# Role of Alpha-lipoic acid(ALA) and statin in vascular smooth muscle cell proliferation

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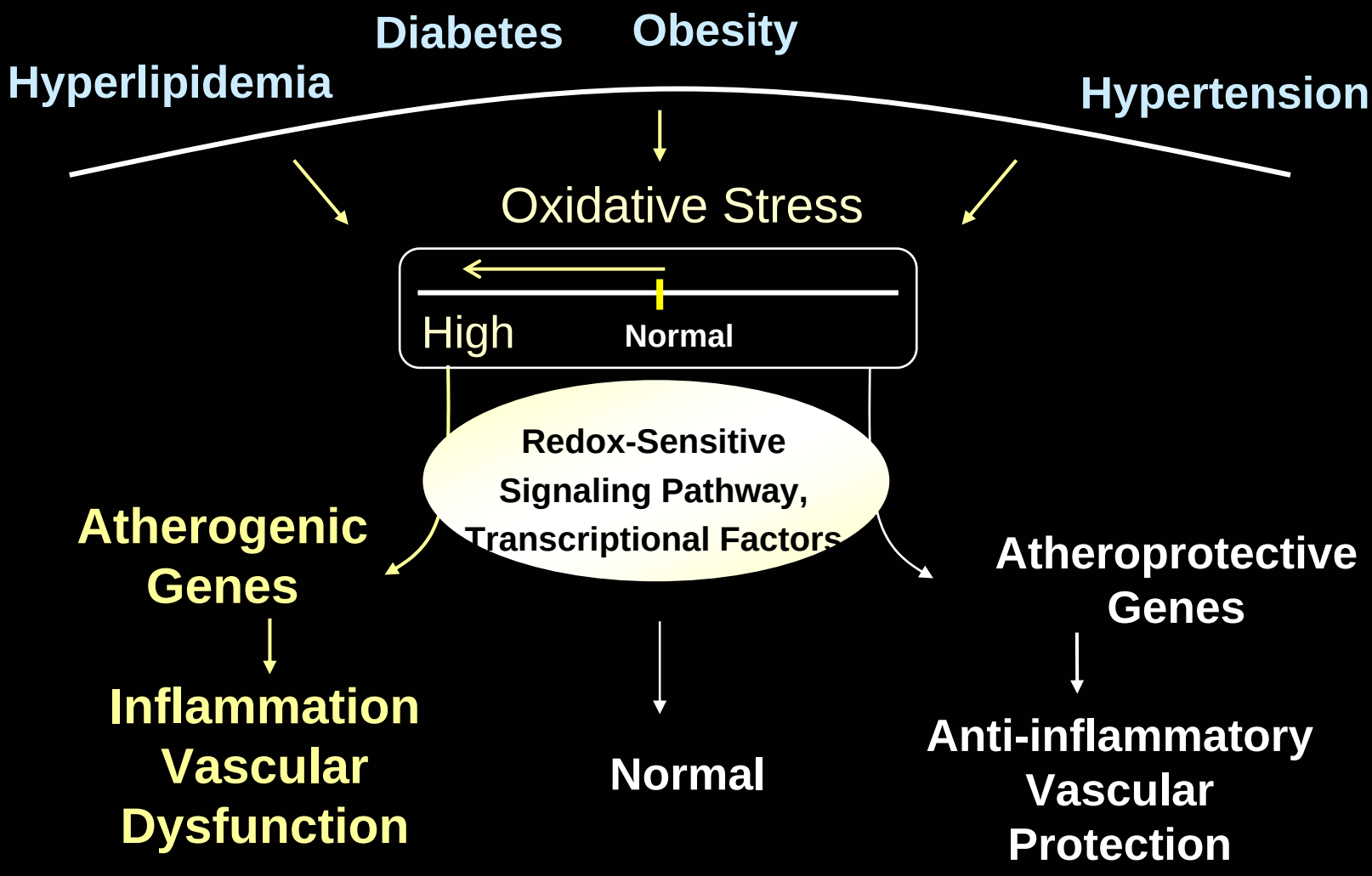
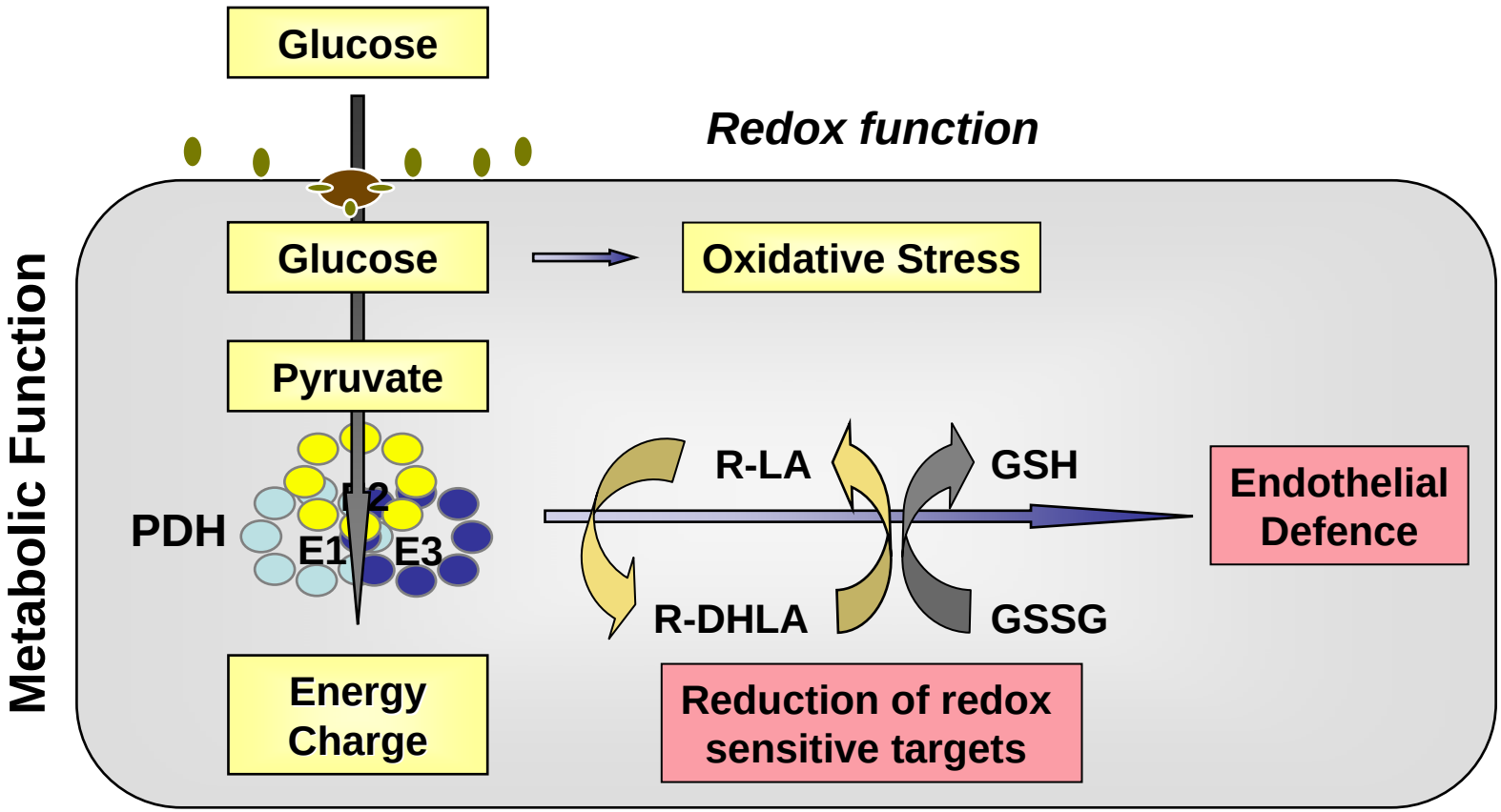
# *Oxidative Stress and Atherosclerosis*



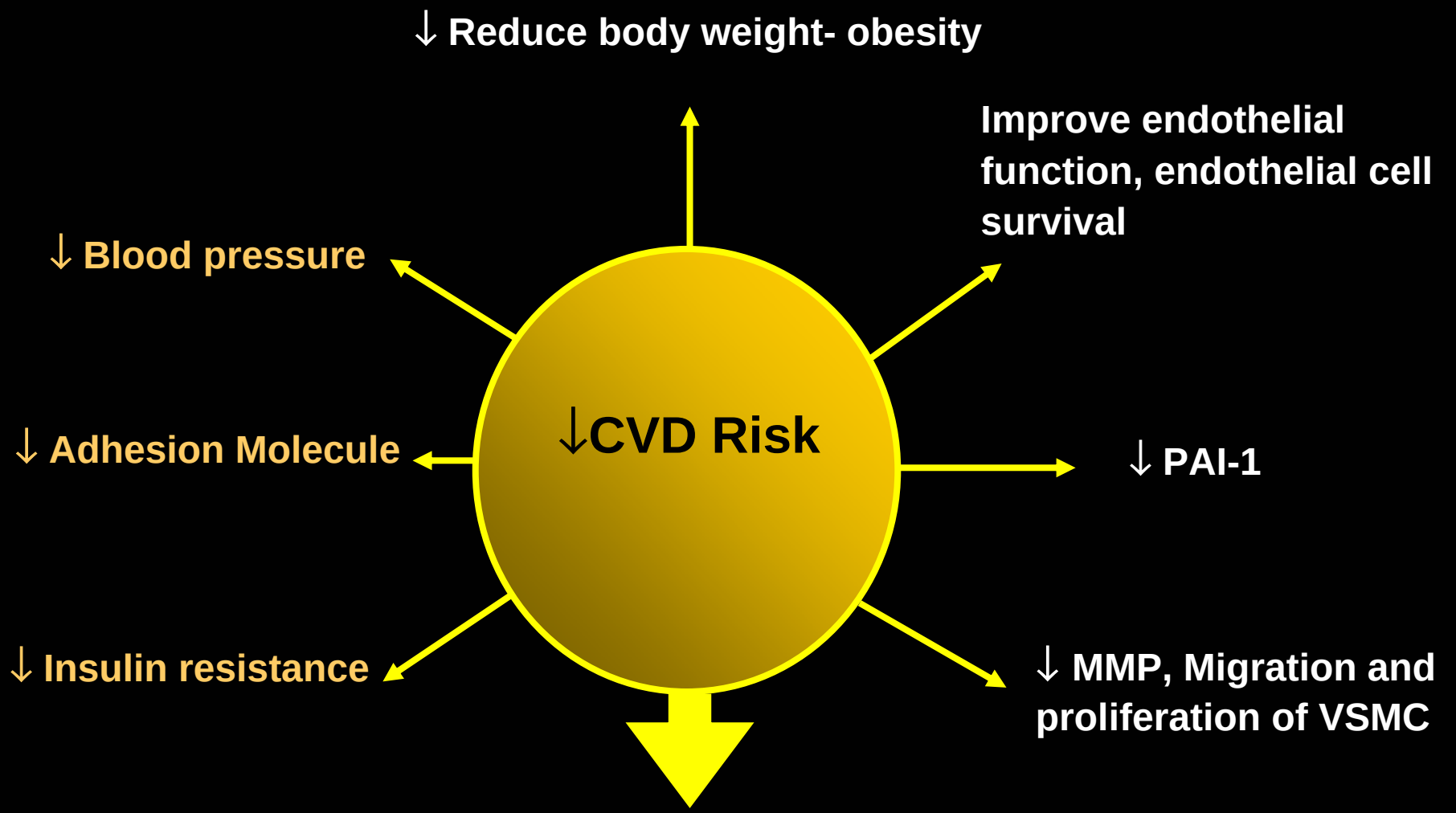

*Kunsch C, Circulation Research. 1999*

# Mode of action

***Alpha-lipoic acid (ALA) is a metabolic cofactor of the pyruvate dehydrogenase complex and a strong antioxidant at the same time***

