Novel function of NADPH oxidase in atherosclerosis

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Department of Life Science
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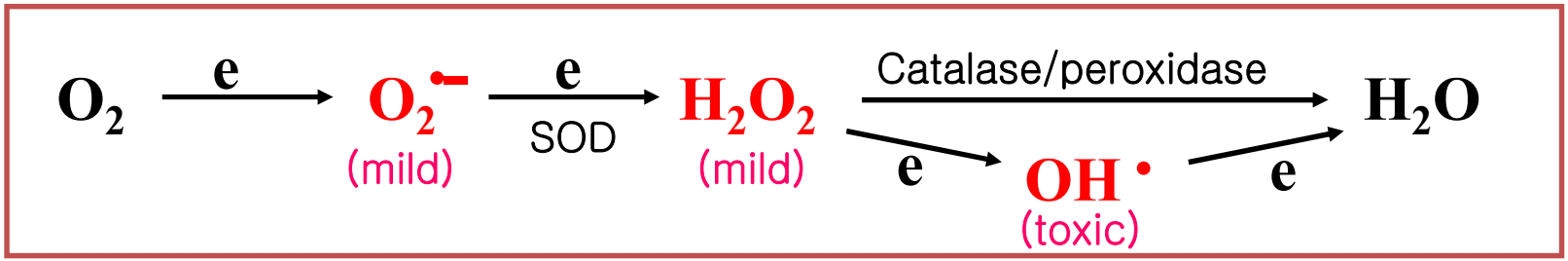
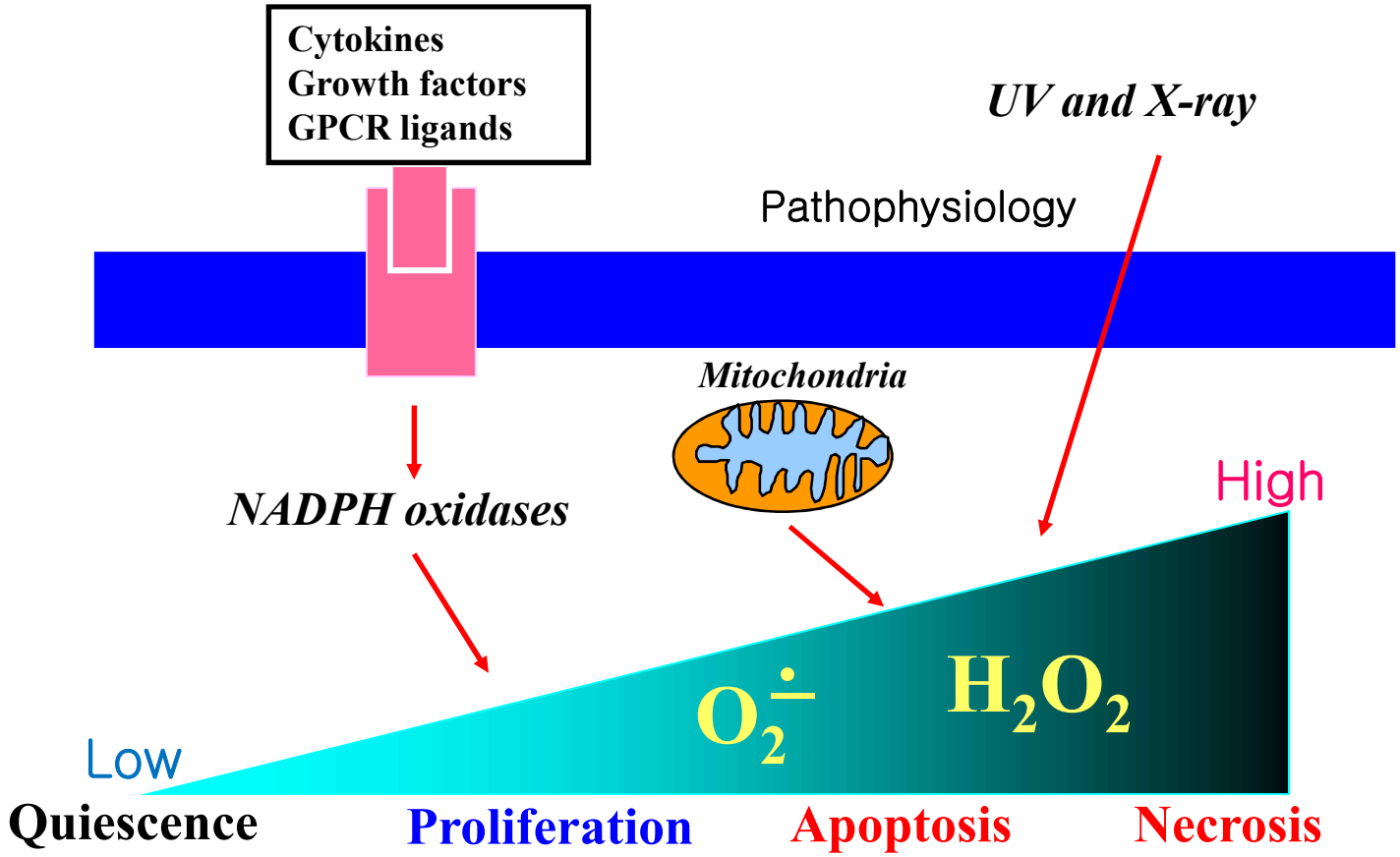
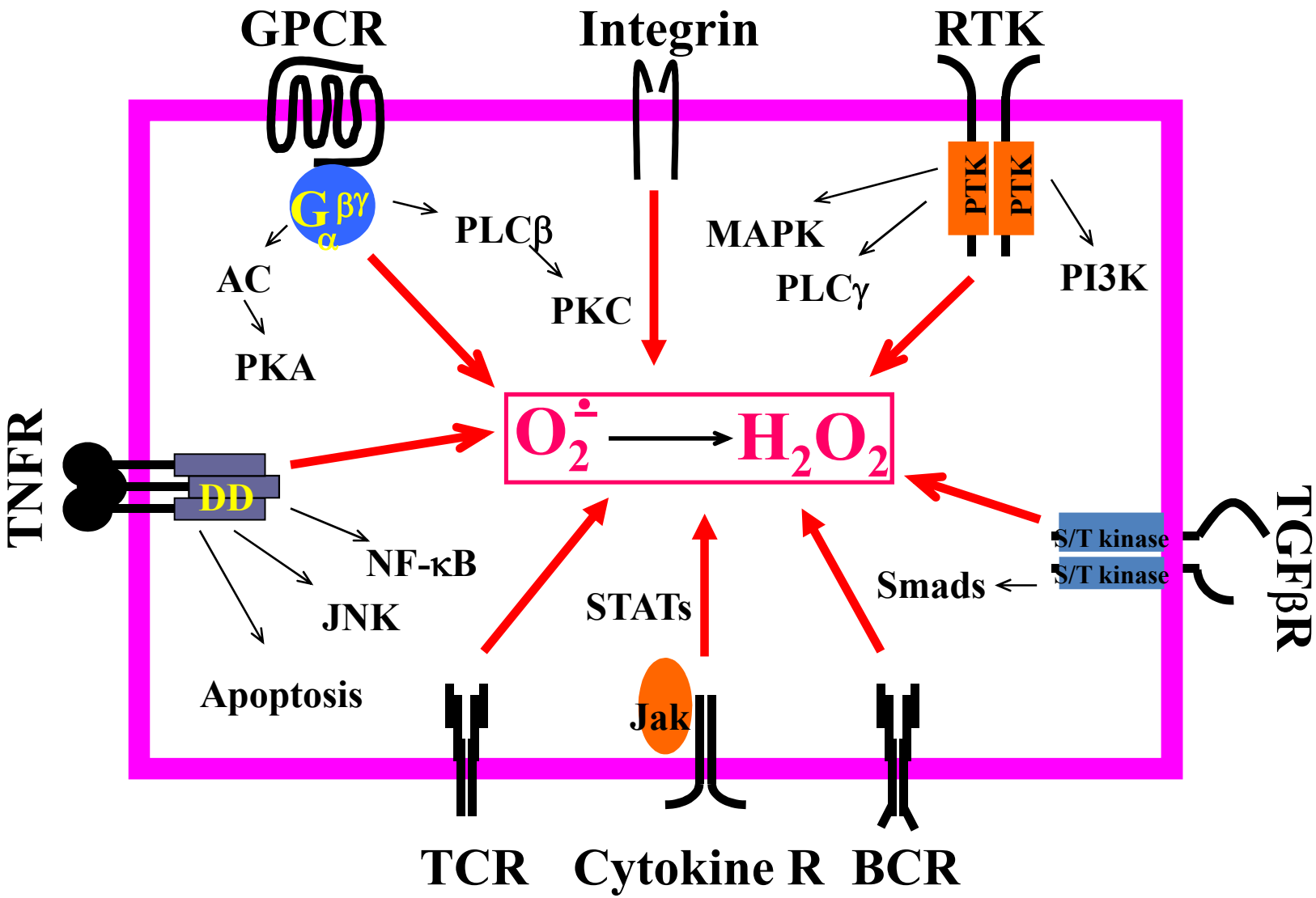
Recent understanding of ROS: act as second messengers

