



# 3<sup>rd</sup> Paris NASH Symposium

French-US Meetings

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Institut Pasteur - Paris

Organized by  
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Virginia Commonwealth University School of Medicine, Richmond, Virginia, US  
Hôpital Saint-Antoine, APHP, Inserm, Université Pierre & Marie Curie, Paris, France

With the partnership of



# Intrahepatic Intracellular Activators of Innate Immunity in NASH



*W. Mehal MD. D.Phil.*

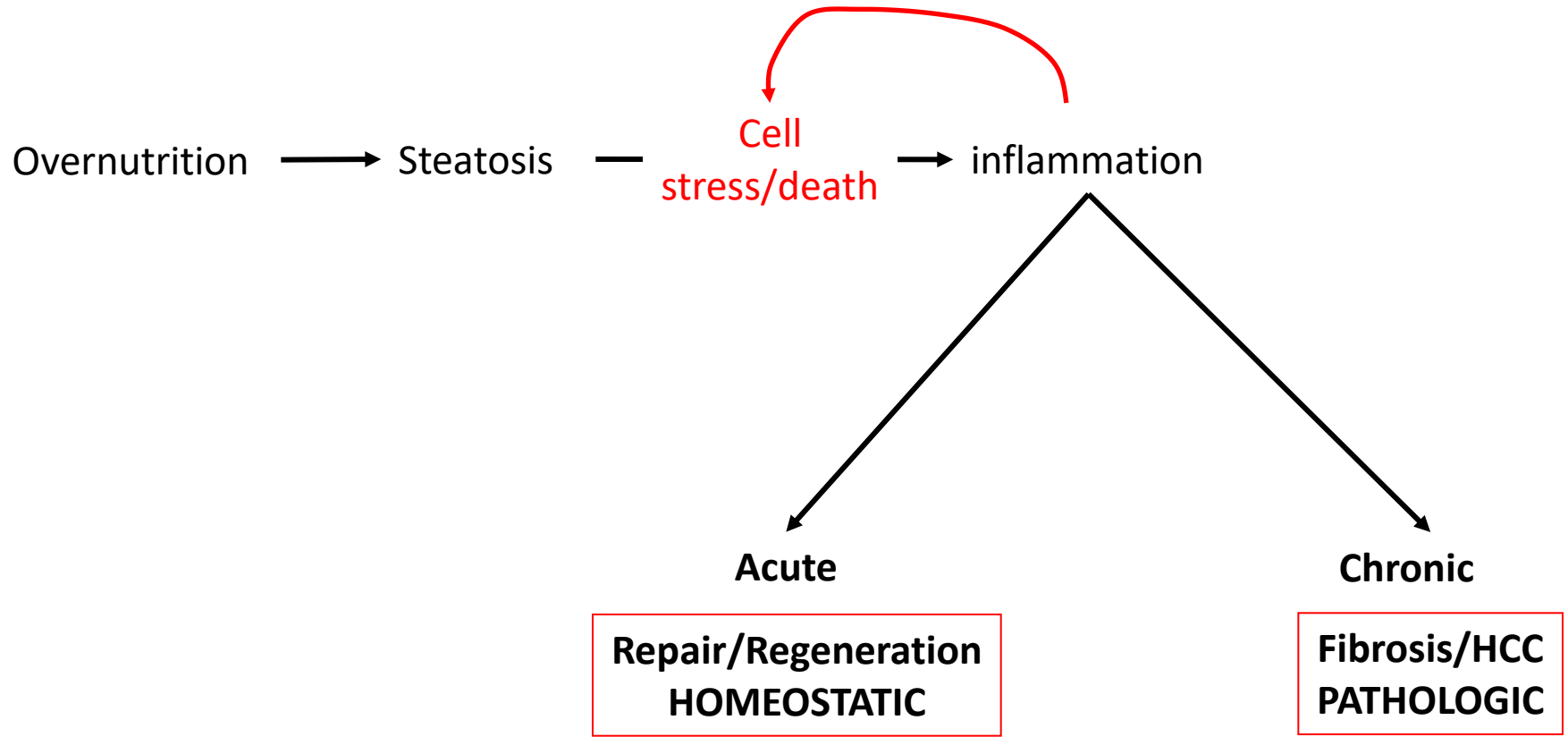
*Director Yale Fatty Liver Program*



## **Overview:**

- 1) How did the concept of sterile inflammation and DAMPs develop?**
- 2) What are DAMPs and what can they do?**
- 3) Are DAMPs important in NASH?**
- 4) What are the therapeutic implications of the biology of DAMPs for NASH?**

# Overview



## Pattern Recognition Receptors (PRRs) and sterile inflammation

Table 2. SUSCEPTIBILITY OF *A/HeJ* and *C3H/HeJ* MICE TO THE LETHAL EFFECT OF ENDOTOXIN

Mouse strain	Endotoxin	$LD_{50}$ ( $\mu\text{g}$ )*
<i>A/HeJ</i>	<i>E. coli</i> 0127 : B8	60
	<i>S. typhosa</i> 0-901	45
<i>C3H/HeJ</i>	<i>E. coli</i> 0127 : B8	2,240
	<i>S. typhosa</i> 0-901	1,020

B. Sultzer  
Nature 1968

**The immune system is designed to detect/respond to non-self**

		mouse/rat		
		712		
C3H/HeJ (700)	...RFHLCLHYRDFI	H	GVAIAANIIQEGFHKS...	(730)
C3H/HeN(700)	...RFHLCLHYRDFI	P	GVAIAANIIQEGFHKS...	(730)
rat (700)	...RFQLCLHYRDFI	P	GVAIAANIIQEGFHKS...	(730)
human (702)	...PFQLCLHYRDFI	P	GVAIAANIIHEGFHKS...	(732)

• •

A. Poltorak et al Science 1998

What about inflammation without a pathogen (sterile inflammation)?



is825-088 www.fotosearch.com



## TOLERANCE, DANGER, AND THE EXTENDED FAMILY\*

*Polly Matzinger*



For many years immunologists have been well served by the viewpoint that the immune system's primary goal is to discriminate between self and non-self. I believe that it is time to change viewpoints and, in this essay, I discuss the possibility that the immune system does not care about self and non-self, that its primary driving force is the need to detect and protect against danger, and that it does not do the job alone, but receives positive and negative communications from an extended network of other bodily tissues.