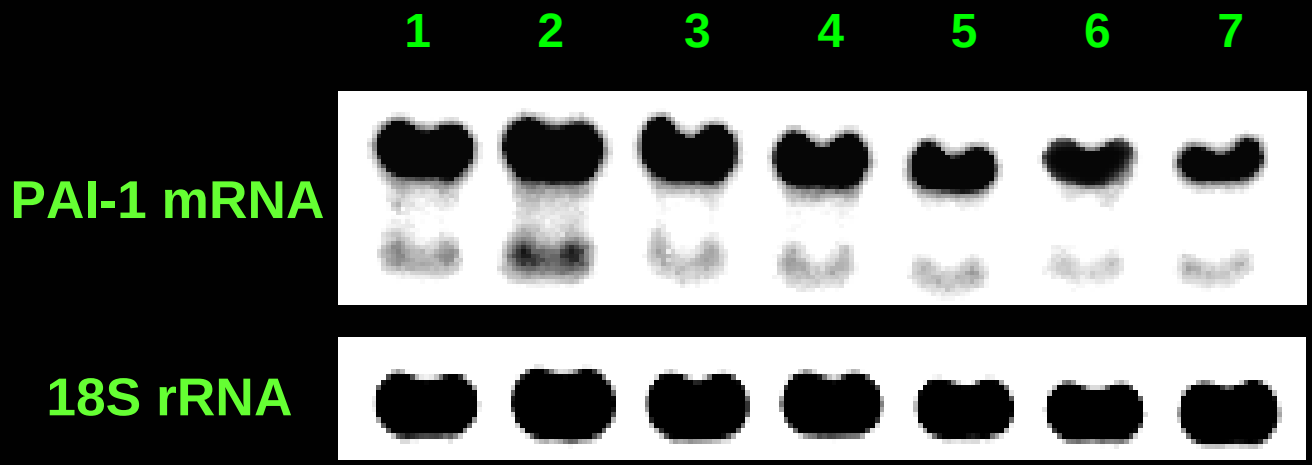


# Alpha lipoic acid has the potential to impact on CV risk



# 1.Effects of Alpha-Lipoic Acid on VSMC PAI-1 expression

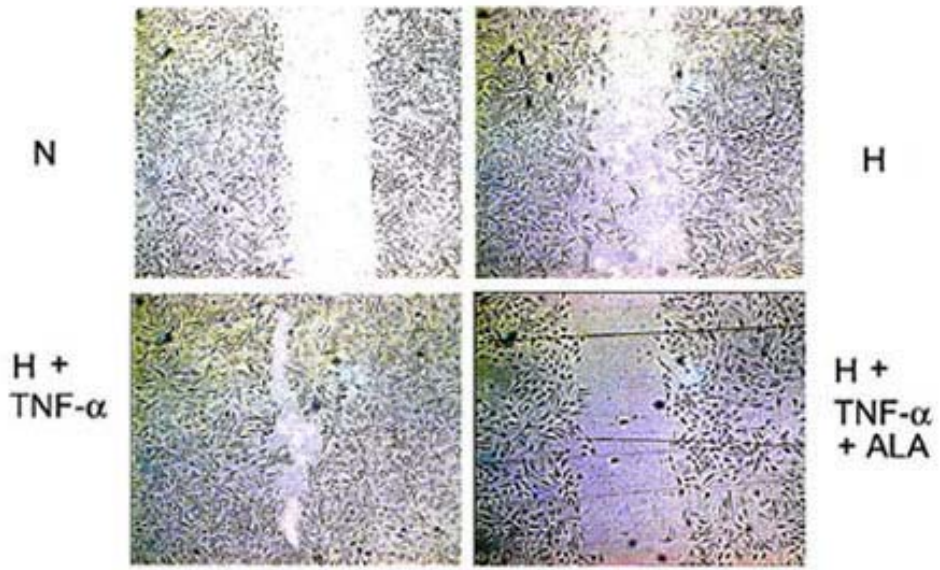
# Effect of α-Lipoic Acid on PAI-1 expression on high glucose condition in VSMC

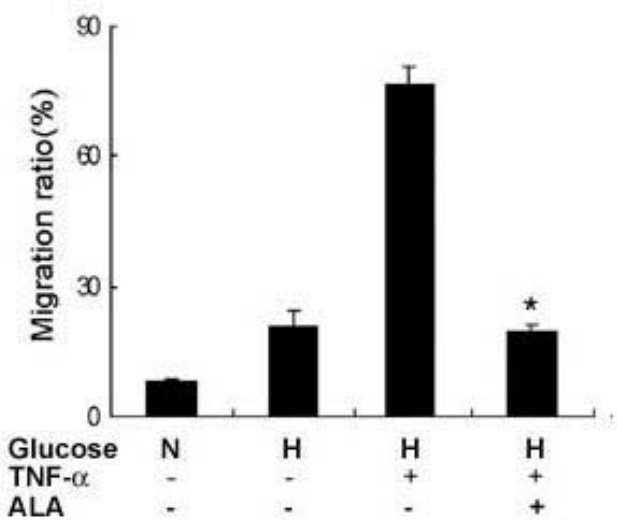



1. Normal glucose (NG)
2. High glucose (HG, 22 mM)
3. High glucose containing 250 μM α-Lipoic acid
4. High glucose containing 500 μM α-Lipoic acid
5. High glucose containing 1 mM α-Lipoic acid
6. High glucose containing 2 mM α-Lipoic acid
7. High glucose containing 4 mM α-Lipoic acid

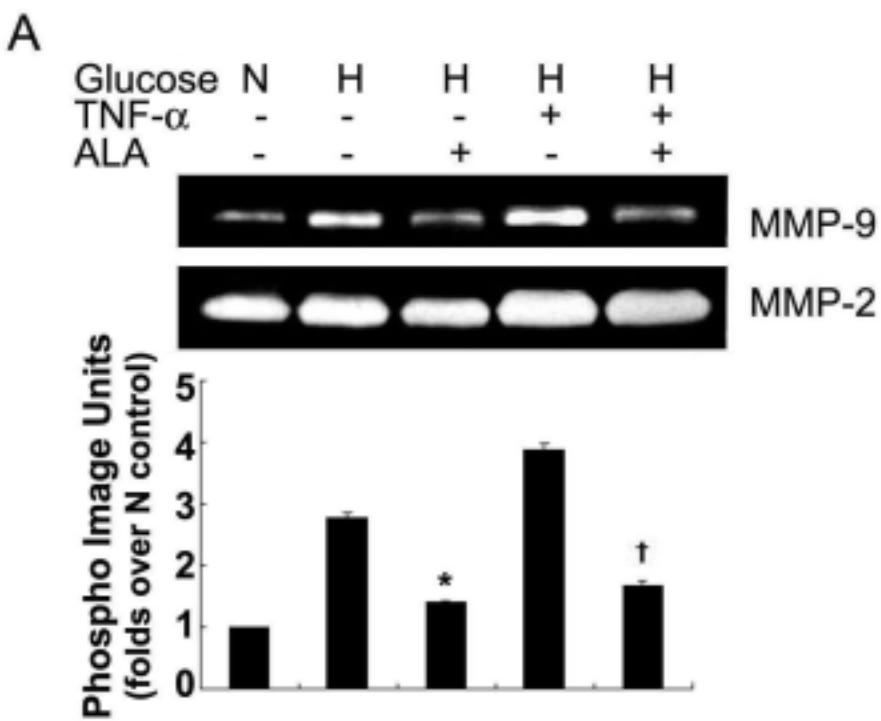
## 2. Effects of Alpha-Lipoic Acid on VSMC migration and MMP expression in Vitro and in Vivo

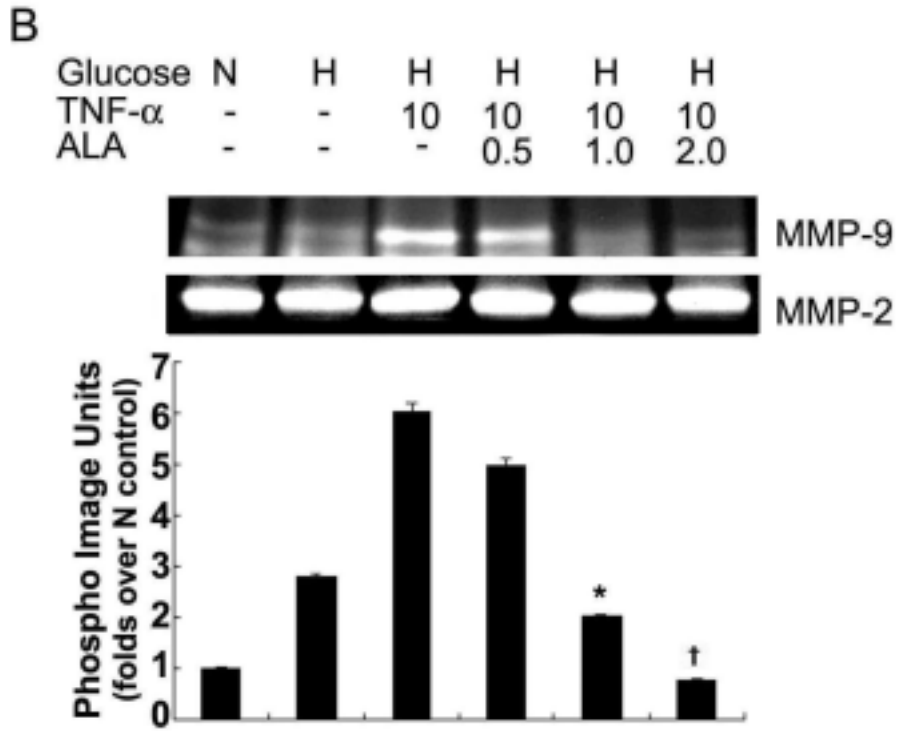
# Inhibitory effect of ALA on migration of VSMCs stimulated by high glucose and TNF-α



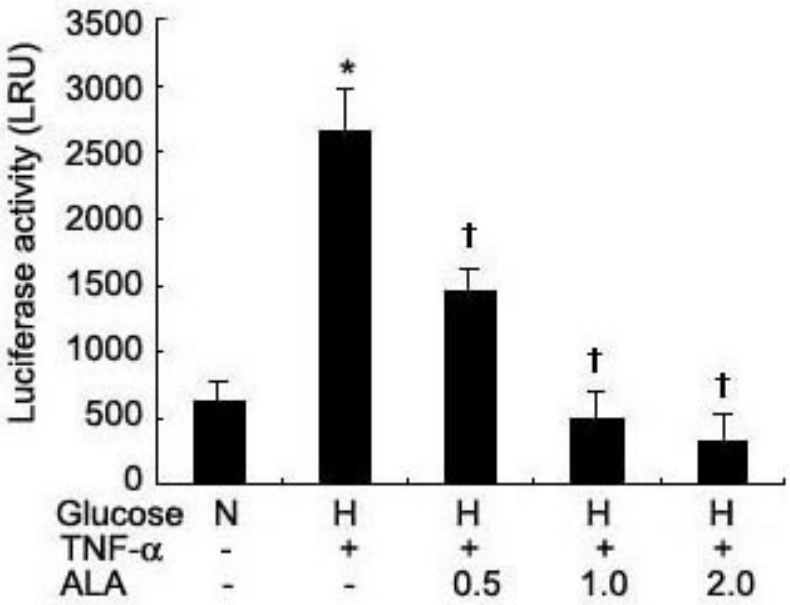



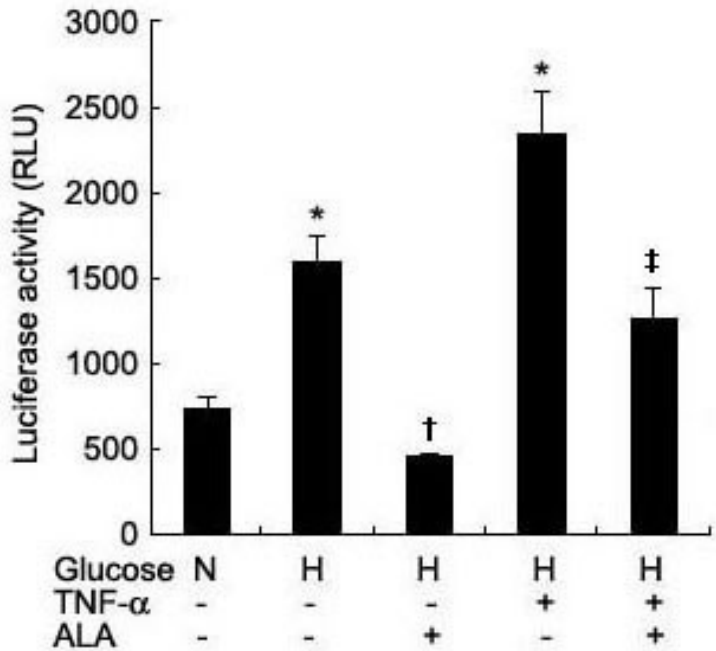
# Effect of ALA on MMP-9 activity



# Effect of ALA on MMP-9 promotor activity



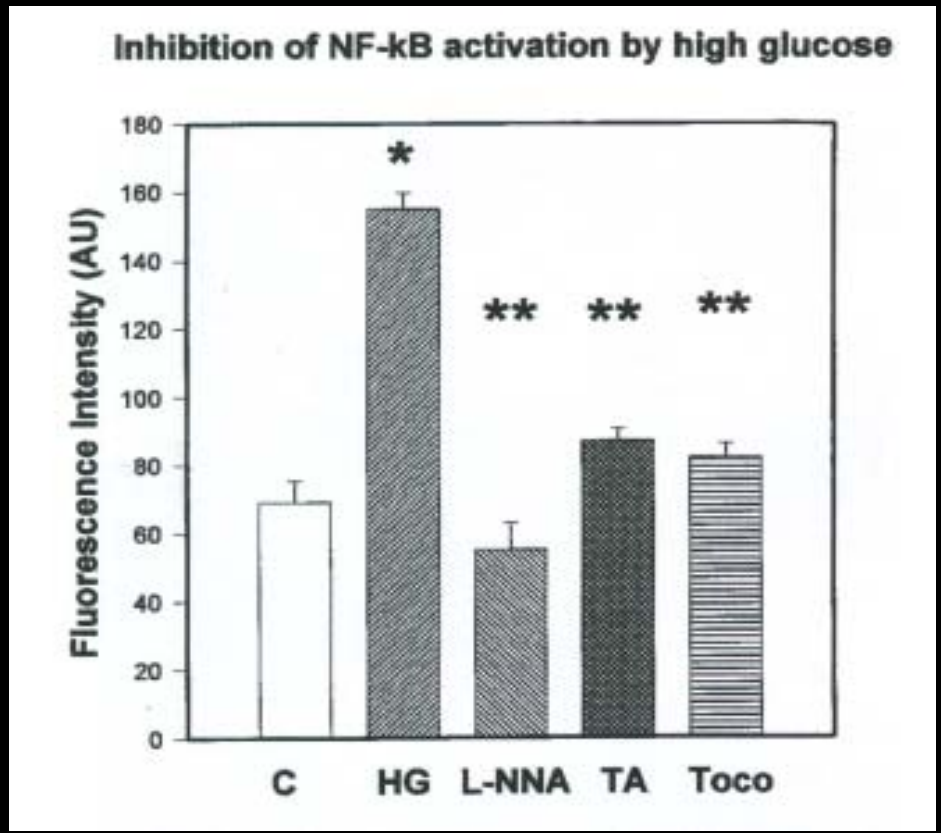
# Effects of α-Lipoic Acid on NF-kB Activity