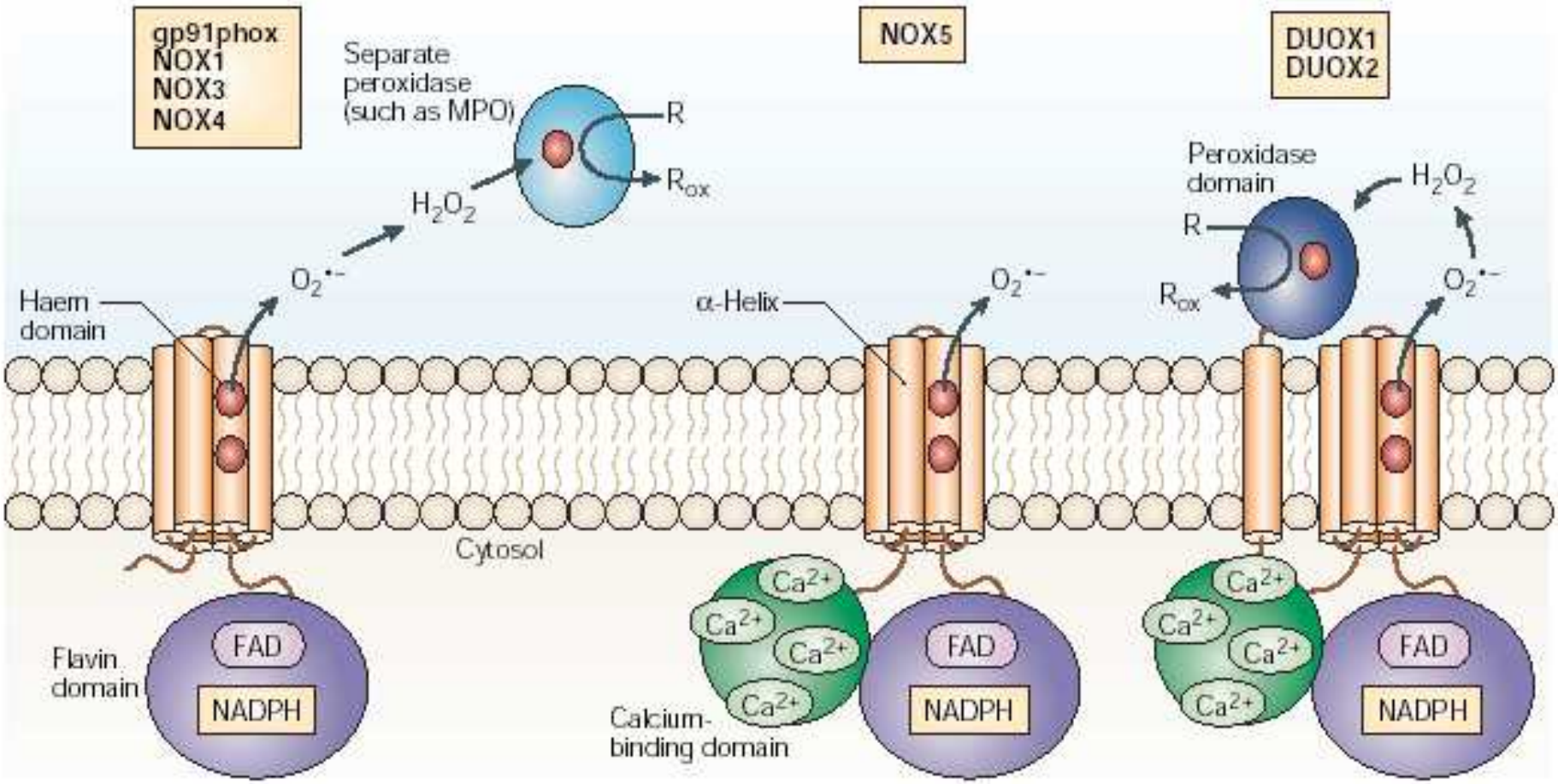






- **ROS production**: temporal (5-10 min) and spatial production
- **Second messenger**: Scavenging of ROS by addition of antioxidants blocks agonist-dependent cell signaling
- It occurs in plasma membrane, not mitochondria: **NADPH oxidase**

Transmembrane topology of NADPH oixdase(Nox) isozymes:
Connection of Nox with various receptors



Lambeth JD, *Nature Rev. Immunol.* 4, 181, 2004

What is atherosclerosis?

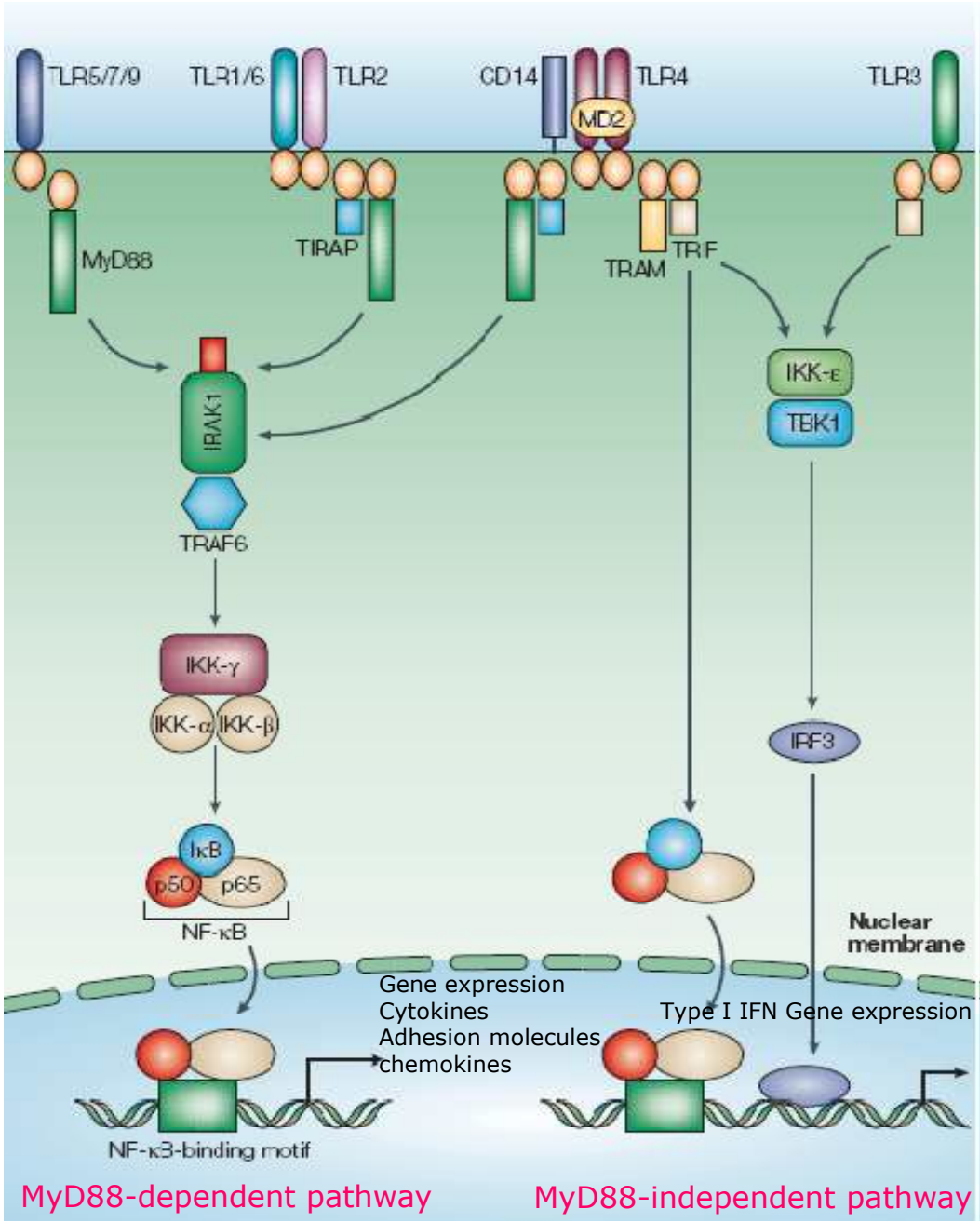
"Atherosclerosis involves the formation in the arteries of lesions that are characterized by inflammation, lipid accumulation, cell death, and fibrosis."
Hansson and Libby Nature Rev. Immunol 6; 508 (2006)

"Inflammatory mechanisms couple dyslipidaemia to atheroma formation."
Libby Nature 420; 868 (2002)

Concerted action of lipid accumulation and inflammation

Inflammation processes in MØ and EC play an important role in initiation and early progression of atherosclerosis

Toll-like receptor signalling.



Receptor	Ligand	Origin of ligand
TLR1	Triacyl lipopeptides	Bacteria and mycobacteria
TLR2	Lipoprotein/lipopeptides Peptidoglycan Lipoteichoic acid	Various pathogens Gram-positive bacteria
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharide	Gram-negative bacteria
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides Lipoteichoic acid	*Mycoplasma* Gram-positive bacteria
TLR7	Single-stranded RNA	Viruses
TLR8	Single-stranded RNA	Viruses
TLR9	CpG-containing DNA	Bacteria and viruses
TLR10	not determined	not determined
TLR11	not determined	Uropathogenic bacteria

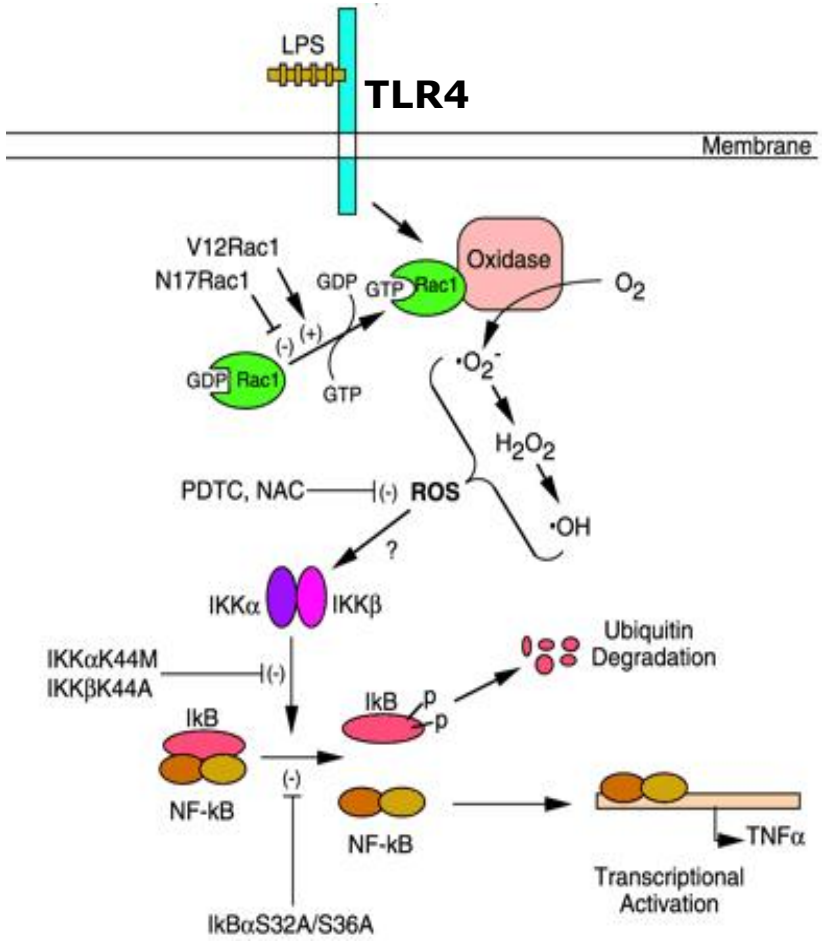
NATURE REVIEWS 2004

TLR4-induced proinflammatory activation in human coronary artery cells

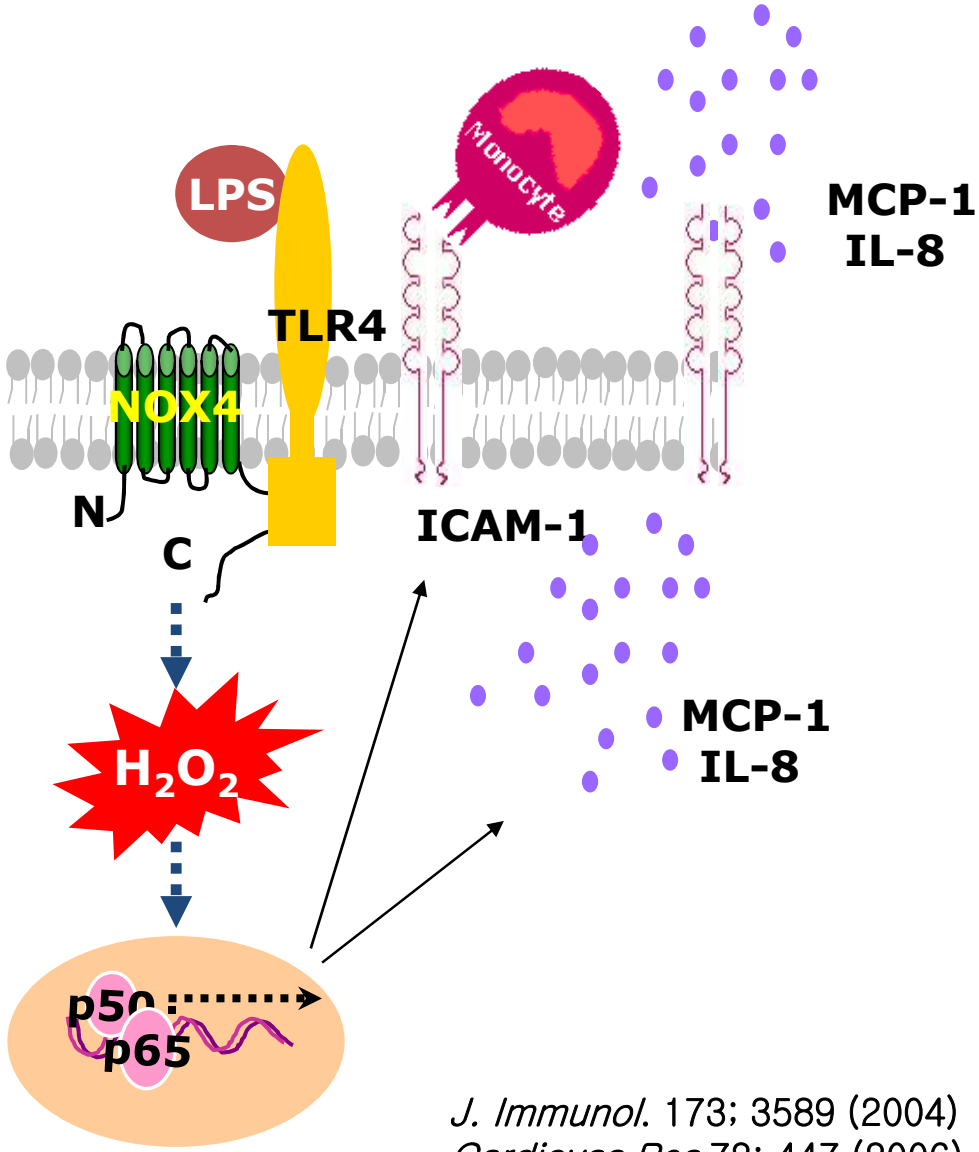
Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways.
Bjorkbacka H *et al.* *Nature Med.* 10:416-21 (2004)

Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E.
Michelsen KS *et al.* *Proc Natl Acad Sci U S A*. 101:10679-84 (2004).

Production of ROS and ICAM-1/MCP-1 play an important role in early event of atherogenesis

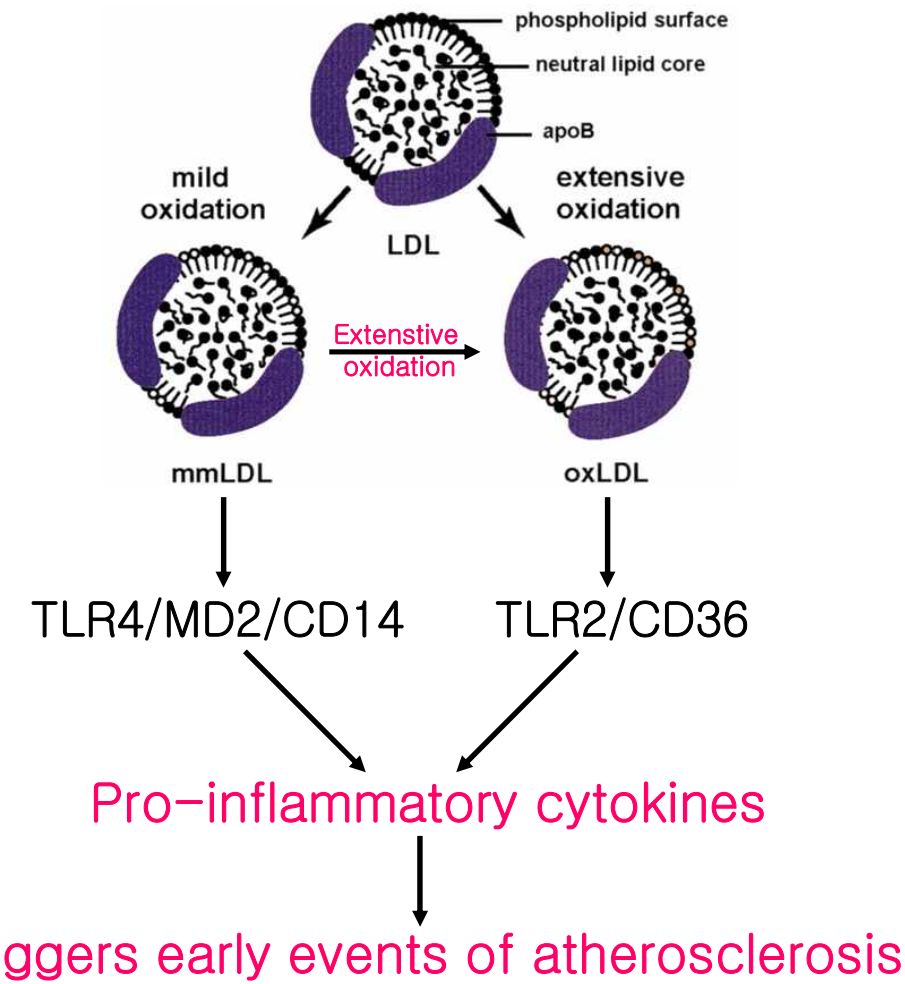


JBC 276; 30188 (2001)



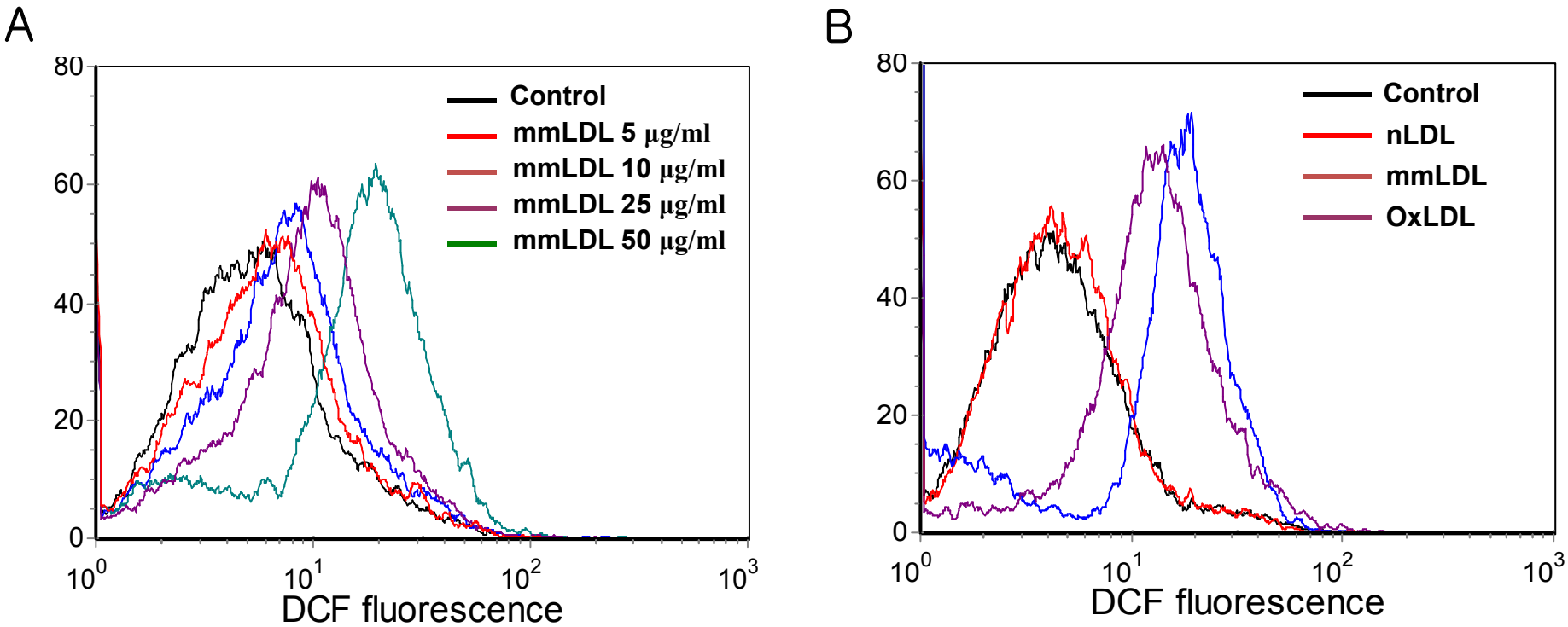
J. Immunol. 173; 3589 (2004)
Cardiovas Res 72; 447 (2006)

What is high risk endogenous molecule causing atherosclerosis?

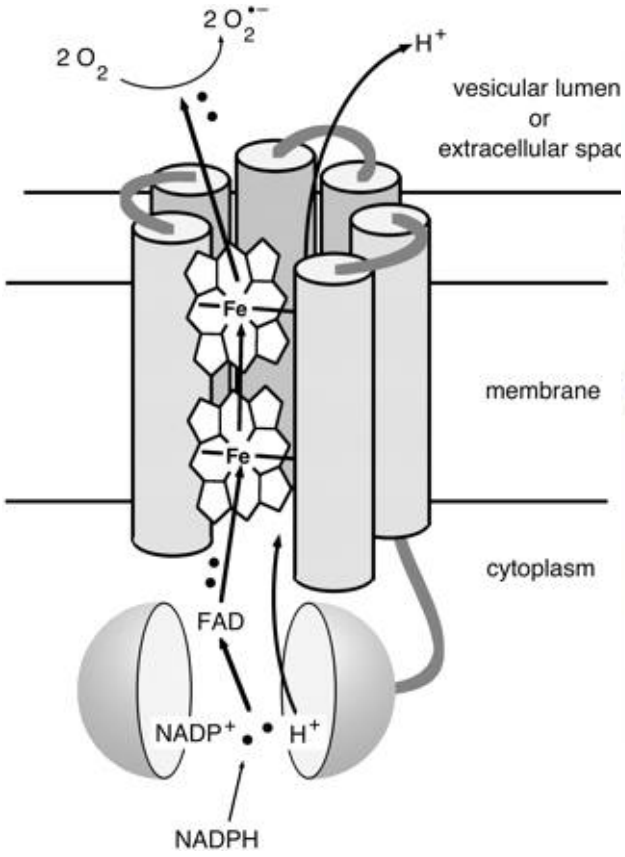


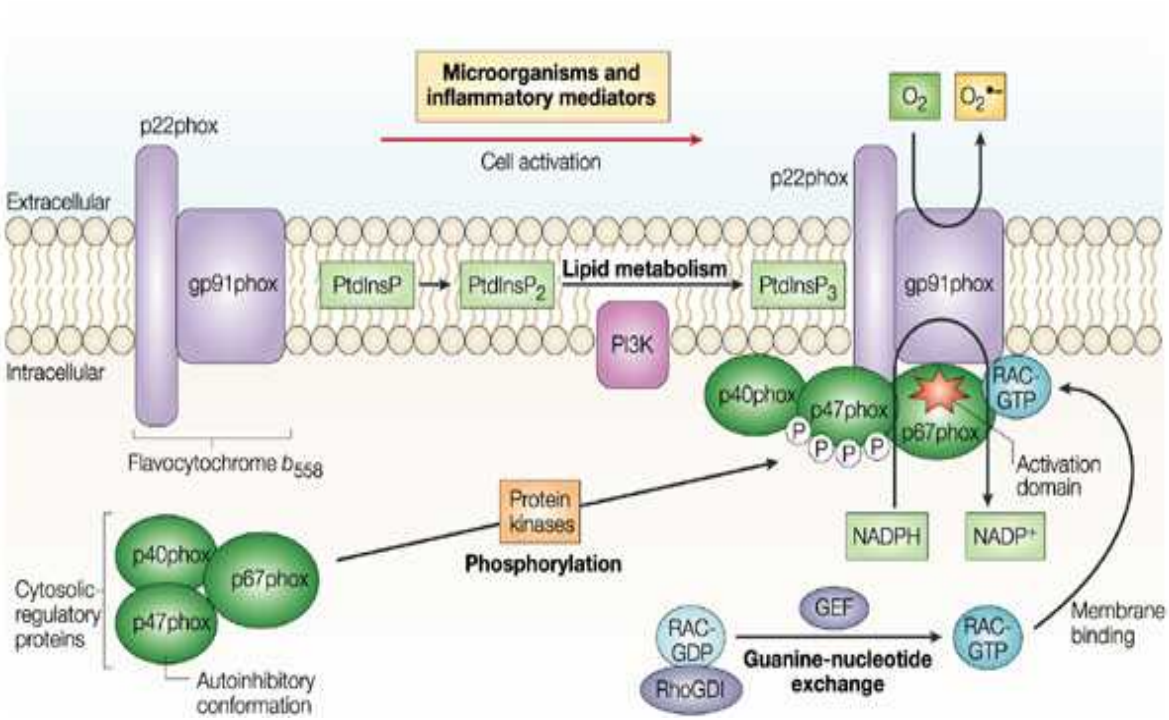
Miller et al., *JBC* 2003; Miller et al., *MBC* 2003; Miller et al., *ATVB* 2005; Boullier et al., *ATVB* 2006