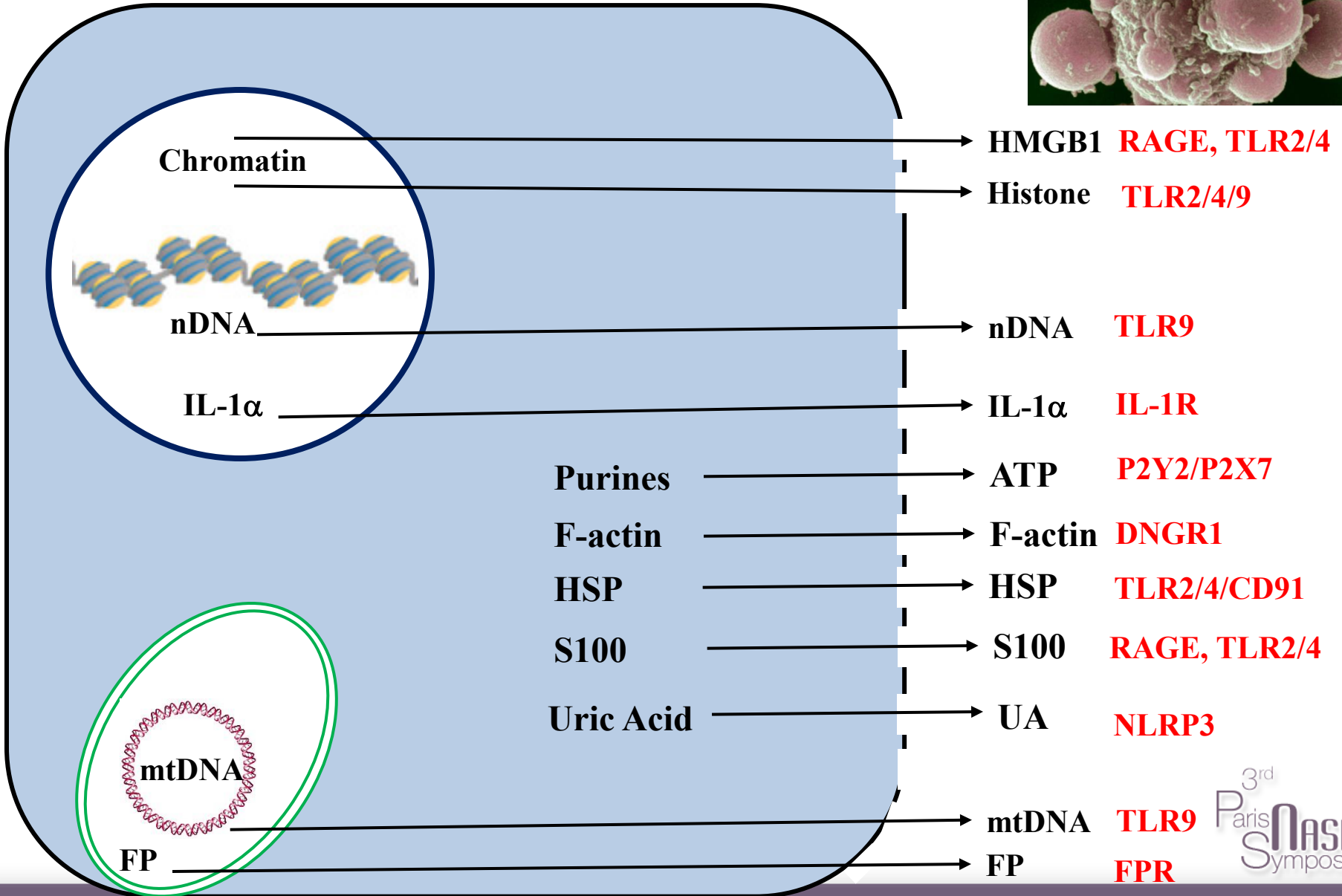
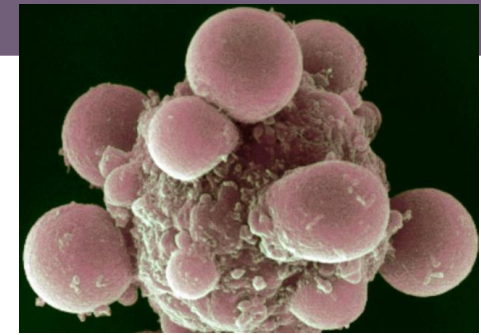
Ann. Rev. Immunology 1994

## Pattern Recognition Receptor (**PRR**)/Ligand Relationships

<b>TLR1</b>	<b>Bacterial lipopeptides</b>
<b>TLR2</b>	<b>Bacterial glycolipids</b> <b>lipopeptides, HSP70, HMGB1</b>
<b>TLR3</b>	<b>Viral double stranded RNA</b>
<b>TLR4</b>	<b>Bacterial LPS</b> <b>fibrinogen, heparan sulfate, hyaluronic acid</b>
<b>TLR5</b>	<b>Flagellin</b>
<b>TLR6</b>	<b>Mycoplasma lipopeptides</b>
<b>TLR7</b>	<b>Viral single stranded RNA</b> <b>self single stranded RNA</b>
<b>TLR8</b>	<b>Viral single stranded RNA</b> <b>self single stranded RNA</b>
<b>TLR9</b>	<b>Bacterial double stranded DNA</b> <b>self double stranded DNA</b>

**PRRs** sense pathogen-associated molecular products (**PAMPs**), **AND** damage-associated molecular products (**DAMPs**)

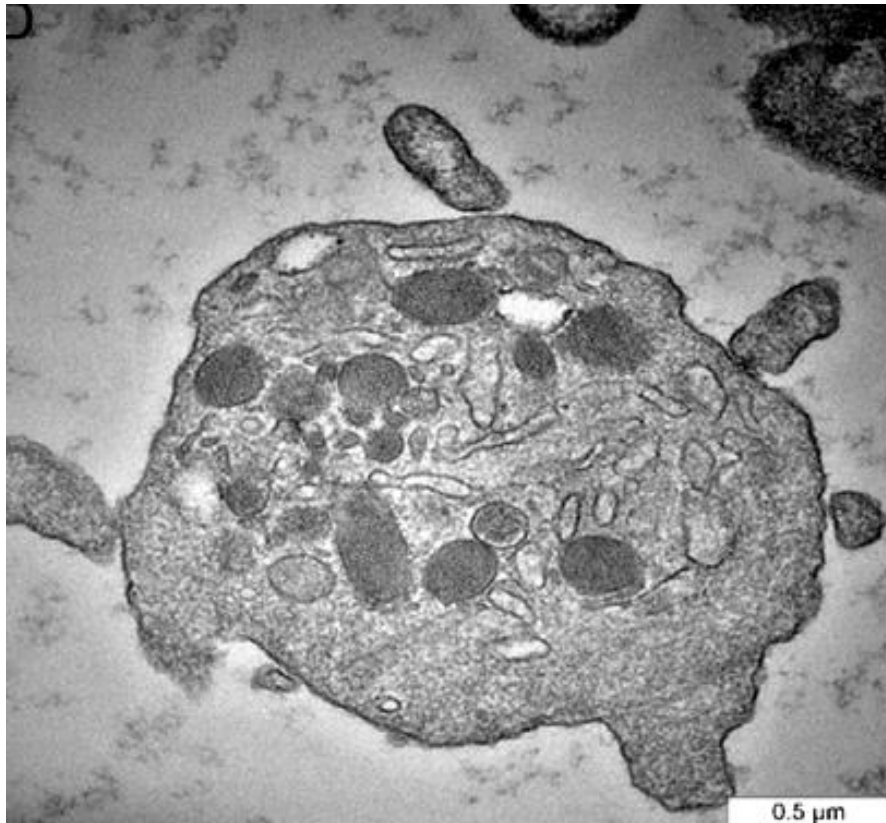
# DAMPs are Released from Necrotic Hepatocytes





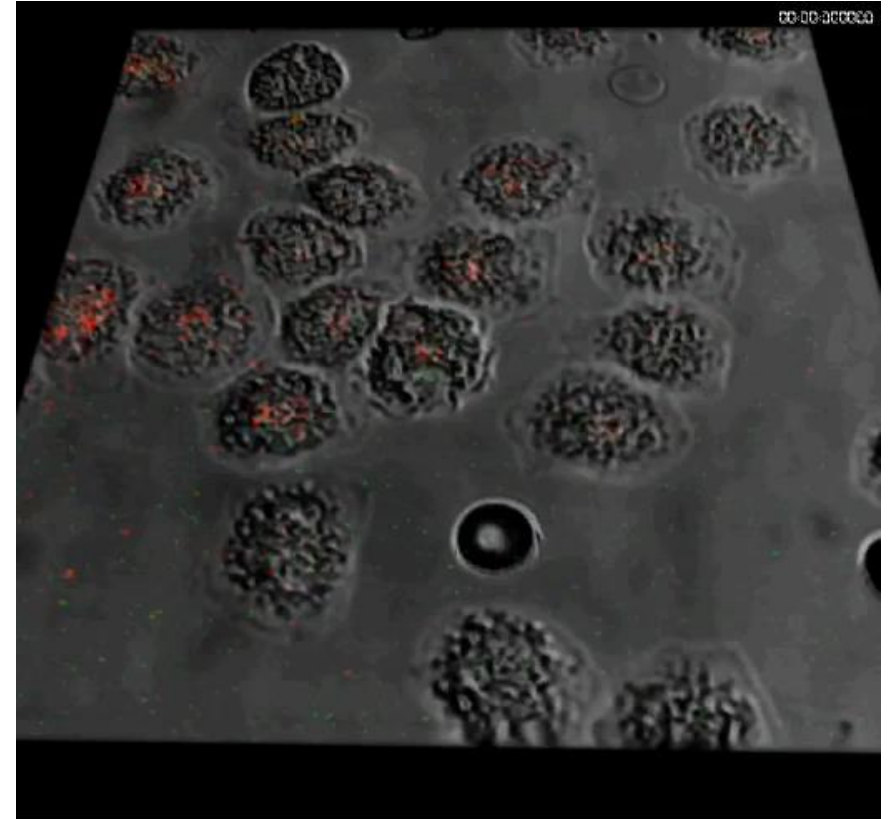
## Hepatocytes are not the only source of DAMPs

