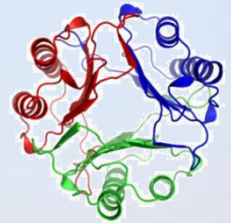


**UNIKLINIK**  
**RWTHAACHEN**

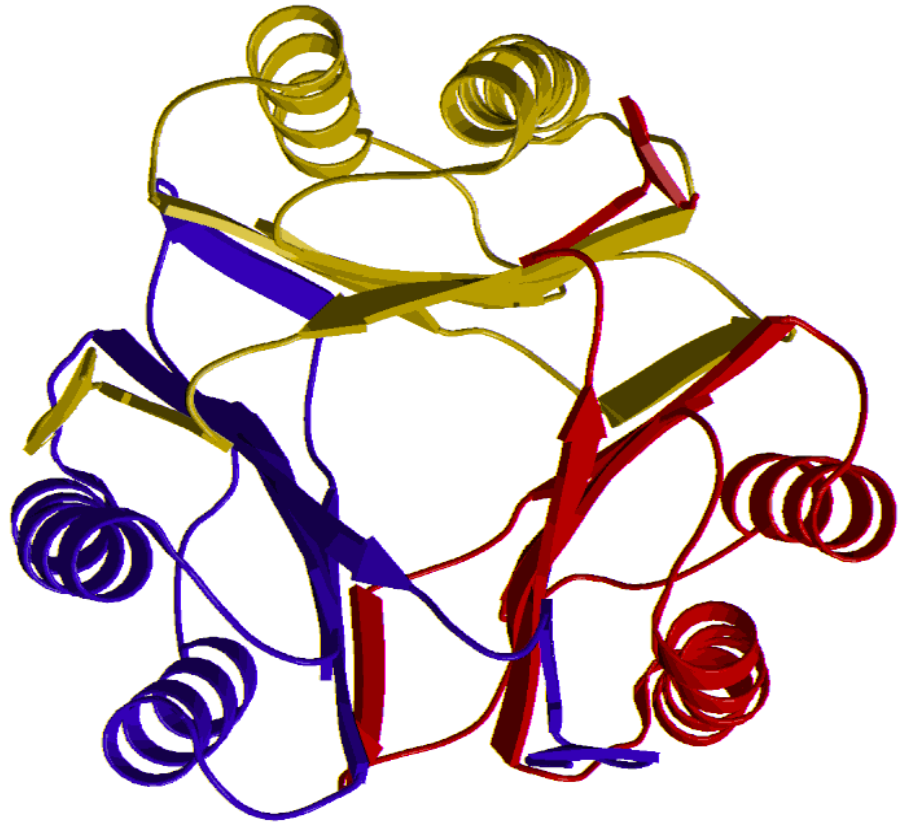
# MIF Family Proteins in Cardiovascular and Inflammatory Disease: Good, Bad or Ugly?

*Jürgen Bernhagen, Aachen, Germany*



receptor

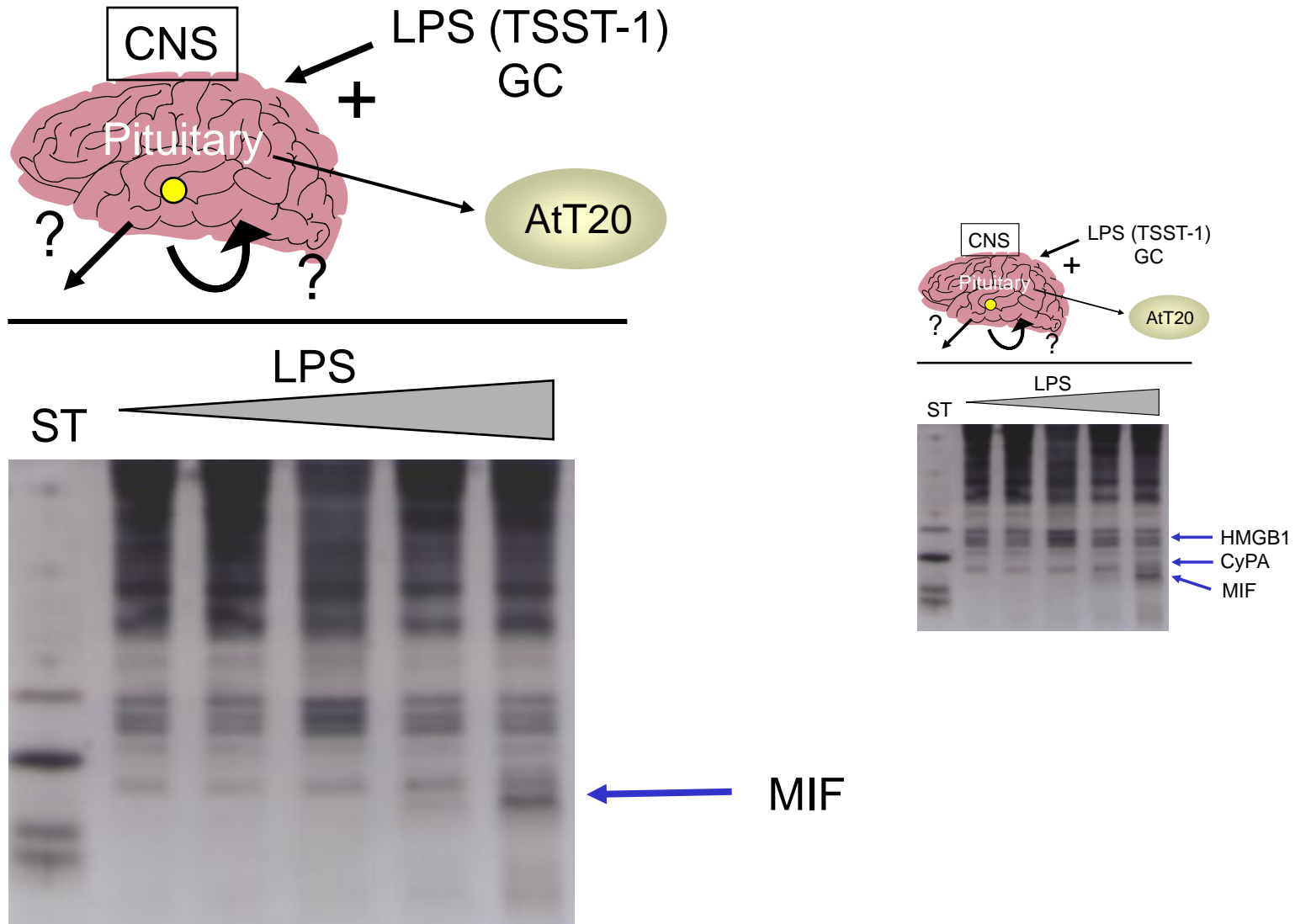
*Talk at SFB-F54-Symposium  
Medical University Vienna  
April 11, 2015*



Crystal structure of MIF

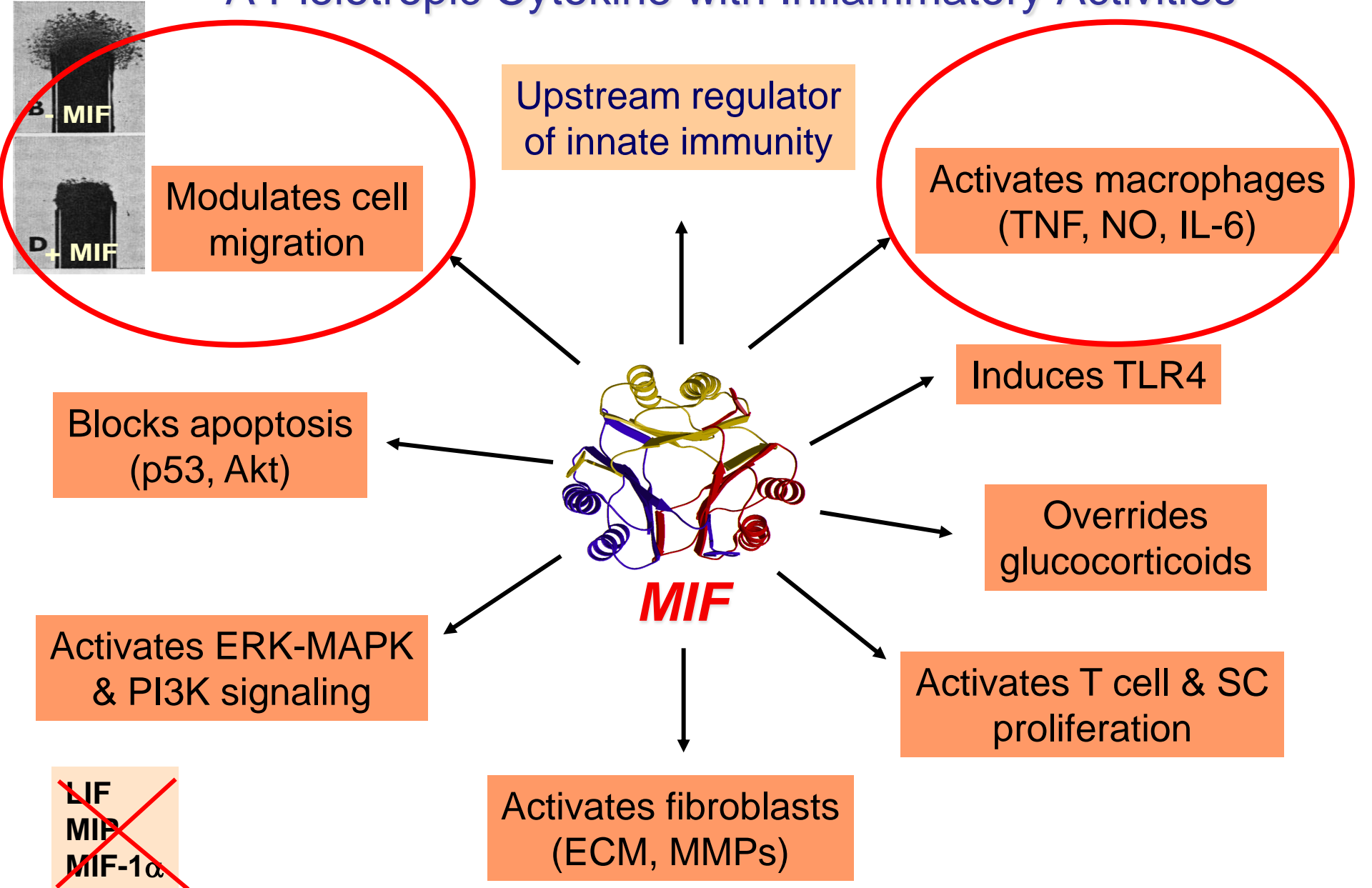


# Identification of **MIF** as Immune-Neuroendocrine Factor



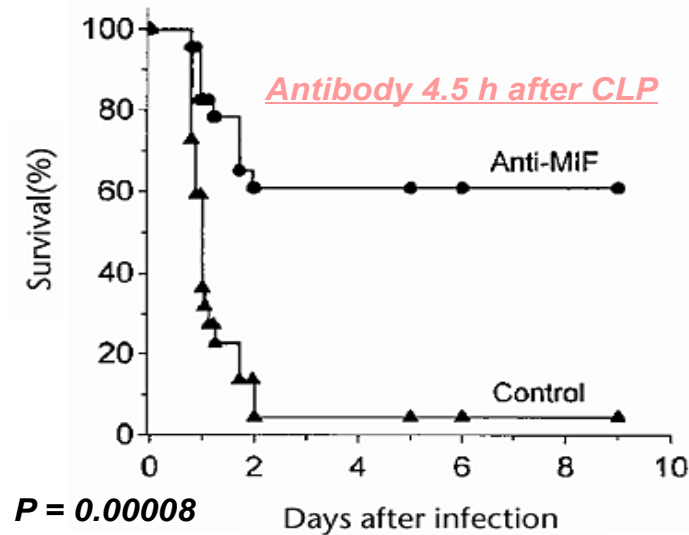
# MIF

## A Pleiotropic Cytokine with Inflammatory Activities



# MIF is a Critical Mediator of Inflammatory Diseases

## Septic shock: *E. coli* peritonitis



Septic shock

Inflammatory lung disease

Rheumatoid arthritis

Inflammation & Cancer

Atherosclerosis

Crohn 's disease / colitis

Myocardial I/R injury

*Bernhagen et al., Nature 1993*

*Calandra, Bernhagen et al., Nature 1995*

*Lan et al., J. Exp. Med. 1997*

*Donnelly et al., Nat. Med. 1998*

*Roger et al., Nature 2001*

*De Jong et al., Nat. Immunol. 2001*

*Burger-Kentischer et al., Circulation 2002*

*Pan et al., Circulation 2004*

*Schober et al., Circulation 2004*

*Morand et al., Nat. Rev. Drug Discov. 2006*

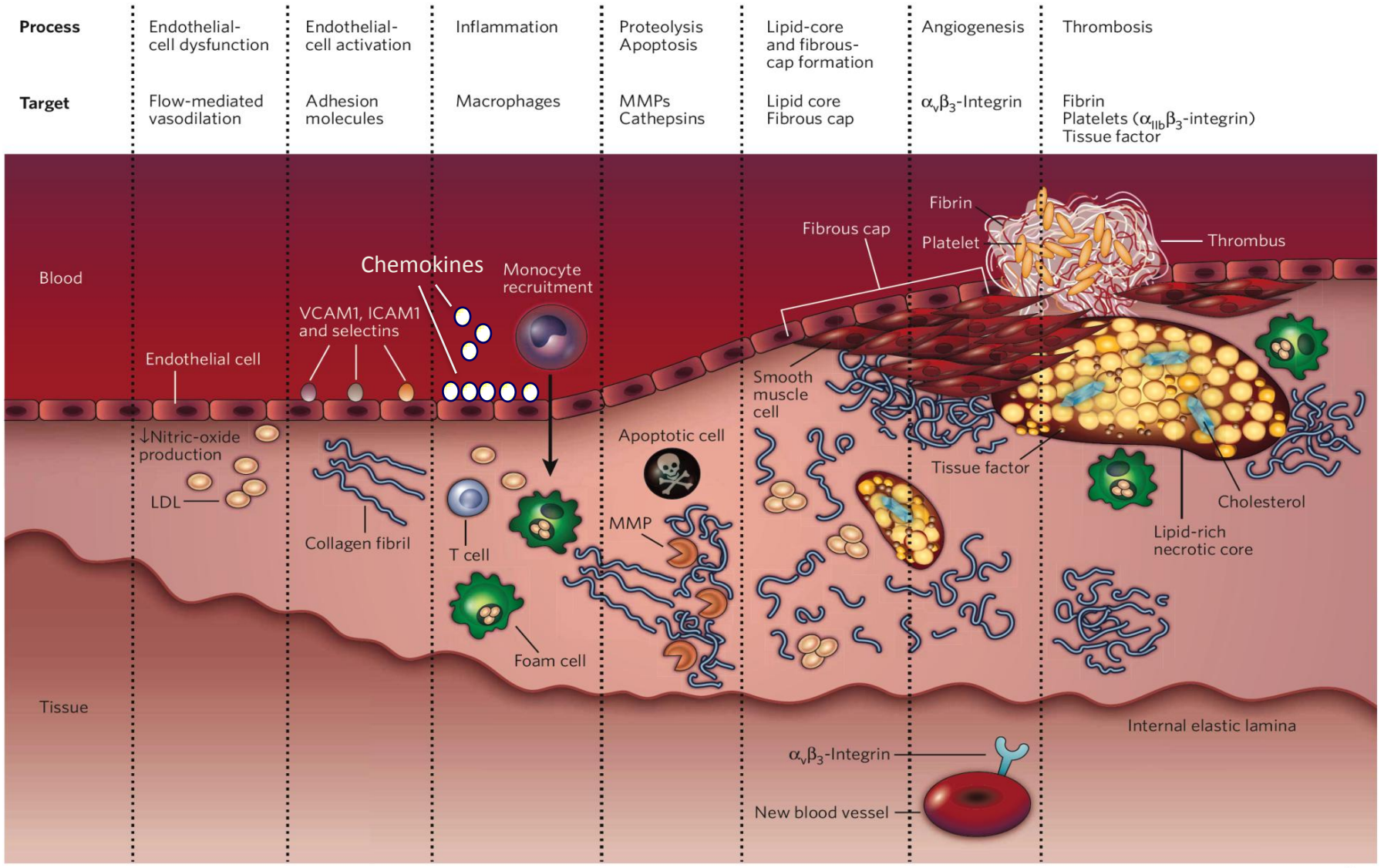
*Bernhagen et al., Nat. Med. 2007*

*Sanchez & Rodriguez-Sosa, J. Diabetic Res. 2014*

.....and many more...

