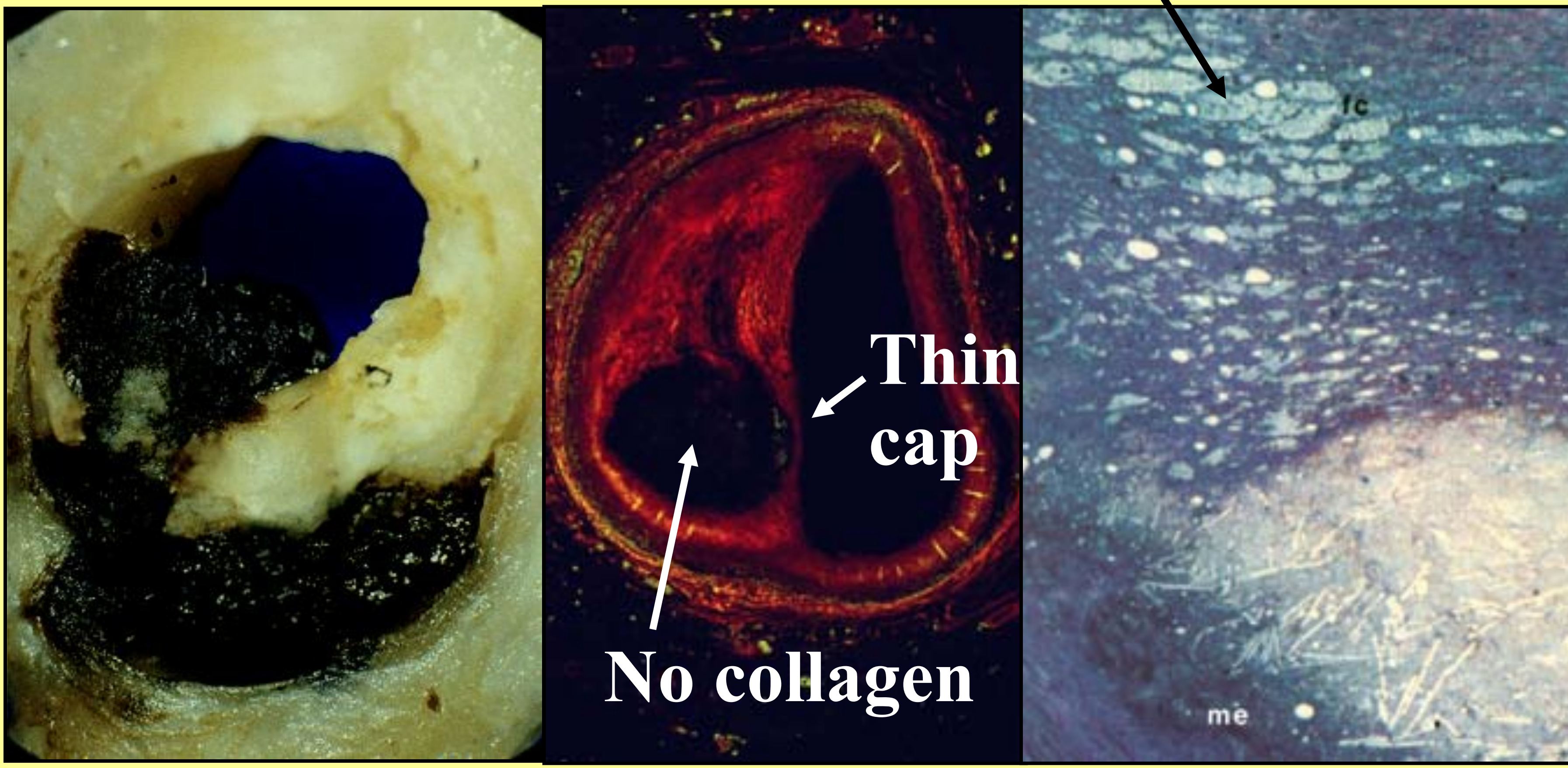
Inflammation and extracellular proteinases

Plaque rupture 75% of MI (heart attack)

