# Immune-Mediated Mechanisms of Atherosclerosis

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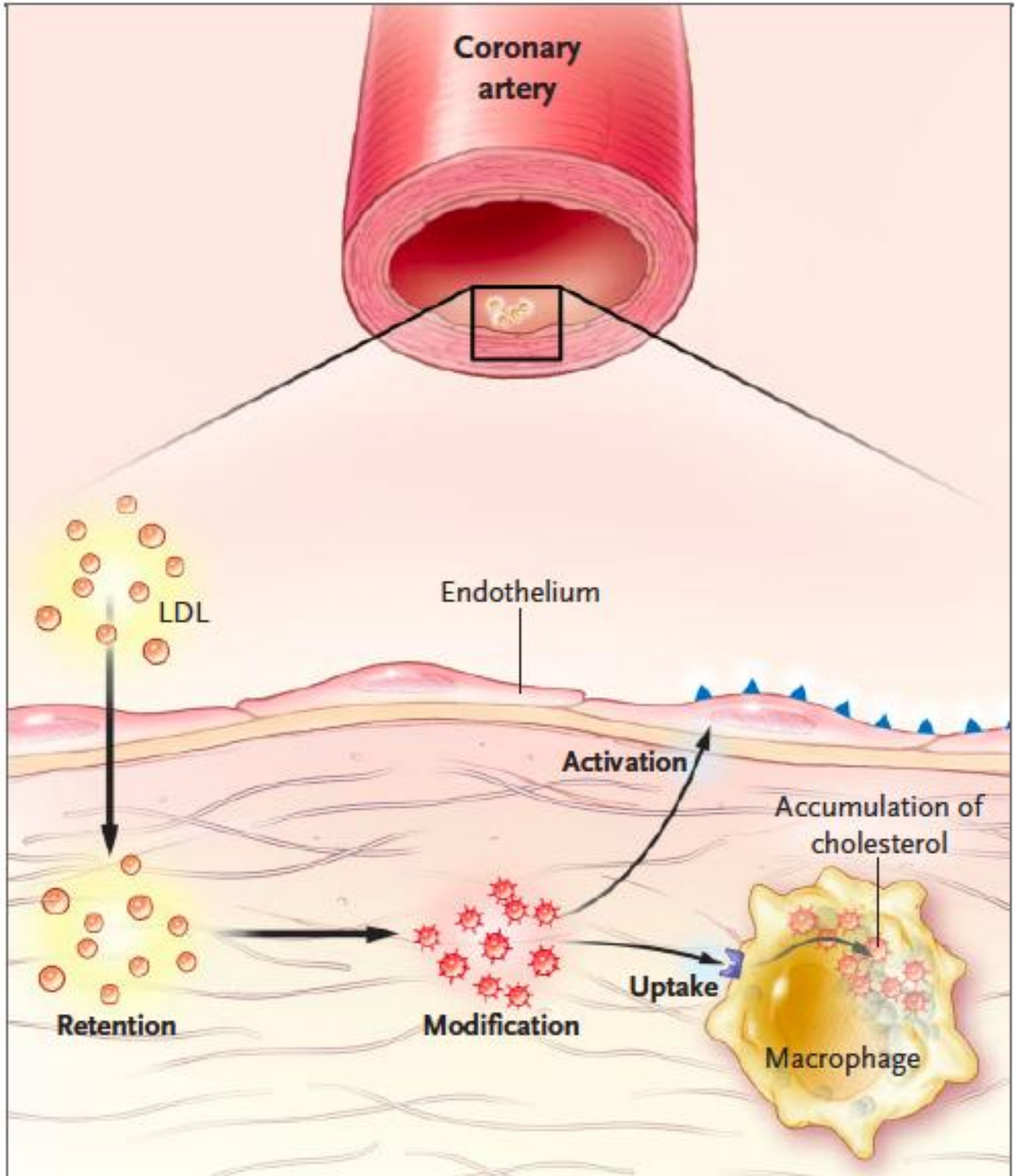
UNIVERSITY OF CAMBRIDGE

Inserm U970

TOLERAGE

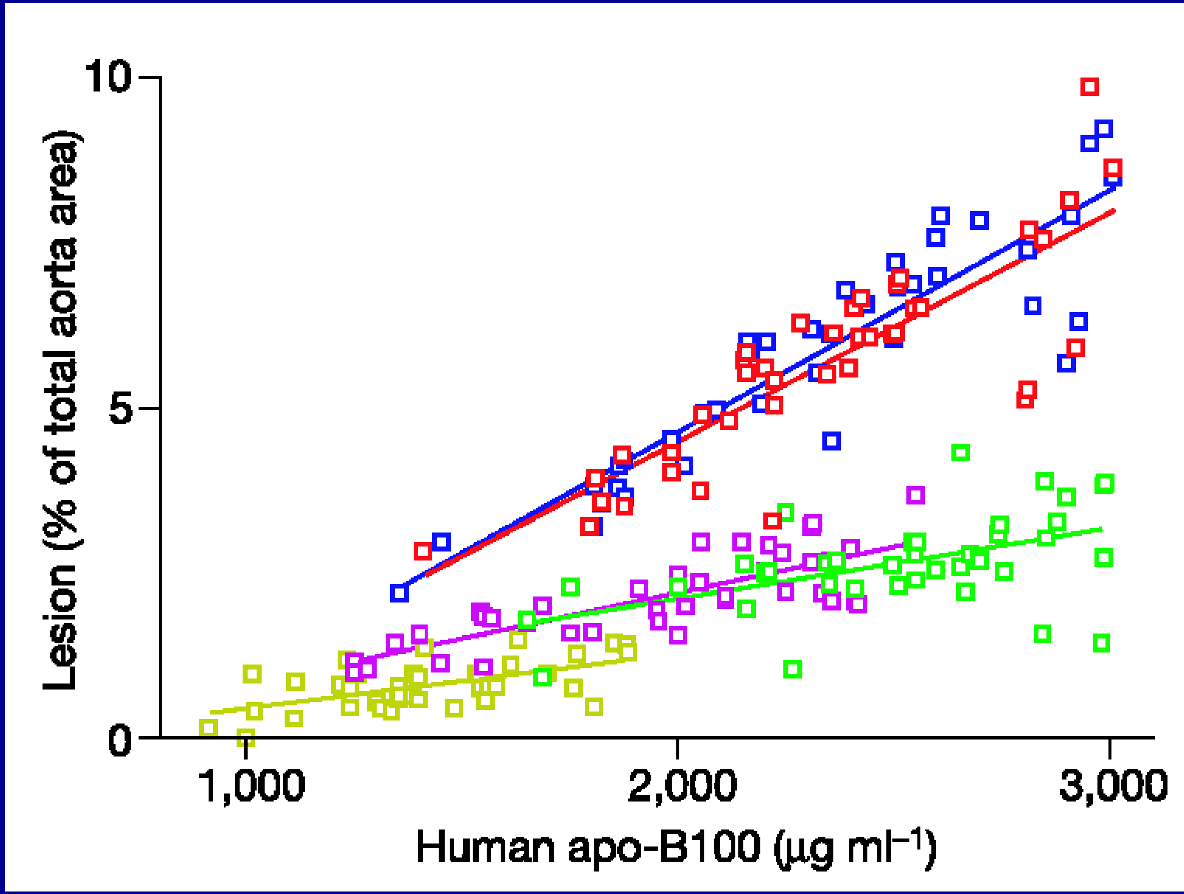
L·I·N·K· LEDUCQ IMMUNOREGULATORY NETWORK

5 erc 2007 - 2012 Years




Hanson GK.
N Engl J Med 2005

# Effect of Subendothelial Retention of Atherogenic Lipoproteins on Atherosclerosis




Wild type-control LDL

Proteoglycan-binding-defective LDL

Skalen et al. Nature, 2002;417:750-754.

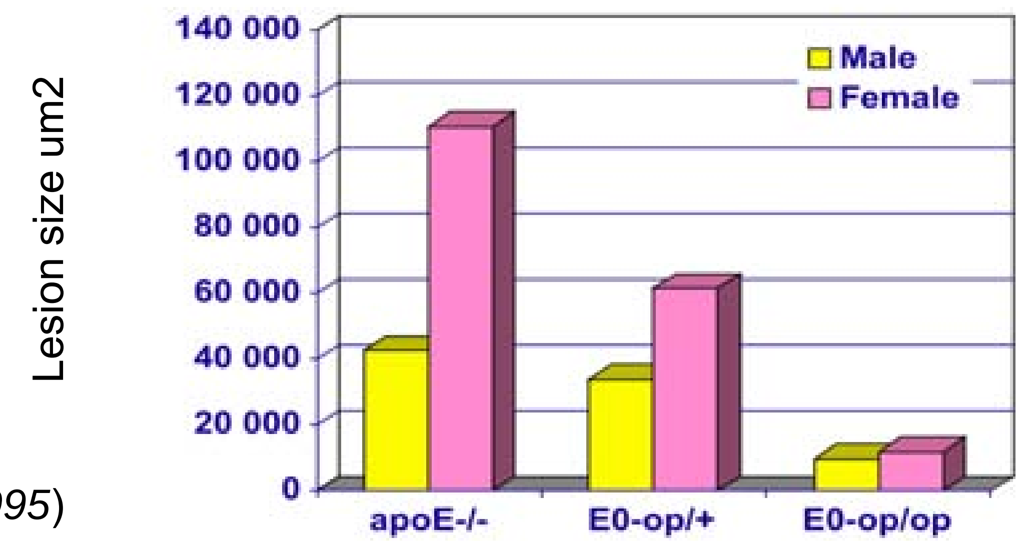
# Crucial role of monocytes/macrophages in atherosclerosis

## M-CSF deficiency inhibits plaque formation (Smith, *PNAS* 1995)

Genetics: Smith *et al.* *Proc. Natl. Acad. Sci. USA 92 (1995)* 8267

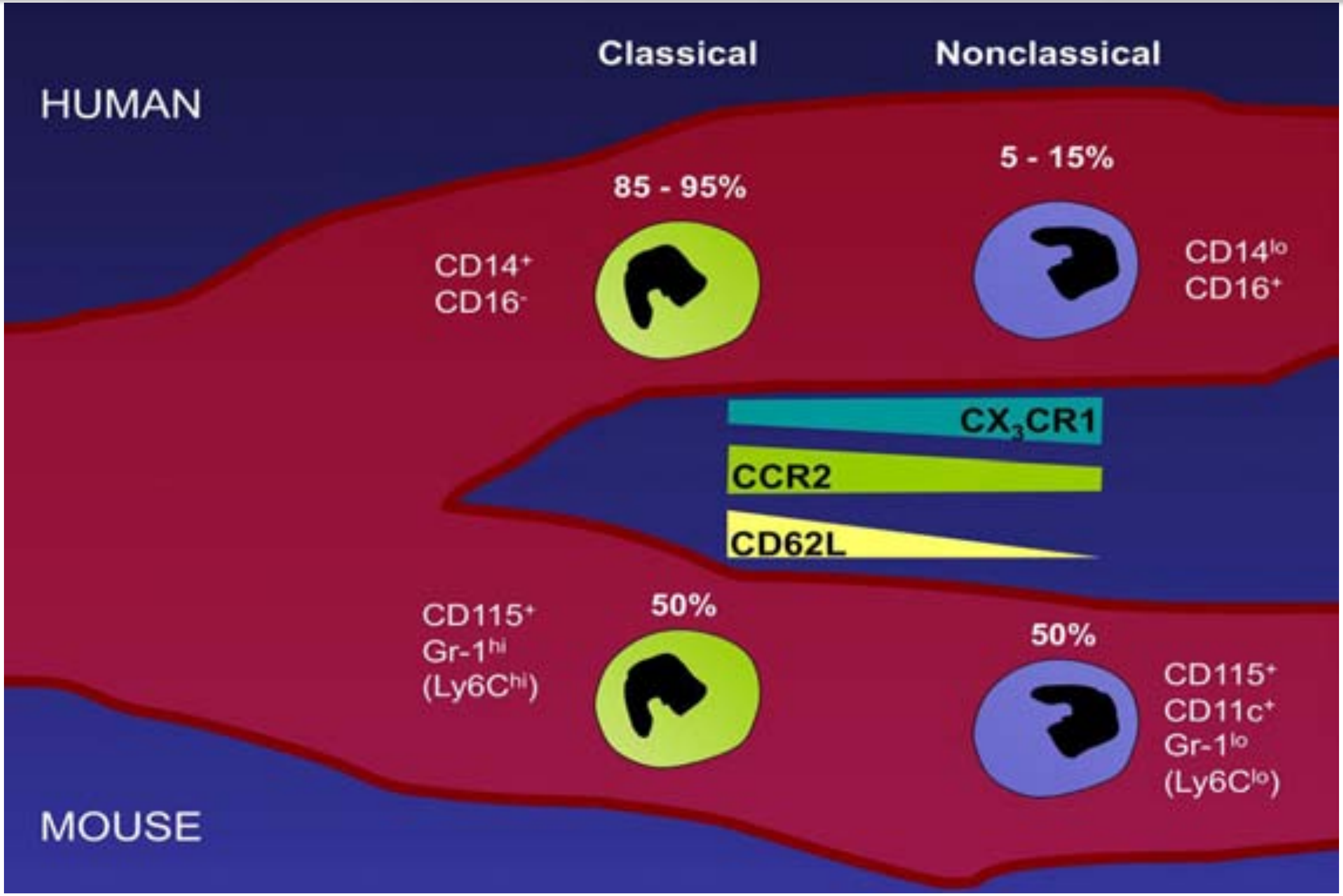
Table 2. Weight, cholesterol, and monocyte differential count by sex and genotype

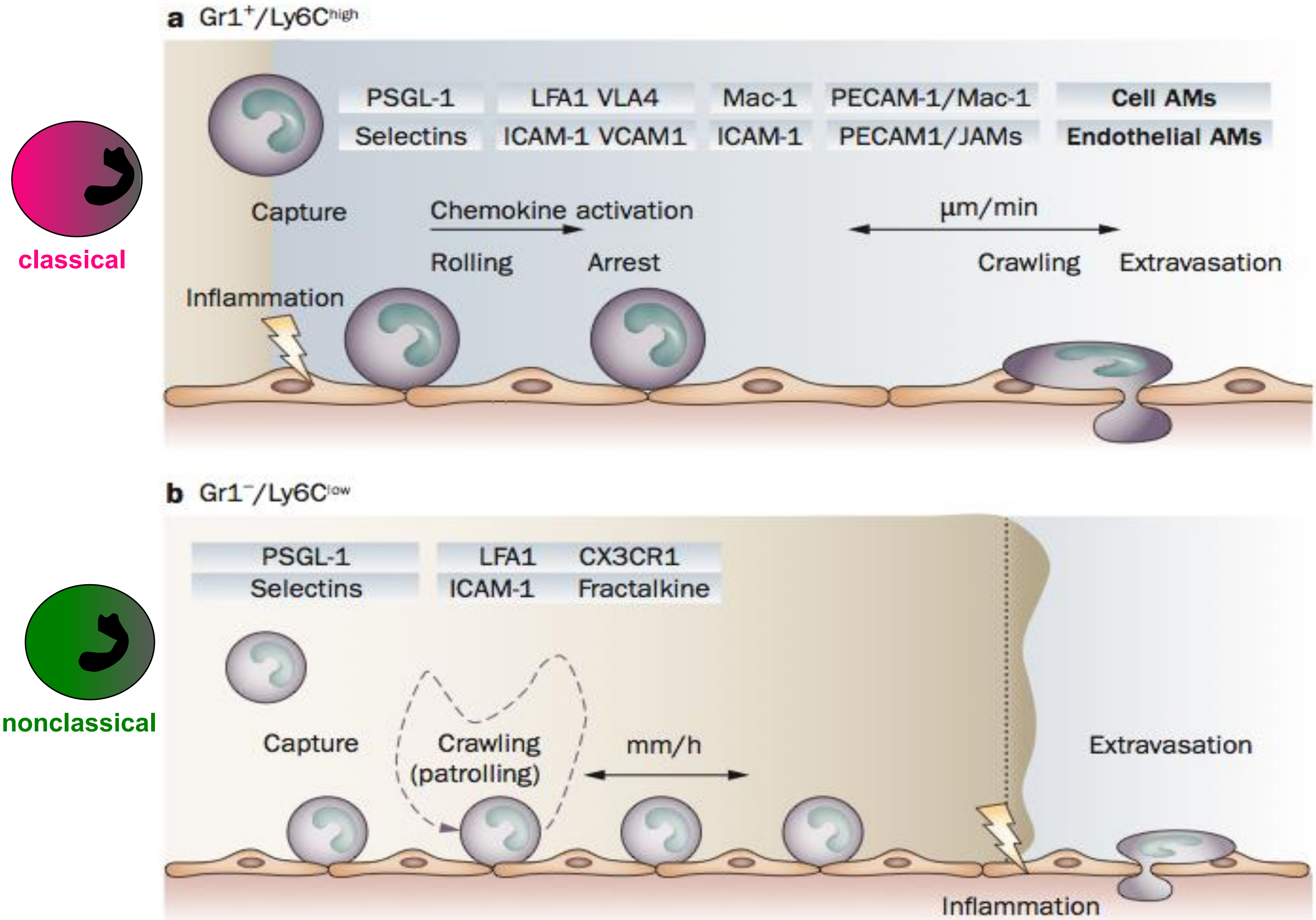
| | Weight, g | | | Total cholesterol, mg/dl | | | Monocyte, % of leukocytes | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| op0/E0 | 20.3 ± 5.3 (6) | 21.3 ± 3.8 (10) | 20.9 ± 4.3* (16) | 1541 ± 651 (6) | 1231 ± 235 (10) | 1347 ± 445* (16) | 3.77 (1) | 4.15 ± 1.50 (3) | 4.05 ± 1.24* (4) |
| op2/E0 | 29.9 ± 3.6 (10) | 26.1 ± 2.9 (16) | 27.5 ± 3.6 (26) | 478 ± 165 (10) | 450 ± 105 (16) | 461 ± 129 (26) | 8.87 ± 0.84 (3) | 9.76 ± 1.06 (5) | 9.43 ± 1.03 (8) |




(Smith et al, *PNAS, 1995*)

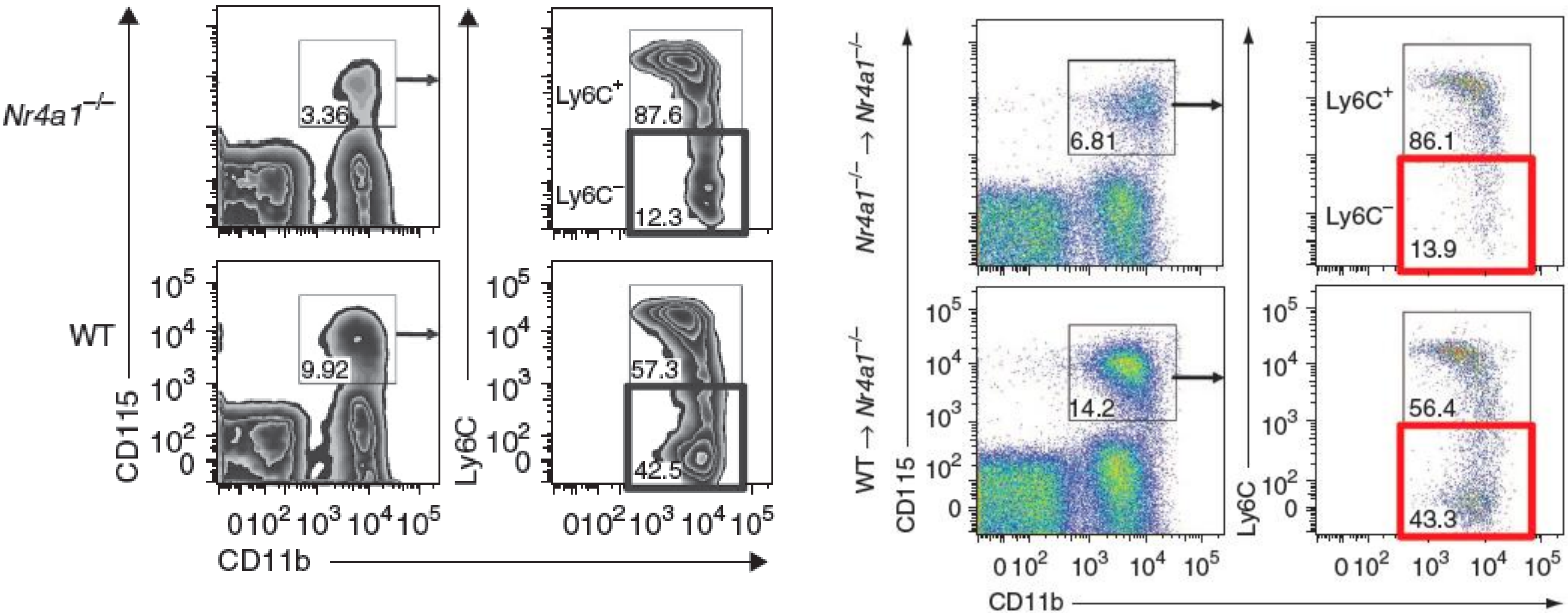
# Monocyte subsets





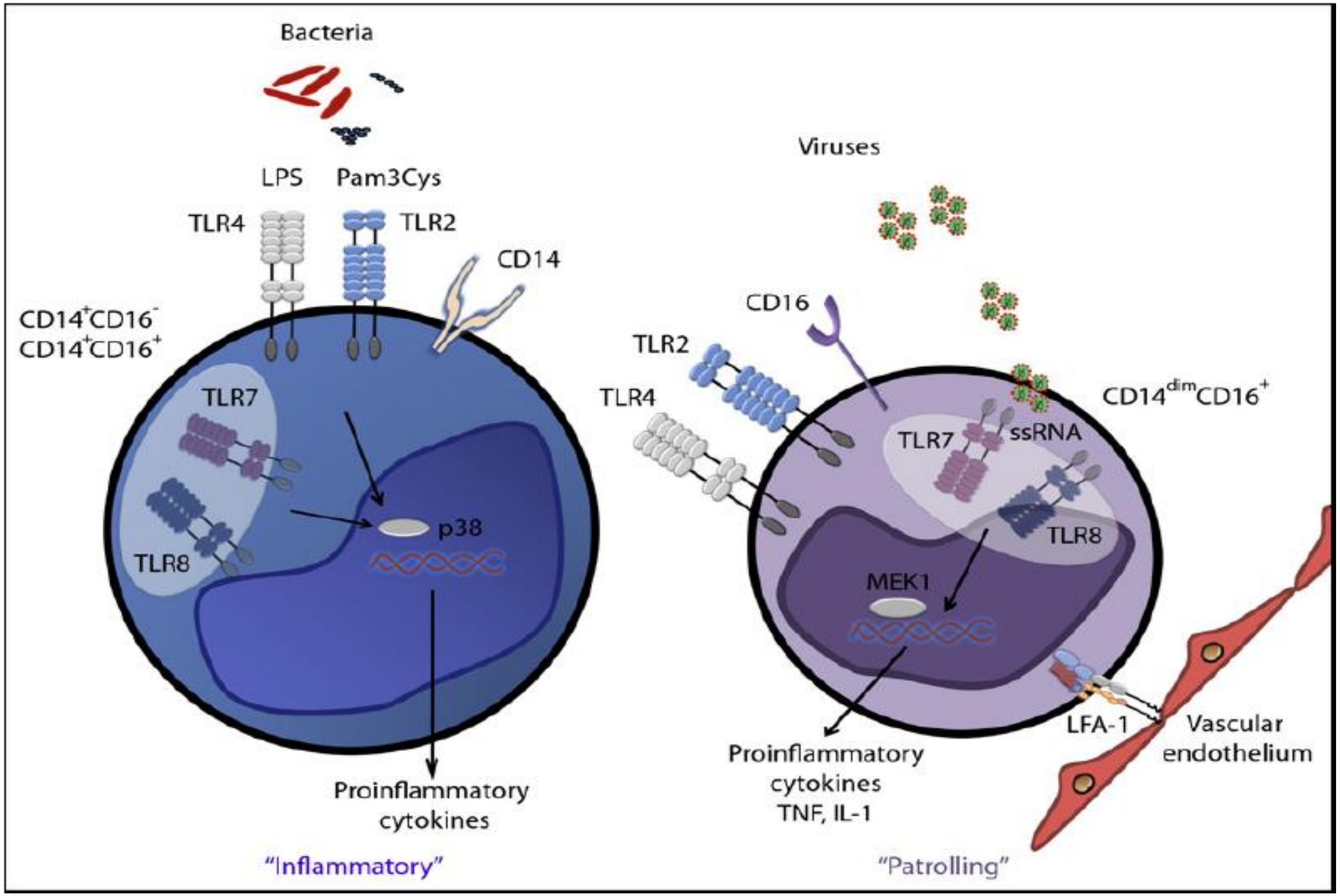

Woollard KJ, Nature Rev Cardiol 2010

# The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C⁻ monocytes



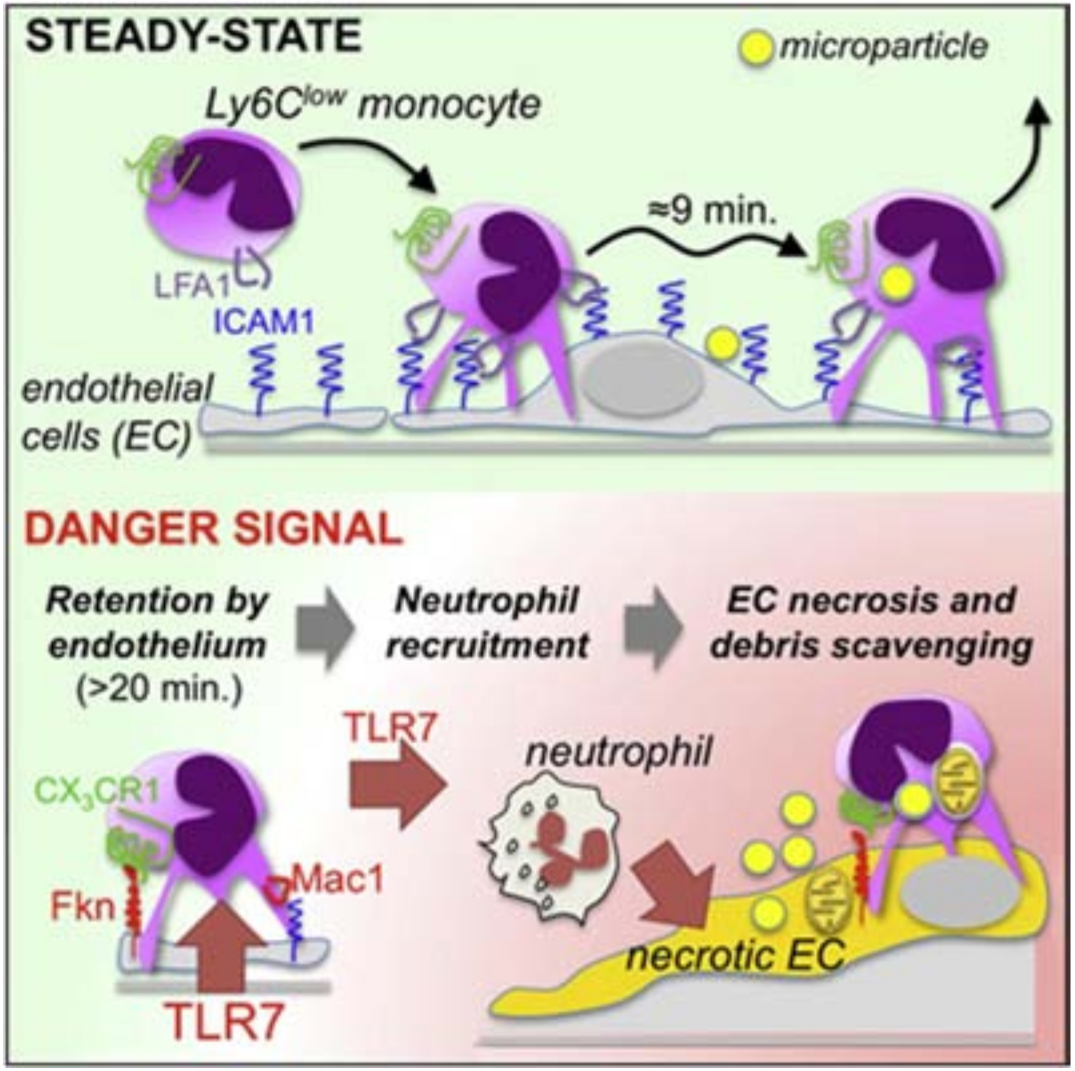

Hannah RN, Nat Immunol 2011

# Human CD14$^{dim}$ Monocytes Patrol and Sense Nucleic Acids and Viruses via TLR7 and TLR8 Receptors

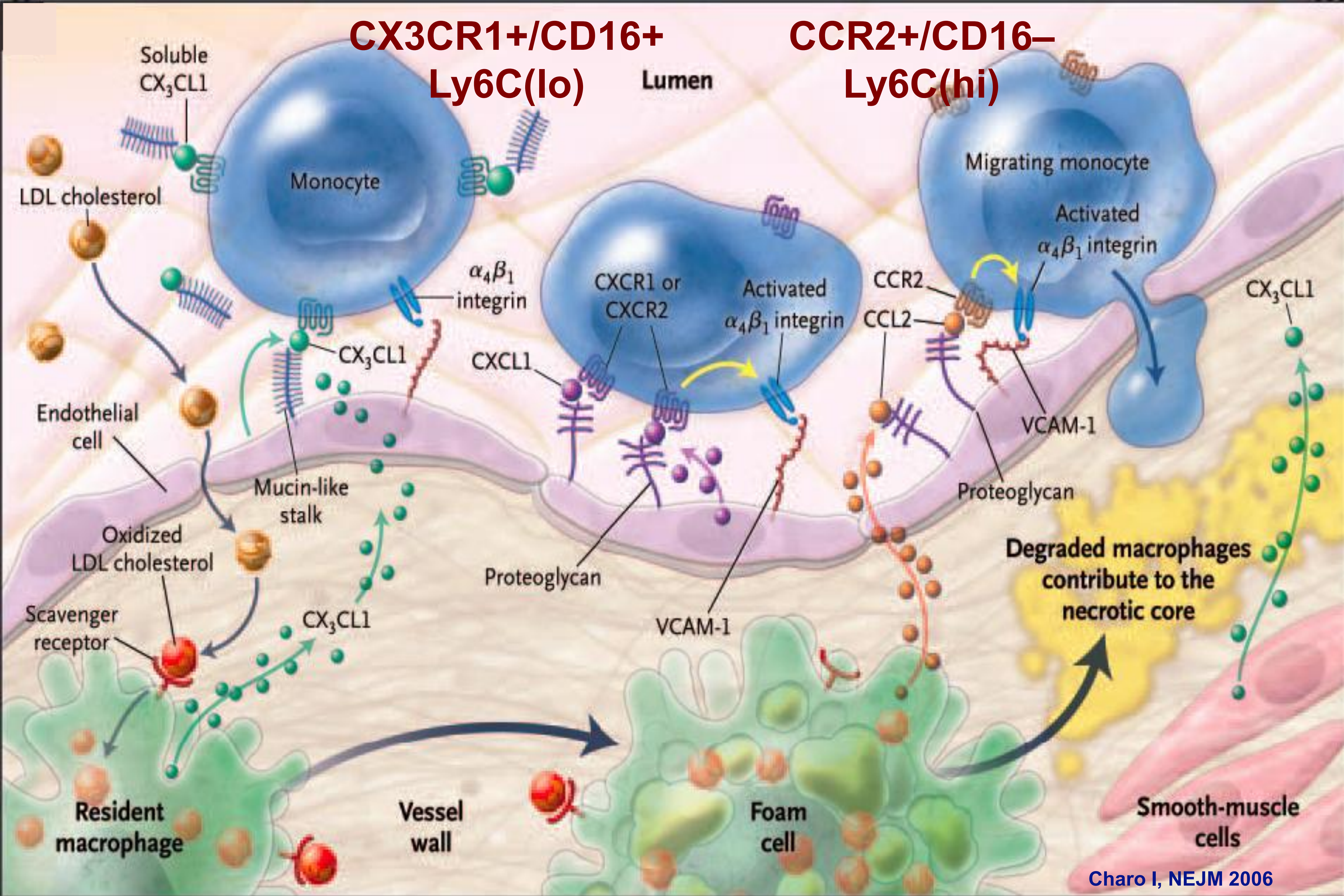



Cros J et al, Immunity 2010

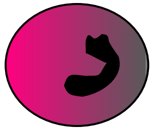
# *Nr4a1*-Dependent Ly6C$^{low}$ Monocytes Monitor Endothelial Cells and Orchestrate Their Disposal



