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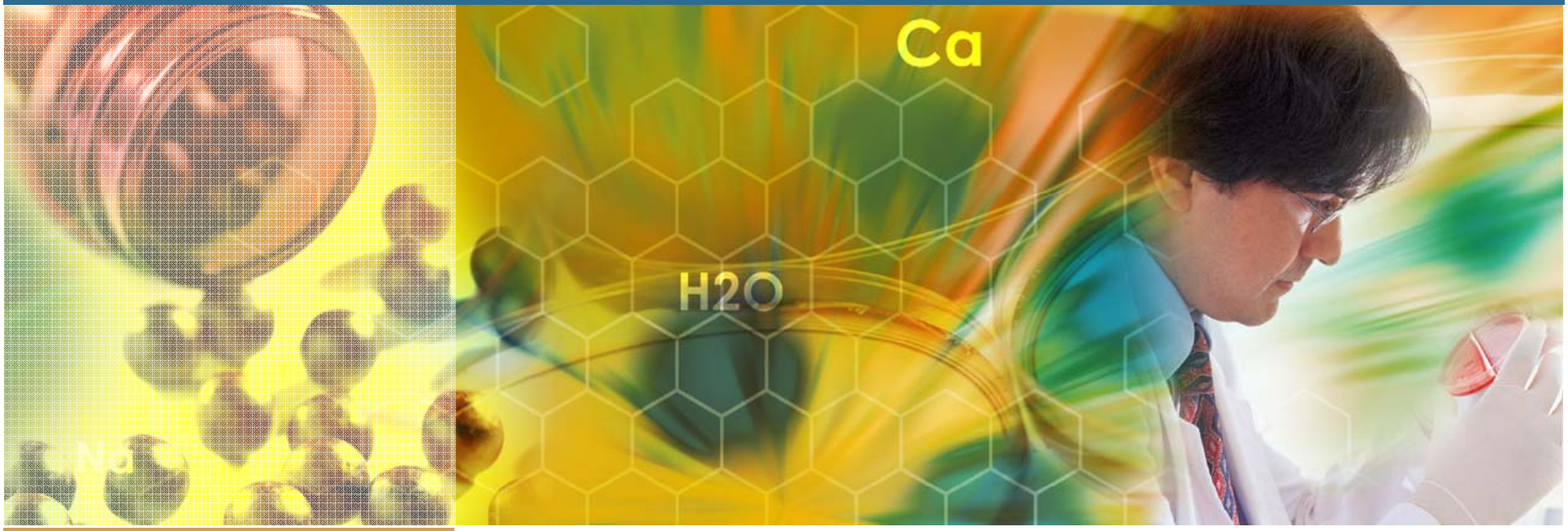
Roles of Interferons and Secretory PLA_2 in Cell Senescence



Jae-Ryong Kim M.D., Ph.D.
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Department of Biochemistry and Molecular Biology
College of Medicine, Yeungnam University

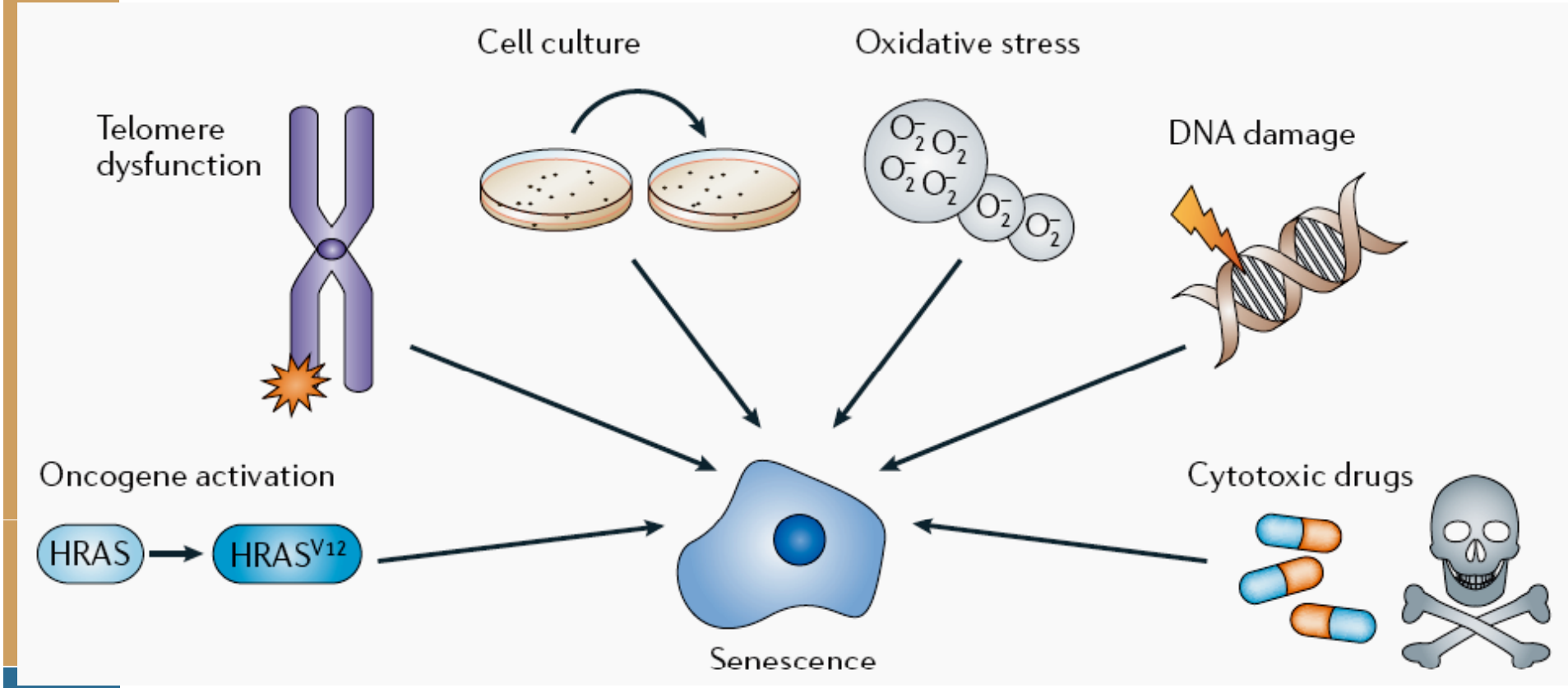
Yeungnam University

Contents

- Characteristics of cellular senescence
- Role of interferons in endothelial cell senescence
- Role of phospholipase A2 in cell senescence

Many roads to senescence in mammalian cells

Cellular senescence: an irreversible arrest of cell proliferation caused by various stresses

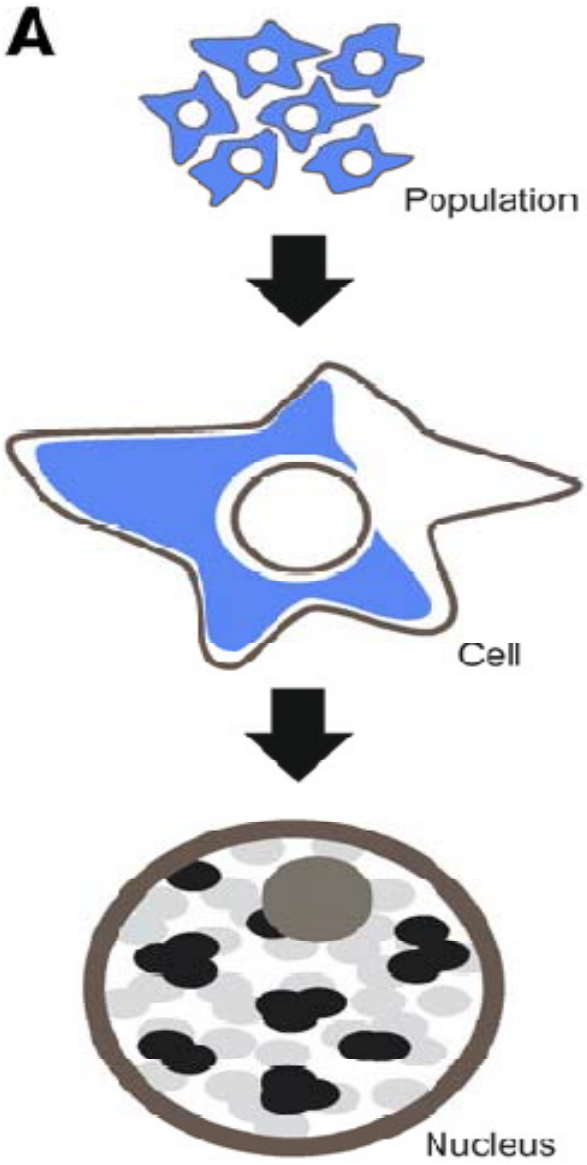


Collado and Serrano, Nature Review Cancer 6:472, 2006

Replicative senescence

- Restricted proliferation of normal cells: Hayflick limit (Hayflick and Moorehead, 1961)
  - Organismal aging
  - Cancer suppression
- Characteristics of senescent cells
  - Enlarged and flattened morphology
  - Resistant to mitogen-induced proliferation
  - Senescence-associated β-galactosidase (SA-β-gal)
  - Altered gene expression: cell cycle regulation, immune and inflammation, cytoskeleton, stress response, metabolism

Senescence biomarkers



- ❖Morphogical changes
  - Larger, flat, granularity
- ❖Biochemical changes
  - SA-β-gal activity
  - Cessation of DNA synthesis
  - p53, p16, γH2AX
- ❖Chromatin changes
  - Senescence-associated heterochromatin foci (SAHF)
  - H3K9 trimethylation

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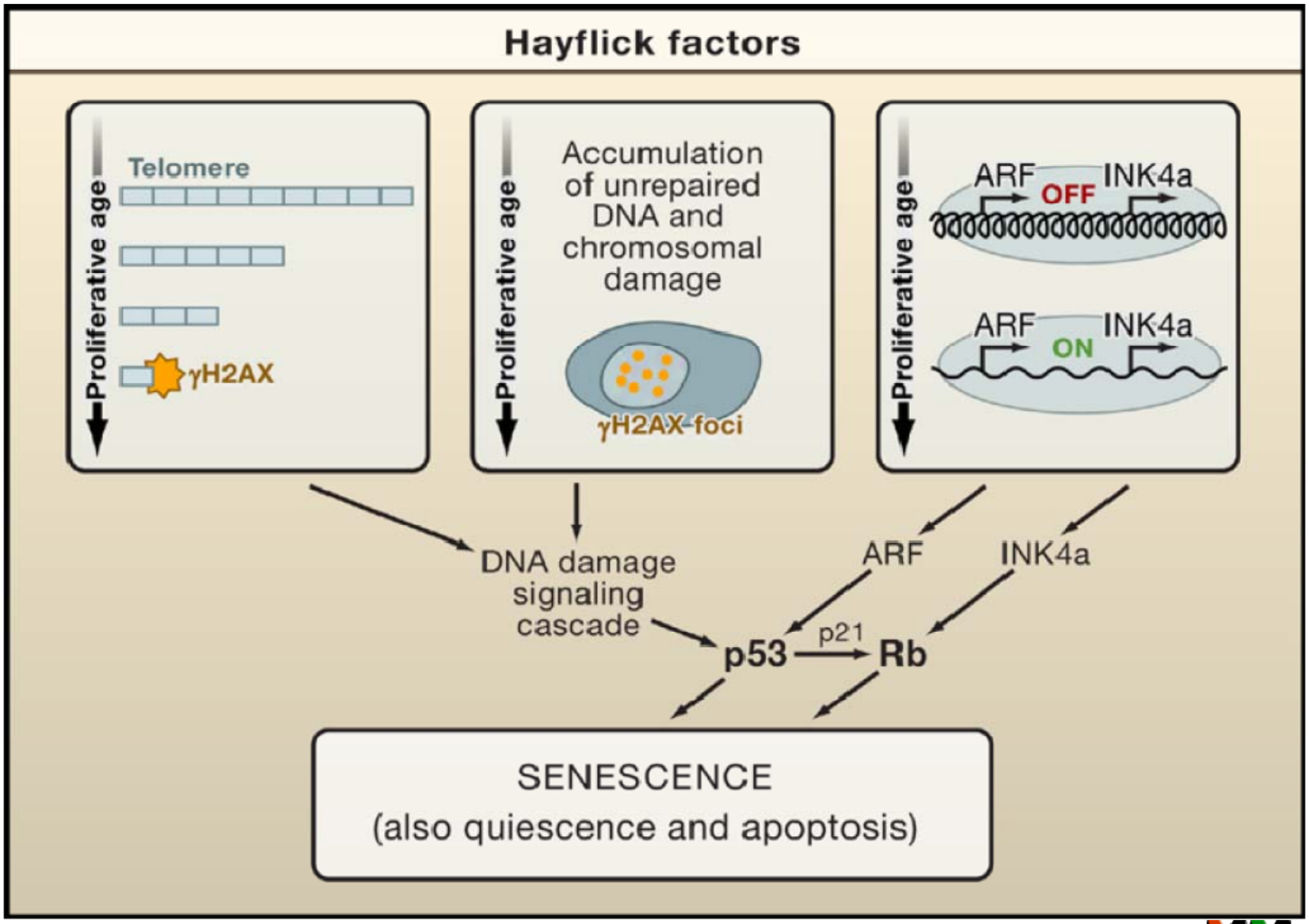
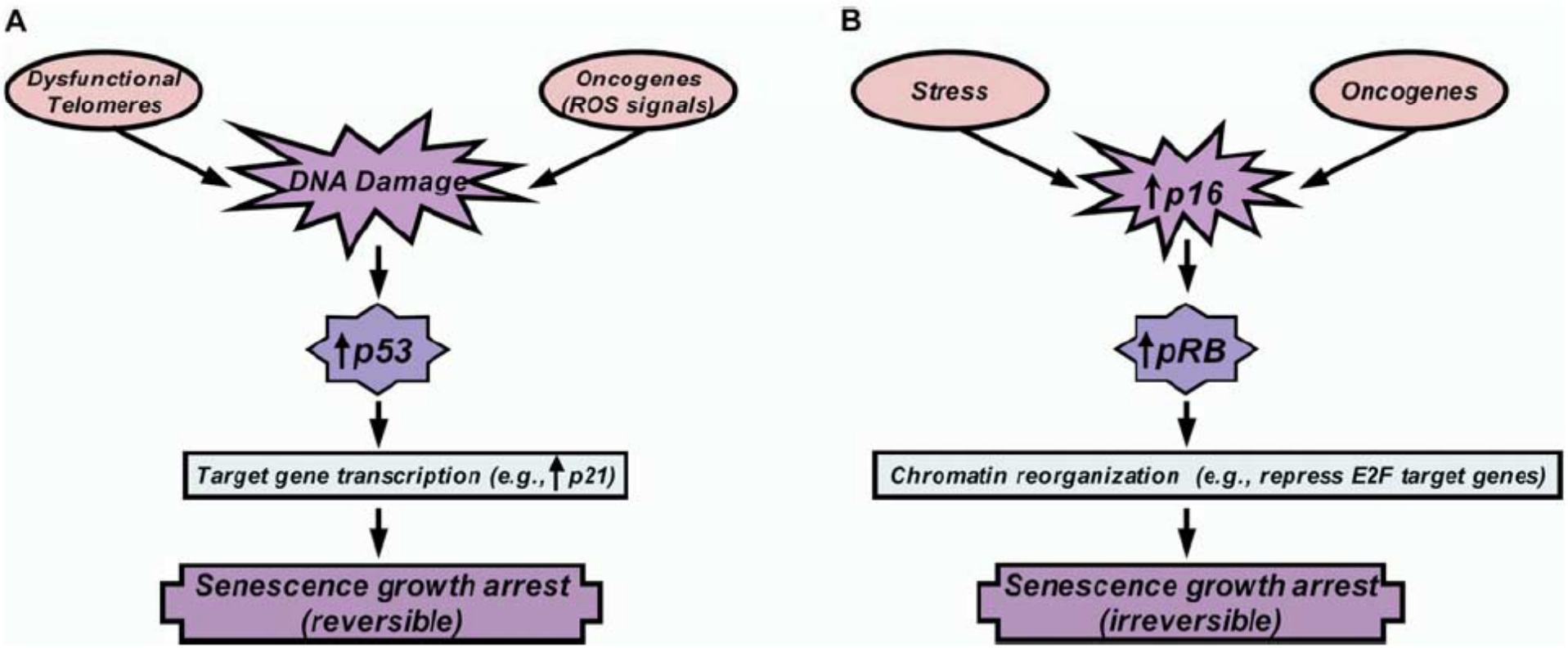
Three Hayflick factors



