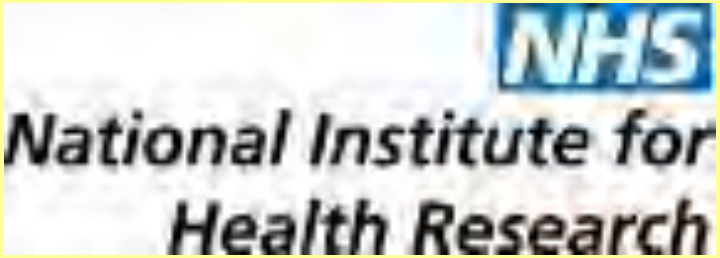
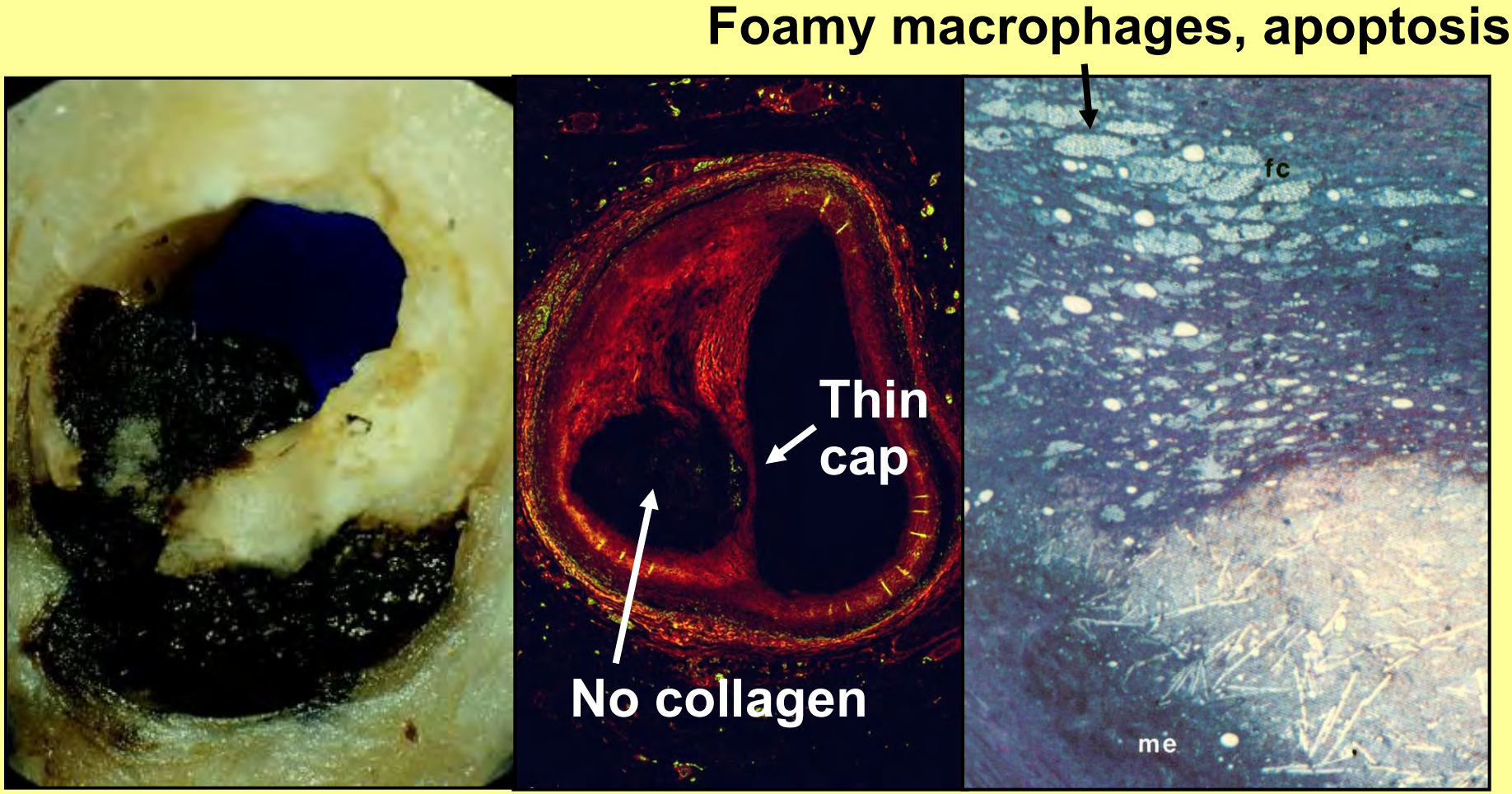
Inflammation and extracellular proteinases



Questions

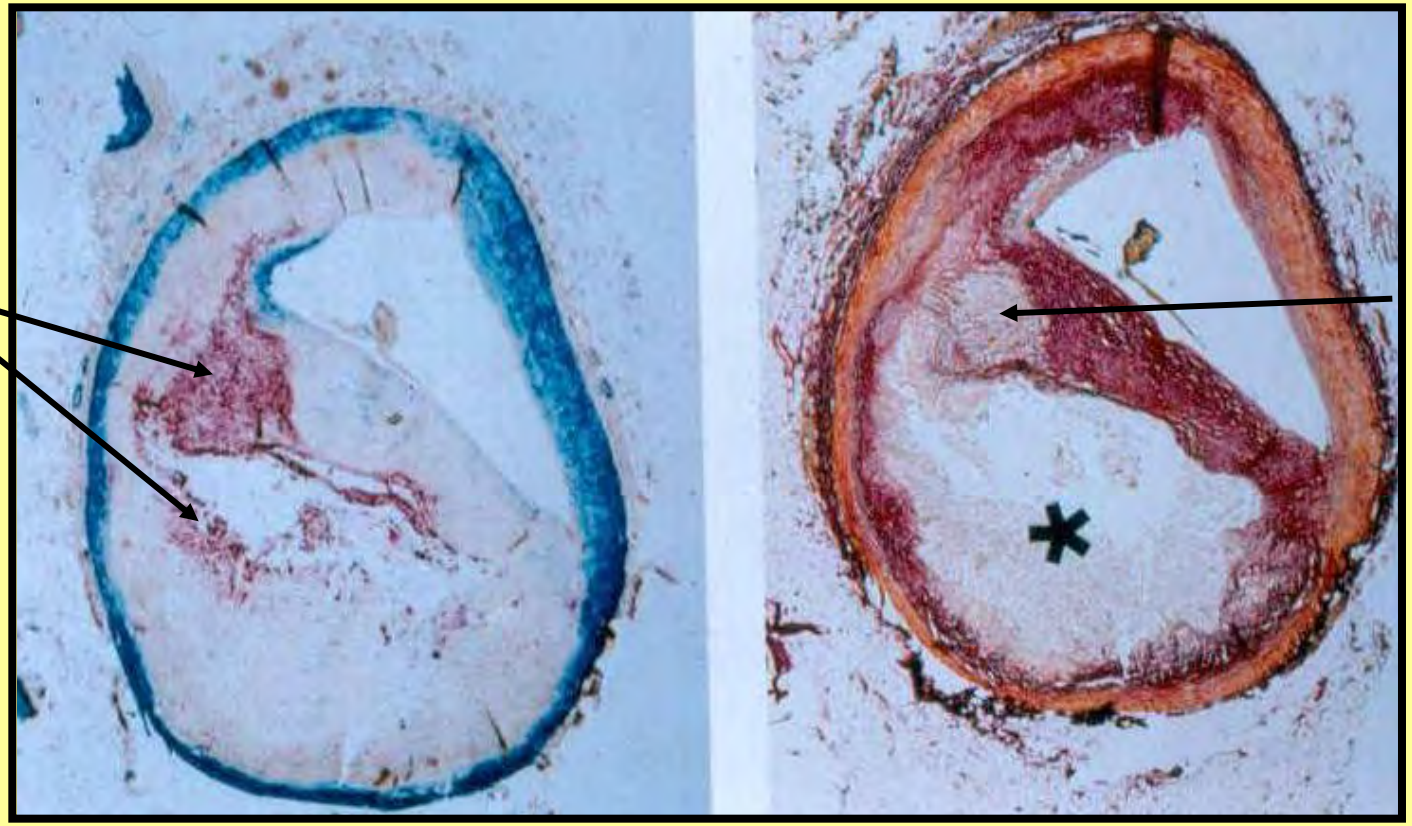
- **Why focus on metalloproteinases?**
- **What turns them on?**
- **What effect does this have in vitro?**
- **What effect does it have in man?**

Plaque rupture 75% of MI



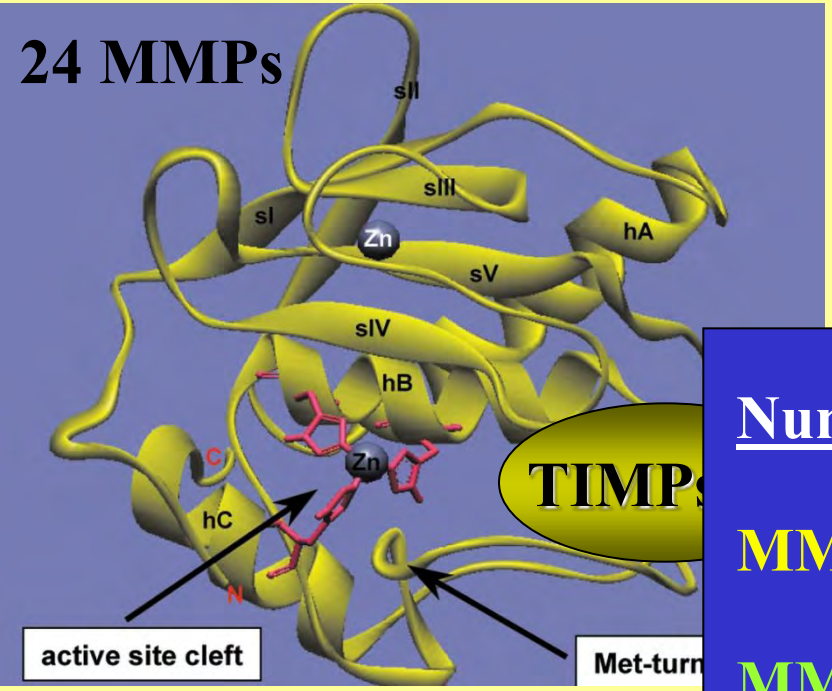
Thin fibrous cap (<65 µm), large lipid core, no collagen, macrophages, T-cells
Davies, M Circulation 1996; Falk, E JACC 2006;47:C7-12

Foam cells make proteases that cause plaque vulnerability



van der Wal Cardiovasc Res 1999;41:334-344

Metalloproteinases and TIMPs



Numbers	MMP class
MMPs 1,8,13	Interstitial collagenases
MMPs (2),9	Gelatinases (A),B
MMPs 3,7,10,11	Stromelysins
MMP 12,19	Metalloelastase +
MMPs 14-7,23,25	Membrane-type MMPs

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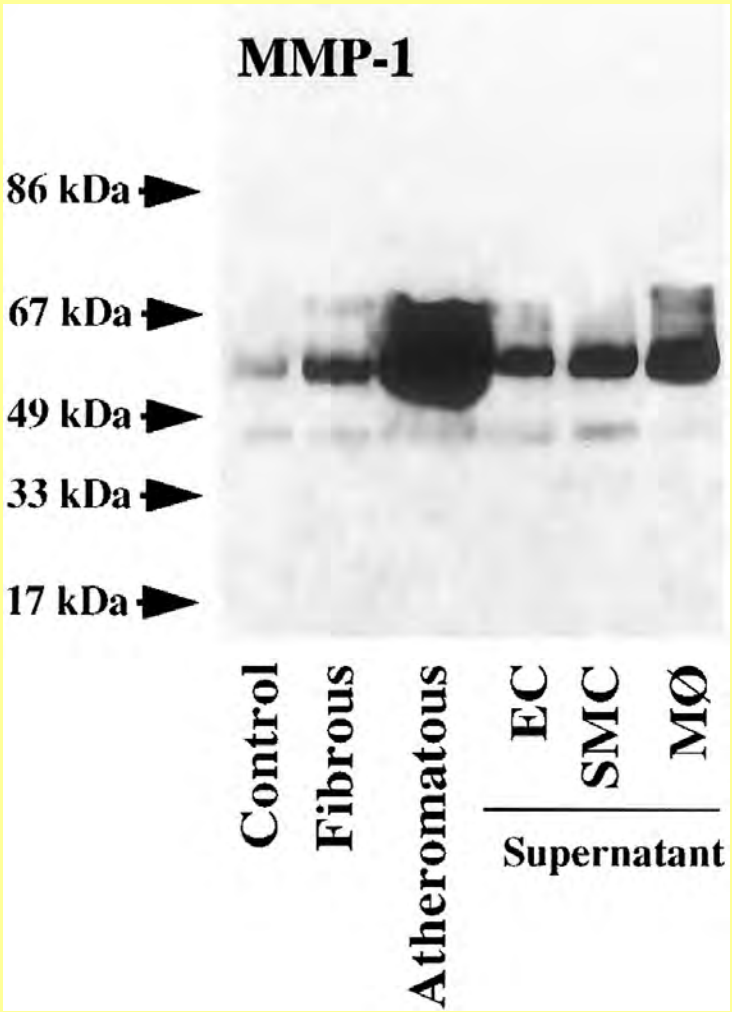
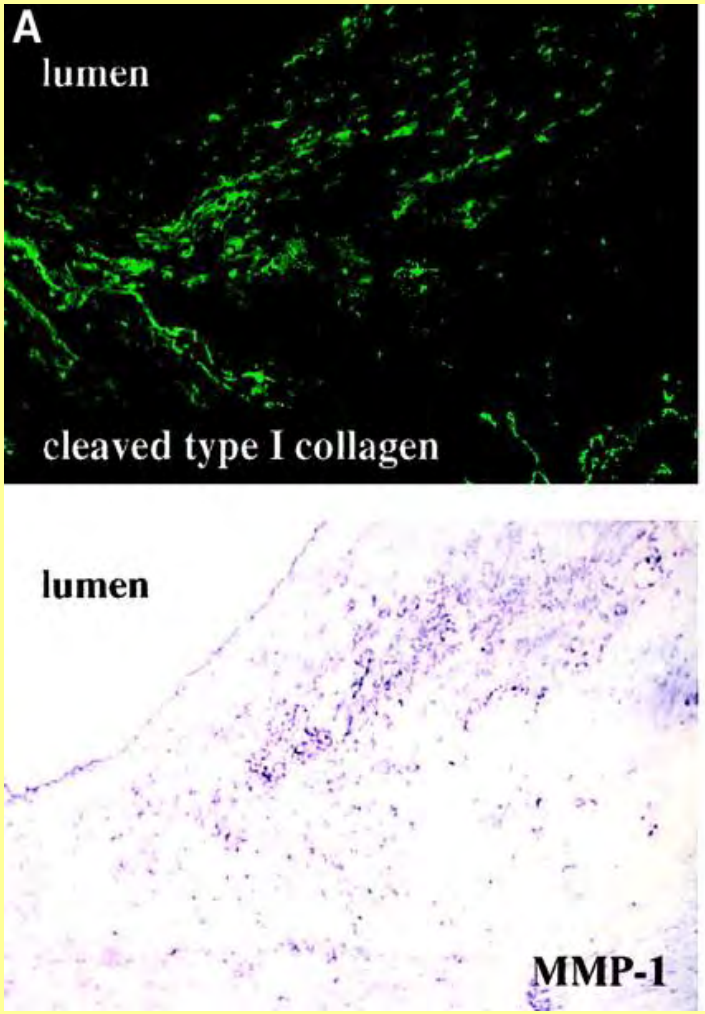
MMPs cause matrix destruction





