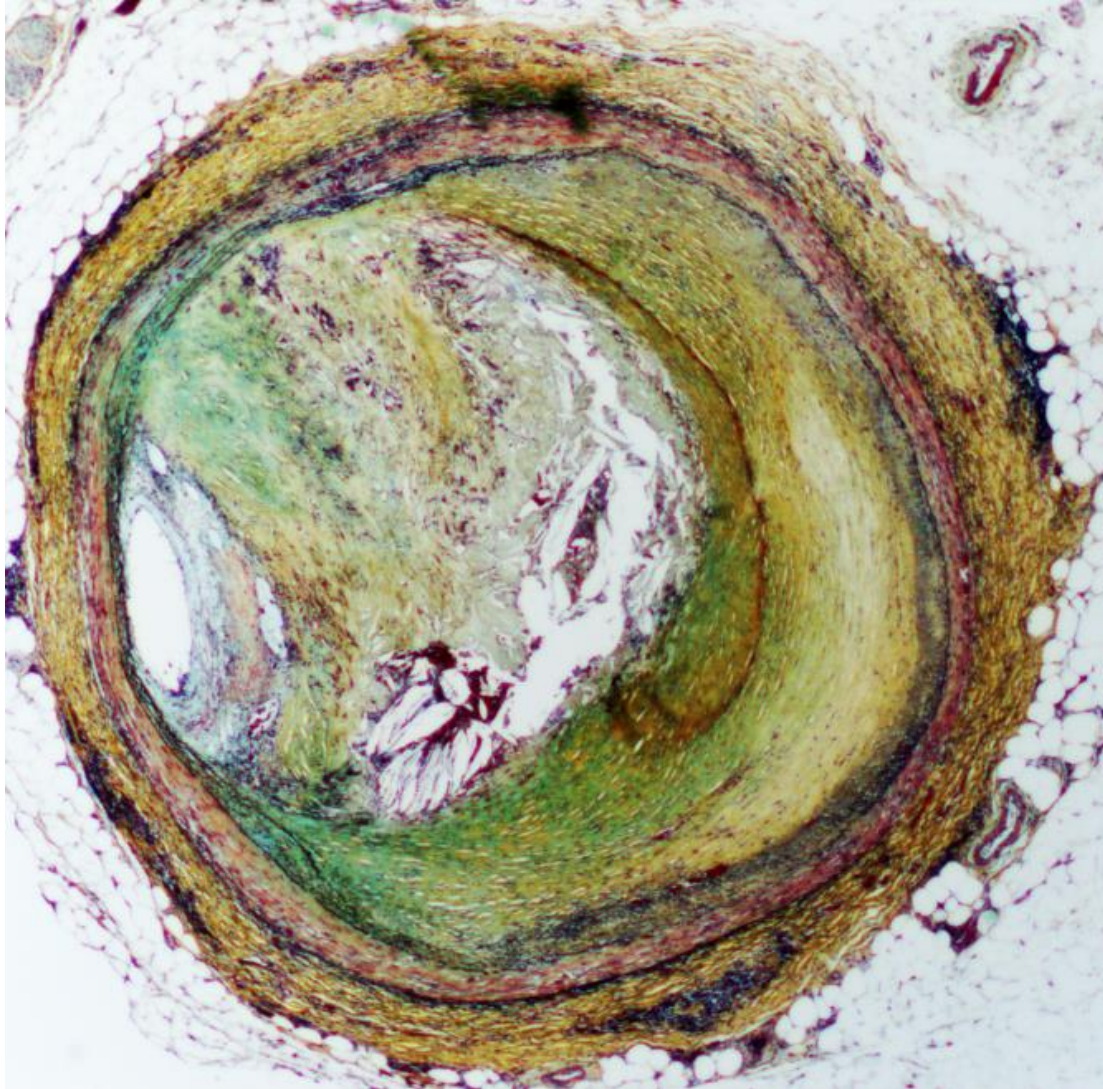


Inhibition of necroptosis reduces pMLKL

G Phosphorylated MLKL

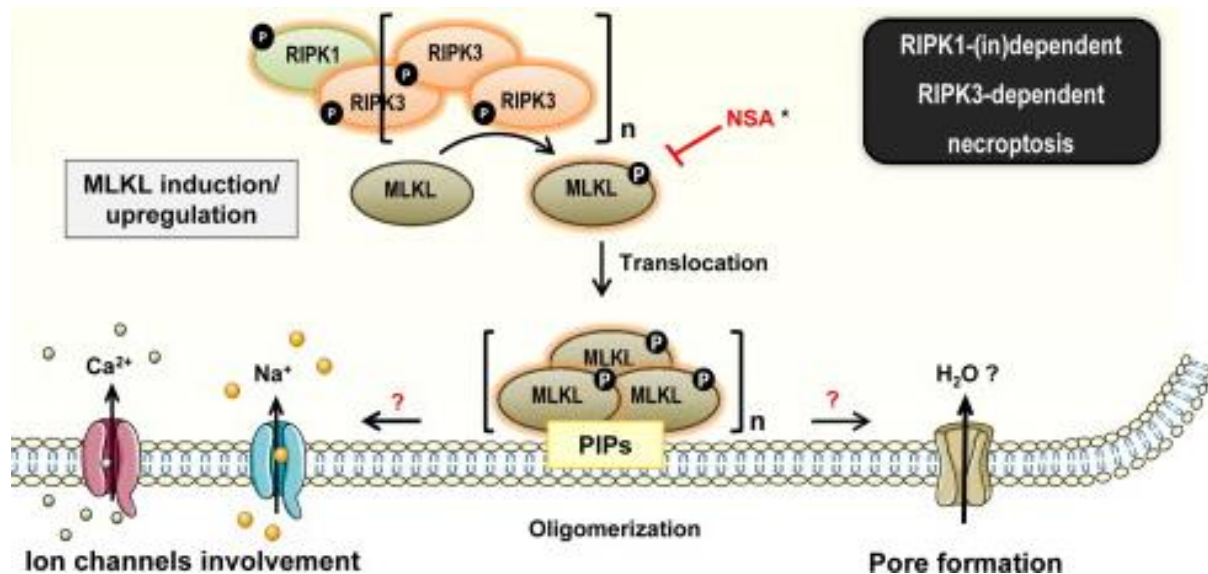


Is necroptosis relevant to human disease?

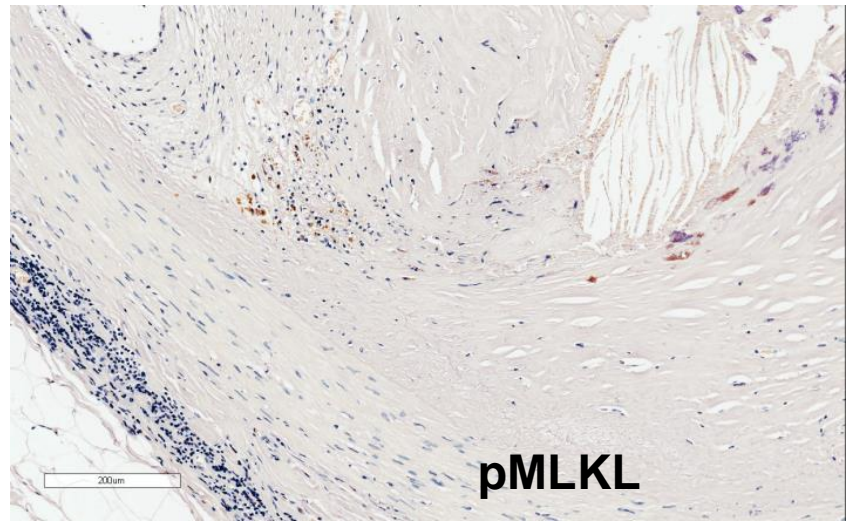
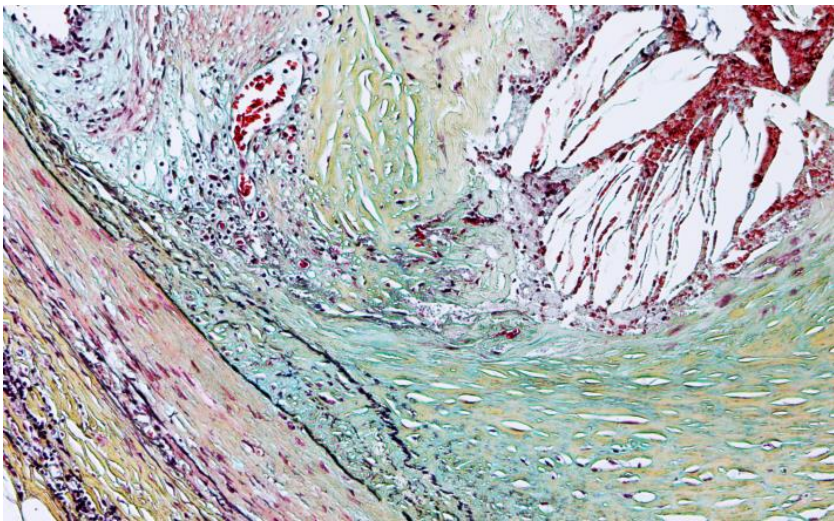
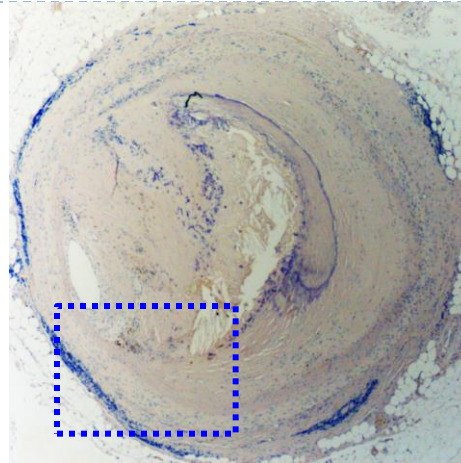
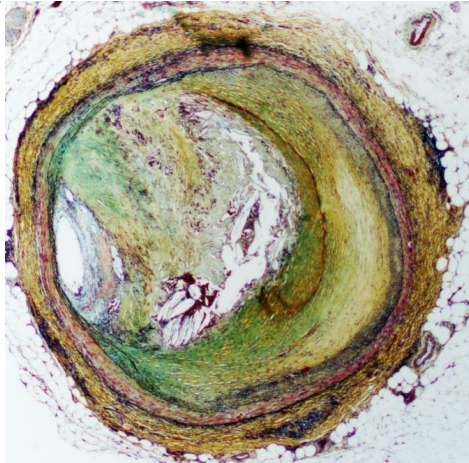


Identifying active necroptosis: challenges

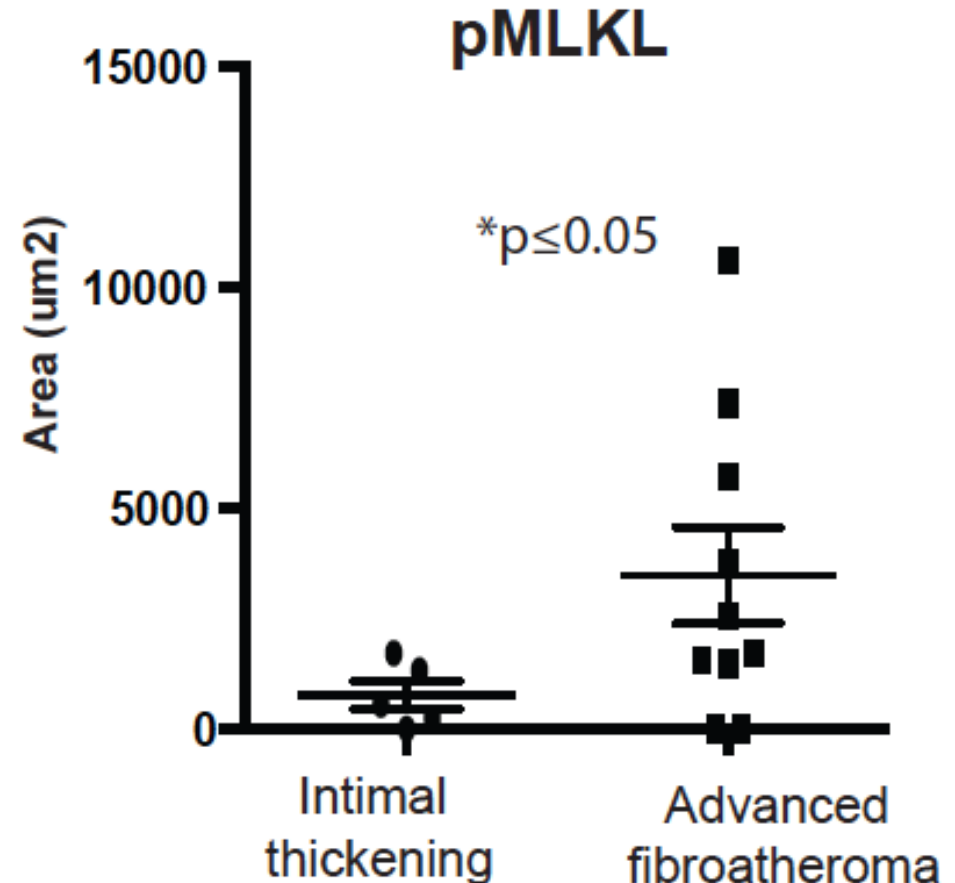
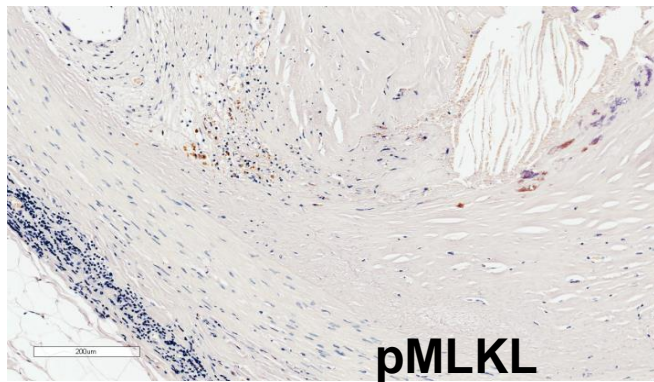
- Expression \neq activation
- Traditional measures of cell death do not distinguish between apoptosis and necroptosis
- Lacking markers specific for necroptosis