Collado et al. Cell 130:223, 2007

Senescent phenotype: reversible?

Campisi J. Cell 120:513, 2005



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Senescence and inflammation

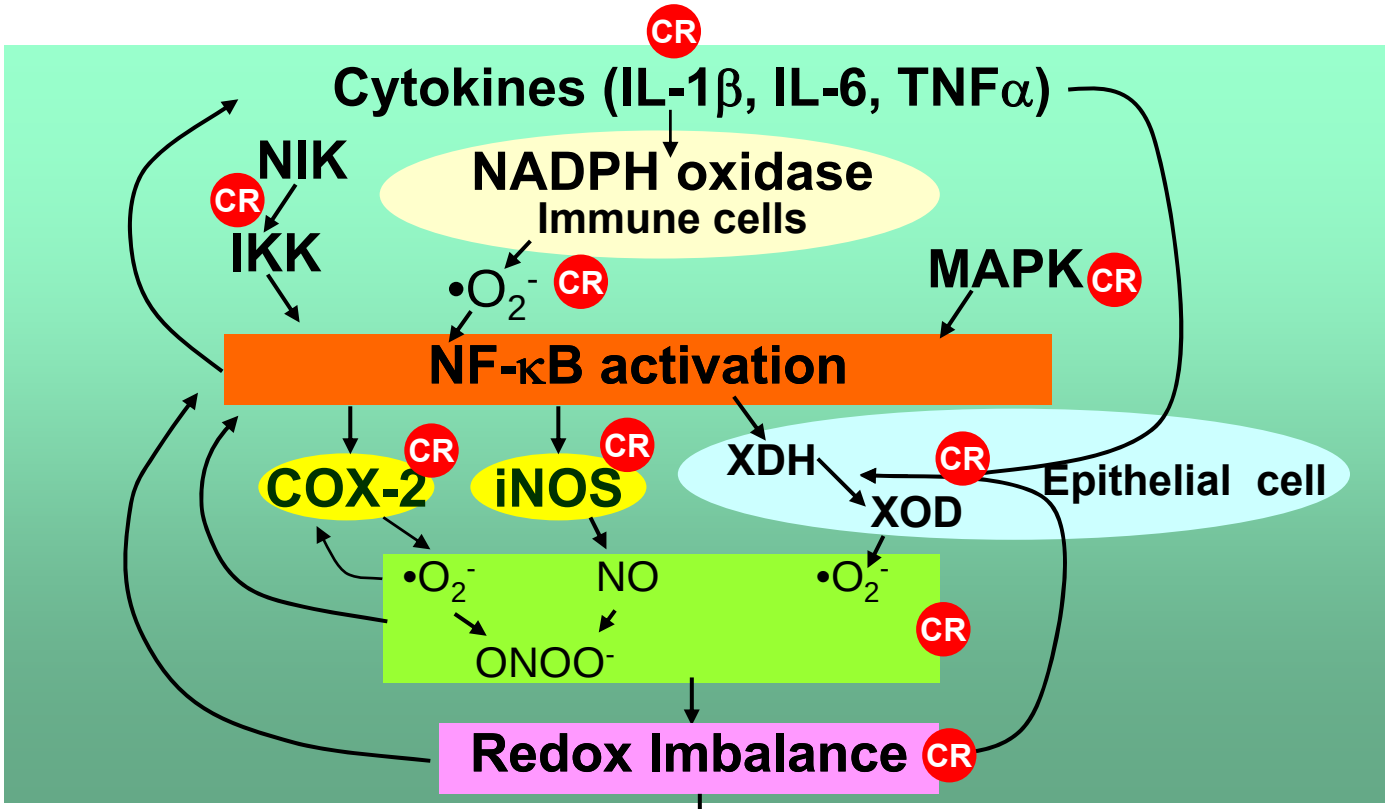
Changes of Inflammatory Parameters during Aging Process

	Parameters	Inflammation	Aging	Calorie restriction
Redox state	ROS/RNS	↑	↑	R
	Catalase, Superoxide dismutase	↓	↓	R
	GSH peroxidase, GSH/GSSG	↓	↓	R
Proinflammatory enzymes	Inducible NO Synthase	↑	↑	R
	Heme oxygenase-1	↑	↑	R
	Cyclooxygenase-2	↑	↑	R
	Xanthine Oxidase	↑	↑	R
Proinflammatory cytokines	IL-1β	↑	↑	R
	IL-6	↑	↑	R
	TNF-α	↑	↑	R
Adhesion molecules	E-selectin	↑	↑	R
	P-selectin	↑	↑	R
	VCAM-1	↑	↑	R
	ICAM-1	↑	↑	R
NF-κB activation	NF-κB DNA binding activity	↑	↑	R
	NIK/IKK activation	↑	↑	R
	Phosphorylation of IκBα	↑	↑	R
	Degradation of IκB in cytoplasm	↑	↑	R
	Nuclear translocation of p65 and p50	↑	↑	R
	NF-κB-dependent gene expression	↑	↑	R
	Active MAPKs (ERK, JNK, p38 MAPK)	↑	↑	R

Chung et al, Microsc Res Tech. 59:264, 2002

Molecular Inflammation Hypothesis of Aging

(Chung, HY)



Chung, H.Y. Rev. Clin. Gerontol. 10, 207 (2000)
Chung, H.Y. Ann. N.Y.Acad.Sci. 928, 327(2001)
Chung, H.Y. Microsc. Res. Techniq (2002)

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Up-regulation of inflammatory genes in replicative senescence of HDFs

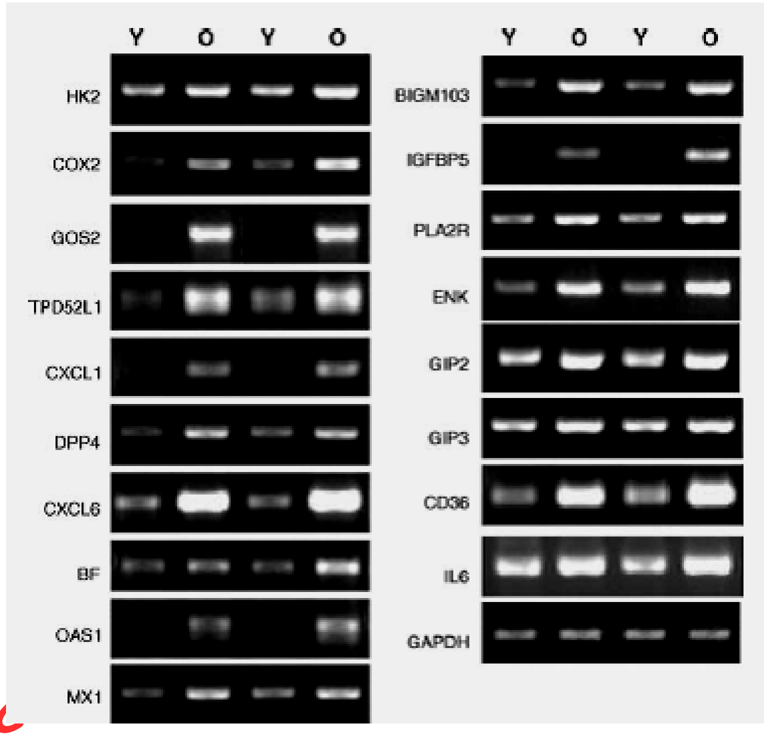
ELSEVIER

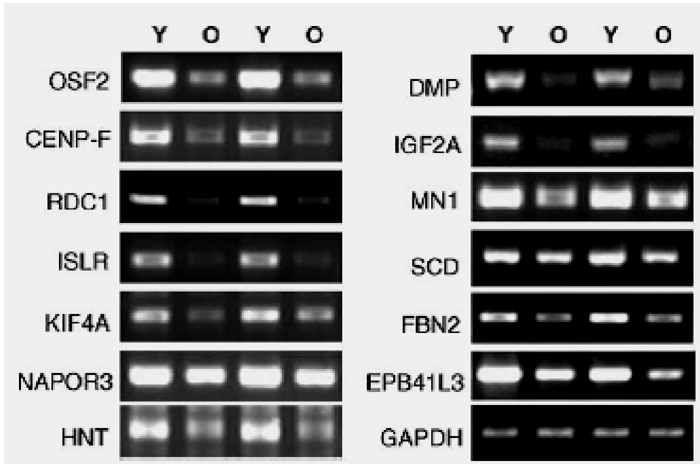
Experimental Gerontology 39 (2004) 1369–1378

www.elsevier.com/locate/expgero

Exploration of replicative senescence-associated genes in human dermal fibroblasts by cDNA microarray technology

In Kyung Yoon[a], Hyun Kyoung Kim[a], Yu Kyoung Kim[a], In-Hwan Song[b], Wankee Kim[c], Seongyong Kim[a], Suk-Hwan Baek[a], Jung Hye Kim[a], Jae-Ryong Kim[a,*]





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Aging Cell (2007) 6, pp535–545

Regulation of replicative senescence by insulin-like growth factor-binding protein 3 in human umbilical vein endothelial cells

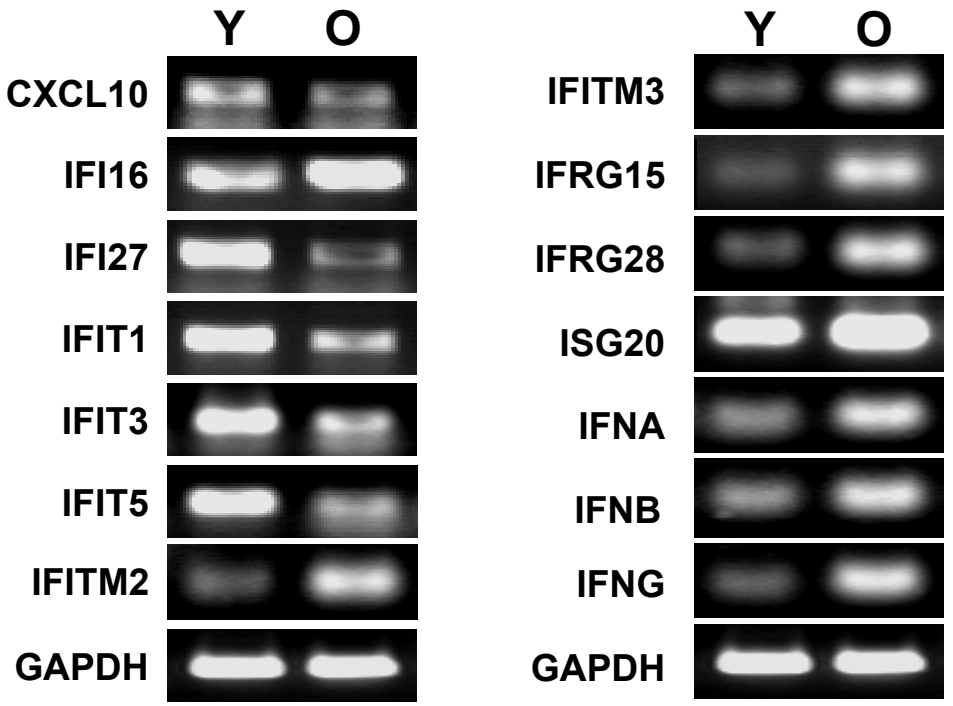
Kwang Seok Kim,[1,2] Min-Sun Kim,[1] Young Bae Seu,[2] Hae Young Chung,[3] Jung Hye Kim[1] and Jae-Ryong Kim[1]

Molecular Biology of the Cell
Vol. 18, 4543–4552, November 2007

Induction of Cellular Senescence by Insulin-like Growth Factor Binding Protein-5 through a p53-dependent Mechanism

Kwang Seok Kim,*†‡ Young Bae Seu,‡ Suk-Hwan Baek,*† Mi Jin Kim,†§ Keuk Jun Kim,†§ Jung Hye Kim,* and Jae-Ryong Kim*†

Interferons and interferon-inducible genes in senescence of human endothelial cells