# Atherosclerosis: a Chronic Inflammatory Disease

## Role for Leukocytes & Chemokines



Approximate  
AHA lesion  
stage

I

II

III

IV

V

VI

Modified after: Sanz & Fayad, Nature 2008

# Chemokines / Chemokine Receptors

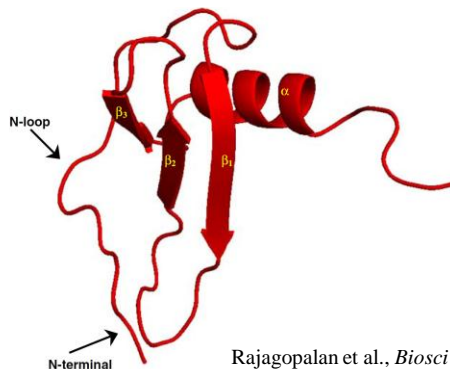
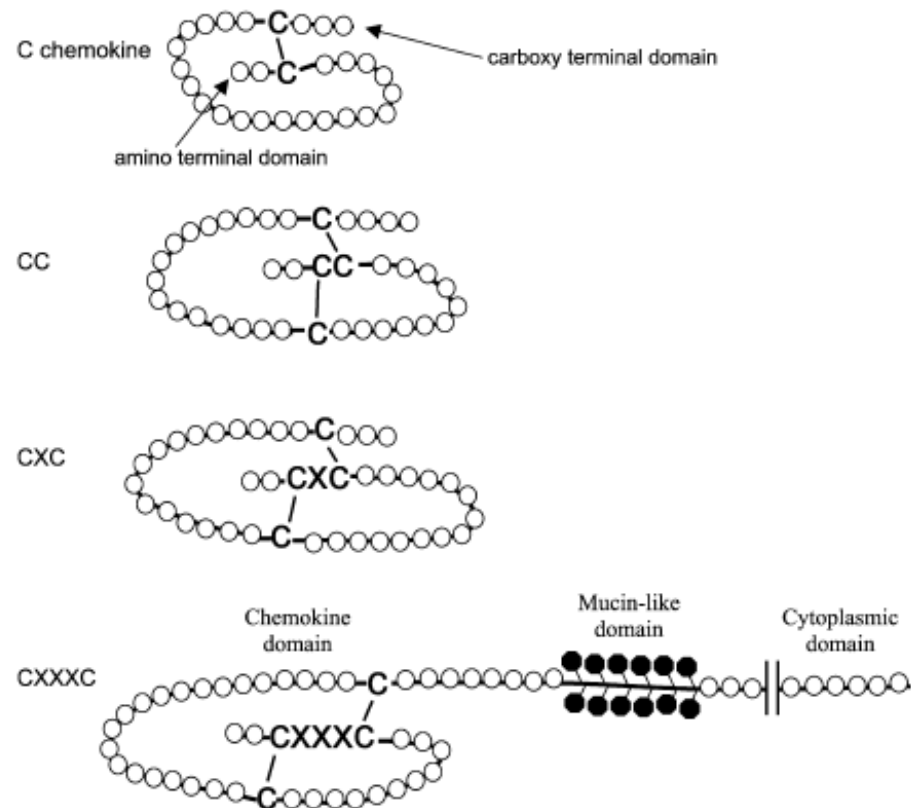
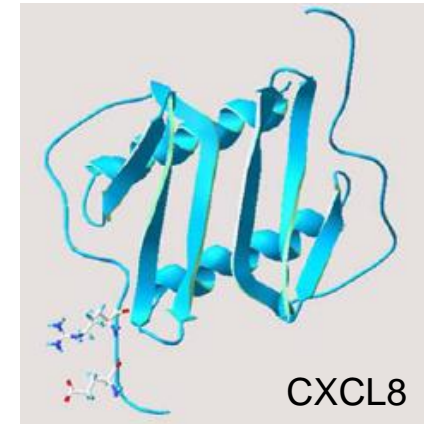
Chemokines = **chemotactic** cytokines

Small (8-10 kDa) polypeptides with a pivotal role in leukocyte migration

50 human chemokine known  
4 families: C, CC, CXC, CXXXC

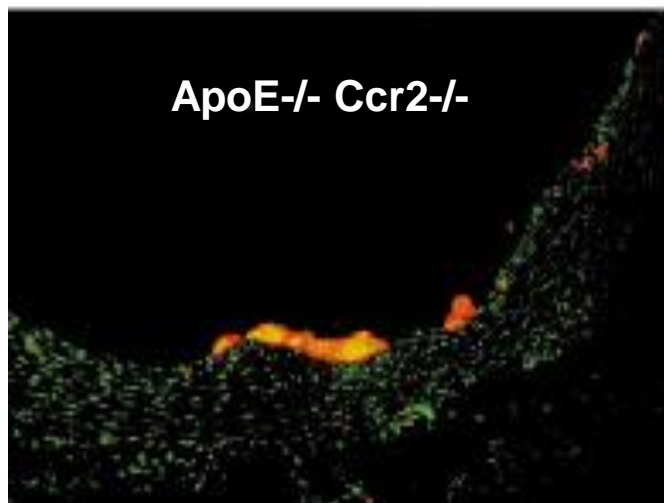
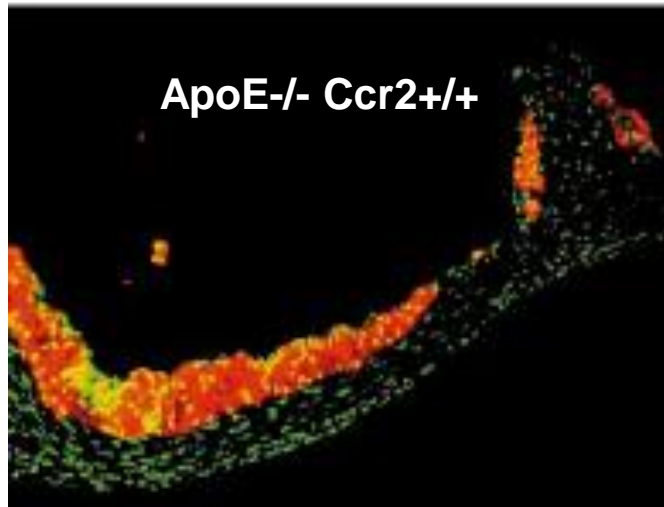
20 chemokine receptors (GPCR)  
CR, CCR, CXCR, CXXXC

⇒ Redundancy on the side of the ligands

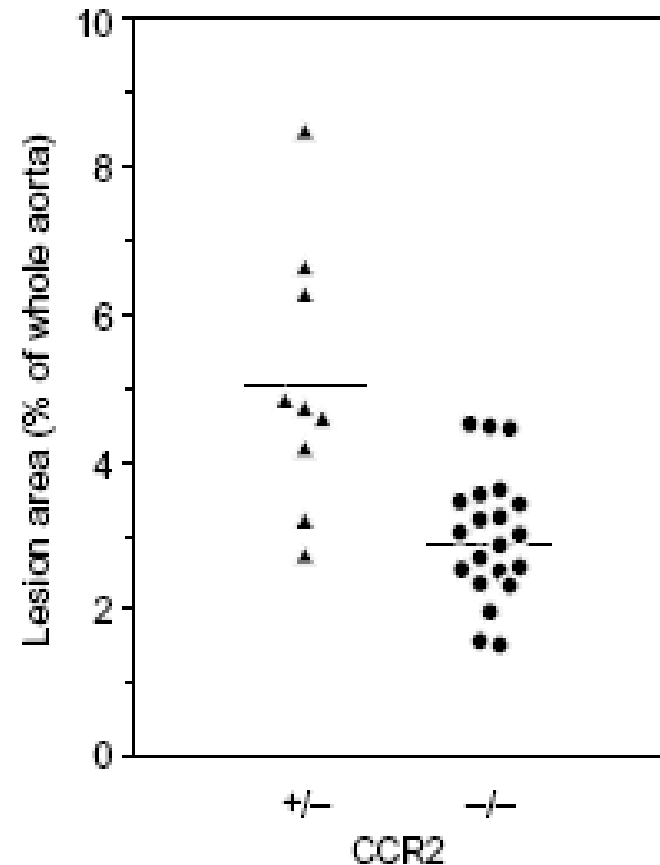


Rajagopalan et al., *Biosci Rep.*, 2006

# *Functional Role of Chemokines in Atherosclerosis* *i.e., the MCP-1/CCR2 Axis in the ApoE<sup>-/-</sup> Mouse Model*



**Macrophage accumulation**

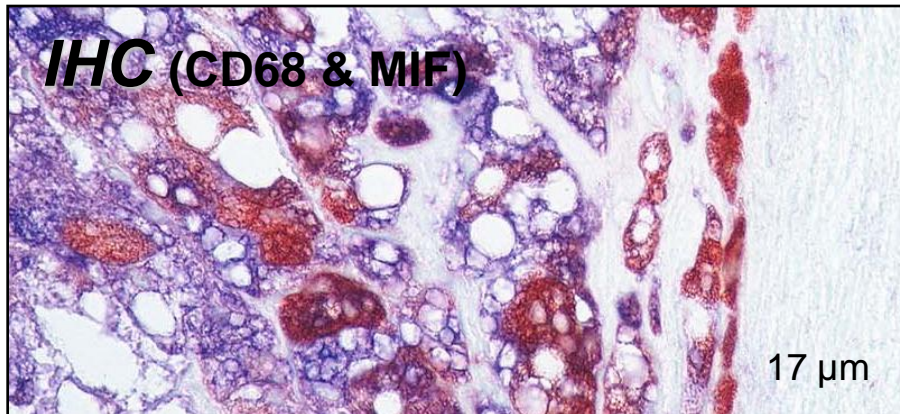


Taken from: Boring et al., Nature 1998  
Decreased lesion formation in CCR2<sup>-/-</sup> mice reveals a role for chemokines in the initiation of atherosclerosis

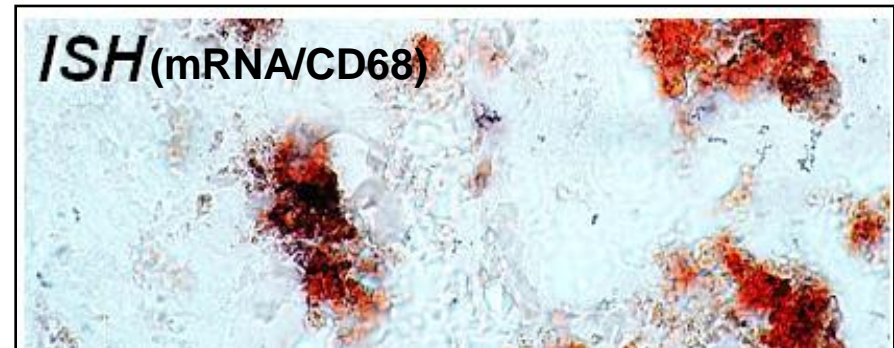
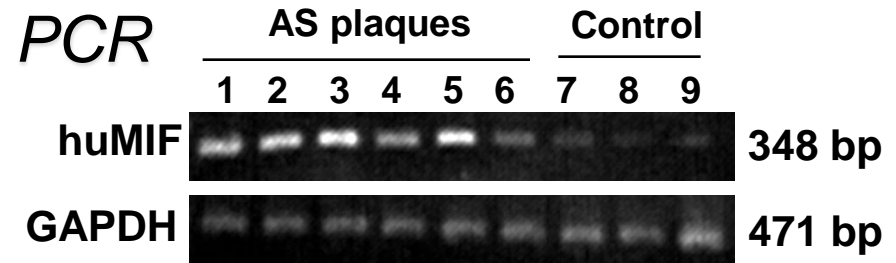


# MIF Expression in Advanced Carotid Atherosclerotic Plaques in Humans

## Expression of MIF in CD68+ M $\Phi$ in a carotid plaque



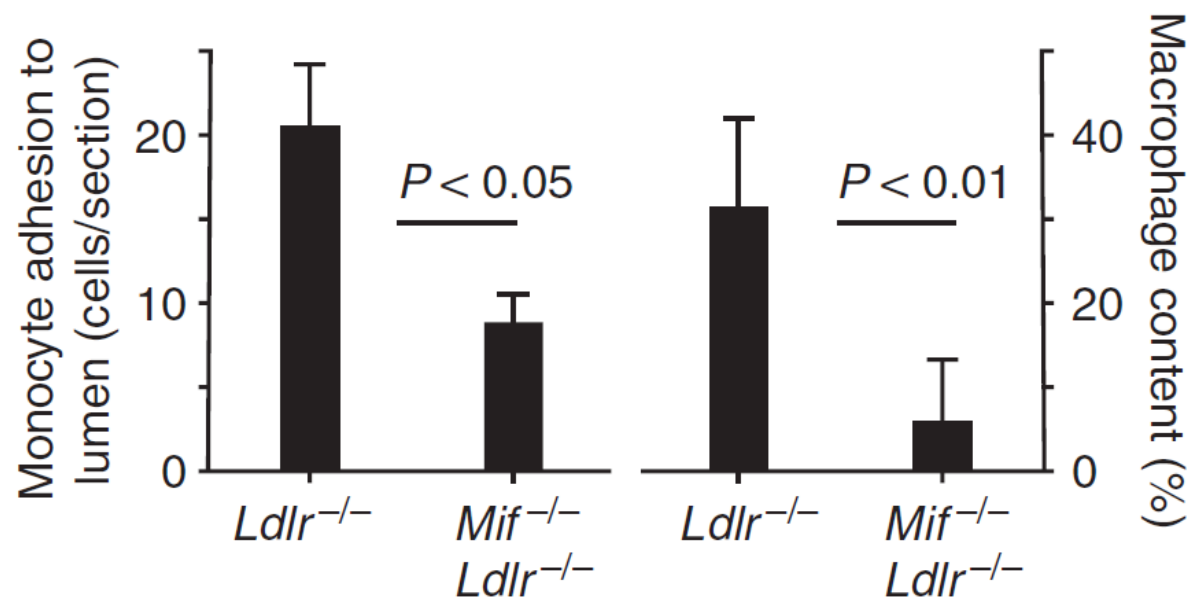
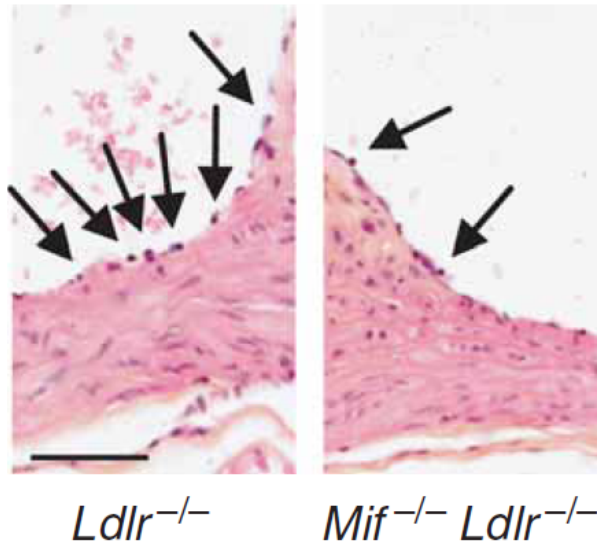
MIF expression in:  
intimal macrophages >> intimal VSMCs  
> medial VSMCs > ECs > T cells



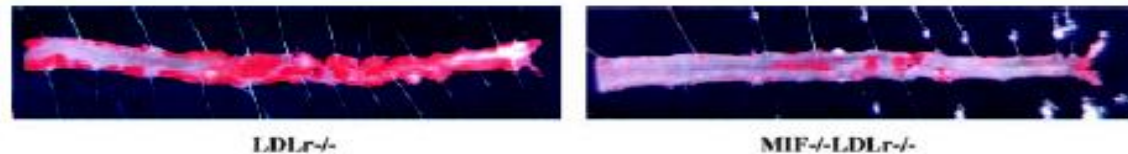
**➔ MIF expression predominantly in M $\Phi$ s and foam cells**

