

# Aging and Osteoarthritis

## The Role of Chondrocyte Senescence

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on Aging

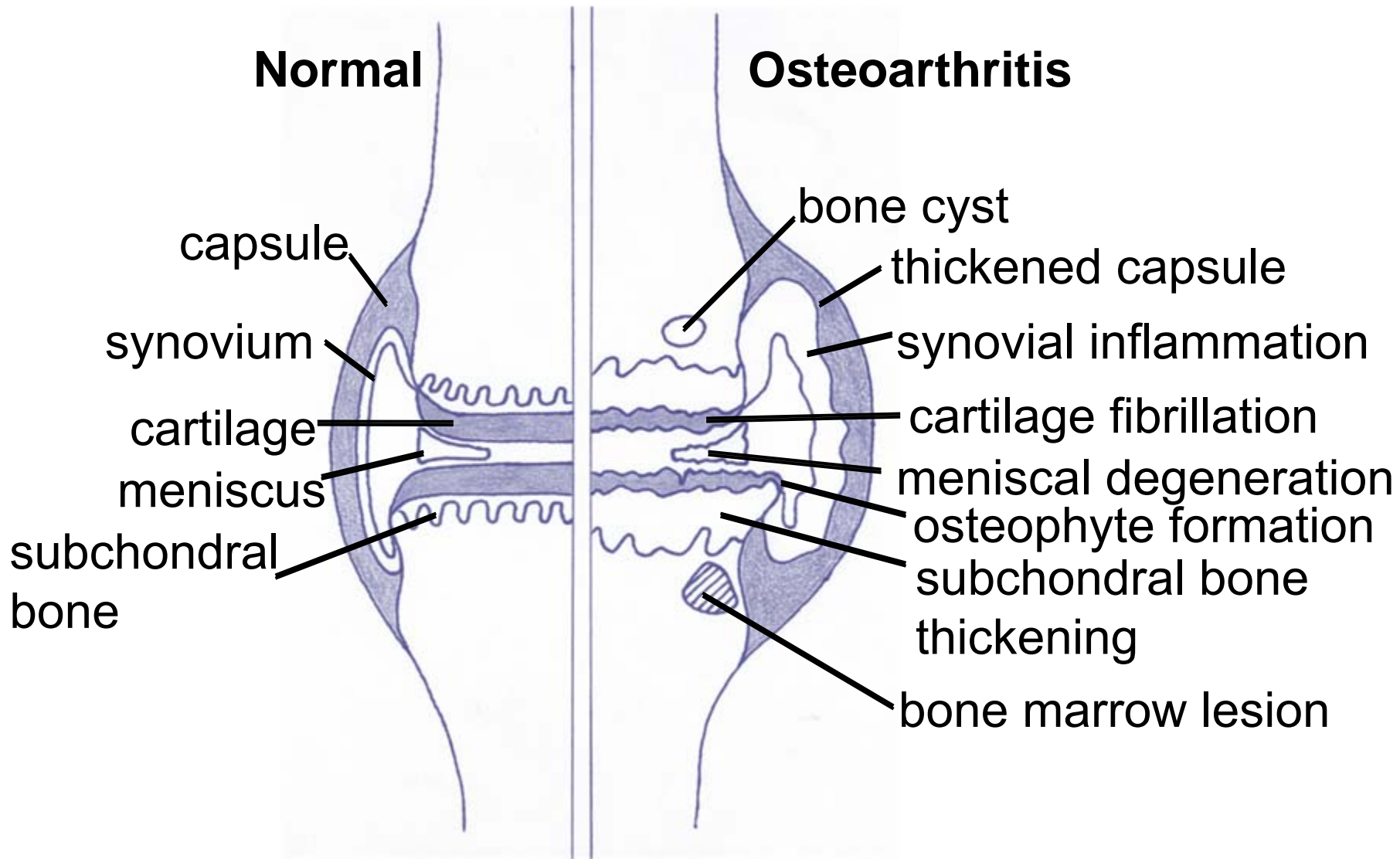


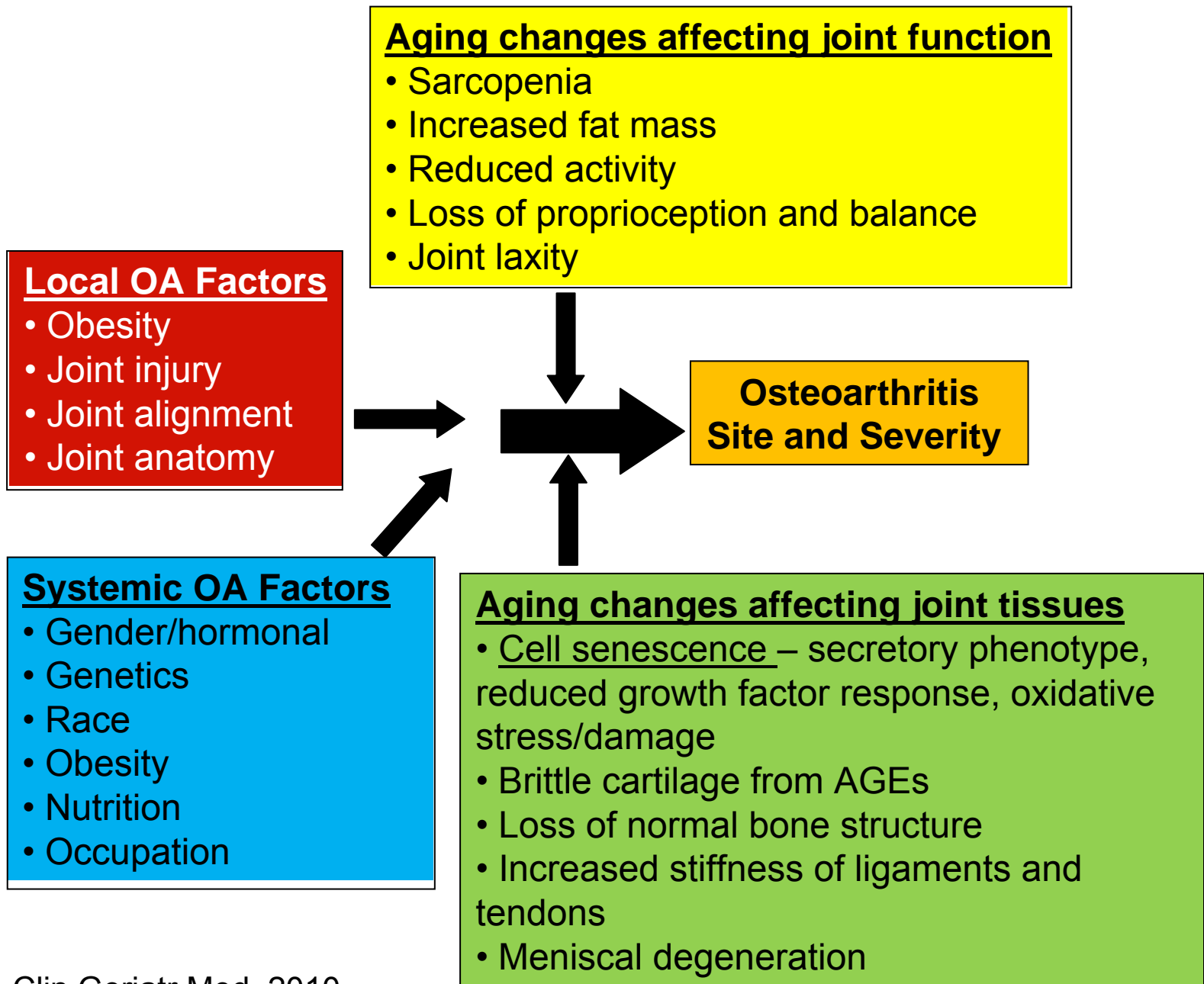
WAKE FOREST

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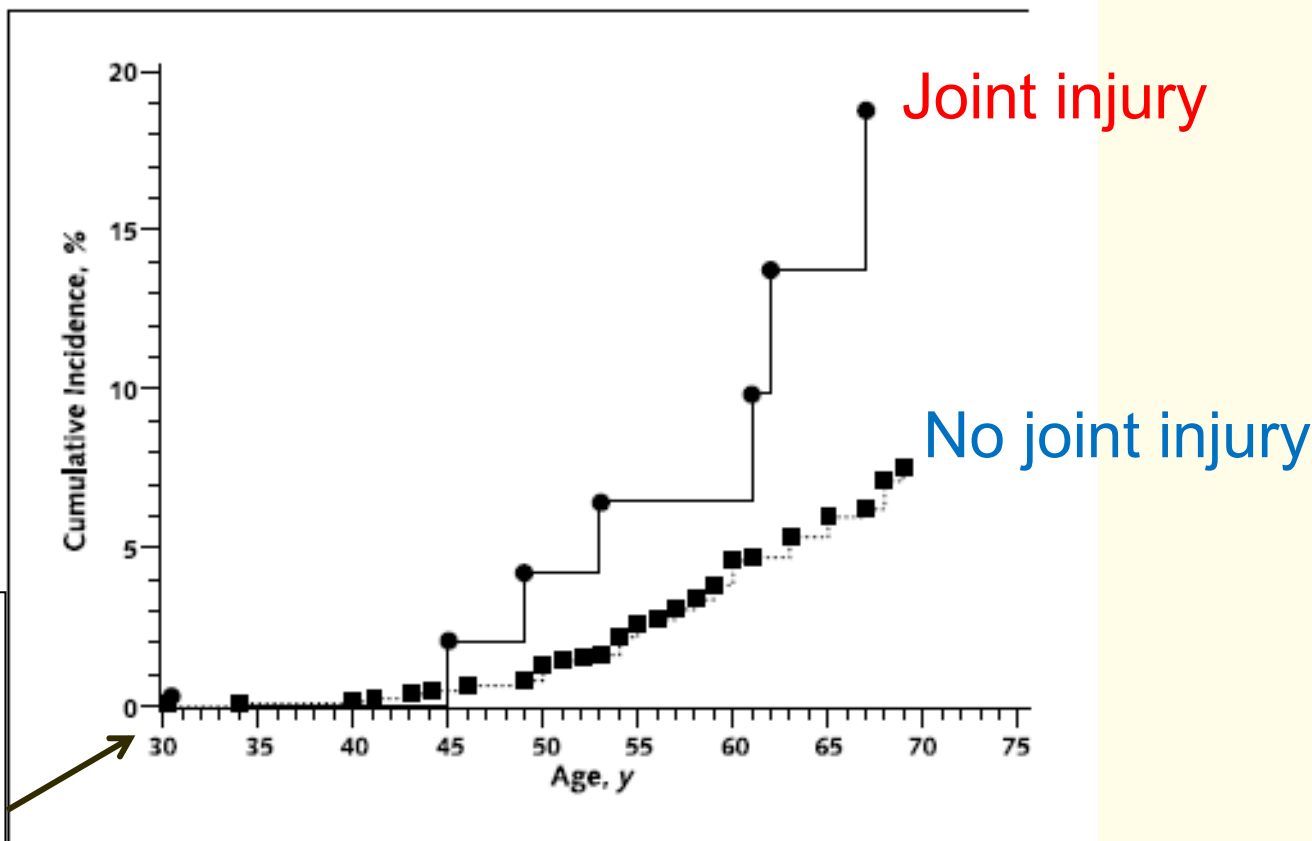
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# Joint injury, aging, and incident OA

Figure. Cumulative incidence of knee osteoarthritis according to injury status at cohort entry.