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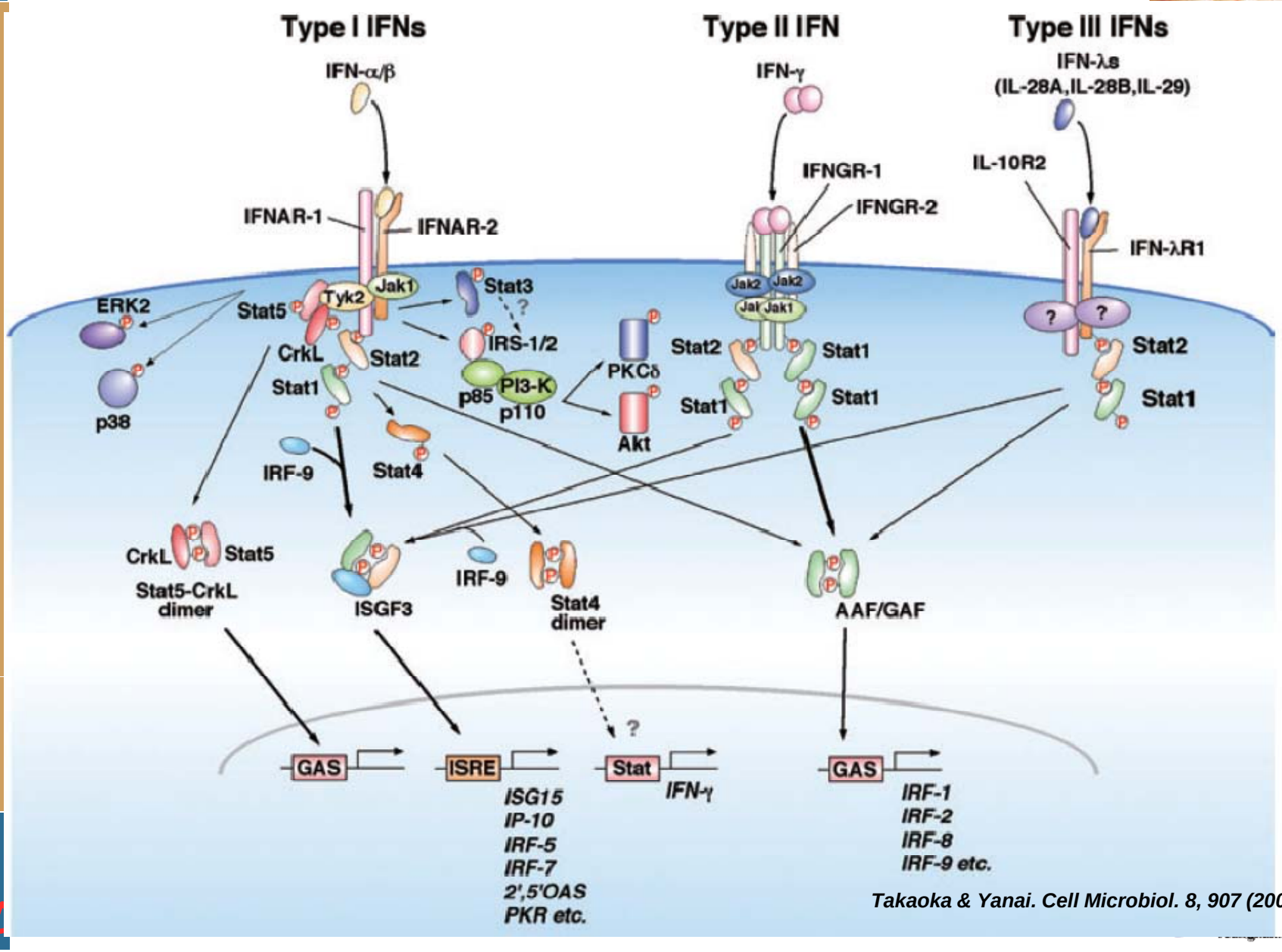
Interferons (IFNs)

- A family of cytokines with antiviral and antiproliferative activity
- Type I, II, and III
  - Type I
    - A major component of the innate immune system
    - IFN-α: dendritic cells, monocytes, B lymphocytes
    - IFN-β: most cells (fibroblasts)
  - Type II
    - IFN-γ: lymphocytes, immunoregulatory functions
  - Type III: IFN-λ

IFN-signaling pathway

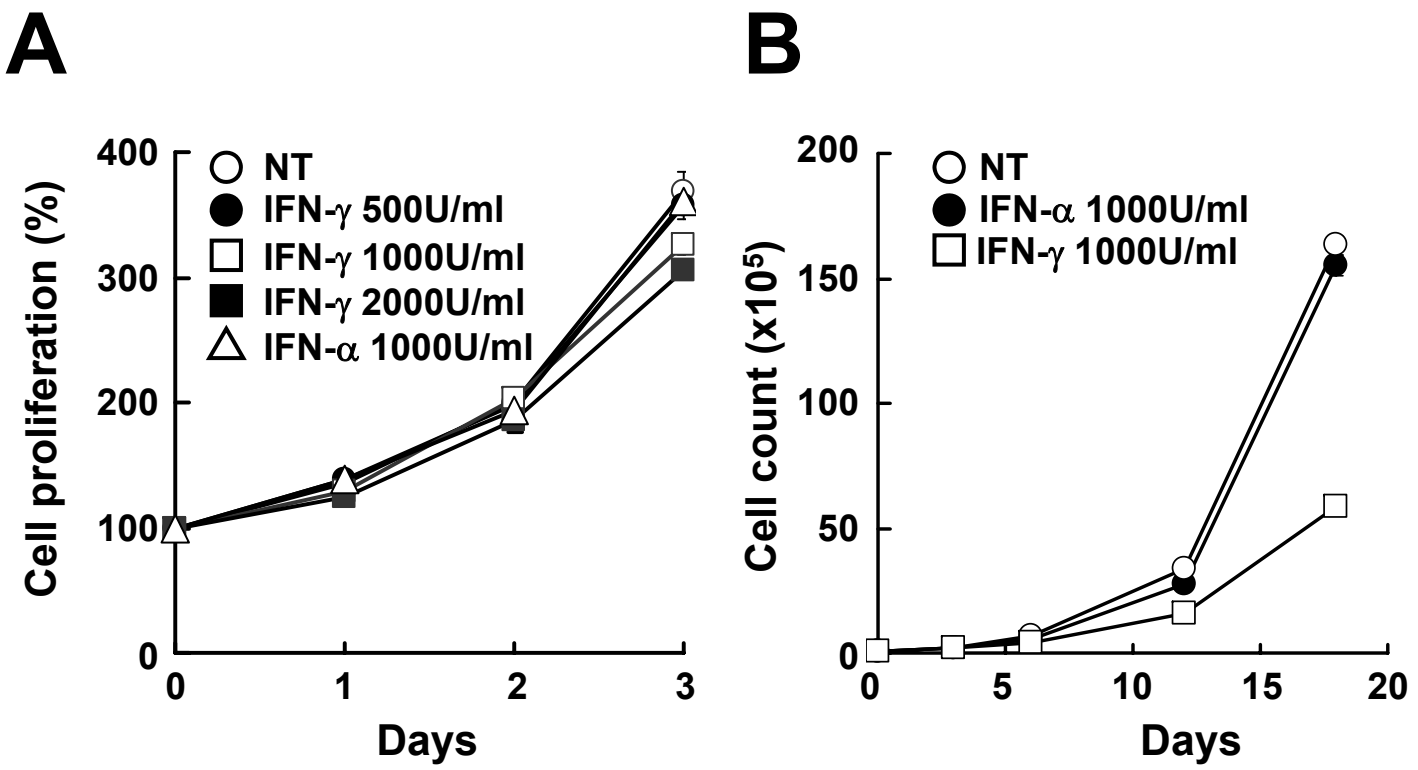


Takaoka & Yanai. Cell Microbiol. 8, 907 (2006)

Interferons and cellular senescence

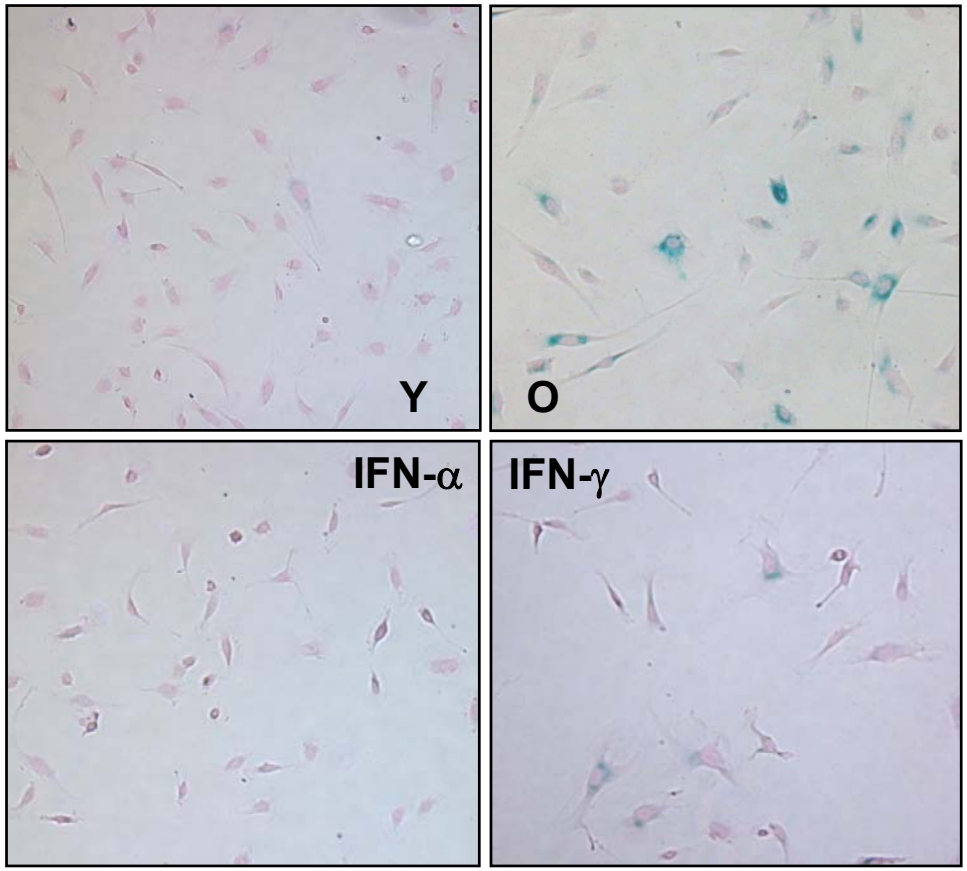
- **IFN-γ and endothelial cell senescence ?**
- **IFI16 (an IFN-inducible gene): up-regulated in old human fibroblasts, and induction of cell senescence by increased levels of IFI16 in old HDFs (Oncogene. 2004; 23: 6209).**
- **IRF3 (interferon regulatory factor 3): induction of cell growth inhibition and cellular senescence through activation of p53 tumor suppressor (Cancer lett. 2006; 242: 215).**
- **Prolonged IFN-β treatment: induction of cell senescence in human fibroblasts by through DNA damage signaling and a p53-dependent pathway (Mol Biol Cell. 2006; 17: 1583).**
- **IFN-α: induction of replicative senescence in endothelial cells after continuous stimulation (Pammer J et al., Lab Invest. 2006; 86: 997).**
- **The levels of p53 mRNA and protein are increased by IFN-α/IFN-β (Nature. 2003; 424: 516), which is an important regulator of tumor suppression as well as cellular senescence.**

Effect of IFNs on endothelial cell proliferation



Induction of cell senescence by IFN-γ in HUVECs

A



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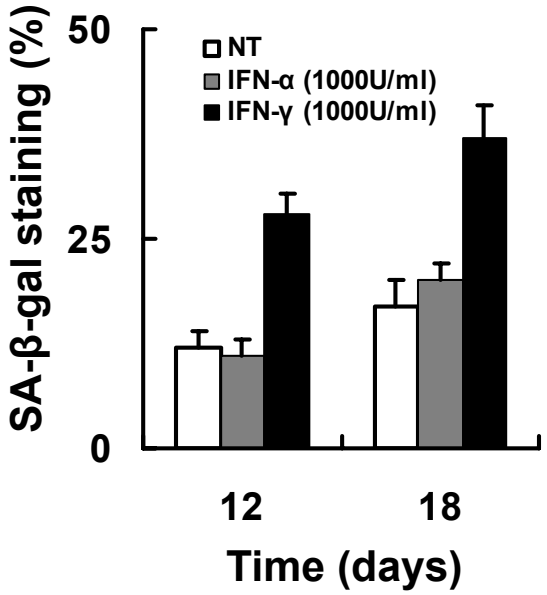
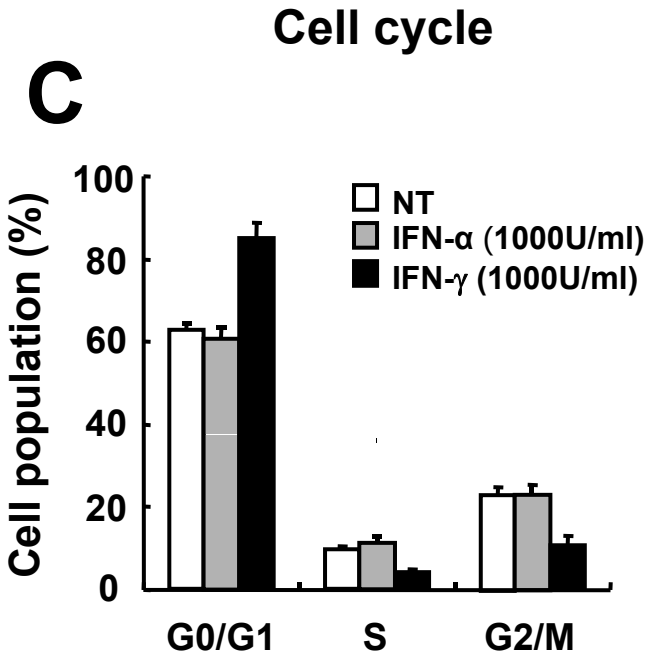
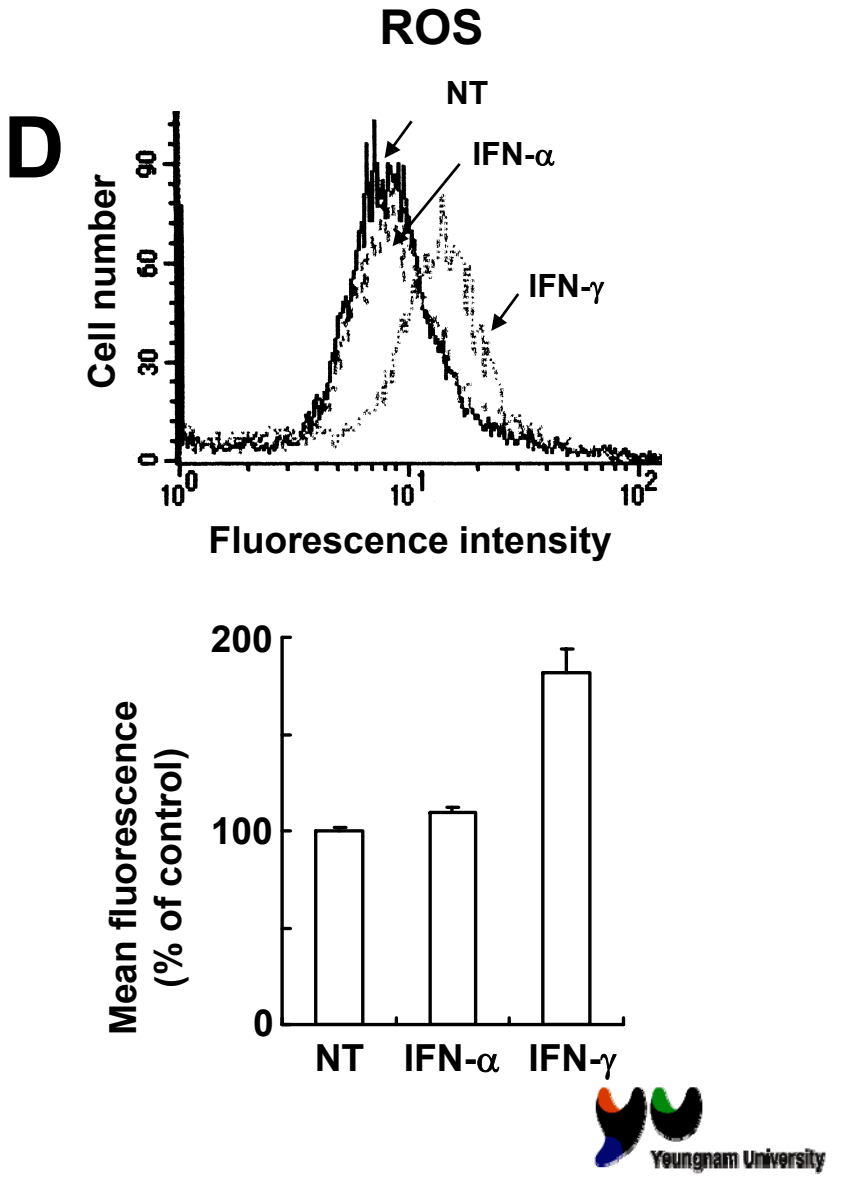