# MIF Deficiency Leads to Reduced Atherosclerotic Lesions and Macrophage Content in Atherogenic Arteries

## LDLR-KO vs. LDLR / MIF -DKO



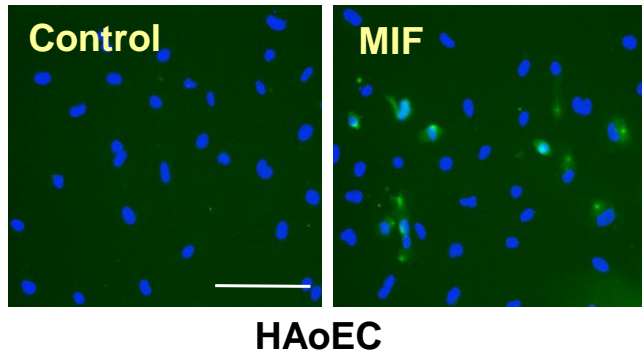
Bernhagen et al., Nature Medicine 2007



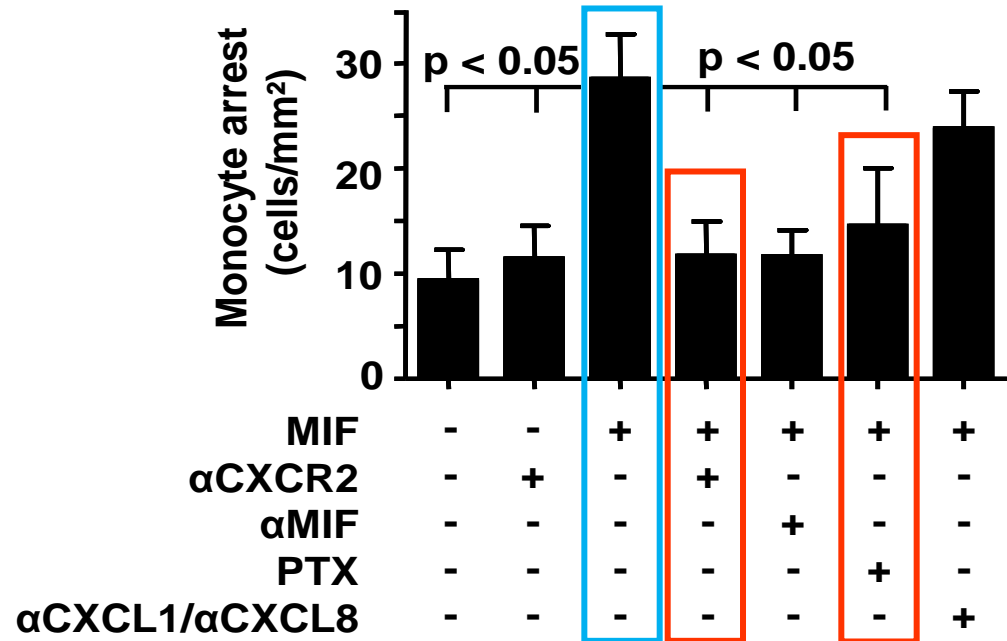
From: Pan et al, Circulation 2004

# MIF has Chemokine-like Activities Dependence on CXCR2

MIF is deposited on ECs



MIF-mediated monocyte arrest on HAoECs  
Blockade by anti-CXCR2 and PTX



# Crucial Paper

*American Journal of Pathology*, Vol. 168, No. 4, April 2006  
Copyright © American Society for Investigative Pathology  
DOI: 10.2353/ajpath.2006.040748

*Vascular Biology, Atherosclerosis and Endothelium Biology*

## Up-Regulated Expression of the CXCR2 Ligand KC/GRO- $\alpha$ in Atherosclerotic Lesions Plays a Central Role in Macrophage Accumulation and Lesion Progression

William A. Boisvert,<sup>\*†</sup> David M. Rose,<sup>‡</sup>  
Kristen A. Johnson,<sup>‡</sup> Maria E. Fuentes,<sup>§</sup>  
Sergio A. Lira,<sup>¶</sup> Linda K. Curtiss,<sup>\*</sup> and  
Robert A. Terkeltaub<sup>‡</sup>

*From the Department of Immunology,\* The Scripps Research Institute, La Jolla, California; Vascular Medicine Research Unit,<sup>†</sup> Brigham and Women's Hospital and Harvard Medical School, Cambridge, Massachusetts; the Department of Medicine,<sup>‡</sup> Veteran's Administration Medical Center, University of California, San Diego, San Diego, California; Roche Biosciences,<sup>§</sup> Palo Alto, California; and the Immunobiology Center,<sup>¶</sup> Mount Sinai School of Medicine, New York, New York*

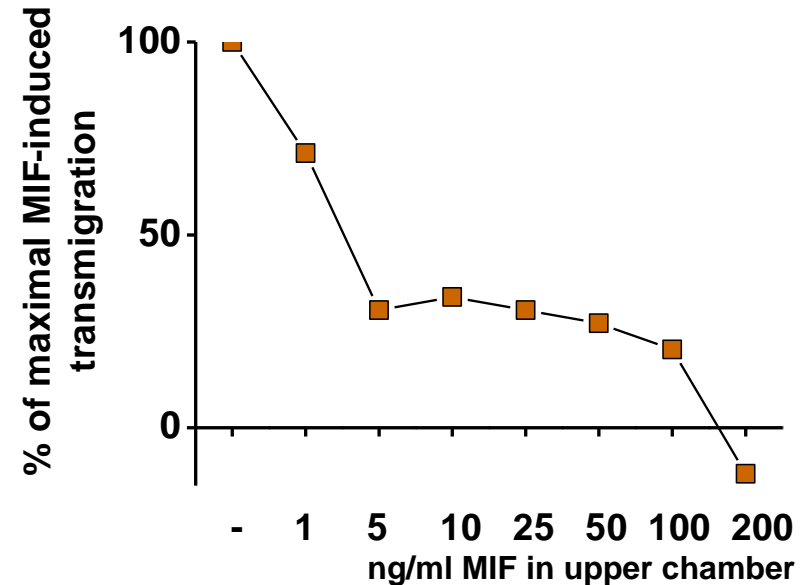
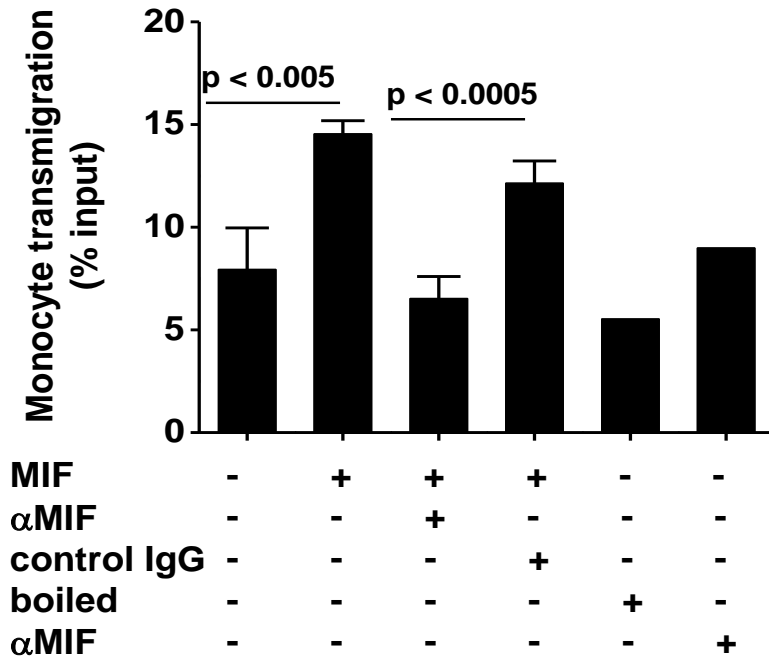
An alternative ligand of (murine) CXCR2 may in part account for the atherogenic role of CXCR2 in atherogenic mouse models

GRO- $\alpha^{-/-}$  mice. The reduction in atherosclerotic lesion size in the LDLR<sup>-/-</sup>, KC/GRO- $\alpha^{-/-}$  mice was approximately half that of the attenuation in lesion size previously seen (and confirmed here) in mice with leukocyte-specific deficiency of CXCR2,<sup>26</sup> suggesting perhaps that CXCR2 ligand(s) other than KC/GRO- $\alpha$  may have compensated somewhat for the loss of KC/GRO- $\alpha$  in enhancing the CXCR2-mediated atherogenic functions of macrophages. Our study also showed that leukocyte-specific

# MIF is a Chemokine with a True Chemotactic Effect

MIF is chemotactic

Checkerboard analysis

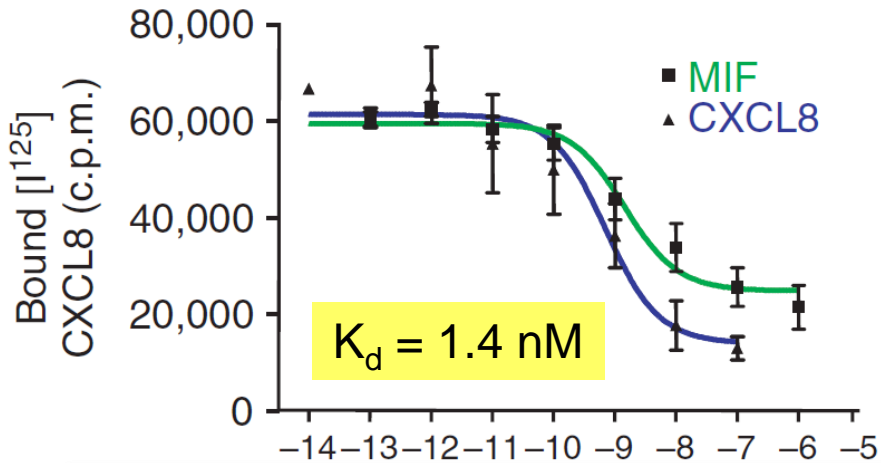


→ Historic „MIF“ effect probably „desensitization“

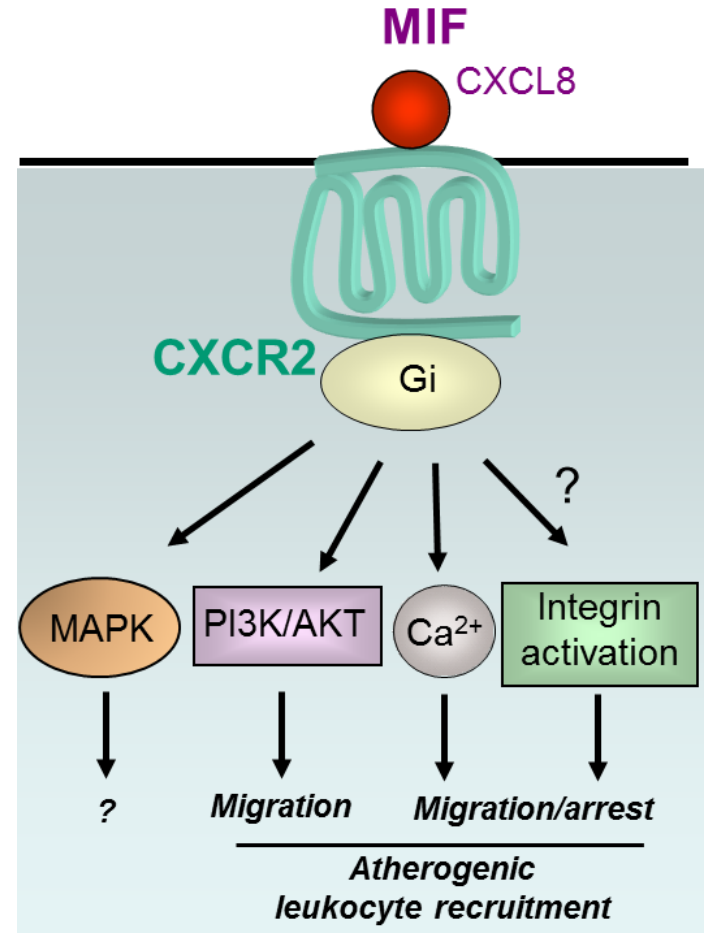
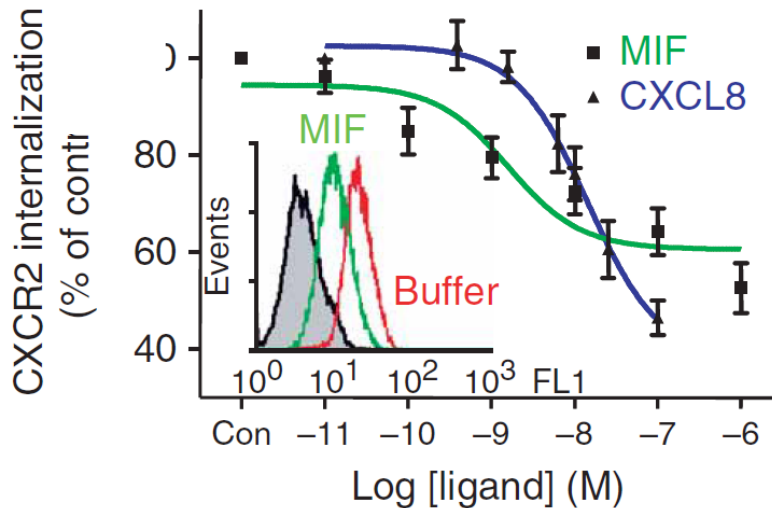


# MIF Directly Binds to CXCR2

## Receptor competition binding assay

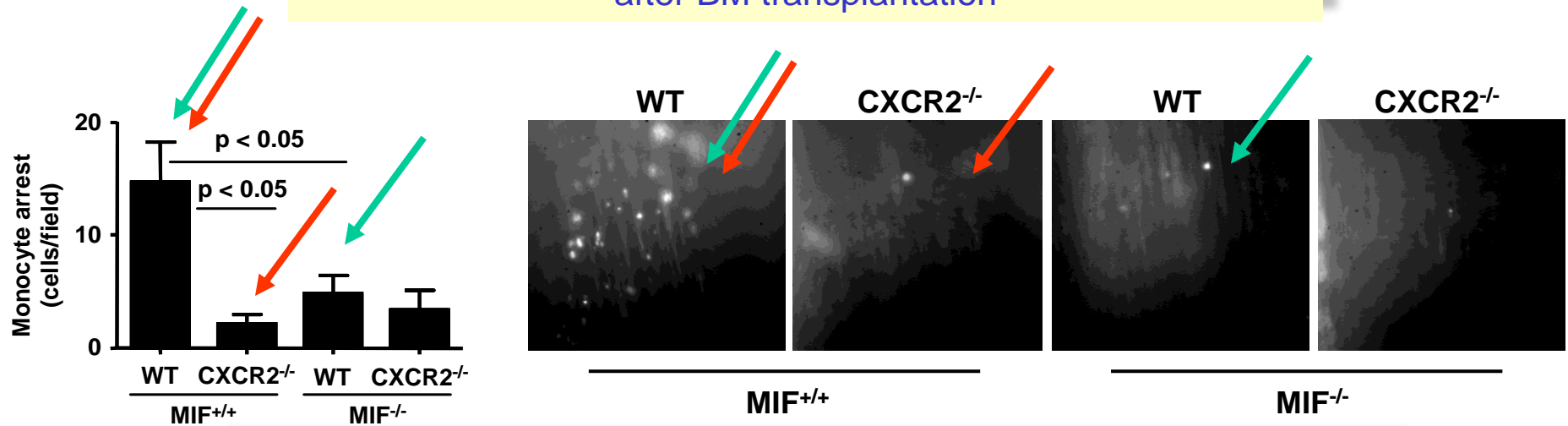


## CXCR2 internalization experiment by FACS

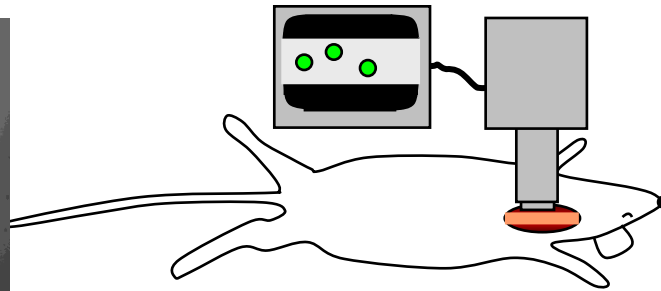


# Role for CXCR2 in MIF-mediated Monocyte Recruitment *in vivo*

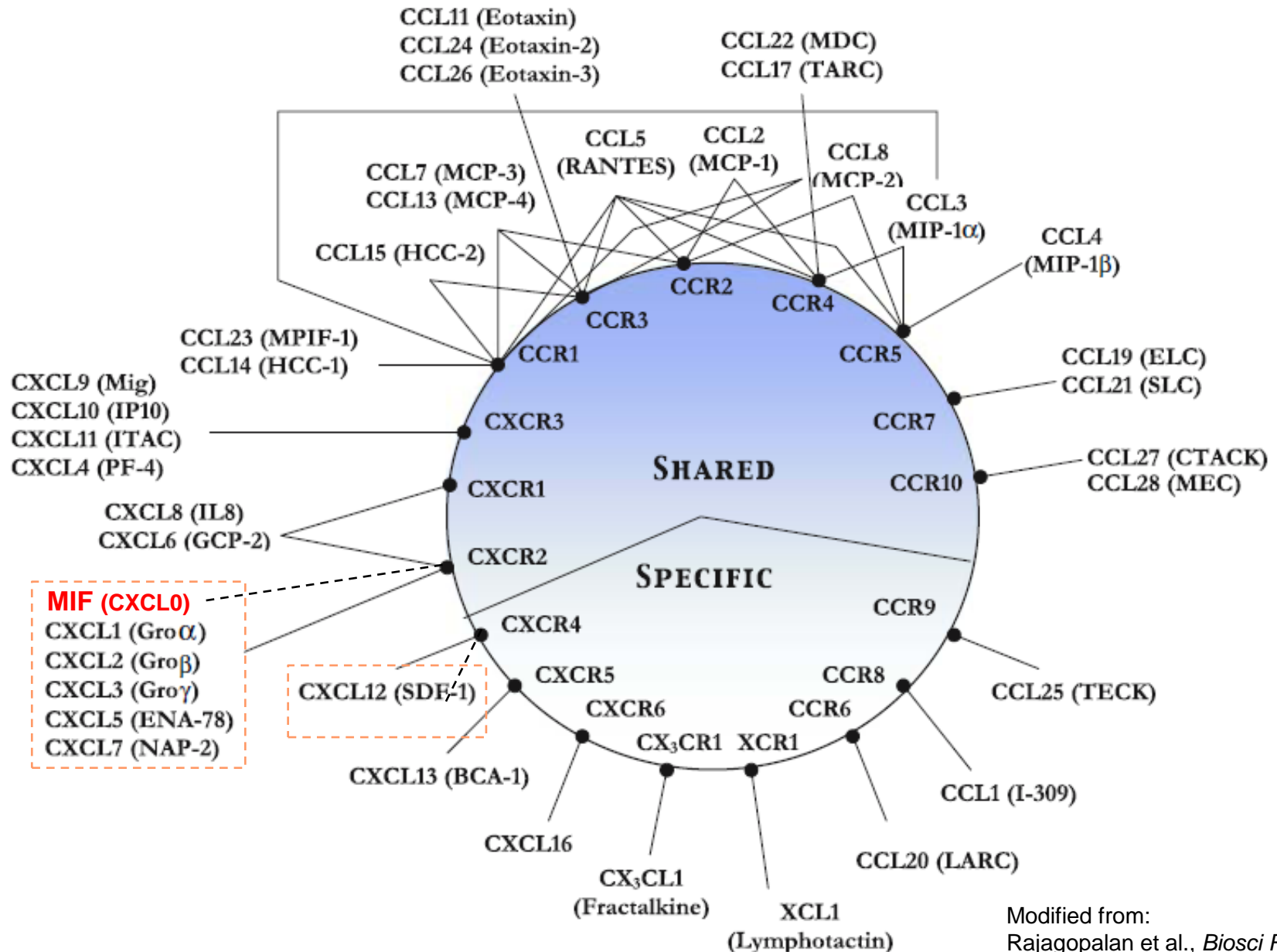
Intravital microscopy in carotids of atherogenic LDLR<sup>-/-</sup> mice after BM transplantation



