



# **The Role of TLR2 in the Pathogenesis of Atherosclerosis**

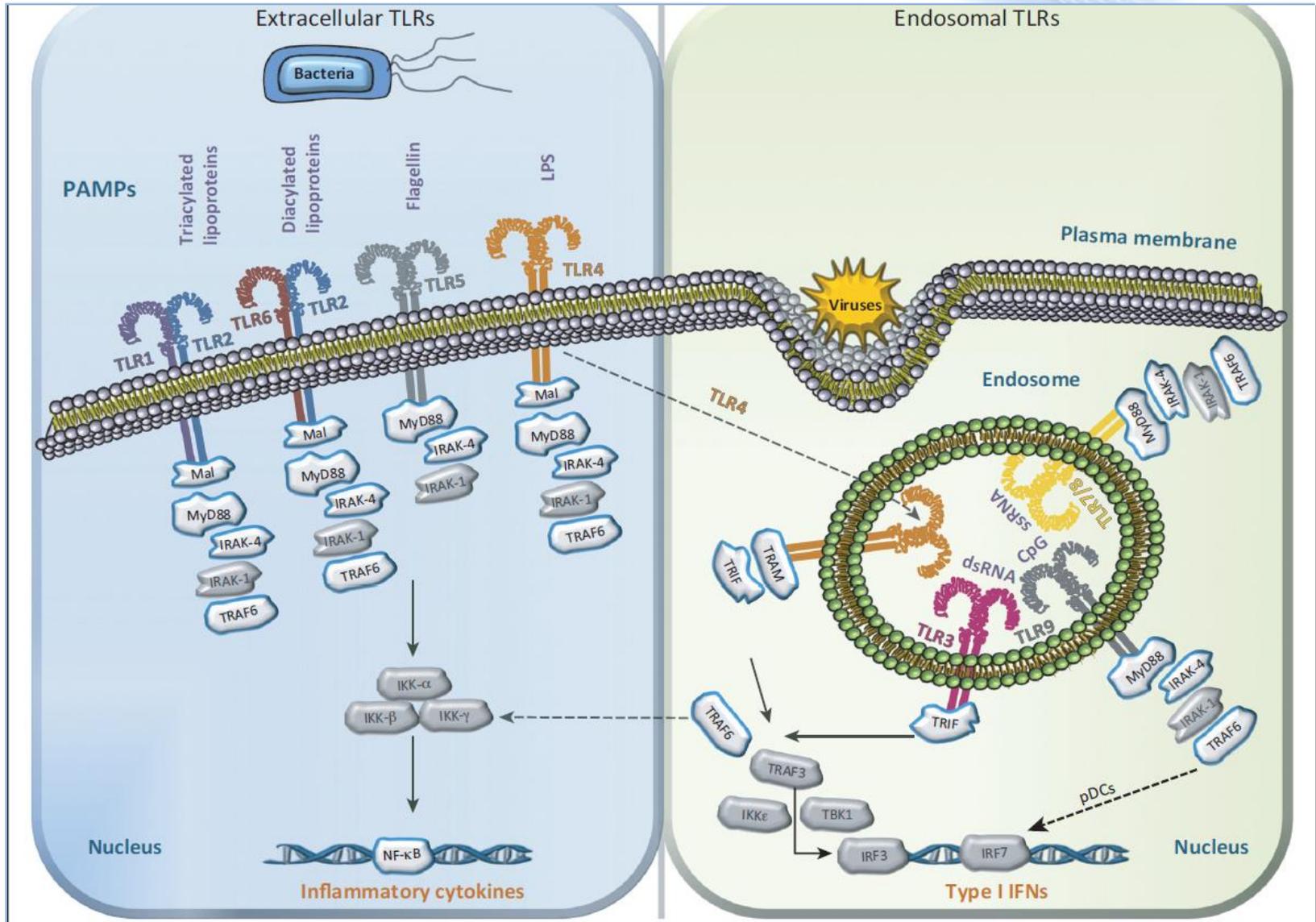
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**Sungha Park**

**Division of Cardiology  
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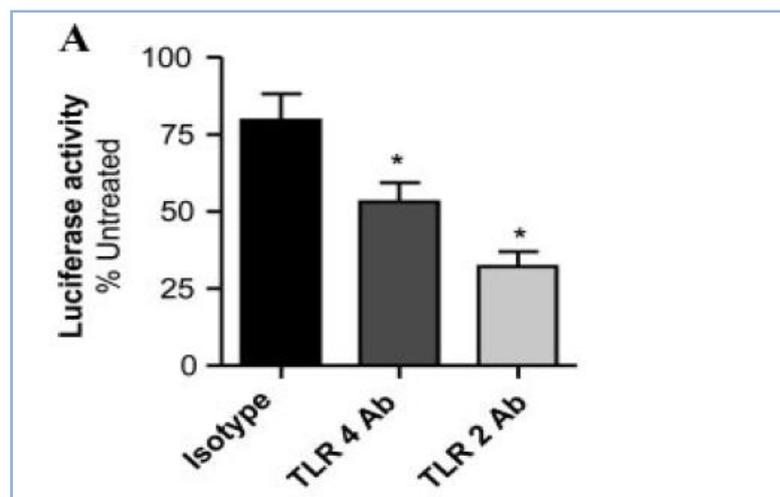
# What are Toll-like receptors?



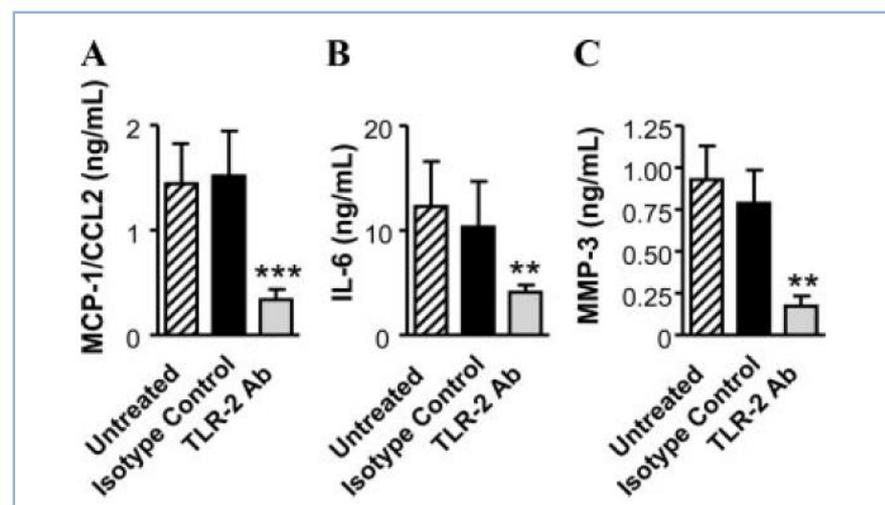
## Toll-Like Receptor-2 Mediates Inflammation and Matrix Degradation in Human Atherosclerosis

Claudia Monaco, MD, PhD, FESC; Scott M. Gregan, BSc; Tina J. Navin, BSc;  
Brian M.J. Foxwell, PhD, DSc, FRCPath<sup>†</sup>; Alun H. Davies, MA, BM, BCh, DM, FRCS, FHEA;  
Marc Feldmann, MB, BS, BSc, PhD, FRCPath, FRCP, FMedSci, FAA, FRS

**TLR-2 and -4 blockade reduces NF- $\kappa$ B activity in human atherosclerosis**



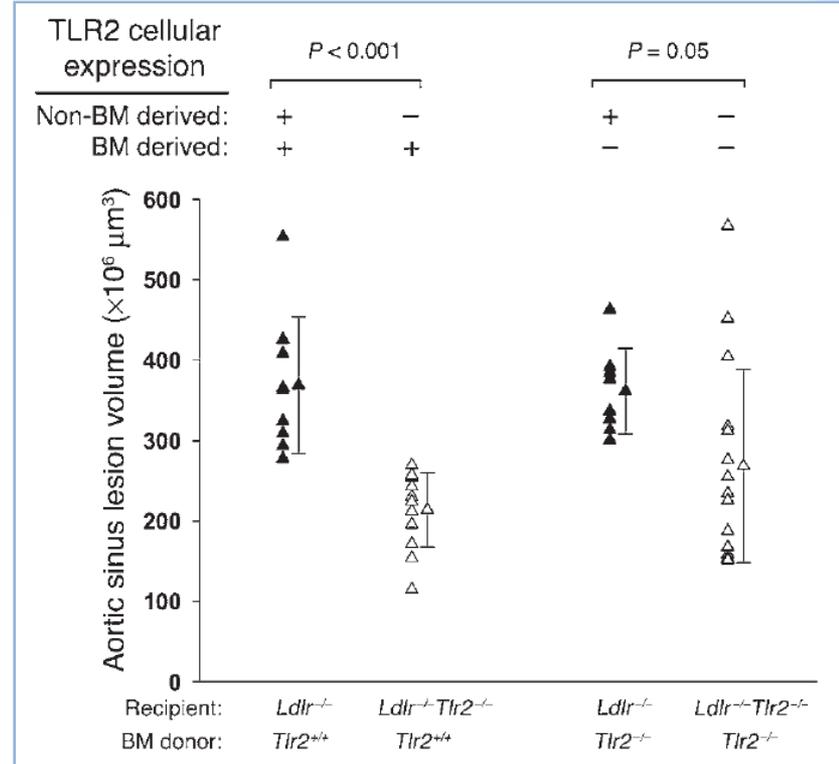
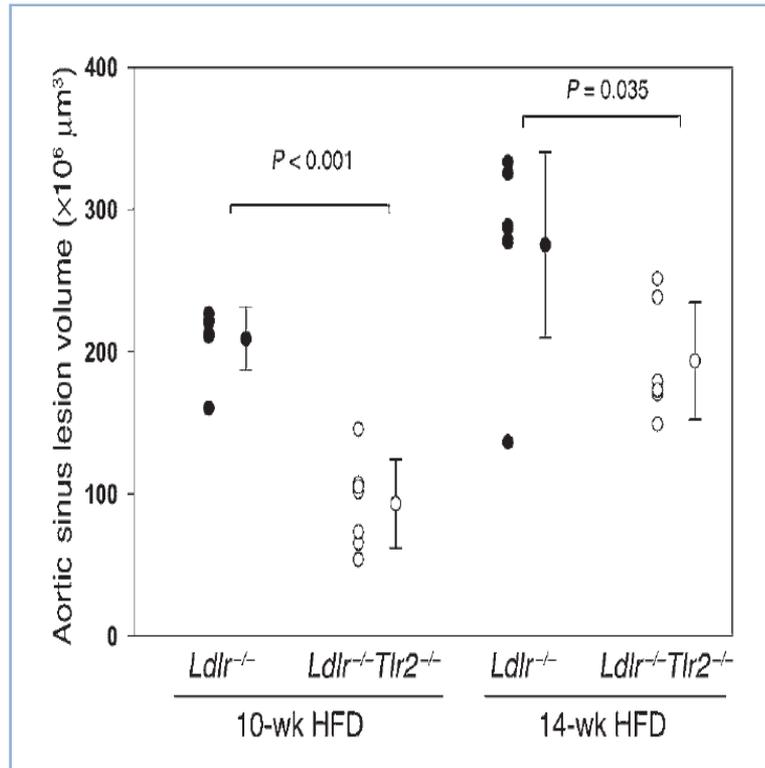
**TLR-2 Blockade Significantly Reduces MMP Production in Human Atheroma Cell Cultures**



# 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.



# Modulation of atherosclerosis in mice by Toll-like receptor 2

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

- **TLR2 KO results in attenuation of atherosclerosis** in LDL receptor KO mice
- **No difference in atherosclerotic plaque area in LDL receptor KO mice with or without BM transfer of TLR2 positive cells**
  - **importance of TLR2 in the vessel itself**
  - **endothelium or VSMC**

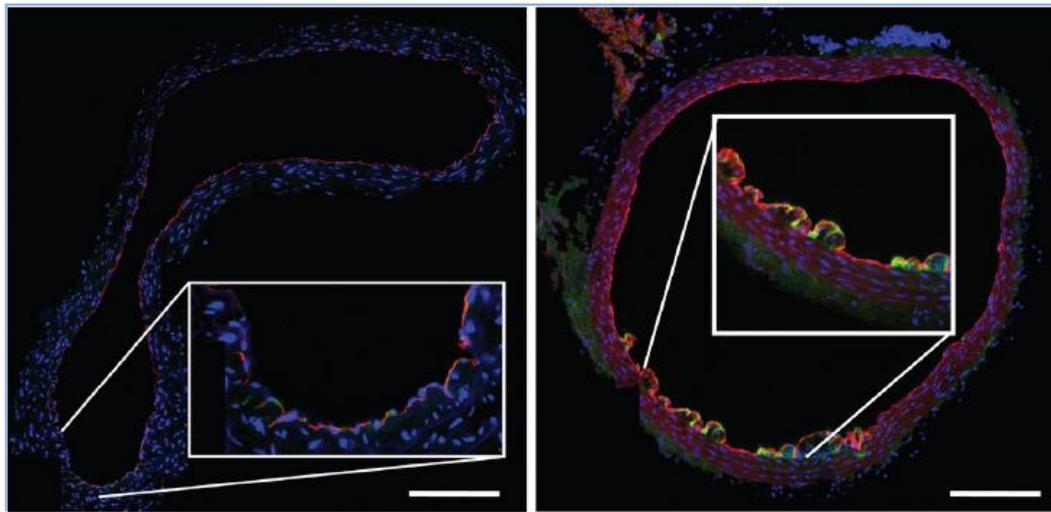
## Increased endothelial expression of Toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events