Induction of cell senescence by IFN-γ in HUVECs





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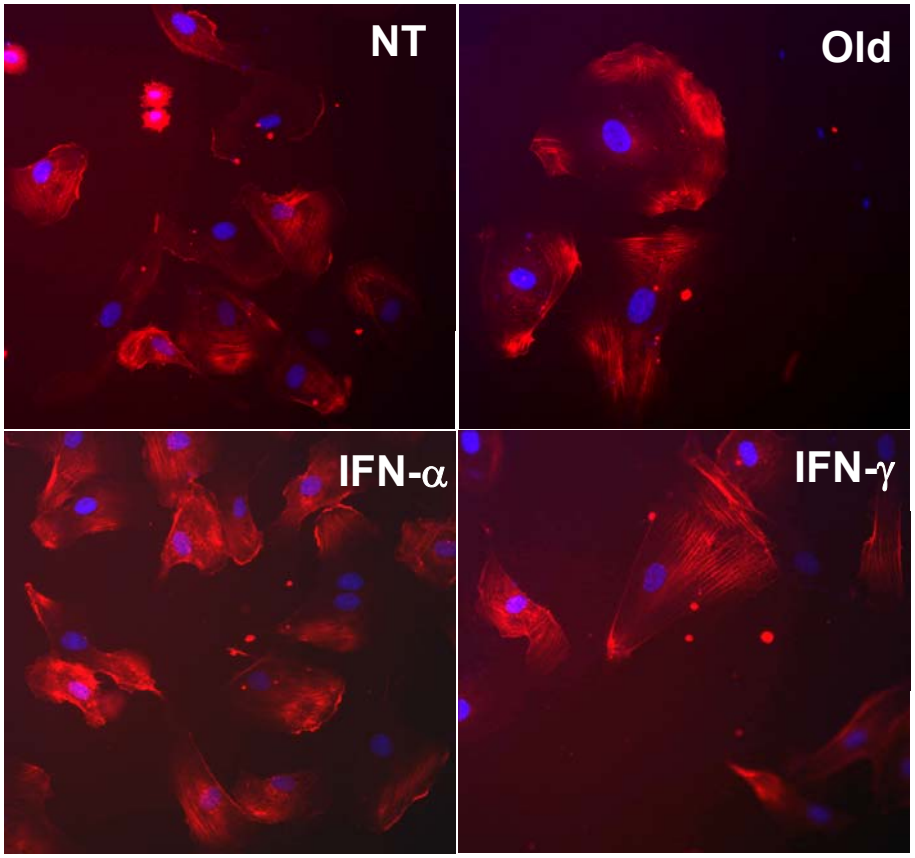
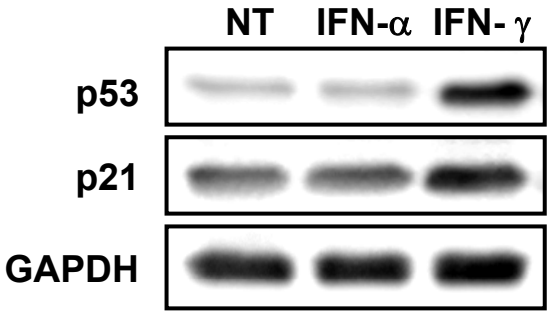
Induction of cell senescence by IFN-γ in HUVECs

E Actin staining



F Western blotting



IFN-γ induces cellular senescence in HUVECs.

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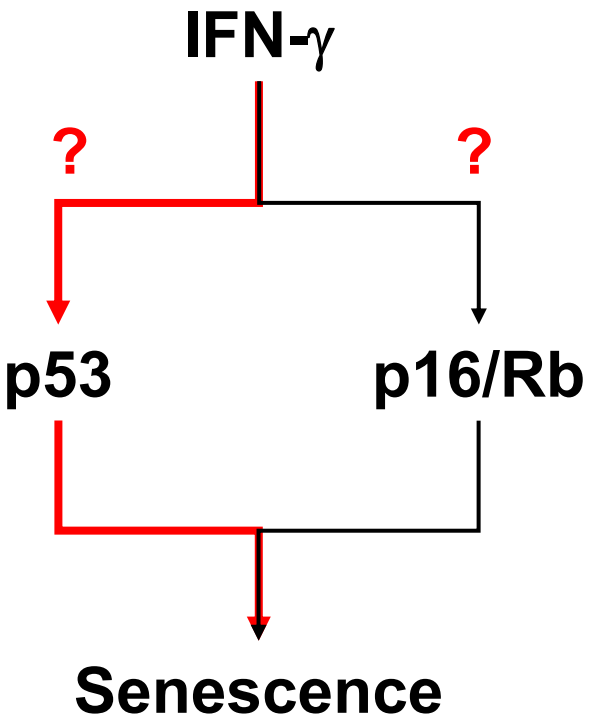
IFN-γ-induced senescence via a p53 signaling pathway



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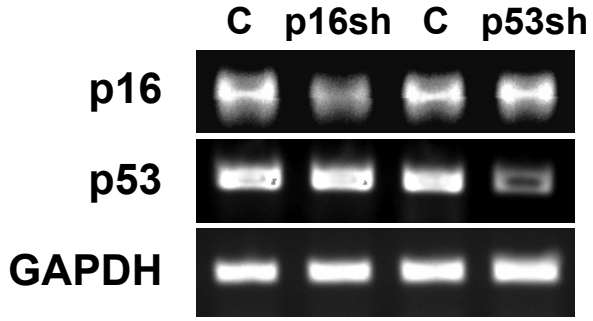

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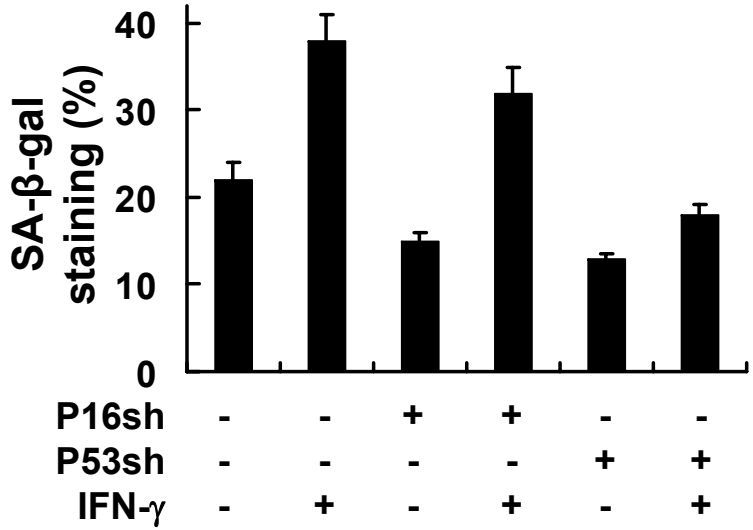

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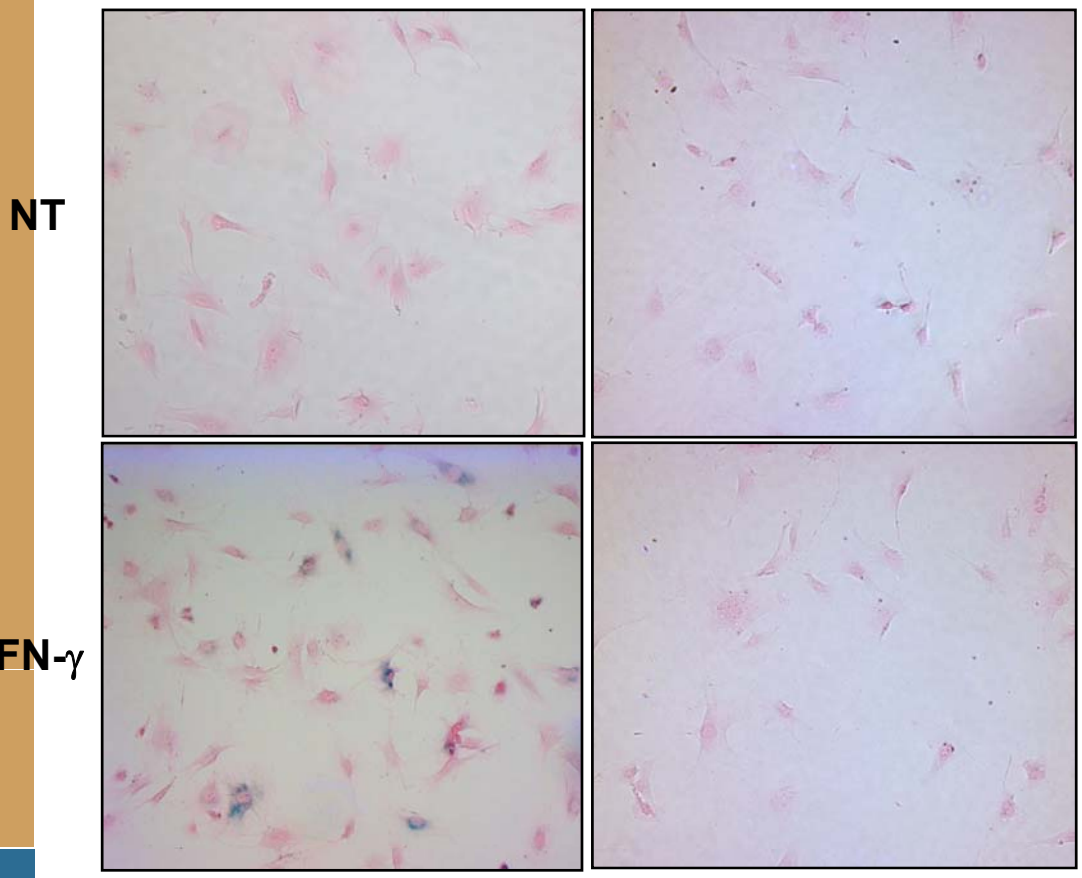
IFN-γ-induced senescence via a p53 signaling pathway

C



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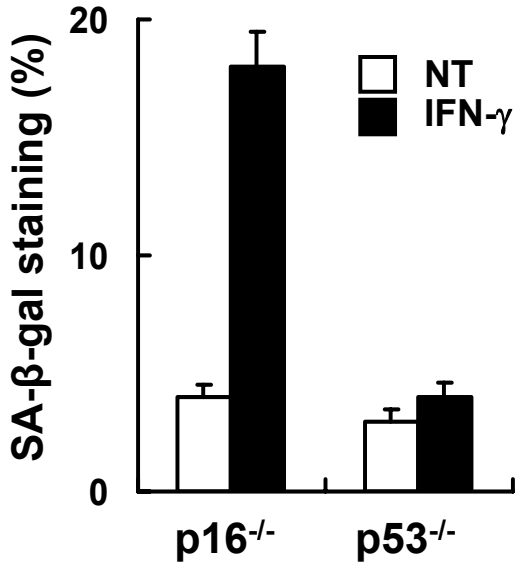