Necroptosis is active in unstable CAD



Necroptosis is active in unstable CAD



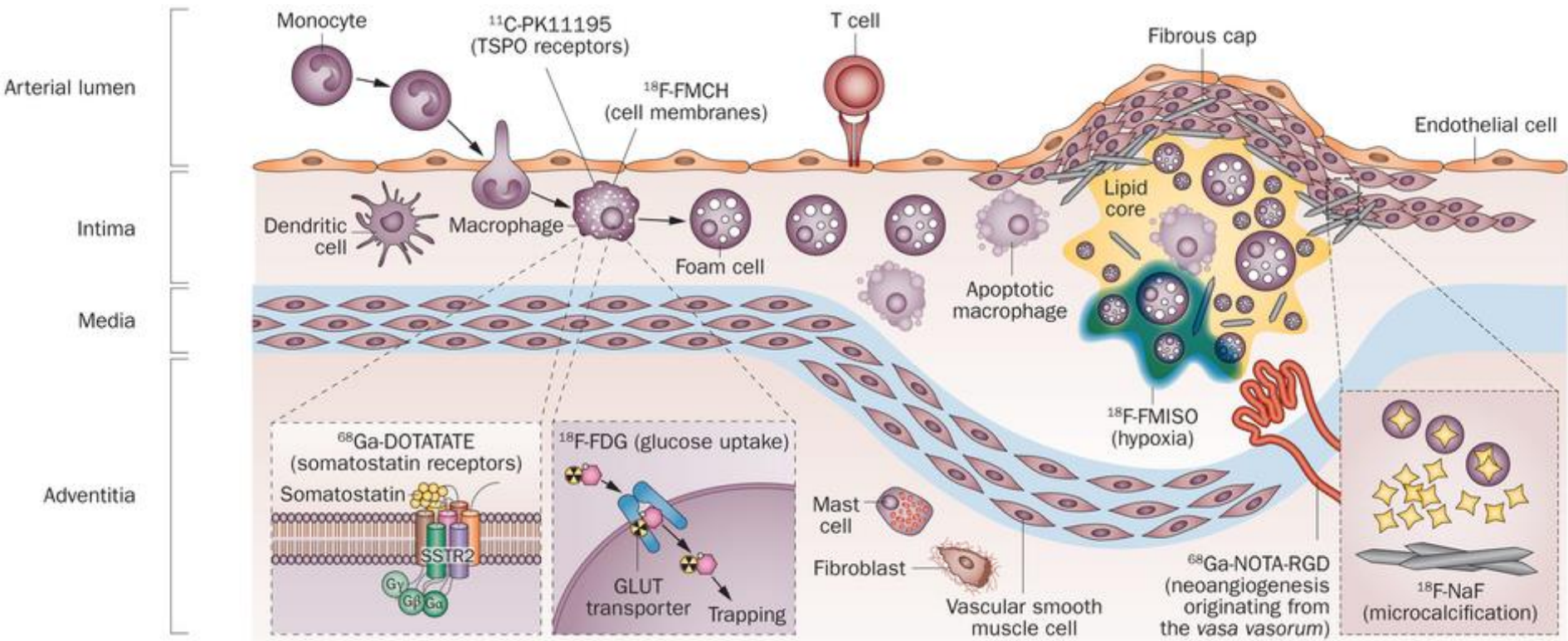
This is the first evidence that necroptosis is activated in vulnerable lesions in humans

What's next?

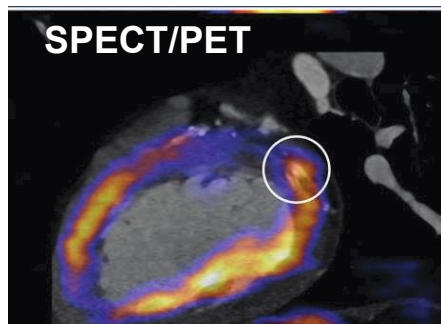
- ▶ Can necroptosis be used as a diagnostic for unstable coronary disease?
- ▶ What are other drivers of necroptosis in the vessel wall?
- ▶ How is necroptosis activated in other inflammatory conditions?
- ▶ How can this be pharmacologically and diagnostically targeted?



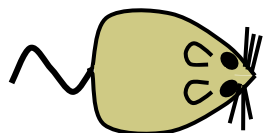
Molecular imaging of atherosclerosis



Can necroptosis serve as an imaging biomarker?



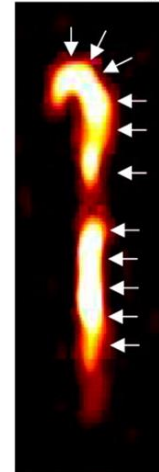
Apoe^{-/-}



radiotracer

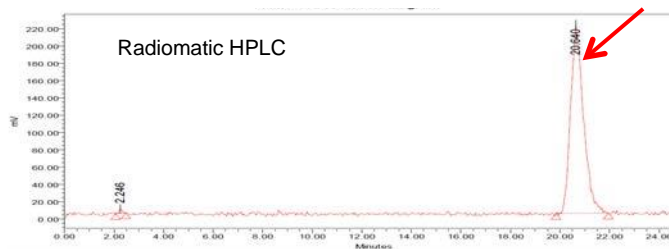
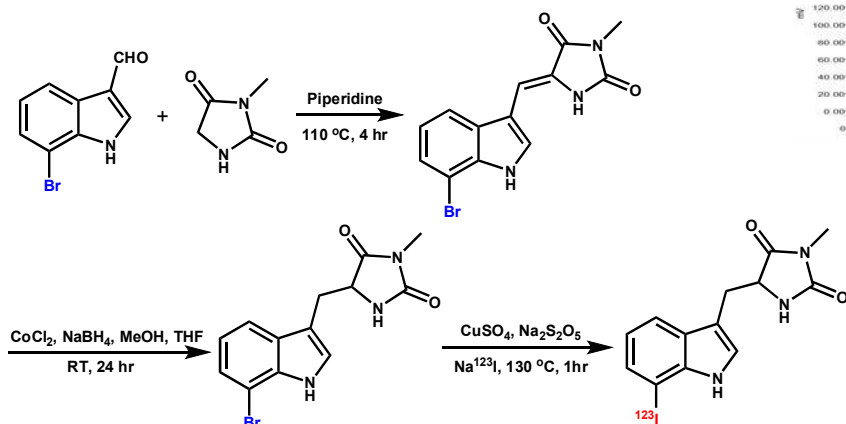


Ex vivo image

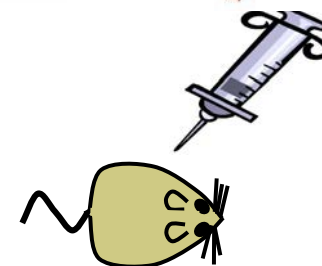
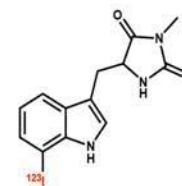


Sadeghi, *J Nucl Med* 2010

Synthesis of 7-¹²³I]-Nec-1



Nec-1 Tracer



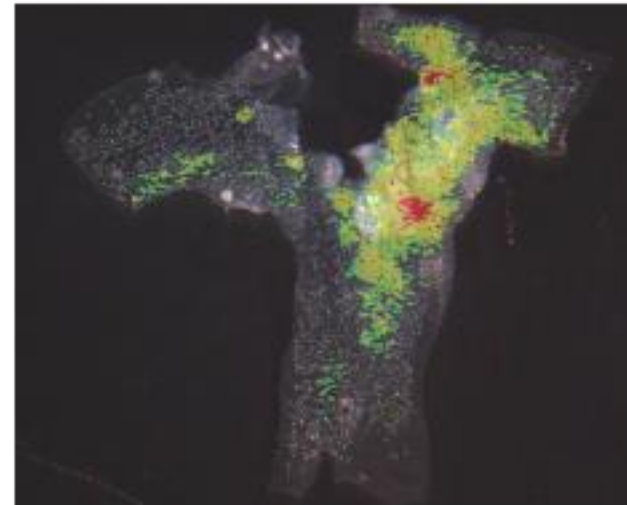
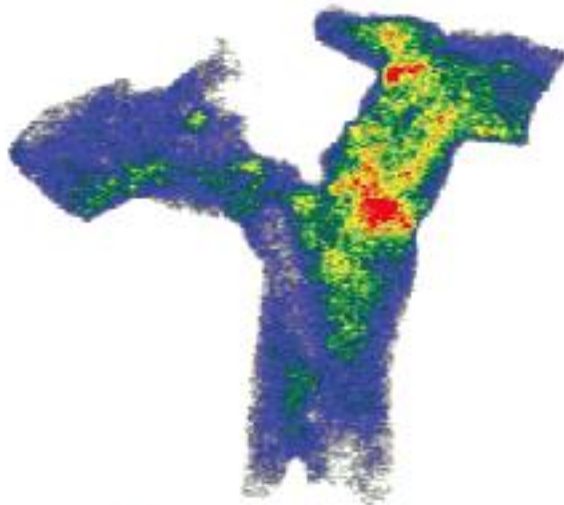
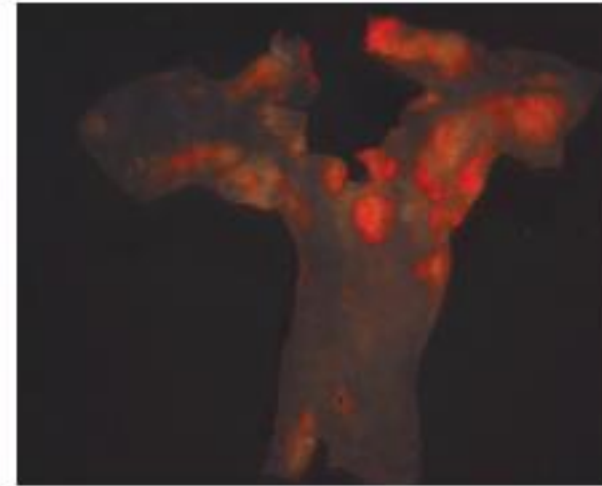
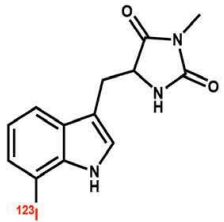
Visualization Atherosclerosis using radiolabeled Nec-1 in ApoE^{-/-} mice

Aortic En face

Oil-Red-O

A

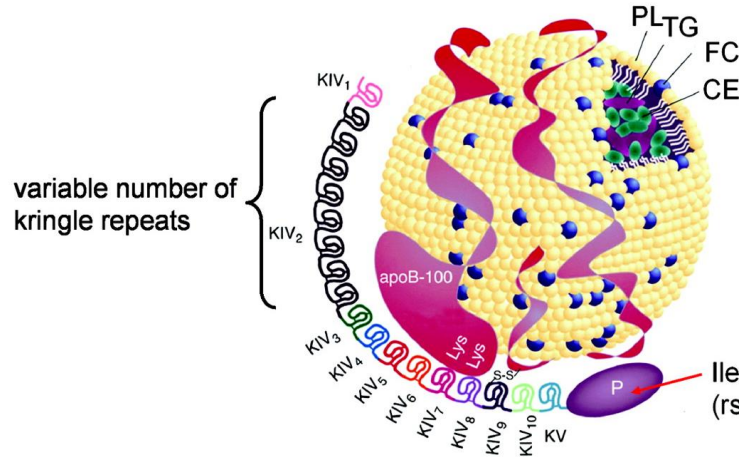
Nec-1 Tracer



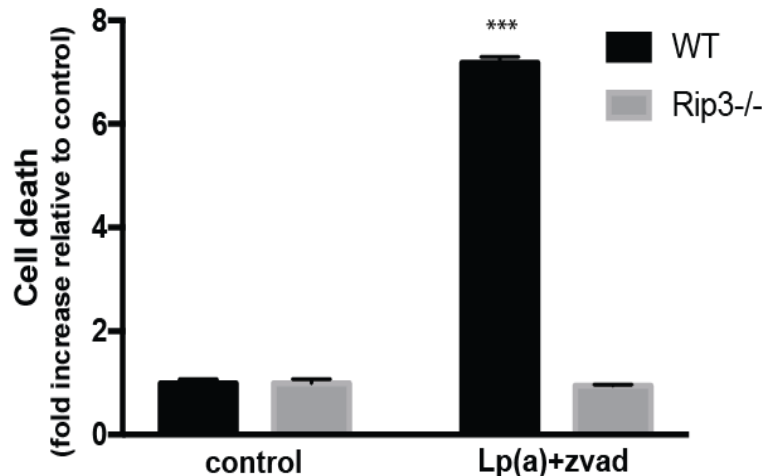
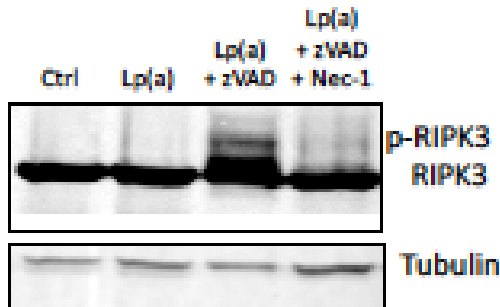
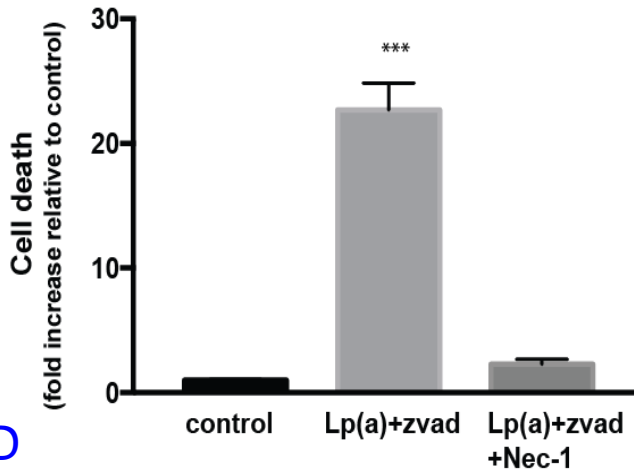
Autoradiogram

Overlay

What are other drives of necroptosis in the plaque?