- ALA decreases AGE induced NFkB activation in cultured endothelial cells

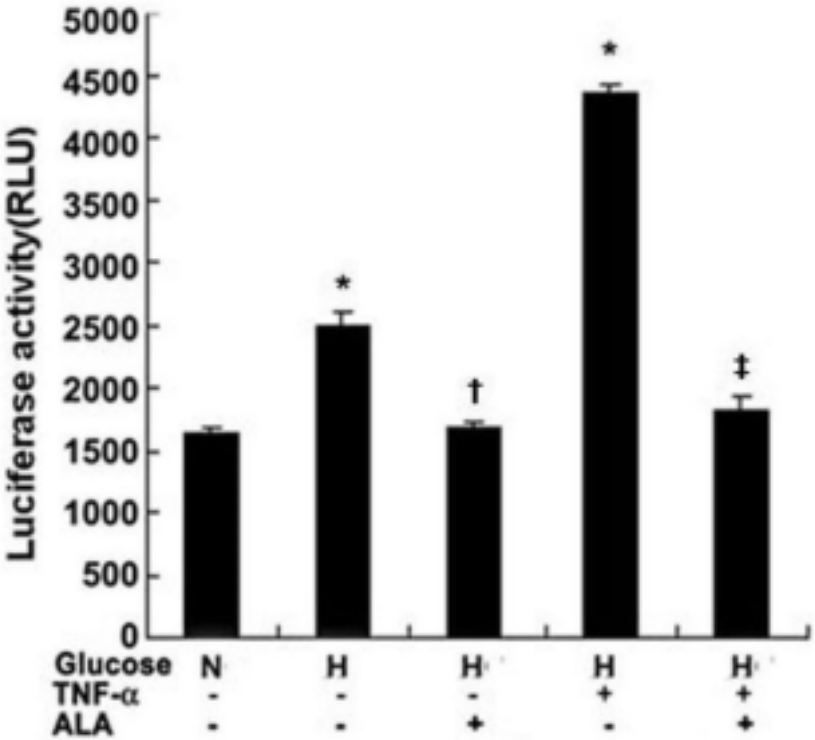
  *Bierhaus A et al, Diabetes, 1997*

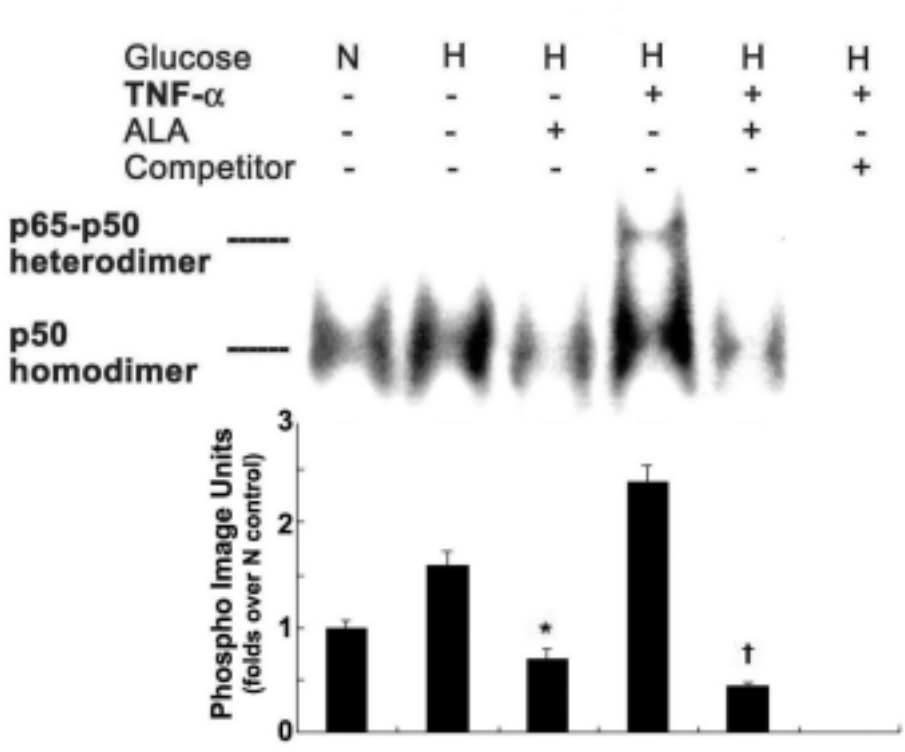
- ALA decreases ROS generation and NFkB activity induced by glucose