Henney, et al. Proc Natl Acad Sci USA, 1991;88:8154-8158.
Sukhova et al Circulation 1999;99:2503-9

In mice, some MMPs stabilize and others destabilize plaques

MMP	Effect
2	More smc (1)
3	More smc/macrophages (2,3)
7	Fewer smc (2)
8	More macrophages/ less smc (4)
9	More smc/macrophages (5,2)
12	More macrophages, apoptosis, aneurysms (5,2)
13	Less collagen (6)
14	Less collagen (6)

1) ATVB 2006;26:1120 2) ATVB 2001;21:1440 3) PNAS 2005;102:15575 4) Circ Res 2009;105:921 5) Circ 2004;109:1408 6) Circulation 2005;112:2708 7) Circ 2008;117:931

Does inflammation turn MMPs on?
A systems approach

Reel, Sala-Newby, Huang and Newby, Br J Pharm, 2011 and unpublished

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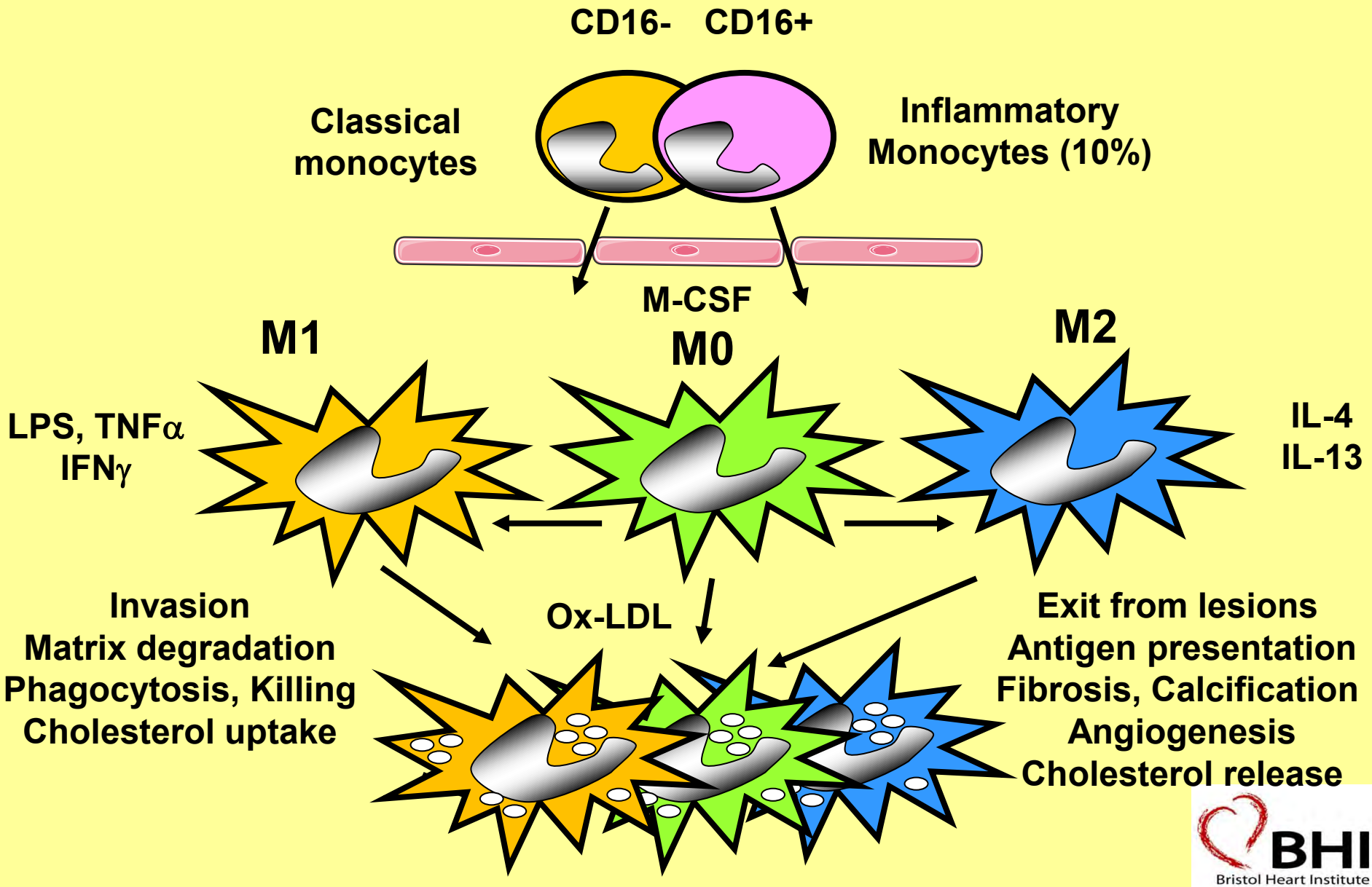
Inflammation and macrophage diversity



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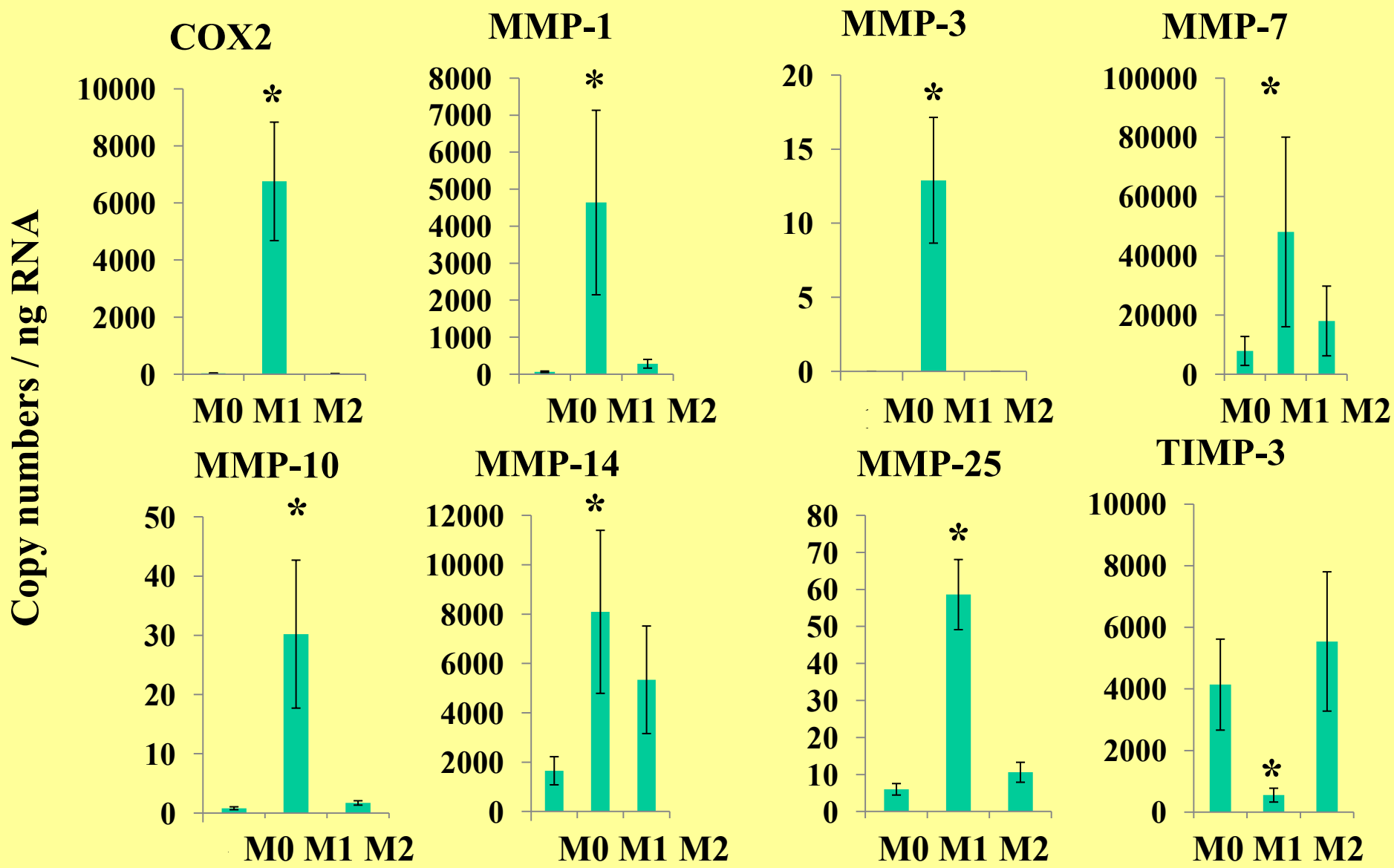
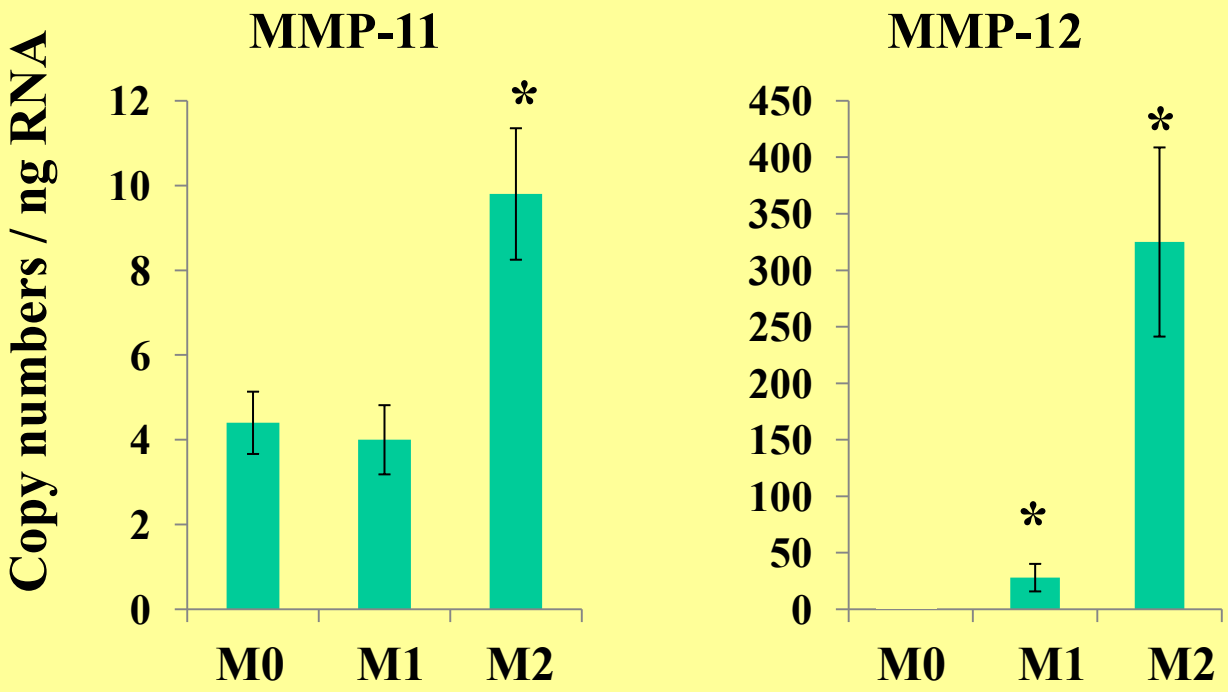
Foam cells stimulated by LPS + IFNγ