1321  
Medical  
students –  
joint  
injuries by  
age 22

Kaplan-Meier plots are shown for participants with joint injury (*circles*) and those without joint injury (*squares*) at cohort entry.  $P = 0.0045$  (log-rank test).

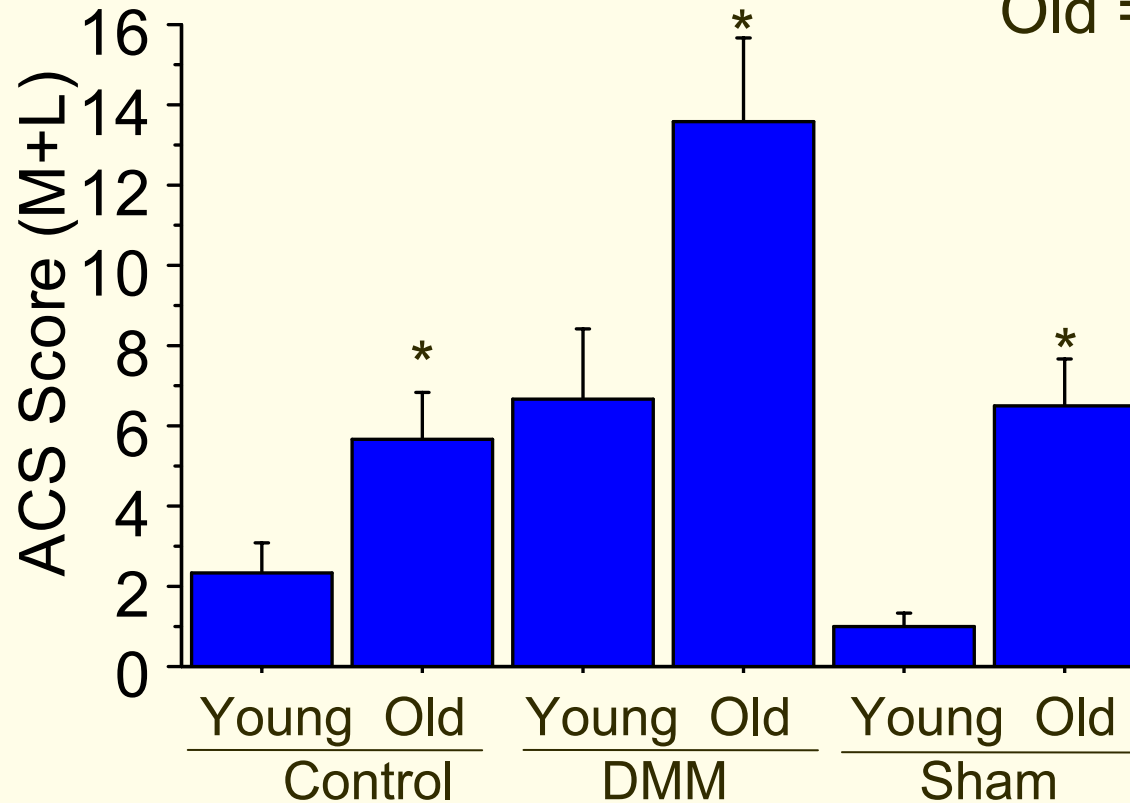
# Aging and “Degenerative Changes” in the Joint



Joints with Collins scores  $>1$  for ankles ( $n = 4005$ ) and knees ( $n = 409$ ) from Loeser & Shakoor, *Rheum Dis Clin N Am*, 2003

# Effect of age on the development of OA in mice

Young = 12 weeks  
Old = 12 months

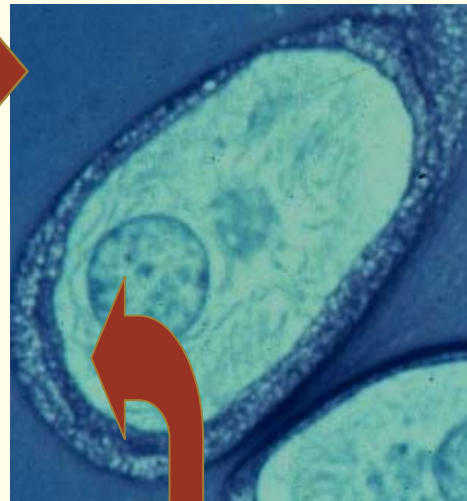
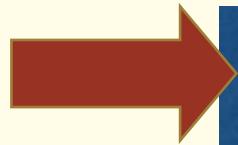


# The OA Chondrocyte: Imbalance of Matrix Synthesis and Degradation

**↑ degradation ± ↓ synthesis**

**↑ synthesis ± ↓ degradation**

**IL-1, IL-6**  
**IL-7, IL-8**  
**TNF $\alpha$ , TGF $\alpha$**   
**Nitric oxide**  
**IL-17, IL-18, LIF,**  
**Oncostatin M**  
**bFGF**  
**S100 proteins**  
**Matrix fragments**



**IGF-I**  
**OP-1**  
**TGF $\beta$**   
**IL-4**  
**BMP-2, CDMPs**



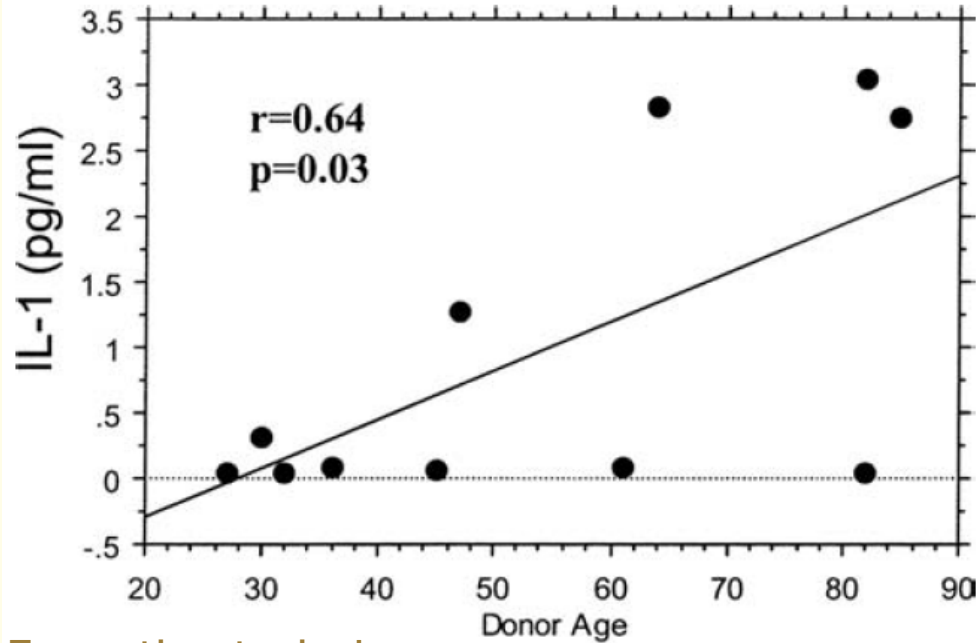
**Matrix metalloproteinases (MMPs)**  
**Aggrecanases, other proteases**  
**Hypertrophic phenotype**

# Senescent-associated secretory phenotype

Freund et al, Trends in Molecular Medicine, 2010

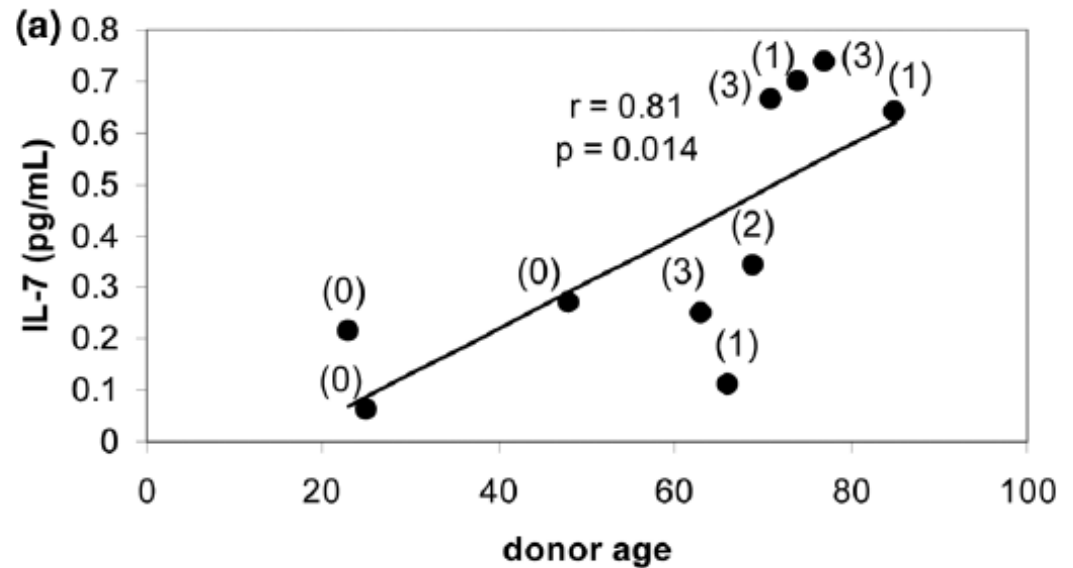
High Increase (4 + fold)	Intermediate increase (2 – 4 fold )	Small increase (below 2 – fold)
GM – CSF	IGFBP - 2	Axl
GRO $\alpha, \beta, \gamma$	IGFBP – 4	bFGF
IGFBP – 7	IGFBP -5	EGF - R
IL – 1 $\alpha$	IL - 13	Fas
IL - 6	IL - 1 $\beta$	G - CSF
IL - 7	MCP - 4	IFN - $\gamma$
IL - 8	MIF	IGFBP – 1
MCP – 1	MIP - 3 $\alpha$	IGFBP – 3
MCP – 2	MMP – 12	IL – 1R1
MIP - 1 $\alpha$	MMP – 13	IL – 6R
MMP – 1	MMP – 14	Leptin
MMP – 10	Oncostatin M	LIF
MMP – 3	Osteoprotegerin	MMP – 2
	RANTES	PA1-1
	TIMP – 2	PA1-2
		SDF-1
		TIMP-1
		TPA
		uPA
		uPAR
		VEGF