mmLDL and oxLDL induce generation of ROS but not nLDL in J774 cells



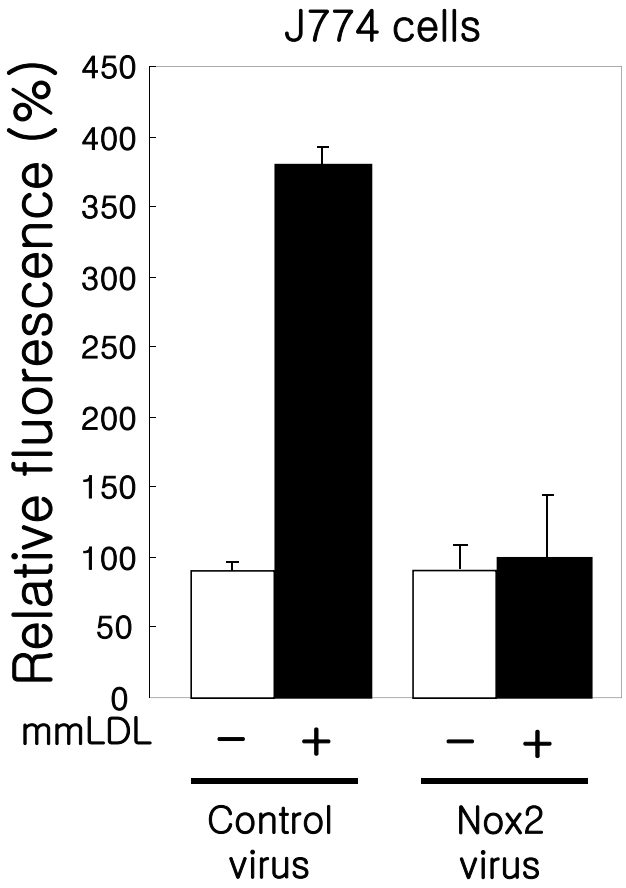
Activation of gp91phox (Nox2) by assembly of regulatory proteins in phagocytes

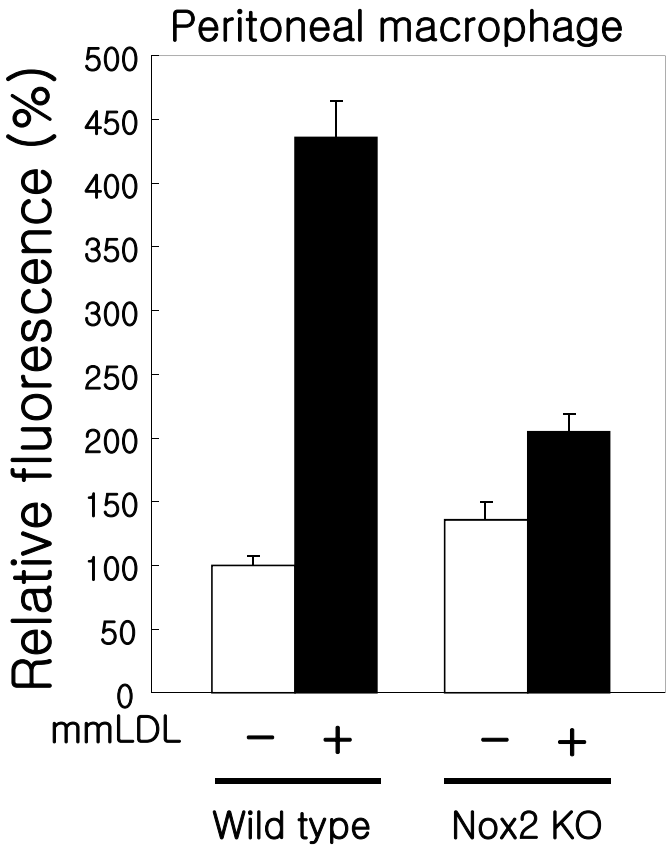


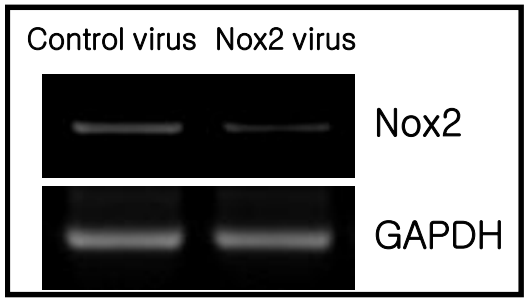


Lambeth JD, *Nature Rev. Immunol.* 4, 181, 2004

Nox2-defective macrophage fails to ROS generation in response to mmLDL







Signaling network by mmLDL in macrophage

mmLDL

↓

TLR4/MD2/CD14 activation

↓

Syk activation

↓

PLCγ-p

↓

PKC

↓

Nox2

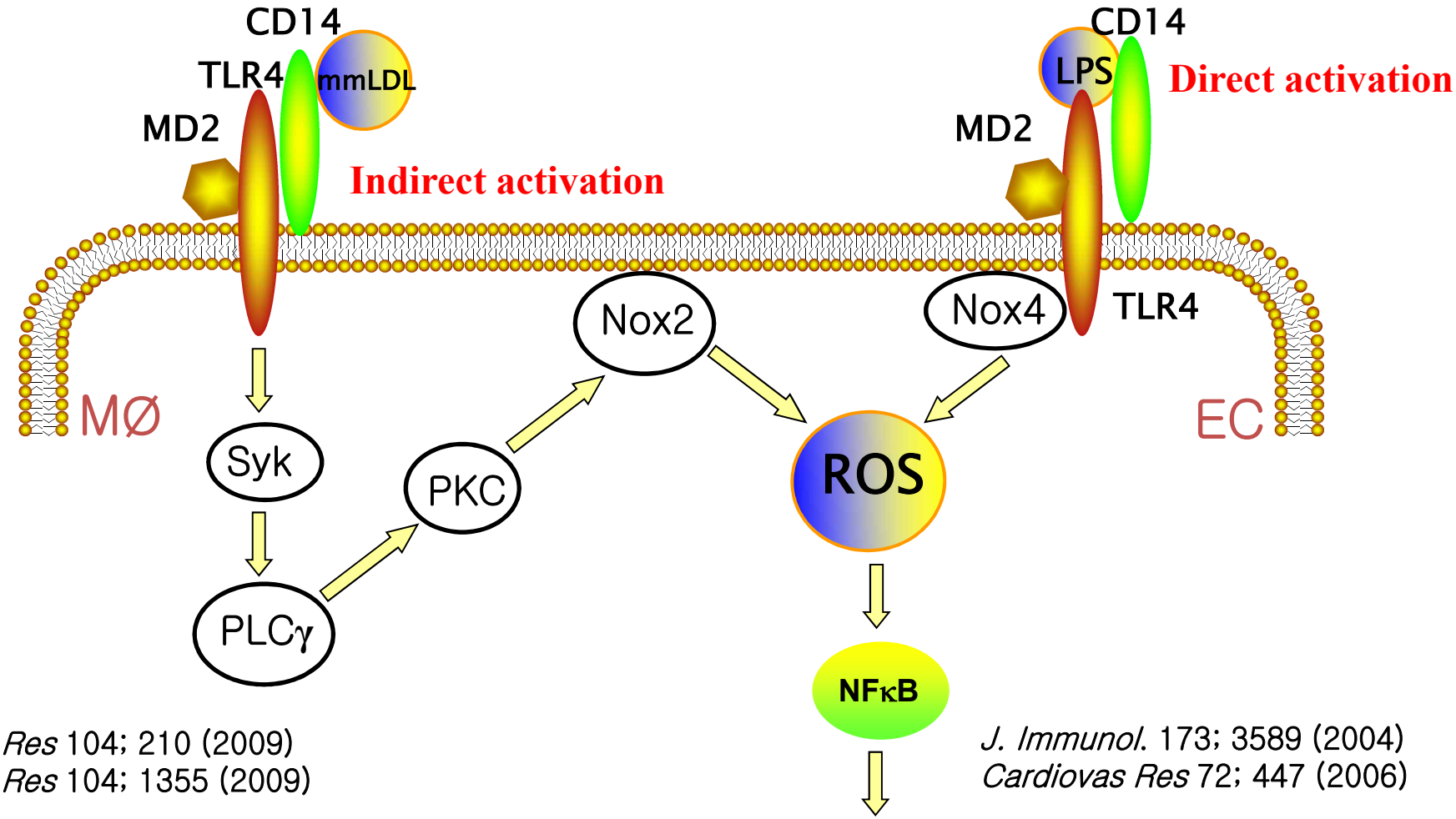
↓

H_2O_2

↓

Pro-inflammatory cytokines production (RANTES, IL-6)
Migration of smooth muscle cell