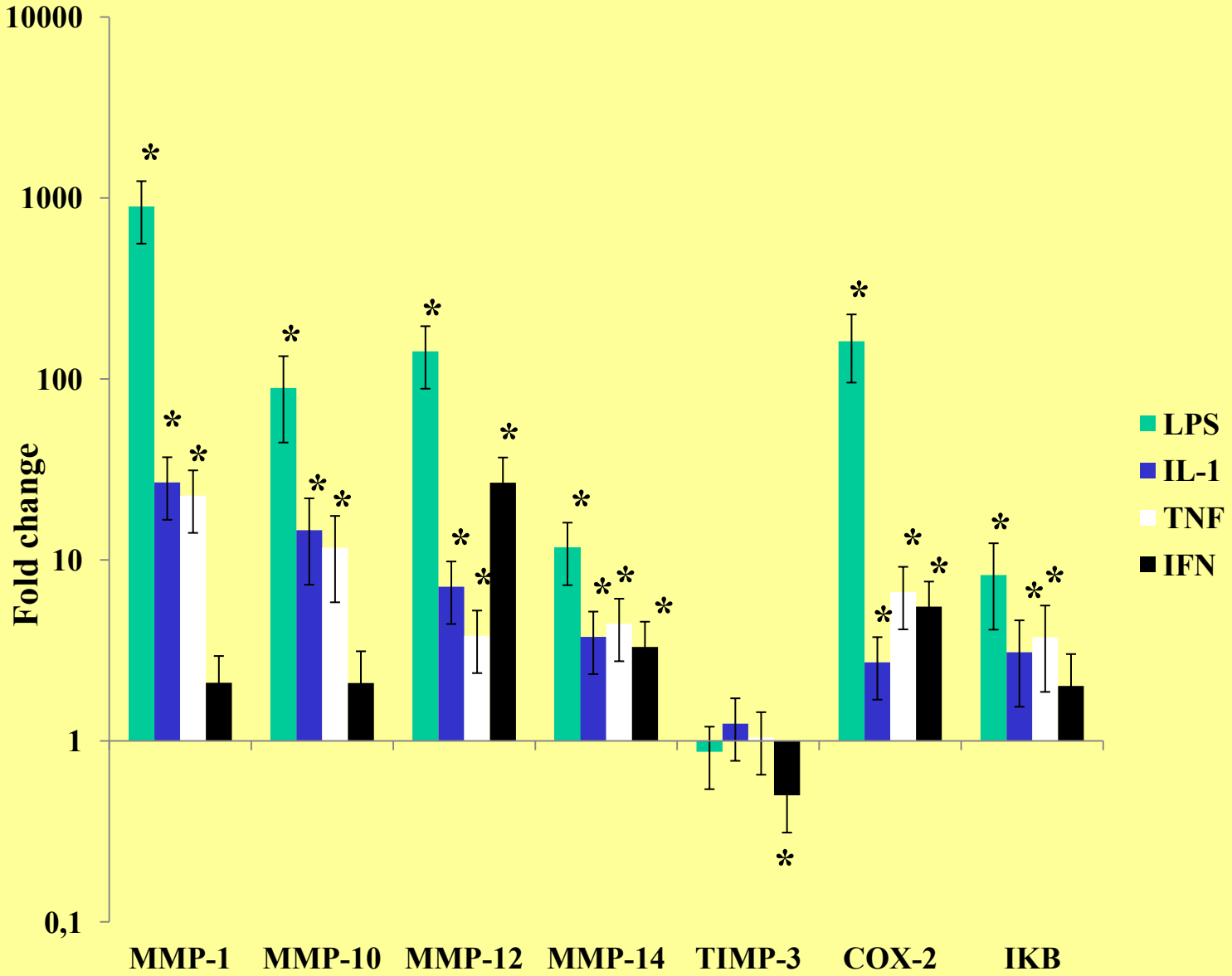
Huang, Sala-Newby et. al. unpublished

Foam cells stimulated by IL-4



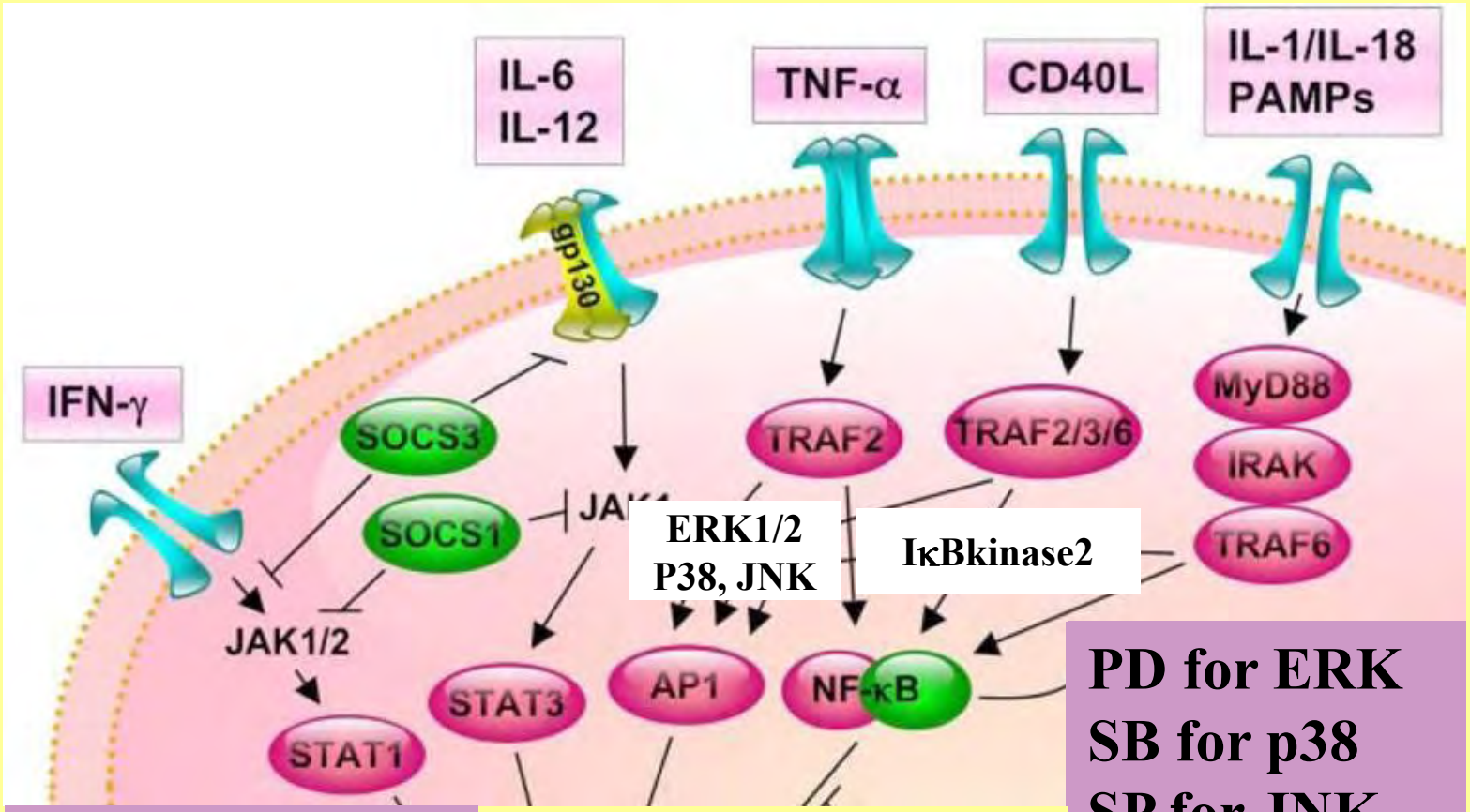
Huang, Sala-Newby et. al. unpublished

Effects of classical activators



Huang, Sala-Newby et. al. unpublished

Pro-inflammatory Signaling Pathways



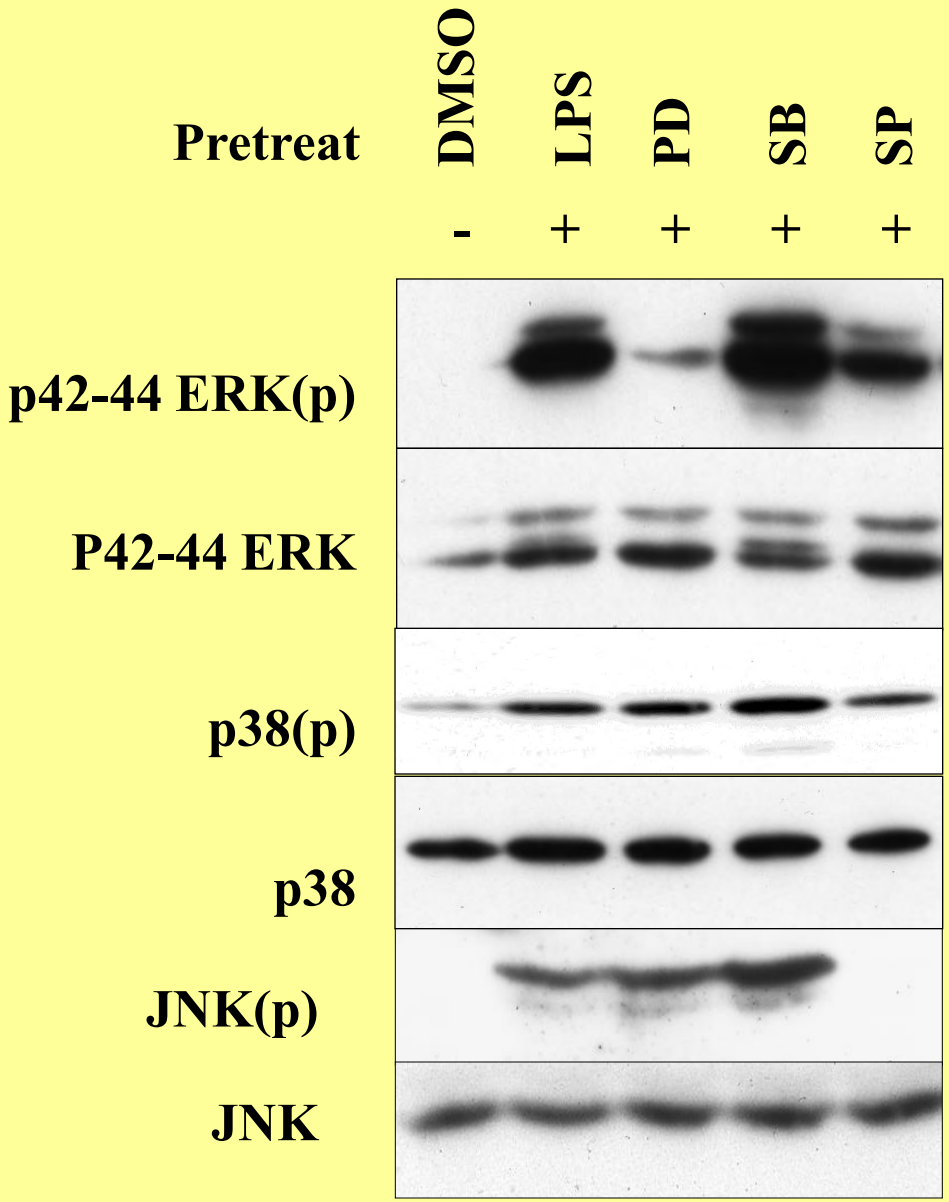
ABSPI for JAK2

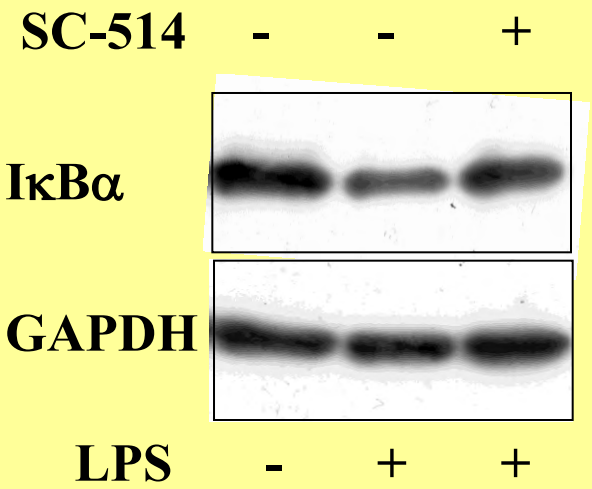
Inflammatory genes
MMPs?

PD for ERK
SB for p38
SP for JNK
SC for IKK2
LY for PI3K

Tedgui & Mallat, Physiol Rev 2006

LPS on MAP and IκB kinases





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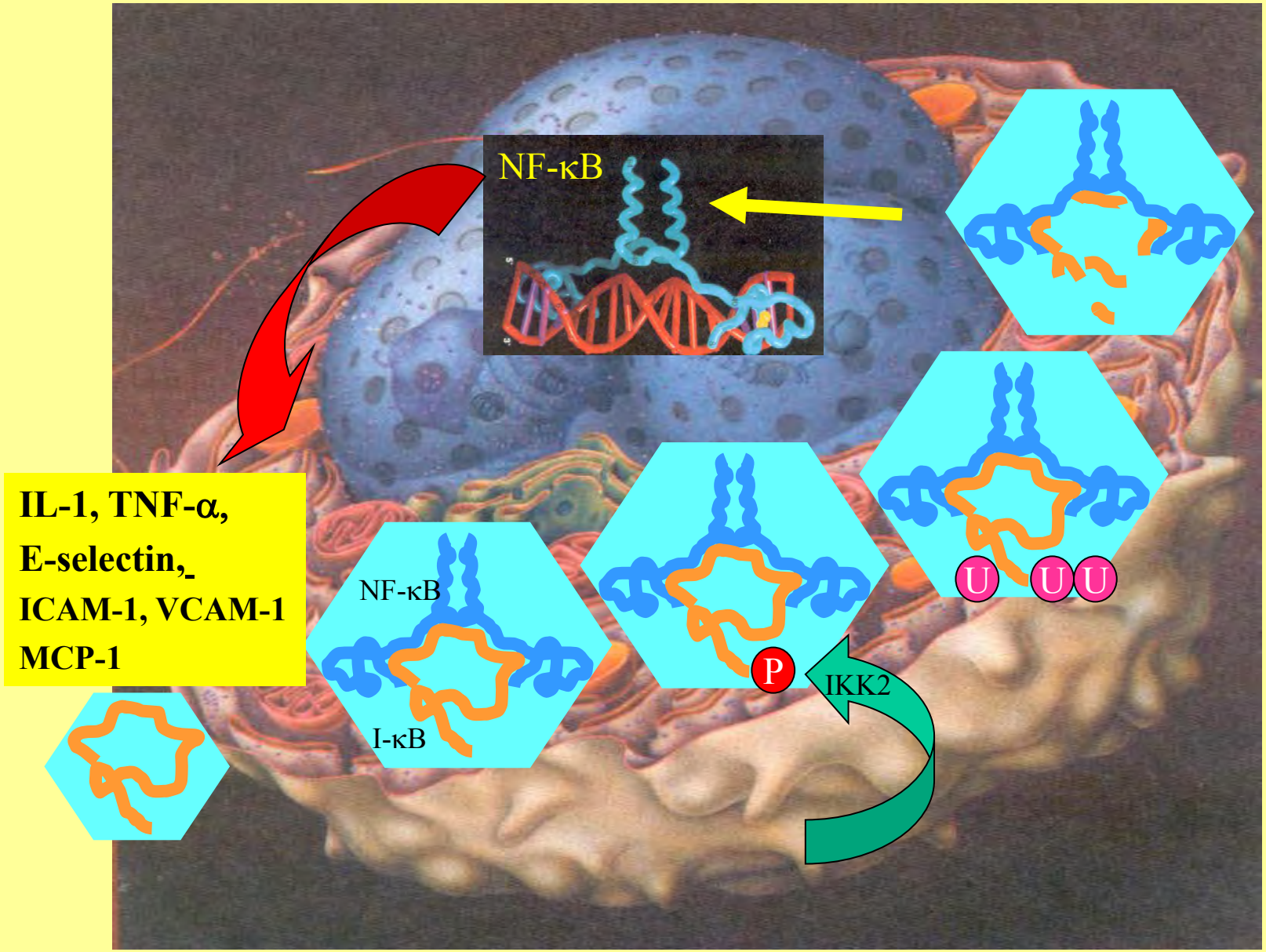
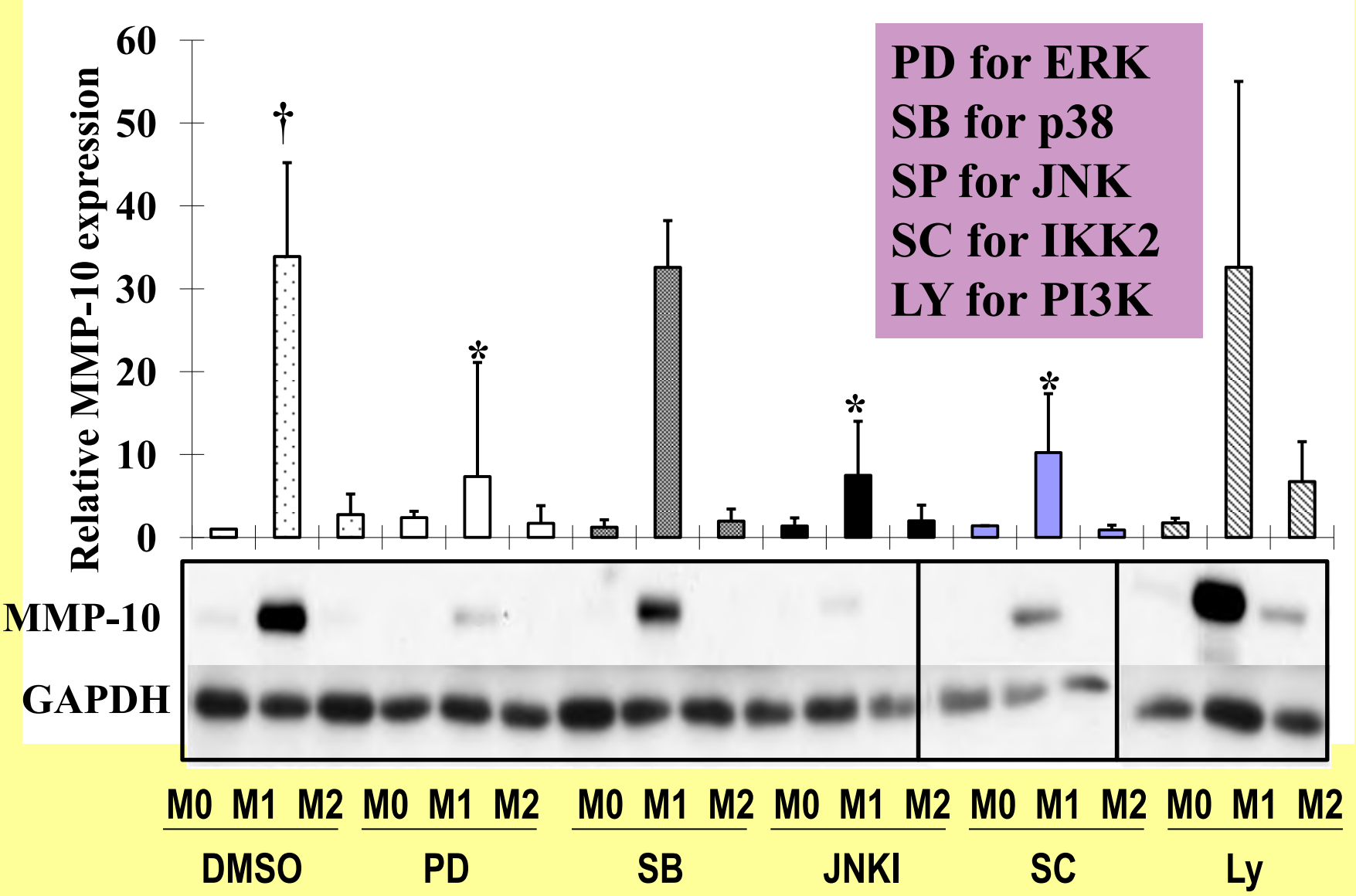
Nuclear factor-κB, NF-κB