*Mif*<sup>+/+</sup> and *Mif*<sup>-/-</sup> mice reconstituted with wild-type or *Cxcr2*<sup>-/-</sup> bone marrow (n=3 each) were stimulated by intraperitoneal injection of TNF- $\alpha$  for 4 h, and the accumulation of leukocytes labeled by intravenous injection of rhodamine was studied in carotid arteries *in vivo*



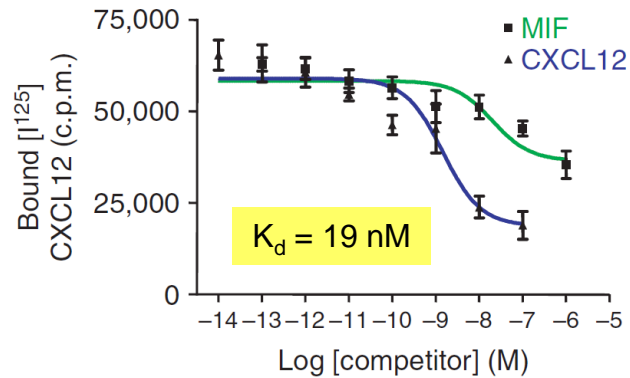
# MIF Adds to Promiscuity in the Chemokine System



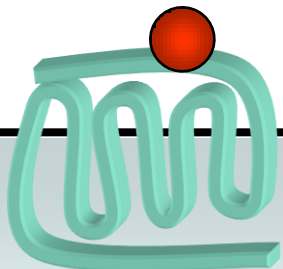


# MIF Interacts with CXCR4 but not with CXCR3

## Receptor competition binding assay

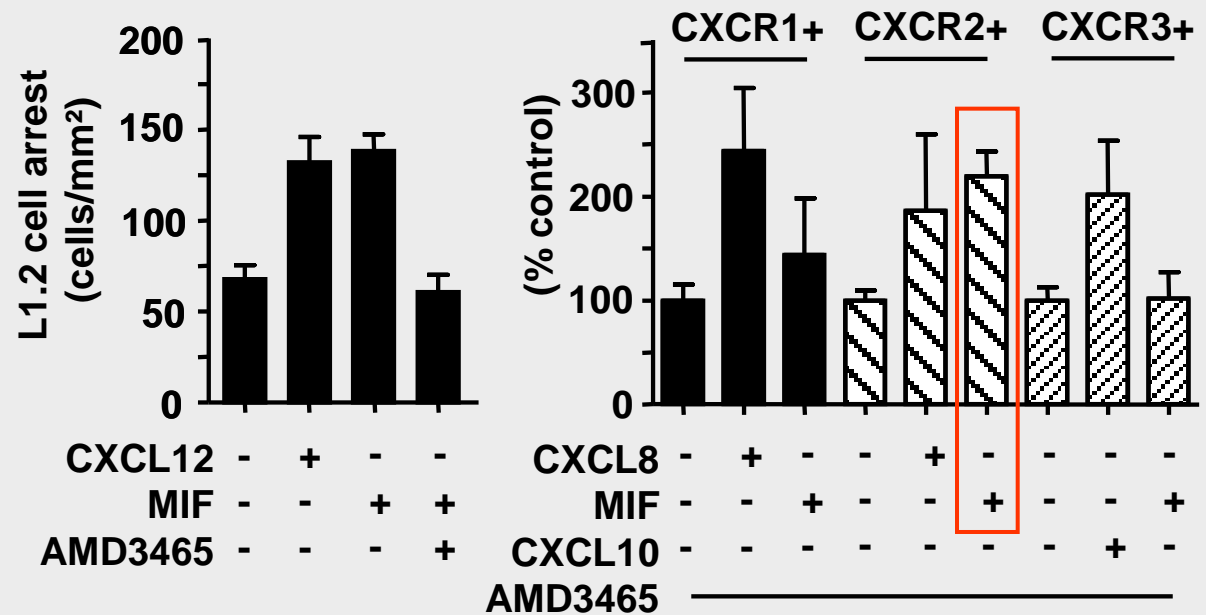


MIF



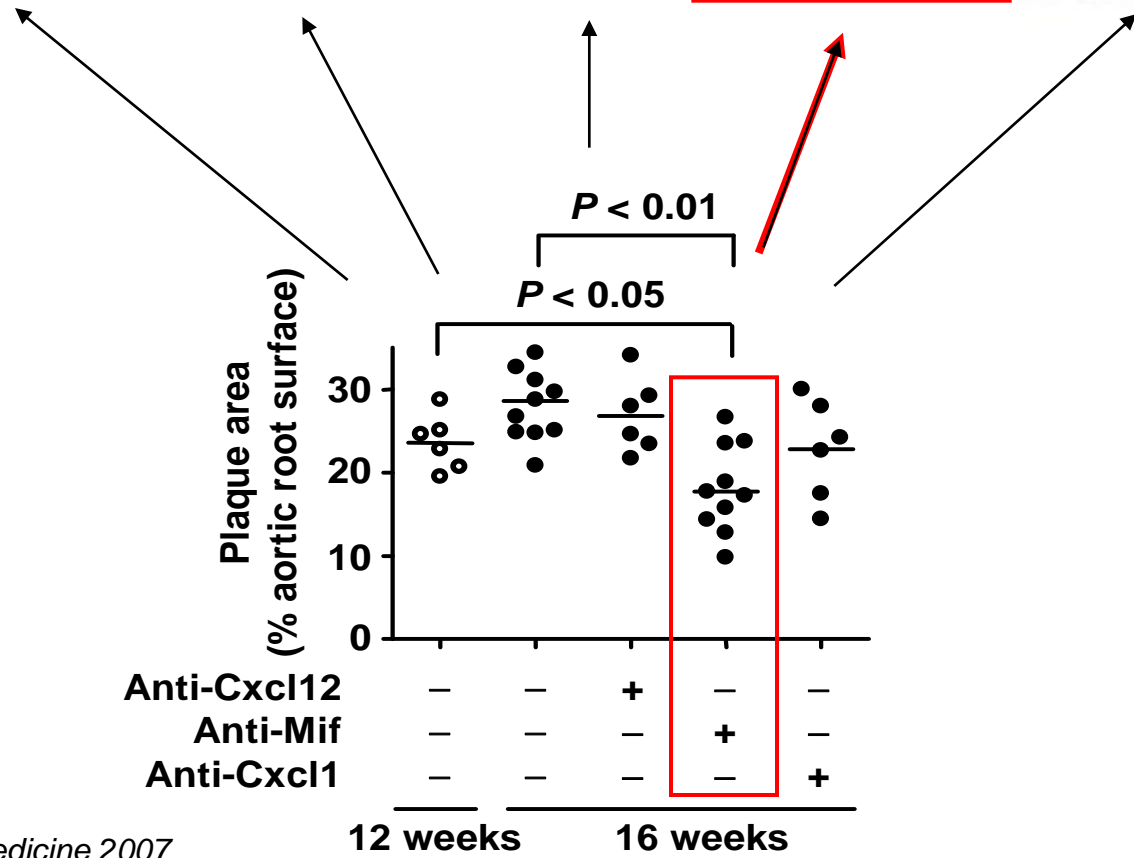
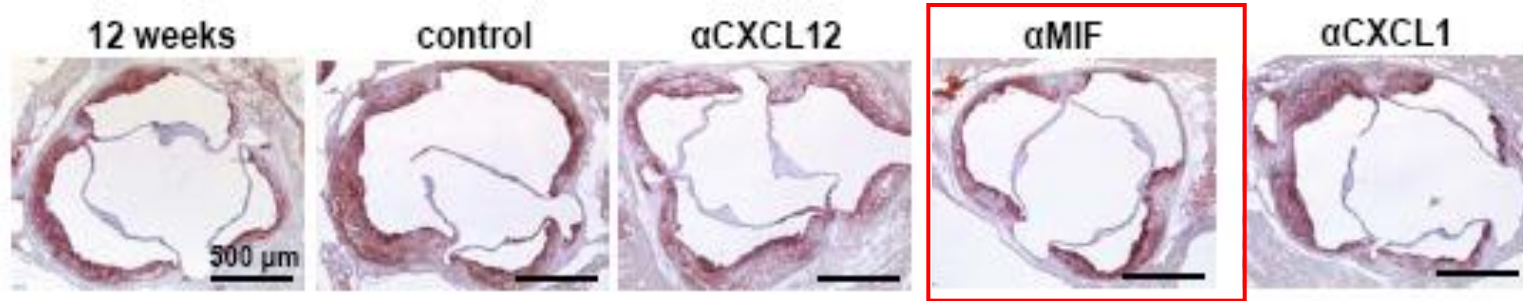
CXCR4

## MIF triggers L1.2 cell arrest on SVECs dependent on CXCR 2 and 4

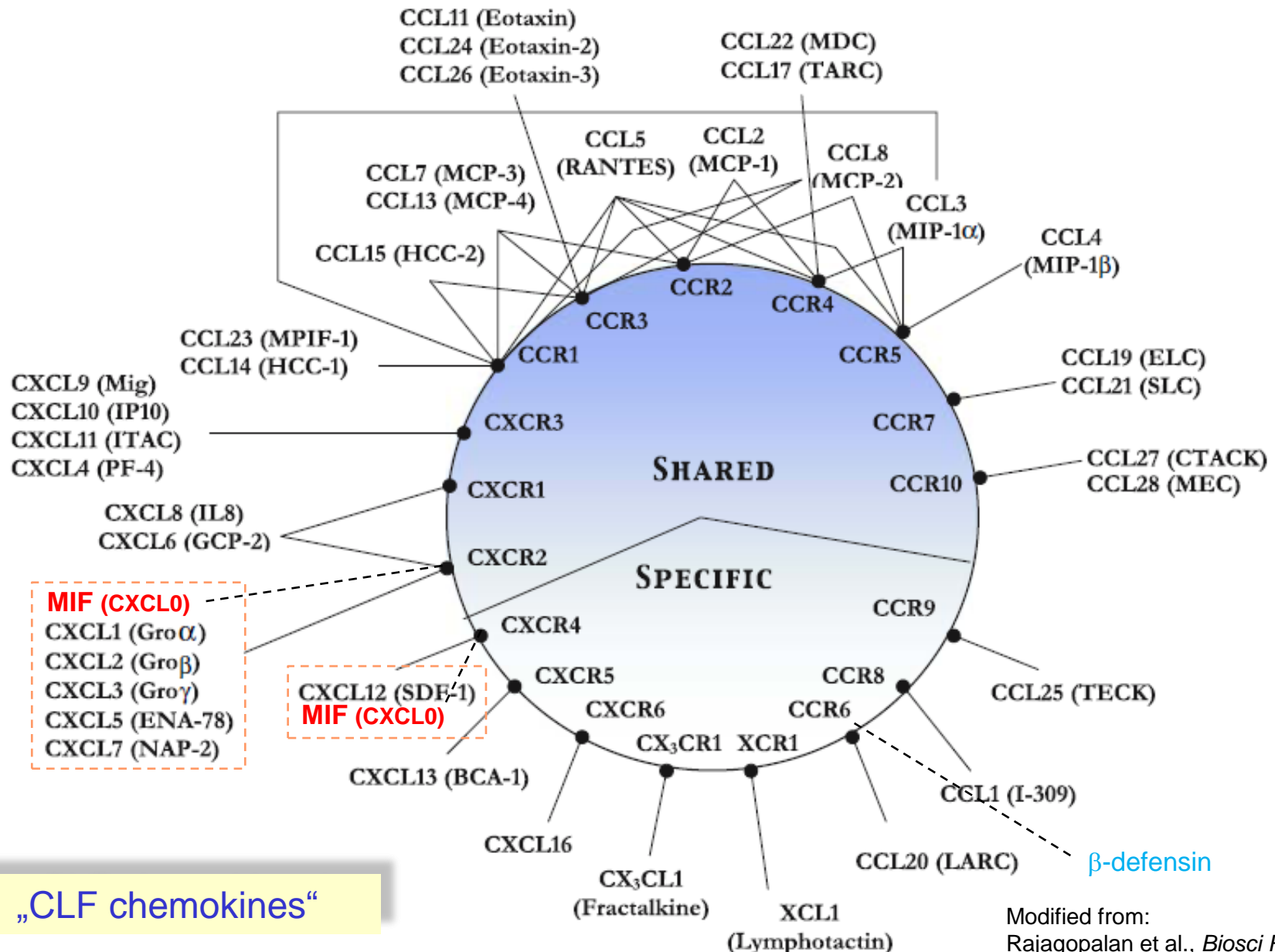


# Potential Role for Anti-MIF Strategy in Plaque Regression

## Dual Action of MIF Through CXCR2 and CXCR4



# MIF Adds to Promiscuity in the Chemokine System



„CLF chemokines“

# Chemokine-Like Function (CLF) Chemokines

- Cytokines with (structural) & functional similarities to chemokines, but that lack the N-terminal cysteine motif
- Interact with classical chemokine receptors
- Examples:
  - $\beta$ -Defensins (CCR6), AaaRS (autoantigenic aminoacyl-tRNA synthetases; CXCR1, CCR3, CCR5), thioredoxin 1-80 (?), HMGB1 (CXCR4)
  - HIV gp120 (CXCR4, CCR5)
  - MIF (CXCR2, CXCR4)
- Overlap with class of alarmins

# The MIF/CXCR2 and MIF/CXCR4 Interface

## Determinants of Interaction with Classical CXC Receptors

MIF / CXCR2

N-like loop  
(47-56)

MIF

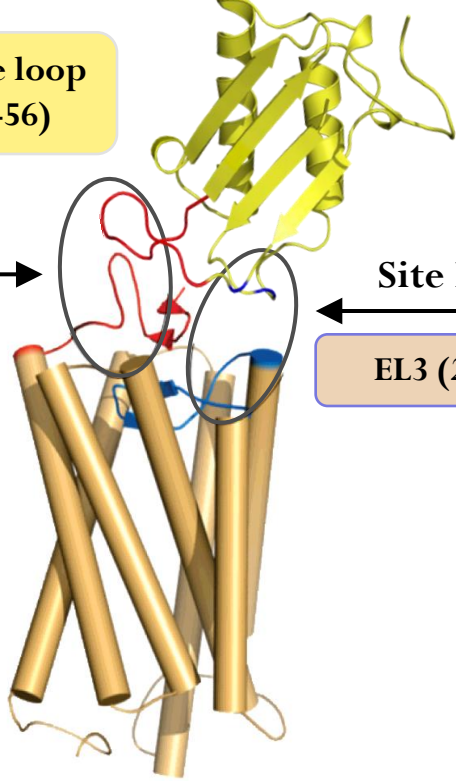
Pseudo (E)LR  
(R12D45)

Site I

Site II

N-term (19-33)  
EL1 (108-120)  
EL2 (184-198)

EL3 (286-290)



CXCR2

MIF / CXCR4

Extended N-like  
loop (47-72)  
+ RLR-motif + D45?

MIF

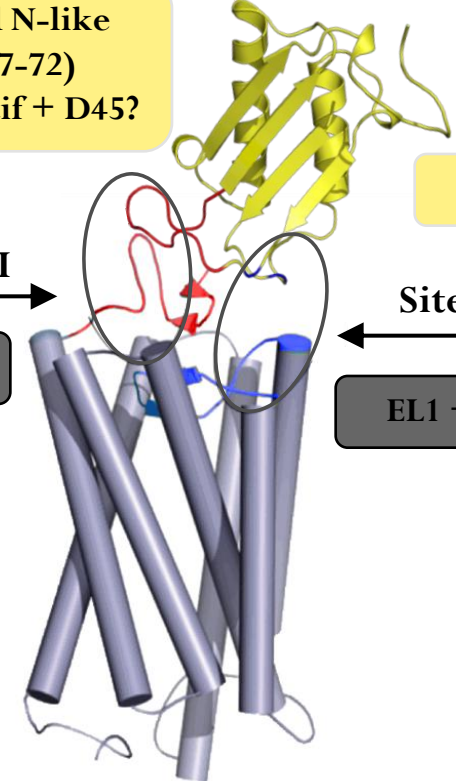
??