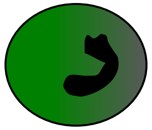

Carlin LM et al, Cell 2013


CX3CR1+/CD16+
Ly6C(lo)
CCR2+/CD16–
Ly6C(hi)
Lumen
Soluble
CX3CL1
LDL cholesterol
Monocyte
$\alpha_4\beta_1$
integrin
CX3CL1
Mucin-like
stalk
Endothelial
cell
Oxidized
LDL cholesterol
Scavenger
receptor
CX3CL1
CXCL1
CXCR1 or
CXCR2
Activated
$\alpha_4\beta_1$ integrin
Proteoglycan
VCAM-1
Migrating monocyte
Activated
$\alpha_4\beta_1$ integrin
CCR2
CCL2
VCAM-1
Proteoglycan
CX3CL1
Degraded macrophages
contribute to the
necrotic core
Resident
macrophage
Vessel
wall
Foam
cell
Smooth-muscle
cells
Charo I, NEJM 2006

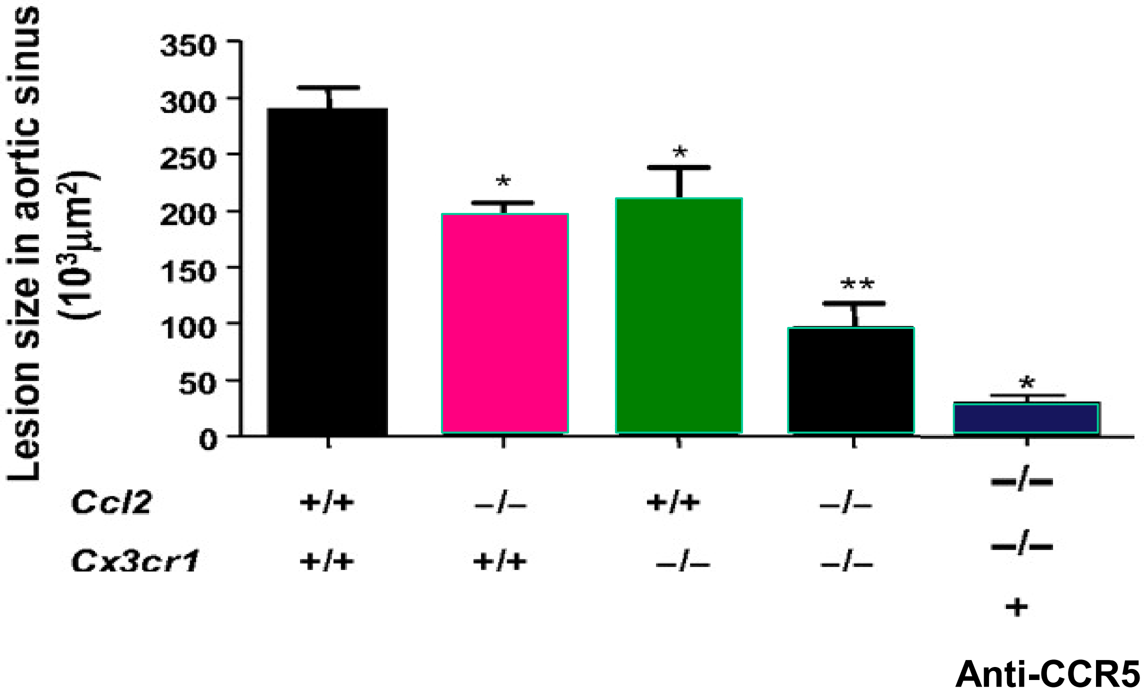
# Additive roles for monocyte subsets in atherosclerosis



**Classical monocytes**
**CCR2$^{hi}$**
CX3CR1$^{lo}$
CCR5$^{+}$



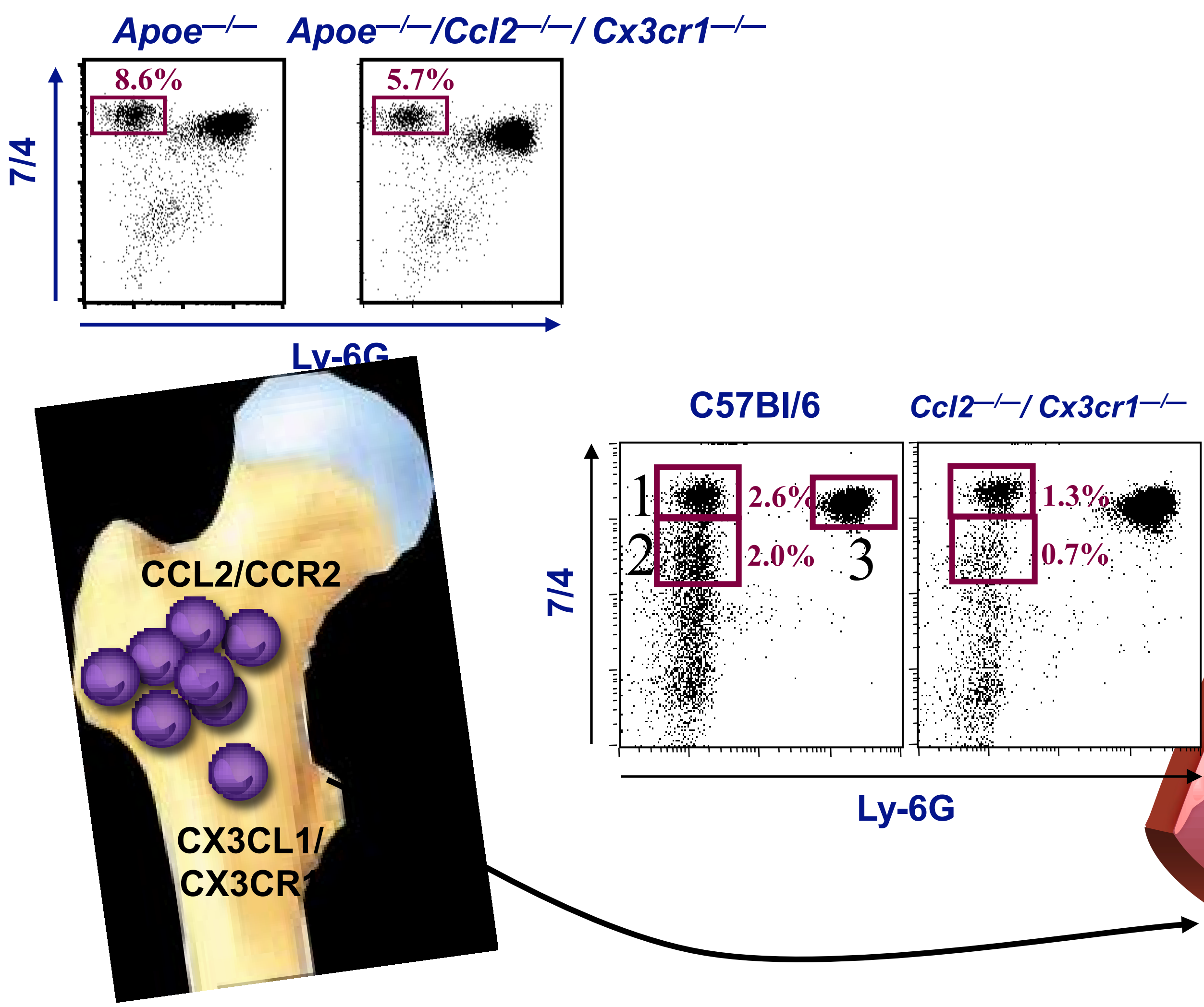
**Non classical monocytes**
CCR2$^{lo}$
**CX3CR1$^{hi}$**
**CCR5$^{+}$**

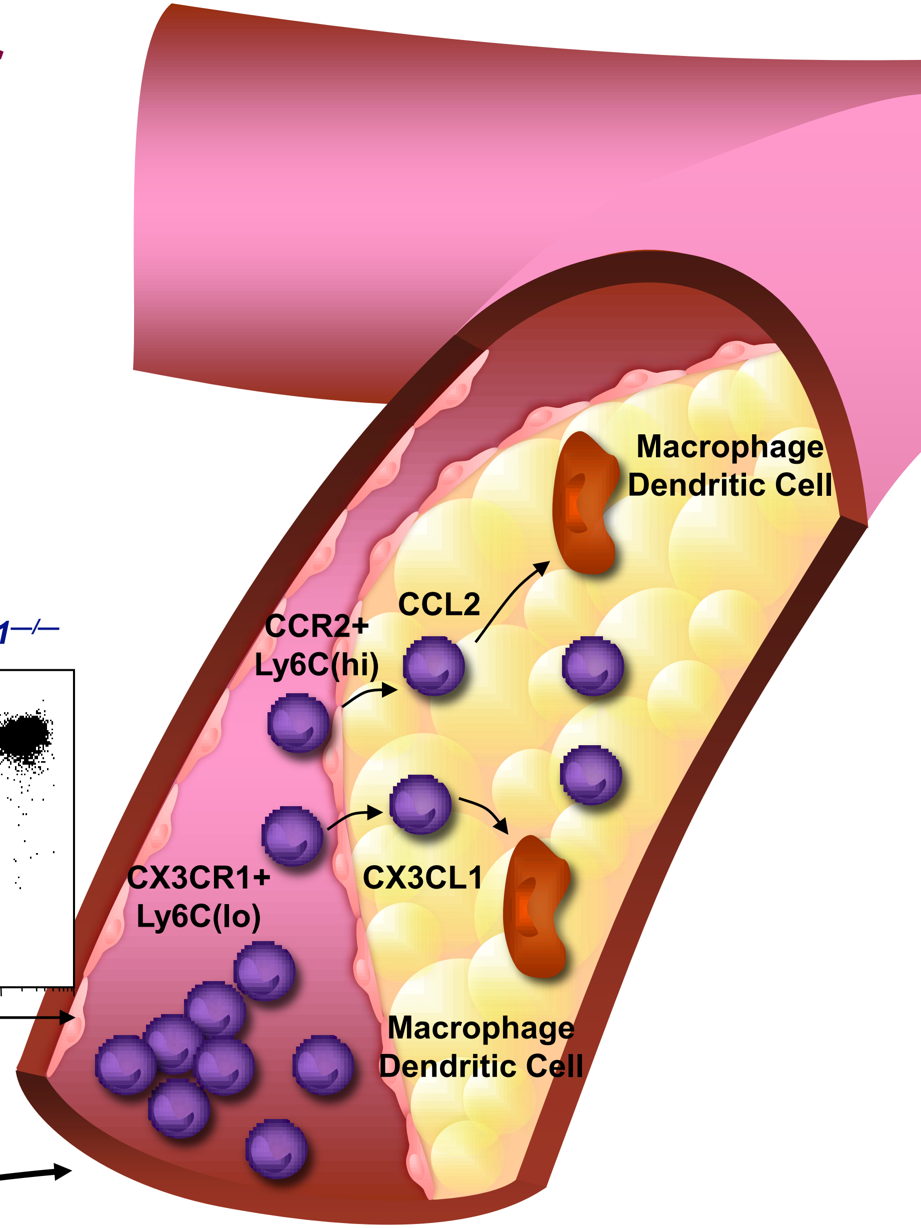



(Combadière et al, *Circulation* 2008)

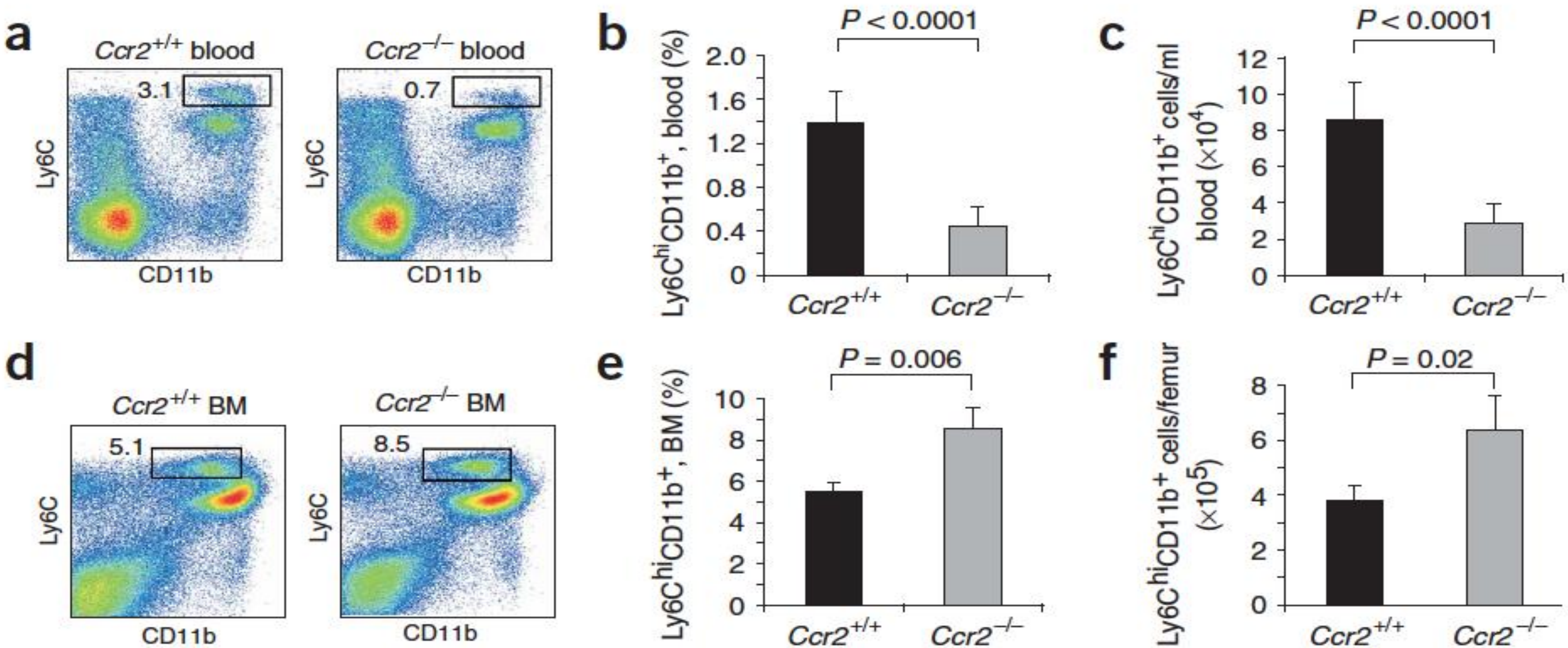
# Chemokines and Atherosclerosis

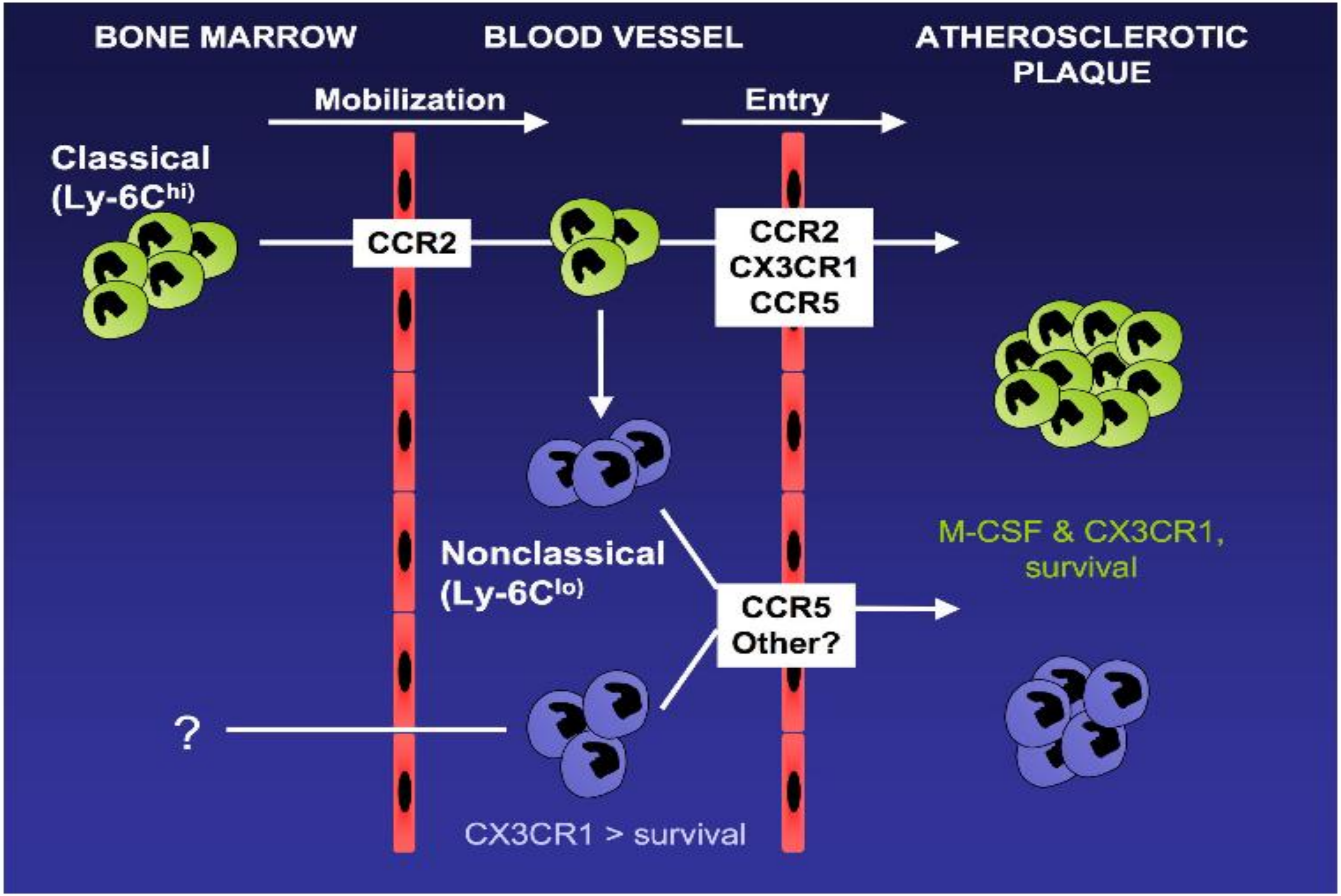
## Control of Monocyte Number in Blood and Bone Marrow



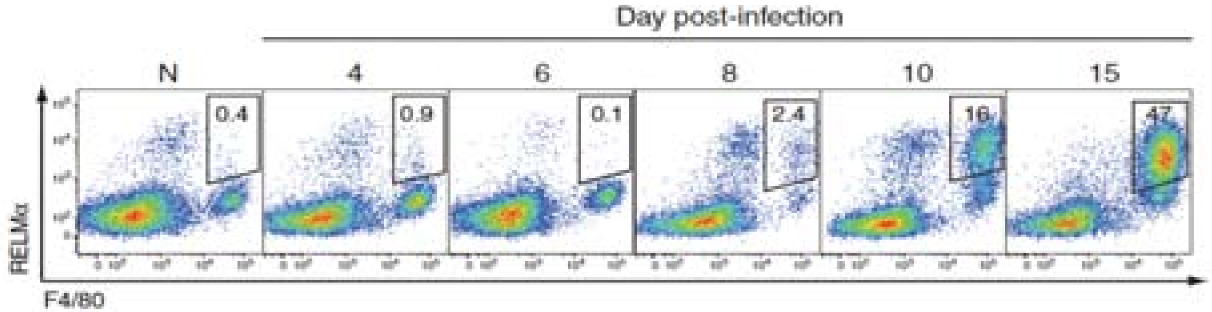
**Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2** (Serbina MV, Nat Immunol 2006)

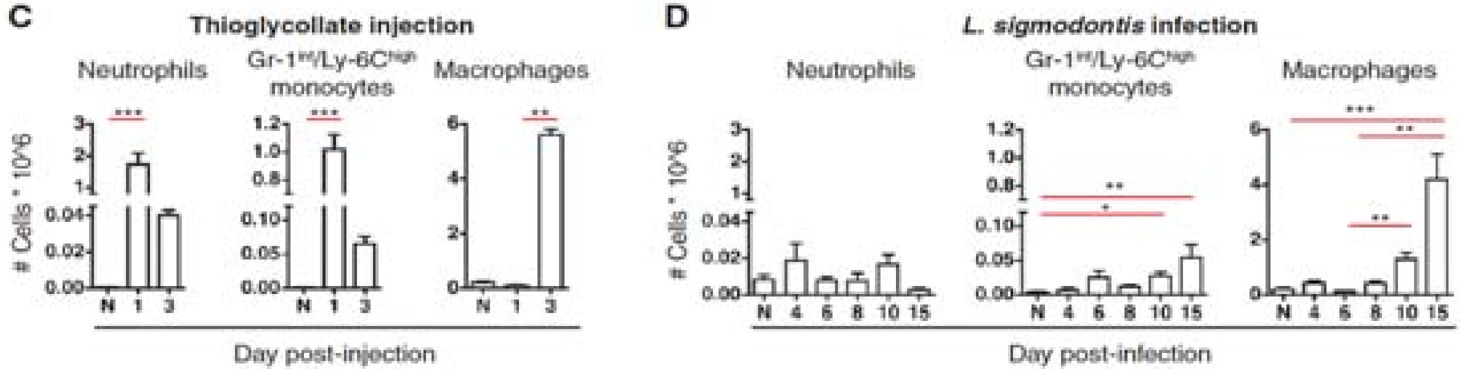


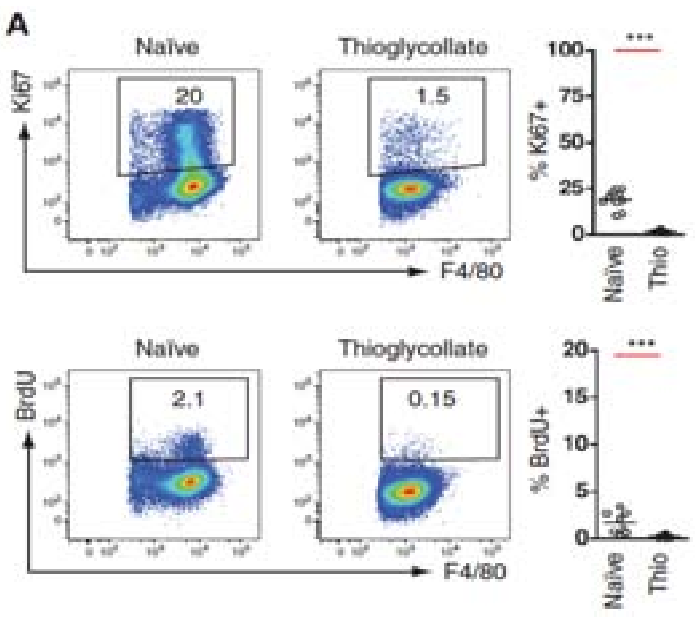

BONE MARROW
BLOOD VESSEL
ATHEROSCLEROTIC PLAQUE
Mobilization
Entry
Classical (Ly-6C^hi)
CCR2
CCR2
CX3CR1
CCR5
Nonclassical (Ly-6C^lo)
CCR5
Other?
M-CSF & CX3CR1, survival
?
CX3CR1 > survival

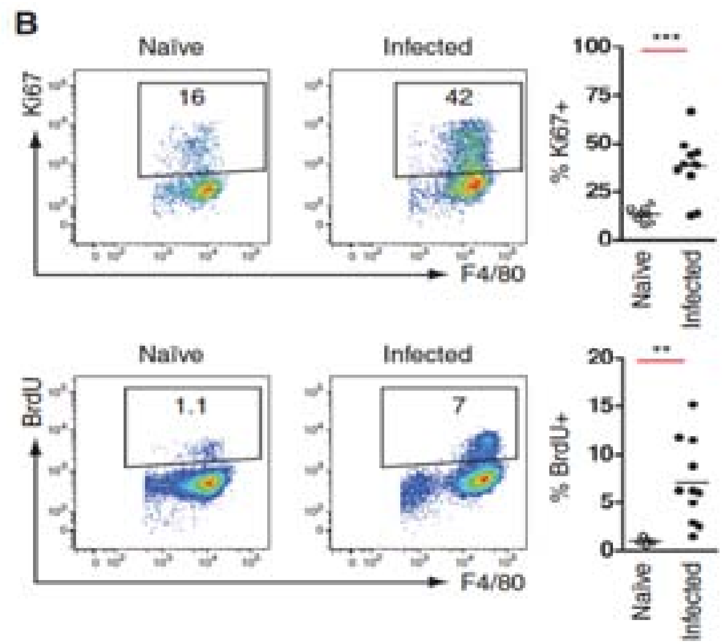
# Local Macrophage Proliferation, Rather than Recruitment from the Blood, Is a Signature of $T_H2$ Inflammation

Stephen J. Jenkins,[1] Dominik Ruckerl,[1] Peter C. Cook,[1] Lucy H. Jones,[1] Fred D. Finkelman,[2-5] Nico van Rooijen,[6] Andrew S. MacDonald,[1] Judith E. Allen[1]*