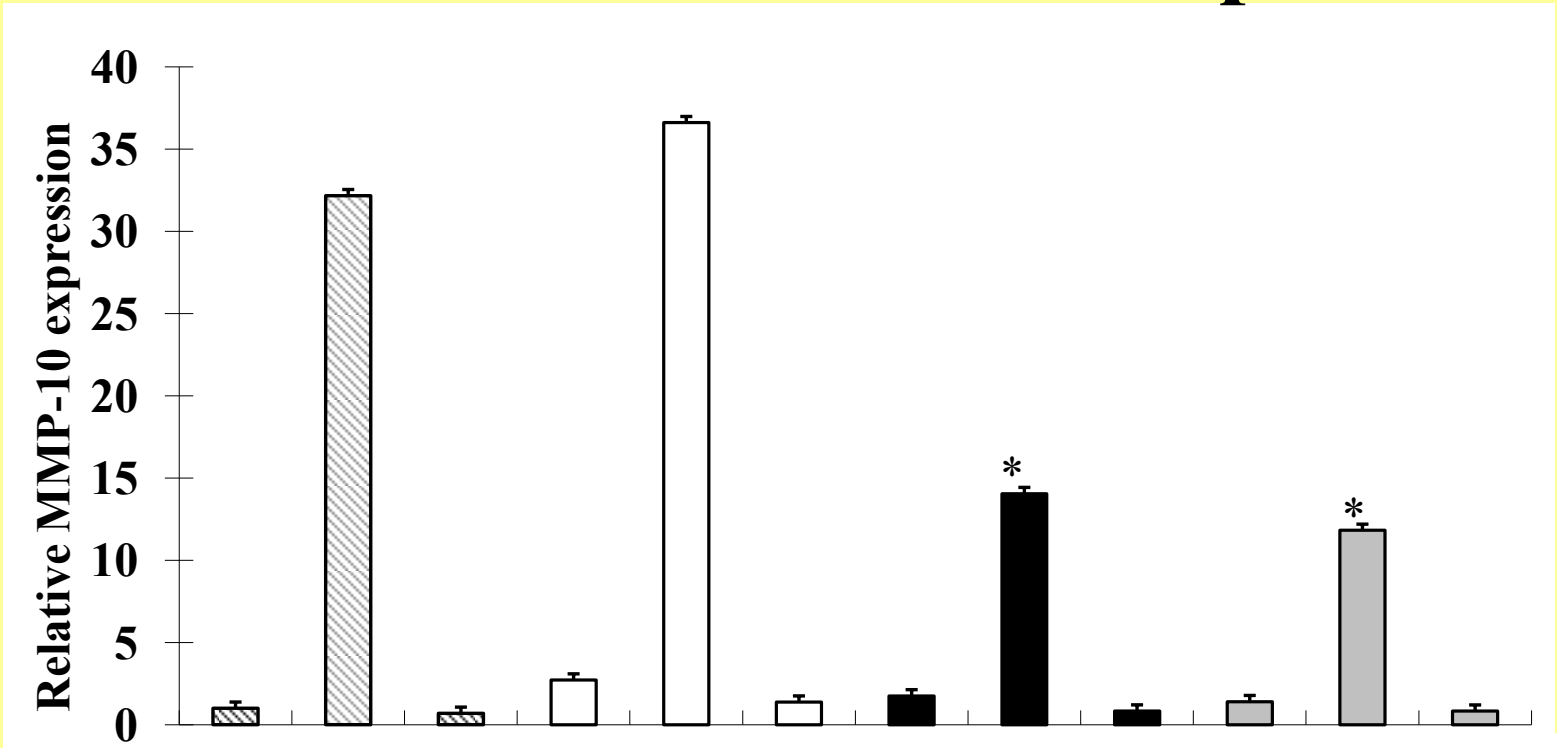


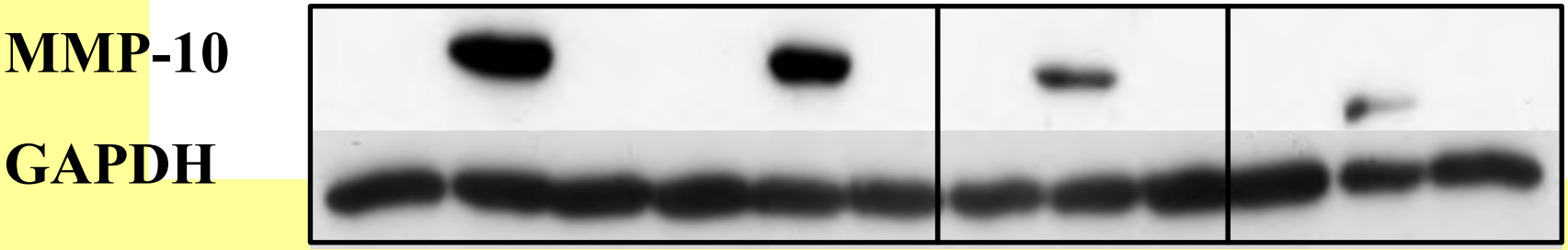
Effect on MMP-10 protein



Huang, Sala-Newby et. al. unpublished

Effect of viruses on MMP-10 protein



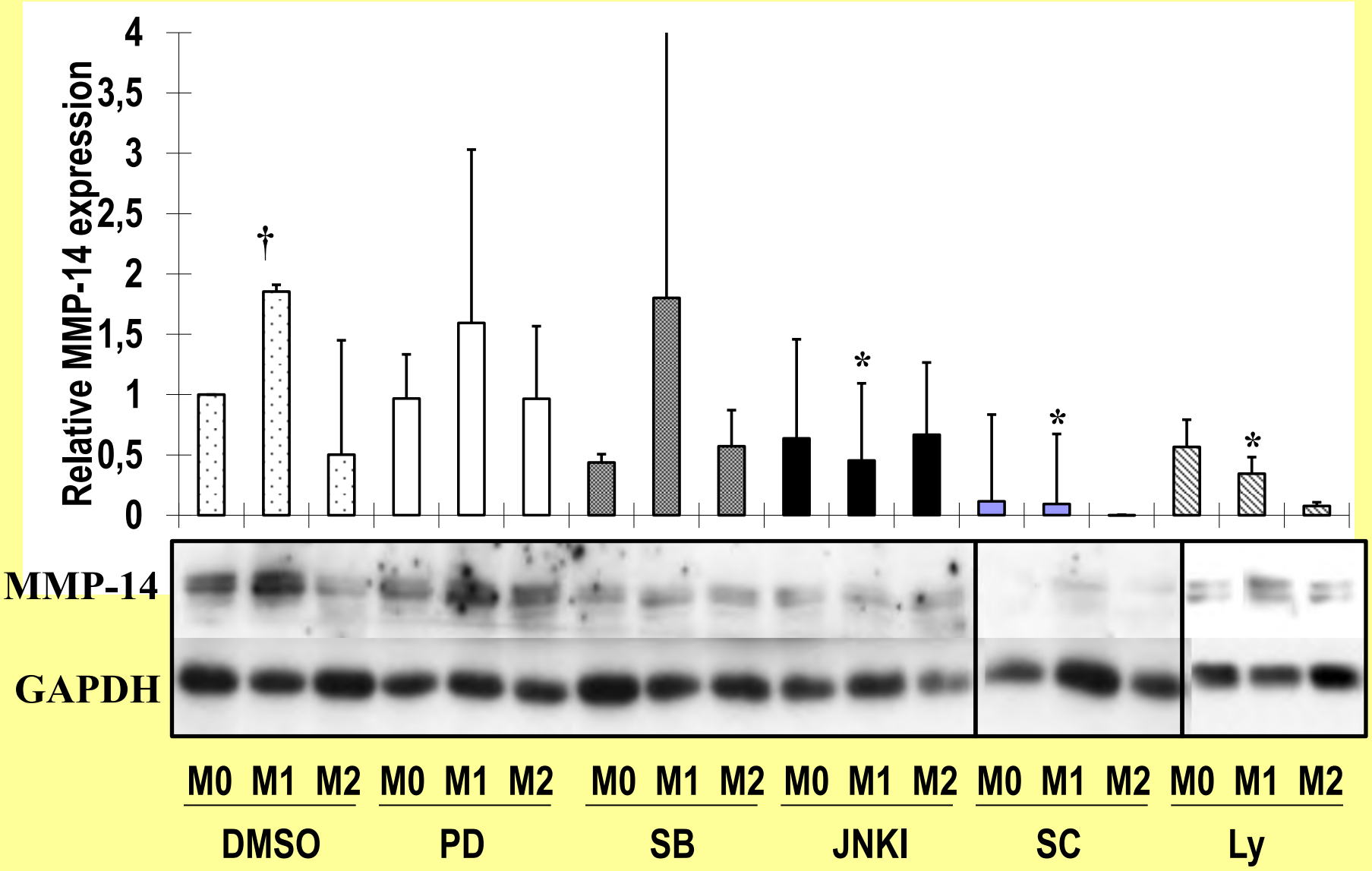


M0 M1 M2 M0 M1 M2 M0 M1 M2 M0 M1 M2

Untransfection β-gal iκB virus dnIKK2 virus

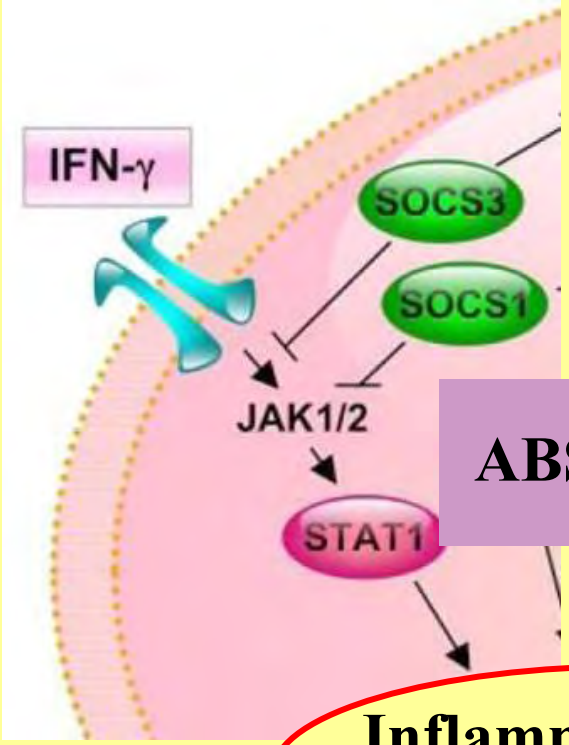
Huang, Sala-Newby et. al. unpublished

Effect on MMP-14 protein



Huang, Sala-Newby et. al. unpublished

IFNγ Signaling Pathway

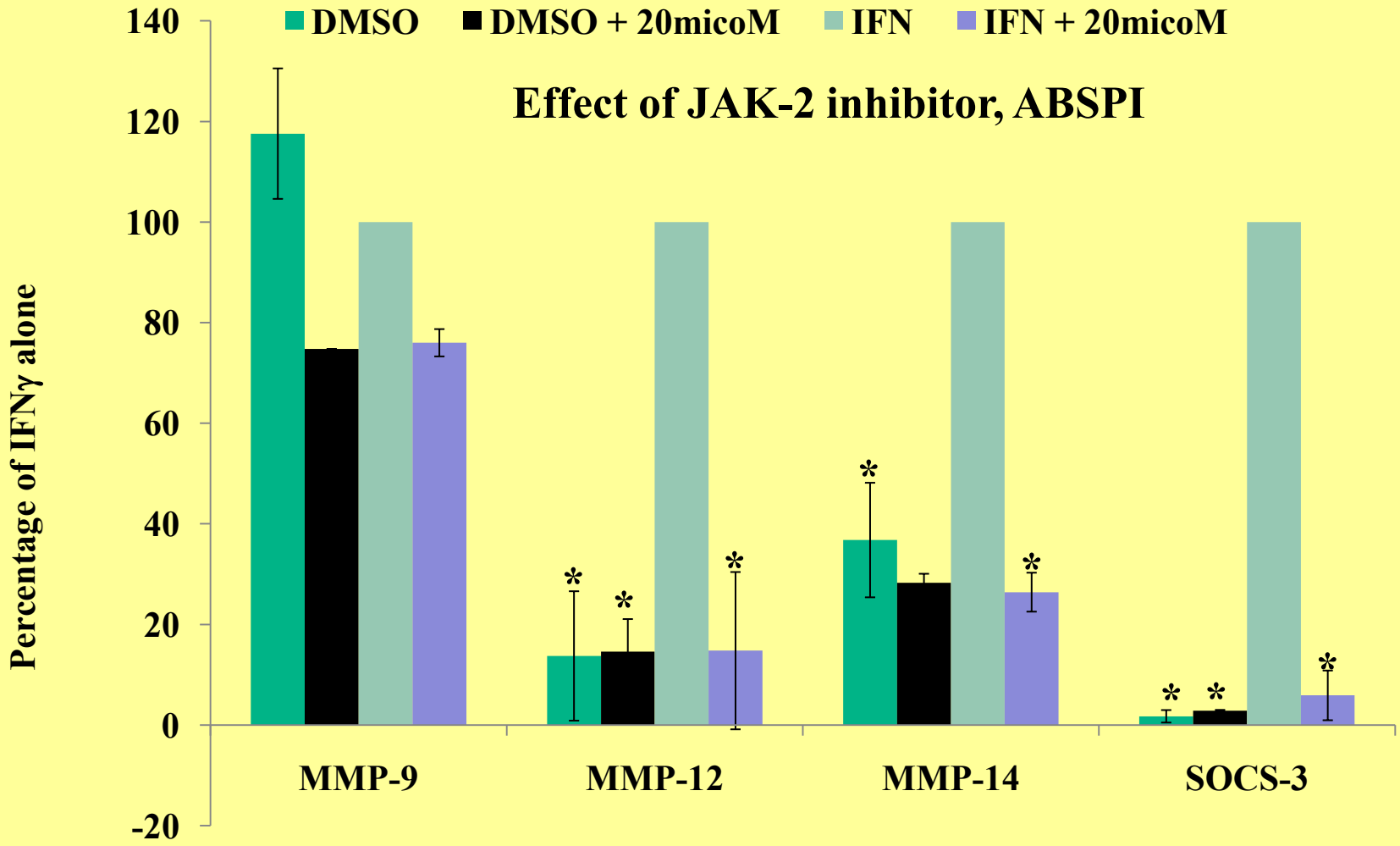


ABSPI for JAK2

Inflammatory genes
MMPs?