Site I

Site II

N-term (1-27?)

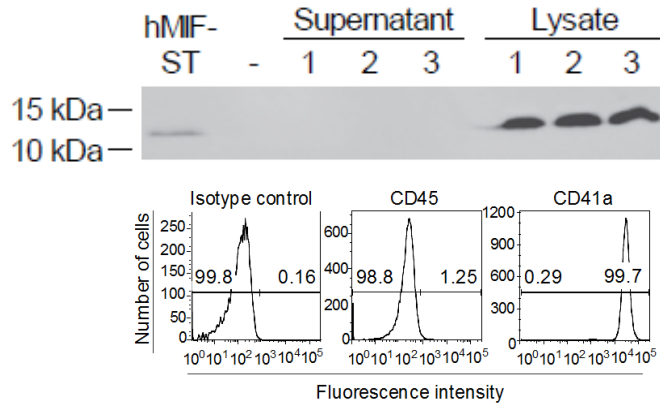
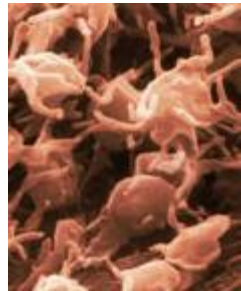
EL1 + EL2



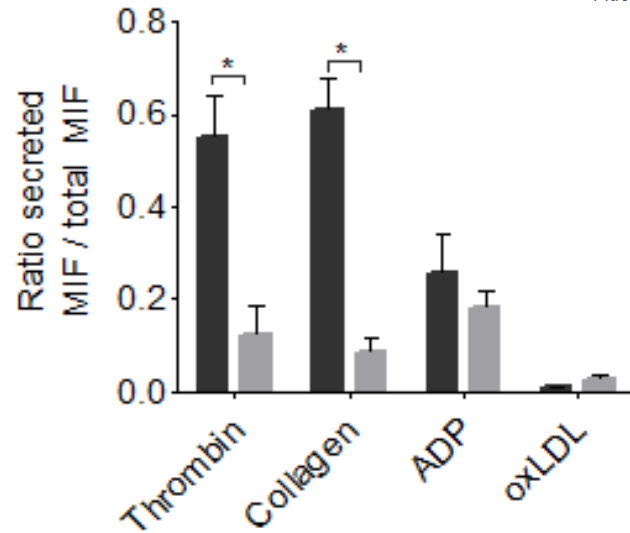
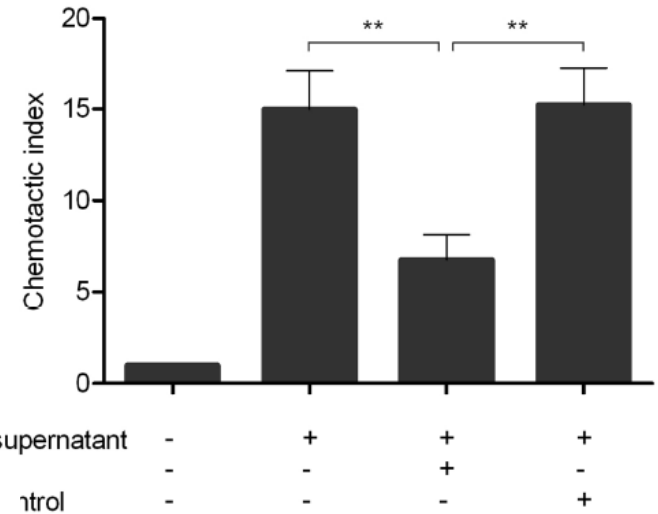
CXCR4

# Platelets and Pericytes Express & Secrete MIF

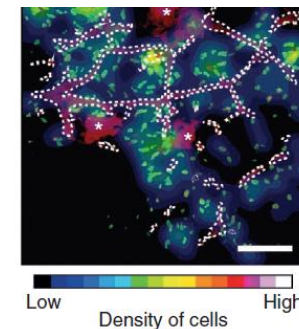
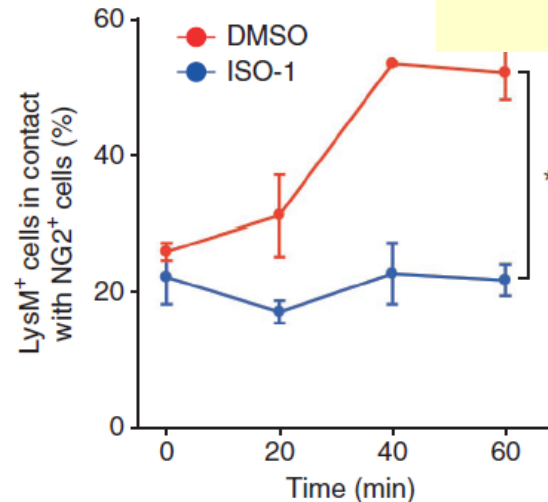
Platelets secrete ~50% of endogenous MIF upon thrombogenic stimuli



Platelet MIF contributes to leukocyte recruitment

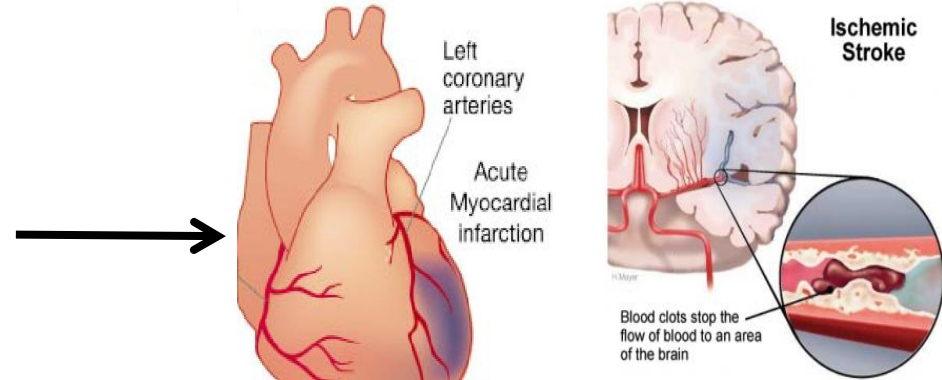
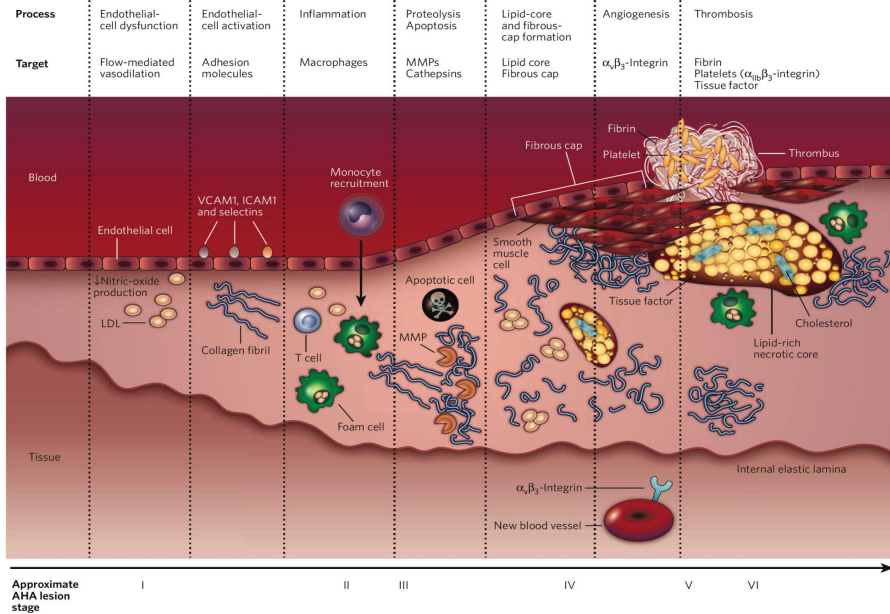


Pericyte-leukocyte contacts are mediated by MIF



Taken from:  
Stark et al., Nat. Immunol. 2012

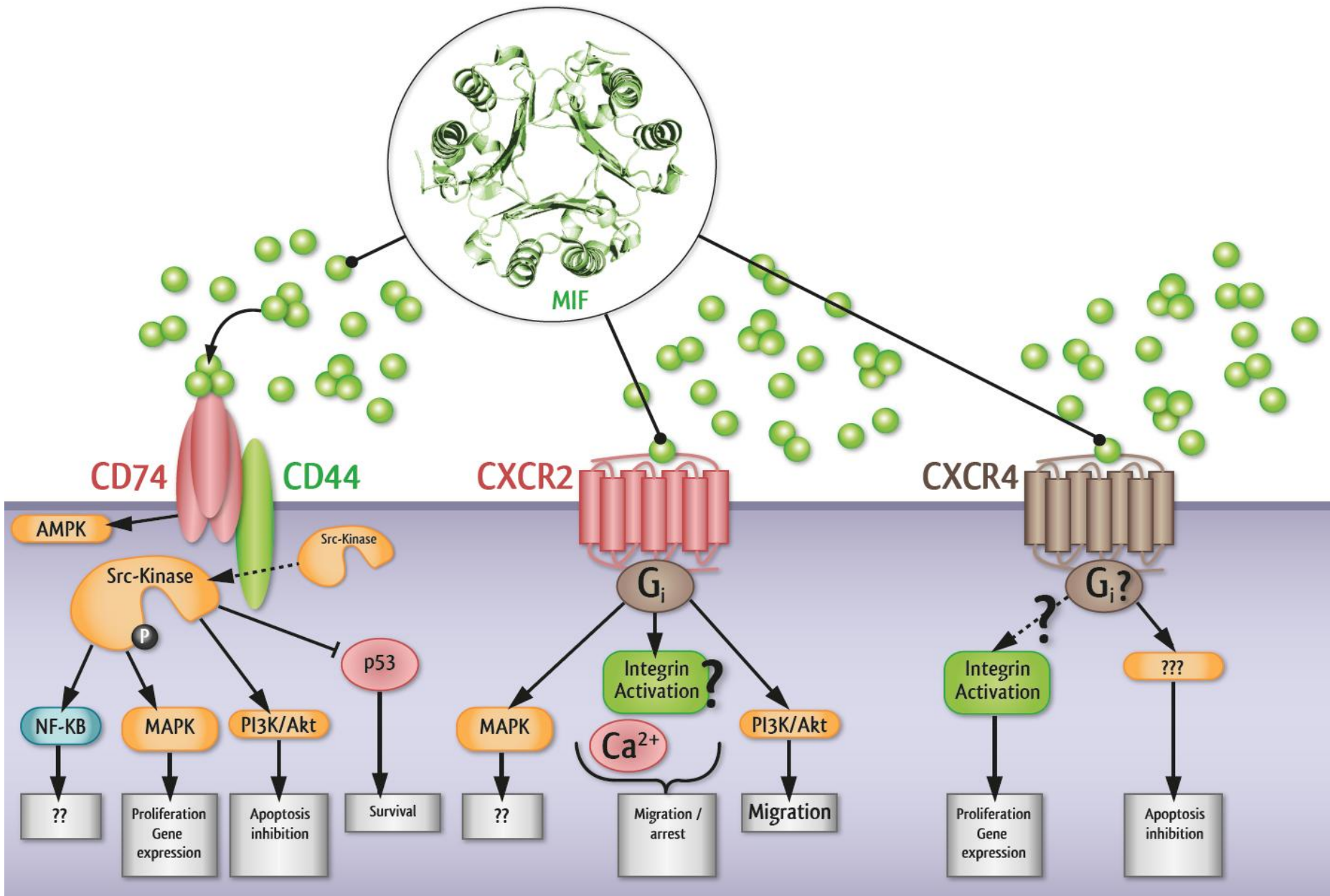
# → Blocking MIF could be a Therapeutic Strategy in Atherosclerosis !!!



Could Blocking MIF also be a Therapeutic Strategy in Acute Myocardial Infarction?

It's not that easy!!!

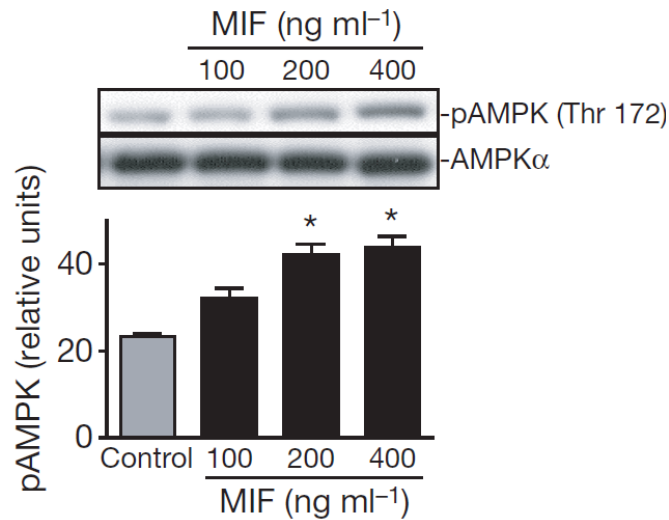
# There is a Third MIF Receptor: *CD74*



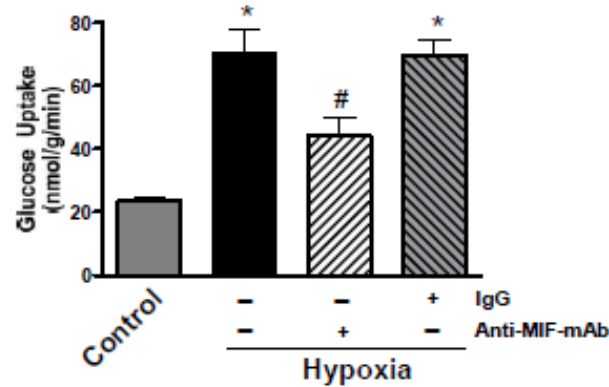


# MIF Protects the Ischemic Heart through Activation of the CD74/AMPK Pathway

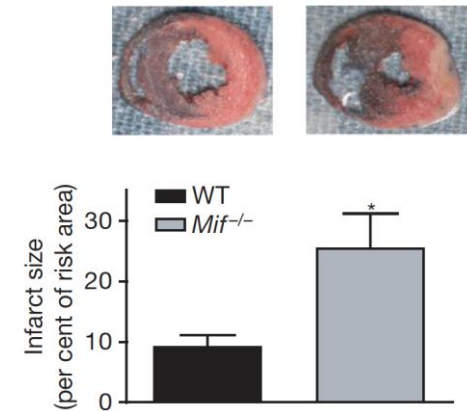
## MIF activates AMPK



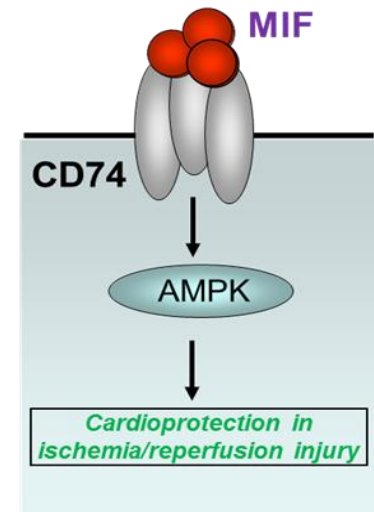
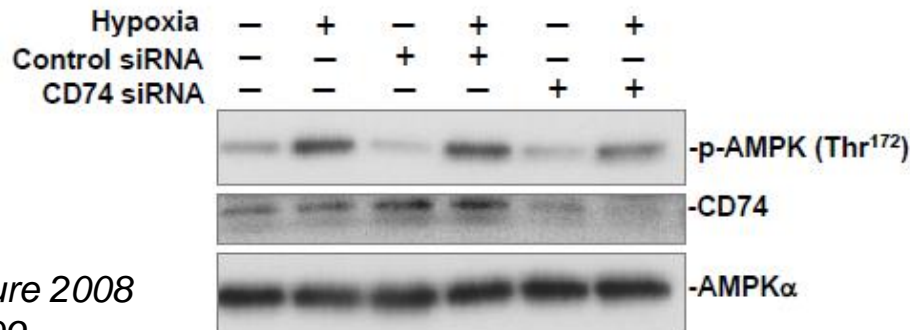
## MIF promotes glucose uptake