Signaling pathways of mmLDL/LPS for macrophage activation

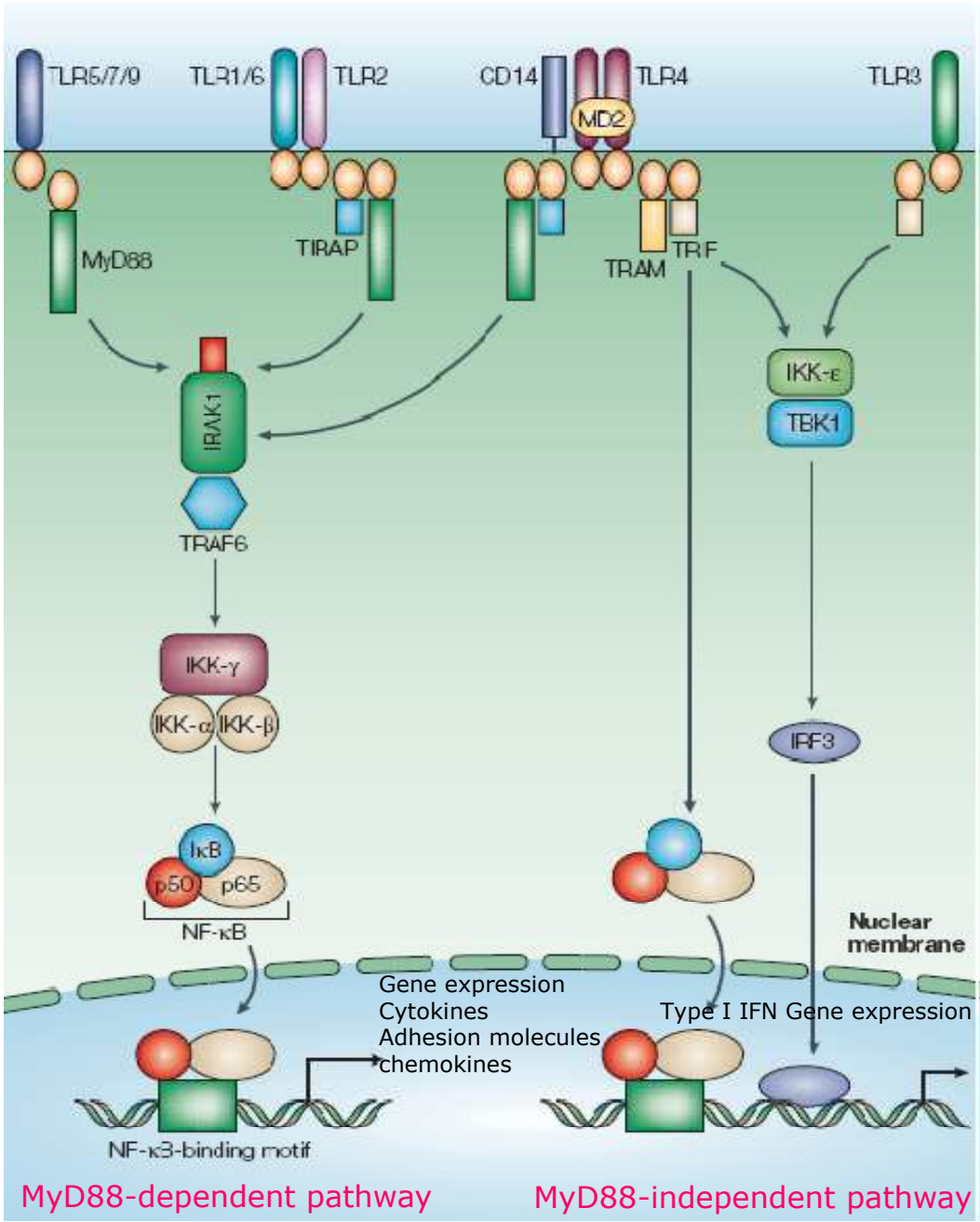


Circ Res 104; 210 (2009)
Circ Res 104; 1355 (2009)

J. Immunol. 173; 3589 (2004)
Cardiovas Res 72; 447 (2006)

Augmented **expression of pro-inflammatory cytokines and stimulated the migration of SMC**

Toll-like receptor signalling.



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TLR10	not determined	not determined
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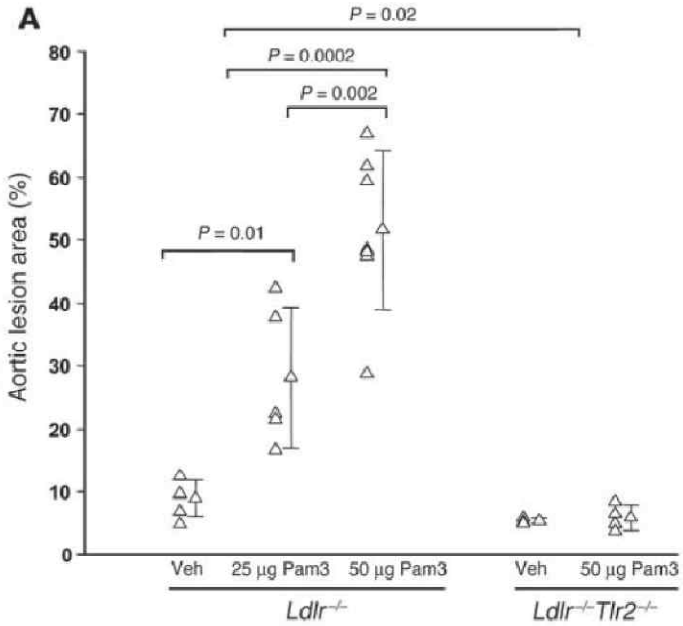
NATURE REVIEWS 2004

Modulation of atherosclerosis in mice by Toll-like receptor 2

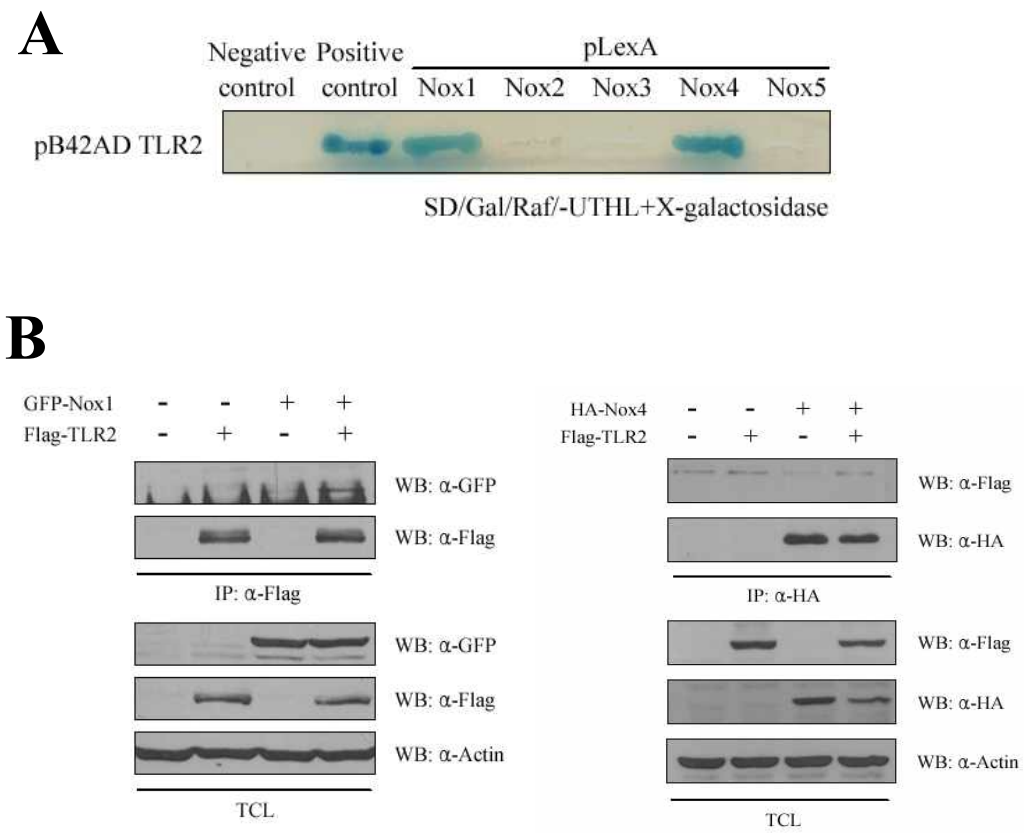
Adam E. Mullick, Peter S. Tobias, and Linda K. Curtiss

Department of Immunology, The Scripps Research Institute, La Jolla, California, USA.



J. Clin. Invest. **115**:3149–3156 (2005)

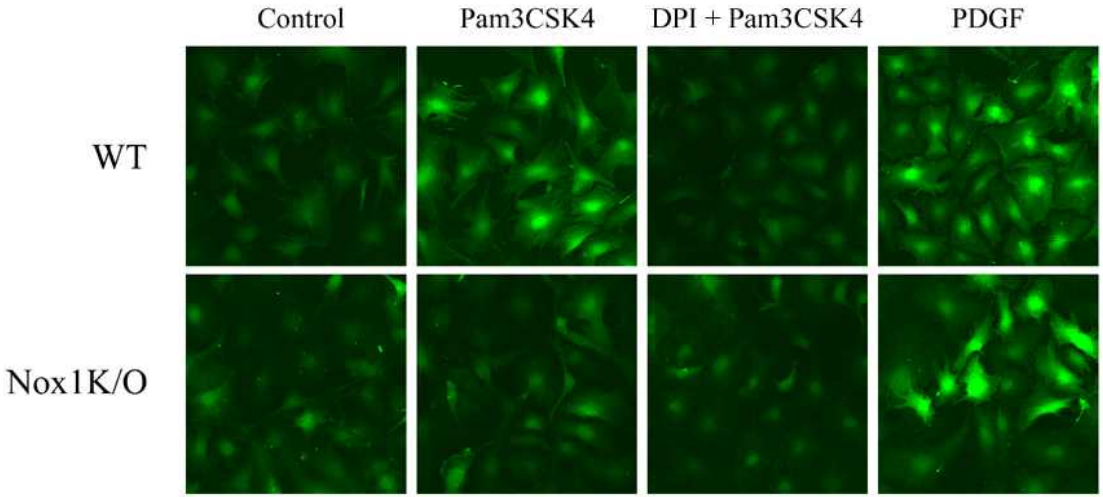
Interaction between TLR2 and ROS generation system



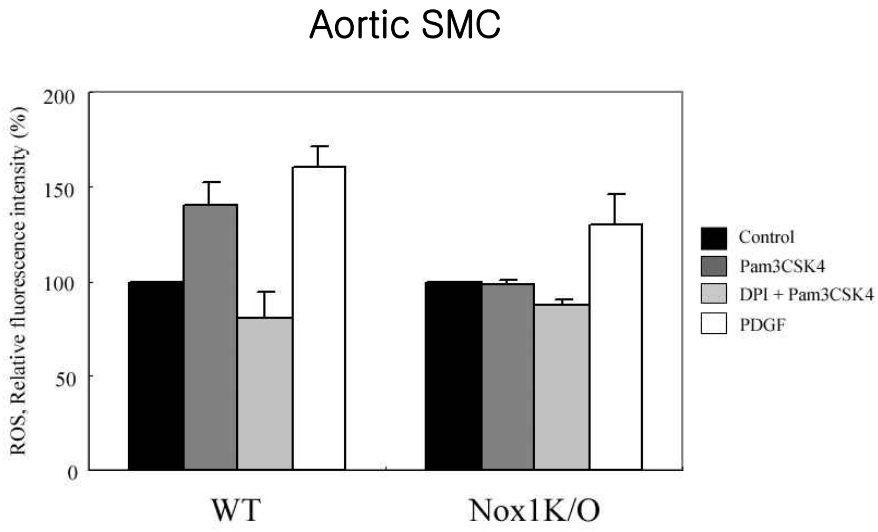
TLR2 interacts with Nox1, but not with Nox4