  *Du X et al, Free Radic Biol Med, 1999*

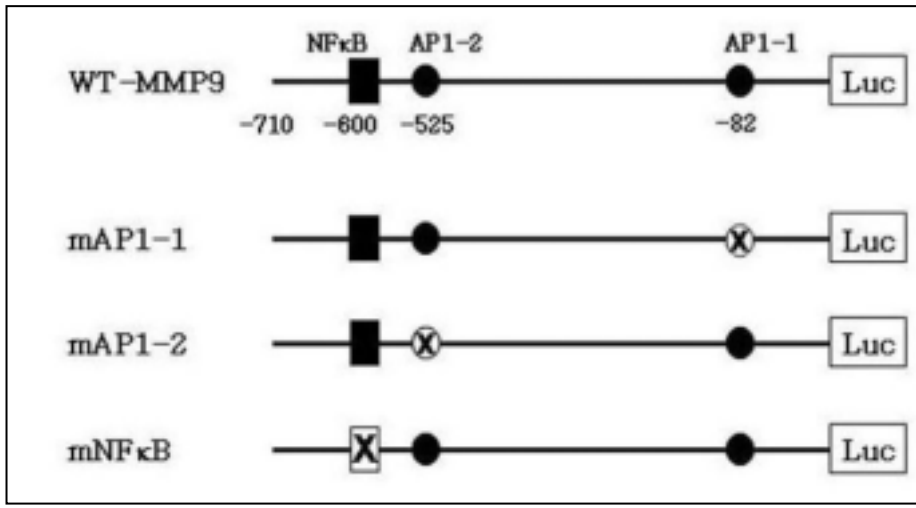


# Effects of ALA on the NF-κB activity in VSMC Cells

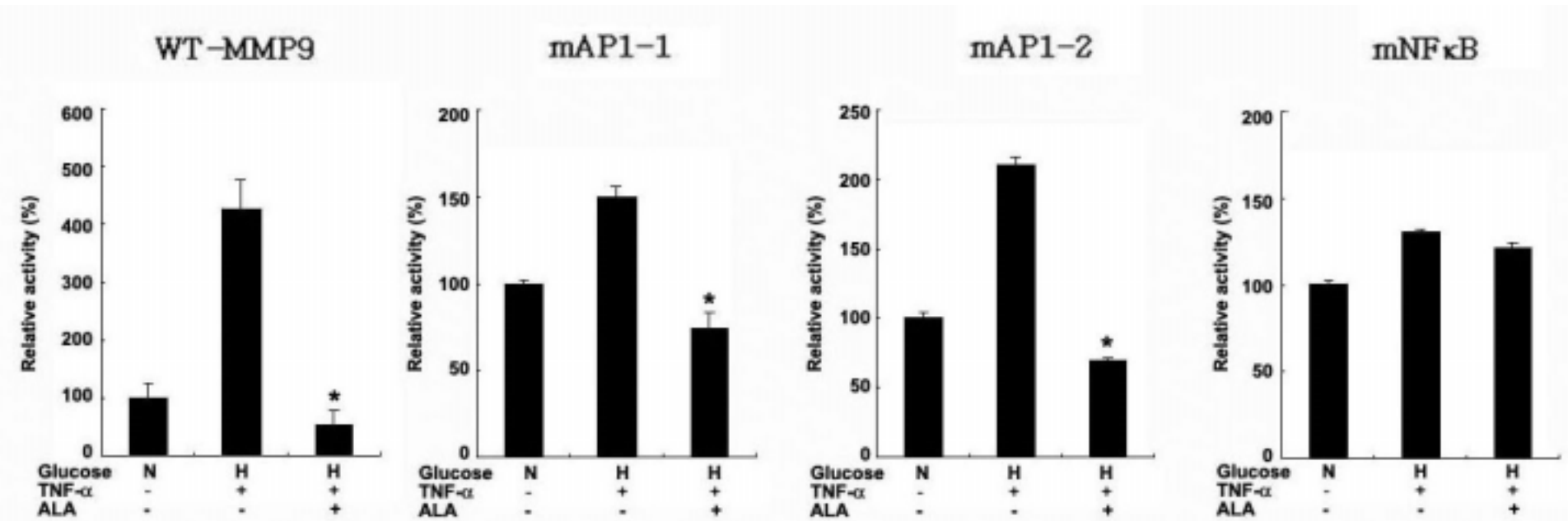


# Effects of ALA on the activities of NF-κB and AP-1




**Schematic structure of wild type and mutant MMP-9 promoter constructs used for promotor assay**



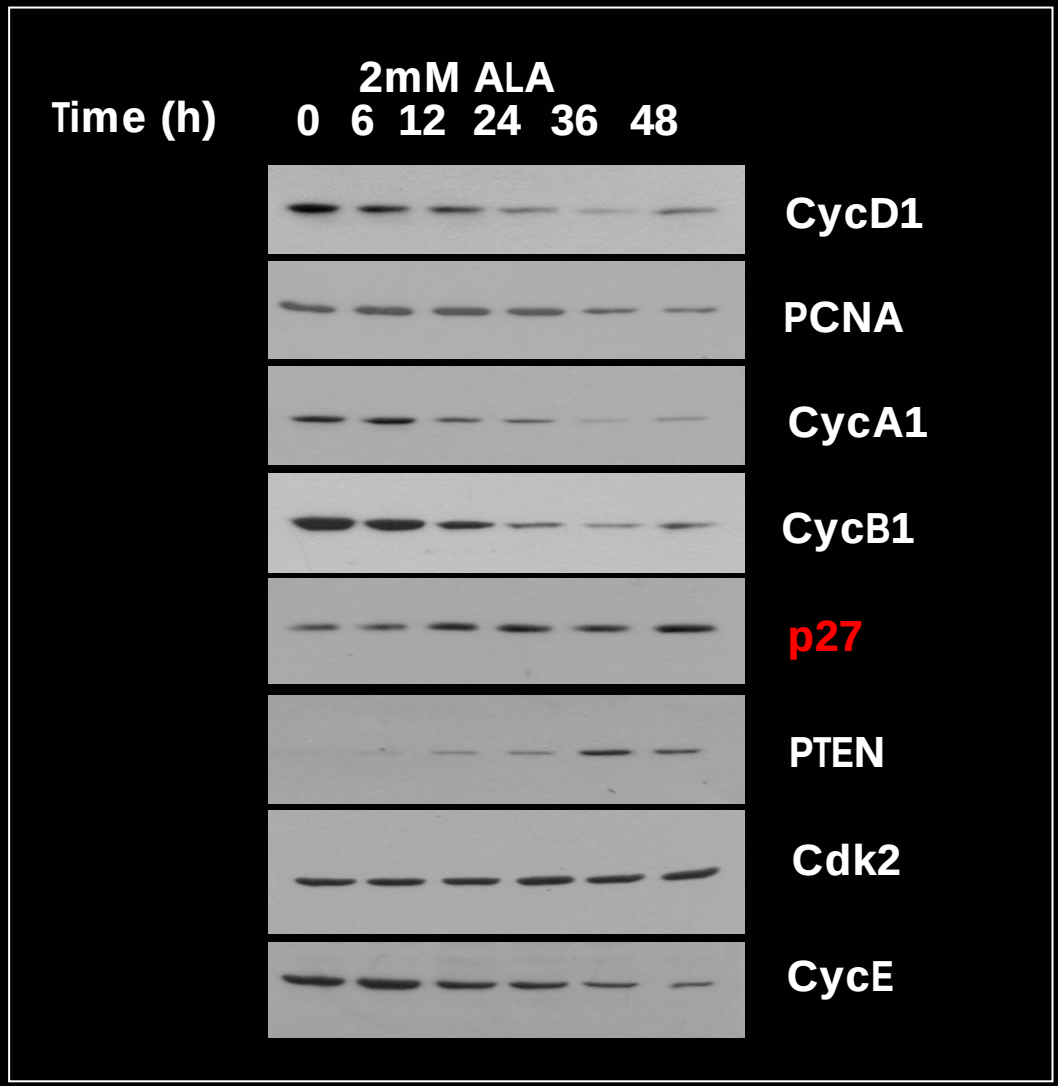
# Summary and Conclusion

- α-Lipoic acid suppress **PAI-1 expression, MMP-9 expression, VSMC migration.**

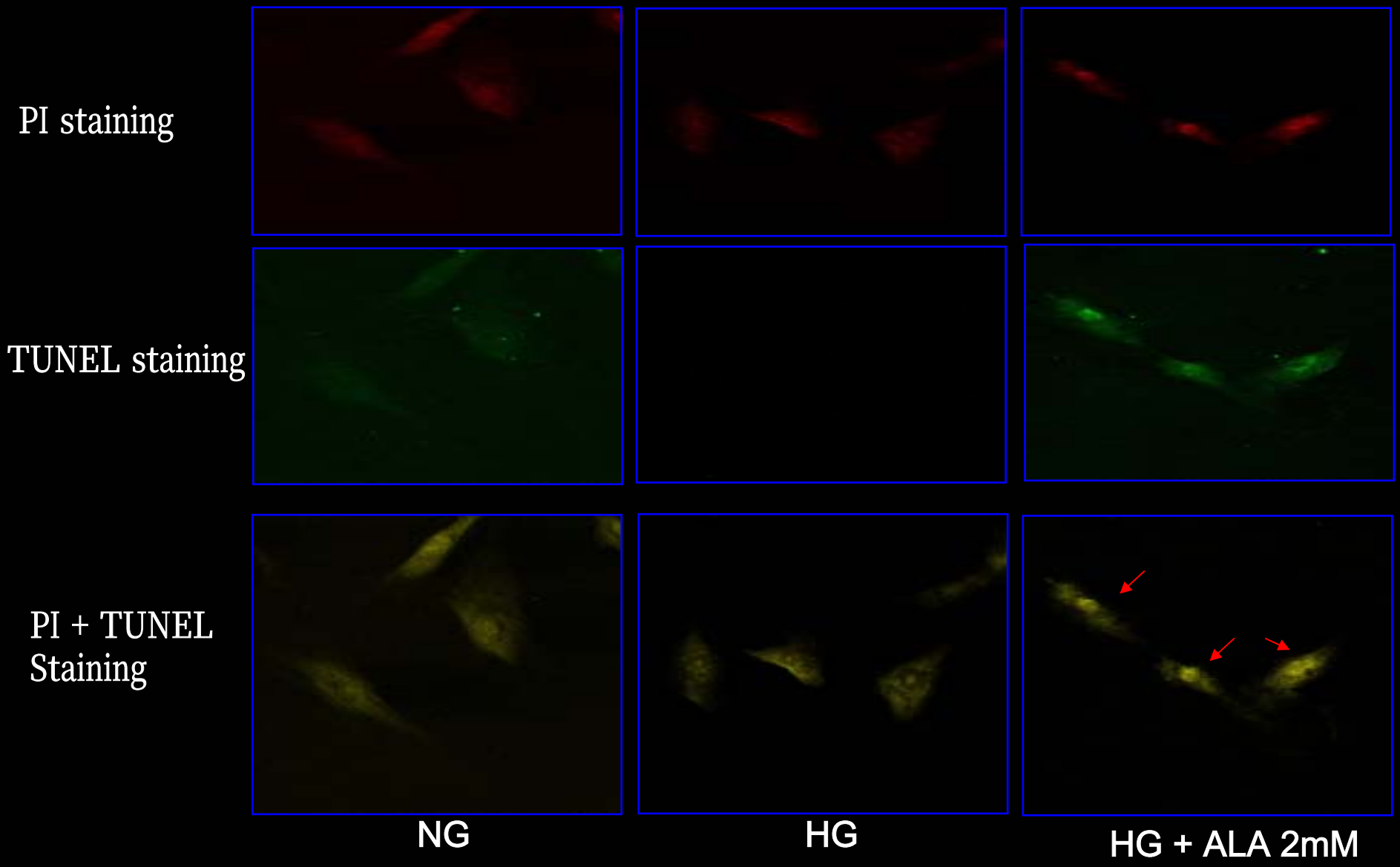
- This effect would be related with blocking **NFκB pathway** which increase expression of genes associated with atherosclerosis including TNF-α, IL-1, IL-6, MCP-1, VCAM-1, ICAM-1, E-selectin, endothelin-1, tissue factor and ets.

# 3. Effects of Alpha Lipoic Acid in Cell Cycle Regulation and Apoptosis

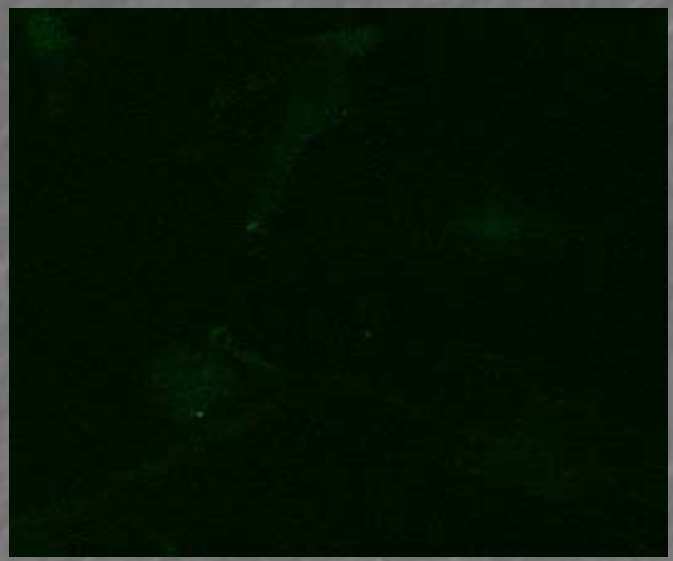
# Cell cycle arrest in ALA treated HASMC



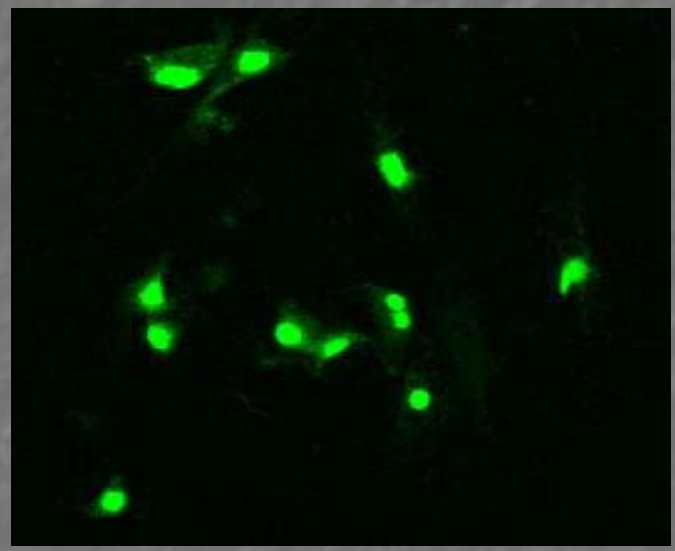
# Apoptotic Effects of ALA in RASMC



# Induce apoptosis by ALA treatment



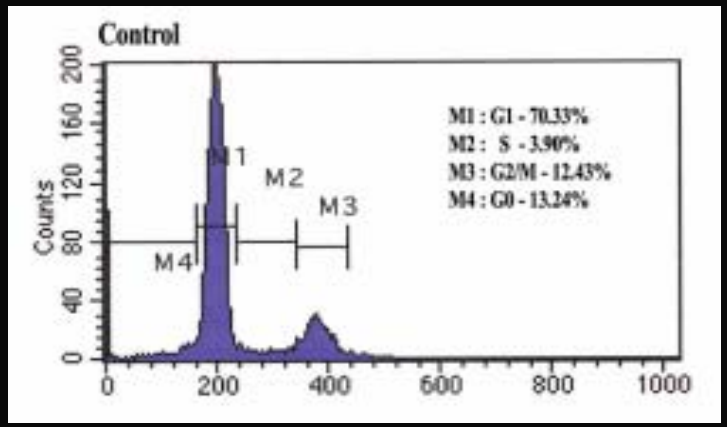
Control

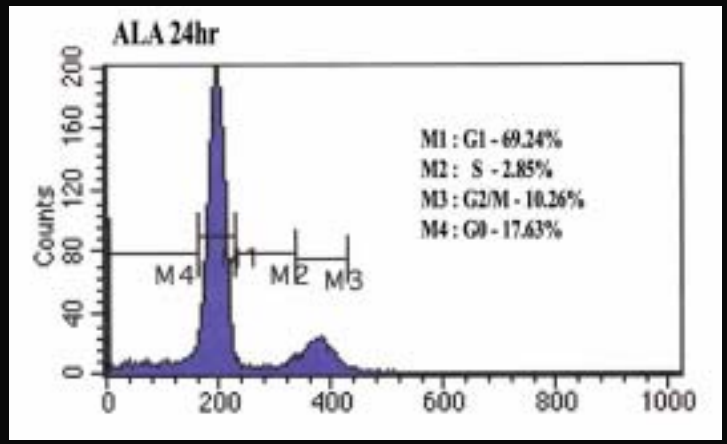


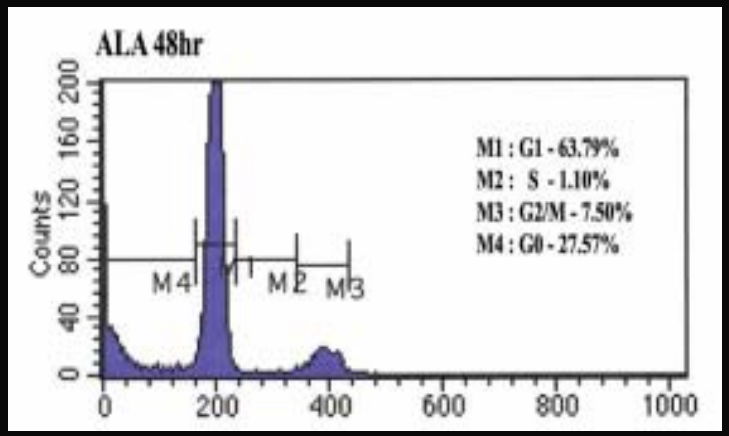
ALA 2mM

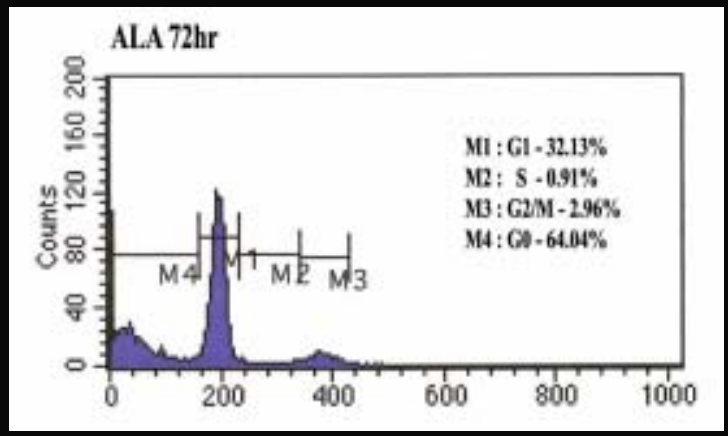
Smooth muscle cells were treated with alpha lipoic acid.
After 20 hours, cells were harvested, fixed, processed and analysed for TUNEL staining.
The apoptotic effect by alpha lipoic acid was shown.

# Induce of apoptosis by ALA treatment



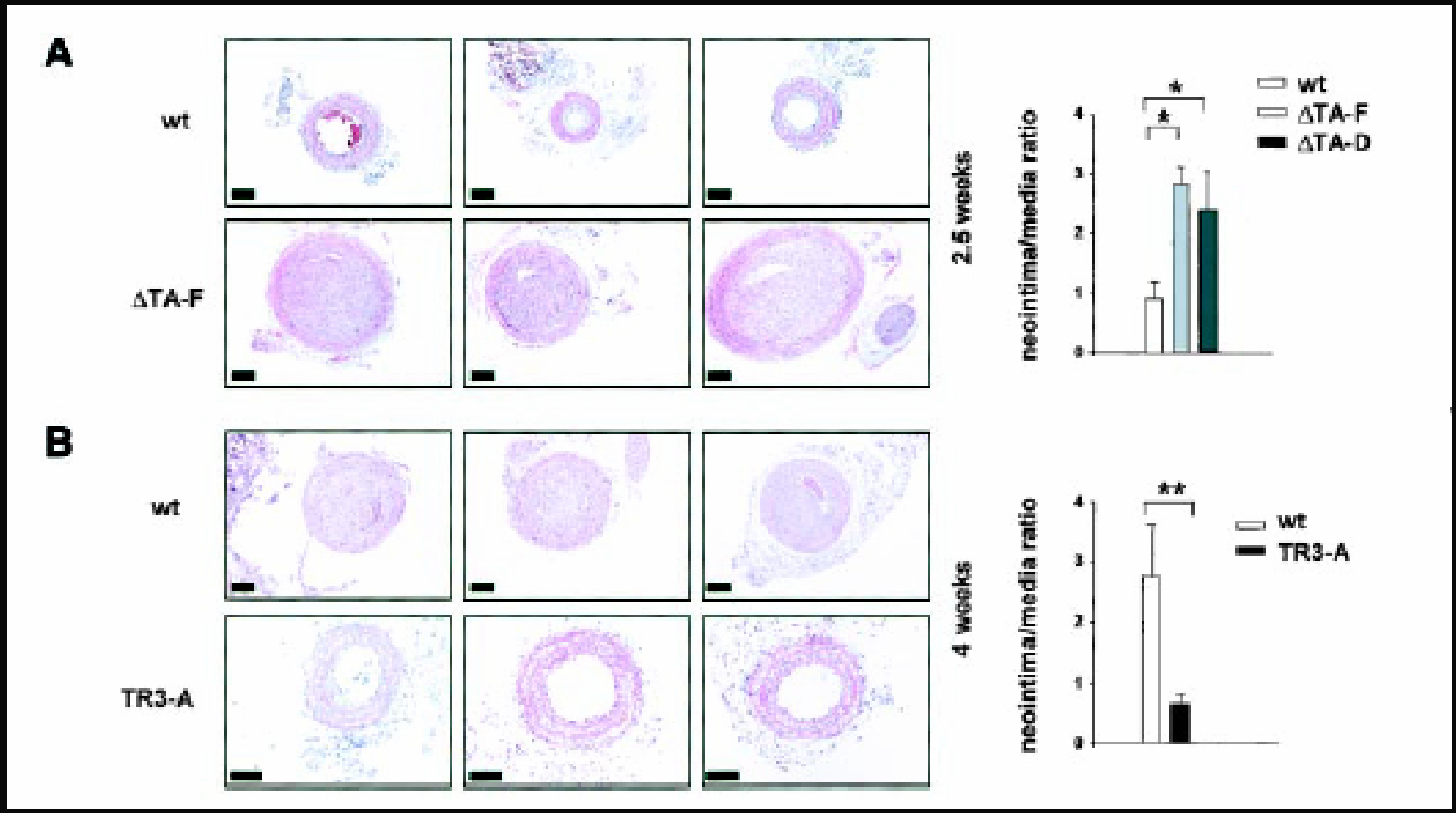




**FACS analysis was performed smooth muscle cells after alpha lipoic acid. Ten thousand cells were sorted. The percentages of cells in G1, S and G2-M phases of the diploid cell cycle are shown. Alpha lipoic acid induced apoptosis.**