# The Chondrocyte – Senescent Secretory Phenotype



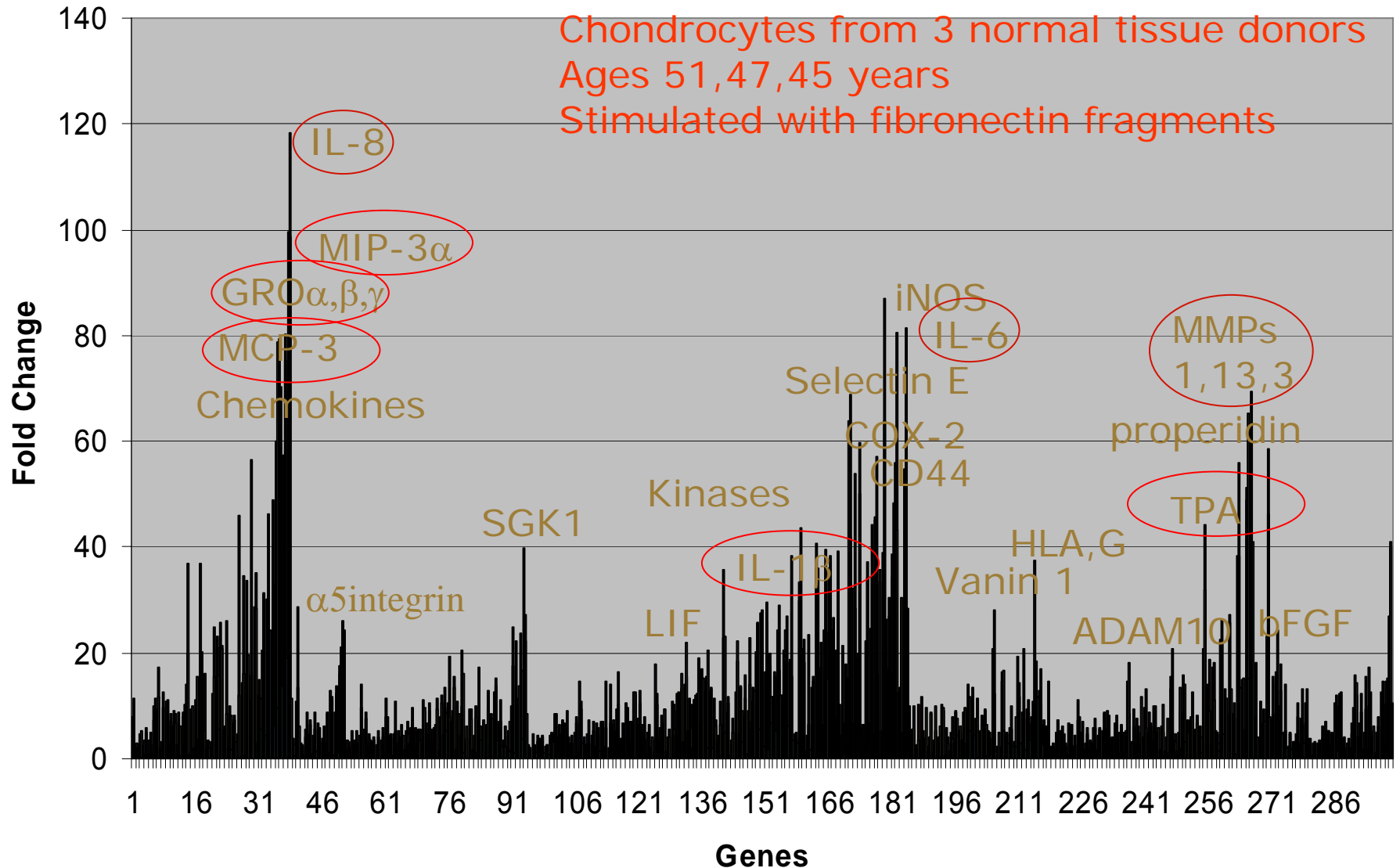
Forsyth et al, J  
 Gerontol Biol Sci,  
 2005

Long et al, Arthritis Res  
 Ther, 2008

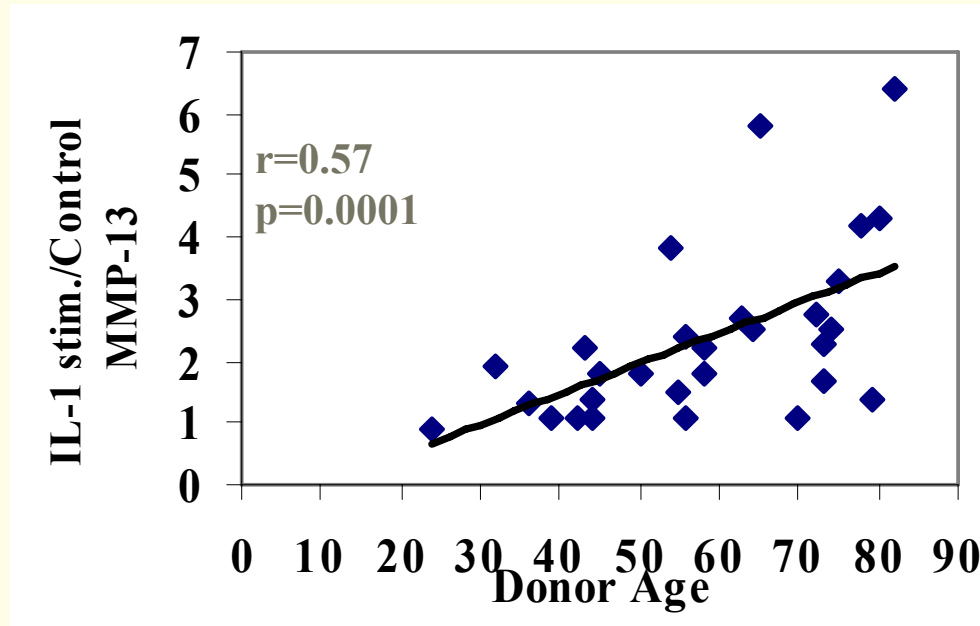
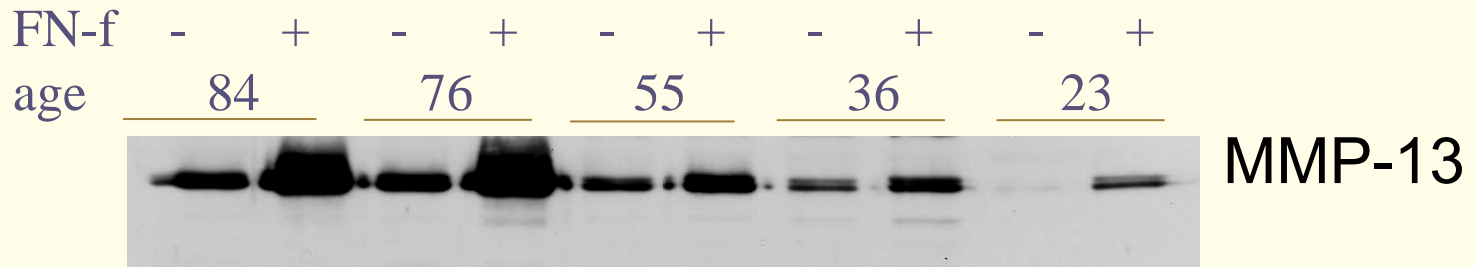