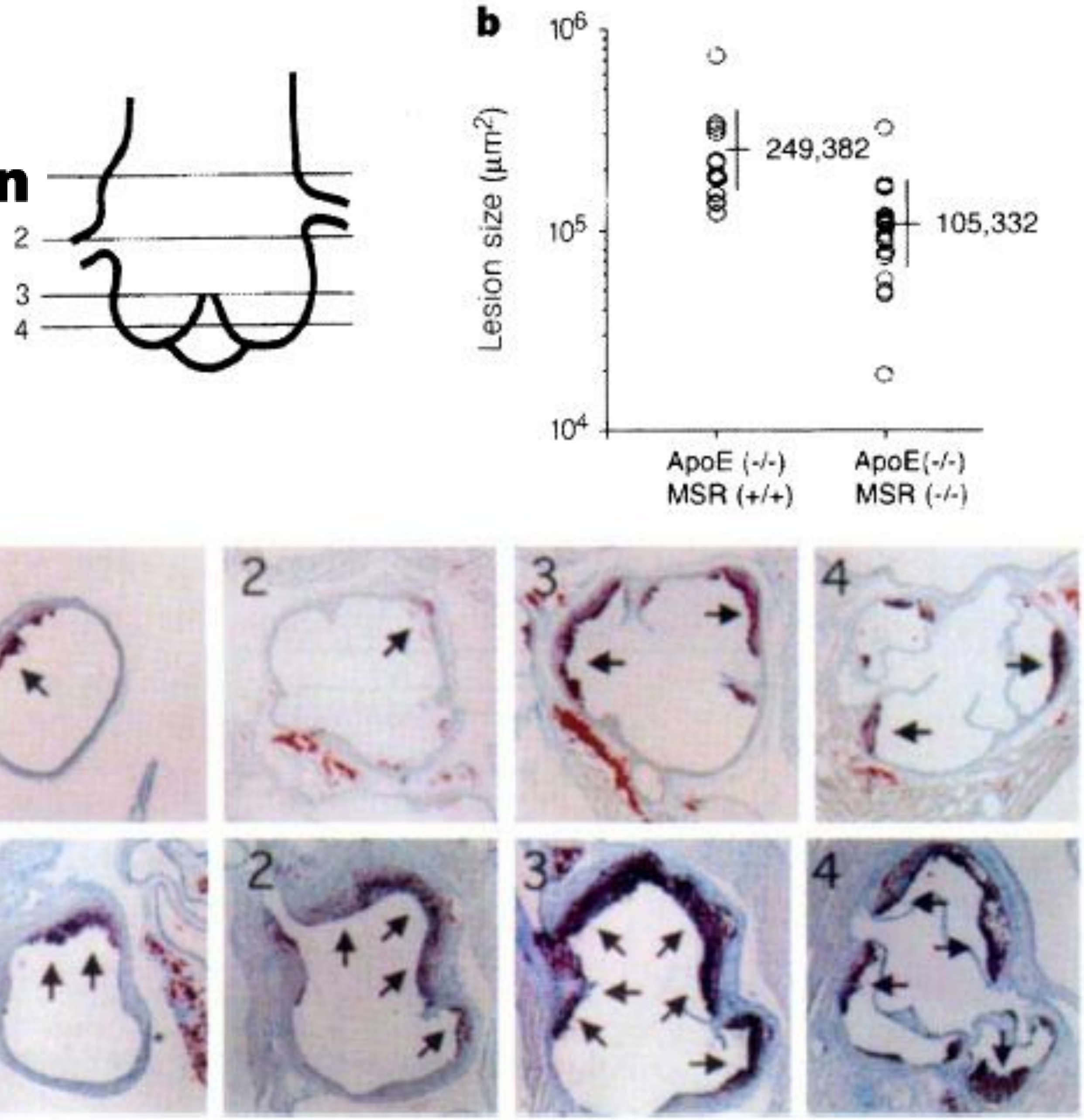


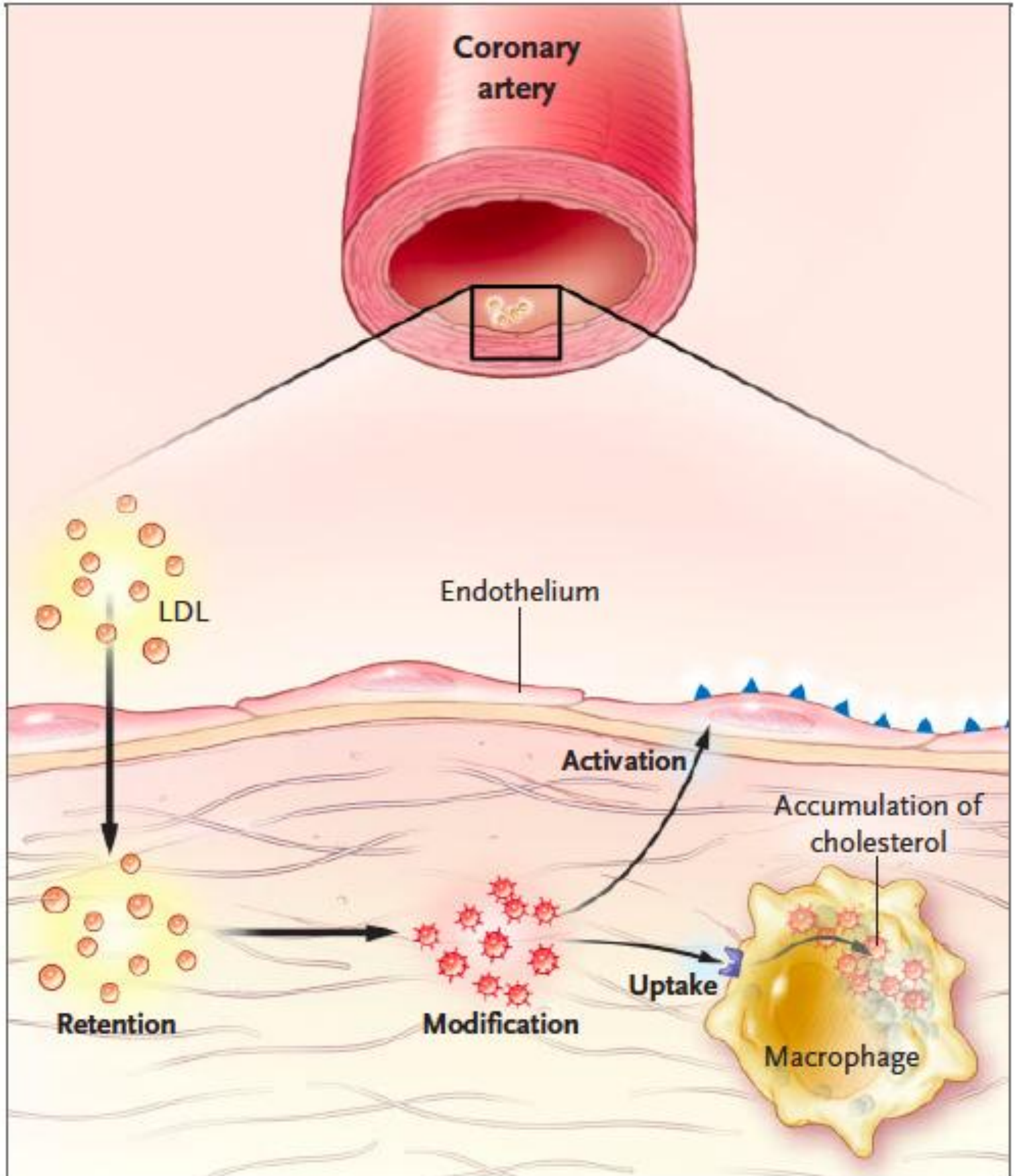



# A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection



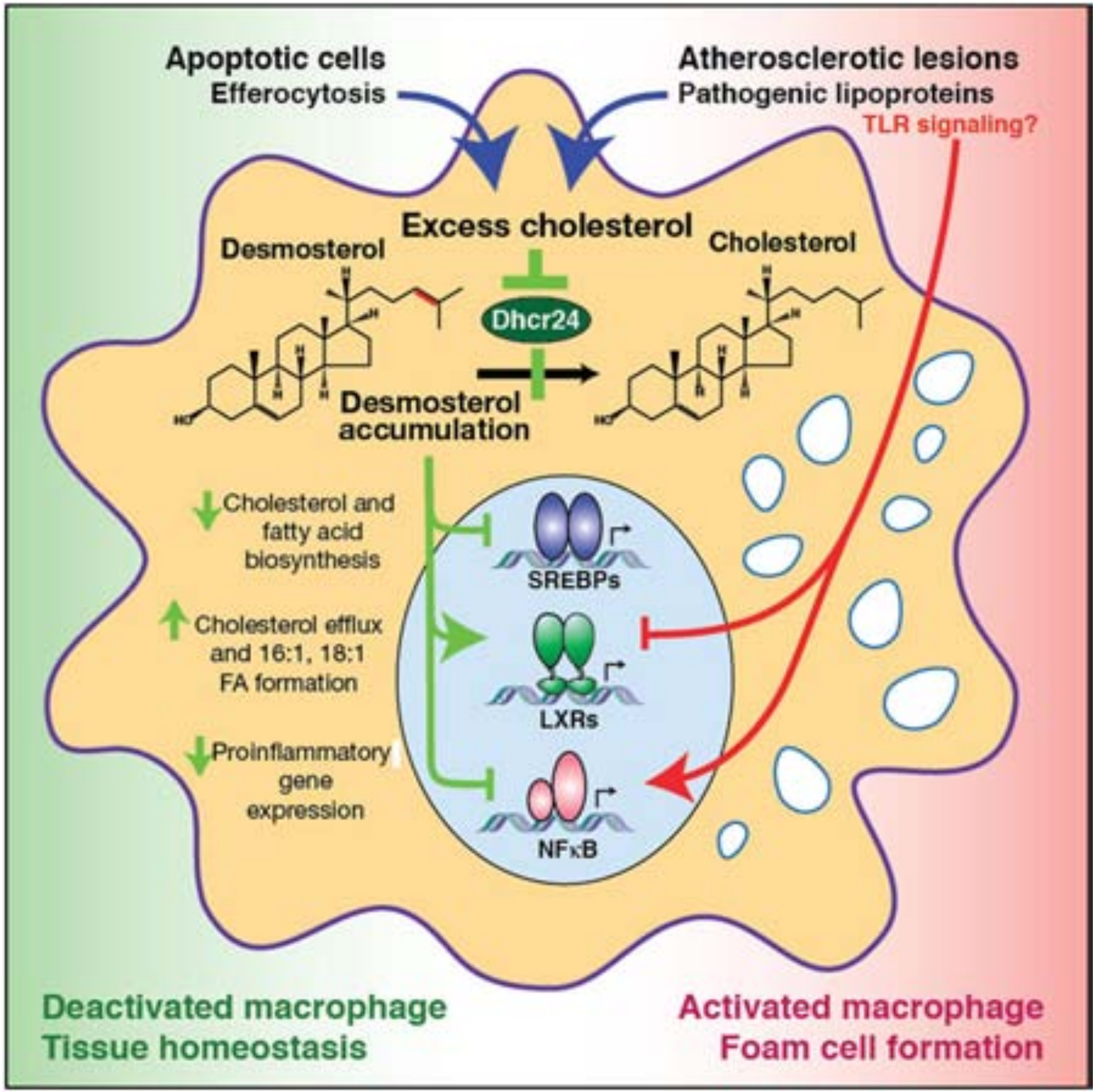

Suzuki H et al, Nature 1997



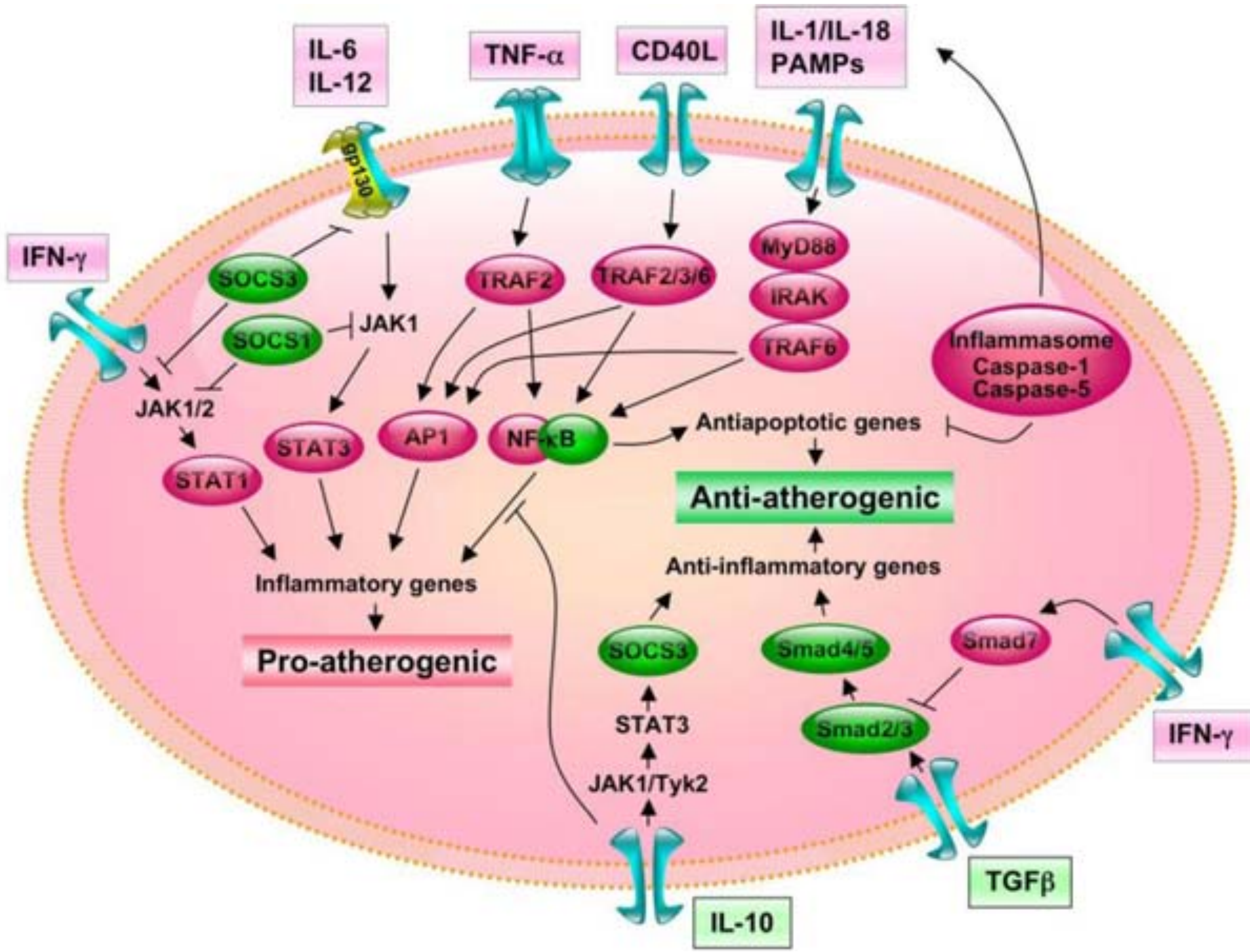

Hanson GK.
N Engl J Med 2005

# Regulated Accumulation of Desmosterol Integrates Macrophage Lipid Metabolism and Inflammatory Responses

Spann NJ et al. Cell 2012



# Pro & Anti-Atherogenic Signaling Pathways

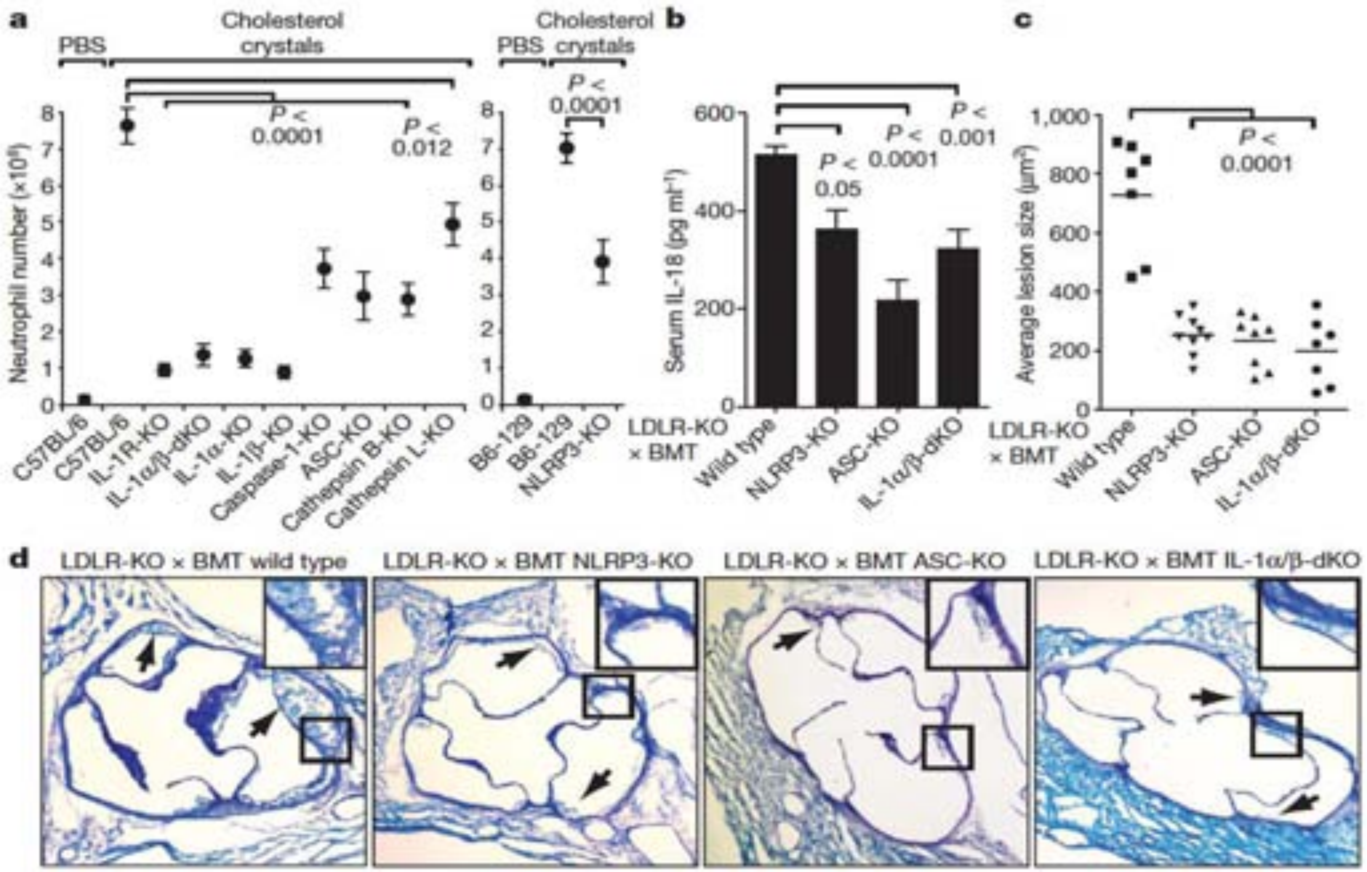



Tedgui & Mallat, Physiol Rev 2006

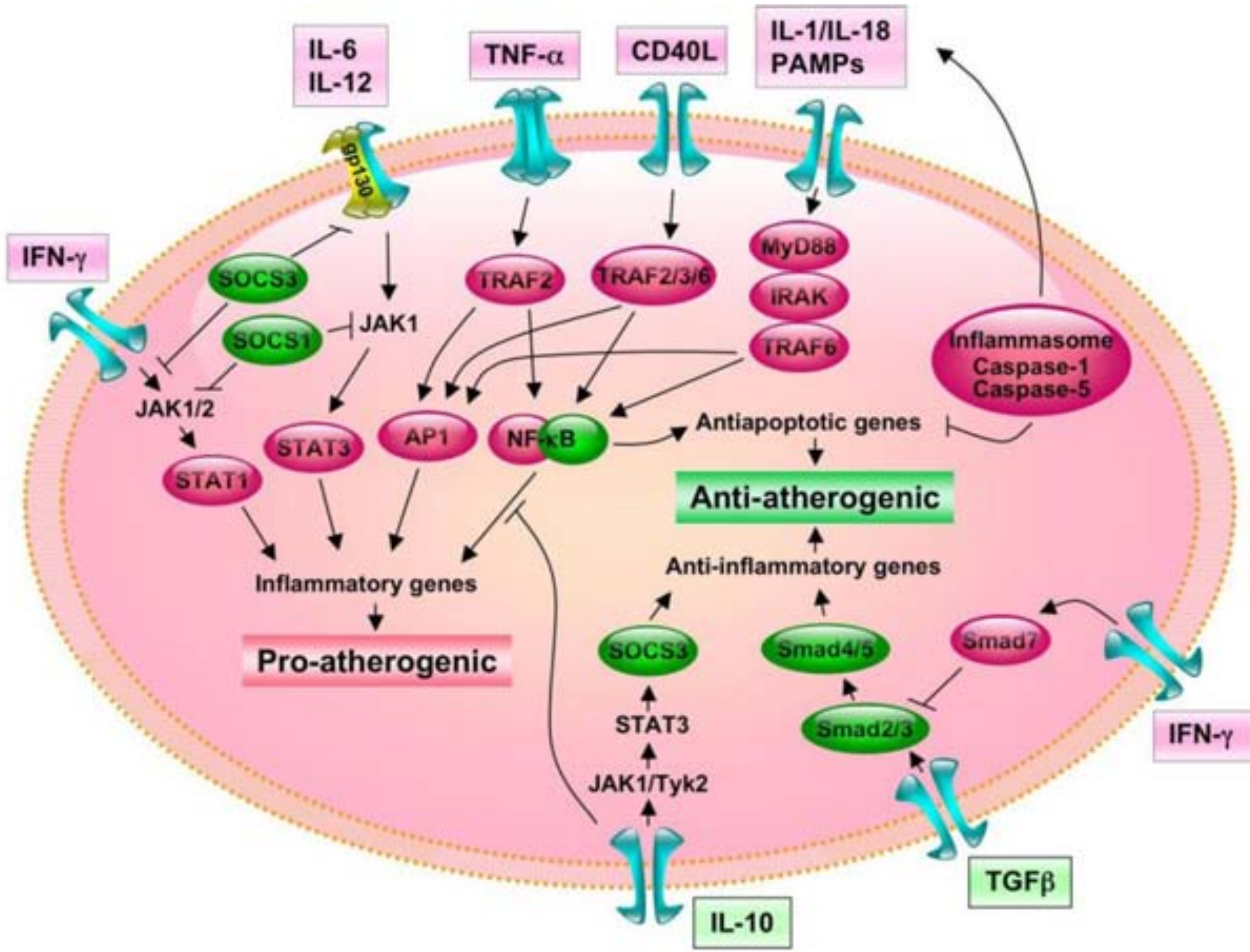
# NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals

nature

Peter Duewell[1,3]*, Hajime Kono[2]*, Katey J. Rayner[4,5], Cherilyn M. Sirois[1], Gregory Vladimer[1], Franz G. Bauernfeind[6], George S. Abela[8], Luigi Franchi[9], Gabriel Nuñez[9], Max Schnurr[3], Terje Espevik[10], Egil Lien[1], Katherine A. Fitzgerald[1], Kenneth L. Rock[2], Kathryn J. Moore[4,5], Samuel D. Wright[11], Veit Hornung[5]* & Eicke Latz[1,7,10]*



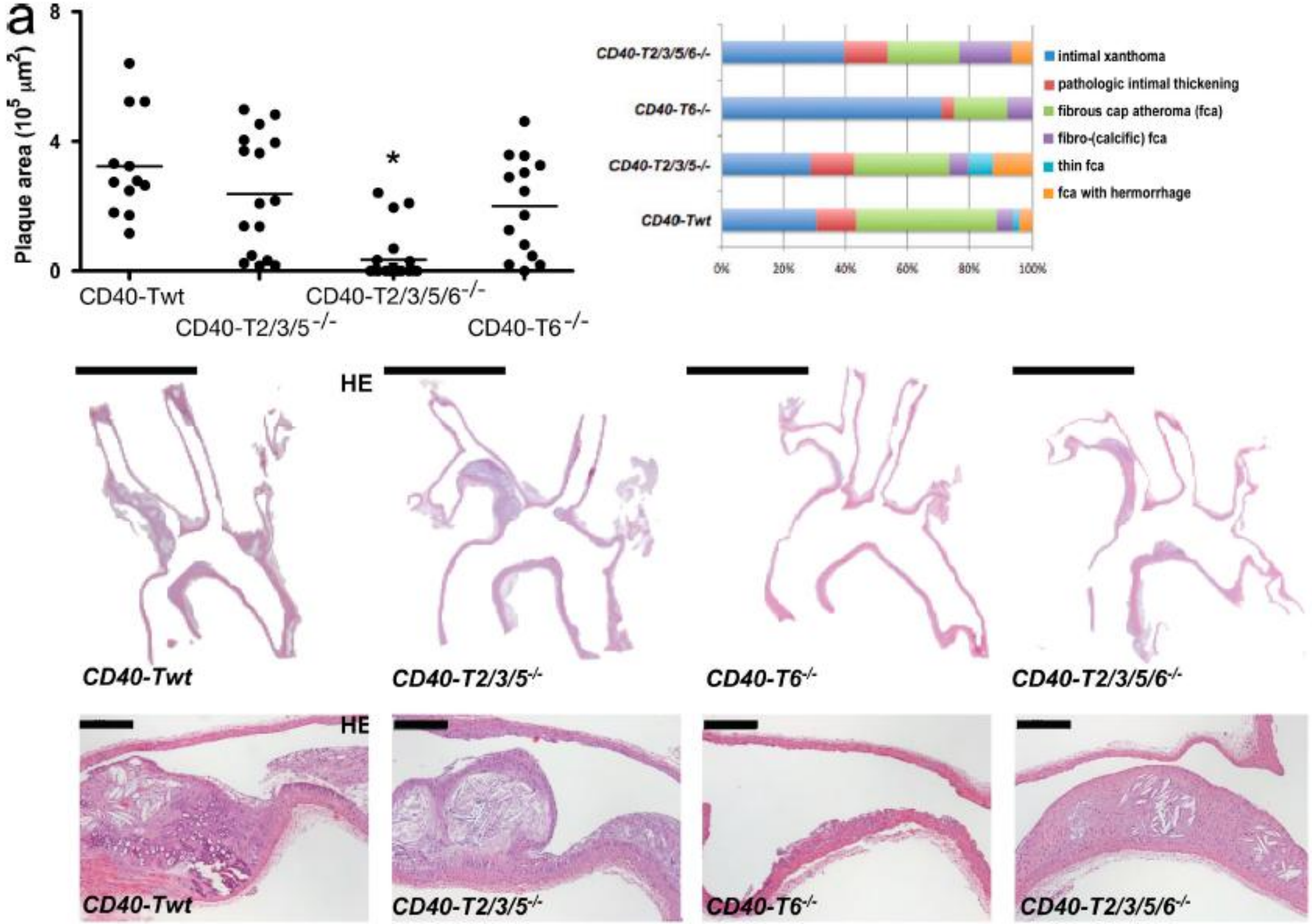
# Pro & Anti-Atherogenic Signaling Pathways

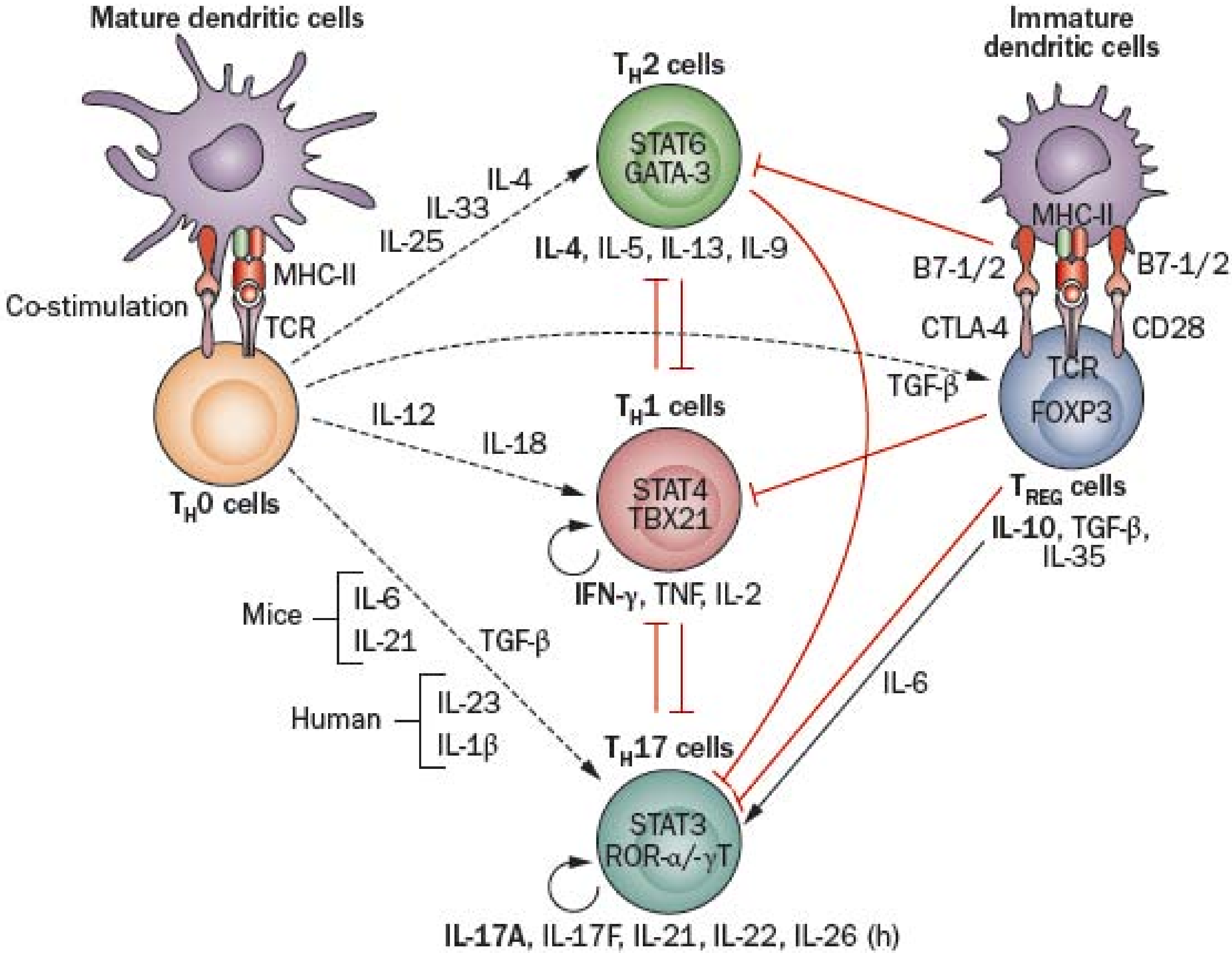



Tedgui & Mallat, Physiol Rev 2006

# Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile

Lutgens E et al,
J Exp Med 2010



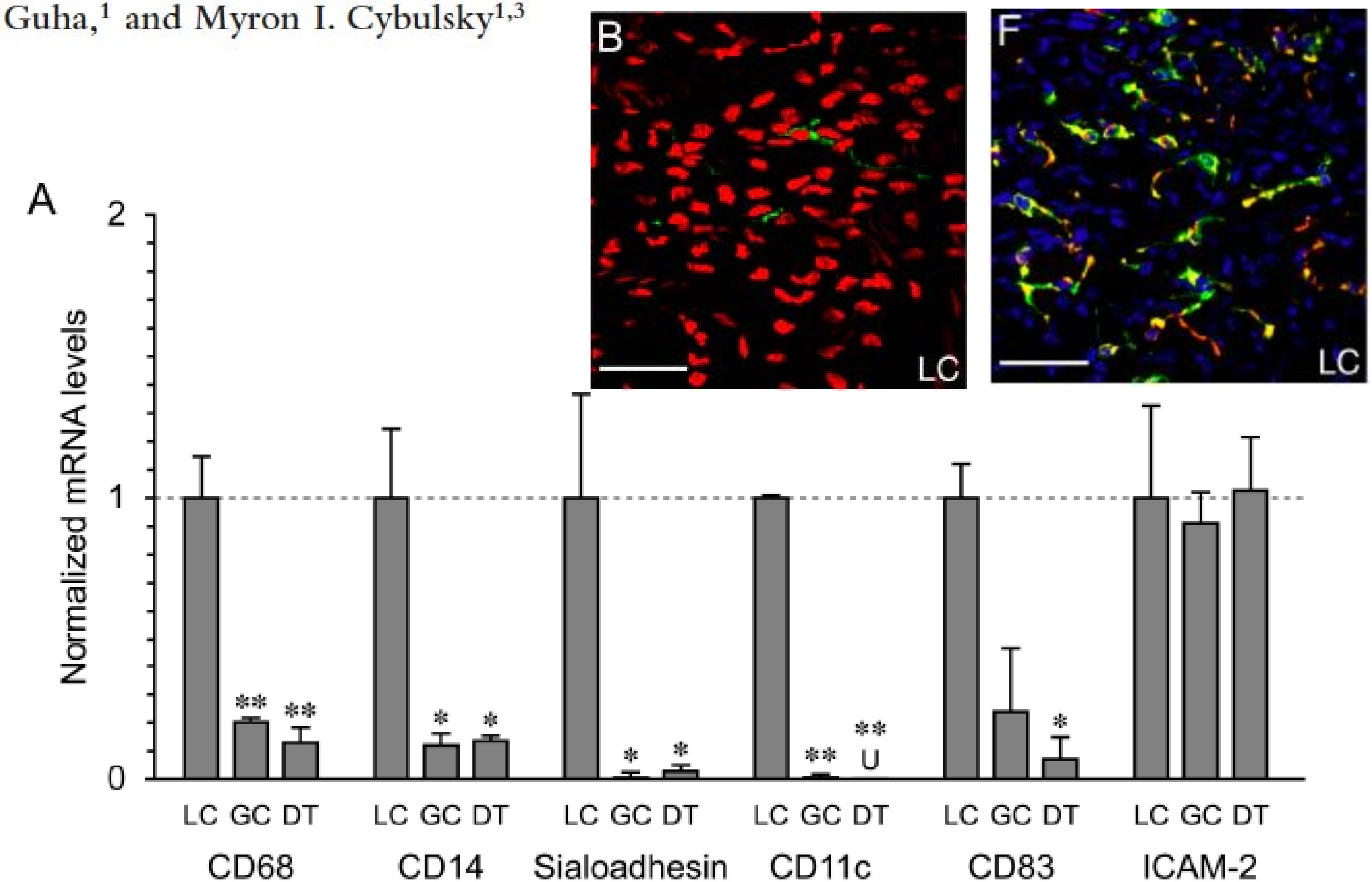



Lahoute C et al., Nat Rev Cardiol 2011

# Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis

JEM 2006

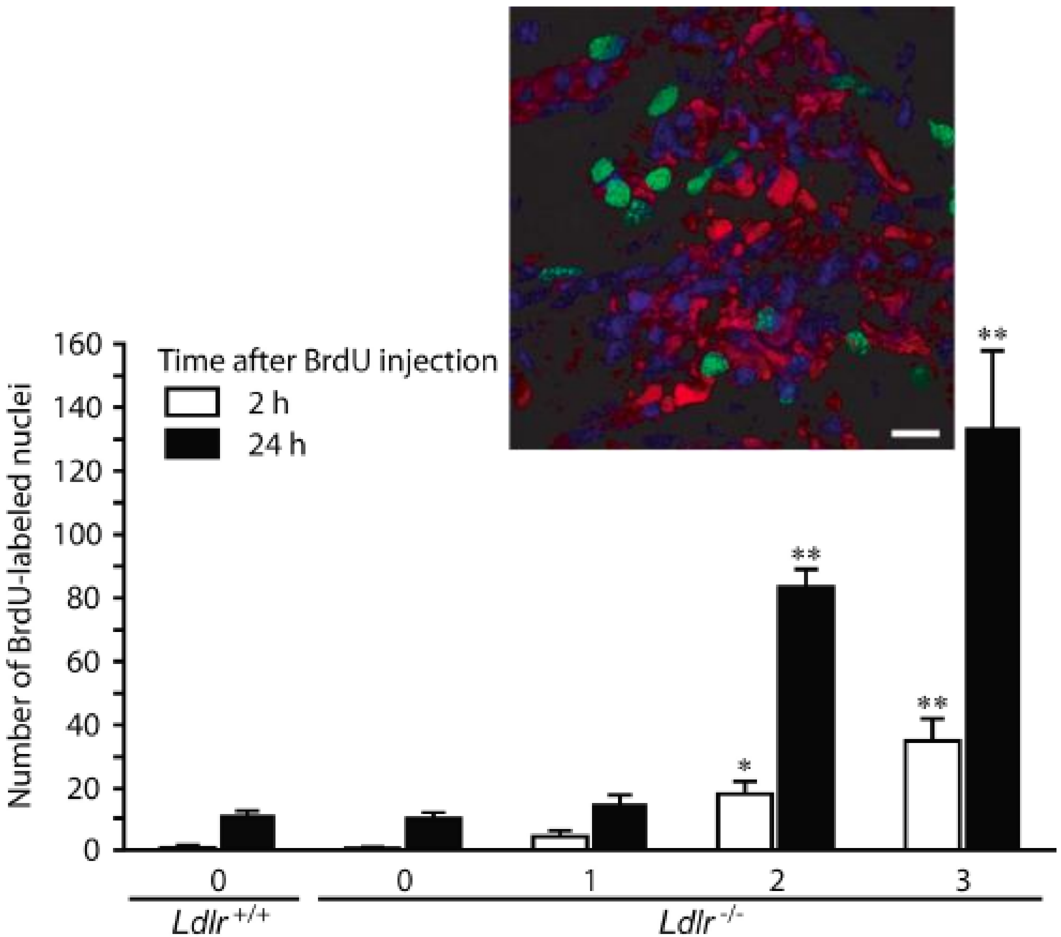
Jenny Jongstra-Bilen,[1,2] Mehran Haidari,[1,3] Su-Ning Zhu,[1,3] Mian Chen,[1,3]
Daipayan Guha,[1] and Myron I. Cybulsky[1,3]