### Platelets



Almhanawi, BH. Porto Biomedical Journal 2017

### Neutrophils



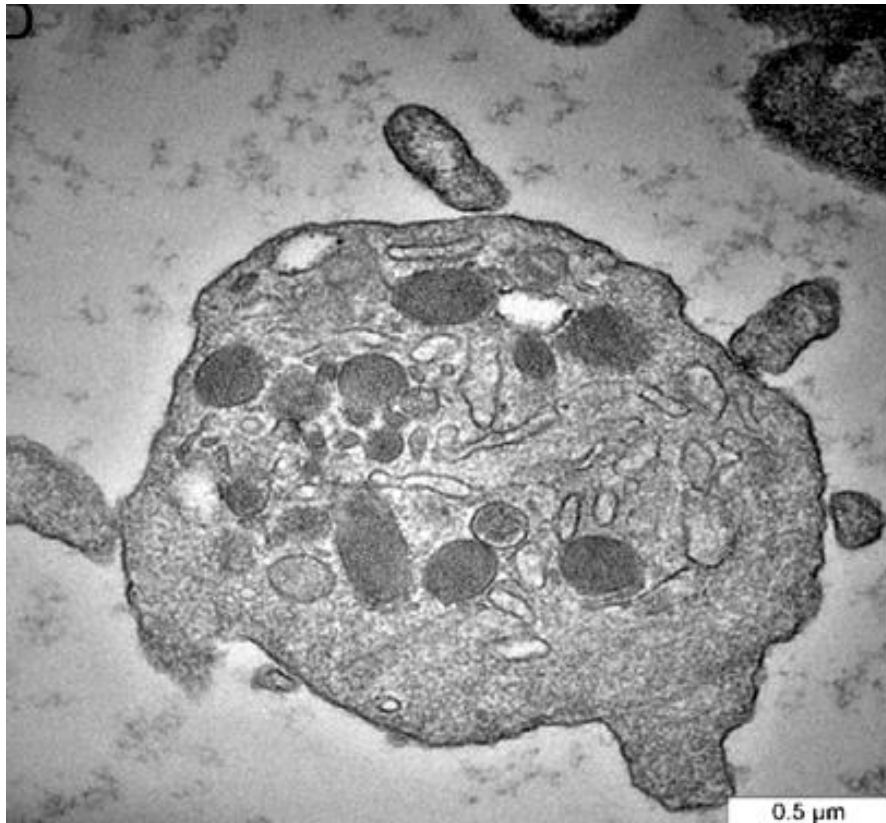
V Brinkmann JCB 2012

Neutrophil elastase: **Green**

Chromatin: **Red**

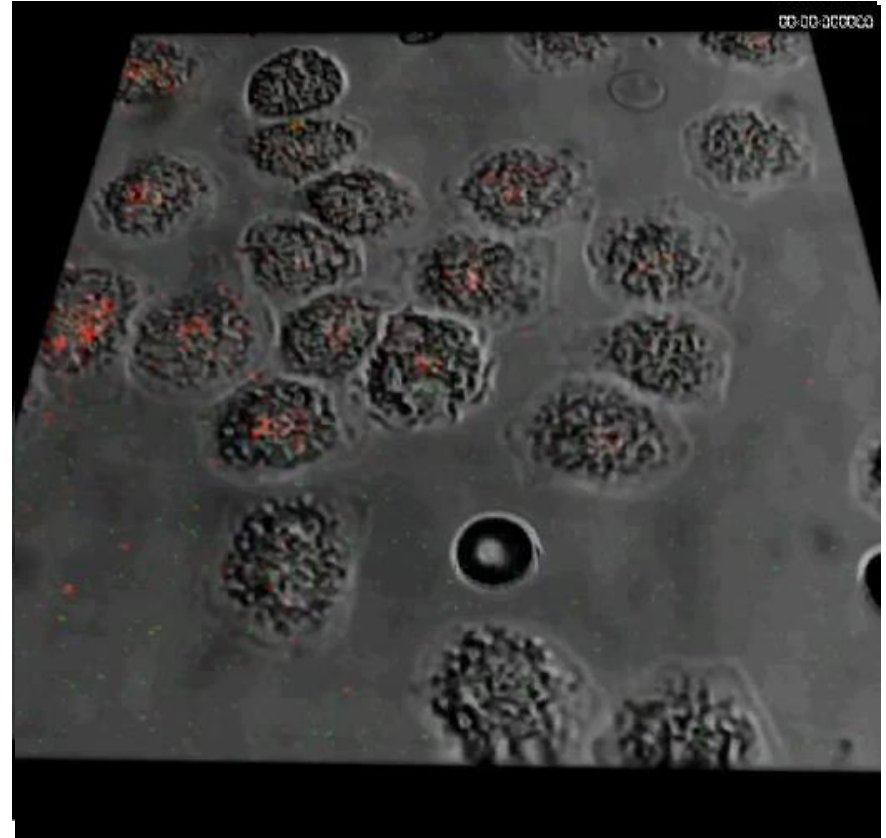
## Hepatocytes are not the only source of DAMPs

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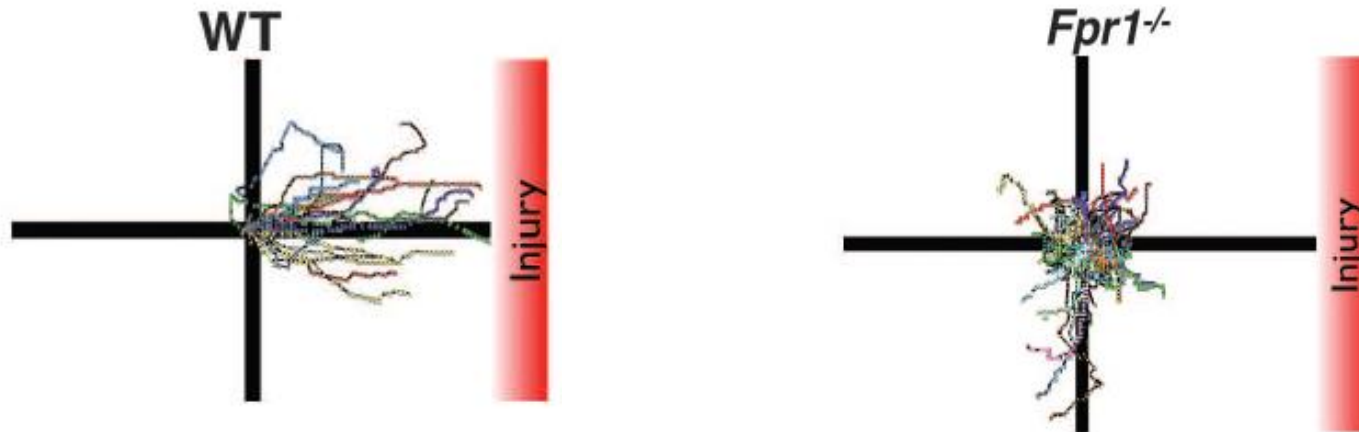
V Brinkmann JCB 2012