Functional analysis of the interaction between TLR2 and Nox1

A



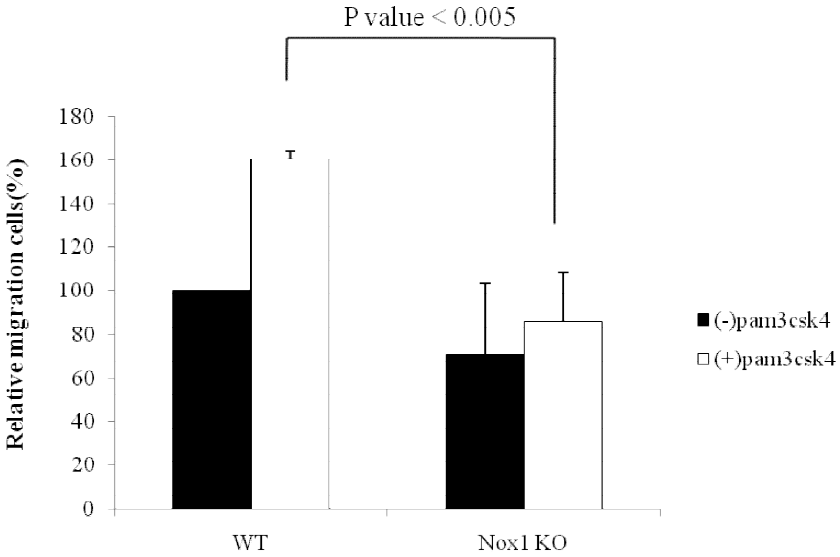
B



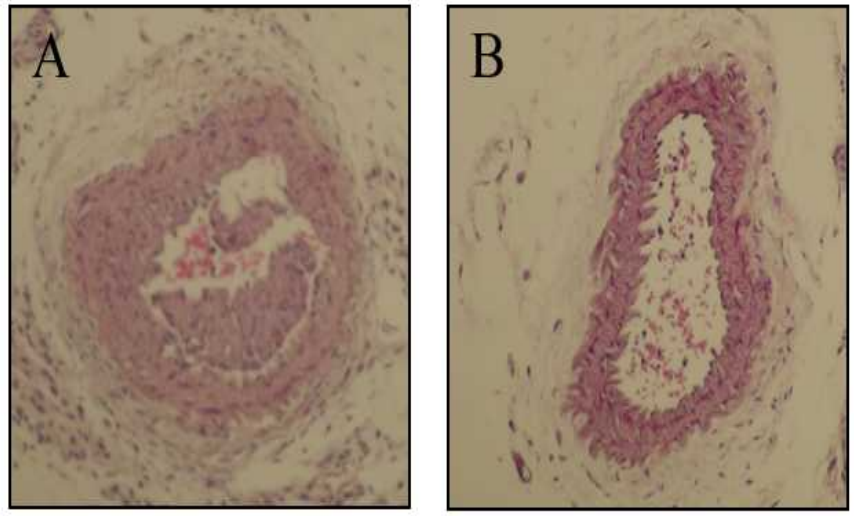
Nox1 is required for Pam3CSK4-induced ROS generation in ASMCs

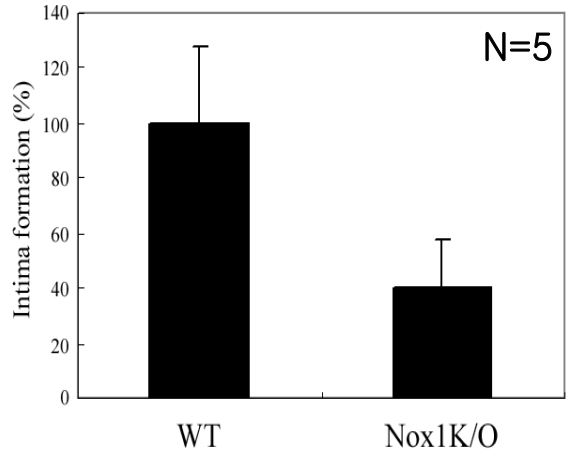
Nox1-dependent SMC migration and restenosis in ligation injury model