Adam E. Mullick,<sup>1</sup> Katrin Soldau,<sup>1</sup> William B. Kiosses,<sup>2</sup>  
Thomas A. Bell III,<sup>1</sup> Peter S. Tobias,<sup>1</sup> and Linda K. Curtiss<sup>1</sup>

<sup>1</sup>Department of Immunology and <sup>2</sup>Core Microscopy Facility, The Scripps Research Institute, La Jolla, CA 92037

Chow-fed *LDLr<sup>-/-</sup>* mice

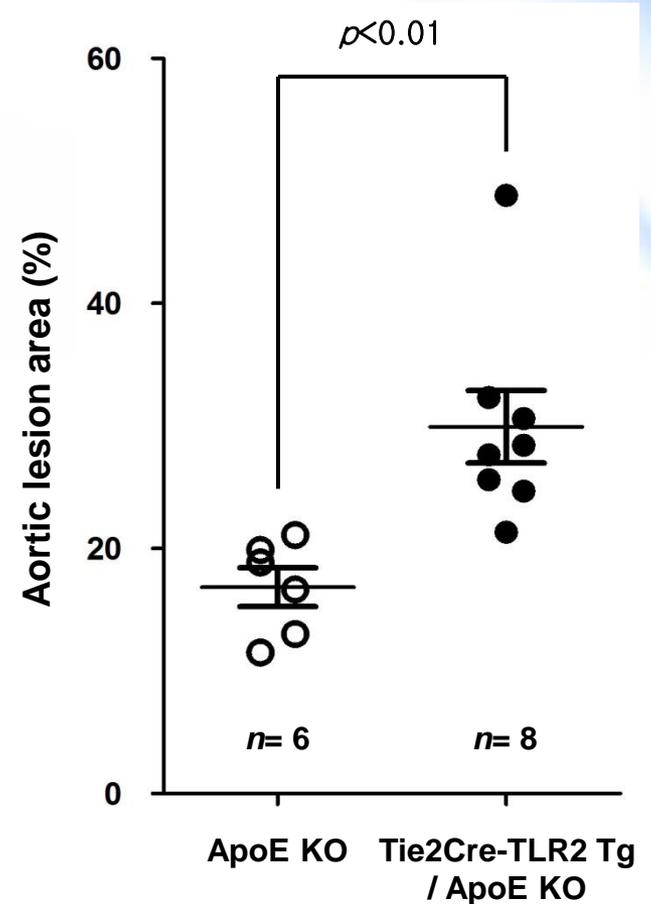
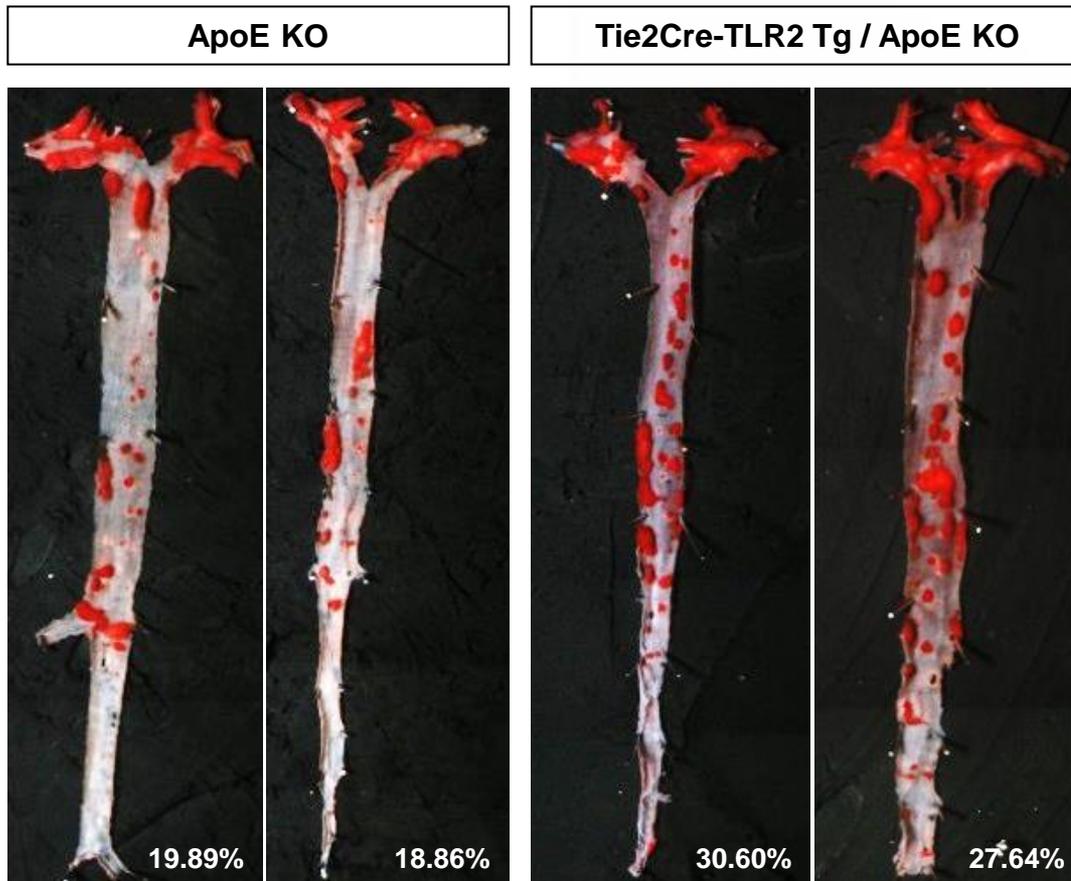
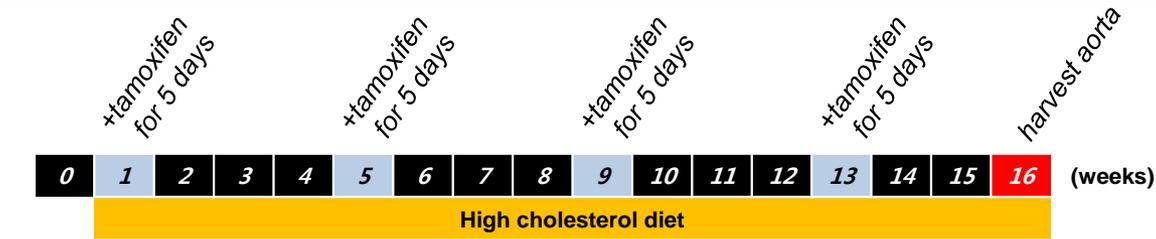
4-wk HFD-fed *LDLr<sup>-/-</sup>* mice



Bright Red: CD31 (endothelial cells)  
Green: TLR2

**Endothelial cell TLR2 expression**  
in the modulation of **key**  
**atherogenic events** during early  
lesion development

# Endothelial cell-specific TLR2 Tg mouse



YUHS data

# Atherosclerosis and Vascular Smooth Muscle Cells

## Vascular Smooth Muscle Cells in Atherosclerosis

Martin R. Bennett, Sanjay Sinha, Gary K. Owens

**Abstract:** The historical view of vascular smooth muscle cells (VSMCs) in atherosclerosis is that aberrant proliferation of VSMCs promotes plaque formation, but that VSMCs in advanced plaques are entirely beneficial, for example preventing rupture of the fibrous cap. However, this view has been based on ideas that there is a homogenous population of VSMCs within the plaque, that can be identified separate from other plaque cells (particularly macrophages) using standard VSMC and macrophage immunohistochemical markers. More recent genetic lineage tracing studies have shown that VSMC phenotypic switching results in less-differentiated forms that lack VSMC markers including macrophage-like cells, and this switching directly promotes atherosclerosis. In addition, VSMC proliferation may be beneficial throughout atherogenesis whereas VSMC apoptosis, cell senescence, and VSMC-derived macrophage formation may be detrimental. We review the effect of embryological origin on VSMC behavior in atherosclerosis, the consequences of phenotypic switching, the evidence for different origin processes that VSMCs undergo in atherosclerosis in regard to plaque formation and lesion progression. We think there is now compelling evidence that a full understanding of VSMC biology is critical to identify therapeutic targets to both prevent and treat atherosclerosis. DOI: 10.1161/CIRCRESAHA.115.306361.)

**Key Words:** atherosclerosis ■ extracellular matrix ■ interleukin ■ platelet-derived growth factor



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doi:10.1093/cvr/cvu171

REVIEW

## Emerging regulators of vascular smooth muscle cell function in the development and progression of atherosclerosis

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Received 13 January 2014; revised 1 July 2014; accepted 14 July 2014; online publish-ahead-of-print 22 July 2014

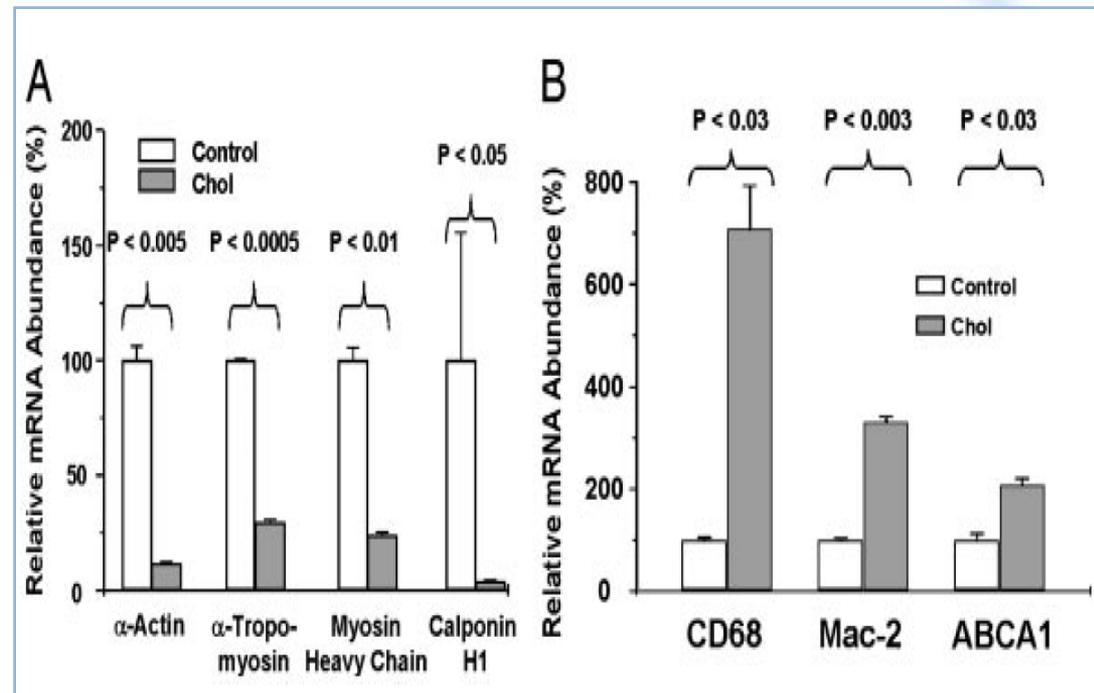
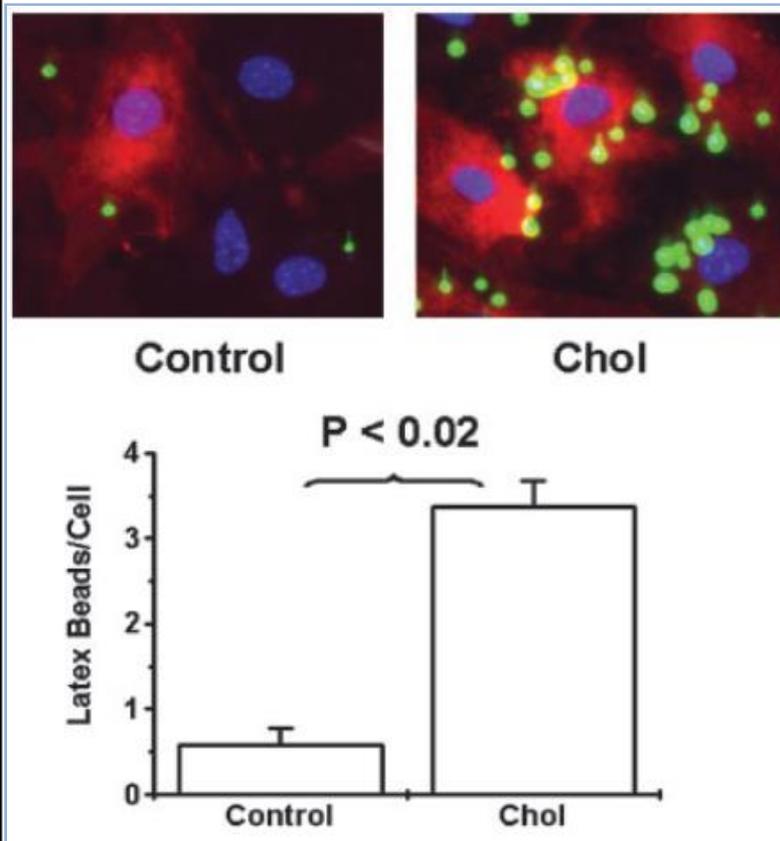
After a period of relative senescence in the field of vascular smooth muscle cell (VSMC) research with particular regards to atherosclerosis, the last few years has witnessed a resurgence, with extensive research re-assessing potential molecular mechanisms and pathways that modulate VSMC behaviour within the atherosclerotic-prone vessel wall and the atherosclerotic plaque itself. Attention has focussed on the pathological contribution of VSMC in plaque calcification; systemic and local mediators such as inflammatory molecules and lipoproteins; autocrine and paracrine regulators which affect cell–cell and cell to matrix contacts alongside cytoskeletal changes. In this brief focused review, recent insights that have been gained into how a myriad of recently identified factors can influence the pathological behaviour of VSMC and their subsequent contribution to atherosclerotic plaque development and progression has been discussed. An overriding theme is the mechanisms involved in the alterations of VSMC function during atherosclerosis.