Foamy macrophages

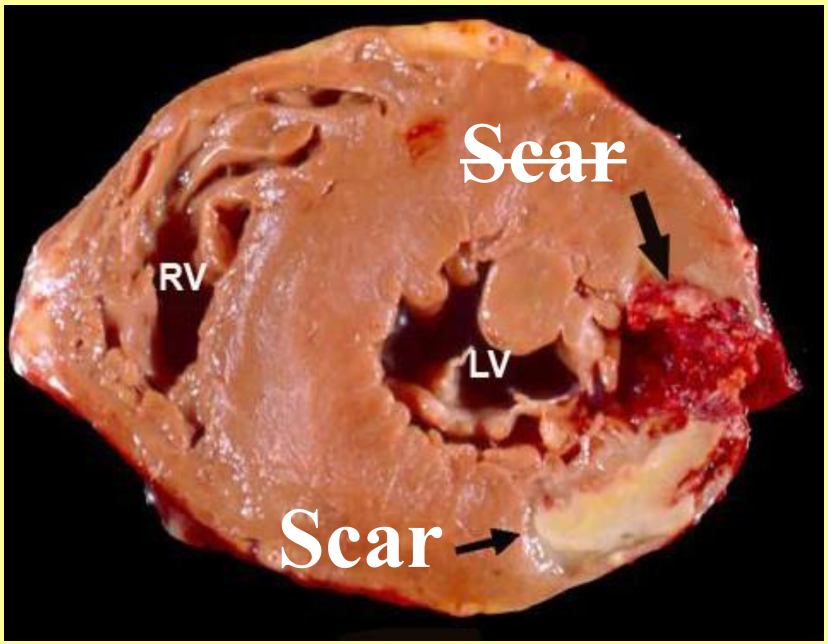


Thin fibrous cap (<65 µm), large lipid core, no collagen, macrophages, T-cells

Davies, M Circulation 1996; Falk, E JACC 2006;47:C7-12

Long-term consequences of MI

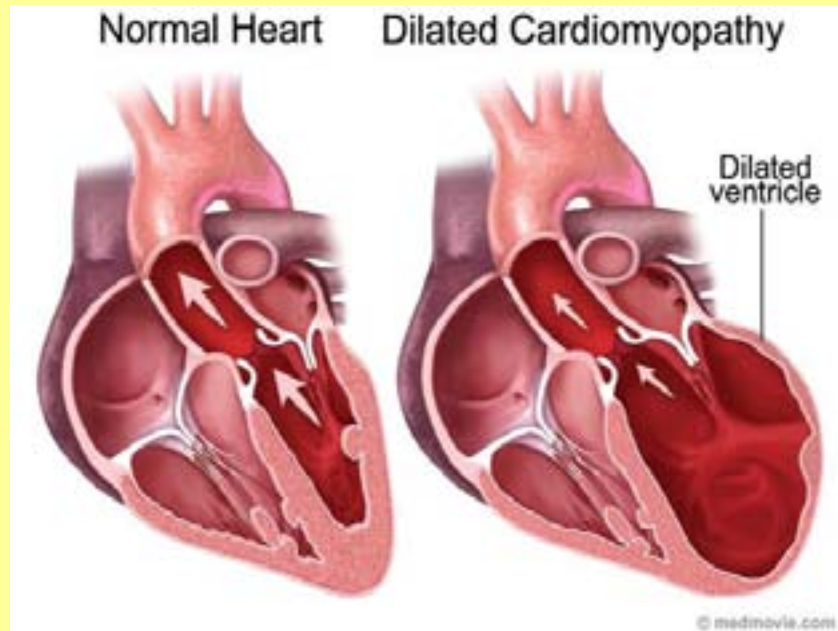
Cardiac rupture



www.readcube.com

Too little
Inflammation/ repair

Heart failure

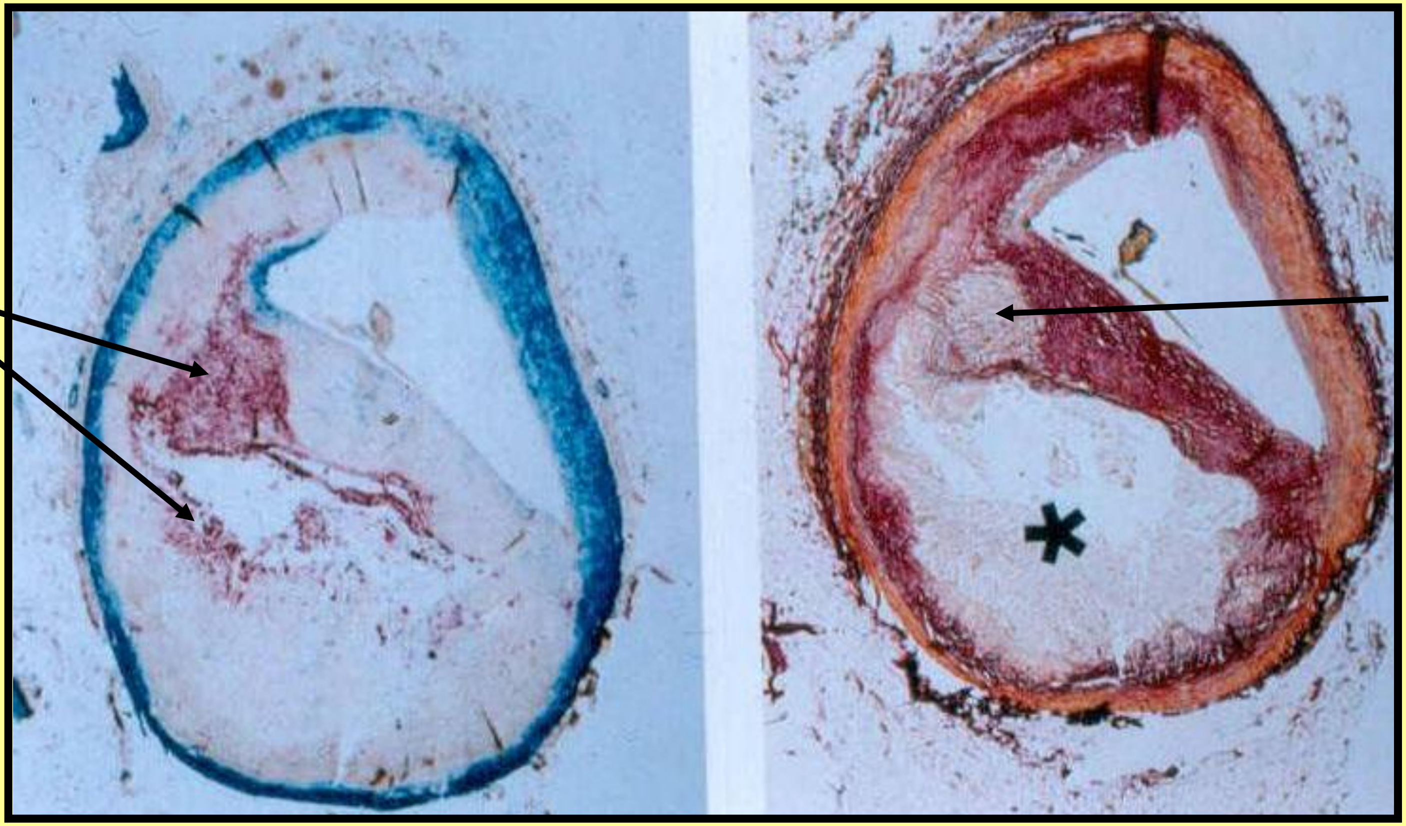


stanfordhospital.org

Too much inflammation

Foam cells make proteases that cause plaque vulnerability

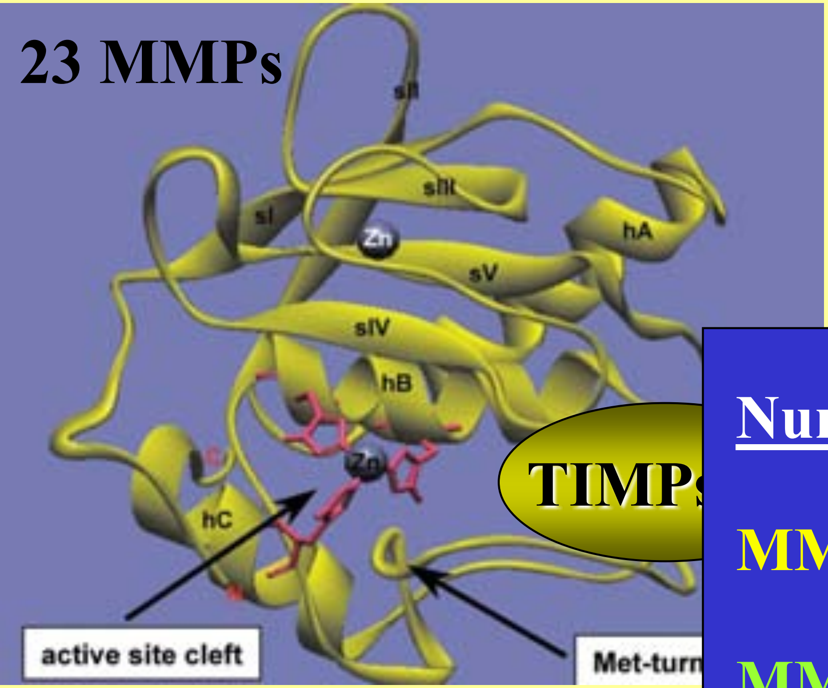
Foam cells



Low collagen

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

Metalloproteinases and TIMPs



Many non-matrix substrates

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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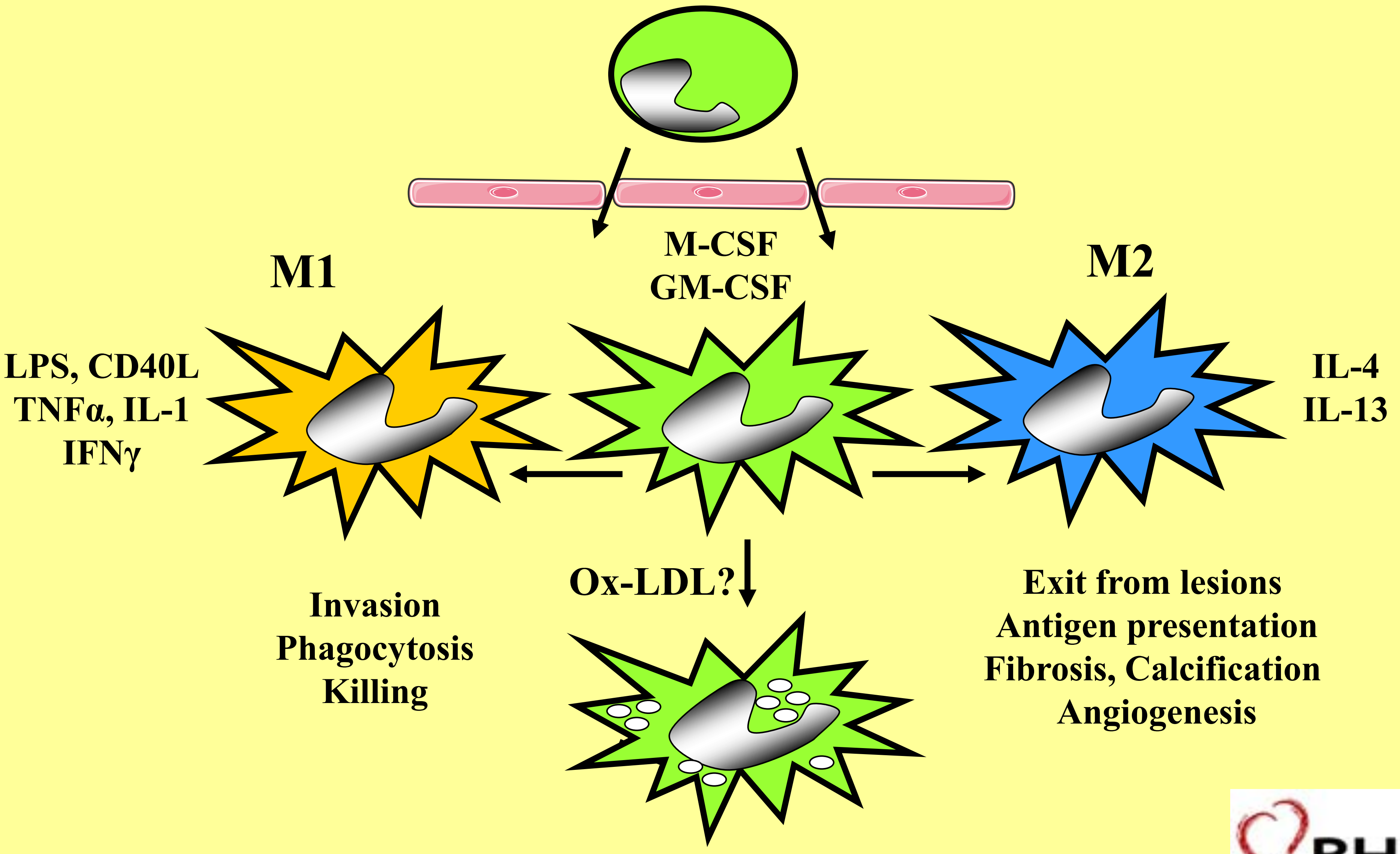
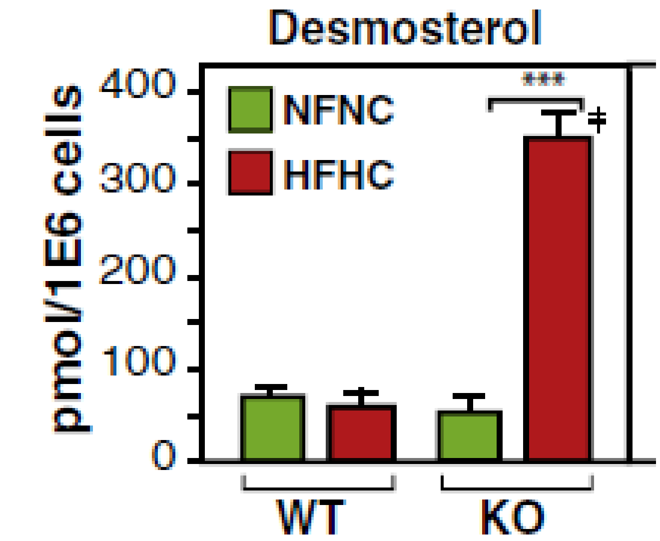
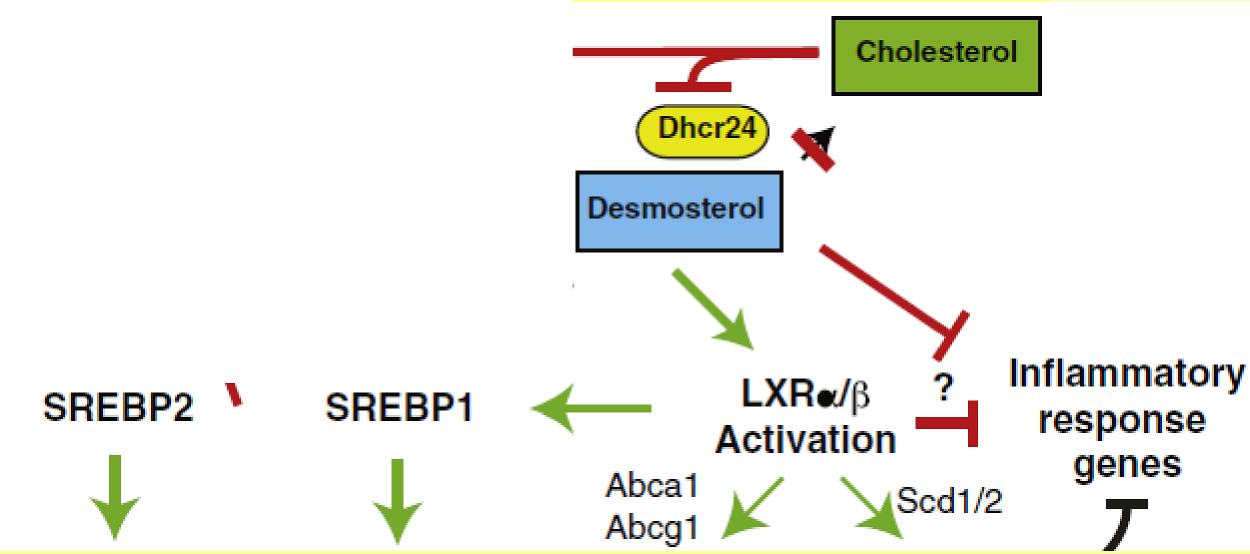
Inflammation and macrophage diversity

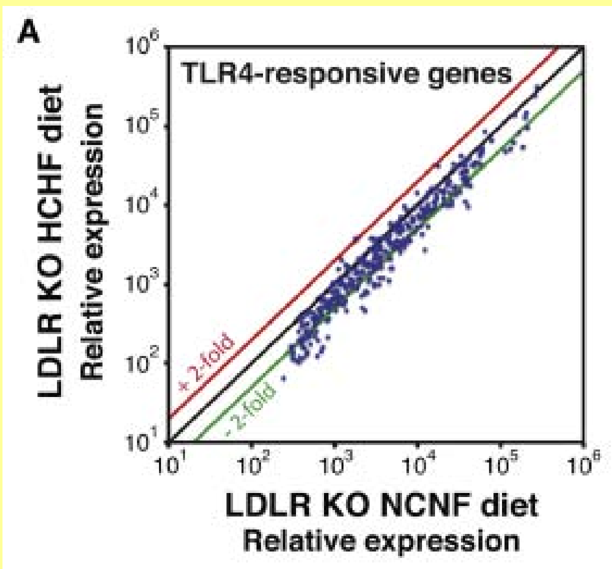


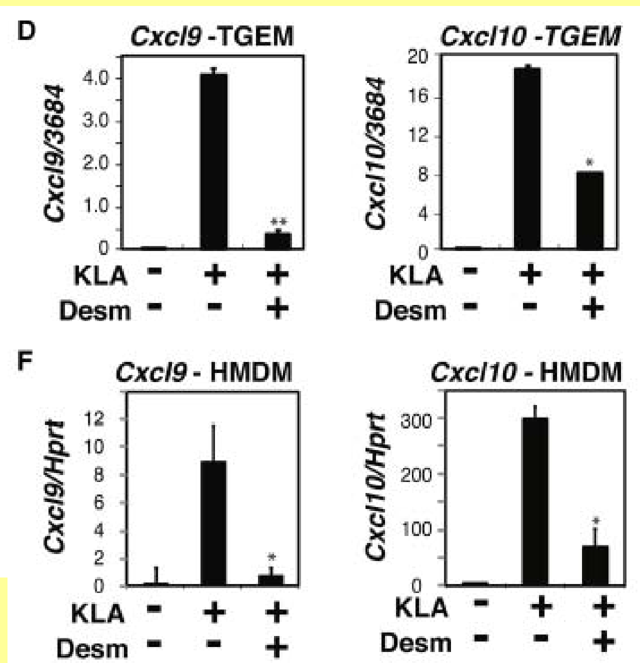
Gordon S, Taylor PR. Nat Rev Immunol. 2005;5(12):953-64.

Effect of foam cell formation







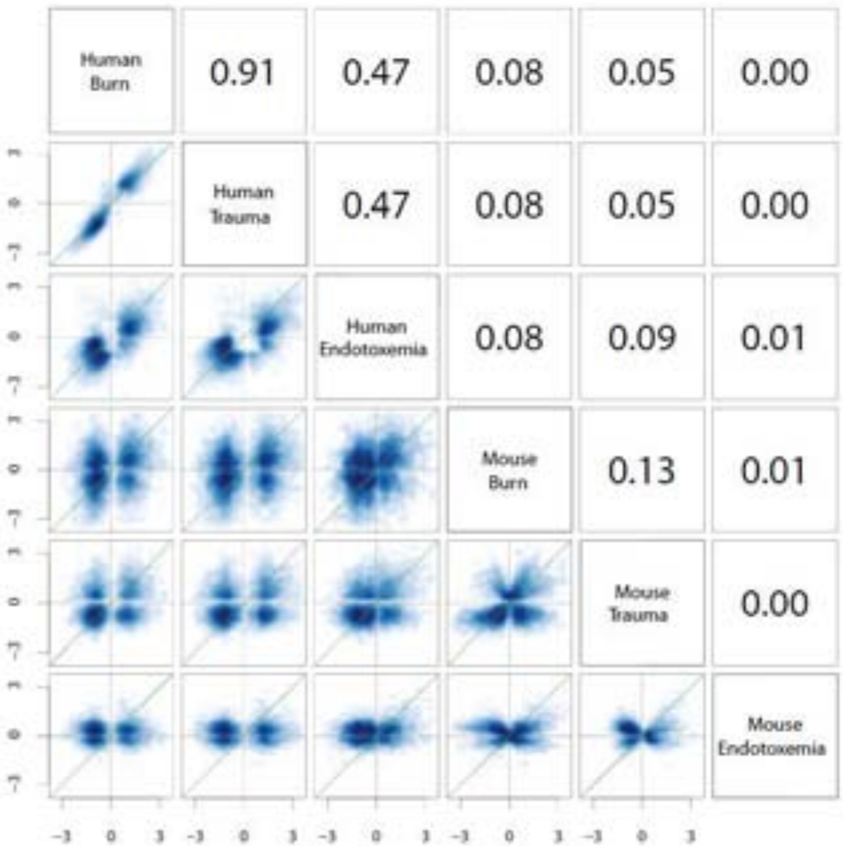


Spann et al. Cell 2012; 151, 138–152

Genomic responses in mouse models poorly mimic human inflammatory diseases

Junhee Seok[a,1], H. Shaw Warren[b,1], Alex G. Cuenca[c,1], Michael N. Mindrinos[a], Henry V. Baker[c], Weihong Xu[a], Daniel R. Richards[d], Grace P. McDonald-Smith[e], Hong Gao[a], Laura Hennessy[f], Celeste C. Finnerty[g], Cecilia M. López[c], Shari Honari[f], Ernest E. Moore[h], Joseph P. Minei[i], Joseph Cuschieri[j], Paul E. Bankey[k], Jeffrey L. Johnson[h], Jason Sperry[l], Avery B. Nathens[m], Timothy R. Billiar[l], Michael A. West[n], Marc G. Jeschke[o], Matthew B. Klein[j], Richard L. Gamelli[p], Nicole S. Gibran[j], Bernard H. Brownstein[q], Carol Miller-Graziano[k], Steve E. Calvano[r], Philip H. Mason[e], J. Perren Cobb[s], Laurence G. Rahme[t], Stephen F. Lowry[r,2], Ronald V. Maier[j], Lyle L. Moldawer[c], David N. Herndon[g], Ronald W. Davis[a,3], Wenzhong Xiao[a,t,3], Ronald G. Tompkins[t,3], and the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program[4]