Neutrophil elastase: **Green**

Chromatin: **Red**

# DAMPs have Multiple Functions in Addition to Initiating Inflammation

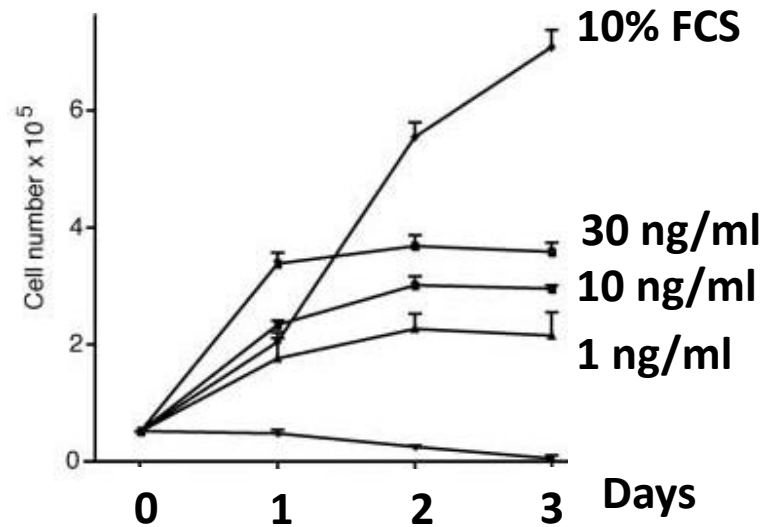
## Migration



McDonald B Science 2010

## Proliferation

### Proliferation of Embryonic Mesangioblasts

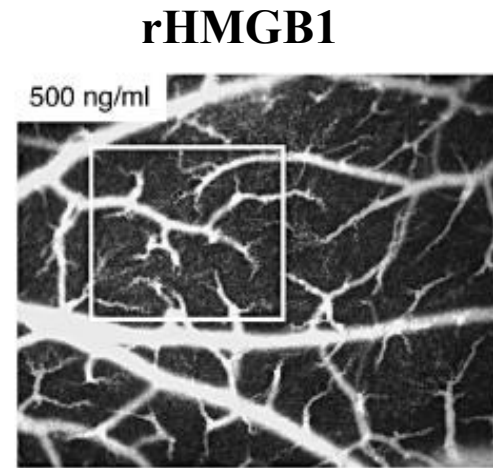
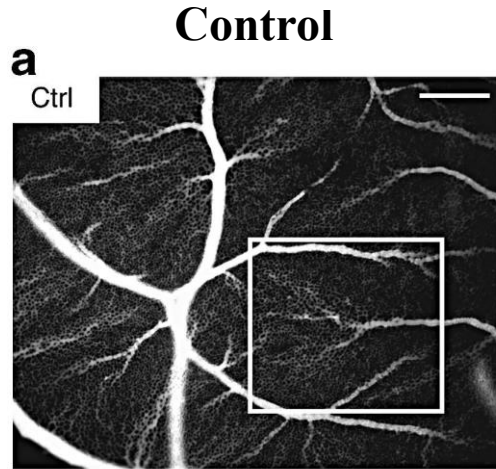


Palumbo R. JCB 2004

# DAMPs have Multiple Functions in Addition to Initiating Inflammation

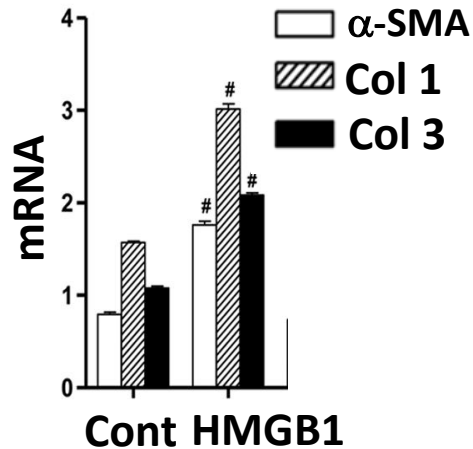
## Angiogenesis

Chorioallantoic membrane

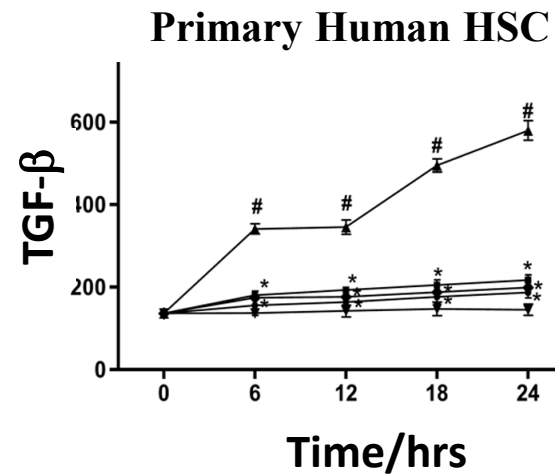


## Fibrogenesis

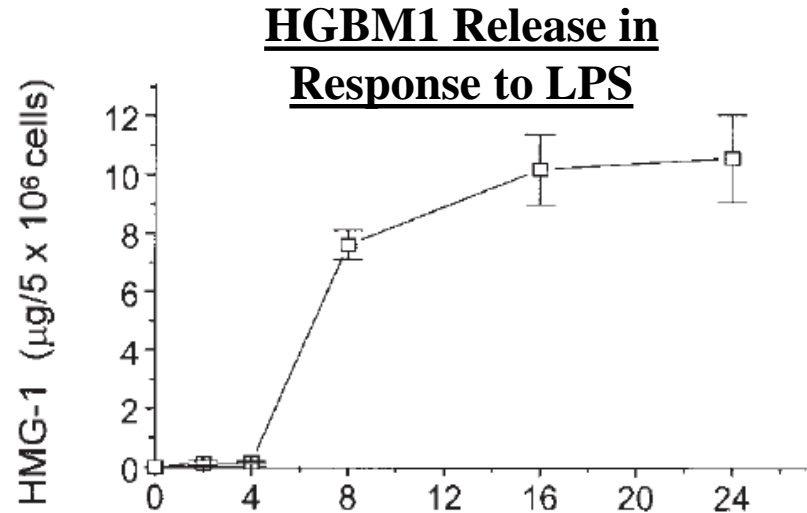
Van Beijnum JR. Oncogene 2013



Wang F. PLOS One 2013

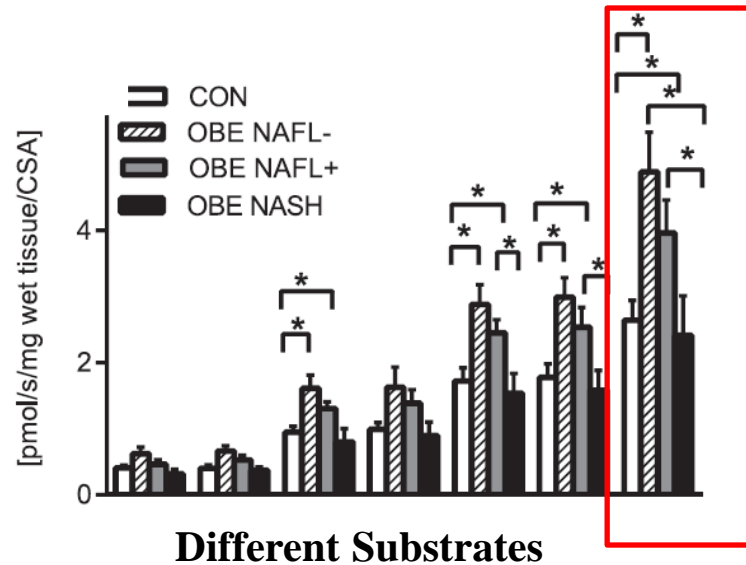


## Cells Under Stress Can Also Release DAMPs

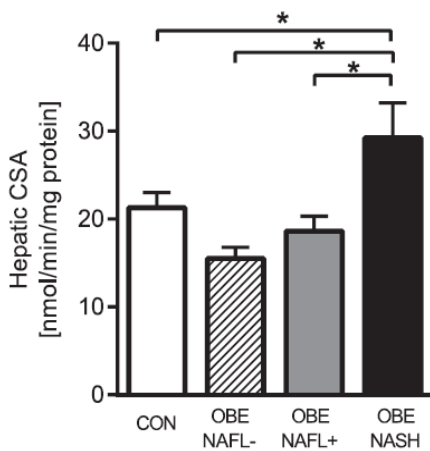


**What is the Hepatocyte Stress/Injury in Human NASH?**

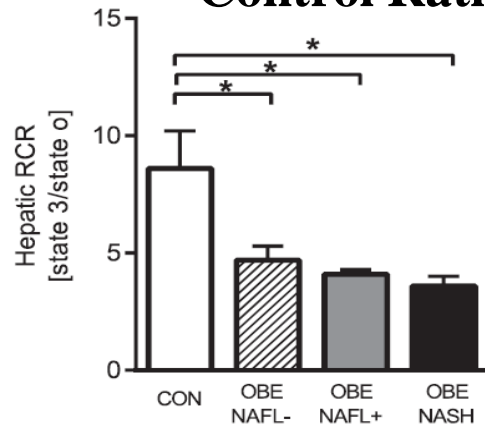
# Loss of Mitochondrial Adaptation in Human NASH