**Keywords** Atherosclerosis • Vascular smooth muscle cells • Migration • Foam cell formation • Calcification • Matrix metalloproteinases

# Transdifferentiation of mouse aortic smooth muscle cells to a macrophage-like state after cholesterol loading

James X. Rong, Mark Shapiro\*, Eugene Trogan\*, and Edward A. Fisher†

*PNAS 2003;100(23):13531-13536*



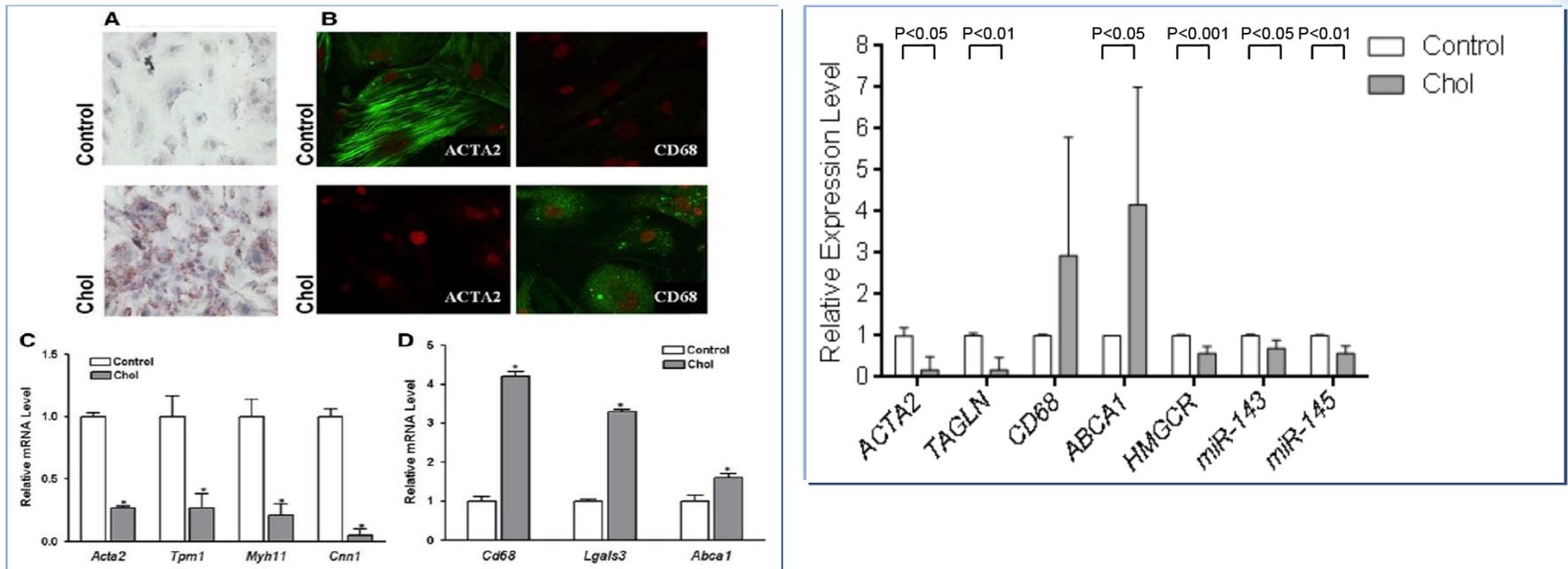
# VSMC in advanced plaques

- > 80% of VSMC derived cells from the plaques of Apo E KO mice lacked common SMC markers such as ACTA2
- > 30% of VSMCS in plaques express macrophage markers such as CD 68, CD11b, F4/80, Mac2
- 40% of foam cells are VSMC derived cells, expressing both SMC(ACTA2) and macrophage markers(CD68)

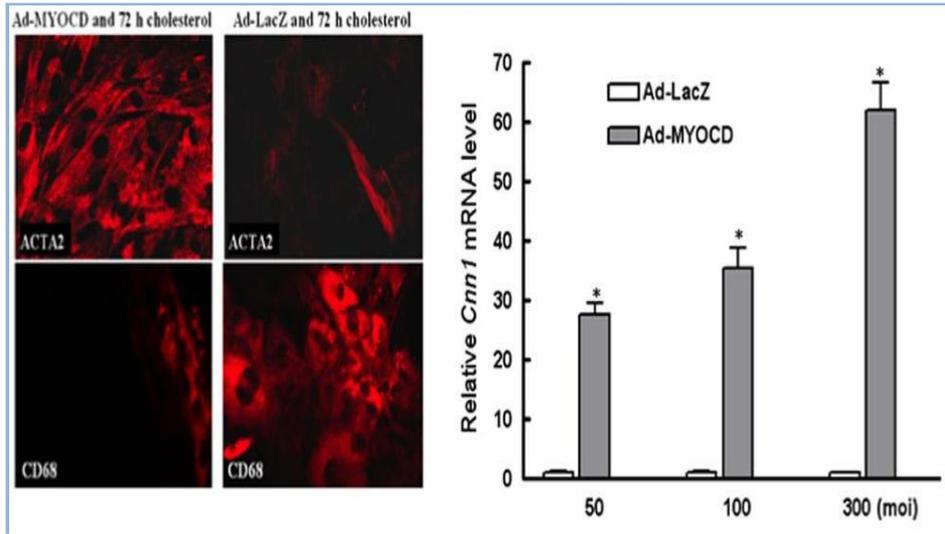
## Cholesterol Loading Reprograms the MicroRNA-143/145–Myocardin Axis to Convert Aortic Smooth Muscle Cells to a Dysfunctional Macrophage-Like Phenotype

Yuliya Vengrenyuk,\* Hitoo Nishi,\* Xiaochun Long, Mireille Ouimet, Nazir Savji, Fernando O. Martinez, Courtney P. Cassella, Kathryn J. Moore, Stephen A. Ramsey, Joseph M. Miano, Edward A. Fisher

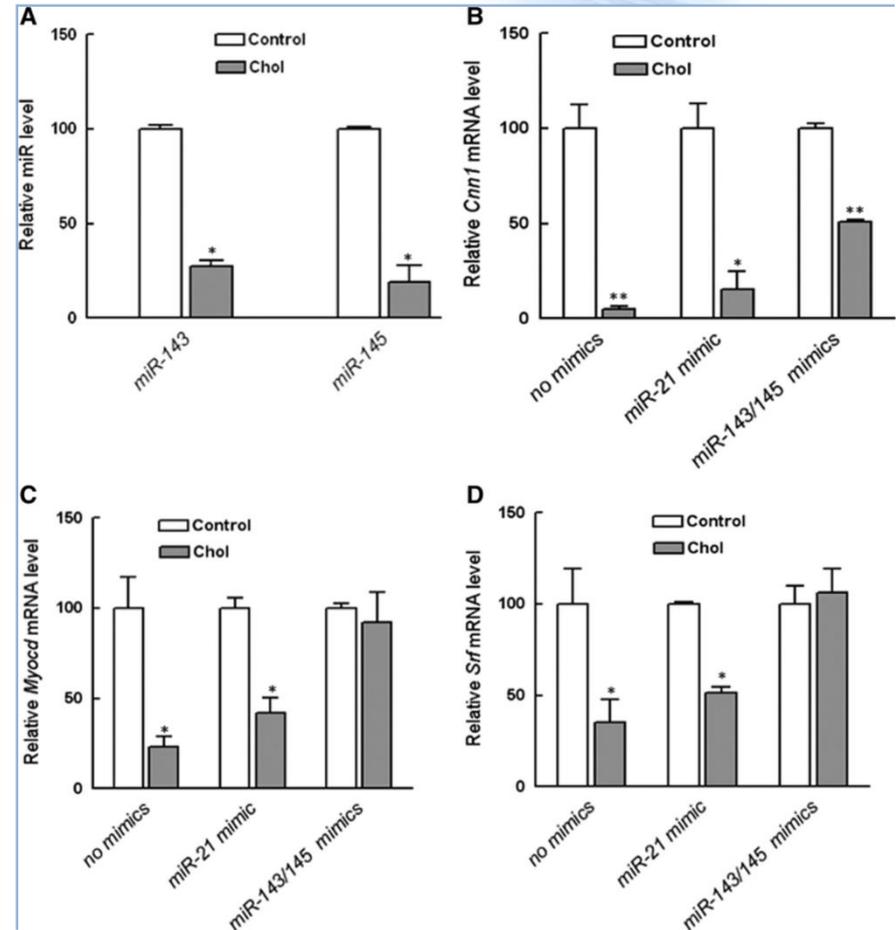
**Cholesterol loading of vascular smooth muscle cell (VSMC) leads to foam cell formation, loss of VSMC characteristics, and emergence of macrophage-like features.**



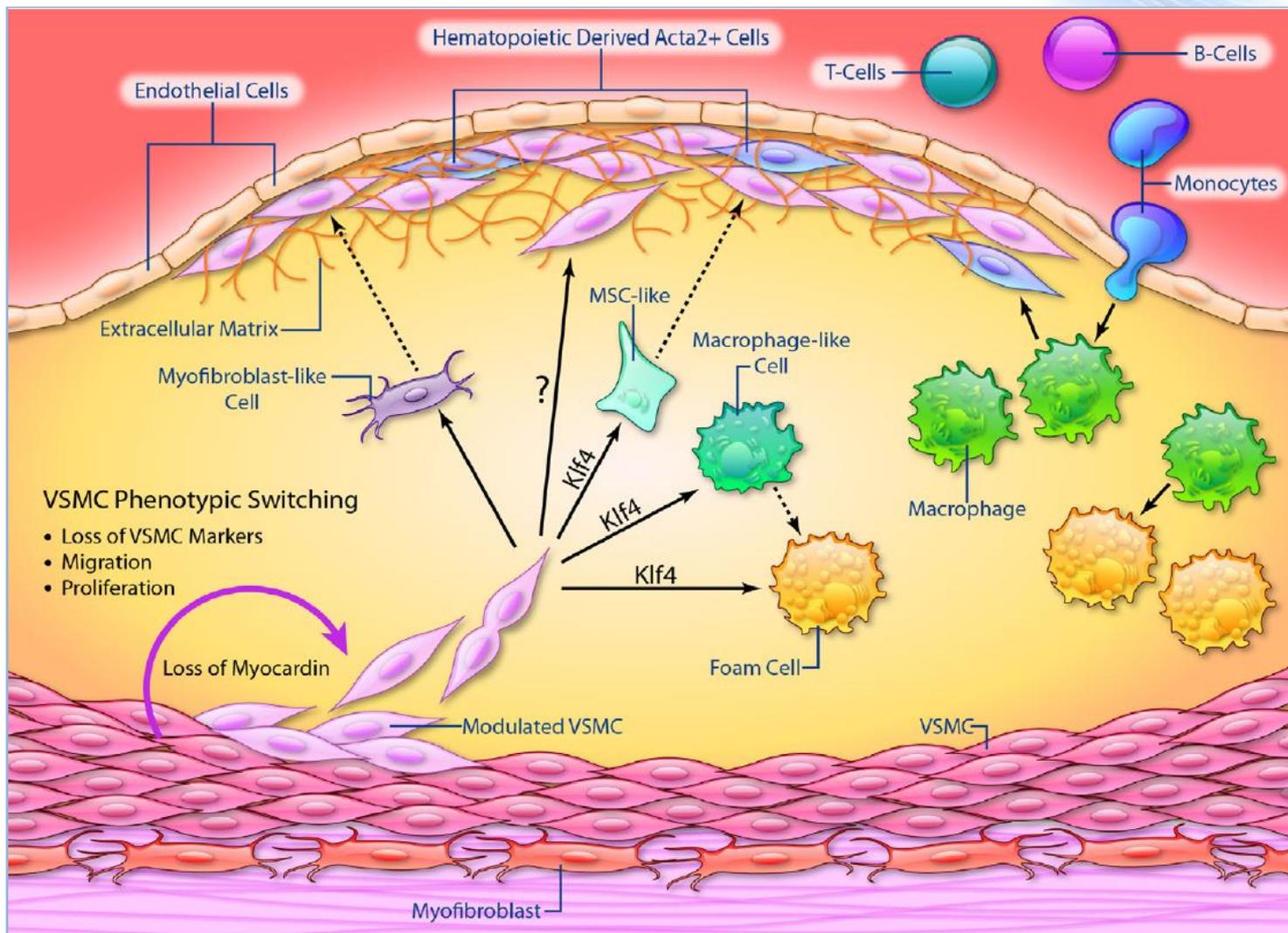
## Overexpression of Myocardin Prevents and Reverses the Loss of VSMC Features After Cholesterol Loading