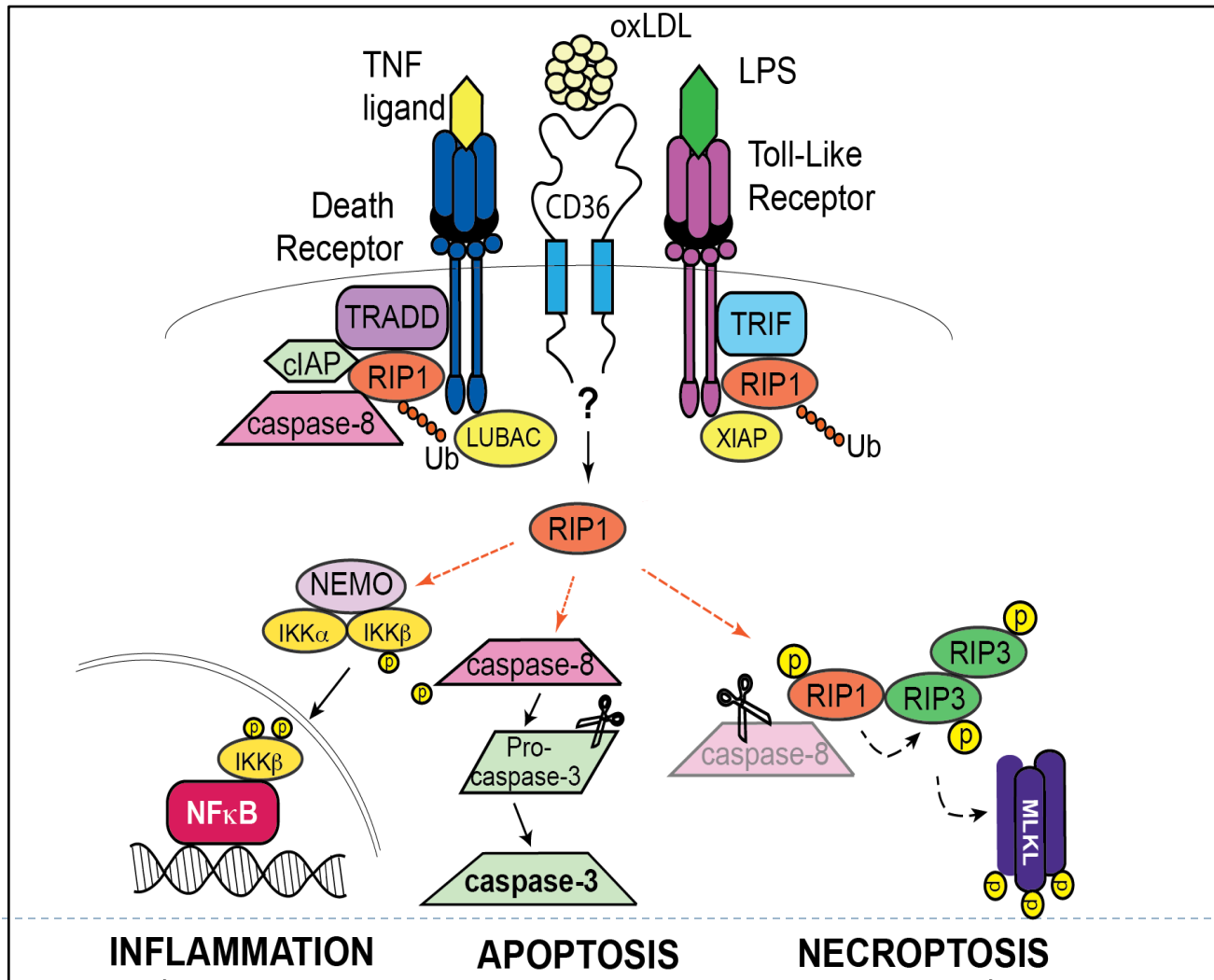


Lipoprotein (a) = strong predictor of CAD



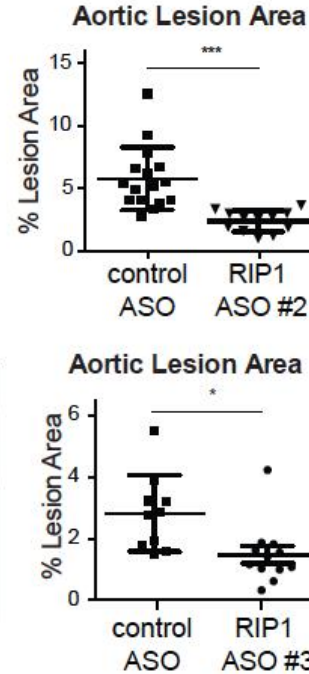
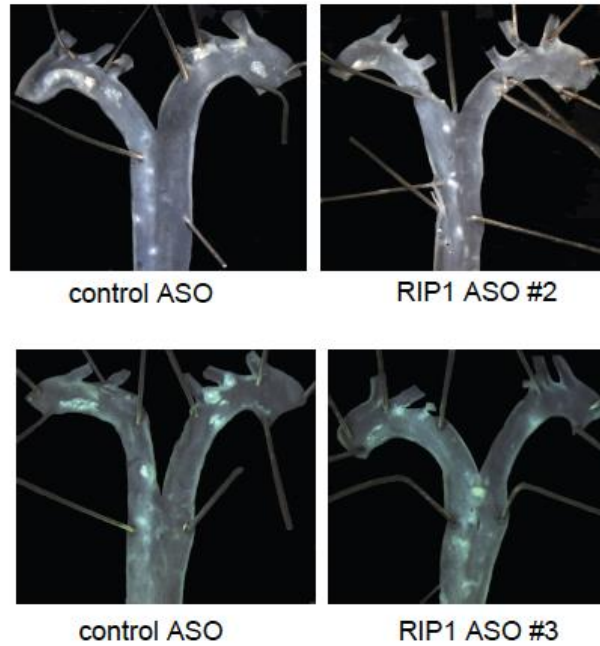
What about upstream of RIP3/MLKL?

RIPK1: A regulator of inflammation, cell survival and necroptosis

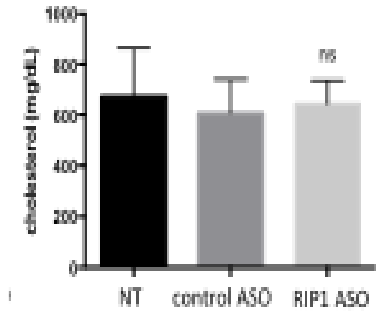


Blocking RIPK1 prevents atherosclerosis

B

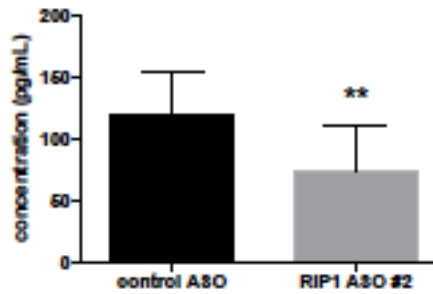


C: Plasma cholesterol

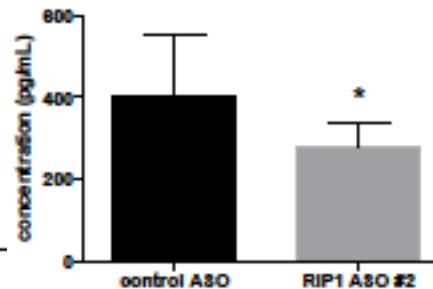


Serum cytokines:

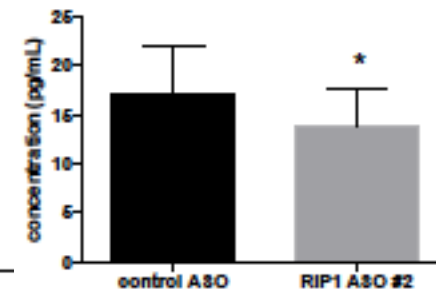
IL-17A



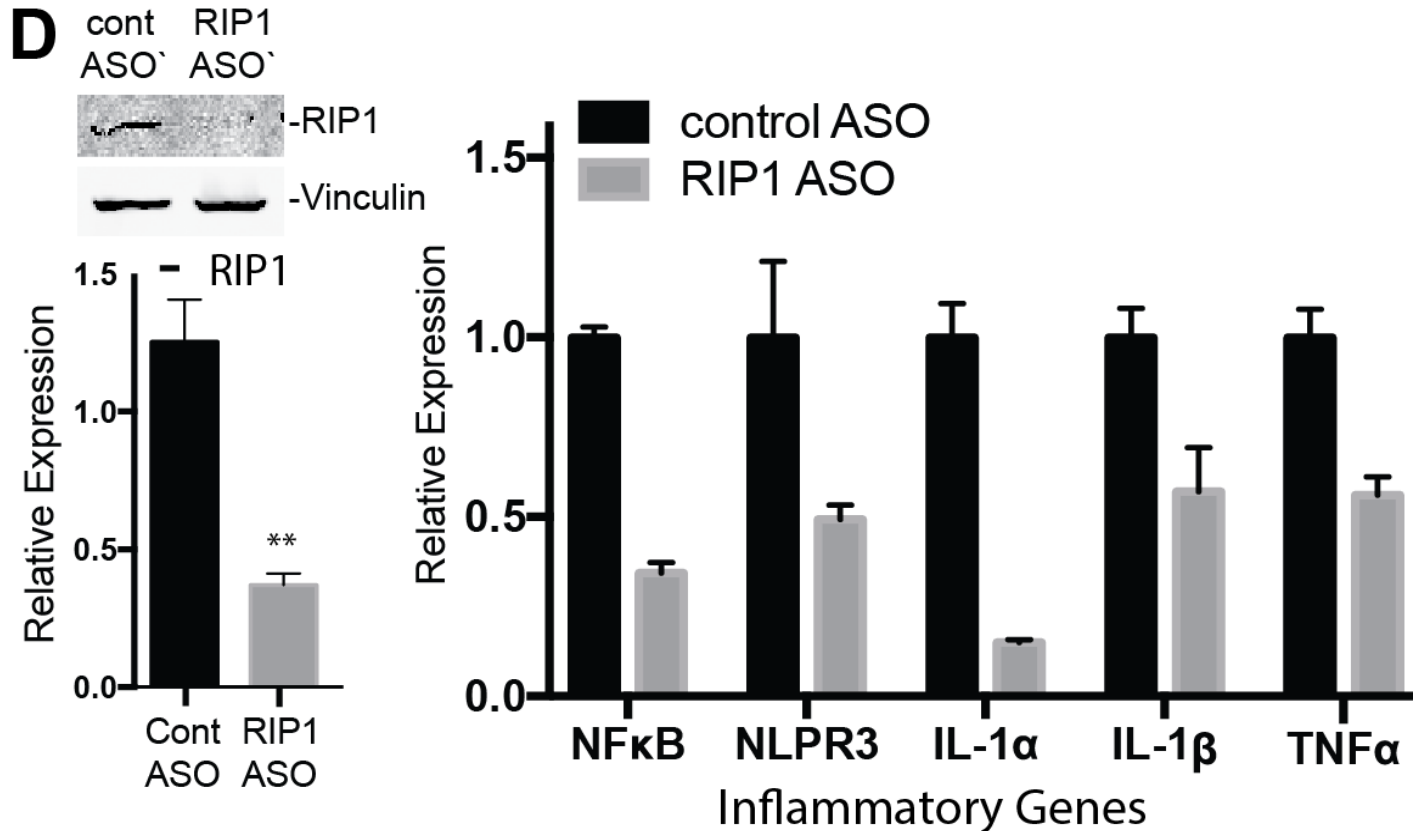
IL-12 (p70)