**Adenovirus-mediated expression of a variant of TR3(Nur77), decreases DNA synthesis and increases p27Kip1 protein expression in cultured SMCs.**

**Protective Function of Transcription Factor TR3 Orphan Receptor(Nur77) in Atherogenesis Decreased Lesion Formation in Carotid Artery Ligation Model in TR3 Transgenic Mice.**

***Circulation*. 2002;106:1530-1535**

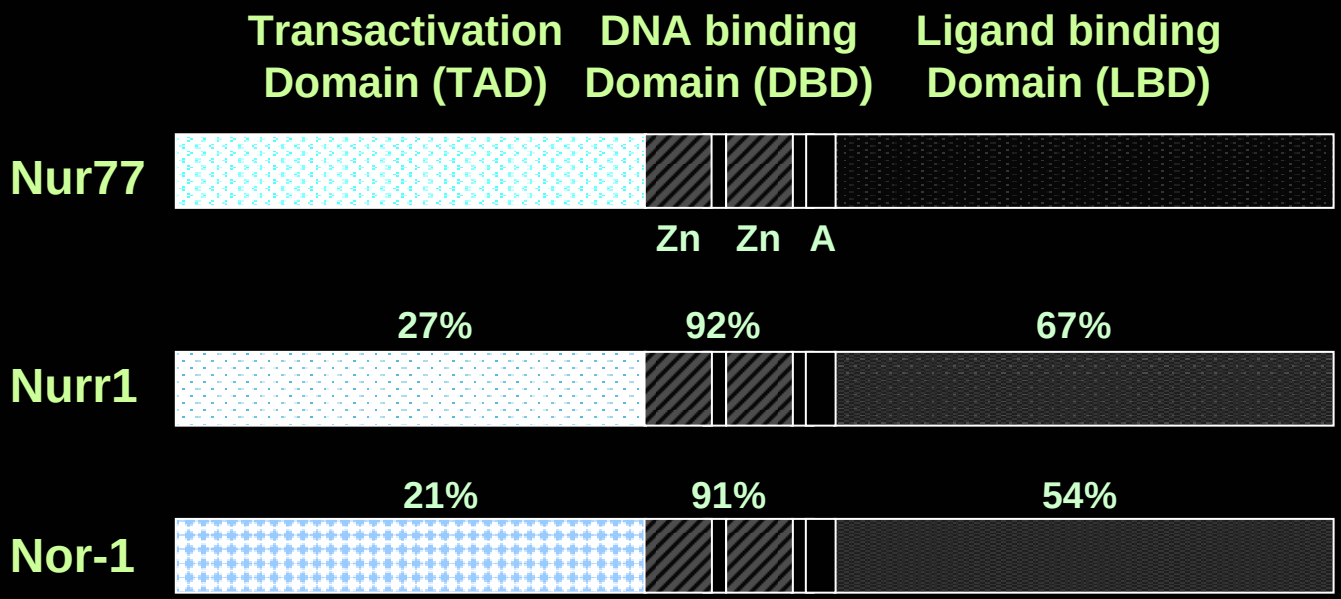



Carotid artery ligation in TR3 and TA transgenic mice.
A, ligated carotid arteries from wild-type and TA-F mice 2.5 weeks after ligation. Neointima/media ratios of ligated left carotid arteries are 3-fold larger in TA-D and TA-F transgenic mice compared with wild-type mice (n4 to 6) (*$P$0.05).
B, left carotid arteries from wild type and TR3-A mice 4 weeks after ligation. Morphometric analyses disclosed that TR3 overexpression results in a 5-fold reduction of neointima/media ratio compared with ratio in wild-type mice(n5 to 8) (**$P$0.01). Bar100 m.

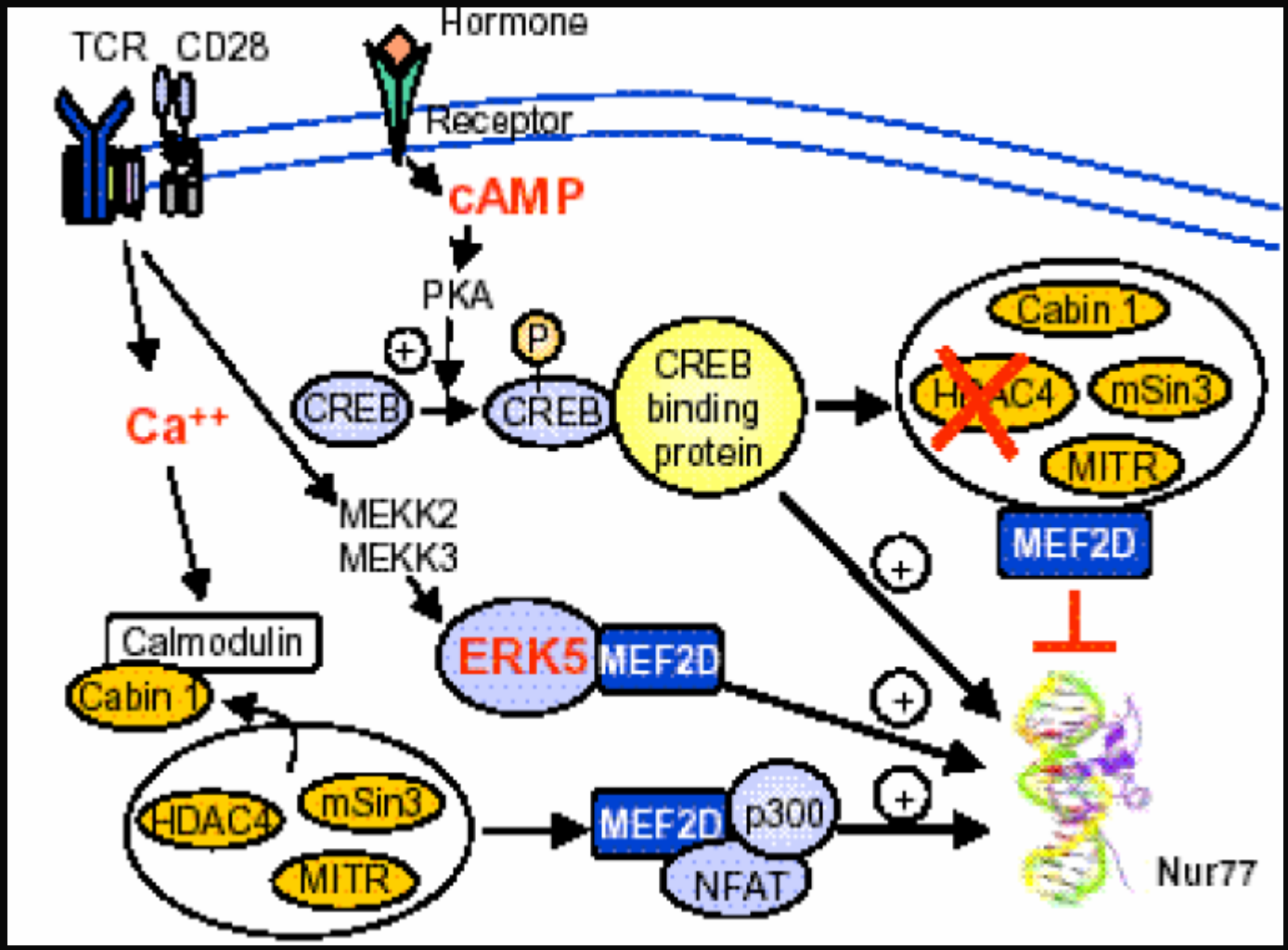
*Circulation*. 2002;106:1530-1535

# Nur77 Family

- Steroid/thyroid hormone receptor superfamily
- Orphan nuclear receptor / Immediate early gene



# Major targets for activation of Nur77 transcription.

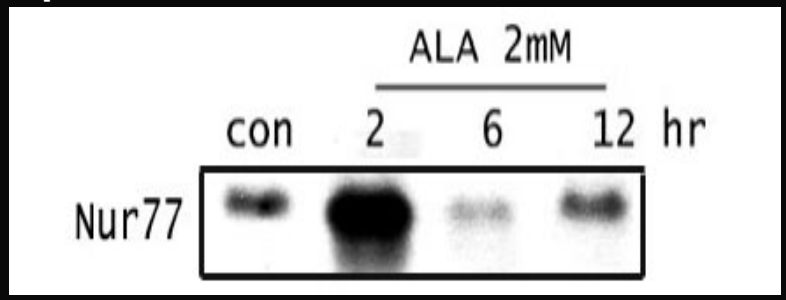


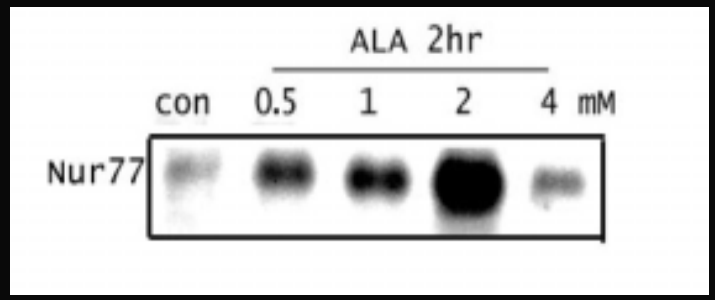

Several critical secondary messengers including cAMP, Ca++, and the activation of ERK, are associated with the activation of Nur77.

**Does ALA treatment can induce Nur77 Expression?**

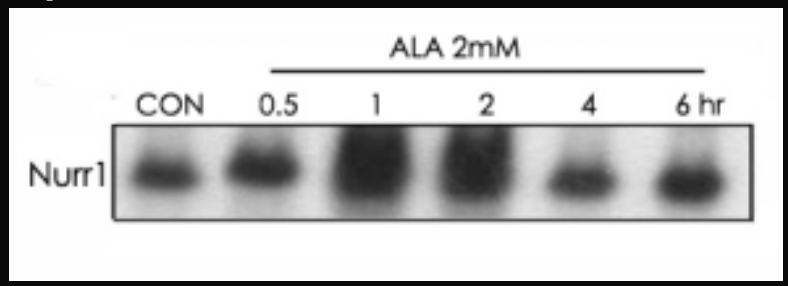
# Expression of Nur77 and Nurr1 in SMC by alpha lipoic acid