## Roles of miR-143/145 in VSMC Phenotypic Changes in Response to Cholesterol Loading

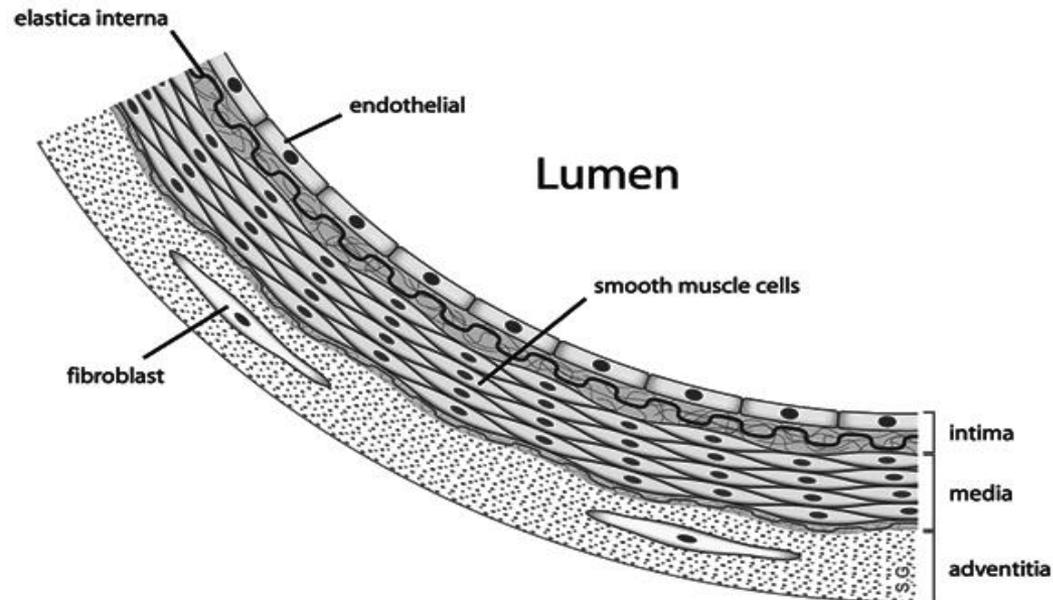


# Atherosclerosis and VSMC phenotypic switching



# TLR2 and vascular smooth muscle cells

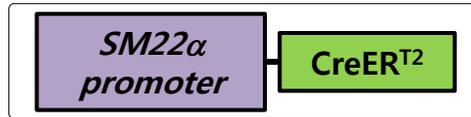
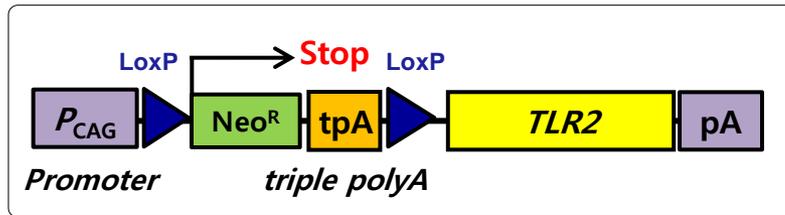
- Does activation of TLR 2 in the VSMC mediate the development and progression of atherosclerosis?
- If so, what is the possible mechanism?



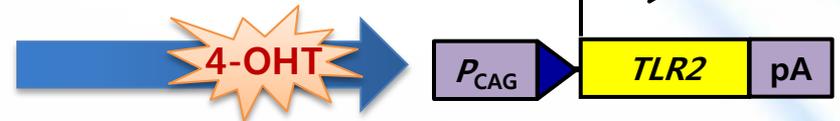
# **Hypothesis**

- **Conditional overexpression of TLR2 in VSMC will result in acceleration of atherosclerosis in Apo E KO mice**
- **TLR2 is important in mediating the phenotypic switch of VSMC into foam cell like cells**

# 혈관특이적 TLR2 과발현 동맥경화 유전자변형 마우스 제작

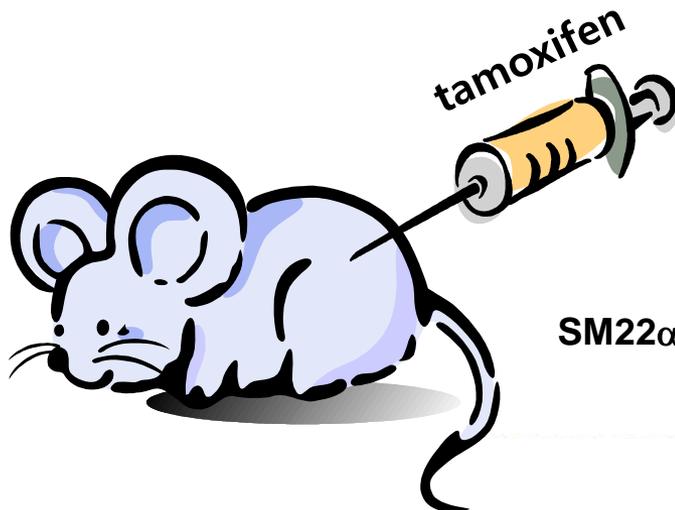


Cross



Major target gene expression

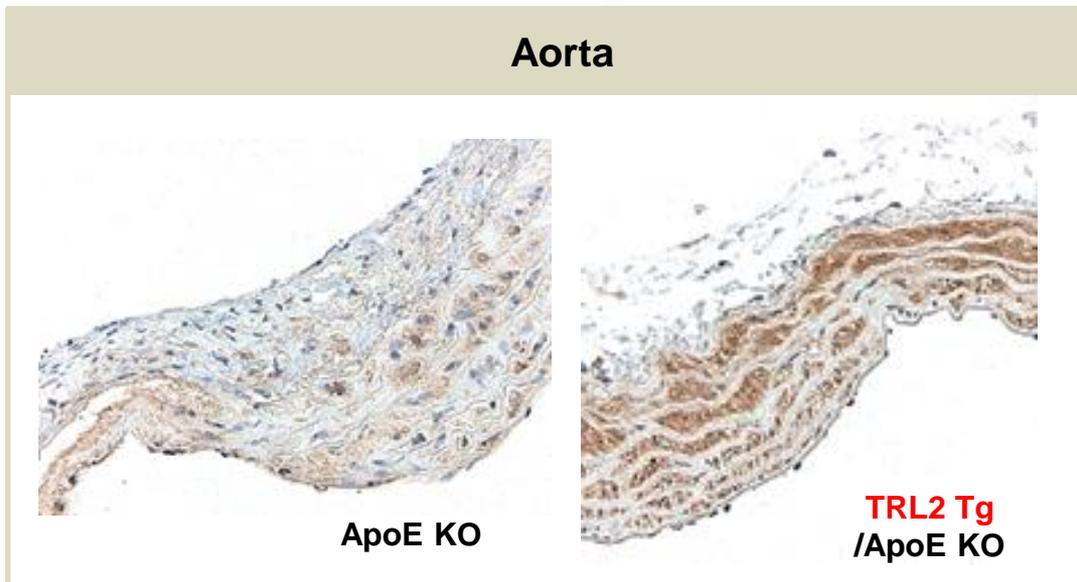
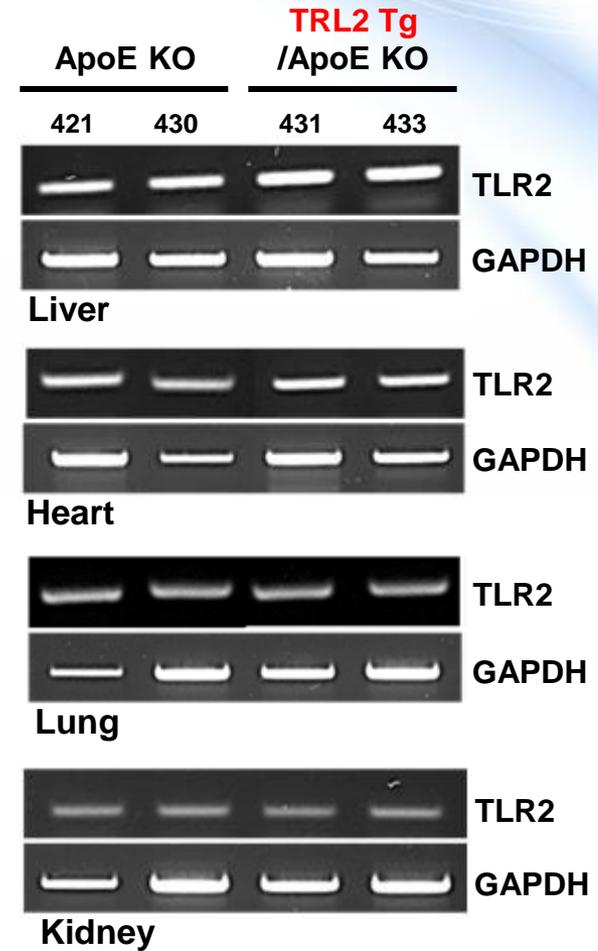
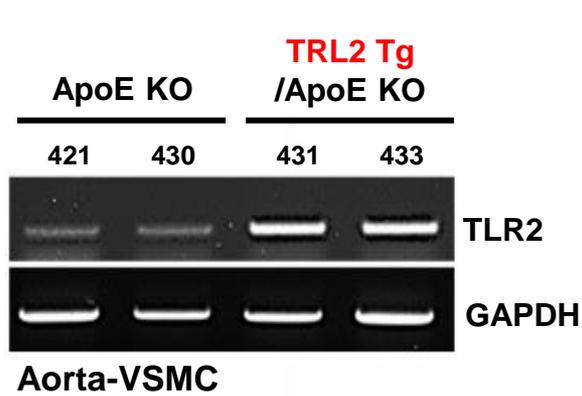
Smooth muscle cell-specific TLR2 overexpression



SM22α-Cre/TLR2 Tg/ApoE KO

혈관특이적 TLR2 과발현 동맥경화 마우스 제작

# SMC-specific TLR2 Tg mouse



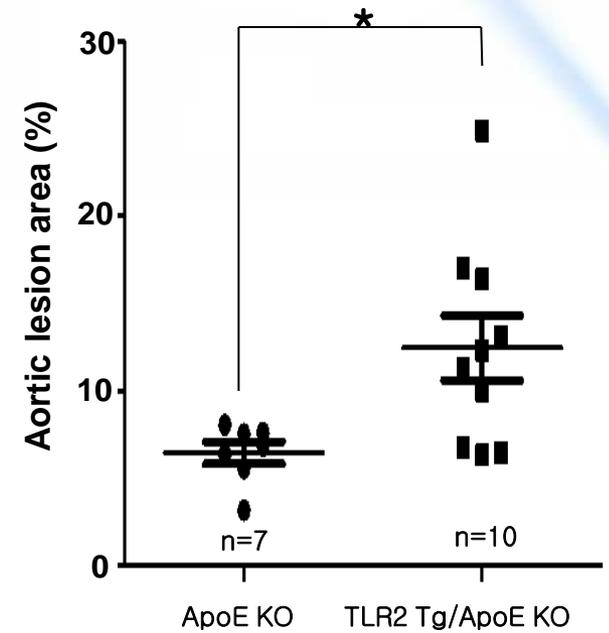
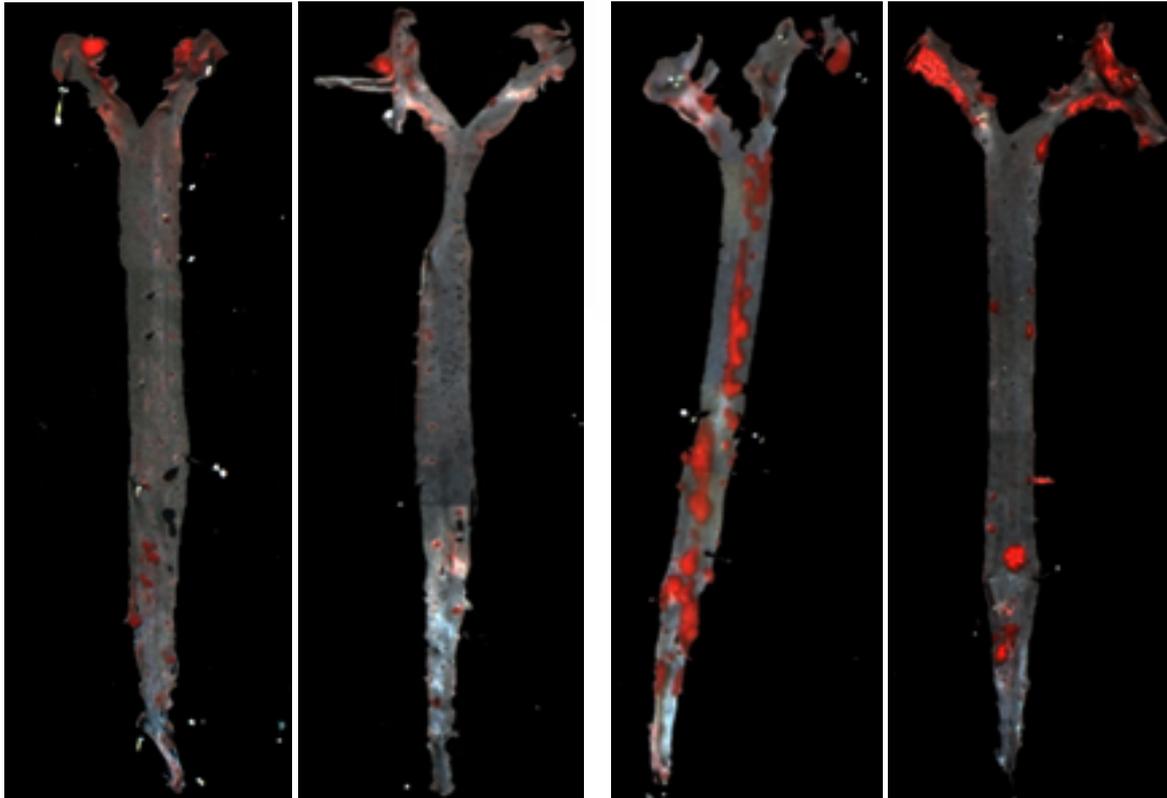
Immunohistochemical staining for TLR2