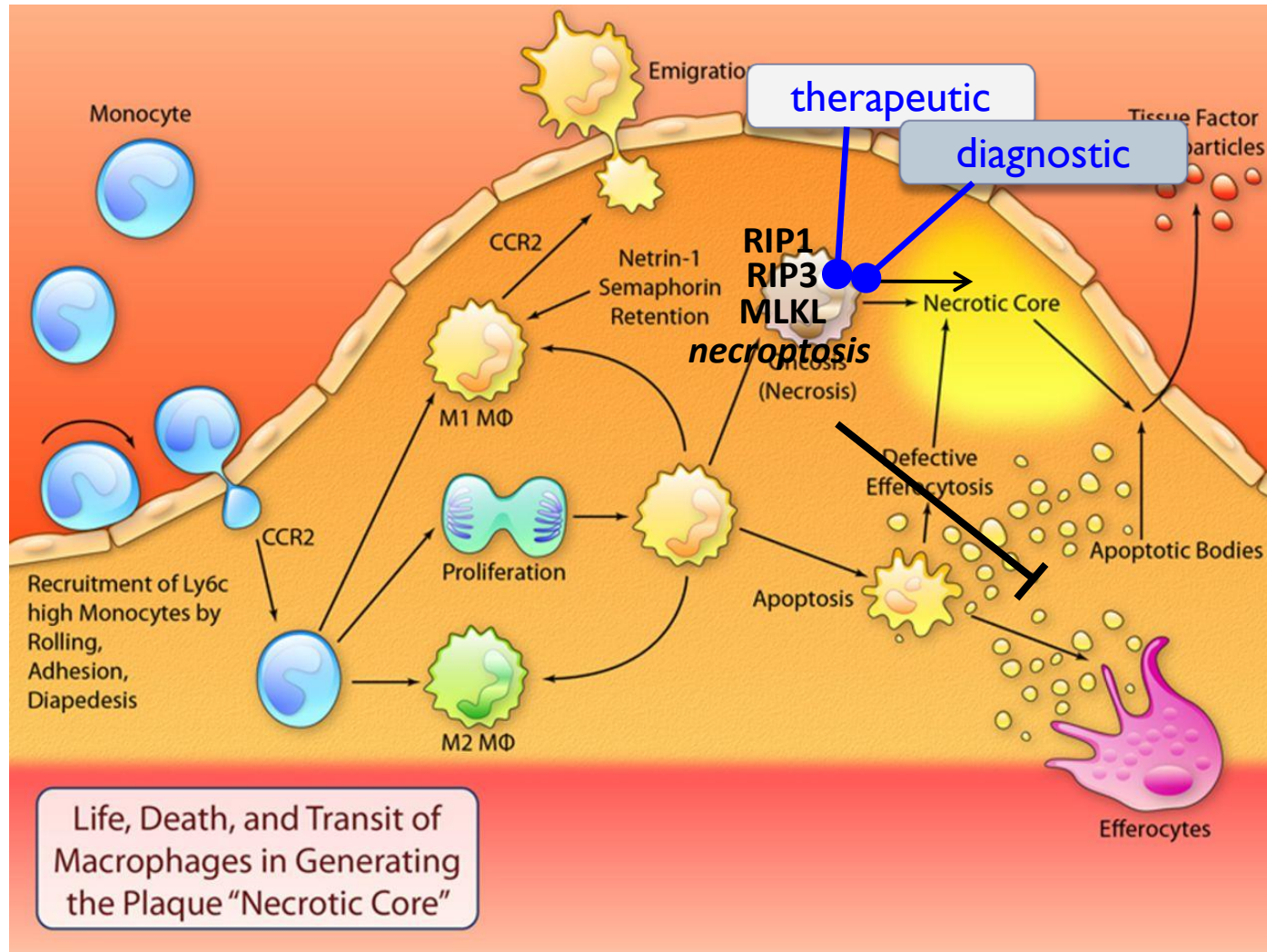
IL-1a



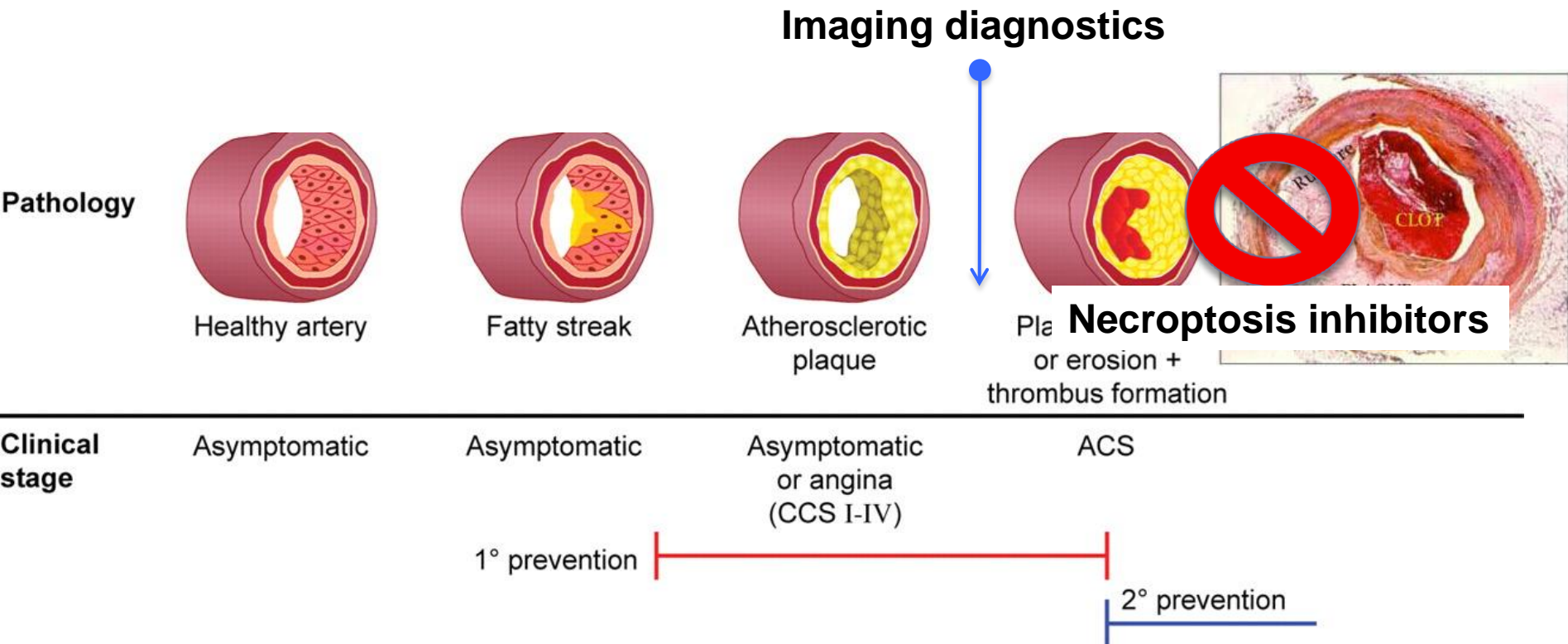
Blocking RIPK1 reduces inflammation in BMDMs



Necroptosis underlies plaque vulnerability



The future?





Bring out yer dead!!



I'm not dead yet! I
feel fine!



UNIVERSITY OF OTTAWA
HEART INSTITUTE
INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

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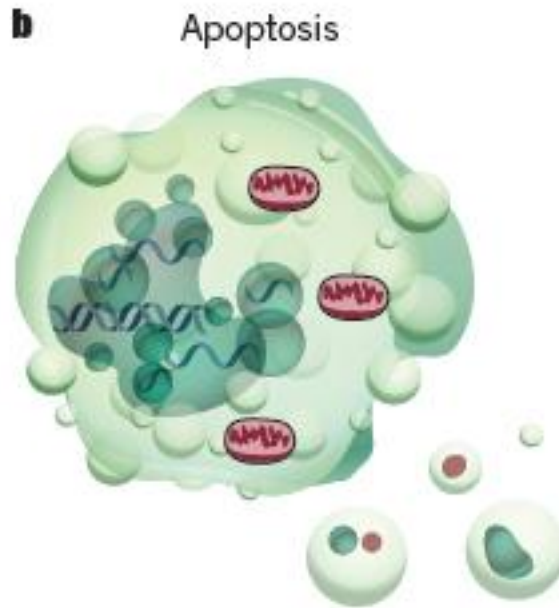
GlaxoSmithKline

Funding sources:

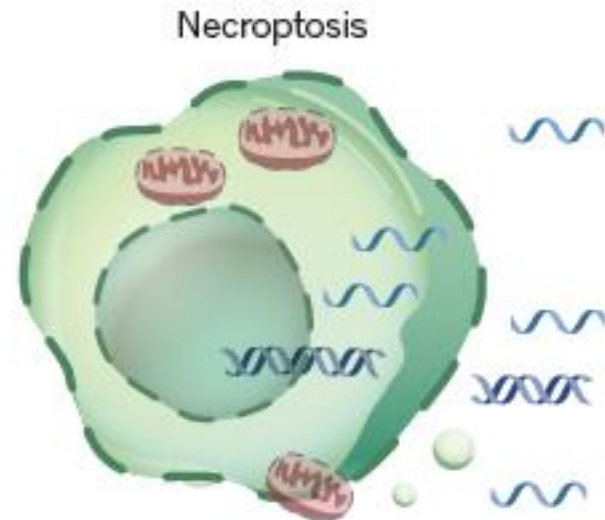




Danger Associated Molecular Patterns (DAMPs)



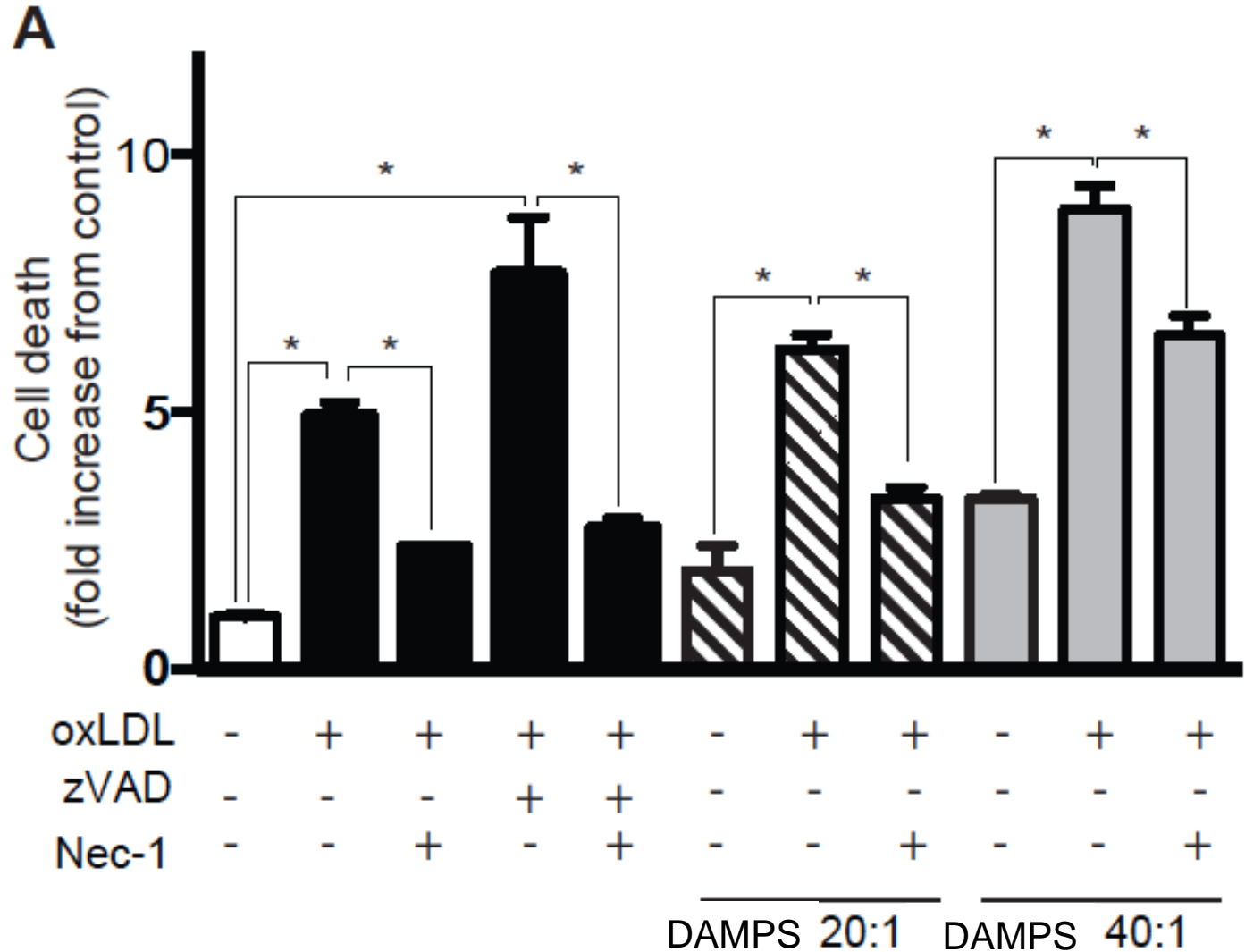
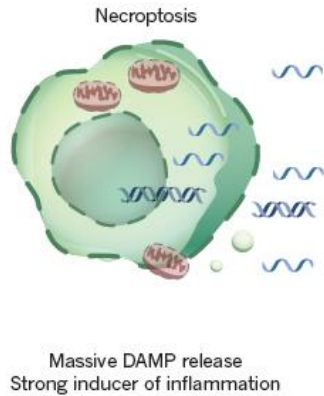
Limited DAMP release
Weak inducer of inflammation



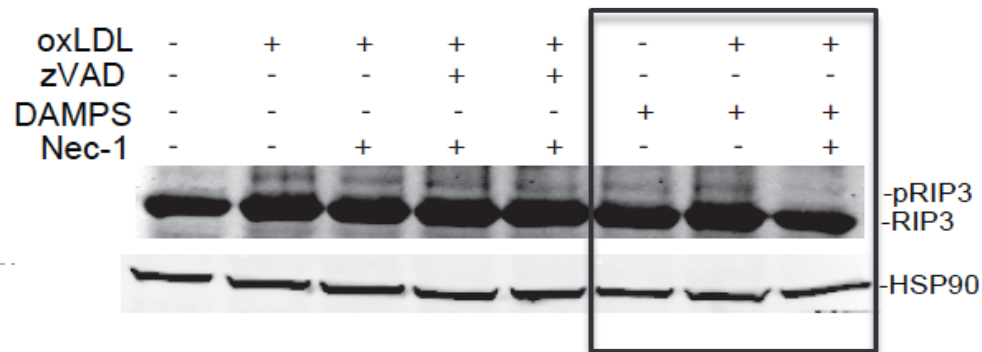
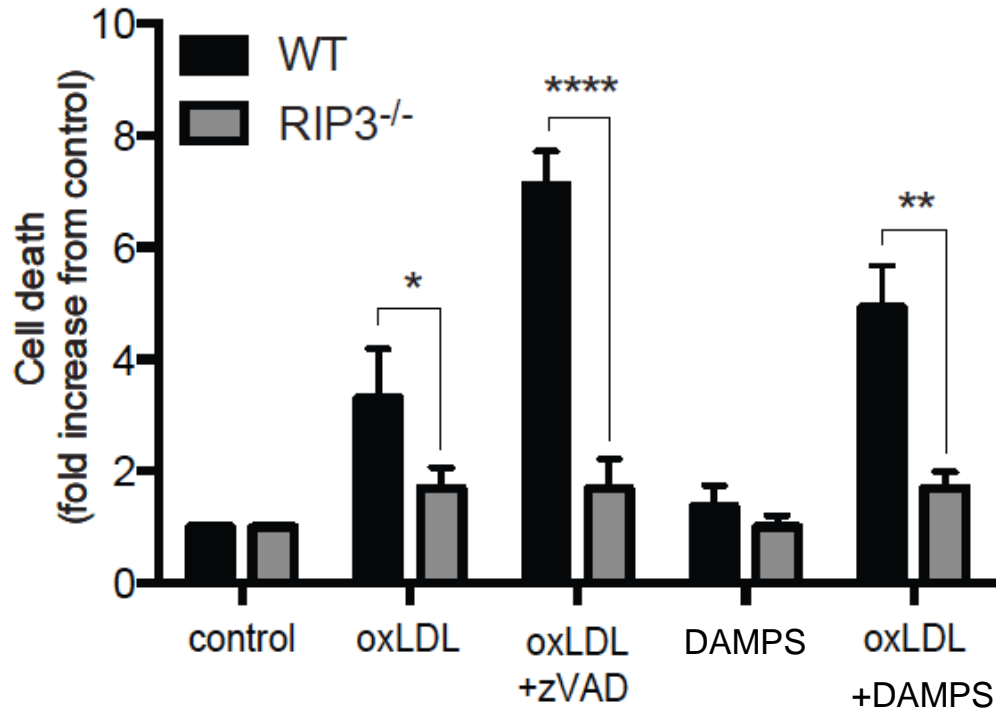
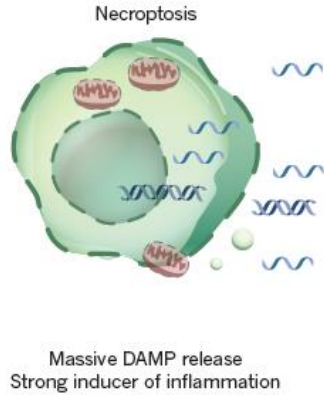
Massive DAMP release
Strong inducer of inflammation