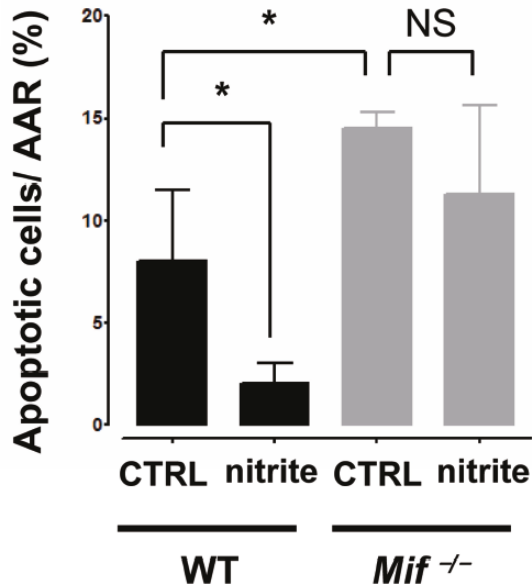
## Increased infarct size of MIF-KO



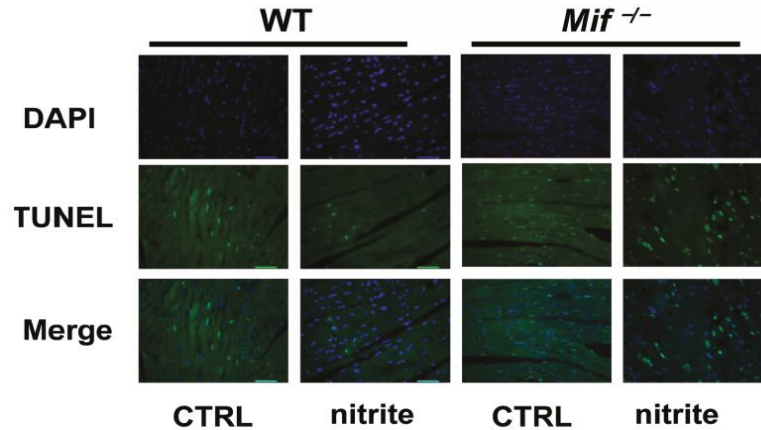
## AMPK activation is mediated by CD74



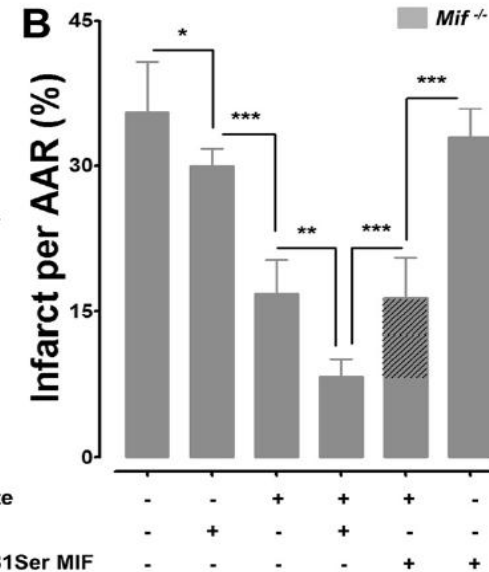
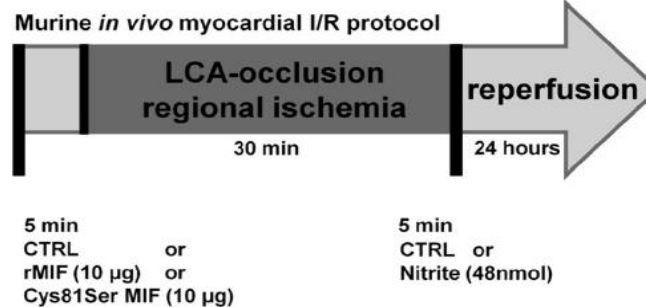
# Enhanced Cardioprotection by S-Nitros(y)lation of MIF



S-NO MIF reduces cardiomyocyte apoptosis

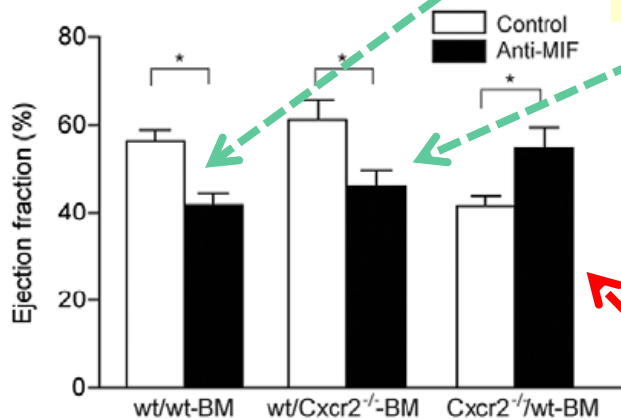
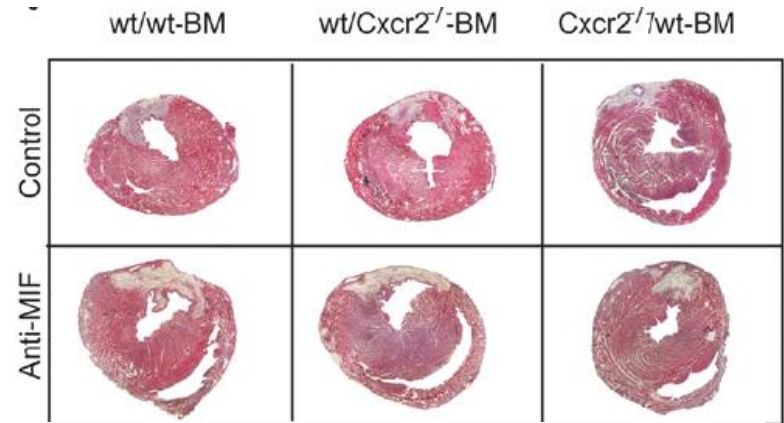
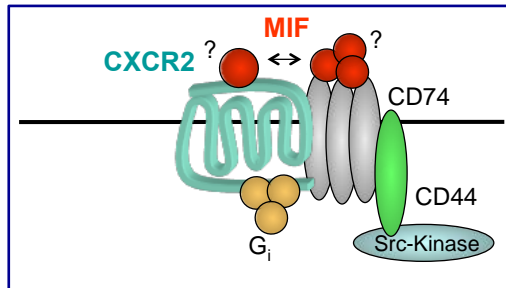


S-NO modification at Cys-81 enhances the cardioprotective effect of MIF in I/R

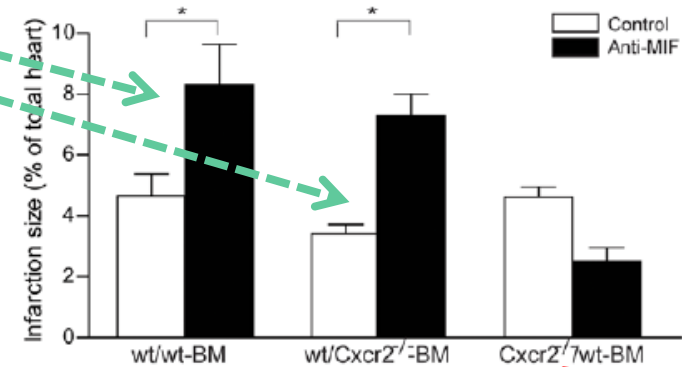


# Dual Effects of the MIF/CXCR2 Axis in Myocardial Infarction/Reperfusion Injury

## Compartmentalized role of MIF/CXCR2 in MI-I/R



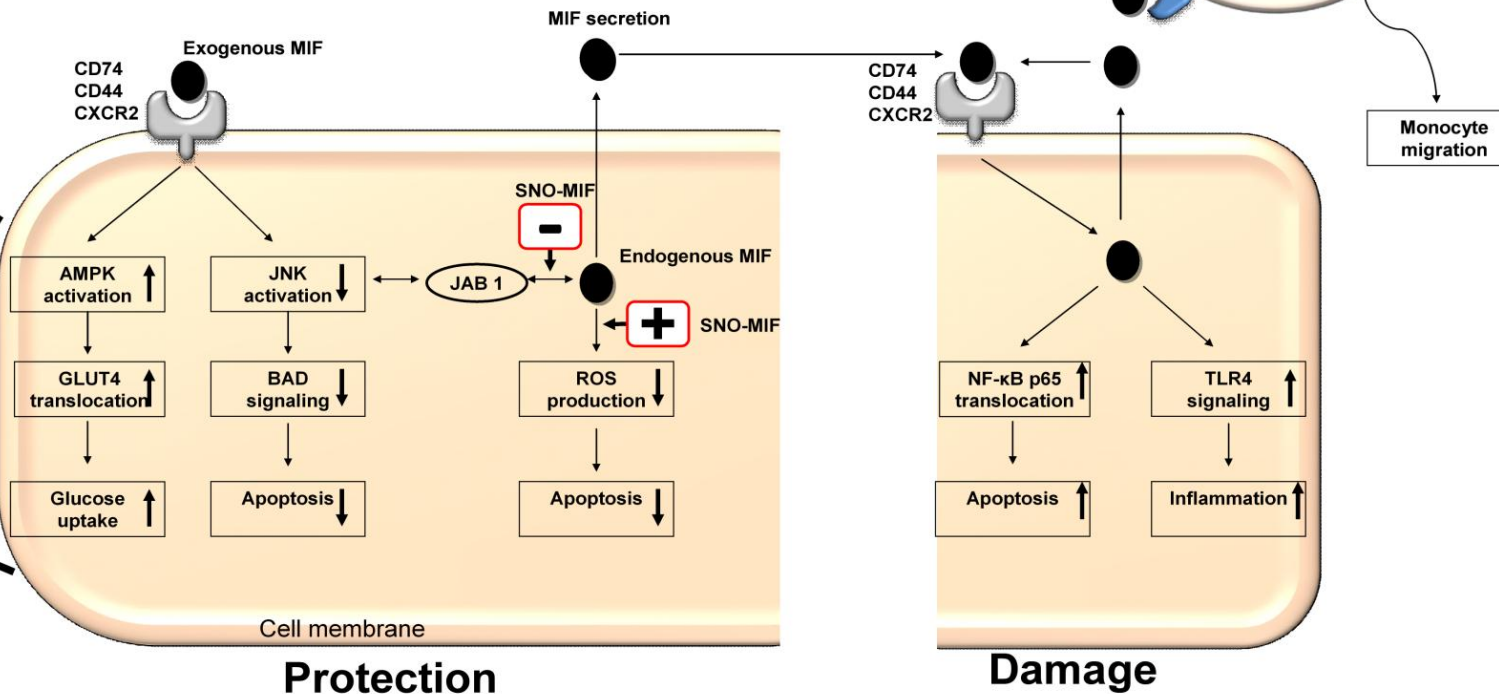
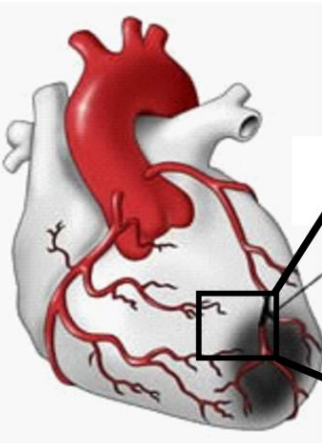
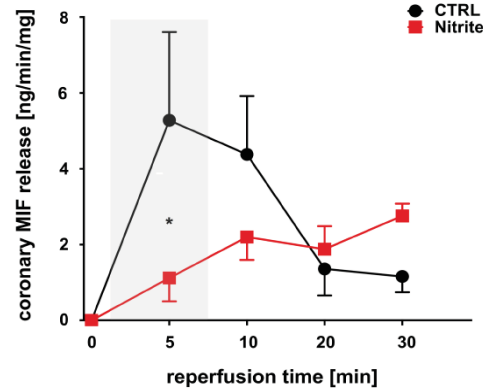
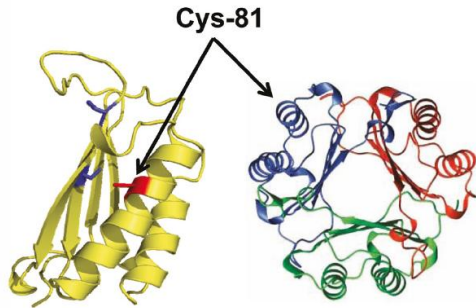
Protection by cardiomyocyte MIF/CXCR2



Exacerbation by leukocyte MIF/CXCR2

# Compartmentalized Effects of MIF in Myocardial I/R

## S-NO modification at Cys-81



Liehn et al., ATVB 2013

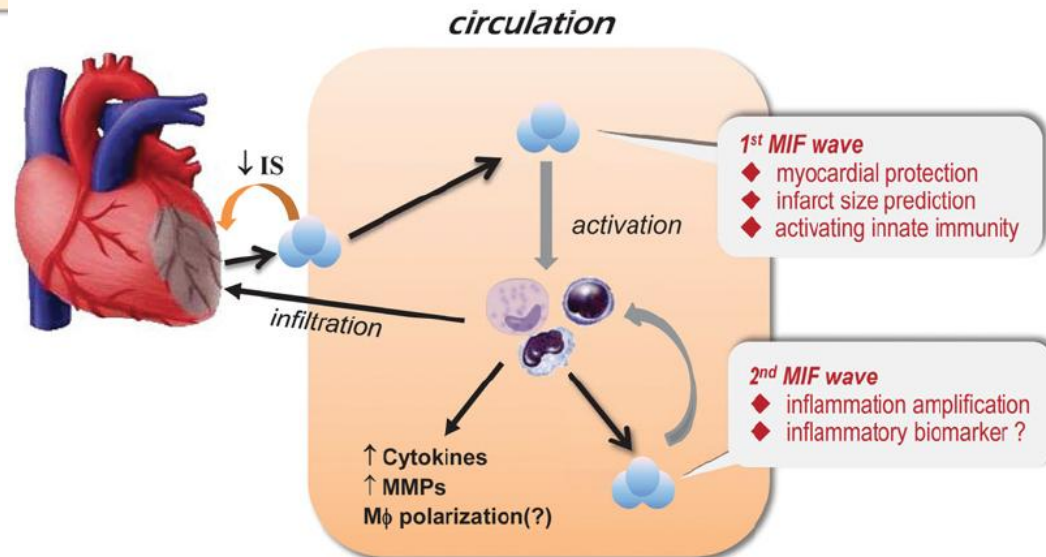
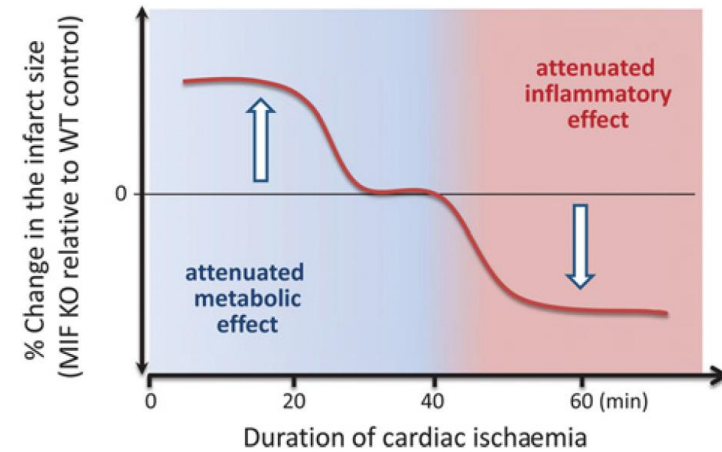
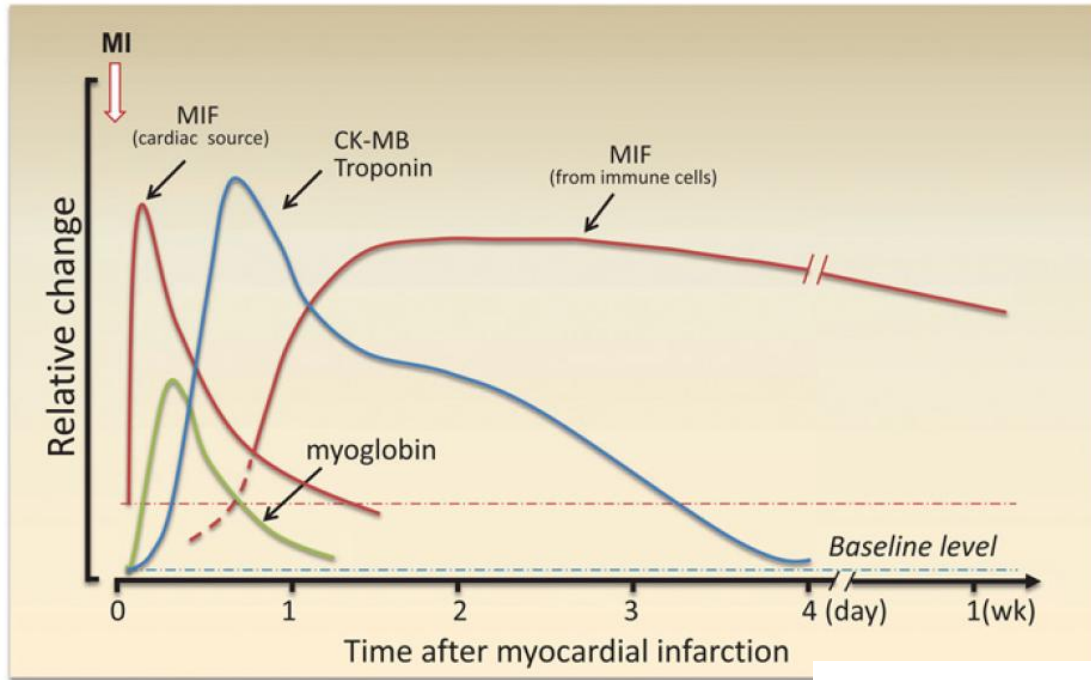
Lüdike et al., Circulation 2012