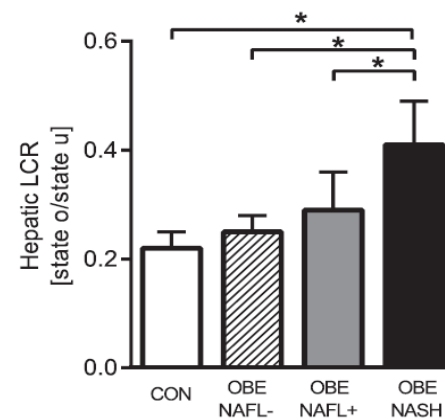
## Mito Mass



## Respiratory Control Ratio





## Leaking Control Ratio

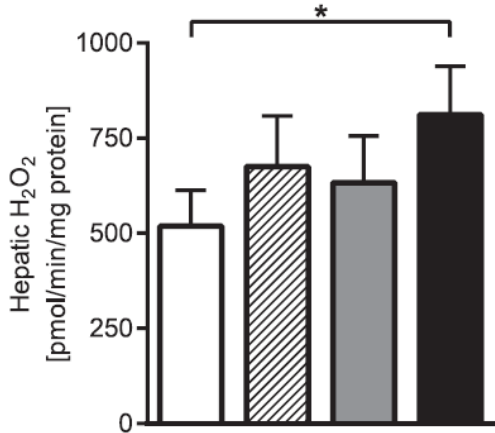


Koliaki C.  
Cell Metab 2015

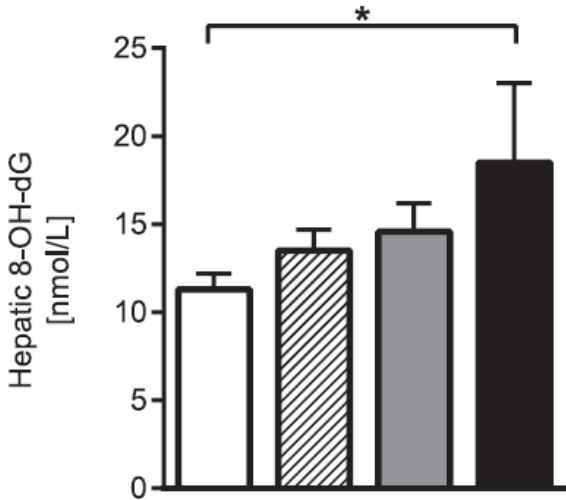
# Increased Mitochondrial ROS Production in Human NASH

-  CON
-  OBE
-  OBE + NAFLD
-  OBE + NASH

## ROS Production



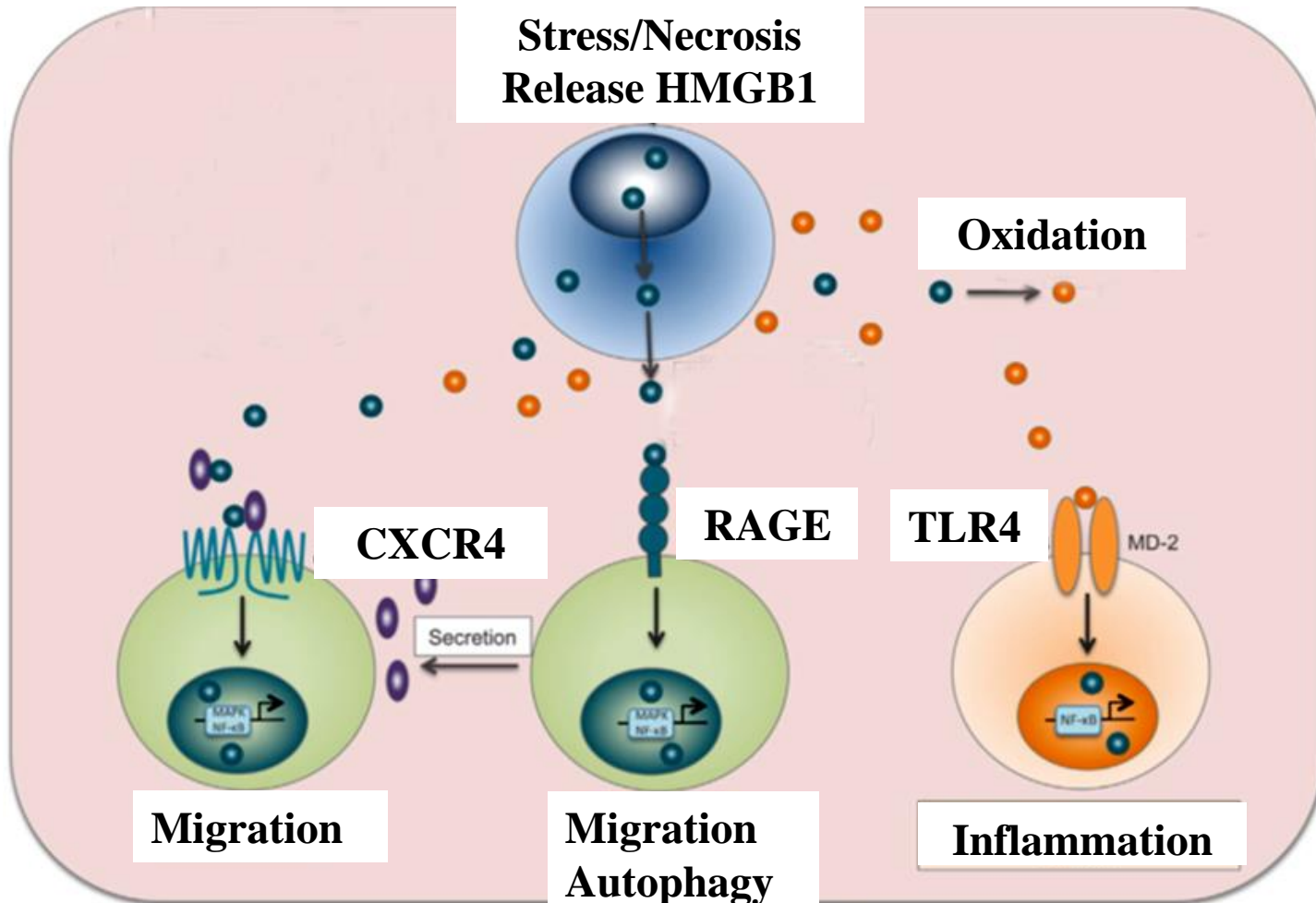
## mtDNA Oxidation



Koliaki C.  
Cell Metab 2015

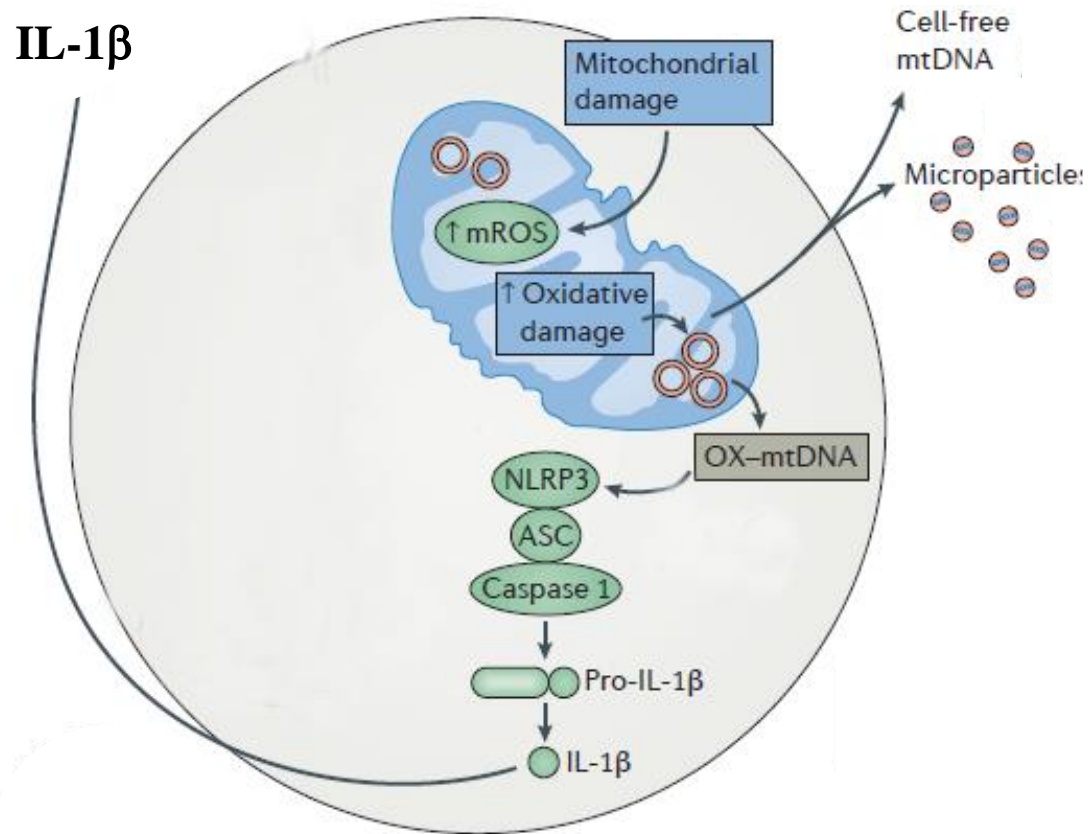
**Key Question: How Do Metabolic Changes Result in Inflammation?**