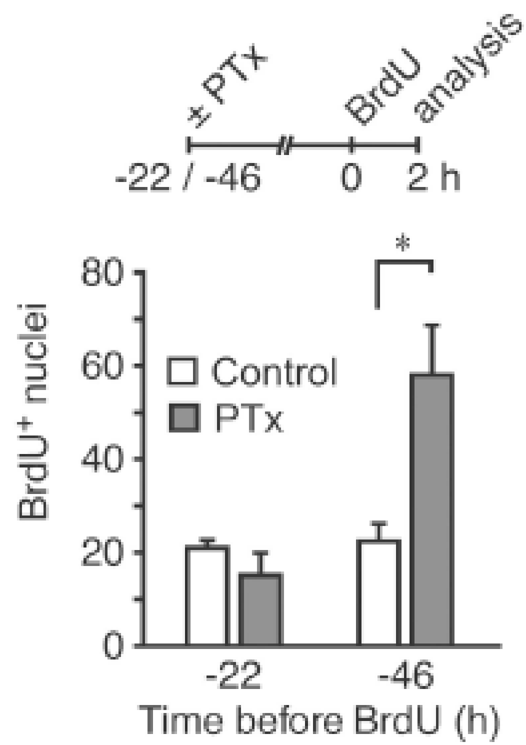


# GM-CSF regulates intimal cell proliferation in nascent atherosclerotic lesions

JEM 2009

Su-Ning Zhu,[1] Mian Chen,[1] Jenny Jongstra-Bilen,[1,2,3] and Myron I. Cybulsky[1,2]

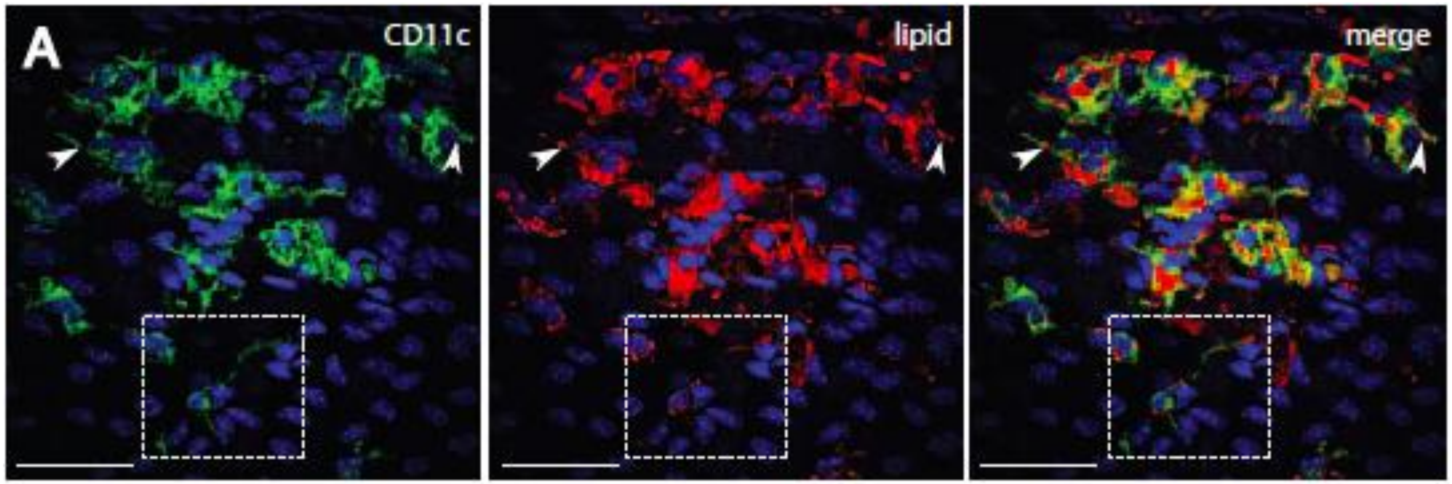


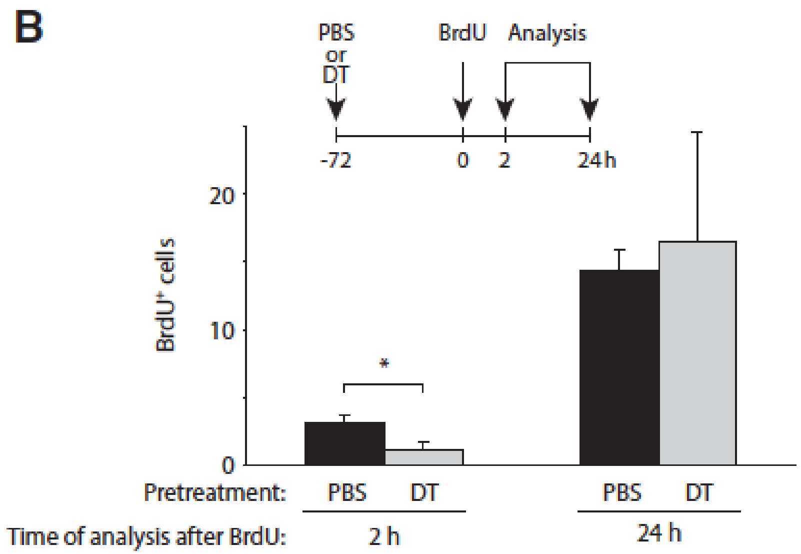



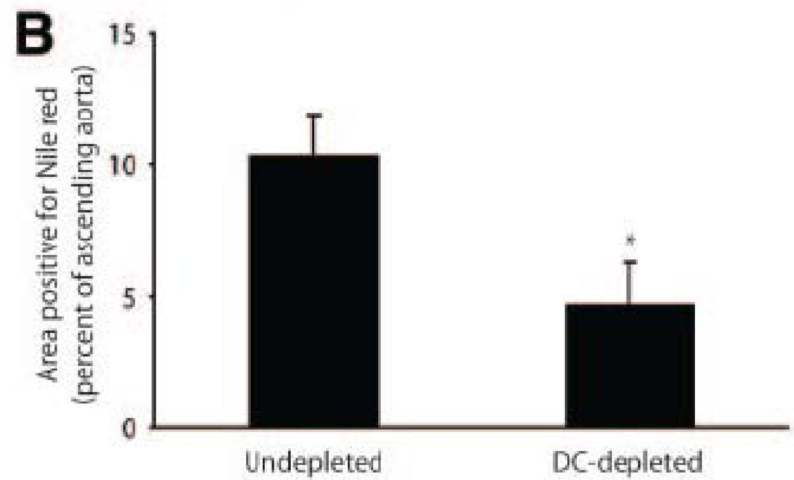
# Resident Intimal Dendritic Cells Accumulate Lipid and Contribute to the Initiation of Atherosclerosis

Kim E. Paulson, Su-Ning Zhu, Mian Chen, Sabrina Nurmohamed, Jenny Jongstra-Bilen, Myron I. Cybulsky

Circulation Research



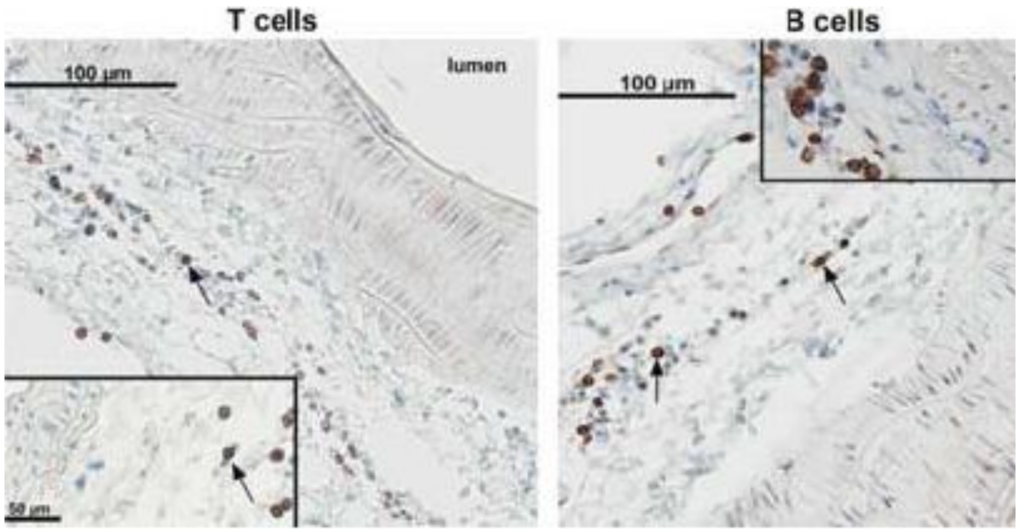



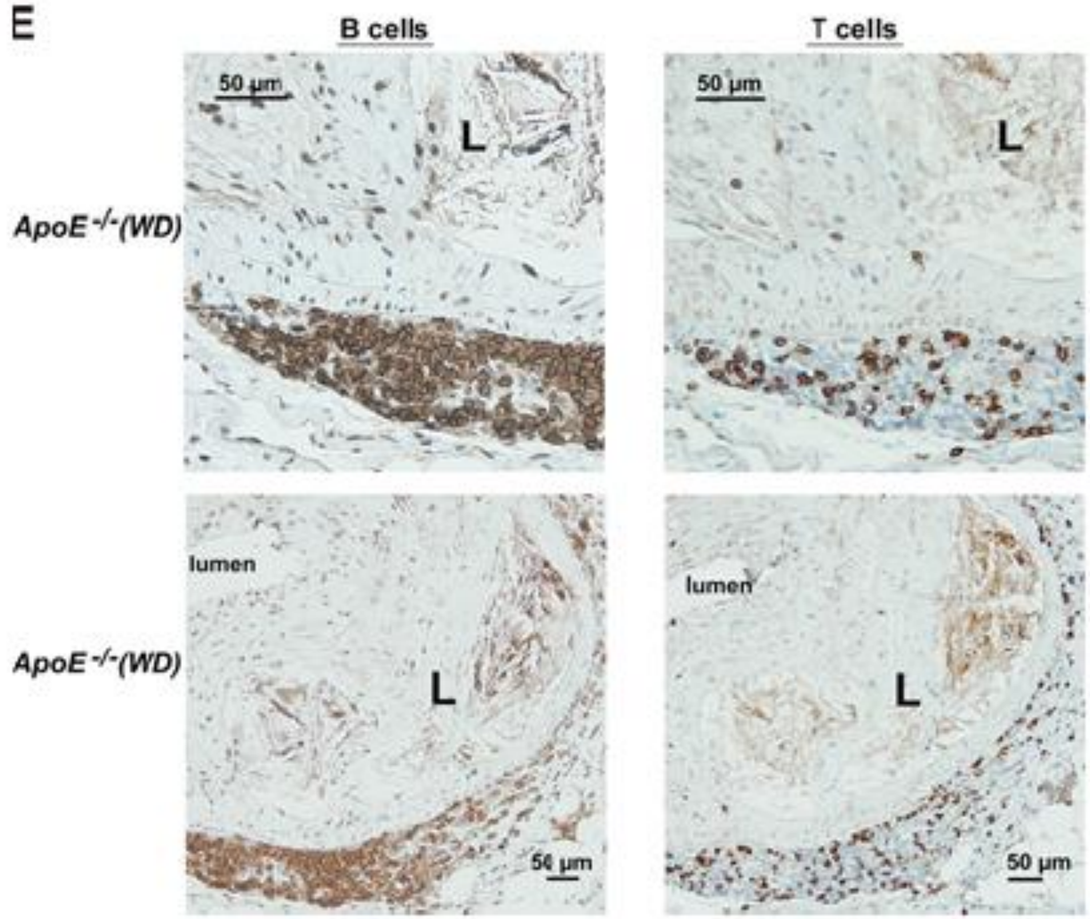



# Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent

JEM 2006

Elena Galkina,[1,4] Alexandra Kadl,[4] John Sanders,[3,4] Danielle Varughese,[4] Ian J. Sarembock,[3,4] and Klaus Ley[1,2,4]

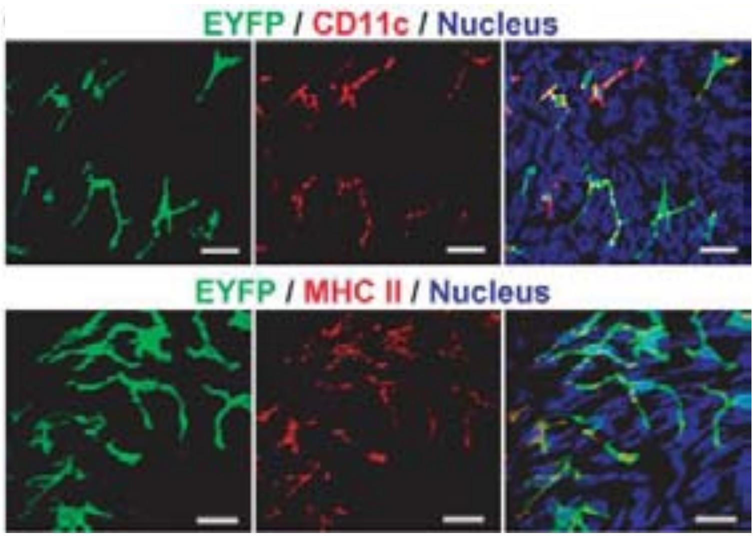


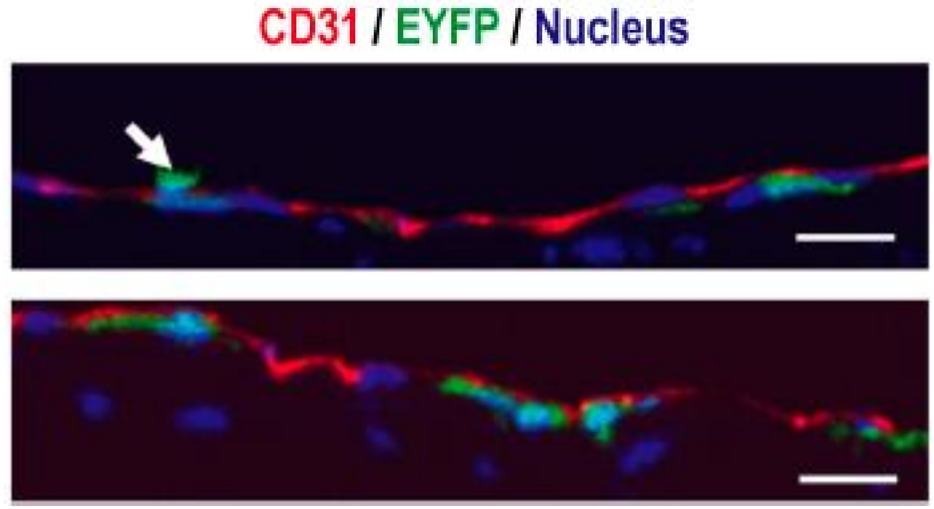



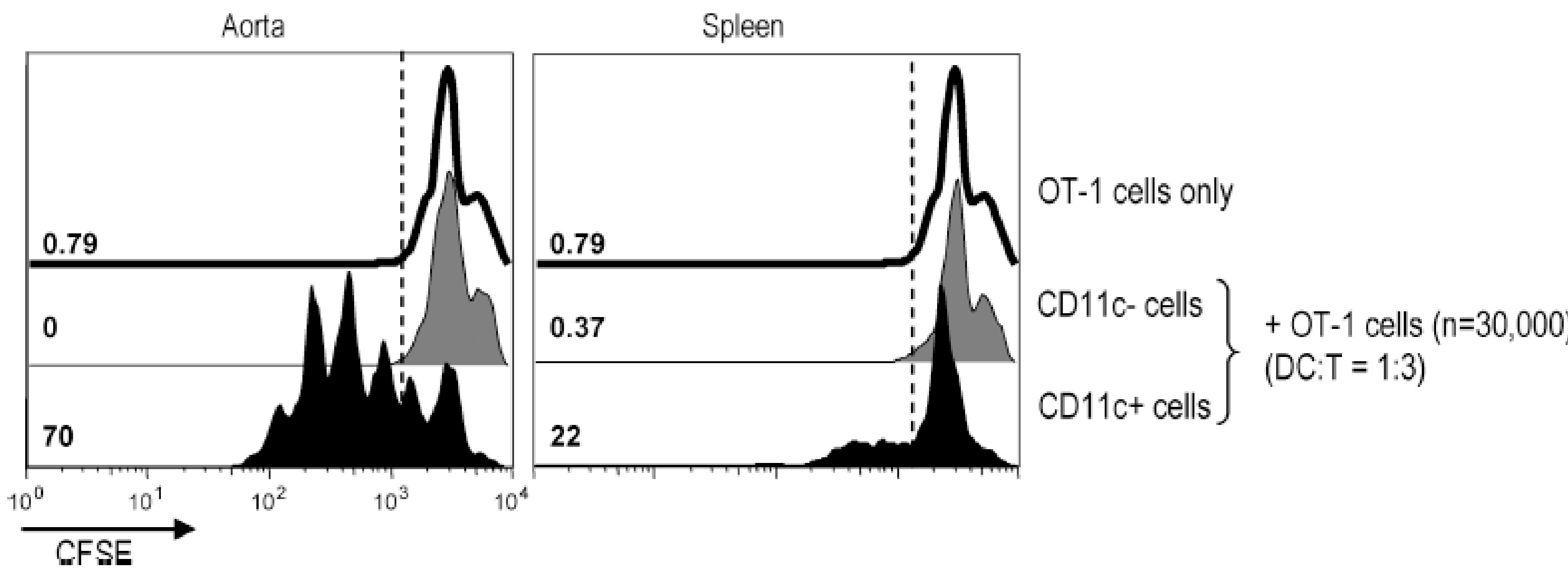
# Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves

JEM

Choi JH et al., 2006

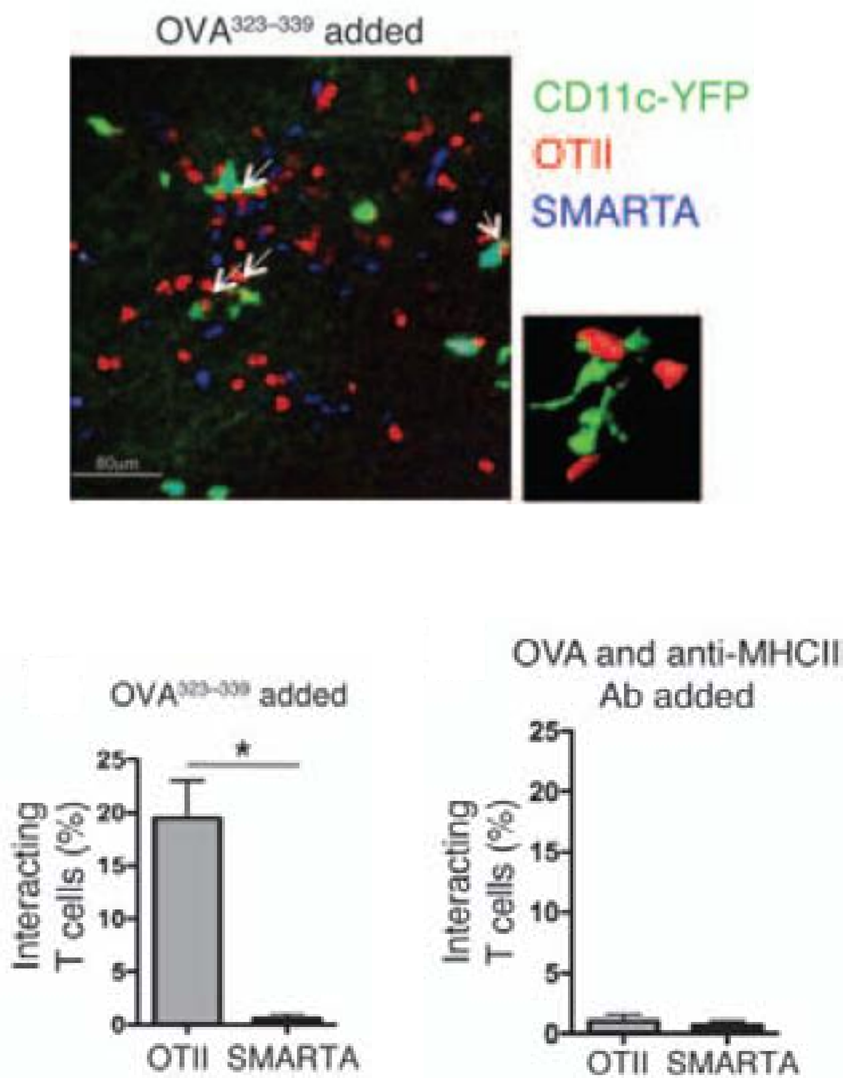


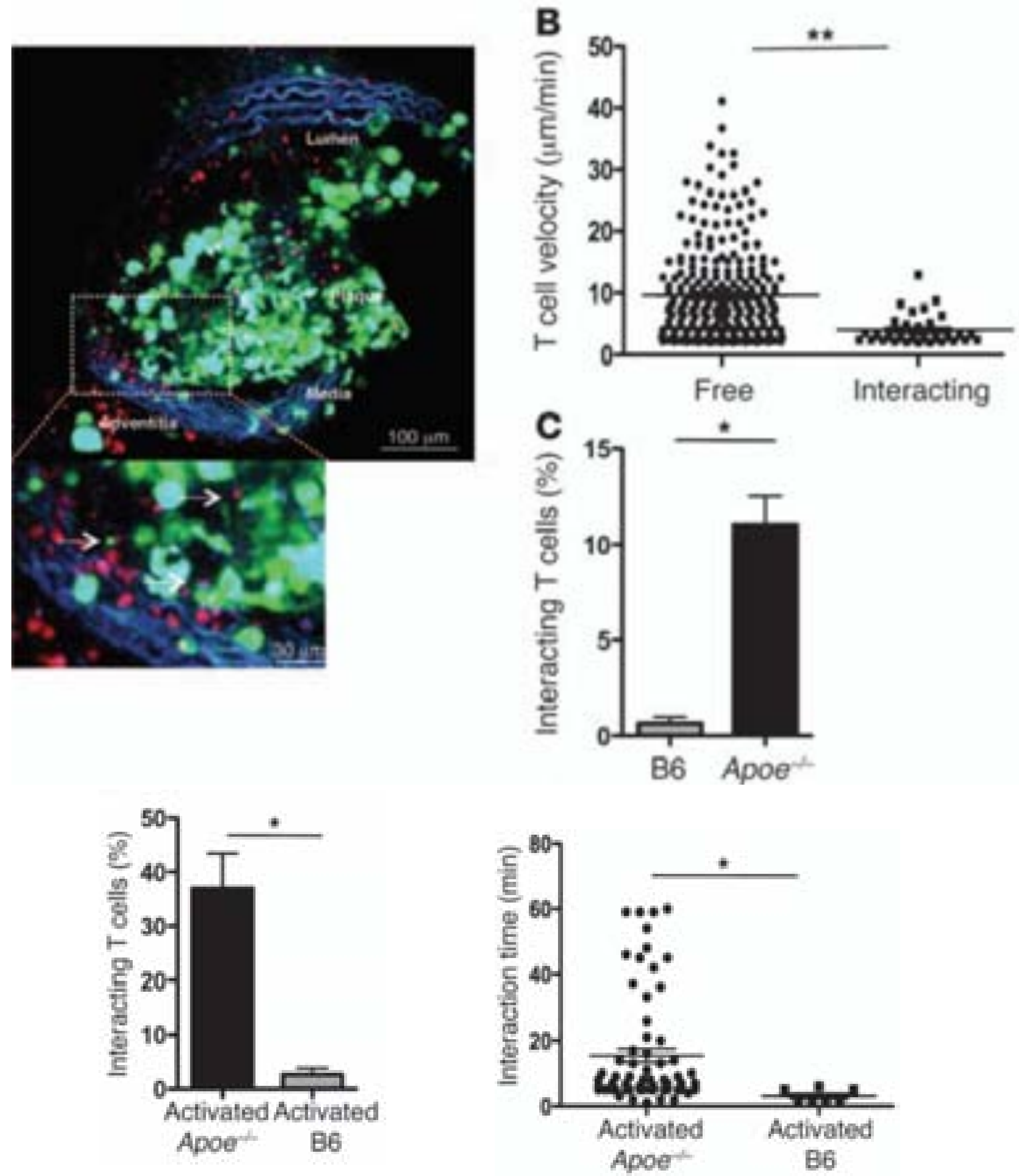



# Dynamic T cell–APC interactions sustain chronic inflammation in atherosclerosis

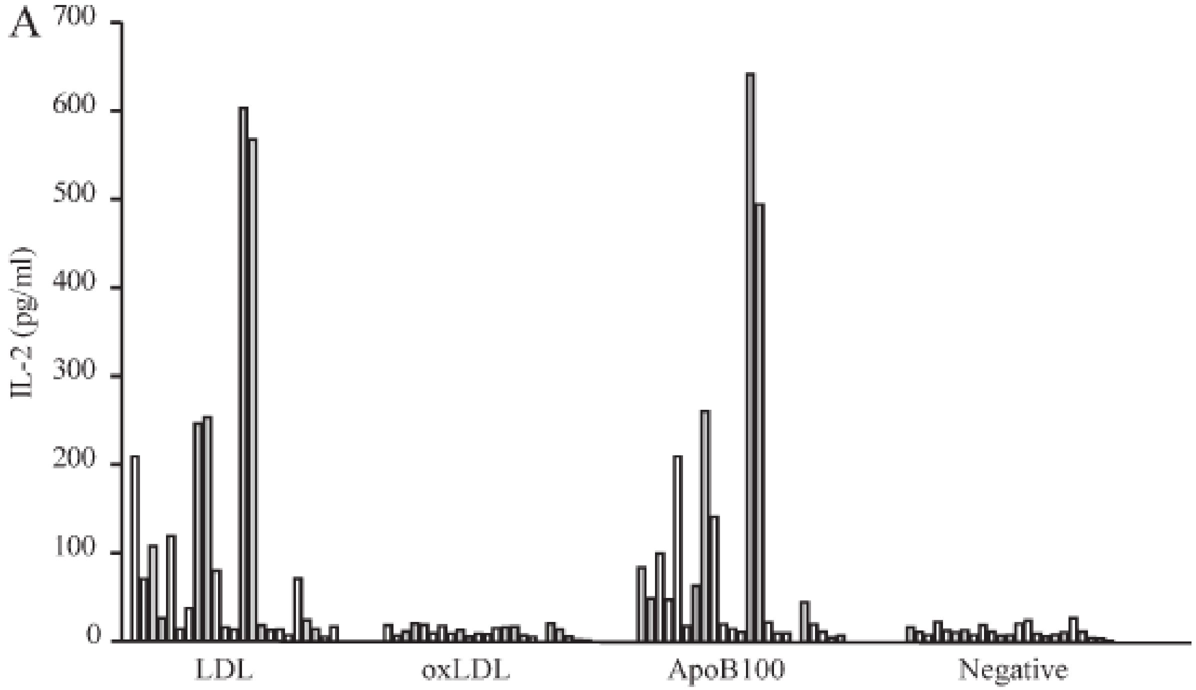
Koltsova EK et al., JCI 2012

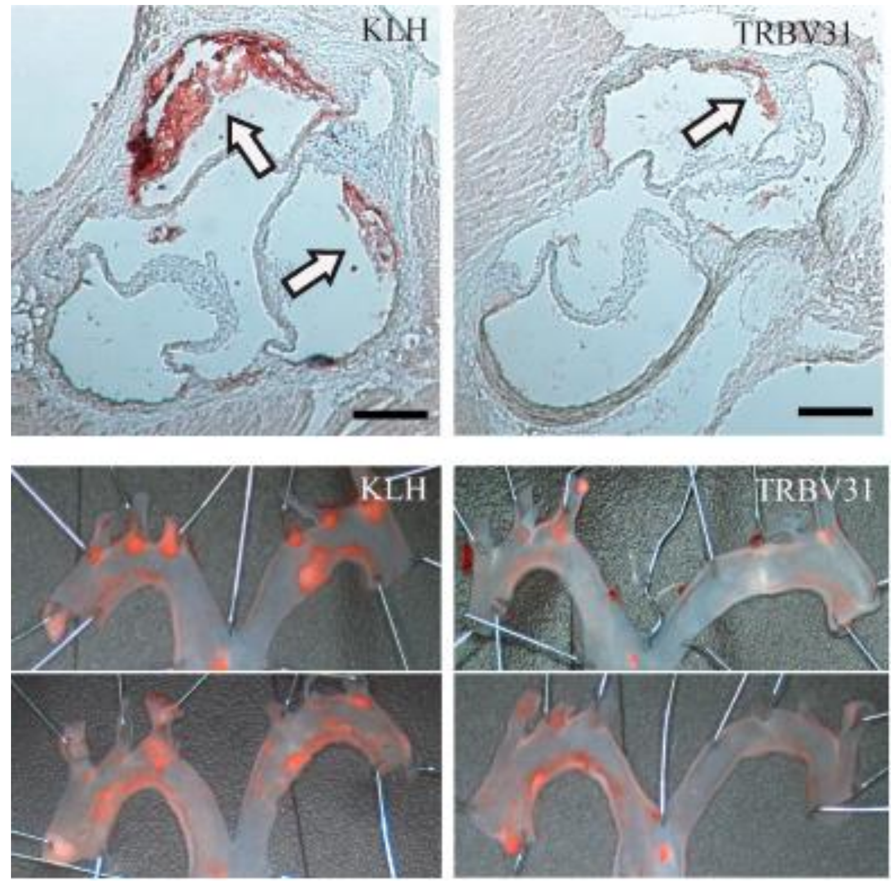


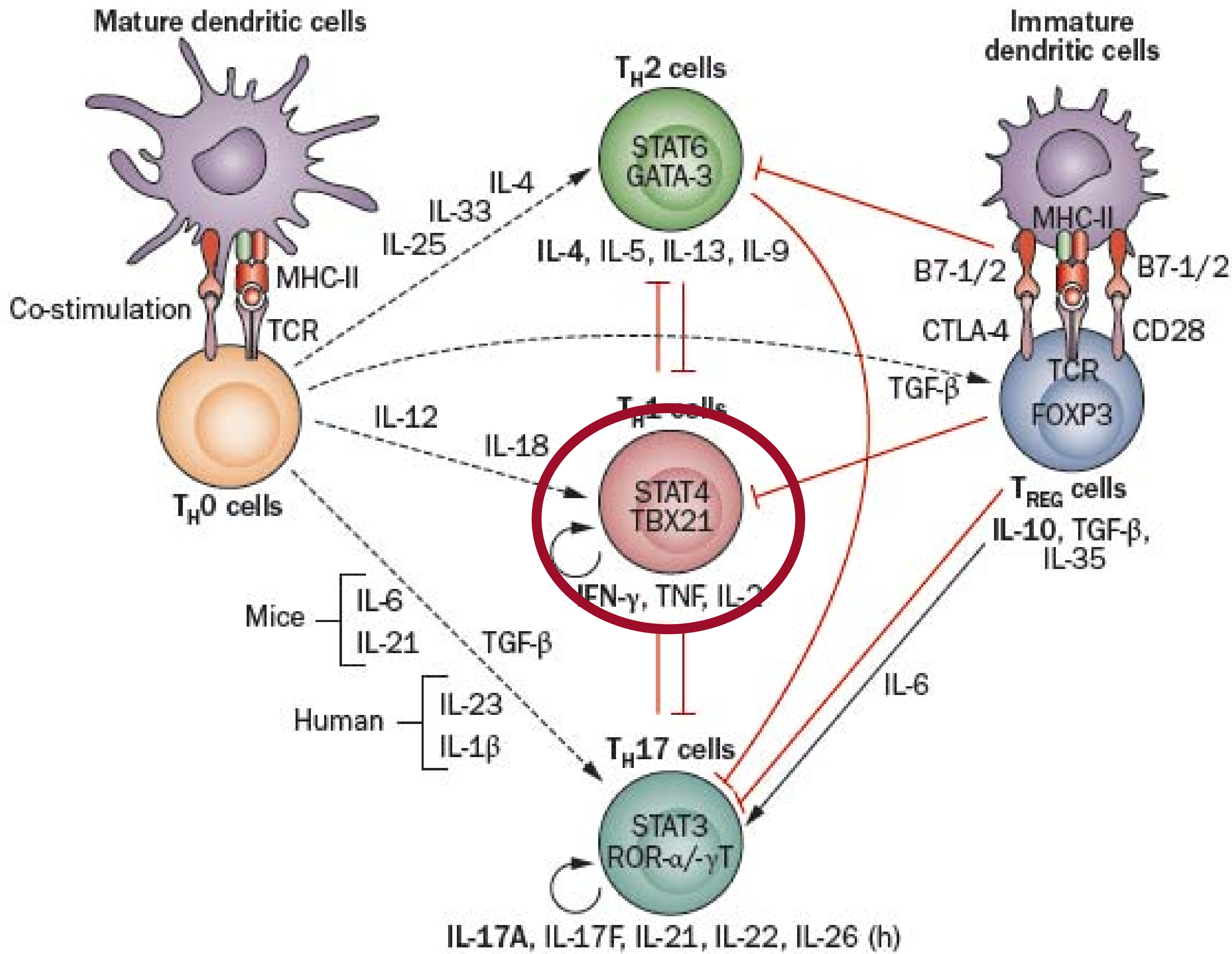



# Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis

Andreas Hermansson,[1] Daniel F.J. Ketelhuth,[1] Daniela Strodthoff,[1] Marion Wurm,[1] Emil M. Hansson,[2] Antonino Nicoletti,[3] Gabrielle Paulsson-Berne,[1] and Göran K. Hansson[1]