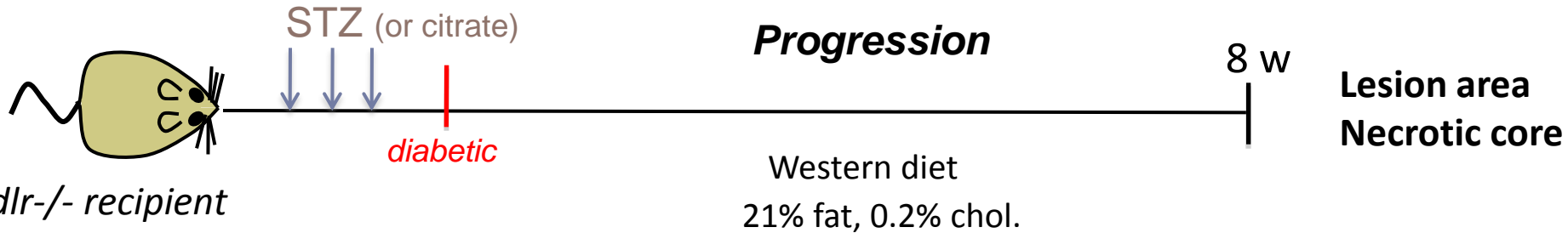
DAMPs exacerbate oxLDL-induced death



DAMPs exacerbate oxLDL-induced death

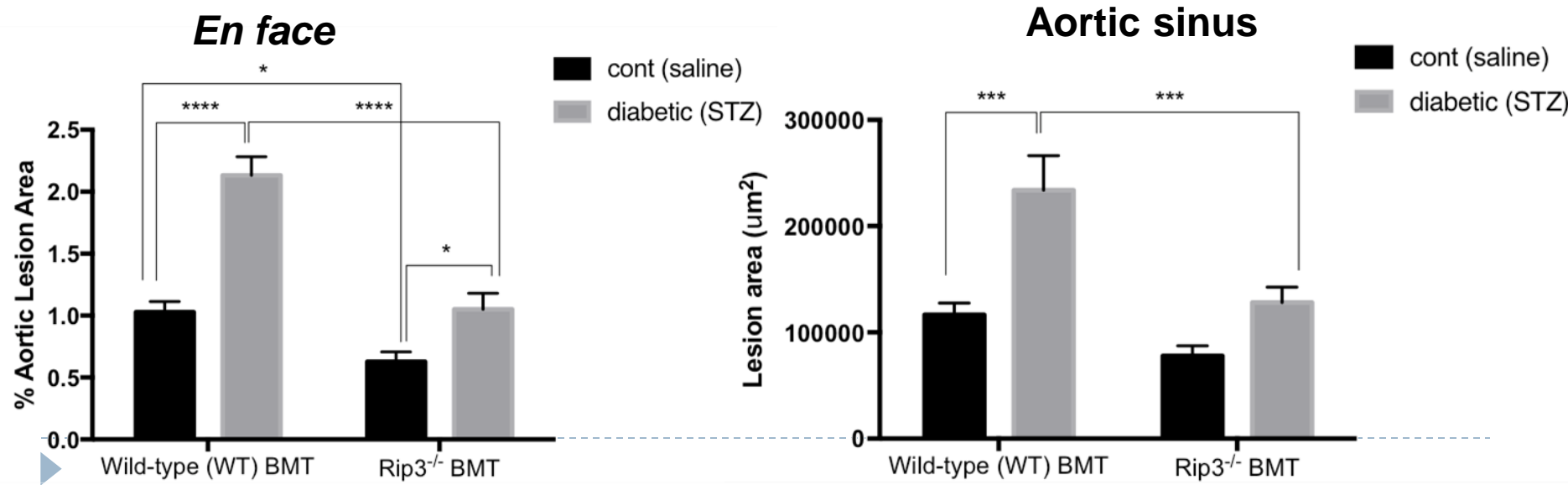


Does necroptosis contribute to accelerated atherosclerosis in diabetes?

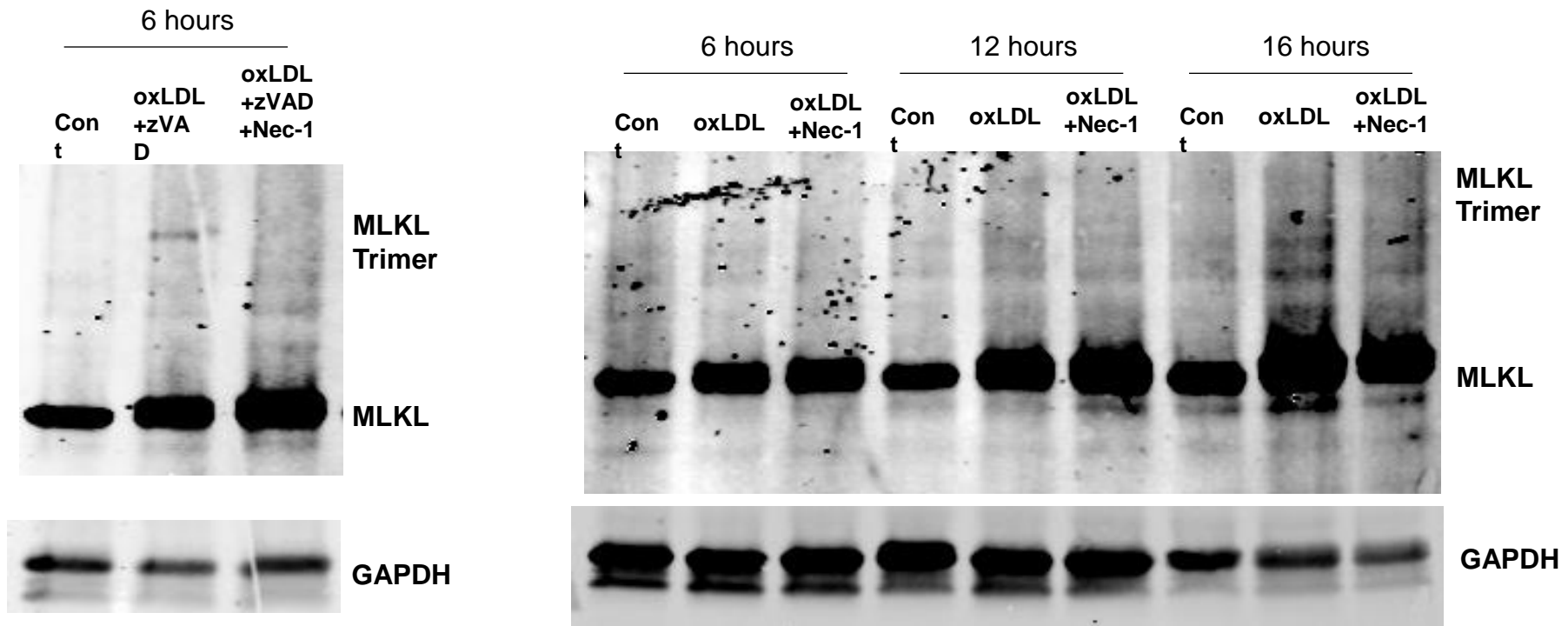


WT BMT

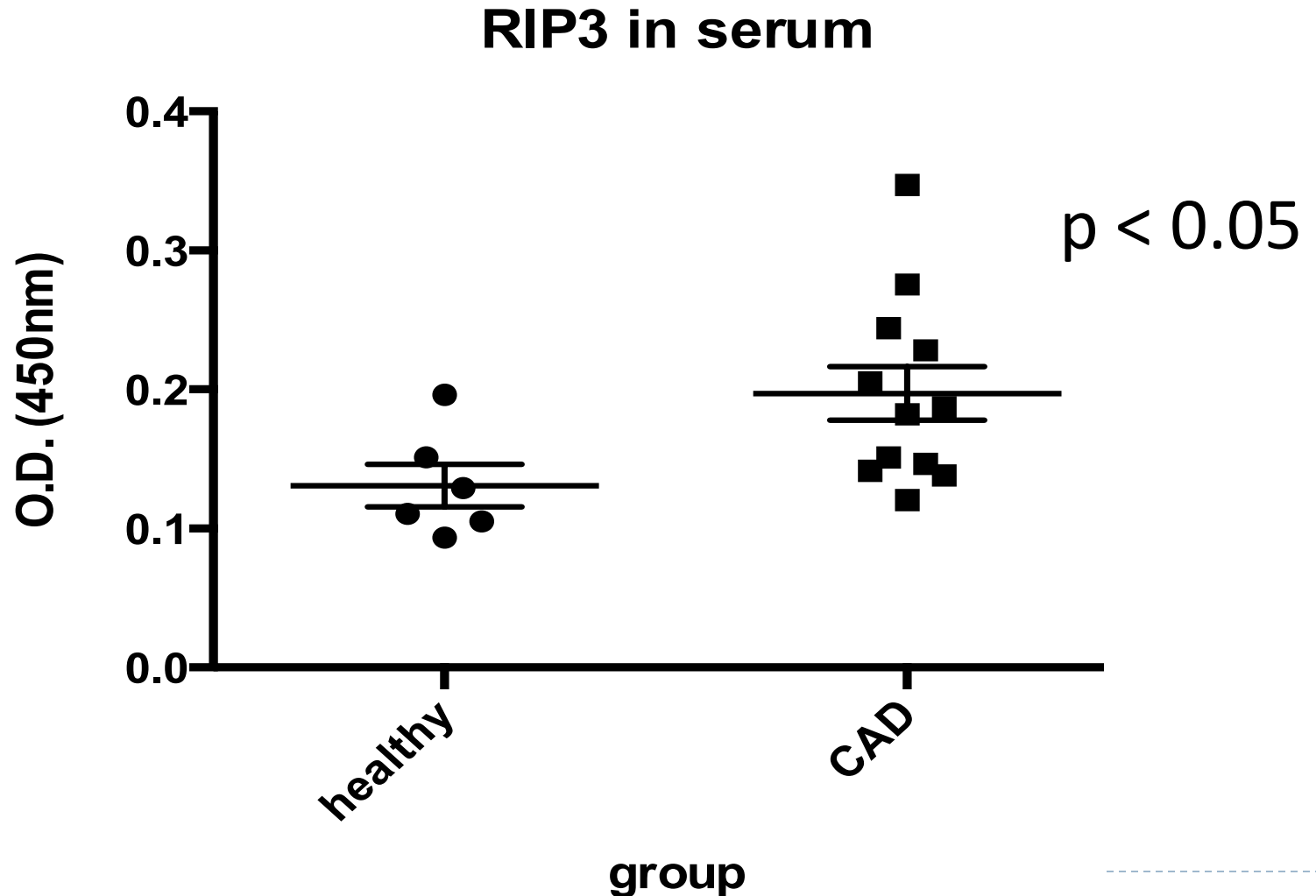
RIP3^{-/-} BMT



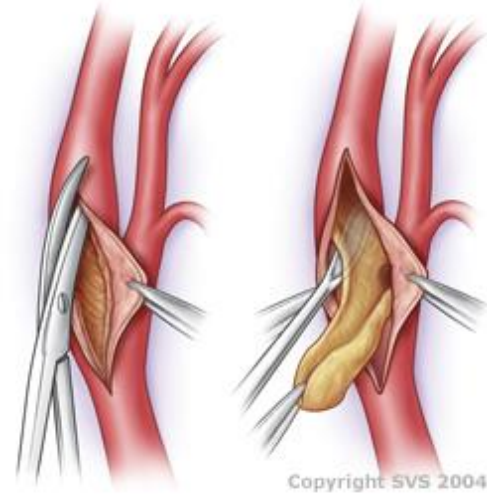
oxLDL induces MLKL trimer formation



Can the necroptotic pathway serve as a biomarker for CAD?



Is necroptosis involved in human atherosclerosis?

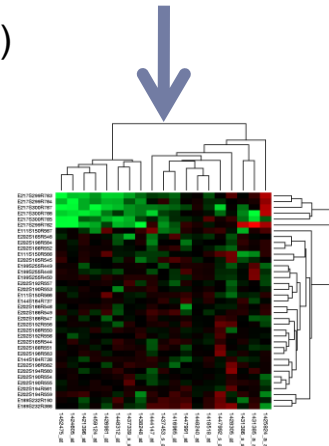


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Table I: Demographics of the microarray patient cohort (*p<0.05)

GENERAL	stable asymptomatic	unstable symptomatic	P value
number of patients	40	87	
age (years, mean)	66.4	72.52	0.0002*
gender (male/female)	39/1	61/26	0.0003*
BMI (mean)	27.5	24.67	ns
SYMPTOM	Not applicable		Not applicable
minor stroke (MS)		32 (36.78%)	
transitory ischemic attack (TIA)		29 (33.33%)	
amaurosis fugax (AF)		26 (29.89%)	