**Modifications of DAMPs (Redox Sensitive) Alters Responses to Them**



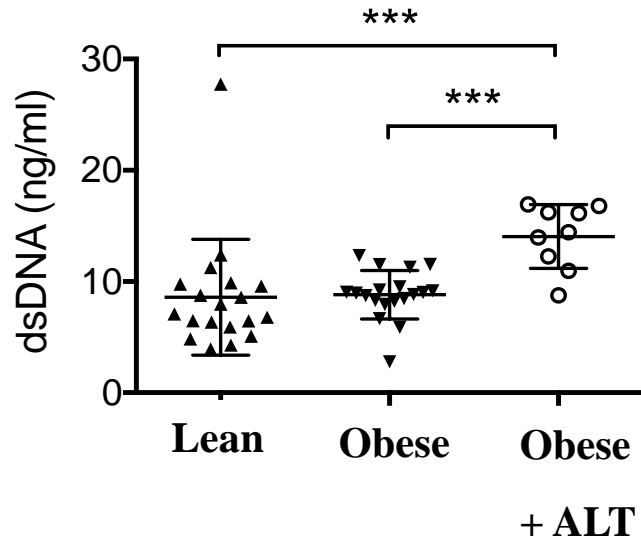


# Modifications of **mtDNA** (redox sensitive) Alters Responses

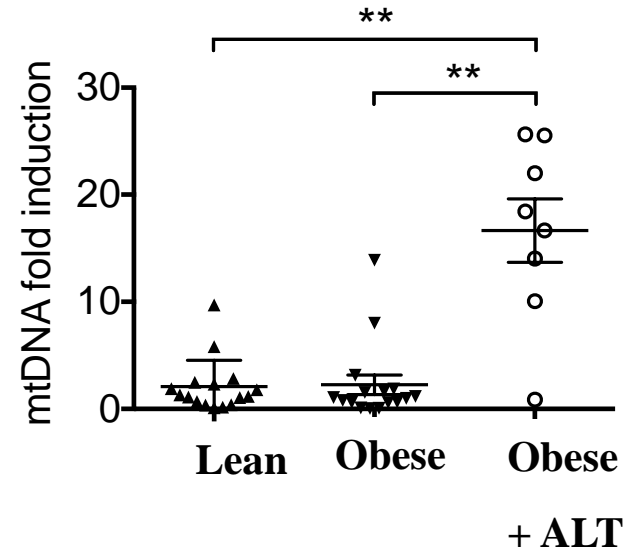


# Increase in **Plasma** mtDNA in Human (and Mouse ) NASH

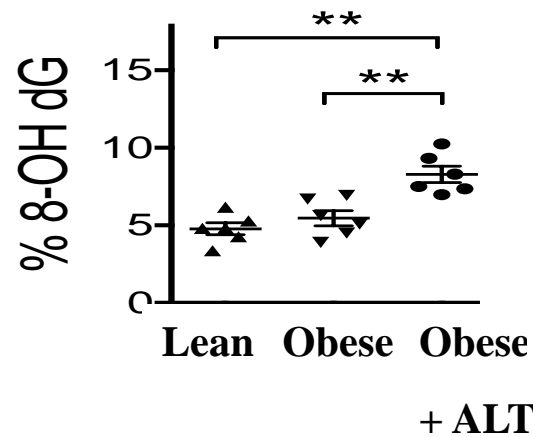
## dsDNA



## mtDNA

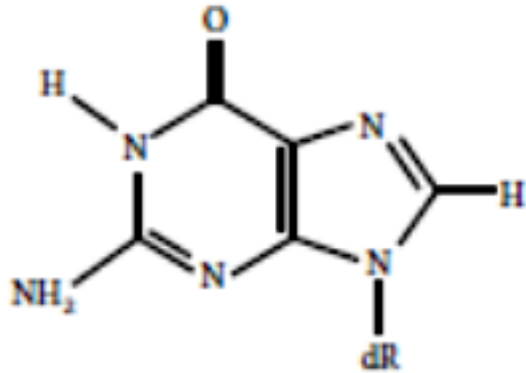


## Oxidized DNA



# Base Editing of mtDNA

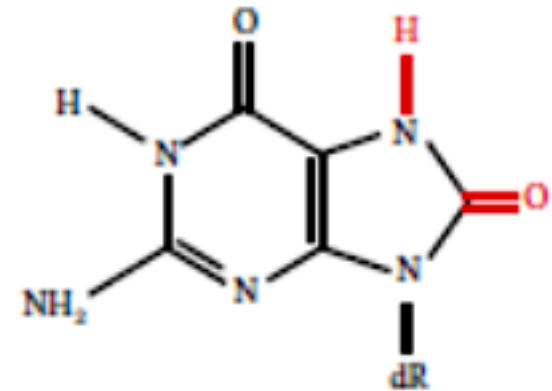
Guanine