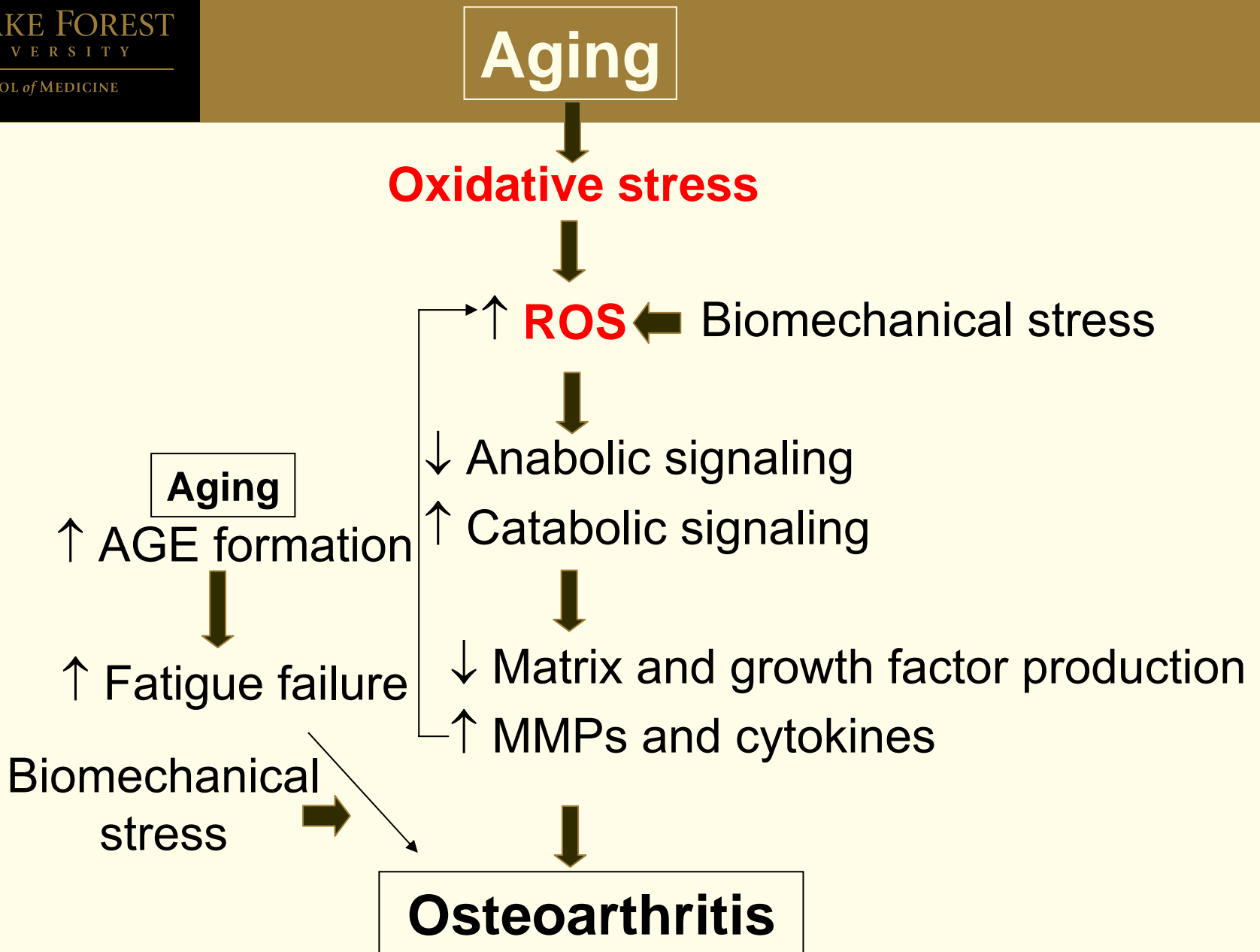
### Affymetrix array

Chondrocytes from 3 normal tissue donors  
 Ages 51,47,45 years  
 Stimulated with fibronectin fragments

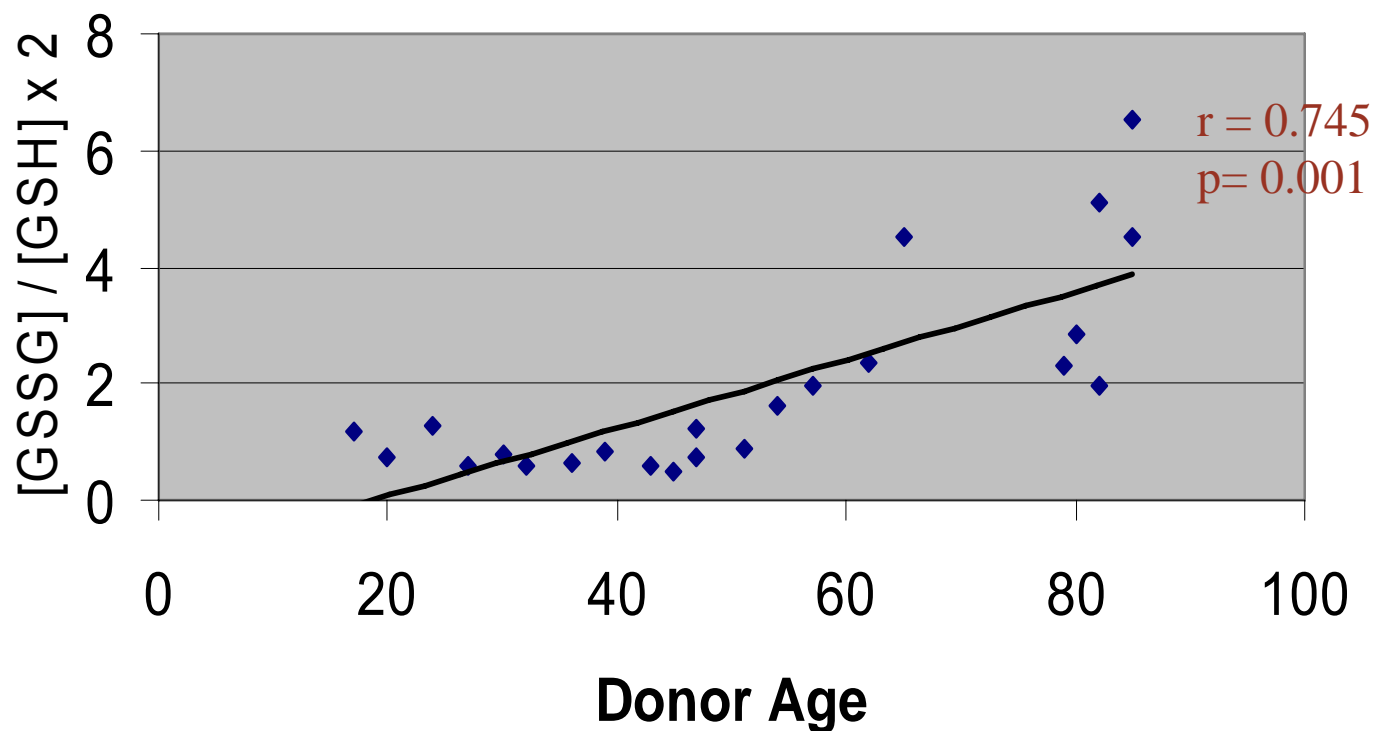


## Effect of age on MMP-13 production when chondrocytes are stimulated with fibronectin fragments or IL-1





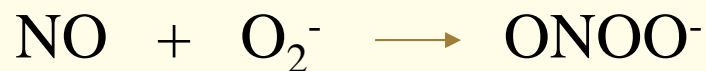
## Ratio of oxidized to reduced glutathione in serum-starved chondrocytes in monolayer



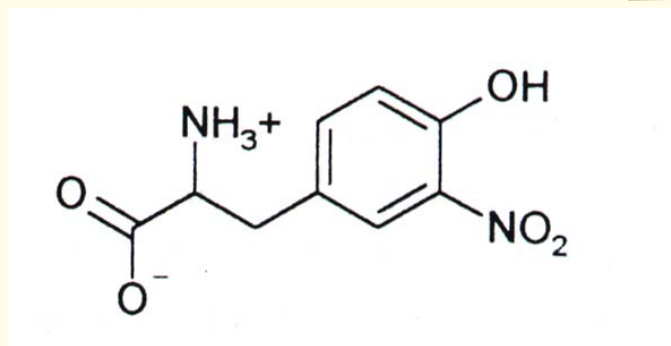
\*Del Carlo & Loeser, Arthritis Rheum, 2003; 48: 3419-3430