+control arteries (disease-free)
n=10

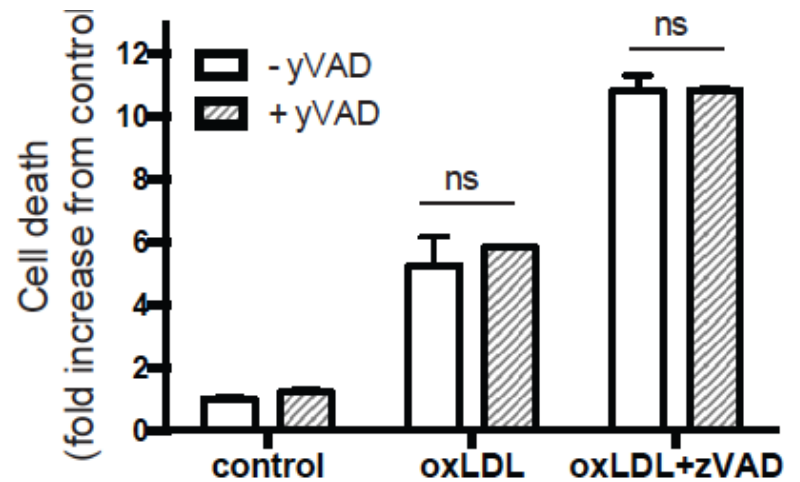
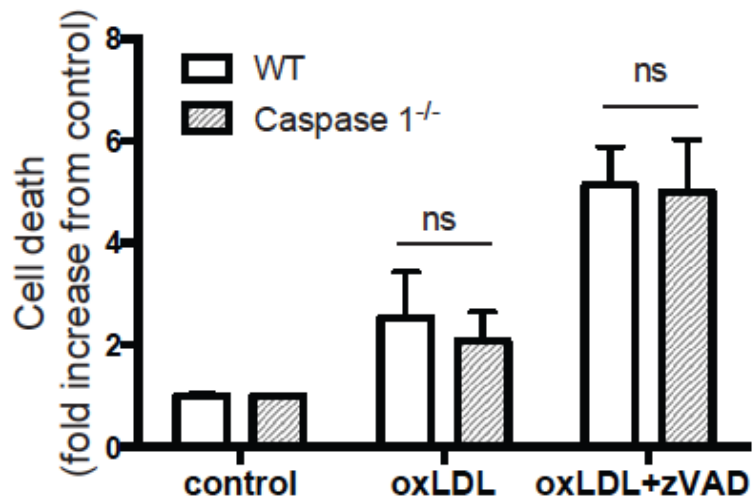
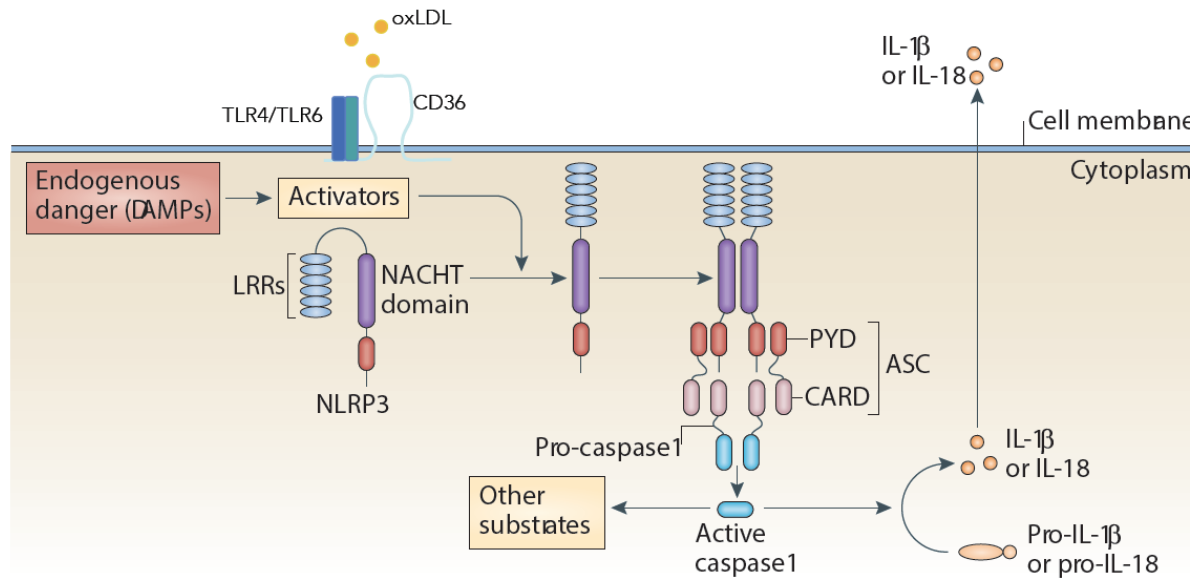


Karolinska
Institutet

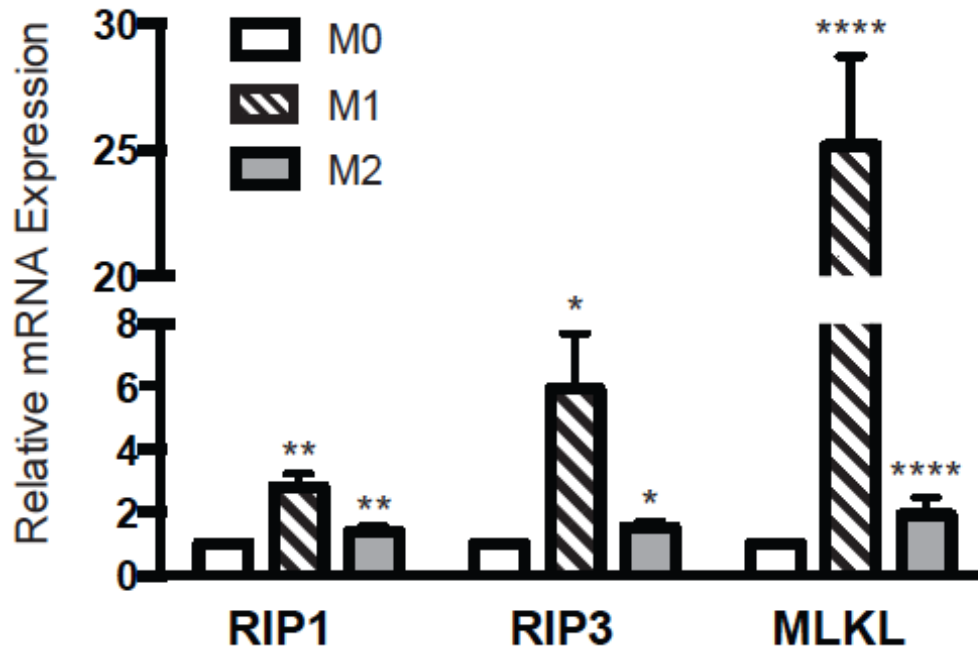
**Biobank of Karolinska
Endarterectomy (BiKE)**

Ljubica Perisic, Lars
Maegdefessel, Ulf Hedin,
Göran K Hansson,

oxLDL-induction of necroptosis is independent of the inflammasome

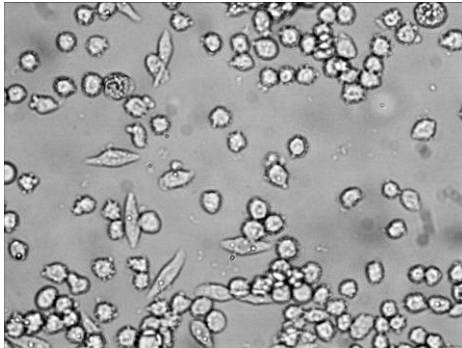


M1 macrophages have higher necroptotic gene expression



METHODS

Macrophages in culture

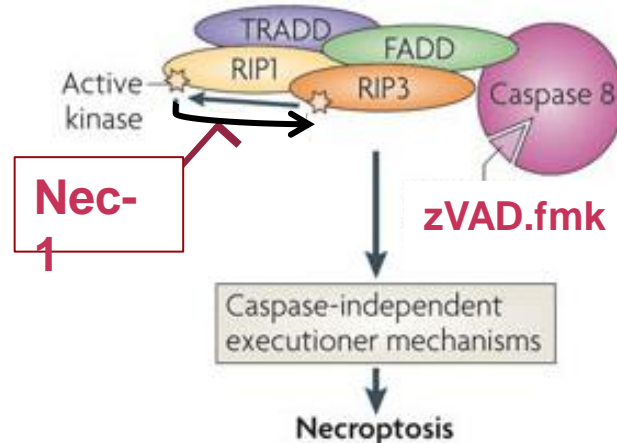


+ oxidized LDL \longrightarrow
24h

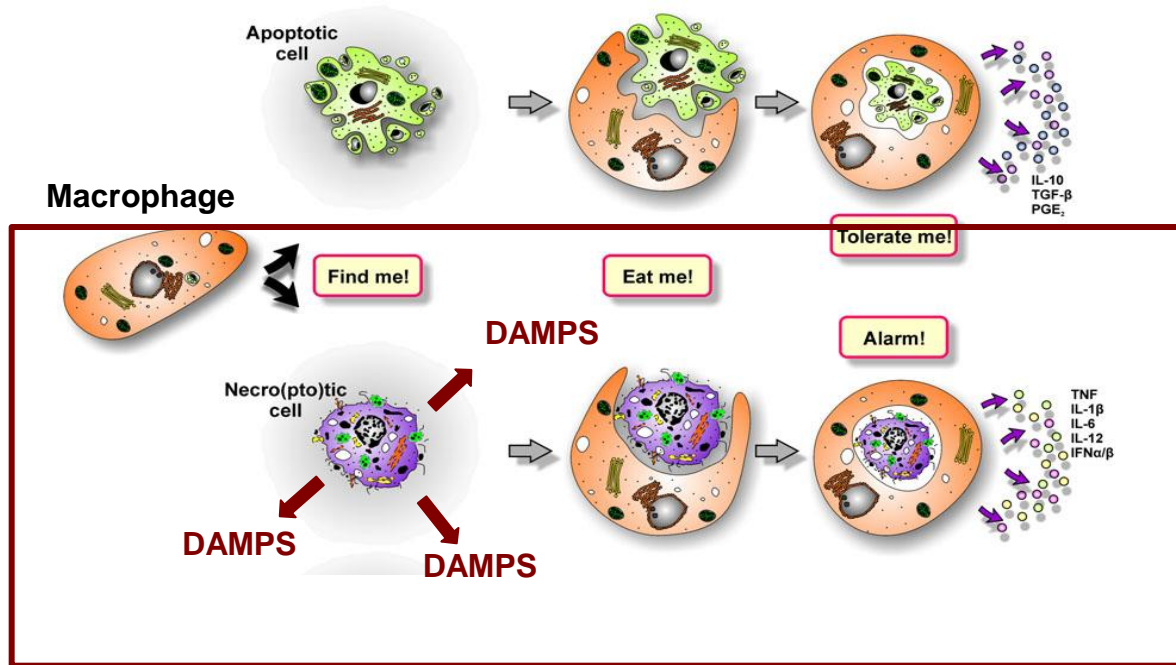
% Cell death

+ **Nec-1** (inhibitor of NECROPTOSIS)

+ **zvad** (inhibitor of apoptosis, \therefore NECROPTOSIS)



Other Ligands that induce Macrophage Necroptosis: DAMPS

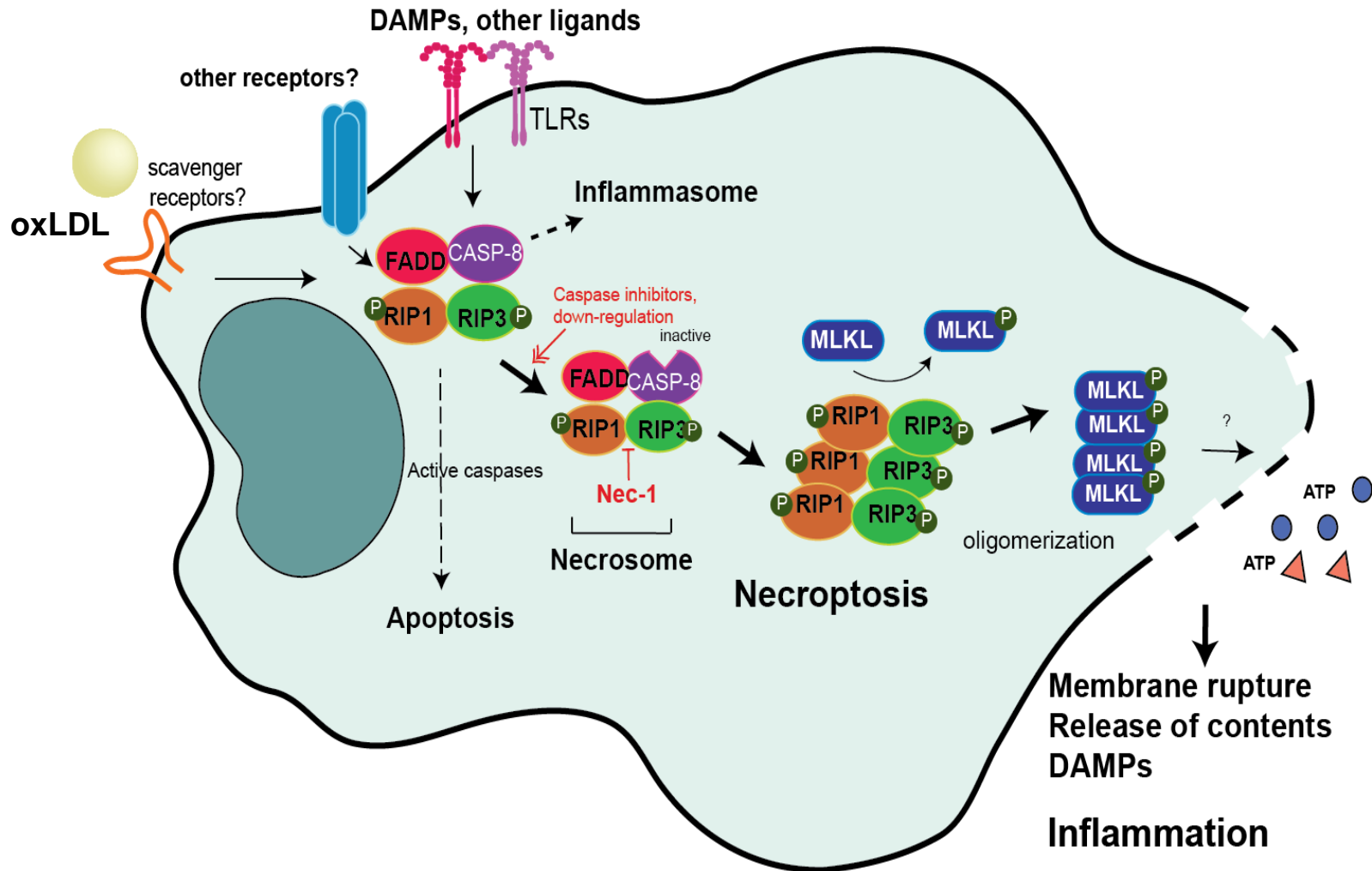


Generated DAMPS from necroptotic cells:

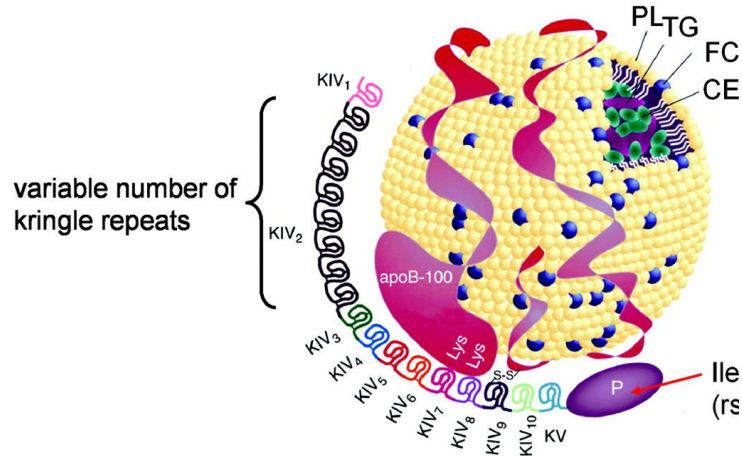
1. Mechanical:
Freeze thaw cycle
2. Chemical Induction:
LPS+zVAD
(biologically validated)