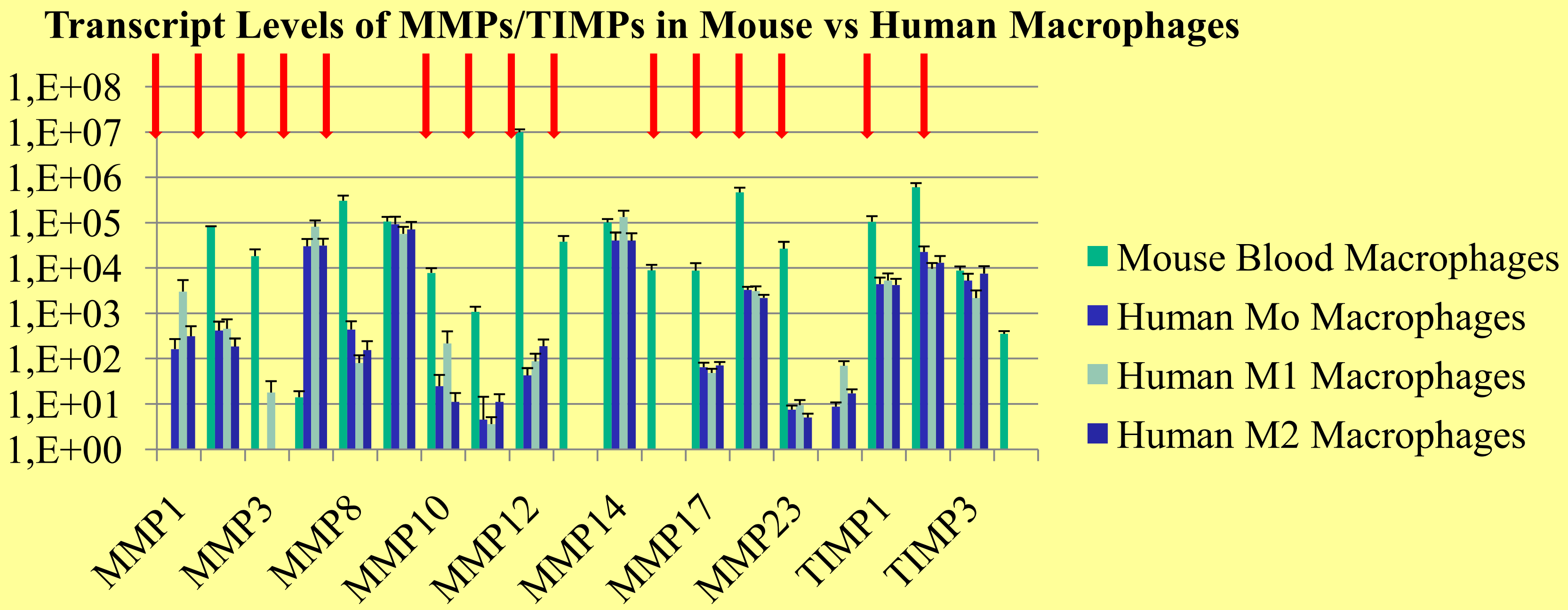
PNAS | February 26, 2013 | vol. 110 | no. 9 | 3507–3512



Are all mouse models of atherosclerosis Dinosaurs?

Many biochemical differences

- **HDL rather than LDL**
- **SAA rather than CRP**
- **MMP-13 rather than MMP-1**



- **Many MMP/TIMPs showed 10-10,000 fold Δ**

Macrophage phenotypes – the future

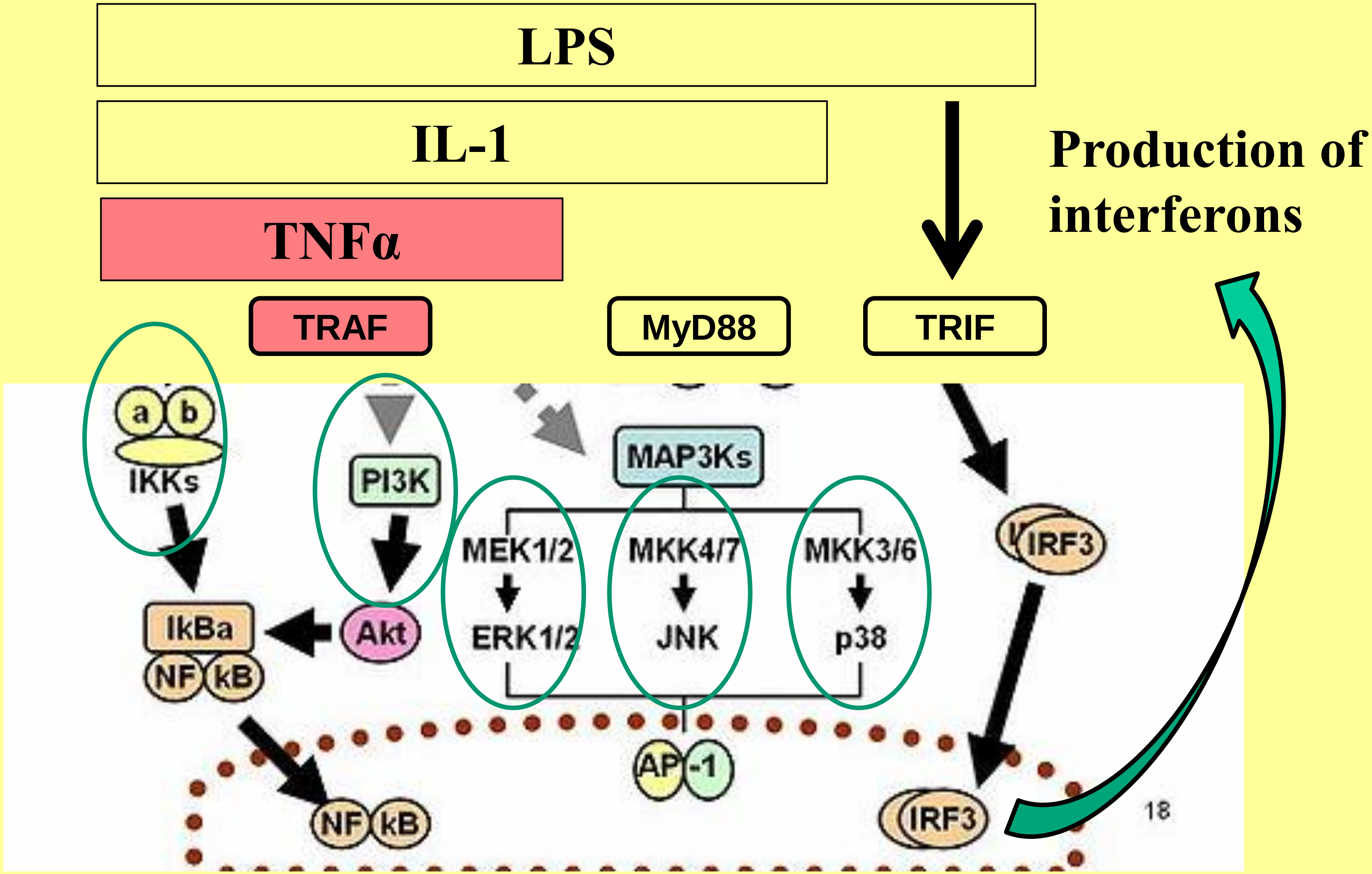
Follow the pathways!

David Hockney

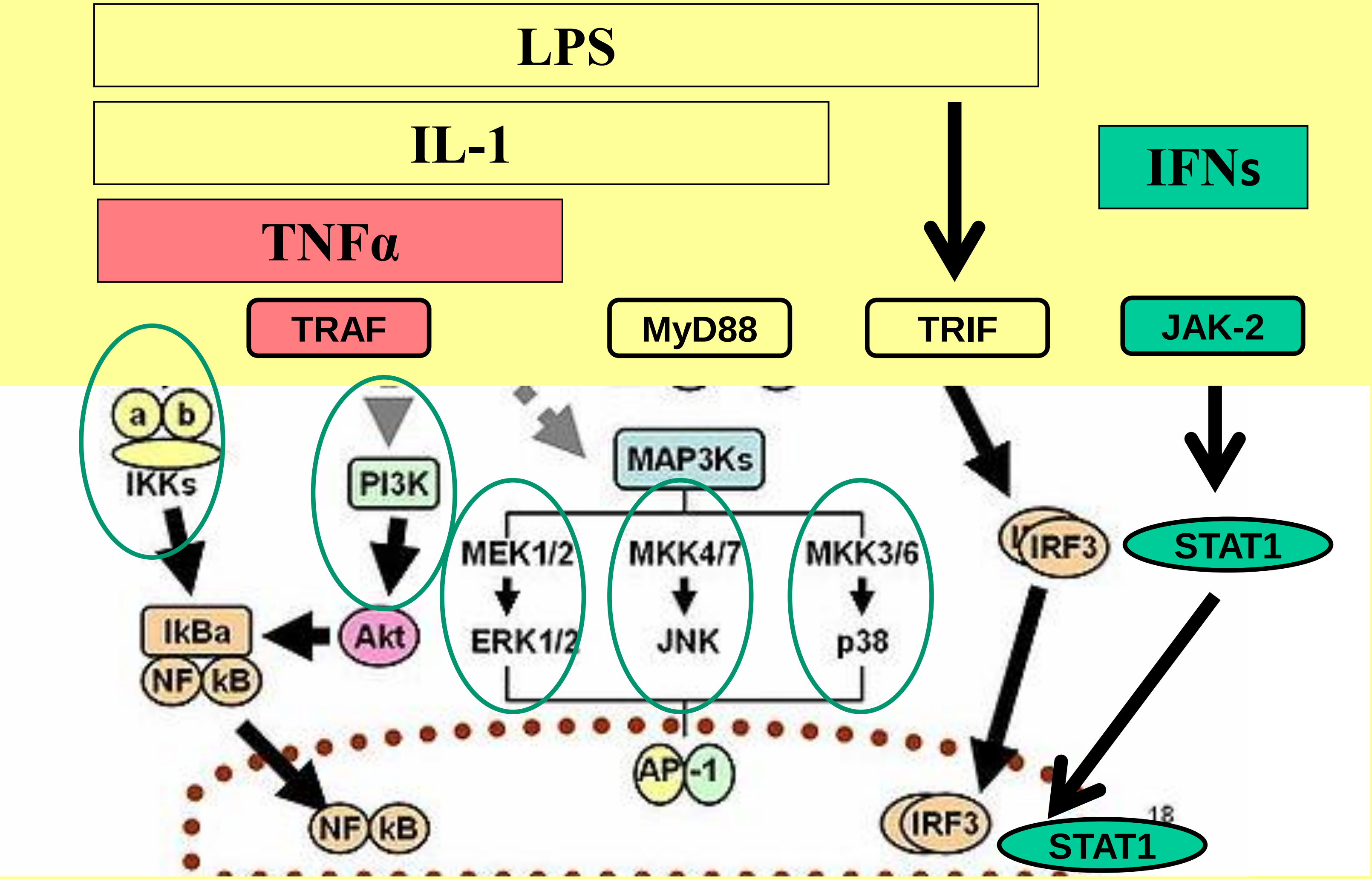
MCSF, GMCSF and OxLDL

Old name	M-Mac	GM-Mac	Foam cell
Proposed name	MM(-CSF)	MGM(-CSF)	FM(OxLDL)
	CSFR1/CD115	CSFR2/CD116	Scavenger Rs
Signal 1	ERKs, PI-3K	ERKs, PI-3K	Free cholesterol, desmosterol
TFs	Pu.1, Egr-1 AP-1	Pu.1, Egr-1 AP-1, STAT-5	LXRα,β SREBPs
Markers	CD206	CD206	CD11c, Foamy appearance
Proposed marker		Nuclear STAT-5P	Adipophilin
	1	2	3