# Nitrotyrosine as a Marker for Oxidative Damage

**nitric oxide + superoxide → peroxynitrite**

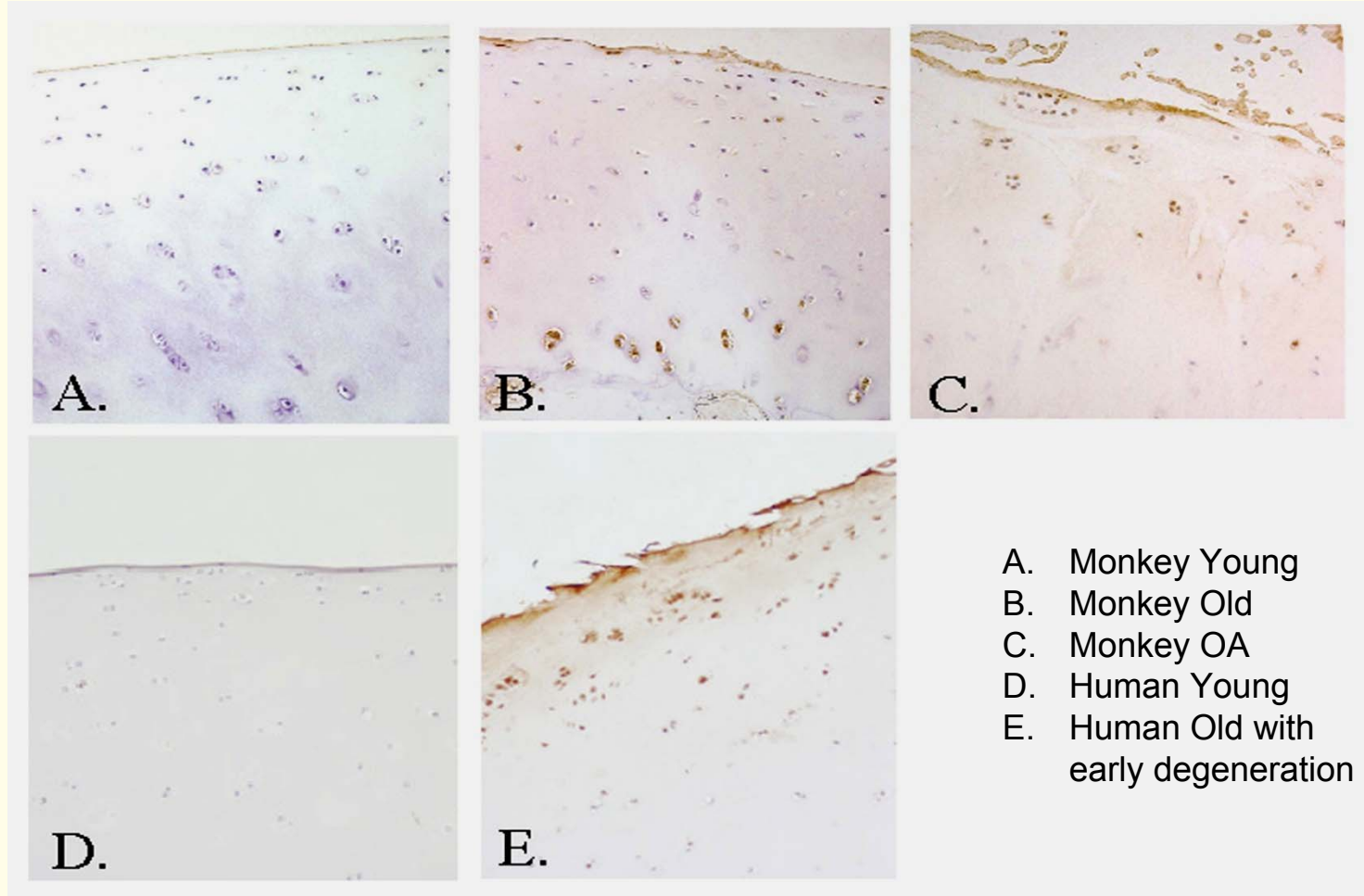


tyrosine residues

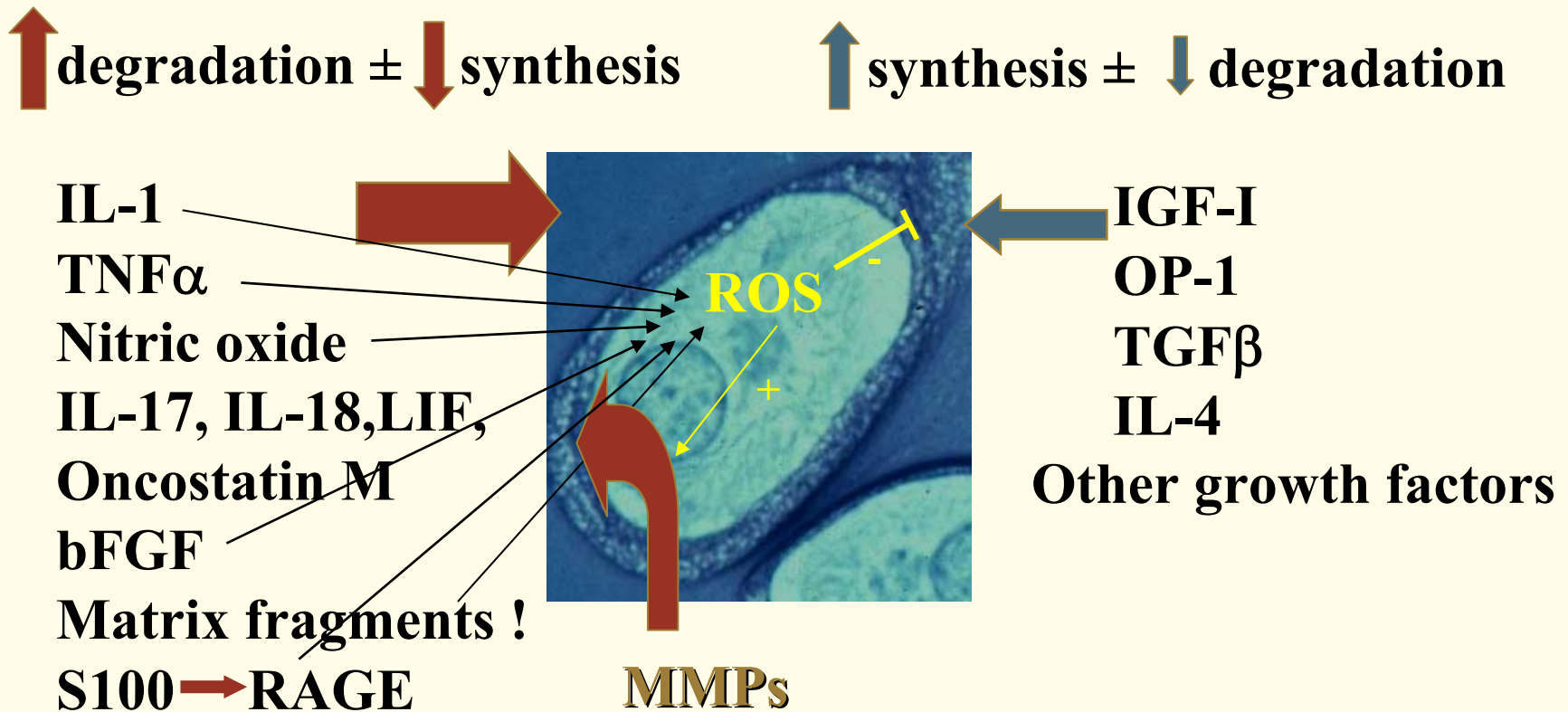


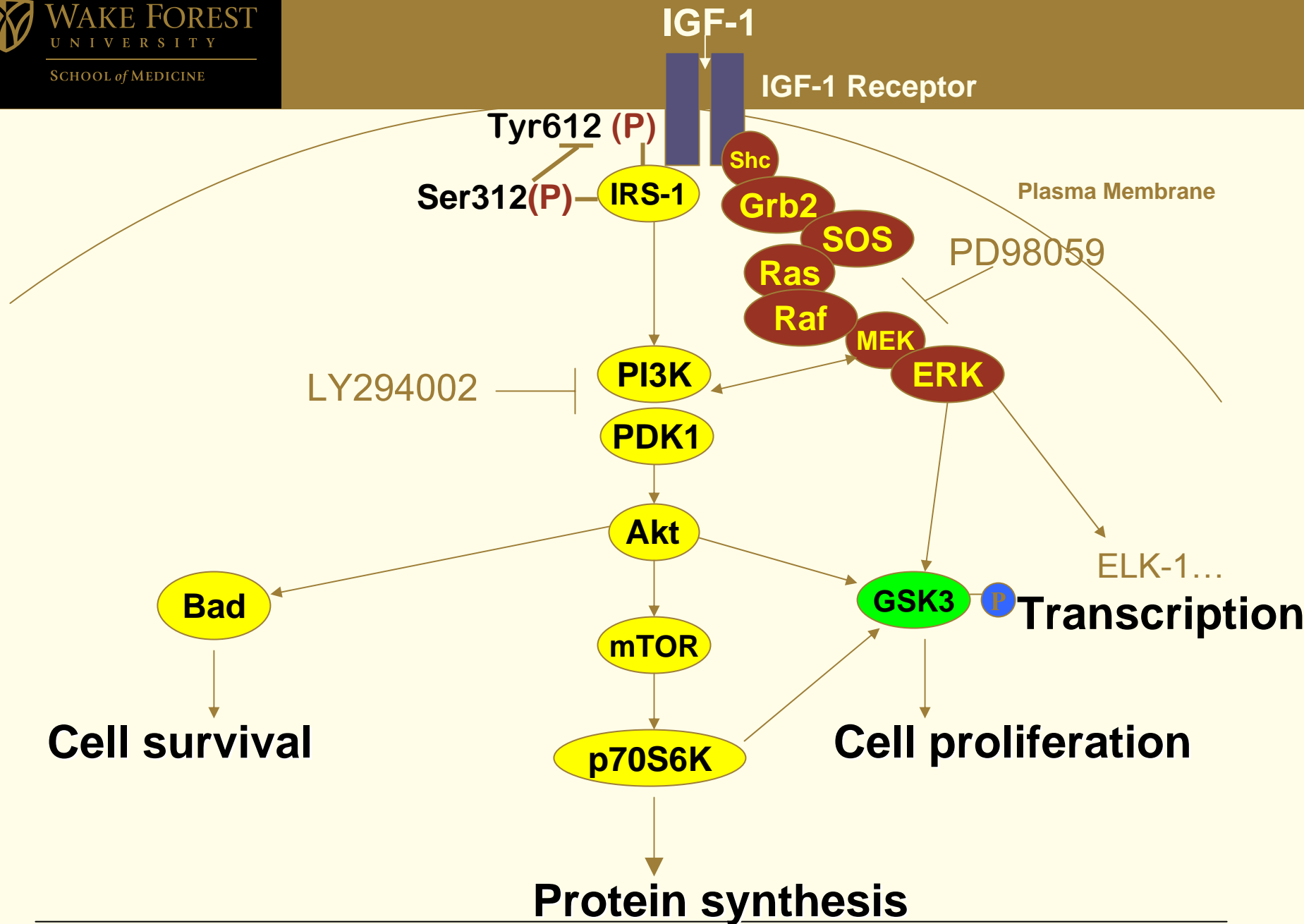
**Nitrotyrosine**

# Does oxidative damage occur in cartilage in vivo? Immunostaining for 3-nitrotyrosine

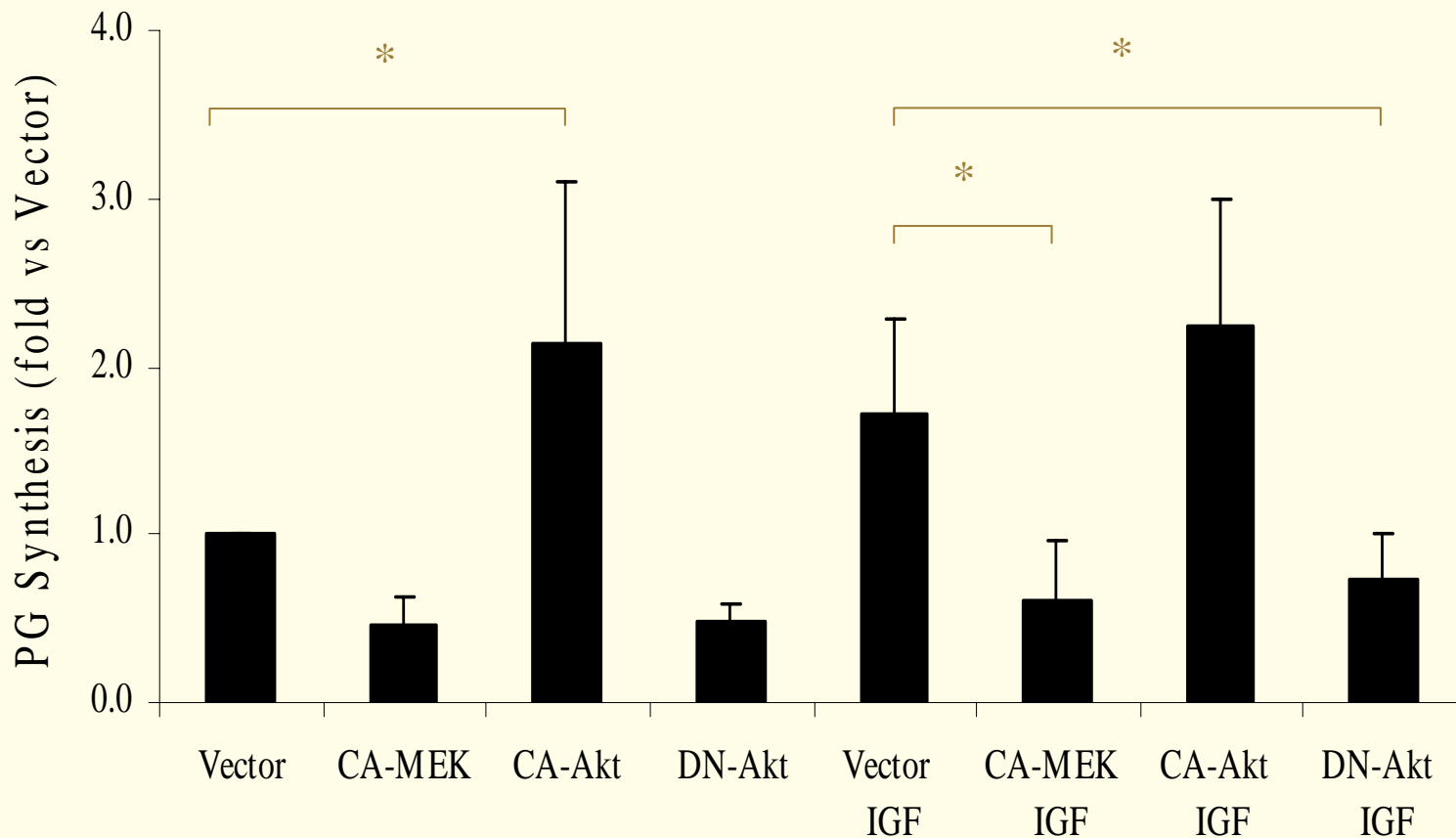