DAMPS = Damage Associated Molecular Patterns

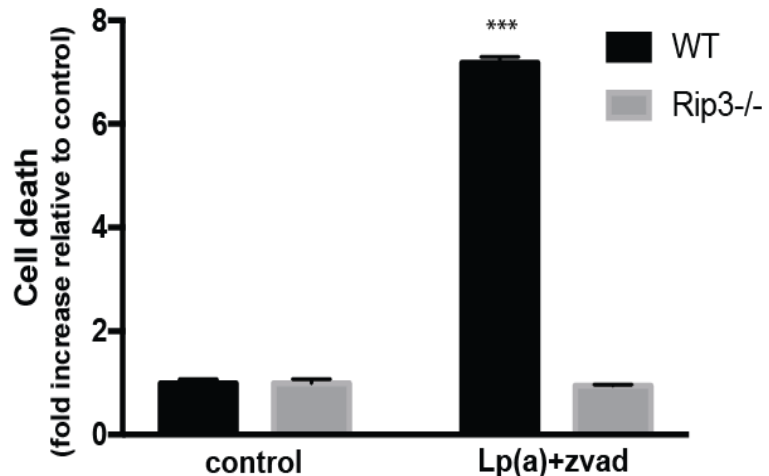
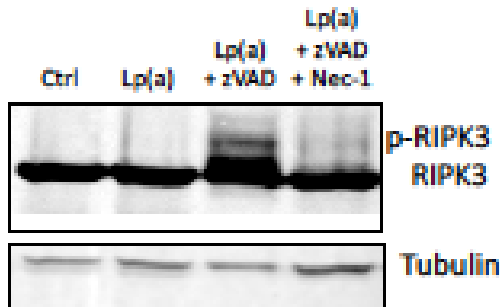
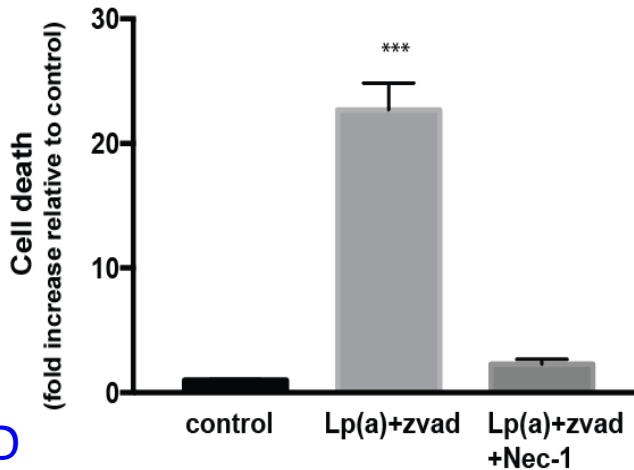
What are other drives of necroptosis in the plaque?



What are other drives of necroptosis in the plaque?



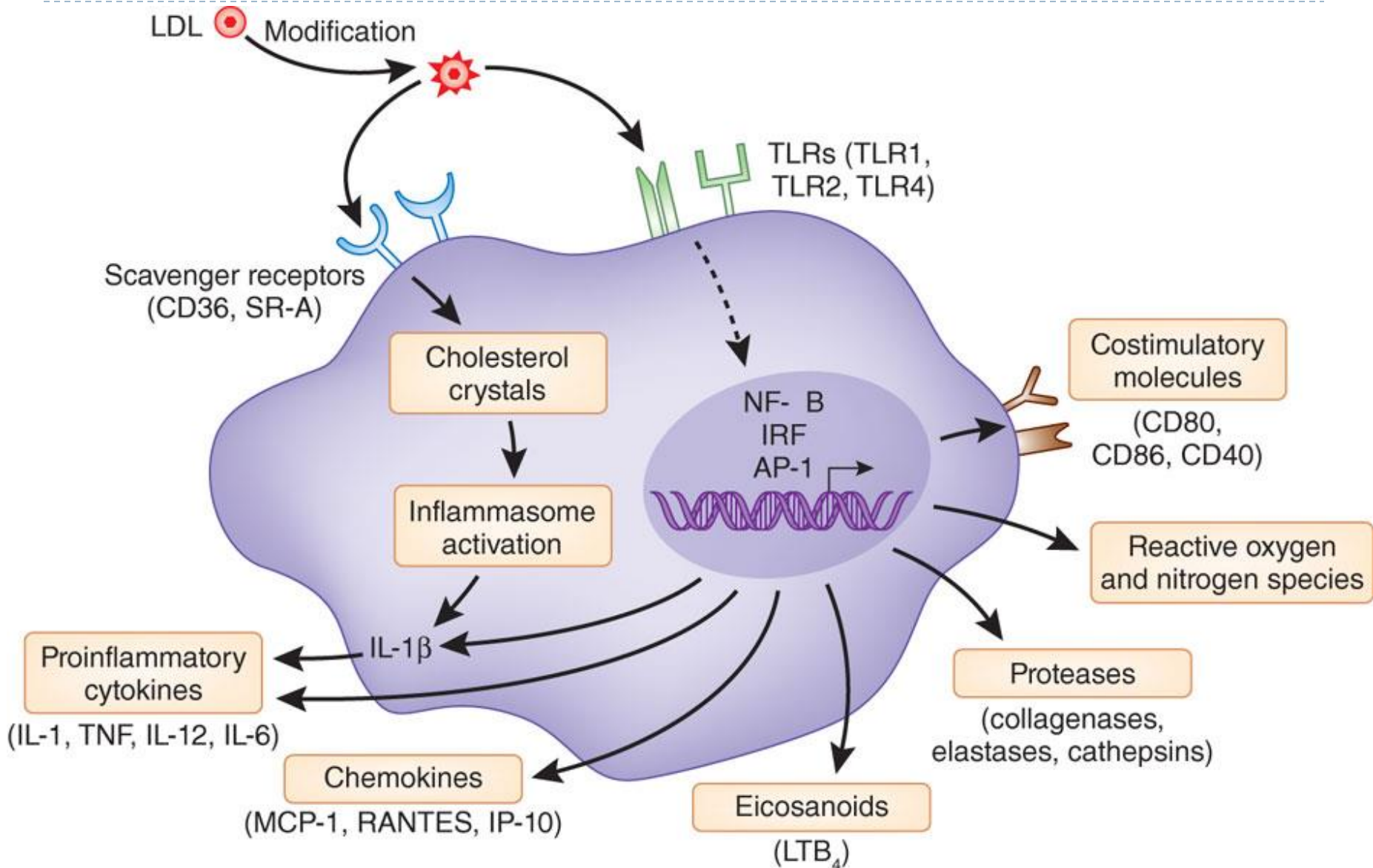
Lipoprotein (a) = strong predictor of CAD



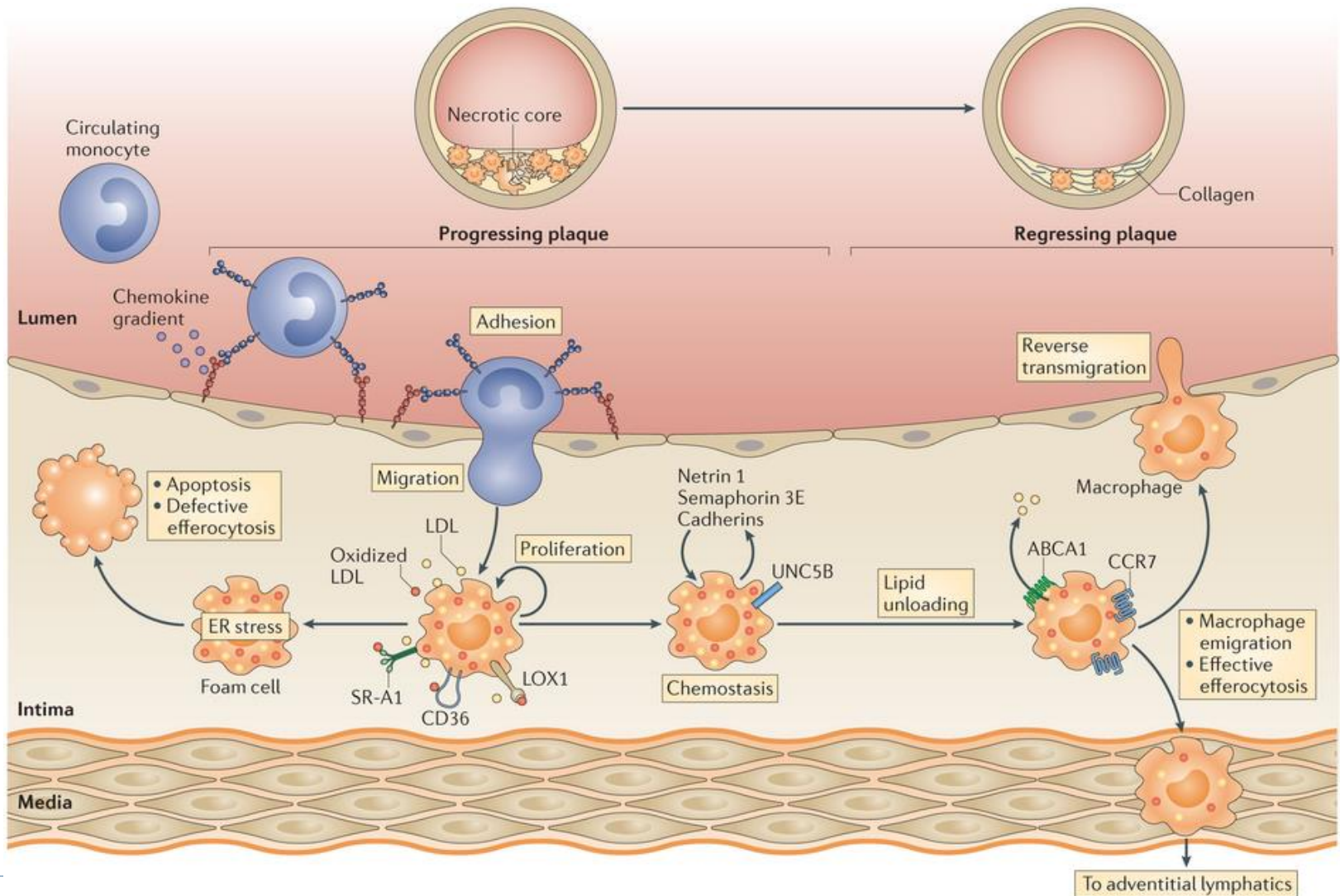
INVOLVEMENT OF OTHER GENES

GENES	ROLE IN NECROPTOSIS
Mixed lineage kinase domain-like (MLKL)	Phosphorylation by RIP3 critical for necroptosis.
Cylindromatosis (CYLD)	Facilitates RIP1 deubiquitination at the TNF receptor1 (TNFR-1) complex
Cyclophilin D	Controls mitochondrial permeability

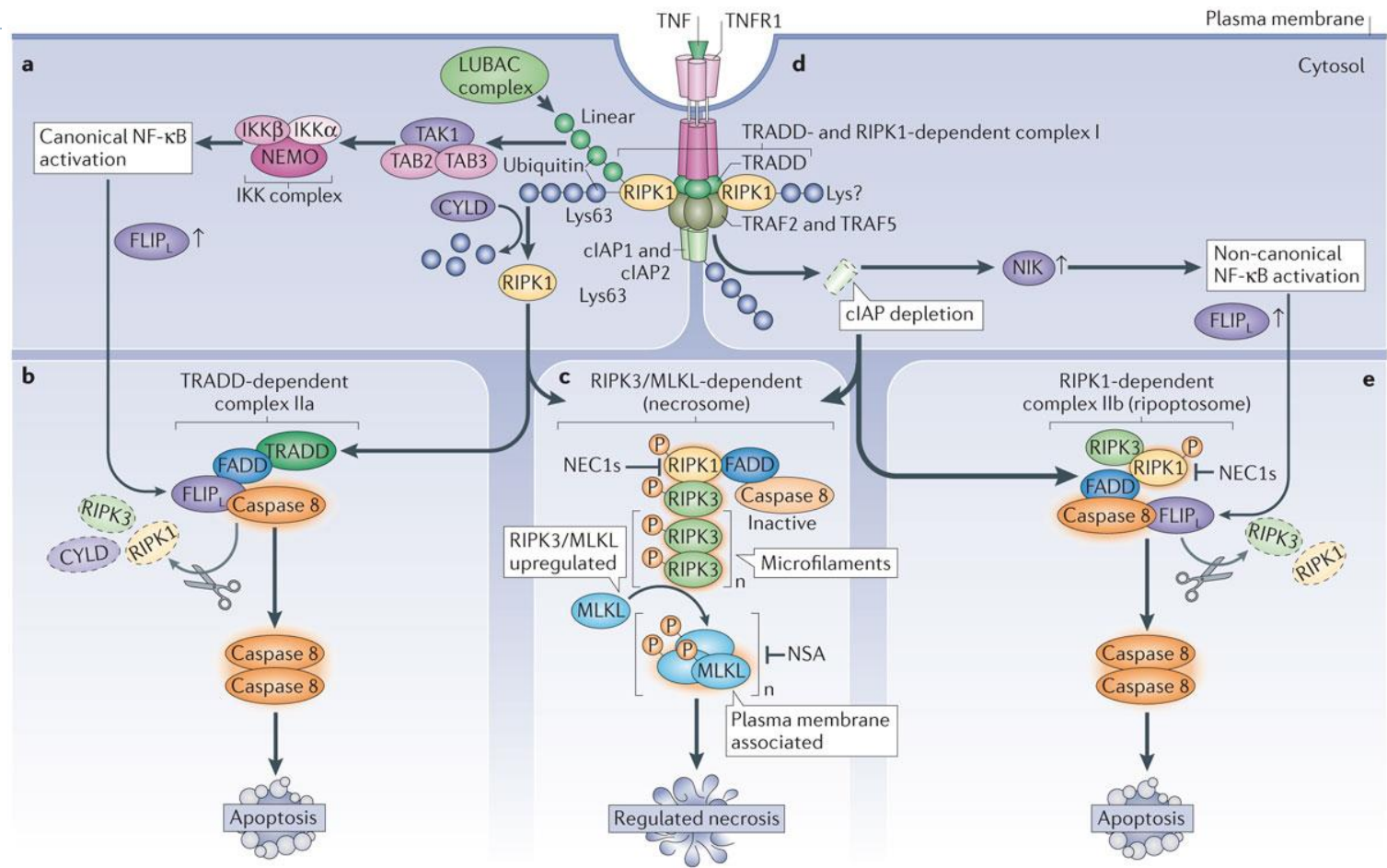
What drives inflammation in the plaque?