Classical activation pathways



Classical activation pathways

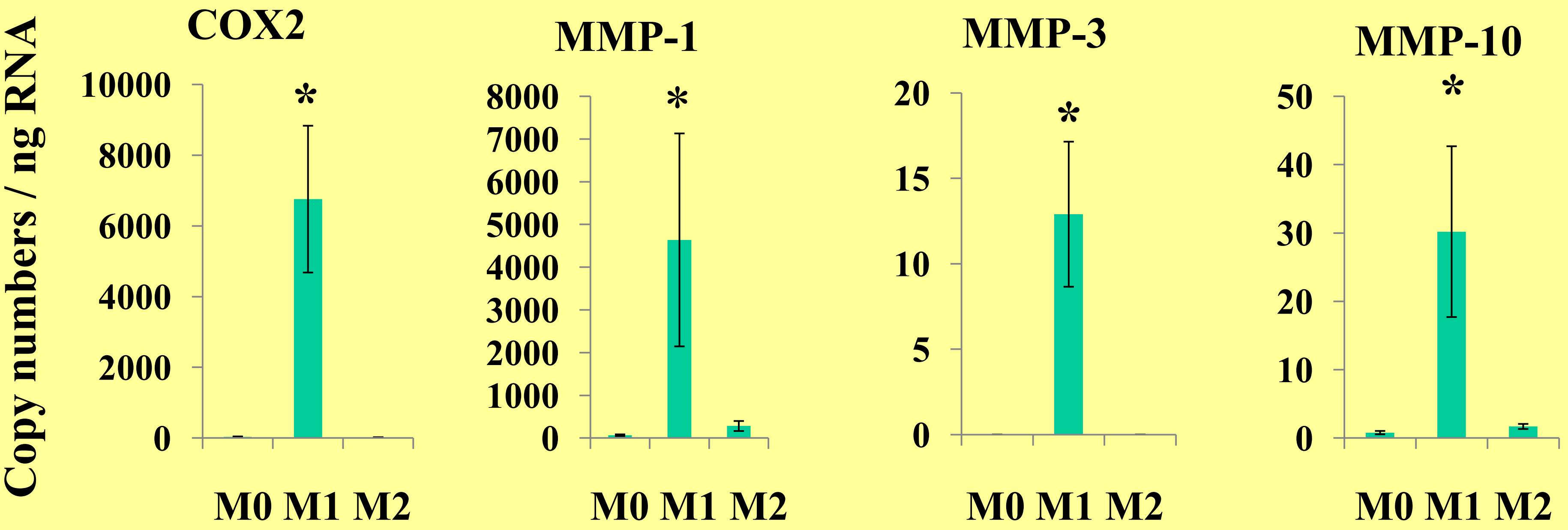


Classically-activated (M1) phenotypes

Proposed name	MTNFα	MCD30L /CD40L /Ox40L	MIL-1	MLPS (TLL)	MIFNγ	(M1) MLPS +IFNγ
Receptor	TNFRSF		IL1R1	TLR-4 (TLRs)	IFNγR1	
Signal 1	TRAFs		Myd88	Myd88 IRF-3	Jak1/2, IRF-3	All
Signal 2	ERKs, PI3K, PKC, IKK2				STAT-1	All
TFs	AP-1, NF-κB				STAT-1	All
Markers?	IκB, COX-2, iNOS				IL12, TLR4, CD14	Both
Proposed marker	Nuclear NF-κB				Nuclear STAT1-P	Both

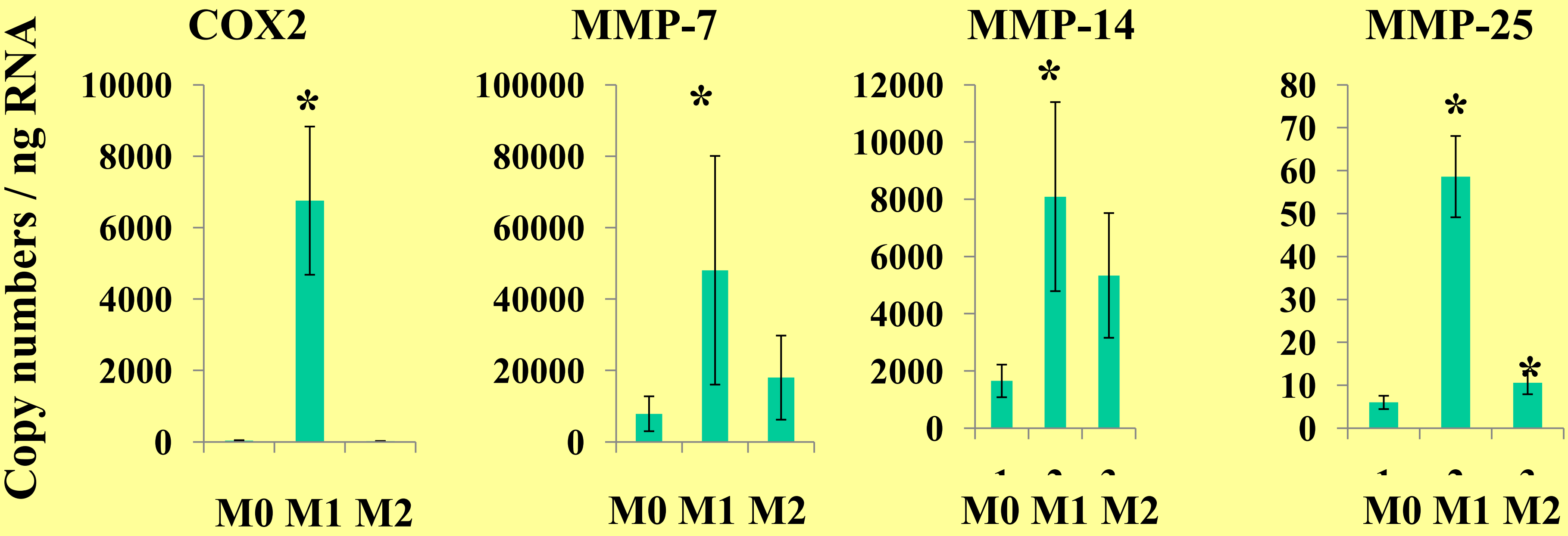
4 5 6

Human Foam cells stimulated by LPS + IFNγ in vitro (M1)



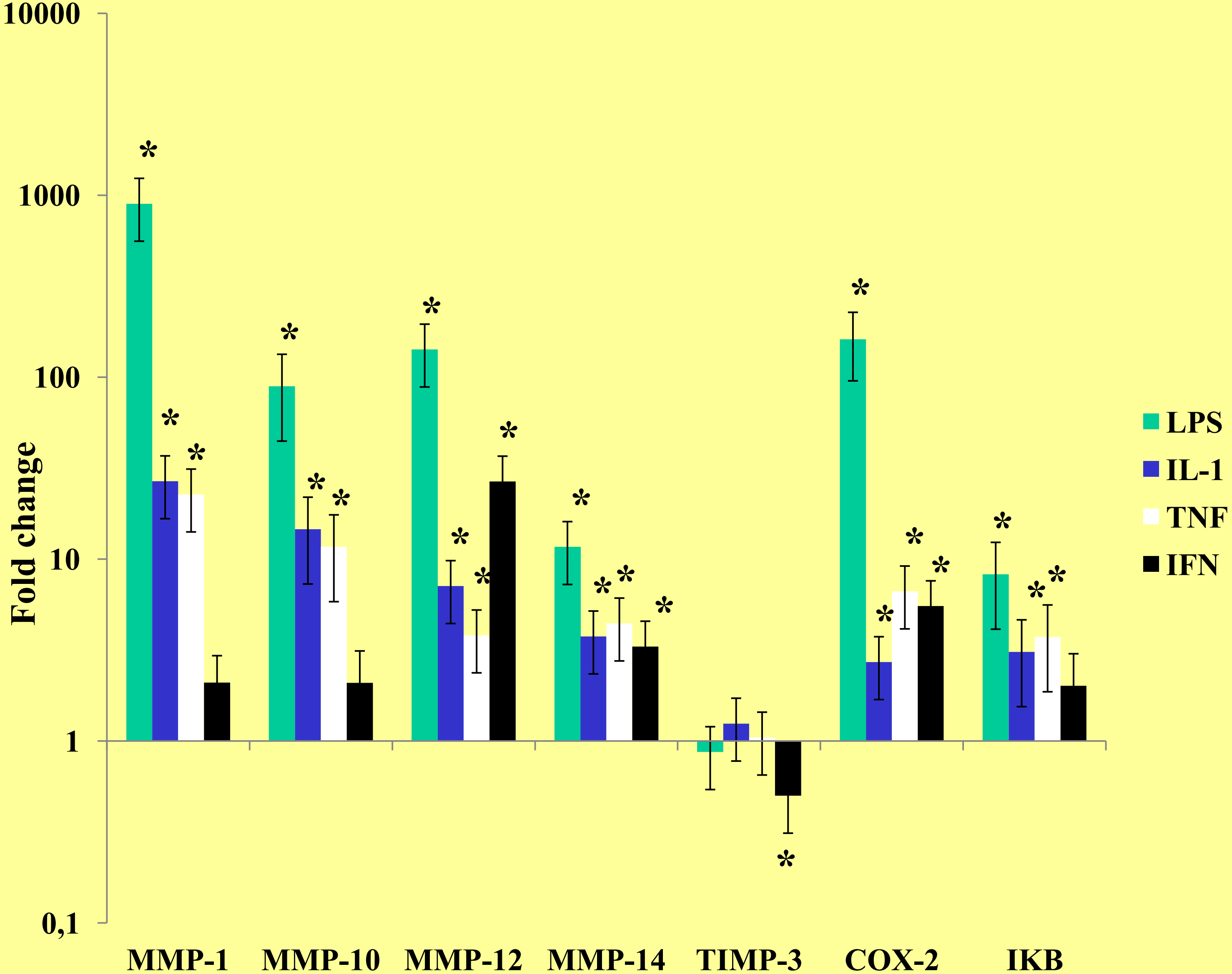
Huang, Sala-Newby et. al. unpublished
Compare Huang, Sala-Newby et. al. PLoS ONE 2012

Human foam cells stimulated by LPS + IFNγ (M1) in vitro



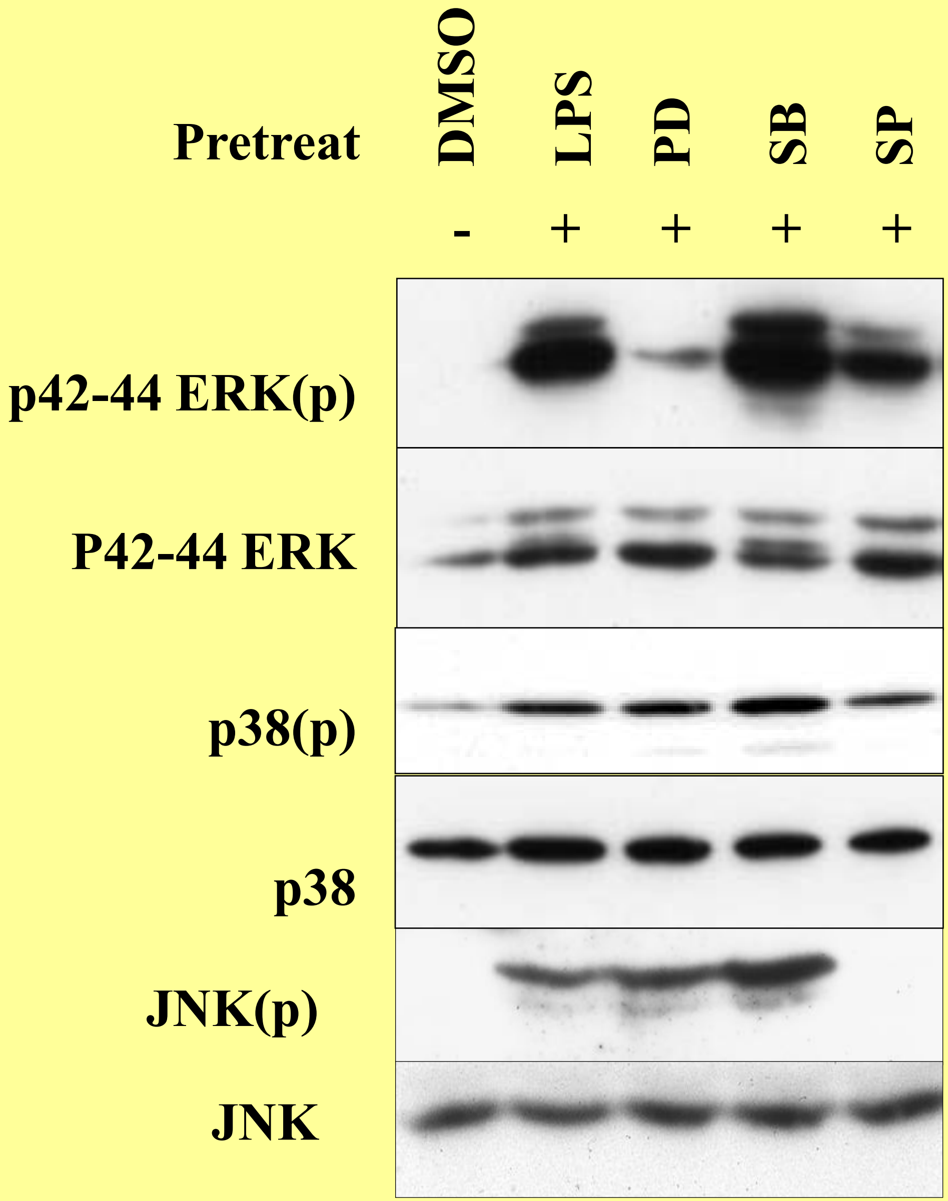
Compare Huang, Sala-Newby et. al. PLoS ONE 2012