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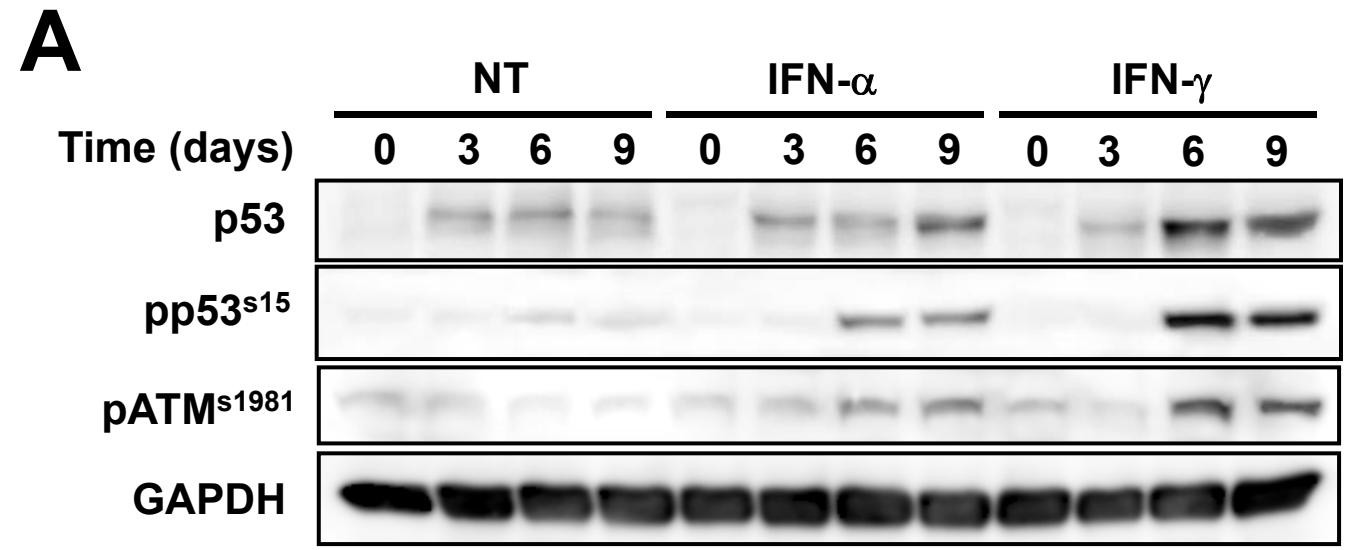
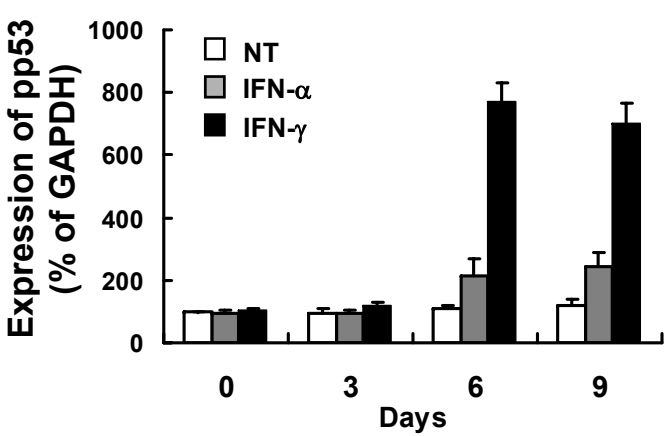
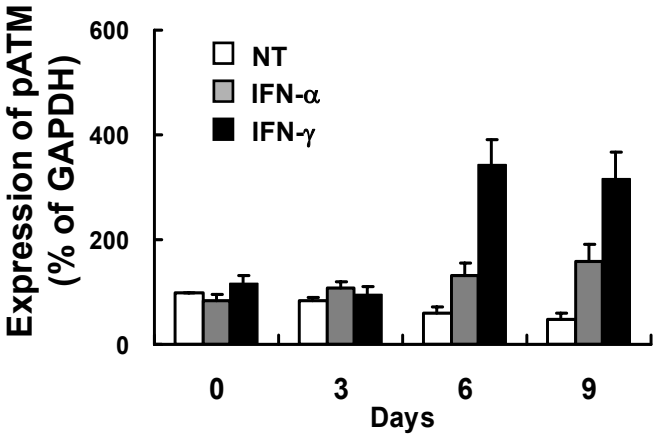
Post-translational modification of p53 by IFN-γ through DNA damage signaling

DNA damage → ATM/ATR → p53 → Senescence







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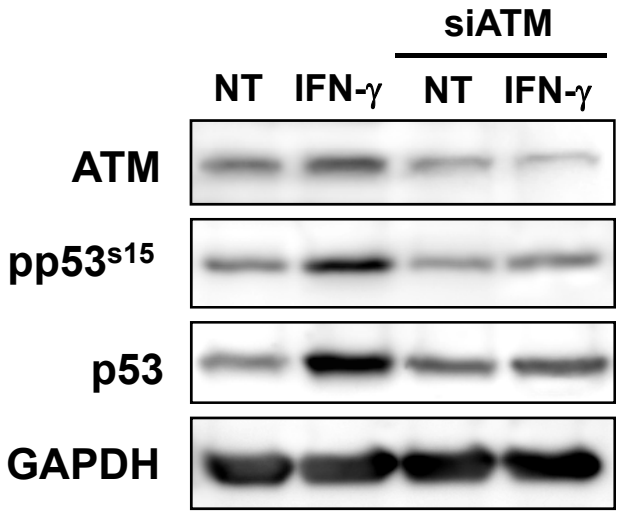

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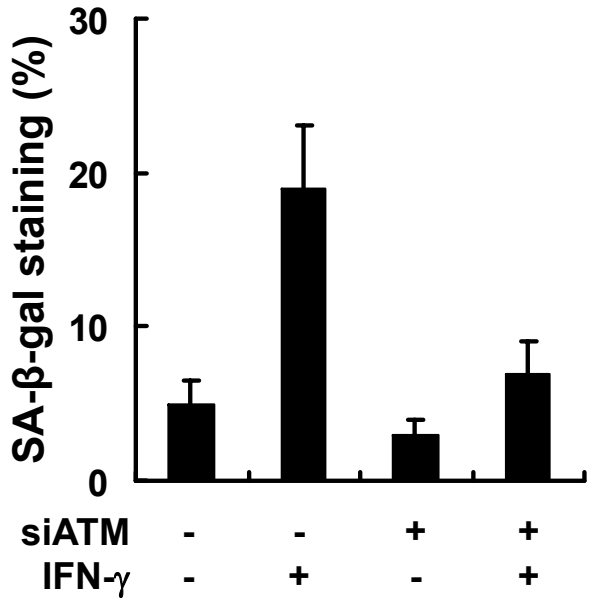

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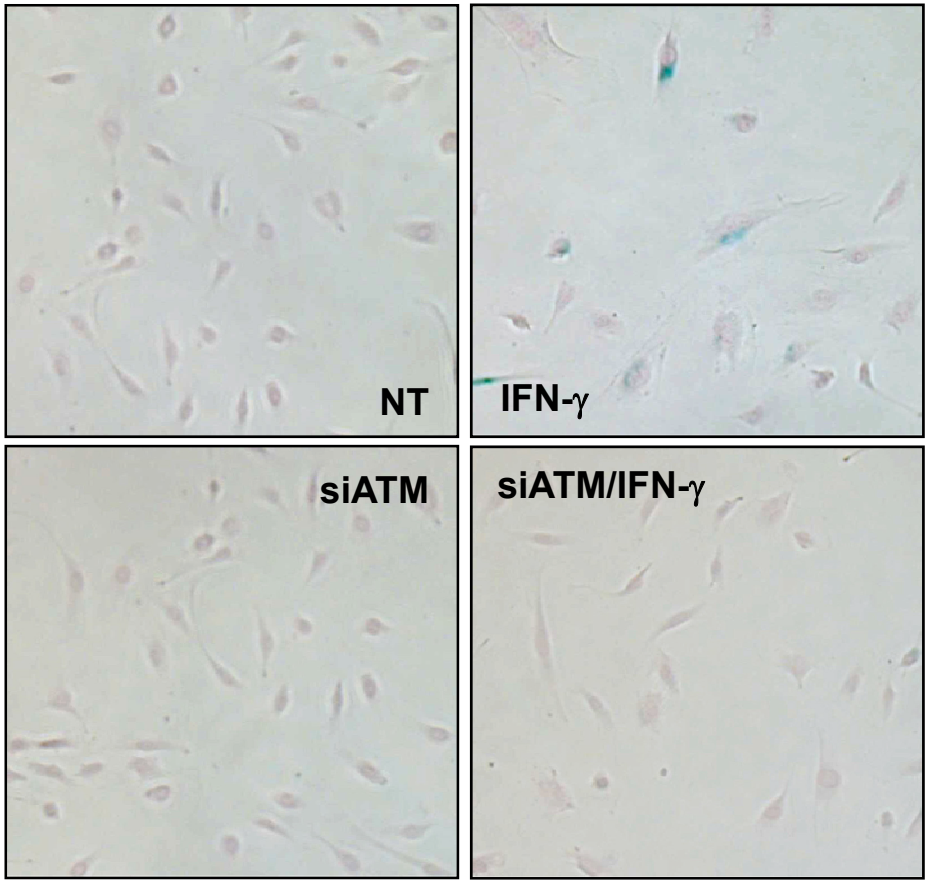

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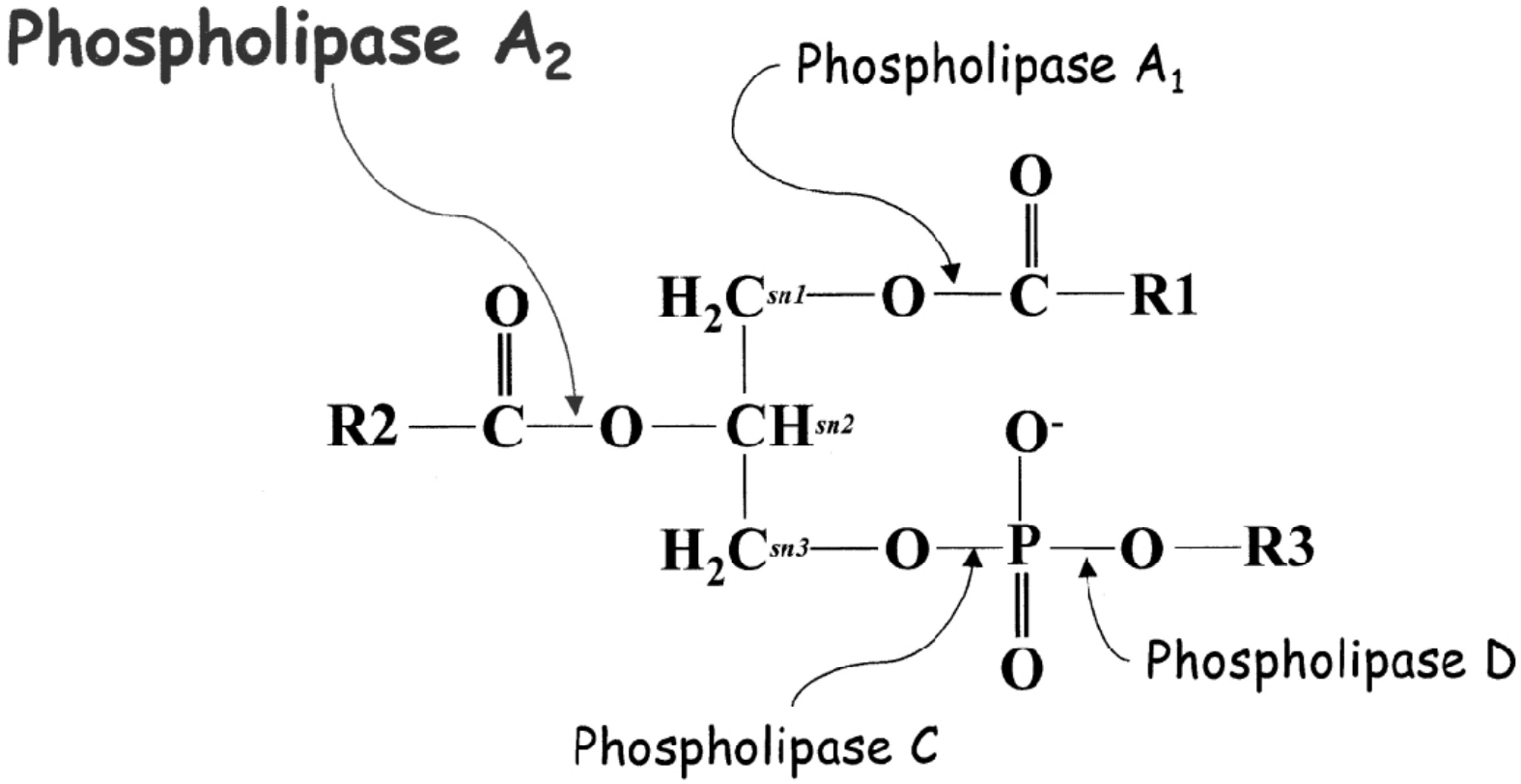
Summary (IFN-γ)

- Prolonged treatment with IFN-γ induced cellular senescence in HUVECs, as confirmed by increased SA-β-gal staining, G0/G1 cell cycle arrest, and up-regulation of p53 and p21 protein levels.
- In contrast to IFN-γ, IFN-α did not induce cellular senescence in HUVECs.
- IFN-γ-induced cellular senescence was observed only in p16-knockdown cells or p16-null MEFs, but not in p53-knockdown cells or p53-null MEFs.
- Knockdown of ATM kinase rescued IFN-γ-induced cellular senescence.
- Therefore, IFN-γ might play an important role in cellular senescence through a p53-dependent DNA damage pathway.