**Expression of Nur77**



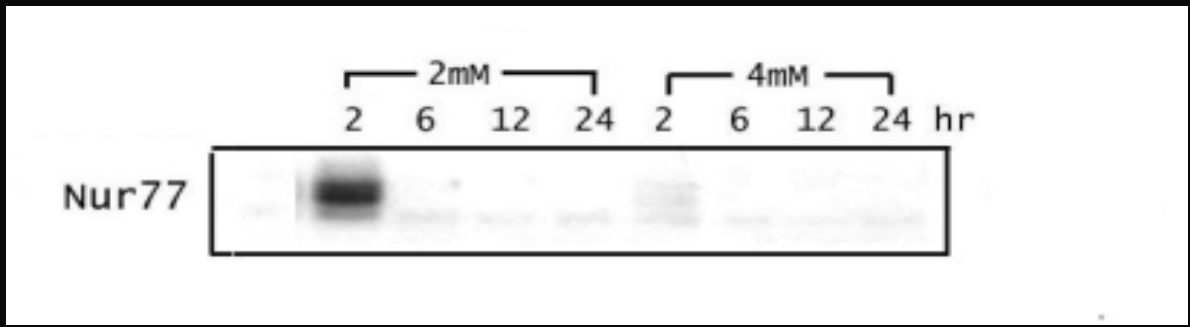

**Expresssion of Nurr1**



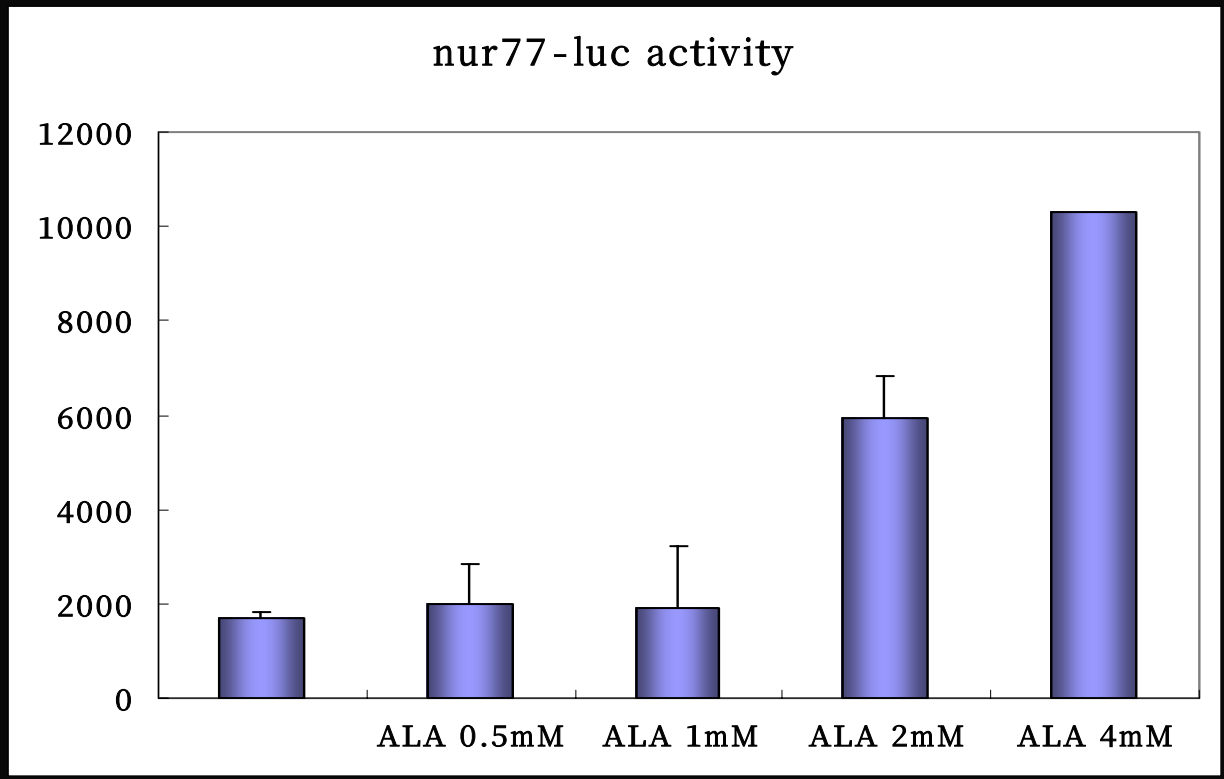

**The rat aortic smooth muscle cells were treated with alpha lipoic acid for  indicated time and dose.**

# Expression of Nur77 in SMC by alpha lipoic acid

**Protein Expression of Nur77**



# Effect of Alpha lipoic acid on Nur77 promoter activity



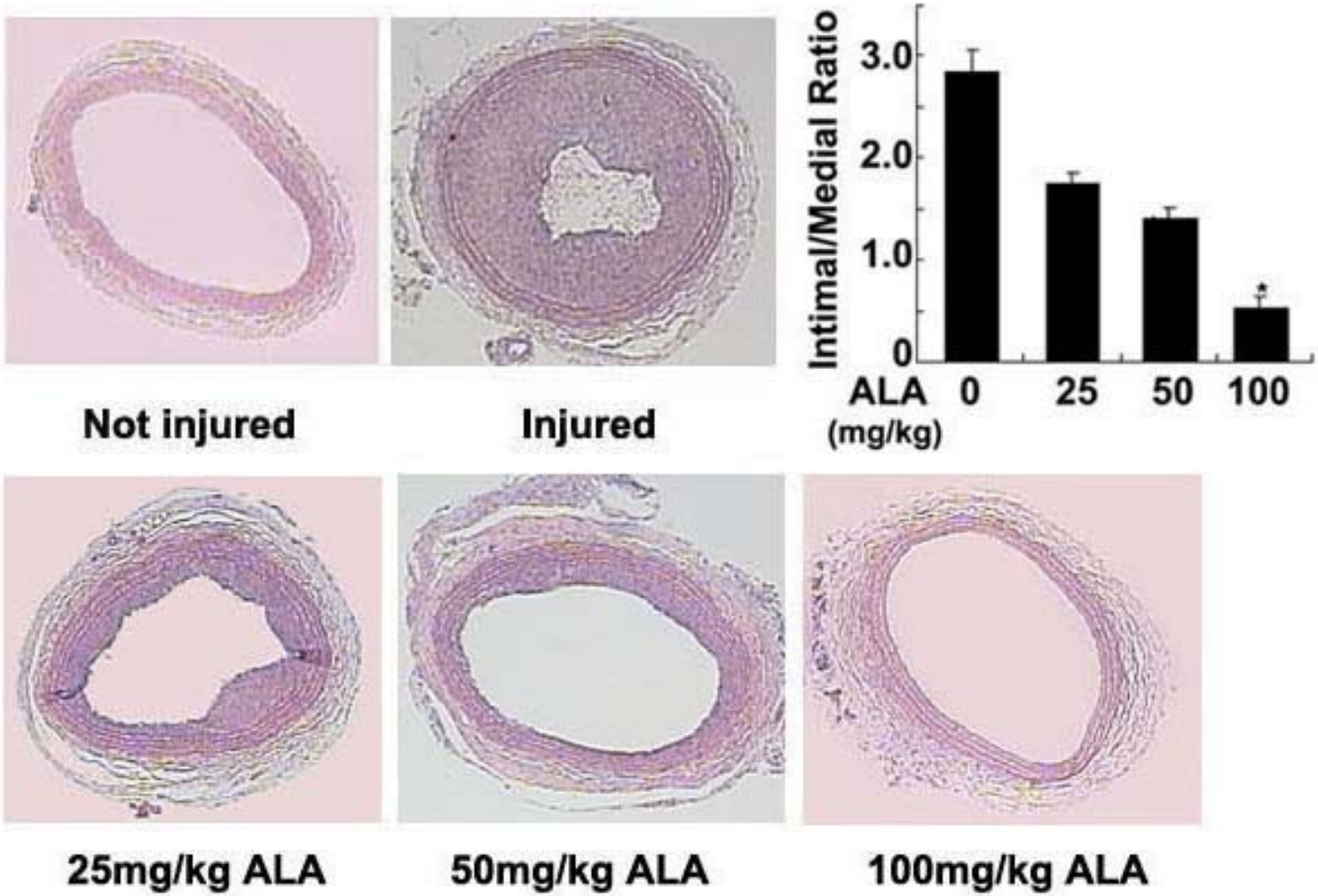

**Effects of ALA on the promoter activity of Nur77-luc promoter in VSMCs. VSMCs were transfected with the Nur77-luciferase reporter plasmid, the cells were treated with 2mM of alpha lipoic acid for 16hrs. The promoter activity was increased by dose dependent manner.**

# Evaluate Effects of α-lipoic acid on Neointimal Formation in Balloon Injury Model


## Study (dose dependent)

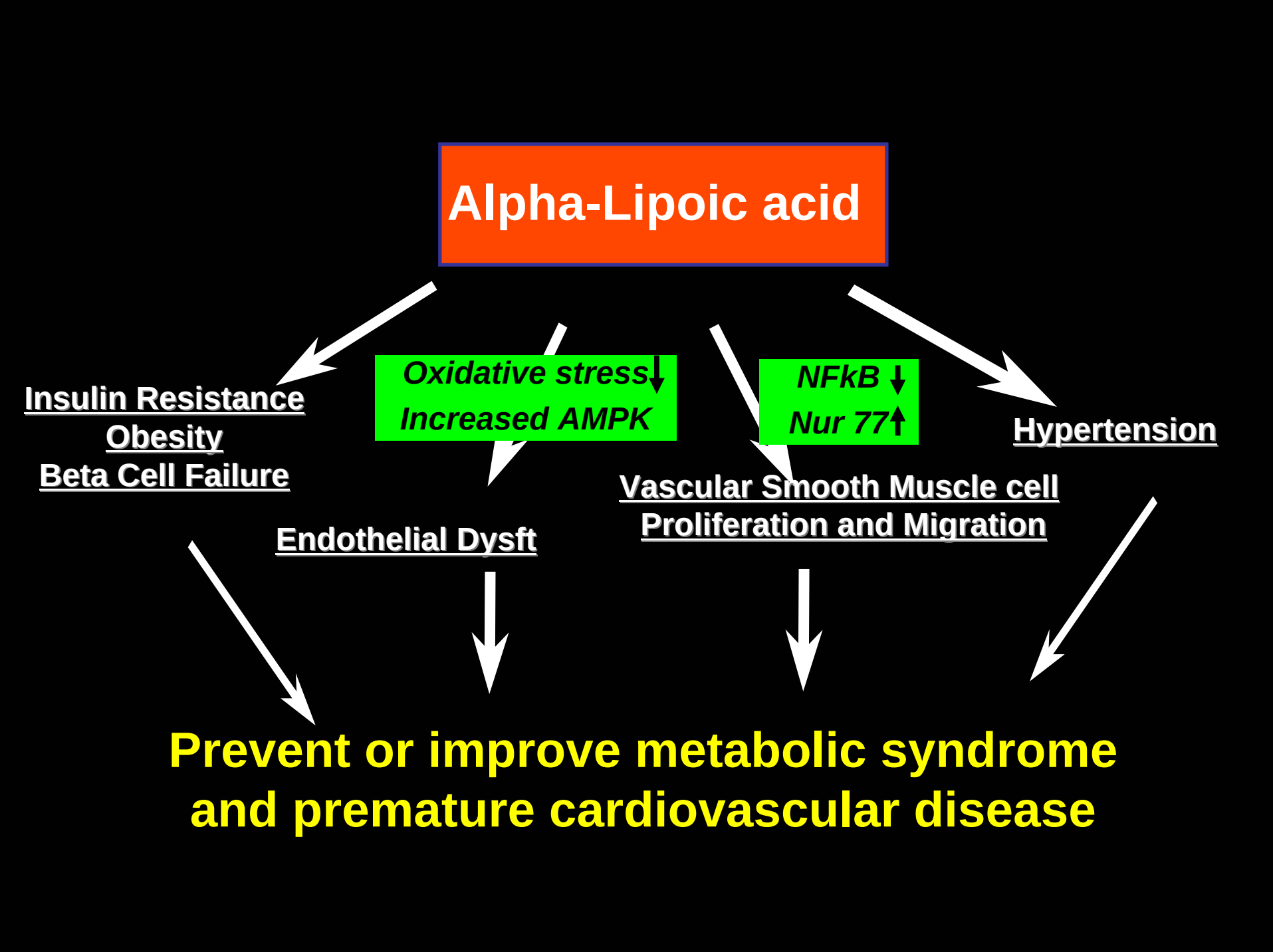
1) Normal control
2) Balloon injury(BI)
3) ALA – BI – ALA (25mg/kg)
4) ALA – BI – ALA (50mg/kg)
5) ALA – BI – ALA (100mg/kg)

# Cross-section of the common carotid artery of control rat and ALA treated rat 14 days after balloon injury



# Summary and Conclusion II

- α-Lipoic acid suppress **PAI-1 expression, MMP-9 expression, RASMC proliferation, migration and neointimal formation.**
- This effect might be related with blocking **NFκB pathway** which increase expression of genes associated with atherosclerosis including TNF-α, IL-1, IL-6, MCP-1, VCAM-1, ICAM-1, E-selectin, endothelin-1, tissue factor.
- Tx with ALA in VSMC induced apoptosis and inhibition of VSMC proliferation, possibly through the increased Nur 77 expression.


Alpha-Lipoic acid
Insulin Resistance
Obesity
Beta Cell Failure
Oxidative stress ↓
Increased AMPK
NFkB ↓
Nur 77 ↑
Hypertension
Endothelial Dysft
Vascular Smooth Muscle cell
Proliferation and Migration
Prevent or improve metabolic syndrome
and premature cardiovascular disease

# Pleiotropic effects of statins

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School of medicine, Keimyung University