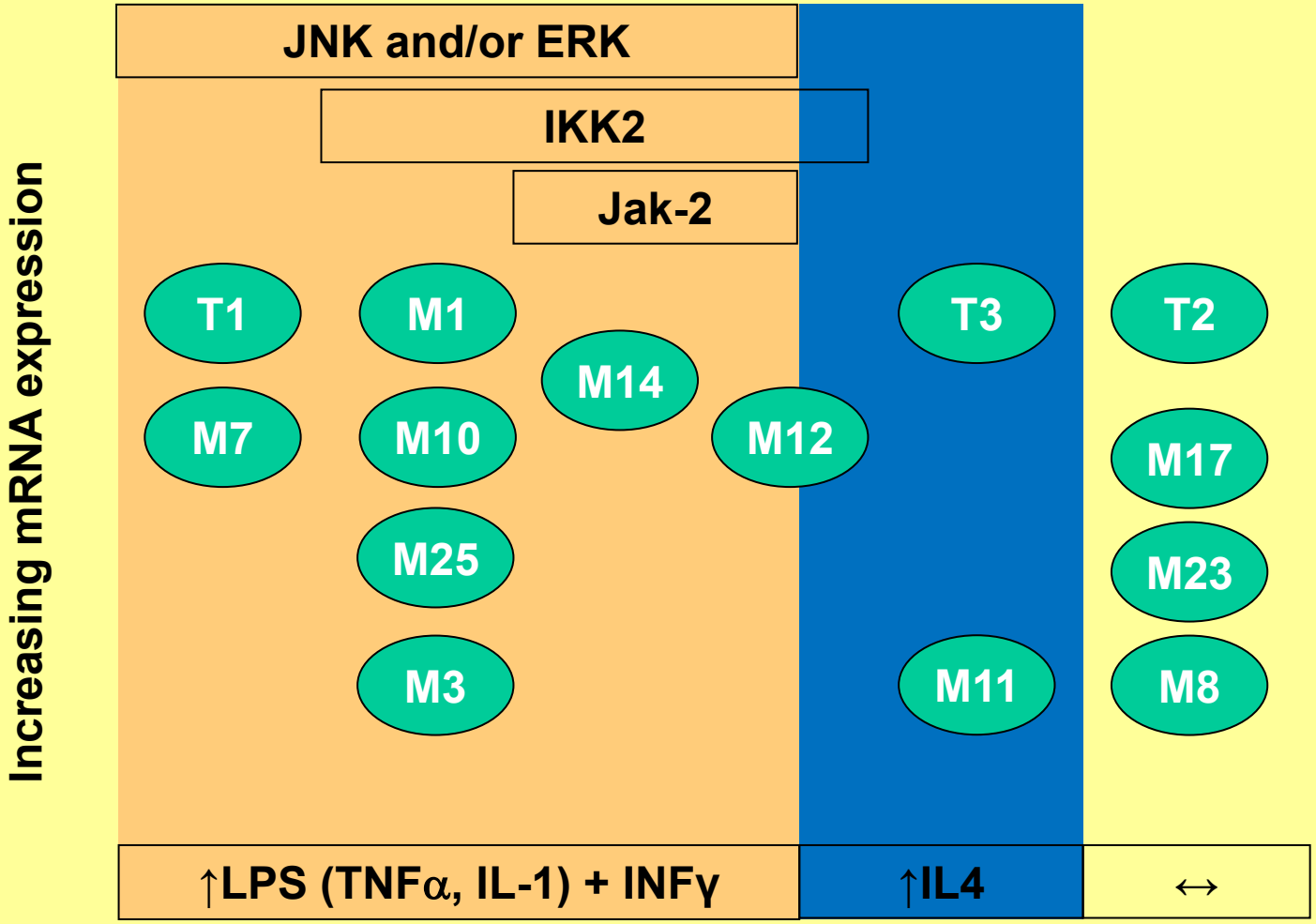
Tedgui & Mallat, Physiol Rev 2006

MMPs stimulated by IFNγ



Huang, Sala-Newby et. al. unpublished

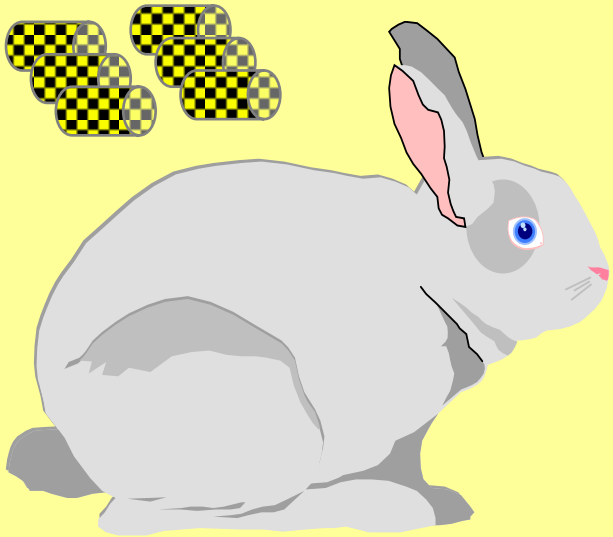
The human macrophage MMP map



Br. J. Pharm. 2011 and unpublished

Which MMPs are turned on in foam cells in vivo?

Foam cell macrophages and non-foamy macrophages from cholesterol or chow-fed rabbits



Chase et al. ATVB 2002;22:765-771

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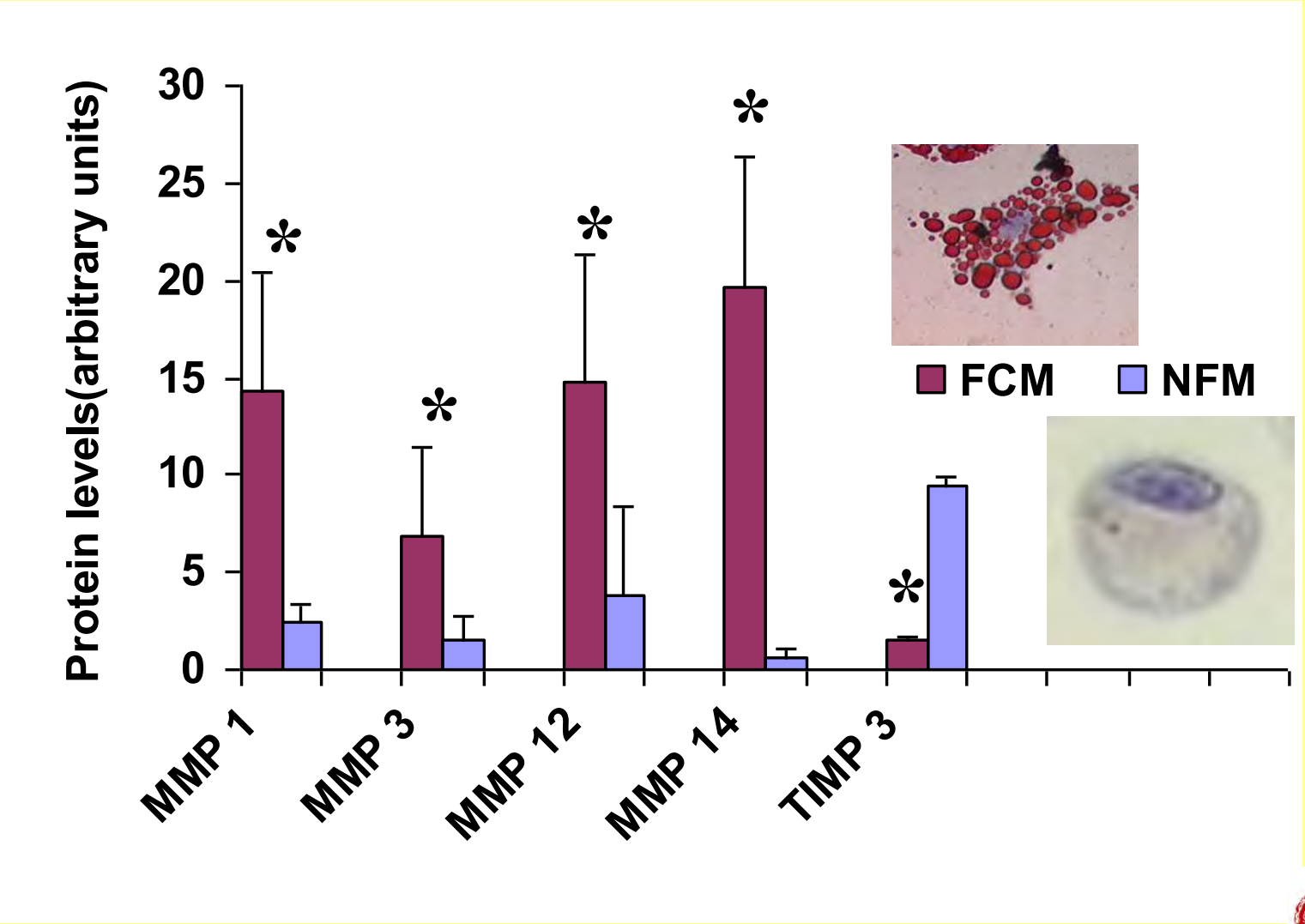
Several MMPs are increased and TIMP 3 decreased in foam cells



Chase et al. ATVB 2002;22:765-771

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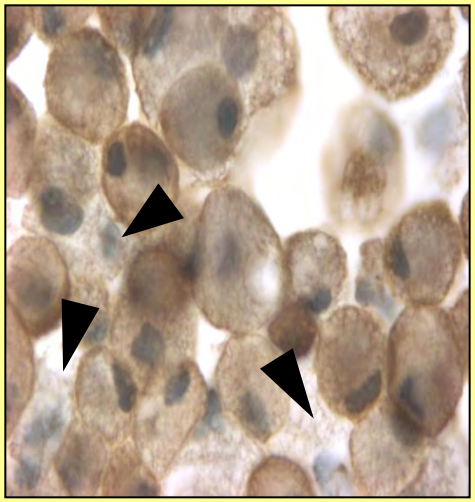
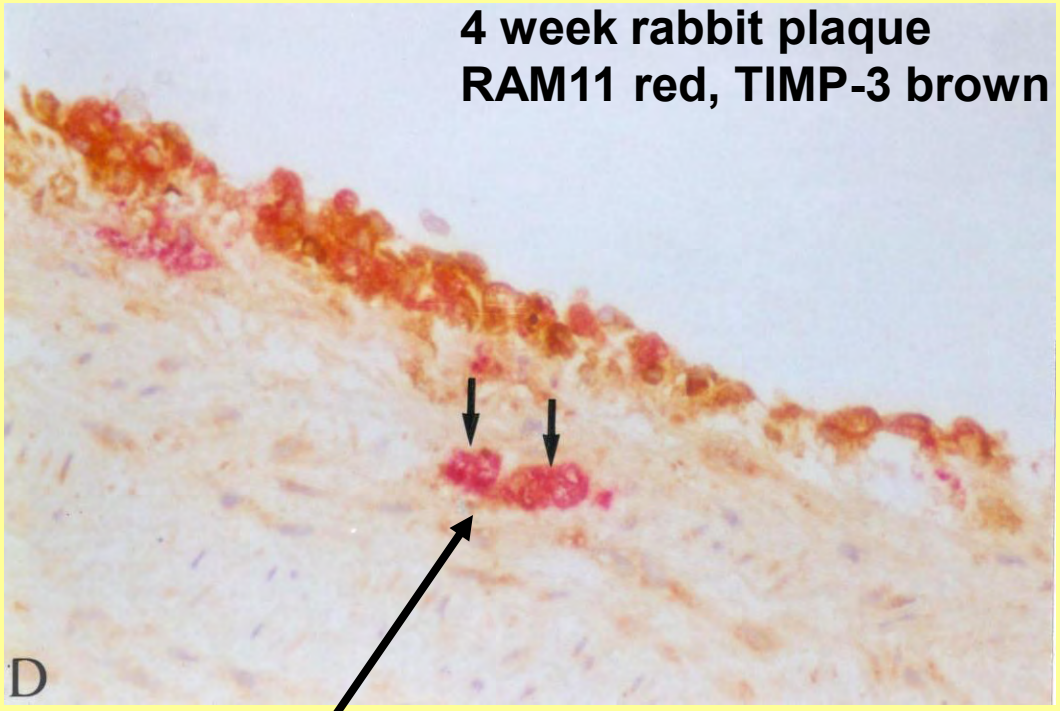
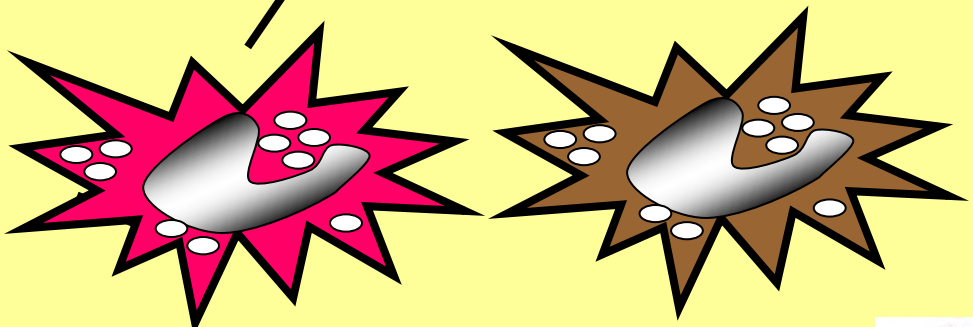
Rabbit foam cells are a mixture of TIMP3+ and TIMP3-