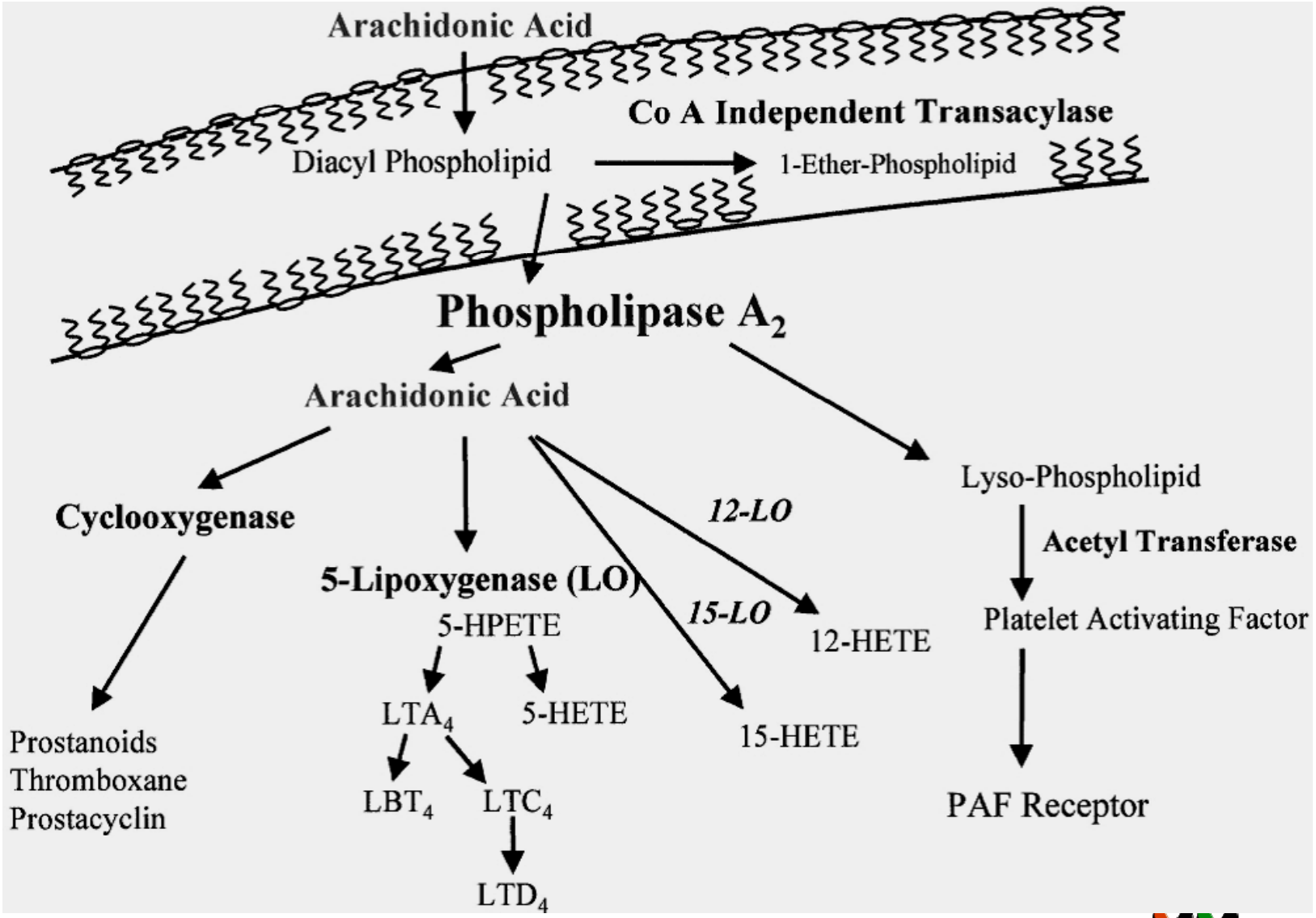
Phospholipase A2

Hydrolysis of the sn-2 fatty acyl bond of phospholipids



Capper and Marshall, Prog Lipid Res. 40:167, 2000

PLA2 and arachidonic acid



Capper and Marshall, Prog Lipid Res. 40:167, 2000

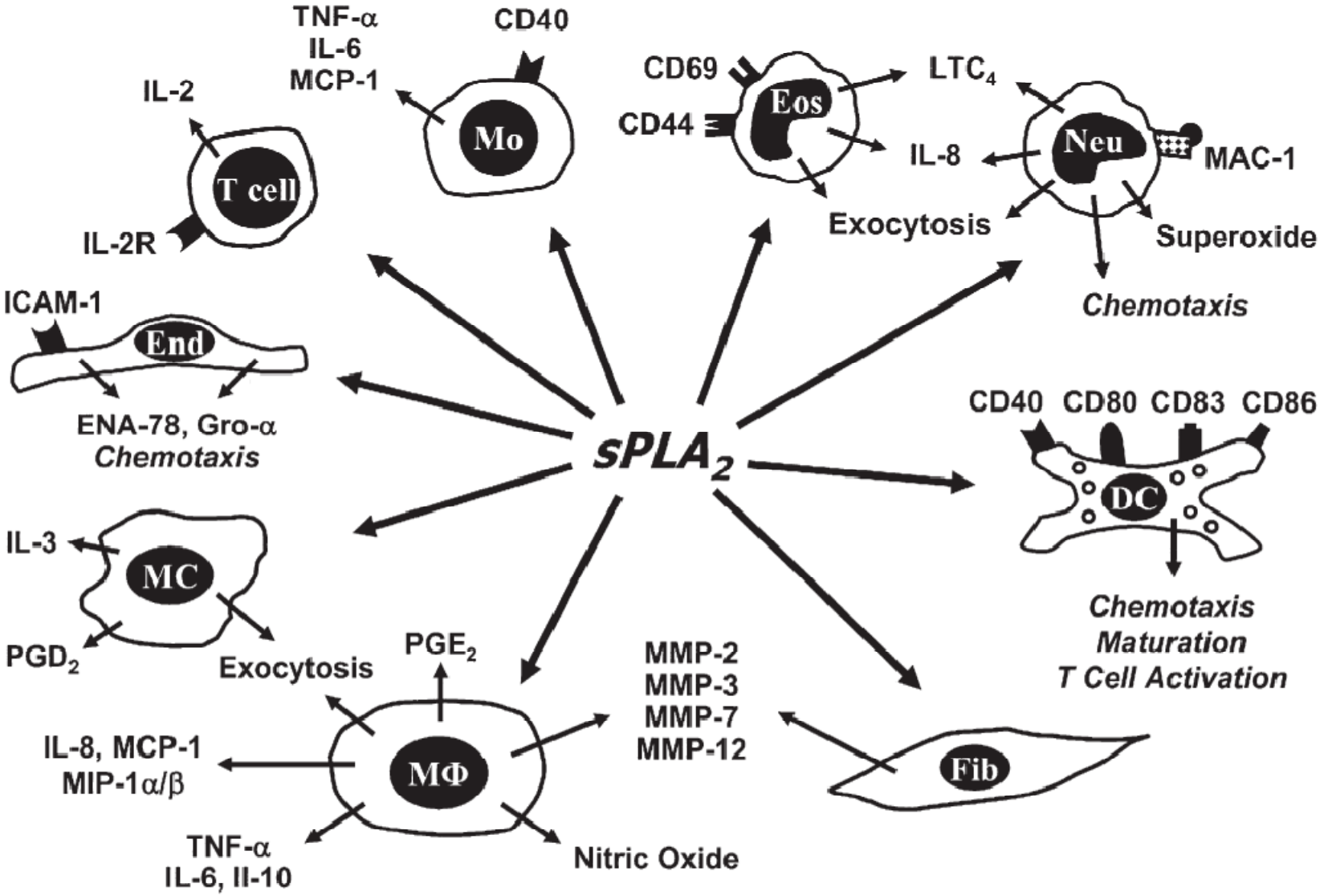
Phospholipase A2

- 5 major families
  - cytosolic PLA2 (cPLA2)
  - secretory PLA2 (sPLA2)
  - Ca^{2+}-independent PLA2s (iPLA2)
  - platelet-activating factor acetylhydrolases (PAFAH)
  - lysosomal PLA2s
- Roles of PLA2
  - Phospholipid digestion and metabolism
  - Host defense
  - Signal transduction
  - inflammation

Secretory PLA2 (sPLA2)

- Ca^{2+}-dependent low-molecular weight (14-18 kDa) enzymes
- Released in plasma and biological fluids of patients with various inflammatory diseases
  - rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel disease, pancreatitis and sepsis
- Increased after treatment of cells with pro-inflammatory cytokines and in diverse pathologic conditions
  - myocardial infarction, viral hepatitis, renal infarction and wound healing

sPLA2-induced effects in inflammatory cells



Triggiani et al. Biochem Biophys Acta 1761:1289, 2006

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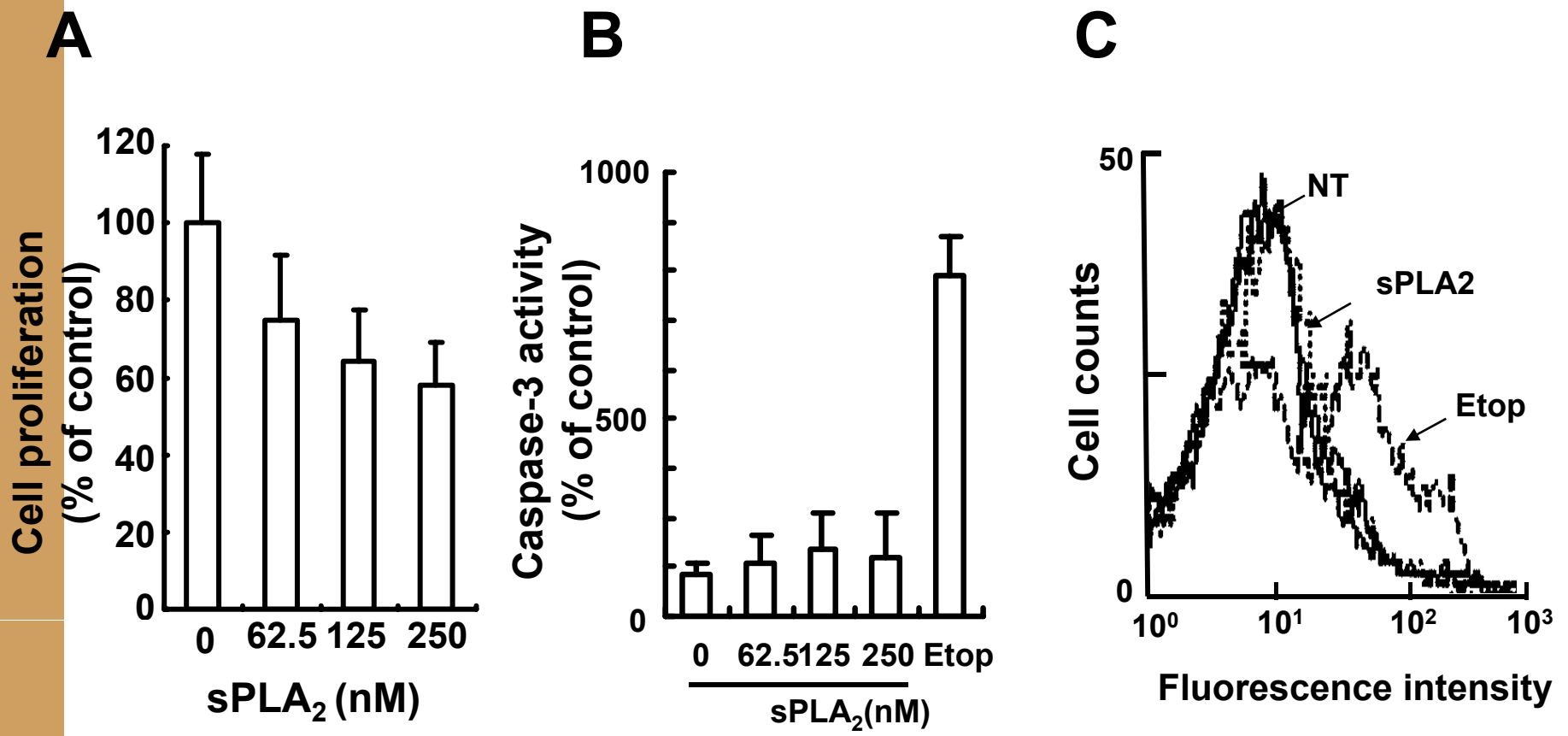
PLA2 and senescence

- **No direct evidence between PLA2 and cell senescence**
  - **COX-2 is up-regulated in fibroblasts during replicative senescence (Han et al., 2004; Yoon et al., 2004; Zdanov et al., 2007).**
  - **AA treatment induces cellular senescence by enhancing COX-2 activity, and inhibition of COX-2 activity by NS-398, or by COX-2 siRNA represses cellular senescence in fibroblasts (Han et al., 2004; Zdanov et al., 2007).**
  - **5-lipoxygenase (LO) activity is increased during senescence-like growth arrest in fibroblasts and overexpression of 5LO promotes cellular senescence via a p53/p21-dependent pathway mediated by ROS (Catalano et al., 2005).**

Inhibition of cell proliferation by sPLA2



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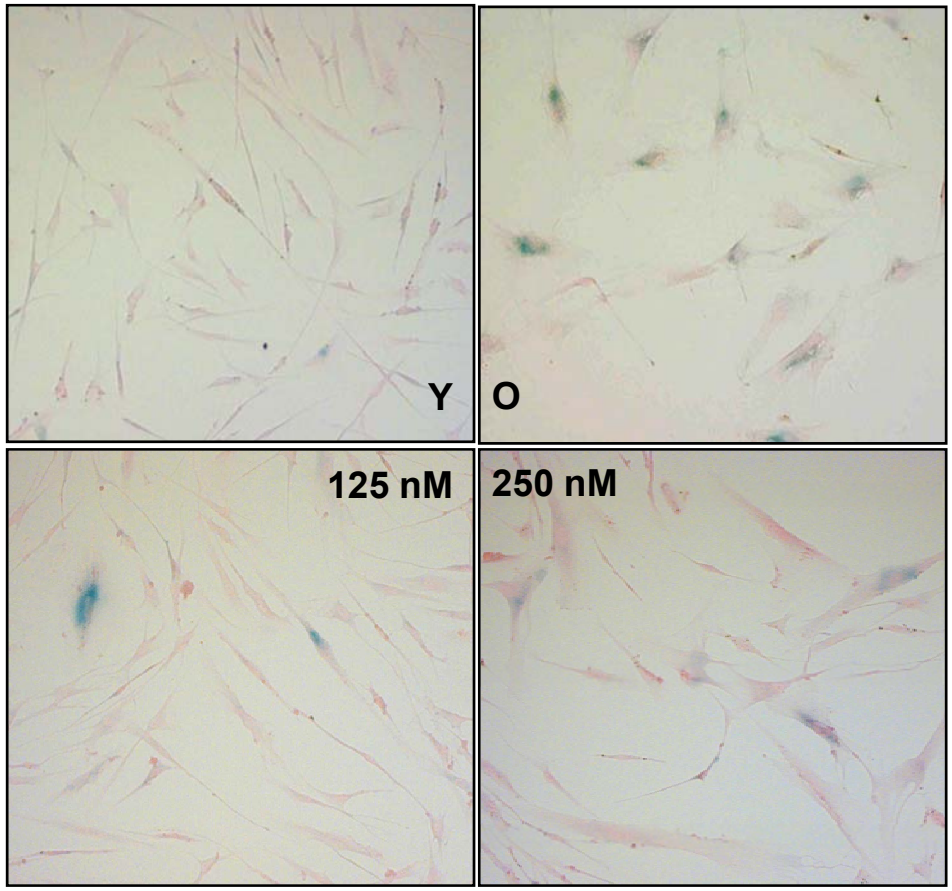
Induction of cell senescence by sPLA2 in HDFs

A



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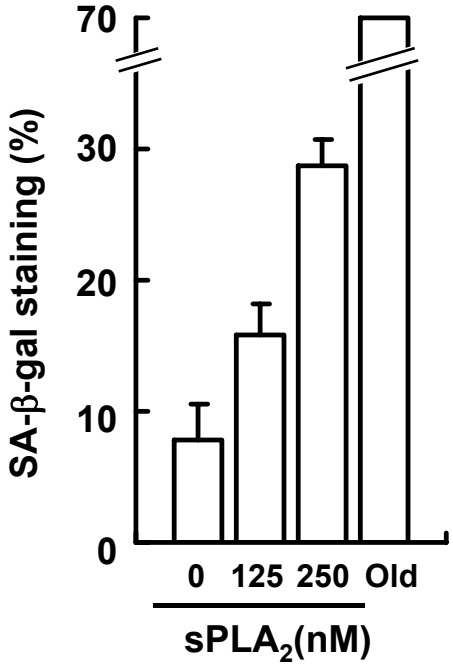