# SMC-specific TLR2 Tg mouse: Atherosclerosis

+ Tamoxifen, + high cholesterol diet

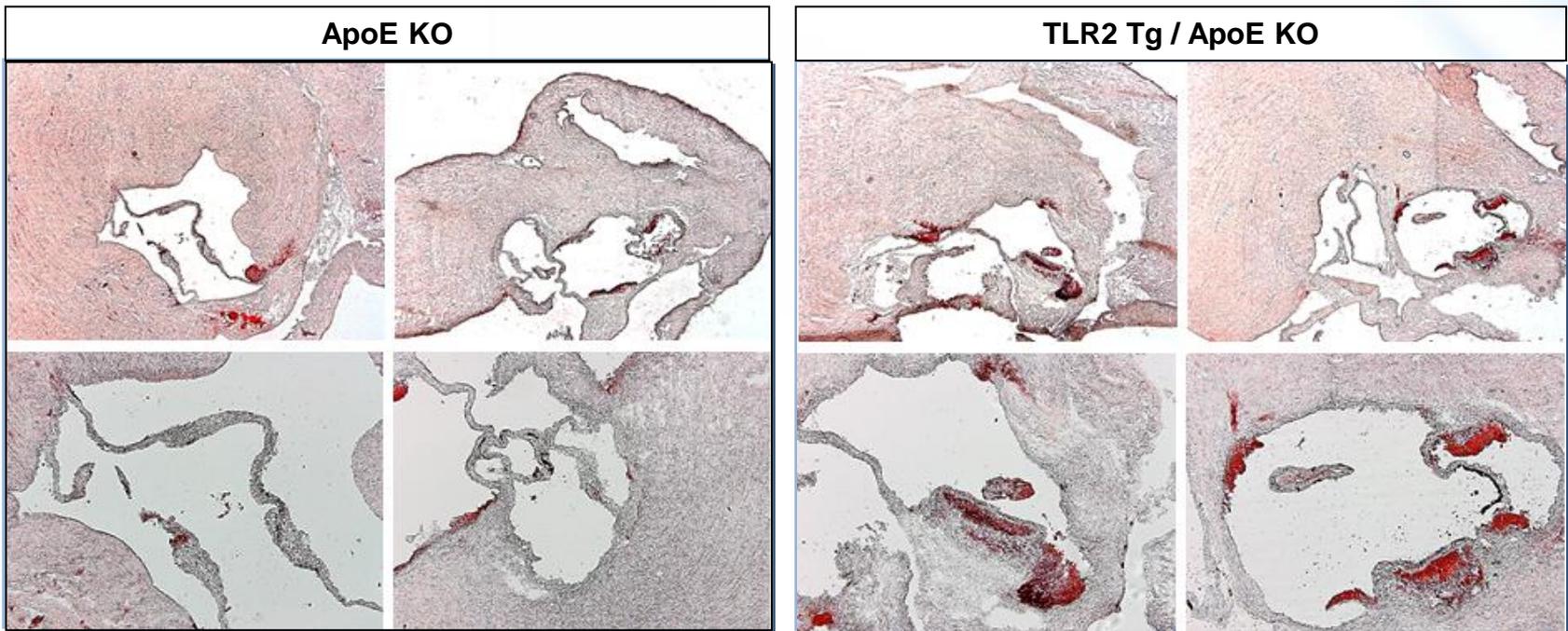
ApoE KO

TLR2 Tg / ApoE KO



# SMC-specific TLR2 Tg mouse: Atherosclerosis

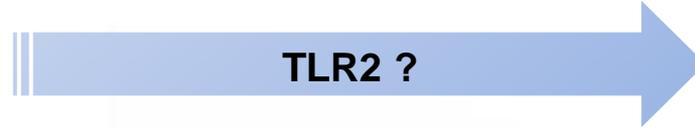
+ Tamoxifen, + high cholesterol diet



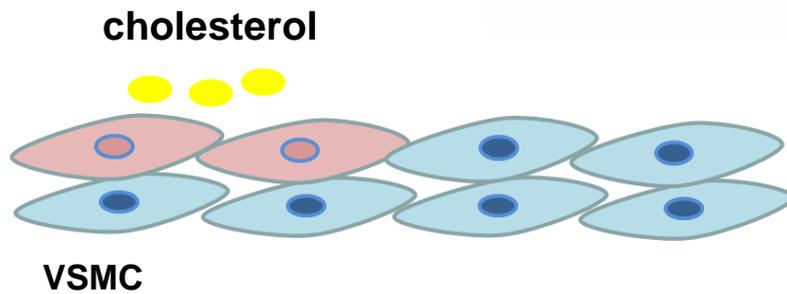
Aortic sinus, Oil-red O staining

# TLR2: Phenotypic switching of VSMC

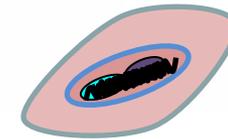
VSMC



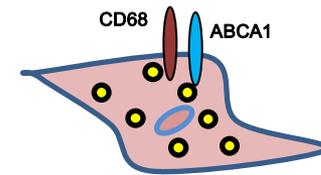
Macrophage-like cells



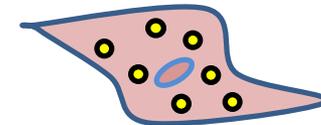
VSMC marker ↓



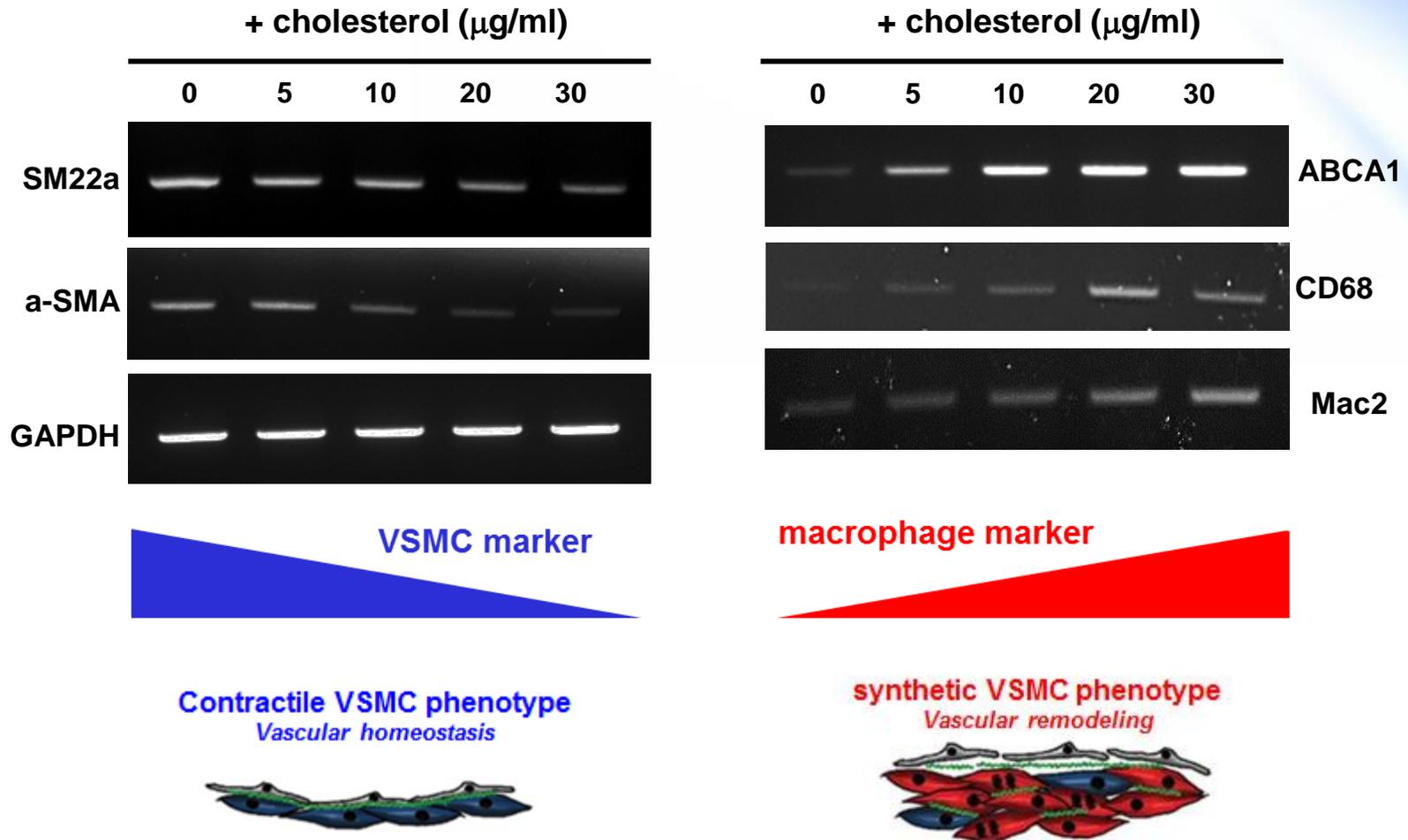
Macrophage marker ↑



Foam cell formation

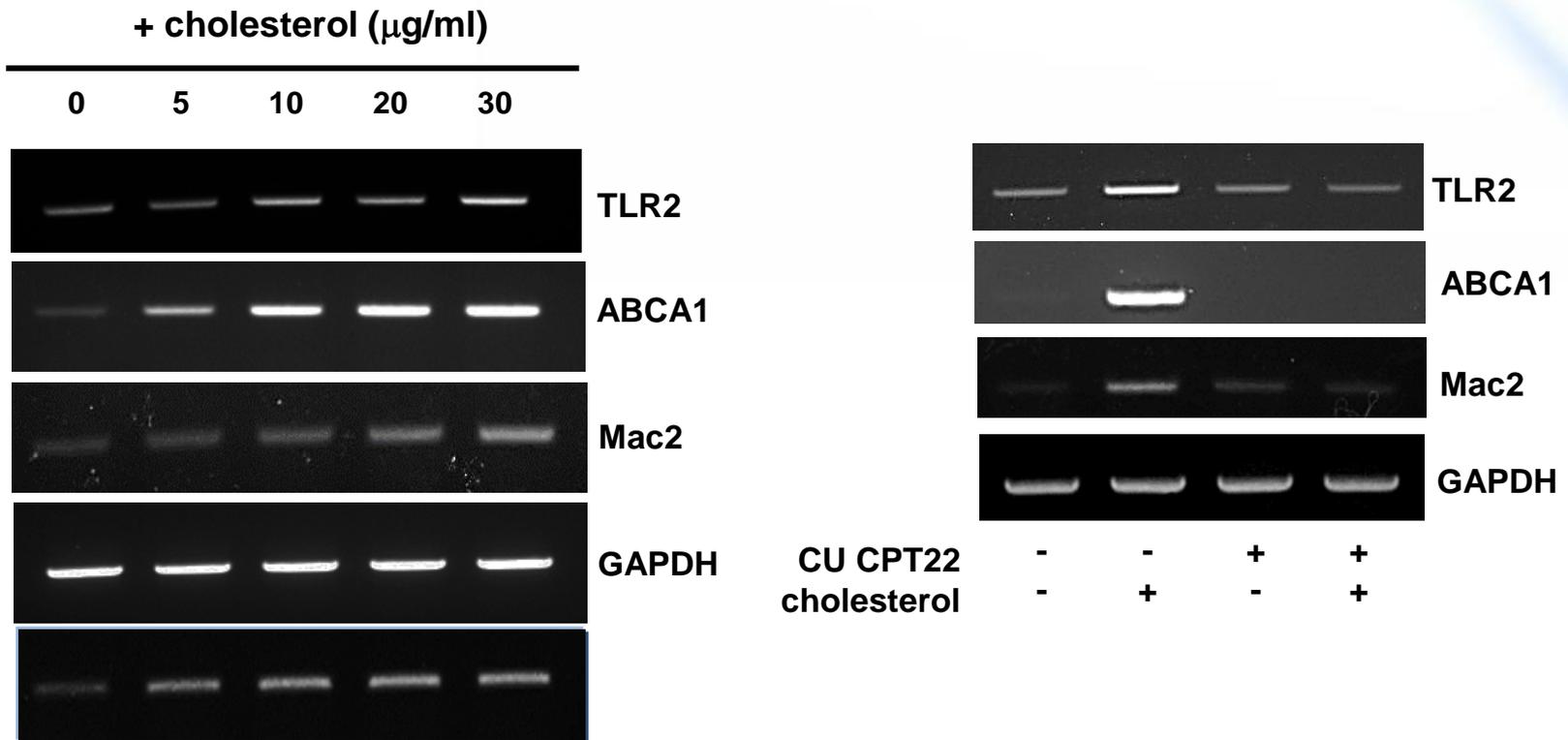


# Phenotypic switching of VSMC

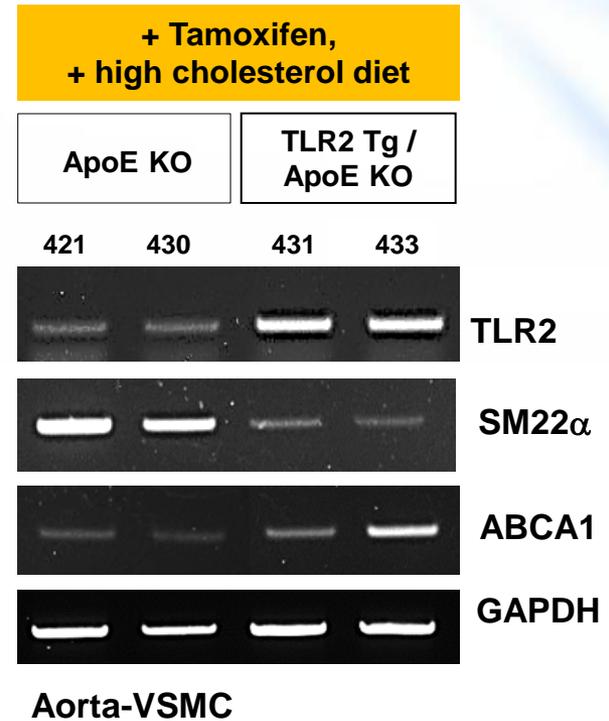
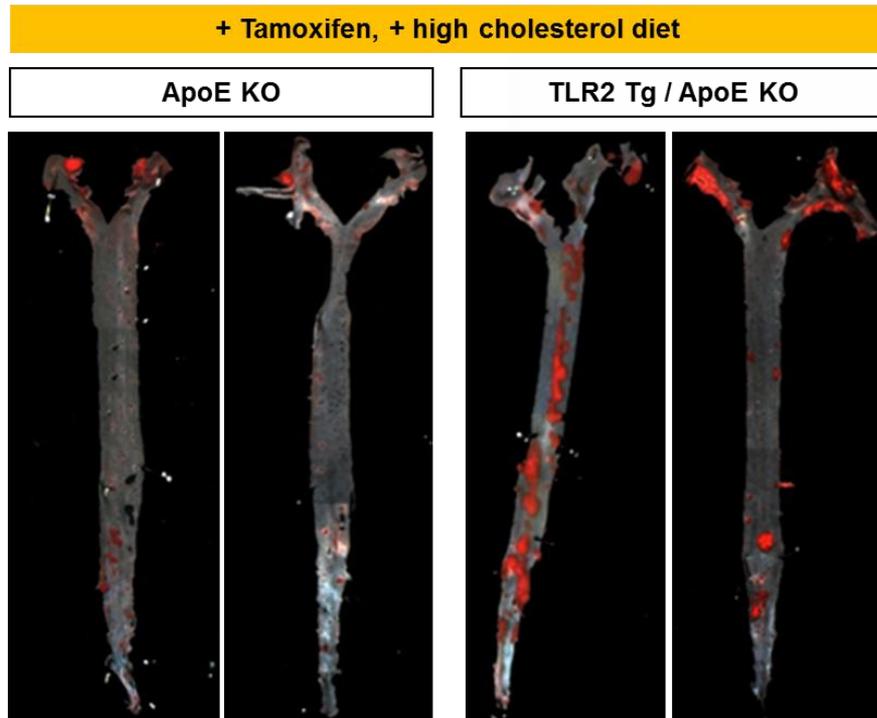


# TLR2: Phenotypic switching of VSMC

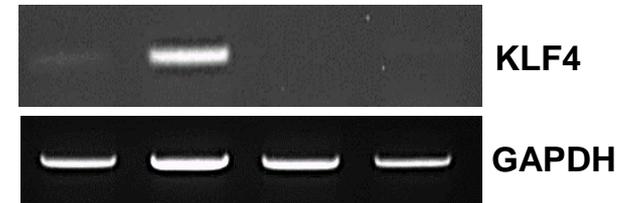
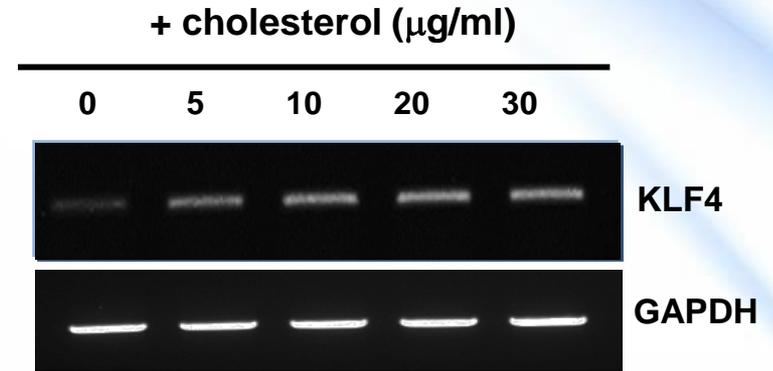
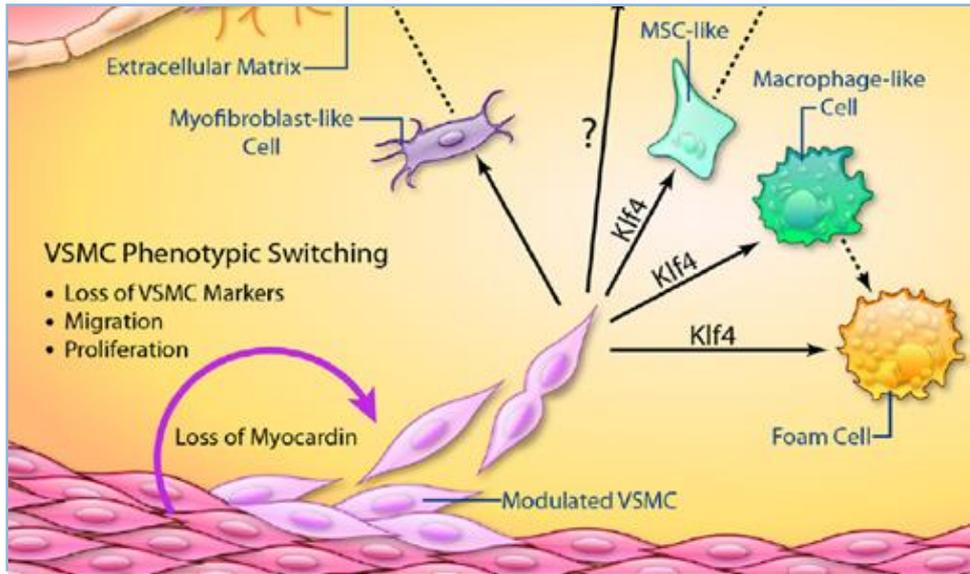
## Effect of TLR2 on cholesterol-induced phenotypic switching of VSMC