Rassaf, Weber, Bernhagen, Cardiovasc Res 2014

Gao et al., JMCC 2011; White et al., PLoS One 2013;

White et al., JMCC 2014

# Phase-specific Roles of MIF in Myocardial I/R?



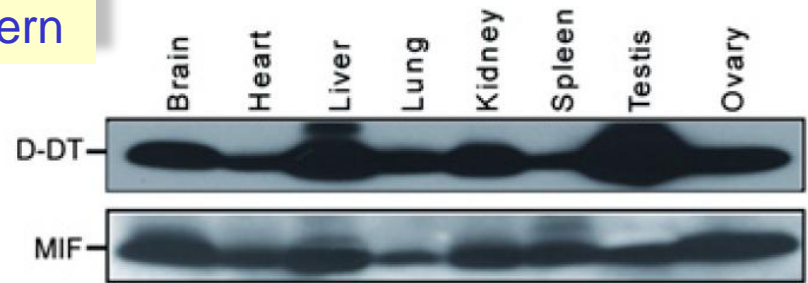
Dayawansa et al., Clin Sci 2014

Rassaf, Weber, Bernhagen, Cardiovasc Res 2014

Kanzler et al., Basic Res. Cardiol. 2013

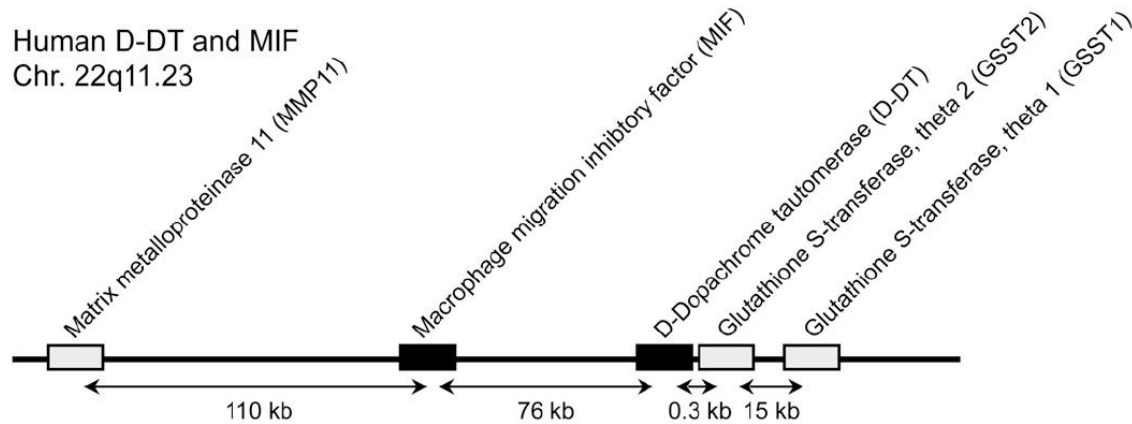
# MIF-2/D-DT is a Cytokine and a Homolog of MIF (1)

Similar expression pattern



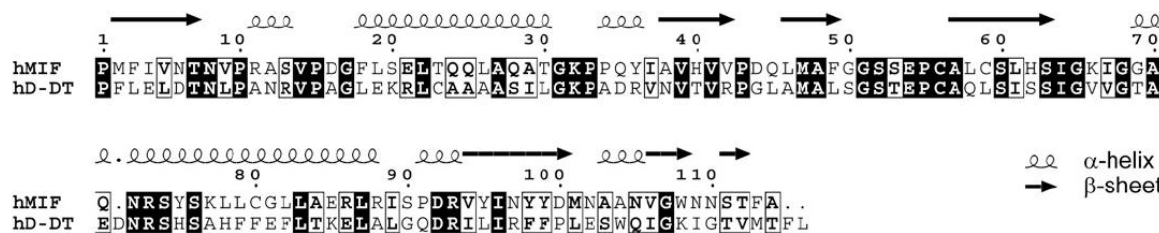
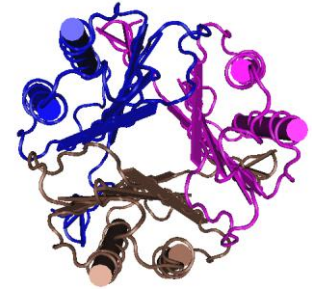
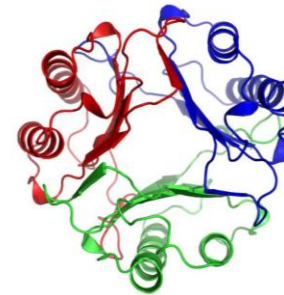
27% sequence identity, same gene cluster and highly similar 3D-architecture

Human D-DT and MIF  
Chr. 22q11.23



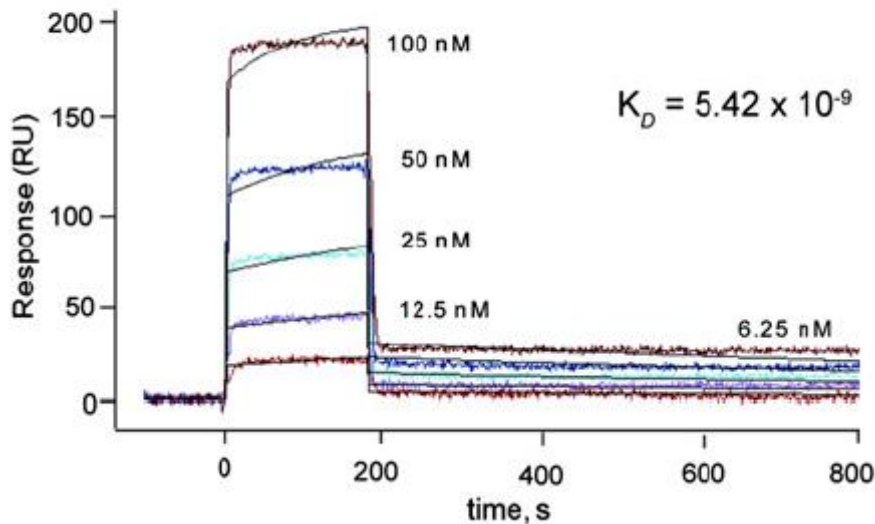
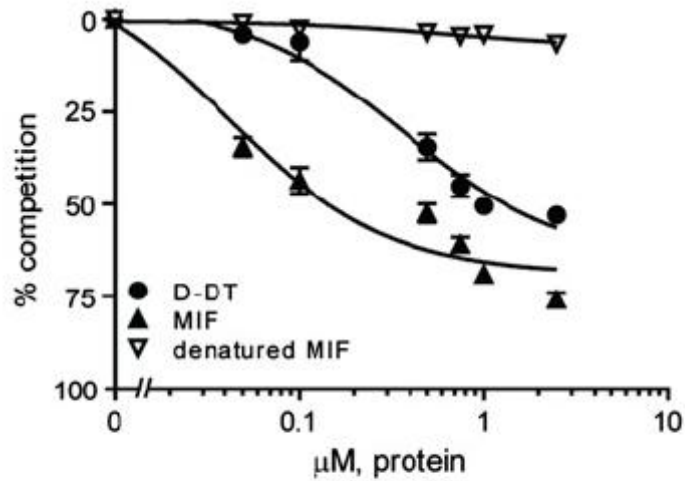
MIF

MIF-2/D-DT

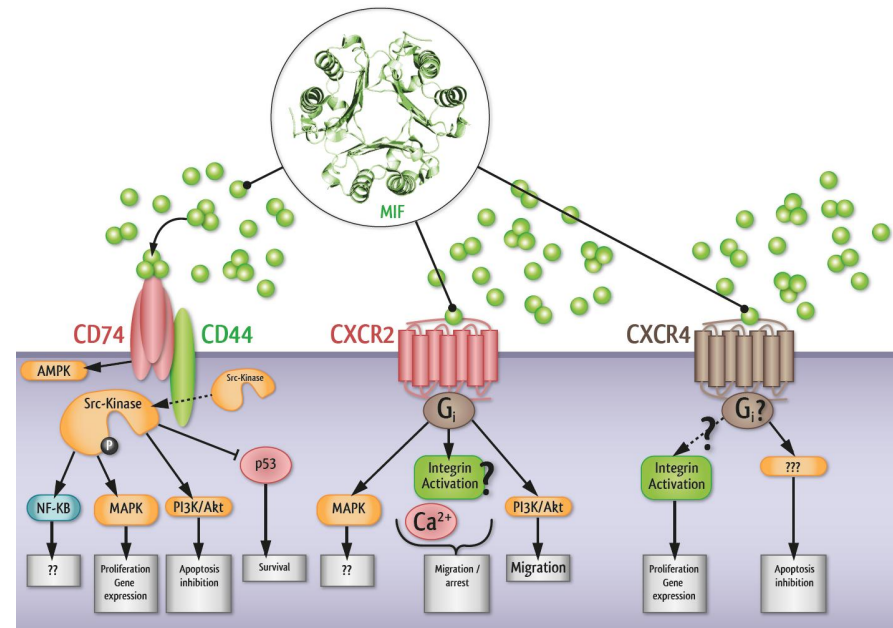


# MIF-2/D-DT is a Cytokine and a Homolog of MIF (2)

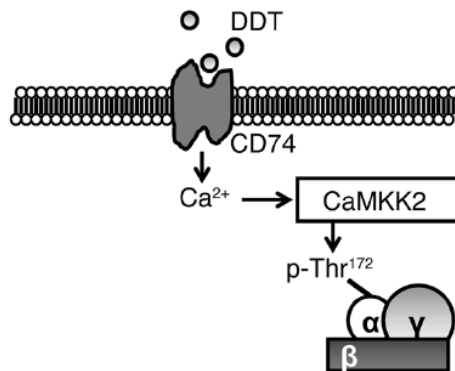
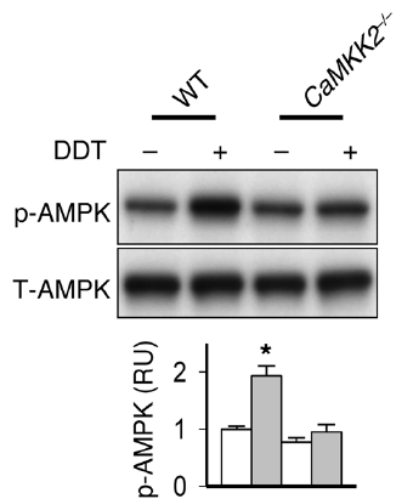
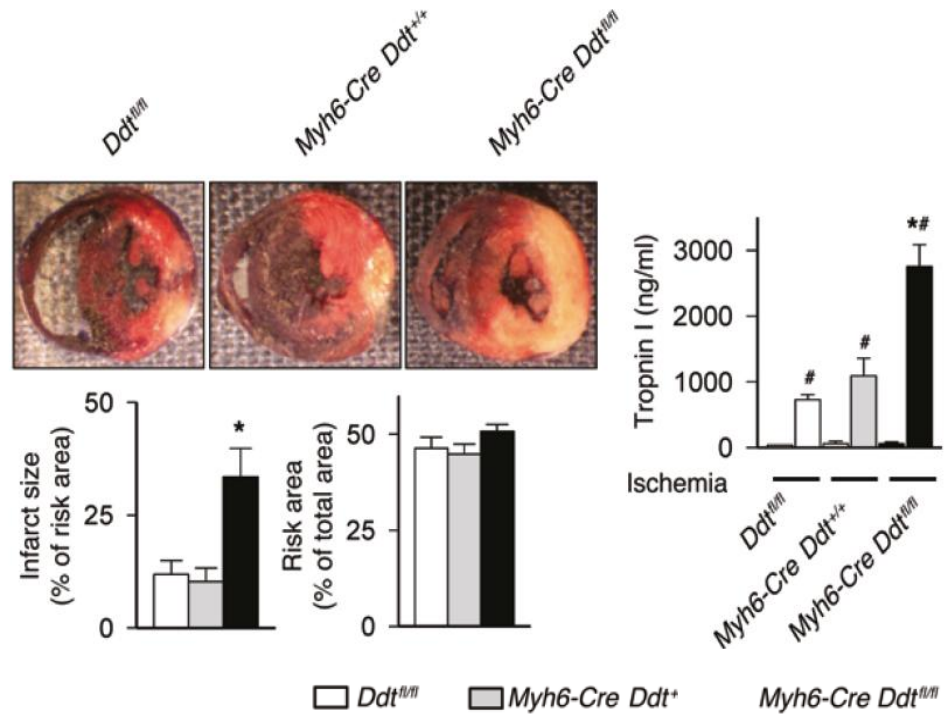
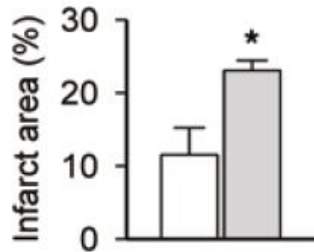
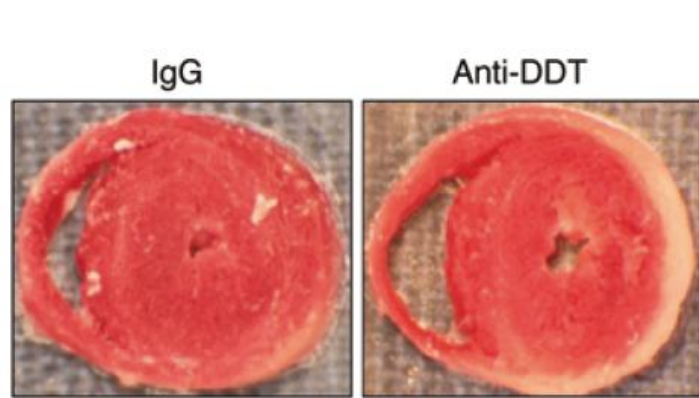
MIF-2 binds to CD74



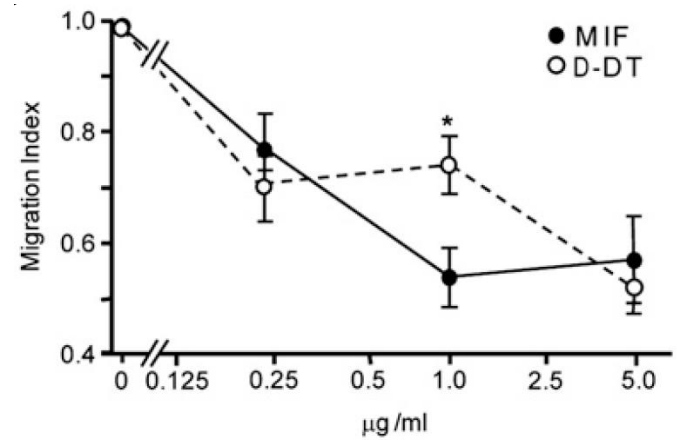
Binding to CXCR2, CXCR4??