# ROS Can Contribute to the Imbalance of Matrix Synthesis and Degradation with Aging



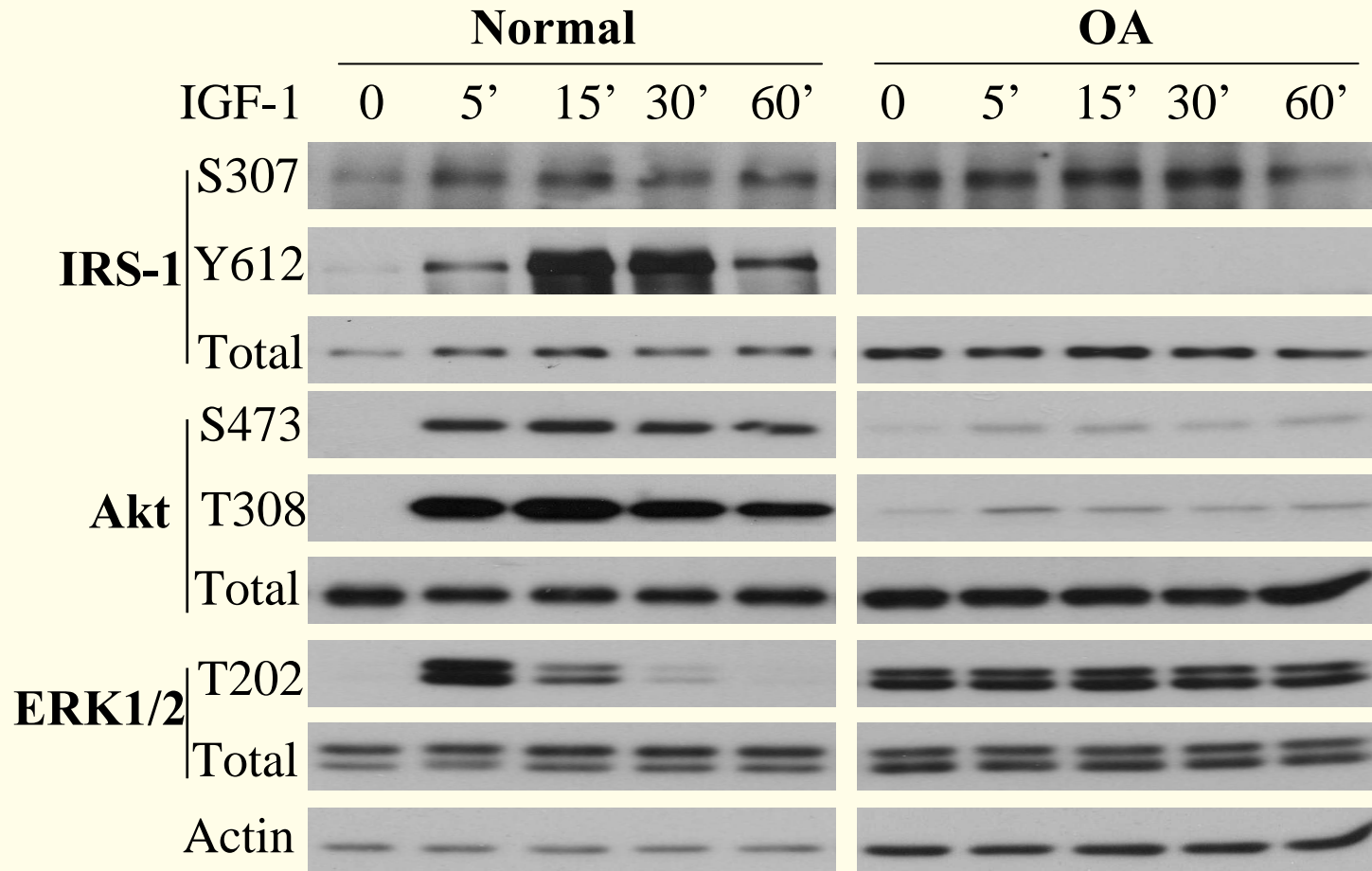


# PI-3 kinase/Akt stimulates while MEK-ERK inhibits PG synthesis

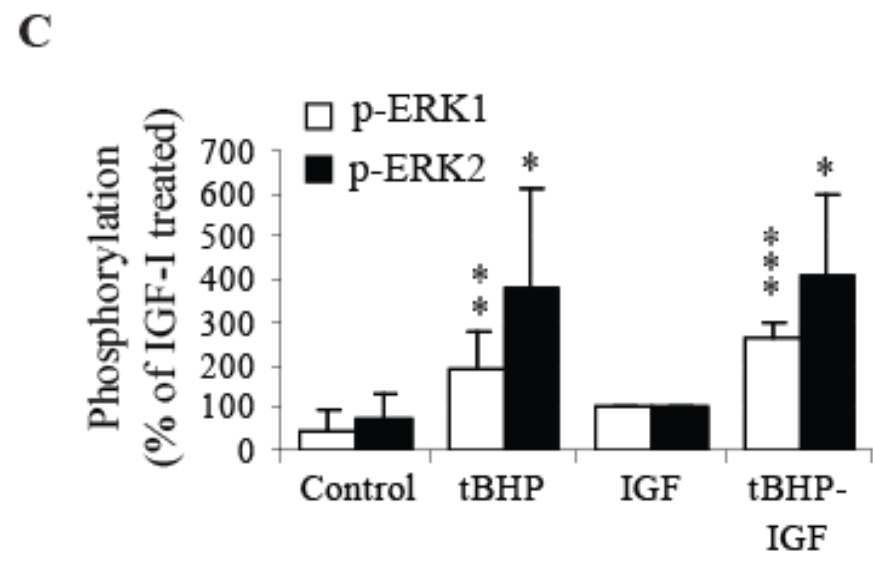
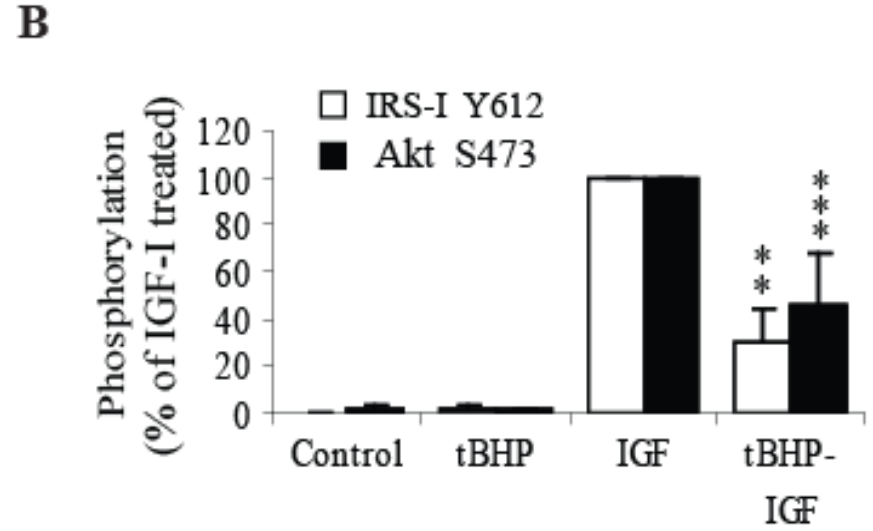
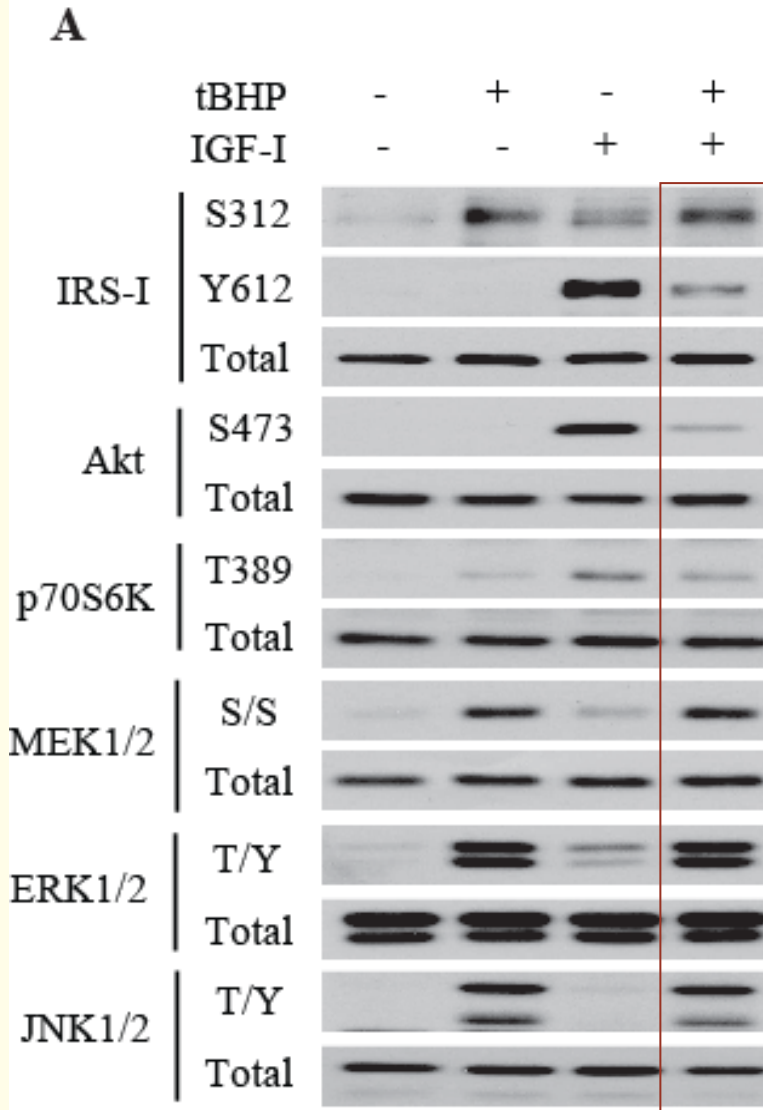