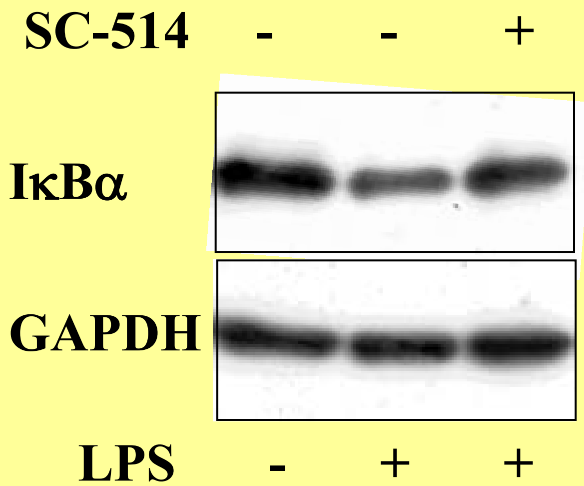
Effects of classical activators



Huang, Sala-Newby et. al. PLoS ONE 2012

LPS on MAP and IκB kinases





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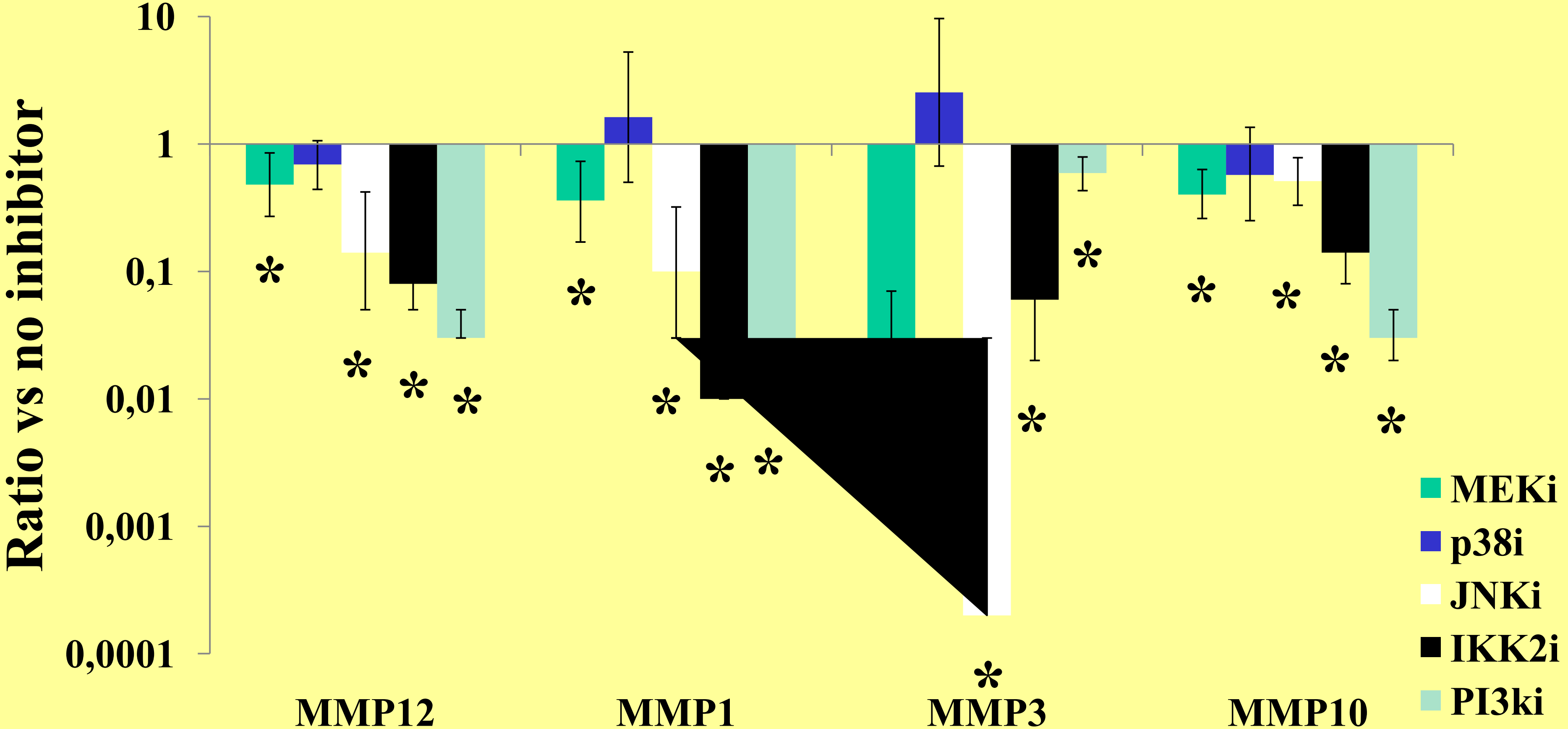
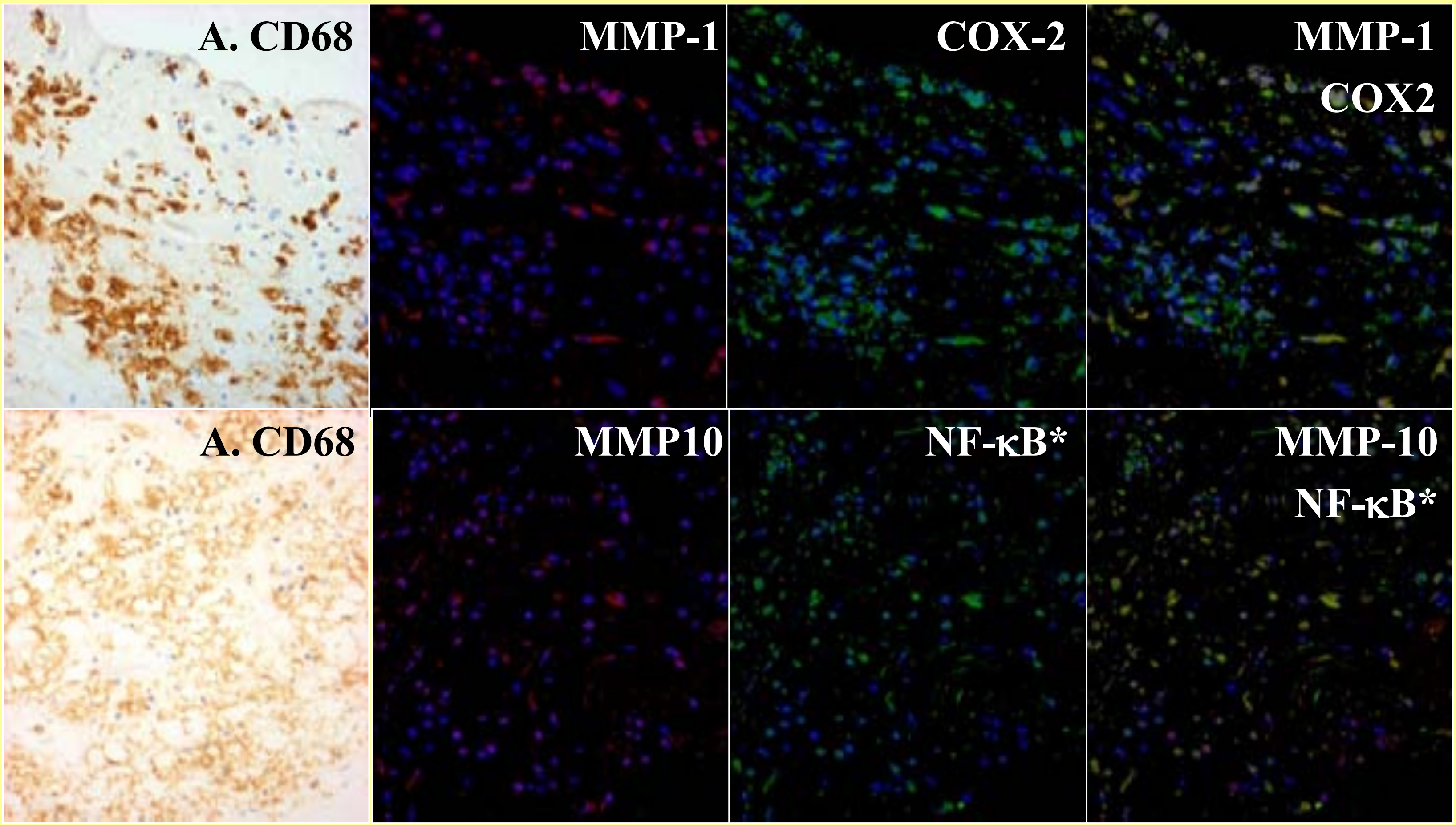
Effect of inhibitors on LPS



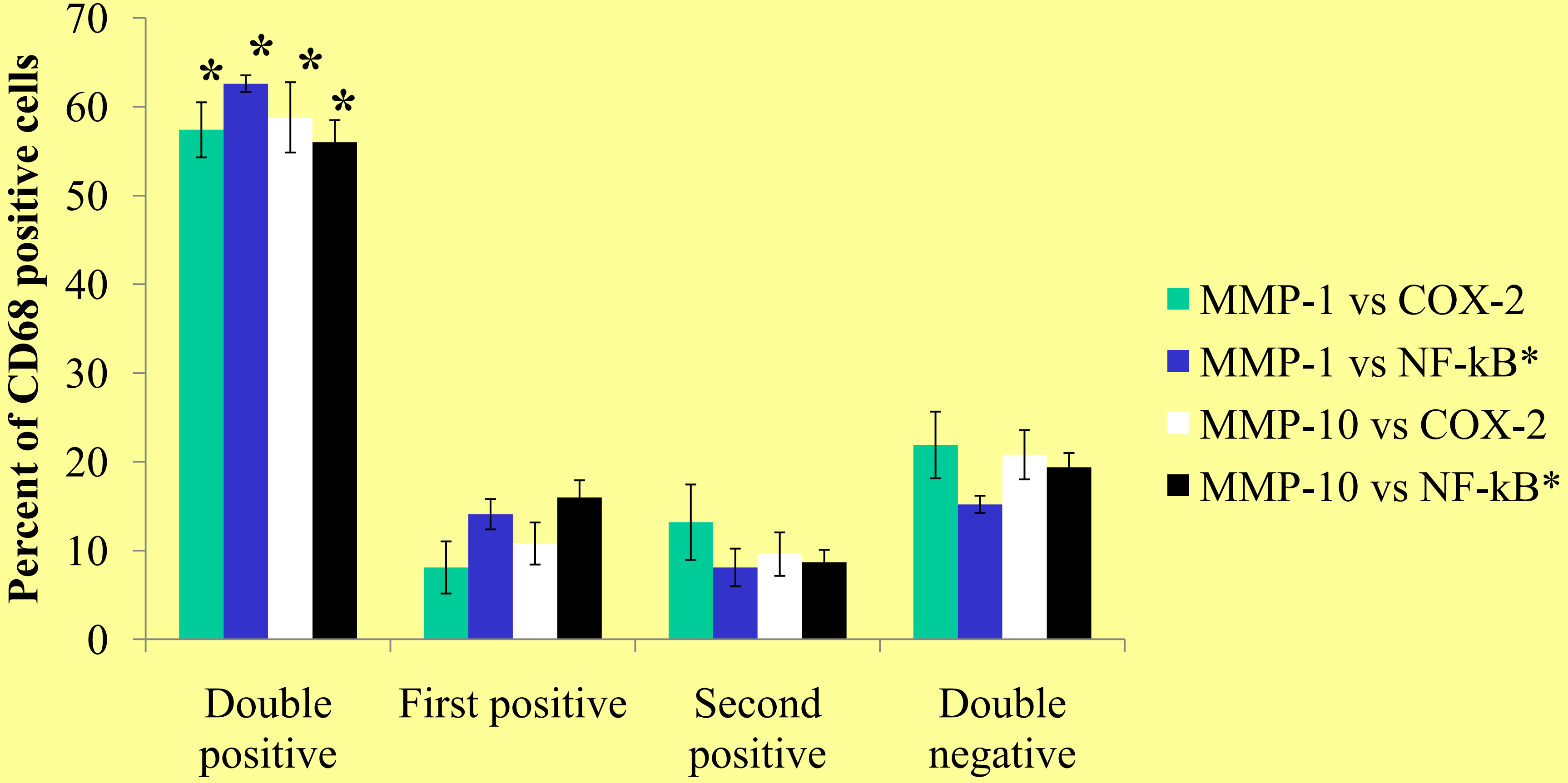
Huang, Sala-Newby and Newby, PLoS One 2012

Co-localisation in human coronary macrophages



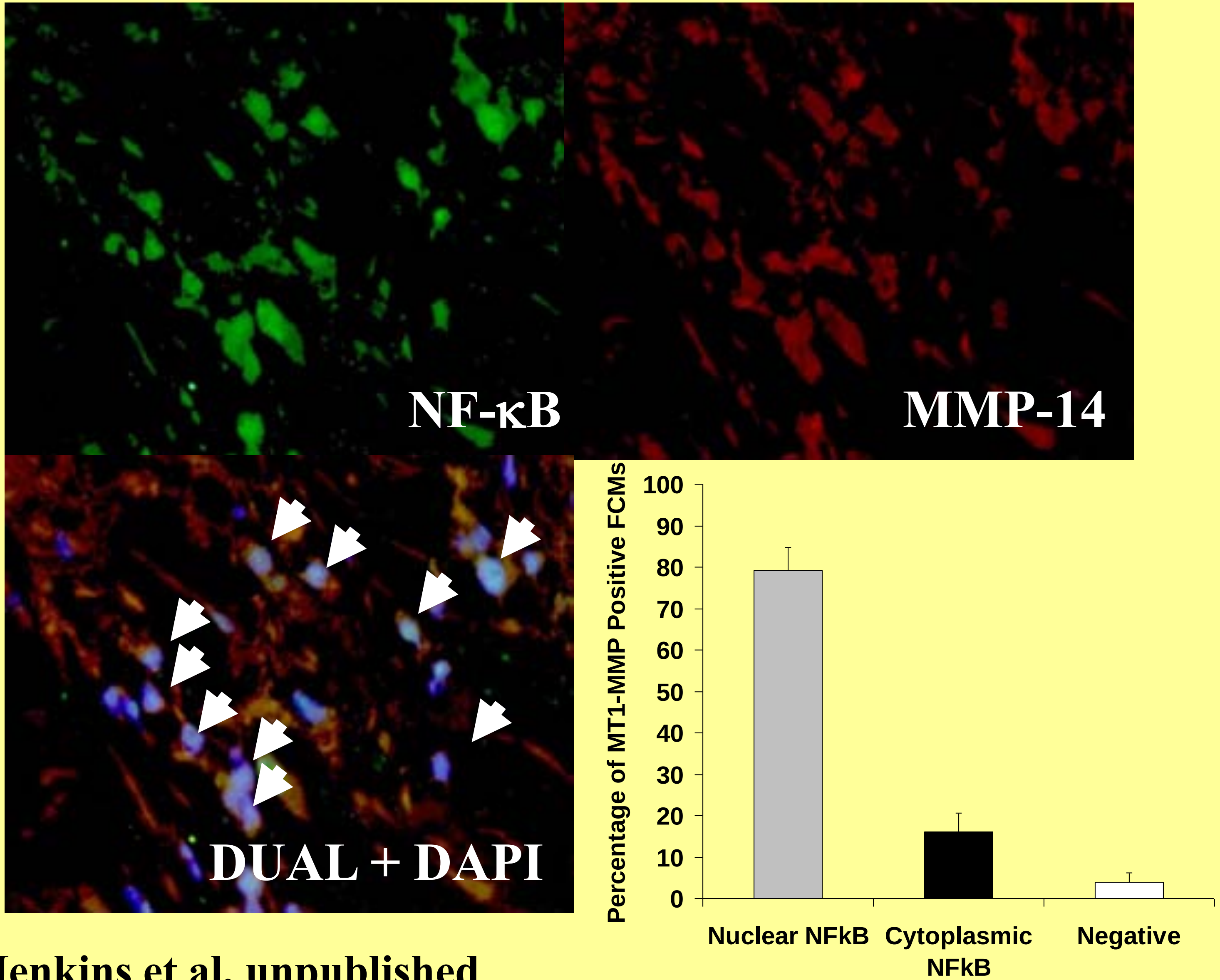
Huang, Sala-Newby and Newby, PLoS One 2012