# MIF-2/D-DT Protects the Heart against Ischemic Injury



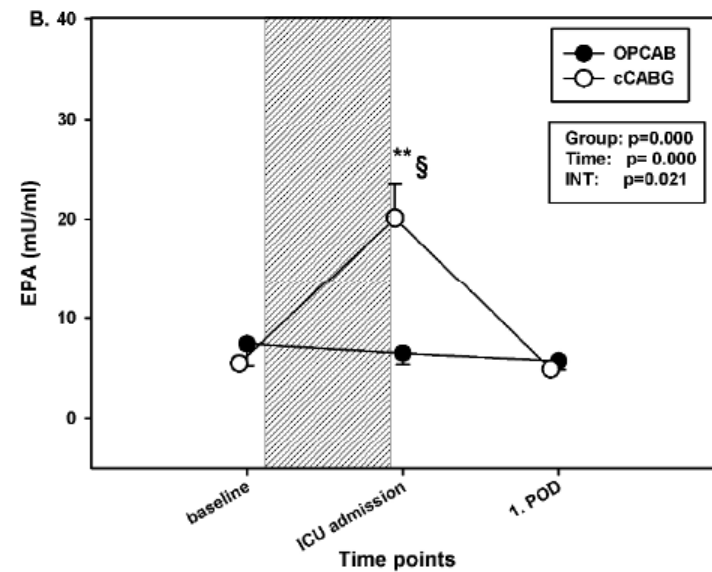
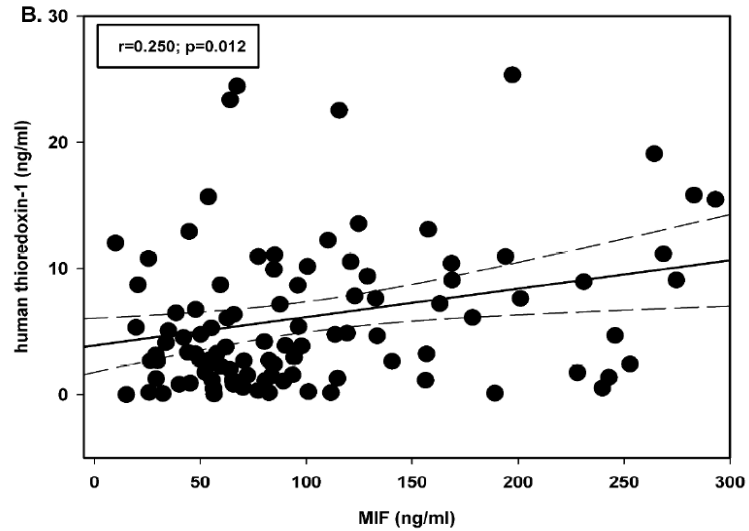
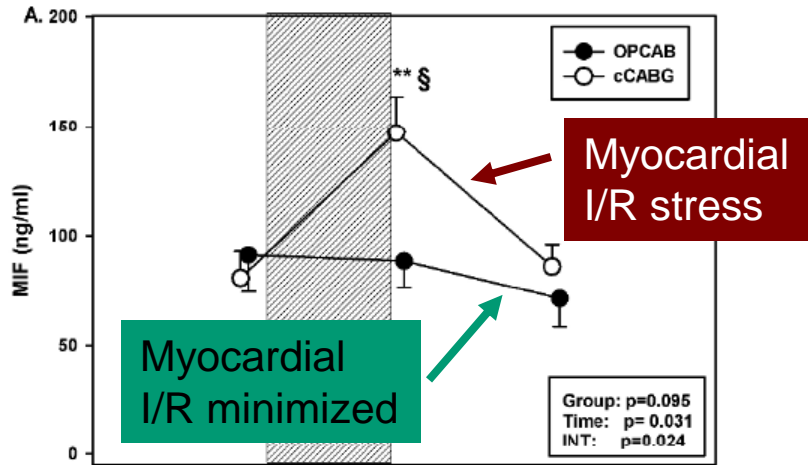
## Differential chemotactic effects



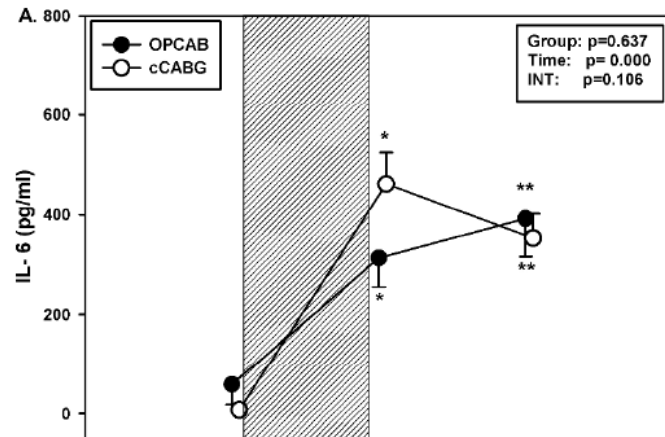


# MIF in Myocardial Infarction: *Cardiac Surgery*

MIF<sub>periop</sub> is induced by I/R stress & enhances anti-oxidative capacity

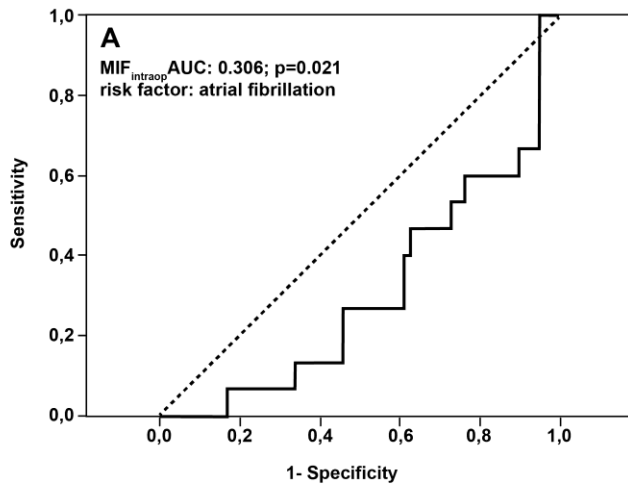


IL-6<sub>periop</sub> is induced independent of I/R stress

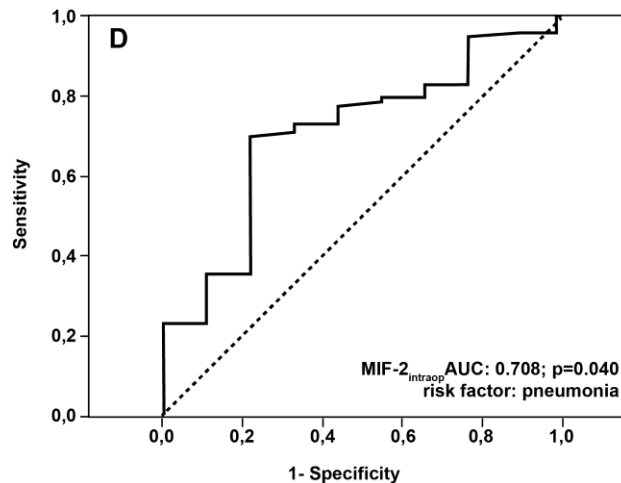
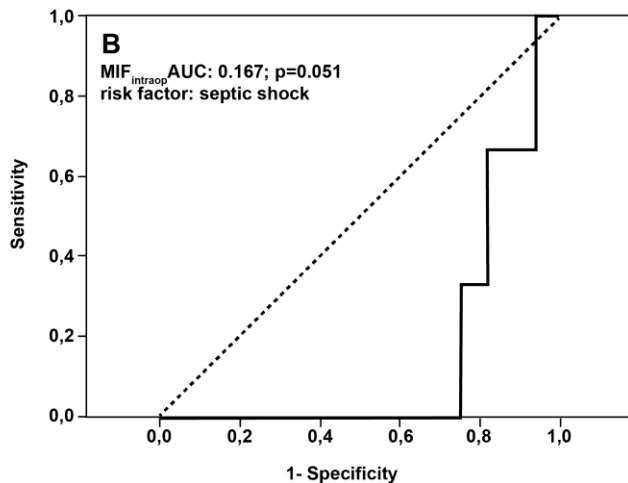
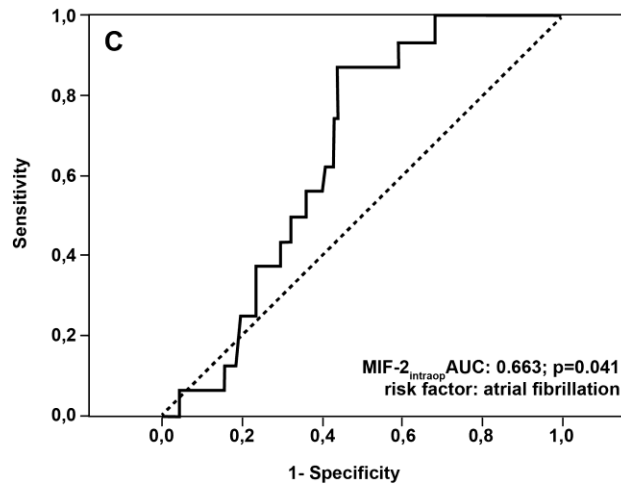


# MIF and MIF-2 predict **OPPOSITE** Clinical Outcomes in the *Cardiac Surgery Patients*

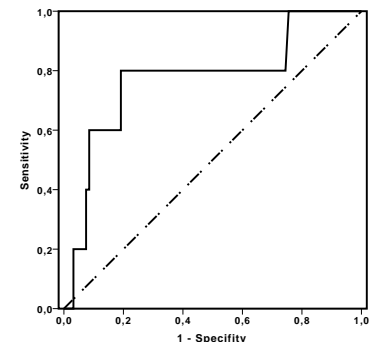
MIF<sub>intraop</sub> predicts for freedom from AF & sepsis



MIF-2<sub>intraop</sub> Prediction of AF & pneumonia



MIF-2<sub>intraop</sub> Prediction of AKI

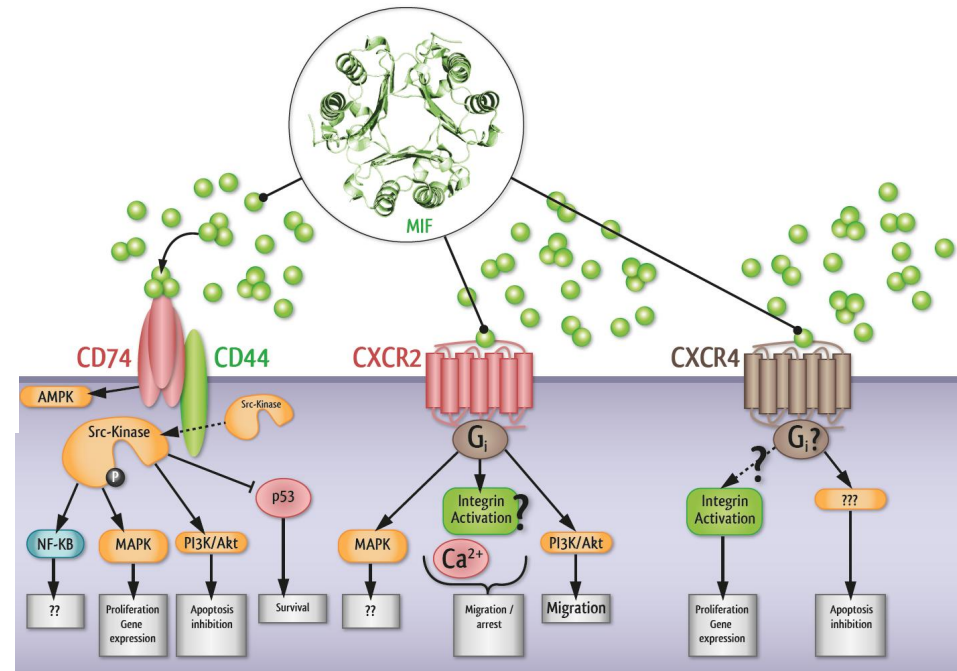
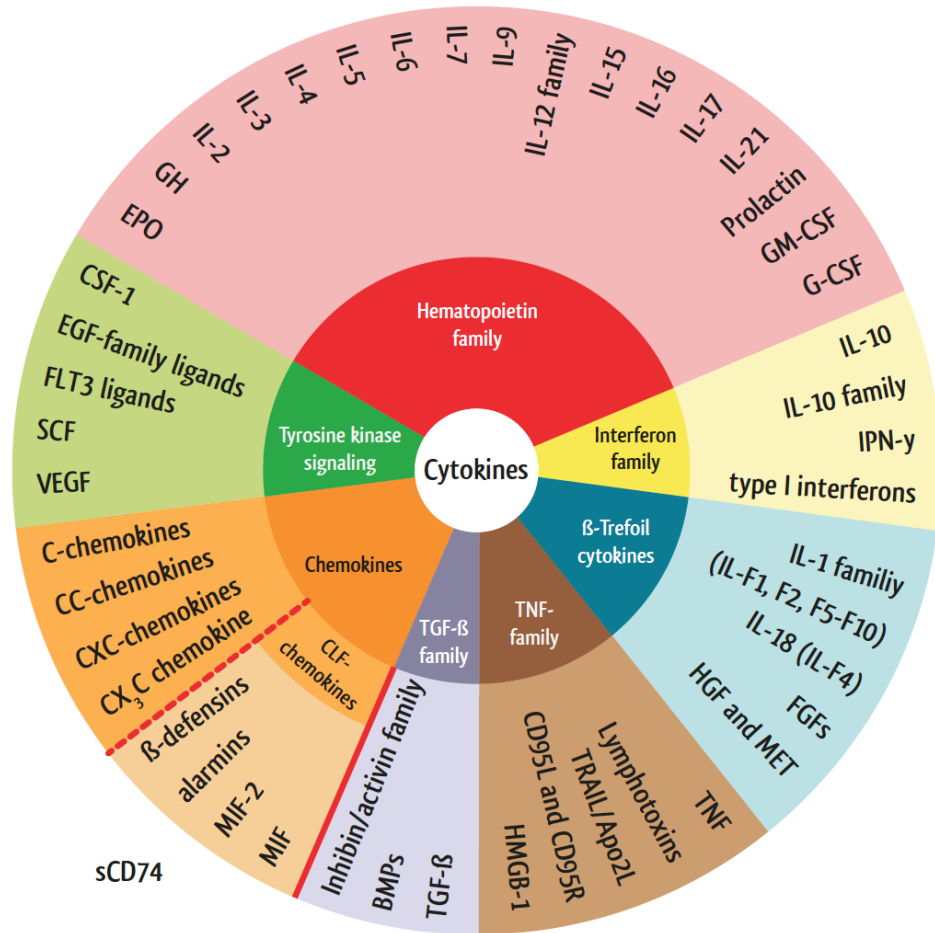


# Cardioprotective and Exacerbating Role of MIF in Myocardial Ischemia/Reperfusion Injury

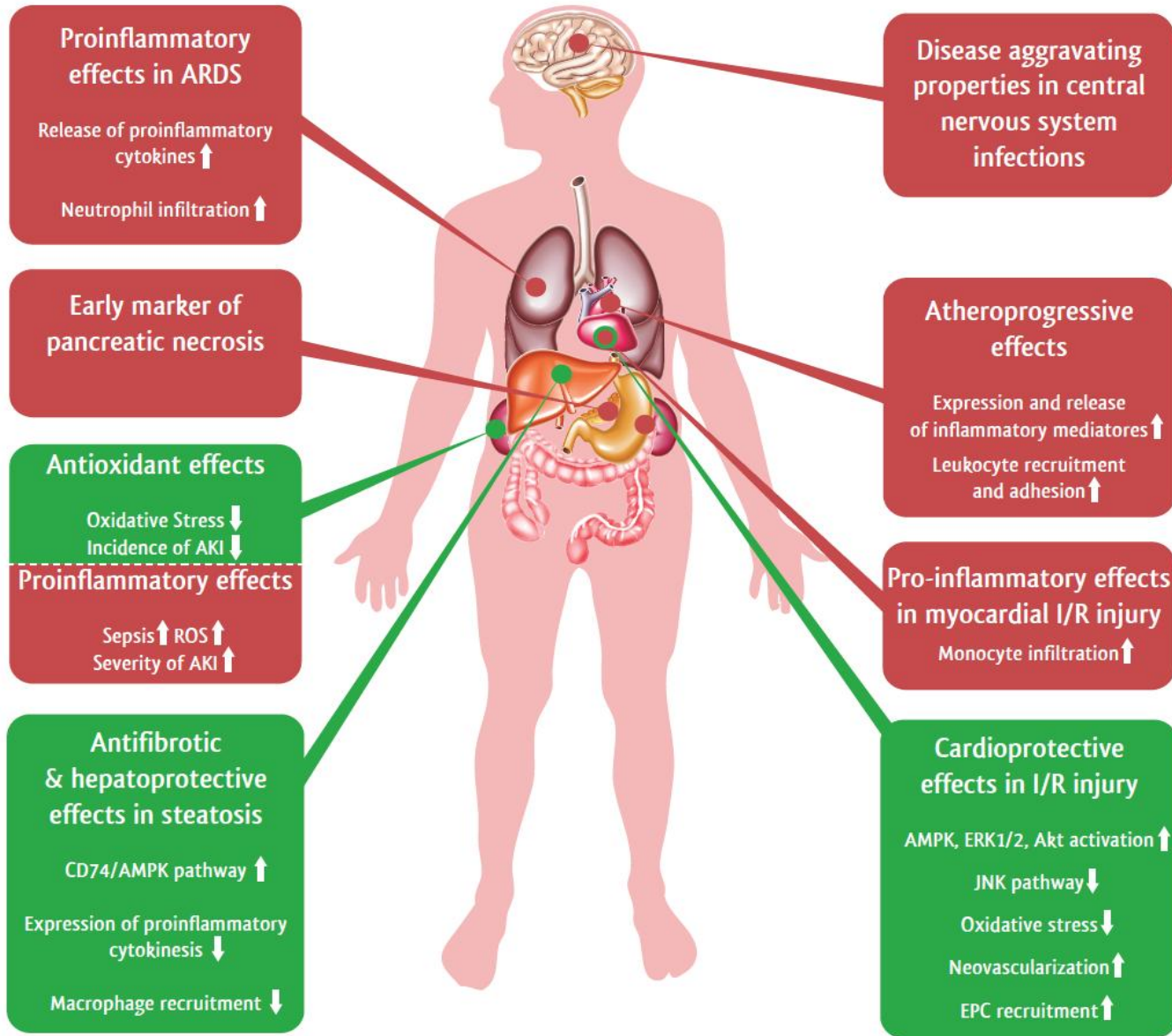
## Complex Role of MIF-2/D-DT in Myocardial Ischemia/Reperfusion Injury

# MIF Family Proteins

## Classification in Cytokine World and Receptors



# MIF Family Proteins in (Cardiovascular) Disease



**Bad**

**Good**

**Pretty**



# MIF Family Proteins are KEY PLAYERS Shaping Decisions in Cardiovascular Disease Tissue Protection vs. Inflammatory Exacerbation

Where/what is the switch??



# Thank you !!

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