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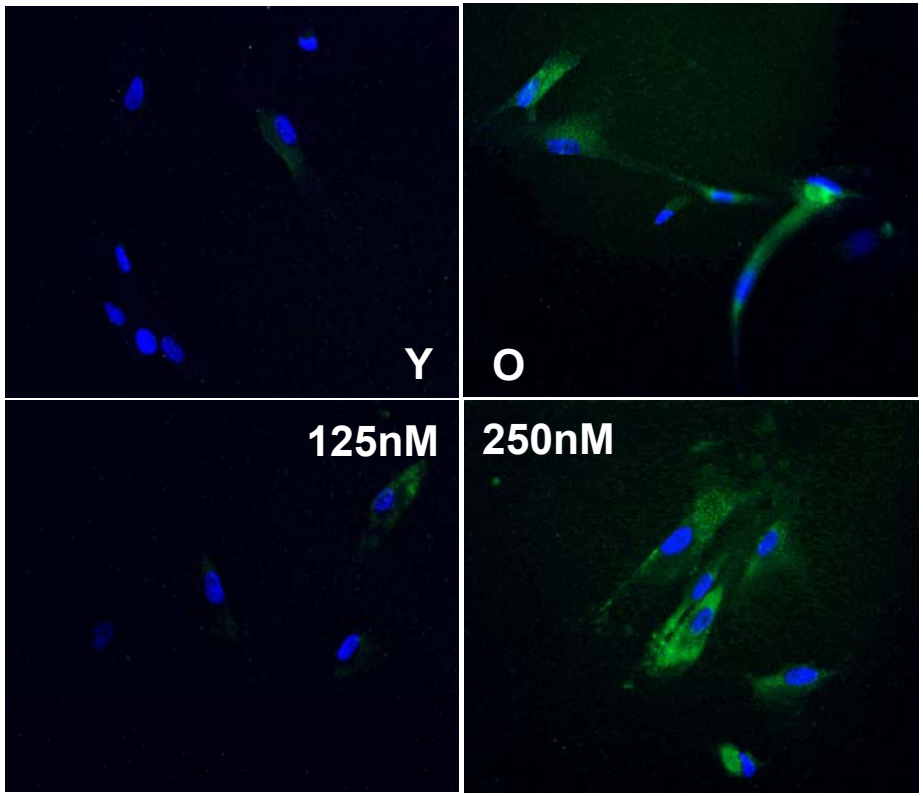
Induction of cell senescence by sPLA2 in HDFs

C



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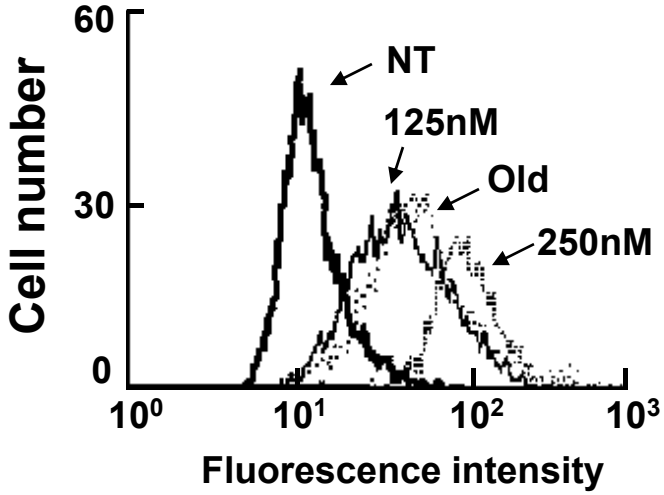

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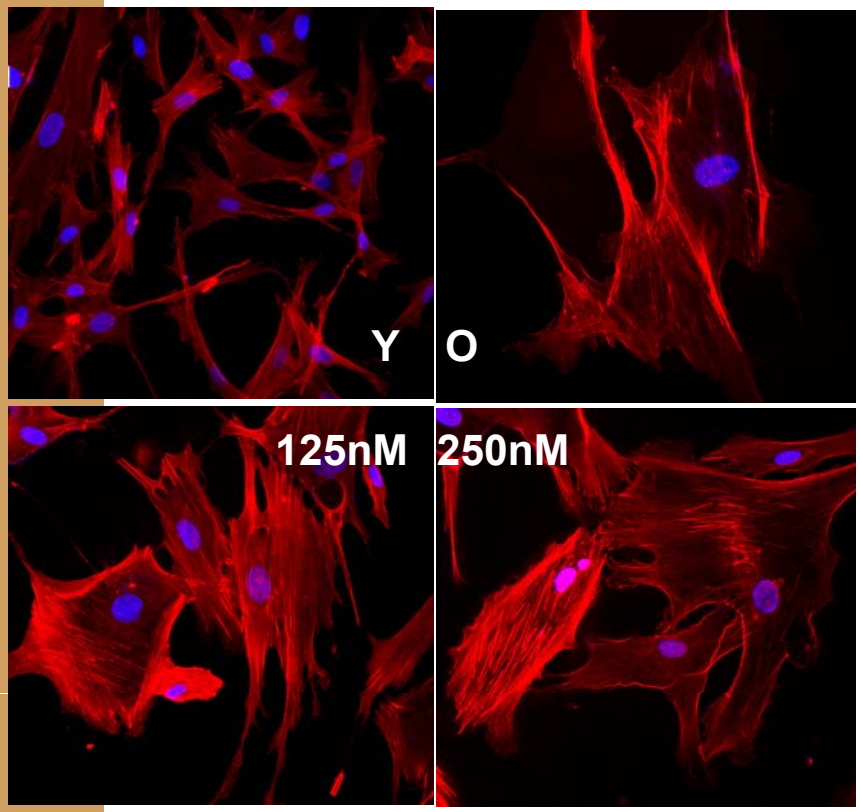
Induction of cell senescence by sPLA2 in HDFs

E



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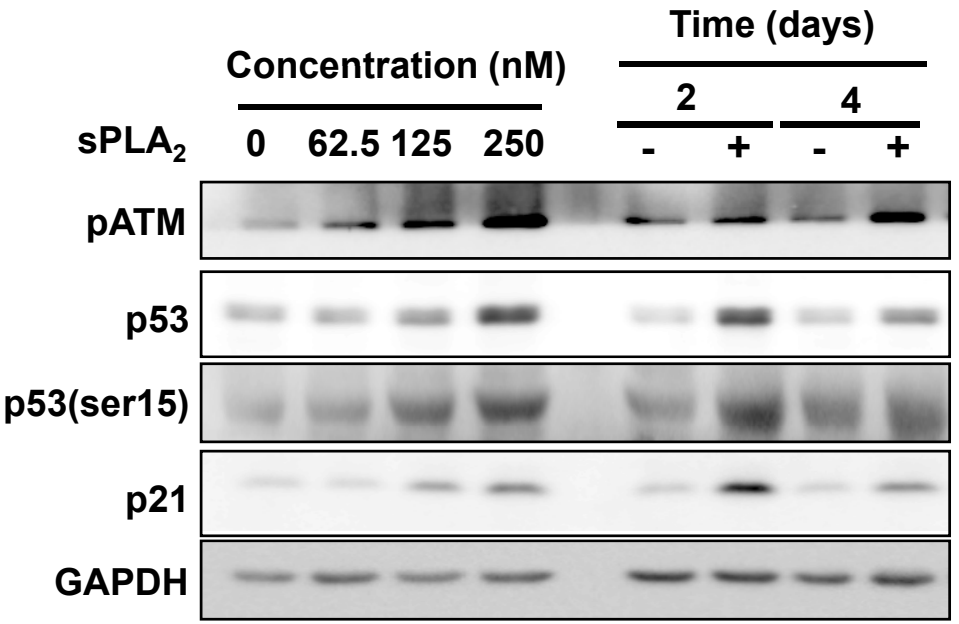

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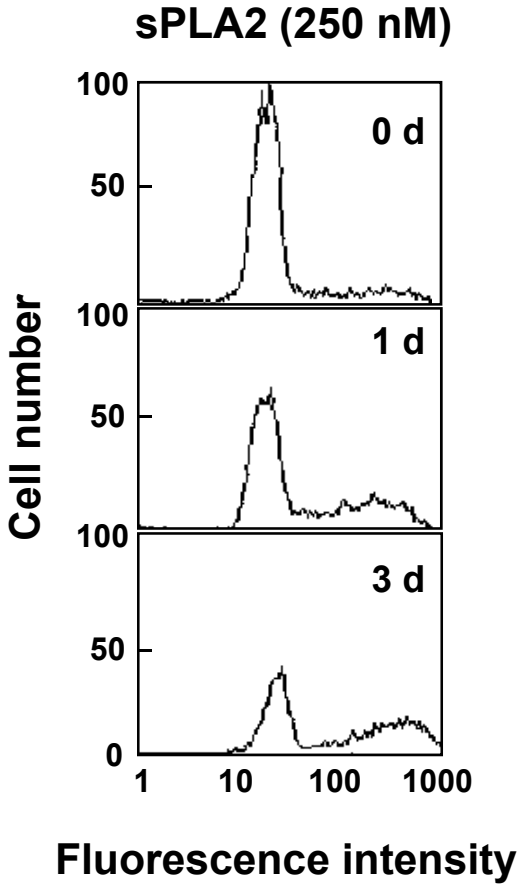
G2 arrest by sPLA2 in HDFs

A



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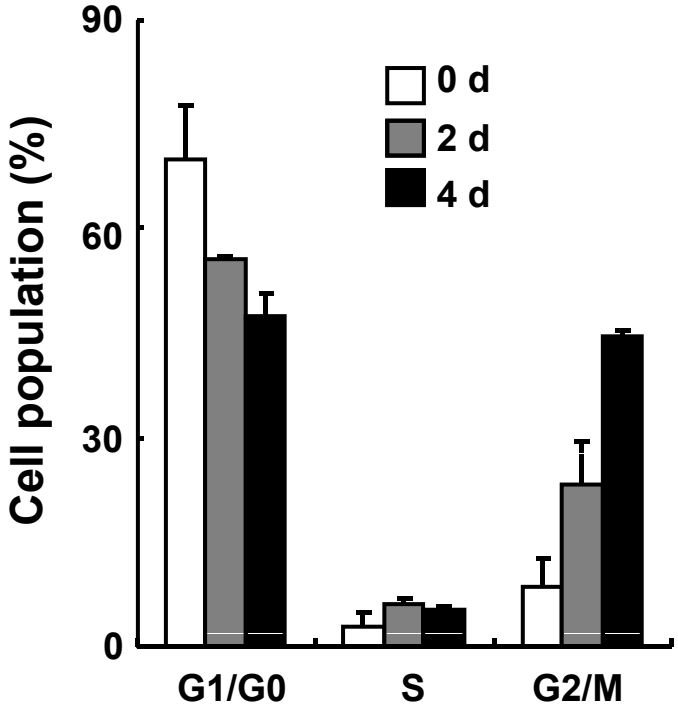
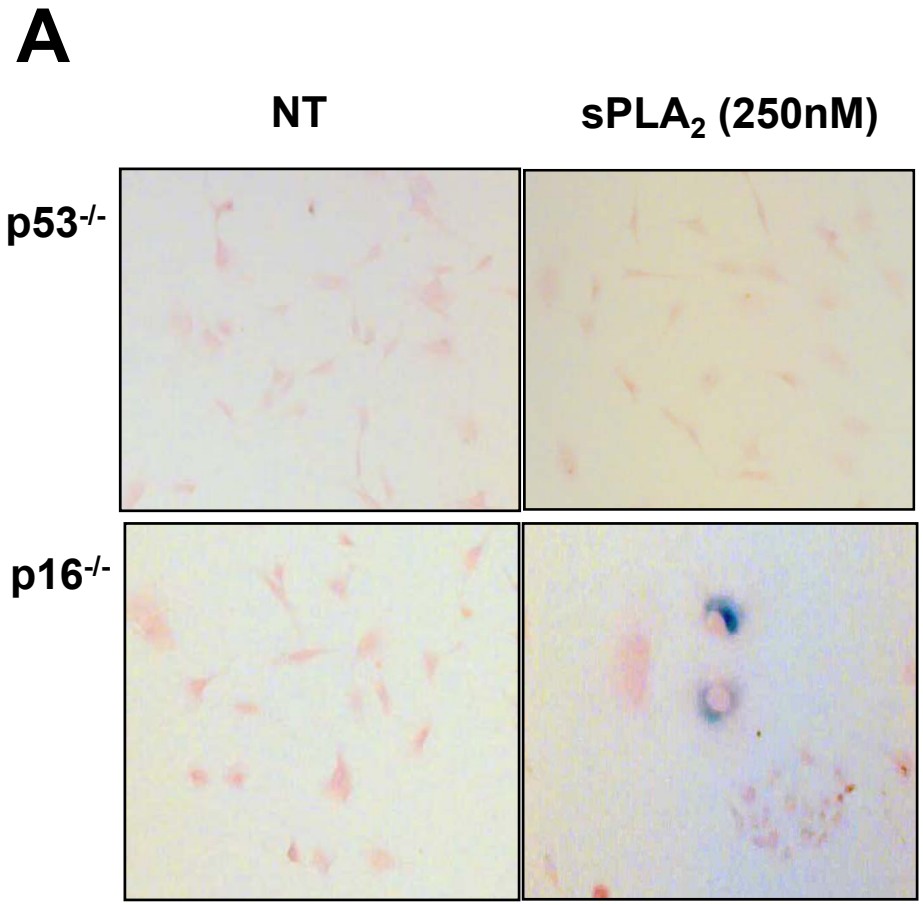
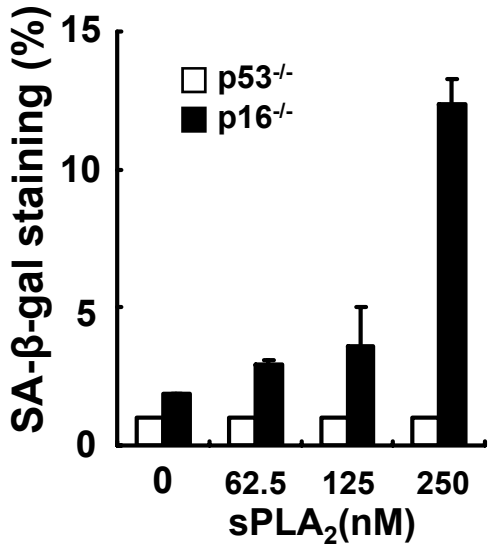