# TLR2: Phenotypic switching of VSMC *in vivo*



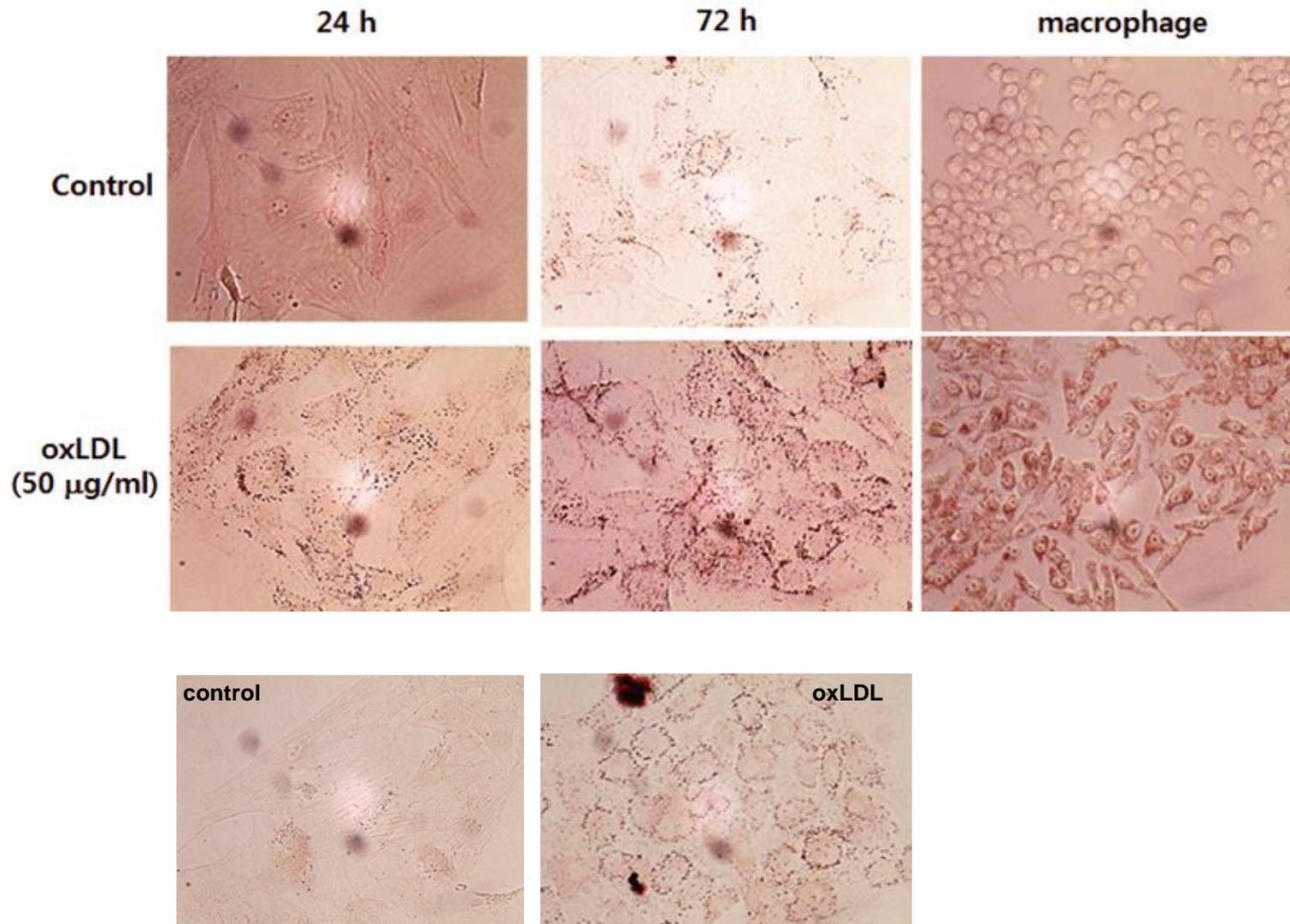
# TLR2: Phenotypic switching of VSMC



<b>CU CPT22</b>	-	-	+	+
<b>cholesterol</b>	-	+	-	+

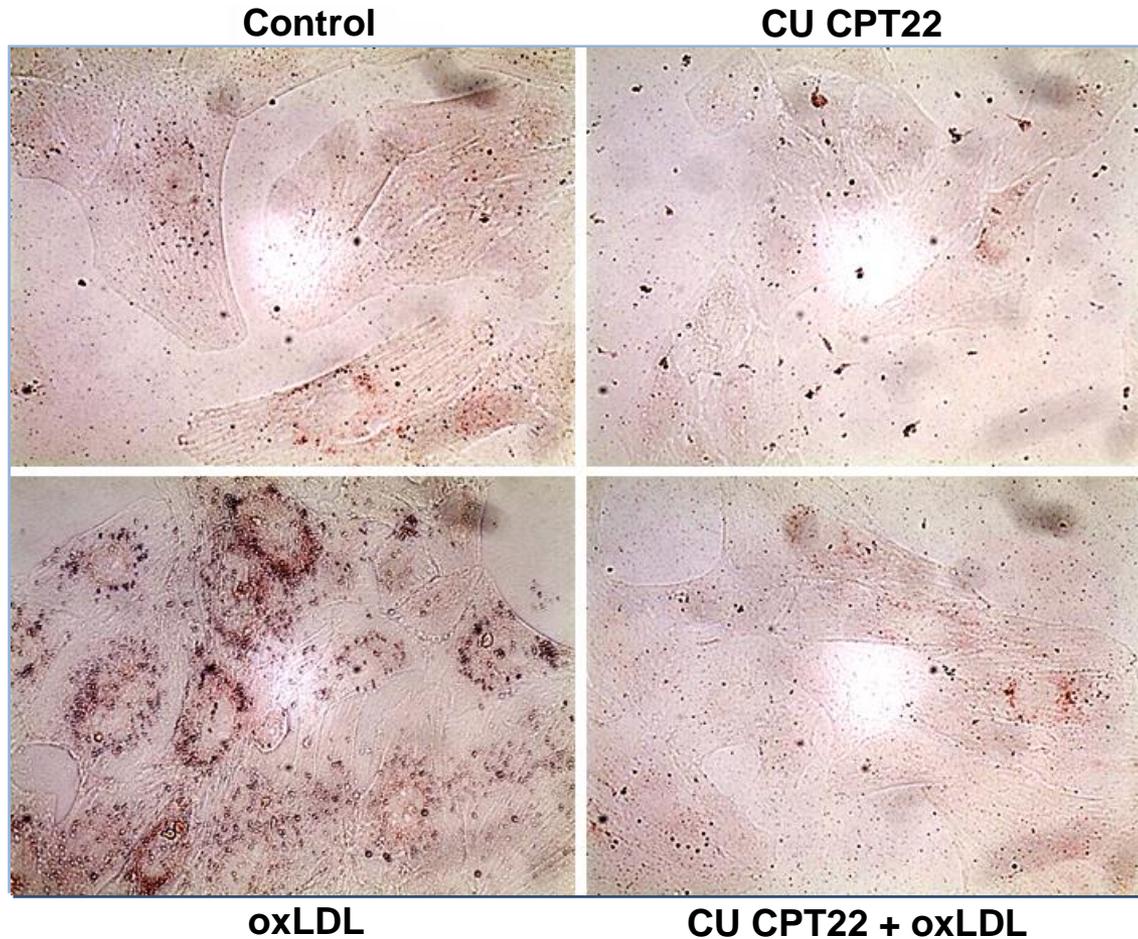
# VSMC foam cell

VSMC from rat aorta



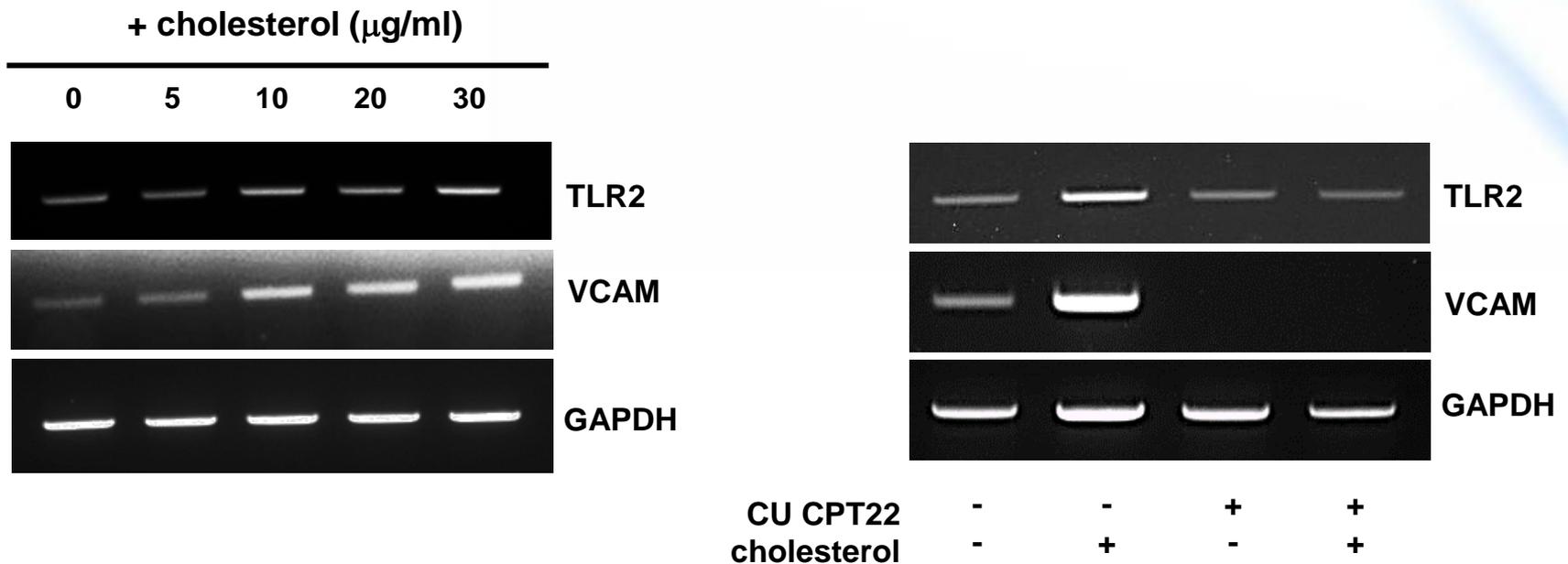
# TLR2: VSMC foam cell

Effect of TLR2 on VSMC foam cell formation

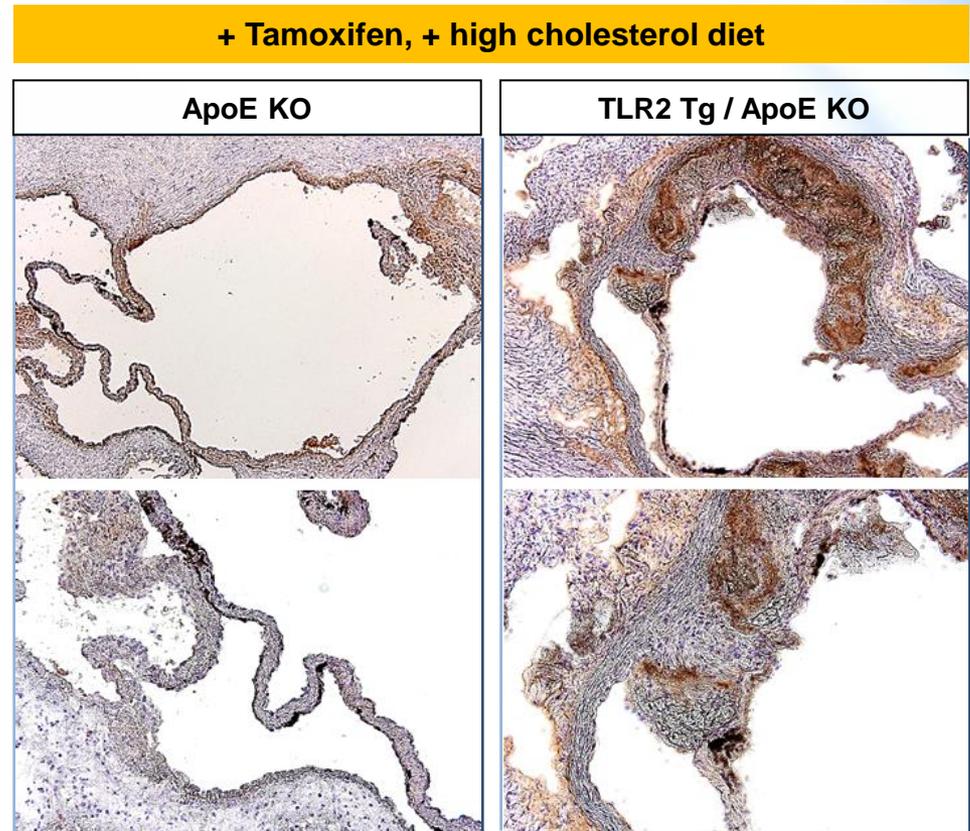
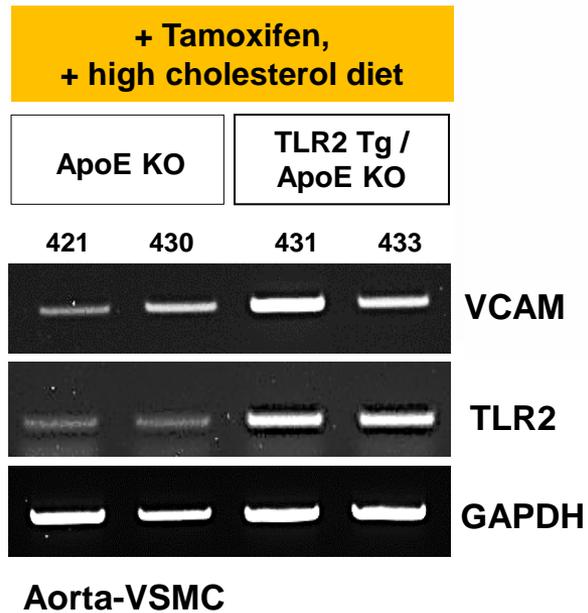


# TLR2: Inflammation of VSMC

## Effect of TLR2 on cholesterol-induced VCAM expression of VSMC

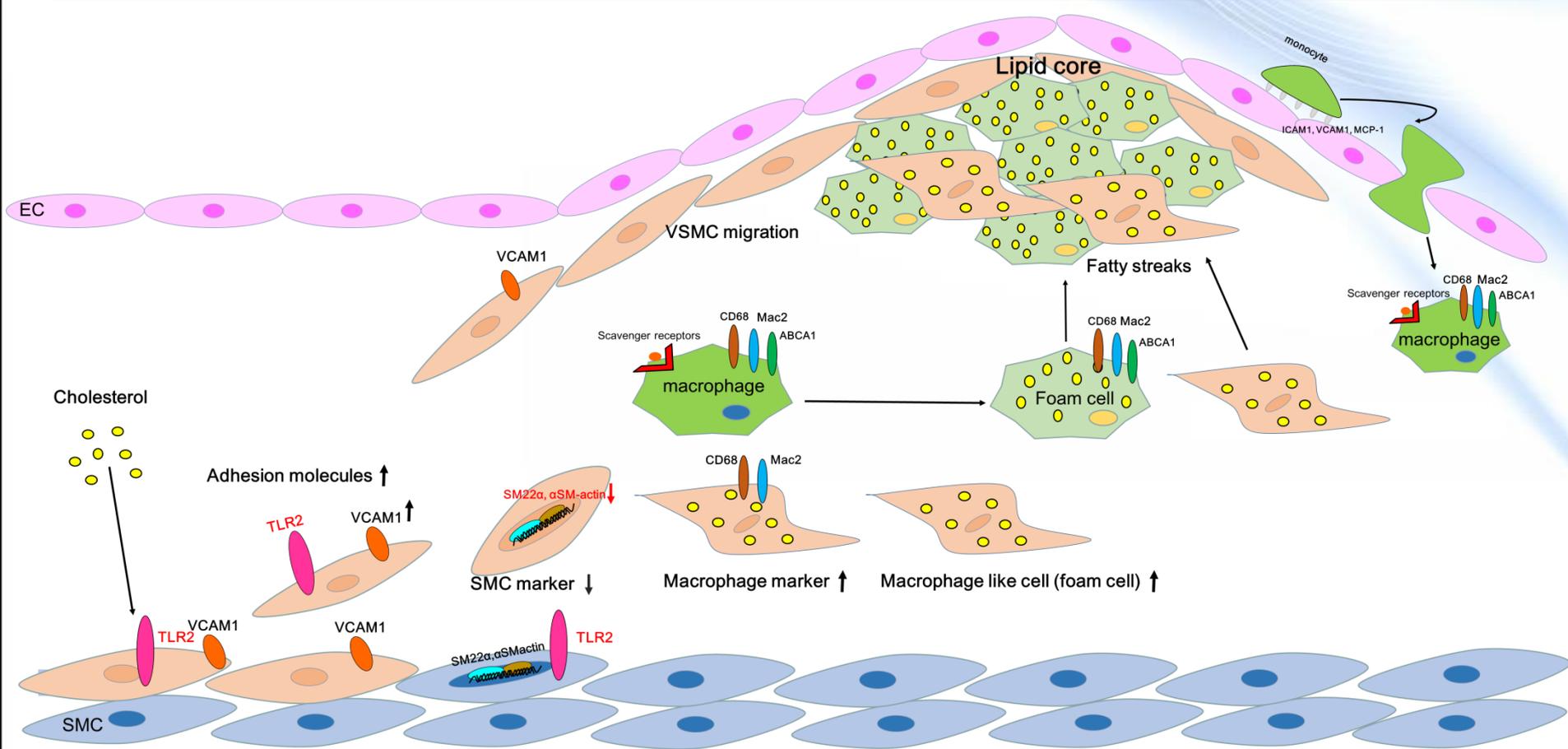


# TLR2: Inflammation of VSMC *in vivo*



Immunohistochemical staining for VCAM

# Summary



- **Conditional overexpression of TLR2 in VSMC results in acceleration of atherosclerosis of ApoE KO mice**