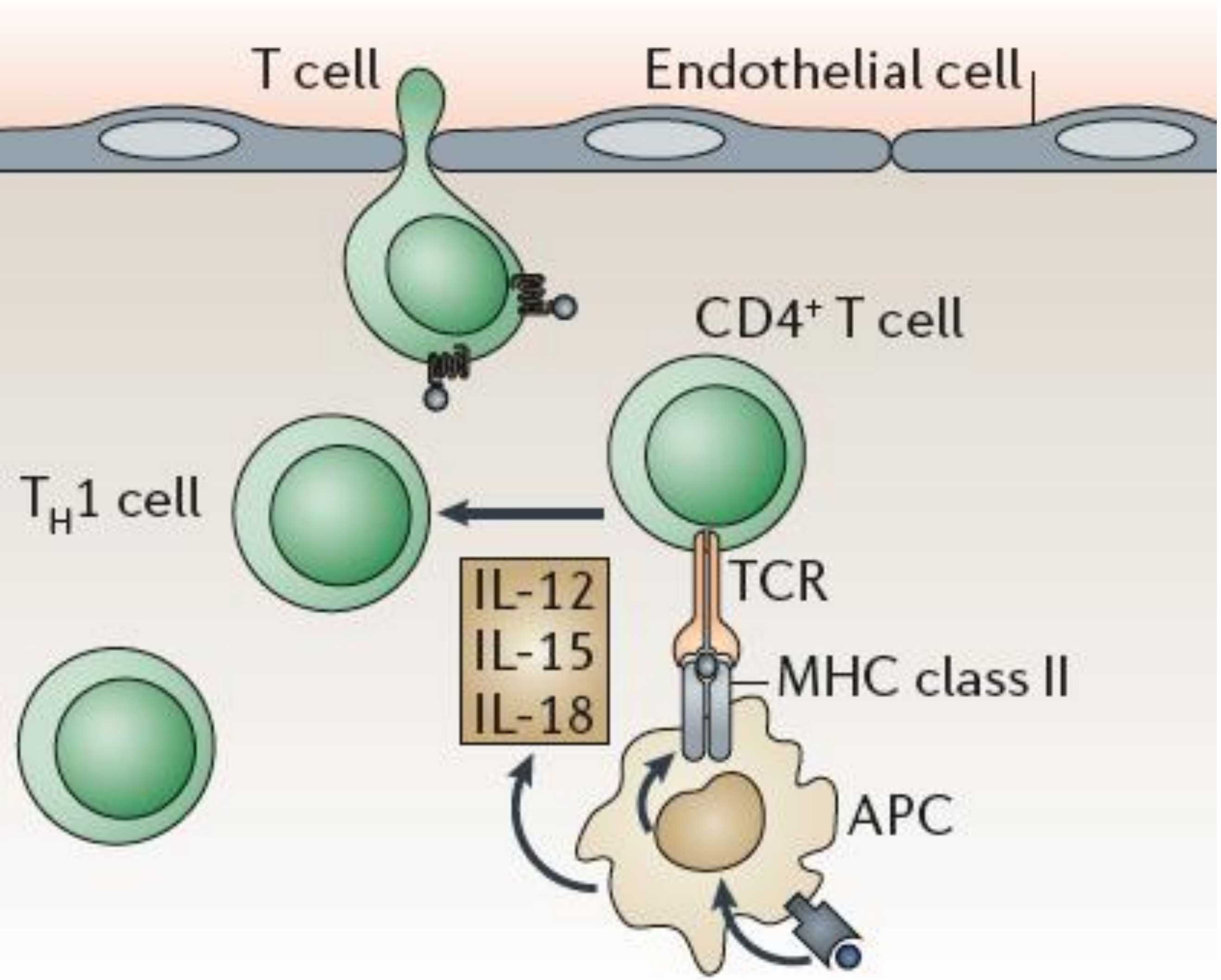


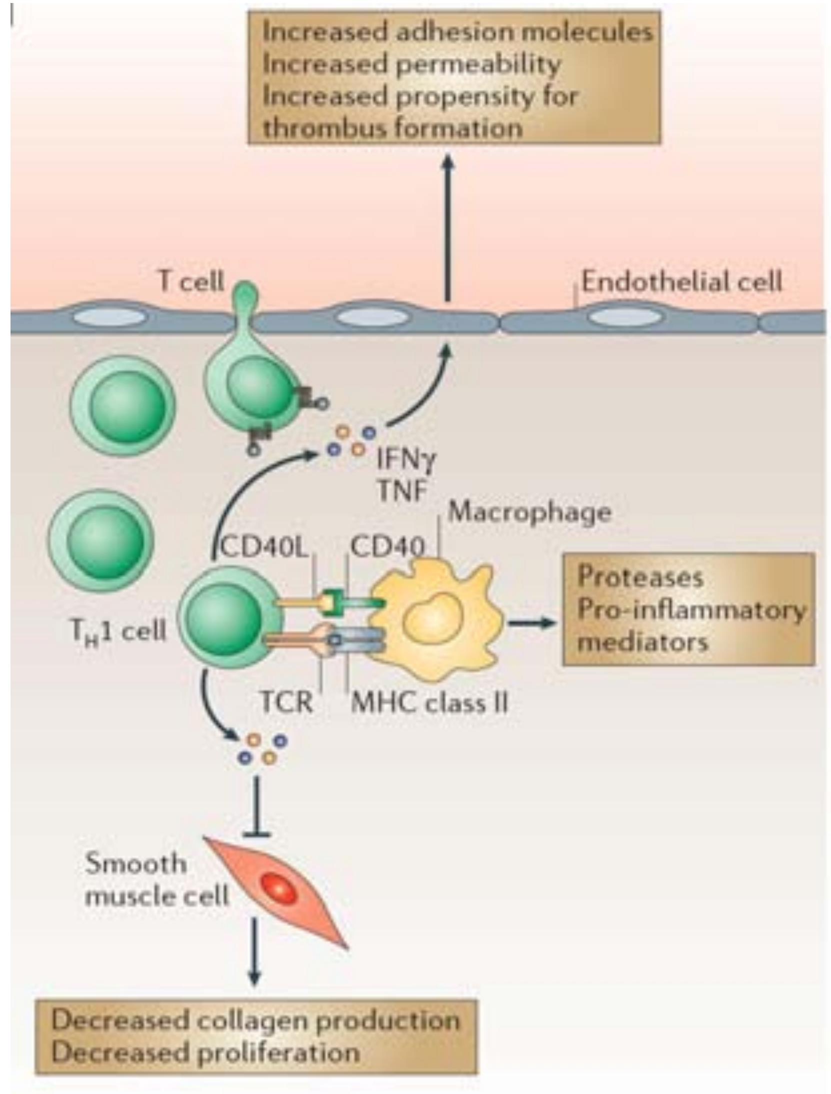





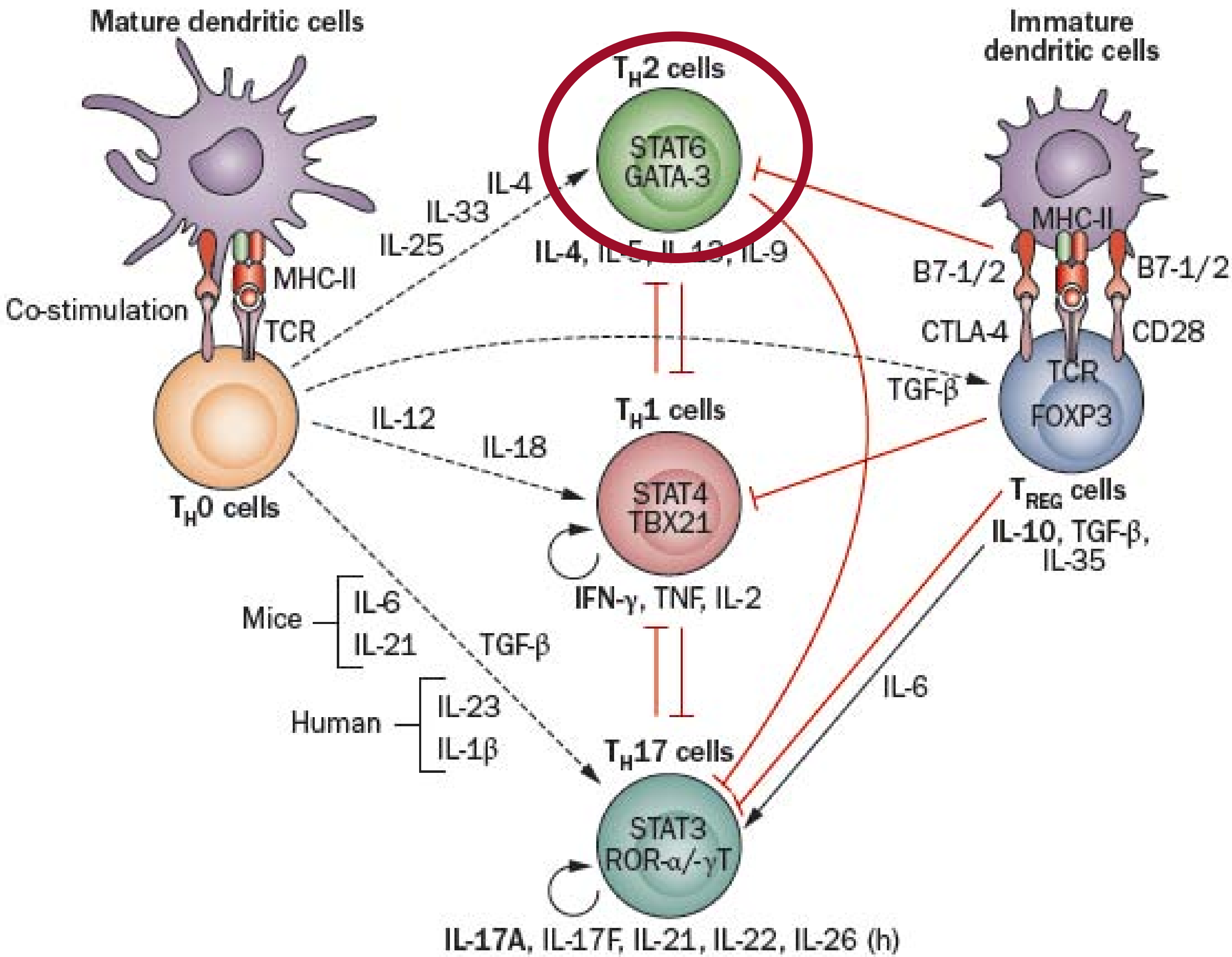

Lahoute C et al., Nat Rev Cardiol 2011

# Recruitment and Activation of Th1 in Atherosclerosis



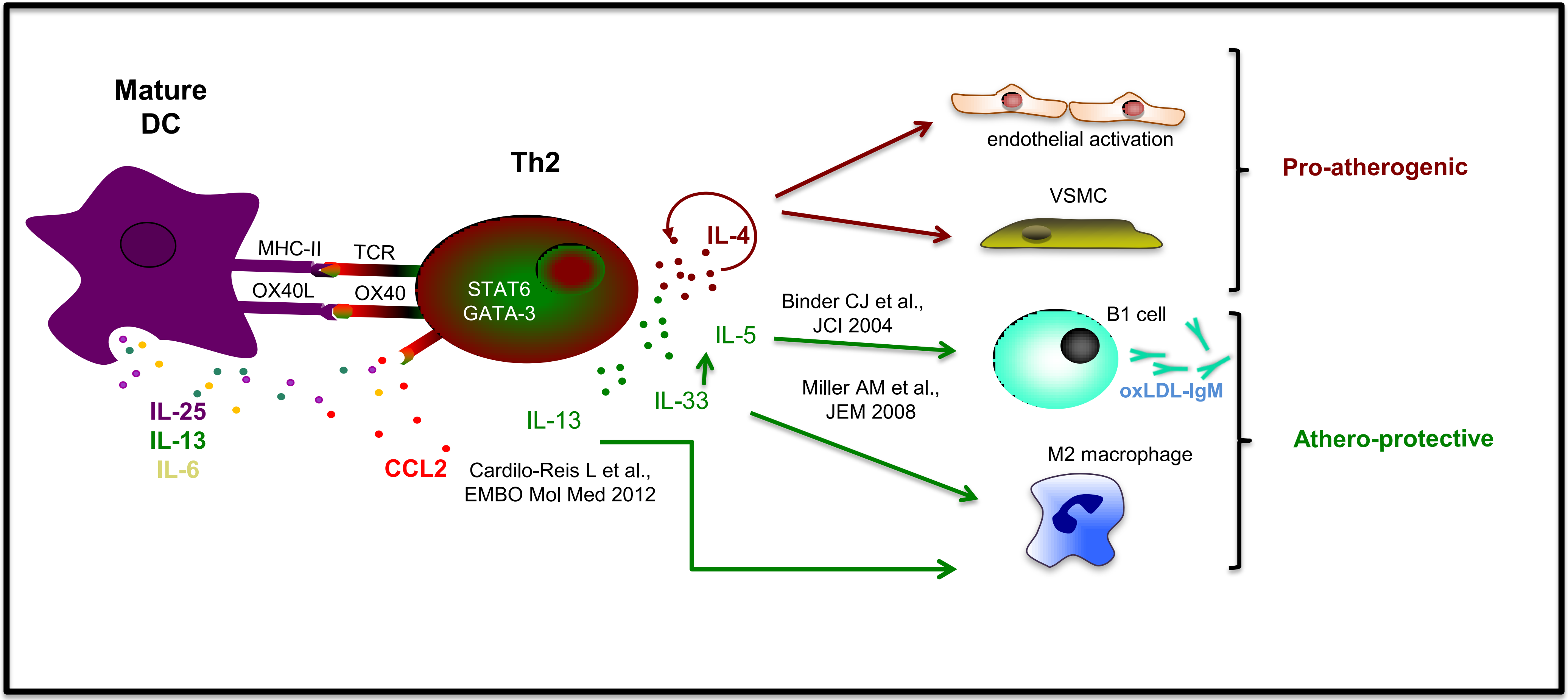




Hansson & Libby, Nat Rev Immunol 2006



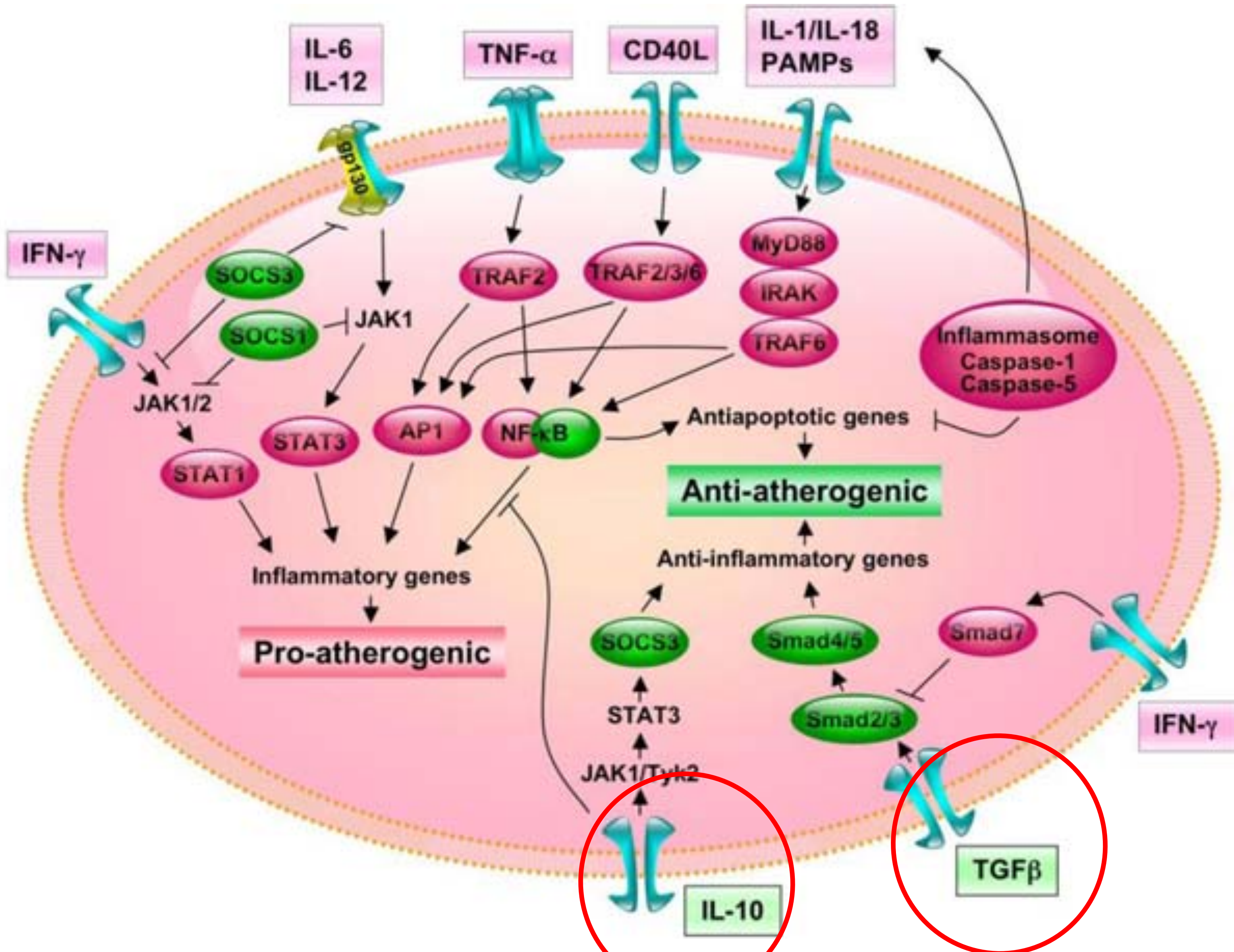

Lahoute C et al., Nat Rev Cardiol 2011

# Potential Role of Th2 Cells in Atherosclerosis



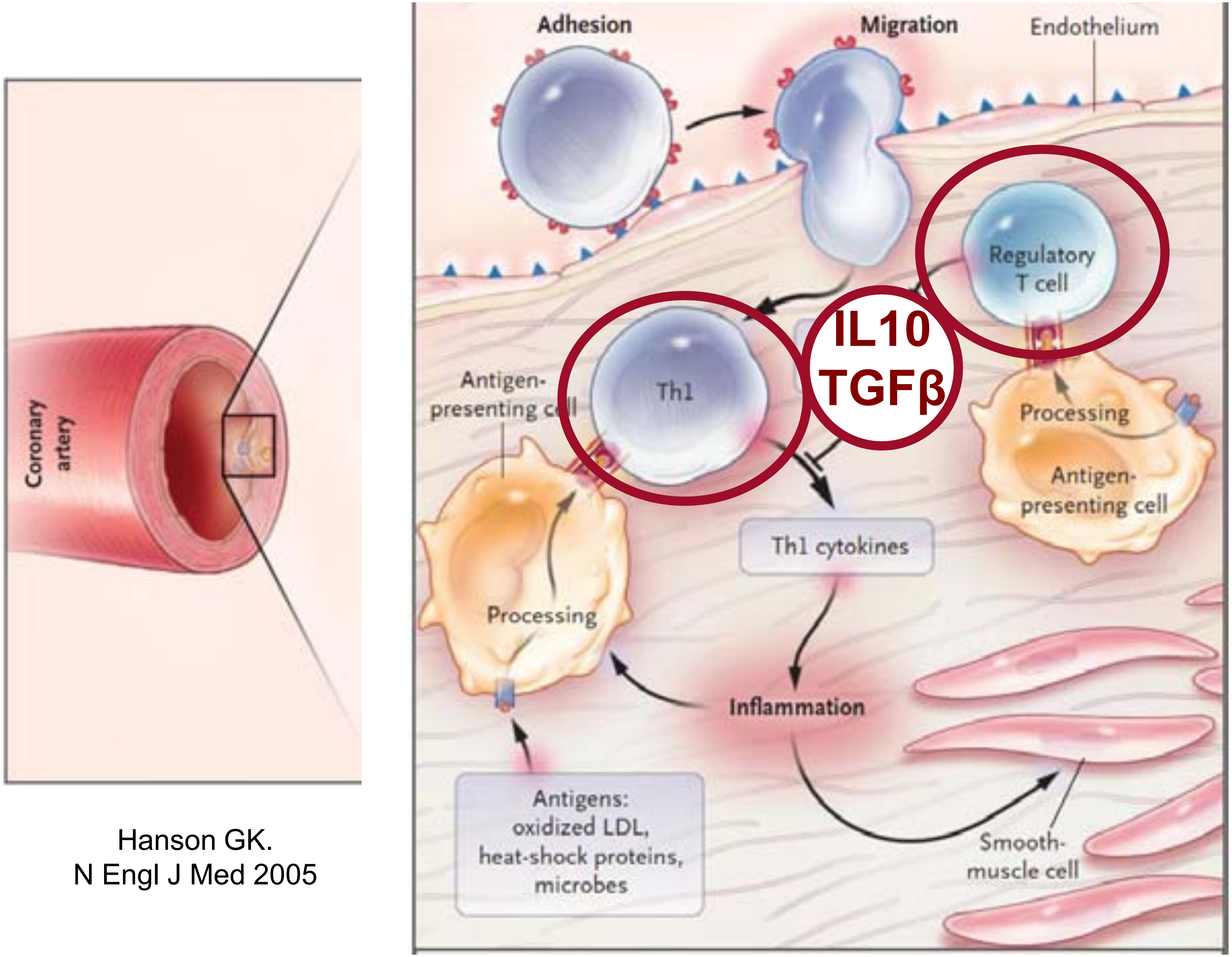

Lahoute C & Mallat Z, 2011

# Pro & Anti-Atherogenic Signaling Pathways




Tedgui & Mallat, Physiol Rev 2006

# Regulatory T cells Control Atherosclerosis




Hanson GK.
N Engl J Med 2005

Circulation
Mallat et al. 2003
Tr1 cells: IL-10

nature medicine
Ait-Oufella et al. 2006
nTreg and TGF-β

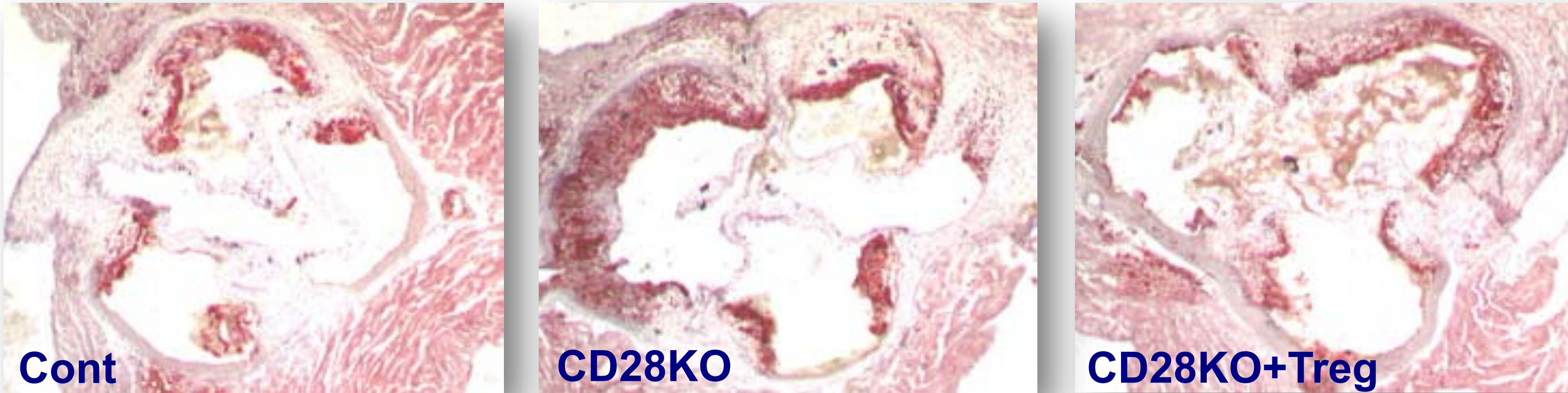
## Types of regulatory T cell: origin, phenotype and function

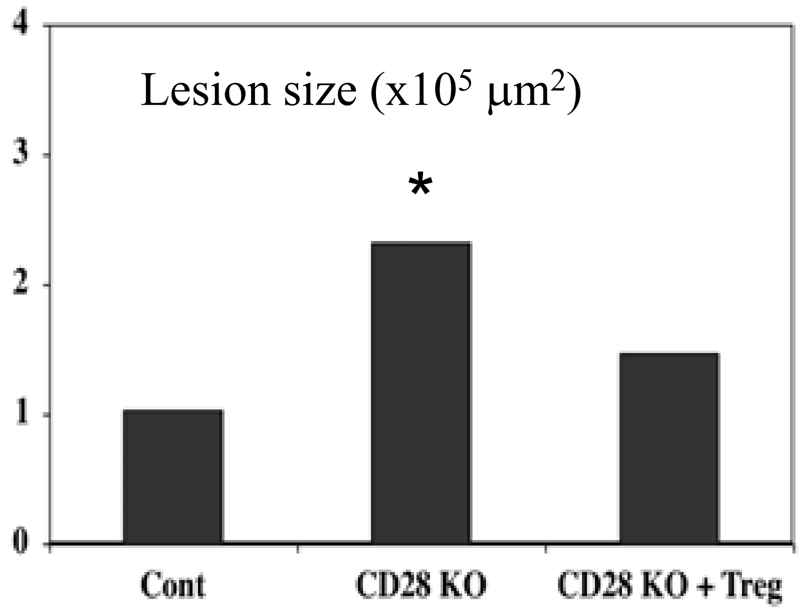
| Cell type | Phenotype* | Mechanism* | Origin |
|---|---|---|---|
| Natural $T_{Reg}$ cells‡ | $FOXP3^+CTLA4^+$ $GITR^+CD45RB^{low}$ $CD62L^{+/-}CD127^{low}$ | Contact dependent, IL-10, TGFβ, CTLA4 and GITR | Thymus |
| Natural $T_{Reg}$ cells (expanded) | $FOXP3^+$ $HLA\text{-}DR^+CD62L^{+/-}$ $CD69^+$ | Contact dependent | Expansion of natural $T_{Reg}$ cells |
| Induced $T_{Reg}$ cells | $FOXP3^+CTLA4^+$ $GITR^+CD45RB^{low}$ | Contact dependent and in some cases TGFβ | Conversion and/or expansion of non-regulatory $CD4^+$ T cells |
| $T_H3$ cells | $FOXP3^{+/-}$ (not well defined) | TGFβ and/or IL-10 | Periphery |
| $T_R1$ cells | $FOXP3^+$ | IL-10 | Periphery |
| $CD8^+$ T cells | $CD28^{+/-}$ | Cell contact, ILT3 and ILT4 | Periphery |