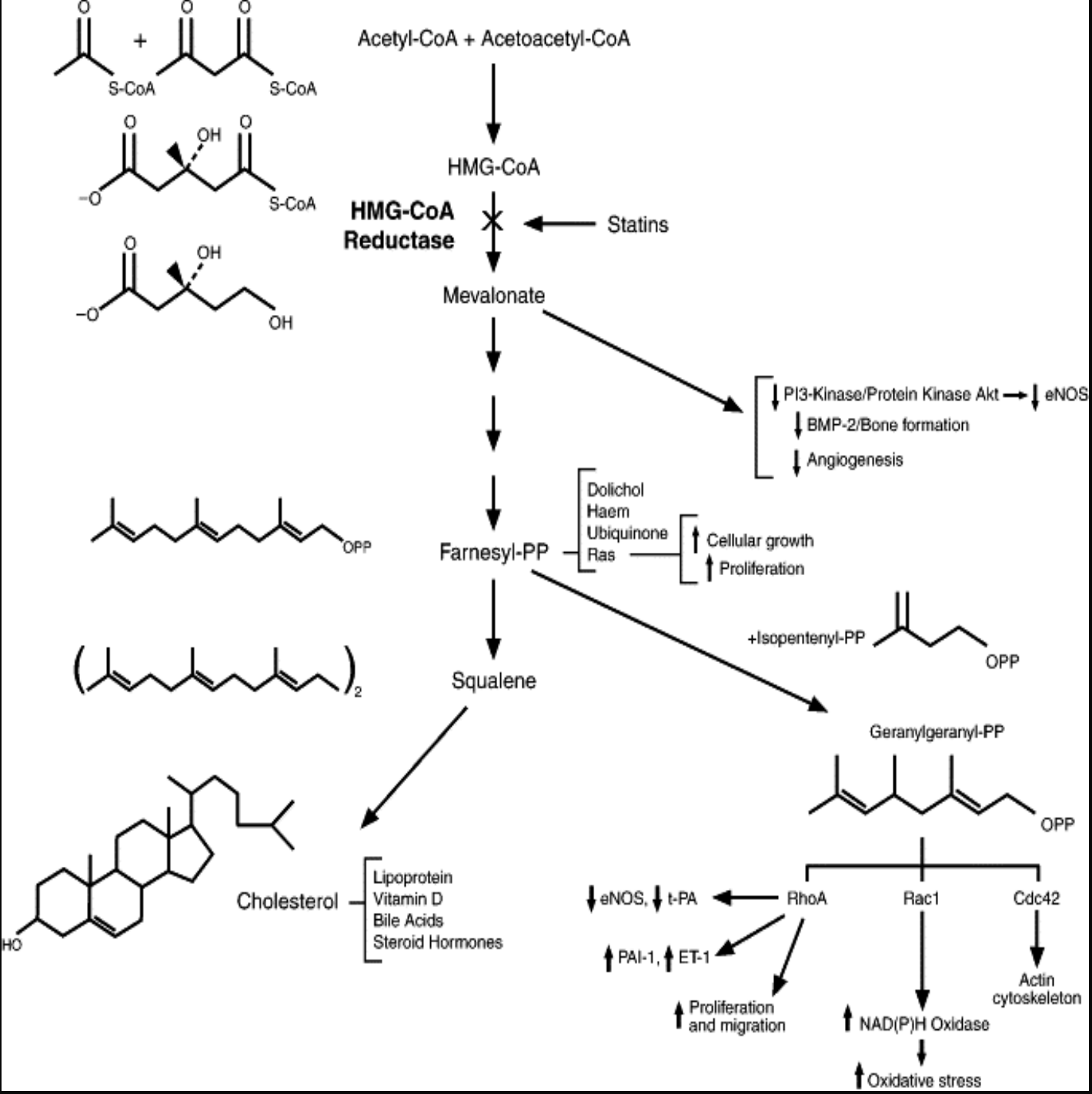



**Fig. 2. Cholesterol biosynthesis pathway. Inhibition of hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase by statins reduces the synthesis of mevalonate, cholesterol and isoprenoids. Reducing these metabolites with statins leads to subsequent changes in bioactive proteins, including the activation of the GTP-binding proteins Rho and Rac.**

**Our study, to clarify whether**

**In *in vivo* Balloon injury Model.**

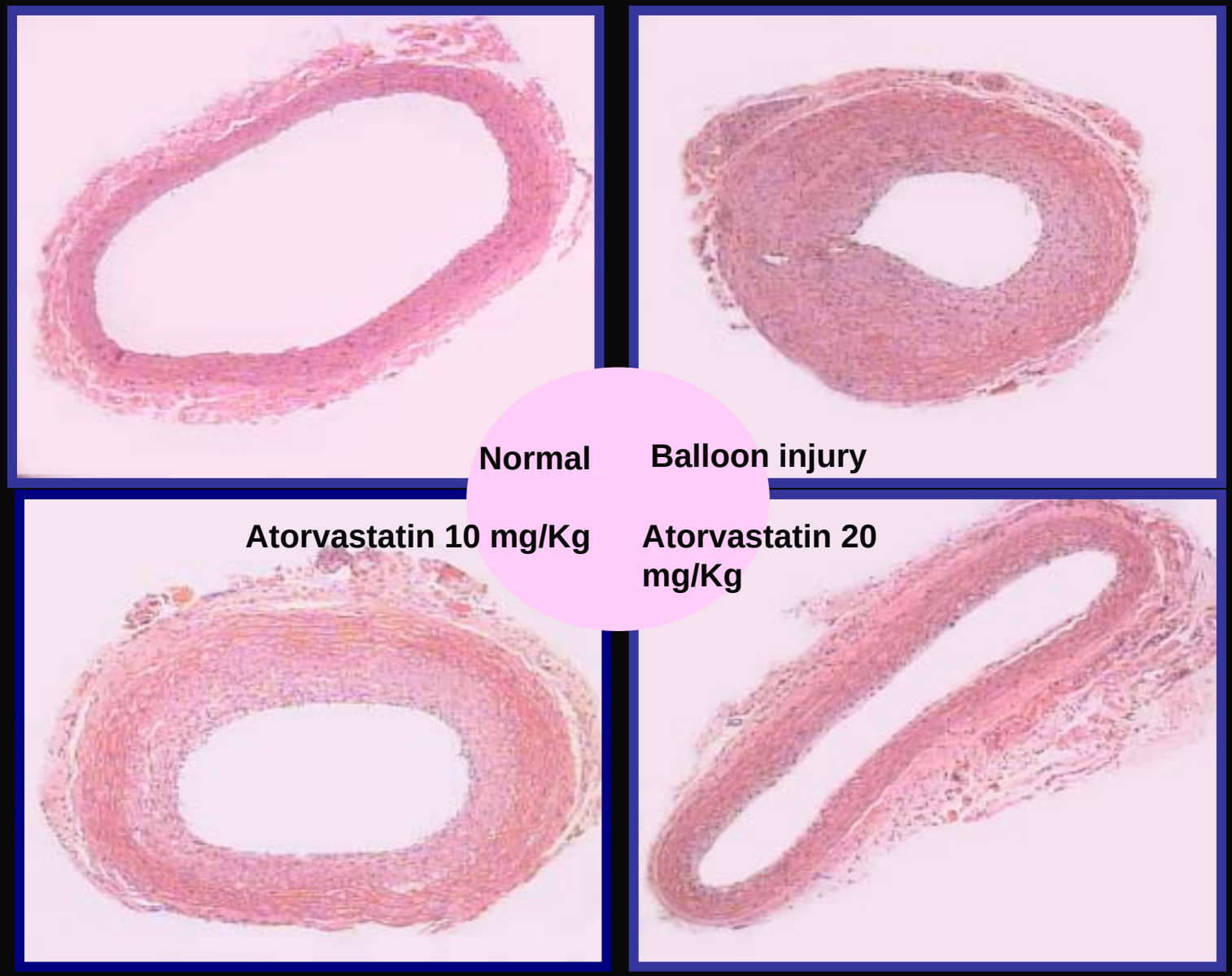
**1.Atorvastatin can suppress neointiaml Formation and *ICAM-1 Expression* in *in vivo* Balloon injury Model.**

**In *in vitro* EC and SMC**

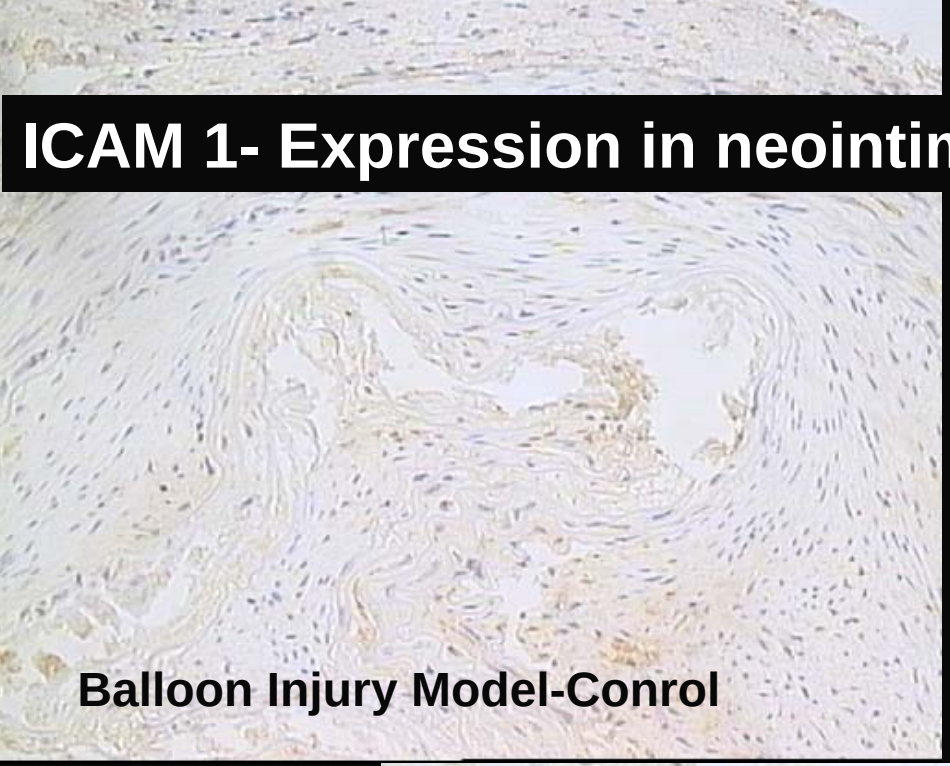
**2. Atorvastatin can suppress IFN-γ induced expression of *ICAM***

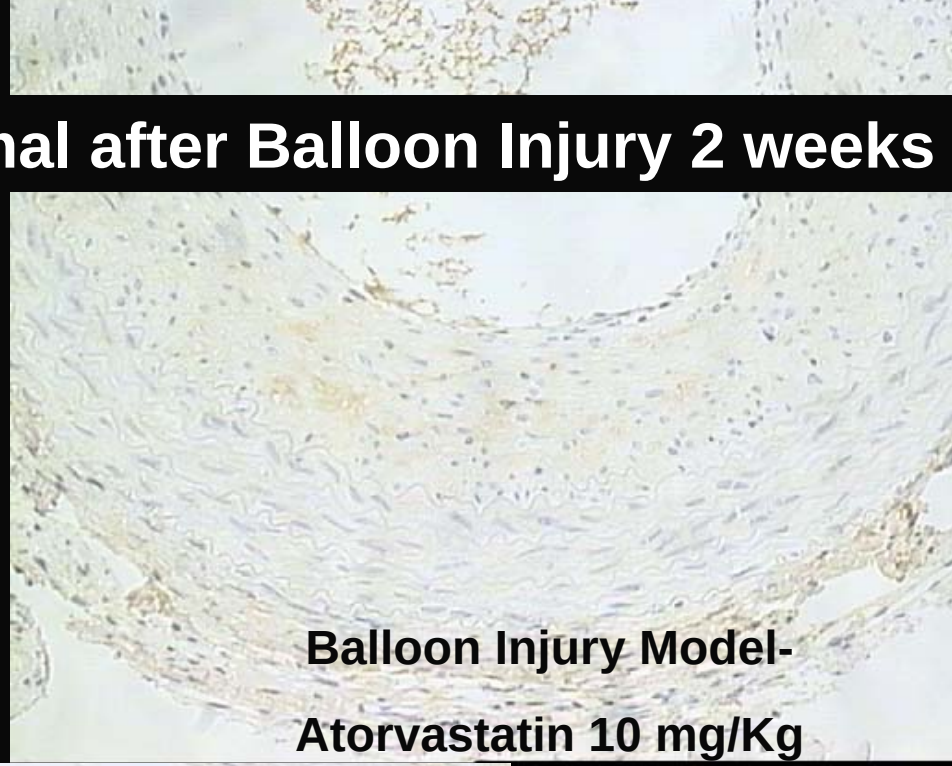
**3. Atorvastatin can modulates MAP kinase activity which suppresses IFN- γ-induced phosphorylation of serine 727 in STAT1.**