- **TLR2 is important in mediating the phenotypic switch of VSMC into macrophage-like cells**

# Further study

- What is the mechanism for the entry of cholesterol into the SMCs? → mediated through CD 36, SR-A, SR-B, Lox-1, CD 68 or TLR2 itself?
- Microarray of the isolated SMCs to determine signaling mechanism associated with TLR2 mediated phenotypic switch

# Acknowledgments



**SIRIC** 세브란스병원  
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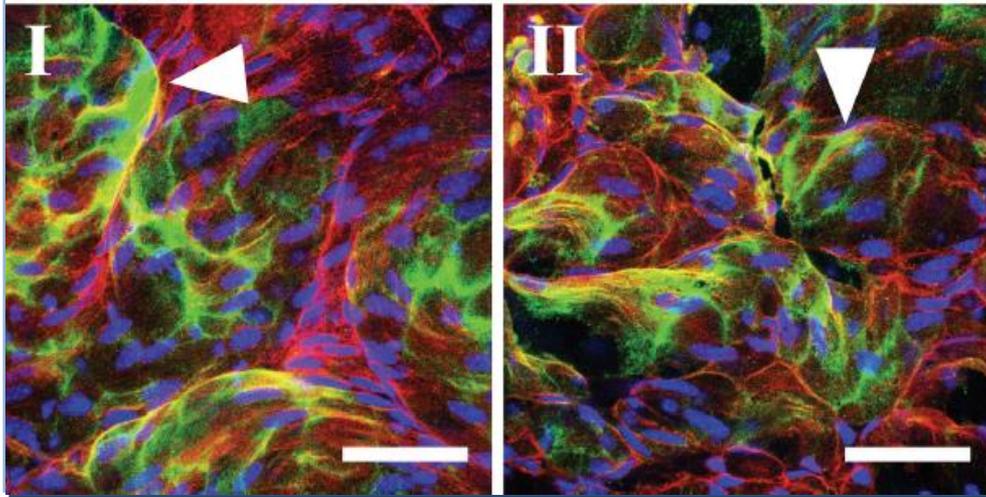
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**MiRan Seo, PhD**  
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**Juyeon Lim**  
**Soyoung Cho**  
**Jisu Jeong**  
**Jiye Lee**  
**Minbum Kim**  
**Soyoung An**  
**Jiyeon Ko**