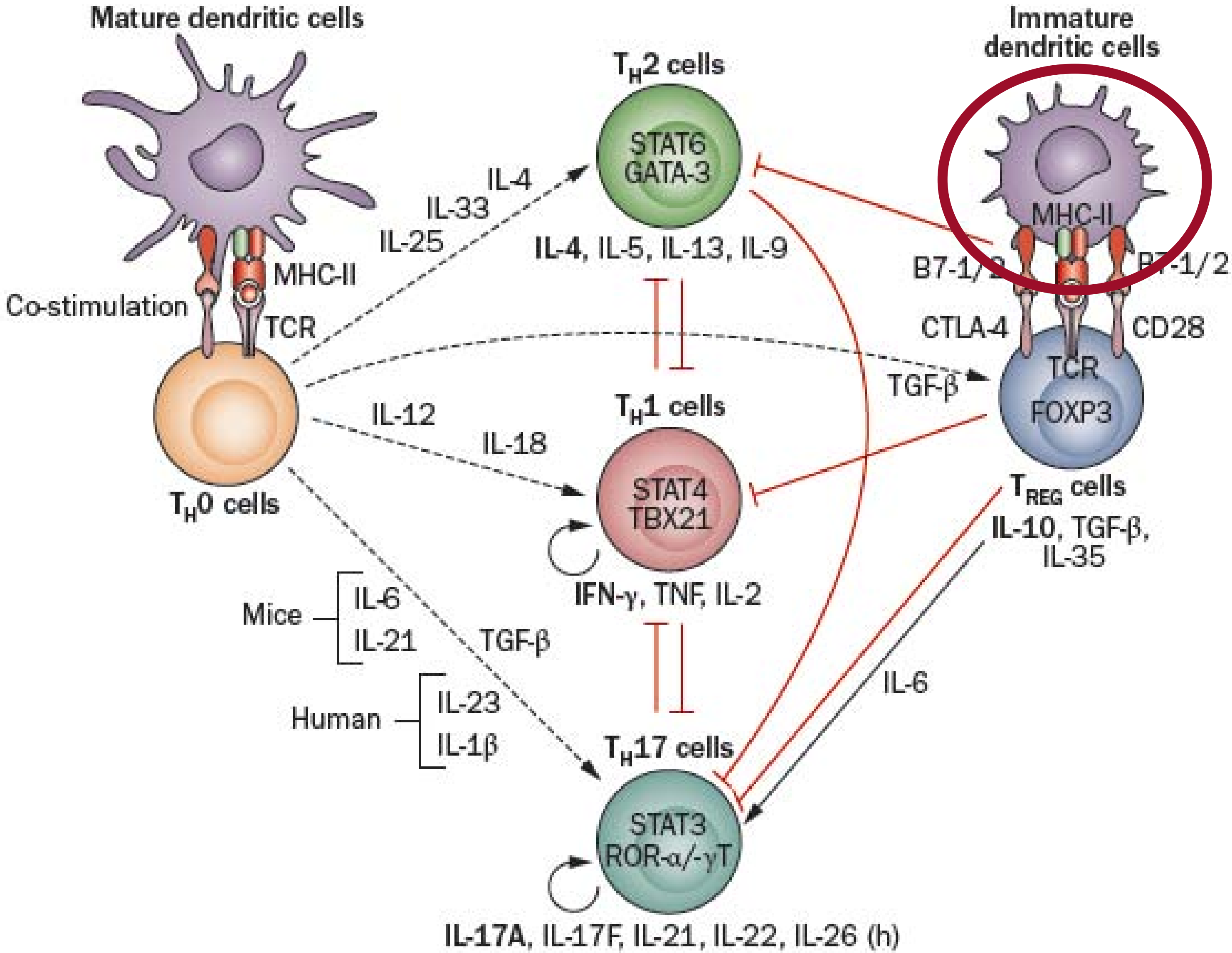
Akbar AN et al., *Nat Rev Immunol*, 2007

# Natural regulatory T cells control the development of atherosclerosis in mice

Ait-Oufella et al. *Nat Med* 2006



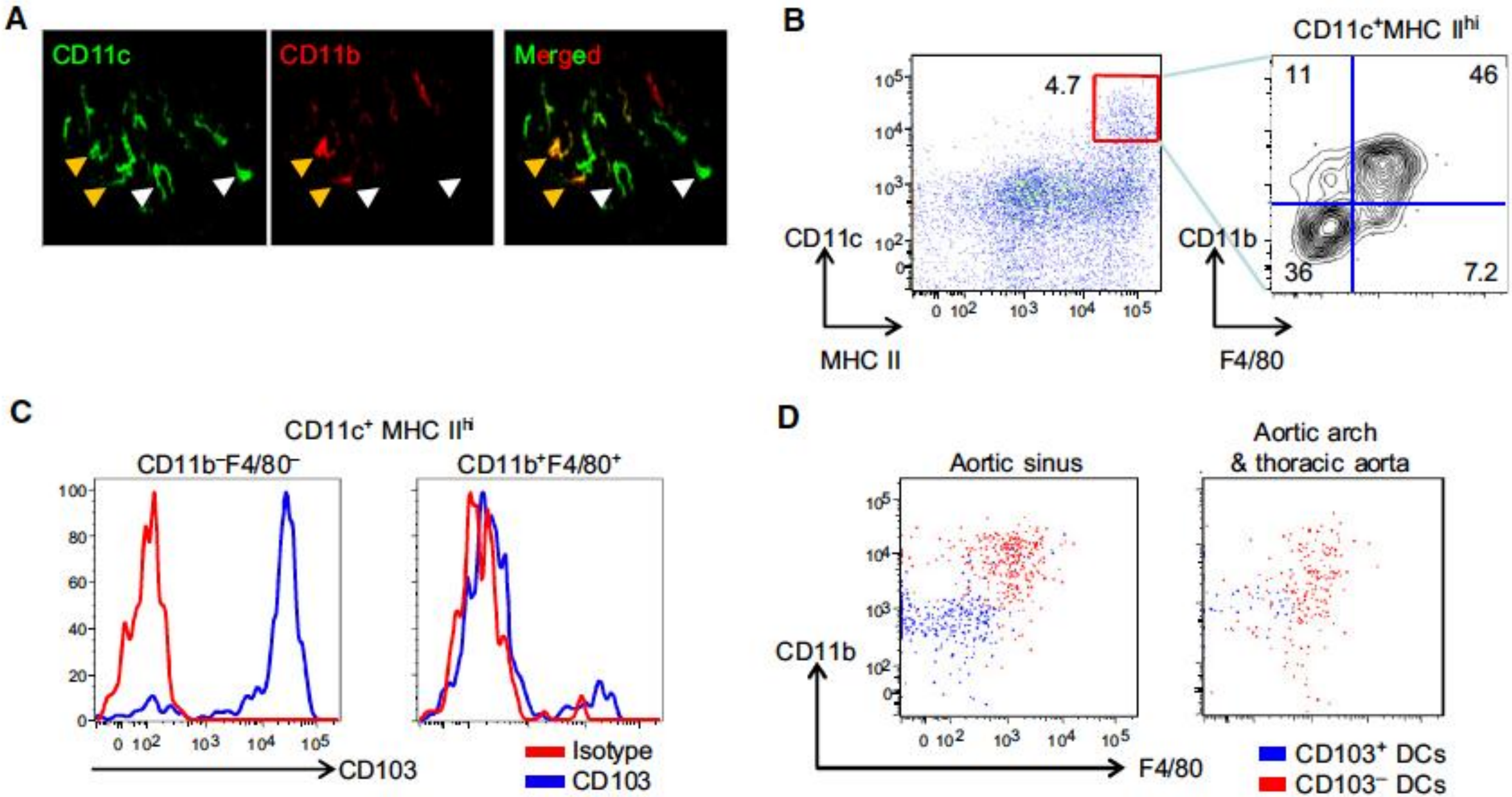






Lahoute C et al., Nat Rev Cardiol 2011

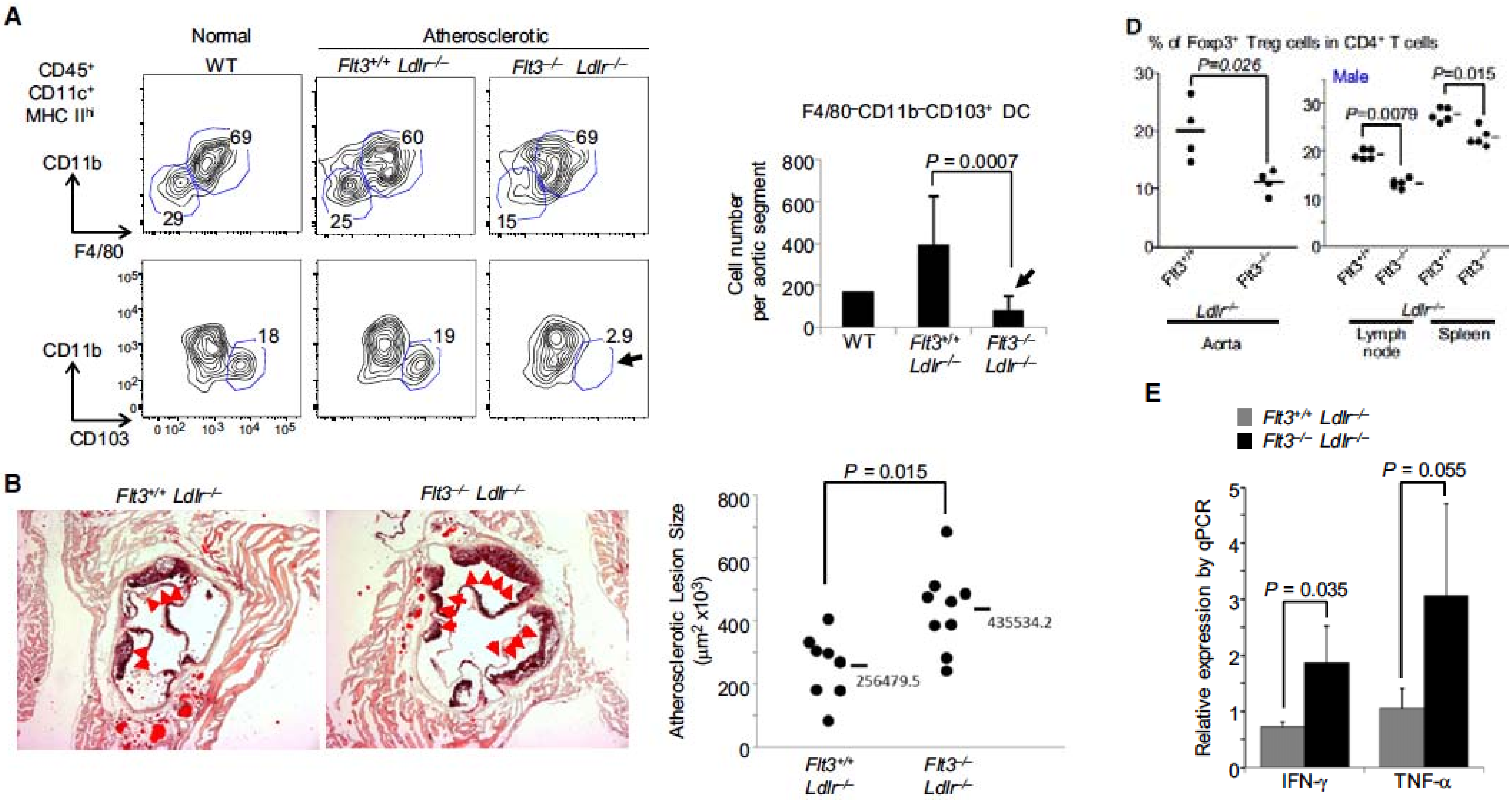
# Flt3 Signaling-Dependent Dendritic Cells Protect against Atherosclerosis

Choi J-H et al., Immunity 2011



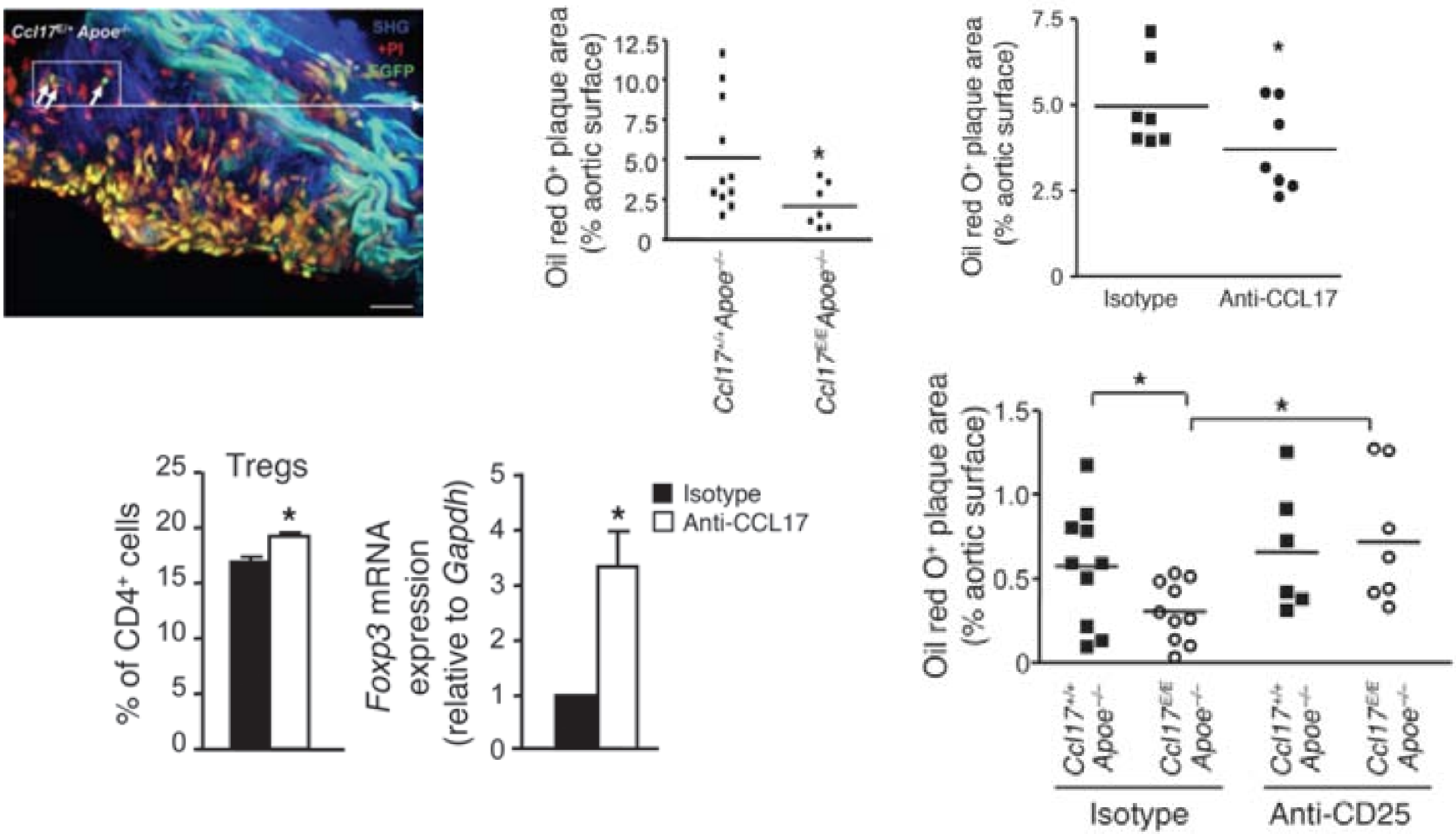
# Flt3 Signaling-Dependent Dendritic Cells Protect against Atherosclerosis

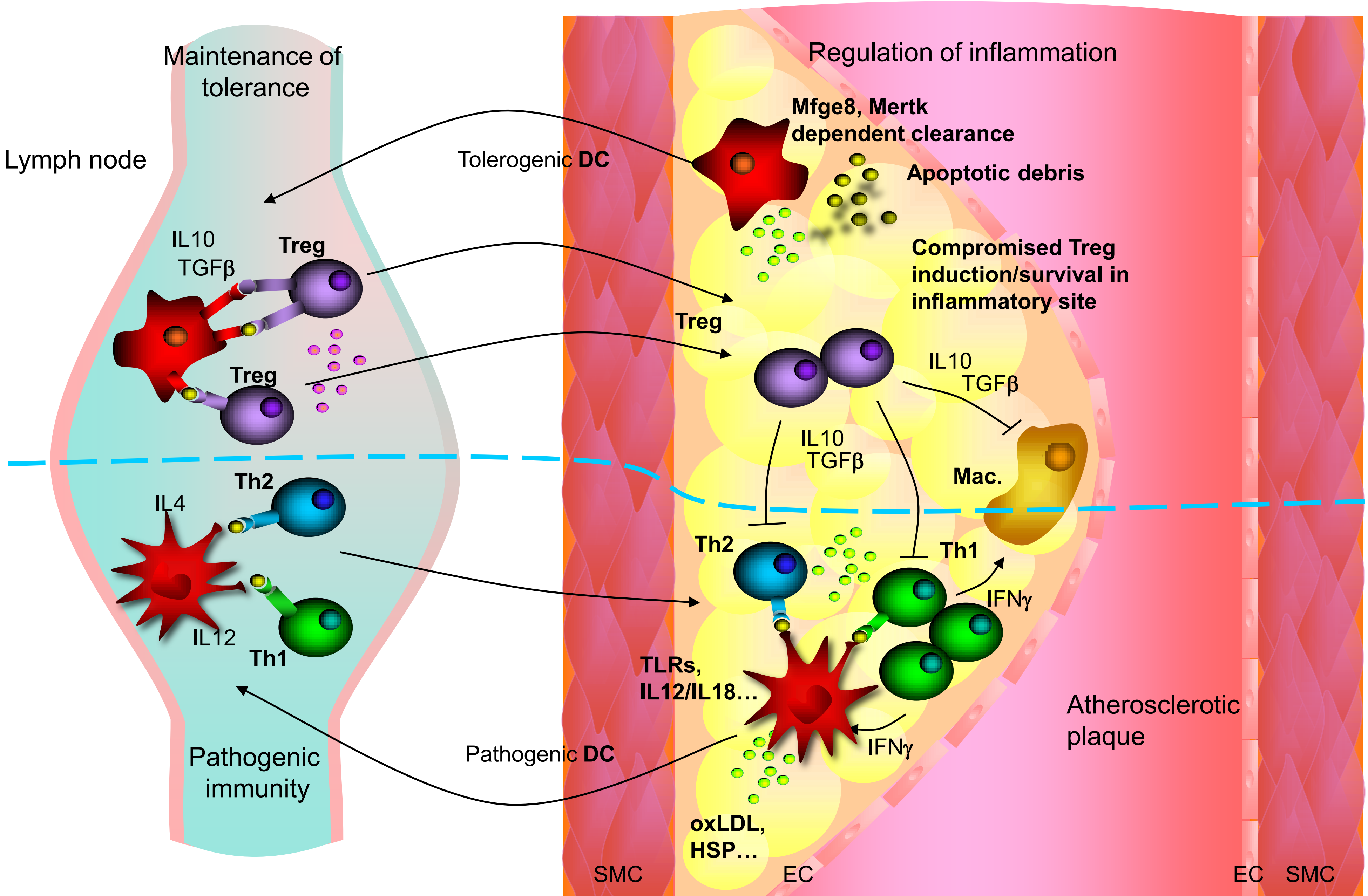
Choi J-H et al., Immunity 2011



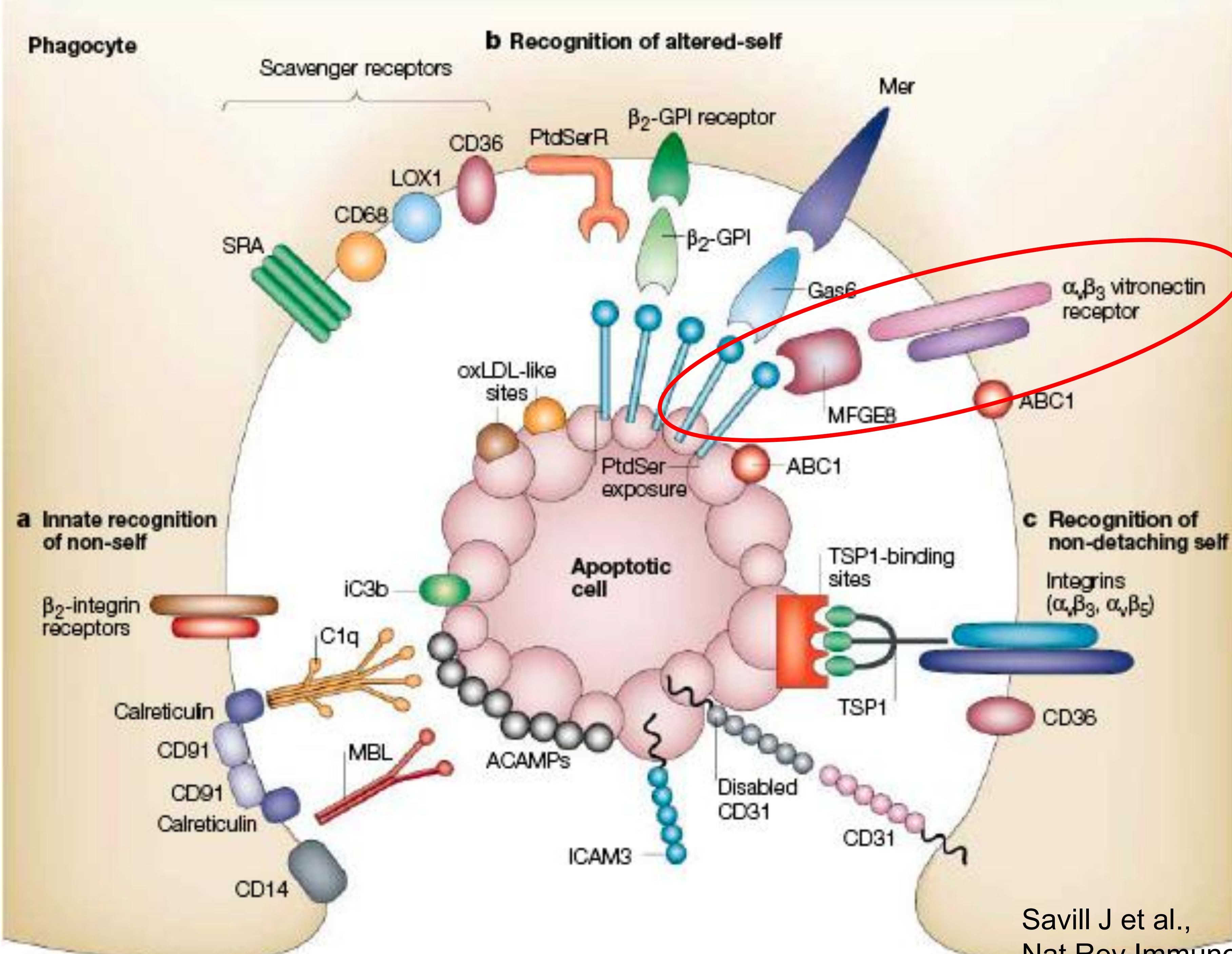
# CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice

Weber C et al.,
*JCI* 2011





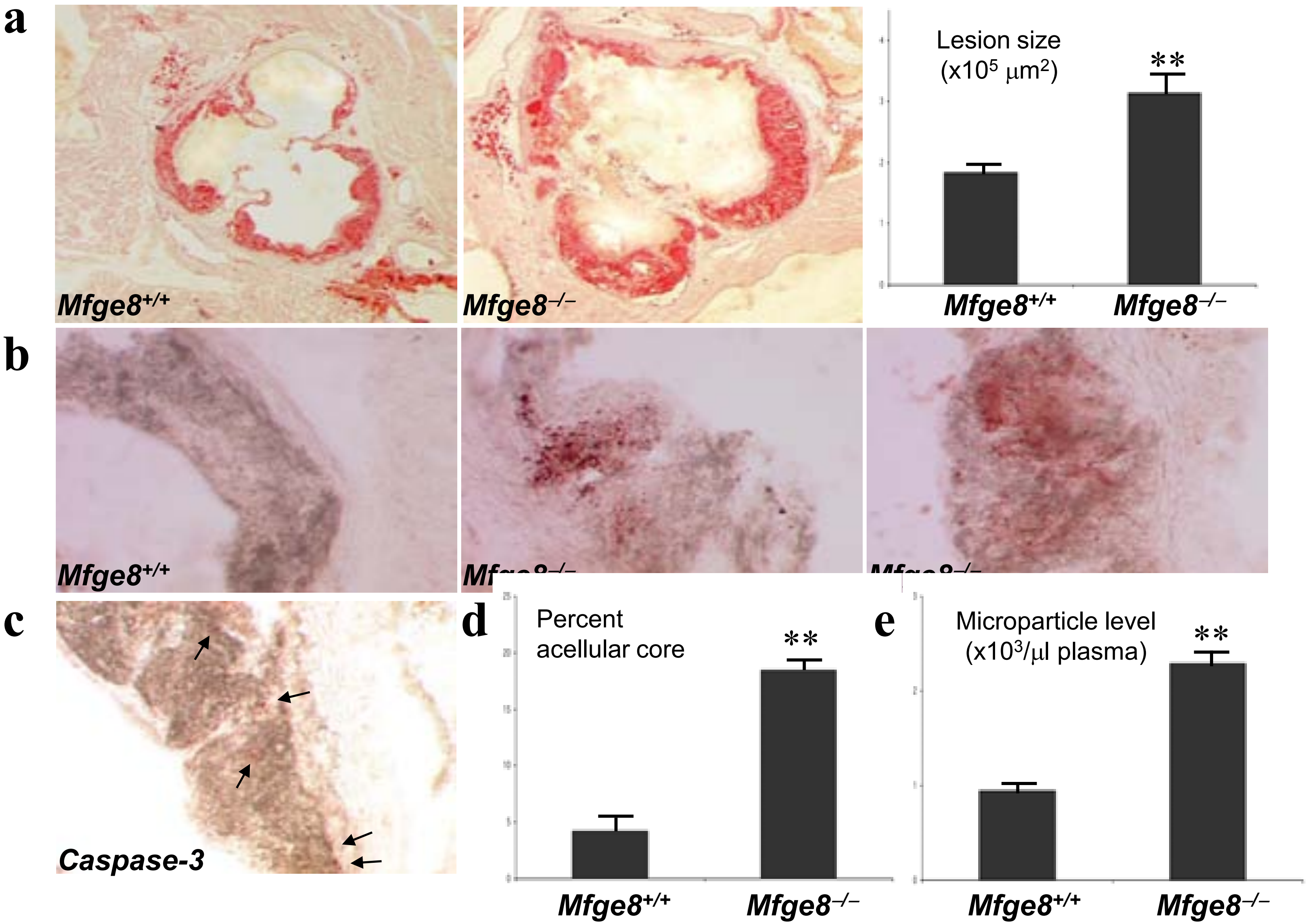

Mallat et al., Physiol Rev 2006; J Lipid Res 2008

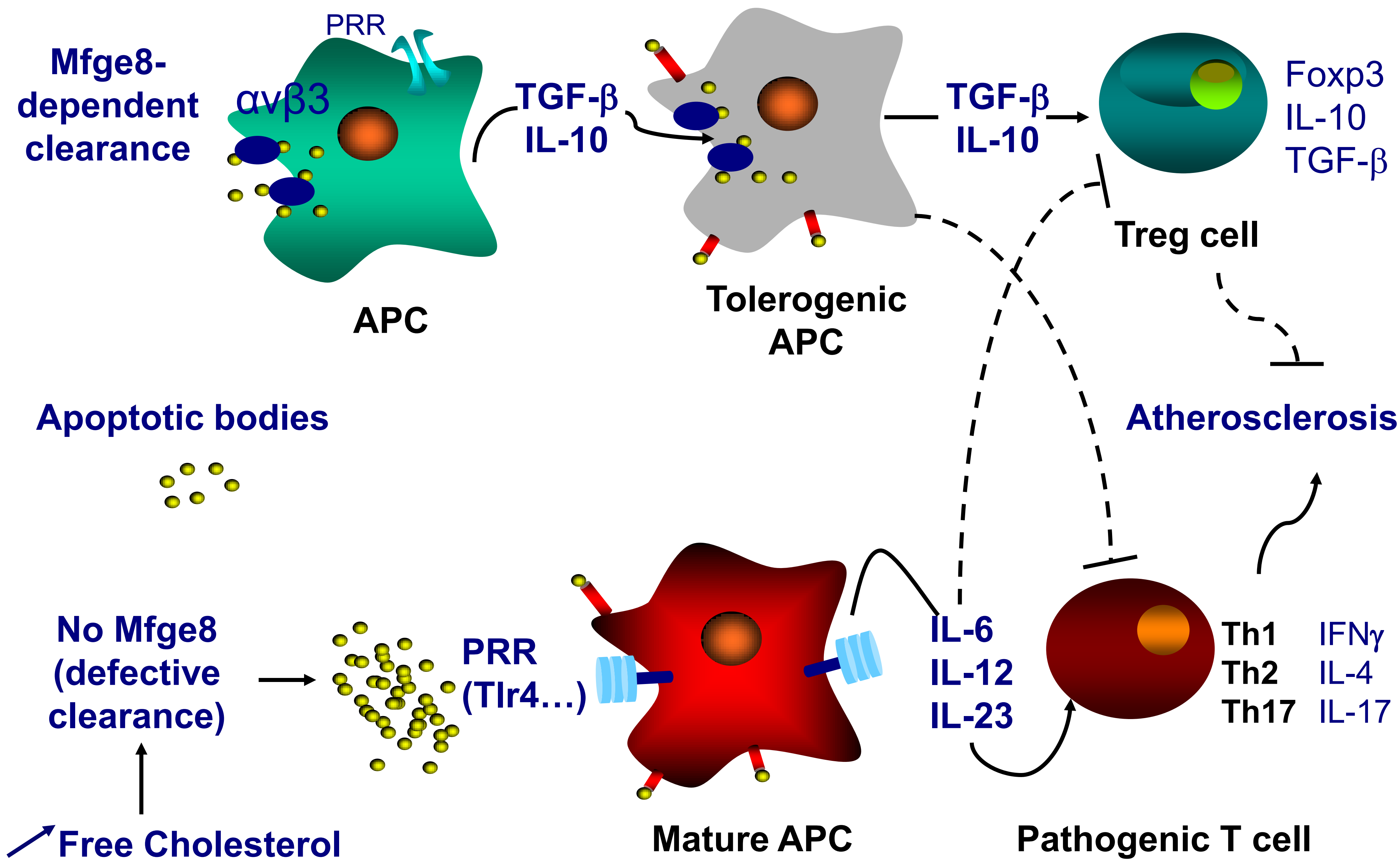



Savill J et al.,
Nat Rev Immunol 2002

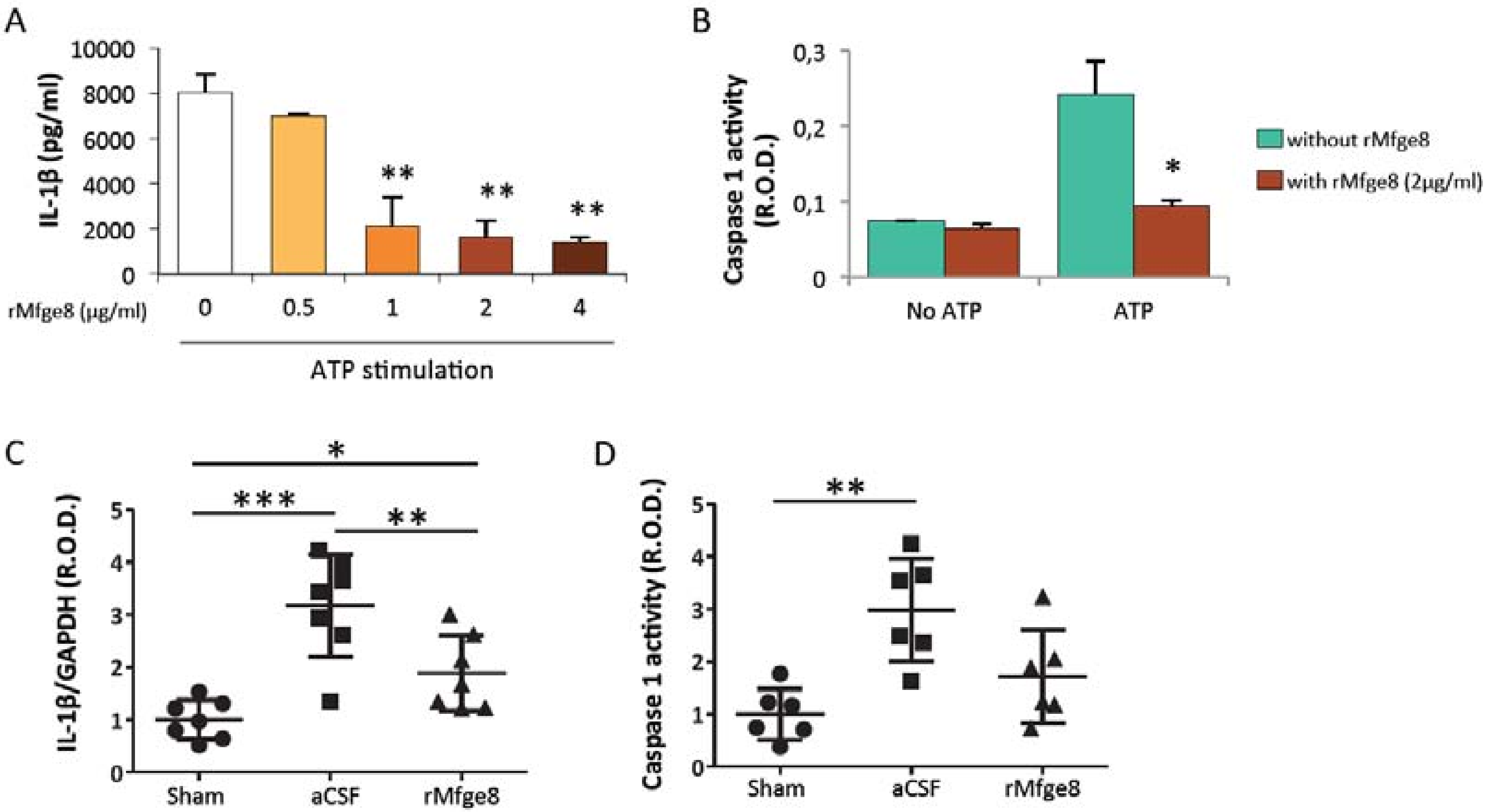
# Lactadherin Deficiency Leads to Apoptotic Cell Accumulation and Accelerated Atherosclerosis in Mice