A



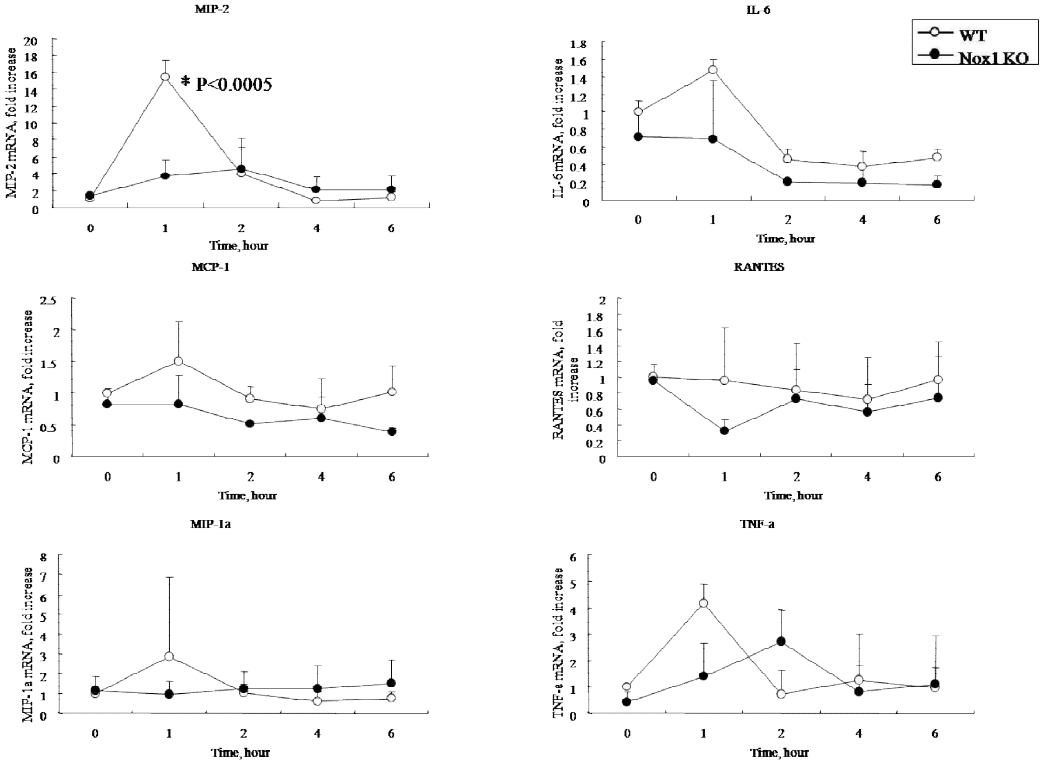
WT **Nox1K/O**



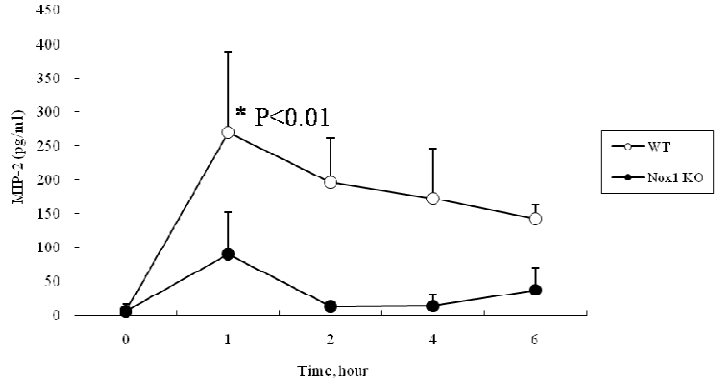


Pam3CSK4-induced of cytokines in murine ASMC

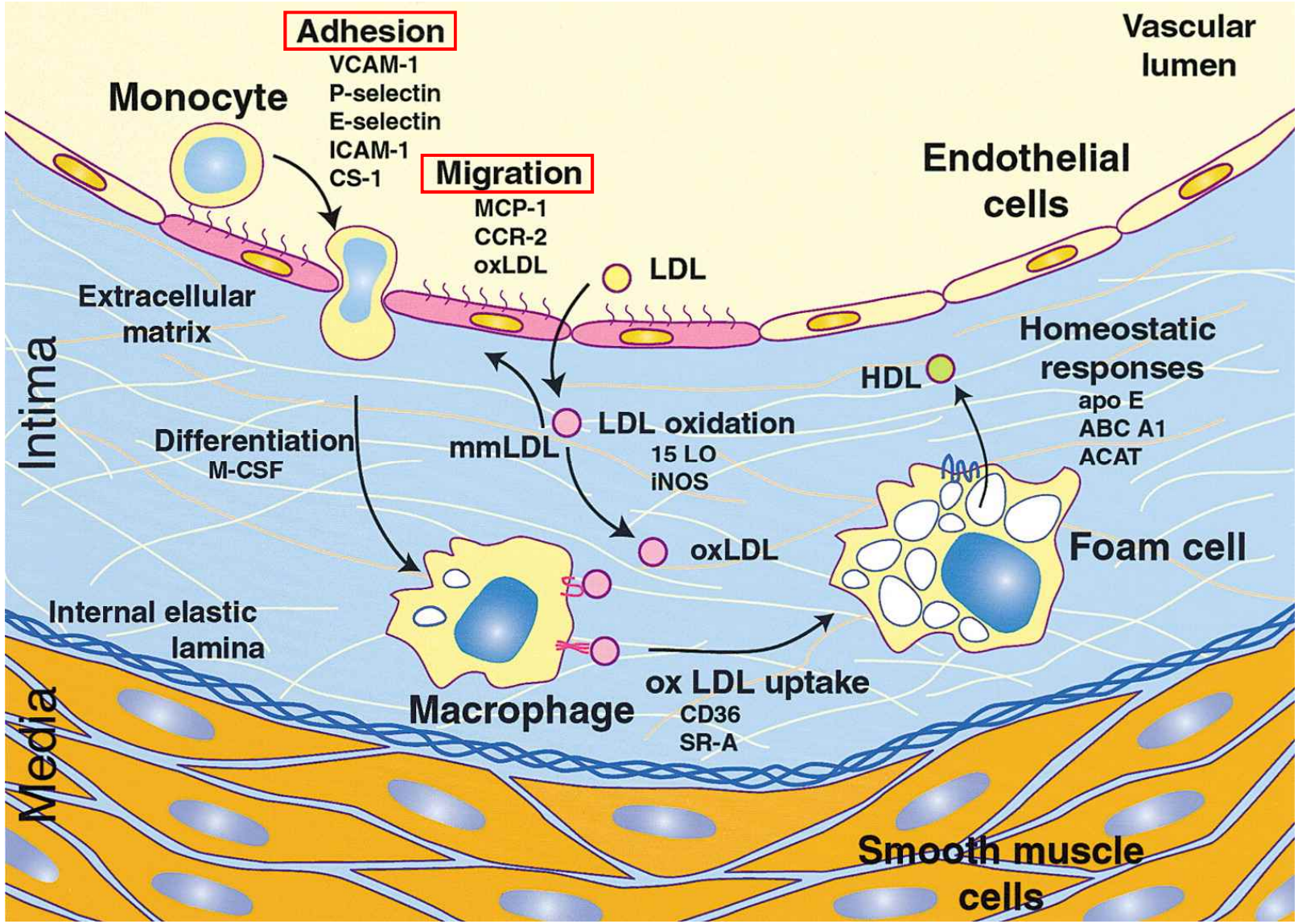
A: Real time PCR



B: ELISA for MIP-2

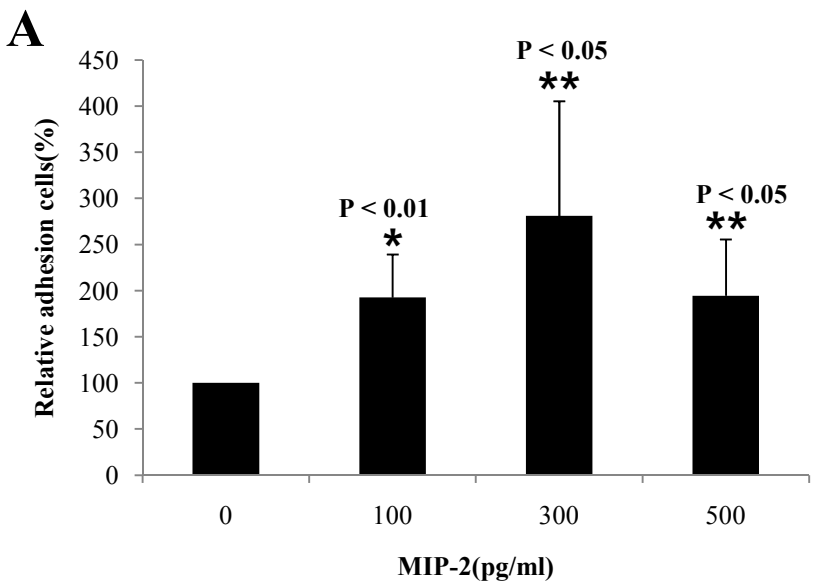


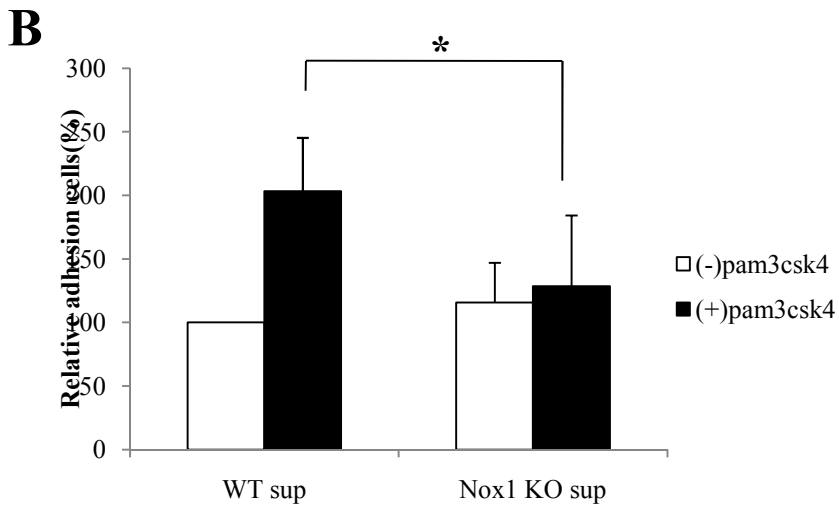
Proinflammation events in atherogenesis



Cell 104: 503-516 (2001)

Pam3CSK4-dependent SMC stimulates U937 monocyte adhesion to HAEC





SMC w/ or w/o Pam3CSK

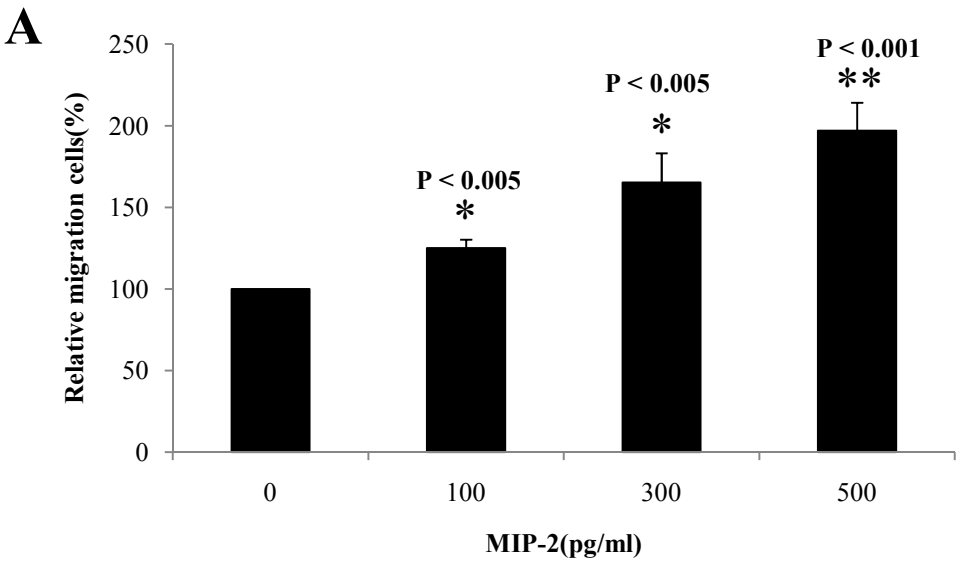
↓

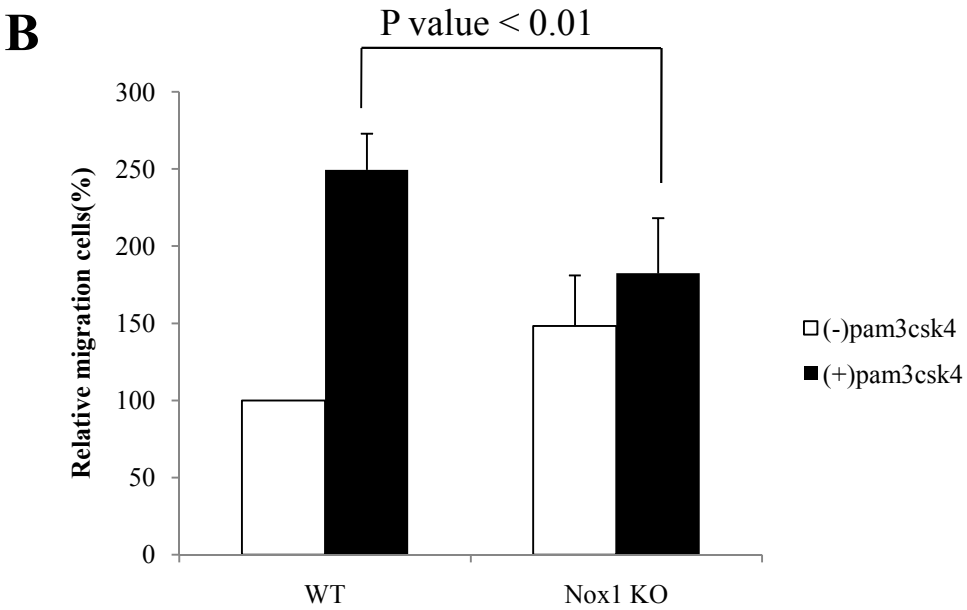
Culture supernatant containing MIP2

↓

Adhesion analysis with HAEC and U937 in Transwell

Pam3CSK4-dependent SMC stimulates U937 transendothelial migration





SMC w/ or w/o Pam3CSK

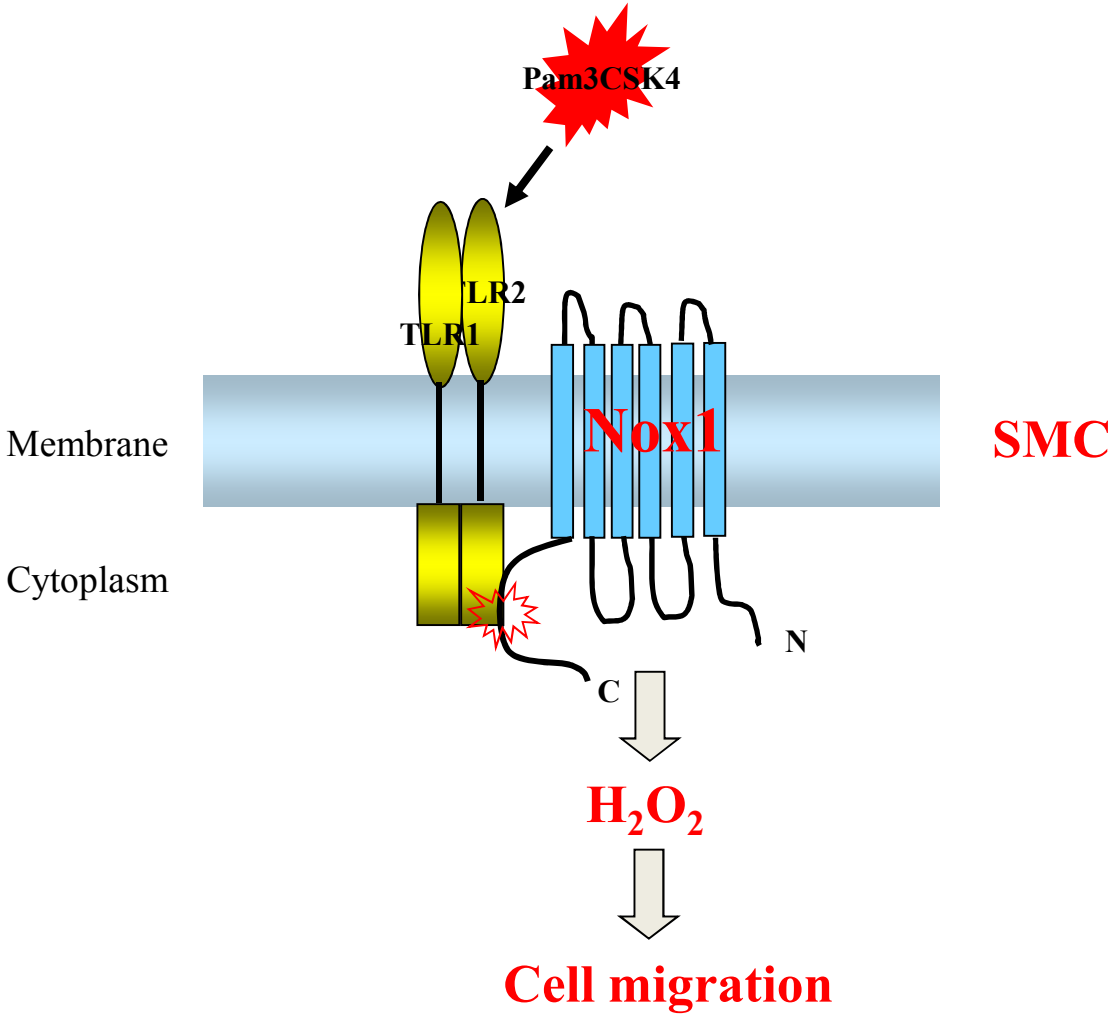
↓

Culture supernatant containing MIP2

↓

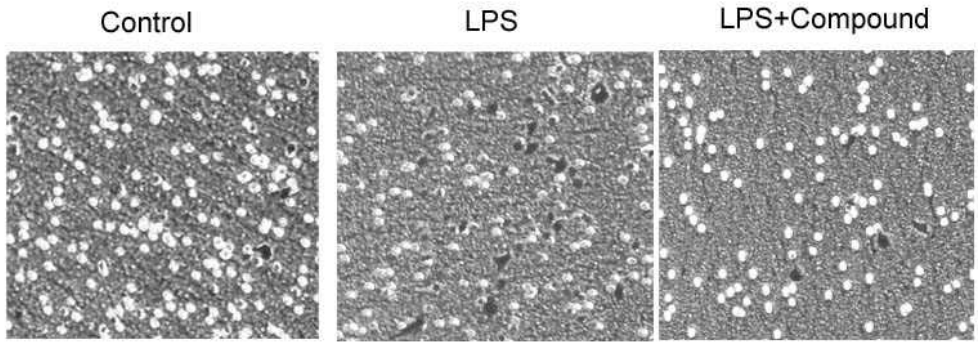
Transmigration analysis with HAEC and U937 in Transwell

Conclusions

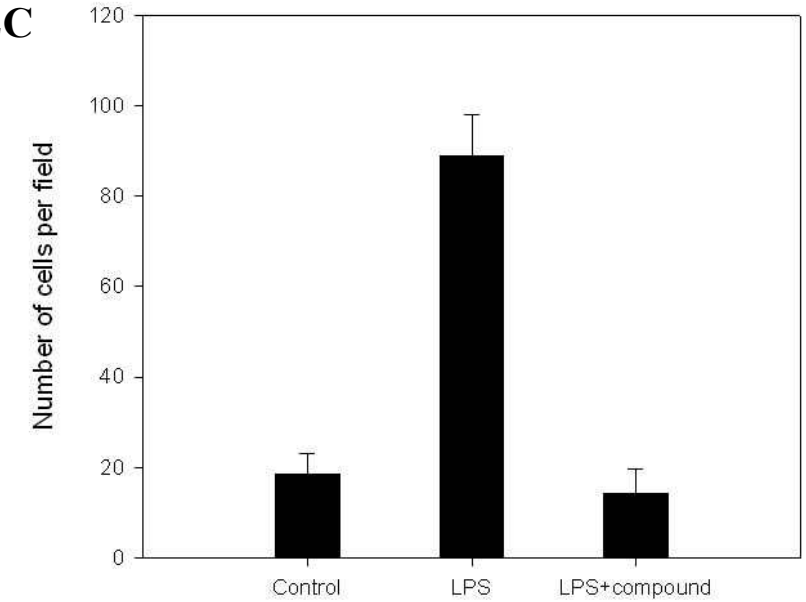


Nox1 plays an important role in TLR2-mediated ROS generation which in turn stimulates SMC migration and vascular remodeling.