Macrophages in atherosclerosis

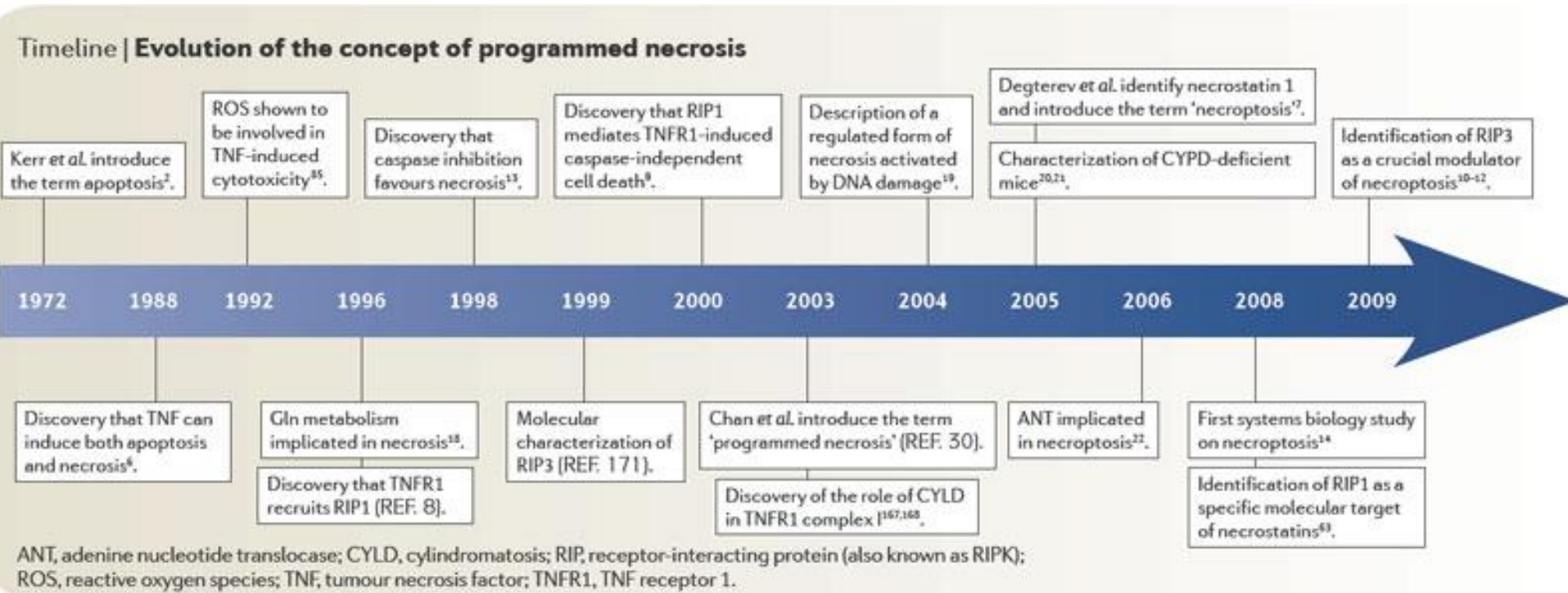


Necroptosis = programmed cell necrosis



Nature Reviews | Molecular Cell Biology

Necroptosis = programmed cell necrosis

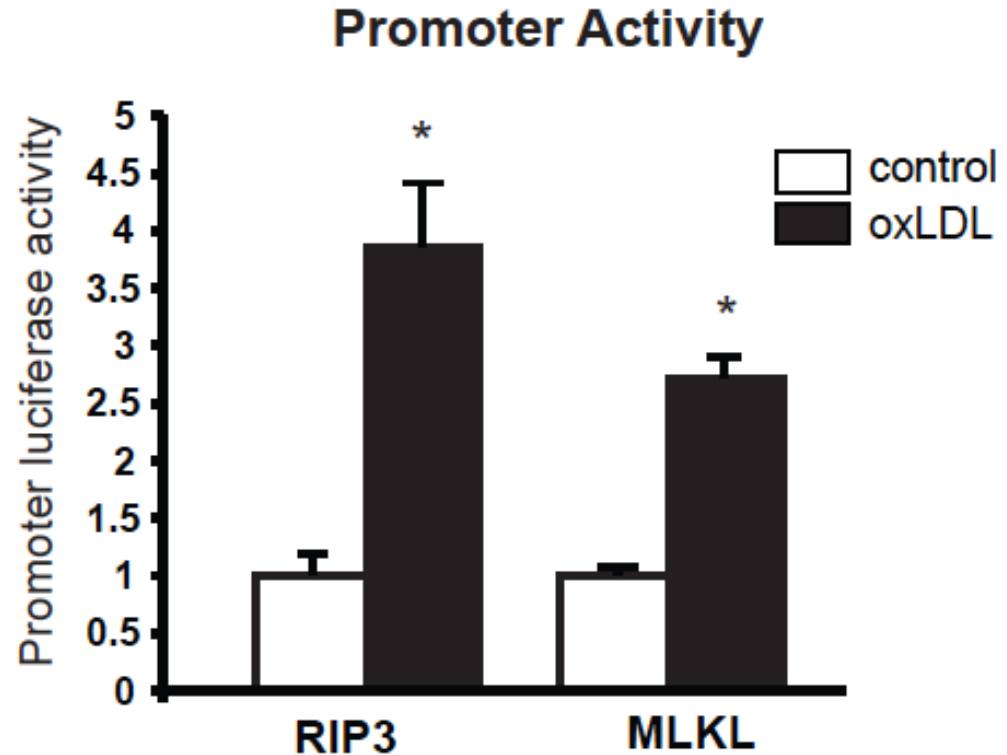
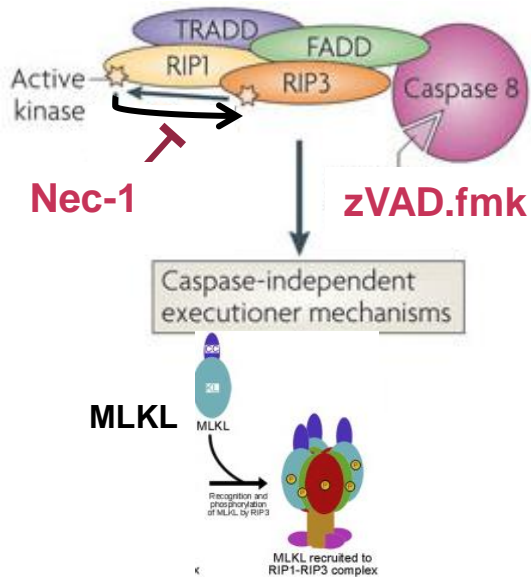


Summary so far

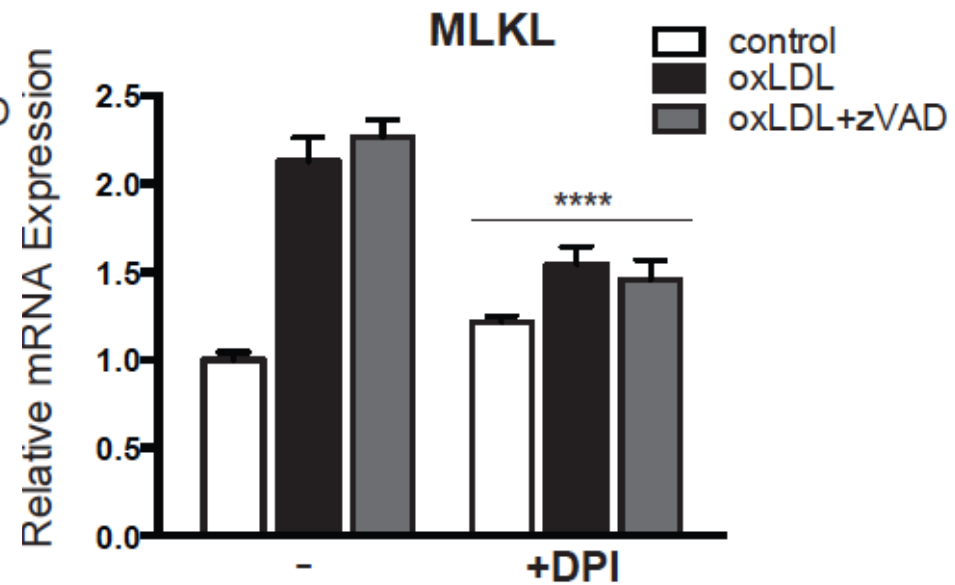
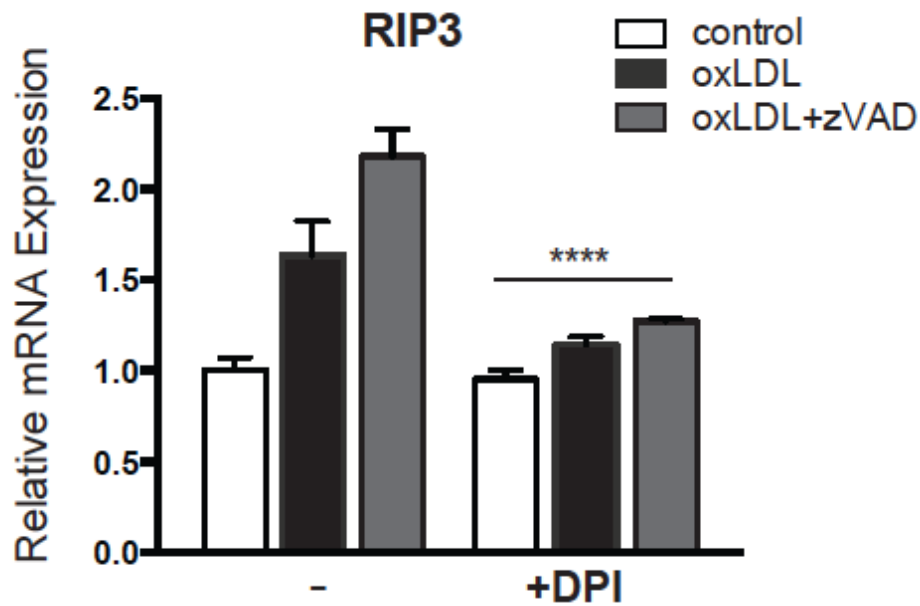
- Macrophages undergo necroptosis:
 - Treatment with “inflammatory” ligand(s)
 - increases cell death, which can be inhibited by Nec-1
 - increases RIP3 phosphorylation
 - increases expression of necroptotic genes (e.g. RIP3, MLKL)
 - amplified necroptotic cell death in the presence of DAMPs
- Necroptosis relevant in atherosclerosis
 - **INTERVENTION** mouse model
 - Associated with plaque vulnerability in patients with carotid atherosclerosis



How do atherogenic ligands drive necroptosis?

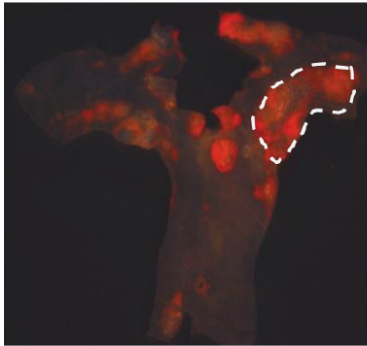


oxLDL induces RIP3 and MLKL via Reactive Oxygen Species

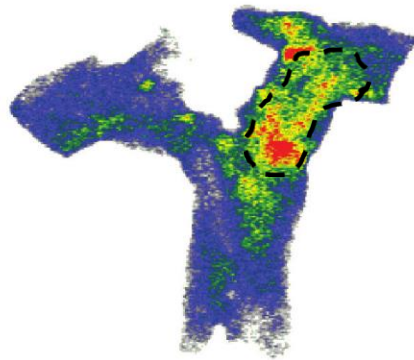


Visualization Atherosclerosis using radiolabeled Nec-1 in ApoE^{-/-} mice

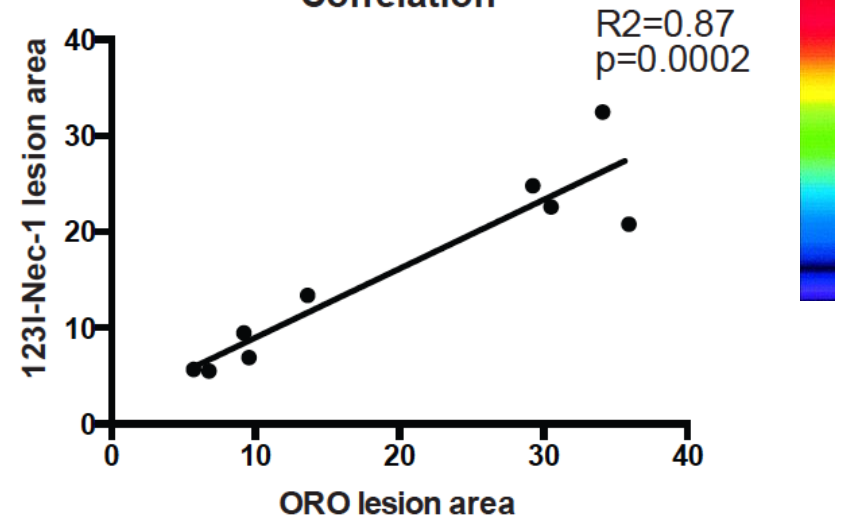
Aortic En face
Oil-Red-O Stain



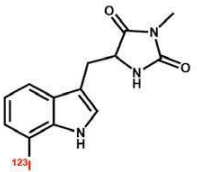
Autoradiogram



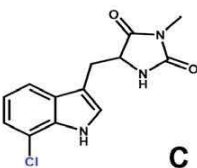
Correlation



Nec-1 Tracer



Blocker



C