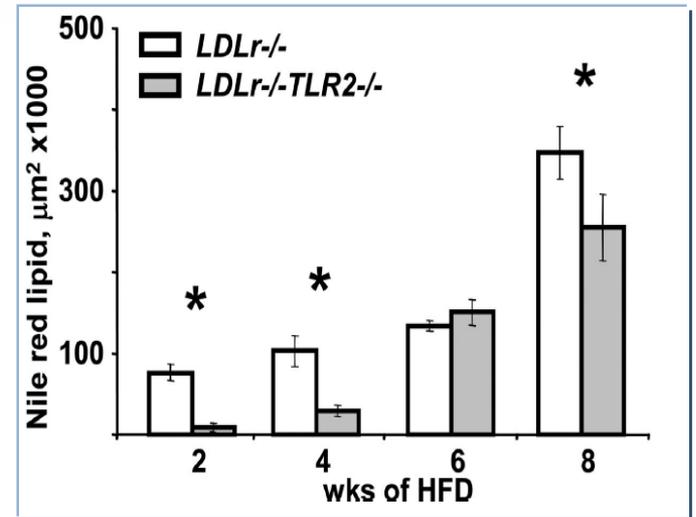
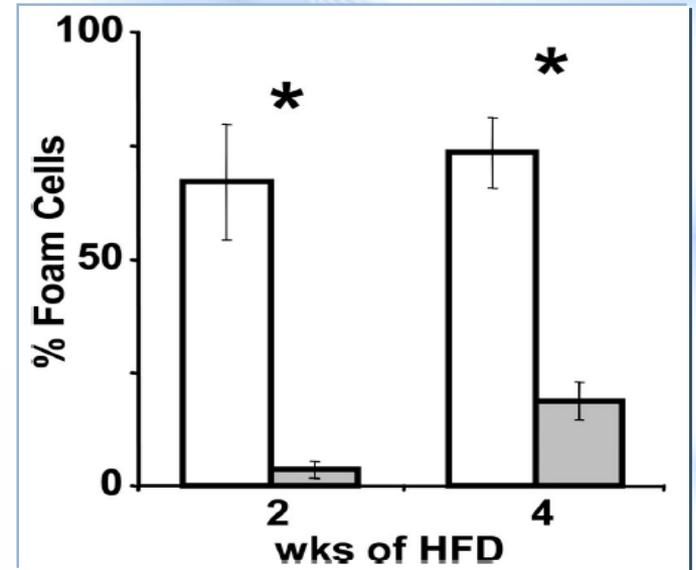
*Thank you very much  
for your attention*



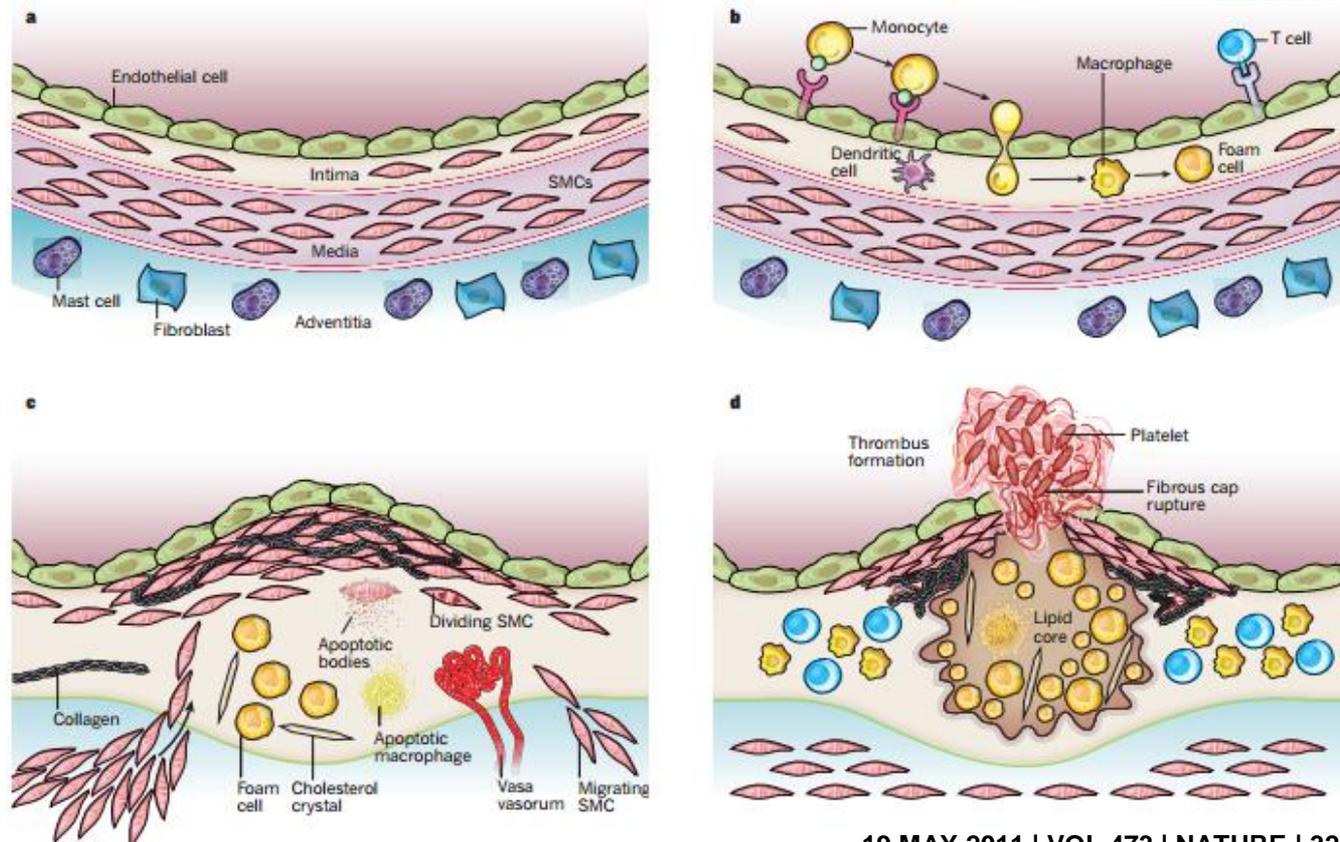
4-wk HFD-fed *LDLr<sup>-/-</sup>* mice



Green: TLR2  
 Red: CD31 (endothelial cells)  
 Blue: Nuclei



# Pathogenesis of Atherosclerosis



19 MAY 2011 | VOL 473 | NATURE | 325

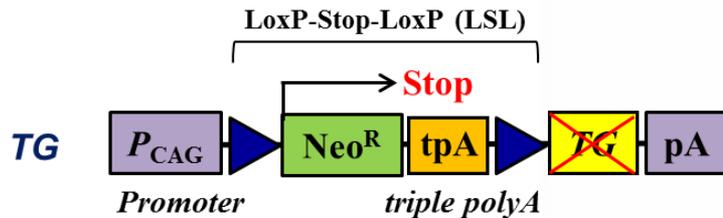
- Endothelial injury (**endothelial dysfunction**)
- **Adherence of monocyte** to endothelial cells
- **Uptake of oxLDL** by macrophage scavenger receptor
- **Foam cell transformation**: accumulation of excess lipid inside the cells
- Recruitment of VSMC (**VSMC phenotype switching**)
- Atherosclerotic plaque formation

# 혈관특이적 TLR2 과발현 동맥경화 유전자변형 마우스 제작

## Conditional TG

Target genes

**TLR2**

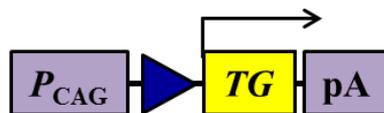


*Sm22 $\alpha$* -CreER<sup>T2</sup>: Smooth muscles

*Tie2*-CreER<sup>T2</sup>: Endothelial cells

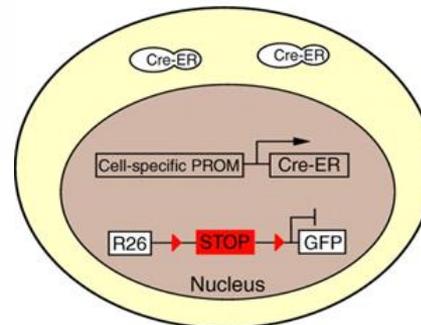


## Spatio-Temporal Gene Regulation

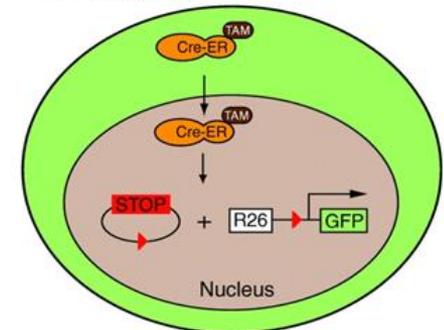


## Inducible Cre system

A No Tamoxifen

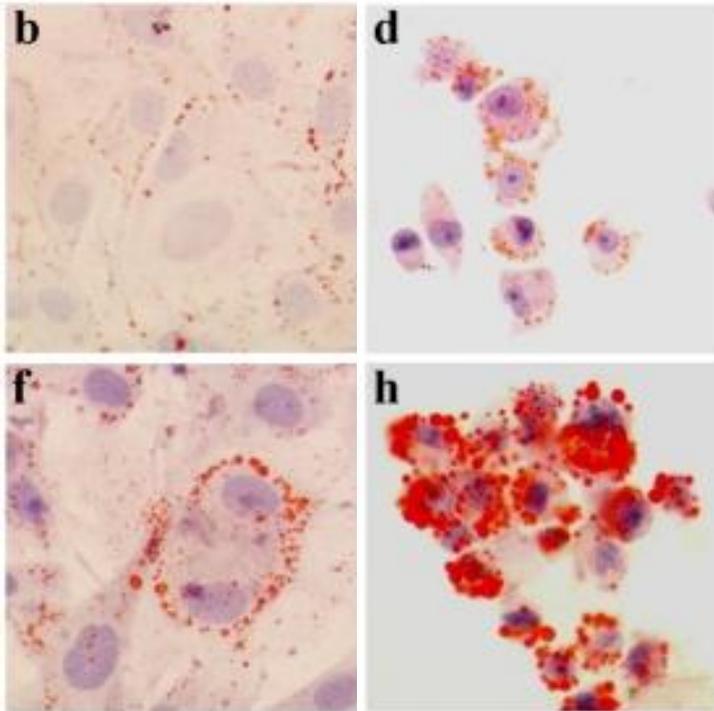


B Tamoxifen

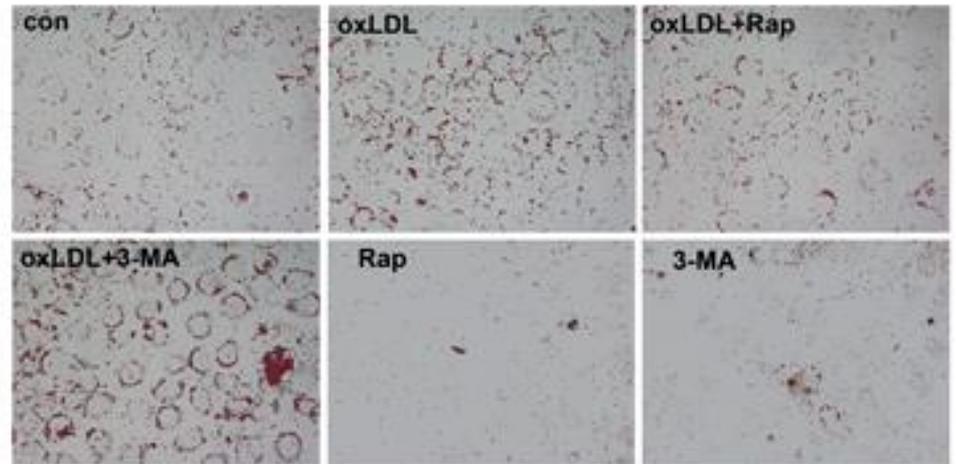


*Development* 2010 137: 1586-1594

# VSMC, oxLDL-induced foam cell formation



*Int J Biol Sci.* 2011;7(7):937-46.



*Cell Death Dis.* 2014 Apr 17;5:e1182.

# Alarmins

Exogenous danger signals  
from microorganisms:  
Pathogen associated  
molecular  
patterns(**Flagellin, LPS**)

Endogenous danger  
signals from tissue  
damage: Damage  
associated molecular  
patterns →  
**HMGB1, S100A12**

Toll like receptor

Receptor for AGE(RAGE)