Oxidative  
damage



8-Oxoguanine

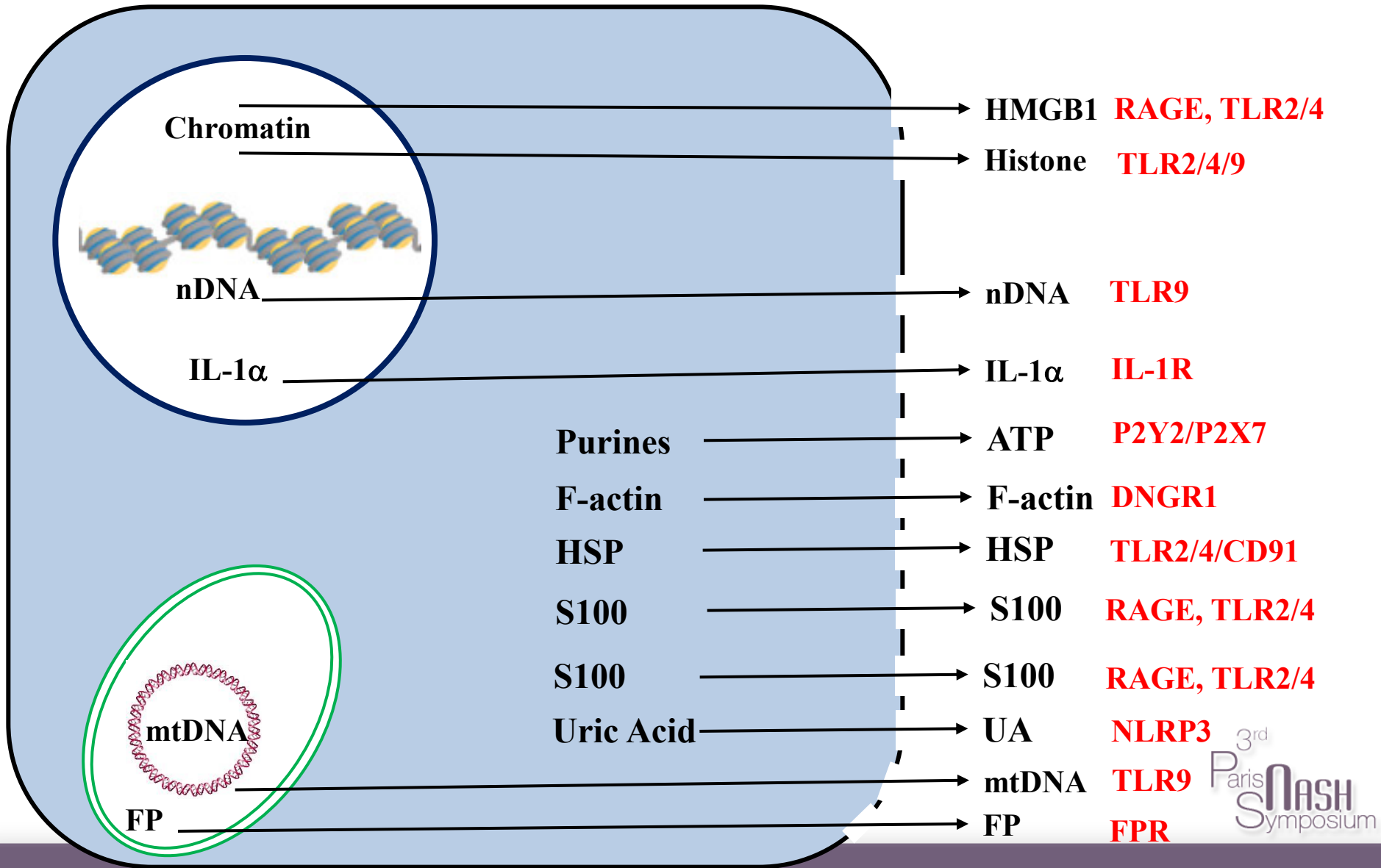


Can form G:T pairing  
Greater activation of TLR9

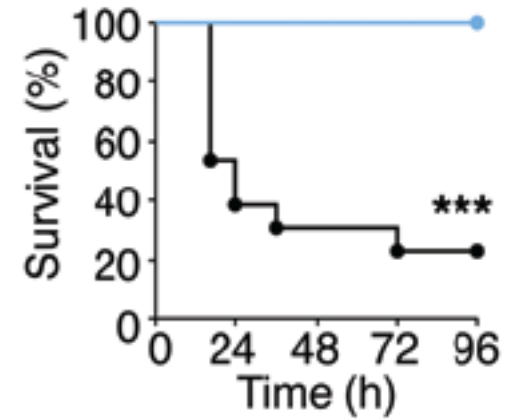
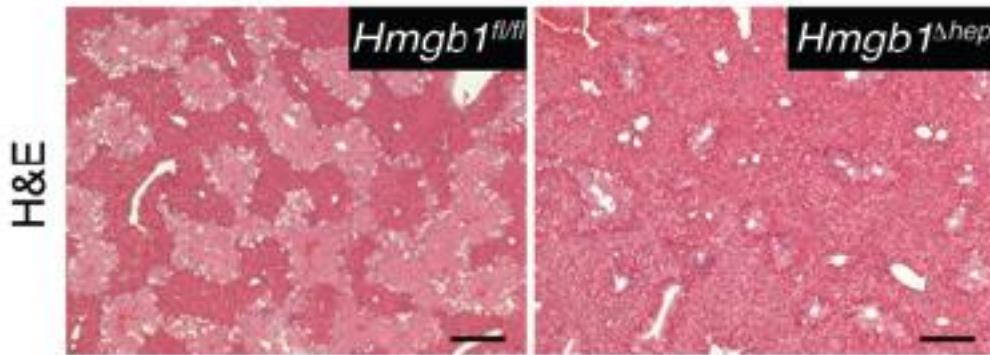
Oxoguanine glycosylase (OGG1)



# What Are the **Therapeutic** Implications of DAMP biology?

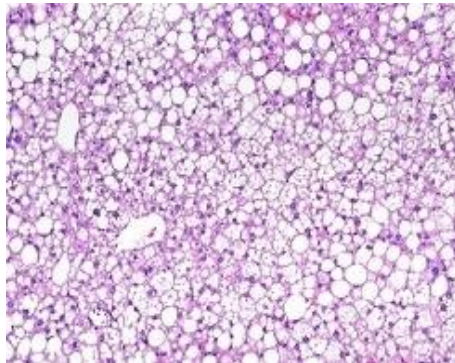


## APAP toxicity and Hepatocyte Specific HMGB1 Deficiency

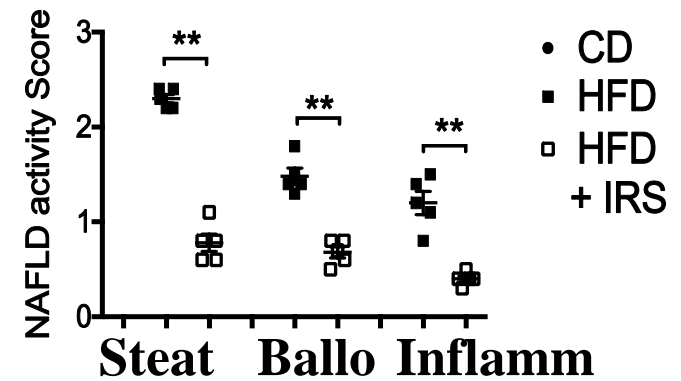
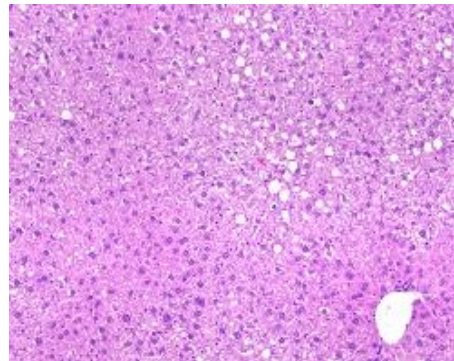