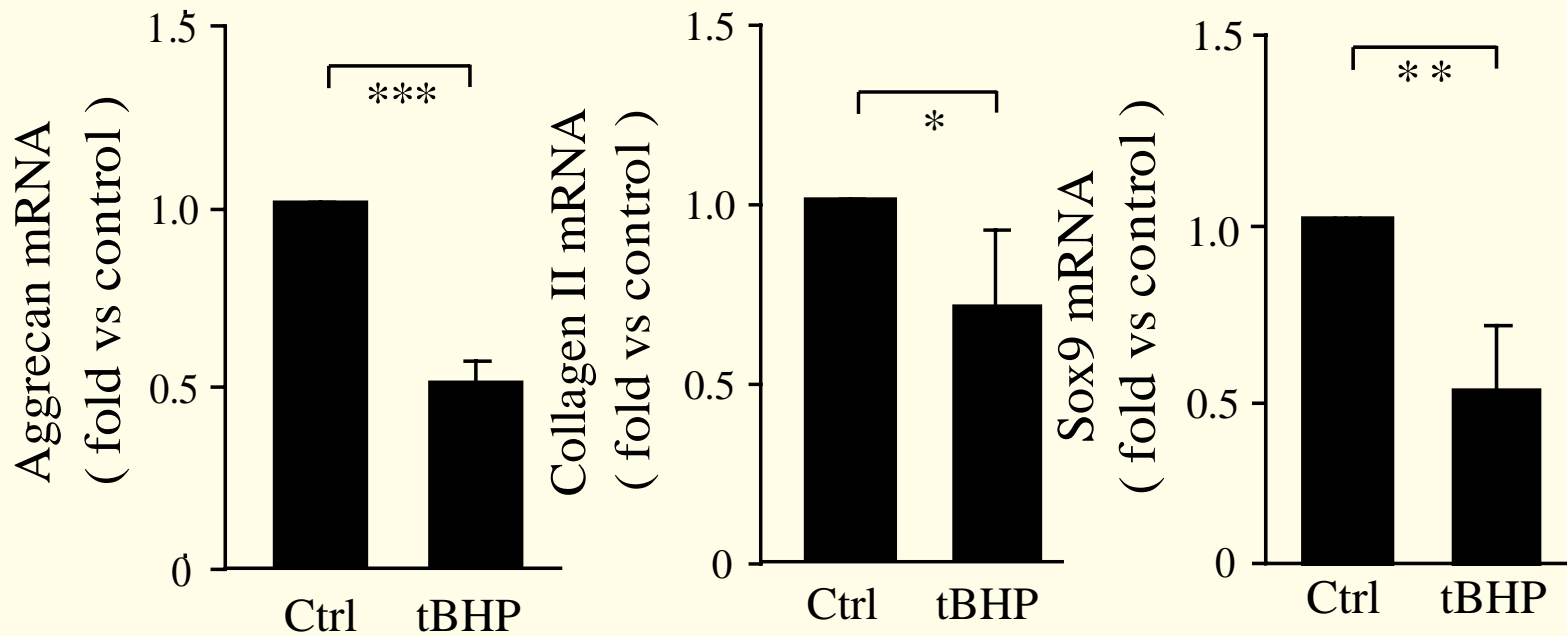
Yin et al, J Biol Chem, 2009

# IGF-1 Signaling: Normal vs OA Chondrocytes

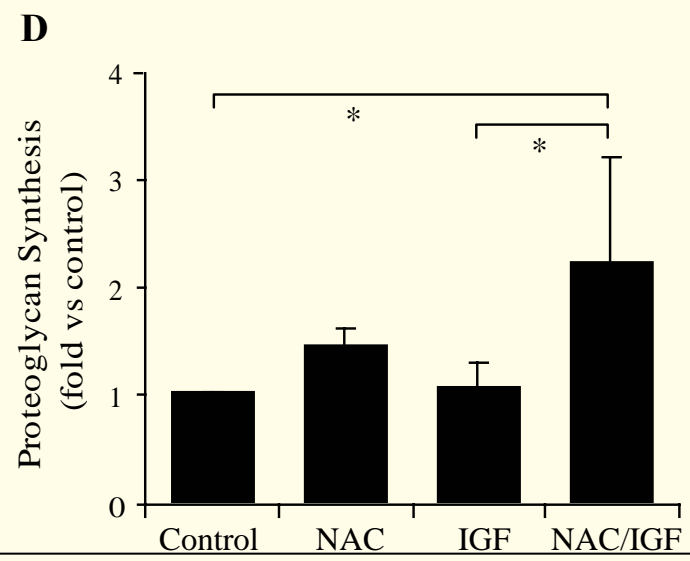
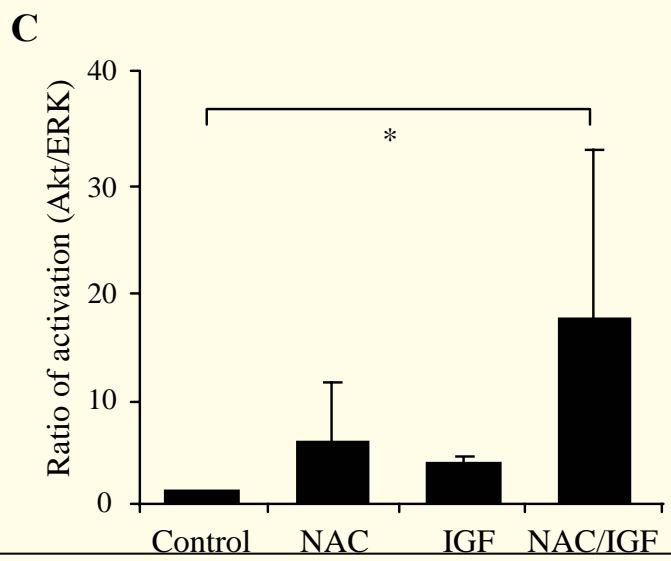
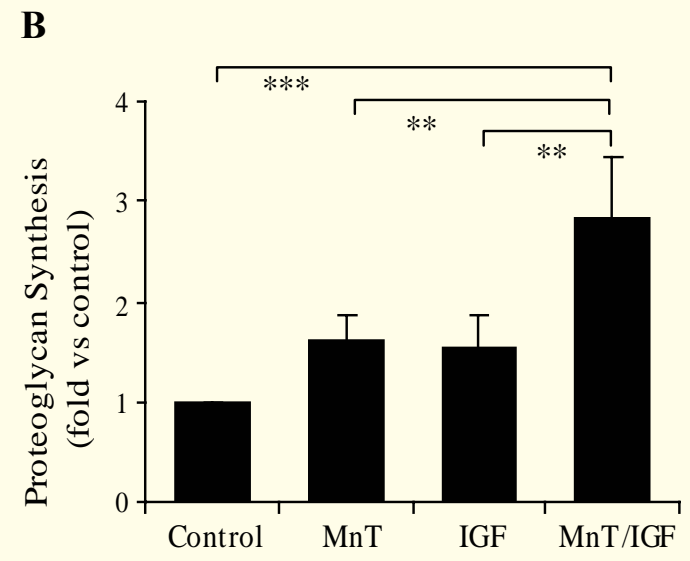
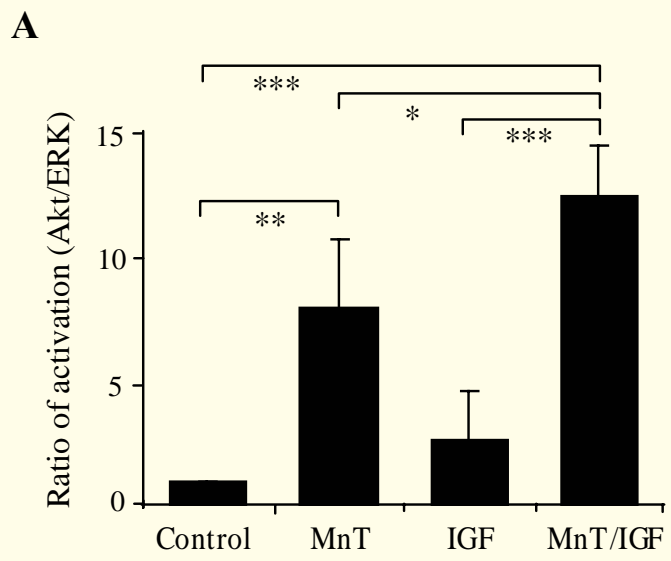