Ait-Oufella et al., Circulation 2007

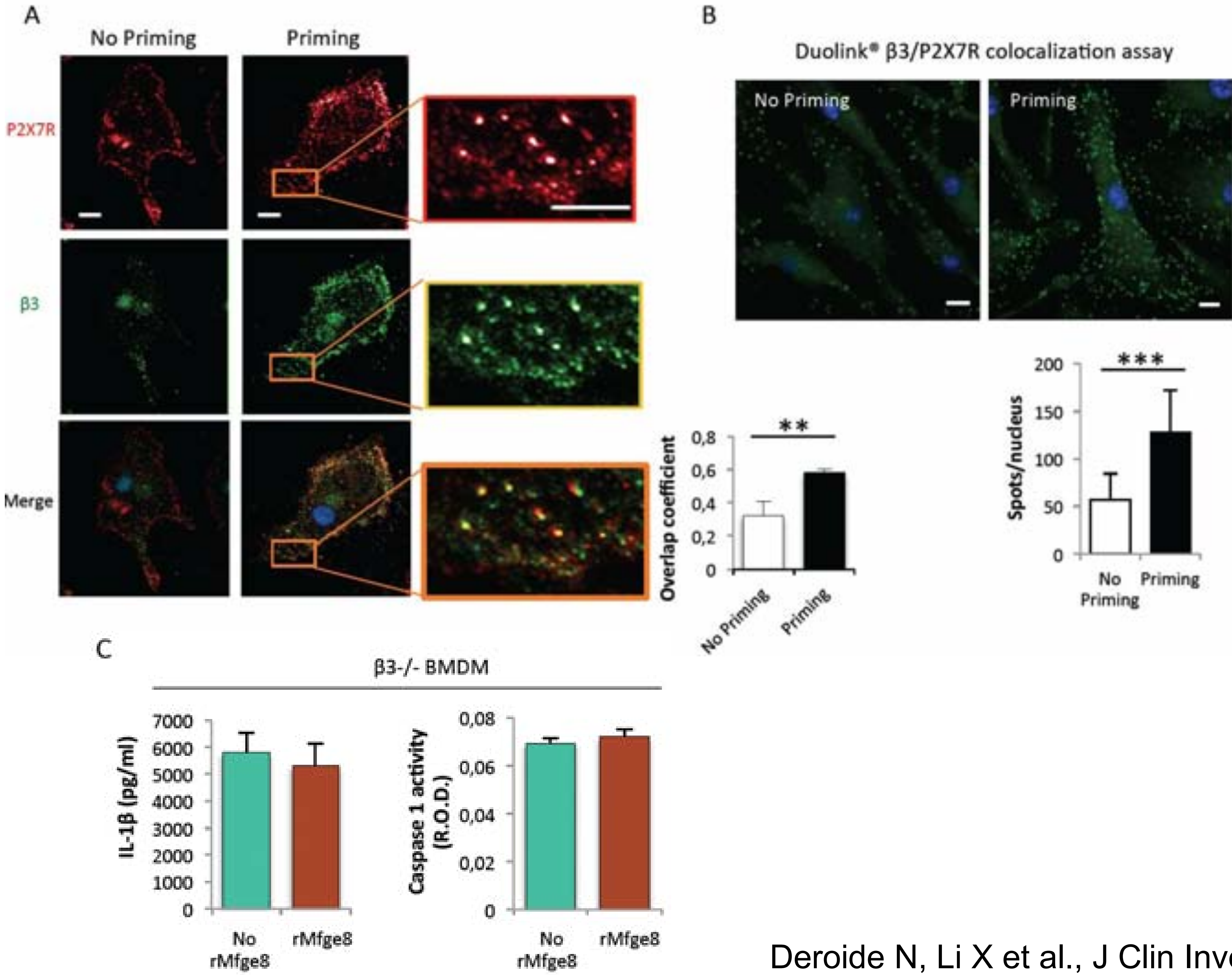



Mfge8-dependent clearance
PRR
αvβ3
APC
TGF-β
IL-10
Tolerogenic APC
TGF-β
IL-10
Foxp3
IL-10
TGF-β
Treg cell
Apoptotic bodies
Atherosclerosis
No Mfge8 (defective clearance)
Free Cholesterol
PRR (Tlr4…)
Mature APC
IL-6
IL-12
IL-23
Th1 IFNγ
Th2 IL-4
Th17 IL-17
Pathogenic T cell

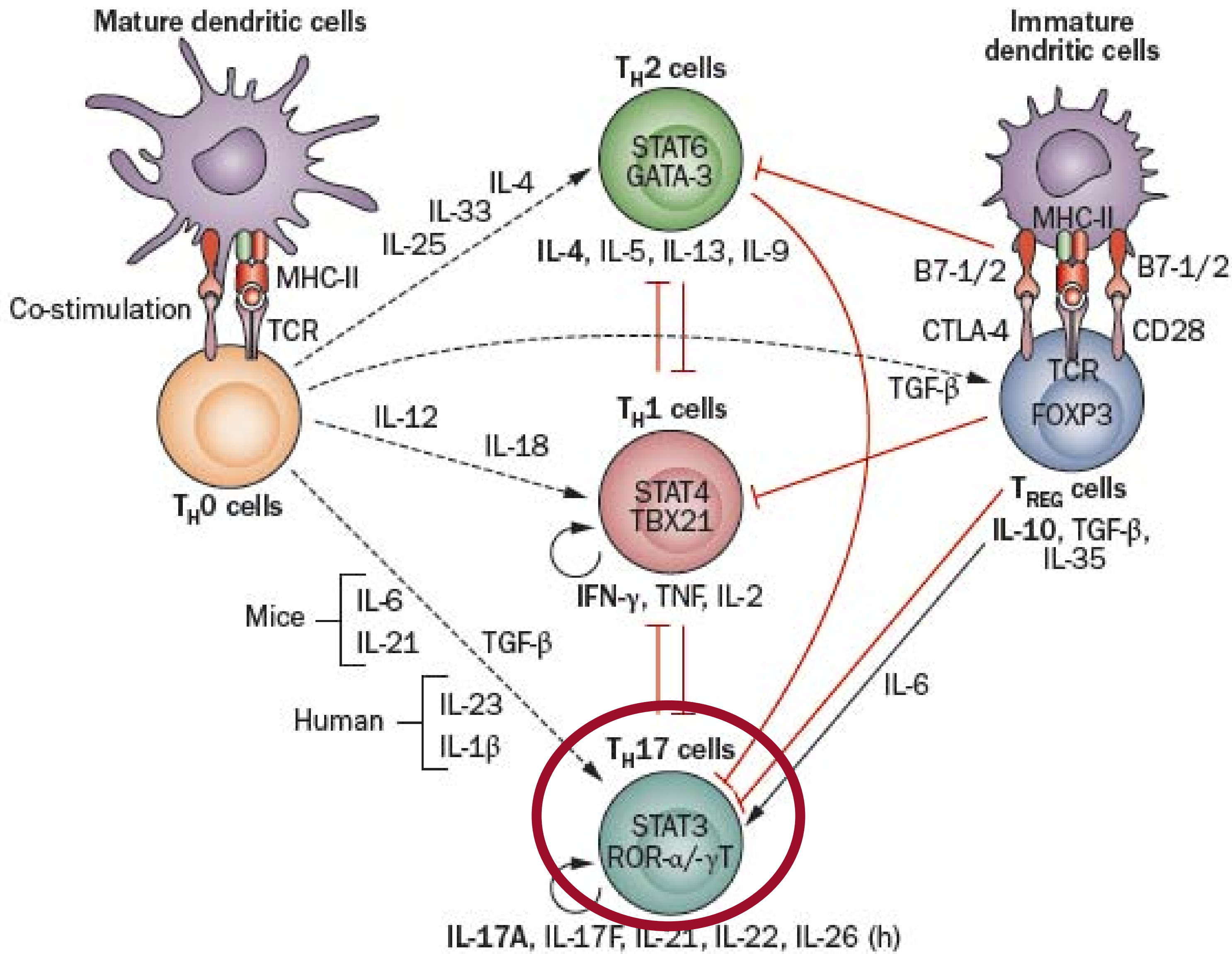
# Mfge8 Inhibits inflammasome-induced IL1β production




Deroide N, Li X et al., J Clin Invest 2013

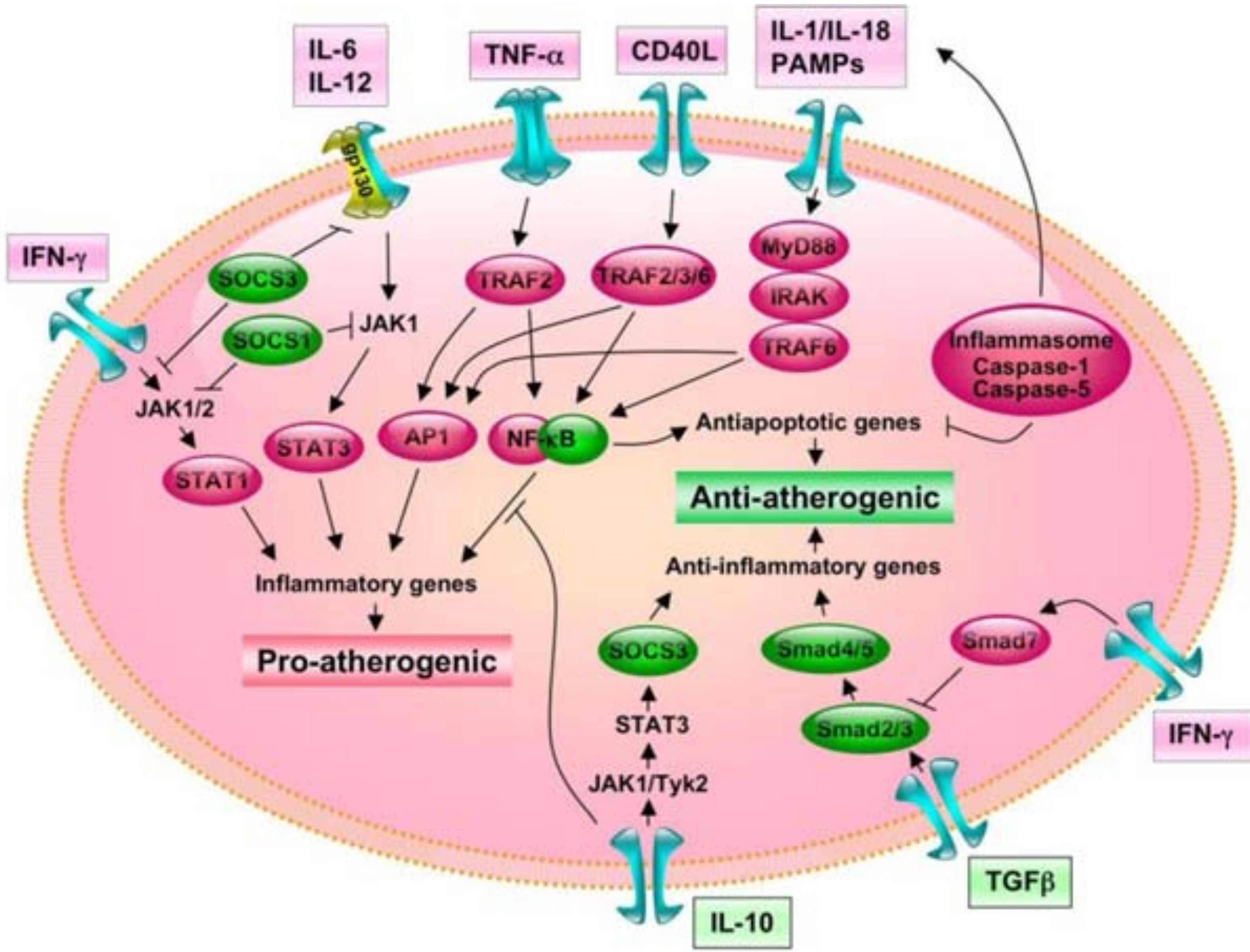



Deroide N, Li X et al., J Clin Invest 2013




Lahoute C et al., Nat Rev Cardiol 2011

# Pro & Anti-Atherogenic Signaling Pathways




Tedgui & Mallat, Physiol Rev 2006

# STAT3 Signaling and the Hyper-IgE Syndrome

## Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome

Yoshiyuki Minegishi[1], Masako Saito[1], Shigeru Tsuchiya[2], Ikuya Tsuge[3], Hidetoshi Takada[4], Toshiro Hara[4], Nobuaki Kawamura[5], Tadashi Ariga[5], Srdjan Pasic[6], Oliver Stojkovic[7], Ayse Metin[8] & Hajime Karasuyama[1]

## *STAT3* Mutations in the Hyper-IgE Syndrome

Steven M. Holland, M.D., Frank R. DeLeo, Ph.D., Houda Z. Elloumi, Ph.D.,
Amy P. Hsu, B.A., Gulbu Uzel, M.D., Nina Brodsky, B.S.,
Alexandra F. Freeman, M.D., Andrew Demidowich, B.A., Joie Davis, A.P.R.N.,

## Impaired $T_H17$ cell differentiation in subjects with autosomal dominant hyper-IgE syndrome

Joshua D. Milner[1]*, Jason M. Brenchley[2]*†, Arian Laurence[3], Alexandra F. Freeman[4], Brenna J. Hill[2], Kevin M. Elias[3,5], Yuka Kanno[3], Christine Spalding[4], Houda Z. Elloumi[4], Michelle L. Paulson[4], Joie Davis[4], Amy Hsu[4], Ava I. Asher[2], John O'Shea[3], Steven M. Holland[4], William E. Paul[1] & Daniel C. Douek[2]

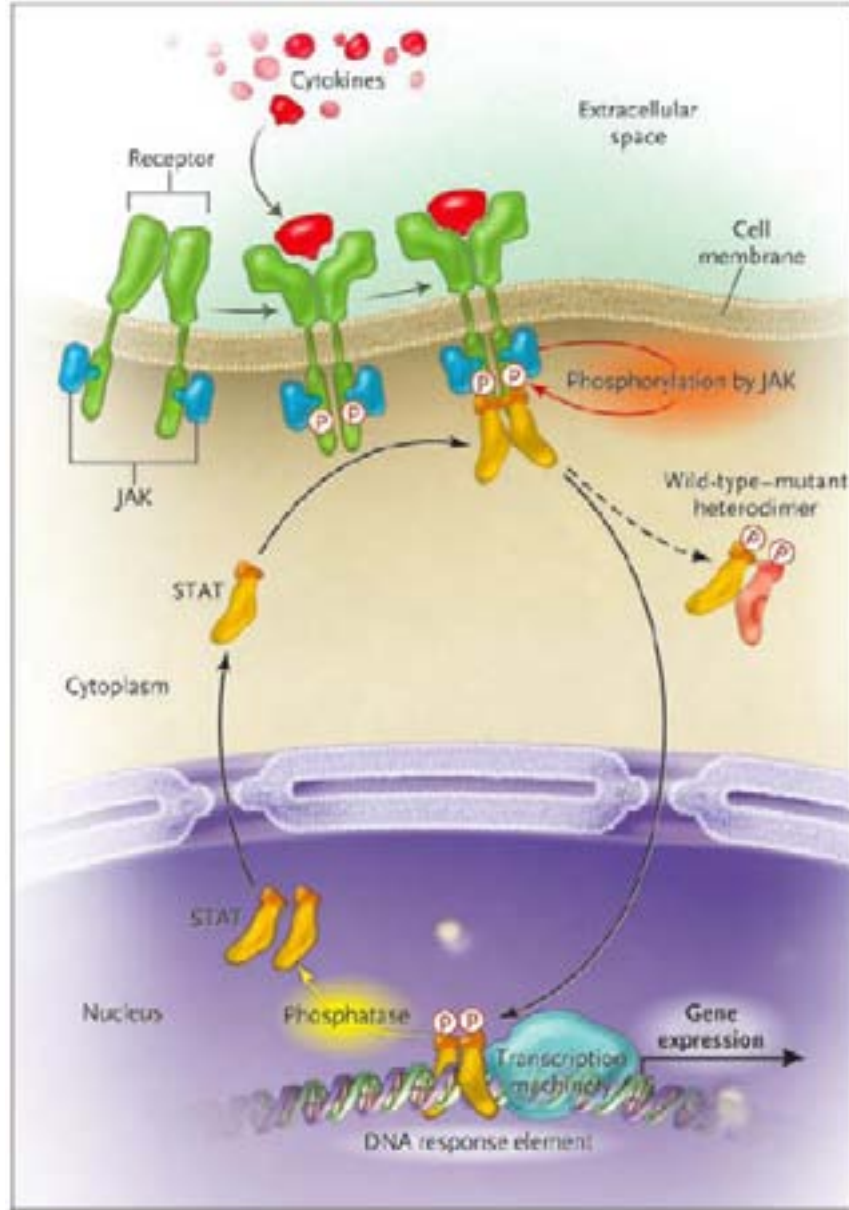
## Mutations in *STAT3* and *IL12RB1* impair the development of human IL-17–producing T cells

Ludovic de Beaucoudrey,[1,2] Anne Puel,[1,2] Orchidée Filipe-Santos,[1,2]
Aurélie Cobat,[1,2] Pegah Ghandil,[1,2] Maya Chrabieh,[1,2] Jacqueline Feinberg,[1,2]
Horst von Bernuth,[1,2] Arina Samarina,[1,2] Lucile Jannière,[1,2] Claire Fieschi,[3]

## Deficiency of Th17 cells in hyper IgE syndrome due to mutations in *STAT3*

Cindy S. Ma,[1] Gary Y.J. Chew,[2,3] Nicholas Simpson,[3] Archana Priyadarshi,[4]
Melanie Wong,[6] Bodo Grimbacher,[8] David A. Fulcher,[7] Stuart G. Tangye,[1]
and Matthew C. Cook[2,3,5]

Levy DE & Loomis CA, NEJM 2007



# Frequent and Widespread Vascular Abnormalities in Human Signal Transducer and Activator of Transcription 3 Deficiency

Marie-Olivia Chandesris, MD; Arshid Azarine, MD, MSc; Kim-Thanh Ong, MD, PhD; Soraya Taleb, PhD; Pierre Boutouyrie, MD, PhD; Elie Mousseaux, MD, PhD; Mélissa Romain, MSc; Erwan Bozec, PhD; Stéphane Laurent, MD, PhD; Nathalie Boddaert, MD, PhD; Caroline Thumerelle, MD, PhD; Isabelle Tillie-Leblond, MD, PhD; Cyrille Hoarau, MD, PhD; Yvon Lebranchu, MD, PhD; Nathalie Aladjidi, MD; François Tron, MD, PhD; Vincent Barlogis, MD; Gérard Body, MD; Marine Munzer, MD; Roland Jaussaud, MD, PhD; Felipe Suarez, MD, PhD; Olivier Clement, MD, PhD; Olivier Hermine, MD, PhD; Alain Tedgui, PhD; Olivier Lortholary, MD, PhD; Capucine Picard, MD, PhD; Ziad Mallat, MD, PhD; Alain Fischer, MD, PhD

***Background***—Signal transducer and activator of transcription 3 (STAT3) deficiency is responsible for autosomal dominant hyperimmunoglobulin E syndrome, characterized by recurrent bacterial and fungal infections, connective tissue abnormalities, hyperimmunoglobulin E, and Th17 lymphopenia. Although vascular abnormalities have been reported in some patients, the prevalence, characteristics, and etiology of these features have yet to be described.

***Methods and Results***—We prospectively screened 21 adult patients with STAT3 deficiency (median age, 26 years; range, 17 to 44) for vascular abnormalities. We explored the carotid arteries with whole-body magnetic resonance imaging angiography, coronary multislice computed tomography, and echo-tracking–based imaging. We also assayed for serum biomarkers of inflammation and endothelial dysfunction. Finally, we studied murine models of aortic aneurysm in the presence and absence of inhibitors of STAT3-dependent signaling. Ninety-five percent of patients showed brain abnormalities (white matter hyperintensities, lacunar lesions suggestive of ischemic infarcts, and atrophy). We reported peripheral and brain artery abnormalities in 84% of the patients and detected coronary artery abnormalities in 50% of the patients. The most frequent vascular abnormalities were ectasia and aneurysm. The carotid intima-media thickness was markedly decreased, with a substantial increase in circumferential wall stress, indicating the occurrence of hypotrophic arterial remodeling in this STAT3-deficient population. Systemic inflammatory biomarker levels correlated poorly with the vascular phenotype. In vivo inhibition of STAT3 signaling or blockade of IL-17A resulted in a marked increase in aneurysm severity and fatal rupture in mouse models.

***Conclusions***—Vascular abnormalities are highly prevalent in patients with STAT3 deficiency. This feature is consistent with the greater susceptibility to vascular aneurysm observed after inhibition of STAT3-dependent signaling in mouse models. (*Circ Cardiovasc Genet*. 2012;5:00-00.)

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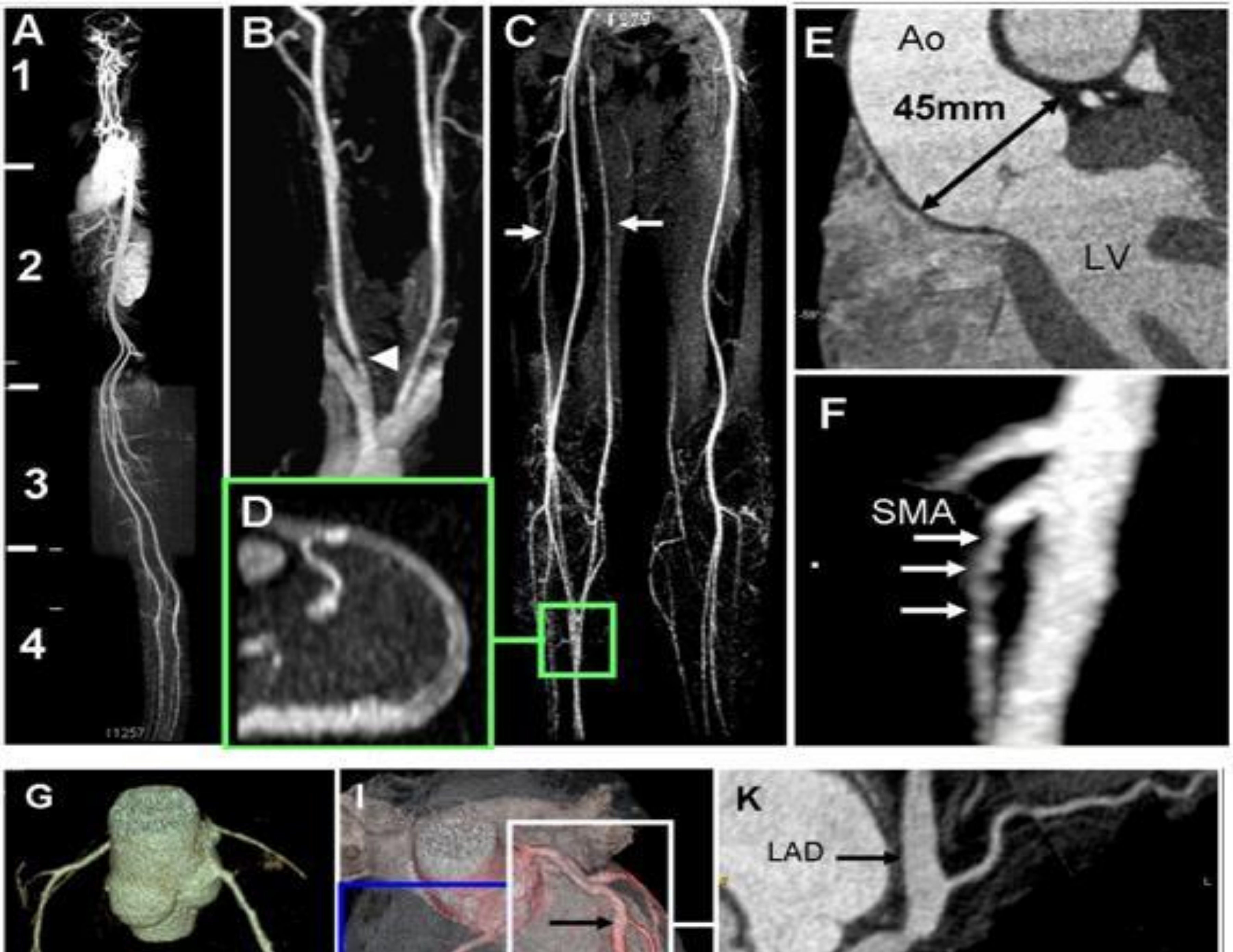

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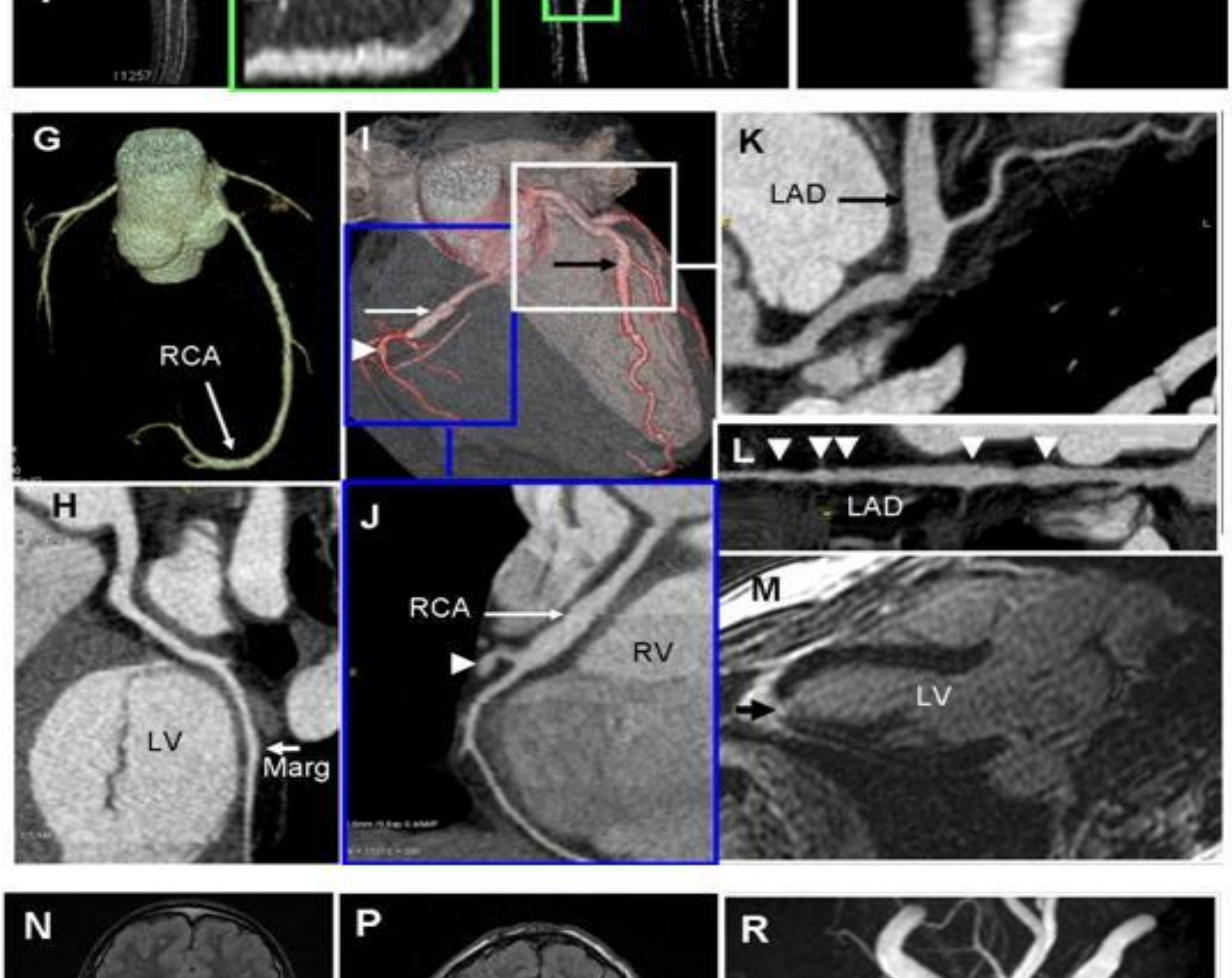