## HFD NASH and a TLR9 Antagonist

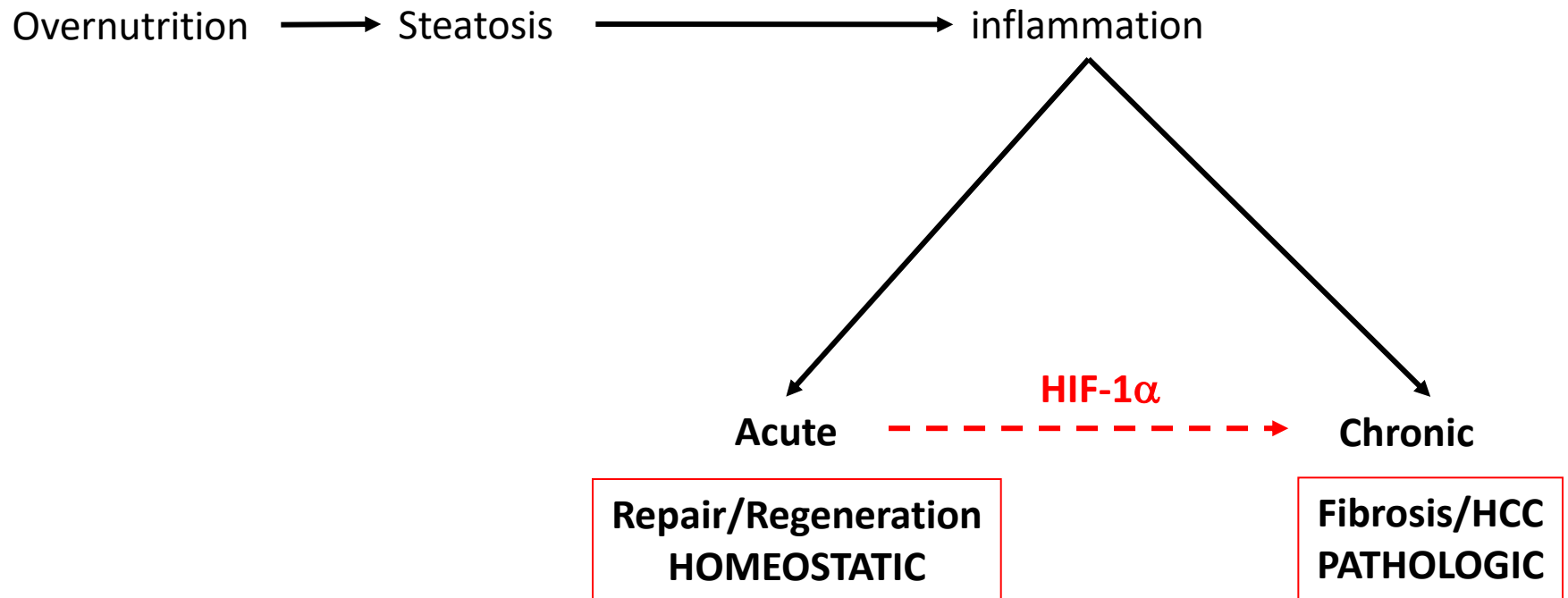
HFD



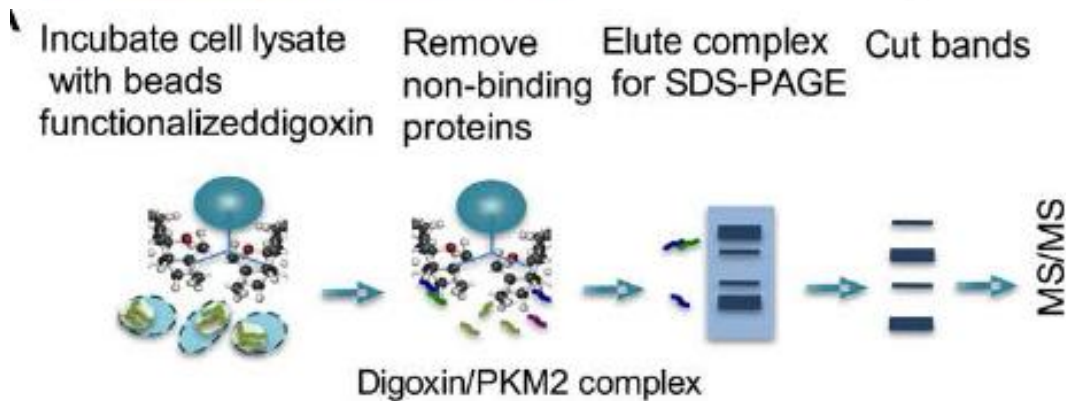
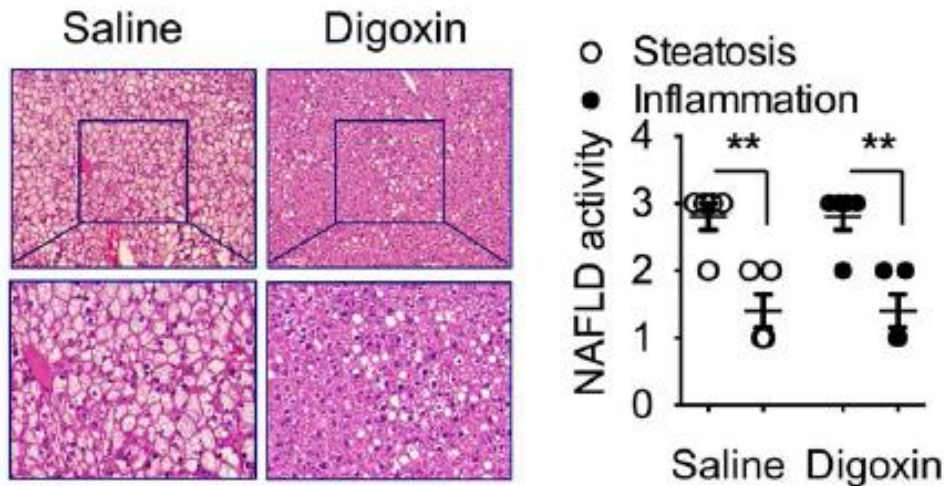
HFD + IRS



## Overview



Digoxin reduces steatosis, and injury in 12 week HFD model of NASH when given from week 8 (dose 1mg/Kg)

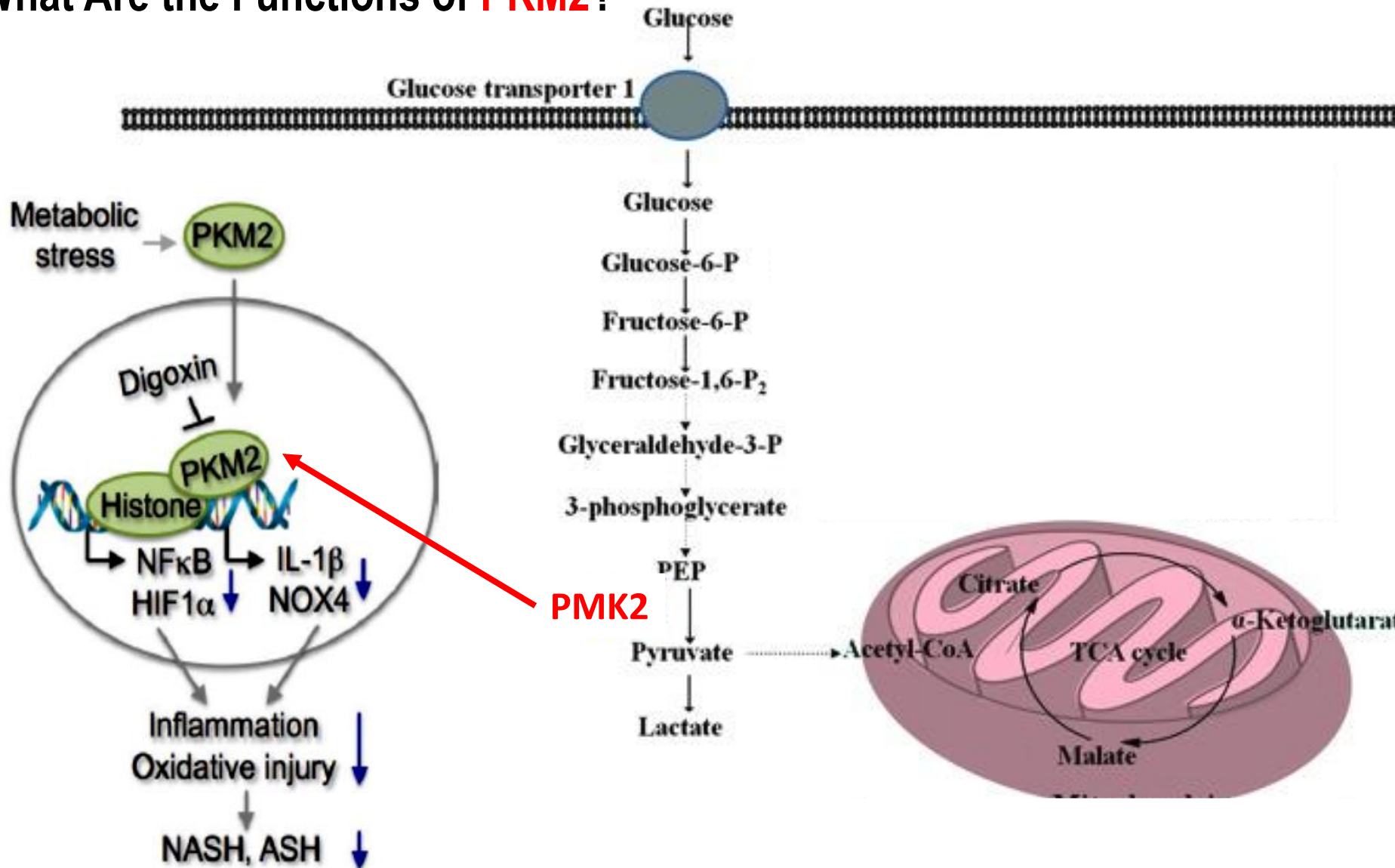


Protein	Score	Peptides	Coverage (%)
PKM2	516	22	45

QHLLIAREAEAAIYHLQLFEELRRLAPITSDPTEAAAVGAVEASFKCCSGAIVLTK

Digoxin binds Pyruvate Kinase M2 (PKM2)

# What Are the Functions of PKM2?





## Summary:

- 1) Sterile Inflammation is ubiquitous in the metabolic syndrome, but the **amplitude** of the injury is greatest in the liver.
- 2) Multiple DAMPs are released by hepatocytes in NASH.
- 3) DAMPs initiate inflammation, proliferation, chemotaxis and fibrogenesis.
- 4) Inhibition of a single DAMP can reverse inflammation and steatosis.
- 5) DAMP pathways are amenable to therapeutic manipulation.
- 6) **Low dose digoxin** binds Pyruvate Kinase M2, inhibits HIF-1 $\alpha$  and protects in NASH