# Effects of oxidative stress on chondrocyte IGF-I signaling

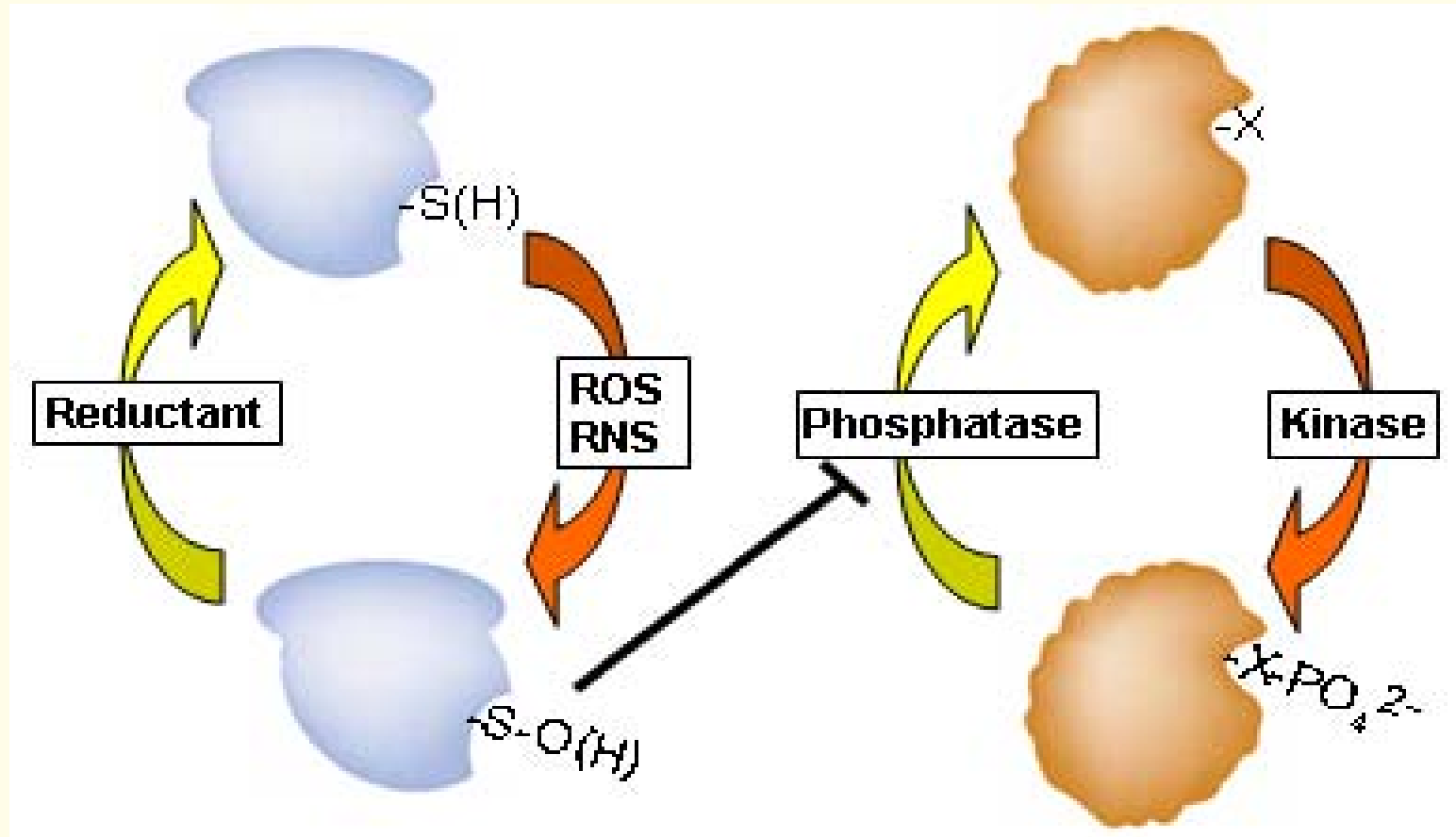




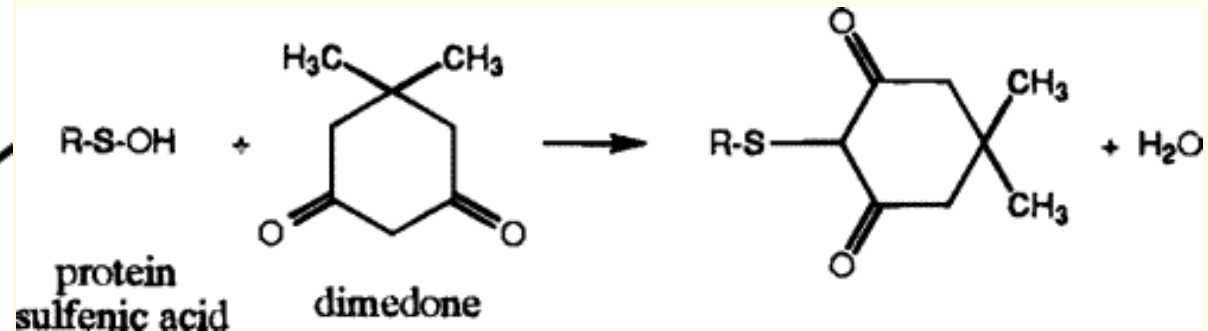
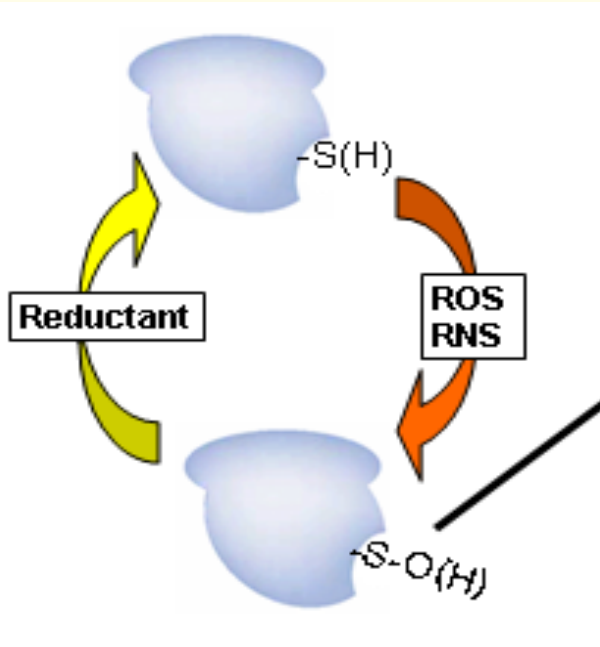
# Antioxidants improve the Akt to ERK activation ratio and improve the OA chondrocyte IGF response



# Redox Signaling: Importance of Cys sulfenic acid formation

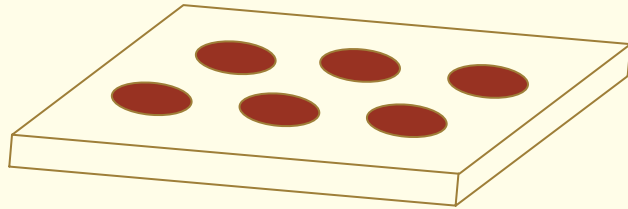


# Use of dimedone to study the role of protein sulfenic acids in redox signaling



# Activity based proteomics: Biotin-dimedone (DCP-biotin) labeling of sulfenic acid-containing proteins

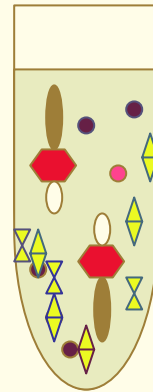
Stimulate cells



Add lysis buffer +  
DCP-biotin

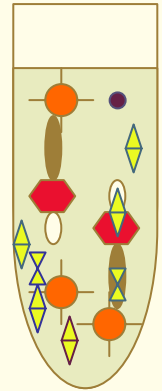


Incubate  
for 30 min  
and  
transfer



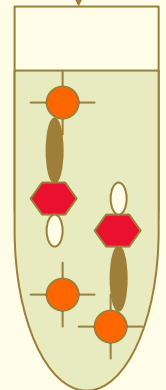
DCP-biotin binds  
proteins w/ SOH

Add  
streptavidin  
beads  
and  
incubate

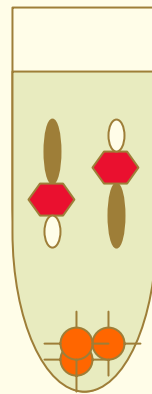


Beads bind biotin  
labeled proteins

Centrifuge and  
wash beads to  
remove  
unbound  
proteins

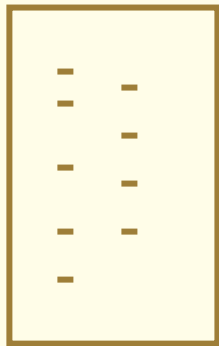


Boil in  
SDS-  
PAGE  
and  
centrifuge  
to elute  
proteins  
from beads



Separate Proteins by SDS-PAGE

Detect by blotting with strepavidin-HRP



- **Reactive oxygen species can inhibit IGF-1-mediated anabolic signaling and promote integrin-mediated catabolic signaling in chondrocytes**
  - **Redox regulation of IGF-1 signaling includes inhibition of the IRS-1/PI3-kinase/Akt pathway and activation of the MEK-ERK MAP kinase pathway**
  - **Redox regulation of integrin signaling stimulated by fibronectin fragments includes the JNK MAP kinase pathway**
  - **Improving the balance of ROS and anti-oxidants in chondrocytes could improve the imbalance of anabolic and catabolic activity that contributes to OA**
-

- **Does adult articular cartilage contain chondroprogenitor cells and if so why don't they replace dead/defective cells ?**
  - **Do secreted proteins from senescent chondrocytes play a central role in OA ?**
  - **Do the aging changes in cartilage reflect similar changes in other joint tissues?**
  - **What is the role of epigenetics ?**
  - **Does age-related oxidative stress play a role in OA?**
-

## Loeser Lab

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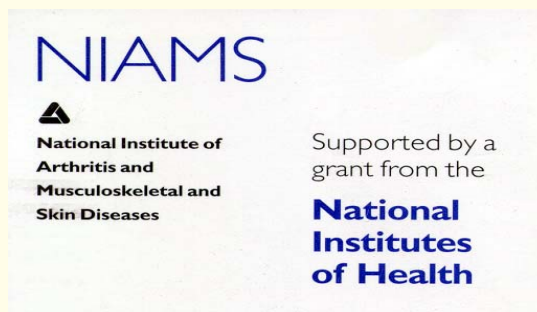
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National Institute  
on Aging ■ ◆ ★ ✨



American Federation for  Research