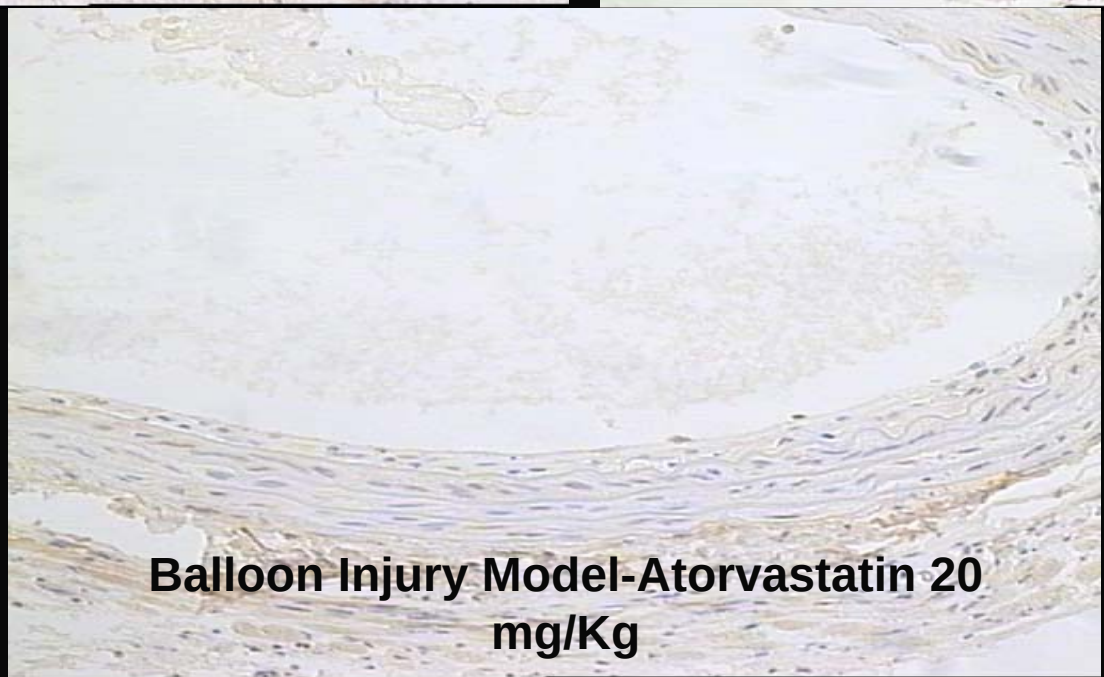
Lee et al, EMM, 34(6), 451-461, 2002


Normal
Balloon injury
Atorvastatin 10 mg/Kg
Atorvastatin 20 mg/Kg

# ICAM 1- Expression in neointimal after Balloon Injury 2 weeks



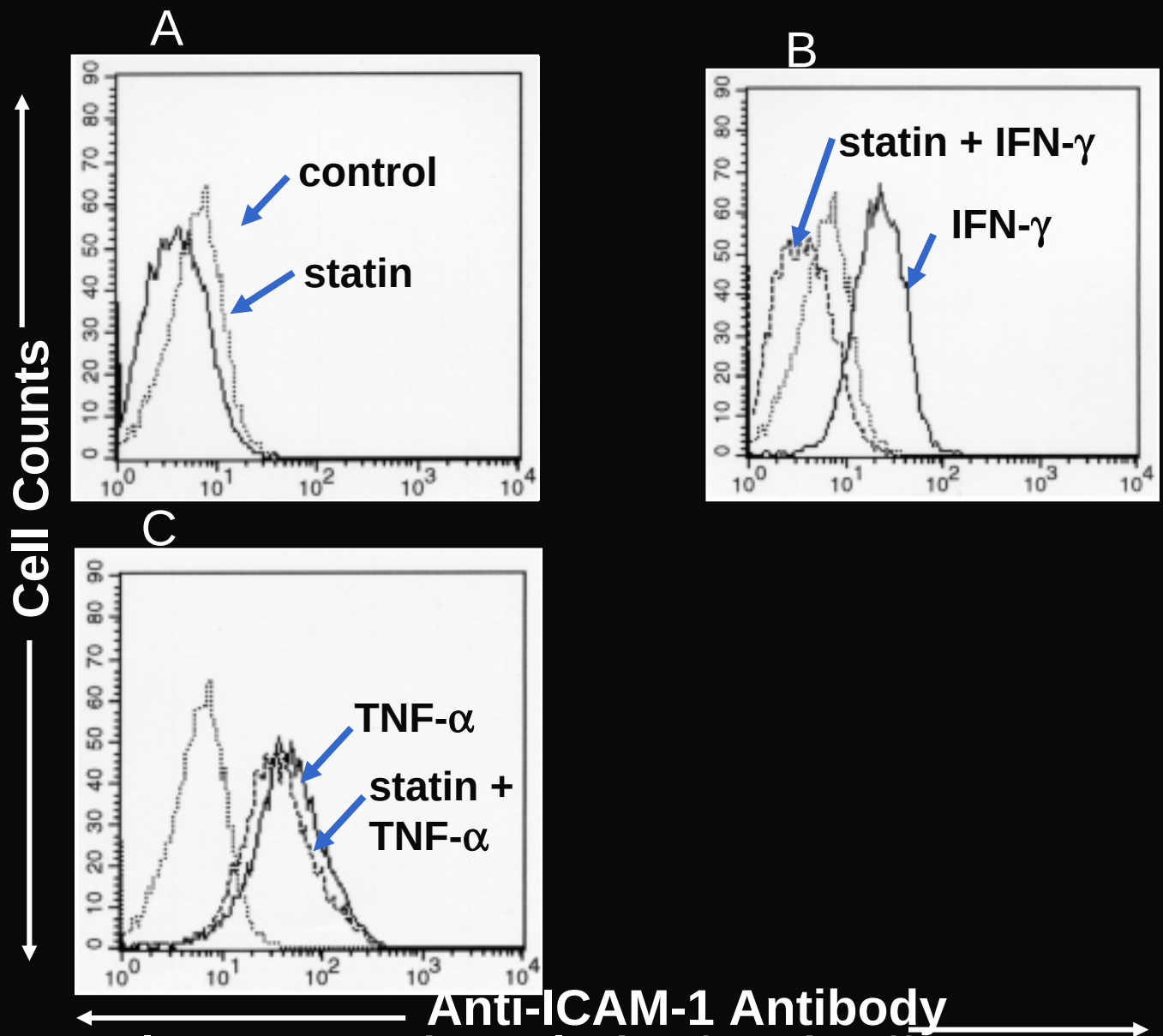



**Figure 1. Flow cytometric analysis of statin effect on ICAM-1 upregulation in response to IFN-γ and TNF-α.**

**Lee et al, EMM, 34(6), 451-461, 2002**

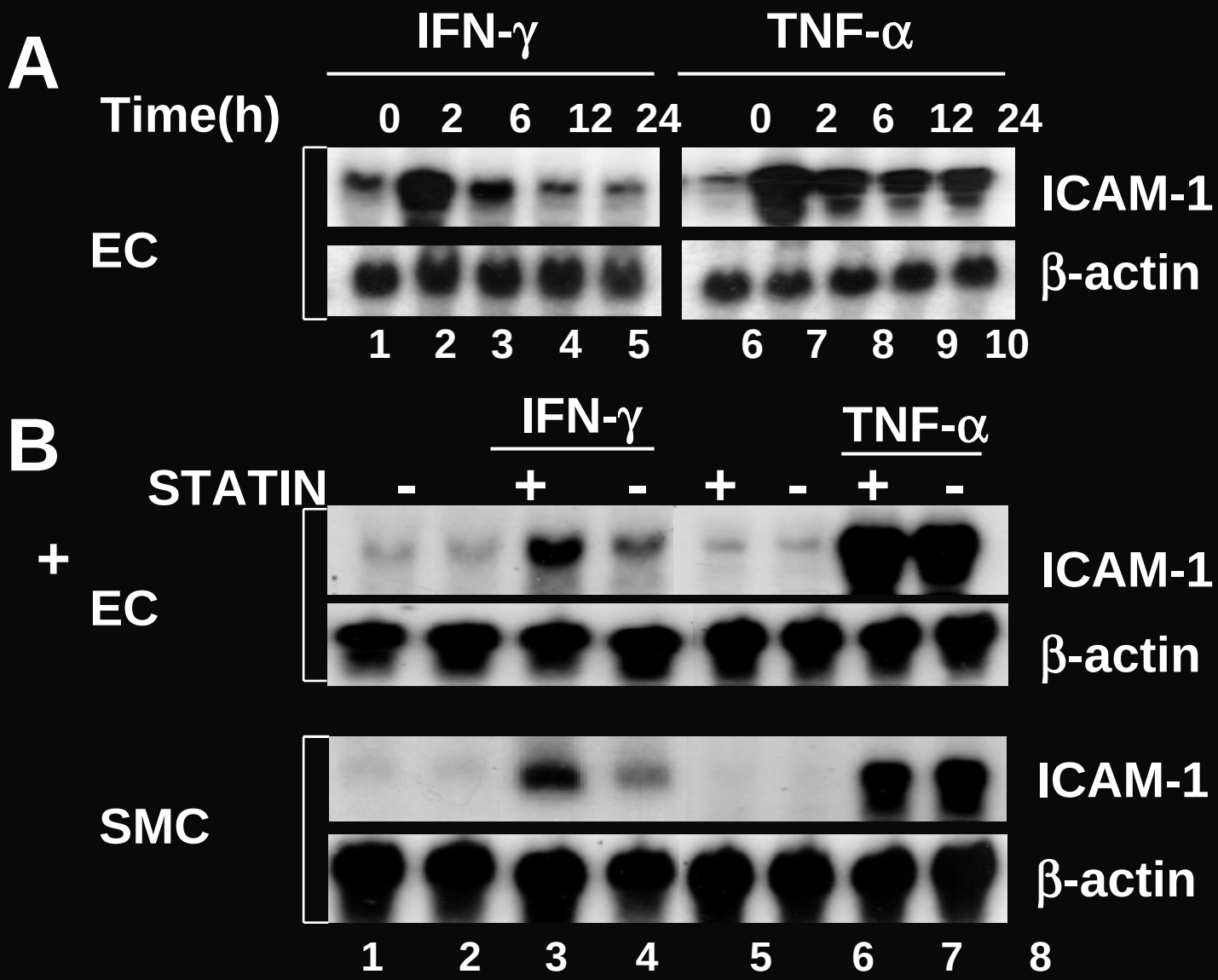



**Figure 2. Effects of statin, IFN-γ and TNF-α on ICAM-1 gene expression in EC and vascular smooth muscle cells.**

Lee et al, EMM, 34(6), 451-461, 2002

**This study demonstrates that atorvastatin inhibits the ability of IFN-γ to induce ICAM-1 in endothelial and vascular SMC.**

**Thus, this finding suggests that statins may be useful as therapeutic agents for atherosclerosis, because they inhibit the action of IFN-γ and they lower serum lipids**

Lee et al, EMM, 34(6), 451-461, 2002

EC SMC.

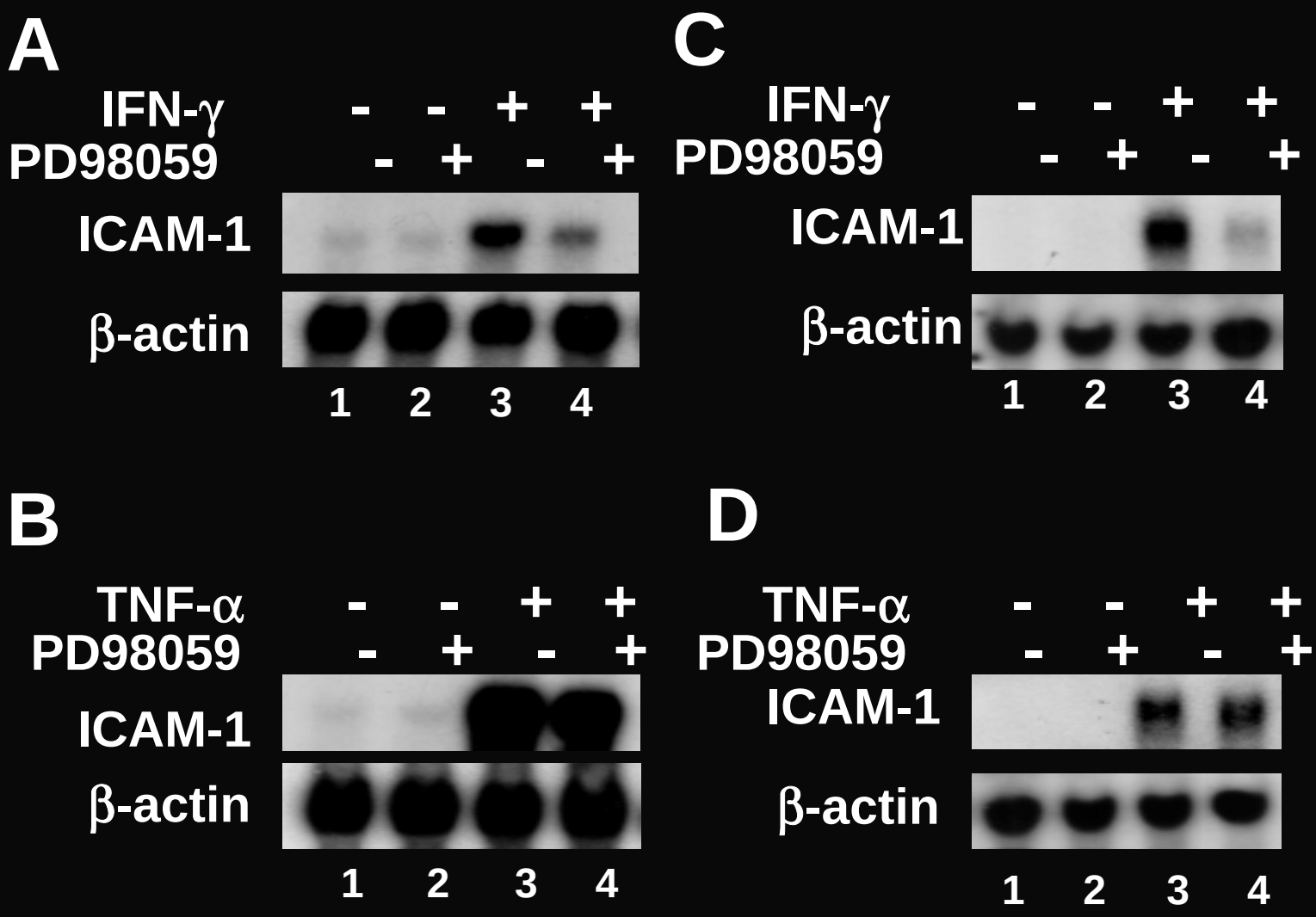



**Figure 3. Effects of PD98059, IFN-γ and TNF-α on ICAM-1 gene expression in EC and SMC.**

Lee et al, EMM, 34(6), 451-461, 2002

# Summary & Conclusion

**In cells treated with atorvastatin and IFN-γ, ICAM-1 was expressed at a lower level than in cells treated with IFN-γ alone. However, atorvastatin does not reduce TNF-α induced expression of ICAM-1.**
**A similar result was observed in cells treated with the MEKK inhibitor PD98059 and IFN-γ.**

# Acknowledgements


**Keimyung University, School of Medicine**

**Endocrinology and Metabolism**

**Institute for Medical Sciences**

Mi-Jung Kim
Hye-Soon Kim
Jong Deok Ahn
Hyo-Jeong Lee
Hye-Jin Kim
Yong-Deuk Kim
Seo-Youn Jung

**University of Ulsan, College of Med.**
**Endocrinology and Metabolism**
**Asan Institute for Life Sciences**

Ki-Up Lee
Joong-Yeol Park M.D
Min Sun Kim

**University of Chunnam.**
**Hormone Research Center**

Heung-sik Choi

**Osaka University**

Y. Kaneda
R. Morishita