Jason Johnson
BHF, IRF





Zaltsmann et al 1999, unpublished and Johnson ATVB 2008; 28:1647

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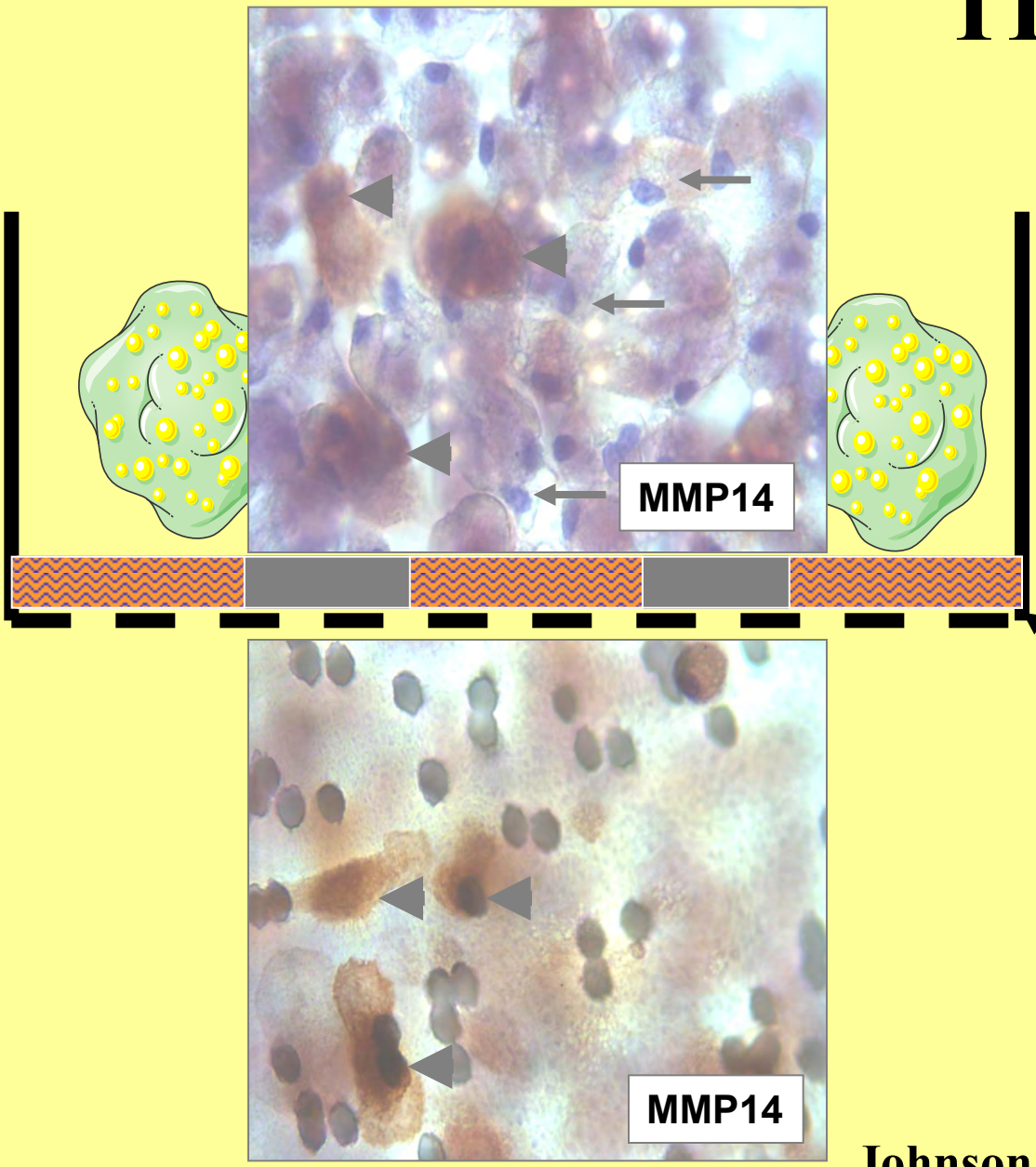
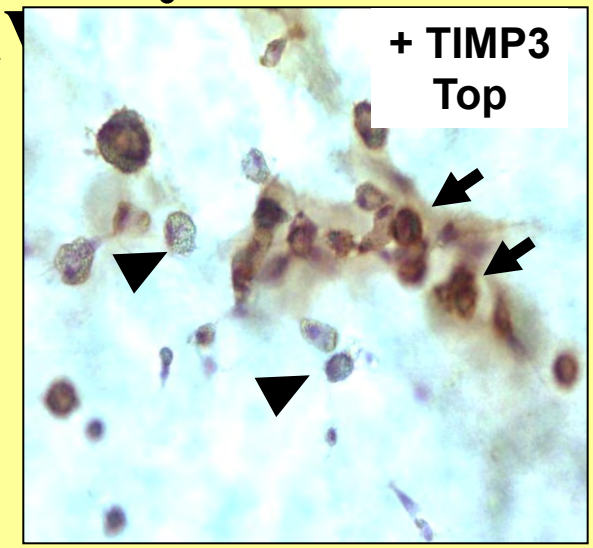
TIMP-3 stops inv





Matrigel layer

Anti-MMP-14 also stops invasion

Johnson ATVB 2008; 28:1647

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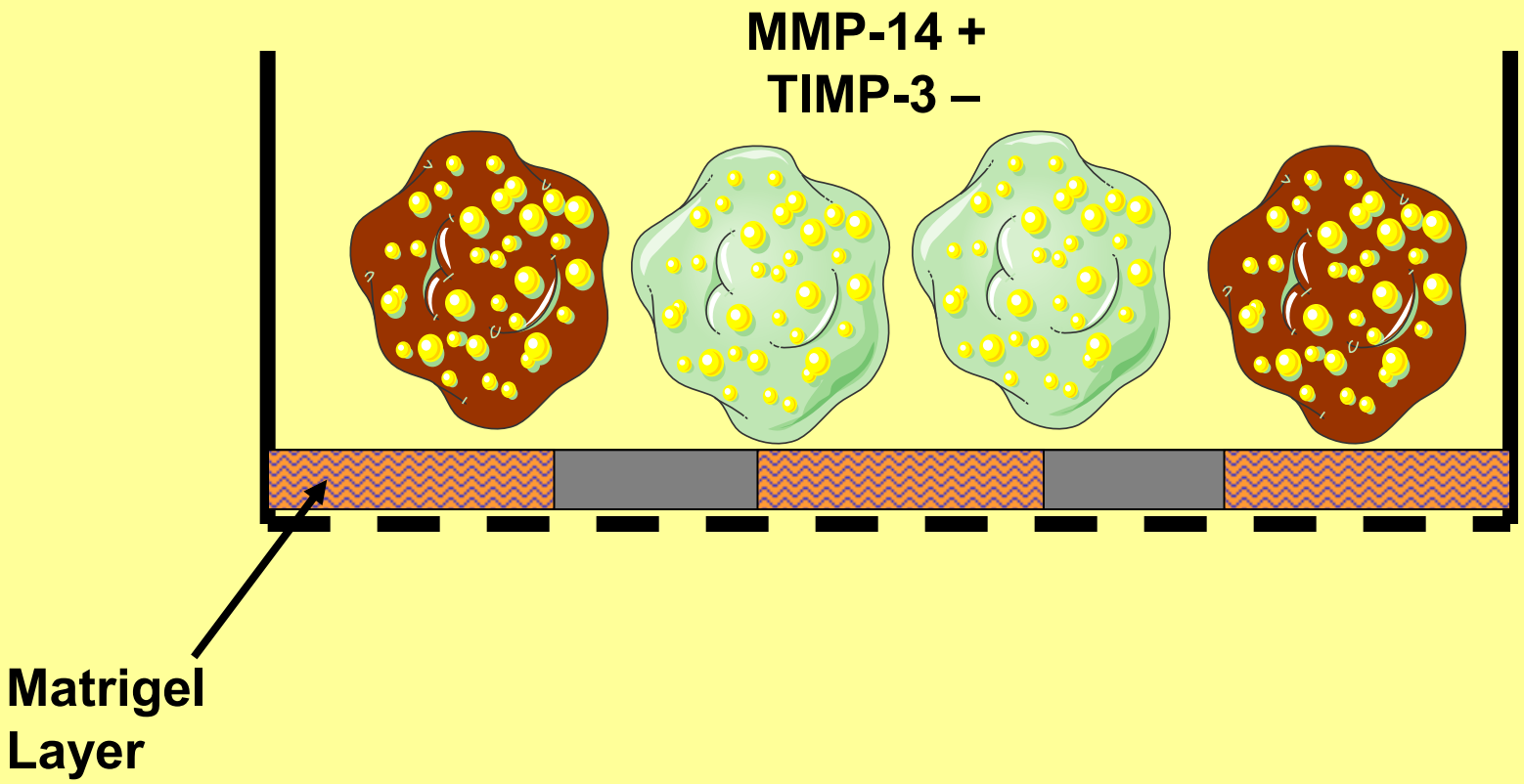
MMP14^{+}TIMP^{-}macrophages invade



Migrated foam cells

Johnson et al ATVB 2008; 28:1647

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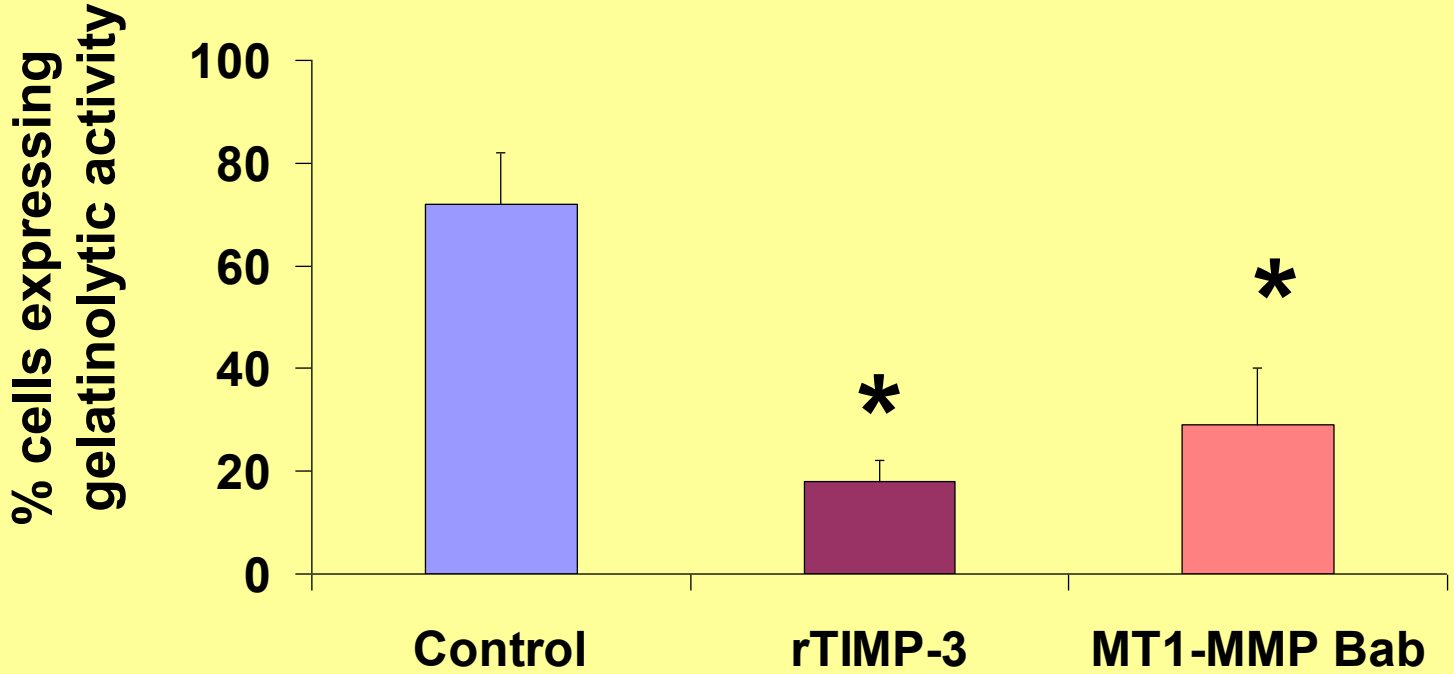
Gelatinolytic activity



Johnson ATVB 2008; 28:1647

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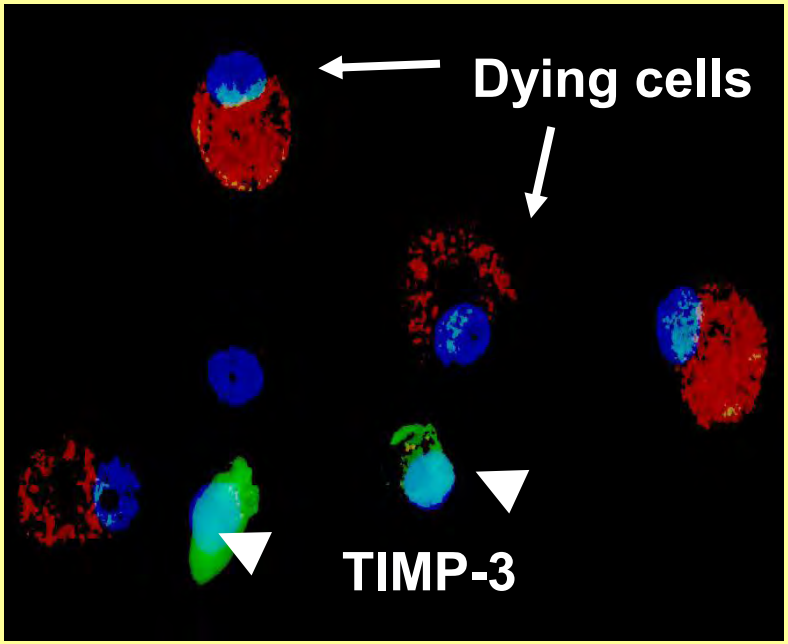
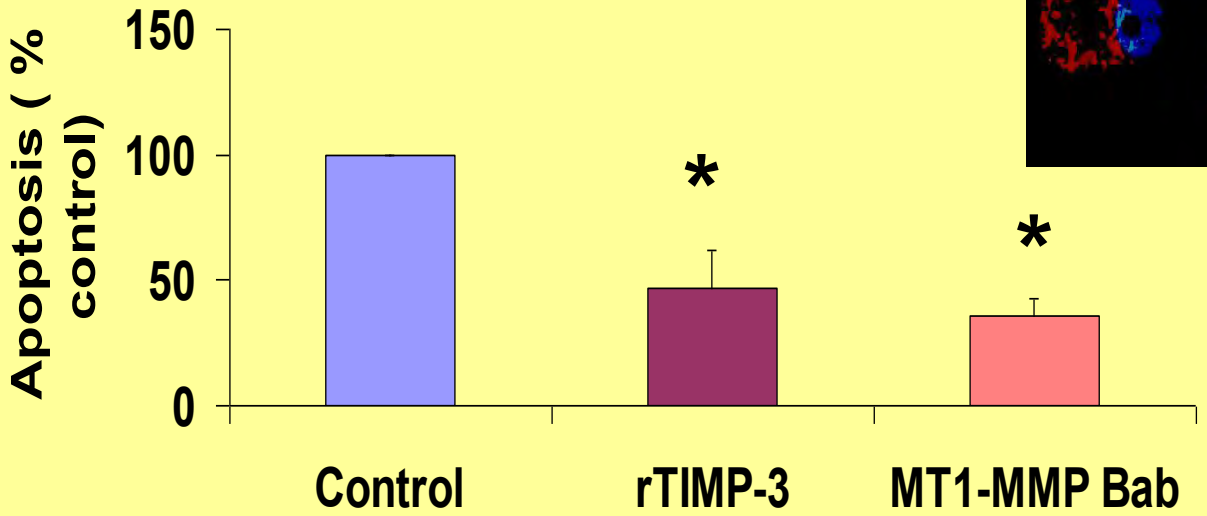
Apoptosis (and proliferation) also greater in MMP-14+, TIMP-3-