Co-localisation in human coronary macrophages



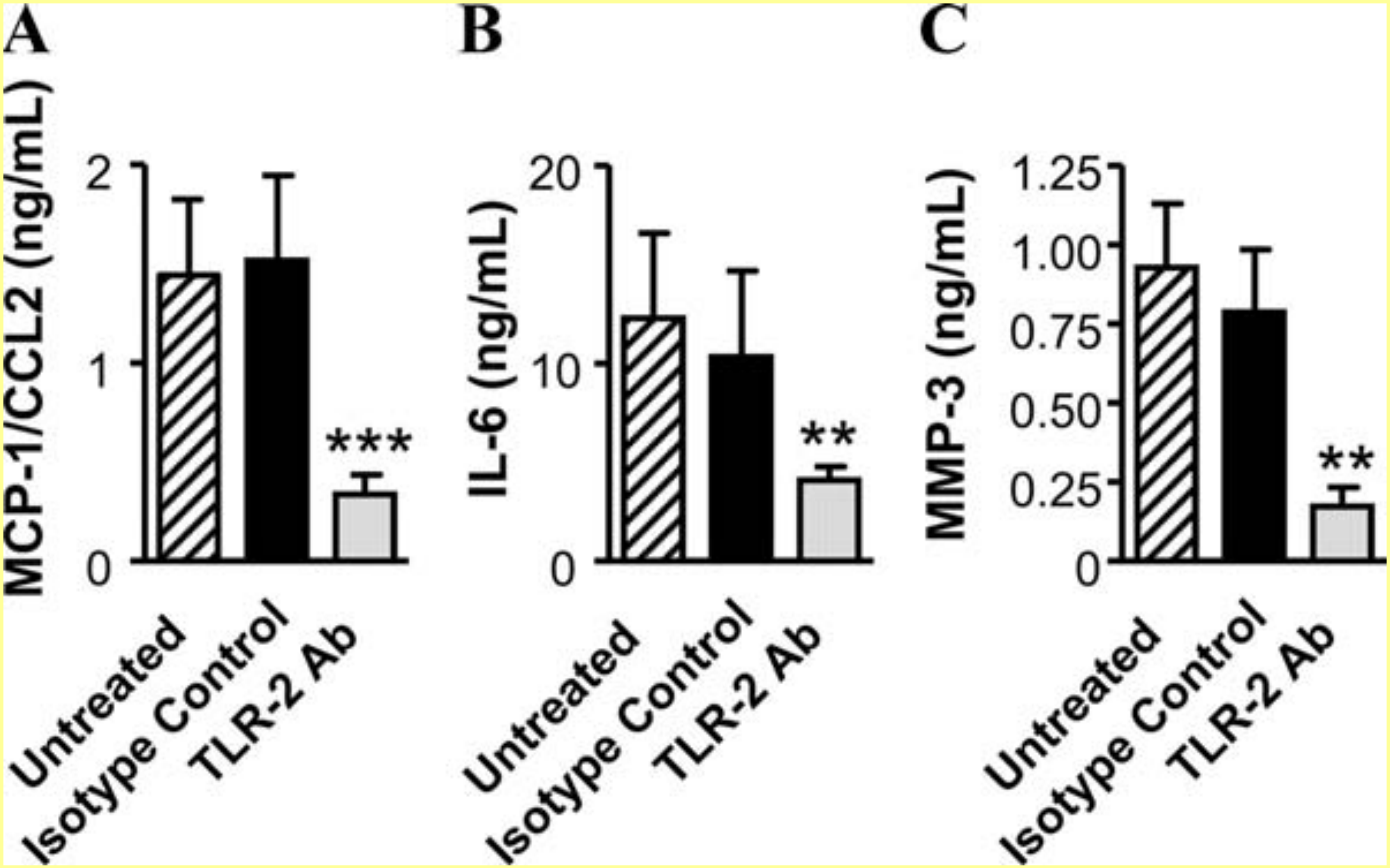
Huang, Sala-Newby and Newby, PLoS One 2012

MMP-14 and NF-κB



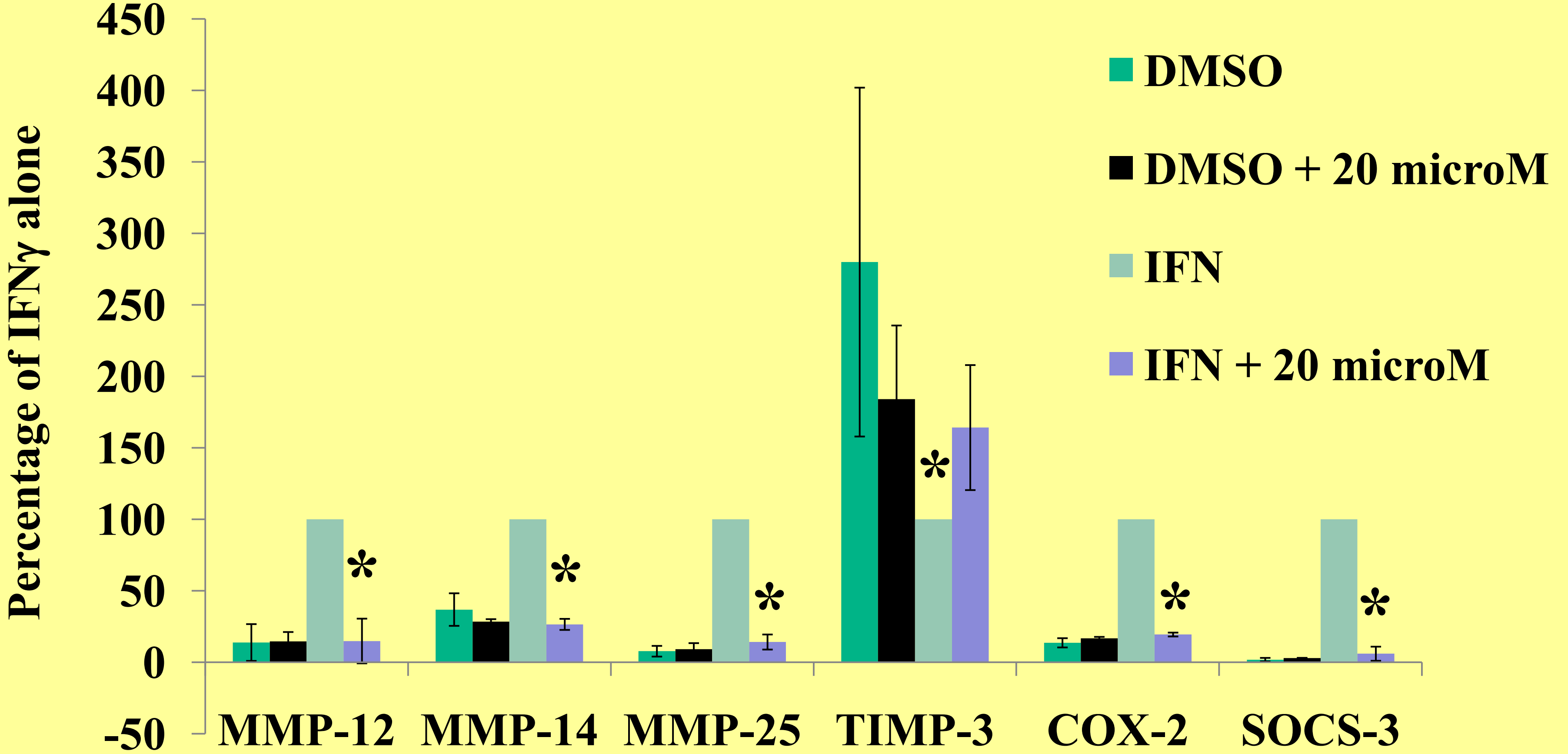
Johnson, Jenkins et al, unpublished

Role of TLR2



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

Effect of JAK-2 inhibitor on IFNγ

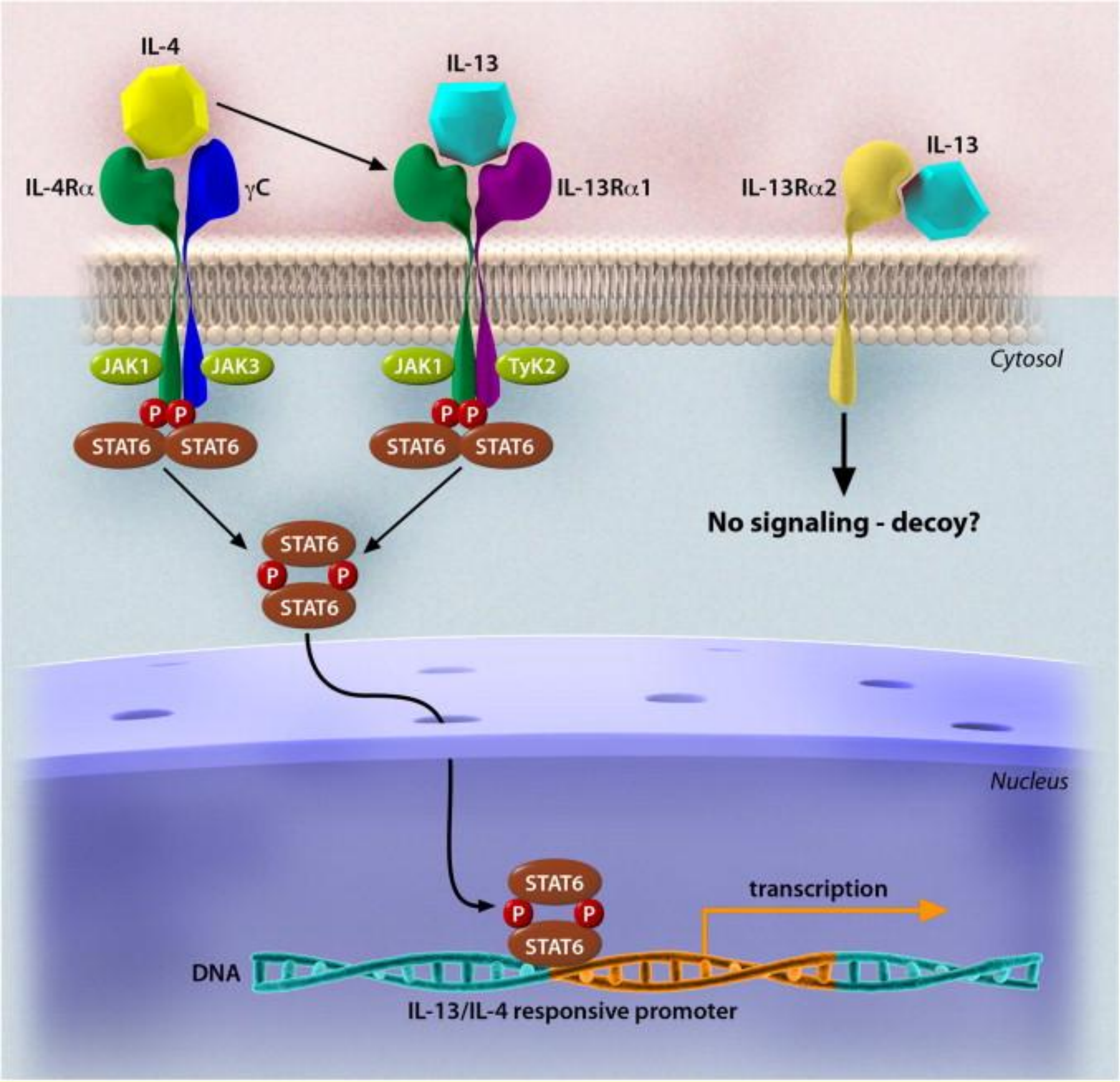


Huang, Sala-Newby and Newby, PLoS One 2012

Conclusions 1

- M1 genes are regulated through NFκB in vitro and co-localize in vivo
- A subgroup may co-localize with Stat-1P (to be shown)