Can Nox isozymes be good drug targets in restenosis or atherosclerosis?

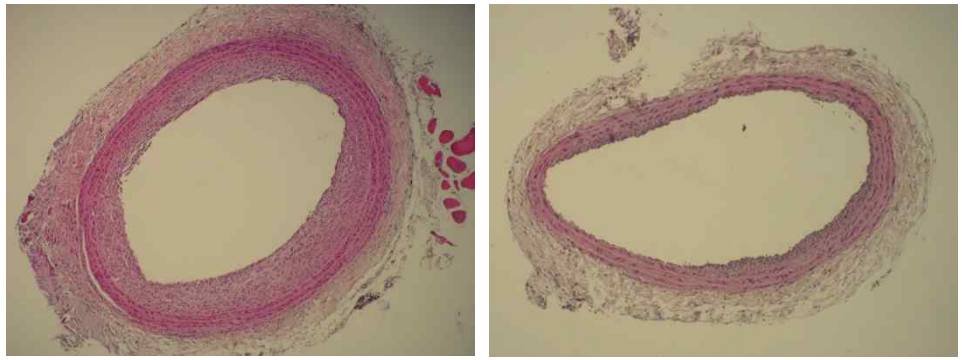


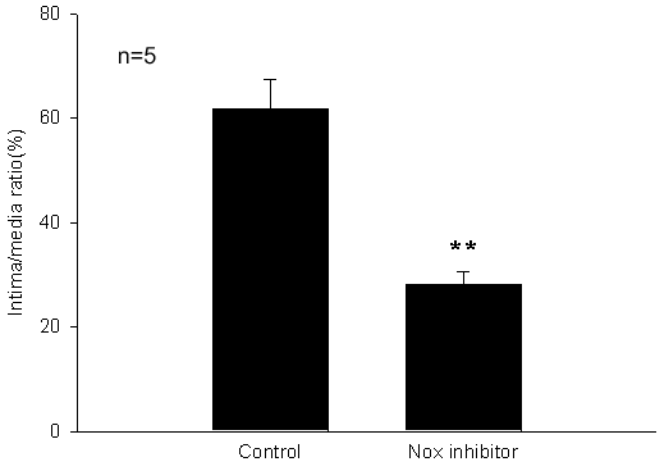
Cell migration assay in EC



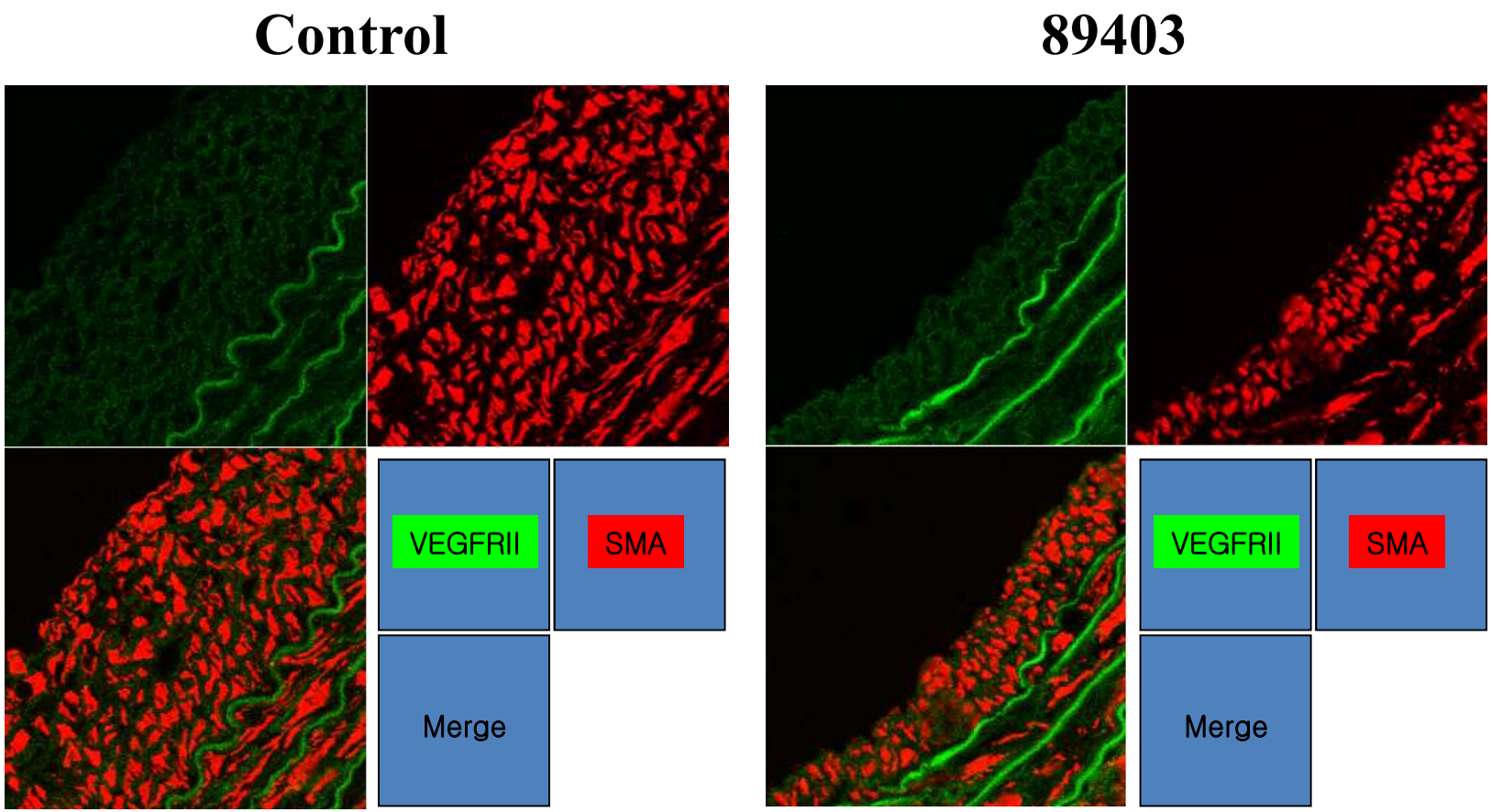
Suppressive effects of Nox inhibitor on balloon-injured neointima formation

Control **Nox inhibitor**

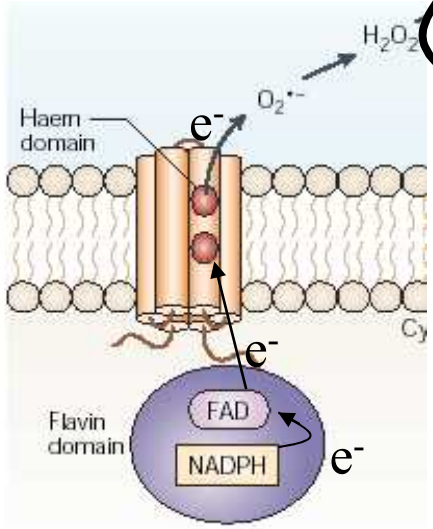


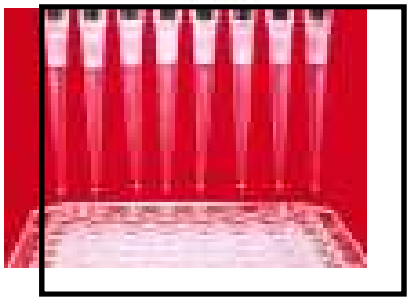


Immunohistochemistry analysis



Development of Nox inhibitor





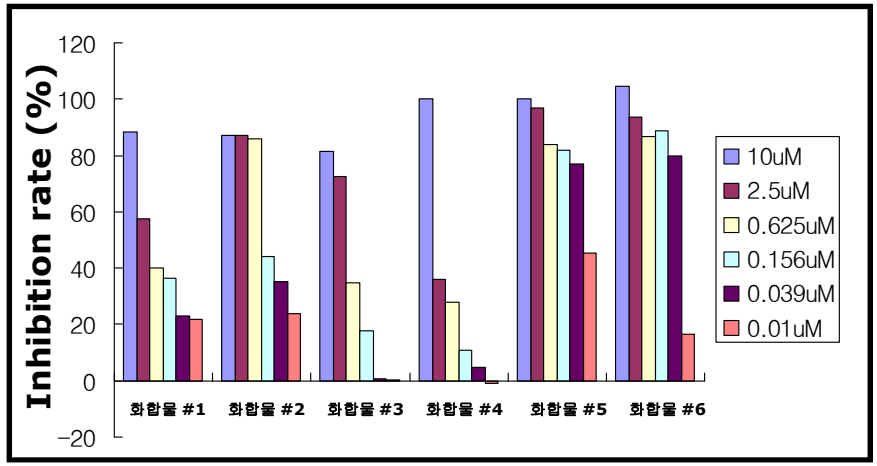
ChemGenomics 25,000

HTS Readout

Lucigenin (red)

Lucigenin (ox)

detect

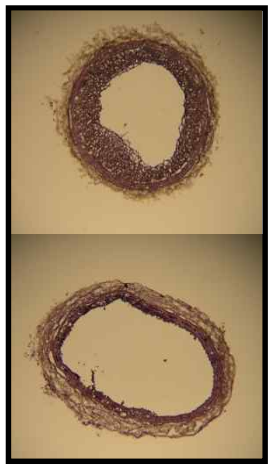


In vivo

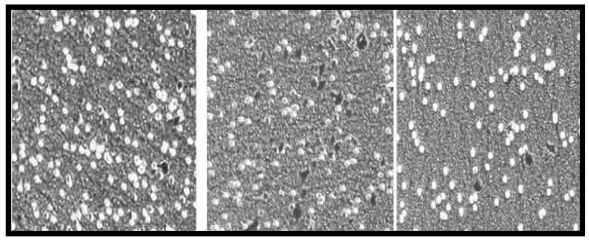
In vitro

Control

inhibitor



Control LPS LPS + hit#5



Cell migration assay

Lead

Acknowledgments

Department of Life Sciences
Ewha Womans University

Jee Hyun Lee: mmLDL/TLR4
Jee Hyun Lee, Sunah Kim: Pam3CSKTLR2
Hye Sun Park: LPS/TLR4

Professor Sang Won Kang

Department of Medicine
UCSD

Dr. Joseph Witztum
Dr. Yury Miller