Johnson ATVB 2008;

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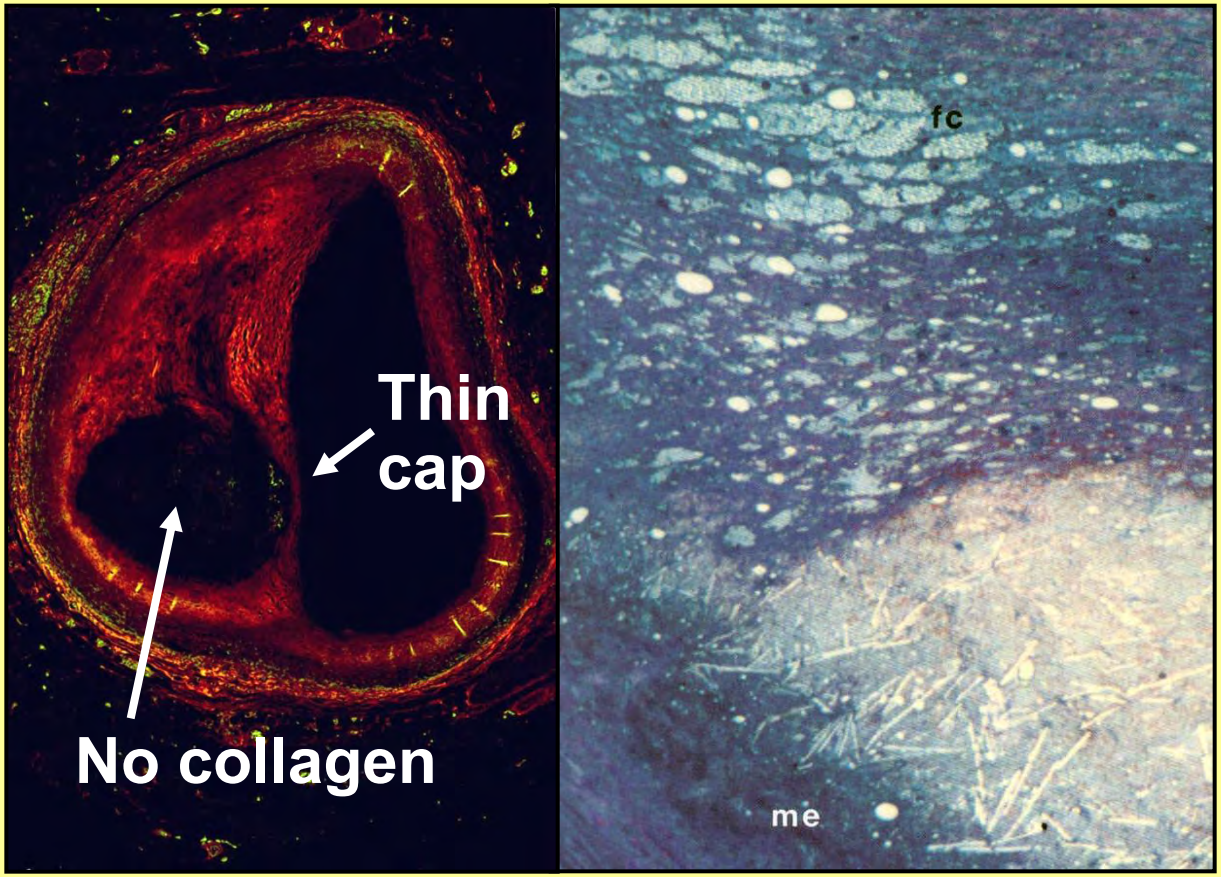
Invasive destructive apoptotic foam cells - cap thinning and core expansion



Does it translate to mice?