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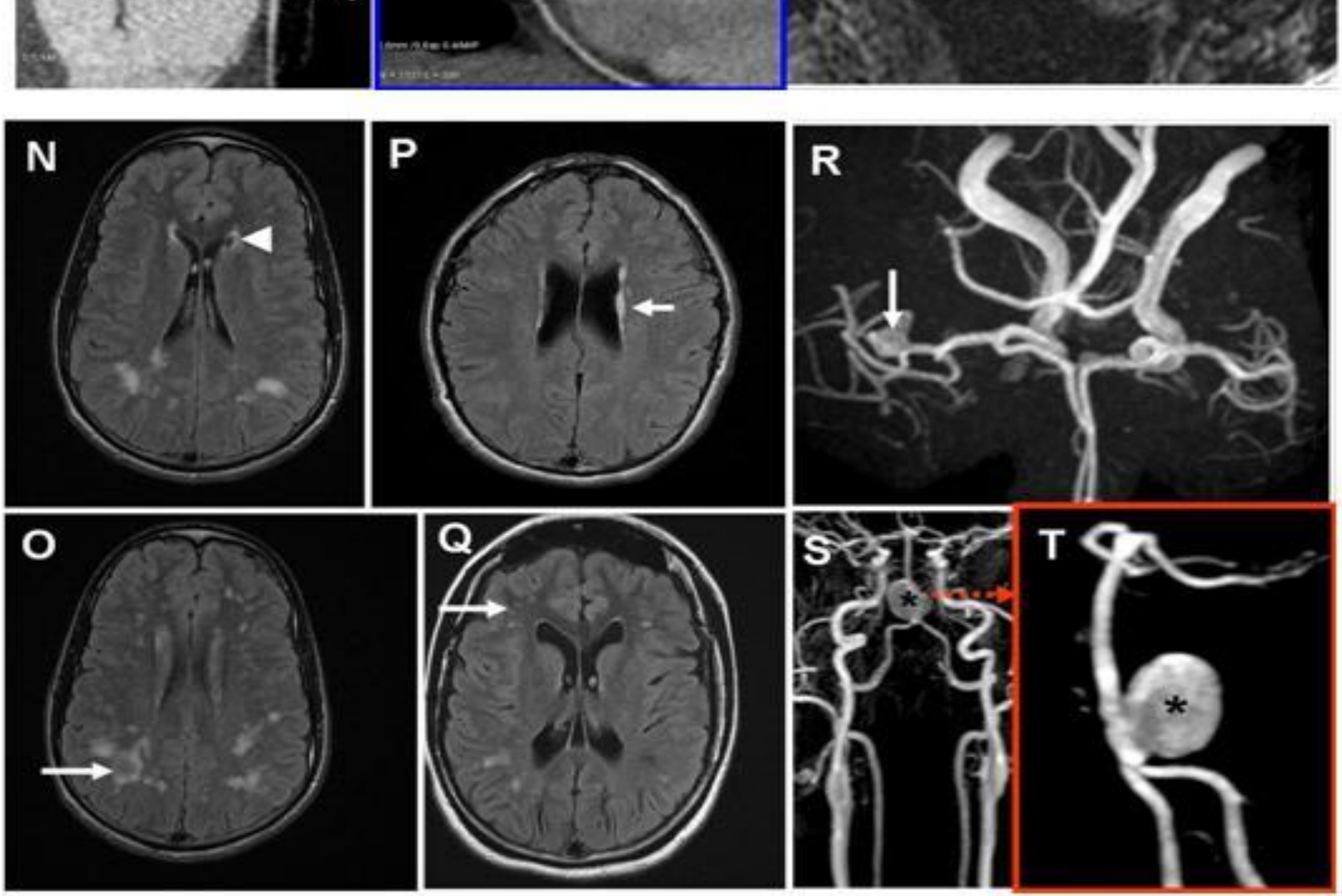

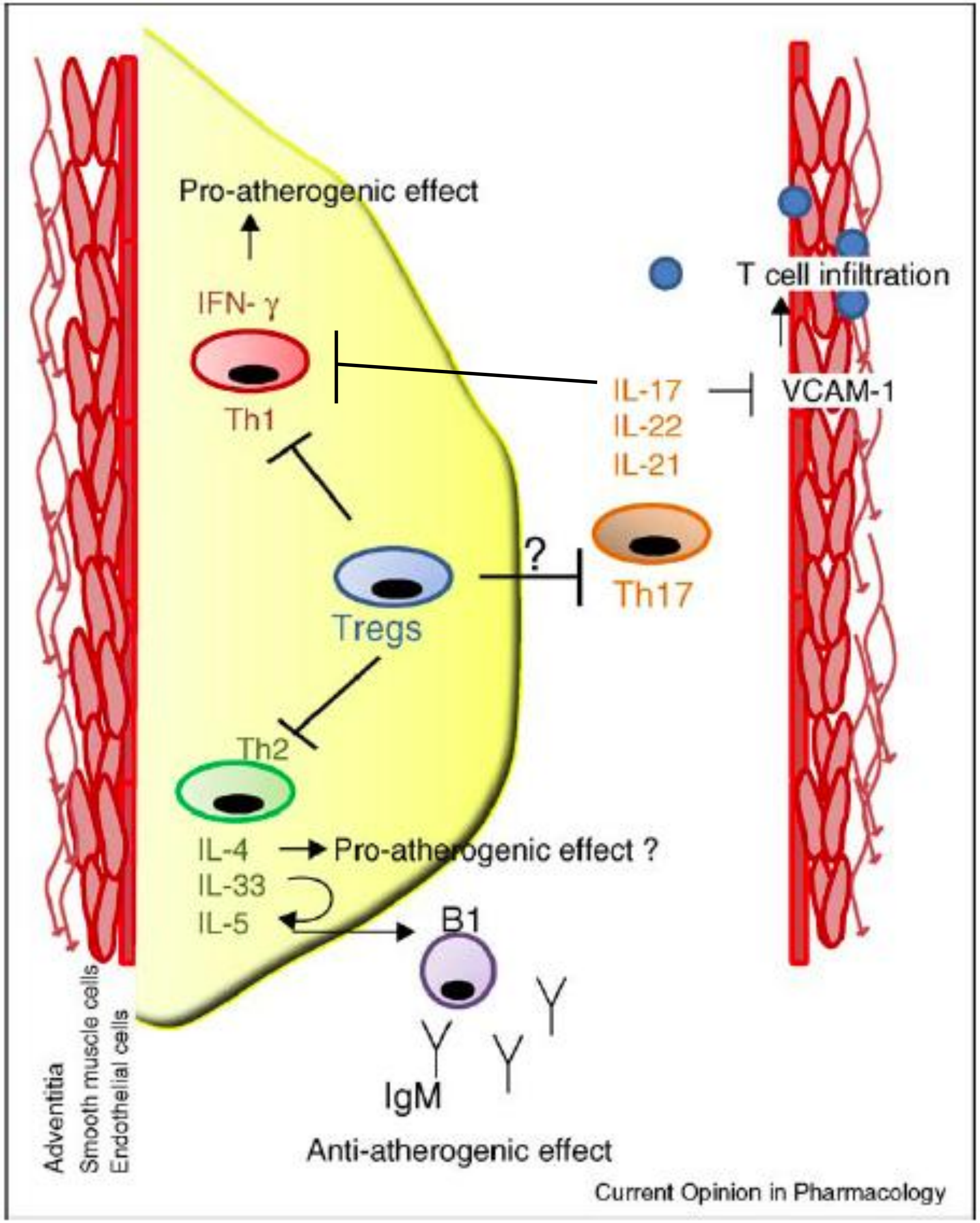


Taleb et al. 2010

**University of Cambridge**
**British Heart Foundation**

Andy SAGE
Deirdre MURPHY
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Leanne MASTERS

Xuan LI
Céline LOINARD
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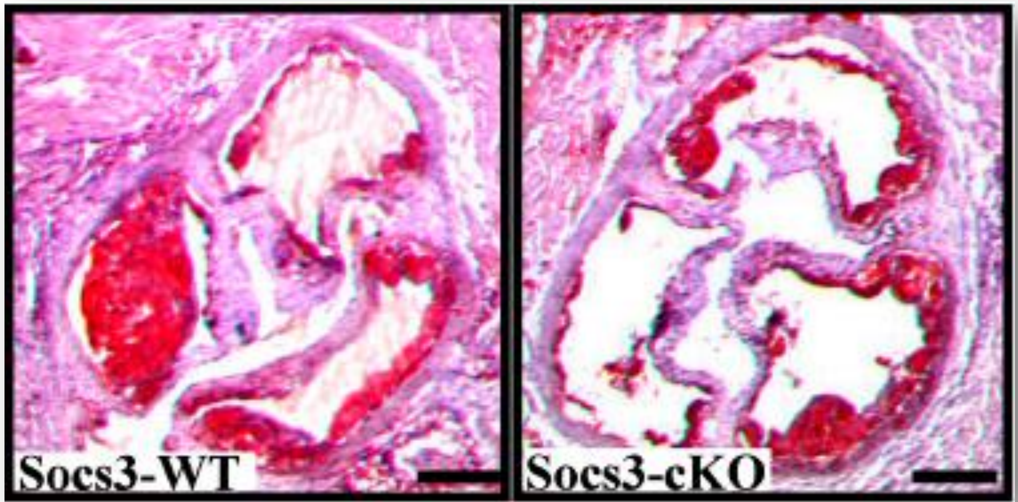
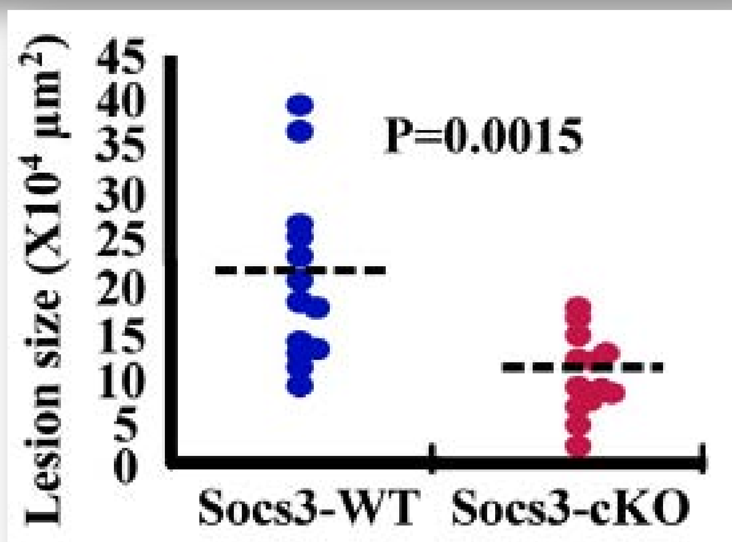
Thomas F. TEDDER

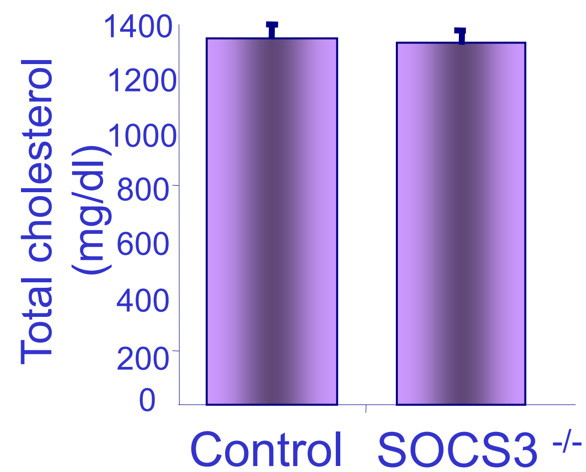
**UCSF, USA**
Israel F. CHARO

**University of Utrecht, The Netherlands**
Gerard PASTERKAMP

# Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis

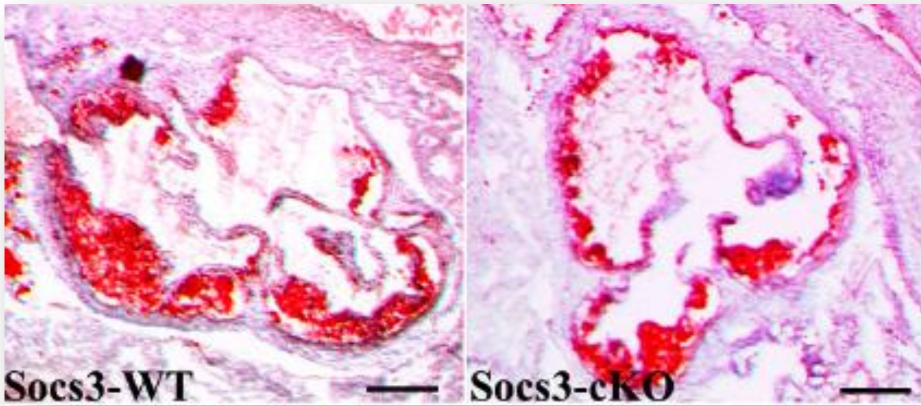


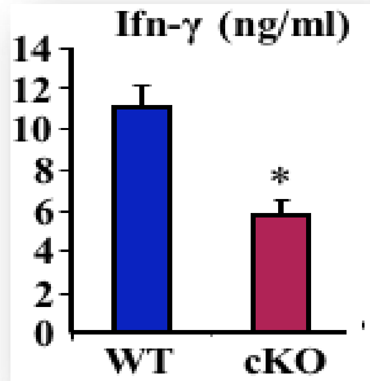



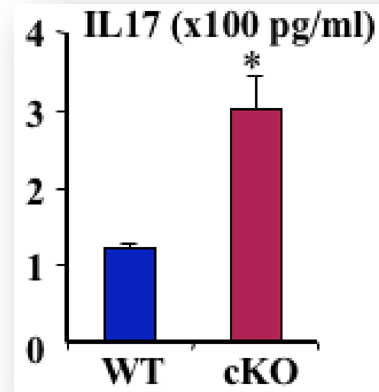



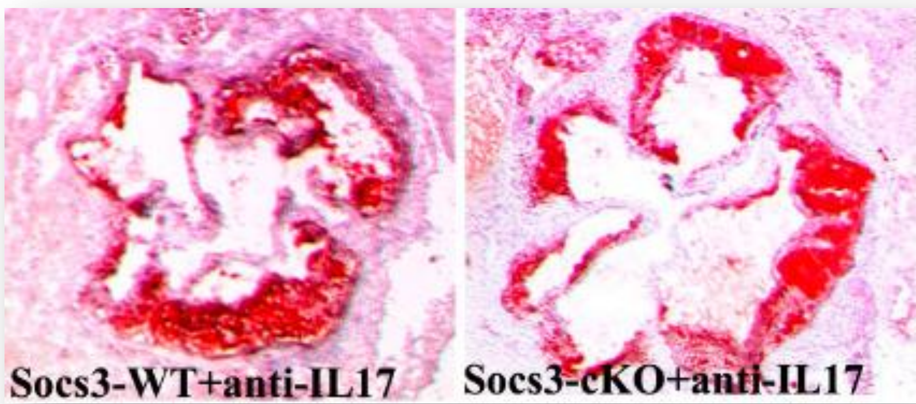

Taleb et al. *J Exp Med*, 2009;206:2067-2077

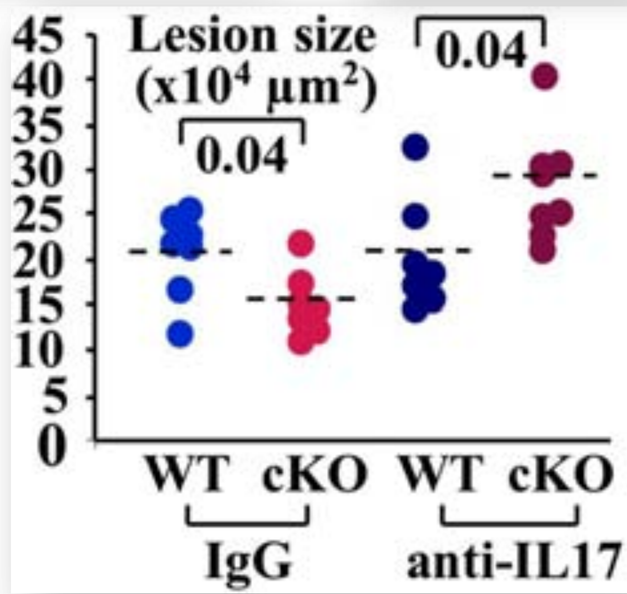
# Neutralization of IL17 Abrogates the Athero-Protective Effect of T Cell-Specific SOCS3 Deletion



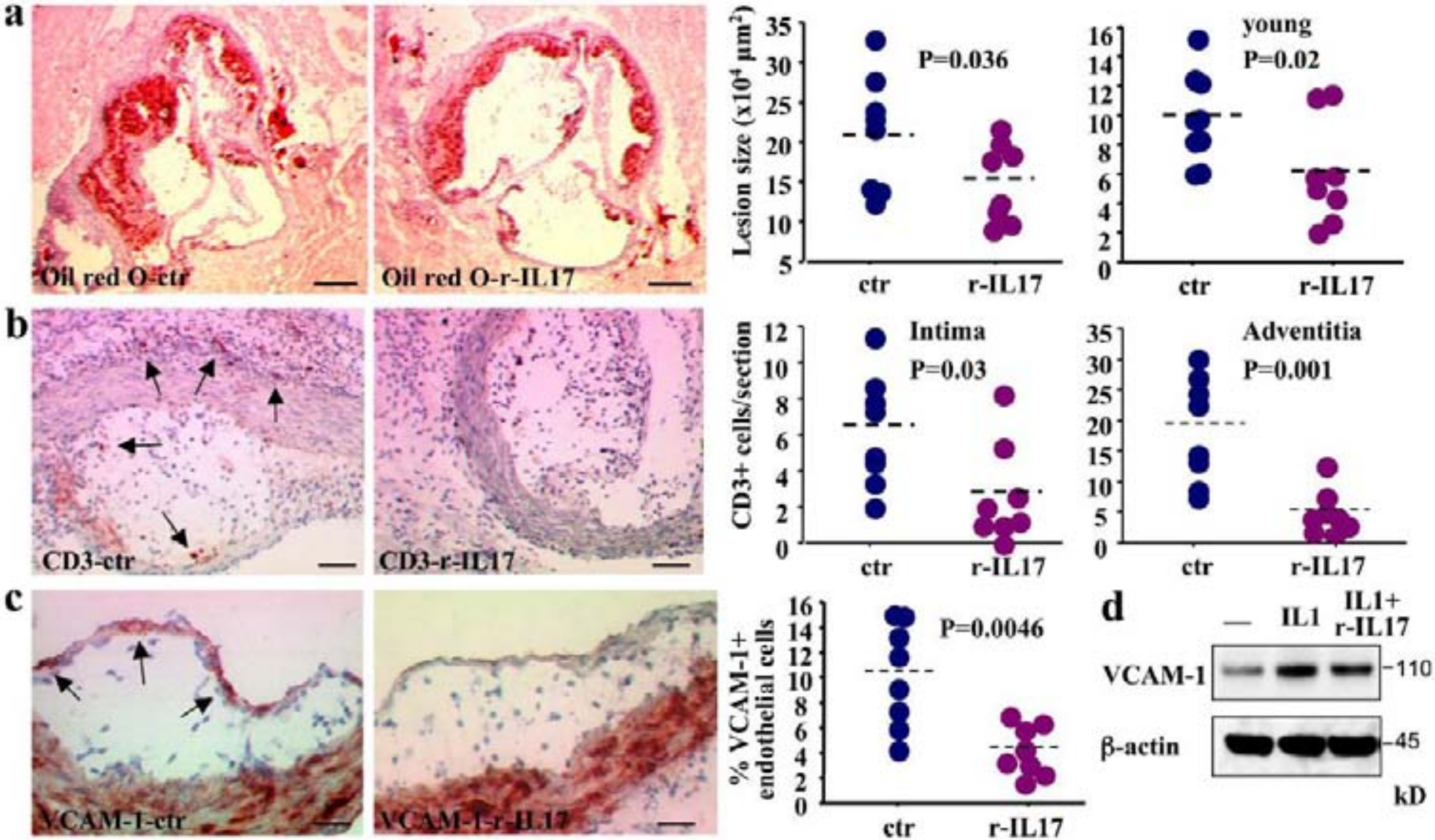



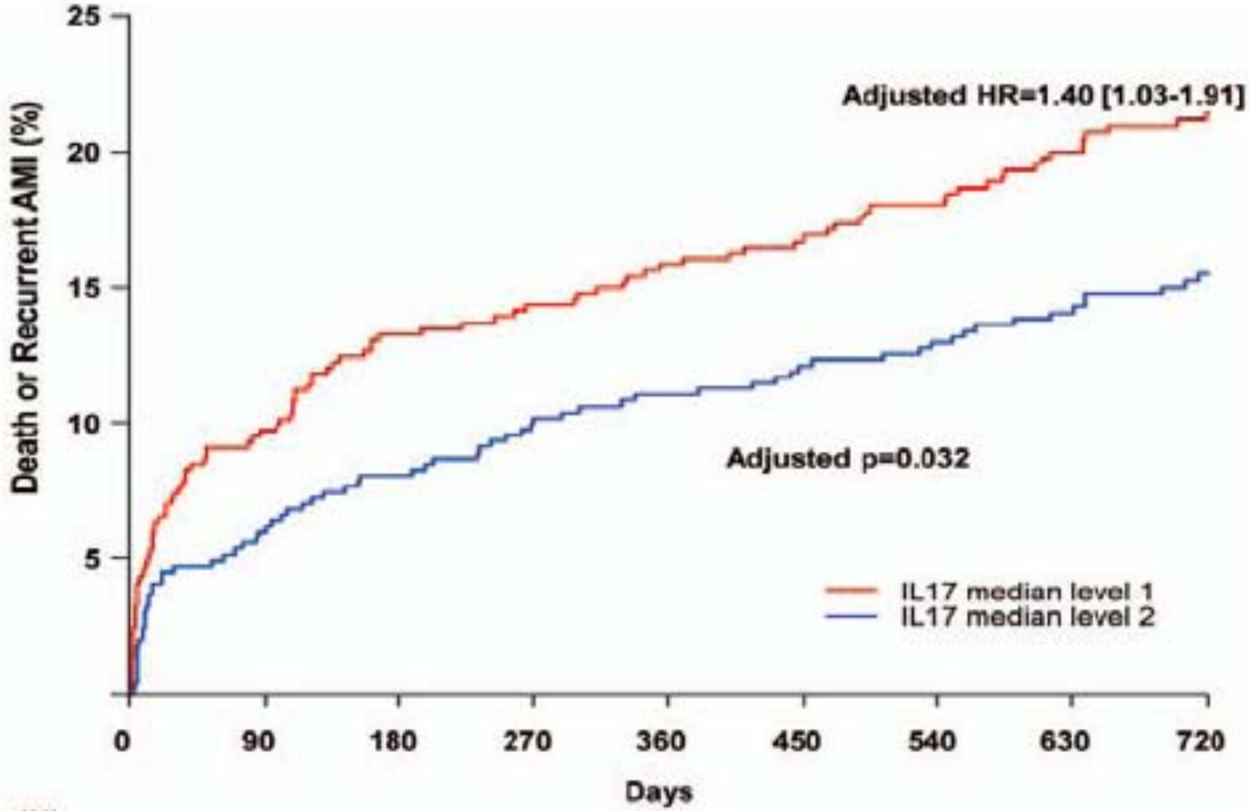

Taleb et al. J Exp Med 2009;206:2067-2077

# IL-17 reduces atherosclerosis in LDLr-/- mice

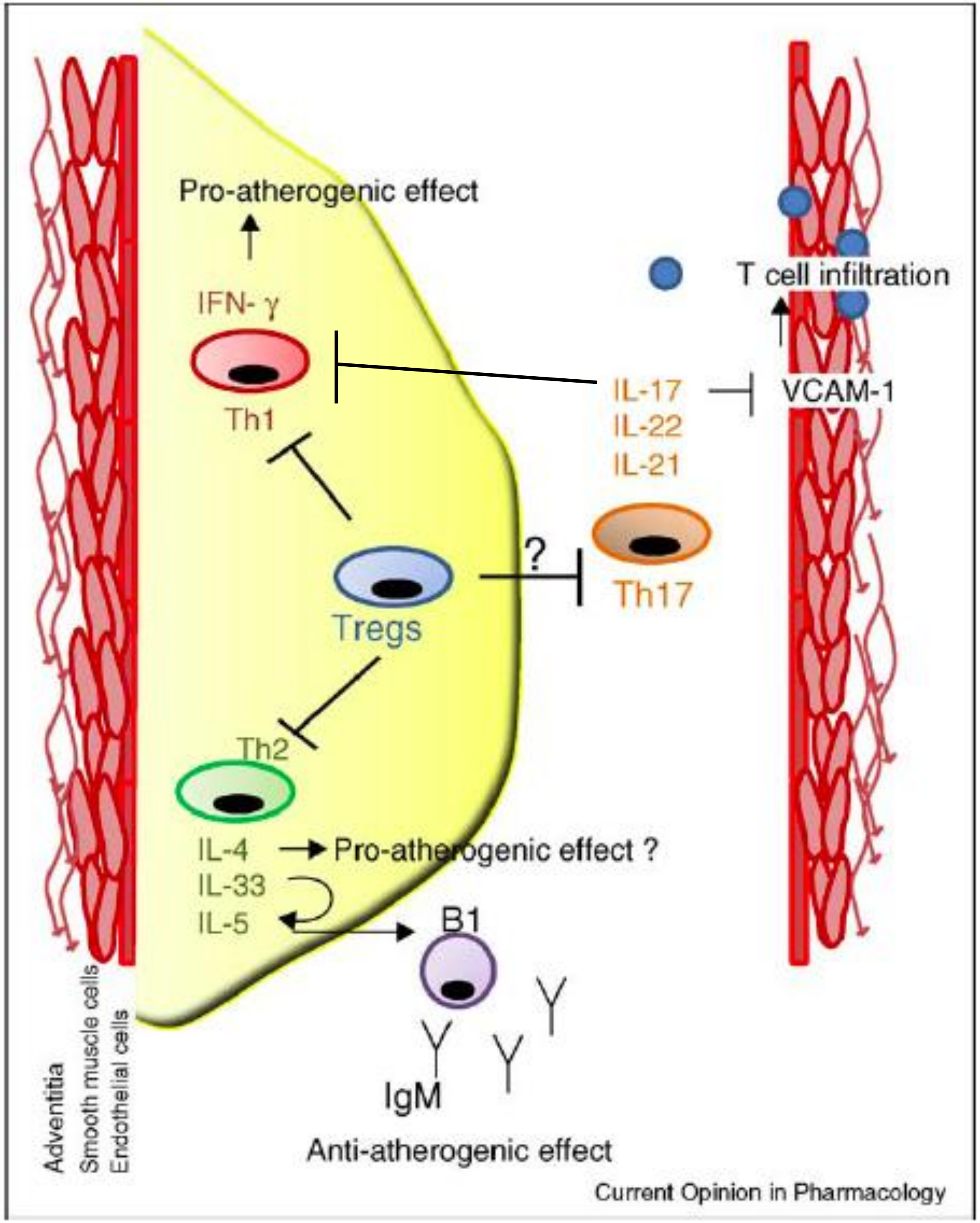


# Circulating levels of interleukin-17 and cardiovascular outcomes in patients with acute myocardial infarction

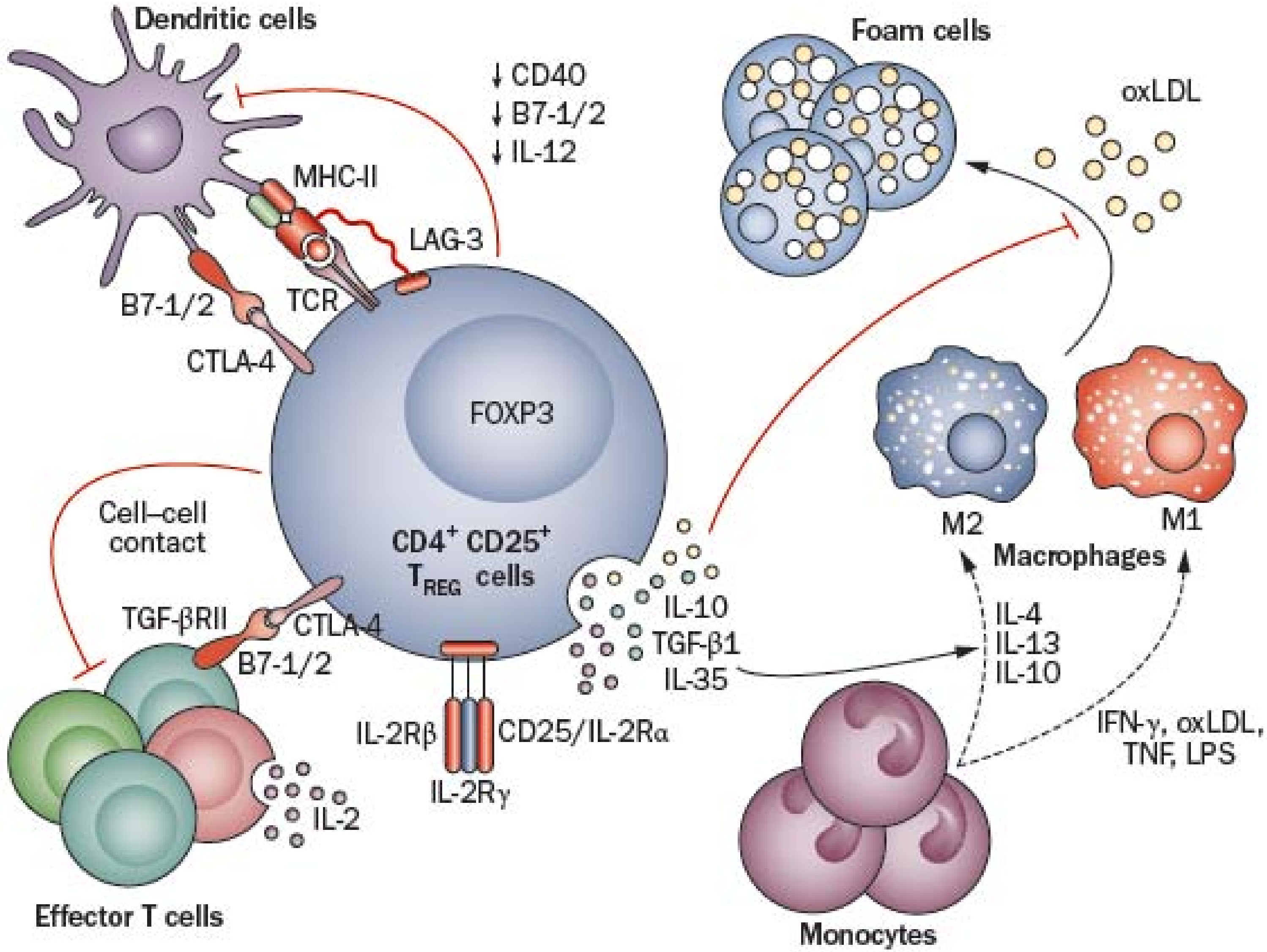
Tabassome Simon[1*], Soraya Taleb[2], Nicolas Danchin[3], Ludivine Laurans[2], Benoit Rousseau[1], Simon Cattan[4], Jean-Michel Montely[5], Olivier Dubourg[6], Alain Tedgui[2], Salma Kotti[1], and Ziad Mallat[2,7*]




| Patients at Risk (N) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *IL17 median level 1* | 490 | 430 | 411 | 402 | 393 | 385 | 377 | 348 | 300 |
| *IL17 median level 2* | 491 | 449 | 439 | 422 | 415 | 409 | 405 | 371 | 321 |




Taleb et al. 2010




Lahoute C et al., Nat Rev Cardiol 2011

Latz E et al,
Nat Rev Immunol 2013