IL-4 and IL-13 signalling

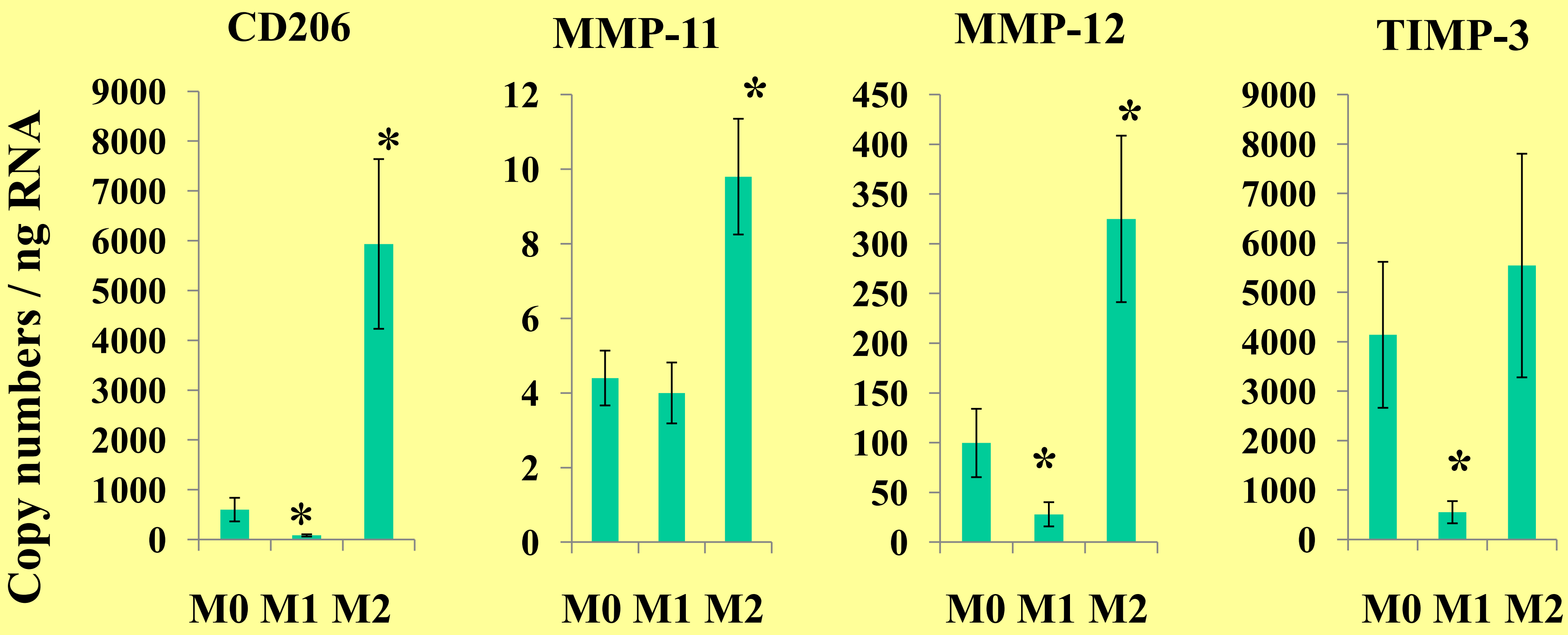


Ingram JL, Journal of Allergy and Clinical Immunology. 2012;130:829-842

Alternatively-activated (M2) phenotypes

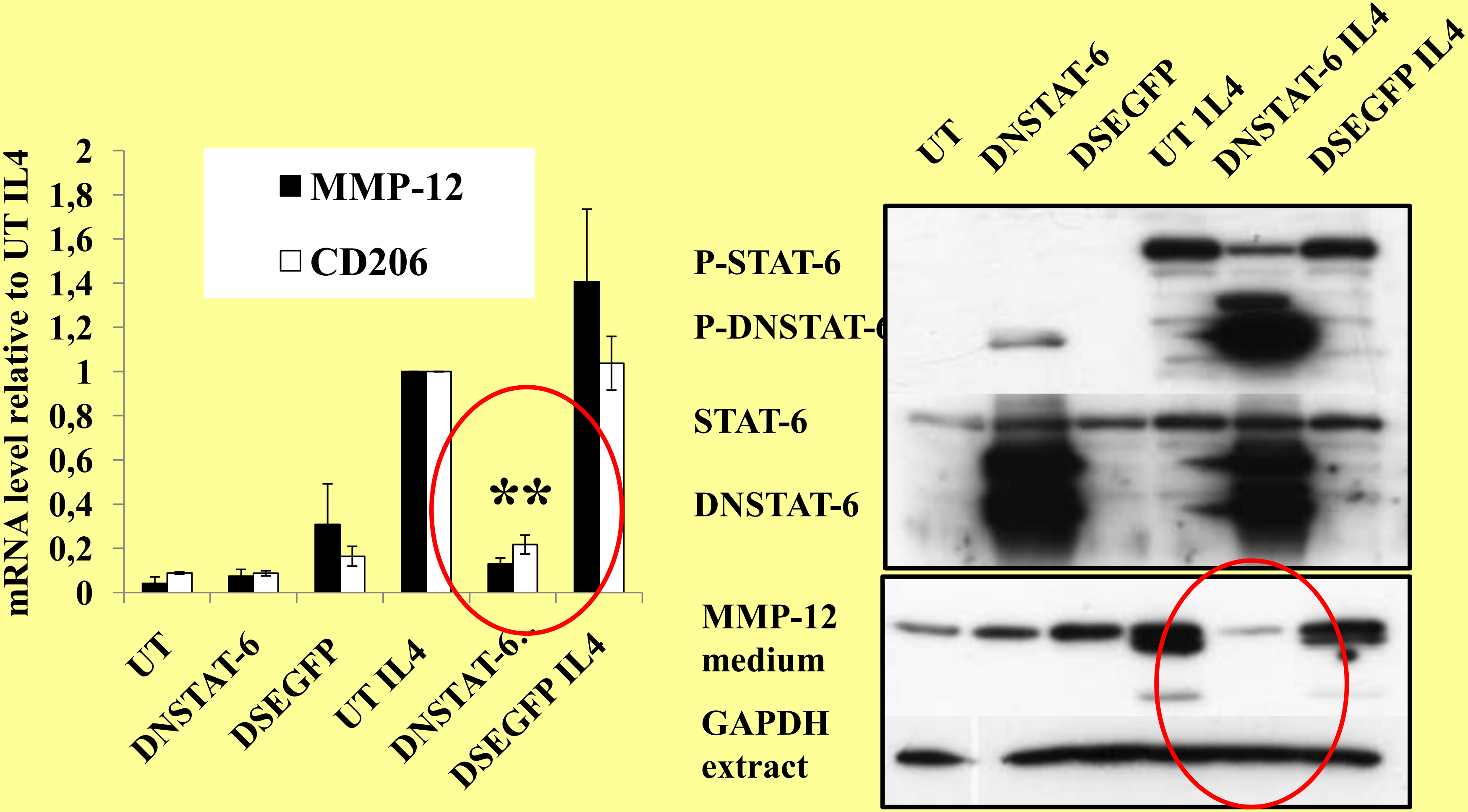
Old name	M2a	M2b	M2c	Fibrocyte
Proposed name	MIL4/13	MNHR	MIL10	MTGFβ
Receptor	IL-4RF	GCR, PPAR, Nur77	IL10R	TGFβRI/II
TFs	STAT-6, KLF4	NHR	STAT-3	SMADs2/3 FosB
Markers	CD206, COX-1, maf-1	CD163	CD163	Collagen, fibronectin
Proposed marker	Nuclear STAT6-P			Nuclear SMAD2-P
	7	8	9	10

Foam cells stimulated by IL-4 (M2)



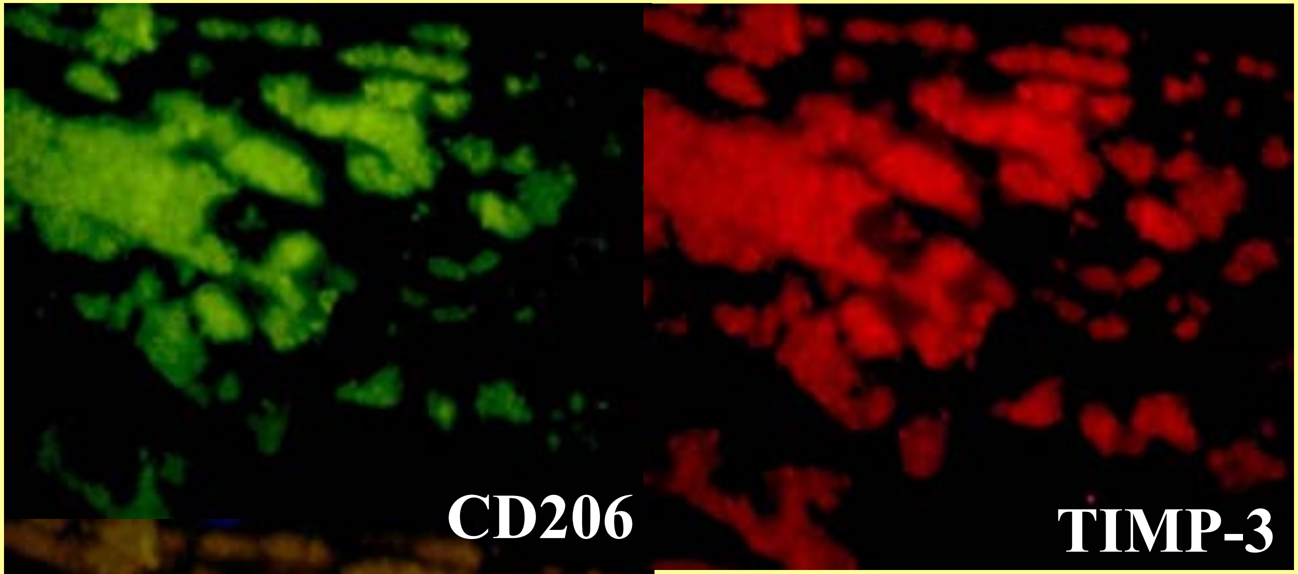
Compare Huang, Sala-Newby et. al. PLoS ONE 2012