Effect of sPLA2 on cellular senescence in p16-/- and p53-/- MEFs





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AVDRC

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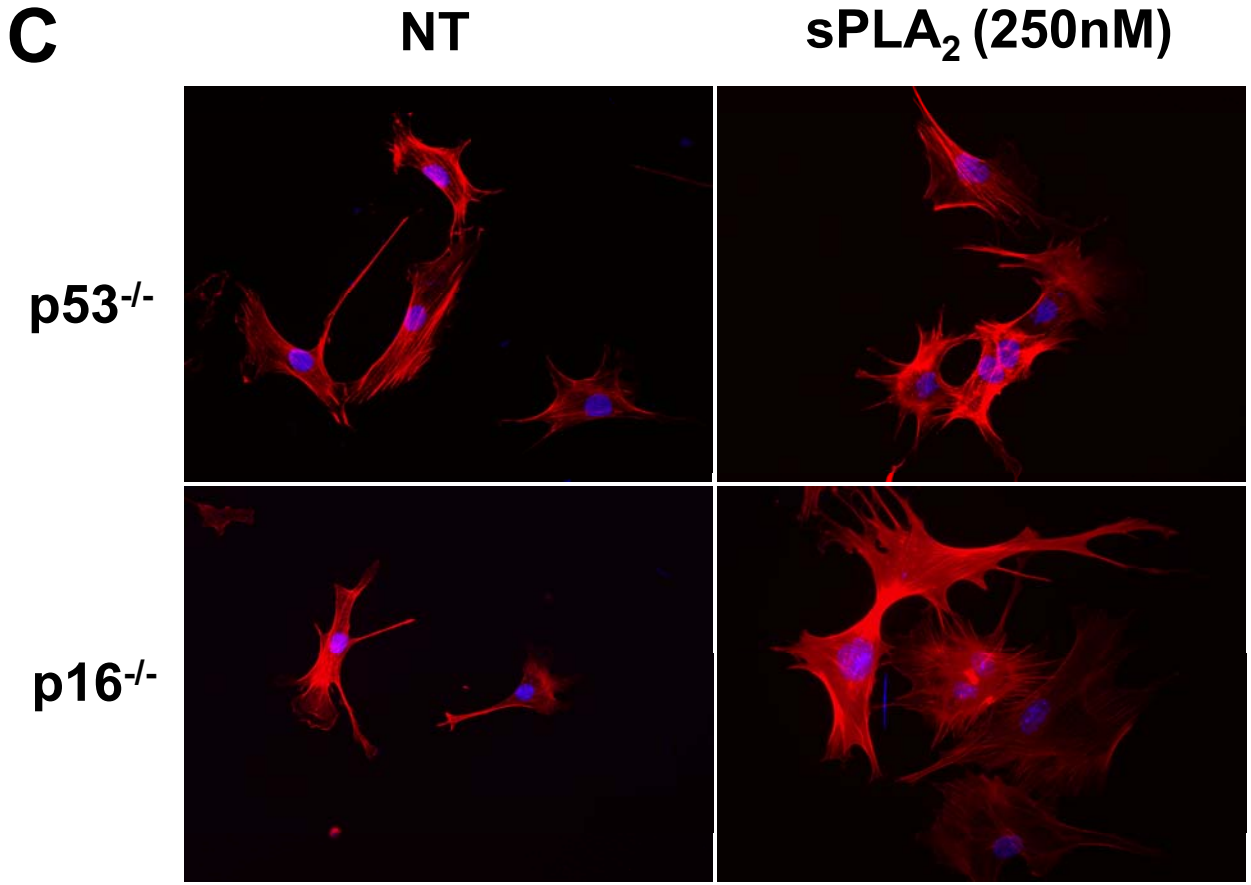
Effect of sPLA2 on cellular senescence in p16-/- and p53-/- MEFs



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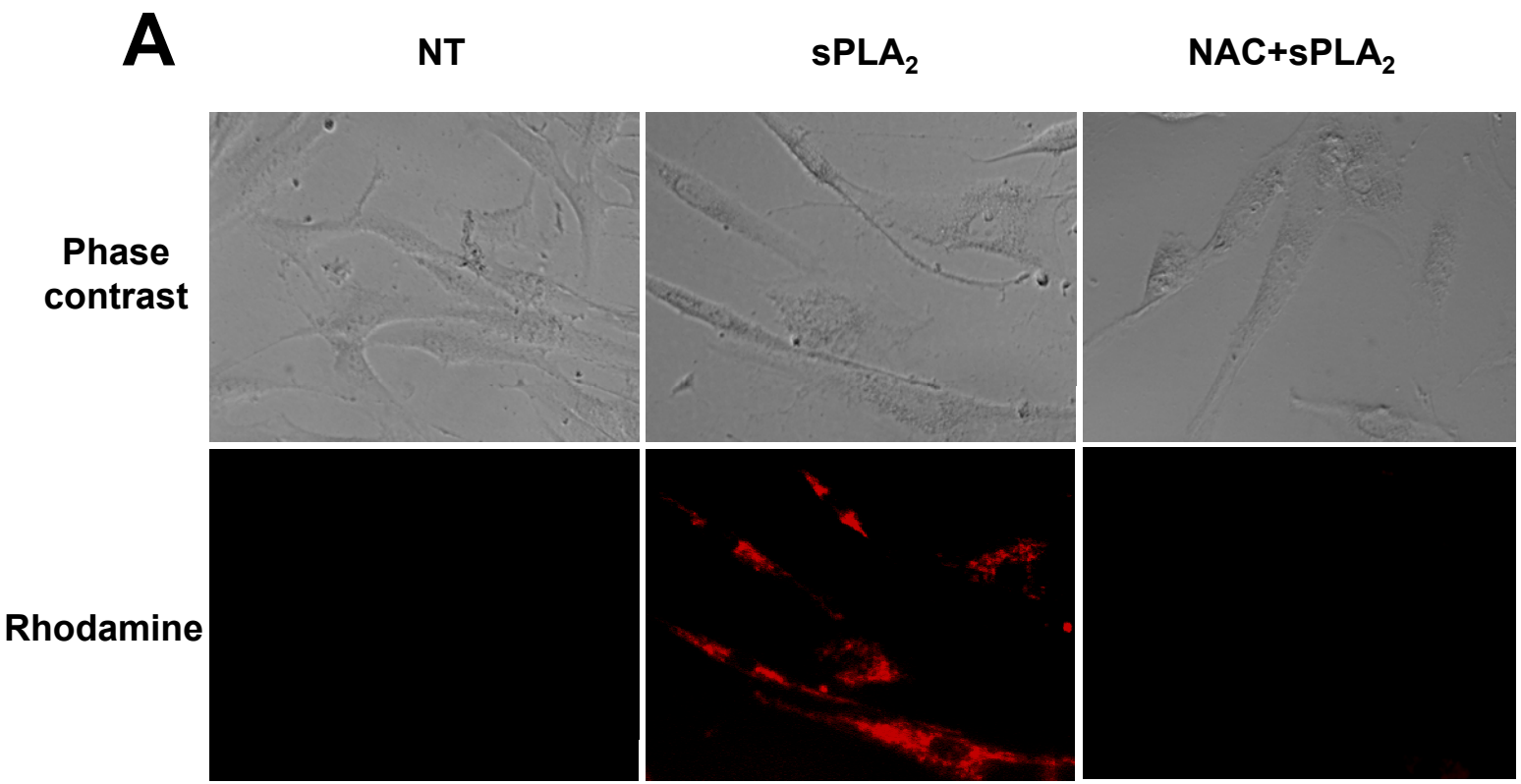
ROS generation by sPLA2 and effect of NAC on the senescence phenotypes of sPLA2-treated cells



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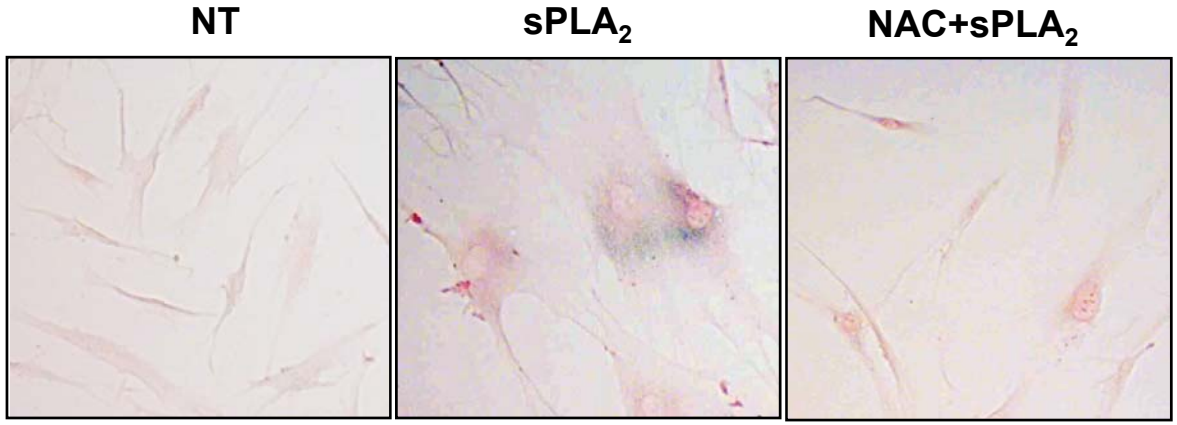

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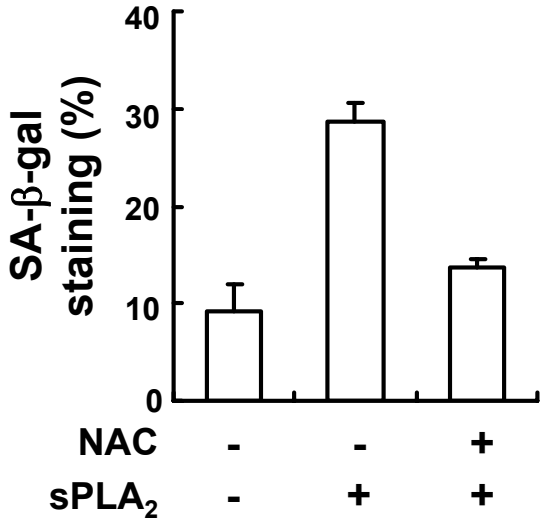

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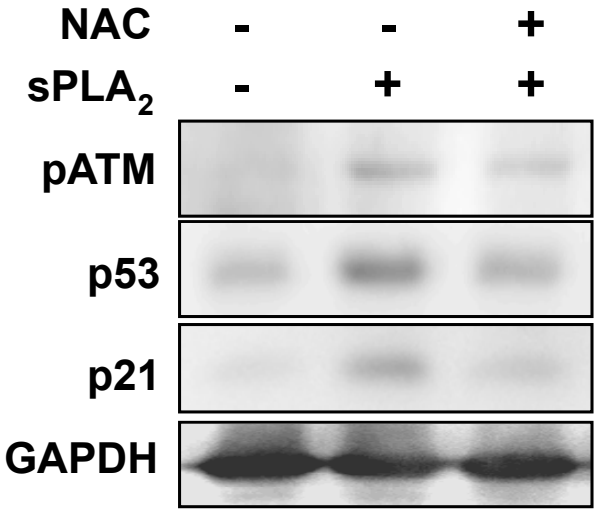

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Summary ($sPLA_2$)

- sPLA2 treatment induces cellular senescence in human dermal fibroblasts (HDFs).
- sPLA2-induced cellular senescence is observed in p16-null MEFs, but not in p53-null MEFs.
- Treatment with sPLA2 increases ROS production, and an antioxidant, N-acetylcysteine, inhibits sPLA2-induced cellular senescence.
- Therefore, sPLA2 plays a role in cellular senescence in HDFs by promoting ROS-dependent p53 activation.

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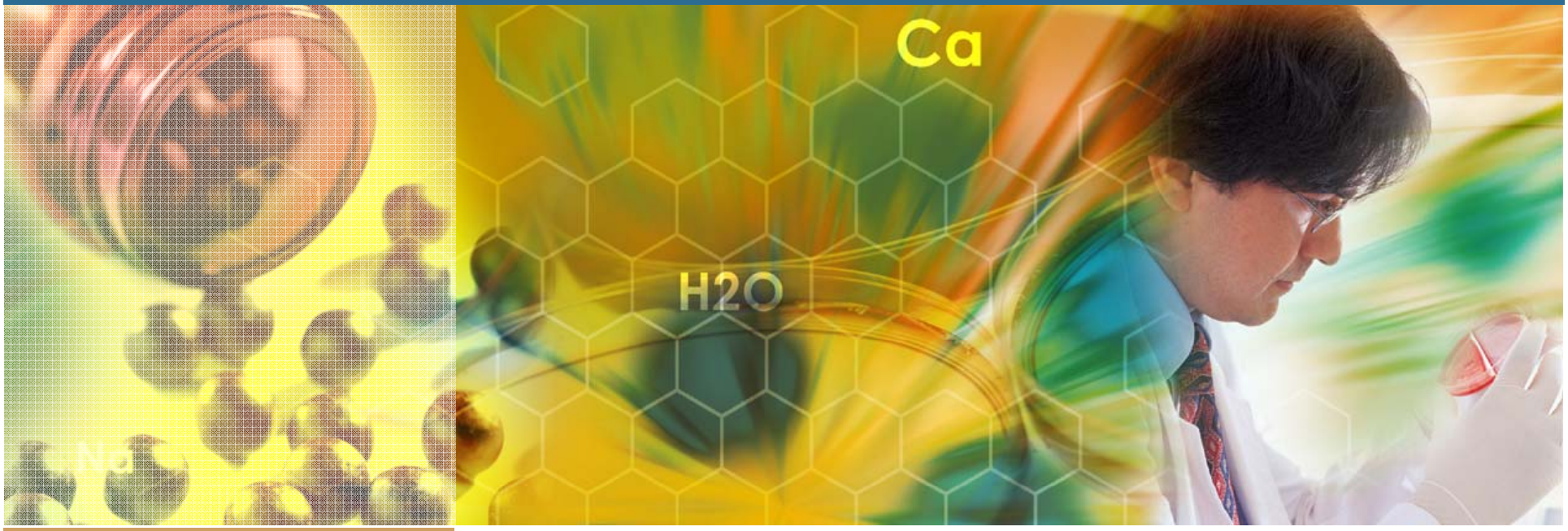
Thank You !



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