- Studies on mouse monocyte macrophages in vitro
- Studies in ApoE null mice

Subcutaneous sponges

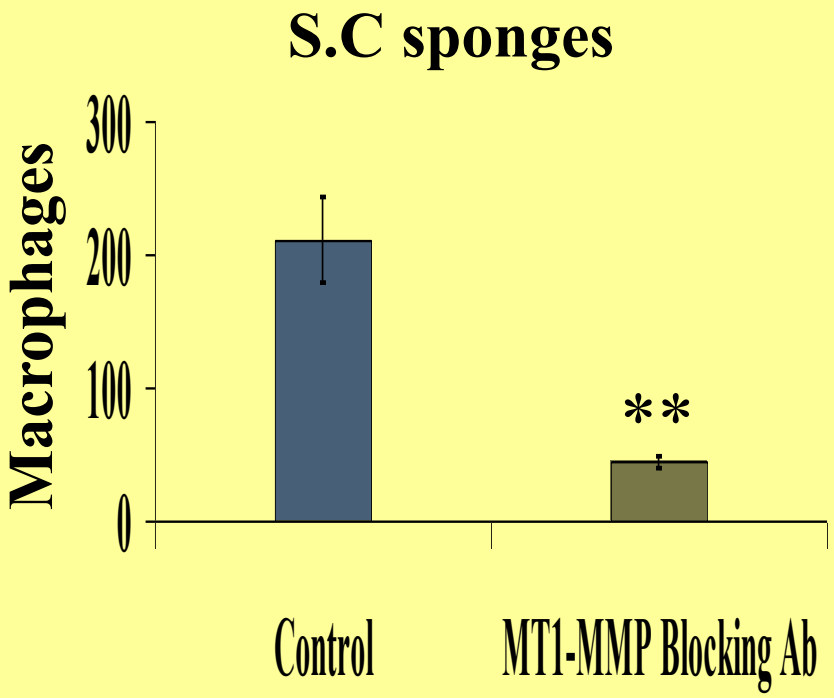
Bracheocephalic arteries

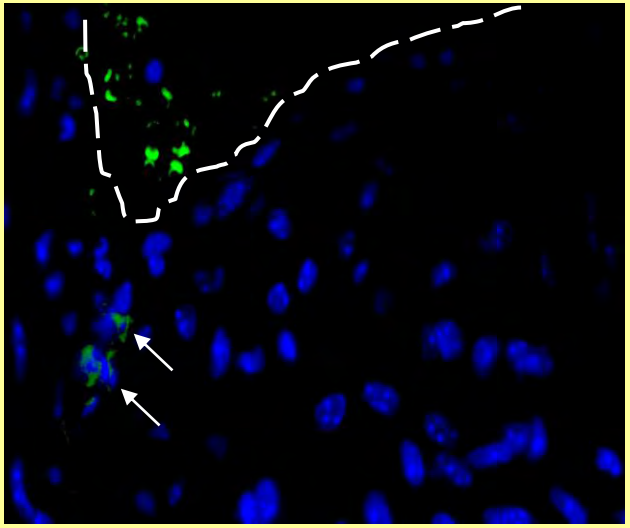
Karina di Gregoli

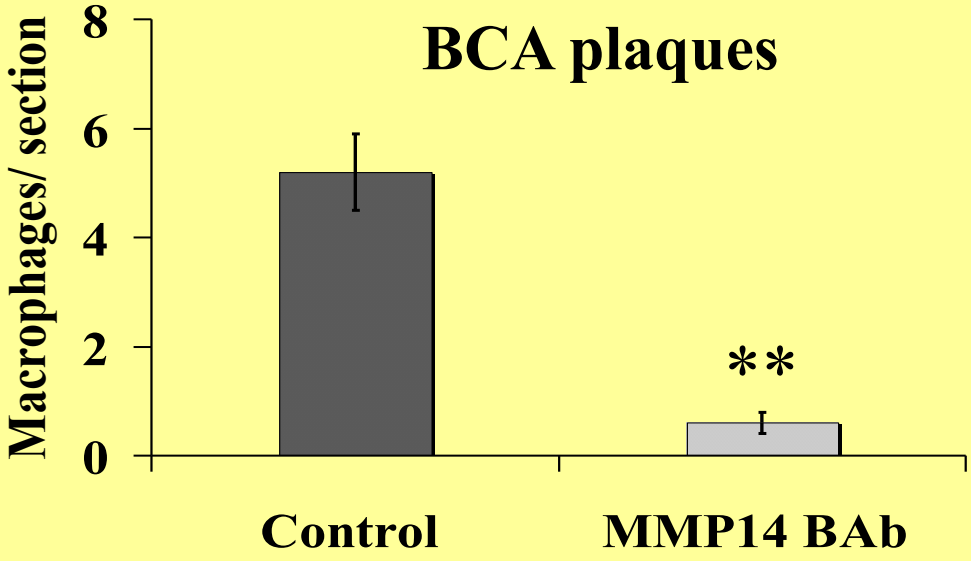
Jason Johnson BHF, IRF

BHI Bristol Heart Institute

Monocyte invasion into s.c sponges and ApoE null plaques

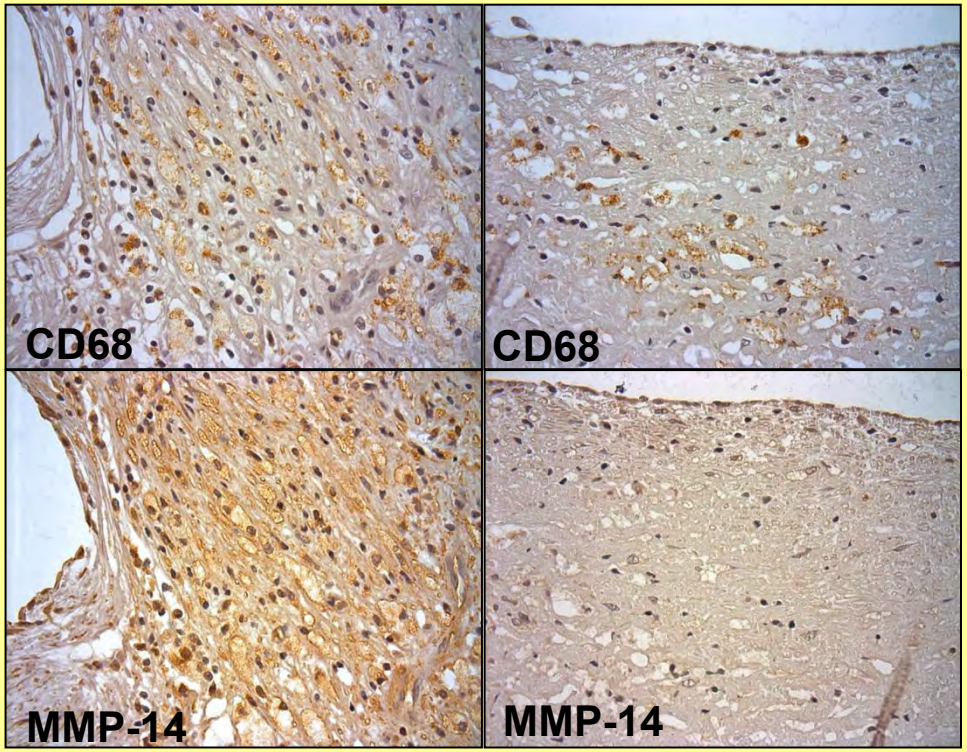


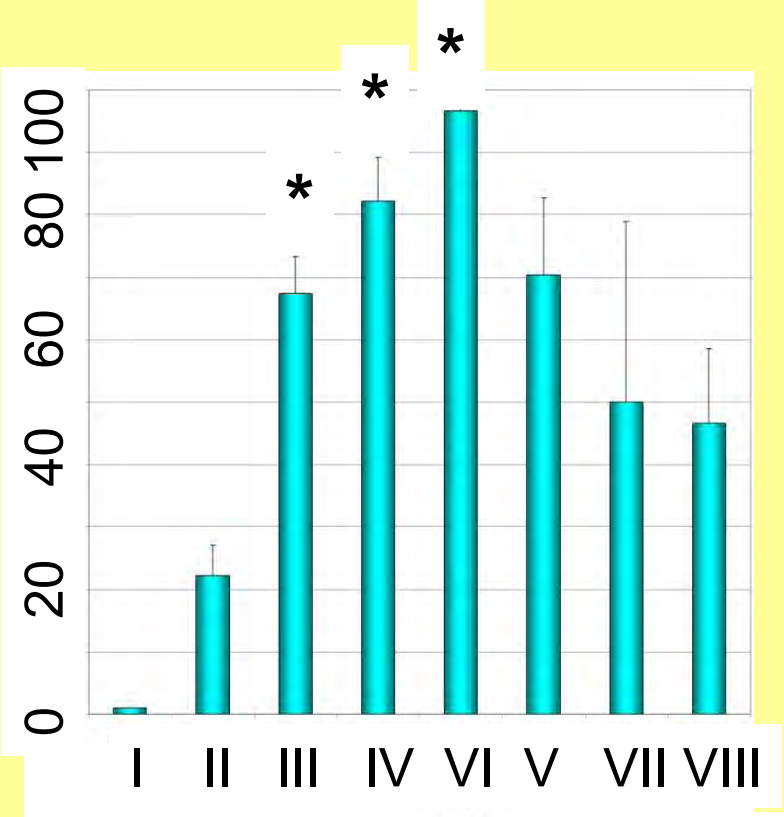




Bristol Coronary Biobank

Nick Jenkins





Jenkins, Johnson, Pasterkamp, Newby, unpublished

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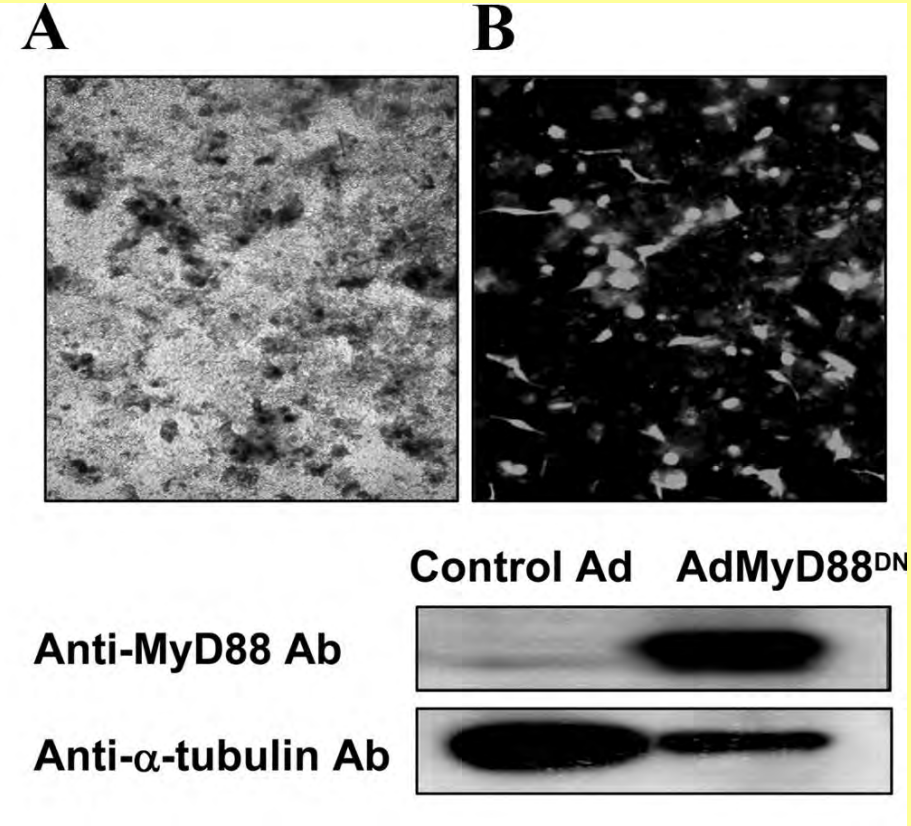
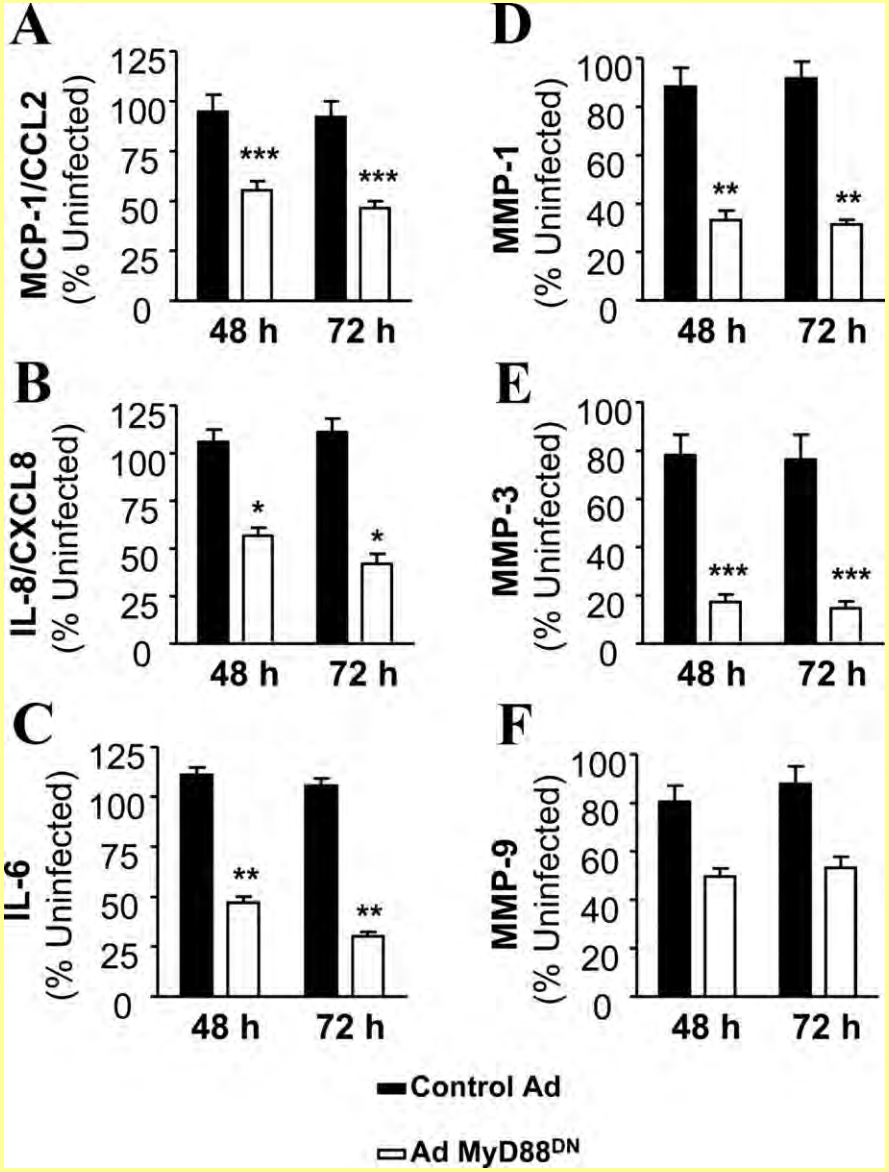
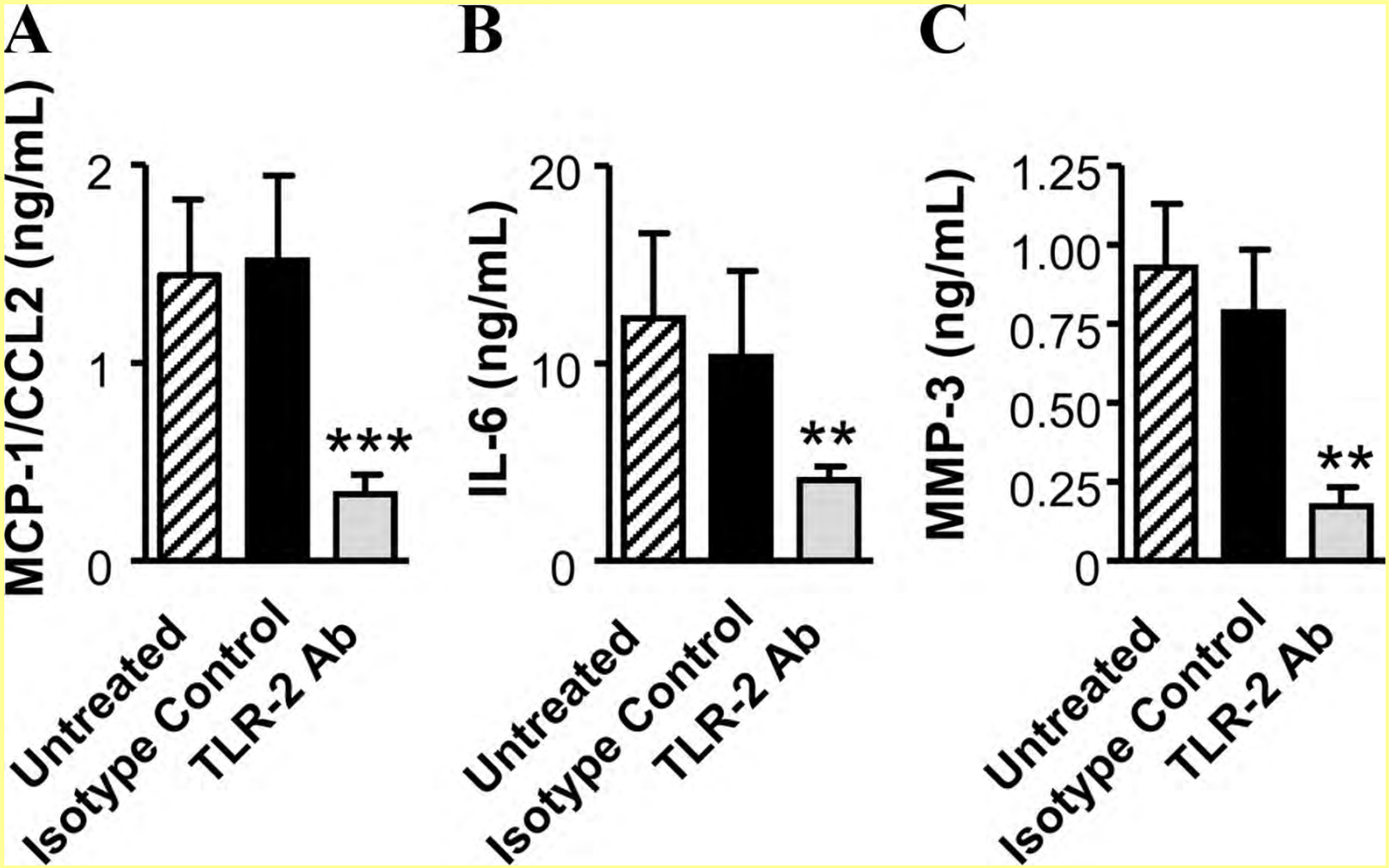
Role of TLR2





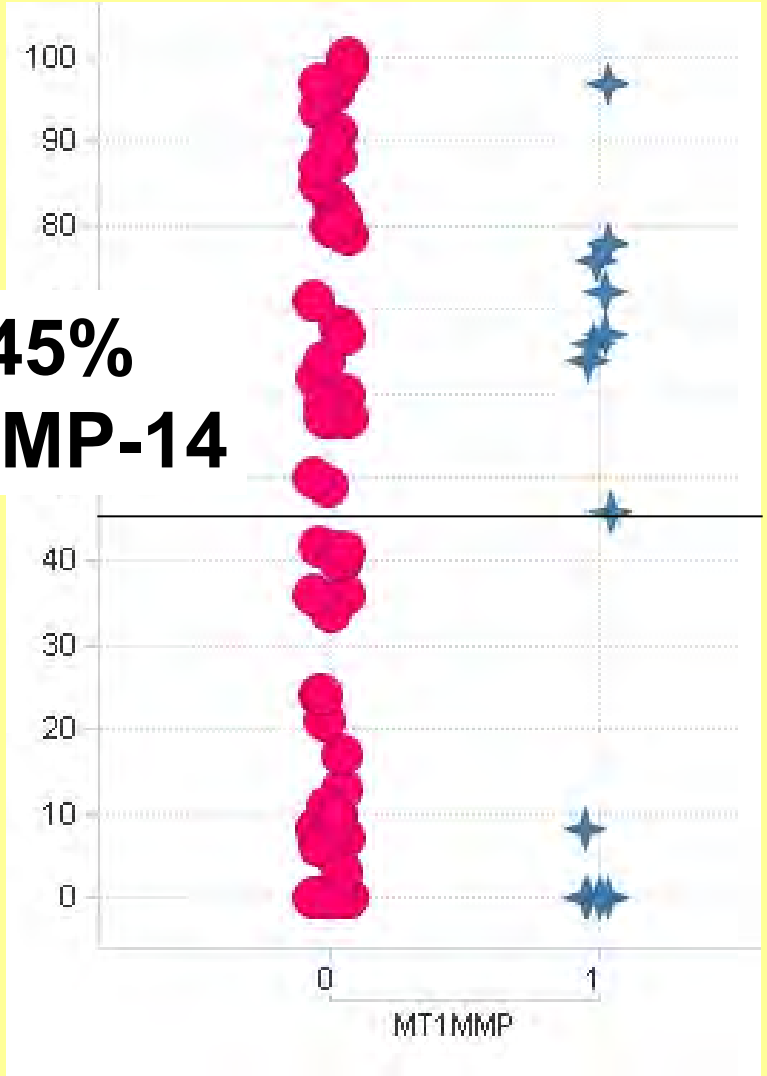
Monaco C. Circulation. 2009;120(24):2462-2469.

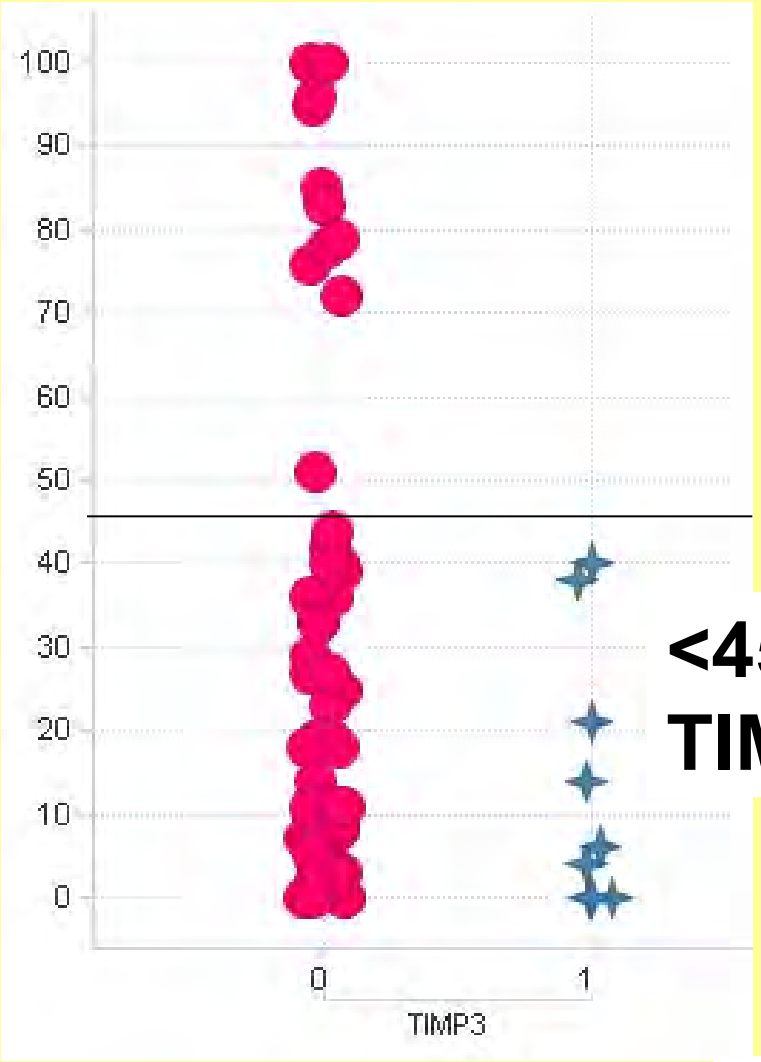
Role of TLR2



Monaco C. Circulation. 2009;120(24):2462-2469.

Subsequent death of patient





Collaboration with Vincent Scholtes and Gerard Pasterkamp

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Conclusions

- **Some metalloproteinases cause instability others not likely**
- **Turned on by inflammation via LPS, TNF, IL-1, CD40L**
- **More selectively by IFNγ and IL-4**
- **More invasive and destructive more likely to undergo apoptosis**
- **Consitent with effects observed in vivo**