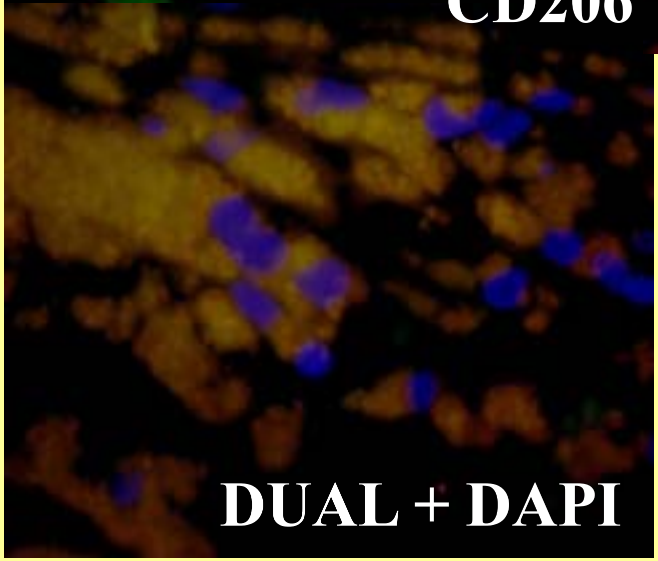
IL-4 up-regulates MMP-12 via STAT6

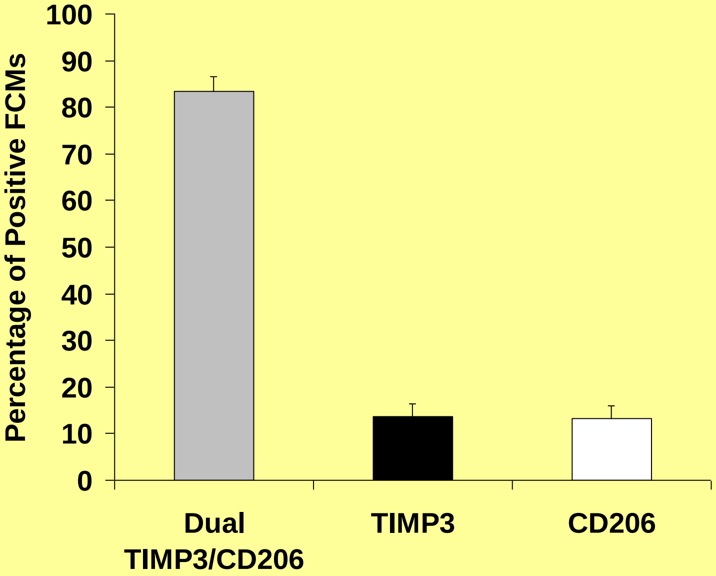


Sala-Newby and Newby, unpublished

TIMP-3 and CD206

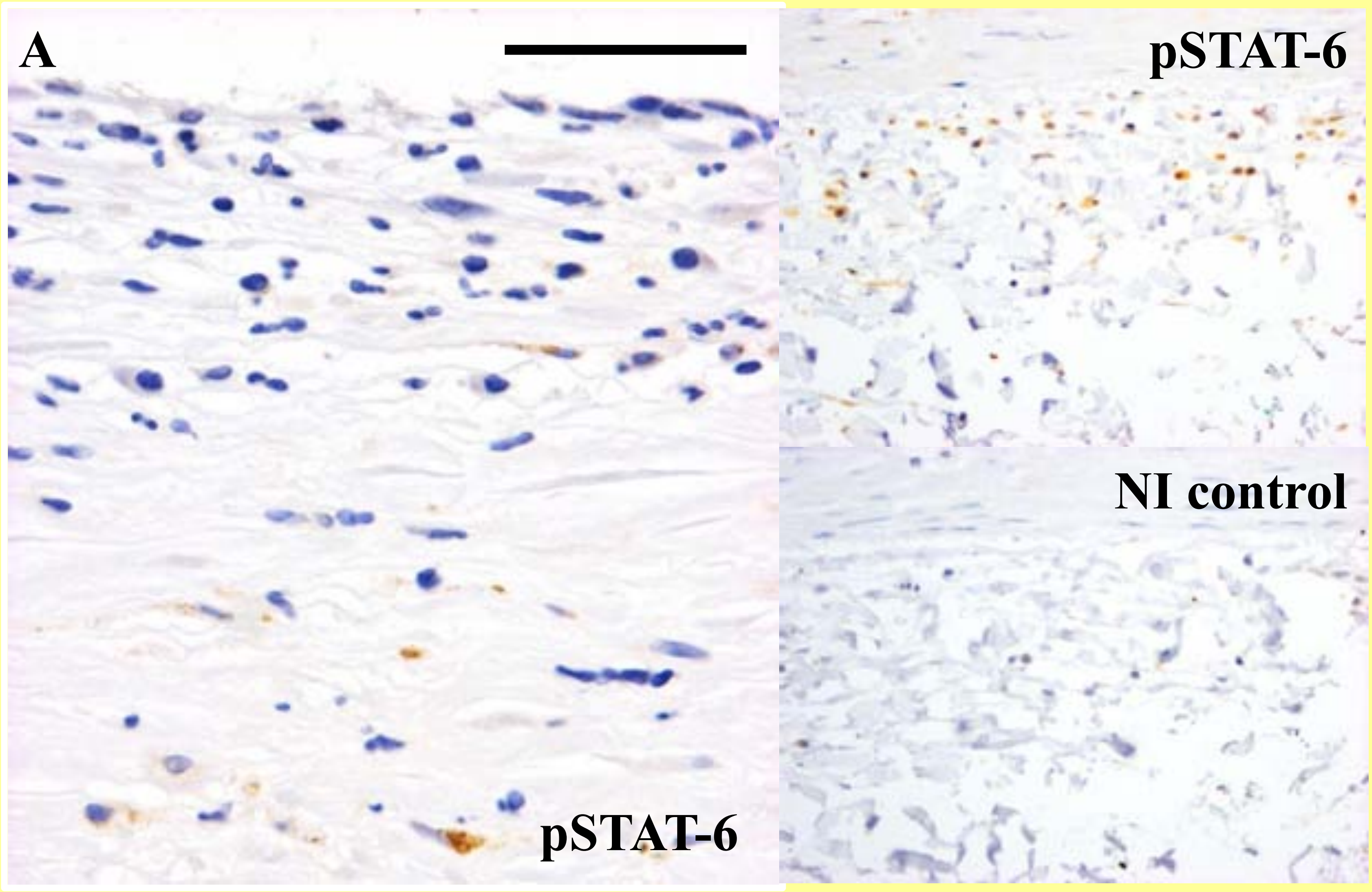






Johnson, Jenkins et al, unpublished

Is IL4/stat6 up-regulating CD206 in plaques?



Johnson and Newby, unpublished

Genomic studies on hypoxic macrophages

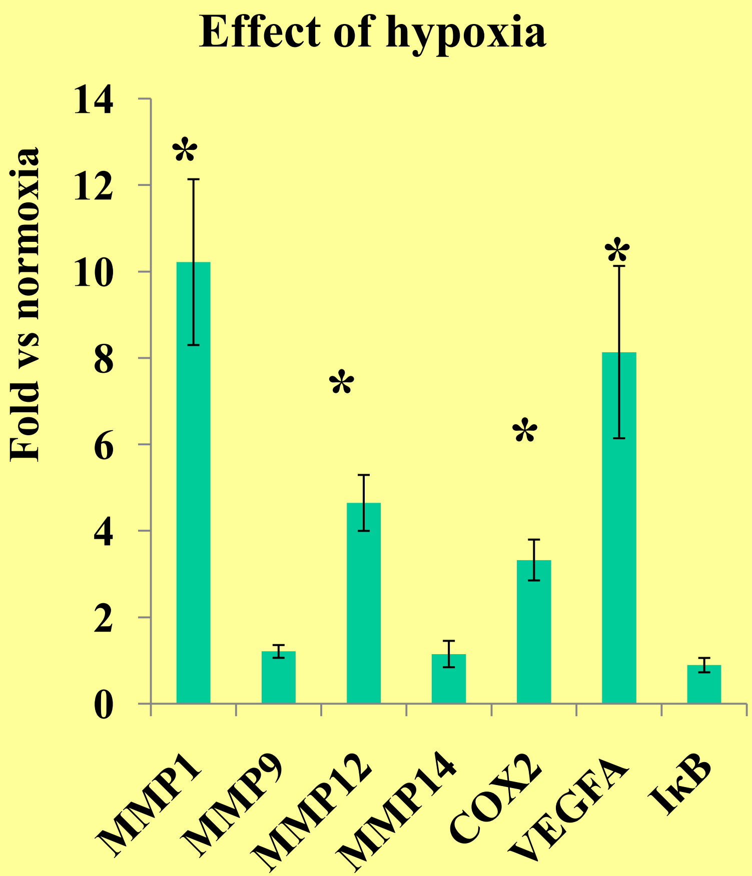
https://www.nextbio.com/b/study/cor.nb?id=12481#corP=13%2C10%2C99.7984072174208

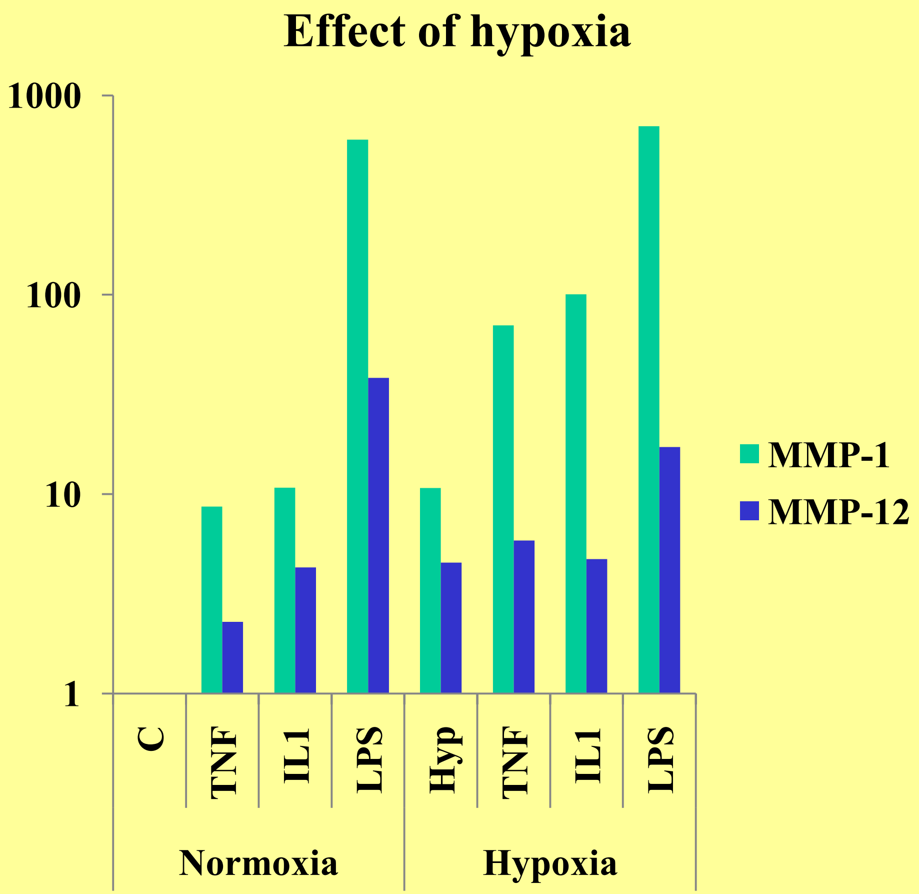
Gene	fold change
SERPINB2	699.5
ANKRD1	343.5
IL1A	212.9
IL1B	148.8
LRRC50	88.6
CA12	84.1
CXCL5	69.5
MMP1	67.6
….	
MMP10	35.8
MMP3	11.6
MMP12	3.93

https://www.nextbio.com/b/study/cor.nb?id=8415

Gene	fold change
DDIT4	104.1
ADM	52.2
SLC2A3	30.7
TSPAN6	27.7
NR4A3	19.2
MT1X	18.7
NEFL	15.6
GSTA2	15
RGS2	14.1
IL8	13.9
….	
MMP12	3.22

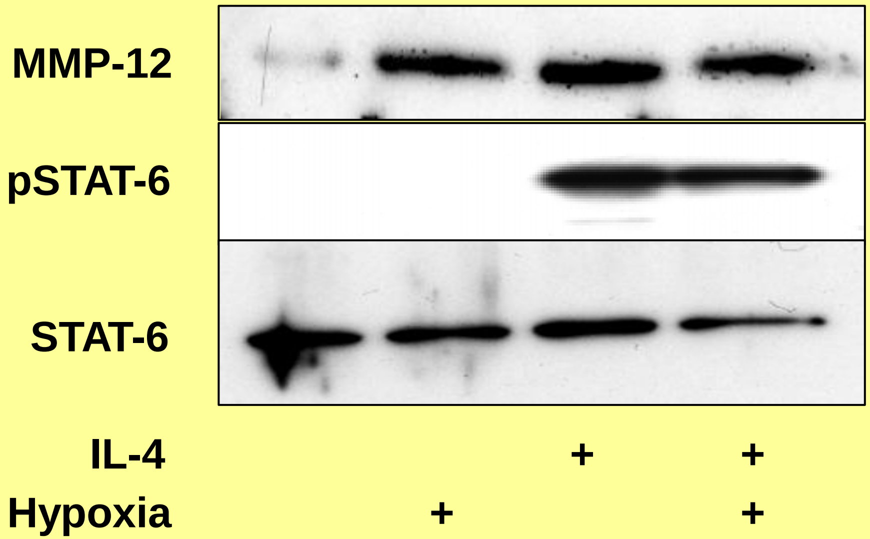
Hypoxia and MMP mRNAs *in vitro*

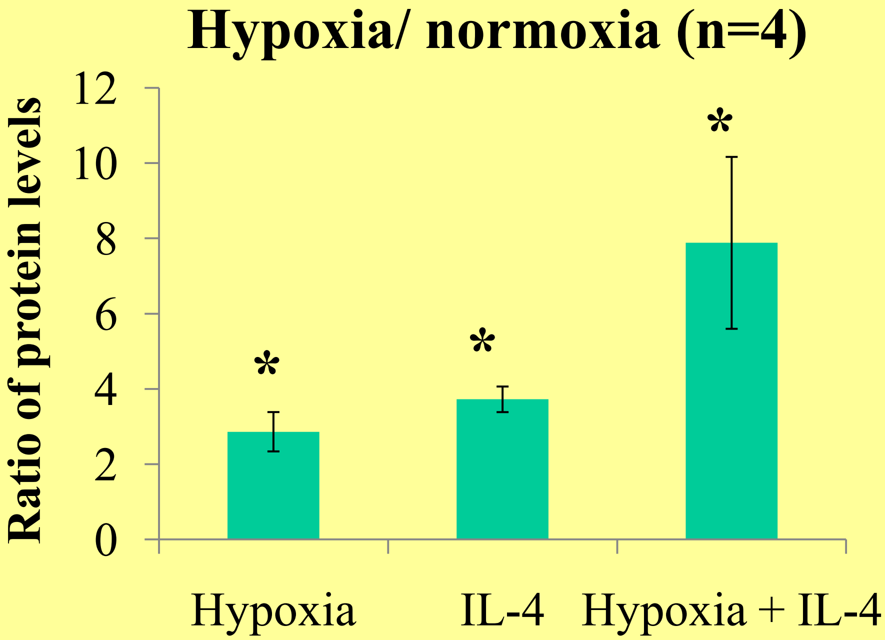




Sala-Newby, et al, unpublished

Hypoxia induced MMP-12 protein independent of Stat-6





Sala-Newby et al, unpublished

Phenotypes related to oxidation

Name	Mox	Mhem	Mhypox
Receptor	ScavengerRs	CD163	
Signal 1	ROS	ROS	HIF1/2α, ERKs, IKK2, PI3K, wnts
TFs	Nrf2, low NF-κB	Nrf2, ATF-1, low NF-κB	HIF1/2α, NF-κB, TCF/Lef
Markers	HO-1	HO-1, CD163, Low MHCII	VEGF, Glut-1
Proposed marker	?	?	Nuclear HIF1α
	14	15	16

Conclusions 2

- ‘M2’ genes are regulated through Stat-6 in vitro but do not necessarily co-localize in vivo
- There are a lot more definable phenotypes than M1 and M2
- Follow the pathways to greater wisdom!

Thanks

BHI
Bristol Heart Institute

NHS
National Institute for Health Research

British Heart Foundation